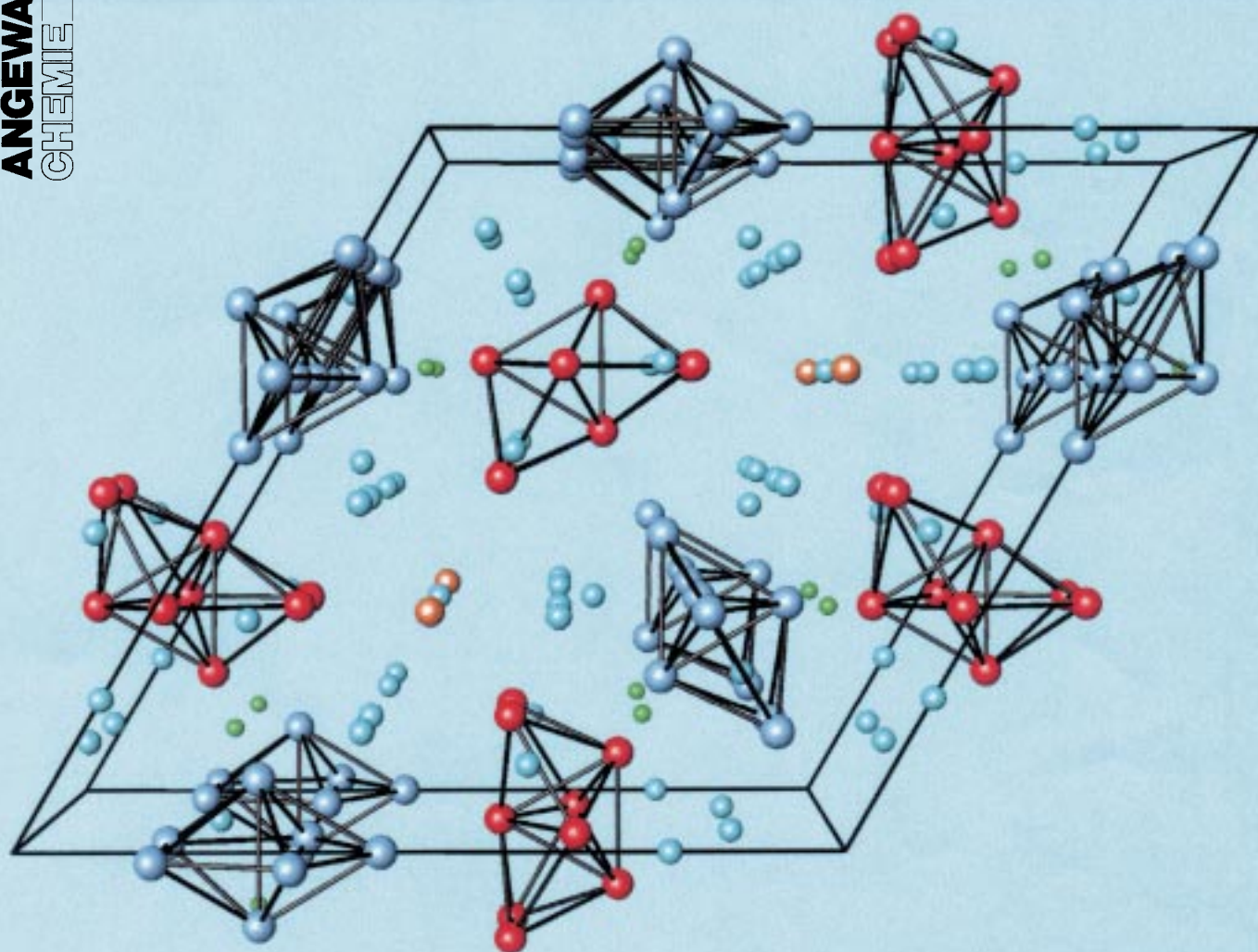
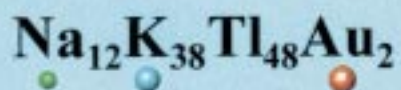


An alkali metal – thallium – gold phase with complex anionic thallium clusters

Polyanionic Clusters and Networks of the Early p-Element Metals in the Solid State: Beyond the Zintl Boundary

John D. Corbett*

Dedicated to Professor Arndt Simon on the occasion of his 60th Birthday

Reduction of p-element (post-transition) metals and metalloids by alkali metals leads to many salts containing polyatomic clusters or network anions of these elements. The earliest solvated examples were referred to as Zintl ions. Synthetic explorations have now established that many of the clusters can in fact be obtained from neat (solvent free) high-temperature reactions of binary to quaternary systems,

particularly for the heavier tetrel (group 14) and triel (group 13) elements. Some synthetic tricks have also proven useful. Electronic guidelines such as Wade's rules, known to account well for other types of electron-deficient cluster bonding, are widely applicable to these compounds, but numerous hypoelectronic (electron-poor) trielide salts have also been discovered. These developments also extend

to related infinite network structures and Zintl (valence) compounds. The Zintl boundary designation traditionally delineated the tetrel elements that form salts with the active metals from those of the triel and earlier elements that were once thought to generate only intermetallic phases. The distinction no longer seems appropriate, at least with regard to some alkali-metal compounds of the triel elements.

It is always difficult to predict the unimaginable

1. Introduction

A great deal is known and understood about metal–metal bonding of the transition metals in well-reduced systems, both as ligated clusters and in extended solids; the number and variety of examples arising in large part because many good bonding d orbitals are available. An analogous chemistry for the pretransition metals appears to be precluded by their diffuse orbitals and weak metal–metal bonds, although the novel delocalized bonding about simple anions in a few metal-rich arrays characterized by Simon is a unique feature of alkali-metal chemistry.^[1] The chemistry of the alkaline-earth metals (including magnesium) seems to be unusually bereft of significant metal–metal bonding features beyond the elements themselves and a few nitride compounds. In contrast, the comparative metal–metal bonding among compounds of the main group p-metals (metametal, metalloids) with the active metals is widespread and anomalous with regard to

traditional examples. On the other hand, aluminum chemistry in intermetallic systems does not correlate well with catenation characteristics of the other p-metals, although this element does show a remarkable solid-state chemistry of its own. Thus, another classification for this review could be in terms of the cluster and network chemistry of the post-transition elements. In this regard there is still little basis for the separation of silicon from germanium chemistry.

The post-transition metals are themselves highly irregular or nonclassical in terms of structures and properties and are often not considered as “real” metals.^[2, 3] Compounds of the heavier p-metals also tend to be relatively electron-poor, to the degree that the ns^2 pairs act increasingly corelike in reduced phases. Perhaps something unusual should have been anticipated with respect to their element–element bonding (catenation) in the electron-rich systems, but as it was not, a surprising length of time passed before these features were discovered or generally recognized. Catenation is, of course, well known for boron, carbon, silicon, and so forth. Indeed, elementary views of the bonding in clusters of the heavier metal analogues in Zintl phases and ions (see below) are based on precepts similar to those developed for the non-metals—namely, Wade's and the octet rules. The former were the first to organize in an empirical manner the compositions, electron counts, and geometries arising with the delocalized electron-deficient cluster bonding in the boron hydrides and boranes,^[4] which in fact have clear and functional parallels in

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“naked” clusters of the heavy p-metals because of the increasing inertness or energetic isolation of the ns^2 pairs.^[5]

The applicability of the octet rule and the occurrence of anionic, homoatomic analogues of some catenated nonmetals were first recognized among alkali-metal “salts” of the heavy metals and metalloids. Such salts are now often termed Zintl (or Zintl–Klemm) or valence phases (broadly expanded to include modern bonding concepts),^[6–11] while polyanions of the heavier metals, once known only in solution with liquid ammonia, are termed Zintl ions.^[11, 12] The further distinction of the Zintl boundary (or Zintl border) was introduced by Laves^[6, 11] as the dividing line between what we chose to call the triel group (group 13, aluminum or gallium family, symbol Tr) and the tetrel group (group 14, silicon family, symbol Tl).^[13] The border itself had been defined earlier by Zintl^[14] as the left-hand limit on the periodic table for elements that allow the formation of 1) monoatomic (8- N) anions (Sb^{3-} , Sn^{4-} , ...) in saltlike structures of compounds richest in an active metal (for example Mg_2Sn in the anti-(CaF_2) structure family) as opposed to alloylike phases,^[13] and 2) polyanions under strongly reducing conditions in liquid ammonia that were later designated^[15] as Zintl ions. All assignments of Zintl’s name to these categories occurred posthumously.

In this article we relate the considerable amount of new chemistry that has been discovered for the earlier p-metals within the last 25 years, with emphasis on the newer developments in the solid state. A review of similar material appeared in 1996.^[16]

2. Traditional Zintl Ions

In the 1930s, Zintl provided the first substantial characterizations of what became termed Zintl ions, although fragmentary evidence regarding the unusual green solutions of lead metal (or its halides) when treated with sodium solutions in liquid ammonia had been known since the 1890s. As reviewed earlier,^[12] his extensive studies led to empirical formulae for a variety of what appeared to be polyanionic salts of the post-transition metals in liquid ammonia, for example Sn_9^{4-} , Pb_9^{4-} , Pb_7^{4-} , Sb_3^{3-} , Bi_5^{3-} , Bi_7^{3-} . Alkaline-earth metals did not afford this chemistry, nor did the potential cluster-forming elements (Ge, Si, Ga, In, or Tl) when

introduced with alkali metals (hereafter noted as A in formulae). However, none of the salts with ammoniated cations was sufficiently stable to yield crystalline products at room temperature. A few Zintl anions were isolated much later as ethylenediamine (en) complexes of the sodium salts according to analytical results, but only an incomplete crystal structure was achieved for $Na_4(en)_7Sn_9$.^[17] The first general route to the isolation of salts of the Zintl anions was accomplished through provision of a stronger chelating agent for the cations, especially [2.2.2]cryptand (“crypt”). We will see later that many of these “ionic” tetrelide clusters, as well as new trielide examples, have in fact been isolated recently as neat binary compounds with the alkali metals, which are closer to the emphasis intended in this article.

2.1. Alkali Metal–Cryptand Salts of Zintl Ions

An updated list of the polyanions isolated from solution and structurally characterized, generally as complexes with cationic sodium or potassium encapsulated in [2.2.2]cryptand, is given in Table 1. (Note that what we list for all of these examples are oxidation state assignments and not the real

Table 1. Zintl anions isolated from molecular solvents with alkali-metal–cryptand cations.

Ion	Symmetry ^[a]	Skeletal p-electrons	Reference
Sb_4^{2-} , Bi_4^{2-}	D_{4h} (arachno)	$2n+6$	[18, 19]
$Sn_2Bi_2^{2-}$, $Pb_2Sb_2^{2-}$, $InBi_3^{2-}$	$\sim T_d$ (nido)	$2n+4$	[20–22]
Sn_5^{2-} , Pb_5^{2-} , Ge_5^{2-}	$\sim D_{3h}$ (closo)	$2n+2$	[23, 24]
Ge_9^{2-} ^[b]	$\sim D_{3h}$ (closo)	$2n+2$	[25]
Ge_9^{2-} } ^[c]	$\sim C_{2v}$ (closo)	$2n+2$	[26]
Ge_9^{4-} }	$\sim C_{4v}$ (nido)	$2n+4$	
Ge_9^{3-}	$\approx C_{2v}$	$2n+1$	[27–29]
Ge_{18}^{6-}	$\sim C_{2h}$ (dimer)	$2n+6$	[22]
Sn_9^{3-}	D_{3h} (closo)	$2n+1$	[30, 31]
Sn_9^{4-} , $In_4Bi_5^{3-}$	C_{4v} (nido)	$2n+4$	[22, 32]
$\infty_1[KSn_9^{3-}]$, $\infty_1[KPb_9^{3-}]$	$\sim C_{4v}+K^+$ (chains)	$2n+4$	[33, 34]
Pb_9^{3-}	$\approx C_{2v}$	$2n+1$	[28, 31, 34]
$TlSn_9^{3-}$ } ^[c]	$\sim D_{3h}$ (closo)	$2n+2$	
$TlSn_9^{3-}$ }	$\sim D_{4h}$ (closo)	$2n+2$	

[a] Neglecting heteroatoms. [b] This structure was first reported as a disordered Ge_{10}^{2-} cluster,^[36] later shown to be false upon reanalysis. [c] Double salts.



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charges.) Considerable expansion of Zintl's original list of polyanions has been possible, while others on his list have not been reproduced, or have been reformulated as other charge types. Note that, relative to the compounds formed in liquid ammonia, inclusion of the cryptand in the reactions expands the cluster-forming elements listed to include germanium, a family of paramagnetic Tt_9^{3-} ions ($Tt = Ge - Pb$), and a couple of substitutional examples involving some of the earlier triel elements. However, no general expansion to include elements further left in the periodic table, beyond the Zintl boundary, has been achieved by forming the complex as a cationic alkali-metal–cryptand salt.

Except for the tetrahedra listed at the beginning of Table 1, all of these polyanions are best described (on comparison with the relatively electron-rich tetrahedra P_4 and As_4) in terms of delocalized electron-deficient bonding, sometimes referred to hereafter as “classical” bonding, which is to include modern views of cluster bonding. These tetrahedra are classified further according to Wade's useful rules and their skeletal p-electron counts, as follows:^[4] the *closo*-deltahedra, of n deltahedral atoms, require $2n+2$ skeletal p-electrons (specifically, Tt_n^{2-} for all tetrelide clusters); *nido* clusters, with their deltahedra missing one vertex, require $2n+4$ electrons (cluster-based electrons of the $n+1$ *closo*-parent remain); and *arachno* polyhedra, missing two vertices, require $2n+6$ electrons. Of course, the same conclusions regarding these special points of electronic stability may be readily obtained from extended Hückel molecular-orbital calculations, spherical-harmonic treatments, and so forth.^[37] In most cases, the ns^2 states mix only weakly into the skeletal bonding orbitals, corresponding instead to nearly inert ns^2 cores.

The important feature is the generality of these simple guidelines for relating geometry to electron count for clusters with notable electronic stabilities. There seem to be no significant exceptions, save for some surprising radicals. A novel, dimeric product of the radical Ge_9^{3-} is shown in Figure 1a. (References will not be repeated in the text or the

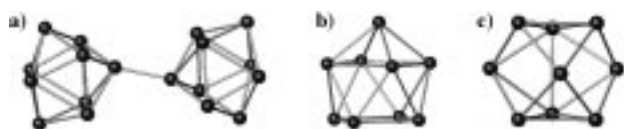


Figure 1. Examples of tetrelide anions, as $[A \subset \text{crypt}]$ salts. a) Ge_{18}^{6-} , the dimer of the Ge_9^{3-} radical. The b) C_{4v} and c) $\sim D_{3h}$ pair in K_4Pb_9 .

Figure captions when these references can be unambiguously found in a Table.) Most of the observed cluster symmetries given in Table 1 are in close agreement with expectations, or have recognizable relationships when characteristic proportions or dihedral angles are considered. On the other hand, the three (or more) nine-atom Tt_9^{n-} species do include geometric uncertainties because of some evident “softness”, disorder of mixed species, or cavity-dependent variations, especially for $n=2, 3$. The Tt_9^{4-} species are fairly close to the uncapped square antiprismatic (*nido*) ideal, whereas the isolated *closo*- Ge_9^{2-} and the exceptional Sn_9^{3-} radical ion are close to D_{3h} symmetry. On the other hand, the anions in $A \subset [2.2.2]$ crypt salts of Ge_9^{3-} ^[27] and Pb_9^{3-} ^[28] that each

contain one unique cluster, exhibit appreciable distortions from C_{2v} symmetry. Three paramagnetic $[A \subset [2.2.2]\text{cryptand}]_3Tt_9$ phases, one each for germanium,^[29] tin, and lead,^[31] contain pairs of independent clusters showing significant (Ge) or some (Sn, Pb) degree of distortion. However, recent reanalysis on the basis of quantitative magnetic studies shows that only one-half of the tin and lead members are paramagnetic.^[38] Clear examples of Tt_9^{2-} have been limited to Ge_9^{2-} , where the first charge assignment was made on the basis of geometry alone,^[26] there being at the time no reason to suspect odd numbered electron clusters. However, Ge_9^{2-} has been recently confirmed, as a cryptand salt, as a single D_{3h} anion.^[25] Recently, several salts of the Sn_9^{4-} and Pb_9^{4-} ions have been obtained by direct reaction of the elements in liquid [18]crown-6.^[39] These results are novel since this complexant does not completely sequester the cations, and several direct alkali-metal–cluster interactions remain.

2.2. Related Group 15 and 16 Cations

It turns out that the list of classical ions in Table 1 may be expanded to include isoelectronic and isosteric cations from electron-richer families shown in Table 2. Limiting stabilities

Table 2. Isoelectronic polycations prepared with weakly basic haloanions ($AlCl_4^-$, $HfCl_6^{2-}$).

Ion	Symmetry	Skeletal p-electrons	Reference
Se_4^{2+} , Te_4^{2+}	D_{4h}	$2n+6$ (arachno)	[40, 41]
Bi_5^{3+}	D_{3h}	$2n+2$ (closo)	[42, 43]
Bi_8^{2+}	$\sim D_{4d}$	$2n+6$ (arachno)	[42, 44]
Bi_9^{5+}	D_{3h}	$2n+4$ (closo)	[45]

of these cations are now defined by the disproportionation reactions to the elements and higher oxidation state compounds, and these reactions are characteristically affected by interactions of the latter with any counteranions present. Accordingly, weakly basic anions are useful for stabilizing the polycations, for instance $AlCl_4^-$ and $HfCl_6^{2-}$, but also fluorinated anions and the like that have analogous importance in superacid systems.^[46] One obvious difference between the anions (Table 1) and cations (Table 2) is the geometric contrast between the conforming uncapped square antiprism of the 22-electron *nido* anion (e.g. Sn_9^{4-}) and the tricapped trigonal prism (D_{3h}) found for the isoelectronic *closo*- Bi_9^{5+} . In fact, the latter structure is an important alternative to the *nido* geometry for a nine atom, 22-electron species, where it has been shown to arise via a 15–20% trigonal elongation from the ideal 20-electron D_{3h} cluster unit, a distortion that stabilizes what is otherwise the a_2'' LUMO.^[30, 47]

2.3. Polytetrelide Anions in Neat Salts

Contrary to the “end of the story” implications of the results shown in Table 1, numerous “neat” salts between “naked” cations and these cluster anions have been shown to exist in the past few years; that is, salts without an added

complexant or solvent. Furthermore, these salts reveal not only numerous new classical and hypoelectronic cluster anions of the trielides (gallium family) but also emphasize the significant increase in stability achieved with tightly packed naked cations over salts with large, complexed cations. The same conditions have more recently been shown to apply to a notable number of the traditional tetrelide (Zintl ion) clusters too. (So far, virtually only the alkali-metal systems have been explored.) Thus, with modern definitions of closed-shell bonding in electron-deficient clusters, we have converged on the common classification of these neat salts of former Zintl ions as Zintl phases, solid state compounds with closed-shell bonding that have characteristics of conventional salts (Section 5). The increased stability of these species—witness the trielide members to be discussed in Section 3—is usually associated with the additional Madelung (electrostatic potential) energy realized with the tighter packing and specific interactions between the alkali-metal cations and the Zintl anions. In fact, it has been known since the early 1960s that the neat 1:1 alkali-metal:tetrelide binary phases (with the exception of lithium) are all stable and that these contain Tt_4^{4-} ions isoelectronic with P_4 , As_4 , and the like.^[9] At the time, the relatively high charge per atom in these anions made this differentiation of Zintl ions and phases reasonable and selective. On the other hand, there is still no evidence that these Tt_4^{4-} tetrahedral species can be extracted into molecular solvents under any conditions, and so these remained as a handful of examples of neat tetrelide salts for many years.

Substantial advancements regarding other tetrelide clusters have taken place recently (Table 3). The accepted view that

$Cs_{12}Pb_{17}$ also exist.^[52] Three other A_4Tt_9 structures are also known. The overall implications are that the earlier solution-state results for the Tt_9^{4-} Zintl ions and subsequent isolation as cryptand derivatives (Table 1) probably came about through the simple solution of one or another of their rather conventional salts. Of course, intercluster or cluster–alloy reactions may have also contributed to the cryptand products. Presently, evidence suggests that most other species (Tt_9^{3-} and Tt_9^{2-} in Table 1) probably originated with redox processes in solution.

Of course, some vagaries of cluster conformation remain. The Si_9^{4-} clusters in monoclinic $Rb_{12}Si_{17}$ occur in four independent asymmetric forms, presumably because of packing effects. One is very close to the characteristic C_{4v} configuration, with one square face defining the base of the uncapped square antiprism, while the other three are similar but more complex. Each of the latter have *two* open “square” bases (contrast Pb_9^{4-} in Figure 1 b), one diagonal thereon corresponding to an elongated prismatic edge of the alternative D_{3h} tricapped trigonal prism. Extended-Hückel MO calculations give similar large HOMO–LUMO gaps for all four configurations, while the three distorted members all have LUMOs that are similar to the extra a_2'' HOMO orbital in Bi_9^{5+} .

The first neat phase discovered was tetragonal Cs_4Ge_9 , which contains four independent cluster anions as well, but these are all very similar C_{4v} examples, virtually the same as found for one cluster in the double-cluster phase $[K \subset \text{crypt}]_6(Ge_9)_2$. The cesium compound is diamagnetic, ruby-red, and semitransparent. Another is the monoclinic

Cs_4Pb_9 with a single cluster that is unambiguously a uncapped square antiprism.

Discoveries of C_{4v} -symmetric Tt_9^{4-} clusters as neat alkali-metal phases are perhaps still exceeded in impact by the elucidation of the structure of diamagnetic K_4Pb_9 and Rb_4Pb_9 , which contains *both* problematic configurations for Pb_9^{4-} —one as a capped antiprism of C_{4v} symmetry, the other an approximately trigonal prism (D_{3h}); see Figure 1 b, c. Heretofore,

the dichotomy involving these two configurations was spread between two examples: the first are the C_{4v} Tt_9^{4-} anions in binary or alkali-metal–cryptand systems; the second like the D_{3h} Bi_9^{5+} cation, known only in the presence of acidic anions such as $HfCl_6^{2-}$ ^[45] but distorted to C_{2v} symmetry with $BiCl_5^{2-}$ and $Bi_2Cl_8^{2-}$.^[59] The choice of views can be critical in distinguishing between these configurations, and distance and dihedral angle considerations are the best means; especially useful considerations are the equal heights of the ideal trigonal prism versus the two equal diagonals and planarity of the capped and open faces of the antiprism. The shape of one Pb_9^{4-} member in K_4Pb_9 (Figure 1 b) is very close to that in the ${}^\infty[KPb_9^{3-}]$ cryptand compound (C_{4v} symmetry)^[34] whereas the C_{2v} version is clearly closer to the two

Table 3. Classical^[a] anionic clusters of the tetrel elements (Tt) that are stable as neat salts.

Ion	Compound	Cluster symmetry ^[a]	Skeletal p-electrons	Reference
Tt_4^{4-}	ATt (A = Na–Cs, Tt = Si–Pb)	T_d (<i>nido</i>)	$2n+4$	[9]
	$BaSi_2$, Na_2BaTt_2 (Tt = Si, Ge)	T_d (<i>nido</i>)	$2n+4$	[48, 49]
$TlSn_3^{5-}$	Na_3TlSn_3	T_d (<i>nido</i>)	$2n+4$	[50]
Si_9^{4-}	$A_{12}Tt_9(Tt_4)_2$ (A = Rb, Cs)	$\sim C_{4v}$ + three distorted	$2n+4$	[51]
Sn_9^{4-}	$A_{12}Tt_9(Tt_4)_2$ (A = K–Cs)	four unique clusters	$2n+4$	[52]
Ge_9^{4-}	A_4Ge_9 (A = Rb, Cs)	C_{4v} (<i>nido</i>)	$2n+4$	[53]
Pb_9^{4-}	Cs_4Pb_9	C_{4v} (<i>nido</i>)	$2n+4$	[54]
	K_4Pb_9 , Rb_4Pb_9	$\sim D_{3h}$ and C_{4v} (two types)	22	[55, 56]
	$A_{12}Pb_9(Pb_4)_2$ (A = Rb, Cs)	$\sim C_{4v}$	$2n+4$	[52]
$ZnGe_8^{6-}$ ^[b]	Cs_6ZnGe_8	$\sim D_{3h}$		[57]
$Cd_3Pb_{16}^{10-}$ ^[b]	K_6CdPb_8	C_{2h} oligomers + Pb_4^{4-}		[58]

[a] Following Wade's rules or equivalent. [b] Not traditional deltahedra.

Zintl ions of the type Tt_9^{4-} could be obtained only via reactive solution of unrelated intermetallic or alloy phases into molecular solvents has been disproven by the Sevov group for all examples. Instead, the A_4Tt_9 compounds or the related $A_{12}Tt_{17}$ species $[=(A^+)_{12}Tt_9^{4-}(Tt_4^{4-})_2]$ with the naked cations (A = K–Cs) are preparable as binary alkali-metal salts by a conventional high-temperature synthesis in tantalum vessels. The absence of an earlier recognition of these neat phases evidently arose from a lack of careful attention to the synthesis and characterization of the solid binary “alloys” and, probably, because of their very poor X-ray diffraction characteristics. Single crystal characterization was first obtained for $Rb_{12}Si_{17}$, while powder diffraction data showed that the isostructural $Cs_{12}Si_{17}$, $A_{12}Sn_{17}$ (A = K–Cs), $Rb_{12}Pb_{17}$, and

examples of Bi_9^{5+} . (Specifically, the divergence from the ideal for the $\sim D_{3h}$ member are 5° – 9° in two dihedral angles and two prism edges (not drawn in the Figure) are 12% longer (0.54 Å) than the third. The angular measures compare with 20° , 22° , and 28° deviations from those parameters of the antiprism.) The Rb_4Ge_9 has also been shown to take up ethylenediamine to give a $\text{Rb}_4\text{Ge}_9 \cdot \text{en}$ structure in which the cations have been partially solvated but not separated from the anion environment.^[60]

It should be noted that additional evidence for Ge_9^{4-} and Sn_9^{4-} species, including these species as neat sodium salts, has been obtained by the von Schnering group from synthetic, X-ray powder diffraction, and vibrational spectroscopic studies but without clear X-ray resolution of the two structure types due to poor crystal quality (twinning, high pseudosymmetry).^[61] (The results from Sevov et al. generally pertain to larger cations.)

2.4. Mixed Element Clusters

There is little doubt that more isoelectronic and substantially isosteric cluster analogues of those in Table 3 will be prepared, and perhaps also some unimaginable species when mixtures of cations (see Section 4) or of p-metals from different groups are employed. A few examples of the latter were noted in Table 1, TlSn_3^{5-} is another in Table 3, and more will be discussed in the next Section. But some attempts will give surprises, accessing different types of cluster structures that are not stable (or have not yet been achieved) under the homoatomic regime. Two early examples, using zinc or cadmium, are noted in Table 3: Attempts to increase the charges on tetrelide clusters, which depend only on the deltahedral type and not on cluster size, gave novel results instead, as condensed or interbridged oligomers in the diamagnetic Cs_6ZnGe_8 and metallic K_6CdPb_8 examples. The first, ZnGe_8^{6-} , can be viewed as two Ge_4 tetrahedra each face-bonded (μ_3) to a central zinc atom to give an effective D_{3h} symmetry, that is, two fused trigonal bipyramids, $\text{Ge}_4\text{ZnGe}_4^{6-}$. The second contains separate Pb_4^{4-} units and $\text{Cd}_3\text{Pb}_{16}^{10-}$ oligomers in a 2:1 ratio: The $\text{Cd}_3\text{Pb}_{16}^{10-}$ consists of pairs of CdPb_8^{6-} units (analogous to ZnGe_8^{6-}) that are further interbridged by a six-coordinate Cd^{2+} again μ_3 -bonded to faces of the end lead groups as $(\text{Pb}_4\text{CdPb}_4)\text{Cd}(\text{Pb}_4\text{CdPb}_4)^{10-}$. Other examples are the similar Cs_6HgPb_8 with a mercury atom bridge between edges of the two lead tetrahedra,^[62] and $\text{Tl}_2\text{Te}_2^{2-}$, which is butterfly, not tetrahedron, shaped.^[63] To date, these contrary geometries all occur with elements differing by two or more groups, but this alone is not a sufficient condition. Inclusion of gold in Tt_4^{4-} and related species generally gives condensation into infinite chains, for example $[\text{Tl}_4\text{Au}^{3-}]_\infty$,^[64, 65] but will not be considered further.

3. Triel Clusters: Beyond the Zintl Boundary

An obvious condition to provide analogues for the electron-poorer trielides is that classical examples have one more negative charge per atom than the comparable tetrelides, that

is, $\text{closo-Tri}_n^{(n+2)-}$ is equivalent to closo-Tt_n^{2-} . In addition, the foregoing Sections have anticipated one other change that is apparently necessary to prepare trielide clusters: sufficiently large Madelung energies in the compact solid state with only more-or-less small, closely packed naked cations. Thus, molecular solvents are (evidently) insufficient, with or without cryptand or equivalent, to separate the ions and yield solutions of the same polyanions. The alloylike structural results that led Zintl to exclude valence-compound prospects for the trielides beyond the Zintl boundary (Section 5), was at the time based on the structure types that these compounds formed with excess magnesium.^[13] With 60 years of subsequent experience, we now know that magnesium is not the best “test” cation; in fact, there are only a few alkaline-earth systems for which there is any information relevant to this review. In any case, the Zintl boundary is now certainly a less general and useful delineation than originally considered for the formation of classical polyanions. For the moment, we will deal primarily with both the classical (Wade’s rule) polyanions and, especially, those that fall short of these requirements and thereby expand our chemistry horizons into a new hypoelectronic cluster regime. Electron localization and relevant properties will be considered later with discussions of Zintl phases (Sections 5 and 6), although it is evident that with trielides we approach the land of intermetallic phases.^[10, 66]

3.1. Classical Examples

The fact that the structure of Na_2Tl , which was (with my encouragement) investigated over 30 years ago, could be analogously formulated as $(\text{Na}^+)_8\text{Tl}_4^{8-}$ in terms of oxidation states^[67] evidently gave the first hint that cluster results comparable to those for the then relatively unknown trielides might be achieved. Clearly, relatively small monovalent cations coupled with the larger, polyanionic atoms in the clusters are important for stability. As with A_4Tt_4 phases ($\text{A} = \text{Li} - \text{Cs}$), it was 25 years later that the smallest extension of the generality of trielide-cluster chemistry first appeared for the comparable Na_8In_4 as well as hypoelectronic examples.^[68–71] At the other extreme, by the early 1980s the alkali-metal–gallium systems were becoming known for their numerous gallium–gallium bonded examples, but only in networks built entirely of what were new interbonded (or fused) gallium clusters and smaller gallium units, often very complex in detail.^[72] At that time, the complex networks of electron-deficient gallium clusters were seemingly unrelated to Zintl phases of the later p-metals, although a couple of insightful articles relating the former to boron chemistry through Wade’s rules had been published.^[73, 74] (Analogous, discrete gallium clusters were not discovered until quite recently.) Notwithstanding, the connection between the cluster chemistry of gallium in networks and that of the heavier, later p-metals was not recognized until the cluster chemistry of the latter and the applicable electronic rules became clearer. In fact, the first investigations on alkali-metal systems of indium were undertaken with other objectives.

Classical examples of trielide clusters, largely discovered along the way to the more novel results, are listed in Table 4.

Table 4. Classical^[a] anionic clusters of the triel elements (Tr) that are stable as neat salts.

Ion	Compound	Cluster symmetry ^[a]	Skeletal p-electrons	Reference
Ti_4^{8-} , In_4^{8-}	Na_2Ti , Na_2In	$\sim T_d$ (<i>nido</i>)	$2n+4$	[67, 68]
	$\text{Na}_{25}\text{K}_9\text{Ti}_{15.33}$	$\sim T_d$ (<i>nido</i>)	$2n+4$	[75]
Ti_5^{7-}	$\text{Na}_2\text{K}_{21}\text{Ti}_{19}$	D_{3h} (<i>closo</i>)	$2n+2$	[76]
	$\text{Na}_{23}\text{K}_9\text{Ti}_{15.33}$	D_{3h} (<i>closo</i>)	$2n+2$	[75]
	$\text{Na}_9\text{K}_{16}\text{Ti}_{18}\text{Cd}_3$	D_{3h} (<i>closo</i>)	$2n+2$	[77]
In_5^{9-}	La_3In_5	C_{4v} (<i>nido</i>)	$2n+2$	[78]
Ga_6^{8-}	$\text{Ba}_5\text{Ga}_6\text{H}_2$	$\sim O_h$ (<i>closo</i>)	$2n+2$	[79]
Ti_6^{8-}	$\text{Na}_{14}\text{K}_6\text{Ti}_{18}\text{M}$ (M = Mg, Zn, Cd, Hg)	T_h	$2n+2$	[80]

[a] Following Wade's rules or equivalent.

These all involve atomic charges that are greater than one, and are found only for the smaller clusters and cations with a higher charge density or under special packing situations. For instance, two examples of trigonal bipyramidal Tr_5^{7-} , the direct analogue of Pb_5^{2-} , were in effect trapped in complex structures along with nonclassical clusters, for example in $(\text{Na}^+)_{21}(\text{K}^+)_{21}(\text{Ti}_9^{9-})(\text{Ti}_5^{7-})_2$ and $(\text{Na}^+)_{23}(\text{K}^+)_{9}(\text{Ti}_5^{7-})_{1.33}(\text{Ti}_3^{7-})_{0.33}(\text{Ti}_4^{8-})_2(\text{Ti}_5^{7-})$. The productive utilization of mixed cations will be elaborated later. The examples of isolated octahedral Tr_6^{8-} units are probably encountered because of particularly favorable lattice packing and the higher charge densities of the cations. More octahedra are certain to be found if enough systems are investigated thoroughly. The octahedral Ga_6^{8-} in $\text{Ba}_5\text{Ga}_6\text{H}_2$ is rigorously D_{3d} (described as approximately O_h in Table 4). This cluster was first reported^[81] for a composition that was difficult to understand, presumably that of a metallic phase $(\text{Ba}^{2+})_5(\text{Ga}_6^{8-})(\text{e}^-)_2$. However, experience has shown that closed-shell Zintl compounds predominate in this sort of chemistry, a condition that has been implicit in nearly all of the examples discussed heretofore and which will be considered later in more detail. Thus, the answer for the apparent imbalance here was found in hydride impurities, namely, reformulated to $\text{Ba}_5\text{Ga}_6\text{H}_2$ with hydride located in the better-sized pair of barium tetrahedra (see Figure 2). Hydrogen



Figure 2. The structure of $\text{Ba}_5\text{Ga}_6\text{H}_2$, with trigonal antiprismatic Ga_6^{8-} and hydride in barium tetrahedra.

contamination is now known to be a particularly insidious problem, even with the best alkaline-earth metals available commercially.^[82, 83]

Finally, the formal In_5^{9-} ion in La_3In_5 is a forecast of probable things to come. In this case, the relatively few higher-charged cations appear insufficient to separate the classical square pyramidal (*nido*) indium clusters well, and some intercluster indium–indium distances remain that are only 11–15 % (0.33–0.48 Å) greater than the average intracluster

bond lengths. (These, in fact, are important in occupied band states.) The experimental result is a metallic compound. (A possible role of the high field La^{3+} has not been examined.) Of course, the metallicity is a property of the weakly bound electrons and the lattice, whereas the well-defined cluster shape is a reflection of more tightly bound valence

electrons. Clearer examples of this isolated cluster type are certain to be found through the utilization of a greater number of cations with lower charges.

3.2. Hypoelectronic Trielide Clusters

Exploratory synthesis efforts within the alkali-metal–triel systems, for indium and thallium especially, have revealed many novel and unanticipated features about their chemistries, not just new classical species, structures, and stoichiometries, but also the presence of evidently the first hypoelectronic clusters relative to Wade's classical rules. For a long time, the minimum for skeletal electron counts in traditional main-group element clusters was taken to be the $2n+2$ electrons for *closo*-deltahedra, but this apparent boundary existed only because this "rule" had never been adequately tested. The trielides provide such a new chemistry not only in homoatomic naked clusters, especially Tr_{11}^{7-} , but also for centered polyhedra such as ZnIn_{10}^{8-} . Indeed, the unusual presence of centered (interstitial) atoms in some clusters is comparable to that requirement for many of the rather distant $\text{M}_6(\text{Z})\text{X}_{12}$ -type clusters of the early transition metals M (interstitial Z, halide X).^[84]

Table 5 summarizes discrete hypoelectronic clusters as 1) homoatomic, 2) substituted, and 3) centered examples. The electronic classification of the last group depends to some extent on the point of view. (Other examples, found in networks such as icosahedral gallium, appear in Section 5.2.) The four new examples in the homoatomic category are shown in Figure 3. The Ti_6^{6-} (a) and Ti_7^{7-} (b) reflect conventional (Jahn–Teller) axial compressions of the conventional octahedron and the uncommon pentagonal bipyramid, respectively. These changes are methods to reduce the relatively large cluster charges on the normal polyhedra (–8 and –9, respectively). A transannular bond is achieved only in Ti_7^{7-} , 0.1–0.2 Å longer than in the remainder of the cluster, although the larger equatorial ring for this example already situates the axial atoms so close that the ideal ($2n+2$) model may not apply. The distortion for each example drives the out-of-phase σ^* (a_2') combination of p_z orbitals on the axial atoms energetically higher and free of occupying electrons. It will be noted later that the structures of K_6Ti_6 and Cs_6Ti_6 are alternatives for the similarly four-bonded, stuffed-diamond structure of NaTi (Section 5.1) since the K^+ and Cs^+ ions are too large to fit comfortably within the (formal) Ti^- network. (The RbTi member does not exist.) The unprecedented Ti_9^{9-}

Table 5. Hypoelectronic clusters of triel elements.

Ion	Compound	Cluster symmetry ^[a]	Skeletal p-electrons ^[b]	Reference
<i>Homoatomic</i>				
Tl ₆ ⁶⁻	KTi	$\sim D_{2h}$	12 (2 <i>n</i>)	[85]
	CsTi	$\sim D_{4h}$	12 (2 <i>n</i>)	[86]
Tl ₇ ⁷⁻	Na ₁₂ K ₃₈ Tl ₄₈ Au ₂	$\sim D_{5h}$ (<i>C</i> _{2v})	14 (2 <i>n</i>)	[87]
	K ₁₀ Tl ₇ ^[c]	$\sim D_{5h}$ (<i>C</i> _{2v})	14 (2 <i>n</i>)	[88]
Tl ₉ ⁹⁻	Na ₅ K ₂₁ Tl ₁₉	<i>C</i> _{2v}	18 (2 <i>n</i>)	[76]
	Na ₁₂ K ₃₈ Tl ₄₈ Au ₂	<i>C</i> _{2v}	18 (2 <i>n</i>)	[87]
Ga ₁₁ ⁷⁻	Cs ₈ Ga ₁₁ ^[d] Cs ₈ Ga ₁₁ Cl	$\sim D_{3h}$	18 (2 <i>n</i> - 4)	[89]
In ₁₁ ⁷⁻	K ₈ In ₁₁ ^[c] Rb ₈ In ₁₁ , Cs ₈ In ₁₁	$\sim D_{3h}$ (<i>D</i> ₃)	18 (2 <i>n</i> - 4)	[69 - 71]
Tl ₁₁ ⁷⁻	A ₈ Tl ₁₁ ^[c] (A = K - Cs)	$\sim D_{3h}$ (<i>D</i> ₃)	18 (2 <i>n</i> - 4)	[90]
	A ₁₅ Tl ₂₇ ^[c] (A = Rb, Cs)	<i>D</i> _{3h}	18 (2 <i>n</i> - 4)	[91]
	K ₁₈ Tl ₂₀ Au ₃ ^[c]	<i>D</i> _{3h}	18 (2 <i>n</i> - 4)	[92]
<i>Substituted</i>				
Tl ₉ Au ₂ ⁹⁻	K ₁₈ Tl ₂₀ Au ₃ ^[c]	<i>D</i> _{3h}	16 (2 <i>n</i> - 6)	[92]
Tl ₈ Cd ₃ ¹⁰⁻	Na ₉ K ₆ Tl ₁₈ Cd ₃ ^[d]	<i>D</i> _{3h}	18 (2 <i>n</i> - 4)	[77]
In ₁₀ Hg ⁸⁻	K ₈ In ₁₀ Hg ^[e]	$\sim D_{3h}$	18 (2 <i>n</i> - 4)	[93]
<i>Centered</i>				
In ₁₀ Zn ⁸⁻	K ₈ In ₁₀ Zn	<i>D</i> _{4d}	18 (2 <i>n</i>)	[94]
Tl ₁₀ Zn ⁸⁻	K ₈ Tl ₁₀ Zn	<i>D</i> _{4d}	18 (2 <i>n</i>)	[95]
Ga ₁₀ Ni ¹⁰⁻	Na ₁₀ Ga ₁₀ Ni	$\approx C_{3v}$	20 (2 <i>n</i>)	[96]
In ₁₀ M ¹⁰⁻	K ₁₀ Tr ₁₀ M (M = Ni - Pt)	$\approx C_{3v}$	20 (2 <i>n</i>)	[97]
Tl ₁₀ M ¹⁰⁻	K ₁₀ Tr ₁₀ M (M = Ni - Pt)	$\approx C_{3v}$	20 (2 <i>n</i>)	[98]
Tl ₁₃ ⁷⁻	Na ₄ A ₆ Tl ₁₃ ^[d] (A = K - Cs)	<i>T</i> _h	25 (2 <i>n</i> +1)	[99]
Tl ₁₃ ¹¹⁻	Na ₃ K ₈ Tl ₁₃	<i>D</i> _{3d}	26 (2 <i>n</i> +2)	[99]
Tl ₁₂ Na ¹³⁻ , Tl ₆ H ⁷⁻	Na ₁₃ K ₆ Tl ₁₈ H	<i>T</i> _h	26, 14 (2 <i>n</i> +2)	[100]
Tl ₁₂ M ¹²⁻ , Tl ₆ ⁸⁻	Na ₁₄ K ₆ Tl ₁₈ M (M = Mg, Zn - Hg)	<i>T</i> _h	26, 14 (2 <i>n</i> +2)	[80]

[a] Neglecting heteroatoms. [b] Skeletal electron counts exclude ns^2 cores, while n values for polyhedra exclude the centered atom, if any. [c] Metallic phase; stoichiometric but not electron precise. [d] Curie–Weiss paramagnetic salt. [e] Hg is disordered over trigonal prismatic sites.

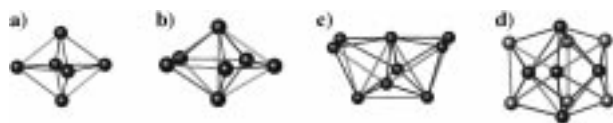
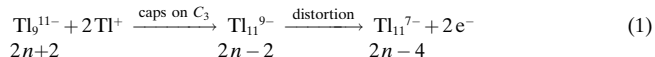


Figure 3. Four unusual trielide anions found in neat salts: a) tetragonally compressed Ti_6^{6-} in CsTi ; b) Ti_7^{7-} in K_{10}Ti ; c) Ti_9^{9-} in $\text{Na}_3\text{K}_{21}\text{Ti}_9$; d) Ti_{11}^{11-} from K_8Ti_{11} , with the atoms defining the trigonal prism drawn lighter.

cluster (Figure 3c) has now been captured in two different compounds (Table 5), as have Tl_5^{7-} and Tl_7^{7-} clusters. The derivation the configuration of Tl_9^{9-} from the common nine-atom polyhedral shapes (D_{3h} or C_{4v}) is fairly tortuous, but the result is readily achieved by removal of four adjoining atoms from the centered Tl_{13}^{11-} icosahedron (see below), which exposes the formerly centered thallium atom (at the top of Figure 3c). The Tl_7^{7-} cluster is also a fragment of the same parent.

The important entrée into new hypoelectronic clusters that are achieved through distortion, and the most common example among the trielides, is In_{11}^{7-} and its descendants, all of which are very close to D_{3h} symmetry. The anion in K_8Tl_{11} is shown in Figure 3d with the trigonal prism highlighted. With the clarity of hindsight, this polyhedron is readily achieved conceptually through first capping the basal faces of the (hypothetically *closo*) tricapped trigonal prism Tl_9^{11-} by two thallium cations, which introduces no new skeletal orbitals or electrons. The two new vertices are at this point rather exposed and of low order in bonding, but a logical axial compression and radial expansion both adds bonding between axial and equatorial capping atoms and empties the formerly

bonding a_2'' orbital within the trigonal prism [Eq. 1]. The a_2'' orbital consists of p_z orbitals that are π bonding within the basal faces but antibonding along the prismatic edges, so that a loss of π bonding upon lateral expansion and increased antibonding effects upon compression make this orbital both energetically high and empty. (The opposite distortion stabilizes the D_{3h} form of the $2n+4$ electron polyhedron Bi_9^{5+} , see Section 2.2.) Hence, the combination of capping and distortion converts the former $(2n'+2)$ Tl_9 polyhedron to the new and more symmetric $(2n-4)$ Tl_{11} cluster with a lower charge ($n=n'+2$). The stability of the seven examples of binary A_8Tl_{11} compounds are logically dependent on the competing, alternative phases present in the various systems, that is, either A_8Tl_{11} or the A_2Tr_3 phase, but not both.^[89] This accounts for at



least some of the surprise associated with the occurrence of the smallest cluster (Ga_{11}^{7-}) with the largest cation (cesium), but this product is irregular in other ways too.

The contrast between cation count ($8+$) and the closed-shell cluster charge ($7-$) in the A_8Tl_{11} phases defies, of course, the traditional belief that their magnitudes should match. Accordingly, the A_8Tl_{11} phases join a limited list of “metallic salts”, of which TiO , CeS , and LaI_2 are simple members. The resistivities of A_8Tl_{11} phases are still moderate (a few hundred $\mu\Omega\text{cm}$), metallike in their temperature dependencies, and their magnetic susceptibilities are characteristic of Pauli paramagnetism (except for Cs_8Ga_{11}). The evident answer is that one extra cation per formula is necessary for good packing in the rhombohedral A_8Tr_{11} lattice; a $[110]$ section for K_8Tl_{11} is shown in Figure 4. More examples of this particular behavior are found in other trielide systems (examples to follow). Two ways have been found to capture the extra electron in A_8Tr_{11} while retaining the basic crystal structure. Substitution of one indium atom by mercury (disordered over the trigonal prismatic sites) in K_8In_{11} yields the diamagnetic product $K_8In_{10}Hg$. Alternatively, a halogen ($X=Cl-I$) added to a variety of A_8Tr_{11} systems becomes bound in a distorted version of an A_8 cavity that was already present in the binary A_8Tr_{11} structure, and this change afforded nine diamagnetic $A_8Tr_{11}X$ phases. In three cases, these ternary halides exist in systems in which the binary

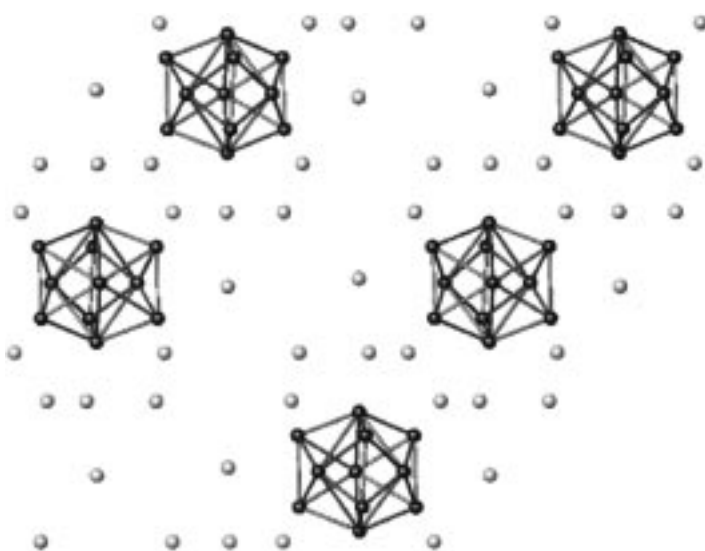


Figure 4. A [110] section of the rhombohedral structure of K_8Tl_{11} .^[69]

phase is evidently not stable (Rb–Ga, Rb–In, Cs–In). We have not been able to reproduce a report of Rb_8In_{11} .^[71]

Two other products of a substitution in the Tl_{11}^{7-} polyhedron have different characteristics and new features. Gold substitutes on the two axial (threefold-symmetric) sites, which would, without modification, give a $Tl_9Au_2^{9-}$ unit that is four electrons short. This is remedied by the addition of two electrons plus the formation of a strong transannular bond ($d_{Au-Au} = 2.96 \text{ \AA}$) between the former capping atoms (see Figure 5a). The remaining thallium framework changes very

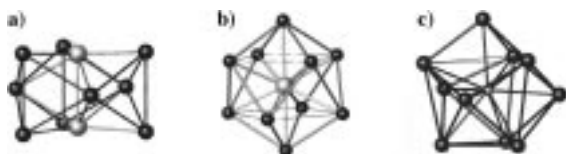


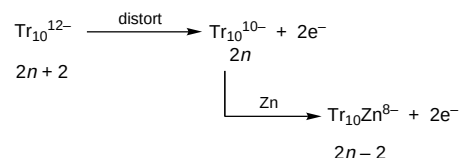
Figure 5. Centered clusters of the trielides: a) $Tl_9Au_2^{9-}$, b) $Tl_{10}Zn^{8-}$, c) $Ga_{10}Ni^{10-}$.

little in this process so the product is essentially a tricapped trigonal prism with centered basal faces. The independent unit in the structure, $(K^+)_{18}(Tl_9Au_2^{9-})(Tl_{11}^{7-})(Au^-)(e^-)$, contains both an isolated and essential Au^- ion and an extra, evidently delocalized, electron. The cation count seems quite certain. On the other hand, a more standard substitution, $Tl_8Cd_3^{10-}$ within $Na_9K_{16}Tl_{18}Cd_3$, puts the heteratoms in the waist-capping positions. This structure also contains a pair of Tl_5^{7-} anions and an extra electron per cell, $(Na^+)_9(K^+)_{16}(Tl_5^{7-})_2(Tl_8Cd_3^{10-})(e^-)$. However, the extra electron for both $Na_9K_{16}Tl_{18}Cd_3$ and Cs_8Ga_{11} does not produce a metallic property. Moreover, no significant change in the cluster dimensions or contact distances appears in either compound, suggesting that electrons are not trapped in antibonding (LUMO, etc.) cluster orbitals, at least on a single unit. The electrons are instead evidently (Mott-)localized, possibly held in some multication cavity, where they yield typical Curie–Weiss magnetic signatures (1.50 and $1.43 \mu_B$, respec-

tively). These phases may be similar to Pr_2I_5 in this character.^[101, 102]

3.3. Centered Trielide Clusters

Another pleasant surprise in polytriellide chemistry is the occurrence of a family of novel centered clusters. Two $Tr_{10}Zn^{8-}$ members ($Tr = In, Tl$) exhibit zinc addition to the center of a standard (D_{4d}) bicapped square (Archimedean) antiprism (Figure 5b), a process that also includes the effects of distortion. The parent cluster again undergoes a tetragonal compression and lateral expansion, presumably to achieve more nearly equivalent $Tr-Zn$ distances, which likewise enlarges the two basal (square) planes and raises the corresponding bonding a_1 molecular orbital to a future LUMO role. Addition of a centered *neutral* zinc atom then causes the loss of one bonding a_1 orbital and two electrons (since both of the totally symmetric s-like orbitals were occupied), and an appreciable stabilization of the remaining orbital through mixing with the zinc 4s orbital (Scheme 1).



Scheme 1. The transition from the ideal Tr_{10}^{12-} cluster to the centered $Tr_{10}Zn^{8-}$ cluster.

Similarly, the 4p orbitals on zinc stabilize existing cluster states while three corresponding antibonding orbitals appear. An alternative view is to retain the electron pair in the weakly stable, distorted Tr_{10}^{12-} unit and to add Zn^{2+} , which stabilizes one and empties another a_1 level. The $2n-2$ electron-count result is in either case the result of the distortion.

The formation of the isoelectronic $Tr_{10}M^{10-}$ clusters ($Tr = Ga, M = Ni$; $Tr = In, Tl, M = Ni, Pd, Pt$) does not give the same geometric result, for reasons that may be rather unfamiliar in molecular chemistry but which appear important in compact ionic compounds in the solid state. The reduced $\sim C_{3v}$ symmetry of the $Tr_{10}M^{10-}$ polyhedron evidently manifests the presence of (or need for) strong interactions of the polyanion with the two additional cations (see below), and the polyhedron is opened to provide additional edges and faces of a roughly tetracapped trigonal prism. In Figure 5c the gallium member $Ga_{10}Ni^{10-}$ is shown with the uncapped basal face at the bottom. The bonding effects in $Ga_{10}Ni^{10-}$ and $In_{10}Ni^{10-}$ show some close parallels with those in the isoelectronic $In_{10}Zn^{8-}$, starting with the similar tetracapped ($2n$) Tr_{10}^{10-} . The principal nickel bonding occurs via the 4s and 4p orbitals, particularly the former, while the nickel d^{10} manifold lies about 5 eV lower, corresponding to a well-reduced Ni^0 core. The average number of trielide neighbors and the $Tr-Tr$ overlap population at each vertex are greater than in the zinc-centered cluster, as is the number of neighboring potassium cations that show rather specific interactions with the cluster at faces, edges and vertices (see

Section 4). Both the nickel- and zinc-centered compounds are diamagnetic. It is interesting to note that three types of 11-atom clusters all have 40 cluster-based electrons when ns^2 cores are included, $\text{Tr}_{10}\text{Zn}^{8-}$, $\text{Tr}_{10}\text{Ni}^{10-}$, and Tr_{11}^{7-} . The Tt_9^{4-} polyhedra are also 40-electron species.

The trielide explorations have also revealed a new family of naked polyhedra, the self-centered (*closo*) Tl_{13} icosahedra. Although these species are, for the most part, not hypoelectronic, they are included in Table 5 with other centered examples. Better precedents of fragments of this polyhedron are the uncentered borane $\text{B}_{12}\text{H}_{12}^{2-}$ and the network of icosahedra interbonded at vertices of α -boron. The nominal $6s^2$ cores on thallium can be viewed as alternates to the exo B–H or B–B bonding functions in the boron compounds. The delocalized bonds in the thallium polyhedra are longer and weaker than for boron, and the observation of only endohedral (centered) examples for such a large polyhedron seems reasonable. The added member can be either a 13th Tl atom, or a cation of a more active metal. (Empty Ga_{12} clusters are also known in cluster networks—see Section 5.2.) As will be detailed later in Section 4.2, the packing of cation neighbors about the approximate icosahedra seems to be of paramount importance.

Among the self-centered examples, the somewhat more versatile structures of the stoichiometric cubic $\text{Na}_4\text{A}_6\text{Tl}_{13}$ (A = K–Cs) contain the odd-electron Tl_{13}^{10-} cluster (Figure 6). Evidently, the particular environment with ten cations, especially the 8/2 Na^+ near



Figure 6. The Tl_{13}^{10-} cluster in $\text{Na}_4\text{K}_6\text{Tl}_{13}$.

neighbors (see below), is structurally more important than achievement of a closed-shell Tl_{13}^{11-} cluster (*closo*- Tl_{12}^{14-} plus a centered Tl^{3+}). The symmetry of the cluster is T_h and the space group $Im\bar{3}$. The unit cell is shown in Figure 7a. The radial distances within the anion are all equal and short, 3.225 Å, while those on the surface distribute between six smaller values, 3.281 Å for the edges bisected by three perpendicular twofold axes and 3.416 Å between these edges. The electron hole in these phases is evidently localized, as they exhibit Curie–Weiss paramagnetism with μ_{eff} values of (Rb) 1.74 and (Cs) 1.90 μ_B , and $g = 2.01$ for both. Provision of other interstitial elements yields an interesting family of mixed octahedral and icosahedral clusters in a related cubic structure (see below) that

retain much of the strong and effective packing of $\text{Na}_4\text{A}_6\text{Tl}_{13}$.

The closed-shell Tl_{13}^{11-} has so far been achieved only as the stoichiometric rhombohedral $\text{Na}_3\text{K}_8\text{Tl}_{13}$, with the cell illustrated in Figure 7b. The lower symmetry $R\bar{3}m$ space group gives a D_{3d} symmetric Tl_{13}^{11-} cluster with two slightly different radial distances and three distinct surface separations: 3.22 Å for those equivalent to the three *trans* bond pairs in Tl_{11}^{10-} and two longer ones, 3.42 and 3.58 Å. This phase has weak Pauli paramagnetism and is a modest metallic conductor, approximately 70 $\mu\Omega\text{cm}$ at 293 K, whereas the $\text{Na}_4\text{A}_6\text{Tl}_{13}$ compounds are poorer metals. These last two features, not uncommon among trielide phases, will be a subject of consideration in Section 6. The phase fields of the two stoichiometric Na–K–Tl cluster compounds $\text{Na}_4\text{K}_6\text{Tl}_{13}$ and $\text{Na}_3\text{K}_8\text{Tl}_{13}$ are well separated and distinct (compare ref. [103]). A novel variation for the former cubic structure $\text{A}_4(\text{A}')_6\text{Tl}_{13}$ has been reported in the phase $\text{K}_6(\text{NaCd})_2(\text{Tl}_{12}\text{Cd})$ by Belin and co-workers^[104] (see also the end of Section 4.2). Insertion of Cd into the Tl_{12}^{14-} clusters alone would leave the system short four electrons per unit cell. In fact, the deficiencies are compensated by random

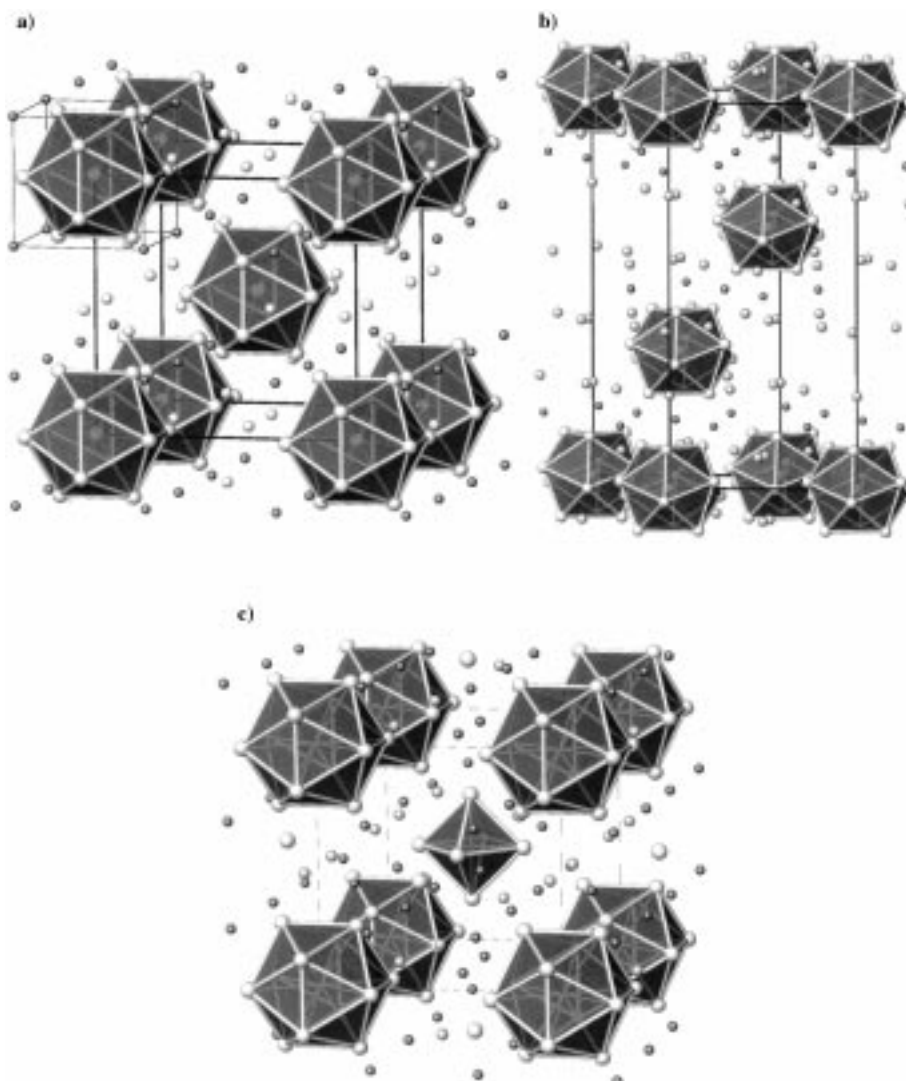


Figure 7. a) The centered cubic unit cell of $\text{Na}_4\text{K}_6\text{Tl}_{13}$, with sodium as the darker isolated atoms; b) the rhombohedral cell of $\text{Na}_3\text{K}_8\text{Tl}_{13}$; c) the primitive cubic cell of $\text{Na}_4\text{K}_6(\text{Tl}_{12}\text{Na})(\text{Tl}_6\text{H})$.

substitution of about 50 % of the four Na^+ sites by Cd^{2+} ($Z = 2$). Support for this novel arrangement is strong, as determined by crystallographic refinement and also by direct analysis of single crystals from a synthesis at that stoichiometry. Although the refinement of cations of post-transition elements on alkali-metal sites has in general raised some concerns (because of the strongly reducing polyanions present), the supporting chemical analyses here and the fact that the product is properly a fairly poor semiconductor make this instance very logical. The cadmium cations, dispersed among an equal number of inner sodium sites (see Section 4.1), provide more communication between the “not-so-naked”^[104] clusters.

The presence of a little adventitious hydrogen in such cluster systems has also given a novel result, as has happened many times before. Reaction of a $2\text{Na}:1\text{K}:3\text{Tl}$ composition yielded a related mixed-cluster compound with a slightly smaller primitive ($Pm\bar{3}$) rather than the body-centered cubic unit cell of $\text{Na}_4\text{A}_6\text{Tl}_{13}$. The structure (see Figure 7c) has a thallium octahedron centering a simple cube of Tl_{12}Na units and the heavy atom composition $\text{Na}_{15}\text{K}_6\text{Tl}_{18}$. The odd-electron stoichiometry seemed amiss since the compound was not Curie–Weiss paramagnetic, and so hydrogen in the form of Tl_6H^{7-} was immediately suspected (only 0.02 wt. % H is needed).^[83] Standard experiments confirmed this; none of this product was encountered when the sealed tantalum reaction container was heated in high vacuum, but it was produced quantitatively on either purposeful addition of a small amounts of H_2 or the use of a sealed silica jacket (which dehydrates slowly on heating) around the reaction container. The presumed formulation is therefore $(\text{Na}^+)_{14}(\text{K}^+)_{6-}(\text{Tl}_{12}\text{Na}^{13-})(\text{Tl}_6\text{H}^{7-})$. (Note that H^- alone in a cation-defined cavity would not be suitable.) The structure can be easily derived from the centered cell composed of $(\text{Na}_4\text{K}_6\text{Tl}_{13})_2$ (Figure 7a) by substitution of one-half of the potassium by sodium about the octahedron, Tl_6H for one Tl_{13} , and Tl_{12}Na for the other Tl_{13} in the primitive cell. Furthermore, other interstitials may substitute in the icosahedral fragment to give an isotopic series that contains empty octahedra and icosahedra centered by a divalent metal, namely $(\text{Na}^+)_{14}(\text{K}^+)_{6-}(\text{Tl}_{12}\text{M}^{12-})(\text{Tl}_6^{8-})$ ($\text{M} = \text{Mg}, \text{Zn}, \text{Cd}, \text{Hg}$, but not $\text{Ca}, \text{Ni}, \text{Eu}, \text{Yb}$). Competition experiments show that the examples with centered divalent cations and empty octahedra are more stable than those with centered sodium and hydrogen atoms.

A fanciful extension of the last cluster structure (Figure 7c) is suggested as it shares an isotopic relationship with $\text{Mg}_2\text{Zn}_{11}$ and $\text{Na}_2\text{Cd}_{11}$, which would be formulated in similar terms as $(\text{M}_{14}\text{M}'_6)(\text{M}_{13})(\text{M}_6)$ ($\text{M} = \text{Zn}, \text{Cd}$; $\text{M}' = \text{Mg}, \text{Na}$).^[80] Clear distinctions between nominal cations and cluster anions are lost as zinc now bridges between the zinc polyhedra that replace the thallium clusters, and so much more homogeneous bonding interactions are present. Thus we have, in the same structure type, basically crossed over the Zintl phase boundary between anion-forming substances in saltlike lattices and alloylike compounds. Some of the similarity is only geometric, but there are certain to be interesting, related bonding features to be deduced in $\text{Mg}_2\text{Zn}_{11}$ and diverse analogues.

4. Cation Effects

4.1. Cluster “Solvation”

Experience with the structures of a wide variety of the present compounds that are characteristically polar or ionic gives compelling indications of both the importance of electrostatic interactions between ions or charged groups and how structures appear to maximize these effects by filling the spaces efficiently. “Nature abhors a vacuum”, in salts anyway.

Close inspection of the diverse neat structures described here reveals that remarkably specific and transferable alkali metal dispositions occur about each cluster anion. The “solvation” environment always consists of cations that, in order of increasing distance from the anion, 1) cap cluster faces, 2) bridge edges, 3) are positioned exo (outward) at vertices. Although these cluster anions must have significant polarizabilities and therefore some covalent interactions with the cations as well, the electrostatic portion presumably dominates. Clearly, the strongest interactions are found with the shorter-ranged face-capping cation sites, within the limits of cation–cation repulsions. I will emphasize the nearest shell or two of cations about the anions, for it is really these “solvated” cluster anions that define our structural building blocks. It should be noted that the cations commonly show specific interactions with two or more clusters, these bridges serving to keep them apart since the polyanions would presumably condense or interbridge directly were they to come in contact. The whole collection of neat cluster structures affords a wide variety of polyanions on which very regular alkali-metal cation positioning can be observed, thereby allowing significant generalizations. The regularities seem surprising, but perhaps earlier comparisons of what we call “solvation” by cations have lacked such a variety of large anions.

Previous tables reveal some specific cation trends. Trielide clusters of the same type must have greater negative charges than related tetrelide members, and it is noteworthy that all neat, classical (Wade’s rule) trielide clusters with charges greater than -1 per atom occur with either sodium or higher charged cations (see Table 4). This is especially noteworthy with the high (formal) charges on tetrahedral Tr_4^{8-} , where the smaller radii of curvature allows better accommodation of a relatively larger number of cations (see below). On the other hand, larger cations may be sufficient for many hypoelectronic (or other) clusters, where charge-per-atom values are all less than or equal to unity (Table 5). Several “odd” phases may be provisionally attributed to these packing effects, especially for paramagnetic Tl_{13}^{10-} in $\text{Na}_4\text{A}_6\text{Tl}_{13}$ where there is no room for the 11th cation (Section 4.2), and in the metallic A_8Tr_{11} phases. A few other electrides are rather inexplicable but presumably related, including $(\text{K}^+)_{10}(\text{Tl}_7^{7-})(\text{e}^-)_3$ and $(\text{K}^+)_{18}(\text{Tl}_{11}^{7-})(\text{Tl}_9\text{Au}_2^{9-})(\text{Au}^-)(\text{e}^-)$. The latter is not obtained without gold, and extra gold does not trap the odd electron, so the presence of a suitable cavity for Au^- is probably the critical point. This and other possible electron trapping means, as by a halogen, have not been widely explored.

4.2. Mixed Cations: A Synthetic Means for Increasing Variety

It is demonstrably true that many more trielide clusters may be isolated in the presence of mixed cations, a new and valuable “synthetic laxative” to expand variety. The interactions in binary systems are self-limiting as all cations are the same. The anions isolated are selectively those for which good packing schemes for their simple “solvates” are possible. Table 6 lists a number of mixed cation phases with their

Table 6. Mixed cation stabilization of new phases and new trielide clusters.^[a]

Formula	Clusters
$\text{Na}_2\text{K}_{21}\text{Tl}_{19}$	Tl_3^{7-} , Tl_9^{9-}
$\text{Na}_3\text{K}_8\text{Tl}_{13}$	Tl_{13}^{11-}
$\text{Na}_4\text{A}_6\text{Tl}_{13}$ (A = K – Cs)	Tl_3^{10-}
$\text{Na}_9\text{K}_{16}\text{Tl}_{18}\text{Cd}_3$	$\text{Tl}_8\text{Cd}_3^{10-}$, Tl_5^{7-}
$\text{Na}_{12}\text{K}_{38}\text{Tl}_{48}\text{Au}_2$	Tl_7^{7-} , Tl_9^{9-}
$\text{Na}_{14}\text{K}_6\text{Tl}_{18}\text{M}$ (M = Mg, Zn, Cd, Hg)	Tl_6^{8-} , $\text{Tl}_{12}\text{M}^{12-}$
$\text{Na}_{15}\text{K}_6\text{Tl}_{18}\text{H}$	Tl_6H^{7-} , $\text{Tl}_{12}\text{Na}^{13-}$
$\text{Na}_{23}\text{K}_9\text{Tl}_{15.33}$	Tl_4^{8-} , Tl_5^{7-} , ($\text{Tl}_7^{7-}/\text{Tl}_5^{5-}$)
$\text{Na}_{26}\text{A}_3\text{In}_{48}$ (A = K – Cs)	network of <i>closo</i> - and <i>arachno</i> - In_{12}^{105}
$\text{Na}_{4.8}\text{K}_{18.2}\text{In}_{39}$	network of <i>closo</i> - In_{12} and open In_{15}^{106}

[a] References to the first eight are in Tables 4 and 5.

cluster contents that have been so achieved in thallium and (less explored) indium systems, all examples containing at least sodium and potassium. In fact, all trielide clusters, excepting Tr_4^{8-} , Tr_6^{6-} , Tr_7^{7-} , and Tr_{11}^{8-} are presently known only in compounds containing mixed alkali-metal ions (or higher charged cation components). Extensions to alkaline-earth metals and lithium should further the variations, although for lithium X-ray diffraction limitations and even special polylithium bonding states^[10] may be encountered. The corresponding effects in the thallium network structures have scarcely been explored, but mixed cations of various types have been reported to yield many new gallium and indium networks.^[107, 108] In general, one can imagine that a large variety of potential clusters may be captured from the starting alloy “soup”, and one or more clusters obtained are subject to the availability of a suitable cavity in a “good” structure. Along this line, neutron diffraction studies on a variety of binary alkali-metal–p-metal melts give clear evidence that polyanions are present in the liquid state. Related indicators are found in thermodynamic properties, resistivities, and the like as a function of composition. Diffraction spectra of K/Tl and Cs/Tl melts also reveal surprising prepeaks, indicating some degree of intercluster ordering as well.^[109, 110]

The following will enumerate the largely unpublished evidence for the specificity and variability of cation environments around some clusters. Four examples are shown in Figure 8. The special face-capping positions sought out by sodium ions are most evident, while the competition of sodium with the larger potassium (rubidium, cesium) ions at other sites is less readily interpreted but must also have to do with cation proportions, space filling, cation–cation separations, and so forth. On the other hand, it appears that sodium cations alone are too efficient at intercluster bonding in the sense that all binary Na/In and Na/Tl phases, except the two high-field Na_8Tr_4 compounds, occur in network structures

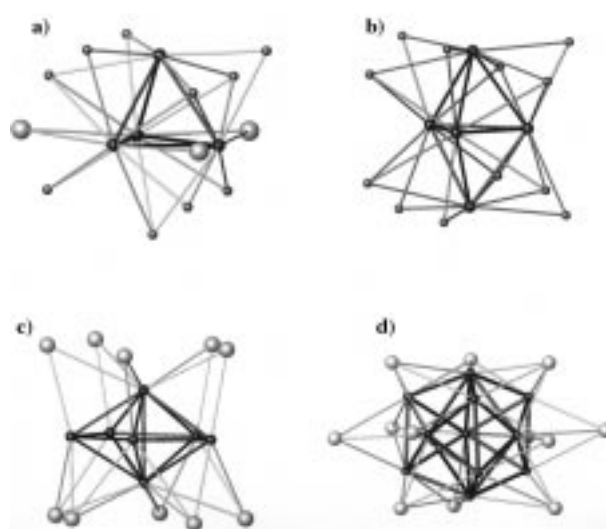


Figure 8. Cation environments about some cluster anions (black), with sodium as the smaller, lighter spheres: a) the $\text{Na}_{10}\text{K}_3\text{Tl}_4$ group in $\text{Na}_{23}\text{K}_9\text{Tl}_{15.33}$; b) $\text{Na}_{12}\text{Tl}_5$ in the same phase; c) the $\text{K}_{10}\text{Tl}_7^{3+}$ group in K_{10}Tl_7 ; d) the $\text{K}_{12}\text{Tl}_{11}$ clusters in K_8Tl_{11} .

in which $\text{Tr}–\text{Tr}$ bonds usually interbridge between clusters, often in complex ways (see below). Some specific examples of cluster solvation follow.

Tr_4^{8-} : In the isostructural Na_2In and Na_2Tl , the nearest neighbor ions at increasing distances from the center of Tr_4^{8-} cap all faces, bridge all but one edge, and are exo at each vertex of each tetrahedron, such that the packing is a little irregular in this orthorhombic structure. In addition, all of the five independent types of sodium act as bridges between two or three clusters with some distribution of these functions.^[68] The more symmetric Tl_4^{8-} component in $\text{Na}_{23}\text{K}_9\text{Tl}_{15.33}$ (see Figure 8a) has ten sodium atoms with a very similar motif on every face and edge, but with three potassium atoms bridging around one basal face and, further out, three more potassium atoms positioned exo at each vertex (not shown).

Tl_5^{7-} : In the same $\text{Na}_{23}\text{K}_9\text{Tl}_{15.33}$, the Tl_5^{7-} unit has all six faces and six edges bonded to sodium, but not the waist edges (see Figure 8b). Further out, there are also potassium atoms positioned exo at each thallium atom: three at each three-bonded apical thallium atom, and two at the less-negative, more-bonded thallium waist atoms (not shown). On the other hand, in the sodium-poorer $\text{Na}_9\text{K}_{16}(\text{Tl}_8\text{Cd}_3)(\text{Tl}_5)_2$, the sodium is face-capping and potassium is edge-bridging on one-half of each trigonal bipyramid, while the reversed roles appear for the other half. There are only exo potassium pairs around the waist. These dispositions are considerable more uniform than those around Tl_5^{7-} in the sodium-poor $\text{Na}_2\text{K}_{21}\text{Tl}_{19}$ where the essential sodium is only tetrahedrally bonded to a short edge on Tl_9^{9-} and to a long equatorial edge on the distorted Tl_5^{7-} .

Tl_7^{7-} : The cation neighbors and thence the presumed relative stabilities of these pentagonal bipyramids anions are distinctly different in K_{10}Tl_7 and $\text{Na}_{12}\text{K}_{38}\text{Tl}_{48}\text{Au}_2$, namely, $\text{K}_{10}\text{Tl}_7^{3+}$ versus $\text{Na}_4\text{K}_6\text{Tl}_7^{3+}$ in the first sphere. In the binary phase (see Figure 8c) potassium bridges edges on one pentagonal prism and caps faces on the other (this is in concert with out-of-plane waist bridging functions, not shown), while in the quaternary phase the cations alternate

face-capping roles in both halves of the prisms in the order K-Na-K-Na-K.

Tr_{11}^{7-} : Clusters in the family of rhombohedral A_8Tr_{11} (see Figure 8d, where the threefold axis is vertical) are surrounded by two independent types of cations. All six triangular faces about the waist (in pairs that share common prism edges) are capped by A2-type cations; each of these cations lies on a parallel threefold axis and thus has the same function in three adjoining clusters. Around each axial cap on the trigonal prism, three times as many A1-type cations (shown smaller in Figure 8) cap all the convex quadrilateral faces (bisected by the bond between capping atoms), bridge each edge of the trigonal pyramid, and are also exo at the three adjoining trigonal prismatic atoms. (The latter are omitted in the Figure 8d.) The same A1 cations also share these functions on adjoining clusters. An identical envelope of cations is found about the Ti_{11}^{7-} cluster in hexagonal $\text{K}_{18}\text{Ti}_{20}\text{Au}_3$.

$\text{Tr}_{10}\text{Zn}^{8-}$: A single type of potassium atoms has three regular functions on this D_{4d} polyhedron (see Figure 5b), collectively capping the eight triangular faces around the waist of each cluster, bridging eight cap-to-antiprism edges in a second, and bonding exo to the eight thallium atoms in the antiprism of a third cluster. The apex atoms thus have bridges on the four adjoining edges but no cation positioned exo, the same relationship as in Tr_{11}^{8-} where these vertices also appear less accessible.

Ti_{13}^{n-} : The three cubic or rhombohedral structures of salts that contain centered icosahedra (see Figure 7) yield the clearest views of the "solvation" and of the roles of cations. Figure 9 shows the cation environments about Ti_{13}^{10-} and Ti_{13}^{11-} as they occur in space groups $I\bar{3}m$ and $R\bar{3}m$, respectively, with the clusters shown as shaded polyhedra. A very

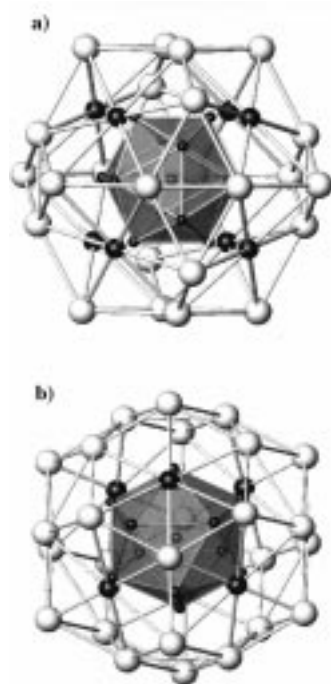


Figure 9. a) The Ti_{13}^{10-} cluster polyhedron in $\text{Na}_4\text{K}_6\text{Ti}_{13}$ with its neighboring eight sodium (small, black) and 24 potassium (larger, white) cations of its "solvation" sphere; b) the same representation of Ti_{13}^{11-} with Na_6K_{26} neighbors (D_{3d} symmetry).

specific positioning of the cations on the icosahedra pertains to the whole series; every face (20) and vertex (12) is capped by a cation to give a fixed solvation sphere of 32 cations about each. Sodium atoms in specific positions are essential, and both their number and orientation define the structures. The eight face-capping sodium atoms (black) in $\text{Na}_4\text{K}_6\text{Ti}_{13}$ (Figure 9a) lie in a regular cube about the cluster (on the cell diagonals) with the other 24 potassium atoms defining a slightly distorted truncated octahedron. In $\text{Na}_3\text{K}_8\text{Ti}_{13}$ (Figure 9b), the sodium atoms define a trigonal antiprism (viewed here almost along the threefold axis). In both cases, the sodium cations are located in the center of chairlike puckered hexagons of potassium cations, and all are also shared with neighboring clusters. (Of course, no alkali metal bonding is implied by the connections drawn, which are only to guide the eye.)

In both structures, each sodium atom caps a triangular face on both the cluster shown and on a neighbor related by the particular symmetry: eight in the case of $\text{Na}_4\text{K}_6\text{Ti}_{13}$ and six in the case of rhombohedral $\text{Na}_3\text{K}_8\text{Ti}_{13}$. These $\text{Ti}_3\text{-Na-Ti}_3$ figures are trigonal antiprismatic and too small to accommodate any other alkali-metal cation. The loss of one of these cations from $\text{Na}_3\text{K}_8\text{Ti}_{13}$ puts more thallium atoms around each cation so that the cavities are larger in $\text{Na}_4\text{K}_6\text{Ti}_{13}$ and, it turns out, rubidium or cesium can be accommodated as well. But there is no room to pack an eleventh cation in $\text{Na}_4\text{K}_6\text{Ti}_{13}$, as the largest cavity remaining is tetrahedral with three cation vertices and a radius of 2.48 Å. Alternatively, the creation of an electron hole in the HOMO to give Ti_{13}^{10-} must simply cost energetically less than disruption of this well-packed structure. In other words, good packing in both structures requires two different sizes of alkali-metal cations in specific ratios, and so the phases appear to be stoichiometric and distinct. The prediction of alternate structural modes is very difficult, if not impossible, but from present evidence we conclude that good structures for any of the known Tr_{12} anions cannot be accomplished with cations of the same size.

Ti_6Z : The substitution of the body-centered cluster in $\text{Na}_4\text{K}_6\text{Ti}_{13}$ by a Ti_6 octahedron to give primitive cubic $(\text{Na}^+)_{14}(\text{K}^+)_{6}(\text{Ti}_{12}\text{Na}^{13-})(\text{Ti}_6\text{H}^{7-})$ (and equivalent), Figure 7c, is accompanied by substitution of six sodium for potassium neighbors around the smaller central cluster with a commensurate decrease in the lattice dimension of about 0.9 Å. Cation dispositions are otherwise very similar in both compounds. The all-sodium environment of the octahedron is shown in Figure 10. Among the three independent sodium atom types, the Na2 atoms still lie on the body diagonals of the cell and cap all faces on the octahedra and on 40 % of the faces on the Ti_{12}Na units that lie at the cell corners. Likewise, pairs of Na3 atoms are positioned exo to each octahedral vertex. The latter cations lie in pairs in pseudotetrahedral sites on each of the cell faces (e. g., at $0, \frac{1}{2}, z$; $0, \frac{1}{2}, \bar{z}$) where they bridge between Ti_6H^{7-} in adjoining cells



Figure 10. The Na^+ ions about Ti_6^{8-} in $\text{Na}_{15}\text{K}_6\text{Ti}_{18}$.

and positions on adjoining $\text{Ti}_{12}\text{Na}^{13-}$ units above and below. These two polyhedra and the space group have uniquely suitable symmetries for this arrangement. Of course, all of these features, especially the mixed cation precondition, apply to the extended family of isotypic $\text{Na}_{14}\text{K}_6(\text{Ti}_{12}\text{M})(\text{Ti}_6)$ ($\text{M} = \text{Mg}, \text{Zn}, \text{Cd}, \dots$) where the now-empty octahedra are slightly expanded while the larger icosahedra are contracted on insertion of formally dipositive cations.

The special stability of the $\text{Na}_4\text{K}_6\text{Ti}_{13}$ packing (Figure 7a) is further emphasized by the discovery of the isostructural $(\text{Na}_2\text{Cd}_2)\text{K}_6\text{Ti}_{12}\text{Cd}^{[104]}$ noted before. Substitution of cadmium in Ti_{12} , as already known in the primitive cubic $\text{Na}_{14}\text{K}_6(\text{Ti}_{12}\text{Cd})(\text{Ti}_6)$ (Section 3.3) and discussed in the previous paragraph, would by itself leave the cluster short by two electrons, but this is nicely compensated for by random replacement of half of the sodium by dicationic cadmium in the closest sites (Figure 9a). The phase is a semiconductor as the outer cadmium ions must serve to enhance the valence band width through intercluster interactions. Substitution of a tetravalent cation in the icosahedron instead, zirconium for instance, could give a diamagnetic product as well.

4.3. A “Good” Structure

There are many features that need to be considered to judge a “good” structure, and some of these judgements derive from subjective experience. A useful pedagogical example is shown in Figure 11, but it is not in any way a

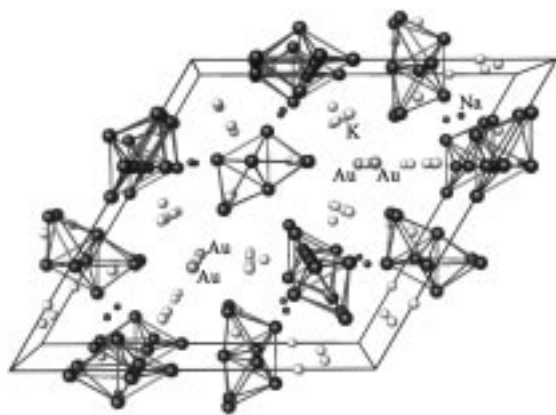


Figure 11. The $\text{Na}_{12}\text{K}_{38}\text{Ti}_{48}\text{Au}_2$ unit cell and its surroundings, with the Ti_9^{9-} and Ti_7^{7-} clusters emphasized. The alkali-metal and gold atoms are represented by small open and medium-sized black spheres, respectively. (This Figure is reproduced as the Frontispiece.)

special one. The phase $\text{Na}_{12}\text{K}_{38}\text{Ti}_{48}\text{Au}_2$ occurs in space group $P\bar{6}m2$, with $(\text{Na}^+)_{12}(\text{K}^+)_{38}(\text{Ti}_7^{7-})_3(\text{Ti}_9^{9-})_3(\text{Au}^-)_2$ as the independent unit (again in terms of oxidation states), there being one such unit in the cell. The phase is stable over a sizable region of the quaternary system and with apparently fixed proportions of the four components, so there is something special about these ions, their sizes, and their packing in this structure. The clusters (black atoms) lie on unit cell faces and exhibit only mirror symmetry together with twofold axes at the plane intersections ($mm2$ or C_{2v}). The potassium and

sodium positions are represented by the larger and smaller open spheres. Only the gold atoms (small solid spheres) lie on special points $\bar{6}$ at $\frac{1}{2}, \frac{2}{3}, 0$, and so forth. Little more can be judged from the illustration alone, and inclusion of bond representations between cations and clusters would make it quite inscrutable.

Closer inspection of distances and local environments is necessary to assess the system well. In a general sense, all contacts between component ions seem reasonable. All cations bridge between clusters, sodium being especially effective at this bridging between faces on pairs of each type of cluster. These sodium connections are in fact chainlike because of the mm symmetry of the clusters. There are four to eight thallium atoms at typical distances about each cation (at somewhat greater distances for potassium), and six to eight cations about each thallium atom except at the less-exposed, face-centered atom on Ti_9^{9-} (i. e., the centered atom in the parent Ti_{13} ; see Figure 3c). The shortest $\text{Ti}-\text{K}$, $\text{Ti}-\text{Na}$, and $\text{K}-\text{K}$ distances are found around the apices in Ti_7^{7-} . Sodium atoms contact most thallium atoms in the structure by bridging four edges on the base of Ti_9^{9-} and capping four faces on Ti_7^{7-} . The 3.50 Å $\text{K}-\text{Au}$ distances within the trigonal antiprismatic K_6Au are comparable to those in other cluster phases containing isolated gold and consistent with the eightfold 3.69 Å interatomic distance in CsAu (CsCl structure). The role of the two gold anions is evidently both to capture two extra electrons in the independent unit and to reduce repulsions within the cation lattice that lies between the clusters. The compound does not form without gold, nor will it take up more gold even though another free K_6 cavity of diameter 3.30 Å exists, presumably because of the lack of another electron. Other candidates to substitute for the gold anion, such as the larger halides, have not been tested.

Both clusters in this case are hypoelectronic ($2n$). It is, of course, necessary that the two clusters and Au^- have electronic structures and valence gaps that are consistent with a common Fermi energy. Further, the requirement of a periodic, three-dimensional structure must also be added to the “good” efficient packing and bonding implied above, all of which makes the overall result even more remarkable. Furthermore, the valence or oxidation state balance exhibited by this phase as well as by a large number of other cluster compounds is impressive and much more frequent than the formation of electrides or truly metallic phases with extra electrons.

5. Network Compounds as Zintl Phases

A further development of the concept of Zintl phases is now necessary beyond that given at the beginning of the review (Section 1). Many network compounds are also found to be valence compounds, even when electron-deficient clusters are involved, CaB_6 being a classic example. These are also collectively and usefully named Zintl phases. This descriptor is generally applied to compounds of active metals with p-metals that appear to have an electronically closed shell in their bonding, a condition that is often deduced from the structure alone.^[111] Originally, Zintl applied this idea to

the maximally reduced monoanions of the main-group elements, and its applicability was later extended to more complex compounds in which the bonding could still be described by octet rules and which might be isostructural and isoelectronic with a following element (Si_4^{4-} , layered As, and so forth).^[7, 8] More modern concepts have been incorporated regarding delocalized or electron-deficient bonding in networks, clusters, and so forth.^[10] All of the neat cluster compounds already considered are in the general sense Zintl phases, save for the few odd examples. However, in this section we are concerned only with those phases exhibiting infinite bonding in one, two, or three dimensions. Some very complex examples are known, especially in gallium systems^[108] and in mixed trielide–tetrelide or other p-metal combinations.^[109] We will limit our considerations to a few network tetrelide and trielide examples that fit with the preceding material.

Clearly the Madelung part of phase stability must be very important here too, and the surface structures of the cations on the anionic networks as well (Section 4). These aspects are more complex in networks and have not been well explored or generalized. The problems of filling space in an efficient way in a three-dimensionally bonded network are more complex than with isolated clusters, and valence closure in some instances may be only approximately achieved. Clearly however, the considerable complexity of many structures reflects a drive to conform to some sort of valence rules.

Many tetrelide networks are achieved in combinations with electron-rich pentels (pnictogens), chalcogens, or the like, but these represent more of classical Zintl phase chemistry^[8, 9, 109] which is not the intent of this article. Addition of cations to the tetrel elements classically gives structures related to those of later elements, for example, CaSi_2 that contains infinite sheets of 3b-Si^- with the arseniclike structure separated by cations.^[7] (In a shorthand often attributed to von Schnering,^[122] 4b-Tr^- or 4b-Tt^- is conveniently used, where 4b means four-bonded Tr^- or Tt^- .) Packing differences in SrSi_2 and LiGe yield analogous 3b-Tt^- lattices with a defect diamond structure and cations in the interstices therein, while those in zig-zag chains of many 2b-Tt^{2-} are found in 1:1 alkaline earth metal salts with the CrB structure.

5.1. Tetrelide Networks

Some more novel tetrelide networks have been realized recently that exhibit different means of cation accommodation. One is the well-known clathrate-I structure $[\text{A}_{\leq 8}(\text{H}_2\text{O})_{46}]$ that was originally assigned to the evident compositions A_8Tt_{46} . This presented problems, since the network of four-bonded tetrelide elements with cations in characteristic cavities apparently has no need or use for the electrons added by the eight alkali-metal atoms. The actual compounds in fact satisfy simple valence rules by incorporation of two specific tetrelide site vacancies ($\square$), which in turn are each accompanied by four neighboring Tt^- sites that absorb the eight electrons from the cations, more explicitly, as $(\text{A}^+)_8(4\text{b-Tt}^0)_{36}(3\text{b-Tt}^-)_8\square_2$.^[112, 113] Numerous isoelectronic, defect-free, and fully four-bonded variations of this have been generated by substitution of eight trielide atoms in the network, namely,

$\text{A}_8\text{Tr}_8\text{Tt}_{38}$ ($\text{A} = \text{K} - \text{Cs}$; $\text{Tr} = \text{Al} - \text{In}$; $\text{Tt} = \text{Ge}, \text{Sn}$)^[114] and with a change in cation as well, $\text{A}^{\text{II}}_8\text{Tr}_{16}\text{Tt}_{30}$ ($\text{A}^{\text{II}} = \text{Sr}, \text{Ba}$; $\text{Tr} = \text{Al}, \text{Ga}$; $\text{Tt} = \text{Si} - \text{Sn}$).^[115] A different proportion of elements from the two groups and another expression of cation accommodation in a four-bonded helical net occurs in $\text{Na}(\text{GaSn}_2)$ which is more clearly described as ${}_\infty[4\text{b-Ga}^-(4\text{b-Sn}^0)_2]$, Figure 12.^[116] A very similar result with a different space group is found for NaInSn_2 .^[117] Anionic tin is

particularly well known for its apparent drive and versatility to form closed-shell anions or networks in valence compounds, as examples witness the mixture of tin monomer and oligomer anions present in $\text{Ca}_{31}\text{Sn}_{20} [\triangle (\text{Ca}^{2+})_{31}(\text{Sn}^{4-})_5(\text{Sn}_2^{6-})_5(\text{Sn}_5^{12-})]$, where all of the tin atoms have simple octet counts.^[118] Likewise, the complex network in $\text{Na}_5\text{Sn}_{13}$ has precisely five 3b-Sn^- per formula unit as expected.^[119] Different variations are found in formal 3b-Tt^- products. A good number of other binary alkali-metal–tetrelide compounds evidently exist judging from phase diagrams,^[120] but their correct compositions and structures are unknown and basically unimaginable.

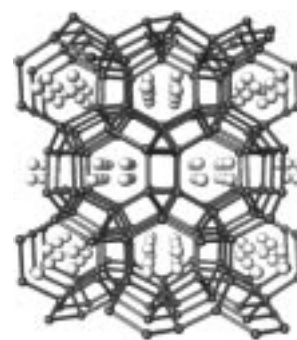


Figure 12. The structure of Na-GaSn_2 with sodium ions (lighter spheres) ordered in zig-zag helical chains of ${}_\infty[\text{GaSn}_2]$. The Ga and Sn atoms are disordered.

5.2. Trielide Networks

Intrinsic to the trielides are greater degrees of interbonding and condensation and, in many cases, incorporation of electron-deficient clusters, but this trend may also be reversed to some degree by the addition of more cations. Nothing gives us the insights needed to predict these often complex structures.

There are many ways in which one can design an anionic four-bonded trielide framework. The most famous is that which is isosteric with cubic-packed diamond and with the elements silicon and gray tin, namely the Zintl phase NaTl ,^[121] shown in Figure 13. The structural problem that remains has to do with cation accommodation. In the case of NaTl , along with NaIn and LiTr ($\text{Tr} = \text{Al} - \text{In}$), the appropriate “stuffed diamond” NaTl -type structure is exhibited since these cations fit well enough in the alternate tetrahedral sites of the parent lattice. Detailed theoretical and property studies also support the original concept of a substantial charge transfer and directed $\text{Tl} - \text{Tl}$ bonding.^[123] On the other hand, this arrangement is ordinarily not possible for KTl and CsTl , presumably due to the size of the cation, and these exist instead as an $(\text{A}^+)_6\text{Tl}_6^{6-}$ cluster (see Table 5).

A different variation occurs in the layered structure exhibited by Cs_2In_3 , Rb_2In_3 ,^[124] and K_2Ga_3 ,^[96] which have a straightforward electronic configuration. That for K_2Ga_3 is shown in Figure 14. The simple construction starts with an ideal Ga_6^{8-} octahedron, with 14 skeletal electrons ($2n+2$) in cluster molecular orbitals above six nominal $4s^2$ core states.

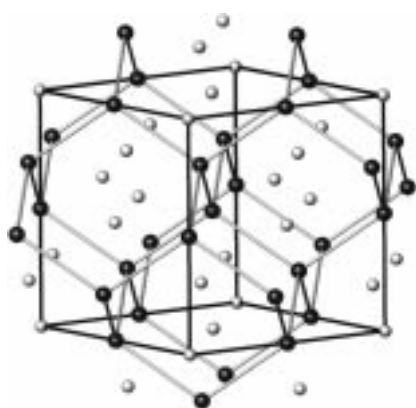


Figure 13. The cubic unit cell of NaTl emphasizing the Tl⁺ (diamond) net.

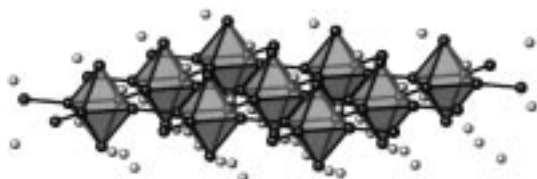


Figure 14. A portion of the layer of interbonded octahedra in K₂Ga₃.

Reduction of the cluster charge and layer generation can be imagined to arise by one-electron oxidation of each of the four 4s² pairs about the waist of the octahedron to give the imaginary tetraradical ion Ga₆⁴⁺. Condensation of these in a planar array then yields the structure shown, the two-electron – two-center intercluster linkages being characteristically shorter than the electron-deficient Ga–Ga “bonds” (edges) within the clusters. Thus K₂Ga₃ is reduced 1 e[−] mol^{−1} from the three-dimensional interbridged network found in CaB₆.

Both versions of known, isolated trielide clusters (see Table 5) as well as new types are found in compounds with network structures. The gallium systems are particularly productive.^[72, 107] Among the more striking are empty *closo*-Ga₁₂ analogues of the Tl₁₂M examples already described. A clear result occurs for Li₂Ga₇,^[125] in which *empty* 12b-Ga₁₂^{2−} clusters are connected directly and through Ga₂^{2−} links, as shown in Figure 15a. The former are also found in K₃Ga₁₃.^[126] Figure 15b shows how a new and smaller cluster variety, *closo*-Ga₈^{2−} dodecahedra, are bound into sheets and these sheets in turn bridged by isolated gallium atoms in AGa₃ (A = K–Cs).^[127] Another novel phase, Na₁₀Ga₆Sn₃, contains Ga₁₂ icosahedra bonded exo to terminal and bridging tin atoms to give chains.^[128] A conventional formulation of this network would leave the structure short one electron per formula unit. A high yield synthesis to confirm the stoichiometry of the crystallographic analysis, particularly for sodium, was not possible. A second phase in this system, Na₅Ga₆Sn₃, contains layers of similarly interconnected Ga₁₂ icosahedra interbonded to disordered Ga₂Sn₃ puckered layers. This is a valence phase according to the X-ray crystallography.^[129] Empty, interbridged In₁₂ clusters, together with other species, are found in A₃Na₂₆In₄₈^[105] and K_{18.2}Na_{4.8}In₃₉,^[106] among others.

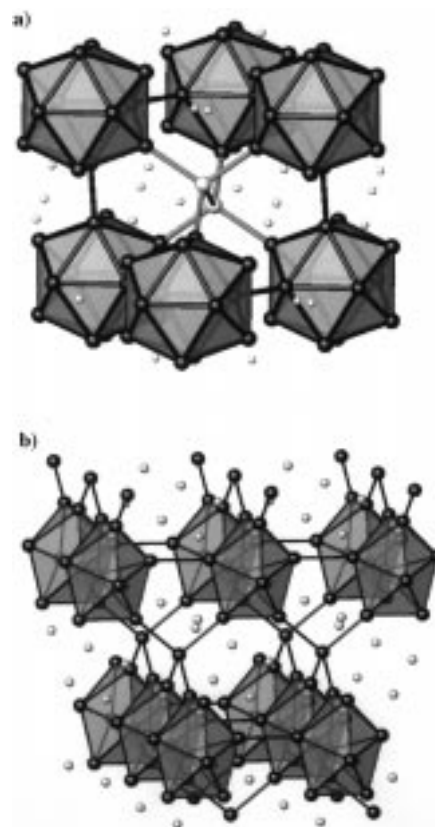


Figure 15. a) A portion of the structure of Li₂Ga₇ with isolated dimeric 6b-Ga₂^{2−} units interlinking sheets of interbridged Ga₁₂ icosahedra. b) The tetragonal structure of KGa₃ with chains of interconnected triangular-faced dodecahedra linked by 4b-Ga[−] atoms.

Other types of rather complex situations have been described and discussed,^[72, 108, 109] while K₃₄Zn₂₀In₈₅^[130] and Na₈K₂₃Cd₁₂In₄₈^[131] are more recent examples.

Another condensed product shown in Figure 16, Na₇In_{11.76},^[132] affords useful lessons about new clusters, bonding, and a close approach to a valence compound in a fairly complex arrangement. Here, *nido* clusters derived from icosahedra ($\sim C_{5v}$ In₁₁) are seen to be linked apex-to-apex as well as each vertex exo bonded through a single bridging atom to ten other clusters, meaning the nominal isolated *nido*-In₁₁^{15−} ($2n+4$) cluster has become In₁₁^{5−}. (The fractional stoichiometry comes about because of $\frac{3}{8}$ vacancies at the apices of these clusters.) The other unit in this structure is a new polyhedron, an elongated (sodium-centered) *closo*-In₁₆ cluster, that requires $2n+4$ skeletal electrons in isolation according to EHMO calculations. These polyhedra are oxidized too through intercluster bond formation at eight vertices, in part via triangular normal valent (3b-In^{2−}) linkages. Finally, MO calculations indicate that intercluster repulsion occurs between certain pairs of nonbonding electron pairs at exo positions on adjoining clusters. The total electron count, calculated to be necessary in consideration of all of these factors and complexities, comes to 99.4% of available electrons according to the refined composition; a remarkable closure that is probably within experimental uncertainties. The drive for closed-shell bonding is evident.

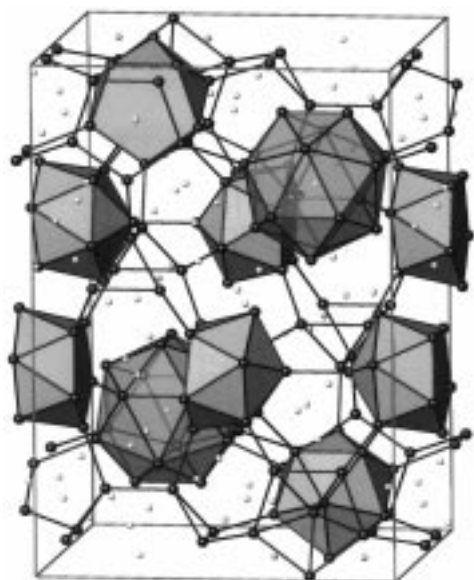


Figure 16. The tetragonal unit cell and surrounding atoms in $\text{Na}_7\text{In}_{11.76}$. Note the interbonded *closo*- In_{16} clusters, *nido*- In_{11} cluster dimers, and In_3 units.

One of the more novel results with thallium to date may be the columnar structure discovered for the composition $\text{Cs}_5\text{Tl}_{11}\text{Cd}_2$,^[133] a piece of which is shown in Figure 17. This is generated from face-sharing, cadmium-centered pentagonal Tl_{10} antiprisms (equivalently, condensed cadmium-centered



Figure 17. A portion of the infinite augmented chains of condensed cadmium-centered Tl_{10} antiprisms in $\text{Cs}_5\text{Tl}_{11}\text{Cd}_2$.

and -capped thallium icosahedra) to which four-bonded thallium “fins” are added on one side. The lopsided rods pack into an hexagonal array, well separated by cesium cations. This phase appears to be a zero-gap semiconductor according to Hückel band theory calculations.

Finally, the “granddaddy” of all trielide cluster condensation products to date must be that found in a couple of complex indium “fulleranes”. The In_{74} building block and the penultimate sodium sphere in the simpler $\text{Na}_{96}\text{In}_{97}\text{Z}_2$ ($\text{Z} = \text{Ni}, \text{Pd}, \text{Pt}$)^[134] are shown in Figure 18. This actually is the outer part of a multiply endohedral unit constituted as $\text{Ni}@\text{In}_{10}@\text{Na}_{39}@\text{In}_{74}$. Note that the innermost $\text{In}_{10}\text{Ni}^{10-}$ unit (not shown because of some disorder) had already been recognized in the form of an isolated cluster (see Table 5), while the outer In_{74} cage has the same conformation as the regular fullerene C_{74} (but not its π -bonding). Not surprising, in light of the cation effects noted earlier (Section 4.1), is the duality exhibited by the intervening Na_{39} complement (represented as open spheres) which places a sodium cation on the inside of every face of the In_{74} unit. (A second, similar shell also occurs on the

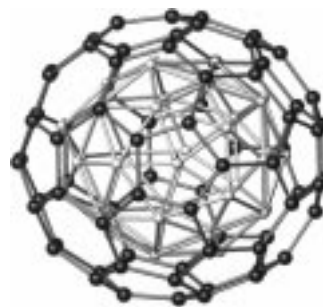


Figure 18. The fulleranelike In_{74} cluster and the endohedral Na_{39} sphere (white spheres) of $\text{Na}_{96}\text{In}_{97}\text{Ni}_2$. The In_{10}Ni core is not shown.

outside of every face.) The complicated part, which will not be detailed here, is the way in which this “stuffed buckyball” is incorporated into a three-dimensional solid. Every surface indium atom gains a fourth neighbor and an electron octet, either by condensation of the In_{74} units into close-packed layers through sharing of the six pentagonal faces around the waist of each unit or through some added In_3 and In_6 decorations bonded near the poles. Additional cations fall between the layers, some defining related $\text{Ni}@\text{In}_{10}@\text{Na}_{32}@\text{In}_{48}@\text{Na}_{12}$ units. The relationship between this complex $\text{Na}_{96}\text{In}_{97}\text{Z}_2$ ($\text{Z} = \text{Ni}, \text{Pd}, \text{Pt}$) and C_{74} are the same as between NaIn (stuffed diamond) and diamond. Furthermore, it differs from the NaIn composition only minutely, largely through the incorporation of a very few nickel, palladium, or platinum core atoms.

A second more complex phase, $\text{Na}_{172}\text{In}_{192}\text{Z}_2$,^[135] exhibits many of the same general features. Two macroclusters, $\text{Ni}@\text{In}_{10}@\text{Na}_{37}@\text{In}_{70}$ and $\text{Na}@\text{In}_{16}@\text{Na}_{39}\text{In}_2@\text{In}_{76}$, are co-condensed into puckered layers as before. All faces on both “fulleranes” are capped on the inside and the outside by sodium atoms except for two indium atoms in the penultimate sphere within the second unit. These are bonded near an open site on In_{76} and serve as tethers by which the innermost $\text{In}_{16}(\text{Na})$ cage unit is suspended! Isn’t chemistry grand!

6. Properties and Philosophy

Implicit in the closed-shell assumption for the assignment of Zintl phases is that these compounds should be diamagnetic semiconductors, although this characteristic has generally been investigated only rarely. As considered before in more detail,^[16] the properties of many of the trielide cluster phases are not so simple but, for some views, this does not always turn out to be of overwhelming importance as far as understanding the chemistry. One major value of the Zintl concept is in testing whether a new compound, its structure, and the electron distribution suggested therefrom are reasonable, whether important features such as impurities are perhaps missing, if something new in bonding is represented, or whether the compound is apparently metallic. Even the last feature is not necessarily fatal to our simple attempts to classify and understand cluster bonding.

A modest survey regarding electrical resistivities, magnetic susceptibilities, or both, of some of these tetrelide and trielide phases^[16] reveals that only a few have resistivities with

appropriately negative temperature coefficients (e.g., Rb_2In_3 , K_8Sn_{44}) while most have resistivities in the range of 200–800 $\mu\Omega\text{cm}$ with positive temperature coefficients. A larger number of phases are, nonetheless, diamagnetic (as opposed to Pauli-like paramagnetic) even after correction for orbital (Langevin) terms, including Na_2Tr , KTl , CsTl , $\text{Na}_2\text{K}_{21}\text{Tl}_{19}$, $\text{K}_{10}\text{In}_{10}\text{Ni}$, $\text{K}_8\text{Tr}_{10}\text{Zn}$, and $\text{Na}_{96}\text{In}_{97}\text{Ni}_2$. On the other hand, a good number of other examples that seem understandable in term of their structures are in fact Pauli-paramagnetic, for instance $\text{Na}_{23}\text{K}_9\text{Tl}_{15,33}$, $\text{Na}_{14}\text{K}_6\text{Tl}_{18}\text{Mg}$, and $\text{Na}_{12}\text{K}_{38}\text{Tl}_{48}\text{Au}_2$. Thus, $\text{Na}_3\text{K}_8\text{Tl}_{13}$ has a positive, approximately temperature-independent paramagnetism (circa $20 \times 10^{-4} \text{ emu mol}^{-1}$) and a resistivity of only about 70 $\mu\Omega\text{cm}$, suggesting it is a poor metal. In no case has any of these phases been characterized well enough to identify, or exclude, a semimetallic (indirect gap) property, a circumstance that would not in the simplest sense contradict Zintl concepts. In making conclusions on the basis of magnetic data, one should remember that many of the metals in this region are themselves diamagnetic because of nontraditional orbital and band properties.^[2]

It is worth noting that essentially all of the phases under consideration here retain other characteristics long attributed to Zintl phases, especially saltlike brittleness, stoichiometric (line) formulations, and melting points above those of the component metals, whereas the magnetic properties suggest an approach to the intermetallic world is also in process.^[3, 10, 66] Nonetheless, important considerations for our chemical understanding are that what we “see” in a structure can be predictably determined by the most tightly bound valence electrons (above ns^2 cores where appropriate), whereas the moderate resistive and Pauli-magnetic properties often found derive from a very small number of the most weakly bound electrons near the Fermi energy, for instance when band dispersion has closed the gap in a near-semiconductor. There is substantial evidence that the former matters more in these considerations; for example, we may usefully classify A_8Tr_{11} , $\text{K}_{18}\text{Tl}_{20}\text{Au}_3$, and so forth with “extra” electrons as “metallic Zintl phases” in which the bonding requirements associated with the clusters seem clear. In a different sense, La_3In_5 (Section 3.1) is a metallic Zintl phase apparently with a closed-shell cluster count but also inadequate intercluster separations. In fact, the square-pyramid clusters persist when the system is made electron richer in the isotypic La_3TrTt_4 and La_3Tt_5 analogues, the internal dimensions of the clusters changing only modestly and as expected from interactions of the LUMO cluster orbitals with the conduction band.^[136] Note that these metallic cluster systems do not react in what some may consider to be an obvious way, in which the metal–metal bonds open upon reduction; rather, many bonded features frequently persist into the intermetallic region.

Nesper has aptly likened these situations to examining a submerged continent observed through an ocean of the conduction electrons, where it is the structure of the former that matters more.^[10] Similarly, metallicity may come about when the nominally highest energy electrons or anions are instead delocalized, such as those in the highly reduced formal Tr^{5-} isolated atoms in both Y_5Ga_3 (Mn_5Si_3 -type) and La_3InC (inverse perovskite),^[16] and the hypoelectronic Ca_3Ga_5 ^[137, 138] and Sr_3In_5 .^[139] A similar result appears to pertain to the π^*

electrons on the dimers in some Cr_5B_3 -type compounds, which otherwise could be simply (or naively) formulated with closed-shell units as in $(\text{Ca}^{2+})_5(\text{Sn}_2^{6-})\text{Sn}^{4-}$. In fact these appear to be closer to multiply bonded dimers after π^* electron delocalization.^[140]

7. Trends and Forecasts

Some clear trends are evident in the foregoing, somewhat evolutionary, presentation. In the overall view, it is generally recognized that the transition from boron to thallium involves major changes in the chemistry, especially from strong and more directed bonds to more diffuse orbitals, more metallike behavior, and longer, weaker bonds. Considerable structural complexities in their compounds with the electropositive metals appear to be a common thread. The first step, an aluminum versus gallium comparison, has been largely ignored for metal-rich systems because there is so little that is comparable. Major differences in their fundamental properties are frequently cited as responsible (ionization energies, ionic and metallic radii, electronegativities, formation enthalpies, etc.) and attributed to the irregular intrusion of the 3d transition elements just before gallium with a resulting decrease in shielding of its valence electrons.^[141] These sizable contrasts between properties of elemental aluminum and gallium do not reveal what the corresponding differences in their chemistry will be.^[3, 66] Aluminum is certainly “harder” chemically and less electronegative, aluminides are generally more refractory, and their compounds with other metals are usually classified as intermetallics. At the same time, aluminides are still near the Zintl border in character and clearly exhibit significant homoatomic bonding, which usually maximizes bonding (overlap-weighted bond populations) within the aluminum framework but does not come near a closed bonding system (gap) electronically. For example, the complex two-dimensional aluminum framework found in $\text{Ca}_{13}\text{Al}_{14}$ ^[142] certainly demonstrates that directional Al–Al bonding is still very effective and important and that manifestations of the Zintl-Klemm concept, especially strong homoatomic bonding, vanish slowly.^[66, 143]

Gallium cluster chemistry has scarcely been described herein because, with few exceptions, so much of it pertains to complex networks, largely unrelated to the behavior of the rest of the family.^[72, 108] In the words of the principal author Belin, “atom defections, atom substitutions, disorder, or nonstoichiometry often obscure the understanding of electronic and bonding requirements [in these networks]”.^[144] The theoretical basis for the fused clusters sometimes encountered is even more problematical.^[145] The transition from gallium to thallium clearly involves considerable enhancement of the stability of isolated clusters, but not to an exclusive degree. Some largely unrelated network structures still appear for indium and thallium (to the extent that the explorations have been comparable for each element), but cluster anions of considerable variety dominate. Some important trends appear to operate in concert in the $\text{Ga} \rightarrow \text{Tl}$ transition—the apparent increase in the relative stability of the ns^2 pair down this series and a parallel decrease expected for the strengths of $\text{Tr}–\text{Tr}$

bonds (the enthalpies of formation of Tr(g) decrease by 36%). Since the imagined process for the formation of networks from clusters is oxidation of the ns^2 pair at each vertex (or some hybridized equivalent) followed by the generation of normal two-electron–two-center intercluster bonds, the expected trends for each step favor the formation of networks for gallium but clusters of thallium.

A pertinent question is often asked: why are the *anionic* states of the earlier p-metals so stable? To our knowledge this property has never been predicted or this strong proclivity well explained beyond the general electron deficiency of these elements and their compounds. One useful, but pretty simple, comparison is with energetic trends relative to the solid elements, that is, the relative energetics of disproportionation of these compounds into the component alkali and trielide metals when all changes in bond energies in the former are neglected. Since the gaseous states of both the sodium and gallium families are noteworthy for their relatively low enthalpies of condensation, there is simply less to be gained on decomposition of these compounds, or less to be required in countervailing bond strengths within the clusters.

Where next? So much of the excitement in exploratory synthesis is the discovery of the unpredictable and unimagined. On the other hand, some simple expectations come from just looking at the periodic table and the literature. Even a cursory look at the structures of the alkali-metal compounds formed by zinc and cadmium reveals numerous examples that can be interpreted as heavily condensed versions of clusterlike structures of the types seen here, a result that is appropriate to the lower electron populations. There must be a intermediate chemistry between these metals and the trielides. In addition, aluminum chemistry roughly along the lines of what has been seen for gallium family elements is too easy an expectation, and it will in fact be different in major and unimagined ways, as is already quite evident.

This article emphasizes the concept that a remarkable variety of homoatomic (and other) bonding types is found in anionic states of the post-transition metals, and that a great number of these diverse species can be understood in terms of stoichiometry, geometry, and electron count by simple applications of the Zintl-Klemm concepts together with, for electron-deficient deltahedra, the essence of Wade's rules. A great deal of room remains for further investigations that range from exploratory syntheses through property studies and theoretical explanations of the considerable variety of new and unprecedented materials. The greatest experimental difficulty in property measurements is handling these very sensitive phases as they are characteristically strong reducing agents and are readily oxidized to the p-metals. A good deal of attention to the detailed electronic and bonding states of these unusual compounds is needed. For instance, understanding bond length variations and differences in these compounds requires detailed calculations, while very few investigations of stability factors have included the important Madelung energy terms. Their boundaries with metallic and intermetallic substances are in much need of characterization, explanation, and understanding.

As for lessons learned, the more or less new, diverse, and important ways in which electron-poor cluster systems may be

stabilized are as follows: 1) distortions of conventional clusters to new bonding regimes; 2) centering of at least main-group elements within clusters; 3) cluster oxidation and formation of cluster networks; 4) utilization of sizable Madelung energy gains in the neat solid state from charged cations that are in intimate contact with anionic clusters or networks; and 5) the serendipitous location of particularly favorable component ions and clusters to achieve very stable three-dimensional structures, a process that at present is only accomplished empirically through synthetic explorations. Opportunities clearly abound!

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Deposition of Data from X-Ray Structure Analyses

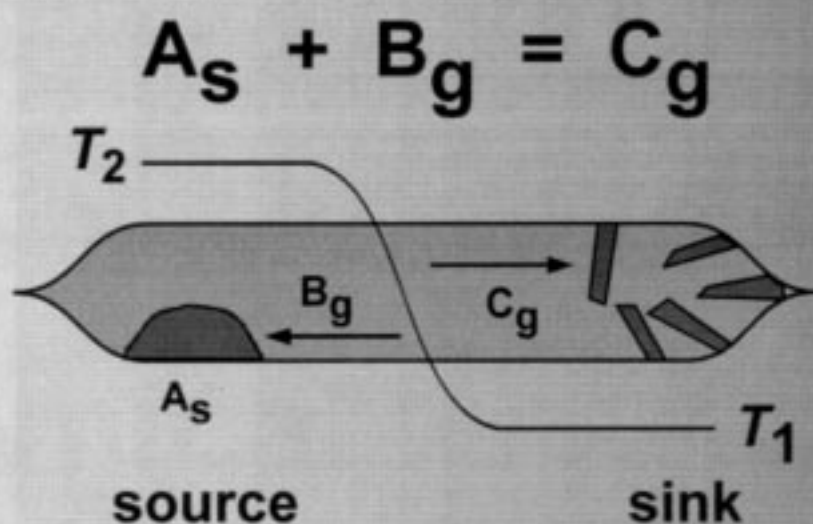
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Chemical Transport Reactions

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Chlorides Fluorides

Silicides Phosphates

Sulfates (Ln, U, Th) / M / O

Ln / M / O / Cl

Niobates

Oxides

Phosphides

Intermetallics

New Results of Chemical Transport as a Method for the Preparation and Thermochemical Investigation of Solids

Reginald Gruehn* and Robert Glaum

Dedicated to Professor Arndt Simon on the occasion of his 60th birthday

Chemical transport experiments are a valuable aid of great potential in the synthesis and thermochemical characterization of solids. Compounds of interest can often only be crystallized by this method. The use of a transport balance allows very detailed observations, particularly of the course of the experiment over time (simultaneous or

sequential deposition of multiple-phase solids). The computer program CVTRANS enables a quantitative thermochemical description of the experiment based on the cooperative transport model. A compilation of recent results illustrates the development and efficiency of the method. The transport of phosphides, anhydrous

phosphates and sulfates, and rare-earth oxide and oxohalide compounds are treated in detail.

Keywords: chemical transport • crystal growth • lanthanides • thermochemistry • transition metals

1. Introduction

Transport phenomena can have various causes. The most familiar is the *physical* transport of energy in various forms; as heat, electricity, or as electromagnetic radiation. Material transport as a physical process is of fundamental significance to chemistry. Important well-known examples include separation and mixing processes, ion migration in electrolytes, or the diffusion of components of an alloy. Physical transport can also be observed in a fridge: humidity migrates by sublimation or distillation of the water to the cooler parts of the unit to form ice. The same process is also utilized for freeze-drying.

In contrast we speak of *chemical* transport when a solid (or liquid) phase (A), that is not normally volatile under the given (temperature) conditions, migrates over a temperature gradient, or more precisely over a thermochemical potential gradient, as a result of a reversible reaction [Eq. (1)] with an added gaseous substance (B) to form a gaseous product (C).



In this way phase A, which is introduced at a defined position in the system, becomes mobile as part of the gaseous compound C and can be deposited at another position, that is at another temperature or at a lower thermochemical potential, by means of the back reaction according to

Equation (1). The resulting “transport” of A to another location can proceed to completeness in a closed system given sufficient time, since the “transport agent” B is not consumed. A low concentration of the transport agent (TA) is usually sufficient. Chemical transport (CT) is therefore not always easy to recognize as such.

The significance of natural, *mineral-forming transport processes*, in which a usually very slow deposition from the gas phase leads to formation of large single crystals of a mineral [e.g. A in Eq. (1)] was recognized for the first time in the middle of the 19th century. In 1846 Bunsen described how Fe_2O_3 can be transported in flowing HCl after studying volcanoes on Iceland.^[1] Please refer to Table 1 for other important experiments originating from this period.

A classical example of chemical transport from a modern viewpoint is the *industrial purification of Ni* developed in 1890 by Mond and Langer, in which the metal is vaporized as nickel tetracarbonyl and then deposited again at slightly higher temperatures.^[5]

Research into chemical transport processes received a new impulse in 1925 by van Arkel and De Boer.^[6, 7] The very intensive developments that continue to the present day began in 1950 with the work of Harald Schäfer and his co-workers. They defined for the first time, reactions as in Equation (1) as “chemical transport reactions” (CTR). Qualitative and quantitative methods of analysis were developed that were easy to understand and easy to use. Further questions on chemical transport were taken up and advanced by scientists from the group of H. Schäfer (H. Wiedemeier, M. Binnewies, R. Gruehn, and his student R. Glaum, see Table 1).

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Table 1. Developments in chemical transport.

Year	Development	Authors
1850–1890	migration of Fe_2O_3 in a HCl flow “artificial formation of minerals” transport of Si and Al via the subchlorides with the addition of gaseous SiCl_4 or AlCl_3 industrial purification of Ni by vaporization as $\text{Ni}(\text{CO})_4$ and decomposition at a slightly higher temperature	Bunsen ^[1] Saint-Claire Deville ^[2] Troost, Hautefeuille ^[3, 4] Mond, Langer ^[5]
1925–1930	vaporization of metals with iodine and decomposition of the iodides over a glowing wire transport of Au with Cl_2	van Arkel, De Boer ^[6, 7] Biltz, Fischer, Juza ^[8]
from 1950	wide-ranging investigation into fundamentals and many systems, review (up to 1962) ^[9] publication list up to 1987 ^[10]	H. Schäfer
from 1960	chalcogenides	R. Nitsche ^[11, 12]
from 1965	thermochemistry of transport; quantitative treatment of the deposition of compounds with a homogeneity range, crystal growth (also under zero gravity)	H. Oppermann, G. Krabbes ^[13]
from 1970	fundamentals and examples	Faktor, Garrett ^[14]
from 1970	chalcogenides, crystal growth (also under zero gravity)	H. Wiedemeier ^[15, 16]
from 1993	CT of intermetallic phases	M. Binnewies ^[17]
from 1975	“transport balance”, ^[18] “cooperative transport model”, ^[19] Transport of anhydrous sulfates ^[20]	R. Gruehn
from 1986	thermodynamic treatment of transport reactions; CT of phosphides and phosphates	R. Glaum ^[21–23]

In addition, a large number of scientists, who cannot all be named here, have contributed to the development of the method since the beginning of the 1960s by using CT for specific investigations and, in particular, to obtain single crystals for X-ray structure analysis. At this point, it should be noted that A. Simon, who was also a student of H. Schäfer, was able to obtain analytically perfect^[24] $\text{NbCl}_{2.33}$ crystals for the first time by means of chemical transport reactions in 1964 and by doing so was able to show conclusively that the composition of NbCl_2 was not correct.^[25]

2. Chemical Transport on a Laboratory Scale

2.1. Experiments in Closed Systems with Temperature Gradients

Regarding CT as an experimental laboratory method leads to restrictions of the subject. Reversible reactions between

gaseous and solid substances as in Equation (1) can take place in *open or closed systems*. Natural processes, such as the aforementioned crystallization of Fe_2O_3 , typically occur in “open systems”. In an industrial plant, which precludes the use of an open system, the reaction gas will be recirculated wherever possible, as with the transport agent CO in the well-known Mond process. Similar laboratory experiments can be considered as so-called “carrier gas experiments”, in which data on the vaporization equilibria can be measured with the help of a flowing gas; in this case a gas circulation loop is not necessary. The possibility to deposit solids as layers of given composition, based on reversible volatility equilibria is extremely important for technical applications. If a solid A is to be deposited by the decomposition of C_g into A_s and B_g in such a process [the reverse reaction according to Eq. (1)], it will often be unnecessary to prepare the required gas component C by a reaction of A_s with B_g [forward reaction Eq. (1)]. Instead, C is introduced directly into the gas flow

Reginald Gruehn was born in 1929 in Dorpat, Estonia. After completing his doctorate in 1962 under the supervision of H. Schäfer at the Anorganisch-chemisches Institut der Westfälischen Wilhelms-Universität Münster, he went on to complete his habilitation in 1969 at the same institute. From 1970 until he became emeritus professor in 1998, he was Professor for Inorganic and Analytical Chemistry at the Department of Chemistry of the Justus-Liebig-Universität, in Giessen. His research interests focus on chemical transport reactions and high-resolution transmission electron microscopy for structure investigations.



R. Gruehn



R. Glaum

Robert Glaum, born in 1960, grew up in Altenstadt/Hessen, studied chemistry at the Justus-Liebig-Universität in Giessen and at the Westfälische Wilhelms-Universität Münster, he completed his doctorate in 1990 with R. Gruehn in Giessen. After a one-year (1993/94) research visit with a Feodor-Lynen stipendium from the Alexander-von-Humboldt Foundation to M. Hitchman (University of Tasmania, Australia), he completed his habilitation in inorganic chemistry in 1999 at the Department of Chemistry of the Justus-Liebig-Universität Giessen. His research interests include the synthesis, structural chemistry, and physical characterization (color, magnetism) of anhydrous transition metal phosphates and the preparative and methodological development of the chemical transport method.

and A_s will be formed at the required position by appropriate adjustment of the pressure and temperature. This procedure, which is referred to as chemical vapor deposition (CVD), is used in many variations for technical purposes. We will not regard this subject further in this review.

In the laboratory, closed systems with *temperature gradients* have proven to be particularly valuable. In our research group, following a procedure already described by Schäfer,^[26] chemical transport experiments in silica glass ampoules ($l \approx 12$ cm, $d \approx 1.5$ cm, wall thickness ca. 1.5 mm) are carried out in electrically heated and electronically regulated two-zone tubular furnaces ($T_{\max} = 1150^\circ\text{C}$). The pressure in the silica glass ampoules should not exceed 10 atm for the given dimensions. The use of other ampoule materials for transport experiments at temperatures far above 1000°C has been described by Kaldis.^[27, 28]

2.2. Objectives and Possibilities of Transport Experiments

A substance that is to be transported is “dissolved” at the temperature of the “source” where it was introduced, according to Equation (1), and because of the reversibility of the reaction at the temperature of the “sink”, deposited once more, usually in a well-crystallized form. *Preparative experiments* can be performed in this way under clear, “defined” conditions, and lead to easily reproducible results, amongst other advantages. When the thermodynamic data of all participating components are known, predictions can be made about the kind of phases produced and about the order of magnitude of the rates of deposition. Often certain predictions and estimations can still be made even if not all the thermodynamic data are available. Also, the results of transport experiments allow the *delimitation of thermodynamic data* of the participating solids or gas molecules.

It is often possible to obtain solids in the sink as pure phases, whereas complete reaction is difficult in conventional solid-state reactions, or necessarily only at much higher temperatures. Transport methods are favorable in comparison to crystallization from solutions or melts because usually only very little “transport agent” is required, which is in most cases gaseous, so that impurities in the crystals can be easily prevented.

2.3. Experiments under Isothermal Conditions

In certain cases, experiments without an externally applied temperature gradient can also be useful.^[29] In such a case, the substance to be crystallized (A_s) is heated together with the transport agent (B_g) in a very short ampoule ($l \sim 3$ cm). If the equilibrium conditions [Eq. (1)] are favorable, a type of “*short-path transport*” between the crystallites or the particles of a powder (A), for example, in connection with a (thermodynamically favorable) particle or crystal growth occurs. The role of an applied gaseous “mineralizer” in small quantities can also be used with advantage to establish equilibrium between the components of a multiple-substance system (several solid phases), particularly when binary or multi-

component phases are to be formed during the experiment from the solid starting materials. In this manner, phase equilibria, for example in a three-phase system, can be investigated under temperature conditions under which no reaction between the reactants would be expected without the presence of the mineralizer. Examples from our research include the *elucidation of the phase relationships* between Ln_2O_3 , LnCl_3 and various metal oxides (see Section 8) and the investigation of the metal/phosphorus/oxygen ($M = \text{Ti, V, Cr, Mn, Co, Ni, Cu, Zn, Nb, Mo, Ta, W}$) systems.^[23] Also in cases where experiments in temperature gradients fail because of an inadequate rate of deposition or separation of the components of a polynary phase, “short-path transport” may be advantageous.

2.4. Deposition of Metastable Compounds from the Gas Phase

It may appear unlikely that *metastable* compounds can be obtained through chemical transport since the reaction is determined by equilibria [such as in Eq. (1)]. But in many cases the equilibria between the various phases possibly occurring at the sink side or between the modifications of one substance will not adjust (quickly enough) at the relatively low temperatures used. With sufficient supersaturation of the gas phase in the sink, deposition of a metastable solid phase is possible through a type of “metastable equilibrium” (see Ostwald’s Rule of Stages), without these phases converting to the thermodynamically stable solid during the course of the experiment. Many examples (Nb_2O_5 modifications,^[30, 31] ternary RE metallates (such as LnTa_3O_9 or $\text{LnNb}_7\text{O}_{19}$, see Section 7 and Table 7), tantalates and niobates of thorium (see Section 7), CoP_2 (see Section 5.3)^[32] have demonstrated that otherwise inaccessible compounds with potentially interesting new crystal structures can be obtained in this way.

2.5. Pathways to Maximum (Thermochemical) Information

Beyond their applications for preparative purposes, chemical transport reactions offer a relatively simple possibility to obtain important thermochemical information for the solids under investigation. Even if this is not the major objective of the experiment, *certain procedures* should be kept in mind when carrying out a reaction to not overlook simple accessible (thermochemical) information. For this purpose, the qualitative and quantitative composition of the equilibrium solids in the sink *and* the source, the migration rate, the order of deposition of the individual phases and, the solubility of the solids in the gas phase (from the difference between the initial and final weights) must be recorded *and* published.

In order to understand the thermodynamic causes of the reaction, one prerequisite is that the reaction is carried out under near equilibrium conditions. To get as close as possible to this state in the experiment, also in the interests of reproducibility, it is recommended that a so-called “*purification transport*” with the “reverse” temperature gradient is

performed before applying the experimental temperature gradient. This can help the various heterogeneous (solid/solid, gas/solid) and homogeneous (gas/gas) equilibria to become established before migration and deposition in the sink begins. If solids with complex compositions are to be synthesized “in situ” during a transport experiment (e.g. TiPO_4 from 4TiO_2 , $4\text{TiP}_2\text{O}_7$, $\text{TiP}^{[33]}$; LaTaO_4 (see Section 7)), several days of purification transports or pre-reaction may be necessary to establish the equilibria. In our experience, setting up the heterogeneous equilibrium between a single-phase solid and the gaseous transport agent takes only minutes (one hour maximum).

Information on the partial pressures that occur (partial pressure differences) can be obtained from the transport rates (TR or $\dot{m}$), if they are measured under the appropriate, carefully selected reaction conditions. However, this data can still contain considerable uncertainties. More precise thermochemical predictions can be obtained in many cases if one avoids looking solely at the desired phase (“single-phase transport”) in the sink, as was recommended in earlier times,^[9] but instead by assuming the presence of a multiple-phase deposit and by including the *appearance* or *disappearance of phases* in the source and the sink in the thermodynamic calculations. In this way it is possible to estimate the thermodynamic data of a previously unknown substance, for example, participating in the reaction.

To stop a transport experiment, the ampoule should always be removed from the oven with the source side first. This procedure ensures that the gas phase condenses onto the remaining starting solid and does not contaminate the phases deposited in the sink.

With the generally used method for chemical transport experiments, the transport rate is obtained as the ratio between the mass of the solid in the sink to the reaction time. With this method, with a multiple-phase solid in the sink, it is only possible to determine a *mean transport rate*. The experiment time can only be definitely determined when solid is still present on the source side of the ampoule when it is taken out of the oven (otherwise migration could have already ended much earlier, without this being recognized).

By means of a measurement arrangement developed in our research group, the so-called transport balance,^[18] to follow the progress of chemical transport experiments over time, the individual observations (quantity of solids, migration rates, sequence of deposition) can be determined even on a sealed ampoule with sufficient accuracy (see Section 3). The program CVTRANS can be used to model the very detailed experimental information obtained (see Section 4).

3. The Transport Balance and the High-Temperature Gas-Phase Balance

With the conventional method of measuring the TR, despite great care when carrying out the experiments, results were obtained for several systems that were only poorly reproducible.^[34] Further investigations showed that the deposition was kinetically inhibited, and could be initiated by coincidental external influences (e.g. CT of tungsten^[35]). In

contrast to the considerations of Schäfer^[9] on the chemical transport of a single-phase solid which coexists with the gas phase only but not with other condensed phases, we were able in the past years to characterize transport systems with much more *complex migration characteristics*. For example in the transport of phosphides with iodine, metal iodides are often formed in addition to the introduced phosphides (see Section 5.3).^[78, 79, 100] Multiple-phase compositions of the solid can also be observed in the transport of CuO with iodine (CuO_s , Cu_2O_s , CuI_l)^[36] or with anhydrous sulfates (e.g. $\text{FeSO}_{4,s}$, $\text{Fe}_2\text{O}_{3,s}$, $\text{Fe}_2(\text{SO}_4)_{3,s}$).^[124–126] A further example is the chemical transport of the various phases occurring in the Cr/O/Cl system, in particular CrOCl ($\text{Cr}_2\text{O}_{3,s}$, CrOCl_s , $\text{CrCl}_{2,s}$, $\text{CrCl}_{3,s}$).^[37, 38] In all these examples, the *transport balance* (HTB, Figure 1) has proven very valuable when performing

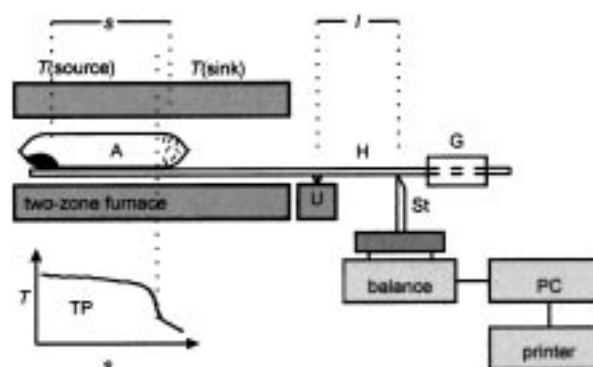


Figure 1. Schematic representation of the transport balance.^[18] A: ampoule, H: balance beam made from two corundum rods, G: counter weight, St: rod for the digital balance, TP: temperature profile, U: pivot of the balance beam, s: transport path (diffusion path).

the experiments. This balance was constructed according to the principles of a beam balance. The migration of a solid within the ampoule (from the end of the supporting arm in the direction of the pivot) leads to a higher loading of the electronic analysis balance. The change in weight, when certain geometric conditions are maintained, corresponds to the mass deposited in the sink and can be recorded with a personal computer. A detailed description of the construction and function of the apparatus can be found in the literature.^[18, 67] Figure 2 shows the example of the *progress over*

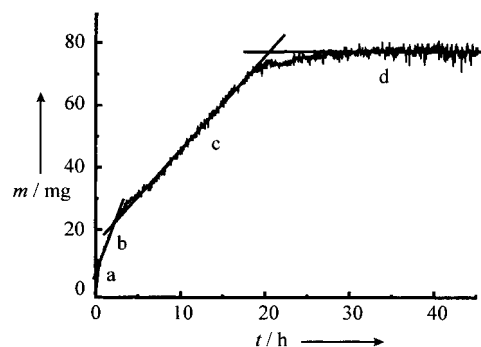
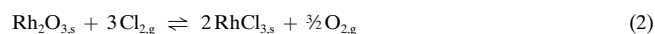


Figure 2. Measurement protocol for the chemical transport of Rh_2O_3 with chlorine.^[105] a) Establishing equilibrium between the solid and the gas phase, b) sublimation of RhCl_3 , c) transport of Rh_2O_3 , d) complete transport.

time of a chemical transport experiment^[105] of Rh_2O_3 with chlorine ($1075 \rightarrow 975^\circ\text{C}$, 75 mg Rh_2O_3 , 54 mg Cl_2 , $V = 20\text{ cm}^3$, $s = 10\text{ cm}$), as recorded with the transport balance. Once equilibrium has been established (section a in Figure 2) three sections with differing slopes (transport rates) can be seen. In section b the deposition of $\text{RhCl}_{3,s}$ ($\dot{m} \approx 8\text{ mg h}^{-1}$, $m \approx 22\text{ mg}$) occurs at a higher rate, then (section c) $\text{Rh}_2\text{O}_{3,s}$ migrates ($\dot{m} \approx 3\text{ mg h}^{-1}$, $m \approx 55\text{ mg}$), until the solid in the source is completely consumed (section d). The measurement arrangement enables the individual transport rates for the two steady states b and c, as well as the masses of the solids to be determined with sufficient accuracy *without* the need to open the ampoule. Individual experiments and also complete measurement series at variable temperature can therefore be performed with a single ampoule. In this way, very detailed and more reproducible data can be obtained than by the conventional method and evaluation of transport experiments.

The amounts of $\text{RhCl}_{3,s}$ observed besides $\text{Rh}_2\text{O}_{3,s}$ during the $\text{Rh}_2\text{O}_3/\text{Cl}_2$ experiment described agree very well with the thermodynamic calculations.^[105] The $\text{RhCl}_{3,s}$ mass is obtained from Equations (2) and (3) and is essentially determined by the thermodynamic stability of the two solids. In contrast, thermodynamic conclusions (for example, about the stability of a deposit) derived from the observed transport rates, can easily be falsified by “non-thermodynamic” influences (kinetics, convection).



An interesting modification of the “transport balance” is the “high-temperature gas-phase balance”.^[39] In this case the shift in the center of gravity of the mass of the ampoule contents (see Figure 1) is not caused by chemical transport in a temperature gradient but by simple isothermal vaporization or decomposition of the solid at one end of the ampoule. The measured loss in mass for the vaporization of potassium iodide (500 mg KI, $l = 12\text{ cm}$, $V = 24\text{ cm}^3$) as a function of the temperature is shown in Figure 3.^[40] The drop in the curve in the nonsaturation region is caused by an incomplete correction of the baseline.

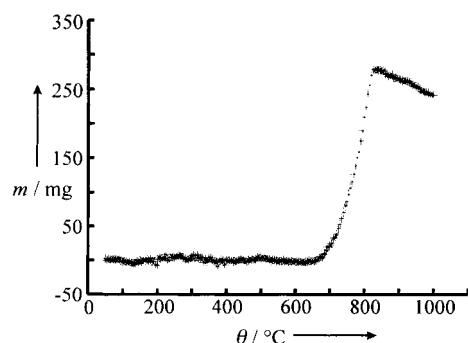


Figure 3. High-temperature gas-phase balance.^[39] Vaporization of KI.^[40] Start of vaporization at about 620°C ; above about 810°C the compound is completely gaseous.

4. Thermodynamic Modeling of Chemical Transport—The Computer Program CVTRANS

A comprehensive summary of the different concepts used to describe chemical transport experiments has recently been provided by Lenz and Gruehn.^[67] Therefore we will limit the description here to the models and procedures used in our research group.

4.1. Modeling Chemical Transport Experiments

Modeling chemical transport experiments has different objectives. Orientational calculations *before* an experiment can give information about the most favorable conditions with respect to temperature and the choice of transport agent. Modeling *after* an experiment, or better after a series of experiments under systematic variation of the reaction conditions, gives information about the composition of the equilibrium gas phases, which is only rarely accessible by direct measurement. It is possible, by means of a critical(!) comparison of the observations (see Section 2.5) with the results of model calculations, to check or at least delimit the thermodynamic data (TD) (enthalpies of formation of VP^[78], CrP and MnP^[79], niobates MNb_2O_6 ,^[159] CrOCl ,^[38] anhydrous sulfates MSO_4 and $\text{M}_2(\text{SO}_4)_3$ ^[124–126]). Overall, thermodynamic analysis leads to a very detailed understanding of the chemical equilibria occurring in such a (transport) system.

The aim of modeling is to attain an exact as possible reproduction of the different observations in a transport system. A differentiation must be made between values that depend exclusively on the thermodynamics of the participating condensed phases and gas molecules, such as the composition of the equilibrium solids and their solubility in the gas phase, and values that are influenced by “non-thermodynamic” effects or even determined by them. *Transport rates* belong to the latter category. These are connected to the partial pressure differences between the source and sink and therefore also with the thermodynamic characteristics of the system according to the diffusion formulation of Schäfer [Eq. (4)]^[9], but can also be considerably influenced by kinetic effects and mass flux by convection.

$$\dot{m} = \frac{a\Delta p_c \bar{T}^{0.8}}{c\sum p} \frac{qD_0}{s} 1.8 \times 10^{-3} \quad [\text{mol h}^{-1}] \quad (4)$$

$\dot{m}$: Transport rate [mol h^{-1}]; a, c : stoichiometric coefficient of the transport equilibrium (for the CT of Rh_2O_3 , [see Eq. (27)], $a/c = 1/2$); $\Delta p_c = p(\text{C})_{\text{source}} - p(\text{C})_{\text{sink}}$, where $p(\text{C})$ is the equilibrium pressure; $\sum p$: total pressure; $\bar{T}$: average temperature of the diffusion path [K]; q : cross-section [cm^2] and s : length [cm] of the diffusion path or the transport path; D_0 : mean diffusion coefficient [cm^2s^{-1}] (values between $0.1^{[9]}$ and $0.025^{[41, 42, 49]}$ have been reported in the literature for D_0 .)

To a limited extent, the influence of convection on the transport rates can be approximated,^[14] but, overall, these calculations are relatively uncertain and the underlying theories of flow dynamics have not yet been adequately

developed.^[67] For the further analyses given here, therefore, the validity of the formulation from Schäfer is taken as a prerequisite.

The calculation of the equilibrium solids and the gas phases in the source and sink are carried out in our method by means of the *minimization of the free enthalpy* of the system ($G_{\min}$ method).^[43, 50] For this the experimental conditions (temperature, ampoule volume, initial weights) are required, as well as the thermodynamic data [$\Delta_f H_T^0$, S_T^0 , and also possibly $C_p(T)$] for all the gas molecules and condensed phases under consideration. The advantage of this procedure over the better known method for the calculation of the equilibrium partial pressures via K_p is, on one hand, in the easy, prerequisite free handling of multiple-phase deposits (the occurrence of certain phases follows from their TD), and, on the other hand, in the simple automation of the calculation (see Section 4.2). In addition to the equilibrium calculations, the *cooperative transport model*^[19, 44] for the calculation of experiments is of fundamental importance. The basic idea of the model is illustrated by the flow diagram in Figure 4. The first

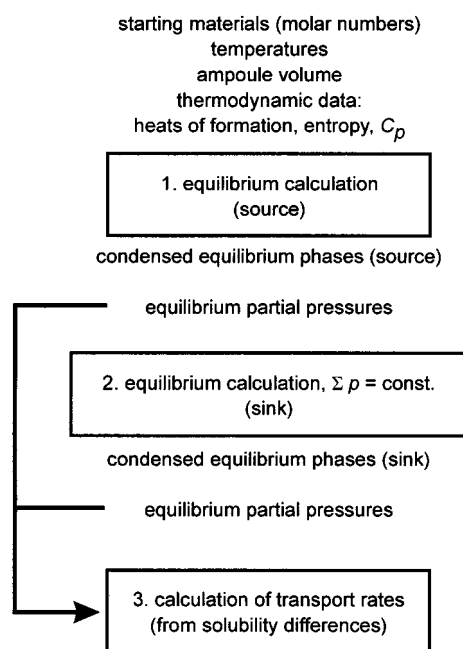


Figure 4. Cooperative transport model. See text for details.

step is an equilibrium calculation (Q_1) for the source temperature. Regarding the experimental conditions and the TD this gives the equilibrium solids and the partial pressures in the source. In a second calculation step, a further equilibrium calculation (S_1) for the temperature of the sink is performed with the *composition of the equilibrium gas phase from Q_1* . One restriction must be taken into consideration: $\Sigma p_{\text{source}} = \Sigma p_{\text{sink}}$ must be fulfilled. From this requirement, it is unavoidable that occasionally an ampoule volume is calculated for S_1 that deviates more or less strongly from that of Q_1 . Schäfer was able to show earlier that this effect does not cause problems when deriving transport rates from calculated partial pressures.^[45]

The great advantage of the cooperative transport model over other models (“ ε relationship”,^[13, 46] “four-stage-model”^[47, 48])^[67] in our experience lies in the easy handling of a transport system, in which the components of a multiple-phase equilibrium solid are transported one after another from the source to the sink; so-called *non-stationary transport behavior*. To model such experiments, a new source calculation, Q_2 , follows on from S_1 . This possibility was first recognized by Schmidt and used to calculate the chemical transport of GeO_2 with hydrogen (solid: $\text{GeO}_2 + \text{Ge}$, $750 \rightarrow 850^\circ\text{C}$).^[49] In contrast to Q_1 , the original weight is not used for Q_2 , but an elemental mass balance that is obtained from the original weight minus the mass of the solid from S_1 . S_2 follows on from Q_2 , then Q_3 , S_3 , and so on. In this way the entire solid in the source is gradually shifted to the sink. In the transport of multiple-phase solids, a sequence of steady states is observed in the calculations, which means that one phase is “dissolved” in one Q_n calculation and then deposited in S_n . As long as the amount of one phase decreases in the source and increases in the sink in each calculation cycle, the composition of the gas phase from the source to the sink remains constant (steady-state behavior). The transition from deposition of one phase to the next is characterized by the non-stationary behavior of the gas phase. A concrete example (Figure 2) shows the already quoted migration of $\text{Rh}_2\text{O}_{3,s}$ after the deposition of $\text{RhCl}_{3,l}$ where the model calculations over several cycles find a dissolution of the oxide in the source but continuing deposition of the chloride in the sink. In this way, the amount of oxygen in the gas phase increases from cycle to cycle until ultimately its activity is sufficiently high to prevent further deposition of chloride in the sink. The steady-state transport of Rh_2O_3 follows this “non-stationary” region in the calculation. *It is only meaningful to report calculated transport rates* when the model calculations have been performed on the basis of partial pressures *in steady-state regions*.

4.2. The Computer Program CVTRANS

The computer program CVTRANS^[21] was developed in our research group for the above-mentioned model calculations. The program was preceded by the less user-friendly programs SOLGASMIX,^[50] EPCBN,^[51, 52] and its derivatives EPC, EPDELT, and EPLAM^[53] that were used for several years in our group. CVTRANS was developed with the programming language Delphi 2.0 for Windows 95/98. In accordance with the Windows interface, almost all the “self-explanatory” inputs are performed with several “windows” (DATA, INPUT, CALCULATION, RESULTS). Even very involved calculations with many cycles or model calculations of entire experimental series are possible in only a few minutes. In addition to the improved user friendliness, the program has several additional routines with which the iteration algorithm for the minimization of G has been improved and stabilized considerably in comparison with similar programs.

The program is available on request from the authors with additional explanations and is also accessible on the Internet.^[21]

5. Metals, Intermetallic Compounds, Silicides, and Phosphides

Because of the direct technical interest, chemical transport for purification and crystallization of many elemental metals and semimetals has been the focus of extensive investigation for some time. Here we will report only on fundamental research, principally from Schäfer et al., (Si,^[54, 55] Ge,^[56–58] Nb,^[80] Ta,^[59] Mo,^[60] W,^[35] Fe,^[61] Co, Ni,^[61] Cu,^[62] Ag, Au, Pt metals^[63]) and review articles on this field of research.^[64, 65] In particular, the gas-phase transport of molybdenum and tungsten in connection with the mode of function of halogen lamps has been repeatedly and thoroughly investigated.^[66–68]

5.1. Intermetallic Phases

Despite the extensive work already done, it was only in the past few years that Binnewies and co-workers investigated the crystallization of alloys and intermetallic compounds by means of chemical transport experiments. Table 2 contains a list of the results obtained. In the systems Mo/W^[69] and Co/Ni,^[70] which show continuous miscibility, and Cu/Ag,^[71] transport behavior similar to that of the pure metals is observed. A new feature is the occurrence of mixed gaseous halides of the metals A and B (e.g. Cu_{3–x}Ag_xI_{3,g}), without the thermodynamics of the vaporization of the metals being greatly altered. The germanides and stannides in the systems Cr/Ge,^[72] Co/Ge,^[73] Ni/Sn,^[74] Ni/Ge, Co/Sn, and Cu/Sn^[17] show modified transport behavior with respect to the elements (see Table 2). The available observations did not permit an extensive

thermodynamic discussion of the participating transport reactions. The examples show, however, the great preparative advantage of the method for the crystallization of intermetallic phases at temperatures much lower than their melting points. It will be interesting in the future to investigate the above-mentioned and also new “intermetallic” transport systems to gain an in-depth understanding of the complex thermodynamic interactions (nonstationary transport behavior, deposition of phases with broad homogeneity ranges, and the control of their compositions).

5.2. Silicides

After the orientational research in the early 1970s (Ti₅Si₃, TiSi, TiSi₂, VSi₂, Cr₃Si, Cr₅Si₃, CrSi, CrSi₂, FeSi^[75, 76]), the work of Krabbes, Oppermann and co-workers, in particular, led to a detailed thermochemical understanding of the migration of this class of substances in a temperature gradient.^[77]

As examples only the heterogeneous equilibria that determine the transport of CrSi_{2,s} will be discussed. With the addition of chlorine or bromine, migration to the cooler side of the ampoule is observed: it is not the halogens themselves that act as the transport agents but the halides SiCl_{4,g} and SiBr_{4,g} that are the result of a reaction prior to Equations (5) and (6).



Table 2. Intermetallic phases. Crystallization by chemical transport experiments. $T_1 \rightarrow T_2$ exothermal reaction, $T_2 \rightarrow T_1$ endothermal reaction ($T_1 < T_2$).

Compound	T [°C]	Transport agent (amount)	$\dot{m}$ [mg h ⁻¹]	Comments	Ref.
Mo/W	1000 → 900	HgBr ₂ (ca. 120 mg)		deposition of mixed crystals of variable composition at T_1	[69]
Cr(Ge)	780 → 880	I ₂	ca. 0.4	transport from a two-phase region Cr(Ge)/Cr ₃ Ge	[72]
Cr ₃ Ge	780 → 880	I ₂	ca. 0.4		[72]
Cr ₅ Ge ₃	780 → 880	I ₂	ca. 0.02	CT of Cr ₁₁ Ge ₈ alongside Cr ₅ Ge ₃	[72]
Cr ₁₁ Ge ₈	780 → 880	I ₂	ca. 0.2	CT of CrGe alongside Cr ₁₁ Ge ₈	[72]
CrGe	780 → 880	I ₂	≈ 0.1	CT of Cr ₁₁ Ge ₁₉ alongside CrGe	[72]
Cr ₁₁ Ge ₁₉	780 → 880	I ₂	ca. 0.3	CT of Ge(Cr) alongside Cr ₁₁ Ge ₁₉	[72]
Co ₅ Ge ₃	900 → 700	I ₂	ca. 4		[73]
CoGe	900 → 700	I ₂		CT from the two-phase region Co ₅ Ge ₃ /CoGe ≈ 4 [a]	[73]
CoSn, Co ₃ Sn ₂	900 → 700	GaI ₃		CT from the two-phase region Co(Sn)/Co ₃ Sn ₂	[17]
Co/Ni	900 → 800	I ₂ /GaI ₃		Co more soluble in the gas phase than Ni, use of a three-zone oven to compensate: 900 (Co) → 940 (Ni) → 800 (sink) ^[b]	[70]
Ni(Ge)	1000 → 800	GaI ₃		CT from the two-phase region Ni(Ge)/Ni ₃ Ge alongside Ni ₃ Ge	[17]
Ni ₃ Ge	1100 → 1000	GaI ₃			[17]
Ni ₅ Ge ₃	900 → 800	GaI ₃		CT from the two-phase region Ni ₃ Ge/Ni ₅ Ge ₃	[17]
Ni ₃ Sn	900 → 700	I ₂ (0.8 atm at 900 °C)	< 0.2		[74]
Ni ₃ Sn ₂	900 → 700	I ₂ (0.8 atm at 900 °C)	< 0.2		[74]
δ-Cu ₄₁ Sn ₁₁	550 → 600	I ₂		SS: ^[c] “Cu ₈₆ Sn ₁₄ ”	[17]
ζ-Cu ₁₀ Sn ₃	550 → 600	I ₂		SS: ^[c] “Cu ₇₅ Sn ₂₅ ”	[17]
Cu(Sn)	550 → 600	I ₂		SS: ^[c] “Cu ₉₁ Sn ₉ ”	[17]
Cu/Ag	600 → 700	I ₂ (150 mg per ampoule)		transport from the two-phase region Cu(Ag)/Ag(Cu); at T_1 (source) melt of CuI/AgI; in the gas phase Cu _{3–x} Ag _x I ₃ ($x = 1, 2, 3$)	[71]

[a] Both Co₅Ge₃ and CoGe have a remarkable phase width; the authors did not give details. [b] No details were given by the authors on the equilibrium solids at 940 °C and 900 °C. [c] Starting solid.

The transport of CrSi_2 with iodine, in contrast, proceeds to higher temperatures as a result of an exothermic reaction [Eq. (7)].



There is a clear analogy to the transport behavior of elementary silicon^[54] with iodine (exothermic at low iodine amounts; endothermic at high iodine quantities with the TA $\text{SiI}_{4,\text{g}}$).

5.3. Phosphides

For several years, we have extensively investigated the chemical transport of transition metal phosphides. As shown in Table 3, in many cases the method permits very good access

to well-crystallized samples (see Figure 5e (β - WP_2), 5h CoP_2 , 5i: CoP_3 , 5r: CrP). In addition to the great preparative advantages, transport experiments on this class of substances have enabled further development of the method (see Sections 3 and 4). Information gained from a critical comparison of all experimental observations (see Sections 2.5 and 3) with the results from thermodynamic model calculations (see Section 4) enable, in addition, a detailed (chemical) understanding of the transport-determining equilibria. In this way, careful selection of the most favorable experimental conditions for the transport of new compounds becomes possible. Our results are summarized in the following section.

In the majority of cases it is possible to *transport transition metal phosphides with iodine*. Depending on the thermodynamic stability of the phosphide and the volatile metal iodide, migration in a temperature gradient can take place by means

Table 3. Chemical transport of phosphides. $T_1 \rightarrow T_2$ exothermic reaction, $T_2 \rightarrow T_1$ endothermic reaction ($T_1 < T_2$).

Phos.-phide	T [°C]	Transport agent (amount per ampoule)	$\dot{m}$ [mg h ⁻¹]	Comments	Ref.
TiP	800 → 900	I ₂ (20–140 mg)	≤ 10		[22, 92]
TiP ₂	650 → 700	I ₂ (150 mg)	ca. 2.2	excess P suppresses TiP	[91]
ZrP	950 → 1050	I ₂ (150 mg)	≤ 20	low temps lead to formation of ZrP ₂	[91]
ZrP ₂	850 → 900 ^[a]		≤ 5	at $T(\text{sink}) \geq 1050^\circ\text{C}$ deposition of ZrP	[91]
VP	810 → 930	I ₂ (255 mg)	ca. 22;	VI _{2,1} in the source; reversal of direction of transport possible (see text)	[78]
VP ₂	800 → 700	I ₂ (ca. 250 mg)	≤ 10	addition of extra phosphorus (ca. 100(!) mg per ampoule) suppresses VP	[91]
VP ₄	700 → 600	I ₂ (ca. 300 mg)	≤ 5	addition of extra phosphorus (ca. 100(!) mg per ampoule) suppresses VP ₂	[246]
NbP	850 → 950	I ₂ (200 mg)	≤ 10	no transport with smaller iodine amounts	[91]
TaP	850 → 950	I ₂ (ca. 200 mg)	≤ 5	no transport with smaller iodine amounts	[91]
CrP	1050 → 950	I ₂ (12–137 mg)	≤ 15	CrI _{2,1} as further deposit at high iodine amounts	[22, 79]
Mo ₃ P	1100 → 1000 or 900 → 800	HgBr ₂ (10 and 130 mg)	≤ 10	Mo ₄ P _{3,5} and MoBr _{2,1} as additional deposits, halide only in experiments with $\bar{T} = 850^\circ\text{C}$	[87]
Mo ₄ P ₃	1050 → 950 and 900 → 800 ^[c]	HgBr ₂ (10–150 mg)	≤ 10		[87]
MoP	1000 → 900	I ₂ (200 mg) + oxygen ^[b]	≤ 3	no transport without O ₂	[87, 91]
	1000 → 900	HgBr ₂ (50 mg)	ca. 3		
MoP ₂	1000 → 900	I ₂ or HgCl ₂ (ca. 200 mg) + oxygen ^[b]	ca. 1 ^[d] ;	excess P otherwise formation of MoP	[247]
WP	1000 → 900	I ₂ (200 mg) + oxygen ^[b]	ca. 1	no transport without O ₂	[91]
WP ₂	1000 → 900	I ₂ (ca. 200 mg) + oxygen ^[b]	ca. 2	slight P excess suppresses formation of WP; large excess suppresses transport	[247]
MnP	1000 → 1100	I ₂ (a few mg)	≤ 2.6	MnI _{2,1} always obtained as additional phase in the source	[22, 79]
Fe ₂ P	1000 → 900	HgBr ₂ (!) (100 mg)	≤ 1	FeP always as additional phase in the source; no transport with iodine	[88]
FeP	800 → 550 ^[a]		≤ 30		[22, 98]
FeP ₂	800 → 700	I ₂ (50 mg)	ca. 0.5		[99]
FeP ₄	700 → 600	I ₂ (70 mg)	ca. 1 ^[d]		[99]
CoP	850 → 750 ^[a]		≤ 10	CoI _{2,1} as additional deposit at higher iodine amounts	[32, 100]
CoP ₂	850 → 650	I ₂ (150 mg)	≤ 2	various other phases: CoP, CoP ₃ , and Co ₂ P ₄ O ₁₂ ; large ΔT favors formation of CoP ₂	[32]
CoP ₃	1000 → 900	I ₂ (≥ 120 mg)	≤ 5	temperature can be varied within limits; CoP (at higher temp.) and rarely CoP ₂ (at lower temp.) as additional solids	[32]
Ni ₅ P ₄	900 → 800	I ₂ (≥ 150 mg)	ca. 0.8		[248]
Cu ₃ P	800 → 900	I ₂ (34 mg)	ca. 5	formation of CuI ₁ at T_1 ; large iodine amounts lead to formation of CuP ₂ alongside Cu ₃ P	[93]
CuP ₂ a)	750 → 650	I ₂ (26–205 mg)	≤ 8	always CuI as additional deposit; also Cu ₂ P ₃ I ₂ at higher iodine amounts	[93, 249]
b)	730 → 630	CuI (20 mg)	ca. 5	Cu ₃ P as additional deposit at T_2 (see text)	[93]
Cu ₂ P ₇	580 → 530	P (> 170 mg), I ₂ (20 mg)	ca. 0.5	CuP ₂ and/or Cu ₂ P ₃ I ₂ as additional deposit	[93, 249]

[a] Temperature and amount of iodine added can be varied over a wide range. [b] In this experiment, oxygen was introduced into the ampoules either as H₂O or by the addition of HgO or MO₃ (M = Mo, W). [c] The deposition of Mo₄P₃ at 800 °C is unusual since this phase is only thermodynamically stable above 910 °C against decomposition to Mo₃P and MoP.^[87] [d] In mg d⁻¹.

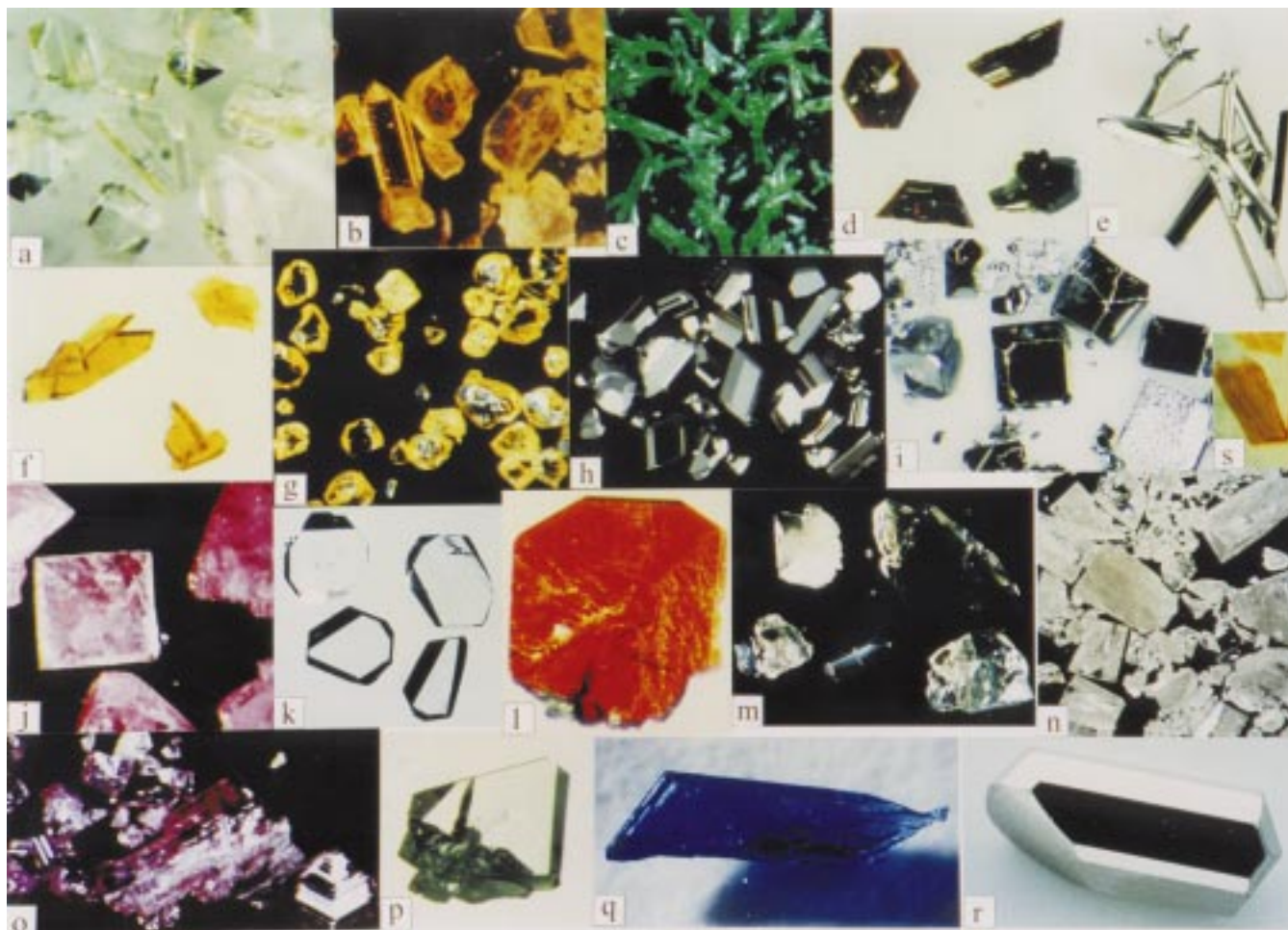
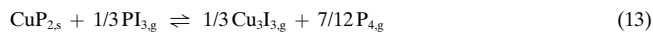
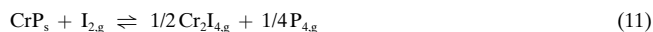
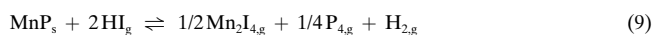


Figure 5. Crystals obtained from chemical transport experiments. a) $\text{Yb}_2\text{Si}_2\text{O}_7$ (crystal size ca. 2 mm), b) NiSO_4 (0.5 mm), c) $\text{Pr}_2\text{Ti}_3\text{O}_8\text{Cl}_2$ (0.3 mm), d) RhPO_4 (0.5 mm), e) $\beta\text{-WP}_2$ (5 mm), f) $\text{Rh}(\text{PO}_3)_3$ (0.4 mm), g) $\text{Sm}_2\text{Ti}_2\text{O}_7$ (0.3 mm), h) CoP_2 (2 mm), i) CoP_3 (2 mm), j) NdNbO_4 (1 mm), k) CuSO_4 (0.5 mm), l) $\text{Pd}_2\text{P}_2\text{O}_7$ (2 mm), m) FeSO_4 (1 mm), n) Ta_2O_5 (0.4 mm), o) $\text{Nd}_4\text{Ti}_9\text{O}_{24}$ (1 mm), p) $\text{CuP}_4\text{O}_{11}$ (4 mm), q) $\text{Cr}_2\text{P}_2\text{O}_7$ (10 mm), r) CrP (8 mm), s) $\text{Ce}_2\text{Ti}_3\text{O}_8\text{Cl}_2$ (0.5 mm).

of an *exothermic* (e.g. VP/I_2 ,^[78] MnP/I_2 ,^[79] $\text{Cu}_3\text{P}/\text{I}_2$ ^[93]) or an *endothermic* (e.g. CrP/I_2 ,^[79] CoP/I_2 ,^[100] CuP_2/I_2 ^[93]) reaction. For the above-mentioned examples, thermodynamic model calculations resulted in the following transport-determining equilibria [Eq. (8)–(13)].



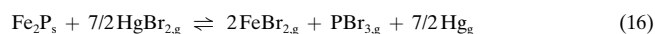
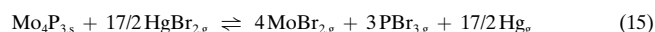
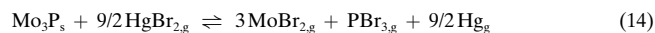
In general, relatively large amounts of iodine of about 100 mg per ampoule are necessary for an effective transport. The failure to observe migration of NbP in earlier experiments of Schäfer^[80] was due to an insufficient quantity of iodine (see Table 3).

Qualitative analysis shows that phosphides with a M:P ratio close to 1:1 apparently give the best results (high transport rates, large crystals). Migration of metal-rich or phosphorus-rich phosphides in temperature gradients, in comparison, is less effective experimentally. In addition, the transport of

metal-rich phosphides ($\text{M:P} \geq 1$) of the early transition metals (Ti, Zr, V, Cr) led to the migration of the respective monophosphides and attack of the walls of the silica ampoules. Various considerations and further experimental results have made the facts understandable.

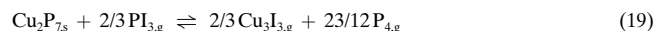
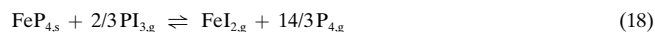
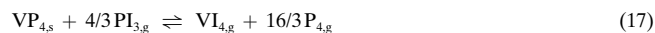
If the activity of the metal component in a phosphide is high, but that of the phosphorus very low (*metal-rich phosphide*), the only reaction is that of the TA (iodine) with the metal to form the volatile metal iodide. It is even possible that its saturation vapor pressure will be reached, so that condensed (s, l) metal halides also occur. Phosphorus becomes enriched in the solid; the simultaneous vaporization of both components of the solid is not possible. Examples for this are the reactions of Cr_{12}P_7 ($\rightarrow\text{CrP}$), Fe_2P ($\rightarrow\text{FeP}$) and Co_2P ($\rightarrow\text{CoP}$) with iodine.^[81] In an alternative but thermodynamically equally possible explanation, the failure to migrate could be caused by the occurrence of phosphorus-rich neighboring phases that limit the *phosphorus existence pressure*. The partial pressures $p(\text{P}_2)$ and $p(\text{P}_4)$ cannot attain sufficiently high values under these conditions (according to Schäfer $\geq 10^{-5}$ atm)^[82] to allow a detectable transport effect. The thermodynamic stability of the phosphorus iodides $\text{P}_2\text{I}_{4,g}$ and $\text{PI}_{3,g}$ ^[83–86] is not sufficient under such conditions to keep phosphorus in the gas phase. These considerations led to the

successful use of $HgBr_2$ as a transport agent for Mo_3P , Mo_4P_3 ^[87] and Fe_2P ^[88]. The chemical transport of the phosphorus part of these phosphides occurs in accordance with Equations (14)–(16) via the much more stable phosphorus tribromide.^[87, 88]



The reversal of these considerations means that, of course, the phosphorus coexistence pressure over a phosphide and its phosphorus-rich neighboring phase must be higher than about 10^{-5} atm if the former is to be transported with iodine.

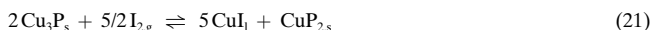
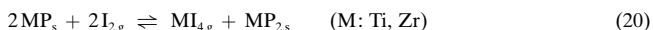
The phosphorus pressure in the ampoule takes on a completely different significance in the *chemical transport of phosphorus-rich phosphides*. At temperatures that allow a sufficient vaporization of the metal component as the iodide, many phosphides with a ratio of M:P < 1:1 (polyphosphides) are only stable at $p(P_2, P_4) > 1$ atm. Despite the relatively low thermodynamic stability of $P_2I_{4,g}$ and $PI_{3,g}$, it is possible under such conditions that the iodine used as the TA can be bound by the phosphorus in the gas phase; it is therefore no longer available for the iodination of the metal component. For this reason, reversible chemical transport is barely possible with many polyphosphides that we know of from the work of Jeitschko^[89] and von Schnering.^[90] Exceptions to this are VP_4 , FeP_4 , and Cu_2P_7 , whose metal components are particularly stable and/or form very volatile iodides, so that transport at relatively low temperatures between 500 and 600 °C with the in situ formed transport agents PI_3 and/or P_2I_4 becomes possible [Eq. (17)–(19)].



The above-mentioned transport systems with phosphides show that the simple case of a single-phase solid that is identical in the source and the sink and that is present during the entire experiment is not always realized. As we have already mentioned, the reaction of a phosphide with the TA (iodine) can lead to the *formation of condensed metal iodides*. In this connection we have made an in-depth investigation of the occurrence of $VI_{2,s}$ (alongside VP)^[78], $CrI_{2,l}$ (alongside CrP)^[79], $MnI_{2,l}$ (alongside MnP)^[79], $CoI_{2,l}$ (alongside CoP)^[100] and CuI_l (alongside Cu_3P and/or CuP_2)^[93]. From the amount of $VI_{2,s}$, it was possible to estimate the enthalpy of formation of VP_s . In other cases it was possible to undertake a reexamination of the relative thermodynamic stability of phosphides and iodides from the mass ratios in the equilibrium solids. For such investigations, the transport balance as described in Section 3 proved to be particularly valuable.

Even if a phosphide does not have a high metal activity, the formation of very stable metal iodides (either in gaseous or condensed form), as described above, can still lead to phosphorus-rich phosphides (incongruent vaporization of the phosphides). In our experiments at sufficiently high iodine quantities, we obtained TiP_2 ^[91] in the presence of TiP ^[92]

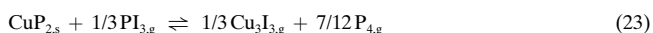
ZrP_2 in the presence of ZrP , and CuP_2 in the presence of Cu_3P (and CuI_l) [Eq. (20) and (21)].



The transport behavior of VP was very revealing for our understanding of the *homogeneous gas-phase equilibrium* between phosphorus and iodine in relation to pressure and temperature. This compound migrates with iodine as the transport agent as the result of an exothermic reaction [Eq. (8)]. Attempts to synthesize VP_2 (800 °C, from the elements, with iodine as mineralizer) resulted in the simultaneous occurrence of the monophosphide and diphosphide and migration of both phosphides to T_1 . Under the transport conditions of $T_2 \rightarrow T_1$, $PI_{3,g}$ does not act as a phosphorus carrier as in Equation (8) or (12) but becomes the transport agent itself [Eq. (22)].



The similarity to the transport behavior of the silicides (Section 5.2) and to that of elemental silicon^[54] is clear. CuP_2 can migrate in temperature gradients both by means of exothermic reactions (in the presence of Cu_3P_s and CuI_l as deposits, low amounts of P and I in the gas phase) and by means of endothermic reactions.^[93] In the latter case, CuI_l and phosphorus vapor ($P_{2,g}$, $P_{4,g}$ but also $PI_{3,g}$ and $P_2I_{4,g}$) are formed from a reaction of $CuP_{2,s}$ and added iodine that occurs prior to transport. With $PI_{3,g}$ as the TA, the vaporization of the remaining $CuP_{2,s}$ is achieved by an endothermic reaction [Eq. (23)].

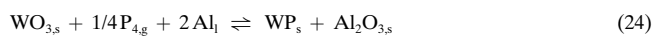


But now some *experimental information*: If a temperature gradient is applied immediately after phosphides have been formed in situ from the metal and phosphorus in a silica ampoule with iodine as the mineralizer, in our experience, there is frequently only a relatively low transport effect to be observed, which is sometimes even completely absent. If, in contrast, a previously synthesized phosphide is used as the starting solid, the transport rates will be much higher and well reproducible. We think that this behavior has two causes. Traces of moisture that have been introduced together with iodine and phosphorus react with the phosphide to form phosphates with the release of hydrogen. This in turn binds the iodine required as the transport agent in the form of HI_g which is less favorable for the transport of phosphides. According to our observations, this effect^[78, 100] is possible with small to intermediate iodine amounts of $m(I_2) \leq 100$ mg per ampoule. In this way the experimental transport rates can be drastically decreased in comparison to those expected for a completely dry system.

It also cannot be excluded that oxygen, which can enter the ampoule by various means (traces of moisture, slight oxidation of the metal used), has an unfavorable influence on the transport of phosphides. This appears to be particularly significant when a phosphide migrates with iodine as a result of an exothermic reaction ($T_1 \rightarrow T_2$). With the simultaneous presence of a phosphate, the phosphide is transported in a coupled transport reaction with the phosphate to the cooler

side of the ampoule ($T_2 \rightarrow T_1$). Examples for such behavior are given in Section 6.3.2.

One good possibility to obtain phosphides is their *synthesis starting from the oxides*. Aluminum^[94] or excess phosphorus^[95] can be used as reduction agents in “one pot” reactions [e.g. Eq. (24)–(26)].



The separation of the phosphides from the other reaction products can be performed by means of chemical transport reactions.

The crystallization of CoP_2 is an example for the deposition of a *metastable* compound from the gas phase. Isothermal tempering of equimolar amounts of CoP and CoP_3 leads only in exceptional cases to the formation of small amounts of CoP_2 . In contrast, with CT, larger amounts of CoP_2 in the presence of CoP and CoP_3 are formed.^[96, 32] The diphosphide can be deposited in the sink as a single phase with the application of an unusually steep temperature gradient $\Delta T = 200^\circ$.^[97]

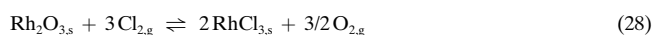
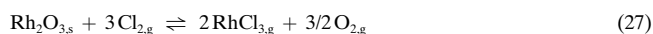
6. Binary and Multicomponent Oxides

The transport behavior of most binary oxides in temperature gradients is already well known from earlier work by Schäfer, Oppermann, and several other authors, and is summarized in review articles.^[101, 102] The transport of complex oxides (sulfates, phosphates, niobates and compounds of the systems $\text{Ln}/\text{TM}/\text{O}/\text{Cl}$) has therefore been of interest to us more recently. It was also possible to gain some new knowledge of the chemical transport of the simple binary oxides.

6.1. Binary Oxides

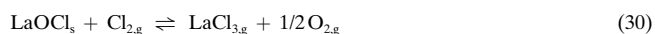
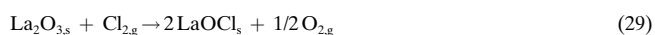
The chemical transport of Cu_2O and CuO with various transport agents is possible due to the exothermic reactions that occur over wide ranges of temperature and transport agent concentration with the deposition of single-phase oxides.^[62, 101, 103, 104] The chemical transport of multiple-phase solids (up to three: CuO_s , Cu_2O_s , CuI_l) and the accompanying time dependence of the transport behavior (“nonstationary behavior”) can be quantitatively understood by means of experiments on the transport balance and extensive thermodynamic modeling.^[36] Calculations on this model system show how precisely the transport behavior of a system can be modeled with reliable thermodynamic data.

Nonstationary behavior was also observed in the first investigation of the migration of Rh_2O_3 in temperature gradients (e.g. $1000 \rightarrow 900^\circ\text{C}$) with the TA chlorine [ca. 1 atm at room temperature (RT)].^[105] Model calculations agreed well with both the experimental composition of the deposit ($\text{Rh}_2\text{O}_{3,s}$, $\text{RhCl}_{3,g}$) and also the transport rates for the two phases as a function of temperature and concentration of chlorine.^[105] The equilibria (27) and (28) fully describe the behavior.



The crystallization of a further precious metal oxide under participation of the gas phase succeeded in the PdO system (e.g. $800 \rightarrow 900^\circ\text{C}$, TA: Cl_2 , 1 atm at RT). The addition of PdCl_2 , which decomposes into $\text{Cl}_{2,g}$ and Pd_s at these temperatures, also promoted the migration of the oxide in a temperature gradient as a result of an exothermic reaction.^[106, 107] Thermodynamic modeling of the experimental observations was, however, not possible. Uncertainties in the thermodynamic characterization of the Pd/Cl system appeared to be responsible.^[127]

In contrast to the migration and deposition of many compounds of the $\text{Ln}/\text{TM}/\text{O}/\text{Cl}$ systems in temperature gradients (see Section 7), the chemical transport of binary La_2O_3 with chlorine is not possible. It is not the high thermodynamic stability of $\text{La}_2\text{O}_{3,s}$ in comparison to $\text{LaCl}_{3,g}$ that is responsible but rather the almost quantitative formation of very stable LaOCl_s [Eq. (29)] from La_2O_3 and chlorine.^[108] The chemical transport of LaOCl_s with chlorine according to Equation (30) is possible, however ($1150 \rightarrow 1000$, 0.1 atm Cl_2 at RT).^[108]



Despite the close chemical relationship between the rare-earth oxides and Sc_2O_3 , the latter can be transported, although at relatively low rates (1 atm Cl_2 at RT; $1100 \rightarrow 1000$). Formation of ScOCl_s under the transport conditions was not observed.^[159]

In contrast to the sesquioxides Ln_2O_3 , cerium dioxide does not react with the transport agent chlorine to form CeOCl_s , but migrates in a temperature gradient (e.g. $1100 \rightarrow 1000^\circ\text{C}$). However, the transport effect is very low as a result of the high stability of CeO_2 ; the strongly endothermic equilibrium [Eq. (31)], which is probably decisive, lies too far to the left.



A more favorable equilibrium state with much higher transport rates can be achieved with the addition of a little carbon, which leads to the formation of CO_2 on the right-hand side.^[109] The pale pink color of the almost colorless cubes of the CeO_2 crystals is probably caused by traces of Pr^{3+} .^[110] With low amounts of additional Ta_2O_5 , the crystals of $\text{Ce}_{1-x}\text{Ta}_x\text{O}_2$ ($x < 0.02$) obtained took on a blue color.^[109] Ta_2O_5 itself migrates with CBr_4 as the TA in a temperature gradient of $900 \rightarrow 1000^\circ\text{C}$ as a result of an exothermic reaction (see Figure 5 n).^[109]

A comparison of thermodynamic estimates with experimental results confirms that chemical transport of ZrO_2 and HfO_2 with chlorine should proceed in accordance with Equation (32).^[111]



TeCl_4 can also be used as the transport agent for the two dioxides.^[102] The use of TeCl_4 as a transport agent has generally proven favorable, particularly in the transport of oxides.^[65] An extensive investigation of the transport behavior of various

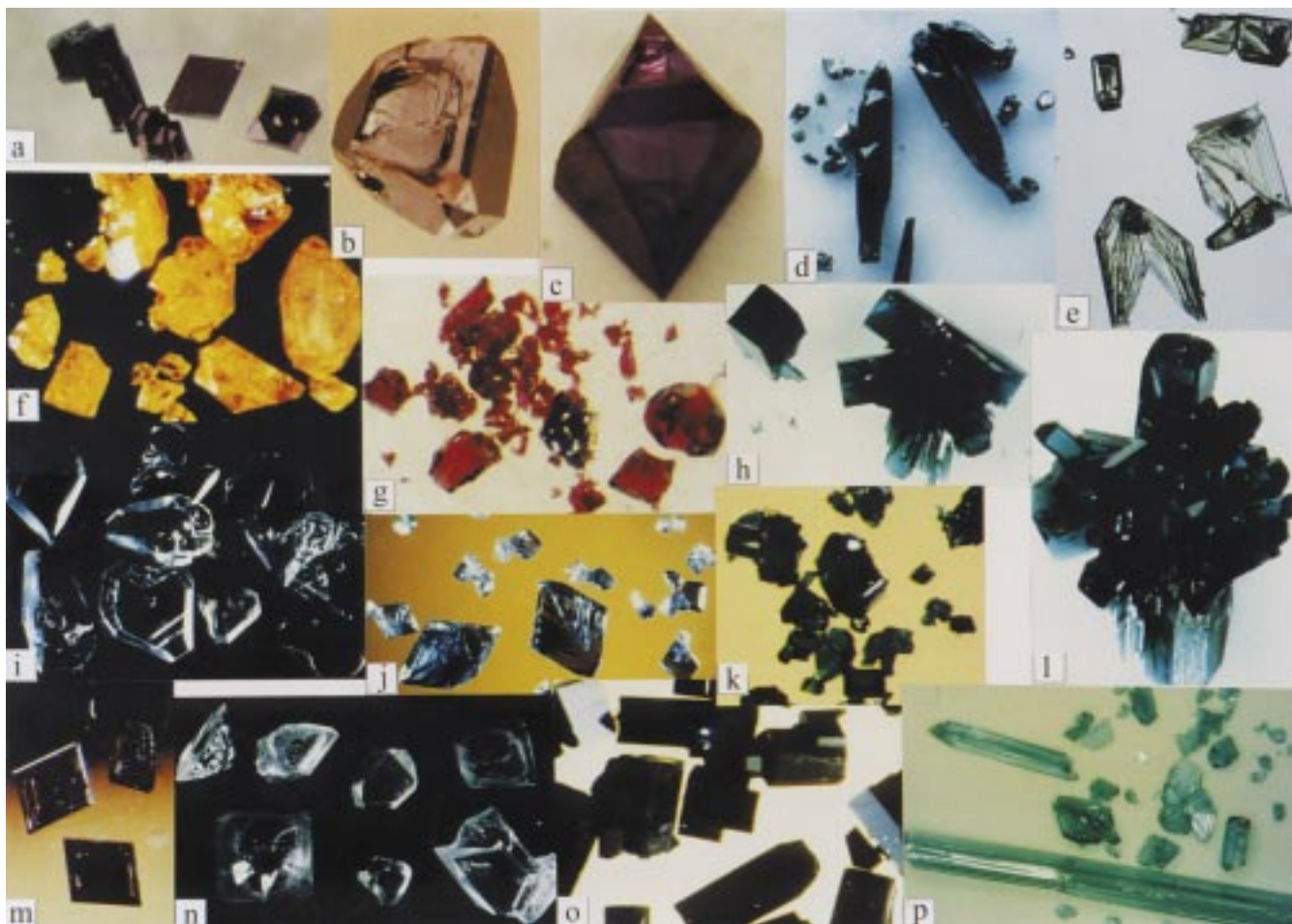


Figure 6. Crystals obtained from chemical transport experiments. a) Mo_4O_{11} (crystal size ca. 3 mm), b) WO_2 (8 mm), c) Mo_4O_{11} (10 mm), d) TiPO_4 (3 mm), e) $\text{Fe}_2(\text{SO}_4)_3$ (1 mm), f) $\sigma\text{-Ni}_2\text{P}_2\text{O}_7$ (1 mm), g) $\text{Co}_2\text{P}_2\text{O}_7$ (0.5 mm), h) VOSO_4 (3 mm), i) ZnSO_4 (1 mm), j) $\text{Ti}(\text{PO}_3)_3$ (0.5 mm), k) $\text{Cr}_2(\text{SO}_4)_3$ (0.2 mm), l) VOSO_4 (3 mm), m) Mo_4O_{11} (3 mm), n) PbSO_4 (1 mm), o) K_xWO_3 (hexagonal tungsten bronze; 1 mm), p) $\text{Cu}_2\text{P}_2\text{O}_7$ (2 mm)

Magnéli phases $\text{Ti}_n\text{O}_{2n-1}$ with the addition of TeCl_4 showed, however, that HCl rather than TeCl_4 was the true TA.^[112] The HCl is formed from the hydrolysis of TeCl_4 by traces of moisture, which are almost unavoidable when working with silica ampoules.^[113] WO_2 (Figure 6b) shows unusually high transport rates with added HgBr_2 ($\sim 1000 \text{ mg h}^{-1}$).^[67, 114] HgBr_2 is also suitable as a TA for Mo_4O_{11} (Figure 6a, c, m).^[115]

Crystals (Figure 6o) of the hexagonal tungsten bronze (HTB) K_xWO_3 were obtained from an unexpected transport reaction with the addition of KBr to WO_3 .^[116]

The intensive examination of the transport behavior of the sesquioxides Cr_2O_3 and V_2O_3 with added chlorine eventually led to the detection of the gas molecules $\text{CrOCl}_{2,g}$ and $\text{VOCl}_{2,g}$.^[117, 118] Extensive experimental studies and subsequent thermodynamic analysis enabled the influence of these molecules on the chemical transport of CrOCl_3 and $\text{V}_2\text{O}_{3,s}$ to be described quantitatively. The vaporization of CrOCl_3 in the presence of small amounts of chlorine follows Equation (33),^[119] whereas for V_2O_3 , Equation (34) applies for the transport after almost quantitative reaction with the chlorine.^[120]



6.2. Sulfates

The crystallization of anhydrous sulfates has long presented a challenge. Most representatives of this class of compounds have a relatively low thermal stability. It is not usually possible to melt the compounds without the loss of $\text{SO}_{3,g}$ (or $\text{SO}_{2,g}/\text{O}_{2,g}$) under normal laboratory conditions. Since crystallization from solution (conc. H_2SO_4) can only be performed for a few anhydrous sulfates, crystallization procedures for these compounds were generally lacking. The use of chemical transport experiments for crystal growth through the gas phase has led to remarkable progress in this field. As shown in Table 4, the range of successfully transported sulfates extends from Ag_2SO_4 ^[121] to VOSO_4 ^[122] and includes many MSO_4 and $\text{M}_2(\text{SO}_4)_3$ compounds. In addition to the great preparative advantages, the in-depth investigations into the transport behavior of sulfates also yielded extensive information on the transport of heterogeneous (solid/gaseous) and homogeneous (gas phase) equilibria. It was shown that *several* transport reactions must often be taken into account for a quantitative description of the transport behavior. This requirement places particular demands on the thermochemical description of the experimental observations. This information takes on an even greater importance

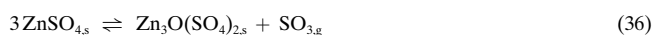
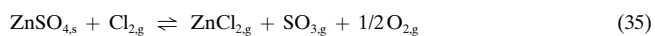
Table 4. Anhydrous sulfates. In all cases, chemical transport occurs as a result of endothermal reaction with the respective transport medium.

Sulfate	Temperatures [°C]	Transport agent	$\dot{m}$ [mg h ⁻¹]	Comments	Ref.
Ag ₂ SO ₄	800 → T ₁	Cl ₂		AgCl forms alongside the sulfate; formation of a low-melting eutectic mixture; T ₁ < 680 °C (m.p. Ag ₂ SO ₄)	[121]
BeSO ₄	700 → 600	Cl ₂ (1 atm at RT)	ca. 1 ^[a]	mixture of BeSO ₄ /BeO as starting material	[250]
MgSO ₄	800 → 700	Cl ₂ (1 atm at RT)	ca. 2 ^[a]		[250]
SnSO ₄			no transport		[121]
PbSO ₄	830 → 730	Cl ₂ or HCl (1 atm at RT), HgCl ₂ or I ₂ (ca. 200 mg per ampoule)	≤ 50		[18, 121]
MnSO ₄	840 → 740	Cl ₂ or HCl (1 atm at RT)		moderate transport rates	[121]
FeSO ₄	650 → 550	NH ₄ Cl (20 mg per ampoule)	ca. 0.5	Fe ₂ O ₃ ^[b] at T ₂	[124]
CoSO ₄	650 → 550	Cl ₂ or HCl (1 atm at RT)	≤ 20	formation of CoCl _{2,l} alongside the sulfate	[121]
NiSO ₄	675 → 560	Cl ₂ or HCl (1 atm at RT)	≤ 10	no formation of NiCl _{2,l} alongside the sulfate ^[c]	[18, 121]
CuSO ₄	700 → 600	various TA (Cl ₂ , HCl, NH ₄ Cl, HgCl ₂ , I ₂)	≤ 10	formation of CuCl alongside the sulfate ^[c]	[121, 251]
Cu ₂ OSO ₄	750 → 650	HgCl ₂ (40 mg per ampoule)	≤ 10		[251]
ZnSO ₄	T ₂ → T ₁	Cl ₂ (1 atm at RT)	≤ 20 ^[d]	T ₁ < 690 °C: N-ZnSO ₄ , T ₁ > 690 °C: H-ZnSO ₄ ; ΔT = 70°; ZnCl _{2,l} alongside the sulfate	[20, 121]
Zn ₃ O(SO ₄) ₂	710 → 620	PbCl ₂ (ca. 5 mg per ampoule)	≤ 10		[123]
CdSO ₄	840 → 740	Cl ₂ or HCl (1 atm at RT)	≤ 40	CdCl ₂ as additional deposit	[121]
HgSO ₄	550 → 470	Cl ₂ or HCl (1 atm at RT)	≤ 20	HgCl ₂ as additional deposit, dissociative sublimation also possible	[121]
Al ₂ (SO ₄) ₃	625 → 525	SOCl ₂ (100 mg per ampoule)	ca. 1	other TAs (Cl ₂ , HCl etc.) give no transport effect ^[c]	[126]
Ga ₂ (SO ₄) ₃	625 → 575	Cl ₂ or HCl	≤ 20 ^[b,d]	Ga ₂ O ₃ at T ₂	[252, 253]
In ₂ (SO ₄) ₃	625 → 575	Cl ₂ or HCl	≤ 20 ^[b,d]	In ₂ O ₃ at T ₂	[253, 254]
Cr ₂ (SO ₄) ₃	690 → 630	Cl ₂ or HCl	≤ 80 ^[b,d]	Cr ₂ O ₃ at T ₂	[255, 256]
Fe ₂ (SO ₄) ₃	775 → 675	Cl ₂ (1 atm at RT)	≤ 10	Fe ₂ O ₃ ^[b,d] at T ₂	[124, 125]
VOSO ₄	525 → 425	Cl ₂ (1 atm at RT)	ca. 5	V ₂ O ₅ alongside VOSO ₄ at higher temperatures	[122]
	550 → 450	HCl (1 atm at RT)	ca. 10	V ₂ O ₅ alongside VOSO ₄ at higher temperatures	[122]

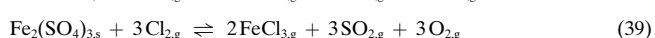
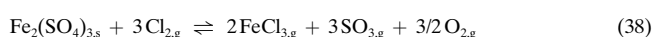
[a] In mg d⁻¹. [b] The amount of the oxide grows with increased temperature. [c] Transport with the addition of PbCl₂ also gives good results. [d] Extensive thermodynamics discussion.

since it has model character for the CT of other complex oxides with a volatile component (phosphates, arsenates, borates).

Suitable transport agents for sulfates are chlorine and HCl, as the overview in Table 4 shows. In individual cases, migration in a temperature gradient has also been observed with the addition of I₂, NH₄Cl, HgCl₂, PbCl₂, PbBr₂, or SOCl₂. The migration of sulfates always occurs by an endothermic reaction (T₂ → T₁). Equations (35)–(37) fully describe the experimental observations for the transport of ZnSO₄ with chlorine (Figure 6i).

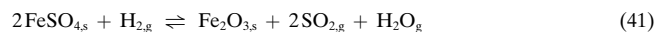
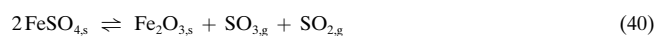


A noteworthy consequence of the interaction of Equations (35) and (36) is the much higher thermal stability of ZnSO₄ in the presence of chlorine.^[20, 123] This effect is less pronounced when HCl is used. After extensive thermodynamic modeling, Equations (38) and (39) were shown to describe the transport of Fe₂(SO₄)₃ with chlorine (Figure 6e) at low temperatures (500–600 °C)

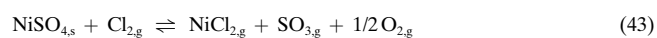


At higher temperatures (600–750 °C), Equation (38) no longer applies. Calculations show that under these conditions

p(SO₃) becomes a function of the temperature according to the (exothermic) homogeneous equilibrium in Equation (37). The oxidizing character of the equilibrium gas phase in the transport of sulfates, even when HCl is used as the TA, can be expressed in various ways. For example, in the CT of FeSO₄ with HCl (Figure 5m), Fe₂O₃ is always present as additional solid [Eq. (40)]. If an equivalent amount of NH₄Cl is used instead of HCl, which decomposes to form HCl, N₂, and H₂, the amount of Fe₂O₃ even increases [Eq. (41)]. Please refer to the literature for an extensive thermodynamic discussion of the transport behavior of iron sulfate.^[124, 125]



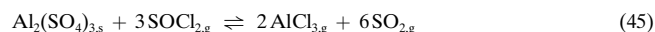
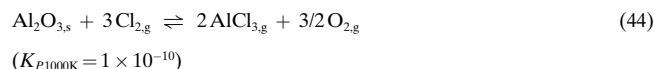
An oxidizing equilibrium gas phase is also the prerequisite for the use of PbCl₂ as the transport agent for several anhydrous sulfates [NiSO₄, CuSO₄^[18] (Figure 5k)]. Chlorine is released in a pre-reaction (e.g. between NiSO₄ and PbCl₂) [Eq. (42)], which involves a “double transformation” and a redox reaction. The chlorine then acts as the true TA for NiSO₄ [Eq. (43)].



With the transport balance, it was possible to detect the three equilibrium solids (PbSO₄, NiSO₄, NiO). It could also

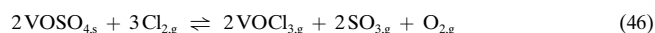
be shown that PbSO_4 (Figure 6n) migrates before NiSO_4 (Figure 5b) in a temperature gradient ($850 \rightarrow 750^\circ\text{C}$), while NiO remained in the source at the end of the experiment.^[18]

It is not possible to vaporize aluminum oxide with chlorine (or other TAs) in a temperature gradient because of the very unfavorable position of the equilibrium [Eq. (44)]. It was therefore all the more surprising that the crystallization of aluminum sulfate, $\text{Al}_2(\text{SO}_4)_3$, succeeded by means of CT ($625 \rightarrow 525^\circ\text{C}$, ca. 50 mg SOCl_2 per ampoule, $\dot{m} \approx 0.6 \text{ mg h}^{-1}$). The use of SOCl_2 as the TA apparently suppressed the formation of free oxygen and thus enabled a favorable positioning of the heterogeneous transport equilibrium [Eq. (45)].^[126]



It has not yet been possible to establish whether the transport of rare-earth sulfates would succeed in a similar fashion. See Table 4 for information on $\text{Cr}_2(\text{SO}_4)_3$ (Figure 6k), $\text{Ga}_2(\text{SO}_4)_3$, and $\text{In}_2(\text{SO}_4)_3$.

Particular mention is made of the possibility of crystallizing VOSO_4 (Figure 6h and 6l) by means of CT at the end of this section. Chlorine has proven valuable as a TA [Eq. (46)].^[122]



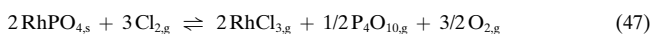
In temperature gradients of $525 \rightarrow 425^\circ\text{C}$, only VOSO_4 occurs as a solid in the source and sink, the TRs lie around ca. 5 mg h^{-1} and increase considerably with higher temperatures ($700 \rightarrow 600^\circ\text{C}$). Then, however, considerable amounts of V_2O_5 are produced in addition to the oxysulfate.^[122]

6.3. Phosphates

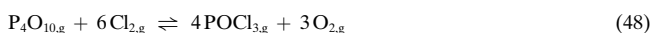
The overview in Table 5 shows the broad applicability of chemical transport reactions for the synthesis, crystallization, and purification of this class of substances. The preparative possibilities are illustrated by the thermally labile $\text{Pd}_2\text{P}_2\text{O}_7$ (Figure 5l),^[127] RhPO_4 (Figure 5d),^[128] and $\text{Rh}(\text{PO}_3)_3$ (Figure 5f),^[127] several low-melting ultraphosphates (e.g. $\text{CuP}_4\text{O}_{11}$ (Figure 5p),^[129] $\text{ZnP}_4\text{O}_{11}$,^[130]) and an series of transition metal phosphates in otherwise difficult accessible (low) oxidation states (e.g. TiPO_4 (Figure 6d),^[33] $\text{Ti}(\text{PO}_3)_3$ (Figure 6j),^[33, 141] $\text{V}_2\text{O}(\text{PO}_4)_2$,^[131] $\text{Cr}_3(\text{PO}_4)_2$,^[132] $\text{Cr}_2\text{P}_2\text{O}_7$ (Figure 5q)^[133]). In addition to the elemental halogens (Cl_2 , Br_2 , I_2), halogen compounds (NH_4X and HgX_2 ; $\text{X} = \text{Cl}$, Br , I), and mixtures P/X ($\text{X} = \text{Cl}$, Br , I) were used. In the case of UP_2O_7 , the chlorides VCl_4 , ZrCl_4 , HfCl_4 , and NbCl_5 also proved to be suitable transport agents.^[134] The best results with respect to transport rate and crystal size of the anhydrous phosphates were achieved, however, with chlorine or mixtures of phosphorus and iodine as transport agents.^[23] See Table 5 for information on $\text{Co}_2\text{P}_2\text{O}_7$ (Figure 6g), $\sigma\text{-Ni}_2\text{P}_2\text{O}_7$ (Figure 6f), and $\text{Cu}_2\text{P}_2\text{O}_7$ (Figure 6p).

6.3.1. Chlorine as the Transport Agent

When chlorine is added, $\text{P}_4\text{O}_{10,g}$ is regarded as the phosphorus-carrying gas molecule, in accordance with the chemical transport of sulfates [Eq. (35), (38), and (43)]. One example is the equilibrium [Eq. (47)] that determines the transport of RhPO_4 .



Thermodynamic analysis of the homogeneous gas-phase equilibrium (48) shows that a notable contribution of phosphorus oxychlorides such as $\text{POCl}_{3,g}$ (or PO_2Cl ,^[135] or polymers of these $(\text{PO}_2\text{Cl})_x$ ^[136]) to the transport of phosphates is unlikely.^[23] From the thermodynamic data from Equation (48), a value of $p(\text{P}_4\text{O}_{10}):p(\text{POCl}_3) = 278$ (1273 K , $p(\text{P}_4\text{O}_{10}) = p(\text{Cl}_2) = 1 \text{ atm}$) is obtained.



$$\Delta_R H(1273 \text{ K}) = 159.2 \text{ kcal mol}^{-1}; \Delta_R S(1273 \text{ K}) = 44.3 \text{ cal mol}^{-1} \text{ K}^{-1};$$

$$\Delta_R G(1273 \text{ K}) = 102.8 \text{ kcal mol}^{-1}; K_p(1273 \text{ K}) = 2.0 \times 10^{-18}$$

6.3.2. Halogens together with Reducing Additives as Transport Agents

As a result of the decreased activity of the metal oxide component in a phosphate, compared with the pure oxides, lower solubility of the phosphates in a chlorine atmosphere is observed. Heterogeneous equilibria such as in Equation (47) tend to lie far over to the side of the reactant and usually lead to low transport rates $\dot{m} < 1 \text{ mg h}^{-1}$.

For this reason Schäfer and Orlovskii used mixtures of P/Cl or P/Br as transport agents for LnPO_4 ($\text{Ln} = \text{La}$, Ce , Pr , Nd).^[137, 168] The release of oxygen was suppressed [Eq. (47)], which resulted in a more favorable equilibrium position [see also the CT of $\text{Al}_2(\text{SO}_4)_3$ with SOCl_2 , Eq. (45)]. In our own investigations on transition metal phosphates with P/Cl or P/Br mixtures as TAs, we observed migration of the phosphates through the gas phase^[22] but at the same time the wall of the silica ampoules was very strongly attacked with the formation of various *silicophosphates* such as $\text{M}_2\text{Si}(\text{P}_2\text{O}_7)_2$ ($\text{M}^{2+} = \text{Mn}$, Fe , Co ,^[138] Ni , Cu , Cd ^[139]), $\text{M}_4\text{P}_6\text{Si}_2\text{O}_{25}$ ($\text{M}^{3+} = \text{Ti}$, V , Cr , Mo , Fe),^[22, 140, 141] $\text{MP}_3\text{SiO}_{11}$ ($\text{M}^{3+} = \text{Ti}$, Cr , Mo),^[141] and $\text{MP}_3\text{Si}_2\text{O}_{13}$ ($\text{M}^{3+} = \text{Ti}$, Cr , Rh)^[81]. In general these experiments were very difficult to reproduce and were not suitable for systematic investigations. It appears that the halogenating effect of the gas mixtures of P/Cl or P/Br is so high that SiO_2 becomes soluble in the gas phase along with the phosphorus-containing solid. The search for a “selectively” *halogenating transport agent* for phosphates finally led to the combination of *iodine with small amounts of metal, phosphorus, or phosphide*.^[22, 23] Iodine without additives as the TA only leads in exceptional cases (TiPO_4 ,^[33] $\text{Cr}_2\text{P}_2\text{O}_7$ ^[133]) to a transport effect. Equilibria such as that formulated in Equation (49) for the CT of orthophosphates require oxidation of O^{2-} by I_2 that is not very meaningful from a chemical viewpoint and are obviously thermochemically very unfavorable.



Table 5. Anhydrous phosphates. Representative selection of the more than 60 compounds that have been crystallized through the gas phase. $\bar{T} = (T_2 + T_1)/2$.

Phosphate	Transport agent	T [°C]	Comments	Appl. ^[a]	Ref.
<i>diphosphates $M_2P_2O_7$ and MP_2O_7</i>					
$Mg_2P_2O_7$	P/I mix	$850 \leq \overline{T} \leq 1000$		+	[133, 146]
$Cr_2P_2O_7$	I_2 or I_2 + a few mg CrP	$1050 \rightarrow 950$		+++	[22, 23, 133]
$Mn_2P_2O_7$	P/I mix	$750 \leq \overline{T} \leq 1050$		+++	[146]
$Fe_2P_2O_7$	I_2 + a few mg FeP	$900 \rightarrow 800$		++	[133]
$Co_2P_2O_7$	a) Cl_2 (1 atm at RT)	$1100 \rightarrow 1000$		+++	[97, 133]
	b) P/I mix	$1000 \rightarrow 900$			
σ - $Ni_2P_2O_7$	I_2 + a few mg Ni_3P_4	$950 \rightarrow 850$		+++	[248]
$Cu_2P_2O_7$	I_2 + a few mg CuP_2	$1000 \rightarrow 900$		++	[133]
$Zn_2P_2O_7$	a) H_2 (0.2 atm at RT)	$920 \rightarrow 820$		++	[133, 257]
	b) NH_4Cl (ca. 10 mg per ampoule)	$920 \rightarrow 820$			
GeP_2O_7 (triclinic)	Cl_2 (0.1 atm at RT)	$700 \leq \overline{T} \leq 1000$		+	[258]
GeP_2O_7 (cubic)	Cl_2 (0.1 atm at RT)	$1140 \rightarrow 1050$			
SnP_2O_7	Cl_2 (0.1 atm at RT)	$980 \rightarrow 840$		+++	[259]
TiP_2O_7	a) P/I mix	$1000 \rightarrow 900$	$Ti(PO_3)_3$ forms with addition of excess P	+++	[33]
	b) I_2 + a few mg TiP	$1000 \rightarrow 900$			
<i>phosphates with transition metals in low oxidation states</i>					
$TiPO_4$	I_2	$1000 \rightarrow 900$	addition of a few mg Ti suppresses the formation of TiP_2O_7 alongside $TiPO_4$	+++	[33, 260]
$V_2O(PO_4)_3$	I_2 + a few mg V	$1000 \rightarrow 900$		+++	[131]
$Nb_2(PO_4)_3$	I_2	$800 \rightarrow 700$	reactants: $NbOPO_4 + P$ (2:1); source: NbP, sink: $Nb_2(PO_4)_3$ and $NbOPO_4$	+++	[261, 262]
$Cr_3(PO_4)_2$	I_2 (in presence of CrP)	$1200 \rightarrow 1150$		++	[132]
<i>transport experiments with chlorine</i>					
α - $CrPO_4$	Cl_2 (0.1 atm at RT)	$1100 \rightarrow 1000$		+	[263]
$FePO_4$	Cl_2	$1100 \rightarrow 1000$		+	[22, 23]
$RhPO_4$	Cl_2 (1 atm at RT)	$1050 \rightarrow 950$		+	[128]
$Pd_2P_2O_7$	Cl_2 (low pressure suppresses $PdCl_2$ in the presence of the phosphate)	$950 \rightarrow 850$		+	[127]
$Pd(PO_3)_2$	Cl_2 (low pressure suppresses $PdCl_2$)	$850 \rightarrow 800$		–	[127]
$Hg_2P_2O_7$	PCl_5 (ca. 20 mg per ampoule)	$550 \rightarrow 500$		+	[264]
<i>miscellaneous phosphates</i>					
$Ti_5O_4(PO_4)_4$	Cl_2 (1 atm at RT, addition of ca. 30 mg $TiPO_4$ per ampoule)	$1000 \rightarrow 900$		+	[265, 266]
$NbOPO_4$ and $NbPO_{5-\delta}$	a) NH_4Cl in the presence of NbP	$1000 \rightarrow 900$			[262]
	b) I_2 in the presence of NbP, 1000 $\rightarrow$ 900, many days of purification transport favors the formation of $NbPO_{5-\delta}$			+++	
WP_2O_7	I_2	$1000 \rightarrow 900$	in situ synthesis from WO_3 and P (W:P = 1:2), WP as a side product	+++	[95]
$WOPO_4$	I_2	$1000 \rightarrow 900$	in situ synthesis of WO_3 and P (W:P = 1:1), WP as a side product	+++	[95]
MPTB ^[b]	$m = 4$: I_2	$900 \rightarrow 800$		++	[95, 148]
	$m = 6$: NH_4Cl (ca. 20 mg per ampoule)	$900 \rightarrow 800$			
$Mn_3(PO_4)_2$	I_2 + a few mg P	$850 \rightarrow 800$		++	[146]
<i>ultraphosphates</i>					
CuP_4O_{11}	I_2 + a few mg P or CuP_2	$600 \rightarrow 500$		+++	[93, 129]
ZnP_4O_{11}	I_2 + a few mg P or ZnP_2	$600 \rightarrow 500$		+++	[130]
CdP_4O_{11}	I_2 + a few mg P	$510 \rightarrow 480$		+++	[130]

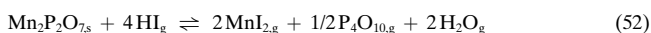
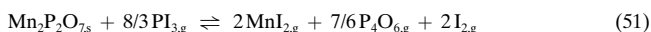
[a] The column "Appl." classifies the experiments according to their preparative applicability: + + + : reversible transport without attack of the ampoule wall, transport rates $\dot{m} > 10 \text{ mg h}^{-1}$, well-formed crystals; –: $\dot{m} < 1 \text{ mg h}^{-1}$, reaction with the ampoule wall, bad reproducibility. + +, + and – used as gradations. [b] MPTB: monophosphate tungsten bronzes $(WO_3)_{2m}(PO_4)_4$.

The great preparative advantage of iodine in combination with reducing additives as the TA (see Table 5) provokes the question of the determining *heterogeneous and homogeneous equilibria* under these experimental conditions. In particular, it is unclear which phosphate-containing gas molecule participates in the transport.

In addition to $P_4O_{10,g}$ and $P_4O_{6,g}$, PO_g , $PO_{2,g}$, and $P_4O_{n,g}$ ($7 \leq n \leq 9$) are also known as gaseous phosphorus oxides.^[142] Apart from P_4O_{10} ,^[143, 144] the thermal behavior of phosphorus oxides is generally insufficiently characterized; data for the enthalpy of formation of $P_4O_{6,g}$ range from -380 to $-512 \text{ kcal mol}^{-1}$.^[144, 145] The thermodynamic stability of the

gaseous phosphorus iodides PI_3 and P_2I_4 has already been discussed in connection with the transport of phosphides (Section 5.3).

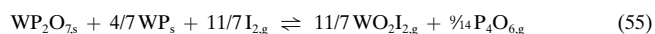
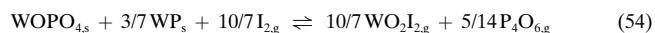
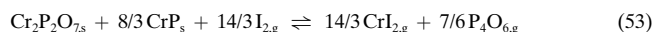
With $\text{PI}_{3,\text{g}}$ as the transport agent and by assuming that migration of the phosphate takes place via P_4O_{10} or P_4O_6 , the equilibria [Eq. (50) and (51)] are formulated for the migration of $\text{Mn}_2\text{P}_2\text{O}_7$ as an example. Similar equilibria with other lower phosphorus oxides also appear to be possible. In addition, transport with HI as the transport agent, which can be produced from phosphorus vapor, iodine, and traces of moisture, seems possible too [Eq. (52)].



A direct “in situ” investigation of the gas phase (MS, IR, Raman) is hardly possible because of the comparatively high pressures and temperatures involved. Thermodynamic calculations to model the behavior of phosphates in chemical transport experiments also fail to yield conclusive proof.^[33, 146] For this reason it is only possible to summarize a few observations that give information on the most important *phosphorus-containing gas molecules*.

It appears the transport of anhydrous phosphates with iodine is only possible when the partial pressure of oxygen is not “low”. This can be achieved experimentally by the addition of small amounts (<10 mg per ampoule) of phosphides, metal, or phosphorus. With the exceptions mentioned above (TiPO_4 , $\text{Cr}_2\text{P}_2\text{O}_7$), the reducing effect of the compound itself is sufficient for transport with iodine to be effective. In this connection, it is worth mentioning that the related phosphates VPO_4 and $\text{Fe}_2\text{P}_2\text{O}_7$ cannot be transported with iodine alone but only with addition of the respective monophosphides.^[22] It is not possible to show quantitatively whether the oxygen partial pressure is always so low in the transport of phosphates with iodine and reducing additives that only low phosphorus oxides are present in the gas phase instead of P_4O_{10} . In several cases at least, migration is possible under conditions which do not permit a sizable P_4O_{10} pressure. For example, $\text{Mn}_3(\text{PO}_4)_2$ migrates with the transport agent mixture of P/I in a temperature gradient of $850 \rightarrow 800^\circ\text{C}$.^[146] The next highest phosphate, $\text{Mn}_2\text{P}_2\text{O}_7$, which decomposes to the orthophosphate, thus determining the P_4O_{10} coexistence pressure, is stable up to its melting point of about 1200°C .^[147] In view of this fact it appears very unlikely that $\text{P}_4\text{O}_{10,\text{g}}$ can function as the essential molecule [$p(\text{P}_4\text{O}_{10}) \geq 10^{-5}$ atm] under the transport conditions for $\text{Mn}_3(\text{PO}_4)_2$. The same applies to the transport of TiO_2 in the presence of TiPO_4 ($1000 \rightarrow 900^\circ\text{C}$, TA: P/I) in which the *lower phosphorus oxides* should be considered as *the carriers of oxygen* and phosphorus.^[33] In addition to these two examples, it can be shown for series of further phosphates in Table 5 by means of model calculations that no heterogeneous equilibria can be formulated with $\text{P}_4\text{O}_{10,\text{g}}$ as the essential phosphorus and oxygen carrier in the gas phase that would explain the observed transport effects.^[23]

If the observed transport of anhydrous phosphates with iodine and reducing additives does not proceed via $\text{P}_4\text{O}_{10,\text{g}}$, the question naturally arises as to which lower phosphorus oxide does take on the dominating role. Observations of the transport of $\text{Cr}_2\text{P}_2\text{O}_7$ with iodine ($1050 \rightarrow 950^\circ\text{C}$) in the presence of an excess of CrP are just as noteworthy in this respect as the transport of WOPO_4 or WP_2O_7 in the presence of WP.^[148] In all three cases, *simultaneous transport* of phosphide and phosphate was observed experimentally. In experiments with the transport balance (see Section 3) it was shown that phosphide and phosphate only migrate in a single steady state from the source to the sink when the two condensed phases are present in a particular molar ratio. If there is more phosphide present in the starting solid, the excess is transported as a second steady state to the sink. This behavior implies a *coupled transport reaction* for the two phases. It is surprising with such a complex gas-phase composition that the experimental ratio of phosphide to phosphate in all three cases agrees very well with the respective calculated ratios which were determined from the assumption of $\text{P}_4\text{O}_{6,\text{g}}$ as the transport-determining molecule in the heterogeneous equilibria [Eq. (53)–(55)]. The formulation of $\text{WO}_2\text{I}_{2,\text{g}}$ and $\text{CrI}_{2,\text{g}}$ as essential metal-carrying molecules is in agreement with many other studies.^[68, 79, 149]



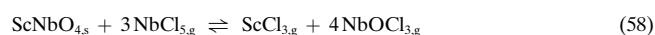
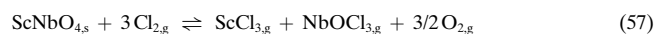
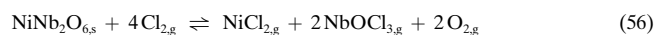
Model calculations on the chemical transport of $\text{Mg}_2\text{P}_2\text{O}_7$ and $\text{Mn}_2\text{P}_2\text{O}_7$ under the same conditions also support the idea of a decisive contribution of “reduced” phosphorus oxides, namely $\text{P}_4\text{O}_{6,\text{g}}$, to the transport of anhydrous phosphates with iodine under reducing conditions.^[146] By taking $\text{P}_4\text{O}_{6,\text{g}}$ into consideration with $\Delta_{\text{B}}H_{298}(\text{P}_4\text{O}_{6,\text{g}}) = -440 \text{ kcal mol}^{-1}$, a value that is used as a fit parameter, it is possible to describe the experiment quantitatively. Both the dependence of the transport rates of $\text{Mg}_2\text{P}_2\text{O}_7$ and $\text{Mn}_2\text{P}_2\text{O}_7$ on the mean temperature and their dependence on the amount of transport agent can be calculated reliably.

6.4. Transition Metal Niobates

The transport behavior of niobates differ in various thermochemical aspects from that of the anhydrous sulfates and phosphates, both of which contain a volatile oxide component (acid anhydride). In contrast, transport of niobates can only take place when both Nb_2O_5 and its bonding partner react with the respective transport agent to form volatile compounds. The heat of neutralization of the formal reaction of formation between the basic (MO , M_2O_3) and the acidic oxides (Nb_2O_5 , SO_3 , P_4O_{10}) is much lower (10–20 kcal per mol Nb_2O_5 with the formation of MNb_2O_6) for niobates^[159] than for sulfates and phosphates. It follows that the activities of the binary components of the niobates do not differ much from those of the pure oxides. The equilibrium partial

pressures in the transport of niobates resemble those observed in the transport of the binary components under similar conditions.^[150, 151, 159] Clear differences are seen however, as expected, in the transport of niobates with a very high content of Nb₂O₅ or the second component.

The determining equilibria for the transport of NiNb₂O₆ or ScNbO₄ with chlorine or NbCl₅ appear to be representative for many other niobates [Eq. (56)–(58)].



Initial experiments and thermodynamic analyses of the transport of niobates and several other ternary oxides have been performed by Emmenegger.^[150, 151] Very extensive experimental investigations (Table 6) and a critical comparison of the detailed thermodynamic model calculations were performed for the systems M/Nb/O (M = Sc,^[152] Hf, Zr,^[111] V, Cr, Mn,^[153] Fe, Co,^[154] Ni,^[155] Cu,^[156, 157] Zn, Cd^[158]) at a later date.^[159] A very unfavorable influence on the transport of niobates was shown for the unavoidable traces of moisture (ca. 10^{−5} mol H₂O per ampoule),^[113] which are desorbed from the wall of the silica ampoule. Thermodynamic modeling shows that this behavior is caused on one hand by an increase in the total pressure. However, the release of oxygen is more important as shown in Equation (59), whereby the heterogeneous equilibria such as in Equation (57) and (58) are shifted to the reactant side.



These effects are certainly significant for all transport experiments with oxides and chlorine as the TA.

The delimitation of the enthalpy of formation for niobates MNb₂O₆ (M = Mn, Fe, Co, Ni, Cu, Zn, Cd) by means of the observations (composition of deposits, transport rates) for the transport experiments is also worth mention.^[159]

7. Ternary Oxides of the Rare Earths, Thorium, and Uranium

Crystals of chalcogenides, in particular the binary and ternary oxides of the rare earths, can only be grown with great effort from melts because of their high melting points.^[160] In addition, complete reaction to a ternary compound can only be achieved at relatively high temperatures (e.g. > 1400 °C) if one of the components is high melting. If crystallization by sublimation is at all possible, it will also require very high temperatures. Phases that are metastable or stable only at fairly low temperatures are hardly accessible in this way. This also applies to compounds such as the multicomponent oxychlorides of the rare earths (Section 8), that are thermally labile and cannot be melted without decomposition. In such cases, preparative chemical transport experiments would be advantageous (Table 7).

For extremely stable oxides such as La₂O₃, chlorine appeared to be promising as a transport agent. However, as a result of the above-mentioned formation of LaOCl_s, [Eq. (29)] in our experiment in a silica ampoule,^[161] the yellow color of the added gaseous chloride (*p*(Cl₂) = 1 atm at RT) disappeared completely and a transport reaction with the formation of LaCl_{3,g} and oxygen did not take place. All other Ln₂O₃ compounds apparently react like La₂O₃, since the oxide of the heaviest rare-earth metal, Lu₂O₃, showed the same behavior in the presence of chlorine (1050 → 950 °C).^[162]

Ternary compounds react in a similar fashion if they are rich in Ln₂O₃. The niobate and tantalate, La₃NbO₇ and La₃TaO₇, for example, are also decomposed by chlorine to form

Table 6. Transition metal niobates. In all cases chemical transport occurs as a result of endothermic reaction with the respective transport medium.

Niobate	Temperatures [°C]	Transport agent	<i>m</i> [mg h ^{−1}]	Comments	Ref.
Sc ₁₁ Nb ₃ O ₂₄	1100 → 1000	Cl ₂		also ScNbO ₄ and “Sc ₁₃ NbO ₃ ” ^[a] at T ₁	[159]
ScNbO ₄	1100 → 1000	a) Cl ₂ b) Cl ₂ /NbCl ₅	ca. 0.02 ≤ 0.6		[152] [152]
TiNb ₁₄ O ₃₇	850 → 750	Cl ₂ (1 atm at RT)		addition of NbCl ₅ and TiCl ₄	[267]
VNbO ₄	1020 → 880	a) NH ₄ Cl b) Cl ₂	ca. 0.1		
				partial oxidation with formation of (V, Nb) ₁₂ O ₂₉ at T ₁ and (V, Nb)O ₂ at T ₂	[159]
CrNbO ₄	1020 → 960	Cl ₂	≤ 1	lower temps (840 → 780) lead to light-green CrNb ₁₁ O ₂₉ at T ₁ ^[b]	[159]
MnNb ₂ O ₄	1020 → 960	Cl ₂	≤ 6		[150, 159]
FeNb ₂ O ₄	1020 → 960	a) NH ₄ Cl b) Cl ₂	≤ 1	larger amounts of TA at T ₂ lead to the formation of (Fe, Nb) ₁₁ O ₂₉ complete oxidation with the formation of FeNbO ₄ and FeNb ₁₁ O ₂₉	[150, 159] [150, 159]
FeNbO ₄	1000 → 900	Cl ₂		FeNbO ₄ (wolframite type); 860 → 640 leads to metastable FeNbO ₄ as the GaNbO ₄ type	[268]
CoNb ₂ O ₄	1020 → 960	a) Cl ₂ b) NH ₄ X (X = Cl, Br, I)	≤ 5 ≤ 2	larger amounts of TA lead to the formation of (Co, Nb) ₁₁ O ₂₉ and/or H-Nb ₂ O ₅ (Co, Nb) ₁₂ O ₂₉ at larger TA amounts	[150, 159, 154] [150, 159, 154]
NiNb ₂ O ₄	1020 → 960	a) Cl ₂ b) NH ₄ Cl	≤ 5 ≤ 0.4	formation of (Ni, Nb) ₁₁ O ₂₉ and/or H-Nb ₂ O ₅ Ni at T ₁	[150, 159, 155] [150, 159, 155]

[a] The phase shows a primitive cubic Guinier diagram; no other characterization was given. [b] The formation of the phase is only possible in a temperature gradient, it is possible that it is metastable towards decomposition to Nb₂O₅ and CrNbO₄.

Table 7. Rare-earth niobates and tantalates a) crystallized for the first time by endothermic chemical transport reactions with chlorine ($p(\text{Cl}_2) = 1$ atm at RT) and b) formed the first time by deposition from the gas phase and only obtainable by this method (probably metastable).

Compound	Temperature [°C]	Explanations	Ref.
LaNbO ₄	1090/1000 ^[a]	mostly deposits with Nb-rich phases (a)	[161, 269]
CeNbO ₄	1000/900	forms alongside Nb-rich phases at $p(\text{Cl}_2, \text{RT}) = 0.5$ atm (a)	[203]
La _{1/3} NbO ₃	1100/1000 ^[b]	defect perovskite (a)	[161]
LaNb ₃ O ₉	1100/1000 ^[b]	monoclinic modification (b) transforms irreversibly at 1300 °C to La _{1/3} NbO ₃	[161, 166]
Ce _{1/3} NbO ₃	1100/1000 ^[b]	exp. with $p(\text{Cl}_2, \text{RT}) \approx 0.5$ atm or with 5 mg NH ₄ Cl; defect perovskite (a)	[203]
CeNb ₃ O ₉	950/900	exp. with 5 mg NH ₄ Cl; monoclinic like LaNb ₃ O ₉ (b)	[203, 166]
PrNb ₃ O ₉	950/900	monoclinic crystals (a) only at $T_2 = 950$ °C! Exp. with 5 mg NH ₄ Cl	[270, 203, 271]
LaNb ₅ O ₁₄	1050/950 ^[b]	(a)	[161, 203, 167]
CeNb ₅ O ₁₄	1100/1000 ^[b]	(a)	[203]
LaNb ₇ O ₁₉	900/800 ^[c]	(b), at 1000 °C irreversible separation into LaNb ₅ O ₁₄ and Nb ₂ O ₅	[203, 272, 182]
CeNb ₇ O ₁₉	850/800	(b), thermal characteristics similar to LaNb ₇ O ₁₉	[203]
PrNb ₇ O ₁₉	800/750	(b), like LaNb ₇ O ₁₉	[203]
NdNb ₇ O ₁₉	800/750	(b) heterogeneous transport product, otherwise like LaNb ₇ O ₁₉	[203]
LaTaO ₄	1050/950 ^[d]	(a)	[161, 273]
CeTaO ₄	(1100/1000)	(a), exothermic react.: 1000 → 1100 °C only with a reducing gas phase CO + Br ₂ from CO ₂ + CBr ₄	[109]
PrTaO ₄	1120/1020	(a)	[209]
NdTaO ₄	1100/1000 ^[e]	(a)	[109]
	980/880 ^[f]	(a)	[109]
La _{1/3} TaO ₃	1090/1000	(a), defect perovskite; single-phase SS is recommended	[161, 108]
LaTa ₃ O ₉	1090/1000	(b), lower $p(\text{Cl}_2)$ as with La _{1/3} TaO ₃ , “bronze-type” structure, transforms irreversibly at 1100 °C to La _{1/3} TaO ₃	[161, 108, 164]
Ce _{1/3} TaO ₃	1100/1000	(a), defect perovskite	[109]
CeTa ₃ O ₉	1090/1000 ^[g]	(b), orthorhombic “bronze-type” structure as with La, alongside Ce _{1/3} TaO ₃ , also monoclinic, bronze-type structure, above 1200 °C both transform irreversibly to Ce _{1/3} TaO ₃	[109, 274]
Pr _{1/3} TaO ₃	1100/1000	(a), orthorhombic defect perovskite	[209]
PrTa ₃ O ₉	1100/1020 ^[h]	(b), difficult to reproduce; monoclinic structure closely related to LaNb ₃ O ₉ ^[i]	[275, 276]
Nd _{1/3} TaO ₃	1100/1000	(a), orthorhombic defect perovskite only in the SS!	[109]
NdTa ₃ O ₉	1100/1000 ^[h,j]	(b), Structure like PrTa ₃ O ₉ ^[i] , transforms irreversibly at 1200 °C to Pr _{1/3} TaO ₃	[109, 275]
“LaTa ₅ O ₁₄ ”	1390/1300	(a), forms above $T \gg 1100$ °C, corresponds to La _{4.67} Ta ₂₂ O ₆₂	[108, 109, 277]
LaTa ₇ O ₁₉	1120/1050 ^[k]	(a), decomposition to LaTa ₅ O ₁₄ + Ta ₂ O ₅ at 1500 °C	[273, 165]
CeTa ₇ O ₁₉	1100/1000 ^[l]	(a)	[108, 163]
PrTa ₇ O ₁₉	1120/1020	(a), lower $p(\text{Cl}_2)$ recommended	[209]
NdTa ₇ O ₁₉	1100/1000 ^[l]	(a)	[108, 163]

[a] LaNbO₄ can also be transported well with HBr (addition of 50 mg NH₄Br).^[269] [b] The appearance of additional, partially Nb-rich phases (LaNb₅O₁₄; H-Nb₂O₅) can be suppressed by a slight temperature gradient.^[269] [c] Instead of Cl₂, the addition of an oxychloride such as LaOCl or La₂NbO₄Cl₃ is advantageous. Powder can also be obtained by isothermal heating with Cl₂. [d] Ta₂O₅-rich phases also occur (e.g. La_{1/3}TaO₃).^[108] [e] Starting solid: monoclinic NdTaO₄. [f] Starting solid: mixture Nd₂O₃:Ta₂O₅ 1:1. [g] SS = CeO₂:Ta₂O₅:Ta 5:7:1. [h] Tribochemical transformation into a modification with higher density that is also monoclinic.^[278] [i] Mainly Ln_{1/3}TaO₃ precipitates. [j] Starting solid = mixture Nd₂O₃:Ta₂O₅ 1:3. [k] Ta₂O₅-poor phases also occur (La_{1/3}TaO₃, LaTa₃O₉).^[273] [l] Higher proportion of LnTa₇O₁₉ with the addition of some (8 mg) V (VCl₄, VOCl₃).

oxychlorides and oxide phases of lower La content.^[109, 161] The titanate Ln₂TiO₅ decompose in transport experiments with Cl₂ to form LnOCl and Ln₂Ti₂O₇ (Ln = La, Pr, Nd, Sm, Eu, Gd) according to Equation (61).^[162]

Ternary oxides of low activity do not react fully to LnOCl [Eq. (29)] when chlorine is present; endothermic equilibria such as those formed with the *rare-earth niobates and tantalates*^[163] become established [Eq. (60)], which allow chemical transport to occur (see also NdNbO₄ (Figure 5j)).^[270]



Therefore in the system La₂O₃/Ta₂O₅, with the exception of LaTaO₄ and La_{1/3}TaO₃, the new, probably metastable phase LaTa₃O₉^[164] and the non-congruent melting LaTa₇O₁₉^[165]

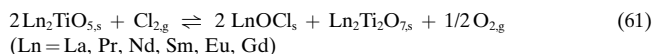
could be crystallized for the first time. The phases LaNb₃O₉^[166] and LaNb₇O₁₉^[167] which occur in the La₂O₃/Nb₂O₅ system could only be obtained by means of chemical transport and are considered to be metastable.

Of the few rare-earth oxo compounds that were crystallized by chemical transport before the ternary oxides listed in Table 6, LaPO₄ deserves a special mention (Section 6.3).^[168] Early efforts to grow crystals of Y₃Fe₅O₁₂ and Gd₃Fe₅O₁₂, in particular, in which HCl, CCl₄, and Cl₂ + TeCl₄ were used as transport agents,^[169, 170] were carried out with a view to the potential physical applications of garnets.

Many of the compounds that can be transported in temperature gradients (Table 6) can also be produced and crystallized by the so-called “short-path transport” method (see Section 2.3); the temperatures used are similar to those of the experiments in a temperature gradient. If compounds cannot

be obtained in this way by the action of a mineralizer but only by deposition in a temperature gradient, that means through a sufficiently supersaturated gas phase, they are considered to be “metastable”.

The phase distribution in the $\text{Ln}_2\text{O}_3/\text{TiO}_2$ system is already widely understood.^[171] Chemical transport experiments, however, had not been performed until the investigations by Hübner.^[162] With Cl_2 as the transport agent, the undesirable side reaction to yield LnOCl [Eq. (29) and Eq. (61)] could be suppressed only for titanates with a ratio of $\text{Ln}/\text{Ti} \leq 1$.



All titanates $\text{Ln}_2\text{Ti}_2\text{O}_7$ from Ln = Nd (Ln = Nd, Sm (Figure 5g), Eu–Lu)^[162] migrate in temperature gradients ($1050 \rightarrow 950^\circ$). Sometimes a small amount of rutile (TiO_2) was deposited as well, probably as a result of reactions with the wall (silica ampoule) which consume Ln_2O_3 . Chlorine proved to be effective as a TA^[162] and also in some cases, HgCl_2 .^[172] The compound $\text{Ce}_2\text{Ti}_2\text{O}_7$, which is sensitive to oxidation, could not be transported with Cl_2 or with HCl ,^[171] but transport did succeed with the addition of NH_4Cl or HgCl_2 ($1050 \rightarrow 950^\circ\text{C}$).^[173]

The crystallization of monoclinic $\text{Nd}_2\text{Ti}_4\text{O}_{11}$ in a short-path transport experiment^[174] is worth mentioning as is that of $\text{Nd}_4\text{Ti}_9\text{O}_{24}$ (Figure 5o), which could be transported in a temperature gradient ($1000 \rightarrow 900^\circ\text{C}$) with chlorine.^[175] Recently the short-path transport of $\text{Pr}_4\text{Ti}_9\text{O}_{24}$ succeeded,^[172] whereas transport reactions with the compounds $\text{La}_4\text{Ti}_9\text{O}_{24}$ and $\text{Ce}_4\text{Ti}_9\text{O}_{24}$, which are also isotypic to $\text{Nd}_4\text{Ti}_9\text{O}_{24}$, could not be realized.^[162]

The new silicate-titanate $\text{Ce}_2\text{Ti}_2\text{SiO}_9$ was produced in the sink ($1050 \rightarrow 900^\circ\text{C}$) when the binary oxides were used as the reactants and SiO_2 was present in excess with NH_4Cl .^[176]

ThO_2 migrates with the addition of Cl_2 or NH_4Cl only with extremely low transport rates. As in the case of La_2O_3 , an unwanted side reaction is the problem, which in this case occurs with the wall of the silica ampoule and leads to the formation of thermodynamically stable ThSiO_4 (α - or at least the β -form). ThSiO_4 also migrates together with small amounts of ThO_2 to the cooler zone (e.g. $1050 \rightarrow 950^\circ\text{C}$).^[177] We are not aware at present of an effective method of chemically transporting ThO_2 .

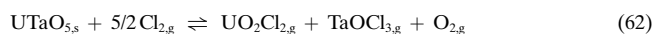
The unwanted effect of the formation of ThSiO_4 can be essentially suppressed in transport experiments with *thorium niobates*, or *tantalates* apparently because of the stability of such compounds. With the help of Cl_2 or NH_4Cl as the TA, it was possible^[177, 178] to deposit the following ternary well-crystallized phases in the cooler zone: $\text{Th}_2\text{Nb}_2\text{O}_9$,^[177] $\text{Th}_2\text{Ta}_2\text{O}_9$,^[179] ThNb_2O_7 ,^[180] ThTa_2O_7 ,^[181] $\text{Th}_2\text{Ta}_6\text{O}_{19}$,^[182] $\text{ThNb}_4\text{O}_{12}$, $\text{ThTa}_4\text{O}_{12}$,^[177] $\text{Th}_4\text{Ta}_{18}\text{O}_{53}$ (TA: $\text{Cl}_2 + \text{TaCl}_5$),^[183] and also α - ThTi_2O_6 (TA: $\text{Cl}_2 + \text{S}$).^[184]

Up to now ThNb_2O_7 , ThTa_2O_7 , and $\text{Th}_2\text{Ta}_6\text{O}_{19}$ could only be obtained by chemical transport or with the help of the mineralizing effect of Cl_2 , since they may be metastable.

A range of transport agents are applicable for UO_2 . In particular TeCl_4 ^[102, 185] and Cl_2 ,^[186, 187] but also Br_2 , $\text{Br}_2 + \text{S}$, I_2 , and HCl have proven useful.^[187]

Mixed crystals $\text{U}_{1-x}\text{Th}_x\text{O}_2$ were deposited with the help of the transport agent HCl .^[188] Transport experiments with the addition of HCl or $\text{Br}_2 + \text{S}$ were successful for U_3O_8 .^[188] It was possible to transport U_4O_9 with Cl_2 , Br_2 , or I_2 .^[189]

In addition, the ternary compounds $\text{UNb}_6\text{O}_{16}$ (TA NH_4Cl),^[190] γ - UNbO_5 ,^[191] and UNb_2O_7 (TA $\text{Cl}_2 + \text{NbCl}_5$),^[192] $\text{U}_4\text{Ta}_{18}\text{O}_{53}$ (TA $\text{Cl}_2 + \text{TaCl}_5$)^[183] and $\text{U}_2\text{Ta}_6\text{O}_{19}$ (TA HCl)^[193] could be crystallized by means of CT. According to the literature,^[189] there is evidence that UOCl_2 or $\text{UO}_2\text{Cl}_{2,g}$ participates in the transport process. For the CT of UTaO_5 , a reaction according to Equation (62) was supported by mass spectrometry experiments.^[193, 194]



8. Polynary Oxychlorides of the Rare Earths

It proved to be extremely productive to undertake an extensive study of the “failure” of Ln_2O_3 to form in the presence of the transport agent chlorine with the formation of LnOCl [Eq. (29)] from ternary and multicomponent compounds.^[109] Ternary oxides can, as the example of Ln_2TiO_5 [Eq. (61)] has already shown, also react with Cl_2 to form LnOCl . But here new, polynary oxychlorides are produced: Starting from Pr_2TiO_5 , a transport experiment with Cl_2 [$p(\text{Cl}_2; \text{RT}) = 1 \text{ atm}$; $1050 \rightarrow 900^\circ\text{C}$] results in the deposition of $\text{Pr}_2\text{Ti}_3\text{O}_8\text{Cl}_2$ alongside PrOCl in the cooler zone.^[162, 195] We also observed similar behavior with Ln_3TaO_7 and Ln_3NbO_7 . The reactions, in which Cl_2 was obviously consumed, proceeded in a more complicated fashion, however, since traces of moisture were also involved (wall of the silica tubes) [Eq. (63)].



When the reaction products are heated (1400°C) in air Equation (63) quantitatively reversed. If H_2O is present in stoichiometric amounts, pure $\text{La}_3\text{TaO}_5(\text{OH})\text{Cl}_3$ is formed in a Cl_2 atmosphere according to Equation (64),^[196, 197] and can be transported in a temperature gradient of $1080 \rightarrow 980^\circ\text{C}$.



In a principally similar fashion, new compounds in the $\text{Ln}/\text{Ti}/\text{O}/\text{Cl}$, $\text{Ln}/\text{V}/\text{O}/\text{Cl}$, $\text{Ln}/\text{Nb}/\text{O}/\text{Cl}$, and $\text{Ln}/\text{Ta}/\text{O}/\text{Cl}$ systems could be identified by deposition and crystallization in temperature gradients followed by structural characterization ($\text{La}_3\text{TiO}_4\text{Cl}_5$,^[198] $\text{Ln}_2\text{Ti}_3\text{O}_8\text{Cl}_2$ (Ln = Ce (Figure 5s),^[172] Pr (Figure 5c), Nd),^[195] LnTiO_3Cl (Ln = Sm–Lu),^[199, 200] $\text{Ln}_3\text{VO}_4\text{Cl}_6$ (La–Nd),^[201] $\text{Ln}_{12,33}\text{V}_6\text{O}_{23}(\text{OH})\text{Cl}_{20}$ (Ln = La, Ce),^[202] $\text{Ln}_3\text{NbO}_4\text{Cl}_6$ (Ln = La,^[163] Ce,^[203] Pr,^[204, 205] Nd^[204]), $\text{Ln}_3\text{TaO}_4\text{Cl}_6$ (La–Nd),^[109, 206] $\text{La}_2\text{NbO}_4\text{Cl}_3$,^[203] $\text{Ln}_2\text{TaO}_4\text{Cl}_3$ (Ln = La, Ce),^[207] $\text{Ln}_3\text{NbO}_5(\text{OH})\text{Cl}_3$ (Ln = La, Pr, Nd),^[204] $\text{Ln}_3\text{TaO}_5(\text{OH})\text{Cl}_3$ (Ln = La, Pr, Nd),^[197, 208, 209] $\text{Ln}_2\text{M}^{4+}\text{NbO}_6\text{Cl}_3$ (Ln = La, Pr, Nd; $\text{M}^{4+} = \text{Ce}$, U; Ln = La, Pr, Nd, Sm–Dy; $\text{M}^{4+} = \text{Th}$),^[204, 210] $\text{Ln}_2\text{M}^{4+}\text{TaO}_6\text{Cl}_3$ (Ln = La–Nd, $\text{M}^{4+} = \text{Ce}$, U; Ln = La–Ho, $\text{M}^{4+} = \text{Th}$),^[208, 210, 211] (Ln, Ce)_{3,25} $\text{MO}_6\text{Cl}_{3,5-x}$

(Ln = La, Ce, Pr; M = Nb, Ta),^[212] (Ln, Ce)_{3.5}MO₆Cl_{4-x} (Ln = La, Pr, Nd, Sm; M = Nb, Ta),^[212] Ln₂Ta₂O₇Cl₂ (Ln = Pr, Nd,^[213] Sm^[214]), Nd_{7.33}Ta₈O₂₈Cl₆^[215]).

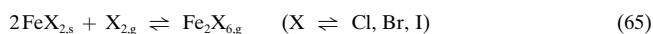
As is known from the given compositions, Ln₂M⁴⁺NbO₆Cl₃ and Ln₂M⁴⁺TaO₆Cl₃, including M⁴⁺ = Th⁴⁺ and U⁴⁺, can be synthesized and transported. This type of compound has the La₃WO₆Cl₃ structure type and also crystallizes (TA = Cl₂) with U⁶⁺ as Ln₃UO₆Cl₃ (Ln = La, Pr, Nd).^[216]

It should be mentioned that it was not possible to synthesize isotypic compounds with other larger or smaller rare-earth ions in some of the compositions given here. Therefore it appeared reasonable that experiments with different rare earths have been carried out to establish the borders of the crystal-chemical “existence ranges”.

9. Chemical Transport of Metal Halides

A large number of the binary metal–halogen compounds such as trihalides (AlX₃, X = Cl, Br, I; FeX₃, X = Cl, Br), tetrahalides (TiX₄, SiCl₄, SnX₄, X = Cl, Br, I), and pentahalides (VCl₅, NbCl₅, TaCl₅) already demonstrate considerable volatility at even slightly raised temperatures. In other cases it can be advantageous for preparative reasons to undertake measures to improve the volatility, for example with the less volatile compounds CrCl₂, CrCl₃, LnCl₃, and chlorides of the alkali and alkaline earth metals.

In individual cases, transport via a gaseous halide in a higher oxidation state was successful [Eq. (65) and (66)]. The compounds FeI₃^[217] and CrCl₄ are, in fact, only known as gas molecules.



Much more frequent are cases in which the greater volatility of a so-called “gas complex” is utilized, whereby mainly AlCl₃, FeCl₃, or GaCl₃ are used as complexing agents, as studied extensively by Schäfer.^[218, 219]

The synthesis and separation of CrCl₂ (350 → 250 °C)^[220] [Eq. (67)] and CrCl₃ (500 → 400 °C)^[221] [Eq. (68)] were improved.

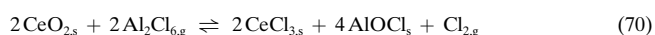
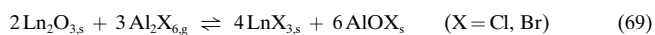


CuCl₂, which releases chlorine when heated, can also be transported in the form of the beautiful blue gas complex CuAl₂Cl₈.^[222]

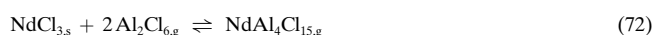
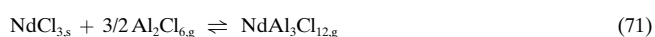
“Gas complexing agents” have also proven useful for the vaporization of rare-earth halides, as the extensive investigation of the phases Eu₅Cl₁₁, Eu₄Cl₉, and Eu₁₄Cl₃₃^[223, 224] in the EuCl₂/EuCl₃ system has shown. It was possible to transport Eu₅Cl₁₁ in the temperature gradient 480 → 545 °C and EuCl₃ at 480 → 380 °C.^[225]

Trihalides cannot, however, be used for the formation of gas complexes, if oxides (O-containing solids) are to be trans-

ported, since the thermodynamic stability of the corresponding oxide, in particular Al₂O₃, would lead to the complete consumption of the trihalide. This reaction behavior can be utilized if the halogenation of rare-earth oxides and, at the same time, the separation of the resulting RE halide LnX₃ (Ln: La–Nd, Sm–Tb; X = Cl, Br) in the form of a suitable gas complex is attempted.^[226, 227] The sesquioxides Ln₂O₃ react with AlX₃ in accordance with Equation (69). If higher rare-earth oxides (e.g. CeO₂, “Tb₄O₇”) are used, additional chlorine is released [Eq. (70)].

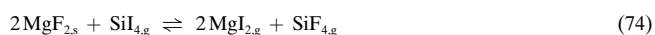
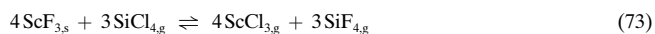


The separation of the RE halides is subsequently achieved by chemical transport with excess AlX₃. For the transport system NdCl₃/Al₂Cl₆, the gas complexes NdAl₃Cl₁₂ and NdAl₄Cl₁₅ have been detected [Eq. (71) and (72)].^[228]



In many cases (e.g. MAl₂Cl₈, M = Cu,^[229] Pd^[230]; LnAl₃Cl₁₂, Ln = Tb, Dy, Ho;^[231] LnAl₃Br₁₂, Ln = La–Nd, Sm, Gd^[227]), it was possible to isolate and crystallize the gas complexes.

Since the volatility of *metal fluorides* is much lower, a suitable transport reaction is of particular interest. The use of silicon halides (Cl, Br, I) as TA enables the utilization of the thermodynamic stability and, at the same time, high volatility of SiF₄ (GeF₄) [Eq. (73)].^[232] Experiments with MgF₂ pointed to a similar transport reaction [Eq. (74)].^[172]



It is not yet clear why MgF_{2,s} migrates in a temperature gradient (1000 → 900 °C) even when mere iodine is added. A reaction in which MgI_{2,g} and F_{2,g} are formed should be excluded with certainty due to the low oxidation capability of iodine.^[172]

10. Summary and Outlook

The broad new fields of application which have been opened up by the transport methods in the past 15 years leave no room for doubt that also in the future, chemical transport reactions will continue to show great potential. Already in this attempt to describe the current situation we have failed, for obvious reasons, to discuss a series of interesting developments from our colleagues and from our own research group. Interesting information on the interpretation of the reaction processes could be obtained from investigations into the crystallization of compounds such as Se₁₇(WCl₆)₂ or Te₆-(WOCl₄)₂ by the research group of J. Beck.^[233, 234] Also the results of chemical transport experiments in non-isothermal plasmas by Veprek show promise for further potential applications in preparative solid-state chemistry.^[235, 236, 237]

Some research which was not discussed here and which is partly still in progress concerns the chemical transport of germanates,^[238, 239] silicates (e.g. Ni_2SiO_4 ^[240] or $\text{Yb}_2\text{Si}_2\text{O}_7$ ^[172] see Figure 5a) and silicate halides such as $\text{Ln}_3\text{SiO}_4\text{Cl}_5$ ^[241] $\text{Ln}_3\text{Si}_2\text{O}_8\text{Cl}$ ^[172] or $\text{Mn}_3\text{SiO}_4\text{F}_2$.^[242] Further new substance classes will be investigated in the coming years, so that the broad applicability of the method to the synthesis, purification, and crystallization of solids will be of great use.

Finally we have also not discussed the many structural investigations of single crystals that were only accessible by the described transport experiments. Information on this important aspect of the research field can be obtained from the cited literature. The potential of the transport method for the synthesis of crystals for physical studies will continue to be considerable. In order to obtain the "large" crystals that are frequently necessary, it is possible that some of the already available procedures could help to prevent the growth defects, additional nuclei formation etc., by means of the "gradient reversal" method described by Scholz,^[243] or by the in situ observation (and control) of crystal growth.^[244, 245]

With our review we would also like to encourage the greater use of chemical transport as an investigative method for the study of equilibrium gas phases and for the development of new methods for the clarification of previously not understood transport processes. In particular, this includes the qualitative and quantitative detection of previously unknown gas molecules, but also the less spectacular thermodynamic characterization of participating solids and gas molecules.

The time dependence of the deposition of compounds with homogeneity ranges (mixed crystals, nonstoichiometric compounds) and their thermochemical description (prediction) should also receive greater attention in the future. Important results in this area have already been provided by Oppermann and Krabbes, and Binnewies. We believe that with suitable transport experiments, information on the thermodynamics of mixed phases can also be obtained. Technically interesting questions such as the directed adjustment of certain mixing or doping ratios make this field particularly interesting.

Up to now the description of the influence of convection on the TR has been difficult. In particular there is the question of how well the "simple" formulations describe the experimental characteristics.

It is certain that the possibility to synthesize and crystallize new classes of compounds through an in-depth understanding of the reactions between the solid and the gas phase will ensure the continuing fascination of the method in the future.

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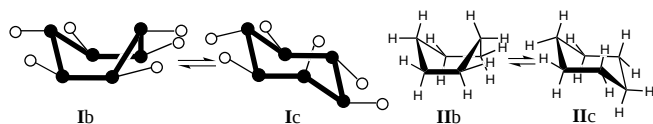
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Water Clusters: Towards an Understanding Based on First Principles of Their Static and Dynamic Properties**

Jesus M. Ugalde, Ibon Alkorta, and José Elguero*

If a single molecule were to be selected as the most important chemical entity for life, most people would agree that this is water. Moreover, water has been used to find a common definition for the Fahrenheit, Celsius, and Kelvin scales through its melting, boiling, and triple points. It is clear that these points are bulk properties and that a single H₂O molecule has neither a melting nor a boiling point. Therefore, water offers a paradigmatic case for studying the transition from quantum reality to classical physics: How many water molecules are necessary for the bulk properties to appear?

Water clusters, discrete or polymeric (ice), can be seen as one of the simplest models connecting molecular to supramolecular chemistry. Consider, for instance, one of the possible water hexamers, **I**, and cyclohexane (**II**), both undergoing boat–chair equilibria (Scheme 1). The study by



Scheme 1. Both a water hexamer (**I**) and cyclohexane (**II**) exist in equilibrium between a boat (b) and chair (c) conformation.

Barton and Hassel of the boat–chair equilibrium of cyclohexane has been one of the cornerstones of conformational analysis.^[1] The water hexamer **I** is the simplest supramolecular analogue of cyclohexane. To concisely represent water clusters (**W_i**) we have used an empty circle for terminal hydrogen atoms, a filled circle for oxygen atoms, a thin line for terminal O–H bonds and a bold line for O–H···O hydrogen bonds (HBs).

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A number of papers have defined our view of water from both the theoretical and experimental points of view;^[2–4] although it has returned as an object of interest in recent years. Another aspect which has also shown spectacular developments concerns the solvated proton (H_{2n+1}O_n)⁺,^[5, 6] but this field will not be dealt with here, nor will supercooled water and molecular dynamic simulations.

The HB is a covalent, not an ionic, bond

In particular, for ice I_h hydrogen atoms form two distinct types of bonds with neighboring oxygen atoms: 1) the shorter (1.00 Å) covalent bond as a typical molecular σ-type bond with a binding energy of 4.8 eV; and 2) the longer (1.75 Å) bond with a binding energy of 0.29 eV, described as a classical, electrostatic interaction between “frozen” charge distributions placed on the two interacting molecules.^[3] However, ab initio calculations on this latter type of bond predict a small but appreciable amount of electron density shared between the hydrogen and oxygen atoms, which is suggestive that the hydrogen bond is at least “partly covalent”.^[2, 7] Nevertheless, this point has been controversial until the results of the beautiful experiment by Isaacs et al.^[8, 9] were published. In essence, this study took advantage of the unique properties of inelastic X-ray scattering^[10] with high momentum transfer, the Compton effect, to obtain information about the Fourier transform of the electron density of the ground state for the low-momentum electrons, namely, electrons located far away from the influence of the nuclei. These electrons set up the electron density responsible for the hydrogen bond. Hence, this experiment enables direct observation of their behavior.

Indeed, a Compton scattering profile measures the electronic momentum density projected onto the scattering vector. Bonds parallel to the scattering vector give rise to oscillations whereas bonds perpendicular to it do not influence the profile. Molecular orientations in ice I_h are such that one hydrogen atom (and bond) lies along each of the tetragonally coordinated neighboring oxygen atoms (see the Bernal–Fowler ice model^[3]). The Compton profile measured along the *c* axis, which is parallel to the bonds along one of the tetrahedral axes and nearly perpendicular to the others, contains a strong signature of the hydrogen bonds. Conversely, in a Compton profile measured in the *ab* plane the signature is weak because the bonds nearly parallel to this

plane are aligned along three different directions. The anisotropies of Compton profiles, being the differences between the measured profiles along different crystalline directions, are exceptionally sensitive to the hydrogen bond. Furthermore, the anisotropies cancel the contributions from isotropic 1s core orbitals of the oxygen atoms.

The experimental anisotropy, obtained as the difference between the Compton profiles with fixed momentum transfer along the c axis and an axis perpendicular to c , shows a periodic variation in intensity as a function of the electron momentum, which corresponds closely to the O–O distance (2.75 Å). Results from a DFT-CGA calculation of ice I_h match the experimental data remarkably well, in contrast to the noticeable disagreement between theory and experiment with a purely electrostatic bonding model for ice. This experiment provides firm support for coherent charge transfer in the hydrogen bond, confirming the prediction of covalency advanced by Pauling in 1935.^[2]

Small Clusters

Obtaining a reliable intermolecular interaction potential for water molecules has been found to be elusive. In this respect, the efforts of Saykally and coworkers are remarkable. They have carried out extensive measurements of Vibration–Rotation Tunneling (VRT) spectra for small water clusters to the hexamer W_6 level.^[11] They have been able to confirm theoretical predictions of quasiplanar ring structures as the most stable form for W_2 – W_5 clusters but not for the W_6 hexamer for which they have found, again in agreement with theoretical predictions, a three-dimensional cage structure as the preferred conformation. Most importantly, their work laid the foundations of a model potential energy function for the intermolecular interaction of water. This potential is dominated by the two-body interaction potential,^[12] but contains also sizeable contributions from three-, four-, and higher many-body interactions which cannot be neglected. Hence, studying higher clusters is critical to obtain information that must be incorporated into the intermolecular potential energy function. Analysis of the VRT data provides very interesting information for the construction of such an accurate model, which includes all the classical and quantum effects together. Notice that such a program, once completed, will result in a very reliable many-body interaction potential, which can eventually be used to calculate the classical partition function through the configuration integral.^[13] This opens the way towards a precise knowledge of all thermodynamic properties of either phase of bulk water as a function of temperature, a remarkable achievement that will deserve close attention in the years to come.

Parallel to the efforts of the Saykally group to understand properties of water clusters, Weinhold is progressing towards a description of the bulk properties of water. He used his QCE (quantum cluster equilibrium) approach,^[7, 14] a mixture of full ab initio calculations and mean-field approximations. He notes that the thermodynamic properties of condensed phases must be simulated by a set of clusters because “the essential continuity of gases and liquids, established by the existence of the gas–liquid critical point, implies that a cluster picture of

dense gases should be *continuously extendible* into the liquid region”.^[14] The balance between enthalpic and entropic contributions, and how strongly selective thermodynamic considerations are among species that might superficially appear to have comparable stabilities, has been clearly established by Weinhold.

Weinhold’s next paper described a model using seven water clusters, from W_2 to W_5 or W_6 ; most properties of the gas–liquid diagram of water are well reproduced.^[15] To reproduce the liquid–solid region proved more difficult and clusters that could adequately represent the four-coordinate, three-dimensional structure of crystalline ice were necessary. Using these clusters (particularly a W_{24} cluster) and a higher level of the theory, Weinhold reproduced the properties of the Bucky-ice phase.^[16] Related studies have shown that there exist procedures to predict the melting transition of water from studies of medium-size clusters such as W_8 and W_6 .^[17]

Figure 1 shows the most significant water clusters, based on the work of several authors. The most stable ones, according to Clary et al.,^[18] are framed. Other minimum energy clusters up to 21 and 24 water molecules can be found in papers by Wales et al.^[19] and Weinhold.^[16]

One of the most important aspects in the structure of small clusters is their topology. The theoretical predictions in this field have been especially helpful to clarify the experimental data. The water dimer W_2 presents an open structure with one water molecule as a HB donor and the other as a HB acceptor (see Figure 1).^[20] The absolute potential energy minimum configurations of W_3 , W_4 , and W_5 are monocyclic, as predicted by theoretical calculations^[21–23] and in agreement with experimental data.^[24] The hydrogen and oxygen atoms involved in a HB are almost coplanar, with each water molecule forming two HBs, one as a donor and the other as an acceptor. The disposition of the free hydrogen atoms results from trying to minimize their interaction, by alternating their positions above and below the molecular plane. In the case of the trimer and pentamer, strict alternation is not completely possible.

The W_6 cluster is the first case where the theoretical calculations proposed the existence of several minima with lower energy than the monocyclic one, such as the cage and the prism (see Figure 1 and note that the represented prism is but one of the several isomers).^[25, 26] The inclusion of the zero-point energy (ZPE) in the calculations has been necessary to properly predict the W_6 global minimum, the cage structure. The experimental data for the W_6 cluster, isolated^[27] or in the presence of benzene^[28] in the gas phase, are in agreement with the calculations. As Saykally pointed out, the cage closely mimics the basic unit of one of the high-density polymorphs of ice, ice VI.^[27] A structure of monocyclic W_6 , with a chair disposition, has been found embedded in the crystalline phase of an nitrogeneous organic compound forming HBs with the surrounding nitrogen atoms.^[29]

The structure of W_7 predicted by ab initio calculations can be described as a cage derived from the W_6 cage minimum by adding one water molecule. The inclusion of the ZPE in this case favored a bicyclic structure. The only experimental data of gas-phase W_7 in the presence of benzene,^[28] indicate the presence of more compact noncyclic structures where the

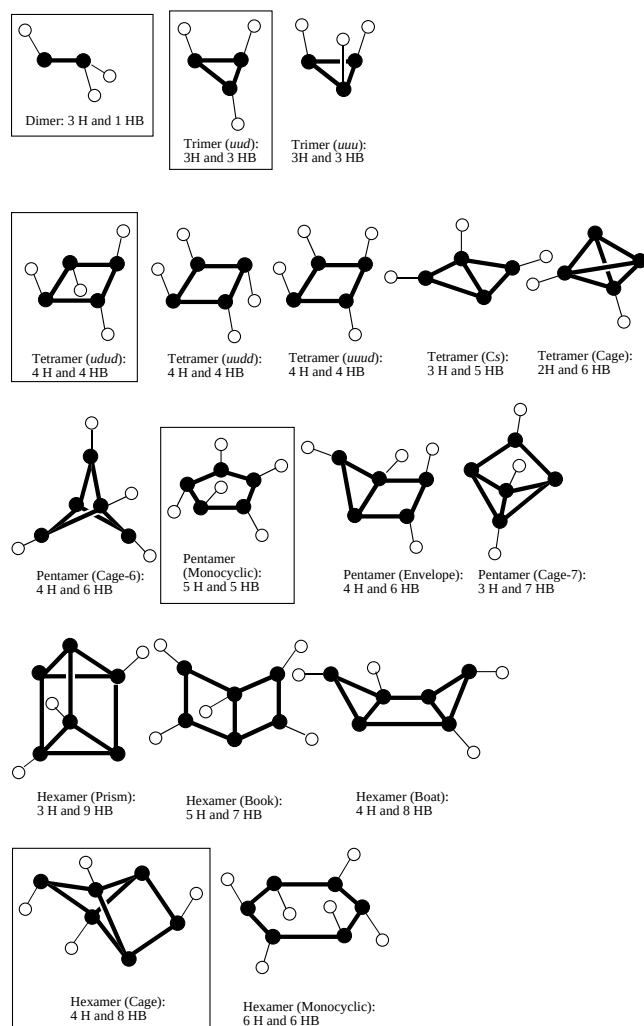
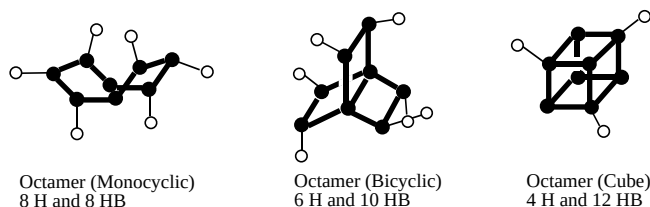


Figure 1. The most stable water clusters W_i ($i=2-6$). The absolute potential energy minimum for each cluster level is framed. ○: terminal hydrogen atoms; ●: oxygen atoms; —: terminal O–H bonds; —: O–H...O (or O...H–O) bonds. The number of terminal free hydrogens (nH) and hydrogen bonds (mHB) is indicated. The orientation of the hydrogen atoms not involved with HB is indicated as above (*up*) and below (*down*) the molecular plane.

majority of the water molecules participate with both hydrogen atoms in HBs. The structure of a crystalline W_7 can be described as a six-membered ring with an isolated water molecule linked to the ring.^[29]

Two configurations of W_8 in the presence of benzene have been characterized in the gas phase and correspond to D_{2d} and S_4 symmetry.^[30] Another W_8 arrangement has been described in an organocobalt crystal.^[31] Here the W_8 configuration corresponds to S_i symmetry which, according to theoretical calculations, is 8.4 kJ mol^{−1} above the D_{2d} and S_4 configurations.

Weinhold has discussed the alternative topology of monocyclic, bicyclic, and cubic octamers (Scheme 2). The main conclusion is that the cubic octamer, in spite of being intrinsically the most stable, is completely negligible in the temperature–pressure region experimentally accessible for bulk water, whereas the monocyclic octamer is important



Scheme 2. The W_8 cluster, in three highly symmetric forms, features differing numbers of hydrogen bonds.

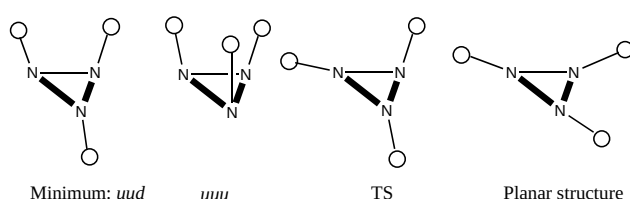
even at low temperatures. The fact “that higher three- or four-coordinate [maximum number of HBs in water] clusters are disfavored at higher T , compared with two-coordinate, closed charge transfer ring forms”, can be recognized from two general factors. First, each three- or four-coordinate site necessitates replacement of nearly free *torsional* motions about a connecting pair of HBs by more strongly hindered (higher frequency) bending or stretching vibrational modes, thereby lowering the vibrational entropy. Second, favorable, cooperative HB directionality patterns apparently cannot be extended indefinitely in higher-coordinate three-dimensional networks, so a significant enthalpy loss per HB occurs even as new hydrogen bonds are being “gained” through coordination.^[15] The influence of temperature has been recognized by Estrín et al. as the reason why molecular-beam electric deflection experiments at high temperature show only monocyclic structures whereas the Saykally VRT experiments at 5 K show cage structures.^[17]

The OH stretching frequencies of W_9 and W_{10} have been compared with the simulations of low-energy structures.^[32] In the case of W_9 , the “basket” structure seems to be the one that most closely resembles the experimental data. A similar basket structure has been found in the $W_8 \cdot Cl^-$ cluster. The simulated spectra of the lowest energy conformers of the W_{10} cluster do not resemble the experimental data. Only a “butterfly-shaped” structure with a minimized potential energy 10 kJ mol^{−1} above the global minimum (1.4 kJ mol^{−1} with inclusion of the ZPE) provides a theoretical vibrational spectrum similar to the experimental one. In addition, a W_{10} cluster has been characterized in a solid-state supramolecular complex.^[33] In this case, the water molecules adopt an ice I_c -like arrangement.

So far, thirteen polymorphic phases of pure solid water were characterized, denoted as I_h , I_c , and II–XI,^[34, 35] and the recently described ice XII.^[36, 37] In addition, two amorphous ice polymorphs (low- and high-density) have been detected.^[38] Phase I_h corresponds to natural ice crystallized in the hexagonal system. The oxygen atoms in this phase adopt a tetrahedral distribution with a 2.74 Å separation and an O–H bond length of 0.985 Å. Each water molecule is involved in four HBs, two as acceptors and another two as donors. The rest of the phases exist at lower temperatures and higher pressures and present a variety of structural characteristics that range from hydrogen atoms centered between the oxygen atoms in ice XI^[39] to substantially bent hydrogen bonds in ices IV, V, and XII. Cooperative effects^[7, 11, 14, 15] and dipole-moment variations are also important in water clusters.^[7, 40]

Dynamic Aspects: Conformational Studies where no HB is Broken

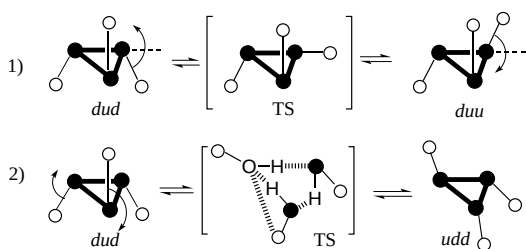
Fowler and Schaefer^[23] have studied the conformation of the water trimer. The minimum energy form has the *uud* structure and C_1 symmetry (see Figure 1), while the *uuu* trimer (bowl structure, C_3) is 3.6 kJ mol^{-1} less stable (Hessian = 2) and, according to Weinhold, practically equivalent for thermodynamic purposes.^[15] The transition state (TS) between two *uud* structures (Hessian = 1) corresponds to the flipping of one OH moiety and is very close in energy to the minimum (C_1 , 1.1 kJ mol^{-1}). Finally, the planar structure which corresponds to the TS of the simultaneous flipping of the three OH moieties (C_{3h}) is 7.0 kJ mol^{-1} (Hessian = 3) less favorable. The closest “molecular” structure is triaziridine (Scheme 3), for which the same kind of structures can be written.



Scheme 3. The structure of triaziridine possesses a number of conformers, which differ in the location of the N–H bonds: above (*u*), below (*d*), or in the N_3 plane, or in some transition state (TS).

Alcamí, Mó, and Yáñez^[41] have studied the triaziridine conformations and calculated (MP4/6-31 + G**) that the *uuu* isomer lies 47 kJ mol^{-1} above the *uud* minimum. Calculations (G2) yield the following four relative potential energies [kJ mol^{-1}]: *uud* 0.0, *uuu* 37, TS_{*uud*→*uud*} 153, and planar structure 545.^[42] Clearly, when comparing molecular and supramolecular structures, it is necessary to take into account that HBs are much more easily deformed and broken than covalent bonds (for example, O–H...O angles of 150° are common) which reduces strain from distortion (if the strain is too high, the HB would break). In consequence, the potential energy surface of the water trimer along all external H–O–O–O out-of-plane coordinates is very flat.

Wales has studied the dynamics of water trimers and pentamers.^[43, 44] He points out that there are two mechanisms by which an *dud* trimer can be transformed into a *duu* or *udd* trimer (Scheme 4): 1) by a single flip; 2) by bifurcation tunneling.



Scheme 4. The W_3 cluster transforms between conformations (notation according to Scheme 3) by two mechanisms: the single flip or a more complex bifurcation tunneling.

tunneling, which entails a significantly larger energy barrier than the single flip. Both have been observed experimentally, in particular, the former has been used by Saykally in his studies of water clusters (see previously). This last author has pointed out that the W_3 trimer is chiral.^[45]

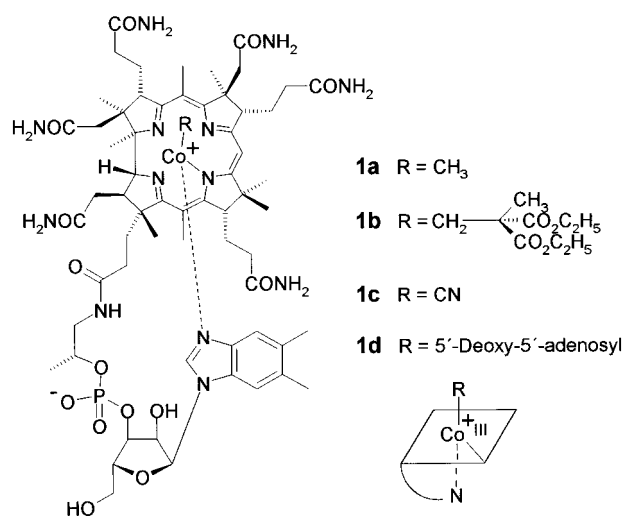
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Unusual Alkylation Reactions in the Biosynthesis of Natural Products and Elucidation of Their Reaction Mechanisms

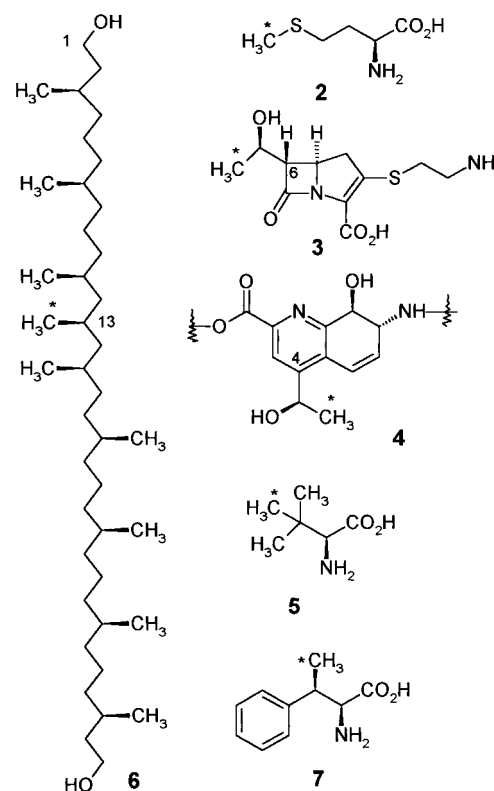
Martina Glasenapp-Breiling and Franz-Peter Montforts*

The vitamin B₁₂ derivative methylcobalamin (**1a**) mediates numerous enzymatic methylation reactions in the biosynthesis of various classes of natural products. The reactions proceed with inversion of configuration with respect to the transferred methyl groups.^[1] The mechanism of a S_N2 reaction was established by investigation of the stereochemical course using chiral methyl groups (CHDT), which were exclusively transferred to reactive nucleophilic centers.



Recently, there have been an increasing number of examples of unusual methylation reactions in which methyl groups originating from methionine **2** were transmitted intact, with overall retention of configuration, to saturated non-activated carbon atoms. At first Floss et al. observed such methylation processes in biosynthetic studies related to thienamycin **3** and thiostrepton. The 6'' methyl group of **3** as

well as the 4'' methyl group of the quinaldine subunit **4** of thiostrepton were transferred from methionine with retention of configuration.^[2] Also the methyl groups of the unusual amino acids *tert*-butylglycine (**5**) and β -methylphenylalanine (**7**) of the peptide antibiotic bottromycin occurring in *streptomyces bottropensis* originate from methionine.^[3]

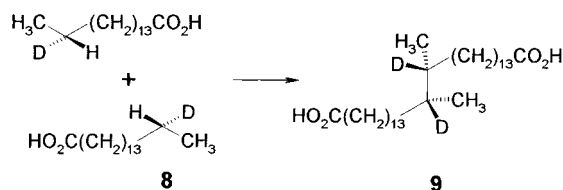


The stereochemical analysis demonstrates that the methyl groups themselves were transferred to valine and phenylalanine with overall retention of configuration and that the methylated carbon atoms experienced inversion. A similar methylation process that occurs with retention at the transmitted methyl group and with inversion at the methylated

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carbon center was observed in biosynthetic investigations of the lipid ethers caldarcheol and isocaldarcheol, which are found in the lipid membrane of archae bacteria.^[4] In the case that the microorganisms were cultivated under stress conditions the normal lipid ethers form homocaldarcheol and homoisocaldarcheol by methylation. The additional methyl groups are located in the 13-position of the bisphytanol **6** which is the non-glyceridic alcohol component of the membrane forming lipids.

Dimerization of palmitic acid **8** leading to diabolic acid **9** in the eubacterium *butyrivibrio fibrisolvens* is closely related to these methylation processes (Scheme 1).^[4] Diabolic acid **9**,

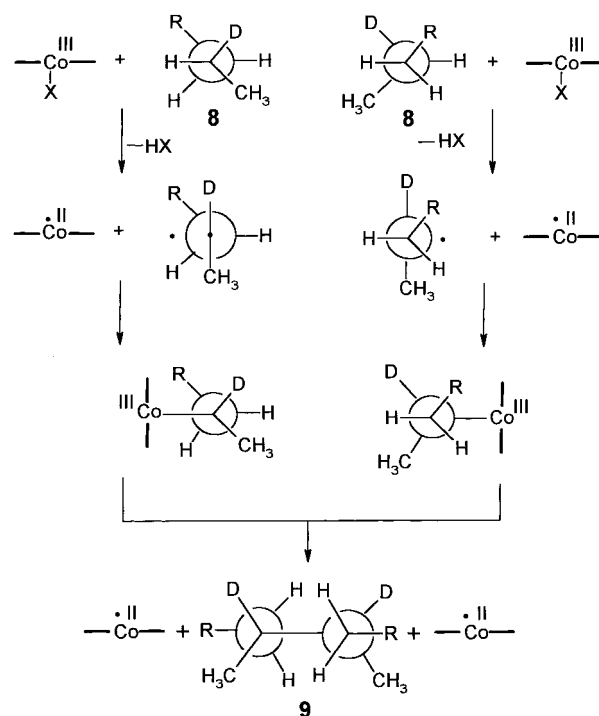


Scheme 1. Dimerization of palmitic acid **8** to diabolic acid **9**.

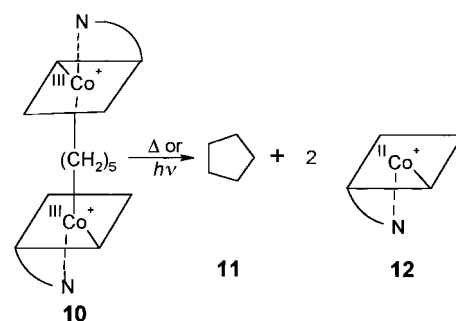
which acts as the key component of the bacterial lipids, is formed from **8** with inversion of configuration at both reacting carbon centers which are non-activated. Investigations using isotopically labeled compounds demonstrate that neither neighboring methyl or methylene groups participate in the formation of the central carbon–carbon bond. A common feature of the bond-forming reactions observed by Arigoni et al. is that they proceed under strictly anaerobic conditions a fact which excludes the participation of oxygen-dependent cofactors related to cytochrome P₄₅₀.

Arigoni et al. proposed a radical reaction mechanism for the formation of diabolic acid **9**, in which, for instance, the coenzyme B₁₂ (**1d**) could be involved. The participation of B₁₂-like coenzymes appears plausible in view of the occurrence of corrinoids in corresponding microorganisms (Scheme 2).^[4] According to the proposed mechanism a radical intermediate is generated by homolysis of a Co–X bond in an alkylated cobalamin followed by hydrogen abstraction from the substrate **8**. The radical intermediate reacts with retention of configuration with the cobalt(II) complex to yield the corresponding alkylcobalamin. Two palmityl cobalamins could then dimerize in an enzymatically controlled stereoselective reaction giving diabolic acid **9**. The evidence of this mechanistic course is supported by a model reaction in which the biscobalamin derivative **10** bridged by a chain of five CH₂ units between the central metal atoms forms cyclopentane **11** on heating or on irradiation (Scheme 3).^[5]

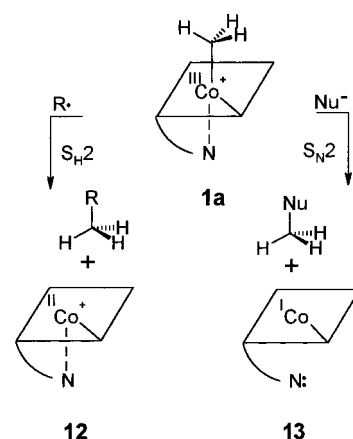
Normally, methylcobalamin (**1a**) methylates nucleophiles according to a S_N2 mechanism. The binding pair of electrons of the cobalt–carbon bond of **1a** is transferred to the central metal ion thus giving the cobalt(I) complex **13** (Scheme 4).^[1, 6] Quite recently Kräutler et al. demonstrated in a thus far unprecedented model reaction the methylation of alkyl radicals by means of methylcobalamin (**1a**) according to a S_H2 reaction. The central cobalt ion is thereby reduced with bond fission to the Co^{II} complex **12**.^[7]



Scheme 2. Proposed mechanism for the formation of **9**.

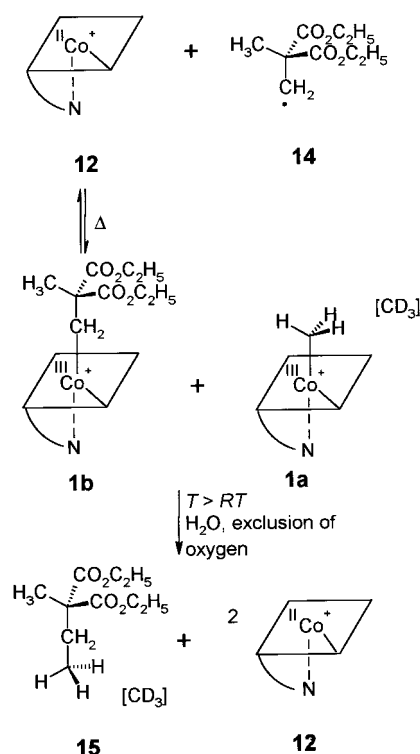


Scheme 3. Model reaction for the reaction process shown in Scheme 2.



Scheme 4. Reaction of methylcobalamin **1a** with radicals and nucleophiles.

To confirm the methylation of a carbon radical experimentally a mixture of methylcobalamin (**1a**) or its tris(deuteriomethyl) derivative and 2',2'-bis(ethoxycarbonyl)propylcobalamin (**1b**) was heated with exclusion of oxygen and light for about 5 h at 70 °C.



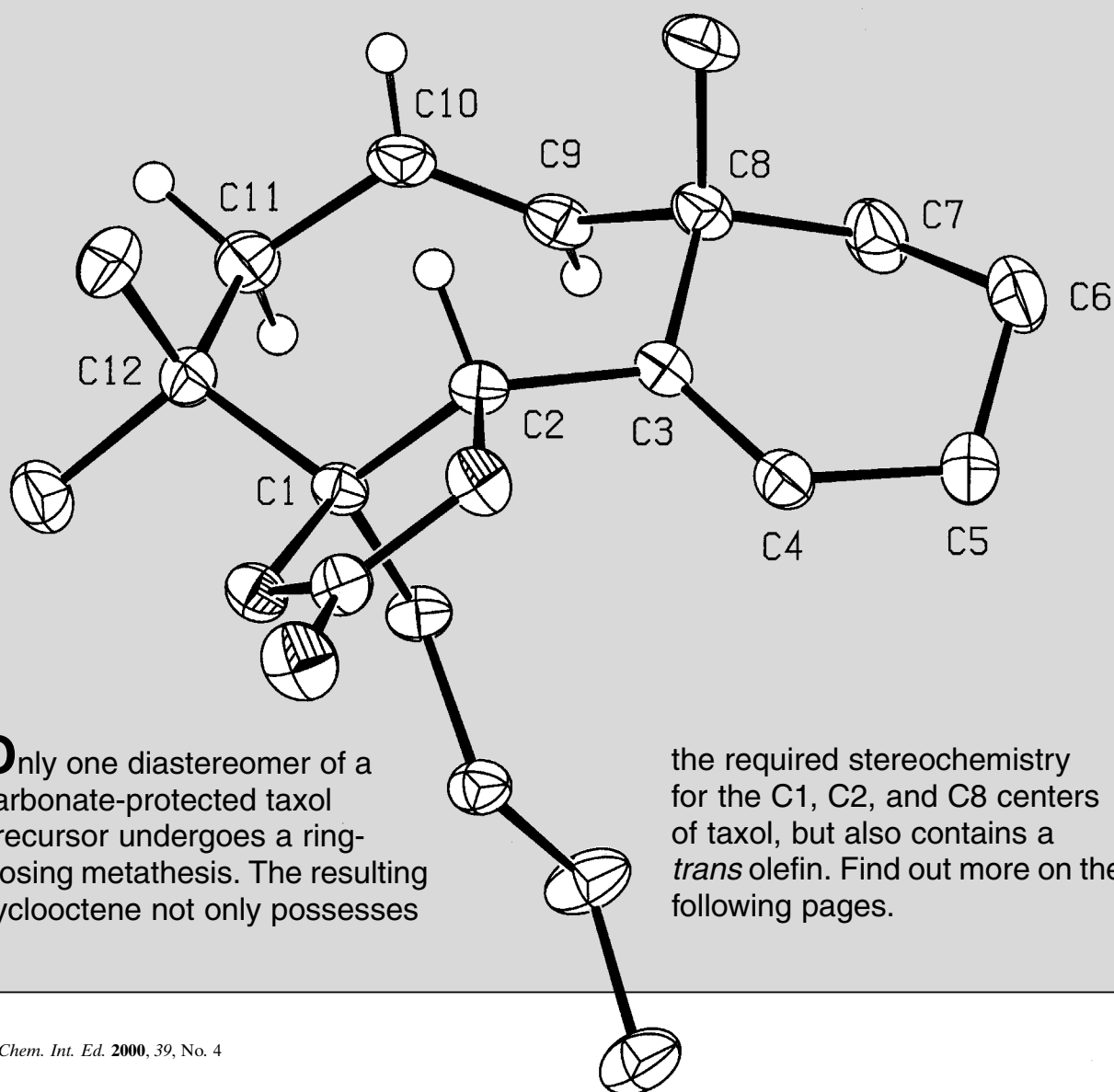
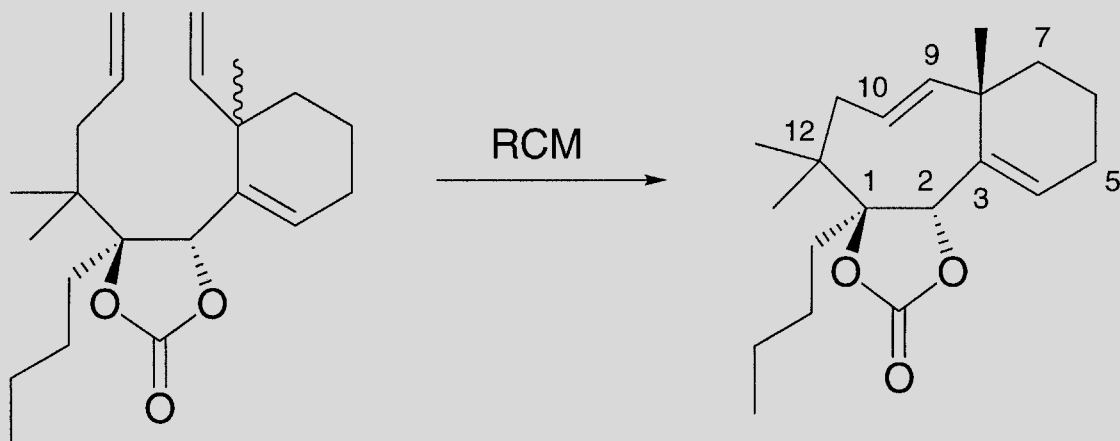
Scheme 5. Experimental proof of the methylation of carbon radicals.

Diethyl 2-ethylmalonate (**15**) or the corresponding deuterated derivative and diethyl 2,2-dimethylmalonate were obtained as reaction products in a 4.7:1 ratio with 70% total yield (Scheme 5). The thermolabile cobalamin **1b** decomposes homolytically even at room temperature to yield cobalamin **12** and the bis(ethoxycarbonyl)propyl radical **14** with a half-life of about 50 min, whereas methylcobalamin (**1a**) decomposes at 130 °C with a half-life of about 4 h. Accordingly, the formation of **15** from **1a** and **1b** can be attributed to a substitution of the cobalt corrin part of **1a** by the alkyl radical **14** generated from **1b**. The estimated homolytic dissociation energy ($\Delta H^\circ \approx -48 \text{ kcal mol}^{-1}$)^[8] for the abstraction of the cobalt-bound methyl group from **1a** by the primary alkyl radical **14** reveals that this reaction step can

be characterized as very exothermic. The reaction can successfully compete with the recombination of radical **14** with the Co^{II} corrin **12** to give **1b**. The enzymatic methyl transfer from **1a** to a carbon radical should proceed with inversion of configuration of the methyl groups when a chiral methyl group is applied. The transfer should show net-retention with regard to the configuration of methionine because prior to this methionine transmits its methyl group to the central metal atom with inversion.

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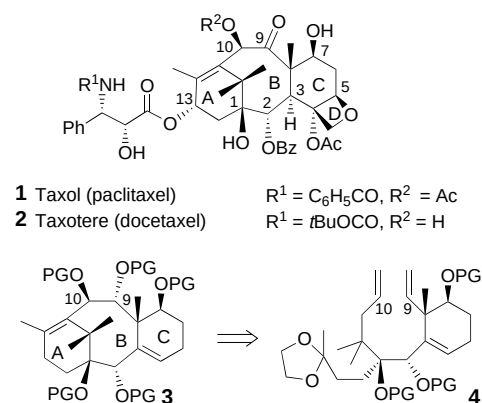
The First *trans*-Cyclooctene Synthesized by Ring-Closing Metathesis (RCM)



Synthesis of Highly Functionalized Cyclooctenes by Ring-Closing Metathesis: Unexpected Formation of a *trans* Isomer**

Damien Bourgeois, Ange Pancrazi, Louis Ricard, and Joëlle Prunet*

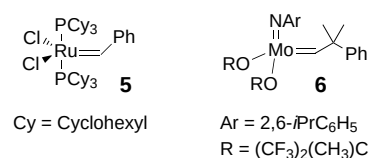
During our studies towards the synthesis of the antitumor agents taxol (**1**)^[1] and taxotere (**2**)^[2] we explored several convergent routes.^[3] One of them relies on a ring-closing metathesis (RCM) to form the eight-membered cycle of this molecule (Scheme 1). This reaction has become a powerful



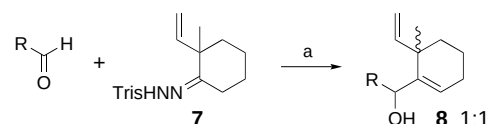
Scheme 1. Retrosynthesis of the ABC ring-system of taxol. See ref. [8] for abbreviations.

tool in organic synthesis, since it involves nonfunctionalized olefins that are usually unreactive towards classical reagents, and is compatible with diverse functional groups.^[4] To the best of our knowledge there are only four reports in the literature of such a reaction for the synthesis of carbocyclic rings of this size.^[5] Herein we present cyclizations of highly functionalized precursors of the BC ring-system of taxol using the Grubbs' ruthenium catalyst **5**^[6] or Schrock's molybdenum catalyst **6**^[7], and also the unexpected formation of a *trans*-cyclooctene in one case.

The closure of the B-ring of taxol between C9 and C10 by RCM presents a double challenge; the high activation enthalpy for the formation of an eight-membered ring has to be overcome, and the neopentyl position at C8 adjacent to one of the olefinic partners might hamper the formation of the intermediate metallacyclobutane, which is already fused

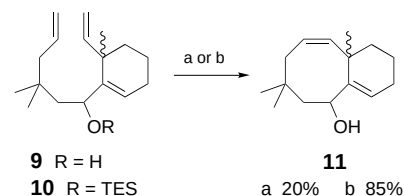


to a highly substituted eight-membered ring. We therefore designed a short route to metathesis precursors **8**, which were synthesized as 1:1 mixture of diastereomers by using a Shapiro reaction on trisylhydrazone **7** and quenching with the appropriate aldehyde (Scheme 2).^[9]



Scheme 2. Preparation of the metathesis precursors. a) 2.1 equiv *t*BuLi, THF, -78°C , 30 min; 0°C , 1 min; 1.2 equiv RCHO, -78°C .

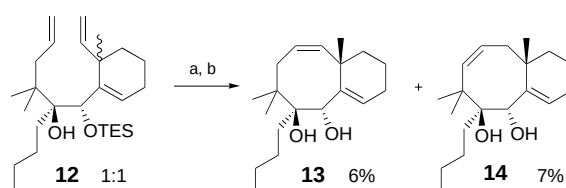
Attempts of ring-closure on alcohol **9** only led to polymers. However, formation of an eight-membered ring was observed when the triethylsilyl ether derivative **10** was heated for eight days in benzene in the presence of the ruthenium complex **5** (Scheme 3). For purification purposes the TES ether was



Scheme 3. Formation of a cyclooctene. a) 10% **5**, 0.02 M, benzene, 80°C , 6 d; TBAF · 3 H₂O, THF; b) 10% **6**, 0.02 M, benzene, 80°C , 3 d; TBAF · 3 H₂O, THF.

cleaved and alcohol **11** was then obtained in 20% yield as a single diastereomer, whose stereochemistry has not been determined. A Thorpe–Ingold effect of the TES group could explain the difference in yields between **9** and **10**,^[10] but a complexation of the catalyst by the free OH group in **9** cannot be excluded. A much better yield was obtained for the RCM reaction with Schrock's catalyst, which gave both diastereomers of **11**.

We then decided to check the feasibility of the RCM using more elaborate models such as alcohol **12**, where the steric hindrance is similar to that of the precursor **4**, a potential precursor for the synthesis of the ABC tricycle (Scheme 4). We assumed that additional substituents would favor the cyclization by reducing the conformational freedom of the molecule. However, reaction of **12** with the ruthenium



Scheme 4. RCM with a more elaborate model. a) 10% **5**, 0.02 M, benzene, 80°C , 8 d; b) TBAF · 3 H₂O, THF.

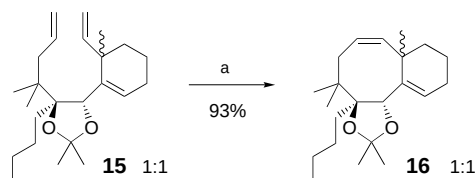
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[**] This work was supported by the CNRS and the Ecole Polytechnique.

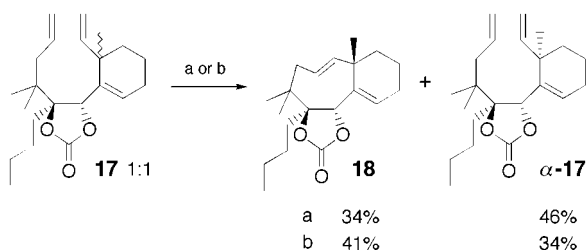
complex **5** in benzene at reflux for eight days produced only minor amounts of two cyclized products.^[11] Removal of the TES ether led to the expected product **13** as a single diastereomer^[12] together with **14**, in which the double bond has shifted from C9-C10 to C10-C11. The structure of **14** was determined by X-ray diffraction analysis.^[13] A similar isomerization was reported by Taylor et al.^[10, 16]

Simple semi-empirical calculations (MM2/PM3) on **12** clearly showed that the substituents at C1 forced the diene in a ground-state conformation in which the two double bonds are far apart. However, a cyclic protecting group as in **15** appeared to place the two olefin residues at a reasonable distance for a metathesis reaction. We were thus disappointed to find only traces of the desired cyclooctene in the unpurified reaction mixture when the Grubbs' catalyst was employed with acetone **15**, even after eight days in benzene at reflux (Scheme 5). However, RCM reaction of **15** with Schrock's molybdenum catalyst **6** led to cyclooctene **16** in 93% yield. Three days were necessary to drive the reaction to completion, but conversion of both isomers was total, giving a 1:1 mixture of diastereomeric cyclooctenes **16** that were easily separable by flash chromatography. Their structures were determined by extensive NMR studies.^[17]



Scheme 5. RCM of compounds bearing an acetone at C1-C2. a) 10% **6**, 0.02 M, benzene, 80 °C, 3 d.

Another suitable protecting group for the C1-C2 diol of taxol precursors such as **3** is a cyclic carbonate.^[18] We thus submitted compound **17** to the Grubbs catalyst **5** in benzene at reflux for 8 days (Scheme 6). Surprisingly, only one diastereomer underwent cyclization, and the resulting product **18** was a *trans*-cyclooctene produced in 34% yield (68% based



Scheme 6. Synthesis of a *trans*-cyclooctene. a) 10% **5**, 0.02 M, benzene, 80 °C, 8 d; b) 10% **6**, 0.02 M, benzene, 80 °C, 3 d.

on the diastereomeric ratio of the starting diene). The other diastereomer of **17** was recovered in 46% yield. The same reaction with the molybdenum catalyst produced 41% of cyclic product **18**, along with 34% of recovered **17** (bearing an α -methyl group at C8).

Structural evidence for **18** was obtained by X-ray diffraction studies^[13] (Figure 1). A coupling constant of 16.7 Hz is observed between the two olefinic protons at C9 and C10 in

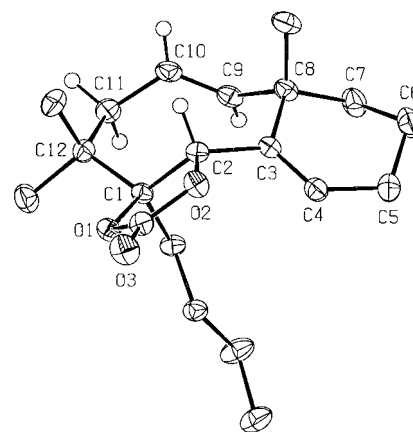


Figure 1. Platon representation of *trans*-cyclooctene **18**.^[19] For the purpose of clarity only the hydrogen atoms on the eight-membered ring are shown.

the ¹H NMR spectrum.^[20] A C8-C9-C10-C11 torsion angle of 139.3° in the solid-state structure indicates a loss of the π character of the double bond. All the stereocenters in **18** (C1, C2, C8) present the configuration required for the synthesis of taxol. Moreover, **18** is the first *trans*-cyclooctene obtained by olefin metathesis. This result is totally unexpected, as olefin metathesis is thought to occur exclusively under thermodynamic control.^[21] In our case, no trace of the more stable corresponding *cis*-cyclooctene was detected. A complexation of the catalyst by the carbonyl moiety might be invoked,^[22] although the involved chelate would be an eight-membered ring. Further studies to provide an explanation of this result are underway.

In conclusion, we have shown that highly functionalized cyclooctenes can be synthesized in very good yields with Grubbs' or Schrock's catalyst. In the specific case of carbonate **17**, only one diastereomer cyclized to give cyclooctene **18**, which not only possesses the required stereochemistry for the C1, C2, and C8 centers of taxol, but also presents a *trans* olefin. This result indicates that RCM does not proceed to complete thermodynamic equilibrium in this case.

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Diastereoselective Pinacol Coupling Reactions of α -Ketoamides Mediated by SmI_2 : Synthesis of Enantiomerically Pure *R* and *S* Quaternary Tartaric Acids**

Sam Min Kim, Il Suk Byun, and Yong Hae Kim*

Multidentate, chiral, C_2 -symmetric ligands are well-known for their ability to impart asymmetry to transition and main group elements.^[1] C_2 -Symmetric diols are among the most frequently applied examples of such molecules, especially in the area of asymmetric catalysis.^[2] Most diol ligands have been derived from C_2 -symmetric molecules which occur naturally in optically pure form (such as tartaric acid).^[2] However, the number of chiral precursors available from natural products is seriously limited.

The pinacol coupling was first described a long time ago,^[3] but this reaction is still a versatile tool for chemists. The intermolecular coupling of various aldehydes or ketones to the corresponding pinacols has been extensively studied.^[4] However, pinacols in an enantiopure form have not really been obtained using this type of coupling.^[5] Although the asymmetric dihydroxylation of olefins mediated by osmium tetroxide has become one of the most useful methods for the preparation of C_2 -symmetric diols,^[6] asymmetric dihydroxylations of tetrasubstituted olefins are extremely rare^[7a] and give low enantioselectivity.^[7]

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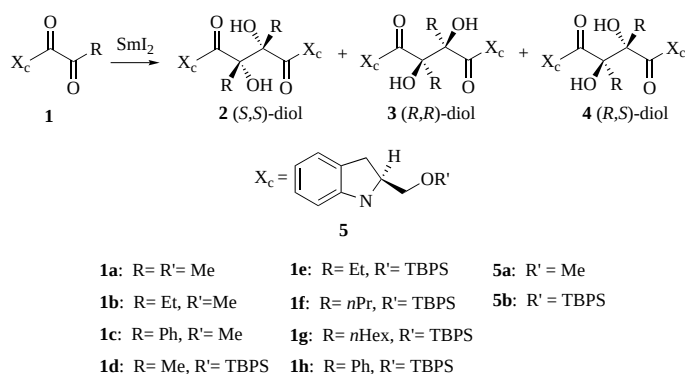
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SmI_2 has been utilized successfully as a one-electron donating agent for diverse organic reactions^[8] including intermolecular^[9] or intramolecular^[10] pinacol coupling reactions of aldehydes. However, the intermolecular coupling of ketones has afforded low diastereoselectivities.^[11]

Chiral 2,3-dialkyltartaric acids could be utilized for designing chiral catalysts or auxiliaries for use in various asymmetric reactions^[2] and as chiral intermediates in the synthesis of natural products. However, to our knowledge, the synthesis of enantiopure 2,3-disubstituted tartaric acid (a quaternary pinacol) has never been reported, although a chiral 2,3-dimethyltartaric acid was obtained by chiral resolution.^[12] A chiral tertiary pinacol can be obtained by using pinacol coupling of ketones by photolysis in a chiral solvent^[11a] or by using a chiral auxiliary,^[11b, c] however, the diastereoselectivities are low.

We report here the coupling of the chiral α -ketoamides **1** in the presence of SmI_2 , HMPA, and *t*BuOH in THF to give pinacol **2** with extremely high diastereoselectivity (>98% *de* in some cases; Scheme 1). This is the first example of such high stereoselectivity for disubstituted tartaric acid derivatives in intermolecular pinacol coupling reactions of α -ketoamides.



Scheme 1. Pinacol coupling reactions of **1a–h** in the presence of SmI_2 , HMPA, and *t*BuOH. TBPS = *tert*-butyldiphenylsilyl, HMPA = hexamethyl phosphoramide.

The results obtained are summarized in Table 1. The coupling of pyruvamide **1a** using SmI_2 in the absence of HMPA and *t*BuOH gave low stereoselectivity (**2:3:4** = 71:~0:29, entry 1). However, the coupling of **1a** in the presence of SmI_2 , HMPA, and *t*BuOH occurred with high diastereoselectivity (**2:3:4** = 95:~0:5, entry 5). Moreover, the coupling of **1e** and **1f** resulted in extremely high diastereofacial selectivity (**2:3:4** = >99:~0:1, entries 13 and 14). HMPA is known to increase the reaction rate and stereoselectivity of SmI_2 -mediated reactions and *t*BuOH is often used as a proton source for these reactions.^[13] The pinacol coupling reaction can form three stereoisomers (*S,S*), (*R,R*), and (*S,R* or *R,S*; *meso* form). The *meso* form was easily confirmed by ¹³C NMR spectroscopy;^[14] however, formation of (*R,R*)-pinacol could not be detected.

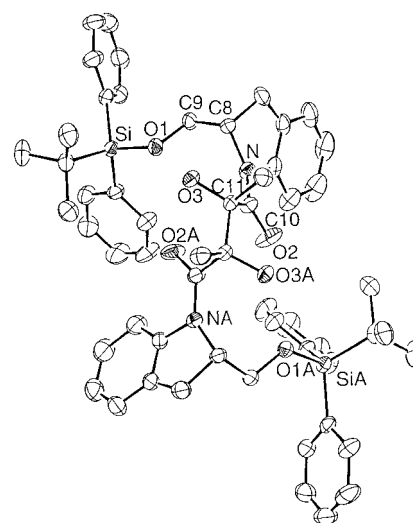
When the *tert*-butyldiphenylsilyl (TBPS) substituent was introduced to give the chiral auxiliary **5b**, the pinacol coupling reactions of **1d–g** had a longer reaction time (5 h) and gave

Table 1. Pinacol coupling of **1a–h**.^[a]

Entry	Substrate	SmI_2 [equiv]	HMPA [equiv]	<i>t</i> BuOH [equiv]	Time [h]	Yield [%]	2:3:4 ^[b]
1	1a	1.5	0	0	0.1	64	71:~0:29
2	1a	2.0	2	4	0.1	51	94:~0:6
3	1a	2.0	4	4	0.1	42	95:~0:5
4	1a	2.0	4	4	1	61	95:~0:5
5	1a	2.0	4	4	2	57	95:~0:5
6	1a	2.0	4	4	5	58	94:~0:6
7	1b	2.0	2	4	0.1	61	95:~0:5
8	1b	2.0	4	4	0.1	39	98:~0:2
9	1c	2.0	4	4	5	– ^[c]	–
10	1d	2.5	4	4	5	69	96:~0:4 ^[d]
11	1e	2.5	0	0	5	87	95:~0:5
12	1e	3.0	4	4	5	87	95:~0:5 ^[e]
13	1e	2.5	4	4	5	79	>99:~0:1
14	1f	2.5	4	4	5	67	>99:~0:1
15	1g	2.5	4	4	5	83	97:~0:3
16	1h	2.5	4	4	5	– ^[c]	–

[a] Reactions carried out at –78°C in THF. [b] Determined by HPLC analysis. [c] The reduction of the α -carbonyl group to the secondary alcohol was the major reaction. [d] After recrystallization the ratio of **2:4** = >99:1. [e] Toluene was used as the solvent.

extremely high diastereoselectivities (**2:3:4** up to >99:~0:1, entries 10–15 in Table 1), probably due to the steric effect of the bulky TBPS moiety. The coupled product **2a** was readily hydrolyzed with 3M HCl in dioxane at 25°C for 4 h to give (*S,S*)-2,3-dimethyltartaric acid (**6a**) (85% yield) together with recovered chiral auxiliary, (*S*)-2-methoxymethylindolinone, (90%) with no loss of chirality. (*S,S*)-2,3-Diethyltartaric acid was obtained by hydrolysis of **2b** and shows an opposite sign of specific rotation ($[\alpha]_D^{25} = -6.37$ ($c = 0.926$, H_2O)) to that of (*S,S*)-2,3-dimethyltartaric acid ($[\alpha]_D^{25} = +13.1$ ($c = 0.598$, H_2O), $[\alpha]_D^{20} = +13.4$ ($c = 4.0$, H_2O)^[12]). As the specific rotation and the absolute configuration of (*S,S*)-2,3-diethyltartaric acid have not been reported previously, its absolute configuration was determined by comparison of the CD spectra of *O,O*-dianisoyl-2,3-diethyltartaric anhydride with that of the previously known *O,O*-dianisoyl-2,3-dimethyltartaric anhydride.^[15] The absolute configuration of **2d** was also determined by X-ray analysis (Figure 1).^[16]



Although the detailed mechanism is not yet clear, the reaction appears to be initiated by formation of a ketyl radical **A** with a one-electron transfer to SmI_2 followed by coupling of the two ketyl radicals (Figure 2). The coupling takes place at the sterically less hindered *si* face and gives the high diastereoselectivities.

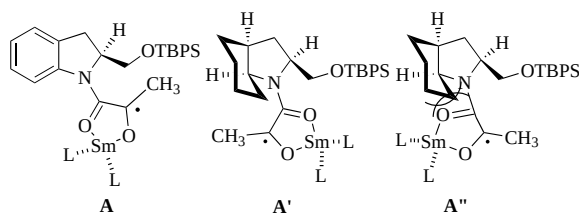
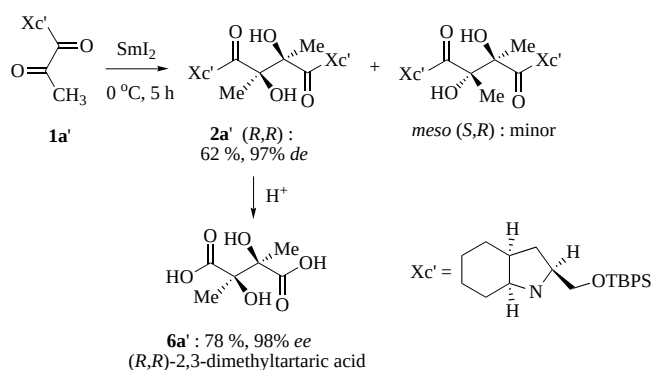


Figure 2. Possible intermediates of the asymmetric pinacol coupling reactions.

In chemical transformations, the synthesis of the individual enantiomers is generally achieved by using chiral sources. However, sometimes natural sources of one of the enantiomers may be limited. Thus, it is desirable and important to obtain both enantiomers by stereocontrolled reactions of prochiral compounds that utilize the same chiral source.^[17] We have found that (2*S*,3*aS*,7*aS*)-*N*-pyruvoyl-2-(*tert*-butyldiphenylsilyloxy)octahydroindoline (**1a'**) (99.8% *ee*),^[18] affords the opposite configuration of **2a'** (*R,R* diol:97% *de*). The ratio of *R,R*:*S,S*:*meso* is 98.5:~0:1.5. The ratio was determined by chiral HPLC analysis with a Daicel OD column. The absolute configuration of **2a'** was determined by comparison of the measured optical rotation of (*S,S*)-2,3-dimethyltartaric acid (**6a'**) ($[\alpha]_{\text{D}}^{20} = -13.2$ ($c = 4.0$, H_2O)), obtained by hydrolysis of **2a'**, with the literature value.^[12]



Scheme 2. Pinacol coupling reaction of compound **1a'** and subsequent hydrolysis to form **6a'**.

The different configurations of the adducts produced can be rationalized by the different intermediates formed from **1a** and **1a'** with SmI_2 . Instead of **A**, the intermediate **A'** is favorable in the case of **1a'**; more so than **A''** due to the steric repulsion between the chair formation of the cyclohexane ring and the amide carbonyl moiety (Figure 2).

In conclusion, we have demonstrated diastereo- and enantioselective pinacol coupling reactions of chiral α -ketoamides mediated by SmI_2 with extremely high diastereoselectivities (>99% *de* in some cases). Enantiopure (*S,S*)- or

(*R,R*)-2,3-dialkyltartaric acid derivatives can now be synthesized for the first time depending on the structure of α -ketoamides. When a similar chiral auxiliary, (*S*)-*N*-pyruvoyl-2-(*tert*-butyldiphenylsilyloxy)proline was used, low diastereoselectivities were obtained.^[19]

Experimental Section

A solution of SmI_2 (0.1M in THF, 6.70 mL) was added dropwise to a solution of (*S*)-*N*-pyruvoyl-2-(methoxymethyl)indoline (78.1 mg, 0.335 mmol), HMPA (1.34 mmol), and *t*BuOH (1.34 mmol) in THF (4 mL) at -78°C . After stirring at -78°C for the appropriate time, the mixture was quenched with 1N HCl solution, extracted with CH_2Cl_2 , dried over MgSO_4 , and then concentrated. The crude product was purified by chromatography to give a solution suitable for HPLC analysis of the mixture of pinacol stereoisomers. The diastereomeric ratio of (*S,S*) and (*S,R*) was determined by HPLC analysis using a chiral column (Daicel OD, 90% *de*). (*S,S*)-Diastereomer **2a** (64% yield): $[\alpha]_{\text{D}}^{18} = +3.08$ ($c = 0.778$ in CH_2Cl_2); ^1H NMR (200 MHz, CDCl_3 , 25°C): $\delta = 1.53$ (s, 6H), 2.96 (d, 2H), 3.1–3.3 (m, 4H), 3.32 (s, 6H), 3.62 (m, 2H), 5.35 (m, 2H), 6.09 (s, 2H), 7.00–7.20 (m, 6H), 7.88 (d, 2H); ^{13}C NMR (CDCl_3): $\delta = 20.12$, 32.43, 59.14, 59.58, 73.67, 80.45, 119.64, 124.96, 125.21, 126.92, 131.53, 142.83, 177.95; IR (NaCl): $\tilde{\nu} = 3350$ (br), 1620.9, 1590.1, 1478.3, 1265.0, 1121.3 cm^{-1} ; MS (70 eV), m/z (%): 468.2221 ($[M^+]$, 84), 306 (48.3), 278 (95.8), 260 (9.4), 235 (24.7), 190 (20.5), 164 (55.1), 163 (13.8), 132 (26.6), 118 (100), 97 (11.6).

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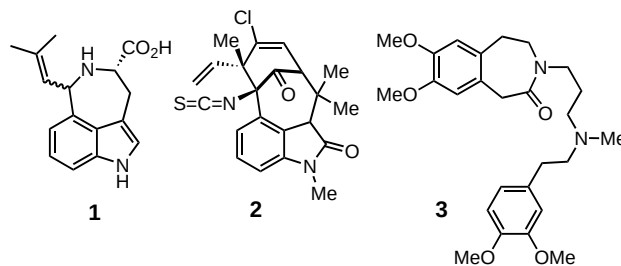
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An Expedient Construction of Seven-Membered Rings Adjoining Aromatic Systems**

Talbi Kaoudi, Béatrice Quiclet-Sire, Stéphanie Seguin, and Samir Z. Zard*

Dedicated to Professor Pierre Potier on the occasion of his 65th birthday

In contrast to the closure of five- and six-membered rings, the direct formation of a seven-membered ring by radical addition to an olefin is a relatively uncommon transformation.^[1, 2] Examples of the even more difficult corresponding cyclization onto an aromatic ring are very rare.^[3] Yet, seven-membered rings adjoining aromatic systems can be found in a variety of biologically active compounds, for example, clavicipitic acid (**1**), a metabolite related to lysergic acid,^[4] the more complex, recently isolated welwitindolinones (e.g. *N*-methylwelwitindolinone C isothiocyanate (**2**)),^[5] and the benzazepine family of drugs^[6] such as Zatebradine (**3**), an antianginal and one of a new group of specific bradycardic agents.^[6c,d]



Construction of such seven-membered rings has relied almost exclusively on ionic chemistry.^[7] Methods based on radicals have been very rarely applied, mainly because the desired cyclization is too slow to compete with other pathways open to the radical intermediate. Premature reduction, for example, is what is usually observed when organotin hydride based procedures are applied.^[1, 2, 8] Indeed, the very few examples involving stannanes are generally low yielding and concern invariably pyrrole- or imidazole-type heteroaromatic structures activated by electron-withdrawing groups.^[3a–d] Compounds with seven-membered (and even larger) rings fused to an aromatic nucleus have been obtained in modest yield by photolysis of suitable chloroamides.^[3e–j] The mechanism here is not clear; formation of exciplexes and electron

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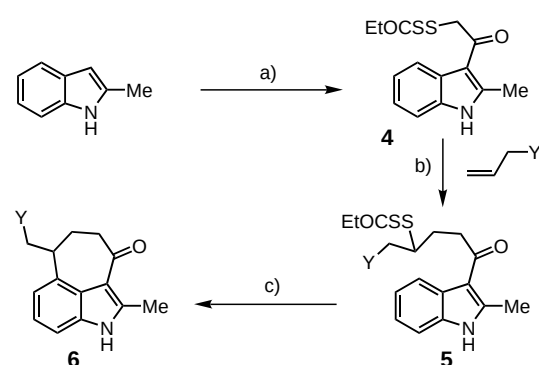
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transfers have been invoked but free radicals may also be involved.^[3f,g] The other instances of the annelation of seven-membered rings to aromatic systems all start by the generation of the radical on the aromatic ring, thereby avoiding the extra activation energy arising from the temporary destruction of aromaticity.^[2]

We have shown that radicals generated from xanthates through either a chain or nonchain process, under tin-free conditions, have a comparatively long effective lifetime and can add to unactivated olefinic traps.^[9] In particular, we could annelate five- or six-membered rings to an aromatic nucleus, providing access to a number of useful structures such as oxindoles, indolines, indanes, and tetralones.^[10] We have now found that the much more difficult formation of seven-membered rings is also feasible through this approach.

Heating a solution of the readily accessible xanthate **4** and allyl acetate (5 equiv) in 1,2-dichloroethane/chlorobenzene (1/1) at reflux in the presence of a small amount of the radical initiator lauroyl peroxide gave the expected adduct **5a** in 77% yield (Scheme 1). When a stoichiometric amount of lauroyl

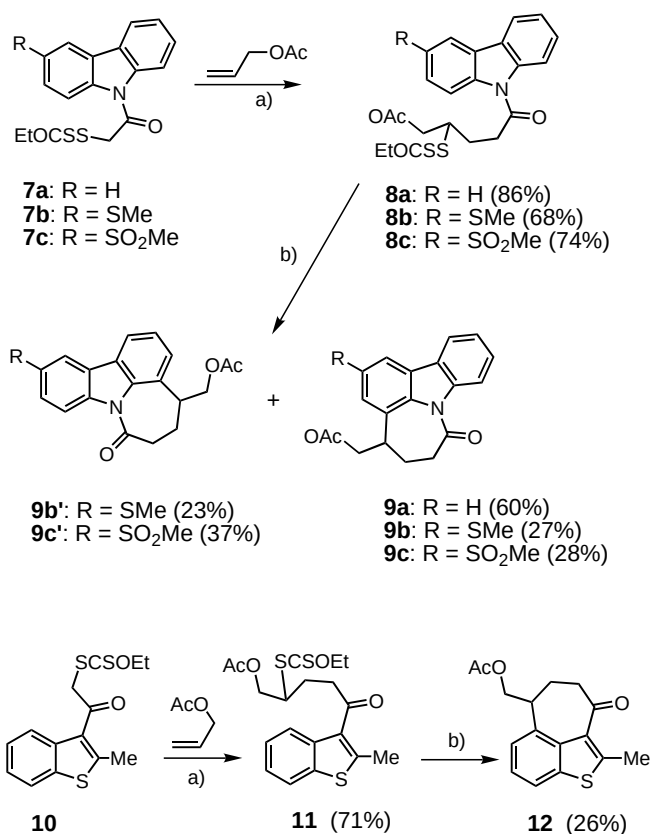


Y = OAc: **5a** (77%), **6a** (54%); Y = Phth: **5b** (88%), **6b** (47%);
Y = CN: **5c** (66%), **6c** (48%); Y = (CH₂)₈OAc: **5d** (88%), **6d** (32%);
Y = CH₂CO₂Et: **5e** (76%), **6e** (35%)

Scheme 1. Xanthate-mediated radical annelation of a seven-membered ring to 2-methylindole. a) ClCH₂COCl, AlCl₃, CH₂Cl₂; EtOCSSK, acetone; b) lauroyl peroxide (0.1–0.2 equiv), ClCH₂CH₂Cl/PhCl (1/1), reflux; c) lauroyl peroxide (1.25 equiv), chlorobenzene, reflux. Phth = phthalimido.

peroxide was added in portions (over 1–2 h) to a boiling solution of **5a** in chlorobenzene, annelation to position 4 of the indole ring took place to furnish **6a** in 54% yield along with a small amount (18%) of a reduced side product (in which the xanthate group in **5a** is replaced with a hydrogen). The same sequence was applied with other olefins to give the tricyclic derivatives **6b–e** in comparable overall yields.

The seven-membered ring may be fused at the nitrogen atom in these heteroarenes. This is illustrated by the synthesis of **9a** (60%), **9b** (27%) and **9b'** (23%), and **9c** (28%) and **9c'** (37%) from precursors **8a–c**, respectively, which were efficiently obtained by addition of the appropriate xanthates **7a–c** to allyl acetate (Scheme 2). The combined yield of the two isomeric products in the case of **9b,b'** and **9c,c'** is acceptable, but there is essentially no regioselectivity. The sulfide and sulfone groups are in a *meta* disposition with respect to site of cyclization and therefore have little influence

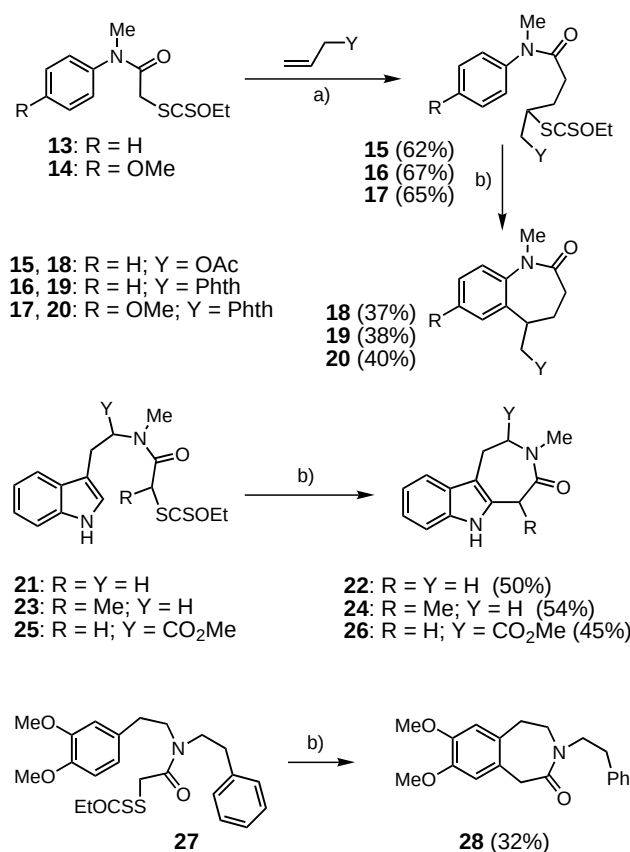


Scheme 2. Examples of xanthate-mediated annelations of seven-membered rings. a) Lauroyl peroxide (0.1–0.2 equiv), ClCH₂CH₂Cl, reflux; b) lauroyl peroxide (1.1–1.3 equiv), chlorobenzene, reflux.

on the reactivity of this site. In the last example in Scheme 2 (conversion **11** → **12**, 26%) the indole moiety was replaced by a benzothiophene ring.

Even simple anilines can be used as the starting substrates, as demonstrated by the examples collected in the top part of Scheme 3. Thus, xanthates **13** and **14** were transformed into benzazepinones **18**, **19**, and **20** with yields of 37, 38, and 40%, respectively, for the cyclization step. We had found in the past that treatment of xanthates **13** and **14** with a peroxide, in the absence of the external olefin, results in the formation of the corresponding oxindoles.^[10a] Clearly, fusion of the five-membered ring to the aromatic nucleus is slower than intermolecular addition to an unactivated olefin. The fact that even a seven-membered ring can be made to close is indeed quite remarkable.

Somewhat simpler structures were obtained by starting from xanthate precursors derived from 2-arylethylamines by standard ionic chemistry (examples **22**, **24**, **26**, and **28** in Scheme 3). Cyclization of the tryptophan derivative **25** to give **26** (45%) is especially noteworthy. The influence of the substituents on the regiochemistry is highlighted by the last transformation (**27** → **28**), in which cyclization occurs preferentially on the more electron-rich aromatic nucleus. The initial radical derived from xanthate **27** is adjacent to the carbonyl group of the amide and therefore moderately electrophilic. Compound **28** is a close analogue of the antianginal drug **3**.



Scheme 3. Further examples of xanthate-mediated annulations of seven-membered rings. a) Lauroyl peroxide (0.2 equiv), ClCH₂CH₂Cl, reflux; b) lauroyl peroxide (1.2 equiv), ClCH₂CH₂Cl, reflux.

In this preliminary account, we have described a new, flexible, and convergent route to a variety of complex aromatic structures containing an adjoining seven-membered ring. No heavy or toxic metals are involved, and the starting materials are cheap and readily available. The generally modest (but unoptimized) yields must be considered in view of the fact that both the inter- and intramolecular radical additions in this sequence are difficult transformations beyond the reach of most other radical methods.

Experimental Section

Typical procedures:

Intermolecular radical addition: A solution of the xanthate (1 mmol) and olefin (3–5 mmol) in degassed 1,2-dichloroethane (1.2 mL; in the case of xanthate **4**, a 1:1 mixture of 1,2-dichloroethane and chlorobenzene was used for solubility reasons) was heated at reflux, and lauroyl peroxide (0.1–0.2 mmol) was added in portions. The reaction was monitored by TLC. The solvent was removed under reduced pressure and the crude residue purified by chromatography on a silica gel column to furnish the desired adducts.

Cyclization reactions: Procedure A: To a refluxing solution of the xanthate adduct (1 mmol) in degassed chlorobenzene (4.4 mL) was added dropwise a chlorobenzene solution of lauroyl peroxide (1.25 mmol in 11 mL) over 1–2 h. The solvent was removed under reduced pressure and the crude residue purified by chromatography on a silica gel column eluting with ethyl acetate/heptane mixtures.

Procedure B: Solid lauroyl peroxide was added portionwise every 1.5 h (0.1 mmol portions for a total of 1.2 mmol) to a solution of the xanthate adduct (1 mmol) in degassed 1,2-dichloroethane (5 mL) heated at reflux.

The solvent was removed under reduced pressure and the crude residue purified by chromatography on a silica gel column eluting with ethyl acetate/heptane mixtures.

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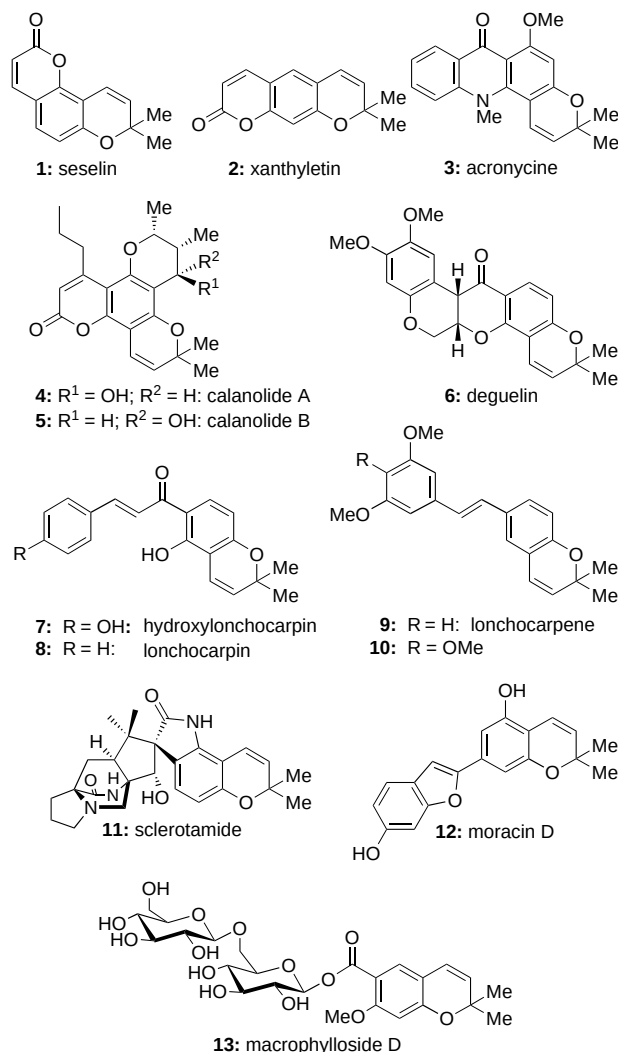
Selenium-Based Solid-Phase Synthesis of Benzopyrans I: Applications to Combinatorial Synthesis of Natural Products**

K. C. Nicolaou,* Jeffrey A. Pfefferkorn, and Guo-Qiang Cao

Combinatorial chemistry is becoming an increasingly important tool in both chemical biology and drug discovery, and its continued expansion is, to a large extent, contingent upon advances in solid-phase organic synthesis (SPOS) as well as in automation and high-throughput screening (HTS) technologies.^[1] Since the demand for libraries of small organic molecules continues to grow, particularly as a result of recent advances in the fields of chemical genetics, genomics, and proteomics, there is an urgent need to develop more efficient strategies for the high-fidelity solid-phase construction of libraries of natural-product-like and drug-like compounds.^[2] Toward this end our research group has been engaged in the development of novel resins as well as improved linking and release strategies.^[3] Recently, we disclosed^[4] the construction of a polystyrene-based selenenyl bromide resin which encompasses the essential features of both a solid-phase reagent^[5a] and a traceless linker.^[5b-k] We envisaged that such a resin should ideally act as a linker that facilitates bond constructions during the loading operation (that is, acts as a reagent) and then serve as a robust tether through subsequent operations until it is ultimately cleaved in a traceless or functionalizing fashion to effect release of the target. The versatility and efficiency of such a method is readily evident since it reduces the extraneous (noncomplexity building) operations typically associated with the loading of a scaffold and also eliminates the problem of residual functionality in the target structure resulting from the linker.^[6] In this and the following communication^[7] we describe our preliminary efforts toward the application of such a linking strategy for the construction of several polycyclic natural products and other medicinally relevant small organic molecules.

As a forum for demonstrating the utility and efficiency of the proposed seamless linking strategy, we chose to target the 2,2-dimethylbenzopyran moiety which is embedded in numerous natural products including flavanoids, coumarins, rotenoids, stilbenoids, chromene glycosides, and others—several of

which have potential applications in medicine.^[8] For example, **1** (Scheme 1),^[9] **2**,^[9] and **3**^[10] exhibit anticancer activities, whereas **4** and **5** are non-nucleoside, HIV-1-specific, reverse transcriptase inhibitors currently in clinical development.^[11] The cubé resin derived agents **6**, **8**, **9**, and related structures



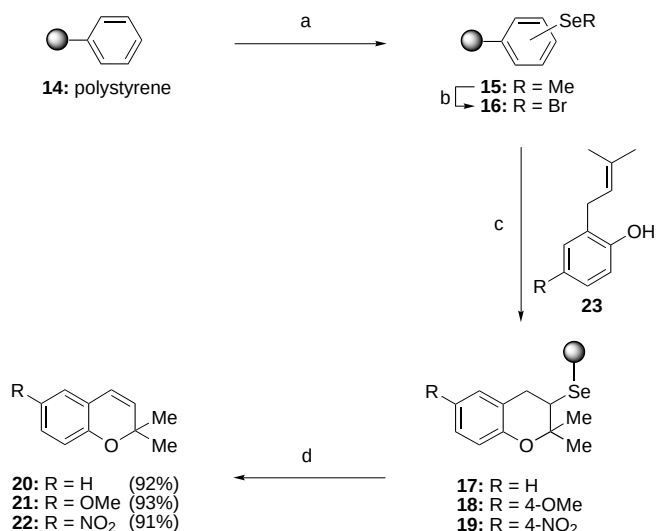
Scheme 1. Representative biologically active natural products containing the 2,2-dimethylbenzopyran moiety.

7 and **10** are under investigation as cancer chemopreventives,^[12] while **11**^[13] and **12**^[14] exhibit insecticidal and antifungal activities, respectively. Furthermore, a growing number of pharmaceutical ligands that incorporate the 2,2-dimethylbenzopyran moiety into their structures have been designed and synthesized (see following paper).^[7] Thus, the development of a practical and reliable method for the solid-phase construction of benzopyrans is of considerable interest and should open the possibility for future combinatorial-based investigations of these as well as other natural and designed molecules.

Initially, we needed to establish the effectiveness of the proposed solid-phase selenium-mediated formation of 2,2-dimethylbenzopyrans,^[15] and we, therefore, undertook a series of preliminary loading and cleavage studies (Scheme 2). As

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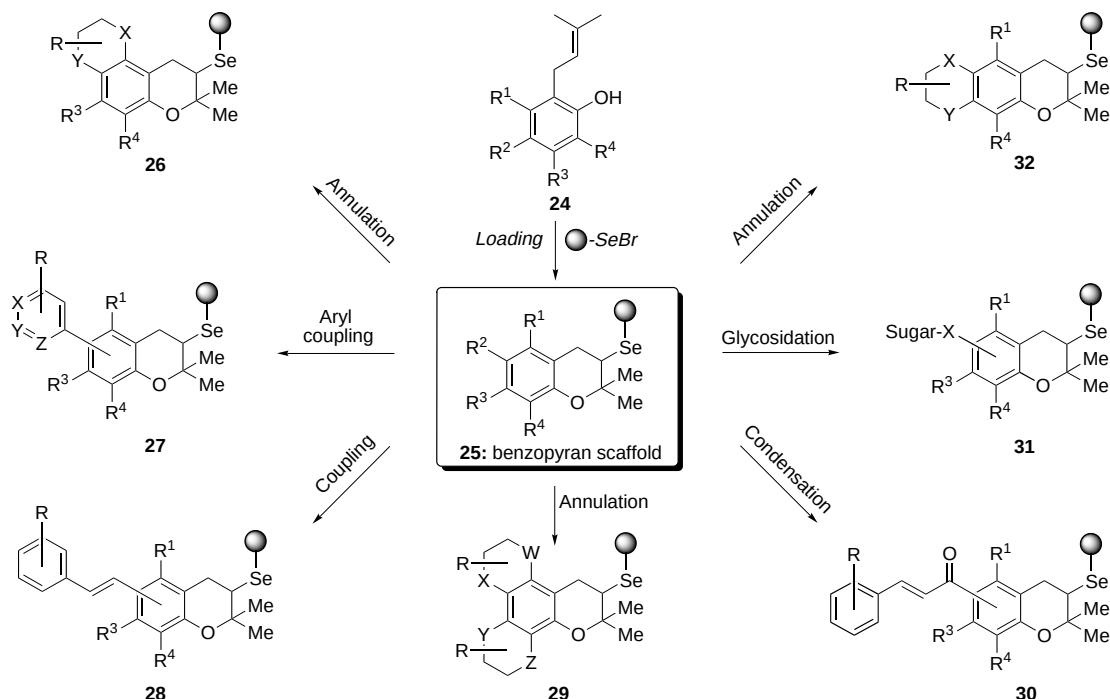


Scheme 2. Preparation of selenium-functionalized resin and preliminary studies on the loading and release of 2,2-dimethylbenzopyrans. a) 1. *n*BuLi (0.6 equiv), polystyrene (1.0 equiv, 1% cross-linked, 100–200 mesh), TMEDA (0.5 equiv), cyclohexane, 65 °C, 4 h; 2. (MeSe)₂ (2.0 mmol g^{−1} polystyrene), THF, 0 °C, 10 min; b) 1. Br₂ (0.9 equiv), CHCl₃, 0 °C, 30 min; 2. EtOH, 70 °C, 1 h; c) phenol **23** (3.0 equiv), 0–25 °C, 30 min; d) 30% H₂O₂ (10.0 equiv), THF, 25 °C, 30 min. TMEDA = *N,N,N',N'*-tetramethylethylenediamine.

previously reported,^[4] selenenyl bromide resin **16** (Scheme 2) was conveniently prepared from commercial polystyrene (**14**) by lithiation followed by treatment with dimethyl diselenide to give methyl selenide **15**, a conjugate whose subsequent oxidation with bromine gave **16** as a dark red polymer.^[4] Simply stirring resin **16** in CH₂Cl₂ at 0 °C with a threefold excess of various *ortho*-prenylated phenols (**23**) resulted in a

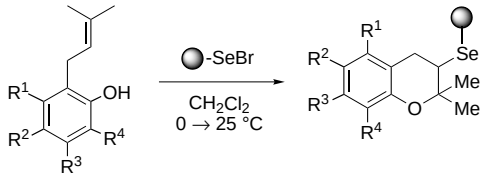
rapid decolorization of the resin (<5 min). These newly loaded resins were then treated with H₂O₂, which resulted in the facile oxidation of the selenide to the corresponding selenoxide. A *syn*-elimination of the selenoxide effected the release of benzopyrans **20–22** in high yield with purities greater than 95%. The high yield and purity of these products suggested that the simultaneous cyclization and loading (cyclo-loading) step proceeded efficiently, regardless of the electronic environment of the phenolic substrate (that is, neutral (**20**), electron rich (**21**), or electron poor (**22**)).

With the effectiveness of the cyclo-loading confirmed, we considered how such technology could be strategically employed for construction of natural products and analogues thereof. The initial loading of various substituted *ortho*-prenylated phenols (**24**) provides functionalized resin-bound benzopyrans (**25**, Scheme 3) for further elaboration towards the skeletons of various 2,2-dimethylbenzopyran-containing natural products. The success of this strategy required not only that the loading step be efficient, but also that it be mild and highly selective so as to tolerate a range of aromatic functionality (R¹–R⁴), preferably without necessitating tedious manipulations of protecting groups. Thus, a series of functionalized *ortho*-prenylated phenols were synthesized and treated with selenenyl bromide resin **16** (Table 1). Encouragingly, the method exhibited broad tolerance toward a wide range of polyfunctionalized aromatic compounds^[16] containing phenols (entries 1–6), methyl ketones (entries 10–12), esters (entries 13, 14), carboxylic acids (entry 15), cyanides (entries 16, 17), nitro groups (entries 18–20), aldehydes (entries 21–26), and halogens (entries 27–35). In all cases the treatment of the resin with a threefold excess of the *ortho*-prenylphenol resulted in nearly quantitative loading and ¹H NMR analysis of the oxidative cleavage products revealed



Scheme 3. Strategy for the construction of resin-bound 2,2-dimethylbenzopyran scaffolds (**25**) and their subsequent solid-phase elaboration to various natural and designed molecular skeletons (**26–32**).

Table 1. Loading of functionalized 2,2-dimethylbenzopyran scaffolds.^[a]



Entry	Substrate preparation ^[b]	R ¹	R ²	R ³	R ⁴	Purity [%] ^[c]
1	B	H	H	H	H	98
2	A	OH	H	H	H	98
3	A	H	OH	H	H	98
4	A	H	H	OH	H	98
5	A	OH	H	OH	H	92
6	A	H	H	OH	OH	98
7	A	H	CH ₂	H	H	98
8	A	H	OMe	OMe	H	98
9	A	OMe	H	H	H	96
10	C	H	Ac	H	H	98
11	A	H	Ac	OH	H	98
12	A	OH	Ac	H	H	98
13	C	H	CO ₂ Me	H	H	92
14	A	H	CO ₂ Me	OH	H	98
15	C	H	CO ₂ H	H	H	96
16	C	H	CN	H	H	98
17	C	H	H	H	CN	98
18	C	H	NO ₂	H	H	98
19	C	H	H	NO ₂	H	94
20	C	H	H	H	NO ₂	96
21	C	H	H	H	CHO	98
22	C	H	CHO	H	H	98
23	A	OH	CHO	H	H	98
24	A	H	CHO	OH	H	88
25	A	OH	CHO	Me	H	98
26	A	Me	CHO	OH	H	98
27	B	Br	H	H	H	98
28	B	H	Br	H	H	98
29	B	H	H	Br	H	98
30	B	H	H	H	Br	91
31	A	H	Br	OH	H	92
32	B	I	H	H	H	94
33	B	H	I	H	H	98
34	B	H	H	I	H	98
35	B	H	H	H	I	98

[a] Loading conditions: selenium bromide resin (1.0 equiv, ca. 1.1 mmol g⁻¹), phenol (3.0 equiv), CH₂Cl₂, 0 → 25 °C, 30 min. [b] Phenolic substrates were prepared in one of three ways: A) Friedel-Crafts alkylation, B) anionic alkylation, or C) aromatic Claisen rearrangement. See reference [16] for representative procedures. [c] Purity was estimated by integration of ¹H NMR signals of crude oxidative cleavage product. Substrate loading was 89–100% in all cases as determined by mass difference of vacuum dried resin. In selected cases the cleavage product was chromatographically purified and the yield after two steps (loading plus cleavage) was determined. These yields ranged from 80–97% (based on an estimated resin functionalization of 1.1 mmol g⁻¹).

the newly constructed benzopyrans to be greater than 95% pure in most cases.

With this collection of functionalized, resin-bound benzopyran platforms in hand, we set out to demonstrate their utility for elaboration toward natural products and combinatorial libraries while concurrently testing the effectiveness of the selenium resin as a robust linker. The first example (Table 2) focused on the construction of several chalcone natural products that are currently being investigated as

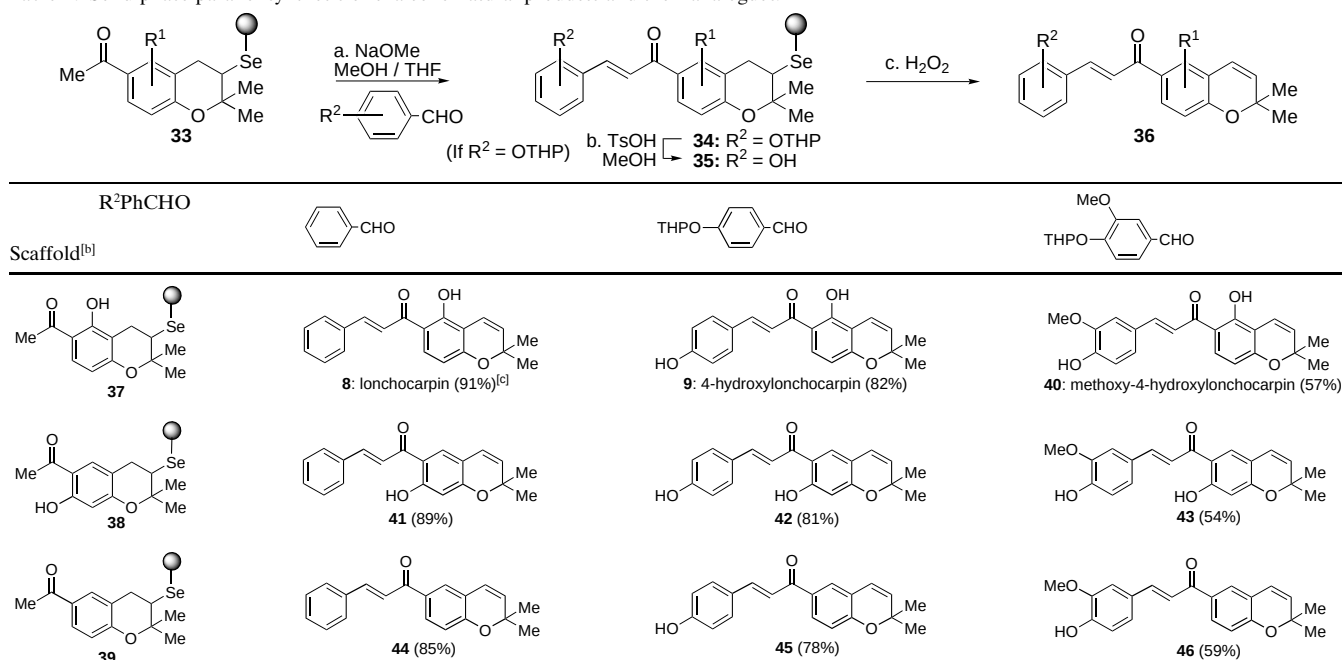
potential chemopreventive agents.^[12] The general synthetic procedure (Table 2) involved the condensation of resin-bound benzopyran methyl ketones (**33**) with substituted aldehydes in the presence of NaOMe.^[17] After condensation and in situ elimination to the chalcone framework (**34**), the hydroxyl substituents (R²) were liberated with TsOH · H₂O (for abbreviations of reagents and protecting groups see legends of schemes) and finally the substrates were subjected to oxidative cleavage with H₂O₂. The condensation of pyran scaffold **37** with benzaldehyde, 4-(THPO)-benzaldehyde, or 3-methoxy-4-(THPO)-benzaldehyde followed by deprotection and cleavage led to the natural products **8**, **9**, and **40** in 91, 82, and 57% overall yields, respectively, based on **37**. Similar sequences with scaffolds **38** and **39** resulted in the construction of analogues **41–46** in yields ranging from 54–89%.

A second example of this method is outlined in Table 3 for the radiofrequency encoded split-and-pool synthesis^[18] of the pyranocoumarin anticancer agents^[9] **1**, **2**, and analogues thereof using IRORI microreactor technology.^[19] In a typical experiment^[20] resin-bound benzopyrans possessing an *ortho*-hydroxy aldehyde moiety (**47**) were treated with either a stabilized phosphonium ylide or a β -ketoester to effect olefination through either a Wittig^[21a] or a Knoevenagel^[21b] reaction, respectively, to provide structures of type **48**. These olefins (presumably isomeric mixtures) were thermally equilibrated in situ to the *Z* isomer, which is situated for lactonization with the adjacent phenolic hydroxyl group to give structures of type **49**, oxidative cleavage of which provided pyranocoumarins of type **50**. The natural products **1** and **2** were obtained in yields of 85 and 92%, respectively when scaffolds **51** and **53** were condensed with (carboxymethoxymethylene)triphenylphosphane in *N,N*-diethylaniline at 165 °C for 3 h^[21a] and subsequently cleaved (Table 3). As in the previous example, the parallel treatment of scaffolds **51–54** with (carboxymethoxymethylene)triphenylphosphane, methyl acetoacetate, phenyl acetoacetate, and diethyl malonate under the conditions illustrated in Table 2, followed by oxidative cleavage, led to the production of a small library of angular and linear pyranocoumarins (**55–68**) in yields ranging from 82 to 97%.

In order to further define the versatility of this selenium-linking strategy we undertook the solid-phase synthesis of two additional targets: the prototypical stilbene **72** (Scheme 4) and the hepta-acetate of the natural product macropylloside D (**13**, Scheme 1)^[22] as these are representative examples of the stilbenoid and chromene glycoside classes of benzopyran-containing natural products. Stilbene **72**, a useful model for the natural product **9** (Scheme 1) and the related stilbene **10** (Scheme 1), was constructed from benzopyran **69** (Scheme 4) by a sequence involving a Takai iodoolefination^[23] to afford vinyl iodide **70** (87%)^[24] and subsequent coupling with *n*Bu₃SnPh in the presence of catalytic amounts of [PdCl₂(PPh₃)₂]^[25] to furnish **71** in 65% yield. Resin-bound stilbene **71** was then cleaved under oxidative conditions to give **72** in 42% overall yield from **69**.

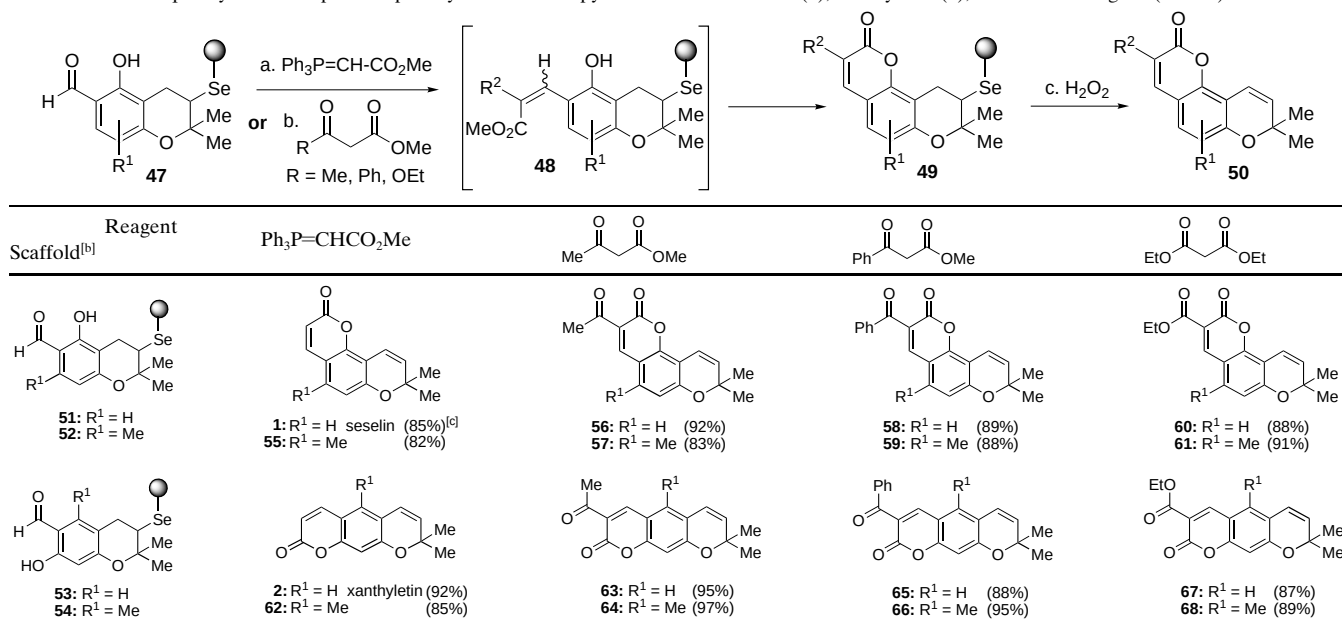
Efforts toward the second target, macropylloside hepta-acetate (**81**, Scheme 5), a derivative of a chromene glycoside isolated from *Gentiana macrophylla*,^[22] commenced from benzopyran **73**. Hence, the phenolic hydroxyl group of **73** was

Table 2. Solid-phase parallel synthesis of chalcone natural products and their analogues.^[a]



[a] Reagents and conditions: a) NaOMe (15.0 equiv), R²C₆H₄CHO (10.0 equiv), THF:MeOH (2:1), 25 °C, 72 h, 60–95 %; b) TsOH · H₂O (1.0 equiv), THF:MeOH (10:1), 25 °C, 2 h, 100 %; c) H₂O₂ (10.0 equiv), THF, 25 °C, 30 min, 54–91 % overall yields based on **37–39**. [b] See Table 1 for preparation of scaffolds. [c] Yields are of chromatographically pure compounds. All compounds were characterized by ¹H NMR spectroscopy and high resolution mass spectrometry (HR-MS). THP = tetrahydropyranyl, Ts = toluene-4-sulfonyl.

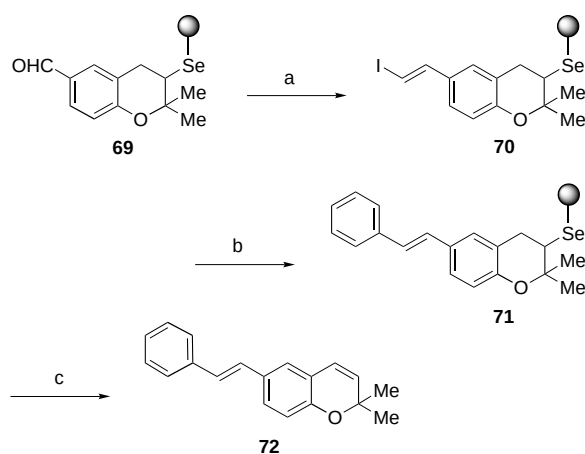
Table 3. Radiofrequency encoded split-and-pool synthesis of the pyranocoumarins seselin (**1**), xanthyletin (**2**), and their analogues (**55–68**).^[a]



[a] Reagents and conditions: a) Ph₃P=CHCO₂Me (5.0 equiv), PhNEt₂, 165 °C, 3 h, 85–95 %; b) β-ketoester (10.0 equiv), piperidine (0.2 equiv), propionitrile, 95 °C, 2 h, 85–100 %; c) H₂O₂, THF, 25 °C, 30 min, 82–95 % overall yield based on **51–54**. [b] See Table 1 for preparation of scaffolds. [c] Yields are of chromatographically pure compounds. All compounds were characterized by ¹H NMR spectroscopy and HR-MS.

first methylated (MeI/NaH, 88 %) ^[24] and the methyl ester of the resulting product **74** then hydrolyzed to reveal the requisite free carboxylic acid **75** ready for the first coupling reaction. In the event, treatment of **75** with trichloroacetimidate **76** ^[26] and BF₃ · Et₂O for 12 h at –40 → 0 °C resulted in

smooth coupling to produce β-glycoside **77** (91 %), which underwent selective deprotection upon treatment with HF · py to afford **78** in 89 % yield. The second coupling between resin-bound substrate **78** and trichloroacetimidate **79** ^[27] proceeded under similar conditions to afford **80** as a



Scheme 4. Solid-phase synthesis of the prototypical stilbene **72**: a) CrCl_2 (16.0 equiv), CHI_3 (5.0 equiv), THF:dioxane (10:1), 0°C , 3 h, 87%; b) $n\text{Bu}_3\text{SnPh}$ (5.0 equiv), $[\text{PdCl}_2(\text{Ph}_3\text{P})_2]$ (0.1 equiv), DMF, 100°C , 10 h, 65%; c) 30% H_2O_2 (0.1 equiv), THF, 25°C , 30 min, 41% over three steps based on **69**.

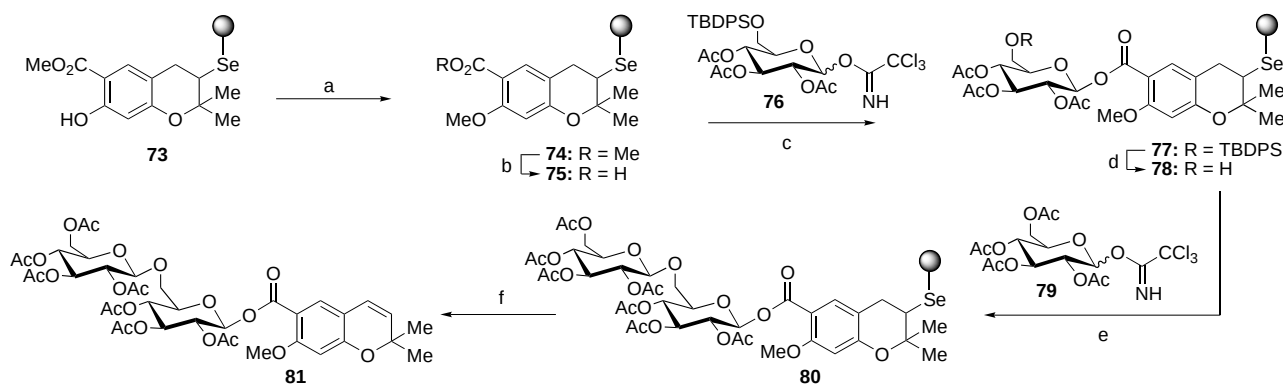
single anomer in 57% yield. Finally, the disaccharide (**80**) was cleaved from the resin to afford hepta-acetylated macrophyllloside (**81**) in 18% yield over six steps based on **73**.

In conclusion, we have described a novel method for the solid-phase construction of 2,2-dimethylbenzopyrans employing a selenium-based linking strategy wherein the loading step constitutes a key ring-forming reaction. Once formed, these resin-bound benzopyrans can be elaborated to a variety of natural products and analogues thereof. Finally, cleavage from the resin is accompanied by introduction of additional functionality in high purity and efficiency. In the following communication^[7] we describe further applications of this strategy, particularly to the construction of various heteroatom-containing small organic molecules of pharmaceutical interest.

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Scheme 5. Solid-phase synthesis of peracetyl macrophyllloside D (**81**): a) NaH (10.0 equiv), MeI (20.0 equiv), DMF, 35°C , 48 h, 88%; b) LiOH (10.0 equiv), THF: H_2O (20:1), 60°C , 12 h, 94%; c) **76** (5.0 equiv), $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (4.0 equiv), 4 Å MS, CH_2Cl_2 , $-40 \rightarrow -0^\circ\text{C}$, 12 h, 91%; d) HF·py (5.0 equiv), THF, $0 \rightarrow 25^\circ\text{C}$, 89%; e) **79** (5.0 equiv), $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (4.0 equiv), 4 Å MS, CH_2Cl_2 , $-40 \rightarrow -10^\circ\text{C}$, 12 h, 57%; f) 30% H_2O_2 (10.0 equiv), THF, 25°C , 30 min, 18% over six steps based on **73**. TBDPS = *tert*-butyldiphenylsilyl.

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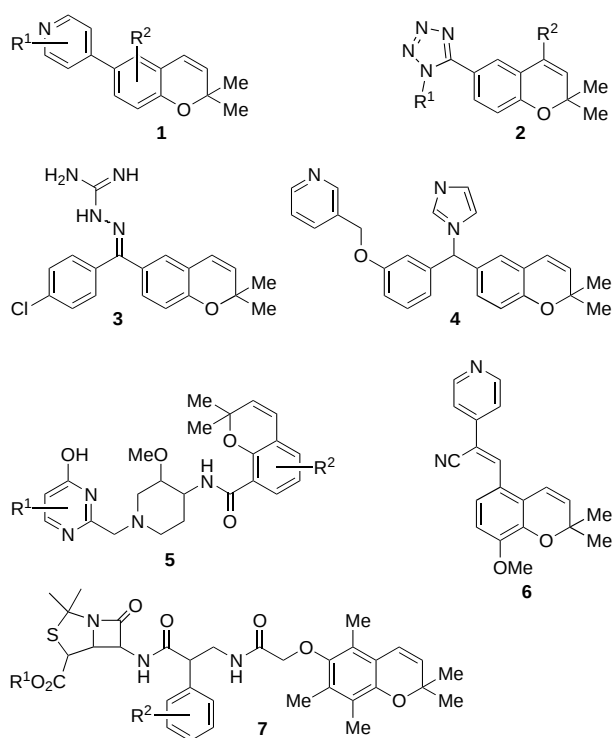
Selenium-Based Solid-Phase Synthesis of Benzopyrans II: Applications to Combinatorial Synthesis of Medicinally Relevant Small Organic Molecules**

K. C. Nicolaou,* Guo-Qiang Cao, and Jeffrey A. Pfefferkorn

In the preceding communication we described a novel selenium-based simultaneous cyclization and loading (cyclo-loading) strategy for the solid-phase combinatorial synthesis of natural products containing the 2,2-dimethylbenzopyran moiety.^[1] Given the vast number and diverse biological activities of these benzopyran natural products, it is reasonable to consider whether this structural motif might be of value in the construction of designed pharmaceutical agents, particularly since natural-product-based mechanistic investigations have revealed that such structures interact with a variety of protein and nucleic acid cellular targets.^[2] Not surprisingly, a search of the patent literature revealed a host of pharmaceutical ligands that contain the 2,2-dimethylbenzopyran skeleton (Scheme 1). Among these are the potassium-channel activators **1** and **2**,^[3] aldosterone biosynthesis inhibitors **3** and **4**,^[4] 5-hydroxytryptamine-3 receptor antagonist **5**,^[5] phosphodiesterase IV inhibitor **6**,^[6] and the ampicillin-derived antibacterial agent **7**.^[7] However, in spite of the potential utility of this substituted benzopyran motif in ligand design, only a limited number of solid-phase methods for its construction has been reported.^[8] Hence we investigated whether our current cyclo-loading approach might be a useful tool in medicinal chemistry for future combinatorial investigations of this class of compounds. Since structures **1**–**7**, unlike the natural products previously described, are structurally quite diverse and possess a variety of heteroatom functionalities, we sought to effect the solid-phase functionalization of several of the previously described resin-bound benzopyrans^[1] with various heteroatom-based functional groups in order to produce scaffolds embodying their structural features. Herein we describe the synthesis of several representative scaffolds as well as a solid-phase synthesis of androsterone biosynthesis inhibitor **4** (Scheme 1) and a small library of analogues using radiofrequency

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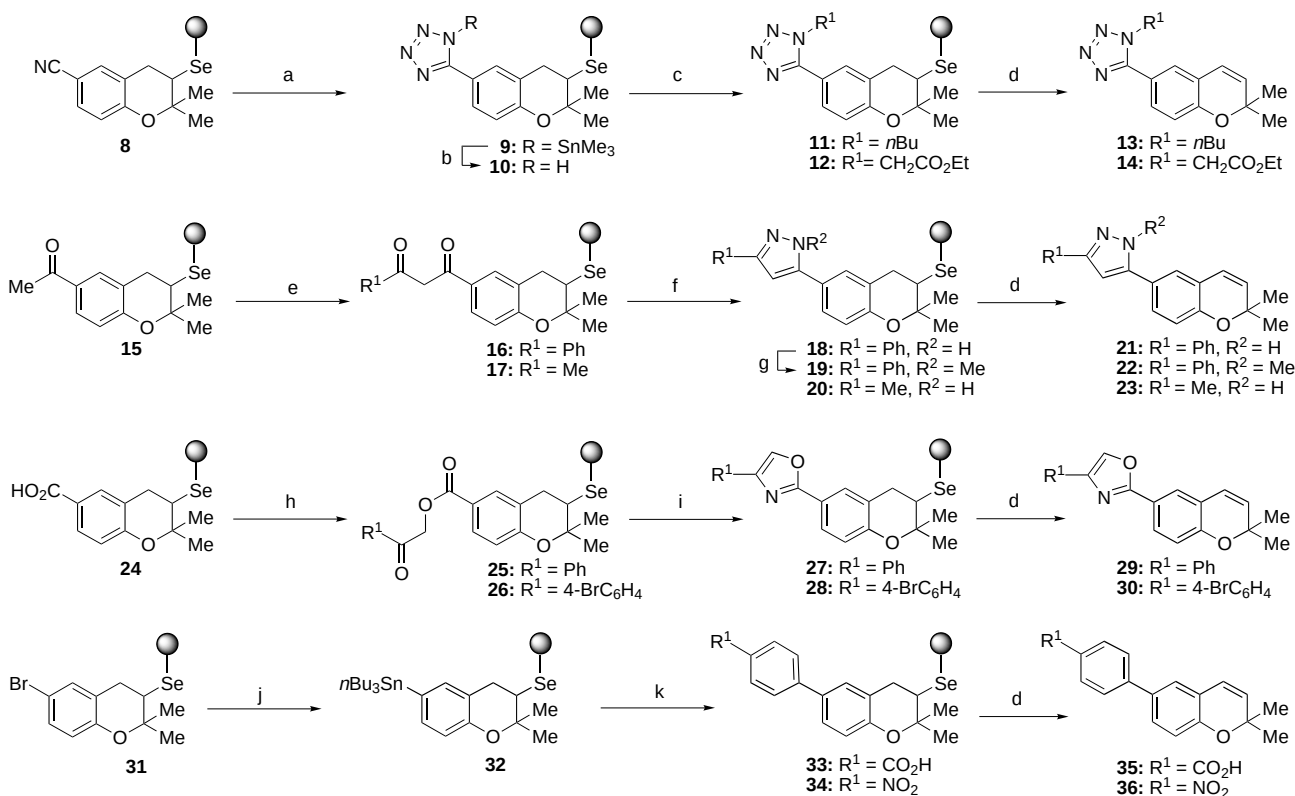


Scheme 1. Representative examples of potential pharmaceutical candidates incorporating the 2,2-dimethylbenzopyran moiety.

directed split-and-pool technology^[9] as an illustration of the wide applicability and efficiency of this strategy.

The initial focus of these efforts was on the construction of several aromatic and heteroaromatic substituted benzopyrans resembling the core structures of the potassium-channel activators **1** and **2** (Scheme 1).^[3] Four prototypes (**13**, **21**, **29**, and **35**, Scheme 2) were constructed, each synthesis commencing from a different resin-bound benzopyran platform (**8**, **15**, **24**, and **31**, respectively). Thus, tetrazoles **13** and **14** were constructed from cyanide **8** by treatment with azido-trimethylstannane at 100 °C to give stannylated tetrazole **9**, which was hydrolyzed with TFA (for abbreviations of reagents and protecting groups see legends in schemes) to provide **10** in quantitative yield^[10] over two steps.^[11] Derivatization of tetrazole **10** was accomplished through alkylation by treatment with an appropriate alkyl halide and Et₃N at 80 °C to give **11** and **12** in 85 and 87 % yields, respectively.^[12] These tetrazoles were then subjected to standard oxidative release conditions (H₂O₂)^[1] to afford **13** and **14** in 52 and 65 % yields, respectively, based on **8**.

In the second example, acetophenone **15** was converted into pyrazoles **21**–**23** (Scheme 2) by initial conversion of the methyl ketone functionality into the corresponding β -dicarbonyl system through formation of an enolate with LHMDS followed by quenching with an appropriate acyl cyanide to afford **16** and **17** in 87 and 75 % yields, respectively.



Scheme 2. Solid-phase synthesis of heterocyclic and aryl-substituted 2,2-dimethylbenzopyrans: a) azidotrimethyltin (5.0 equiv), toluene, 110 °C, 12 h, 100%; b) TFA (2.0 equiv), THF:H₂O (10:1), 25 °C, 2 h, 100%; c) R¹Br (10.0 equiv), Et₃N (20.0 equiv), MeCN, 80 °C, 12 h, 85–87%; d) H₂O₂ (10.0 equiv), THF, 25 °C, 30 min, **13** (52%), **14** (65%), **21** (65%), **22** (58%), **23** (49%), **29** (42%), **30** (46%), **35** (62%), **36** (75%); e) LHMDS (3.0 equiv), R¹C(O)CN, THF, –78 °C, 1 h, 88–75%; f) hydrazine hydrate (10.0 equiv), AcOH (0.01 equiv), THF:MeOH (2:1), 65 °C, 3 h, 87–65%; g) MeI (10.0 equiv), NaH (5.0 equiv), THF, 40 °C, 12 h, 95%; h) R¹C(O)CH₂Br (10.0 equiv), DBU (20.0 equiv), DMF, 80 °C, 12 h, 100%; i) acetamide (10.0 equiv), BF₃·Et₂O (1.0 equiv), xylenes, 140 °C, 48 h, 50–55%; j) *n*BuLi (10.0 equiv), THF, –78 °C, 2 h; then, *n*Bu₃SnCl (15.0 equiv), THF, –78 → 0 °C, 1 h, ca. 100%; k) [Pd₂dba₃P(2-furyl)₃] (0.05 equiv), 4-R¹C₆H₄I (5.0 equiv), DMF, 100 °C, 12 h, 85–90%. TFA = trifluoroacetic acid, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

Condensation of **16** and **17** with hydrazine hydrate and catalytic amounts of acetic acid gave resin-bound pyrazoles **18** and **20** in 87 and 65 % yields, respectively.^[13] These pyrazoles could also be further derivatized through alkylation as demonstrated by the conversion of **18** → **19** upon treatment with MeI/NaH in DMF at 60 °C. Finally, the constructed heterocycles were cleaved from the resin under oxidative conditions to afford **21**–**23** in 65, 58, and 49% overall yields, respectively, based on **15**.^[14]

The third example involved conversion of benzoic acid **24** into oxazoles **29** and **30** (Scheme 2). Hence, alkylation of **24** was effected by treatment with several α -bromo ketones and DBU in DMF at 80 °C to afford **25** and **26** in quantitative yields. Heterocycle formation was accomplished by treatment of **25** and **26** with acetamide and BF₃·Et₂O in xylenes at 140 °C^[15] to give **27** and **28** (50 and 55 % yields, respectively), which were then cleaved under standard oxidative conditions to afford oxazoles **29** and **30** in 42 and 46 % yields over three steps based on **24**.

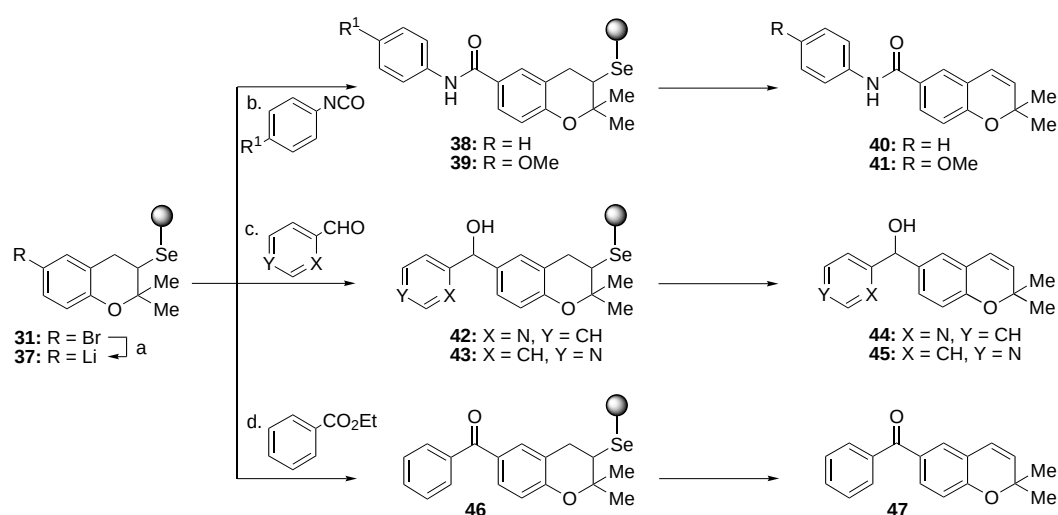
In the fourth example bromobenzopyran **31** was converted into the corresponding tri-*n*-butylstannane **32** through a halogen–metal exchange with *n*BuLi at –78 °C followed by quenching with *n*Bu₃SnCl (Scheme 2).^[16] Tri-*n*-butylstannane **32** was then treated with several aryl iodides under palladium(0)-catalyzed coupling conditions^[17] to afford structures **33** (90 %) and **34** (85 %), which were subsequently cleaved to give **35** and **36** in excellent yields and purities. Interestingly, while these electron-deficient aryl iodides coupled quite effectively, attempts to employ either unsubstituted or electron-rich aryl iodides resulted, so far, in only low yields of the desired coupling products.

The facility of the latter halogen–metal exchange prompted us to consider additional reaction manifolds of the resin-bound lithiobenzopyran (derived from **31**) for the construction of other potentially useful substituted benzopyran structures, particularly amides, alcohols, and benzophenones (for example, **40**, **44**, and **47**, Scheme 3).^[16] Bromobenzopyran

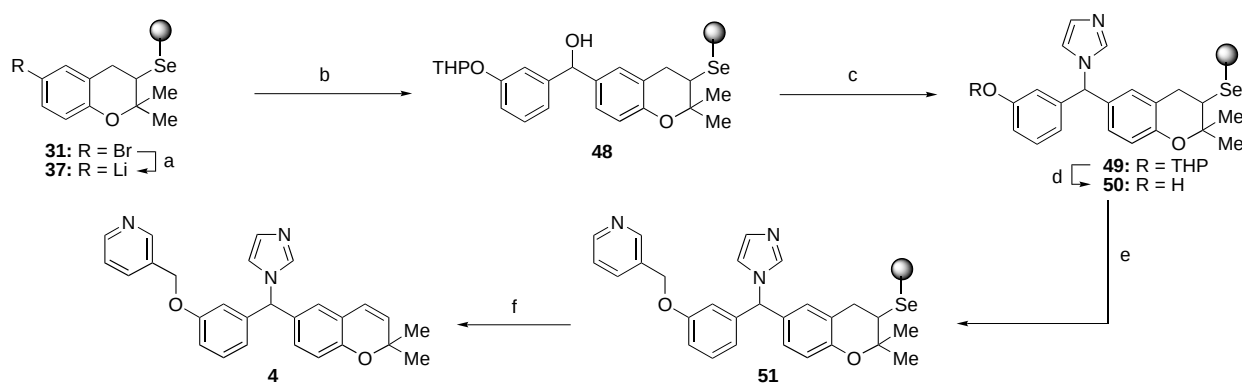
31 was once again converted into the corresponding lithium anion and then treated with a series of electrophiles. For example, the quenching of **37** with aryl isocyanates led to amides **38** and **39** in yields greater than 95 %. The addition of aromatic aldehydes followed by warming to 25 °C provided access to benzylic alcohols **42** and **43** in 90 and 88 % yields, respectively, whereas quenching with ethyl benzoate allowed direct access to benzophenones such as **46** in 82 % yield.

As a final illustration of how the current benzopyran cycloaddition-loading strategy could be useful in expediting drug discovery efforts, we set out to demonstrate a solid-phase synthesis of aldosterone biosynthesis inhibitor **4**^[4] along with a small library. The initial synthesis of **4** commenced with the described halogen–metal exchange of **31** (Scheme 4) to give **37**, which was then treated with 3-(THPO)-benzaldehyde and allowed to warm from –78 to 25 °C over 12 h to afford **48** in 99 % yield over two steps. The benzylic alcohol of **48** was then displaced with imidazole employing standard Mitsunobu conditions to afford tetracycle **49** in 85 % yield. After removal of the THP protecting group, phenol **50** was treated with 3-pyridylmethanol under Mitsunobu conditions to afford **51** (95 % yield) from which the targeted compound **4** was released by treatment with H₂O₂ (75 % yield over six steps based on **31**). Application of this route using IRORI Micro-Kans and radiofrequency encoded split-and-pool technology^[9] allowed for the construction of a small library of related compounds (**57**–**64**, Scheme 5).^[18] In general, the lithio species (**37**, Scheme 5) was treated with aromatic aldehydes to furnish alcohols **52**, which underwent Mitsunobu reaction with a variety of amines (R³NH) to give structures **53**. Substrates containing a THP-protected phenol were then deprotected and the phenolic hydroxyl groups were reacted with benzyl alcohols (R⁵OH) under Mitsunobu conditions to give structures **55**, which were subsequently oxidatively cleaved to provide benzopyrans **57**–**64**.

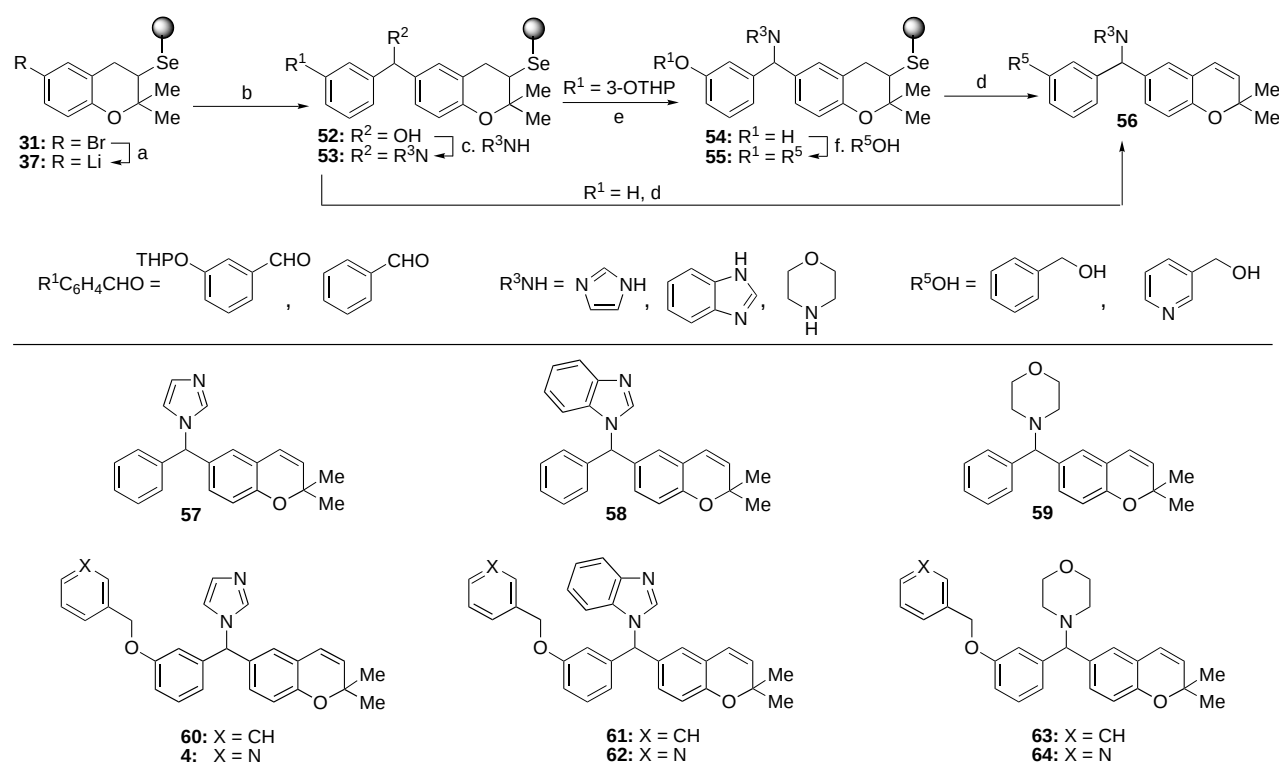
The chemistry described in this and the preceding communication^[1] demonstrate the versatility and applicability of a



Scheme 3. Solid-phase synthesis of substituted 2,2-dimethylbenzopyrans: a) *n*BuLi (10.0 equiv, 1.6 M in hexane), THF, –78 °C, 2 h; b) phenyl isocyanate (15.0 equiv), –78 → 25 °C, 30 min, 90–95 % over two steps; c) aryl aldehyde (15.0 equiv), –78 → 25 °C, 12 h, 88–90 % over two steps; d) ethyl benzoate (15.0 equiv), –78 → 25 °C, 30 min, 85 % over two steps; e) H₂O₂ (10.0 equiv), THF, 25 °C, 30 min.



Scheme 4. Solid-phase synthesis of the aldosterone biosynthesis inhibitor **4**: a) *n*BuLi (10.0 equiv, 1.6 M in hexane), THF, -78°C , 2 h; b) 3-(THPO)- $\text{C}_6\text{H}_4\text{-CHO}$ (20.0 equiv), THF, $-78 \rightarrow 25^{\circ}\text{C}$, 12 h, 99% over two steps; c) Ph_3P (10.0 equiv), DEAD (10.0 equiv), imidazole (20.0 equiv), CH_2Cl_2 , $0 \rightarrow 25^{\circ}\text{C}$, 48 h, 85%; d) $\text{TsOH} \cdot \text{H}_2\text{O}$ (1.0 equiv), THF:MeOH (10:1), 25°C , 2 h, 100%; e) Ph_3P (20.0 equiv), DEAD (20.0 equiv), 3-pyridylmethanol (20.0 equiv), CH_2Cl_2 , 25°C , 20 h, 95%; f) H_2O_2 (10.0 equiv), THF, 25°C , 15 min, 75% over six steps based on **31**. DEAD = diethyl azodicarboxylate, $\text{TsOH} \cdot \text{H}_2\text{O} = p$ -toluenesulfonic acid monohydrate.



Scheme 5. Solid-phase radiofrequency encoded split-and-pool synthesis of structural analogues (**58–65**) of aldosterone biosynthesis inhibitor **4**. Reagents and conditions: a) *n*BuLi (10.0 equiv, 1.6 M in hexane), THF, -78°C , 2 h; b) $\text{R}^1\text{C}_6\text{H}_4\text{CHO}$ (10.0–20.0 equiv), THF, $-78 \rightarrow 25^{\circ}\text{C}$, 12 h, 80–90% over two steps; c) Ph_3P (10.0 equiv), DEAD (10.0 equiv), R^3NH (20.0 equiv), CH_2Cl_2 , $0 \rightarrow 25^{\circ}\text{C}$, 48 h, 75–85%; d) H_2O_2 (10.0 equiv), THF:MeOH (10:1), 25°C , 2 h, (ca. 100%); e) $\text{TsOH} \cdot \text{H}_2\text{O}$ (1.0 equiv), THF:MeOH (10:1), 25°C , 2 h, (ca. 100%); f) Ph_3P (20.0 equiv), DEAD (20.0 equiv), R^5OH (20.0 equiv), CH_2Cl_2 , 25°C , 20 h, 90–95%.

new solid-phase, seamless linking strategy for the rapid construction of complex structures. The reported selenium-based method makes use of both the loading and cleavage steps to impart complexity and functionality into the target molecules and can be used to produce both natural products and designed molecules efficiently and in a specific or combinatorial fashion. The practical nature of the steps involved, the fact that no selenium compounds are released into the solution phase, and the high efficiency and purity in which the final products are obtained should make the

reported strategy a favorite method for combinatorial synthesis to be applied in chemical biology studies and the drug discovery process.

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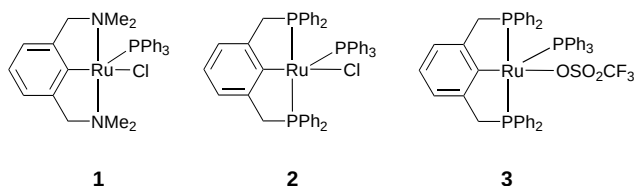
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Hydrogen-Transfer Catalysis with Pincer-Aryl Ruthenium(II) Complexes**

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Complexes of pincerlike aryl ligands such as **1–3** belong to a new class of homogeneous catalytic systems that contain a stable metal–carbon σ bond.^[1, 2a] Such organometallic complexes have already been used in a number of metal-mediated organic transformations, for example, dehydrogenation,^[2a] asymmetric allylic alkylation,^[2b] asymmetric aldol condensation,^[2c,d] Heck reactions,^[1b] atom-transfer radical polymerization (ATRP), and Kharasch addition (ATRA).^[2e,f] Additionally, the terdentate pincer ligand stabilizes complexes with unusual geometries or oxidation states.^[2f–i]



Reduction by means of hydrogen-transfer reactions has recently attracted much attention because of its practical simplicity and potential use at ambient pressure.^[3] Furthermore, the use of an alternative source of hydrogen may result in different reactivity patterns.^[4] Coordination complexes containing bidentate ligands are normally used as catalysts in hydrogen-transfer reactions.^[3] Herein we report that the pincer-type arylruthenium(II) complexes **1–3**,^[5] containing the monoanionic, terdentate bis(amino)aryl $[\text{C}_6\text{H}_3(\text{CH}_2\text{-NMe}_2)_2\text{-2,6}]^-$ (NCN) and bis(phosphanyl)aryl $[\text{C}_6\text{H}_3(\text{CH}_2\text{-PPh}_2)_2\text{-2,6}]^-$ (PCP) ligands, are highly active catalysts for the reduction of various ketones to the corresponding alcohols with *i*PrOH as the hydrogen source and KOH as the promoter [Eq. (1)]. Representative types of ketones were chosen to evaluate the performance of **1–3** in such processes. Dialkyl (aliphatic and cyclic), alkyl aryl, and diaryl ketones were all

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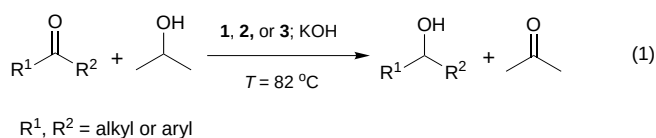


Table 1. Reduction of ketones by **1–3** with *i*PrOH/KOH as the reducing agent.^[a]

Entry	Substrate	Product	Complex	Conversion [%] ^[b] (<i>t</i> [h])	TOF [h ^{−1}] ^[c]
1			1	> 98 (3.3)	1100
2			2 ^[d]	> 98 (1.8)	10000
3			2 ^[e]	> 98 (2.5)	900
4			3 ^[d]	> 98 (1.3)	27000
5			1 ^[f]	70 (44.0)	36
6			3	90 (0.5)	9000
7			1	90 (90.0)	83
8			3	> 98 (108.0)	100
9			1	90 (4.5)	1000
10			3	90 (1.5)	2000

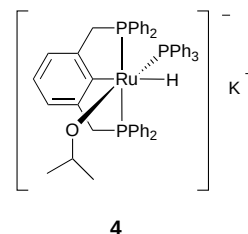
[a] The reactions were carried out on a 200-mmol scale in 200 mL of *i*PrOH under N₂ with [KOH]/[Ru] = 20. Unless otherwise noted, [Ru] = 0.1 mol % and *T* = 82 °C. [b] The conversion is equal to the yield of the product and was determined by gas chromatography. [c] Turnover frequency (moles of ketone converted to alcohol per mole of catalyst per hour) at 50 % conversion. [d] [Ru] = 0.01 mol %. [e] Reaction performed at 25 °C, [Ru] = 0.25 mol %. [f] Deactivation of the catalyst occurs.

reduced in a good to high yield by **1–3** (Table 1). The turnover frequencies (TOF) at 50 % conversion were higher with the [Ru(PCP)] complexes than with [Ru(NCN)].

In the case of cyclohexanone, more than 98 % conversion and a TOF of 1100 h^{−1} were attained by using **1** (0.1 mol %) without removal of the acetone formed in the reaction (entry 1). The use of only 0.01 mol % of **2** or **3** under the same conditions resulted in higher TOFs of up to 10000 h^{−1} and 27000 h^{−1} (entries 2 and 4, respectively). These values are superior to those obtained with Ru^{II} complexes that contain only monodentate phosphane ligands, such as [RuCl₂(PPh₃)₃],^[6] [RuCl(H)(PPh₃)₃], and [Ru(H)₂(PPh₃)₄].^[7, 8]

The experiments with **1–3** show a number of common features. Pretreatment of **1–3** with *i*PrOH containing KOH was essential for high catalytic activity. At a KOH/Ru molar ratio of 20/1, only traces of aldol products were formed.^[9] Their formation increased at higher KOH concentration, as did the reaction rate. When a solution of the complexes in *i*PrOH/KOH was exposed to air before the addition of the ketone substrate, no reaction occurred, and paramagnetic Ru^{III} complexes were formed. The rate of transfer-hydrogenation increased with increasing temperature, and the highest TOFs were obtained under reflux conditions (82 °C). However, **2** was able to reduce cyclohexanone at a reasonable rate even at room temperature (entry 3). In situ monitoring of **3** by ³¹P NMR spectroscopy under catalytic conditions showed the absence of free PPh₃ or C₆H₄(CH₂PPh₂)₂ – **1,3**.

Neutral ruthenium(II) hydrido complexes have been claimed as the actual catalytic species in reductions mediated by low-valent Ru complexes.^[10, 11] We have observed that when **3** is heated to reflux in *i*PrOH/KOH in the absence of a ketone substrate, a ruthenium hydrido species is formed in solution; it was assigned the ionic structure **4** on the basis of NMR spectroscopic data. Anionic ruthenium hydrido complexes have been used as catalyst precursors in the reduction of ketones by H₂,^[12] and their participation in hydrogen abstraction from alcohols has been proposed.^[13] However, to the best of our knowledge, this is the first time that such a species was detected in the course of a hydrogen-transfer process in *i*PrOH.^[14]



These observations indicate that: 1) the structural unit [Ru(PCP)PPh₃] is retained in the course of the catalytic cycle, 2) a ruthenium hydride is most probably the true catalyst in this process, and 3) the ruthenium hydrido complex **4** represents a resting state of the catalyst before the addition of the substrate. We regard this species as the reservoir that delivers the actual catalyst once the substrate is added. Although this ruthenium hydrido complex does not correspond to the known [Ru(H)(PCP)PPh₃],^[5c] the latter may play some role in the catalysis.

In conclusion, the pincer-aryl ruthenium(II) complexes **1–3** form active catalysts in the reduction of ketones by hydrogen transfer in *i*PrOH. Under these conditions, the σ Ru–C bond is stable and the [Ru(PCP)PPh₃] fragment is preserved. We are currently testing the performance of chiral Ru^{II} complexes of pincer-aryl ligands in this reaction.

Experimental Section

Typical procedure for the catalytic hydrogen-transfer reaction: A suspension of **3** (0.0197 g, 0.02 mmol) and KOH (10 mL, 0.2 M in *i*PrOH) in *i*PrOH (30 mL) was heated to reflux under nitrogen for 1 h. The resulting brown solution was quickly transferred to a solution of the ketone (200 mmol) in *i*PrOH (170 mL), and the mixture was subsequently heated to 82 °C. The acetone formed was not distilled off. Yields were determined by GC analysis with *n*-undecane as internal standard.

4: ¹H NMR (200 MHz, C₆D₆, 25 °C, TMS): δ = −8.50 (br dt, ²J(H,P) = 18, 20 Hz, 1H, RuH), 1.20 (brs, 6H, CH₃), 3.68 (brd, ²J(H,H) = 14 Hz, 2H, PCH), 4.00 (brs, 1H, CH–O), 4.30 (doublet of virtual triplets (dvt), ²J(H,H) = 14 Hz, ³J(H,P) not resolved, 2H, CH–P), 6.50–7.60 (m, 34H, ArH) 7.70–7.80 (m, 4H, *o*-H of PCP); ³¹P{¹H} NMR (81 MHz, C₆D₆, 25 °C, 85 % H₃PO₄): δ = 47.31 (t, 1P, PPh₃), 71.80 (d, ²J(P,P) = 19 Hz, 2P, PCP).

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Synthesis and Characterization of an Open-Framework Antimony(III) Phosphate: [H₃N(CH₂)₂NH₃]_{1.5}[(SbO)₂(SbF)₂(PO₄)₃]**

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Nanoporous aluminosilicate zeolites find technological applications in areas such as catalysis, separations, and ion exchange.^[1] After Flanigen et al. reported a series of open-framework aluminophosphates (AlPO₄s),^[2] others successfully incorporated transition and other main group metals into open frameworks.^[3] Unlike the zeolites and many AlPO₄s, which are based upon corner-sharing [XO₄] tetrahedra, many of the newer materials are based on other polyhedra that give rise to new topologies. For example, several open-framework tin(II) phosphates^[4] were prepared in which the stereochemically active electron lone pair associated with each Sn^{II} center leads to trigonal [SnO₃] and square pyramidal [SnO₄] units. One of these materials^[4f] is truly nanoporous and exhibits reversible dehydration and ion-exchange properties. We therefore extended the search for open frameworks into the Sb₂O₃/P₂O₅ system.

Open frameworks containing Sb^{III} include the antimony(III) sulfides^[5] and the mineral cetineite and related synthetic phases.^[6] While a number of Sb^V [7] and mixed-valent Sb^{III}/Sb^V [8] phosphates have been made, until recently the only known materials based solely on [Sb^{III}O_x] and [P^VO₄] polyhedra were condensed SbPO₄^[9] and layered SbO(H₂PO₄)·H₂O.^[10] Layered antimony(III)fluoride phosphates have also been synthesized: KSbF₂(HPO₄)^[11] and MSbFPO₄·nH₂O (M = NH₄, Na, K, Rb, Cs, CN₃H₆, n = 0–1.5)^[12, 13], as well as a chain-type structure: CsSbF₃(H₂PO₄).^[13] The three-dimensional structure of a new Sb^{III}-based material, NaSb₃O₂(PO₄)₂,^[14] although not microporous, suggested that Sb^{III} could be incorporated into an open framework phosphate. By using a structure-directing agent (SDA) under hydrothermal synthesis conditions^[15] we synthesized the material described below.

The asymmetric unit of [H₃N(CH₂)₂NH₃]_{1.5}[(SbO)₂(SbF)₂(PO₄)₃] contains 29 crystallographically independent non-hydrogen atoms (Figure 1). As in many other open frameworks for which fluorine is used as a mineralizer,^[16–19] fluorine is incorporated into the structure. This framework is based upon a network containing: 1) pseudo-octahedral [SbO₅E] and [SbO₄FE] units (E = lone pair), 2) pseudo-trigonal-bipyramidal [SbO₄E] and [SbO₃FE] units (the lone pairs of electrons occupy axial and equatorial positions in the

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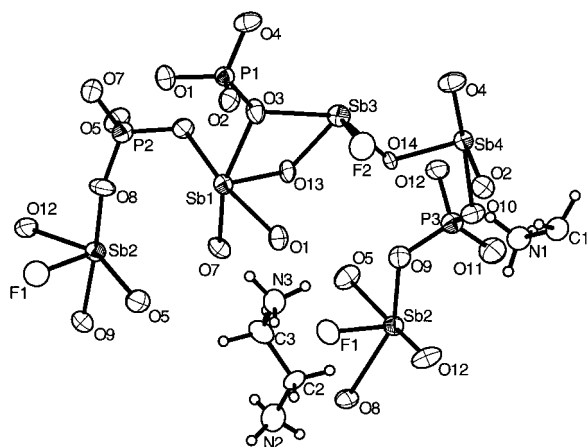


Figure 1. Asymmetric unit of $[\text{H}_3\text{N}(\text{CH}_2)_2\text{NH}_3]_{1.5}[(\text{SbO})_2(\text{SbF})_2(\text{PO}_4)_3]$ (50% probability thermal ellipsoids).

octahedra and bipyramids, respectively, when Sb–O bonds up to 2.7 Å are taken into account), and 3) tetrahedral $[\text{PO}_4]$ units. The bond lengths and angles agree with those of reported Sb^{III} phosphates and fluoride phosphates (av P–O 1.537 Å, av O–P–O 109.51°; for SbX_5E , av Sb–O 2.202 Å, av adjacent X–Sb–X 85.1°; for SbX_4E , av Sb–O 2.135, av adjacent X–Sb–X 83.4°; av Sb–F = 1.964 Å).^[9–14]

The polyhedra combine to form layers built from four-rings, which are made from two Sb and two P atoms bridged by oxygen atoms (an Sb_2P_2 ring), and six-rings (Sb_3P_3) (Figure 2). $[\text{SbO}_3\text{FE}]$ polyhedra connect the layers by sharing the axial

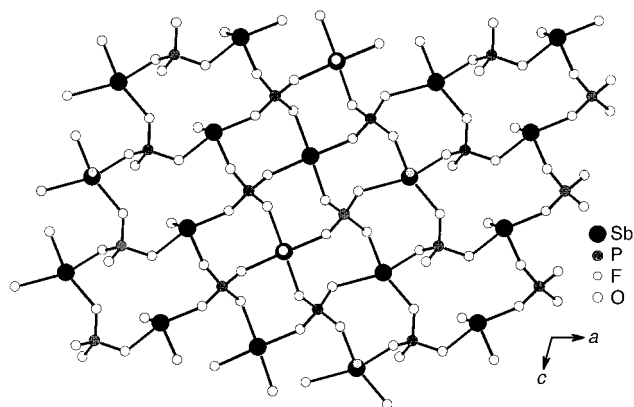


Figure 2. View of $[\text{H}_3\text{N}(\text{CH}_2)_2\text{NH}_3]_{1.5}[(\text{SbO})_2(\text{SbF})_2(\text{PO}_4)_3]$ down the b axis, showing the four- and six-rings that make up the layers.

oxygen atoms of the $[\text{SbO}_5\text{E}]$ polyhedra to give eight- and twelve-ring channels that run along the $[100]$ and $[001]$ directions, respectively (Figure 3). Diprotonated ethylenediamine (en) resides in the channels and provides for charge compensation. Each N atom of the en dication is within 3 Å of two framework O atoms (av 2.841 Å); this distance is indicative of hydrogen bonding. Fluorine atoms are directed into both channels: the F atoms of $[\text{SbO}_4\text{FE}]$ lie in the twelve-rings and those of $[\text{SbO}_3\text{FE}]$ in the eight-rings. Sb_2P_2 rings are also found in layered Sb^{III} fluoride phosphates, in which $[\text{SbO}_3\text{F}_2\text{E}]$ ^[9] or $[\text{SbO}_4\text{FE}]$ ^[10, 11] octahedra and $[\text{PO}_4]$ polyhedra combine to form layers separated by small cations.

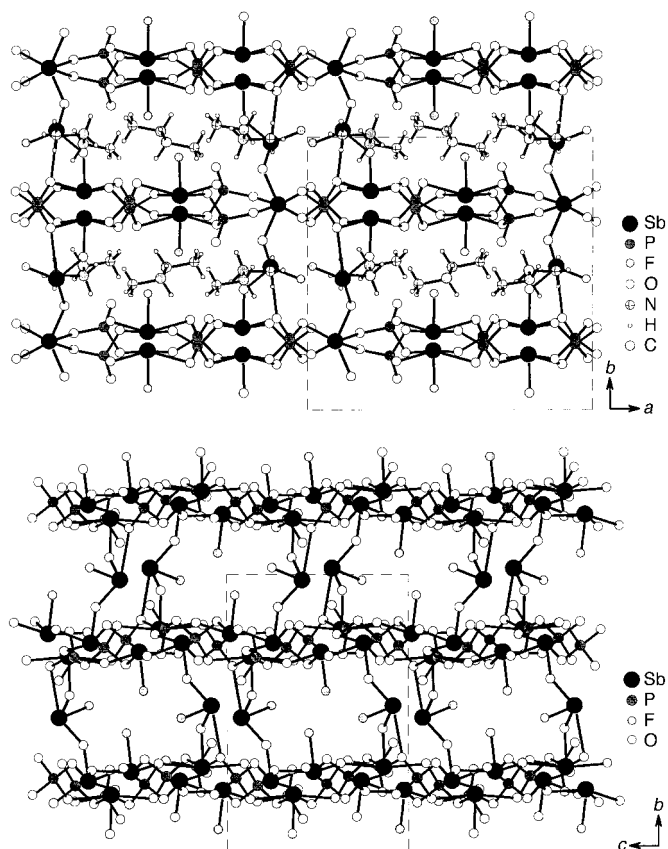


Figure 3. Top: View of $[\text{H}_3\text{N}(\text{CH}_2)_2\text{NH}_3]_{1.5}[(\text{SbO})_2(\text{SbF})_2(\text{PO}_4)_3]$ down the c axis, showing the twelve-ring channels and diprotonated en. The unit cell is outlined. Bottom: View of $[\text{H}_3\text{N}(\text{CH}_2)_2\text{NH}_3]_{1.5}[(\text{SbO})_2(\text{SbF})_2(\text{PO}_4)_3]$ down the a axis, showing the eight-rings lined with fluorine atoms (en omitted for clarity).

When ethylene glycol (eg) is replaced by water in the present synthesis, an unreported material forms: $[\text{H}_3\text{N}(\text{CH}_2)_2\text{NH}_3]_{0.5}\text{SbF}(\text{PO}_4)$ (monoclinic, space group $P2_1/c$, $a = 6.5417$, $b = 14.9877$, $c = 9.2193$ Å, $\beta = 134.769^\circ$). This material, which will be described in detail elsewhere, contains diprotonated en between inorganic layers that are identical to those found in the $\text{M}[\text{SbFPO}_4] \cdot n\text{H}_2\text{O}$ family.^[12, 13]

The product of the present synthesis appears pure, as determined by comparing the experimental with the simulated X-ray powder diffraction pattern. However, elemental analysis results (Sb 51.9, P 9.1 (Sb/P 1.46), F 5.2, C 4.1, N 4.7, H 2.0%; calcd: Sb 56.2, P 9.9 (Sb/P 1.33), F 4.1, C 3.9, N 4.5, H 1.6%) do not rule out the possibility of the presence of an impurity phase. Differential thermogravimetric analysis was performed under flowing O_2 . An exothermic peak and a mass loss of about 10% at about 300 °C corresponds to the removal of the organic substance (calcd 9.9%). This is immediately followed by a mass loss of about 14%, accompanied by an exothermic peak, which is complete by around 500 °C. Powder diffraction indicates the final material to be mainly SbPO_4 , with a small amount of Sb_2O_3 (Sb/P = 1.25 from elemental analysis). We have so far been unable to remove the organic component without loss of the open framework, but are investigating ion-exchange methods to achieve this end.

The small number of reported materials based on Sb^{III} /P^V^[9, 10, 14] or Sb^{III} /P^V/F^[13–15] contain a large variety of polyhedra

([SbO₅E], [SbO₄FE], [SbO₃F₂E], [SbO₂F₃E], [SbO₄E], [SbO₃FE]) and secondary building units (Sb₂P₄^[14] Sb₂P₂,^[9, 11–14] Sb₄P₂,^[10, 14] Sb₃P₃,^[14] and Sb₅P₃^[14] rings), as well as the new Sb₆P₂ and Sb₈P₄ rings of the title compound. We have recently discovered additional molecular, chain, layered, and three-dimensional materials belonging to this large family. In addition to their novel architectures, Sb^{III}-based materials, unlike closed-shell zeolites and AlPO₄s, may have redox behavior or other interesting properties associated with their lone pairs of electrons.

Experimental Section

Hydrofluoric acid (49 wt %, Fisher) was added to antimony(III) oxide (Aldrich), and the mixture was stirred to give a clear solution. Phosphoric acid (85 wt %, Fisher), en (Aldrich), eg (Fisher), and water, were added with stirring to give a mixture with composition 1Sb:0.6P:3.1F:1.0en:11H₂O:37eg. This was sealed in a PTFE-lined stainless steel autoclave and heated at 135 °C under autogeneous pressure for 5 d. The crystalline product was filtered, washed with ethanol, and dried at 90 °C in air. A single crystal (0.08 × 0.04 × 0.03 mm) was mounted on a glass fiber, and room temperature X-ray diffraction data were collected with a Siemens SMART CCD diffractometer equipped with a normal focus 2.4-kW sealed X-ray tube (MoK_α radiation, λ = 0.71073 Å) operating at 45 kV and 35 mA. About 1.3 hemispheres of intensity data were collected in 1321 frames with ω scans (width 0.30°, exposure time 10 s per frame). A total of 8328 reflections were collected in the range 2.8 < 2θ < 46.6° and merged to give 2648 unique reflections. Unit cell parameters were determined by a least-squares fit of 1724 reflections: monoclinic, space group P2₁/c (no. 14), a = 14.8222(14), b = 13.7657(13), c = 9.3022(9) Å, β = 105.341(2)°, V = 1830.4(3) Å³, Z = 2, M = 935.09, ρ_{calcd} = 3.393. Absorption correction was performed with the program SADABS.^[20] Positions of Sb and P atoms were determined by direct methods with SHELXS-97.^[21] Other non-hydrogen atoms were located in subsequent difference Fourier maps. Bond-valence parameters^[22] unambiguously distinguished bridging atoms. The final structure model was established by full-matrix least-squares refinement against |F|² with SHELXTL-PLUS.^[21] The hydrogen atoms were placed in calculated positions and held in the riding model. The final reliability factors obtained in the fitting of 265 parameters with 1809 independent reflections with I > 2.0σ(I) were R₁(F) = 0.0387, wR(F²) = 0.0843, S = 0.985. The final difference Fourier map had maximum and minimum peaks of 0.936 and −1.325 e Å^{−3}. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-133133. 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 p(O₂)-Responsive MRI Contrast Agent Based on the Redox Switch of Manganese(II/III) – Porphyrin Complexes

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Contrast in magnetic resonance imaging (MRI) is largely determined by differences in the relaxation times of protons in tissues. Therefore, contrast agents (CAs) for this diagnostic methodology have mainly been sought among the coordination compounds of metal ions with several unpaired electrons,

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proportional to the applied $p(\text{O}_2)$, thus providing a very useful tool for reporting the relative amount of oxygen present in the region of interest in a magnetic resonance image.

The relaxometric response may be modulated, according to the requirements of a given functional protocol, by introducing suitable substituents on the aromatic moieties of the porphyrin ring to control the thermodynamics and kinetics of the $\text{Mn}^{\text{II}}/\text{Mn}^{\text{III}}$ switch. Moreover one may envisage several ways to attain longer τ_{R} values—either by dealing with covalent macromolecular conjugates or by forming supramolecular adducts by interlocking of molecular components other than those used in this work.

Experimental Section

The formation of $[(\text{Mn}^{\text{II}} \text{ tpps})^+]$ from $[(\text{Mn}^{\text{III}} \text{ tpps})^3-]$ (Porphyrin Products, UT, USA) and $\text{Na}_2\text{S}_2\text{O}_3$ was monitored by UV spectroscopy following the growth of the band at 428 nm and the disappearance of the band at 466 nm, typical for the +3 oxidation state. The binding affinity of the two complexes with poly- β -CD was determined by measuring the water proton relaxation rates of a solution of the complex (about 0.2 mM) with increasing amounts of the poly- β -CD substrate (0.2–14 mM). The data obtained were fitted with those calculated on the basis of the established theory of proton relaxation enhancement. The $1/T_1$ NMRD curves were obtained on a fully automatic Field-Cycling Relaxometer (Stelar, Mede, PV, Italy) in the frequency range of 0.01–12 MHz. Data at higher frequencies were obtained on a Stelar Spin-Master Relaxometer (20 MHz) and on a Jeol EX-90 (90 MHz). The titrations with poly- β -CD and the assessment of $[(\text{Mn}^{\text{II}} \text{ tpps})^+]$ as a pO_2 -responsive agent were carried out at 20 MHz and 298 K.

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Electrophilic Aromatic Substitution Reactions of a Metallabenzene: Nitration and Halogenation of the Osmabenzene $[\text{Os}\{\text{C}(\text{SMe})\text{CHCHCHCH}\}\text{I}(\text{CO})(\text{PPh}_3)_2]^+ \text{PF}_6^-$

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It was established at the outset, with the isolation of the first metallabenzenes,^[1] that these compounds exhibit physical properties that are consistent with the presence of an aromatic ring system. The osmabenzene $[\text{Os}(\text{CSCHCHCHCH})(\text{CO})(\text{PPh}_3)_2]$ displays ring planarity, equal Os–C distances intermediate between normal Os–C single and double bonds, C–C distances comparable to that found for benzene, and NMR chemical shifts appropriate for an aromatic system. The related iridabenzene $[\text{Ir}(\text{CHCMeCHCMeCH})(\text{PET}_3)_3]$,^[2] which has been extensively studied, shows similar physical properties.^[3] A recently reported, but differently substituted iridabenzene, $[\text{Ir}(\text{CPhCPhCHCHCH})(\text{CO})(\text{PPh}_3)_2]$, again shows physical properties consistent with aromaticity.^[4]

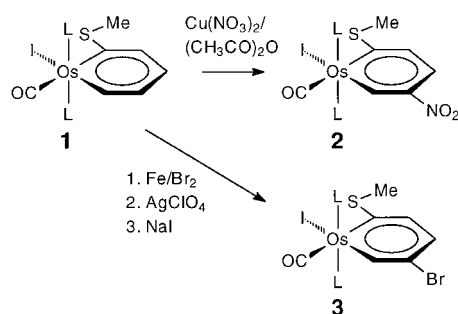
Despite the considerable weight of physical evidence pointing towards aromaticity for metallabenzenes, the reaction chemistry so far reported does not entirely reflect this. For example the iridabenzene $[\text{Ir}(\text{CHCMeCHCMeCH})(\text{PET}_3)_3]$ has a propensity to undergo cycloaddition reactions rather than electrophilic substitutions.^[3a] Other metallabenzenes are unstable with respect to rearrangement to cyclopentadienyl–metal complexes.^[5] One hint of more conventional aromatic behavior is given by the formation of η^6 complexes with other metal–ligand fragments, for example, $[\{\eta^6\text{-CH=CMeCH=CMeCH-Ir}(\text{PET}_3)_3\}\text{Mo}(\text{CO})_3]$.^[6] Several related metallabenzene η^6 complexes have also been described.^[7]

Here we describe: 1) the first examples of electrophilic aromatic substitution of a metallabenzene, specifically both mononitration and monohalogenation of the osmabenzene $[\text{Os}\{\text{C}(\text{SMe})\text{CHCHCHCH}\}\text{I}(\text{CO})(\text{PPh}_3)_2]$ (**1**) and 2) structure determinations of both the nitrated and brominated products, which reveal 3) the operation of a *para*-directing effect of the SMe ring substituent.

Treatment of the brown osmabenzene $[\text{Os}(\text{CSCHCHCHCH})(\text{CO})(\text{PPh}_3)_2]$,^[1, 8] with MeI produces the blue osmabenzene **1**, which bears an SMe substituent on the carbon atom adjacent to the osmium center (Scheme 1). The geometry of **1** has been confirmed by an X-ray crystal structure determination of its blue chloride analogue.^[9] When **1** is subjected to the mild nitrating conditions offered by $\text{Cu}(\text{NO}_3)_2/\text{acetic anhydride}$ and the crude reaction product

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Scheme 1. Electrophilic aromatic substitution reactions of **1** ($\text{L} = \text{PPh}_3$).

purified by chromatography on silica gel, the blue-purple, mononitrated product, $[\text{Os}\{\text{C}(\text{SMe})\text{CHCHC}(\text{NO}_2)\text{CH}\}\text{I}(\text{CO})(\text{PPh}_3)_2]$ (**2**), can be isolated in 5% yield (see Scheme 1). The presence of the nitro group is revealed by bands in the IR spectrum at 1526 and 843 cm^{-1} (see *Experimental Section* for spectroscopic data for **1–3**). At the same time the frequency of the $\tilde{\nu}(\text{CO})$ band increases from 1939 cm^{-1} in **1** to 1955 cm^{-1} in **2**. In the ^1H NMR spectrum the resonances for the remaining three ring protons are consistent with the introduction of the nitro group *para* to the SMe group. This structural assignment is confirmed by the X-ray crystal structure of **2** (Figure 1).^[10] Presumably the location of the nitro group is dictated by the directing effect of the SMe group as would be the case for a simple benzene derivative. The structure shows that both the nitro group and the SMe group lie in the plane of the osmabenzene ring.

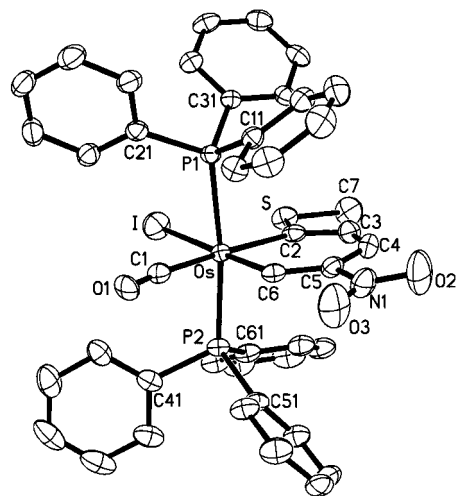


Figure 1. X-ray crystal structure of **2** (ORTEP). Selected bond lengths [Å] and angles [°]: Os–C1 1.908(8), Os–C2 2.129(7), Os–C(6) 2.011(7), Os–P(1) 2.4005(16), Os–P(2) 2.4021(16), Os–I 2.8158(6), C2–C3 1.411(11), C3–C4 1.347(11), C4–C5 1.411(10), C5–C6 1.336(10), N1–C5 1.476(9), N1–O2 1.231(9), N1–O3 1.218(9), C2–S 1.702(7); C1–Os–C6 84.0(3), C2–Os–C6 87.1(3), C2–Os–I 90.9(2), C1–Os–I 98.1(2), P1–Os–P2 172.78(6).

In the same fashion, when **1** is treated with bromine/iron powder, bromination occurs, and the green complex $[\text{Os}\{\text{C}(\text{SMe})\text{CHCHCBrCH}\}\text{I}(\text{CO})(\text{PPh}_3)_2]$ (**3**) is obtained in an acceptable 40% yield (after chromatography). During this reaction some of the osmium-bound iodide is exchanged for bromide giving the mixture $[\text{Os}\{\text{C}(\text{SMe})\text{CHCHCBrCH}\}(\text{Br}/\text{I})(\text{CO})(\text{PPh}_3)_2]$. The osmium-bound halide in this mixture is

labile and easily removed by the addition of silver perchlorate. Subsequent addition of excess sodium iodide gives pure **3**. Again the site of bromination is *para* to the SMe group as revealed by NMR spectroscopy (see below) and confirmed by X-ray crystal structure determination.^[10] The molecular structure of **3** is shown in Figure 2. Chlorination of **1**, in the same ring position, has also been achieved through treatment of **1** with PhICl_2 (20% yield, the product is blue-green).^[9]

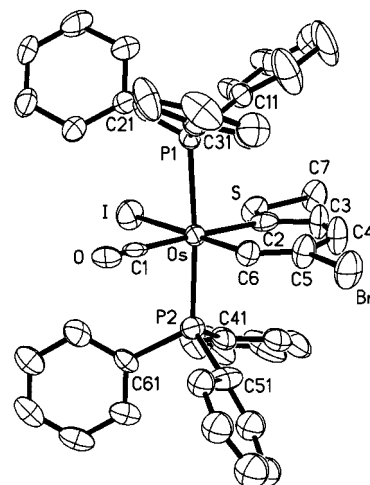


Figure 2. X-ray crystal structure of **3** (ORTEP). Selected bond lengths [Å] and angles [°]: Os–C1 1.936(13), Os–C2 2.128(9), Os–C6 2.039(9), Os–P1 2.392(2), Os–P2 2.408(2), Os–I 2.8021(9), C2–C3 1.418(13), C3–C4 1.367(14), C4–C5 1.390(14), C5–C6 1.320(13), Br–C(5) 1.968(10), C2–S 1.702(10); C1–Os–C6 84.6(4), C2–Os–C6 87.2(4), C2–Os–I 91.7(3), C1–Os–I 96.6(3), P1–Os–P2 175.27(8).

These results indicate that osmabenzene **1** shows the defining chemical characteristic of aromaticity in undergoing the classical electrophilic aromatic substitution reactions of nitration and halogenation. Furthermore, normal directing (and presumably activating) substituent effects are apparent.

Experimental Section

1: $[\text{Os}\{\eta^2\text{-C}(\text{S})\text{CHCHCHCH}\}(\text{CO})(\text{PPh}_3)_2]$ (200 mg, 0.238 mmol) was dissolved in methyl iodide (25 mL) and the solution heated under reflux for 90 min. Addition of ethanol (20 mL) and removal of the methyl iodide under reduced pressure afforded teal-blue colored crystals of the title complex, which were recrystallized from dichloromethane/ethanol to give pure **1** (176 mg, 75%). M.p. 175°C ; ^1H NMR (400 MHz, CDCl_3 , 24°C , TMS) (the numbering scheme for the C and H atoms and the atoms of the metallacycles in **1–3** is shown in Figures 1 and 2): $\delta = 1.55$ (s, 3H; SCH₃), 6.21 ("t", $^3J(\text{H,H}) = 8.5$ Hz, 1H; H5), 6.60 (d, $^3J(\text{H,H}) = 8.9$ Hz, 1H; H3), 6.96 ("t"d, $^3J(\text{H,H}) = 8.2$, $^4J(\text{H,H}) = 1.8$ Hz, 1H; H4), 12.74 (d, $^3J(\text{H,H}) = 9.0$ Hz, 1H; H6), 7.28–7.54 (m, 30H; PPh₃); $^{13}\text{C}\{^1\text{H}\}$ NMR (100.6 MHz, CDCl_3 , 24°C , TMS): $\delta = 23.20$ (s; SCH₃), 121.62 (s; C3), 123.82 (s; C5), 145.85 (s; C4), 191.67 (t, $^2J(\text{C,P}) = 11.1$ Hz; CO), 221.58 (t, $^2J(\text{C,P}) = 6.3$ Hz; C6), 237.45 (t, $^2J(\text{C,P}) = 9.1$ Hz; C2), 126.91 (t', $^{111}2,4J(\text{C,P}) = 10.1$ Hz; *ortho*-PPh₃), 129.35 (s; *para*-PPh₃), 133.29 (t', $^{13}J(\text{C,P}) = 53.3$ Hz; *ipso*-PPh₃), 134.74 (t', $^{3,5}J(\text{C,P}) = 11.1$ Hz; *meta*-PPh₃); IR (Nujol): $\tilde{\nu} = 1933$ (vs CO), 1523, 1384, 1347, 1246, 1220, 1146, 924, 840, 770, 618 cm^{-1} ; positive-ion FAB-MS: m/z : 981.0623; $\text{C}_{43}\text{H}_{37}\text{IOOsP}_2\text{S}$ requires 981.0622; elemental analysis calcd for $\text{C}_{43}\text{H}_{37}\text{IOOsP}_2\text{S}$: C 52.65, H 3.80; found: C 52.56, H 4.08.

2: Copper(II) nitrate (45.0 mg, 0.238 mmol) was dissolved in acetic anhydride (12 mL) under nitrogen and the solution cooled to 0°C . Complex **1** (200 mg, 0.238 mmol) was added, and the slurry was stirred at 0°C for 2 h. The ice bath was removed, and the green suspension was stirred for 1 h at room temperature. A mixture of sodium acetate (1 g) in

water (15 mL) and ice (40 g) was added, and the reaction mixture was stirred for a further 2 h during which time a black, oily solid separated out. The solution was removed by decantation, and the oily solid was dried in a vacuum desiccator. The dry residue was redissolved in dichloromethane (1 mL), placed on a silica gel column (2.5 cm $\times$ 1.5 cm), and eluted with dichloromethane. The fast-moving purple band was collected, ethanol was added, and the dichloromethane was removed under reduced pressure to yield green crystals. Recrystallization from dichloromethane/ethanol yielded pure **2** as purple crystals (12 mg, 5%). ^1H NMR (400 MHz, CDCl_3 , 24 °C, TMS): δ = 1.84 (s, 3H; SCH_3), 7.00 (d, $^3J(\text{H,H})$ = 9.9 Hz, 1H; H3), 7.95 (dd, $^3J(\text{H,H})$ = 9.8, $^3J(\text{H,H})$ = 3.4 Hz, 1H; H4), 14.00 (m, 1H; H6), 7.20–7.56 (m, 30H; PPh_3); $^{13}\text{C}\{^1\text{H}\}$ NMR (100.6 MHz, CDCl_3 , 24 °C, TMS): δ = 24.70 (s; SCH_3), 124.10 (s; C3), 138.90 (s; C4), 144.24 (s; C5), 212.54 (t, $^2J(\text{C,P})$ = 10.6 Hz; CO), 222.69 (t, $^2J(\text{C,P})$ = 6.0 Hz; C6), 127.32 (t', $^{2,4}J(\text{C,P})$ = 9.1 Hz; *ortho*- PPh_3), 130.00 (s; *para*- PPh_3), 132.01 (t', $^{1,3}J(\text{C,P})$ = 54.3 Hz; *ipso*- PPh_3), 134.41 (t', $^{3,5}J(\text{C,P})$ = 10.0 Hz; *meta*- PPh_3); IR (Nujol): $\tilde{\nu}$ = 1955 (vs CO), 1526 (NO_2), 1299, 1276, 1237, 874, 843 (NO_2), 745 cm^{-1} ; positive-ion FAB-MS: m/z : 1024.0479; $\text{C}_{43}\text{H}_{36}\text{INO}_3\text{OsP}_2\text{S}$ requires 1024.0473; elemental analysis calcd for $\text{C}_{43}\text{H}_{36}\text{INO}_3\text{OsP}_2\text{S}$: C 50.34, H 3.53, N 1.36, S 3.13; found: C 50.63, H 3.58, N 1.78, S 3.03.

3: Complex **1** (200 mg, 0.238 mmol) was dissolved in carbon tetrachloride (50 mL), and iron powder (1.0 mg, 0.018 mmol) was added. A solution of Br_2 (12.2 mg, 0.238 mmol) in carbon tetrachloride (10 mL) was added dropwise to the blue solution. The reaction flask was heated on a water bath at 40 °C for 1 h. The carbon tetrachloride was removed in vacuo, and the green solid was dissolved in dichloromethane (1 mL) and placed on a silica gel column (2.5 cm $\times$ 1.5 cm). The blue-green fractions were collected, and AgClO_4 (49.4 mg, 0.238 mmol) was added. The solution was stirred for 10 min, during which time a flocculant yellow solid appeared. The solution was then filtered through Celite, and NaI (71 mg, 0.48 mmol) in ethanol/water (10 mL/1 mL) was added. Removal of the dichloromethane, addition of ethanol (20 mL), and subsequent recrystallization of the crude product from dichloromethane/ethanol yielded dark green crystals of pure **3** (105 mg, 45%). ^1H NMR (400 MHz, CDCl_3 , 24 °C, TMS): δ = 1.65 (s, 3H; SCH_3), 6.79 (d, $^3J(\text{H,H})$ = 9.5 Hz, 1H; H3), 7.15 (dd, $^3J(\text{H,H})$ = 9.4, $^3J(\text{H,H})$ = 3.0 Hz, 1H; H4), 12.61 (d, $^3J(\text{H,H})$ = 2.9 Hz, 1H; H6), 7.20–7.52 (m, 30H; PPh_3); $^{13}\text{C}\{^1\text{H}\}$ NMR (100.6 MHz, CDCl_3 , 24 °C, TMS): δ = 20.28 (s; SCH_3), 110.56 (s; C5), 126.14 (s; C3), 147.75 (s; C4), 190.73 (t, $^2J(\text{C,P})$ = 11.1 Hz; CO), 210.81 (t, $^2J(\text{C,P})$ = 5.5 Hz; C6), 240.31 (t, $^2J(\text{C,P})$ = 9.1 Hz; C2), 127.36 (t', $^{2,4}J(\text{C,P})$ = 10.1 Hz; *ortho*- PPh_3), 129.67 (s; *para*- PPh_3), 132.27 (t', $^{1,3}J(\text{C,P})$ = 55.3 Hz; *ipso*- PPh_3), 134.39 (t', $^{3,5}J(\text{C,P})$ = 10.1 Hz; *meta*- PPh_3); IR (Nujol): $\tilde{\nu}$ = 1943 (vs CO), 1401, 1367, 1238, 1187, 922, 800, 668 cm^{-1} ; positive-ion FAB-MS: m/z : 931.0697; $\text{C}_{43}\text{H}_{36}\text{BrOOSp}_2\text{S}$ requires 931.0730 [$\text{M}^+ - \text{I}$]; elemental analysis calcd for $\text{C}_{43}\text{H}_{36}\text{BrIOOSp}_2\text{S} \cdot 0.5\text{CH}_2\text{Cl}_2$: C 47.40, H 3.38; found: C 47.46, H 3.03.

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- [10] Data for the X-ray structure analyses: **2**: crystals from benzene/ethanol, $\text{C}_{43}\text{H}_{36}\text{INO}_3\text{OsP}_2\text{S} \cdot 1.5\text{C}_6\text{H}_6$ (M_r = 1142.99); crystal dimensions 0.24 $\times$ 0.24 $\times$ 0.04 mm³; triclinic; space group $P\bar{1}$; a = 12.2283(1), b = 13.7785(2), c = 14.1391(1) Å, α = 80.144(1), β = 77.314(1), γ = 78.930(1)°, Z = 2, V = 2260.06(4) Å³, ρ_{calcd} = 1.680 g cm⁻³, T = 203 K; $2\theta_{\text{max}}$ = 56°; 22095 total reflections of which 10023 were unique (R_{int} = 0.0268); Siemens SMART CCD diffractometer, $\text{MoK}\alpha$ radiation (λ = 0.71073 Å); empirical absorption correction (μ = 3.66 mm⁻¹, $T_{\text{min}}/T_{\text{max}}$ = 0.473/0.867). The structure was solved by Patterson and Fourier techniques and refined by full-matrix least squares on F^2 . The asymmetric unit contains one molecule of **2** and 1.5 molecules of benzene. R_1 = 0.0477 for the 8702 observed data ($I > 2\sigma(I)$) and wR_2 = 0.1334 for all data; GOF(F^2) = 1.027. **3**: crystals from dichloromethane/ethanol, $\text{C}_{43}\text{H}_{36}\text{BrIOOSp}_2\text{S}$ (M_r = 1059.73); crystal dimensions 0.21 $\times$ 0.12 $\times$ 0.08 mm³; orthorhombic; space group $Pbca$; a = 14.211(2), b = 22.270(5), c = 24.315(3) Å, Z = 8, V = 7695(2) Å³, ρ_{calcd} = 1.829 g cm⁻³, T = 203 K; $2\theta_{\text{max}}$ = 47°; 31267 total reflections of which 5529 were unique (R_{int} = 0.0795); Siemens SMART CCD diffractometer, $\text{MoK}\alpha$ radiation (λ = 0.71073 Å); empirical absorption correction (μ = 5.33 mm⁻¹, $T_{\text{min}}/T_{\text{max}}$ = 0.401/0.675). The structure was solved by Patterson and Fourier techniques and refined by full-matrix least squares on F^2 . The asymmetric unit contains a single molecule of **3**, in which one phenyl unit of one triphenylphosphane is disordered. R_1 = 0.0437 for the 4190 observed data ($I > 2\sigma(I)$) and wR_2 = 0.1086 for all data; GOF(F^2) = 1.061. Programs used: SHELXS-97 (structure solution) and SHELXL-97 (structure refinement). Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication nos. CCDC-133773 (**2**) and CCDC-133774 (**3**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [11] The denotation t' indicates that the signal has apparent multiplicity; $^{nm}J(\text{C,P})$ is the sum of the two coupling constants $^mJ(\text{C,P})$ and $^nJ(\text{C,P})$ as explained in: S. M. Maddock, C. E. F. Rickard, W. R. Roper, L. J. Wright, *Organometallics* **1996**, *15*, 1793–1803.

Highly Regio- and Stereoselective Cycloreductions of 1,6- and 1,7-Enynes Activated with a Carbonyl Functionality**

Chang Ho Oh,* Hyung Hoon Jung, Joo Sung Kim, and Seung Woo Cho

The cyclization of enynes or halodienes has been widely utilized in the synthesis of structurally complex natural and synthetic products.^[1] Various 1,6- and 1,7-enynes, aided by suitable palladium catalysts, cycloisomerize to the corresponding dienes,^[2] which could further serve as valuable

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such as Mn^{II} (five unpaired electrons) and Gd^{III} (seven unpaired electrons).^[1, 2] The potential ability of a paramagnetic complex to act as a CA for MRI is first tested in vitro by measuring its relaxivity (r_1), which represents the relaxation enhancement of solvent water protons in the presence of the paramagnetic complex at a concentration of 1 mM. The administration of CAs has entered into several diagnostic protocols and has proved particularly useful in the assessment of organ perfusion and abnormalities in the blood–brain barrier or in kidney clearance. The next generation of CAs will have high tissue specificity and the ability to act as reporters of the physico–chemical environment where they are distributed. Such systems may be claimed as “smart” CAs; examples include paramagnetic complexes whose relaxivity is dependent on pH, temperature, or even enzymatic activity.^[3]

Among the various parameters that characterize a biological environment, the partial pressure of oxygen ($p(\text{O}_2)$) appears to be relevant in a number of pathological conditions including strokes and tumors. The availability of CAs whose relaxivity is dependent upon $p(\text{O}_2)$ would be useful to obtain a better separation of arterial and venous blood as well as to develop novel applications of functional MRI (f-MRI). The latter methodology is based on the contrast which is dependent on blood oxygenation levels; this contrast arises chiefly from the magnetic properties of deoxyhemoglobin, which influences the transverse relaxation time of water protons.^[4] We have already tackled the problem of designing a CA which acts as a reporter of the blood oxygenation level by suggesting two Gd^{III} chelates which are able to recognize T and R forms of human hemoglobin.^[5, 6] Unfortunately, the intracellular nature of these CAs sets severe limitations on the development of practical MRI applications. Thus, we resort to systems which may yield the same information while remaining confined in the extracellular space or in direct contact with the extracellular fluid when they bind to outer cell membranes.

The simplest design of $p(\text{O}_2)$ -responsive CA is based on a complex whose metal ion can switch between two redox states characterized by different relaxation properties. A very efficient system could be provided by $\text{Eu}^{\text{II}}/\text{Eu}^{\text{III}}$ complexes, for which relaxivities analogous to Gd^{III} would be expected for the lower oxidation state. However, it is not straightforward to control the stabilization of this oxidation state.

For this reason, we resort to manganese complexes for which modulation of the redox switch between the $+2/+3$ oxidation states appears more amenable. As a model for this application we have considered Mn^{II} and Mn^{III} complexes with 5,10,15,20-tetrakis-(*p*-sulfonatophenyl) porphinate (tpps), whose relaxometric properties have been widely investigated.^[7, 8] Moreover, this system shows a high affinity to tumor cells and, because of this property, it has been considered as a potential candidate for photodynamic therapy of cancer.^[9]

The complex $[(\text{Mn}^{\text{II}} \text{tpps})^{4-}]$ is easily formed by treating $[(\text{Mn}^{\text{III}} \text{tpps})^{3-}]$ with dithionite. A simple inspection of the $1/T_1$ nuclear magnetic resonance dispersion (NMRD) profiles of the two complexes from 0.01 to 90 MHz (Figure 1) suggests that their relaxivities are only very different at low frequencies. Whereas this observation may suggest further applica-

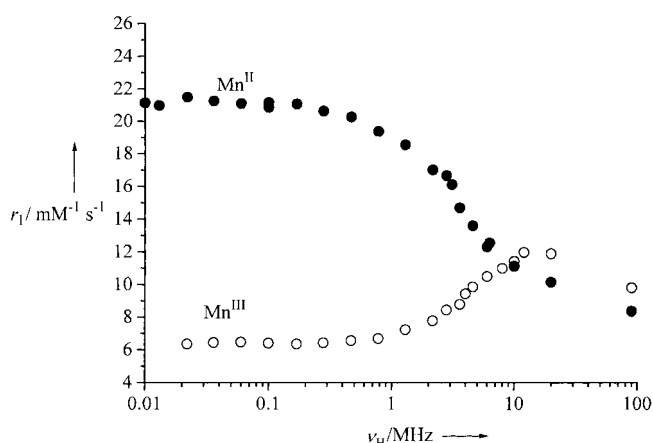
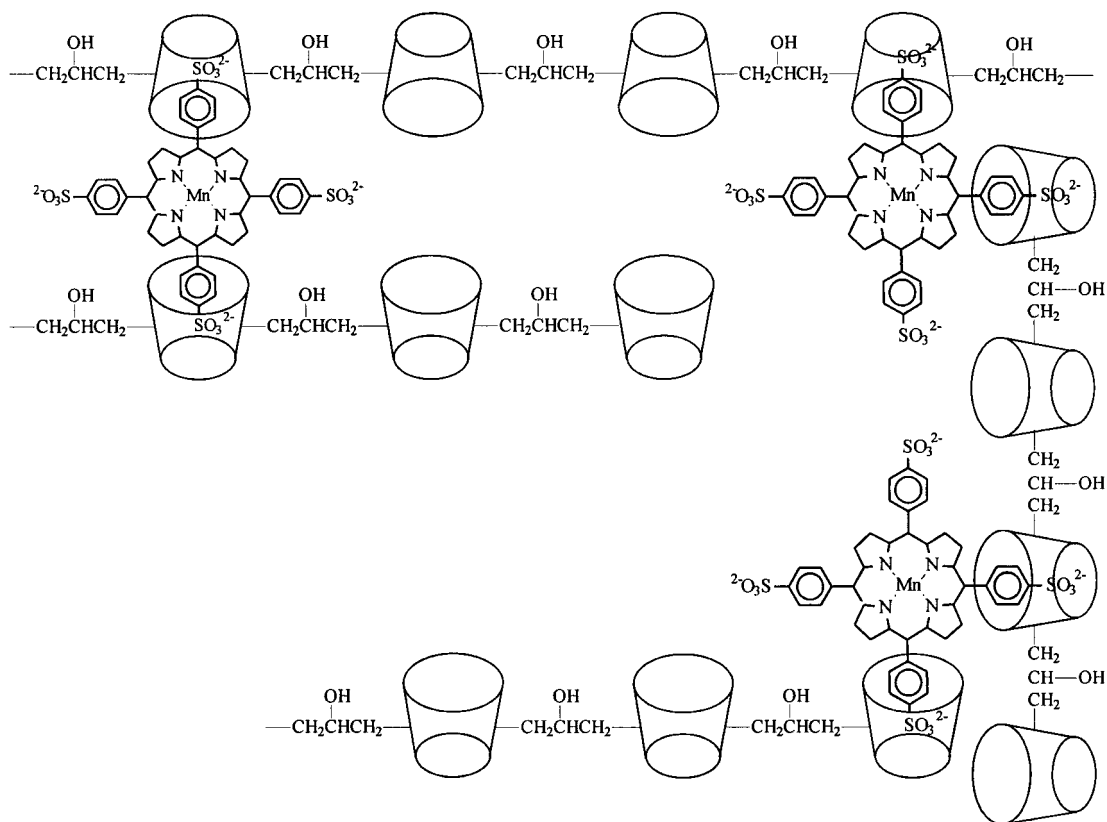


Figure 1. The $1/T_1$ NMRD profile of aqueous solutions (1 mM) of $[(\text{Mn}^{\text{II}} \text{tpps})^{4-}]$ (●) and $[(\text{Mn}^{\text{III}} \text{tpps})^{3-}]$ (○) at pH 7 and 25 °C.

tions in the low-field MRI systems, it is evident that, at the magnetic field strengths currently employed in the commercial MRI tomographs (0.5–1.5 T), the relaxivities of the two complexes are too close to be exploited for the proposed application. However, it is important to recall that the similar relaxivity values shown by the two systems arise from quite different relaxation pathways.^[7, 8] In fact, the relaxivity shown by $[(\text{Mn}^{\text{III}} \text{tpps})^{3-}]$ is dominated by the electronic relaxation time, whereas the relaxivity of $[(\text{Mn}^{\text{II}} \text{tpps})^{4-}]$ is controlled by the rotational motion of the complex (τ_R). On this basis, it was straightforward to pursue a better separation of r_1 values for the two complexes by forming an adduct with a macromolecular substrate; this would only cause a relaxation enhancement for the Mn^{II} system, whose relaxivity is determined by τ_R . This procedure has been widely explored for Gd^{III} chelates to pursue high relaxivities at the imaging fields. Usually the macromolecular adduct is formed through the noncovalent interaction between a suitable functionalized Gd^{III} chelate and serum albumin.^[1, 2] In principle, one may exploit an analogous interaction for $[(\text{Mn}^{\text{II/III}} \text{tpps})^{4/3-}]$ complexes, but we decided to work with a more defined system due to the involved chemistry of the porphyrin complexes.^[10]

It has been recently reported that, in order to inhibit dimerization reactions of porphyrins, each complex can be encapsulated into cyclodextrin (CD) hosts through the formation of strong interactions between the substituted phenyl rings of TPPS and the cavity of the CD molecule.^[11] Although it has been claimed that β -CD can form 1:4 complexes with $[(\text{Zn}^{\text{II}} \text{tpps})^{4-}]$ and $[(\text{Fe}^{\text{III}} \text{tpps})^{3-}]$,^[12] molecular modeling studies suggest that only two β -CDs can bind simultaneously to one porphyrin molecule.^{[13], [14]}

To pursue a higher enhancement for $[(\text{Mn}^{\text{II}} \text{tpps})^{4-}]$, a poly- β -CD was employed in place of the monomeric β -CD.^[15] The commercially available, water-soluble poly- β -CD used in this work has an average molecular weight of 6000 Da and is a mixture of oligomers containing three to nine CD units. We expected that each poly- β -CD would bind with more than one paramagnetic complex, thus yielding τ_R values much higher than those attainable for the adducts containing only two β -CD units. Furthermore, the close proximity of several β -CD cavities should favor the interaction with the complex to result



Scheme 1. Schematic representation of the supramolecular adduct formed from $[(\text{Mn}^{\text{II}} \text{tpps})^{4-}]$ and the poly- β -CD.

in a high stability of the overall supramolecular adduct (Scheme 1).

In Figure 2, the titration of 0.3 mM solutions of $[(\text{Mn}^{\text{II}} \text{tpps})^{4-}]$ (●) and $[(\text{Mn}^{\text{III}} \text{tpps})^{3-}]$ (○) with poly- β -CD is reported. Analysis of the data gave an nK_A value (where n is the number of the interacting sites) of $9.7 \times 10^4 \text{ M}^{-1}$ and a relaxation enhancement factor of 4 for the $[(\text{Mn}^{\text{II}} \text{tpps})^{4-}]$ complex. The interaction strength of Mn^{III} complex was similar but, as expected, there was only a very small relaxation enhancement for the macromolecular adduct. It appears reasonable to assume that the latter enhancement arises from second- and/or outer-sphere water protons. It follows that the

formation of macromolecular adducts with poly- β -CD allows us to deal with r_1 values of $40.8 \text{ mm}^{-1} \text{ s}^{-1}$ and $15.2 \text{ mm}^{-1} \text{ s}^{-1}$ for $[(\text{Mn}^{\text{II}} \text{tpps})^{4-}]$ and $[(\text{Mn}^{\text{III}} \text{tpps})^{3-}]$, respectively.

The ability of $[(\text{Mn}^{\text{II}} \text{tpps})^{4-}]$ to act as a $p(\text{O}_2)$ -responsive agent has been tested in vitro by measuring the relaxation rates of solutions containing a fixed amount of the complex (0.125 mM) and variable amounts of added oxygen gas in a sealed NMR tube.

The results (Figure 3) clearly indicate that in the presence of 40 torr of O_2 (after 5 min and with vigorous shaking) there has been a complete oxidation of $[(\text{Mn}^{\text{II}} \text{tpps})^{4-}]$ into $[(\text{Mn}^{\text{III}} \text{tpps})^{3-}]$. Below this value the observed relaxivities appear

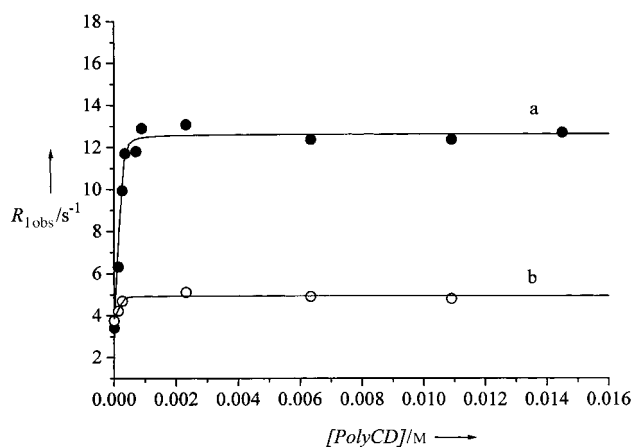


Figure 2. Plot of the water proton relaxation rate of solutions (0.2 mM) of $[(\text{Mn}^{\text{II}} \text{tpps})^{4-}]$ (●) and $[(\text{Mn}^{\text{III}} \text{tpps})^{3-}]$ (○) as a function of the poly- β -CD concentration (20 MHz, 25 °C, pH 7).

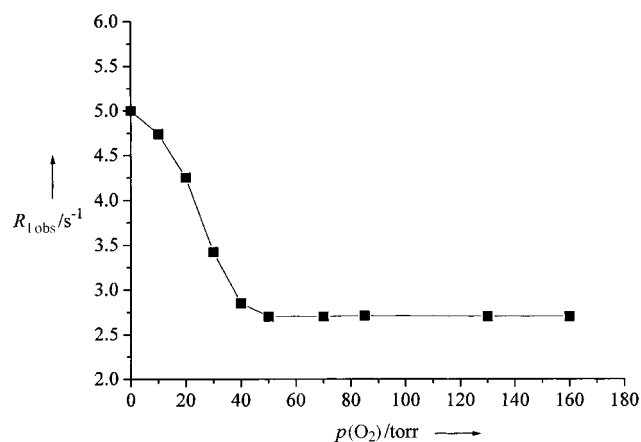


Figure 3. Plot of $p\text{O}_2$ -dependence of the water proton relaxation rate of solutions (0.125 mM) of $[(\text{Mn}^{\text{II}} \text{tpps})^{4-}]/[(\text{Mn}^{\text{III}} \text{tpps})^{3-}]$ (20 MHz, 25 °C, pH 7).

proportional to the applied $p(\text{O}_2)$, thus providing a very useful tool for reporting the relative amount of oxygen present in the region of interest in a magnetic resonance image.

The relaxometric response may be modulated, according to the requirements of a given functional protocol, by introducing suitable substituents on the aromatic moieties of the porphyrin ring to control the thermodynamics and kinetics of the $\text{Mn}^{\text{II}}/\text{Mn}^{\text{III}}$ switch. Moreover one may envisage several ways to attain longer τ_{R} values—either by dealing with covalent macromolecular conjugates or by forming supramolecular adducts by interlocking of molecular components other than those used in this work.

Experimental Section

The formation of $[(\text{Mn}^{\text{II}} \text{ tpps})^+]$ from $[(\text{Mn}^{\text{III}} \text{ tpps})^3-]$ (Porphyrin Products, UT, USA) and $\text{Na}_2\text{S}_2\text{O}_3$ was monitored by UV spectroscopy following the growth of the band at 428 nm and the disappearance of the band at 466 nm, typical for the +3 oxidation state. The binding affinity of the two complexes with poly- β -CD was determined by measuring the water proton relaxation rates of a solution of the complex (about 0.2 mM) with increasing amounts of the poly- β -CD substrate (0.2–14 mM). The data obtained were fitted with those calculated on the basis of the established theory of proton relaxation enhancement. The $1/T_1$ NMRD curves were obtained on a fully automatic Field-Cycling Relaxometer (Stelar, Mede, PV, Italy) in the frequency range of 0.01–12 MHz. Data at higher frequencies were obtained on a Stelar Spin-Master Relaxometer (20 MHz) and on a Jeol EX-90 (90 MHz). The titrations with poly- β -CD and the assessment of $[(\text{Mn}^{\text{II}} \text{ tpps})^+]$ as a pO_2 -responsive agent were carried out at 20 MHz and 298 K.

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Electrophilic Aromatic Substitution Reactions of a Metallabenzene: Nitration and Halogenation of the Osmabenzene $[\text{Os}\{\text{C}(\text{SMe})\text{CHCHCHCH}\}\text{I}(\text{CO})(\text{PPh}_3)_2]^+]$ **

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Scott D. Woodgate, and L. James Wright*

It was established at the outset, with the isolation of the first metallabenzene,^[1] that these compounds exhibit physical properties that are consistent with the presence of an aromatic ring system. The osmabenzene $[\text{Os}(\text{CSCHCHCHCH})(\text{CO})(\text{PPh}_3)_2]$ displays ring planarity, equal Os–C distances intermediate between normal Os–C single and double bonds, C–C distances comparable to that found for benzene, and NMR chemical shifts appropriate for an aromatic system. The related iridabenzene $[\text{Ir}(\text{CHCMeCHCMeCH})(\text{PET}_3)_3]$,^[2] which has been extensively studied, shows similar physical properties.^[3] A recently reported, but differently substituted iridabenzene, $[\text{Ir}(\text{CPhCPhCHCHCH})(\text{CO})(\text{PPh}_3)_2]$, again shows physical properties consistent with aromaticity.^[4]

Despite the considerable weight of physical evidence pointing towards aromaticity for metallabenzene, the reaction chemistry so far reported does not entirely reflect this. For example the iridabenzene $[\text{Ir}(\text{CHCMeCHCMeCH})(\text{PET}_3)_3]$ has a propensity to undergo cycloaddition reactions rather than electrophilic substitutions.^[3a] Other metallabenzene are unstable with respect to rearrangement to cyclopentadienyl–metal complexes.^[5] One hint of more conventional aromatic behavior is given by the formation of η^6 complexes with other metal–ligand fragments, for example, $[\{\eta^6\text{-CH=CMeCH=CMeCH-Ir}(\text{PET}_3)_3\}\text{Mo}(\text{CO})_3]$.^[6] Several related metallabenzene η^6 complexes have also been described.^[7]

Here we describe: 1) the first examples of electrophilic aromatic substitution of a metallabenzene, specifically both mononitration and monohalogenation of the osmabenzene $[\text{Os}\{\text{C}(\text{SMe})\text{CHCHCHCH}\}\text{I}(\text{CO})(\text{PPh}_3)_2]$ (**1**) and 2) structure determinations of both the nitrated and brominated products, which reveal 3) the operation of a *para*-directing effect of the SMe ring substituent.

Treatment of the brown osmabenzene $[\text{Os}(\text{CSCHCHCHCH})(\text{CO})(\text{PPh}_3)_2]$,^[1, 8] with MeI produces the blue osmabenzene **1**, which bears an SMe substituent on the carbon atom adjacent to the osmium center (Scheme 1). The geometry of **1** has been confirmed by an X-ray crystal structure determination of its blue chloride analogue.^[9] When **1** is subjected to the mild nitrating conditions offered by $\text{Cu}(\text{NO}_3)_2/\text{acetic anhydride}$ and the crude reaction product

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water (15 mL) and ice (40 g) was added, and the reaction mixture was stirred for a further 2 h during which time a black, oily solid separated out. The solution was removed by decantation, and the oily solid was dried in a vacuum desiccator. The dry residue was redissolved in dichloromethane (1 mL), placed on a silica gel column (2.5 cm $\times$ 1.5 cm), and eluted with dichloromethane. The fast-moving purple band was collected, ethanol was added, and the dichloromethane was removed under reduced pressure to yield green crystals. Recrystallization from dichloromethane/ethanol yielded pure **2** as purple crystals (12 mg, 5%). ^1H NMR (400 MHz, CDCl_3 , 24 °C, TMS): δ = 1.84 (s, 3H; SCH_3), 7.00 (d, $^3J(\text{H,H})$ = 9.9 Hz, 1H; H3), 7.95 (dd, $^3J(\text{H,H})$ = 9.8, $^3J(\text{H,H})$ = 3.4 Hz, 1H; H4), 14.00 (m, 1H; H6), 7.20–7.56 (m, 30H; PPh_3); $^{13}\text{C}\{^1\text{H}\}$ NMR (100.6 MHz, CDCl_3 , 24 °C, TMS): δ = 24.70 (s; SCH_3), 124.10 (s; C3), 138.90 (s; C4), 144.24 (s; C5), 212.54 (t, $^2J(\text{C,P})$ = 10.6 Hz; CO), 222.69 (t, $^2J(\text{C,P})$ = 6.0 Hz; C6), 127.32 (t', $^{2,4}J(\text{C,P})$ = 9.1 Hz; *ortho*- PPh_3), 130.00 (s; *para*- PPh_3), 132.01 (t', $^{1,3}J(\text{C,P})$ = 54.3 Hz; *ipso*- PPh_3), 134.41 (t', $^{3,5}J(\text{C,P})$ = 10.0 Hz; *meta*- PPh_3); IR (Nujol): $\tilde{\nu}$ = 1955 (vs CO), 1526 (NO_2), 1299, 1276, 1237, 874, 843 (NO_2), 745 cm^{-1} ; positive-ion FAB-MS: m/z : 1024.0479; $\text{C}_{43}\text{H}_{36}\text{INO}_3\text{OsP}_2\text{S}$ requires 1024.0473; elemental analysis calcd for $\text{C}_{43}\text{H}_{36}\text{INO}_3\text{OsP}_2\text{S}$: C 50.34, H 3.53, N 1.36, S 3.13; found: C 50.63, H 3.58, N 1.78, S 3.03.

3: Complex **1** (200 mg, 0.238 mmol) was dissolved in carbon tetrachloride (50 mL), and iron powder (1.0 mg, 0.018 mmol) was added. A solution of Br_2 (12.2 mg, 0.238 mmol) in carbon tetrachloride (10 mL) was added dropwise to the blue solution. The reaction flask was heated on a water bath at 40 °C for 1 h. The carbon tetrachloride was removed in vacuo, and the green solid was dissolved in dichloromethane (1 mL) and placed on a silica gel column (2.5 cm $\times$ 1.5 cm). The blue-green fractions were collected, and AgClO_4 (49.4 mg, 0.238 mmol) was added. The solution was stirred for 10 min, during which time a flocculant yellow solid appeared. The solution was then filtered through Celite, and NaI (71 mg, 0.48 mmol) in ethanol/water (10 mL/1 mL) was added. Removal of the dichloromethane, addition of ethanol (20 mL), and subsequent recrystallization of the crude product from dichloromethane/ethanol yielded dark green crystals of pure **3** (105 mg, 45%). ^1H NMR (400 MHz, CDCl_3 , 24 °C, TMS): δ = 1.65 (s, 3H; SCH_3), 6.79 (d, $^3J(\text{H,H})$ = 9.5 Hz, 1H; H3), 7.15 (dd, $^3J(\text{H,H})$ = 9.4, $^3J(\text{H,H})$ = 3.0 Hz, 1H; H4), 12.61 (d, $^3J(\text{H,H})$ = 2.9 Hz, 1H; H6), 7.20–7.52 (m, 30H; PPh_3); $^{13}\text{C}\{^1\text{H}\}$ NMR (100.6 MHz, CDCl_3 , 24 °C, TMS): δ = 20.28 (s; SCH_3), 110.56 (s; C5), 126.14 (s; C3), 147.75 (s; C4), 190.73 (t, $^2J(\text{C,P})$ = 11.1 Hz; CO), 210.81 (t, $^2J(\text{C,P})$ = 5.5 Hz; C6), 240.31 (t, $^2J(\text{C,P})$ = 9.1 Hz; C2), 127.36 (t', $^{2,4}J(\text{C,P})$ = 10.1 Hz; *ortho*- PPh_3), 129.67 (s; *para*- PPh_3), 132.27 (t', $^{1,3}J(\text{C,P})$ = 55.3 Hz; *ipso*- PPh_3), 134.39 (t', $^{3,5}J(\text{C,P})$ = 10.1 Hz; *meta*- PPh_3); IR (Nujol): $\tilde{\nu}$ = 1943 (vs CO), 1401, 1367, 1238, 1187, 922, 800, 668 cm^{-1} ; positive-ion FAB-MS: m/z : 931.0697; $\text{C}_{43}\text{H}_{36}\text{BrOOSp}_2\text{S}$ requires 931.0730 [$\text{M}^+ - \text{I}$]; elemental analysis calcd for $\text{C}_{43}\text{H}_{36}\text{BrIOOSp}_2\text{S} \cdot 0.5\text{CH}_2\text{Cl}_2$: C 47.40, H 3.38; found: C 47.46, H 3.03.

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- [10] Data for the X-ray structure analyses: **2**: crystals from benzene/ethanol, $\text{C}_{43}\text{H}_{36}\text{INO}_3\text{OsP}_2\text{S} \cdot 1.5\text{C}_6\text{H}_6$ (M_r = 1142.99); crystal dimensions 0.24 $\times$ 0.24 $\times$ 0.04 mm³; triclinic; space group $P\bar{1}$; a = 12.2283(1), b = 13.7785(2), c = 14.1391(1) Å, α = 80.144(1), β = 77.314(1), γ = 78.930(1)°, Z = 2, V = 2260.06(4) Å³, ρ_{calcd} = 1.680 g cm⁻³, T = 203 K; $2\theta_{\text{max}}$ = 56°; 22095 total reflections of which 10023 were unique (R_{int} = 0.0268); Siemens SMART CCD diffractometer, $\text{MoK}\alpha$ radiation (λ = 0.71073 Å); empirical absorption correction (μ = 3.66 mm⁻¹, $T_{\text{min}}/T_{\text{max}}$ = 0.473/0.867). The structure was solved by Patterson and Fourier techniques and refined by full-matrix least squares on F^2 . The asymmetric unit contains one molecule of **2** and 1.5 molecules of benzene. R_1 = 0.0477 for the 8702 observed data ($I > 2\sigma(I)$) and wR_2 = 0.1334 for all data; GOF(F^2) = 1.027. **3**: crystals from dichloromethane/ethanol, $\text{C}_{43}\text{H}_{36}\text{BrIOOSp}_2\text{S}$ (M_r = 1059.73); crystal dimensions 0.21 $\times$ 0.12 $\times$ 0.08 mm³; orthorhombic; space group $Pbca$; a = 14.211(2), b = 22.270(5), c = 24.315(3) Å, Z = 8, V = 7695(2) Å³, ρ_{calcd} = 1.829 g cm⁻³, T = 203 K; $2\theta_{\text{max}}$ = 47°; 31267 total reflections of which 5529 were unique (R_{int} = 0.0795); Siemens SMART CCD diffractometer, $\text{MoK}\alpha$ radiation (λ = 0.71073 Å); empirical absorption correction (μ = 5.33 mm⁻¹, $T_{\text{min}}/T_{\text{max}}$ = 0.401/0.675). The structure was solved by Patterson and Fourier techniques and refined by full-matrix least squares on F^2 . The asymmetric unit contains a single molecule of **3**, in which one phenyl unit of one triphenylphosphane is disordered. R_1 = 0.0437 for the 4190 observed data ($I > 2\sigma(I)$) and wR_2 = 0.1086 for all data; GOF(F^2) = 1.061. Programs used: SHELXS-97 (structure solution) and SHELXL-97 (structure refinement). Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication nos. CCDC-133773 (**2**) and CCDC-133774 (**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).
- [11] The denotation t' indicates that the signal has apparent multiplicity; $^{nm}J(\text{C,P})$ is the sum of the two coupling constants $^mJ(\text{C,P})$ and $^nJ(\text{C,P})$ as explained in: S. M. Maddock, C. E. F. Rickard, W. R. Roper, L. J. Wright, *Organometallics* **1996**, *15*, 1793–1803.

Highly Regio- and Stereoselective Cycloreductions of 1,6- and 1,7-Enynes Activated with a Carbonyl Functionality**

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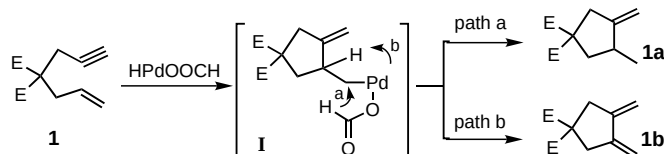
The cyclization of enynes or halodienes has been widely utilized in the synthesis of structurally complex natural and synthetic products.^[1] Various 1,6- and 1,7-enynes, aided by suitable palladium catalysts, cycloisomerize to the corresponding dienes,^[2] which could further serve as valuable

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synthetic intermediates.^[3] Similar palladium catalysts, combined with silanes as hydrogen-atom donors, have been employed to reduce the alkylpalladium intermediates formed from enynes to give the corresponding mono-olefins.^[4] These mono-olefins are the same products as those formed from a Bu₃SnH-mediated radical cyclization followed by destannylation.^[5] Another approach leading to these products is through the cycloisomerization of 1,6-dienes.^[6] We have reported that the alkylpalladium formates, where the alkyl group is derived from a coordinated enediyne, undergo direct reduction by the formate ligand prior to β -elimination of the alkyl species to give the cycloreduced products.^[7] Recently, we have found that the alkylpalladium intermediate **1**, formed during catalyzed enyne cyclizations, is reductively cleaved by the formate ligand (Scheme 1).^[8]



Scheme 1. Enyne **1** coordinates to the palladium-based catalyst, and intramolecular attack by the formyl group (path a) leads to cycloreduction and product **1a**; alternatively β -hydride elimination (path b) leads to product **1b**.

In order to explore the synthetic potential of this process, our efforts were directed at palladium-catalyzed cycloreductions, assisted with formic acid, for a wide variety of carbonyl-conjugated enynes.^[9] In this context, Lu and Wang have reported coupling reactions of alkynes with α,β -unsaturated carbonyl compounds to form γ,δ -unsaturated carbonyl compounds.^[10] Herein we report our success in forming γ,δ -unsaturated carbonyl compounds by palladium-catalyzed reactions of 1,6- and 1,7-enynes in which the alkyne moieties are conjugated with a carbonyl group.

Enyne **2a** (see Table 1) was chosen as a model substrate. Palladium catalysts "HPdOOCH" were prepared in situ by mixing commercially available palladium compounds (2–5 mol %) with 1.5–3.0 equivalents of formic acid based on the amount of **2a**. Palladium compounds investigated were Pd(OAc)₂, [Pd(PPh₃)₄], [Pd₂(dba)₃], [PdCl₂(PPh₃)₂], [PdCl₂(dppe)], and [Pd₂Cl₂(π -C₃H₅)₂] under various conditions. Among these palladium compounds, Pd(OAc)₂ and [Pd(PPh₃)₄] were shown to be the most effective. Common solvents such as toluene, acetonitrile, and DMF were also tested. We established the optimal conditions as: toluene as solvent, palladium acetate as catalyst (5 mol %), triphenylphosphane as ligand (10 mol %), and formic acid as a reductant (1.5 equiv). The palladium-catalyzed cycloreduction of the enyne **2a** was expected to give the corresponding α,β -unsaturated compound **3a** according to the proposed mechanism (path a) shown in Scheme 1. Under the optimized conditions, enyne **2a** seemed to be initially cyclized to the dienolate **4a**, which was eventually converted to the γ,δ -unsaturated compound **5a** without forming any detectable amount of the expected product **3a**. This initial result prompted us to further examine the possibility of a straight-forward approach to γ,δ -unsaturated carbonyl compounds.

We prepared the easily accessible enynes **2b–j** and followed their cyclizations under the above-mentioned conditions [Eq. (1)], and the results are summarized in Table 1.

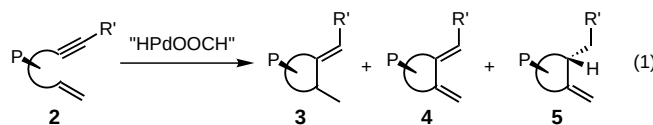


Table 1. Palladium-catalyzed cycloreductions of enynes according to Equation (1).^[a]

Substrates	R'	T [°C], t [h]	Products	Yield
 2a COCH(CH ₃) ₂ 2b COOEt		60, 3	 5a	81
 2c COOEt 2d CoPh 2e COCH(CH ₃) ₂ 2f CONEt ₂ 2g CONPh ₂		65, 2	 5c	65 ^[c]
 2h COPh 2i CONPh ₂		60, 2 80, 0.5	 5h	72
 2j COOEt		60, 12	 5j	40 ^[d]

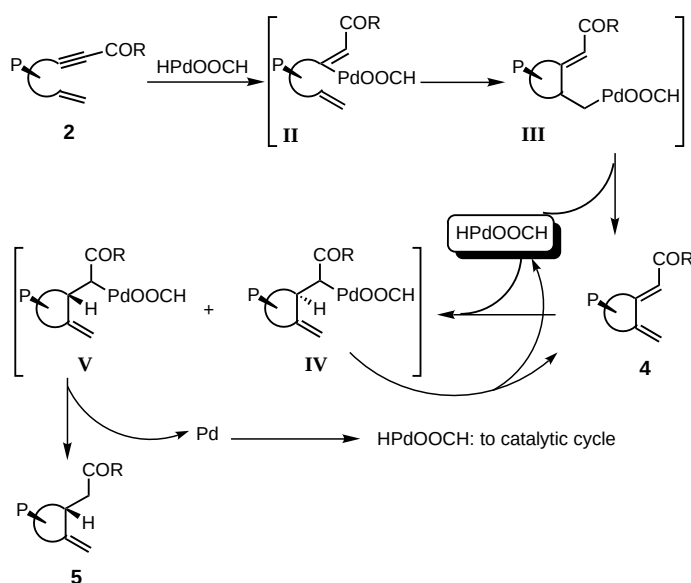
[a] Catalyst: 5 mol % Pd(OAc)₂, 10 mol % PPh₃, 1.5–3.0 equiv HCOOH; substrate **2**: 0.3 M in toluene. [b] Isolated as a 1:5 mixture of the products **3b** and **5b**. [c] About 20% of its epimer was present. [d] Its epimer epi-**5j** (9%) was also isolated.

Several features are noteworthy. First, we demonstrated that this method allows regioselective cycloreductions of carbonyl-conjugated enynes **2a–j** to the corresponding γ,δ -unsaturated carbonyl compounds **5a–j** in high yields. Second, aliphatic and aromatic ketone, ester, and amide groups assisted the initial hydropalladation under mild conditions: all reactions were completed within 2–10 h at 60–80 °C. Even the highly sterically hindered enyne **2j** underwent cycloreduction to the corresponding products **5j** and its epimer epi-**5j** in 40% and 9% yields, respectively. Finally, we should emphasize the excellent stereoselectivity, showing that the enynes **2d–i** were cycloreduced to the γ,δ -unsaturated carbonyl compounds **5d–i** along with their epimers in diastereomer ratios ranging from 4:1 to greater than 99:1, as determined by ¹H NMR analyses of the crude products.

When a catalytic amount of formic acid (10 mol %) was employed for the enyne **2g**, we isolated the corresponding diene **4g** in 72% yield.^[11] In a separate experiment, addition of a stoichiometric amount of formic acid to the diene **4g** in the presence of the palladium catalyst was shown to furnish the γ,δ -unsaturated product **5g** in 64% yield. These observations suggested that the reaction involves two steps: palladium-catalyzed cycloisomerization to the corresponding diene **4**, followed by reduction into the γ,δ -unsaturated products **5** mediated by hydropalladium formate. It is worth

noting here that hydrogenation of 1,3-dienes conjugated with a carbonyl group, such as **4**, have been studied by Cortese and Heck.^[12] They found that, with 1.1 equiv of formic acid and a palladium catalyst, the conjugated dienone β -ionone was mainly reduced to the corresponding γ,δ -unsaturated enone, while methyl sorbate was mainly reduced to methyl 2-hexenoate along with the side-product methyl 5-hexenoate.

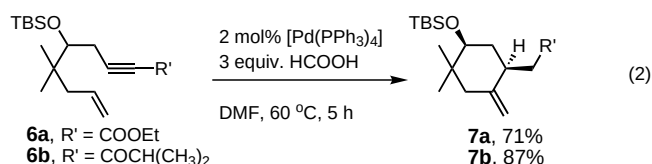
The relative configurations of representative products (**5c**, **5i**, and **5j**) were unambiguously determined by NOESY experiments. These NMR experiments were also undertaken to clarify some mechanistic details of the cycloreductions, and why, in contrast to our original expectation, they do not lead to the thermodynamically more stable products. One might speculate that hydropalladation of the diene **4** gave a diastereomeric mixture of the intermediates **IV** and **V** (Scheme 2). While the intermediate **V** formed by attack at



Scheme 2. Carbonyl-conjugated enyne **2** offers two possibilities to coordinating to the palladium-based catalyst (**II**, **III**) to form the carbonyl-conjugated diene **4** after β -elimination. Diene **4** may then in turn coordinate to the catalyst to form two diastereoisomers (**IV**, **V**). Intermediate **IV** β -eliminates back to **4** and the catalyst, whereas the reductive cleavage of **V** produces **5** regio- and stereoselectively.

the more-hindered face of the carbonyl-conjugated diene **4** would undergo reductive cleavage to the thermodynamically stable product **5**, the intermediate **IV** resulting from attack at the less-hindered face might undergo the reverse reaction, β -elimination, to reproduce the carbonyl-conjugated diene **4**. Repeating the above processes could result in high stereoselectivity during formation of product **5**.

We have extended this method to the 1,7-enynes **6a** and **6b**, activated with electron-withdrawing ester and ketone groups, respectively. These enynes, under the above reaction conditions, underwent cycloreductions in moderate yields. Optimization experiments led to a dramatically improved reaction efficiency [Eq. (2)]. Enyne **6a** was converted to the γ,δ -unsaturated ester **7a** as a single diastereomer in 71% yield.^[13] When a catalytic amount of formic acid was used under similar conditions, we also detected the corresponding diene,



analogous to **4**, as a major product that was reduced further to the γ,δ -unsaturated ester **7a** upon addition of an equivalent of formic acid to the reaction mixture. Likewise, the 1,7-enyne **6b**, activated with a ketone group, furnished the desired γ,δ -unsaturated ketone **7b** in 87% yield. It was proven by NOESY experiments that product **7b** has the more stable *cis* configuration.

In conclusion, we have shown that cycloreductions of 1,6- and 1,7-enynes, in which the alkyne unit is conjugated with an electron-withdrawing group, provide an entry to γ,δ -unsaturated compounds like **5** and **7** in a highly regio- and stereoselective manner. Mechanistically, the reaction proceeds in two consecutive steps: β -elimination to the carbonyl-conjugated dienes **4**, then regio- and stereoselective reduction to the γ,δ -unsaturated carbonyl compounds **5**. The present study should be of considerable use for syntheses of γ,δ -unsaturated carbonyl compounds and also should be of importance for mechanistic insight into enyne cyclizations.

Experimental Section

Typical procedure for cyclization of enyne **2a**: In a 5 mL test tube the enyne **2a** (38.0 mg, 0.15 mmol), PPh_3 (3.9 mg, 0.015 mmol), $\text{Pd}(\text{OAc})_2$ (1.7 mg, 0.0075 mmol), and dry toluene (0.5 mL) were added. The resulting mixture was treated with HCOOH (10 μL , 0.26 mmol) under an Ar atmosphere. The mixture was stirred for 10 min at room temperature and for 3 h at 60 °C. The reaction mixture was concentrated and separated by silica gel chromatography (eluent: ethyl acetate/hexane (5/95)) to give the cyclized product **5a** (31.1 mg, 81%) as a colorless oil. ^1H NMR (400 MHz, CDCl_3): δ = 4.85 (d, J = 2.0 Hz, 1H), 4.69 (d, J = 2.0 Hz, 1H), 3.34 (s, 3H), 3.38 (s, 3H), 3.24 (s, 4H), 3.00 (m, 1H), 2.74 (dd, J = 16.8, 5.2 Hz, 1H), 2.61 (sept, J = 6.8 Hz, 1H), 2.48 (dd, J = 16.8, 8.4 Hz, 1H), 2.28 (s, 2H), 1.96 (dd, J = 12.8, 8.0 Hz, 1H), 1.12 (d, J = 6.8 Hz, 3H), 1.11 (d, J = 6.8 Hz, 3H), 1.11 (dd, J = 12.8, 6.4 Hz, 1H); ^{13}C NMR (100 MHz, CDCl_3): δ = 217.20, 154.36, 105.12, 75.63, 74.76, 59.33, 59.28, 45.76, 45.53, 41.13, 39.43, 38.51, 37.42, 18.33, 18.28; FT-IR (neat): $\tilde{\nu}$ = 2962, 2928, 2875, 1711, 1650, 1455, 1206, 1158, 1107 cm^{-1} ; HRMS for $\text{C}_{15}\text{H}_{26}\text{O}_3$ (M^+): calcd: 254.1882; found: 254.1880.

Characterization data for the products **5a–j** and **7a, b** and NOESY experimental data for **5c**, **5i**, **5j**, and **7b** is found under the Supporting Information.

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 [13] All products **5a–j** and **7a, b** were characterized by ^1H and ^{13}C NMR, FT-IR, and high resolution mass spectra, and were shown to be stereochemically pure (detection limits >99:1) unless otherwise noted.

Guest-Templated Selection and Amplification of a Receptor by Noncovalent Combinatorial Synthesis**

Mercedes Crego Calama, Peter Timmerman, and David N. Reinhoudt*

Template-directed synthesis, using molecular recognition to favor the formation of one particular product, plays an essential role in important biological processes, such as protein synthesis and self-replication. It has been widely used in a variety of macrocyclization reactions^[1] and (in)organic self-assembly processes.^[2] Molecular imprinting of synthetic receptors by guest templation, a process first realized in polymers,^[3] has recently received enormous attention as a way to direct the molecular evolution of a dynamic mixture of synthetic receptors.^[4–7] Thermodynamic equilibration of these receptor systems is achieved through the reversible formation of *covalent* bonds, namely, *cis–trans* isomerization,^[4] transesterification,^[5, 8] or disulfide bond formation.^[6] Here we describe the first example of guest-templated selection of a

receptor in a dynamic *noncovalent* combinatorial mixture, in which the different receptors equilibrate through the reversible formation of multiple hydrogen bonds.

Recently we have reported^[9] the noncovalent synthesis^[10] of a family of hydrogen-bonded assemblies $\mathbf{1}_3 \cdot (\text{DEB})_6$ (DEB = 5,5-diethylbarbituric acid), consisting of nine different components held together by 36 cooperative hydrogen bonds. Structural diversity at the supramolecular level can be generated in an extremely simple way, by mixing the various components **1** (**a, b, ...N**; Figure 1) under thermodynamically controlled conditions.^[11] In this way a library of 220 different noncovalent assemblies can be synthesized simply by assembling a mixture of ten different components derived from **1**.^[12] The composition of these libraries is statistical as determined for the model system $\mathbf{1a}_{3-n} \cdot \mathbf{1b}_n \cdot (\text{DEB})_6$ ($n = 0–3$), which exists as a 1:1:3:3 mixture of homomeric ($\mathbf{1a}_3 \cdot (\text{DEB})_6$ and $\mathbf{1b}_3 \cdot (\text{DEB})_6$) and heteromeric ($\mathbf{1a} \cdot \mathbf{1b}_2 \cdot (\text{DEB})_6$

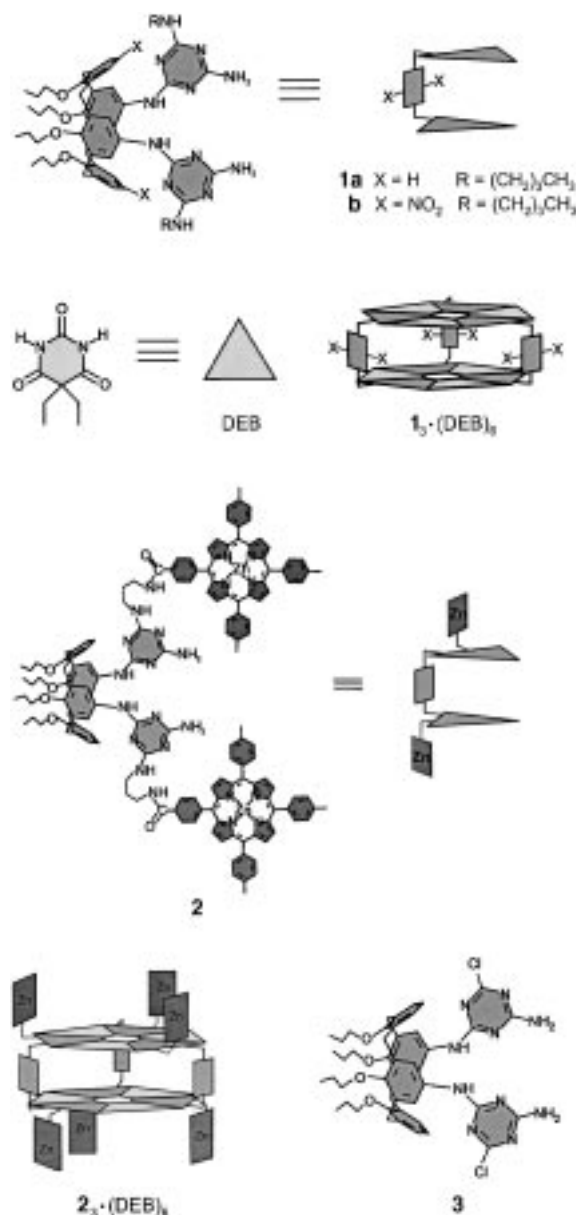


Figure 1. Molecular structures and schematic representations of the molecular components **1–3** and the hydrogen-bonded assemblies $\mathbf{1}_3 \cdot (\text{DEB})_6$ and $\mathbf{2}_3 \cdot (\text{DEB})_6$.

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and $1a_2 \cdot 1b \cdot (DEB)_6$ assemblies. However, none of the assemblies in this mixture contains a functional binding site for guest complexation.

To study the guest-templated selection of receptor $2_3 \cdot (DEB)_6$ in the noncovalent combinatorial mixture $2_{3-n} \cdot 1b_n \cdot (DEB)_6$ ($n=0-3$, Figure 2), we synthesized dimelamine **2**, with two opposed zinc-porphyrin moieties.^[13] It is known from previous work by Sanders and Anderson^[14] that zinc porphyrins complex pyridine derivatives with moderate affinity ($K_a = 3 \times 10^3 M^{-1}$ in $CDCl_3$) and that the binding of tripyridines to cyclic multiporphyrin arrays is much stronger ($K_a \approx 10^{10} M^{-1}$ in $CDCl_3$) as a result of cooperativity between the porphyrin centers.^[15] Similarly, we anticipated that all four assemblies in the dynamic mixture $2_{3-n} \cdot 1b_n \cdot (DEB)_6$ ($n=0-3$) will bind the tripyridine **4** with very different affinities depending on the number of preorganized zinc-porphyrin centers that cooperate in the binding process. The addition of **4** to a statistical mixture (1:3:3:1) of the assemblies $2_{3-n} \cdot 1b_n \cdot (DEB)_6$ ($n=0-3$) will then shift the thermodynamic equilibrium of the mixture towards the strongest **4**-binding assembly, the homomeric $2_3 \cdot (DEB)_6$, thereby reducing the concentrations of the heteromeric assemblies $2 \cdot 1b_2 \cdot (DEB)_6$ and $2_2 \cdot 1b \cdot (DEB)_6$ (see Figure 2). In this way, the tripyridine **4** serves as a guest molecule that drives the chemical evolution of assembly $2_3 \cdot (DEB)_6$ in the dynamic mixture $2_{3-n} \cdot 1b_n \cdot (DEB)_6$ ($n=0-3$).

First we studied the hydrogen-bond directed formation of the all-porphyrin assembly $2_3 \cdot (DEB)_6$ alone and its binding to **4**. Assembly $2_3 \cdot (DEB)_6$ contains two identical binding sites, one on top and one on the bottom of the assembly, each composed of three zinc-porphyrin moieties. Mixing of free **2**, the ^1H NMR spectrum of which mainly shows broad resonances (Figure 3A), with 2.0 equivalents of DEB in $CDCl_3$, leads to the clean self-assembly of $2_3 \cdot (DEB)_6$ as judged from the characteristic signals at $\delta = 13.3$ and 14.1 for

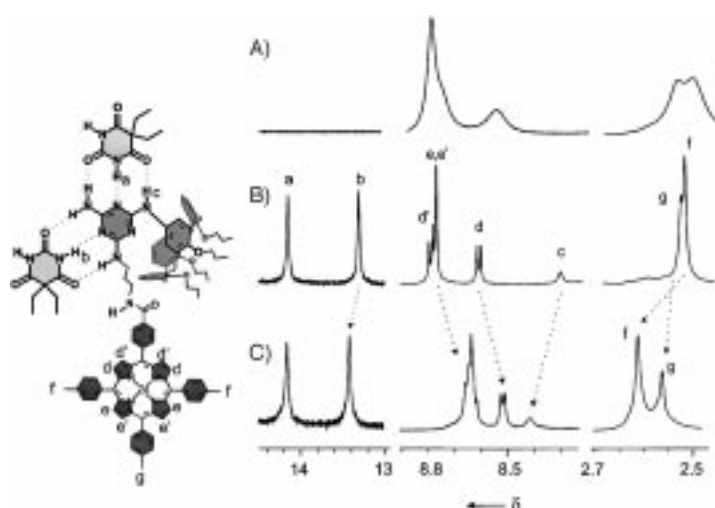


Figure 3. Parts of the ^1H NMR spectra of A) free **2**; B) assembly $2_3 \cdot (DEB)_6$; C) assembly $2_3 \cdot (DEB)_6$ plus 2.0 equiv of **4**. All spectra were recorded at 300 MHz in $CDCl_3$ at room temperature.

the hydrogen-bonded NH_a and NH_b protons (Figure 3B). Complexation studies of $2_3 \cdot (DEB)_6$ with **4** showed the quantitative formation of the corresponding complex $2_3 \cdot (DEB)_6 \cdot 4_2$ and clearly proved the 1:2 stoichiometry. For example, addition of 1.4 equivalents of **4** to assembly $2_3 \cdot (DEB)_6$ causes several signals in the ^1H NMR spectrum to shift, but the signals of the resulting spectrum are severely broadened, most probably as a result of the slow exchange between assembly $2_3 \cdot (DEB)_6$ and the 1:2 complex $2_3 \cdot (DEB)_6 \cdot 4_2$. However, when 2.0 equiv of **4** are present, a well-defined spectrum with sharp peaks is obtained in which some of the porphyrin and dimelamine proton signals are shifted significantly (Figure 3C). The NH_b and NH_c proton signals both shift downfield ($\Delta\delta = 0.10$ and 0.14, respectively), while the NH_a proton signal is unaffected. In contrast, all

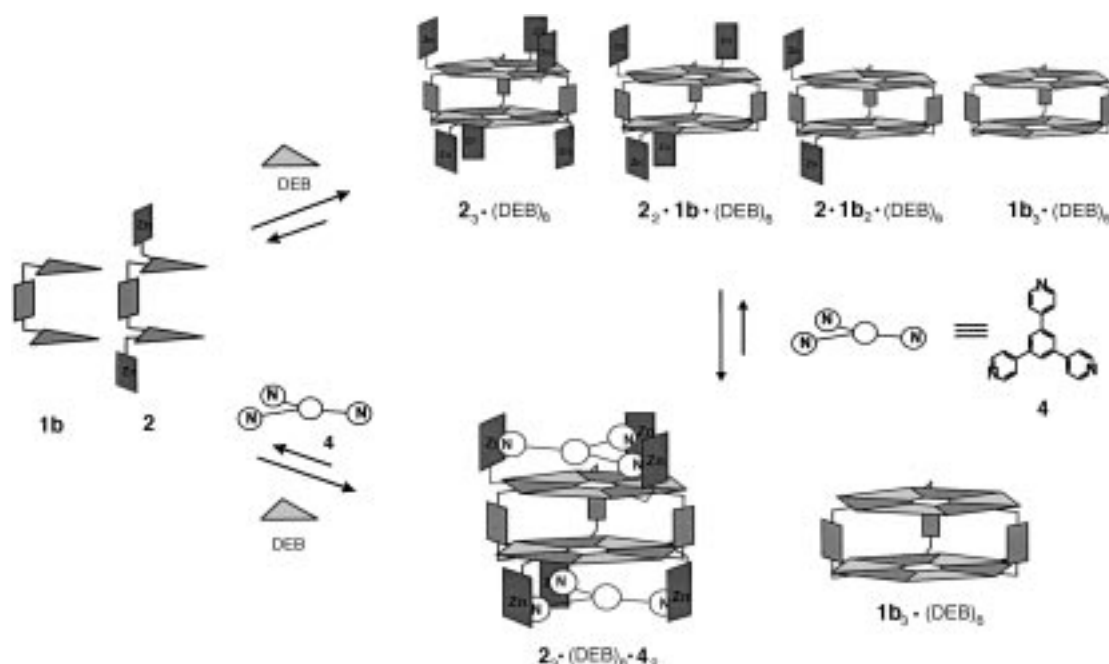


Figure 2. Schematic representation of the self-assembly of the 4-component dynamic mixture $1b_{3-n} \cdot 2_n \cdot (DEB)_6$ ($n=0-3$) and posterior templated selection of the best receptor upon the addition of guest molecule **4**.

signals of the β -pyrrol protons ($H_{d,e}$) of the porphyrin moieties shift upfield ($-\Delta\delta = 0.10$ – 0.15). Moreover, the chemically nonequivalent H_e protons show an AB-pattern in the complex $2_3 \cdot (\text{DEB})_6 \cdot 4_2$, whereas the spectrum of the free assembly $2_3 \cdot (\text{DEB})_6$ exhibited only one single frequency at $\delta = 8.78$. Apparently, in the presence of guest molecule **4** the chemical difference between the two H_e protons is larger. Finally, the complexation of **4** causes a $\Delta\delta = 0.12$ ($\delta = 2.50 \rightarrow 2.62$) and $\Delta\delta = 0.04$ ($\delta = 2.51 \rightarrow 2.55$) shift for the H_f and the H_g proton signals, respectively, resulting in an exchange of their relative position in the spectrum. Addition of more than two equivalents of **4** does not shift any of the proton signals further than in the 1:2 complex $2_3 \cdot (\text{DEB})_6 \cdot 4_2$, indicating that the affinity of **4** for assembly $2_3 \cdot (\text{DEB})_6$ is very high indeed.

Subsequent to the formation studies, we investigated to what extent **4** can shift the thermodynamic equilibrium in the dynamic mixture $2_{3-n} \cdot 1b_n \cdot (\text{DEB})_6$ ($n = 0$ – 3). The mixture was prepared by mixing equimolar solutions of the individual assemblies $1b_3 \cdot (\text{DEB})_6$ and $2_3 \cdot (\text{DEB})_6$ in CDCl_3 at room temperature. The H_c – H_e ($\delta = 8.0$ – 9.0) and the H_f , H_g proton signals ($\delta = 2.5$ – 2.7) clearly illustrate the mixing process (Figure 4A–C).^[16] For the H_c proton signal of the mixture $2_{3-n} \cdot 1b_n \cdot (\text{DEB})_6$ ($n = 0$ – 3) at least five signals ($\delta = 8.3$ – 8.5) are present, while this proton appears as a single resonance at $\delta = 8.32$ for the pure homomeric assembly $2_3 \cdot (\text{DEB})_6$ (Figure 4A). Similarly, the H_d , H_e and the H_f , H_g protons of the mixture give rise to multiple resonances. Integration of the various proton signals clearly proves the almost statistical

composition of the mixture (30% versus 25% homomeric, 70% versus 75% heteromeric).^[17]

The addition of two equivalents of **4** to the dynamic mixture $2_{3-n} \cdot 1b_n \cdot (\text{DEB})_6$ ($n = 0$ – 3) shifts the thermodynamic equilibrium of the mixture towards the maximized formation of the strongest receptor $2_3 \cdot (\text{DEB})_6$. Evidence for this comes from the complete disappearance of the proton signals for the heteromeric assemblies $2 \cdot 1b_2 \cdot (\text{DEB})_6$ and $2_2 \cdot 1b \cdot (\text{DEB})_6$ (Figure 4D). From the five H_e proton resonances at $\delta = 8.3$ – 8.4 , only two signals for the homomeric assemblies $1b_3 \cdot (\text{DEB})_6$ and $2_3 \cdot (\text{DEB})_6 \cdot 4_2$ (marked with $\blacktriangle$) are present after addition of **4**. Similarly, three of the four resonances for the H_d protons at $\delta = 8.6$ – 8.8 disappear and only the single doublet at $\delta = 8.58$ for the 1:2 complex $2_3 \cdot (\text{DEB})_6 \cdot 4_2$ is observed. Finally, the six resonances for the H_f , H_g proton signals present in the library convert to the two singlets at $\delta = 2.55$ and 2.62 (Figure 4D) of the complex $2_3 \cdot (\text{DEB})_6 \cdot 4_2$ (Figure 4E).^[18] The assembly $1b_3 \cdot (\text{DEB})_6$ does not display any proton signals in this region (Figure 4B). These results clearly show that guest molecule **4** efficiently templates the formation of the strongest binding assembly in the library, $2_3 \cdot (\text{DEB})_6$, by shifting the thermodynamic equilibrium in the four-component mixture $2_{3-n} \cdot 1b_n \cdot (\text{DEB})_6$ ($n = 0$ – 3) from an almost statistical composition (ca. 1:3:3:1) of all possible assemblies, towards a 1:1 mixture of the homomeric assemblies $1b_3 \cdot (\text{DEB})_6$ and $2_3 \cdot (\text{DEB})_6$.

In conclusion, we have described the guest-templated selection and amplification of the strongest binding receptor in a dynamic mixture of hydrogen-bonded assemblies. These noncovalent receptors are reminiscent of antibodies in the sense that they contain both a constant region, consisting of three functionalized calix[4]arenes dimelamines subunits held together in a noncovalent fashion, and a variable region generated by the introduction of a variety of substituents on the six melamine units.^[19] Current work in our group is aimed at the covalent capture of supramolecular libraries, a novel strategy to enable characterization by MALDI-TOF MS and HPLC.

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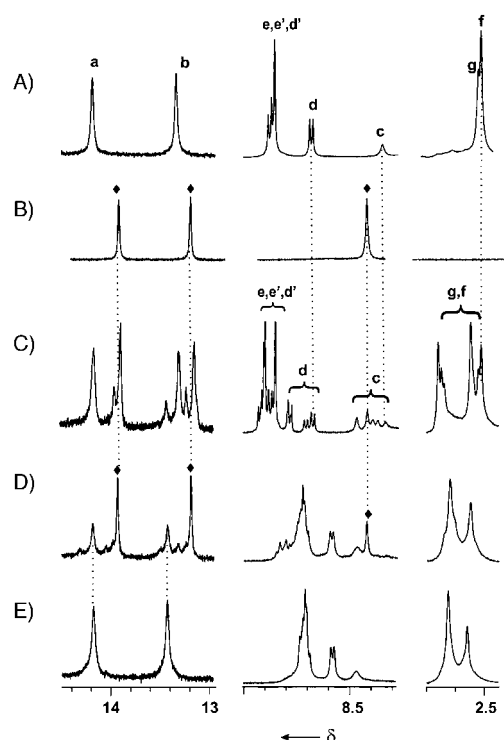


Figure 4. Parts of the ^1H NMR spectra of A) assembly $2_3 \cdot (\text{DEB})_6$; B) assembly $1b_3 \cdot (\text{DEB})_6$; C) 1:1 mixture of assemblies $1b_3 \cdot (\text{DEB})_6$ and $2_3 \cdot (\text{DEB})_6$; D) 1:1 mixture of assemblies $1b_3 \cdot (\text{DEB})_6$ and $2_3 \cdot (\text{DEB})_6$ after addition of two equivalents of **4**; E) assembly $2_3 \cdot (\text{DEB})_6$ plus two equivalents of **4**. All spectra were recorded at 300 MHz in CDCl_3 at room temperature. The signals marked with $\blacklozenge$ are from $1b_3 \cdot (\text{DEB})_6$ protons.

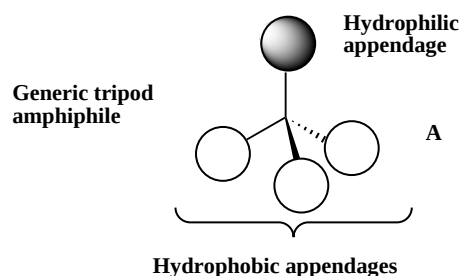
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- [17] The experimental distribution was determined by integration of the proton signals in the $\delta = 2.5$ –2.7 region ($\text{H}_{\text{f,g}}$). The calculated distribution of 25/75% reflects the threefold higher probability for formation of the heteromeric assemblies.
- [18] The additional small signals present in spectrum 4D ($\delta = 13$ –15, around $\delta = 8.8$, and the two shoulders of the singlet at $\delta = 2.62$) are due to the presence of a slight excess of **4**, giving rise to the formation of small amounts of the heteromeric assembly $1\text{b}_2 \cdot 2 \cdot (\text{DEB})_6 \cdot 4_2$. Evidence for this comes from the fact that the intensity of these signals increases when **4** is present in larger excess.
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Rigid Amphiphiles for Membrane Protein Manipulation**

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The shape of an amphiphile strongly influences self-association in solution^[1] and in liquid crystalline phases,^[2] as well as interactions with self-assembled structures such as lipid bilayers.^[3] In recent years several groups have examined unusual amphiphile topologies.^[4,5] We and others, for example, have explored amphiphiles in which hydrophilic groups project on one side of an approximately planar hydrophobic unit (“contrafacial amphiphiles”).^[4] Here we introduce a related family of molecules based on a rigid quaternary carbon center, “tripod amphiphiles” (**A**), and present evidence that these amphiphiles can solubilize the two non-homologous membrane proteins bacteriorhodopsin (BR) and bovine rhodopsin (Rho) in a stable monomeric state.



Intrinsic membrane proteins perform many crucial functions, including transport, catalysis, photosynthesis, respiration, and signal transduction. The detailed study of membrane protein structure requires that the protein be isolated in a soluble native-like conformation, which in turn requires the use of a synthetic amphiphile (a detergent) to shield large

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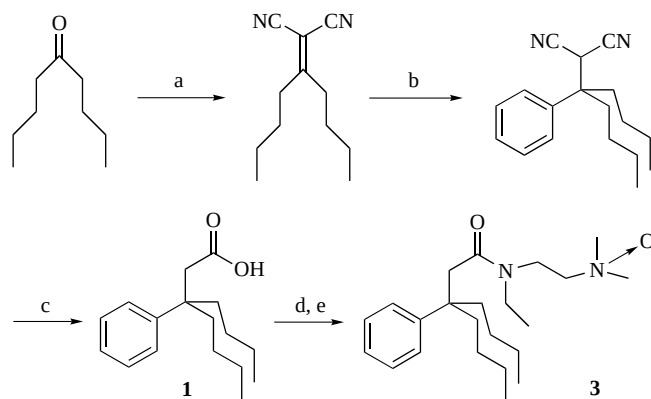
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hydrophobic patches on the protein surface. X-ray crystallography is the best method for obtaining high-resolution structural information on membrane proteins; however, crystallizing membrane proteins has proven to be a significant obstacle to this form of analysis.^[6] Thus, an amphiphile must perform two functions if it is to support crystallization: 1) solubilize the membrane protein in its native form for at least several weeks (long enough for the growth of high-quality crystals), and 2) contribute to the crystalline lattice stability.^[7] Our results show that the first criterion can be fulfilled by a tripod amphiphile, which is an important step toward our long-term goal of using these molecules to facilitate the structural analysis of membrane proteins.

Our interest in tripod amphiphiles was motivated by the hypothesis that crystallization of complexes of membrane proteins and amphiphiles would be promoted by increasing amphiphile rigidity.^[8] The detergents used for the crystallization of membrane proteins usually contain very flexible linear alkyl chains; these detergent molecules remain disordered even when high-quality crystals form.^[7] Tripod amphiphiles are designed to be more conformationally rigid than conventional detergents while retaining the ability to bind to and shield the large hydrophobic surfaces displayed by membrane proteins.^[6] Tripod amphiphiles are built around a quaternary carbon atom that bears one polar and three nonpolar appendages. The quaternary carbon atom introduces a substantial conformational restriction^[9] relative to the linear alkyl chains common among detergents.

Our synthetic route to tripod amphiphiles is modular, as illustrated by the synthesis of acid **1** outlined in Scheme 1, which allows for the facile variation of the hydrophilic and

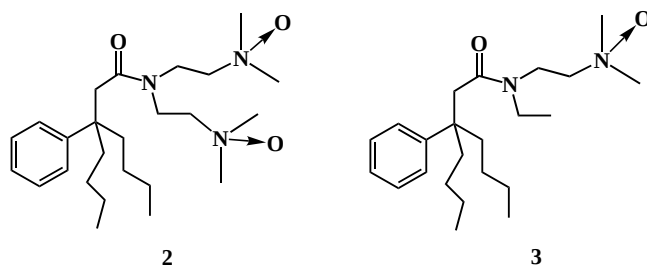


Scheme 1. Synthetic route leading to tripod amphiphile **3**: a) $\text{CH}_2(\text{CN})_2$, AcOH, NH_4OAc , benzene, reflux; b) PhMgBr , CuI, diethyl ether, 0°C ; c) KOH, ethylene glycol, reflux; d) 1) oxalyl chloride, benzene, 0°C ; 2) N -ethyl- N' , N' -dimethylethylenediamine, diethyl ether, 0°C ; e) $m\text{CPBA}$, chloroform, -10°C . $m\text{CPBA}$ = *meta*-chloroperbenzoic acid.

hydrophobic regions.^[10] The conformational flexibility of tripod amphiphiles can be tuned by the choice of hydrophobic appendages. This tunability is important, since a minimum level of amphiphile flexibility is thought to be crucial for membrane protein solubilization.^[11] Conventional detergents used for membrane protein manipulation nearly all bear nonionic or zwitterionic hydrophilic moieties; we selected the N -oxide group, which could be readily introduced through derivatization of **1**, for our initial studies.

We utilized BR solubilization as an initial screen to identify promising tripod amphiphiles. BR is an excellent model system for evaluating membrane protein manipulation methods; several groups have used this protein for solubilization and crystallization studies.^[12] BR assembles naturally into two-dimensional crystalline domains in the *Halobacterium salinarum* membrane, and this BR–lipid assembly (“purple membrane”) is readily isolated. Four conventional detergents have been reported to solubilize BR from the purple membrane: Triton X-100, octylglucoside, nonylglucoside, and octylthioglucoside.^[12] (Many other detergents denature BR.) Triton X-100 is the most effective solubilizer, but there are no reports of any membrane protein being crystallized in the presence of Triton X-100, perhaps because this detergent is heterogeneous and/or because the oligoether portion is highly flexible. Several additional amphiphiles, such as dodecylmalto-side, C_{12}E_8 , reduced Triton X-100, “amphipols”,^[13] CHAP-SO^[14] (a zwitterionic cholate derivative), and a “peptitergent”^[15] (a 24 residue peptide that adopts an amphiphilic α -helix), have been reported to maintain the solubility of BR after extraction from purple membrane with nonylglucoside or TX-100 followed by detergent exchange.^[12] Recently, Landau and Rosenbush demonstrated that high-quality crystals of BR could be obtained by crystallization from a lipidic cubic phase. The crystals obtained provided a high-resolution, three-dimensional crystal structure ($2.8 \text{ \AA}$).^[16]

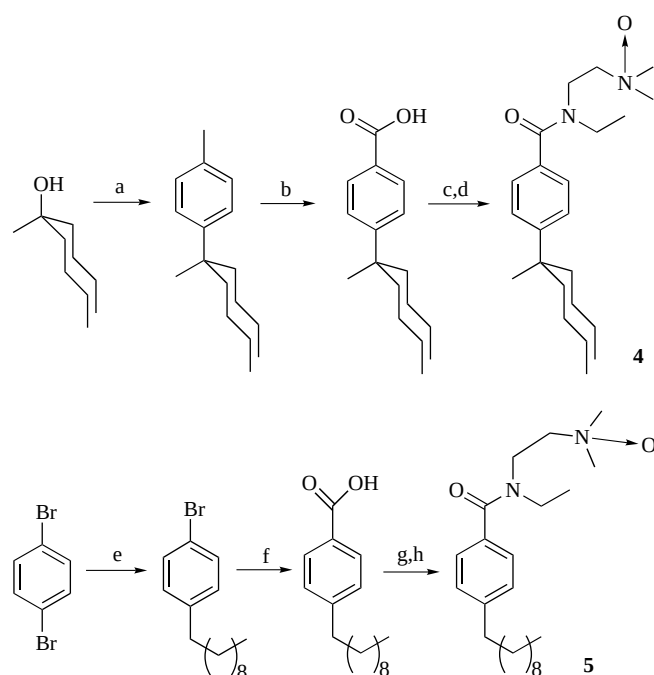
Preliminary experiments suggested that bis- N -oxide **2** was able to solubilize BR from the purple membrane, but subsequent efforts revealed that very pure samples of **2** did not have this capacity. We discovered that heating of **2** during the initial preparation had inadvertently induced a small proportion to undergo Cope elimination. Since the product after double Cope elimination (an N,N -divinyl amide) is probably not soluble in water, we speculated that the intermediate mono- N -oxide was the BR-solubilizing agent. We prepared **3**, a more accessible analogue of the monovinyl intermediate, to test this hypothesis. Tripod amphiphile **3** proved to be very effective at solubilizing BR from the purple membrane. Compound **3** is quite stable if stored at room temperature or below.



Solubilization was carried out by gently rocking a suspension of purple membrane in 25 mM aqueous phosphate buffer, pH 6.9, that contained 100 mM NaCl and tripod amphiphile **3**. The extent of BR solubilization was quantified by measuring the absorbance at 560 nm after the sample had been centrifuged to remove residual purple membrane and other insoluble material. Intact BR was extracted effectively from the purple membrane by solutions containing **3** at or above its

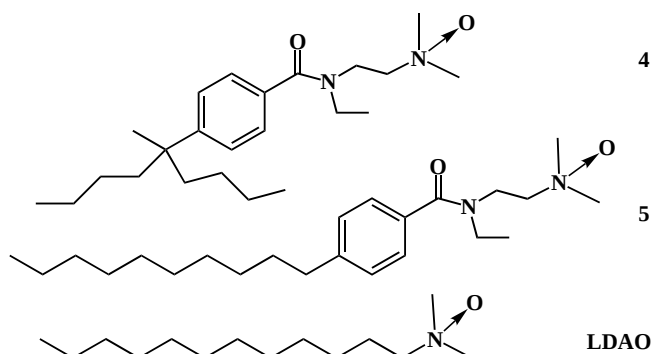
critical micelle concentration (estimated to be 3 mM by the 1,6-diphenylhexatriene solubilization method^[17]). The rate of solubilization was evaluated by following the disruption of the purple membrane by circular dichroism. The close and regular spacing of BR molecules in the purple membrane lattice gives rise to exciton coupling between adjacent retinal Schiff base chromophores; this coupling is abolished when BR is solubilized.^[18] Triton X-100 required more than 3 h for complete solubilization of BR, while solubilization by **3** was complete within 10 minutes. Analytical ultracentrifugation indicated that BR solubilized with 3 mM **3** (near the CMC) sediments as a dimer or higher oligomer, while BR solubilized with 6–12 mM **3** sediments as a monomer. BR solubilized with **3** shows little bleaching when stored at 4 °C (<10%) for 10 days.

We synthesized **4** and **5** (Scheme 2),^[19] which are isomers of **3**, in order to probe the relationship between amphiphile structure and BR solubilization. These isomers represent an



Scheme 2. Synthesis of two constitutional isomers of **3**: a) TiCl_4 , toluene, RT; b) hydroxyphthalimide, $[\text{Co}(\text{acac})_2]$, O_2 , AcOH, RT; c) 1) oxalyl chloride, cat. DMF, benzene, 0 °C; 2) *N*-ethyl-*N*,*N'*-dimethylethylenediamine, diethyl ether, 0 °C; d) *m*CPBA, chloroform, –10 °C; e) $[\text{PdCl}_2(\text{dppf})]$, *n*-decylMgBr, THF/diethyl ether; f) 1) *t*BuLi, diethyl ether, –78 °C; 2) dry CO_2 ; g) 1) oxalyl chloride, benzene, 0 °C; 2) *N*-ethyl-*N*,*N'*-dimethylethylenediamine, diethyl ether, 0 °C; h) *m*CPBA, chloroform, –10 °C.

incremental transformation of the tripod amphiphile architecture into a more conventional detergent architecture, similar to that of lauryldimethylamine oxide (LDAO) with a polar N-oxide group at one end of a linear hydrophobic chain. Amphiphile **4** has a CMC of approximately 0.7 mM, and **5** has a CMC of about 0.1 mM (a CMC of about 2 mM has been reported for LDAO^[20]). Like LDAO, **4** and **5** denature BR when exposed to purple membrane preparations. Thus, within the isomer series **3**–**5**, the tripod amphiphile architecture of **3** confers unique behavior with regard to BR.



The promising behavior of **3** toward BR prompted us to examine the solubilization of Rho with the amphiphile. Rho has been a subject of widespread interest because this protein is a G-protein-coupled receptor and therefore represents a large class of important signaling proteins.^[21] The behavior of Rho with conventional detergents has been extensively studied, but no Rho–detergent complex has yet provided high quality three-dimensional crystals.^[22] Only low-resolution structural information is presently available for Rho, which was obtained from two-dimensional crystals.^[23]

Solubilization of Rho was carried out using rod outer segments (ROS) purified from bovine retina.^[24] A mixture of ROS and 30 mM **3** was incubated for 30 min in 10 mM acetate buffer, pH 6.9, at room temperature. After centrifugation to remove membrane fragments, the supernatant was found to contain 90% intact Rho, as judged by an absorbance at 500 nm.^[25] Rho solubilized by **3** showed less than 10% bleaching after storage for 20 days at 4 °C in the dark.

These results indicate that we have accomplished an important first step toward creating amphiphiles that can be used to crystallize membrane proteins. Growth of high-quality crystals is widely acknowledged to be the bottleneck in the structural analysis of intrinsic membrane proteins.^[6] Low molecular weight amphiphiles are essential for crystallization, but relatively little effort has been devoted to the synthesis and evaluation of alternatives to commercially available detergents. Since the tripod skeleton is easily modified, this new class of amphiphiles could prove to be very useful for membrane protein manipulation. We are currently initiating collaborative crystallization efforts.^[26]

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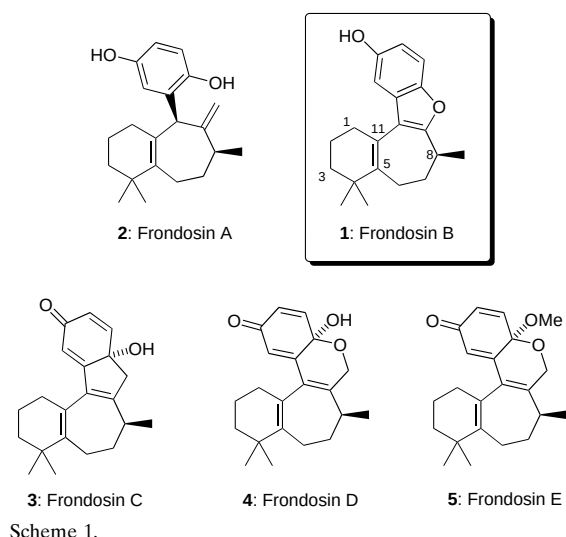
The Total Synthesis of Frondosin B**

Masayuki Inoue, Alison J. Frontier, and Samuel J. Danishefsky*

Interleukin-8 (IL-8), a chemoattractant for neutrophils, is produced by macrophages and endothelial cells.^[1] IL-8 promotes the accumulation and activation of neutrophils and has been implicated in a wide range of acute and chronic inflammatory disorders. Accordingly, blockade of IL-8-mediated chemotaxis represents a possible opportunity for the development of novel pharmacological agents. Frondosins A-E were recently isolated from the sponge *Dysidea frondosa* (Scheme 1). These compounds, which bear a casual relationship to one another, inhibit the binding of IL-8 to its receptor in the low micromolar range.^[2a] The structures and relative stereochemistries of the frondosins were determined primarily by NMR spectroscopy. Their unifying feature is the presence of a bicyclo[5.4.0] ring system attached to variously permuted hydroquinone moieties.^[3] A team from the National Cancer Institute (NCI) also isolated frondosins A and D from the HIV-inhibitory organic extract of the marine sponge.^[2b, 4] It is worth noting that these compounds have optical rotations with different but opposite absolute values. Thus, the frondosins may occur as scalemic mixtures. Further biological studies of the frondosins could spur fruitful

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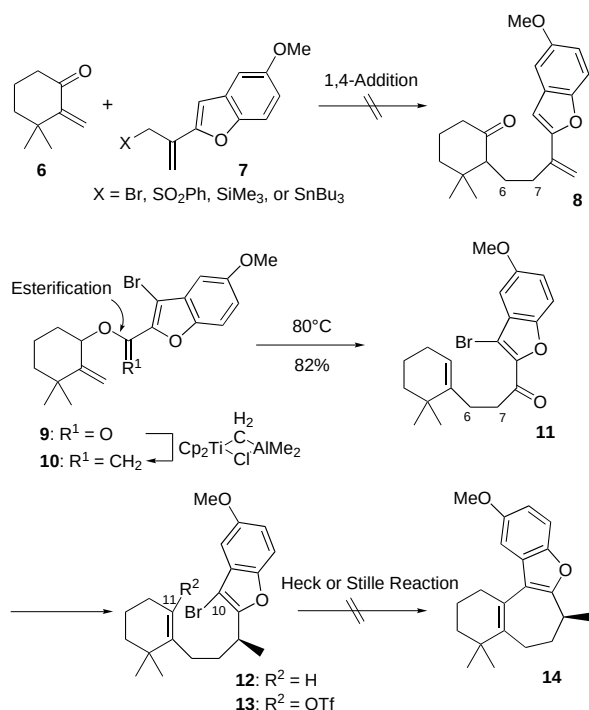
[**] This work was supported by the National Institutes of Health (Grant Numbers: AI16943 and CA08748). M.I. gratefully acknowledges the Uehara Memorial Foundation for a postdoctoral fellowship. A.J.F. gratefully acknowledges the American Chemical Society Organic Division and Merck & Co. for Graduate Fellowship support. We thank Dr. George Sukenick of the MSKCC NMR Core Facility and Vinka Parmakovic of the Columbia University Mass Spectral Facility for mass spectral analyses.



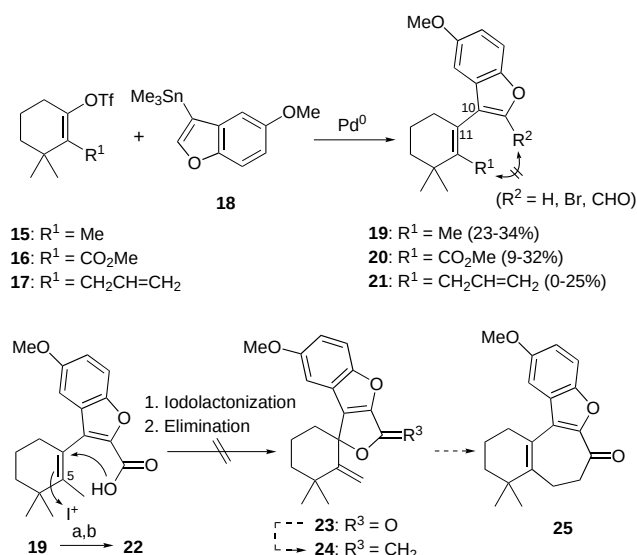
investigations into IL-8 inhibitory activity and thereby offer new opportunities for the development of novel anti-inflammatory agents. These considerations prompted a synthetic venture directed toward the frondosins. In the opening phase of the inquiry we focused on frondosin B (**1**),^[2a] which contains an intriguing benzofuran ring system fused to a norsesquiterpenoid (14 carbon) framework. The other frondosins exhibit a 15 carbon atom framework. Herein we describe a highly efficient total synthesis of (+)-frondosin B.

We considered and evaluated a variety of strategies that seemed to hold promise for particularly concise total syntheses of frondosin B. In reality, many seemingly straightforward programs could not be implemented. Of course we emphasize that our failures with many attractive routes to frondosin B do not necessarily rule out possibilities for success by others who pursue these or related approaches.^[5] In our case, there was much learned from these forays. Indeed, the plan by which the total synthesis of frondosin B was achieved evolved from appraisal of earlier setbacks described below and more fully elsewhere. Our first generation strategy was based on achieving a preliminary union of the C6-C7 bond, which would pave the way for ring formation by connecting C10 and C11 (Scheme 2). Early indications of the difficulties, presumably steric in nature, associated with bond formation in the region of the geminal methyl groups were seen in our inability to couple a variety of agents of the type **6** and **7** to reach **8**. Eventually, successful solution to the C6-C7 union was accomplished by intramolecular means (**9**→**11**). However numerous attempts to achieve transition metal induced closure of the C10-C11 bond (compare inter alia substrates **11**–**13**) by intramolecular Heck or Stille reactions were to no avail.^[6]

Our second generation strategy contemplated the reverse order of steps, that is, initial union between C10 and C11 followed by cyclization through merger of C6 and C7. This plan was undercut by a series of unanticipated difficulties, which attended the proposed transition metal mediated coupling reactions en route to seco compounds of the type **19**–**21** (Scheme 3).^[7] The few cases in which small-scale reactions produced these products in low yields could not be



Scheme 2. First generation strategy.



Scheme 3. Second generation strategy. a) POCl₃, DMF, (CH₂Cl)₂, reflux, 36% (65% based on recovered starting material), b) AgNO₃, aq NaOH, 75%.

scaled up to practical levels. Furthermore, no subsequent closures in which R² on benzofuran could be joined with R¹ on the dimethylcyclohexane ring could be achieved, despite testing several permutations.

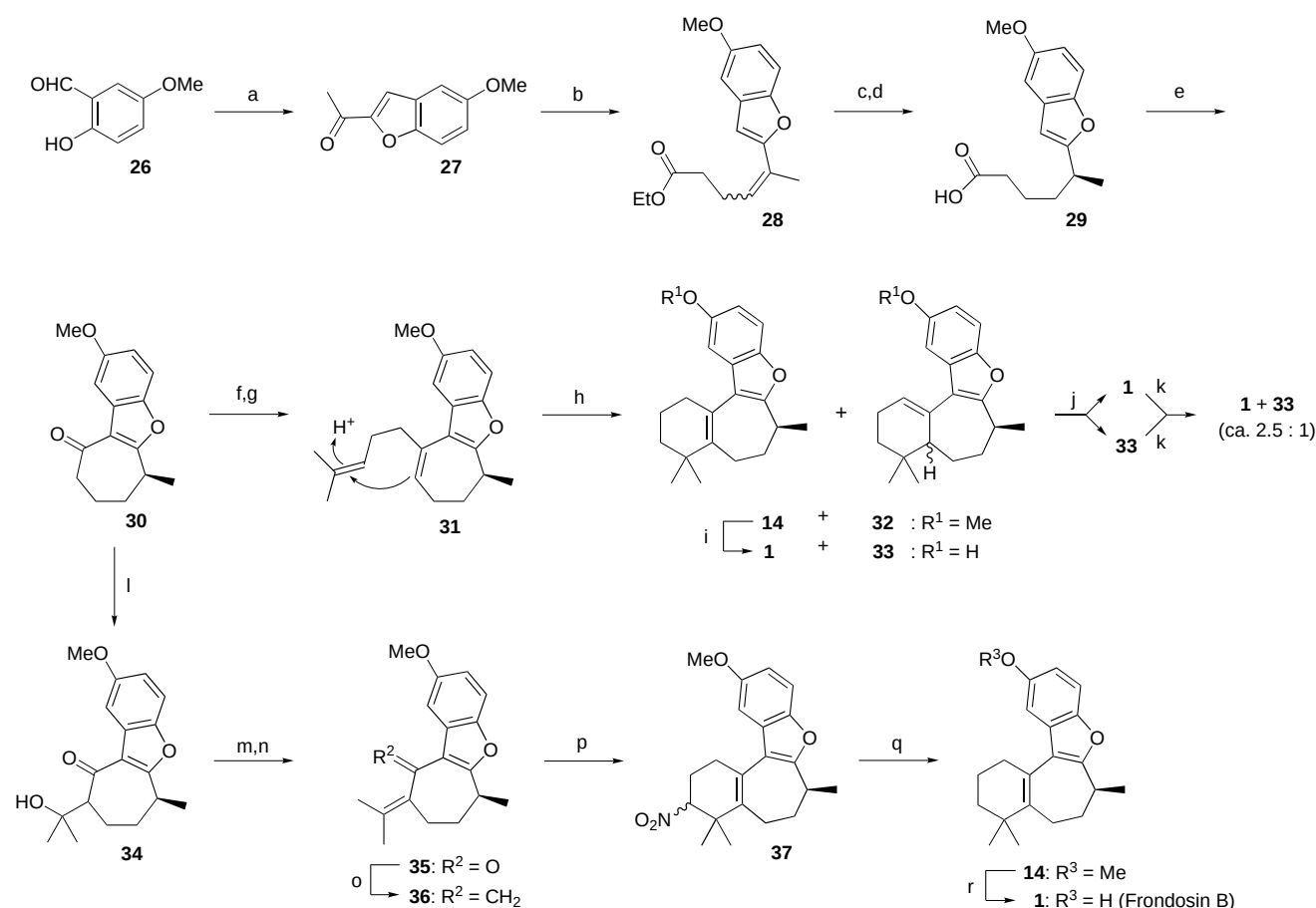
One such C10-C11 coupling that could marginally be conducted on small scale was that of **15** + **18**→**19**. The latter was converted, as shown, into **22**. The thought was to reach **25** via **23** and **24**. Unfortunately, this program failed at the stage of the projected iodolactonization (**22**→**23**), once again reflecting the difficulties of attack by a viable iodonium equivalent at C5, even in the presence of a proximal, and presumably participating carboxylate function.

As a consequence of these difficulties, it was decided to target an intermediate structure of the type **30** and defer construction of the A ring corresponding to the 4,4-dimethylcyclohexene moiety until the last phase of the effort. Commercially available 5-methoxysalicylaldehyde (**26**) was converted into **30** by way of **27**^[8]–**29** (Scheme 4). Homoprenylation of **30**,^[9] gave rise to a labile adduct that underwent dehydration to afford **31**. Finally, treatment of **31** with formic acid gave an 81 % yield of a mixture of isomers.^[10] The major product (56 %) was in fact *O*-methylfrondosin B (**14**). However, this compound was accompanied (ca. 24 %) by a 1:1 mixture of the stereoisomers corresponding to **32**. The ratio of **14**:**32** and the ratio of the stereoisomers **32** seemed to be virtually independent of the acidic conditions employed to bring about cyclization of **31**. Demethylation of the anisole moiety was accomplished on the mixture of **14** and **32** with BBr_3 . After considerable efforts it proved possible to achieve separation of the products by preparative HPLC to afford fully synthetic and homogeneous frondosin B (**1**) as well as diastereomers **33**.

It was then found that the treatment of either of these materials with *p*-TsOH in benzene created the same 2.5:1 mixture of **1**:**33**. These data seem to confirm that the mixture

of double bond isomers (**14** and **32** or **1** and **33**) that we obtain reflects thermodynamic equilibration and that this equilibration is quite rapid under strongly acidic conditions. Accordingly, to achieve control in our synthesis, it would be necessary to construct the dimethylcyclohexene from **30** without recourse to acidic catalysis in the critical steps leading to or following formation of the fully mature A ring.

Given these constraints, which were now properly recognized, we proceeded as follows. Condensation of **30** (via its zinc enolate) with acetone led to **34**^[11] and thence, following dehydration, to **35**. Upon methylenation by the Tebbe method,^[12] **36** was in hand. Remarkably, a Diels–Alder reaction with nitroethylene occurred at 80 °C to afford **37** as a mixture of nitro stereoisomers.^[13] Reductive de-nitration^[14] of **37** produced the previously mentioned **14** with no contamination from double bond isomers. Finally, de-methylation of **14** was carried out with sodium ethyl sulfide.^[15] Frondosin B (**1**) was produced free of double bond isomers under these basic conditions. Although a trace specimen sample of the natural product that we received had entirely decomposed, we could still unambiguously establish the nature of our synthetic endpoint by independent spectroscopic analysis and comparisons with published data.



Scheme 4. Third and fourth generation strategies. a) Chloroacetone, K_2CO_3 , 2-butanone, 80 °C, 72 %; b) $\text{Br-Ph}_3\text{P}^+(\text{CH}_2)_3\text{CO}_2\text{Et}$, $\text{NaN}(\text{SiMe}_3)_2$, THF, 0 °C → RT, 87 %; c) H_2 , Pd/C, EtOH, RT; d) LiOH, THF/MeOH/ H_2O , 100 % (over 2 steps); e) $(\text{COCl})_2$, CH_2Cl_2 , reflux, then SnCl_4 , $-78 \rightarrow -10$ °C, 67 %; f) 4-methyl-3-pentenylmagnesium bromide, CeCl_3 , THF, -78 °C; g) CDCl_3 , 93 % (over 2 steps, endo:exo = 6:1); h) HCOOH , RT, 81 %, (**14**:**32** = ca. 2.5:1); i) BBr_3 , CH_2Cl_2 , -78 °C → RT, 98 %, (**1**:**33** = ca. 2.5:1); j) HPLC separation (silica gel, 5 % ethyl acetate/hexane); k) *p*-TsOH, benzene, 80 °C; l) $\text{LiN}(\text{SiMe}_3)_2$, ZnCl_2 , acetone, THF, -78 °C → -40 °C, 81 % (96 % based on recovered starting material); m) MsCl , Et_3N , CH_2Cl_2 , 0 °C, 88 %; n) NaOMe , MeOH, 0 °C, 96 %; o) Tebbe reagent, pyridine, THF, -40 °C, 97 %; p) excess nitroethylene, di-*tert*-butyl pyridine, 79 %; q) tri-*n*-butyltin hydride, AIBN, toluene, 110 °C, 58 %; r) NaSEt , DMF, reflux, 94 %. AIBN = azobisisobutyronitrile.

In future studies we shall study other frondosins with a view to total synthesis, determination of absolute configuration, and biological evaluation.

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Self-Assembled Porphyrin Polymers**

Ulrike Michelsen and Christopher A. Hunter*

Supramolecular chemistry offers an attractive strategy for the construction of functional and responsive polymeric architectures.^[1] Thermodynamically reversible noncovalent interactions such as hydrogen bonds and metal-ion coordination can be used to induce the self-assembly of suitably functionalized monomers into polymeric structures.^[2, 3] However, the degree of polymerisation in such systems, N , depends critically on the stability constant of the monomer–monomer interaction, K .^[3, 4]

$$N \approx (K [\text{monomer}])^{1/2} \quad (1)$$

Most studies of supramolecular polymer systems have been limited to the solid or liquid-crystalline states, where the value of $[\text{monomer}]$ is maximized and the requirement for a strong monomer–monomer interaction is relaxed.^[2] However, if K is sufficiently large, it is possible to generate very high molecular weight polymers in solution.^[3] These systems have all the physical properties of covalently bound polymers, but in addition are sensitive to both changes in environmental conditions and the presence of small molecules which alter the stability of the monomer–monomer interactions, and hence the degree of polymerisation may be changed in a reversible manner.^[1]

We have previously used the coordination of zinc porphyrins by pyridine ligands to assemble small cyclic oligomers.^[5] Thus, zinc porphyrins, covalently linked to an appropriate pyridine side arm, form macrocyclic dimers with stability constants in the range $K = 10^6$ – 10^8 M^{-1} in chloroform (Figure 1 a). These systems represent ideal building blocks for the formation of supramolecular polymers: Equation (1) predicts a degree of polymerisation of the order of $N = 10$ to 100 at millimolar monomer concentrations. Here, we show that this self-assembly strategy can indeed be used to generate soluble, high molecular weight porphyrin polymers. The rich photo- and electrochemical properties of the monomeric units make such polymers interesting from the point of view of charge storage and transport, solar energy conversion, and nonlinear optics.^[6]

Figure 1 illustrates the approach. Changing the metal from zinc, which is five coordinate in a porphyrin, to cobalt, which is six coordinate, increases the number of vacant ligand binding sites from one to two,^[7] and attachment of a second pyridine side arm yields a monomer with two identical

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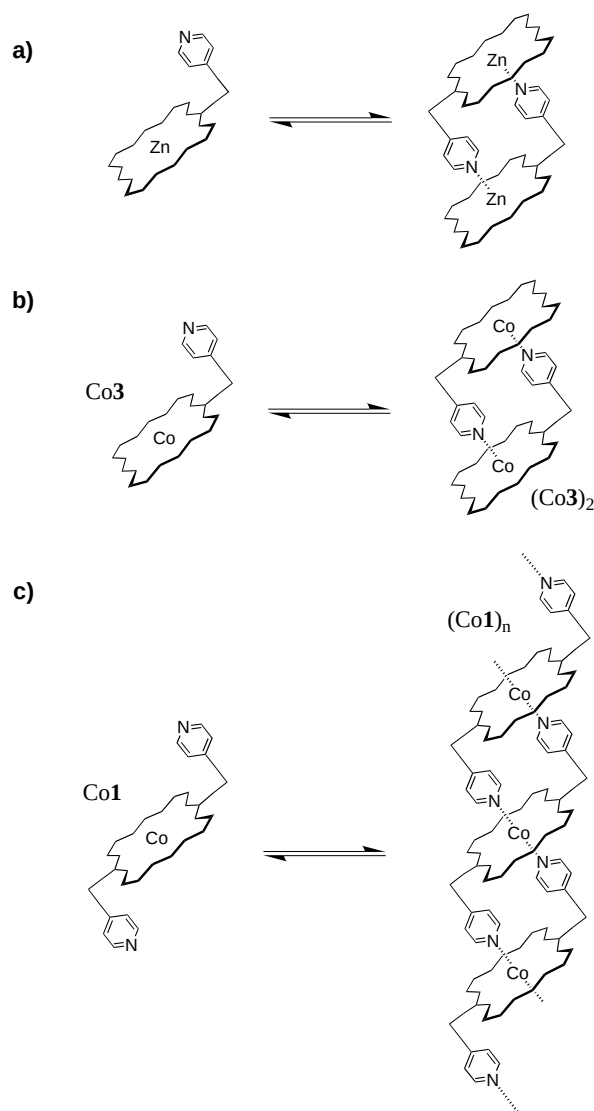
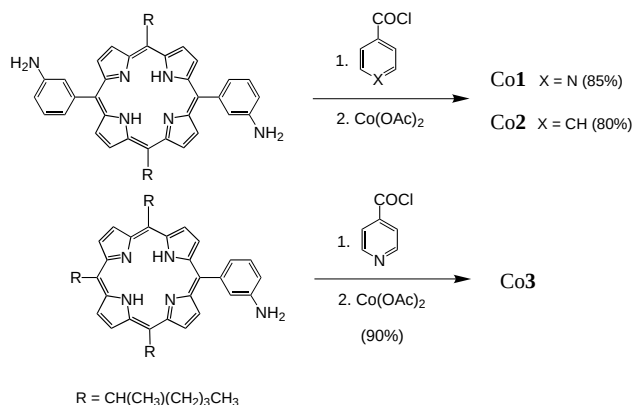


Figure 1. a) Self-assembly of a zinc porphyrin dimer via coordination of a covalently attached pyridine ligand. b) Self-assembly of a cobalt porphyrin dimer via coordination of a covalently attached pyridine ligand. This leaves a vacant ligand binding site on the six-coordinate cobalt center. c) Self-assembly of a cobalt porphyrin polymer via coordination of two covalently attached pyridine ligands, one on each face of the porphyrin.

dimerisation sites on the two faces of the porphyrin. This bifunctional analogue should polymerize through the same monomer–monomer interaction found in the zinc system (Figure 1c).

The synthesis of a suitable monomer for supramolecular polymerisation, Co1, is shown in Scheme 1.^[8] In addition, we prepared two control compounds: Co2 which is not capable of self-assembly, because both pyridine ligands have been replaced by noncoordinating phenyl groups, and Co3 which can only assemble to give dimers, because it carries one pyridine ligand rather than two (Scheme 1). The incorporation of 2-ethylhexyl side chains was essential to ensure good solubility in organic solvents. Since cobalt(II) porphyrins are paramagnetic,^[9] the products were characterized at the free base stage, H₂1, H₂2, and H₂3, and all gave satisfactory spectroscopic data. The metallation reaction of H₂1 with cobalt acetate was sensitive to concentration: the solubility of



Scheme 1. The required aminoporphyrins were prepared from the corresponding nitroporphyrins using literature procedures.^[8]

Co1 is much lower than that of the corresponding free base porphyrin H₂1 and the control compounds Co2 and Co3. At concentrations greater than 10 mM, Co1 precipitated from the reaction mixture and could only be redissolved in coordinating solvents such as pyridine which is consistent with the polymerisation process illustrated in Figure 1c.

The UV/Vis absorption spectrum of the monomeric reference porphyrin Co2 has a Soret band at 415 nm. Addition of pyridine at millimolar concentrations resulted in a red shift of the Soret absorption to 444 nm.^[10] The Soret band of both Co1 and Co3 is at 440 nm at micromolar porphyrin concentrations.^[9] This implies that cooperative self-assembly takes place in these systems giving rise to coordinated cobalt porphyrins at concentrations significantly lower than usually required for cobalt porphyrin–pyridine complexation.

Mass spectrometry, using soft ionization techniques, FAB, electrospray, and MALDI, provided no evidence for self-assembly: the only significant signals were due to the monomer units of Co1, Co2, and Co3. A weak signal corresponding to (Co3)₂ was detected, but it is clear that the coordination bonds are not stable under mass spectrometry conditions.^[5]

The ¹H NMR spectra of the cobalt porphyrins were difficult to assign, because of the paramagnetic broadening associated with the cobalt(II) center and the complexity of the spectra. However, pulsed-gradient spin-echo (PGSE) NMR diffusion experiments did provide some insight into the relative sizes of the self-assembled complexes.^[11] Figure 2 shows the change in

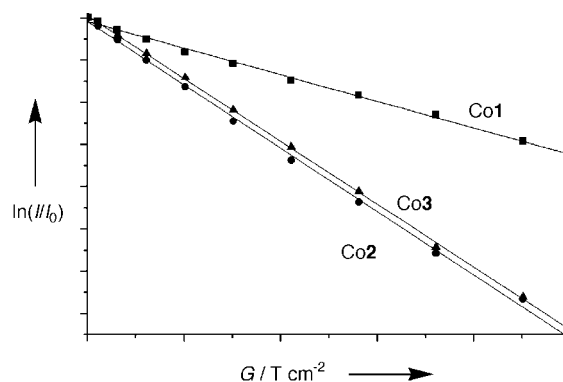


Figure 2. Variation in the intensity of the ¹H NMR signals of the aromatic protons of cobalt porphyrins ($\ln(I/I_0)$) in PGSE experiments, as a function of the field gradient (G). The measurements were carried out on a Bruker AMX-600 using 7 mM porphyrin solutions in chloroform/methanol (10/3).

intensity of the aromatic signals as a function of the field gradient. Clearly the diffusion coefficient of Co1 is much smaller than that of Co3 or Co2, which suggests that Co1 forms a high molecular weight polymer (Figure 1c). Porphyrin Co3 appears to be marginally larger than the monomeric Co2 as expected for simple dimerisation (Figure 1b).

The best method for characterising these self-assembled complexes proved to be size-exclusion chromatography (SEC). The monomeric reference porphyrin Co2 exhibits a retention time of 16.6 min, while the self-assembled dimer of Co3 emerges after 15.7 min (Figure 3). For the self-assembled

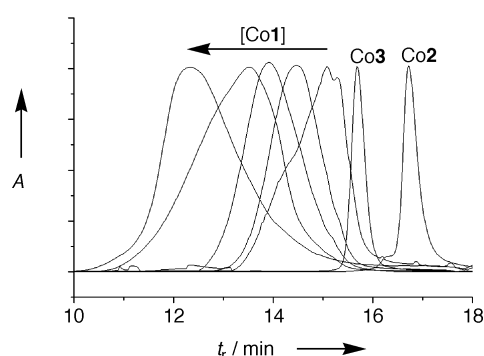


Figure 3. Normalized SEC trace (5 μm mixed-bed column) for Co1 at various concentrations between 55 and 7000 μM , Co2 and Co3 (t_r : retention time; A: absorbance at 440 nm).

polymer of Co1, a single peak was observed, but the retention times ranged from 12 to 15 minutes depending on the concentration of the monomer (Table 1). No peak corresponding to the monomeric reference compound could be detected. This again indicates the formation of stable, high molecular weight polymers of Co1.

The SEC results were calibrated by titrating Co3 into Co1. The monofunctional Co3 has exactly the same binding site as Co1 and so acts as a chain stopper, which can be used to control the mean molecular weight of the Co1 polymer (Figure 4).^[3] For the Co1/Co3 mixtures, the length of the polymer decreases with increasing amounts of chain stopper Co3, and when the proportion of Co3 is large, it is possible to assign clearly resolved SEC peaks to dimer, trimer, tetramer, and so forth (Figure 4). The molecular weights of these oligomers follow the expected logarithmic relationship with the retention time, and this calibration curve was used to determine the size of the pure Co1 polymers (Table 1).

Table 1. Relationship between the concentration of the monomer and the mean molecular weight of the corresponding Co1 polymers determined by SEC.

[M] [μM]	t_r [min]	M_w [g mol ⁻¹]	N
7000	12.4	136 000	95
2200	13.5	82 500	58
550	13.9	26 700	19
275	14.5	23 100	16
55	15.1	17 600	12

[M] is the monomer concentration, t_r is the SEC peak retention time, M_w is the mean molecular weight, and N is the corresponding degree of polymerisation (calibration based on the chain stopper experiment illustrated in Figure 4).

In pure samples of Co1, the degree of polymerization N clearly increases with the concentration of the monomer, as expected from Equation (1): the results in Table 1 give a monomer–monomer association constant of $K = 1.0 \pm 0.5 \times 10^6 \text{ M}^{-1}$. The polydispersity index of the polymers is between

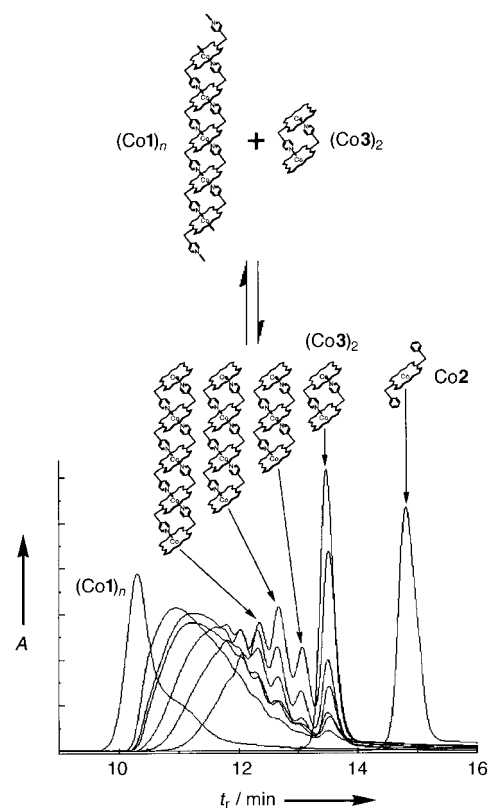


Figure 4. Co3 acts as a chain stopper for the Co1 polymers. The SEC traces (two 3 μm mixed-bed columns) for mixtures of Co1 and Co3 shift to shorter retention times as the proportion of Co1 increases. The pure Co1 polymer lies within the exclusion volume of the column, but this assembly is clearly larger than the mixtures. The trace for the monomeric reference compound Co2 is included for comparison (t_r : retention time; A: absorbance at 440 nm).

1.5 and 2.5, as expected for an equilibrium polymerisation.^[4] A solubility limit is reached at about 10 mM, at which point the longer polymers start to precipitate. Nevertheless, this self-assembly approach can be used to generate soluble porphyrin polymers of about 100 units long with an mean molecular weight of 136 kDa. This is significantly larger than the porphyrin systems obtained from covalent reactions which have only achieved modest degrees of polymerization ($N = 10–15$).^[6c]

This work demonstrates that it is possible to design large, self-assembling ordered arrays of rigid dye molecules using simple coordination chemistry. A variety of different polymer architectures can be obtained by varying the length and orientation of the pyridine ligand, and this could be used to control the communication between the porphyrin chromophores in the material. Investigations of the photophysical and electrochemical properties of these polymers are in progress.

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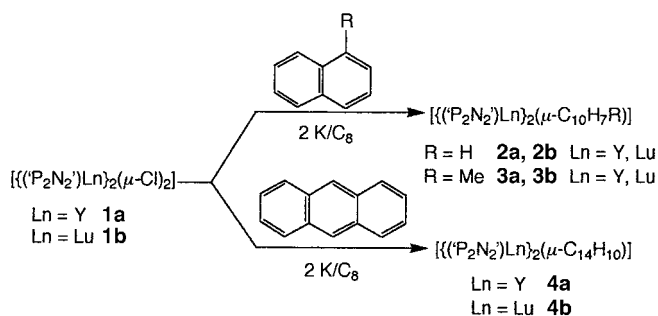
Dinuclear π Complexes of Yttrium and Lutetium with Sandwiched Naphthalene and Anthracene Ligands: Evidence for Rapid Intramolecular Inter-Ring Rearrangements**

Michael D. Fryzuk,* Laleh Jafarpour, Francesca M. Kerton, Jason B. Love, and Steven J. Rettig

The coordination of aromatic hydrocarbons to transition metals is of considerable interest in organometallic chemistry not only from the point of view of bonding but also in organic

synthesis.^[1] In particular, benzene and substituted benzenes coordinated to $\text{Cr}(\text{CO})_3$ have played a central role in this area. Less well developed, but potentially useful, are studies of the coordination of polycyclic arenes such as naphthalene and anthracene to metal atoms.^[2–17] These aromatic systems can act as ligands for Group 3 and lanthanide elements, although the intrinsic electropositive nature of the metal ions means the bonding is best described as ionic, with the arene ligands formally carrying considerable negative charge.^[4–6, 10, 11, 17, 18] Here we describe some dinuclear yttrium and lutetium complexes of naphthalene and anthracene, for which the solution and solid-state structures can only be correlated by invoking rapid intramolecular inter-ring migration of the metal-complex fragment.

A previous report described the synthesis of $[(\text{P}_2\text{N}_2')\text{Y}]_2(\mu\text{-Cl})_2$ (**1a**; $\text{P}_2\text{N}_2' = \text{PhP}(\text{CH}_2\text{SiMe}_2\text{NSiMe}_2\text{CH}_2)_2\text{PPh}$)^[19] and its ability to couple aryl units to generate π -bound bis-arene moieties.^[20] Given that yttrium could bind to an arene ligand, we examined the formation of yttrium and lutetium complexes with polycyclic aromatic compounds. The reaction of **1a** with potassium graphite (KC_8) in toluene/diethyl ether (75/25) in the presence of naphthalene generated the dark blue π -naphthalene complex $[(\text{P}_2\text{N}_2')\text{Y}]_2(\mu\text{-C}_{10}\text{H}_8)$ (**2a**) in moderate yield (Scheme 1). The ^1H NMR spectroscopic data



Scheme 1. Syntheses of polyarene complexes of yttrium and lutetium.

show only two sets of coupled resonances for the C_{10}H_8 moiety, which demonstrates that the bridging naphthalene unit is symmetrically bound; in addition, the $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum shows equivalent phosphane donors on both yttrium(III) centers. A similar reaction with the lutetium complex **1b** (Scheme 1) produced the dark purple dinuclear naphthalene complex **2b**.

The crystal structure^[21] of **2a** is shown in Figure 1. Each $\text{Y}(\text{P}_2\text{N}_2')$ unit binds in an η^4 fashion on the opposite sides of the different rings of the naphthalene unit. The naphthalene moiety is distorted slightly with elongated bond lengths, and the rings have a nonplanar step conformation.

A similar reaction with 1-methylnaphthalene ($\text{MeC}_{10}\text{H}_7$) gave $[(\text{P}_2\text{N}_2')\text{Ln}]_2(\mu\text{-}\eta^4\text{-}\eta^4\text{-MeC}_{10}\text{H}_7)$ ($\text{Ln} = \text{Y}$, **3a**; $\text{Ln} = \text{Lu}$, **3b**). The ^1H NMR spectrum of **3a** shows signals for the seven inequivalent protons on the naphthalene unit and a singlet for the 1-Me protons. The $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum shows equivalent phosphane donors (a doublet at $\delta = 21$, $^1J(\text{Y}, \text{P}) = 60 \text{ Hz}$). The solid-state molecular structure^[21] of **3a** is very similar to that of **2a**; the two yttrium ions are bound to opposite faces of the different rings. However, in this case the methyl group is disordered over all four equivalent positions (centrosymmetric). Nevertheless, since the behavior in solution of the

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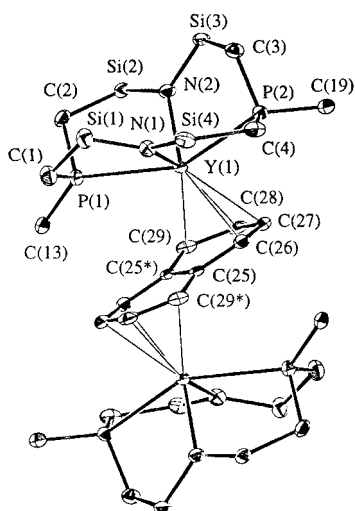
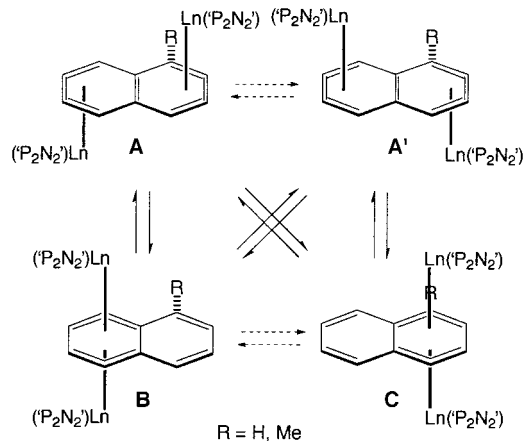


Figure 1. Molecular structure of **2a**. Selected bond lengths [Å] and angles [°]: Y(1)–P(1) 2.8918(7), Y(1)–P(2) 2.8239(7), Y(1)–N(1) 2.308(2), Y(1)–N(2) 2.320(2), Y(1)–C(26) 2.692(2), Y(1)–C(27) 2.684(3), Y(1)–C(28) 2.686(3), Y(1)–C(29) 2.652(3); P(1)–Y(1)–P(2) 150.32(2), N(1)–Y(1)–N(2) 99.03(7), P(1)–Y(1)–C(29) 79.93(6), P(2)–Y(1)–C(28) 99.47(7), N(1)–Y(1)–C(26) 95.57(8), N(2)–Y(1)–C(27) 124.61(7).

1-methylnaphthalene complex **3** is similar to that of **2**, particularly the equivalence of the phosphane donors in the $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum, a fluxional process must be operative or the signals of the different phosphane donors are incidentally coincident. A reasonable fluxional process is shown in Scheme 2; to interconvert **A** and **A'** directly would require each $\text{Ln}(\text{P}_2\text{N}_2)$ unit to migrate simultaneously from one



Scheme 2. Possible fluxional process occurring in naphthalene complexes: **2a**, **2b**, **3a**, and **3b**.

naphthalene ring to the other. A perhaps more likely alternative is that each $\text{Ln}(\text{P}_2\text{N}_2)$ fragment moves independently, thereby interconverting **A** and **A'** via the intermediates **B** and **C**. Attempts to freeze out this process at low temperatures were unsuccessful; ^1H NMR and $^{31}\text{P}\{^1\text{H}\}$ NMR spectra at -90°C showed slight broadening but no evidence for lower symmetry structures, even for the 1-methylnaphthalene complexes **3a** and **3b**.

The reaction of anthracene with **1a** in the presence of KC_8 leads to the formation of a dinuclear complex with π -bound anthracene ($\text{C}_{14}\text{H}_{10}$) having the formula $[(\text{P}_2\text{N}_2)\text{Y}]_2(\mu\text{-C}_{14}\text{H}_{10})$

(**4a**). The ^1H NMR spectrum is particularly informative, since one sees three signals for the coordinated anthracene unit that are shifted upfield relative to free anthracene. The highly symmetric nature of the NMR pattern and the fact that the $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum shows equivalent phosphane donors suggest two possible configurations (Figure 2): a structure in which the $\text{Y}(\text{P}_2\text{N}_2)$ units are bound to opposite faces of the middle ring of anthracene (**E**), or one in which each $\text{Y}(\text{P}_2\text{N}_2)$ moiety is bound to one of the outside rings and on opposite faces (**D**).

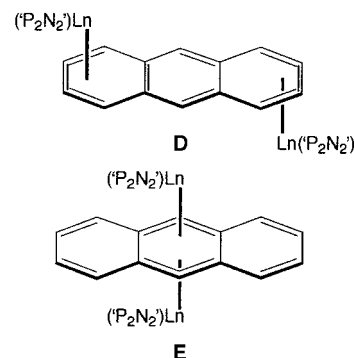


Figure 2. Postulated structures of **4a** on the basis of NMR spectroscopic data.

To our surprise, the solid-state molecular structure^[21] (Figure 3) showed that the two yttrium-containing fragments are bound to an outside ring and the middle ring on opposing faces, that is, neither of the proposed symmetric solution

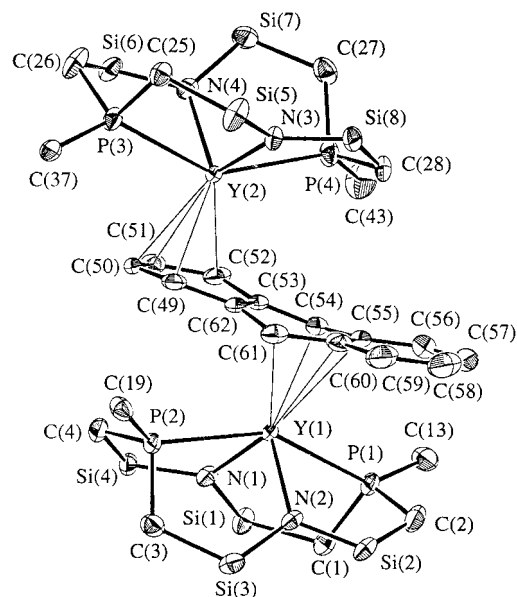
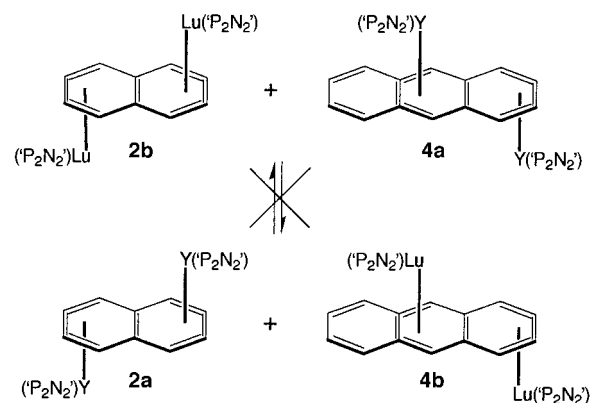


Figure 3. Molecular structure of **4a**. Selected bond lengths [Å] and angles [°]: Y(1)–P(1) 2.8469(9), Y(1)–P(2) 2.8335(9), Y(1)–N(1) 2.295(2), Y(1)–N(2) 2.290(2), Y(2)–P(3) 2.9052(9), Y(2)–P(4) 2.8250(9), Y(2)–N(3) 2.284(2), Y(2)–N(4) 2.284(3), Y(1)–C(54) 2.731(3), Y(1)–C(55) 2.832(3), Y(1)–C(60) 2.794(3), Y(1)–C(61) 2.646(3), Y(2)–C(49) 2.721(3), Y(2)–C(50) 2.655(3), Y(2)–C(51) 2.677(3), Y(2)–C(52) 2.724(3); P(1)–Y(1)–P(2) 151.03(3), N(1)–Y(1)–N(2) 96.45(8), P(3)–Y(2)–P(4) 145.40(3), N(3)–Y(2)–N(4) 104.29(9), P(1)–Y(1)–C(54) 81.61(7), P(2)–Y(1)–C(61) 80.30(8), N(1)–Y(1)–C(55) 134.94(9), N(2)–Y(1)–C(60) 104.00(9), P(3)–Y(2)–C(50) 79.62(8), P(4)–Y(2)–C(52) 81.63(8), N(3)–Y(2)–C(49) 100.09(9), N(4)–Y(2)–C(51) 103.81(9).

structures **D** or **E** corresponds to the solid-state structure. This solid-state structure is similar to that of a lithium anthracenide complex.^[22] Despite the difference in size between yttrium and lithium, the anthracene moiety is distorted to the same extent, as evidenced by C–C bond length variations and deviations from planarity. To rationalize the solution and solid-state data, a fluxional process similar to that shown in Scheme 2 for the naphthalene complexes can be envisioned, wherein the two $\text{Ln}(\text{P}_2\text{N}_2)$ units migrate along the top and the bottom of the anthracene ligand via intermediates such as **E**, the anthracene analogue of **B** (or **C**), and perhaps **D**, although we have no evidence for this particular stereoisomer.

The facile inter-ring migratory behavior displayed by these yttrium and lutetium complexes is not found for the majority of naphthalene and anthracene complexes of the transition metals. For example, activation barriers for inter-ring migrations in $[(\eta^4\text{-C}_{10}\text{H}_8)\text{Cr}(\text{CO})_3]$ and $[(\eta^4\text{-C}_{14}\text{H}_{10})\text{Cr}(\text{CO})_3]$ were determined to be in excess of 25 kcal mol^{-1} .^[8] In contrast, the only system to the best of our knowledge to exhibit fast inter-ring rearrangements is the Ni^0 complex $[\text{Ni}(\eta^2\text{-C}_{10}\text{H}_8)(\text{dippe})]$ ($\text{dippe} = 1,2\text{-bis}(\text{diisopropylphosphanyl})\text{ethane}$),^[2] for which a $\Delta G^\ddagger$ of 15 kcal mol^{-1} was estimated. Presumably, the formally d^{10} nickel center does not engage in strong π back-donation to the naphthalene ring, and this facilitates both intra- and inter-ring migration.

Consideration of these dinuclear lanthanide π -complexes as having covalent bonding between the Y or Lu and the polycyclic aromatic hydrocarbon is probably unrealistic. However, if we consider the bonding to be electrostatic in nature, then the motion can be regarded as the migration of the cation $[\text{Ln}(\text{P}_2\text{N}_2)]^+$ over the polycyclic aromatic dianions. This then raises an important question: do these ion pairs dissociate in solution? We devised a crossover experiment to test this (Scheme 3): a mixture of the lutetium naphthalene



Scheme 3. Crossover experiment indicating no ion dissociation in solution.

complex **2b** with the yttrium anthracene complex **4a** was monitored as a function of time. The complementary experiment with **2a** and **4b** was also carried out to ensure that thermodynamic factors were not preventing crossover. No evidence of intermolecular migration of a $[\text{Ln}(\text{P}_2\text{N}_2)]^+$ ion between naphthalene and anthracene was obtained, since only the starting materials were observed by $^{31}\text{P}\{^1\text{H}\}$ NMR spectroscopy throughout this experiment. In addition, no

mixed-metal complexes were detected. The $^{31}\text{P}\{^1\text{H}\}$ NMR spectroscopic data also demonstrated that no exchange occurs with free naphthalene or anthracene.

The solubility^[23] of these complexes in hydrocarbons is a result of the ancillary ' P_2N_2 ' ligand attached to the lanthanide(III) ion and suggests that these complexes will serve as versatile reducing agents and synthons for other organo-metallic derivatives of f-block elements.

Experimental Section

General procedure for **2–4**: Toluene (30 mL) and diethyl ether (10 mL) were added to a mixture of **1** (0.5 g, 0.38 mmol), C_8K (0.11 g, 0.84 mmol), and polycyclic aromatic compound (0.57 mmol) at room temperature. The reaction mixture, which turned dark blue for **2a**, **3a**, and **4a**, and dark purple for **2b**, **3b**, and **4b**, was stirred for 4 d. The solvent was then evaporated to dryness, the residue extracted with toluene, and the extract filtered through celite and dried in vacuo to yield a dark blue or dark purple semicrystalline material (60 %). Crystals suitable for X-ray analysis were grown by slow evaporation of solutions in toluene.

2a: Elemental analysis calcd (found) for $\text{C}_{58}\text{H}_{92}\text{N}_4\text{P}_4\text{Si}_8\text{Y}_2$: C 50.78 (50.67), H 6.76 (6.79), N 4.08 (4.01); ^1H NMR ($[\text{D}_6]$ benzene, 200 MHz, 298 K): $\delta = 7.40$ (m, 4H, *o*-H), 7.00 (m, 6H, *m/p*-H, phenyl), 4.50 (m, 4H, naphthalene), 2.80 (m, 4H, naphthalene), 1.40 (AB m, 4H, ring CH_2), 1.20 (AB m, 4H, ring CH_2), 0.50 (s, 6H, ring SiMe_2), 0.25 (s, 6H, ring SiMe_2); $^{31}\text{P}\{^1\text{H}\}$ NMR ($[\text{D}_6]$ benzene, 81 MHz, 298 K): $\delta = -26.00$ (d, $J_{\text{Y-P}} = 87.2\text{ Hz}$); $^{13}\text{C}\{^1\text{H}\}$ NMR ($[\text{D}_6]$ benzene, 75.45 MHz, 298 K): $\delta = 139.2$ (m, *ipso*- $\text{C}_6\text{H}_5\text{P}$), 133.9 (s, *ipso*-C, naphthalene), 131.1 (t, $J_{\text{C-P}} = 8\text{ Hz}$, *o*- $\text{C}_6\text{H}_5\text{P}$), 129.5 (s, *m/p*- $\text{C}_6\text{H}_5\text{P}$), 128.8 (s, *m/p*- $\text{C}_6\text{H}_5\text{P}$), 116.8 (s, CH, naphthalene), 92.9 (s, CH, naphthalene), 19.0 (s, PCH_2), 7.3 (s, $\text{Si}(\text{CH}_3)_2$), 7.0 (s, $\text{Si}(\text{CH}_3)_2$). No $\text{Y}^{89}\text{-C}^{13}$ coupling was observed in this complex or in **3a** or **4a** as the half-height peak width was approximately 10 Hz for all signals.

2b: Elemental analysis calcd (found) for $\text{C}_{58}\text{H}_{92}\text{N}_4\text{P}_4\text{Si}_8\text{Lu}_2$: C 45.12 (45.18), H 6.01 (6.01), N 3.63 (3.59); ^1H NMR ($[\text{D}_6]$ benzene, 200 MHz, 298 K): $\delta = 7.20$ (m, 4H, *o*-H), 6.90 (m, 6H, *m/p*-H, phenyl), 4.50 (m, 4H, naphthalene), 2.80 (m, 4H, naphthalene), 1.30 (AB m, 4H, ring CH_2), 1.10 (AB m, 4H, ring CH_2), 0.20 (s, 6H, ring SiMe_2), 0.10 (s, 6H, ring SiMe_2); $^{31}\text{P}\{^1\text{H}\}$ NMR ($[\text{D}_6]$ benzene, 81 MHz, 298 K): $\delta = -18.37$; $^{13}\text{C}\{^1\text{H}\}$ NMR ($[\text{D}_6]$ benzene, 75.45 MHz, 298 K): $\delta = 138.3$ (m, *ipso*- $\text{C}_6\text{H}_5\text{P}$), 134.0 (s, *ipso*-C, naphthalene), 132.4 (t, $J_{\text{C-P}} = 8\text{ Hz}$, *o*- $\text{C}_6\text{H}_5\text{P}$), 129.4 (s, *m/p*- $\text{C}_6\text{H}_5\text{P}$), 128.8 (s, *m/p*- $\text{C}_6\text{H}_5\text{P}$), 115.5 (s, CH, naphthalene), 93.3 (s, CH, naphthalene), 20.1 (s, PCH_2), 6.5 (s, $\text{Si}(\text{CH}_3)_2$), 6.0 (s, $\text{Si}(\text{CH}_3)_2$).

3a: Elemental analysis calcd (found) for $\text{C}_{59}\text{H}_{94}\text{N}_4\text{P}_4\text{Si}_8\text{Y}_2$: C 51.14 (51.45), H 6.84 (6.94), N 4.04 (3.89); ^1H NMR ($[\text{D}_6]$ benzene, 500 MHz, 298 K): $\delta = 7.50$ (m, 4H, *o*-H), 7.10 (m, 6H, *m/p*-H, phenyl), 4.78 (AB m, 1H, 1-methylnaphthalene), 4.60 (AB m, 3H, 1-methylnaphthalene), 4.53 (AB m, 1H, 1-methylnaphthalene), 3.00 (AB m, 1H, 1-methylnaphthalene), 2.90 (AB m, 1H, 1-methylnaphthalene), 2.60 (AB m, 1H, 1-methylnaphthalene), 2.15 (s, 3H, CH_3 , 1-methylnaphthalene), 1.40 (AB m, 4H, ring CH_2), 1.20 (AB m, 4H, ring CH_2), 0.50 (s, 6H, ring SiMe_2), 0.25 (s, 6H, ring SiMe_2); $^{31}\text{P}\{^1\text{H}\}$ NMR ($[\text{D}_6]$ benzene, 202.5 MHz, 298 K): $\delta = -25.96$ (d, $J_{\text{Y-P}} = 87.9\text{ Hz}$); $^{13}\text{C}\{^1\text{H}\}$ NMR ($[\text{D}_6]$ benzene, 75.45 MHz, 298 K): $\delta = 139.2$ (s, *ipso*-C, 1-methylnaphthalene), 134.5 (m, *ipso*- $\text{C}_6\text{H}_5\text{P}$), 131.4 (t, $J_{\text{C-P}} = 7.2\text{ Hz}$, *o*- $\text{C}_6\text{H}_5\text{P}$), 129.2 (s, *m/p*- $\text{C}_6\text{H}_5\text{P}$), 128.8 (s, *m/p*- $\text{C}_6\text{H}_5\text{P}$), 125.6 (s, CH, 1-methylnaphthalene), 117.6 (s, CH, 1-methylnaphthalene), 116.6 (s, CH, 1-methylnaphthalene), 114.7 (s, CH, 1-methylnaphthalene), 112.8 (s, CH, 1-methylnaphthalene), 101.8 (s, CH, 1-methylnaphthalene), 95.6 (s, CH, 1-methylnaphthalene), 31.8 (s, *ipso*-C, 1-methylnaphthalene), 19.0 (s, PCH_2), 14.3 (s, CH_3 , 1-methylnaphthalene), 7.4 (s, $\text{Si}(\text{CH}_3)_2$), 7.2 (s, $\text{Si}(\text{CH}_3)_2$).

3b: Elemental analysis calcd (found) for $\text{C}_{59}\text{H}_{94}\text{N}_4\text{P}_4\text{Si}_8\text{Lu}_2$: C 44.20 (44.50), H 6.23 (6.09), N 3.68 (3.44); ^1H NMR ($[\text{D}_6]$ benzene, 500 MHz, 298 K): $\delta = 7.20$ (m, 4H, *o*-H), 6.90 (m, 6H, *m/p*-H, phenyl), 4.78 (AB m, 1H, 1-methylnaphthalene), 4.60 (AB m, 3H, 1-methylnaphthalene), 4.53 (AB m, 1H, 1-methylnaphthalene), 3.00 (AB m, 1H, 1-methylnaphthalene), 2.70 (AB m, 2H, 1-methylnaphthalene), 2.25 (s, 3H, CH_3 , 1-methylnaphthalene), 1.20 (AB m, 4H, ring CH_2), 1.10 (AB m, 4H, ring CH_2), 0.20 (s, 6H, ring SiMe_2), 0.1 (s, 6H, ring SiMe_2); $^{31}\text{P}\{^1\text{H}\}$ NMR ($[\text{D}_6]$ benzene,

202.5 MHz, 298 K): $\delta = -18.28$; $^{13}\text{C}\{^1\text{H}\}$ NMR ($[\text{D}_6]$ benzene, 75.45 MHz, 298 K): $\delta = 139.4$ (t, $J_{\text{C-P}} = 7.2$ Hz, *ipso*-C₆H₅P), 131.4 (t, $J_{\text{C-P}} = 7.0$ Hz, *o*-C₆H₅P), 129.5 (s, *m/p*-C₆H₅P), 129.2 (s, *m/p*-C₆H₅P), 125.6 (s, CH, 1-methylnaphthalene), 121.4 (s, CH, 1-methylnaphthalene), 115.7 (s, CH, 1-methylnaphthalene), 113.1 (s, CH, 1-methylnaphthalene), 94.5 (s, CH, 1-methylnaphthalene), 91.0 (s, CH, 1-methylnaphthalene), 88.7 (s, CH, 1-methylnaphthalene), 31.2 (s, *ipso*-C, 1-methylnaphthalene), 19.0 (s, PCH₂), 14.0 (s, CH₃, 1-methylnaphthalene), 7.7 (s, Si(CH₃)₂), 7.2 (s, Si(CH₃)₂).

4a: Elemental analysis calcd (found) for (C₆₂H₉₄N₄P₄Si₈Y₂): C 52.45 (52.29), H 6.53 (6.39), N 3.95 (4.16); ^1H NMR ($[\text{D}_6]$ benzene, 500 MHz, 298 K): $\delta = 7.50$ (m, 4H, *o*-H), 7.10 (m, 6H, *m/p*-H, phenyl), 5.55 (AB m, 4H, anthracene), 4.43 (AB m, 4H, anthracene), 3.00 (s, 2H, anthracene), 1.40 (AB m, 4H, ring CH₂), 1.20 (AB m, 4H, ring CH₂), 0.50 (s, 6H, ring SiMe₂), 0.25 (s, 6H, ring SiMe₂); $^{31}\text{P}\{^1\text{H}\}$ NMR ($[\text{D}_6]$ benzene, 202.5 MHz, 298 K): $\delta = -27.29$ (d, $J_{\text{Y-P}} = 88.05$ Hz); $^{13}\text{C}\{^1\text{H}\}$ NMR ($[\text{D}_6]$ benzene, 75.45 MHz, 298 K): $\delta = 140.7$ (s, *ipso*-C, anthracene), 139.2 (m, *ipso*-C₆H₅P), 132.9 (t, $J_{\text{C-P}} = 8$ Hz, *o*-C₆H₅P), 130.8 (t, $J_{\text{C-P}} = 8$ Hz, *m*-C₆H₅P), 129.5 (s, *p*-C₆H₅P), 119.7 (s, CH, anthracene), 109.0 (s, CH, anthracene), 89.6 (s, CH, anthracene), 19.4 (s, PCH₂), 7.0 (br, Si(CH₃)₂).

4b: Elemental analysis calcd (found) for (C₆₂H₉₄N₄P₄Si₈Lu₂): C 46.72 (46.59), H 5.94 (6.04), N 3.51 (3.45); ^1H NMR ($[\text{D}_6]$ benzene, 500 MHz, 298 K): $\delta = 7.20$ (m, 4H, *o*-H), 6.90 (m, 6H, *m/p*-H, phenyl), 5.65 (AB m, 4H, anthracene), 4.60 (AB m, 4H, anthracene), 3.25 (s, 2H, anthracene), 1.20 (AB m, 4H, ring CH₂), 1.10 (AB m, 4H, ring CH₂), 0.20 (s, 6H, ring SiMe₂), 0.10 (s, 6H, ring SiMe₂); $^{31}\text{P}\{^1\text{H}\}$ NMR ($[\text{D}_6]$ benzene, 202.5 MHz, 298 K): $\delta = -19.04$; $^{13}\text{C}\{^1\text{H}\}$ NMR ($[\text{D}_6]$ benzene, 75.45 MHz, 298 K): $\delta = 140.2$ (s, *ipso*-C, anthracene), 139.7 (t, $J_{\text{C-P}} = 7.0$ Hz, *ipso*-C₆H₅P), 131.3 (t, $J_{\text{C-P}} = 8$ Hz, *o*-C₆H₅P), 129.3 (t, $J_{\text{C-P}} = 7.5$ Hz, *m*-C₆H₅P), 128.8 (s, *p*-C₆H₅P), 119.5 (s, CH, anthracene), 110.9 (s, CH, anthracene), 89.5 (s, CH, anthracene), 17.8 (s, PCH₂), 7.8 (s, Si(CH₃)₂), 7.2 (s, Si(CH₃)₂).

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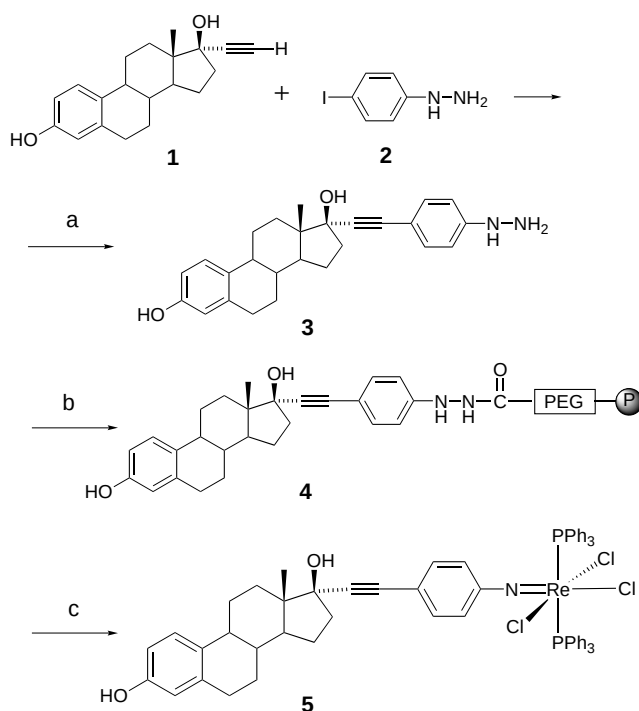
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Solid-Supported Hydrazine Substrate For Labeling Estradiol Ligands with Rhenium**

Jeffrey B. Arterburn,* Kalla Venkateswara Rao, and Marc C. Perry

Receptor-targeted radiopharmaceuticals offer great promise for the diagnostic imaging and therapy of tumors and other disease sites. Technetium-99m is readily available in nuclear medicine clinics throughout the world for diagnostic imaging applications, and the β -emitting radioisotopes of its congener, rhenium-186/188, are suitable for irradiating small to medium-sized tumors.^[1] Radiolabeled bioligands such as steroids, peptides, and antibodies are capable of binding to receptors expressed by cancer cells, providing the selectivity needed for diagnostic and therapeutic applications.^[2–4] The estrogen and progesterone steroid hormone receptors found in approximately two-thirds of breast tumors are suitable targets for steroid-based radiopharmaceuticals.^[5] Radiopharmaceuticals with high specific activity are required, and the removal of all excess *unlabeled* ligand is essential to avoid competitive saturation of the binding sites of the ligand receptor. Herein we demonstrate a new strategy for labeling with rhenium using an organoimido-forming reaction of a polymer-supported hydrazine, which simultaneously establishes the steroid–radioisotope linkage and releases the labeled steroid product into solution, thereby facilitating complete removal of all unlabeled ligand by simple filtration. The approach outlined here is uniquely amenable to the specific problem of developing “instant kits” for labeling low-capacity receptor ligands, and the technology is suitable for adaptation to a wide variety of different structural classes of ligands.

The 17 α position of estradiol was selected as the site for appending the linking organoimido group, following the examples of organometallic steroid derivatives which exhibit high receptor binding affinities.^[6–8] We have previously synthesized highly functionalized organoimido complexes from substituted 1-acetyl 2-phenyldiazane (hydrazine derivatives) using carrier free trichlorooxobis(triphenylphosphane) rhenium(v), [ReOCl₃(PPh₃)₂].^[9, 10] Our approach required a convenient method for attaching pendant phenylhydrazine moieties to ethynylestradiol (**1**). The desired hydrazine **3** was obtained directly using a palladium-catalyzed coupling^[11] of ethynylestradiol (**1**) with 4-iodophenyl hydrazine (**2**) in diethylamine at ambient temperature in 87% yield (Scheme 1). The free hydrazine **3** was attached to Tentagel carboxy resin (loading capacity = 0.26 mmol g^{−1}) using (1-ben-



Scheme 1. a) 5 mol % Pd(OAc)₂, 10 mol % CuI, PPh₃, NHEt₂, 25 °C, 3 h, 87%; b) Tentagel carboxy resin, PyBOP, NEt₂Pr₂, CH₂Cl₂, 25 °C, 20 h, 100% (based on loading capacity = 0.26 mmol g^{−1}); c) [ReOCl₃(PPh₃)₂], PPh₃, CH₂Cl₂, 40 °C, 3 h, 82%.

zotriazolyl)oxy tris(pyrrolidino) phosphonium hexafluorophosphate (PyBOP) and diisopropylethylamine in dichloromethane to give the corresponding solid-supported acetyl hydrazine derivative **4**. This reaction was monitored using FT-IR spectroscopy to follow the change in the carbonyl stretch from the free carboxylic acid (1737 cm^{−1}) to the carbohydrazide (1660 cm^{−1}), and the appearance of the characteristic aryl C–H bend at 1611 cm^{−1} from the estradiol.

The organoimido-forming labeling reaction of solid-supported acetyl hydrazine **4** with [ReOCl₃(PPh₃)₂] (2.86 mM in CH₂Cl₂) and triphenylphosphane occurred readily in solution to produce the air- and moisture-stable complex **5** as an olive-colored solid in 82% yield (Scheme 1). The product exhibited a single ³¹P NMR signal due to the coordinated triphenylphosphane ligands at δ = −20.4 and displayed a characteristic UV/Vis absorption spectrum with maximum at λ = 370 nm (ϵ = 16200, CH₂Cl₂).

The previous example demonstrates the efficient reactivity of the polymer-supported hydrazines. The specific requirements for radiolabeling with rhenium and technetium involve highly dilute conditions, therefore a series of labeling reactions were carried out using the polymer-supported hydrazine **4** and dilute solutions of [ReOCl₃(PPh₃)₂] from 10^{−5} to 10^{−6} M (Table 1). The formation of the organoimido complex **5** was followed spectroscopically by observing the absorption maximum at λ = 370 nm. The half lives for the labeling reactions ($t_{1/2}$ = 2 h) were unchanged over a 100- to 1000-fold excess of the support **4** relative to rhenium concentration. The yields of the reaction were similar when a solution of [ReOCl₃(PPh₃)₂] prepared in situ from potassium perrhenate was used (entry 3, Table 1).^[10] The organoimido

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Table 1. Rhenium labeling using the solid-supported hydrazine **4**.

Entry	Concentration [10 ⁻⁵ M]	Conditions ^[a]	Time [h]	Yield [%]
1	2	A	2	53
			5	72
2	0.2	B	2	50
			5	70
3	9.3	C	2	54
			5	70
4	2	D	2	55
			5	65

[a] Reactions carried out in CH₂Cl₂ (10 mL) at 40 °C. Reactant ratios used: A: **4**/PPh₃/[ReOCl₃(PPh₃)₂] = 100/100/1; B: **4**/PPh₃/[ReOCl₃(PPh₃)₂] = 1000/1000/1; C: **4**/PPh₃/KReO₄ = 100/100/1; D: **4**/HPPPh₃Cl/Bu₄NReO₄ = 100/100/1.

complex **5** was also prepared using a one-pot procedure starting with tetrabutylammonium perrhenate and triphenylphosphane hydrochloride in dichloromethane (entry 4, Table 1).

These examples illustrate a new strategy for labeling estradiol ligands with rhenium using a solid-supported hydrazine substrate, and this chemistry should also be successful for preparing technetium analogs.^[12] The ability to use perrhenate and pertechnetate salts for labeling is particularly advantageous, since these species are obtained directly from the radionuclide generators. The efficiency and convenience of this approach can be extrapolated to a new generation of rhenium and technetium complexes for diagnostic and therapeutic applications in nuclear medicine. Further studies that are currently in progress involve evaluation of the receptor binding affinity and in vivo stability of these estradiol derivatives, and the extension of this technology to other low-capacity receptor systems.

Experimental Section

4: To a suspension of Tentagel Carboxy resin (1.0 g, 0.26 mmol) in dichloromethane (50 mL) was added PyBOP (405 mg, 0.78 mmol) followed by hydrazine **3** and diisopropylethylamine (0.3 mL). The resulting suspension was stirred at 25 °C for 20 h. The reaction product was filtered to give the yellow polymer support **4**. FT-IR (KBr): $\tilde{\nu}$ = 3443, 2869, 1652, 1611, 1104 cm⁻¹.

5: A suspension of **4** (110 mg, 0.0286 mmol), triphenylphosphane (7.5 mg, 0.0286 mmol), and [ReOCl₃(PPh₃)₂] (23.8 mg, 0.0286 mmol) in dichloromethane (10 mL) was heated at 40 °C for 3 h. The reaction mixture was filtered, and washed thoroughly with dichloromethane. The combined organic layers were concentrated, and the product was precipitated from dichloromethane/hexanes and recrystallized to provide the complex **5** (28 mg, 82 %) as olive-green crystals containing CH₂Cl₂. Elemental analysis for C₆₂H₅₇Cl₃NO₂P₂Re · 0.5 CH₂Cl₂: calcd: C 60.23, H 4.61, N 1.12; found: C 60.29, H 4.66, N 1.50. Spectral data is provided in the Supporting Information.

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Regioselective Lactonization of Tetrasialic Acid**

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Polysialic acids (PSAs) are polymers of *N*-acetylneuraminic acid. Depending on their glycosidic linkages, these sugar polymers exist in nature as α -2,8-, α -2,9-, and α -2,8/2,9-linked polysaccharides.^[1] PSAs have been reported to demonstrate many important biological functions.^[2] For example, α -2,8-PSA is mainly linked to the neural cell adhesion molecule (N-CAM). This homopolymer of sialic acid has been implicated in reducing N-CAM adhesion; removal of the PSA increases the adhesive capability of N-CAM.^[3] In addition, α -2,8- and α -2,9-PSAs are the capsular polysaccharides of, respectively, serogroups B and C of *Neisseria meningitidis*, a leading worldwide cause of meningitis and rapidly fatal sepsis in otherwise healthy individuals.^[4]

Structural diversities of PSAs are even more complicated with the possibility of PSA lactonizations. For α -2,8-PSA, the C-2 carboxylic acid of one residue can condense with the C-9 hydroxyl group of an adjacent residue to generate a δ -lactone under acidic conditions. Such δ -lactonizations have also been

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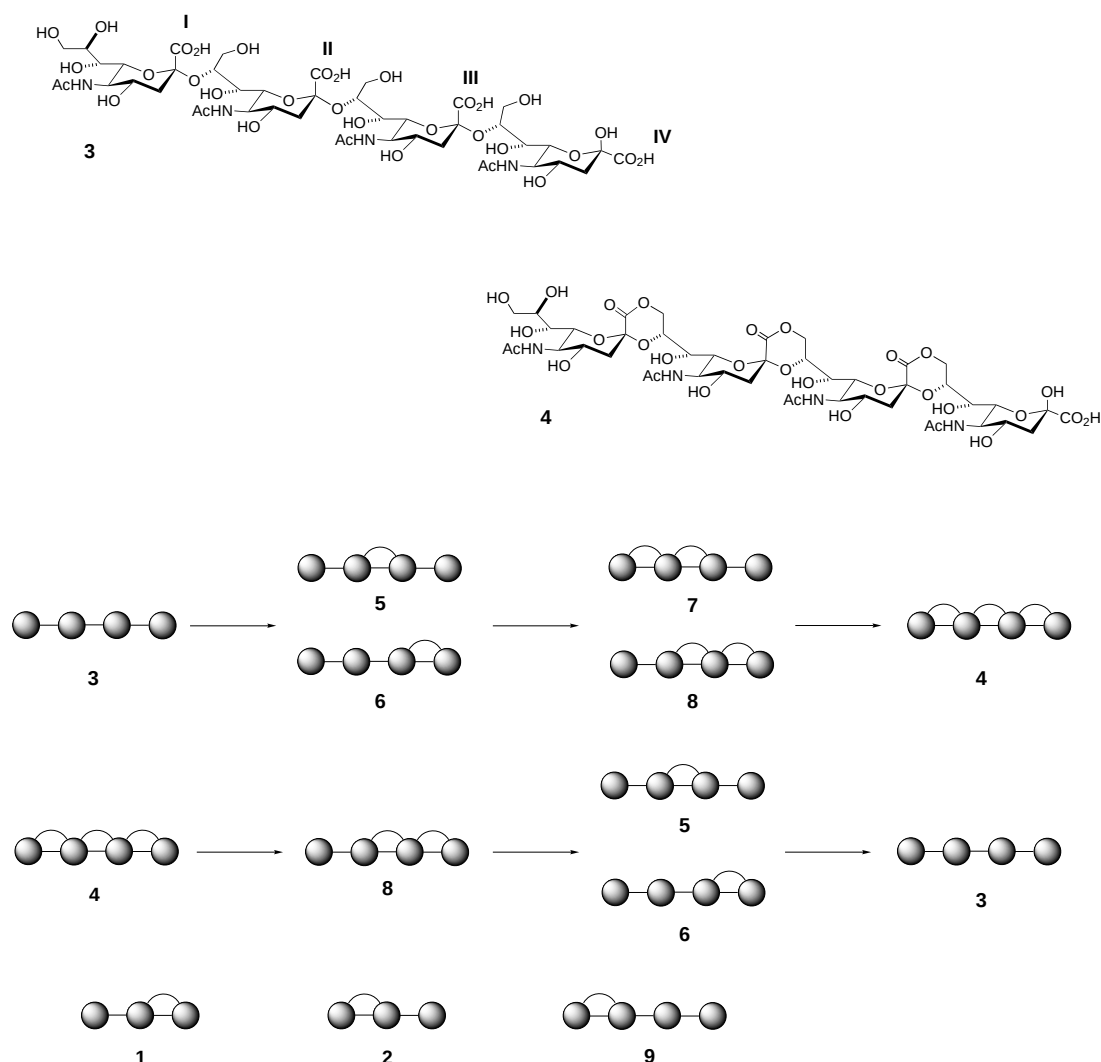
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observed in α -2,8/2,9-PSA.^[5] Some unusual properties of PSAs can be attributed to the occurrence of these PSA lactones. For instance, the formation of ganglioside lactones has been found in nature and was suggested to play an important biological role.^[6] Moreover, in vivo lactonizations of the serogroup B PSA of *Neisseria meningitidis* are proposed to explain the poor immunogenicity shown in humans. In contrast, the serogroup C PSA of the same organism is a better immunogen since it is difficult for the α -2,9-homopolymer of the sialic acid to undergo lactonization due to the steric hindrance of the C8 hydroxyl group.^[5, 7]

Recently, we have demonstrated the use of capillary electrophoresis (CE) for elucidating the lactonization of α -2,8-trisialic acid.^[8] It was found that formation of the fully lactonized trimer occurred by the pathway in which monolactone **1** is the dominant intermediate (Scheme 1). The other monolactone **2** was the sole intermediate observed during the hydrolysis of the fully lactonized trimer. Meanwhile, it has been suggested by Zhang and Lee that initial lactonizations of α -2,8-oligosialic acids occur between internal sialic acid

residues.^[9] To further clarify whether regioselectivity exists in the lactonization of oligo/polysialic acids with a degree of polymerization (DP) greater than three and to compare lactonizations between different linkages of PSAs, we report here our investigations into α -2,8-tetrasialic acid and its 2,9-analogue.

α -2,8-Tetrasialic acid **3** was treated with glacial acetic acid to initiate lactonization which was then monitored by CE. As shown in Figure 1, six peaks in the CE chromatograms can be grouped into four categories (A to D). Peak A with the lowest mobility is the starting material, free tetrasialic acid (**3**); this was proved by co-injection with an authentic standard. Peak D is recognized as the fully lactonized tetramer **4** with the lowest charge density. Obviously, peaks A and D showed their predominant presence in the initial and final stages of lactonization, respectively (Stages 1 and 5) in Figure 1). Based on the retention times measured on CE chromatograms, it is reasonable to suggest that groups B and C correspond to the mono- and dilactonized species, respectively. These signals could not be identified without enzymatic



Scheme 1. Structures of α -2,8-linked sialic acid tetramer **3** and fully lactonized tetramer **4** as well as simplified representations of trimeric monolactones **1** and **2** and the reaction pathways of lactonization (upper) and delactonization (lower). Sialic acid residues are represented by the spheres; the reducing termini are always on the right side. Sites of lactone rings are marked by the curves between spheres. Compound **5** was the major monolactonized tetramer with **6** as the minor one in both pathways. The tetrameric monolactone **9** was not detected in the lactonization.

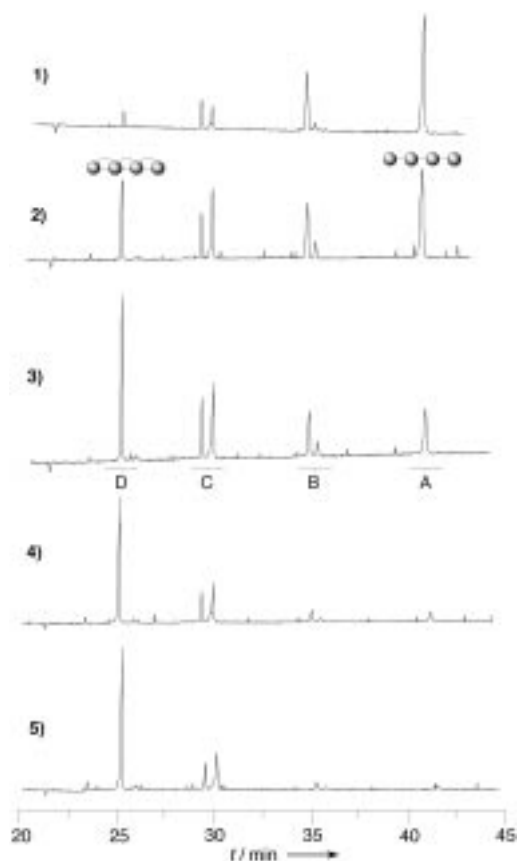


Figure 1. CE chromatograms of the lactonization of **3** in glacial acetic acid at different reaction times (Stage 1) = 10 min, 2) = 60 min, 3) = 3 h, 4) = 8 h, and 5) = 16 h).

hydrolysis by neuraminidase, the progress of which is depicted in Figure 2. Peaks a to d were identified as sialic acid monomer, trimeric monolactone **2**, fully lactonized trimer, and lactonized dimer, respectively, by co-injection studies and comparison of retention times with authentic standards.

The formation of **2** (peak b) and lactonized dimer (peak d) should result from the enzymatic cleavage of the tetrameric monolactones **5** and **6**, respectively, because neuraminidase cleaves sialic acid residues exclusively from the nonreducing end and stops when the sialic acid residue at the nonreducing end is lactonized. Due to the results of the enzymatic hydrolysis and the intensity changes of the peaks (Figure 2), peaks e and f (group B in Figure 1) are concluded to be the tetrameric monolactones **5** and **6**, which can be hydrolyzed by neuraminidase. Monolactones **5** and **6** can further lactonize to give the dilactonized tetramers as peaks g and h (group C in Figure 1). Peak h is identified as compound **7** because of its resistance to neuraminidase hydrolysis. Compound **8**, with the two consecutive lactone rings in the reducing terminal, is assigned to peak g due to its rapid disappearance by hydrolytic cleavage by neuraminidase. The hydrolysis of **8** results in the formation of the fully lactonized trimer, as shown by the appearance and increasing intensity of peak c. Based on the results illustrated in Figures 1 and 2, the lactonization pathway of the sialic acid tetramer can be deduced (Scheme 1). At first, the free tetramer **3** was lactonized to generate **5** as the major monolactone and **6** as the minor one. The monolactones

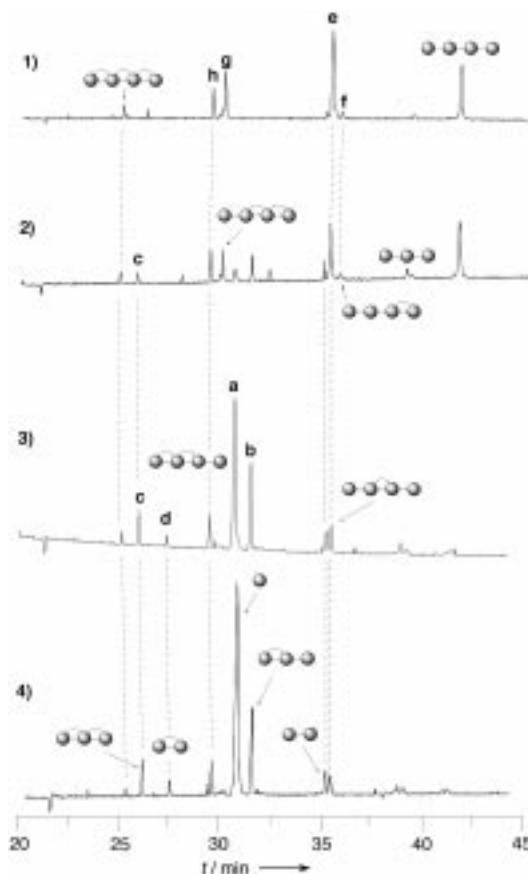


Figure 2. CE chromatograms of the hydrolysis of the mixed lactonization products by neuraminidase at different times of incubation (Stage 1) = 0 min, 2) = 10 min, 3) = 1 h, and 4) = 2 h).

were further lactonized to form dilactones **7** and **8**, respectively, and finally to form the fully lactonized tetramer **4**.

These results confirmed the previous proposal that the internal sialic acid residues in oligo/polysialic acids are more inclined to lactonize^[9] since **5** was formed as the major product in the initial stage of lactonization. Our lactonization experiments were conducted in glacial acetic acid so all carboxylic acids should be in the protonated form. Inspection of the molecular structure of tetrasialic acid (**3**) indicates that the internal carboxylic acids (II and III, Scheme 1) confront more dipole–dipole repulsion than the external ones. Carboxylic acids II and III therefore have less freedom and this explains why they show more tendency to lactonize, which leads to the formation of **5** and **6**, respectively. Since the carboxylic acid in the reducing terminal (IV) is in the equatorial position due to the anomeric effect,^[10] carboxylic acid II is expected to be more confined and easier to lactonize than III; this was proved by the observation that **5** was the predominant monolactone and **6** was formed in only trace quantities at the beginning of lactonization. On the other hand, the tetrameric monolactone **9** (Scheme 1) was undetected in our investigation. Such a result is consistent with the observation that lactone formation is faster at the reducing terminal than at the nonreducing end.

The fully lactonized tetramer **4** was treated with 0.05 M (NH₄)₂CO₃ to understand how it is hydrolyzed under alkaline conditions. As shown in Figure 3, the CE analysis indicated

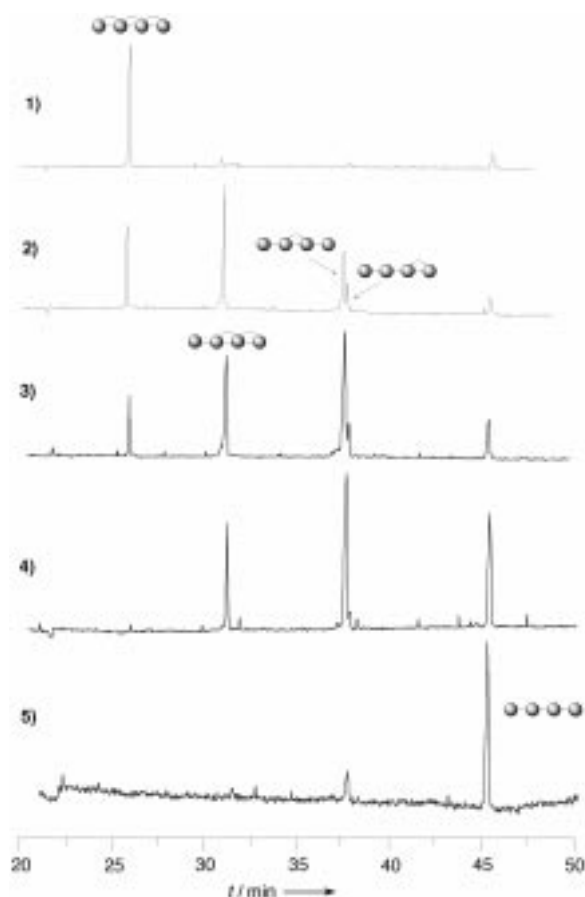


Figure 3. CE chromatograms of the hydrolysis of **4** under alkaline conditions at different reaction times (Stage 1) = 0 min, 2) = 10 min, 3) = 1 h, 4) = 4 h, and 5) = 16 h).

that dilactone **8** was generated first and followed by the mixture of the two monolactones **5** and **6**, with **5** being produced preferentially. All the peak assignments were verified by co-injections of the samples from both lactonization and enzyme hydrolysis and further confirmed by mass spectrometry analyses. It was intriguing that the intensity ratio of two tetrameric monolactones changed as a function of hydrolytic times with **5** gradually becoming predominant. The different stabilities of the two monolactones under alkaline hydrolysis may reasonably explain this observation.

The same studies were carried out for α -2,9-tetrasialic acid. Except for the extremely slow rate of lactonization (Figure 4), similar patterns of lactone formation and hydrolysis were observed, which was not surprising based on our explanations mentioned above.

In comparison with a similar investigation using high performance anion-exchange chromatography, our analysis using CE affords a superior method with high resolution to identify each lactonized species as well as sialic acid oligomers. In fact, this is the first report to demonstrate the progress of lactonization of sialic acid oligomers and present clear evidence of the initial lactonization taking place at the internal positions. The study of lactonization of oligosialic acids could provide a better understanding of the natural lactonization of PSAs which may represent an on/off mechanism for a physiological function.^[11]

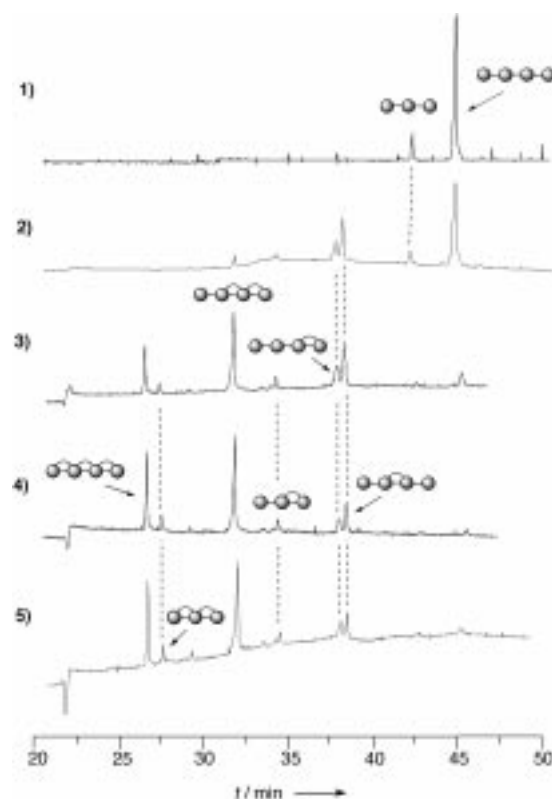


Figure 4. CE chromatograms of the lactonization of α -2,9-linked sialic acid tetramer in glacial acetic acid at different reaction times (Stage 1) = 0 day, 2) = 1 day, 3) = 2 days, 4) = 3 days, and 5) = 6 days). The tetramer was contaminated with a small amount of the trimer so that trimeric monolactone and dilactone were also observed. The structures of the α -2,9-linked sialic acid oligomers and lactonized species are equivalent to the α -2,8-linked species except for the glycosidic linkage.

Experimental Section

N-Acetylneuraminic acid tetramer ($[\rightarrow 8\text{Neu}5\text{Aca}2\rightarrow]_4$) was obtained as the gift from Prof. Yasuo Inoue (Institute of Biological Chemistry, Academia Sinica, Taiwan) and NGK Biochemical Ltd. (Handa, Japan). α -2,9-tetrasialic acid was isolated from meningococcal serogroup C and obtained from Prof. T.-Y. Liu (Institute of Biological Chemistry, Academia Sinica, Taiwan). Neuraminidase from *Anthrobacter ureafaciens* was purchased from Sigma (St. Louis, USA). The treatment of α -2,9-tetrasialic acid was the same as that of α -2,8-tetrasialic acid except for longer reaction times.

Lactonization of 3: Free tetramer **3** (25 μg) was incubated in glacial acetic acid (1 mL) at room temperature. The reaction was stopped by freezing with liquid nitrogen and then dried immediately in a SpeedVac concentrator (Savant, USA) to remove acetic acid. Dried samples were dissolved in doubly distilled water and an aliquot (5 μL) of the mixture was analyzed by high performance capillary electrophoresis (HPCE).

Preparation of 4: Free tetramer **3** (25 μg) was left in glacial acetic acid (1 mL) at room temperature for 8 h, frozen with liquid nitrogen, and then dried immediately in a SpeedVac concentrator (Savant, USA) to remove acetic acid.

Delactonization of 4: Trilactone **4** (50 μg) was dissolved in 0.05 M ammonium carbonate (500 μL) at 37 $^\circ\text{C}$. Aliquots were withdrawn at periods of 20, 40, and 80 min, frozen with liquid nitrogen, and then dried in a SpeedVac concentrator (Savant, USA). Dried samples were dissolved in doubly distilled water and an aliquot (5 μL) of the mixture was analyzed by HPCE.

Chromatographic Conditions: Capillary electrophoreses (CE) were performed on a Beckman capillary electrophoresis system (P/ACE 2100) using a fused silica capillary (118 cm $\times$ 75 μm) and carried out by applying 20 kV

at 25 °C. Phosphate buffer (50 mM, pH 8.0) was used as the running buffer. The chromatograms were monitored by UV absorption at 200 nm. Samples were injected into the capillary by high pressure nitrogen (20 psi) for 3 s. The capillary was regenerated by washing with 0.1 M NaOH for 5 min after purging with doubly distilled water for 3 min.

Neuraminidase Hydrolysis: Partially lactonized samples (10 µg) in ammonium acetate buffer (100 mM, pH 5) were digested with neuraminidase (1 mU) from *Anthrobacter ureafaciens* in 20-µL CE vials at room temperature. The progress of hydrolysis was monitored at each time interval by HPCE.

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Octahedral Coordination of an Iodide Ion in an Electrophilic Sandwich**

Hans Lee, Martin Diaz, Carolyn B. Knobler, and M. Frederick Hawthorne*

Until now, no discrete six-coordinate iodide (–1 oxidation state) sandwich species has been reported. A limited number of octahedral complexes such as $[\text{I}^{\text{VII}}\text{F}_6]^+$,^[1] $[\text{I}^{\text{VII}}(\text{OH})_6]^+$,^[2] and $[\text{I}^{\text{V}}\text{F}_6]^{-3}$ (distorted octahedral) with iodine in a formal +7 or +5 oxidation state are known. Multidentate Lewis acidic hosts form four- to six-coordinate iodine species, with iodine in a formal –1 oxidation state.^[4, 5] The field of anion host–guest chemistry offers prospects for molecular recognition, anion transport, and catalysis.^[4–6]

Penta-, tetra-, and tridentate organomercury receptors form complexes with halide anions and a variety of neutral electron-rich species. The cyclic pentameric $[(\text{CF}_3)_2\text{CHg}]_5$ coordinates two halide anions (Cl^- , Br^- , I^-), one above and one below the center of the cavity.^[7, 8] Similarly, tetrameric 12-mercuracarborand-4 ($\text{C}_2\text{B}_{10}\text{H}_{10}\text{Hg}_4$) can also bind two iodide ions, while the smaller chloride and bromide ions usually reside within the cavity to produce 1:1 host–guest complexes.^[9] Trimeric perfluoro-*o*-phenylenemercury coordinates halide ions in a 1:1 stoichiometric ratio of trimer:halide. The halide ions are situated above and below the cavity to form an infinite bent polydecker structure with the composition $[(\text{o-C}_6\text{F}_4\text{Hg})_3\text{X}]^-$ ($\text{X} = \text{Cl}$, Br , I) and a distorted octahedral geometry around the anion.^[8, 10]

Here we report the synthesis and structural characterization of the first discrete octahedral sandwich complex $[\mathbf{1}_2 \cdot \text{I}]^-$, composed of two electroneutral trimeric *B*-hexamethyl-9-mercuracarborand-3 $[\mathbf{9}, 12-(\text{CH}_3)_2-\text{C}_2\text{B}_{10}\text{H}_8\text{Hg}]_3$ (**1**) receptors which simultaneously coordinate an iodide ion in a sandwich fashion.

The reaction of LiI with **1** results in the sandwich complex $[\mathbf{1}_2 \cdot \text{I}]^-$ in 82 % yield. $\text{Li}[\mathbf{1}_2 \cdot \text{I}]$ is an air- and moisture-stable crystalline solid. The ^1H , ^{13}C , and ^{11}B NMR spectra revealed that $[\mathbf{1}_2 \cdot \text{I}]^-$ has a highly symmetrical structure, with chemical shifts nearly identical to those of the empty host **1**.^[11] The ^{199}Hg NMR spectrum of $[\mathbf{1}_2 \cdot \text{I}]^-$ in acetone exhibits a singlet at $\delta = -957$, with no evidence of the empty host **1** ($\delta = -1158$).^[11] A downfield shift relative to the signal of **1** is diagnostic of coordination of a guest to the mercury atoms of **1**.^[9] The ^{199}Hg NMR chemical shifts of similar complexes of organomercury compounds exhibit strong dependencies on the nature of the solvent and the concentration of the complex.^[12] $\text{Li}[\mathbf{1}_2 \cdot \text{I}]$ has a ^{199}Hg NMR chemical shift which is essentially independent of concentration at room temperature. This observation suggests that the solid-state structure of $\text{Li}[\mathbf{1}_2 \cdot \text{I}]$ is maintained in solution.^[9] The negative-ion fast atom bombardment (FAB) mass spectrum exhibits a peak centered at m/z 2351, with the

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correct isotopic pattern for $[\text{I}_2 \cdot \text{I}]^-$, and an anion envelope at m/z 1239, which corresponds to $[\text{I} \cdot \text{I}]^-$. The diiodide complex $[\text{Li}(\text{I} \cdot \text{I}_2)]^-$ is not observed by negative-ion FAB-MS or ^{199}Hg NMR spectroscopy. The $[\text{I} \cdot \text{I}]^-$ species at m/z 1239 is presumed to arise from cleavage of $[\text{I}_2 \cdot \text{I}]^-$, since only a single sharp signal is observed in the ^{199}Hg NMR spectrum of the latter.

A single crystal of $[\text{Li}(\text{H}_2\text{O})_4][\text{I}_2 \cdot \text{I}] \cdot 2\text{CH}_3\text{CN}$, grown from acetonitrile/acetone, was selected for an X-ray diffraction study (Figure 1).^[13] The centrosymmetric trimeric complex

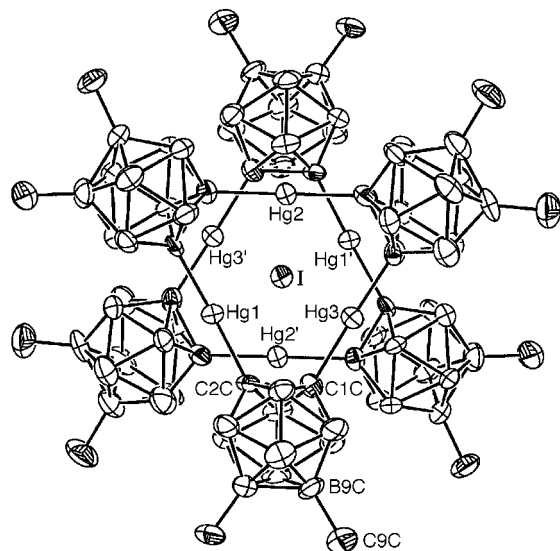


Figure 1. Structure of $[\text{I}_2 \cdot \text{I}]^-$ (ORTEP plot; hydrogen atoms omitted for clarity). Selected distances [Å] and angles [°]: $\text{Hg1} \cdots \text{I}$ 3.2492(5), $\text{Hg2} \cdots \text{I}$ 3.2549(5), $\text{Hg3} \cdots \text{I}$ 3.2728(5); $\text{Hg1-I-Hg1}'$ 180, Hg1-I-Hg2 69.95(1), Hg1-I-Hg3 69.07(1), Hg2-I-Hg3 69.81(1), $\text{Hg1-I-Hg2}'$ 110.05(1), $\text{Hg1-I-Hg3}'$ 110.93(1), $\text{Hg2-I-Hg3}'$ 110.19(1).

$[\text{I}_2 \cdot \text{I}]^-$ contains an iodide ion coordinated to six mercury atoms. The lithium ion is located on an inversion center and is coordinated to the oxygen atoms of four water molecules. The two trimeric hosts of $[\text{I}_2 \cdot \text{I}]^-$ are inverted with respect to one another and separated by 4.902 Å. The iodide ion is located almost equidistant from each of the six mercury atoms ($\text{Hg} \cdots \text{I}$ 3.2492(5), 3.2549(5), 3.2728(5) Å) at less than the sum of the van der Waals radii (3.89 Å).^[14, 15] $[\text{I}_2 \cdot \text{I}]^-$ has shorter $\text{Hg} \cdots \text{I}$ distances than the cyclic trimeric perfluoro-*o*-phenylenemercury iodide complex mentioned before (3.331–3.487 Å)^[16] and the *B*-octamethyl-12-mercuracarborand-4 diiodide complex (3.438(4), 3.335(3) Å).^[17] The three mercury atoms of **1** in $[\text{I}_2 \cdot \text{I}]^-$ are arranged in a near-equilateral triangle ($\text{Hg} \cdots \text{Hg}$ 3.7285(8), 3.6975(7), 3.7352(7) Å; Hg-Hg-Hg 59.39(1), 60.21(1), 60.39(1)°). The $\text{Hg} \cdots \text{I}$ interactions and steric hindrance of the opposing icosahedra contribute to the observed deviation from linearity of the C-Hg-C angles (169.0(5) to 171.0(5)°) relative to those of the uncomplexed host **1** (172.7(6)–174.9(6)°).^[11] The Hg-C-C-Hg torsion angles indicate the coplanarity of these four atoms, with values equal to or smaller than 1°.

The coordination of the iodide ion in $[\text{I}_2 \cdot \text{I}]^-$ must arise from the interaction of its filled p orbitals with empty mercury p orbitals that have the proper orientation to form three

equivalent $p_{\text{Hg}}-p_{\text{I}}-p_{\text{Hg}}$ three-center, two-electron bonds and a sandwich structure (Figure 2).

Experimental Section

Li $[\text{I}_2 \cdot \text{I}]$: A solution of **1**^[11] (0.50 g, 0.45 mmol) in acetone (40 mL) was treated with LiI (0.15 g, 1.12 mmol) at room temperature for 12 h. The solvent was removed under vacuum, and the residual solid was washed with water and then extracted with diethyl ether (3 × 25 mL). The combined organic phase was dried over anhydrous magnesium sulfate and filtered. The solvent was removed under vacuum to give $\text{Li}[\text{I}_2 \cdot \text{I}]$ in 82% yield. $[\text{Li}(\text{H}_2\text{O})_4][\text{I}_2 \cdot \text{I}] \cdot 2\text{CH}_3\text{CN}$ is formed on repeated recrystallization from acetonitrile/acetone; m.p. > 300 °C. ^1H NMR (400 MHz, acetone, 25 °C): δ = 3.0–1.0 (B–H), 0.05 (CH_3); $^{13}\text{C}\{^1\text{H}\}$ NMR (90 MHz, $[\text{D}_6]\text{acetone}$, 25 °C): δ = 89.4 (carborane-C), 0.81 (brs, CH_3); $^{11}\text{B}\{^1\text{H}\}$ NMR (160 MHz, $[\text{D}_6]\text{acetone}$, 25 °C, external $\text{BF}_3 \cdot \text{Et}_2\text{O}$): δ = 10.7, –3.3, –8.0 (2:2:6); $^{199}\text{Hg}\{^1\text{H}\}$ NMR (89.6 MHz, $[\text{D}_6]\text{acetone}$, 25 °C, external 1.0 M PhHgCl in $[\text{D}_6]\text{DMSO}$): δ = –1187^[12] upfield from neat Me_2Hg : δ = –957; negative-ion FAB-MS: m/z (%): 2351 (5) $[\text{I}_2 \cdot \text{I}]^-$, 1239 (100) $[\text{I} \cdot \text{I}]^-$.

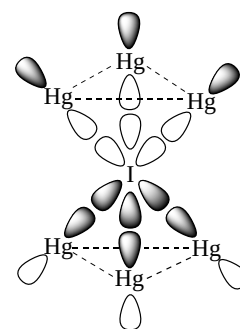


Figure 2. Orbital representation of $[\text{I}_2 \cdot \text{I}]^-$ illustrating the three-center, two-electron bonds.

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values based approximately on the value for the attached atom. The final discrepancy index was $R = 0.063$, $R_w = 0.171$ ($w = 1/\sigma^2(|F_o|)$) for 6469 independent reflections with $I > 2\sigma(I)$. The largest peak maximum and minimum on a final difference electron density map were 2.34 (near Hg) and $-1.72 \text{ e } \text{\AA}^{-3}$. Data were processed with the UCLA crystallographic package and SHELX93. 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-135263. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road,

Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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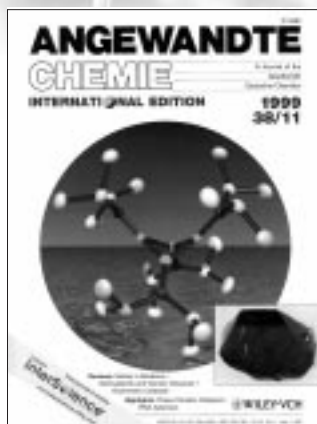
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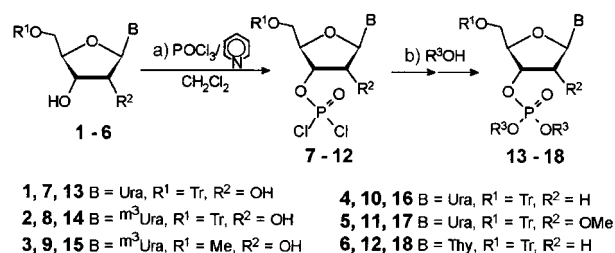
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Medium-Controlled Intramolecular Catalysis in the Direct Synthesis of 5'-O-Protected Ribonucleoside 2'- and 3'-Dialkylphosphates**

Christo D. Roussev,* Gabriela D. Ivanova, Emilia K. Bratovanova, and Dimiter D. Petkov

Understanding of the mechanisms of enzyme action has progressed considerably in recent years due to rapid developments in crystallography, enzymology, and chemistry. The introduction of substrate mimics^[1] has become a powerful tool for the elucidation of the mechanistic aspects of RNA and enzyme catalysis. Model reactions in aprotic organic solvents are considered to accurately mimic the environment of the active site in the enzyme, which contains little or no water and where intramolecular catalysis would be favoured. In aprotic media, hydrogen-bonding interactions of the reagents with the solvent are absent or weak, which allows the catalytic or inhibitory effects on the reaction rates by hydrogen-bond donors and acceptors to be studied. Research into structure–activity relationships and solvent effects has shown that the reaction mechanisms can be dramatically altered by changing reactant substituents and/or the reaction medium.^[2]

We describe here the first example of solvent-induced, intramolecular electrophilic catalysis by a *cis*-vicinal hydroxyl group. The phosphate transfer reaction in the synthesis of 5'-O-protected ribonucleoside 2'- and 3'-dialkylphosphates **13**–**15** is accelerated, relative to the corresponding 2'-deoxy- and 2'-O-protected derivatives **16**–**18** (see Scheme 1 and Table 1; isomeric mixtures of 2'- and 3'-compounds **13**–**15** were formed



Scheme 1. Two-step synthesis of the 5'-O-protected ribonucleoside derivatives. Compounds **7**–**9** and **13**–**15** are formed as an isomeric mixture of the 2'- and 3'-derivatives in ratio ca. 65:35. For simplicity the 2'-derivatives are not shown. Ura = uridine, Tr = triphenylmethyl, Thy = thymine.

but only the 3'-derivatives are shown). In the accelerated reactions, medium-strength selective microsolvation controls the participation of the adjacent hydroxyl group, 2'-OH (or 3'-OH), and prevents the usual, entropically favoured attack of the internal nucleophile. This promotes external substitution

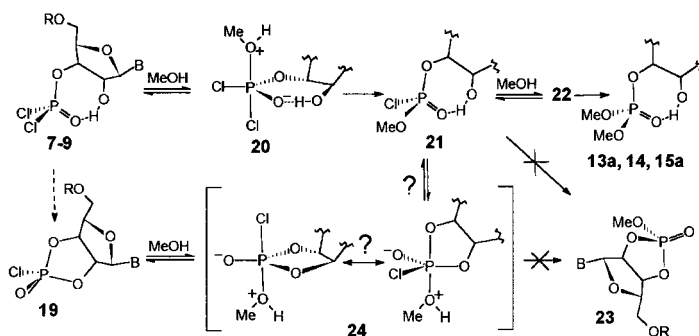
Table 1. Half-life data ($\tau_{1/2}$) showing the acceleration of the phosphate transfer reaction in the syntheses of the 5'-O-ribonucleoside derivatives

Products	R ³ OH (see Scheme 1)	Yield [%]	$\tau_{1/2}$ [min] ^[a]
13a	CH ₃ OH	77	5.5
13b	CH ₃ CH ₂ OH	68	12
14	CH ₃ OH	79	7.5
15a	CH ₃ OH	73	6.5
15b	Ph(CH ₂) ₃ OH	69	14.5
16	CH ₃ OH	98	267
17	CH ₃ OH	96	290
18	CH ₃ OH	97	261

[a] $\tau_{1/2}$ is measured after the addition of R³OH in the second step of the reaction (Scheme 1).

at the phosphorus atom and provides different phosphoryl transfer mechanisms in the aqueous and organic media. If the solvent characteristics of the enzyme active centers are better mimicked by nonaqueous solvents^[3], these findings may provide relevant information as to the possible role of intramolecular catalysis in enzymatic reactions. This approach also provides a rapid, simple, and cost-effective synthetic pathway to the ribonucleoside 2'- and 3'-dialkylphosphates **13**–**15**. These compounds could be used to mimic the neutral, ionic RNA form^[4, 5] in model reactions.

The triesters **13**–**18** were synthesized in two steps as shown in Scheme 1. The first step was phosphorylation of the 5'-O-protected nucleosides **1**–**6** by POCl₃/pyridine in dry CH₂Cl₂ (see the Experimental Section); this was complete (as judged by RPLC and ³¹P NMR) within five minutes—the time necessary to record the first ³¹P NMR spectrum. The second step was the alcoholysis of the formed 5'-O-protected nucleoside phosphodichloridates **7**–**12** with R³OH. This step took only 20–40 minutes for compounds **7**–**9**, but had to be left overnight for the 2'-deoxy-derivatives **10** and **12**^[4–7] and the 2'-O-methyl-derivative **11**.^[8] Since little of the cyclic monochloridate **19**, the cyclic triester **23** (Scheme 2) or any of the possible diphosphorylated byproducts were detected on the ³¹P NMR spectra, we tentatively assumed that the presence of the free *cis*-vicinal hydroxyl group enhanced the two consecutive alcoholyses of the ribonucleoside dichlorophosphates **7**–**9**. Our interpretation is consistent with intramolecular hydrogen-bonding participation of the adjacent hydroxy functionality, general acid catalysis induced by the aprotic medium, which promotes external alcoholysis in the ground-



Scheme 2. Possible mechanisms for the formation of the 5'-O-ribonucleoside derivatives. According to Westheimer's guidelines, the first result of nucleophilic attack on **19** should be the structures **24** which are expected to be high-energy structures.

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state structures **7–9** and **21** and/or the pentacoordinated phosphorane intermediate/transition-state structures **20** and **22** (structure **22** is similar to **20** but after substitution of one of the chlorine atoms with the methoxy group).

It is known that 2'-OH nucleophilic assistance causes RNA to undergo rapid hydrolysis in neutral aqueous media and under physiological conditions.^[9, 10] Here we point out the distinction between this mechanism, which involves nucleophilic participation of the vicinal hydroxyl group, and our proposed mechanism, in which the hydroxyl group activates an ester by intramolecular general acid catalysis. In polar solvents which can act as hydrogen-bond donors, such as water, the hydrogen-bond acceptor (the phosphate residue) and the intramolecular donor (the adjacent OH group) are solvated separately. Thus, such a medium prevents the intramolecular hydrogen bonding and internal nucleophilic attack at the phosphorus atom by the adjacent OH group is the only possible reaction. On the other hand, in nonpolar, aprotic organic solvents the free vicinal hydroxyl group is capable of acid catalysis^[11, 12] as a hydrogen-bond donor, as explained above.

During the substitution reaction, the six-membered ring formed through hydrogen bonding may adopt the diequatorial position, and protonation of the former phosphoryl oxygen atom stabilizes the intermediate/transition state **20**. This promotes the external alcoholysis of the ribonucleoside 3'(and/or 2')-di/monochlorophosphates **7–9** and **21**. The same selective microsolvation by the adjacent hydroxyl group in aprotic solvents has been shown to significantly enhance acyl transfer reactions and is known as the Henbest–Kupcham effect.^[13] One example of this is the ester aminolysis of 2-hydroxyphenyl benzoate in acetonitrile relative to that of 2-methoxyphenyl benzoate.^[14]

We assumed that the key intermediate structures in the reaction scheme are the derivatives **7–9**. To prove this we undertook ³¹P NMR studies. We monitored the first and second steps of the reaction for 5'-O-trityluridine (**1**) relative to those of 5'-O-trityl 2'-deoxyuridine (**4**) and 5'-O-trityl 2'-methoxyuridine (**5**) with the alcohol being MeOH (Scheme 1). During the first step, the resonances assigned to **7** (3': $\delta_P = 22.4$, d, $^3J_{PH} = 12$ Hz; 2': $\delta_P = 20.03$, d, $^3J_{PH} = 10$ Hz; ratio ca. 65:35), **10** ($\delta_P = 6.7$, d, $^3J_{PH} = 11$ Hz), and **11** ($\delta_P = 8.4$, d, $^3J_{PH} = 10$ Hz) indicated a quantitative conversion five minutes after the reaction had been started. The multiplicity of these signals in the ³¹P–¹H coupled spectrum and the $^3J_{PH}$ for **7**, **10** and **11** are similar and, taken in conjunction with the bigger downfield shift ($\Delta\delta_P = 15.7$ and 14) of **7** relative to **10** and **11**, this strongly supports the proposed structure **7** (Scheme 2), involving intramolecular hydrogen bonding. This interpretation is further supported by the existence of a weak hydrogen bond in the ground state of the triester product **13a**, for which a relatively small downfield shift ($\Delta\delta_H = 1.66$) was observed in ¹H NMR spectrum for the proton of the 2'(3')-hydroxyl group.

The cyclization mechanism, however, is not completely avoided. The absorption assigned to the cyclic monochloridate **19** ($\delta_P = 20.01$, t, $^3J_{PH} = 9.2$ and 8.4 Hz)^[15] appeared as one signal despite the chirality at the phosphorus atom. The intensity was not over 6–7 % and the signal on the ¹H NMR

spectrum overlaps with those assigned to the 2'-isomer of **7**, but on ³¹P–¹H coupled spectrum they are clearly distinguishable.

The very strong evidence supporting our interpretation that the cyclization route via **19** is almost completely avoided with our conditions was found when the first step of the reaction was prolonged for one hour at room temperature (Scheme 1). There was no change in the intensities, the multiplicities or the ratio of the signals (³¹P NMR) for the 3'- and 2'-isomers of **7**. The intensity of the cyclic monochloridate **19** remains the same (ca. 7–8 %) as at the beginning of the reaction instead of increasing, which one would expect if the nucleophilic participation of the *cis*-vicinal hydroxyl was not avoided.

When the first-step reaction mixtures were treated with MeOH (Scheme 1) and the ³¹P NMR spectrum monitored, the signals for **7**, **10** and **11** immediately disappeared (5 min—the time necessary to record the first spectrum). New absorptions for the products **13a**, **16**, and **17** emerged simultaneously at $\delta = 1.11$ and 0.92 (2'- and 3'-isomers in ratio ca. 35:65), 0.65, and 0.8 respectively in {¹H} decoupled spectra. After 15 min the intensities of the resonances assigned to **13a** indicated a quantitative yield, while those of **16** and **17** indicated 12.5 % and 11 % conversion only. No evidence supporting the formation of the cyclic triester **23** (Scheme 2) was found, even after 24 hours reaction time.

The kinetic parameters, presented in Table 1, were obtained by RPLC analysis of aliquots withdrawn regularly from the reaction mixture after the addition of the R³OH. The time-dependent mole fractions of the products from the reaction were calculated from their respective peak areas and the half-life data ($\tau_{1/2}$) were then extracted from the kinetic curves. Yields presented for the ribo-triesters **13–15** are for the combined 3'- and 2'-isomers (ratio of ca. 65:35 as judged by RPLC).

By using aprotic solvents for these studies we hoped to mimic the conditions in the enzyme active sites where water molecules are either restricted or excluded. Our interpretation of the results is that the nonaqueous media change the nature of participation of the *cis*-vicinal hydroxyl group from nucleophilic to electrophilic. The in situ, easy-to-handle phosphorylation procedure described here eliminates the difficulties connected with the introduction of protecting groups and the use of complicated phosphorylating agents. Thus, the method may be of general use for the introduction of phosphoryl groups in nucleoside chemistry.

Experimental Section

Materials and Methods. Analytical RPLC was carried out on a NUCLEOSIL 100-5C₁₈ column (12.5 cm × 4.6 mm) with isocratic elution (10–55 % CH₃CN, depending on ROH, in 20 mM K₂HPO₄/KH₂PO₄ buffer, pH 7.0). Detection was by UV absorbance at 254 nm. Preparative HPLC was carried out using a semi-preparative NUCLEOSIL 100-5C₁₈ column (25 cm × 8 mm) with isocratic elution (20–68 % CH₃CN in the same buffer). ¹H and ³¹P NMR spectra were recorded on a BRUKER Avance DRX 250 spectrometer with TMS as an internal standard or 85 % H₃PO₄ as an external standard at room temperature.

General procedure. Pyridine (4 mmol) was added dropwise to a cooled (5 °C) solution of POCl₃ (1 mmol) in dry CH₂Cl₂ (4 mL). After the initial fuming had subsided, the resulting clear solution was added to a precooled mixture (5 °C) of 5'-O-protected nucleoside (0.2 mmol) and pyridine

(4 mmol) in dry CH_2Cl_2 (4 mL) and the reaction was stirred at room temperature for 5–10 min. The appropriate alcohol (4 mmol) was added and stirring continued until the reaction was completed (as judged by RPLC and ^{31}P NMR). The reaction mixture was then evaporated to dryness under reduced pressure (maximum 40°C), dissolved in acetonitrile, and applied to a semi-preparative RP-HPLC column. Appropriate fractions (2'- and 3'-isomers can be collected separately) were evaporated immediately (reduced pressure, maximum 40°C), dried several times by co-evaporation with dry acetonitrile or CH_2Cl_2 , and kept in a desiccator. The pure samples of the triesters **13–18** were prepared by dry extraction from the inorganic buffer salts in acetonitrile or CH_2Cl_2 . ^1H and ^{31}P NMR spectra and chromatographic properties of all isolated compounds were identical with the corresponding phosphotriesters obtained by an alternative procedure (see references [4–6]).

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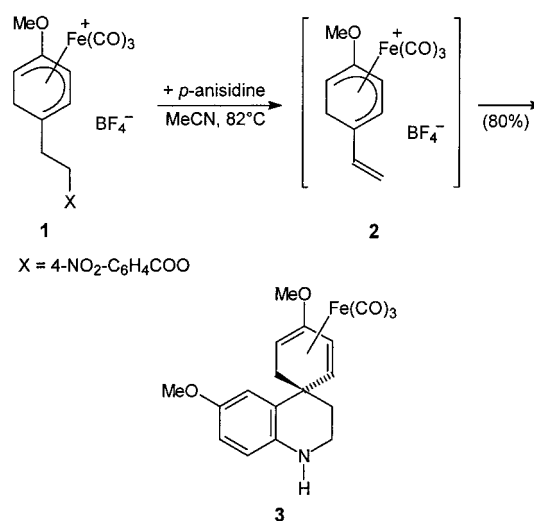
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Iron-Mediated Diastereoselective Spiroannulations with Vinylogous Urethanes – A Novel Access to Spiroannulated Carbo- and Heterocycles**

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Tricarbonyl(η^4 -1,3-diene)iron complexes are widely used in organic synthesis;^[1] applications include stereoselective spirocyclization.^[2] A few years ago, we reported the diastereoselective iron-mediated spiroannellation of arylamines to spirotetrahydroquinolines.^[3, 4] The reaction of the iron complex salt **1** with *p*-anisidine provided the tricarbonyliron complex **3** containing a spiro[quinoline-4,1'-cyclohexane] framework (Scheme 1).^[3] Through a deuterium labelling



Scheme 1. Iron-mediated spiroannulation of *p*-anisidine.

study, the one-pot spiroannellation was recently shown to proceed via the intermediate tricarbonyliron-complexed 1-vinyl-4-methoxycyclohexadienyl cation **2**.^[5] Moreover, it was demonstrated that arylamines which are more nucleophilic in the *ortho*-amino position show a regioselectivity reversal in their reaction with **1** and afford the spiro[quinoline-2,1'-cyclohexane] framework.^[4] The iron-mediated spiroannellation of arylamines was applied to the synthesis of a tetracyclic

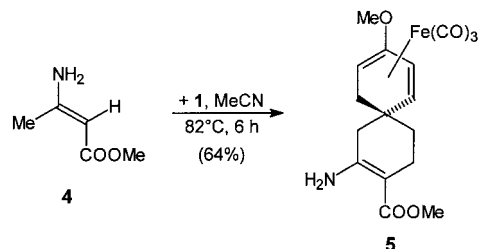
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substructure of the discorhabdin alkaloids.^[6] Here, we describe a remarkable extension of the scope of the iron-mediated spiroannulation by using vinylogous urethanes as substrates for the reaction with the complex salt **1**.

The reaction of methyl β -aminocrotonate **4** with **1** in acetonitrile under reflux afforded diastereoselectively the spiro[5.5]undecane derivative **5** (Scheme 2). The chemical



Scheme 2. Iron-mediated spiroannulation of methyl β -aminocrotonate **4**.

shift for the signal of the spiro carbon atom at $\delta = 36.27$ in the ^{13}C NMR spectrum indicated immediately that a carbocyclic, rather than a heterocyclic, ring system had been formed (Table 1). The structure (with respect to the orientation of the methyl β -aminocrotonate unit) and configuration (with respect to the spiroannulation) of the product **5** were determined by comparison of the ^{13}C NMR data with those of the compounds described below.

Table 1. Selected spectroscopic data of the spirocyclic tricarbonyliron complexes **5**, **7**, **8**, **10a**, and **10b**.

5: ^{13}C NMR and DEPT (75 MHz, CDCl_3): $\delta = 20.33$ (CH_2), 34.92 (CH_2), 36.27 (C), 38.42 (CH_2), 47.94 (CH_2), 50.48 (CH_3), 53.47 (CH), 54.45 (CH_3), 60.77 (CH), 64.19 (CH), 91.41 (C), 140.66 (C), 155.52 (C), 170.44 (C=O), 211.16 (3 CO)

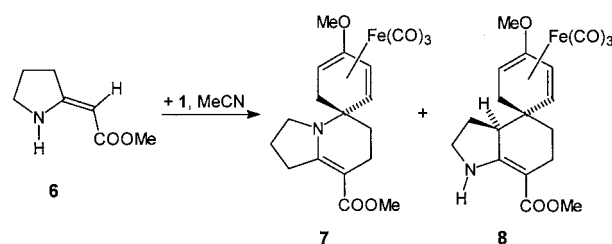
7: ^{13}C NMR and DEPT (100 MHz, CDCl_3): $\delta = 19.36$ (CH_2), 20.87 (CH_2), 33.34 (CH_2), 35.38 (CH_2), 38.87 (CH_2), 47.99 (CH_2), 50.21 (CH_3), 51.80 (CH), 53.10 (CH), 54.63 (CH_3), 57.94 (C), 64.72 (CH), 88.62 (C), 140.63 (C), 158.81 (C), 168.94 (C=O), 210.67 (3 CO); elemental analysis for $\text{C}_{19}\text{H}_{21}\text{FeNO}_6$ (%): calcd: C 54.96, H 5.10, N 3.37; found: C 54.92, H 5.06, N 3.51

8: ^{13}C NMR and DEPT (100 MHz, CDCl_3): $\delta = 19.96$ (CH_2), 27.09 (CH_2), 32.09 (CH_2), 36.49 (CH_2), 38.57 (C), 44.44 (CH_2), 50.27 (CH_3), 51.71 (CH), 53.90 (CH), 54.43 (CH_3), 60.76 (CH), 64.42 (CH), 85.23 (C), 140.58 (C), 161.36 (C), 170.08 (C=O), 211.26 (3 CO)

10a: ^{13}C NMR and DEPT (100 MHz, CDCl_3): $\delta = 20.41$ (CH_2), 22.94 (CH_2), 23.02 (CH_2), 32.04 (CH_2), 36.98 (CH_2), 40.01 (C), 41.36 (CH_2), 45.86 (CH), 50.32 (CH_3), 54.11 (CH), 54.43 (CH_3), 60.95 (CH), 66.45 (CH), 87.98 (C), 139.96 (C), 159.80 (C), 170.90 (C=O), 211.44 (3 CO); elemental analysis for $\text{C}_{20}\text{H}_{23}\text{FeNO}_6$ (%): calcd: C 55.96, H 5.40, N 3.26; found: C 56.21, H 5.41, N 3.49

10b: ^{13}C NMR and DEPT (100 MHz, CDCl_3): $\delta = 20.67$ (CH_2), 23.42 (CH_2), 36.76 (CH_2), 39.29 (C), 41.11 (CH_2), 41.32 (CH_2), 46.18 (CH), 50.31 (CH_3), 53.17 (CH), 53.72 (CH), 54.40 (CH_3), 66.15 (CH), 86.78 (C), 140.06 (C), 160.19 (C), 170.98 (C=O), 211.42 (3 CO); elemental analysis for $\text{C}_{20}\text{H}_{23}\text{FeNO}_6$ (%): calcd: C 55.96, H 5.40, N 3.26; found: C 56.10, H 5.38, N 3.49

Spiroannulation of the 2-(methoxycarbonylmethylene)pyrrolidine **6**^[7] with the complex salt **1** in acetonitrile provided the spiro[indolizidine-5,1'-cyclohexane] **7** and the spiro[indole-4,1'-cyclohexane] **8** (Scheme 3). In this case, the ratio of the two products was dependent on the reaction time and the temperature. At lower temperatures and shorter reaction



Scheme 3. Synthesis of the spiroindolizidine **7** and the spiroindole **8**.

times the spiroindolizidine **7** was generally preferred. Reaction for 10 h at room temperature and subsequently 13 h under reflux afforded **7** and **8** in 80 % yield and a ratio of 6:1. After 4 d in acetonitrile under reflux the spiroindole **8** was formed predominantly (78 % yield, ratio of **7** to **8** = 1:5). A temperature-induced reversal of the regioselectivity of the spiroannulation was already found for the reaction of arylamines with **1**.^[4, 6]

The regioselectivity for the spiroannulation of **6** leading to the spiroindolizidine **7** becomes clear by the chemical shift for the signal of the spiro carbon atom at $\delta = 57.94$ (Table 1), characteristic for a 1-azaspiro[5.5]undecane framework.^[4] The configuration of **7** was deduced from the steric requirements of the tricarbonyliron fragment. The ^{13}C NMR spectrum of the spiroindole **8**, with a signal for the spiro carbon atom at $\delta = 38.57$ (Table 1), indicated that **8** contains a spiro[5.5]undecane core related to **5**. Although **8** has an additional stereogenic center, it was obtained surprisingly as a single diastereoisomer. The configuration of the spiroindole **8** was unequivocally confirmed by an X-ray crystal structure analysis (Figure 1).^[8] This analysis shows that compound **8** was

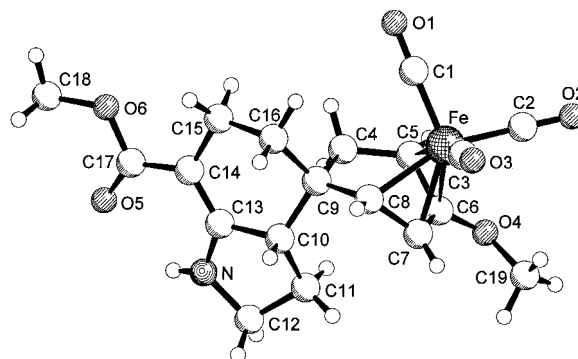
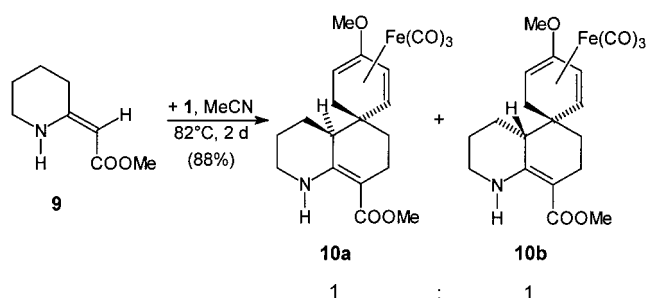


Figure 1. Molecular structure of **8** in the crystal. Selected bond lengths [Å]: Fe–C5 2.122(2), Fe–C6 2.103(2), Fe–C7 2.042(2), Fe–C8 2.065(2), C5–C6 1.417(3), C6–C7 1.406(3), C7–C8 1.431(3).

formed by an approach of **6** to **1** from the face *anti* to the tricarbonyliron fragment. Moreover, it determines the configuration of the newly generated additional stereogenic center C10 adjacent to the spiro carbon C9 (crystallographic numbering). The angular proton at C10 is oriented *syn* relative to the terminal carbon of the coordinated 4'-methoxycyclohexa-2',4'-diene moiety (C8).

The spiroannulation of the homologous 2-(methoxycarbonylmethylene)piperidine **9**^[9] with the complex salt **1** in acetonitrile for 2 d under reflux afforded the two diastereoisomeric spiroquinoline complexes **10a** and **10b** in a 1:1 ratio and 88 % yield (Scheme 4).

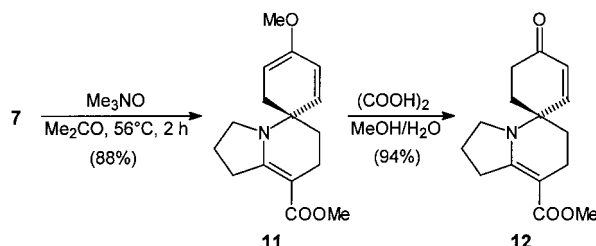


Scheme 4. Synthesis of the spiroquinolines **10**.

The structural assignment of the spiroquinolines **10** is based on the characteristic ^{13}C NMR spectra with signals for the spiro carbon atoms at $\delta = 40.01$ for **10a** and $\delta = 39.29$ for **10b** (Table 1), which suggest again a spiro[5.5]undecane subunit. The X-ray crystal structure determinations of **10a** and **10b** unambiguously confirmed that both compounds have a spiro[quinoline-5,1'-cyclohexane] framework with the vinyl-ous urethane oriented *anti* relative to the tricarboxyliron fragment. Both complexes differ only in the configuration of the additional stereogenic center C10 (Figure 2).^[8] Thus, compound **10a** has a stereochemical arrangement related to **8**,

in which the angular proton at C10 is *syn* relative to the terminal carbon atom (C8) of the coordinated 4'-methoxycyclohexa-2',4'-diene moiety, while in **10b** this stereochemical relationship is *anti*.

In order to demonstrate the utility of the spirocyclic tricarbonyliron complexes for further synthetic transformations, the spiroindolizidine **7** was smoothly converted to the corresponding free ligand **11** by reaction with anhydrous trimethylamine *N*-oxide in acetone under reflux (Scheme 5).^[10] Hydrolysis of the enol ether using oxalic acid in aqueous methanol^[2b] provided the spirocyclohexenone **12**, which represents a promising substrate for additional functionalization by nucleophilic additions.



Scheme 5. Transformation of complex **7** to the spirocyclohexenone **12**.

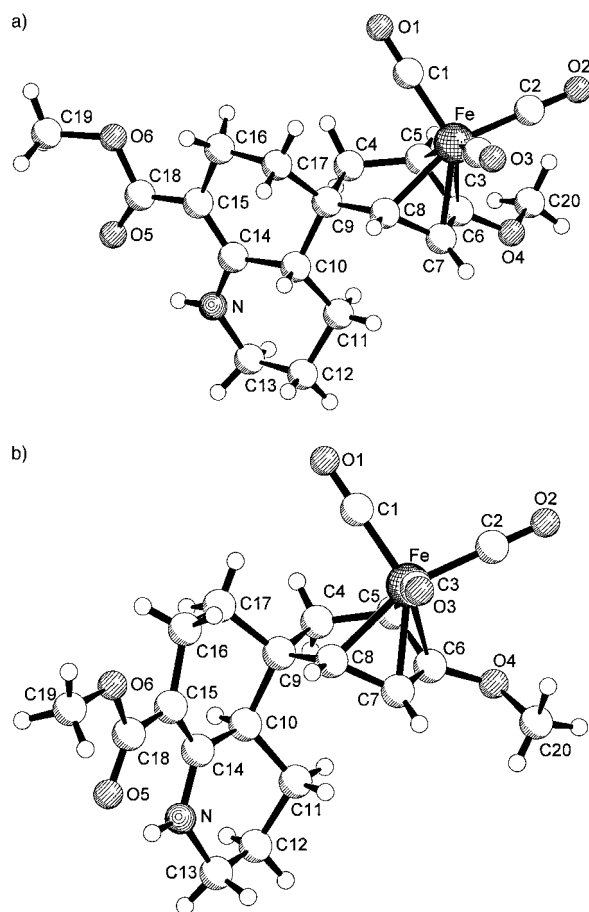


Figure 2. a) Molecular structure of **10a** in the crystal. Selected bond lengths [Å]: Fe–C5 2.110(3), Fe–C6 2.111(3), Fe–C7 2.042(3), Fe–C8 2.088(3), C5–C6 1.417(4), C6–C7 1.402(4), C7–C8 1.427(4). b) Molecular structure of **10b** in the crystal. Selected bond lengths [Å]: Fe–C5 2.115(2), Fe–C6 2.102(2), Fe–C7 2.048(2), Fe–C8 2.082(2), C5–C6 1.419(3), C6–C7 1.402(2), C7–C8 1.434(2).

In conclusion, the method described above offers an easy access to a broad range of carbo- and heterospirocyclic frameworks for the synthesis of biologically active compounds. Taking advantage of the asymmetric catalytic complexation of the appropriate diene precursor for **1**, enantioselective syntheses of the products are feasible.^[11]

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293(2) K, θ range: 1.71–25.99°; 3966 independent reflections; full-matrix least-squares refinement; R indices [$I > 2\sigma(I)$]: $R_1 = 0.0408$, $wR_2 = 0.1036$; max. residual electron density: 0.431 e Å⁻³. **10b**: C₂₀H₂₃FeNO₆, $M_r = 429.24$ g mol⁻¹, monoclinic, space group $C2/c$, $\lambda = 0.71073$ Å, $a = 19.630(10)$, $b = 15.928(11)$, $c = 15.375(5)$ Å, $\beta = 124.12(2)^\circ$, $V = 3979.8(37)$ Å³, $Z = 8$, $\rho_{\text{calcd}} = 1.433$ g cm⁻³, $\mu = 0.773$ mm⁻¹, $T = 293(2)$ K, θ range: 1.79–27.50°; 4473 independent reflections; full-matrix least-squares refinement; R indices [$I > 2\sigma(I)$]: $R_1 = 0.0384$, $wR_2 = 0.1075$; max. residual electron density: 0.372 e Å⁻³. Programs: G. M. Sheldrick, SHELXS-86, Göttingen, Germany, 1986; G. M. Sheldrick, SHELXL-93, Göttingen, Germany, 1993; E. Keller, SCHAKAL-97, Freiburg im Breisgau, Germany, 1997. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-134504 (**8**), -134505 (**10a**), and -134506 (**10b**). 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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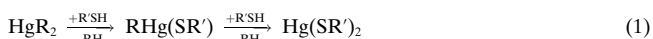
Intracellular Degradation of Diorganomercury Compounds by Biological Thiols—Insights from Model Reactions**

Henry Strasdeit,* Angelika von Döllen, Wolfgang Saak, and Michaela Wilhelm

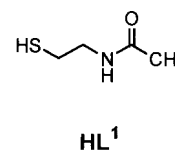
Dimethylmercury, HgMe₂, the simplest diorganomercury compound, is classified as a "super poison"—only a few drops on the skin is fatal for an adult.^[1,2] It is noteworthy that several months pass after exposure before the first characteristic symptoms of severe damage to the central nervous system (CNS) appear. In contrast, the onset of the toxic action of diphenylmercury, HgPh₂, is rapid and is associated with a different symptomology; it resembles inorganic Hg^{II} salts.^[3] At a molecular level these results may only be explained piecemeal, although the extent and rate of degradation of HgR₂ compounds to RHg⁺ and finally to Hg²⁺ would appear to play a central role.^[4]

We have occupied ourselves with the question as to the possible nature of the degradation reactions in humans from

the viewpoint of complexation chemistry. The polar character of Hg–C bonds (Hg^{δ+}–C^{δ-}), which has been confirmed inter alia by quantum-mechanical calculations on HgMe₂ and HgPh₂,^[5] and the protic conditions of most biological compartments suggest that reactions with Brønsted acids are likely. Because of their acidity, their frequent intracellular occurrence, and in particular because of the high thermodynamic stability of the Hg–S bond ("thiophilia" of Hg^{II}), biological thiols have been assumed to be the most important reaction partners [Eq. (1)].

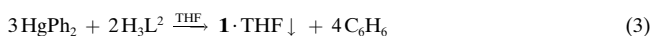
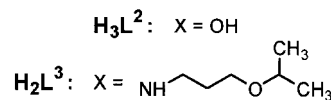
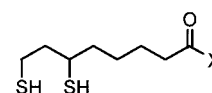


Indeed, we have been able to isolate sequentially the two dearylation stages as PhHgL¹ and HgL¹ from reactions of HgPh₂ with the thiol *N*-acetylcysteine HL¹ (HgPh₂:HL¹ = 1:2, room temperature, tetrahydrofuran (THF), see the Experimental Section), a model for the biological monothiols glutathione and coenzyme A. Moreover, it was also possible to detect the symmetrization of PhHgL¹ by NMR spectroscopy in [D₇]N,N-dimethylformamide [Eq. (2)].



Accordingly, HgL¹ need not necessarily be formed by the direct attack of HL¹ on PhHgL¹. Alternatively HL¹ could react solely with HgPh₂, first with that initially available and later with that provided by symmetrization. These results show that HgPh₂ can be completely dearylated by a suitable thiol under mild conditions.^[6]

An analogous reaction of HgPh₂ with the coenzyme dihydrolipoic acid H₃L² also led to the two dearylation stages. However, in this case they appeared unexpectedly as components of the same complex. This complex, [Hg(PhHg)₂(HL²)₂] (**1**) was isolated in good yield as the poorly soluble THF adduct. [Eq. (3), see the Experimental Section].



The formation of **1**·THF is essentially independent of the stoichiometric ratio of the reagents. HgPh₂ also formed a complex of analogous composition with the amide H₂L³, which we synthesized as a sterically equivalent model for the dihydrolipoyl-lysyl side chain (swinging arm) in enzymes. This excludes the possibility that the carboxyl functionality of dihydrolipoic acid is critical for the formation of **1**. The crystal structure analysis^[7] showed that **1** is a trinuclear, centrosymmetric complex (Figure 1). Each of the two peripheral mercury atoms Hg1 and Hg1' is bound to a phenyl group and to a thiolate sulfur atom; the S–Hg–C arrangement

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[**] Coordination Chemistry of Lipoic Acid and Related Compounds, part 3. This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. Part 2: ref. [11].

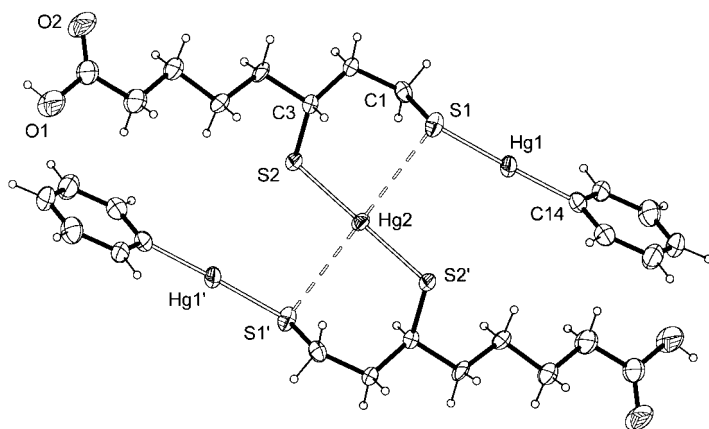


Figure 1. Crystal structure of **1** (thermal ellipsoids at the 50% probability level). Selected distances [Å] and angles [°]: Hg1–S1 2.356(2), Hg1–C14 2.068(7), Hg2–S1 3.259(2), Hg2–S2 2.328(2), S1–C1 1.825(7), S2–C3 1.850(6); S1–Hg1–C14 172.7(2), S1–Hg2–S1' 180.0, S1–Hg2–S2 89.80(5), S1–Hg2–S2' 90.20(5), S2–Hg2–S2' 180.0, Hg1–S1–Hg2 89.88(6), Hg1–S1–C1 103.3(2), Hg2–S1–C1 80.4(2), Hg2–S2–C3 103.1(2).

deviates marginally from linearity. In contrast, the central mercury atom Hg2 is coordinated solely to sulfur atoms. The primary coordination is here exactly linear (S2–Hg2–S2'). In addition there are two secondary bonds to S1 and S1', each with a length of 3.259(2) Å,^[8] so that overall a planar [2+2] coordination is present.

In contrast to HgPh₂, HgMe₂ reacts extraordinarily slowly with thiols. If HgMe₂ and an equivalent amount of the dithiol H₂L³ are mixed in [D₈]dioxane at 37 °C (physiological temperature), [MeHg(HL³)] is first detectable by ¹H NMR spectroscopy after two weeks, and only 2% of the HgMe₂ is converted into MeHg⁺ after seven weeks. Regardless of the thiol used, we were unable to observe cleavage of the second methyl group!

The low solubility of HgMe₂ and HgPh₂ in water made it necessary to use organic solvents; in addition the concentrations used were comparatively high. Although our results have therefore limited applicability to biological systems, they do support the hypothesis that intracellular degradation of diorganomercury compounds could occur primarily through thiols in the human body. Thus both the first phase of the long latency period in HgMe₂ poisoning (degradation to MeHg(SR')) and the similarity of the toxic action of HgPh₂ to that of inorganic Hg(II) salts (more rapid degradation to Hg(SR')₂) become understandable. Our findings give no indication of an involvement of biological thiols in the demethylation of MeHg⁺, for example in the CNS.^[4] However, it cannot be excluded on the basis of these results alone that under physiological conditions MeHg⁺ can also be cleaved slowly by thiols, particularly if the effects of additional donors are taken into consideration.^[9]

Experimental Section

Caution! Organomercury compounds, especially methylmercury compounds are extremely toxic. All direct contact must be strictly avoided by suitable protective measures such as the wearing of special gloves.^[1, 2]

For the synthesis of H₂L³ the imidazolidine was first prepared from lipoic acid and *N,N'*-carbonyldiimidazole^[10] and then converted into the amide with

3-isopropoxypropylamine. The subsequent reduction to the dithiol was carried out as described for the synthesis of H₂L² from lipoic acid.^[11] All reactions of Hg compounds with thiols were carried out in an N₂ atmosphere.

Reaction of HgPh₂ with *N*-acetylcysteamine: A solution of HgPh₂ (355 mg, 1.00 mmol) and HL¹ (238 mg, 2.00 mmol) in THF (30 mL) was allowed to stand for 48 h and then concentrated to 10 mL. After a few days white, feathery needles of PhHgL¹ (120 mg, 0.30 mmol) were filtered off. The filtrate was evaporated to dryness in order to remove the benzene formed. The residue was redissolved in THF. The solution was concentrated to 5 mL, and white, microcrystalline HgL₂¹ (80 mg, 0.18 mmol) was isolated after a few days. First precipitation (= PhHgL¹): IR (KBr): $\tilde{\nu}$ = 727 (s), 698 (m), 341 cm⁻¹ (m), the characteristic band of HgL₂¹ (see below) was absent; ¹³C{¹H} NMR (75.5 MHz, [D₇]N,N-dimethylformamide): δ = 23.03 (CH₃), 27.76 (CH₂S), 45.28 (CH₂N), 128.4 (*p*-C), 129.0 (*m*-C), 137.6 (*o*-C), 164.3 (*ipso*-C), 170.3 (C=O); as a result of symmetrization [Eq. (2)] additional signals of HgPh₂ could be observed after a few hours: δ = 127.9 (*p*-C), 128.6 (*m*-C), 138.7 (*o*-C), 171.8 (*ipso*-C). Second precipitation (= HgL₂¹): IR (KBr): $\tilde{\nu}$ = 357 cm⁻¹ (m, Hg–S^[12]), the characteristic bands of PhHgL¹ (see above) were absent. The band at 357 cm⁻¹ can be shifted to 366 cm⁻¹. According to the elemental analysis HgL₂¹ was also present in these precipitations, apparently in the form of a second, previously unknown modification: elemental analysis calcd for C₈H₆HgN₂O₂S₂: C 21.99, H 3.69, Hg 45.91, N 6.41, S 14.68; found: C 22.09, H 3.53, Hg 47.60, N 6.67, S 14.55.

Synthesis of **1 · THF:** A stirred solution of HgPh₂ (1.06 g, 3.0 mmol) in THF (40 mL) was treated with a solution of H₂L² (0.42 mg, 2.0 mmol) in THF (10 mL). After the mixture had been stirred at room temperature for 48 h, most of the solvent was removed under vacuum. The product precipitated as a colorless, finely crystalline powder, which was isolated, washed with THF, and dried briefly under vacuum. A second fraction was isolated after further concentration. Yield: 0.94 g (76%). IR (KBr): $\tilde{\nu}$ = 2926 (m), 1709 (vs), 1427 (m), 1252 (m), 1235 (m), 729 (s), 698 (m), 448 cm⁻¹ (m); elemental analysis calcd for C₃₂H₄₆Hg₃O₅S₄: C 30.98, H 3.74, Hg 48.50, S 10.34; found: C 31.10, H 3.83, Hg 48.52, S 10.39.

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supplementary publication no. CCDC-134036. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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An Unprecedented [C₂+C₁+C₁] Coupling of an Alkynyl, an Allenylidene, and a CO Unit: The Formation of a Highly Unsaturated Cyclobutenone Derivative in the Coordination Sphere of Rhodium(I)**

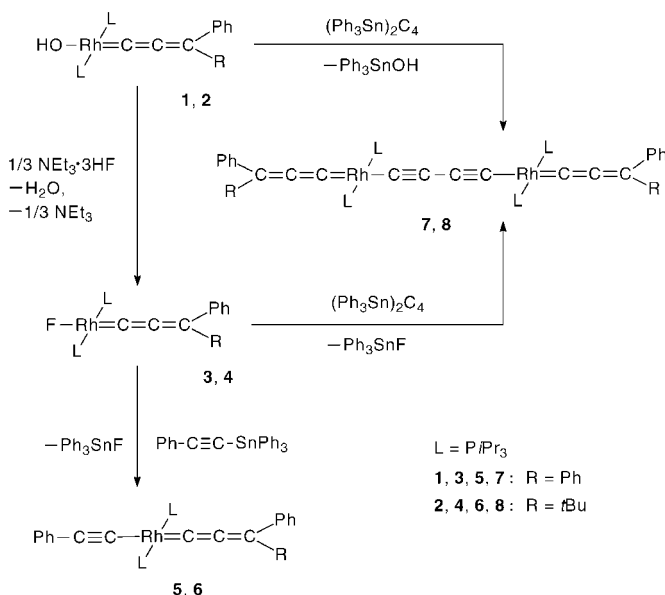
Juan Gil-Rubio, Birgit Weberndörfer, and Helmut Werner*

Dedicated to Professor Heinrich Vahrenkamp on the occasion of his 60th birthday

Metal-assisted C–C coupling reactions belong to the most important processes in organometallic chemistry.^[1] Following our work on the synthesis of metal vinylidene compounds from alkynes, we recently reported that the square-planar rhodium complexes *trans*-[RhR(=C=CR¹R²)(PiPr₃)₂] (R = methyl, vinyl, phenyl, alkynyl) undergo an intramolecular C–C coupling reaction in the presence of CO to give *trans*-[Rh{C(R)=CR¹R²}(CO)(PiPr₃)₂].^[2] A related reaction occurs between the allenylidene compounds *trans*-[RhX(=C=C=CR¹R²)(PiPr₃)₂] (X = OAr, OC(O)Me, N₃) and CO to give the alkynyl complexes *trans*-[Rh(C≡C–CXR¹R²)(CO)(PiPr₃)₂], resulting from the migration of the group X to the γ-C atom of the allenylidene ligand.^[3] While earlier attempts from our laboratory to obtain alkyl, vinyl, or arylrhodium(I)

complexes of the general composition *trans*-[RhR(=C=C=CR¹R²)(PiPr₃)₂] failed,^[4] we have now succeeded with the preparation of the corresponding alkynyl(allenylidene) compounds *trans*-[Rh(C≡CPh)(=C=C=CR¹R²)(PiPr₃)₂], one of which undergoes an unprecedented [C₂+C₁+C₁] coupling reaction with CO to give a highly unsaturated cyclobutenone derivative.

The synthetic route to the new alkynylrhodium(I) complexes **5** and **6** is outlined in Scheme 1. In order to coordinate the C₂ unit to the metal center, the key to success was the use



Scheme 1. Synthesis of mono- and dinuclear rhodium(I) complexes **5**–**8**.

of the mild transmetalating reagent PhC≡CSnPh₃^[5] and the highly reactive fluoro compounds **3** and **4**. While the latter could not be prepared by salt metathesis from *trans*-[RhCl(=C=C=C(R)Ph)(PiPr₃)₂] and KF or TlF, respectively, they were obtained in excellent yield by treatment of the hydroxo derivatives **1** and **2** with one equivalent of NEt₃·3HF in benzene. The fluoro complexes are green-yellow (**3**) and green (**4**) crystalline solids that can be stored at room temperature under argon and are stable for several days in solution in C₆D₆. The ¹⁹F NMR spectra of **3** and **4** show the expected pattern of signals with ¹⁰³Rh–¹⁹F and ³¹P–¹⁹F couplings, the ²J(P,F) values of about 19 Hz being characteristic for a *cis* disposition of the fluoro and the phosphane ligands.^[6]

The reactions of **3** and **4** with PhC≡CSnPh₃ resulted in the formation of the alkynyl complexes **5** and **6** together with a white, poorly soluble by-product which was identified as Ph₃SnF by comparison of its IR spectrum with that of an authentic sample prepared from Ph₃SnCl and KF.^[7] Although compounds **5** and **6** (which are violet and brown crystalline solids, respectively) are more sensitive than the related fluoro or hydroxo derivatives, they have been fully characterized on the basis of their analytical and spectroscopic data. Besides the single resonance in the ³¹P NMR spectra with a ¹J(Rh,P) coupling constant that is typical for a *trans* disposition of both phosphorus atoms, the most characteristic features are the

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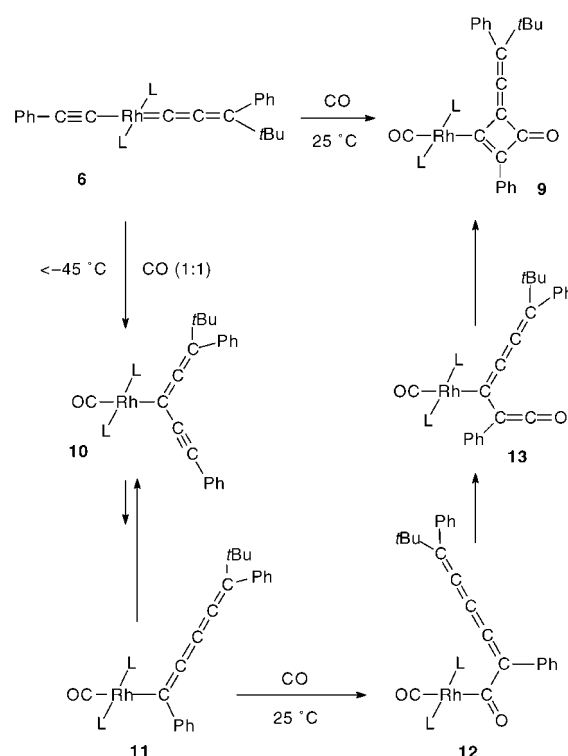
[**] This work was supported by the Deutsche Forschungsgemeinschaft (Grant SFB 347), the Fonds der Chemischen Industrie, and the European Commission (Contract ERBFMBICT960698).

low-field signals for the allenylidene and the alkynyl ligands in the ^{13}C NMR spectra of **5** and **6**. In particular, the resonances for the α -carbon atoms of the allenylidene and the alkynyl units (both doublets of triplets) can be clearly assigned owing to the large $^1J(\text{Rh},\text{C})$ coupling constants of about 53 and 40 Hz, respectively.

The high reactivity of the fluororhodium(i) compounds **3** and **4** is not only illustrated by the formation of **5** and **6** but also by the synthesis of the dinuclear complexes **7** and **8** (Scheme 1). However, these compounds were not only formed by treatment of **3** and **4** with $\text{Ph}_3\text{SnC}\equiv\text{CC}\equiv\text{CSnPh}_3$, but even more conveniently by using the hydroxo derivatives **1** and **2** as starting materials. In this case the by-product is Ph_3SnOH , which can be easily separated from the less soluble complexes **7** and **8** by washing with acetone. The ^{13}C NMR spectrum of compound **8** (which is more soluble in organic solvents than **7**) displays the expected signals for the allenylidene and butadiynediyl ligands with the chemical shifts and coupling constants similar to those of **6**. Additional structural support is provided by the mass spectra of **7** and **8**, which show in both cases the peaks of the molecular ions and the fragments $[\text{Rh}(\text{C}_3(\text{R})\text{Ph})(\text{P}i\text{Pr}_3)_2]^+$, respectively. In this context we note that dinuclear transition metal complexes containing a “naked” C_4 bridge were recently prepared by Gladysz et al.,^[8] Lapinte et al.,^[9] Bruce et al.,^[10] and others (including us)^[5, 11] and investigated as promising candidates for use in material science.^[12]

Since we knew that reactions of the vinylidene complexes $\text{trans}[\text{Rh}(\text{C}\equiv\text{CR})(=\text{C}=\text{CR}^1\text{R}^2)(\text{P}i\text{Pr}_3)_2]$ with CO give rise to a migratory insertion of the vinylidene ligand into the $\text{Rh}-\text{C}$ σ bond,^[2] we were interested to find out whether an analogous reaction of **6** with CO would also occur. However, when a stream of CO was passed through a suspension of **6** in hexane at room temperature, a quantitative conversion of the alkynyl derivative to the cyclic compound **9** took place (Scheme 2). The result of the single-crystal X-ray diffraction study of **9** confirmed (Figure 1)^[13] that quite unexpectedly two CO molecules were involved in the formation of the novel C–C coupling product. One carbonyl group is coordinated to the metal, while the other forms a four-membered ring together with the two alkynyl carbon atoms and the α -carbon atom of the allenylidene unit. The cyclobutenone ring is σ -bonded to the rhodium center by the α -C atom of the original alkynyl ligand. The phenyl group at C3, the cyclobutenone ring, and the cumulene unit are nearly coplanar, defining a plane which lies almost perpendicular to the coordination plane around the metal center. The dihedral angle between the two planes $[\text{P}1, \text{P}2, \text{Rh}, \text{C}1, \text{C}2]$ and $[\text{C}2, \text{C}3, \text{C}4, \text{C}5]$ amounts to $89.7(1)^\circ$. Owing to the molecular symmetry and the slow rotation around the $\text{Rh}-\text{C}$ σ bond, the two phosphorus nuclei are chemically inequivalent and thus the ^{31}P NMR spectrum of **9** displays a multiplet corresponding to the AB part of an ABX system. We therefore assume that the molecular structure of **9** which was found in the crystal is also preserved in solution.

A possible mechanism for the conversion of **6** to **9** is shown in Scheme 2. In the initial step, the starting material **6** reacts with one molecule of CO to give the intermediate **10**, which contains a C_5 chain resulting from the coupling of the allenylidene and the alkynyl ligands. By carrying out the



Scheme 2. Synthesis and mechanism of the formation of **9**. $\text{L} = \text{P}i\text{Pr}_3$.

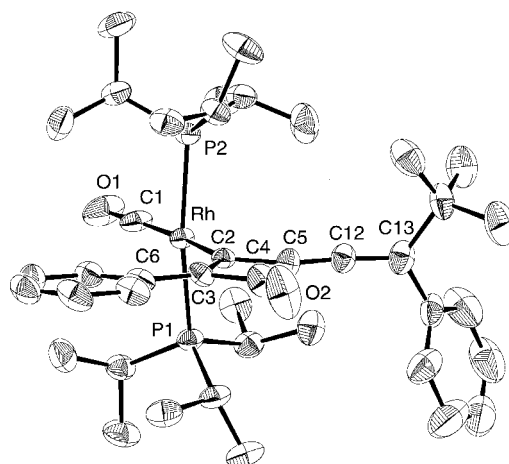


Figure 1. Molecular structure of **9** in the crystal. Selected bond lengths [Å] and angles $^\circ$: Rh–C1 1.840(3), Rh–C2 2.030(3), Rh–P1 2.3439(9), Rh–P2 2.3347(9), O2–C4 1.209(3), C2–C3 1.392(4), C2–C5 1.511(4), C3–C4 1.458(4), C3–C6 1.459(4), C4–C5 1.523(4), C5–C12 1.298(4), C12–C13 1.320(4); C1–Rh–C2 173.38(13), C2–Rh–P1 90.83(8), C1–Rh–P2 89.06(10), C1–Rh–P1 89.72(10), C2–Rh–P2 91.51(8), P2–Rh–P1 170.14(3), C2–C3–C4 93.6(2), O1–C1–Rh 175.6(3), C3–C2–Rh 132.7(2), C5–C2–Rh 135.8(2), C3–C2–C5 91.5(2), O2–C4–C3 137.4(3), O2–C4–C5 134.0(3), C3–C4–C5 88.5(2), C12–C5–C2 140.9(3), C12–C5–C4 132.7(3), C2–C5–C4 86.4(2), C5–C12–C13 172.2(3).

reaction of **6** with one equivalent of CO at low temperature, **10** could be isolated as a yellow, thermally unstable solid. The IR and ^{13}C NMR data of **10** are similar to those of the related four-coordinate compound $\text{trans}[\text{Rh}\{\eta^1\text{-C}(\text{CH}=\text{CH}_2)=\text{C}(\text{CPh}_2)(\text{CO})(\text{P}i\text{Pr}_3)_2\}]$ which was obtained by treatment of $[\text{Rh}(\eta^3\text{-CH}_2\text{CHC}=\text{C}=\text{CPh}_2)(\text{P}i\text{Pr}_3)_2]$ with CO.^[4] The intermediate **10** (which in C_6D_6 in the presence of CO affords quantitatively the cyclobutenone derivative **9**) could inter-

convert with the isomer **11** through the interaction of the C≡C bond with the rhodium center. An analogous equilibrium has recently been observed between *trans*-[Rh(η¹-C(C≡CH)=CH₂)(CO)(PiPr₃)₂] and *trans*-[Rh(η¹-CH=C=C=CH₂)(CO)(PiPr₃)₂].^[14] In a subsequent step, the reaction of **11** with a second molecule of CO could give the acyl complex **12** which undergoes an isomerization to **13** by an 1,3-shift of the [Rh(CO)(PiPr₃)₂] fragment. The cyclobutenone derivative **9** could finally be generated by an intramolecular [2+2] cycloaddition from **13**. Although to the best of our knowledge there is no precedent in organometallic chemistry for such an isomerization reaction of **12** to **13**, an analogous rearrangement has been observed in the thermal interconversion of acylallenes to vinylketenes by flash vacuum thermolysis.^[15] Regarding the formation of unsaturated four-membered rings which are connected to an organometallic entity, we note that the groups of Davison,^[16] Kolobova,^[17] Hughes,^[18] and Fischer^[19] already reported the synthesis of cyclobutenylidene complexes by [2+2] cycloaddition of either cationic or neutral vinylidene–metal precursors and metal alkynyl complexes. However, in this case the generated four-membered ring does not contain an exocyclic C–C double bond.

Experimental Section

All experiments were carried out under argon. The NMR data of the phenyl and *tert*-butyl groups are omitted for clarity. Abbreviations: v = virtual coupling; *N* = ³*J*(PH) + ⁵*J*(PH) or ¹*J*(PC) + ³*J*(PC); w = weak; m = medium; s = strong.

3: A solution of **1** (458 mg, 0.73 mmol) in benzene (20 mL) was treated with NEt₃·3 HF (40 μL, 0.25 mmol) at room temperature. The reaction mixture was stirred for 1 h and evaporated to dryness in vacuo. The residue was extracted four times with 20 mL-portions of pentane. The extract was concentrated to about 4 mL in vacuo and then stored for 2 h at –25 °C. A green-yellow microcrystalline solid precipitated which was washed three times with 3 mL-portions of pentane (–50 °C) and dried; yield 386 mg (84 %); m.p. 112 °C (decomp); IR (nujol): $\tilde{\nu}$ = 1871 (C=C=C), 475, 458 (Rh–F) cm^{–1}; ¹H NMR (400 MHz, C₆D₆): δ = 2.74 (m, 6H, PCHCH₃), 1.34 (dvt, *N* = 13.5, ³*J*(H,H) = 6.4 Hz, 36H, PCHCH₃); ¹³C{¹H} NMR (100.6 MHz, C₆D₆): δ = 254.3 (dt, ²*J*(Rh,C) = 14.2, ³*J*(P,C) = 6.4 Hz, Rh=C=C), 230.6 (m, Rh=C), 154.7 (s, Rh=C=C=C), 23.5 (vt, *N* = 19.3 Hz, PCHCH₃), 20.2 (s, PCHCH₃); ¹⁹F NMR (188.3 MHz, C₆D₆): δ = –188.8 (dt, ¹*J*(Rh,F) = 23.0, ³*J*(P,F) = 20.2 Hz); ³¹P{¹H} NMR (162.0 MHz, C₆D₆): δ = 41.5 (dd, ¹*J*(Rh,P) = 140.7, ²*J*(P,F) = 18.7 Hz).

Compound **4** was prepared in a similar way from **2** (274 mg, 0.45 mmol) and NEt₃·3 HF (24.4 μL, 0.15 mmol); green crystals; yield 245 mg (89 %); m.p. 102 °C (decomp); IR (nujol): $\tilde{\nu}$ = 1890, 1879 (C=C=C), 473, 467, 449 (Rh–F) cm^{–1}; ¹H NMR (400 MHz, C₆D₆): δ = 2.55 (m, 6H, PCHCH₃), 1.29 (dvt, *N* = 13.5, ³*J*(H,H) = 6.4 Hz, 36H, PCHCH₃); ¹³C{¹H} NMR (100.6 MHz, C₆D₆): δ = 250.5 (m, Rh=C=C), 239.0 (m, Rh=C), 154.9 (s, Rh=C=C=C), 23.1 (vt, *N* = 18.1 Hz, PCHCH₃), 20.1 (s, PCHCH₃); ¹⁹F NMR (376.4 MHz, C₆D₆): δ = –181.7 (dt, ¹*J*(Rh,F) = ²*J*(P,F) = 20.3 Hz); ³¹P{¹H} NMR (162.0 MHz, C₆D₆): δ = 43.1 (dd, ¹*J*(Rh,P) = 141.6, ²*J*(P,F) = 19.5 Hz).

5: A solution of **3** (285 mg, 0.45 mmol) in pentane (25 mL) was treated with PhC≡CSnPh₃ (203 mg, 0.45 mmol) and stirred for 2 h at room temperature. A dark red-violet suspension was formed, which was filtered through a cotton pad. The filtrate was evaporated to dryness in vacuo and the residue was extracted four times with 10 mL-portions of pentane. The combined extracts were concentrated to about 8 mL in vacuo and then stored for three days at –25 °C. A dark red-violet microcrystalline solid precipitated which was washed three times with 3 mL-portions of pentane (–78 °C) and dried; yield 260 mg (81 %); m.p. 128 °C (decomp); IR (nujol): $\tilde{\nu}$ = 2059 (C≡C), 1869 (C=C=C) cm^{–1}; ¹H NMR (400 MHz, C₆D₆): δ = 3.01 (m, 6H,

PCHCH₃), 1.40 (dvt, *N* = 13.5, ³*J*(H,H) = 7.0 Hz, 36H, PCHCH₃); ¹³C{¹H} NMR (100.6 MHz, C₆D₆): δ = 249.1 (dt, ²*J*(Rh,C) = 10.2, ³*J*(P,C) = 5.1 Hz, Rh=C=C), 233.5 (dt, ¹*J*(Rh,C) = 53.9, ²*J*(P,C) = 17.3 Hz, Rh=C), 158.6 (d, ²*J*(Rh,C) = 8.1 Hz, Rh=C=C), 156.0 (t, ⁴*J*(P,C) = 2.6 Hz, Rh=C=C=C), 118.6 (dt, ¹*J*(Rh,C) = 40.7, ²*J*(P,C) = 19.0 Hz, Rh=C=C), 25.9 (vt, *N* = 19.3 Hz, PCHCH₃), 20.8 (s, PCHCH₃); ³¹P{¹H} NMR (162.0 MHz, C₆D₆): δ = 41.5 (d, ¹*J*(Rh,P) = 134.0 Hz).

Compound **6** was prepared in a similar way from **4** (296 mg, 0.48 mmol) and PhC≡CSnPh₃ (218 mg, 0.48 mmol); brown crystals; yield 242 mg (72 %); m.p. 79 °C (decomp); IR (nujol): $\tilde{\nu}$ = 2064 (C≡C), 1890 (C=C=C) cm^{–1}; ¹H NMR (400 MHz, C₆D₆): δ = 2.82 (m, 6H, PCHCH₃), 1.34 (dvt, *N* = 13.2, ³*J*(H,H) = 6.2 Hz, 36H, PCHCH₃); ¹³C{¹H} NMR (100.6 MHz, C₆D₆): δ = 257.6 (dt, ¹*J*(Rh,C) = 51.9, ²*J*(P,C) = 16.8 Hz, Rh=C), 236.1 (dt, ²*J*(Rh,C) = 12.2, ³*J*(P,C) = 5.8 Hz, Rh=C=C), 156.3 (t, ⁴*J*(P,C) = 3.0 Hz, Rh=C=C=C), 153.5 (d, ²*J*(Rh,C) = 9.2 Hz, Rh=C=C), 123.4 (dt, ¹*J*(Rh,C) = 39.7, ²*J*(P,C) = 19.3 Hz, Rh=C=C), 25.8 (vt, *N* = 19.3 Hz, PCHCH₃), 20.8 (s, PCHCH₃); ³¹P{¹H} NMR (162.0 MHz, C₆D₆): δ = 43.9 (d, ¹*J*(Rh,P) = 133.9 Hz).

7: A solution of **1** (251 mg, 0.40 mmol) in THF (10 mL) was treated with Ph₃SnC≡CC≡CSnPh₃ (149 mg, 0.20 mmol) at –78 °C. Under continuous stirring, the mixture was slowly allowed to warm to room temperature and stirred for 7 h. The resulting dark blue suspension was concentrated to about 0.5 mL in vacuo and acetone (5 mL) was added. The solution was filtered and the filtrate was evaporated to dryness in vacuo. The residue was washed five times with 5 mL-portions of acetone and dried; yield 217 mg (85 %); m.p. 50 °C (decomp); IR (nujol): $\tilde{\nu}$ = 1947 (C≡C), 1860 (C=C=C) cm^{–1}; ¹H NMR (200 MHz, CD₂Cl₂): δ = 2.77 (m, 12H, PCHCH₃), 1.21 (dvt, *N* = 13.4, ³*J*(H,H) = 6.4 Hz, 72H, PCHCH₃); ³¹P{¹H} NMR (81.0 MHz, CD₂Cl₂): δ = 42.5 (d, ¹*J*(Rh,P) = 132.9 Hz); ESI MS (CH₂Cl₂/CH₃CN): *m/z*: 1275 [MH⁺], 613 [Rh(C₃Ph₃)(PiPr₃)₂]⁺.

Compound **8** was prepared in a similar way from **2** (310 mg, 0.51 mmol) and Ph₃SnC≡CC≡CSnPh₃ (190 mg, 0.25 mmol) in benzene (10 mL) at room temperature; blue-violet solid; yield 202 mg (65 %); m.p. 59 °C (decomp); IR (nujol): $\tilde{\nu}$ = 1952 (C≡C), 1888, 1878 (C=C=C) cm^{–1}; ¹H NMR (400 MHz, [D₈]THF): δ = 2.69 (m, 12H, PCHCH₃), 1.21 (dvt, *N* = 13.5, ³*J*(H,H) = 6.4 Hz, 72H, PCHCH₃); ¹³C{¹H} NMR (100.6 MHz, [D₈]THF): δ = 254.2 (dt, ¹*J*(Rh,C) = 51.9, ²*J*(P,C) = 17.3 Hz, Rh=C), 235.3 (dt, ²*J*(Rh,C) = 11.2, ³*J*(P,C) = 6.1 Hz, Rh=C=C), 156.0 (t, ⁴*J*(P,C) = 3.1 Hz, Rh=C=C=C), 149.8 (m, Rh=C=C), 133.7 (dt, ¹*J*(Rh,C) = 39.7, ²*J*(P,C) = 19.3 Hz, Rh=C=C), 25.9 (vt, *N* = 20.3 Hz, PCHCH₃), 20.8 (s, PCHCH₃); ³¹P{¹H} NMR (162.0 MHz, [D₈]THF): δ = 44.4 (d, ¹*J*(Rh,P) = 133.9 Hz). ESI MS (CH₂Cl₂/CH₃CN): *m/z*: 1234 [M⁺], 593 [Rh(C₃(*t*Bu)Ph)(PiPr₃)₂]⁺.

9: A slow stream of CO was passed through a suspension of **6** (168 mg, 0.24 mmol) in hexane (6 mL) for 30 s at room temperature. The flask was sealed and the mixture was stirred for 3 h. The resulting yellow-orange suspension was concentrated to about 3 mL in vacuo and stored at –25 °C for 3.5 h. A yellow solid precipitated which was washed twice with 2-mL portions of pentane (–78 °C) and dried; yield 172 mg (90 %); m.p. 161 °C (decomp); IR (nujol): $\tilde{\nu}$ = 1955 (s), 1908 (w) (CO and C=C=C), 1734 (s), 1717 (s) (C=O), 1596 (m), 1512 (m) (C=C) cm^{–1}; ¹H NMR (400 MHz, C₆D₆): δ = 2.17, 1.94 (both m, 6H, PCHCH₃), 1.26, 1.00, 0.92, 0.88 (all m, 36H, PCHCH₃); ¹³C{¹H} NMR (100.6 MHz, C₆D₆): δ = 241.7 (dt, ¹*J*(Rh,C) = 33.2, ²*J*(P,C) = 15.3 Hz, Rh=C), 198.1 (dt, ¹*J*(Rh,C) = 53.9, ²*J*(P,C) = 15.0 Hz, Rh=CO), 189.9 (s, C=O), 181.3 (d, ³*J*(Rh,C) = 2.7 Hz, C=C=C), 163.4 (t, ³*J*(P,C) = 3.6 Hz, Rh=C=CPh), 129.5 (d, ²*J*(Rh,C) = 1.8 Hz, C=C=C), 122.0 (s, C=C=C), 27.0, 26.6 (both vdd, *J* = 15.3 and 5.4 Hz, PCHCH₃), 20.9, 20.4, 19.6, 19.5 (all s, PCHCH₃); ³¹P{¹H} NMR (162.0 MHz, C₆D₆): AB part of an ABX system, δ_A = 47.3, δ_B = 48.2 (¹*J*(Rh,P_A) = ¹*J*(Rh,P_B) = 135.7 Hz, ²*J*(P_A,P_B) = 235.7 Hz).

10: Gaseous CO (ca. 0.1 mmol) was introduced into a schlenk flask containing a suspension of **6** (69 mg, 0.01 mmol) in pentane (10 mL) at –78 °C. Under continuous stirring, the mixture was allowed to warm to –45 °C, stirred for 25 min and then evaporated to dryness in vacuo to give a yellow solid; IR (nujol): $\tilde{\nu}$ = 2159 (m) (C≡C), 1969 (w), 1947 (s), 1902 (w), 1875 (w) (CO and C=C=C) cm^{–1}; ¹³C{¹H} NMR (100.6 MHz, C₆D₆): δ = 208.3 (t, ³*J*(P,C) = 5.1 Hz, C=C=C), 195.6 (dt, ¹*J*(Rh,C) = 60.0, ²*J*(P,C) = 15.8 Hz, Rh=CO), 104.3 (s, Rh=C=C=C), 97.9 (dt, ¹*J*(Rh,C) = 25.4, ²*J*(P,C) = 12.2 Hz, Rh=C), 94.8, 92.0 (both s, C≡C), 26.0 (br.m, PCHCH₃), 20.7, 20.1 (both br.m, PCHCH₃).

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- [13] Data for the X-ray structure analysis of **9**: Crystals from acetone, $C_{41}H_{61}O_2P_2Rh$ ($M_r = 750.75$); crystal size $0.20 \times 0.20 \times 0.15 \text{ mm}^3$; monoclinic, space group $P2_1/c$ (no. 14), $a = 11.891(1)$, $b = 13.328(1)$, $c = 25.797(3) \text{ Å}$, $\beta = 98.29(1)^\circ$, $Z = 4$, $V = 4045.6(7) \text{ Å}^3$, $\rho_{\text{calcd}} = 1.233 \text{ g cm}^{-3}$; $T = 173(2) \text{ K}$; $2\theta = 54.20^\circ$; 33 008 reflections measured, 8328 were unique ($R_{\text{int}} = 0.0696$), and 4799 observed ($I > 2\sigma(I)$); IPDS (STOE), MoK_{α} radiation ($\lambda = 0.71073 \text{ Å}$), graphite monochromator; Lp correction. The structure was solved by Patterson method (SHELXS-97; G. M. Sheldrick, *Acta Crystallogr. Sect. A* **1990**, *46*, 467) and refined with the full-matrix, least-squares method (SHELXL-97; G. M. Sheldrick, a program for crystal structure refinement, Universität Göttingen, **1993**); $R_1 = 0.0357$, $wR_2 = 0.0629$ (for 4799 reflections with $I > 2\sigma(I)$), $R_1 = 0.0804$, $wR_2 = 0.0711$ (for all 8328 data); data-to-parameter ratio 18.10; residual electron density $+0.483/-1.224 \text{ e Å}^{-3}$. One phenyl group consisting of the atoms C18 to C23 is disordered; two alternative positions were refined anisotropically with restraints on the anisotropic displacement parameters with the occupancy factors 57.5% and 42.5%. 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-134997. 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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Dialkylaminoethyl-Functionalized *ansa*-Zirconocene Dichlorides: Precatalysts for the Regulation of the Molecular Weight Distribution of Polyethylene**

Christian Müller, Dieter Lilge, Marc Oliver Kristen,* and Peter Jutzi*

Dedicated to Professor Reinhard Schmutzler on the occasion of his 65th birthday

The synthesis of metallocene derivatives which can be used as precatalysts in the methylalumoxane (MAO)-activated polymerization of α -olefins is a rapidly growing research area. Special attention is directed to the design of the metal coordination sphere: variations in the substitution pattern and in the connection of cyclopentadienyl (Cp) ligands may be utilized for synthesizing tailor-made single-site catalysts. Thus, polymer parameters such as stereochemical microstructure (tacticity), molecular weight distribution, crystallinity, and copolymer incorporation can be influenced systematically.^[1, 2] This new generation of catalysts therefore is playing an important role in the development of new polyolefin-based materials.

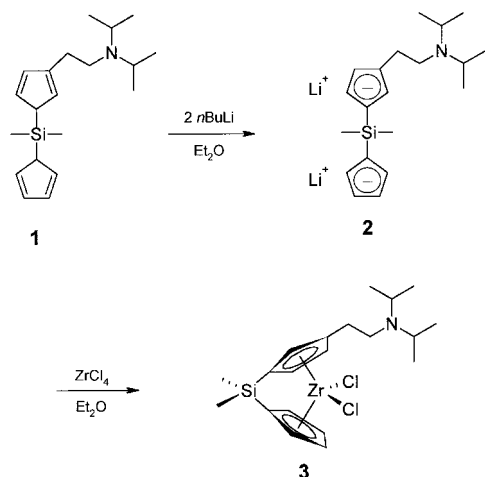
The molecular weights of polymers produced with $[Cp_2ZrCl_2]/MAO$ catalysts range between 100 000 and 1 000 000 g mol^{-1} for polyethylenes and between 200 and 1000 g mol^{-1} for polypropylenes. Polymers made by the conventional Ziegler–Natta process have broad molecular weight distributions with high polydispersities of $M_w/M_n = 5$ –10, whereas single-site metallocene catalysts usually produce polyolefins with a unimodal molecular weight distribution and polydispersities of about 2. With respect to these features, there are no differences between unbridged, bridged, or Cp-substituted systems.^[1a, 3]

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We report here on the polymerization of ethylene with amino-functionalized *ansa*-zirconocene dichlorides, activated with MAO.^[4] These systems provide the possibility of regulating the molecular weight distribution of polyethylene systematically. Using only one precatalyst, high-molecular-weight as well as low-molecular-weight polyethylene with a classical unimodal molecular weight distribution is obtained. Furthermore, it is possible to produce polyethylenes with variable amounts of high- and low-molecular weights; these polymers show bimodal molecular weight distributions.

The silicon-bridged, diisopropylaminoethyl-functionalized zirconocene dichloride **3** was synthesized according to Scheme 1, and obtained as a beige, air- and moisture-sensitive solid in 43 % yield. The polymerization of ethylene with the



Scheme 1. Synthesis of **3**.

catalyst **3**/MAO (Zr:Al = 1:1000) was performed at $T = 30, 45, 50, 60,$ and 90°C . The polyethylene samples were analyzed by gel permeation chromatography (GPC). Table 1 gives a summary of the polymerization results with regard to activity of the catalyst, M_w , M_n , and viscosity of the polymers. The

Table 1. Results of the ethylene polymerization with the catalyst system **3**/MAO.

$T [^\circ\text{C}]$	M_w	M_n	M_w/M_n	Yield [g]	Activity ^[a]	$\eta^{[b]}$ [dl g ⁻¹]
30	1 821 768	144 989	12.6	0.80	16	10.70
45	1 193 864	72 504	16.5	1.50	30	6.80
50	584 946	42 869	13.6	2.50	50	4.78
60	389 131	22 306	17.5	4.45	89	3.75
90	54 731	12 939	4.2	19.7	394	1.00

[a] Activity in kg_{PE} (mol_{Zr} h bar)⁻¹, [b] viscosity in decaline at $T = 180^\circ\text{C}$.

molecular weight distribution as a function of the polymerization temperature is shown in Figure 1. The unimodal distribution at $T = 90^\circ\text{C}$ is typical for the polymerization with single-site catalysts. Lowering the temperature to $T = 60^\circ\text{C}$ resulted in the formation of a high-molecular-weight “shoulder”, with a decrease in the low-molecular-weight area. This effect was intensified at a polymerization temperature of $T = 50^\circ\text{C}$. At $T = 45^\circ\text{C}$, the bimodal distribution is obvious; the maxima are located at $M = 45\,000\text{ g mol}^{-1}$ and $1\,430\,000\text{ g mol}^{-1}$, respectively. At $T = 30^\circ\text{C}$, high-molecular-weight poly

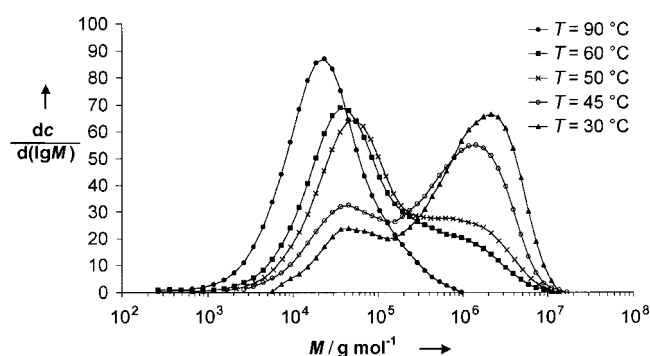
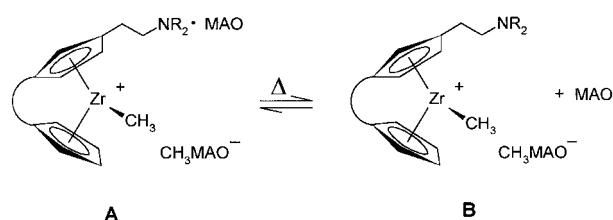


Figure 1. Molecular weight distribution of polyethylene as a function of the polymerization temperature.

ethylenewas formed predominantly, while the low-molecular-weight fraction was decreased dramatically. At $T = 20^\circ\text{C}$, high-molecular-weight polyethylene was obtained, which could not be analyzed by GPC. As judged from the trend in Figure 1, one would expect that this polymer shows a unimodal molecular-weight distribution again.

We have demonstrated that it is possible to produce polyethylenes with different amounts of low- ($M = 30\,000 - 60\,000\text{ g mol}^{-1}$) as well as high-molecular-weight ($M = 1\,000\,000 - 2\,500\,000\text{ g mol}^{-1}$) polymer chains by varying the polymerization temperature and using a catalyst system consisting of an amino-functionalized *ansa*-zirconocene dichloride and MAO.^[5] Thus, polymer properties can be influenced more effectively than by using conventional single-site catalysts.

We propose that the dialkylaminoethyl functionality plays an important role in the polymerization process: the cocatalyst MAO not only acts as a methylating agent and as a Lewis acid to build the catalytically active, cationic complex, but it can also coordinate to the nitrogen atom of the dialkylaminoethyl group.^[6] This results in the formation of the active species **A**, which produces high-molecular-weight polyethylene (Scheme 2). In the high-temperature region the catalytically active species **B** is formed in which the MAO does not



Scheme 2. Suggested mechanism for the generation of the catalytically active species **A** and **B**.

coordinate to the amino group any more. **B** produces low-molecular-weight polyethylene. An equilibrium between **A** and **B** leads to the formation of a bimodal molecular-weight distribution in the polymerization process. Chain propagation and termination processes are obviously influenced by the differently structured side chain in **A** and **B**.^[7] The assumption of a reversible temperature-dependent adduct formation ($\text{N} \rightarrow \text{MAO}$) is supported by ^1H and ^{27}Al NMR spectroscopic investigations on a model system: a solution of diisopropyl-

amine and MAO (N:Al = 1:2) in [D₈]toluene as solvent shows a temperature-dependent equilibrium between amine·MAO adducts and dissociated species in the temperature range between $T = -80^{\circ}\text{C}$ to $+80^{\circ}\text{C}$.

The 3-position of the dialkylaminoethyl substituent as well as the *ansa* bridge seem to be essential for the results reported here: only in this constellation is the amino group directed to the open sector of the catalytically active center; thus, chain propagation and chain termination processes can be effected by altering the donor functionality. This assumption is proven by the fact that unbridged, amino-functionalized zirconocene dichlorides as precatalysts do not show this phenomenon in the polymerization of olefins at all.^[8]

The results reported here are not restricted to the system **3**/MAO. In combination with MAO the carbon-bridged, dimethylaminoethyl-functionalized zirconocene dichloride **4**^[4] produces polyethylene which shows a bimodal molecular weight distribution at $T = 50^{\circ}\text{C}$.^[9] For the system **4**/MAO, it was additionally shown by Schulz–Zimm statistical methods^[10] that the observed distribution was generated by a superposition of two independent distributions: the experimentally obtained data and the curve-fit, calculated from two different single-site catalysts ($M_w/M_n = 2.9$ and 2.3, respectively), are in good agreement (Figure 2). Although a single-component precatalyst was used for the polymerization process, two catalyst systems have to be present which produce a polyethylene with a bimodal molecular weight distribution in the selected temperature region (Table 2).

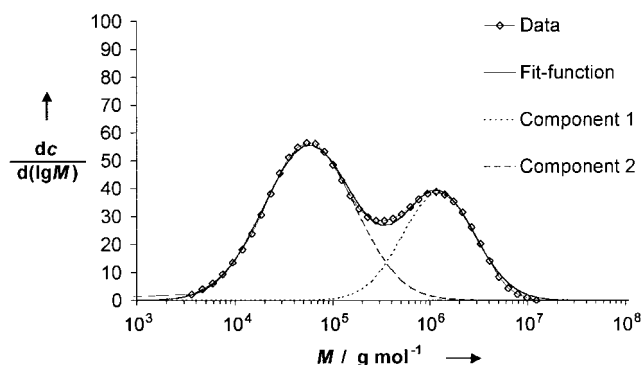
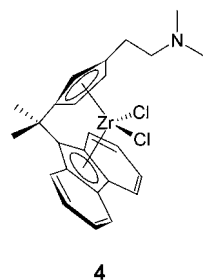


Figure 2. GPC curve of a polyethylene sample in comparison to a calculated molecular weight distribution consisting of two independent components; catalyst system: **4**/MAO, polymerization temperature: $T = 50^{\circ}\text{C}$.

Table 2. Experimentally obtained data and calculated molecular weight parameters M_w and M_w/M_n of polyethylene; catalyst system **4**/MAO, polymerization temperature $T = 50^{\circ}\text{C}$.

	M_w	M_w/M_n	Amount
data	824884	15.2	—
calculated-fit	817849	16.1	—
component 1	1850057	2.3	41 %
component 2	88049	2.9	59 %

Similar effects should be generally possible in other dialkylaminoethyl-functionalized and sterically fixed catalyst systems.

Experimental Section

All manipulations were carried out under purified argon atmosphere using standard Schlenk techniques. The solvents were commercially available, purified by conventional methods, and distilled immediately prior to use.

3: *n*BuLi (4.5 mL, 7.0 mmol) was added dropwise at 0°C to a solution of **1**^[11] (1.08 g, 3.4 mmol) in diethyl ether (10 mL). A white suspension of the dilithium salt **2** was formed immediately. The mixture was stirred for 18 h at room temperature. Subsequently, compound **2** was filtered off, dried in vacuo (1.00 g, 3.0 mmol; 89 %), and then suspended again in diethyl ether (10 mL). ZrCl_4 (0.70 g, 3.0 mmol) was added slowly at -60°C . The yellow suspension was allowed to warm to room temperature and stirred for a further 18 h. The solvent was removed in vacuo and the residue was extracted with toluene (20 mL). After filtration the solvent was again removed in vacuo. The remaining yellow oil solidified by addition of pentane (10 mL). The orange solid was recrystallized from dimethoxyethane/acetonitrile (10:1). Compound **3** was obtained as a beige, air- and moisture-sensitive solid (0.61 g, 1.3 mmol, 43 %); M. p. 124°C ; ^1H NMR (500.1 MHz, C_6D_6 , 25°C): $\delta = 0.14$ (s, 3H; SiCH_3), 0.15 (s, 3H; SiCH_3), 0.91 (d, $^3J(\text{H,H}) = 6.3$ Hz, 6H; CHCH_3), 0.92 (d, $^3J(\text{H,H}) = 6.3$ Hz, 6H; CHCH_3), 2.64–2.68, 2.89–2.96 ($2 \times$ m, 6H; CHCH_3 , CH_2CH_2), 5.37 (pt, $^3,^4J(\text{H,H}) = 2.5$ Hz, 1H; C_5H_3), 5.44 (m, 1H; C_5H_4), 5.57 (m, 1H; C_5H_4), 5.59 (pt, $^3,^4J(\text{H,H}) = 2.5$ Hz, 1H; C_5H_3), 6.70 (pt, $^3,^4J(\text{H,H}) = 2.5$ Hz, 1H; C_5H_3), 6.78 (m, 1H; C_5H_4), 6.85 (m, 1H; C_5H_4); $^{13}\text{C}\{^1\text{H}\}$ NMR (125.8 MHz, C_6D_6 , 25°C): $\delta = -5.7$ (SiCH_3), 20.9, 21.0 (NCHCH_3), 32.3 (NCH_2CH_2), 45.5 (NCH_2CH_2), 48.1 (NCH), 107.2, 107.5, 113.6, 113.8, 114.4, 114.8, 127.3, 128.3, 128.5, 141.9 (CpC). $^{29}\text{Si}\{^1\text{H}\}$ NMR (99.4 MHz, C_6D_6 , 25°C): $\delta = -13.8$; MS (CI): m/z (%): 476 (13) [M^+], 114 (100) [$(\text{C}_5\text{H}_7)_2\text{NCH}_2^+$]; elemental analysis (%): $\text{C}_{20}\text{H}_{31}\text{Cl}_2\text{NSiZr}$ (475.68): calcd: C 50.51, H 6.57, N 2.94; found: C 50.21, H 6.96, N 2.76.

General procedure for the polymerization of ethylene: A 1 L-Büchi autoclave, equipped with mass-flow and pressure-controller was filled under argon with toluene (194 mL) and MAO solution (3 mL; 4.9 % aluminum, $\rho \approx 0.9 \text{ g mL}^{-1}$, 1 mmol Al = 0.6 mL). The interior was pressurized with ethylene (5 bar) and thermostated to the desired temperature. The ethylene pressure was kept constant during the polymerization process. Compound **3** (4.8 mg, 1×10^{-5} mol) was preactivated for 10 min with MAO solution (3 mL) and injected into the autoclave. The polymerization process was terminated after 1 h by injecting 2-propanol (20 mL). The suspension containing the polymer was poured into methanol/HCl (200 mL; 5:1) and stirred for 18 h. After filtration the polymer was dried in the air.

Determination of the molecular weight distribution: The polyethylenes were analyzed by high-temperature gel permeation chromatography using a Waters 150 C apparatus with an IR detector at $\lambda = 3.5 \mu\text{m}$; the solvent was 1,2,4-trichlorobenzene at 135°C . Calibration was performed by using narrow and broad distributed linear polyethylene standards.

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Real Space Observation of a Chiral Phase Transition in a Two-Dimensional Organic Layer**

Matthias Böhringer,* Wolf-Dieter Schneider, and Richard Berndt

Chiral discrimination between mirror-image stereoisomers can lead to the spontaneous separation of a racemic mixture into enantiomerically pure phases.^[1] In three-dimensional systems the formation of these conglomerates is the exception rather than the rule; most racemic mixtures crystallize as racemates with the unit cell composed of an equal number of molecules with opposite chirality or as random solid solutions. It was originally proposed by Stewart and Arnett that confinement to two dimensions (2D) enhances chiral discrim-

ination between enantiomers.^[2] Most racemic 2D systems investigated experimentally so far, with respect to spontaneous breaking of the mirror symmetry, are Langmuir films of amphiphilic molecules with one or more asymmetric carbon atoms.^[2–7] Atomic force microscopy (AFM) observations by Eckhardt et al. of enantiomorphous supramolecular structures evolving from a racemic mixture of a chiral amphiphile have been interpreted in terms of chiral segregation.^[4] However, chiral packing of achiral molecules has also been observed to cause enantiomorphous structures.^[8–12] Therefore, the observation of chiral supramolecular structures alone does not prove chiral phase separation.

A straightforward proof for chiral segregation is the direct determination of the chirality of individual molecules. Scanning-tunneling microscopy (STM) has been successfully applied to determine the conformation and chirality of individual molecules.^[13, 14] Lopinski et al. used STM to determine the absolute configuration of isolated chiral centers.^[14] The same technique was employed by de Feyter et al. to identify the positions of asymmetric carbon atoms within homochiral domains of a chiral terephthalic acid derivative.^[15] In the present paper, we report the first real space observation of a coverage-driven chiral phase transition from a conglomerate to a racemate at low and high molecular coverages, respectively. The chirality of individual molecules is determined from high resolution STM images which closely reflect the asymmetric electronic structure of the molecules. From the STM data, models are deduced for the distinct chiral and achiral phases, revealing the driving force for chiral symmetry breaking.

The substrate used is reconstructed Au(111) which is comprised of uniaxial domains of alternating face centred cubic (fcc) and hexagonal close packed (hcp) stacking of the surface atoms (Figure 1a). The domain walls form a herringbone pattern visible as bright stripes in the STM topographs.

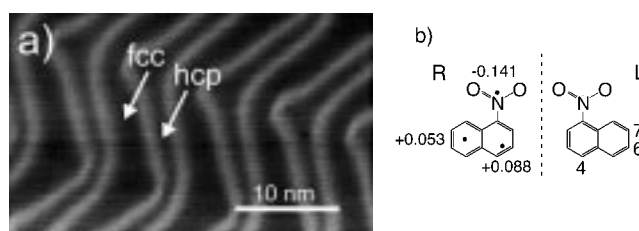


Figure 1. a) STM topograph of the reconstructed Au(111) surface. Broad and narrow dark stripes correspond to domains with surface atoms in fcc or hcp positions, respectively. The transition regions (domain walls) separating fcc and hcp regions appear as bright stripes. b) 1-Nitronaphthalene (NN) in a planar adsorption geometry on Au(111) is 2D chiral. Molecules with the nitro group attached to the left/right carbon ring are denoted as L/R-enantiomers. The nitro group has a net negative charge; the two carbon rings are positively charged.^[17] The centres of mass of the respective charges are marked with dots.

1-Nitronaphthalene (NN, Figure 1b) adsorbs with the naphthalene system parallel to the Au(111) surface.^[16] This geometry imposes a chirality onto the NN molecules which is not present in the gas phase. An adsorbed molecule and its mirror image can not be superimposed by rotation and translation within the surface plane (Figure 1b). On the

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Au(111) surface equal amounts of left- and right-handed (L and R, respectively) NN molecules are present; these form a racemic mixture.

Below a temperature of 100 K the NN molecules aggregate into distinct structures whose dimensionality depends on the coverage. At 50 K and coverages below 0.25 monolayers (ML), monodisperse zero-dimensional molecular clusters are observed.^[17, 18] In a medium coverage range, one-dimensional (1D) structures prevail. These are straight molecular double chains within the fcc domains (Figure 2a, 0.3 ML) and, at higher coverages, also within the hcp domains (Figure 2b, 0.4 ML). Moreover, zig-zag patterns are observed within fcc domains at 0.4 ML coverage. Finally, at full monolayer coverage 1D and 2D periodic molecular structures coexist on the surface (Figures 2c, d).

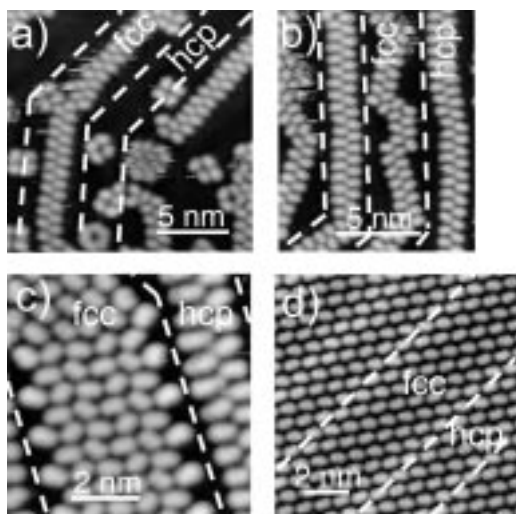


Figure 2. Molecules are imaged as bright ellipsoids (long axis ≈ 1 nm). a) STM image recorded at 0.3 ML coverage of NN. Straight molecular double chains follow the fcc domains, while a few small aggregates are observed within the hcp domains. Domain walls separating fcc and hcp domains are indicated by broken lines. b) STM image recorded at 0.4 ML coverage of NN. The linear structure is observed within hcp domains as well. In fcc domains, chains now form a zig-zag pattern while the domain walls are largely free of molecules. c) and d) STM image recorded at monolayer coverage. 1D structures within fcc and hcp domains (c) coexist with a 2D periodic phase (d).

Although the electronic structure of NN is strongly asymmetric owing to the nitro group, the molecule is imaged as a rather symmetrical ellipsoid over a wide range of tunneling parameters and the enantiomers cannot be distinguished (Figure 2 and Figure 3a). However, when occupied sample states are probed at a tunneling voltage of $V = -2.3$ V, intramolecular structure is resolved which reflects the asymmetry of the molecule and enables enantiomeric discrimination. In Figure 3b each molecule appears as three bright maxima and a fourth very weak maximum. This intramolecular contrast pattern shows the expected handedness.

The charge density calculated for the highest occupied molecular orbital (HOMO) of the NN molecule 5 Å above

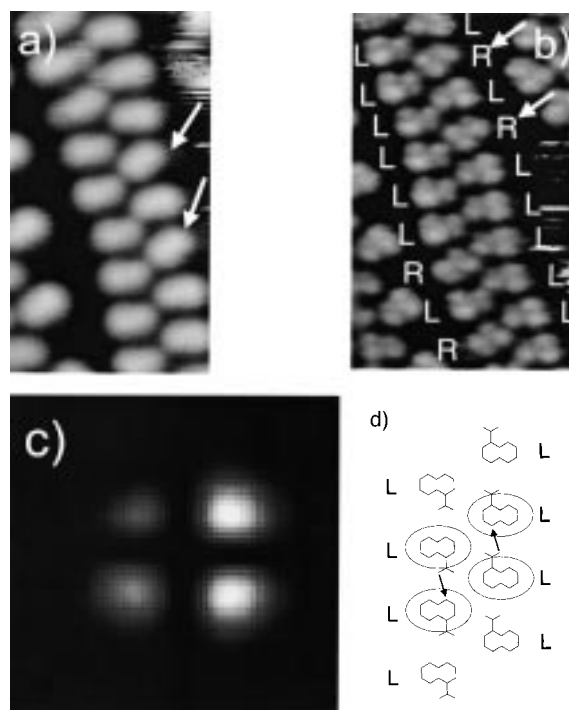


Figure 3. a) Segment of an NN double chain as typically imaged at low tunneling voltages (-1.2 V). b) At high negative tunneling voltage (-2.3 V) each molecule appears as three bright maxima and one very weak maximum. This intramolecular contrast pattern reflects the asymmetry of the NN molecules and allows for the discrimination between the NN enantiomers. Defect free, straight sections of the double chains are composed of exclusively one enantiomer. Molecules with opposite chirality (marked by arrows) are rotated. c) Charge density of the NN HOMO, calculated 5 Å above the molecular plane for an L type molecule (from Ref. [17]). The weakest maximum corresponds to the part of the naphthalene system where the nitro group is attached. The chirality is attributed to the molecules by comparison with this calculated charge density. d) Model of the enantiopure double chain structure (from Ref. [17]). Arrows denote the hydrogen bonds. The ellipsoids indicate the shape of the molecules observed in low-resolution images.

the molecular plane (Figure 3c) closely resembles the observed submolecular structure.^[17] The weakest maximum is associated with the part of the naphthalene system which carries the substituent. This pattern may be understood in terms of the strong charge-pulling effect of the nitro group on the NN π -electron system. From this agreement we conclude that, at a bias voltage of -2.3 V, the submolecular image contrast is dominated by an NN HOMO-derived sample state. At lower bias voltage, hybridization with substrate states results in the nearly symmetrical appearance of the molecules.^[19] The high-resolution data in Figure 3b enables a direct determination of the handedness of each molecule and leads to the model reproduced in Figure 3d. This model has been confirmed by molecular dynamics simulations on the basis of long range electrostatic interactions and short range van der Waals interactions between the NN molecules.^[17] Within each single strand the molecules are arranged in a “head to tail” configuration with hydrogen bonds between a negatively charged oxygen atom and the “back” hydrogen atom on the carbon atom C4 of a neighbouring NN molecule. The second strand is rotated by 180° and shifted by half a period such that opposite charges are close to each other. From the high-

resolution images and the theoretical modeling we conclude that straight, defect-free segments of the double chains consist of exclusively one NN enantiomer (L type in Figure 3b), thus representing a 1D conglomerate.

Sporadically, molecules in the L conglomerate are exchanged with a R enantiomer (arrows in Figure 3b). These molecules are rotated by $\approx 25^\circ$ (arrows in Figure 3a). This specific rotation allows for hydrogen bonding to the back hydrogen atom of the adjacent molecule (Figure 4a). At low coverages we find less than 2% of these defects in double chains, corresponding to an enantiomeric excess $> 96\%$.

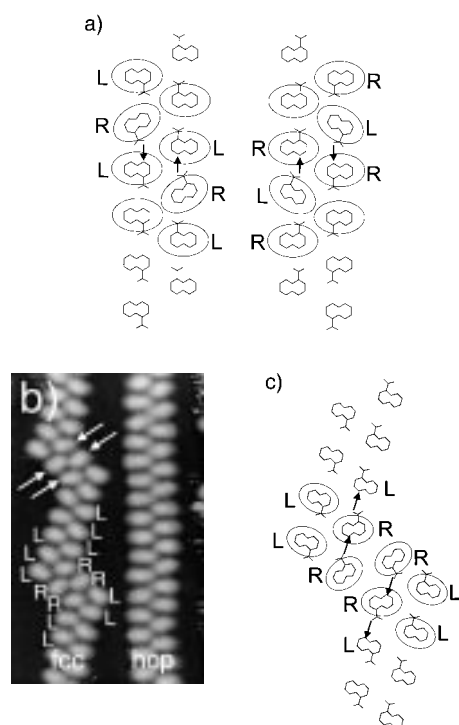


Figure 4. a) Model of an L type double chain with some isolated molecules of opposite chirality incorporated into the otherwise enantiopure structure. b) Typical geometry of the zig-zag structure observed within fcc domains at 0.4 ML coverage. The zig-zag geometry in the upper and lower half of the image is identical. Arrows mark rotated molecules which terminate straight segments. c) Model of the zig-zag structure. Minimal segments of chains with opposite chirality are incorporated into the otherwise enantiopure double chains. The attribution of L and R to the molecules in (b) corresponds to this model.

Moreover, one observes that molecules with opposite chirality can introduce kinks into the otherwise straight molecular chains. The zig-zag arrangement within the fcc domains shown in Figure 2b indicates that with increasing coverage more and more molecules with opposite handedness are built into the double chains, corresponding to a gradual transition from a conglomerate to a racemate. In the typical zig-zag geometry, straight segments terminate with two rotated molecules (arrows in Figure 4b). This structure can be modeled by introducing minimal segments of opposite chirality into the otherwise enantiopure double chains (Figure 4c).

Packing even more NN molecules onto fcc sites results in the structure shown in Figure 2c. Rows composed of five

molecules are stacked along the fcc domains and in this low resolution image adjacent rows appear enantiomorphous. Indeed, the corresponding high resolution image (Figure 5a) reveals that neighboring rows are composed of opposite enantiomers. The surface unit cell is therefore comprised of an

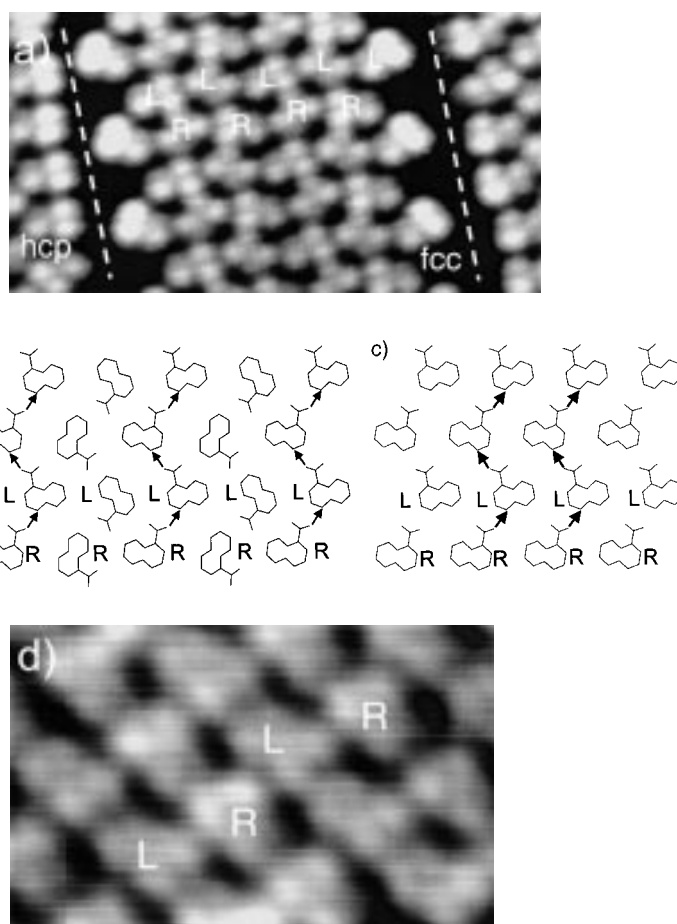


Figure 5. a) High-resolution image of the densely packed structure in the fcc domains of Figure 2c. Along the fcc domain, molecules in adjacent rows show opposite chirality. The structure therefore represents a quasi-1D racemate. In the hcp domains enantiopure molecular double chains persist but with an increased density of chiral defects. b) Model of the structure in the fcc domains. Half of the molecules form hydrogen bonds (arrows) similar to the "head to tail" geometry described for the double chain structure. However, contrary to the enantiopure double chains the hydrogen bonds now occur between molecules of opposite chirality. c) Rotation of half of the molecules in (b) by $\pm 120^\circ$ results in a racemate structure where all molecules take part in hydrogen bonding. d) High resolution images of the 2D structure shown in Figure 2d are consistent with the model reproduced in (c).

equal number of both enantiomers so that the structure is a 1D racemate. Half of the molecules form hydrogen bonds similar to the "head to tail" geometry described above (arrows in the model Figure 5b). Here, however, hydrogen bonds occur between molecules of different chirality. For the other molecules, the negative oxygen atoms point towards (positive) hydrogen atoms on the carbon atoms C6 and C7 of adjacent NN molecules. Rotation of these molecules by approximately $\pm 120^\circ$ results in the racemate structure depicted in Figure 5c where all molecules form "head to tail" hydrogen bonds to neighbouring molecules of opposite

chirality. This structure is consistent with the high-resolution image (Figure 5d) of the 2D periodic molecular arrangement shown in Figure 2d. At monolayer coverage it coexists with the 1D racemate in fcc domains and the 1D conglomerate in hcp domains (Figure 2c). The average density of molecules in the 1D and 2D structures (Figure 2c, d) are identical. In Figure 2c the lower density of molecules in the double rows and the depletion of the domain walls from molecules is compensated for by a more dense packing in the fcc domains.

The structural transformations described above represent a transition from a conglomerate at low coverages into a racemate at high coverage. They may be interpreted in terms of two driving forces. First, electrostatics favor interactions among identical enantiomers. Second, adsorption is strongest on fcc sites of the reconstructed Au(111) surface, weaker on hcp sites, and weakest on the transition regions between fcc and hcp domains (domain walls). As a consequence of these trends, at low molecular densities NN assembles into straight enantiopure chains which are located in fcc domains (Figure 2a). At higher coverages, the length of the double chains in the fcc domains increases through the formation of a zig-zag structure. This, however, costs electrostatic energy since opposite enantiomers have to be incorporated into the otherwise homochiral chains. Likewise, electrostatically optimal, straight, enantiopure chains are formed at energetically less favorable hcp sites. No kinks are introduced into the chains within hcp domains because any zig-zag would expel molecules from these narrow domains into unfavorable sites on top of the domain walls. At monolayer coverage, a 2D periodic racemic structure with dominating heterochiral interactions is formed, which does not follow the surface reconstruction (Figure 2d). This phase is unstable and can decay into a 1D racemate within fcc domains and a 1D conglomerate within hcp domains (Figure 2c).

Our observations show similarities with a scenario described by Andelman and de Gennes.^[20] For amphiphiles in an adsorption geometry where three of the four distinct substituents of an asymmetric carbon atom are confined to two dimensions, spontaneous chiral segregation was predicted, provided that there are strong electrostatic interactions between two oppositely charged groups and a third neutral in-plane substituent which interacts equally with the two charged groups. The predicted ground state structure is an enantiopure 2D hexagonal lattice. In the case of NN, at low coverages, the enantiopure double chain structure is likewise determined by the strong interaction between the opposite charges localized at the oxygen atoms and the hydrogen atom on the carbon atom C4. However, unlike the tripodal molecules discussed in Ref. [20], an additional net positive charge is localized at the second carbon ring of the naphthalene system (Figure 1b) which prevents the formation of the predicted homochiral 2D structure. Rather, as long as the density of molecules is low, a linear 1D structure occurs where the positive charge of the second carbon ring is expelled to the periphery. At high NN coverage, the strong competition in the electrostatic interactions between all three groups is expected to result in frustration effects.^[20] The interaction with the reconstructed Au(111) substrate may then become important in destabilizing the 2D periodic, heterochiral, monolayer

structure with respect to a mixture of a 1D heterochiral and a 1D homochiral phase.

In summary, we have observed in real space a coverage-driven transition from a conglomerate to a racemate for the 2D chiral molecule 1-nitronaphthalene on Au(111). High resolution STM images directly reveal the chirality of the adsorbed molecules and lead to models for the chiral and achiral phases. The observed spontaneous breaking of the chiral symmetry at low coverages has been discussed within an electrostatic model for the intermolecular interactions.

Experimental Section

The experiments were performed under ultra high vacuum (base pressure $< 1 \times 10^{-10}$ mbar) with a custom-built low-temperature scanning tunneling microscope.^[21] Clean Au(111) surfaces were obtained by repeated sputter-anneal cycles. Purified NN was admitted to the vacuum chamber by a leak valve from a gas handling line (≈ 350 K) onto the sample at 295 K. The amount of NN dosed onto the surface was controlled by the increase of the total pressure inside the vacuum chamber ($2-5 \times 10^{-8}$ mbar) and the time of exposure (4–20 s). Subsequently, the sample was cooled to 50 K and constant-current STM images were recorded. For experiments at distinct coverages the sample was warmed up, cleaned, redosed and cooled to 50 K as before.

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The Novel Hybrid Antitumor Compound Premithramycinone **1** Provides Indirect Evidence for a Tricyclic Intermediate of the Biosynthesis of the Aureolic Acid Antibiotic Mithramycin**

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Dedicated to Professor Axel Zeeck on the occasion of his 60th birthday

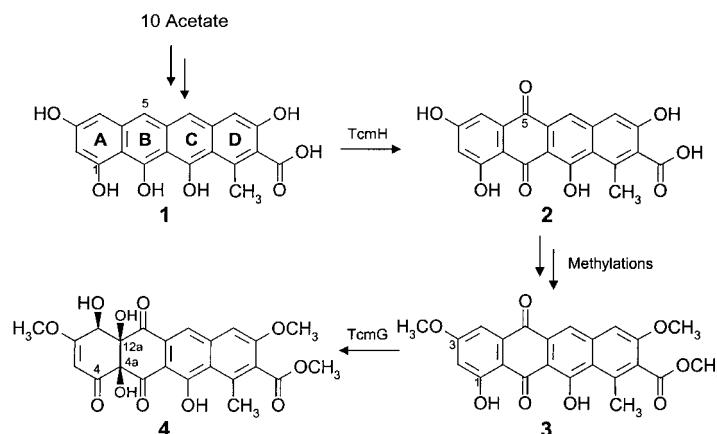
Since the pioneering work of Hopwood et al. in 1985^[1] the use of recombinant DNA technology has offered interesting perspectives for the generation of novel “unnatural” or “hybrid” natural products through the manipulation of biosynthetic gene clusters (combinatorial biosynthesis). Combinatorial biosynthesis is now emerging as a potential tool for the production of structural biodiversity. Gene disruption and recombination experiments are also suitable for gaining insight into biosynthetic pathways.^[2]

Our work has been focused on post-polyketide synthase (PKS) tailoring steps, mainly oxidoreductase and group transfer steps, which occur late in the biosynthetic pathways and are often responsible for the conversion of biologically inactive intermediates into active products.^[3]

We describe here a recombination of the mithramycin PKS genes with oxygenase-encoding genes from the tetracenomycin gene cluster. The experiments were not only aimed at the generation of novel biologically active hybrid natural products, but also were designed to intercept a labile, early intermediate of the mithramycin biosynthesis and to provide evidence for its structure.

The oxygenases TcmG and TcmH are responsible for the introduction of four oxygen atoms in the biosynthesis of

tetracenomycin C **4**. TcmH catalyzes the oxidation of the first tetracyclic anthrone intermediate tetracenomycin F1 **1** into the naphthacenequinone tetracenomycin D3 **2**. TcmG is responsible for the very last step of this biosynthesis, the conversion of tetracenomycin A2 **3** into tetracenomycin C **4** (Scheme 1), through which three further oxygen atoms are introduced.^[4]



Scheme 1. Oxygenation reactions of the tetracenomycin biosynthetic pathway.

Mithramycin **8** is the most important member of the aureolic acid antitumor drugs.^[5] During the past three years, we have increased the knowledge regarding the biosynthesis of this class of antibiotics tremendously. A large number of genes of the mithramycin gene cluster have been characterized and various intermediates have been identified.^[6–9] The earliest known intermediates are premithramycinone **6** and its 4-demethylated derivative **5**,^[7] which is converted into premithramycin B **7** through several methyl and glycosyl transfer steps. The latter is finally converted into mithramycin **8** by an oxidative opening and a ketoreduction step (Scheme 2).^[6a, 8]

The premithramycinones **5** and **6** resemble tetracenomycin F1 **1** in that they are also tetracyclic biosynthetic intermediates and rings C and D of **5** and **6** are identical to rings A and B of **1**. Thus, it should be possible to intercept the mithramycin biosynthesis through the influence of one or both oxygenases from the tetracenomycin C gene cluster in order to produce premithramycin or mithramycin analogues, in which the normally aromatic rings are highly functionalized, as in **4**. If successful, this “interception experiment” should also provide further insight into the mithramycin biosynthetic pathway.

To test this hypothesis, we initially used the premithramycinone/4-demethylpremithramycinone accumulating mutant strain *Streptomyces argillaceus* M7D1^[6c, 7] as a host to test whether these compounds can be modified by the foreign oxygenases TcmG and/or TcmH. In a second experiment (see below), we used *S. argillaceus* M7O2^[9] as a mutant host strain, in which the oxygenase MtmOII is blocked. This strain accumulates an unknown labile intermediate of the mithramycin biosynthetic pathway which should be intercepted by the foreign oxygenases. Both experiments led unexpectedly to the same hybrid product. Plasmid pWHM1026 contains the

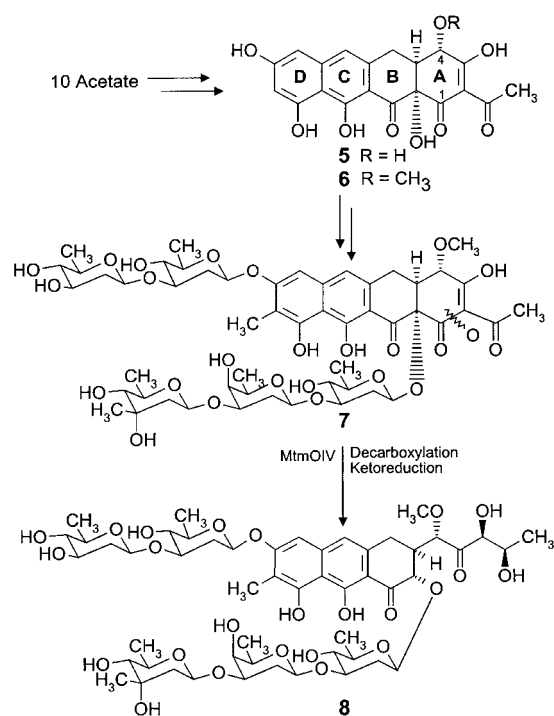
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Scheme 2. The biosynthetic pathway of mithramycin **8** and the important intermediates premithramycinone **6** and premithramycin B **7**.

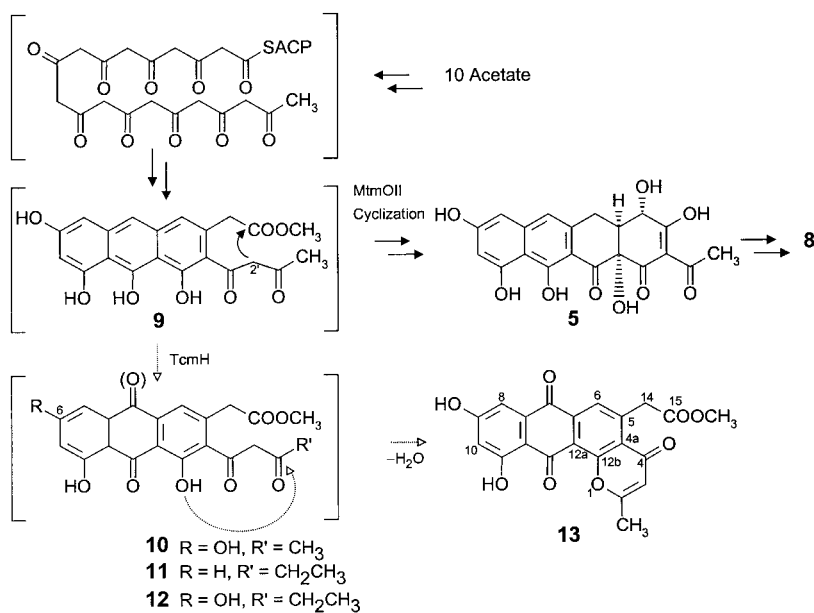
entire **4** gene cluster.^[10] A 2.5-kB *NcoI*-*BglII* DNA fragment (both sites blunt-ended) from pWHM1026, which contained the *tcmG* and *tcmH* genes, was subcloned into the *Bam*HI site (blunt-ended) of the plasmid pIAGO^[11] under the control of the erythromycin resistance promoter, *ermE*. This construct (pFLOGH) was used to transform protoplasts of mutant M7D1. The resulting new hybrid strain *S. argillaceus* M7D1 (pFLOGH) initially accumulated mainly premithramycinone **6** when grown on R5 solid medium but, after 7 days of incubation, a new yellow compound was visible which differed from the known M7D1-products **5** and **6**. This compound was subsequently called premithramycinone H. The bathochrome shift of its UV spectrum ($\lambda_{\text{max}} = 290, 442 \text{ nm}$; **6**: $\lambda_{\text{max}} = 280, 418 \text{ nm}$) indicates a quinone moiety. This was confirmed by the typical decoloration reaction with sodium hydrosulfide and the NMR data (Table 1). The structure elucidation was complicated, since the ^1H NMR data show almost only singlets (few long-range couplings) and the ^{13}C NMR spectrum consists of a large number of quaternary signals. The FAB mass spectrum resolved the molecular formula of $\text{C}_{21}\text{H}_{14}\text{O}_8$. This and the few detectable H_H and C_H long-range couplings allow the structure for premithramycinone H to be determined unambiguously as **13** (Table 1).

Premithramycinone H **13** is a shunt product formed by spontaneous cyclization of anthraquinone **10**, which is probably derived from tricyclic intermediate **9** (Scheme 3). The oxygenase TcmH oxidizes anthrone **9** (shown as the enol in

Table 1. NMR Data of Premithramycinone H **13**.^[a]

Position	^1H	^{13}C	$^nJ_{\text{C-H}}$ Observable in HMBC spectrum	^4J Coupling with: ^[b] ($^4J_{\text{H,H}}$ [Hz]) ^[c]
2		167.5		
3	6.31 (d)	112.7	2-C; 4a-C; 13-C	13-H ₃ (0.5)
4		178.5		
4a		126.6		
5		144.2		
6	7.96 (s)	126.6	4a-C; 7-C; 12a-C; 14-C	14-H ₂ (nd) ^[c]
6a		134.5		
7		182.1		
7a		136.5		
8	7.07 (d)	108.5	7-C; 10-C; 11a-C	10-H (2)
9	11.37 (s, OH)	165.4		
10	6.61 (d)	109.4	8-C; 9-C; 11-C; 11a-C	8-H (2)
11	12.85 (s, OH)	165.4	10-C; 11-C; 11a-C	
11a		111.1		
12		185.7		
12a		122.4		
12b		156.0		
13	2.44 (d, 3H)	20.4	2-C; 3-C	3-H (0.5)
14	4.31 (s, 2H)	42.0	4a-C; 5-C; 6-C; 15-C	6-H (nd) ^[c]
15		172.1		
15-OCH ₃	3.58 (s, 3H)	52.1		

[a] In $[\text{D}_6]\text{DMSO}/1\%$ trifluoroacetic acid at 11.74 T. [b] Determined from H_H -COSY spectrum. [c] nd = not determinable in the 1D ^1H NMR spectrum.



Scheme 3. Normal biosynthetic pathway of mithramycin **8** (solid arrows) and the new shunt pathway to premithramycinone H **13** (dotted arrows) mediated by foreign oxygenase TcmH.

Scheme 3) into the anthraquinone **10**. The anthraquinone formation stabilizes **9** and changes its properties. This oxidation reaction is accompanied by the attack of the phenolic 1-OH group at the 3'-keto group (dotted arrow), while in the normal mithramycin biosynthetic pathway the interchain cyclization between C-2' and the ester carbonyl (solid arrow), which is catalyzed by an Mtm cyclase, presumably MtmX, dominates.^[6a, 12] Intermediate **10** closely resembles **11**^[12b] and other early intermediates (such as compound 58B **12**) of the biosynthetic pathways of anthracyclines

(for example doxorubicin).^[13] Compound **9** resembles 12-deoxyaklanonic acid, a proposed intermediate of the doxorubicin biosynthesis,^[13a] and also tetracenomycin F2.^[14a] Like the latter in the tetracenomycin pathway, **9** represents the earliest discovered intermediate of the mithramycin biosynthetic pathway for which evidence now exists. Premithramycinone **H 13** is a hybrid natural product which is formed under the influence of the foreign oxygenase TcmH; the other heterologously expressed oxygenase TcmG has no effect.^[14b] TcmG fails to react on intermediate **10**, although this resembles its natural substrate tetracenomycin A2 **3** in respect of two rings (see above). Thus, for an interaction of TcmG with compound **10**, a methylated 6-OH group is probably necessary (this is the equivalent of the 3-OCH₃ group of **3**).

Since premithramycinone **H 13** does not contain the hydroxyl groups that are supposed to be introduced by the MtmOII oxygenase to form 4-demethylpremithramycinone **5**, it might be expected that TcmH could act on a nonhydroxylated mithramycin intermediate prior to **5**. M7D1 (pFLOGH) was able to either hydroxylate this hypothetical intermediate **9** through the action of MtmOII oxygenase generating **5** and **6** or to generate premithramycinone **H 13** by the action of TcmH. For this reason we tested a possible action of TcmH on the intermediate accumulated by mutant M7O2.^[9] The M7O2 mutant was transformed with pFLOGH and, interestingly, **13** was also accumulated by this recombinant strain. This supports the hypothesis of a tricyclic intermediate **9** since the oxygenase MtmOII, which is supposed to convert **9** or a corresponding tetracyclic intermediate,^[9] cannot act once anthraquinone **10** is formed through the action of TcmH. In contrast to mutant M7D1 (pFLOGH) which also produces the premithramycinones **5** and **6**, mutant M7O2 (pFLOGH) solely accumulates premithramycinone **H 13**. This suggests that the proposed tricyclic intermediate **9** is likely to be the true substrate of oxygenase MtmOII and in the normal mithramycin biosynthetic pathway the oxygenation step catalyzed by MtmOII occurs prior to the fourth cyclization to 4-demethylpremithramycinone **5**. This is in contrast to our earlier assumptions, in which we favored a tetracyclic compound to be the substrate of MtmOII.^[9] Since it was not yet possible to isolate the unstable compound accumulated by the M7O2 mutant, this interception experiment provides the first indirect information regarding the MtmOII substrate. The pyranone formation of **13** from **10** seems unlikely due to the poor nucleophilicity of the *peri*-hydroxy groups in anthraquinones.^[14c] Thus, quinone **10** might not be a true intermediate of the shunt pathway to **13**, and TcmH may interact simultaneously with or even after the pyranone formation. This also means for the normal pathway to mithramycin **8** that cyclization to **5** can only occur after the action of MtmOII on the tricyclic intermediate **9**. The M7O2 mutant is not affected in its deoxysugar biosynthesis or glycosyl transfer (in contrast to the M7D1 mutant) and it has the full capability to synthesize and transfer the mithramycin sugars. However, no glycosylated derivatives of **13** could be detected, probably due to a lack of substrate flexibility of the Mtm glycosyl transferases.

Like mithramycin **8**, premithramycinone **H 13** showed antibacterial activity against *Micrococcus luteus* and *Strepto-*

myces albus. The anticancer activity (see Table 2)^[15] is in almost the same range as that of mithramycin, in contrast to premithramycinone **6** which shows no antibacterial and only very weak antitumor activities.

Table 2. Proliferation inhibition assays with four different cancer cell lines (IC₅₀ [$\mu\text{g mL}^{-1}$]).^[15]

	13	8	6
Squamous cell carcinoma KB	11.7	10	29.3
Melanoma SK-Mel 30	15.3	9.6	— ^[a]
Non-small cell lung carcinoma A549	19.1	12.9	25.2
Breast carcinoma MDA-MB 231	15.2	12.3	37.7

[a] No inhibition ($> 40 \mu\text{g mL}^{-1}$).

Experimental Section

Microorganisms and culture conditions: *S. argillaceus* M7D1^[6c] and M7O2^[9] were used as hosts for the transformations. *Escherichia coli* XL1-Blue^[16] was used as the host for subcloning and was grown at 37 °C in TSB medium (trypticase soya broth, Oxoid). Growth of the different clones in solid medium was in modified R5 agar medium (R5A).^[6d]

DNA manipulation: Plasmid-DNA preparations, restriction endonuclease digestions, alkaline phosphatase treatments, ligations, and other DNA manipulations were according to standard procedures for *E. coli*^[16d] and *Streptomyces*.^[16c] Transformation of protoplasts of the two mutants followed standard procedures^[16c, d] and transformants were selected for resistance to thiostrepton (25 mg L⁻¹).

Production and isolation of premithramycinone **H**: Strain M7D1 (pFLOGH) was grown on R5 agar plates for 17 days at 28 °C. Strain M7O2 (pFLOGH) was grown on modified R5 liquid medium^[6d] for 3 days at 30 °C. In both cases, 50 mg L⁻¹ thiostrepton was added to the medium. The cultures were extracted four times with EtOAc/0.1 % HCO₂H, concentrated in vacuo until the volume was approximately 20 mL and clarified by centrifugation. HPLC analysis of the extract revealed a main product that was purified by preparative HPLC (Column: μ Bondapak C18 radial compression cartridge, PrepPak Cartridge, 25 $\times$ 100 mm, Waters; eluent: MeOH (Gradient from 50 to 80 % methanol in 30 min)/0.1 % trifluoroacetic acid (TFA) in H₂O; flow rate: 10 mL min⁻¹). The purified material was adjusted to pH 4.0 with 0.2 M potassium phosphate buffer (pH 7.0) and diluted fourfold with water. This solution was applied to a solid-phase extraction column (Lichrolut RP-18, Merck), washed with water to eliminate salts, eluted with methanol and dried in vacuo. A final purification was achieved with Sephadex LH-20 (MeOH/TFA 99:1).

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A Metalloid Al₁₄ Cluster with the Structure of a “Nano-Wheel”***

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Dedicated to Professor Heinrich Vahrenkamp on the occasion of his 60th birthday

Solutions of metastable aluminum(II) halides^[1] can be prepared by condensation of the high-temperature molecules AlX (X = Cl, Br, I) with suitable donor-containing solvents. The thermodynamically favored disproportionation of these compounds to aluminum metal and the trihalide can be controlled kinetically by the choice of halide, donor, and temperature. Thus, with NEt₃ as donor a planar Al₄ species (for example, Al₄Br₄·4NEt₃^[2]) is obtained, whereas with THF the polyhedral subhalide Al₁₂(AlBr₂·THF)₁₀·2 THF forms as a result of “internal” disproportionation.^[3]

An additional variation of the disproportionation is achieved by replacement of the halide with suitable bulky substituents. Use of the N(SiMe₃)₂ group proved to be especially successful: Reaction of LiN(SiMe₃)₂ with a solution of AlI provided a Al₇₇R₂₀^{2–} compound with the largest *metalloid* cluster yet characterized by diffraction methods. This compound can be viewed as an intermediate on the way

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to aluminum metal.^[4] Recently, utilizing the more reactive AlCl solutions, two further *metalloid* clusters with the same substituents—Al₇R₆[−]^[5] and Al₁₂R₈[−]^[6]—could be isolated as intermediates in the formation of the Al₇₇ unit.

In these clusters (Al₇, Al₁₂, Al₇₇) the number of direct metal–metal contacts exceeds the number of metal–ligand interactions. We classify such species as *metalloid* clusters,^[5] to distinguish them from the variety of “metal clusters” included in Cotton’s original definition.^[7] In the search for further intermediates on the way to the metal, the title compound [Al₁₄(N(SiMe₃)₂)₆I₆Li(OEt₂)₂]⁺[Li(OEt₂)₄][−]·Tol (**1**; Tol = toluene), a mixed (I, N(SiMe₃)₂) metalloid cluster, could be isolated and structurally characterized for the first time.

Compound **1** was synthesized by slight variation of the reaction conditions under which the Al₇₇ cluster was formed. After addition of solid LiN(SiMe₃)₂ to a pretreated AlI·Et₂O solution, the mixture was warmed to 55 °C several times and slowly cooled. After a few weeks **1** crystallized at +7 °C as dark red-brown platelets. The novel formation of the mixed substituted compound **1** appears to be plausible, as only the less reactive AlI reacts in the way described here. Treatment of the more reactive AlCl with the same starting material LiN(SiMe₃)₂ leads to Al₇R₆[−] and Al₁₂R₈[−] clusters with complete substitution of the halide. It is possible that a Al₇R₃X₃[−] species is a common precursor that is rapidly substituted in the case of X = Cl. For X = I the substitution is so slow that dimerization to form **1** is kinetically preferred.

Figure 1 presents the results of the X-ray structure analysis^[8] of **1**. The [Li(OEt₂)₄]⁺ ion, which fills the voids resulting from packing of Al₁₄^{2−} cluster units, is not shown.^[9]

The main structural unit is represented by two staggered, approximately Al-centered Al₆ rings. The central Al atoms deviate somewhat from the planes of the rings and are separated by 2.728 Å. The other Al–Al distances range from 2.570 Å (between Al atoms with iodine ligands) to 2.910 Å

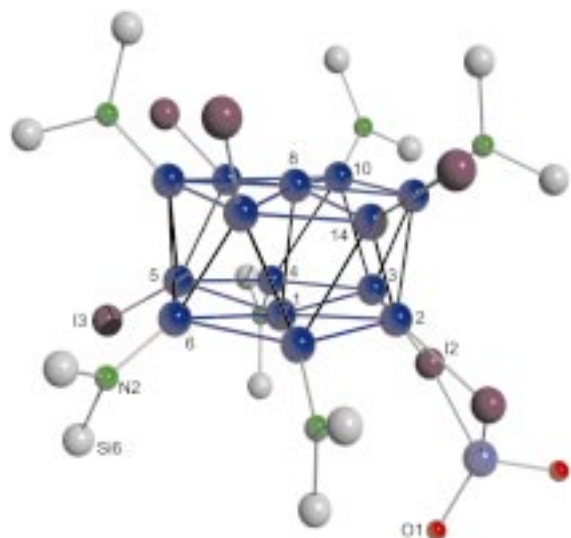


Figure 1. Molecular structure of **1**; for reasons of clarity the C and H atoms have been omitted. Blue: Al atoms. Selected distances [Å]: Al1–Al5 2.623(2), Al1–Al4 2.758(2), Al2–Al3 2.597(2), Al4–Al5 2.763(2), Al1–Al8 2.728(2), Al4–Al10 2.910(3), Al2–Al14 2.570(2), Al6–N2 1.855(5), Al5–I3 2.550(2), Al3–I2 2.636(2).

(between Al atoms with N(SiMe₃)₂ ligands); that is, the values are in the range expected from other polyhedral or metalloid Al clusters.^[10]

In the case of the Al₇R₆[−] cluster **2** as well as **1** the average oxidation state for the Al atoms is 0.71, which is in contrast to 0.23 for the Al₇₇ cluster. The disproportionation of the initially monovalent Al species to aluminum metal is therefore equally far advanced for **2** and **1**. To be able to discuss the bonding situation in the two species with aluminum in the same average oxidation state, we carried out density functional theory (DFT) calculations^[11] on the model compounds Al₇R₆[−] (**2a**; R = NH₂) and Al₁₄R₁₂^{2−} (**1a**; R = NH₂). These calculations indicated that the dimerization **2a**→**1a** is exothermic (−275 kJ mol^{−1}). The geometrical parameters of **2a** and **1a**, which are shown in Figure 2, correspond at a first glance to the

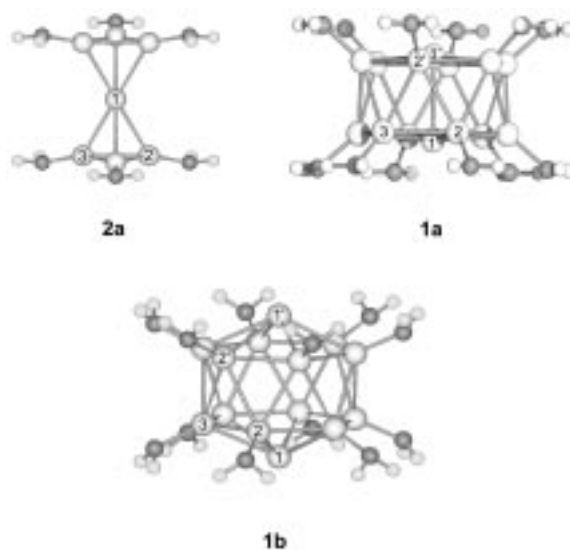


Figure 2. The model compounds Al₇(NH₂)₆[−] (**2a**), Al₁₄(NH₂)₁₂^{2−} (**1a**), and polyhedral Al₁₄(NH₂)₁₂^{2−} (**1b**). The following characteristic Al–Al distances [Å] were obtained by DFT calculations:^[10] **2a**: Al1–Al2 2.760, Al2–Al3 2.543; **1a**: Al1–Al2 2.721, Al2–Al3 2.713, Al2–Al2′ 2.690, Al1–Al1′ 2.693; **1b**: Al1–Al2 2.908, Al2–Al3 2.638, Al2–Al2′ 2.808, Al1–Al1′ 4.903.

experimentally determined values for **2** and **1**. Additionally, calculations were carried out on the isomer **1b**, which is also shown in Figure 2. In this case the central Al–Al distance is highly elongated and an approximately polyhedral structure results, as found for M₁₄ Frank–Kasper polyhedra, for example Mg₂₃(Al,Zn)₄₉.^[12] However, the central atom is missing in **1b**.

Although the polyhedral structure of **1b** leads one to expect a bonding situation as described by Wade for a *precloso* or *nido* structure,^[13] the calculations showed that **1b** is clearly destabilized with respect to **1a** (+123 kJ mol^{−1}); that is, a metalloid cluster is energetically favored over a polyhedral cluster.^[14] As already discussed for other metalloid clusters (Al₇,^[5] Al₁₂,^[6] Al₇₇,^[4] Ga₂₂^[15]), the experimental and theoretical findings presented here confirm that Wade’s rules are not appropriate for describing bonding situations of this type.

To obtain a deeper understanding of the exceptional bonding situation in **2** and **1**, we calculated the ²⁷Al NMR shifts for the central (**2a**, **1a**) and apical Al atoms (**1b**).^[16] The

results for **2a** ($\delta = 652$), **1a** ($\delta = 358$), and **1b** ($\delta = -313$) show—as already discussed for **2**^[5]—that for **2a** and **1a**, but not for polyhedral **1b**, the central Al atoms approach the bonding situation in aluminum metal ($\delta = 1640$).^[17]

The geometry of the partially preformed metal structure present in metalloid clusters is rationalized for **1** in Figure 3. Closest packing as in the metal structure results from rotation by 30° and subsequent translation.

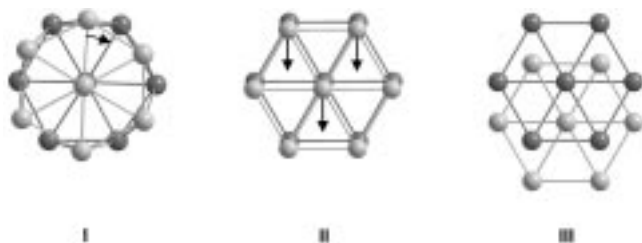


Figure 3. Rotation of the upper ring by 30° about the z axis—which incorporates the two central Al atoms of the two six-membered rings in the Al_{14} cluster (**I**)—leads to AA stacking, as found in hexagonal-primitive packing (**II**). If the upper ring is shifted in the layer of the page by $(\frac{1}{4}, \frac{1}{4}, 0)$ —based on the basis vectors of the aluminum metal lattice—one obtains AB stacking (**III**), which represents one section of the closest packing of aluminum metal.

The results presented here have again demonstrated the large synthetic potential of AlX/AIR solutions for the experimentally challenging preparation of metalloid clusters in a well defined form. These clusters should probably play a key role in understanding the mechanism of metal formation.

Experimental Section

Gaseous AlI₃ (40 mmol) was condensed at -196°C with toluene (64 mL) and diethyl ether (16 mL) according to the method described in ref. [1]. An aliquot (6 mL) of the ca. 0.30 M dark red-brown $\text{AlI}_3 \cdot \text{Et}_2\text{O}$ solution was concentrated at -78°C under vacuum and dried. The residue was dissolved in toluene (12 mL), and the solution obtained was again concentrated to 6 mL and added to donor-free $\text{LiN}(\text{SiMe}_3)_2$ (150 mg). The reaction mixture was allowed to warm to -25°C within 2 d. After a further 2 d at 7°C , the amide had dissolved and LiI precipitated, which was subsequently redissolved in Et_2O (1 mL). The solution was warmed for 1.5 h at 55°C and then kept at room temperature for 1 d. This temperature cycle was repeated several times, and then the warm solution was allowed to cool slowly within 3 d to room temperature. During this process disproportionation of Al^I species led to the formation of aluminum metal. The filtered solution was again warmed to 55°C and allowed to cool slowly. After 3 d at 7°C , 1 h at -80°C , and several weeks at 7°C , **1** crystallized as dark red-brown platelets that are chemically and mechanically very labile (yield: ca. 2%).

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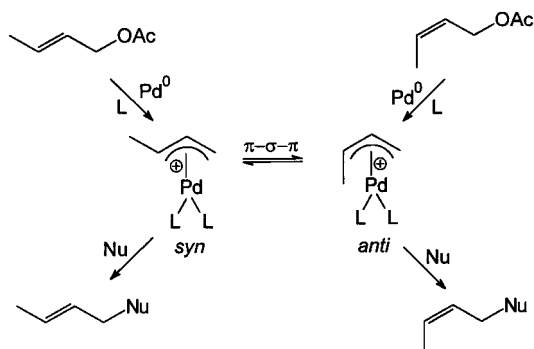
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- [8] Crystal structure analysis of $[\text{Al}_{14}[\text{N}(\text{SiMe}_3)_2]_6\text{Li}(\text{OEt}_2)_2]^- [\text{Li}(\text{OEt}_2)_4]^+ \cdot \text{Tol}$ (**1**): STOE-IPDS “Image Plate” diffractometer, 55 kV, 50 mA, $\text{MoK}\alpha$ radiation ($\lambda = 0.71073$), measurement temperature 200(2) K. Crystal dimensions $0.8 \times 0.1 \times 0.1$ mm, monoclinic I, space group $P2_1/n$, $a = 14.5638(5)$, $b = 37.546(5)$, $c = 23.266(4)$ Å, $\beta = 94.775(7)^\circ$, $V = 12678(1)$ Å³, $Z = 4$; $\rho_{\text{calc}} = 1.390$ Mg m⁻³, $\mu_{\text{Mo}} = 1.720$ mm⁻¹, $\theta_{\text{min}} = 1.93^\circ$, $\theta_{\text{max}} = 25.99^\circ$; of 78033 reflections measured, 24071 were independent, $16967 > 2\sigma(I)$, $5127 > 3\sigma(I)$; 788 parameters, empirical absorption correction, $R_1 = 0.0623$, $wR_2 = 0.1595$; max./min. residu electron density 1.805/−0.968 e Å⁻³. The structure was solved by direct methods and refined against F^2 for all observed reflections. Software programs used: Shelxs-86, Shelxl-93 (G. M. Sheldrick, Universität Göttingen). 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-136893. 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 asymmetric unit contains an $[\text{Al}_{14}[\text{N}(\text{SiMe}_3)_2]_6\text{Li}]^{2-}$ ion which is coordinated to a $[\text{Li}(\text{OEt}_2)_2]^+$ ion through two of the I ligands. Several of the methyl groups are disordered over two positions. Furthermore, a Li⁺ ion coordinated to four Et₂O molecules was found along with a toluene molecule whose atoms could be refined with common isotropic temperature factors. All hydrogen atoms were placed in calculated positions and refined according to a riding model.
- [9] As a supplement to the structure analysis, the presence of an even number of electrons could be confirmed by the lack of an ESR signal. The ESR measurement on crystalline **1** was carried out by Dr. B. Pilawa, Physikalisches Institut, Universität Karlsruhe.
- [10] For purposes of comparison the Al–Al distances [Å] of selected substances are listed: $[\text{Al}_7[\text{N}(\text{SiMe}_3)_2]_6]^-$ 2.540 (in the $[\text{Al}_3[\text{N}(\text{SiMe}_3)_2]_3$ ring; cf. Figure 2) and 2.737 (Al–Al contacts to the central Al atom);^[5] $[\text{Al}_{12}[\text{N}(\text{SiMe}_3)_2]_8]^{2-}$ 2.542–2.759;^[6] $\text{Al}_{12}(\text{AlBr}_2 \cdot \text{THF})_{10} \cdot 2\text{THF}$ 2.526–2.762;^[3] Al_{metal} 2.860.
- [11] All quantum-chemical calculations were carried out with the RIDFT module (B-P86 functional) of the program TURBOMOLE with SV(P) basis sets. a) TURBOMOLE: O. Treutler, R. Ahlrichs, *J. Chem. Phys.* **1995**, *102*, 346; b) B-P86 functional: A. D. Becke, *Phys. Rev. A* **1998**, *38*, 3098; J. P. Perdew, *Phys. Rev. B* **1996**, *33*, 8822; c) RIDFT: K. Eichkorn, O. Treutler, H. Öhm, M. Häser, R. Ahlrichs, *Chem. Phys. Lett.* **1995**, *242*, 652; K. Eichkorn, F. Weigend, O. Treutler, R. Ahlrichs, *Theor. Chem. Acc.* **1997**, *97*, 119.
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- [13] An analysis of the MOs of **1b** (however, for the simplified $\text{Al}_{14}\text{H}_{12}^{2-}$ species) indicated that the 3s electrons of the apical Al atoms are not involved to an appreciable extent in the bonding of the Al_{14} framework; that is, with 28 framework electrons ($12 \times 2 + 2 \times 1 + 2$) a *precloso* structure would be expected.
- [14] The MO analysis for **1a** showed that this stabilization with respect to **1b** results from bonding interactions of the 3s electrons of the central and apical Al atoms with the Al_{14} framework.
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- [16] The calculated ²⁷Al NMR shifts are normalized to the shift of Al_4H_4^- . The following ²⁷Al NMR shifts were calculated for the Al atoms that carry ligands: $\delta = 205$ (**2a**), 61 (**1a**), 205 (**1b**). The calculations were carried out with the MPSHIFT module of the program TURBOMOLE with DFT (B-P86 functional). The implementation with use of the efficient TURBOMOLE routines was based on the GIAO method (gauge invariant atomic orbitals). a) GIAO with DFT: G. Schreckenbach, T. Ziegler, *J. Phys. Chem.* **1995**, *99*, 606; GIAO with SCF: M. Häser, R. Ahlrichs, H. P. Baron, P. Weiss, H. Horn, *Theor. Chim. Acta* **1992**, *83*, 455; b) TURBOMOLE: ref. [10].
- [17] An ²⁷Al NMR shift was also measured in this range ($\delta = 1650$) for nanosized Al particles (ca. 3 nm): S. Faber, Dissertation, Saarbrücken, **1996**.

Palladium-Catalyzed Allylic Alkylations without Isomerization—Dream or Reality?*

Uli Kazmaier* and Franz L. Zumpe

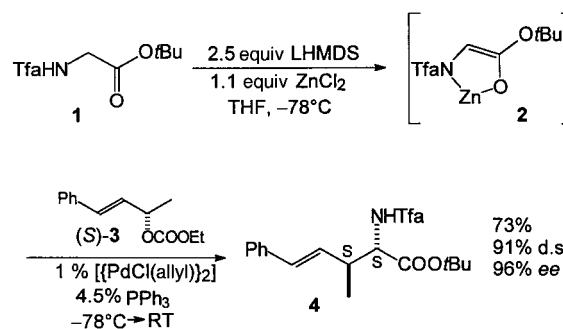
The palladium-catalyzed allylic alkylation has developed into one of the standard reactions in organic synthesis.^[1] The reaction proceeds via π -allyl–palladium complexes—the configuration of the products obtained strongly depends on the configuration of these intermediates.^[2] Thus, the oxidative addition of palladium(0) to (*E*)-allyl acetates and carbonates leads to *syn*-configured allyl complexes (Scheme 1), which



Scheme 1. Isomerization of π -allyl–palladium complexes.

react with nucleophiles (Nu) to provide the corresponding *E*-substitution products. The corresponding *anti* complexes are generated by the attack of palladium on *Z* substrates. Subsequent reaction with nucleophiles should result in *Z*-configured products, provided that no π - σ - π isomerization occurs.^[3] This isomerization causes a fast interconversion of the π -allyl complexes, normally with preference for the *syn* complex. Exceptions can only be observed if steric interactions either between the substituents in the allylic substrate^[4] or between the allyl moiety and the ligands on palladium^[5] destabilize the *syn* complex. As a result, the *anti* complex is enriched, and reactions with nucleophiles give rise to an increased amount of *Z*-substitution product. However, selective conversion of (*Z*)-allyl substrates with retention of the olefin geometry still remains an unsolved problem.^[6] Suppression of the π - σ - π isomerization is therefore an essential requirement for solving this problem. By using very reactive nucleophiles we were able to suppress the π - σ - π isomerization for the first time.

In the course of our investigations concerning the synthesis of unsaturated amino acids,^[7] we found chelated ester enolates of amino acids (**2**) to be efficient nucleophiles in palladium-catalyzed allylic substitutions (Scheme 2).^[8] Be-



Scheme 2. Allylation of chelated amino acid ester enolates with (*E*)-allyl carbonate **3**. Tfa = trifluoroacetyl; LHMDS = lithium bis(trimethylsilyl)-amide.

sides the generally used soft nucleophiles, such as malonates, only a few examples on the use of nonstabilized enolates, like those of ketones^[9] or esters,^[10] have been described so far. Therefore, these chelated enolates considerably enlarge the spectrum of potential nucleophiles. A further advantage is that conversions of these enolates (**2**) directly lead to the formation of unsaturated amino acid derivatives (**4**),^[11] which can be further modified at the double bond in the side chain.^[12] Based on the high reactivity of **2**, the conversions already occur under very mild reaction conditions at -78°C , and therefore very good selectivities are normally obtained. Thus, treatment of the amino acid ester **1** with the chiral allylic carbonate (*S*)-**3** (97% *ee*) provides the corresponding amino acid (2*S*,3*S*)-**4** as a single regioisomer. The reaction proceeds in a highly diastereoselective fashion and with perfect chirality transfer. The analogous allyl acetates can be used as well, but the results obtained are in general a little worse.

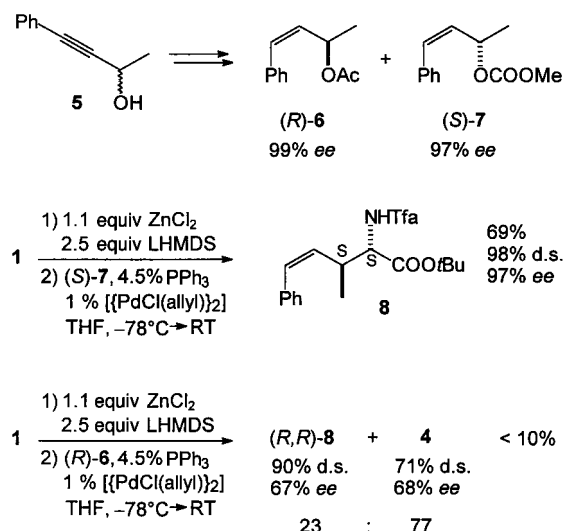
Especially the very mild reaction conditions prompted us to investigate the reaction of **2** with *Z*-configured allylic substrates. Owing to the rather low reaction temperature, we hoped to remain under the energy barrier of the π - σ - π isomerization. The (*Z*)-allyl substrates **6** and **7**, which are analogous to **3**, were obtained from racemic propargylic alcohol **5** (Scheme 3): Compound **5** was subjected to a kinetic enzymatic resolution, which provided the (*R*)-acetate and the (*S*)-alcohol, each with high enantiomeric excess. The chiral alcohol was converted into the carbonate, and subsequent Lindlar hydrogenation of the two acetylenic derivatives provided the substrates (*R*)-**6** and (*S*)-**7**.

Very interesting results were obtained for the reaction of these substrates with **1** (Scheme 3). The reaction with (*S*)-**7** almost exclusively yielded the desired *Z*-substitution product (2*S*,3*S*)-**8** (less than 1% of the *E*-substitution product). The excellent selectivities (98% d.s., 97% *ee*) even surpassed the very good results obtained with (*E*)-carbonate **3**. Also in this case, the *anti*-configured product was formed preferentially. In contrast, the reaction with (*R*)-**6** furnished a *E/Z* mixture in very low yield. The selectivities were markedly worse than those obtained with the carbonate. The major isomer was identical to the product **4** resulting from the reaction with (*S*)-**3**.

This difference in product formation can be explained by a higher reactivity of the allylic carbonate **7**. This substrate already reacts at -78°C , and apparently at this temperature

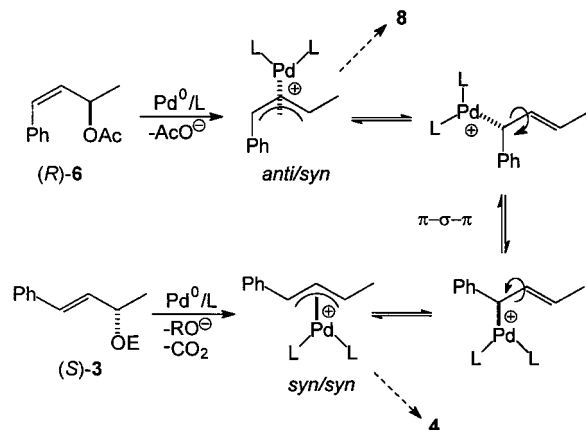
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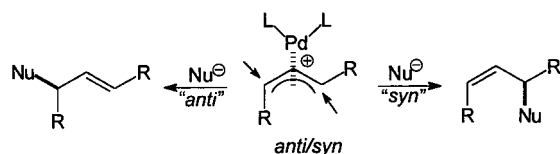


Scheme 3. Reactions of (Z)-allylic substrates (R)-6 and (S)-7.

the π - σ - π isomerization does not occur. In contrast, the reaction of the acetate **6** requires a higher temperature. In this case, the reaction probably starts in the same temperature range as the isomerization, and partial conversion from the primarily formed *anti*/*syn* complex into the more stable *syn*/*syn* complex can be observed (Scheme 4). The same complex is also formed from the (S)-carbonate **3**.^[13]

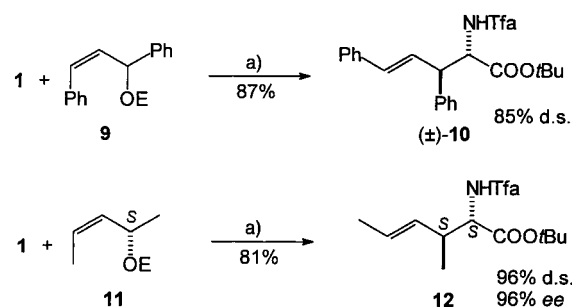

 Scheme 4. Mechanism of the π - σ - π isomerization.

In the examples investigated so far, the regioselectivity of the nucleophilic attack has never been a problem, since the product with the conjugated double bond was formed exclusively. After we had succeeded in suppressing the isomerization by using allyl carbonate (S)-7, we turned to further interesting questions: What about (Z)-allylic carbonates with the same substituents at the allylic termini? Which position is more reactive, the “*anti*” or the “*syn*” position (Scheme 5)?


 Scheme 5. Reaction pathways of *anti*/*syn* complexes.

The clarification of these questions is of major interest, because symmetrically substituted allyl substrates are normally used in asymmetric catalyzed reactions. From these substrates achiral π -allyl–palladium complexes are formed, independent of the configuration of the substrate, and nucleophilic attack on these complexes can be controlled, for example, by chiral ligands.^[14] If enantiomerically pure allylic substrates are used, the chiral information gets lost during the reaction. However, if it is possible to suppress the π - σ - π isomerization during the reaction of (Z)-configured substrates, and if one of the two allylic positions is clearly more reactive, it should be possible to generate optically active compounds with these substrates as well.

We started our investigations of regioselectivity with the diphenyl derivative **9** (Scheme 6). Reaction of **9** under standard conditions surprisingly provided the *E*-configured *anti* product **10** exclusively in high yield. No *Z* isomers could


 Scheme 6. Stereoselective reaction of (Z)-allyl carbonate **9**. a) See Scheme 3 and the Experimental Section for reaction conditions. E = ester group.

be detected. This indicates that the *anti* position is decisively more reactive,^[5] and that attack at the *syn* position is negligible. The diastereoselectivity was significantly higher than for the reaction of the corresponding (*E*)-allyl carbonate (65% d.s.); the same product was formed in both cases. Clearly no appreciable π - σ - π isomerization is involved in the process. To exclude their participation completely, we investigated the reaction of the chiral carbonate (S)-**11** (99% ee), which was obtained by the method used to synthesize **6** and **7**,^[15] with **1** (Scheme 6). Also in this case, the *E*-substituted product **12** was obtained exclusively in high yield. The diastereoselectivity was excellent, and the nearly complete transfer of chirality verifies the reaction pathway via the *anti*/*syn* complex and not the *syn*/*syn* complex, which would inevitably lead to racemization. Again, the π - σ - π isomerization could be suppressed almost completely.

Experimental Section

The protected amino acid ester **1** (1 mmol) was dissolved in THF (4 mL). At -78°C a freshly prepared solution of LHMDS (2.5 mmol) in THF (2 mL) was added. After 30 min a solution of ZnCl_2 (1.1 mmol) in THF (2 mL) was added with stirring. Then 30 min later a solution of $[\text{PdCl}(\text{allyl})_2]$ (1 mol %), PPh_3 (4.5 mol %), and the corresponding allyl ester (1.5 mmol) in THF (2 mL) was added. The solution was stirred overnight and allowed to warm to room temperature. Subsequently, the solution was diluted with diethyl ether and hydrolyzed with 1N KHSO_4 solution. The aqueous phase was extracted twice with diethyl ether, and the combined organic layers were dried over anhydrous Na_2SO_4 . After evaporation of the

solvent in vacuo the crude product was purified by flash chromatography (hexanes/ethyl acetate).

8: M.p. 78–79 °C (hexanes/diethyl ether); $[\alpha]_D^{25} = +3.5$ ($c = 2.6$, CHCl_3); ^1H NMR (300 MHz, CDCl_3): $\delta = 1.21$ (s, 9H), 1.66 (d, $J = 4.7$ Hz, 3H), 3.59 (m, 1H), 4.74 (dd, $J = 8.6$, 8.6 Hz, 1H), 5.60–5.69 (m, 2H), 6.88 (d, $J = 8.6$ Hz, 1H), 7.19–7.34 (m, 5H); ^{13}C NMR (75 MHz, CDCl_3): $\delta = 17.54$, 27.36, 52.86, 56.75, 82.84, 115.52, 126.05, 127.18, 127.49, 128.20, 129.49, 138.83, 156.37, 168.80; elemental analysis calcd for $\text{C}_{18}\text{H}_{22}\text{F}_3\text{NO}_3$: C 60.50, H 6.20, N 3.92; found: C 60.42, H 6.44, N 3.99.

12: Colorless oil; $[\alpha]_D^{25} = +21.7$ ($c = 1.0$, CHCl_3); ^1H NMR (300 MHz, CDCl_3): $\delta = 1.03$ (d, $J = 6.9$ Hz, 3H), 1.45 (s, 9H), 1.65 (d, $J = 6.3$ Hz, 3H), 2.75 (m, 1H), 4.42 (dd, $J = 8.7$, 4.7 Hz, 1H), 5.24 (dd, $J = 15.3$, 7.7 Hz, 1H), 5.52 (dq, $J = 15.3$, 6.3, 1.0 Hz, 1H), 6.65 (brs, 1H); ^{13}C NMR (75 MHz, CDCl_3): $\delta = 16.36$, 17.63, 27.75, 39.37, 56.96, 82.83, 115.56 (q, $J = 288$ Hz), 128.03, 129.30, 156.74 (q, $J = 37.2$ Hz), 168.83; elemental analysis calcd for $\text{C}_{13}\text{H}_{20}\text{F}_3\text{NO}_3$: C 52.88, H 6.83, N 4.74; found: C 52.85, H 6.66, N 4.71.

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A Stable, Conspicuously Active, Water-Soluble Pd Catalyst for the Alternating Copolymerization of Ethene and CO in Water**

Göran Verspui, Frank Schanssema, and Roger A. Sheldon*

Alternating copolymers of olefins and CO are relatively new, low-cost materials with interesting physical properties.^[1] The discovery of active cationic palladium catalysts containing flexible *cis*-chelating diphosphanes has made the preparation of these copolymers commercially feasible.^[2] Especially catalyst systems prepared in situ from a Pd precursor, a sterically demanding diphosphane such as 1,3-bis(di(2-methoxyphenyl)phosphino)propane (D-*o*-APPr), and an excess of a suitable Brønsted acid exhibit excellent catalyst performance in the ethene/CO copolymerization in organic solvents such as methanol.^[3]

The current, increasing interest in sustainable technologies has intensified the research activity in organometallic catalysis in aqueous media over the past few years. Water is an environmentally friendly, economically attractive, and safe reaction medium for a variety of reactions catalyzed by homogeneous transition metal catalysts.^[4]

We and others previously reported that the alternating copolymerization of ethene and CO can be performed efficiently in water by employing Pd complexes of water-soluble diphosphanes.^[5] At 90 °C and 60 bar the aqueous Pd/DPPPr-S/HOTs (DPPPr-S = 1,3- $\text{C}_3\text{H}_6(\text{P}(\text{C}_6\text{H}_4\text{-}m\text{-SO}_3\text{Na})_2)_2$, HOTs = *p*-toluenesulfonic acid) system exhibits catalyst ac-

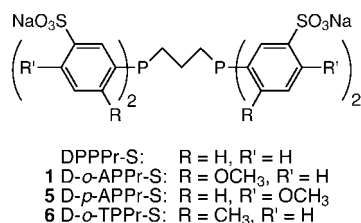
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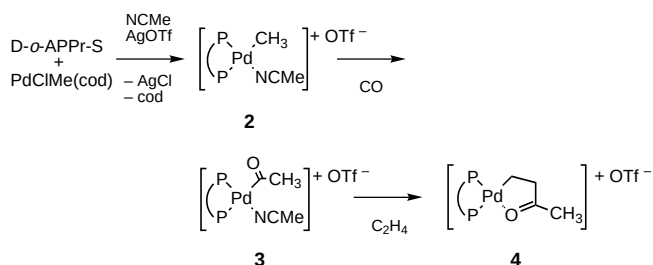
Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

tivities up to 7.7 kg copolymer per g Pd per hour.^[6] Despite the excellent catalyst performance, the catalyst precursor is rather unstable and decomposition of the catalyst during the copolymerization is frequently observed.

To develop a more stable catalyst system, we turned to the synthesis of a water-soluble analogue of *D-o*-APPr. Initial attempts to introduce sodium sulfonate groups to the aryl rings under conventional sulfonation conditions (30 % oleum) were unsuccessful due to the decomposition of the ligand. We developed a much milder sulfonation procedure which afforded the tetra-sulfonated diphosphane 1,3-C₆H₄[P(C₆H₃-2-OMe-5-SO₃Na)₂]₂ (*D-o*-APPr-S) **1** in high purity and moderate yield (see supporting information).



Stirring **1** with [PdCl(CH₃)(cod)] (cod = cyclooctadiene) in methanol and abstraction of the chloro ligand with AgOTf and NCMe afforded [Pd(CH₃)(*D-o*-APPr-S)(NCMe)]OTf (**2**), which was isolated as an air-stable white solid. At lower temperatures, this compound reacts readily with CO and ethene to afford the intermediates **3** and **4** (Scheme 1); the ¹H and ³¹P NMR spectra are consistent with those of related



Scheme 1. The synthesis of **2–4**.

complexes in organic media.^[7] In contrast with **2**, **3** is unstable in solution and decomposes to Pd-black. Compound **4** is more stable in aqueous solution but at room temperature it undergoes a slow hydrolysis reaction to afford 2-butanone. NMR experiments with ¹³CO revealed that the oxygen atom of the carbonyl group in **4** is coordinated to the palladium center, resulting in a five-membered chelate ring.

Compound **2** was tested in the aqueous alternating copolymerization of ethene and CO, and indeed, the reaction proceeded smoothly in neat water. At 90 °C and 60 bar of an ethene/CO mixture (1:1) an activity of 32.2 kg copolymer per gram Pd per hour was reached (Table 1)—four times faster than the previously reported Pd/DPPPr-S/HOTs system. This corresponds to an average turnover frequency (TOF = mol ethene per mol Pd per hour) of >61000 h⁻¹, the highest activity reported for a water-soluble Pd catalyst so far. When

Table 1. Alternating copolymerization of ethene and CO in water catalyzed by Pd/*D-o*-APPr-S.

Run ^[a]	Catalyst	Additive [mmol]	Activity	M_n [kg g Pd ⁻¹ h ⁻¹]	M_n/M_w [kg mol ⁻¹]
1 ^[b]	2	–	32.2	69.2	1.9
2	Pd/ <i>D-o</i> -APPr-S	–	24.4	64.4	2.1
3	Pd/ <i>D-o</i> -APPr-S	HOTs (1.0)	18.6	59.7	2.0
4	Pd/ <i>D-o</i> -APPr-S	NaOH (3.2)	0.0	–	–
5 ^[c]	Pd/ <i>D-o</i> -APPr-S	<i>D-o</i> -APPr-S	24.4	n.d. ^[d]	n.d. ^[d]

[a] Reaction conditions: [Pd] = 17.6 × 10⁻⁶ mol L⁻¹, 142 mL water, *D-o*-APPr-S/Pd = 1.0, 90 °C, 60 bar ethene/CO (1/1), 1000 rpm, reaction time: 60 min. [b] 0.00125 mmol of **2**; [Pd] = 8.8 × 10⁻⁶ mol L⁻¹. [c] *D-o*-APPr-S/Pd = 2.0, reaction time: 65 min. [d] n.d. = not determined.

the catalyst was prepared in situ by adding an aqueous solution of **1** to [Pd(OTs)₂(NCMe)₂] somewhat lower catalyst activities (25.0 kg g Pd⁻¹ h⁻¹) were observed.

In contrast with Pd/DPPPr-S, the Pd/*D-o*-APPr-S catalyst is air stable in solution; only a minor decrease in catalyst activity was observed (from 16.0 to 15.6 kg g Pd⁻¹ h⁻¹, at 70 °C and 40 bar) when an aqueous solution of **2** was stirred in air for 16 h. The addition of a Brønsted acid cocatalyst is not required for a higher catalyst stability and activity. In fact, the addition of some HOTs led to a lower copolymer production. When a small amount of NaOH was added no reaction was observed, analogous to our previously studied Pd/DPPPr-S/HOTs system. We suggest that in the case of in situ prepared catalysts, the copolymerization starts with the formation of a palladium–hydrido complex,^[8] which is presumably not formed in basic media.

Quantitative ¹³C NMR spectroscopy confirmed the perfectly alternating ethene/CO distribution in the copolymer, and the absence of end group signals indicated a very high molecular weight. Size-exclusion chromatography confirmed the high average molecular weight; M_n = 124.9 kg mol⁻¹ for the copolymer sample obtained with Pd/*D-o*-APPr-S/HOTs at 70 °C and 40 bar, a weight six times higher than that of the copolymer obtained with Pd/DPPPr-S/HOTs under the same reaction conditions (Table 2). Even higher molecular weights were obtained when the Brønsted acid cocatalyst was omitted.

We also synthesized the *para*-methoxy isomer of **1**, *D-p*-APPr-S (**5**), and *D-o*-TPPr-S (**6**) containing *ortho*-CH₃ groups and tested them in the aqueous alternating copolymerization. Compared to the Pd/DPPPr-S/HOTs system the catalysts prepared in situ from **5** or **6** are considerably less active. The sharp contrast between ligands **1** and **5** suggests that the rate-

Table 2. The alternating copolymerization of ethene and CO with various water-soluble catalysts.

Run ^[a]	Catalyst	Additive [mmol]	Activity	M_n [kg g Pd ⁻¹ h ⁻¹]	M_n/M_w [kg mol ⁻¹]
1	Pd/DPPPr-S	HOTs (1.0)	2.61	21.8	2.0
2 ^[b]	Pd/ <i>D-o</i> -APPr-S	HOTs (1.0)	8.22	124.9	1.9
3	Pd/ <i>D-p</i> -APPr-S	HOTs (1.0)	1.67	55.9	2.0
4	Pd/ <i>D-o</i> -TPPr-S	HOTs (1.0)	1.71	3.9	1.4
5 ^[c]	Pd/ <i>T-o</i> -APTS	HOTs (1.0)	0.00	–	–

[a] Reaction conditions: [Pd] = 70.4 × 10⁻⁶ mol L⁻¹, 142 mL water, ligand/Pd = 1.0, 70 °C, 40 bar ethene/CO (1/1), 1000 rpm, reaction time: 120 min. [b] [Pd] = 14.1 × 10⁻⁶ mol L⁻¹, reaction time: 60 min. [c] Catalyst decomposition, *T-o*-APTS = P(C₆H₃-2-OCH₃-5-SO₃Na)₃.

enhancing effect of the *ortho*-methoxy substituents is steric in nature. This is confirmed by the fact that no decrease in catalyst activity was observed when an excess of **1** was used, in contrast with the Pd/DPPPr-S/HOTs system, where the reaction is almost completely inhibited when two equivalents of ligand are added.^[5b] Apparently, catalytically inactive [Pd^{II}(D-*o*-APPr-S)₂] complexes cannot be formed with **1**.

The presence of the *ortho*-methyl groups in **6** did not induce a similar rate-enhancing effect, which suggests that the effect of the *ortho*-methoxy substituents in **1** is not only steric. The oxygen atoms might coordinate to the palladium atom at some stages during the reaction to stabilize energetically less favorable configurations.^[9] Nevertheless, a permanent coordinative bond with the oxygen atom was not observed in the NMR spectra of **2–4**. When the monodentate P(C₆H₃-2-OCH₃-5-SO₃Na)₃ was used as ligand, catalyst decomposition took place, showing that the copolymerization does not proceed in the presence of a P ~ O chelate alone, but requires a P ~ P ligand.

Although the alternating copolymerization of ethene and CO is initially homogeneous, in a later stage the growing polymer precipitates and the reaction might proceed heterogeneously. Indeed, when we recovered the Pd/D-*o*-APPr-S catalyst by filtering off the product, only traces of copolymer were formed in a second run. This indicates that the catalyst was still attached to the polymer chain, which is precipitated on the rest of the product. Nonetheless, by introducing a “termination procedure” (see supporting information) 61 % of the catalyst activity could be restored.^[10]

The Pd/D-*o*-APPr-S catalyst was also active in a supported aqueous phase (SAP) system, in which the copolymer itself is used as a support for an aqueous catalyst solution, which is distributed as a thin film over the surface. Such a “gas-phase process” minimizes the amount of solvent needed in the process and avoids fouling of the reaction vessel. In this SAP system, under nonoptimized conditions (90 °C, 60 bar), catalytic activities of 21.3 kggPd⁻¹h⁻¹ were reached.

In conclusion, we have developed a simple, highly active, air-stable, and environmentally friendly catalyst for the alternating copolymerization of ethene and CO.

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Generation-Dependent Intramolecular CT Complexation in a Dendrimer Electron Sponge Consisting of a Viologen Skeleton

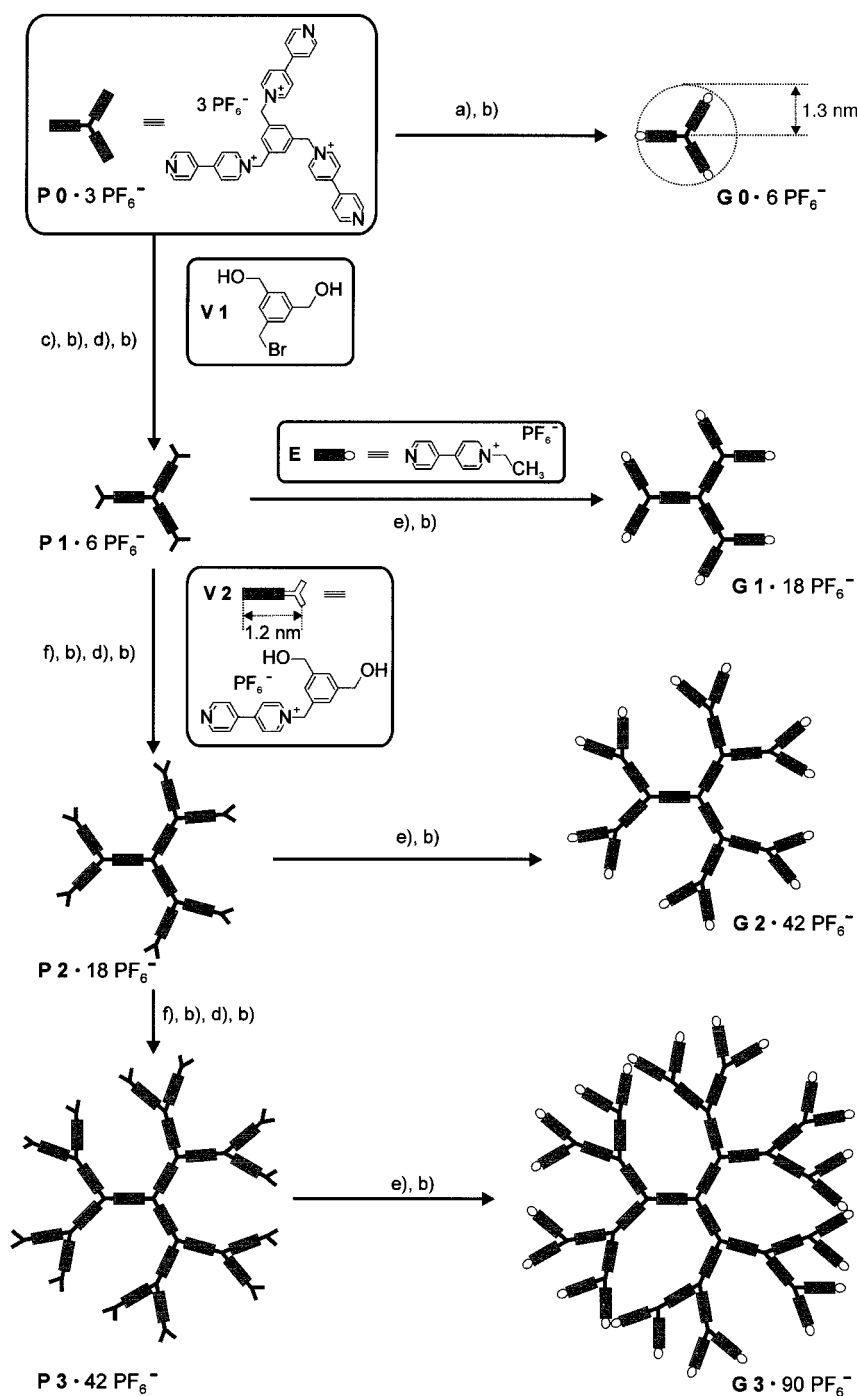
Susanne Heinen and Lorenz Walder*

In the last two decades efficient methodologies for the cascade synthesis of dendrimers have been developed. Nowadays the main research activities are focused on dendritic effects resulting from the topology and the interaction of built-in molecular functionalities.^[1] Redox-active subunits play an important role in this respect. They may be located: a) in the core,^[2, 3] b) in the periphery,^[4–7] or c) in the core, the branches, and the periphery of the dendrimer.^[8–11] The resulting dendritic effects concern redox protein mimetics,^[3] redox gradients,^[11] charge transfer (CT) complex-based conductivity,^[7] electron sponges,^[4] and redox sensors.^[12]

We present here a new homologous series of electroactive dendrimers belonging to class (c). The dendrimers consist of a viologen skeleton that contains up to 45 electrochemically accessible 4,4'-bipyridinium subunits which cause electron sponge properties. Electrochemical measurements revealed an excellent correlation between the hydrodynamic radius and the generation number of the dendrimers. Furthermore, an electrochemically switchable and generation-dependent CT complex was observed for the first time in the cation radical oxidation state.

The synthesis of the dendrimers of generations zero to three follows the “divergent method with preformed branching units” (Scheme 1).^[13] The well-known trifunctional initiator core **P0**·3PF₆⁻ consists of a mesityl derivative linked to three 4,4'-bipyridinium units.^[14] The three peripheral nitrogens react quantitatively with primary benzyl or alkyl bromides or iodides. 1,3-Di-(hydroxymethyl)-benzyl bromide (**V1**)

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Scheme 1. Synthesis of the dendritic polycations **G0** to **G3**; a) EtI/MeCN; b) $\text{NH}_4\text{PF}_6/\text{H}_2\text{O}$; c) **V1**/MeCN; d) HBr/AcOH; e) **E**/MeCN; f) **V2**/MeCN.

plays the role of the “preformed branching unit”. The resulting hexol was activated by conversion into the corresponding hexabromide **P1** · 6 PF_6^- . The direct reaction of **P0** · 3 PF_6^- with 1,3,5-tris(bromomethyl) benzene failed because of inter- and intramolecular crosslinking. The branching unit **V2**, equipped with a reactive pyridine nitrogen and two benzylic alcohols, was used for the preparation of the precursors **P2** · 18 PF_6^- and **P3** · 42 PF_6^- . The higher dendrimer precursors were grown from **P1** · 3 PF_6^- by repetitive application of the reaction steps (f), (b), and (d). In order to solubilize the intermediates it was necessary to exchange the counter ion

after each reaction step (b). The dendrimer **G0** · 6 PF_6^- was available from its precursor via alkylation with ethyl iodide followed by ion exchange, whereas the dendrimers **G1** to **G3** were obtained from the reactions of *N*-ethyl-4,4'-bipyridinium hexafluorophosphate (**E**) with the corresponding precursors in good yields as hexafluorophosphate salts. The products were pure by NMR spectroscopy and by elemental analysis, apart from a variable water content (see Experimental Section). MALDI-TOF mass spectrometry experiments were not successful, probably because of the high charge present in these compounds.^[15]

The dendrimers **G0** to **G3** exhibit two chemically reversible waves in cyclic voltammetry (DMF/0.1M TBAPF₆, TBA = *t*BuN⁺), similar to those of monomeric dibenzyl viologen, with peak separations in the range of 70–80 mV at scan rates up to 100 mV s⁻¹. The shape of the second wave indicates adsorption of the neutral compound on the electrode surface. The redox centers within the dendrimers do not exhibit strong intramolecular interactions, except for a weak coulombic destabilization of the dicationic oxidation state, resulting in a trend towards more positive E_1^0 values with increasing generations (*n*) (Table 1). The number of exchanged electrons per molecule (n_{exp}), as measured by preparative electrolysis coulometry, agrees with the calculated value (n_{theor}) to within 95 % (Table 1). The diffusion limited currents (i_l) for the reduction of dendrimers **G0** to **G3** were obtained with a rotating disk electrode; they fulfil the Levich equation ($i_l \sim \omega^{1/2}$, ω = angular velocity) and were used to calculate the diffusion coefficient D_{exp} (Table 1 and Figure 1 a).^[16] From the diffusion coefficients the hydrodynamic radii r_h (Table 1) were calculated with the Stokes equation, with the assumption of hard spheres. However, according to MM⁺ calculations^[17] even **G3** is ellipsoidal rather than spherical ($r_{1\text{calcd}}$ and $r_{2\text{calcd}}$, Table 1). The experimental radii

(r_h) lie between the large and small calculated radii of the ellipsoid. In Figure 1b the experimental radii are plotted against the generation number. A linear dependence with a slope of 0.86 nm generation⁻¹, a y-intercept of 1.16 nm, and an excellent correlation factor R^2 of 0.9968 is observed. The value from the slope corresponds to 72 % of the theoretical length of the mesityl bipyridinium inserted per generation (ca. 1.2 nm).^[17]

A novel, dendrimer generation-dependent and electrochemically switchable CT complexation was observed. All dendrimers show reversible behavior under spectroelectro-

Table 1. Physical Chemical Data and Simulation Results

	G0	G1	G2	G3
M [g mol ⁻¹]	1542.7	4658.1	10888.9	23350.4
E_1^0 [mV] ^[a]	-310	-295	-275	-252
n_{exp} ^[b]	2.9	9.1	20.4	43.4
n_{theor} ^[b]	3	9	21	45
D_{exp} [cm ² s ⁻¹] ^[c]	2.05×10^{-6}	1.23×10^{-6}	8.8×10^{-7}	6.5×10^{-7}
r_h [nm] ^[d]	1.2 ± 0.1	2 ± 0.2	2.8 ± 0.2	3.8 ± 0.2
$r_{1\text{calcd}}, r_{2\text{calcd}}$ [nm] ^[e]	1.3, 0.4	2.6, 1.8	3.7, 2.6	4.6, 3.7
P_{tot} [%] ^[f]	7.4	23.6	25.1	29.3
P_{per} [%] ^[g]	(11.2)	36	44	55

[a] First reduction potential determined by spectroelectrochemistry (DMF/0.1M TBAPF₆, against Ag/AgCl). [b] Number of electrons transferred per molecule theoretically (n_{theor}) and as determined by electrolysis (n_{exp}). [c] Diffusion coefficient from the diffusion limited current with the rotating disk electrode according to the Levich equation.^[16] [d] Hydrodynamic radius from D_{exp} according to the Stokes equation. [e] Large and small radii of the ellipsoid from MM⁺ simulations.^[17] [f] Degree of pimerization (from analysis of the visible spectra at 537 and 605 nm) with respect to all viologen centers. [g] Degree of pimerization (from analysis of the visible spectra at 537 and 605 nm) with respect to the viologen centers in the outermost dendrimer shell.

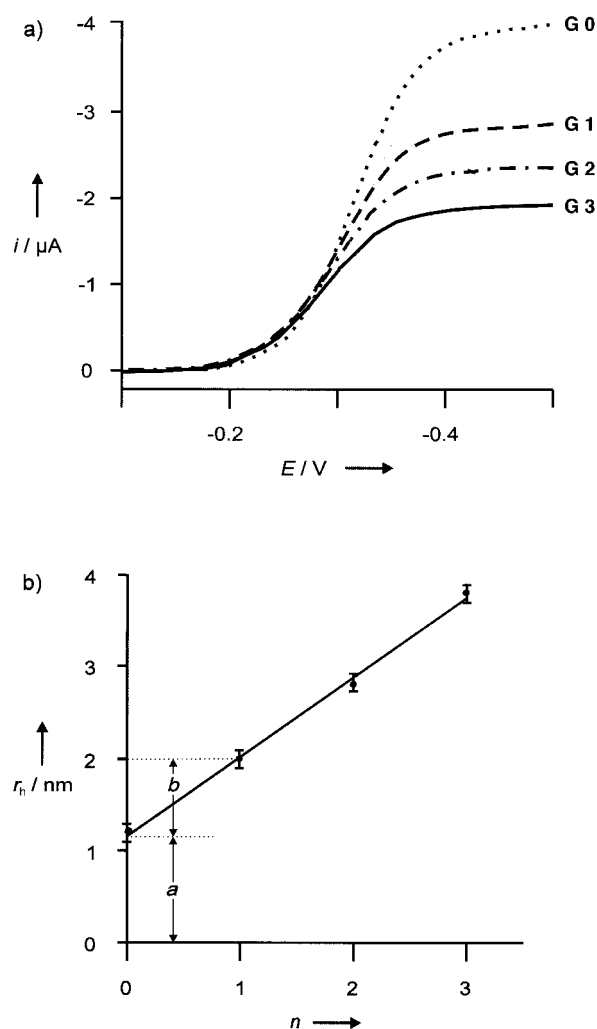


Figure 1. a) Current–potential curves for the reduction of **G0** to **G3** with the rotating disk electrode at constant viologen subunit concentration ($n_{\text{theor}}c(\text{dendrimer})$). From the plateau currents the diffusion coefficients were calculated. b) Hydrodynamic radii r_h [nm] as a function of the generation number n ; y-intercept $a = 1.16$ nm, slope $b = 0.86$ nm generation⁻¹.

chemical conditions (preparative electrolysis in combination with a flow-through cuvette); they show complete decoloration at 0 V and blue to violet coloration if reduced at -0.48 V against Ag/AgCl. The absorption bands at 370, 540, and 800–1100 nm in the visible spectrum indicate the presence of “pimers”, CT complexes between two radical cations.^[18] The degree of pimerization in the reduced **G0** to **G3** was evaluated by quantitative comparison of the cation radical dimer spectra with data from literature for pure pimers and pure monomeric radical cations (Table 1, Figure 2).^[18] Surprisingly, the degree

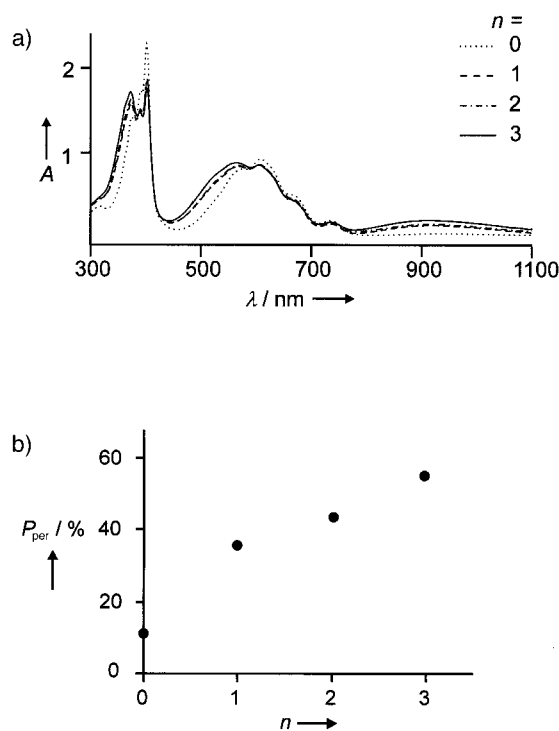


Figure 2. a) Generation-dependent pimerization: UV–Visible spectra of the 3-, 9-, 21- and 45-electron reduced **G0**, **G1**, **G2**, and **G3** at $n_{\text{theor}}c(\text{dendrimer}) = 3.8 \times 10^{-4}$ M (Spectroelectrochemical cell with a length of 2 mm). b) Degree of pimerization in the outermost peripheral shell from analysis of the adsorption at 537 and 605 nm as a function of the generation number n .

of pimerization (P_{tot}) grows with rising generation numbers (Figure 2). From the literature it is known that pimerization is strongly distance dependent. As the relative intensity of the CT adsorption bands showed no influence on the concentration of the dimers, pimerization must be related to intramolecular interactions of cation radicals. We believe that the probability for a cisoid conformation of two *meta*-positioned viologen radical cations in the outermost dendrimer shell—a prerequisite for pimer formation—becomes increasingly larger for higher generation numbers. In accordance with this model, an approximately linear increase of the degree of pimerization related to the outermost shell (P_{per}) with the generation number is observed (Figure 2b).

We are currently exploring the possibilities for using these observed CT properties in combination with association reactions for molecular recognition/signal transduction.

Experimental Section

Conditions for ^1H NMR data: 250 MHz, CD_3CN , 25 °C, TMS; for ^{13}C NMR data: 63 MHz, CD_3CN , 25 °C, TMS.

G0·6PF₆⁻: ^1H NMR: δ = 8.92 (d, $^3J(\text{H,H})$ = 7.0 Hz, 12H), 8.39 (d, $^3J(\text{H,H})$ = 7.0 Hz, 12H), 7.66(s, 3H), 5.83 (s, 6H), 4.68 (q, $^3J(\text{H,H})$ = 7.3 Hz, 6H), 1.65 (t, $^3J(\text{H,H})$ = 7.3 Hz, 9H); ^{13}C NMR: δ = 151.1, 150.1, 146.2, 145.8, 135.3, 132.3, 127.8, 127.6, 64.0, 58.2, 16.0; elemental analysis calcd for $\text{C}_{45}\text{H}_{48}\text{N}_6\text{P}_6\text{F}_{36}$ ·1 H₂O: C 34.63, H 3.23, N 5.39; found C 34.85, H 3.58, N 5.36.

G1·18PF₆⁻: ^1H NMR: δ = 8.93–8.90 (m, 36H), 8.42–8.36 (m, 36H), 7.66(s, 9H), 7.64(s, 3H), 5.83 (s, 24H), 4.67(q, $^3J(\text{H,H})$ = 7.3 Hz, 12H), 1.65 (t, $^3J(\text{H,H})$ = 7.3 Hz, 18H); ^{13}C NMR: δ = 151.1, 151.0, 150.1, 146.3, 146.2, 145.8, 135.3, 132.2, 127.9, 127.8, 127.6, 64.0, 58.2, 16.0; elemental analysis calcd for $\text{C}_{138}\text{H}_{138}\text{N}_{18}\text{P}_{18}\text{F}_{108}$ ·3 H₂O: C 35.18, H 3.08, N 5.35; found C 35.13, H 3.21, N 5.32.

G2·42PF₆⁻: ^1H NMR: δ = 8.93–8.90 (m, 84H), 8.41–8.39 (m, 84H), 7.66 (br.s, 30H), 5.83 (s, 60H), 4.67(q, $^3J(\text{H,H})$ = 7.3 Hz, 24H), 1.64 (t, $^3J(\text{H,H})$ = 7.2 Hz, 36H); ^{13}C NMR: δ = 151.1, 151.0, 150.1, 146.2, 145.7, 135.3, 135.2, 132.2, 132.1, 127.9, 127.8, 127.6, 64.0, 58.1, 15.9; elemental analysis calcd for $\text{C}_{324}\text{H}_{318}\text{N}_{42}\text{P}_{42}\text{F}_{252}$ ·5 H₂O: C 35.45, H 3.01, N 5.36; found C 35.23, H 3.01, N 5.33.

G3·90PF₆⁻: ^1H NMR: δ = 8.93–8.90 (m, 180H), 8.42–8.39 (m, 180H), 7.67 (br.s, 66H), 5.83 (s, 132H), 4.67(q, $^3J(\text{H,H})$ = 7.3 Hz, 48H), 1.64 (t, $^3J(\text{H,H})$ = 7.2 Hz, 72H); ^{13}C NMR: δ = 151.1, 151.0, 146.2, 145.7, 135.3, 135.2, 132.2, 127.9, 127.8, 127.6, 64.0, 58.1, 15.9; elemental analysis calcd for $\text{C}_{696}\text{H}_{678}\text{N}_{90}\text{P}_{90}\text{F}_{540}$ ·23 H₂O: C 35.18, H 3.07, N 5.30; found C 35.02, H 3.18, N 5.23.

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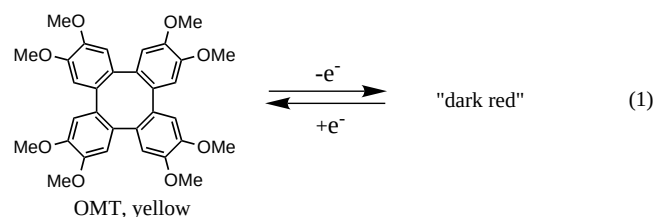
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A Redox-Controlled Molecular Switch Based on the Reversible C–C Bond Formation in Octamethoxytetraphenylene**

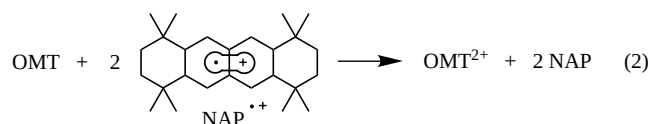
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The design and synthesis of organic derivatives for molecular devices such as sensors, switches, electrical conductors, ferromagnets, electronic circuits, and nonlinear optical materials has been actively pursued in recent years.^[1–3] Especially noteworthy are organic materials that exhibit changes in physical properties such as magnetism, conductivity, and optical response when triggered by external stimuli such as heat, light, or electrical potentials.^[4] One of the approaches in designing such materials is to exploit electronic interactions in macrocyclic or supramolecular assemblies consisting of multiple redox-active components.^[5] We now introduce a methoxylated tetraphenylene derivative as a potential molecular switch based on its dramatic color change upon the application of an electrical potential.

Octamethoxytetraphenylene^[6] (OMT) was prepared from a readily available 1,2-dimethoxybenzene derivative (veratrole) in excellent yield using standard synthetic procedures (see Experimental Section). This saddle-shaped macrocycle with cofacial phenylene groups (established by X-ray crystallography) shows a striking electrochromic behavior with a color change from yellow to red, which is completely reversible over multiple redox cycles [Eq. (1)].^[7]



The dramatic color change upon electrooxidation of OMT can also be achieved by a variety of chemical oxidants such as aromatic radical cations,^[8] nitrosonium cation,^[9] or triethyloxonium hexachloroantimonate.^[10] For example, a blue solution of an octahydronaphthacene radical cation^[11] (NAP^{•+}) turns dark red immediately upon the addition of neutral OMT [Eq. (2)]. In order to establish the stoichiometry of this



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redox reaction, a dichloromethane solution of NAP^{+} ($\lambda_{\text{max}} = 672 \text{ nm}$, $\epsilon = 9300 \text{ M}^{-1} \text{ cm}^{-1}$) was treated with incremental amounts of neutral OMT and the resulting UV/Vis spectral changes are shown in Figure 1. The three well-defined isosbestic points ($\lambda_{\text{max}} = 350, 420$, and 610 nm) clearly establish the concomitant reduction of NAP^{+} and oxidation of neutral OMT. That the final spectrum remains unchanged upon the addition of OMT beyond 0.5 equivalents establishes the redox stoichiometry as shown in Equation (2).^[12]

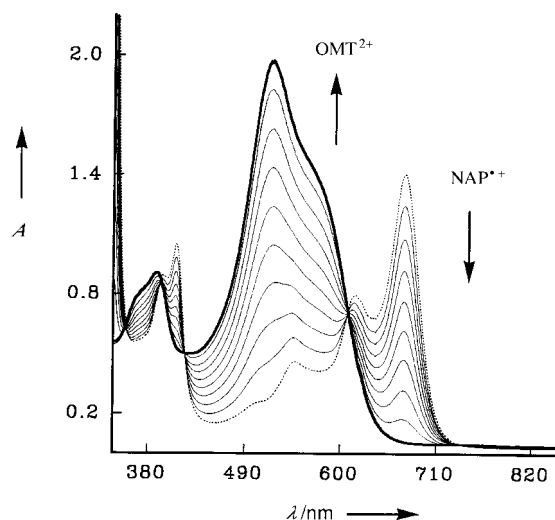


Figure 1. Spectral changes attendant upon an incremental addition of neutral OMT ($2.2 \times 10^{-4} \text{ mmol}$) to a 0.15 mM solution of NAP^{+} ($4.5 \times 10^{-4} \text{ mmol}$) in dichloromethane at 25°C .

The resulting dark-red product is thus identified as octamethoxytetraphenylene dication (OMT^{2+}) with an intense absorption band at $\lambda_{\text{max}} = 521 \text{ nm}$ and a high molar absorptivity of $\epsilon_{521} = 31\,000 \text{ M}^{-1} \text{ cm}^{-1}$ (in dichloromethane). Similarly, the simple dissolution of a 2:1 mixture of the well-known one-electron oxidant $\text{NO}^+\text{SbCl}_6^-$ and neutral OMT in dichloromethane at -20°C immediately results in a red solution with an identical absorption spectrum; and the quantitative formation of OMT^{2+} according to Equation (3) is determined spectrophotometrically.^[13]



It is important to note that the transformation of OMT to OMT^{2+} is initiated by well-known one-electron oxidants [see Eq. (2) and (3)]. As such, the oxidation of OMT to its dication can also be achieved by photoinduced electron transfer (PET) using tetrachlorobenzoquinone (chloranil; $E_{\text{red}}^0 = 0.02 \text{ V}$ vs. SCE) as the electron acceptor. For example, the pale-yellow mixture of neutral OMT and chloranil in dichloromethane in the presence of trifluoroacetic acid (ca. 1%) is stable in the dark for prolonged periods; however, upon a brief exposure to a mercury lamp it immediately develops the characteristic red color of the dication.^[14] Note that the use of strongly oxidizing 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ; $E_{\text{red}}^0 = 0.60 \text{ V}$ vs. SCE) instead of chloranil, under otherwise identical conditions, affords the dication OMT^{2+} even in the dark in excellent yield.^[15]

The intensely colored OMT^{2+} dication is highly stable in dichloromethane at ambient temperature and the dark-red single crystals can be readily isolated after precipitated by the slow diffusion of toluene into the solution at -23°C . X-ray crystallography establishes the hexacyclic structure of the novel dicationic OMT^{2+} (Figure 2). Comparison of the

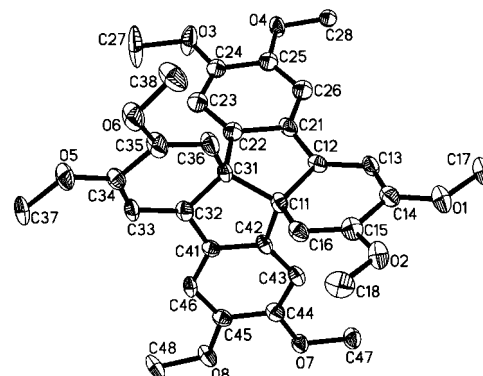


Figure 2. ORTEP diagram showing the fused hexacyclic structure of OMT^{2+} (hydrogen atoms and the SbCl_6^- anion are omitted for clarity).

structures of neutral and dicationic OMT reveals the change from the saddle-shaped eight-membered ring in OMT to the *cis*-fused bicyclo[3.3.0]octane framework of OMT^{2+} . The two-electron oxidation of OMT leads to the spontaneous formation of a C–C bond between the *ipso*-carbons C1 and C5 of opposite phenylene rings ($\text{C1}=\text{C5} = 1.56 \text{ \AA}$, Figure 3^[16]). The C–C bond formation is accompanied by (1) the sp^2 to sp^3 rehybridization of C1 and C5^[17] and (2) the formation of a pair of cationic cyclohexadienyl rings which show considerable quinoidal distortion.^[18]

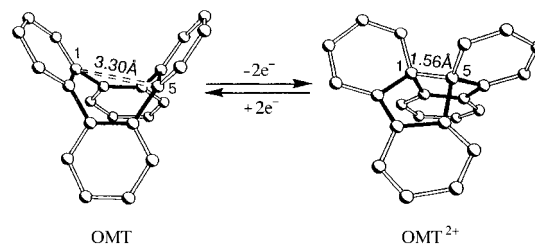


Figure 3. Structural changes in the oxidation of OMT to OMT^{2+} with concomitant bond formation between C1 and C5 of the OMT ring. Atoms C1 and C5 are represented in Figure 2 as C11 and C31, respectively.

The reversibility of the redox-controlled structural change (Figure 3) is established by voltammetric analysis. Thus, oxidation of OMT shows an irreversible cyclic voltammogram (Figure 4a) in which the anodic peak at $E_{\text{ox}} = 1.25 \text{ V}$ (vs. SCE) corresponds to the oxidation of OMT to OMT^{2+} , whereas the cathodic peak at $E_{\text{red}} = 0.25 \text{ V}$ (vs. SCE) on the return scan represents the reduction of OMT^{2+} to OMT.^[7] Such an assignment is further verified by the reduction of dark-red OMT^{2+} (prepared from two equivalents of NO^+PF_6^-) which leads to an irreversible voltammogram with identical peak potentials as shown in Figure 4b. The cyclic voltammograms

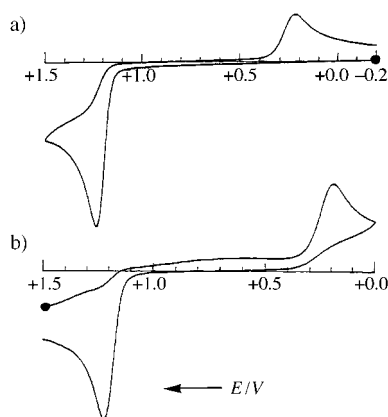
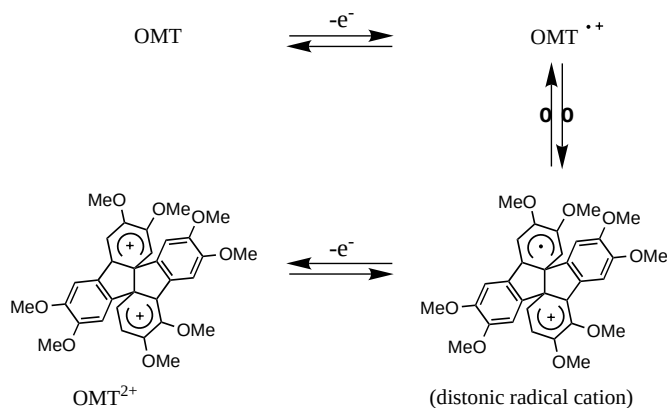


Figure 4. a) The initial positive-scan cyclic voltammogram of 5 mM neutral OMT and b) the initial negative-scan cyclic voltammogram of 5 mM $\text{OMT}^{2+}(\text{PF}_6^-)_2$ at 25 °C in anhydrous dichloromethane containing 0.2 M $n\text{Bu}_4\text{N}^+\text{PF}_6^-$ at a scan rate of $\nu = 200 \text{ mV s}^{-1}$.

in Figure 4 can both be repeated at least 100 times without any sign of degradation, and this electrochemical behavior is symptomatic of a chemically reversible but electrochemically irreversible redox system.^[19]

The cyclic voltammograms in Figure 4 can be readily simulated^[20] according to an ECE mechanism (Scheme 1)^[21] based on the spectroscopic and structural data. Thus, one-electron oxidation of OMT generates its radical cation which undergoes an intramolecular C–C bond formation to yield a distonic radical cation.^[22] It is the distonic nature of this rearranged radical cation which facilitates the removal of a second electron at a much lower potential^[23] and leads to the formation of stable dication OMT^{2+} .



Scheme 1. Proposed mechanism of the oxidation of OMT to its corresponding dication.

The facile, electrochemically controlled interconversion between OMT and its dication (Figure 3) via the two-electron redox change thus satisfies the three most important criteria for the construction of molecular switches, namely bistability, reversibility, and ready detection owing to the accompanying intense color changes.

Experimental Section

Synthesis of OMT: Crystalline 2,2'-dibromo-3,4,3',4'-tetramethoxybiphenyl was readily prepared from 1,2-dibromo-3,4-dimethoxybenzene (synthesized from veratrole) and *n*-butyllithium using a standard procedure^[24]

[m.p. 151–152 °C; ^1H NMR (300 MHz, CDCl_3): $\delta = 3.87$ (s, 6H), 3.92 (s, 6H), 6.77 (s, 2H), 7.12 (s, 2H); ^{13}C NMR (75 MHz, CDCl_3): $\delta = 56.31, 56.36, 114.07, 115.22, 134.13, 148.15, 149.26$; GC-MS: $m/z = 432$ (M^+)]. Following a literature procedure,^[25] 2,2'-dibromo-3,4,3',4'-tetramethoxybiphenyl (7.58 g, 17.5 mmol) was suspended in anhydrous diethyl ether (200 mL) and the mixture was cooled to –78 °C. A 2.5 M solution of *n*-butyllithium in hexane (15.5 mL, 38.6 mmol) was added dropwise and the dark yellow mixture was stirred for 1 h. Anhydrous cupric chloride (4.7 g, 35 mmol) was added and the resulting green mixture was slowly warmed to room temperature and stirred overnight. Aqueous workup afforded a yellow residue which was filtered through a pad of silica gel with ether/dichloromethane (1/1) as the eluent. The resultant product (ca. 10% 2,3,6,7-tetramethoxybiphenylene together with OMT) is further purified by recrystallization from ether to afford pure OMT as pale yellow prisms (1.7 g). Yield 46%, m.p. 260–262 °C (ether); ^1H NMR (300 MHz, CDCl_3): $\delta = 3.82$ (s, 24H), 6.66 (s, 8H); ^{13}C NMR (75 MHz, CDCl_3): $\delta = 55.99, 112.42, 134.02, 147.85$; GC-MS: $m/z = 544$ (M^+); elemental analysis: calcd for $\text{C}_{32}\text{H}_{32}\text{O}_8$: C 70.57, H 5.92; found: C 70.28, H 5.52.

X-ray crystallography: The intensity data were collected on a Siemens SMART diffractometer equipped with a CCD detector using MoK_α radiation ($\lambda = 0.71073 \text{ \AA}$) at –150 °C. The structures were solved by direct methods^[26] and refined by full-matrix least-squares procedures.

Crystal data for OMT [$2\text{C}_{32}\text{H}_{32}\text{O}_8 \cdot \text{C}_{16}\text{H}_{16}\text{O}_4$]. A suitable crystal ($0.8 \times 0.6 \times 0.5 \text{ mm}$) of OMT and 2,3,6,7-tetramethoxybiphenylene as a 2:1 complex was obtained by recrystallization from ethanol/dichloromethane at 0 °C. $M_r = 1361.44$, triclinic, space group $P\bar{1}$, $a = 11.2651(2)$, $b = 12.4218(3)$, $c = 13.3150(2) \text{ \AA}$, $\alpha = 96.350(1)^\circ$, $\beta = 109.833(1)^\circ$, $\gamma = 92.559(1)^\circ$, $\rho_{\text{calcd}} = 1.303 \text{ Mg m}^{-3}$, $V = 1735.20(6) \text{ \AA}^3$, $Z = 1$. The total number of reflections measured were 25533, of which 15301 reflections were symmetrically nonequivalent. Final residuals were $R1 = 0.0718$ and $wR2 = 0.1288$ for 15286 reflections with $I > 2\sigma(I)$.

Crystal data for OMT^{2+} [$\text{C}_{32}\text{H}_{32}\text{O}_8 \cdot 2\text{SbCl}_6^- \cdot 2\text{CH}_2\text{Cl}_2$]. A suitable crystal ($0.3 \times 0.2 \times 0.15 \text{ mm}$) was obtained by a slow diffusion of hexane into a dichloromethane solution of dark-red OMT^{2+} at –23 °C. $M_r = 1383.33$, monoclinic, space group $P2_1/c$, $a = 17.1786(10)$, $b = 10.0095(5)$, $c = 29.460(2) \text{ \AA}$, $\beta = 90.577(1)^\circ$, $\rho_{\text{calcd}} = 1.814 \text{ Mg m}^{-3}$, $V = 5065.4(5) \text{ \AA}^3$, $Z = 4$. The total number of reflections measured were 30818, of which 12612 reflections were symmetrically nonequivalent. Final residuals were $R1 = 0.0741$ and $wR2 = 0.1431$ for 12563 reflections with $I > 2\sigma(I)$.

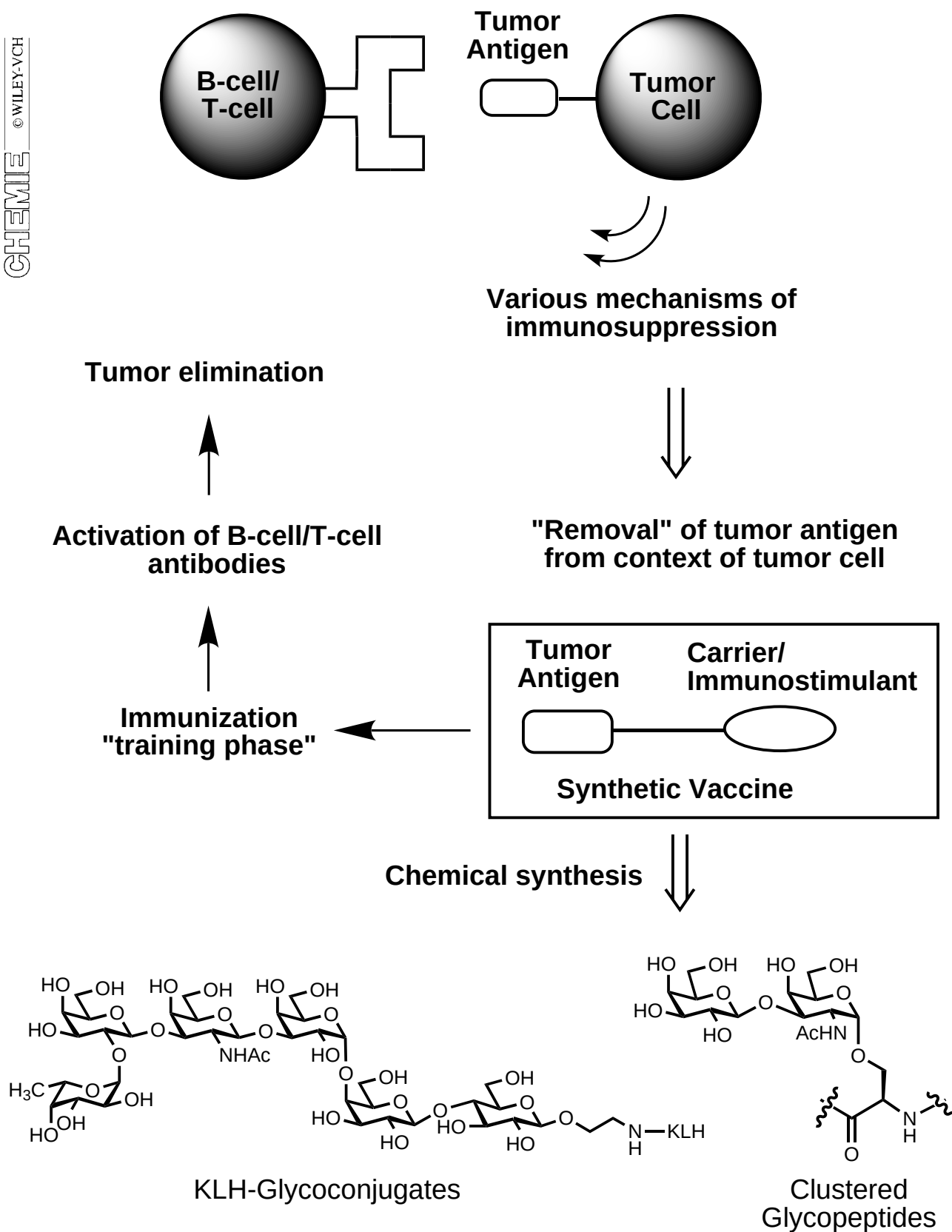
Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-133990 and CCDC-133991. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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Development of Carbohydrate-Based Anticancer Vaccines



From the Laboratory to the Clinic: A Retrospective on Fully Synthetic Carbohydrate-Based Anticancer Vaccines**

Samuel J. Danishefsky* and Jennifer R. Allen

Dedicated to Dr. Paul Marks

This review provides an account of our explorations into oligosaccharide and glycoconjugate construction for the creation and evaluation of vaccines based on carbohydrate-centered tumor antigens. Our starting point was the known tendency of transformed cells to express selective carbohydrate motifs in the form of glycoproteins or glycolipids. Anticancer vaccines derived from carbohydrate-based antigens could be effective targets for immune recognition and attack. Obtaining significant quantities of such structures from natural sources is, however, extremely difficult. With the total synthesis of tumor-associated carbohydrate antigens accomplished, we began to evaluate at the clinical level whether the human immune system can respond to such fully synthetic

antigens in a focused and useful way. Toward this goal, we have merged the resources of chemistry and immunology in an attack on the problem. The synthesis and immunoconjugation of various tumor-associated carbohydrate antigens and the results of such constructs in mice vaccinations will be described. For fashioning an effective vaccine, conjugation to a suitable immunogenic carrier was necessary and conjugates of KLH (keyhole limpet cyanin) have consistently demonstrated the relevant immunogenicity. Pre-clinical and clinical studies with synthetic conjugate carbohydrate vaccines show induction of IgM- and IgG-antibody responses. Another approach to anticancer vaccines involves the use of clustered glycopeptides as targets for immune attack. Initial attention has

been directed to mucin related O-linked glycopeptides. Synthetic trimeric clusters of glycopeptides derived from the Tn-, TF- and Lewis^x-antigens, appropriately bioconjugated, have been demonstrated to be immunogenic. The hope is that patients immunized in an adjuvant manner with synthetic carbohydrate vaccines would produce antibodies reactive with cancer cells and that the production of such antibodies would mitigate against tumor spread, thereby enabling a more favorable survival and "quality of life" prognosis.

Keywords: carbohydrates • drug research • glycoconjugates • tumor therapy • vaccines

1. Introduction

The first successful immunization procedure to protect against infectious disease is most commonly credited to Edward Jenner, in recognition of his published findings on the use of cowpox vaccination in 1798 (vaccinia, from the Latin vacca, meaning cow). It was, however, nearly 80 years later

that a better comprehension of the mechanisms involved in such phenomena paved the way for the development of modern vaccines by Eulrich, Koch, Metchnikoff, Pasteur, von Behring, and others.^[1] A vaccine to induce an anticancer immune response has been a long-standing vision in medicine.^[2] However, in contrast to classical viral or bacterial vaccines, which have generally been used to protect from future infections, and conventional cancer therapies, which focus on excision or killing of malignant cells, cancer vaccines are thus far perceived as a mode of treatment subsequent to the detection of the disease. Our emphasis is on the development of vaccine strategies to provide enhanced protection against tumor reoccurrence and metastasis when the tumor burden has been rendered minimal through surgery, radiation, or chemotherapeutic treatment.

Tumor immunotherapy is based on the theory that tumors possess specific antigens that can be recognized when

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[**] Frequently used abbreviations are listed in the appendix.

presented to or processed by a properly trained immune system. The connection of our laboratory to this field arose from the fact that malignant cells are commonly characterized by the appearance of large and unusual oligosaccharide motifs on their cell surfaces which distinguish them from their normal cell counterparts.^[3] The developmental reasons for formation of these oligosaccharide patterns are not completely understood, but the end result is the expression of distinct, cell-surface bound glycolipid or glycoprotein conjugates.^[4] Moreover, advances in monoclonal antibody (mAb) technology, immunohistology, and synthetic and structural chemistry have allowed the characterization of a number of carbohydrate-based tumor-associated antigens, providing a series of interesting structures.^[5] The exploitation of tumor-associated carbohydrate ensembles to trigger active immunotherapy against cancer cells which express counterpart structures on their surfaces has since been the focus of much investigation, including that in our laboratory. In fact carbohydrate antigens, when administered as a coherent vaccine, have proven to be targets for immune recognition and immune attack.^[6, 7]

2. Immunological Considerations

Tumor-bearing hosts fail to reject malignant cells, even in experimentally induced animal tumor models in which the

cells express detectable levels of immunogenicity.^[8] A conceptual objection can be raised against attempting immunotherapy in humans by using cancer-associated antigens: How can antigens induce an effective immune response when the same antigens expressed by the cancer cells have not done so? Apparently, there exist mechanisms that allow tumor-presented antigens to be seen as "self" by the immune system. These mechanisms, which facilitate tumor growth, have been the subject of much discussion and experimentation.^[9] Numerous reports have identified suppressive mechanisms during the growth of immunogenic tumors which serve to provide a rationale for the paradoxical growth of tumors in immunocompetent hosts.^[10] In addition, it has been documented that some tumors lack, or shed, crucial recognition molecules which then provides a selective advantage for tumor cells.^[11] The goal in the development of anticancer vaccines is to break the tolerance which the immune system has for antigens expressed mainly or exclusively by the tumor. Accordingly, the idea of using synthetically derived cell-free glycoconjugates as versions of immunostimulatory antigens in the development of antitumor vaccines merits a sustained investigation.

The development of increasingly effective vaccines requires follow-up assays to evaluate relevant immunogenicity. These feedbacks serve to guide the process of vaccine construction and testing. With the development of serological typing systems for defining cell-surface antigens, immunologists now

Under the tutelage of his father, Samuel J. Danishefsky was exposed, at an early age, to the elements of logical thought and critical analysis through the study of the Talmud. He received a B.S. degree at Yeshiva University (1956). In keeping with the example of an older brother (Isadore), he took an interest in chemistry in college. A life long fascination with organic chemistry followed from absorption with two introductory treatments of the subject—one by Raymond Brewster and the other by Louis and Mary Fieser. This exposure led him to pursue graduate studies at Harvard University where he received a Ph.D. (1962) under the direction of Professor Peter Yates. From 1961–1963 he was an NIH sponsored Postdoctoral Fellow at Columbia University under the mentorship of Gilbert Stork. His first independent academic position, which started in 1963, was at the University of Pittsburgh where he became Professor in 1971 and University Professor in 1979. In 1980 he moved to Yale University and served as chairman of the department from 1981–1987. He was named Eugene Higgins Professor in 1984 and Sterling Professor in 1990. In 1993 he returned to New York as Professor of Chemistry at Columbia University and as Kettering Professor and first Head of the Laboratory for Bioorganic Chemistry at the Memorial Sloan–Kettering Cancer Center. His research interests have been in the areas of synthetic strategy, cytotoxic natural products, and, most recently, fully synthetic carbohydrate based tumor antigens. In 1996 he shared the Wolf Prize in Chemistry with Gilbert Stork.



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J. R. Allen

Jennifer Allen was born as Jennifer Lowe in Cleveland, Ohio. She received her A.B. degree in Chemistry in 1992 from Miami University in Oxford, Ohio, and her Ph.D. from Duke University in 1998. Her doctoral research, under the supervision of Ned A. Porter, examined the synthesis and applications of unsymmetrically labeled alkyl hydroperoxides. Particular emphasis was on mechanistic studies of the [2,3]-allylperoxyl rearrangement. As a postdoctoral fellow with Professor Danishefsky she is attempting to fashion new anticancer vaccines via fully synthetic carbohydrate-based tumor antigens. Her areas of interest include glycosidic rearrangements for the construction of glycosides, glycoconjugates, and glycomimetics and studies which allow for a better understanding of the biological phenomena in which carbohydrates participate.

have assays of requisite sensitivity and specificity which are comparable to those used in the monitoring of vaccines against infectious diseases.^[12] Furthermore, effective assays capable of detecting T-lymphocyte responses, while still lagging behind, may soon provide an opportunity for evaluation of the progress with T-cell responsive antigens. Thus, immunogenicity to cancer vaccines can be established through the use of well-defined tumor antigens, whose structure and quantitative expression levels on tumors and normal cells *in vivo* are known.

There are grounds for both optimism and caution in using cancer vaccines which contain carbohydrate antigens as major stimulants. Carbohydrate-based vaccines have thus far been unsuccessful in inducing detectable T-cell immunity. As a result, sole reliance on carbohydrate antigens for immunotherapy of cancer can be limiting.

In general, immune responses against carbohydrate antigens are largely restricted to inducement of antibodies. Antibodies used in this way could provide a mechanism for eradication of circulating tumor cells (in the blood stream) and micrometastases, thus providing protection from tumor reoccurrence. The mechanism of protection could involve attack and lysis, mediated by other blood plasma substances, with the cell-surface carbohydrate antigens as targets; however, there may be other operative mechanisms. Experimentation involving administration of monoclonal antibodies against carbohydrate antigens supports this mechanism and treatment in mouse models has progressed to the point where micrometastases can apparently be eliminated.^[13] Cancer patients in clinical studies have also responded favorably to natural or passively administered antibodies, resulting in prolonged disease-free survival and prognosis.^[14] With repeated occurrences of metastases controllably minimized as a consequence of high levels of circulating antibodies, aggressive local therapies followed by vaccination could result in long-term control over even metastatic cancers. It is clear that there is potential for immunotherapy of cancers using monoclonal antibodies.^[15]

There is another potentially complicating feature in the use of carbohydrate-based tumor antigens in cancer vaccines. Cancer and normal cells growing in tissue culture generally show minimal levels of expression of such antigens. The immense difficulties associated with their purification from such sources render them virtually nonavailable as homogeneous starting materials for a clinical program. Accordingly, it falls to organic chemists to play a key role in the development of such cancer vaccines. The first role of the synthetic chemist is that of solving purity and availability problems if the program is to have any chance of advancing to a clinical setting. Moreover, chemistry must play a major role in the conjugation phase, which is decisive in upgrading a synthetic antigen to a vaccine. Although they do show minimal activity, most tumor antigens, including carbohydrate tumor antigens, are generally poor immunogens. Rather they require an appropriate immunogenic carrier to achieve an optimal response.^[16] Therefore, an additional challenge to cancer vaccine strategies is the successful delivery of the synthetic tumor antigens in a favorable molecular context for eliciting a therapeutically useful immunological response. Collectively,

there is an ample basis for studying carbohydrate antigen-based vaccines to promote the production of antibodies in a clinical setting.

Our laboratory has been engaged in synthesizing complex oligosaccharides and glycoconjugates for some time. In addition to their aforementioned role as tumor antigens, carbohydrates can influence many biological events. They mediate a variety of functions including inflammation, control of growth and differentiation, cell–cell adhesion,^[17] as well as being determinants in blood group typing.^[18] Given these facts and given our synthetic advances in the preparation of complex carbohydrates, the next logical step in our program was to explore the total synthesis of carbohydrate-based tumor antigens as part of a coherent vaccinology program. From our viewpoint, emphasis was placed on the assembly of carbohydrate-based vaccine constructs that would otherwise be unavailable from natural sources or from other isolation methods.

At the Sloan–Kettering Institute, our laboratory is in active collaboration with the Laboratory for Tumor Vaccinology and the Laboratory for Tumor Antigen Immunochemistry and is closely associated with various clinical facilities in the Hospital of the Memorial Sloan–Kettering Cancer Center (MSKCC). This review will report on our continuing chemical studies into the development of synthetic methodology of general applicability for the preparation of carbohydrates, in the form of both glycolipids and glycopeptides, which mimic components of the cell surface of tumor cells. This progress has allowed us to assemble a number of vaccine constructs containing tumor-associated antigens which have been clinically evaluated and are in ongoing human trials (Figure 1). We emphasize that this account is not meant to be a formal and exhaustive treatment of the whole subject. Rather it should be viewed as a retrospective on our own activities, stressing not only the chemistry which lay at the heart of the effort, but also the way in which the chemistry was interfaced with allied disciplines to move the program forward. There are certainly others also currently working in the same direction using synthetic conjugates and we point the reader to accounts of their important work.^[19, 96, 97]

We describe here the synthesis and immunological evaluation of neoglycoconjugates which contain the following antigens: the MBr1 antigen Globo-H (**1**), the adenocarcinoma antigen KH-1 (**2**), the blood group determinant and ovarian cancer antigen Lewis^x (**3**), the major and minor N3 antigens associated with gastrointestinal cancer (**4a** and **4b**), and the small cell lung carcinoma antigen fucosyl GM₁ (**7**). In addition, we have investigated clustered mucin-related structures of O-linked antigens containing Lewis^x, the Tn (**5**) and TF (**6**) antigens. All of the vaccine constructs reported here have been synthesized in appropriate bioconjugatable form and been through proper mouse immunization studies. This has led to a “proof of principle” clinical evaluation of three of our fully synthetically derived vaccines (Globo-H in prostate and breast cancer trials, Lewis^x–KLH conjugate in ovarian cancer trials and Tn/TF clustered, O-linked mucin models in prostate cancer trials. The Globo-H–KLH conjugate is poised to enter phase II and phase III human clinical trials). The case histories of these constructs will be described

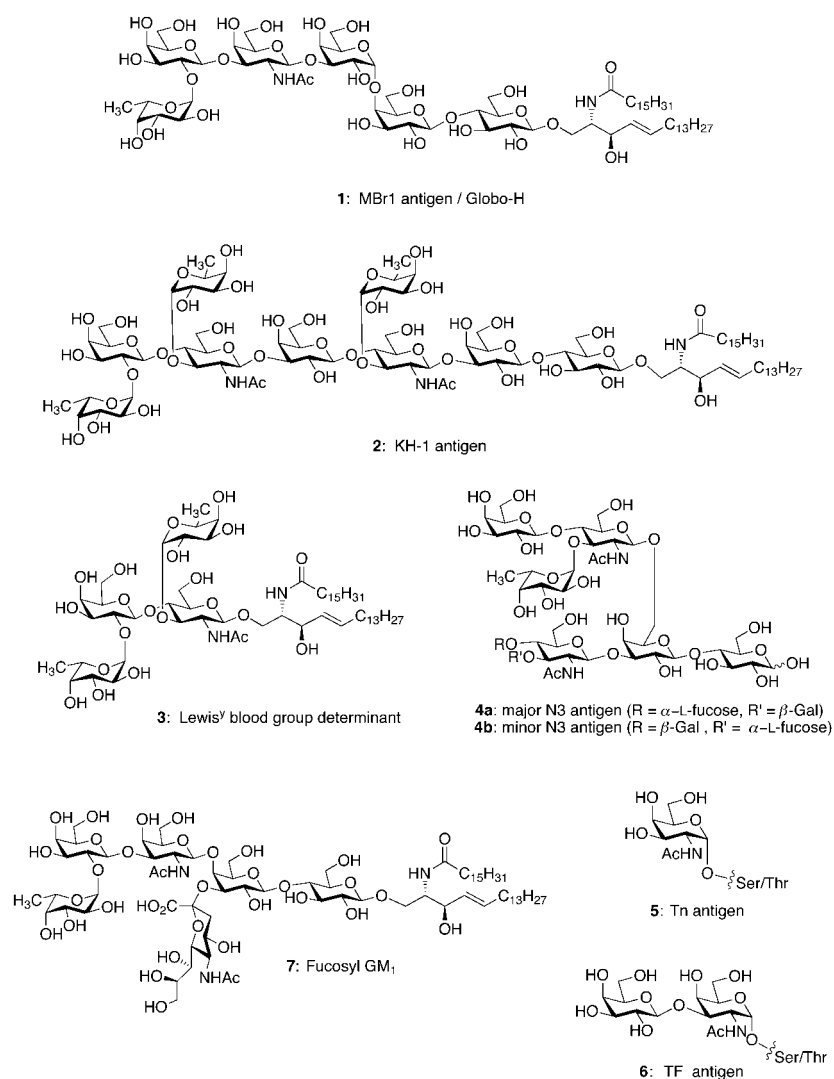


Figure 1. Structures of tumor associated antigens described in this account.

with emphasis on the rationale followed for progression from the drawing board through the lab and on to immunocharacterization en route to mouse vaccinations which terminate, in favorable cases, with clinical trials. In terms of the final structures, these represent the most complex, fully synthetically derived constructs ever to be clinically evaluated.

3. Synthetic Considerations

Synthesis is first and foremost an eminently practiced subject with primary emphasis on issues of conciseness and efficiency. Thus the best syntheses are the ones which address these goals most successfully. However, in pursuing this program we also sought a unifying framework around which to organize our thinking. In this connection we turned to the glycal assembly methods which have been developed over many years in our laboratory. The logic and details of glycal assembly have been amply reviewed.^[20] In this document we do not review the matter further except by illustration.

Several issues merit particular consideration when contemplating the synthesis of complex glycoconjugate-containing

substructures, such as those in Figure 1. From the standpoint of synthetic economy, it is beneficial to gain significant relief from the protecting group manipulations which have come to dominate traditional syntheses of complex branched oligosaccharides. It is in this area that we perceived a great advantage in using glycal building blocks to rapidly buildup the oligosaccharides of carbohydrate-based tumor antigens.

Another determining issue for the vaccine project involved establishing a linker domain through a spacer unit, for purpose of creating a functional immunogen. Attachment of an appropriate immunostimulant to the carbohydrate would follow. Although the optimal spacer-linker combinations are not well-established to date, the overall goal is that the molecular recognition of the synthetic tumor antigen by the immune system is not compromised by the conjugation of the carbohydrate domain to an effective biocarrier.^[21] In this context, we began with an exploration to fashion the carbohydrate antigen attached to an appropriate carrier protein (keyhole limpet hemocyanin, KLH). The conjugation strategy which we elected relies on the protocol of Bernstein and Hall,^[22] which calls for reductive coupling of a glycoside, which terminates in a glycoaldehyde, with the intended protein carrier, presumably at the ϵ -amino acid residues of exposed lysines. In a second-generation synthetic plan, we envisioned an option for clustering of the antigens via suitable peptide couplings to generate mucin-like structures.^[23] A general method for solving the stereochemical issues associated with the construction of

α -serine/threonine O-linked oligosaccharides was developed with the help of a "cassette" approach (see Section 9). With the glycopeptides, we also explored different immunostimulatory moieties in order to circumvent the requirement for conjugation of the complex construct to a carrier protein.

In summary, the preclinical goals at the outset of the vaccine program, with respect to the various tumor antigens investigated, were the following:

- Total synthesis of the desired tumor-associated antigen, chemically rigorous proof of structure as well as homogeneity, and, when appropriate, synthesis of relevant truncated congeners as probes of epitope specificity.
- Incorporation of an appropriate spacer group so that the immunological integrity of the antigens is retained.
- Covalent contact with an immunostimulant or carrier protein to generate a fully functional vaccine.
- Mouse immunization and follow-up evaluation of immune response.

Petition for human clinical trials would have to await the attainment of these goals. The interface of the glycal assembly logic with these strategies and goals is depicted in the paradigm shown in Figure 2.

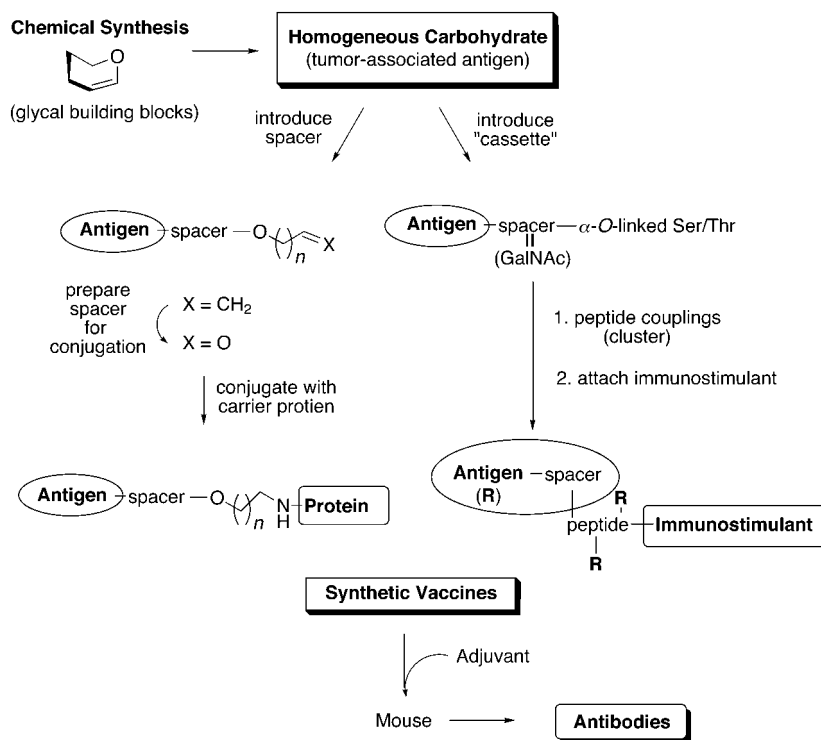


Figure 2. General approach to synthetic carbohydrate vaccines.

4. The Lewis^y – KLH Glycoconjugate

4.1. Synthesis of the Le^y – KLH Glycoconjugate

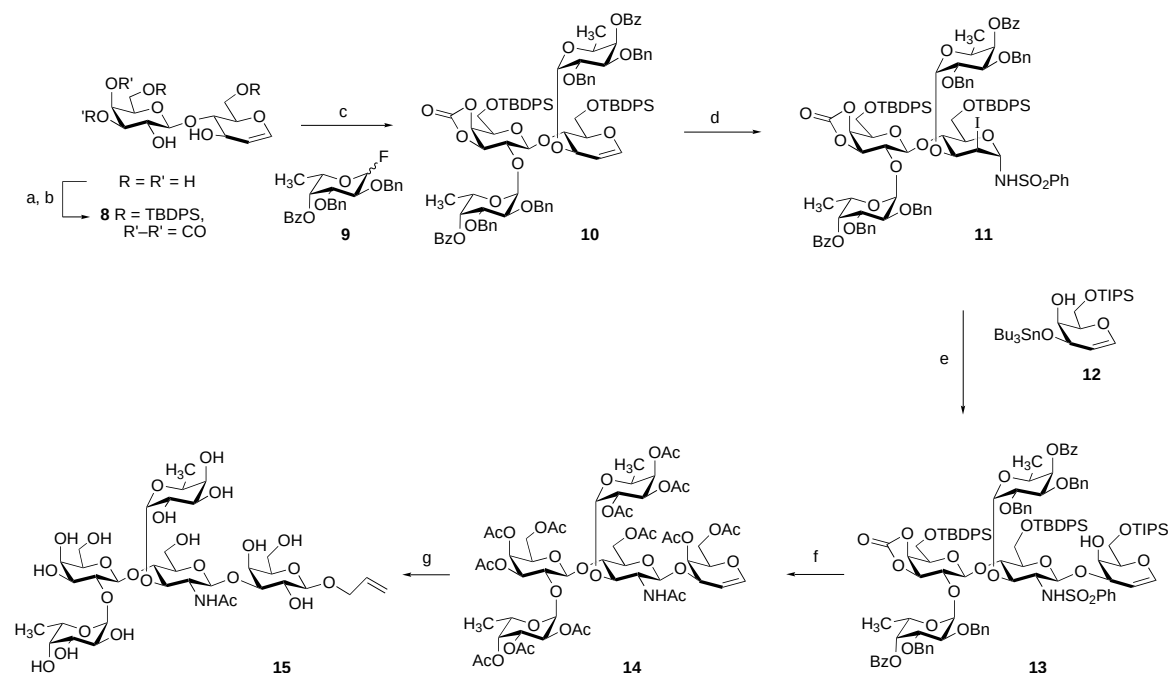
In 1995 our synthesis of neoglycoconjugates of Lewis^y (Le^y) and Lewis^b (Le^b) blood group determinants and of H-Type I and H-Type II oligosaccharides was described.^[24] The Le^y determinant was of particular interest to us because it had

been previously identified as an important epitope for eliciting antibodies against colon and liver carcinomas.^[25] It has also recently been implicated as a marker in metastatic prostate cancer and was found to be overexpressed in ovarian tumors.^[26]

In keeping with the objectives outlined above, the initial goal was the synthesis of a bioconjugatable precursor of the Le^y epitope **3** (Figure 1) for incorporation into a coherent vaccine. The successful fashioning and implementation of the synthesis, organized around Le^y, was a critical development en route to future vaccine constructs. In addition, it serves as a guiding paradigm for the use of glycal assembly methods and logic.

The pentasaccharide containing the Le^y specificity was prepared as shown in Scheme 1. The route took full advantage of the *N*-acetylglucosamine backbone in the target. Readily available lactal^[27] was silylated at the two primary sites. This selective protection phase was followed by cyclic carbonate formation through the *cis*-C3',C4' sites to give **8**. The resulting exposure of the C3 and C2' hydroxyls was of particular significance in this

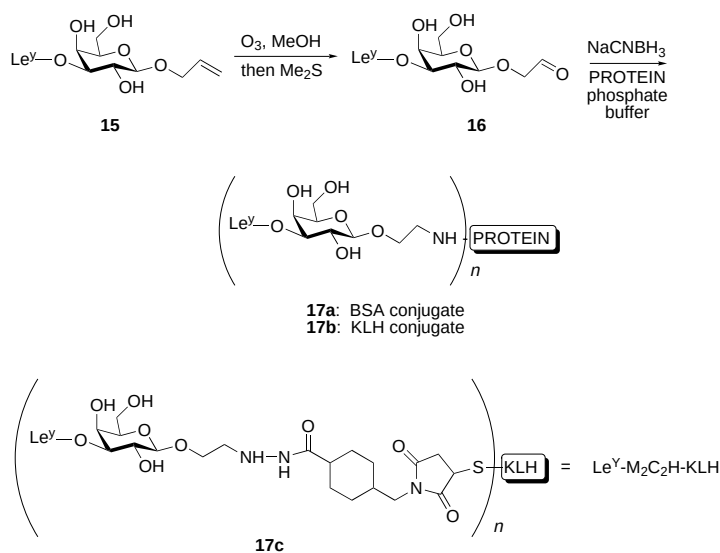
synthesis. Thus, the stage was now set for the bis-fucosylation of **8** to provide access to the Le^y-series tetrasaccharide. In the event, bis-fucosylation^[28] of acceptor **8** was carried out with fluorinated donor **9**,^[29] thereby providing the Le^y tetrasaccharide as a glycal, **10**. The nonparticipatory benzyl ether at C2 and a potentially participating benzoate at C4 of donor **9** had conspired to provide the required α -selectivity. Next, the glycal functionality was readied to act as an azaglycosylation



Scheme 1. Synthesis of the Lewis^y allyl glycoside. a) TBDPSCl, imidazole, DMF, 84 %; b) Carbonyl diimidazole, THF, 58 %; c) AgClO₄, SnCl₂, DTBP, Et₂O, 51 %; d) PhSO₂NH₂, I(coll)₂ClO₄, 99 %; e) AgBF₄, THF, 75 %; f) 1. TBAF, THF; 2. Na/NH₃, MeOH; 3. Ac₂O, pyridine, 37 %; g) 1. DMDO, CH₂Cl₂; 2. allyl alcohol, ZnCl₂, THF; 3. NaOMe, MeOH, 72 %; 3 steps.

donor^[30] by using our previously developed iodosulfonamidation protocol. Use of the iodosulfonamide **11** to glycosylate the galactal tin ether **12** was subsequently achieved with silver tetrafluoroborate to give the pentasaccharide glycal **13**. The desired amino functionality and β -selectivity were installed in the rollover with the galactal acceptor. Global deprotection followed by peracetylation afforded the derivative **14**. This compound later proved to be an extremely valuable intermediate, quite aside from its role en route to **15** (see O-linked system, vide infra).

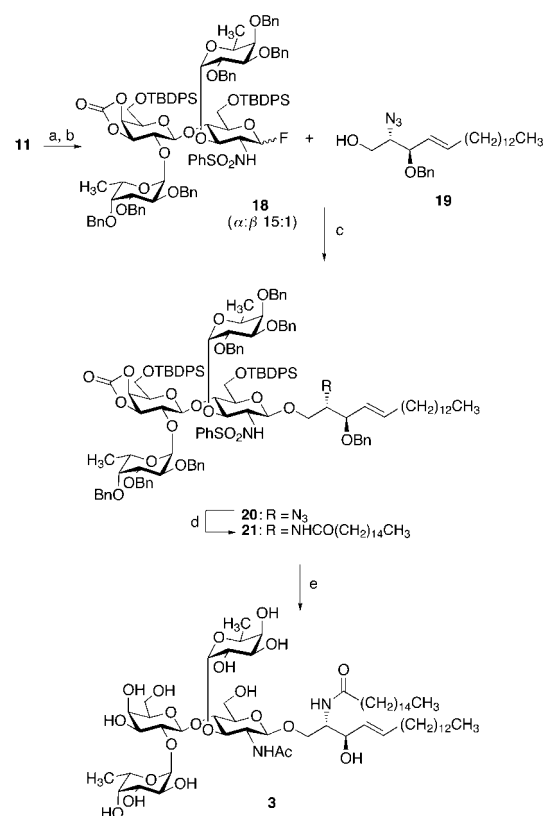
With the glycal **14** in hand, the necessary chemistry for conjugation to the carrier protein was implemented. Treatment of **14** with dimethyldioxirane,^[31] followed by opening of the resulting epoxide with allyl alcohol selectively gave the desired allyl glycoside. Removal of the ester protecting groups with catalytic sodium methoxide gave the fully deprotected Le^y pentasaccharide **15** ready for the conjugation phase. In the event, **15** was ozonolyzed in MeOH at -78°C and subjected to a workup with dimethyl sulfide to yield the uncharacterized aldehyde intermediate **16**, as shown in Scheme 2.



Scheme 2. Conjugation of allyl glycoside **15**.

As previously mentioned, the reductive amination protocol of Bernstein and Hall^[22] was applied to the case at hand with bovine serum albumin (BSA) in phosphate buffer (pH 8.0) to afford the protein conjugate **17a** which was purified by exhaustive dialysis. Analysis^[32] following cleavage of the glycosidic linkages with TFA showed the expected sugar composition: 2 parts galactose, 2 parts fucose and 1 part glucosamine. Analyses of the carbohydrate:protein ratio showed the substitution of an average of 15 Le^y moieties per carrier molecule. Subsequent studies of the installation of carrier protein KLH to give **17b** by an analogous reductive amination procedure provided glycoconjugates with an average of 287 Le^y moieties per molecule of KLH.

To complete the glycolipid total synthesis objective, the ceramide-linked glycoconjugate of the Le^y epitope was also prepared. Returning to the iodosulfonamide **11**, conversion into the fluoro donor **18** was accomplished in a two-step procedure, as shown in Scheme 3.^[33] Donor **18** (15:1 α/β) was



Scheme 3. Synthesis of the Lewis^y sphingoglycolipid. a) THF/H₂O, TEA, Ag₂CO₃; b) DAST, THF; c) [Cp₂ZrCl₂], AgOTf, CH₂Cl₂, 57%; d) H₂/Lindlar's catalyst, palmitic anhydride, 22%; e) 1. TBAF, THF; 2. Na/NH₃; MeOH; 3. Ac₂O, Pyridine; 4. NaOMe, MeOH.

coupled to compound **19**, the well known azidosphingosine precursor,^[34] using a mixed metal promotion system involving zirconocene dichloride and silver triflate to yield the Le^y sphingosine **20**. Reduction of the azide followed by installation of the ceramide with palmitic anhydride to the resulting amine was accomplished, albeit in low yield, to give **21**. Other conditions to reduce the azide in this system remain to be explored, although it will be seen that similar conditions with alternative oligosaccharides proved to be quite successful. Global deprotection of **21** as before yielded the known Le^y glycolipid conjugate **3**.

The successful synthesis of the Le^y constructs **17a**, **17b**, and **3** marked the beginnings of our immunological collaborations and set the stage for further investigations into the synthesis of antigenic glycoconjugates. In addition, in conjunction with the described studies, the synthesis and bioconjugation of a Lewis^b hexasaccharide was also completed using analogous methodology.^[24] Thus with these synthetic endeavors, it was clear that we had secured sufficient protocols for protein conjugation with carbohydrates prepared by glycal assembly methods (see Scheme 2).

4.2. Mouse Immunizations with Synthetic Le^y – KLH Glycoconjugate

With a view to developing Le^y-based vaccines, we examined the immunogenicity of the Le^y conjugates in mice. The

production of antibodies as a direct result of immunization was of interest. A critical question to be addressed was whether antibodies thus elicited would show binding reactivity with cancer cell lines expressing the Le^y epitope. Determination of lysis to such cancer cells would also be important. Demonstration of positive results would constitute a significant starting point for human trials. Needless to say, a critical control would demonstrate that the induced antibodies were unreactive with Le^y negative cell lines and other controls. The results of the mouse immunization studies that were obtained are summarized below.^[35] As in all of our studies, the vaccines were coadministered with the immunological adjuvant QS-21.^[36]

In addition to synthetic conjugates **17a** and **17b**, a related synthesis^[37] provided a maleido-derivatized KLH conjugate **17c** (see Scheme 2). Immunization of groups of mice with the three conjugates, together with the adjuvant QS-21, showed that the Le^y oligosaccharide linked directly to KLH **17b** was the most efficient for eliciting both IgG and IgM antibody responses to natural forms of Le^y epitopes carried on mucins and glycolipids.^[38] As determined by an ELISA (enzyme linked immunosorbant assay), the IgM antibody responses were typically much higher in titer than the IgG responses. The antisera obtained following immunization with **17b** were also tested, using an immune adherence assay, for their reactivity with Le^y expressing tumor cells. It was found that the antisera from the Le^y–KLH immunized mice strongly bound Le^y-positive cell lines (MCF-7) and not Le^y-negative cell lines (SK-MEL-28).

Cytotoxicity tests for antibody-dependent complement-mediated lysis were also carried out and the results are shown in Table 1. Again, immune adherence assays were used to detect complement-binding antibodies (mainly IgM) which were subsequently found, in the presence of human complement, to be cytotoxic to Le^y-positive MCF-7 cell lines. Furthermore, the antibodies detected by this assay were not cytotoxic to SK-MEL-28 cell lines.

In summary, these studies showed that mice immunized with **17b**, together with QS-21, produced high titers of both IgG and IgM antibodies capable of reacting with epitopes carried on tumor cells expressing Le^y. These studies provided the basis of protocols which were drafted to guide a phase I clinical trial. Following approval from various regulatory bodies, resynthesis of the vaccine under conditions and specifications suitable for human trials was necessary. We then moved onto phase I trials using human patients with ovarian cancers. The goal of the phase I trials was to test the

safety of the vaccine and any antibodies that may be produced in response to the vaccine. The results of these studies have now been evaluated and show positive serological findings. These clinical results will be presented separately.

5. The MBr1 Antigen Globo-H

5.1. Synthesis of Glycoconjugates Containing the MBr1 Antigen Globo-H

Globo-H **1** (Figure 1) is a hexasaccharide which was isolated in submilligram quantities as a ceramide-linked glycolipid from the human breast cancer cell line MCF-7 by Hakomori et al.^[39] It is expressed at the cancer cell surface as a glycolipid, and possibly a glycoprotein. Another advance, which sparked interest in this antigen, was its immunocharacterization via monoclonal antibody (mAb) MBr1 by Colnaghi and co-workers, where the antibody had been obtained from mice immunized with intact MCF-7 cell lines.^[40] The isolation of **1** from these cell lines and its binding to MBr1 were therefore taken to implicate this glycolipid as a breast tumor antigen. In addition, Globo-H was more recently immunocharacterized (mAb VK-9) by Lloyd et al.^[41] Subsequent immunohistological analysis with MBr1 found that the antigen was also expressed in other types of carcinomas including colon, lung, ovary, and small cell lung cancers.^[42] Globo-H has also been detected in the majority of the carcinomas of the pancreas, stomach, uterine endometrium, and, in particular, was found to be expressed in both primary and metastatic prostate cancer specimens.

There is also evidence that there are cell surface carbohydrates, assumed to be Globo-H, which react with the MBr1 antibody on normal breast, pancreas, small bowel, and prostate tissues. The antigen in these tissues is, however, predominantly localized where access to the immune system is restricted.^[43] Therefore, results using the criterion of immunohistology, which are based on the binding of MBr1 to define the presence of Globo-H as a total structural entity in a particular tissue, should be viewed with caution.

As a result, only experimentation would reveal whether Globo-H is a useful antigen in mouse immunizations and would be of value in cancer treatment in the adjuvant setting. Indeed, at this stage only synthesis could confirm that **1** is the actual antigen recognized by MBr1 and only synthesis could provide sufficient quantities for the immunization studies. Even before we progressed far in the total synthesis, it was recognized that mapping studies with truncated versions of **1**,^[44] to investigate which portions of **1** are necessary for binding, would also be of interest to probe these intimate questions in more detail.^[45] Collectively, these findings provided the rationale for evaluating Globo-H as a candidate target antigen and rendered it an important synthetic target. At the outset of the Globo-H program, no total syntheses of the antigen had been accomplished.^[46]

We now relate the key steps in our total synthesis of constructs corresponding to the carbohydrate sector of **1**. We go on from there to describe the construction of the proposed

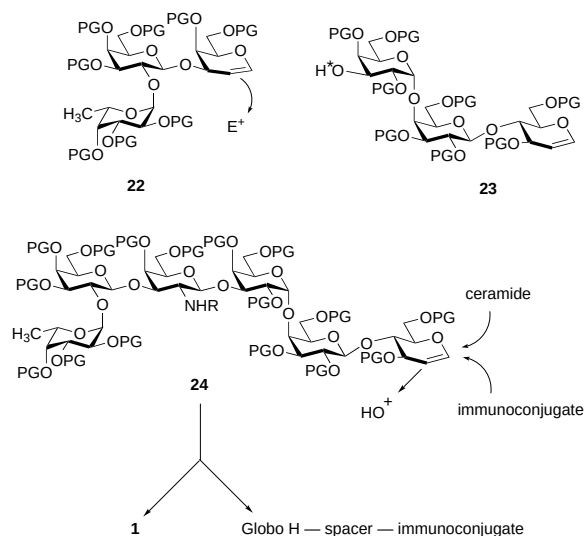
Table 1. Cytotoxicity assay of antisera on cultured cells.

Antibody or antiserum	MCF-7 ^[a] (Le ^y +)	SK-MEL-28 ^[a] (Le ^y –)
mouse 1 (Le ^y -KLH)	1:80	< 1:10
mouse 2 (Le ^y -KLH)	1:40	< 1:10
mouse 3 (Le ^y -M ₂ C ₂ H-KLH)	1:40	< 1:10
mouse 4 (Le ^y -M ₂ C ₂ H-KLH)	1:40	< 1:10
3S193 (anti-Le ^y)	0.5 µg mL ⁻¹	> 10 µg mL ⁻¹
R24 (anti-GD3)	> 10 µg mL ⁻¹	0.62 µg mL ⁻¹

[a] Greatest dilution giving detectable lysis; no lysis (< 1:10) was observed in pre-immunization sera.

vaccine containing the MBr1 antigen. The serological data starting from mouse immunizations and progression through a recently completed phase I trial are reported.

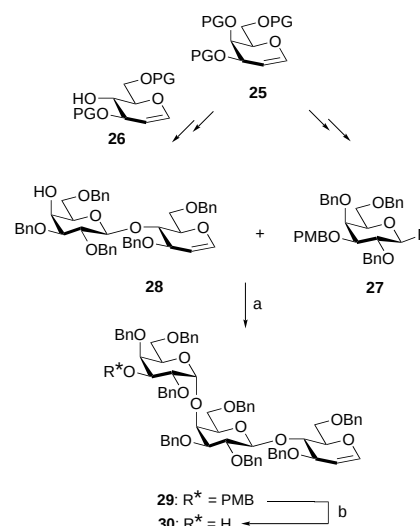
In studying the structure of Globo-H **1** with respect to a venture in chemical synthesis, we were mindful of an important requirement. Our first sub-goal would be, as usual, an academic type solution that might produce adequate quantities (5–10 mg) for proof of structure, immunocharacterization, conjugation, and mouse vaccinations. However, the synthesis eventually had to be capable of producing much larger amounts if the serological findings were positive and if the intent were to move toward clinical trials. We came to favor a disconnection into two trisaccharides, as shown in Scheme 4. In anticipation of a [3+3] coupling to bring the two



Scheme 4. Retrosynthesis of the MBr1 antigen, **1**. E⁺ = electrophile (acetamido equivalent), PG = protecting group.

trisaccharides together, glycal **22** emerged eventually to play the role of donor. Glycal **23**, with the appropriate oxygen distinguished at C3 of the galactal residue at the nonreducing end of the trisaccharide, would function as the acceptor. As in the synthesis of Le^y, system **22** would be converted to an azaglycosylation donor upon activation of its glycal linkage. The desired amino functionality would result through glycosylation with **23**. In the forward sense, formation of hexasaccharide **24** now allows for introduction of the ceramide moiety via the glycal linkage to yield the natural form of Globo-H, as well as formation of a suitable immunoconjugate through a linker sector to create a vaccine construct.

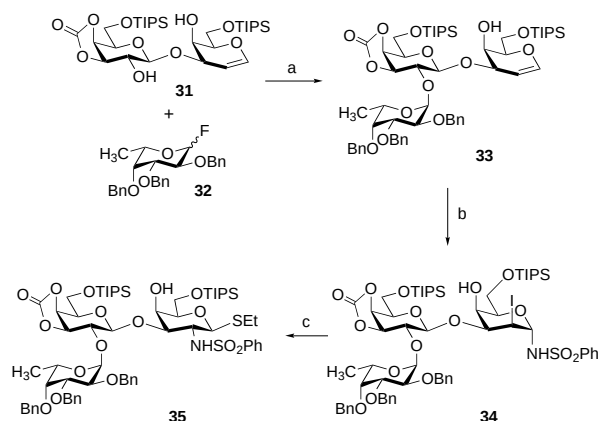
The plan charted in Scheme 4 was implemented^[47] and the first total synthesis of **1** was reported in 1996.^[48] We begin with the synthesis of the acceptor trisaccharide corresponding to **23** (Scheme 5). Both fluoro donor **27** and lactal acceptor **28** were obtained using galactal **25** and glucal **26** type building blocks. Donor **27** contains the differentially protected PMB ether at C3. In the subsequent [2+1] coupling, acceptor **27** and donor **28** were combined to provide the trisaccharide glycal **29** using a modified version of the conditions described by Mukaiyama



Scheme 5. Synthesis of the trisaccharide acceptor. a) AgClO₄, SnCl₂, DTBP, Et₂O, 54 %; b) DDQ, CH₂Cl₂, H₂O, 86 %.

et al.^[28] and Nicolaou and co-workers.^[49] Oxidative deprotection of the PMB ether afforded **30** which was poised for coupling with the appropriate trisaccharide donor.

Construction of the donor commenced with disaccharide **31** (itself available by two galactal building blocks of type **25**), as shown in Scheme 6. Regioselective fucosylation of the C2 equatorial hydroxyl with donor **32** provided the trisaccharide



Scheme 6. Construction of the trisaccharide donor. a) AgClO₄, SnCl₂, DTBP, Et₂O, 47 %; b) I(coll)₂ClO₄, PhSO₂NH₂, 4 Å molecular sieves, THF; c) EtSH, LHMDS, DMF, −40 → 0 °C; 40 % over 2 steps.

glycal **33**. The latter was converted to iodosulfonamide **34** under standard conditions. Even after careful and repeated investigation, it was found that union of acceptor **30** with trisaccharide substrates, such as **34**, could not be accomplished by direct azaglycosylation in a serviceable yield. Although this type of merger with simpler substrates provided a very powerful entry to somewhat simpler constructs (see Le^y), the method proved to be unreliable with severely hindered acceptors.

Fortunately, a solution did present itself. We had earlier explored the conversion of iodosulfonamides into more generally competent donors which might be effective in

otherwise difficult cases.^[30] These findings were applied to the case at hand, in the hope that a successful coupling would result in the formation of the β -configured product via sulfonamide participation. Following this lead, iodosulfonamide **34** was treated with lithium ethanethiolate to afford the requisite thioethyl donor **35**.

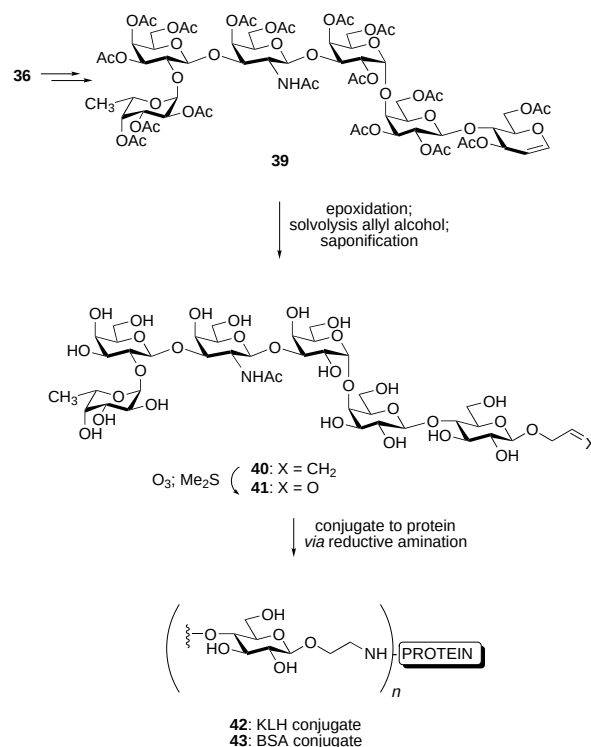
The cornerstone of the synthesis is depicted in Scheme 7. The critical coupling of **30** and **35** was effected by the action of stoichiometric MeOTf as promoter of the thioethyl donor, to give hexasaccharide glycal **36**. This was a splendid demonstration that the two-stage formation of *trans*-2-sulfonamido- β -thioglycosides from glycals could be very useful for the coupling of complex fragments when direct glycosylation employing the corresponding iodosulfonamide fails.

To yield the naturally occurring globoside, the properly configured hexasaccharide was epoxidized, treated with sphingosine precursor **19**,^[34] and acetylated (Scheme 7) to yield **37**. Reduction of the azide using H_2 /Lindlar's catalyst in the presence of palmitic anhydride provided ceramide **38**, in excellent yield. Global deprotection using standard procedures, followed by acetylation of the crude mixture, and saponification yielded glycosphingolipid **1**, whose spectral properties at the various anomeric centers were in complete agreement with those published for the natural material. A detailed proton NMR spectrum of the purified MBr1 antigen from tissue collection had not previously been available due to lack of material. However, our ^1H and ^{13}C spectra as well as mass spectral measurements, in conjunction with the spectra which reflected the synthetic progression, provided convincing support as to the chemical structure of the final product.

At the immunocharacterization level, synthetic compound **1** was shown by ELISA and immune thin layer chromatography assays to bind to monoclonal antibody MBr1. Inhibition studies revealed that preincubation of MBr1 with **1** completely inhibits MBr1 reactivity with human breast cancer cell line MCF-7. Thus, synthetic glycosphingolipid **1** contains the same antigenic epitope with which MBr1 reacts on breast cancer cells.

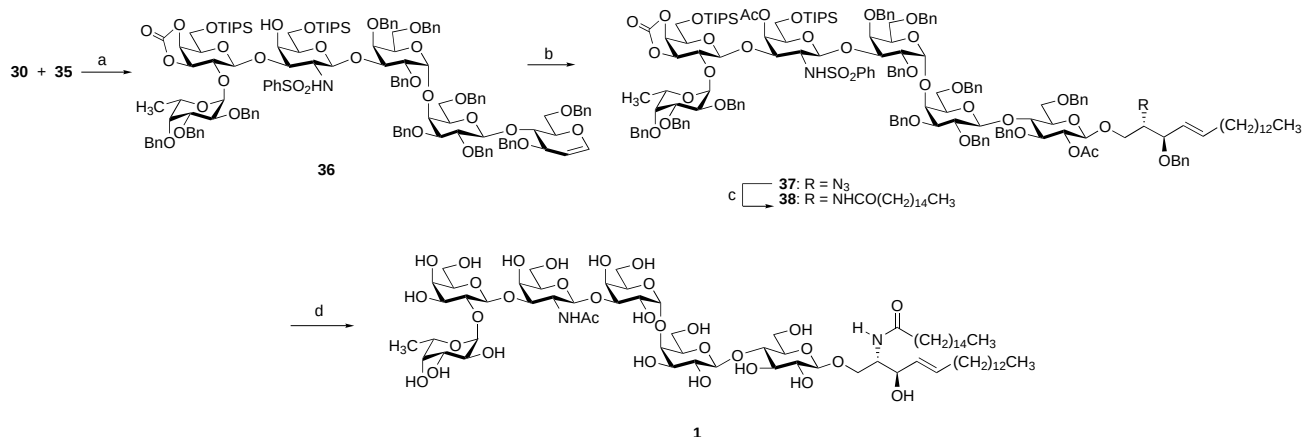
We next turned our attention to fashioning a functional vaccine which contained the synthetic antigen. In accordance

with our previous studies, we constructed the corresponding allyl glycoside to allow for carrier protein conjugation (Scheme 8). For this purpose we returned to hexasaccharide glycal **36**. Deprotection of **36** and re-acetylation, provided the peracetate of the glycal, **39**. Epoxidation of **39** with dimethyldioxirane, solvolysis with allyl alcohol, and exhaustive saponification gave the allyl glycoside **40**, which now contains



Scheme 8. Synthesis of the Globo-H-KLH vaccine construct.

the access point to reach the fully functional vaccine. As before, ozonolysis of the allyl linker sets the stage ($\rightarrow$ **41**) for reductive coupling to KLH^[22] thus yielding the immunogenically functional oligosaccharide-protein conjugate **42**. Carbohydrate:protein analysis^[32] of such material routinely

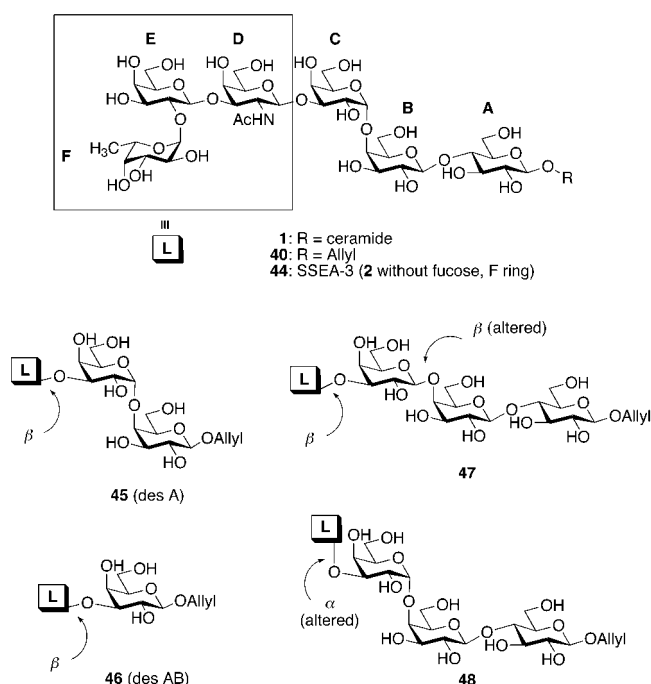


Scheme 7. Coupling of **30** with **35** and the last steps of the synthesis of MBr-1 antigen Globo-H **1**. a) MeOTf, Et₂O/CH₂Cl₂ (2:1), 70 %; b) 1. DMDO, CH₂Cl₂; 2. **19**, ZnCl₂, THF, 53 %; 3. Ac₂O, pyridine, 95 %; c) H₂, Lindlar's catalyst, palmitic anhydride, EtOAc, 90 %; d) 1. TBAF, THF; 2. NaOMe, MeOH; 3. Na/NH₃; 4. Ac₂O, pyridine, DMAP; 5. NaOMe, MeOH.

reveals approximately 350 Globo-H epitopes per molecule of KLH. Similarly, we investigated conjugates containing BSA where 17 carbohydrate units per protein were introduced (see 43).

5.2. Synthesis of Truncated Structures Corresponding to Globo-H

With a viable synthesis in hand, we could extend the methodology to truncated versions of **1**. Such compounds might be more readily synthesized and might perhaps also be recognized by Globo-H directed antibodies.^[45] We were much influenced in these synthetic studies by the fact that SSEA-3 **44** (Scheme 9), which lacks the terminal fucose residues, fails



Scheme 9. Truncated versions of **40**.

to bind the MBr1 antibody.^[50, 51] Although the fucose moiety appears to be crucial, it was of interest to investigate which truncated or isomeric versions of **1** that contain the fucose group but lack residues from the reducing end of the carbohydrate domain would be recognized by the MBr1 antigen. Accordingly, through total chemical synthesis we assembled the allyl glycosides **45–48** by glycal assembly methods (Scheme 9).^[44] The hexasaccharide core contained in **1** is maintained in compounds **47** and **48**, but in each case a glycosidic linkage is altered relative to the natural antigen. It should be noted that addressing the molecular recognition of the Globo-H antigen at such a detailed level (α versus β linkages) through truncated probe structures was only possible by the power of chemical synthesis and persistence of dedicated colleagues.

With the four allyl glycosides available in sufficient quantities and rigorously defined in terms of structural integrity, we carried out binding studies to MBr1 antibody using MCF-7 cell lines as the targets for binding. Increasing

amounts of glycosides (0.05 μg –500 μg) were used and the results from an individual inhibition experiment are demonstrated in Table 2. Compounds **45–47** show significant binding to MBr1, suggesting that the binding domain is localized in the CDEF ring system and that the terminal fucose is indeed

Table 2. Inhibition of monoclonal antibody MBr1 binding to an MCF-7 cell line by synthetic antigens **45–48** as well as **40** and **44**.

	40	44	45	46	47	48
IC ₅₀ [μM] ^[a]	16	> 500 ^[b]	10	26	27	200

[a] Concentration for 50 % inhibition. [b] IC₅₀ was not reached.

critical for this particular antibody. In addition, the stereochemistry of the glycosidic linkage joining rings C and D also appears to be critical for binding to MBr1 antibody (No binding occurs with **48**). Apparently, once the properly configured CDEF domain has been presented, the nature of the glycosidic linkage joining rings B and C is of minimal consequence (see compound **47**). Thus, it does appear as though analogues of **2** can be recognized by the MBr1 antibody. However, immunoassessment in a polyclonal setting would be of greater importance.

5.3. Immunological Studies Pertaining to Globo-H Based Vaccines

We next turned our attention to a detailed immunocharacterization of this important Globo-H antigen and its analogues. Systems **42** and **43** were starting points for these investigations. As before, the initial stages toward development of a Globo-H based vaccine started with mouse vaccination studies^[52] to confirm the immunogenicity of the synthetic glycoconjugates. The goal was to demonstrate that the synthetic vaccine conjugate combination activates the mouse immune system to produce antibodies that bind to human cancer cells expressing the epitope around which the carbohydrate region was organized.

Serological responses were analyzed by ELISA to determine antibody titers and the cell-surface reactivity of the resulting antibodies was assayed by flow cytometry and immune adherence assays. The ability of these sera to mediate complement lysis was also assessed. Both KLH and BSA conjugates (**42** and **43**, respectively) were initially investigated. As in the case of the Le^x based vaccine as well as other examples, the KLH-conjugate was superior in terms of immunogenicity when administered with the adjuvant QS-21.^[53]

In the animal studies with vaccine **42**, sera from all mice provided high titer IgM and IgG responses against Globo-H antigen (median titers 1/128,000 and 1/2560, respectively; Figure 3). A critical finding was that these sera reacted with Globo-H positive cancer cells (MCF-7) and, in a control experiment, failed to react with the Globo-H negative B78.2 melanoma cells. Serological studies also revealed that these antibodies were highly effective at inducing complement-mediated cytotoxicity. The percentage of lysis with antibodies

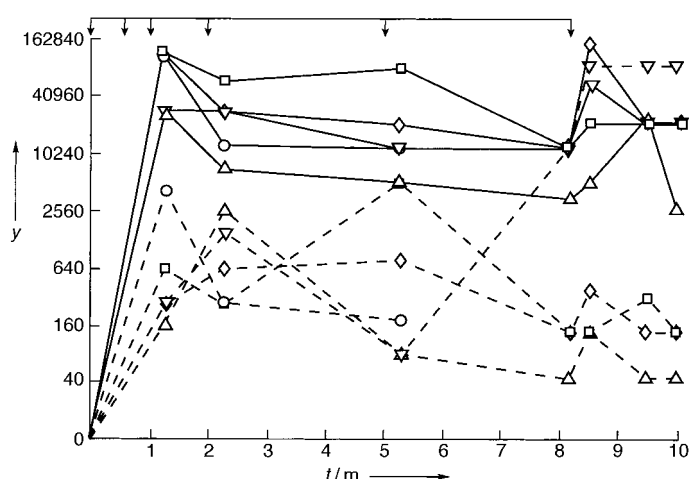


Figure 3. Time Course of the Antibody Titers in 5 Mice ($\diamond$, $\square$, $\triangle$, ∇) vaccinated with Globo-H–KLH conjugate **42** and QS-21. The reciprocal titer against Globo-H (determined by ELISA) is on the Y axis, the time t in months is on the X axis. —: IgM titer, ---: IgG titer. The vaccination times are indicated by the vertical arrows.

induced by the Globo-H–KLH conjugate (**42**) was 48%. For comparison, reference experiments with monoclonal antibody MBr1 showed 72% complement-induced cytotoxicity. These immunogenicity results then provided the basis for further examination of the vaccine construct **42** in tandem with QS-21. To date, the carbohydrate moiety of the vaccine in question is the most complex synthetic antigen brought to the stage of clinical evaluation.

Upon discovering these favorable serological and cell-surface reactivity results in vaccination with mice, various protocols were proposed for the use of fully synthetic Globo-H vaccines in a clinical setting. Following resynthesis and re conjugation in an appropriate setting, a study was launched. The study involved prostate cancer patients who had relapsed following prostatectomy or radiation therapy.

It should be emphasized at this point, that in progressing from a murine to a human setting for vaccinations, potential risks had to be faced. Human sera and cell-surface glycoproteins present related structures (in the form of Lewis blood group determinants and, indeed, very low levels of globosides). Hence, in the human clinical setting, there are potential issues of immunotolerance or possibly autoimmune damage to be addressed. Such considerations were not pertinent to the mouse immunizations. A focused humoral antitumor response using the fully synthetic carbohydrate vaccine would be a satisfactory proof of principle.^[54] These phase I trials were designed to test the safety of the vaccine and to investigate the proper doses of the vaccine conjugate and QS-21.

In the initial trial, five patients with progressive and recurrent prostate cancer received the KLH conjugate vaccine **42**, containing 30 μ g of Globo-H plus 100 μ g QS-21, according to defined clinical protocols.^[55] Their sera were submitted for detailed analysis and evaluation. Post-vaccination IgG and IgM titers against Globo-H ceramide, as determined by ELISA, are depicted in Figure 4. Subsequent to vaccination, all five patients produced a strong IgM response, while two concurrently generated a high IgG

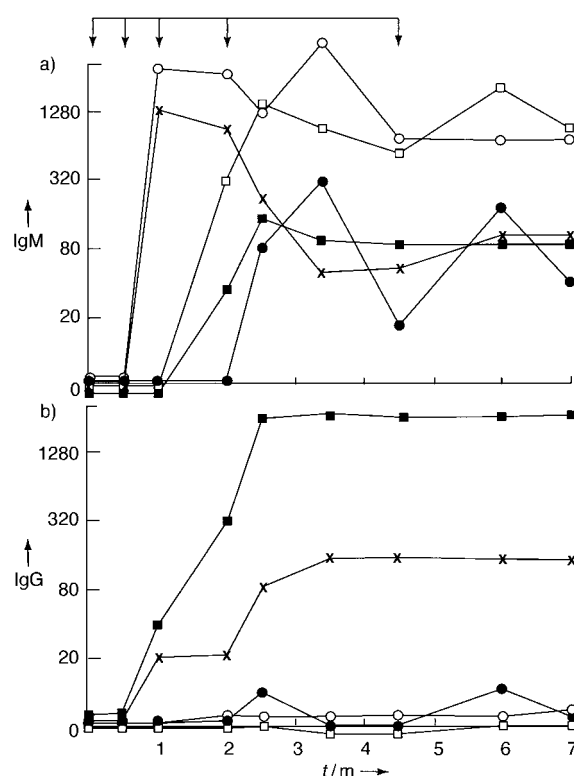


Figure 4. Time Course of the Antibody Titers in Five Patients ($\bullet$, $\times$, $\circ$, $\square$, and $\blacksquare$) immunized with Globo-H–KLH conjugate **42** and QS-21. a) IgM titer; b) IgG titer. The reciprocal titer against Globo-H (determined by ELISA) is on the Y axis, the time t in months is on the X axis. The vaccination times are indicated by the vertical arrows.

response. The specificity of these antibodies for Globo-H in prostate cancer cell extract from tumor or biopsy, as well as breast cancer biopsy specimens, was analyzed by immune thin layer chromatography revealing that the post-vaccination sera recognized both synthetic and tumor derived Globo-H ceramide. By contrast, the sera failed to react with melanoma biopsy specimen extracts which contain various glycolipids but are Globo-H negative.

With post-vaccination sera available, inhibition assays were carried out to determine the specificity of the antiGlobo-H antibodies in the immunized patients. Synthetic Globo-H ceramide **1** and the structurally related antigens **45–48** (Scheme 9), which were previously prepared by total synthesis, were surveyed for inhibition by using IgM and IgG ELISAs and the results are shown in Figure 5. Although the truncated oligosaccharide isomers were recognized to some extent, synthetic **1** inhibits antiGlobo-H reactivity most efficiently. As a control, unrelated glycolipids and synthetic Le^y–allyl glycoside **15** showed no IgM response (Figure 5a). The antisera which demonstrated IgM ELISA activity in the IgG assay (Figure 5b). Both synthetic hexasaccharides **1** and **40** as well as pentasaccharide **45** effectively inhibited binding in this assay. Apparently, in the polyclonal setting, IgG antibodies from the two sera mainly recognize an epitope area encompassing on average five reducing terminal carbohydrate units.

The lack of recognition of Le^y antigen **15** and various probe structures, even in the polyclonal regime, also reflected a

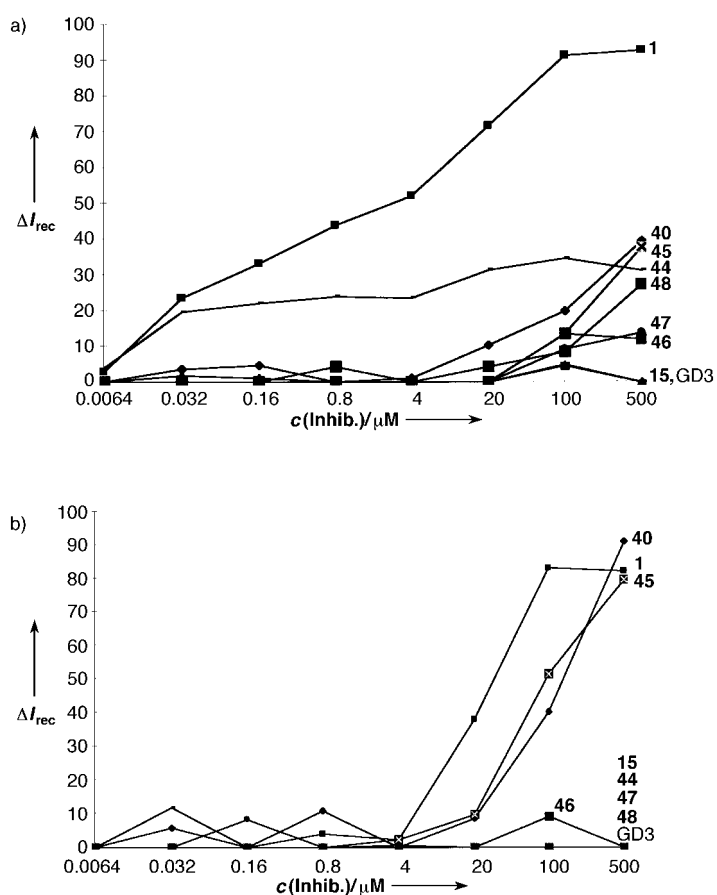


Figure 5. Analysis of the specificity of antiGlobo-H antiserum by inhibition assays. The ELISA reactivity of the serum with Globo-H ceramide when inhibited with compounds **1**, **15**, **40**, **44–49**, and GD3 (GD3 = NeuAc α 2 $\rightarrow$ 8NeuAc α 2 $\rightarrow$ 3Gal β 1 $\rightarrow$ 4Glc β 1–1'-cer) is shown. a) IgM antibody response; b) IgG antibody response. (ΔI_{rec} = % Inhibition; $c(\text{Inhib.})$ = Concentration of inhibitor.)

disiplined response against portions of Globo-H. Clearly, the specificity for **1** arises from the difference in the structural and stereochemical connectivity of the antigenic subunits. The results demonstrate that a fucosylated structure is required for an optimal antiGlobo-H response (see structure **44**).

Encouraged by these findings, we pursued the critical question as to whether antibodies elicited by the KLH vaccine construct **42** could recognize the antigen in its natural context, that is to say, the cell surface. This type of recognition is obviously a crucial milestone in the progression and development of antitumor vaccines. Preincubation of sera with Globo-H positive (MCF-7) cell lines produced more than a 50% decrease in binding against Globo-H ceramide, indicating that much of the antibody was recognized and bound to the tumor cell surface. No decrease in binding activity was observed following incubation with Globo-H negative (SK-MEL-28) melanoma cells. Furthermore, cell-surface reactivity of the antiGlobo-H antibodies was tested by flow cytometry and the results showed an increase for IgM and, to a lesser extent, IgG antibodies.

A final element of the initial preclinical serological evaluation following vaccination with **42**, was to test for the ability of the resultant sera, containing antiGlobo-H anti-

bodies to mediate complement-dependent cytotoxicity (CDC). Following relevant control experiments, results showed that three of the five post-vaccination sera exhibited strong CDC to MCF-7 cells. These results are also encouraging because complement-induced lysis of relevant cancer cells would seem to be associated with decreased tumor outgrowth and would favor longer survival.^[7a]

With the successful demonstration that the vaccine construct **42** combined with the adjuvant QS-21 is safe in humans and induces specific antibodies against tumor cells which carry the same antigenic structure contained in the vaccine on their cell surface, we progressed to a larger patient trial. The full phase I trial was completed by 18 patients with progressive and reoccurring prostate cancer. The clinical details of the trial can be found in the literature.^[56] All immunized patients exhibited good IgM responses against Globo-H confirming its immunogenicity in prostate cancer patients with a broad range of stages and tumor burdens. Pre- and post-immunization sera from the 18 patients were evaluated for the ability to mediate complement lysis. IgM antibodies induced in this trial were able to react with tumor cells as demonstrated by flow cytometry and, in nine cases, induced complement-mediated lysis of Globo-H expressing cell lines (Figure 6).

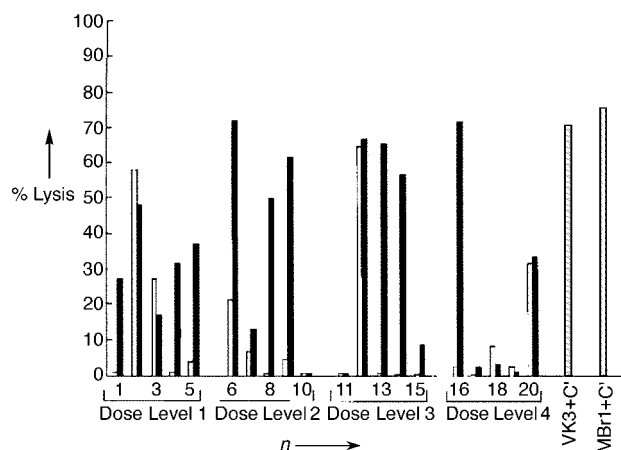


Figure 6. Complement lysis of MCF-7 tumor cells by pre- and post-immunization sera from 18 evaluable patients. Positive controls included MCF-7 cells with mAb VK9 (IgG) and MBr1 (IgM) with complement. White: before immunization, Black: after immunization, Grey: controls. n = Number of patients.

In addition to inducing proper immunogenicity, another important finding was advanced and addressed in the full patient sampling. Prostate cancer is, in principle, a unique disease to study because a highly specific biomarker, the Prostate Specific Antigen (PSA), is available. This allows the disease to be monitored at low tumor burdens where the vaccine therapies of interest to us are more likely to be effective. Measurement of PSA levels in several patients of this trial could indicate that a treatment effect could occur after completion of the vaccine therapy. Currently, the vaccination seems to bring about a decline of the slope in the plot of log PSA concentration versus time after treatment compared with values before treatment.

All patients showed PSA rises during the first 26 weeks of treatment, although in some cases PSA rate of rise appeared

to slow during the course of immunization. However, the potentially important, although preliminary, finding was that as patients continued to be observed for the six to nine-months post-treatment, favorable changes in the PSA slopes occurred in most patients who presented an initial non-metastatic state. By the criteria of declining PSA slopes, it could be argued that some actual treatment effect was occurring even after only three months. However, given the small sample size, these findings are highly tentative. An example of the PSA criterion is depicted in Figure 7.

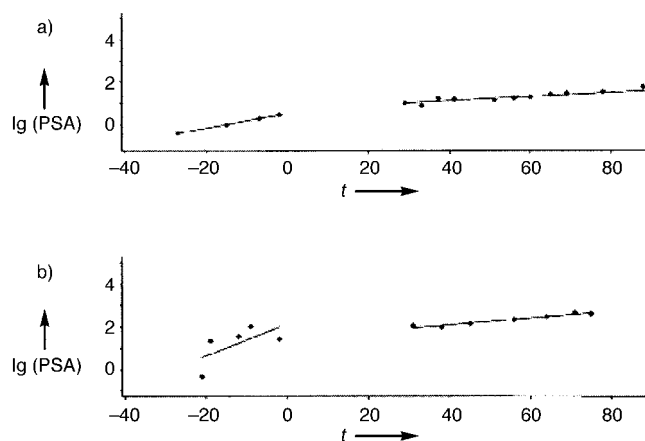


Figure 7. Decline of the slope of the logarithm of PSA concentration against time t after treatment of the patients in comparison with values before treatment. These patients continue to be radiographically free of disease more than 80 weeks after treatment and show stable PSA slopes. a) Slope before = 0.034, Slope after = 0.009; b) before = 0.075, after = 0.016.

Five patients from this trial, who continue to receive booster vaccinations, have stable PSA slope profiles in the absence of any radiographic evidence of disease after two years. In two of the five, the PSA slopes are decreasing. We emphasize that the concept of using PSA slope profiles for assessment of early treatment effects in biological therapies such as vaccines must await further evaluation in phase II and phase III trials and at present is not a reliable diagnostic.

As noted at the outset, Globo-H has been found to be expressed on a variety of tumors. Thus, we have hopes for use of a Globo-H based vaccine in other cancers as well. A vaccine containing the Globo-H antigen **42** has also been administered to breast cancer patients in a phase I clinical trial, according to defined clinical protocols. Data from the vaccinations are beginning to be correlated. Initially they appear to be encouraging at the serological level. A full report will be provided in due course.

With respect to further advancement of Globo-H based vaccines, we have obtained and organized clinical data to the point where we are planning a 200 patient trial on prostate cancer. Such a trial would use the fully synthetic vaccine construct **42** containing the Globo-H antigen, possibly in the context of a polyvalent setting which would include a menu of tumor antigens. With synthesis efforts proving increasingly successful,^[57] a fully definitive 1000 patient trial involving Globo-H is also being organized to begin sometime this year.

6. Synthesis of the KH-1 Antigen

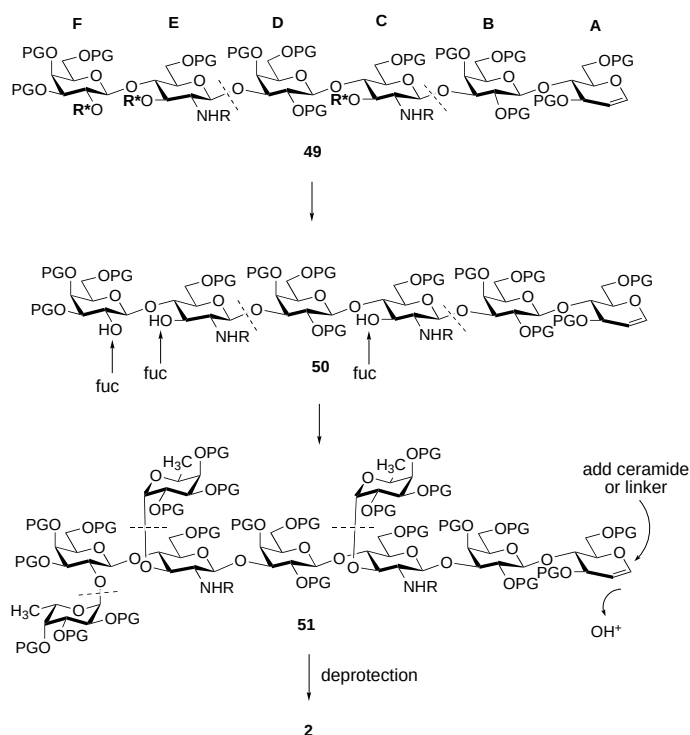
The glycolipid KH-1 **2** (see Figure 1) is perhaps the most formidable carbohydrate-based tumor antigen thus far characterized.^[58] The antigen was isolated from human colonic adenocarcinoma cells by using antibodies generated against the classical Le^y determinant (**3** in Figure 1). System **2** has been present on the cell surface of all adenocarcinoma cells thus far studied. Furthermore, its presence has never been detected in normal colonic extracts. Obviously, the chances of success from the point of view of vaccinology will be higher with the greater specificity of the carbohydrate domain of the antigen.

Monoclonal antibodies were raised against this antigen and found to bind specifically to compound **2**. Based on these studies, Hakomori et al.^[59] postulated that the KH-1 antigen is a highly specific marker for malignancy and premalignancy involving colonic adenocarcinoma. Recently, an X-ray crystal structure of an antitumor antibody BR96 in complex with the nonanoate ester derivative of Le^y tetrasaccharide was reported by Jeffrey et al.^[60] The view of the antibody–Le^y complex provided by this determination suggested that the BR96 antibody has unused binding capacity which might also recognize structures larger than the Le^y tetrasaccharide (such as the KH-1 antigen).

We were attracted to the KH-1 antigen in terms of chemical synthesis for several reasons. At the outset, a total synthesis of **2**, and bioconjugatable precursors, would represent the construction of another extremely complex tumor antigen.^[61] Embarking on such a task also provided the opportunity and setting to evaluate important strategic advances in terms of synthetic economy in oligosaccharide synthesis. Furthermore, our interests were not limited to **2**, but included congeners that would also be bioconjugated to the appropriate carrier systems.^[62] Difficulties associated with isolation and separation of complex carbohydrates from human colonic cancer tissue have been such that compound **2** has not been available for evaluation. Only through total synthesis could workable quantities of such chemically defined complex systems be obtained to allow such evaluations.

Our synthesis of the KH-1 antigen **2** was reported in early 1998.^[63] Not only was a total synthesis achieved, but the always important issue of strategy in oligosaccharide synthesis was suitably addressed as well. The synthetic plan put forth is outlined below.

Considerable thought was invested in gaining maximum relief from blocking group manipulations and obtaining optimal conciseness, a consequence which clearly becomes increasingly more difficult with increased branching and complexity in the oligosaccharide target. From these perspectives, we came to favor a plan that would build a hexasaccharide (**49**, Scheme 10), so differentiated in terms of its protecting patterns, as to allow for the simultaneous unveiling of the three free hydroxyl groups destined for fucosylation at a strategic point of our choosing. As the featured asset of our synthesis, the three fucosylations would then be conducted concurrently (see **50**–**51**). Requirement for three kinds of blocking groups in **49** was therefore necessary; one at the nitrogen centers, another for the three proposed fucosylation



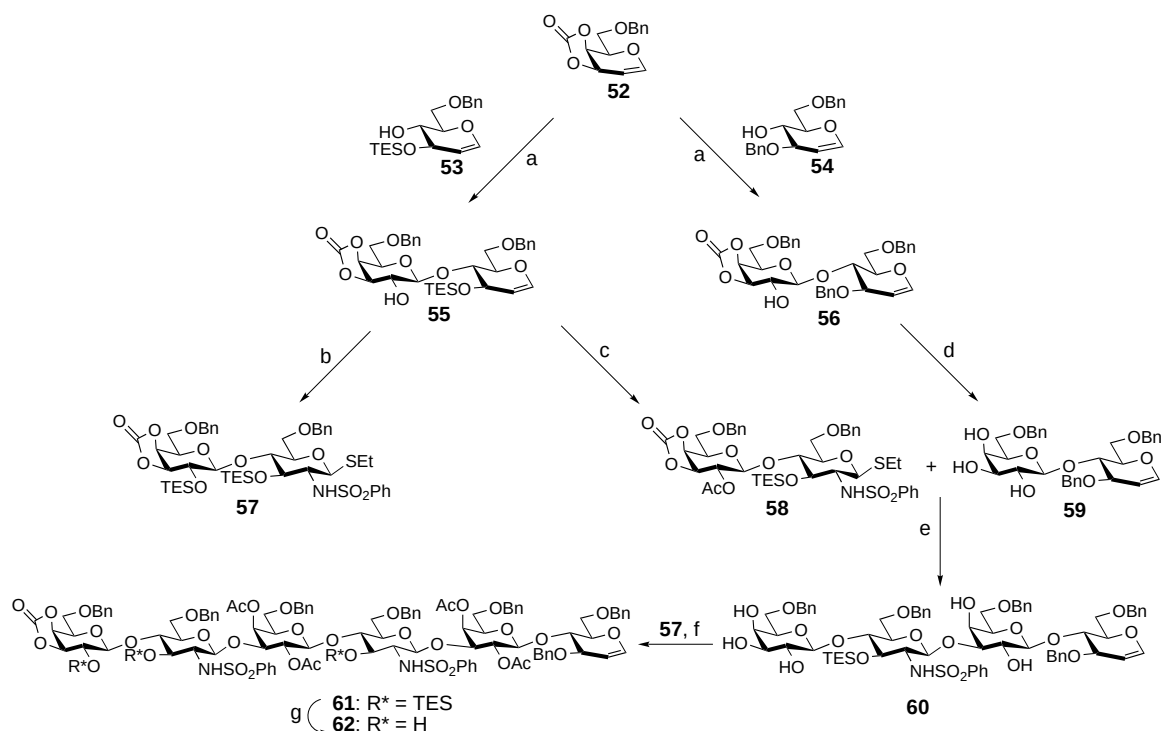
Scheme 10. Planned synthesis of KH-1 **2**. PG=generalized hydroxyl protecting group, R=nitrogen protecting group, R*=unique oxygen protecting group.

sites (R*) and a third for the remaining hydroxyls. As we have successfully demonstrated before, the terminal glycal func-

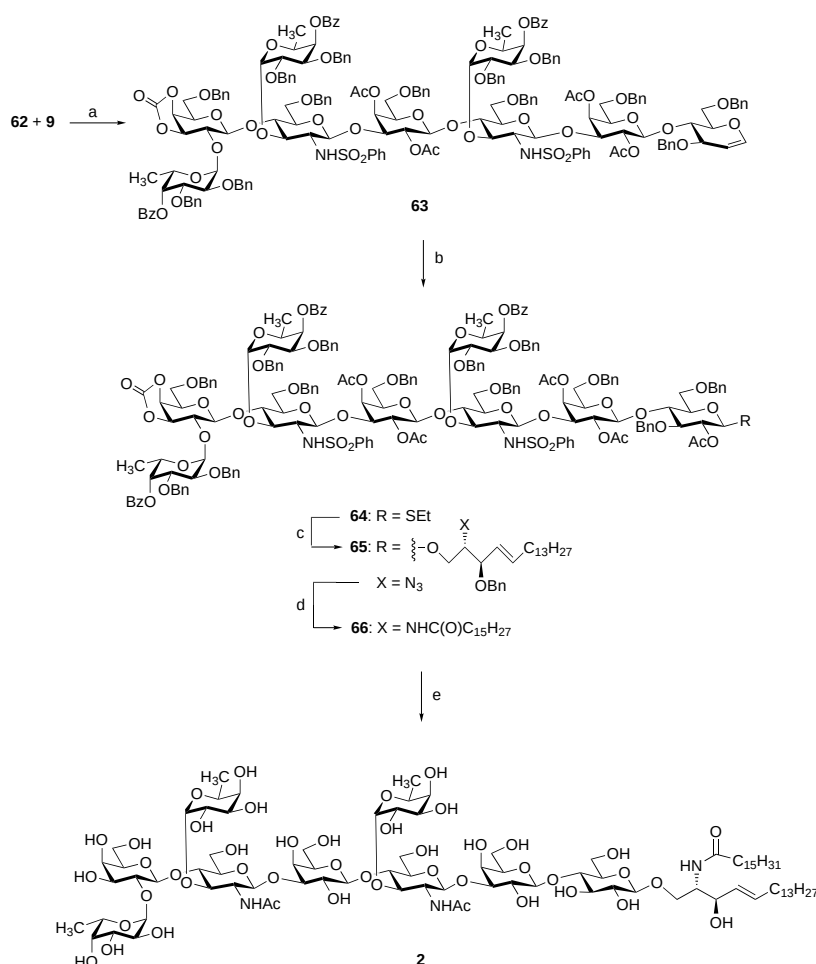
tionality in **51** could then be used to provide access to the native KH-1 antigen **2** or to bioconjugates, en route to evaluable antiadenocarcinoma vaccines. The synthesis based on such a plan is depicted in Scheme 10.

Suffice it to say, the construction of the hexasaccharide glycal which corresponds to structure **49** was a large undertaking in itself. The synthesis of differentially protected glycal **61** is presented in Scheme 11 without detailed discussion. It is possible to benefit from the conciseness of using glycal building blocks **52**, **53**, and **54** for rapid construction of large oligosaccharides, as shown in Scheme 11. Deprotection of **61** to yield **62** then allowed for the culminating stage of the plan to be put in place.

As hoped for, it proved possible to install the three α -L-fucose residues in a single synthetic step via fluoro donor **9** thereby affording the nonasaccharide glycal **63** in an exemplary yield (Scheme 12). From this point, the protocols required to reach **2** and related systems were much influenced by our earlier work in the Globo-H and Le^x series. The first step in the introduction of the ceramide side chain to reach the natural KH-1 antigen **2**, was the epoxidation of glycal **63**. While this reaction seemed to occur smoothly, attempts to use the epoxide directly as a glycosyl donor with acceptor **19** gave low yields of coupled products. Accordingly, we turned to the application of a recently developed variation of the glycal epoxy donor method.^[64] This protocol started with epoxidation of **63** with dimethyldioxirane, followed by thiolation of the resulting epoxide and further acetylation, thus leading to acetate **64**. With the expectation of effective neighboring group participation by the C2 acetoxyl function available to



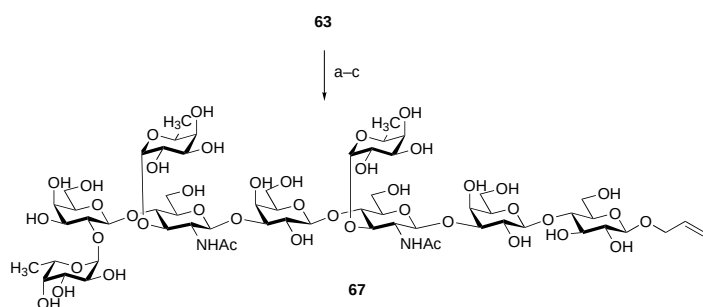
Scheme 11. Synthesis of hexasaccharide **62**. a) 1. DMDO, CH₂Cl₂; 2. **53** or **54**, ZnCl₂, THF, 65% for **55** and 55% for **56**; b) 1. TESOTf, Et₃N, CH₂Cl₂, 92%; 2. I(coll)₂ClO₄, PhSO₂NH₂, 4 Å molecular sieves, CH₂Cl₂, 90%; 3. LHMDs, EtSH, DMF, 90%; c) 1. Ac₂O, Et₃N, DMAP, CH₂Cl₂, 95%; 2. I(coll)₂ClO₄, PhSO₂NH₂, 4 Å molecular sieves, CH₂Cl₂, 90%; 3. LHMDs, EtSH, DMF, 4. Ac₂O, Et₃N, DMAP, CH₂Cl₂, 85%; d) K₂CO₃, MeOH, 80%; e) 1. MeOTf, DTBP, Et₂O/CH₂Cl₂ (2:1), 4 Å molecular sieves, 60%; 2. Ac₂O, Pyridine, DMAP, CH₂Cl₂, 95%; g) TBAF/AcOH, 93%.



Scheme 12. Total synthesis of the KH-1 antigen **2**. a) $\text{Sn}(\text{OTf})_2$, Toluene/THF (10:1), 4 Å molecular sieves, 60%; b) 1. DMDO, CH_2Cl_2 ; 2. EtSH , CH_2Cl_2 , H^+ (cat.); 3. Ac_2O , Pyridine, CH_2Cl_2 ; 60% over 3 steps; c) **19**, MeOTf , $\text{Et}_2\text{O}/\text{CH}_2\text{Cl}_2$ (2:1), 4 Å molecular sieves, 55%; d) Lindlar's catalyst, H_2 , palmitic anhydride, EtOAc , 85%; e) 1. Na/NH_3 , THF then MeOH ; 2. Ac_2O , Et_3N , DMAP, CH_2Cl_2 ; 3. MeONa , MeOH , 70% over 3 steps.

guide a β -glycosidation, compound **64** was treated with **19** under the agency of methyl triflate. This process indeed led to the formation of glycoside **65**, in greatly improved yield. From this point, the required methodology was quite familiar to us and the total synthesis of the KH-1 antigen **2** was completed as shown in Scheme 12.

For immunological studies, the allyl glycoside was again the goal system. For this purpose, we returned to glycal **63**.



Scheme 13. Synthesis of allyl glycoside **67**. a) 1. Na/NH_3 , then MeOH ; 2. Ac_2O , pyridine, DMAP; b) 1. DMDO, CH_2Cl_2 ; 2. allyl alcohol; c) NaOMe , MeOH , 60% over 3 steps.

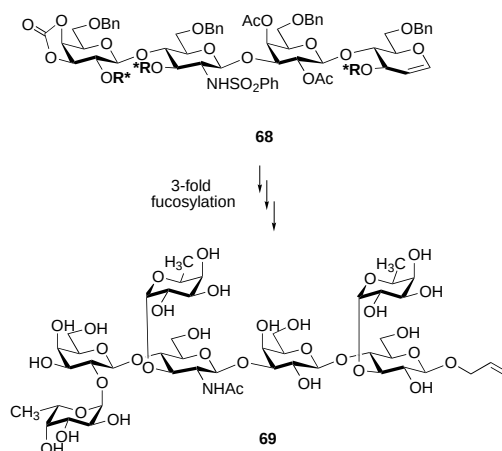
Removal of the protective groups followed by esterification gave the peracetylated glycal. Epoxidation under standard conditions and solvolysis with allyl alcohol installed the necessary spacer region. Exhaustive deacetylation yielded fully deprotected nonasaccharide **67** (Scheme 13) which was then conjugated to KLH as in the previous vaccine preparations.

The structures of the products **2** and **67** were fully substantiated by mass spectroscopy, by self-consistent NMR analysis and, in the case of **2**, by comparison with the available fragmentary published data. A direct material comparison was not possible because of the nonfeasibility of obtaining the KH-1 antigen from natural sources. Owing to this synthesis, access to the KH-1 system is no longer an impassable problem.

In preparing for immunological investigations, it would be helpful to determine the specificities of various antibodies to the structural features of the KH-1 antigen. Toward that end, it was of interest to generate truncated structures in which segments of the molecule would be deleted. In our first such effort, we directed our attentions to a construct in which the three fucose residues, as well as an *N*-acetyl function, would be retained (**69**, Scheme 14). However, the reducing terminal *N*-acetylglucosamine substructure would be deleted. Again, we relied on a three fold fucosylation procedure to install the required linkages and the remaining steps in the conversion of fucosylated **68** to construct **69** ran parallel to those used in the synthesis of **67** (Scheme 14). Indeed, the

strategy of threefold fucosylation to achieve conciseness in the synthesis was successfully demonstrated.

With all of the goals from a chemical standpoint realized, the focus on the KH-1 antigen shifted to issues of immunology and vaccinology. Mice were immunized with the fully



Scheme 14. Synthesis of a truncated version of **2**.

synthetic KH-1 vaccine containing **67** which was administered together with QS-21 as an immunoadjuvant. The mice responded to the vaccine with strong antibody titers. Studies of the immunological properties of the murine antibodies so elicited, with a view to possible clinical trials, are well underway and the results will be disclosed shortly.^[65]

7. Future Vaccine Prospects

7.1. Synthesis of the Small Cell Lung Carcinoma Antigen Fucosyl GM₁

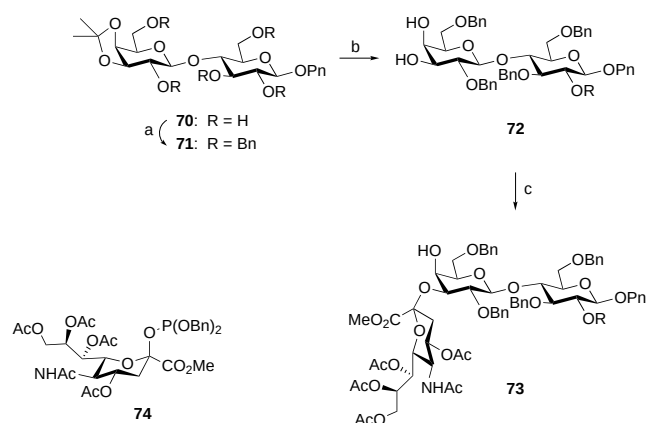
In bringing the synthesis of this review to a close, we update the reader on our recently completed, but as yet unpublished, total syntheses of the fucosyl GM₁ and N3 antigens. Clearly, in approaching these studies we were much influenced by our previous successes. The biological and chemical rationale for addressing these targets will be described only briefly. They integrate these syntheses into the context of our ongoing vaccinology program.

The glycolipid fucosyl GM₁, **7** (Figure 1), has been identified as a highly specific marker associated with small cell lung cancer (SCLC) cells.^[66, 67] At the outset of our investigations, no total syntheses of **7** had been accomplished. Because of the tumor specificity which antigen **7** displays,^[68] we were encouraged not only to perform a total synthesis of the requisite hexasaccharide core, but to devise a synthesis capable of generating material for possible clinical evaluation as well. Our recent efforts towards the synthesis of the pentenyl glycoside of fucosyl GM₁ are described below.^[69]

Recognition that the three saccharides at the nonreducing end of **7** are identical to the DEF portion contained in Globo-H **22** (Scheme 4) disposed us to think in terms of another [3+3] coupling. Using this disconnection however, would require a potentially difficult merger using a trisaccharide, which contains a protected sialic acid residue, as the acceptor. We assumed this risk mindful of our earlier precedent, however imperfect, from the Globo-H series. We also drew inspiration from another precedent from our laboratory, practice in the assembly of GM₁ using a related sulfonamidoglycosylation.^[70] For vaccine development involving fucosyl GM₁, we envisaged the installation of a functionalized glucopyranoside at an early stage in the synthesis, rather than relying on manipulation of the fully mature oligosaccharide.

The synthesis of the requisite trisaccharide acceptor is shown in Scheme 15. Pentenyl lactoside^[71] was converted to its thermodynamic C3',C4'-acetonide to give **70** and subsequently perbenzylated to give the differentially protected **71**. Removal of the acetonide protecting group revealed two hydroxyl groups to give **72**. Diol **72** would successively act as an acceptor in, first, a sialylation reaction with **74** and, secondly, in the [3+3] coupling. Reaction of phosphite donor **74**^[72] with TMSOTf in the presence of **72** gave the trisaccharide **73** as the only observable trisaccharide product.

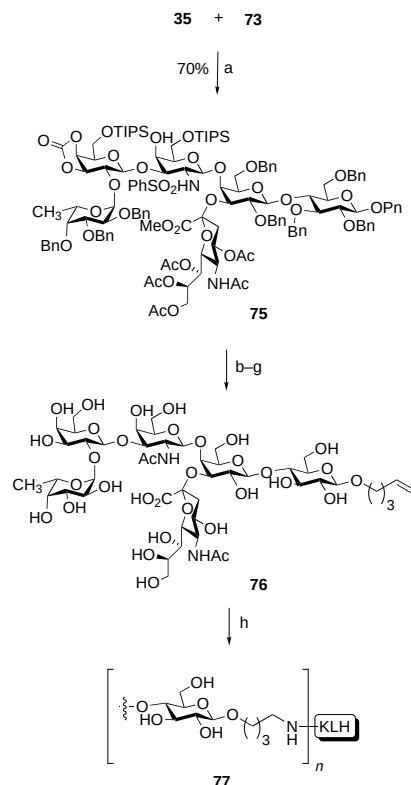
Trisaccharide donor **35** is readily accessible from the Globo-H synthesis (see Scheme 6). Coupling of **35** to **73** under the action of MeOTf proceeded to give the hexasaccharide **75** in good yield, again demonstrating the power of the azaglyco-



Scheme 15. Synthesis of **73**. a) BnBr, NaH, DMF, 84%; b) AcOH/H₂O (4:1), 90%; c) **74**, TMSOTf, EtCN, 4 Å molecular sieves, −40 °C, 75%.

sylation sequence (Scheme 16). Hexasaccharide **75** was deprotected under standard conditions to yield the pentenyl glycoside of fucosyl GM₁ **76**. Our assignment of the structure **76** was based on NMR analysis of intermediates en route to the final structure and was supported by high resolution mass spectrometry. This constituted the first total synthesis of the fucosyl GM₁ hexasaccharide core.

Also of notice in this synthesis was that the pentenyl glycoside modification allowed for a much more efficient synthesis of potential conjugation precursors because late



Scheme 16. Synthesis of **77**. a) MeOTf, CH₂Cl₂/Et₂O, 0 °C, 70%; b) TBAF, AcOH, THF; c) NaOMe, MeOH; d) NaOH, THF; e) Na/NH₃, THF, −78 °C, then MeOH; f) Ac₂O, pyridine, DMAP, CH₂Cl₂, 46% over 5 steps; g) Steps c and d, 96%; h) 1. O₃, MeOH; Me₂S; 2. KLH, NaCNBH₃, phosphate buffer.

stage adjustments (in some cases rather costly) were effectively avoided. We are actively pursuing this strategy for immunoconjugation in other cases.^[73]

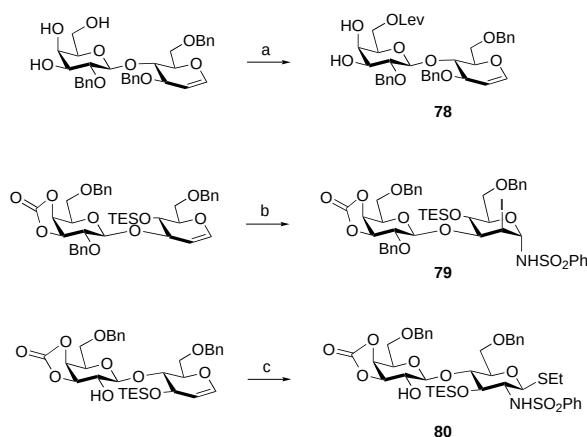
Fucosyl GM₁ pentenyl glycoside **76** has been immunoconjugated to carrier protein KLH to give glycoconjugate **77**. Importantly, as was shown with this result, the pentenyl linker apparently served as well as the allyl linker for conjugation purposes. Initial control studies with **76** and mouse vaccination studies with KLH-conjugate **77** are currently underway.

7.2. Synthesis of the N3 Antigens

The presence of anti-N3 antibodies in the serum of early stage gastrointestinal (GI) cancer patients has been shown to correlate with the development of GI cancer. We, as well as others,^[74] have high hopes that a suitably conjugated version of this antigen could be used to detect the onset of even minuscule amounts of the N3 antibody. A group in our laboratory has recently completed a total synthesis of the N3 major and N3 minor antigens **4a** and **4b** (Figure 1), as well as the more biomedically versatile allyl glycosides.^[75]

As described earlier (Scheme 12), central to our synthesis of KH-1 was the concept of polyfucosylation of a suitably protected acceptor. The structural features of N3 antibodies (namely a high degree of branched fucose residues) suggested that a similar approach could be invoked on a hexasaccharide core. It was also soon recognized that the required hexasaccharide core could be obtained from three disaccharide building blocks using glycal assembly methods.

The challenge of appropriate protection and deprotection sequences played a determining role in achieving this goal. The disaccharide building blocks needed for N3 major and N3 minor antibodies are shown in Scheme 17. Disaccharide **78** contains a differentiating levulinate (4-oxopentanoate) protecting group at C6', formed by a selective acylation. Synthesis of the iodosulfonamide **79** was achieved from the corresponding glycal, itself available from glycal building blocks. The required differentiating protecting group in **79** is the C4 silyl ether. Finally, donor **80** originated from the corresponding



Scheme 17. Synthesis of the disaccharide building blocks **78**–**80**. a) Levulinic acid, CMPI, Et₃N, dioxane, 91%; b) I(coll)₂ClO₄, PhSO₂NH₂, CH₂Cl₂, 90%; c) 1. Step b, 94%; 2. LHMDs, EtSH, DMF, 85%.

lactal derivative and contains a C3 silyl ether for appropriate unmasking.

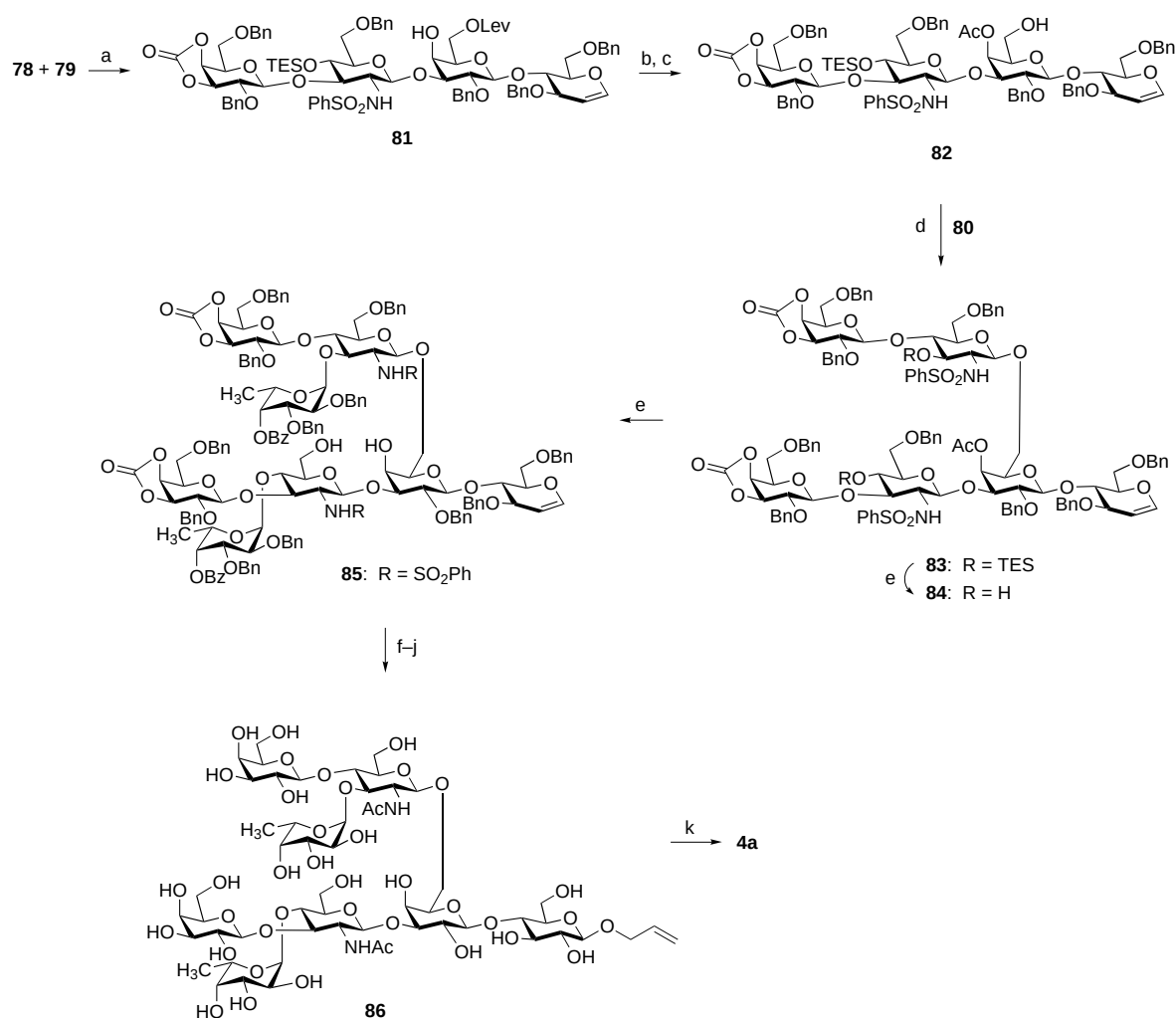
We relate here the synthesis of the major N3 antigen **4a**. (Scheme 18). Iodosulfonamide donor **79** was coupled directly to the stannane derived from **78** to yield the tetrasaccharide **81**. To allow for incorporation of the second disaccharide subunit, the resulting free hydroxyl group was acetylated, followed by the selective unmasking of the C6' levulinate. Coupling of **82** with donor **80** gave the hexasaccharide core **83** as a glycal. Clearly, a significant amount of design was required to reach this structure. This concept was exemplified by subsequent removal of the two silyl protecting groups in **83** to reveal the two new acceptor sites for the expectant fucosylation. Indeed we were pleased when bisfucosylation of diol **84** using fluoro donor **9** provided the fully protected octasaccharide **85**. The sequence of deprotection, epoxidation, solvolysis with allyl alcohol and deacetylation were performed to yield bioconjugatable allyl glycoside **86**. Removal of the allyl group completed the synthesis of **4a**, as a mixture of α - and β -isomers. Following a similar course, the minor N3 antigen **4b** and its allyl glycoside were also synthesized starting from disaccharide blocks **78** and **80**. Further evaluation of the fully synthetic N3 antigens is in progress and the results of these inquiries will be disclosed in due course.

8. Antigen Clusters as Carbohydrate-Based Vaccines

As a second-generation synthetic investigation in our program, we began to undertake the development of synthetic methodology of general applicability for the preparation of glycopeptide-based vaccine constructs that mimic the cell-surface of tumor cells. Our initial focus has been on mucin-related O-linked glycopeptides. The remainder of this account will emphasize our efforts to synthesize and demonstrate that trimeric clusters of glycopeptides, appropriately bioconjugated, are immunogenic as judged by antibody production and that these clustered constructs warrant further investigation as glycopeptide-based anticancer vaccines. In fact, one construct is beginning phase II human clinical trials.

Mucins, which comprise a family of large glycoproteins expressed on cells of epithelial tissues, carry large glycodomains in clustered modes.^[76] Mucin amino acid sequences possess a very high percentage of serine and threonine residues, often found in contiguous arrays ranging in number from two to five. In most cases, the details of the occupancy of such blocks of serine and threonine subunits are not known in detail.^[77] Despite a large variety of mucin glyco-structures, the modality wherein the first residue, an *N*-acetylgalactosamine moiety, is linked to a serine or threonine residue via an α -linkage appears to be broadly conserved (Figure 8). The glycophorin family of α -O-linked carbohydrates is a well-known and studied class of tumor antigens.

The Tn antigen **5** (Figure 1) represents the simplest member of the glycophorin family, as shown in Figure 8. This antigen, as well as the related Thomsen–Friedenreich disaccharide (TF) antigen **6** (Figure 1) is quite common in



Scheme 18. Synthesis of **4a**. a) $(\text{Bu}_3\text{Sn})_2\text{O}$, benzene, **78**; then **79**, AgBF_4 , THF, 4 Å molecular sieves, 67%; b) Ac_2O , DMAP (cat.), pyridine, 88%; c) H_2NNH_2 , pyridine, AcOH, 83%; d) TBAF, AcOH, 93%; e) **9**, $\text{Sn}(\text{OTf})_2$, DTBP, 4 Å molecular sieves, toluene/THF (10:1), 76%; f) Na/NH_3 , then MeOH; g) Ac_2O , pyridine, DMAP, 58% over 2 steps; h) DMDO, CH_2Cl_2 ; i) allyl alcohol, 41% over 2 steps; j) NaOMe , MeOH, 88%; k) PdCl_2 , wet MeOH, quantitative yield.

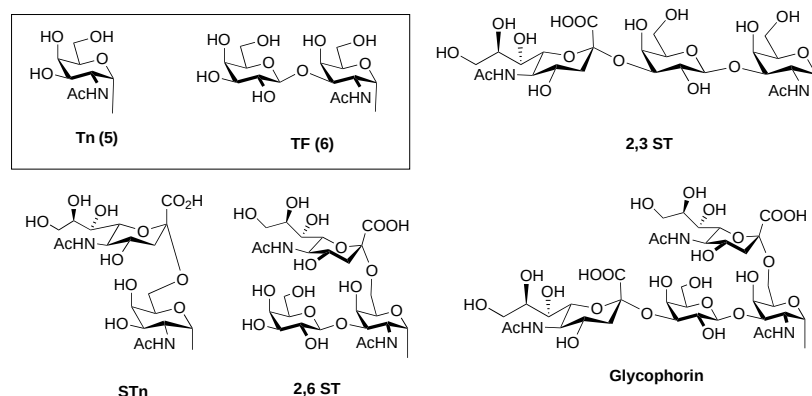


Figure 8. The glycophorin family of α -O-linked antigens.

carcinoma malignancies, particularly of the colon and prostate.^[78] These simple carbohydrate antigens have been synthesized and their immunogenicity in conjugate vaccines confirmed.^[79] For example, antibody titers against STn have been reported to correlate with improved prognosis in breast cancer patients.^[80] Comparable studies with more complex

carbohydrates have rarely been described, thus we developed a clear interest in the synthesis of large clustered forms of these antigens.

9. The Cassette Approach

The Tn and TF antigens have previously been prepared by a number of methods,^[81] however at the time of our investigations, there remained a long-standing problem in glycopeptide synthesis.^[82] The crux of the difficulty has been the problematic character of synthesizing carbohydrate domains O-linked to the key amino acids, serine and threonine, with strong stereochemical control in the formation of the α -glycosidic linkage. After much study with more complex targets, we were unable to provide a generally reliable protocol that would deliver the required amino acid to fully mature substrates of our choosing with high α -selectivity.

The glycopeptide assembly method that we have consequently developed and come to rely on is that of “cassette” modality rather than a maximally convergent approach.^[83] In the cassette strategy, we build an *N*-acetylgalactosamine synthon stereospecifically O-linked to a serine (or threonine) residue with a differentiable acceptor site on the GalNAc. This construct serves as a general insert (cassette) that is joined to a target saccharide bearing a glycosyl donor function at its reducing end. In this way, we avoid the need for direct coupling of the serine side chain hydroxyl group to a fully elaborated, already complex saccharide donor. The clear advantage of this method is the need to only solve the very difficult O-linkage problem once for a given “reducing end” and to exploit that capability for building on the desired clustered system. Figure 9 demonstrates the logic of the cassette strategy. It is with this strategy in mind that we set out to synthesize clustered Tn antigen immunogenic structures and also put the cassette model to the test with the simplest case of clustered TF antigenic structures. With a successful venture, clusters containing other members of the glycophorin family of antigens would ultimately be within reach.

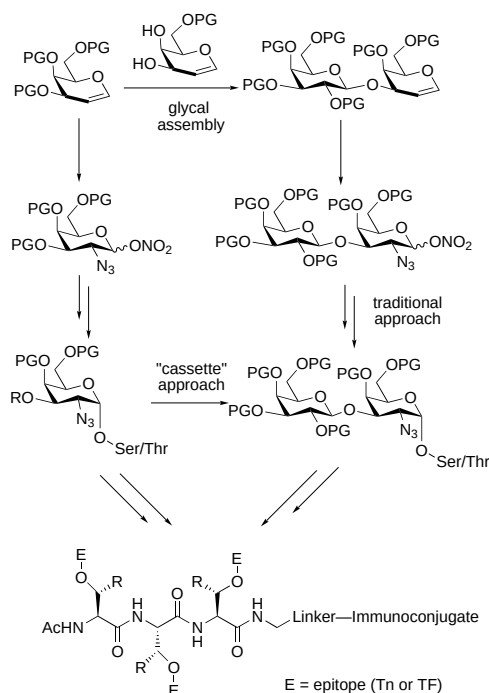
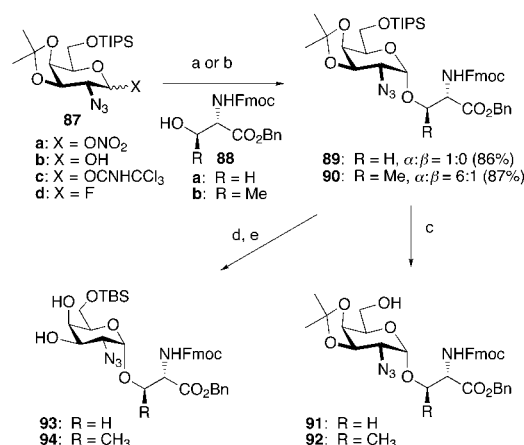


Figure 9. The cassette method for glycopeptide synthesis.

The most appealing approach we have found to the desired α -O-linked cassettes is shown in Scheme 19.^[84] Donors **87c** and **87d** were generated from anomeric alcohol **87b**, itself available by azidonitration^[85] of the corresponding glycal to give **87a**. In the case of serine derived acceptor, the glycosylation ratio apparently gave only the α -product **89**. In the synthesis of the threonine product **90** only a small amount of β -product was noted. Both preparations proceeded in excellent yield. Thus, the idea then emerged to use **89** and **90** as general inserts (cassettes) to be installed toward the end



Scheme 19. Synthesis of cassettes **91–94**. a) R = H: **87c**, TMSOTf, THF, -78°C ; b) R = Me: **87d**, [Cp₂ZrCl₂], AgOTf, CH₂Cl₂, c) TBAF, AcOH, THF, 94–100%; d) I₂/MeOH (63–81 %); e) TBSCl, imidazole, DMF, 64–85 %.

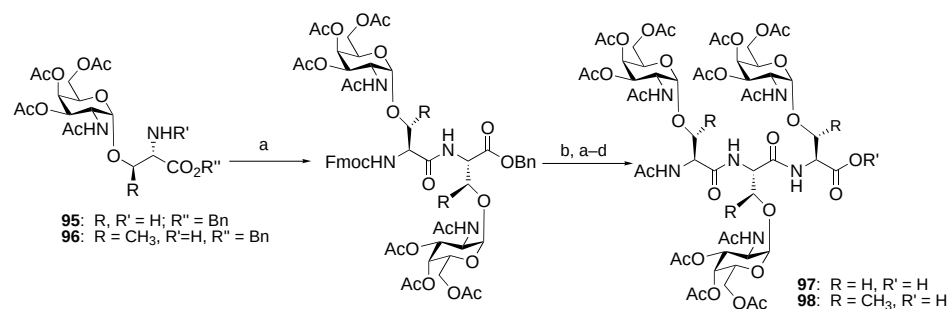
of a complex synthesis. However, to implement this strategy, a variety of orthogonally protected modules for further use as glycosyl acceptors were required. Accordingly, with the transformations shown in Scheme 19, ready access to position 6 acceptors **91** and **92**, or position 3 acceptors **93** or **94** was achieved.

10. Tn-Clustered Immunoconjugates

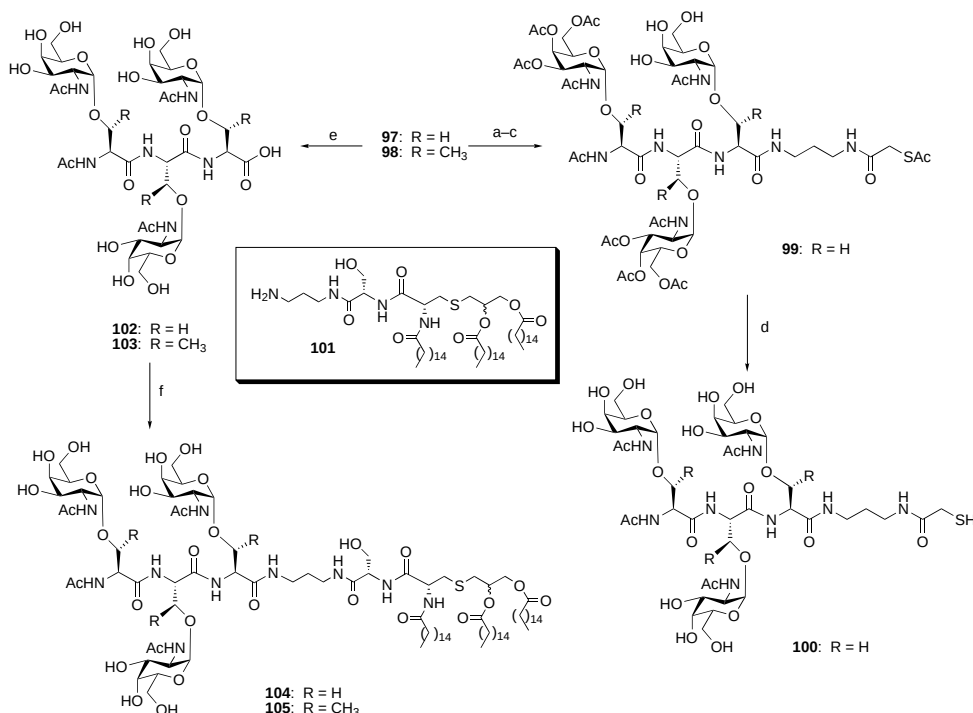
10.1. Synthesis of Tn-Clustered Immunoconjugates

With respect to synthesizing a Tn cluster, we turned to compounds **95** and **96**.^[86] Due to the lack of reliable information regarding which serine or threonine residues within a contiguous array constitute an optimal epitope, a sequence of three consecutive Ser/Thr residues was chosen for initial evaluation.^[87] Following standard peptide coupling procedures the synthesis of the trimeric clusters **97** and **98** was completed (Scheme 20). In bringing the synthesis to completion, the amino terminus was capped and the remaining carboxy terminus was freed to allow for further modification. For the purposes of creating a functional vaccine, such modification included conjugation to a synthetic lipopeptide as the immunological activator, or to an immunogenic carrier protein as in previous studies.

The two pathways that were followed for eventual conjugation are shown in Scheme 21. The first pathway involved attachment of a suitable linker for conjugation with a carrier protein. In a slightly different protocol with regard to our previous constructs, we investigated the mercaptoacetamide unit for this purpose.^[88] Acid **97** was coupled with *tert*-butyl-*N*-(3-aminopropyl)carbamate with the agency of IIDQ. This step was followed by removal of the previously introduced Boc cap, and coupling with *S*-acetylthioglycolic acid pentafluorophenyl ester. The resulting fully protected glycopeptide **99** was then subjected to methanolysis under carefully controlled conditions (pH $\approx$ 9, degassed MeOH) to give clustered **100** which was now ready to be conjugated to the appropriate carrier (KLH or BSA).



Scheme 20. Synthesis of the trimeric Tn cluster. a) **95/96** (where R' = Fmoc, R'' = H), IIDQ, CH₂Cl₂, 85–97%; b) 20% morpholine in DMF, 90–100%; c) Ac₂O, CH₂Cl₂, 70–76%; d) Pd/C, H₂, MeOH, H₂O, 85–95%.



Scheme 21. Preparation of the clustered immunoconjugates **100**, **104**, and **105**. a) H₂N(CH₂)₃NHBoc, IIDQ, CH₂Cl₂; b) TFA, CH₂Cl₂; c) SAMA-(OPfp), DIEA, CH₂Cl₂, 81%; d) NaOMe, MeOH (degassed), 85%; e) NaOH, MeOH, 95%; f) **101**, NHS, EDC, DMF, DIEA or HOAt, HATu, DMF, collidine, 35–40%.

For the synthesis of a fully synthetic lipopeptide, we followed the method of Tokoyuni et al. for attaching tripalmitoyl-*S*-glycerylcysteinylserine (Pam₃Cys **101**).^[89] Pam₃Cys has proven to be a potent macrophage and B lymphocyte activator, and has been pioneered for purposes similar to ours by Tokoyuni and co-workers with one to three epitopes of serine Tn.^[90] First, careful saponification of **97** or **98** with NaOMe/MeOH gave the fully deprotected glycopeptides **102** or **103**. Coupling with amine **101** using either the NHS or HOAt/HATU methods^[91] then afforded glycolipids **104** or **105**.

10.2. Discussion of Early Immunological Results

The initial experiments were to evaluate the antibody response to vaccination of mice with either Tn(cluster)-

lipopeptide **104** or more conventional Tn(c)-KLH or -BSA conjugates. The preparation of these conjugates started with the previously described **100**, which was covalently linked with carrier proteins.^[92] For KLH, about 317 clusters per protein were introduced, while BSA showed only 7 clusters per protein.

These conjugates plus the adjuvant QS-21, Tn(c)-pam **104** in intralipid, or **104** in intralipid plus QS-21 were used to vaccinate groups of five mice. All of these constructs proved to be immunogenic. The median IgM and IgG ELISA titers against Tn(c)-pam in sera are shown in Table 3. Although sera of mice immunized with **104** in conjunction with QS-21 failed to show strong reaction, construct **100** conjugated with KLH induced high IgM and moderate IgG titers.

The cell surface reactivities of anti-Tn(c) antibodies were also evaluated using Tn(c) positive LS-C colon cancer cells and Tn(c) negative LS-B colon cancer cells. As before, measurements involved flow cytometry assays and complement-dependent cytotoxicity (CDC) assays. Sera from mice vaccinated with **100**-KLH or **100**-BSA with QS-21 showed clear IgM reactivity with LS-C colon cancer cells by flow cytometry. Significant IgG reactivity was also seen.

Again, the arguments for a progression into human clinical trials were much augmented with these studies. A phase I human trial using **100**-KLH in a prostate cancer trial has just been completed and has produced extremely positive serological results. As a result, the synthetic construct **100**-KLH is now planned to enter phase II clinical trials in a multivalent context.

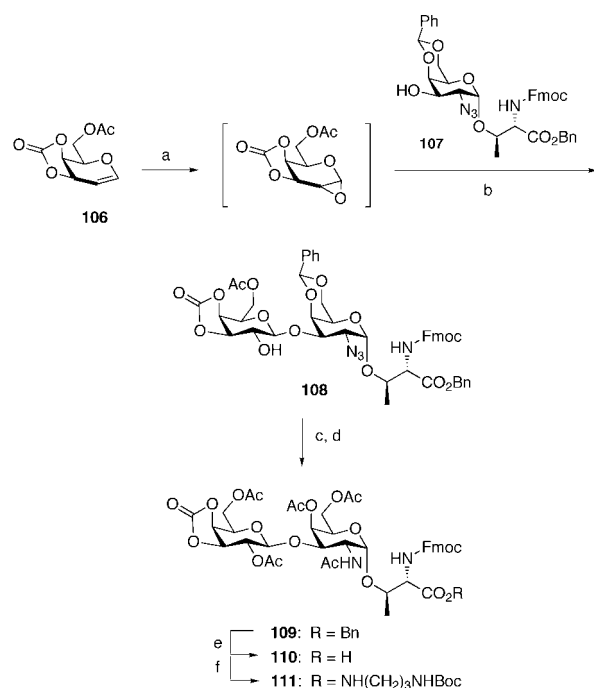
Table 3. ELISA Antibody Titers Against Tn(c).^[a]

Mice Vaccine	Vaccination			
	Before	Before	After 3rd immunization	After 3rd immunization
	IgM	IgG	IgM	IgG
104	0	0	1350	150
104 + QS-21	0	0	1350	50
100 - KLH	0	0	12150	450
100 - BSA	0	0	1350	150

[a] All titers are medians for groups of five mice.

11. Synthesis of Clustered TF Antigen

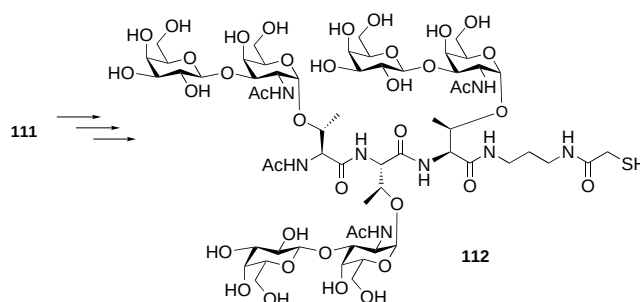
With the successful immunization of clustered antigens well demonstrated, we next directed our attention to the synthesis of clustered TF disaccharide. Early efforts revealed that constructing the α -O-linkage on the fully mature disaccharide was plagued by poor selectivity. Thus, an ideal situation for application of the cassette methodology presented itself. To implement the strategy, we turned to our glycal assembly logic to simplify the construction. The idea was to use the glycosylated amino acid with the required α -O-linkage in place as the glycosyl acceptor. As shown in Scheme 22, the



Scheme 22. Cassette coupling to provide TF disaccharide **108**. a) DMDO, CH_2Cl_2 , 0°C ; b) **107**, ZnCl_2 , THF, $-78^\circ\text{C} \rightarrow \text{RT}$, 97%; c) 1. $\text{AcOH}/\text{H}_2\text{O}$ (4:1), 70°C , 3 h; 2. Ac_2O , DMAP, TEA, CH_2Cl_2 , 93%; d) $\text{CH}_3\text{C}(\text{O})\text{SH}$, 19 h, 87%; e) Pd/C , H_2 , 2 h, quantitative yield; f) HOAt, HATU, collidine, DMF, 84%.

epoxide derived from glycal **106** proved to be a powerful donor in reaction with cassette **107** to afford the β -linked disaccharide **108**. At this point, we decided to attach the protected diamine linker first (see **111**), and then proceed on to clustering.^[93] Similar processing of intermediates as in the synthesis of the Tn cluster produced the trimeric TF cluster **112** (Scheme 23).

Following the methodology provided in the syntheses described here, our cassette strategy in combination with the glycal assembly methods have also culminated in the synthesis of two other clustered motifs containing glycophorin family



Scheme 23. Synthesis of the TF cluster.

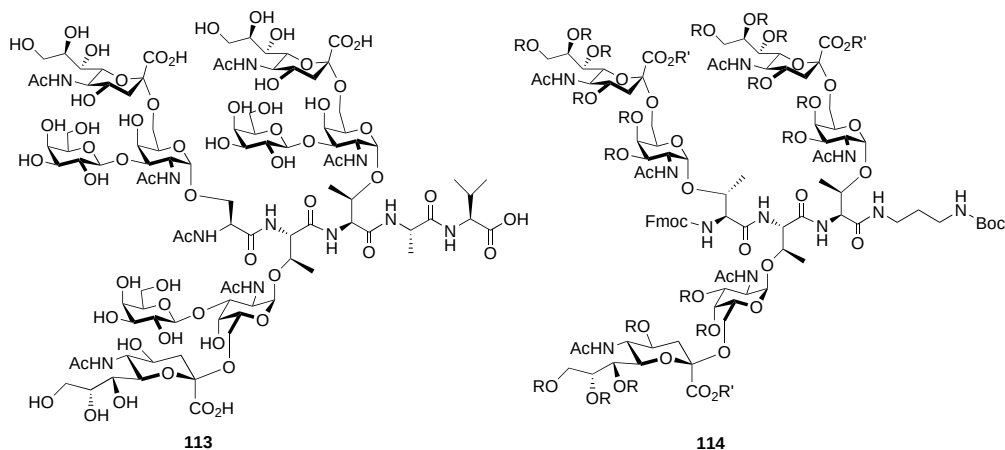
members. Structures **113**^[94] and **114**^[95] containing the 2,6-STF^[96] and STn groups,^[97] respectively, have been synthesized (Scheme 24). Conjugation and immunological evaluation of these constructs is currently well under way.

12. The Lewis^y-Clustered Glycopeptide

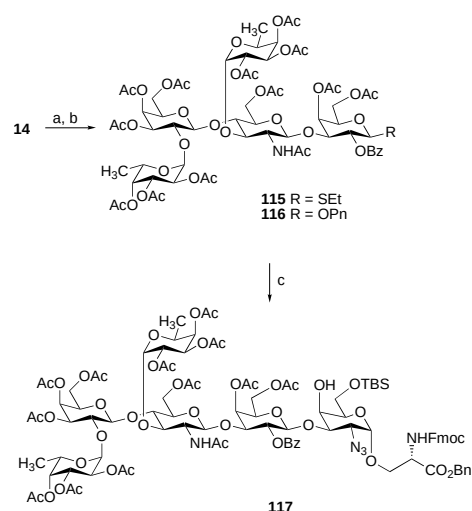
12.1. Synthesis of Lewis^y-Clustered Glycopeptide

The complexity of many issues to be overcome in pursuit of a fully synthetic homogeneous blood group determinant in a clustered setting presented a clear challenge to the science of chemical synthesis. We embarked on this challenge by confronting the synthesis of the clustered Lewis^y determinant.^[98] As always with glycopeptide clusters, we were also interested in providing for installation of a flanking sequence through the carboxy terminus culminating in the immunostimulatory Pam₃Cys moiety. Given the range of protecting groups necessary to support such a synthesis, this requirement proved to be a major element of the undertaking.

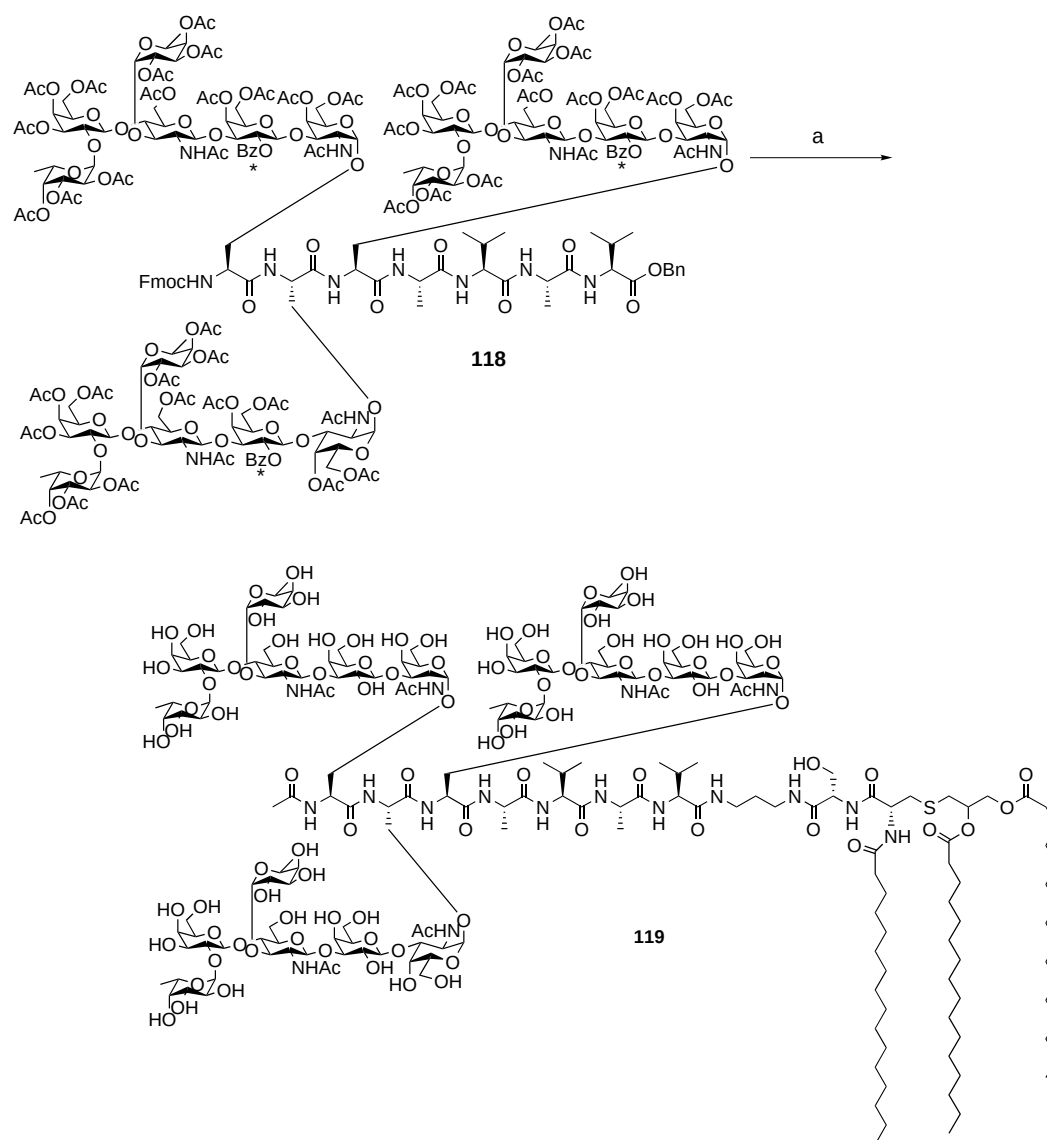
In keeping with the cassette strategy, we returned to the glycal **14** which contains the Le^y specificity (Scheme 1). Upon suitable activation of the glycal for glycosylation, serine cassette **93** (Scheme 19) was called upon to play the acceptor. As shown in Scheme 25, glycal **12** was epoxidized in the usual way and converted to the thioethyl donor **115**. In subsequent studies, the pentenyl glycoside donor **116**^[99] was also prepared by reaction of the epoxide with pentenyl alcohol.^[100] The C2



Scheme 24. 2,6-STF and STn clusters **113** and **114**.



Scheme 25. Cassette coupling to give the Le^Y pentasaccharide. a) 1. DMDO, CH₂Cl₂, 0°C; 2. EtSH, TFAA, 40–50%, or PnOH, ZnCl₂, THF, –78°C, 83%; b) BzCl, pyridine, CH₂Cl₂, DMAP, 40–50% for R = SEt, 97% for R = OPn; c) **93**, NIS, TfOH, 4 Å molecular sieves, CH₂Cl₂, 79% for R = SEt, 83% for R = OPn.



Scheme 26. Conversion of heptapeptide **118** to antigenic construct **119**. a) 1. Morpholine, Ac₂O; 2. H₂, Pd/C; 3. hydrazine hydrate; 4. **101**, HOAt, HATU.

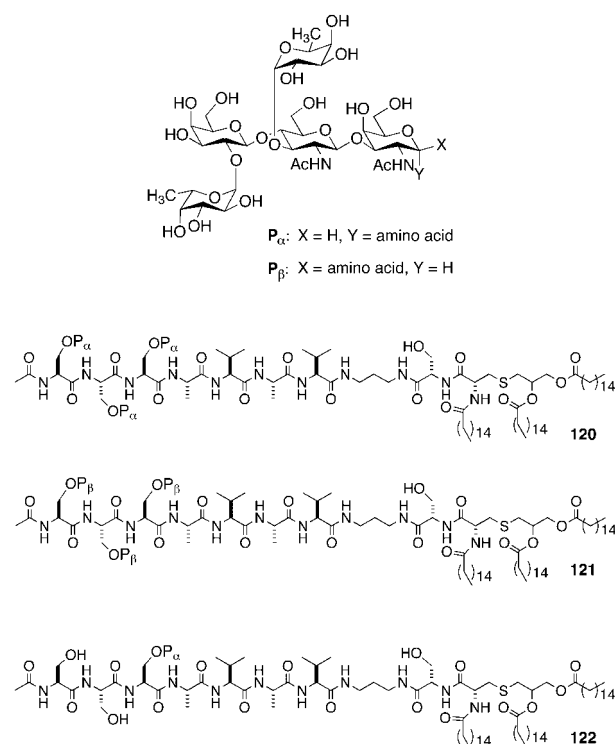
benzoate protecting group was installed to minimize orthoester formation and to allow for the desired β -selectivity in the glycosylation. In the [5+1] cassette coupling event, using NIS/TfOH for promotion,^[101] hexasaccharide **117** bearing the required serine α -O-linked to the complex carbohydrate domain was obtained in outstanding yield.

The cassette linked construct **117** was then advanced through the peptide assembly phase. Iterative peptide couplings, guided by our previous cluster syntheses, provided the trimeric cluster **118** (Scheme 26). In advancing **118** to become a functional immunoconjugate, the Fmoc-protecting group was removed and the free amine was capped by acetylation. Hydrogenolytic cleavage of the benzyl ester then exposed the C-terminal carboxyl. In the culminating global deprotection step, treatment with hydrazine hydrate in methanol smoothly cleaved the acetate and benzoate esters to afford the fully deprotected glycopeptide. The success of the hydrazinolysis step was crucial, since the benzoate protecting groups on the three galactose spacers (see asterisks) insulating the determinant from the serine residues, had resisted typical deprotection conditions.^[102] Finally, the lipid amine (Pam₃Cys) **101** was

coupled to the acid terminus of the heptapeptide under the conditions shown to afford the synthetic antigenic construct **119**, thus completing this total synthesis of a mucin like cluster of fully synthetic Le^y epitopes.

12.2. Immunogenic Consequences of Clustered Le^y Glycopeptide

Through total synthesis efforts, three additional pentasaccharide-based constructs lacking the internal galactose were prepared through a conceptually related route (Scheme 27): a trisubstituted lipopeptide **120** which retains the α -GalNAc linkage of **119**, a similar construct with a β -linked GalNAc **121**, and a singly Le^y-substituted lipopeptide **122**. In this route, when we did not follow the cassette logic, the glycopeptide



Scheme 27. Synthetic Clustered Probe Structures.

synthesis was nonstereospecific and therefore allowed for isolation of both α - and β -glycosyl serine stereoisomers. Thus, through total synthesis, including nonstereospecific total synthesis, we had the means to probe the cell surface architecture of tumor cells. The immunological evaluations conducted with the hexasaccharide construct **119** and the series of pentasaccharide constructs **120–122** were designed to make comparisons between the isomeric structures.

An ELISA was used to determine the immunological reactivities to anti-Le^y antibody 3S193^[103] of lipoglycopeptide constructs containing Le^y **120–122**, as well as the control compound Le^y-ceramide **3** (Figure 10). This antibody had been elicited by tumor cells that presumably display the cell surface mucin motif. Of the synthesized constructs, the α -O-linked hexasaccharide **120** and the β -O-linked glycopeptide containing Le^y **122** were the most reactive and were com-

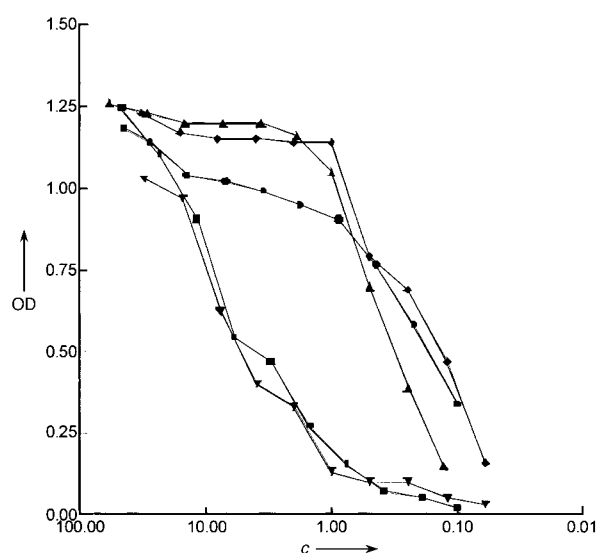


Figure 10. Reactivity of synthetic Le^y hexa- and pentasaccharide lipoglycopeptides with mouse anti-Le^y monoclonal antibody 3S193 as determined by ELISA. ♦: **119**, ■: **120**, ▲: **121**, ▼: **122**, ●: Le^y-ceramide (**3**). The optical density *O* is shown against the antigen titer in ng per test sample.

parable to the Le^y-ceramide control **3**. The α -O-linked monomer and trimeric constructs **122** and **120**, respectively, showed similar reactivity to one another, but were significantly less well bound than the control. These results suggest that the constructs having a β -linkage for the attachment of the terminal pentasaccharide most closely resemble the tumor-expressed, cell-surface Le^y antigen against which the antibody 3S193 was elicited.

In the next phase, mice were immunized with the Le^y-pentasaccharide constructs without adjuvant and the antisera were tested against Le^y-ceramide, Le^y-mucin, and Le^y-expressing tumor cells to examine the effects of antigen structure on immunogenicity and the tumor cell reactivity of the antibody response. Clustering of the glycodomain was found to be crucial for antibody production against natural substrates. The α - and β -O-linked trimeric structures **120** and **121** are highly immunogenic with levels of antibody response to Le^y-ceramide and Le^y-mucin comparable to Le^y-KLH, whereas the immunological response of the monomeric construct **122** to the same targets was poor. The same trend was observed in FACS analysis of cell surface reactivity. Antisera produced against the clustered motifs each bound Le^y positive tumor cells more efficiently than the monomeric structure (74 % versus 54 %).

Interestingly, the natural glycosidic linkage to the amino acid that is found in mucin glycoproteins is not critical for antibody production to Le^y-bearing glycolipids and mucin, as **120** is equally as immunogenic as **121**. Also noteworthy is that antibody response to the lipopeptide constructs was primarily IgM, whereas Le^y-KLH produced IgG as well as IgM antibodies. It appears that the Pam₃Cys immunomodulating unit stimulated only B cells in this study. Nonetheless, this study represents the first demonstration that immunization with synthetic antigens having clustered structures, without use of carrier proteins, mimics immunizations with cells or natural antigens. Future results in this area will be forthcoming.

13. Future Directions

It has been known for some time that specific types of glycolipids or glycoproteins, which may be detectable in normal cells by immunohistology, are more highly expressed in tumors. Furthermore, high levels of expression on tumor cells causes an antibody response, consequently rendering the cell-surface glycoconjugate recognizable as a tumor-associated antigen. The idea of such glycoconjugates as tumor-associated antigens is the basis for using carbohydrates in the development of antitumor vaccines.

Cancer carbohydrate antigens such as TF, Tn, KH-1, Le^x, and Globo-H are suitable targets for both active and passive immunotherapies because they have been carefully characterized as being over-expressed at the surface of malignant cells in a variety of cancers. In addition, they have been immunocharacterized by suitable monoclonal antibodies and therefore have relevant serological markers available for immunological studies. We have conducted such studies with the hope that patients immunized in an adjuvant setting with synthetic carbohydrate vaccines would produce antibodies reactive with cancer cells and that the production of such antibodies would mitigate against tumor spread so enabling a more favorable prognosis.

With respect to the mucin-like constructs and the cassette strategy, while complexities will undoubtedly be encountered on a case-to-case basis, we believe that the results shown here constitute validation and broad demonstration that the required chemistry can be achieved in the general case. The synthesis of **119** is a particularly striking example in which we have achieved success. In fact, we are in the process of applying the cassette methodology to the synthesis of antigenic structures containing the Globo-H epitope. Increasingly sophisticated and, we hope, increasingly realistic cell-surface molecular mimics can now be assembled and evaluated, both in regard to spectroscopy^[104] and immune recognition.

In conclusion, it is clear that chemical synthesis has met the challenge of complex glycoconjugate synthesis and will continue to do so. The ability to probe intricate structural and, in due course, mechanistic questions with regard to anticancer vaccine development will, accordingly, grow. Although the studies which may be particularly important with regard to cancer vaccines are still in their infancy, the necessary complex materials required for further preclinical and clinical studies are becoming increasingly available. It is to be hoped that the creative interfacing of complex target-oriented synthesis and immunology will bring with it clinical benefits.

Abbreviations

Ac	acetyl
Bn	benzyl
Boc	<i>tert</i> -butoxycarbonyl
BSA	bovine serum albumin
Bz	benzoyl
CDC	complement-dependent cytotoxicity

CMPI	2-chloro-1-methylpyridinium iodide
coll	collidine (2,4,6-Trimethylpyridine)
Cp	cyclopentadienyl
DAST	diethylaminosulfurtrifluoride
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMDO	3,3-dimethyldioxirane
DTBP	di- <i>tert</i> -butylpyridine
EDC	<i>N'</i> -(3-dimethylaminopropyl)- <i>N</i> -ethylcarbo-diimide
ELISA	enzyme-linked immunosorbant assay
Fmoc	fluoren-9-ylmethoxycarbonyl
HATU	<i>N</i> -[(dimethylamino)-1 <i>H</i> -1,2,3-triazole[4,5- <i>b</i>]-pyridin-1-ylmethylene]- <i>N</i> -methylmethanaminium hexafluorophosphate
HOAt	7-aza-1-hydroxy-1 <i>H</i> -benzotriazole
IIDQ	2-(2-methylpropoxy)-1(2 <i>H</i>)-quinoline carboxylic acid-(2-methylpropyl)ester
KLH	keyhole limpet hemocyanin
Lev	levulinate (4-oxopentanoate)
LHMDS	lithium bis(trimethylsilyl)amide
mAb	monoclonal antibody
NHS	<i>N</i> -hydroxysuccinimide
NIS	<i>N</i> -iodosuccinimide
OTf	trifluoromethanesulfonate
Pam ₃ Cys	tripalmitoyl- <i>S</i> -glycerylcysteinylserine
PMB	<i>para</i> -methoxybenzyl
Pn	pentenyl
PPTS	pyridinium- <i>p</i> -toluolsulfonate
PSA	prostate specific antigen
SAMA-(OPfp)	<i>S</i> -acetylmercaptoacetic acid pentafluorophenyl ester
SCLC	small cell lung cancer
TBAF	tetrabutylammoniumfluoride
TBDPS	<i>tert</i> -butyldiphenylsilyl
TBS	<i>tert</i> -butyldimethylsilyl
TEA	triethylamine
TES	triethylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
TIPS	triisopropylsilyl
TMS	trimethylsilyl

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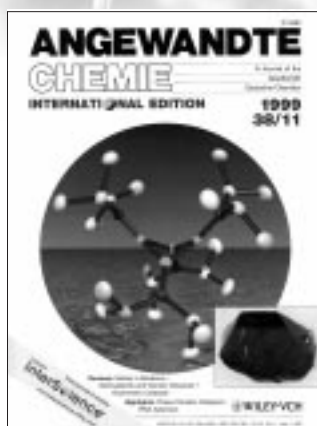
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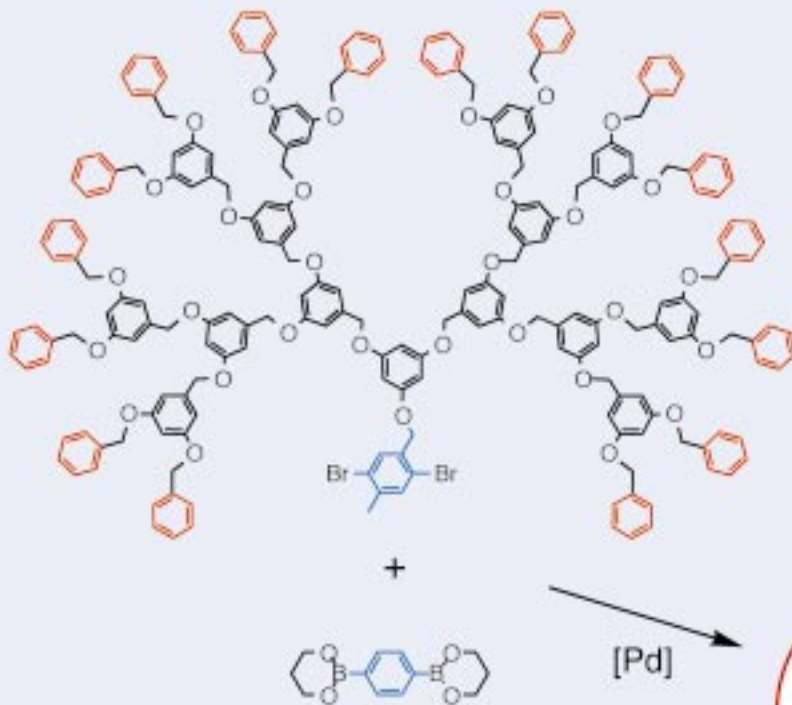


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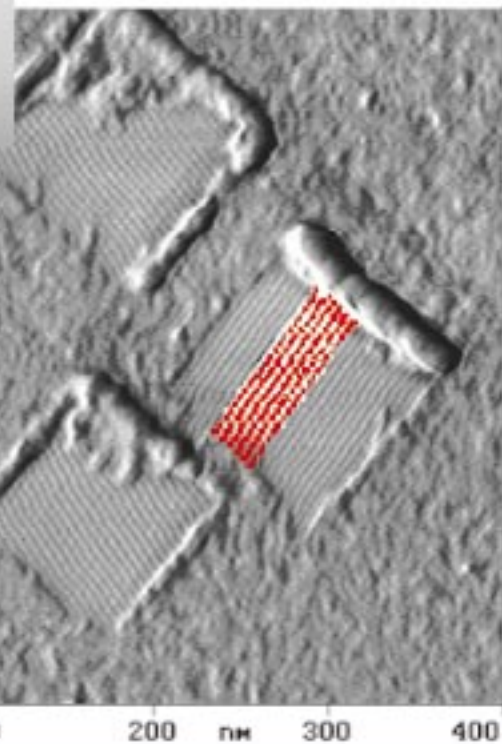
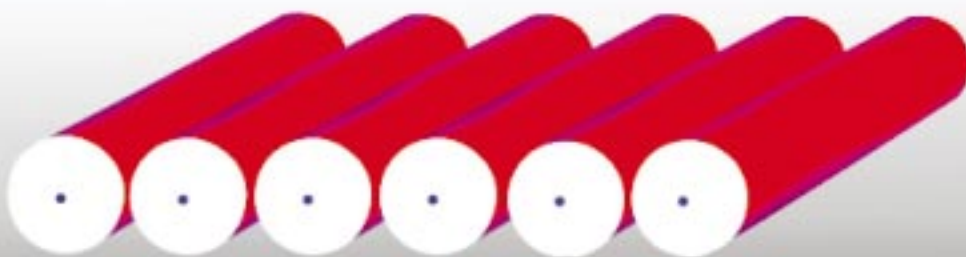
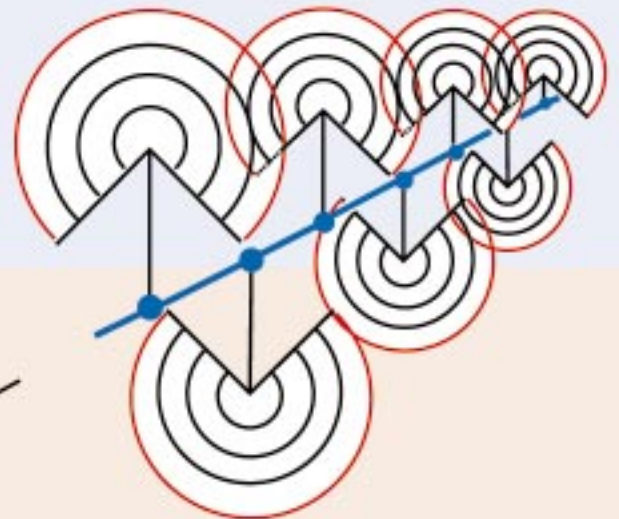
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Dendronized Polymers: Synthesis, Characterization, Assembly at Interfaces, and Manipulation

A. Dieter Schlüter* and Jürgen P. Rabe*

Dedicated to Gerhard Wegner on the occasion of his 60th birthday

Dendrimers are presently one of the most intensely studied classes of compounds because of their unusual structure. They can be described as a jungle of entangled branches traversed by winding trails which lead to sweet fruits and bright blossoms. On these trails one can reach the thicket's interior as well as find a way out. Expressed less lyrically, this thicket stands for regularly branched, densely packed structures, and the trails represent voids and channels not filled by bent back branches but by solvent. The fruit and blossoms are photochemically, electrochemically, or synthetically addressable units, catalytically active sites, etc., and the back and forth on the trails stands for transport processes. In a mathematical sense dendrimers

are enveloped by an interface, which defines what is either in or out. This interface is shaped like a sphere if the trails are filled to bursting. Otherwise dendrimers are more flattened like amoeba, especially if in contact with a surface. The high density of the functional groups, the expansion of these compounds to a range of several nanometers, the existence of usable "surface" and transport possibilities in and with them have made dendrimers interesting candidates for many applications. This review describes how dendrimer construction and polymer synthesis were combined and used to move from fully or flattened spherical shapes to cylindrical ones. The shape-inducing influence of dendritic substituents can be driven to create nano-

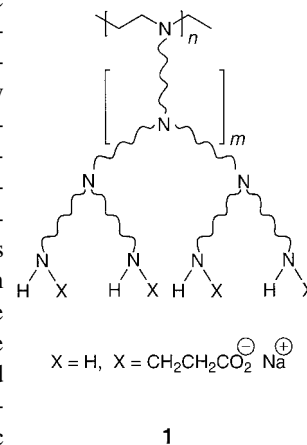
objects with a cylindrical shape, which not only considerably widens the range of applications for the dendrimer class but also opens up new perspectives for supramolecular and polymer chemistry. Because of the sheer size of the described objects and complexity of shape-related properties, research in this area must necessarily be interdisciplinary. This article tries to mirror this by giving special attention not only to synthesis but also the characterization and behavior of these compounds in bulk and at interfaces. Furthermore, potential application fields are described.

Keywords: dendrimers • interfaces • nanostructures • polycondensations • scanning force microscopy

1. Introduction

Practically all dendrimers^[1] known today have cores with a few functional groups to which the corresponding number of dendrons (dendritic wedges) are attached. The fact that these dendrons are connected to one another by a small, almost dotlike molecule results in considerable steric congestion around the core. This congestion is a unique structural feature

and has led to the viewing of dendrimers, specifically those of high generation, as molecular boxes or containers^[2] and as entities that assume a spherical shape to which a "surface" can be assigned. The term surface may, of course, only be applied with care. Since their discovery some 20 years ago, dendritic macromolecules have stimulated an almost explosive research effort and many synthetic, analytical, and application-related issues have been addressed.^[1] Even industrially applicable syntheses were developed.^[3] During this stormy process research has almost exclusively been focused on dendrimers with small cores, in spite of a US patent entitled "Rod-like Dendrimer" filed by Tomalia and Kirchoff in 1987.^[4] There the dendrimers are like **1**, with polymeric



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cores, and were proposed as being useful in the production of molecular composites and as crystallinity modifiers for polymeric materials.^[4] It took several more years before first (published) steps were undertaken to obtain these dendrimers.^[5] It is immediately apparent that these compounds not only complement dendrimers with small cores under structural aspects, but as a consequence of the structural differences they should also have unique properties (Sections 4 and 5). A simple reason for this slow development may be that in the beginning of dendrimer research spherical dendrimers were simply considered a more appropriate and perhaps more important target. Judging from the increasing number of publications, this view is presently undergoing some modification. Another reason may be seen in a reluctance to begin working on a seemingly more complex area than small-core dendrimers, where chemists are already being confronted with considerable synthetic and analytical difficulty.^[6]

This article shows that the synthetic and analytical problems associated with dendronized polymers are not outrageously more complex than for small-core dendrimers, and that the difficulties can be overcome. It tries to draw a comprehensive picture of what has been done in the field of dendrimers with polymeric cores, putting emphasis on synthetic issues, on accurate molecular weight determination, and, finally, on the aggregation behavior of these intriguing macromolecules both in the bulk and at solid and fluid interfaces. The article starts with a short section summarizing some of the ideas why dendronized polymers make up a challenging project and where these unusual molecules are expected to have impact on the natural sciences.

The macromolecules treated here may be considered as either dendrimers with polymeric core or alternatively dendronized polymers (or polymers with appendent dendrons) depending on whether one sees them from the vantage point of an organic or macromolecular chemist (Figure 1). The first view is somewhat misunderstandable, because dendrimers are normally considered monodispers (which they certainly are not^[6]) whereas polymeric dendrimers are intrinsically polydispers. At best the polymeric core may have a narrow molecular weight distribution. Not considered here are polymers which do not carry dendrons at every repeat unit, but only at a few, for example at the two termini,^[7] as well as work on surface coatings with dendrons or hyperbranched molecules.^[8] The references to dendrimers with a dotlike core are kept at a minimum, and the reader is referred to pertinent reviews and monographs.^[1, 9] A long^[10] and a short review^[11] on dendronized polymers are available. The present review covers also the many important developments in synthesis and characterization that have taken place in this rapidly moving area. Attempts to stiffen polymer chains by attaching flexible, straight oligomers to each repeat unit are not covered in this article due to space limitations.^[12]

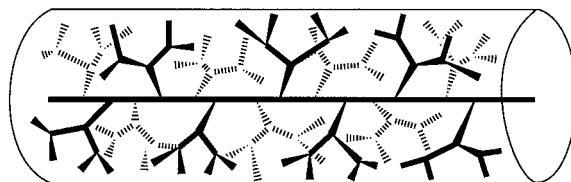


Figure 1. Cartoon representation of a dendronized polymer whose dendritic layer is so densely packed around the backbone that the backbone stretches out and a molecular cylinder with a "surface" is generated.

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A. D. Schlüter



J. P. Rabe

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2. Dendronized Polymers—What Are They Like and What Are They Good For?

Dendronized polymers are formally comb polymers,^[13] with the combs' teeth being dendrons. What makes them so unique that one does not normally refer to them as such? Mainly it is the complex interplay of dendron size, distance between dendrons, and backbone flexibility or rigidity together with several interactions (e.g. dendron/dendron, dendron/backbone, solvent/dendron). All these factors sum up to a slower decrease in density on going from the interior to the exterior than in common comb polymers where the teeth are linear chains.^[14] This unusual density profile leads to new and rather fascinating properties of otherwise conventional polymers. The first issue to mention here is the shape of the polymer and its dependence on the substitution with dendrons. Depending on the dendrons' structure, size, and attachment density along the backbone, conventional polymer backbones such as polyacrylate or polystyrene can attain conformations all the way from random-coil to fully stretched linear: A flexible, (cooked!) spaghetti-like polymer can be converted into a rigid (i.e., with a high bending modulus) rod just by proper substitution with large dendrons. This stiffening of the backbone is caused by steric repulsion between the pendant dendrons. For that reason the whole matter is referred to as shape control by implementation of steric strain. In this sense steric strain may be compared to hydrogen bonding and π,π stacking as the corresponding shape-determining factors in proteins or DNA. In the extreme case, which will be described in Section 5, the dendritic layer around the polymer backbone is so dense that a macromolecule can turn into a molecular cylinder with defined dimensions: Its length is determined by the degree of polymerization and its diameter is roughly two times the dendron extension. In this case the dendrons spread away from each other to keep steric repulsion between them at a minimum.

Dendronized polymers can, however, also be designed so that there is an attractive interaction between dendrons that leads to shape control. This requires two features: a) dendrons with mesogenic properties and b) their loose attachment to the backbone, which leaves the dendrons sufficient freedom to find the optimum packing. This kind of shape control driven by the mesogenic properties of the dendrons can lead to highly ordered materials in the solid state, as will be described in Section 4.

Let us come back to rigid polymers with a tightly packed dendritic layer around the backbone. What is the importance of such cylindrical objects? Two issues which immediately come to mind here are rigidity and surface. In contrast to conventional polymers their diameter is on the order of a few nanometers rather than a few angstroms. As a consequence the rigidity is so high that the persistence length of an individual chain is not on the order of 10 nm, but may instead exceed 50 nm. Thus, exceptionally high bending moduli can be expected, despite the fact that the polymers are still single-stranded. Dendritic rods could therefore potentially be used for constructions on the nanometer scale, very much like an architect builds frames for houses or bridges from plywood. The first steps in this direction would be to isolate individual

rods, to determine their dimensions, and to move them about. Developments in this direction will be described in Section 5. So far individual molecules have been adsorbed to atomically flat surfaces. The high modulus results in a very strong tendency of these rods to form large, ordered, two-dimensional arrays in which the individual rods are packed parallel to each other like tree trunks of a raft, the only difference being that the dendritic "trunks" have a length distribution. Such large, parallel-ordered, nanometer-scale objects on surfaces are interesting for a number of applications, for example as polarizers, polarized emitters, or orienting surfaces in liquid crystal displays.

Apropos tree trunks and cylindrical surface: This may be also a starting point to think about dendritic rods as supports with defined curvatures to which catalytically active components could be attached. In a way cylinders of the size discussed here bridge the gap between homogeneous and heterogeneous catalysis. They may combine the advantages of both. Attachment of catalytically active groups to the surface at more or less constant distances may well provide candidates, for example, for applications in flow reactors. Spherically shaped dendrimers equipped with transition metal complexes have already been successfully employed for such purposes.^[15]

At a first glance, the fact that the tobacco mosaic virus also has a cylindrical shape^[16] is a somewhat artificial link to the biosciences, but this striking similarity may nevertheless help initiate thought about the potential biological importance of dendritic rods and derivatives thereof.

In Section 5.2 we describe first steps towards another important application of dendritic cylinders. Common amphiphiles such as dodecylsulfonate can form micelles, bilayers, and other interesting and sometimes even commercially relevant supramolecular aggregates.^[17] These amphiphiles normally are a few nanometers long and have diameters of a few angstroms. If it were possible to decorate the surface of a dendronized polymer with one hydrophilic and one hydrophobic block that is segregated perpendicularly to the backbone, giant amphiphiles would result, which may lead to a big jump in the size of the corresponding aggregates. Alternatively, if it were possible to decorate the cylinders not cross-but lengthwise, the resulting amphiphiles would have the unique polarity distribution of some ion channel membrane proteins, and could be used as coatings to reverse surface polarities or for various applications as colloids.

Last but not least, the issues of synthesis and structural analysis ought to be mentioned. Before chemists can enter into all the exciting options mentioned above, dendronized polymers need to be synthesized and sufficiently characterized on both the organic chemistry and polymer level. Without going into details the main synthetic problems include a) steric repulsion and thus incomplete reactions; b) autocatalytic decomposition of sensitive dendrons because of their exceptionally high functional group density; c) high molar mass and sometimes large differences in the molar mass of reactants, which makes it difficult to meet correct stoichiometry; and d) purification of high molar mass reactants. These factors may appear almost trivial, but can brew together to a seething mixture which renders a

controlled synthesis quite an endeavour and challenge. Additionally, the size of the molecules involved makes the tools of organic chemistry absolutely insufficient. Additional methods like small-angle neutron scattering (SANS), scanning force microscopy (SFM), X-ray diffraction, and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry need to be employed to really prove what one proposes to have.

3. Synthesis

3.1. General Considerations

There are two principally different synthetic routes to dendronized polymers (Scheme 1). In the first route, the polymer which becomes the core in the final product serves as starting material. The anchor groups of this polymer are used to either divergently or convergently attach a dense sequence of dendrons (attach-to route, route A). In the second route, monomers already carrying dendrons are subjected to polymerization or polycondensation (macromonomer route, route B). The intrinsic problems of both routes become more serious and limiting, the more sterically demanding the dendrons are. An obviously critical issue associated with route A is achieving complete coverage of the backbone anchor groups with dendrons. Even if a very efficient coupling chemistry is available, a large excess of dendron may be required to drive the reaction to completion. This excess may, in turn, make it difficult to purify the product. If large dendrons are to be attached, steric hindrance also comes into play for two reasons: a) the shielding of unchanged anchor

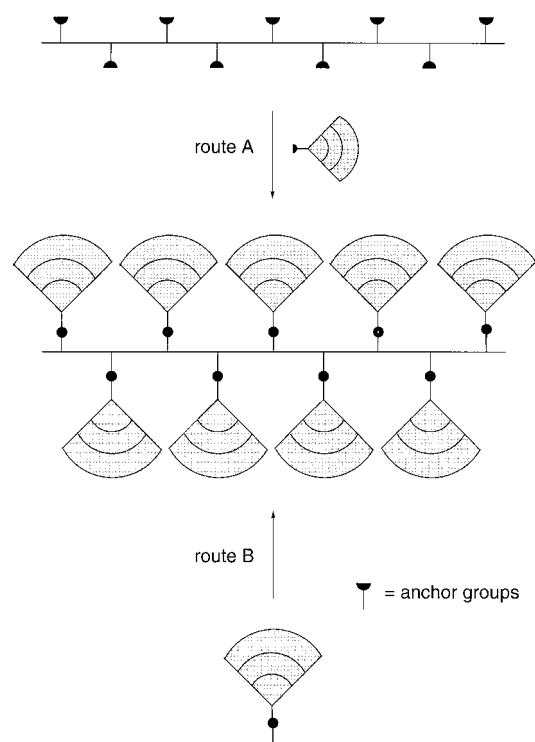
groups on the backbone by dendrons already attached in close proximity and b) the conformation of the dendron, which may lead to a self-shielding of the functional group at the focal point through which the attachment ought to take place. Both factors lead to a decrease in the rate of attachment, if they do not suppress the process entirely. This decrease of rate can favor side reactions which may not just involve themselves or the solvent but also the already attached dendrons.

The problems associated with route B also have something to do with steric hindrance. Here the critical point is the steric demand of both monomer and chain end. Incoming monomer will only be connected to the chain end if the steric hindrance is not too high. Otherwise this process will be slowed or even rendered impossible. Depending on the kind of polyreaction applied, this may lead to termination of the reactive chain end and/or to side reactions of the monomer, such as loss of coupling functionality, as in some polycondensations, or auto-initiation, specifically in radical polymerizations. From this discussion it can be extracted that the basic problems for both routes are: incomplete coverage (route A) and low molecular weight dendronized polymer (route B).

Besides hurdles to synthesis there are also hurdles to analysis. Dendronized polymers tend to have repeat units (RU) of considerable molecular weight. Repeat units with 1, 2, or even 3 kDa are no exception. Such high molecular weights sometimes render structural characterization difficult, because the proportion of backbone to dendron atoms becomes so unfavorable that NMR spectroscopy reaches its limits. For example, sometimes the degree of attachment (coverage) simply cannot be determined with sufficient accuracy, because the signal intensity of the unchanged anchor groups is too weak for comparison with reference signals in the spectrum. NMR characterization may occasionally be further complicated by large differences in the relaxation times of backbone and dendron nuclei. Thus, integrals of NMR signals are rendered unreliable if a sufficient pulse delay time is not applied.

Another problem with dendronized polymers is their molecular weight determination (Section 3.3). Gel permeation chromatography (GPC) is a quick and easy method to roughly estimate the molar mass of a polymer.^[18] This estimation can only be reasonably used as long as the hydrodynamic volumina of the polymer under consideration and the polymer used for calibration purposes, typically polystyrene (PS), are in the same range. The hydrodynamic volume of dendronized polymers strongly deviates, however, from polystyrene, and GPC results should be treated with care. Additionally, facile aggregation of these dendrimers is sometimes encountered, which leads to further complications. Other methods of molecular weight determination such as light scattering have to be used and measures taken to avoid aggregation.

Looking back over what has happened regarding synthesis of dendronized polymers since 1992, when one of the authors (A.D.S.)^[5a] and Percec et al.^[5b] independently started working in this field, one has to arrive at the conclusion that the attach-to route (route A) is inferior to the alternative macromonomer approach (route B). There is no reported case of route A in which complete coverage of a polymeric core with



Scheme 1. The two routes (A and B) to dendronized polymers. The dendrons shown are of generation three (G3).

dendrons of higher generation than G2 was achieved. Additionally the polymeric cores used (mostly of the poly(*para*-phenylene) (PPP) type) did not have very high molecular weights. For route B, however, many cases of high molecular weight G2 compounds and one case of a G3 compound are known. Furthermore, the case of a high molecular weight G4 compound was reported very recently. It is for that reason that this article concentrates on the latter case. For a recent treatment of representatives of route A, the reader is referred to another review.^[10]

3.2. Overview of the Macromonomer Approach

The synthesis of dendronized polymers from macromonomers (route B) has been developed broadly in recent years. A reason for this is the advantage that polymers, if they can be obtained at all by this method, necessarily carry the predetermined number of dendrons at the backbone. The above questions of dendron perfection and degree of coverage in divergent and convergent syntheses do not play a role. This only holds true, of course, if the dendrons are compatible with the polymerization conditions, a prerequisite which is fulfilled in many of the cases reported. The polymerization procedures used can be divided into a) radically initiated and transition metal catalyzed chain growth and (b) step-growth polymerizations (polycondensations). Figure 2 gives an overview of the macromonomer structures reported to date and groups them into the subclasses a and b depending on the respective type of polyreaction. Fréchet-type dendrons^[19] are drawn in an abbreviated form, which is explained in Figure 3. The monomers are polymerized by radical polymerization (**2**;^[5b, 20] **3**,^[21] **4**,^[22] **5**,^[23] **6**,^[24] **7**,^[25, 26] **8**,^[27] **9**,^[28] **10**,^[24] **11**,^[29] and **12**,^[30]), insertion polymerization (**13**)^[31], ring-opening metathesis polymerization (ROMP)^[32] (**14**)^[33, 34], Suzuki polycondensation (SPC)^[35] with **17** (**15**)^[36] and **16**)^[37] and **23** (**20**,^[38] **21**,^[39] and **22**)^[39], polyaddition with **19** under polyurethane formation (**18**)^[40], and Heck coupling of **24** with **25**.^[41] Dendronized oligomers with enediine RUs were also prepared.^[42]

Some features of the dendronized polymers obtained from the macromonomers of Figure 2 will become important later in this article and are therefore emphasized in the following: a) Most of the macromonomers carry G1 or G2 dendrons. The only exceptions are **12**(G3), **16**(G3), and **18**(G3, G4) (see also Section 3.4). b) Many dendrons are of the benzyl ether type whose terminal phenyl rings are either unsubstituted or have between one and three long alkoxy or fluoroalkoxy chains. The corresponding polymers are important for a variety of reasons, but are unreasonable candidates, of course, whenever chemical modification becomes an issue. c) This is where dendrons **7**, **8**, **10**, **11**, and **13**, which carry protected functional groups (hydroxy, amino, trimethylsilyl (TMS))^[43] in the periphery, come into play. The corresponding polymers are the starting point for the important aspect of surface modification (Section 6). d) Finally, dendron **8**(G2) has one unpolar and two polar branching units, and dendron **11**(G2) is completely unpolar except for its terminal, protected amine groups. These structural features are viewed in the context of synthesizing dendronized polymers with specific polarity

patterns, which will become an important matter in the future but will not be discussed in this review.

In contrast to common organic reactions, the concentration of the reaction medium plays an absolutely important role in the polymerization of macromonomers. This may go so far that below a certain concentration polymerization is not only slowed down but rather rendered impossible. This question will therefore be addressed in the following in some detail using monomer **8**(G2) as a representative example.^[27] For this monomer the dependence of molar mass on concentration was investigated systematically for $c_m = 34\text{--}64\%$, where c_m is the concentration of monomer in weight percent. As can be seen from Figure 4 (p. 872), there is only a narrow concentration window in which polymerization proceeds satisfactorily. Below $c_m = 45\%$ the monomer practically does not polymerize at all; molar masses are in the oligomer regime and yields are poor. Molar masses and yields increase sharply between $c_m = 45$ and 53% , whereby the masses pass through a maximum. For concentrations higher than $c_m = 53\%$ yields remain more or less constant; molar masses, however, decrease quickly until a plateau is reached. Similar results were observed by Tsukahara et al. for macromonomers with oligostyrene side chains.^[44] Additionally they observed threshold molarities $[M]$, at which the steep increase occurs rapidly, and shifts to higher values as the molecular weight of the macromonomer decreases. For monomers with roughly $12\,500$ and $4\,500\text{ g mol}^{-1}$ they observed threshold molarities of $[M] = 0.04$ and 0.1 , respectively. Unfortunately the molarity of the solutions of monomer **8**(G2) could not be determined with sufficient accuracy for concentrations higher than $59\text{ wt}\%$, rendering a sound comparison of the above experiments with those reported by Tsukahara et al. impossible. Qualitatively, however, monomer **8**(G2) lies in the trend.

A possible explanation for the observed maximum may be that after a few growth steps the oligomer adopts a spherical shape with the radical chain end somewhere in its interior. At low molarity, this results in a sharp concentration gradient from the interior of the sphere to the surrounding solution, against which the monomer has to diffuse to achieve growth; in other words, the concentration gradient protects the radical chain end. As the monomer concentrations in solution and in the spheres (here: monomer = pendant dendron) become similar, a gradient no longer exists and further growth can take place. At even higher concentrations the viscosity of the medium increases, which limits the mobility of large monomers and thus favors termination.^[45] For a kinetic analysis of the polymerization of G1 macromonomers, which led the authors to assume that their monomers self-assemble into molecular “reactors” prior to polymerization, see the work by Percec et al.^[46] A recent review on poly(macromonomer)s is also available.^[47]

This example makes impressingly clear how important it is to use unusually high concentrations and to carefully investigate the optimum concentration range. One simply does not get any polymeric material otherwise. This may be falsely attributed to detrimental steric congestion as the monomer approaches the reactive chain end and lead to the wrong impression that polymerization of a certain monomer is intrinsically impossible.

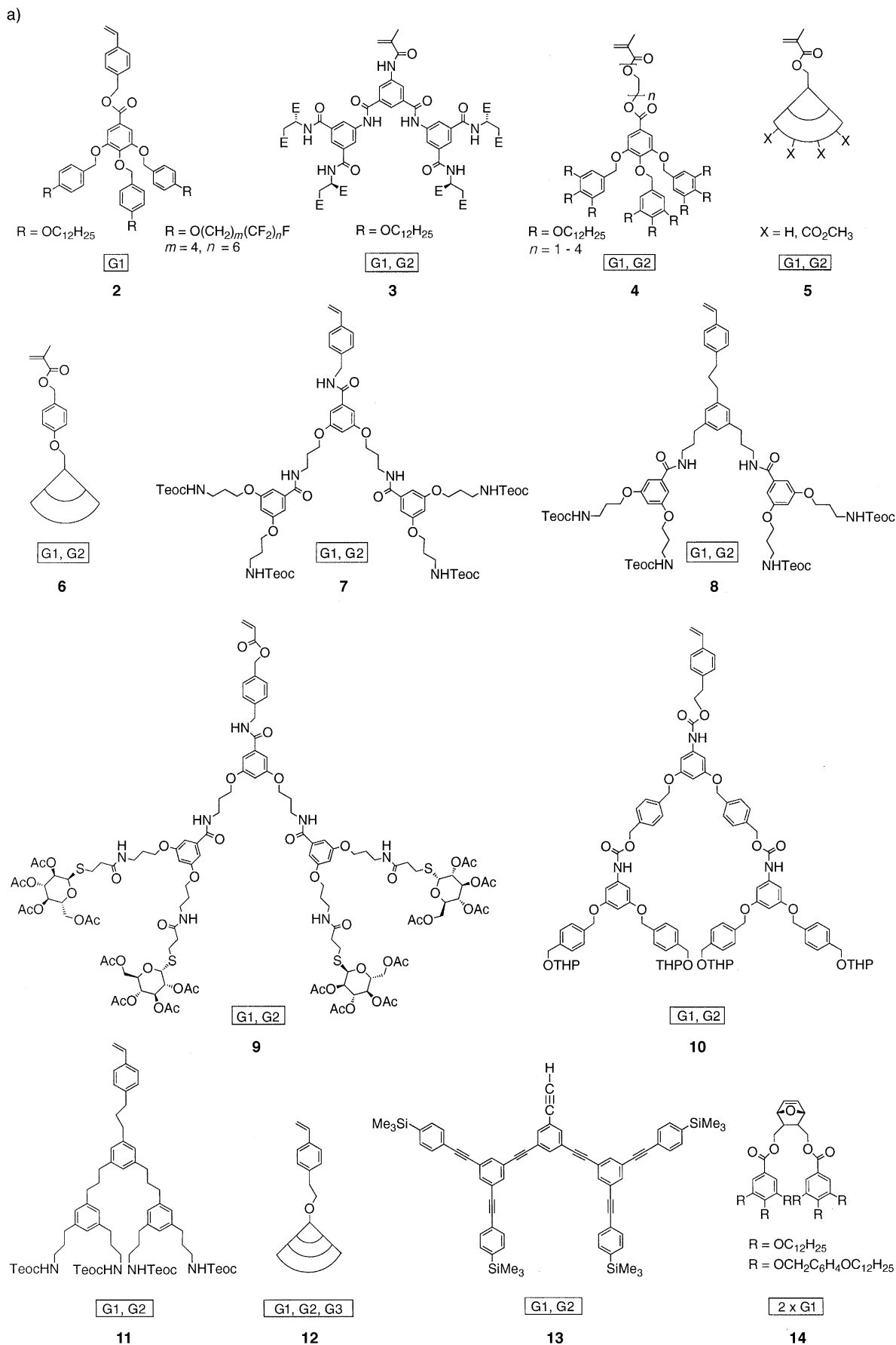


Figure 2. Structures of macromonomers used to obtain dendronized polymers by chain-growth (a) and step-growth procedures (b). THP = tetrahydropyranyl.

b)

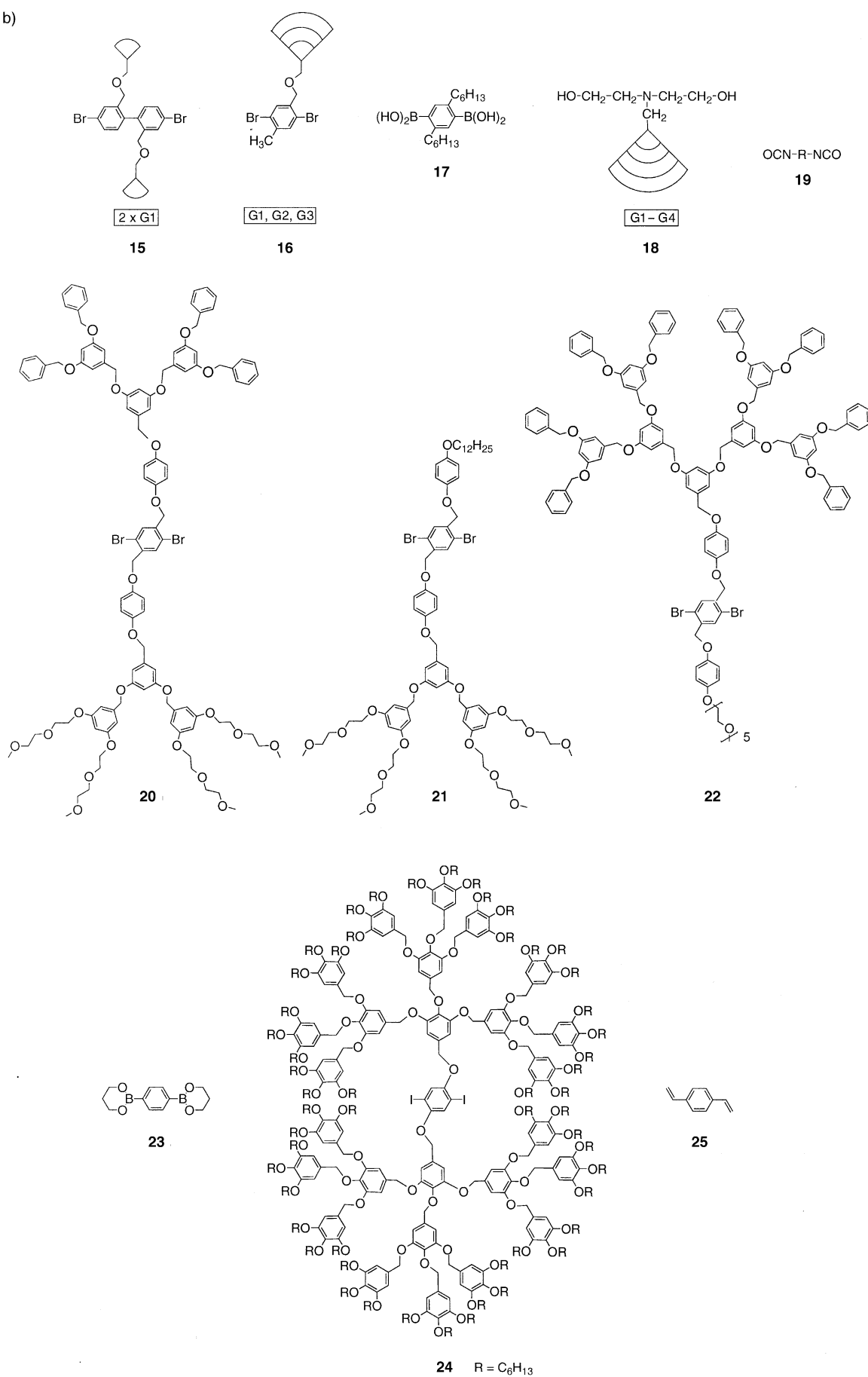


Figure 2. (Continued)

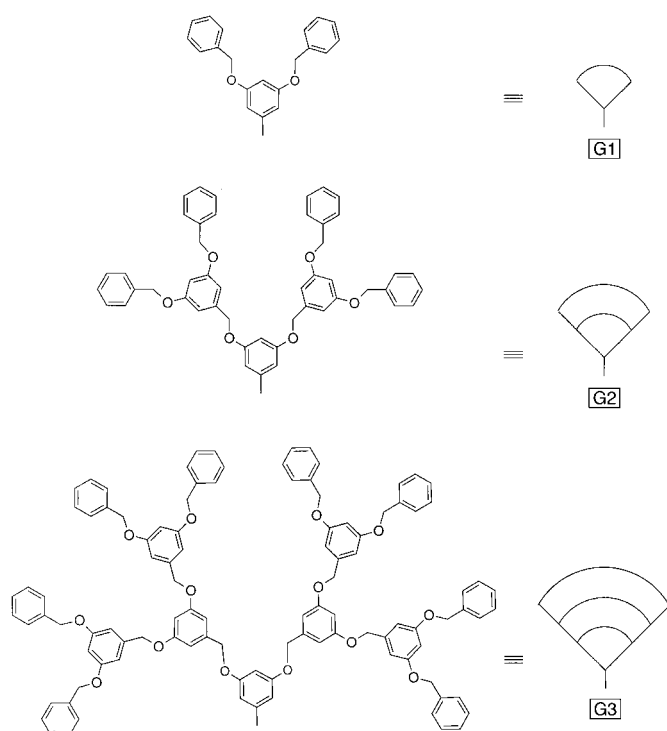
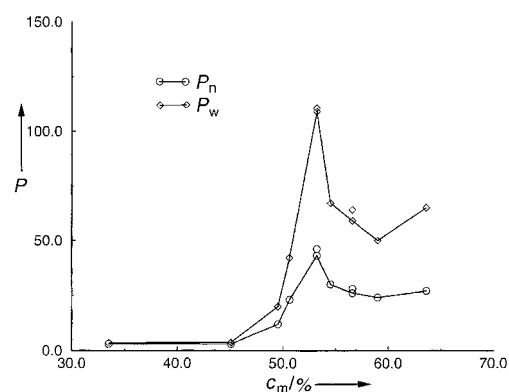


Figure 3. Fréchet-type dendrons (G1–G3).

Figure 4. Dependence of the degree of polymerization (P_n , P_w) on the monomer concentration (c_m in weight %) for the polymerization of monomer **8**(G2).

3.3. Molar Mass Determination

Table 1 contains the molar masses of most of the prepared polymers. In practically all cases these values were obtained from GPC versus a polystyrene (PS) standard. As pointed out in Section 3.1 this is not an appropriate method for determining the molar mass of dendronized polymers. The values given

Table 1. Dendronized monomers, polymerization conditions, and molecular weights (determined by GPC) of the polymers obtained. The molecular weights are not comparable (see text).

Monomer	Conditions	$M_n \times 10^{-3}$	M_w/M_n	P_n	Yield [%]
2 ($R = OC_{12}H_{25}$)	AIBN, 60 °C, benzene	25–69 ^[a]	1.7–2.5	23–58	44–72
2 [$R = O(CH_2)_m(CF_2)_nF$]	AIBN, 60 °C, benzene	23	1.2	12	65
3 (G1)	AIBN, 60 °C, DMF	23	3.9	43	90
3 (G2)	AIBN, 60 °C, DMF	–	–	≤ 6 ^[b]	53
4 ^[c]	AIBN, 60 °C	187	2.4	84	–
5 (G1)	AIBN, 60 °C	9	2.6	24	70–80
5 (G2)	AIBN, 60 °C	6	2.3	7	60
6 (G1)	AIBN, 40 °C	270	2.2	544	75
6 (G2)	DBPO, 60 °C	83	2.2	90	90
7 (G1)	AIBN, 50 °C	110	2.9	166	97
7 (G2)	DBPO, 60 °C	48	2.2	40	79
8 (G2)	<i>t</i> BPB, 90 °C, toluene	65	2.4	46	93
9 (G1)	AIBN, 55 °C, benzene	71	2.2	55	92
9 (G2)	<i>t</i> BPB, 90 °C, toluene	12	1.03	5	46
10 (G2)	<i>t</i> BPB ^[d] , 90 °C	85	3.3	51	90
11 (G1)	AIBN, 55 °C, toluene	38	2.0	60	85
11 (G2)	<i>t</i> BPB, 90 °C, toluene	50	1.9	39	86
12 (G1)	DBPO, 60 °C, toluene	25	1.9	56	60
12 (G2)	DBPO, 70 °C, toluene	33	3.9	38	93
12 (G3)	<i>t</i> BPB ^[d] , 90 °C	62	6.7	36	70
13 (G2)	$[Rh(C_7H_8Cl)_2]$, NEt ₃	960	1.4	975	36
14 ($R = OCH_2C_6H_4OC_{12}H_{25}$)	$[RuCl_2(CHPh)](PCy_3)_2$, 22 °C, CH ₂ Cl ₂	87	1.3	120	84
15 + 17	$[Pd(PPh_3)_4]$, 2 N NaOH, toluene	18	3.8	18	80–85
16 (G1) + 17	$[Pd(PPh_3)_4]$, 2 N NaOH, toluene	19	3.6	29	90
16 (G2) + 17	$[Pd(PPh_3)_4]$, 2 N NaOH, toluene	21	2.8	19	89
16 (G3) + 17	$[Pd(PPh_3)_4]$, 2 N NaOH, toluene	52	5.3	27	96
18 (G2) + 19 ^[c]	— ^[d]	17	3.0	17	98
18 (G3) + 19 ^[c]	— ^[d]	21	4.2	12	97
18 (G4) + 19 ^[c]	— ^[d]	24	3.0	7	92
20 + 23	$[Pd\{P(p\text{-tolyl})_3\}_3]$, 2 N NaHCO ₃ , THF	38	2.3	20	98
21 + 23	$[Pd\{P(p\text{-tolyl})_3\}_3]$, 2 N NaHCO ₃ , THF	28	2.0	20	96
22 + 23	$[Pd\{P(p\text{-tolyl})_3\}_3]$, 2 N NaHCO ₃ , THF	21	1.9	10	94
24 + 25	$[Pd\{P(p\text{-tolyl})_3\}_3]$, 2 N NaHCO ₃ , THF	35	1.6	12	–

[a] Dependant on the length of the ethylene oxide spacer, [b] Determined by MALDI-TOF mass spectrometry. [c] A related monomer based on acrylate gives higher molecular weights. [d] Without solvent. [e] $R = (CH_2)_6$. AIBN = azobisisobutyronitrile, DBPO = dibenzoyl peroxide, *t*BPB = *tert*-butyl perbenzoate.

should therefore be treated at best as rough guesses (see below in this Section), and a direct comparison of different polymers is impossible. This also holds true for polymers with the same backbone but dendrons from different generations. Nevertheless, some general conclusions can be drawn:

- Monomers with more complex structures, like **3**, or those in which the dendron is bound too tightly to the polymerizable group, like **5**, do not give high molecular weight materials.^[46]
- Rh-catalyzed acetylene polymerization of **13** and Ru-catalyzed ROMP of **14** give the best results, along with a few of the radical polymerizations (**6**(G1), **4**).
- There is a trend to decreasing molecular weights with increasing size (generation) of dendron.
- The yields are not always high, which means that the corresponding polymerizations do not proceed well or that oligomeric products are formed, which are removed during workup. The latter point relativates some of the molecular weight values given.

Despite the importance of an accurate molar mass determination of dendronized polymers, a systematic study on this matter is not yet available. The most extensive investigation is one where GPC molar mass of five polymers obtained from monomers **7**(G1), **7**(G2), **12**(G1), **12**(G2), and **12**(G3) are compared with those obtained from small-angle neutron scattering (SANS).^[48] Both fractions and representative samples of these polymers were employed. Table 2 summarizes the results. In all cases the SANS values are higher than

Table 2. Molar masses of dendronized polymers determined by GPC and SANS.

Polymer	$M_n \times 10^{-3}$		$M_n(\text{SANS})/M_w(\text{GPC})$	Length [nm]
	(GPC) ^[a]	(SANS)		
P7 (G1)	277	437 ^[b]	1.6	3.8
P7 (G2)	84	275 ^[b]	3.3	5.0
P12 (G1)	178	276 ^[c]	1.6	3.1
P12 (G3)	710	2530 ^[c]	3.6	4.6
P12 (G3)	59	233 ^[c]	3.9	5.1

[a] In THF. [b] In CD₃OD. [c] In C₆D₆.

those from GPC, and the factor by which GPC underestimates the real molar masses varies between 1.6 and 3.9. A qualitative explanation for this finding is to be seen in the much larger mass per unit length of dendronized polymers than the calibration standard polystyrene. The true molar mass should therefore be higher than the GPC molar mass. There is, however, an opposing effect which has to do with the stiffening of the polymer chain imposed by the spatially demanding substituents. This leads to an increased hydrodynamic volume, causing the GPC molar mass to become larger than the actual molar mass. For the cases shown in Table 2 the latter effect obviously does not overcompensate the former. Whether this will still be the case for polymers with G4 substituents awaits to be seen. The SANS measurements also revealed that polymer **12**(G3) is a rigid rod whose persistence length is on the order of the contour length.

The degree of polymerization (P_n) of polymer **P16/17**(G3), a representative of a step-growth polymer (Figure 2b) obtained from monomers **16**(G3) and **17**, was also investigated

carefully in order to learn about the effectiveness of step-growth procedures (here SPC^[35]) in the case of sterically demanding monomers. Its P_n could not be directly determined because of unsurmountable problems with aggregation. Through some chemical modification, however, an average $P_n = 110$ was finally obtained. According to Carother's equation,^[49] this P_n results when each coupling step proceeds with a conversion of 99.1%. From a synthetic point of view this finding is truly remarkable. It shows the enormous potential of SPC, even for cases where steric congestion is considerable, and has initiated further developments, which are described in the following Section.

3.4. Some Aspects of the Molecular Structure and Recent Progress in Suzuki Polycondensation (SPC)

As discussed in Section 2, one objective for research of dendronized polymers was to use the decoration with dendrons as a means to stiffen the backbone to the point that it is fully stretched. This goal should be reached when the individual dendrons are evenly distributed around the backbone and tightly packed at the van der Waals distance. Of course, the fully stretched conformation will also be reached with somewhat less tight packing, provided solvent molecules are diffused into the dendritic layer through osmotic effects. Both cases lead to a rigid rod with a cylindrical envelope; however, only in the first case would this shape be practically independent from the surrounding medium. In the second the dendritic layer would collapse under conditions where the solvent diffuses out (e.g. under vacuum). The question of tightness of packing is essential for synthesis. Generally speaking, the synthesis becomes more difficult, the less spatial mobility the reaction partners have to attain the mechanistically required relative conformation in the transition state of the reaction (here: growth step). To evaluate the feasibility of a certain polymerization on grounds of steric interactions, it would be ideal to have a detailed picture of the spatial changes around the reaction center on going along the reaction coordinate. Since it is practically impossible to obtain this with a reasonable amount of computational effort, only some molecular dynamics (MD) calculations of final products were performed. Additionally some polymers were visualized by computer-generated three-dimensional images in order to help the synthetic chemist to assess whether or not a macromonomer undergoes polymerization.

The MD simulations were performed in vacuo with polymers **P12**(G3)^[50] and **P16/17**(G3),^[51] which carry the same G3 dendrons but differ by the average distance between the dendrons' anchor groups (**P12**(G3): 2.50 Å, **P16/17**(G3): 8.25 Å) and the flexibility of the backbone (Figure 5). Unfortunately solvent molecules could not be considered due to the enormous number of atoms already present in the polymers. Figure 6 shows the minimum conformations obtained. Whereas the dendritic layer of **P12**(G3) is quite compact, that of **P16/17**(G3) is much more open and loose. The diameters of **P12**(G3) and **P16/17**(G3) are on average approximately 4.4 nm and 2–4 nm, respectively. This difference in compactness is certainly to a large extent due to the

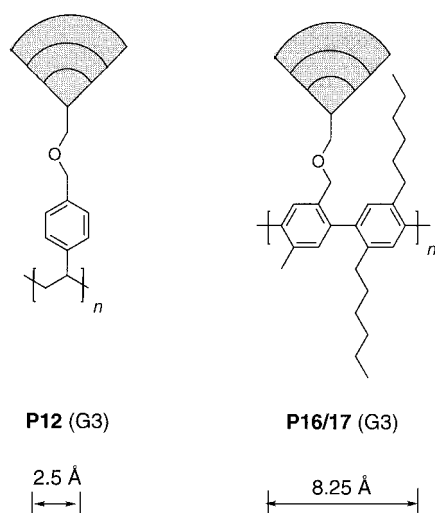


Figure 5. Polymers **P12(G3)** and **P16/17(G3)** along with the lengths of their repeat units (2.5 and 8.25 Å, respectively), which is required to qualitatively assess the average distance between attached dendrons.

different average distance of the dendrons' anchor groups at the backbones, but the different stiffnesses of the backbones

also play a role here. Whereas the contour length of **P16/17(G3)** of the starting conformation upon equilibration remains practically constant, that of **P12(G3)** is reduced by some 30 % when the minimum conformation is reached. This shows that the dense packing of the latter polymer can be partially attributed to some backbone coiling. It is reasonable to assume that it is exactly this small, still available space which rendered the polymerizations feasible. Considerations regarding preaggregation of polymerizable dendrons into ordered cylindrical arrays prior to polymerization through the aggregate^[46] do not seem to be applicable here.

Figure 7 shows computer-generated structures of **P16/17(G1)** through **P16/17(G4)**. Though these structures are not fully energy minimized, it is quite obvious that there is space between consecutive dendrons even for the G4 case. This space together with the surprisingly successful polymerization of monomer **16(G3)** supported the idea to also try SPC with monomer **16(G4)**.

The attempt to subject a G4 monomer to SPC met with trivial but nonetheless serious synthetic difficulty, and it was not clear for a long time whether or not such a polycondensation could be brought about. Scheme 2 shows the finally

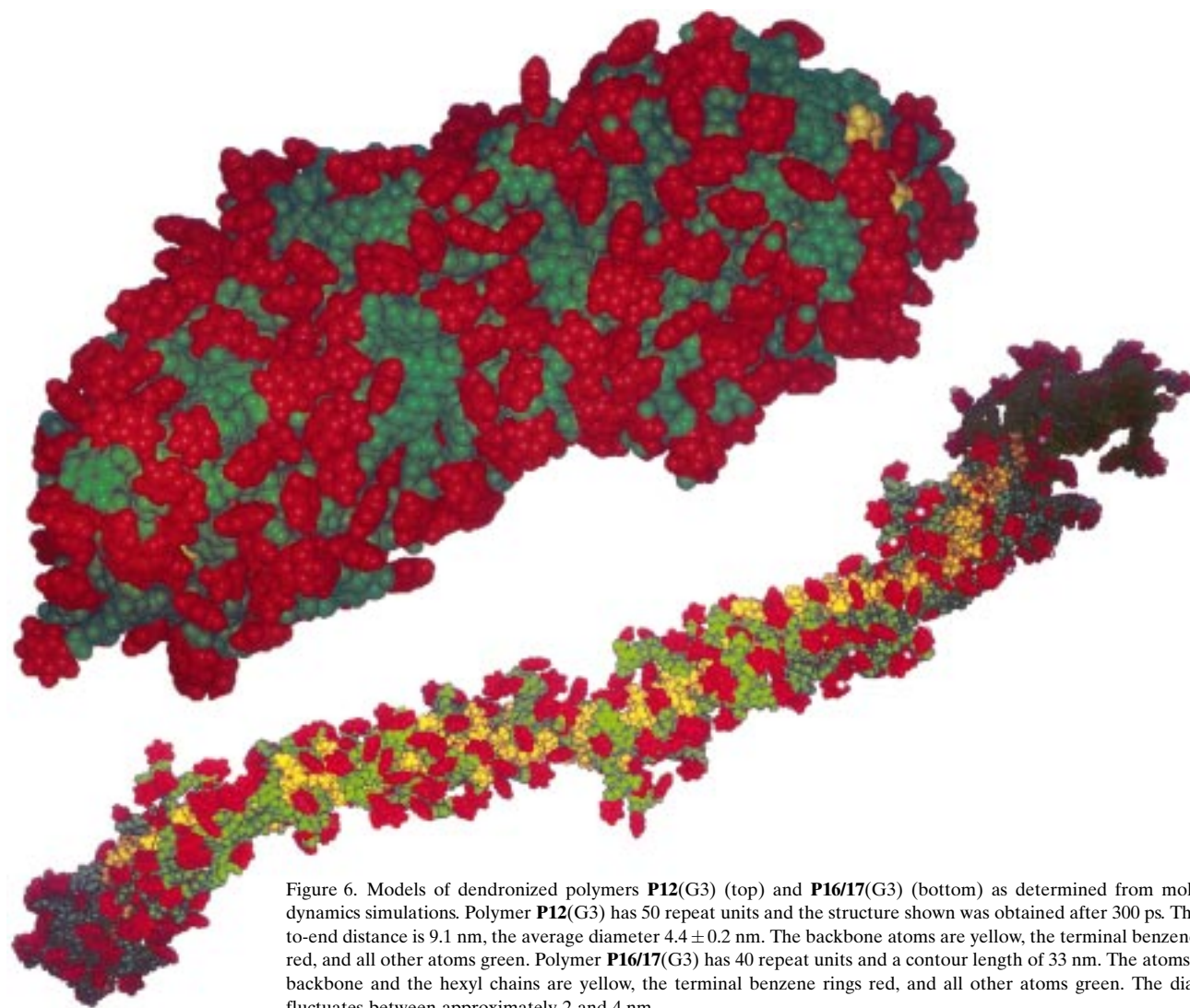


Figure 6. Models of dendronized polymers **P12(G3)** (top) and **P16/17(G3)** (bottom) as determined from molecular dynamics simulations. Polymer **P12(G3)** has 50 repeat units and the structure shown was obtained after 300 ps. The end-to-end distance is 9.1 nm, the average diameter 4.4 ± 0.2 nm. The backbone atoms are yellow, the terminal benzene rings red, and all other atoms green. Polymer **P16/17(G3)** has 40 repeat units and a contour length of 33 nm. The atoms of the backbone and the hexyl chains are yellow, the terminal benzene rings red, and all other atoms green. The diameter fluctuates between approximately 2 and 4 nm.

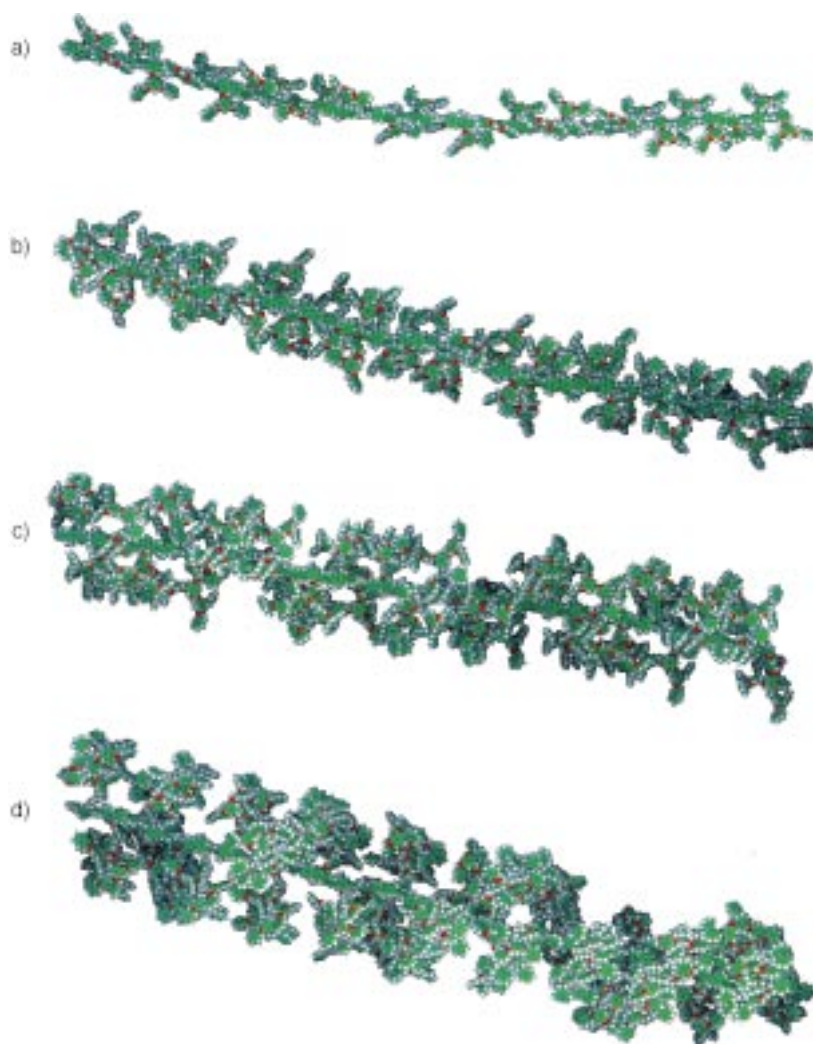


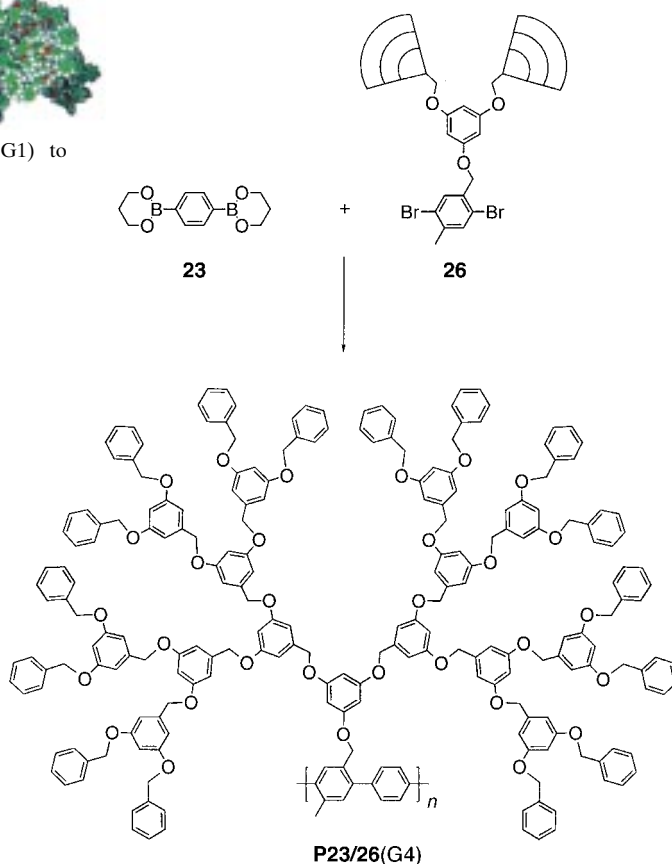
Figure 7. Computer-generated images of dendronized polymers **P16/17**(G1) to **P16/17**(G4) (a–d).

successful case.^[52] The molecular weight difference between the two components **23** and **26** is so considerable (**23**: 246 g mol⁻¹; **26**: 3540 g mol⁻¹) that it was difficult to meet in practice the 1:1 stoichiometry strictly required for polycondensations. For that purpose monomer **26** was prepared on the 20-g scale. This allowed SPC on a relatively large scale, ensuring sufficient stoichiometry control. In a series of optimization experiments conditions were finally found which gave the corresponding polymer **P23/26**(G4)^[53] with $P_n = 25$ and $P_w = 125$ (according to GPC) on a gram scale. Presently fractions of this material with $P_n = 10, 75, 150$, and 300 (GPC) are being investigated by SANS in order to determine their true molar mass.^[54] Though it is still unclear what the actual molar mass of this polymer is, these data clearly show that SPC works even for sterically enormously loaded G4 monomers.

4. Behavior in the Bulk

The bulk properties have been investigated for polymers **P2**(G1 and G2),^[22] **P4**(G2)^[22] (**P2**, **P4**: R = OC₁₂H₂₅), and to

some extent **P18/19**(G4)^[41] and **P24/25**.^[42] All four structures have flexible backbones with a non-compact sequence of dendrons. In the first two cases and in the last one, the dendrons have liquid crystalline properties, whereas in the third case they do not. Polymer **P2**(G1) self-assembles into a tubular supramolecular structure exhibiting enantiotropic columnar hexagonal (Θ_h) phases. These phases are characterized by differential scanning calorimetry, wide- and small-angle X-ray scattering, thermal optical polarized microscopy, and molecular modeling.^[5b] The structure model proposes the stratum of the column to be formed by the backbone and the linking segments melted and segregated in the center of the column with their melted dendrons radiating towards the column periphery (Figures 8 and 9). When a second-generation dendron is used as in **P2**(G2) and **P4**(G2) an additional interesting feature is observed:^[22] As the degree of polymerization increases, the dendrimer's shape shifts from spherical to cylindrical. This is accompanied by the backbone going from a random-coil to the extended conformation. This phenomenon provides the unique possibility for molecular engineering of polymer shapes, backbone conformation, and properties, and is a great way to show the interplay and impact of the above-mentioned interactions.



Scheme 2. Synthesis of **P23/26**(G4) from monomers **23** and **26** following a Suzuki polycondensation protocol.

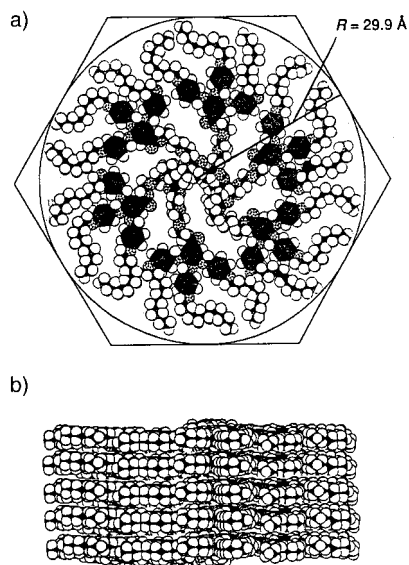


Figure 8. Schematic representation of a supramolecular cylinder of dendronized polymer **P2**(G1) ($R = \text{OC}_{12}\text{H}_{25}$, $n = 3$) in the Θ_h mesophase: a) Top view of a cylinder containing six repeat units in a stratum; the alkyl tails are melted to match the average column radius determined by X-ray scattering experiments. b) Side view of a cylinder containing 30 repeat units of the polymer assembled with melted alkyl tails. (Reproduced with permission from reference [5b].)

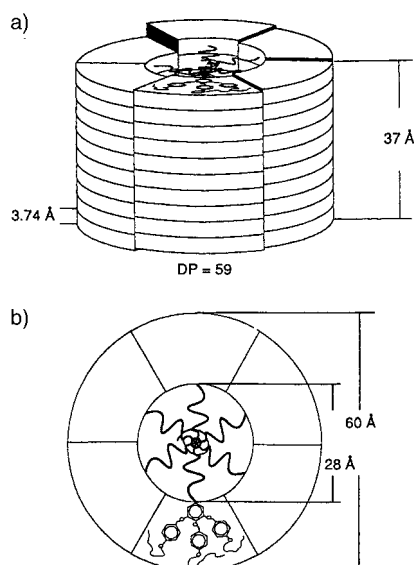


Figure 9. An idealized representation of the supramolecular cylinder formed by self-assembly from a single chain of dendronized polymer **P2**(G1) ($R = \text{OC}_{12}\text{H}_{25}$, $n = 3$) in the hexagonal columnar phase, assuming one single backbone per cylinder; drawn to proportion under consideration of existing data: a) side view; b) top view. (Reproduced with permission from reference [5b].)

Spherical and extended architectures of these dendrimers are basically proven by X-ray diffraction and can also be visualized by a scanning force microscopical (SFM) investigation using bilayers on a mica substrate.

Though polymer **P18/19**(G4) does not show any liquid crystallinity, it nevertheless forms supramolecular structures in the bulk.^[40] This was concluded from X-ray diffraction

patterns obtained from Si plates coated with this polymer and monomer **18**. A series of Bragg peaks for the polymer showed the existence of an ordered structure. Based on MD calculations and additional small-angle X-ray scattering experiments this structure was proposed to be body-centered cubic.

Polymer **P24/25** self-orders in solvent-cast films with the backbones parallel to the substrate and a strong solvent dependence for the degree of ordering.^[41] Spacings of 22–26 Å are observed by X-ray diffraction, indicating interdigitation of the dendritic side chains. Polymer **P24/25** also forms thermotropic nematic liquid crystalline phases. With optical microscopy Schlieren textures are observed for thin films cast from solution.

5. Behavior at Solid and Fluid Interfaces

The behavior of dendronized polymers on solid substrates and at air/water interfaces is of interest from various points of views (see Section 2). One reason is that the preparation of single dendronized polymers or molecular layers thereof on solid substrates allows molecular resolution imaging by scanning force microscopy (SFM).

The behavior of the dendronized polymers at interfaces is determined not only by intra- and intermolecular forces, but also by interfacial forces.^[55] The latter include a rather short-range van der Waals-type force on the nanometer scale, which is due to the different dielectric properties of the two adjacent phases. This force is always attractive, and therefore favors a high molecular segment density at the interface, which in turn favors two-dimensional crystallization since crystals exhibit usually higher densities than less ordered phases. Counteracting is the influence of entropy: Immobilizing flexible molecules in 2D crystals at interfaces reduces the entropy, since the molecules lose translational as well as conformational entropy. For the adsorption from a liquid mixture this favors the adsorption of the largest molecules, since the loss of translational entropy is counted per particle, while the van der Waals energy gained is proportional to the mass. Entropy effects also favor the adsorption of the most rigid molecules, since they lose the least conformational entropy. As a result the large and rigid rods will be preferably adsorbed from a liquid molecular mixture. In addition, there are short-range chemical forces, which are specific for the particular chemical species at the interface, and may be attractive or repulsive. Well-known consequences of these forces are epitaxial layering on crystalline substrates and the ordering of amphiphilic molecules at the air/water interface.

In the following, the behavior of two classes of dendronized polymers at interfaces will be discussed: those with one type of dendrons (“homophilic systems”) and those with a hydrophobic and a hydrophilic dendron at each repeat unit (“amphiphilic systems”).

5.1. Homophilic Systems

Dendronized polymers may be imaged by scanning force microscopy either in ordered ultrathin films or as individual

molecules on solid supports. SFM^[56, 57] is a powerful tool for investigating morphology, molecular packing, and molecular dynamics at surfaces with a resolution on the molecular scale. Examples include synthetic and biological polymers^[58–60] as well as spherical dendrimers.^[61, 62] Of particular interest for rather soft organic materials is the operation in the “intermittent contact” or “tapping” mode,^[57] which minimizes wear during imaging.

Ultrathin layers of homophilic dendrimers can be prepared by solution casting (slow layer formation) or spin-coating (fast layer formation) from organic solvents. The SFM images of **P12**(G3) were obtained for thin (in the range of 20–30 nm) solution-casted layers on highly oriented pyrolytic graphite (HOPG, Figure 10).^[50] They reveal a remarkably high degree

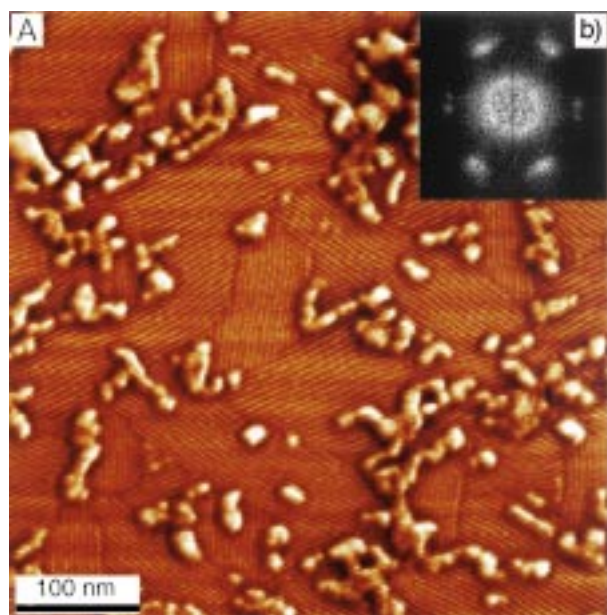


Figure 10. a) Phase-contrast SFM image (tapping-mode) of an ultrathin layer of **P12**(G3) on HOPG.^[50] The rows are attributed to molecules which are predominantly oriented parallel to the surface as well as to each other. Their orientation within the surface plane also reflects the threefold symmetry of the substrate, as indicated by the Fourier transform (b).

of order: One observes domains which consist of periodic arrays of rows with a periodicity of $D = 5.0 \pm 0.5$ nm. For comparison, MD simulations (Figure 6, top) and SANS measurements reveal rod diameters of $D = 4.4 \pm 0.2$ nm and $D = 5.1 \pm 0.5$ nm, respectively. These numbers are very similar to the distances between the rows, suggesting that the rows can be attributed to molecules which are predominantly oriented parallel to the surface. They are grouped in anisotropic domains whose sizes vary between 20 and 200 nm (parallel to the director axis) and 20 and 100 nm (perpendicular to the director axis). A reliable correlation of individual chain lengths with domain size is not possible. Despite the high resolution of the image, only the chain ends at the grain boundaries can be clearly identified. In contrast, those in the interior may be obscured if two dendrimers with the same director axis are in tight contact and oscillate somewhat along this axis.^[50] Large-scale SFM images show that the films exhibit terraces with height differences Δh

between adjacent terraces of 4.2 ± 0.2 nm. This is close to $5.0 \text{ nm} \cos 30^\circ = 4.3$ nm, which one would expect for a model of close-packed cylinders with a diameter of 5 nm (Figure 11).

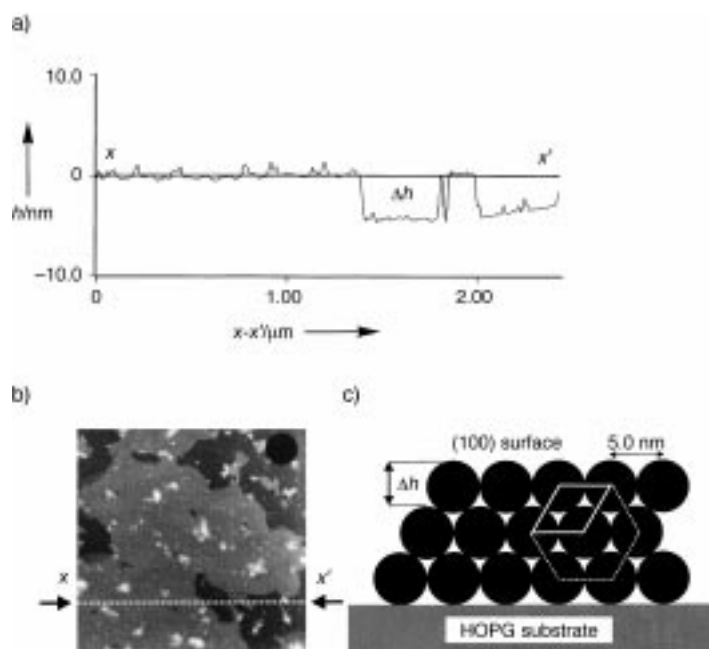


Figure 11. Some SFM results of ultrathin films of **P12**(G3) on HOPG and a packing model.^[50] a) Cross-sectional profile along $x-x'$ as indicated in b). The height difference between adjacent terraces has the dimension of a monolayer ($\Delta h = 4.2 \pm 0.2$ nm). b) Large-scale SFM image ($2.4 \times 2.4 \mu\text{m}^2$) of molecular terraces. c) Schematic model of close-packed molecularly defined cylinders. See text for further details.

Interestingly, the different domains in Figure 10 exhibit three molecular orientations at $60 \pm 8^\circ$ relative to each other, indicating that even in a layer with a thickness of about five monolayers the top layer reflects the threefold symmetry of the graphite substrate. While this symmetry is stable with time, reorientations by 120° do occur. For the case of a similar polymeric dendrimer, **P16/17**(G3), this is demonstrated in Figure 12.^[51]

These results show that the ultrathin layers of the described dendronized polymers offer the possibility to orient the polymeric backbones along the substrate axes, and to access molecular dimensions in arrays by SFM imaging. To determine the properties of single molecules, such as persistence lengths or Young's or bending moduli, it is desirable to prepare submonolayers with isolated single molecules on the surface. A convenient preparation for this is spin-coating HOPG from a dilute solution. The SFM images of **P2**(G1) (Figure 13)^[63] reveal single molecules stretched out on the surface with sharp kinks of typically 120° , which then reflect the symmetry of the substrate. The sharpness of the kinks indicates that this polymer is quite flexible. The SFM images may also be used to determine the distributions of contour lengths. They revealed that **P2**(G1)—that is, a polymer with a side group that is not very spatially demanding—appeared to be at least two times shorter than the contour length expected on the basis of size-exclusion chromatography and static light scattering. This was attributed to a disordered helixlike conformation of the polymeric backbone, and can be con-

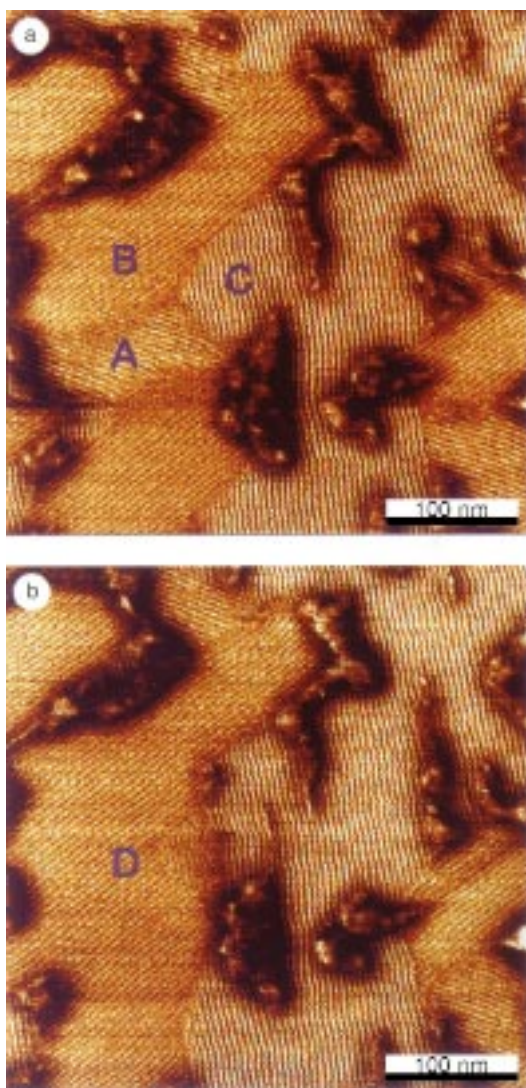


Figure 12. Phase-contrast SFM images (tapping-mode) of an ultrathin film of **P16/17(G3)** on HOPG.^[51] The rows are attributed to molecules which are predominantly oriented parallel to the surface as well as to each other. Their orientation within the surface plane also reflects the threefold symmetry of the substrate (three molecular orientations denoted A, B, and C in a)). Images a) and b) were recorded at the same position at an interval of 10 min. Domain A reorients in the same direction as domain B, resulting in a new domain D.

trasted to the case of **P4(G1)** with a spatially more demanding side group, whose contour lengths agreed fairly well with the expectation.^[63] This shows that bulky dendrons force the backbone into extension and that there is a degree of freedom for less bulky dendrons, namely, the conformation of the flexible polymer backbone, which may be used to control the contour length of the dendronized polymer (e.g., by contraction through helix formation).

Another means to stiffen a polymer is by Coulombic charges along the backbone. Whereas **P11(G1)** is quite flexible (Figure 14a), the corresponding positively charged derivative without the trimethylsilylethoxycarbonyl (Teoc) protecting group is rather stiff (Figure 14b).^[65] The latter polymers are therefore, similar to the polymers with bulky dendrons **P12(G3)** and **P16/17(G4)**, suitable candidates

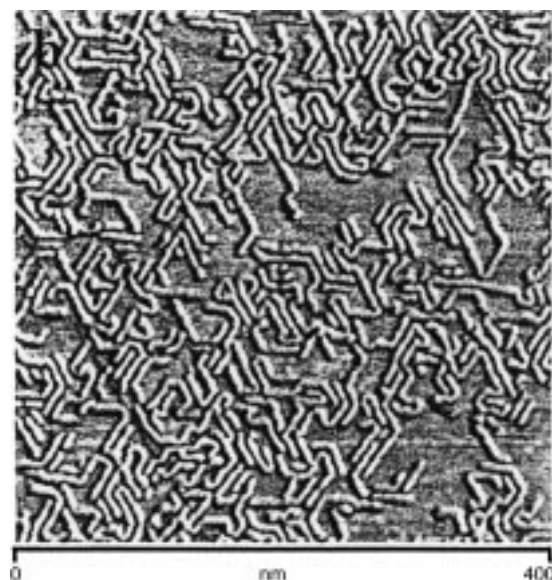


Figure 13. SFM image (tapping-mode) of **P2(G1)** on HOPG.^[63] It shows that the molecular orientations reflect the symmetry of the substrate, and that the molecules are flexible enough to be frequently bent by 120°. The strong tendency for the single molecule to align along lattice axes of the substrate has been attributed to the orientation of the pendant alkyl chains.^[63, 64]

for one-by-one manipulation with the SFM tip, just like rolling trees on the ground.

Another example for the manipulation of dendronized polymers with the SFM is shown in Figure 15, which was obtained as follows: First about one monolayer of a rather low molecular weight fraction (on the order of ten monomers on average according to GPC with polystyrene standard) of **P16/17(G4)** was spin-coated on HOPG. The result is an apparently disordered surface. Scanning with the SFM-tip, however, orients the molecules almost perfectly uniformly in the whole scan window, which can be 1 μm^2 or more, and is limited apparently only by the domain size of the underlying HOPG, which is typically on the order of 10 μm but also varies.

5.2. Amphiphilic Systems

Amphiphilic dendronized polymers may lead to amphiphilic cylinders which, depending on the surrounding medium, could segregate lengthwise into two different halves (Figure 16). This structural motif is rather unique. In nature it can be found in some ion channel membrane proteins, which means that amphiphilically dendronized polymers are of interest as models for such proteins.^[66–68] They may also serve as novel and giant constituents of self-aggregated assemblies and should show interesting behavior at interfaces. A good candidate is a dendronized polymer whose repeating units are equipped with two sterically demanding substituents, one hydrophobic and the other hydrophilic.^[69] This area was entered with the three differently equipped poly(*para*-phenylene)s **P20/23**, **P21/23**, and **P22/23**, which differ in the relative spatial demand of their polar and nonpolar substituents (Figure 16).

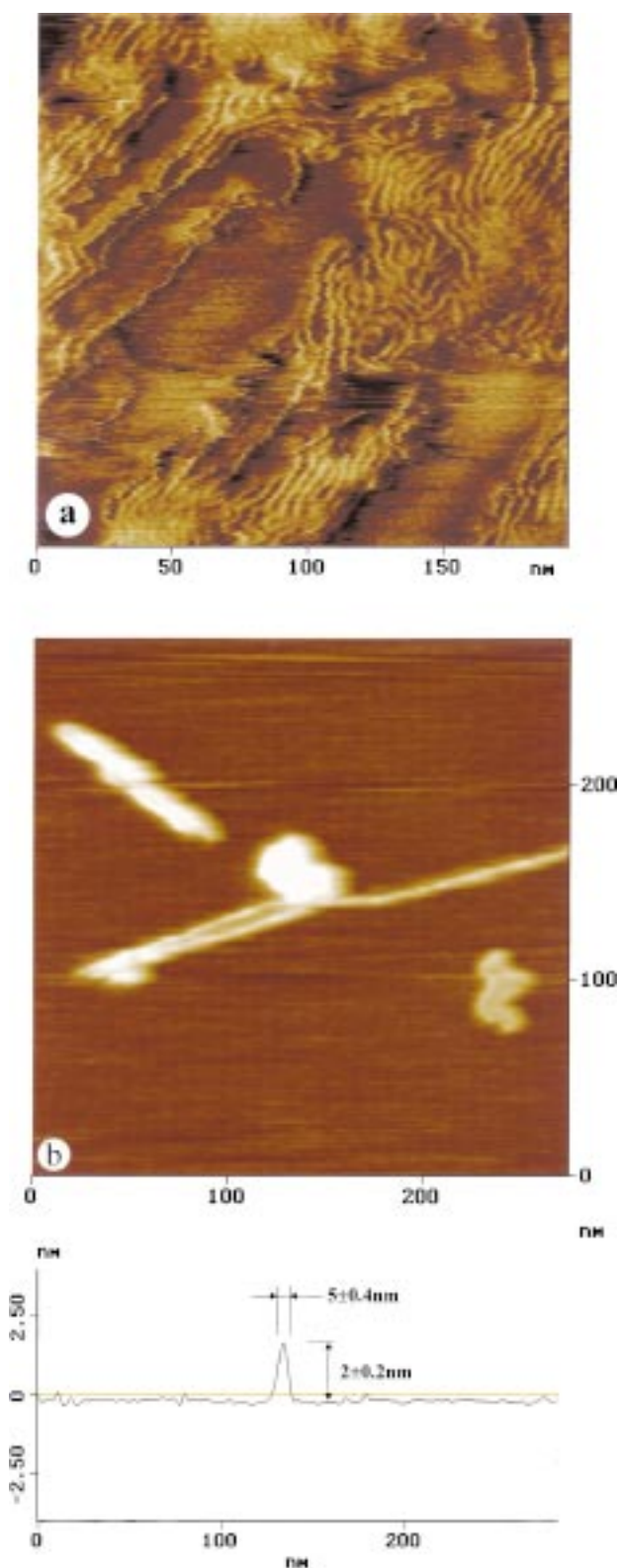


Figure 14. SFM images (tapping-mode) of two low-generation dendronized polymers: a) **P11**(G1) (phase image: $0-30^\circ$) and b) its unprotected and quaternized (charged) counterpart on HOPG (height image: $0-8$ nm).^[65] Whereas the neutral **P11**(G1) is rather flexible, as evidenced also by the fact that the exact conformation changes on the time scale of the measurement, the charged species appears very rigid, as judged by the straightness of the feature going from left to right over more than 200 nm. The two kinks in this feature may indicate that it consists of several individual rods that are roughly 100 nm long. The height profile was taken perpendicular to the x axis at 200 nm.

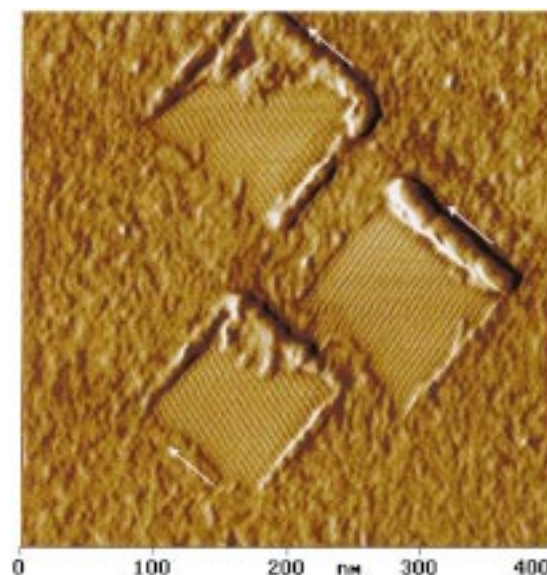


Figure 15. Phase-contrast SFM image (tapping-mode) of a monolayer of oligomeric **P23/26**(G4) after scanning of three areas.^[65] It reveals that upon scanning the molecules are perfectly oriented along one of the three symmetry axes of the substrate.

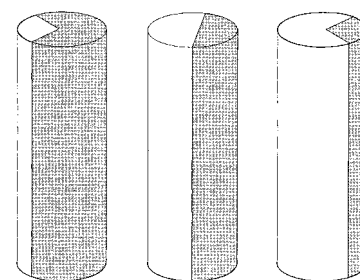


Figure 16. Cartoon representations of (not yet fully realized) amphiphilically equipped dendritic cylinders with varying spatial demands of polar and nonpolar domains. The lengthwise segregation of these domains is interesting for surface modifications and for attaining unprecedented aggregates in solution.

There are different ways to prepare ultrathin layers from amphiphilic polymers: One method is to form Langmuir monolayers at the air/water interface followed by transfer to solid substrates using the Langmuir–Blodgett technique. Another way is spin-coating, as for the homophilic systems.

5.2.1. Langmuir and Langmuir–Blodgett (LB) layers

Molecules based on amphiphilic dendrons have been investigated at the air/water interface, and stable monolayers were found for some of these systems.^[70] In a recent, elegant study by Frank and Hawker et al.^[71] the behavior of hydrophobic Fréchet-type dendrons carrying hydrophilic ethyleneoxy (EO) chains at their focal point was studied at this interface by systematically varying the dendron size (G3 to G5) and chain length. Longer hydrophilic chains increased the stability of the Langmuir films for G3 and G4 by roughly 3.1 and 4.1 mN m⁻¹, respectively, per additional EO unit. On going from the lower to the higher generations the shape of the dendrons changes from vertically elongated to more flat.

The collapse pressures ranged from approximately 6 mNm^{-1} for G3 with one EO group to 25 mNm^{-1} for G3 with six EO groups.

Langmuir monolayers of monomer **20** and polymer **P20/23** have been prepared at the air/water interface.^[38] The surface pressure–area isotherms exhibited at room temperature reveal stable monolayers. The monolayers of **20** display very good reversibility for compression, decompression, and repeating cycles, with an area of about 0.73 nm^2 per molecule at 20 mNm^{-1} . These data are consistent with a monolayer structure in which the four EO chains per water-soluble dendron are close packed and oriented perpendicular to the monolayer, thereby defining a minimum area per molecule. In comparison, polymer **P20/23** exhibits a 10% larger area per RU ($0.82 \text{ nm}^2 \text{ RU}^{-1}$) upon the first compression with a hysteresis in the first decompression and a shift to a more reversible isotherm and a smaller area per RU in the second cycle ($0.77 \text{ nm}^2 \text{ RU}^{-1}$). The good agreement between the areas per molecule for monomer **20** and the per repeat unit of polymer **P20/23** indicates a structure for the polymer monolayer in which the rodlike polymer molecules are oriented with their long axes within the monolayer plane and close-packed. Moreover, the hydrophilic EO chains are on the side of the polymer that faces the water subphase, while the more hydrophobic dendron faces the air side of the polymer. This picture is supported by a control experiment with a closely related polymer in which the hydrophilic dendron of **P20/23** is replaced by another G2 Fréchet dendron (structure not shown). Under the same conditions this polymer does not form a stable monolayer at the air/water interface.

Polymers **P21/23** and **P22/23** and the corresponding monomers **21** and **22** were also investigated at the air/water interface.^[39] With the exception of **P22/23** they all form stable monolayers on the Langmuir trough, which can be transferred by the Langmuir–Blodgett technique to mica. Whereas the transferred films of **21** and **22** are unstable, **P21/23** forms stable, rather smooth monolayers whose morphology remains unchanged for at least a week. The fact that both monomers are stable on water but not on mica reflects the discrepancy of the spatial demand of the hydrophilic and hydrophobic parts. Since the polymer **P21/23** is stable on mica, the covalent attachment of the amphiphilic repeat units can stabilize an LB layer. On the other hand, the fact that **P22/23** does not form a stable monolayer, not even on water, indicates that this polymer, with its amphiphilic imbalance, does not behave like an amphiphile under these conditions.

A quantitative comparison of the collapse pressures of the Curtis–Hawker amphiphiles^[71] with the ones described here cannot be made. There are substantial structural differences, even if this comparison is restricted to the monomers **20–22**. Additionally the Curtis–Hawker amphiphiles have EO chains with terminal (polar) hydroxy functionalities, whereas the monomers have (unpolar) methoxy groups. The collapse pressures are, however, in a similar range.

Usually, no SFM images with molecular-scale resolution were obtained for the LB layers, in contrast to the case of the analogous homophilic systems prepared by spin-coating. Therefore spin-coating has been employed as an alternative preparation method also for the amphiphilic systems.^[65]

Figure 17 displays an SFM image of a spin-coat monolayer of **P20/23** on HOPG, which reveals rather straight rods. Again, their orientation within the plane is not random, but it reflects the threefold symmetry of the substrate. Within experimental error, the length distribution of the rods agrees with the values

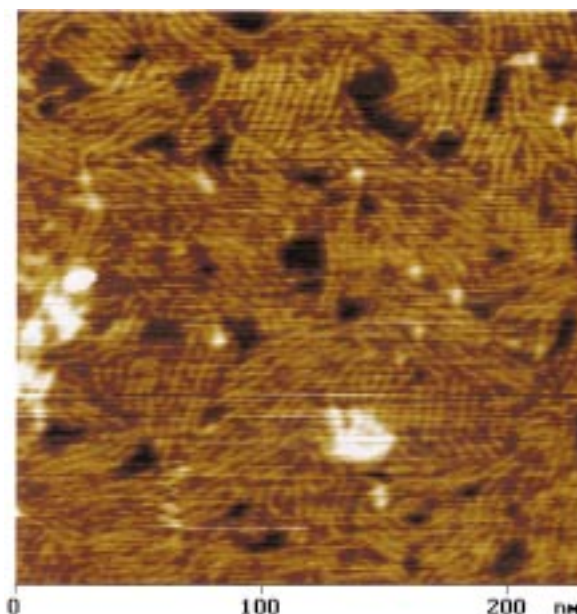


Figure 17. Molecular-resolution SFM height image of spin-coated amphiphiles **P20/23** on HOPG.^[65]

determined by size-exclusion chromatography. However, the distance between the rods is $4.4 \pm 0.5 \text{ nm}$, which is larger than one would expect for close-packed molecules. A possible explanation is a well-defined aggregation of the amphiphilic rods, for example into dimers which exhibit hydrophobic surfaces that are in contact with both the rather hydrophobic substrate and the ambient.

6. Modification of Cylinder “Surfaces”

Controlled surface modification of dendritic nanorods is a challenging goal for both synthesis and many application-related issues. Some initial experiments have already been undertaken. The essential steps are:

- Synthesis of monomers with protected functional groups in the periphery. The protection is required for compatibility with the polymerization conditions.
- Polymerization of these monomers and complete deprotection of the peripheral functional groups of the resulting polymer. The resulting deprotected polymer needs to be soluble.
- Derivatization of the deprotected polymer with the desired groups or functional units.

Step a has been solved for at least up to generation two. Monomers **7**, **8**, **10**, and **11** in Figure 2 are good examples of this. These monomers have all been polymerized to the corresponding high molar mass materials **P7**, **P8**, **P10**, and **P11** (step b) and their functional groups deprotected. For the use

of the somewhat uncommon Teoc protecting group, see reference [25]. Figure 18 shows ^1H NMR spectra of monomer **7**(G2), the corresponding polymer **P7**(G2), and its deprotected counterpart. The deprotection can obviously be driven to completion. This is important because remaining protected

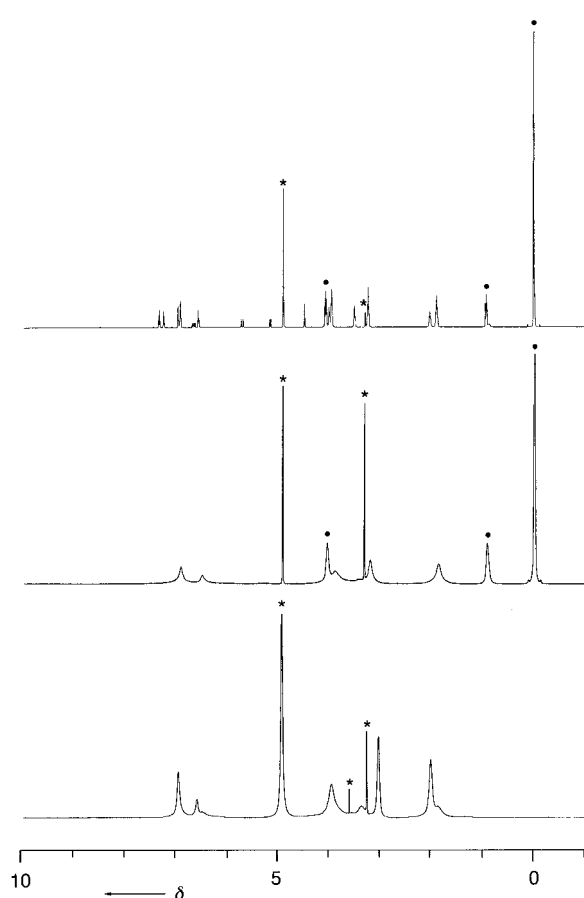
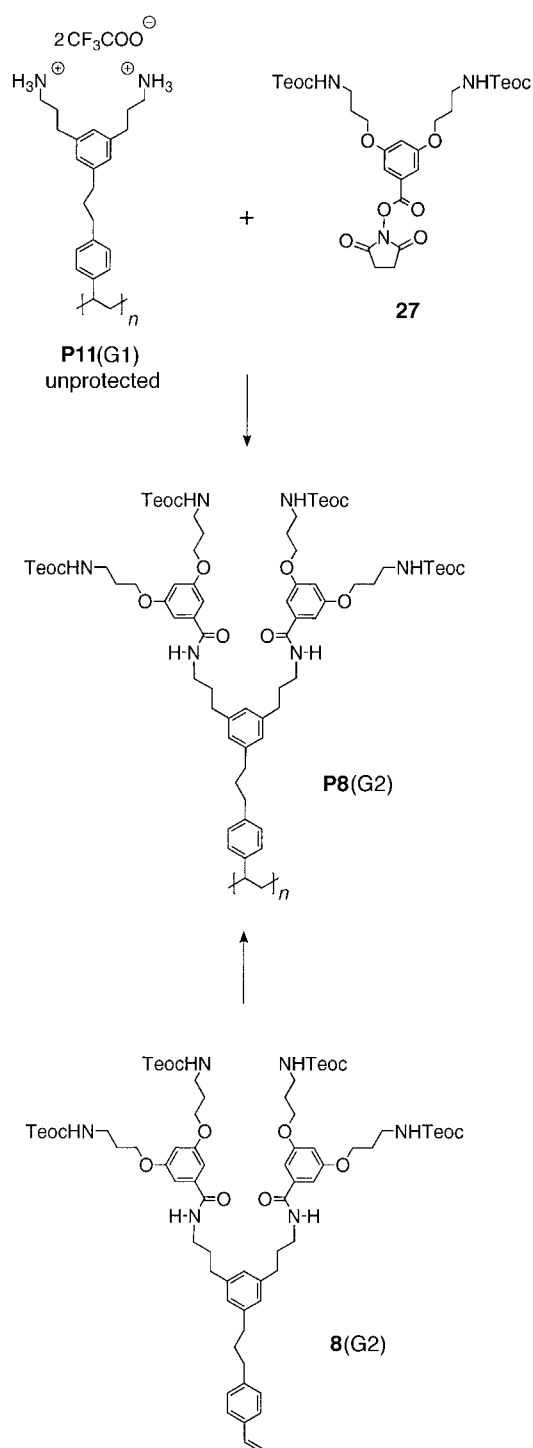


Figure 18. The ^1H NMR spectra of monomer **7**(G2), the corresponding polymer **P7**(G2), and its deprotected counterpart (from top to bottom) illustrate the structure control during polymerization and the degree to which deprotection of the polymer can be achieved. The signals of the Teoc protecting group (●) and the solvent (*) are marked.

functional groups are an integral part of the polymer and cannot be removed by purification as in common organic chemistry. Step c is the least developed. Scheme 3 shows a synthetic sequence in which the deprotected G1 polymer **P11**(G1) is allowed to react at the amine groups with the active ester of the protected G1 dendron **27** to give polymer **P8**(G2). The structure of this polymer was proven by all common means and additionally by independent preparation from monomer **8**(G2).^[29] Comparison of the ^1H NMR spectra (500 MHz) of samples obtained from the two routes proves that the coverage with **27** is virtually complete. This result is nice, but the chemistry is certainly not yet where it should be. The starting material was just a G1 polymer, which is anything else but a cylindrically shaped nanoobject, even if one takes the stiffening effect caused by the two positive charges of the ammonium functionalities into consideration. It is positive, however, that a polymer with roughly $P_n = 80\text{--}100$, which translates into 160–200 amine groups, could be completely



Scheme 3. Two different synthetic routes to polymer **P8**(G2) starting from **P11**(G1) with subsequent dendronization or from macromonomer **8**(G2).

covered with G1 dendrons. This experiment has initiated studies of more complex systems.

Attachment under amide formation was selected because it allows easy use of almost any interesting component—such as amino acids, sugars, or short DNAs—instead of dendrons. An alternative way to get to modified dendronized polymers is to polymerize modified dendronized monomers. For example, monomers **9**(G1 and G2) give sugar-coated polymers **P9**.^[28]

7. Summary and Outlook

High molecular weight dendronized polymers are synthetically accessible. If certain prerequisites are taken into account, even the sterically demanding macromonomers **12**(G3), **16**(G3), and **26**(G4) can be employed, which widens the scope of both radically initiated polymerizations as well as Suzuki polycondensations. The successful synthesis of polymers such as **P12**(G3) and **P23/26**(G4) allows investigation of the impact of the dendritic decoration on the backbones' coiling behavior and addresses the important question of whether or not steric congestion can be used as a tool to force molecules into a certain shape, which is more or less independent of the surrounding medium (in solution, adsorbed on surfaces, spread at solid/liquid interfaces, and in the solid state). SFM and SANS measurements as well as MD simulations reveal that, for example, polymer **P12**(G3) is remarkably stiff and can be viewed as a cylindrically shaped molecular object with a defined diameter of approximately 5 nm and a persistence length on the order of the contour length (50 nm). One may even be inclined to assign a "surface" to this polymer. Driven by the idea of generating molecular nanoobjects with functional surfaces just by polymerizing appropriately equipped monomers, researchers made the first steps to decorate these surfaces with both functional and polar/unpolar groups in order to engineer properties and explore possible fields of application.

Research on nanoobjects of the kind described is a truly interdisciplinary enterprise, and progress depends upon tight cooperation between synthetic chemists, experimental physicists, and theoreticians. Some future directions will be a) to develop synthesis to the point where several rigid objects with various surface functionalities are available and their lengths distributions are somewhat better under control, b) to isolate individual cylinders on surfaces and determine quantitatively molecular properties such as bending moduli, c) to try to move these objects about on surfaces for patterning and construction purposes, and d) to start investigations into their properties along the lines described in Section 2.

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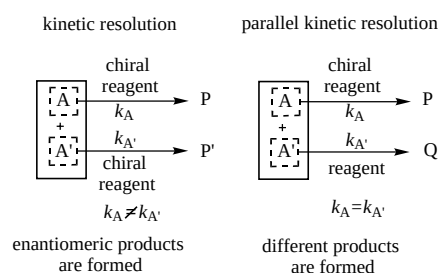
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Parallel Kinetic Resolutions

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Kinetic resolution of a 50:50 mixture of two enantiomeric substrates (A and A') with a single chiral reagent to give enantiomerically enriched compounds (P or P') is well documented.^[1] For resolution to occur the reaction rates must be unequal ($k_A \neq k_{A'}$), and for efficiency the reaction must be stopped at some stage before completion. At best, when only one enantiomer reacts, for example, reaction of A and A' with a chiral reagent gives a maximum of 50 % product P (derived from A) and 50 % recovered A', both of which are enantiomerically pure (Scheme 1). For this to occur, the



Scheme 1. Schematic representation of kinetic resolution and parallel kinetic resolution.

selectivity factor s ($k_A/k_{A'}$) needs to be greater than 200.^[1] Selectivities such as these are above that for most chemical kinetic resolutions, and even above that of some enzymes, such as lipase esterase.^[2] However, problems do arise depending on this inherent difference in selectivity and can have dramatic consequences on the yield and enantiomeric excess of the product P and the recovered substrate A'. This is in part due to the build-up of the less reactive enantiomer—its concentration is much greater than that of the more reactive enantiomer A owing to preferential removal of the latter. As the resolution approaches completion, the two enantiomers react equally due to the balance between this inherent rate and the available concentration.^[1]

One way of preventing this concentration effect and the resulting dominance of enantiomer A' near the end of the

resolution is to remove it in parallel during the course of the resolution. Ideally, its rate of reaction is similar to that of the other enantiomer. This has led to a new strategy termed parallel kinetic resolution^[3] (PKR; Scheme 1).

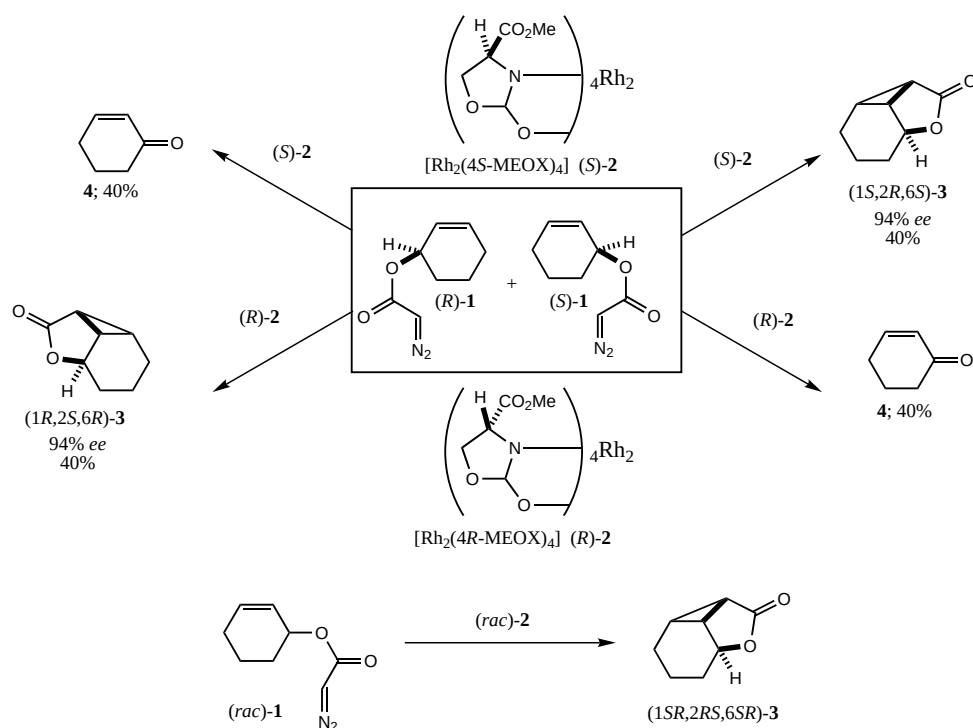
The concept of such a strategy is not new, in fact, the theoretical data^[4] have been available for many years. It has been shown that the selectivity factor s can be significantly lower for a parallel resolution than for a traditional kinetic resolution to achieve the same result. For example, an s factor of 49 for a parallel resolution corresponds to an s factor of 200 for traditional a kinetic resolution, where the products are isolated in 49 % yield (out of a maximum 50 %) and with 96 % *ee*.

One of the first examples to illustrate the usefulness of this strategy surfaced in the intramolecular cyclopropanation of racemic secondary allylic diazoacetates (Scheme 2).^[5] Treatment of (*rac*)-**1** with the catalyst tetrakis(4*S*-methoxycarbonyl-2-oxyoxazolidinyl)dirhodium(II) ($[\text{Rh}_2(4\text{S-MEOX})_4]$), (*S*)-**2** gave the tricyclic ketone (1*S*,2*R*,6*S*)-**3** in 40 % yield with an enantiomeric excess of 94 %. Surprisingly, the fate of the other enantiomer (*R*)-**1** is accounted for by the by-product 2-cyclohexenone (**4**), which was formed by intramolecular hydride abstraction with subsequent ketene loss. Furthermore, it has been shown that the chiral catalyst selectively removes just one enantiomer in the resolution by converting it into the ketone, and consequently the concentration effect is removed. Both enantiomers of the catalyst are available, and thus either enantiomer of the tricyclic ketone **3** can be synthesized efficiently. It is also worthy of note that in the case of a mutual kinetic resolution, using $[\text{Rh}_2(\text{cap})_4]$ (dirhodium(II)-caprolactamate) as the catalyst, (*rac*)-**1** gives exclusively (1*S*,2*R*,6*S*)-**3** in near perfect yield without formation of the by-product **4** (bottom of Scheme 2), which is due to the complementary recognition.

Another similar example involved the removal of the less reactive enantiomer by employing a stereorandom $\text{S}_{\text{N}}2$ substitution. Mischitz and Faber have shown that basic hydrolysis of the racemic epoxide (*rac*)-**5** occurs with the *Rhodococcus* sp immobilized enzyme (SP 409) to give the diol (*S*)-**6** in 40 % yield with a modest enantiomeric excess of 72 % (Scheme 3).^[6] However, conducting the reaction in the presence of an additional nonnatural nucleophilic azide (N_3^-) increased the *ee* value of this diol to greater than 90 %. It appears that this additional nucleophile removes the less reactive enantiomer (*R*)-**5** by a noncatalyzed $\text{S}_{\text{N}}2$ reaction.

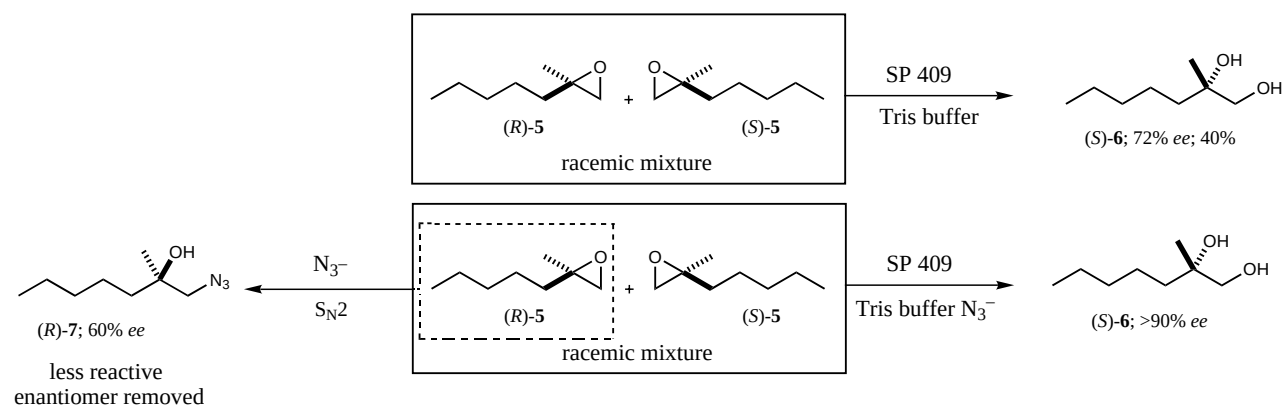
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Scheme 2. Intramolecular cyclopropanation of *(rac)*-1.

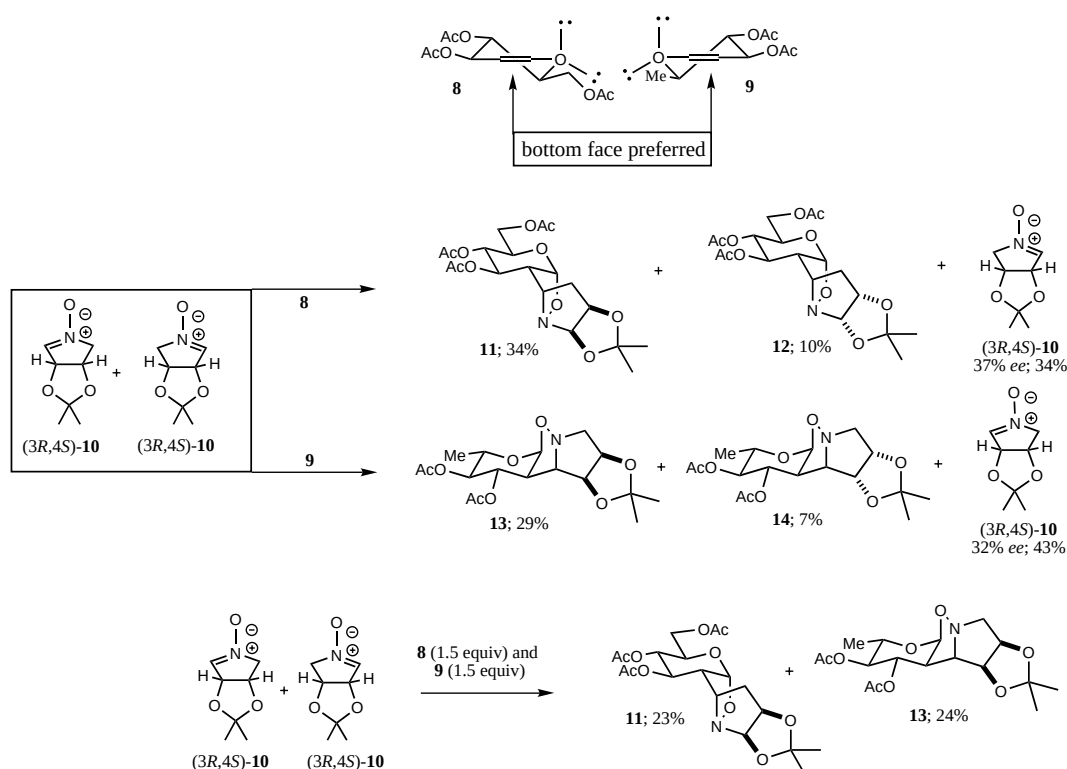
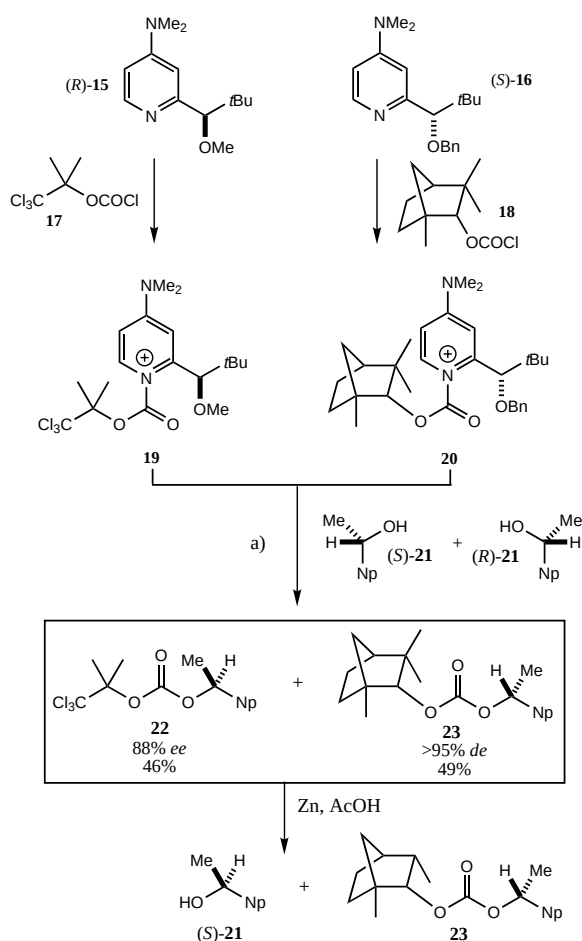
The azide alcohol *(R)*-7 was isolated with over 60% *ee*. The lower *ee* value for this alcohol is not unusual as the reaction was uncatalyzed and clearly the azide reacts equally with both enantiomers of epoxide **5**, but preferentially with the one present in larger concentration.

In an extension of this strategy Goti, Brandi et al. used the two quasi-enantiomeric dihydropyrans **8** and **9** as complementary reagents to selectively remove both enantiomers simultaneously (Scheme 4).^[7] They have shown that with a traditional kinetic resolution procedure the racemic *syn*-dihydroxypyrroline *N*-oxide **10** can be partially resolved by 1,3-dipolar cycloaddition to recover **10** as an enantiomer in either configuration with a modest enantiomeric excess (37–43% *ee*) depending on which dihydropyran is used. In the parallel kinetic resolution, racemic nitron **10** was treated with a slight excess of **8** and **9**, both of which displayed

Scheme 3. Basic hydrolysis of *(rac)*-5 with (bottom) and without (top) additional nonnatural nucleophilic azide (N_3^-). Tris = tris(hydroxymethyl)-aminomethane.

complementary selectivity and thus afforded two distinct and separable adducts **11** and **13**. Since the two competing reactions have similar rates, the optimum 50:50 substrate ratio was maintained, and therefore the maximum inherent selectivity was preserved throughout. The *exo* adducts **11** and **13** were formed exclusively in the expected 50:50 ratio by “matched” interactions as a result of 1,3-dipolar cycloaddition on the more electron rich bottom face of the dihydropyran. No minor diastereoisomeric adducts were observed, indicating that a near perfect match in relative rates was achieved. These adducts were further converted into quasi-enantiomeric imino-C-diasaccharides.^[7]

By far, the most elegant application of this strategy has been developed by Vedejs and Chen with a chiral DMAP acyl transfer reaction.^[3] Activation of the quasi-enantiomeric pyridines *(R)*-**15** and *(S)*-**16** with the hindered chloroformates **17** and (+)-fenchyl chloroformate (**18**) gave the acyl transfer agents **19** and **20** (Scheme 5), which have previously been shown to have opposite enantioselectivity. The alkyl substituent of these chloroformates (trichlorobutyl and fenchyl) is very important since it is transferred to the resolved alcohol, and because the substituents are obviously different the chloroformates can be used to enable product separation. The fact that the fenchyl group in **23** is chiral is probably irrelevant to the selectivity. Addition of equimolar amounts (1.1 mol equiv) of the separately formed pyridinium salts **19** and **20**, combined with an excess of $MgBr_2$ and Et_3N , to a solution of racemic 1-(1-naphthyl)ethanol (**21**, 1 mol equiv) gave the mixed carbonates **22** (46% yield, >88% *ee*) and **23** (near

Scheme 4. Use of complementary reagents **8** and **9** for the resolution of **10**.Scheme 5. a) Et_3N (3 equiv), MgBr_2 (2.25 equiv). In the case of **19** only the S enantiomer reacts, and for **20** only the R enantiomer. Np = 1-naphthyl.

perfect 49% yield, 95% ee). The separation of the products was made much simpler by treatment of the mixture with Zn in acetic acid (which chemoselectively removed the trichlorobutyl protecting group) to give the more readily separable, resolved alcohol (S)-**21** and the fenchyl carbonate **23**. The stereoselectivity was exceptional, and the two mixed carbonates were isolated in near perfect yield and with high enantiomeric excess. These quasi-enantiomeric chiral DMAP equivalents **15** and **16** have furthermore been shown to be fully recyclable.

It is unsurprising that such examples of efficient parallel kinetic resolution are rare—this is primarily due to the infancy of this strategy. It is clear that this resolution procedure is far more efficient than traditional methods at improving the enantiomeric excess of the required product. As a disadvantage, both types of resolutions can only give a maximum yield of 50% for a minimum of two possible products. As an alternative, the overall yield can be improved to 100% by conducting a dynamic kinetic resolution (DKP).^[8] This strategy also relies on removal of the less reactive enantiomer—in this case by recycling through racemization—rather than direct derivatization with an additional complementary chiral reagent, as in a parallel kinetic resolution.

From these studies it has been shown that for a successful and efficient parallel kinetic resolution the following guidelines need to be adhered to: 1) derivatization with both complementary chiral reagents has to occur without mutual interference, 2) the two reactions need to occur at a similar rate and have complementary stereocontrol, and 3) the reactions must afford distinct and easily separable products.

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Selective Oxidations of Linear Alkanes with Molecular Oxygen on Molecular Sieve Catalysts—A Breakthrough?

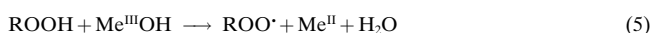
Martin Hartmann* and Stefan Ernst

Hydrocarbons that are oxidized at the terminal, that is, in the α - or 1-, position are important feedstocks for the chemical and pharmaceutical industry. Nevertheless, the selective oxidation of alkanes at their terminal methyl groups is still a challenge in modern catalysis research. It is well known that some enzymes are capable of performing selective terminal oxidations, however, without the necessary stability for use in the conditions employed for pure inorganic catalysts. In principle, selective partial oxidations are easier to control when hydrogen peroxide or organic hydroperoxides (for example, *tert*-butylhydroperoxide) are used as oxygen donors, although from an economic point of view, the use of molecular oxygen, that is, air or O₂, is preferred.

Recently, Thomas and co-workers^[1,2] succeeded in the preparation of a pure inorganic catalyst that allows for the oxidation of *n*-alkanes at the terminal carbon atoms with high selectivity using molecular oxygen in a liquid-phase reaction. These catalysts belong to the group of zeolite-like, crystalline and microporous aluminophosphates, in which small amounts of manganese or cobalt ions are introduced as redox centers into the zeolite framework.^[3,4] Essential for this group of catalysts is the fine tuning of the pore diameter, which enables the terminal C atom of the *n*-alkane to approach the catalytically active site. The especially selective catalysts have the AlPO₄-18 (AEI) topology. These are eight-ring molecular sieves with pore openings formed by eight TO₄ tetrahedra (T = Al, P, Co, Mn) and a pore diameter of about 0.38 nm. With such catalysts it seems feasible that the terminal carbon atom of the reactants approach the active site (that is, the transition metal ions in tetrahedral positions) and are oxidized at the end of the hydrocarbon chain to give the respective alcohols, ketones, and carboxylic acids. The catalytic results are explained by an oxidation mechanism involving radical intermediates, whose selectivities are superimposed by shape selectivity, that is, certain products or intermediates are not

formed as a result of the restricted space in the zeolite channels or cages.

From mechanistic considerations, oxidation reactions can be divided into three categories: 1) autoxidation via a free-radical chain reaction; 2) oxidation of the substrate coordinated to the metal ion followed by reoxidation of the reduced metal, and 3) catalytic oxygen transfer.^[5] The dominance of one of these mechanisms depends on the reaction conditions (gas or liquid phase, reaction temperature, pressure) and especially on the nature of the metal and the oxidizing agent used. Cobalt- and manganese-containing compounds are known to be highly efficient catalysts for the liquid-phase autoxidation via free radicals (mechanism 1) with oxygen as the oxidizing agent. Intermediates along this reaction path are alkoxy and alkylperoxy radicals [Eq. (1–6)]:



The initiation reaction [Eq. (1)] is typically very slow; once formed, the radicals quickly react with dissolved oxygen to form peroxy radicals (ROO[•]) [Eq. (2)]. Such peroxy radicals are stabilized in different ways, for example, by formation of a hydroperoxide [Eq. (3)]. The main role of the metal ions is to catalyze the homolytic decomposition of the intermediate hydroperoxide (ROOH) according to Equations (4) and (5). As a result of this decomposition, metal ions generate chain-initiating radicals, which form alcohols as oxidation products according to Equation (6). Only those metals that exist in two oxidation states with comparable stability are active catalysts for the hydroperoxide decomposition. Besides Cu⁺/Cu²⁺ and Fe²⁺/Fe³⁺, Co²⁺/Co³⁺ and Mn²⁺/Mn³⁺ are suitable. Until now,

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reactions of the type described (namely, the metal ion catalyzed decomposition of hydroperoxides) have been predominantly catalyzed by dissolved metal salts. Handling and reprocessing large amounts of metal-containing solutions, however, causes the well-known environmental problems. Furthermore, free-radical chain reactions do not yield terminal oxyfunctionalized alkanes preferentially because of the decreasing relative stability of tertiary to secondary to primary carbon atoms. Therefore, a major goal in research on catalytic oxidations in the liquid phase is the discovery of catalysts that suppress the autoxidation by free radicals and catalyze the oxidation by mechanism 2 (see above). Catalytic oxidations via oxometal intermediates are the main reaction path for the oxidation of hydrocarbons in the gas phase. This mechanism is called the Mars van Krevelen mechanism.^[5]

In this context, the above mentioned publications from the group of J. M. Thomas are worth mentioning.^[1] These authors report the oxidation of linear alkanes (namely, *n*-pentane, *n*-hexane, *n*-octane) with molecular oxygen under mild reaction conditions (373–403 K; 1.5 MPa). They employed cobalt- and manganese-containing aluminophosphate catalysts of structural type 18 (AEI; structure code of the International Zeolite Association (IZA)) and 36 (ATS) with respective pore diameters of 0.38 nm and 0.65×0.75 nm (Figure 1). The structure of AlPO_4 -18 consists of perpendicularly arranged 8-ring channels, while AlPO_4 -36 possesses a one-dimensional channel system with 12-ring channels. The addition of cobalt and manganese salts to the synthesis gel and an appropriate choice

of synthesis parameters results in the formation of molecular sieves, in which cobalt and manganese ions (≈ 4 atom % of the Al^{III} ions)^[1] are isomorphously substituted in tetrahedral positions. The regioselectivity observed for the oxidation of *n*-hexane on CoAPO -18 and MnAPO -18 is remarkable: after a reaction time of 24 h 60 % of the oxidation products are oxyfunctionalized at the terminal carbon atom and an additional 36 % are oxidized in the 2-position (hexan-2-ol, hexan-2-one). Interestingly, the corresponding acid (1-hexanoic) is formed with a high selectivity of 54 %. Analogous results have been obtained for the oxidation of *n*-octane, while the oxidation of *n*-pentane predominantly results in the formation of pentan-2-one and pentan-3-one. In contrast to the autoxidation in a homogeneous (liquid) phase, a dramatic enhancement in the regioselectivity of the reaction is observed for *n*-alkanes with sufficiently long alkyl chains to give products oxyfunctionalized in the 1-position. Thomas et al. demonstrated that these exceptionally high selectivities for the oxidation of terminal carbon atoms are only achieved with cobalt- or manganese-containing aluminophosphates of AEI structure. Similar regioselectivities are not observed over molecular sieves with larger pore diameters, for example, with AlPO_4 -5 or AlPO_4 -36 structures. These experimental findings are rationalized by Thomas et al.^[1, 2] as being a consequence of the narrow pore openings of molecular sieves with the AlPO_4 -18 (AEI) structure, which allow access of only slender hydroperoxide intermediates to the pore interior, where they can interact with the active metal site in an “end-on” configuration. The molecular sieve acts in a selective way, analogous to the protein mantle of an enzyme or the bulky ligand of a metal complex.^[6, 7] The mode of operation of these novel oxidation catalysts requires, however, that the cobalt and manganese ions remain incorporated in the zeolite framework and do not leach into the reaction mixture, where they work in the dissolved form (homogeneous catalysis). Experimental evidence that no leaching occurs is often difficult to produce, because metal ions in solutions may already possess sufficient catalytic activity at ppm concentrations.^[5, 8] To test for leaching, the catalyst is filtered from the hot reaction mixture and if the catalyzed reaction stops as a consequence of the catalyst removal, then it can be considered as a first hint of true heterogeneous catalysis. (In this context, it is important to point out that termination of the reaction after filtration of the cold reaction solution is not unambiguous evidence for the presence of a heterogeneously catalyzed reaction. Cooling down the reaction mixture may result in the heterogenization of the (previously dissolved) metal ions as a result of adsorption on the solid, that is, the molecular sieve.) On the basis of the results (termination of the reaction after filtration of the hot reaction mixture) reported by Thomas et al., one can conclude that the transition metal cations remained in the aluminophosphate framework. Nevertheless, the authors explicitly point out elsewhere that the conversion has to be kept low ($< 10\%$) to minimize the danger of leaching by polar reaction products (particularly carboxylic acids), which are formed in large amounts.

With respect to the reaction mechanism, Thomas et al. provide experimental data^[1] that suggest the participation of

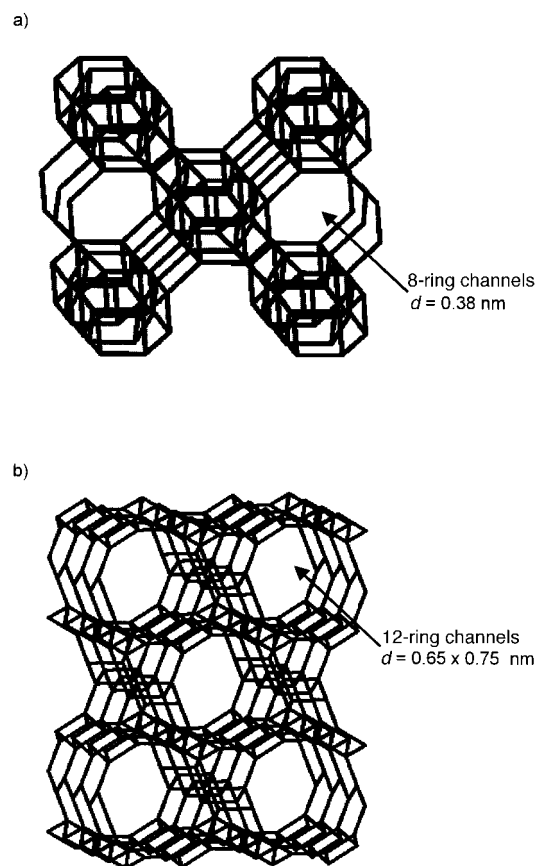


Figure 1. Structure of the aluminophosphates AlPO_4 -18 (a) and AlPO_4 -36 (b).

free radicals in the reaction: Addition of a free-radical initiator (*tert*-butylhydroperoxide) largely reduces the otherwise observed induction period; addition of a free-radical scavenger (hydroquinone) largely reduces the reaction rate.

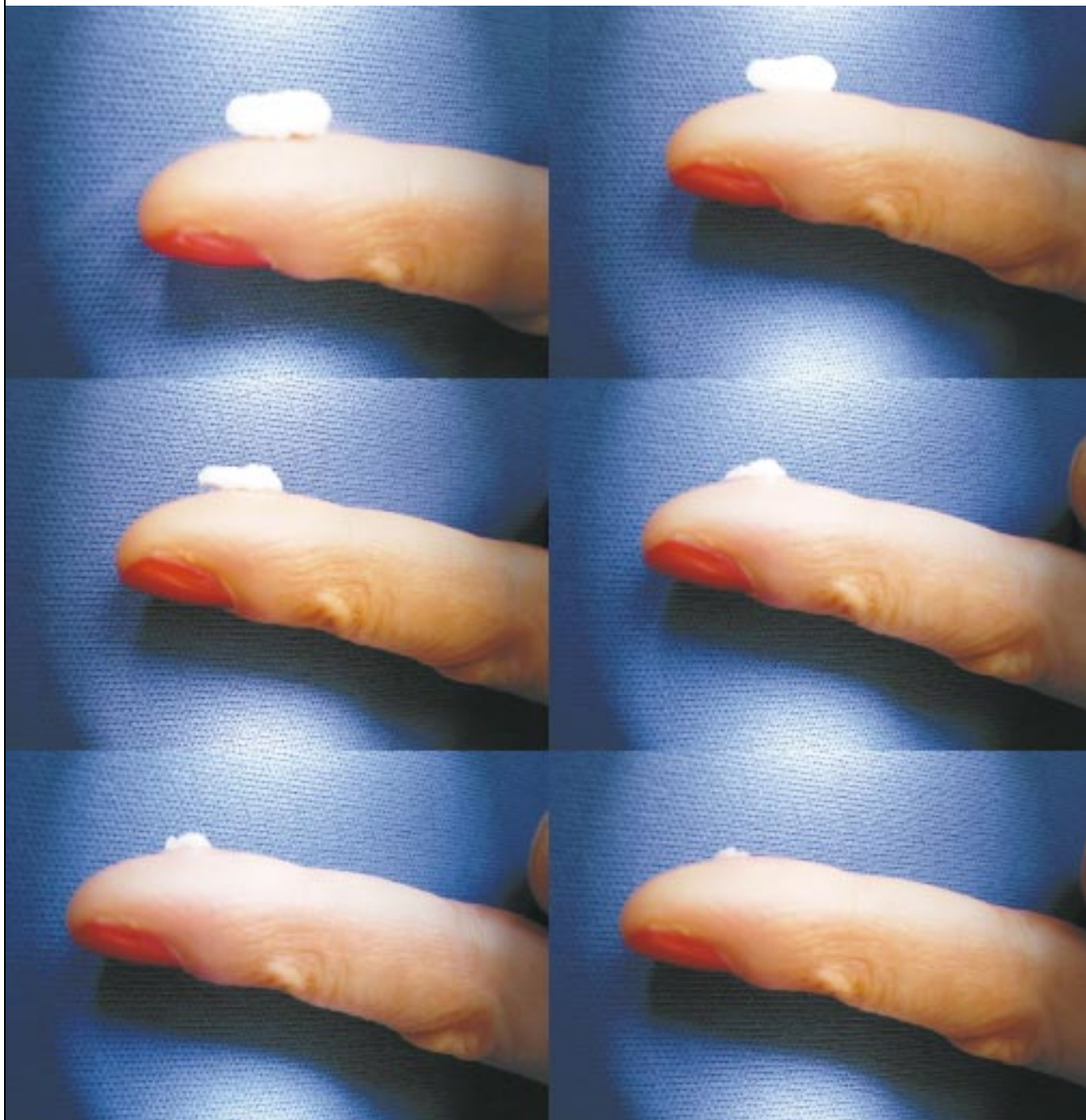
Only limited propositions can be made concerning the long-term stability of these catalysts. From the published conversion/reaction time curve, it follows that the reaction stops at a 6 to 10% conversion of the *n*-alkanes. A possible explanation for the observed catalyst deactivation, besides leaching of the metal into the solution, is the blocking of the catalytically active sites by polar reaction products. The experimental finding that a regeneration of the catalyst by washing with methanol and subsequent calcination at 550 °C is possible, supports the assumption of a blocking of the active centers or pores rather than a loss of active sites by leaching.

The catalytic materials based on crystalline, microporous cobalt- and manganese-containing aluminophosphates proposed by Thomas et al. represent a promising approach to the development of novel catalysts for the selective oxyfunctionalization of linear alkanes with molecular oxygen in the liquid phase. Meanwhile, it has been shown that such catalysts are also suitable for the epoxidation of alkenes with air^[9] and the Baeyer–Villiger oxidation of ketones to lactones.^[10] Although many questions (leaching, long-term stability, structure of the active site,^[11] uniformity of the active sites towards

activity, and selectivity, etc.) require further investigations, the recently presented results of Thomas et al. open new horizons for the selective oxidation of hydrocarbons with O₂ in the liquid phase.

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Carbonic Acid (H_2CO_3) on the Finger Tip



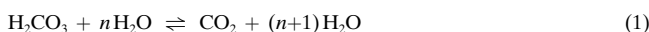
Decay of carbonic acid (H_2CO_3) in an ice matrix on warming from the temperature of liquid nitrogen (77 K) on the finger tip of Ingrid Kohl over a period of about two minutes. More about this on the following pages.

On the Surprising Kinetic Stability of Carbonic Acid (H₂CO₃)*

Thomas Loerting, Christofer Tautermann,
Romano T. Kroemer, Ingrid Kohl,
Andreas Hallbrucker, Erwin Mayer, and
Klaus R. Liedl*

The hydration of carbon dioxide is one of the most fundamental reactions in biological systems.^[1–3] The reaction partner, namely carbonic acid (H₂CO₃), has received considerable attention both from an experimental^[4–8] and theoretical viewpoint.^[9–13] Synthesis of carbonic acid—one of the most fundamental compounds in inorganic and organic chemistry as well as in biochemistry—proved to be rather elusive. For a long time even the observation of intermediary formed carbonic acid appeared unlikely due to the apparently rapid decay. Despite the belief of the nonexistence of carbonic acid, chemists recently succeeded first in recognizing the stability of carbonic acid^[4, 5] and then in isolating and characterizing^[6, 7] pure carbonic acid. It was even possible to sublime and recondense the solid and therefore prove the stability of gas-phase carbonic acid.^[8] Quantum-chemical investigations revealed the gas-phase dimer of carbonic acid to be energetically similar to its constituents, water and carbon dioxide,^[13] whereas the monomer is thermodynamically unstable.^[9, 12, 14]

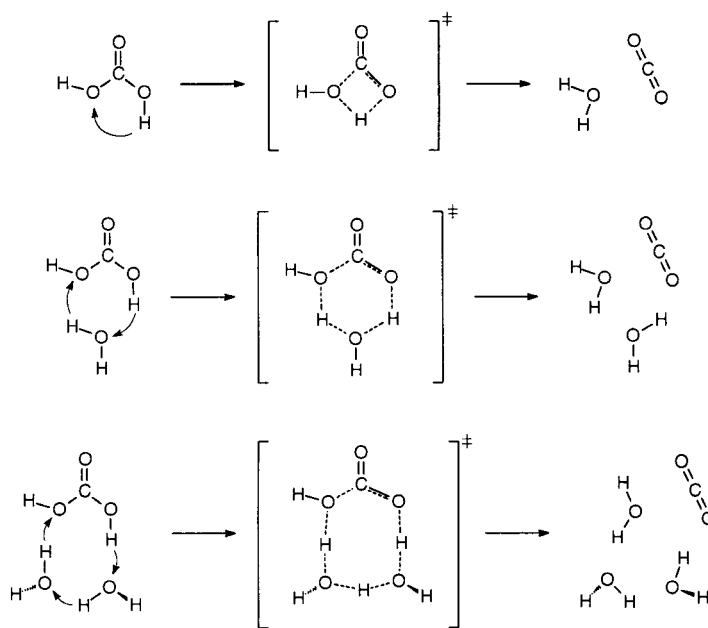
Here, we elucidate the influence of water molecules on the decomposition rate, that is the kinetic stability of carbonic acid in the presence of water [Eq. (1), $n = 0–2$].



We address the issue of the kinetic stability of carbonic acid in terms of transition state theory (TST) based on Eyring's concepts.^[15] The exact calculation of reaction rates requires thorough investigation of at least three completely different contributions: a) the energy differences between the reactants, the transition state, and the products, b) a multidimensional description of possible paths connecting reactants and products, and c) an accurate description of quantum effects, such as tunneling and zero point energies, arising from the dual particle-wave nature of the atoms. These three criteria are considered within the canonical variational transition state theory (TST) formalism used herein^[15] with corrections for multidimensional tunneling effects developed by Truhlar and co-workers.^[16–20] Recently, we confirmed the methodological details required to obtain chemically meaningful results.^[21, 22] For the benchmark double proton transfer reaction in carboxylic acid dimers we reached nearly quanti-

tative agreement to results of T₁ NMR spin-lattice relaxation spectroscopy, inelastic neutron scattering experiments, and fluorescence line narrowing in terms of reaction rate constants between 0 and 400 K, apparent activation energy curves, crossover temperatures, kinetic isotope effects, and vibrational and electronic ground-state tunneling-splittings.^[23]

The stationary points of the reaction hypersurfaces investigated here using $n = 0–2$ water molecules are depicted in Scheme 1. The geometries agree well with the results of previous calculations^[9–12, 14, 24–29] as well as with structures



Scheme 1. Geometries of the stationary points of the decomposition of carbonic acid in the presence of zero ($n = 0$), one ($n = 1$), and two ($n = 2$) water molecules as found at MP2/aug-cc-pVDZ level of theory.^[43]

obtained from microwave spectroscopy.^[30, 31] The reaction barrier for the decomposition of isolated carbonic acid of 44 kcal mol^{−1} is a first indication of its kinetic stability (Figure 1), which we established by accurate molecular quantum mechanics.^[32] The catalytic effect, that is the lowering of the reaction barriers due to the participation of a

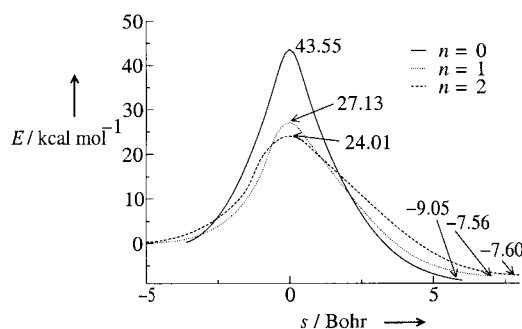


Figure 1. Energy along the classical reaction path obtained from a CCSD(T)/aug-cc-pVDZ//MP2/aug-cc-pVDZ///B3LYP/6-31+G(d)^[44–46] interpolation of the decomposition of carbonic acid in the presence of zero ($n = 0$), one ($n = 1$), and two ($n = 2$) water molecules.

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single water molecule,^[14] amounts to 16 kcal mol^{-1} . The movement of the nuclei within the reaction is described by the reaction path, which is classically the path of lowest possible energy connecting reactants with products (minimum energy path, MEP). However, in the case of the decomposition of carbonic acid, the reaction can be divided in two sharply separable parts: a) approach of oxygen atoms without variation of the O–H bond length, and b) transition of hydrogen atoms, while oxygen atoms remain in their positions. Noticeably, this reaction path clearly deviates from the classical MEP, because part b is highly amenable to tunneling due to the low mass of the moving protons. The combination of these tunneling paths and the precise energy barriers (see Figure 1) directly leads to the reaction rate constants depicted in Figure 2. These plots show non-Arrhenius behavior,

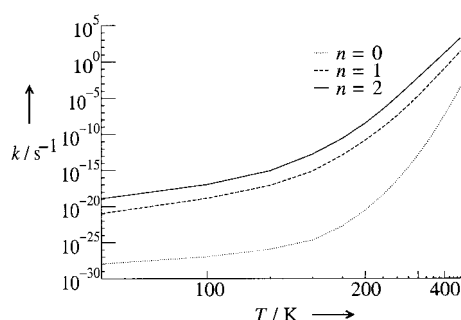


Figure 2. Arrhenius plot for the decomposition of carbonic acid in the framework of transition state theory including non-adiabatical multidimensional tunneling effects. The reaction path is calculated at B3LYP/6-31 + G(d)^[46] level of theory and interpolated to MP2/aug-cc-pVDZ^[43] geometries, CCSD(T)/aug-cc-pVDZ//MP2/aug-cc-pVDZ energies and MP2/aug-cc-pVDZ ($n=0$) or B3LYP/aug-cc-pVDZ ($n=1,2$) frequencies at the stationary points.

emphasizing that quantum effects are indeed very important in these reactions. Especially at low temperatures, where tunneling plays the most important role, there is a big difference to rates determined from classical TST alone. For instance, through tunneling, the uncatalyzed reaction at 150 K is a factor of 10^{20} faster than in the classical case. The low rates (Figure 2) for the decomposition of the carbonic acid monomer in the gas phase are in good agreement with the experiment, which demonstrates the kinetic stability at temperatures between 180 K and 220 K.^[8] The decomposition rate of $1.2 \times 10^{-13} \text{ s}^{-1}$ at 300 K, which corresponds to a half-life of 0.18 million years, indicates that carbonic acid is kinetically stable at room temperature. However, an autocatalytic process taking place after decay of the first molecule will lower this half-life in bulk H_2CO_3 significantly. The released water molecules form $\text{H}_2\text{CO}_3/\text{H}_2\text{O}$ complexes that decay much faster (Figure 2). Nevertheless, if synthesized free of water, carbonic acid is expected to be stable over a wide temperature range for the above-mentioned reasons.

On the other hand, carbonic acid in aqueous solution decomposes quickly.^[33] This phenomenon is directly explained by Figure 2. The addition of a single water molecule to the carbonic acid molecule increases the rate of decomposition by a temperature-dependent factor of 10^7 – 10^9 , that is the half-life is reduced to 10 h at 300 K. The addition of a second water

molecule further accelerates the reaction by a factor of 10^2 – 10^3 , yielding a half-life of 119 s at 300 K. Together these two accelerations amount to the remarkable factor of 50 billion at 300 K. This is the reason why most chemists believe “carbonic acid” to be unstable. But instead of H_2CO_3 (“carbonic acid”) they actually mean “carbon dioxide dissolved in water” (which leads to an equilibrium of 1 % H_2CO_3 and 99 % loosely hydrated CO_2 as well as to acidic behavior because of the $\text{H}_2\text{CO}_3/\text{HCO}_3^-$ acid base pair).^[34–38] The half-life of 30 days (two additional water molecules) at 225 K is consistent with the experimental fact that crystalline and amorphous H_2CO_3 are kinetically stable in an ice matrix. The experimentally determined decomposition rate to carbon dioxide and water in neutral aqueous solution of $18 \pm 7 \text{ s}^{-1}$ at room temperature^[33, 39] is just 3000 times faster than the calculated one using $n=2$. This good agreement suggests that the decomposition mechanism involves at least two catalytically active water molecules besides the electrostatic effect of the bulk environment.

The remarkable kinetic stability of gaseous H_2CO_3 in the absence of water has implications for its formation and detectability in outer space. Solid carbonic acid forms on proton irradiation not only of cryogenic $\text{CO}_2/\text{H}_2\text{O}$ ice mixtures,^[6] but also of pure solid CO_2 .^[40] The latter route appears promising for generating gaseous H_2CO_3 because, once formed in the solid state in the absence of water, it can vaporize without decomposition. The ratio of the monomer/dimer mixture of gaseous carbonic acid depends on temperature.^[8] In order to search for its existence in outer space in the gas phase, laboratory infrared spectra of the gaseous species are required for comparison with, for example, unidentified infrared bands.^[41] Because of hydrogen bonding pronounced spectral changes are expected for many normal modes in going from the monomer to the dimer and the solid, in a similar manner to that observed for formic acid.^[42] Therefore, infrared spectra of solid carbonic acid reported so far^[6–8] seem unsuitable for the detection of gaseous carbonic acid in outer space.

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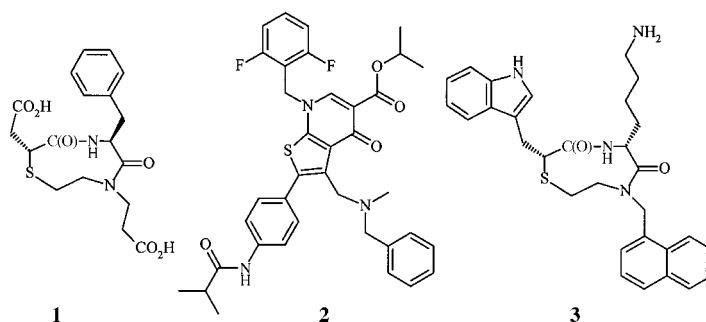
Are β -Turn Mimetics Mimics of β -Turns?

Gerhard Müller,* Gerhard Hessler, and Helene Y. Decornez

Dedicated to Dr. Pol Bamelis on the occasion of his 60th birthday

At the molecular level almost all important physiological and pathophysiological processes in the human organism are modulated by peptide–protein and protein–protein interactions. Consequently for pharmaceutical research these recognition phenomena represent attractive target systems for therapeutic intervention in a broad spectrum of disease states.^[1] From the viewpoint of structural chemistry β -turns have been attributed a special importance as carriers of molecular recognition since within the context of pharmacophore arrangement they allow a sterically controlled presentation of two to four interaction-mediating amino acid side chains.^[2] In medicinal chemistry the retention of the orientation of the amino acid side chains with simultaneous replacement of the peptide backbone by nonpeptide druglike structures, so-called “ β -turn mimetics”, has progressed to become an established topic in the structural design of peptide mimetics.^[3]

The relevance of the design element “ β -turn” with variable decoration in the molecular periphery has recently been convincingly confirmed by combinatorial chemistry and classical medical chemistry. This work resulted in, for example, high-affinity and selective peptidomimetic $\alpha_4\beta_1$ -integrin antagonists such as **1**,^[4] LHRH antagonists such as **2**,^[5] and somatostatin antagonists such as **3**.^[6]



Here we take up the problem of the conformational compatibility of β -turn mimetics with the secondary structural elements that are to be imitated, and we introduce a simulation procedure with which is possible to evaluate not

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only the compatibility, but also the turn-inducing potential of a designed β -turn mimetic. This evaluation can be made in advance of a possibly cumbersome chemical synthesis.

We have investigated a series of β -turn mimetics with respect to the preferred conformation by deterministic (molecular dynamics, MD) and stochastic (Monte Carlo, MC) molecular mechanics simulation procedures. All dipeptide mimetics were built into the $i + 1 - i + 2$ turn position of a cyclic hexapeptide analogue (Figure 1) that already exhibited a tendency to form and stabilize a β, β' -turn pattern because of its remaining tetrapeptide sequence (-Ala-D-Ala-Ala-Ala-). It is known from cyclic hexapeptides of this structural type that amino acids in the D-configuration preferably occupy the $i + 1$ position of β II' turns and thus induce the corresponding turn pattern.^[7]



Figure 1. Construction principle of the cyclic hexapeptide analogues cyclo(- β TM-Ala-D-Ala-Ala-Ala-) with which the β -turn mimetics were investigated by MD simulation for structural compatibility. β TM (β -turn mimetic) indicates the position of the mimetic; all starting conformations were generated so that the illustrated β, β' -turn pattern was preinduced.

By MD simulation over 100 ps the stability of the β, β' -turn pattern was investigated under explicit treatment of an aqueous solvent environment for the turn mimetics under discussion.

The unexpected conformational heterogeneity of dipeptide modules described as β -turn mimetics, as identified by the methods introduced here, is demonstrated on the basis of compounds **4**–**8**. Whereas the β, β' -turn pattern remains stable over the trajectory of the MD simulation for mimetics **4**–**6** and **8**, analysis of the MD simulation of the *ortho,ortho'*-disubstituted biphenyl derivative **7** reveals a significant incompatibility with the turn geometry that should actually be stabilized (Figure 2).

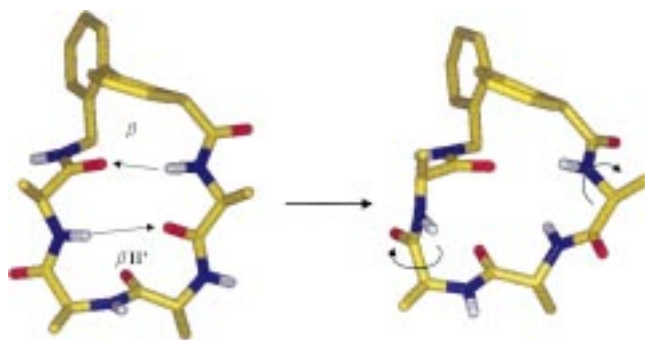
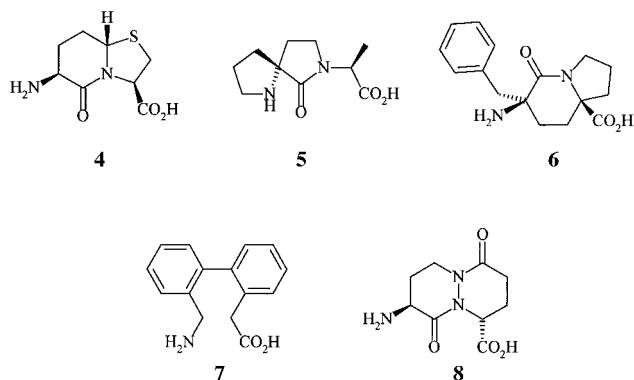


Figure 2. Starting conformation (left) of the cyclic hexapeptide analogue cyclo(7-Ala-D-Ala-Ala-Ala-) with a β, β' -turn structure (transannular hydrogen bonds are indicated as arrows) that was relinquished during the MD simulation. The mimetic **7** clearly destabilized the regular turn conformation in favor of the conformation illustrated on the right. The rotations around bonds indicated by arrows (right) lead to the loss of the β II'-turn in the lower part of the molecule.

To obtain a differentiated image of the conformational characteristics of the turn mimetics investigated here the potential for turn induction (adequate requirement) of each mimetic was investigated by computer methods in addition to the compatibility (necessary requirement for a mimetic). The preferred conformations of all dipeptide analogues that were inserted as a central building block into the model linear peptide sequence Ac-Ala-Ala- β TM-Ala-Ala-NHMe was investigated by MC-based simulated annealing protocol. Thus, the direct consideration of the influence of a minimal flexible peptidic environment is guaranteed. The stochastic scanning of the conformational space spanned by all rotatable bonds gave for each turn mimetic a conformer ensemble consisting of 1000 structures, which was analyzed on the basis of geometric turn diagnostic parameters (Figure 3).

The distribution profile determined from the conformer ensemble, illustrated in Figure 3, clearly shows that the potential for turn induction decreases in the order **6** > **5** > **4** > **8** > **7**. In terms of turn induction, the β -turn mimetic BTD **4** (bicyclic turned dipeptide), the “classic” of all mimetics, was actually surpassed by the spiro compound **5** and the β VI turn mimetic **6** ($i + 1 - i + 2$ *cis*-amide), which may be seen from the sharp maxima of the distribution profile of the geometric descriptors analyzed (Figure 3). The structural convergence in the turn region of mimetics **5** and **6** derived from the MC calculations is illustrated by the conformation families shown in Figure 4.

The conformational analysis of the hydrazide derivative **8** based on MC gives an extremely interesting and unexpected finding: Apart from the only moderately populated conformation family corresponding to the design in which the mimetic occupies the central positions ($i + 1 - i + 2$) of the β -turn being mimicked, a second, clearly energetically more favored, preferred conformation was identified. In this conformation **8** stabilizes a regular β -turn, which in the basic turn pattern is displaced by a backbone position in the direction of the N terminus (Figure 5).

A comparison of the distribution profile for the topology that should actually be stabilized (**8** in Figure 3) with the distribution profile of the arrangement displaced by one

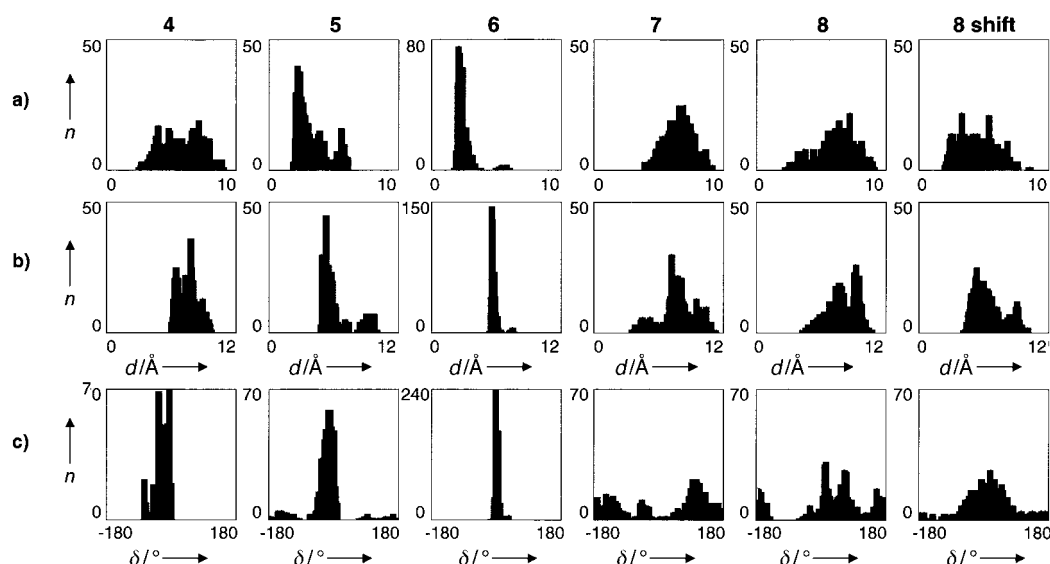


Figure 3. Statistical evaluation of the conformation ensemble produced by MC simulation. The following distribution profiles are illustrated: a) donor-acceptor distance ($(\text{HN}_{i+3})-(\text{C}=\text{O})_i$) of the turn-stabilizing hydrogen bond;^[9] b) the $\text{Ca}_i-\text{Ca}_{i+3}$ distance characteristic of β -turns (ideal value: 4.1–4.8 Å), according to Lewis et al.;^[10] c) pseudodihedral angle encompassing four consecutive C_α atoms of the turn-forming amino acids or dipeptide analogues (ideal value: $50^\circ > \theta > -50^\circ$). The ideal values are taken from the geometries of known β -turns^[2a] (βI , $\beta\text{I}'$, βII , $\beta\text{II}'$, βVia , βVib).

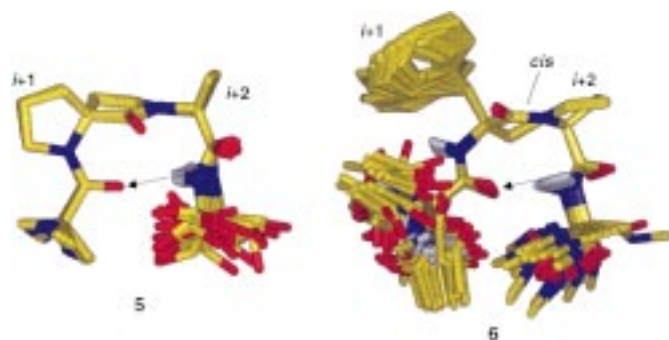


Figure 4. Superimposition of representative examples from the most highly populated conformation families derived from MC calculations for the β -turn mimetics **5** (left) and **6** (right) built into the model peptide. The reference atoms for the superimposition are the heavy atoms of the peptide-analogous backbone of the mimetic, including the atoms of the inwardly and outwardly directed amide bond. For reasons of clarity only the heavy atoms (C: yellow, N: blue, O: red) and amide protons (white) are shown.

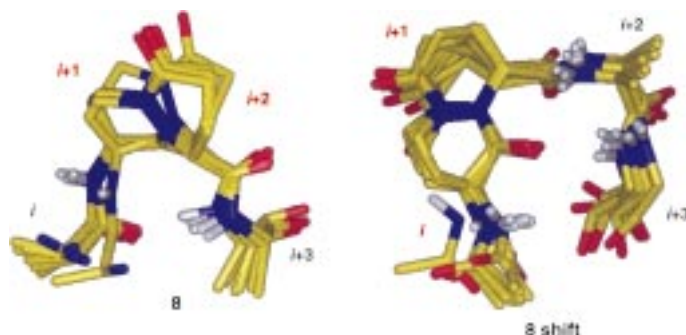


Figure 5. Structural comparison of the turn topologies identified by MC and stabilized by the mimetic **8**. In the less populated conformation family (left) the mimetic adopts the central position ($i+1-i+2$) of the β -turn, whilst in the most highly populated family (right) the turn positions $i-i+1$ are imitated by the mimetic.

position (**8shift** in Figure 3) suggests that the latter geometry is the more plausible turn arrangement (Figure 5).

In view of the results obtained from the simulation procedures for the predictive assumption of the turn-inducing potential, we suggest that the design of potential turn mimetics should be supported by the computer methods described here, for clearly not all of the compounds categorized as β -turn mimetics are to be described as such. The value of the simulation procedure lies in the early identification and elimination of “false-

positive” β -turn mimetics, that is, those compounds which were designed as turn mimetics but exhibit no corresponding compatibility in a three-dimensional structural context. Moreover, the molecular modeling method described here is suitable for the ranking of structure mimetics that in recent years have become established as attractive peptide mimetic templates for the rational design of combinatorial libraries but are to some extent cumbersome to synthesize.

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Novel Type of Thermostable Channel Clathrate Hydrate Formed by Heptakis(2,6-di-*O*-methyl)- β -cyclodextrin $\cdot$ 15H₂O—A Paradigm of the Hydrophobic Effect**

Thammarat Aree, Helga Hoier, Burkhard Schulz, Günter Reck, and Wolfram Saenger*

The α -, β -, γ -cyclodextrins (CDs) are macrocycles composed of six, seven, or eight glucose units linked by $\alpha(1 \rightarrow 4)$ glycosidic bonds.^[1] They adopt the shapes of hollow, truncated cones with both rims covered by OH groups: O6-H at the narrower side and O2-H and O3-H at the wider side (Figure 1; for atom-numbering scheme see Figure 2 a). Since all glucose units are oriented *syn*, O2-H and O3-H of adjacent glucose units may form intramolecular O2 $\cdots$ O3' hydrogen bonds which contribute to the conformational stability of the CD macrocycles. If dissolved in water, the CDs show a *positive* solubility coefficient as they are more soluble in hot than in cold water and they crystallize as high hydrates with water molecules located inside/outside the central cavities in ratios 2/4 in α -CD $\cdot$ 6H₂O,^[2] 6.5/5.5 in β -CD $\cdot$ 12H₂O,^[3] and 12/5 in γ -

CD $\cdot$ 17H₂O.^[4] In these hydrates, CD hydroxy groups and water molecules form irregular cooperative O-H $\cdots$ O hydrogen-bonded networks.^[5]

The solubility of the CDs in water is reversed when their glucose units are methylated at the 2- and 6- or the 2-, 3-, and 6-hydroxy groups. (In the following, the methylated CDs are denoted as DIMEA for hexakis(2,6-di-*O*-methyl)- α -CD; TRIMEG for octakis(2,3,6-tri-*O*-methyl)- γ -CD etc.) The solubility coefficients are now *negative*, the methylated CDs being more soluble in cold water (0.2–0.6 g mL⁻¹) than in hot water (<0.05 g mL⁻¹)^[6] where they crystallize (at 50–70 °C) as anhydrous DIMEA,^[7] anhydrous DIMEB,^[8] anhydrous TRIMEA,^[9] TRIMEB $\cdot$ H₂O,^[10] and TRIMEG $\cdot$ 2H₂O.^[11] The crystal structures show common features: a) two or three of the O6-CH₃ groups are rotated “inward” to close the cavity from the O6 side and reduce its volume; b) the cavity is either empty or filled by the O6-CH₃ group of an adjacent molecule (“self-inclusion”); c) none, one, or two water of hydration molecules are located in voids between the methylated CDs.

By contrast, TRIMEG and DIMEB crystallize from cold water (18 °C) in heavily hydrated forms, (4TRIMEG) $\cdot$ 19.3H₂O^[12] and DIMEB $\cdot$ 15H₂O (this work); in one case,

DIMEB $\cdot$ 2H₂O was crystallized,^[13] but this could not be reproduced. Since it was of interest to determine the hydration pattern of DIMEB $\cdot$ 15H₂O and to compare it to (4TRIMEG) $\cdot$ 19.3H₂O,^[12] the X-ray structure analysis described below was carried out.

In crystalline DIMEB $\cdot$ 15H₂O (see Experimental Section), all glucose units of DIMEB adopt the common ⁴C₁ chair conformation and are oriented *syn*; they are stabilized in this conformation by systematic interglucose O3-H $\cdots$ O2' hydrogen bonds with O $\cdots$ O distances in the range 2.81(1)–3.04(1) Å (Figures 1 and

2 a). All O2-CH₃ groups point “away” from the cavity, and the O6-CH₃ groups of glucose units 2, 4, and 6 are rotated “toward” the cavity and close it from this side (Figure 1). The cavity is not filled by water but by the O6-CH₃ group of an adjacent DIMEB molecule related by the twofold screw axis parallel to *a*, giving rise to infinite stacks of DIMEB molecules.

The hydration of these stacks by the 15H₂O molecules is remarkable. The O3-H groups of DIMEB form systematic hydrogen bonds to seven water molecules with O $\cdots$ O distances between 2.81(1) and 2.89(1) Å (Figures 1 and 2 a). Together with eight additional water molecules, a hydrogen-bonded, regular network of water molecules is constructed that encapsulates the DIMEB molecules in the form categorized as clathrate hydrate channel structure.^[14] The network is

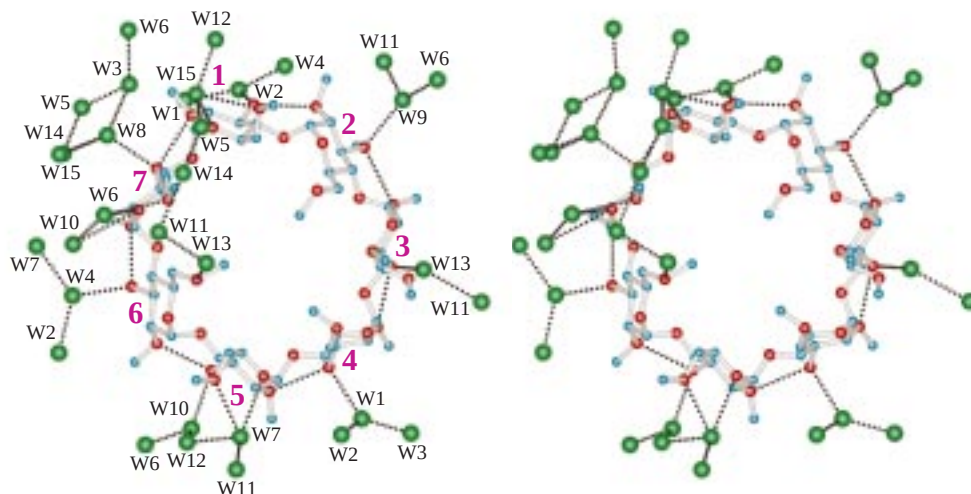


Figure 1. Stereodiagram showing the molecular structure of DIMEB and its hydrogen bonding to water molecules in DIMEB $\cdot$ 15H₂O. Carbon, oxygen, and water oxygen atoms as cyan, red, and green spheres, respectively; the seven glucose residues are labeled with red numbers, for atom-numbering see Figure 2 a. Dotted lines indicate O-H $\cdots$ O hydrogen bonds.

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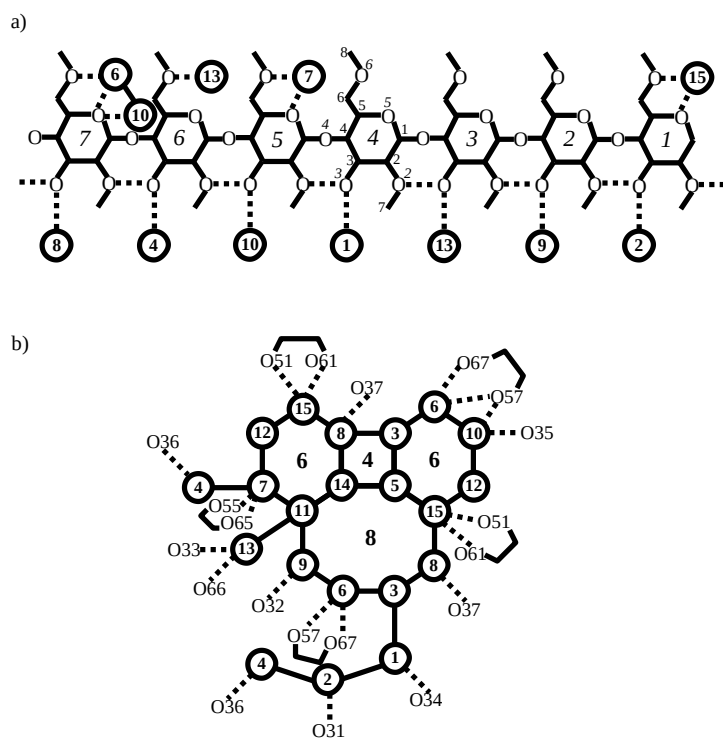


Figure 2. Schematic presentation of a) the hydration of DIMEB, glucose units are numbered sequentially 1 to 7, atom-numbering scheme is given for glucose unit 4; methyl-C atoms attached to O2 and O6 are assigned as C7 and C8, respectively. Strong hydrogen bond donors/acceptors O3-H are systematically hydrated, the weak O5, O6 acceptors only partially (glucose units 1, 5, 6, and 7), and O2 and O4 never. b) The water network showing edge-sharing polygons with one tetra-, two hexa-, and one octagon ($4^16^28^1$) described by **4**, **6**, **8**; water molecules are denoted as circled numbers; dashed and solid lines indicate hydrogen bonds $O_w \cdots O_{\text{DIMEB}}$ and $O_w \cdots O_w$, respectively. The chain W1-W2-W4 and the individual water molecule W13 are not involved in the formation of these polygons.

formed by a repeating motif described as $4^16^28^1$ (Figure 2b) which by the operation of the twofold screw axes (Figures 3a and 3b) yields corrugated sheets parallel to the *ac* plane; they are linked by chains of water molecules W1-W2-W4 into channels that harbor the stacks formed by DIMEB molecules.

The clathrate hydrate channel structure of DIMEB · 15H₂O is highly specific as no other methylated CD has the same constellation of O3-H groups that is required to anchor and stabilize the network of 15 water molecules. This clathrate hydrate is unique in four respects: a) there are no water pentagons that usually dominate clathrate and semiclathrate structures;^[14] b) in contrast to all other clathrate hydrates where either no or only few hydrogen bonds are formed between guest and clathrate water molecules, DIMEB · 15H₂O abounds with such interactions (see Figures 2a, 2b, and the broken pink sticks in Figures 3a, 3b). Of the 15H₂O, only five are *not* hydrogen-bonded to DIMEB, the other ten being in contact with the seven O3-H groups and with the O5 and O6 atoms, (see Figures 1, 2a, and 2b); c) a total of 15 hydrogen bonds anchors DIMEB tightly and specifically to the clathrate hydrate network and gives rise to high thermal stability: upon heating, a crystal-to-crystal phase transition occurs at 110°C with changes of unit cell constants *b* and *c* of −17.0% and 30.7%, respectively, and increase of unit cell

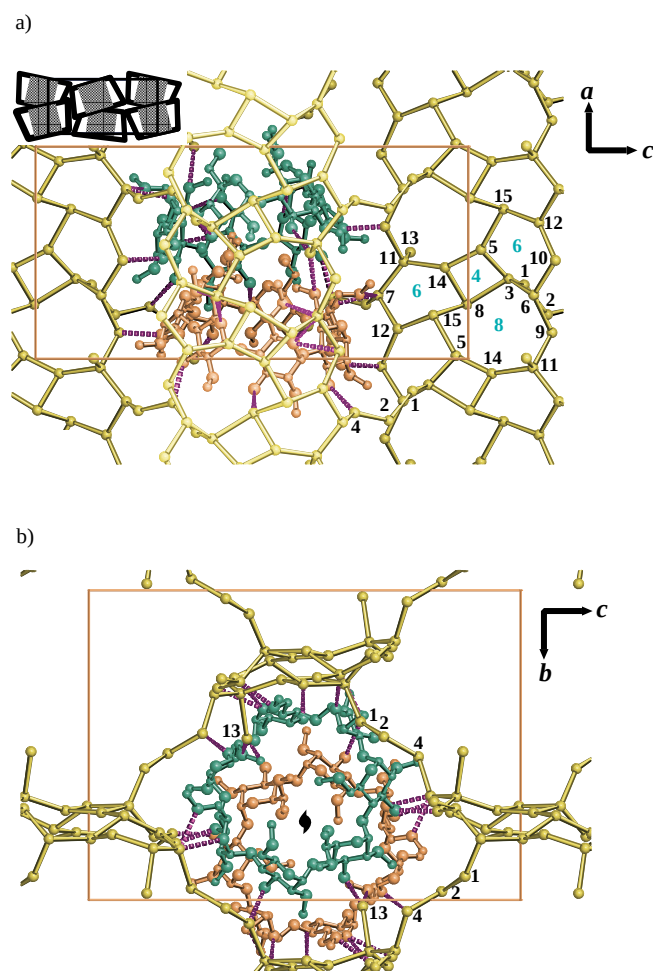


Figure 3. Clathrate hydrate channel structure in the crystal lattice of DIMEB·15H₂O in views on *ac* (a) and *bc* planes (b). Two DIMEB molecules related by the twofold screw operation ($\bullet$) parallel to *a* are shown in green and orange ball-and-stick representations; larger spheres are oxygen and smaller spheres, carbon atoms. Water molecules (labeled with black numbers) and hydrogen bonds between water molecules are shown as yellow balls and sticks, respectively. Broken pink sticks indicate hydrogen bonds between oxygen atoms of DIMEB and water molecules. The repeating unit (4^62^81) of the network formed from water molecules is indicated by cyan numbers ($O_w \cdots O_w$ distances 2.75(1)–3.26(2) Å). This motif is extended by the 2_1 operation parallel to *a* forming a “ribbon” in the *ac* plane (ribbons closer to the viewer (bright yellow) and further away (dark yellow) (a)). Each “ribbon” is connected by water chains W1-W2-W4 to other ribbons symmetry related by the operation of twofold screw axes parallel to *c*, giving rise to clathrate hydrate channels which accommodate the stacks of DIMEB (b). The inset in (a) shows a schematic presentation of crystal packing (water molecules omitted).

volume by 8.5 %, indicating structural changes; at 280 °C the X-ray diffraction pattern breaks down because crystals decompose (see Experimental Section)—by contrast, the most stable known clathrates (of the alkylammonium type) melt in the range -20 to 30 °C;^[14] d) DIMEB is the largest guest found thus far hosted by a clathrate hydrate.

Compared with DIMEB, the fully methylated TRIMEG has lost the strong hydrogen bonding donor/acceptor functionality of the O3-H groups; they are replaced by the weak O3-CH₃ acceptors so that no intramolecular O3-H...O2' hydrogen bonds can form. This explains why in (4TRIMEG)·

19.3H₂O crystallized from cold water^[12] each of the four independent TRIMEG molecules shows two diametrically opposed glucose units flipped from the common *syn* to *anti* orientation; their O6 atoms are now close enough to permit hydrogen bonding to a bridging water molecule. Additional water molecules are located inside and outside the cavities, hydrating the four TRIMEG individually: three to five O_{TRIMEG}...O_w hydrogen bonds connect TRIMEG to water clusters of different sizes and shapes.

It is conceivable that the structures of DIMEB · 15H₂O and (4TRIMEG) · 19.3H₂O represent snapshots of hydrated DIMEB and TRIMEG in cold aqueous solutions, similar to those found for trimethylamine decahydrate in crystalline and liquid states.^[15] This would correspond to Pauling's clathrate hydrate model of liquid water^[16] and to Jeffrey's "liquid analogy" to clathrate hydration.^[17] If aqueous solutions of DIMEB or TRIMEG are heated, we envisage that hydration water molecules become more mobile and the macrocycles more flexible so that the hydration networks break down, leading to aggregation and crystallization of DIMEB anhydrate^[8] and of TRIMEG · 2H₂O,^[11] the two water molecules being accommodated in intermolecular voids. As this particular behavior can be attributed, in essence, to the hydrophobic effect,^[18] the methylated CDs may serve as well-defined, easily obtainable objects to study it in more depth.

Experimental Section

DIMEB (Cyclolab/Budapest) crystallized from aqueous solution (0.3 g mL⁻¹) at 18 °C as pentadecahydrate (C_{56.25}H_{97.75}O₃₅ · 15H₂O), orthorhombic space group *P*2₁2₁2₁, *a* = 14.1632(5), *b* = 20.8278(7), *c* = 29.2611(10) Å, *M*_r = 1604.35, *V* = 8.631.7(5) Å³, *Z* = 4, ρ_{calcd} = 1.214 g cm⁻³. A crystal of 0.4 × 0.5 × 2.0 mm³ was sealed in a quartz capillary, and 37113 X-ray reflections were collected at room temperature to 0.9 Å resolution on a Bruker-AXS CCD area detector using graphite-monochromated MoK_α radiation (λ = 0.71073 Å); semiempirical absorption correction from ψ scans (μ = 0.11 mm⁻¹) and data reduction (with programs SAINT^[19] and SHELXTL^[20]) yielded 6760 unique reflections. The structure was determined by direct methods (SHELXS-97^[21]) and refined by full-matrix least-squares on *F*² (SHELXL-97^[22]). All non-hydrogen atoms were treated anisotropically. Most of hydrogen atoms of the β-CD skeleton and some of the water molecules could be located and were refined isotropically—positions of the methyl and hydroxy hydrogen atoms were calculated according to the "riding model";^[22] at glucose unit 2, a methyl group was located with an occupancy factor of 0.25 (due to impurity as verified by mass spectrometry, not shown). All 15 water sites were found fully occupied. The refinement of 1110 parameters converged at a final *R* = 0.069, *wR* = 0.177 for 5725 data with *F*² > 2σ(*F*²),^[23] the highest peak and deepest hole are 0.32 and -0.23 e Å⁻³ in the final difference electron density.

For thermal analysis, a DIMEB · 15H₂O crystal was mounted in an Enraf-Nonius heating device installed on a Mar Research image plate. The same diffraction pattern with 5° oscillation range was taken at 10 °C intervals from 20 to 310 °C using CuK_α radiation from an Enraf Nonius FR571 X-ray generator with rotating anode. Unit cell constants evaluated with DENZO^[24] showed a phase transition at 110 °C (changes of unit cell parameters *b* and *c* to 17.284 and 38.243 Å), and decomposition (loss of X-ray diffraction) above 280 °C. For differential scanning calorimetry (DSC), powdered DIMEB · 15H₂O (10 mg) was enclosed in an aluminum pan and heated in a Netsch Instruments apparatus from -100 to 350 °C. Phase transition occurred at 111 °C and decomposition above 280 °C. Figure 1 was drawn with MOLSCRIPT^[25] and rendered with RASTER3D,^[26] Figures 3a and 3b with SCHAKAL88.^[27]

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Synthesis and Functional Studies of a Membrane-Bound THF-Gramicidin Cation Channel**

Anna Schrey, Andrea Vescovi, Andrea Knoll, Corry Rickert, and Ulrich Koert*

Channel-mediated transport of ions through lipid bilayers is a particularly important molecular function.^[1] Synthetic ion channels can contribute to the understanding of the active conformations of biological ion channels.^[2, 3] As a result of the ability of tetrahydrofurans (THFs) to complex cations, a channel approach based on oligo-THFs^[4] and oligo-THF-amino acids^[5] was developed. Herein we report on the synthesis of a functional cation channel with a biomimetic channel entrance and exit where THF-amino acids are incorporated as building blocks.

Our biomimetic concept originates at gramicidin A, an ion channel active pentadecapeptide with the sequence HCO-L-Val₁-Gly₂-L-Ala₃-D-Leu₄-L-Ala₅-D-Val₆-L-Val₇-D-Val₈-L-Trp₉-D-Leu₁₀-L-Trp₁₁-D-Leu₁₂-L-Trp₁₃-D-Leu₁₄-L-Trp₁₅-CONHCH₂-CH₂OH.^[6] The active conformation of the gramicidin channel in lipid bilayers is a H-bonded head-to-head dimer consisting of two right-handed, single-stranded $\beta^{6,3}$ -helices.^[7] The helix turns forming the channel entrance and exit each contain four L-tryptophan residues. It is proposed that the indole side chains of the tryptophan groups contribute to the stabilization of the channel structure by H-bonding to the polar lipid head groups and through electrostatic dipole interactions with the surface potential of the membrane.^[8]

Our goal is to construct a unimolecular ion channel that would have the advantage of better structural characterization and functional control relative to a self-assembled channel consisting of numerous subunits. THF-gramicidin hybrids **1** and **2** were chosen as target compounds.

Compound **1** contains the heptapeptide part (L-Trp-D-Leu)₃-L-Trp, a tetra-THF-peptide part, a linker derived from tartaric

acid, and the pentadecapeptide known from gramicidin A. The (S,S)-tartrate unit has been used successfully for a covalently linked head-to-head gramicidin dimer.^[9] Both ends of **1** are terminated by ethanolamine units protected by lipophilic *tert*-butyldiphenylsilyl (TBDPS) groups. Compound **2** possesses free ethanolamine end groups. An INSIGHT/DISCOVER generated structural model of **2** is shown in Figure 1. In this structure the four THF-amino acids continue the gramicidin β -helix.

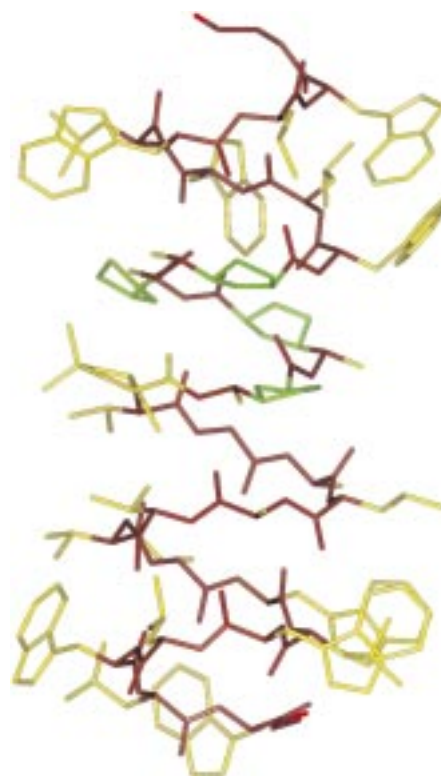
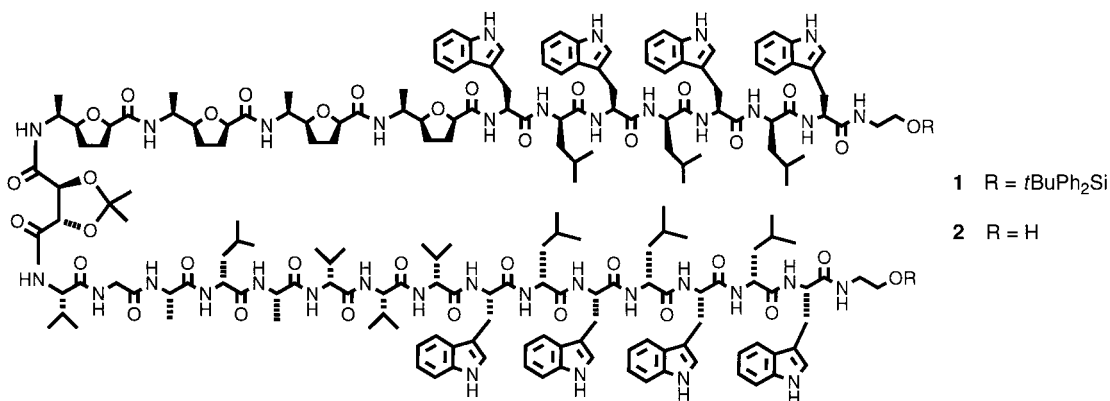


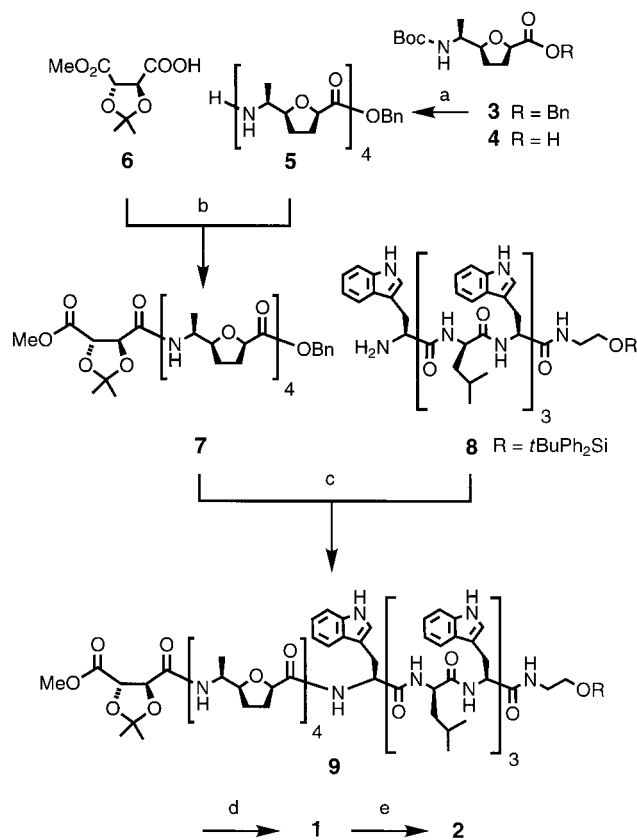
Figure 1. Structural model (INSIGHT/DISCOVER) of the ion channel formed by compound **2**. Peptide backbone is shown in brown, side chains and tartrate linker in yellow, THF rings in green.



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The starting point of the synthesis of **1** were the L-alanine-derived^[10] Boc-THF-amino acid building blocks **3** and **4** (Scheme 1). Both were connected via a dimer intermediate to THF-tetrapeptide **5**. Coupling of the acetonide-protected monomethyl tartrate (**6**) to **5** gave compound **7**. Hydrogenolysis of the benzyl ester of **7** afforded a carboxylic acid, which could be linked with the heptapeptide building block



Scheme 1. Synthesis of **1** and **2**. a) 1. CF₃COOH (5% in CH₂Cl₂), 30 min; 2. **4**, HOBT, EDC, Et₃N, CHCl₃, 12 h, 80%; 3. dipeptide, Pd(10%)/C, H₂, CH₃OH:H₂O (5:1), 25 °C, 1 h or CF₃COOH (5% in CH₂Cl₂), 30 min; 4. N- and C-deprotected dipeptides, HOBT, EDC, Et₃N, CHCl₃, 12 h, 81%; 5. CF₃COOH (5% in CH₂Cl₂), 30 min; b) HOBT, EDC, Et₃N, CHCl₃, 12 h, 86%; c) 1. **7**, Pd(10%)/C, H₂, CH₃OH:H₂O (5:1), 25 °C, 3 h; 2. **8**, 2,6-lutidine, HATU, DMF, 0 → 25 °C, 6 h, 80%; d) 1. LiOH, THF:H₂O (2:1), 25 °C, 30 min; 2. pentadecapeptide building block, 2,6-lutidine, HATU, DMF, 0 → 25 °C, 4 h, 60%; e) *n*Bu₄NF, THF, 25 °C, 2 h, 15% after column chromatography. HOBT = 1-hydroxybenzotriazole, EDC = *N*-(3-dimethylaminopropyl)-*N*-ethyl-carbodiimide hydrochloride, HATU = *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetra-methyluronium hexafluorophosphate.

8^[11] to yield compound **9**. After hydrolysis of the methyl ester of **9**, a peptide coupling with the pentadecapeptide L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-CONHCH₂-CH₂OTBDPS^[11] afforded target compound **1** (MALDI-MS: found: [M+Na]⁺ = 4215). A fluoride-mediated cleavage of the TBDPS groups gave compound **2** (MALDI-MS: found: [M+Na]⁺ = 3741).

The ability of **1** and **2** to function as channels was studied using conductance measurements^[12] on synthetic lipid bilayers (see Experimental Section). Compound **1** is incorporated into the membrane and acts as a functional cation channel. The single channel measurements (Figure 2a) display more than one conductance level. Level I with 15.4 pS and an average open time of 500 ms accounts for up to 30% of the channel events. Level II with 19.7 pS is found less often; its contribution to the overall current, however, is the highest as a result of its long open time of up to 15 s. The appearance of multiple channel levels suggests the existence of more than one channel-active conformation in the membrane. The

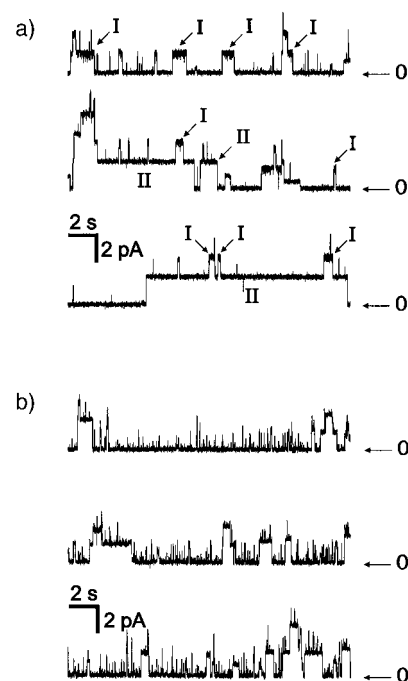


Figure 2. Typical single channel currents through soybean lecithin membranes in 1 M KCl at a membrane potential of +100 mV in the presence of 0.01 μM **1** (a) and **2** (b). The numbers indicate the closed channel (0), the conductance level with the highest frequency (I), and that with the highest open probability (II).

channels exhibit an Eisenman-I selectivity^[13] towards monovalent cations (NH₄⁺ > Cs⁺ > K⁺ > Na⁺, Table 1). If the passage of the ion through the channel requires a partial loss of the hydration sphere, then a strong influence of the dehy-

Table 1. Conductance and relative permeabilities of **1** and **2** to various cations.

Electrolyte/channel	Conductance ^[a]	Permeability ^[b]
NH ₄ Cl/ 1	35.7	9.8
NH ₄ Cl/ 2	42.3	10.9
CsCl/ 1	30.5	4.7
CsCl/ 2	34.0	5.4
KCl/ 1	20.2	2.7
KCl/ 2	23.1	3.1
NaCl/ 1	9.5	1.0
NaCl/ 2	16.3	1.0

[a] All values in pS, corresponding to the regression slope of the current–voltage relationship for the highest single-channel conductance level. [b] Values relative to Na⁺. Determined from the reversal potentials according to Goldmann, Hodgkin, and Katz. See ref. [1c].

dration energy and the weak binding sites in the channel would lead to the observed order of Eisenman-I selectivity.^[1c] Figure 3 shows the linear current–voltage relationships over the range of –100 mV to +100 mV for compound **1** with various cations.

The single channel measurements of compound **2** (Figure 2b) also exhibit multiple-conductance levels with Eisenman-I selectivity for monovalent cations, however, the open times are remarkably shorter. The removal of the lipophilic TBDPS groups results in a shortening of the channel dwell times.

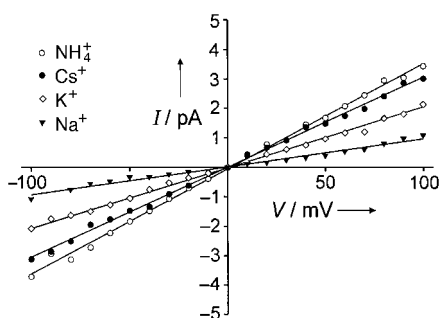


Figure 3. Current–voltage relationships for single channels of **1** in soybean lecithin membranes in the presence of various electrolytes. Only the highest conductance levels were considered.

The present work reports on the synthesis and functional characterization of a novel cation channel. The structural characterization of the active conformation of the THF-gramicidin hybrids in the membrane by isotope labeling and solid-phase NMR is currently under investigation and will be reported elsewhere.

Experimental Section

Planar lipid membranes were prepared by painting a solution of soybean lecithin (45% Avanti Polar Lipids) in *n*-decane (25 mg mL⁻¹) over a cuvette aperture with a diameter of 0.15 mm.^[14] The membrane surface was 0.01 to 0.02 mm² assuming a specific membrane capacity of 0.4 μF cm⁻². All experiments were performed at ambient temperature. The electrolyte solutions with a concentration of 1 M each were unbuffered. The probes, dissolved in methanol, were added to the *trans* side, to give a final concentration of 0.01–0.03 μM. Current detection and recording was performed using a patch-clamp amplifier Axopatch 200, a DigiData A/D converter, and the pClamp 6 software (Axon Instruments). The acquisition frequency was 5 kHz. The data were filtered with an analogue filter at 100 Hz for further analysis.

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Simple Synthesis of Tetra-Acceptor-Substituted Alkenes by the Formal Dehydrodimerization of Malonates**

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*Dedicated to Professor Bernd Giese
on the occasion of his 60th birthday*

Acceptor-substituted alkenes are important precursors for Diels–Alder reactions, Michael additions, or polymerizations. Due to their electronic properties, ethylenetetracarboxylates exhibit an especially high reactivity, but are difficult to prepare. Traditional syntheses start from halomalonates and sodium or mesoxalic acid,^[1] whereas modern methods by the dimerization of malonates require an excess of oxidant or the use of expensive azo compounds.^[2] Furthermore, disadvantages of all methodologies are the moderate yields. Herein we describe a simple new access to tetra-acceptor-substituted alkenes by the formal dehydrodimerization of malonates in only two steps. The synthesis is characterized by cheap reagents and high yields.

During the course of our investigations on transition metal mediated radical reactions,^[3] we succeeded in the addition of dimethyl malonate (**1a**) to various alkenes. To further improve the yields and due to the mild conditions, we became

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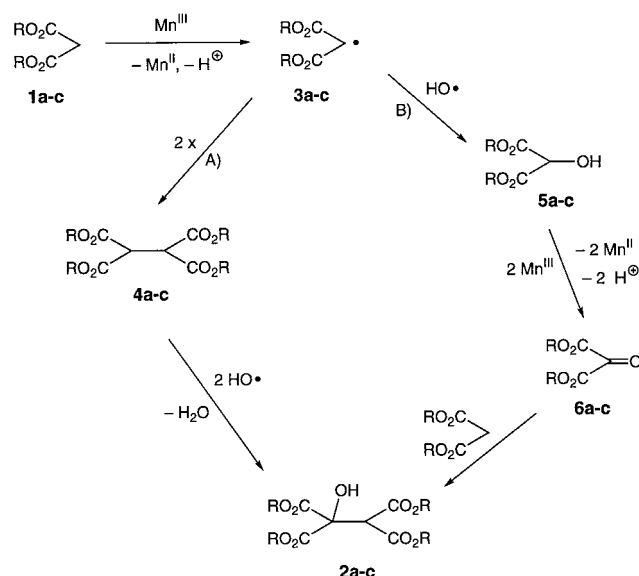
interested in reactions under ultrasonic irradiation. Such reactions have attracted much attention in organic synthesis^[4] and were recently applied for transition metal mediated radical reactions.^[5] However, our experiments did not afford the expected addition products, but instead the alcohol **2a** in high yield. To further optimize this surprising reaction, dimethyl malonate (**1a**) was irradiated without alkenes under various conditions (Table 1).

The initial experiments were performed in the presence of ceric(IV) ammonium nitrate (CAN; entries 1 and 2), the reagent of choice for the radical reactions we investigated recently.^[3c-e] However, the desired alcohol **2a** was isolated in only moderate yield at low conversion, which can be rationalized by the direct oxidation of the solvents.^[4] Manganese(III) acetate dihydrate (abbreviated here as Mn(OAc)₃) in acetic acid afforded better results (entry 3), although the conversions were not satisfactory. Finally, the optimal conditions were found by the addition of acetic anhydride and potassium acetate, which accelerate the enolization of CH-acidic compounds and, therefore, the generation of radicals (entry 4).^[3] Furthermore, reactions with catalytic amounts of oxidant were realized (entries 5–7), since manganese(II) can be reoxidized to manganese(III) in acetic acid during ultrasonic irradiation.^[5a] With only 0.05 equiv of Mn(OAc)₃, the conversion dropped remarkably (entry 7), but unconverted malonate **1** could be reisolated by distillation. Besides manganese(III) acetate, CAN and potassium permanganate can serve as cheap oxidants, but again for such reactions the conversion is lower (entries 8 and 9). Finally, control experiments clearly indicated that both manganese salts and ultrasound are essential for the formation of the alcohol **2a** (entries 10 and 11). After the successful optimization of the reaction conditions with dimethyl malonate (**1a**), the diethyl ester **1b** and the sterically more hindered diisopropyl ester **1c** afforded the desired products **2b** and **2c** in excellent yields as well (entries 12 and 13).

Table 1. Ultrasonic reactions of the malonates **1**.^[a]

Entry	Ester (R =)	Oxidant [equiv]	Solvent	Conversion [%] ^[b]	Yield of 2 [%] ^[c]
1	1a (Me)	CAN [1.0]	MeOH ^[d]	20	45
2	1a (Me)	CAN [1.0]	CH ₃ CN ^[d]	20	43
3	1a (Me)	Mn(OAc) ₃ [1.0]	HOAc	40	90
4	1a (Me)	Mn(OAc) ₃ [1.0]	HOAc/Ac ₂ O ^[e]	85	96
5	1a (Me)	Mn(OAc) ₃ [0.4]	HOAc/Ac ₂ O ^[e]	90	98
6	1a (Me)	Mn(OAc) ₃ [0.1]	HOAc/Ac ₂ O ^[e]	90	98
7	1a (Me)	Mn(OAc) ₃ [0.05]	HOAc/Ac ₂ O ^[e]	60	93
8	1a (Me)	CAN [0.1]	HOAc/Ac ₂ O ^[e]	60	90
9	1a (Me)	KMnO ₄ [0.1]	HOAc/Ac ₂ O ^[e]	65	98
10	1a (Me)	–	HOAc/Ac ₂ O ^[e]	–	–
11	1a (Me)	Mn(OAc) ₃ [0.1]	HOAc/Ac ₂ O ^[e,f]	–	–
12	1b (Et)	Mn(OAc) ₃ [0.1]	HOAc/Ac ₂ O ^[e]	85	98
13	1c (iPr)	Mn(OAc) ₃ [0.1]	HOAc/Ac ₂ O ^[e]	80	98

[a] Reaction of 5.0 mmol of malonate **1** in 40 mL of solvent at 80 °C for 3 h, irradiation with a BANDELIN sonotrode HD 200 (50 % intensity). [b] Unconverted malonate **1** was reisolated by distillation. [c] Yield based on converted malonate **1**. [d] Reaction at 50 °C. [e] Addition of 3.0 g of potassium acetate. [f] Reaction without ultrasound.

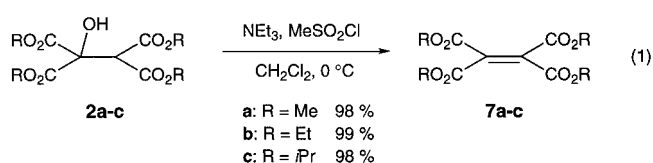


Scheme 1. Proposed mechanism for the formation of alcohols **2**.

The formation of the alcohols **2** can be rationalized by two different mechanistic pathways (Scheme 1). In the first step, the malonyl radicals **3** are generated from the malonates **1**, an electron transfer reaction that is strongly accelerated by ultrasound.^[4, 5] The resulting high concentration of radicals might lead to a dimerization (pathway A). Finally, the oxidation of the dimers **4** to the observed products **2** by hydroxy radicals, which are always formed by ultrasonic reactions,^[4] is conceivable. Alternatively, the malonyl radicals **3** might be trapped directly by hydroxy radicals to form the alcohols **5** (pathway B), which are further oxidized to the mesoxalic esters **6** under the reaction conditions. This hypothesis is in accordance with examples for the acceleration of the oxidation of alcohols by manganese salts and ultrasound.^[6] Finally, the excess of malonates **1** and ketones **6** should afford the products **2** by a Knoevenagel reaction.

To differentiate between these two mechanistic pathways, the dimer **4b** was synthesized independently^[1a] and then irradiated in acetic acid/acetic anhydride in the presence of Mn(OAc)₃. Even after 6 h no conversion was obtained, which excluded pathway A. Further evidence for pathway B was found by control experiments with diethyl malonate (**1b**) and diethyl mesoxalate (**6b**), which react smoothly to the alcohol **2b** under irradiation. Therefore, ultrasound not only accelerates the generation of radicals and the oxidation to the mesoxalates **6**, but also the Knoevenagel reaction, which is in accordance with literature results.^[7]

To demonstrate the potential of the herein described ultrasonic reactions for the synthesis of tetra-acceptor-substituted alkenes, we investigated the dehydration of the alcohols **2**. Triethylamine and methanesulfonyl chloride were found to be the reagents of choice; they afforded the desired alkenes **7** quantitatively under mild conditions [Eq. (1)].



In summary, malonates, which are cheap and commercially available C_3 building blocks, were transformed into tetra-acceptor-substituted alkenes in only two steps in excellent yields. The procedure is characterized by simple reagents, especially since only a catalytic amount of $Mn(OAc)_3$ is required. Ultrasonication was the method of choice as it accelerates the radical reactions as well as the Knoevenagel reactions. Since radicals can be generated from various CH-acidic substrates by manganese(III), the present methodology offers promising prospects for the synthesis of other acceptor-substituted alkenes.

Experimental Section

A solution of malonate **1** (5.0 mmol), potassium acetate (3.0 g, 31 mmol), and acetic anhydride (1.0 mL, 11 mmol) in acetic acid (40 mL) was heated to 80 °C. Manganese(III) acetate dihydrate (270 mg, 1.0 mmol) was added, and the mixture was irradiated (BANDELIN sonotrode HD200, 50 % intensity) for 3 h. After dilution with water (200 mL), the solution was extracted with dichloromethane (3 × 50 mL) and the combined organic phases were washed with a saturated sodium hydrogen carbonate solution (2 × 50 mL) and brine (50 mL). After drying (sodium sulfate) and concentration under vacuum, the unconverted malonate was removed by Kugelrohr distillation. The remaining alcohol **2** can directly be used for the next step or recrystallized from ethanol. A solution of the alcohol **2** (10.0 mmol) in dichloromethane (40 mL) was cooled to 0 °C, and triethylamine (3.0 g, 30 mmol) and a solution of methanesulfonyl chloride (2.3 g, 20 mmol) in dichloromethane (10 mL) were added dropwise at this temperature. After 2 h 10 % hydrochloric acid (50 mL) was added carefully and the combined organic phases were washed with a saturated sodium hydrogen carbonate solution (2 × 50 mL) and brine (50 mL). After drying (sodium sulfate) and concentration under vacuum the residue was crystallized from isopropanol. The alkenes **7** were isolated in analytically pure form in 98–99 % yield.

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Direct Evidence for Trivalent Cationic Conduction in Nd^{3+} - β'' - Al_2O_3 **

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Dedicated to Prof. Arndt Simon
on the occasion of his 60th birthday

The ionic conduction properties of monovalent $M^{+}\beta''$ - Al_2O_3 compounds ($M = Na, Ag, Cu$) have been known for decades.^[1, 2] Also the migration of ions in oxides with divalent metals, $M^{2+}\beta''$ - Al_2O_3 ($M = Ca, Sr, Pb$), has been confirmed and identified.^[3–5] On the other hand, the issue of whether or not $M^{3+}\beta''$ - Al_2O_3 crystals behave as conductors with mobile M^{3+} cations as charge carriers has not yet been clarified and is still being controversially discussed. The main point of the criticism concerns the high charge of the trivalent cations. It is assumed that the resulting high Columbic interaction with the surrounding anionic host lattice prevents a corresponding current transport. Here, new experiments and results are presented for $Nd^{3+}\beta''$ - Al_2O_3 crystals which directly identify and confirm the trivalent cation transport in $M^{3+}\beta''$ - Al_2O_3 .

About two decades ago, the Na^{+} ions in $Na^{+}\beta''$ - Al_2O_3 crystals were replaced by trivalent lanthanide cations (Ln^{3+}).^[6] The realization of these ion-exchange experiments already indicates that the trivalent cations are capable of noticeable diffusive migration at comparatively low temperatures.^[7] However, the final evidence that these cations behave as charge carriers in an electric potential gradient and migrate macroscopic distances within the crystal could not be given.

Previous characterizations of $Ln^{3+}\beta''$ - Al_2O_3 compounds are mainly based on impedance spectroscopy^[8–11] or X-ray diffraction experiments^[12–15] in which the measured impedances and electron density distributions are interpreted as the mobility of the trivalent cations. However, critics have often expressed doubt that these methods actually convey con-

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duction over trivalent cations. Alternative explanations for the experimental data are possible owing to a fundamental problem of ion-exchange reactions: It is impossible to achieve 100 % ion exchange of the Na^+ ions by other cations. Thus, the resulting $\text{Nd}^{3+}/\beta''\text{-Al}_2\text{O}_3$ crystals always contain, to a certain extent, remaining nonexchanged Na^+ cations, which influence the conductivity measurements and which may lead to a misinterpretation of the data. Furthermore, the presence of divalent Mg^{2+} cations in the conduction planes must be assumed. Magnesium oxide is used during the synthesis of $\text{Na}^+/\beta''\text{-Al}_2\text{O}_3$ in order to stabilize the metastable $\beta''\text{-Al}_2\text{O}_3$ phase. The main fraction of the MgO is built into the spinel blocks of the crystal structure, but a partial incorporation of Mg^{2+} cations into the conduction planes cannot be excluded. In such a case, the Mg^{2+} cations are also assumed to be mobile and would consequently influence the electrical conductivity measurements.

Therefore, with respect to the presence of different cations within the conduction planes, experiments are needed to *directly* identify the migrating charge carriers. For this purpose, direct current (dc) electrolysis has turned out to be the most appropriate method. It has already been successfully used to identify the phenomenon of trivalent cationic conduction in compounds crystallizing in the $\text{Sc}_2(\text{WO}_4)_3$ structure type.^[16] In these experiments, the sample to be investigated is exposed for a given length of time to a constant potential difference, which forces the mobile cations to migrate from the anode towards the cathode. Through the use of nonreversible (ion-blocking) electrodes, characteristic cation redistributions occur at the electrodes (e.g. cation depletion at the anode, cation accumulation at the cathode), which can be measured by usual physical methods.

For the identification of trivalent cationic conduction in $\text{M}^{3+}/\beta''\text{-Al}_2\text{O}_3$, the choice of the appropriate electrolysis temperature is of significant importance. An interpretation of the conductivity data as a function of temperature can be found in the literature for $\text{M}^{3+}/\beta''\text{-Al}_2\text{O}_3$ ($\text{M} = \text{La}, \text{Nd}, \text{Pr}$).^[15] Accordingly, the ionic conductivity at low temperatures ($T < 250^\circ\text{C}$) is based only on the remaining nonexchanged Na^+ cations. At higher temperatures, however, the trivalent cations also become mobile, generating a mixed cationic conduction. This transition from pure Na^+ conduction to mixed $\text{Na}^+/\text{Nd}^{3+}$ conduction occurs at about 250°C and can be recognized by an apparent change in the activation energies E_a ($E_a(T > 250^\circ\text{C}) > E_a(T < 250^\circ\text{C})$) in the corresponding Arrhenius plots.

Following this model, two electrolysis experiments were performed below and above the transition temperature. The cation distribution patterns at the two temperatures were expected to differ significantly, and thus give rise to a clear identification of Nd^{3+} conduction. The experimental procedure for the electrolysis is schematically shown in Figure 1. Several $\text{Nd}^{3+}/\beta''\text{-Al}_2\text{O}_3$ crystals, prepared by ion exchange of $\text{Na}^+/\beta''\text{-Al}_2\text{O}_3$ with NdCl_3 ,^[17] were placed between two non-reversible (ion-blocking) platinum electrodes,^[18] and a constant dc voltage was applied.^[19, 20]

As shown in Figure 1c, the crystals were then cut perpendicular to the platelet plane—that is, perpendicular to the conduction planes in which the cation conduction occurs.

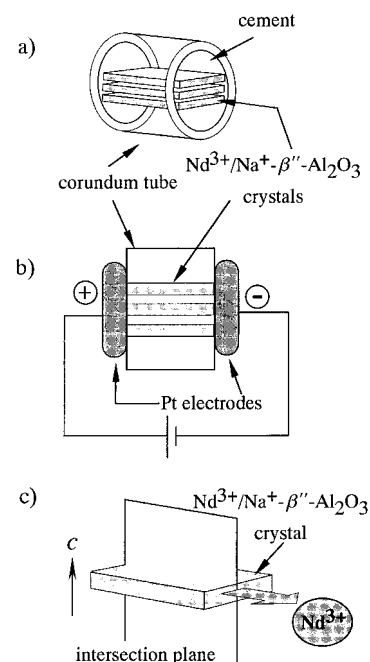


Figure 1. Schematic representation of the pellets, the electrolysis experiments, and the preparation of samples for EMPA. a) Several $\text{Nd}^{3+}/\beta''\text{-Al}_2\text{O}_3$ crystals are fixed in a corundum tube with a cement-like binder; b) electrolysis cell containing nonreversible Pt electrodes, which are arranged perpendicular to the conduction planes of the crystals; c) intersection plane perpendicular to the conduction planes of $\text{Nd}^{3+}/\beta''\text{-Al}_2\text{O}_3$. The corresponding cross-section (from anode to cathode) was characterized by EPMA.

Concentration profiles of the entire cross-section of the sample were measured by electron probe microanalysis (EPMA) for the elements sodium, neodymium, aluminum, and magnesium. EPMA was also used for the characterization of the anodic and cathodic surfaces as well as for the determination of the chemical composition of the crystals before the electrolysis. The average composition of the $\text{Nd}^{3+}/\beta''\text{-Al}_2\text{O}_3$ crystals used (averaged over ten samples) was found to be $\text{Na}_{0.04}\text{Nd}_{0.57}\text{Mg}_{0.52}\text{Al}_{10.43}\text{O}_{17.04}$ with a degree of exchange of 97.6%.^[21] Despite “complete” ion exchange, 2–3 % (based on the original sodium content) of the Na^+ cations still remain within the samples.

The concentration profiles of the elements Na and Nd are shown in Figure 2 for the electrolysis of a $\text{Nd}^{3+}/\beta''\text{-Al}_2\text{O}_3$ crystal at 250°C . The sodium concentration is significantly increased at the cathodic side in comparison to the uniform distribution within the bulk of the sample. The neodymium distribution is also uniform in the inner part of the crystals, whereas at the cathodic side only a comparatively slight increase can be observed. It should be noted that the concentration profile of Na, Nd, Mg, and Al for a non-electrolyzed $\text{Nd}^{3+}/\beta''\text{-Al}_2\text{O}_3$ sample (not shown) is uniform over the whole cross-section without any significant fluctuations, even at the electrodes.

The increase in the Na content at the cathode is interpreted in terms of mobile Na^+ cations that migrate toward the cathode during electrolysis. Owing to the ion-blocking properties of the platinum electrodes, the Na^+ cations accumulate. On the other hand, the Nd^{3+} cations are not

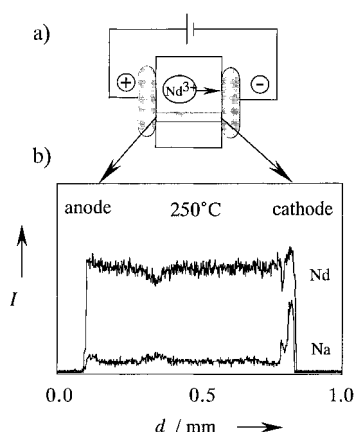


Figure 2. a) Schematic representation of the electrolysis cell; b) concentration profiles over the entire cross-section (from anode to cathode) of a $\text{Nd}^{3+}\text{-}\beta''\text{-Al}_2\text{O}_3$ crystal after electrolysis at 250 °C.

sufficiently mobile at this low temperature, and a comparable increase in the Nd concentration at the cathode does not occur. It can be concluded that at temperatures below 250 °C Na^+ cations represent the exclusive or main charge-carrying species.

A different result is obtained for the electrolysis of a $\text{Nd}^{3+}\text{-}\beta''\text{-Al}_2\text{O}_3$ crystal at 600 °C, that is, significantly higher than the transition temperature. The measured concentration profiles for Na, Nd, and Mg are displayed in Figure 3. Like in Figure 2, a positive sodium concentration gradient appears at the cathodic side of the sample. However, in contrast to the low-temperature electrolysis, a remarkable increase in concentration can be recognized for the trivalent Nd^{3+} cation at the cathode. This differs significantly from the data of the low-temperature electrolysis. In accordance with the aforementioned model of ion conduction in $\text{M}^{3+}\text{-}\beta''\text{-Al}_2\text{O}_3$,^[15] it can be stated that the trivalent Nd^{3+} cations are mobile at higher temperatures ($T > 250$ °C). Both Na^+ and Nd^{3+} cations are forced by the potential gradient to migrate through the crystal, leading to the corresponding accumulation at the Pt cathode.

Table 1 compares the averaged data from elemental analysis (by EPMA) taken at different sites (punctual analysis) on the anodic and cathodic surfaces of a $\text{Nd}^{3+}\text{-}\beta''\text{-Al}_2\text{O}_3$ sample before and after high-temperature electrolysis. In comparison to the nonelectrolyzed sample, a clear increase

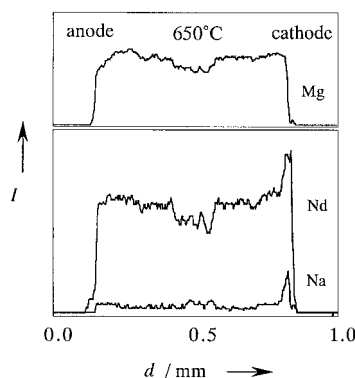


Figure 3. Concentration profiles of Na, Nd, and Mg over the entire cross-section (from anode to cathode) of a $\text{Nd}^{3+}\text{-}\beta''\text{-Al}_2\text{O}_3$ crystal after electrolysis at 650 °C. The notable decrease in intensity in the middle part of the crystal is caused by a large hole in the investigated section of the sample.

Table 1. Cationic elemental analysis (EPMA) of the cathodic and anionic surfaces of a $\text{Nd}^{3+}\text{-}\beta''\text{-Al}_2\text{O}_3$ sample before and after electrolysis at 650 °C. The data are given in atomic percent (at-%) and correspond to the average value of eight measurements at different sites on the sample.

Element:	Na	Nd	Mg	Al
before electrolysis (cathode and anode)	0.4	4.9	4.5	90.2
after electrolysis (cathode)	0.7	28.9	2.5	67.9
after electrolysis (anode)	0	2.3	4.8	92.9

in the Na and Nd concentration on the cathode surface and a corresponding decrease at the anodic side can be recognized.

All of the discussed observations clearly indicate the presence of mobile Nd^{3+} ions (and Na^+ ions) in $\text{Nd}^{3+}\text{-}\beta''\text{-Al}_2\text{O}_3$. At the anodic side of the pellets the sample is decomposed, releasing Nd^{3+} and Na^+ cations. Following the potential gradient, these cations migrate from the anode through the solid electrolyte to the cathodic side of the sample. The resulting Nd^{3+} and Na^+ accumulation reflects the corresponding increase in concentration from the EPMA measurements. On the other hand, at the anodic side of the sample mainly aluminum and magnesium oxides are left.^[22] Such a redistribution of, for example, neodymium would not occur if the Nd^{3+} ions are not mobile within the $\text{Nd}^{3+}\text{-}\beta''\text{-Al}_2\text{O}_3$ -matrix. It is not possible to explain the results only on the basis of electrolytic decomposition and/or chemical reactions between the electrolyte and the atmosphere without assuming mobile charge carriers. Thus, these observations are the first direct and clear indication for trivalent cation conduction in $\beta''\text{-Al}_2\text{O}_3$.

With respect to the comparatively uniform concentration profile of magnesium over the entire sample cross-section at low and high temperatures (Figure 3), it can be additionally stated that these cations are not mobile. The measurements indirectly confirm the exclusive incorporation of Mg^{2+} into the spinel blocks of the crystal structure. A comparable result could also be obtained for aluminum (the concentration profile is not shown here), indicating the nonmobility of Al^{3+} . Additional experiments, for example polarization measurements or measurements of conductivity as a function of O_2 partial pressure, have been performed in order to characterize the conduction properties of $\text{Nd}^{3+}\text{-}\beta''\text{-Al}_2\text{O}_3$ more thoroughly. However, the detailed description of these experiments is beyond the scope of this article and therefore not included here. It can nonetheless be stated that the electrical conduction in $\text{Nd}^{3+}\text{-}\beta''\text{-Al}_2\text{O}_3$ is neither generated nor influenced by electronic or O^{2-} charge carriers.

In conclusion, $\text{Nd}^{3+}\text{-}\beta''\text{-Al}_2\text{O}_3$ can be characterized as a solid electrolyte in which the electrical current is transported by mobile trivalent cations. However, the influence of the remaining nonexchanged Na^+ cations, which are present in a minor concentration, on the conduction behavior cannot be neglected. The Na^+ ions represent the predominant charge carriers at low temperatures, whereas at higher temperatures the Nd^{3+} ions also contribute to the current transport, generating a mixed cationic conduction. These investigations demonstrate for the first time that highly charged trivalent cations are mobile within the $\beta''\text{-Al}_2\text{O}_3$ matrix. For future studies the ambitious goal remains to quantitatively identify

the current transport by trivalent charge carriers. This challenge can be managed, for example, with “Tubandt electrolysis” experiments.

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- [18] The crystals were fixed by insertion into a corundum tube and filling of the remaining spaces with a hardening liquid cement.
- [19] The applied dc voltage amounted to 6 V in both experiments. The sample was electrolyzed for 130 d in the case of the low-temperature electrolysis ($T = 250^\circ\text{C}$), and for 16 d in the case of the high-temperature electrolysis ($T = 650^\circ\text{C}$).
- [20] With the use of nonreversible electrodes, the applied voltage must exceed the decomposition voltage of the compound in order to generate a constant flux of charge carriers. In the case of $\text{Nd}^{3+}/\beta''\text{-Al}_2\text{O}_3$, the decomposition voltage was determined in advance to be about 1.5–2 V.
- [21] The degree of exchange refers to the original Na^+ content of the $\text{Na}^+/\beta''\text{-Al}_2\text{O}_3$ crystals used.
- [22] The transference numbers for the electrolysis cannot be quantitatively determined because the absolute amount of transported charge carriers is not known. The EPMA data only represent the relative cation distribution on the surface. Transported cations which are located beneath the surface are not detected.

Extremely Strong s^2 – s^2 Closed-Shell Interactions**

Ralf Wesendrup and Peter Schwerdtfeger*

Closed-shell interactions (CSI) range from extremely weak van der Waals forces, as for the helium dimer (dissociation energy, $E_d = 9.06 \times 10^{-2} \text{ kJ mol}^{-1}$),^[1,2] to strong aurophilic interactions (E_d up to 30 kJ mol^{-1}).^[3] The strength of the latter lie almost in the order of covalent bonds, and strong CSI have recently been reviewed by Pyykkö.^[4] The bonding situation in these compounds is described by an equal number of electrons in bonding and antibonding molecular orbitals. This gives rise to a bond order of zero and explains why CSI are generally so weak.

Unusually strong “closed-shell interactions” have recently been reported for the d^{10} – s^2 system Au^+/Hg ($E_d = 179 \text{ kJ mol}^{-1}$, Table 1),^[5] and for the related cationic gold rare gas (Rg) compounds RgAu^+ ($E_d = 126 \text{ kJ mol}^{-1}$).^[6] These

Table 1. Bond lengths r_e [Å] and dissociation energies E_d [kJ mol^{−1}] (not corrected for zero-point vibration) at various levels of theory.

	AuM		AuM		AuM [−]	
	r_e	E_d	r_e	E_d	r_e	E_d
<i>M</i> = Hg ⁺ [a]						
R-HF	2.700	119.4	–	–	3.293	13.4
R-MP2	2.528	197.2	2.630	56.3	2.838	58.1
R-CCSD	2.590	168.5	2.743	28.8	3.018	30.4
R-CCSD(T)	2.581	179.2	2.711	37.6	2.967	36.8
NR-HF	3.066	89.8	–	–	3.631	15.4
NR-MP2	2.794	145.9	2.883	41.7	3.016	59.3
<i>M</i> = Ba						
R-HF	2.979	125.7	3.131	138.8	3.322	105.4
R-MP2	2.711	319.0	2.848	292.0	2.998	163.3
R-CCSD	2.811	263.2	2.951	238.9	3.126	136.1
R-CCSD(T)	2.789	286.2	2.930	255.7	3.102	142.9
NR-HF	3.490	62.7	3.595	54.7	3.856	70.7
NR-MP2	3.055	169.1	3.146	138.4	3.325	127.4

[a] Results for AuHg⁺ are taken from ref. [5].

cationic dimers fulfill the formal criterion for CSI since they consist of two interacting closed-shell fragments, the d^{10} gold cation and a closed-shell atom. However, significant charge transfer from the neutral atom to the gold cation at the equilibrium distance and low-lying alternative dissociation channels indicate a contribution from open-shell configurations to the bonding. Even though the degree of covalency in RgAu^+ and related species remains subject to discussion,^[7] these compounds are clearly different from the aforementioned examples. Their highest occupied molecular orbitals

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(HOMO) are of bonding character and the resulting bond order is one, in line with the high dissociation energies. However, addition of two electrons to the low-lying antibonding σ^* orbital would result in a bond order of zero and lead to the corresponding anionic gold compounds.^[6]

Anionic diatomic closed-shell compounds have not been studied extensively,^[8] and only few gold-containing examples were addressed so far.^[5,6] In a study on alkaline-earth/alkali metal dimers Bauschlicher, Jr. et al reported the notable dissociation energy of 26 kJ mol⁻¹ for the anion MgNa⁻.^[9] A simple MO picture cannot explain why this s^2-s^2 interaction gives rise to that particular stability and why MgNa⁻ is even more stable than its neutral analogue MgNa. In the anion however, the negative charge at the Group 1 element gives rise to a dipole interaction (charge-induced dipole interaction,^[10]

CID). Such interactions are responsible for the stability of weakly bound species such as HeH⁻, NeH⁻, or ArH⁻.^[11] In addition, dispersion forces (D) are increased in the anionic molecule because the polarizability of the atomic anion is substantially higher than that of the neutral atom. These two forces can be expressed by Equation (1) using the London expression^[12] for the dispersion force.

$$V^{A+B}(R) \cong V_{\text{CID}}^{\text{AB}}(R) + V_{\text{D}}^{\text{AB}}(R) = -\frac{1}{2} \frac{\alpha_{\text{A}}^{\text{B}} \alpha_{\text{B}}^{\text{A}}}{R^4} - \frac{3}{2} \frac{\alpha_{\text{A}}^{\text{B}} \alpha_{\text{B}}^{\text{A}} E_{\text{A}} E_{\text{B}}}{R^6 E_{\text{A}} + E_{\text{B}}} \quad (1)$$

A similar effect can be expected for anionic gold compounds. Owing to the strong relativistic contraction of its singly occupied 6s orbital gold possesses a very high electron affinity and diatomic gold compounds are characterized by unusually short bond lengths.^[13] According to Equation (1) these factors should make the gold anion with a relatively small ionic radius the ideal candidate for strong CID and dispersion interactions. We therefore studied the series AuM⁺, AuM, and AuM⁻ for the s^2 atoms M = Hg and Ba by Hartree–Fock (HF), second-order Møller-Plesset (MP2), and coupled-cluster (CCSD(T)) methods.^[14]

Successively filling up the antibonding $\sigma^*(6s_{\text{Au}}-6s_{\text{Hg}})$ orbital along the series AuHg⁺, AuHg, and AuHg⁻ has the expected influence on the geometry (Table 1). The calculated bond lengths increase from AuHg⁺ to AuHg⁻ in connection with a decreasing vibrational frequency at the correlated level. For the dissociation energy however, the trend is different. At the CCSD(T) level E_{d} drops drastically from 179 kJ mol⁻¹ for AuHg⁺ to 38 kJ mol⁻¹ for AuHg, but it remains almost constant from AuHg to AuHg⁻ at 37 kJ mol⁻¹. At the HF level neutral AuHg is even unbound and only the ionic species are stable. While it has been pointed out that removing an electron from a bonding orbital can increase the bond strength,^[15] AuHg serves as an example for the reverse effect.^[9] The bonding of AuHg⁻ at coupled cluster level is

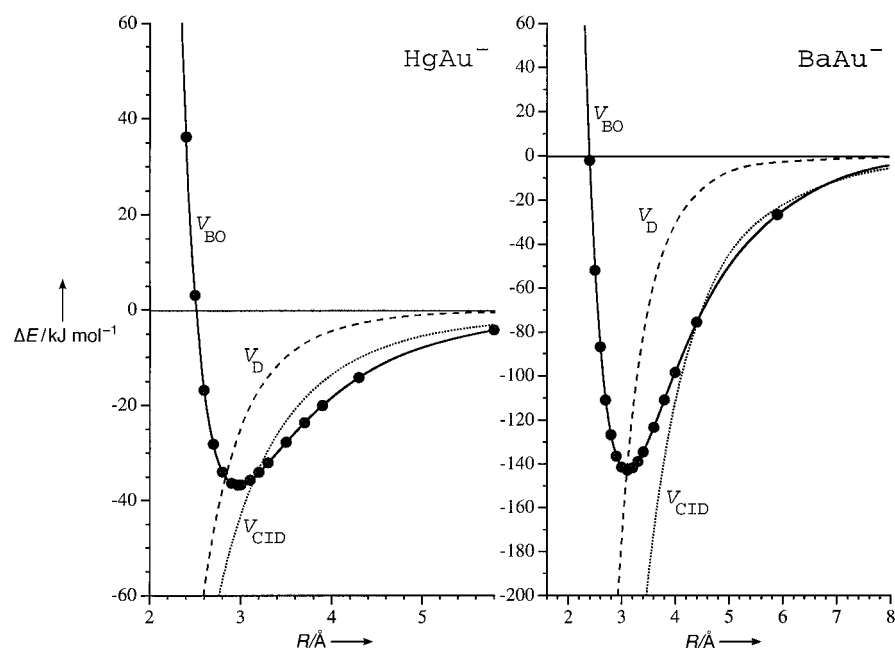


Figure 1. Born–Oppenheimer potential energy curves $V_{\text{BO}}(R)$ for AuHg⁻ and AuBa⁻. Also shown from the top to the bottom are the curves for the dispersion interaction $V_{\text{D}}(R)$ and the charge-induced dipole interaction $V_{\text{CID}}(R)$.

analyzed in Figure 1 in terms of CID and dispersion interactions. Calculated relativistic CCSD(T) polarizabilities were used for Au⁻ and Hg and experimental values for the ionization energy of Au⁻ and Hg, as listed in Table 2. It is evident from the greater slope of the $V_{\text{CID}}(R)$ curve that the interaction in AuHg⁻ is predominantly of charged-induced nature. In contrast to anionic alkaline-earth/alkali metal dimers dispersion forces are of minor importance,^[9] which explains why AuHg⁻ is bound at the HF level in comparison with AuHg where dispersion prevails.^[16]

Table 2. Calculated (CCSD(T)) ionization energies IE [eV] and static dipole polarizabilities α [au] for Au/Au⁻, Ba, and Hg compared with experimental values or those from benchmark calculations.^[34]

	CCSD(T)		Reference	
	IE	α	IE	α
Au	9.14	34.9	9.23	36.1 ^[a]
Au ⁻	2.22	89.2	2.31	92.0 ^[a]
Ba	5.16	278.6	5.21	268 ± 22
Hg	10.32	33.9	10.44	33.9

[a] Calculated value.

Ba is believed to have the highest polarizability of all neutral closed-shell atoms,^[17] roughly three times that of Hg (Table 2). Neglecting higher order induction terms in the weak perturbation one would accordingly expect an approximately three times higher dissociation energy for AuBa⁻ at bond lengths similar to those in AuHg⁻. We note in passing that the much lower polarizability of Xe ($\alpha_{\text{D}} = 27.3$ au)^[18] explains why calculations for the related AuXe⁻ yield only a small interaction energy of 8 kJ mol⁻¹ at a relatively long bond length of 4.18 Å.^[6] In fact, the dissociation energy of AuBa⁻ is almost four times higher than in AuHg⁻ and amounts to 143 kJ mol⁻¹ (Table 1) at the coupled-cluster level; a value

that is usually associated with covalent bonds. The Mulliken population analysis of AuBa[−] based on the MP2 density reveals a slight participation from Au(6p_o) and Ba(5d_o) to the two σ-HOMOs thus reducing the Pauli-repulsion between the s² electron pairs. Besides this mere polarization effect the orbital population in the molecular fragments is practically unchanged from the population in separated Au[−] and Ba. This rules out large contributions of excited higher states. Only a negligible charge transfer is observed in AuBa[−] (Mulliken charge $q_{\text{Ba}} = -0.04$) in line with the electron affinities of Au ($E_{\text{Au}} = 2.31$ eV)^[19] and Ba ($E_{\text{Ba}} = 0.22$ eV).^[20] These findings clearly demonstrate that AuBa[−] represents the strongest closed-shell interaction reported so far. As in AuHg[−] the interaction is mainly of CID character (Figure 1, using IP = 5.16 eV for Ba).^[21] This is confirmed by the fact that even at the HF level AuBa[−] is bound by more than 100 kJ mol^{−1}. Moreover, a plot of lg(−ΔE) over lg(R) for the potential energy curve ΔE(R) of AuBa[−] yields an almost perfect linear behavior and excellent agreement with the corresponding $V_{\text{CID}}(R)$ curve at distances of $R > 4$ Å.

The situation for the neutral and cationic molecules is distinctly different. Both AuBa⁺ and AuBa exhibit a dissociation energy greater than 250 kJ mol^{−1} and clearly lie in the order of other covalently bound gold main group atom dimers.^[22] In Table 3 we report vibrational–rotational constants for studied AuM compounds (M = Hg, Ba) for future spectroscopic measurements. The constants were derived from extended Morse potentials fitted to the CCSD(T) single points. Fit parameters for the Morse curves are also listed in Table 3.

Finally, one should consider if there are related charged diatomic compounds with similarly high dissociation energies. Preliminary MP2 calculations for a few selected M₁M₂⁺ species (M₁ a Group 2 or 12 element and M₂ a Group 3 or 13 element) give $r_e = 2.201$ Å and $E_d = 174.0$ kJ mol^{−1} for BeB⁺, $r_e = 3.283$ Å and $E_d = 24.9$ kJ mol^{−1} for BaB⁺ (dissociating into Ba⁺ and B), $r_e = 3.342$ Å and $E_d = 27.5$ kJ mol^{−1} for HgTl⁺,^[28] $r_e = 4.395$ Å and $E_d = 4.2$ kJ mol^{−1} for BaTl⁺ (dissociating into Ba⁺ and Tl). Similarly to AuHg⁺ a high dissociation energy is only obtained if the ionization poten-

tials of M₁ and M₂ become comparable in size thus resulting in large metal p-orbital participation and strong covalent bonding character. As a particular example we further mention the doubly charged HeBe²⁺ with a very short bond length of $r_e \approx 1.40$ Å and a dissociation energy of $E_d \approx 86$ kJ mol^{−1}.^[23]

For selected anionic (Group 1/11)–Ba diatomics preliminary MP2 calculations give the following bond lengths and dissociation energies: $r_e = 3.689$ Å and $E_d = 94.6$ kJ mol^{−1} for CuBa[−], $r_e = 3.757$ Å and $E_d = 83.9$ kJ mol^{−1} for AgBa[−], $r_e = 4.431$ Å and $E_d = 49.2$ kJ mol^{−1} for LiBa[−] and $r_e = 5.902$ Å and $E_d = 32.8$ kJ mol^{−1} for CsBa[−].^[24] The corresponding values for AuBa[−] at the same computational level are $r_e = 3.458$ Å and $E_d = 112.9$ kJ mol^{−1}. It is evident that the bond lengths in the alkali metal containing dimers are too long to allow for efficient CID interactions which results from the diffuse and highly polarizable nature of the alkali metal anions. The less pronounced difference of AuBa[−] to its lighter homologues CuBa[−] and AgBa[−] can be attributed to relativistic effects. The relativistic contraction of the doubly occupied 6s orbital in Au[−] reduces the ionic radius and allows a comparably short bond in AuBa[−]. This relativistic bond contraction of $\Delta_R r_e = 0.173$ Å (at the MP2 level, see Table 1) greatly increases the CID interaction and overcompensates the relativistic reduction of the Ba polarizability ($\Delta_R \alpha_D = 37$ au).^[25] It is this unique combination of a relativistically contracted short bond length and the large dipole polarizability of Ba which makes the extraordinarily strong s²–s² interaction in AuBa[−] possible.

Computational Details

The nonrelativistic (NR) and scalar relativistic (R) energy-adjusted small-core pseudopotentials of the Stuttgart group were used to replace the inner 46 electrons for Ba and the inner 60 electrons for Au and Hg.^[26] Large uncontracted (11s10p9d4f) correlation-consistent Gaussian type basis sets were used to describe the valence electrons. The basis sets were generated by numerically optimizing (9s9p6d4f) subsets of exponents at the second-order Møller-Plesset level (MP2) which were augmented by (2s1p3d) sets of diffuse functions.^[27] Basis sets obtained by this procedure can successfully suppress of what is known as the basis set superposition error (BSSE).^[28] The generated basis sets were first tested for the accurate description of atomic polarizabilities and ionization energies as these properties are crucial for binding in the anionic molecules. A finite field

Table 3. Spectroscopic constants as derived from the CCSD(T) potential energy curves; harmonic frequency ω_e [cm^{−1}], anharmonicity constant $\omega_e x_e$ [cm^{−1}], rotational constant B_e [10^{−1} cm^{−1}], centrifugal distortion constant D_e [10^{−8} cm^{−1}], vibration–rotational coupling constant α_e [10^{−4} cm^{−1}]. Parameters in atomic units for extended Morse fits to the CCSD(T) single points are also included. The extended Morse potential is defined as $V(R) = \sum_i A_i e^{-a_i(R-r_e)}$.

	AuBa ⁺	AuBa	AuBa [−]	AuHg ⁺ [a]	AuHg	AuHg [−]
ω_e	149	123	94	139	103	64
$\omega_e x_e$	0.56	0.18	0.21	0.36	0.84	0.50
B_e	0.268	0.242	0.216	0.253	0.228	0.191
D_e	0.366	0.376	0.455	0.330	0.436	0.680
α_e	0.130	0.533	0.660	0.47	1.447	1.464
a_1	1.1810×10^{-1}	1.0450×10^{-1}	7.0600×10^{-2}	1.4330×10^{-1}	1.4895	2.0976
A_1	2.9900×10^{-1}	2.8580×10^{-1}	3.5040×10^{-1}	2.5460×10^{-1}	7.6900×10^{-2}	-5.9379×10^{-3}
a_2	1.6583	1.5691	2.6038×10^{-6}	2.2378	1.8186	1.1075
A_2	-2.5500×10^{-2}	-1.9300×10^{-2}	-8.3000×10^{-1}	-1.3600×10^{-2}	-6.3300×10^{-2}	1.2300×10^{-2}
a_3	-5.1600×10^{-2}	-6.6243×10^{-3}	-1.6300×10^{-2}	−2.6014	1.6910×10^{-1}	9.0250×10^{-1}
A_3	-1.6480×10^{-1}	-1.6930×10^{-1}	1.4502	2.8678×10^{-6}	7.3691×10^{-4}	-1.0100×10^{-2}
a_4	−2.0292	−1.8889	-3.2913×10^{-5}	2.0395	−	4.4480×10^{-1}
A_4	1.1655×10^{-6}	4.8447×10^{-7}	-6.3510×10^{-1}	-4.3644×10^{-4}	−	1.7600×10^{-2}

[a] Results for AuHg⁺ are taken from ref. [5].

model using external fields of 0.000, 0.001, 0.002 and 0.004 au was used to calculate the static dipole polarizabilities for Ba, Au/Au⁺ and Hg. Table 2 shows the excellent agreement of our CCSD(T) properties with available reference data.

Calculations were performed at Hartree–Fock (HF), MP2, CCSD and CCSD(T) level using the programs Gaussian98^[29] and Aces II.^[30] At least 20 single points were calculated to describe the potential curves and corrected for the BSSE using the counterpoise correction by Boys and Bernardi.^[31] The corrected potential curves were fitted by an extended Morse potential (Table 3).^[32] Spectroscopic parameters were derived from a numerical Numerov–Cooley procedure as implemented in MOLCAS3.^[33]

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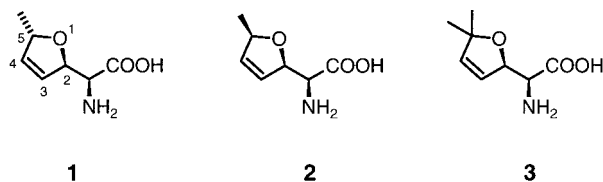
A General Approach to L-(+)-Furanomycin and Some Stereoisomers and Analogues Using Furoisoxazoline Intermediates**

Peter Jan Zimmermann, Iva Blanarikova, and Volker Jäger*

In 1967 Katagiri et al. reported on the isolation of the antibiotic α -amino acid L-(+)-furanomycin (**1**) from the fermentation broth of *Streptomyces threomyceticus* (ATCC 15795).^[1] L-(+)-Furanomycin shows considerable activity against different bacteria, for example *E. coli*.^[2] Later, **1** was found to be charged to isoleucine tRNA by isoleucyl-tRNA synthetase from *E. coli* and incorporated into protein.^[3]

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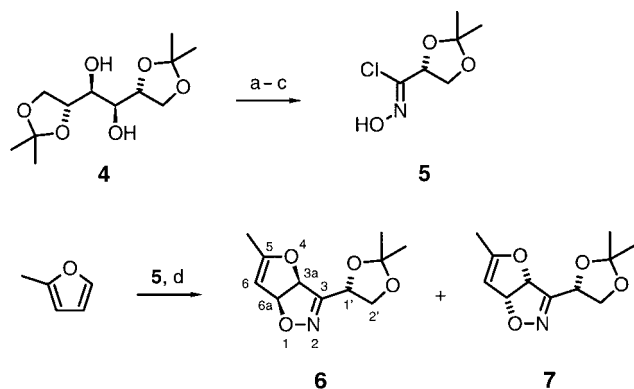
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Originally, **1** was assigned the $\alpha S,2R,5R$ configuration (**2**).^[2, 4] Later, this was revised in favor of (+)-($\alpha S,2R,5S$)- α -amino-2-(2,5-dihydro-5-methyl)furan-2-acetic acid (**1**), based on an unambiguous synthesis from D-glucose and an X-ray structure analysis of the *N*-acetyl derivative.^[5, 6]

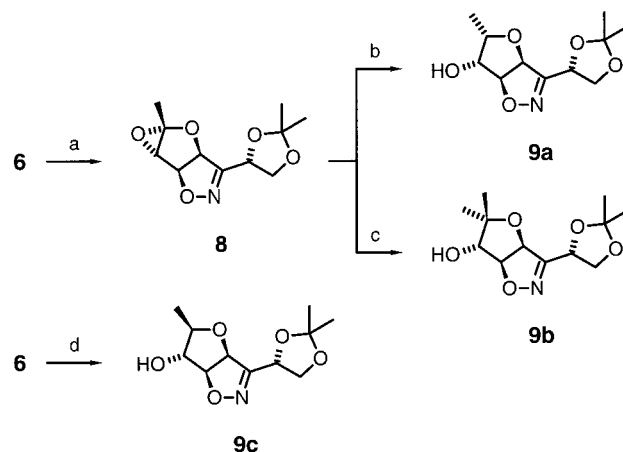
Over the last 20 years several strategies for the synthesis of **1** and its stereoisomers were advanced, starting from various carbohydrate precursors,^[5, 7] substituted furans,^[4, 5a] or dimethyl L-tartrate.^[8] The new approach presented here is based on our earlier work, in which the 1,3-dipolar cycloaddition of nitrile oxides to furan was studied in view of synthesizing polyhydroxypiperidines and amino sugars.^[9] The new route is illustrated by the preparation of **1–3**. The cycloaddition products obtained from 2-methylfuran and nitrile oxide (furo[2,3-*d*]isoxazolines) and the derived epoxide served as key intermediates; twofold deoxygenation led to the C=C bond at a late stage, when the relative configuration of the remaining stereocenters had already been established.

The known hydroxamic acid chloride **5** was prepared in three steps from diisopropylidene mannitol (**4**).^[10, 11] On treatment of the nitrile oxide precursor **5** with triethylamine, 1,3-dipolar cycloaddition to 2-methylfuran was effected to give a mixture of the diastereomeric furoisoxazolines **6** and **7** (d.r. 60:40), which were separated by MPLC and isolated on a multigram scale in 40 and 27% yield, respectively (Scheme 1).^[12]



Scheme 1. a) NaIO_4 ; b) $\text{HONH}_2 \cdot \text{HCl}$; ^[10b] c) *N*-chlorosuccinimide, ^[11a] 78% from **4**; d) NEt_3 (1.2 equiv), 2-methylfuran, room temperature (RT), 5 d; d.r. 60:40 (crude product); MPLC separation, **6** 40%, **7** 27%.

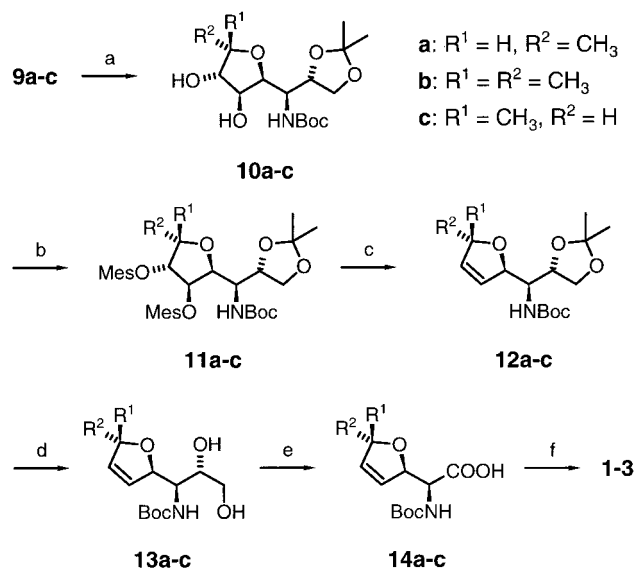
The major diastereomer **6** served as an entry to the L-amino acids **1–3** (with **7**, the D-enantiomers are obtained). First, epoxidation of the dihydrofuran **6** with dimethyldioxirane (DMDO)^[13] led to the (unexpectedly) stable, isolable enol ether epoxide **8**, a derivative of a 1,2-anhydrofuranose, in quantitative yield with high selectivity (d.r. > 95:5; Scheme 2).^[14] Reductive opening^[15] of the epoxide ring in **8** resulted in a highly regio- and *endo*-stereoselective hydride



Scheme 2. a) DMDO (2.0 equiv, 0.1M in acetone),^[13] CH_2Cl_2 , 0°C, 3 h, quant.; b) NaBH_3CN (2.0 equiv), H_2O (1.0 equiv), THF, 16 h, **9a** 74%; c) MeMgBr (1.5 equiv, 3.0M), THF, CH_2Cl_2 , 15 min, **9b** 83%; d) 1. $\text{BH}_3 \cdot \text{THF}$ (2.0 equiv, 1.0M), THF, 0°C, 10 h; 2. $\text{Me}_3\text{NO} \cdot 2\text{H}_2\text{O}$ (7.0 equiv), diglyme, 0 $\rightarrow$ 150°C, 20 h, **9c** 73%. DMDO = dimethyldioxirane.

transfer to give the alcohol **9a** with the required 2,5-*trans* configuration at the tetrahydrofuran ring. After screening of several metal and borohydride reagents, the best results were obtained with sodium cyanoborohydride in THF in the presence of one equivalent of water. On the other hand, the dimethyl compound **9b** was derived from regioselective opening of the epoxide ring of **8** with methylmagnesium bromide.^[16] As an alternative method to introduce the hydroxy group onto the 6-position of the heterobicycle, regioselective hydroboration was considered first:^[17] Reaction of **6** with $\text{BH}_3 \cdot \text{THF}$ and subsequent oxidation by trimethylamine *N*-oxide dihydrate^[18] led to the alcohol **9c**, the 5-epimer of **9a**, with high *exo* selectivity (> 90:10; Scheme 2).

Next, elaboration of the amino group in the side chain and of the C=C bond in the dihydrofuran ring had to be accomplished. As expected from earlier work,^[9] reduction of the isoxazolines **9a–c** with lithium aluminium hydride in THF/diethyl ether led to the corresponding 1,3-amino alcohols with high *syn*-(*erythro*) selectivity (> 95:5 from NMR analyses). From these, the *N*-Boc-protected amino diols **10a–c** were obtained (Scheme 3). Introduction of the double bond was then effected by twofold mesylation and subsequent twofold mesylate removal.^[19] The diols **10a–c** were first converted into the corresponding dimesylates **11a–c**,^[20] which were then treated with sodium naphthalenide in THF^[19b] to smoothly afford the 2,5-dihydrofurans **12a–c**. Hydrolysis of the acetonide group with dilute trifluoroacetic acid proceeded without cleavage of the carbamate to yield the diols **13a–c**. From these, oxidative cleavage with sodium periodate provided the corresponding *N*-Boc-amino aldehydes, which were immediately treated with buffered sodium chlorite^[21] to furnish the optically pure, *N*-protected amino acids **14a–c** in excellent yield. Finally, deprotection of **14a–c** with 6N hydrochloric acid, followed by ion-exchange chromatography and further purification by crystallization from water/acetone, provided the free α -amino acids **1–3**. All analytical and spectroscopic data of L-(+)-furanomycin (**1**) thus obtained, and of the known 5-epimer **2**,



Scheme 3. a) 1. $LiAlH_4$ (4.0 equiv), THF, diethyl ether, $0^\circ C \rightarrow RT$, d.r. > 95:5, 2. Boc_2O (1.5–2.0 equiv), dioxane/ H_2O , $0^\circ C \rightarrow RT$, 16 h, **10a** 71 %, **10b** 65 %, **10c** 70 %; b) CH_3SO_2Cl (3.0 equiv), pyridine, $0^\circ C \rightarrow RT$, 16 h, **11a** 95 %, **11b** 92 %, **11c** 97 %; c) Na/naphthalene, THF, $0^\circ C \rightarrow RT$, 15 min, **12a** 85 %, **12b** 81 %, **12c** 75 %; d) $CF_3COOH/MeOH/H_2O$, $0^\circ C \rightarrow RT$, 16–30 h, **13a** 88 %, **13b** 86 %, **13c** 93 %; e) 1. $NaIO_4$ (1.2 equiv), $MeOH/H_2O$ 1:1, $0^\circ C$, 15–20 min, 2. $NaClO_2$ (1.5–2.0 equiv), NaH_2PO_4 (1.5–2.0 equiv), $tBuOH$, 2-methyl-2-butene, RT, 2–3 h, **14a** 89 %, **14b** 93 %, **14c** 93 %; f) 1. 6 N HCl, $0^\circ C$ or RT, 2–16 h, 2. Dowex 50 WX-8 (H^+), 1 N NH_3 , 3. crystallization from H_2O /acetone, **1** 61 %, **2** 60 %, **3** 56 %. Boc = *tert*-butoxycarbonyl, Mes = methanesulfonyl (Mesyl).

were in complete agreement with those previously reported.^[22]

The results shown here present a new, practical, and stereoselective approach to L-(+)-furanomycin (**1**) in 14 steps with an overall yield of 6.3 %. Noteworthy features are the use of glyceronitrile oxide as a chiral glycine equivalent,^[11a] and the establishment of the relative configuration of the three stereocenters by means of intermediate tetrahydrofuran-3,4-diols. Compared with known syntheses, the scheme outlined here offers the advantage of a short and concise access to four of the eight stereoisomers of **1**. Further, for each case, various structural analogues of **1** can be prepared from the key epoxide **8** (a promising intermediate for other ventures too) by simple variation of the nucleophile (Grignard reagent). The biological evaluation of this group of compounds is pending and will be reported in due course.

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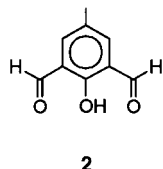
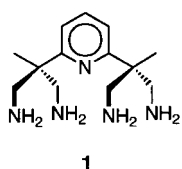
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Spontaneous Assembly of a Schiff Base Tetramacrocycle**

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Dedicated to Professor Akio Yamamoto on the occasion of his 70th birthday

Tetrapodal pentadentate ligands, in which a four-legged donor set is supported by a framework containing a central donor atom, are rare.^[1] We have set out to explore the potential of such ligands as head groups in functional transition metal complexes, and recently introduced a polyamine of this topology (pyN₄, **1**).^[2] Further derivatization aims to append a “pocket” to the tetrapodal ligand, and mono-, di- and tetrafunctionalized derivatives of **1** have been obtained by Schiff base condensation with monocarbonyl compounds.^[1] As an extension of this project, we chose to study the reactions of **1** with dialdehydes and selected a classic representative,^[3] 2,6-diformyl-4-methylphenol (**2**), as reagent.^[4]



The condensation of dialdehyde **2** with polyfunctional amines has been used for the synthesis of macrocycles and cryptands^[5] having some of the topologies shown schematically in Scheme 1.^[4] For good yields, the reactions usually rely on a templating agent (main group, transition metal, or lanthanoid ions; protons), and only a few direct condensations^[6] have been reported. Mono- and polymacrocycles whose individual rings each incorporate two molecules of **2** prevail (Scheme 1 a, d); only very few rings containing three^[6]



- a) $n = 1$: [2 + 2]
b) $n = 2$: [3 + 3]
c) $n = 3$: [4 + 4]
d) [2 + 3]
e) [2 + 4]
f) [3 + 6]

Scheme 1. Topologies of documented (a–d, f) and hypothetical (e) macrocycles from the Schiff base condensation of polyamines with the dialdehyde 2,6-diformyl-4-methylphenol (**2**). Only the reactive centers are shown; full circles: polyamine; open circles: dialdehyde. The expressions in brackets refer to the number of polyamine and dialdehyde molecules that combine in each macrocyclization.

(Scheme 1 b) or four^[4k] (Scheme 1 c) of these dialdehyde units are known.

One possible condensation reaction between one equivalent of **1** and two equivalents of **2** is the “tetrapode capping”^[7] reaction (Scheme 1 e), in which two pyN₄ head groups would be anchored in close proximity. Such a reaction involves six fragments with a total of 16 reactive centers, and hence its probability is very low.^[7, 8] There is precedent, however, for the condensation of 1,3-diaminopropane (which is a structural subunit of **1**) with **2** in the presence of acid to yield a [2 + 2] macrocycle (cf. Scheme 1 a).^[9] We therefore performed the reaction in the presence of protons as a potential templating agent.

The condensation of **1** and **2** in the ratio 1:2 in refluxing methanol, in the presence of two equivalents of HBr, yielded an orange precipitate (**3**) as the only product. Its IR spectrum (KBr) showed strong absorptions in the aldimine region ($\tilde{\nu}$ = 1665, 1643 cm^{−1}), indicating Schiff base formation. No residual aldehyde bands ($\tilde{\nu}$ = 1682, 1666 cm^{−1}; (C=O)_{str}) were observed. Elemental analysis data of this material indicated a definite 1:2 condensation product, containing two equivalents of HBr. NMR spectra of **3** were broad and badly resolved; however, anion exchange with sodium perchlorate gave a microcrystalline solid (**4**) whose ¹H and ¹³C NMR spectra showed excellent resolution (see below).

The X-ray structure analysis of a mixed Br[−]/PF₆[−] salt of the protonated condensation product ([LH₆]Br₂(PF₆)₄, **5**; see Experimental Section)^[10] revealed a [3 + 6] condensation species (cf. Scheme 1 f) whose molecular structure is shown in Figure 1: three molecules of **1** and six molecules of **2** have combined in the presence of six protons (by inference from the number of anions) to give a macrocycle in which three 20-membered cyclic subunits are strung together into an alternating single and double strand with 42 member atoms along each loop. Each phenolbis(aldimine) unit is planar, suggesting two protons in an N⋯H⋯O⋯H⋯N hydrogen-bonded arrangement which is unipositive and involves two iminium and one phenoxide group. The molecule is chiral, and crystallizes as a racemic mixture.

The molecule has noncrystallographic threefold rotational symmetry and possesses a central cavity of truncated conical shape, with a wide and a narrow opening. The intermolecular distances do not suggest significant hydrogen-bonding between the cation and any of the counterions. The three cyclic subunits containing the bis(aldimine) bridges have conforma-

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Supporting information for this article (¹³C data for compound **3**) is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

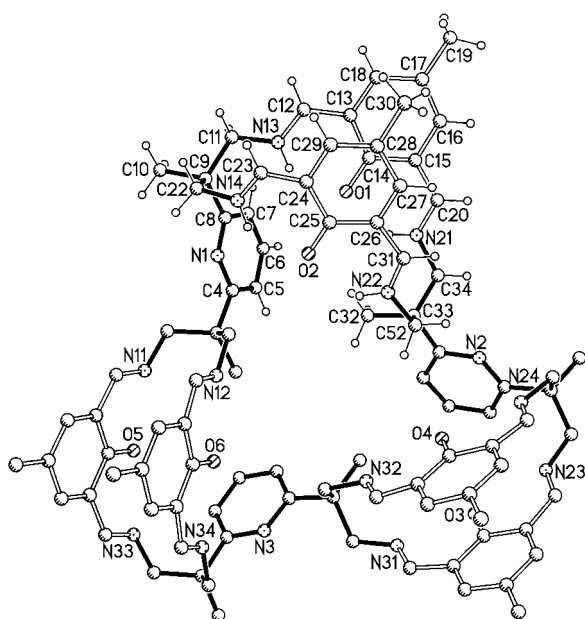


Figure 1. Molecular structure of the tetramacrocyclic cation $[LH_6]^{6+}$ obtained from the condensation of polyamine **1** (solid bonds) and dialdehyde **2** (open bonds) in the presence of acid. See text for the location of charges. For correlation with the NMR data, hydrogen atoms and the numbering scheme are partly shown. The proton numbers follow the numbers of their parent carbon or nitrogen atoms.

tions reminiscent of the conformation of calixarenes, virtually identical to that of the $[2+2]$ condensation product of 1,3-diaminopropane and **2**.^[9] $\pi-\pi$ -stacking interactions induce a parallel orientation of the phenyl rings, with interplanar distances averaging 3.7 Å.

The chiral structure of the cation in $[LH_6]Br_2(PF_6)_4$ (**5**) is completely compatible with the ^1H and ^{13}C NMR spectra of the perchlorate salt **4**. A threefold symmetry axis interconverts the three pyridine as well as the three pairs of dialdehyde-derived spacer units. The “inner” and “outer” dialdehyde spacers exhibit different chemical shifts: Owing to the inherent symmetry of the tetramacrocycle, the methine protons and carbon atoms, the NH protons, etc. are pairwise nonequivalent, that means a total of four NH chemical shifts, four methine chemical shifts, etc. are observed. The proton and carbon resonances were assigned by standard 2D NMR methods (COSY, ROESY, HETCOR, COLOC, HMBC), as well as by the newly developed 1D pulse sequence DPGFSE-ROE.^[11a] The numbering of the protons follows the numbering of their parent C and N atoms (Figure 1). The starting point for the assignment of the ^1H NMR signals is the proton H11g which is *gauche* with respect to its vicinal NH proton (H13): H11g is very close (2.18 Å) to H7 on the adjacent pyridine ring and exhibits, as the only one of all the geminal CH_2 protons, a correspondingly intense cross peak in the ROESY spectrum. The remaining assignment is straightforward (see Figures 1 and 2, and Experimental Section). Of the eight resonances found for the geminal CH_2 protons, four are split into doublets and four are split into triplets (Figure 2a). This is due to the geometry relative to the adjacent NH protons: coupling with NH ($^3J(H-C-N-H) \approx ^2J(H-C-H)$) is observed only for the CH_2 proton which is *anti*, not for the one in the *gauche* position (Karplus equation).

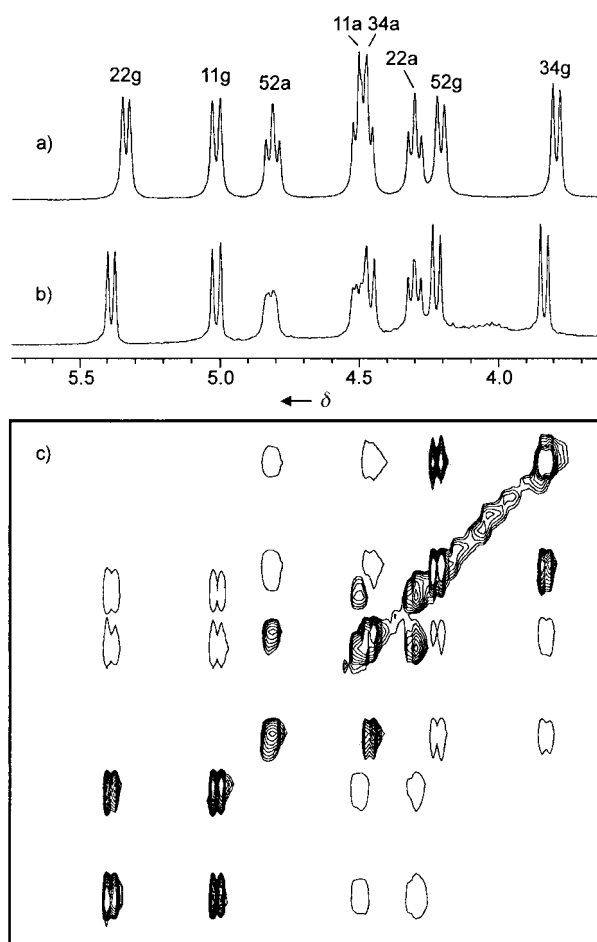
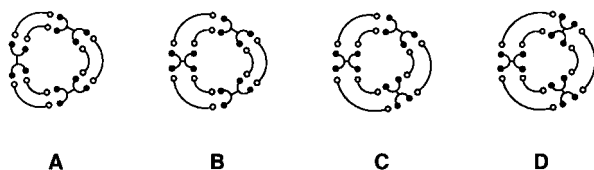


Figure 2. a) ^1H NMR spectrum of **4** ($[D_6]DMSO$, +25 °C): Resonance region of the geminal CH_2 protons H11, H22, H34 and H52. The numbering is based on the numbering of the parent carbon atoms in Figure 1. Symbols a and g denote *anti* and *gauche* positions, respectively, relative to the vicinal NH proton. b) Same as a), at +100 °C. c) ROESY spectrum of **4** ($[D_6]DMSO$, +100 °C). Cross peaks with more than one contour are positive (same phase as diagonal peaks) and are due to chemical exchange. Cross peaks with only one contour are negative and originate from NOE.

Unusual dynamic phenomena are observed in the ^1H NMR spectra at elevated temperatures. At +70 °C, the ROESY spectrum shows chemical exchange of the NH protons, a quite normal observation for iminium ions. However, at still higher temperatures (≥ 100 °C), there is also pairwise chemical exchange of the methyl groups on the dialdehyde-derived aromatic rings, of the non-aromatic methine protons, and of the geminal CH_2 protons (Figure 2b). Interestingly, no exchange is observed for the protons of the CH_3 groups on the pyridine-derived spacer (C10, C32). Moreover, there is only pairwise exchange (Figure 2c) of the “inner” *gauche/anti* CH_2 protons with their “outer” counterparts, for example, $H11_{gauche} \rightleftharpoons H22_{gauche}$, $H11_{anti} \rightleftharpoons H22_{anti}$. We therefore conclude that the underlying dynamic process must be an inversion of the whole molecule in umbrella-like fashion, leading to its enantiomer. The relative positions of the methyl groups in the pyridine spacers do not change during this process, that is methyl group C10 remains pointing “outward”, while C32 remains pointing “inward”. As is deduced from the chemical shift separation of corresponding exchanging groups in the

^1H NMR spectra, the barrier for the inversion process must be higher than 17 kcal mol^{-1} .^[11b]

As shown in Scheme 2, four topologies are conceivable for the macrocyclic product of the condensation reaction at hand: **A**, the topology that is actually found, and **B–D**, which differ from **A** and from one another by an increasing number of



Scheme 2. Four topologies are conceivable for the product of the [3+6] condensation of **1** and **2**: **A–D** differ from one another by an increasing number of pyN_4 units being oriented “sideways” with respect to the large ring.

pyN_4 units that are oriented “sideways” with respect to the large ring. To estimate the relative stabilities of **A–D**, we performed molecular dynamics simulations at constant temperature using the MM3 force field developed by Allinger et al.^[12] After the samples had been heated to 300 K, the different topologies were equilibrated for 1 ns. Averaging over the potential energy showed that **A** is by far the most stable. Increasing the ring size of an increasing number of circular subunits by incorporating a progressive number of pyN_4 fragments into the large ring “sideways” results in a stepwise increase of the relative energies. Details of these simulations in combination with NMR spectroscopic results will be reported elsewhere.

The assembly of **3** is remarkable in that it is a high-yielding (>90%) single-batch nine-component condensation that leads to a cyclic oligomer in which 12 new bonds have been formed. The specificity with which the cyclization proceeds is quite extraordinary. Protons can be assumed to play a templating role in the formation of the three smaller macrocyclic rings in much the same way as in the [2+2] condensation of 1,3-diaminopropane with 2,6-diformyl-4-methylphenol.^[9] While bromide ion acting as an electrostatic template in the closure of the protonated large ring cannot be ruled out, its role is expected to be minor since Br^- can be removed completely by anion exchange. A more decisive factor may be simultaneous or sequential amination formation between the 1,3-diaminopropane subunits of three molecules of **1** and the carbaldehyde functionalities of three molecules of **2**, leading to the kinetically controlled closure of a single-stranded large ring containing six six-membered cyclic aminals. In the presence of protons, these aminals may open and incorporate three additional dialdehyde molecules to give the more thermodynamically stable macrocycle containing cyclic Schiff base subunits. The intermediacy of aminals in the formation of Schiff base macrocycles is documented in the literature,^[13] and a bis(aminale) derivative of **1** obtained from the reaction with two equivalents of ferrocenecarbaldehyde has been isolated and structurally characterized.^[14]

The tetramacrocycle described here is compartmental^[4b] and contains 21 potential donor atoms (15 N and 6 O atoms). In the solid-state structure of the metal-free macrocycle, the

orientation of three of these (the pyridine N atoms) is divergent, but the conformation of the molecule may readjust upon metal coordination. Also, reduction of the imine moieties is expected to make the framework more flexible.

Experimental Section

$[\text{LH}_6]\text{Br}_6$, **3**: **1**·4HBr·MeOH (2.59 g, 4.26 mmol) was suspended in anhydrous methanol (100 mL) under an atmosphere of dry dinitrogen. Sodium metal (0.20 g, 8.53 mmol) was added with stirring, and the suspension gradually became a clear solution. After the evolution of H_2 had ceased, a solution of 2,6-diformyl-4-methylphenol (1.40 g, 8.53 mmol) in methanol (200 mL) was added in one portion, and the reaction mixture was refluxed for 15 h. During this time the color of the solution changed from yellow through orange to red, and an orange precipitate formed. The mixture was allowed to cool and then filtered under dinitrogen, the solid was washed with methanol, and dried in vacuo (2.59 g, 91%). Elemental analysis data indicated the correct composition, but ^1H and ^{13}C NMR spectra were poorly resolved. Anion exchange with ClO_4^- gave an analytically pure compound whose spectra showed excellent resolution at room temperature.

$[\text{LH}_6](\text{ClO}_4)_6$, **4**: Compound **3** (0.25 g, 0.12 mmol) was suspended in methanol (25 mL), and water (ca. 2 mL) was added to give an almost clear solution. After filtration, NaClO_4 (0.18 g, 1.44 mmol) dissolved in the minimum amount of methanol was added in one portion. Compound **4** precipitated immediately, was isolated by filtration, washed with methanol, and dried in vacuo (0.27 g, 95%). Correct elemental analysis for $\text{C}_{93}\text{H}_{105}\text{N}_{15}\text{O}_{30}\text{Cl}_6 \cdot 3\text{CH}_3\text{OH} \cdot 3\text{H}_2\text{O}$; IR (KBr): $\tilde{\nu} = 1665$, 1643 cm^{-1} ($\text{C}=\text{N}$)_{str}; 1088 (ClO_4); MS (FAB, p-NBA): m/z (%): 1524, $[\text{LH}_6]^+$ (100), 1826 $[(\text{LH}_6)(\text{ClO}_4)_3]^+$ (40); ^1H NMR (500 MHz, $[\text{D}_6]\text{DMSO}$, +25 °C; the numbering follows the C and N atom-numbering in Figure 1; “anti” and “gauche” refer to positions relative to vicinal NH protons): $\delta = 14.31$ (t, 3H, 12.0 Hz, H13), 13.51 (t, 3H, 12.2 Hz, H21), 13.13 (t, 3H, 12.5 Hz, H22), 13.07 (t, 3H, 12.5 Hz, H14), 8.80 (d, 3H, 12.0 Hz, H12), 8.79 (d, 3H, 13.0 Hz, H23), 8.74 (d, 3H, 13.6 Hz, H20), 8.67 (d, 3H, 13.6 Hz, H31), 8.37 (t, 3H, 8.6 Hz, H6), 8.19 (d, 3H, 8.6 Hz, H5), 8.00 (d, 3H, 8.6 Hz, H7), 7.45 (d, 3H, 3.1 Hz, H18), 7.30 (d, 3H, 3.1 Hz, H29), 7.29 (d, 3H, 3.1 Hz, H16), 7.11 (d, 3H, 3.1 Hz, H27), 5.33 (d, 3H, 12.0 Hz, H22_{gauche}), 5.01 (d, 3H, 14.6 Hz, H11_{gauche}), 4.81 (t, 3H, 12.5 Hz, H52_{anti}), 4.50 (t, 3H, 10.7 Hz, H11_{anti}), 4.48 (t, 3H, 10.1 Hz, H34_{anti}), 4.31 (t, 3H, 12.0 Hz, H22_{anti}), 4.21 (d, 3H, 13.0 Hz, H52_{gauche}), 3.79 (d, 3H, 13.6 Hz, H34_{gauche}), 2.16 (s, 9H, H19), 1.98 (s, 9H, H30), 1.72 (s, 9H, H32), 1.46 (s, 9H, H10).

$[\text{LH}_6]\text{Br}_2(\text{PF}_6)_4$, **5**: Single crystals of a methanol solvate of this composition were obtained by slow diffusion of a methanolic solution of NaPF_6 into a methanolic solution of **3**.

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Discovery of a New Efficient Chiral Ligand for Copper-Catalyzed Enantioselective Michael Additions by High-Throughput Screening of a Parallel Library**

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Umberto Piarulli,* and Simona Ceccarelli

The 1,4-addition of organometallic reagents to α,β -unsaturated carbonyl compounds is an important process for C–C bond formation in organic synthesis.^[1] A number of chiral stoichiometric reagents have been described during the last few years which allow enantioselective additions,^[2] while the development of chiral catalysts has been comparably slower. A prominent position in this rapidly expanding field is occupied by the copper-catalyzed, chiral-ligand-accelerated, 1,4-addition of organozinc reagents.^[3] In particular, chiral phosphoramidites,^[3b] phosphites,^[3c–f] and aminophosphanes^[3g] were used as ligands in the addition to cyclic enones with very good enantioselectivities (up to 98% *ee*).^[3b] On the other hand, chiral sulfonamides, which have proved effective in various catalytic asymmetric processes, were reported to catalyze the conjugate addition of organozinc reagents to cyclic enones^[4a] only with marginal enantioselectivity (up to 31% *ee*).^[4b]

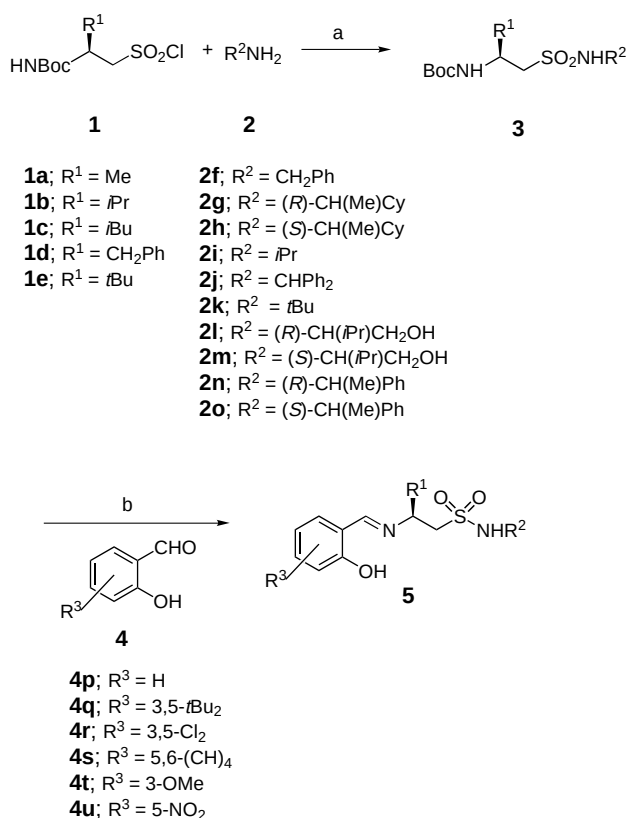
We have developed a new family of chiral Schiff base ligands of general structure **5**, which contain a set of different metal binding sites (a phenol, an imine, and a secondary sulfonamide), with the expectation that such a multidentate array would favor the formation of organometallic complexes with well-organized spatial arrangements, and with the goal of obtaining ligands for asymmetric catalysis capable of broad applicability. Ligands **5** were easily obtained (Scheme 1) by condensation of salicylaldehydes with enantiomerically pure β -amino sulfonamides. Sulfonamides **3** were in turn synthesized by coupling different primary amines with sulfonyl chlorides **1**, prepared in high yields from L- α -amino acids by a straightforward synthetic protocol.^[5] At the beginning of this work, a few model ligands **5** were prepared and tested, which proved effective in accelerating the copper-catalyzed (5% Cu(OTf)₂; Tf = F₃CSO₂) conjugate addition of diethylzinc to

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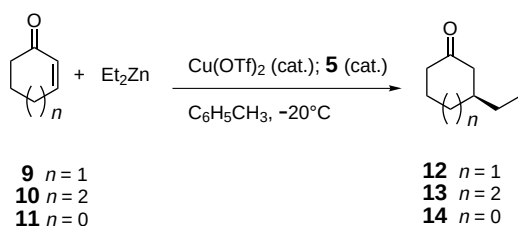
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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.



Scheme 1. Synthesis of the library of ligands **5**. a) **1** (1.2 equiv), **2** (1.0 equiv), **6** (2.0 equiv), **7** (0.2 equiv), CH_2Cl_2 , 20°C , 3 h; then **8** (3.0 equiv), 3 h, 86 %; b) **3** (1.0 equiv), $\text{TFA}:\text{CH}_2\text{Cl}_2$ (1:3), 20°C , 30 min; evaporation; **4** (0.9 equiv), **7** (3.0 equiv), CH_3OH , 20°C , 24 h, 88 %.

cyclohexenone (**9**). The enantiomeric excesses were only moderate or poor, ranging from 28 % with catalytic (5.5 %) **5dfp** ($\text{R}^1 = \text{CH}_2\text{Ph}$, $\text{R}^2 = \text{CH}_2\text{Ph}$, $\text{R}^3 = \text{H}$) to 48 % with **5dfq** ($\text{R}^1 = \text{CH}_2\text{Ph}$, $\text{R}^2 = \text{CH}_2\text{Ph}$, $\text{R}^3 = 3,5\text{-}t\text{Bu}_2$) (Scheme 2).^[6] At this stage, we considered a combinatorial approach for tuning the ligand structure and improving the results,^[7,8] taking advantage of the ligand synthesis scheme, a sequence of coupling steps with different monomers (Scheme 1), which is well-suited for the generation of diversity.



Scheme 2. Enantioselective conjugate addition of Et_2Zn to enones **9–11** catalyzed by $\text{Cu}(\text{OTf})_2/\mathbf{5}$. Screening of the library of ligands **5**.

For the synthesis of the ligand library, we used solution-phase parallel synthesis and solid-phase extraction (SPE) techniques to scavenge excess reagents and reaction by-products, and avoid chromatography.^[8,9] For the formation of sulfonamides **3** (Scheme 1), the reaction of excess sulfonyl chlorides **1** (1.2 equiv) with amines **2** (1.0 equiv) was run in

dichloromethane in the presence of methyl trimethylsilyl dimethylketene acetal (MTDA, **6**) (2.0 equiv)^[5] and a catalytic amount (0.2 equiv) of polymer-bound “4-dimethylaminopyridine”^[10] (**7**) to catalyze the coupling reaction and scavenge the liberated HCl. Apart from the polymer, which is removed by filtration, the only by-products were trimethylsilyl chloride and methyl isobutyrate. These are volatile and are removed with the solvent. After all the amine had been consumed, the excess sulfonyl chloride was removed by reaction with solid-phase-bound [tris(2-aminoethyl)amine]^[9c] (**8**) (3.0 equiv) and subsequent filtration. The *tert*-butoxycarbonyl (Boc) protecting group was then cleaved with 25 % $\text{CF}_3\text{CO}_2\text{H}$ (TFA) in CH_2Cl_2 , and the resulting amine trifluoroacetate salts (1.0 equiv) were treated in MeOH with aldehydes **4** (0.9 equiv) in the presence of **7**^[10] (3.0 equiv) to yield the target Schiff bases **5** in 76 % overall yield (average); these were pure enough to be used in the ligand-catalyzed reactions.

A multisubstrate^[8,11] high-throughput screening of the library was realized by performing the conjugate addition reactions on an equimolar mixture of 2-cyclohexenone (**9**) and 2-cycloheptenone (**10**, each 0.1 mmol), using 5.5 mol % ligand **5** (0.011 mmol) and 5 mol % $\text{Cu}(\text{OTf})_2$ (0.010 mmol) in toluene at -20°C . The reactions were quenched after 5 h, and the crude reaction mixtures were directly analyzed for conversion and enantiomeric excess by gas chromatography using a chiral capillary column, under conditions where the four peaks of the two enantiomeric pairs show baseline separation (time for each analysis: 15 min).

In the construction of the library, the choice of the building blocks (sulfonyl chlorides, amines, and aldehydes) is crucial. A test library of 60 compounds (one sulfonyl chloride **1d**, ten amines **2f–o**, and six aldehydes **4p–u**) was built to study the role of R^2 and R^3 by maximizing their diversity. This first set of ligands was screened under the conditions described above, and the results revealed some interesting features. 1) Poor enantioselectivities ($\leq 40\%$ *ee*) were obtained with amines **2k–m** (irrespective of the aldehyde), and with aldehydes **4t** and **4u** (irrespective of the amine). 2) Enantioselectivities with amines **2n** and **2o** were lower than those obtained with amines **2g** and **2h**. 3) The stereocenter bearing R^1 controls the absolute configuration of the reaction product, while the stereocenter on R^2 (when present) tunes the selectivity.

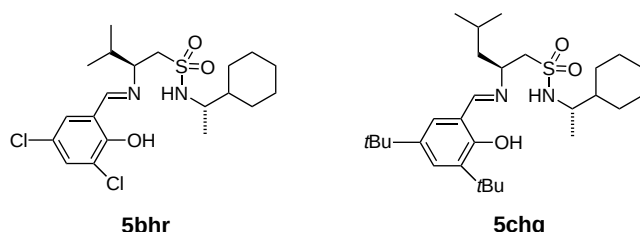
From this analysis a new library of 100 terms was designed, containing five sulfonyl chlorides (**1a–e**), five amines (**2f–j**), and four aldehydes (**4p–s**). From the screening of this library, **5bhr** ($\text{R}^1 = i\text{Pr}$; $\text{R}^2 = (S)\text{-CH(Me)Cy}$; $\text{R}^3 = 3,5\text{-Cl}_2$) was identified as the best ligand for 2-cyclohexenone (82 % *ee*) and 2-cycloheptenone (81 % *ee*). These results confirm the value of the combinatorial approach: it would have been very difficult to identify this ligand for the two different substrates if a “rational” or a “positional scanning”^[12] approach were followed. The data in Table 1 clearly show the importance of the mutual influences of the substituents $\text{R}^1\text{–R}^3$ in the fine tuning of the ligand structure.

Optimization of the reaction conditions was performed on the single enones **9–11** using the best ten ligands shown in Table 1, and taking into consideration the catalyst loading, the concentration, the reaction temperature, and the solvent.

Table 1. High-throughput screening of the library of ligands **5**: best ten results.

Entry	Ligand	R ¹	R ²	R ³	ee (12) [%]	ee (13) [%]
1	5bhr	<i>i</i> Pr	(<i>S</i>)-CH(Me)Cy	3,5-Cl ₂	82	81
2	5ehq	<i>t</i> Bu	(<i>S</i>)-CH(Me)Cy	3,5- <i>t</i> Bu ₂	80	79
3	5biq	<i>i</i> Pr	<i>i</i> Pr	3,5- <i>t</i> Bu ₂	76	72
4	5ejq	<i>t</i> Bu	CHPh ₂	3,5- <i>t</i> Bu ₂	74	75
5	5chr	<i>i</i> Bu	(<i>S</i>)-CH(Me)Cy	3,5-Cl ₂	73	74
6	5chq	<i>i</i> Bu	(<i>S</i>)-CH(Me)Cy	3,5- <i>t</i> Bu ₂	72	71
7	5aiq	Me	<i>i</i> Pr	3,5- <i>t</i> Bu ₂	69	76
8	5ehs	<i>t</i> Bu	(<i>S</i>)-CH(Me)Cy	(CH) ₄	71	71
9	5bjq	<i>i</i> Pr	CHPh ₂	3,5- <i>t</i> Bu ₂	70	69
10	5chr	<i>t</i> Bu	(<i>S</i>)-CH(Me)Cy	3,5-Cl ₂	71	67

Under the best conditions (2.75 mol % **5**, 2.5 mol % Cu(OTf)₂, toluene/hexane 80/20, 5 h), **5bhr** was identified as the best ligand for cyclohexenone **9** and cycloheptenone **10**



(at –20 °C), giving **12** in 90 % ee and **13** in 85 % ee, with 100 % conversion in both cases and 93–95 % yield (isolated product). Compound **5chq** (Figure 1) was recognized as the best ligand for cyclopentenone (**11**) (at 0 °C), giving **14** in 80 % ee albeit in a low yield (25 %).

In conclusion, we have developed a parallel library of new Schiff base chiral ligands **5** and optimized their use in the enantioselective conjugate addition of Et₂Zn to cyclic enones by a high-throughput screening approach. Work is in progress to extend the scope of ligands **5** in other enantioselective reactions.

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The Melting Point Alternation in α,ω -Alkanediols and α,ω -Alkanediamines: Interplay between Hydrogen Bonding and Hydrophobic Interactions**

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Dedicated to Professor Paul Rademacher on the occasion of his 60th birthday

Hydrogen bonding and hydrophobic interactions are ubiquitous in biological structures, be they lipids, proteins, or nucleic acids.^[1] The interference between these two kinds of interactions in such natural systems is obvious, but hard to study and difficult to perceive owing to their inherent complexity. An understanding of such interference has important implications in biological and material phenom-

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ena.^[2] Described here are the structural details of α,ω -alkanediols and α,ω -alkanediamines (named diols and diamines hereafter) which are characterized by hydrogen bonding and hydrophobic interactions. These systems represent a balance between structural simplicity and interaction complexity, which makes the study of interference between interactions a feasible exercise.^[3]

The melting point alternation in n -alkanes and most end-substituted n -alkanes has been known for decades.^[4, 5] Physical properties such as solubilities and sublimation enthalpies that are related to the solid state also exhibit an alternating pattern, whereas those related to the liquid state show monotonic behavior.^[5] n -Alkanes are solely held together by hydrophobic interactions in the solid state, and we have shown recently that the melting point alternation in n -alkanes can be explained in terms of a simple geometrical model.^[6] We have now undertaken a structural study of the diols $\text{HO}-(\text{CH}_2)_n-\text{OH}$ ($n=2-10$) and the diamines $\text{H}_2\text{N}-(\text{CH}_2)_n-\text{NH}_2$ ($n=2-8$). Most of these are liquids at room temperature, and their single crystals have been grown in situ using a miniature zone melting procedure with an IR laser.^[7] The melting points were recorded by differential scanning calorimetry and no indications of phase transitions were found. The melting point alternation is shown in Figure 1a. The X-ray data (Table 1) for all the compounds were collected at the same temperature (130 K) to allow a comparison of calculated densities.^[8, 9] The density gives a measure of compactness in packing and in a homologous series it may be correlated with the melting point.^[6] Figure 1b shows that the odd-numbered members (named odd hereafter) are more poorly packed than the even-numbered members (named even hereafter) in both series. The even diols with $n \geq 4$ form a layerlike network (space groups $P2_1/n$ and $P2_1/c$), and the odd members form a three-dimensional structure (space group $P2_12_12_1$). This result is against the intuition that a three-dimensional network should have a higher melting point than a two-dimensional network.

The end groups in the diols and diamines form hydrogen bonds, and the alkyl chains participate in hydrophobic interactions. Therefore our analysis seeks to find the relevant

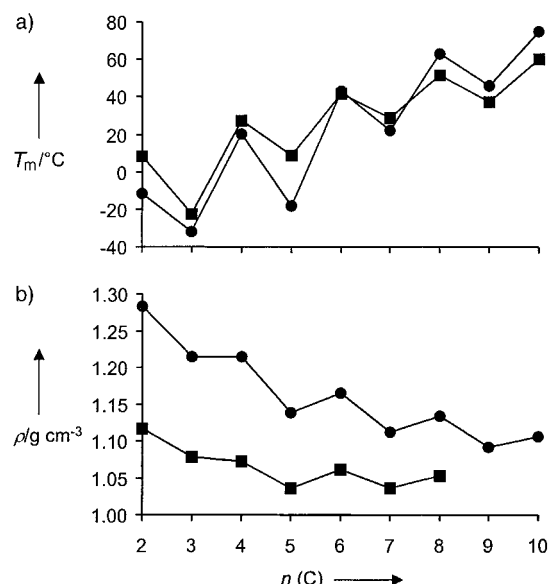


Figure 1. Alternation in the melting point T_m (a) and density ρ (b) in diols (●) and diamines (■) with increasing number of C atoms $n(C)$.

requirements for hydrogen bonding and hydrophobic interactions and establish their mutual dependence. The common packing pattern in both even and odd n -alkanes is a columnar structure wherein the molecules are stacked such that CH_2 groups of successive molecules are intergrooved (Figure 2).^[6] Typically, such a close packing of the CH_2 groups occurs at an intermolecular separation of 4.7 Å.

Since OH groups act as hydrogen-bond donors and acceptors simultaneously, they form several types of supramolecular synthons,^[3] the most common of which is a chain pattern.^[10] If $\text{O}-\text{H}\cdots\text{O}$ chains are formed at both ends of the molecule, a layer structure consisting of molecules in columns can be anticipated (Figure 3a).^[11] Given the $\text{O}-\text{H}\cdots\text{O}$ geometry, successive molecules in a column are then separated by 5.1 Å, a distance that is too long for the hydrophobic packing observed in n -alkanes. However, the layer structure of the even diols circumvents this problem in an exquisite fashion

Table 1. Important structural information of diols and diamines.

Compound	Space group	Z	Lattice parameters				C_k^*	Lattice energy [kcal mol ⁻¹]	
			a [Å]	b [Å]	c [Å]	β [°]		H-bond	van der Waals
$\text{HO}(\text{CH}_2)_2\text{OH}$	$P2_12_12_1$	4	5.0130	6.9149	9.2710	90	0.693	-7.675	-5.735
$\text{HO}(\text{CH}_2)_3\text{OH}$	$P2_1/n$	4	4.9396	7.9436	10.6007	90.097	0.698	-7.623	-7.282
$\text{HO}(\text{CH}_2)_4\text{OH}$	$P2_1/n$	4	5.0135	13.7880	7.4635	107.275	0.708	-7.713	-10.935
$\text{HO}(\text{CH}_2)_5\text{OH}$	$P2_12_12_1$	4	5.3234	6.6991	17.0398	90	0.672	-7.772	-11.014
$\text{HO}(\text{CH}_2)_6\text{OH}$	$P2_1/n$	4	7.8676	5.0554	16.9653	93.454	0.714	-7.536	-15.018
$\text{HO}(\text{CH}_2)_7\text{OH}$	$P2_12_12_1$	4	5.1698	6.8442	22.3214	90	0.680	-7.528	-15.889
$\text{HO}(\text{CH}_2)_8\text{OH}$	$P2_1/n$	2	4.7995	5.0846	17.5408	90.315	0.710	-7.726	-19.813
$\text{HO}(\text{CH}_2)_9\text{OH}$	$P2_12_12_1$	4	5.1118	6.9409	27.4620	90	0.682	-7.577	-21.028
$\text{HO}(\text{CH}_2)_{10}\text{OH}^{[a]}$	$P2_1/c$	2	4.799	5.113	21.029	93.37	0.714	-7.028	-23.898
$\text{H}_2\text{N}(\text{CH}_2)_2\text{NH}_2$	$P2_1/c$	2	5.0467	7.1552	5.4746	115.363	0.717	-3.579	-8.421
$\text{H}_2\text{N}(\text{CH}_2)_3\text{NH}_2$	$Cmc2_1$	4	12.6191	6.1297	5.9074	90	0.694	-4.982	-9.413
$\text{H}_2\text{N}(\text{CH}_2)_4\text{NH}_2$	$Pbca$	4	6.5516	5.7382	14.5275	90	0.698	-5.224	-12.358
$\text{H}_2\text{N}(\text{CH}_2)_5\text{NH}_2$	$Cmc2_1$	4	17.7088	6.2282	5.8572	90	0.688	-4.972	-13.657
$\text{H}_2\text{N}(\text{CH}_2)_6\text{NH}_2$	$Pbca$	4	6.7607	5.6643	19.0030	90	0.698	-4.923	-17.316
$\text{H}_2\text{N}(\text{CH}_2)_7\text{NH}_2$	$Cmc2_1$	4	22.8133	6.3654	5.7511	90	0.677	-4.938	-18.223
$\text{H}_2\text{N}(\text{CH}_2)_8\text{NH}_2$	$Pbca$	4	6.8928	5.6101	23.5274	90	0.692	-4.932	-22.387

[a] The packing fraction (C_k^*) and lattice energies given for this compound^[2a] are extrapolated to 130 K.

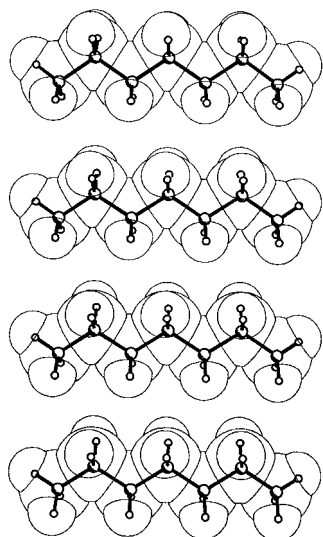


Figure 2. Columnar structure in *n*-heptane revealing the typical intergrooving pattern of CH₂ groups in *n*-alkanes.

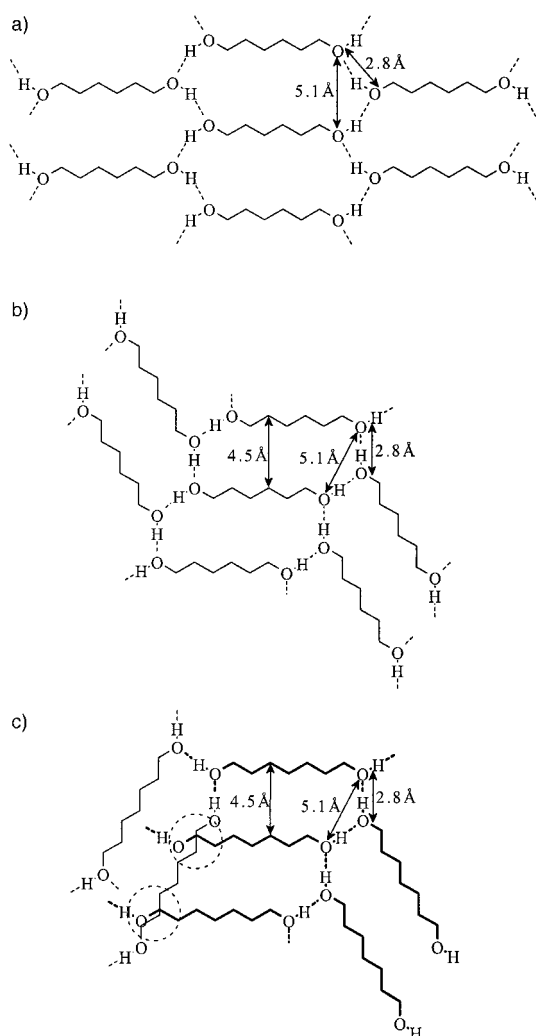


Figure 3. a) Hypothetical layer structure sustained by O–H...O hydrogen-bonded chains in diols (shown here for an even diol). b) Offset of even diol molecules within a column driven by the intergrooving pattern of hydrophobic interactions. The magnitude of the offset (2.4 Å) is the same as the distance between alternate CH₂ groups. c) Geometrical hindrance of molecular offset in odd diols (conflicting areas are highlighted in dashed circles). The O–H...O chains can only be formed at one of the ends.

(Figures 3 b and 4). The molecules are offset along their length such that the α -CH₂ groups fit into the penultimate grooves, and adjacent columns are inclined to preserve the O–H...O chains. The offset also allows the β -CH₂ groups to fit into the supramolecular grooves formed by CH₂–O–H...O (Figure 4), leading to a dense packing with hydrocarbon chains separated by 4.5 Å (c.f. 4.7 Å in *n*-alkanes).

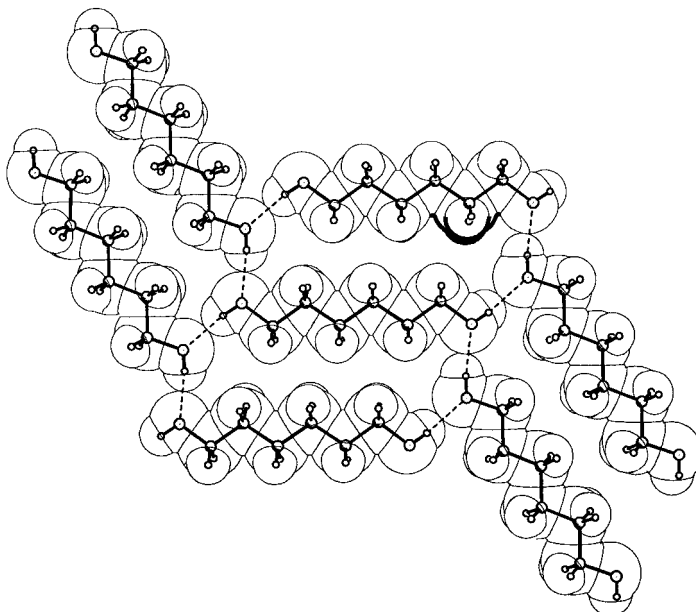


Figure 4. Layer structure in even diols illustrated with the structure of 1,6-hexanediol. Notice the offset of molecules within the columns and the fit of β -CH₂ groups into the supramolecular grooves formed by CH₂–O–H...O (marked with thick lines).

However, a similar offset packing is not possible in odd diols. Whereas the all-*trans* conformation in even diols leads to an antiparallel projection of the two C–O groups, in odd diols it projects the two C–O groups in the same direction, thus precluding the formation of O–H...O chains at opposite ends of the molecule (Figure 3 c). In fact, in the odd diols one of the C–O groups adopts a *gauche* conformation, leading to a three-dimensional network (Figure 5)^[12] in which the packing of the hydrophobic moiety is not as effective as in the even diols. The odd diols are thus less densely packed than the even ones.^[13]

Like the OH groups, the NH₂ groups also act as hydrogen-bond donors and acceptors simultaneously, and extend to a chain pattern in the diamine structures.^[10] The layer structure in even diamines is similar to that in even diols (Figure 6), but the N...N separation (3.2 Å) is generally longer than the O...O separation (2.8 Å). Thus, adjacent molecules are now spaced at 5.2 Å, a distance at which the hydrophobic packing pattern of the even diols or *n*-alkanes would leave much empty space. The hydrocarbon chain therefore swivels with respect to the fulcrum of the hydrogen-bonded chain (around the main molecular axis by 45°) to fill the gaps (compare Figures 4 and 6).

As described for odd diols, odd diamines cannot adopt the offset packing. Nevertheless, unlike odd diols, odd diamines

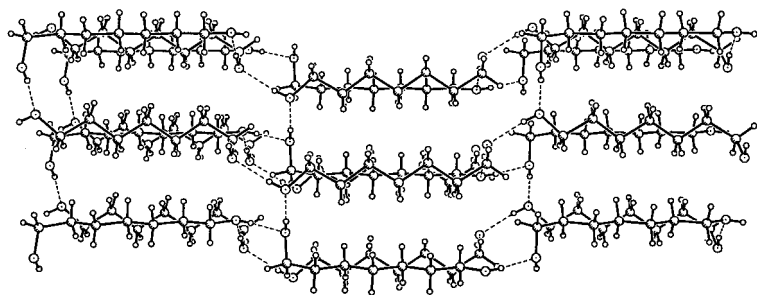


Figure 5. Crystal structure of 1,7-heptanediol illustrating the three-dimensional networking of O–H...O hydrogen bonds in odd diols. Notice that one of the C–O groups adopts a *gauche* conformation.

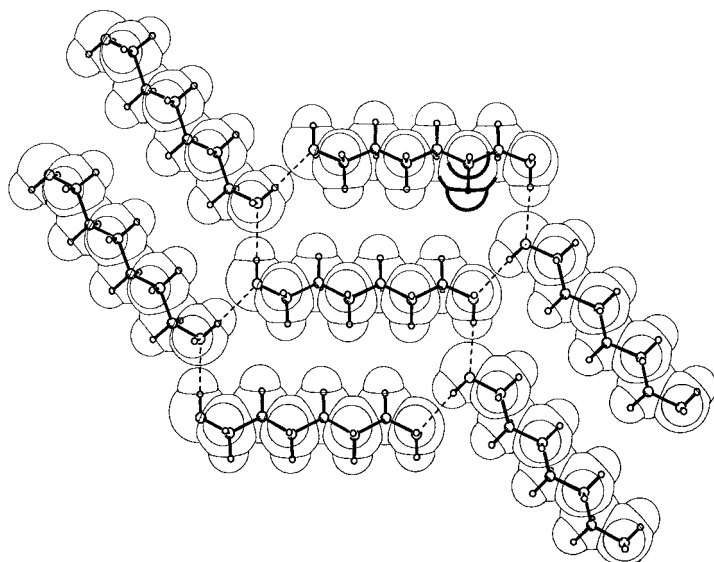


Figure 6. Layer structure in even diamines illustrated with the structure of 1,6-hexanediamine. Notice the offset of molecules within the columns and the fit of β -CH₂ groups into the supramolecular grooves formed by CH₂–N–H...N (marked with thick lines). Attention is also called to the swiveling of molecules with respect to the layer plane (compare with Figure 4).

form a layer structure with N–H...N chains, but without the offset of the molecules within a column (Figure 7). This layer is analogous to the hypothetical layer depicted in Figure 3a. Adjacent molecules within a column are now separated by 5.7 Å. Even after the swiveling of the hydrocarbon chain, the

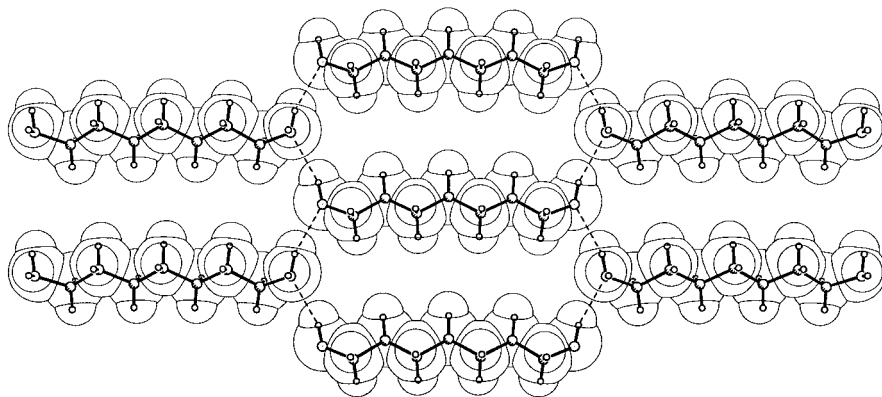


Figure 7. Layer structure in odd diamines illustrated with the structure of 1,7-heptanediamine. Notice the similarity to Figure 3a and the absence of the offset as well as the swiveling of molecules with respect to the layer plane (compare with Figure 6).

odd diamines are less densely packed than the even ones.^[13] We note that the interlayer packing in even and odd diamines is similar, with the second NH group forming a longer N–H...N hydrogen bond between the layers.

Lattice energy calculations have been made for all the structures reported here.^[14] The contributions from hydrogen-bond and van der Waals interactions are recorded in Table 1. While hydrogen bond energies are similar within each series, the van der Waals energies are greater for even members and show an alternation similar to that of the melting points in both series. It is therefore the difference in hydrophobic packing that causes the alternation of melting points.

The packing patterns in 1,2-ethanediol, 1,3-propanediol, and 1,2-ethanediamine are different from those described here because in these lower members the hydrogen bonds overrule the hydrophobic interactions.

The analysis of the crystal structures of diols and diamines has revealed the interplay between two important intermolecular interactions that are possible in these amphiphilic compounds, namely, hydrogen bonding and hydrophobic interactions. While hydrogen bonding and hydrophobic interactions operate in consonance in even members, and therefore culminate in dense packing, they run into geometrical conflicts in odd members, leading to looser packing. Accordingly, the odd members of the diol and diamine series have relatively lower densities and melting points than the even members.^[15, 16]

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- [13] This is further corroborated by the fact that the calculated packing fractions for even diols and even diamines are systematically higher than those for the corresponding odd members (Table 1) and exhibit an alternating trend similar to that of the melting points and densities.
- [14] We have used the Dreiding-II force field built-in the crystal packer module of the Cerius² program. All calculations were performed as the snapshots of crystal structures. The overall result is the same even after the crystal structures were minimized.
- [15] One of the C–O groups adopts a strained *gauche* conformation in odd diols in the solid state, which also contributes to the lowering of the melting points of odd diols.
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Bridged Cyclic Oligoribonucleotides as Model Compounds for Codon–Anticodon Pairing**

Ronald Micura,* Werner Pils, and Karl Grubmayr

Dedicated to Professor Albert Eschenmoser
on the occasion of his 75th birthday

The base sequences of genes are translated with complementary mRNA and the assistance of tRNAs into protein sequences. This process takes place at the ribosome.^[1] Thereby, the formation of specific Watson–Crick base pairs between codon (mRNA) and anticodon (tRNA) is crucial. The structure of the corresponding pairing complex is similar to a double helical A-form RNA.^[2,3] Although in general,

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complementary oligoribonucleotides in aqueous buffer solutions acquire such a geometry, a duplex consisting of solely three base pairs is too weak to allow determination of its pairing properties through routine UV and CD spectroscopic methods.^[4] The development of new model compounds with increased three base pair duplex stabilities is the aim of the work presented here. In particular, stacking interactions which are known, or assumed to be, of significance during codon–anticodon pairing are taken into account.

We based the modeling of a codon–anticodon pairing complex on the crystal structure of a tRNA which codes for phenylalanine^[5] and on the assumption of A-type helix formation with respect to the codon–anticodon interaction (Figure 1).^[2–4] With regard to the stereochemical situation it can be deduced that a structurally related model must not be restricted to the three base pairs themselves. The modified purine base 37 at the 3'-end of the anticodon propagates the A-form helix and ideally interferes with the first base pair of the codon–anticodon core duplex by base stacking. In a comparable manner, base stacking is conceivable at the opposite end of the helix which comprises the base at the 3'-end of the codon.^[6] In contrast, the bases which are attached to the core duplex through the 5'-end are unable to interfere by base stacking.

According to the structural features described above, two types of chemical constitutions (**I** and **II**) are justifiable as appropriate model compounds. They also fit the criteria described for the different pairing modes of tRNA and mRNA within the P-Site and the A-Site of the ribosome.^[2, 3] Both types **I** and **II** contain 1-methylguanosine (*m¹G*) as the representative for the modified tRNA purine bases at position 37^[7] and, optionally, in model compounds of type **II**, the first base of the following codon is taken into account (Figure 2). The characteristic structural feature of the model compounds is the covalent bridging of short complementary nucleotide sequences by hexa- or heptaethylene glycol units.^[8] The linkage spans from a 5'-phosphate group to the corresponding opposite 3'-phosphate group of the ribose backbone and, therefore, does not directly affect base pairing.

In accordance with the modeling we chose an anticodon sequence motif of tRNA which codes for phenylalanine, namely 5'-GAAM¹G,^[9] and synthesized the macrocycles **1–7** (shown in Table 1) following a solid-phase approach which was developed for the preparation of cyclic oligoribonucleotides in general.^[10] All compounds were characterized by MALDI-TOF mass spectrometry and their pairing properties investigated.

The temperature-dependent trace of UV absorption at 265 nm shows sigmoid melting profiles for the macrocycles **1–7** in aqueous buffer systems (Figure 3). The corresponding melting transitions are independent of concentration and are observed in a temperature region that guarantees a reliable determination of thermodynamic parameters.^[11] The stability

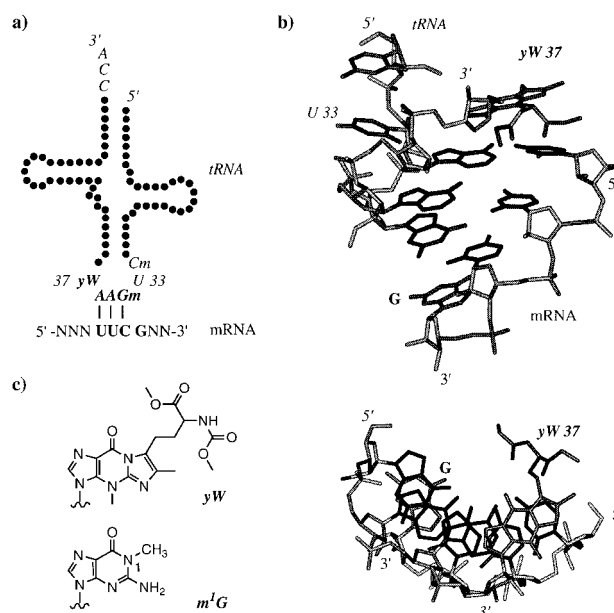


Figure 1. Codon–anticodon pairing of tRNA^{Phe} from *Saccharomyces cerevisiae* (tRNA symbols in *italic* style). a) Sequence motif: wybutosine (yW), 2'-O-methylguanosine (Gm), 2'-O-methylcytosine (Cm). b) Modeling: views perpendicular (above) and parallel (below) to the helix axis; the nonpairing bases wybutosine (tRNA) and guanosine (mRNA) at the 3'-ends of the codon–anticodon duplex interfere by base stacking. c) Structural formula of tRNA^{Phe} purine bases at position 37; 1-methylguanosine (*m¹G*).^[3, 5]

of the minihelices is of entropic origin and is displayed in the high absolute values of the entropic terms which reach up to 100 cal mol^{−1} K^{−1} (Table 1).

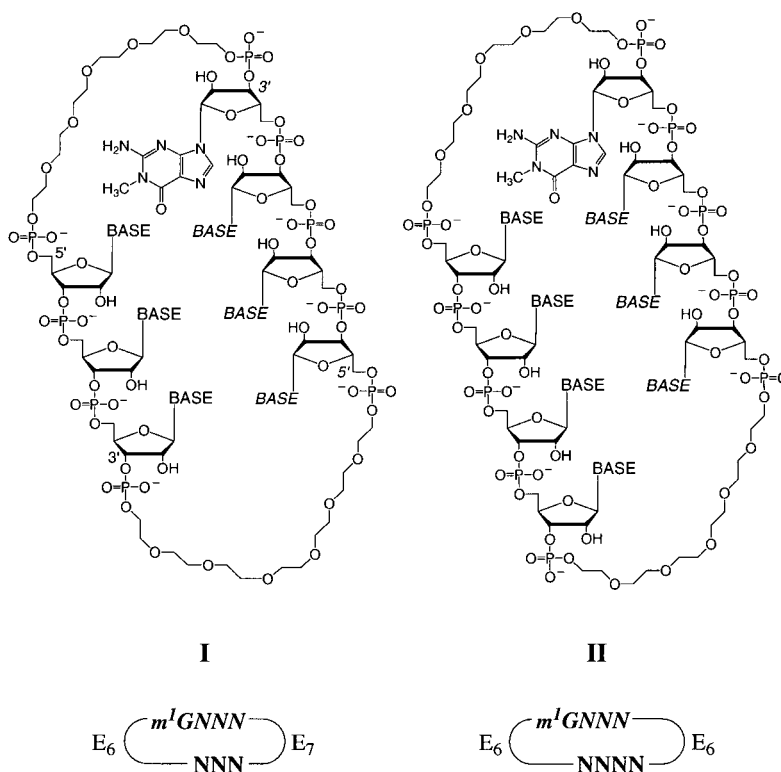


Figure 2. Constitutional types **I** and **II** of cyclic model compounds for the codon–anticodon pairing. N, N = nucleotides, E₆ = hexaethylene glycol phosphate, E₇ = heptaethylene glycol phosphate.

Table 1. Selected cyclic oligoribonucleotides containing the sequence motif of the tRNA^{Phe} codon–anticodon pairing.

Constitution type	No.	Base sequence	T_m ^[a] [°C]	ΔH° ^[c] [kcal mol ⁻¹]	Thermodynamic data ^[b] ΔS° [cal mol ⁻¹ K ⁻¹]	ΔG_{298}° [kcal mol ⁻¹]	MALDI-TOF-MS [M+H] ⁺ (theor.)	[M+H] ⁺ (obs.)
I	1	E ₇ $\begin{array}{c} \text{AAAG} \\ \text{UUC} \end{array}$ E ₇	36.0	–22.7 (–19.3)	–73.4 (–51.5)	–0.8	2698	2698
	2	E ₆ $\begin{array}{c} \text{GAAG} \\ \text{UUC} \end{array}$ E ₇	41.2	–26.8 (–24.8)	–85.3 (–66.5)	–1.4	2999	2999
	3	E ₆ $\begin{array}{c} m^1\text{GAAG} \\ \text{UUC} \end{array}$ E ₇	42.6	–27.6	–87.4	–1.5	3013	3011
II	4	E ₆ $\begin{array}{c} m^1\text{GAAG} \\ \text{UUCG} \end{array}$ E ₆	49.9	–33.3	–103.1	–2.6	3314	3313
II	5	E ₆ $\begin{array}{c} m^1\text{GAAG} \\ \text{UUCU} \end{array}$ E ₆	43.8	–30.1	–95.3	–1.7	3275	3276
II	6	E ₆ $\begin{array}{c} m^1\text{GAAG} \\ \text{UUUG} \end{array}$ E ₆	18.0	–17.8	–61.1	+0.4	3315	3316
II	7	E ₆ $\begin{array}{c} m^1\text{GAAG} \\ \text{UUUU} \end{array}$ E ₆	16.5	–16.1	–55.6	+0.5	3276	3278

[a] Melting temperatures T_m ; 4 μM , 1.0 M NaCl, 0.01 M Na₂HPO₄, pH 7.0. [b] Determination of ΔH° and ΔS° from plots of α against T . See ref. [11] and ref. [12], p. 1119. The analysis by differentiation of the melting profiles according to ref. [11a], p. 1610 gave similar values. [c] The terms in brackets provide the ΔH° and ΔS° contributions within a (bimolecular) duplex calculated on the basis of free-energy parameters tabulated for single standard base pairs. See ref. [11b], p. 30, p. 34.

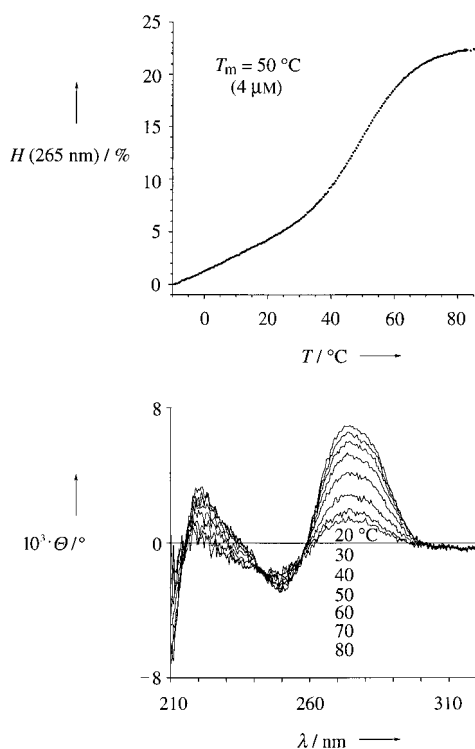


Figure 3. Temperature dependence of the base pairing of cy-rGAAm¹G-E₆-UUCG-E₆ **4** (4 μM , 1.0 M NaCl, 0.01 M Na₂HPO₄, pH 7). Above: UV melting profile. Below: CD spectra. H = hyperchromicity, ΔT = 0.7 K min⁻¹.

The comparison of the thermodynamic stabilities of pairing complexes that belong to the different constitutional types **I** and **II** is reasonable despite their different ring size. A detailed study concerning this matter suggests that differences in stability which originate from conformational changes of the linkers or from different linker lengths are small when compared to the stabilization which arises from base stacking

by an additionally attached nucleotide.^[8] Therefore, it can be outlined that the pairing complex which consists of the three base pairs UUC/GAA is significantly stabilized by nonpairing, dangling bases at one or both 3'-ends (Table 1; **1–5**). In general, the extent of stabilization is higher with purine bases than pyrimidine bases (Table 1; **4, 6** compared with **5, 7**). This might be of interest with respect to evolution of tRNA structures as tRNA bases at position 37 are, without exception, of purinic structure. Moreover, their chemical modifications often enable additional interstrand stacking and, therefore, potentially enhance duplex stabilities. In nature, these principles seem to be best realized in the tricyclic base wybutosine (Figure 1),^[13] however even the frequently occurring 1-methylguanosine causes a definite stabilization of our model compounds compared to the unmodified guanosine (Table 1; **3** compared with **2**).

1-Methylguanosine has another characteristic feature in common with modified tRNA bases at position 37: its 1-methyl group hampers base pairing according to the Watson–Crick mode. For compound **7**, this property is the reason for retaining the reading frame of UUU/GAA; the incorporation of guanosine instead of 1-methylguanosine would most likely cause slippage from the intended pairing complex of three base pairs towards one consisting of the corresponding four base pairs.^[14]

This principle of cyclic bridging through flexible linkers offers the availability of very short, but highly stable, double helical RNAs. The physico chemical and structural properties of these compounds can be easily ascertained with standard methods used in the field of oligonucleotide chemistry. In particular, double helices of three base pairs represent model compounds for codon–anticodon interactions and enable the estimation of any intrinsic codon–anticodon pairing stabilities. The results presented show that the conception of codon–anticodon pairing as an interaction between solely trinucleotidic sequences is insufficient. With respect to

stabilities, the nucleobases close to the 3'-ends have to be taken into account. Biological phenomena that concern the accuracy of ribosomal translation should, particularly, be newly analysed in terms of their codon context. In this sense, we are extending the current studies towards a systematic comparison of coded and recoded codon-anticodon complexes encountered during ribosomal tRNA slippage, in order to reveal differences in the base-stacking patterns as a determinant for frameshift events.^[15]

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A Convenient and General Tin-Free Procedure for Radical Conjugate Addition**

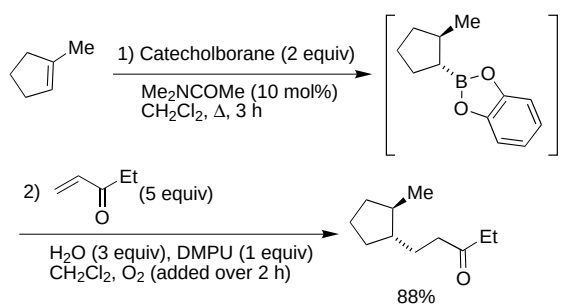
Cyril Ollivier and Philippe Renaud*

Radical reactions are becoming an extremely useful tool in organic synthesis, particularly for the formation of carbon-carbon bonds in intra- and intermolecular processes.^[1] The very rapid development of these reactions could be attributed to the emergence of highly efficient ways to conduct them. Among these methods, the tin hydride mediated addition of radicals to activated alkenes has played a major role.^[2] However, the application of this reaction for the synthesis of pharmaceuticals is severely limited by the toxicity of the tin reagents and by the difficulty in removing traces of organotin residues from the final products. Therefore, alternative ways of running radical reactions are under intensive investigation.^[3] Recently, we have reported a modified version of the Brown-Negishi reaction^[4] where efficient hydroborations with catecholborane and radical additions to enones and enals were performed in a one-pot procedure (Scheme 1).^[5a] This oxygen-initiated reaction proved to be efficient with enones and enals. However, other classical radical traps such as unsaturated esters, amides, and sulfones failed to react. Herein, we present an efficient procedure to run one-pot hydroboration for radical addition to any kind of activated alkenes. The reaction is based on the use of a Barton carbonate as radical chain transfer reagent (RCTR).^[6]

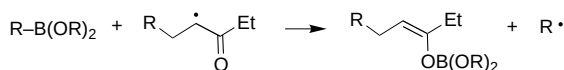
The failure of our modified version of the Brown-Negishi reaction with classical radical traps such as acrylate moieties was interpreted as a consequence of an inefficient propagation step resulting from the reaction between the radical adducts and *B*-alkylcatecholboranes. This inefficiency is caused by the lower density of unpaired electrons at the

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[**] Organoboranes in Radical Reactions. Part 4. This work was generously supported by the Fonds National Suisse de la Recherche Scientifique. We thank Valéry Weber for performing some preliminary experiments. Parts 1–3: see ref. [5].

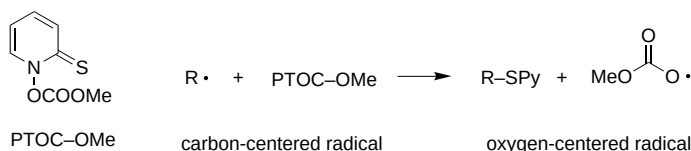


Propagation step:



Scheme 1. Modified Brown–Negishi reaction involving *B*-alkylcatecholboranes.^[5a]

oxygenatom of these radicals relative to ketone–enolate and aldehyde–enolate radicals.^[7, 8] Based on this assumption, we thought that, for radical traps other than enones and enals, the use of a chain transfer reagent able to convert the carbon-centered radical adduct into an oxygen-centered radical was necessary. The Barton carbonate PTOC-OMe (PTOC = pyridine-2-thione-*N*-oxycarbonyl),^[9] easily prepared by reacting the commercially available sodium salt of *N*-hydroxypyridine-2-thione and methyl chloroformate, should possess the necessary reactivity to act as RCTR (Scheme 2). The yellow solution of the Barton carbonate PTOC-OMe is stable in the dark and is expected to furnish, upon irradiation with a standard lamp, the methoxycarbonyloxyl radical (MeO-COO•). Decarboxylation of this radical to afford the methoxyl radical cannot be excluded; however, this process is known to be slow.^[10]



Scheme 2. The radical chain transfer reagent (RCTR) PTOC-OMe converts a carbon-centered into an oxygen-centered radical.

In a preliminary study, we have checked that the methoxycarbonyloxyl radical, generated by the simple irradiation of PTOC-OMe with a 150 W tungsten lamp, was able to react with *B*-alkylcatecholborane and to propagate efficiently the chain reaction. A one-pot procedure starting from the alkene was examined [Eq. (1)]. After hydroboration with catecholborane,^[11] the in situ generated alkylborane was treated with three equivalents of PTOC-OMe and the solution was irradiated with a standard 150 W lamp for 12 h at 10 °C; the results are summarized in Table 1. Under these conditions,

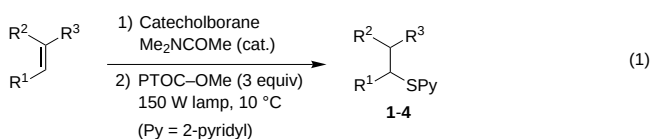


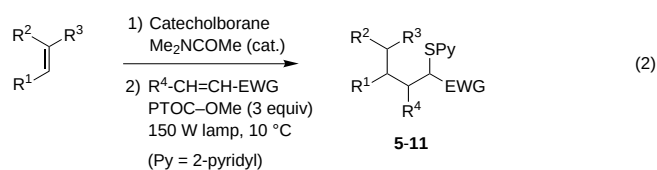
Table 1. Preparation of sulfur-containing derivatives by the hydroboration procedure shown by Equation 1.

Entry	Alkene	Product	Yield ^[a] [%]	Stereoselectivity
1			74 (50 ^[b])	
2			62	d.r. = 82:18 ^[c]
3			75	d.r. = 90:10 ^[c]
4			52	

[a] All reactions used 3 mmol of the olefin. [b] Using 1 equiv of PTOC-OMe. [c] Only major isomer is shown.

sulfur-containing derivatives were easily prepared from primary and secondary alkyl radicals in 62 % to 75 % yield (Table 1, entries 1–3). Use of only one equivalent of PTOC-OMe led to a decrease of the yield (Table 1, entry 1); however, the cyclohexyl 2-pyridyl sulfide **1** was still isolated in 50 % yield proving that the propagation step of the reaction is quite effective. The radical nature of the reaction was proved by the reaction with 2-carene, which gave the cyclohexene derivative **4** resulting from the ring opening of the intermediate cyclopropylmethyl radical (Table 1, entry 4).

Based on these encouraging results, a similar reaction procedure involving hydroboration with catecholborane, irradiation in the presence of five equivalents of an activated alkene as radical trap, and three equivalents of PTOC-OMe as mediator was examined [Eq. (2)]. Despite the greater complexity of this reaction sequence relative to the reaction depicted in Equation 1, we were pleased to notice that the yields were similar, and in several cases even higher, than in the absence of radical traps (Table 2).



In the first set of experiments, hydroboration of cyclohexene followed by reaction with different radical traps such as methyl acrylate, dimethyl fumarate, *N*-phenyl maleimide, or phenyl vinyl sulfone was examined (Table 2, entries 1–4). Yields between 70 % (methyl acrylate) and 94 % (dimethyl fumarate) were obtained. Interestingly, only small amounts of products from the direct reaction of the cyclohexyl radical with PTOC-OMe were observed when using a simple one-pot procedure. Indeed, all reagents are mixed together before irradiation and slow addition of the PTOC-OMe is not required. Similar results were obtained with phenylcyclopentene (Table 2, entry 5). In this case, the *trans* isomer of **9** is formed with a good diastereoselectivity (*trans*:*cis* 97:3). A

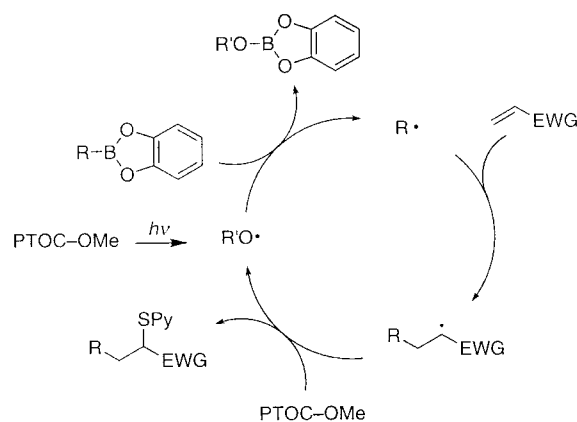
Table 2. Hydroboration and conjugate addition according to Equation 2.

Entry	Alkene	Radical trap	Product	Yield ^[a] [%] (direct addition)	Stereoselectivity
1				70 (14)	
2				94 (≤ 2)	d.r. 77:23 ^[b]
3				81 (≤ 2)	d.r. > 98:2
4				75 (15)	
5				61 (21)	<i>trans</i> : <i>cis</i> 97:3 ^[c]
6				69 (15)	<i>cis</i> : <i>trans</i> 90:10 ^[c]
7				66 (≤ 2)	d.r. 53:47

[a] All reactions were run on 3 mmol scale. [b] Relative configuration of the major isomer attributed by analogy to related reactions, see ref. [12]. [c] Mixture (1:1) of isomers relative to the center bearing the *S*-pyridyl group.

primary alkyl radical was also generated from β -pinene and added to methyl acrylate with similar efficiency (Table 2, entry 6). In this example, the *cis/trans* stereochemistry was controlled during the hydroboration step. In entry 7, the first example of cyclization reaction starting from a diene is presented. The interesting chemo- and regioselective hydroboration of the terminal double bond followed by an intramolecular radical addition furnished the cyclopentane **11** in 66% yield. This cyclization procedure starting from a diene is particularly attractive due to the extremely easy preparation of the starting material. Indeed, no reactive radical precursor such as a bromide or an iodide has to be prepared before running the cyclization reaction. As already mentioned, the yields observed in the conjugate additions compare favorably with the simpler processes described in Equation 1. This could be rationalized by taking into account the polar effects in the chain reaction. The general mechanism of the conjugate addition is depicted in Scheme 3.

Irradiation of the PTOC-OMe initiates the reaction by providing a methoxycarbonyloxyl or methoxyl radical ($R'O^\bullet$). This radical reacts with the borane to give the nucleophilic alkyl radical $R^\bullet$ that adds to the radical trap. The radical adduct has some electrophilic character due to its substitution by an electron-withdrawing group (EWG) and therefore reacts rapidly with the electron-rich thiocarbonyl group of the PTOC-OMe. This mechanism parallels nicely the tin hydride mediated reaction: The abstraction of a halide atom by the tin radical is replaced by the reaction of an alkoxy radical with a *B*-alkylcatecholborane. The three radicals involved in



Scheme 3. Radical chain mechanism for the conjugate addition of *B*-alkylcatecholboranes to activated olefins (R = alkyl group; EWG = electron withdrawing group; $R'O^\bullet$ = $MeOCO^\bullet$, $MeO^\bullet$).

the chain reaction all possess a different reactivity to allow efficient fulfillment of the selectivity criteria formulated by Giese.^[1a, 2d]

In conclusion, we believe that the present procedure represents a very valuable alternative to the tin hydride mediated reaction for the formation carbon–carbon bonds through a radical process. Good yields of the intermolecular reaction could be achieved in a simple one-pot procedure. Slow addition of the reagents is not necessary when activated radical traps are used. Moreover, the process is nonreductive and furnished products bearing a *S*-pyridyl group that can be

removed or transformed into a variety of functional groups as amply demonstrated by Barton.^[13] Since radical precursors are generated through a hydroboration step, this procedure could take advantage of the chemo- and regioselectivity of the hydroboration and offers a very simple and efficient method for the preparation of highly functionalized systems through inter- and intramolecular radical addition. Applications of this approach to cascade reactions starting from a polyene are currently under investigation. Finally, preparation of optically active materials through enantioselective hydroboration of prochiral alkenes should represent valuable entries into the synthesis of enantiomerically enriched compounds.

Experimental Section

Sulfur inclusion into *B*-Alkylcatecholboranes: Catecholborane (0.64 mL, 6.0 mmol) was added dropwise at 0 °C to a solution of the olefin (3.0 mmol) and *N,N*-dimethylacetamide (28.0 µL, 0.3 mmol) in CH₂Cl₂ (2.0 mL). The mixture was heated under reflux for 3 h. Methanol (0.15 mL, 3.6 mmol) was added at 0 °C and the mixture was stirred for 15 min at room temperature. The CH₂Cl₂ was evaporated under vacuum with strict exclusion of O₂.^[14] A yellow solution of PTOC-OMe (9.0 mmol), freshly prepared by stirring for 1 h in the dark the sodium salt of *N*-hydroxypyridine-2-thione (1.41 g, 9.45 mmol) and methyl chloroformate (0.7 mL, 9.0 mmol) in benzene (15 mL), was added to the *B*-alkylcatecholborane followed by 1,3-dimethyl hexahydro-2-pyrimidone (DMPU; 0.36 mL, 3.0 mmol). The reaction mixture was irradiated at 10 °C with a 150 W tungsten lamp for about 14 h, and treated with 1N NaOH (20 mL). The aqueous layer was extracted with CH₂Cl₂ and the combined organic phases were washed with a saturated NaCl solution (30 mL), dried with MgSO₄, and concentrated in vacuo. The crude product was purified by flash chromatography (hexane/EtOAc).

Conjugate addition: Catecholborane (0.64 mL, 6.0 mmol) was added dropwise at 0 °C to a solution of the olefin (3.0 mmol) and *N,N*-dimethylacetamide (28.0 µL, 0.3 mmol) in CH₂Cl₂ (2.0 mL). The reaction mixture was heated under reflux for 3 h. Methanol (0.15 mL, 3.6 mmol) was added at 0 °C and the mixture was stirred for 15 min at room temperature. A yellow solution of PTOC-OMe (9.0 mmol), freshly prepared as previously described, was added to the *B*-alkylcatecholborane followed by the activated alkene (15 mmol) in benzene (15 mL) and DMPU (0.36 mL, 3.0 mmol). In the case of dimethyl fumarate as the alkene, CH₂Cl₂ (15 mL) was used instead of benzene. The reaction mixture was irradiated at 10 °C with a 150 W tungsten lamp for about 14 h, and treated with 1N NaOH (20 mL). The aqueous layer was extracted with CH₂Cl₂ and the combined organic phases were washed with a saturated NaCl solution (30 mL), dried with MgSO₄, and concentrated in vacuo. The crude product was purified by flash chromatography (hexane/EtOAc).

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“Naked” Phosphorus as a Bent Bridging Ligand**

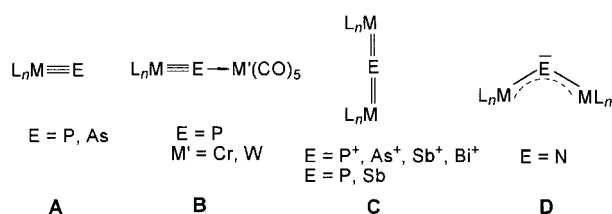
Peter Kramkowski and Manfred Scheer*

*Dedicated to Professor Dirk Walther
on the occasion of his 60th birthday*

Only a small number of the complexes containing pnico-genido (E^{3–}) ligands of the heavier Group 15 elements (E = P, As, Sb, Bi)^[1] are known in which the ligands display low coordination numbers 1 and 2. Only in 1995 were the first complexes of type **A**,^[6] with terminal ligands and coordination number 1, synthesized and structurally characterized with the compounds [(Ar'RN)₃Mo≡P] (Ar' = 3,5-C₆H₃Me₂, R = C(CD₃)₂CH₃)^[2] and [(N₃N')M≡E] [N₃N' = N(CH₂CH₂NSiMe₃)₃; E = P, M = W, Mo;^[3] E = As, M = W,^[4] Mo^[5]]. We recently found the asymmetric linear coordination mode **B** with coordination number 2 in the complexes [(N₃N')M≡E → ML_m] (ML_m = GaCl₃,^[7] M(CO)₄, M = Cr, W^[5]) and [(RO)₃W≡P → M(CO)₅] (M = Cr, W; R = *t*Bu^[8], Ar (2,6-Me₂C₆H₃)^[9]). Symmetrical linearly bridged complexes of

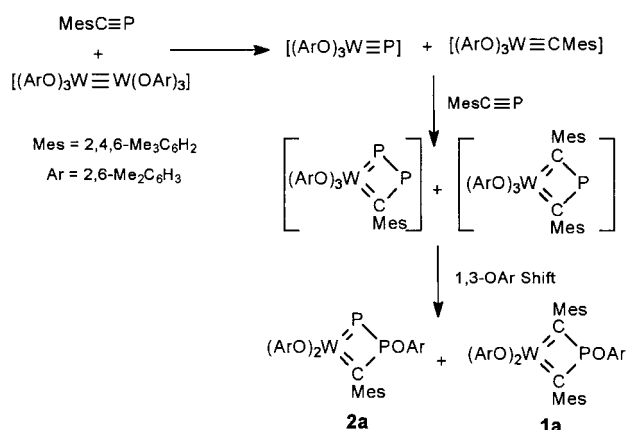
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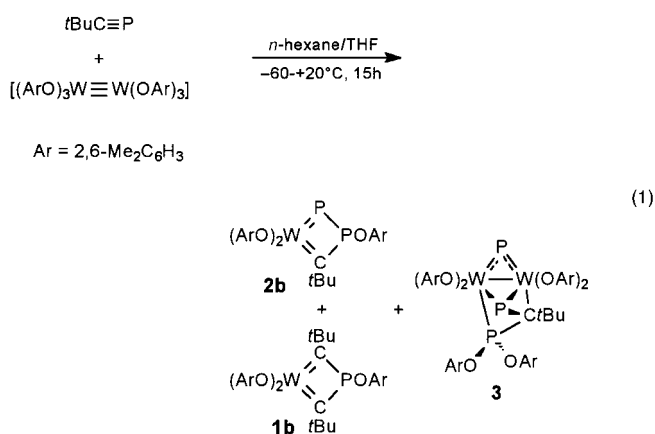
type **C** of the heavier Group 15 elements are so far exclusively known for cationic complexes, which were prepared by Huttner and co-workers.^[10] Neutral compounds of this type are only known for $E = P$ in the complexes $[(Cp_2Zr)_2(\mu-P)]$ and $[(R'RN)_3Mo)_2(\mu-P)]$ ($R = Ph$; $R' = tBu$), synthesized in the groups of Stephan^[11] and Cummins^[12], respectively. We recently synthesized the first neutral example for antimony in the complex $[(N_3N')W=Sb=W(N_3N')]$ (**C**) [$N_3N' = N(CH_2CH_2NCH_2tBu)_3$].^[13] The nonlinear bent bridging coordination mode **D** of the coordination number 2, however, has not yet been observed for the heavier Group 15 elements.^[11] So far this coordination mode has been found exclusively for nitrogen,^[14] for example, in complexes of vanadium^[15] and tantalum^[16]. We report here the synthesis, structure, and properties of the first complex with a bent bridging phosphorus ligand of coordination number 2.

In our studies on the synthesis of phosphido complexes of type **B** with the formula $[thf(ArO)_3W≡P → M(CO)_5]$ using the three-component reaction between $[W_2(OAr)_6]$ ($Ar = 2,6-Me_2C_6H_3$)^[17] and $R'C≡P$ ($R' = tBu, Mes$; $Mes = 2,4,6-Me_3C_6H_2$) in the presence of $[M(CO)_5thf]$ ($M = Cr, W$),^[9] we have also investigated the corresponding two-component systems $[W_2(OAr)_6]/R'C≡P$.^[18] Whereas, for $MesC≡P$ only the four-membered ring derivatives **1a** and **2a** were obtained as a consequence of the reaction of the initially formed phosphido complex and alkylidyne complex, respectively, with further phosphalkyne and subsequent 1,3-OAr shift (Scheme 1), in the case of $tBuC≡P$ the complex **3** of type **D**



Scheme 1.

with a bent phosphorus ligand [Eq. (1)] is obtained in addition to the cyclic complexes **1b** and **2b**. The dark red crystalline complex **3** is extremely sensitive towards hydrolysis and is readily soluble in *n*-pentane, toluene, and THF. The molecular ion peak is observed in the mass spectrum of **3**.



The formation of **3** could occur by the dimerization of two phosphido complex units $[(ArO)_3W≡P]$ with simultaneous addition of a further phosphalkyne. This intermediate is stabilized by 1,3-ArO shifts from the W atoms to the P atom of the additionally attached phosphalkyne with formation of a tungsten-tungsten bond. The postulated dimerization of two $[(ArO)_3W≡P]$ intermediates is also observed for the isolable phosphido complex $[(ArO)_3W≡P → W(CO)_5]$, leading to the planar four-membered ring derivative **4**.^[9]

The molecular structure of **3**^[19] (Figure 1) reveals a four-membered ring of the atoms W1, P1, W2, and P2, which is folded along the W1–W2 bond (2.8162(8) Å) (dihedral angle: 151.4°) and further coordinated by a *t*BuCP unit (C1 and P3). The atom C1 is connected to the tungsten atom W2 and to the phosphorus atoms P1 and P3

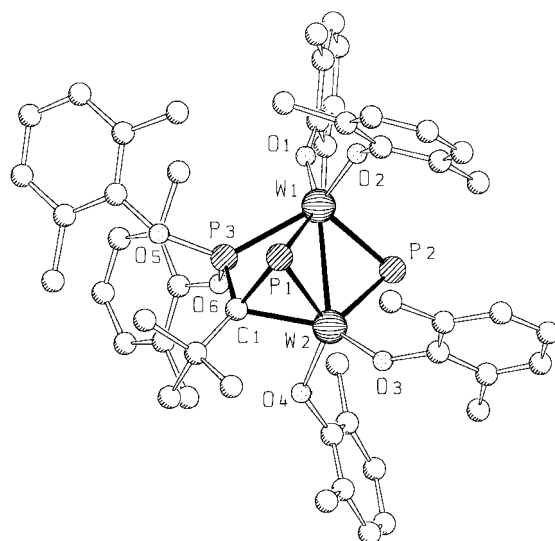
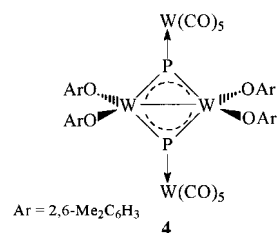
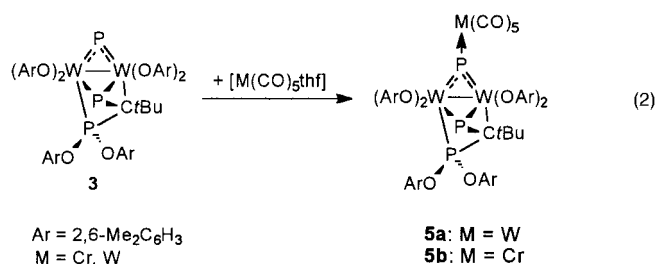


Figure 1. Molecular structure of **3** (hydrogen atoms are omitted for clarity). Selected bond lengths [Å] and angles [°]: W1–P2 2.301(2), W2–P2 2.284(2), W1–P1 2.483(2), W2–P1 2.483(2), W1–P3 2.475(2), W1–W2 2.8162(8), W2–C1 2.200(7), P1–C1 1.860(7), P3–C1 1.743(6); W1–P2–W2 75.78(6), W1–P1–W2 70.22(5), W1–P3–C1 95.1(2), P1–W1–P2 100.98(7), P1–W2–P2 103.68(7), C1–P1–W2 60.4(2), C1–P1–W1 91.9(2), P3–C1–W2 99.0(3).

through single bonds. The lone pair of electrons on the phosphorus atom P3 coordinates to the W1 atom, as indicated by the P–W distance of 2.475(2) Å. The predominant structural feature of **3** is the substituent-free phosphorus atom P2 that bridges the atoms W1 and W2. The bond lengths of the phosphorus atom P2 to the atoms W1 and W2 are 2.301(2) and 2.284(2) Å, respectively, with an angle W1–P2–W2 of 75.78(6)°. Comparison with the structural data for the complex **4**, in which the symmetrical phosphido ligand has the coordination number 3 ($d(\text{W–P})$ 2.2805(17) and 2.2806(17) Å; $\angle(\text{W–P–W})$ 76.69(7)°), demonstrates the insignificant structural influence of the additional coordination of the phosphido phosphorus atom to the W(CO)₅ moiety in **4**. Presumably the planar geometry in **4** is responsible for a shortening of the W–P bonds. The differently substituted and therefore electronically different W atoms could be the reason for the asymmetrical phosphido bridge of the P2 atom in **3**.

The ¹H-coupled as well as the ¹H-decoupled ³¹P NMR spectra of **3** show three singlets. The signal at $\delta = 831.8$ can be assigned to the naked phosphorus atom P2. In comparison with the P atom coordinating to a W(CO)₅ moiety in **4**, the signal appears shifted down field by almost 300 ppm (**4**: $\delta = 558.2$). A similar difference in the chemical shifts, combined with a high-field shift in the case of additional coordination of the P atom is observed for the phosphido complexes [(*t*BuO)₃W≡P] ($\delta = 845.0$) and [(*t*BuO)₃W≡P → W(CO)₅] ($\delta = 546.0$).^[8] Furthermore, as a result of the two ArO substituents, the signal of the phosphorus atom P3 in **3** is detected at high field ($\delta = 97.5$). However, this effect is less distinct than with the P atoms bearing single ArO substituents in the WC₂P and WCP₂ four-membered ring complexes **1b** ($\delta = -151.5$) and **2b** ($\delta = -87.1$), respectively. Only the signal of the phosphorus atom P3 shows coupling to tungsten (¹J(W,P) = 216 Hz). No coupling to the tungsten atoms was observed for either of the other singlets, including that of atom P1 at 140.4 ppm.

The bent bridged phosphorus atom P2 in **3** should be able to coordinate through its lone pair of electrons to Lewis acid complex fragments such as [M(CO)₅thf]. Thus, **3** reacts with [M(CO)₅thf] (M = W, Cr) to give the complexes **5a, b** [Eq. (2)]. The signals of **3** could no longer be observed in the ³¹P NMR spectrum of the crude mixture of reaction (2).



The ³¹P NMR data of **5a, b** support the coordination of the bridging atom P2 to the corresponding M(CO)₅ group (M = Cr, W) with a significant high field shift of 180 ppm (M = W: 650.5 ppm) and 115 ppm (M = Cr: 715.0 ppm), respectively, for the resonance signal of this phosphorus atom. A pair of satellites with a coupling constant of 210 Hz (**5a**) and 197 Hz

(**5b**), respectively, appears on this signal, which has to be assigned to the coupling with the tungsten atoms of the W(OAr)₂ moieties. No couplings to the W(OR)₂ groups were found, either in complex **4**^[9] with the coordination number 3 on the phosphido phosphorus atom or in its *t*Bu-substituted analogues.^[8] This highlights the different geometry at the tungsten alkoxide groups of **5** in comparison with those of **3** and **4**, respectively. All other chemical shifts of the phosphorus atoms in **5a, b** are slightly different from those in **3** (a down field shift of 10–12 ppm in each case).

The cause of the so far unique formation of the bent bridged P₁ ligand of **3** is the predominant tendency of the phosphorus atom to incorporate its lone pair of electrons into the bonding mode to form either the coordination number 3 or the linear coordination modes **B** or **C**. Thus, in the complex **3** optimum conditions for the realization of this coordination mode **D** are present: 1) the high tendency of (RO)₃W fragments to eliminate RO groups under formation of a W–W bond^[9] and 2) optimal steric parameters of the substituents at the tungsten alkoxide and on the phosphalkyne, which prevent a subsequent reaction with the alkoxide dimer to form [(RO)₃W]₂(μ₃-P) (R = 2,6-Me₂C₆H₃), as we had observed for R = neopentyl.^[8] Furthermore, the reaction procedure is also very important; long reaction times at very low temperatures give the metathesis product [(ArO)₃W≡P] the possibility to react with a second molecule and *t*BuC≡P simultaneously to give the complex **3**. The exclusive reaction of the phosphido complex with *t*BuC≡P leads to complex **2b**. Therefore, the accessibility of complexes of type **D** for the heavier homologues of phosphorus seems to be only possible after the first synthesis of the corresponding arsa- and stibaalkynes.

Experimental Section

1a and **2a**: [W₂(OAr)₆]^[20] (Ar = 2,6-Me₂C₆H₃) (550 mg, 0.5 mmol) was dissolved in toluene (25 mL) and frozen with liquid nitrogen. A solution of MeC≡P^[21] (Mes = 2,4,6-Me₃C₆H₂) (162 mg, 1 mmol) in *n*-hexane (20 mL) was added at –78 °C. The reaction mixture was allowed to warm to room temperature over a period of 15 h. All volatile materials were then removed under high vacuum. The reddish brown residue was characterized by ³¹P{¹H} NMR spectroscopy, which revealed the presence of **1a** and **2a**. No separation of **1a** and **2a** was achieved either by fractional crystallization or by column chromatographic work-up on silica gel and Al₂O₃, respectively. ³¹P{¹H} NMR (101.256 MHz, [D₆]benzene, 298 K, 85% H₃PO₄ ext.): **1a**: $\delta = -154.8$ (s); **2a**: $\delta = 434.2$ (d, ¹J(P,P) = 442 Hz), –89.5 (d, ¹J(P,P) = 442 Hz).

3: [W₂(OAr)₆] (Ar = 2,6-Me₂C₆H₃) (550 mg, 0.5 mmol) was dissolved in THF (15 mL) and cooled to –60 °C. To this solution was added a solution of *t*BuC≡P^[22] (100 mg, 1 mmol) in *n*-pentane (15 mL) over 3 h. The reaction mixture was allowed to warm to room temperature over a period of 15 h. After removal of the solvent, the residue was extracted with *n*-pentane (30 mL) and filtered through celite. After the volume of solvent had been reduced to about 10 mL, dark red crystals of **3** (75 mg, 12%) were obtained. The solid reaction residue was dissolved in toluene (30 mL) and filtered through celite. Fractional crystallization failed to separate the four-membered ring derivatives **1b** and **2b**. ³¹P{¹H} NMR (101.256 MHz, [D₆]benzene, 298 K, 85% H₃PO₄ ext.): **1b**: $\delta = -151.5$ (s); **2b**: $\delta = 446.0$ (d, ¹J(P,P) = 449 Hz), –87.1 (d, ¹J(P,P) = 449 Hz). **3**: ³¹P{¹H} NMR (101.256 MHz, [D₆]benzene, 298 K, 85% H₃PO₄ ext., P_A = P2, P_B = P1, P_C = P3): $\delta = 831.8$ (P_A: s), 140.4 (P_B: s), 97.5 (P_C: s; ¹J(W,P) = 216 Hz); ¹H NMR [250.113 MHz, [D₆]benzene, 298 K, TMS): $\delta = 6.58$ –7.16 (18H, aromatic protons) 2.50 (12H, Me), 2.05 (12H, Me), 1.36 (9H, *t*Bu); EI-MS

(70 eV; 140 °C): m/z (%): 1256 (1) [M^+], 1014 (4) [$M^+ - 2\text{OAr}$], 772 (10) [$M^+ - 4\text{OAr}$].

5a, b: Complex **3** (50 mg, 0.04 mmol) was added to a solution of $[\text{M}(\text{CO})_5\text{thf}]$ (0.06 mmol; $M = \text{W}, \text{Cr}$) in THF (25 mL) and stirred for 24 h at room temperature. Then all of the volatiles were removed under high vacuum and the residue was recrystallized from toluene to give the dark red complexes **5a** (45 mg; 71%) and **5b** (35 mg; 60%), respectively. $^{31}\text{P}\{^1\text{H}\}$ NMR (101.256 MHz, $[\text{D}_6]\text{benzene}$, 298 K, 85% H_3PO_4 ext.): **5a** ($M = \text{W}$): $\delta = 650.5$ (s, $^1J(\text{W,P}) = 210$ Hz), 152.8 (s), 107.2, (s, $^1J(\text{W,P}) = 233$ Hz); **5b** ($M = \text{Cr}$): $\delta = 715.0$ (s, $^1J(\text{W,P}) = 197$ Hz), 153.7 (s), 108.0 (s, $^1J(\text{W,P}) = 233$ Hz).

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A Quadruply Stranded, Alkaline Earth Metal Containing Helical Catenate: A Charge-Neutral Heterotopic Homodinuclear [2]Catenane**

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Dedicated to Professor Dietmar Seyferth on the occasion of his 70th birthday

Organized supramolecular architectures such as catenates, helicates, and metallamacrocycles have long attracted considerable interest, both for their intrinsic aesthetic appeal and the challenge associated with their preparation. Successful synthetic strategies have often relied on templating effects of transition metal ions, π donor–acceptor interactions, or favorable hydrogen bonding.^[1]

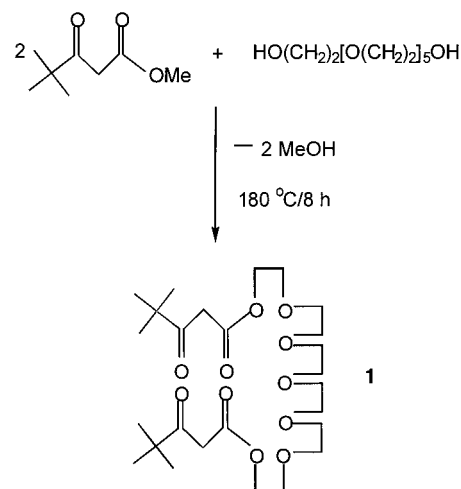
Employment of metals in the self-assembled formation of catenates is restricted, typically, to transition metal ions, whose preferred coordination geometry results in selective binding to specific sites of the ligand. In the formation of dinuclear catenates, segmental ligands often are employed, leading to heterodinuclear complexes in which each metal ion is attached to a portion of the ligand according to its stereochemical preferences.^[2, 3] Due to the somewhat rigid coordination requirements of transition metals, metal ions will bind selectively to only one functionality present within the ligand. Moreover, metallamacrocycles with two distinct binding environments have been prepared to bind both hard and soft metal ions in the same molecule.^[4, 5] In addition to being synthesized from neutral ligands by templated syntheses, such structures generate a high positive charge as their size increases.

As one component of an on-going research project related to coordination chemistry of alkaline earth metals, we examined ligands that would accommodate barium's high size-to-charge ratio. The reaction described here is completely self-templated, resulting in a charge-neutral species. Such helicates and catenanes are, if not unknown, very rare compositions.

The novel multidentate ligand 2,2,28,28-tetramethyl-3,5,25,27-tetraoxo-6,9,12,15,18,21,24-heptaaxanonacosane (**1**, see Scheme 1) consists of a hexaethylene glycol central segment with a β -ketoester functionality at each terminus. It possesses nine oxygen atoms with potential coordination capabilities (for steric reasons, the ester oxygen atoms are not

considered as binding sites), and, following deprotonation, forms a dianionic ligand. Its structure and concept resemble the bis(β -diketone)-substituted oligoethylene glycol prepared by Kokube and co-workers,^[4, 6] as well as the noncyclic crown ether arrangements of Vögtle et al.^[7] Prior to the crystal structure resolution of the title compound, it was presumed that **1** would form a mononuclear complex with barium, in analogy to the reported noncyclic crown ether–rubidium structure of 1,20-bis(8-quinolyloxy)-3,6,9,12,15,18-hexaoxaicosane · RbI.^[7] In this complex, the ligand wraps around the metal ion in a spiral fashion, giving rise to a mononuclear compound. The bis(β -diketone)oligoethylene glycol ligands of Kokube et al. form dinuclear metallamacrocycles, with a transition metal ion bound by the diketones to generate a “crown ether” pocket, which additionally encapsulates an alkali metal of the appropriate size. The crystal structure of the complex consisting of the dianion of 2,4,17,19-tetraoxo-6,9,12,15-tetraoxaicosane, copper(II). K⁺(picrate)^[6] shows a nearly planar arrangement of the diketone oxygen atoms around the Cu^{II} ion, as well as a K⁺ ion, which resides slightly off-center in the O₆ pseudo crown ether cavity formed by the oligoether section of the ligand.

The bis(ketoester) oligoether ligand **1** was prepared by a transesterification of hexaethylene glycol with two equivalents of methyl 4,4-dimethyl-3-oxopentanoate (Scheme 1). This procedure follows that of Kellogg et al.,^[8] who previously reported the preparation of bis(ketoesters) of shorter glycols.



Scheme 1. Synthetic route leading to the bis(ketoester) oligoether ligand **1**.

Complex **2** became accessible through treatment of a slurry of Ba(NH₂)₂ in toluene with one equivalent of **1**. The NMR spectrum of **2** shows a shift in the α -H resonance from $\delta = 3.54$ to $\delta = 4.67$, consistent with the deprotonation of the ketoester. Accordingly, the ethylene resonances have become broad multiplets, which indicates a loss of symmetry in the ligand as well as the presence of diastereotopic protons. X-ray quality single crystals of **2** were grown by a slow evaporation of a solution in CHCl₃/benzene. The molecular structure of **2** (Figure 1), determined by X-ray diffraction, exhibits several unique features. The two ligands bind through their terminal ketoester functionalities to one barium atom each, forming

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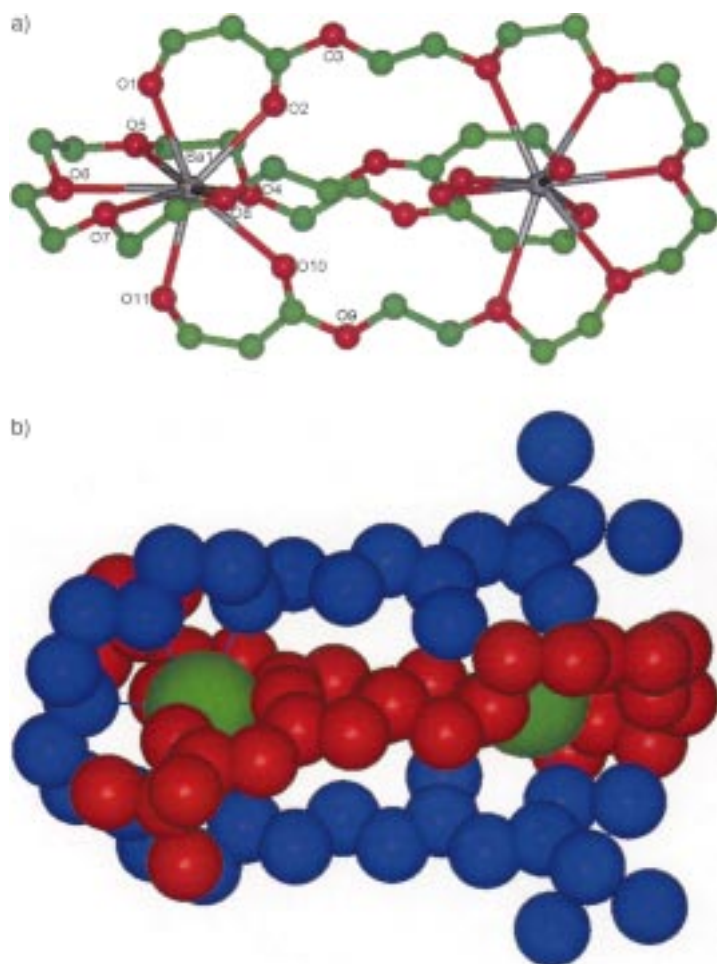


Figure 1. a) An ORTEP plot representation (gray: barium, green: carbon, red: oxygen) and b) a space-filling model of **2** (green: barium, red: one ligand, blue: the second ligand). All *t*Bu groups have been omitted for clarity. Selected interatomic distances [Å]: Ba1–O1 2.639(9), Ba1–O2 2.693(7), Ba1–O4 2.926(7), Ba1–O5 2.934(7), Ba1–O6 2.968(7), Ba1–O7 2.919(8), Ba1–O8 3.016(8), Ba1–O10 2.786(8), Ba1–O11 2.615(7).

two interlocked metallamacrocycles. The [2]catenane contains two nine-coordinate barium cores bound by four oxygen atoms stemming from two ketoester groups of one ligand, and additionally encapsulated by five oxygen atoms from the polyether chain of the second ligand. The complex displays idealized C_2 symmetry, with the C_2 rotational axis bisecting the Ba1–Ba1A vector. Average Ba–O_{keto} interatomic distances of 2.627 Å (O1, O11) are slightly shorter than the corresponding distances for the Ba–O_{ester} interactions (O2, O10: 2.740 Å). Both barium atoms reside in the center of the crown ether like pocket, interacting with five oxygen atoms of the polyether chain with Ba–O interatomic distances ranging from 2.919 to 3.016 Å. Furthermore, the catenane **2** exhibits a most unusual left-handed helical twist (25.9(4)°).

Compound **2** is a rare example of a quadruply stranded helicate;^[9] another example is [Co₅(μ₅-tpda)₄(NCS)₂],^[10] a linear, pentanuclear transition metal complex. Oligo(multidentate) ligands can complex with two or more identical metal ions, hence forming a variety of homonuclear architectures, such as double and triple helices. In addition, the employment of multidentate ligands possessing designed different binding

sites allows for the preparation of heteronuclear metal complexes. In general, however, it is difficult to prepare homodinuclear complexes from such ligands, as a consequence of the somewhat rigid coordination requirements of most metal ions. One example of a homodinuclear helicate displaying metal ions in different coordination environments is made up of four- and two-coordinate Cu^I ions.^[11] The lanthanide complex (HH)₂(HT)-[Ln₂(L)₃]⁶⁺ (HT = head to tail; HH = head to head; L = 2-{6-[1-(3,5-dimethoxybenzyl)-1H-benzimidazol-2-yl]-pyridin-2-yl}-1,1'-dimethyl-5,5'-methylene-2'-(5-methylpyridin-2-yl)bis[1H-benzimidazole]) constitutes, to date, the only reported example of a homonuclear heterotopic triple helicate.^[12] Increased coordinative flexibility of Ba²⁺ permits coordination to the pseudo crown ether portion of the designed catenand as well as to both of the anionic ketoester terminal moieties, making complex **2** a rare example of a heterotopic, homodinuclear catenane.

The first organometallic catenane, published in 1993 by Gruter et al.,^[13] consisted of two interlocking crown ether and diarylalkyl rings surrounding a magnesium ion. The NMR evidence suggested an equilibrium between the interlocking ring structure and a second, noncatenated structure, in which the crown ether is bound to the magnesium in a side-on fashion. However, no crystal structures were available for these compounds. Since the first magnesium complex in 1993, no other alkaline earth containing catenates have been reported. To the best of our knowledge, complex **2**, therefore, also represents the first crystallographically characterized catenane containing a Group 2 element.

Experimental Section

1: Methyl 4,4-dimethyl-3-oxopentanoate (10 g, 63 mmol) and hexaethylene glycol (8.92 g, 31.0 mmol) were heated at 180 °C for 8 h under stirring. Subsequently, the formed methanol was removed by distillation and the residue was treated with benzene. The organic solution then was washed with brine, saturated Na₂CO₃, and water and dried over MgSO₄. Following solvent evaporation, **1** was isolated as a colorless and viscous liquid. Yield 11.16 g (67 %). Elemental analysis calcd for C₂₆H₄₆O₁₁: C 58.41, H 8.67; found: C 58.12, H 8.67; ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 4.24 (t, 8H; CH₂), 3.67 (t, 8H; CH₂), 3.60 (s, 16H; CH₂), 3.59 (s, 16H; CH₂), 3.54 (s, 4H; CH), 1.12 (s, 36H; CH₃); ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 208.21, 168.01, 70.67, 69.03, 64.33, 63.19, 44.78, 43.87, 27.46, 26.11.

2: Barium metal (0.365 g, 2.65 mmol) in 50 mL of toluene was dissolved in liquid ammonia at –78 °C. After excess NH₃ was allowed to evaporate at ambient temperature, the slurry in toluene was refluxed and then degassed under argon. Compound **1** (1.42 g, 2.65 mmol) was added through a syringe to the off-white slurry, which initially turned white, and then milky gray. The release of a small amount of heat and gas was registered. The mixture was stirred overnight at ambient temperature and then filtered to separate a white powder from a pale yellow solution. Following refluxing in toluene, the formed colorless microcrystalline powder was filtered, washed with hexane, and dried under vacuum. X-ray quality crystals were formed as small thin needles by slow evaporation of a solution in CHCl₃/benzene (1/2) under a stream of argon. Yield 1.73 g (49 %). C,H analysis calcd for Ba₂O₂₂C₅₂H₈₈: C 42.16, H 5.66; found: C 43.2, H 5.2; ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 4.67 (s, 4H; CH), 4.21 (m, 16H; CH₂), 3.87–3.55 (m, 32H; CH₂), 1.07 (s, 36H; CH₃); ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 196.77, 170.81, 75.33, 70.98, 59.53, 40.32, 28.98.

Crystal data for **2** (Ba₂O₂₂C₅₂H₈₈): *M*_r = 1339.918, crystal dimensions 0.340 × 0.136 × 0.102 mm, orthorhombic, space group *P*2₁2₁2, *a* = 19.4469(3), *b* = 19.5347(2), *c* = 8.2529(2) Å; *V* = 3135.19(10) Å³, *Z* = 2, ρ_{calcd} = 1.419 g cm^{–3}, Siemens SMART CCD diffractometer, 1.48 < θ < 25.00°, MoKα radiation (λ = 0.71073 Å), ω scans, *T* = 173(2) K; of 16334

measured reflections, 6519 were independent and 5518 observed with $I > 2\sigma(I)$, $-25 < h < 24$, $-25 < k < 24$, $-6 < l < 10$; $R_1 = 0.0659$, $wR_2 = 0.1503$, $GOF = 1.258$ for 373 parameters, $\Delta\rho_{\max} = 2.335 \text{ e}\cdot\text{\AA}^{-3}$. 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, $\mu = 1.318 \text{ mm}^{-1}$, min./max. transmission 0.6627/0.8773. The coordinates of the hydrogen atoms were calculated in SHELXL using an appropriate riding model with varied thermal parameters. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-136903. 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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Copper Chalcogenolate Complexes as Precursors to Ternary Nanoclusters: Synthesis and Characterization of $[\text{Hg}_{15}\text{Cu}_{20}\text{S}_{25}(\text{nPr}_3\text{P})_{18}]^{**}$

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The chemistry and physics of binary semiconductor nanoclusters and colloids have been the focus of much attention due to the interesting electronic properties displayed by these nanometer sized “Q-particles”.^[1] The use of bis-silylated reagents $\text{E}(\text{SiMe}_3)_2$ ($\text{E} = \text{S}, \text{Se}, \text{Te}$) as a chalcogen source has led to the synthesis of near monodisperse XII–XVI particles^[2] and a wealth of structural chemistry in copper chalcogenide complexes including the nanocluster $[\text{Cu}_{146}\text{Se}_{73}(\text{PPh}_3)_{30}]$.^[3] It has also been shown that the monosilylated chalcogen reagents RESiMe_3 and ArESiMe_3 can yield mixed metal–chalcogenide–chalcogenolate complexes via subsequent chalcogen–carbon bond cleavage of the first formed metal–chalcogenolate cluster complexes.^[4]

The importance of ternary semiconductor solids^[5] prompts us to develop a general route into related nanometer sized ternary clusters.^[1a] Brennan and co-workers have recently described the formation of ternary lanthanide–chalcogenolates^[6] and Kanatzidis et al. have illustrated the utility of molecular copper–indium chalcogenolates for the generation of ternary solids.^[7] We are developing suitable reagents for ternary nanocluster synthesis by targeting the reactive metal–silylchalcogenolate complexes $[\text{L}_n\text{M}–\text{ESiMe}_3]$, which can be used as a source of “metallachalcogenolate” (metal chalcogenide, $\text{M}–\text{E}^-$) fragments for the synthesis of polynuclear complexes of the type $[\text{L}_n(\text{M}'\text{ME})_x]$.

Despite the propensity for chalcogenolate ligands to adopt bridging coordination modes, terminal coordination can be promoted by using bulky substituents about the chalcogen center^[8] or by restricting the number of vacant coordination sites about the metal.^[9] Herein we describe the synthesis of the tetrahedral copper–chalcogenolate complexes $[(\text{nPr}_3\text{P})_3\text{Cu}–\text{ESiMe}_3]$ (**1a**, $\text{E} = \text{S}$; **1b**, $\text{E} = \text{Se}$; **1c**, $\text{E} = \text{Te}$) and demonstrate their utility in ternary nanocluster synthesis with the formation of $[\text{Hg}_{15}\text{Cu}_{20}\text{S}_{25}(\text{nPr}_3\text{P})_{18}]$ **2** in high yield.

When CuOAc is treated with four equivalents of nPr_3P and one equivalent of $\text{E}(\text{SiMe}_3)_2$ at -40°C , complexes **1a–c** are

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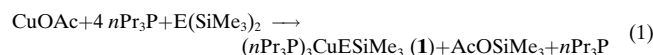
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isolated in good yields as viscous oils at room temperature [Eq. (1)]. The oils readily solidify as colorless crystals when cooled slowly to -5°C . Although relatively stable as neat liquids under inert atmosphere (**1c** is the least stable), the copper–chalcogenolate complexes rapidly undergo a series of condensation reactions to form copper–chalcogenide clusters if allowed to stand in solution.^[10]



The molecular structure of $[(n\text{Pr}_3\text{P})_3\text{Cu-SSiMe}_3]$ (**1a**, Figure 1)^[11] illustrates the tetrahedral coordination geometry of the copper(I) center and the pendant SiMe_3 moiety bonded to the sulfur atom [$\text{Cu-S} = 2.397(1)$, $\text{S-Si} = 2.078(1)$ Å; $\angle \text{Cu-S-Si} = 129.20(5)^{\circ}$].

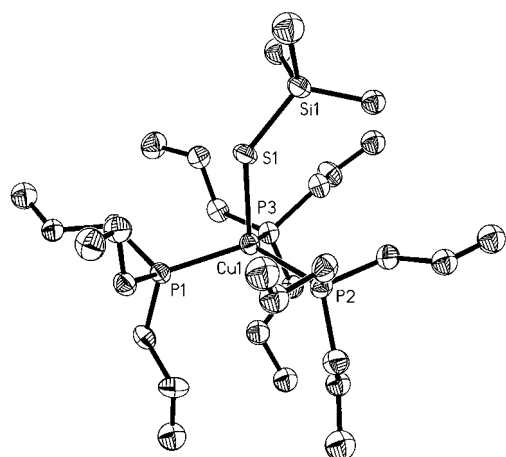
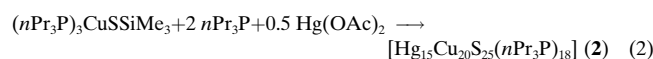


Figure 1. The molecular structure of $[(n\text{Pr}_3\text{P})_3\text{Cu-SSiMe}_3]$ (**1a**, hydrogen atoms omitted, thermal ellipsoids are drawn at the 40% probability level). Selected interatomic distances [Å] and angles $^{\circ}$: Cu-P 2.284(1)–2.3011(9), Cu1-S1 2.397(1), S1-Si1 2.078(1); P-Cu1-P 111.77(4)–119.87, P-Cu1-S1 100.06(3)–109.77(4), Si1-S1-Cu1 129.20(5). Each carbon atom around Si1 was disordered over two sites.

Due to the labile nature of the phosphine ligands bonded to the copper center, in conjunction with the expected reactivity of the S-SiMe_3 moiety,^[12] we reasoned that **1a** would serve as a good source of “metallathiolate” Cu-S^- when reacted with a second metal salt. Thus, when **1a** is treated with 0.5 equivalents of $[(n\text{Pr}_3\text{P})_2\text{Hg}(\text{OAc})_2]$ at -30°C , orange crystals of $[\text{Hg}_{15}\text{Cu}_{20}\text{S}_{25}(n\text{Pr}_3\text{P})_{18}]$ (**2**) form in good yields within a few hours [Eq. (2)].



An X-ray analysis^[11] provided full structural details (Figure 2) and illustrates the intimate mixing of the three core elements. The molecule resides on a crystallographic $\bar{3}$ site with the three-fold axis along the Hg2-S5 vector. There is some disorder about the central sulfide ion S5 where atoms Hg3 and Cu3 are each statistically distributed over six equivalent centers (see supplementary material). Twenty-five sulfide (S^{2-}) ions bridge the Cu and Hg centers. There are no unusually short Cu-Hg contacts. The Hg atoms adopt either near linear (Hg1 ; $\angle \text{S1-Hg1-S2} = 177.0(3)^{\circ}$) or trigonal coordination

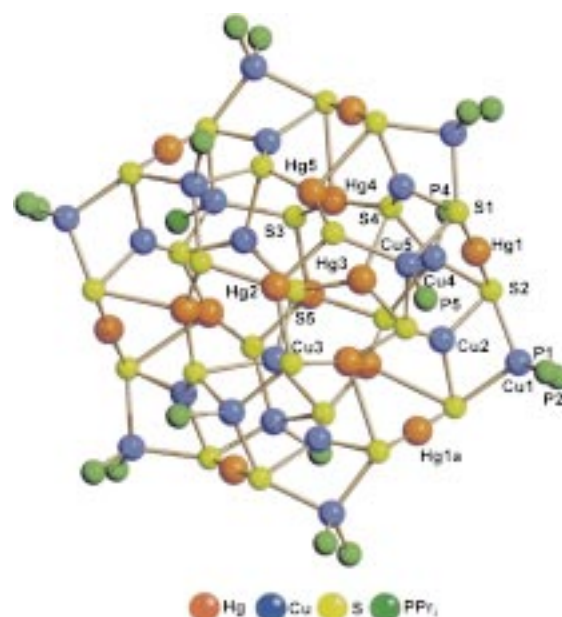


Figure 2. The molecular structure of $[\text{Hg}_{15}\text{Cu}_{20}\text{S}_{25}(n\text{Pr}_3\text{P})_{18}]$ (**2**, carbon atoms omitted for clarity). Selected bond lengths and angles are discussed in the text.

dination (Hg2-5 ; sum of the angles = $352.5-358.5^{\circ}$), although for atoms Hg3–5, the third Hg-S contact is markedly longer (2.73–2.98 Å) than the other two (2.22(1)–2.45(1) Å). The copper centers have coordination numbers three (Cu2-3) and four (Cu1 , 4–5). None of the fifteen Hg atoms is bonded to a phosphine ligand, whereas metal centers Cu1 and its symmetry equivalents are each bonded to two phosphorus centers and sites Cu4 and Cu5 also form single Cu-P bonds. Cu-Cu and Hg-Hg contacts are 2.54 and 3.35 Å, respectively, which is consistent with oxidation states +1 (Cu) and +2 (Hg) for the d^{10} metal centers. The 25 sulfur ligands bridge either three (S3, S5) or four (S1, S2, and S4) metal centers. The molecule contains a 0.7×1.2 nm CuHgS core that is enveloped in a tri- n -propylphosphine ligand shell.

Using this strategy we have now isolated several CuHgE ($\text{E} = \text{S}, \text{Se}, \text{Te}$) nanoclusters and these findings will be reported shortly.^[10] Interestingly, varying the ratio of **1a**: $\text{Hg}(\text{OAc})_2$: $n\text{Pr}_3\text{P}$ has yet to afford polynuclear HgCuS complexes other than **2**. The approach of using a silylated “CuE” precursor has allowed the intimate mixing of the three core elements at low temperatures, with a general distribution of the metal centers within the (nonbonded) S_{25} polyhedron. The strategy outlined above for the synthesis of phosphane-stabilized copper-mercury sulfide clusters offers a powerful route into a variety of ternary clusters and colloids.

Experimental Section

Synthesis of 1: CuOAc (1.84 g, 14.5 mmol) was dissolved in ether (25 mL) with four equivalents of $n\text{Pr}_3\text{P}$ to give a colorless solution. The solution was cooled to -40°C and one equivalent of $\text{E}(\text{SiMe}_3)_2$ was added to give a clear pale yellow solution after stirring. The reaction mixture was allowed to warm up slowly to -5°C , with stirring. After three hours at this temperature, the solvent was removed in vacuo to leave a white solid. The solid was redissolved in cold ether (5 mL) and cooled to -30°C . Colorless crystals of **1** formed within a few hours in high yield. Crystals of **1** are low melting solids ($\approx 5^{\circ}\text{C}$). Whilst **1a** and **1b** are stable at room temperature, **1c** decomposes rapidly to yield elemental tellurium. X-ray quality crystals

of **1a–c** were obtained in each case, although only the structure of **1a** is reported here. Satisfactory elemental analyses were obtained for **1a**, **1b**, and **2**. **1a**: Yield > 90%; $^{31}\text{P}\{^1\text{H}\}$ NMR (-50°C , $[\text{D}_8]\text{toluene}$) $-21.5(\text{s})$. **1b**: Yield 80%; $^{31}\text{P}\{^1\text{H}\}$ NMR (-50°C , $[\text{D}_8]\text{toluene}$) $-27.0(\text{s})$. **1c**: Yield > 90%; $^{31}\text{P}\{^1\text{H}\}$ NMR (-50°C , $[\text{D}_8]\text{toluene}$) $-29.9(\text{br.s})$. Synthesis of **2**: Mercuric acetate (0.63 g, 1.98 mmol) was dissolved with $n\text{Pr}_3\text{P}$ (0.64 mL, 4 mmol) in THF (15 mL) and cooled to -65°C . This clear solution was added to a freshly prepared solution of **1a** with excess $n\text{Pr}_3\text{P}$. Upon warming to -30°C , the solution became bright orange and, within several hours at this temperature, orange crystals (0.62 g, 60%) of **2** appeared.

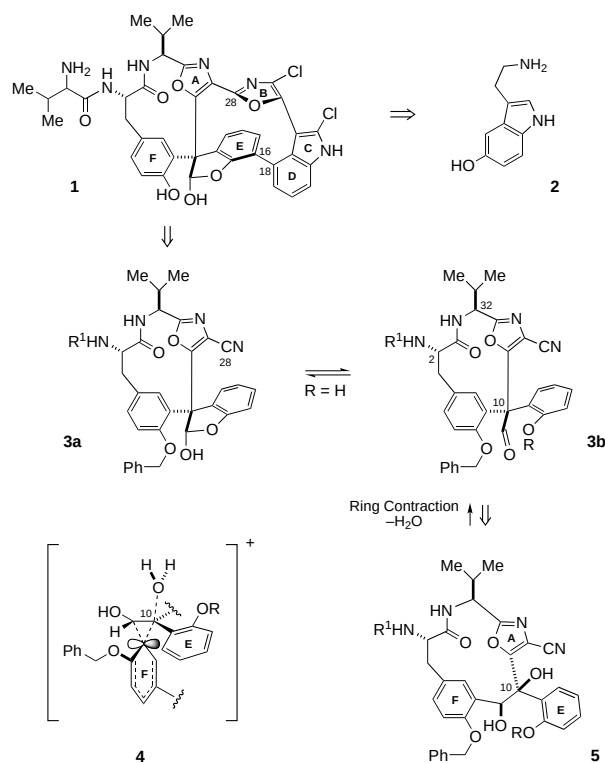
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- [11] X-ray structure analyses: Enraf-Nonius Kappa CCD diffractometer (MoK_α radiation). Crystal data for $\text{C}_{30}\text{H}_{27}\text{P}_3\text{CuS}_2\text{Si}$ **1a**: colorless polyhedron, $M_r = 649.48$, triclinic, space group $P\bar{1}$, $a = 10.9010(2)$, $b = 11.1233(2)$, $c = 17.3734(2)$ Å, $\alpha = 92.8890(11)$, $\beta = 97.3160(11)$, $\gamma = 105.1140(7)^\circ$, $V = 2009.48(6)$ Å³, at 200 K, $Z = 2$, $\rho_{\text{calcd}} = 1.073$ g cm⁻³, $\mu = 0.761$ mm⁻¹, $2\theta_{\text{max}} = 41.6^\circ$, 11 440 reflections collected, 4058 independent ($R_{\text{int}} = 0.037$). The structure was solved by direct methods and refined on F^2 using SHELXTL software. Data were corrected for Lorentz and polarization effects. No absorption corrections were performed. All atoms (with the exception of disordered carbon sites, see supplementary material) were refined anisotropically and hydrogen atoms were included as riding on their respective carbon atoms, 307 parameters). Final $R = 0.0357$ ($wR_2 = 0.0851$) and $\text{GoF} = 1.058$. Largest difference peak and hole = 0.34 and -0.25 e Å⁻³, respectively. For $\text{C}_{162}\text{H}_{378}\text{P}_{18}\text{Cu}_{20}\text{S}_{25}\text{Hg}_{15}$ **2**: orange polyhedron, $M_r = 7965.25$, rhombohedral, space group $R\bar{3}$, $a = 23.5814(9)$, $c = 43.376(2)$ Å, $V = 20889.1(15)$ Å³, at 200 K, $Z = 3$, $\rho_{\text{calcd}} = 1.900$ g cm⁻³, $\mu = 10.044$ mm⁻¹, $2\theta_{\text{max}} = 40.0^\circ$, 8646 reflections collected, 4339 independent ($R_{\text{int}} = 0.0744$). The structure was solved and refined as for **1a** except C atoms were refined isotropically, P–C and C–C distances were each refined with common values, and H atoms were not included. Final $R = 0.0709$ ($wR_2 = 0.2167$) and $\text{GoF} = 1.102$. Largest difference peak and hole = 1.11 and -1.21 e Å⁻³, respectively. Atoms Cu3 and Hg3 were statistically distributed over the six equivalent positions ($\bar{3}$) around S5. 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-135160 and -135161. 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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Stereocontrol in Pinacol Ring-Contraction of Cyclopeptidyl Glycols: The Diazonamide C_{10} Problem**

Xin Chen, Lothar Esser, and Patrick G. Harran*

Diazonamide A (**1**) is a unique product of marine invertebrate secondary metabolism reported to inhibit the growth of a human colorectal carcinoma ($\text{IC}_{50} < 15 \text{ ng mL}^{-1}$ against HCT-116) in vitro.^[1] Nearly a decade after this discovery, the biochemical events mediating this activity, as well as the effects of the molecule on other cell types and tissues, remain unknown. Mode of action studies have been limited by a shortage of natural material^[2] and the difficulties inherent in efforts to reconstitute the structure through synthesis.^[3] The diazonamide network of linked aromatic/heteroaromatic rings maintains elements of axial chirality (constrained to a single atropisomeric form) around a densely functionalized triarylacetaldehyde core **3b** (Scheme 1). Recently, we



Scheme 1. Diazonamide synthesis: primary disconnections and strategy for control of stereochemistry at C_{10} .

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proposed that atropisomer selection in this case could be made dependent on the configuration of a preassembled core; **3a** and/or **3b**.^[4] Stepwise annulation of a B/C/D segment onto compounds **3**, initiated with acylation of serotonin (**2**) and culminating in selective formation of the C₁₆–C₁₈ biaryl linkage, would generate a skeleton in which the relative rotational orientation of rings A–E paralleled that observed in **1**.

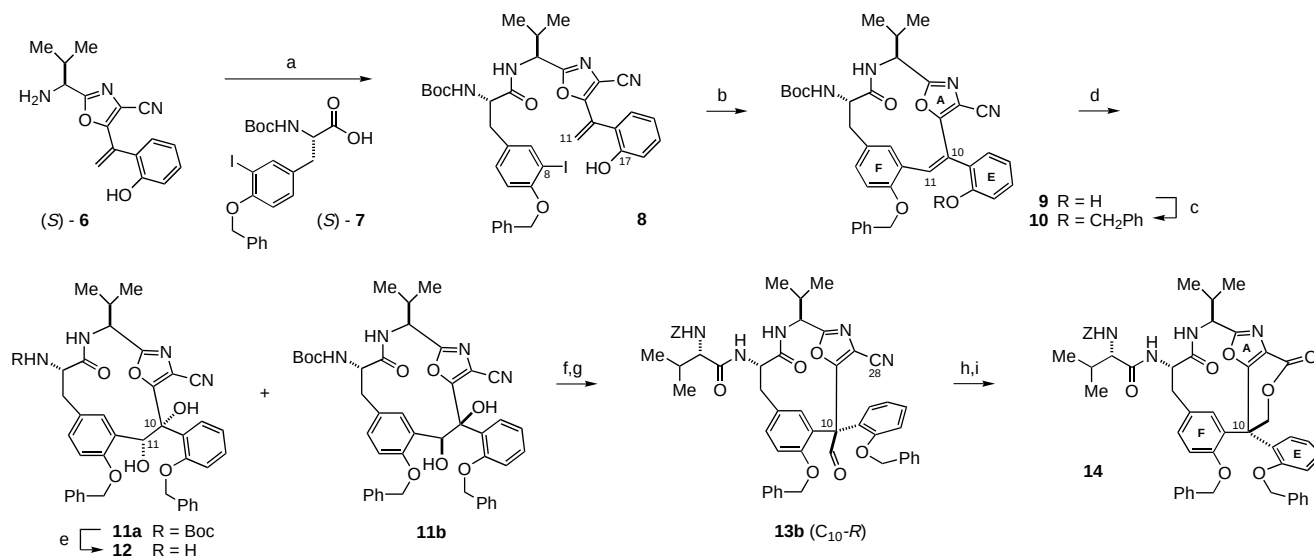
To begin along this pathway, lactam **9** (Scheme 2) was prepared^[4] with the intent that the triarylethylene component serve as a latent equivalent of acetaldehyde **3b**. Glycol **5**, an oxidized congener of **9**, was targeted as a direct precursor to **3b** in that acidolysis of **5** could generate aldehyde **3b** (Scheme 1) by a ring-contracting pinacol rearrangement. Provided kinetic control over the process was established, rearrangement through the geometry of a bridging phenonium ion^[5] **4** (Scheme 1) would communicate C₁₀ stereochemistry (with inversion) from **5** to **3b** independent of the relative configurations at C₂ and C₃₂. We outline here an implementation of this strategy as well as additional maneuvers in preparation for the final stages of a diazonamide total synthesis.

Lactam **9** is derived from two primary fragments with a catalyzed cyclization forming the C₈–C₁₁ bond (**8**→**9**, Scheme 2). The sequence remains as described in the literature^[4] except in the execution of the Heck endocyclization. The original procedure used 10 mol % of [Pd₂(dba)₃] and 2 equivalents of Ag₃PO₄ (refluxed in THF for 11 h) convert **8** into **9** in 66 % yield. This method was, however, limited as the reaction would often stall (with deposition of metallic palladium) and would invariably generate by-products at high conversion. Incorporation of 2-(di-*tert*-butylphosphanyl)biphenyl into the mixture (1:1 Pd atom/phosphane)^[6] generates a more robust catalyst (ostensibly not more active) that permits the same transformation to be executed in higher

yield with less metal. Notably, all other phosphane additives which were examined inhibit the reaction.

With **9** in hand, the E-ring phenol was etherified and attention turned towards dihydroxylating the C₁₀–C₁₁ olefin. Compound **10** resisted attempts at catalyzed oxidation, both for dihydroxylation and epoxidation. Fortunately, stoichiometric osmylation^[7] was effective and afforded stable osmium glycolates which could be purified by chromatography. Low-temperature treatment with H₂S^[8] subsequently liberated isomeric diols **11a** and **11b** (60 % combined yield) although assignment of the relative stereochemistries of **11a**, **b** proved difficult. Efforts to predict the facial bias of lactam **10** towards achiral reagents by calculation of conformational preference, or to directly assign configuration with spectroscopic data, were inconclusive. In fact, the solid-state structure of **9**^[9] readily superimposed onto the lowest energy conformers calculated for its benzylated derivative **10** (Figure 1a) and ¹H NMR did not detect, in either **9** or **10**, conformational equilibria within the range –20 °C to 60 °C. However, while the slightly cup-shaped topography of lactam **10** appeared to favor oxidation from the β-face (as drawn); its “*exo*”-orientated F-ring benzyloxy substituent seemed to shield along this same trajectory.

A crystal of **11a** suitable for X-ray diffraction finally allowed an unambiguous assignment.^[9] The result (Figure 1b) was instructive in several ways. Compound **11a** was the major diol isomer (*ds* = 5:1) and its C₁₀ hydroxyl group was α-oriented. If the original proposition that diols of this type would rearrange with inversion at the migrating terminus was correct, α hydroxyl group stereochemistry at C₁₀ in **11a** would translate into unnatural *S* stereochemistry at C₁₀ in pinacol products (such as C₁₀ *epi*-**3b**). Moreover, the C₈–C₁₁–C₁₀–O₉ torsional angle in **11a** is 172.2° and the plane of the F-ring is roughly perpendicular to the ring containing atoms C₁₁, C₁₀, and O₉—a favorable arrangement for ring-contraction via



Scheme 2. a) TBTU, DIPEA, DMF, RT (89 %); b) [Pd₂(dba)₃] (3 mol %), 2-(di-*tert*-butylphosphanyl)biphenyl (6 mol %), Ag₃PO₄, THF (0.025 M), 90 °C, 69 %, 82 % based on recovered **8**; c) DEAD, PPh₃, PhCH₂OH, THF, 97 %; d) 1. OsO₄, DMAP (2 equiv), *t*BuOH/H₂O, RT; 2. H₂S, THF, –50 °C, 57–60 %, **11a**:**11b** = 5:1; e) *p*TsOH (1 equiv), toluene, 95 °C, 1 h, 90 %; f) *p*TsOH (3.0 equiv), toluene, 95 °C, 2.2 h; g) *N*-Z-L-Val-OH, TBTU, DIPEA, DMF, 25 % from **11b**; h) NaBH₄, MeOH/THF, –10 °C, 82 %; i) (+)-10-camphorsulfonic acid (1 equiv), PhH, 45 °C, 24 h, 57 %. TBTU = *O*-Benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium tetrafluoroborate, DIPEA = *N,N*-diisopropylethylamine, RT = room temperature, dba = dibenzylideneacetone, DEAD = diethylazodicarboxylate, DMAP = 4-dimethylaminopyridine, Z = benzyloxycarbonyl, Val = valine, Boc = *tert*-butoxycarbonyl.

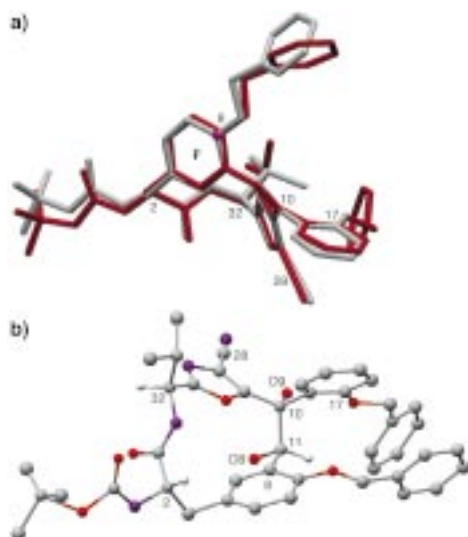
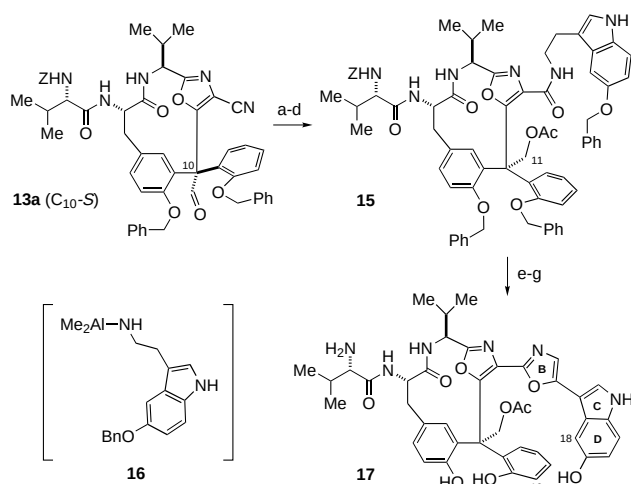


Figure 1. a) Rigid superimposition (RMSD root mean square deviation = 0.131 Å) of lactam ring atoms in the X-ray structure of **9**^[9] (blue) and the lowest energy conformer calculated for its benzylated derivative **10** (red). The diagram is oriented to highlight topography in the macrocycle. b) X-ray structure of **11a**^[9]. Hydrogen atoms are partially omitted for clarity. Calculations performed and images generated with MacroModel V6.0.

charge-delocalized species ent-**4** (Scheme 1) upon O₉ protonation.

Experiments to test this idea encountered an unexpected hydrolytic stability of both **11a** and **11b**. The C₂ *tert*-butyl carbamate of either molecule was cleanly decomposed (>90% yield of corresponding amino diols) using trifluoroacetic acid, HCl or, preferably, *p*TsOH (*p*-toluenesulfonic acid). More forcing conditions were required to initiate pinacol chemistry. For example, the product obtained from reacylation of the C₂ amine of **12** with *N*-Z-L-Val-OH (TBTU, DIPEA, DMF, 85% yield from **11a**) underwent rearrangement in the presence of 2.5 equivalents of anhydrous *p*TsOH at 95 °C. A 35% yield of aldehyde **13a** was afforded (Scheme 3) along with one major by-product (≈35%) which data indicates is a ketone resulting from either C₁₁ hydride migration or semi-pinacol rearrangement. Importantly, a single aldehyde isomer was observed in crude ¹H NMR spectra and the X-ray structure of crystalline **13a** showed that the stereochemistry at C₁₀, derived from **11a**, was indeed *S*.^[9] Stereochemical fidelity was also observed in the rearrangement of **11b**. In this case, Boc removal and pinacol rearrangement were carried out in one flask (3.0 equivalents *p*TsOH, toluene, 95 °C) and the resultant crude residue acylated with *N*-Z-L-Val-OH (TBTU, DIPEA, DMF) to furnish aldehyde **13b** in 25% overall yield (Scheme 2). One major, as yet unidentified, by-product also accompanied **13b** although there was no evidence for contamination with **13a**. When subjected to strong acid (1.5 equivalents *p*TsOH, toluene, 95 °C) **13a** and **13b** did not interconvert or produce more than a trace of putative ketone by-products.

These results corroborated the working model for stereochemical control in the glycol rearrangement and represent the first synthesis of an intact diazonamide triarylacetaldehyde. Recently, the conversion of **13b** into a form amenable



Scheme 3. a) NaBH₄, MeOH/THF, −10 °C, 85%; b) (+)-10-camphorsulfonic acid (1.4 equiv), PhH, 60 °C, 6.5 h, 81%; c) **16**, toluene/CH₂Cl₂, RT, 88%; d) Ac₂O, pyridine, CH₂Cl₂, 0 °C, 95%; e) DDQ, THF/H₂O, RT, 92%; f) PPh₃, (CCl₃)₂, Et₃N, CH₂Cl₂, RT, 94%; g) Pd/C (10%), HCO₂H, Et₃N, MeOH/H₂O, RT, 90%. DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

for completion of the diazonamide polycycle has been achieved. A primary concern was how to unmask and activate the C₂₈ carboxylate. This issue had been examined in earlier intermediates with no indication that hydrolysis of the nitrile would be viable. A reduction/oxidation sequence was likewise problematic. The solution came with an assisted hydrolysis using, as an internal nucleophile, the alcohol generated from **13b** by NaBH₄ reduction. Warming this material with (+)-10-camphorsulfonic acid (C₆H₆, 45 °C, 24 h) produced lactone **14**—presumably via imide hydrolysis during workup. The lability of this lactone ring allows incorporation of serotonin derivatives, by neutral amidation, and quick entry into late stages of diazonamide total synthesis. In the more abundant C₁₀ epimeric series, elaborations have been explored in some detail. For example, the corresponding lactone derived from **13a** combines with dimethylaluminum amide **16**^[10] to afford, after acylation, δ -acetoxy amide **15** (Scheme 3). Four-electron oxidation at the indole benzylic position, dehydration of the incipient β -keto amide^[3e], and exhaustive hydrogenolysis subsequently generates **17**—a structure appropriately functionalized to examine C₁₆–C₁₈ biaryl synthesis by oxidative phenolic coupling.

Experimental Section

13b: Anhydrous *p*TsOH (14 mg, 0.081 mmol) was added to a solution of **11b** (51.1 mg, 0.067 mmol) in dry toluene (4.5 mL) under N₂. The resultant suspension is heated to 95 °C. Two additional portions of *p*TsOH (13 mg each) are added after 60 and 90 min, respectively, while stirring is continued at 95 °C. The mixture is cooled to room temperature, diluted with EtOAc (10 mL), washed with sat. NaHCO₃ (2 × 5 mL), and dried over Na₂SO₄. Following concentration in vacuo, the crude residue (49.9 mg) is dissolved in dry DMF (0.6 mL) and cooled to 0 °C. *N*-Z-L-Val-OH (17 mg, 0.068 mmol), *i*Pr₂NEt (12 μ L, 0.069 mmol) and TBTU (22 mg, 0.069 mmol) are added successively. The incipient solution is brought to RT over 40 min, diluted with EtOAc (5 mL), washed with water and brine, dried over Na₂SO₄, and concentrated in vacuo. Purification by flash chromatography (EtOAc/benzene 3/7) affords **13b** (14.6 mg, 25%) as a white film; *R*_f = 0.27 (EtOAc/benzene, 3:7); [α]_D²⁵ = −36.0° (*c* = 1.0, CHCl₃); IR (film): $\tilde{\nu}$ = 3290,

2962, 2246, 1708, 1647, 1537, 1500, 1249, 1024, 753 cm⁻¹; ¹H NMR (400 MHz, CDCl₃, 60 °C): δ = 9.86 (s, 1H), 7.45 (td, *J* = 7.6, 1.6 Hz, 1H), 7.33 (d, *J* = 4.4 Hz, 4H), 7.29 (m, 1H), 7.22 (m, 6H), 7.15 (m, 4H), 7.04 (m, 2H), 6.97 (t, *J* = 7.4 Hz, 1H), 6.87 (d, *J* = 8.4 Hz, 2H), 6.44 (d, *J* = 8.0 Hz, 1H), 6.11 (d, *J* = 7.2 Hz, 1H), 5.80 (s, 1H), 5.25 (d, *J* = 8.8 Hz, 1H), 5.21 (d, *J* = 12.0, 1H), 5.14 (d, *J* = 12.0 Hz, 1H), 5.08 (s, 2H), 4.93 (s, 2H), 4.64 (t, *J* = 7.6 Hz, 1H), 4.25 (td, *J* = 10.0, 2.4 Hz, 1H), 3.98 (t, *J* = 8.0 Hz, 1H), 3.13 (t, *J* = 12.4 Hz, 1H), 2.61 (d, *J* = 11.2 Hz, 1H), 2.08 (m, 2H), 1.04 (d, *J* = 6.8 Hz, 3H), 0.94 (m, 9H); ¹³C NMR (75 MHz, CDCl₃): δ = 195.8, 172.1, 171.2, 164.6, 158.7, 157.2, 156.5, 154.3, 136.4, 136.0, 135.9, 132.5, 131.5, 130.8, 130.4, 129.3, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9, 127.4, 127.2, 122.5, 121.7, 113.6, 113.4, 112.9, 111.7, 71.0, 67.3, 62.4, 60.3, 56.3, 55.3, 53.7, 51.0, 37.9, 31.7, 30.0, 19.6, 19.3, 18.1; MS (Positive electrospray) for C₃₂H₃₂N₅O₈ [M+H]⁺: calcd: 874.4, found: 874.4; HRMS (FAB) for C₃₂H₃₂N₅O₈ [M+H]⁺: calcd: 874.3815, found: 874.3797.

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Electrochemical Transduction of Liposome-Amplified DNA Sensing**

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*Dedicated to Professor Heinz Dürr
on the occasion of his 65th birthday*

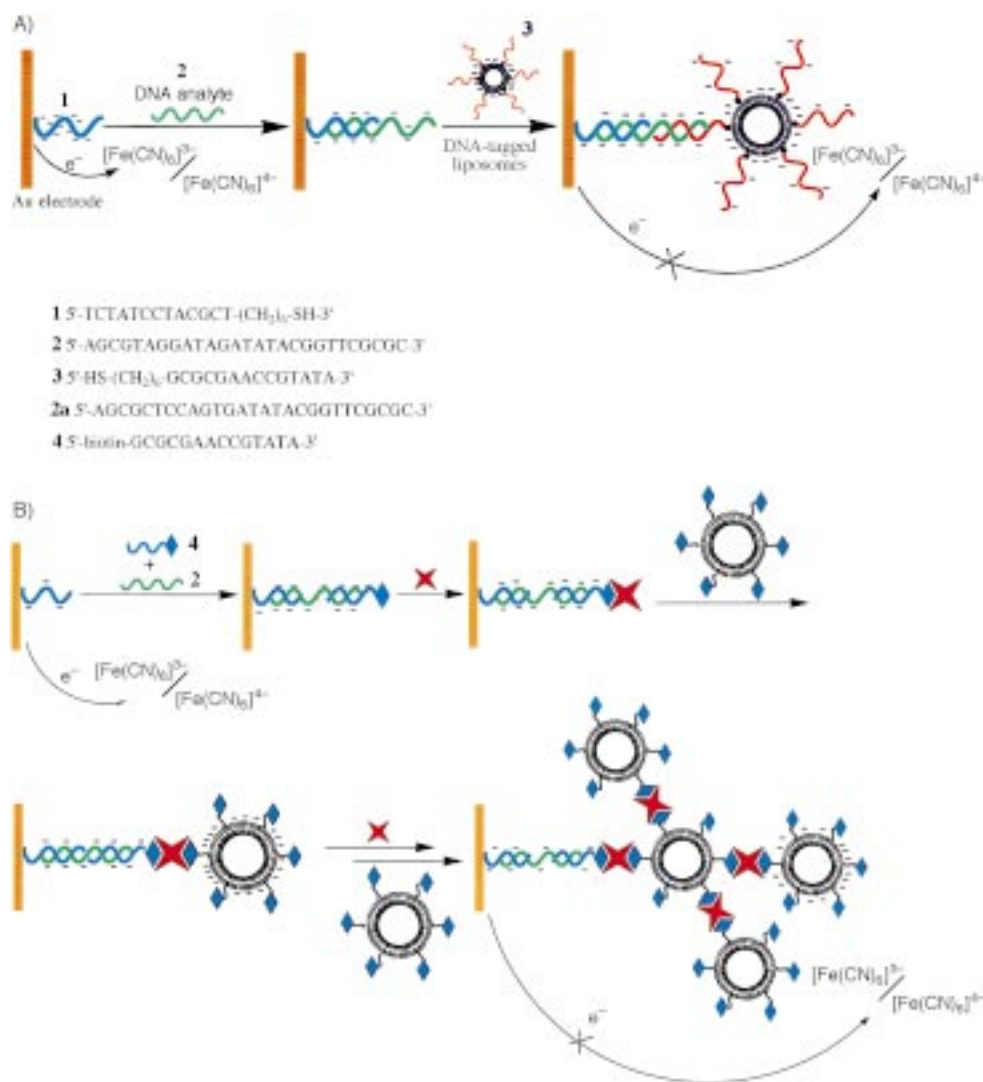
The development of DNA sensors has recently attracted substantial attention in connection with research efforts directed at gene analysis, the detection of genetic disorders, tissue matching, and forensic applications.^[1, 2] Optical detection of DNA was accomplished by the application of fluorescence-labeled oligonucleotides,^[3] or by the use of surface plasmon resonance (SPR) spectroscopy.^[4] Electronic transduction of oligonucleotide–DNA recognition events, and specifically the quantitative assay of DNA, are major challenges in DNA-based bioelectronics.^[5] Electrochemical DNA sensors based on the amperometric transduction of the formation of double-stranded oligonucleotide–DNA assemblies have been reported.^[6] Also, electrostatic attraction or intercalation of transition metal complexes^[7] or dyes^[8] was used for the voltammetric probing of the production of double-stranded oligonucleotide assemblies. Microgravimetric quartz crystal microbalance (QCM) analyses were also applied to sense the formation of double-stranded oligonucleotide–DNA complexes on surfaces.^[9]

Two fundamental issues that need to be addressed for the development of DNA sensors relate to the specificity and sensitivity of the sensing devices. We have reported on a general method to amplify biorecognition and sensing events by the biocatalyzed precipitation of an insoluble product on the electrode support.^[10–12] Enzyme electrodes,^[10] immunosensors,^[11] and specifically DNA sensors^[12] were developed by this amplification route. We have also described specific DNA sensing by the application of a three-component double-stranded sensing assembly consisting of a primer oligonucleotide that is complementary to the mutation domain, the analyte DNA, and a labeled oligonucleotide for assaying and amplifying of the recognition event.^[12] Here we report on a novel method for the amplification of oligonucleotide–DNA biorecognition events using functionalized liposomes.^[13] The DNA sensing events are transduced electrochemically, using Faradaic impedance spectroscopy. Electronic DNA sensors of unprecedented specificity and sensitivity have been organized, and they exhibit a 10⁵- to 10⁶-fold enhancement of sensitivity over previous electrochemical DNA sensors.^[6, 14]

One DNA sensing configuration is outlined in Scheme 1 A. The sulfanylhhexyl oligonucleotide **1** was assembled as a monolayer on an Au electrode. The surface coverage (1.1 × 10⁻¹¹ mol cm⁻²) was determined by the electrochemical meth-

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Scheme 1. A) Amplified electrochemical sensing of an analyte DNA using oligonucleotide-functionalized liposomes and Faradaic impedance spectroscopy as a means of transduction. Oligonucleotides **1**, **2**, and **3** are represented by blue, green, and red wavy lines, respectively. B) Electrochemical sensing of an analyte DNA using a biotinylated oligonucleotide, avidin, liposome labeled with biotin as an amplification conjugate and Faradaic impedance spectroscopy as a means of transduction. Green wavy lines: oligonucleotide **2**, blue wavy lines: oligonucleotide **4**, blue diamonds: biotin, red stars: avidin.

od of Tarlov et al.,^[15] and comparable results were obtained by QCM analyses. The resulting monolayer-functionalized electrode was allowed to interact with the analyte DNA **2** (15 min hybridization, 25 °C) to yield the double-stranded assembly on the electrode surface. The resulting electrode interface was then treated with the liposome labeled with oligonucleotide **3** (15 min, 25 °C). Oligonucleotide **3** is complementary to the residual base sequence of the analyte **2**. Thus, a liposome-linked, three-component, double-stranded assembly—consisting of the primer **1**, the analyte **2**, and the liposome tagged with **3**—is generated on the electrode support. The **3**-labeled liposomes are negatively charged in order to eliminate nonspecific adsorption of the liposomes onto the sensing interface. The oligonucleotide-functionalized liposome was prepared by assembling liposomes composed of phosphatidic acid, phosphatidyl choline, maleimide-phosphatidylethanolamine, and cholesterol (labeled with ³H-cholesterol, 45 Ci mol⁻¹) in a ratio of 79:20:1:0.1. The liposomes

were modified with **3** (4 °C, 20 h) and purified by chromatography (Sephadex G-75).

The surface coverage of the liposome with **3** (50–60 oligonucleotide units per liposome) was determined by allowing the resulting liposome suspension to react with Oligreen (Molecular Probes) and following the fluorescence intensity of the resulting liposome suspension at $\lambda_{\text{ex}} = 480$ nm. The size of the liposomes was determined by dynamic light scattering experiments to be (220 ± 20) nm. The liposomes associated with the electrode support represent “giant” negatively charged interfaces that electrostatically repel a negatively charged redox probe solubilized in the electrolyte solution. Therefore, the biorecognition event between the primer **1** and the analyte DNA **2** is amplified by the generation of a highly charged microenvironment that repels the electroactive probe, $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$, in solution. This electrostatic repulsion of the redox probe introduces a barrier for interfacial electron transfer, and results in an interfacial electron transfer resistance that can be assayed by Faradaic impedance spectroscopy.^[16]

Figure 1 A shows the impedance spectra (in the form of a Nyquist plot, Z_{im} vs. Z_{re}) of the oligonucleotide-functionalized electrode (curve a), after hybridization with the analyte DNA **2** (bulk concentration 5×10^{-6} M, curve b), and after interaction with the probing **3**-functionalized liposome (curve c). While a bare Au electrode exhibits an electron transfer resistance of 0.5 kΩ, the association of the primer **1** onto the conducting support increases the electron transfer resistance to 3 kΩ. This is attributed to the electrostatic repulsion of the redox label, $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$, which results in a barrier for the interfacial electron transfer. The formation of the double-stranded assembly with the analyte DNA increases the electron transfer resistance to $R_{\text{et}} = 4.5$ kΩ. This is consistent with the fact that the higher negative charge formed on the surface as a result of hybridization enhances the electrostatic repulsion of the electroactive species in solution. Binding of the **3**-modified liposome introduces a very high electron transfer resistance corresponding to 15 kΩ. This is attributed to the formation of a negatively charged

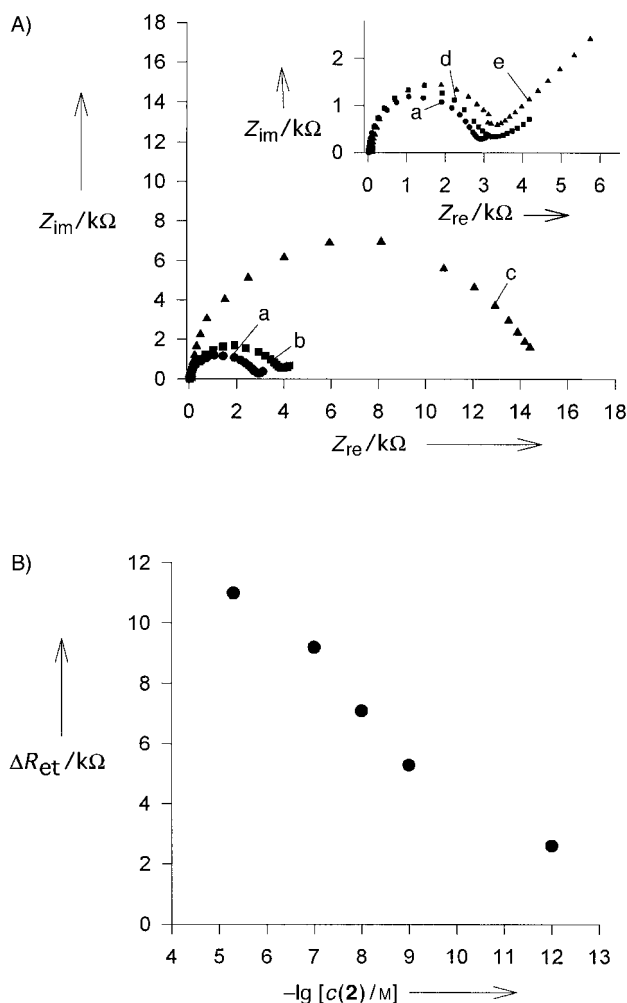


Figure 1. A) Faradaic impedance spectra (Nyquist plots, Z_{im} = imaginary impedance, Z_{re} = real impedance) of a) the 1-functionalized Au electrode, b) after interaction of the sensing electrode with **2** (5×10^{-6} M; 15 min, 25 °C) and c) after interaction with the 3-functionalized liposome (lipid concentration 0.2 mM, 15 min). Inset: Faradaic impedance spectra of a) the 1-modified electrode; d) after interaction with **2a** (5×10^{-6} M); e) after treatment with 3-functionalized liposomes (lipid concentration 0.2 mM). All measurements were performed using 0.2 cm² Au electrodes (roughness factor 1.2–1.5) in a 0.1 M phosphate buffer (pH 7.2) that contained $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ (5×10^{-3} M, 1:1); bias potential 0.180 V vs. SCE, frequency range 100 mHz–10 kHz, alternate voltage 5 mV. B) Changes in the electron transfer resistance of the 1-functionalized electrode upon treatment with the analyte DNA **2** in different concentrations and secondary amplification with the 3-functionalized liposomes. ΔR_{et} corresponds to the difference in the electron transfer resistance after amplification with the 3-functionalized liposome and the resistance of the 1-modified electrode.

microinterface upon association of the liposome to the double-stranded assembly. The resulting charged interface strongly repels the redox label from the electrode interface, resulting in a high electron transfer resistance. A control experiment where only **3** binds to the double-stranded assembly of the primer oligonucleotide and the analyte DNA introduces only a small increase in the electron transfer resistance ($R_{et} = 4.7$ k Ω), indicating that the negatively charged liposome indeed amplifies the electrostatic repulsion of the redox label. A further control experiment involved an attempt to sense the DNA with **2a**, which includes a six-base mutation relative to the analyte DNA **2**.

Curve d of Figure 1 A Inset shows the impedance spectrum of the functionalized electrode after treatment with the mutant **2a**, and curve e shows the impedance spectrum of the resulting electrode after treatment with the 3-functionalized liposome. The interfacial electron transfer resistances are almost unchanged, implying that the sensing interface is selective for the analyses of **2**. The results also indicate that no nonspecific association of **2a** or the 3-functionalized liposomes on the electrode takes place. This is attributed to the electrostatic repulsions existing between these components and the sensing interface. The extent of increase in the electron transfer resistances upon the binding of the analyte DNA, and the secondary association of the 3-modified liposome, is controlled by the bulk concentration of the analyte DNA (Figure 1B). The lower sensitivity limit for analyzing the analyte DNA is 1.2×10^{-12} M (or 6×10^{-16} mol in the analyte sample). The results shown in Figure 1 were fully reproducible in a series of 25 electrodes. For the lowest sensitivity limit the value $\Delta R_{et} = (2.5 \pm 0.2)$ k Ω (signal-to-noise (S/N) > 4) was found.

Scheme 1 B shows a second configuration for the amplified sensing of the analyte DNA using biotin-labeled, negatively charged liposomes. The 1-modified electrode was allowed to react with the analyte DNA **2** (15 min hybridization, 25 °C), which was pretreated with the biotinylated oligonucleotide **4** (complementary to one segment of the target DNA). This process results in a three-component double-stranded assembly on the electrode consisting of the primer **1**, the target DNA **2**, and the biotin-labeled oligonucleotide **4**. Association of avidin (8 min) and then the biotin-tagged liposome (8 min) results in the formation of a negatively charged interface that amplifies the primary DNA recognition event by the electrostatic repulsion of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ and the enhancement of the interfacial electron transfer resistance. This sensing configuration enables further amplification of the biorecognition event by the multiple reaction of the resulting array with avidin and then with the biotinylated liposomes to yield a dense array of the negatively charged liposomes. The biotin-functionalized liposomes are composed of phosphatidyl choline, phosphatidylethanolamine, cholesterol (partially labeled with ³H, 45 Ci mol⁻¹), and biotinylated phosphatidylethanolamine in a ratio of 80:20:0.1:0.1. The average coverage corresponds to 550 biotin units per liposome. The biotin-labeled liposomes were purified by gel chromatography (DEAE Sephadex A-25). The size of the liposomes was determined by dynamic light scattering to be (180 ± 40) nm.

Figure 2 A shows the impedance spectra of the array in the different steps of modification. The 1-functionalized interface exhibits an electron transfer resistance of 3 k Ω (curve a). Upon the formation of the double-stranded assembly with the analyte DNA **2**, complexed to the biotinylated oligonucleotide **4**, the electron transfer resistance increases to 4.8 k Ω (curve b). Association of avidin to the interface further increases the electron transfer resistance to 7.6 k Ω as a result of the hydrophobic, insulating, features of the protein (curve c). Association of the biotin-labeled liposome to the surface substantially increases the electron transfer resistance ($R_{et} = 14.8$ k Ω , curve d). The enhanced electron transfer resistance is due to the electrostatic repulsion of the electro-

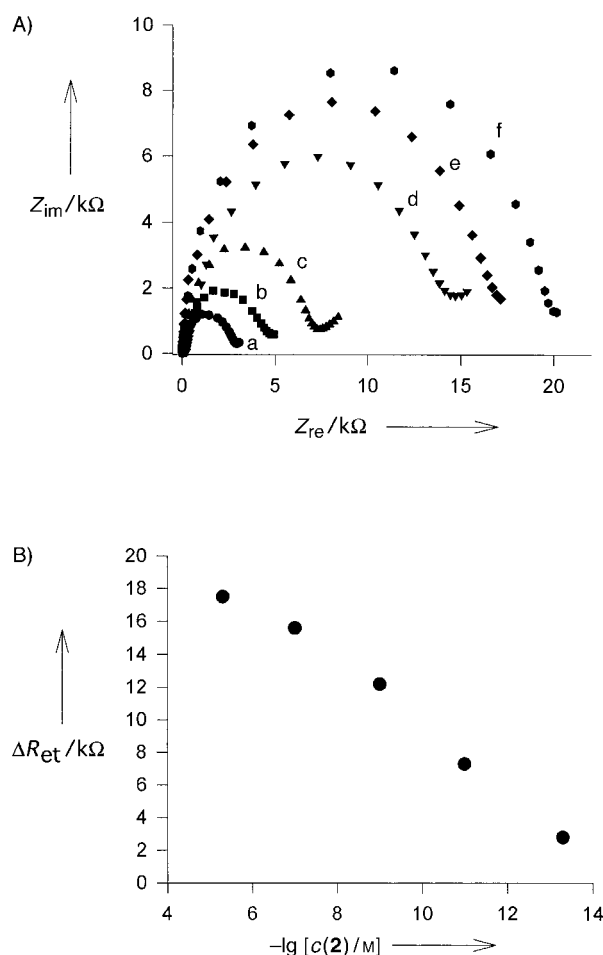


Figure 2. A) Faradaic impedance spectra (Nyquist plots) of a) the 1-functionalized Au electrode; b) after interaction of the sensing electrode with **2** (5×10^{-6} M), which was pretreated with **4** (1×10^{-5} M, interaction time 30 min, 25°C); c) after treatment of the resulting electrode with avidin ($2.5 \mu\text{g mL}^{-1}$); d) after interaction with the biotinylated liposomes (8 min, lipid concentration 0.25 mM); e) after treatment of the interface for a second time with avidin ($2.5 \mu\text{g mL}^{-1}$); f) after interaction of the interface for a second time with the biotinylated liposomes. All impedance experiments were performed under the conditions detailed in the legend to Figure 1 A. B) Calibration curve corresponding to the changes in the electron transfer resistance of the sensing electrode upon interaction with the analyte DNA **2** in different concentrations and enhancement of the sensing processes by a double-step avidin/biotinylated liposome amplification path. ΔR_{et} corresponds to the difference in the electron transfer resistance after a double-step avidin/biotinylated liposome amplification and the electron transfer resistance of the 1-functionalized electrode.

active species in solution by the charged membrane interface. The sensing of the target DNA can be further amplified by the application of a second step of association of the avidin/biotinylated liposomes, which enhances the electron transfer resistances to 17 k Ω and 20 k Ω , respectively (curves e and f).

In a control experiment, the sensing interface was allowed to interact with the noncomplementary DNA **2a** (see Scheme 1 A; 5×10^{-6} M), which was pretreated with **4**, and subsequently treated with avidin and the biotinylated liposome. A minute increase in the electron transfer resistance corresponding to $R_{et} = 3.4 \text{ k}\Omega$ was observed, which is attributed to the nonspecific adsorption of avidin to the sensing interface. The increase in the electron transfer resistance at the electrode upon binding of avidin and the biotin-labeled

liposome is controlled by the bulk concentration of the analyte DNA in the sample (Figure 2 B). With a double-step pathway with amplification of the avidin/biotin-labeled liposome, analyte DNA concentrations as low as 5×10^{-14} M (or 2.5×10^{-17} mol in the analyte sample) can be determined. Although the results shown in Figure 2 represent the data for a single set of five electrodes, the data are fully reproducible, and for the lowest detectable concentration the value $\Delta R_{et} = (2.5 \pm 0.5) \text{ k}\Omega$ ($S/N > 3$) was observed for a series of 30 electrodes. The amplified transduction of DNA analyses by the functionalized liposomes, and with use of the two configurations, exhibits excellent reproducibility.

In conclusion, we have demonstrated novel methods to amplify oligonucleotide–DNA recognition events on electrodes using negatively charged labeled liposomes. The highly sensitive sensing processes were transduced by Faradaic impedance measurements, and the sensing interfaces revealed selectivity in the analysis of the target DNA. The possibility of organizing microelectrode arrays on transducers paves the way to develop electrochemical DNA chips for the multi-component DNA analyses.^[17]

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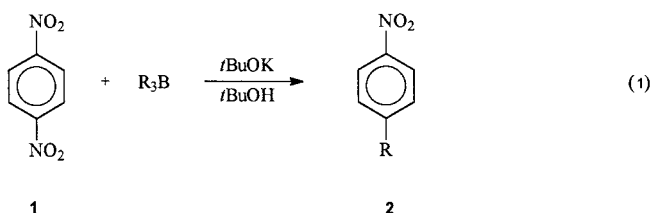
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Alkylation of Nitroaromatics with Trialkylborane**

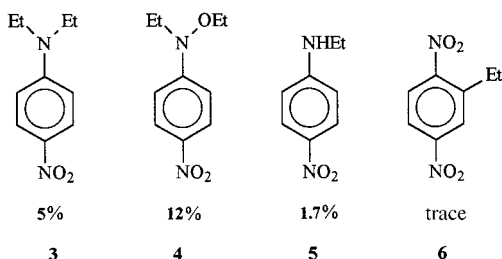
Anna Shifman, Namasivayam Palani, and Shmaryahu Hoz*

Aromatic substitution is one of the most thoroughly studied reactions.^[1] Yet in spite of the wealth of mechanistic knowledge and numerous synthetic applications, this paper reports a facile and hitherto unknown alkylation procedure for nitroaromatics.

When *p*-dinitrobenzene (**1**) is treated with trialkylborane in the presence of potassium *tert*-butoxide in *tert*-butyl alcohol at room temperature for five minutes, it furnishes *p*-alkylnitrobenzene (**2**, Equation 1) in high yield. Equivalent amounts of



the three components—**1**, trialkylborane, and base—were found to be essential for the completion of the reaction. When the amount of trialkylborane or base is reduced below this ratio, the substrate is consumed in an amount equivalent to the reagent present in the minimum concentration. Since an excessive amount of base results in the formation of *p*-*tert*-butoxynitrobenzene, a small excess (approximately 10%) of the borane reagent was usually employed. When the reaction was performed with triethylborane, by-products **3–6** were isolated in addition to 70% of **2** (R = Et, Scheme 1).



Scheme 1. By-products obtained in the reaction of *p*-dinitrobenzene with triethylborane.

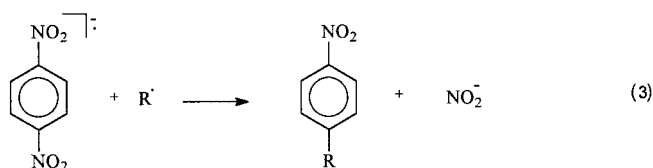
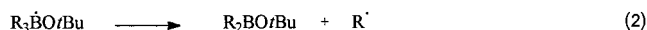
The reaction can also take place in THF. However, the yields are slightly lower with the major difference being the presence of approximately 15% of *p*-*tert*-butoxynitrobenzene amongst the products. Using two equivalents of triethylborane increased the yield to 85% and no *p*-*tert*-butoxynitro-

benzene was formed. Temperature sensitivity was also observed. When the reaction was conducted at temperatures below 25 °C the rate decreased drastically. At 0 °C, for example, the reaction does not progress, to a significant extent, even after 2 hours.

Using tributylborane, the yield of *p*-butylnitrobenzene was also in the range of 70–80% and no isomerization on the butyl chain was observed. However, the reaction failed with triphenylborane. Even after 24 hours no *para* phenylation was observed. When the substrate was changed to *p*-nitrobenzaldehyde and the previous reaction conditions were employed in THF, only 7% of **2** (from departure of CHO) was observed after 12 hours.

Aluminum, the homologue of borane, was found to be much less reactive. Treatment with triethylaluminum, under identical reaction conditions gave only 19% (NMR yield) of *p*-ethylnitrobenzene after 12 h at room temperature although over 40% of **1** was consumed.

The reaction is unlikely to be a radical or a radical–anionic chain mechanism of a type similar to the *S*_{rn}1 reaction^[2] since it also proceeds smoothly in 2-propanol, a solvent which rapidly reacts with free radicals. In addition, no inhibition of the reaction by 5 mol% galvinoxyl is observed and the reaction is not catalyzed by the laboratory light. On the other hand, *p*-dinitrobenzene is known to be a powerful electron acceptor^[3] and trialkylboranes are known to yield very rapidly, with a rate constant of 10⁸ M^{−1} s^{−1}, alkyl radicals when reacted with oxy-radicals.^[4] Based on the nature of the reactants and the aforementioned results a tentative nonchain mechanism can be suggested where all the steps take place in a complex or in a solvent cage. In this mechanism, the alkyl radical formed (Equation 2) reacts with the radical anion of *p*-dinitrobenzene (Equation 3) initially generated by an electron transfer from an anionic species present in the reaction mixture.



Experimental Section

Typical procedure: Compound **1** (84 mg, 0.5 mmol) in 1 mL of dry *t*BuOH was injected into a flask which contained a solution of *t*BuOK (56 mg, 0.5 mmol) and trialkylborane (0.55 mmol) in *t*BuOH (5 mL) under argon. After 5 to 10 min at room temperature, the reaction was treated with dilute aqueous HCl (3%), extracted several times with CH₂Cl₂, and dried over anhydrous sodium sulfate. After purification by flash column chromatography, the yield of **2** was 70–80%.

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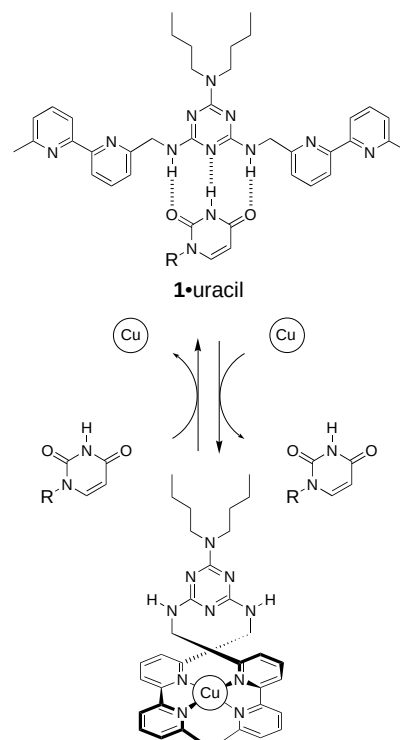
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Metal Ions as Allosteric Inhibitors in Hydrogen-Bonding Receptors**

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Although there are many examples of artificial systems that display positive allosterism,^[1] where the induced conformational change increases the receptor's binding efficacy, there are few reports of simple negative allosteric receptors,^[2] where the initial interaction with the cofactor results in a decrease in the receptor's binding affinity for its substrate. Of those that have been described, none, as far as we are aware, involve the destruction of well-ordered multipoint hydrogen-bond surfaces that are specific for biologically interesting guest species.

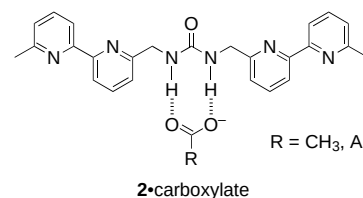
We report here how two novel receptors inhibit substrate binding in the presence of Cu^I . Receptor **1** is based on the triaminotriazine scaffold, which presents a donor–acceptor–donor hydrogen-bond surface well suited to act as a host for imide guests such as uracil (Scheme 1) and thymine.^[3] When **1** is exposed to the metal ion, the two bipyridine arms swing towards each other and form a metal–ligand coordination compound. It is the Lewis acid that acts as the allosteric inhibitor, as its complexation to the chelating ligands forces two of the triazine's exocyclic C–N bonds to rotate, distorting the hydrogen-bonding surface. When the metal ion is



Scheme 1. Exchange of a substituted uracil and Cu^I in receptor **1**. $\text{R} = \text{C}_4\text{H}_9$.

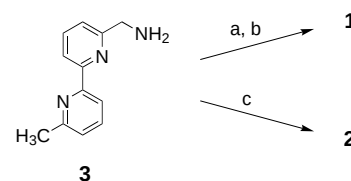
extracted from the coordination pocket, the original hydrogen-bonding surface is reconstructed. This reactivates the receptor.

In a similar fashion, urea **2** presents a donor–donor hydrogen-bond recognition site for carboxylate guests.^[4] It can exist in its active, natural form in the absence of Lewis acids, or it can be deactivated upon copper complexation.



Both receptors can be conveniently prepared from the readily available 6-aminomethyl-6'-methyl-2,2'-bipyridine (**3**)^[5] as outlined in Scheme 2. All new species were characterized by multinuclear NMR spectroscopy, IR spectroscopy, and mass spectrometry.

The binding efficacy of receptors **1** and **2** to their substrates was evaluated by analyzing the changes that occurred in the



Scheme 2. Reagents and conditions: a) cyanuric chloride, Et_3N , THF, 72%; b) Bu_2NH , THF, 52%; c) 1,1'-carbonyldiimidazole, THF, 75%.

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^1H NMR spectra upon titrating solutions of *N*-butyluracil with **1**, and solutions of **2** with tetrabutylammonium acetate or tetrabutylammonium *p*-methylbenzoate. The significant downfield shift ($\Delta\delta > 2$) observed for the imide NH of uracil (2 mM in $[\text{D}_8]\text{toluene}$) as it was treated with aliquots of a solution of **1** (30 mM in $[\text{D}_8]\text{toluene}$) is indicative of effective hydrogen bonding. A similar downfield shift was observed for the urea NH in **2** (5 mM in $[\text{D}_6]\text{DMSO}$) when it was titrated with tetrabutylammonium acetate or tetrabutylammonium *p*-methylbenzoate (100 mM in $[\text{D}_6]\text{DMSO}$). Data sets from both experiments correlate well with calculated curves using 1:1 binding models,^[6] and give association constants K_a of $600 \pm 10 \text{ M}^{-1}$ for **1**, $50 \pm 4 \text{ M}^{-1}$ for **2** with tetrabutylammonium acetate, and $55 \pm 2 \text{ M}^{-1}$ for **2** with tetrabutylammonium *p*-methylbenzoate. These values are in good agreement with those reported for similar host–guest complexes.^[3, 4]

X-ray quality single crystals of **1**·Cu were isolated by slowly diffusing diethyl ether into a solution of the coordination compound in CH_3CN . The crystal structure^[7] highlights the metal's role in the negative allosteric process (Figure 1). The

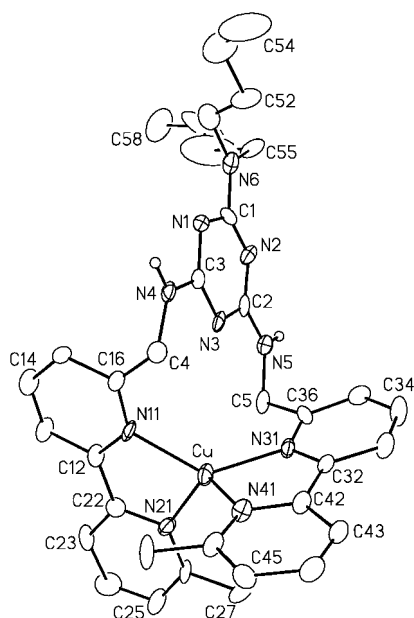


Figure 1. Molecular structure of **1**·Cu in the crystal. The PF_6^- counterion and all hydrogen atoms, except for those on N4 and N5, have been removed for clarity. The thermal ellipsoids are drawn at the 20% probability level.

C2–N5 and C3–N4 bonds must rotate by 180° from their original position in the active hydrogen-bonding receptor in order to form the copper complex. In the crystal structure, the torsional angles C4–N4–C3–N1 and C5–N5–C2–N2 are 175° and 179° , respectively. This reduces the recognition elements to two identical, but weakly bonding, donor–acceptor hydrogen-bond surfaces (N1, H(N4) and N2, H(N5)).

The ability of the metal to shut down the recognition process for both **1** and **2** was evaluated by repeating the titration experiments, but replacing the active receptors with preformed complexes **1**·Cu and **2**·Cu. The solvent was changed to $\text{CD}_3\text{CN}/\text{CDCl}_3$ in the case of **1**·Cu to ensure solubility of all components. The insignificant change ($\Delta\delta < 0.1$) in the chemical shift of the imide NH of uracil upon

the addition of a solution of **1**·Cu in $\text{CD}_3\text{CN}/\text{CDCl}_3$ (1/1) signifies the existence of only very weak hydrogen bonds (Figure 2).^[8] This small movement of the chemical shifts can be attributed to the association of uracil to the less effective

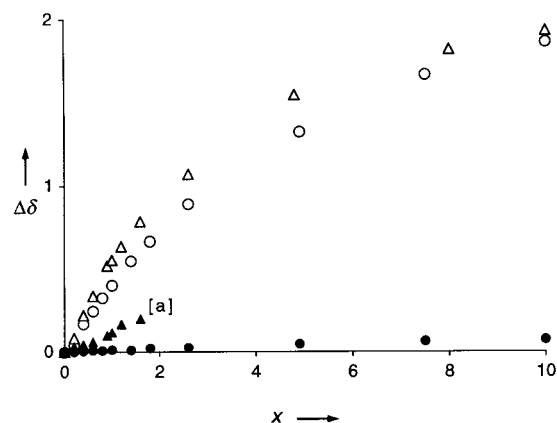


Figure 2. Titration of *N*-butyluracil with **1**·Cu (○) and **1**·Cu (●) in $\text{CD}_3\text{CN}/\text{CDCl}_3$ (1/1), and of **2** with tetrabutylammonium acetate (Δ) and **2**·Cu with tetrabutylammonium acetate (▲) in $[\text{D}_6]\text{DMSO}$, as followed by ^1H NMR spectroscopy. The observed changes in chemical shift ($\Delta\delta$) are plotted against equivalents of titrant (x). The symbol [a] indicates a peak that broadens into the baseline.

donor–acceptor sites in **1**·Cu. Despite the existence of two of these sites in the coordination compound, the reduced inherent binding affinity of recognition surfaces presenting only two hydrogen bonds couples with the presence of the *N*-butyl groups to prevent the facile approach of the substrate. These factors produce a significantly less effective receptor.

The metal-induced allosteric inhibition is also effective when the coordination compound **1**·Cu is prepared in situ, again diagnosed by the relative position of the chemical shift of the imide NH in the ^1H NMR spectra. The addition of one equivalent of **1** to a solution of *N*-butyluracil in $\text{CD}_3\text{CN}/\text{CDCl}_3$ (1/1) produces an immediate downfield shift of the signal for this hydrogen atom ($\Delta\delta = 0.4$). This signal returns to its original position when $[\text{Cu}(\text{CH}_3\text{CN})_4](\text{PF}_6)_2$ is added directly to the NMR sample. In the aromatic region of the spectrum, peaks that correspond to those for the isolated **1**·Cu complex in this solvent replace all original signals. Subsequent addition of an excess of the stronger coordinating ligand neocuproine (2,9-dimethyl-1,10-phenanthroline, dmph)^[1a, 9] results in the immediate disappearance of these signals and the regeneration of those corresponding to the hydrogen-bonded **1**·uracil complex.

A similar trend is observed for the NH protons of preformed **2**·Cu when this complex is titrated with a solution of tetrabutylammonium acetate in $[\text{D}_6]\text{DMSO}$ (Figure 2). In this case, signals for the urea NH could not be followed throughout the entire concentration range of the titration, as they quickly broadened to become indistinguishable from the baseline. The observed trend up to the addition of 1.6 equiv of substrate, however, proves successful metal-induced allosteric inhibition. Another complication was apparent when more than one equivalent of the carboxylate was added. At this molar ratio, signals corresponding to the hydrogen-bonded

2·acetate^[10] complex began to appear and became significantly more intense as the concentration of tetrabutylammonium acetate was increased. This observation illustrates a competition between the complexation of Cu^I to the bipyridine ligands and the binding of acetate for the hydrogen-bond surface in **2**.

The addition of 0.5 equiv of tetrabutylammonium acetate to a solution of **2** in [D₆]DMSO produces a small but significant downfield shift for the urea NH proton ($\Delta\delta = 0.2$), indicative of hydrogen bonding. When 1 molar equiv of [Cu(CH₃CN)₄](PF₆) is added directly to the NMR sample, **2**·Cu is immediately generated at the expense of **2**·acetate, and the signal for the urea NH returns to the original position for **2**. Unlike in the case of **1**, the NH signal does not shift significantly downfield when the sample is treated with an excess of neocuproine. This can be attributed to the preference of the acetate ion to act as a counterion for the harder copper cation and not the softer tetrabutylammonium cation. This favorable ion pairing effectively prevents the acetate from acting as a guest for receptor **2**. This phenomenon was also observed for samples of the noncoordinating *N,N'*-dimethylurea when they were treated under identical conditions.

Experimental Section

1: Receptor **1** was prepared in two steps from **3**, cyanuric chloride, and di-*n*-butylamine following an adapted procedure of Whitesides et al.^[11] (37% overall yield): m.p. 88–90 °C (decomp.); ¹H NMR (300 MHz, CDCl₃): $\delta = 8.23$ (t, $J = 7.8$ Hz, 4H), 7.62–7.73 (m, 4H), 7.26 (d, $J = 7.8$ Hz, 2H), 7.11 (d, $J = 7.5$ Hz, 2H), 5.99 (brs, 2H), 4.74 (d, $J = 5.7$ Hz, 4H), 3.42 (t, $J = 7.5$ Hz, 4H), 2.60 (s, 6H), 1.47 (brs, 4H), 1.23 (brs, 4H), 0.83 (brs, 6H); ¹³C NMR (100.5 MHz, CDCl₃): $\delta = 157.9, 155.8, 155.5, 137.4, 137.1, 124.3, 123.3, 121.2, 119.4, 118.4, 46.8, 46.2, 30.1, 24.7, 20.2, 14.0$ (16 of 18 carbon atoms); MS-EI: m/z : 602.36 [M^+].

2: A solution of **3** (0.12 g, 0.6 mmol) in dry THF (40 mL) was added dropwise to a solution of 1,1'-carbonyldiimidazole (0.049 g, 0.3 mmol) under argon and stirred overnight. Evaporation and trituration of the residue in ethyl acetate and then in water afforded **2** as a white solid (95 mg, 75%): m.p. 240–242 °C (decomp.); ¹H NMR (300 MHz, [D₆]DMSO): $\delta = 8.23$ (t, $J = 7.2$ Hz, 4H), 7.85 (t, $J = 7.8$ Hz, 2H), 7.79 (t, $J = 7.8$ Hz, 2H), 7.33 (d, $J = 7.5$ Hz, 2H), 7.28 (d, $J = 7.5$ Hz, 2H), 6.89 (t, $J = 3.0$ Hz, 2H), 4.43 (d, $J = 6.0$ Hz, 4H), 2.60 (s, 6H); ¹³C NMR (100.5 MHz, [D₆]DMSO): $\delta = 189.2, 159.3, 158.2, 157.4, 154.5, 137.6, 137.3, 123.4, 121.0, 118.4, 117.6, 45.1, 24.2$; MS (ES, positive ion): m/z : 424.19 [M^+].

1·Cu and **2**·Cu: The Cu^I complexes were prepared by stirring a mixture of **1** or **2** (1.03 equiv), [Cu(CH₃CN)₄](PF₆) (1 equiv), and CH₃CN for 1 h under argon. Filtration and evaporation afforded the copper complexes as red solids in quantitative yield.

1·Cu: ¹H NMR (300 MHz, CD₃CN): $\delta = 8.270$ (d, $J = 7.8$ Hz, 2H), 8.233 (d, $J = 8.1$ Hz, 2H), 8.057 (t, $J = 8.1$ Hz, 2H), 8.025 (t, $J = 7.8$ Hz, 2H), 7.619 (d, $J = 7.8$ Hz, 2H), 7.446 (d, $J = 7.5$ Hz, 2H), 6.127 (brs, 2H), 4.657 (d, $J = 5.7$, 1H), 4.607 (d, $J = 5.7$ Hz, 1H), 4.090 (d, $J = 6.3$ Hz, 1H), 4.040 (d, $J = 6.3$ Hz, 1H), 3.511–3.415 (m, 2H), 3.269–3.171 (m, 2H), 2.104 (s, 6H), 1.476–1.377 (m, 4H), 1.253–1.154 (m, 4H), 0.878 9t, $J = 7.2$ Hz, 6H); ¹³C NMR (100.5 MHz, CD₃CN): δ (17 of 18 carbon atoms) = 167.0, 162.0, 158.5, 152.6, 151.9, 139.5, 139.3, 126.8, 125.4, 121.5, 120.4, 49.6, 46.6, 30.7, 24.9, 20.8, 14.2; UV/Vis (CH₃CN): $\lambda_{\max}(\epsilon)$: 455 nm (3900); MS (ES, positive ion): m/z : 665.29 [M^+].

2·Cu: ¹H NMR (300 MHz, CD₃CN): $\delta = 8.278$ (d, $J = 7.8$ Hz, 2H), 8.220 (d, $J = 8.1$ Hz, 2H), 8.110 (brs, 2H), 7.997 (t, $J = 7.8$ Hz, 2H), 7.677 (brs, 2H), 7.448 (d, $J = 7.8$ Hz, 2H), 6.308 (brs, 2H), 4.318 (brs, 2H), 3.893 (brs, 2H), 2.077 (s, 6H); ¹³C NMR (100.5 MHz, CD₃CN): $\delta = 159.9, 158.9, 158.6, 152.7,$

152.3, 140.2, 139.5, 127.0, 124.8, 121.9, 120.7, 47.4, 25.2; UV/Vis (CH₃CN): $\lambda_{\max}(\epsilon)$: 448 nm (3900); MS (ES, positive ion): m/z : 487.13 [M^+].

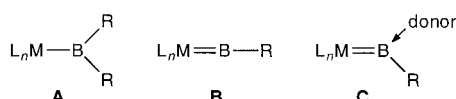
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- [6] The data were analyzed by nonlinear regression using Craig Wilcox's HOSTEST program (University of Pittsburgh, Pittsburgh, PA, USA). All titrations were performed in triplicate.
- [7] Crystal data for **1**·Cu (C₃₅H₄₆CuF₆N₁₀O): $M_r = 831.33$, triclinic, space group $P\bar{1}$, $a = 14.278(2)$, $b = 15.202(2)$, $c = 20.190(3)$ Å, $\alpha = 70.922(4)$, $\beta = 78.889(4)$, $\gamma = 69.360(3)^\circ$, $V = 3862.5(10)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 1.430$ g cm⁻³, $\mu = 0.679$ mm⁻¹, crystal dimensions $0.37 \times 0.14 \times 0.02$ mm, $T = 193$ K; of 14 674 unique reflections, 2803 were observed ($F_o^2 \geq 2\sigma(F_o^2)$), $R_1 = 0.0763$, $wR_2 = 0.1869$, $S = 0.753$, largest difference peak and hole 0.558 and -0.584 e Å⁻³, Bruker P4/RA/SMART 1000 CCD diffractometer, graphite-monochromated MoK α radiation ($\lambda = 0.71073$ Å). 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-133769. 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 Base-Stabilized Terminal Borylene Complex of Osmium Derived from Reaction between a Dichloroboryl Complex and 8-Aminoquinoline**

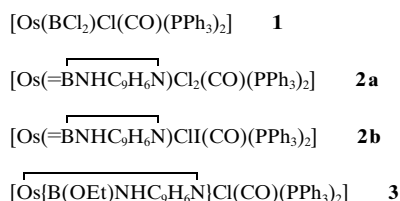
Geoffrey J. Irvine, Clifton E. F. Rickard, Warren R. Roper,* Alex Williamson, and L. James Wright*

Two classes of terminal boron ligand have been structurally characterized, namely, boryl (**A**)^[1] and borylene (**B**).^[2] A theoretical analysis of the bonding characteristics of terminal borylene ligands shows that these ligands, which are isolobal with CO, have both good σ -donor and π -acceptor properties.^[3] However, because of the high electrophilicity of the boron



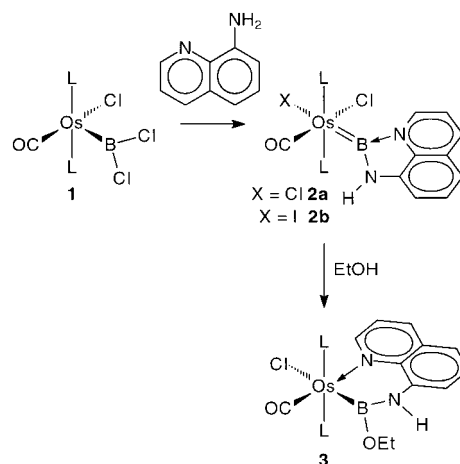
center, complexes of these ligands are likely to be stable only when sterically protecting and electron-releasing substituents are present on the boron atom, for example, $N(SiMe_3)_2$ or $\eta^5-C_5Me_5$ (Cp^*).^[2] Alternative strategies for stabilizing borylene complexes include placing the borylene ligand in a bridging situation between two metal centers,^[4] utilizing very electron rich metal–ligand fragments, or coordination of a Lewis base to the boron atom, that is, formation of base-stabilized borylene complexes (**C**). Similar base-stabilized silylene complexes are a prominent feature of metal–silicon chemistry.^[5]

Here we describe the synthesis of the dichloroboryl–osmium complex **1** as a precursor, which reacts with 8-aminoquinoline to provide **2a**, the first stable internally base-stabilized terminal borylene complex; the structure determination of the iodide derivative **2b**; and the further reaction of **2a** with ethanol to give the structurally characterized osmium-tethered ethoxyboryl complex **3**.



Following the established route to five-coordinate boryl complexes of osmium(II),^[6] treatment of $[Os(Ph)Cl(CO)(PPh_3)_2]$ with $HBCl_2 \cdot OEt_2$ produces bright yellow **1** (see the

Experimental Section). The ^{11}B NMR spectrum for **1** exhibits a broad signal at $\delta = 52.5$. Complex **1** is a versatile synthon for many other boryl derivatives by virtue of the two good leaving groups on boron. For example, reaction with catechol ($catH_2$) gives $[Os(Bcat)Cl(CO)(PPh_3)_2]$, and treatment with ethanol gives $[Os\{B(OEt)_2\}Cl(CO)(PPh_3)_2]$.^[1c] Reaction of **1** with 8-aminoquinoline gives the poorly soluble, red, base-stabilized borylene complex **2a** (Scheme 1). The more soluble,



Scheme 1. Synthesis of base-stabilized borylene complexes and their reaction with ethanol. Complex **2b** is formed by treatment of **2a** with Bu_4NI . $L = PPh_3$.

deep purple complex **2b**, in which the chloro ligand *trans* to boron is replaced with iodide, was formed by treatment of **2a** with tetrabutylammonium iodide. Both the ^1H and ^{13}C NMR spectra of **2b** (see the Experimental Section) are as expected for this formulation, but the signals for the borylene fragment and for the two triphenylphosphane ligands indicate the presence of two isomers. These isomers are interpreted as arising from two different orientations of the base-stabilized borylene ligand. The existence of these two isomers is also evident in the crystal structure, in which it is manifested as a disorder between the mutually *trans* Cl and CO ligands. The ^{11}B NMR spectrum of **2b** exhibits a broad signal at $\delta = 51.7$.

The higher solubility of **2b** allowed crystals suitable for X-ray crystal structure determination to be obtained.^[7] The molecular structure of **2b** is depicted in Figure 1. The overall geometry about osmium is approximately octahedral with mutually *trans* triphenylphosphane ligands and the iodo ligand *trans* to the borylene ligand. Hence, the chloro ligand *trans* to the borylene group was replaced by iodide ion, and this reflects a strong *trans* influence of the borylene ligand. The five-membered ring of the base-stabilized borylene ligand is almost coplanar with the equatorial ligand plane (angle between plane of borylene ligand and plane containing Os, Cl, I, CO is $8.3(2)^\circ$) and is sandwiched between two phenyl rings of the mutually *trans* triphenylphosphane ligands. The distances between the five-membered ring of the borylene ligand and the centroids of each of the two phenyl rings are only 3.271(3) and 3.192(5) Å. This arrangement suggests the existence of π – π stacking interactions between the three

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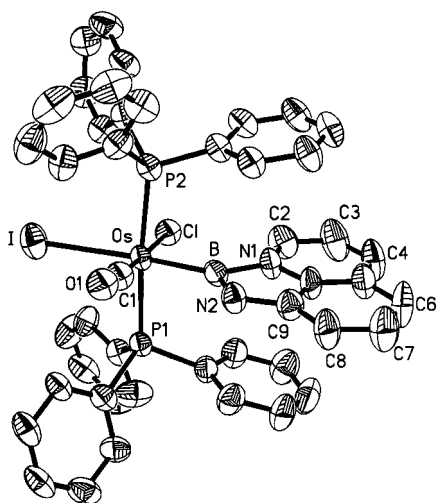
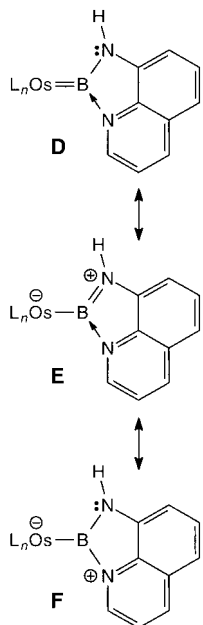


Figure 1. Molecular structure of **2b** (ORTEP) in the crystal (only one isomer shown). Selected bond lengths [Å] and angles [°]: Os–B 2.055(8), Os–Cl 1.763(18), Os–I 2.8340(6), Os–Cl 2.476(7), Os–P1 2.3989(18), Os–P2 2.3833(18), B–N1 1.571(10), B–N2 1.440(10); C1–Os–B 84.3(6), C1–Os–I 85.8(5), C1–Os–Cl 179.0(5), I–Os–B 170.2(2), I–Os–Cl 93.62(12), P1–Os–P2 174.82(6), Cl–Os–B 96.2(3), Os–B–N1 131.6(5), Os–B–N2 129.1(5), B–N2–C9 113.6(6), B–N1–C10 109.8(6), N1–C10–C9 109.3(6), N2–C9–C10 108.0(6).

rings. The Os–B distance of 2.055(8) Å is the shortest of the range of Os–B distances we have observed in six-coordinate boryl complexes with either O or N substituents on boron. This distance is still longer than might be anticipated for a formal Os=B bond, but a pronounced shortening would not be expected for a base-stabilized borylene complex, since the valence-bond structures depicted in Scheme 2 (among others) must be considered. Base-stabilized silylene complexes also exhibit metal–silicon distances typical of single bonds rather than double bonds.^[5] The two Os–B–N angles are approximately equal (ca. 130°), but the two B–N distances are markedly different (B–N2, 1.440(10), B–N1, 1.571(10) Å). The B–N2 distance is shorter than average and suggests a contribution from **E**. The B–N1 bond is also shorter than the average of all B–N(pyridine type) interactions reported in the CCSD. This is consistent with the presence of a particularly electrophilic boron center.

A theoretical study^[3] on terminal borylene complexes drew attention to the expected electrophilicity of the boron atom in these complexes. Even the base-stabilized borylene complex **2a** displays this electrophilic character in undergoing a ready reaction with ethanol to form the ethoxy(amino)boryl complex **3**, experimental details of which will be published elsewhere. In this reaction the quinoline nitrogen atom shifts from boron to osmium to form an osmium-tethered boryl ligand (see Scheme 1). The structure of **3** is



Scheme 2. Valence-bond structures for a base-stabilized osmium-borylene complex.

depicted in Figure 2. The Os–B distance of 2.072(3) Å is not significantly longer than the corresponding distance in **2b**. The B–O and B–N distances and Os–B–O and Os–B–N angles are all as expected. The Os–Cl distance of 2.6022(7) Å is the longest so far recorded.^[8]

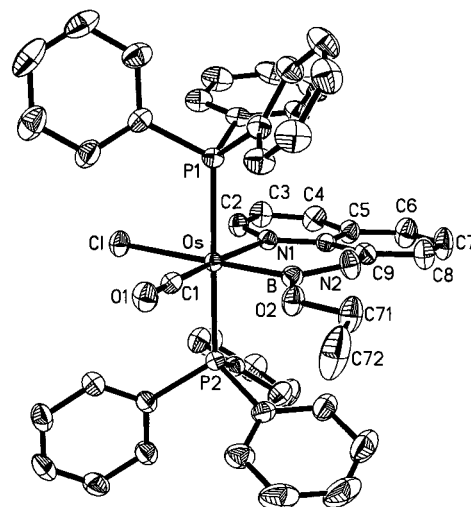


Figure 2. Molecular structure of **3** (ORTEP) in the crystal. Selected bond lengths [Å] and angles [°]: Os–B 2.072(3), Os–Cl 1.843(3), Os–N1, 2.235(2), Os–Cl 2.6022(7), Os–P1 2.3799(7), Os–P2 2.3755(7), B–O2 1.401(4), B–N2 1.439(4); C1–Os–B 86.45(12), C1–Os–N1 174.50(10), C1–Os–Cl 93.19(8), N1–Os–B 88.05(10), N1–Os–Cl 92.31(6), P1–Os–P2 176.86(2), Cl–Os–B 179.31(8), B–N2–C9 132.2(2), Os–N1–C10 127.61(17), Os–B–O2 121.2(2), Os–B–N2 123.5(2).

The results reported here indicate that dichloroboryl ligands are potential precursors for numerous base-stabilized borylene ligands by reaction with potentially chelating hydroxyl and amino compounds.

Experimental Section

1: To a red suspension of [Os(Ph)Cl(CO)(PPh₃)₂] (100 mg, 0.117 mmol) in benzene (15 mL) was added a solution of HBCl₂·OEt₂ in diethyl ether (0.15 mL, 1.0 M, 0.15 mmol). The mixture was then stirred at reflux temperature for 20 min. The resultant yellow solution was reduced to about 1 mL in vacuo, and hexane (20 mL) was added to precipitate a yellow solid, which was collected on a sintered glass crucible and washed with hexane. The product was purified by first placing it under vacuum for 30 min, then dissolving in benzene, filtering, reducing the solvent volume to about 1 mL and precipitation with hexane to give pure **1**. Yield 85 mg (85 %); ¹H NMR (400 MHz, CDCl₃, 25 °C, TMS): δ = 7.36–7.46 (m, 18 H, PPh₃), 7.55–7.60 (m, 12 H, PPh₃); ¹³C{¹H} NMR (100 MHz, CDCl₃, 25 °C, TMS): δ = 128.37 (t', ^{2,4}J(C,P) = 11 Hz, *o*-PPh₃), 130.67 (s, *p*-PPh₃), 130.73 (t', ^{1,3}J(C,P) = 53 Hz, *ipso*-PPh₃), 134.46 (t', ^{3,5}J(C,P) = 11 Hz, *m*-PPh₃), 182.32 (t, ²J(C,P) = 9 Hz, CO); ¹¹B NMR (128 MHz, C₆D₆, 25 °C, BF₃·OEt₂): δ = 52.5; IR (Nujol): $\tilde{\nu}$ [cm^{−1}] = 1937 vs (CO), 824 s (B–Cl); elemental analysis calcd for C₃₇H₃₀BCl₂OOSp₂: C 51.68, H 3.52, Cl 12.37; found: C 51.87, H 3.79, Cl 12.34.

2a: A solution of **1** (85 mg, 0.0988 mmol) in benzene (10 mL) was added to 8-aminoquinoline (35 mg, 0.243 mmol) to produce a bright orange suspension, which was filtered through a sintered glass crucible. The orange solid was washed with EtOH, toluene, and hexane to give **2a** as a microcrystalline red solid. Yield 90 mg (87 %); ¹H NMR (400 MHz, CD₂Cl₂, 25 °C, TMS): major isomer: δ = 6.82 (m, 1 H, NC₉H₆NH), 7.30 (m, 1 H, NC₉H₆NH), 7.49 (m, 1 H, NC₉H₆NH), 8.28 (s, 1 H, NC₉H₆NH), 8.40 (m, 1 H, NC₉H₆NH), 8.57 (m, 1 H, NC₉H₆NH); minor isomer: δ = 6.60 (s, 1 H, NC₉H₆NH), 6.79 (m, 1 H, NC₉H₆NH), 7.46 (m, 1 H, NC₉H₆NH), 8.38 (m, 1 H, NC₉H₆NH), 10.31 (m, 1 H, NC₉H₆NH); overlapping signals of the two

isomers: $\delta = 6.95-7.04$ (m, PPh_3), $7.15-7.28$ (m, $\text{NC}_9\text{H}_6\text{NH}$), $7.82-7.90$ (m, PPh_3); ^{13}C and ^{11}B NMR spectra were not obtained due to low solubility; IR (Nujol): $\tilde{\nu} [\text{cm}^{-1}] = 1902\text{vs}$ (CO); ^1H NMR shows 1.0 equiv of C_6H_6 present as solvent of crystallization; elemental analysis calcd for $\text{C}_{46}\text{H}_{37}\text{BCl}_2\text{N}_2\text{OOSp}_2 \cdot \text{C}_6\text{H}_6$: C 59.72, H 4.14, N 2.67; found: C 59.87, H 4.22, N 2.64.

2b: A solution of Bu_4NI (110 mg, 0.298 mmol) in CH_2Cl_2 (5 mL) was added to a suspension of **2a** (90 mg, 0.093 mmol) in CH_2Cl_2 (15 mL), and the resultant mixture stirred for 40 min to give a dark red solution. Reduction of the solvent volume to about 1 mL in vacuo and addition of hexane gave an orange solid. The solid was collected on a sintered glass crucible and washed with EtOH, toluene, and hexane to give pure **2b** as a deep purple solid. Yield 93 mg (94 %); ^1H NMR (400 MHz, CD_2Cl_2 , 25°C , TMS): major isomer: $\delta = 6.87$ (m, 1H, $\text{NC}_9\text{H}_6\text{NH}$), 7.52 (m, 1H, $\text{NC}_9\text{H}_6\text{NH}$), 8.25 (s, 1H, $\text{NC}_9\text{H}_6\text{NH}$), 8.40 (m, 1H, $\text{NC}_9\text{H}_6\text{NH}$), 8.46 (m, $\text{NC}_9\text{H}_6\text{NH}$), 8.65 (m, 1H, $\text{NC}_9\text{H}_6\text{NH}$); minor isomer: $\delta = 6.82$ (m, 1H, $\text{NC}_9\text{H}_6\text{NH}$), 7.23 (m, 1H, $\text{NC}_9\text{H}_6\text{NH}$), 7.49 (m, 1H, $\text{NC}_9\text{H}_6\text{NH}$), 8.27 (s, 1H, $\text{NC}_9\text{H}_6\text{NH}$), 8.57 (m, 1H, $\text{NC}_9\text{H}_6\text{NH}$), 10.11 (m, 1H, $\text{NC}_9\text{H}_6\text{NH}$); overlapping signals of both isomers: $\delta = 6.97-7.04$ (m, PPh_3), $7.29-7.35$ (m, $\text{NC}_9\text{H}_6\text{NH}$), $7.75-7.87$ (m, PPh_3); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CD_2Cl_2 , 25°C , TMS): major isomer: $\delta = 127.48$ (t'), $^{24}\text{J}(\text{C},\text{P}) = 10$ Hz, $o\text{-PPh}_3$), 129.58 (s, $p\text{-PPh}_3$), 135.04 (t' , $^{3,5}\text{J}(\text{C},\text{P}) = 10$ Hz, $m\text{-PPh}_3$); minor isomer: $\delta = 127.65$ (t' , $^{24}\text{J}(\text{C},\text{P}) = 10$ Hz, $o\text{-PPh}_3$), 129.45 (s, $p\text{-PPh}_3$), 134.59 (t' , $^{3,5}\text{J}(\text{C},\text{P}) = 10$ Hz, $m\text{-PPh}_3$); signals from other C atoms not observed due to low solubility; ^{11}B NMR (128 MHz, CD_2Cl_2 , 25°C , $\text{BF}_3 \cdot \text{OEt}_2$): $\delta = 51.7$; IR (Nujol): $\tilde{\nu} [\text{cm}^{-1}] = 1909\text{m}$, 1892s , 1876s (CO); UV/Vis (CH_2Cl_2): $\lambda_{\text{max}} = 521.5$ nm; elemental analysis for $\text{C}_{46}\text{H}_{37}\text{BClIN}_2\text{OOSp}_2$: calcd: C 52.17, H 3.52, N 2.65; found: C 52.42, H 3.69, N 2.73.

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- [7] Data for the X-ray structure analyses: **2b**: crystals from dichloromethane/benzene; $\text{C}_{46}\text{H}_{37}\text{BClIN}_2\text{OOSp}_2 \cdot 0.5 \text{C}_6\text{H}_6$, $M_r = 1098.13$; crystal dimensions $0.28 \times 0.25 \times 0.08$ mm; monoclinic; space group $\text{C}2/c$; $a = 30.0757(4)$, $b = 20.6367(2)$, $c = 17.1417(2)$ Å, $\beta = 111.176(1)^\circ$, $Z = 8$, $V = 9920.8(2)$ Å³, $\rho_{\text{calcd}} = 1.470$ g cm^{−3}; $T = 203$ K; $2\theta_{\text{max}} = 55^\circ$; of 30013 total reflections, 10794 were unique ($R_{\text{int}} = 0.0259$); Siemens SMART CCD diffractometer, $\text{MoK}\alpha$ radiation ($\lambda = 0.71073$ Å); empirical absorption correction ($\mu = 3.346$ mm^{−1}, $T_{\text{min}}/T_{\text{max}} = 0.454/0.775$). The structure was solved by Patterson methods and refined by full-matrix least-squares on F^2 on all data. The asymmetric unit cell contains one molecule of **2b**, in which the chloro and carbonyl ligands are disordered equally between the two sites, and half a molecule of benzene. $R_1 =$

0.0518 for the 8660 observed data ($I > 2\sigma(I)$) and $wR_2 = 0.1712$ for all data. Goodness of fit on F^2 1.078. **3**: crystals from dichloromethane/toluene/*n*-hexane; $\text{C}_{48}\text{H}_{42}\text{BClN}_2\text{O}_2\text{OsP}_2 \cdot \text{C}_7\text{H}_8$, $M_r = 1069.37$; crystal dimensions $0.40 \times 0.28 \times 0.22$ mm; monoclinic; space group $P2_1/n$; $a = 16.6920(2)$, $b = 17.7366(1)$, $c = 17.6239(2)$ Å, $\beta = 106.510(1)^\circ$, $Z = 4$, $V = 5002.65(9)$ Å³, $\rho_{\text{calcd}} = 1.420$ g cm^{−3}; $T = 200$ K; $2\theta_{\text{max}} = 54^\circ$; of 29951 total reflections, 10719 were unique ($R_{\text{int}} = 0.0220$); Siemens SMART CCD diffractometer, $\text{MoK}\alpha$ radiation ($\lambda = 0.71073$ Å); empirical absorption correction ($\mu = 2.708$ mm^{−1}, $T_{\text{min}}/T_{\text{max}} = 0.410/0.587$). The structure was solved by Patterson and Fourier methods and refined by full-matrix least-squares on F^2 on all data. The asymmetric unit cell contains one molecule of **3** and one molecule of toluene; $R_1 = 0.0247$ for the 9473 observed data ($I > 2\sigma(I)$) and $wR_2 = 0.0630$ for all data. Goodness of fit on F^2 1.053. Programs used: SHELXS-97 (structure solution) and SHELXL-97 (structure refinement). 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-135052 (**2b**) and CCDC-135053 (**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).

[8] Longest of the 629 determinations found in the CCSD.

[9] t' denotes that the signal has apparent multiplicity; $^{n,m}\text{J}(\text{C},\text{P})$ is the sum of the two coupling constants $^n\text{J}(\text{C},\text{P})$ and $^m\text{J}(\text{C},\text{P})$, as explained in S. M. Maddock, C. E. F. Rickard, W. R. Roper, L. J. Wright, *Organometallics* **1996**, *15*, 1793–1803.

Orientation-Controlled Monolayer Assembly of Zeolite Crystals on Glass and Mica by Covalent Linkage of Surface-Bound Epoxide and Amine Groups**

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Efforts have been made during the last decade to develop zeolite thin films for separative, catalytic, and chemical sensor applications.^[1–10] For practical purposes, they have usually been prepared on various substrates or supports since they are extremely fragile. The chemical methods to assemble zeolite thin films on substrates can generally be classified into two groups, namely, “direct growth”^[1–5] and “postsynthetic crystal attachment”.^[6, 7] The former group usually involves in situ growth of the zeolite crystals or films on the substrates by immersing them into the synthesis gel.^[1–4] For best results, the substrates are often chemically modified or pretreated with colloidal seed crystals. The unique pulsed laser ablation of preformed supported zeolites can also be classified into this group.^[5] In the latter group, preformed zeolite crystals are chemically attached on substrates by ionic or covalent bonding by use of appropriate organic additives.^[6, 7] For these

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methods, the organic functional groups are usually tethered onto either the zeolites or the substrates by covalent bonding, proceeded by subsequent chemical linkage between the tethered functional groups and the unmodified surface. However, the coverage and orientation of the zeolite crystals attached onto the substrates by this “one-component modification” were rather poor. In fact, the ability to uniformly align zeolite crystals on substrates during the assembly is essential for the applications of zeolite thin films as the hosts for quantum dots, magnetic particles, nonlinear optical materials,^[8, 9] or chemical sensors.^[11] It has been reported that zeolite crystals tend to physically adhere onto substrates with their flat faces facing the substrate when the substrate is dip coated into a colloidal zeolite solution.^[2] This phenomenon led to uniform alignment of cubic zeolite A crystals on substrates with a face parallel to the plane of the substrate.^[2] For zeolites with lower symmetry, particularly for those that possess significant net intrinsic dipole moments due to one of the axes being longer than the other two, the application of strong electric fields is highly effective in aligning the crystals with the longest axis parallel to the direction of the electric field.^[10] We now report a novel “two-component modification” strategy that leads to monolayer assembly of zeolite A and ZSM-5 crystals onto glass and mica with very high surface coverage and also uniform alignment. This new approach involves independent tethering of two different functional groups onto the zeolite and the substrate, followed by covalent linking of the two tethered functional groups as schematically illustrated in Scheme 1.

Visual observation of the zeolite-coated glass plate prepared by the method shown in Scheme 1 revealed that the entire glass plate (18 × 18 mm) was uniformly covered with white zeolite A crystals. A thin layer of zeolite particles maintained adhesion onto the glass substrate even after sonication for 30 s in toluene. Omitting the surface anchoring of any of the silyl compounds in the binding procedure resulted in essentially empty glass plates scarcely covered with physisorbed zeolite crystals; these few crystals immediately fell off the glass plate during sonication. This result strongly indicates that facile formation of amine–hydroxide bridges indeed proceeds between terminal amine and epoxide groups, which leads to effective assembly of zeolite A crystals on the

glass substrate. The efficacy of uniform coverage of the entire glass surface seemed to depend on factors such as the uniformity of the chemical composition, the pretreatment of the glass substrate, the degree of coverage of the corresponding organic compound on the glass and zeolite, and the size and morphology of the zeolite crystals.

The scanning electron micrograph (SEM) of the glass plate coated with zeolite A crystals revealed that almost the entire plate was covered by the zeolite crystals with high-density packing, as shown in Figure 1 A. Observation at a higher

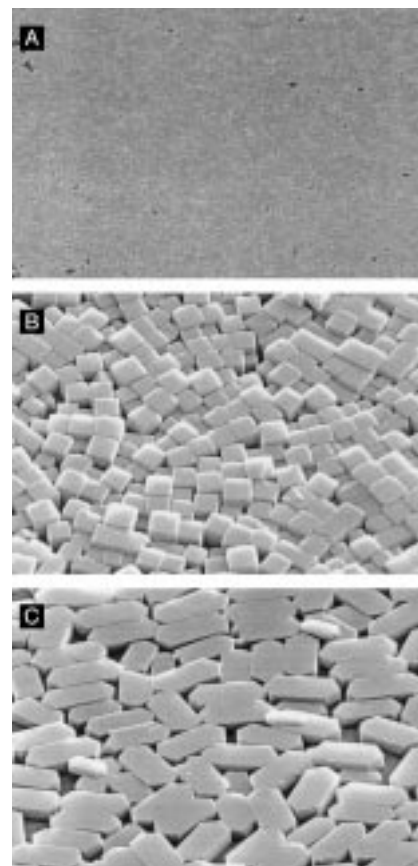
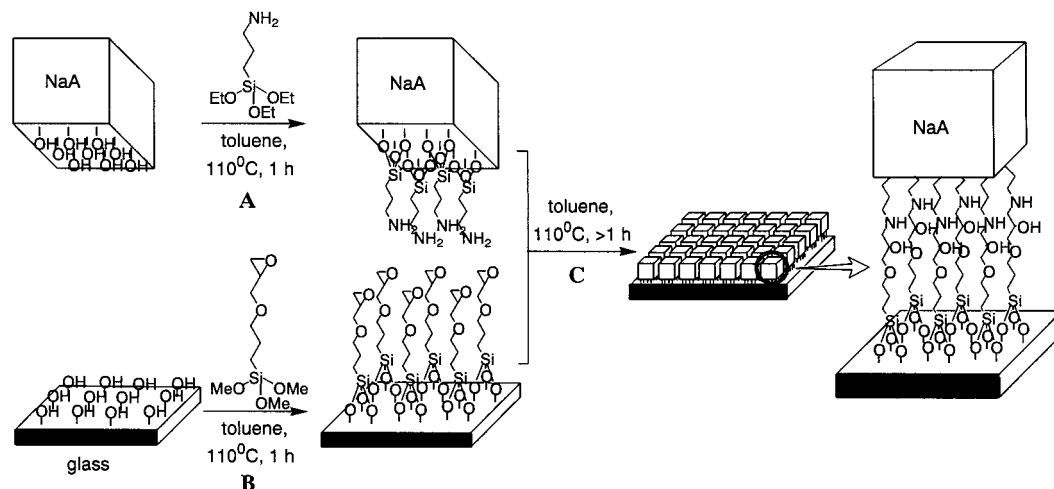


Figure 1. Scanning electron micrographs showing the monolayers of zeolite A crystals bound to a glass substrate at magnifications of × 200 (A) and × 12 000 (B), and ZSM-5 crystals at a magnification of × 5 000 (C).



Scheme 1. The procedure to assemble zeolite A monolayer on a glass substrate.

magnification (Figure 1 B) revealed that only a monolayer of the crystals was assembled on the glass. Moreover, this image shows the striking feature that all the zeolite crystals are aligned with a face parallel to the glass substrate, a perfect one-dimensional orientation.

Most interestingly, the zeolite A crystals showed a strong tendency to pack closely during the attachment onto the substrate with the side faces in contact. This phenomenon indicates that a 3-aminopropyl-covered zeolite A crystal, once mounted on the substrate, tends to act as a template for positioning the next crystal. Although further study is necessary, we propose for the moment that such a close packing arises due to a strong face-to-face interaction between the 3-aminopropyl-covered faces of two separate zeolite A crystals through a large number of hydrogen bonds between the terminal NH_2 groups. Consistent with our proposal, the commonly observed close packing of the physisorbed, unmodified zeolite crystals on substrates during dip coatings has also been attributed to hydrogen bonding between the surface hydroxyl groups of zeolites.^[2]

Figure 1 C shows the SEM image of a monolayer of ZSM-5 crystals similarly assembled on a glass substrate. This figure demonstrates that the method described above works equally well even for large ($\approx 5 \mu\text{m}$) zeolite crystals. This image further shows that all the ZSM-5 crystals assemble with their b axis perpendicular to the glass surface despite that the rectangular-shaped side faces can, in principle, also be attached onto the surface. This preferential orientation might occur because ZSM-5 crystals can have better contact with the glass substrate through their substantially larger $[010]$ face than through their smaller side faces. It was also noticed that the intercrystalline void spaces increase upon changing from zeolite A to ZSM-5, presumably due to the lower symmetry and increased size of ZSM-5 relative to zeolite A. The orthorhombic CoAPO-34 and SAPO-34 molecular sieve crystals ($\approx 7 \mu\text{m}$) also efficiently assembled onto the glass substrate with similar high-density, monolayer packing by the same method. The SEM images of the assembled molecular sieves (not shown) revealed that these crystals also align with a face parallel to the substrate. However, no preference for a particular face for these crystals was observed, presumably due to less substantial differences in the area of the three faces. The attachment method was also effective for freshly cut mica as the substrate, although the coverage was usually a bit less than onto glass.

The XRD pattern of the zeolite A-coated glass showed only five lines that correspond to $[200]$, $[600]$, $[800]$, $[1000]$, and $[1200]$ planes of the zeolite A crystals (Figure 2a). The reflection for $[400]$ expected at $2\theta = 14.40^\circ$ seems to be lost in the background due to an intrinsically very low intensity (less than 0.5% of the $[200]$ reflection).^[12] Such a simple pattern contrasts strongly with the more common, complex pattern of the randomly oriented zeolite A crystal powder shown in the inset. Likewise, only five lines appeared from the monolayer of ZSM-5 crystals due to $[020]$, $[040]$, $[060]$, $[080]$, and $[0100]$ reflections (Figure 2b).

The strength of bonding between zeolite A crystals and substrates seems to vary and is sensitively dependent on the assembly procedure. That a large number of zeolite A crystals

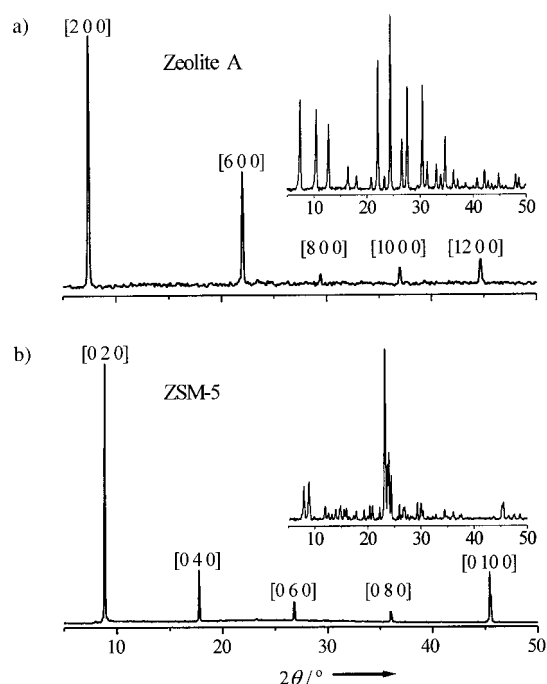


Figure 2. X-ray powder diffraction patterns of a) zeolite A and b) ZSM-5 monolayers showing the aligned state of crystals on glass substrates. Insets: XRD patterns for randomly oriented powders of the zeolites.

remain adhered to the glass substrate even after sonication as long as 5 min suggests the bonding to be fairly strong. Interestingly, SEM images of the extensively sonicated samples revealed that most of the surviving zeolite particles had become severely fractured. This result contrasts with the fact that all the zeolite A particles retain their original cubic morphology under the same treatment conditions as a free powder. The larger ZSM-5, CoAPO-34, and SAPO-34 crystals tend to be more easily removed by sonication.

This report introduces a novel strategy to assemble monolayers of zeolite A and ZSM-5 onto glass and mica substrates with a perfect one-dimensional orientation through covalent binding between two independently tethered, terminal functional groups, in this case epoxide and amine. This method uniquely provides strong zeolite–substrate bonding, high surface coverage, uniform orientation, and easy control of the monolayer assembly. In a broader sense, this report demonstrates a novel strategy to efficiently organize micrometer-sized nanoporous inorganic crystals into functional macroscopic structure.

Experimental Section

Zeolite A, ZSM-5, CoAPO-34, and SAPO-34 were synthesized according to the literature procedures. The synthetic templates were not removed from the zeolite or molecular sieve crystals prior to assembly. The washed and dried zeolite or molecular sieve (0.2 g) was treated with 3-aminopropyltriethoxysilane (2 mm, 10 mL, 110°C , 1 h) under argon. This led to 3-aminopropylsilyl groups covalently bound onto the zeolite or molecular sieve surface (see Scheme 1A). The presence of the surface-bound 3-aminopropylsilyl groups was confirmed by purple coloration of the surface when treated with 1,2,3-triketohydrindene (ninhydrin) at room temperature.^[13] The diffuse-reflectance UV/Vis spectrum of the purple-coloured zeolite showed an adsorption at 570 nm indicating the formation of diketohydrindylidenediketohydrinamine. Control experiments with

fresh zeolites undoped with aminopropylsilyl groups did not develop the characteristic purple color.

Independently, a piece of thin glass (18 × 18 mm²) was treated with [3-(2,3-epoxypropoxy)propyl]trimethoxysilane (2 mm) in toluene (10 mL, 110 °C, 1 h) under argon to assemble a layer of 3-(2,3-epoxypropoxy)propylsilyl groups on the glass surface (see Scheme 1B). The presence of surface-bound terminal epoxy groups attached on the glass was confirmed by pale-red coloration of the surface when treated with an aminated azo dye (Fat Brown RR) at 110 °C in toluene.^[14] The UV/Vis spectrum of the glass showed a broad band with the absorption maximum at ≈440 nm. Undoped glasses gave negligible intensities in the ≈440 nm region.

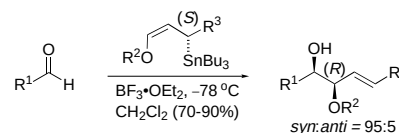
The coated glass plate was inserted into a suspension of 3-aminopropylsilyl-coated zeolite powders in toluene (0.1 g, 10 mL) and the mixture was refluxed (> 1 h) under argon (see Scheme 1C). The zeolite-coated opaque glass was then removed from the reaction mixture and washed extensively with toluene. The zeolite-coated glass was subsequently sonicated in toluene for 20 s to remove physisorbed zeolite crystals on the chemically bound first layer.

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Addition of Enantioenriched γ -Oxygenated Allylic Stannanes to *N*-Acyl Iminium Intermediates: A New Synthesis of *syn*-Amino Alcohol Derivatives**

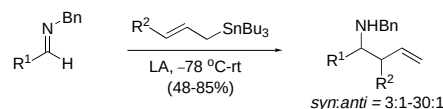
James A. Marshall,* Kevin Gill, and Boris M. Seletsky

Some years ago we discovered a facile route to mono-protected *syn*-1,2-diol derivatives through BF₃-promoted addition of enantioenriched γ -oxygenated allylic stannanes to aldehydes (Scheme 1).^[1] We had hoped to extend these



Scheme 1. A facile route to monoprotected *syn*-1,2-diol derivatives. R¹ = alkyl, alkenyl; R² = methoxymethyl (MOM), tributylsilyl (TBS), benzyl-oxyethyl (BOM); R³ = alkyl.

additions to imines, along the lines reported by Keck and Enholm (Scheme 2),^[2] but were unsuccessful in these attempts. No detectable β -amino ether adducts were formed, even at room temperature. We attributed these failures to the lower reactivity of oxygenated allylic stannanes relative to their nonoxygenated allyl and crotyl counterparts.^[3]



Scheme 2. Addition of allylic stannanes to imines. R¹ = *c*-C₆H₁₁, Ph, *s*Bu, *i*Pr; R² = H, Me; Lewis acid LA = BF₃·OEt₂, TiCl₄. Bn = benzyl, rt = room temperature.

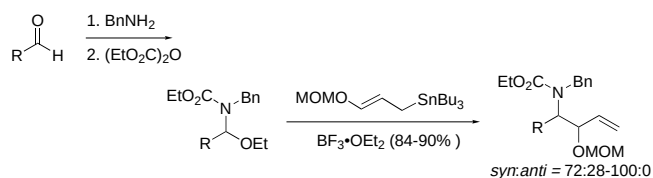
A report by Yamamoto and Schmid, describing the addition of a γ -OMOM allylic stannane to several *N*-acyliminium intermediates from Hiemstra and Speckamp^[4] (Scheme 3), prompted our examination of this alternative route to β -amino alcohol derivatives.^[5] In fact, addition of the racemic (*Z*)- γ -oxygenated allylic stannanes **2a** and **2b**^[1] to the *N*-acyliminium precursor **1**, derived from isovaleraldehyde,^[5] proceeded in high yield to afford the desired adducts (Table 1). Unfortunately, a mixture of *syn* and *anti* isomers **3** and **4** was obtained from these additions.

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

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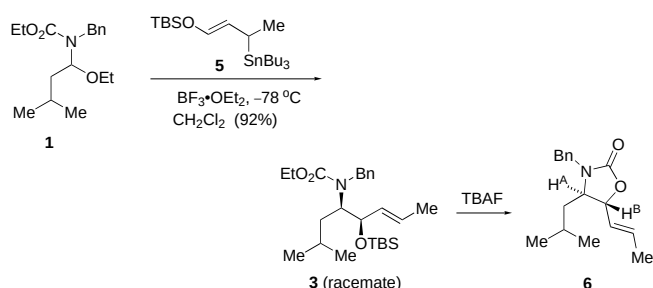
Scheme 3. Addition of γ -OMOM allylic stannanes to N -acyliminium intermediates. R = *i*Pr, *i*Bu.

Table 1. Additions of racemic allylic stannanes **2** to **1**, the N -benzyl- N -carboxyiminium salt of isovaleraldehyde.

R	LA	Yield [%] ^[a]	3:4
TBS (2a)	BF ₃ ·OEt ₂	72	86:14
MOM (2b)	BF ₃ ·OEt ₂	72	84:16
MOM (2b)	BF ₃ ·OEt ₂	92 ^[b]	84:16
MOM (2b)	TiCl ₄	82	86:14

[a] Yield of **3** and **4**. [b] Lewis acid added to premixed stannane and carbamate.

Interestingly, the racemic (*E*)- γ -OTBS allylic stannane **5** afforded only the *syn* adduct **3** when treated with the ethoxy carbamate **1** (Scheme 4).^[6] The relative stereochemistry of this adduct is assigned by analogy with related additions to aldehydes and in consideration of the coupling constant ($J_{A,B}$ = 5.9 Hz)^[7] of the indicated *trans*-oxazolidinone protons of **6**,^[5] into which **3** is readily converted. While this result was encouraging, it failed to meet our needs for a route to enantioenriched amino alcohol adducts, as we have not yet been able to devise a synthesis of enantioenriched (*E*)- γ -oxygenated allylic stannanes such as **5**.



Scheme 4. Reaction of (*E*)- γ -OTBS allylic stannane **5** with ethoxy carbamate **1**. For product **6**, $J_{A,B}$ = 5.9 Hz. TBAF = tetrabutylammonium fluoride.

We next examined additions of the racemic (*Z*)- γ -OTBS allylic stannane **2a** to the ethoxy carbamate derived from isovaleraldehyde and (*R*)- α -phenethylamine in the hopes of effecting a kinetic resolution by using a twofold excess of the stannane.^[8] The result was a nearly 1:1 mixture of the diastereomeric *syn* adducts and a modest 8% enantioenrichment of the recovered stannane, favoring the *R* enantiomer (*R*)-**2a**. Interestingly, the *syn:anti* ratio of the diastereomeric adducts was found to be 98:2, as opposed to 86:14 previously

observed with the benzyl analogue **1** of the *N*- α -phenethyl carbamate. Thus it appeared that branching at the benzylic center caused a marked improvement in the diastereoselectivity of the addition.

Supposing that this improvement was the consequence of increased bulk or restricted rotation of the *N*-benzylic group in the N -acyliminium intermediate, we examined the *o*-methoxybenzyl derivative **7a** and the enantioenriched (*S*)- γ -silyloxy allylic stannane **2a** (Table 2, entry 1). The results were quite promising: the *syn* adduct **8aa** predominated over the *anti* adduct by over 95:5. The relative stereochemistry of the *syn* adduct was confirmed through its one-step conversion into the *trans*-oxazolidinone **9a**.^[5] The OMOM adduct **8ab** afforded the identical oxazolidinone (entry 2) by a two-step procedure involving sequential treatment with aqueous acid followed by base. The absolute configuration of the adducts was initially assigned by analogy to the well-studied additions of stannanes **2a** and **2b** to aldehydes.^[1]

Table 2. Additions of enantioenriched allylic stannanes (*R*)-**2a** and **2b** to N -acyliminium salts.

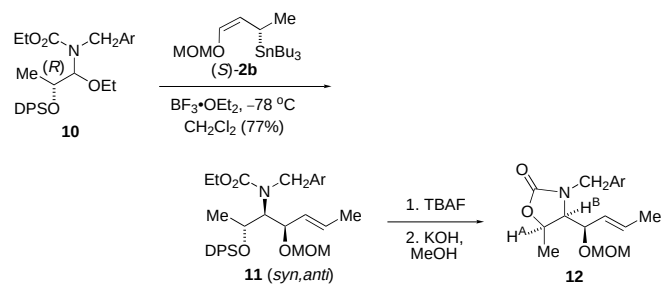
Entry	R ¹	R ²	8	Yield [%] 9
1	<i>i</i> Bu (7a)	TBS (2a)	90 (8aa)	91 (9a)
2	<i>i</i> Bu (7a)	MOM (2b)	91 (8ab)	88 (9a)
3	C ₆ H ₁₃ (7b)	TBS (2a)	71 ^[c] (8ba)	93 (9b)
4	C ₆ H ₁₃ (7b)	MOM (2b)	66 (8bb)	82 (9b)
5	<i>c</i> -C ₆ H ₁₁ (7c)	TBS (2a)	67 ^[d] (8ca)	94 (9c)
6	<i>c</i> -C ₆ H ₁₁ (7c)	MOM (2b)	78 (8cb)	72 (9c)
7	2-furyl (7d)	TBS (2a)	73 (8da)	94 (9d)
8	2-furyl (7d)	MOM (2b)	65 (8db)	decomp.

[a] For R² = TBS: TBAF, THF. [b] For R² = MOM: 1. HCl, H₂O, THF, 2. KOH, THF, MeOH. [c] R² = H. [d] A mixture was used: 42% of R² = H and 25% of R² = TBS.

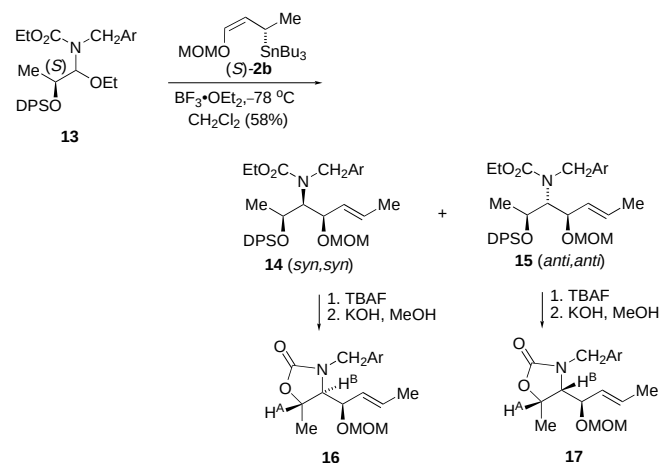
Additions to the N -acyliminium intermediates from carbamates **7b–7d** were equally selective (entries 3–8). The diastereomeric *anti* adducts made up less than 5% of the product, based on GC analysis. This analysis also revealed that the *ee* value of adduct **9a** was over 95%. We were unable to separate the enantiomers of adducts **9b–9d** by this technique or by HPLC on several columns. However, we were able to determine *ee* values (> 95%) of adducts **9b** and **9c** through removal of the *N*-*o*-methoxybenzyl groups with ceric ammonium nitrate^[9] and conversion of the derived oxazolidinones to the *N*-*tert*-butoxycarbonyl (BOC) amino alcohols by addition of (BOC)₂O and cleavage of the resulting *N*-BOC oxazolidinones with aqueous K₂CO₃.^[10] The *N*-BOC alcohols

obtained through this sequence were analyzed as their *O*-methyl mandelic esters.^[11] The ¹H NMR spectra of the *R* and *S* derivatives confirmed both the *ee* values (>95%) and the assigned absolute configuration of these compounds.

The matching/mismatching characteristics of the addition were examined with the OMOM allylic stannane (*S*)-**2b**^[12] and the *N*-(*o*-methoxybenzyl)carbamates **10** and **13** derived from the DPS ether derivatives of (*R*)- and (*S*)-lactic aldehyde (Schemes 5 and 6; DPS = Si*t*BuPh₂).^[13] As expected from



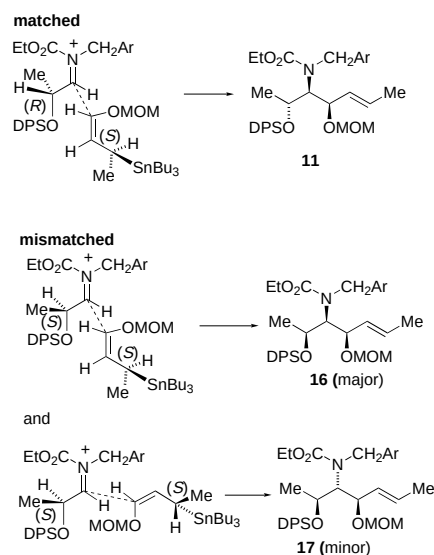
Scheme 5. Reaction of **2b** with **10**. For product **12**, *J*_{A,B} = 7.2 Hz. Ar = *o*-MeOC₆H₄.



Scheme 6. Reaction of **2b** with **13**. Adducts **14** and **15** are formed as a 60:40 mixture. For product **16**, *J*_{A,B} = 3.6 Hz. For product **17**, *J*_{A,B} = 7.5 Hz. Ar = *o*-MeOC₆H₄.

analogous additions to aldehydes,^[1] the *R/S* combination is the matched pairing and affords the *syn,anti* adduct **11** as the exclusive product (Scheme 5). The *S/S* combination led to a 60:40 mixture of the *syn,syn* and *anti,anti* adducts **14** and **15** (Scheme 6). The relative stereochemistry of these adducts was confirmed through ¹H NMR analysis of the derived oxazolidinones **12**, **16**, and **17**. The observed diastereoselectivity is consistent with a preferred antiperiplanar acyclic transition state (Scheme 7).

The present findings extend the utility of nonracemic γ -oxygenated allylic stannanes to the preparation of enantioenriched *syn*- β -amino alcohol derivatives.^[14] These products are potential precursors of α -amino- β -hydroxy acids, β -amino- α -hydroxy acids, amino and aza sugars, and related natural products of biological interest.^[15] The reason for the unprecedented enhanced diastereoselectivity of the *o*-methoxybenzyl derivatives is at present obscure. Neither steric nor

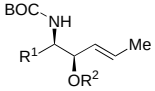


Scheme 7. Possible transition states for matched and mismatched pairing of allylic stannane (*S*)-**2a** and the Speckamp iminium intermediates from ODPS-protected lactic aldehyde.

electronic factors offer likely explanations, as reactions with the *o*-methyl- or *p*-methoxybenzyl analogues of **7** both gave approximately 80:20 mixtures of *syn* and *anti* adducts. The issue is a complex one and may depend upon *E/Z* preferences of the intermediate acyliminium ion.^[4] It is also possible that internal hydrogen bonding between the *o*-methoxy oxygen atom and the acidic acyliminium hydrogen atom gives rise to a rotationally restricted transition state.^[16] Further studies with other nucleophilic reagents and *ortho*-substituted benzylic imines are clearly warranted. However, mechanistic rationale aside, the influence of *ortho*-methoxy substitution on diastereoselectivity is unexpected and of significant practical import to the success of the present methodology. It is worth noting that although other routes to enantioenriched β -amino alcohols are known,^[17] few proceed with simultaneous creation of a carbon–carbon bond and two contiguous stereogenic centers.^[18,19]

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Scheme 8. $R^1 = \text{C}_6\text{H}_{13}$ or $c\text{-C}_6\text{H}_{11}$; $R^2 = (R)\text{- or } (S)\text{-PhCH(OMe)CO}_2\text{H}$. CAN = cerium ammonium nitrate, BOC = butoxycarbonyl, DMAP = dimethylaminopyridine.

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Sterically Controlled Pathways in the Reaction of 2,4,6-Tris(isopropyl)benzenesulfonyl Azide and $[\text{Pd}_2\text{Cl}_2(\text{dppm})_2]$ **

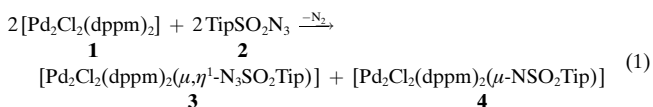
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Organic azides often react with metal complexes with extrusion of N_2 , affording imido (nitrene) derivatives.^[1] The azide moiety is not necessarily cleaved, however, as illustrated by earlier studies on hydridoosmium and dimeric molybdenum complexes.^[2] Several aryl and cyclohexyl azide complexes revealing various modes of coordination have recently been characterized structurally and chemically.^[3]

Our previous work on the phosgene-free synthesis of isocyanates has shown that arenesulfonyl azides react with $[\text{Pd}_2\text{Cl}_2(\text{dppm})_2]$ (**1**, dppm = bis(diphenylphosphanyl)methane), resulting in the formation of arylsulfonylimido A-frame adducts.^[4, 5] These novel reactions show good

selectivities with most sulfonyl azides, except for the 2-nitro derivative, which gave $[\text{Pd}_2\text{Cl}_2(\text{dppm})_2(\text{N}_3\text{SO}_2\text{C}_6\text{H}_4\text{-2-NO}_2)]$ as a by-product.^[5a] To elucidate the steric effects of azide ligands, we conducted studies with 2,4,6-tris(isopropyl)benzenesulfonyl azide (TipSO_2N_3 , **2**). The results of these investigations, together with crystallographic data on the parent azide **2**, are presented here.

Reaction of $[\text{Pd}_2\text{Cl}_2(\text{dppm})_2]$ with **2** affords the azide complex **3** and the nitrene complex **4** in 75 and 25% yield, respectively [Eq. (1); ^1H NMR, see the Experimental Section], involving a very bulky bridging imido ligand. The



molecular structures of the complexes formed are shown in Figures 1 and 2. The products are typical A-frame adducts with an extended boat conformation of the $\text{Pd}_2\text{P}_4\text{C}_2$ ring. The Pd–Pd distances are about 0.6 Å longer than in $[\text{Pd}_2\text{Br}_2(\text{dppm})_2]$, ruling out metal–metal bonding.^[6]

The redistribution of valence electrons induced by coordination can be best visualized by comparing bond lengths in free and coordinated sulfonyl azide. Although N3 is disordered in **2** (structure not shown, the numbering corresponds to that of **3**), the short N1–N2 and N2–N3 bonds (1.213(3) and 1.14 Å) indicate multiple bonding, in line with structural data for other sulfonyl azides.^[7] Decreased bond orders as a result of complexation are clear from the N2–N3 and N1–N2 distances of 1.248(5) and 1.340(5) Å in **3**. The structural features of **3** are consistent with **2** reacting as a 1,3-dipole ($\text{Ar-SO}_2\text{-N}^--\text{N}=\text{N}^+$), producing a zwitterionic structure with the negative charge delocalized on the N1–N2 and N1–S1 bonds. The azide-to-sulfonyl electron transfer shortens the

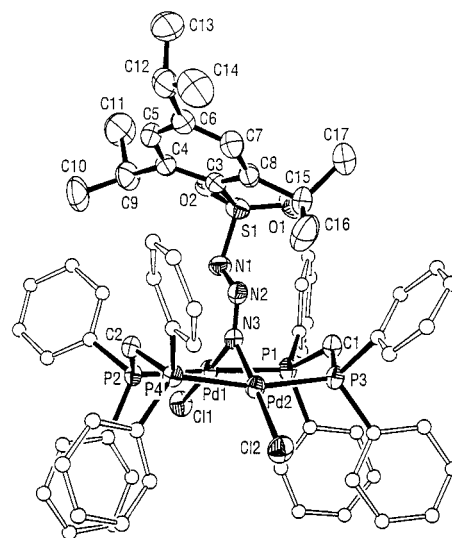


Figure 1. Molecular structure of **3** without hydrogen atoms. Selected interatomic distances [Å] and angles [°]: Pd1...Pd2 3.315(1), Pd1–N3 1.984(4), Pd2–N3 1.972(4), N2–N3 1.248(5), N1–N2 1.340(5), N1–S1 1.627(4), S1–C3 1.808(5), S1–O_{av} 1.437; Pd1–N3–Pd2 113.8(2), Pd1–N3–N2 126.0(3), Pd2–N3–N2 120.2(3), N1–N2–N3 114.7(4); C–H...O close contacts: H9...O2 2.325, C9–H9...O2 114.4, H15...O1 2.205, C15–H15...O1 125.2.

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[**] dppm = bis(diphenylphosphanyl)methane. This work was supported by the Hungarian Research Fund (OTKA grant 16213).

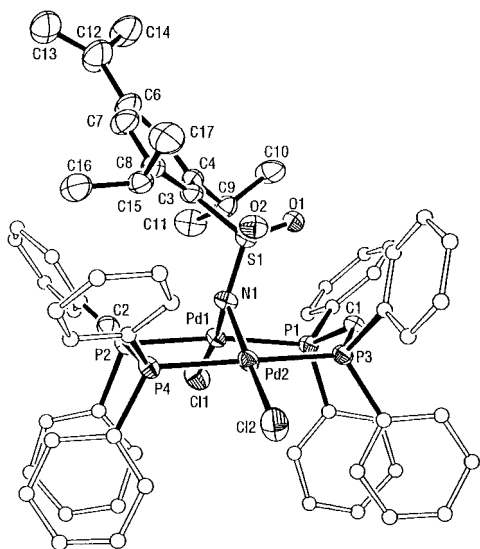


Figure 2. Molecular structure of **4** without hydrogen atoms. Selected interatomic distances [Å] and angles [°]: Pd1...Pd2 3.268(1), Pd1-N1 2.010(2), Pd2-N1 2.039(2), N1-S1 1.579(2), S1-C3 1.810(3), S1-O_{av} 1.451; Pd1-N1-Pd2 107.7(1), Pd1-N1-S1 126.5(1), Pd2-N1-S1 119.3(1); C-H...O close contacts: H9...O1 2.334, C9-H9...O1 113.5, H15...O2 2.248, C15-H15...O2 118.2.

N1-S1 distance (**2**: 1.728(2), **3**: 1.627(4) Å) and lengthens the S1-C bond (**2**: 1.773(2), **3**: 1.808(5) Å). Spectroscopic data for **3** and the azide adduct derived from 2-NO₂C₆H₄SO₂N₃, suggest that the coordination modes of the azide ligands in these complexes are identical.^[5a]

Comparison of bonding within the N₃ unit in **3** and in other azide adducts of known structures reveals a variety of metal-azide interactions. The most significant structural discrepancy can be observed with the V and Ta complexes, where the N-N bond close to the metal center is longer than that near the aromatic ring.^[3a,b]

¹H NMR spectroscopy gives an azide-to-nitrene product ratio (**3**:**4**) of about 3:1. For 2-NO₂C₆H₄SO₂N₃, about 15% azide complex was detected, but with other sulfonyl azides only the nitrene complex was observed in the raw reaction mixtures. This implies that the azide and imido adducts have a common intermediate, which, under sterically favorable conditions, collapses to the nitrene complex.^[5a] Owing to repulsive interactions of the *ortho* substituents with the phenyl groups, this intermediate may be stabilized by μ,η^1 bridging of the Pd atoms. It seems plausible that the bulkiness of the isopropyl groups leads to the predominant formation of the azide adduct **3**.

The important steric effect of the *o*-isopropyl groups is apparent from the unusual ¹H NMR spectrum of **4**. The methylene protons of **4** appear as four separate resonances, in contrast with the doublet/quintet multiplicity observed in other sulfonylimido adducts.^[5a] Variable-temperature NMR spectra in CDBr₃ show that rotation around the S-N axis is feasible, but the expected coalescence of methylene resonances does not occur up to 125 °C. These spectra clearly indicate that rotation of the isopropyl groups about the C4-C9 and C8-C15 bonds is also hindered. Of the three methyl doublets, the resonance at δ = 0.72 can be attributed to the methyl groups of the *o*-isopropyl groups pointing toward the sulfonyl

moiety. The upfield shift of the signals for the other pair of methyl groups to δ = 0.21 can be ascribed to the anisotropic effect of equatorial phenyl moieties. Although the resonances at δ = 0.72 and 0.21 tend to merge upon warming of the sample, the coalescence temperature is again higher than 125 °C. Presumably, steric hindrance by the *o*-isopropyl groups makes an intermediate unfavorable in which both the γ - and α -nitrogen atoms are coordinated, the obvious prerequisite for azide group cleavage. Thus, **3** is the major product from **2**.^[2b, 3c, 8]

The sensitivity of **3** to light in both the solid state and in solution requires exclusion of sunlight during manipulations. However, **3** is surprisingly stable in the dark: Its UV/Vis spectrum in CH₂Cl₂ does not change over a period of 22 h at room temperature. Also, **3** remained unchanged overnight when the solution contained five equivalents of 4-nitrobenzenesulfonyl azide, ruling out thermal equilibration with its constituents **1** and **2** (should any free **1** form by thermal dissociation, reaction with 4-NO₂C₆H₄SO₂N₃, the most reactive sulfonyl azide studied, would immediately generate the corresponding nitrene complex). Although full conversion of **1** with **2** does occur over a period of about three days, the thermal stability of **3** seems to warrant the conclusion that it is not an intermediate along the reaction path leading to nitrene adduct **4**.

Experimental Section

Reaction of [Pd₂Cl₂(dppm)₂] with **2**: Compounds **1** and **2** were prepared by known procedures.^[9, 10] The synthesis and isolation of the azide complex should be carried out under protection from sunlight. A solution of **1** (362 mg, 0.34 mmol) and **2** (290 mg, 0.94 mmol) in CH₂Cl₂ (7 mL) was stirred in a capped Schlenk tube for 4 d at 20–25 °C. Evaporation under vacuum and repeated washing with hexane was followed by column chromatography (Kieselgel 60 F₂₅₄; CH₂Cl₂/EtOAc 20/1), which resulted in yellow **3** (262 mg) and red **4** (84 mg), the latter being eluted first.

3: ¹H NMR (400 MHz, CDCl₃): δ = 0.72 (d, 12 H, *J* = 6.7 Hz; *o,o'*-iPr), 1.30 (d, 6 H, *J* = 6.9 Hz; *p*-iPr), 2.46 (dq, 2 H, *J*_{HH} = 13.4, *J*_{HP} = 3.0 Hz; CH₂), 2.84 (dq, 2 H, *J*_{HH} = 13.4, *J*_{HP} = 5.1 Hz; CH₂), 2.92 (sept, 1 H, *J*_{HH} = 6.9 Hz; *p*-iPr), 4.05 (sept, 2 H, *J*_{HH} = 6.7 Hz; *o,o'*-iPr), 6.97 (s, 2 H; *i*Pr₃C₆H₂), 7.0–8.0 (m, 40 H; P-C₆H₅); ³¹P NMR (162 MHz, CDCl₃): AA'BB' spectrum; δ_A = 12.7, δ_B = 14.6; FT-IR (KBr): 1289 (ν_{as}, SO₂), 1144 cm⁻¹ (ν_s, SO₂); UV/Vis: λ (ε): 330 (24000), 424 nm (5300).

4: ¹H NMR (400 MHz, CDCl₃): δ = 0.21 (d, 6 H, *J* = 6.9 Hz; *o,o'*-iPr), 0.72 (d, 6 H, *J* = 6.3 Hz; *o,o'*-iPr), 1.15 (d, 6 H, *J* = 6.9 Hz; *p*-iPr), 2.43 (brm, 1 H; CH₂), 2.71 (brm, 1 H; CH₂), 2.75 (sept, 1 H, *J* = 6.9 Hz; *p*-iPr), 3.52 (brm, 1 H; CH₂), 4.07 (sept, 2 H, *J* = 6.6 Hz; *o,o'*-iPr), 5.93 (brm, 1 H; CH₂), 6.66 (s, 2 H; *i*Pr₃C₆H₂), 6.9–8.1 (m, 40 H; P-C₆H₅); ³¹P NMR (162 MHz, CDCl₃): poorly resolved AA'BB'-type multiplet centered at δ = 6.4 and 6.8; FT-IR (KBr): 1217 (ν_{as}, SO₂), 1090 cm⁻¹ (ν_s, SO₂).

X-ray structure analyses: Enraf-Nonius CAD4 diffractometer, graphite monochromator, *T* = 293(2) K. Intensity data were corrected for crystal decay for **2** and **3**. All structures were solved by direct methods; hydrogen atoms were placed at calculated positions. An empirical psi-scan absorption correction was applied to the data in all cases. The structures were refined by anisotropic full matrix refinement on *F*² for all non-hydrogen atoms with all unique reflections. 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-132472 (**2**), -132473 (**3**), and -132474 (**4**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk). Selected crystal data: **2** (C₁₅H₂₃N₃O₂S): *M*_r = 309.42, colorless prism, monoclinic, space group *P*₂₁/*n*, *a* = 5.997(1), *b* = 21.456(2), *c* = 13.579(1) Å, β = 95.88(1)°, *V* = 1738.0(4) Å³, *Z* = 4. **3**

(C₆₅H₆₇Cl₂N₃O₂P₄Pd₂S·CH₂Cl₂): *M_r* = 1446.78, yellow prism, triclinic, space group *P*1̄, *a* = 13.311(2), *b* = 14.002(1), *c* = 19.661(3) Å, *α* = 95.07(1), *β* = 106.45(2), *γ* = 105.35(1)°, *V* = 3335.8(8) Å³, *Z* = 2. 4 (C₆₅H₆₇Cl₂N₃O₂P₄Pd₂S·CH₂Cl₂): *M_r* = 1418.76, red prism, monoclinic, space group *P*2₁/c, *a* = 19.037(1), *b* = 15.689(1), *c* = 21.576(2) Å, *β* = 97.92(1)°, *V* = 6382.7(8) Å³, *Z* = 4.

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Formation of the Azadisulfite Dianion [O₂S(μ-NPh)SO₂]^{2−} by Twelffold Insertion of SO₂ into the Mg–N(Ph) Bonds of [(thf)MgNPh]₆**

Justin K. Brask, Tristram Chivers,* and Masood Parvez

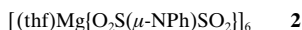
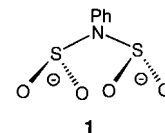
The insertion of a sulfur dioxide molecule into a metal–carbon σ bond is a widely studied reaction.^[1] For example, SO₂ reacts with organomagnesium reagents to give, upon hydrolysis, sulfinic acids.^[1a, 2] The facile insertion of SO₂ into the M–O bonds of the polymeric metal alkoxides [M(OMe)₂]_n (M = Ca, Mg) yields the corresponding methylsulfites.^[3]

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Sulfur dioxide also undergoes insertion into M–NR₂ linkages (e.g., Me₃SnNMe₂).^[4] Despite the recent interest in both transition metal^[5] and main group imido chemistry,^[6–8] the reaction of SO₂ with an “MNR” group has not been reported. Divalent main group imides are normally oligomers, for example the hexagonal prism [(thf)MgNPh]₆,^[6a] and the outcome of the reaction of these clusters with SO₂ is not readily predictable. Here we describe the generation of the novel azadisulfite anion [O₂S(μ-NPh)SO₂]^{2−} (**1**) by the reaction of SO₂ with [(thf)MgNPh]₆. To our knowledge this is the first report of the double insertion of SO₂ into a single functional group. We also describe the product of the reaction of [(thf)MgNPh]₆ with *t*BuNSO, in which the [(thf)₂MgNPh]₂ dimer is trapped by cycloaddition with two molecules of *t*BuNSO.

When SO₂ gas is bubbled into a slurry of [(thf)MgNPh]₆^[6a] in THF, an immediate reaction occurs to give a yellow solution and, subsequently, a pale yellow precipitate. The product **2** is insoluble in diethyl ether, *n*-hexane, and *n*-pentane, sparingly soluble in THF and toluene, but soluble in



benzene. Elemental analyses and ¹H/¹³C NMR spectra of **2** are consistent with the retention of the 1:1 ratio of THF:Ph ligands and the uptake of two SO₂ molecules per MgNPh unit. The X-ray crystal structure analysis^[9] of **2** confirmed these conclusions and revealed that a hexameric arrangement is maintained (Figure 1).

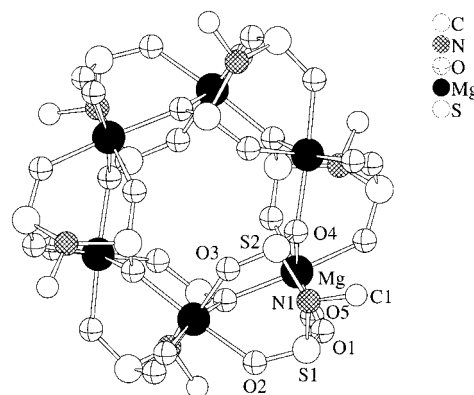
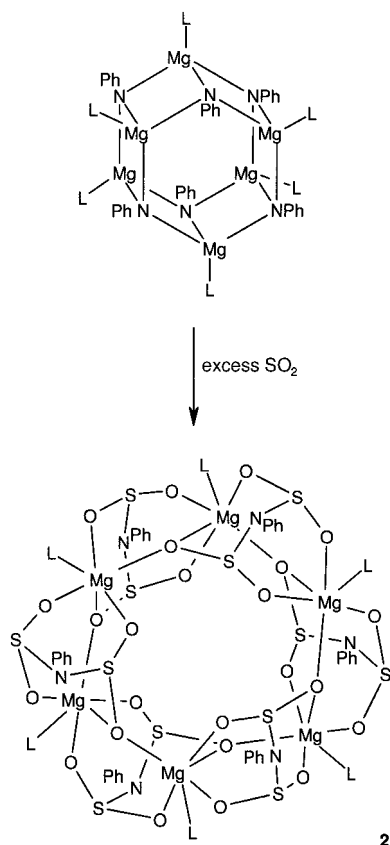


Figure 1. Molecular structure of **2**. For clarity, only the oxygen atoms O5 of the THF molecules and the *ipso*-C atoms of the phenyl groups are shown. Mean values and ranges of bond lengths [Å]: S–O 1.52, 1.479(17)–1.553(16), S–N 1.73, 1.72(2)–1.74(2), Mg–O(*λ*²) 2.03, 2.00(2)–2.046(18), Mg–O(*λ*³) 2.17, 2.151(18)–2.194(17), Mg–O(THF) 2.068(17).

Complex **2** contains a 48-atom Mg₆S₁₂N₆O₂₄ quaternary cluster core with *S*₆ molecular symmetry. It can be viewed as the result of the insertion of twelve SO₂ molecules into the Mg–NPh bonds of [(thf)MgNPh]₆ (Scheme 1), which generates the novel azadisulfite dianion [O₂S(μ-NPh)SO₂]^{2−} (**1**).^[10] Each of these dianions bis-chelates two Mg²⁺ cations, and one oxygen atom exhibits monodentate coordination to a third Mg²⁺ ion. Thus the fundamental building block in the cluster is the adamantane-like Mg₂S₂O₅N unit **3**; each of these units is



Scheme 1. Formation of **2** (L = thf) from [(thf)MgNPh]₆.

fused to two neighboring units through shared Mg–O edges.^[11] A THF ligand completes the octahedral coordination at each Mg²⁺ ion.

For comparison, the reaction of [(thf)MgNPh]₆ with the heteroallene *t*BuNSO, which is isoelectronic with SO₂, was also investigated. In THF solution this reaction produces the magnesium diazasulfite **4**. The ¹H NMR spectrum of **4** in [D₈]THF showed signals for *t*Bu, Ph, and THF ligands in the ratio 1:1:2, consistent with the uptake of one *t*BuNSO molecule per MgNPh unit.



X-ray crystal structure analysis of **4**^[12] revealed a dimeric structure in which the (MgNPh)₂ dimer (one face of the hexagonal prism in [(thf)MgNPh]₆^[6a] is trapped by cycloaddition to two molecules of *t*BuNSO to give the Mg²⁺ salt of the pyramidal diazasulfite dianion [OS(*Nt*Bu)(NPh)]²⁻ (Figure 2). Previously reported dilithium diazasulfites [Li₂{OS(*Nt*Bu)(NR)}]₆ (R = *t*Bu, SiMe₃) form hexameric 36-atom Li₁₂S₆N₁₂O₆ clusters.^[13] The structure of the centrosymmetric Mg₂S₂O₂N₄ cluster core in **4** is reminiscent of the “deck-chair” arrangement of the Sb₃N₆Li core in [Sb₃(Ncy)₄(NMe₂)₂Li] (cy = cyclohexyl).^[14] The mean Mg–N distance in the planar four-membered Mg₂N₂ ring of 2.262 Å is significantly longer than the corresponding distance of 2.06 Å in the hexagonal-prismatic [(thf)MgNPh]₆^[6a] presumably

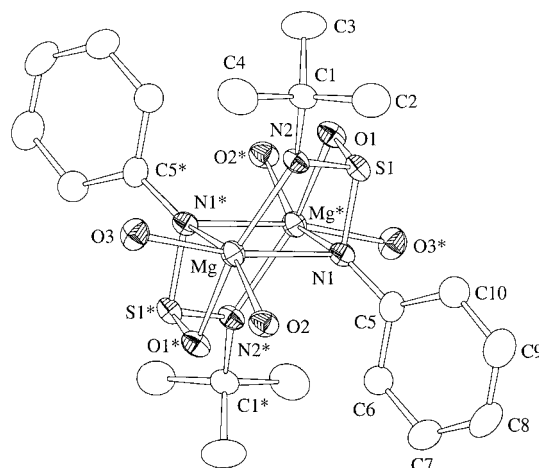


Figure 2. Molecular structure of **4**. For clarity, only the oxygen atoms of the THF molecules are shown, and H atoms are omitted. Displacement ellipsoids are plotted at the 40% probability level. Selected bond lengths [Å] and angles [°]: Mg1–O1* 2.065(4), Mg1–O2 2.117(4), Mg1–O3 2.156(4), Mg1–N1 2.295(4), Mg1–N1* 2.229(5), Mg1–N2 2.123(4), S1–N1 1.672(4), S1–N2 1.610(4), S1–O1 1.541(4); O1*–Mg1–N2 158.76(17), O2–Mg1–N1* 157.13(16), O3–Mg1–N1 170.09(17), O1–S1–N1 96.8(2), O1–S1–N2 110.6(2), N1–S1–N2 96.7(2). Symmetry transformation used to generate equivalent atoms marked with an asterisk: $-x+1, -y, -z+1$.

reflecting the higher coordination number (λ^6) of Mg²⁺ in **4**. The octahedral geometry about the magnesium center is significantly distorted by the formation of two chelate rings, (N1–Mg1–N2 67.33(15), N1*–Mg1–O1* 67.98(15)°). The geometry at the three-coordinate N2 atoms is exactly planar. The S–N and S–O distances are similar to those reported for dilithium diazasulfites.^[13] Unlike the dilithium derivatives,^[15] however, the magnesium diazasulfite **4** does not form colored radicals upon exposure to oxygen (ESR spectroscopy). In contrast to the reaction with SO₂, **4** is the only product formed when [(thf)MgNPh]₆ is treated with an excess of *t*BuNSO. On the other hand the reaction of [(thf)MgNPh]₆ with only six equivalents of SO₂ gives a 1:1 mixture of **2** and unconverted [(thf)MgNPh]₆.

In summary, the reaction of the magnesium imide [(thf)MgNPh]₆ with sulfur dioxide provides the first example of the formal double insertion of two SO₂ molecules. The [PhN(SO₂)₂]²⁻ ion **1** so generated is a potentially interesting multidentate ligand.

Experimental Section

All manipulations were carried out under anaerobic and anhydrous conditions.

2: Dry sulfur dioxide gas was bubbled into a stirred slurry of [(thf)MgNPh]₆^[6c] (2.00 g, 1.78 mmol) in THF (60 mL) at 23 °C for 10 min. The reaction mixture became clear yellow and then cloudy. Removal of all volatile materials under vacuum and subsequent washing of the yellow residue with *n*-pentane (2 × 50 mL) yielded, after drying, pale yellow **2** (2.84 g, 1.50 mmol, 84%); m.p. 140 °C (decomp.). Elemental analysis calcd for C₁₀H₁₃MgNO₃S₂: C 38.05, H 4.15, N 4.44; found: C 37.85, H 4.06, N 4.67; ¹H NMR (C₆D₆, 23 °C): δ = 7.81 (m, 2H, *o*-H), 7.03 (m, 2H, *m*-H), 6.86 (m, 1H, *p*-H), 3.84 (m, 4H, (CH₂)₂(CH₂)₂O), 1.25 (m, 4H, (CH₂)₂(CH₂)₂O); ¹³C{¹H} NMR (C₆D₆, 23 °C): 139.3 (s, *ipso*-C), 129.4 (s, *o*-C), 129.2 (s, *m*-C), 126.7 (s, *p*-C), 68.8 (s, (CH₂)₂(CH₂)₂O), 25.2 (s, (CH₂)₂(CH₂)₂O).

4: A solution of *t*BuNSO^[16] (0.50 g, 4.20 mmol) in THF (15 mL) was added slowly to a stirred slurry of [(thf)MgNPh]₆^[6c] (0.79 g, 0.70 mmol) in THF

(45 mL) at 23 °C. The clear yellow reaction mixture was stirred for 4 h, and a precipitate formed. Removal of all volatile materials under vacuum and subsequent washing of the yellow residue with *n*-pentane (2 × 30 mL) yielded, after drying, powdery white **4** (1.32 g, 1.74 mmol, 83 %); m.p. 190 °C (decomp.). Elemental analysis calcd for C₁₈H₃₀MgN₂O₃S: C 57.07, H 7.98, N 7.40; found: C 57.35, H 7.77, N 7.59; ¹H NMR ([D₈]THF, 23 °C): 7.23 (br, 2H, *o*-H), 6.86 (m, 2H, *m*-H), 6.33 (m, 1H, *p*-H), 3.58 (m, 8H, (CH₂)₂(CH₂)₂O), 1.74 (m, 8H, (CH₂)₂(CH₂)₂O), 1.34 (s, 9H, C(CH₃)₃); ¹³C{¹H} NMR ([D₈]THF, 23 °C): 154.0 (s, *ipso*-C), 128.4 (s, *o*-C), 115.0 (s, *m*-C), 96.8 (s, *p*-C), 67.9 (s, (CH₂)₂(CH₂)₂O), 52.4 (s, C(CH₃)₃), 34.3 (s, C(CH₃)₃), 26.4 (s, (CH₂)₂(CH₂)₂O).

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Controllable Orientation of Helical Poly(L-glutamic acid) Rods through Macro-dipole Interaction on Gold Surfaces and Vectorial Electron Transfer

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Helix orientation at interfaces is one of the key factors to fabricate biofunctional synthetic polymer systems that mimic natural polymers such as proteins. The orientation of segments in membrane proteins is known to govern functions such as molecular recognition and electrochemical communication, including electron transfer. In this context, there has been considerable interest in constructing perpendicularly oriented polypeptide monolayers on aqueous or solid substrates. It has been indicated that α helices prefer to lie flat on every possible interface.^[1] Efforts have been made to orient α helices perpendicular to the surface of the water or solid substrates,^[2–5] by incorporation of functional groups onto one terminus of polypeptide helices. Such groups under consideration include quaternary ammonium groups^[6, 7] to interact with anionic templates, a crown ether moiety,^[8–10] or a sulfur atom^[11–14] to attach to a gold surface.

In this communication, we describe the reversible variation of the orientation of a disulfide-modified poly(L-glutamic acid) (PLGA-SS) α helix assemblies attached to a gold surface and their enhanced molecular packing based on the interaction of helix macroscopic dipoles (“macro-dipoles”). We

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have investigated the macrodipole-induced formation of aggregates of α helical polypeptides on water^[15] and gold substrates.^[13a] The macrodipole of helical polypeptides, resulting from the accumulation of dipole moments of the component peptide bonds, produces an electrostatic potential directed from the N-terminus to the C-terminus.^[16, 17] Such an electrostatic potential plays an important role in the higher-order structures and functions of proteins.^[18–20] Fox et al.^[21, 22] have demonstrated that the macrodipole influences the efficiency of photoinduced, intramolecular fluorescence quenching. In our previous report,^[13b] we have provided a direct evidence for helix–helix macrodipole interaction by exploring an attractive interaction between the PLGA-SS monolayers on gold and redox-active PLGA derivatives (PLGA-Fc-N and PLGA-Fc-C) as “guest” helices (Figure 1). Now we report on the extension of this PLGA-helix system to the control of the orientation and molecular packing of the helix monolayers and also on mediator functions of the redox-active guest helices.

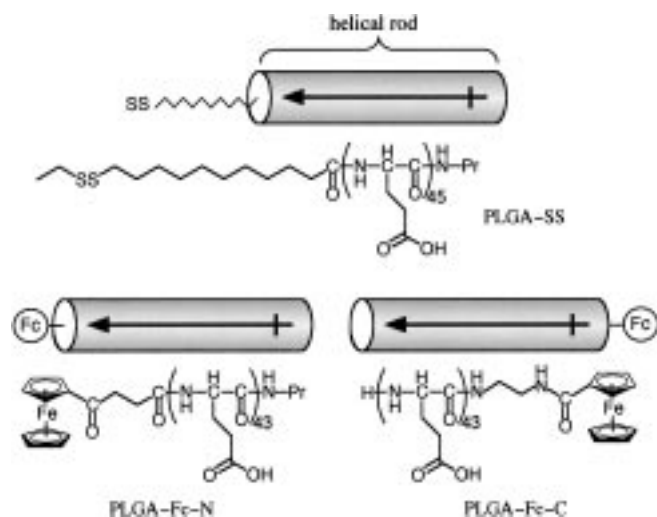


Figure 1. Molecular structures for the monolayer-forming PLGA disulfide derivative (PLGA-SS) and the ferrocenyl-modified, redox-active PLGA guests (PLGA-Fc-N, PLGA-Fc-C) used in this study. The corresponding schematic models of the helical rods are also shown. Arrows indicate the moment of the helix macrodipole.

We have previously developed a technique^[13b] for the lateral molecular distribution of PLGA-SS onto a gold surface. By such a technique, the molecular packing within the resulting monolayer can be readily varied by adjusting the conformational size of the PLGA segments through the solution pH.

To examine the orientation of helical rods, two kinds of self-assembled monolayers of PLGA-SS were prepared on gold substrates: one of them is the densely packed monolayer prepared at pH 4.5, in which the PLGA segments take a α helix structure (monolayer **I**); the other is the more loosely packed monolayer prepared at pH 9.0, in which the PLGA segments take an expanded coil conformation (monolayer **II**). After reaching adsorption equilibrium, both of the monolayers were immersed into water at pH 3.5 to convert the conformation of the PLGA segments completely to the α

helix structure. In addition, as previously reported, monolayer **II** has suitable cavities for accepting guest PLGA helices (PLGA-Fc-N or PLGA-Fc-C).^[13b] Figures 2a and 2b display

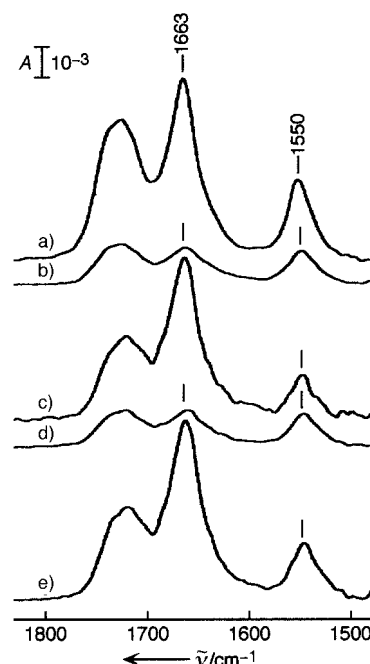


Figure 2. RA-FTIR spectra of the PLGA-SS monolayers: a) prepared at pH 4.5 and then treated with water of pH 3.5 (monolayer **I**); b) prepared at pH 9.0 and then treated with water of pH 3.5 (monolayer **II**); c) monolayer **II** complexed with PLGA-Fc-N; d) monolayer **II** after desorption of PLGA-Fc-N; and e) monolayer **II** re-complexed with PLGA-Fc-C.

reflection–absorption (RA) FTIR spectra of the C=O stretching region for monolayers **I** and **II**, respectively. The differences in absorption are remarkable, reflecting differences in surface coverage. In the amide I and amide II regions (1700–1500 cm^{−1}), both spectra exhibit two bands at 1663 and 1550 cm^{−1}, which are assigned to the α helix structure.^[23, 24] The additional C=O stretching band, due to COOH groups of the side chain of PLGA, is observed at 1730 cm^{−1}. Enriquez et al.^[25] demonstrated that the nature of the order and average orientation of the polypeptide α helix in the films can be inferred from RA-FTIR data. By using the ratio of the integrated intensities of the amide I and amide II regions, $D = A_I/A_{II}$, the average tilt of the helix axis from the surface normal, $\langle \theta \rangle$,^[26] was evaluated to be 40° and 51° for monolayers **I** and **II**, respectively (Table 1). Such a difference in the tilting angle can be reasonably interpreted by accounting for the

Table 1. Ratio D of the integrated intensities of the amide I to amide II regions from RA-FTIR spectra and the average tilt angle of the helix axes from the surface normal ($\langle \theta \rangle$).^[a]

System	D	$\langle \theta \rangle$ [°]
monolayer I	2.8	40 ± 3
monolayer II	1.7	51 ± 3
monolayer II with PLGA-Fc-N	5.8	25 ± 3
monolayer II without PLGA-Fc-N	1.5	55 ± 3
monolayer II with PLGA-Fc-C	5.2	27 ± 3

[a] The $\langle \theta \rangle$ values were estimated from the D values,^[26] with $\tilde{\nu}(\text{amide I}) = 1663$, $\tilde{\nu}(\text{amide II}) = 1550$ cm^{−1}. Details are described in the text.

molecular packing density in the monolayers; for the more densely packed monolayer **I**, the helices are obliged to orient more perpendicularly to the surface due to crowding with the helical rods in comparison to the less densely packed monolayer **II**. The rigid PLGA-SS helical rods, anchored to the gold surface through a flexible methylene spacer, will lie as flat as possible on the substrate, as observed for the loosely packed monolayer **II**. It should be noted that also the helices of monolayer **I** are tilted from the surface normal in spite of their high packing density. In this monolayer, the helices must be assembled into a parallel packing. These helical peptides are known to carry a macrodipole moment along the helix axis.^[15] Thus, a parallel packing of the helices is energetically unfavorable, and as a result the helical rods in monolayer **I** achieve a tilting angle of 40° to compensate for such an energetic disadvantage.

Figure 2c shows the RA-FTIR spectrum of monolayer **II** after complexation with PLGA-Fc-N. In the previous study,^[13b] we demonstrated that monolayer **II** is able to accept both PLGA-Fc-N and -C guests at a 1:1 composition through macrodipole interaction. From the value of the ratio *D*, the tilting angle of the helices is calculated to be 25° (Table 1), which is significantly enhanced in the perpendicular orientation of helical rods, compared with the 51° tilt found before complexation. This result suggests that by complexation of monolayer **II** with the PLGA-Fc-N helix, a monolayer in a head-to-tail antiparallel orientation, which is energetically more favorable, is spontaneously formed, and consequently achieves a nearly perpendicular helix orientation. This effect is shown schematically in Figure 3a and 3b. When this complexed monolayer was treated with water at pH 9.0, the PLGA segments underwent a conformational change from α helix to random coil, then the monolayer released the guest

PLGA-Fc-N into the bulk aqueous phase because of the loss of the helix macrodipole. An RA-FTIR spectrum of the monolayer after the pH-induced desorption of PLGA-Fc-N is similar to the original monolayer (Figure 2d and 2b), and consequently the tilting angle returns to the original value of monolayer **II** (Table 1). The spectrum of Figure 2e arises when the other guest, PLGA-Fc-C, is incorporated into monolayer **II**. The calculated tilting angle of the helices (Table 1) again approaches the perpendicular orientation.

The molecular packing of these monolayers before and after binding of the guest PLGAs was subsequently evaluated as an electrochemical barrier toward the redox couple of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$. Figure 4a shows a cyclic voltammogram (CV) for the gold surface (electrode) modified with monolayer **II**, in comparison with that for a bare electrode. The

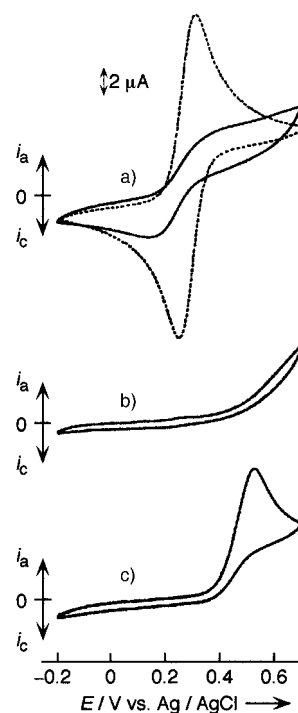


Figure 4. Cyclic voltammograms of gold electrodes modified with monolayer **II** in the presence of 3 mM $[\text{Fe}(\text{CN})_6]^{4-}$ (scan rate 100 mVs⁻¹): a) bare gold electrode (---); b) after complexation with PLGA-Fc-N; and c) after complexation with PLGA-Fc-C.

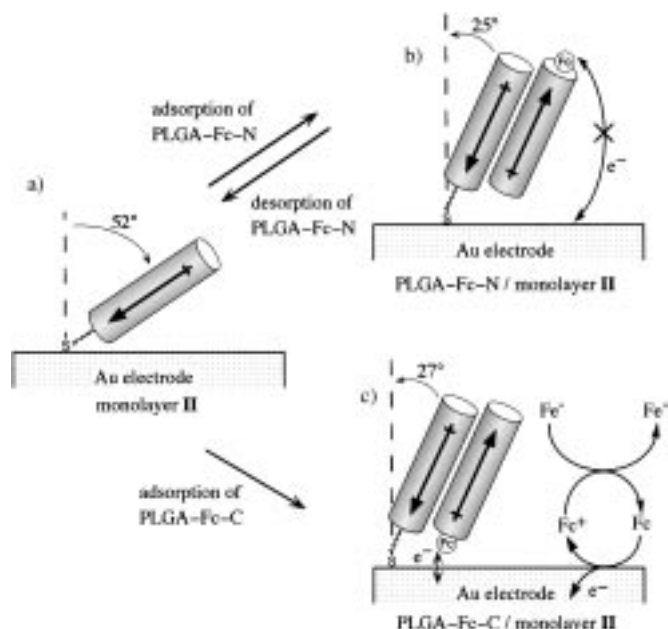


Figure 3. Illustration of the proposed mechanism for the reversible variations in PLGA helix rod orientation upon adsorption and desorption of guest helices and for the electron mediation function of the ferrocenyl moiety on gold substrates: a) pure monolayer **II**; b) monolayer **II** complexed with PLGA-Fc-N; and c) monolayer **II** complexed with PLGA-Fc-C.

peaks due to the redox reaction of $\text{Fe}^{\text{III}} \leftrightarrow \text{Fe}^{\text{II}} + \text{e}^-$ are observed but their peak currents are considerably suppressed in comparison with those of the bare electrode. This implies that a monolayer was formed while having a void for the probe ion to diffuse to the electrode surface. When this electrode was immersed into an aqueous solution with PLGA-Fc-N at pH 3.5 to adsorption equilibrium, the peaks for the $\text{Fe}^{\text{III}} \leftrightarrow \text{Fe}^{\text{II}} + \text{e}^-$ reaction were found to disappear completely (Figure 4b), indicating that the electrochemical blocking ability of the monolayer was markedly enhanced because of binding of the PLGA-Fc-N guest, which has the ferrocenyl moiety at the N-terminus which is, by the macrodipole interaction, oriented away from the surface. Figure 4c displays a CV curve after binding of the guest PLGA-Fc-C, which has the ferrocenyl moiety at the C-terminus which is oriented towards the surface. In this case, there is also no peak that corresponds to the redox reaction of $\text{Fe}^{\text{III}} \leftrightarrow \text{Fe}^{\text{II}} + \text{e}^-$, reflecting the same blocking ability as observed for the electrode bound with the PLGA-Fc-N monolayer. As reported previously,^[13b] when the CV analysis had been performed for this electrode covered with a PLGA-Fc-C monolayer under the same conditions except for absence of $[\text{Fe}(\text{CN})_6]^{4-}$, two peaks had appeared at around +0.5 V (vs. Ag/AgCl) due to one-electron oxidation and reduction of the ferrocenyl moiety of the bound PLGA-Fc-C. Furthermore, the peak current (*i*) acquired at different potential scan rates

(ν) was found to vary linearly with the scan rate, indicating that PLGA-Fc-C was bound to the monolayer **II** and not subject to diffusion to the electrode. In the presence of $[\text{Fe}(\text{CN})_6]^{4-}$ there appears, however, an oxidation peak for the ferrocenyl moiety at around +0.5 V (vs. Ag/AgCl), with a remarkably large current compared with that observed in the absence of $[\text{Fe}(\text{CN})_6]^{4-}$ at the same scan rate, and no reduction peak was observed. This electrode, when the scan rate was varied at a constant concentration of $[\text{Fe}(\text{CN})_6]^{4-}$ (3 mM), yields a linear i versus $\nu^{1/2}$ plot, to show the catalyzed reaction is sufficiently fast such that the process is controlled by $[\text{Fe}(\text{CN})_6]^{4-}$ diffusion.^[28] From these results, it can be assumed that the immobilized ferrocenyl moiety facing the electrode surface behaves successfully as an electron-transfer mediator and the direction of electron transfer must be vectorial as schematically illustrated in Figure 3. To further support this hypothesis, the dependence on the $[\text{Fe}(\text{CN})_6]^{4-}$ concentration was examined under conditions similar to those in Figure 4c (data not shown). The oxidation peak of the ferrocenyl moiety grew upon increasing the concentration of $[\text{Fe}(\text{CN})_6]^{4-}$, as expected for a mediated process.^[29]

In conclusion, the orientation of helix rods and the precise location of a redox moiety in self-assembled monolayers have been demonstrated to be controlled through the helix–helix macrodipole interaction. The presented polypeptide assembly system must be a useful model of electron-transfer functions in cell membranes.

Experimental Section

Materials: poly(L-glutamic acid) derivatives PLGA-SS, PLGA-Fc-N, and PLGA-Fc-C were prepared as previously described^[13b] and characterized by MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight) mass spectrometry.

Fabrication of PLGA-SS-modified surfaces: A gold disk electrode (BAS, surface 0.02 cm²) was cleaned by sonication in a 0.1 M aqueous KOH solution for a few minutes, careful polishing with diamond and alumina pastes (mean particle sizes 1 μm and 0.05 μm , respectively), and rinsing with Milli-Q purified water. Gold substrates, on clean glass slides, used for RA-FTIR were prepared by thermal evaporation of gold at a pressure of ca. 10^{-6} Torr and cleaned by rinsing with CHCl_3 and Milli-Q water prior to use. The monolayers were prepared by immersing the gold substrates into aqueous solutions containing PLGA-SS (1 mM) at pH 4.5 or pH 9.0 until adsorption equilibrium was reached, followed by incubating in water (pH 3.5).

Measurements: Cyclic voltammetry was performed at 25 °C with a CV-1B cyclic voltammograph (BAS). A standard three-electrode configuration was used with the monolayer sample on gold as the working electrode, Ag/AgCl (3 M NaCl) as the reference electrode, and a platinum wire as the counter electrode. $\text{K}_4[\text{Fe}(\text{CN})_6]$ was obtained commercially and used as received. The solutions containing 1 M KCl as a supporting electrolyte were deoxygenated by an N_2 purge. FTIR was performed on a Nicolet System 800 spectrometer with a mercury-cadmium-tellurium (MCT) detector. The RA-FTIR measurements used 1024 scans of interferogram accumulations with the bare gold substrate as reference. The optical path

was purged with dry air before and during measurements. A reflection attachment, at an incident angle of 80°, together with a polarizer was used.

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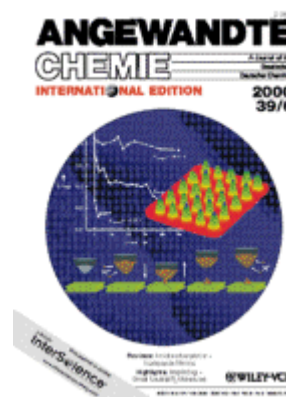
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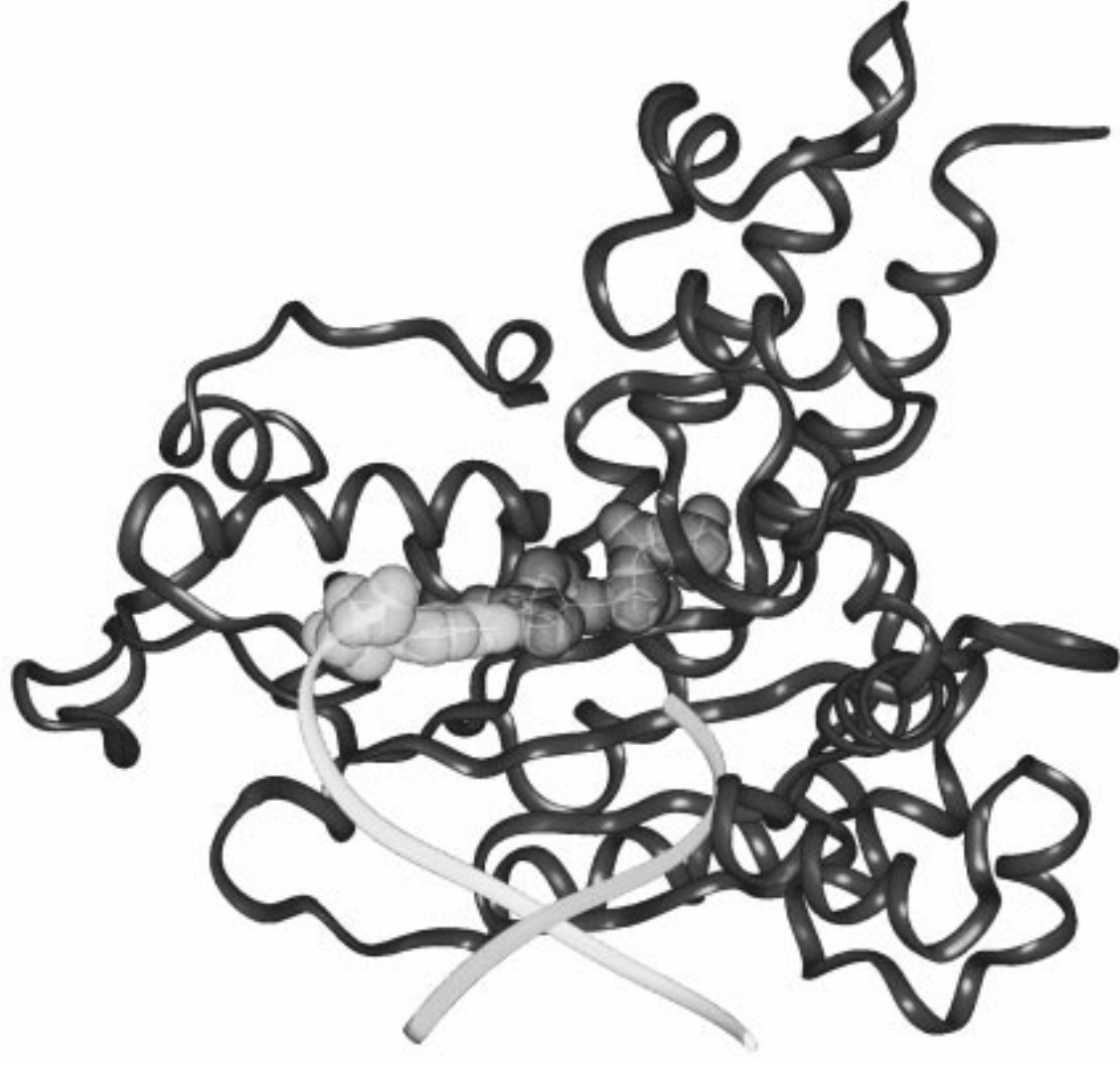
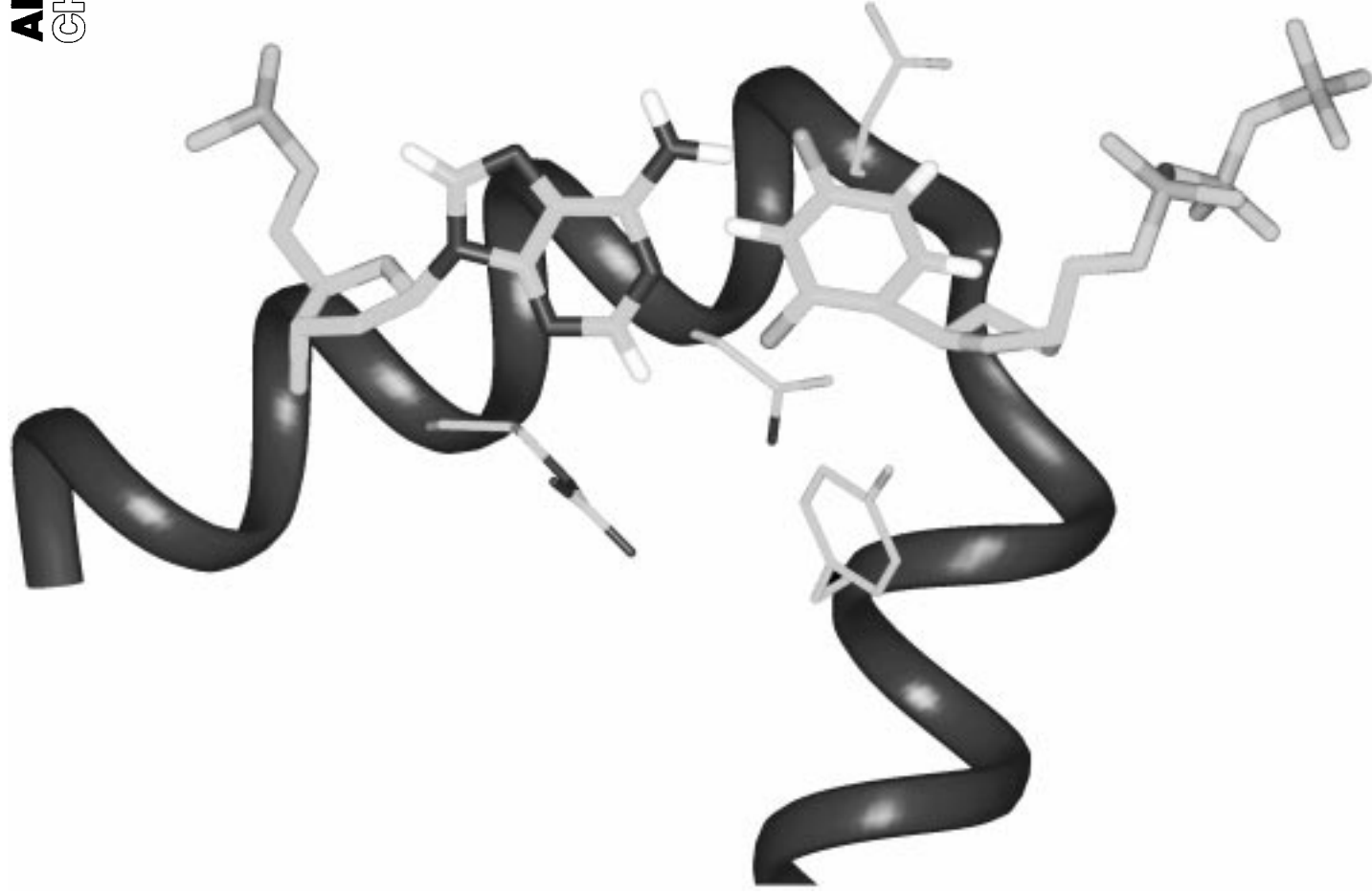
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- $$D\left(\frac{A_{\text{I}}}{A_{\text{II}}}\right) = K \frac{\frac{1}{2}(\sin \langle \theta \rangle \sin 39^\circ)^2 + (\cos \langle \theta \rangle \cos 39^\circ)^2}{\frac{1}{2}(\sin \langle \theta \rangle \sin 75^\circ)^2 + (\cos \langle \theta \rangle \cos 75^\circ)^2} \quad (1)$$
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Cover Picture

Dieter M. Kolb, Gerald E. Engelmann, Jörg C. Ziegler

The cover picture shows, on the right, a field of 25 copper clusters on a gold(111) electrode surface. The clusters were deposited from solution with the tip of a scanning-tunneling microscope. The clusters were first isolated from the electrolyte onto the tip and selectively transferred onto the surface by the tip being in proximity to the substrate (see under for a sketch of the mechanism). The metal clusters are composed of approximately 100 atoms and are unexpectedly stable against anodic dissolution. As the picture on the left illustrates, the clusters also remain stable for a long time against a positive Nernst potential. In the background, one sees a section of the gold surface with the copper clusters.





Mimicking the Structure and Function of DNA: Insights into DNA Stability and Replication

Eric T. Kool,* Juan C. Morales, and Kevin M. Guckian

The physical and chemical factors that allow DNA to perform its functions in the cell have been studied for several decades. Recent advances in the synthesis and manipulation of DNA have allowed this field to move ahead especially rapidly during the past fifteen years. One of the most common chemical approaches to the study of interactions involving DNA has been the use of DNA base analogues in which functional groups are added, deleted, blocked, or rearranged. Here we describe a different strategy, in which the polar natural DNA bases are replaced by nonpolar aromatic molecules of the

same size and shape. This allows the evaluation of polar interactions (such as hydrogen bonding) with little or no interference from steric effects. We have used these nonpolar nucleoside isosteres as probes of noncovalent interactions such as DNA base pairing and protein–DNA recognition. We have found that, while base-pairing selectivity does depend on Watson–Crick hydrogen bonding in the natural pairs, it is possible to design new bases that pair selectively and stably in the absence of hydrogen bonds. In addition, studies have been carried out with DNA polymerase enzymes to investi-

gate the importance of Watson–Crick hydrogen bonding in enzymatic DNA replication. Surprisingly, this hydrogen bonding is not necessary for efficient enzymatic synthesis of a base pair, and significant levels of selectivity can arise from steric effects alone. These results may have significant impact both on the study of basic biomolecular interactions and in the design of new, functionally active biomolecules.

Keywords: base pairing • DNA polymerase • hydrogen bonds • nucleobases

1. Introduction

1.1. Overview

We describe a series of studies involving a new class of nucleoside mimics. These molecules, called nonpolar nucleoside isosteres, imitate the shape of the natural nucleosides and nucleotides as closely as possible, but they lack Watson–Crick hydrogen-bonding ability. This makes them especially valuable as mechanistic tools for the study of the importance of these noncovalent bonds in the various functions of nucleic acids.

These studies have led to some surprising observations about the mechanism whereby polymerase enzymes copy a

strand of DNA. The results have potentially important implications in the basic scientific study of biochemical and biophysical structure and function, and may lead to practical applications as well. In this paper, we first briefly review the basic structure formed by DNA and the functions that it serves in a cell. Then we describe how the structure and functions of DNA can be mimicked successfully by consideration of simple steric factors. Finally, we discuss the implications and possible future directions for this work.

1.2. The Structures and Functions of DNA

The three dimensional structure of the DNA double helix was correctly elucidated by Watson and Crick more than four decades ago.^[1] The central features of this structure include an anionic sugar–phosphate backbone that winds around the outside of the cylindrical helix, and neutrally charged nucleobases that occupy the center.^[2] The paired bases are roughly perpendicular to the helical axis, and are stacked in van der Waals contact with one another. The right-handed twist of the backbone (in B-form DNA) defines two grooves in the helix (the major and the minor) which are lined by the

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sugar–phosphate backbone (serving as the walls of the grooves) and the edges of the bases (which act as floors).

The chemical moieties that make up the primary structure of DNA are shown in Scheme 1. The phosphodiester backbone includes 2'-deoxyribose residues which serve to carry and orient the DNA bases. The backbone as a whole is highly polar because of the negative charge carried on each phosphate diester at neutral pH. The nucleobases themselves are aromatic nitrogen heterocycles comprised of monocyclic pyrimidines and bicyclic purines. They are generally quite polar along their edges, carrying multiple substituents such as amino, imino, and carbonyl groups.

The primary function of DNA is the storage of genetic information; the three billion base pairs in each human cell encode the synthesis of approximately 100 000 proteins. It is, of course, crucial that not only are these proteins synthesized faithfully, but also that this genetic information is transferred accurately from generation to generation in a line of organisms. Central to these abilities is the double helical structure of DNA. Its antiparallel, C₂-symmetrical structure with complementary hydrogen-bonded base pairs provides an extraordinarily effective means to carry out storage and transfer of the encoded messages.

DNA also acts as a structural scaffold for the arrangement of multiprotein complexes in a cell. This water-soluble scaffold is inherently rather rigid in a local sense, but proteins can use their binding energy to remodel the duplex by bending, winding, or unwinding it. In addition, chemists are currently making use of the structural properties of DNA to build artificial scaffolds for use in materials science and biomedical applications.^[3–11]

1.3. Testing the Paradigms for DNA Stability and Replication

The aim of this paper is to examine some of the most basic questions of DNA structure and function. To begin to answer such questions, the chemist turns naturally to the idea of using nucleoside analogues. However, the large majority of existing analogues of natural DNA bases have significant structural changes relative to their natural congeners. Even a seemingly small perturbation such as alkylation of a base leads to significant steric changes in the molecule. For example, methylation of a hydrogen-bonding group might decrease a

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E. T. Kool



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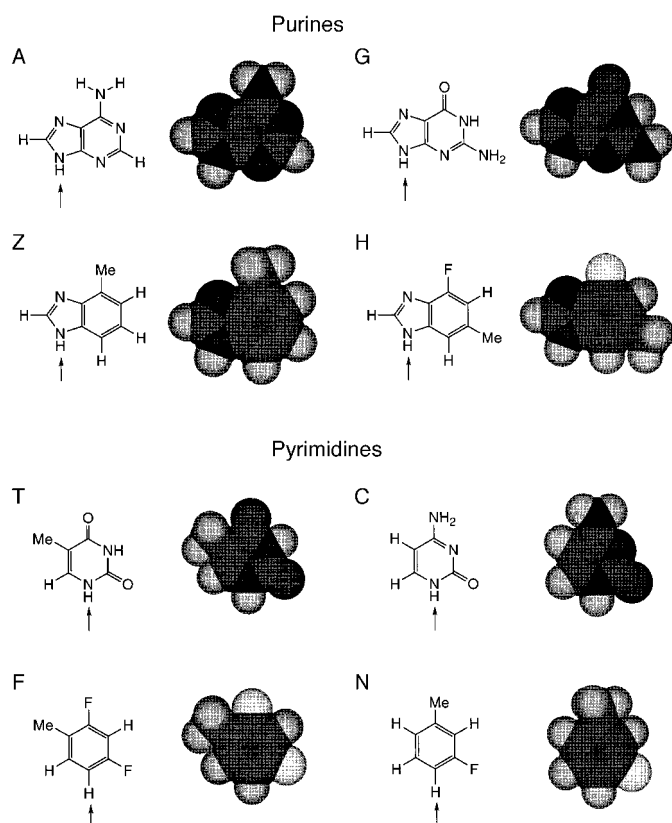


K. M. Guckian

research interests fall in the field of bioorganic chemistry, and include the design of molecules which can recognize DNA and RNA sequences and structures, and of molecules that mimic biological functions. More than 70 research publications to date describe this work, and he has presented over 120 invited lectures. He has received several awards in recognition of his work, including the Arthur C. Cope Scholar Award of the American Chemical Society, an Alfred P. Sloan Foundation Fellowship, the Young Investigator Award of the US Office of Naval Research, the US Army Research Office Young Investigator Award, the Arnold and Mabel Beckman Foundation Young Investigator Award, an American Cyanamid Faculty Award, and the Camille and Henry Dreyfus Foundation Teacher–Scholar Award.

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Kevin M. Guckian was born and raised in Rochester, NY (USA). He obtained his B.Sc. in chemistry from Oswego State University in 1995. Since 1995 he has studied the structure and function of DNA using nonpolar base analogues under the supervision of Prof. E. T. Kool at the University of Rochester. His research interests focus on protein–DNA interactions.



Scheme 1. Chemical formulae and space-filling models of the four natural bases and their corresponding nonpolar isosteres. Arrows indicate point of attachment of deoxyribose.

protein's affinity for the DNA, but the reason for this could be either loss of the hydrogen bond or steric repulsion.

For this reason, we designed a series of compounds that are meant to mimic DNA base structure but to lack Watson–Crick hydrogen-bonding ability.^[12, 13] These “nonpolar nucleoside isosteres” (Scheme 1) are simple compounds and are generally prepared in short syntheses. In this design, substituted benzenes mimic the pyrimidine bases, and indoles or benzimidazoles replace the purines. Of the four shown in Scheme 1, we consider two to be nearly perfect isosteres (H—an analogue of G, and F—an analogue of T). We consider N and Z less ideal, because in these a nitrogen in the aromatic ring is replaced by the sterically larger C–H group. This adds approximately 0.7 Å of steric bulk to the molecules at this position. Nonetheless, these are the closest possible isosteres available which lack the ability to form hydrogen bonds.

1.4. Possible Outcomes

The intended use for these molecules was to substitute them for the natural nucleosides in DNA, and evaluate whether they function properly. One possible outcome of such experiments is negative; that is, the molecular mimics may lack essentially all ability to function properly. However, it is sometimes difficult to draw confident conclusions when a negative result is found, because it is usually true that in changing a molecule, more than one property is affected.

Probably more useful and important is the second possible outcome, retention of activity. For example, deletion of a hydrogen-bonding group in DNA might conceivably result in little loss of stability or activity. In such a case one can be somewhat more confident in concluding that the hydrogen bond was probably not energetically important to the stabilization of a ground or transition state. It is notable that similar analyses have been carried out for years in site-directed mutagenesis studies of proteins.

The basic tenets of science become paradigms when they have been repeatedly tested and questioned. Here we ask some of these questions again.

Are hydrogen bonds necessary for stabilization of the DNA helix? Since Watson and Crick's elucidation of the duplex structure, it has been generally accepted that the hydrogen bonds between the bases are critical to the stability of DNA. A Watson–Crick pair has two or three such bonds, and these add approximately 0.5–1.8 kcal mol^{−1} of stabilization per base pair of DNA.^[14–17] The uncertainties in this value arise from the fact that the experimentally measured value varies greatly depending on the molecular context. Of course, this relatively low energetic stabilization reflects the competition with water, because breaking each hydrogen bond in a base pair (by separating the bases) also means the simultaneous formation of two hydrogen bonds with water molecules. In the gas phase, where such competition does not occur, experiments show that individual hydrogen bonds add stabilization of 6–7 kcal mol^{−1} with the kinds of functional groups found in DNA.^[18, 19] Although DNA has been studied for decades, the role of hydrogen bonds in stabilization of the helix and selective base-pair formation is still unclear.

Is stacking of the aromatic bases less important to helix stability than hydrogen bonds? In general, the number of hydrogen bonds in the base pairs have received more attention than base stacking in explaining DNA function. However, in B-form DNA, neighboring base pairs make contact over approximately 115 Å², by overlap of one π -system with the next (Figure 1). The formation of a helix from a random coil therefore involves removing as much as 230 Å²

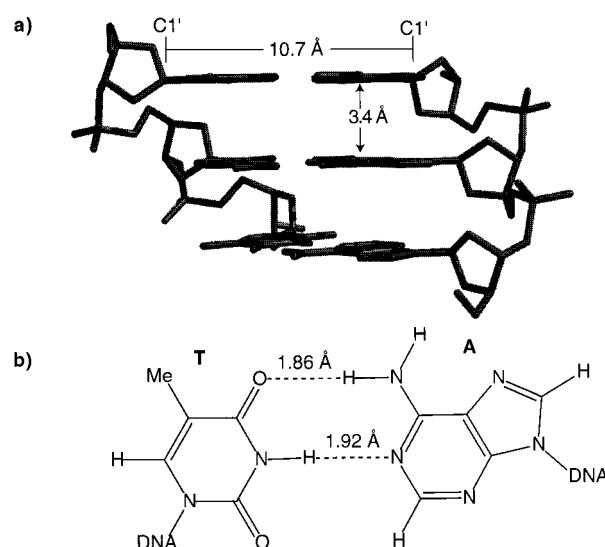


Figure 1. a) Model of a three base-pair B-form DNA duplex with distances between strands and bases. b) Watson–Crick A–T base pair.

of base π -surface area from the solvent per pair, assuming that no such stacking occurs in the single-stranded coil. The specific noncovalent forces that stabilize this stacking are relatively poorly understood. Although permanent electrostatic effects, induced dipole attractions, and solvophobic effects may all play a role, the relative contributions of these effects are still not clear.^[20–26]

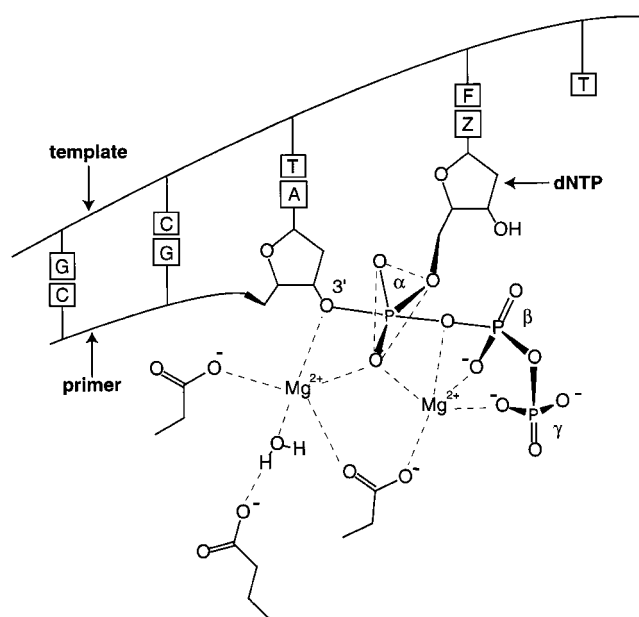
Are hydrogen bonds necessary for the specificity of base pairing? Hydrogen bonds are widely believed to be one of the most important factors in the specificity of biomolecular recognition. Again, if one examines the existing data on natural and modified DNAs, one would tend to support this paradigm. For example, synthetic DNA duplexes have been widely studied for the thermodynamics of stabilization. When a complementary base pair is mutated to a noncomplementary (mismatched) one, the energetic penalty is quite large. For example, a T–C mismatch destabilizes the helix by approximately 3.2–5.8 kcal mol^{–1} relative to a T–A matched pair.^[27–29] However, if only hydrogen bonding is disrupted by mismatches such as T–C, then why does it destabilize the DNA so much? It is reasonable to assume that the stacking of the bases continues to stabilize even with this mismatch. One possible answer to this question might be that the T–C pair might lead to structural distortion of the DNA beyond that pair alone. Indeed, structural studies of mismatched pairs in DNA, such as G–T, and T–T pairs,^[30–32] suggests that they may well distort the DNA. Significantly, they are usually hydrogen bonded, but to form the orientation necessary for hydrogen bonding, the pair usually adopts a geometry different than in the normal pairs, thus distorting the DNA backbone as well.

Are Watson-Crick hydrogen bonds essential to DNA replication? The enzymatic replication of DNA occurs with high efficiency (approximately 20–1000 nucleotides per second) and with very high fidelity (with an error only once in 10³–10⁵ nucleotide insertions).^[33–40] The idea that Watson–Crick hydrogen bonds are primarily, even solely, responsible for these effects has been taught in biochemistry and molecular biology classes for decades. Commonly used textbooks in these fields nearly always cite the high specificity of hydrogen bonds in explaining DNA synthesis fidelity.^[41, 42] However, as mentioned above, several forces are involved in the stabilization of DNA in the absence of enzymes. It would seem reasonable to assume that these may also affect the stability of the transition state of the polymerase catalyzed reaction (Scheme 2). Thus, in addition to hydrogen bonds, what are the effects of permanent dipolar or electrostatic attractions in the stacking of the incoming nucleotide? What are the effects of solvation on this process? And importantly, what are the effects of steric size and shape in the polymerase active site?

2. Mimicking the Primary Structure

2.1. Shape and Conformation

To address such questions more directly, we have studied nonpolar nucleoside isosteres, which mimic the shape of the



Scheme 2. Schematic representation of the transition state of the DNA polymerase reaction. A detailed description of the two metal ion mechanism is given by Steitz and co-workers.^[142, 143]

natural bases more closely than the previously known analogues (Scheme 1). The structures of these nucleosides have been studied computationally and experimentally. X-ray crystal structures of dF and dZ have been published, with comparisons made to their natural counterparts dT and dA.^[43, 44] The structure of dF^[43] in the crystal is remarkably close to that of thymidine^[45, 46] (Figure 2). The two have identical sugar puckers and very similar glycosidic torsional angles. The structure of dZ differs somewhat from that of deoxyadenosine in the solid state.^[44] The two sugars have different conformations (although both are skew conformers), and the glycosidic orientation of dZ is *syn*, while that of dA is *anti*.

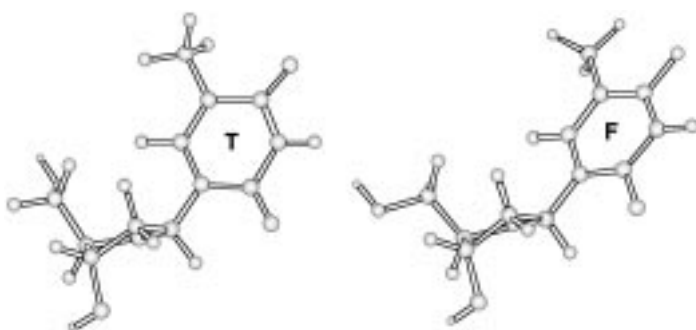


Figure 2. Single-crystal X-ray structure of difluorotoluene deoxynucleoside (right) compared to the published structure of thymidine (left).^[45, 46]

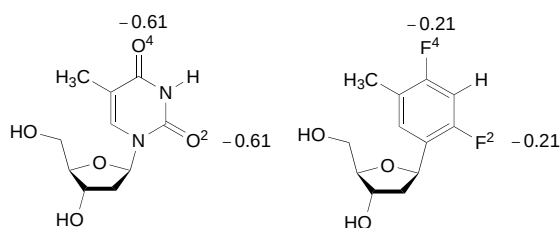
More relevant to the biochemical activities are the solution structures of these analogues, which have also been studied in D₂O by NMR spectroscopic methods. Here it was shown that analogue dF has a sugar conformation quite similar to that of dT in solution, with dF being classified as 90–100% in the skew conformation and dT as 70%.^[43] Both nucleobases are

oriented *anti*, or away from the sugar, as nucleosides normally are in DNA. In solution, the sugars of dA and dZ are almost identical (60% and 70% south conformation, respectively), and the bases are both *anti*. Thus, in solution both analogues are very nearly perfect in their steric and conformational mimicry of the natural nucleosides.

2.2. Electronic Factors

There has been some discussion in the literature as to whether organofluorine compounds can form hydrogen bonds with good hydrogen-bond donors.^[47, 48] This question is of particular interest in the present case, because difluorotoluene might conceivably form two weakened hydrogen bonds with adenine. These would be a C–F...H–N and a C–H...N interaction. However, Thalladi et al. have concluded that fluorines attached to sp² carbons are extremely poor as hydrogen-bonding acceptors.^[49] Similarly, studies in crown ether systems have concluded that, if such bonds exist at all, they must be very weak.^[50]

A number of computational studies have addressed the electronic properties of the nonpolar nucleoside analogues, especially for the difluorotoluene compound.^[51–54] For example, a few studies have examined the partial charge on F4, which is analogous to O4 on T (one of the Watson–Crick hydrogen-bonding groups). The calculated partial charge on F4 is –0.21, while for the analogous O4 it is –0.61 (Scheme 3). Based on simple electrostatic (point charge)



Scheme 3. Calculated partial charges for difluorotoluene and thymidine. Data were taken from Wang and Houk.^[53]

calculations, one might expect the F–A pair to be approximately one third as stable in enthalpic terms as T–A in the gas phase. Ab initio calculations of the relative F–A and T–A pair stabilities also fall in this range, and so the pair is predicted to be quite weak.^[52, 54] In addition, it has been pointed out that screening of the electrostatic attractions by water's high dielectric constant would lead to a significant reduction in this attractive force.^[53] Most of the theoretical studies have concluded that this weak noncovalent interaction between F and A is not sufficient to explain later results in DNA replication (see Section 5.3).

2.3. Do Nonpolar Nucleoside Isosteres Form Hydrogen Bonds?

Despite the calculations cited above, the question of whether analogues such as F can undergo base pairing

remains an interesting one for experimental testing. The polarity of dT and dF were compared by water–octanol partitioning studies (unpublished results). The results showed that dT is quite hydrophilic, preferring water over octanol ($\lg P = -1.27$; P = partition coefficient). By contrast, dF is lipophilic, preferring octanol over water by a similar margin ($\lg P = 1.39$). Since the sugar is the same in the compounds, it actually masks an even larger difference between the bases T and F. Thus, if F were proficient at hydrogen bonding with water, it should have shown a preference for partitioning in the aqueous phase.

A more direct study of the hydrogen-bonding question was carried out in deuteriochloroform, by measuring any complexes formed between 9-ethyladenine and difluorotoluene or, for comparison, a uracil derivative, U.^[55] Complexation was observed by following chemical shifts of adenine in response to the addition of increasing concentrations of F or U. The results showed that while U is indeed able to form a complex, no differences in chemical shifts were observed in the presence of F, even at the highest concentrations studied. Thus, even in chloroform, where hydrogen-bonded complexes are much more stable than in water, we find no evidence for hydrogen bonding by F.

Also relevant are some observations made in an NMR spectroscopy study of duplex DNAs containing T or F paired opposite A (discussed in Section 3).^[56] We observed that, for the natural pair, the H3 proton of T was shifted downfield by approximately 3.0 ppm on forming the duplex from the single strands. This is attributed to the effects of hydrogen bonding with the N1 of the adenine opposite. By contrast, we observed that the H3 of F in the same situation was actually shifted slightly upfield (by 0.15 ppm) on forming the duplex from the single-stranded state at 3 °C. Overall, NMR spectroscopy shows no evidence for hydrogen bonds of either the C–H...N or F...H–N type between F and A.

3. Mimicking secondary structure

The observation that analogues such as dF and dZ mimic the structures of the natural nucleosides well in solution does not guarantee that this is the case in the context of double helical DNA. This is especially true in the light of experimental observations (discussed in Section 4.1) which show that the pairing properties of the nonpolar and polar compounds in DNA are very different. One might well expect that a substitution that is very destabilizing to DNA (as is true for some F→T substitutions) might also cause it to be structurally distorted. We were therefore interested in carrying out a structural study of a short DNA duplex containing pairs with F or Z.

This was carried out by use of 2D-NMR spectroscopic methods combined with restrained molecular dynamics and energy minimization.^[56] The duplex studied was a twelve base-pair sequence corresponding to the binding site of a known transcription factor. The data showed, surprisingly, that this duplex is structurally identical to what one would expect with a T–A pair at the same site (Figure 3). Thus, although the F–A pair is destabilizing to the DNA (see Section 4.1.2),

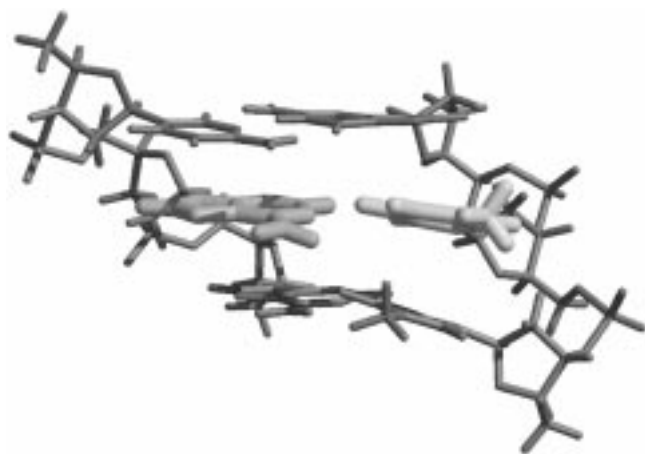


Figure 3. Side view of the central three base pairs of a twelve base-pair duplex which contains an adenine–difluorotoluene pair in the center. Analogue F is on the right.^[55]

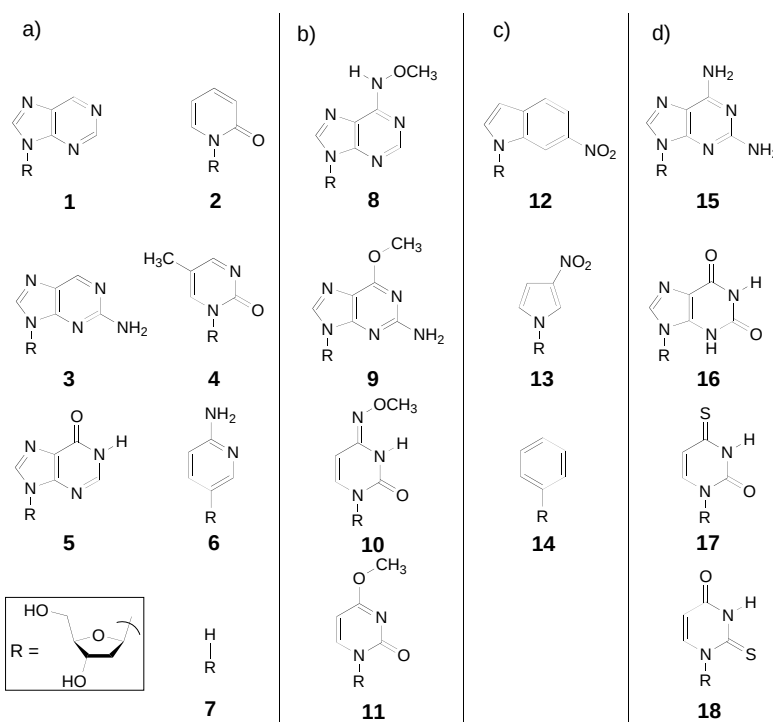
it adopts a geometry virtually indistinguishable from that of a T–A pair. Later studies were carried out with the same duplex sequence, but in which both T and A were exchanged for F and analogue Z (unpublished results). Those studies have also indicated very little structural distortion of the DNA.

4. Noncovalent Interactions and DNA Stability

4.1. Thermodynamic Consequences of Removal of Hydrogen Bonds

4.1.1. Nonisosteric Base Analogues

Many analogues which delete, block, modify, or rearrange the hydrogen-bonding groups of natural DNA bases have been studied in the past.^[57–68] Some of these analogues are shown in Scheme 4. Deletion of hydrogen-bond donor or acceptor groups (compounds **1–6**),^[57–60] or deletion of the entire base (abasic site, **7**),^[57] has been found to result in a destabilization of the DNA (Table 1), although with some variability. The only exception is the compound 2-aminopurine (**3**),^[61] which maintains good stability, presumably due to the fact that two hydrogen bonds can still be made without disrupting the Watson–Crick geometry. A second class of analogues has been prepared by blocking a hydrogen-bond donor or acceptor group (compounds **8–11**),^[62–65] examples are O-methylated versions of thymine and guanine. These also destabilize DNA, as do the members of the third group (compounds **12–14**), analogues that do not resemble the purine or pyrimidine bases.^[66–68] Examination of the pairing data generated for the compounds that completely remove the hydrogen bonds between the bases (Table 1) shows that all the analogues do appear to destabilize the DNA. Upon examining the data closely, one observes that the loss of stability on making these substitutions is surprisingly large.



Scheme 4. Nucleoside analogues with altered, blocked, or rearranged hydrogen-bonding groups.

Is this destabilization due to the lack of hydrogen bonds? Or are other factors affecting the stability of the DNA? Two possible explanations must be considered. First, there may be a significant degree of structural distortion with these analogues, which influences not only the pair in question but also the DNA around it. This distortion might well arise from the fact that the analogues are sterically different than the natural molecules, leading to steric or geometric penalties. A second answer may be that solvation effects may be energetically costly; this effect is discussed in Section 4.3, 5.6, and in the following sections.

Another class of analogues contain modifications in their hydrogen-bonding ability (compounds **15–18**).^[58, 69] For example, a carbonyl group is switched with an amino group, a C=O bond is replaced by a C=S bond, or an amino group is added. Some of these modified bases, like 2,6-diaminopurine (**15**),^[69] or 2-thiothymine (**18**)^[58] appear to stabilize the DNA, due to

their retention of the normal Watson–Crick hydrogen bonds and maintaining the correct geometry, whereas others are slightly destabilizing.

One further class of analogues are those that rearrange hydrogen-bond donors and acceptors to form new base pairs. Two new Watson–Crick base pairs have been designed with a hydrogen-bonding pattern different from that of A–T and G–C pairs (Scheme 5). These rearrangements were found to not significantly affect the DNA stability.^[70, 71]

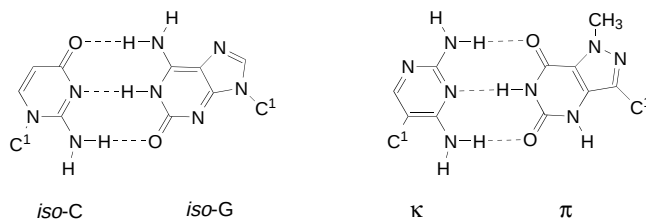
4.1.2. Isosteric Nonpolar Base Analogues.

The nonpolar nucleoside isosteres, analogues unlike those discussed above, do not change the steric size of the DNA

Table 1. Melting temperatures for DNA duplexes which contain non-isosteric base analogues.

Base Pair X–Y	T_m [°C]	ΔT_m [°C]
A–T ^[a,b]	42.5	–
A–C	36.0	6.5
A– ϕ	26.0	16.5
G– ϕ	30.0	12.5
C– ϕ	26.0	16.5
T– ϕ	28.0	14.5
A–B ^[a,b]	32.0	10.5
G–B	29.0	13.5
C–B	25.5	17.0
T–B	29.0	13.5
G–C ^[c,d]	45.4	–
O ⁶ meG–A	19.4	26.0
O ⁶ meG–G	22.6	22.8
O ⁶ meG–C	25.2	20.2
O ⁶ meG–T	21.9	23.5
A–T ^[e,f]	35.0	–
A–C	12.0	23.0
N–A	18.0	17.0
N–G	19.0	16.0
N–C	13.0	22.0
N–T	29.0	6.0

[a] Measured in the sequence d(AACCAGTACGXTGAG)·d(CTCAYCGTACTGGTT) in a buffer which contained 9.5 mM NaCl, 2 mM HEPES and 20 mM EDTA (pH 7.0). B is compound **14**. [b] Data taken from Millican et al.^[66] [c] Measured in the sequence d(GGTTXTTGG)·d(CCAAYAACC) in a buffer which contained 100 mM NaCl, 0.1 mM EDTA and 10 mM phosphate (pH 7.0). O⁶meG is compound **9**. [d] Data taken from Gaffney and Jones.^[64] [e] Measured in the sequence d(CGXGCGC)·d(GCCYCCG) in a buffer which contained 150 mM NaCl and 50 mM phosphate (pH 7.0). N is compound **1**. [f] Data taken from Eritja et al.^[57] HEPES = 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid, EDTA = ethylenediaminetetraacetate.

Scheme 5. Structures of novel base pairs with rearranged hydrogen-bonding patterns as reported by Benner and coworkers.^[70, 90, 91]

base; thus the importance of hydrogen bonding can be probed in the absence of significant steric effects. We therefore carried out studies of the stability of DNA duplexes containing analogues F or Z.^[44, 55] The data are shown in Table 2.

Both of these analogues are quite destabilizing to duplex DNA. The single substitutions with F or Z result in a loss of 4–5 kcal mol^{–1} in stability, which is a significant fraction of the entire free energy of the duplex. In addition, we observed that both these analogues were almost entirely nonselective in their base pairing, giving roughly equal amounts of destabilization when paired with any of the four natural bases. This latter result suggests that hydrogen bonds do play a significant role in the selectivity of base pairing of natural bases. Thymine shows a selectivity of 3–4 kcal mol^{–1} in pairing with A rather than T, C, or G, while molecule F, which has the same size and shape, shows little or no selectivity. This observation also

Table 2. Free energies and melting temperatures for DNA duplexes which contain isosteric nonpolar bases F and Z.

Base Pair X–Y ^[a]	T_m [°C]	ΔT_m [°C]	$-\Delta G_{25}^0$ [kcal mol ^{–1}]
T–A ^[b]	39.4	–	12.3
T–C	26.4	13.0	8.7
T–G	30.7	8.7	9.3
T–T	27.1	12.3	8.9
F–A ^[b]	21.4	18.0	7.4
F–C	25.0	14.4	8.2
F–G	23.0	16.4	8.0
F–T	20.3	19.1	7.3
F–F	28.6	10.8	8.9
A–T ^[c]	39.8	–	12.4
Z–A	25.3	14.5	8.0
Z–C	24.4	15.4	7.9
Z–G	23.8	16.0	7.6
Z–T	20.8	19.0	7.5
Z–F	30.3	9.5	8.9

[a] Measured in the sequence d(CTTTCXTTCTT)·d(AAGAAY-GAAAAG) in a buffer which contained 100 mM NaCl, 10 mM MgCl₂ and 10 mM Na-PIPES (pH 7.0). [b] Data taken from Schweitzer and Kool.^[55] [c] Data taken from Guckian et al.^[44] Na-PIPES = sodium piperazinebis-(ethanesulfonate).

strongly argues that F is not capable of forming a measurably hydrogen-bonded complex with A in this context.

At a second glance, these data prompt a new question: why are these analogues so destabilizing to the helix? Simple removal of the base pair in question actually results in less destabilization than observed with F–A, for example.^[72] This suggests that the net interaction between F and A (or T and Z) is actually repulsive by more than 2 kcal mol^{–1} in water. One possible explanation is that these analogues stack very poorly, this however does not seem to be the case (see Section 4.2). Another experiment shows that mutating an F–A pair to an F–Z or F–F pair actually is a little less destabilizing.^[44, 55] This suggests that a reason for the energetic penalty of pairing F with A is the cost of removing the waters of solvation strongly bound to A.^[73, 74] Since the 2D-NMR spectroscopically determined structure of F opposite A in DNA shows the two next to one another in the center of the duplex, this suggests that approximately two solvating waters might be lost on forming this duplex, with little energetic compensation (since F cannot hydrogen bond well with A). Of course, another contributing factor to the destabilization is the lack of hydrogen bonds between F and A. A third explanation is that the increased flexibility of these nonpolar base pairs (due to the lack of hydrogen bonds) may disrupt stacking of neighboring base pairs. The structure discussed above containing the F–A pair shows that the neighboring T–A base-pair signal broadens at a lower temperature than others in the sequence, indicating increased movement at this base pair.

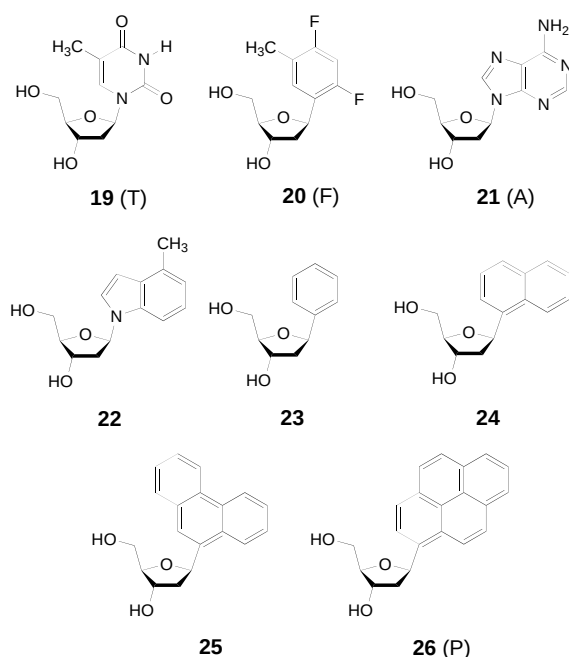
4.2. Forces Contributing to Aromatic Stacking in DNA

Of course, stacking of the bases is a very significant factor in the stabilization of the double helix, and the phenomenon is still relatively poorly understood from a noncovalent bonding standpoint. Several research groups have investigated the stacking phenomenon and the results have spurred a debate in

the literature.^[20–26, 75, 76] Some have asserted that electrostatic interactions are a primary driving force for base stacking, while others cite the importance of hydrophobic effects. However, while there has been much discussion on the topic, there has been relatively little study of stacking in the context of DNA itself. For this reason we undertook a quantitative study of the relative stacking abilities of natural DNA bases and nonnatural analogues in the context of double-stranded DNA.^[77] The thermodynamic comparisons were carried out by use of a self-complementary six base pair duplex having the sequence 5'-dXCGCGCG in each strand. When the expected B-form duplex is formed, this leaves residue X as an overhanging (dangling) base that can stack on the helix but cannot pair with the opposite strand. Similar studies have been carried out previously with the natural bases in RNA.^[78] In the context of DNA, however, there exists (until recently) only one published example of a dangling thymidine in which full thermodynamic parameters were reported,^[79] while in another study only T_m values for the four natural bases were reported.^[80] In all previous work no unnatural analogues were studied.

The bases studied in our experiments^[77] are shown in Scheme 6, and the data are given in Table 3. Of the natural bases, A stacks most strongly, but interestingly, both of the nonpolar isosteres F and Z stack more strongly than any of the natural bases. This is in marked contrast to their strong destabilization when F and Z are in the middle of a duplex opposite a natural base.^[44, 55] Clearly, the structural context is important for explaining these differences.

A simple series of unsubstituted aromatic hydrocarbons was studied for comparison, and it was found that all of them stack relatively strongly, with increasing size generally leading to increasing stacking ability. The overall relative stacking abilities of this series of molecules do not correlate well with polarizability or with electrostatic polarization. Instead, we



Scheme 6. Structures of natural and nonnatural nucleosides studied for stacking propensity.^[77]

Table 3. Free energies and melting temperatures for DNA duplexes which contain a dangling base at the 5' end.

Dangling residue X ^[a,b]	T_m [°C]	ΔT_m [°C]	$-\Delta G_{37}^0$ [kcal mol ⁻¹] ^[c]
–	41.0	–	8.1
thymine	48.1	7.1	9.2
difluorotoluene	54.4	13.4	10.7
trimethylbenzene ^[d]	51.4	9.7	9.7
cytosine	46.2	5.2	9.1
adenine	51.6	10.6	10.1
guanine	51.5	10.5	9.4
4-methylindole	54.6	13.6	11.1
pyrrole	46.6	4.9	8.9
benzene	48.3	7.3	9.4
naphthalene	56.2	15.2	10.9
phenanthrene	57.3	16.3	10.7
pyrene	64.1	23.1	11.4

[a] Measured in the sequence d(XCGCGCG) · d(GCGCGCX) in a buffer which contained 1 M NaCl and 10 mM Na-Phosphate (pH 7.0). DNA concentration: 5.0 μ M. [b] Data taken from Guckian et al.^[77]. [c] Values obtained by plotting $1/T_m$ against $\ln(C_T)$ (C_T = total concentration) with data from at least five concentrations. [d] DNA concentration: 6 μ M.

concluded that the most important single factor in this series is the hydrophobic effect, with the largest and most nonpolar molecules giving the best stacking.

Some useful conclusions and applications arise from these data. First, it seems that the natural DNA bases do not stack very strongly, and it is simple to find compounds that are more efficient at stacking. In retrospect, this seems reasonable, since the DNA helix must be unwound at various points during gene expression and replication. A second observation is that the relatively nonpolar amino acid side-chains of phenylalanine and tryptophan would be expected to stack well with DNA (see the phenyl and indole nucleoside data in Table 3), and such side-chains would therefore make good intercalators in protein–DNA recognition.^[81, 82] Finally, a third lesson to be taken from the results is that a useful and simple way to stabilize nucleic acid helices is to place a strongly stacking group at the end. Indeed, the pyrene nucleoside is seen to increase the melting temperature of the test sequence by 23 °C.

The ability to stack strongly can be useful in the stabilization of structures beyond simple double helices. Hairpin secondary structures contain elements of helix as well as loops bridging the ends. The residues in loops often undergo stacking even when they are not strongly contributing to pairing interactions,^[83] and so increasing the stacking ability can stabilize looped structures. This has been demonstrated in the context of loops bridging DNA double helices.^[84] Table 4 shows the results of one such study. Pentanucleotide loops were used in a duplex sequence, and the effects of replacing the loop residues with nonpolar nucleosides were investigated. The results showed that in all cases the replacement leads to increased stability of the structures.

4.3. The Importance of Solvation Effects in Base Pairing and Stacking

Small nonpolar analogues such as F and Z can stabilize or destabilize DNA structures, depending on where they are substituted. They are generally quite proficient at stacking

Table 4. Free energies and melting temperatures for DNA duplexes which contain tetranucleotide loops.

DNA sequence ^[a-c]	T_m [°C]	ΔT_m [°C]	$-\Delta G_{60}^0$ [kcal mol ⁻¹]
5' -GCAATTGA ^T T 3' -CGTTAACG ^T T	67.7	–	1.3
5' -GCAATTGA ^F F 3' -CGTTAACG ^F F	78.5	10.8	3.6
5' -GCAATTGA ^A A 3' -CGTTAACG ^A A	62.9	–	0.4
5' -GCAATTGA ^D D 3' -CGTTAACG ^D D	67.2	4.3	1.1

[a] Measured in buffer which contained 100 mM NaCl and 10 mM phosphate (pH 7.0). DNA concentration: 5.0 μ M. [b] D is compound **22**. [c] Data taken from Ren et al.^[84]

species, but are very poor as base-pairing species. Interestingly, however, there is one situation in which residues such as F and Z appear to pair well. When F, for example, is placed opposite A at the end of a duplex, the resulting pair is actually more stabilizing than a natural T–A base pair (unpublished results). We attribute this stabilization to the stronger stacking of F relative to T, without a penalty for desolvation of A. At the end of the helix we surmise that water is able to remain hydrogen bonded to the polar groups on A.

Thus we attribute a number of effects—strong stacking, poor pairing in the center of the duplex, and stable pairing at the end—to solvation effects in water. The strong stacking of nonpolar F and Z (and aromatic hydrocarbons) results from the favorable avoidance of solvent-exposed surface area by adopting the stacked structure. It may be expected that strongly stacking residues may actually stack even in the single-stranded state, which then lowers the entropic cost of duplex formation. It is well known that a significant degree of single-strand stacking occurs within runs of purines in DNA,^[85–87] and we have seen that nonpolar nucleosides stack even better than the purines.^[77] When a nonpolar “base” is present in the middle of a strand, it may hide both its flat faces from solvent by stacking with the two neighboring bases. At the end, it can still remove one of its faces from solvent by stacking.

An interesting question arises at this point: if only the flat π -surfaces of the nonpolar analogues are removed from solvent upon stacking, then why are the π -surfaces of the natural bases not able to stack with the same affinity? Are not all aromatic π -surfaces the same in solvation and stacking ability? Clearly the edges of the natural bases are much more polar than those of the nonpolar isosteres, but one would guess that the edges of all bases remain exposed to solvent in single-stranded DNA. This implies that the flat surfaces of the aromatic DNA bases are more polar than the analogous surfaces of the nonpolar isosteres, and the data imply that the flat surfaces of DNA bases are more favorably solvated than those of the very nonpolar molecules. It would appear, therefore, that solvent molecules align dipoles to interact more favorably (or less unfavorably) with the local bond dipoles that exist at the flat π -surfaces of the natural bases. In

the nonpolar analogues these dipoles are generally small or are absent.

Almost all of the nonpolar nucleoside analogues exhibit strong destabilization in the center of a DNA duplex when paired opposite natural DNA bases, as mentioned above. This is very likely due in part to the cost of desolvation of the polar edge of the natural base^[74] when the nonpolar base is forced against it in the helix. This is despite the very strong stacking of the nonpolar molecules, which is apparently not large enough to compensate for the cost of desolvation.

5. Testing DNA Replication Mechanisms

5.1. Previous Studies

This work has been aimed at investigating the precise noncovalent interactions that govern the formation of base pairs. The work described above focuses on the inherent properties of DNA bases and analogues in forming base pairs. Judging by the results with analogues F and Z, it appears that Watson–Crick hydrogen bonds do add a significant level of stabilization to DNA duplexes, and that they are very important to the selectivity of base pair formation. Due to their lack of hydrogen-bonding groups, F and Z show 1) inherently strong destabilization in their interaction with natural bases, and 2) very little, if any, pairing selectivity.

Once the inherent properties of the nonpolar nucleoside mimics were determined, we began using them to test the mechanisms governing the insertion of nucleotides into DNA by polymerase enzymes. When this work began, it was widely believed that Watson–Crick hydrogen bonds are essential for replication of a DNA base pair by polymerases. For example, recent editions of biochemistry and molecular biology textbooks argue that hydrogen bonds alone may explain the observed fidelity.^[41, 42]

If one examines the data gathered by use of modified nucleosides up to 1995, one can find a number of examples of nucleosides such as *O*-6-methylguanosine (*O*⁶meG, **9**; Scheme 4). This compound has been altered from natural guanine by methylation. This blocks Watson–Crick hydrogen bonding by the carbonyl, and changes N1 from a hydrogen-bond donor to an acceptor. Studies with the Klenow fragment of *E. coli* DNA polymerase I, perhaps the most well studied of DNA polymerases, have shown that this compound is a poor substrate.^[88] When placed in a template strand of DNA, the enzyme inserts nucleotides opposite with efficiency ($v_{\max}/K_m$; $v_{\max}$ = maximum rate of reaction, K_m = Michaelis constant) that is 5000 times less than insertion of C opposite unmodified G (Figure 4). Moreover, when nucleotides are inserted, there is a tendency for insertion of both C and T.

It has been common to attribute these changes in the polymerase substrate properties of compounds like *O*⁶meG to their altered hydrogen-bonding properties. Indeed, since hydrogen bonding to cytosine is blocked, this might be a viable explanation for such a result. However, one must also consider other factors as possible explanations. Specifically, the steric size and shape of the molecule is altered by such substitution; the methyl group adds 2–3 Å of bulk to the *O*⁶

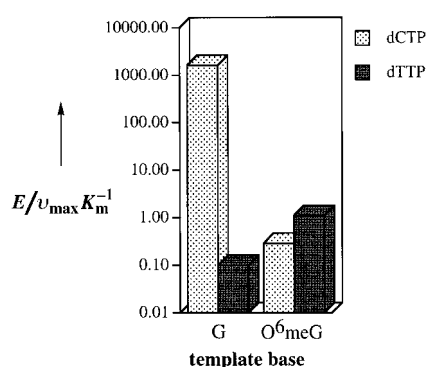
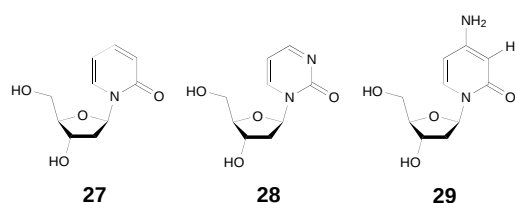


Figure 4. Histogram showing the steady state insertion of dCTP and dTTP opposite O⁶meG and, for comparison, G. Data was taken from Levy and Spratt.^[88]

position, and the loss of the proton removes approximately 0.7 Å of bulk at N1. Since both the hydrogen bonding and the sterics are affected, one cannot be confident in attributing the polymerase activities with this compound to only one of these noncovalent interactions.

A previous series of studies was carried out to address the importance of hydrogen bonds in DNA replication.^[89] It involved new pyrimidine analogues that were missing a single hydrogen-bonding group. These analogues are shown in Scheme 7. Primer extension experiments were carried out



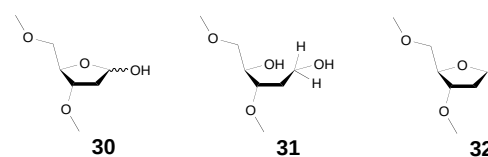
Scheme 7. Deoxycytidine analogues studied by Strazewski and Tamm^[89] for their ability to be accepted as polymerase substrates.

with these analogues both in the template and as nucleoside triphosphates. As triphosphates none of these compounds were found to extend the primer. In the template, however, compound **29** (a hydrogen bonding impaired analogue) was found to code for dCTP incorporation. Unfortunately, no quantitative data are available for these interesting analogues, and so we must rely on qualitative observations. The general conclusion made from this work is that the chief factor governing polymerase activity is “the number and strength of hydrogen bonds” between bases in a pair.^[89] As we have pointed out, however, some of the steric changes made to these analogues may confuse the situation somewhat. Lastly, some of the changes lead to possible tautomerization of the DNA base, which confuses both the issues of hydrogen bonding and steric effects. In addition, partial deletion of hydrogen-bonding groups also raises issues of solvation that may lead to problems (see Section 4.3).

Another important set of studies has been carried out by Benner and co-workers,^[70, 90, 91] who have made analogues following a different strategy. They have designed pairs in which hydrogen bonds are not deleted, but are rather rearranged (Scheme 5) to be different from that in A–T

and C–G (purine–pyrimidine geometry is maintained). Two such base pairs have been shown qualitatively to be replicated efficiently by DNA polymerase (Klenow enzyme). This is a nice example suggesting the possibility that base pairs other than the natural ones might be used to expand the genetic code. These analogues contain hydrogen bonds and maintain Watson–Crick geometry; therefore they do not allow us to address the importance of hydrogen bonds and geometric effects in replication.

Other potentially relevant modified nucleotides that can help address the importance of hydrogen bonds in DNA replication are the abasic nucleosides, such as **30–32** (Scheme 8).^[92–94] Naturally occurring abasic sites are one of the most common forms of DNA damage and are formed by alkylation damage to DNA bases followed by hydrolysis of the glycosidic bond, base excision repair, and spontaneous hydrolytic loss of purines.^[95–97]



Scheme 8. Structure of the abasic site (**30**) and abasic site analogues (**31**, **32**).

Abasic sites have been termed “non-instructional” because of their lack of hydrogen-bonding information.^[98, 99] Interestingly, it has generally been observed that deoxyadenosine is preferentially inserted opposite abasic sites in a template strand by most DNA polymerases.^[94, 100–103] This default choice by the enzyme is commonly referred to as the “A rule”. The A rule has been variously explained by an inherent preferential interaction with A by the enzyme, or by the lower polarity or stronger base stacking ability by A relative to the other nucleotides. Kinetics studies have shown that polymerases insert dA opposite abasic sites with efficiency approximately three to four orders of magnitude lower than for adenine opposite T, which would tend to argue that hydrogen bonding does provide a significant benefit. However, the abasic site may lead to DNA distortion,^[104–107] which could also explain the low efficiency. Also of interest is the magnitude of selectivity observed for inserting A opposite abasic sites. The kinetics experiments show that the selectivity is actually rather low, with an approximately tenfold preference for dA rather than dG insertion.^[92] Although abasic sites do provide interesting information on what happens without hydrogen bonds, the lack of any base structure to test steric effects, and the large structural perturbation caused by the lack of a base makes it difficult to judge the effects of sterics and hydrogen bonding.

To summarize, in the general literature on polymerase mechanisms and fidelity, Watson–Crick pairing has most commonly been cited as the important factor which drives incorporation of a nucleotide and governs the specificity of this process.^[41, 42] It is worth noting that some workers have also pointed out the relevance of maintaining a geometry similar to standard base pairs.^[108–110]

5.2. Predictions of the Hydrogen-Bonding Model

Before experiments with DNA polymerases were carried out, we considered what the standard model would predict. A purely hydrogen-bonding model for replication would suggest that analogues such as F would be very poor substrates for polymerases. In addition, if they were made to be substrates under forcing conditions, they would be expected to be handled with little or no selectivity. This would not be surprising, since the inherent property of F is very unstable and nonselective pairing. Indeed, such thermodynamic base-pairing measurements (done in the absence of enzymes) are routinely carried out as predictors of polymerase activity with the same base pair.

5.3. Studies with Nonpolar Thymine Analogue F

The difluorotoluene analogue was placed in synthetic DNA templates and tested for its ability to be accepted by a DNA polymerase. For the initial studies we carried out experiments with the Klenow fragment of *E. coli* DNA polymerase I, which is among the best studied of DNA polymerases.^[34, 111–113] Immediately we observed a number of surprising results. In an experiment with all four nucleotides present, the polymerase proceeded past F with no apparent hesitation, and one of the natural nucleotides was inserted directly across from F (Figure 5).^[114] Single nucleotide additions were then carried out, and these revealed that it was adenine that was selectively inserted. Quantitative steady-state kinetics were then measured for the A–F pair, and it was found that efficiency of the base-pair synthesis was only approximately fourfold lower than that of the natural pair (Figure 6). Even more surprisingly, the selectivity of adenine insertion (rather than the other nucleotides) was nearly as high as that of the natural pair. Since we were not able to measure any hydrogen-bonding ability for F, we proposed that effects other than hydrogen bonding should be considered as important to polymerase activity. Clearly the enzyme enforces properties on F that it does not display on its own in the absence of enzymes. We proposed at the time that steric effects might be sufficient to explain the results.^[114]

Of course, when such a surprising result is observed, then alternative explanations must be considered. For example, one might conclude that since F does not hydrogen bond well, it acts essentially as a non-instructional base, and the enzyme simply follows the A rule when it selectively inserts A. We pointed out, however, that the A–F pair is considerably more efficiently processed than occurs with the A rule, and that the A–F pair does not cause strong pauses as the A–abasic pair does^[114]. Nonetheless, the best way to disprove the A rule hypothesis is to turn the base pair around. We therefore synthesized the nucleoside triphosphate derivative of F, and studied its insertion by the polymerase.^[115] It was found that insertion was still highly efficient, and that the selectivity was as high as for the natural pair (Figure 7). We concluded that the A rule could not be invoked here. We proposed that hydrogen bonding in DNA synthesis was not as important as

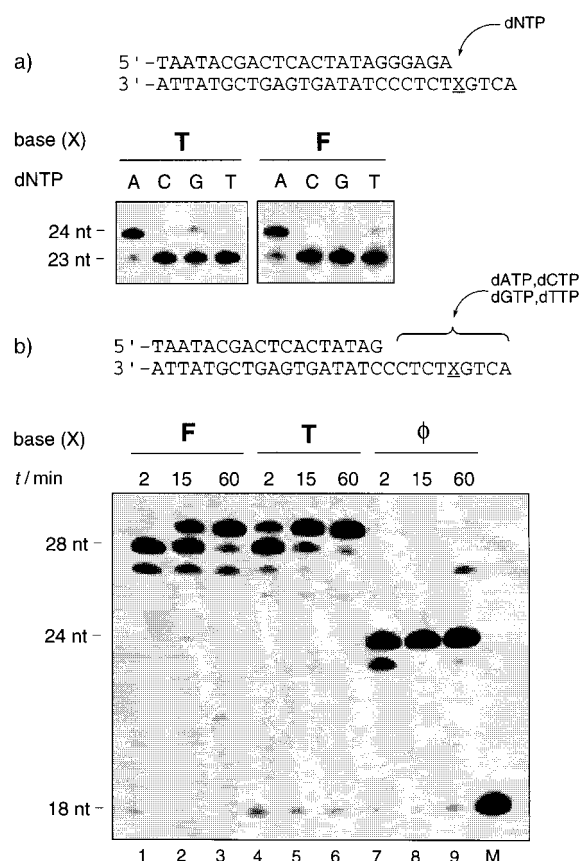


Figure 5. Polymerase insertion of nucleotides opposite difluorotoluene, thymine, or an abasic site. a) Autoradiogram showing single nucleotide insertions of the four natural bases on 23/28 nucleotide primer–template duplexes (d(TAATACGACTCACTATAGGGAGA)–d(ACTGXTCTCCCTATAGTGAGTCGTATTA)) which contain either thymine or difluorotoluene in position X.^[114] b) Autoradiogram showing running-start multiple nucleotide insertion experiments on 18/28 nucleotide primer–template duplexes (d(TAATACGACTCACTATAG)–d(ACTGXTCTCCCTATAGTGAGTCGTATTA)), with mixtures of the four natural nucleosides.^[114]

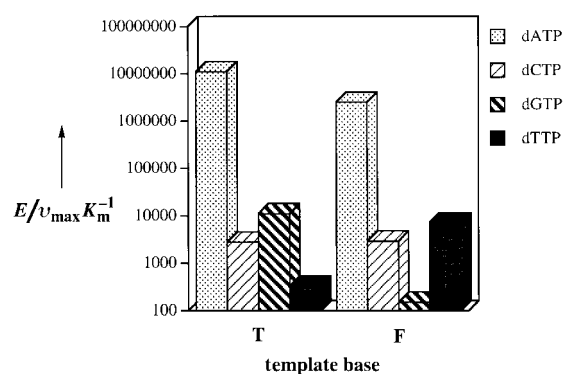


Figure 6. Histogram of fidelity of nucleotide insertion opposite T and isostere F using the primer–template shown in Figure 5a.^[114]

commonly believed, and that perhaps steric effects might play a significant role.

It was later shown that the F–A pair could replace every T–A pair in a 48 base-pair duplex, with faithful synthesis occurring in six different DNA contexts.^[116] Ironically, the DNA being synthesized was less stable than the natural DNA by a significant amount. Clearly, however, the DNA polymerase does not sense this destabilization until after the transition

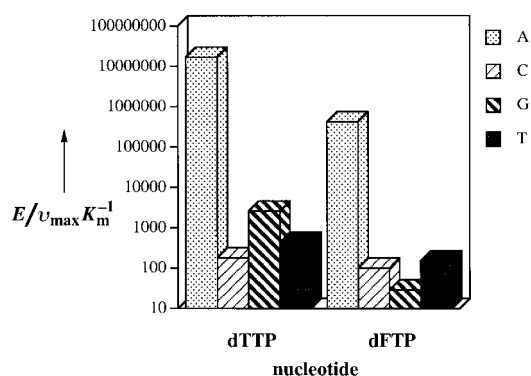


Figure 7. Histogram of selectivity of dTTP and dFTP insertion opposite the four natural bases using the primer–template shown in Figure 5a.^[115]

state for nucleotide insertion. Interestingly, the DNA polymerase does apparently sense destabilization when multiple F–A pairs are being synthesized in a row.^[116] We suspect that this might arise from the enforced desolvation of the F–A pairs when they become buried further into the active site.

Later studies have also tested various polymerase enzymes for their ability to process the F–A pair (unpublished results). It was found that *Taq* polymerase, T7 DNA polymerase, and HIV reverse transcriptase can all handle the pair qualitatively very well. Quantitative data are not yet available.

5.4. Studies with Nonpolar Adenine Analogue Z

The results with analogue F raised questions about generality and mechanism. Would similar results be found for other nucleoside isosteres? Or were the fluorine substituents responsible for the special activity of F? Indeed, one possible explanation for the high efficiency of the F–A base pair in replication is that F forms a hydrogen-bonded pair with A. In addition, another possible influence on the selectivity of the F–A pair might arise from solvation effects; since A is the least strongly solvated of the natural bases, this would favor its pairing with a nonpolar analogue such as F.

Thus, we carried out a series of replication experiments with a new analogue, Z (Scheme 1), which has a 4-methylbenzimidazole group replacing adenine in dA.^[44] Models suggest that it can fit opposite F in a DNA duplex with only a relatively small structural distortion arising from the bulk of the H1 proton on Z (a group that is absent in A). Both qualitative and quantitative experiments were carried out with the Klenow fragment enzyme. Consistent with the previous results with analogue F, we observed relatively efficient base-pair synthesis when Z was present in a template or as a deoxynucleoside triphosphate (Figures 8, 9). For example, F and Z are replicated very well against one another, despite the indisputable lack of hydrogen bonds between the two. Also interesting is the fact that analogue Z is not as well inserted opposite another Z. This provides an example of selectivity in the absence of hydrogen bonding. Thus we concluded that efficient base-pair synthesis by this polymerase does not require Watson–Crick hydrogen bonding.^[117] In addition, steric effects alone (in the absence of these hydrogen

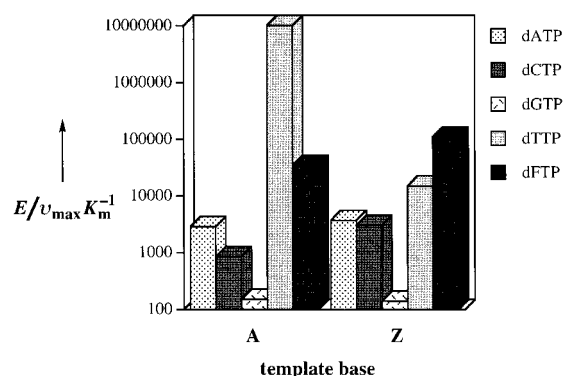


Figure 8. Histogram of fidelity of nucleotide insertion opposite A and isostere Z using the primer–template shown in Figure 5a.^[117]

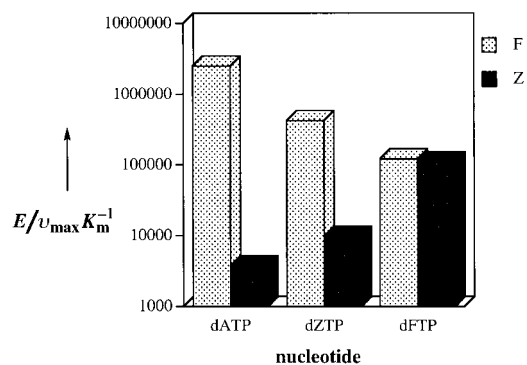


Figure 9. Histogram of selectivity of dATP, dZTP, and dFTP insertion opposite isosteres Z and F using the primer–template shown in Figure 5a.^[117]

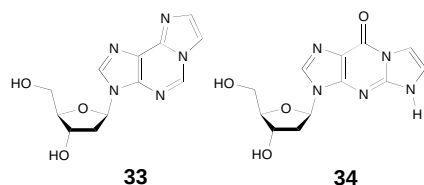
bonds) can result in significant pairing selectivity. Importantly, the selectivity observed with analogues F and Z (using the Klenow fragment enzyme) is much higher than the very low or nonexistent pairing selectivity observed by these “bases” in the absence of the enzyme.^[44] Thus, it appears that the enzyme provides an active site which closely conforms to the Watson–Crick duplex DNA geometry, and which strongly restricts the flexibility of the DNA backbone and bases.

In our studies with analogues F and Z we observed one way in which the members of this pair behave very differently than the natural bases T and A. It was seen that dFTP is actually inserted relatively efficiently opposite another F in the template (Figure 9).^[115, 117] This is in contrast to the analogous natural pair, T–T, which is synthesized 900-fold less efficiently by the KF enzyme. Possible reasons for this difference are discussed in Section 5.7.

5.5. Other Hydrogen Bonding Impaired Nucleotides

The activity of DNA polymerases in handling other hydrogen bonding impaired nucleotides has been recently reviewed,^[118] and so the data will be discussed here only briefly. Previous to the work with difluorotoluene and benzimidazole analogues, no polymerase studies had been carried out with nucleotide analogues that completely lack hydrogen-bonding ability. The ethenoadenine and ethenoguanine deoxynucleoside adducts **33** and **34**, respectively, are potentially of interest

(Scheme 9), but ϵ G **34** can still potentially form one Watson–Crick bond,^[119] and ϵ A **33** can form hydrogen-bonded complexes by adopting a *syn* glycosidic orientation.^[120] As a result, the only entirely convincing results in the absence of hydrogen



Scheme 9. Structures of 1,6-*N*-ethenodeoxyadenosine **33** and 1,2-*N*-ethenoguanine **34**, which are formed upon alkylation of dA and dG with vinyl chloride.

bonds between the bases comes from the previously mentioned work with abasic sites^[92–94] and the nonpolar analogues described above. Interestingly, there exists one isolated 1973 report of the study of benzimidazole deoxynucleoside being incorporated into the DNA in cells.^[121] No studies were carried out with a purified polymerase or with an isolated DNA sequence, and so no quantitative data are available for that analogue. Another interesting report was published in 1984, in which benzene deoxynucleoside was incorporated into synthetic DNA.^[66] No polymerase work was carried out. Interestingly, the workers reported that it formed very unstable pairs with natural bases, but this was attributed to poor stacking ability, something that we know now not to be the case.^[77]

5.6. Discussion of Possible Explanations

The work with the dF and dZ analogues provokes an important and provocative question: Is it true that Watson–Crick hydrogen bonds are completely dispensable in efficient and specific replication of DNA base pairs? Before this can be safely concluded, we must consider alternative explanations that might conform to our results. Perhaps analogue F functions correctly because it is in fact forming efficient hydrogen bonds with A.^[51] However, it seems clear that this is not the case. First, we see that there is no measurable complex between difluorotoluene and adenine even in a solvent that strongly encourages such bonding.^[55] Second, dF is highly hydrophobic, while dT is the opposite, highly hydrophilic. Third, F shows no tendency to base pair with adenine, even when forced to be opposite it in a DNA helix. Fourth, F shows no measurable selectivity in pairing with A, while thymine displays 3–4 kcal mol^{−1} of selectivity in the same context. Fifth, although calculations suggest that there might be one weakened hydrogen bond between F and A in the gas phase,^[52, 54] there appears to be general agreement among most computational scientists that in water there will not be nearly enough favorable interaction to explain our results.^[52–54, 122] Finally, hydrogen bonds are completely ruled out in cases such as the pair between F and Z, which is still processed relatively efficiently by the enzyme.

A second explanation for why adenine is preferentially inserted opposite F starts with the opposite assumption, that F is nonpolar and does not form hydrogen bonds. One might argue that F is another non-instructional lesion, analogous to abasic sites. Once again, experiments do not support this. First, when A is inserted opposite abasic sites there is a strong pause, while no such pause is observed for A insertion opposite F.^[114] Second, the insertion of A opposite F is two orders of magnitude more efficient than insertion of A opposite abasic sites, and the specificity opposite F is two orders of magnitude higher. So in these respects, F is clearly not behaving in a manner analogous to other non-instructional lesions in DNA. Probably the most convincing result, however, comes when the F–A pair is turned around.^[115] The data show that F is very well inserted opposite A, and once again it is processed with very high specificity. In this case adenine is not being inserted, and the A rule cannot be invoked. For these reasons, this second explanation does not hold water.

The final alternative explanation to be considered is that solvation effects might favor enzymatic pairing of F with A and vice versa. Indeed, calculations suggest that the Watson–Crick edge of adenine is less strongly solvated than the analogous parts of the other DNA bases.^[74] For this reason, it might cost less energy to enzymatically pair F and A together as opposed to F with T, C, or G. However, if this explanation were correct, then why does F not show selective pairing with A in the absence of enzymes? In addition, when analogue Z is studied in the template strand, the polymerase selectively inserts T,^[117] although it is among the most strongly solvated of the four bases.^[74] Thus one suspects that most of the available results do not support this solvation effect as the most important answer, superseding steric effects. In any case, probably the best way to rule out such effects is to look at pairs in which neither partner carries strongly solvated hydrogen-bonding groups. Thus, we find that the pair of F with Z is indeed handled with good efficiency and with significant selectivity (the results discussed in Section 5.4).

Thus, overall we feel confident in stating that hydrogen bonds are not necessary to achieve highly efficient base-pair synthesis, and that significant levels of selectivity can be achieved in the complete absence of these bonds. However, it is important to note that the replication of Z opposite F is not entirely as efficient as a natural base pair, and that the misinsertion of Z opposite Z is more favorable than the analogous A opposite A.^[117] Thus, one should not yet rule out a smaller favorable effect of hydrogen bonding on efficiency and especially specificity.

5.7. The Steric Exclusion Model for DNA Replication

The results in Section 5.6 led us to propose a model for replication of DNA base pairs which does not rely on Watson–Crick hydrogen bonds to explain efficiency and selectivity. In this steric exclusion model,^[118] the polymerase active site is relatively rigid and presents a binding pocket of specific size and shape. This active-site pocket has four possible shapes, since it is defined both by the structure of the

polymerase and that of the template base immediately downstream of the primer. The incoming nucleotide is fixed in position for polymerization (phosphodiester bond formation) by the binding of the triphosphate moiety and by proper fit of its DNA base. The forces that support binding of the nucleoside triphosphate at the transition state are binding of the triphosphate and sugar, and stacking of the base on the primer 3' end base. In the simple steric exclusion model, hydrogen bonding between the two bases does not add much energy of stabilization at the transition state. The rationalization for this is that both the template base and the incoming nucleotide base are already hydrogen bonded to water prior to the reaction, and so there is little stabilization to be gained by exchanging these for Watson–Crick bonds.

How does the steric exclusion model address the issue of fidelity of DNA synthesis, that is, the high selectivity in choosing to insert the correct nucleotide rather than the other three? Again, while the steric exclusion model does not rule out additional contributions from other factors such as Watson–Crick hydrogen bonding, it is worthwhile to consider how steric effects alone might function to give high selectivity. It is clear that the assumption of a rigid active site which provides a close fit to duplex DNA geometry^[39, 123–131] will provide an explanation for why purines are not misinserted opposite other purines; they are simply too large to fit opposite one another (Figure 10). In addition, one can use a steric exclusion argument to explain why purine–pyrimidine mismatches (G–T and A–C) are not well synthesized. If one tries to enforce a standard base-pair geometry on them, there will be steric overlap of two protons in either type of pair. In addition, if a wobble geometry is adopted, then the active site will again provide a steric clash between the protein and the base pair. Thus, for these types of mismatches a simple steric exclusion argument fits the data well.

However, there appears to be a serious shortcoming in this simplistic model: how does one explain why the sterically small pyrimidines are not misinserted opposite other pyrimidines? Clearly, the low frequency of these mispairings^[132] deserves an explanation, and steric effects as described at the beginning of this section would not account for this, since two pyrimidines can easily fit into the space normally occupied by a pyrimidine paired with a purine. Here we suggest that one must take into consideration the tightly bound solvent molecules that associate themselves strongly with the polar groups on DNA bases. Each of these groups is hydrogen bonded to at least one water molecule. One such hydrogen bond will be bound by 6–7 kcal mol^{−1} in terms of gas phase enthalpy.^[18, 19] While such a water molecule is exchanging rapidly with others in solution, the solvating water is essentially never lost because of this energetic cost of breaking the bond. As a result, the only time that a solvating water is lost in aqueous solution is when it is

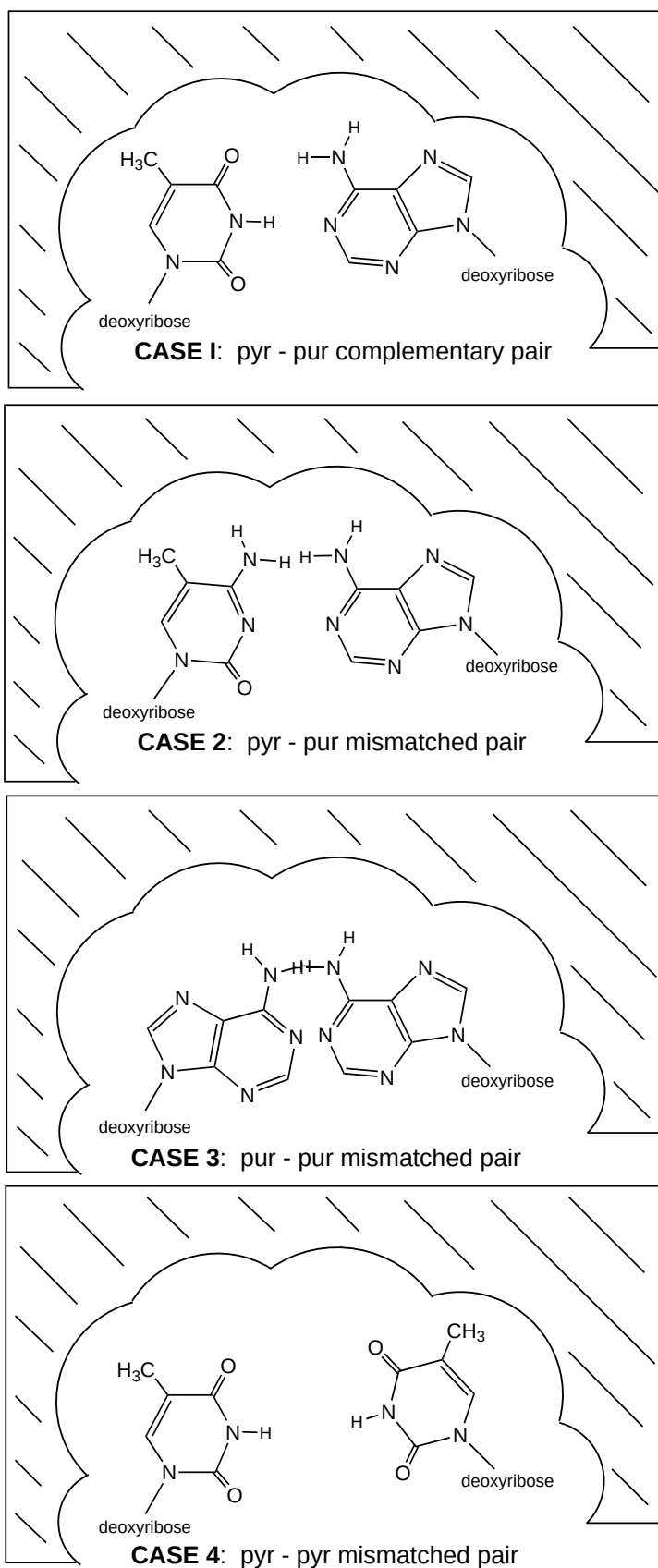


Figure 10. Representation of steric clashes that could lead to the exclusion of mismatches from the polymerase active site in at least three of the four possible classes of mismatches. The polymerase DNA binding cleft tightly surrounds the template, leaving an active-site cavity for the triphosphate, defined by the polymerase and the template base.

simultaneously exchanged for another complementary, energetically equivalent hydrogen bond.

We argue that the strongly bound waters of solvation along the Watson–Crick pairing edge of a natural DNA base serve to make the base sterically larger than we commonly draw it (Figure 11). Therefore, pyrimidines (indeed, all bases) are

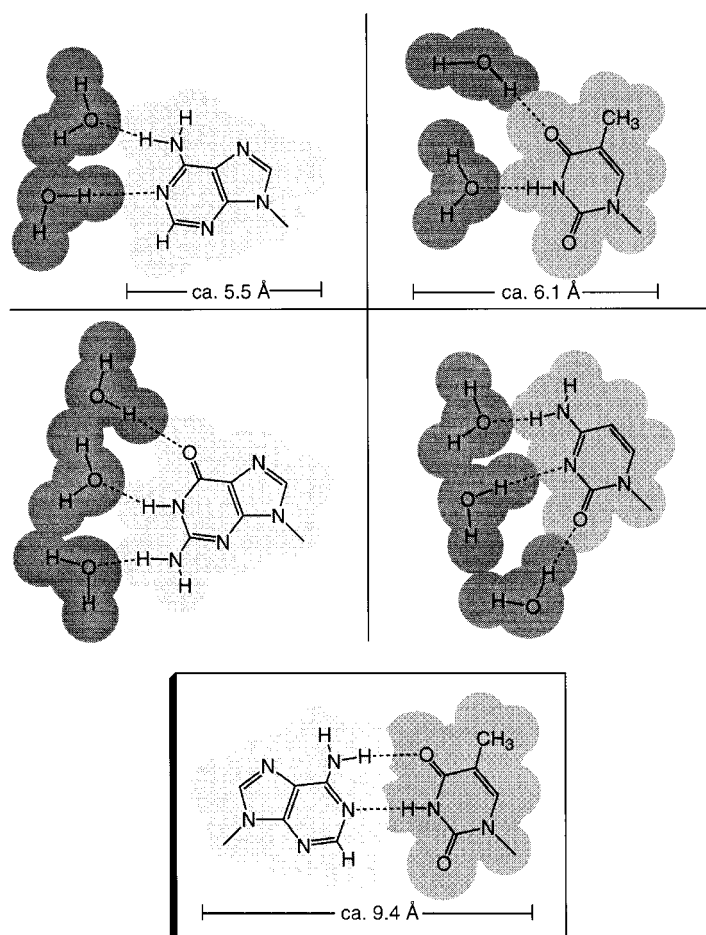


Figure 11. Schematic representation of possible geometries of solvating waters on the four DNA bases. Also shown are the estimated sizes of the van der Waals surface of a solvated pyrimidine and purine base; this shows how solvation greatly increases the size of the DNA bases in water. A solvated pyrimidine may be two-thirds the size of an entire base pair!

larger than we commonly envision them, and this added steric size can have energetically important effects at the transition state for DNA polymerization. Thus, it is argued that pyrimidines are not misinserted opposite other pyrimidines because they are sterically too large. They will not give up these waters (to become small enough to fit) because they cannot exchange them for energetically equivalent hydrogen bonds.

Is there any evidence available for the steric effect of waters of solvation? Here we describe two pieces of experimental data, one with polymerases and one with small molecules. First, we consider the classic measure of the steric size of a functional group in organic chemistry: the *A* value, which is measured for substituted cyclohexanes. The *A* value represents the magnitude of steric clashes with axial protons, and it has been recognized for some time that polar groups give

larger values in protic than in aprotic solvents.^[133] For example, the alcohol of cyclohexanol has an *A* value of 0.52 in aprotic solvent and 0.87 in protic solvent. A similar trend is seen for the amine of cyclohexylamine, which has an *A* value of 1.2 in aprotic solvent and 1.6 in protic solvent.

The second piece of evidence supporting the influence of solvating waters on the steric size of DNA bases comes from study of our nonpolar isosteres. As mentioned above, we observe that F is inserted opposite another F 900-fold more efficiently than T is inserted opposite another T.^[115] We attribute the facile F→F base-pair synthesis to the fact that F is small enough to be accommodated opposite another F residue. Conversely, T with its waters of solvation is too large to fit opposite another T (which is also solvated).

The “jigsaw puzzle” prediction

This steric exclusion model for DNA polymerase activity leads to some testable predictions about new experiments that could be performed.^[118] First, it predicts that in general it will be possible to replicate base pairs between nonpolar, non hydrogen bonding residues as long as they are sterically well matched in the double helix. The chief factors in their efficiency will be their avoidance of steric clashes and their stacking ability. Second, at least some measure of selectivity among various non hydrogen bonding bases will be achievable by careful selection of their molecular shapes. If a selective pair that does not suffer from self-pairing (as occurs with F) is the goal, then a self-exclusive shape must be part of the design. Thus, a successful base pair will be matched like two adjacent pieces of a jigsaw puzzle. Finally, the shape exclusion hypothesis predicts that purine and pyrimidine shapes will not be a requirement for efficient synthesis; any flat aromatic shape should be acceptable as long as the pair fits within the overall context of duplex DNA.

5.8. Second Generation Base-Pair Design

Figure 12 shows the structures involved in a new base pair that tests some of the above predictions. This pair, P– ϕ , is a somewhat extreme example testing the concept that pyrimidine and purine shapes might not be required for polymerase

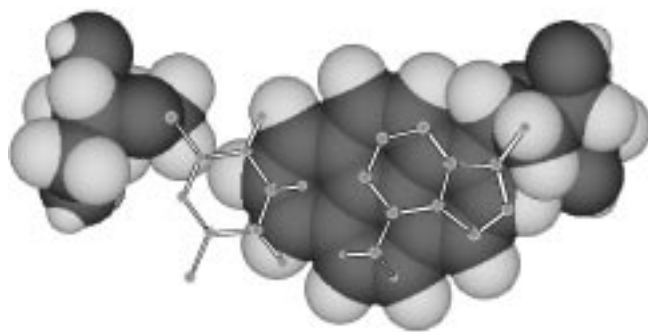


Figure 12. Space-filling model of a pyrene abasic pair within the context of B-form duplex DNA. For comparison a Watson Crick A–T base pair is superimposed on top of the space-filling model.

activity. The pair consists of pyrene deoxynucleoside **26** and an abasic nucleoside **32**. In this pair, one partner is sterically as small as possible (the “base” is a proton), and the other is nearly as large as possible, filling the void left when a base is removed. This novel example has now been tested, both as a base pair in the absence of enzymes,^[72] and more recently, as a substrate for DNA synthesis by polymerases.^[134]

Table 5 shows data illustrating the pairing properties of pyrene and the abasic nucleoside^[72] in a DNA duplex without

Table 5. Free energies and melting temperatures for DNA duplexes which contain pyrene and abasic sites.

Base pair X–Y ^[a]	T_m [°C] ^[b]	ΔT_m [°C]	$-\Delta G_{37}^0$ [kcal mol ⁻¹] ^[c]
T–A	43.2	–	12.6
P–A	38.7	4.5	10.8
P–C	37.6	5.6	10.5
P–T	36.4	6.8	9.6
P–G	38.2	5.0	10.7
P– ϕ	41.0	2.2	11.6
P–P	42.9	0.3	11.8
ϕ –A	22.0	21.2	7.0
ϕ –C	20.9	22.3	6.7
ϕ –T	18.0	25.2	6.2
ϕ –G	23.1	20.1	7.2

[a] Measured in the sequence d(CTTTCXTTCTT)·d(AAGAAAG) in a buffer which contained 100 mM NaCl, 10 mM MgCl₂ and 10 mM Na-PIPES (pH 7.0). DNA concentration: 5.0 μ M. Error in T_m is ± 5 %. [b] Data taken from Matray and Kool.^[72] [c] Values obtained from the fit of at least five denaturation curves.

enzymes. Interestingly, the pyrene–abasic “pair” is nearly as stabilizing as an A–T pair in the same sequence. This is surprising because abasic sites are notoriously destabilizing to DNA when paired opposite natural bases^[66] (see examples in the Table). As a result of the P– ϕ stability, one notes that the abasic site actually shows high selectivity in its pairing preference for pyrene over natural bases. On the other hand, pyrene shows only a small preference for pairing with ϕ rather than the natural bases. We presume that in the latter case, pyrene is intercalated between bases on the opposite strand (structural studies are underway to address this question).

We then examined the more difficult questions: would the unusually large, non hydrogen bonding pyrene nucleotide behave as a substrate for DNA polymerases? Would the abasic site act as a viable template? And would the efficiency be low or high? Would any selectivity be observed, since there are no hydrogen bonds in the pair? To test these questions we synthesized the deoxynucleoside 5'-triphosphate derivative of P (dPTP), as well as template DNAs containing the abasic analogue ϕ . These were studied with the Klenow DNA polymerase as well as T7 DNA polymerase.

Surprisingly, the results show that dPTP is, in fact, a substrate for DNA polymerases (Figure 13).^[134] More surprisingly, it is a very good substrate, with efficiency approaching that of a natural base pair, and a very selective one, being inserted much more efficiently opposite ϕ than opposite natural bases or another pyrene (Figure 14). Once again, the polymerase is clearly enforcing properties of pyrene base that it does not display in DNA without an enzyme.



Figure 13. a) Structure of dPTP, which was shown to be an efficient substrate for DNA polymerases. b) Autoradiogram showing single nucleotide insertions of the four natural nucleotides and dPTP opposite the natural bases or an abasic site. For sequences see Figure 5a.^[134]

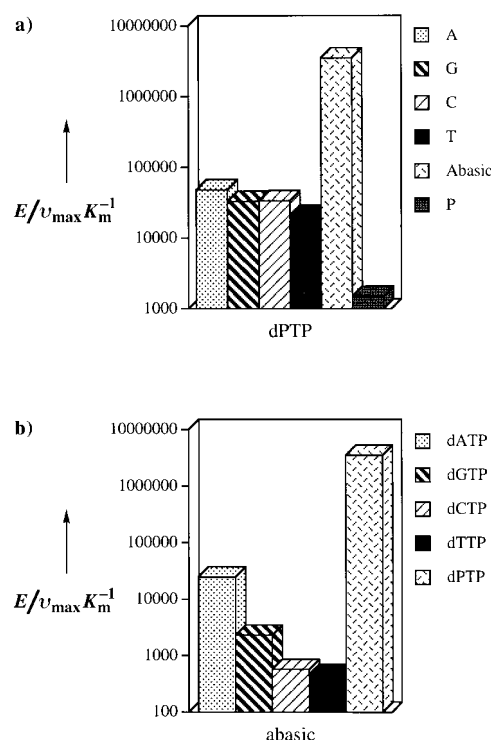


Figure 14. Histograms showing the steady state efficiency of insertion of dPTP: a) Selectivity for insertion of dPTP, showing preferential insertion opposite the abasic site rather than the four natural bases or pyrene itself.^[134] b) Fidelity of insertion of the four natural nucleotides and dPTP on the primer–template shown in Figure 5a where X is the abasic site.^[134]

The clear conclusions of this work are, first, that hydrogen bonds are not required to reach very high base-pair synthesis efficiencies. Second, significant levels of selectivity can be achieved without hydrogen bonds; in the present case, they apparently arise from simple steric effects alone. Third, base structures other than ones resembling purines and pyrimidines can still be very effective as substrates, as long as they are sterically matched against an appropriate partner. This bodes well for the design of new base pairs in the future.

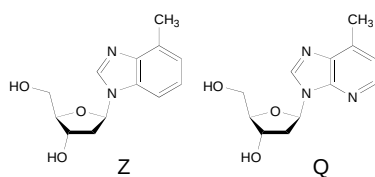
It should be pointed out that the P– ϕ pair is quite different from a natural pair in at least one important way. After the pyrene is inserted, the DNA synthesis is halted; that is, the

strand is extended no further.^[134] It is not yet known why this happens, but it may be due in part to some property of the abasic site. For example, extension of the DNA strand containing an abasic residue also proceeds very poorly even when a natural base (adenine) is inserted opposite it.^[135] In the pyrene study, we took advantage of this chain termination after pyrene insertion by using it as a DNA sequencing agent for detecting abasic sites in DNA.

5.9. Other Polymerase Effects

The results described in this review tend to support the idea that Watson–Crick hydrogen bonds are not essential to DNA replication, at least for an isolated base pair. In some ways, this makes good sense, because such bonds are internal to the DNA helix and so their presence or absence cannot be easily sensed structurally by the enzyme. Recent X-ray crystal structures have shown, however, that polymerase enzymes do, in fact, make use of hydrogen bonds between the protein and the DNA.^[123, 128, 129, 131, 136] These interactions occur between hydrogen-bond donor side-chains and acceptor groups in the DNA minor groove (this is referred to as the “minor groove binding track”).^[137]

This fact may help explain why some nonpolar base analogues, such as the benzimidazole analogue Z, are inserted well but then give significant inhibition of further chain elongation.^[117] We proposed that moving Z downward in the active site (so that the next nucleotide can be inserted) causes the hydrogen-bond donor in the active site to lose its hydrogen bond with the previous base. In effect, it becomes desolvated, which is energetically costly, and this causes the enzyme to dissociate. We proved this by synthesizing a new analogue, Q, which is the same as Z (it still lacks Watson–Crick hydrogen-bonding groups) but has one nitrogen engineered back into the minor groove (Scheme 10). This modified base is a very good substrate, both for insertion and for further chain elongation (Figure 15).^[138]



Scheme 10. Structure of the nonpolar isostere Q and, for comparison, the previously described nonpolar isostere Z. These analogues are used to probe DNA minor groove interactions with polymerases.

6. Conclusions

In the absence of enzymes, it appears that hydrogen bonding may indeed add a significant degree of stabilization to existing DNA helices, and that it does add a strong component of pairing specificity. However, it appears that base stacking is also a very important factor for stabilization of the double helix, even for the natural DNA bases, which

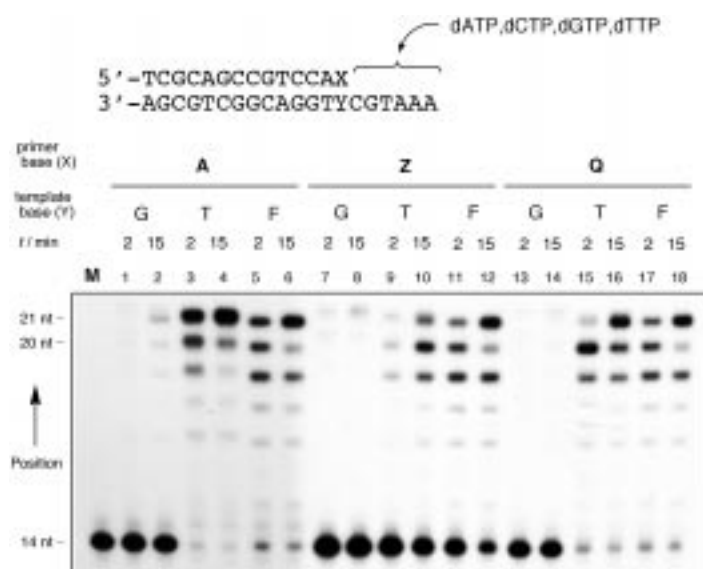


Figure 15. Autoradiogram showing minor groove interaction effects on extension of normal and modified base pairs.^[138] Note the higher efficiency of primer extension with Q (lanes 15–18) as compared with Z (lanes 9–12).

stack relatively weakly as compared to other less polar molecules.

In DNA replication, we conclude that Watson–Crick hydrogen bonds are not required to replicate a base pair with very high efficiency. In addition, significant selectivity can also be achieved from steric effects alone. It remains to be seen, however, whether steric effects in the absence of hydrogen bonding can give the very high levels of fidelity observed for normal hydrogen-bonded base pairs. Regardless, it is clearly true that one must pay close attention to steric and geometric factors in the DNA if one is to understand how base pairs are replicated, and if one aims to design new replicable base pairs.

Moreover, it is clear that polymerase enzymes closely enforce geometric constraints on DNA beyond what it feels from its own backbone constraints. Thus, base-pairing stabilities in the absence of enzyme correlate poorly with the ability to be replicated by an enzyme. This means that even base pairs that are very unstable and nonselective in DNA alone may be handled with high specificity and efficiency by an enzyme.

7. Future Work

There remains much work to be done in this field. Synthesis of a number of new analogues is warranted, to test the effects of subtle changes in molecular shape on selectivity in base-pair synthesis. In addition, studies with a greater number of polymerases would test the generality of the effects observed. Testing other classes of polymerases, such as RNA polymerases and reverse transcriptases, would also be of interest.

These analogues could also be valuable in the study of other activities of polymerase enzymes. For example, extension of base pairs is distinct from insertion of base pairs, and this involves different interactions with the enzyme. In addition,

the proofreading (mismatch correction) activity of polymerases could also be tested. In this case, a similar question can be addressed: is it hydrogen bonding or base-pair shape, which governs the rate at which a base pair is removed by the proofreading apparatus of the polymerase?^[139, 140]

Other interactions of proteins and DNA are also of interest. Since the difluorotoluene–adenine pair does not distort the DNA,^[56] the analogue could be very useful in probing the importance of hydrogen bonding and solvation on protein recognition of the DNA structure. In addition, it could serve as an NMR structural probe; analogue F contains two fluorines that are located in the center of each groove of the DNA and this provides a useful ¹⁹F probe for NOE studies.^[56]

DNA repair mechanisms are also currently under intensive study. For example, the base excision repair enzymes remove mismatched DNA bases by “flipping” them out of the helix and hydrolyzing the C–N glycosidic bond.^[141] Analogues such as F would not be hydrolyzable by such enzymes, and so they might serve as specific inhibitors in synthetic DNA substrate sequences.

In addition to using such analogues as tools for studying mechanism and structure, we envision a number of potentially useful practical applications in biomedicine. For example, the use of analogues such as the pyrene nucleoside P might give strong stabilization of helices because of its robust stacking ability.^[77] Hybridization is widely used in high-throughput genomics strategies.

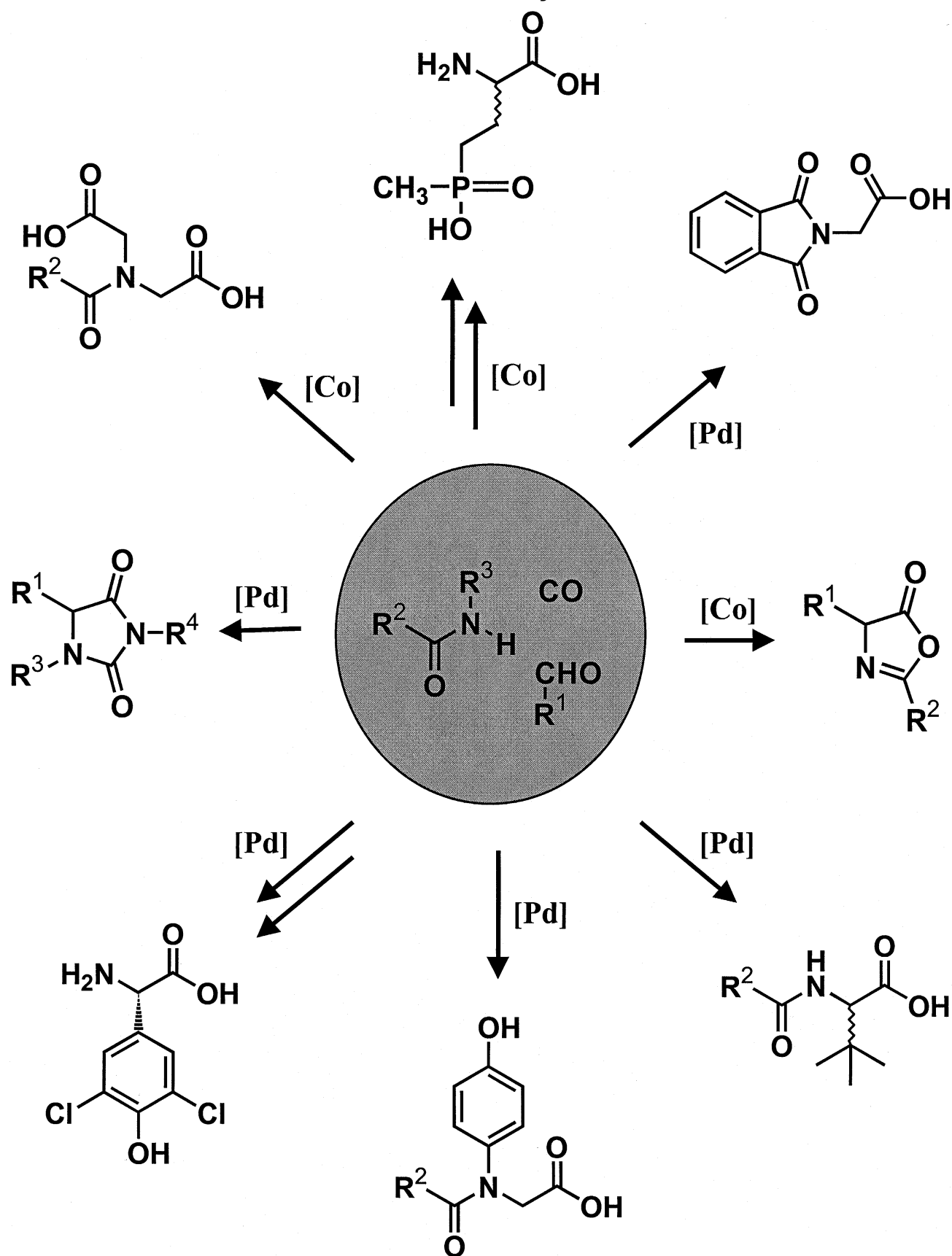
We are grateful to the past and present workers in the Kool laboratory who have contributed to this research. Special recognition in this regard is deserved by Dr. Barbara Schweitzer, Dr. Sean Moran, Dr. Dongyu Liu, Dr. Tracy Matray, Dr. Rex Ren, Dr. Narayan Chaudhuri, and Robin Carroll. This work was supported by the National Institutes of Health (GM52956). JCM thanks the Spanish Ministry of Education for a postdoctoral fellowship.

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An Atom-Economic Route to Amino Acid Derivatives through Amidocarbonylation



Through these cobalt- and palladium-catalyzed three-component reactions of aldehydes, amides, and carbon monoxide, *N*-acyl- α -amino acid derivatives can be obtained very efficiently

Amidocarbonylation—An Efficient Route to Amino Acid Derivatives

Matthias Beller* and Markus Eckert*

Dedicated to Dr. H. Wakamatsu on the occasion of his 70th birthday

Atom efficient, multicomponent reactions that lead to high-value products from inexpensive starting materials are of both economic and ecological interest for industrial organic synthesis. α -Amino acids are amongst the most important compounds in chemistry and biology. As well as their biochemical significance as building blocks of peptides and proteins, α -amino acids are also becoming increasingly interesting as fine chemicals. Possibly one of the key reactions in the preparation of these compounds is transition metal catalyzed amidocarbonylation, where the α -amino acid framework is constructed in a single step from an

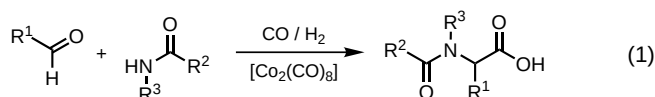
aldehyde, an amide, and carbon monoxide. This article gives a current overview of transition metal catalyzed amidocarbonylation reactions used in the synthesis of α -amino acids derivatives. A classification and summary of the significant features of this three component reaction is first presented together, with an historical introduction. This section is followed by two sections on cobalt- and palladium-catalyzed amidocarbonylation. A discussion of the mechanism of each of the different amidocarbonylation variants form an introduction. Overviews on further synthetic development of the methodology, such as the domino re-

action with an amidocarbonylation step and the expansion of the range of starting materials, form the main topics of both variants. The potential of the method is demonstrated with the help of examples of special synthetic utility (for example, the preparation of arylglycines). Finally, possibilities for future developments in transition metal catalyzed amidocarbonylation reactions are proposed on the basis of the current state of knowledge.

Keywords: amidocarbonylation • amino acids • cobalt • homogeneous catalysis • palladium

1. Introduction

Amidocarbonylation has great potential for the industrial synthesis of racemic *N*-acylamino acids because of its atom economy and range of application. *N*-acyl- α -amino acids are formed in one step from the reaction of an aldehyde, an amide, and carbon monoxide in the presence of cobalt or palladium catalysts [Eq. (1)].



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Amidocarbonylation is the only transition metal catalyzed multicomponent reaction that constructs the amino acid framework directly from simple, inexpensive starting materials. In the past the reaction has undergone many new developments, principally in the area of in situ aldehyde generation. Several review articles on cobalt-catalyzed amidocarbonylation have appeared over the last decade either as book contributions or in journals.^[1] In addition to a description of the historical aspects and a comparison with related methods, this contribution presents a review of the more recent developments in cobalt-catalyzed amidocarbonylations and, for the first time, in palladium-catalyzed amidocarbonylations.

The newly awakened interest in this reaction reflects the fact that C_1 chemistry (here with CO), which in the past has mainly made its appearance in the synthesis of bulk chemicals, is receiving greater attention in the area of fine and specialty chemicals for a number of economic and ecological reasons.^[2]

The structural motif of the *N*-acyl- α -amino acids is found in a large number of compounds from the most diverse areas of applications. Examples include the pharmaceuticals captopril

and *N*-acetylcysteine, the herbicide Flamprop-isopropyl, the anionic sarcosinate tensides, and simple dipeptides such as the sweetener aspartame (Figure 1).

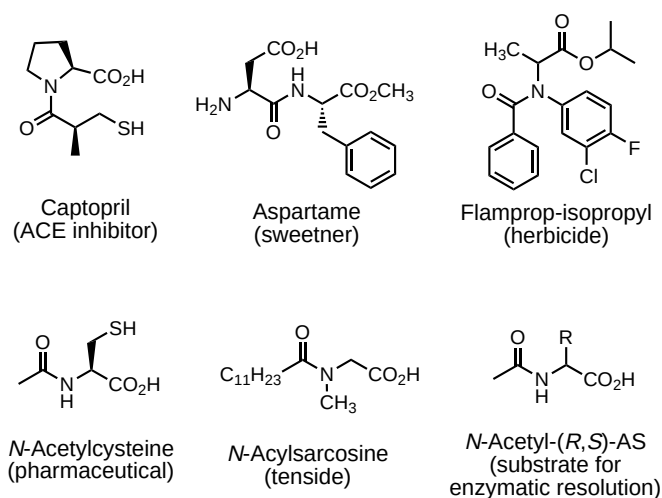


Figure 1. Examples of the applications of *N*-acyl- α -amino acids

Increasing environmental awareness and cost pressures make it necessary^[3] to question and rethink existing chemical procedures used for the synthesis of *N*-acylamino acids, such as the acylation of free amino acids by the Schotten–Baumann method or cascade reactions involving an initial Strecker reaction followed by hydrolysis and subsequent acylation with anhydrides.^[4]

Racemic *N*-acylamino acids are of considerable importance for the preparation of both natural and unnatural, enantiomerically pure amino acids. The development of synthetic chemical routes to optically active amino acids remains even now one of the great challenges in amino acid chemistry, as is demonstrated by numerous publications in recent years.^[5] The trend is shifting in a consequent manner from diastereoselective variants to the use of asymmetric catalytic methods. The target of enantioselectivity, which has been the focus in the past, is only one factor of many that have to be considered in respect of industrial processes. Thus, enzymatic separation of racemic *N*-acylamino acids (for example, *N*-acetylvaline or *N*-acetylmethionine) is still currently the method of choice if amino acids are to be prepared on a large scale, despite many known asymmetric catalytic procedures.

With the background of an increasing demand for different amino acids, this review article aims to demonstrate the potential of amidocarbonylation to utilize readily accessible raw material sources and generate a wealth of useful compounds, as well as highlight future development possibilities.

1.1. Historical Background

At the beginning of the 1970s, two routes were discovered, starting at the same time from quite different directions, for the formation of *N*-acylamino acids by the reaction of an aldehyde, an amide, and carbon monoxide. Whereas Wakamatsu et al. at Ajinomoto first discovered cobalt-catalyzed

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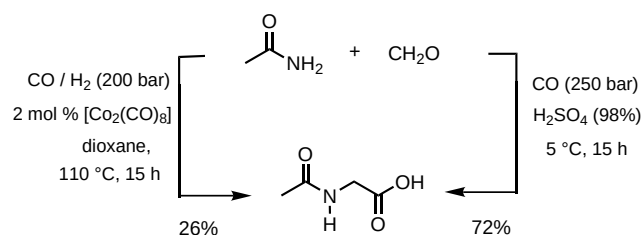
Makus Eckert, born 1971 in Köln, Germany, studied chemistry at the Universität Köln and the Technische Universität München from 1991 to 1996 as Stipendiat of the Studienstiftung des deutschen Volkes. From 1996 to 1999 he studied for his doctorate thesis on palladium-catalyzed amidocarbonylation with M. Beller. Research appointments lasting several months led him to the Chemistry Department, Pharmaceutical Division, Bayer Corp., West Haven, CT, USA, and the Institute for Physical and Chemical Research at Y. Wakatsuki in Wakashi, Japan. Since 1999 he has been working at Bayer AG in Leverkusen in the Department of Fine Chemicals.



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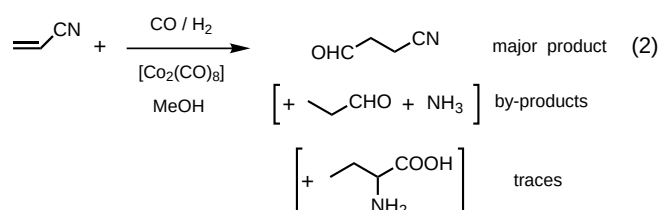
amidocarbonylation in 1970 during investigations into the oxo process of acrylonitrile.^[6] Witte and Seeliger developed a corresponding Koch carbonylation in sulfuric acid (Scheme 1) in 1971 at Hüls.^[7]



Scheme 1. The discovery of amidocarbonylation.

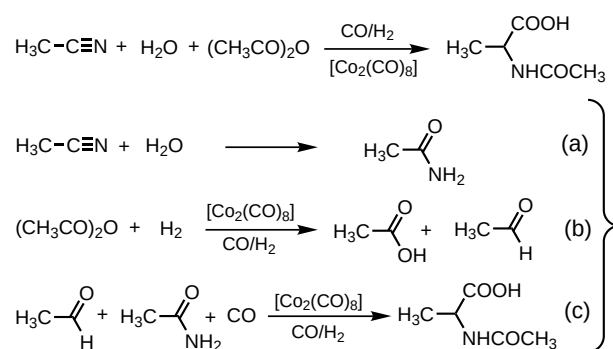
1.1.1. The Wakamatsu Reaction

The development of the oxo process for the preparation of 3-cyanopropionaldehyde from acrylonitrile in the central research laboratories of Ajinomoto was the essential step in the discovery of today's well-known amidocarbonylation reaction. As early as 1962 the hydroformylation of acrylonitrile with methanol as the solvent had been used successfully on a commercial scale.^[8] Closer investigations into the formation of propionaldehyde as a by-product in the industrial process showed small amounts (2.5 %) of α -aminobutyric acid were present in stoichiometric reactions of acrylonitrile with $[\text{HCo}(\text{CO})_4]$ in addition to propionaldehyde and ammonia [Eq. 2].



It was first assumed that an insertion reaction of CO into acrylonitrile was involved, and it became clear that an investigation of the reaction of acetonitrile, synthesis gas, water, and acetic anhydride under cobalt catalysis was necessary, however, no formation of alanine was observed. Surprisingly this reaction was first made possible by an additional hydrolysis of the reaction solution with hydrochloric acid, albeit in low yield. Wakamatsu concluded that the reaction did not proceed through CO insertion into the nitrile, but occurred by prior hydrolysis of the nitrile to the amide (Scheme 2a) and the known reduction of acetic anhydride^[9] to acetaldehyde and acetic acid (Scheme 2b), therefore, in the final instance, a reaction of acetaldehyde, acetamide, and carbon monoxide occurred (Scheme 2c).

The reaction of acetaldehyde and acetamide with synthesis gas under cobalt catalysis led consequentially to *N*-acetylalanine in high yield. Today this reaction is generally known under the name of amidocarbonylation. At the time Wakamatsu published only a short communication,^[6] and it was Pino et al. who first confirmed in 1979 that the reaction was of general applicability and led to *N*-acyl- α -amino acids in high yields.^[10]



Scheme 2. Historical aspects of the discovery of amidocarbonylation.

1.1.2. The Amidomethylation of Carbon Monoxide

The reaction of cationic α -amidoalkyl compounds with nucleophilic carbon atoms of aromatics, olefins, acetylenes, and active methylene groups is well known. In 1971 Witte and Seeliger at the Chemische Werk Hüls Troisdorf GmbH (now Degussa-Hüls AG) were successful in using the weak nucleophile carbon monoxide in such reactions for the first time.^[7, 11] The *N*-acyliminium ion, formed in situ by reaction of an amide and paraformaldehyde, is converted into *N*-acylglycine derivatives with carbon monoxide (250 bar) in concentrated sulfuric acid at 5 °C. Methylols, bisamides, and methoxymethylene compounds are also suitable precursors of *N*-acyliminium ions in this reaction, and since the carbonylation of a functionalized carbenium ion occurs in sulfuric acid to form the carboxylic acid, it can in principle also be considered as a Koch carbonylation.^[12] This amidocarbonylation variant has hitherto found no further application because of the severe reaction conditions and the structural limitation of glycine derivatives.

1.1.3. Palladium-Catalyzed Amidocarbonylation

In contrast to other carbonylation reactions it is surprising that for almost two decades cobalt carbonyls have been used exclusively as catalysts in amidocarbonylations. It was only in 1987 during a search for patent-free catalyst systems for amidocarbonylation^[13] that Jägers et al. at Hoechst AG discovered that palladium halides could function as precatalysts and catalyze the reaction. A closer reading of the first amidocarbonylation patent^[6b] would have enabled this discovery to have been made much sooner! As early as 1970 the admittedly over-stoichiometric reaction of acetaldehyde with acetamide and synthesis gas in the presence of 1.67 equivalents of palladium dichloride to form alanine (2.5 % yield relative to Pd) was described in a patent example. In a comprehensive examination of amidocarbonylation by palladium catalysis we were able to develop this method further and to establish it as a method for the synthesis of numerous *N*-acylamino acid derivatives.^[14]

1.2. Reactions Related to Amidocarbonylation

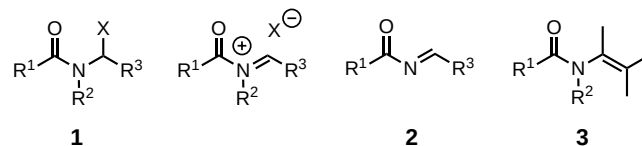
For both a better understanding of the amidocarbonylation variants and for future developments it is useful to consider

reactions that are mechanistically related to amidocarbonylation. On the basis of the initial equilibria, the method represents a reaction of an *N*-acyliminium ion (or *N*-acyliminium ion precursor) with a nucleophile, here carbon monoxide. Thus a relationship with the classical α -amidoalkylation procedures is established. If only the key metal-catalyzed step is considered, the amidocarbonylation can be classified as a transition metal catalyzed carbonylation (Scheme 3).

Since amidocarbonylation represents a carbonylation of a C–X compound (with X = OH, OMe, hal, etc.), general conclusions can be drawn from methanol carbonylation (Monsanto process)^[16] or benzoic acid synthesis from chlorobenzene.^[17] However, there is also a relationship with transition metal catalyzed carbonylation of aldehydes. There are surprisingly few examples of this type of reaction. In addition to the nickel-catalyzed carbonylation of formaldehyde to glycolic acid^[18] mentioned in Scheme 3, the palladium-catalyzed carbonylation of an electron-rich benzaldehyde derivative to form the phenylacetate ester is of interest.^[19]

If amidocarbonylation is regarded as an amidoalkylation of carbon monoxide,^[12, 20] the emphasis lies in the formation of

electrophilic species of general structure **1**, wherein X represents a leaving group (Figure 2).^[11, 20, 21] If the residue R² is hydrogen, HX elimination to the acylimine **2** frequently occurs, although enamides **3** can also function as α -amidoalkylation reagents,^[22] usually after a corresponding protonation at the terminal C atom.

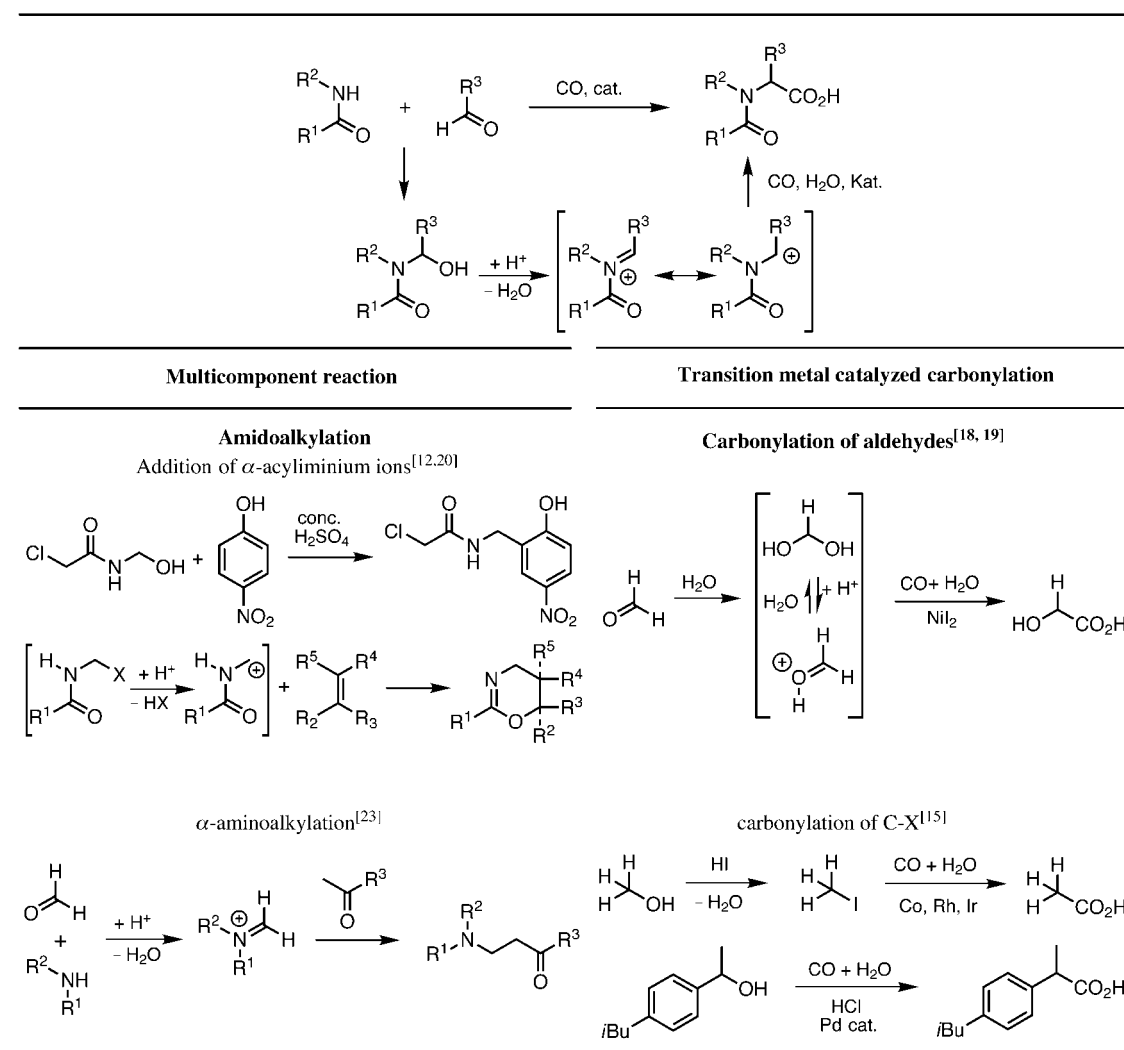


X = OH, OR, hal, NHCOR, NR₂

Figure 2. Intermediates in α -amidoalkylations.

The most well known nucleophiles in such α -amidoalkylations are aromatics and olefins.^[12] Electron rich arenes are converted into benzylamides and alkenes into dihydrooxazines in a cycloaddition reaction (Scheme 3).

Since the reactive α -amidoalkyl species are generally formed in situ from an aldehyde and an amide amidocarbon-



Scheme 3. Classification of amidocarbonylation.

ylation counts amongst the three component reactions (3-CR).^[23] Familiar reactions are the Mannich and Biginelli reactions as well as the Strecker or Asinger reactions. Common to all is the reaction of an initially formed α -aminoalkyl species with a nucleophile. Within this context the cobalt-catalyzed amidocarbonylation can with some justification be called the “Wakakatsu 3-component reaction” (W-3CR).

This different categorization of amidocarbonylation as a multicomponent reaction on the one hand and a carbonylation reaction on the other merges once again on the basis of the usual mechanistic classification; for the success of such a reaction the initial equilibria must be followed by an irreversible final step, which is the (usually transition metal catalyzed) carbonylation step in the case of amidocarbonylation.

2. Cobalt-Catalyzed Amidocarbonylation

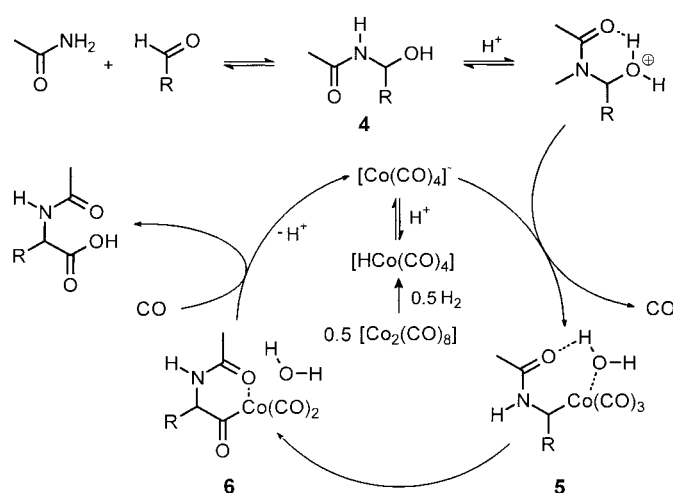
Cobalt-catalyzed amidocarbonylation is generally carried out at 70–160 °C, with synthesis gas pressures of 50–200 bar in solvents such as dioxane, THF, 1,2-dimethoxyethane (DME), ethyl acetate, acetone, or benzene. Dicobalt octacarbonyl $[\text{Co}_2(\text{CO})_8]$ in amounts of 1–5 mol % usually functions as the catalyst precursor. The CO/H_2 ratio varies between 1/1 and 4/1, the concentration of the starting materials in the solutions is usually 0.1–3 mol/L⁻¹, and the amide may be used in excess.

2.1. The Mechanism of Cobalt-Catalyzed Amidocarbonylation

In the first step of the amidocarbonylation reaction a nucleophilic addition of the amide to the aldehyde occurs with formation of an *N*- α -hydroxyalkyl amide **4**,^[10, 24] which can react with another amide molecule to form the bisamide in an equilibrium reaction or, depending upon the substitution pattern, can eliminate HX (X = OH, NRCOR) to form the corresponding *N*-acylimine or *N*-acylenamine (Scheme 4).^[23–25]

In the cobalt-catalyzed carbonylation step it is assumed that the active catalyst species, the tetracarbonylcobaltate $[\text{Co}(\text{CO})_4]^-$, forms the corresponding cobalt alkyl complex **5** with the protonated *N*-hydroxyalkyl amide in a nucleophilic substitution. The water formed possibly remains within the ligand sphere of the cobalt catalyst.^[26] The actual generation of the cobaltate anion from $[\text{Co}_2(\text{CO})_8]$ can take place in a number of ways, for example, a) in a reduction with hydrogen when $[\text{HCo}(\text{CO})_4]$, a strong acid, is formed, or b) in the absence of hydrogen by a disproportionation with a base (amide) to $[\text{CoB}_6][\text{Co}(\text{CO})_4]_2$,^[27] where, however, the addition of an acid, for example, sulfuric acid, is necessary for the protonation.

The cobalt alkyl complex **5** is transformed into the cobalt acyl complex **6** by CO insertion. This product can be opened to the oxazolonium salt by intramolecular nucleophilic attack of the carbonyl oxygen atom of the amide group on the



Scheme 4. Postulated mechanism of cobalt-catalyzed amidocarbonylation.

acyl–cobalt bond^[28] or by intermolecular nucleophilic attack of water on the acyl bond with formation of the carboxylic acid.^[26] In both cases the active catalyst species $[\text{Co}(\text{CO})_4]^-$ or $[\text{HCo}(\text{CO})_4]$ is regenerated and is then available for the next cycle.

2.2 Method Developments in Cobalt-Catalyzed Amidocarbonylation

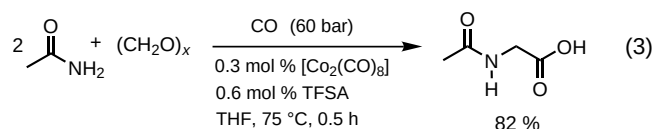
The emphasis of investigations to establish amidocarbonylation in the area of organic chemistry has without doubt hitherto laid in the exploitation of new starting materials by means of the in situ generation of the aldehyde component. In addition to aldehydes it is now possible to use olefins, acetals, epoxides, and allyl alcohols as well as alcohols and benzyl chloride as starting materials and treat them with amides and CO to form *N*-acylamino acids by amidocarbonylation.

2.2.1. Aldehydes

Aldehydes are the most important starting materials for amidocarbonylation. Numerous aldehydes have been used successfully in amidocarbonylation for the synthesis of natural and unnatural amino acid derivatives. However, cobalt catalysts are only capable of converting formaldehyde and aldehydes that possess at least one α -hydrogen atom. In the former case diamidocarbonylation is known, which leads, for example, to *N*-acylamino diacetic acids from a primary amide and two equivalents of formaldehyde.^[29] Such reactions have a potential use in the industrial production of glufosinate intermediates. It is only with formaldehyde that cyclic amides such as 2-pyrrolidinone may be amidocarbonylated in moderate yield.^[30]

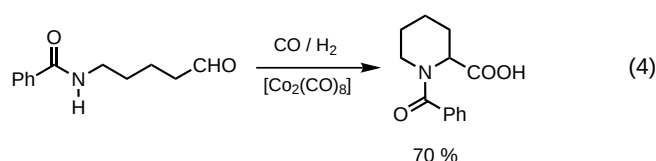
In order to carry out amidocarbonylation under mild conditions and in high yield the ligands were varied, acid co-catalysts were used, and a two-stage reaction procedure was developed. Lin et al. investigated the effect of different ligands in the synthesis of *N*-acetyl glycine. This study showed that basic ligands such as PBu_3 allowed amidocarbonylation to

be carried out at a lower pressure (55 bar). Ligands such as Ph_2SO or succinonitrile held the catalyst in solution such that the selectivity of the reaction and the recovery of the cobalt catalyst could be improved.^[31] Thus, in the presence of succinonitrile the yield of *N*-acetylglycine could be increased from 63 to 78 %, and the Co recovery from <10 to 80 %. A successful reaction procedure at lower temperature (60–90 °C) and with a higher rate of reaction was achieved by the addition of a strong acid ($\text{p}K_{\text{a}} < 3$) as co-catalyst. Moreover, under these conditions Drent et al. succeeded in carrying out amidocarbonylation with carbon monoxide instead of synthesis gas [Eq. (3); TFSA = trifluorosulfonic acid].^[32]

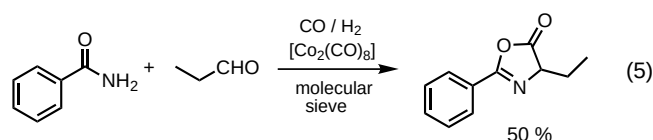


In 1994 a two-stage process was developed at Hoechst AG for the reaction of paraformaldehyde with *N*-methyl amides, which allowed the reaction to be carried out under very mild conditions. In the first stage methylol was formed by acid catalysis (80 °C), which was carbonylated in the second stage by cobalt catalysis at 50–70 °C and 10–50 bar (see sarcosinate preparation, Scheme 9) to afford the glycine derivative.^[33] The success of this reaction procedure is explained by the first stage of methylol formation having a higher activation energy than the carbonylation stage, and the cobalt catalyst is considerably more stable at the lower temperatures of the acidic conditions.^[1b] In addition, carbonylation at the lower temperatures leads to a conversion with significantly higher selectivity such that the yield of the industrially interesting fatty acid sarcosinates increases from 87^[31] to 98 %.

The preparation of ^{13}C -labeled *N*-acylamino acids^[34] and the intramolecular amidocarbonylation of aldehydes demonstrates the preparative usefulness of the reaction. For example, it was possible to synthesize *N*-benzoylpipecolinic acid in 70 % yield from the aldehyde in this way [Eq. (4)].^[28]



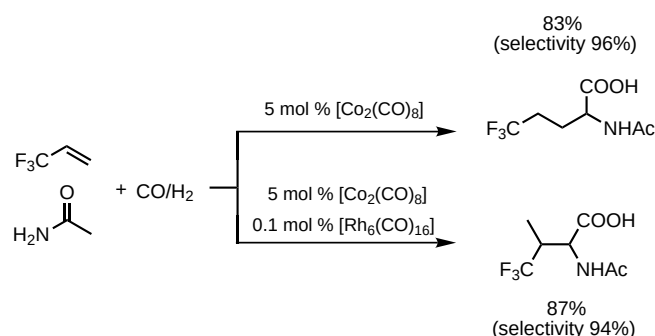
Another variant that should be mentioned here is the amidocarbonylation of aldehydes in the presence of dehydrating agents such as molecular sieves. In this way oxazolones can be obtained directly in moderate yield, as Izawa et al. demonstrated with the preparation of 2-phenyl-5-ethyl-oxazolone [Eq. (5)].^[28]



2.2.2. Olefins

Olefins are usually suitable as raw materials and are available commercially in a wide variety so that the combination of hydroformylation and amidocarbonylations with in situ aldehyde formation is of considerable importance. The hydroformylation stage can be catalyzed by cobalt carbonyl, rhodium carbonyl, or bimetallic Co-Rh complexes.

Although Wakamatsu et al. had suggested the use of olefins as early as 1974,^[35] it was Stern et al. who first described this domino reaction.^[36] 2-(Acetylamino)tetradecanoic acid was obtained from dodecene and acetamide in 73 % yield on a greater than 100-g scale (conditions: 672 g dodecene, 256 g acetamide, 1.6 L dioxane, 1.05 g $[\text{Co}_2(\text{CO})_8]$, 10 g $\text{Co}(\text{OAc})_2$, 100 bar CO/H_2 (1/1), 100 °C, 5 h). The question of *n*/*iso* regioselectivity was studied by Ojima et al. in investigations into the amidocarbonylation of trifluoropropene in which they synthesized *N*-acetyltrifluoronorvaline in the presence of 5 mol % $[\text{Co}_2(\text{CO})_8]$ (yield 83 %, selectivity 96 %).^[37] The addition of 0.1 mol % $[\text{Rh}_6(\text{CO})_{16}]$ to the cobalt catalyst system achieved a selectivity reversal to *N*-acetyltrifluorovaline (yield 87 %, selectivity 94 %), which showed that the regioselectivity was determined at the hydroformylation stage (Scheme 5). IR studies of the reaction showed that the bimetallic complex $[\text{CoRh}(\text{CO})_7]$ represented the active catalyst species.^[38]



Scheme 5. Effect of rhodium carbonyl complexes on the amidocarbonylation of 3,3,3-trifluoropropene.

The positive effect of chelating phosphane ligands on the yield of domino hydroformylation–amidocarbonylation was demonstrated by Lin and Knifton in the reaction of tetradecene. The addition of 1 mol % of 1,3-bis(diphenylphosphanyl)propane (dppp) to 2 mol % of $[\text{Co}_2(\text{CO})_8]$ enabled an increase in the yield of *N*-acetyl- α -aminohexadecanoic acid from 68 to 95 % to be achieved.^[31]

The use of functionalized olefins leads in many cases to interesting *N*-acylamino acid derivatives (Table 1). In addition to the proteinogenic amino acids lysine, glutamic acid, and proline, cyano, polyoxyethylene, and *O*-acetyl-functionalized derivatives are accessible by amidocarbonylation. Diamidocarbonylation products may also be synthesized in moderate yields from terminal diolefins.^[31]

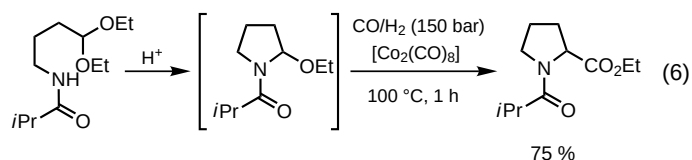
2.2.3. Acetals, Epoxides, and Allyl Alcohols

Acetals as simple protected aldehyde equivalents are either hydrolyzed in situ under the reaction conditions and react in

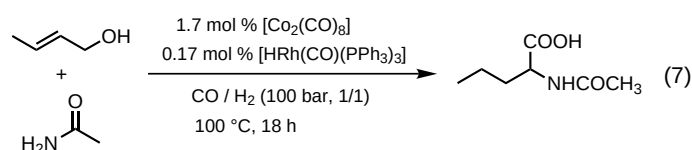
Table 1. Amidocarbonylation of functionalized olefins.

Entry	Functionalized olefin	Main product	Yield	Application
1			76 %	lysine ^[39]
2		 	n.a. 69 %	glutaminic acid ^[28] proline ^[28]
3			85 %	glutamate precursor ^[40]
4			85 %	polyamide ^[31a]
5			n.b.	tenside ^[31a]
6			85 %	polyamide esters ^[31a]

an analogous manner to the aldehydes, or they lead to the corresponding *N*-acylamino acid esters by water removal.^[41] Mechanistically this variant of amidocarbonylation proceeds via *N*-acyl- α -alkoxy amines. The use of acetals is of particular advantage if other functional groups in the starting material make a protective group necessary as, for example in intramolecular amidocarbonylations, or if the respective ester is the desired product (however, a certain amount of the free acid is also formed) [Eq. (6)].^[28]



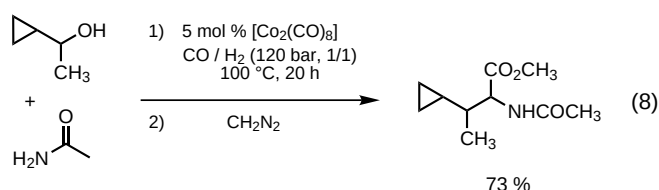
The in situ generation of the aldehyde can be achieved by a preceding rearrangement reaction as is the case in the use of epoxides and allyl alcohols. Epoxides, such as styrene oxide or propene oxide, may be rearranged into aldehydes by Lewis acid catalysis and then amidocarbonylated as demonstrate successfully by Ojima et al. with [Ti(OiPr)₄] or [Al(OiPr)₃] as co-catalysts (see also phenylalanine preparation; Section 2.3).^[37] Transition metal complexes such as [HRh(CO)(PPh₃)₃], [Fe₂(CO)₉], [RuCl₂(PPh₃)₃], and [PdCl₂(PPh₃)₂] have proved to be suitable for the in situ isomerization of allyl alcohols to enols, which then tautomerize to aldehydes [Eq. (7)].^[42]



2.2.4. Alcohols and Benzyl Chloride as Substrates in Amidocarbonylation

Alcohols that form stable carbenium ions may be hydrocarbonylated to the corresponding aldehyde under oxo conditions and then amidocarbonylated. Yukawa et al. demonstrated the preparation of *N*-acetyl-*O*-methyltyrosine in 50 % yield from 4-methoxybenzyl alcohol.^[43] Similarly, 1-cyclopropylmethanol was converted into *N*-acetyl-3-cyclopropylalanine by a carbonylation – amidocarbonylation sequence [Eq (8)].^[44]

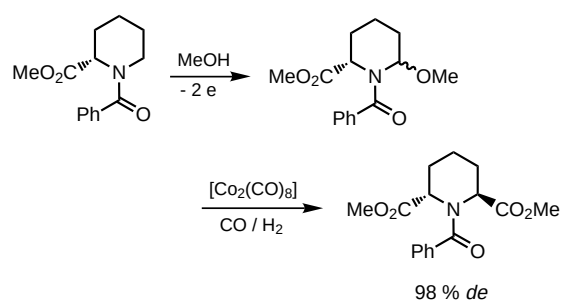
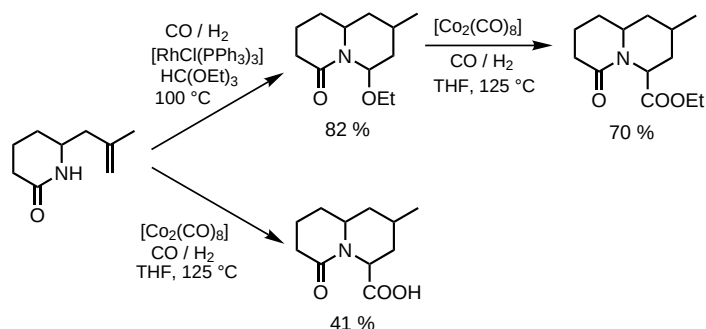
A new variant of amidocarbonylation uses benzyl chloride as the starting material to afford *N*-acetylphenylalanine in a domino hydrocarbonylation – amidocarbonylation sequence. By the shrewd selection of the individual reaction parameters de Vries et al. succeeded in achieving good yields (up to 79 %) of *N*-acylarylalanines, although high catalyst concentrations (12.5 mol % [Co₂(CO)₈], high pressures (275 bar), and the addition of NaHCO₃ are necessary.^[45] Critical factors here include the chloride ions that are formed in the first stage and which deactivate the catalyst.



2.2.5. Carbonylation of *N*- α -alkoxyamides

N- α -Alkoxyamides are intermediates in the amidocarbonylation of acetals, which can be isolated or separately synthesized. They may be carbonylated in good yields to a number of interesting *N*-acylamino acids. Different methyl esters of *N*-acylamino acids were prepared in good yields by Izawa by the two-stage synthesis involving the anodic oxidation of cyclic amides to α -methoxyamides and subsequent cobalt-catalyzed carbonylation.^[28] Particularly interesting is the high diastereoselectivity that was observed in the preparation of dimethyl *trans*-piperidine-2,6-dicarboxylate from optically active pipecolinic acid by this route (Scheme 6). This reaction currently represents the only example of an amidocarbonylation which takes place with high diastereoselectivity.

Ojima et al. prepared bicyclic *N*- α -ethoxyamides for mechanistic investigation by rhodium-catalyzed hydroformylation in the presence of triethyl orthoformate, which they were able to carbonylate to the corresponding ethyl esters in good or moderate yields by cobalt catalysis (Scheme 7).^[26] The corresponding *N*-acylamino carboxylic acids could also be obtained directly in a cobalt-catalyzed domino hydroformylation – amidocarbonylation reaction.

Scheme 6. Preparation of *trans*-piperidine-2,6-dicarboxylic acid.

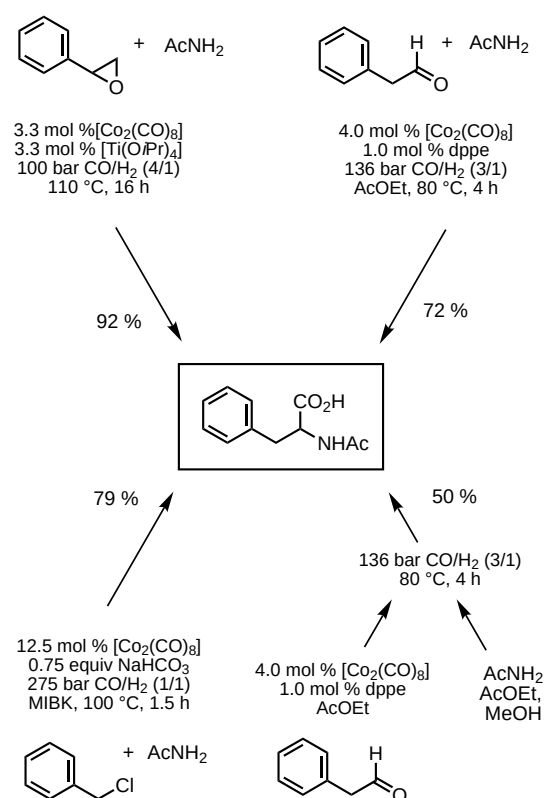
Scheme 7. Synthesis of cyclic quinolizidine derivatives.

2.3. Synthetic Applications of Cobalt-Catalyzed Amidocarbonylation

2.3.1. *N*-Acetyl-D,L-phenylalanine

N-Acetyl-D,L-phenylalanine is a precursor of L-phenylalanine, the key intermediate in the synthesis of aspartame (the methyl ester of L-aspartyl-L-phenylalanine) a sweetener developed by Ajinomoto and Searle which is 140 times sweeter than saccharose.^[46] Currently L-phenylalanine is produced by tyrosine fermentation. However, in an enzymatic aspartame process developed by the Holland Sweetener Company D,L-phenylalanine, which can be prepared advantageously by chemical means, can also be used as the starting material.^[47] More recently, procedures starting from phenylacetaldehyde, benzyl chloride, and styrene have been proposed as alternative chemical synthetic methods for the amidocarbonylation step. The product may be obtained in 92% yield by a Lewis acid catalyzed rearrangement of styrene oxide to phenylacetaldehyde followed by amidocarbonylation.^[37] The use of benzyl chloride in a domino hydrocarbonylation–amidocarbonylation sequence has given *N*-acetylphenylalanine in 79% yield after optimization of the conditions by the addition of NaHCO₃, although greater catalyst amounts and higher pressure were needed (Scheme 8).^[45]

At the forefront of investigations into batch and continuous processes based on phenylacetaldehyde is the question of how to keep the cobalt catalyst in solution. Dppe has emerged as the ligand of choice, and yields of 50–72% have been achieved.^[49] If the different amidocarbonylation methods for the preparation of phenylalanine are compared from an economic standpoint, it is clear that only those processes that start from benzyl chloride (<1 US \$ per kg) or styrene

Scheme 8. Cobalt-catalyzed amidocarbonylation to *N*-acetyl-D,L-phenylalanine. dppe = 1,2-bis(diphenylphosphanyl)ethane, MIBK = methyl isobutyl ketone.

oxide (>3 US \$ per kg) have any chance of realization. Phenylacetaldehyde, at a price of about 14 US \$ per kg, is clearly too expensive to be used industrially as a starting material. Styrene oxide is preferred to benzyl chloride as the starting material because of the lower incidence of side products. The disadvantages of the cobalt-catalyzed process are currently the low catalyst productivity and the space–time yields. These problems must be overcome by process improvements for a technical synthesis to be realized.

2.3.2. Sarcosinates

The long-chain *N*-acyl derivatives of sarcosine (*N*-methylglycine) belong to the group of anionic tensides that are used as components of surfactants, soaps, and emulsifiers because of their low hardening sensitivity and good dermatological compatibility.^[46] Hitherto, sarcosinates were prepared industrially on a scale in excess of 10 000 tonnes per year by a Schotten–Baumann reaction of a fatty acid chloride with the sodium salt of sarcosine.^[4]

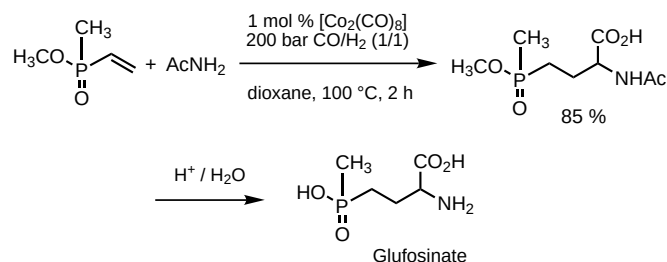
Although secondary amides generally give poorer results in amidocarbonylation, it has been possible to prepare sarcosinates successfully in a number of processes by the reaction of a fatty acid *N*-methylamide with paraformaldehyde. Lin and Knifton were able to produce *N*-methylauroylglycine in two hours in 87% yield under severe conditions (200 bar CO/H₂ (3/1), 120 °C with 3 mol % [Co₂(CO)₈]).^[31e] As already mentioned, Hoechst AG has developed an improved, two-

stage process for the preparation of a number of *N*-acylsarcosines (Scheme 9) in which yields in excess of 98 % of the target product have been achieved under very mild conditions (see Section 2.2.1).^[33] In particular, a method was developed to recover the cobalt catalyst used. The water-soluble cobalt salt was precipitated as cobalt hydroxide and then converted into a cobalt-containing melt with the *N*-acylsarcosine. The cobalt carbonyl catalyst was regenerated from the melt with synthesis gas (120 bar) at 90 °C and then reused.^[33b] The process outlined here was used successfully in a 1-L to a 250-L scale, and as a result sarcosinates have been prepared on a ton scale in a pilot plant.

2.3.3. Glufosinate

Glufosinate is a naturally occurring hydroxyphosphoryl-amino acid with herbicidal and antibiotic properties and is marketed as a nonselective herbicide under the name BASTA. An above average growth in the product is expected in the coming years because of the development of genetically modified, BASTA-resistant plants. Consequently new and efficient methods for the synthesis of glufosinate are of considerable interest. Syntheses starting from the respective olefins, aldehydes, or acetals of the methyl phosphinate are known for the production of BASTA by amidocarbonylation. Jägers et al. (Hoechst AG) were successful with the conversion of 3-[butoxy(methyl)phosphoryl]propylaldehyde diethylacetal into the *N*-acetyl-amino acid in 79 % yield (1.8 mol % $[\text{Co}_2(\text{CO})_8]$, 120 bar CO/H_2 (1/1), 120 °C) in one hour.^[50] Glufosinate may also be obtained in an

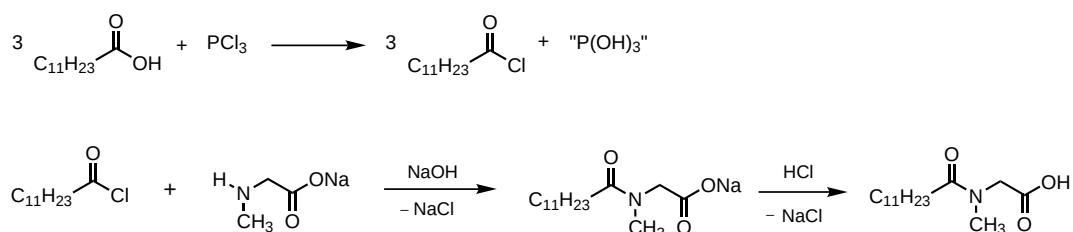
overall yield of 80 % by a domino hydroformylation–amidocarbonylation sequence with subsequent hydrolysis (Scheme 10).^[51]



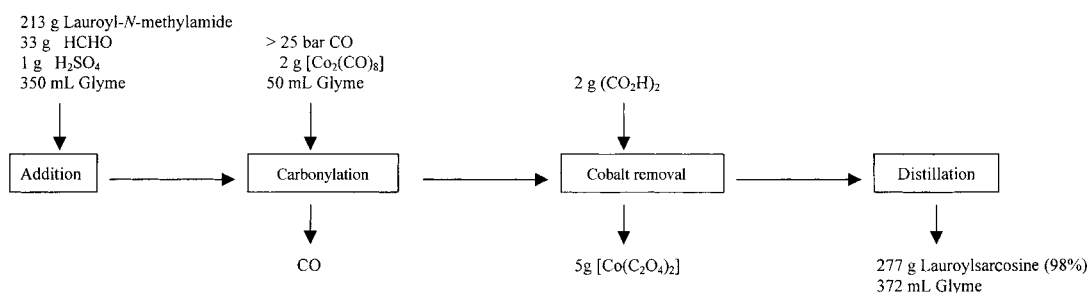
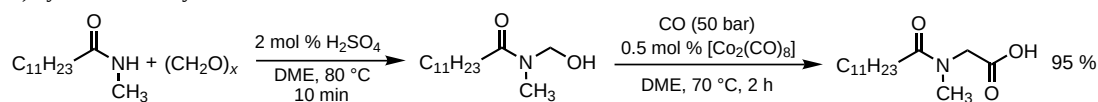
Scheme 10. Glufosinate preparation by amidocarbonylation.

In spite of numerous synthetically interesting possibilities for the use of amidocarbonylation in the production of *N*-acylamino acids, as far as we are aware sarcosinates have only been synthesized on a pilot plant scale. This observation is attributable in part to inadequate catalytic activity (turnover number (TON) < 100) as well as in most cases to the very high pressures (100–200 bar), which make the reaction economically unattractive. However, the realization of an adaptation of the Strecker synthesis, with subsequent Schotten–Baumann acylation to complete the amidocarbonylation, as an atom-efficient process (theoretically 100 %) for the preparation of a number of *N*-acylamino acids is worthwhile from an ecological viewpoint. In principle, two routes for the further improvement and investigation of amidocarbonylation, and thus a better utilization of the great potential of this method in the future, are conceivable. Firstly, the development of the

a) classical:



b) by amidocarbonylation:



Scheme 9. Two-stage amidocarbonylation in the synthesis of *N*-acylsarcosines. DME = 1,2-dimethoxyethane.

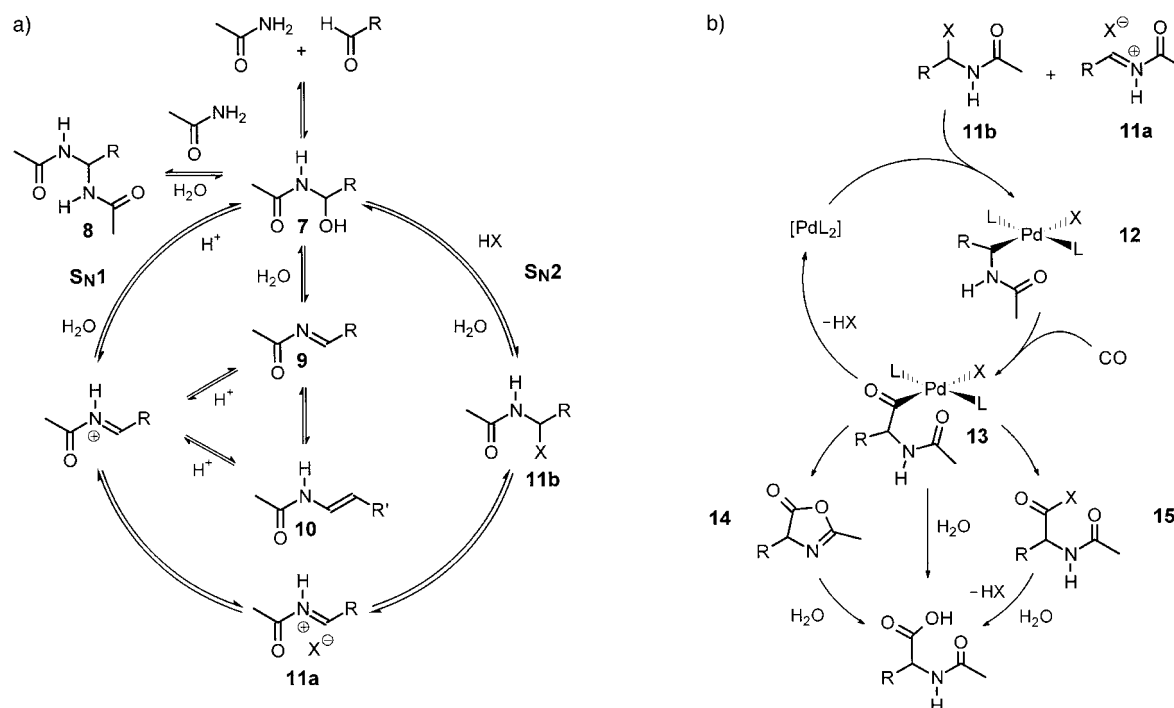
two-stage *N*-acylsarcosine process has illustrated that the scope for further optimization of the cobalt-catalyzed route is far from being exhausted. Secondly, the search for alternative catalyst systems has hitherto been greatly neglected, and the development of new transition metal catalyst systems for amidocarbonylation will reveal new pathways for further synthetic applications.

3. Palladium-Catalyzed Amidocarbonylation

In comparison to the cobalt catalyst variants, milder reaction conditions are generally used for amidocarbonylation in the presence of a palladium catalyst. Temperatures of 70–130 °C and carbon monoxide pressures of 10–60 bar are used. The presence of halide ions as co-catalysts have proved to be essential, and typically 10–35 mol% of LiBr or *n*Bu₄NBr are used. A synergistic effect, in which a doubling in the reaction rate without loss of specificity, is achieved by the addition of catalytic amounts of strong acids, for example 1 mol% H₂SO₄. Of the many different dipolar, aprotic solvents *N*-methylpyrrolidone (NMP) has proved to be particularly suitable. DMF, DMAc, or acetonitrile may also be used as solvents. The use of both palladium(0) (for example, [Pd₂(dba)₃], [Pd(PPh₃)₄]), and palladium(II) compounds (for example, PdBr₂, Pd(OAc)₂) as catalyst precursors is also possible. Under optimal conditions catalyst turnover numbers of 60000 (turnover frequency (TOF) > 1000 h⁻¹) may be achieved in the synthesis of *N*-acetyl-leucine by palladium-catalyzed amidocarbonylation. Thus, palladium catalysts have an activity greater than the cobalt systems by a factor of 10–100.^[14, 52]

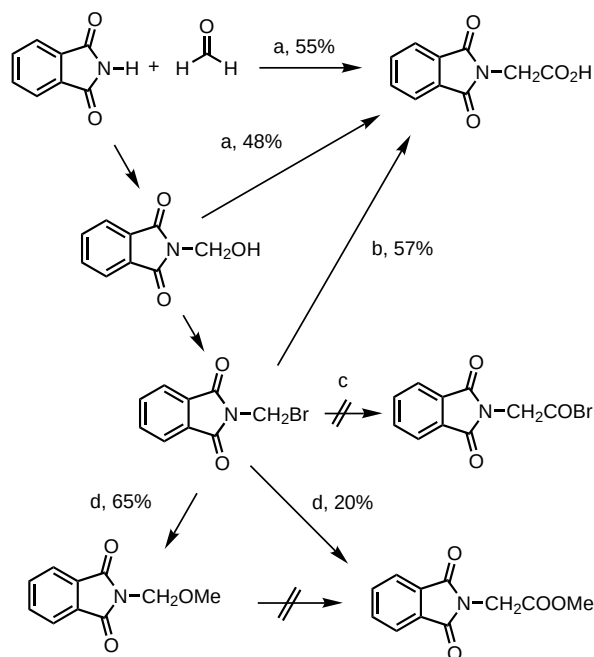
3.1. The Mechanism of Palladium-Catalyzed Amidocarbonylation

For a mechanistic discussion of palladium-catalyzed amidocarbonylation it is useful here to take as a basis the classification of amidocarbonylation into a multicomponent reaction and transition metal catalyzed carbonylation (Scheme 3), as described in Section 1.2. The initial equilibria, in which the starting materials and intermediates are in equilibrium, do not differ fundamentally from those of cobalt-catalyzed amidocarbonylation. However, additional critical intermediates are formed here through the involvement of halide ions (Scheme 11 a). After the initial step involving the formation of a α -hydroxyamide **7** by the nucleophilic attack of the amide on the carbonyl group followed by the generation of a bisamide **8**, an *N*-acylimine **9**, or enamide **10**, there is the possibility of forming a α -haloamide **11b** or the corresponding ion pair **11a**.^[53] This intermediate probably plays a critical role in palladium-catalyzed amidocarbonylation since active palladium(0) species can insert into these halogen–carbon bonds in an oxidative addition. This assumption finds support in that the presence of halide ions are essential for successful conversion. Because of the predominantly reductive conditions (CO, aldehyde, phosphane), the Pd^{II} precursors are readily converted into the active Pd⁰ species. Palladium(0) complexes such as [Pd(PPh₃)₄] or [Pd₂(dba)₃] are also ideally suitable as catalyst precursors for amidocarbonylation. The resulting Pd^{II}-alkyl complex **12** is subsequently transformed into the corresponding acyl complex **13** by a CO insertion. The cleavage of this compound probably occurs through the direct nucleophilic intermolecular opening of the complex by water. However, an intramolecular formation of an oxazolone derivative with subsequent hydrolysis cannot be ruled out in all cases.



Scheme 11. Postulated mechanism of palladium-catalyzed amidocarbonylation: a) initial equilibria; b) palladium-catalyzed carbonylation step.

The use of stable reaction intermediates has been studied in the mechanistic investigation of the amidocarbonylation of phthalimide with formaldehyde to give *N*-phthaloylglycine by palladium catalysis. Particularly rewarding was the carbonylation of the α -haloamide intermediate *N*-(bromomethyl)-phthalimide, which led to the carboxylic acid or the methyl ester only in the presence of a nucleophile such as water or methanol, respectively, through which it was possible to confirm that an intermolecular opening of the acyl complex had occurred (Scheme 12).



Scheme 12. Mechanistic investigations of palladium-catalyzed amidocarbonylation with the phthalimide-formaldehyde system. a) 0.25 mol % $\text{PdBr}_2/2\text{PPh}_3$, 35 mol % LiBr , 1 mol % H_2SO_4 , 60 bar CO, 12 h; b) 1 mol % $\text{PdBr}_2/2\text{PPh}_3$, 1 mol % H_2SO_4 , 1 equiv H_2O , 60 bar CO, 12 h; c) 0.25 mol % $\text{PdBr}_2/2\text{PPh}_3$, 1 mol % H_2SO_4 , 60 bar CO, 12 h; d) 1 mol % $\text{PdBr}_2/2\text{PPh}_3$, 1 mol % H_2SO_4 , 1 equiv MeOH, 60 bar CO, 12 h.

It was possible to isolate the palladium(II) alkyl complex and the corresponding acyl complex by the oxidative addition of the *N*-(bromomethyl)phthalimide to $[\text{Pd}(\text{PPh}_3)_4]$.^[54] Both complexes may be used successfully as a catalyst. In a high pressure NMR study it was also possible to follow the critical step of CO insertion leading to the formation of the acyl complex. Thus all critical steps of the palladium-catalyzed carbonylation cycle were now fully understood for the example of the amidocarbonylation of phthalimide with formaldehyde, and it was possible for the intermediates to be isolated so that the mechanism proposed in Scheme 11 could be underpinned.

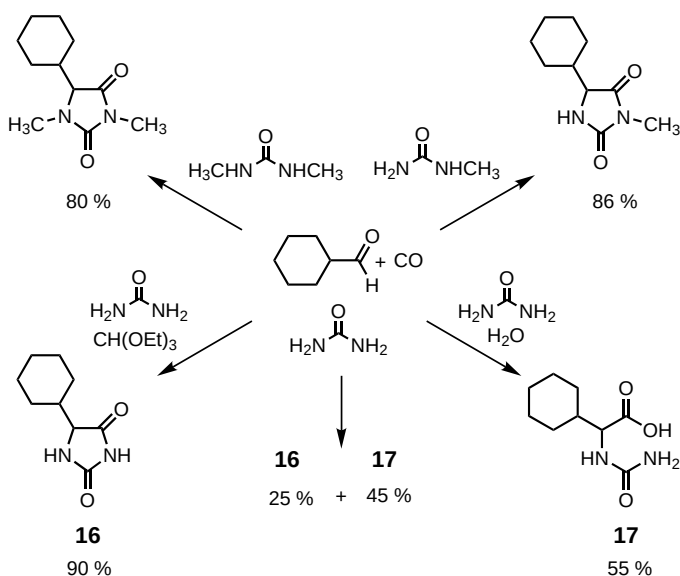
3.2. Developments in the Methodology of Palladium-Catalyzed Amidocarbonylation

Whereas in cobalt-catalyzed amidocarbonylations the implementation of in situ aldehyde generation stands almost

without exception in the foreground, in amidocarbonylation by palladium catalysis important developments in amide variation have been undertaken, the main highlights of which are described in the following section.

3.2.1. Ureidocarbonylation to Substituted Hydantoins

Depending on the nature of the starting material, the use of urea derivatives as amide components has made it possible to isolate the actual amidocarbonylation products, the *N*-ureidoamino acids. However, the substituted hydantoin is usually obtained with high selectivity.^[55] Hydantoins are very important in both production of amino acids and in research of pharmaceutically active, low molecular weight heterocycles.^[56] Until recently only 1,3-unsubstituted hydantoins were accessible in an efficient multi-component synthesis, the Bucherer–Bergs reaction.^[57] Not only were 1,3-unsubstituted hydantoins accessible in ureidocarbonylation, but for the first time hydantoins varyingly substituted by a wide range of substituents (H, alkyl, aryl) in the 1-, 3-, and 5-position were also available in good yield and selectivity.^[55] In conversions with free urea, control of selectivity leads to the hydantoin by the addition of a dehydration agent, or to the *N*-ureidoamino acid in the presence of an equivalent of water (Scheme 13).



Scheme 13. Palladium-catalyzed ureidocarbonylation.

When monosubstituted ureas are used, 3-substituted hydantoins are obtained selectively, while with *N,N'*-disubstituted ureas the 1,3-substituted hydantoins are obtained (Table 2).^[58] The acylpalladium(II) complex formed by CO insertion can first react intermolecularly with water to form the *N*-ureidocarboxylic acid, which then cyclizes,^[59] or it can be opened directly with the intramolecular formation of the hydantoin.^[60]

One important advantage of ureidocarbonylation lies in the practicability of the reaction, thus, for example 47 g (89%) of 5-cyclohexyl-1,3-dimethylhydantoin can be formed without complications in a 300-mL autoclave from 29 g of cyclo-

Table 2. Palladium-catalyzed ureidocarbonylation to substituted hydantoins.^[a]

$\text{R}^1\text{CHO} + \text{R}^2\text{NH}-\text{C}(=\text{O})-\text{NHR}^3 \xrightarrow[\text{NMP, 12h}]{\text{CO (60 bar) [Pd], 35 mol \% LiBr, 1 mol \% H}_2\text{SO}_4} \text{R}^1\text{C}(=\text{O})\text{N}(\text{R}^2)\text{N}(\text{R}^3)\text{C}(=\text{O})$						
Entry	R ¹	R ²	R ³	T [°C]	Yield [%] ^[b]	TON
1		CH ₃	H	80	75	300
2		C ₂ H ₅	H	100	51	204
3			H	100	64	256
4			H	100	50	200
5		C ₂ H ₅	C ₂ H ₅	100	89	356
6		CH ₃	CH ₃	120	61	244
7 ^[c]	H			130	93	372
8	H	CH ₃	CH ₃	100	73	292
9		CH ₃	CH ₃	100	85	340
10		CH ₃	CH ₃	100	79	316

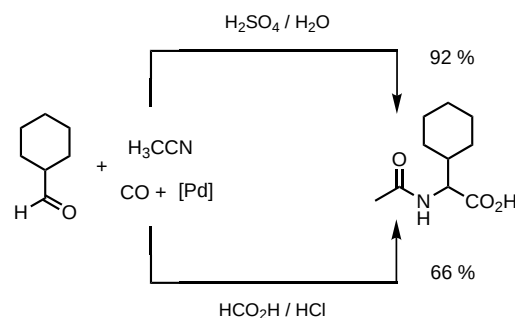
[a] In each case a 25.0 mL solution of the urea (1.0 M) and the aldehyde (1.0 M) in *N*-methylpyrrolidinone (NMP), 0.25 mol % [PdBr₂(PPh₃)₂] prepared in situ, 35 mol % LiBr, and 1 mol % H₂SO₄ were treated with 60 bar carbon monoxide for 12 h. [b] Yield of the isolated product. [c] Reaction time 48 h.

hexanal, 22 g of dimethylurea, and carbon monoxide (60 bar, 0.16 g PdBr₂, 0.32 g PPh₃, 4.5 g LiBr, 0.25 g H₂SO₄, 100 °C, 24 h).

3.2.2 Amidocarbonylation of Nitriles

The use of acetamide currently stands in the way of the use of amidocarbonylation in the production of high tonnage *N*-acylamino acids such as *N*-acetyl-D,L-valine or methionine. Unlike the raw material costs of various aldehydes and amines, it is clear that the amide components are frequently the cost-determining factor in the raw material costs. The corresponding nitriles may be regarded as economically interesting amide equivalents. For this reason the amidocarbonylation of nitriles was developed in which the amide is generated in situ.^[61] A one-pot process with selective hydrolysis of the nitriles to the amides, and subsequent palladium-catalyzed amidocarbonylation has been carried out successfully by a number of routes. The dropwise addition of the

nitrile to a concentrated sulfuric acid/water mixture^[62] gave different *N*-acylamino acids in very good yields after amidocarbonylation (Scheme 14).



Scheme 14. Amidocarbonylation of nitriles.

In addition, *N*-acylamino acids may be prepared conveniently by passing a stream of HCl through a nitrile/formic acid solution^[63] followed by carbonylation of the aldehydes formed.^[64]

3.2.3. Miscellaneous

The special significance of halide ions as co-catalysts for palladium-catalyzed amidocarbonylation suggests that low-melting halide salts could be used as a reaction medium. The use of salt melts is known in the literature for hydrogenation, hydroformylation, olefin dimerization, and the Heck reaction.^[65] Investigations have shown that the use of quaternary ammonium or phosphonium salts, such as *n*Bu₄NBr or *n*Bu₄PBr, as the solvent give activities and selectivities as high as NMP in palladium-catalyzed amidocarbonylation.^[66] The use of halide-free 1-ethyl-3-methylimidazolium hexafluorophosphate as the solvent has demonstrated that the presence of halide ions cannot be omitted, even in ionic liquids. In the reaction of cyclohexanal with acetamide in *n*Bu₄NBr, a catalyst turnover number of 20000 (TOF 330 h⁻¹) can be achieved with 0.001 mol % of [PdBr₂(PPh₃)₂].

For a process-orientated palladium-catalyzed amidocarbonylation reaction it is important that the salt and solvent waste is kept as low as possible for each product. This may be achieved by, for example, the successful multiple use of the same product-saturated mother liquor from which the product crystallizes on cooling after the reaction.

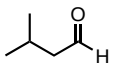
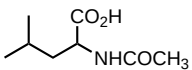
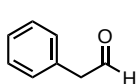
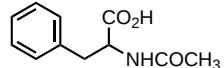
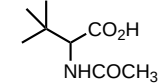
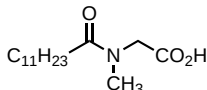
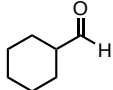
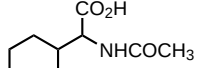
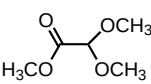
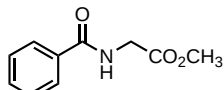
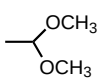
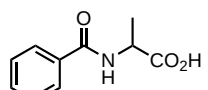
With the use of palladium on active carbon as the active catalyst it was possible to carry out amidocarbonylation in the presence of heterogeneous catalysts for the first time.^[67] The otherwise system-inherent disadvantage of problematic catalyst separation in homogeneous catalytic reactions could be solved simply and efficiently in this heterogenization. The possibility of continuous process control in which the active catalyst remains constantly in the reaction is opened up by this variant. Almost quantitative conversions in palladium-catalyzed amidocarbonylation at only 10 bar CO pressure are not only of great interest for technical realization, but for the first time also permits a broad preparative utilization of amidocarbonylation in glass autoclaves, which are available in many laboratories.^[68]

3.3. Synthetic Applications of Palladium-Catalyzed Amidocarbonylation

Amongst the synthetic applications of palladium-catalyzed amidocarbonylation, the examples discussed in greater detail here are those that are not amenable to cobalt catalysis, or have not otherwise been described. These include amidocarbonylation of aldehydes without α -H atoms, investigations into chemoenzymatic, two-stage processes for optically pure amino acids, and the preparation of *N*-substituted amino acids. In addition to these special applications (see below), numerous substrates have been used successfully in palladium-catalyzed amidocarbonylation, a few of which are listed in Table 3.

Of these, the high-yielding reaction to give *N*-acetyl-*tert*-leucine, which when enantiomerically pure is employed as a chiral auxiliary and as a building block in pharmaceutically active molecules,^[69] is of special interest. The *N*-acetyl derivative of cyclohexylglycine, also an important unnatural amino acid, was obtained almost quantitatively with the palladium on active carbon catalyst (entry 5).^[68] Acetals in the presence of one equivalent of water can also be used in place of the aldehyde in palladium-catalyzed amidocarbonylation (entries 6 and 7), and when methylglyoxal dimethylacetal was used a concomitant decarboxylation afforded methyl glycinate.^[68]

Table 3. Palladium-catalyzed amidocarbonylation of aldehydes and acetals.^[a]

Entry	Aldehyde/acetal	<i>N</i> -Acyl- α -amino acid	Yield [%] ^[b]
1			99
2 ^[c]			78
3			95
4	CH ₂ O		85
5 ^[d]			98
6 ^[e]			42
7 ^[e]			86

[a] In each case a 25.0 mL solution of the urea (1.0 M) and the aldehyde (1.0 M) in *N*-methylpyrrolidinone (NMP), 0.25 mol % [PdBr₂(PPh₃)₂] prepared in situ, 35 mol % LiBr, and 1 mol % H₂SO₄ were treated with 60 bar carbon monoxide for 12 h. [b] Yield of isolated product. [c] 2 equiv aldehyde. [d] With 1 equiv H₂O. [e] Catalyst: 0.5 mol % Pd/C.

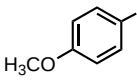
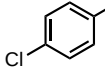
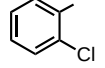
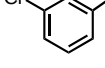
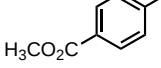
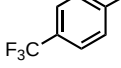
3.3.1. Functionalized Arylglycines

The products of amidocarbonylation of benzaldehydes, *N*-acyl- α -arylglycines, occur as substructures in many different pharmacologically active compounds.^[70] Owing mainly to its occurrence in a group of highly effective glycopeptides, such as vancomycin, β -avoparcin, and chloropectin I,^[71] it has been one of the synthetically most investigated series of amino acids over the last two years.^[72]

An atom-efficient synthesis by transition metal catalyzed amidocarbonylation has until now proved elusive since the classical cobalt catalysts cannot be extended to the carbonylation of aldehydes without an α -H atom. Instead benzylamides are formed as the product. Systematic studies have shown that amidocarbonylation of benzaldehydes in the presence of palladium catalysts allows the synthesis of many functionalized, racemic *N*-acetyl- α -arylglycines in very good yields (Table 4).^[73]

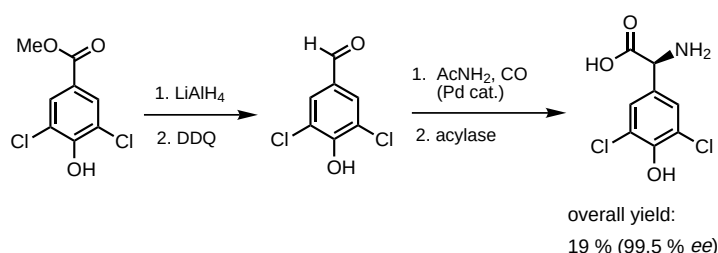
It has also been found that substituents exert a significant electronic influence on the reactivity of the aldehyde.^[74] Derivatives with electron-donating substituents react faster than those with electron-withdrawing substituents, although satisfactory yields could be achieved synthetically by an increase in the reaction temperature and reaction time. Further investigations with heteroaromatic aldehydes have

Table 4. Palladium-catalyzed amidocarbonylation of aromatic aldehydes.

$\text{H}_3\text{C}-\text{C}(=\text{O})-\text{NH}_2 + \text{O}=\text{C}-\text{H} \xrightarrow[\text{LiBr/H}_2\text{SO}_4/\text{NMP}]{\text{CO (60 bar), PdBr}_2/2\text{PPh}_3, 12\text{ h}} \text{H}_3\text{C}-\text{C}(=\text{O})-\text{NH}-\text{CH}(\text{Ar})-\text{COOH}$						
Entry	Ar	σ_p ^[a]	(100 °C, 12 h) yield [%] ^[b]	(100 °C, 12 h) TON ^[c]	(120 °C, 15 h) yield [%] ^[b]	(120 °C, 15 h) TON ^[c]
1		-0.27	75	300	—	—
2		-0.17	86	344	95	380
3		0.30	65	260	—	—
4		—	56	224	—	—
5		—	63	252	—	—
6		0.39	52	208	89	356
7		0.54	42	168	82	328
8		—	—	—	42 ^[d]	168

[a] Hammett constants for *para*-substituents. [b] Yield of isolated product. [c] TON [mol product/(mol cat.)]. [d] 60 h, 125 °C.

also given interesting heteroaryl glycines by this preparative route. The relevance of the palladium-catalyzed amidocarbonylation of benzaldehyde to the synthesis of natural products has also been demonstrated by the preparation in good to very good yields of 4-hydroxyarylglycines, which occur in cyclic glycopeptide antibiotics. In the case of the exemplary synthesis of the central amino acid of chloropeptin I,^[75] (*S*)-3,5-dichloro-4-hydroxyphenylglycine, it was also possible to confirm the advantage of the two-stage combination of palladium-catalyzed amidocarbonylation and enantioselective, enzymatic hydrolysis by the acylase *Aspergillus spp.* in the synthesis of enantiomerically pure α -arylglycines. This synthesis is three steps shorter than the known literature method for similar derivatives, but with the same yield (Scheme 15).^[76]



Scheme 15. Synthesis of the central amino acid of chloropeptin I by palladium-catalyzed amidocarbonylation.

3.3.2. Chemoenzymatic Two-stage Processes for Optically Active Amino Acids

In the past many elegant procedures have been developed for the preparation of enantiomerically pure α -amino acids.^[5, 77] In spite of broad applicability and selectivity, many procedures have proved to be problematic when the methods have been applied to the 100-g scale or above. Furthermore, simple estimates of raw material costs show that even the most recent catalytic procedures are a factor of 10–100 more expensive than the route composed of a combination of multi-

component reaction (Strecker reaction/acetylation or amidocarbonylation) with enzymatic separation of the racemate. The sequence of chemical preparation of racemic *N*-acetyl-amino acids followed by enantioselective enzymatic hydrolysis is therefore one of the most versatile and industrially used methods for the preparation of optically active amino acids.^[78] In this context the recent, more detailed description of the two-stage, chemoenzymatic process composed of amidocarbonylation and acylase-catalyzed racemate separation is of interest.^[79]

Different types of acylase differ not only in their activity and acceptance of the amino acid residue but are also to a large extent dependent upon the nature of the acyl residue.^[80] Thus, in addition to acetyl derivatives, phenacetyl, methoxyacetyl, and formyl derivatives have been synthesized by palladium-catalyzed amidocarbonylation and hydrolyzed enantiospecifically with different acylases (AA, PKA, PA; Table 5).^[80]

In this chemoenzymatic, two-stage synthesis it was possible to adapt the amidocarbonylation step for the use of different acylases. Thus, depending upon the derivative, it is possible to optimize the reaction for the best possible rate of hydrolysis and substrate tolerance by the selective coordination of the acyl residue with the acylase, which makes this method more attractive for the synthesis of enantiomerically pure amino acids. In this way it was also possible to prepare novel, *N*-acyl-functionalized amino acids by means of amidocarbonylation and to show that palladium-catalyzed amidocarbonylation is doubly convergent, that is, not only is an enormous breadth of substrate diversity tolerated in the aldehyde and hence in the amino acid residue, but equally a useful permutation in the amide moiety was accepted.

3.3.3. *N*-Substituted Glycines

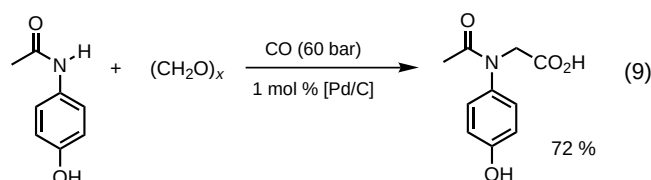
As already mentioned, the amidocarbonylation of secondary amides—especially those with sterically demanding

Table 5. Synthesis of enantiomerically pure α -amino acids by palladium-catalyzed amidocarbonylation and enzymatic hydrolysis.

entry	yield [%] ^[b] (TON)	Amidocarbonylation ^[a] (<i>R,S</i>)- <i>N</i> -acyl-AS		enzyme ^[c]	Enzymatic hydrolysis	
		R ¹	R ²		(<i>S</i>)-AS yield [%] (<i>ee</i> [%])	(<i>R</i>)- <i>N</i> -acyl-AS yield [%] (<i>ee</i> [%])
1	75 (300)		CH ₃	AA	40.4 (≥99.5)	45.6 (94.4)
2	75 (300)		CH ₃	PKA	32 (≥99.5)	40 (85.6)
3	83 (332)		Bz	PA	38 (≥99.5)	49 (≥94)
4	83 (332)		CH ₂ OCH ₃	PKA	43.9 (98.8)	47.2 (96.8)

[a] Conditions: 25 mL NMP (1M in aldehyde and acetamide), 0.25 mol % [PdBr₂(PPh₃)₂], 35 mol % LiBr, 1 mol % H₂SO₄, 60 bar CO, 120 °C, 12 h. [b] Yield of isolated product. [c] AA = *Aspergillus spp.* acylase, PKA = pig liver acylase.

residues—are significantly more difficult to carry out than the corresponding reactions of primary amides. However, it has been possible to prepare a series of interesting *N*-substituted *N*-acetylgylicines by reaction with formaldehyde.^[67] Particularly good results have been achieved with a catalyst of palladium on activated carbon at 130°C and extended reaction times (60 h). For example, the respective reaction of paracetamol gave a 70% yield of *N*-acetyl-*N*-(4-hydroxyphenyl)glycine [Eq. (9)], the analogue of *N*-acetyl-4-hydroxyphenylglycine, which may also be prepared in very good yields by palladium-catalyzed amidocarbonylation.



N-Substituted glycines are building blocks of an important class of peptide mimics, the peptoids.^[81] By the synthesis of different alkyl- and aryl-substituted glycines it was possible to show that palladium-catalyzed amidocarbonylation offers a simple route to the monomers of this medically important group of active compounds

4. Summary and Prospects

Since its discovery in 1971 cobalt-catalyzed amidocarbonylation has experienced numerous developments. Of particular importance here are the development of domino sequences such as the domino hydroformylation–amidocarbonylation reaction to give *N*-acylamino acids directly from olefins. Such reactions are prime examples of the increasingly discussed development of “sustainable” synthetic methods (“green chemistry”),^[82] since more complex, industrially interesting products are formed in a 100% atom economic manner from simple building blocks.

With the establishment of palladium-catalyzed amidocarbonylation, critical advances have been made in the recent years with the attainment of higher catalyst activities under milder conditions. With respect to functional-group tolerance too, palladium-catalyzed variants are currently superior to the cobalt-catalyzed reaction. Synthetically, the spectrum could thus be widened by the preparation of functionalized arylglycines and hydantoins as well as the amidocarbonylation of nitriles. Today, standardized reactions with a catalyst of palladium on activated carbon and a carbon monoxide pressure of 10 bar simplifies the applicability of the methodology.

Although numerous elegant new synthetic methods are available for the preparation of enantiomerically pure amino acids, the development of economically practicable procedures on a scale in excess of 100 g are still a challenge. With this background, chemoenzymatic two-stage procedures of amidocarbonylation with subsequent enzymatic separation of the racemate offers an interesting route to enantiomerically

pure, natural and unnatural amino acids on a multi-gram scale.

What are the future challenges in the area of amidocarbonylation procedures? Without doubt these include the development of asymmetric amidocarbonylation. We have succeeded in making the first step in this direction by the use of chiral phosphane ligands such as 1-diphenylphosphanylmethylbenzene in the palladium-catalyzed amidocarbonylation of isovaleraldehyde to *N*-acetylleucine with 10% *ee*.^[83] Other research groups have also recently observed enantiomeric excesses in palladium-^[84] and cobalt-catalyzed amidocarbonylations,^[85] although they are still unsatisfactory. The search for “efficient” ligands has only just begun. Although amidocarbonylation has been known for almost 30 years, another area of stereoselective methodology has been neglected. With one exception no diastereoselective conversions have been described. In the area of diastereoselective reactions, di- and oligopeptide preparations appear to us to be of particular interest for the future. Such reactions are fundamentally possible, but here too the task still remains to explore the applicability of the method systematically.

Although a large number of *N*-acylamino acids are easy to synthesize with palladium and cobalt catalysts, there are still aldehydes that are not amenable to amidocarbonylation, or are only unsatisfactorily so (for example, carbohydrate aldehydes, indole aldehydes, iodo- and bromo-substituted aldehydes, a few arylacetaldehydes). The use of ketones as carbonyl components would also be very interesting for the preparation of α -quaternary *N*-acylamino acids. Perhaps totally new catalyst systems will have to be used for these “problem cases”. When it is considered that carbonylation reactions related to amidocarbonylation can be catalyzed not only by metal complexes of Co and Pd, but also, for example by Rh and Ir complexes, it should be fundamentally possible to develop alternative catalysts for amidocarbonylation.

A larger, industrial scale cobalt- or palladium-catalyzed amidocarbonylation is still awaited. Whereas the cobalt-catalyzed variant has been able to prove its industrial feasibility on a pilot plant scale, to our knowledge the more recent palladium-catalyzed variant has been only developed to a 2-L scale. Technical questions and the problem of the co-catalyst remain to be solved in the future.

In conclusion it remains to be said that amidocarbonylation, a three-component reaction with a terminal carbonylation step, is but one example of a transition metal catalyzed multicomponent reaction. In the development of such multicomponent reactions with the participation of a transition metal catalyzed step we see great potential for the development of totally new reactions, which will lead to greater creation of wealth by the formation of interesting products from simple starting materials.

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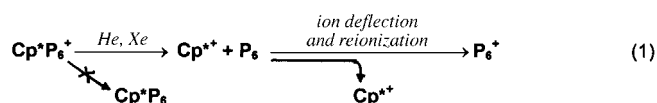
Small Neutral P_n Molecules

Otto J. Scherer*

Among the stable and well-studied element modifications of phosphorus are, besides the „parent structure“ of white, tetrahedral P_4 , the modifications resulting from thermolysis, namely, black phosphorus (double layers of chairlike P_6 rings), violet or Hittorf's phosphorus (P_2 - P_8 - P_2 - P_9 repeating units that form tubes of five-membered rings, which, in a simplified way, can be described as being arranged crosswise like wood piles),^[1] and red phosphorus, whose controversial structure was only recently predicted by theory by Häser et al.^[2] (P_{10} - P_2 repeating units) and structurally confirmed in $(CuI)_3P_{12}$ by Pfützner et al.^[3] as helical P_{10} - P_2 strands embedded in a matrix of CuI layers.

There have been numerous attempts to experimentally characterize the small molecules P_3 , P_4 , P_5 , and P_6 as neutral species. In this respect, the research groups of Schwarz (Berlin) and Jutzi (Bielefeld) have now achieved a major breakthrough^[4] with the detection of neutral P_6 in the gas phase by neutralization–reionization (NR) mass spectrometry.^[5]

Starting from the structurally characterized P_6 benzvalene derivative Cp^*P_6 ^[6] ($Cp^* = \sigma$ -bound C_5Me_5), whose EI mass spectrum shows $Cp^*P_6^+$ and Cp^{*+} as the major fragments along with the molecular ion, P_6^+ and P_n^+ ($n=1-5$) are detected in the following order of intensity $P_4^+ \gg P_3^+ > P_2^+ \approx P_6^+ \gg P_5^+ \approx P^+$ [Eq. (1)] by means of the neutral fragment reionization method (N_fR), a variation of the NR method.



Extensive theoretical investigations^[7] have established the following order for the stability of the five P_6 isomers (Figure 1):^[7a] (a) > (b) > (c) > (d) $\gg$ (e). On the basis of this finding along with the P_6 benzvalene framework of $Cp_2^*P_6$ the authors propose a benzvalene structure ((a) in Figure 1) for the neutral P_6 molecule detected in the gas phase.

As a general rule, neutral and charged P_n molecules are more stable the fewer double bonds the molecule contains

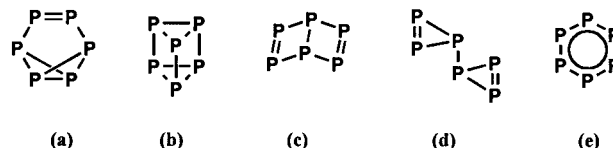
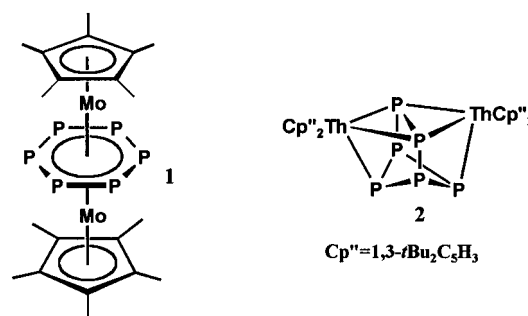


Figure 1. The P_6 isomers isoelectronic to the five $(CH)_6$ valence isomers (isolobal relationship: $P \leftrightarrow CH$) of benzene. P_6 benzvalene (a), P_6 prismane (b), P_6 Dewar benzene (c), P_6 bicyclopropenyl (d), and P_6 benzene (e).

and the larger the value of n is. The slightly higher stability of P_6 benzvalene over P_6 prismane (Figure 1) can be explained when additional strain and resonance energies are taken into consideration.^[7b]

To characterize unstable molecules, one can either turn to matrix techniques or to coordinative stabilization; it is also possible in certain cases to achieve kinetic stabilization by using sterically demanding substituents. Pertinent examples from the organometallic chemistry of phosphorus are the compounds **1** and **2**, whose P_6 ligand in the molybdenum triple-decker sandwich complex **1** contains the all-phosphorus analogue of benzene as the middle deck,^[8a,b] whereas the dinuclear thorium complex **2** contains an open-edge P_6 benzvalene unit.^[8c]



Neutral P_5 has so far not been detected. Of the structures calculated (Figure 2), the P_4 butterfly framework (f) (cf. P_6

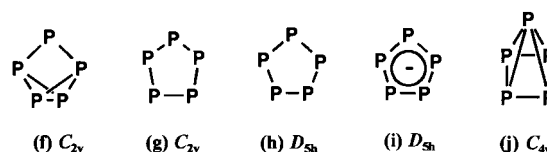


Figure 2. Calculated geometries (f), (g), (h), and (j) of neutral P_5 as well as planar, aromatic $cyclo-P_5^-$ (i).

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benzvalene, (a) in Figure 1) bridged by one P atom represents the most stable P_5 structure, followed by (g) (slightly distorted planar P_5 ring) and the almost energetically equivalent structures of the undistorted P_5 ring (h) and of the square-pyramidal P_5 (j).^[7a]

If for P_5 one goes from the 25 valence electron (VE) radical to the 26 VE anion ((i) in Figure 2) by introduction of a negative charge, then, as shown by Baudler et al.,^[9a] the all-phosphorus analogue of the cyclopentadienide ion (*cyclo*- $P_5^- \triangleq \text{cyclo } C_5H_5^-$) can be prepared in solution in the form of the MP_5 salts ($M = \text{Li, Na}$) and characterized; *cyclo*- P_5^- is almost as aromatic as the $C_5H_5^-$ analogue.^[10] As for the P_6

benzene (see complex 1), the 6π -electron system of *cyclo*- P_5^- can also be coordinatively stabilized in the form of the unusually stable (cf. the isoelectronic ferrocene, $[Cp_2Fe]$) sandwich complex **3**^[9b,a].

Of the calculated structures for neutral P_4 ^[7a], as expected the P_4 tetrahedron ((k) in Figure 3) of white phosphorus is by far the most stable form. It is followed by the P_4 butterfly (roof-type) form (l) and then by the P_4 rectangle (m). A P_4 zigzag chain comprising two weakly bound P_2 units is a particularly unstable form.

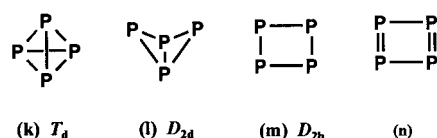


Figure 3. Calculated geometries (k)–(m) of neutral P_4 as well as planar tetraphosphacyclobutadiene (n).

All P_4 structure alternatives shown in Figure 3 can be coordinatively stabilized in the form of complexes with 17-VE L_nM fragments ($2 \times$ terminal for (l)), 16-VE L_nM fragments (μ -bridging for (l), and terminal for (k)) and 14-VE L_nM fragments (type (n)) as tetraphosphacyclobutadiene complex **4**.^[11]

For the neutral 15-VE P_3 radicals theoretical studies^[7a] report the isosceles P_3 triangle ((o) in Figure 4) to be slightly more stable than the equilateral triangle (p), and the linear arrangement (q) is significantly more unstable.

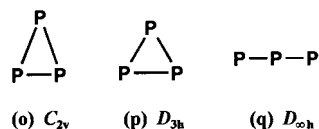


Figure 4. Calculated geometries (o)–(q) of the neutral P_3 radical.

The equilateral P_3 triangle (p) can be stabilized both in triple-decker complexes $[L_nM(P_3)ML_n]^{n+}$ as well as in tri-phosphametallatetrahedranes $[L_nMP_3]$ with one 15-VE L_nM fragment.^[12] Bent P_3 ligands are found in polynuclear complexes.^[11]

If white phosphorus P_4 is heated to 1000–1500 °C, one finds exclusively the equilibrium $P_4 \rightleftharpoons 2P_2$ and no indication of other P_n species.^[13] P_2 , $:P \equiv P:$, a well-characterized molecule

both theoretically^[7] and in the gas phase, displays diverse ligand properties similar to those of acetylene $HC \equiv CH$ to which it is the isoelectronic and isolobal.^[11]

The extension of the use of neutralization-reionization mass spectrometry for the detection of new neutral P_n allotropes will be dependent primarily on the availability of suitable P_n starting materials bearing easily removable substituents or ligands—a new challenge for preparative phosphorus chemistry. A worthwhile goal besides P_5 ^[9a,b] (provided that suitable precursors can be found) would be P_8 *cuneane*, which according to calculations^[7, 14] is the most stable P_8 isomer. As an almost ubiquitous P_8 building block it is a structural motif of Hittorf's phosphorus, is discussed for the clusters P_{25}^+ , P_{33}^+ , P_{41}^+ , and P_{49}^+ , whose P_n^+ frameworks possibly consist of nP_8 *cuneane* building blocks ($n = 3–6$) each with an additional P cap,^[14] and could be complex-chemically stabilized as a P_8 *cuneane* with two open-edges.^[15] If one goes from the small to the larger neutral P_n molecules, then P_{20} , the all-phosphorus analogue of the Paquette's $(CH)_{20}$ dodecahedron (a platonic solid with 12 pentagons, 20 vertices, and 30 edges) represents a modification of phosphorus, the search for which should not be a search for utopia, particularly since theory predicts that P_{20} lies energetically only slightly above the value for $5 \times P_4$ units.^[16, 7d]

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Imprinted Polymers with Memory for Small Molecules, Proteins, or Crystals**

Börje Sellergren*

Introduction

Molecular recognition is associated with biological processes such as the immuno response, ligand–receptor interactions, and enzyme catalysis. The ability of biological hosts to strongly and specifically bind to a particular molecular structure is a key factor in the biological machinery. Well-known examples are the sensitivity of the immuno response,^[1] where antibodies are generated in response to minute amounts of a foreign antigen, or the energy saved by enzymes due to their ability to stabilize the transition state of the reaction to be catalyzed.^[2] With biological examples as models, chemists hope to be able to mimic these properties for various applications.^[3–5] For instance stable recognition elements capable of strongly and selectively binding molecules could be used in robust, sensitive analytical methods for the analysis of trace levels of compounds in complex matrices. Alternatively such recognition elements could be used to separate undesirable compounds from foods or biological fluids, for targeted delivery of drugs, or for demanding separations at a preparative level in the fine-chemical industry.

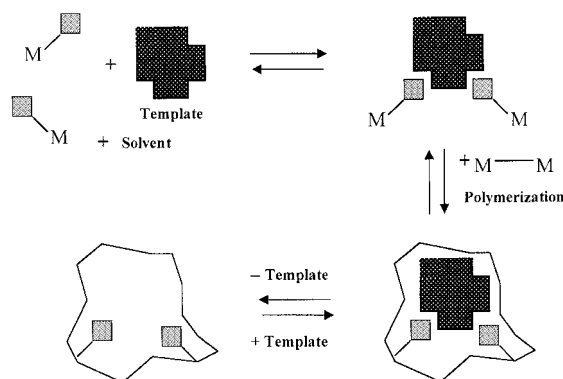
Robust molecular recognition elements with antibody-like ability to bind and discriminate between molecules or other structures can today be synthesized using molecular imprinting techniques.^[6, 7] This includes the synthesis of cross-linked polymers in the presence of templates which may be small molecules, biological macromolecules, microorganisms, or whole crystals (Scheme 1). Functional monomers that can be linked covalently or noncovalently to the template bind the

template to the monomer or growing polymer during synthesis and, after template removal, provide the subsequent binding interactions (Table 1). Removal of the template from the formed polymer thus generates a structure complementary to the template structure or to an analogous structure. This simple and appealing concept has for more than 50 years^[8] attracted chemists from several disciplines in their search for solutions to their particular problems. Nevertheless advances in the field have been slow and real applications that can be commercially exploited are still lacking. However, in view of some recent promising developments this may be about to change. Combinatorial techniques for rapid, parallel synthesis and testing of large groups of materials promise, for instance, to accelerate the development of new recognition elements significantly.^[9, 10] Furthermore clever design of linkers to position the binding-site functional groups at distances allowing subsequent rebinding by electrostatic interactions has resulted in materials that exhibit excellent recognition properties for highly interesting targets such as peptides^[11] and steroids.^[12] Also larger molecules such as proteins can now be recognized by surface imprinting techniques.^[13] Thus, template imprinting is not restricted to low molecular weight targets. Recent contributions have shown that even imprinting of whole cells^[14] and inorganic crystals^[15] generates structures capable of efficient recognition of the corresponding template structure. Finally, imprinting of metal ions for sequestration or sensing^[7] and molecularly imprinted enzyme mimics^[4] are burgeoning areas, but outside the scope of this contribution. This highlight will summarize some of the most promising recent developments for the generation of templated binding sites and then provide connections to potential applications.

Noncovalent Imprinting for the Recognition of Small Molecules

The most successful noncovalent imprinting systems are based on commodity acrylic or methacrylic monomers, cross-linked with ethyleneglycol dimethacrylate (EDMA). Methacrylic acid (MAA) is, to date, the most widely used functional monomer, as can be seen from the numerous examples in the literature.^[7, 16] The most common procedure applied to imprinting with L-phenylalanine anilide (L-PA) is outlined in Scheme 2.^[17, 18]

The broad applicability of MAA as a functional monomer is related to the fact that the carboxylic acid group serves well as a hydrogen-bond and proton donor and as a hydrogen-bond acceptor.^[19] In aprotic solvents such as acetonitrile, carboxylic acids and amine bases form contact hydrogen-bonded assemblies, where the association strength for a given acid increases with the basicity of the base.^[20] Thus templates containing Brønsted basic or hydrogen-bonding functional groups are potentially suitable templates for the MAA/EDMA system.^[10, 17, 18] Furthermore, hydrogen bonds can form with templates containing acid,^[21] amide,^[22] or functionalized



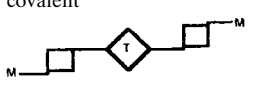
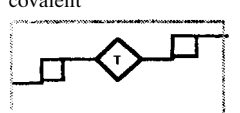
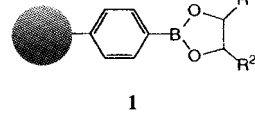
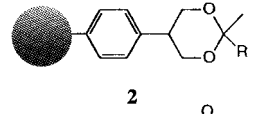
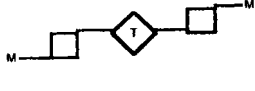
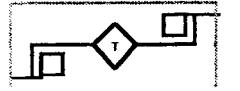
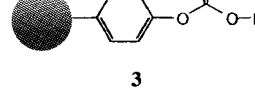
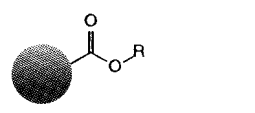
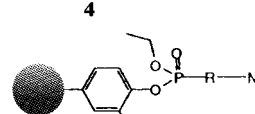
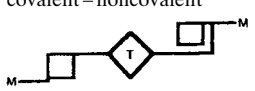
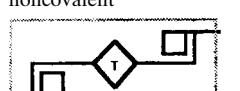
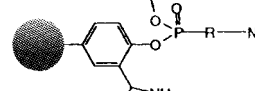
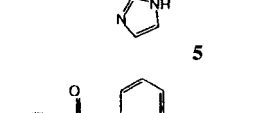
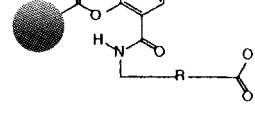
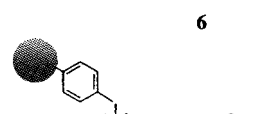
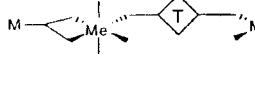
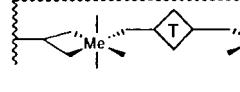
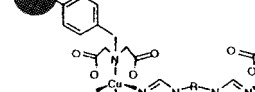
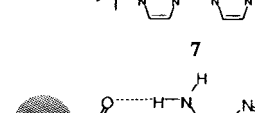
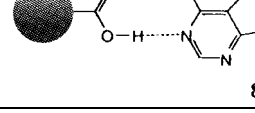
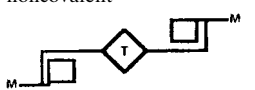
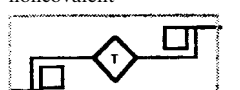
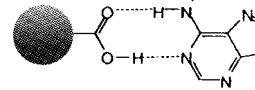
Scheme 1. Imprinting of network polymers with various templates. The template can be a small molecule, protein, cell, or crystal.

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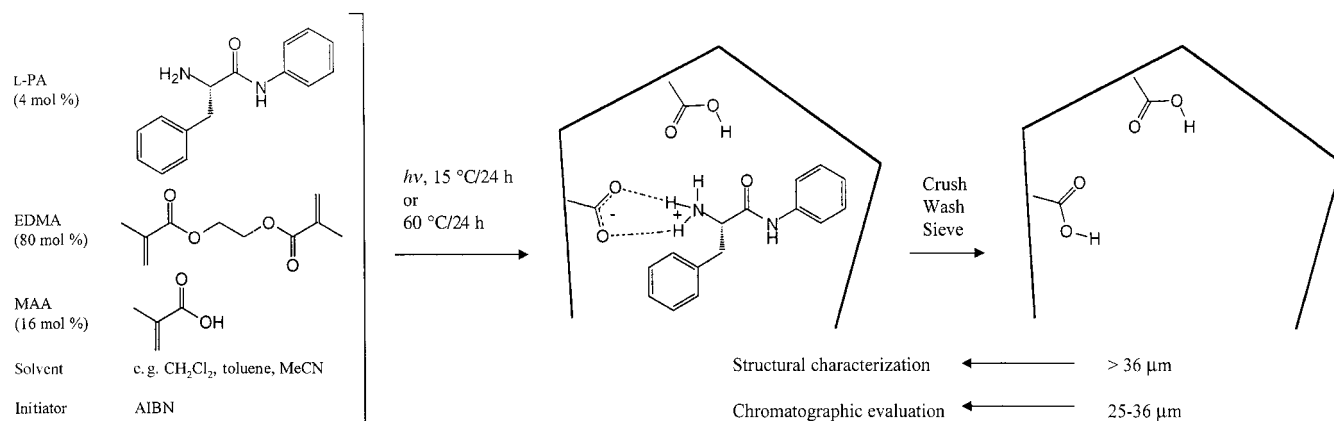
[**] The author is grateful to Dr. Andrew Hall and Dr. Gunter Büchel for linguistic advice.

HIGHLIGHTS

Table 1. Approaches for imprinting small molecules.^[a]

Synthesis	Rebinding	Attachments	Ref.
covalent	covalent		[40]
			
			
			
covalent	noncovalent		[12]
			
			
			
			
covalent – noncovalent	noncovalent		[42]
			
			
			
			
			
metal ion mediated	metal ion mediated		[43]
			
			
			
			
noncovalent	noncovalent		[23]
			
			

[a] T = template, M = polarizable group.

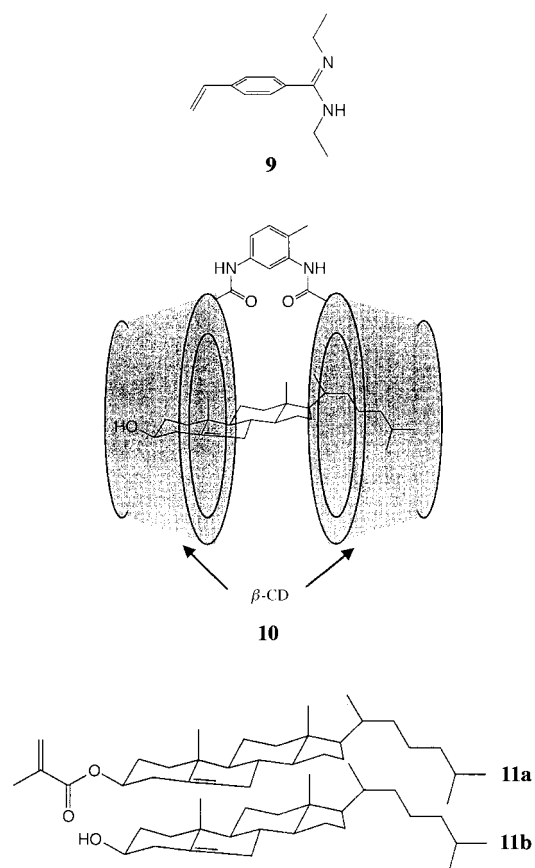


Scheme 2. Imprinting protocol for the recognition of L-phenylalanine anilide (L-PA). AIBN = azobisisobutyronitrile, EDMA = ethyleneglycol dimethacrylate, MAA = methacrylic acid.

nitrogen heterocycles,^[23, 24] the latter two being particularly stable. However, other criteria are that the template is soluble in the most common solvents or monomers used in the imprinting step, that it is available in preparative amounts, and that it is stable under the conditions of the polymerization and does not undergo reactions with free radicals. Provided that these criteria are fulfilled the resulting molecularly imprinted polymers (MIPs) usually exhibit a significant and selective rebinding of the template when compared to non-imprinted control polymers.

The potential for a given monomer template pair to produce templated sites can be predicted by measuring the stability constants, for example by spectroscopic techniques, in a homogeneous solution mimicking the monomer mixture prior to polymerization.^[17, 25] This can ultimately be used as a preliminary screening procedure to search for suitable functional monomers. With 9-ethyladenine (**8**, see Table 1) as template a comparison can be made between the solution stability of the complex and the binding constant and site density obtained in experiments for batch rebinding to the imprinted material. 9-Ethyladenine associates with butyric acid in chloroform at three sites with estimated association constants of 114, 41, and 5 M^{-1} respectively.^[26] Assuming similar association constants between MAA and this template prior to polymerization the major part of the template would be present in the complexed form. The corresponding imprinted polymer prepared in chloroform exhibited an adsorption isotherm that could be fitted to a bi-Langmuir adsorption model, resulting in a class of high-energy binding sites with a binding constant (K_a) in chloroform of 76000 M^{-1} , similar to the binding energies observed for designed synthetic receptors of the same molecule.^[23] Also of interest is the fact that more than 30% of the added template resulted in high-affinity binding sites (site density $n = 20\text{ }\mu\text{mol g}^{-1}$). Usually, however, the yield is considerably lower, often in the range of only a few percent. Subtracting the binding to a reference polymer imprinted with benzylamine from the binding to a polymer imprinted with 9-ethyladenine gave a mono-Langmuir isotherm ($K_a\text{ }79000\text{ M}^{-1}$), thus indicating that the sites probed in this experiment are isolated and relatively uniform. The remaining sites interact nonselectively with solutes binding to carboxylic acids and limit the degree of separation that can be achieved.

In spite of the pronounced recognition observed for a number of important targets with the use of MAA as functional monomer, recognition of any given target molecule requires access to functional monomers targeted towards structural features specific for particular compounds or classes of compounds. Based on the structural features of the templates that generate good sites in the MAA system, an interesting possibility would be to incorporate these structures in new functional monomers for the recognition of carboxylic acids. This concept is somewhat similar to the reciprocity concept in the design of chiral stationary phases. Thus, Wulff et al. synthesized *N,N'*-diethyl-*p*-vinylbenzamidinium (**9**) and showed that this monomer could be used to generate high-fidelity sites for the molecular recognition of carboxylic acids as well as sites exhibiting catalytic esterolytic activity.^[27] The binding is strong enough here to provide



efficient recognition also in aqueous media. Furthermore, since the functional monomer is quantitatively associated with the template prior to polymerization, the nonspecific binding is minimized. Functional-group complementarity is thus one basis for the choice of functional monomer. In the case of templates with low polarity and with few functional groups that can interact with functional monomers, it may be beneficial to use amphiphilic monomers stabilizing the monomer template assemblies by hydrophobic and van der Waals forces. Thus cyclodextrins have been used to template binding sites for cholesterol (**10**, $\beta\text{-CD} = \beta\text{-cyclodextrin}$)^[28] or to enhance the selectivity in the imprinting of amino acids.^[29] Monomers based on bile acids or cholesterol (**11a**) have also been used to generate binding sites for cholesterol (**11b**).^[30] In a comparison of a number of common adsorbents, the adsorbents imprinted with cholesterol exhibited the largest uptake of cholesterol from liquids mimicking intestinal fluids at physiologically relevant cholesterol concentrations.

In summary the simple mix-and-bake procedure described here can be used to generate molecular recognition elements for a large variety of small molecules. However, important challenges remain in the molecular imprinting of larger complex biomolecules.^[36]

Molecular Recognition of Oligopeptides

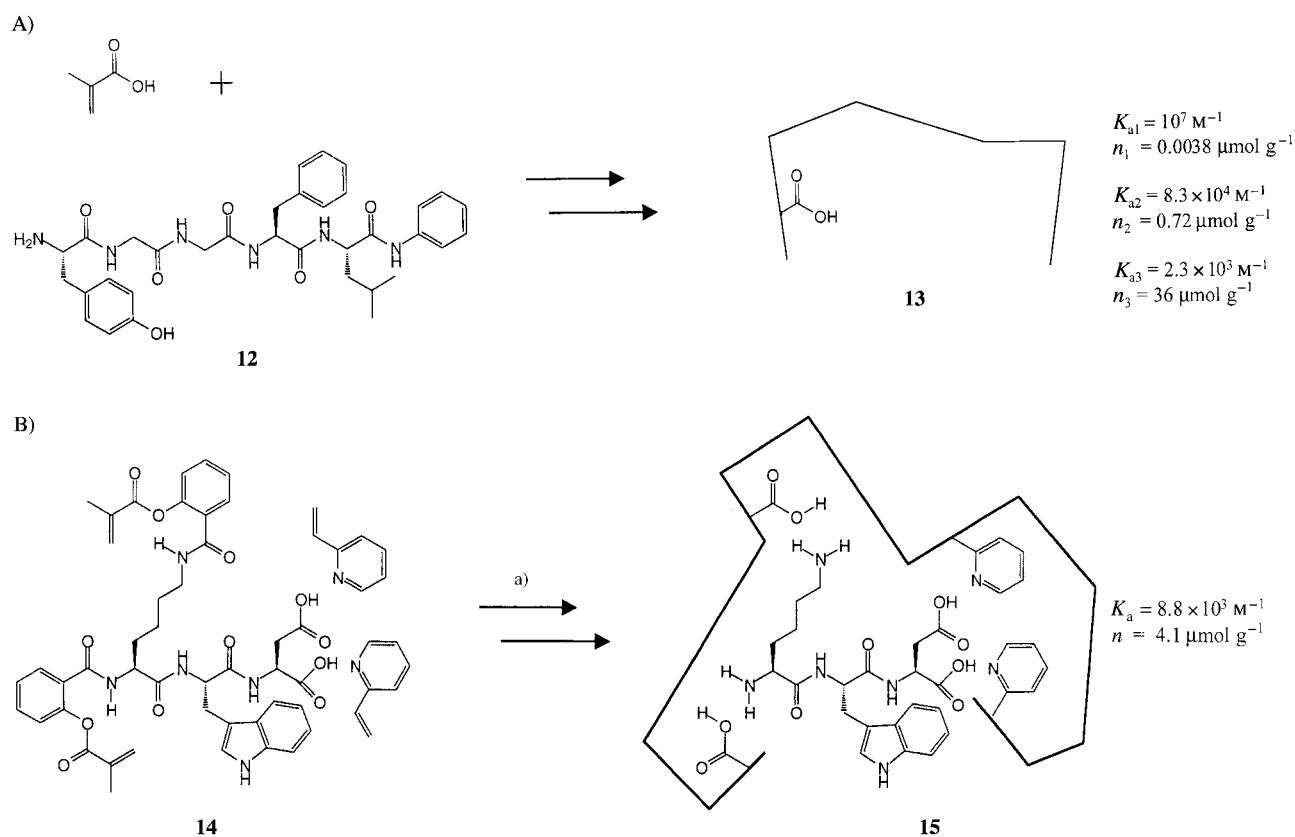
Stable molecular recognition elements designed towards amino acid sequences and isomers of peptides could potentially be used for demanding separations of peptide mixtures, in peptide sensors, in therapeutic applications, or as biological

model systems. Amino acid amides and smaller peptides have been used as templates in the MAA/EDMA protocol (Scheme 2), resulting in materials showing strong and selective binding of the amino acid or peptide target. Thus Leu-enkephalin anilide (**12**) was imprinted to generate a binding site for Leu-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH) (**13**).^[22] The resulting material exhibited high binding constants for **13** in organic as well as in organic/aqueous solvents (Scheme 3A). The high affinities for the peptide target allow these materials to be used as antibody substitutes in competitive assays and for other analytical applications. However, as noted above the nonspecific binding to these materials is considerable and the density of high-energy binding sites is low. This essentially limits their use to analytical applications. Solubility is another problem. Many peptides are only sparingly soluble in commonly used monomer–solvent systems. Therefore, addition of polar solvents prior to polymerization or the use of more soluble analogues may be required. The former results in destabilization of the functional monomer–template assemblies, which are held together by electrostatic interactions, and a lower yield of templated sites. The latter, on the other hand, may result in a site lacking complementarity to the target peptide.

One possible way to circumvent these problems is to attach the template covalently to the functional monomer and, after polymerization and template removal, allow the rebinding to take place by noncovalent association (see above). This is

feasible in the case of a hydrolyzable ester (such as **4**, Table 1),^[31] amide, or imine bond,^[32] where subsequent rebinding can take place by electrostatic interactions between the respective amine and carboxylic acid. After disappointing results in terms of template removal, binding capacity, and selectivity, this approach has not been pursued further. Reasons for the poor results were the intrinsic stability of the monomer–template bonds and an incorrect positioning of the functional group for optimal noncovalent recognition. One solution to these problems was introduced by the group of Whitcombe and Vulfson.^[12] They used easily cleavable sacrificial spacers between the monomer and the template (**3** and **6**, Table 1) to correctly position functional groups for rebinding.

So far the approach has been used to generate recognition elements for cholesterol, polychlorinated biphenyls (PCBs), and peptides. As an example of the latter, the tripeptide Lys-Trp-Asp (**15**, Scheme 3B) was used as template.^[11] It was covalently linked to MAA through two salicyl groups connected to the lysine amino groups and to the MAA carboxylic acid group (**14**). The lability of this linker would result in a high recovery of template with all resulting carboxylic acid groups associated with templated sites. Intramolecular hydrogen bonding between the amide and ester group of the linker would position the amino group at a hydrogen-bond distance from the carboxylic acid group after cleavage of the template. Furthermore, it was anticipated that



Scheme 3. Synthesis of peptide-selective polymers according to A) Andersson et al.^[22] or B) Klein et al.^[11] based on noncovalent or covalent linkages, respectively, of the MAA groups to the template prior to polymerization. Association constants K_a and site densities n obtained from batch equilibrium rebinding experiments using A) Leu-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH, **13**) or B) **15** are given. Solvents: A) EtOH/20 mM sodium citrate (pH 4.5) 10/90 (v/v), B) acetonitrile/water 4/1 (v/v); a) 1. divinylbenzene (DVB), initiator, 2. UV, polymerization, 3. NaOH, aq. MeOH, 4. addition of Lys-Trp-Asp.

the aryl group of the linker would create the space necessary for template removal and rebinding. Finally the covalent strategy to recognize the lysine group was combined with the addition of 2-vinylpyridine to complement the carboxylic acid groups of the aspartic acid residue. The resulting monomer assemblies were copolymerized with an excess of divinylbenzene (DVB) by UV initiation, and the template was cleaved from the polymer by refluxing the polymer in methanolic aqueous NaOH, resulting in 58 % template removal. The polymer was then tested in a batch equilibrium rebinding experiment and compared to a control polymer prepared by replacing the template with an equimolar amount of MAA. The results demonstrated that this strategy templated the peptide. Particularly noteworthy was the amount of bound template in comparison with structurally similar peptides in aqueous/organic solvents. However, in view of the binding to the control polymer at the applied concentrations the results should be interpreted with caution. The amount adsorbed nonspecifically to the control polymer—although much lower than the amount adsorbed to the peptide-imprinted polymer—correlated to some extent with the amount adsorbed to the imprinted polymer.

It is interesting to compare the covalent imprinting strategy with the noncovalent one described above. Although the polymers synthesized by the covalent strategy exhibit pronounced recognition of the template peptide, it is unclear whether they contain sites capable of rebinding the template with the affinities shown by the materials prepared by the noncovalent strategy. This may be decisive for the applications, analytical or preparative, in which the respective materials will be used.

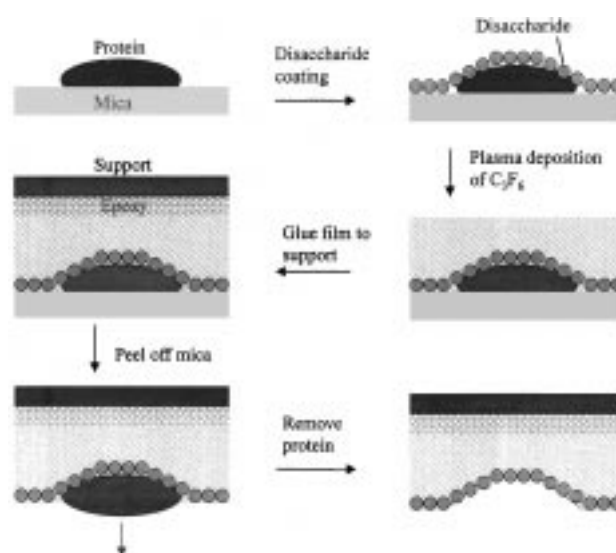
Molecular Recognition of Proteins

The design of stable molecular recognition elements for proteins is, due to the complexity of these macromolecules, a difficult task, but attractive in view of the great need for such tools in biotechnology for down-stream processing, in therapeutics for drug delivery, for bioanalysis in sensors, and in diagnostics.^[5] Affinity separations of proteins can be achieved using antibodies, enzymes, or receptors capable of recognizing protein epitopes. However, the limited stability of these recognition elements precludes the development of robust and cheap methods to recognize or separate this class of compounds. The recent development of stable cross-linked peptide-recognition elements prepared by screening of phage peptide libraries may be an exception in this regard. Otherwise separation of proteins using nonbiological affinity techniques is based on the presence of certain functional groups. Thus protein surface thiol groups, imidazoles, diols in glycoproteins, or hydrophobic side-chain amino acids can be targeted using ligands immobilized on solid supports.^[5] The level of discrimination that can be achieved is limited to the amount of surface-exposed functional groups, and these techniques cannot be used to differentiate between closely related proteins. In this regard, molecular imprinting would allow the functional groups to be spatially distributed at positions complementary to the distribution of the surface functional groups of the protein. However, the structure and

function of proteins are sensitive to solvent, temperature, pH, and ionic strength. New imprinting techniques considering these factors need to be developed.

Previous examples of the preparation of protein-imprinted gels or coatings^[33] are all limited with respect to selectivity, binding capacity, site accessibility, or robustness.

However, an interesting approach to confine the imprinted sites to biocompatible hydrophilic surfaces was recently published by the group of Ratner.^[13] They adsorbed the template protein (plasma proteins: bovine serum albumine (BSA), immunoglobuline G (IgG), ribonuclease (RNase), or lysozyme (LSZ)) to a mica surface. This substrate was chosen because it is atomically flat and because proteins are adsorbed with minimal denaturation and conformational changes. Thus only the template protein causes changes in surface topography. A 1–5 nm layer of a nonreducing disaccharide was then spin-coated on the surface followed by plasma deposition of a 10–30 nm layer of a fluoropolymer (Scheme 4). The



Scheme 4. Protein surface imprinting technique based on carbohydrate–protein interactions.

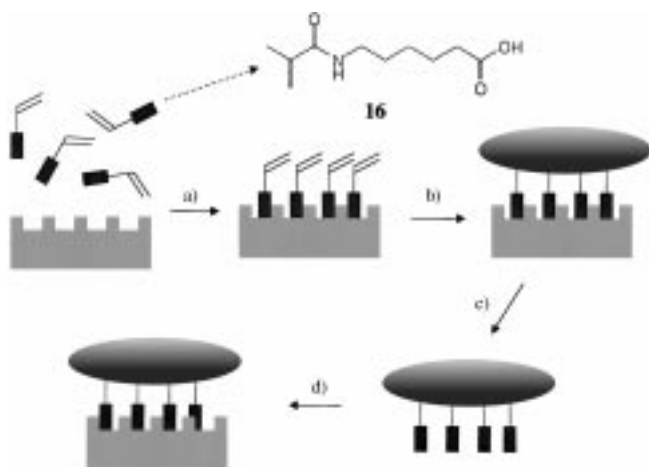
resulting film was epoxyglued to glass, the mica sheet peeled off, and the protein removed by treatment of the surface in aqueous NaOH/NaOCl. This resulted in a sugar-coated surface containing templated pits. Through the use of surface analytical techniques sensitive to elemental composition (ESCA) and mass distribution (TOF-SIMS), it could be shown that the protein was effectively removed after this treatment. The resulting surfaces, when examined with atomic force microscopy (AFM), revealed pits of approximately the size and shape of the protein used as template. Furthermore, in competitive adsorption experiments the surfaces exhibited a preference for the protein used as template. Even though all the templated surfaces adsorbed all proteins nonspecifically, the protein used as template was only displaced at elevated concentrations of competing protein (Table 2). Since only one procedure was used for the synthesis of all templated surfaces, this technique may become generally applicable to the imprinting of proteins.

Table 2. Results from competitive adsorption experiments showing the ratio of competing protein to the substrate radiolabelled protein at which the binding of the substrate was reduced by 50%.

Substrate	Displacer	Displacer:substrate at 50% displacement			
		BSA im-printed	IgG im-printed	LSZ im-printed	RNase im-printed
BSA	IgG	30.2	3.1	–	–
IgG	BSA	0.9	3.4	–	–
RNase	LSZ	–	–	0.2	4.0
LSZ	RNase	–	–	31.0	1.2

Imprinting of Crystals for Structure-Directed Nucleations

Nature uses proteinaceous templates to direct the crystallization of inorganic materials into structures that deviate from the spontaneously formed structure.^[34] Mimicking these templates may lead to a new class of biomaterials with superior properties.^[35] In a recent report the group of Vulfson showed that imprinting of inorganic crystals resulted in polymeric templates capable of directing crystallizations into structures that would not form in solution.^[15] Thus a network copolymer of divinylbenzene (DVB) and 6-methacryloylamidohexanoic acid (**16**) was prepared by free radical polymerization in the presence of crystals of calcite (rhombohedral, ca. 4 μm) suspended in chloroform (Scheme 5). Additionally two control polymers were prepared, one in the absence of crystals and one in the presence of crystals but without the functional monomer. Otherwise the control polymers were



Scheme 5. Process for the imprinting of crystals and subsequent use of the polymer for directing crystal nucleation of CaCO_3 . a) Incubation in chloroform, b) DVB, polymerization, c) HCl, aq. MeOH, template removal, d) supersaturated CaCO_3 solution.

prepared identically to the crystal-imprinted polymer. After polymerization the polymer monolith was ground to give particles about 100 μm in size, and the template crystal removed by washing the polymers with acidified aqueous methanol. After this treatment no polymer-bound calcite could be detected by scanning electron microscopy (SEM), X-ray powder diffraction, or IR spectroscopy, indicating that the wash procedure effectively removed the crystals.

Nucleation experiments were then performed by mixing aqueous solutions of Na_2CO_3 (0.8 mM) and CaCl_2 (1.0 mM)

and allowing nucleation to take place over 24 hours. The polymer surfaces were then washed with water, and the surfaces investigated by SEM. The surface of the imprinted polymer contained considerably more calcite crystals than the surface of the control polymers. Moreover, their morphology was different from the crystal formed spontaneously in solution and resembled the original template morphology. Powder X-ray diffraction experiments confirmed that the crystals contained more than 96% calcite. Interestingly, even under conditions favoring the formation of the alternative structure aragonite (95 °C), calcite was still formed at the imprinted sites. By potentiometric titrations it was shown that the number of accessible carboxylic acid groups in the imprinted polymer ($39 \mu\text{mol g}^{-1}$) was much higher than in the control polymer ($10 \mu\text{mol g}^{-1}$), but lower than the theoretical number ($79 \mu\text{mol g}^{-1}$). Furthermore, it was shown that the crystals adsorbed a large amount of the acid functional monomer in chloroform. It can then be assumed that the templated sites are enriched in accessible carboxylic acid groups.

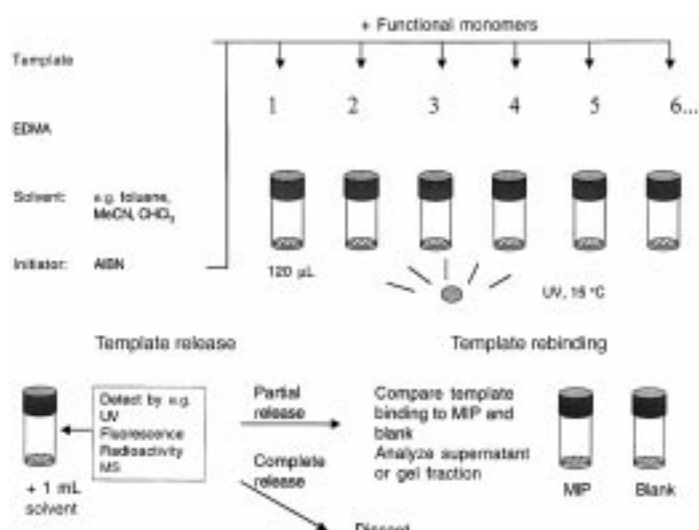
To what extent are accessible and densely packed acid groups capable of the same structural control? In a previous contribution by Tremel and co-workers it was observed that self-assembled monolayers of ω -carboxylic and sulfonic acid functionalized alkanethiols on gold can exert a similar level of structural control.^[36] Unfortunately in the work of Vulfson et al. aragonite imprinted polymers did not enhance the nucleation of aragonite, which would have provided additional strong evidence for the proposed mechanism.

In the context of the crystal imprinting concept, some similarities are seen with a recently revived proposal^[37] for the mechanism of recognition exhibited by noncovalently imprinted polymers.^[38] This suggests that template remaining in the polymer after template removal is capable of acting as a nucleation site for the template in the rebinding experiment. Thus the actual binding sites are complementary to small clusters of the template. This is an interesting theory that can explain the adsorption behavior in some systems (note especially the use of cholesterol monomers to imprint cholesterol (**11**)),^[30] particularly at low site occupancy. However, in contrast, the commonly observed bi- or tri-Langmuir adsorption isotherms^[39] indicate a true receptor behavior (for example, in the case of **8**).^[23]

Rapid Synthesis and Screening of Large Groups of Polymers

A key to the development of new imprinted polymers is the identification and optimization of the main factors affecting the structure and molecular recognition properties of the materials. These factors can be the type and the relative amounts of functional monomer and cross-linking monomer, the polymerization initiator, the temperature, the pressure, or the solvent of polymerization.^[16] The presently used monolith procedure—that is, synthesis of the polymer as a block that needs to be crushed and sieved—is time-consuming, and a careful optimization of all the factors affecting the material's properties is therefore not feasible. One way around this problem, as suggested in two recent reports by the groups of Takeuchi^[9] and Sellaergren,^[10] is to scale down the batch size to

about 50 mg and perform the synthesis in small vials adopted for automated handling of samples (Scheme 6). Since this batch size leaves the polymer as a layer on the bottom of the vials, intralayer diffusion of template is fast enough for the polymer to be analyzed in situ without workup. This significantly speeds up the synthesis and analysis of the materials. By automation of this procedure with simple equipment for sample handling, it is estimated that over 100 materials can be processed and analyzed in parallel within 24 hours. Based on the previous examples the method is



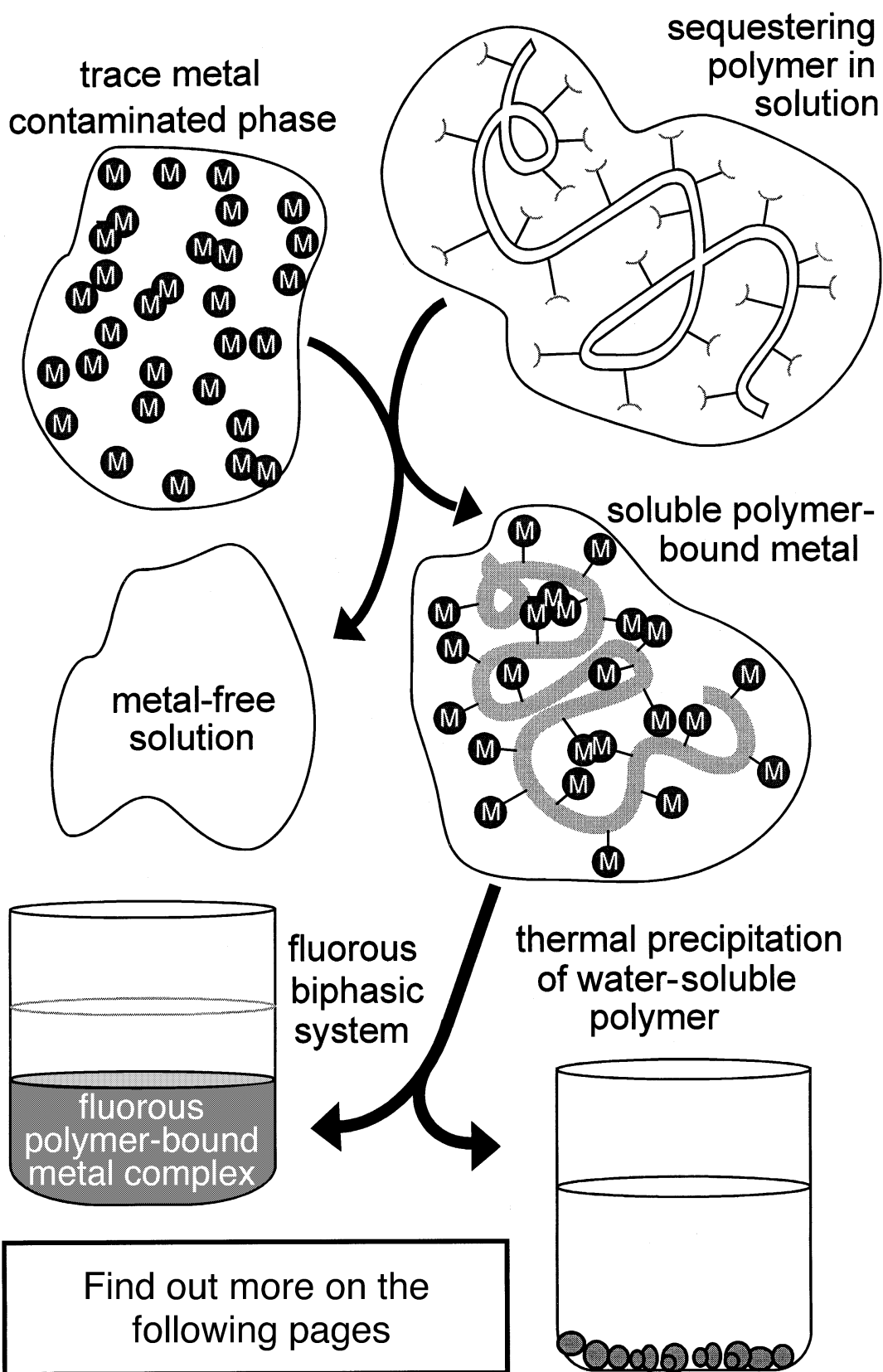
Scheme 6. Combinatorial imprinting technique suitable for automation.

reproducible and has been further validated by comparing the selectivity of the small-scale batch with a corresponding normal-scale batch of materials. Therefore, a rational synthetic approach for MIPs may soon be a reality.

Final Remarks

Of the above-discussed systems, the imprinting of small molecules has, to date, reached the highest level of maturation. Nevertheless, some limitations still hamper real applications of the materials. One of these concerns the recognition of templates at either the low or high end of the polarity scale. In the former case the problem is mainly related to the lack of monomers that provide sufficiently stable monomer–template assemblies. In the latter case, poor solubility in commonly used organic monomer–solvent systems can be expected. This is true for a number of important target biomolecules. Further problems are the low yield of high-affinity binding sites and the resulting nonspecific binding, poor site accessibility, and a lack of practical and economic methods for manufacturing the materials. Finally, even if these problems can be solved, a challenging competition with alternative biological and synthetic recognition elements can be expected. The ultimate test of the usefulness of the imprinted materials will be whether they can be synthesized with comparable recognition properties at a lower cost and in a shorter time than their counterparts.

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Sequestration of Trace Metals Using Water-Soluble and Fluorous Phase-Soluble Polymers**

David E. Bergbreiter,* Nirmal Koshti, Justine G. Franchina, and Jonathon D. Frels

For several years now, our group has actively pursued the design and synthesis of soluble, polymer-bound ligands for transition metal catalysis.^[1–5] Here we describe the extension of this chemistry to the design of functional polymers that are soluble in aqueous or fluorous phases, and that recover and remove trace metals from aqueous or organic solutions.

Soluble polymeric catalysts prepared from poly(*N*-isopropylacrylamide) have a lower critical solution temperature (LCST) and quantitatively phase separate from aqueous solution on heating. We have used this property to design “smart”, water-soluble, reusable catalysts.^[1, 3] We also showed that fluoroacrylate-bound reagents are selectively soluble in the fluorous phase of a fluorous biphasic system.^[6] This work shows that these two types of polymers can be used as soluble ion-exchange resins for removal of trace metal from water or organic solvents, respectively.

The studies reported here focus on the recovery of iron using polymeric hydroxamic acid ligands. Hydroxamic acids complex Fe^{III} to form red complexes (Scheme 1). The intense color of these complexes facilitates the determination of iron remaining in solution using UV/Vis spectroscopy.

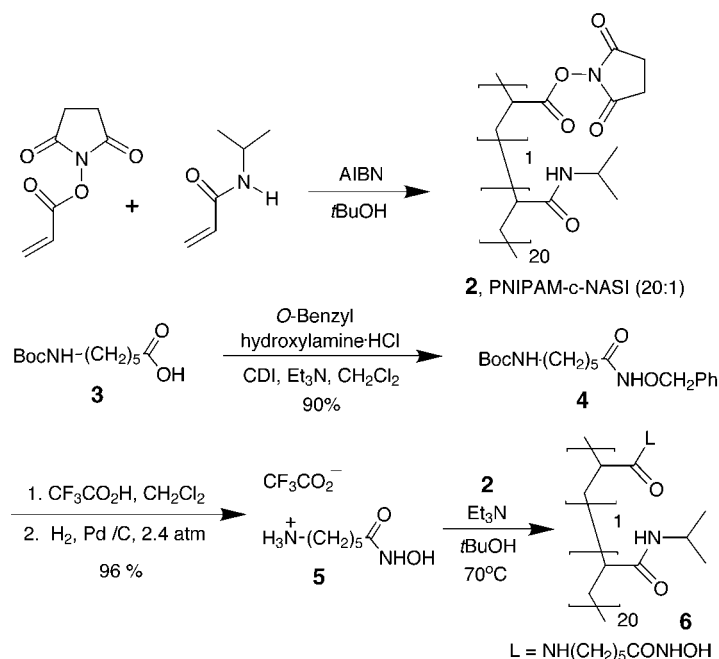
The first polymeric ligand we studied was prepared by addition of **5** to a 20:1 poly(*N*-isopropyl acrylamide)-*co*-(*N*-acryloxy succinimide) copolymer (**2**, PNIPAM-*c*-NASI; Scheme 2). The polymer-bound hydroxamic acid **6** so formed had an LCST of 31 °C. A solution of 200 mg of **6** and 15.5 ppm of Fe^{III} in 25 mL of water formed a red hydroxamate complex that precipitated above 31 °C leaving a colorless supernatant. Addition of an excess of **5** to the supernatant produced a colorless solution (*A* < 0.002), showing that over 99% of the Fe^{III} had been removed from solution by thermal sequestration by **6** above the LCST of **6**. Inductively coupled plasma (ICP) analysis of the supernatant affirmed this result, showing that the 15.5 ppm Fe^{III} concentration was reduced to 116 ppb.

Substrates or catalysts attached to PNIPAM have little reactivity above their LCST.^[2] Likewise, Fe^{III} complexed to **6** does not readily exchange Fe^{III} with **5** above the LCST of **6**.

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Scheme 2. Synthesis of a poly(*N*-isopropylacrylamide)-bound hydroxamic acid. AIBN = azobisisobutyronitrile, Boc = *tert*-butoxycarbonyl, CDI = *N,N'*-carbonyl diimidazole.

When an aqueous red suspension of 200 mg of **6** containing 18.5 ppm of Fe^{III} at 50 °C was stirred with 5 mg of **5** at 50 °C for 12 h, no detectable Fe^{III} was seen in the supernatant. The failure of **5** to bind Fe^{III} at 50 °C when soluble **5** was mixed with insoluble Fe^{III}-containing **6** shows that Fe^{III} is not readily leached from the precipitated polymer even when soluble ligands are present.

Figure 1 shows that **6** (the PNIPAM-bound hydroxamic acid) recovers Fe^{III} from water even in the presence of **5**. This occurs if a single polymeric hydroxamic ligand on the Fe^{III} is enough to insure precipitation of the hydroxamate–Fe^{III} complex. The results in Figure 1 show that more than 99% of the Fe^{III} is sequestered by **6** if the ratio of the **5**:**6** is less than 1.

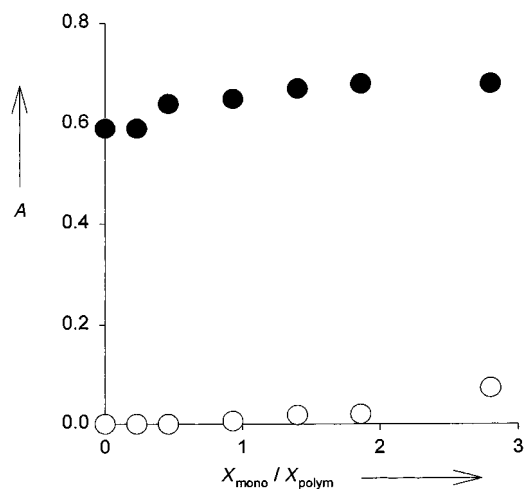
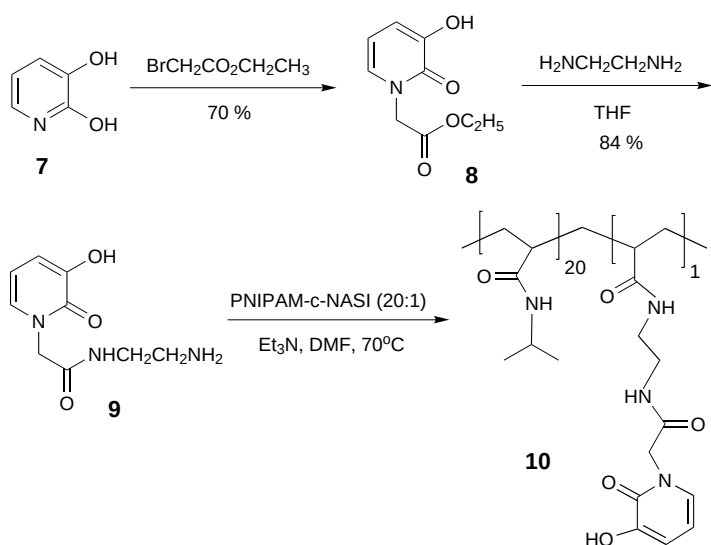


Figure 1. Absorbance *A* at 439 nm of a solution with varying ratios of **5** and **6** in a 15 ppm Fe^{III} solution (●) and of its supernatant after precipitation of **6** (○).

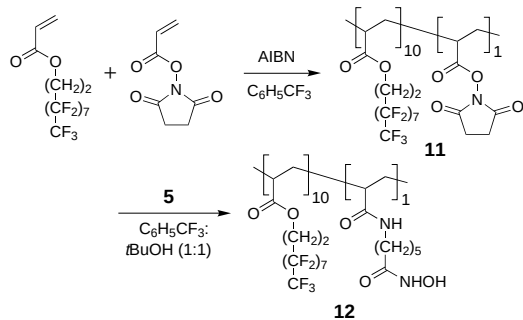
PNIPAM copolymers containing other ligands also bind Fe^{III} or Cu^{II} . 3-Hydroxy-2-pyridinone ligands on PNIPAM were prepared as shown in Scheme 3. Imidazole-containing



Scheme 3. Synthesis of polymer-bound 3-hydroxy-2-pyridinone.

copolymers were prepared by copolymerization of vinyl imidazole and **1**.^[7, 8] Experiments like those with **5** showed that 0.15 g of **10** in 25 mL of water binds 14.4 ppm of Fe^{III} (at 25 °C: $A = 1.109$, $\lambda_{\text{max}} = 578 \text{ nm}$). Heating results in precipitation of **10** and over 99% of the Fe^{III} , based on the residual absorbance of less than 0.002 for the supernatant after treatment with **9**. Similarly, 1.23 g of a 10:1 NIPAM–vinyl imidazole copolymer binds 98% of a 269.2 ppm Cu^{II} solution (CuSO_4) on heating above the LCST (35 °C). Addition of imidazole to the supernatant resulted in a residual absorbance of 2% at 658 nm.

While PNIPAM copolymers recover trace metals from water, similar LCST behavior cannot be used at a convenient temperature to recover trace metals from an organic solvent. To address this issue, we prepared a fluorous phase soluble hydroxamic acid polymer (Scheme 4). Prior work has shown the utility of fluorous biphasic chemistry for separations involving organic solvents,^[9, 10] and we have reported that derivatives of polymers like **11** are fluorous phase soluble polymer supports.^[6] In this case, amidation of **11** by **5** in trifluorophenylmethane yields the fluorous phase soluble, metal-binding polymer **12**. When 140 ppm of **12** were



Scheme 4. Synthesis of the fluorous phase soluble polymer **12**.

dissolved in 10 mL of FC-77 (a perfluorinated ether) and then shaken with 10 mL of CH_3OH containing 28 ppm of Fe^{III} for 15 min, a red fluorous phase formed. Centrifugation and liquid/liquid separation of the fluorous phase resulted in a CH_3OH phase that contained no detectable Fe^{III} , based on the absence of a detectable absorbance on addition of **5** to this solution (>99.5% Fe^{III} extraction).

Treatment with ethylenediaminetetraacetate (EDTA) regenerates these soluble polymer complexing agents. When an aqueous solution of Fe^{III} -containing **6** was stirred with disodium EDTA, the solution became colorless. Competitive binding of Fe^{III} by EDTA reformed metal-free **6**, which was isolated by two thermal precipitation/dissolution cycles. Recovered **6** was as effective as the original **6** at sequestering Fe^{III} from water. Likewise, when the red fluorous phase containing the Fe^{III} complex of **12** was shaken with a solution of disodium EDTA in CH_3OH , the fluorous phase decolorized. Once decolorized, the fluorous phase soluble **12** was successfully used again to remove Fe^{III} from a fresh Fe^{III} solution in CH_3OH .

We have shown that near quantitative metal recovery from aqueous solution is possible with water-soluble PNIPAM copolymers that by design contain metal-binding agents. Chelated metal does not readily leach from the precipitated polymer. A variety of ligands can be employed. Similar fluorous phase soluble polymers also complex trace amounts of Fe^{III} from organic solutions in a fluorous biphasic system. In both cases the iron-complexing agents are regenerable. These soluble ion-exchange resins provide two simple ways to complex and remove trace amounts of metal from solution. In water, the resin is recovered as a solid after mild heating. In organic solvents, a fluorous phase containing a fluorinated polymer is used under fluorous biphasic conditions with separation being effected by liquid/liquid phase separation. Such materials are alternatives to the existing arsenal of cross-linked, insoluble ion-exchange resins and may be useful in other solvents too (e.g. supercritical CO_2). The polymers described here function differently and under milder conditions than the thermally regenerable Sirotherm resins.^[11] Further studies of this chemistry continue in our group to establish the generality and limitations of these techniques.

Experimental Section

The PNIPAM copolymers were prepared from **1** and *N*-acryloxysuccinimide (NASI) or vinyl imidazole using AIBN initiation in *t*BuOH.^[2, 7, 12] Amines containing hydroxamic acid or 3-hydroxy-2-pyridinone were prepared using unexceptional chemistry, and then coupled to the active ester groups on **2**. In a typical metal-binding experiment, 200 mg of **6** were added to 25 mL of an aqueous solution containing 15.5 ppm ferric chloride to give a red solution at 25 °C ($A = 0.65$, $\lambda = 439 \text{ nm}$). Heating to 40 °C produced a red precipitate. Centrifugation (above 35 °C) yielded a clear solution whose absorbance at 439 nm was less than 0.002 after treatment with **5**. Hydroxamic-containing fluorous phase soluble polymers were prepared from a previously reported reactive fluoroacrylate copolymer (prepared by AIBN-initiated polymerization of NASI and 1*H*,1*H*,2*H*,2*H*-heptafluorodecyl acrylate).^[6, 13]

Detailed experimental procedures for the synthesis of **6**, **10**, and **12** are available in the Supporting Information.

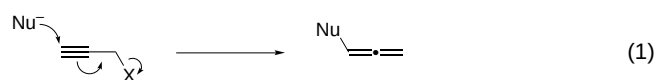
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π -Allylpalladium-Mediated Catalytic Synthesis of Functionalized Allenes**

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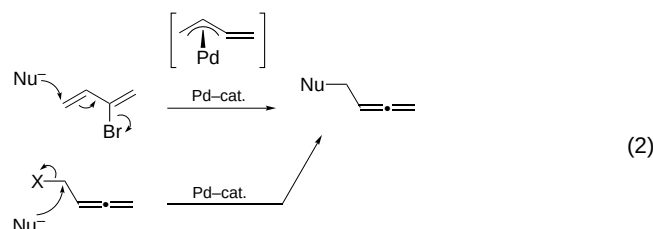
Allenenes have attracted attention as useful synthons for synthetic organic chemistry.^[1] The interesting reactivity of allenenes originates mainly from their unique strained structure, and thus continuous efforts have been made to construct the 1,2-dienic moieties. Standard methods of allene synthesis are based on the S_N2' -type substitution of propargylic compounds [Eq. (1)].^[1, 2] However, the products from these synthetic



methods are often contaminated with the corresponding propargylic compounds or 1,3-dienes, and purification steps become problematic. Recently, some highly selective synthetic methods for the formation of allenenes were reported,^[3] however, the yields of the allenenic products were not always satisfactory. In addition, these procedures require reactive reagents, such as Grignard reagents or organolithium species, and are unable to handle substrates or products with

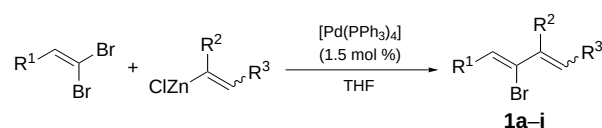
susceptible functional groups. We describe herein a novel synthetic method for synthesizing a variety of functionalized allenenes.

Our strategy is a formal S_N2' substitution of 2-bromo-1,3-butadienes, which is catalyzed by a palladium complex [Eq. (2), top]. The reaction proceeds under very mild conditions via a π -allylpalladium intermediate^[4] and gives



the clean products in excellent yields. The reaction described here has some analogies to the Pd-catalyzed functionalization of allenyl esters with “soft” carbon nucleophiles reported by Gore and co-workers in 1984 [Eq. (2), bottom].^[5] They suggested an analogous intermediate to the one established in this report. However, the substrates they employed possess 1,2-dienic moieties and thus, tedious procedures are required to construct the allenic skeletons prior to the functionalization step, which greatly reduces the synthetic usefulness of the reaction. The advantages of our approach described here are: 1) easy access to the 1,3-dienyl substrates, which enables us to introduce a wide range of substituents in the substrates and the allenic products, and 2) a variety of nucleophilic reagents, including N, O, and P nucleophiles, can be used. The reaction also represents a rare example of a transition metal catalyzed transformation to give allenenes.^[6]

The substrates, (*Z*)-2-bromo-1,3-butadienes (**1**), are readily available in 63–90 % yield by palladium-catalyzed regio- and stereoselective cross-coupling of 1,1-dibromo-1-alkenes^[7] with the corresponding vinylzinc reagents (Scheme 1).^[8] The



Scheme 1. Palladium-catalyzed selective cross-coupling of dibromoalkenes with organozinc reagents.

choice of the organometallic reagent is important for this step; while $(CH_2=CH)ZnCl$ gives the coupling product in satisfactory yield, the less-reactive vinyltin reagent $Bu_3Sn(CH=CH_2)$ gives the same product in very low yield (<20 %). More basic Grignard reagents enhance the elimination of HBr from **1** to give a considerable amount of $R^1C\equiv C-CH=CH_2$ as a by-product. When R^1 is an alkyl substituent, a second cross-coupling proceeds to a certain extent to give a triene, $R^1CH=C(CH=CH_2)_2$, as a by-product ($\approx 15\%$). The triene does not affect the allene formation, thus, a mixture of **1** and the triene can be employed in the following step without further purification.

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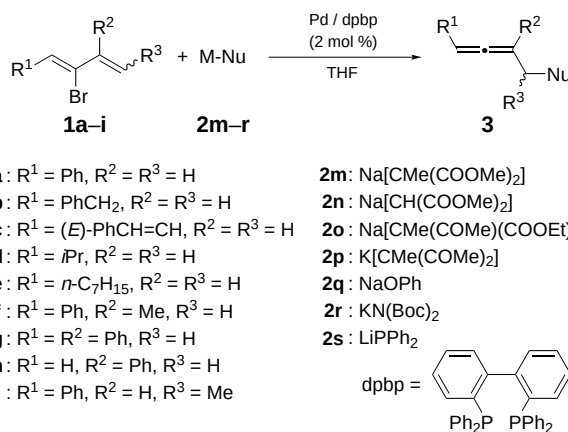
Our initial studies on the formation of the allenes focused on finding a suitable phosphane ligand for the palladium precursor, $[\text{PdCl}(\eta^3\text{-C}_3\text{H}_5)]_2$, so that the reaction of (Z)-2-bromo-1-phenyl-1,3-butadiene (**1a**) and $\text{Na}[\text{CMe}(\text{COOMe})_2]$ (**2m**) would occur (see Scheme 2). Although the reactions produced dimethyl 2-(4-phenyl-2,3-butadienyl)-2-methylpropane-1,3-dioate (**3am**) cleanly as we expected, the palladium–phosphane complexes initially examined did not have satisfactory catalytic activity. The catalytic efficiency of each phosphane ligand was evaluated by the conversion of **1a** to **3am** after 2 h at 20 °C in the presence of 2 mol % of the Pd catalysts: dppe gave less than 1 % conversion, dppp 4 %, dppb 12 %, dppf 11 %, and PPh_3 less than 1 %.^[9] The reaction was efficiently catalyzed by a palladium–dppb^[9] complex generated in situ from $[\text{PdCl}(\eta^3\text{-C}_3\text{H}_5)]_2$ and 1.1 equivalents (with respect to Pd) of dppb. The dppb system shows 75 % conversion in 2 h under the same conditions employed for the other Pd-phosphane catalysts. Prolonged reaction with the Pd–dppb catalyst at room temperature gives **3am** in 91 % yield (entry 1 in Table 1). The reaction proceeds very cleanly to give the allenic compound as the sole organic product.

Table 1. Palladium-catalyzed synthesis of allenes **3** from bromodiene **1** and nucleophile **2**.^[a]

Entry	Bromodiene	Nucleophile	T [°C]	t [h]	Yield [%] ^[b]
1	1a	2m	23	12	91 (3am)
2	1a	2n	23	12	79 (3an) ^[c]
3	1a	2o	23	12	93 (3ao) ^[d]
4	1a	2p	23	6	96 (3ap)
5	1a	2q	23	24	83 (3aq)
6	1a	2r	23	12	89 (3ar)
7	1a	2s	0	3	62 (3as) ^[e]
8	1b	2m	23	12	91 (3bm)
9	1c	2m	23	12	92 (3cm)
10	1d	2m	23	6	88 (3dm)
11	1e	2m	23	6	93 (3em)
12	1f	2m	23	12	90 (3fm)
13	1g	2m	23	12	95 (3gm)
14	1h	2m	23	6	93 (3hm)
15	1i ^[f]	2m	23	60	80 ^[g] (3im) ^[h]

[a] Reaction was carried out in THF in the presence of 2 mol % of the catalyst generated from $[\text{PdCl}(\eta^3\text{-C}_3\text{H}_5)]_2$ and dppb. [b] Yield of isolated product after column chromatography on silica gel. [c] Doubly reacted products, *rac*- and *meso*-**3an'**, were isolated in 19 % yield (based on **1a**) as a *rac/meso* = 47/53 mixture. [d] Mixture of two diastereomers (50/50). [e] The product was oxidized during an aerobic work-up and isolated as a corresponding phosphane oxide. [f] Mixture of two isomers (*E/Z* = 5/1). [g] 18 % of **1i** was recovered (*E/Z* = 6/1). [h] Mixture of two diastereomers (50/50).

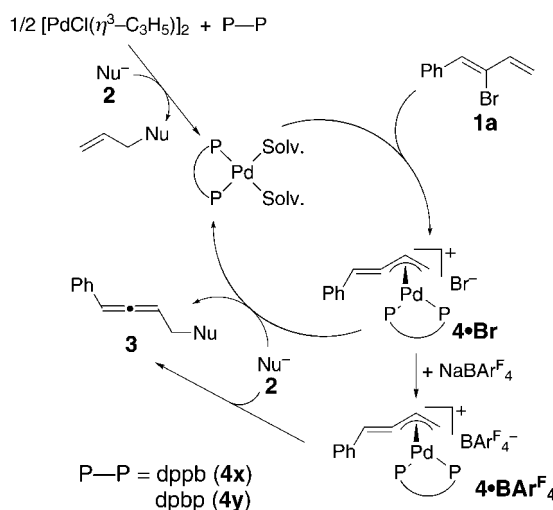
The scope of the present reaction is broad and representative examples are shown in Scheme 2 and Table 1. The reaction works well with several types of “soft” nucleophiles, including phenoxide (Table 1, entry 5), amide (entry 6), and phosphide (entry 7), while “hard” nucleophiles, such as PhMgBr and PhZnCl , produce conjugated 1,3-dienes instead of allenes.^[5, 10] A wide range of substitution patterns are acceptable to the dienyl substrates. The substrates with benzyl, β -styryl, primary or secondary alkyl groups, or hydrogen as R^1 undergo the reaction (entries 8–11, 14). Substitution at the R^2 position puts the substituent at the other end of the allenic skeleton in the final product (entries 12–14). The



Scheme 2. π -Allylpalladium-mediated catalytic synthesis of functionalized allenes.

R^3 substituent, however, reduces the reactivity of the dienyl substrate. Reaction of the diene **1i**, which is a mixture of two isomers (*E/Z* = 5/1), with **2m** shows only 80 % conversion even after 60 h, and 18 % of **1i** is recovered (*E/Z* = 6/1) from the reaction mixture (entry 15). The choice of the leaving group in the dienyl substrate is critical for the success of the reaction. While bromide **1a** reacts smoothly with the nucleophiles in the presence of the Pd catalyst, the corresponding acetate, (*E/Z*)- $\text{PhCH=C}(\text{OAc})\text{-CH=CH}_2$, is totally inert under the same conditions.

The reaction patterns described above indicates formation of (methylene- π -allyl)palladium^[11] species **4** as a key intermediate, and we propose a catalytic cycle shown in Scheme 3. Isolation of the palladium species was achieved as follows: An



Scheme 3. Catalytic cycle of the allene synthesis reaction (Solv. = solvent).

equimolar mixture of $[\text{Pd}(\eta^3\text{-C}_3\text{H}_5)(\text{dppb})]\text{Cl}$, generated in situ from $[\text{PdCl}(\eta^3\text{-C}_3\text{H}_5)]_2$ and dppb, and **2m** in THF was stirred at room temperature for a short period (<1 min) to give a dark orange solution. The addition of a stoichiometric amount of **1a** to the solution changed the color to yellow on formation of **4x·Br** (Scheme 3). Successive anion exchange with NaBARF_4 ^[12] in dichloromethane afforded a crude product

as a 1:1 mixture of $4\mathbf{x} \cdot \mathbf{BAR}_4^F$ and $\text{CH}_2=\text{CHCH}_2\text{CMe}(\text{COOMe})_2$ (by ^1H NMR spectroscopy). The crude mixture was purified by column chromatography over SiO_2 with CHCl_3 as the eluent to give $4\mathbf{x} \cdot \mathbf{BAR}_4^F$ as a yellow viscous oil. Although all attempts to obtain single crystals of $4\mathbf{x} \cdot \mathbf{BAR}_4^F$ have been unsuccessful because of its low crystallinity, the coordination of the benzylideneallyl moiety in a η^3 -fashion was established by various NMR measurements (see Supporting Information). Similarly, an analogous methylene- π -allylpalladium complex with dpbp ($4\mathbf{y} \cdot \mathbf{BAR}_4^F$) was isolated and the NMR measurements of $4\mathbf{y} \cdot \mathbf{BAR}_4^F$ clarify that it exists as a mixture of two diastereomers, which originate from atropisomerism of the coordinating dpbp ligand. As expected, a stoichiometric reaction of the isolated $4\mathbf{y} \cdot \mathbf{BAR}_4^F$ with $2\mathbf{m}$ in THF gave the allene $3\mathbf{am}$ in 67% yield. Although the yield is relatively low compared to the catalytic reaction, presumably as a result of moisture in the oily complex, the nucleophilic attack on the benzylideneallyl moiety is highly selective and $3\mathbf{am}$ is the sole product from the reaction. The observations all strongly support the proposed catalytic cycle.

In summary, we have developed a general and efficient new method for the palladium-catalyzed conversion of 1,1-dibromo-1-alkenes to substituted functionalized allenes. We also established the catalytic cycle of the allene formation reaction, including the isolation and the characterization of the methylene- π -allylpalladium key intermediate. The experimental ease and the high yield of this method should enhance its attractiveness.

Experimental Section

1 (general procedure): A typical procedure is given for the preparation of **1a**. A solution of $\text{PhCH}=\text{CBr}_2$ (5.65 g, 21.6 mmol) and $[\text{Pd}(\text{PPh}_3)_4]$ (400 mg, 0.35 mmol) in THF (40 mL) was added at 0°C by cannula transfer to a suspension of $(\text{CH}_2=\text{CH})\text{ZnCl}$ in THF, prepared from dry ZnCl_2 (3.41 g, 25.0 mmol) and vinylmagnesium bromide (0.95 M in THF, 25 mL, 24 mmol). After the addition, the mixture was stirred for 1 h at room temperature. The mixture was diluted with *n*-hexane and filtered. The solvents were removed under reduced pressure and then the residue was extracted with hexane. The hexane solution was evaporated, and the residue was purified by column chromatography on silica gel with *n*-hexane as the eluent to give **1a** (3.78 g, 84% yield) as a colorless oil. All the spectral data of this material were consistent with those reported previously.^[13]

3 (general procedure): The reaction conditions and results are summarized in Table 1. A typical procedure is given for the preparation of **3am** (entry 1, Table 1). $[\text{PdCl}(\eta^3\text{-C}_3\text{H}_5)]_2$ (1.5 mg, 8.2 μmol /Pd), dpbp (4.7 mg, 9.0 μmol), and **1a** (89.0 mg, 426 μmol) were dissolved in THF (5 mL) and the solution was added to **2m** (80.1 mg, 476 μmol) by cannula transfer under nitrogen. The mixture was stirred at room temperature for 12 h, then filtered through a short pad of SiO_2 to remove precipitated inorganic salts. The silica gel pad was washed three times with a small amount of Et_2O and the combined solution was evaporated to dryness under reduced pressure. The yellow residue was purified by chromatography on silica gel (*n*-hexane/ Et_2O = 1/1) to give the allene **3am** (106 mg, 91%) as a colorless oil. ^1H NMR (500 MHz, CDCl_3): δ = 1.51 (s, 3H), 2.70 (dd, J = 2.4, 7.7 Hz, 2H), 3.70 (s, 3H), 3.72 (s, 3H), 5.46 (dt, J = 6.3, 7.7 Hz, 1H), 6.14 (td, J = 2.4, 6.3 Hz, 1H), 7.17–7.20 (m, 1H), 7.24–7.31 (m, 4H); ^{13}C NMR (125 MHz, CDCl_3): δ = 19.97, 35.60, 52.60, 52.64, 53.78, 89.37, 94.62, 126.82, 126.99, 128.59, 134.14, 172.11, 172.13, 206.91. Elemental analysis calcd for $\text{C}_{16}\text{H}_{18}\text{O}_4$: C 70.06, H 6.61; found: C 70.18, H 6.76.

4 · BAR₄^F (general procedure): The complexes were isolated with dpbp ($4\mathbf{x} \cdot \mathbf{BAR}_4^F$) or with dpbp ($4\mathbf{y} \cdot \mathbf{BAR}_4^F$) and a typical procedure is given for the preparation of $4\mathbf{x} \cdot \mathbf{BAR}_4^F$. A mixture of $[\text{PdCl}(\eta^3\text{-C}_3\text{H}_5)]_2$ (81.7 mg, 447 μmol /Pd), dpbp (193 mg, 453 μmol), and **2m** (80.1 mg, 476 μmol) was

placed in a Schlenk flask and dry THF (5 mL) added under nitrogen. The mixture was stirred at room temperature for 30 s, then **1a** (104 mg, 497 μmol) was added to the flask by means of syringe. After stirring the orange-yellow solution for 3 h at room temperature, all the volatiles were removed under reduced pressure. $\text{NaBAR}_4^F \cdot \text{OEt}_2$ (500 mg, 521 μmol) and CH_2Cl_2 (5 mL) were then added to the residue and the mixture was stirred for 30 min at room temperature. The mixture was filtered and the filtrate was evaporated to dryness under reduced pressure. The yellow residue was purified by column chromatography on silica gel with CHCl_3 as the eluent to give the complex $4\mathbf{x} \cdot \mathbf{BAR}_4^F$ (598 mg, 88% yield) as a yellow viscous oil. ^1H NMR (500 MHz, CDCl_3): δ = 1.68–1.78 (m, 1H), 1.87–2.04 (m, 3H), 2.51–2.55 (m, 2H), 2.61–2.74 (m, 2H), 3.30 (dd, J = 8.3, 14.6 Hz, 1H), 4.42 (dd, J = 5.1, 8.3 Hz, 1H), 5.25 (ddd, J = 3.1, 7.8, 11.6 Hz, 1H), 5.77 (ddd, J = 3.1, 8.3, 14.6 Hz, 1H), 6.93 (m, 2H), 7.22–7.27 (m, 5H), 7.34–7.56 (m, 21H), 7.62–7.64 (m, 1H), 7.72 (br, 8H); ^{31}P NMR (202 MHz, CDCl_3): δ = 14.3 (d, J = 45.3 Hz, 1P), 30.3 (d, J = 45.3 Hz, 1P).

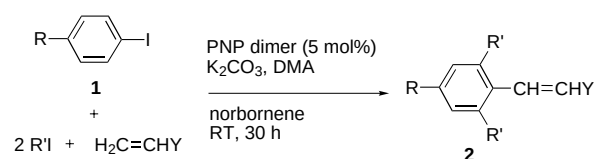
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A New Route to Fused Aromatic Compounds by Using a Palladium-Catalyzed Alkylation–Alkenylation Sequence**

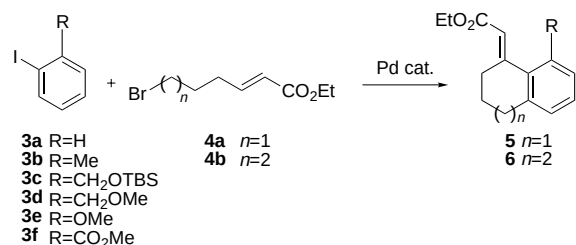
Mark Lautens* and Sandrine Piguel

Selective aromatic substitution is a long-standing goal in organic chemistry.^[1] Recently, a new method involving a palladium-catalyzed process based on a sequential double aromatic substitution and an intermolecular Heck reaction leading to *o,o'*-disubstituted vinylarenes **2** was described (Scheme 1).^[2] The potential of this strategy is truly remarkable since it allows the formation of three new carbon–carbon bonds in a one-pot process.



Scheme 1. Palladium-catalyzed synthesis of *O,O'*-dialkylvinylarenes. DMA = *N,N*-dimethylacetamide; PNP dimer = *cis,exo*-2-phenylnorbornyl-palladium chloride.^[3]

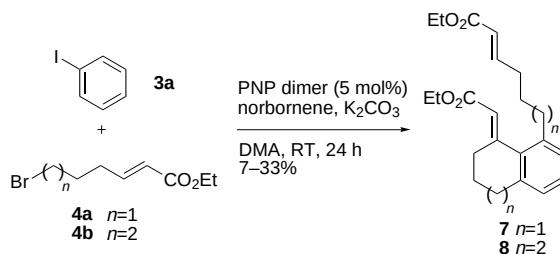
With the goal of synthesizing various fused carbocycles, we sought to extend and modify this domino sequence by using a difunctional acceptor so that an intramolecular Heck reaction can follow the *o*-alkylation, resulting in a new route to fused aromatic compounds starting from iodoaryl compounds (Scheme 2).^[4] Herein, we report our first results for the synthesis of benzocarbocycles with six- and seven-membered rings.



Scheme 2. Palladium-catalyzed synthesis of various carbocycles. TBS = *tert*-butyldimethylsilyl.

In our initial studies we used iodobenzene (**3a**) and ethyl (*E*)-6-bromohept-2-enoate (**4a**) as starting materials.^[5] By using the procedure described by Catellani and co-workers,^[2] the six-membered-ring compound **7** was isolated in a yield ranging from 7 to 33% as a single stereoisomer. This

palladium-catalyzed process involved the formation of three new carbon–carbon bonds in one pot through a double aromatic substitution in the *ortho*- and *ortho'*-positions followed by an intramolecular Heck reaction (Scheme 3). However, poor yields and irreproducible results were obtained largely because of variability in the activity of the catalyst.



Scheme 3. Synthesis of carbocycles with six- and seven-membered rings from iodobenzene (**3a**).

It was necessary to find improved conditions, and toward this end ligand effects as well as different palladium sources and solvents were examined. The reaction of iodobenzene (**3a**) with ethyl (*E*)-6-bromohept-2-enoate (**4a**) served as a model for these studies. Among the different phosphanes and palladium sources we tried, tri-2-furylphosphane and Pd(OAc)₂ afforded the most encouraging results; the bicyclic compound **7** was obtained in 24% yield as a single stereoisomer and in reproducible yield (Scheme 3).^[6] Other catalysts such as [Pd(PPh₃)₄], [Pd(dppf)Cl₂]/HgCl (dppf = 1,1'-bis(diphenylphosphanyl)ferrocene), [Pd₂(dba)₃] (dba = *trans,trans*-dibenzylideneacetone), Pd(OAc)₂/PPh₃, Pd(OAc)₂/PnBu₃, Pd(OAc)₂/tri-*o*-tolylphosphane, and Pd(OAc)₂/P(OMe)₃ either promoted no reaction or gave only traces of the expected compound.^[7] An investigation of different solvents revealed that acetonitrile was the solvent of choice. Under the same conditions, the yield increased from 24% in DMA to 43% in refluxing MeCN whereas, in other solvents (CH₂Cl₂, THF, toluene) only traces of **7** were isolated.

We next investigated the scope of the reaction using *ortho*-substituted aryl iodides since the resulting products are of much broader interest. Under the optimized conditions (Pd(OAc)₂ (10 mol%), tri-2-furylphosphane (20 mol%), norbornene (2 equiv), K₂CO₃ (2 equiv), MeCN, reflux), we could effect the palladium-catalyzed reaction of a wide array of *ortho*-substituted aryl iodides with **4a** (*n* = 1) and ethyl (*E*)-7-bromohept-2-enoate (**4b**; *n* = 2) leading to carbocycles with six- and seven-membered rings, respectively, in moderate to excellent yields (Scheme 2, Table 1).^[8] The overall sequence with *ortho*-substituted aryl iodides involves one aromatic substitution in the *ortho'*-position followed by an intramolecular Heck reaction. Therefore, two new carbon–carbon bonds were formed in a one-pot reaction and the bicyclic compounds were isolated as single *E*-configured stereoisomers as determined by NOE experiments.

The yield of the reaction is strongly influenced by the *ortho*-alkyl substituent R. In fact, when R = alkyl, the reaction is very efficient and the six- and seven-membered rings were isolated in 85 and 62% yield, respectively (entry 1, Table 1).

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Table 1. Effects of *ortho*-substitution and bases on the yield of the reaction shown in Scheme 2.^[a]

Entry	R	<i>n</i>	K ₂ CO ₃ Yield [%] ^[b]	Cs ₂ CO ₃ Yield [%] ^[b]
1	Me	1	85	90
		2	62	83
2	CH ₂ OTBS	1	41	60
		2	33	47
3	CH ₂ OMe	1	20	35
		2	6	25
4	OMe	1	29	26
		2	41	93
5	CO ₂ Me	1	— ^[c]	— ^[c]
		2	— ^[c]	— ^[c]

[a] Conditions (optimized): Pd(OAc)₂ (10 mol %), tri-2-furylphosphane (20 mol %), norbornene (2 equiv), K₂CO₃ (2 equiv), MeCN, reflux, 12 h. [b] Yield of isolated product. [c] No reaction.

With an electron-withdrawing group (entry 5), no reaction occurred and with an electron-donating group (entry 4), the yield was moderate. When R = CH₂OTBS, the yields were moderate, however, when the silyl group was replaced by a methyl group, the yield was even lower (entries 2 and 3). This lack of reactivity might be explained by a complexation between the oxygen atom and the palladium center, which renders the palladium catalyst unavailable for further reaction. We suppose that this phenomenon is minimized in the case of R = CH₂OTBS because of the steric bulk of the silyl group.

It is also known that some other parameters (such as bases or additives) might influence the yield.^[9, 10] Therefore, we surveyed a few bases to optimize the reaction. The same transformation could now be achieved in higher yields by using Cs₂CO₃ instead of K₂CO₃ (Table 1). In both cases (*n* = 1, 2), excellent yields were obtained when R = alkyl (entry 1). Although the yield was still low when R = CH₂OMe, it was improved from 20 to 35% for the six-membered-ring carbocycle and from 6 to 25% for the seven-membered-ring carbocycle (entry 3). This poor yield was due to dimerization of the iodo compound, a pathway we had not previously observed.^[11, 12] When R = OMe, the yield increased to 93% for the formation of the seven-membered-ring carbocycle. As before, no reaction occurred when there was an electron-withdrawing substituent on the iodoaryl compound. The difference in reactivity between Cs₂CO₃ and K₂CO₃ might be explained by the better solubility of Cs₂CO₃.

Finally, under our best conditions (Pd(OAc)₂ (10 mol %), tri-2-furylphosphane (20 mol %), norbornene (2 equiv), Cs₂CO₃ (2 equiv), MeCN, reflux), the sequence of double aromatic substitution and intramolecular Heck reaction was carried out with iodobenzene (**3a**) and **4a** or **4b** and provided compounds **7** and **8** as single stereoisomers in 59 (*n* = 1) and 64% (*n* = 2) yield, respectively (Scheme 3).

We have developed a new approach for the synthesis of carbocycles, which proceeds by a palladium-catalyzed process based on a sequential aromatic substitution and intramolecular Heck reaction of iodoaryl compounds and involves the formation of at least two new carbon–carbon bonds. Under optimized conditions we can accomplish the sequence with a variety of iodoaryl substrates that have different chain lengths

of the alkenyl group, leading to six- and seven-membered-ring carbocycles as single stereoisomers. Application of these methods to the synthesis of five-membered rings and heterocyclic compounds is currently in progress in our laboratory.

Experimental Section

The general procedure is described for the case of 2-iodotoluene and ethyl (*E*)-6-bromohex-2-enoate. Under argon, a flame-dried round-bottom flask equipped with a condenser was charged with 2-iodotoluene (20 μL, 0.157 mmol), ethyl (*E*)-6-bromohex-2-enoate (138 mg, 0.624 mmol), Cs₂CO₃ (105 mg, 0.322 mmol), norbornene (30.2 mg, 0.32 mmol), tri-2-furylphosphane (7.5 mg, 0.032 mmol), Pd(OAc)₂ (4 mg, 0.017 mmol), and acetonitrile (1.5 mL). The reaction mixture was heated under reflux for 12 h and then quenched with a saturated aqueous ammonium chloride solution. The organic layer was separated and the aqueous layer was extracted with diethyl ether. The combined organic layers were washed with brine and dried over MgSO₄. Evaporation of the solvent and flash chromatography on silica gel of the residue with 1–3% diethyl ether in hexanes afforded the six-membered-ring compound **5b** (32.6 mg, 90%) as a colorless oil. ¹H NMR (400 MHz, CDCl₃, 25 °C, TMS): δ = 7.1 (m, 2H; =CH(Ar)), 6.9 (m, 1H; =CH(Ar)), 5.9 (t, ³*J* = 2 Hz, 1H; =CHCO₂Et), 4.2 (q, ²*J* = 7.2 Hz, 2H; OCH₂), 3.1 (td, ²*J* = 7 Hz, ³*J* = 2 Hz, 2H; CH₂), 2.6 (br t, ²*J* = 6.1 Hz, 2H; CH₂), 2.47 (s, 3H; CH₃), 1.7 (m, 2H; CH₂), 1.31 (t, ³*J* = 7.2 Hz, 3H; CH₃); ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 166.9, 154.7, 141.8, 136.3, 134.9, 129.5, 127.8, 125.2, 118.2, 59.7, 30.2, 28.2, 21.7, 21.6, 14.3; MS (EI, 70 eV): *m/z* (%): 185 (100) [*M*⁺ – EtO]. HR-MS: calcd for C₁₅H₁₈O₂ [*M*]⁺: 230.1311; found: 230.1306.

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Towards the Design of Open-Framework Cluster Materials: A Novel Layered Niobium Oxochloride with a Honeycomblike Structure**

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Michael W. Essig, and Abdessadek Lachgar*

In memory of Jean Rouxel

Open-framework compounds have numerous technological applications as heterogeneous catalysts, ion exchangers, and as molecular sieves.^[1] Research in this field focuses on the preparation of open-framework materials containing redox-active centers which leads to a considerable increase of their catalytic potential.^[2] The use of metal clusters as redox-active centers is of special importance due to the remarkable catalytic properties of cluster compounds compared to mononuclear metal complexes.^[3] Our systematic investigation of reduced niobium oxochlorides containing octahedral Nb₆ clusters led to the preparation of the layered material described herein. Its crystal structure is based on an octahedral Nb₆ cluster core coordinated by eight inner chloride, four inner oxide, and six outer chloride ligands: [(Nb₆Cl₈O₄)Cl₆]³⁻ (Figure 1). These clusters share outer chloride ligands to form

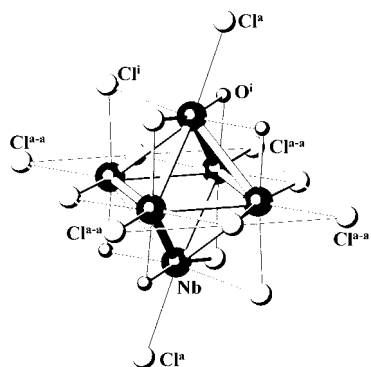


Figure 1. View of the [(Nb₆Cl₈O₄)Cl₆]³⁻ cluster unit in **1** highlighting the plane containing outer chloride ligands (Cl^{a-a}) that connect the clusters into a two-dimensional network.

layers characterized by large six-membered rings. The ring openings contain units of [Ti₂Cl₉]³⁻ dimers surrounded by thallium cations. The two-dimensional character of this material is favored by the arrangement of the oxide and chloride ligands which results in an anisotropic charge distribution around the Nb₆ cluster core.

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The novel, layered niobium oxochloride [(Ti₅(Ti₂Cl₉))₃[(Nb₆Cl₁₂O₄)₃(Ti₃Cl₄)₂]] (**1**) crystallizes in the space group *P*3̄c1, with two formula units per unit cell. Its structure^[4] is composed of layers shown in Figure 2. The basic cluster unit

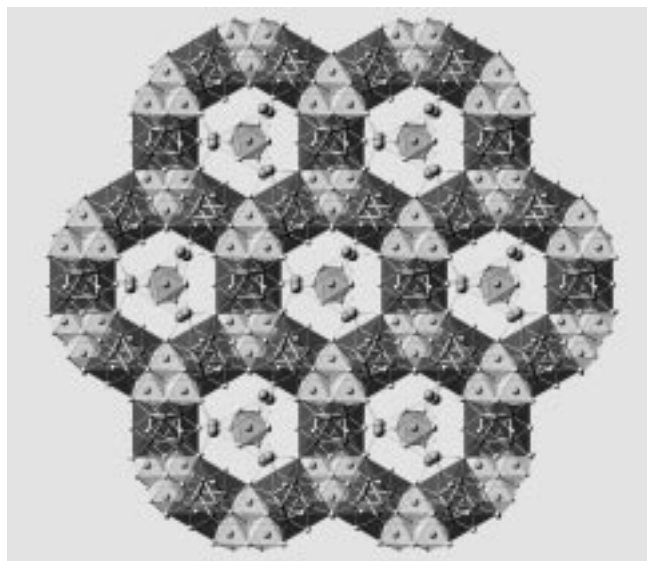


Figure 2. A projection of one layer in **1** on the *ab* plane. Dark gray octahedra of [(Nb₆Cl₈O₄)Cl₆]³⁻ clusters and light gray [Ti₃Cl₇O₆] trimers form a honeycomblike framework with cavities containing [Ti₂Cl₉]³⁻ dimers surrounded by Tl ions.

is a Nb₆ octahedron in which every edge is bridged by a chloride or oxide ligand ("inner" Clⁱ and Oⁱ ligands), and six other chloride ligands attach to the Nb₆ octahedron in apical positions ("outer" ligands, Cl^a). The cluster has the same ligand arrangement and similar bond distances as those found in Ti₂Nb₆Cl₁₄O₄^[5] (selected bond lengths [Å]: Nb–Nb 2.8164(10)–2.9824(9), Nb–Clⁱ 2.427(2)–2.484(2), Nb–Cl^a 2.585(2)–2.652(2), Nb–O 1.992(5)–2.033(5)). Each cluster shares four outer chlorines (Cl^{a-a} in Figure 1) with four adjacent clusters to form a two-dimensional network which generates six- and three-membered rings similar to those found in hexagonal tungsten bronzes (HTB).^[6] Additional linkages between clusters within the same layer are provided by [Ti₃Cl₇O₆] trimers^[7] (Ti–Ti 3.792(3) Å) built from three [TiCl₄O₂] octahedra that share one vertex (Figure 3 a). Each trimer connects three clusters through inner and outer

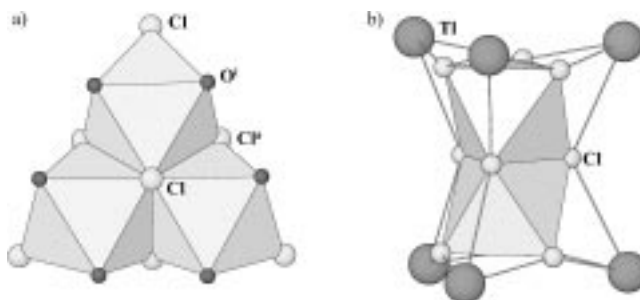


Figure 3. a) [Ti₃Cl₇O₆] trimer built from three [TiCl₄O₂] octahedra that share one vertex; b) [Ti₂Cl₉]³⁻ dimer surrounded by thallium ions.

chloride ligands. The $[\text{Ti}_3\text{L}_{13}]$ units are common in reduced titanium chlorides and are prone to metal–metal bond formation.^[8] For example, $\text{Na}_2\text{Ti}_3\text{Cl}_8$ ^[8a] contains $[\text{Ti}_3\text{Cl}_{13}]$ trimers, and undergoes a phase transition resulting in the formation of triangular titanium clusters and a decrease in Ti–Ti distances from 3.717 to 2.996 Å.

The openings of the six-membered cluster rings contain $[\text{Ti}_2\text{Cl}_9]^{3-}$ dimers surrounded by Ti^+ ions (Figure 3b). The dimers are formed from two $[\text{TiCl}_6]$ octahedra sharing one face (Ti–Ti 2.977(8) Å) and are aligned along the [001] direction. Each dimer is surrounded by five Ti^+ ions statistically distributed over six crystallographic sites. Similar $[\text{Ti}_2\text{Cl}_9]^{3-}$ dimers surrounded by A^+ ions are found in the series $\text{A}_3\text{Ti}_2\text{Cl}_9$ (A = Cs, In).^[9]

Adjacent layers are stacked perfectly on top of each other and differ only in the orientation of Ti^+ and $[\text{Ti}_2\text{Cl}_9]^{3-}$ ions. The ring openings in individual layers merge into channels parallel to the [001] direction.

Layered frameworks similar to that found in **1** are present in non-cluster compounds such as $\text{K}[\text{Al}(\text{OH})_2]_3(\text{SO}_4)_2$ (alunite),^[10] $\text{K}_3(\text{SbO}_2)_3(\text{PO}_4)_2$,^[11] and $(\text{NH}_4)(\text{VO}_2)_3(\text{SeO}_3)_2$.^[12] Their anionic frameworks have the general formula $\text{M}_3\text{T}_2\text{O}_x^{n-}$ and consist of HTB-type layers made up of corner-sharing $[\text{MO}_6]$ octahedra and $[\text{TO}_3]$ or $[\text{TO}_4]$ polyhedra. In the structure of **1**, the $[\text{Nb}_6\text{Cl}_8\text{O}_4]$ cluster core plays the role of the metal M, outer chloride ligands correspond to oxide ligands of $[\text{MO}_6]$, and $[\text{Ti}_3\text{Cl}_7\text{O}_6]$ trimers correspond to the $[\text{TO}_4]$ or $[\text{TO}_3]$ units. In contrast to **1**, the layers in these $\text{M}_3\text{T}_2\text{O}_x^{n-}$ examples are shifted with respect to each other resulting in the absence of channels.

The formation of the HTB-type cluster framework in **1** is favored by the presence of $[\text{Ti}_2\text{Cl}_9]^{3-}$ units which play the role of a template around which the six-membered cluster ring is assembled. A similar templating effect was observed in the case of $\text{Nb}_7\text{S}_2\text{I}_{19}$,^[13] in which triangular $[\text{Nb}_3\text{I}_{10}\text{S}]$ clusters form a graphite-like network stabilized by $[\text{NbI}_5]$ molecules. Attempts to remove $[\text{NbI}_5]$ led to the decomposition of the phase.^[13a] This decomposition is probably due to the fact that the size of the ring opening fits only one iodide ligand. In the case of **1**, however, a cluster-ring opening can accommodate an entire $[\text{Ti}_5(\text{Ti}_2\text{Cl}_9)]^{2+}$ unit. Removal of $[\text{Ti}_2\text{Cl}_9]^{3-}$ dimers or the whole $[\text{Ti}_5(\text{Ti}_2\text{Cl}_9)]^{2+}$ template could be possible due to the electronic flexibility of the framework. Attempts to remove or exchange these units are underway.

Compound **1** contains 50 valence electrons per formula unit. The observed intracluster bond distances and the analogy with other niobium oxochloride cluster compounds,^[5, 14] indicate that each cluster has 14 electrons, and the remaining eight electrons belong to eight titanium atoms, which corresponds to all titanium atoms being present in the oxidation state +3. The magnetic measurements^[15] (Figure 4) show Curie behavior with an effective magnetic moment of $3.96 \mu_B$ per formula unit which corresponds to 5.2 unpaired electrons.^[16] This value is consistent with the model of noninteracting Ti^{3+} ions in the $[\text{Ti}_3\text{Cl}_7\text{O}_6]$ trimers (two trimers per formula unit), and antiferromagnetically coupled Ti^{3+} ions in the $[\text{Ti}_2\text{Cl}_9]^{3-}$ dimers (one dimer per formula unit) as is the case in $\text{Cs}_3\text{Ti}_2\text{Cl}_9$.^[17]

Compound **1** represents the first example of a layered

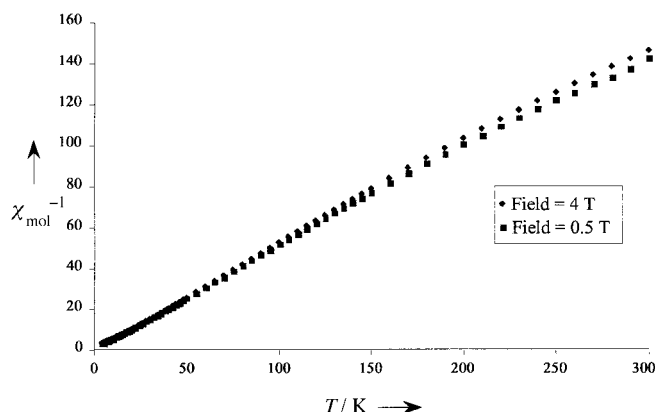


Figure 4. Reverse molar magnetic susceptibility χ_{mol}^{-1} (molemu⁻¹) of **1** over the temperature range 2–300 K.

HTB-type framework in cluster chemistry. Its layered structure confirms the relationship between the ligand arrangement in the cluster unit and the cluster framework dimensionality. These correlations and the structural analogies with noncluster framework prototypes can serve as a basis for future design of open-framework compounds containing octahedral clusters.

Experimental Section

Compound **1** was initially obtained from the composition $\text{TiTi}_2\text{Nb}_6\text{Cl}_{15}\text{O}_4$ corresponding to the addition of one equivalent of TiCl_3 to the starting stoichiometry of the parent compound $\text{Ti}_3\text{Nb}_6\text{Cl}_{14}\text{O}_4$. After the composition of the new phase was established from the crystal structure analysis, the compound $[\{\text{Ti}_5(\text{Ti}_2\text{Cl}_9)\}\{\text{Nb}_6\text{Cl}_{12}\text{O}_4\}_3\{\text{Ti}_3\text{Cl}_7\text{O}_6\}_2]$ was obtained quantitatively from stoichiometric amounts of Nb powder, Ti foil, TiCl_3 , Nb_2O_5 , and NbCl_5 . The mixture (handled under an inert atmosphere) was placed in a silica tube, sealed under vacuum, heated for 4 d at 720 °C, and cooled to 500 °C over 4 d, followed by radiative cooling to room temperature. The product was obtained as black hexagonal plates up to 2 mm in diameter.

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- atom. The $[\text{Ti}_2\text{Cl}_9]$ units are present about 90% of the time, so that the net stoichiometry is $\text{Ti}_{4.87(16)}\text{Ti}_{7.81(3)}\text{Nb}_{18}\text{Cl}_{51.51(16)}\text{O}_{12.40(4)}$. The highest residual electron-density peak ($1.724 \text{ e}\text{\AA}^{-3}$) is located between the two titanium atoms that form the dimers. Anisotropic refinement of all atoms except for O7 converged to $R_1 = 0.064$, $wR_2 = 0.102$. Further details of the crystal structure investigation may be obtained from the Fachinformationszentrum Karlsruhe, 76344 Eggenstein-Leopoldshafen, Germany (fax: (+49) 7247-808-666; e-mail: crysdata@fiz-karlsruhe.de) on quoting the depository number CSD-410776.
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Molecular Beacons: A Novel Approach to Detect Protein–DNA Interactions**

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Protein–DNA interactions are known to play an important role in a wide variety of biological processes. Binding of proteins to genomic DNA is involved in various biological processes including DNA replication, transcription, recombination, and repair.^[1] To study these protein–DNA binding

events, a variety of techniques such as band shift and footprinting assays, affinity chromatography, fluorescence analysis, and microscopy visualization have been developed.^[2–5] While all of these techniques provide insight into one specific aspect of protein–DNA binding, optical and fluorescence methods offer significant advantages in analytical sensitivity and simplicity. Here we describe a new approach to study protein–DNA binding and to quantify proteins using a recently developed oligonucleotide probe that serves as a molecular beacon (MB).^[6–11]

The molecular beacon is designed in such a way that it can report the presence of a specific complementary nucleic acid in homogeneous solutions.^[6] The molecular beacon is labeled with a fluorophore at one end and a nonfluorescent quencher at the opposite end. In solution, the molecular beacon forms a hairpin loop which brings the fluorophore and quencher close enough to allow quenching to occur by fluorescence resonance energy transfer. Upon hybridization to a complementary sequence, the hairpin loop is broken and the fluorophore and quencher are separated, resulting in the restoration of fluorescence. The molecular beacon approach has great advantages over other DNA probes, including the ability to detect single-base mismatches, the capability of studying biological processes in real time, and the possibility for in vivo analysis. There have been several applications of molecular beacons, such as sensitive monitoring of the polymerase chain reaction,^[7] real-time detection of DNA–RNA hybridization in living cells,^[8] DNA mutation analysis,^[9] and even detection of pathogenic retroviruses.^[10] Recently, a biotinylated single-stranded DNA (ssDNA) molecular beacon was designed and used for DNA-hybridization studies at a liquid–solid interface and for the development of ultrasensitive DNA sensors.^[11] Molecular beacons hold great promise in studies in genetics and disease mechanisms as well as in disease diagnostics. So far, however, all applications have been based on the hybridization between a molecular beacon and DNA or RNA molecules. The use of a molecular beacon for studying protein–DNA interactions could be of great benefit for understanding the many important biological processes that require ultrasensitive and specific protein detection. Herein, we report for the first time the interactions between proteins and a molecular beacon.

The molecular beacon synthesized for this study is shown in Figure 1. It was designed with tetramethylrhodamine (TAM-RA; Molecular Probes, Eugene, OR) as the fluorophore and 4-(4'-dimethylaminophenylazo)benzoic acid (DABCYL; Molecular Probes) as the quencher. The molecular beacon was synthesized by using DABCYL-derivatized controlled pore glass (CPG) as the starting material. The synthesis was started from the 3' end. The nucleotides were added sequentially by using standard cyanoethylphosphoramidite chemistry. The 5' end nucleotide is conjugated to a $(\text{CH}_2)_6\text{-NH}$ linker arm, producing a primary amine group at the 5'-end. This amino group was used to attach tetramethylrhodamine. The product was purified by gel filtration chromatography and reverse-phase high-pressure liquid chromatography (RP-HPLC). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry was used to confirm the synthesis of the designed molecular beacon (data not shown). In the absence

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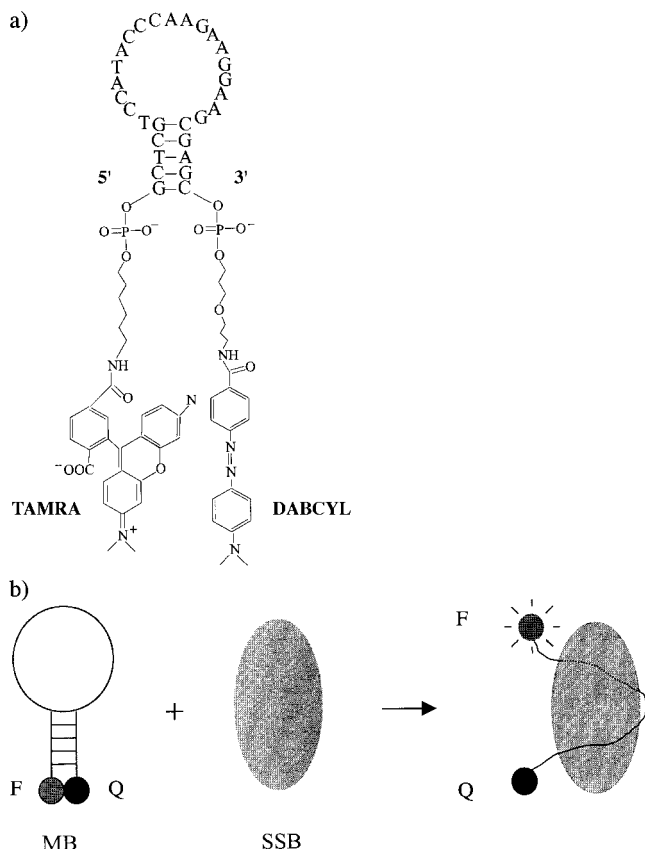


Figure 1. a) The structure of the molecular beacon used in this study. The MB was designed as a 19-mer loop sequence flanked on the 5'- and 3'- ends with 5-mer long complementary sequences. The oligonucleotide has a stem-and-loop structure, stabilized by a five base-pair duplex formed by intramolecular hybridization of the complementary ends. The fluorophore (TAMRA) and quencher (DABCYL) are attached to the 3'- and 5'- ends of the oligonucleotide by $(\text{CH}_2)_6\text{-NH}$ and $(\text{CH}_2)_3\text{-O-(CH}_2)_3\text{-NH}$ linker arms, respectively. b) Schematic representation of the light-generating mechanism for the molecular beacon (MB) that occurs upon binding to proteins (SSB). See text for details. F = fluorophore; Q = quencher; the ellipsoid represents a protein molecule.

of the target sequence, the molecular beacon adopts a hairpin structure, including a stem of five base pairs and a loop of 19 bases. The stem keeps the fluorophore (TAMRA) and quencher (DABCYL) in close proximity (Figure 1 a), causing the fluorophore to be quenched by energy transfer. Any conformational reorganization that opens the stem should lead to the separation of the fluorophore and the quencher, and therefore to the restoration of fluorescence. The conformational state of a molecular beacon is thus directly reported by its fluorescence: In the closed state, the molecular beacon is not fluorescent; in the open state, when the fluorophore and the quencher molecules are apart, it emits intense fluorescence. It has been reported that fluorescence from molecular beacons can be restored by different approaches: hybridization to their complementary DNA (cDNA) and denaturation by either heat or high pH.^[6–10] In addition, it is known that the binding of proteins to DNA or RNA molecules can disturb and rearrange their conformation.^[12] Therefore we expect that the interactions between protein and MB may result in the spatial separation of the

quencher and the fluorophore part of the molecular beacon, which would restore the MB fluorescence (Figure 1 b).

To test this principle, a well-characterized *E. coli* protein, single-stranded DNA binding protein (SSB; Promega, Madison, WI), was chosen to study the interactions between proteins and molecular beacons. SSB consists of four identical 18.9 kDa subunits, binds with high affinity to ssDNA, and plays an essential role as an accessory protein in DNA replication, recombination, and repair.^[13] As controls, histone (HIS, type II-AS; Sigma, St. Louis, MO), a protein which mainly binds double-stranded DNA (dsDNA), and bovine serum albumin (BSA, non-acetylated; Sigma), a protein which does not bind DNA, were tested for their effect on the molecular beacon. Perfect and mismatched complementary sequences were used for hybridization experiments with the MB in order to compare the effect of DNA hybridization and protein binding on the fluorescence restoration of the molecular beacon. The titration of the molecular beacon with proteins and complementary DNAs was carried out on a spectrophotometer (F-112A, SPEX Industries). Samples were excited at 558 nm and detected at 580 nm. The fluorescence was measured at room temperature (25 °C) after mixing the samples with a pipette for about five seconds in a 100 μL cuvette cell. The buffer (10 mM Mg^{2+} , 50 mM NaCl, 20 mM Tris-HCl, pH 7.8) was used for both protein binding and hybridization experiments.

We observed that in solution the interaction between the molecular beacon and SSB is rapid and tight. Fluorescence detection was used to probe the interaction between the protein and the molecular beacon. SSB was added to the MB solution, and the intensity of the fluorescence of the mixture was monitored. The time scans showed that SSB quickly interacts with MB, resulting in significant fluorescence enhancement (Figure 2). A control experiment with free

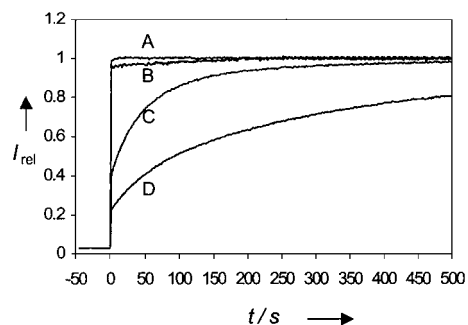


Figure 2. Relative fluorescence intensity time scans of the binding of the MB to SSB and cDNA, respectively, at two different molar ratios: A, SSB:MB = 4:1; B, SSB:MB = 1:1; C, cDNA:MB = 4:1; D, cDNA:MB = 1:1. Before $t = 0$ s, the fluorescence of the MB solution was detected; from $t = 0$ s on, the fluorescence of the mixture of the MB with the binding reagent (either SSB or cDNA) was monitored. The cDNA sequence is 5'-CTTCTTCTTGGGTATGGA-3'.

TAMRA in the same buffer solution revealed that the fluorescence intensity was not influenced on addition of SSB (data not shown). This demonstrates that the enhancement in fluorescence intensity is indeed due to the conformational change of the molecular beacon: The binding of SSB to the MB causes the separation between the fluorophore and

the quencher, and consequently leads to the restoration of MB fluorescence (Figure 1b). The reaction, as monitored by the changes in fluorescence intensity, reaches a stable plateau within ten seconds. This indicates that the binding reaction is fast and that the equilibrium can be reached quickly. We have also performed similar time scan experiments by using a variety of concentrations of complementary DNA under optimal binding conditions. These analyses reveal that the interaction between the SSB and the MB occurs much faster than that between the cDNA and the molecular beacon. Also, it is worth noting that there is no obvious change in the binding rate when the molar ratio of SSB to MB decreases from 4:1 to 1:1, while the same change in molar ratio for cDNA to MB results in a significant binding rate difference. This rapid protein binding reaction could provide the basis for a quick protein assay.

We titrated MB with SSB solutions, and the resulting fluorescence titration curve is shown in Figure 3. At low SSB concentrations, there is a linear relationship between the fluorescence intensity of the solution and the concentration of

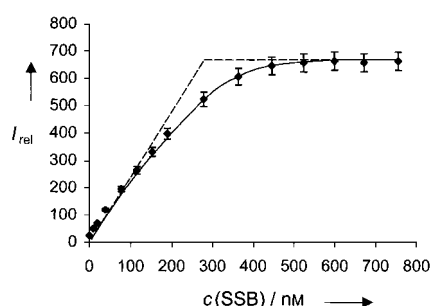


Figure 3. Continuous fluorescence titration of the molecular beacon with SSB. Each fluorescence value ($\blacklozenge$) at a certain SSB concentration is the average of ten readings of the fluorescence after the binding has reached equilibrium (corresponding to the plateau part of the time-scan curve shown in Figure 2). The dilution effect has been considered in data processing. The concentration of the MB was 250 nM. The stoichiometric ratio of MB to SSB was determined by the intersection point of two straight lines (—) extended, respectively, from the initial linear part and the plateau part of the titration curve (---). The results indicate that one SSB molecule binds to one MB molecule.

SSB. When the SSB concentration exceeds $40 \mu\text{g mL}^{-1}$, the intensity reaches a plateau. The stoichiometry for the binding of SSB to MB can be estimated based on the saturation concentration data shown in Figure 3. By extending the linear and the plateau parts of the curve, an intersection point is obtained. There is no additional fluorescence enhancement when more SSB is added beyond this point. Therefore, this point should give the stoichiometry of the binding reaction, which is estimated to be 1:1 (MB:SSB tetramer). Based on sedimentation experiments, one SSB tetramer can bind either four d(pT)_8 or two d(pT)_{16} deoxyoligonucleotides, or one d(pT)_{30-40} oligonucleotide.^[14] Among all polynucleotides, SSB has the highest affinity to poly(dT).^[14-16] It is thus not surprising that one SSB can only bind one molecule of the MB which has 29 mixed bases. We have determined the binding constant K_b of SSB–MB based on the stoichiometry information and the data presented in Figure 3. Under our experimental conditions, K_b is determined to be $2.0 \times 10^7 \text{ M}^{-1}$.

This binding constant is within the range of previously reported results ($6 \times 10^5 - 5 \times 10^8 \text{ M}^{-1}$).^[13, 14] That the binding constant of the molecular beacon fits well in this range suggests that the stem structure as well as the fluorophore and the quencher at the two ends of the molecular beacon do not significantly influence the binding affinity between SSB and MB. This clearly demonstrates that the MB described here is a suitable oligonucleotide probe for the study of protein–DNA interactions.

Our results further show that the MB can be used to detect SSB sensitively under optimal conditions. The concentration detection limit for SSB under our experimental conditions is calculated to be $2 \times 10^{-10} \text{ M}$. It is worth noting that all the experiments described herein were carried out with a spectrometer equipped with a mercury lamp. It is known that the use of a laser usually results in an enhancement in detection limit of three orders of magnitude.^[17] We thus expect that for protein analysis a sufficiently low detection limit will be achieved with better optical detection and laser excitation systems.

Our results also indicate significant differences in enhancement of fluorescence intensity of the molecular beacon by three different proteins and two DNA molecules. Figure 4 shows that the cDNA and SSB cause a similar enhancement in fluorescence intensities upon interaction with the molecular

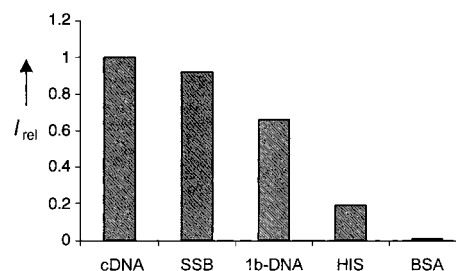


Figure 4. Relative fluorescence enhancement occurring upon binding of the molecular beacon to various DNA and protein molecules. The concentrations used were: cDNA, $1 \mu\text{M}$; SSB, $40 \mu\text{g mL}^{-1}$; 1b-DNA, $4 \mu\text{M}$; HIS, $200 \mu\text{g mL}^{-1}$; BSA, $400 \mu\text{g mL}^{-1}$; MB, 250 nM. The concentrations of the proteins and DNA molecules are high enough to saturate the molecular beacon in all binding studies. The perfect cDNA sequence is shown in Figure 2. The one base-pair mismatch complementary sequence is: 5'-CTTCCTTCTGGGATATGGA-3' (the underlined base represents the mismatch).

beacon. This indicates that the conformational change induced by SSB binding is comparable to that observed when the cDNA hybridizes to the MB. The enhancement brought about by the SSB is higher than that caused by binding of a cDNA molecule containing one base mismatch (1b-DNA). The reason that the fluorescence enhancement by SSB is slightly lower than that caused by interaction with cDNA might lie in the conformational difference of the “stretched” MB molecule in the two types of complexes. The molecular beacon is straightened by the formation of a dsDNA structure on the loop when cDNA is used. As a result, the spatial separation between the fluorophore and the quencher is the largest, while that for the SSB–MB complex will be slightly smaller since the ssDNA may still coil around the SSB.^[13] We also noticed that interaction of the molecular beacon with histone caused a much smaller fluorescence enhancement,

whereas for BSA, no apparent enhancement was detected. This is because SSB specifically binds to ssDNA with high affinity, while histone mainly binds to dsDNA. There has been no report suggesting any significant DNA binding to BSA. By intelligent molecular beacon design, this difference in fluorescence enhancement, which can be converted into a difference in bioassay sensitivity, might serve as the basis for the use of molecular beacons in specific and sensitive protein bioanalysis.

Our results demonstrate that there is a direct relationship between the restoration of molecular beacon fluorescence intensity and the DNA binding capability of the protein. Even though nonspecific DNA binding proteins are used here, the fact that different proteins do have different binding efficiencies with the molecular beacon in this first study is very encouraging. The molecular beacon used in this report lacks sequence specificity with regard to binding to particular proteins. Thus the next step is to develop MBs which can recognize certain proteins with high selectivity. There have been many recent studies aimed at designing functionalized single-stranded DNA or RNA molecules (aptamers) for selective protein binding studies and for enzymatic applications.^[18–20] Therefore, it is likely that aptamer-based molecular beacons can be developed for specific protein binding studies and for protein analysis with excellent selectivity.

In summary, we have opened a new way for studying protein–DNA interactions and for protein bioanalysis by using molecular beacons. The interaction between single-stranded DNA binding protein and molecular beacon results in significant restoration of the fluorescence of the molecular beacon. The fluorescence enhancement brought by SSB and by complementary DNA is very comparable. The molar ratio of the binding between SSB and MB is 1:1 with a SSB–MB binding constant of around $2.0 \times 10^7 \text{ M}^{-1}$. The detection limit of SSB is $2.0 \times 10^{-10} \text{ M}$. Preliminary results also show that there are significant differences in MB binding affinity by different proteins, which will constitute the basis for highly selective bioassay of a variety of proteins. The new approach is potentially useful for the study of protein–DNA/RNA interactions because of its high selectivity, speed, and convenience. The result also opens the possibility of using easily obtainable and designer DNA molecules for genomics and proteomics and for new drug development.

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Novel Zinc Fluorescent Probes Excitable with Visible Light for Biological Applications**

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Zinc is an essential component of many enzymes and transcription factors (for example, Zn^{II} in carbonic anhydrase and zinc finger proteins).^[1] Synaptic vesicles in excitatory nerve terminals contain high concentrations of chelatable Zn^{II},^[2] which is released by neuronal activity^[3] and may modulate the *N*-methyl-D-aspartate (NMDA) receptor.^[4]

Thus, Zn^{II} plays an important role in various biological systems. However, little is known about the cellular regulation of Zn^{II} in comparison with that of Ca^{II}. Chemical tools for measuring Zn^{II} in living cells (like fura-2 for Ca^{II}) are required. *N*-(6-Methoxy-8-quinolyl)-*P*-toluenesulfonamide (TSQ) was the first fluorescent probe to be developed for Zn^{II}: Its fluorescent intensity is increased by Zn^{II}, but not by Ca^{II} or Mg^{II}.^[5] TSQ is used for histochemical staining of Zn^{II} in tissue sections.^[6] Zalewski et al. developed a water-soluble TSQ derivative, Zinquin, which can, as an ethyl ester, be used to stain Zn^{II} in living cells.^[7] However, TSQ and derivatives are excited at UV wavelengths, which may cause cell damage, and is subject to interference by autofluorescence from pyridine nucleotides, among others.^[8]

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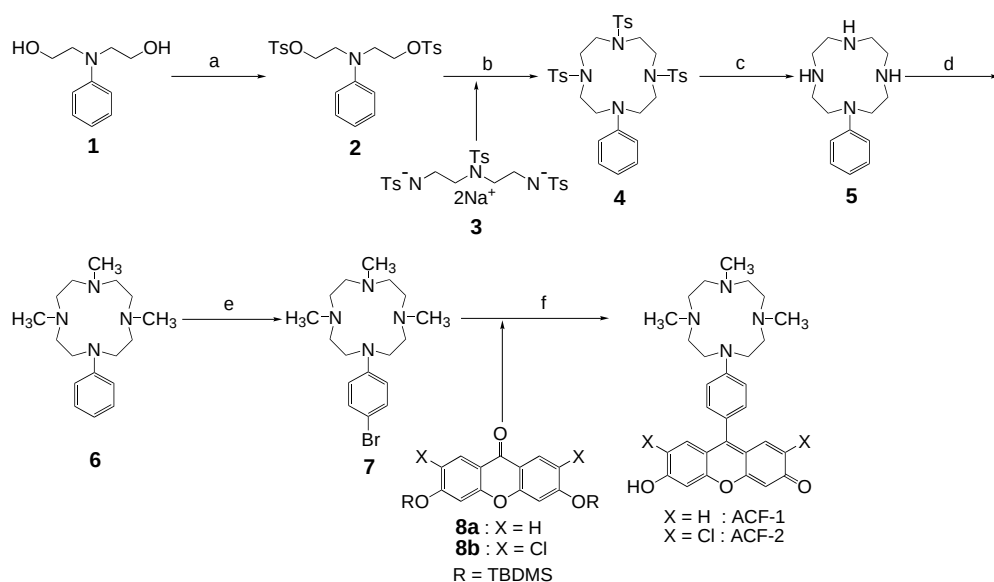
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Fluorescein is one of the most widely used fluorophores in biology as, for example, a label in immunofluorescence studies. Fluorescein is advantageous in that it has a high quantum yield of fluorescence in aqueous solutions and its excitation wavelength is in the visible range. We have designed and synthesized two new probes for Zn^{II} , 6-hydroxy-9-[4-(4,7,10-trimethyl-1,4,7,10-tetraazacyclododecan-1-yl)]-phenyl-3*H*-xanthen-3-one (ACF-1; azacrownfluorone) and its 2,7-dichloride derivative (ACF-2), as shown in Scheme 1. The fluorophore of ACF-1 is a fluorescein derivative, 6-hydroxy-9-phenylfluorone. This fluorophore is directly linked to a macrocyclic polyamine, which strongly complexes with transition metals such as Zn^{II} and Cu^{II} .

When an amino group is attached to the phthalic ring of fluorescein, the fluorescence is quenched. This quenching is due to photoinduced electron transfer (PET), which is the intramolecular quenching mechanism of an amine group in fluorophore–amine conjugates.^[9] However, when the amino group is replaced by a weaker electron-donating group (e.g. an amido group), the molecule fluoresces because the PET is hindered. This hindrance is caused by a reduction of the HOMO energy compared to that of the amino moiety.^[10] On

the basis of this concept, our group has developed two types of fluorescent probes: diamino fluoresceins (DAFs) for nitric oxide (NO)^[11] and 9-[2-[3-(carboxy)-9,10-diphenyl]anthryl]-6-hydroxy-3*H*-xanthen-3-ones (DPAXs) for singlet dioxygen ($^1\text{O}_2$).^[12] When NO is treated with DAFs, the diamino group is transformed to a triazole, resulting in lowering the HOMO level and an increase in the fluorescent intensity. Similarly, when Zn^{II} binds to a macrocyclic polyamine, it too may hinder PET and enhance the fluorescence.

Excitation and emission spectra of ACF-1 (5 μM) at pH 7.5 (100 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) buffer) in the presence of various concentrations of Zn^{II} are shown in Figure 1. Upon addition of Zn^{II} (5 μM), the fluorescent intensity of ACF-1 was increased 14-fold with no significant change in the position of excitation and emission maxima ($\lambda_{\text{ex}} = 495 \text{ nm}$, $\lambda_{\text{em}} = 515 \text{ nm}$). Under the same conditions, the fluorescent intensity of ACF-2 was



Scheme 1. The structure and synthesis of ACFs: a) TsCl , Py , 0°C , 91%; b) DMF , 100°C , 62%; c) Na , $n\text{BuOH}$, reflux, 67%; d) HCHO , NaBH_3CN , MeOH , rt, 26%; e) Br_2 , dioxane, 0°C , 90%; f) $t\text{BuLi}$, 2 MeTHF , -150°C , (ACF-1 (22%) and ACF-2 (1.2%)). $\text{Ts} = \text{CH}_3\text{C}_6\text{H}_4\text{SO}_2$.

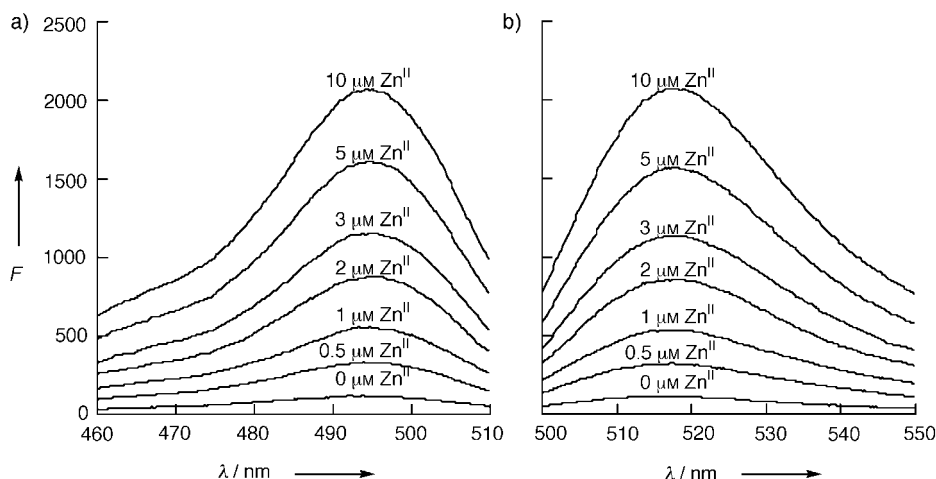


Figure 1. a) Excitation spectra (emission at $\lambda_{\text{em}} = 515 \text{ nm}$) and b) emission spectra (excitation at $\lambda_{\text{ex}} = 495 \text{ nm}$) of ACF-1 (5 μM) in the presence of Zn^{II} from 0–10 μM . These spectra were measured at pH 7.5 (100 mM HEPES buffer). F = Fluorescent intensity (arbitrary units).

increased 26-fold ($\lambda_{\text{ex}} = 505 \text{ nm}$, $\lambda_{\text{em}} = 525 \text{ nm}$). ACFs were more sensitive than the existing zinc fluorescent probe excitable with visible light, Newport Green,^[13] whose fluorescent intensity increased only 3.3-fold (Figure 2). The detection limit of the ACFs is 500 nM of Zn^{II} under these conditions, with a signal-to-noise ratio of 3.0, using 5 μM of ACFs. At pH 10.0 (100 mM 3-(cyclohexylamino) 1-propanesulfonic acid (CAPS) buffer), the fluorescent intensities of the ACFs increased linearly with Zn^{II} concentration up to 1:1 Zn^{II} :ACF mole ratio and remained at a plateau at increasing zinc levels (data not shown), indicating that ACFs and Zn^{II} form a 1:1 complex.

The fluorescent intensities of ACF-2 (5 μM) with various metal cations at pH 7.5 are shown in Table 1. The fluorescent intensities were not increased by addition of various cations found at high concentrations in cells, such as K^+ and Ca^{II} . These results were due to the poor complexation of alkaline

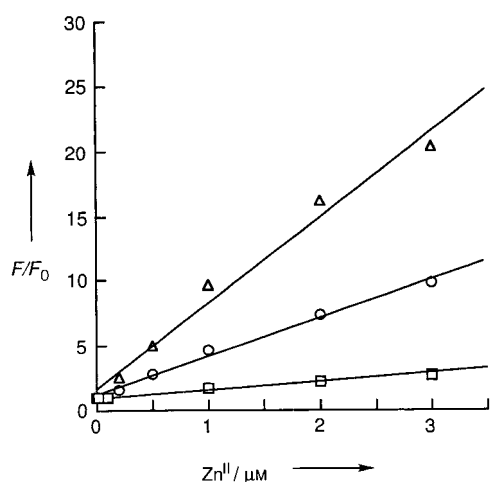


Figure 2. Fluorescent intensity of zinc fluorescent probes (5 μM) in the presence of various concentrations of Zn^{II}. The fluorescent intensities of ACF-2 (Δ: λ_{ex} = 505 nm; λ_{em} = 525 nm), ACF-1 (○: λ_{ex} = 495 nm; λ_{em} = 515 nm), and Newport Green (□: λ_{ex} = 505 nm; λ_{em} = 535 nm) were measured at pH 7.5 (100 mM HEPES buffer). *F* = Fluorescent intensity. *F*₀ = Fluorescent intensity of each fluorescent probe without metal.

Table 1. Relative fluorescent intensities of ACF-2 (5 μM) with various biologically important metal cations.

Entry	Metal cation	Relative intensity ^[a]
1	none	1.0
2	5 μM Zn ^{II}	24.3
3	5 mM Na ^I	1.2
4	5 mM K ^I	1.4
5	5 mM Ca ^{II}	1.0
6	5 mM Mg ^{II}	1.2
7	5 μM Fe ^{II}	1.1
8	5 μM Fe ^{III}	1.0
9	5 μM Cu ^{II}	0.3
10	5 μM Ni ^{II}	0.9
11	5 μM Co ^{II}	1.1
12	5 μM Mn ^{II}	1.1
13	5 μM Cd ^{II}	6.3
14	5 μM Zn ^{II} + 5 mM Na ^I	25.3
15	5 μM Zn ^{II} + 5 mM K ^I	25.4
16	5 μM Zn ^{II} + 5 mM Ca ^{II}	24.6
17	5 μM Zn ^{II} + 5 mM Mg ^{II}	23.1

[a] The fluorescent intensities (λ_{ex} = 505 nm, λ_{em} = 525 nm) were measured at pH 7.5 (100 mM HEPES buffer).

metals or alkaline earth metals with macrocyclic polyamines like the ACFs. Addition of transition metals such as Cu^{II} and Fe^{II} did not increase the fluorescent intensity. Although the binding constants of Cu^{II} to macrocyclic polyamines are generally larger than that of Zn^{II},^[14] the Cu^{II}–ACF complexes did not fluoresce possibly because of a quenching mechanism.^[15] Addition of Cd^{II} increased the fluorescence intensity slightly but its concentration in cells is much lower than that of Zn^{II}, so, in practice, Cd^{II} would not significantly affect the measurement of Zn^{II}. The fluorescence of ACF-2 (5 μM) and Zn^{II} (5 μM) was not affected by high concentrations (5 mM) of Na^I, K^I, Ca^{II} and Mg^{II}. Similar results were obtained with ACF-1.

Czarnik et al. developed fluorescent probes whose acceptors for Zn^{II} are also macrocyclic polyamines.^[16] At pH 10, the fluorescent intensity of their 9-(1,4,7,10-tetraazacyclododec-

yl)methylanthracene increased 14-fold upon the addition of Zn^{II}. However, under neutral conditions the nitrogen atom in this molecule, which is linked with the anthracene moiety through a methylene bridge, is protonated and the fluorescent intensity increased without addition of Zn^{II}. This result was due to the elimination of the PET upon protonation.^[17] To avoid this, we bound the macrocyclic polyamine directly to the fluorophore in the design of our ACFs. The fluorophore–nitrogen atom binding results in lower p*K*_a values and consequently the fluorescent intensity of the ACFs at pH 7 was comparable to that at around pH 10. The ACF–Zn^{II} complex thereby fluoresces at a physiological pH.

In summary, our fluorescent probes for Zn^{II}, the ACFs, are excited by visible light, and can selectively detect Zn^{II} under physiological conditions. ACFs are expected to be a useful tool for measuring Zn^{II} concentrations in the living cells.

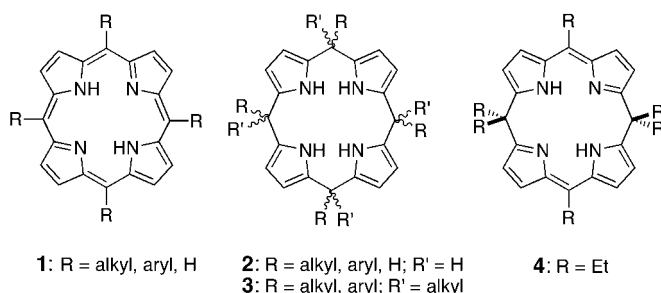
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Calixphyrins: Novel Macrocycles at the Intersection between Porphyrins and Calixpyrroles**

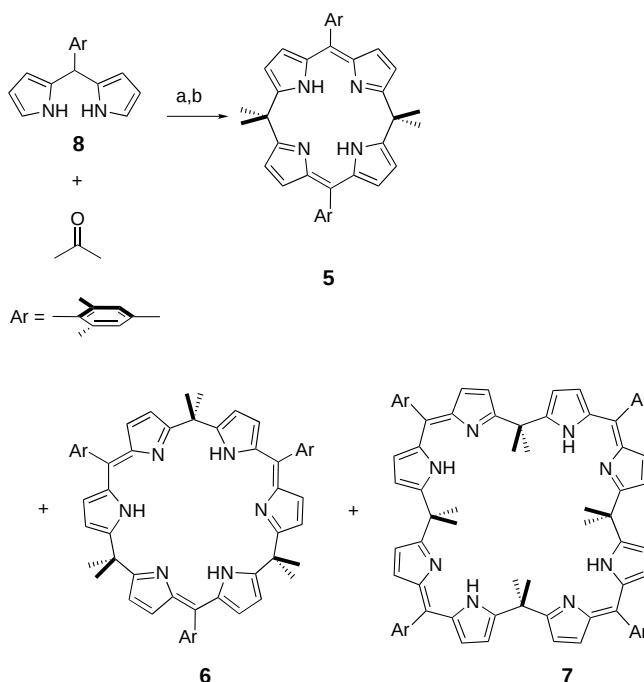
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A salient feature of porphyrins (**1**) is full conjugation throughout the macrocyclic skeleton, a property that contributes inter alia to their stability and special aromatic nature.^[1] Such conjugation is absent in porphyrinogens (**2**),^[2] the tetrapyrrolic precursors from which porphyrins are generally obtained through a net six-electron oxidation process, and in



the *meso*-octaalkylated porphyrinogen analogues, the calix[4]pyrroles (**3**).^[3, 4] In the case of the latter, the alkyl groups enforce sp³ hybridization at the linking *meso*-like positions and generally prevent oxidation to the corresponding porphyrins or other intermediate conjugated products. Recently, however, Floriani and co-workers have shown that, through an unusual sequence of organometallic rearrangements, the partial (formal four-electron) oxidation of calix[4]pyrroles could be achieved to produce, among other products, hexaalkylporphodimethenes (**4**).^[5] Porphodimethenes^[2, 6] are fascinating molecules that lie at the interface between porphyrin and calixpyrrole chemistry. Unfortunately, in spite of Floriani's recent work, these kinds of materials are still not easy to prepare.^[2, 6] Herein, we report a simple, rational

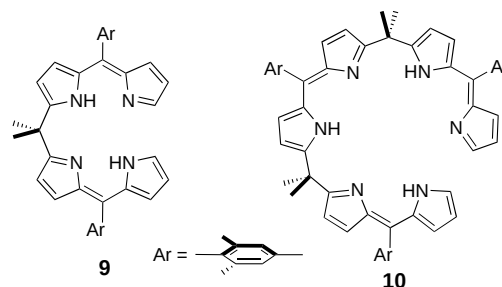
synthesis of diaryl tetraalkyl porphodimethenes (**5**; Scheme 1), as well as two higher-order homologues, **6** and **7**, that are to the best of our knowledge without precedent in the literature. These molecules, which contain both dialkyl-substituted sp³-hybridized bridging carbon atoms and *meso*-like dipyrrolylmethene subunits, bear analogy to both the



Scheme 1. Preparation of calix[4]pyrhyrin (**5**) and its higher homologues **6** and **7**. Reagents: a) acid catalyst (TFA, MSA, *p*-TSA, HCl, HBr, or PPA); b) DDQ oxidation.

calix[*n*]pyrrole^[3, 4, 7] and the porphyrin/expanded porphyrin series of macrocycles.^[1, 8] To underscore this analogy, we propose that compounds **5–7** be referred to as calix[4]-, calix[6]-, and calix[8]pyrhyrins, respectively, and that the term calix[*n*]pyrhyrin be used in a generic sense to describe this kind of hybrid macrocycle.

The synthesis of **5–7** is summarized in Scheme 1. Briefly, these compounds are prepared in good yields by the acid-catalysed coupling of mesityldipyrrolylmethane (**8**)^[9] with acetone at room temperature, followed by 4,5-dichloro-3,6-dioxo-1,2-benzenedinitrile (DDQ) oxidation.^[10] In some cases small amounts of the acyclic dimer **9** and trimer **10** were also formed. The reaction proceeds smoothly in a range of solvents and in the presence of various Brønsted acids, including trifluoroacetic acid (TFA), methane sulfonic acid (MSA),



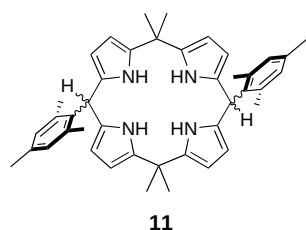
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4-methylphenylsulfonic acid (*p*-TSA), HCl, HBr, and polyphosphoric acid (PPA).^[11] However, the total and relative yields were strongly dependent on the specific conditions employed. For instance, reaction conditions of acetone as solvent, trifluoroacetic acid as catalyst, and 24 h mixing prior to oxidation proved optimal to produce all three calixphyrins; under these conditions calixphyrins **5–7** were obtained in 44 %, 23 %, and 9 % yield, respectively, along with the acyclic dimer **9** (2 %) and acyclic trimer **10** (1 %). In contrast, when pure dichloromethane as the solvent and trifluoroacetic acid as the catalyst were employed, the calix[4]phyrin **5** was obtained nearly exclusively.

Although unstable, the porphyrinogen-like intermediates that lead to **5–7** could be characterized readily using high-resolution mass spectrometry (HR-MS). For the case of **5**, the presumed porphyrinogenlike precursor **11** was isolated under



an inert atmosphere and subjected to ¹H and ¹³C NMR analysis. With this critical precursor **11** identified, the course of the subsequent four-electron oxidation step was elucidated. In particular, upon conversion from **11** to **5**, the NH signal shifted significantly (from $\delta = 7.54$ to 13.70) in the ¹H NMR spectrum, while the mesityl-bearing *meso*-carbon signal shifted from $\delta = 30$ to 164 in the ¹³C NMR spectrum. Dramatic color changes were also seen. Whereas calixphyrins **5–7** are all orange to red-yellow and have maximal UV/Vis absorbances at 418, 438, and 404 nm, respectively, the mixture of porphyrinogen-like precursors was colorless.

Compounds **5–7** were readily separated from each other and from acyclic and other impurities by column chromatography. They were fully characterized by HR-MS, UV/Vis, and NMR (¹H and ¹³C) spectroscopy, combustion analysis, and, in the case of **5** and **7**, single crystal X-ray diffraction analysis.^[12]

The solid state structures of **5** and **7** are shown in Figures 1 and 2, respectively. Inspection reveals that neither system is planar. Indeed, both have marked kinks enforced by their dialkyl-substituted sp³-hybridized bridging elements. However, both systems also display regions of local planarity corresponding to the dipyrrolylmethene links. Thus, as their names imply, they are hybrid molecules that resemble both the aromatic porphyrins and their nonplanar, formally six-electron reduced calixpyrrole analogues. Interestingly, the metal-free form of **5** is far more bent and less planar than the bis-lithium complex of its analogue **4** prepared by Floriani.^[5] In this latter case, metal coordination presumably enforces a high degree of local and long-range planarity with the result that surprisingly small pyrrole-methylene-pyrrole dihedral angles of 12.5° are observed.

While only a single conformer is observed in the X-ray structures of **5** and **7**, molecular modeling suggested that **5** is

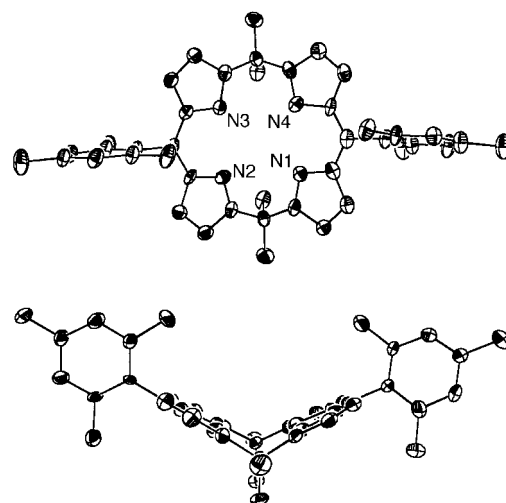


Figure 1. Top and side views of the solid-state structure of **5**. The molecule adopts a winglike conformation, in which the pyrrole-methylene-pyrrole dihedral angles are 55.4°. Thermal ellipsoids are at the 30 % probability level.

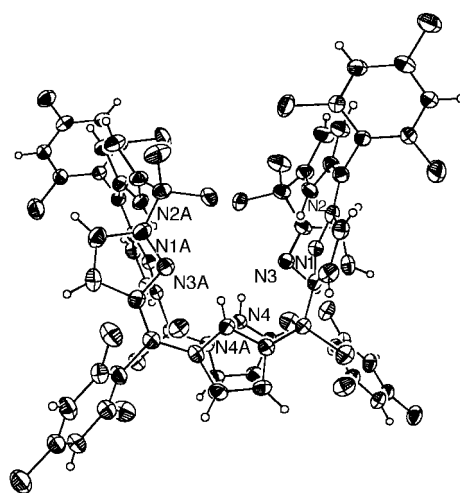


Figure 2. View of the solid-state structure of **7**. The molecule adopts a partially closed bowl-like conformation, wherein each of the pyrrole groups are oriented in different directions to its neighbors. Thermal ellipsoids are at the 30 % probability level.

likely to be relatively rigid, whereas the higher homologues **6** and **7** might be fluxional in solution. The NMR spectra of **5–7** recorded in [D₁]chloroform (see Supporting Information) supported this conclusion. Whereas sharp peaks are observed in the ¹H NMR spectrum of **5**, broad peaks were seen for the *meso*-CH₃ signals of **6** as well as those ascribed to the 2- and 6-CH₃-aryl protons of this prototypic calix[6]phyrin, with similar trends also observed in the ¹³C NMR spectra. As expected for a fully fluxional molecule, no significant broadening of any signals was seen in either the ¹H or ¹³C NMR spectra of calix[8]phyrin **7** at room temperature. However, at low temperature fully resolved sharp signals were observed for both this calixphyrin and its smaller congener **6**.

These hybrid calixpyrrole-porphyrin macrocycles are prototypical of what might be a large class of partially oxidized, mixed condensation products.^[13, 14] On account of the rich pyrrolic functionality, they show promise as receptors of

exceptional utility. While further study is needed to detail the substrate-binding properties of **5–7**, preliminary work confirmed, as expected in light of Floriani's prior studies,^[5] that **5** forms complexes with Cu^{II} and Zn^{II}, while its higher homologue **6** coordinates trivalent Co^{III} and Ru^{III} ions. All three calixpyrins bind anions, as judged from changes in the UV/Vis spectra when dichloromethane solutions of **5–7** are treated with tetrabutylammonium salts of various anions. Further, peaks corresponding to the proposed calixpyrin–anion complexes are observed by mass spectrometric analysis.

In conclusion, the first rational syntheses of hybrid calixpyrrole–porphyrin macrocycles, porphodimethene (calix[4]pyrin) and its homologues, calix[6]pyrin and calix[8]pyrin, are reported. Taken together with binding studies, these findings suggest that the calixpyrins could provide a useful complement to the calixpyrrole,^[3, 4, 7] porphyrin,^[1] and expanded porphyrin^[8] systems that are known for their rich cation-coordination and anion-binding chemistries.

Experimental Section

A solution of dipyrromethane **8** (0.1 mmol)^[9] in dry solvent (acetone, dichloromethane, ethanol, or acetonitrile; 20 mL) and acetone (10–100 molar excess), was degassed by bubbling with argon for 10 min. Catalytic acid (usually TFA (0.03 mL) but also MSA, *p*-TSA, HCl, HBr, PPA) was added. The reaction mixture was stirred under argon for 16–48 h and followed by removing samples for HPLC analysis. Because of the presumed instability of the porphyrinogen-like intermediates, they were not generally isolated. However, for spectroscopic identification, the reaction mixtures were dried in vacuo, redissolved in deoxygenated dichloromethane/hexane (1/4) and filtered through degassed silica gel, all under argon, before being evaporated to dryness again. Compound **11** (the precursor to **5**) was obtained in relatively pure form in this way. For the oxidation procedure, DDQ was added to the initial reaction flask when dichloromethane was used as the solvent. For other solvents, the reaction mixture was evaporated to dryness and redissolved in dichloromethane before addition of DDQ. The product was purified by column chromatography (packing: silica gel; eluent: hexane, with dichloromethane (0–50 %) gradient). Single crystals of **5** and **7** were grown from dichloromethane/methanol. Metal complexes were prepared from acetate or acetylacetonate salts of the metal cation in question using either chloroform or a chloroform/methanol mixture as the solvent.^[15]

5: ¹H NMR (500 MHz, CDCl₃, 25 °C): δ = 1.87 (s, 12 H, *meso*-CH₃), 2.09 (s, 12 H, 2,6-(CH₃)₂C₆H₂), 2.32 (s, 6 H, 4-(CH₃)C₆H₂), 6.11 (d, ³J(H,H) = 4 Hz, 4 H, β-pyrrole CH), 6.17 (d, ³J(H,H) = 4 Hz, 4 H, β-pyrrole CH), 6.86 (s, 4 H, C₆H₂), 13.70 (br s, 2 H, NH); ¹³C NMR (125 MHz, CDCl₃, 25 °C): δ = 20.11, 21.07, 29.44, 38.15, 76.74, 77.25, 114.06, 126.49, 127.56, 133.53, 136.99, 137.03, 139.04, 140.21, 164.62; UV/Vis (CH₂Cl₂): λ_{max} (ε) = 302 (15 726), 418 (72 037); HR-MS(CI) for C₄₂H₄₃N₄ ([M + H]⁺): calcd: 605.3644; found: 605.3648; elemental analysis for C₄₂H₄₄N₄ (604.36): calcd: C 83.40, H 7.33, N 9.26; found: C 83.31, H 7.35, N 9.23.

Copper(II) complex of **5**: UV/Vis (CH₂Cl₂): λ_{max} 478 nm; HR-MS(CI) for C₄₂H₄₃N₄Cu ([M + H]⁺): calcd: 666.278872; found: 666.278880; elemental analysis for C₄₂H₄₂N₄Cu (665.28): calcd: C 75.70, H 6.53, N 8.41; found: C 75.41, H 6.27, N 8.22.

Zinc(II) complex of **5**: UV/Vis (CH₂Cl₂): λ_{max} 452 nm; HR-MS(CI) for C₄₂H₄₃N₄Zn ([M + H]⁺): calcd: 667.277918; found: 667.278392; elemental analysis for C₄₂H₄₂N₄Zn (666.28): calcd: C 75.49, H 6.34, N 8.38; found: C 75.12, H 6.22, N 8.18.

6: ¹H NMR (500 MHz, CDCl₃, 25 °C): δ = 1.75 (m, 18 H, *meso*-CH₃), 2.10 (s, 18 H, 2,6-(CH₃)₂C₆H₂), 2.30 (s, 9 H, 4-(CH₃)C₆H₂), 6.26 (d, 12 H, β-pyrrole CH), 6.85 (s, 6 H, C₆H₂), 13.22 (br s, 3 H, NH); ¹³C NMR (125 MHz, CDCl₃, 25 °C): δ = 14.11, 20.32, 20.70, 21.09, 22.65, 25.28, 27.81, 29.70, 31.58, 34.52, 34.67, 39.22, 76.75, 77.26, 115.33, 127.12, 127.61, 133.33, 137.05, 138.36, 161.88; UV/Vis (CH₂Cl₂): λ_{max} (ε) = 274 (7470), 438 (11 229), 490 (1243); HR-MS(CI) for C₆₃H₆₇N₆ ([M + H]⁺): calcd:

907.542722; found: 907.543616; elemental analysis for C₆₃H₆₆N₆ (906.54): calcd: C 83.40, H 7.33, N 9.26; found: C 83.25, H 7.28, N 9.31.

Cobalt(III) complex of **6**: HR-MS(CI) for C₆₃H₆₄N₆Co ([M + H]⁺): calcd: 963.452444; found: 963.453567; elemental analysis for C₆₃H₆₃N₆Co (962.45): calcd: C 78.56, H 6.59, N 8.73; found: C 78.37, H 6.39, N 8.59.

Ruthenium(III) complex of **6**: HR-MS(CI) for C₆₃H₆₄N₆Ru ([M + H]⁺): calcd: 1006.423594; found: 1006.420271; elemental analysis for C₆₃H₆₃N₆Ru (1005.42): calcd: C 75.27, H 6.32, N 8.36; found: C 75.06, H 6.21, N 8.18.

7: ¹H NMR (500 MHz, CDCl₃, 25 °C): δ = 1.52 (m, 24 H, *meso*-CH₃), 2.12 (m, 24 H, 2,6-(CH₃)₂C₆H₂), 2.31 (m, 12 H, 4-(CH₃)C₆H₂), 6.12 (d, ³J(H,H) = 4 Hz, 4 H, β-pyrrole CH), 6.16 (d, ³J(H,H) = 4, 4 H, β-pyrrole CH), 6.21 (m, 4 H, β-pyrrole CH), 6.22 (m, 4 H, β-pyrrole CH), 6.86 (m, 8 H, C₆H₂), 13.22 (br s, 4 H, NH); ¹³C NMR (125 MHz, CDCl₃, 25 °C): δ = 1.01, 20.13, 20.47, 21.09, 27.58, 29.44, 29.70, 38.85, 76.74, 77.25, 77.56, 127.61, 133.50, 137.07, 138.05; UV/Vis (CH₂Cl₂): λ_{max} (ε) = 404 (3345), 428 (3080), 448 (2900); HR-MS(CI) for C₈₄H₈₉N₈ ([M + H]⁺): calcd: 1209.721021; found: 1209.720281; elemental analysis for C₈₄H₈₈N₈ (1208.72): calcd: C 83.40, H 7.33, N 9.26; found: C 83.14, H 7.18, N 9.14.

9: ¹H NMR (500 MHz, CDCl₃, 25 °C): δ = 1.81 (m, 6 H, *meso*-CH₃), 2.09 (m, 12 H, 2,6-(CH₃)₂C₆H₂), 2.33 (m, 6 H, 4-(CH₃)C₆H₂), 6.09 (m, 2 H, β-pyrrole CH), 6.15 (m, 2 H, β-pyrrole CH), 6.42 (d, ³J(H,H) = 4.5 Hz, 2 H, β-pyrrole CH), 6.51 (d, ³J(H,H) = 4.5 Hz, 2 H, β-pyrrole CH), 6.89 (m, 4 H, C₆H₂), 7.12 (m, 2 H, α-pyrrole CH), 12.5 (br s, 2 H, NH); HR-MS(CI) for C₃₉H₄₀N₄ ([M + H]⁺): calcd: 565.3331; found: 565.3337.

10: ¹H NMR (500 MHz, CDCl₃, 25 °C): δ = 1.71 (12 H, *meso*-CH₃), 2.12 (m, 18 H, 2,6-(CH₃)₂C₆H₂), 2.18 (s, 9 H, 4-(CH₃)C₆H₂), 6.04 (m, 3 H, β-pyrrole CH), 6.18 (m, 3 H, β-pyrrole CH), 6.33 (m, 3 H, β-pyrrole CH), 6.46 (m, 3 H, β-pyrrole CH), 6.86 (m, 6 H, C₆H₂), 7.12 (m, 2 H, α-pyrrole CH), 12.30 (br s, 3 H, NH). HR-MS(CI) for C₆₀H₆₂N₆ ([M + H]⁺): calcd: 867.5114; found: 867.5125.

11: ¹H NMR (500 MHz, CDCl₃, 25 °C): δ = 1.58–1.62 (m, 12 H, *meso*-CH₃), 1.96–2.01 (m, 12 H, 2,6-(CH₃)₂C₆H₂ broadened due to rotation), 2.25 (s, 6 H, 4-(CH₃)C₆H₂), 5.67 (s, 2 H, *meso*-CH), 5.97 (m, 8 H, β-pyrrole CH), 6.78 (s, 4 H, C₆H₂), 7.54 (br s, 4 H, NH); ¹³C NMR (125 MHz, CDCl₃, 25 °C): δ = 20.51, 20.75, 38.30, 76.75, 77.26, 106.48, 108.63, 116.11, 130.31, 131.22, 134.52, 136.55, 137.57; HR-MS(CI) for C₄₂H₄₈N₄: calcd: 608.3879; found: 608.3879.

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- [10] An alternative approach to preparing porphodimethenes and related systems, involving the mixed condensation of an aldehyde, ketone, and pyrrole, was also tested. As expected, such a reaction generates a statistical mixture of possible products.
- [11] Lewis acids were found to be far less efficacious as catalysts to produce only trace quantities of products.
- [12] Crystal structure analyses were measured on a Nonius Kappa CCD using graphite-monochromatized MoK α radiation ($\lambda = 0.71073$ Å) at -120°C . **5**: C₄₂H₄₄N₄. Orange plates and lathes grown from CH₂Cl₂, monoclinic, space group C2/c, $Z = 12$, $a = 26.618(3)$, $b = 13.546(2)$, $c = 29.934(3)$ Å, $\beta = 104.765(6)^\circ$, $V = 10437(2)$ Å³, $\rho_{\text{calc}} = 1.16$ g cm⁻³, $F(000) = 3888$. A total of 15391 reflections were measured, 9097 unique ($R_{\text{int}} = 0.11$). The structure was refined on F^2 to $R_w = 0.218$, $R = 0.145$ (3471 reflections with $F_o > 4\sigma(F_o)$), and GoF = 2.02 for 623 refined parameters. There are two crystallographically independent molecules per asymmetric unit. One molecule lies around a crystallographic two-fold rotation axis. **7**: C₈₄H₈₈N₈·CH₂Cl₂. Orange lathes and needles grown from CH₂Cl₂, orthorhombic, space group Pccn, $Z = 4$, $a = 26.183(2)$, $b = 11.1017(8)$, $c = 25.1343(13)$ Å, $V = 7305.9(9)$ Å³, $\rho_{\text{calc}} = 1.18$ g cm⁻³, $F(000) = 2760$. A total of 10948 reflections were measured, 5795 unique ($R_{\text{int}} = 0.10$). The structure was refined on F^2 to $R_w = 0.182$, $R = 0.098$ (2372 reflections with $F_o > 4\sigma(F_o)$), and GoF = 1.34 for 416 refined parameters. The tetrameric calixpyrrole lies around a crystallographic two-fold rotation axis. 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-134665 and 134666. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [13] A reaction analogous to that of Scheme 1 but employing a bipyrrrole, rather than dipyrromethane derivative, produced, inter alia, a cyclotrimer and a cyclotetramer under a variety of reaction conditions. These compounds will be discussed in a subsequent report.
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Synthesis of Geometrically Well Defined, Molecularly Thin Polymer Films**

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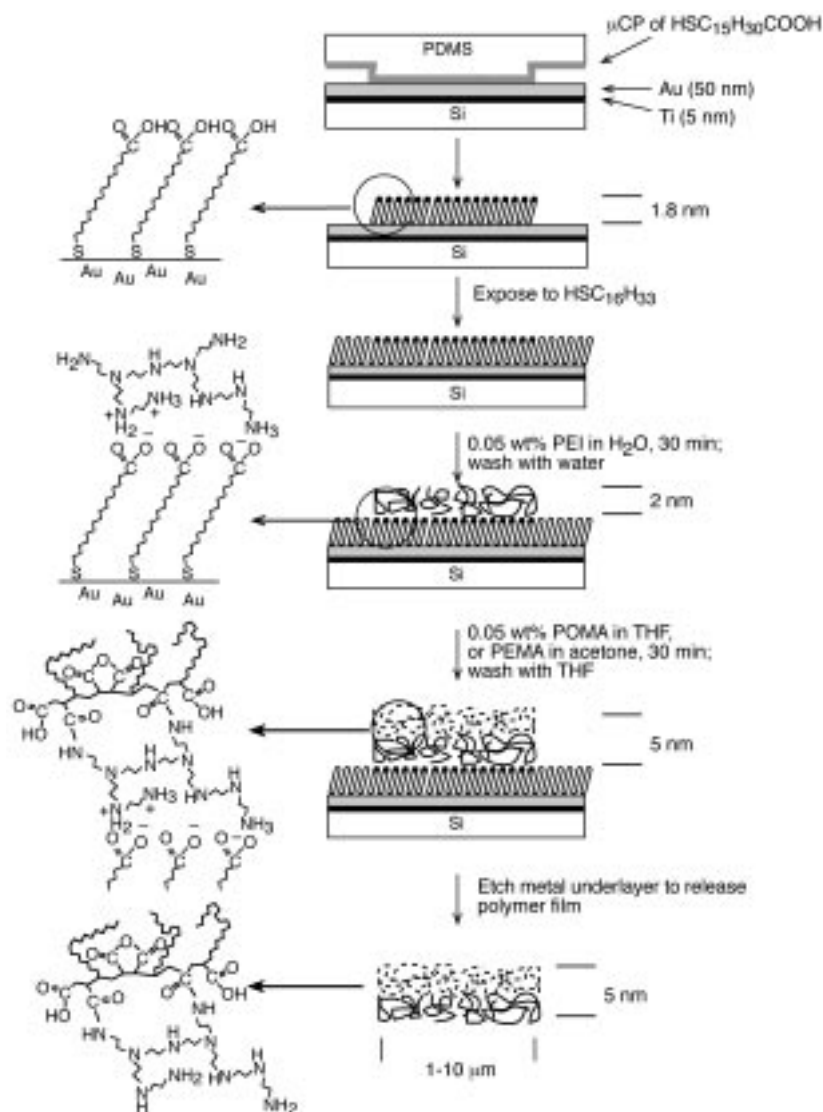
Here we demonstrate a general method for the fabrication of molecularly thin polymer films with well-defined (in-plane) dimensions. The strategy for the synthesis of these materials starts with a sequence of reactions on a patterned self-assembled monolayer (SAM). This pattern, defined by microcontact printing, determines the size and shape of the polymer films on the micrometer scale. The out-of-plane thickness and composition is defined on a nanometer scale by the sequence of chemical reactions.

These molecularly thin polymer films with in-plane cross-linking are a subset of the class of “two-dimensional polymers”.^[1] Synthetic routes to two-dimensional polymers include polymerization of reactive monomers in SAMs^[2] and lipid bilayers,^[3] at oil–water^[4] and air–water interfaces,^[5] and polymerization of monomers that form self-assembled layered structures.^[6] Although certain of these approaches have generated two-dimensional polymeric structures, they have not controlled their lateral (in-plane) dimensions. Our strategy for the fabrication of two-dimensional polymers relies on the work of Grainger et al.,^[7] Crooks et al.,^[8] and others,^[9] who examined covalently bound polymer mono- and multilayers on surfaces. Rubner et al.^[10] and Decher^[11] also examined structures made up of electrostatically associated layers of polymers. Möhwald et al. described covalently linked hollow shell multilayers^[12] and Hammond et al. studied electrostatically deposited multilayers on patterned SAMs.^[13] We do not know of any attempts to release these polymeric multilayers into suspension following assembly on a surface.

We began the fabrication of the two-dimensional polymers by using microcontact printing (μCP)^[14] to pattern a SAM; this patterned SAM served as a template for the growth of the polymer films (Scheme 1). The synthesis involves four steps: 1) patterning a gold surface by μCP with COOH- and CH₃-terminated SAMs;^[15] 2) deposition of an amine-containing polymer (polyethyleneimine, PEI) by ionic adsorption onto the CO₂⁻-terminated areas;^[16] 3) cross-linking the adsorbed PEI covalently by allowing it to react with a polymeric anhydride (poly(octadecene-*alt*-maleic anhydride), POMA, or poly(ethylene-*alt*-maleic anhydride), PEMA);^[17] and 4) releasing the film from the surface by dissolving the gold substrate completely in 5 mM sodium dodecylsulfate (SDS)/30% aqua regia/0.5% HF in water.^[18] We examined polymeric films patterned into hexagons with 10- μm sides and 10-

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Scheme 1. Schematic outline of the process used to obtain patterned thin polymer films. Fluorescence-labeled polymer films were synthesized by treating fluoresceinamine with the bilayers (PEI/POMA) prior to release from the surface. Printing was achieved with a poly(dimethylsiloxane) (PDMS) stamp.

μm central holes, in sheets with $10 \times 10 \mu\text{m}$ square holes, and in $20 \mu\text{m}$ wide lines. To aid in characterizing the polymer films we coupled fluoresceinamine to the PEI/POMA bilayer.^[19] Polymer films capped with this fluorophore showed green fluorescence.

Ellipsometry on homogeneous, unpatterned films of polymer prepared by the procedure of Scheme 1 (excluding microcontact printing and final release) gave a thickness of $1.5\text{--}2.0 \text{ nm}$ for the film formed by reaction with PEI, and $4\text{--}5 \text{ nm}$ for the bilayer formed by reaction with POMA.^[20]

The thickness of the polymer bilayers, as determined by atomic force microscopy (AFM), is approximately 5 nm .^[20] The layers appeared to be flat and continuous, that is, without holes or defects at the scale we could observe ($\approx 150 \text{ nm}$). The PEI is highly branched, and after covalent cross-linking by POMA or PEMA, the resulting two-dimensional polymer is a tightly interwoven network.

We released the thin films from the support by dissolving the metal underlayer in a mixture of aqua regia, HF, and SDS in water. We believe that the etching of the metal underlayer does not significantly damage the films, because the polymers did not dissolve or change appearance after prolonged exposure to the etchant. We added SDS to the etching solution because it seems to provide extra stability to the polymer films in suspension, perhaps by forming a mono- or bilayer on the top and bottom. This SDS layer could also aid the suspension of the polymer sheets. We were not able to release the sheets under mild conditions (for example, aqueous solutions with high ionic strengths). We speculate that the size of the sheets, the cooperative effect of a large number of electrostatic interactions between the polymers and the surface, the insolubility of the polymer sheets, spontaneous amide formation between the SAM and the polymer layer, and other unknown pinning points might contribute to the strong adhesion of the polymer sheets to the surface.

For characterization, the released polymeric structures were deposited on a surface and examined by AFM and optical microscopy. X-ray photoelectron spectroscopy (XPS) on redeposited thin films showed only traces of Ti and Au; this observation is compatible with the idea that the structures we observe are free polymers, unsupported by metallic films. We estimate that the molecular weight of a 5 nm thick film is around $3 \times 10^6 \text{ Da } \mu\text{m}^{-2}$.^[21]

We released hexagonal sheets with $10\text{--}20 \mu\text{m}$ sides and central holes $10 \mu\text{m}$ in diameter. Figure 1a shows an optical image of these hexagons after they had been transferred to a glass slide. The films have a clearly recognizable hexagonal shape. Some narrow creases are visible; the center sheet was apparently folded and deformed, perhaps during deposition on the surface. The sheetlike structures in Figure 1b remained mostly flat and extended in suspension. The original $10 \times 10 \mu\text{m}$ square holes in the sheet are deformed but recognizable. The fiberlike structure on the left of the image is probably a rolled-up sheet. The square pattern in the background is the image left from the patterned surface. Figure 1c shows an AFM image of a sheet with $10 \times 10 \mu\text{m}$ square holes that was released and redeposited without adding SDS to the etching solution. We performed this experiment to measure the thickness of the polymer films with AFM. The AFM measurements gave a thickness for the polymer layer of approximately 10 nm . This value is in satisfactory agreement with the thickness of 5 nm estimated before release from the surface. The difference might be due to solvation by a thin

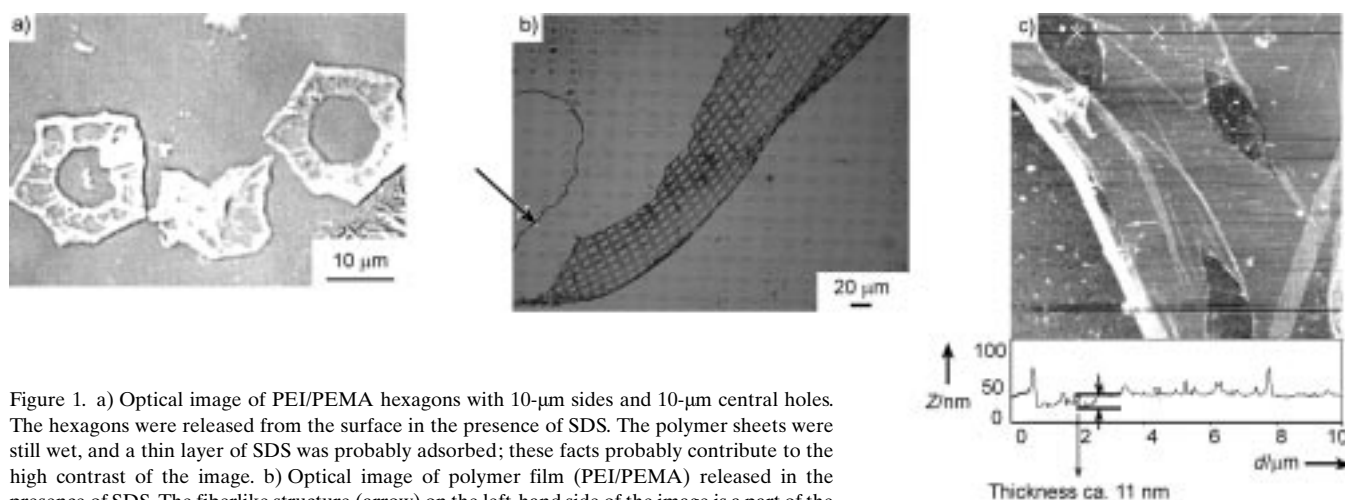


Figure 1. a) Optical image of PEI/PEMA hexagons with 10- μm sides and 10- μm central holes. The hexagons were released from the surface in the presence of SDS. The polymer sheets were still wet, and a thin layer of SDS was probably adsorbed; these facts probably contribute to the high contrast of the image. b) Optical image of polymer film (PEI/PEMA) released in the presence of SDS. The fiberlike structure (arrow) on the left-hand side of the image is a part of the sheet that crumpled. The faded pattern in the background is a remainder of the patterning of the surface by μCP . c) AFM image of a two-dimensional sheet of polymer film (PEI/POMA) with (deformed) $10 \times 10 \mu\text{m}^2$ holes.

layer of adsorbed water, reorganization of the polymers in the film on release from the surface, or cumulative uncertainties in the measurements.

The influence of SDS on the stability of the released films became clearer in a series of experiments (Figures 2a–d) in which we released 20 μm wide lines in the absence of SDS. Figure 2a shows an AFM image of a released polymer film that was deposited onto a surface. The original polymer lines folded after release from the surface. The height profile shows that the thinnest parts of the polymer films are approximately 8–10 nm thick. The profile suggests that the variations in thickness along the line scan correspond to the pleating of layers that are 8–10 nm thick. This thickness is realistic when relief of strain in the film (with lateral shrinking and perpendicular thickening) upon release from the substrate is taken into account. Figure 2b shows an optical image of partially released 20- μm lines. On the right and left, the two-

dimensional polymer lines are still attached to the substrate and remain flat. In the center, the polymers are suspended and folded. Fluorescence images of polymer sheets capped with fluorescein (Figure 2c) confirm that the structures observed under the microscope (Figure 2d) are indeed polymers.

The thin polymer films described here are the first examples of a new class of two-dimensional organic polymers, characterized by well-defined shape and lateral dimensions, by thicknesses of 5–20 nm, and by controllable composition in the direction perpendicular to the plane of the film. The synthesis of the polymers on the surface ensures high dimensional reproducibility (or, in the context of macromolecular science, monodispersity). The molecular weight can be controlled by means of the μCP process, either by the pattern used (for example, by the shape of the polymer) or by the number of layers used in the fabrication of the polymer (for example, PEI/POMA, PEI/POMA/PEI/POMA, etc.). The

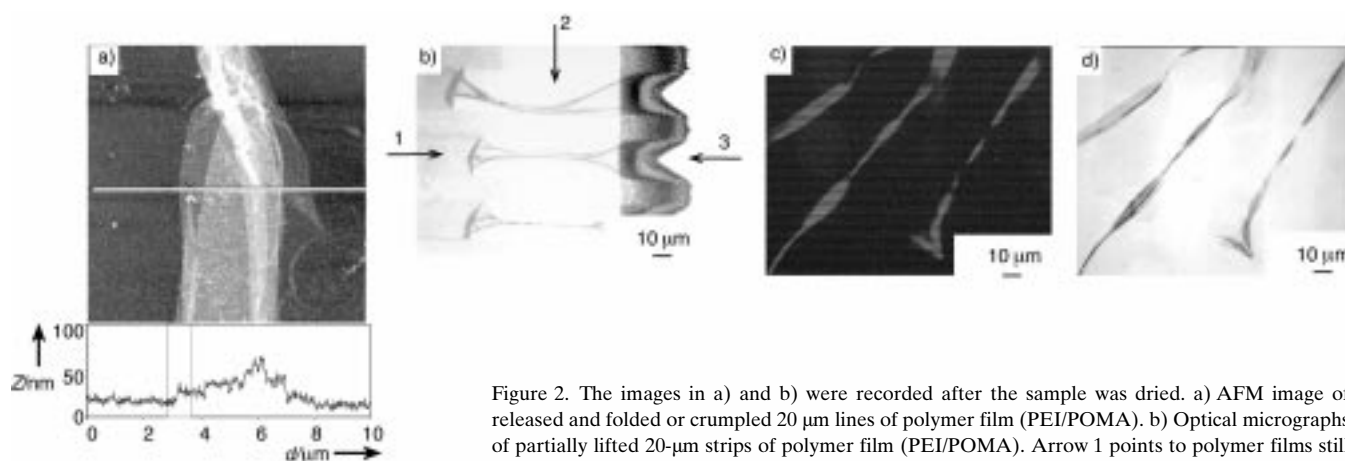


Figure 2. The images in a) and b) were recorded after the sample was dried. a) AFM image of released and folded or crumpled 20 μm lines of polymer film (PEI/POMA). b) Optical micrographs of partially lifted 20- μm strips of polymer film (PEI/POMA). Arrow 1 points to polymer films still attached to the surface; the section indicated by arrow 2 has detached from the support and redeposited on the surface; arrow 3 points to a part of the metal underlayer that was unetched. The images in c) and d) were taken from wet samples. c) Fluorescence image of released 20- μm lines of fluorescence-labeled polymer film (PEI/POMA) floating in water. d) Optical image of the same polymer films as in (c).

composition of the polymer sheets depends on the type of polymers used and the presence or absence of a capping layer. The patterns used here resulted in polymer films that can be classified either as materials or as macromolecules. More familiar systems that combine macroscopic and molecular dimensions are liposomes, SAMs, and Langmuir–Blodgett films.^[22] The smallest patterns that can now be formed by μ CP are approximately $0.01 \mu\text{m}^2$; the two-dimensional polymers derived from these patterns will have $M_r \approx 100$ MDa and would begin to approach the molecular weight of very large soluble polymers such as polyacrylamide (20 MDa)^[23] and λ -phage DNA (32 MDa).^[24] This study represents a first step towards the fabrication (rather than synthesis) of polymers with well-defined nanosize shapes and dimensions. The combination of (nano)lithographic techniques and surface chemistry will allow the fabrication of a wide range of different shapes and chemical functionalities for these macromolecules.

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- [17] The patterned substrate was placed in a 0.05 wt % solution of PEI (branched, $M_r = 750000$) in isopropyl alcohol for 20 min. The surface was briefly (< 30 s) sonicated in water and dried under a stream of nitrogen. This polymer layer was cross-linked by reacting the amine-terminated surface with a 0.05 wt % solution of POMA ($M_r = 30000$) in THF, or PEMA ($M_r = 100000$ – 500000) in acetone. The excess polymer was removed by briefly (< 30 s) sonicating the sample in THF or acetone and washing with CH_2Cl_2 .
- [18] We added 0.5 % HF to remove the underlying film of Ti that was used to promote adhesion of gold to SiO_2 .
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- [21] The volume of a 5-nm thick $1 \mu\text{m}^2$ film is $5 \times 10^{-15} \text{ cm}^3$. The density of PEI is 1.05 g cm^{-3} , and the density of POMA is 0.97 g cm^{-3} . Assuming an average density for the polymer film of 1.01 g cm^{-3} , the molecular weight is $1.01 \times 6.02 \times 10^{23} \times 5 \times 10^{-15} \approx 3 \text{ GDa}$.
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Surface-Enhanced Resonance Raman Scattering as a Novel Method of DNA Discrimination**

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Sensitive and fast analytical methods for the identification of specific DNA sequences and fragments are a prerequisite to exploit the advantages of the new understanding of DNA with the completion of the human genome map. The most frequently used analytical methods employ fluorescence spectroscopy to detect a labeled nucleic-acid probe.^[1, 2] Detection of the fluorophore confirms the presence of a specific base sequence. The main disadvantage of this method for an oligomer mixture is identification of differently labeled sequences due to the inherently broad fluorescent signals. Differentiation is possible by using fluorophores with very different emission profiles^[3] or by time-resolved fluorescence detection instead.^[4] However, these methods require special fluorophores and complex equipment that still cannot easily

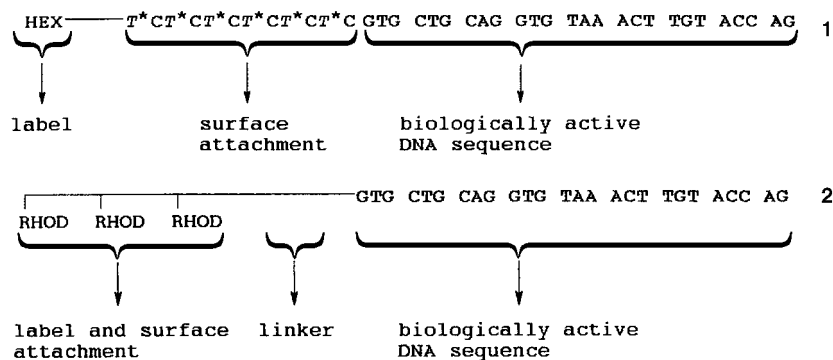
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resolve mixed fluorophores in varying proportions. These factors are key limitations in the development of the fast and discriminatory assays now required. Surface-enhanced resonance Raman scattering (SERRS) has comparable sensitivity to fluorescence but also a unique advantage in that the scattered light consists of sharp, molecule-specific vibrational bands.^[5–10] Thus, it has the potential to enable discrimination of a mixture of probes and to facilitate the development of more powerful DNA analysis. We report the identification of two labeled oligonucleotide probes in a mixture as an illustration of the potential power of SERRS in nucleic acid analysis. We also show how we can use SERRS to analyse varying proportions of the oligonucleotides. Additionally, specially designed oligonucleotides are required to achieve the necessary sensitivity. We report here a new type of oligonucleotide probe design and compare it to the only previous example.

To obtain effective SERRS, an analyte containing a chromophore must adsorb onto a suitably roughened metal surface, usually silver or gold.^[6] The surface reported herein is an aggregated silver colloid, since it gives good sensitivity and has been used previously with DNA.^[11] The aggregation is required to obtain maximum enhancement from the surface plasmons. The oligonucleotides are negatively charged and will not adsorb onto the silver colloid unless additional moieties, allowing adsorption, are attached.^[11] Also, to provide a resonance contribution, a chromophore compatible with the exciting laser must exist. Thus, a positive charge for attachment to the surface and a suitable chromophore are required in the oligonucleotide.

Two 26mer oligonucleotides were synthesized. Oligonucleotide **1** used the fluorophore 2,5,2',4',5',7'-hexachloro-6-carboxyfluorescein (HEX) as the visible chromophore. HEX is negatively charged and does not have a strong affinity for the metal surface. The surface attachment was achieved by



incorporation of six modified nucleobases, 5-(3-aminopropyl)-2'-deoxyuridine (T*), at the 5'-terminus next to the HEX label. Oligonucleotide **2** is labeled with positively charged rhodamine 6G (RHOD) that also adsorbs onto the metal surface. Further, to assist the detection of **2**, three RHOD labels were used. The three RHOD labels were attached at the 5'-terminus through aminoethyl-2'-deoxyribose groups separated from the rest of the oligonucleotide by a hexaethylene glycol spacer.

The two oligonucleotide probes gave efficient Raman scattering from the surface and distinctly different signal patterns (Figure 1). Both labeled oligonucleotides were

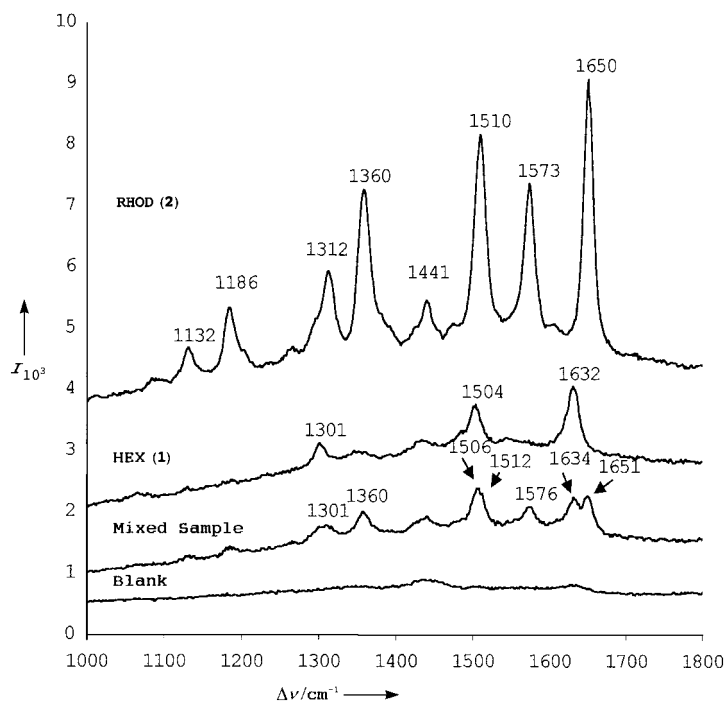


Figure 1. SERRS spectra **1** and **2** separately and mixed in a 5:1 ratio of **1:2**.

present at 8×10^{-13} moles, illustrating the sensitive nature of detection by this method. The spectrum from a 5:1 (**1:2**) mixed sample indicates that the two labels are easily discriminated. Figure 2 illustrates this for different molar ratios, selecting for illustration the quadrant aromatic stretches of both dyes at 1632 cm^{-1} for **1** and 1650 cm^{-1} for **2**. The oligonucleotides produce approximately equal scattering intensity in this region at a ratio of 5:1 (**1:2**).

The figures clearly show that the rhodamine 6G-labeled oligonucleotide **2** gives a better signal-to-noise ratio than the HEX-labeled oligonucleotide **1** by an amount greater than that expected with the use of three RHOD labels as opposed to one HEX. The cross section capture of the two dyes for SERRS is not known, but in part the difference in sensitivity is due to the design of the oligonucleotides. In the case of **1**, the HEX label itself does not adhere to the silver surface but is held in proximity by the adsorption of the nucleotide to the surface. In the case of **2**, the attachment group is on the chromophore and consequently the label adsorbs directly onto the surface. This latter method is likely to provide more effective surface enhancement. Extension products generated by the action of DNA polymerase have also been detected by SERRS. Further investigation into the use of these probes for meaningful biological analysis is currently underway.

This study illustrates the ability of SERRS to discriminate different oligonucleotide probes in mixtures without requiring

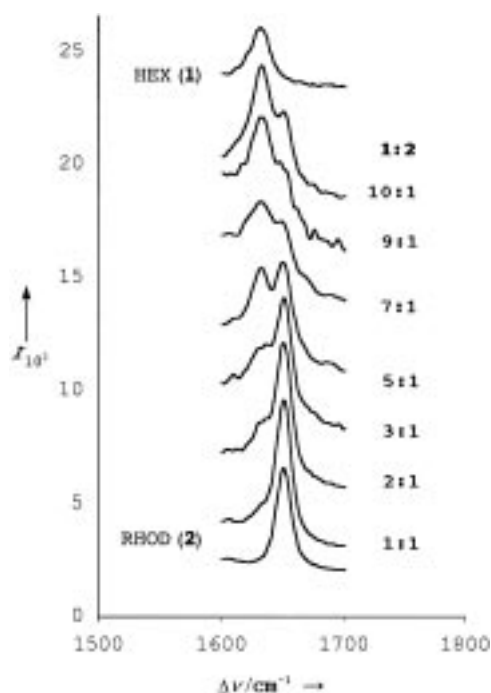


Figure 2. The signal corresponding to the quadrant aromatic stretches of the labeled oligonucleotides, as two single samples and in a mixture.

separation and in varying proportions. In addition, these initial experiments indicate that oligonucleotide probe design is crucial and there is considerable potential for further development particularly as both nonfluorescent and fluorescent chromophores can be used as SERRS labels. The modified oligonucleotide probes can also still be used as substrates for polymerases, therefore demonstrating that they remain biologically active. These results prove that, by considering the chemistry involved, SERRS can feature in the development of more effective DNA assays allowing the development of assay formats not possible by conventional methods.

Experimental Section

A solution of modified oligonucleotide (20 μL , $4 \times 10^{-8} \text{ M}$) was premixed on ice with an aliquot of spermine tetrahydrochloride (20 μL , $8 \times 10^{-2} \text{ M}$). Water (500 μL) and citrate-reduced silver colloid (500 μL) were added to this solution. Analysis used a Renishaw 2000 Raman Microprobe with excitation provided by a Spectra-Physics Model 2020 argon-ion laser (100 mW, $\lambda = 514.5 \text{ nm}$). The detector was a charge-coupled device (CCD). Samples were analysed over 1 s in a plastic micro-titre plate using a X10 objective with the grating centred at 1400 cm^{-1} .

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α -Oxymethyl Ketone Enolates for the Asymmetric Mannich Reaction. From Acetylene and *N*-Alkoxy carbonylimines to β -Amino Acids**

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The reactions of enolizable carbonyl compounds with azomethine functions, usually referred to as Mannich-type reactions (also termed aza-aldol reactions), result in the formation of β -amino acids, ketones, or aldehydes.^[1] These reactions are conceptually equivalent to the aldol reactions, but, in sharp contrast, they have been substantially less developed.^[2, 3, 4] There are two reasons that can justify this situation: firstly, the poorer electrophilicity of the azomethine function relative to that of the carbonyl function, and secondly, the preference of enolizable azomethines to undergo α -deprotonation rather than addition.^[5] To date, there are two main ways to approach these problems. One strategy lies in the use of activated forms of azomethines,^[1, 2] and the other in the use of trialkylsilylenol ethers or *O*-(trialkylsilyl)ketene acetals as nucleophiles.^[3, 6] The later strategy has led to outstanding advances in enantioselective Mannich reactions^[7] catalyzed by chiral ruthenium,^[8a-b] palladium,^[8c-e] and copper^[8e-g] complexes. Some highly diastereoselective methods involving chiral azomethines are also known,^[6, 9] but in most

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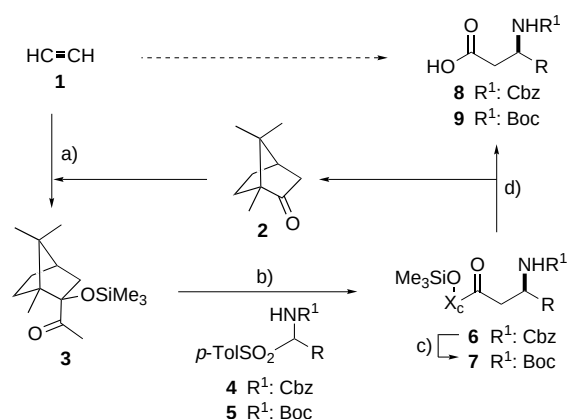
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instances, the chiral auxiliary is often difficult to recover. Despite these achievements, however, there are very few known diastereoselective methods involving chiral enolates,^[1] which are almost paramount in the parent aldol reactions. Most importantly, the stereoselectivity attained with the hitherto known α -unsubstituted chiral enolates is still rather inadequate.^[10, 11] We report here on a new diastereoselective Mannich-type reaction that helps to solve the above problems.

The lithium enolate of the methyl ketone **3**, the latter being readily prepared from acetylene (**1**) and (1*R*)-(+)-camphor (**2**),^[12] reacted, in the presence of an excess of LDA, with the carbamates **4** or **5**^[13, 14] to afford the corresponding adducts **6/7** in good yields^[15] and, in both cases, with diastereomeric ratios greater than 96:4 (Scheme 1). In general carbamates **5** provided the adducts **7** essentially as single diastereomers



Scheme 1. Asymmetric Mannich-type synthesis of β -amino acids. a) ref. [12]; b) LDA (3.5 equiv), THF, -78°C , 1 h then **4** or **5** (2 equiv), THF, -78°C , 15 min; c) H_2 (1 atm), Pd/C, EtOAc, RT, $(\text{Boc})_2\text{O}$; d) $(\text{NH}_4)_2\text{Ce}(\text{NO}_3)_6$ (3 equiv), $\text{CH}_3\text{CN}/\text{H}_2\text{O}$, 0°C , 16 h. Boc = *tert*-butoxycarbonyl, Cbz = benzyloxycarbonyl, LDA = lithium diisopropylamide, Tol = tolyl.

(Table 1), while the carbamates **4** led to slightly lower reaction diastereoselectivities, typically 98:2 for carbamates with linear side chains, and 96:4 for those bearing branched side chains. For stereochemical correlation purposes the adducts **6** were converted into the *N*-Boc derivatives **7** in a one-pot procedure.^[16] The assigned configuration for the adducts was established by a single-crystal X-ray structure analysis of the β -amino ketones **7a** and **7j**.^[17] The configuration for the other adducts was assigned by analogy.

The excellent diastereoselectivity attained with *N*-[1-aryl-sulfonyl]alkyl carbamates derived from either nonenolizable or enolizable aldehydes is of particular interest in that it provides, through oxidative cleavage of the acyloin moiety, β -alkyl- β -amino acids.^[18] For example, the β -amino acid **8a**, the *N*-Cbz β -homovaline **8d**, and the *N*-Boc- β -homophenylglycine **9j** were formed in yields of 81, 86, and 80%, respectively, by prolonged exposure of the adducts **6a**, **6d**, and **7j** to a threefold excess of ammonium cerium nitrate (CAN) in acetonitrile/water. Thus, β -amino acids appropriately protected at the amino function are obtained in an enantiomerically pure form,^[19] along with the recovery of the starting (1*R*)-(+)-camphor.^[20] This result is also particularly important because it makes it economically viable to use the more expensive

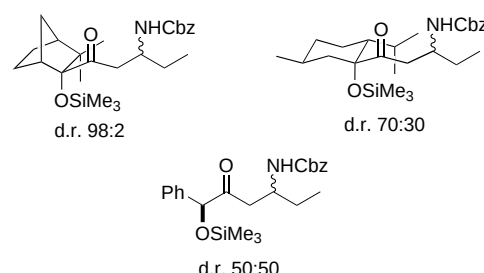
Table 1. Mannich-type synthesis of β -amino ketones **6** and **7**.^[a]

	R	R ¹	Product 6/7		
			d. r. ^[b]	yield[%] ^[c]	m. p. [$^{\circ}\text{C}$]
a	CH_3CH_2	Cbz	98:2	94	98–99
		Boc	> 98:2 ^[d]	71	120
b	$\text{CH}_3(\text{CH}_2)_2$	Cbz	98:2	66	82–84
		Boc	> 99:1	60	61–62
c	$\text{CH}_3(\text{CH}_2)_3$	Cbz	> 98:2 ^[d]	80	104–105
		Boc	> 99:1	60	77–78
d	$(\text{CH}_3)_2\text{CH}$	Cbz	98:2	82	87–88
		Boc	> 99:1	60	92–94
e	PhCH_2CH_2	Cbz	98:2	90	86–89
f	$(\text{CH}_3)_2\text{CHCH}_2$	Cbz	96:4	65	oil
g	$\text{BnOCH}_2\text{CH}_2$	Cbz	97:3	55	103–105
h	$\text{c-C}_6\text{H}_{11}$	Cbz	98:2	81	133–136
i	$\text{c-C}_6\text{H}_{11}\text{CH}_2$	Cbz	96:4	54	105–106
j	Ph	Boc	> 99:1	72	104–105
k	$4\text{-Cl-C}_6\text{H}_4$	Cbz	> 98:2 ^[d]	60	

[a] Reactions carried out on a 4 mmol scale. [b] Diastereomeric ratios determined by HPLC analysis, using a LiChrospher 100 RP-18 column and mixtures of $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ as eluant ($T = 40^{\circ}\text{C}$). [c] Yields of **6** and **7** after purification of the crude product by column chromatography and/or crystallization from *n*-hexane. [d] Determined by ^{13}C NMR spectroscopy.

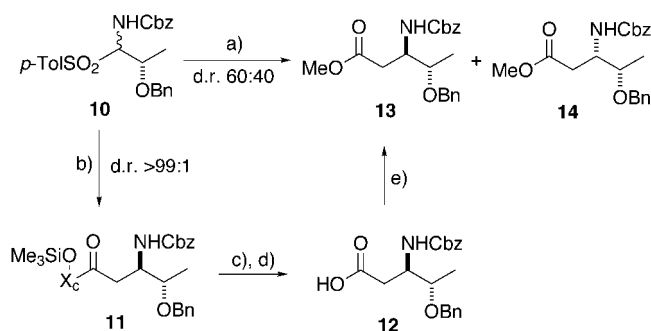
(1*S*)-(-)-camphor isomer, which would lead to the corresponding enantiomeric β -amino acids. Both (*R*)- and (*S*)- β -amino acids are found in natural products^[18b] and are also very interesting as potential building blocks for β -lactam antibiotics^[21] and for β -peptides.^[22]

To gain further insight into the importance of the present enolate model, three representative chiral α -oxymethyl ketones were also examined. Scheme 2 illustrates that of



Scheme 2. Mannich-type adducts from some selected α -silyloxymethyl ketones and carbamate **4a**. The configuration at the newly created stereogenic center was not determined. The diastereomeric ratios were determined by HPLC.

these ketones only that derived from (1*R*)-(-)-camphenilone was comparable in terms of efficiency to **3**. Therefore, owing to the advantageous preparation and low cost of the methyl ketone **3** it should be, from a practical standpoint, the reagent of choice for highly diastereoselective “acetate” aza-aldol reactions. In this respect, the example of double asymmetric induction^[23] depicted in Scheme 3 demonstrates the potential scope of **3**. Carbamate **10**, which shows essentially no diastereofacial selectivity against the lithium enolate of methyl acetate, reacted with the lithium enolate of **3** to produce **11**, essentially as the sole isomer, in 70% yield. This result was also consistently reproducible when mixtures of **10** with different epimeric compositions were employed.^[24] In addition, desilylation of **11** and subsequent oxidative cleavage of the acyloin moiety in the resulting intermediate afforded



Scheme 3. Diastereoselective synthesis of β -homo-*allo*-threonine **12**. a) $\text{CH}_3\text{CO}_2\text{Me}$, LDA (3.5 equiv), THF, -78°C , 1 h then **10**, 15 min; b) **3**, LDA (3.5 equiv), THF, -78°C , 1 h then **10**, 15 min; c) TBAF, THF, RT, 5 min; d) $(\text{NH}_4)_2\text{Ce}(\text{NO}_3)_6$ (3 equiv), $\text{CH}_3\text{CN}-\text{H}_2\text{O}$, 0°C , 1 min; e) $\text{Me}_3\text{-SiCHN}_2$, $\text{MeOH}/\text{C}_6\text{H}_6$. TBAF = tetrabutylammonium fluoride.

the *N*-Cbz-*O*-benzyl- β -homo-*allo*-threonine **12** in 75 % yield. The diastereomeric purity of this compound was established by both ^{13}C NMR and HPLC analyses of the corresponding methyl ester **13**, and comparison with the chromatograms from the mixture of **13/14**. The determination of isomeric ratios, the preparation procedures, and the analytical data for compounds **6a–j**, **7a–b**, **7j**, **8a**, **8d**, **9j**, **11**, and **12** can be found in the Supporting Information.

In summary, an asymmetric Mannich-type reaction that involves the use of acetylene and α -sulfonyl carbamates as the consumable organic materials is described for the first time.

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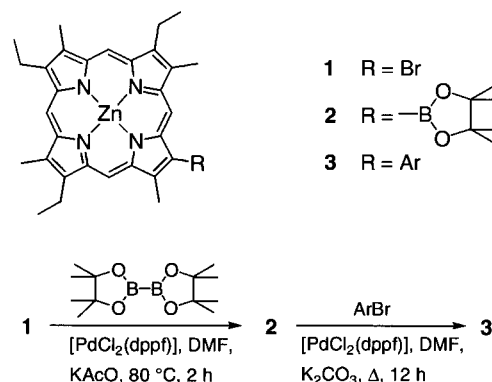
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- [19] The enantiomeric purity of the final β -amino acids was determined by HPLC analyses of their methyl ester derivatives, using a chiralcel OD column and mixtures of *n*-hexane/isopropanol as the eluent. Comparison of these chromatograms with those corresponding to the racemic β -amino acid esters, the latter prepared by the reaction of the lithium enolate of methyl acetate and the corresponding *N*-1[*p*-(tolylsulfonyl)alkyl] carbamate, revealed an *ee* $\geq 99\%$ for the cases studied.
- [20] (+)-Camphor was typically recovered, after filtration of the crude material through a Sep-pak cartridge and evaporation of the solvent, in 75–80 % yield, and showed an optical rotation value of $[\alpha]_D^{25}$ (EtOH, *c* = 1.0) = +41.5 ($[\alpha]_D^{25}$ (EtOH, *c* = 1.0) = +42.2 for the starting camphor, purchased from Aldrich).
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- [24] Apparently, these results indicate that the present Mannich-type reaction proceeds through prior formation of the corresponding *N*-alkoxycarbonylimine.

Facile Synthesis of β -Derivatized Porphyrins—Structural Characterization of a β - β -Bis-Porphyrin**

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Linked porphyrin complexes have been a popular structural motif for studies of electron and energy transfer. The ability to tune the electronic properties of porphyrins by their catenation is central to controlling the efficiencies and rates of these transfers,^[1–6] engendering applications in areas ranging from the design of “molecular wires” and optoelectronic materials to the development of biomimetic models of photosynthetic reaction centers and light-harvesting antenna complexes.^[7–12] Modification of porphyrins is more common at the *meso*-position than at the β -position, which confronts more lengthy syntheses and lower product yields. This is especially true for bis-porphyrins where several new emerging methodologies for direct *meso* coupling^[13–16] contrast a limited set of approaches for the synthesis of β - β linked bis-porphyrins.^[16b, 17] Porphyrin coupling chemistry has changed in recent years with the emergence of metal-catalyzed cross-coupling methods, which have been employed to link bis-porphyrins via phenyl and ethynyl spacers at *meso*-*meso*-, *meso*- β - and β - β -positions.^[7, 8] The direct coupling of porphyrins by such methods, however, has not yet been achieved. We now show the utility of metal-mediated cross-coupling for the general synthesis of β -derivatized porphyrins, including the preparation and first structural characterization of a bis-porphyrin unit directly linked at the β -position.

Scheme 1 shows the strategy developed for the direct β - β coupling of the porphyrin rings. Air- and water-stable **2** was isolated in 76% yield by applying the method of Miyaura et al.^[18] to couple the pinacol diboronate with bromoporphyrin **1**.^[19] Porphyrin **2** can also be prepared according to Masuda's method where the pinacol borane is the transmetalating reagent.^[20, 21] The same boronate has recently been applied to synthesize porphyrins derivatized at the *meso*-position^[22] in yields similar to that reported here for β -derivatization. Cross-coupling **1** and **2** under typical Suzuki reaction conditions affords the previously reported bis-porphyrin **3a**,^[17, 23] but prepared here more conveniently and in higher yield (62%).



a: Ar = (7,12,17-triethyl-3,8,13,18-tetramethylporphyrinato)zinc(II); **b:** Ar = Ph; **c:** Ar = *p*-CH₃Ph; **d:** Ar = 9-Anthryl; **e:** Ar = *p*-OHCPPh
Scheme 1. Synthesis of **3a–e**.

The crystal structure of the porphyrin dimer **3a**^[24] (Figure 1) is unique inasmuch as no porphyrin system directly linked by a carbon–carbon bond at the β -position of the rings has been heretofore structurally characterized. Connection

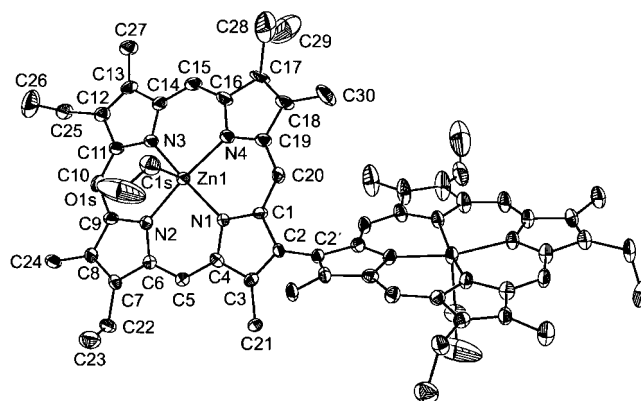


Figure 1. ORTEP representation of **3a** with hydrogen atoms omitted for clarity. Thermal ellipsoids are drawn at the 30% probability level.

at the less sterically encumbered β -position results in a dihedral angle (51.7°) (defined by four N_{pyrrolyl} atoms of each porphyrin) between the two porphyrin macrocycles that is considerably smaller than that observed between the phenyl and porphyrin rings in tetraphenylporphyrin (TPP),^[25] other crystallographically characterized TPP type compounds^[26, 27] and, most pertinently, than two porphyrins directly linked by a C–C bond at their *meso*-positions (dihedral angle of 65 to 84°).^[14] The unique bridging C2–C2' bond (1.46 Å) is also shorter than the C–C bond of the *meso*-*meso* coupled bis-porphyrin (*d* = 1.51 Å) and the sp³–sp³ distance of a recently reported β - β linked bis-chlorin (*d* = 1.61 Å), which is typical of a single bond.^[28] These bond length comparisons suggest greater electronic conjugation between two porphyrin macrocycles when linked at their β -positions.

Figure 2a compares the electronic absorption spectra of the monomeric subunit (zinc(II) etio-I porphyrin) and **3a**. The

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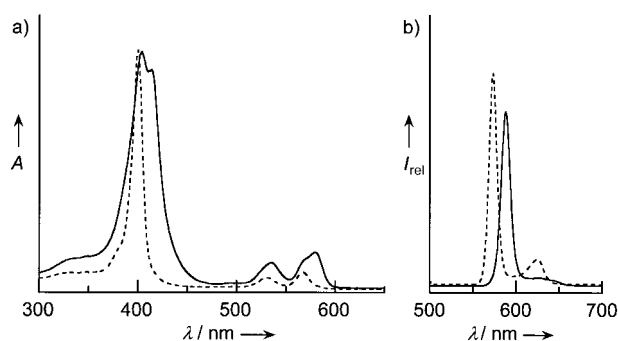


Figure 2. a) Absorption and b) emission spectra of **3a** (—) and zinc(II) etio-I porphyrin (monomer) (---) in CH_2Cl_2 at 22°C .

Soret (B) and Q band absorptions are clearly perturbed upon β -catenation of the monomer subunits. The B band maximum of **3a** is red-shifted relative to that of the monomer. A concomitant broadening and splitting of the B band is indicative of exciton splitting within the bis-porphyrin superstructure.^[29] A similar splitting is also reflected in the Q band absorptions to lower energy. The difference in energy (ΔE) of 539 cm^{-1} between the two maxima of the split B band (and an analogous $\Delta E = 395\text{ cm}^{-1}$ for the split Q band) is smaller than that of bis-porphyrins connected by a C–C bond at the *meso*– β - ($\Delta E = 1265\text{ cm}^{-1}$) and *meso*–*meso*- ($\Delta E = 2100\text{ cm}^{-1}$) positions.^[16]

The crystal structure of **3a** provides critical insight into the trend in ΔE for the three cases. Exciton splittings arise from allowed transitions (B_y and B_x) resulting from the constructive vector sum of transition dipole moments of coplanar bis-porphyrin subunits in a parallel (μ_x) and in-line (μ_y) disposition. For the case of the *meso*–*meso* bis-porphyrin system, the in-line transition dipoles are always aligned^[14] and only the parallel transition moment varies with rotation about the connecting C–C bond. Conversely, the crystal structure of the β – β linked bis-porphyrin **3a** shows that the μ_x and μ_y transition dipole moments assume an oblique angle due to the fanning of the two porphyrin subunits from each other. In the situation of oblique transition dipoles, the exciton splitting energy will be attenuated owing to the angular dependence of the transition moment. Although the orientation of the porphyrin rings in a *meso*– β system has not been crystallographically determined,^[16b] the observation that the exciton splitting energy lies between the β – β and *meso*–*meso* bis-porphyrin systems suggests that the two porphyrins are also obliquely disposed but to a lesser degree than observed for **3a**.

Excitation into the absorption manifold of **3a** results in the intense luminescence bands characteristic of $Q(0,0)$ and $Q(0,1)$ transitions (Figure 2b). As with the absorption profile, the emission bands are red-shifted with respect to monomer emission and a splitting of the lowest energy emission band is clearly evident ($\Delta E = 345\text{ cm}^{-1}$). The luminescence red shift, consistent with increased conjugation of the bis-porphyrin pair, is reflected in a slightly shorter lifetime than that of the monomer ($\tau_{\text{obs}}(\mathbf{3a}) = 1.55(1)\text{ ns}$ and $\tau_{\text{obs}}(\text{zinc(II) etio-I porphyrin}) = 1.61(1)\text{ ns}$).

The β -derivatized boronate **2** is a general building block for modification of porphyrins at the β -position. Cross coupling between **2** and various aryl halides was carried out in DMF

in the presence of a catalytic amount (5–10 mol%) of $[\text{PdCl}_2(\text{dppf})]$ ($\text{dppf} = 1,1'$ -bis(diphenylphosphanyl)ferrocene) and 10 equivalents of aqueous K_2CO_3 at 80°C under an inert atmosphere to give the corresponding β -substituted porphyrins in high yields.^[30] Reaction of bromobenzene and *p*-bromotoluene with **2** affords the phenyl and tolyl β -derivatized porphyrins **3b** and **3c**, respectively, in yields that are similar to that obtained for the coupling between **1** and phenyl boronic acid or *p*-tolyl boronic acid. Although molecules such as anthryl porphyrin **3d** may be synthesized by reacting water-sensitive dichlorozinc anthracene reagents with **1**,^[31] the air and water stability of **2** makes the approach reported here superior for preparing this type of compound in high yields. Furthermore, **2** reacts smoothly with aryl halides bearing electrophilic functional groups; coupling of **2** with 4-bromobenzaldehyde leads to the formation of **3e** with the reactive aldehyde group remaining intact, thus demonstrating the suitability of **2** for a wide range of substrates. Along these lines, utilization of β -boronate **2** as a coupling precursor provides a general alternative to recent methods relying on arylboronic acids^[32] and boronates,^[9] with the advantage that it is more tolerant to base-sensitive functional groups.

A structurally characterized *meso*–*meso*, *meso*– β , β – β bis-porphyrin series provides a solid foundation from which to interpret trends in the observed electronic spectra of directly coupled porphyrins. The ability to differentially perturb electronic structure by the site of substitution about the porphyrin periphery has important reactivity issues. As we have recently shown, substituents at the β -position that are in direct electronic communication with the porphyrin ring can profoundly influence the rates of electron transfer.^[33] In view of the rich chemistry of boronic acid esters and borates,^[34] further application of **2** will undoubtedly provide unique porphyrin architectures that will further advance our understanding of the spectroscopic and the electron/energy transfer properties of catenated porphyrin supermolecules.

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- [24] Crystallographic data: $\text{C}_{63}\text{H}_{74}\text{N}_8\text{O}_3\text{Zn}_2$ from $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$, $M_r = 1122.04$, monoclinic, space group $C2/c$, $a = 14.58560(10)$, $b = 21.5321(3)$, $c = 19.6353(3) \text{ \AA}$, $\beta = 104.5520(10)^\circ$, $V = 5968.81(13) \text{ \AA}^3$, $Z = 4$, $\rho_{\text{calcd}} = 1.249 \text{ g cm}^{-3}$, $F(000) = 2368$, $\lambda(\text{MoK}\alpha) = 0.7107 \text{ \AA}$, crystal dimensions $0.5 \times 0.25 \times 0.10 \text{ mm}^3$. A total of 8840 reflections were collected at -90°C using a Siemens diffractometer equipped with a CCD detector in the θ range of 1.72 to 20.00° , of which 2788 were unique ($R_{\text{int}} = 0.0570$). The structure was solved by the Patterson heavy atom method in conjunction with standard difference Fourier techniques. Hydrogen atoms were placed in calculated positions using a standard riding model and were refined isotropically. A methanol solvent molecule was found to be disordered and was modeled by standard procedures. The largest peak and hole in the difference map were 0.957 and $-0.424 \text{ e \AA}^{-3}$, respectively. The least-squares refinement converged normally giving residuals of $R = 0.0749$ and $wR^2 = 0.1983$. 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-133672. 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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- [29] Standard exciton considerations predict that B_y should blue-shift and B_x should red-shift about the unperturbed B band. In **3a**, both transitions red-shift relative to the B band of the monomer. The same situation occurs for the *meso*- β and the *meso*-*meso* bis-porphyrins of ref. [16b]). These red shifts indicate that the exciton coupling in porphyrins linked by a single bond is augmented by other factors; one likely factor is electronic coupling between porphyrin subunits across the C-C bond.
- [30] **3b**-**e**: **3b**, 91 % yield. UV/Vis (CH_2Cl_2): $\lambda_{\text{max}} = 402, 530, 570 \text{ nm}$; $^1\text{H NMR}$ (300 MHz, CDCl_3 , 25°C): $\delta = 1.73$ (m, 9H; CH_3), 3.39, 3.41, 3.50, 3.64 (s, 3H each; CH_3), 3.86 (m, 6H; CH_2), 7.70–8.22 (m, 5H; Ar-H), 9.41, 9.53, 9.74, 9.84 (s, 1H each; *meso*-H); FAB HRMS for $\text{C}_{36}\text{H}_{36}\text{N}_4\text{Zn}$: calcd: 588.2223; found: 588.2230. **3c**, 93 % yield. UV/Vis (CH_2Cl_2): $\lambda_{\text{max}} = 402, 533, 569 \text{ nm}$; $^1\text{H NMR}$ (300 MHz, CDCl_3 , 25°C): $\delta = 1.75$ (m, 9H; CH_3), 2.72 (s, 3H; CH_3), 3.39 (s, 6H; CH_3), 3.44, 3.66 (s, 3H each; CH_3), 3.86 (m, 6H; CH_2), 7.68, 8.08 (dd, $^3J(\text{H,H}) = 14.5 \text{ Hz}$, 2H; Ar-H), 9.40, 9.50, 9.73, 9.84 (s, 1H each; *meso*-H); FAB HRMS for $\text{C}_{37}\text{H}_{36}\text{N}_4\text{OZn}$: calcd: 602.23879; found: 602.23908. **3d**, 88 % yield. UV/Vis (CH_2Cl_2): $\lambda_{\text{max}} = 404, 534, 570 \text{ nm}$; $^1\text{H NMR}$ (300 MHz, CDCl_3 , 25°C): $\delta = 1.74$ (t, $^3J(\text{H,H}) = 7.5 \text{ Hz}$, 3H; CH_3), 1.85 (t, $^3J(\text{H,H}) = 7.3 \text{ Hz}$, 3H; CH_3), 1.92 (t, $^3J(\text{H,H}) = 7.5 \text{ Hz}$, 3H; CH_3), 3.07, 3.41, 3.56, 3.65 (s, 3H each; CH_3), 4.08 (m, 6H; CH_2), 7.17 (m, 2H; Ar-H), 7.53 (m, 2H; Ar-H), 7.81 (d, $^3J(\text{H,H}) = 8.8 \text{ Hz}$, 2H; Ar-H), 8.30 (d, $^3J(\text{H,H}) = 8.9 \text{ Hz}$, 2H; Ar-H), 8.86 (s, 1H; Ar-H), 9.48, 10.00, 10.06, 10.34 (s, 1H each, *meso*-H); FAB HRMS for $\text{C}_{44}\text{H}_{40}\text{N}_4\text{Zn}$: calcd: 688.25444; found: 688.25432. **3e**, 82 % yield. UV/Vis (CH_2Cl_2): $\lambda_{\text{max}} = 406, 533, 574 \text{ nm}$; $^1\text{H NMR}$ (300 MHz, CDCl_3 , 25°C): $\delta = 1.72$ (m, 9H; CH_3), 3.36 (s, 3H; CH_3), 3.40 (s, 6H; CH_3), 3.59 (s, 3H; CH_3), 3.84 (m, 6H; CH_2), 8.34 (dd, $^3J(\text{H,H}) = 14.9 \text{ Hz}$, 2H; Ar-H), 9.43, 9.50 (s, 1H each; *meso*-H), 9.62 (s, 2H; *meso*-H), 10.35 (s, 1H; CHO); FAB HRMS for $\text{C}_{37}\text{H}_{36}\text{N}_4\text{OZn}$: calcd: 616.21806; found: 616.21786.
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One-Pot Synthesis of Antigen-Bearing, Lysine-Based Cluster Mannosides Using Two Orthogonal Chemoselective Ligation Reactions**

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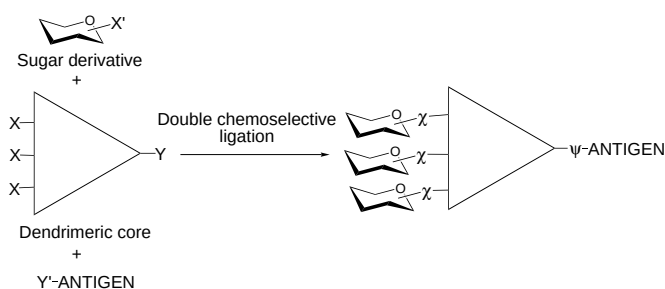
Dendritic cells (DCs) are well-recognized for playing a crucial role in the control of immunity. These professional antigen-presenting cells act both as initiators and modulators

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of the immune response.^[1] In particular, they are able to stimulate T-lymphocyte response by presenting antigens bound to major histocompatibility complex class II molecules.^[2] DCs utilize at least two distinct mechanisms to accomplish antigen uptake, one involves internalization by fluid-phase mediated macropinocytosis and the other follows association with a mannose receptor.^[3, 4] In fact, mannosylation of peptide or protein antigens results in a 200–10 000 fold enhanced potency for the stimulation of peptide-specific T-cell clones, when compared with their non-mannosylated peptides.^[4] This suggests that mannosylation of antigens leads to selective targeting and a subsequent superior presentation by DCs, which may improve the efficacy of vaccines and permit the use of lower doses. The mannose receptor preferentially selects microorganisms or molecules possessing several sugar residues such as D-mannose, *N*-acetyl-D-glucosamine, or L-fucose,^[5] which is consistent with the cluster effect.^[6] Thus, the targeting of the mannose receptor requires the preparation of multivalent ligands, such as cluster glycosides or glycodendrimers.^[7]

The sole antigen-bearing cluster mannoses prepared to date have been synthesized following a recurrent strategy.^[4] Thus, we have sought to develop a convergent approach which, in addition to improved yields and purities, would allow us to vary independently the nature of the glycosides, antigens, and dendrimeric cores, giving access to a large number of constructs for the rapid optimization of the antigen presentation (Scheme 1). Our modular strategy relies on: a) the synthesis of dendrimeric cores bearing two sets of



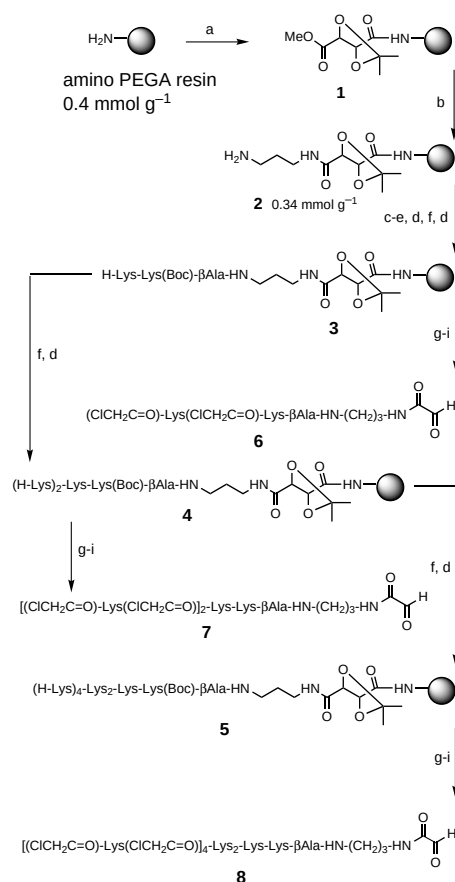
Scheme 1. Double ligation strategy to antigen-associated glycodendrimers. X = ClCH₂CO[−], X' = HS[−], Y = CHOCO[−], Y' = NH₂–NH[−], χ = –SCH₂CO[−], ψ = =N–NH[−].

chemocompatible functional groups; b) the derivatization of mannoses and antigens with complementary functional groups; c) the assembly of the purified, fully deprotected fragments in a one-pot procedure using two successive orthogonal chemoselective ligation reactions.

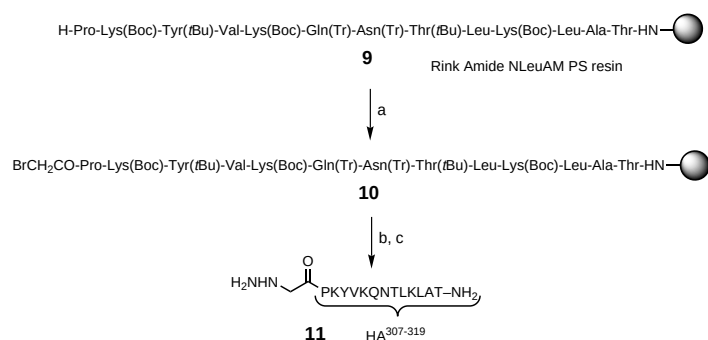
The chemoselective ligation, which refers to the coupling of fully deprotected molecular fragments through two mutually and uniquely reactive functional groups in an aqueous environment, has found broad applications.^[8] Despite the potential usefulness of assembling macromolecules using a double ligation reaction, Kent's preparation of the cMyc-Max factor, a heterodimeric transcription factor, provides a unique example where two successive oxime and thioester ligations were performed in a one-pot procedure, to the best of our knowledge.^[9] We decided to examine the reaction of *N*-

chloroacetylated L-lysiny cores,^[10] modified with a glyoxylyl function, with *N*-terminal hydrazinopeptide antigens and 2-thioethyl α, D-mannopyranoside **13**^[11] using combined hydrazone/thioether ligation.

Thus, di-, tetra-, and octavalent lysiny cores **6**, **7**, and **8**, whose lysine ε-amino group near the C-terminus was left for further derivatization with a fluorescent probe,^[12] have been prepared with the Fmoc/*tert*-butyl solid phase peptide strategy (SPPS, Scheme 2).^[13] The solid support was a polyethylene glycol–dimethylacrylamide copolymer resin (PEGA), derivatized with a novel (+)-dimethyl 2,3-*O*-isopropylidene-D-tartrate-based linker.^[14] This commercially available tartrate derivative was first partially saponified and then coupled to the amino resin. The intermediate **1** was further transacylated using 1,3-diaminopropane to give **2**. The following steps



Scheme 2. Synthesis of glyoxylyl *N*-(chloroacetyl)lysiny cores. a) Dimethyl 2,3-*O*-isopropylidene-D-tartrate (46 equiv), H₂O (4 equiv), DBU (4 equiv), RT, 1 h then addition to amino resin (1 equiv), BOP (4 equiv), DMF, RT, 40 min; b) 1,3-diaminopropane (7.8 equiv), DMF, RT, 1 h; c) Fmoc-βAla-OH (4 equiv), HBTU/HOBt/*i*PrNEt₂ (4:4:12 equiv), NMP, 40 min; d) piperidine/NMP 20:80, 20 min; e) Fmoc-L-Lys(Boc)-OH (4 equiv), HBTU/HOBt/*i*PrNEt₂ (4:4:12 equiv), NMP, 40 min; f) Fmoc-L-Lys(Fmoc)-OH (4 equiv/NH₂), HBTU/HOBt/*i*PrNEt₂ (4:4:12 equiv/NH₂), NMP, 40 min; g) ClCH₂COOH (8 equiv/NH₂), DIC (4 equiv/NH₂), DMF, RT, 1 h; h) TFA/anisole/H₂O 95:2.5:2.5, RT, 2 h; i) NaIO₄ (6 equiv), AcOH/H₂O 1:2.4, RT, 2 min; 18, 31 and 23.5% overall yields for compounds **6**, **7** and **8**, respectively. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, RT = room temperature, BOP = 1-benzotriazolyl-*o*-tris(dimethylamino)phosphonium hexafluorophosphate, Fmoc = 9-fluorenylmethoxycarbonyl, HBTU = *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, HOBt = 1-hydroxy-1*H*-benzotriazole, NMP = *N*-methylpyrrolidone, Boc = *tert*-butoxycarbonyl, DIC = diisopropylcarbodiimide, TFA = trifluoroacetic acid.



Scheme 3. Preparation of the hydrazine-modified antigen **11**. a) Bromoacetic acid (8 equiv), DIC (4 equiv), DMF, 20 min then added to peptidyl resin, DMF, RT, 1 h; b) BocNHNH₂ (4 equiv), *i*PrNEt₃ (6 equiv), DMF, RT, overnight; c) TFA/anisole/H₂O 95:2.5:2.5, RT, 3 h, 40 %.

comprised conventional SPPS to furnish peptidyl resins **3–5**, the simultaneous removal of the Boc and isopropylidene protecting groups, and finally a solid phase periodic oxidation allowing both separation of the product from the solid support and the formation of the glyoxylyl group. The cores were obtained in 18–31 % yield, after several washings of the resin with H₂O and RP-HPLC purification.

The synthesis of an α -hydrazinoacetyl modified epitope, HA^{307–319}, was undertaken as shown in Scheme 3. Compound **11** was obtained with the Fmoc/*tert*-butyl strategy;^[15] acylation of peptidyl resin **9** at the N-terminus with bromoacetic acid and then displacement of the bromine with commercial *tert*-butylcarbazate to install the hydrazine moiety^[16] gave compound **10**. Hydrazinoantigen **11** was finally obtained in 40 % overall yield as sole product following deprotection and cleavage from the resin by acidic treatment.

Having all the building blocks hand, we next examined their ability to give the target molecules using a one-pot double orthogonal reaction (Scheme 4). Compound **6** was coupled with hydrazinoantigen **11** in DMF/citrate phosphate buffer (pH 5.2)^[17] to furnish cleanly the corresponding

hydrazone fragment (as detected by RP-HPLC). The chloroacetyl moieties were reacted with the mannosyl derivative **13** at pH 8–8.5,^[18] to give construct **14** in 43 % isolated yield. Cores **7** and **8** were reacted similarly to give the antigen-bearing clusters **15** and **17** in 40 and 18 % yields, respectively (Figures 1 and 2). Compound **17** was obtained in a much lower yield compared with the first two generations, as indicated by the complex RP-HPLC trace obtained following the double ligation (Figure 3d). The mannose derivative **13** was found to react easily with simple *N*-chloroacetylated lysinyl trees.^[11] Thus, we envisioned performing the thioetherification at once, since the glyoxylyl group should be stable and, therefore, not interfere during the substitution reaction. As expected, the thioetherification proceeded smoothly to give intermediate **16** (Figure 3a), which was further treated with **11** (Figures 3b and 3c). The presence of the desired tree **17**, together with reformed disulfide **12**, was detected almost exclusively in the crude mixture by RP-HPLC, which contrasted with the profile observed when the alternative route was applied (compare Figures 3c and 3d). We were actually able to obtain the fully assembled construct **17** in a considerably improved yield (51 %). Following semipreparative RP-HPLC purification, all

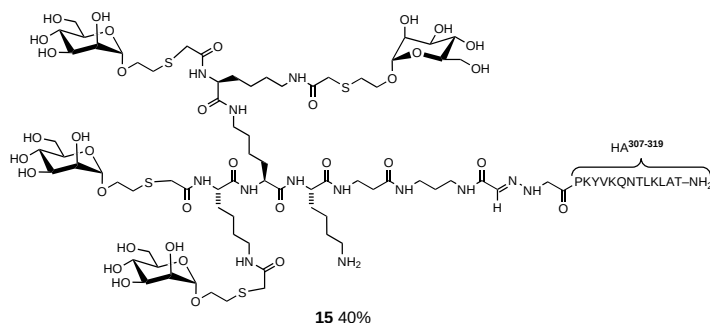
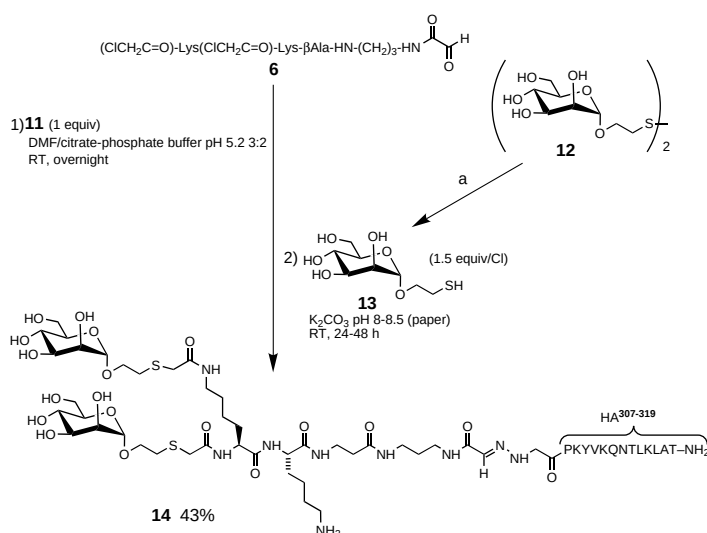


Figure 1. Product of orthogonal hydrazone/thioether ligation reaction between glyoxylyl *N*-(chloroacetyl)lysiny core **7**, the hydrazinopeptide antigen **11**, and the thio-sugar derivative **13**.



Scheme 4. Orthogonal hydrazone/thioether ligation reaction between glyoxylyl *N*-(chloroacetyl)lysiny core **6**, the hydrazinopeptide antigen **11**, and the thio-sugar derivative **13**. a) *n*Bu₃P (1 equiv), *n*PrOH/H₂O 1:1, RT, overnight.

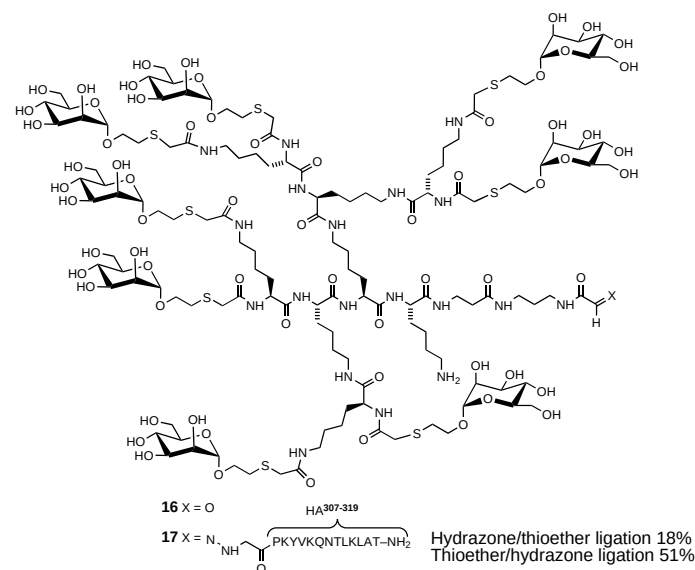


Figure 2. Product of orthogonal hydrazone/thioether ligation reaction between glyoxylyl *N*-(chloroacetyl)lysiny core **8**, the thio-sugar derivative **13**, and the hydrazinopeptide antigen **11**.

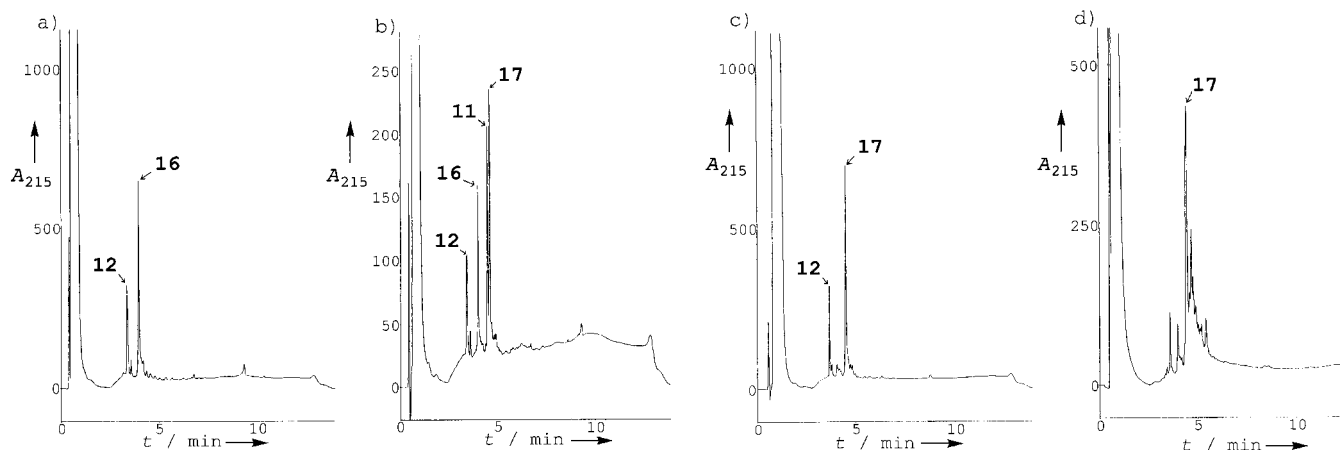


Figure 3. RP-HPLC chromatograms of crude mixtures from: a) the thioether ligation of core **8** with **13** after 36 h; b) and c) the second ligation with hydrazinoantigen **11** after 30 min and overnight, respectively; d) sequential hydrazine/thioether ligation to give **17**. Chromatographic conditions: TSK gel (TosoHaas) C18 (110 Å, 2 µm, 4.6 × 50 mm). Flow rate 2 mL min⁻¹, 50 °C. Buffer A: 0.05 % aqueous TFA; buffer B: 0.05 % TFA in CH₃CN/H₂O 80:20. Gradient: 0 % B for 5 min, 0–100 % B over 10 min, 100 % B for 1 min, 0 % B for 2 min.

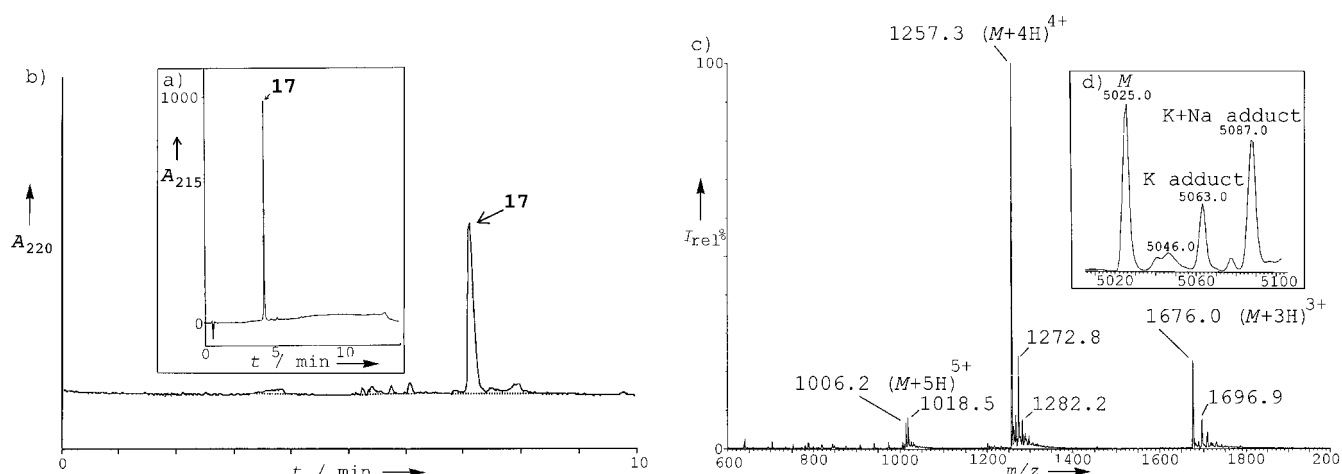


Figure 4. a) RP-HPLC chromatogram of pure compound **17**. b) CZE profile of compound **17**. Conditions: 75 µm × 500 mm fused silica capillary, with a 20 mA current and a 30 kV field in an Applied Biosystems Model 270A-HT system (Foster City, USA); separation was performed at 50 °C using a 50 mM sodium borate (pH9) migration buffer. c) Positive ESI-MS of compound **17**. Flow rate 5 µL min⁻¹ at a concentration of 5 pmol mL⁻¹ in 0.2 % HCO₂H in CH₃CN/H₂O 50:50. d) ESI-MS true mass scale of compound **17**.

compounds were characterized by electrospray ionization mass spectrometry (ESI-MS) and capillary zone electrophoresis (CZE) as illustrated for **17** (Figure 4).

Experimental Section

Synthesis of **17** with the thioether/hydrazine ligation procedure: Compound **12** (5.75 mg, 6 equiv) was dissolved in *n*PrOH/H₂O (1:1, 500 µL) and treated with *n*Bu₃P (3.0 µL, 6 equiv) at room temperature overnight under nitrogen. The reaction was monitored by RP-HPLC. The mixture was concentrated under reduced pressure for 20 min and then, dissolved in H₂O (50 µL) under N₂. Compound **8** (3.9 mg, 2 µmol) was dissolved in DMF (600 µL) and added to this solution. The apparent pH was adjusted to 8.0–8.5 by addition of solid potassium carbonate. The mixture was stirred for 36 h at room temperature and monitored by RP-HPLC. Citrate/phosphate buffer (350 µL) and modified HA^{307–319} peptide **11** (4.05 mg, 1 equiv) were then added to the mixture and the pH adjusted to 5.2 by addition of aqueous 1N HCl. The mixture was further stirred for 12 h at room temperature, diluted with H₂O, frozen and lyophilized. Compound **17** (5.46 mg, 51 %) was obtained as a white powder following RP-HPLC purification and lyophilization.

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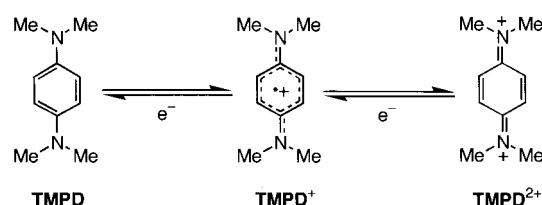
The Tetraaza[1.1.1.1]*m,p,m,p*-cyclophane Dication: A Triplet Diradical Having Two *m*-Phenylenediamine Radical Cations Linked by Twisted Benzenes**

Akihiro Ito, Yukiharu Ono, and Kazuyoshi Tanaka*

Many kinds of cyclophanes have been synthesized so far for the purpose of investigating the peculiar electronic and spectroscopic properties, which result from the interesting stereochemistry, as well as the effective binding properties for use as host molecules in molecular recognition chemistry.^[1] Although carbon is the most common bridging element in useful macrocyclic compounds such as porphyrins^[2] and calixarenes,^[3] other elements such as nitrogen, oxygen, silicon, phosphorus, and sulfur can be used to obtain additional chemical and physical properties. Indeed, over the past few

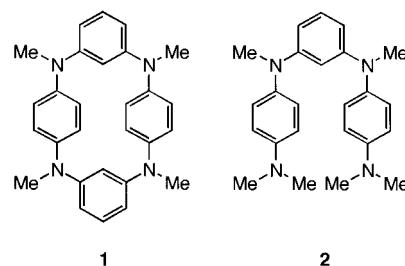
years, a considerable number of studies have been devoted to the syntheses of various heteroatom-bridged [1_n]cyclophanes.^[4]

We are interested in aza-bridged cyclophanes^[5] in connection with the chemistry of high-spin organic molecules; several cyclophane-based high-spin molecules have been prepared to date.^[6] Chemically and thermally stable poly-radicals often contain heteroatoms that serve as spin-bearing sites^[7] and are connected to each other through *m*-phenylene units, which have proven to be effective ferromagnetic couplers.^[8] For example, a *p*-phenylenediamine (PD), such as *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD; Scheme 1), can be readily converted by one-electron oxidation into a stable semiquinone radical (Wurster's blue), in contrast to the instability of ammonium radicals. Therefore, the use of this type of radical as a spin-bearing unit is pertinent to the exploitation of novel high-spin organic molecules.^[9]



Scheme 1. Conversion of TMPD into the stable semiquinone radical (Wurster's blue) as well as the dication.

When PD is to be employed as a spin-bearing site, **1** is a promising precursor of high-spin azacyclophanes with the smallest ring size possible. In addition, **1** can be regarded as *m*-phenylene-tethered version of **2**. We have already reported

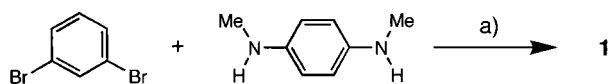


the spin preference of **2**²⁺ and also pointed out the presence of several conformational isomers.^[10] On the other hand, **1** is anticipated to retain only one conformation in which the two PD moieties are juxtaposed. Such a conformation is not feasible for **2** because of steric repulsion between the methyl groups. Here, we report on the synthesis, structure, and redox properties of cyclophane **1**. Furthermore, the electronic structure of its dication is described on the basis of EPR and quantum-chemical studies.

Palladium-catalyzed condensation of *m*-dibromobenzene with *N,N'*-dimethyl-*p*-phenylenediamine gives the desired **1** in an abysmal yield of 0.28%^[11] (Scheme 2), but in a one-pot reaction. The UV/Vis spectrum of **1** in cyclohexane measured at room temperature shows two absorption maxima at $\lambda_{\text{max}} = 236$ and 302 nm, which are slightly blue-shifted to those of

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Scheme 2. Synthesis of **1**: a) NaOtBu, $[\text{PdCl}_2[\text{P}(o\text{-tolyl})_3]_2]$, toluene, 100 °C, 16 h, 0.28 %.

TMPD.^[12] Colorless plates were obtained by slow evaporation of a dilute solution of **1** in ethyl acetate at room temperature. The X-ray structure analysis (see the Experimental Section) designates an alternate *meta-para*-linked macrocyclic structure for **1** (Figure 1). Cyclophane **1** has an approximate C_{2v} symmetry, and the *m*-phenylene and *p*-phenylene planes are inclined in the range of 13–21° and 57–59°, respectively, to the plane defined by the four nitrogen atoms.

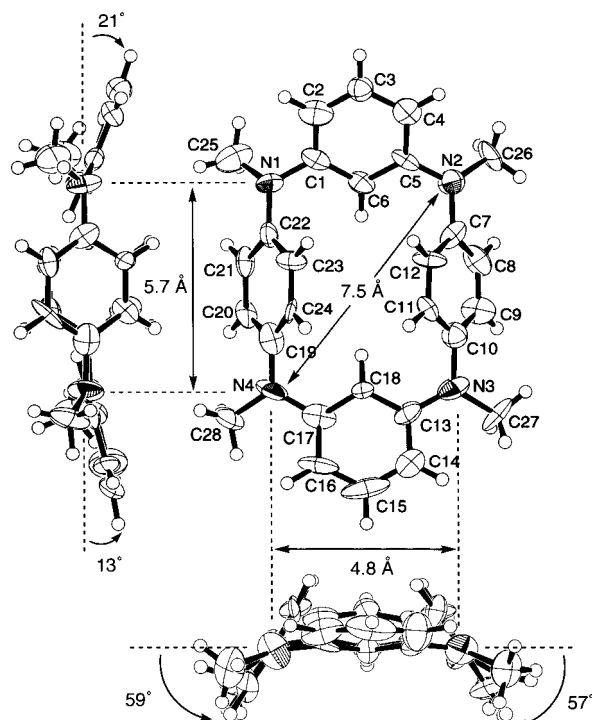


Figure 1. Side (top left and bottom right) and plan views (top right) of the molecular structure of **1** in the solid state. Thermal ellipsoids have been drawn at the 50 % probability level.

The density functional theory (DFT) calculations^[13] indicate that the C_{2v} -symmetric structure is favored for a model compound of **1** in which the methyl groups at N are replaced by H (**1_{NH}**). These results are in accordance with the X-ray crystal structure, except for a small inclination angle of the *m*-phenylene plane (3.0°).^[14] More importantly, **1_{NH}** (and **1**) can be classified as a coextensive non-Kekulé molecule, judging from its frontier orbitals (HOMO and HOMO – 1), which are composed of two nonbonding MOs (**2a₂** and **3b₁**) of *m*-phenylenediamine and a HOMO (e_{1g}) of benzene (Figure 2). Hence, when these two frontier MOs are singly occupied, a strong exchange interaction is expected; the energies of the triplet state of **1_{NH}²⁺** (and **1²⁺**) lie well below those of the corresponding singlet state. In fact, the singlet–triplet energy difference, ΔE_{S-T} , for **1_{NH}²⁺** is estimated to be 2.2 kcal mol^{–1}, indicating a ground-state triplet. The C_{2v} -symmetric structure

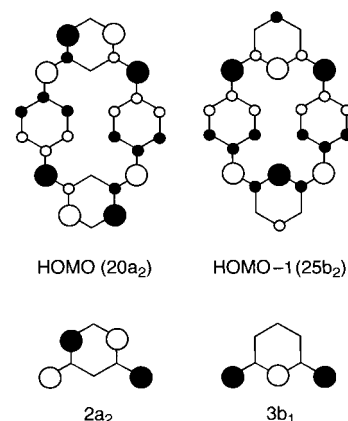


Figure 2. Schematic representation of the frontier orbitals for **1_{NH}** on the basis of BPW91/6-31G* calculations. These orbitals are depicted after the expansion of the nonplanar structure onto the plane of the page. Two nonbonding MOs (**2a₂** and **3b₁**) of *m*-phenylenediamine are also shown at the bottom.

of **1_{NH}** remains unchanged even in **1_{NH}²⁺**, and moreover two PD moieties are close to the semiquinone structure, judging from the optimized bond lengths of **1_{NH}²⁺**.

The cyclic voltammogram of **1** in acetonitrile clearly shows four redox couples at $E^{or} = -0.03$, +0.16, +0.47, and +0.62 V (vs. ferrocene/ferrocenium (Fc/Fc⁺)), which correspond to **1⁰/1⁺**, **1⁺/1²⁺**, **1²⁺/1³⁺**, and **1³⁺/1⁴⁺**, respectively (Figure 3). Whereas a quasi-two-electron transfer takes place in **2²⁺ → 2⁴⁺**, the

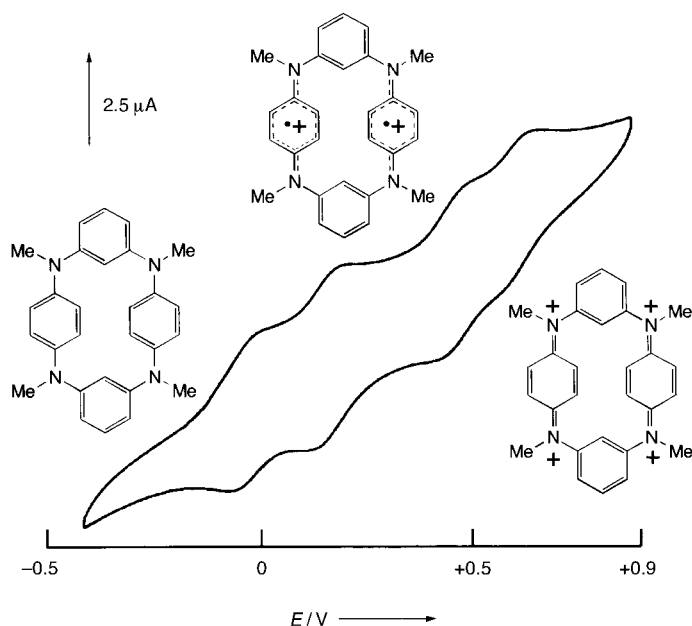


Figure 3. Cyclic voltammogram of **1** in MeCN at 25 °C (scan rate 100 mV s^{–1}).

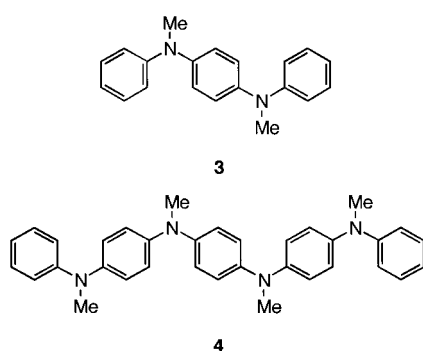
corresponding redox processes for **1** are two separate single-electron transfers, as expected from the macrocyclic structure of **1**. The redox potentials of **1** are summarized in Table 1 together with those of related compounds under the same conditions. Interestingly, the first and second redox potentials of **1** are approximately 0.16 V higher than those of **2**. The decrease of electron-donating ability in **1** is ascribed to the *m*-

Table 1. Redox potentials of **1** and related compounds.^[a]

Compd	$E_1^{o'}$ [V]	$E_2^{o'}$ [V]	$E_3^{o'}$ [V]	$E_4^{o'}$ [V]
1	−0.03 (−0.12 ^[b])	0.16 (0.14 ^[b])	0.47 (0.50 ^[b-d])	0.62
2	−0.19	−0.01	0.44 ^[d]	
3 ^{[b], [15]}	0.05	0.55		
4 ^{[b], [15]}	−0.09	0.20	0.64 ^[d]	
TMPD	−0.29	0.29		

[a] Conducting salt 0.1M *n*Bu₄NClO₄ in MeCN, potentials given versus Fc/Fc⁺, Pt electrode, 25 °C, scan rate 100 mV s^{−1}. [b] Measured in CH₂Cl₂. [c] Redox behavior such as the formation and dissolution of chemisorbed hydride and oxide layers on the polycrystalline Pt electrode is observed in this two-electron transfer (C. H. Hamann, A. Hamnett, W. Vielstich, *Electrochemistry*, Wiley-VCH, Weinheim, 1998, p. 222). [d] Quasi-two-electron oxidation.

phenylene tether. In addition, the donor ability of **1** is comparable to that of a linear *N*-methylated oligoaniline tetramer (**4**) rather than the dimer (**3**).^[15]



Chemical oxidation of **1** (1 mM) with less than one equivalent of tris(4-bromophenyl)ammonium hexachloroantimonate^[16] in *n*-butyronitrile at −78 °C yields a blue solution which shows an ESR spectrum (−157 °C) with a poorly resolved hyperfine structure resulting from ¹⁴N and ¹H nuclei at the *N*-methyl groups. The *g* value of 2.0026 suggests fairly large spin densities at the *p*-phenylene units, since nitrogen-centered radicals are typically characterized by large deviation from the *g* value of the free electron (2.003–2.004).

When **1** in *n*-butyronitrile (1 mM) is treated with two equivalents of the same oxidant at −78 °C in the presence of 1 % (v/v) trifluoroacetic acid, the corresponding dark blue di(cation radical) **1**²⁺ is obtained. The EPR spectrum of **1**²⁺ at −157 °C (Figure 4) shows a definite six-line pattern characteristic of a randomly oriented non-axial triplet molecule, in contrast to the case of **2**²⁺. Corroborating evidence for the triplet state of **1**²⁺ is obtained from the resonance at half-field for the forbidden transition $\Delta M_S = \pm 2$ (inset of Figure 4). The generated dication is stable for several weeks in solution at −78 °C, but decays gradually at higher temperature (lifetime ca. 5 min at room temperature); the resulting solution exhibits an EPR spectrum similar to that observed for **1**⁺ at −157 °C.

Nevertheless, **1**²⁺ is far more stable than **2**²⁺. From the peak-to-peak linewidth of three pairs of bands, the zero-field parameters of the dipolar electron–electron interaction can be obtained for **1**: $|D| = 77.6$ G (0.0073 cm^{−1}) and $|E| = 7.5$ G (0.0007 cm^{−1}). The value of $|D|$ corresponds to an average distance between the two radical centers of about 7.1 Å within

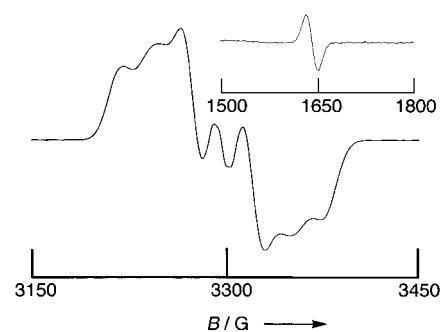


Figure 4. The X-band EPR spectrum of **1**²⁺ in *n*-butyronitrile/trifluoroacetate (99/1 v/v) at −157 °C. The central line is due to some doublet impurity. Inset: The resonance for $\Delta M_S = \pm 2$ at −157 °C.

the point dipole approximation. This value is, however, larger than the distance between the centers of two *p*-phenylene moieties estimated from the X-ray structure analysis (ca. 5 Å). The average distance is rather close to the distance between the centers of the two *m*-phenylene moieties (ca. 7 Å). In fact, a reasonable *D* value of 0.0068 cm^{−1} calculated for **1**_{NH}²⁺ is obtained on the assumption that the largest dipole–dipole interaction is along the direction connecting the two *m*-phenylene moieties.^[17] Hence, **1**²⁺ can be regarded as two *m*-PD radical cations linked by two *p*-phenylenes rather than two *p*-PD radical cations linked by two *m*-phenylenes. Note that the midpoints of the pairs of bands do not coincide, indicating anisotropy of the *g* factor: $g_{xx} = 2.0021$, $g_{yy} = 2.0008$, $g_{zz} = 2.0028$.^[17]

To elucidate the spin multiplicity of **1**²⁺ in the ground state, we have carried out variable-temperature EPR measurements (4–100 K). The intensity of the resonance for $\Delta M_S = \pm 2$ demonstrates a linear correlation to the reciprocal of the temperature, indicating a triplet ground state or degeneracy of singlet and triplet states for **1**²⁺. Consequently, it follows from these results and the calculated ΔE_{S-T} value for **1**_{NH}²⁺ that the ground state of **1**²⁺ is triplet.

In summary, we have described the triplet state of the new azacyclophane-based dication **1**²⁺. Moreover, it was confirmed that the stabilization of the di(cation radical) based on Wurster's blue is, to some extent,^[19] achieved by macrocyclization due to the *m*-phenylene tether, which is lacking in the conformationally unrestricted **2**²⁺. Cyclophane **1**²⁺ is a prototype of one-dimensional high-spin ladderlike polymers, so that further work on its oligomer model is now in progress.

Experimental Section

1: A mixture of *N,N'*-dimethyl-*p*-phenylenediamine (2.7 g, 20 mmol), 1,3-dibromobenzene (4.8 g, 20 mmol), NaOtBu (5.4 g, 56 mmol), and [PdCl₂(*p*-tolyl)₃]₂ (0.61 g, 0.78 mmol) in toluene (180 mL) was heated under an argon atmosphere at 100 °C for 19 h according to the reported procedure.^[20] After the usual workup including removal of brown intractable polymeric solid, the crude product was purified by medium-pressure liquid chromatography (MPLC) on SiO₂ (*n*-hexane/ethyl acetate (7/1) as eluent). A fraction (*R*_f = 0.5) afforded **1** as a white solid (12 mg, 0.028 mmol, 0.28 %) after recrystallization from ethyl acetate. M.p. 210 °C (decomp.); ¹H NMR (400 MHz, CDCl₃, 25 °C, TMS): δ = 3.26 (s, 12 H), 5.83 (t, ⁴*J*(H,H) = 2.2 Hz, 2 H), 6.45 (dd, ³*J*(H,H) = 8.3, ⁴*J*(H,H) = 2.2 Hz, 4 H), 6.95 (s, 8 H), 7.23 (t, ³*J*(H,H) = 8.3 Hz, 2 H); ¹³C NMR (100.5 MHz, CDCl₃, 25 °C, TMS): δ = 39.63, 106.18, 125.49, 130.05, 130.05, 144.09, 150.33; UV/Vis (cyclohexane): $\lambda_{\max}$ (lg ϵ) = 236 (4.62), 302 nm (4.57); FT-IR

(KBr): $\tilde{\nu}$ = 698, 761, 837, 880, 1348, 1510, 1591, 2811, 2876, 2957; HR-MS: m/z calcd: 420.2314 [M^+]; found: 420.2332.

Crystal data for **1** ($C_{28}H_{28}N_4$): M_r = 420.56, monoclinic, space group $P2_1/n$ (no. 14), a = 8.822(4), b = 17.577(7), c = 15.200(6) Å, β = 106.43(3)°, V = 2260(1) Å³, T = 296 K, Z = 4, ρ_{calcd} = 1.235 g cm⁻³, $\mu(\text{MoK}\alpha)$ = 0.74 cm⁻¹; of 3911 total reflections, 3689 were independent; R = 0.084 and R_w = 0.090 for 861 reflections with $I > 2\sigma(I)$. The structure was solved by direct methods and refined with the full-matrix least-squares method of the TEXSAN program (version 1.9, Molecular Structure Corporation). 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-133430. 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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Nanoscale Container Structures and Their Host–Guest Properties**

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Molecule-within-molecule complexes can be formed irreversibly, as in carcerands,^[1] or reversibly, as in self-assembled capsules.^[2] In between, hemicarcerands—covalently sealed container molecules with larger portals—offer a possibility for a guest to move more freely in and out.^[3] Open-ended hosts, cavitands, also show promise for molecular recognition,^[4] and recently we demonstrated that higher kinetic stability for their complexes, caviplexes, can be achieved when intramolecular hydrogen bonds control the cavity's size and shape.^[5]

Here we report that these cavitands can be fused together in a way that leads to novel molecule-within-molecule complexes. The hosts (**1**, see Figure 1) balance entropic advantages of unimolarity^[6] with dynamic qualities of hydrogen bonding. Above all, they accommodate guests of nanoscale dimensions.

The new structure **1** (Figure 1) consists of two deepened, resocinarene-based cavitands connected by an extended aromatic spacer. It is among the largest of synthetic unimolecular hosts, featuring cavity dimensions of about $23 \times 10 \text{ \AA}$ and an internal volume of approximately $800 \text{ \AA}^3$.^[7] The molecule may be thought of as a hemicarcerand, but the cavity is held in place by a seam of intramolecular hydrogen bonds rather than covalent bonds. Accordingly, access to the cavity is facile under ambient conditions, and the uptake and release of guests is reversible.

The synthesis of **1** draws on the regioselective functionalization of resocinarenes^[8] and the modular deepening of cavitands.^[9] Partial bridging of the hydroxyl groups in **2** with 1,2-difluoro-4,5-dinitrobenzene led to **3**, a cavitand with three extended walls (Scheme 1). The NO_2 groups were reduced (H_2 , Raney Ni, toluene) and then immediately acylated with propanoyl chloride. The remaining two resorcinol hydroxyl groups were also acylated under these conditions, but they were deprotected with NH_2NH_2 in toluene/EtOH to give hexaamide diol **4**. Reaction of **4** with another equivalent of 1,2-difluoro-4,5-dinitrobenzene followed by reduction with H_2

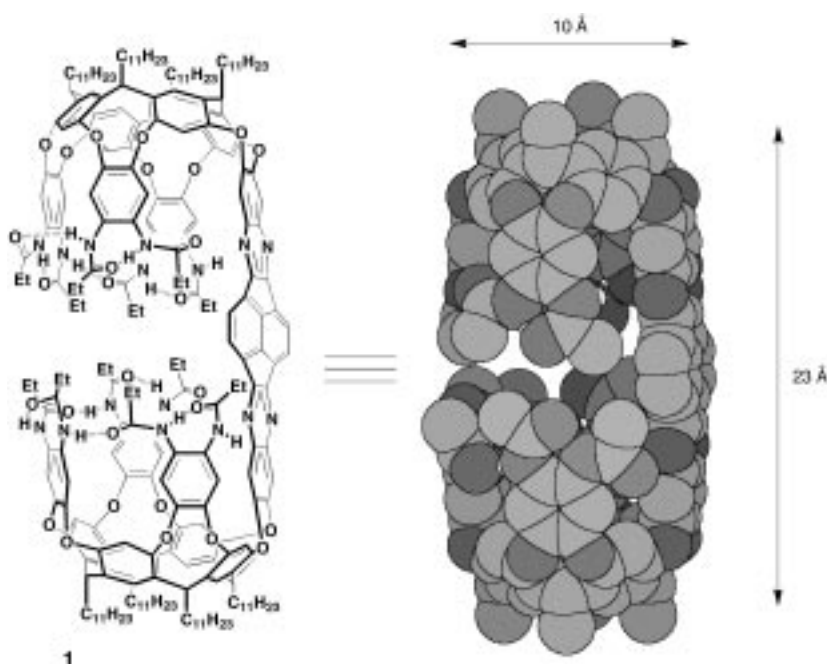


Figure 1. Molecular host **1** and its energy-minimized structure (Amber* force field, MacroModel 5.5 program). Long alkyl chains and CH bonds have been omitted for clarity.

and Raney Ni gave diamine **5**. Heterocyclization of **5** with 1,2,5,6-tetraoxopyracene gave the C-shaped **1** and its S-shaped isomer (not shown), which were separated by chromatography.^[10, 11]

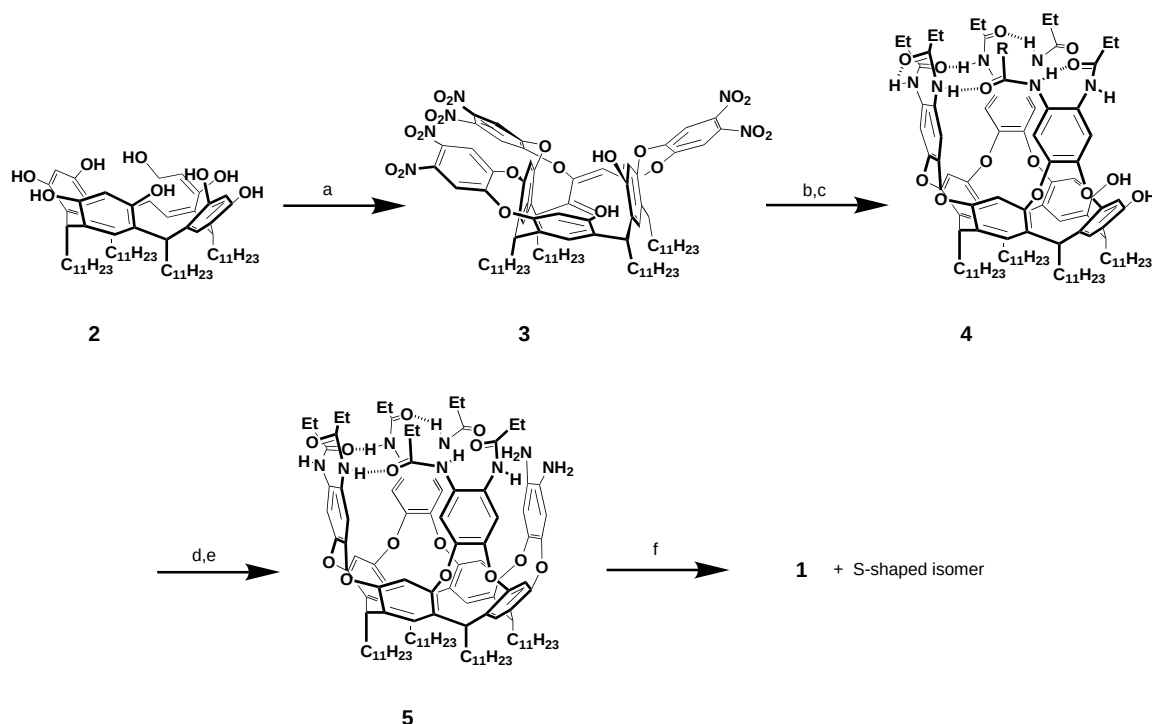
The ^1H NMR spectra of **1** are sharp in $[\text{D}_6]\text{benzene}$ and $[\text{D}_8]\text{toluene}$, but broad in $[\text{D}_{10}]\text{p-xylene}$ at room temperature (Figure 2), suggesting that rates for guest exchange are sensitive to solvent size. The NH resonances are observed far downfield at $\delta = 8$ to 11 in the spectra, and the corresponding FT-IR spectra show stretching absorptions for hydrogen-bonded NH at $\tilde{\nu} = 3244 \text{ cm}^{-1}$ ($[\text{D}_8]\text{toluene}$). Accordingly, the secondary amides of each cavitand form a seam of five intramolecular hydrogen bonds, and modeling implicates an additional interaction of this type between one NH group and its neighboring quinoxaline nitrogen atom. These hydrogen bonds help to maintain the vase-like shape shown in Figure 1. Each cycloenantiomeric cavitand in **1** can possess either clockwise or counterclockwise arrangements of the head-to-tail amides (Figure 2). The presence of two seams in **1** results in diastereomeric relationships, and multiple NH resonances appear in the ^1H NMR spectra: In $[\text{D}_6]\text{benzene}$ and $[\text{D}_8]\text{toluene}$ at least ten singlets are seen downfield of $\delta = 8$, while twelve are expected for two diastereomers undergoing slow exchange. Since two NH protons do not participate in the cyclic arrays they appear more upfield, in the aromatic region of the spectra.

The internal dimensions of **1** were estimated using a series of molecular rulers **6–10**,^[12] guest molecules of well-defined length (Scheme 2). The cavities in **1** are similar to those in self-folding octaamido cavitands **11**,^[5] which showed high affinity to adamantanes as guests. Therefore, we acylated 1-adamantanamine with acyl chlorides of varying length.

The rigid, long (ca. $18 \text{ \AA}$) guests **6** and **7** form kinetically stable complexes. Admittedly, the criteria for kinetic stabil-

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Scheme 1. Synthesis of **1** and its precursors **3–5**: a) 1,2-difluoro-4,5-dinitrobenzene (3 equiv), Et₃N (16 equiv), DMF, 70 °C, 16 h, 57 %; b) Raney Ni, H₂, toluene/MeOH (3/1), 40 °C, 12 h, > 90 %; c) C₂H₅C(O)Cl (ca. 10 equiv), pyridine (18 equiv), CH₂Cl₂, –78 °C, 1 h; then NH₂NH₂/H₂O (10 equiv), toluene/EtOH (1/1), 85 °C, 4.5 h, 13 %; d) 1,2-difluoro-4,5-dinitrobenzene (2 equiv), Et₃N (10 equiv), DMF, 70 °C, 15 h, 65 %; then Raney Ni, H₂, toluene, 40 °C, 3 h, 65 %; e) 1,2,5,6-tetraoxopyracene (0.5 equiv), AcOH_{glacial}/THF (1/100), reflux, 15 h, 55 % C-shaped and S-shaped isomers (ca. 1:1 ratio).

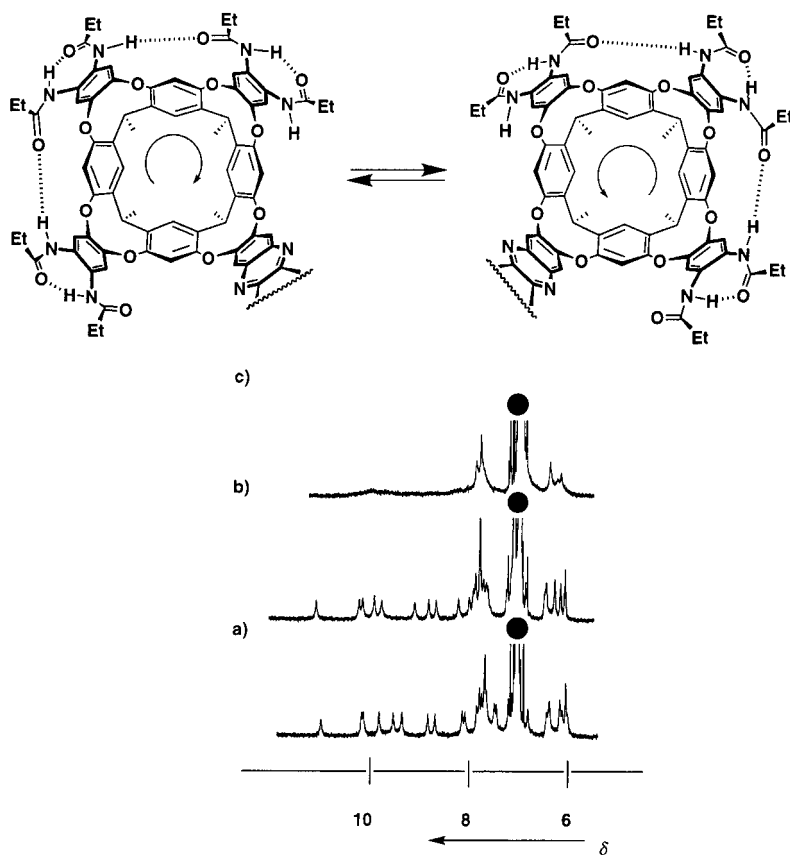
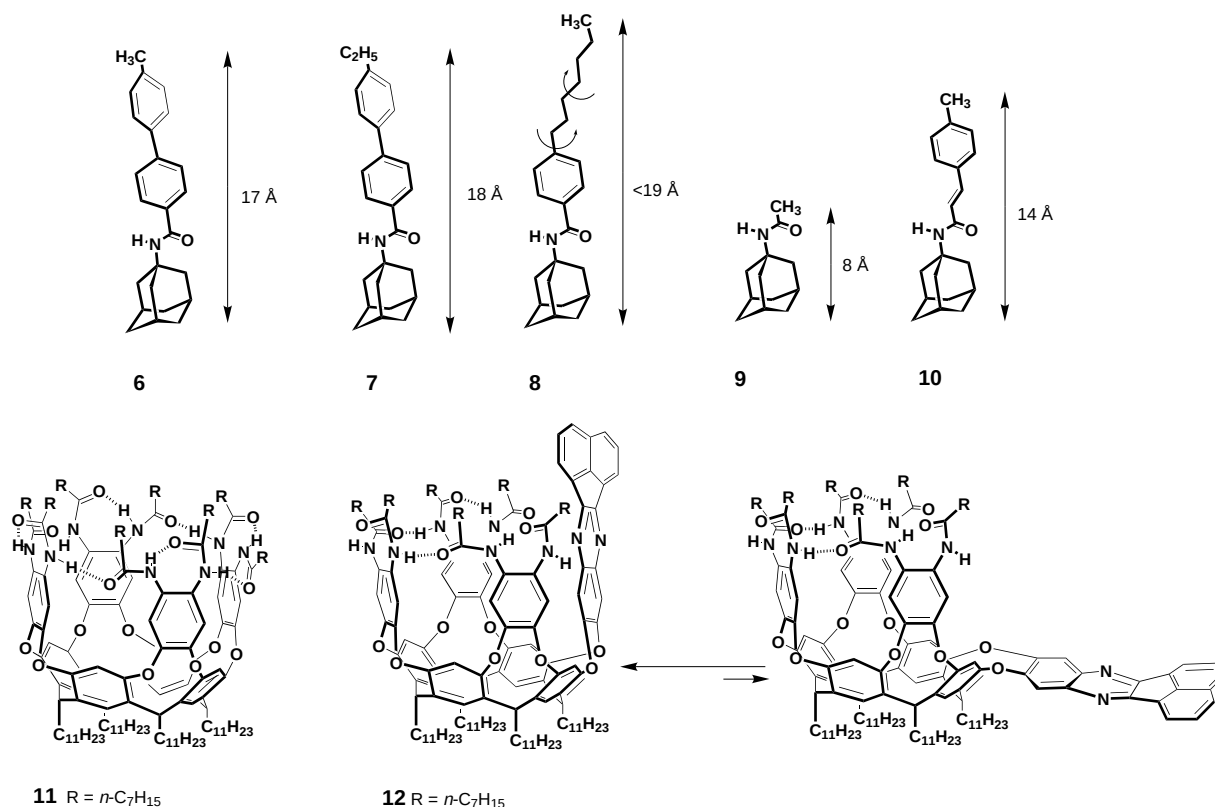


Figure 2. Top: Schematic representation of two cycloenantiomeric cavities in **1**. Bottom: Portion of the ¹H NMR spectra (600 MHz, 295 K) of **1** in a) [D₆]benzene, b) [D₈]toluene, and c) [D₁₀]p-xylene. The amide C(O)-NH signals are shown in the region δ = 8–11. The solvent signals are marked with ●.

ity—slow exchange on the NMR time scale at room temperature for measurement with a 600-MHz spectrometer—are arbitrary, but the behavior of **1** is unprecedented for unimolecular cavities of such dimensions.^[7] Specifically, four adamantyl signals and resonances for the CH₃ or C₂H₅ groups of complexed **6** and **7** were clearly observed *upfield* of δ = 0 (Figure 3). This feature is characteristic of guests in a highly shielded environment.^[1–3]

Through integration, association constants K_a of $500 \pm 50 \text{ M}^{-1}$ ($-\Delta G^{295} = 3.6 \pm 0.1 \text{ kcal mol}^{-1}$) and $270 \pm 100 \text{ M}^{-1}$ ($-\Delta G^{295} = 3.2 \pm 0.2 \text{ kcal mol}^{-1}$) were calculated for the 1:1 complexes **1·6** and **1·7**, respectively. With the more flexible guest **8** and/or shorter (<14 Å) adamantanes **9** and **10** (Scheme 2), slow exchange between the free and complexed guest species was the case only at lower temperatures (<280 K in [D₈]toluene). Moreover, two equivalents of the smallest guest **9** were bound.

The energetics of the exchange process depend on the seam of hydrogen bonds that encircle the host structure. For example, addition of [D₇]DMF to solutions of the complexes in [D₈]toluene causes the upfield guest signals to disappear. [D₇]DMF competes for hydrogen bonds that stabilize the complex and may also compete for the cavity. At temperatures of 320 K or higher, exchange rates become fast on the



Scheme 2. Guests **6**–**10** and model cavitands **11** and **12**. The proposed conformational mobility in cavitand **12** is depicted.

NMR time scale. A model structure representing half of the cavity in **1** was found in **12** (Scheme 2), which is prepared by reaction between **5** and 1,2-acenaphthenequinone. Compound **12** does not form kinetically stable complexes with adamantanes **6**–**10** at room temperature.

Conformational changes in the host seem unavoidable during the process of guest exchange, as openings must be created that allow for guest substitution. The fast exchange of smaller guests from **1** and **12** may result from their “odd” walls. These can flip open and expose the resident guest to displacement (Scheme 2). This conformational mobility may be contrasted with octaamide **11**.^[5] This cavitand features a complete seam of hydrogen bonds and forms kinetically stable complexes with small guests, even at higher temperatures (up to 330 K). For exchange of the longer, more rigid guests, one opening is not enough: A second wall must also open, and the increased energy required slows the process.

The ROESY spectrum of complex **1**·**6** yielded the exchange rate constant of $k \approx 0.5 \text{ s}^{-1}$ (at 295 K). The reaction is faster than for Cram’s hemicarceplexes with sizeable guests ($k = 1 \times 10^{-2} \text{ s}^{-1}$, $T \geq 373 \text{ K}$),^[3] but slower than that of the open-ended cavitand **11** ($k \approx 2 \text{ s}^{-1}$ at 295 K).^[5, 13]

Carcerands and cryptophanes produced the earliest molecule-within-molecule complexes.^[1] Their kinetic stability allowed the direct observation of reactive intermediates,^[14] while their cramped quarters permitted trapped guests to show isomeric orientations.^[15] Self-assembled capsules allow the encapsulation of more than one guest^[12, 16] and they are useful as reversibly formed chambers for bimolecular reactions.^[17] The features described above for molecule **1** fulfill

the promise of hybrid properties expected, and place **1** in a unique position among the cavity-forming structures.

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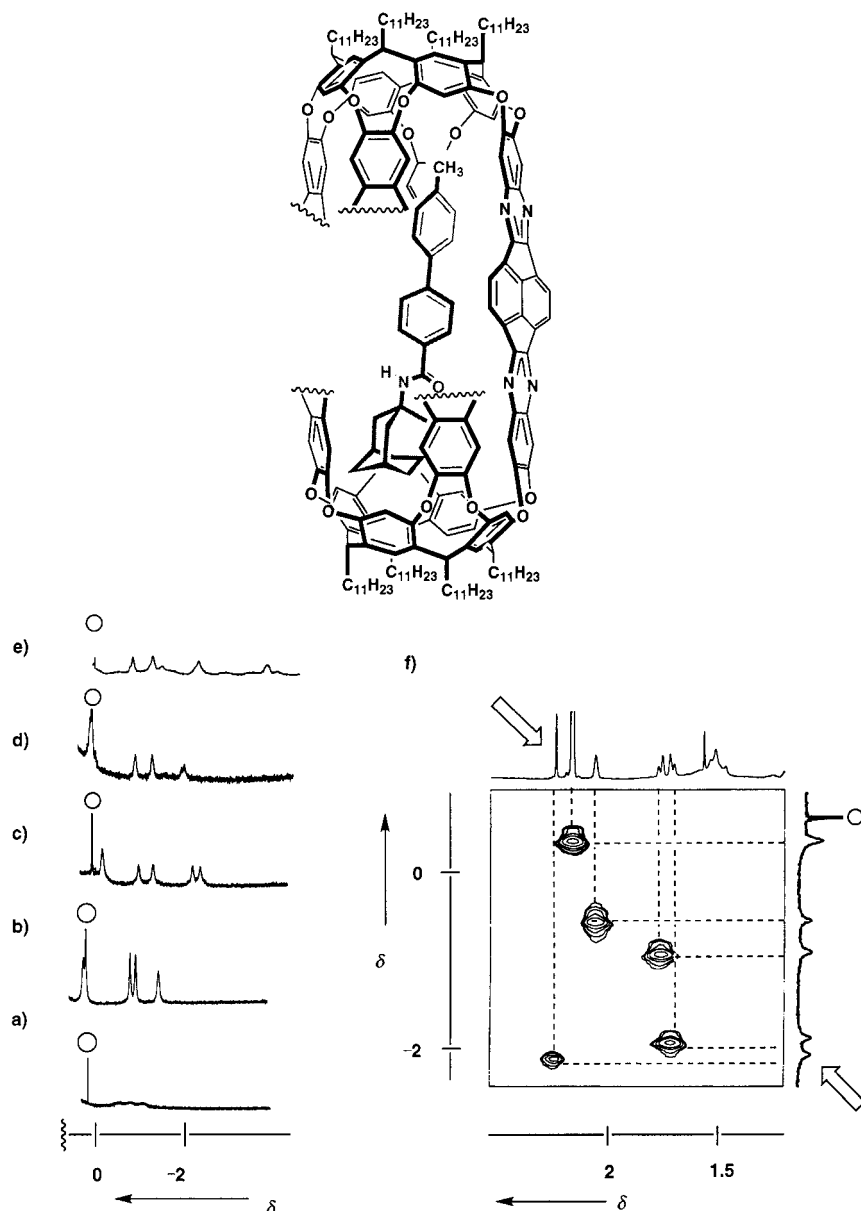


Figure 3. Upfield portions of the ^1H NMR spectra of the complexes (600 MHz, $[\text{D}_8]\text{toluene}$): a) **12**·**10** at 295 K. b) **12**·**10** at 220 K. In the downfield region, all six NH singlets of cavitant **12** are clearly seen now at $\delta = 8.5$ –11, as expected for the cyclic arrangement of the hydrogen bonds. c) The C-shaped **1**·**6** at 295 K. d) The S-shaped **1**·**6** at 280 K. No upfield signals were observed at 295 K. e) The C-shaped **1**·**7** at 295 K. f) Fragment of the ROESY spectrum of the **1**·**6** complex (295 K). The resonances for exchangeable CH_3 groups of free and encapsulated **6** are indicated. The internal standard singlet is marked with $\circ$.

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Design of Small Molecules That Recognize RNA: Development of Aminoglycosides as Potential Antitumor Agents That Target Oncogenic RNA Sequences**

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Thomas J. Tolbert, and Chi-Huey Wong*

Aminoglycosides are a structurally diverse family of amino-sugar and aminocyclitol derivatives that are currently the only well-characterized class of small molecules that selectively bind RNA,^[1] including sites on prokaryotic 16S rRNA which is believed to be the locus of aminoglycoside antibiotic activity.^[2] Of particular relevance to our own studies were the recent observations that aminoglycoside–RNA interactions could be designed and could potentially be used to modify gene expression.^[3] These results suggest that methods for synthesizing libraries of aminoglycosides or aminoglycoside mimetics may have wide application for inhibiting oncogenic RNA transcripts. We have chosen the breakpoints of the Bcr–Abl and PAX3–FKHR translocations as model mRNA sequences for studying aminoglycoside–mRNA interactions (Figure 1).

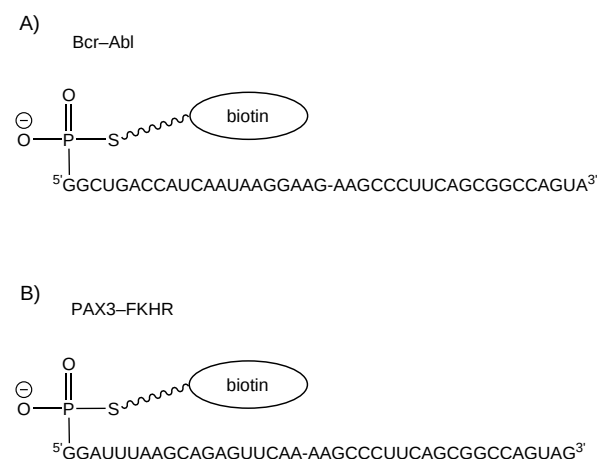


Figure 1. Biotinylated 5'-monophosphorothioates mimicking the breakpoints of the Bcr–Abl (A) and PAX3–FKHR gene translocations (B).

Bcr–Abl was the first oncogenic fusion protein to be discovered in the late 1980s. It is expressed in nearly all cases of chronic myelogenous leukemia, and in 25 % of patients with acute lymphocytic leukemia.^[4, 5] Wild-type Bcr and Abl are signal transduction proteins. Wild-type Abl is a tyrosine

kinase apparently involved in response to DNA damage, cell cycle progression, and cellular adhesion. Bcr is a serine/threonine kinase which is believed to be involved in signal transduction.^[6] The chimeric protein Bcr–Abl is a constitutively active signaling molecule which switches on many pathways by phosphorylating several different proteins, such as Ras,^[7] resulting in uncontrolled cellular growth.

The other oncogenic fusion protein, PAX3–FKHR, was discovered in a usually fatal pediatric cancer, alveolar rhabdomyosarcoma.^[8] In this translocation, the 5'-domain of one transcription factor, PAX3, is fused to the 3'-domain of another transcription factor, FKHR.^[9] This generates a new chimeric transcription factor which contains the DNA-binding domain of PAX3 and the potent transcription activation domain of FKHR. The chimeric transcription factor is essentially hyperactive and leads to overexpression of many proteins.

As previously described, Bcr–Abl and PAX3–FKHR arise from chromosomal translocations.^[4, 5, 9] Translocations such as these are ubiquitous in many leukemias and some solid tumors. Therefore, these sequences can serve as a model for the inhibition of many other oncogenes. Importantly, the breakpoints of gene fusions are unique sequences not found in the wild-type genes and therefore are a specific target sequence. Based on previous successes with antisense agents, it has been shown that inhibiting expression of these oncogenes inhibits the growth of the cancer.^[10] Thus, it is reasonable that similar results can be achieved with an aminoglycoside-based approach, that is, by using aminoglycosides as anticancer agents.

Biotinylated Bcr–Abl and PAX3–FKHR (Figure 1) were prepared chemoenzymatically with a previously reported method.^[11] DNA templates for Bcr–Abl and PAX3–FKHR were transcribed in vitro with T7-polymerase-catalyzed transcription in the presence of guanosine 5'-monophosphorothioate to yield the respective 5'-phosphorothioate. A biotin–iodoacetamide linker was used to biotinylate the 5'-phosphorothioate of the RNA. The biotinylated RNAs were purified by polyacrylamide gel electrophoresis (PAGE) and immobilized on a streptavidine-coated surface plasmon resonance (SPR) sensor chip.

In order to establish that aminoglycosides may be good candidates for binding Bcr–Abl and PAX3–FKHR RNAs, the K_d values of several commercially available aminoglycosides (Figure 2) were determined by using SPR spectroscopy.^[11] The results are shown in Table 1. The binders showing the highest affinity were the 4,5- and 4,6-linked deoxystreptamines. The order of binding was as follows: neomycin B > paromomycin = tobramycin > kanamycin = gentamicin. These 4,5- and 4,6-linked aminoglycosides bind Bcr–Abl and PAX3-FKHR RNAs in the low micromolar range. Neamine, the simplest aminoglycoside, showed a K_d value of 25 μ M for binding to Bcr–Abl and PAX3-FKHR RNAs. It was somewhat surprising that ribostamycin, neamine 5-O-linked with ribose, showed a fourfold lower binding affinity to the RNAs than neamine. This result suggests that the aminoglycoside–RNA binding was sensitive to modification at the 5-position of neamine and that it involved more than a simple electrostatic interaction.

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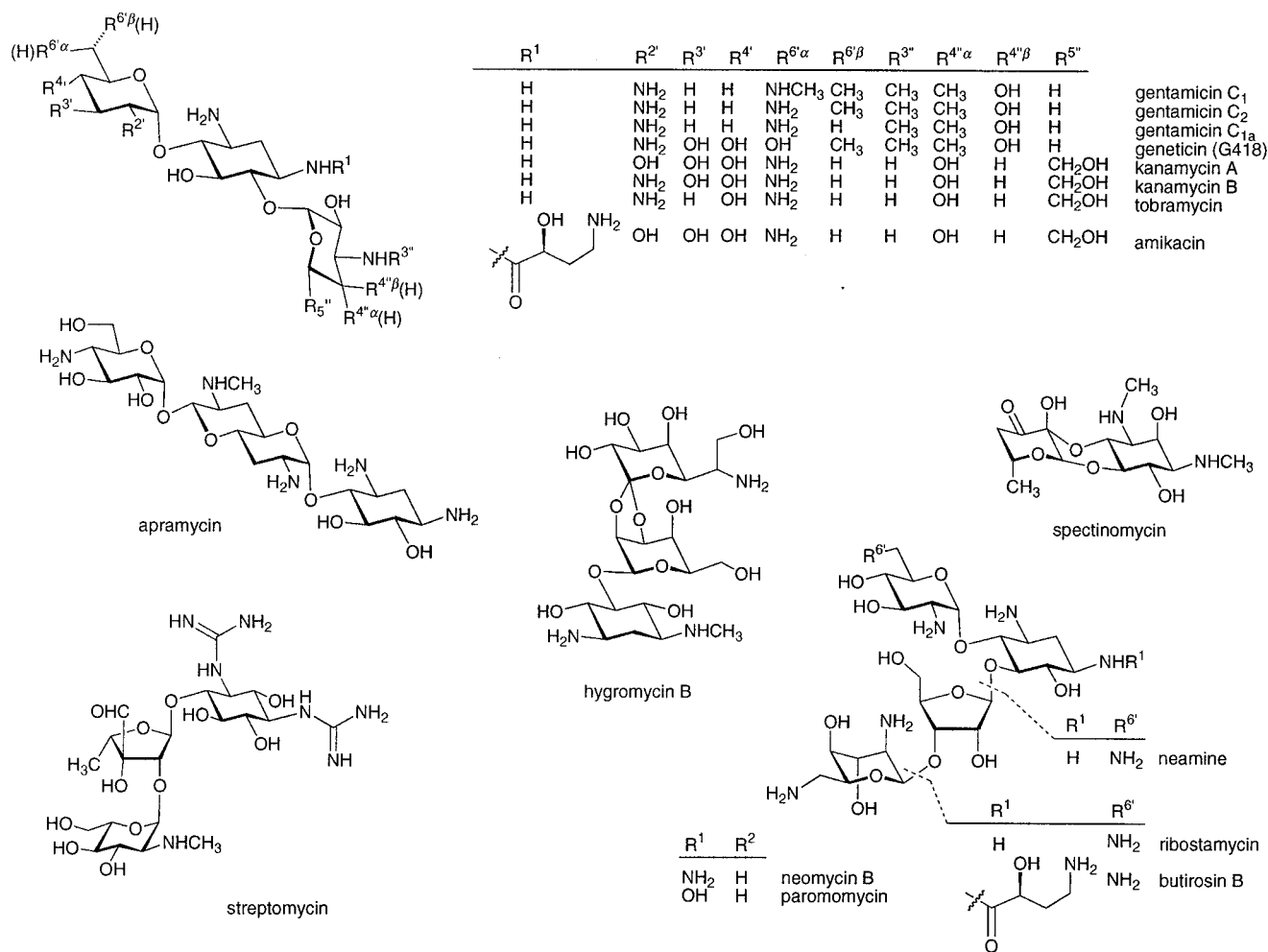


Figure 2. The structures of various commercially available aminoglycosides.

Table 1. Determination of approximate K_d values for naturally occurring aminoglycosides binding to Bcr2–Abl2 and PAX3–FKHR mRNAs.^[a]

Compound	K_d [μ M] (Bcr–Abl)	K_d [μ M] (PAX3–FKHR)
neomycin	1.5	1.5
paromomycin	1.7	1.8
kanamycin B	2.5	5.0
tobramycin	3.6	1.8
gentamicin	3.0	6.0
apramycin	20	20
ribostamycin	80	90
kanamycin A	10	20
amikacin	10	15
neamine	17	20
butirosin B	15	25
geneticin (G418)	20	30
streptomycin	20	25
hygromycin B	> 100	> 100
spectinomycin	> 100	> 100

[a] Solution conditions: 150 mM NaCl, 10 mM HEPES (pH 7.4), 3.4 μ M EDTA.

We then investigated the RNA-binding properties of synthetic aminoglycoside libraries that use neamine as a core RNA-binding motif. Neamine was chosen as a core structural element because the motif was found to be conserved in the naturally occurring aminoglycosides that bind the Bcr–Abl

and the PAX3–FKHR RNAs with the highest affinity. Furthermore, neamine contains the β -hydroxyamine motif which is commonly found in aminoglycosides and essential for interaction with the phosphodiester group and the Hoogsteen face of guanine in RNA (Figure 3).^[12]

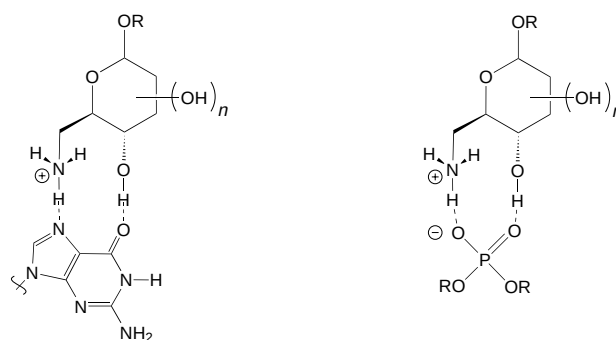
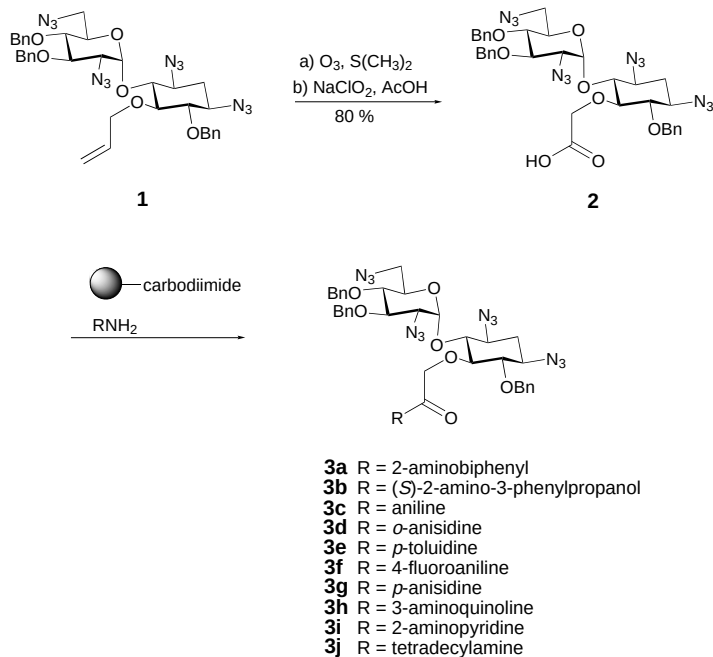


Figure 3. Proposed mode of complexation for hydroxyamines with phosphodiester groups (right) and the Hoogsteen face of purines (left; guanine is shown as an example).

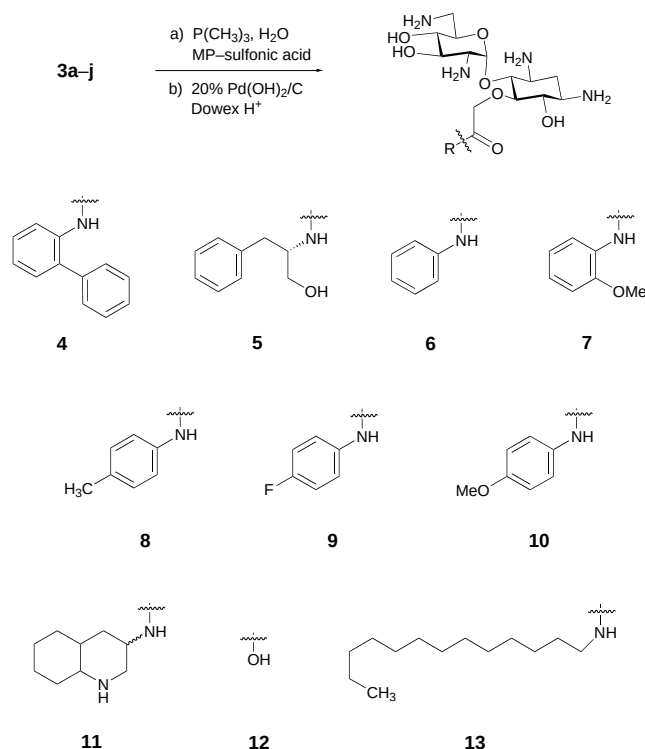
To exploit aminoglycosides as small-molecule inhibitors of translation, methods for preparing large libraries of aminoglycoside mimetics were necessary. The protected 5-*O*-carboxyethylneamine **2** was synthesized from a protected

and allylated neamine (**1**) by a two-step oxidation procedure (Scheme 1). Attempts to prepare the acid **2** directly by oxidation of the allylated neamine **1** with $\text{RhO}_2/\text{NaIO}_4$ were only modestly effective. The allylated neamine **1** was easily oxidized to an intermediate aldehyde with ozone followed by reductive workup. The crude aldehyde was oxidized to the acid **2** with sodium chlorite in carbon tetrachloride/acetic acid (1:1). The carboxyethylneamine **2** was used as a core starting material for parallel synthesis.



Scheme 1. Parallel synthesis of a 5-*O*-alkylated neamine library prepared by the resin-bound activated ester. Bn = benzyl.

The carboxyethylneamine **2** was distributed into a parallel synthesizer (Quest 210) and activated with cyclohexylcarbodiimide bound to a macroporous polystyrene (MP) resin. A ratio of two equivalents of resin to one equivalent of acid and 0.8 equivalents of amine was used to synthesize a library of protected neamine amides. Various aromatic and aliphatic amines were coupled to the neamine core through the resin-bound activated ester to form neamine amides **3a-j** (Scheme 1). The amides **3a-j** were isolated by filtration and were >95 % pure, as determined by NMR spectroscopy. The amides were deprotected by a two-step reduction. Simultaneous reduction of the azides and benzyl protecting groups was not feasible by hydrogenolysis owing to the formation of complex product mixtures. Therefore, the azides were reduced under classical Staudinger conditions. The resulting amines were captured from solution with a resin-bound sulfonic acid scavenger (MP-TsOH; Ts = toluene-4-sulfonyl). Impurities were removed by washing and the free amine was released from the resin by elution with 2 M NH_3 in methanol (Scheme 2). The amines were pure, as determined by TLC analysis, and were subjected to hydrogenolysis with 20 % Pd(OH)_2 on carbon under an H_2 atmosphere. The reaction mixtures were filtered, concentrated, and purified by



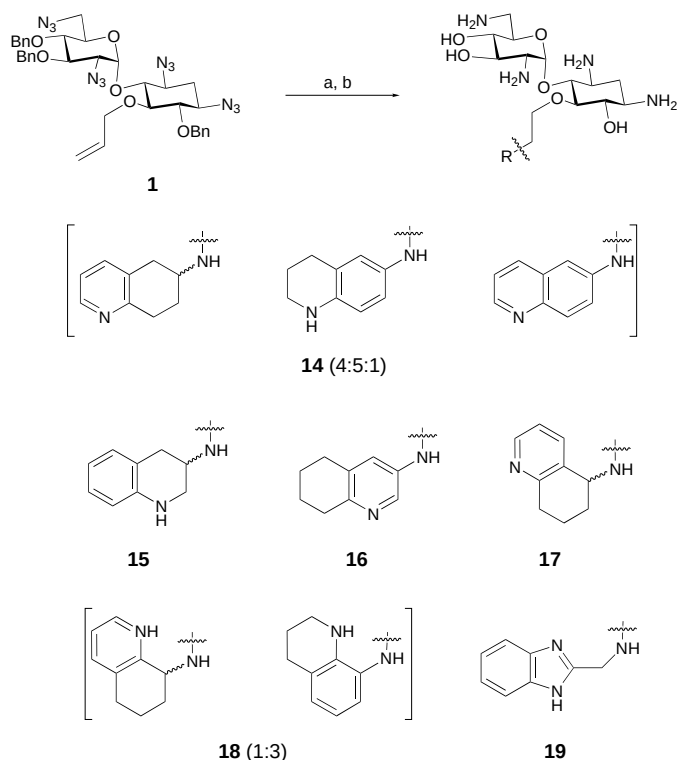
Scheme 2. Catch-and-release deprotection/purification strategy for the synthesis of aminoglycoside libraries (e.g., **4–13**).

cation exchange chromatography to provide the aminoglycosides **4–13** (Scheme 2).

Several other aromatic and heteroaromatic analogues of neamine were prepared by reductive amination. Benzimidazole as well as several isomeric aminoquinolines were chosen because of their potential ability to intercalate or form hydrogen bonds with nucleotides. The allylated neamine **1** was oxidized to an aldehyde as previously described^[13] (Scheme 3). The intermediate aldehyde was used for reductive aminations with several different heteroaromatic amines. The azide protecting groups were reduced with hydrazine and Raney nickel followed by debenzoylation with 20 % Pd(OH)_2 on carbon to provide aminoglycosides **14–19**. The aminoquinolines **14–18** were partially reduced; however, these mixtures were separable by ion exchange chromatography in most cases.

Also included in the study were the aminoglycoside mimetics **20–26** (Figure 4). These congeners contain various polyamines varied at the 5-*O*-position of neamine. They can be expected to enhance aminoglycoside–RNA binding through hydrogen bonding or electrostatic interactions with the negatively charged nucleotides. These analogues were prepared as previously reported.^[13]

The compounds with the highest binding affinity were the polyamine analogues **20–26** and the 6-aminoquinoline **14** which consisted of a 5:4:1 phenylene diamine/pyridine/quinoline mixture (Table 2). Aminoglycoside binding and selectivity was also modulated when the aromatic groups at the 5-*O*-position of neamine were varied for compounds **4–13**. The *p*-anisidine analogue **10** showed a fivefold preference for the Bcr–Abl transcript compared to PAX3–FKHR RNA. Sev-



Scheme 3. Parallel synthesis of a 5-*O*-alkylated neamine library (**14**–**19**) prepared by reductive amination. a) 1. O_3 , $\text{S}(\text{CH}_3)_2$; 2. amine, AcOH, NaCNBH_3 , 51–75 %; b) 1. Raney nickel, N_2H_4 , EtOH; 2. H_2 (1 atm), 20 % $\text{Pd}(\text{OH})_2/\text{C}$, AcOH/ H_2O , 23–54 %.

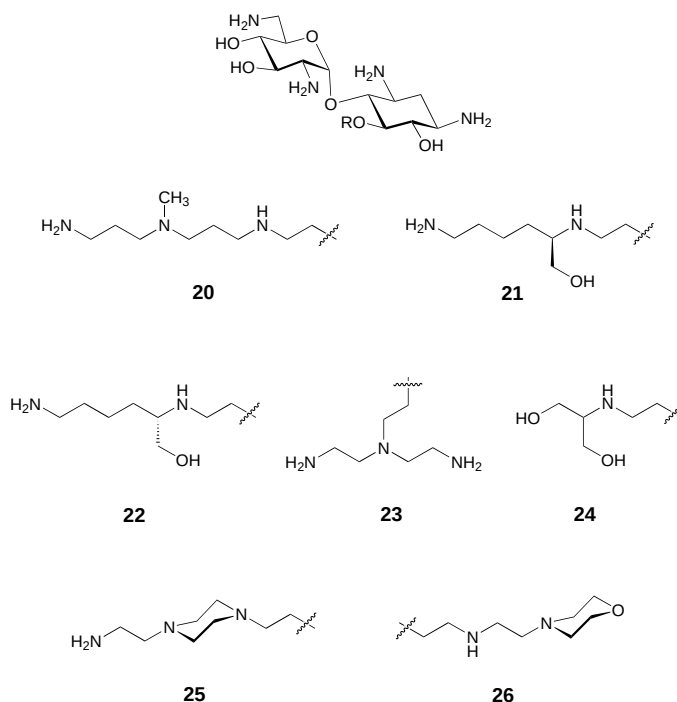


Figure 4. A 5-*O*-alkylated neamine library (**20**–**26**) based on polyamines.

eral of the aminoglycosides studied showed nanomolar binding affinities with RNA. The 6-aminoquinolines showed the highest preference for the Bcr–Abl transcript, eightfold over PAX3–FKHR. The additional binding for these compounds

Table 2. Determination of approximate K_d values for aminoglycoside mimetics binding to Bcr2–Abl2 and PAX3–FKHR mRNAs.^[a]

Compd	K_d [μM] (Bcr2–Abl2)	K_d [μM] (PAX3–FKHR)
4	10	7.2
5	26	8.7
6	5.0	1.7
7	20	8.0
8	5.0	3.0
9	10	2.5
10	5.0	1.0
11	5.0	6.0
12	> 100	> 100
13	6.7	6.0
14	0.52	4.0
15	2.3	4.8
16	2.1	2.6
17	3.2	5.0
18	18	11
19	5.1	6.8
20	0.36	0.50
21	0.61	1.0
22	0.70	1.0
23	1.1	2.0
24	5.8	9.0
25	13	11
26	7.0	5.7

[a] Solution conditions: 150 mM NaCl, 10 mM HEPES (pH 7.4), 3.4 μM EDTA.

can be partially explained as arising from enhanced electrostatic interactions or, in the case of the aminoquinolines, through intercalation with RNA. However, the increase in binding was not the same for both RNA sequences studied. The greater selectivity exhibited by the 6-aminoquinolines for binding to Bcr–Abl may result from an interaction with a specific conformation or structural domain in that sequence. Notably, the GC content for both sequences is quite similar, 54 % and 51 % for Bcr–Abl and PAX3–FKHR, respectively, and the sequences are of similar length. Therefore, it is unlikely that the observed preference for aminoglycoside **14** binding Bcr–Abl results from a difference in the nucleotide composition of the RNA. It is likely, however, that the difference in binding selectivity for Bcr–Abl results from the high degree of secondary structure in Bcr–Abl, while the PAX3–FKHR sequence has significantly less secondary-structure content based on structure calculations.

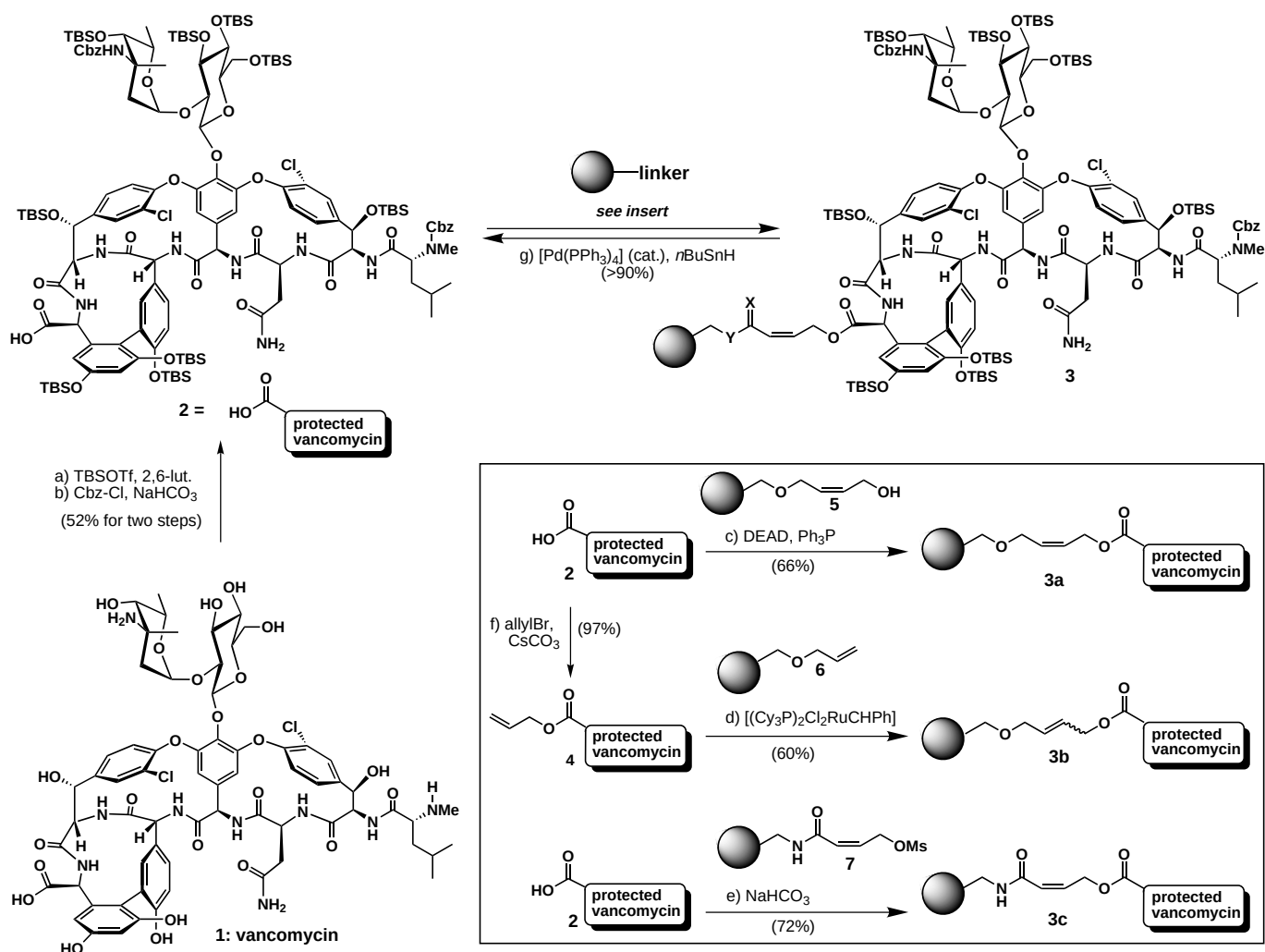
These results demonstrate for the first time that aminoglycosides act as high-affinity small-molecule ligands for the oncogenic Bcr–Abl and PAX3–FKHR single-stranded mRNA breakpoints. Based on the affinities of these synthetic aminoglycosides for RNA, it is reasonable to believe that aminoglycosides can potentially act as regulators of gene expression. The future direction of this work should focus on validating the use of aminoglycosides as inhibitors of gene expression in cell culture and on generating larger libraries of aminoglycosides in order to find more highly selective ligands for target mRNAs.

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New Selenium-Based Safety-Catch Linkers: Solid-Phase Semisynthesis of Vancomycin**

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The generation of molecular diversity through solid-phase combinatorial chemistry has become an important component of the drug discovery process.^[1] An enduring challenge in the design of solid-phase synthetic sequences is the selection of an appropriate linker^[2] that is stable to the proposed chemistry, yet which can be readily and practically cleaved. Solid-phase synthesis of natural products offers a particularly good platform to evaluate linkers because of the multitude of functionality often present. Vancomycin^[3] (**1**, Scheme 1), renowned for its activity against methicillin-resistant *Staphylococcus aureus* (MRSA), has been used for the past forty years to treat Gram-positive bacterial infections. The emergence of vancomycin-resistant *enterococci* strains (VRE) has raised serious health concerns and prompted a renewed vigor in the field of glycopeptide antibiotics.^[3, 4] Researchers from Eli Lilly have demonstrated that modificat



Scheme 1. Protection of vancomycin, as well as loading onto and cleavage from allyl ester resins. Reagents and conditions: a) TBSOTf (excess), 2,6-lutidine (120 equiv), CH₂Cl₂:DMF (10:1), 23 °C, sonication, 8 h; b) CbzCl (5.0 equiv), NaHCO₃ (10 equiv), 1,4-dioxane: H₂O (3:1), 23 °C, 3 h, 52 % over two steps; c) **5** (7.0 equiv), DEAD (7.0 equiv), Ph₃P (7.0 equiv), THF, -15 °C, 1.5 h, 66 %; d) **6** (10.0 equiv), [(Cy₃P)₂Cl₂RuCHPh], (2 × 0.1 equiv), benzene, 65 °C, 15 h, 60 %; e) **7** (4.0 equiv), NaHCO₃ (10.0 equiv), 4-Å molecular sieves, 23 °C (vacuum), 0.5 h; DMF, 65 °C, 18 h, 72 %, f) allylBr (10.0 equiv), NaHCO₃ (10.0 equiv), 4-Å molecular sieves, 23 °C (vacuum), 0.5 h; 8 h, 97 %. Cbz = benzyloxycarbonyl, Cy = cyclohexyl, DEAD = diethyl azodicarboxylate, DMF = *N,N*-dimethylformamide, lut. = lutidine, TBS = *tert*-butyldimethylsilyl.

stability, as well as mild loading and cleavage conditions compatible with silyl protecting groups. Our first efforts focused on photolabile^[7] linkers because of their mild cleavage; however, an acceptable compromise between loading and cleavage efficiency could not be reached. We then turned our attention to allylic ester linkers.^[8] As shown in Scheme 1, protected vancomycin **2** was loaded in good yield using Mitsunobu, cross metathesis, or alkylation techniques. All three linkers were cleaved in excellent yield under the very mild palladium(0)-mediated allyl transfer conditions.^[9] However, the cleavage efficiency of the linkers in **3a** and **3b** was dramatically reduced after exposure to the Lewis acidic conditions utilized in the glycosidation, presumably as a result of a [3,3] sigmatropic rearrangement^[10] to give a secondary allylic ester. The allylic acrylate linker in **3c**, although stable to the glycosidation conditions, was found to be susceptible to premature cleavage and deactivation, which was assumed to arise from a nucleophilic attack on the acrylate functionality. It soon became clear that despite the attractiveness of their

mild and efficient cleavage, the linkers in **3a–c** were not suitable for application to the vancomycin problem.

In the search for alternative strategies to solve the problem at hand we turned our attention to our recently reported preparation of polymer-bound selenenyl bromide.^[11] We reasoned that the facile oxidation/elimination of the polymer-bound phenylseleno group could be used to mask an allylic functionality (Figure 1), thus serving as a pro-allyl safety-catch linker. Since the presence of residual tin can be detrimental to purification and biological assays we further envisioned the use of polymer-bound tin hydride^[12] for the removal of the allyl group. Thus, the proposed strategy retains the critical, but cumbersome elements on the resin, and provides a measure of waste control and disposal. To test the scope of this proposal, we prepared resins **9–11** (Scheme 2). Thus, LiBH₄ reduction of the polymer-bound phenyl selenenyl bromide **8** afforded the corresponding lithiated selenium species, which was quenched with either 1,3-diiodopropane or 3-iodopropanol to furnish resins **9** or **10**, respectively. Treat-

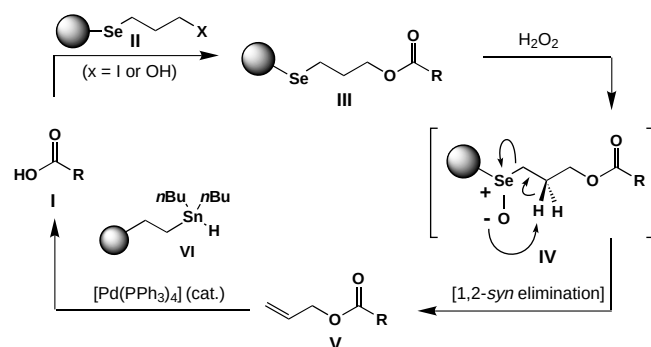
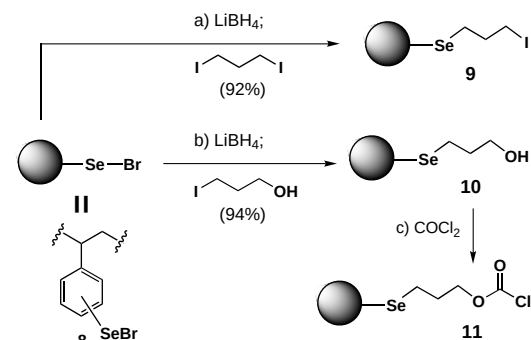
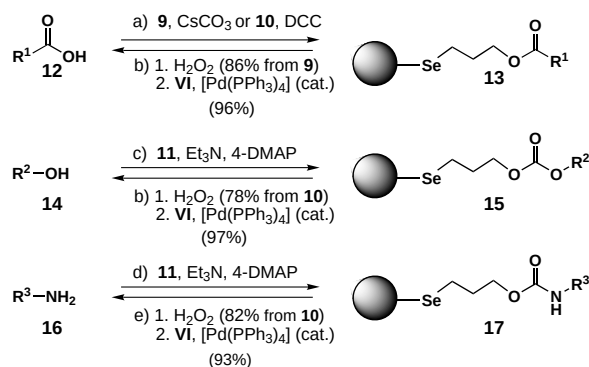


Figure 1. General concept of the selenium-based resin as a pro-allyl or pro-alloc safety-catch linker.



Scheme 2. Synthesis of pro-allyl and pro-alloc selenium linkers. Reagents and conditions: a) LiBH_4 (1.5 equiv), THF, 0°C , 0.5 h, wash, 1,3-diiodopropane (4.0 equiv), THF:DMF (1:1), 23°C , 5 h, 92%; b) LiBH_4 (1.5 equiv), THF, 0°C , 0.5 h, wash, 3-iodopropanol (4.0 equiv), THF:DMF (1:1), 23°C , 5 h, 94%; c) COCl_2 (4.0 equiv), THF, 23°C , 5 h. Yields are calculated on the basis of the mass gain of the polymer. Alloc = allyloxy-carbonyl.

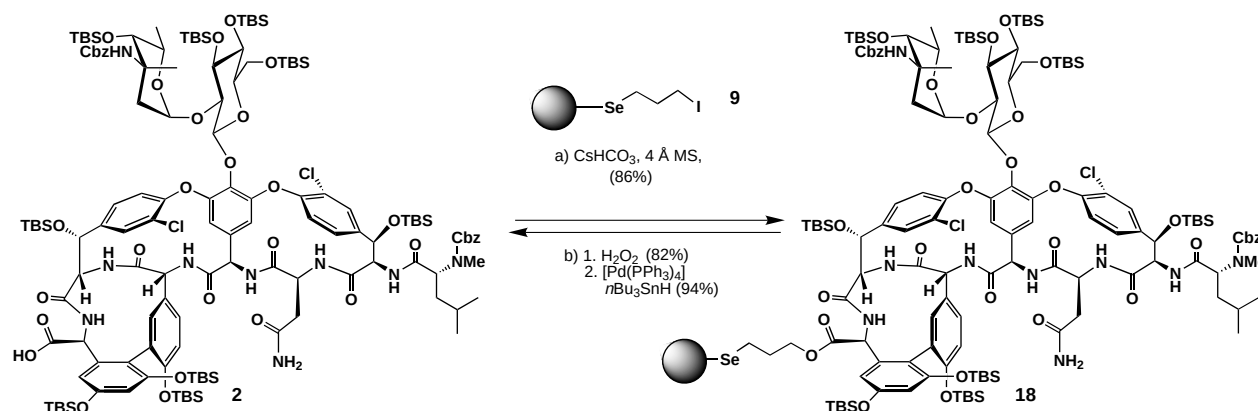
ment of resin **10** with phosgene afforded polymer-bound chloroformate **11**. We were gratified to find that the pro-allyl ester **13** (Scheme 3) was formed smoothly by either alkylation or esterification of resins **9** and **10**, respectively. Furthermore, formation of pro-alloc derivatives **15** and **17** also proceeded smoothly (Scheme 3). Treatment of polymer-bound pro-allyl derivative **13** as well as pro-alloc compounds **15** and **17** with H_2O_2 , followed by exposure to catalytic amounts of $[\text{Pd}(\text{PPh}_3)_4]$ and polymer-bound tin hydride **VI** (Figure 1)



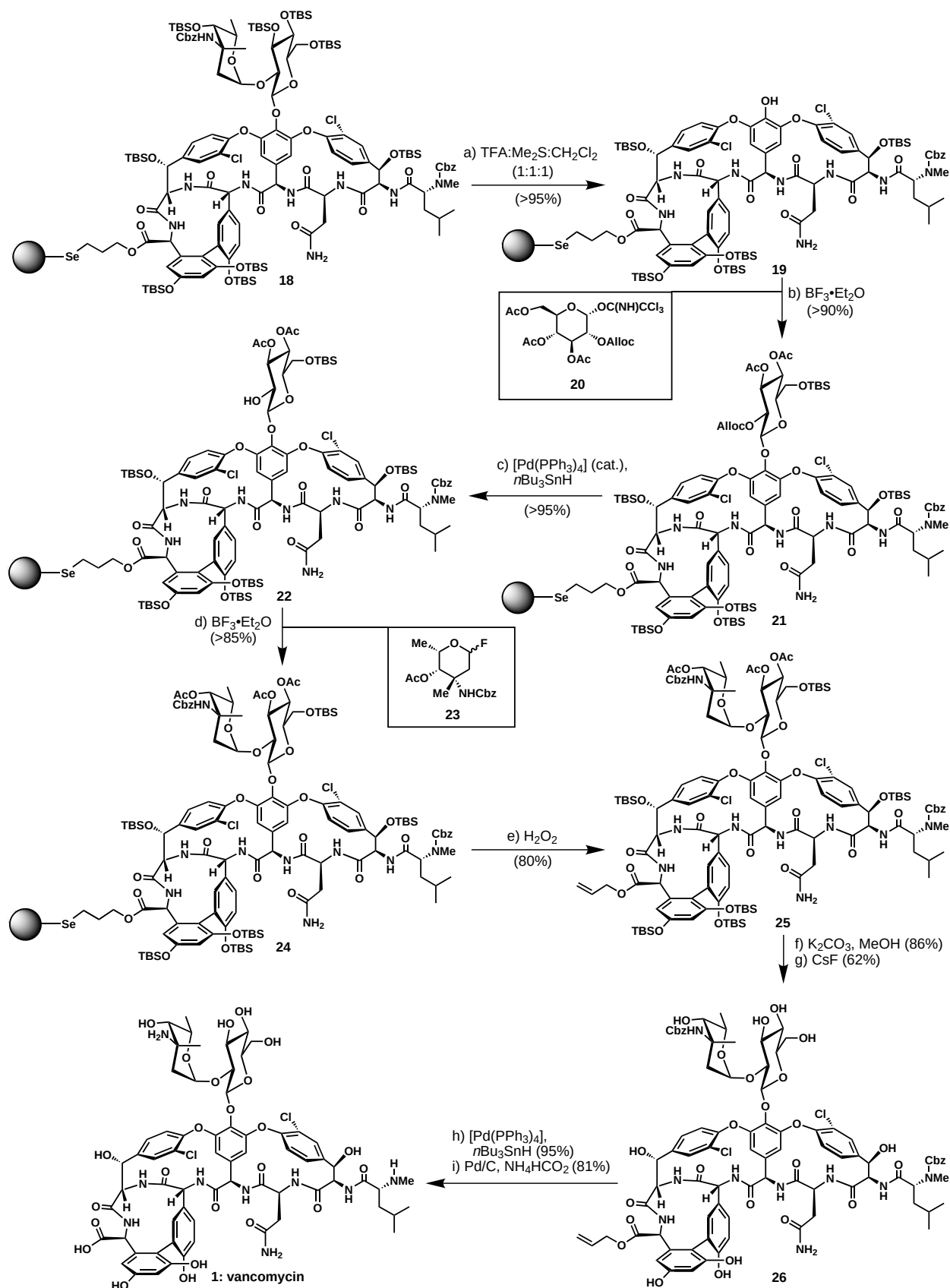
Scheme 3. Loading onto and cleavage from pro-allyl and pro-alloc selenium resins. Reagents and conditions: a) **12** (4.0 equiv), CsCO_3 (4.0 equiv), DMF, 50°C , 5 h or **12** (4.0 equiv), DCC (4.0 equiv), DMF, 23°C , 15 h; b) 1. H_2O_2 (2.0 equiv), THF, 23°C , 2 h; Me_2S (4.0 equiv), 60°C , 10 h, 86% from **9** and 78% from **10**; 2. **VI** (3.0 equiv), $[\text{Pd}(\text{PPh}_3)_4]$ (0.1 equiv), CH_2Cl_2 , 23°C , 0.5 h, 96% for **12** and 97% for **14**; c) **11** (4.0 equiv), Et_3N (5.0 equiv), 4-DMAP (0.1 equiv), 23°C , 6 h; d) **11** (4.0 equiv), Et_3N (5.0 equiv), 4-DMAP (0.1 equiv), 23°C , 3 h; e) 1. H_2O_2 (2.0 equiv), THF, 23°C , 2 h; Me_2S (4.0 equiv), 60°C , 10 h, 82% from **10**; 2. **VI** (3.0 equiv), $[\text{Pd}(\text{PPh}_3)_4]$ (0.1 equiv), AcOH (4.0 equiv), CH_2Cl_2 , 23°C , 0.5 h, 93%. R^1 = 4-bromophenyl, R^2 = 4-iodobenzyl, R^3NH_2 = methoxyvaline, DCC = *N,N'*-dicyclohexylcarbodiimide, 4-DMAP = 4-dimethylaminopyridine.

furnished carboxylic acid **12**, alcohol **14**, and amine **16** in yields of 86, 78, and 82%, respectively. More importantly, loading and cleavage of protected vancomycin derivative **2** proceeded smoothly via intermediate **18** (Scheme 4) and without the previously discussed limitations of the allyl linkers shown in Scheme 1.

The now readily available polymer-bound vancomycin derivative **2** was utilized to demonstrate an efficient sequence of deglycosidation and re-glycosidation reactions leading to vancomycin (**1**, Scheme 5). Thus, the disaccharide moiety was hydrolyzed from **2** under acidic conditions to provide polymer-bound phenol **19**, which was glycosylated with the imidate **20**^[6a] ($\text{BF}_3 \cdot \text{Et}_2\text{O}$) to afford glycoside **21**. Deprotection of the C-2 alloc group using palladium(0)-mediated allyl transfer conditions afforded compound **22** (see Table 1) in excellent yield. The polymer-bound monosaccharide **22** was then subjected to a second glycosidation reaction with



Scheme 4. Loading and cleavage of the vancomycin scaffold using a pro-allyl safety-catch linker. Reagents and conditions: a) **9** (8.0 equiv), CsHCO_3 (5.0 equiv), DMF, 23°C (vacuum), 0.5 h, 50°C , 5 h, 86%; b) 1. H_2O_2 (2.0 equiv), THF, 23°C , 2 h; Me_2S (4.0 equiv), 23°C , 48 h, 82%; 2. $[\text{Pd}(\text{PPh}_3)_4]$ (0.1 equiv), $n\text{BuSn}_3\text{H}$ (4.0 equiv), CH_2Cl_2 , 0.5 h, 94%. The loading yield is based on the mass gain of the polymer.



Scheme 5. Solid-phase semisynthesis of vancomycin **1**. Reagents and conditions. a) TFA:Me₂S:CH₂Cl₂ (1:1:1), 23 °C, 3 h, >95%; b) **20** (6.0 equiv), BF₃·Et₂O (9.0 equiv), 4-Å molecular sieves, -40 → 0 °C, 12 h, >90%; c) [Pd(PPh₃)₄] (0.1 equiv), *n*Bu₃SnH (10.0 equiv), CH₂Cl₂, 23 °C, 2 h, >95%; d) **23** (4.0 equiv), BF₃·Et₂O (6.0 equiv), -40 → 0 °C, 4 h, >85%; e) H₂O₂, THF, 23 °C, 48 h, 80%; f) K₂CO₃, MeOH, 23 °C, 6 h, 86%; g) CsF, DMF, 23 °C, 15 h, 62%; h) [Pd(PPh₃)₄], *n*Bu₃SnH, DMF, 23 °C, 1 h, 95%; i) 10% Pd/C, NH₄HCO₂, H₂O:AcOH (1:1), 23 °C, 4 h, 81%. Ac = acetyl, TFA = trifluoroacetic acid. Yields for the reactions carried out on a solid phase are estimates based on ¹H NMR, MS, and TLC analysis of the crude cleavage product.

Table 1. Selected physical data for compounds **22** and **25**.

22 (oxidative cleavage product): $R_f = 0.39$ (silica gel, 5% MeOH in CH_2Cl_2); ^1H NMR (600 MHz, CD_3OD, 330 K): $\delta = 7.53$–7.48 (m, 2H), 7.43–7.38 (m, 2H), 7.38–7.27 (m, 7H), 7.27 (s, 1H), 7.08 (d, $J = 2.0$ Hz, 1H), 7.01 (dd, $J = 9.5$, 2.0 Hz, 1H), 6.78 (d, $J = 8.0$ Hz, 1H), 6.41 (d, $J = 1.5$ Hz, 1H), 6.39 (d, $J = 1.5$ Hz, 1H), 5.98–5.90 (m, 1H), 5.90–5.87 (m, 1H), 5.79 (s, 1H), 5.56–5.17 (m, 9H), 5.01–4.84 (m, 6H), 4.73–4.50 (m, 6H), 4.25–4.18 (m, 1H), 4.11 (s, 1H), 3.89–3.84 (m, 1H), 3.74–3.68 (m, 1H), 3.78–3.70 (m, 1H), 3.60 (dd, $J = 11.0$, 3.2 Hz, 1H), 2.93 (s, 3H, NCH_3), 2.55–2.40 (m, 2H), 2.03 (s, 3H, COCH_3), 2.00 (s, 3H, COCH_3), 1.78–1.75 (m, 1H), 1.53–1.49 (m, 2H), 1.01 (s, 9H, $t\text{BuSi}$), 0.94 (s, 9H, $t\text{BuSi}$), 0.93–0.91 (m, 6H), 0.89 (s, 9H, $t\text{BuSi}$), 0.77 (s, 9H, $t\text{BuSi}$), 0.73 (s, 9H, $t\text{BuSi}$), 0.63 (s, 9H, $t\text{BuSi}$), 0.23 (s, 6H, CH_3Si), 0.18 (s, 3H, CH_3Si), 0.12 (s, 3H, CH_3Si), 0.10 (s, 3H, CH_3Si), 0.09 (s, 3H, CH_3Si), 0.09 (s, 3H, CH_3Si), 0.08 (s, 3H, CH_3Si), 0.04 (s, 3H, CH_3Si), 0.03 (s, 3H, CH_3Si), –0.08 (s, 3H, CH_3Si), –0.09 (s, 3H, CH_3Si); MS (ES^+): calcd for $\text{C}_{110}\text{H}_{160}\text{Cl}_2\text{N}_8\text{O}_{26}\text{Si}_6$ [$M + \text{H}^+$]: 2247; found: 2247. 25: $R_f = 0.40$ (silica gel, 5% MeOH in CH_2Cl_2); ^1H NMR (600 MHz, CD_3OD, 330 K): $\delta = 7.65$–7.45 (m, 3H), 7.40–7.20 (m, 12H), 7.10–7.08 (m, 2H), 7.03 (m, 2H), 6.80 (d, $J = 0.5$ Hz, 1H), 6.78 (d, $J = 0.5$ Hz, 1H), 6.44 (d, $J = 2.0$ Hz, 1H), 6.40 (s, 1H), 5.91 (m, 1H), 5.83–5.78 (m, 2H), 5.64–5.45 (m, 2H), 5.44–5.28 (m, 5H), 5.26–5.10 (m, 5H), 5.01 and 4.88 (AB, $J = 12.5$ Hz, 2H), 4.64 (m, 2H), 4.18–4.07 (m, 2H), 3.85–3.80 (m, 2H), 3.64 (m, 1H), 2.94 (s, 3H, NCH_3), 2.48 (m, 2H), 2.03 (s, 3H), 2.05–1.98 (m, 5H), 1.92 (s, 3H), 1.55 (brs, 3H), 1.29 (s, 3H), 1.09 (m, 3H), 1.02 (s, 9H, $t\text{BuSi}$), 0.97 (s, 9H, $t\text{BuSi}$), 0.93 (m, 6H), 0.88 (s, 9H, $t\text{BuSi}$), 0.82 (s, 9H, $t\text{BuSi}$), 0.79 (s, 9H, $t\text{BuSi}$), 0.71 (s, 9H, $t\text{BuSi}$), 0.24 (s, 3H, CH_3Si), 0.23 (s, 3H, CH_3Si), 0.19 (s, 3H, CH_3Si), 0.18 (s, 3H, CH_3Si), 0.13 (s, 3H, CH_3Si), 0.12 (s, 3H, CH_3Si), 0.10 (m, 6H, CH_3Si), 0.04 (s, 3H, CH_3Si), 0.03 (s, 3H, CH_3Si), –0.06 (s, 3H, CH_3Si), –0.08 (s, 3H, CH_3Si); MS (ES^+): calcd for $\text{C}_{127}\text{H}_{181}\text{Cl}_2\text{N}_9\text{O}_{31}\text{Si}_6\text{Na}$ [$M + \text{Na}^+$]: 2592; found: 2592.

glycosyl fluoride **23**^[6a] ($\text{BF}_3 \cdot \text{Et}_2\text{O}$) to deliver fully protected polymer-bound vancomycin derivative **24**, which was cleaved with H_2O_2 to release allyl derivative **25** (see Table 1). Finally, vancomycin (**1**) was obtained from **26** through a deprotection sequence involving deacetylation ($\text{K}_2\text{CO}_3/\text{MeOH}$), desilylation (CsF), deprotection of the carboxylic acid group ($[\text{Pd}(\text{PPh}_3)_4]/n\text{Bu}_3\text{SnH}$ ^[13]), and Cbz cleavage (10% Pd/C , NH_4HCO_2) via intermediate **26**. It is important to note that the four-step deprotection sequence is carried out without work-ups and requires only two purifications (at the stages where **26** and **1** are formed). An interesting solvent effect was observed during the course of studying the desilylation of this series of compounds. Namely, it was found that the phenolic silyl groups could be cleanly removed without affecting the other secondary silyl-protected hydroxyl groups on the core of vancomycin by carrying out the CsF -induced deprotection in CH_3CN rather than DMF.

In conclusion, we have prepared a new set of selenium-based safety-catch linkers for carboxylic acids, alcohols, and amines. The utility of one of these linkers was demonstrated with a solid-phase semisynthesis of vancomycin (**1**). The developed technology and sequence has potential for the construction of vancomycin libraries for biological screening purposes starting from the readily available vancomycin scaffold.

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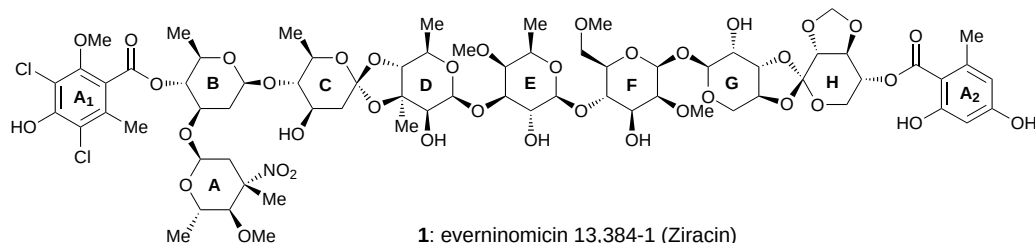
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1,2-Seleno Migrations in Carbohydrate Chemistry: Solution and Solid-Phase Synthesis of 2-Deoxy Glycosides, Orthoesters, and Allyl Orthoesters**

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In the field of antibiotics lead compounds derived from natural sources, for example, vancomycin and penicillin, provide a starting point from which a library of analogues can be synthesized and screened for biological activity. The naturally derived everninomicin 13,384-1 (Ziracin, **1**),^[1] a potent antibiotic against methicillin-resistant *staphylococci* and vancomycin-resistant *enterococci*, is rapidly advancing through clinical trials as a new weapon against multidrug resistant bacteria. With the total synthesis of this antibiotic now accomplished,^[2] the feasibility of constructing semisynthetic analogues for biological investigations through solution and solid-phase combinatorial chemistry can be put to the test. Furthermore, the synthesis of libraries of compounds representing novel regions, such as the 2-deoxy glycoside and orthoester moieties, of this complex molecule for general screening purposes is deemed highly desirable and important. Herein we report a general method for the stereocontrolled construction of 2-deoxy glycosides and orthoesters, both in solution and on a solid support, which is facilitated by a 1,2-selenium migration.

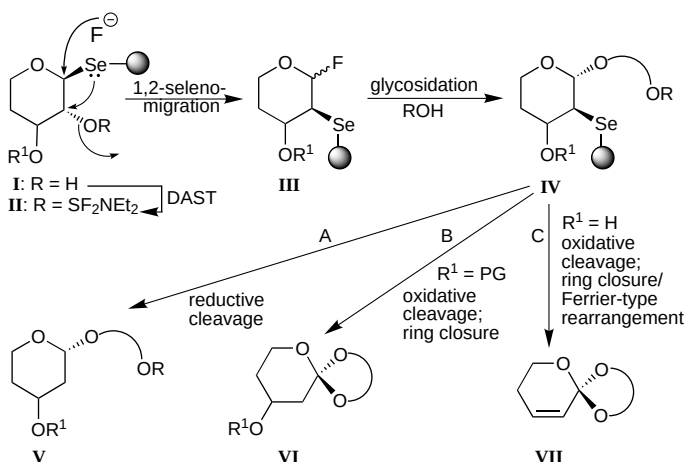
Based on the observation of the 1,2-phenylseleno migration^[3] and its application to the synthesis^[2] of everninomicin's



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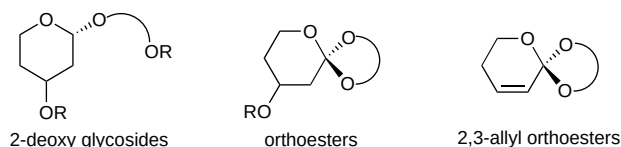
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orthoester moieties in solution together with recognizing the potential use of our previously synthesized polystyrene-based selenium resin,^[4] we designed the chemistry shown in Scheme 1. A general method employing this novel 1,2-selenium migration reaction was envisioned for both the



Scheme 1. General concept for the stereoselective synthesis of 2-deoxy glycosides (**V**), orthoesters (**VI**), and allyl orthoesters (**VII**) via 1,2-selenium migrations. PG = protecting group.

solution (● = phenyl) and solid-phase (■ = polystyrene) synthesis of 2-deoxy glycosides, 2-deoxy orthoesters, and 2,3-allyl orthoesters from structures of general type **I**. Thus, treatment of the readily available 2-hydroxy-1-seleno glycoside (**I**) with diethylaminosulfur trifluoride (DAST)^[5] should result in a stereospecific 1,2-migration of the selenium group, with simultaneous installation of a fluoride group at C-1, to furnish **III**. Exposure of these reactive donors (**III**) to various alcohols in the presence of Lewis acids should then selectively afford the desired α -glycosides **IV** through participation of the seleno group. From **IV**, one of the following three paths may be followed: path A should furnish the 2-deoxy glycosides **V** in



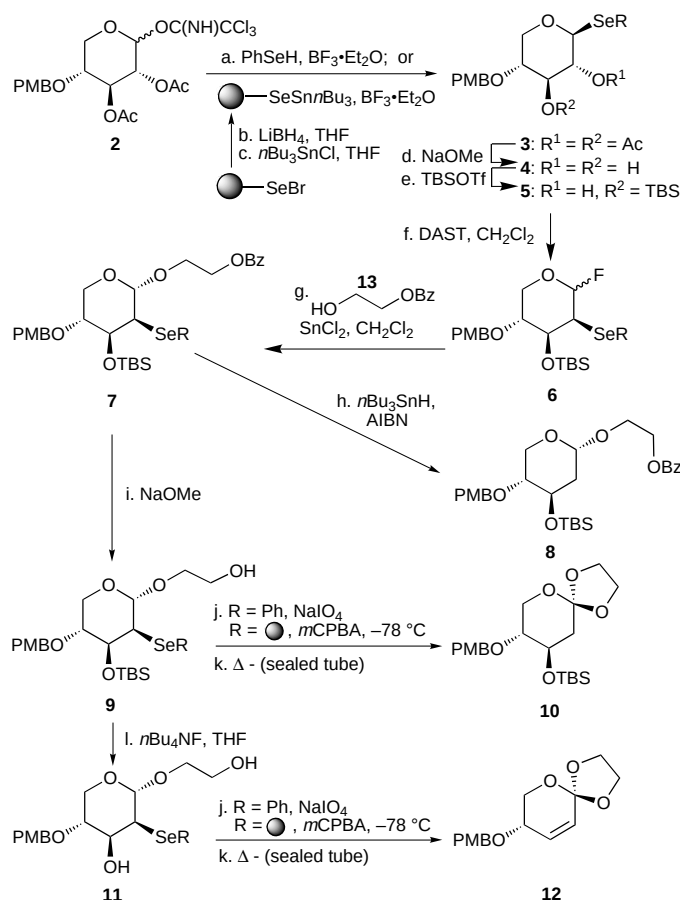
high yield, via radical deselenation, facilitated with $n\text{Bu}_3\text{SnH}$; path B would first require removal of the protecting group R, followed by oxidation of the selenium to the selenoxide and heating to promote the *syn*-elimination and cyclization, to furnish 2-deoxy orthoesters (**VI**); and path C would require that both protecting groups (R and R¹) be removed before subsequent oxidation and heating, and should afford the 2,3-

allyl orthoesters (**VII**) through a Ferrier-type rearrangement.^[6]

As a demonstration of the efficiency and utility of the 1,2-seleno-migration chemistry, we employed three different carbohydrate donors which were coupled with three alcohols of varying complexity, to afford a small library of 2-deoxy glycosides and orthoesters, followed by the formation of three 2,3-allyl orthoesters. Scheme 2 illustrates the method and conditions used for one example, while Tables 1 and 2 provide combined yields for all the examples. Solution-phase β -seleno glycosides such as **3** were prepared by treating the respective trichloroacetimidate donors^[7] (**2**, Scheme 2) with freshly prepared PhSeH^[8] in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$, using C-2 esters as participating agents to control the anomeric ratio. The sugar was further manipulated to remove the ester groups to afford **4** followed by selective silylation to furnish **5**. Treatment of the 2-hydroxy compound with DAST facilitated the 1,2-selenophenyl migration affording the 2-seleno-1-fluoro donor **6** in excellent yield. The donor was coupled with alcohol **13** in the presence of SnCl_2 to selectively form the α -glycoside **7** in 95% yield. Formation of the 2-deoxy glycoside (**8**) was achieved by exposure to $n\text{Bu}_3\text{SnH}/\text{AIBN}$ via radical cleavage of the Se–C bond. Deprotection of the ester from **7** to afford **9**, followed by oxidation of selenium to the selenoxide, and heating in a sealed tube^[9] led to the formation of the 2-deoxy orthoester **10** in 87% yield. On the other hand, removal of the silyl group from **9** to afford diol **11**, followed by oxidation to the selenoxide and heating in a

sealed tube as described above, led to elimination of the C-2 hydroxyl group with simultaneous migration of the double bond and formation of the orthoester to afford the 2,3-allyl orthoester **12** in good yield (79%). Table 1 provides the combined yields (66–95%) for the formation of the glycosides from the glycosylation of the donors **16**, **17**, and **6** with alcohols **13**, **14**, and **15**, as well as the formation of the corresponding 2-deoxy glycosides. Table 2 provides yields for the formation of the orthoester for each glycoside as well as for the examples of 2,3-allyl orthoesters. In order to compare the stereochemistry of the 2-deoxy orthoester and the 2,3-allyl orthoester (**42** and **43**, Table 2, see Table 3 for spectroscopic data), **42** was transformed into its respective 2,3-allyl orthoester by first removal of the TBS group (TBAF, THF, 95%) followed by dehydration (Martin sulfurane, CHCl_3 , 50°C, 1 h, 80%). Examination of the NMR spectrum of each orthoester revealed that the allyl orthoester derived from **42** (three step procedure) had the opposite configuration of **43** (one-pot procedure). Thus, while the stereochemistry during 2-deoxy orthoester formation (**42**) is reported to be controlled by the anomeric effect (the alcohol approaches the ketene acetal from an axial direction so as to maximize the anomeric effect), in the case of the 2,3-allyl orthoester (**43**), the incoming alcohol must come from the top face or same side as the leaving hydroxyl group. A more detailed investigation of this stereochemical effect is on-going.

Satisfied with the results of the solution-phase chemistry, we now proceeded to apply this technology to solid-phase



Scheme 2. Solution and solid-phase synthesis of 2-deoxy glycosides (**8**), 2-deoxy orthoesters (**10**), and 2,3-allyl orthoesters (**12**). Reagents and conditions: R = Ph: a) 2.0 equiv of PhSeH (0.5 M solution in CH_2Cl_2), 1.5 equiv of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ CH_2Cl_2 , -78°C , 2 h, 90%; b) 2.5 equiv of NaOMe, MeOH, 25°C , 3 h, 95%; c) 1.1 equiv of TBSOTf, 1.3 equiv of 2,6-lutidine, CH_2Cl_2 , -78°C , 1 h, 90%; d) 1.5 equiv of DAST, CH_2Cl_2 , 0°C , 0.5 h, 100%; e) 1.5 equiv of $\text{HOCH}_2\text{CH}_2\text{OBz}$, 1.5 equiv of SnCl_2 , CH_2Cl_2 , 0°C , 3 h, 95%; f) 10 equiv of $n\text{Bu}_3\text{SnH}$, 0.1 equiv of AIBN, benzene, 80°C , 1 h, 90%; g) 1.5 equiv of NaOMe, MeOH, 25°C , 3 h, 95%; h) 10 equiv of $n\text{Bu}_3\text{SnH}$, 0.1 equiv of AIBN, benzene, 80°C , 1 h, 95%; i) 5.0 equiv of NaOMe, MeOH, 25°C , 3 h, >95%; j) 3.0 equiv of $m\text{CPBA}$, CH_2Cl_2 , -78°C , 10 min; k) vinyl acetate:toluene:diisopropylamine (2:2:1), sealed tube, 140°C , 12 h, >85%; l) 5.0 equiv of $n\text{Bu}_4\text{NF}$, THF, 25°C , 1 h, >95%. R = polystyrene: a) 3.0 equiv of **2**, 1.5 equiv of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ CH_2Cl_2 , -78°C , 2 h, >90%; b) 1.5 equiv of LiBH_4 , THF, 25°C , 2 h, 100%; c) 20 equiv of $n\text{Bu}_3\text{SnCl}$, THF, 25°C , 2 h, 100%; d) 5.0 equiv of NaOMe, MeOH, 25°C , 3 h, >95%; e) 1.1 equiv of TBSOTf, 1.3 equiv of 2,6-lutidine, CH_2Cl_2 , -78°C , 1 h, >80%; f) 3.0 equiv of DAST, CH_2Cl_2 , 0°C , 0.5 h, 100%; g) 10 equiv of $\text{HOCH}_2\text{CH}_2\text{OBz}$, 3.0 equiv of SnCl_2 , CH_2Cl_2 , 0°C , 3 h, >89%; h) 10 equiv of $n\text{Bu}_3\text{SnH}$, 0.1 equiv of AIBN, benzene, 80°C , 1 h, 95%; i) 5.0 equiv of NaOMe, MeOH, 25°C , 3 h, >95%; j) 3.0 equiv of $m\text{CPBA}$, CH_2Cl_2 , -78°C , 10 min; k) vinyl acetate:toluene:diisopropylamine (2:2:1), sealed tube, 140°C , 12 h, >85%; l) 5.0 equiv of $n\text{Bu}_4\text{NF}$, THF, 25°C , 1 h, >95%. Yields from the solid support reported here represent purity as determined by NMR spectroscopy, see Tables 1 and 2 for overall yields determined by cleavage. TBSOTf = *tert*-butyldimethylsilyl trifluoromethanesulfonate; PMB = *p*-methoxybenzyl; AIBN = 2,2'-azobisisobutyronitrile; DAST = diethylaminosulfur trifluoride; *m*CPBA = *meta*-chloroperoxybenzoic acid; $\bullet$ = polystyrene.

Table 1. Solution and solid-phase synthesis of 2-seleno glycosides and 2-deoxy glycosides.^[a]

Acceptors =	Donors =				
		13	14	15	
16a: R = Ph (100%) 16b: R = (100%)		18a: Y = SePh (95%) 18b: Y = Se- (94%) 19: Y = H (30%, >78%)	22a: Y = SePh (87%) 22b: Y = Se- (93%) 23: Y = H (15%, >69%)	28a: Y = SePh (71%) 28b: Y = Se- (91%) 29: Y = H (11%, >64%)	
17a: R = Ph (100%) 17b: R = (100%)		20a: Y = SePh (92%) 20b: Y = Se- (91%) 21: Y = H (32%, >80%)	24a: Y = SePh (86%) 24b: Y = Se- (94%) 25: Y = H (18%, >71%)	30a: Y = SePh (70%) 30b: Y = Se- (95%) 31: Y = H (13%, >66%)	
6a: R = Ph (100%) 6b: R = (100%)		7a: Y = SePh (94%) 7b: Y = Se- (90%) 8: Y = H (30%, >78%)	26a: Y = SePh (85%) 26b: Y = Se- (93%) 27: Y = H (17%, >70%)	32a: Y = SePh (66%) 32b: Y = Se- (92%) 33: Y = H (11%, >65%)	

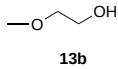
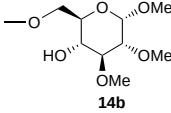
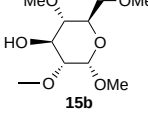
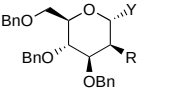
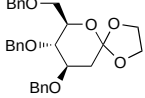
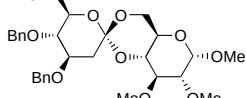
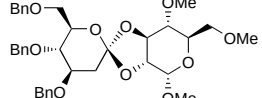
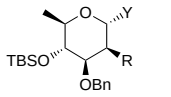
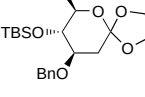
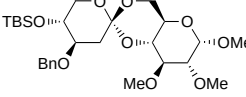
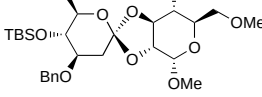
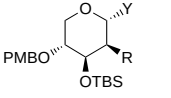
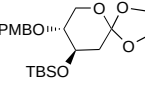
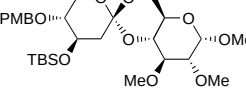
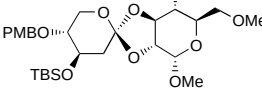
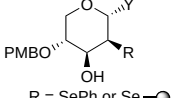
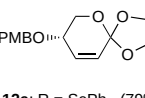
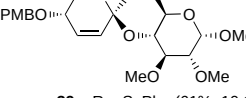
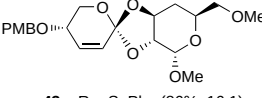
[a] Yields of solid-phase glycosylations reported as the overall yield of 2-deoxy glycoside released from the resin (overall yield, average yield for each step (0.78⁵ = 0.30)). [b] *n*Bu₃SnH, AIBN, benzene, 80 °C, 1 h; = polystyrene.

chemistry. Before attempting the coupling between the resin and sugars, we required a resin-bound selenol in a form that could be manipulated in air (PhSeH rapidly oxidatively dimerizes in air, and must be kept under argon at all times). It was found that the *n*Bu₃Sn ether of the resin-bound selenol was easy to prepare, fairly stable to air, and quite reactive in couplings. This reagent was readily prepared from the previously reported selenium bromide resin^[4] (Scheme 2) by lithiation (LiBH₄, THF) followed by quenching with excess *n*Bu₃SnCl to form a colorless, odorless resin, which could be quickly filtered in air. Treatment of this resin with three equivalents of the trichloroacetimidate donors in the presence of BF₃·Et₂O led to excellent loading of the sugar (as determined by IR spectroscopy and cleavage with *n*Bu₃SnH). Of particular interest was the observation that cleavage of the resin-bound 1- or 2-seleno glycosides with *n*Bu₃SnH/AIBN regenerated the *n*Bu₃SnSe resin, which could be filtered off and re-used in subsequent coupling reactions. As in the solution-phase chemistry, the C-2 esters were removed under basic hydrolysis (NaOMe, THF/MeOH), followed by exposure to DAST to precipitate the 1,2-migration of the seleno resin and yield the resin-bound 1-fluoro-2-seleno donors (**16b**, **17b**, and **6b**, Table 1). The filtrate from the DAST reaction was monitored for products of elimination; surprisingly, very little, if any, was observed. The fluoro donors were exposed to 10 equivalents of alcohols **13**, **14**, and **15** in the presence of SnCl₂ to afford the primary alcohols (**18b**, **22b**, **20b**, **24b**, **7b**, **26b**, >70 %, Table 1) and secondary alcohols (**28b**, **30b**, **32b**,

>60 %, Table 1). The 2-deoxy glycosides were prepared as described above by cleavage with *n*Bu₃SnH/AIBN (Table 1).^[10] Deprotection of the esters was smoothly effected by basic hydrolysis (NaOMe, THF/MeOH), setting the stage for formation of the orthoester. Several methods for oxidizing the selenium to the selenoxide were investigated, including *m*CPBA in CH₂Cl₂ at –78 °C, H₂O₂ in THF/H₂O at 0 °C, and O₃ in toluene at –78 °C. Unlike the solution-phase selenoxides, it was observed that the resin-bound selenoxide was more prone to eliminate at room temperature and therefore necessitated the use of lower temperatures in the oxidation step. Thus, treatment with *m*CPBA in CH₂Cl₂ at –78 °C followed by rapid filtration and transfer to a sealed tube was found to give the cleanest results. The selectivity in the stereochemistry of the orthoester formed on a solid support paralleled that observed in the solution-phase chemistry. Furthermore, the 2,3-allyl orthoesters were again prepared by first removal of the silyl group followed by oxidation and orthoester formation.^[10]

In conclusion, we have investigated the scope of the 1,2-selenium migration in carbohydrates and have found that 2-deoxy glycosides, orthoesters, and allyl orthoesters^[11] can be readily prepared with good selectivities and in high yield. We have also described a novel solid-phase construction of carbohydrate orthoesters employing a selenium-based linking strategy, where the selenium atom serves to link, couple, and facilitate orthoester formation in an efficient and traceless manner. The described technology can now be expanded to

Table 2. Solution and solid-phase synthesis of 2-deoxy orthoesters and 2,3-allyl orthoesters.^[a]

Y =			
			
R = SePh or Se- 	34a: R = SePh (87%) 34b: R = Se-  (12%, >74%)	36a: R = SePh (83%, 3.2:1) 36b: R = Se-  (11%, >73%, 2.2:1)	40a: R = SePh (75%, 15:1) 40b: R = Se-  (2%, >57%, 15:1)
			
R = SePh or Se- 	35a: R = SePh (85%) 35b: R = Se-  (20%, >80%)	37a: R = SePh (81%, 2.7:1) 37b: R = Se-  (5%, >65%, 1:1)	41a: R = SePh (73%, 12:1) 41b: R = Se-  (3%, >61%, 12:1)
			
R = SePh or Se- 	10a: R = SePh (82%) 10b: R = Se-  (15%, >76%)	38a: R = SePh (82%, 2:1) 38b: R = Se-  (8%, >70%, 1:1)	42a: R = SePh (72%, 10:1) 42b: R = Se-  (7%, >68%, 10:1)
			
R = SePh or Se- 	12a: R = SePh (79%) 12b: R = Se-  (19%, >81%)	39a: R = SePh (61%, 10:1) 39b: R = Se-  (6%, >70%, 10:1)	43a: R = SePh (86%, 16:1) 43b: R = Se-  (5%, >69%, 16:1)

[a] Yields of orthoesters derived from solution: (combined yield for deprotection, oxidation, and orthoester formation, as well as the ratio of the orthoester diastereoisomers). Yields of orthoesters derived from solid support (overall yield for the sequence from the selenium bromide resin was determined by the weight of released product, average yield for each step (0.74^a = 0.12), ratio of orthoester diastereoisomers. (PS = polystyrene).

Table 3. Selected physical properties of compounds **42** and **43**.

42: R_f = 0.35 (silica, 50 % Et₂O in hexanes); α_D^{25} = +15.3 (c = 1.30, CHCl₃); IR (thin film) $\tilde{\nu}_{\max}$ = 2931, 2856, 1613, 1514, 1468, 1383, 1320, 1250, 1203, 1104, 963, 920, 838, 778 cm⁻¹; ¹H NMR (600 MHz, CDCl₃, 10:1): δ = 7.24 (d, J = 8.5 Hz, 2H, PMB), 6.85 (d, J = 8.5 Hz, 2H, PMB), 5.07 (d, J = 3.0 Hz, 1H, H-1'), 4.65 and 4.51 (AB, J = 11.4 Hz, 2H, CH₂Ar), 4.03 (t, J = 9.6 Hz, 1H, H-3'), 3.98–3.92 (m, 1H, H-3), 3.82 (dd, J = 10.1, 3.1 Hz, 1H, H-2'), 3.78 (s, 3H, OMe), 3.77 (dd, J = 11.2, 4.8 Hz, 1H, H-5), 3.61 (brs, 2H, H-6'), 3.55 (t, J = 11.4 Hz, 1H, H-5), 3.52–3.48 (m, 2H, H-4', H-5'), 3.49 (s, 3H, OMe), 3.41 (s, 3H, OMe), 3.39 (s, 3H, OMe), 3.33 (ddd, J = 9.1, 9.1, 4.1 Hz, 1H, H-4), 2.13 (dd, J = 13.0, 5.0 Hz, 1H, H-2), 1.91 (dd, J = 13.0, 10.0 Hz, 1H, H-2), 0.86 (s, 9H, *t*BuSi), 0.08 (s, 3H, MeSi), 0.07 (s, 3H, MeSi); ¹³C NMR (150 MHz, CDCl₃): δ = 159.2, 130.6, 129.4, 120.3, 113.7, 97.2, 78.7, 78.5, 75.2, 72.9, 71.4, 70.7, 70.6, 62.9, 59.3, 58.7, 55.5, 55.2, 40.5, 30.3, 25.7, 18.0, –4.6, –4.8; HR-MS (MALDI): calcd for C₂₈H₄₆O₁₀SiNa [M +Na⁺]: 593.2758; found: 593.2755.

43: R_f = 0.32 (silica, 100 % Et₂O); α_D^{25} = +70.0 (c = 0.17, CHCl₃); IR (thin film) $\tilde{\nu}_{\max}$ = 2983, 2922, 2862, 1617, 1512, 1451, 1384, 1248, 1182, 1077, 1017, 918, 819 cm⁻¹; ¹H NMR (500 MHz, CDCl₃, 10:1): δ = 7.25 (d, J = 8.6 Hz, 2H, PMB), 6.86 (d, J = 8.6 Hz, 2H, PMB), 6.15 (dd, J = 10.0, 4.1 Hz, 1H, H-3), 5.77 (dd, J = 10.0, 1.1 Hz, 1H, H-2), 5.06 (d, J = 3.0 Hz, 1H, H-1'), 4.59 and 4.51 (AB, J = 11.7 Hz, 2H, CH₂Ar), 4.31 (t, J = 9.5 Hz, 1H, H-3'), 4.14–4.11 (m, 2H, H-3, H-5), 3.88–3.85 (m, 1H, H-4), 3.81 (s, 3H, OMe), 3.65–3.63 (m, 2H, H-6'), 3.59 (dd, J = 10.1, 3.0 Hz, 1H, H-2'), 3.61–3.53 (m, 2H, H-4', H-5'), 3.50 (s, 3H, OMe), 3.46 (s, 3H, OMe), 3.41 (s, 3H, OMe); ¹³C NMR (125 MHz, CDCl₃): δ = 159.2, 130.5, 130.2, 129.3, 128.4, 125.5, 116.6, 113.8, 97.4, 77.7, 76.2, 71.3, 70.7, 69.9, 66.8, 65.7, 59.3, 58.8, 55.7, 55.2, 30.3; HR-MS (MALDI): calcd for C₂₂H₃₀O₉Na [M +Na⁺]: 461.1787; found: 461.1789.

the combinatorial synthesis of novel semisynthetic everninomicins and other carbohydrate libraries for biological screening.

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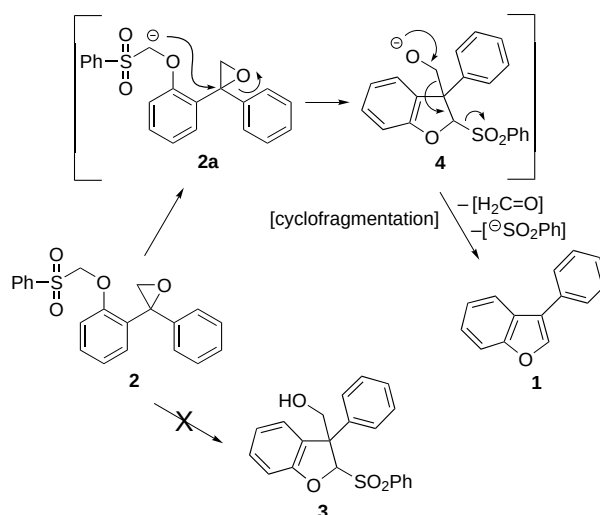
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- [10] Yields from the solid-support synthesis were determined from the weight of cleaved product and are reported as an overall yield for the sequence based upon the initial loading of the selenium bromide resin (see ref. [4]).
- [11] All new compounds exhibited satisfactory spectral and exact mass data.

Solution and Solid-Phase Synthesis of Functionalized 3-Arylbenzofurans by a Novel Cyclofragmentation–Release Pathway**

K. C. Nicolaou,* Scott A. Snyder, Antony Bigot, and Jeffrey A. Pfefferkorn

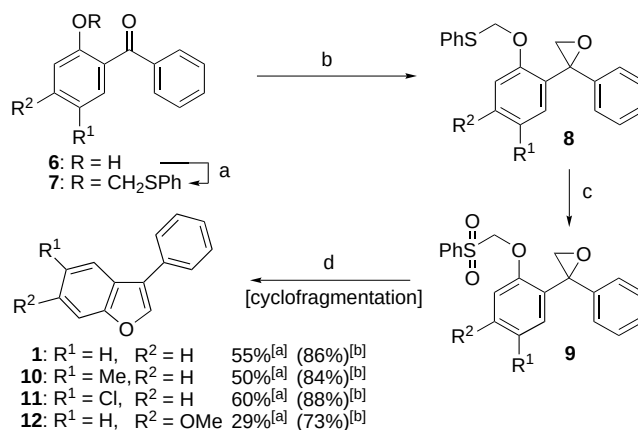
The 3-arylbenzofuran nucleus is a central component of a diverse class of heterocyclic natural and synthetic products that possess a broad range of biological activities.^[1] During the course of recent synthetic investigations we discovered a novel reaction cascade leading to 3-phenylbenzofuran (**1**, Scheme 1), the core structure of the 3-arylbenzofuran class. This serendipitous observation occurred as a result of attempts to convert epoxide **2** (see Scheme 2 for its preparation) into **3** by deprotonation of the methylene group adjacent to the sulfone followed by selective opening of the epoxide ring.^[2] Surprisingly, rather than providing the desired system **3**, the only product observed was benzofuran **1**. The proposed mechanism for this transformation, a novel cyclofragmentation pathway, is outlined in Scheme 1, in which after the initial generation of the alkoxide intermediate **4** from a 5-*exo*-trig cyclization, collapse to **1** occurs by the concomitant expulsion



Scheme 1. The cyclofragmentation pathway to 3-arylbenzofurans (**1**).

of both formaldehyde and a phenylsulfinate anion.^[3, 4] Given the importance of 3-arylbenzofurans in biology and medicine, we sought to test the generality of this reaction cascade for the preparation of more highly functionalized systems. Herein, we report the exploration of the scope of this technology, both in solution and on solid support, which led to the generation of a diverse family of 3-arylbenzofurans and the streamlining of the method for use in combinatorial chemistry.

We initially alkylated three commercially available 2-hydroxybenzophenones (**6**) with chloromethylphenyl sulfide to generate **7** (Scheme 2). Subsequent epoxidation with trimethylsulfonium iodide^[5] followed by *m*CPBA-mediated oxida-



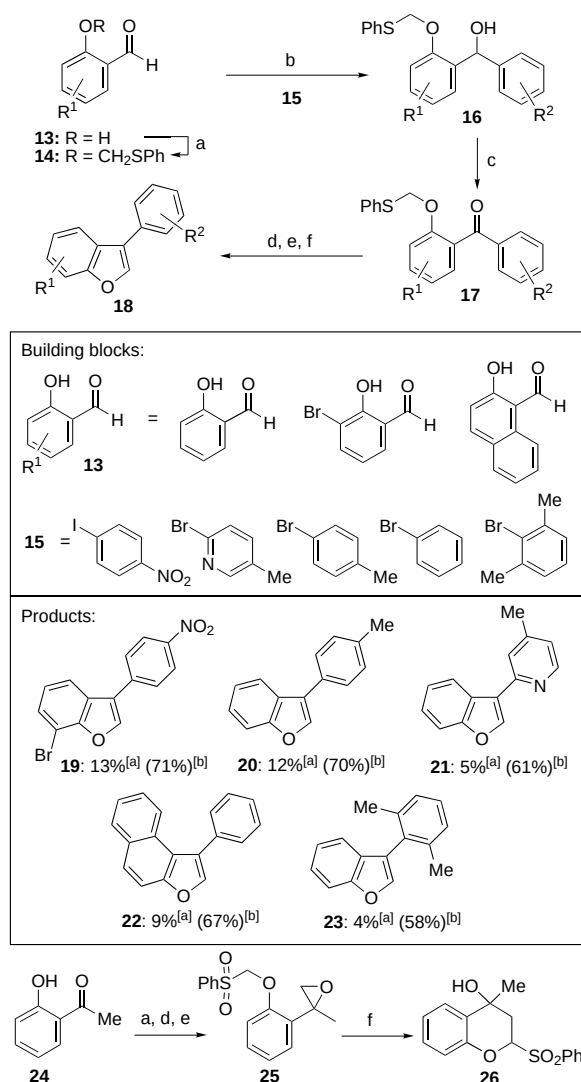
Scheme 2. Synthesis of 3-arylbenzofurans (**1**, **10–12**) from 2-hydroxybenzophenones. a) ClCH₂SPh (1.3 equiv), K₂CO₃ (1.5 equiv), DMF, 50 °C, 5 h; b) trimethylsulfonium iodide (1.5 equiv), KO^tBu (1.0 M in THF, 1.5 equiv), DMSO, 0 °C, 10 min; c) *m*CPBA (2.5 equiv), NaHCO₃ (2.5 equiv), CH₂Cl₂, 25 °C, 2 h; d) KO^tBu (1.0 M in THF, 2.5 equiv), DMF, 0 °C, 5 min. *m*CPBA = *meta*-chloroperbenzoic acid. [a] Overall yield after four steps. [b] Average yield per step.

tion of the sulfide gave **9** in high overall yields. Gratifyingly, treatment of these sulfones with either KO^tBu in DMF (–57 °C or 0 °C) or LDA in THF (–78 °C) resulted in the exclusive formation of the desired 3-arylbenzofurans **10–12**,^[6] thereby establishing the potential of this reaction to generate substituted benzofurans.

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We next undertook a more generalized approach to the construction of the requisite benzophenone precursor so that further diversity could be introduced onto the 3-phenylbenzofuran scaffold (Scheme 3). Initial alkylation of 2-hydroxybenzaldehydes (**13**) with chloromethylphenyl sulfide



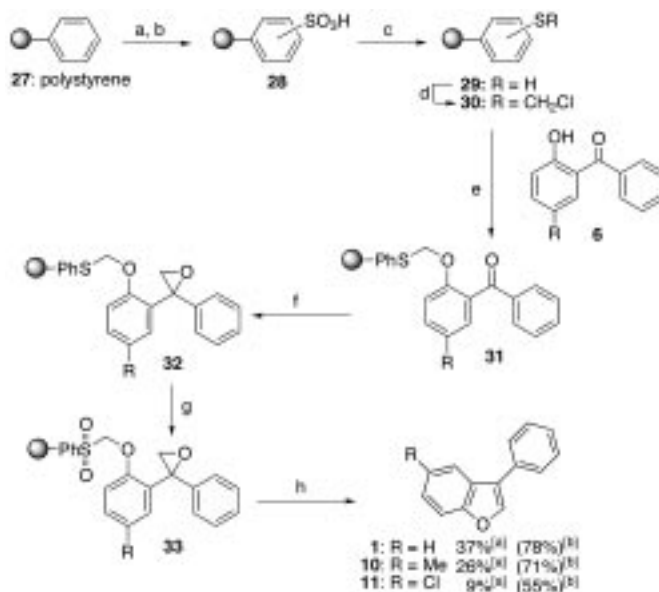
Scheme 3. Synthesis of 3-arylbenzofurans (**19–23**). a) ClCH₂SPh (1.3 equiv), K₂CO₃ (1.5 equiv), DMF, 50 °C, 5 h; b) aryl halide (1.1 equiv), *n*BuLi (1.6 m in hexanes, 1.1 equiv), THF, –78 °C; then **14**, –78 °C, 5 min; c) MnO₂ (10.0 equiv), CH₂Cl₂, 25 °C, 12 h; d) trimethylsulfonium iodide (1.5 equiv), KO^tBu (1.0 m in THF, 1.5 equiv), DMSO, 0 °C, 10 min; e) *m*CPBA (2.5 equiv), NaHCO₃ (2.5 equiv), CH₂Cl₂, 25 °C, 2 h; f) KO^tBu (1.0 m in THF, 2.5 equiv), DMF, 0 °C, 5 min. [a] Overall yield after four steps. [b] Average yield per step.

followed by nucleophilic attack or the aldehyde using an aryl lithium species afforded benzhydrols **16**. Subsequent selective oxidation of the bisbenzylic secondary alcohol with MnO₂ provided the desired functionalized benzophenones (**17**), which were then transformed to 3-arylbenzofuran derivatives **19–23** by using the protocol described above. Rather than performing a serial coupling of each benzaldehyde and aryl lithium building block, we instead sought to test only the diversity that could be incorporated onto the final scaffold.

Not only were both electron-withdrawing and electron-donating groups tolerated, but pyridyl (**21**),^[7] naphthyl (**22**), and sterically hindered analogues such as xylene derivative **23** were also readily obtained.

To verify that both aryl groups were required for regioselective epoxide opening, an attempt was made to generate 3-methylbenzofuran starting from 2-hydroxyacetophenone (**24**, Scheme 3). As expected, after elaboration to the corresponding sulfone (**25**), base treatment led solely to **26** (as a diastereomeric mixture at the quaternary center), the exclusive product of a 6-*endo*-trig cyclization. The only other instance in which such a product was observed was during the final reaction to generate naphthyl analogue **22**, where intramolecular nucleophilic attack at the quaternary carbon center of the epoxide is conformationally less favored than in other cases, and resulted in the formation of equal amounts of both **22** and the 6-*endo*-trig congener.

Given the generality of this method for the construction of 3-arylbenzofurans in solution, we hoped to adapt the strategy to a solid-phase approach, thereby facilitating its use in combinatorial synthesis.^[8] Examination of the proposed cyclofragmentation mechanism (Scheme 1) suggested that the requisite solid support might be tethered to the scaffold through the thiophenyl functionality. The advantage of such a strategy would be threefold: 1) linking at this position allows for incorporation of full diversity on either aromatic ring; 2) since the phenylsulfonate moiety is ultimately expelled



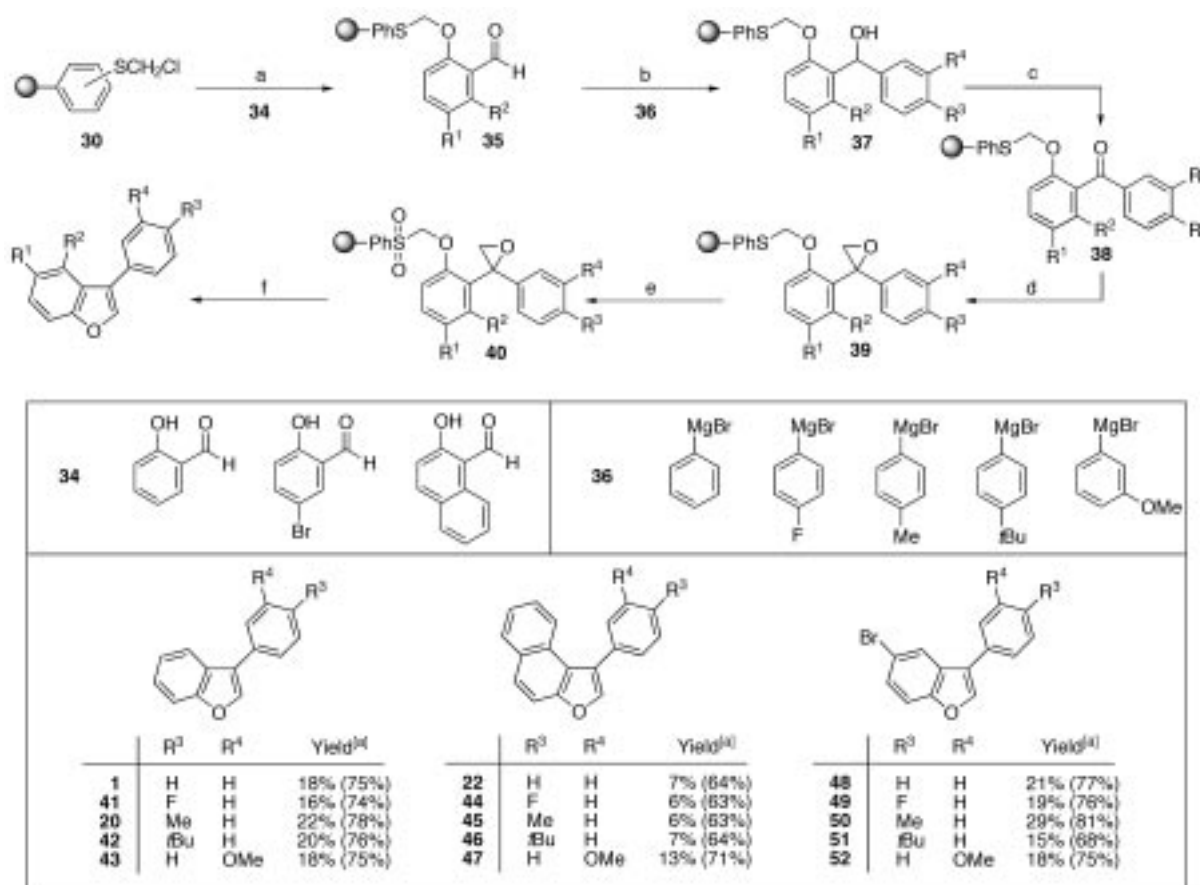
Scheme 4. Preparation of the chloromethyl sulfide resin and preliminary studies on the loading and cyclofragmentation release of 3-arylbenzofurans. a) *n*BuLi (1.2 equiv), polystyrene (1.0 equiv, 1% DVB cross-linked, 100–200 mesh), TMEDA (1.0 equiv), cyclohexane, 65 °C, 4 h; b) SO₃·NMe₃ (1.5 equiv), THF, 0 → 25 °C, 8 h; c) I₂, (5.0 equiv), Ph₃P (15.0 equiv), benzene, 80 °C, 8 h; d) DBU (3.0 equiv), BrCH₂Cl, 25 °C, 12 h; e) benzophenone **6** (10.0 equiv), Cs₂CO₃ (5.0 equiv), DMF, 95 °C, 12 h; f) trimethylsulfonium iodide (10.0 equiv), KO^tBu (1.0 m in THF, 10.0 equiv), THF:DMSO (1:1), 0 → 25 °C, 2 h; g) *m*CPBA (10.0 equiv), NaHCO₃ (15.0 equiv), CH₂Cl₂, 25 °C, 12 h; h) KO^tBu (1.0 m in THF, 10.0 equiv), DMF, 0 → 25 °C, 15 min. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DVB = divinylbenzene; TMEDA = *N,N,N',N'*-tetramethylethylenediamine. [a] Overall yield of isolated product based on a resin loading of 0.21 mmol g^{–1}. [b] Average yield per step.

during product formation, substrates will undergo traceless release; 3) linking through the aryl sulfone imparts a fail-safe feature to the cleavage step, since only the desired benzofuran skeleton can undergo release, thereby ensuring that the final products will be isolated in high purity.^[9, 10]

To test this solid-phase strategy we required the polymer-bound equivalent of the previously used chloromethylphenyl sulfide, which we envisaged could be constructed from a polymeric phenylthiol.^[11] Thus, polystyrene was lithiated^[12] and then quenched with $\text{SO}_3 \cdot \text{NMe}_3$ ^[13] to provide sulfonic acid resin^[14] **28** (Scheme 4, see page 1094), which was subsequently reduced to the free thiol resin **29** upon treatment with $\text{I}_2/\text{Ph}_3\text{P}$.^[15] Resin **29** became dark orange upon standing in air for two hours, presumably as a result of oxidative cross-linking; therefore, freshly prepared **29** was immediately alkylated with ClCH_2Br in the presence of DBU^[16] to provide **30** as a stable resin that could be stored under ambient conditions. To confirm the viability of our linking/cleavage strategy, we loaded the previous 2-hydroxybenzophenones (**6**) onto the chloromethylsulfide resin to give structures **31**, which were subsequently elaborated to sulfones **33** by utilizing the same methodology as described above. Gratifyingly, the 3-phenylbenzofurans **1**, **10**, and **11** were cleanly released from the solid support in greater than 95% purity upon treatment with KOtBu , with no other side-products observed.

With the fidelity of the tethering strategy confirmed, we sought to generalize this method to accommodate greater structural diversity. Hence, a series of functionalized salicylaldehydes (**34**) were loaded onto resin **30** (Scheme 5). The resulting resin-bound aldehydes were then treated with a series of arylmagnesium bromides (**36**) to give **37** and subsequently selectively oxidized with IBX^[8e] to afford benzophenones **38**. Interestingly, although the controlled use of an aryllithium reagent in solution readily provided benzhydrols such as **16** (Scheme 3), large excess of the lithium reagent failed to generate **37** (Scheme 5). On the basis of additional solution studies, the extra base probably deprotonates the methylene group adjacent to the sulfide moiety, leading to several rearrangement side-products. With **38** in hand, sulfur ylide epoxidation followed by *m*CPBA oxidation gave resin-bound sulfones **40** via **39** as before, which provided 3-arylbenzofurans **1**, **20**, **22**, and **41–52** upon treatment with KOtBu . As expected, all cleavage products were remarkably clean, presumably since the product of any structures other than **40** remained bound to the resin.

In conclusion, a novel synthetic methodology leading to 3-arylbenzofurans has been developed both in solution and on solid support. The cascade pathway for the generation of the benzofuran system is particularly well-suited to solid-phase synthesis, which permits the efficient generation of a large



Scheme 5. Solid-phase parallel synthesis of 3-arylbenzofurans (**1**, **20**, **22**, **41–52**). a) **34** (10.0 equiv), Cs_2CO_3 (5.0 equiv), DMF, 95 °C, 12 h; b) **36** (10.0 equiv), $-20 \rightarrow 0^\circ\text{C}$, 3 h; c) IBX (10.0 equiv), THF:DMSO (1:1), 25 °C, 2 h; d) trimethylsulfonium iodide (10.0 equiv), KOtBu (1.0 M in THF, 10.0 equiv), THF:DMSO (1:1), $0 \rightarrow 25^\circ\text{C}$, 2 h; e) *m*CPBA (10.0 equiv), NaHCO_3 (15.0 equiv), CH_2Cl_2 , 25 °C, 12 h; f) KOtBu (1.0 M in THF, 10.0 equiv), DMF, $0 \rightarrow 25^\circ\text{C}$, 15 min. IBX = 1-hydroxy-1,2-benziodoxol-3(1*H*)-one. [a] Overall isolated yield based on a resin-loading of 0.21 mmol g⁻¹; average yield per step is given in parentheses.

number of compounds in high purity for potential applications in chemical biology and medicinal chemistry. Continuing efforts are aimed towards the development of additional carbonyl-based coupling strategies to extend the scope of this method into new structural classes.

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tion) would result in a structure incapable of undergoing the cyclofragmentation–release cascade.

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Novel Ruthenium Building Blocks for the Efficient Modular Construction of Heterobimetallic Molecular Squares of Porphyrins**

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The construction of structurally well defined, highly ordered supramolecular systems containing porphyrins and metalloporphyrins is of prime interest. By virtue of the

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inherent useful photochemical and redox properties of the porphyrin molecule, such arrays are being investigated for modeling the photosynthetic system^[1] and for developing new photonic materials and light-harvesting devices.^[2] The metal-mediated self-assembly approach, which exploits the formation of coordination bonds between peripheral basic site(s) on the porphyrins and metal centers, has provided many large discrete assemblies of porphyrins in good yields.^[3]

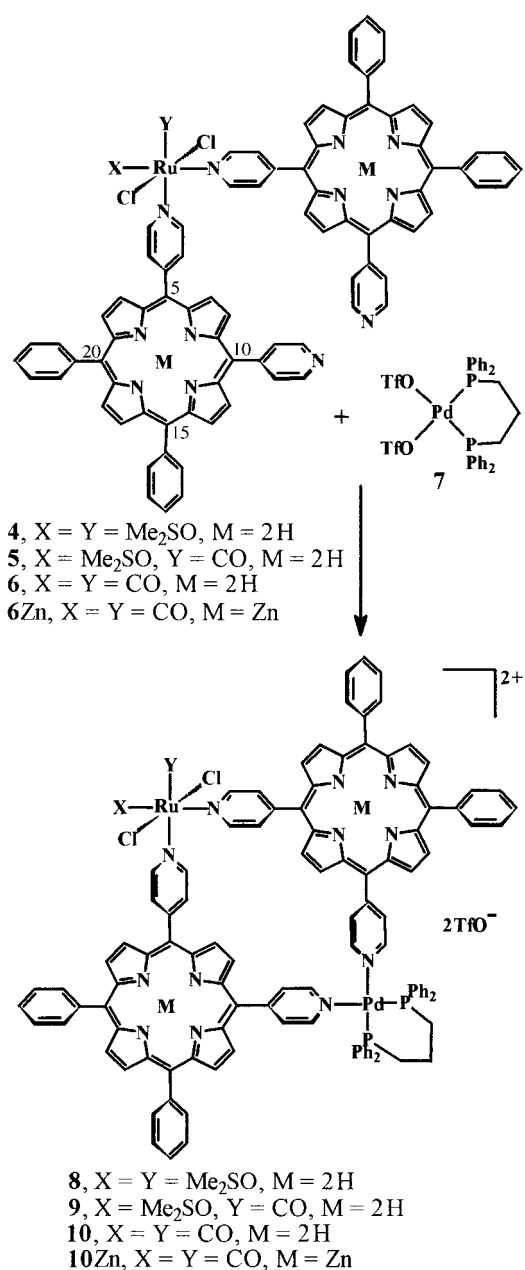
Among the various architectures reported for such porphyrin aggregates, there are only a few examples of molecular squares, and they are all homometallic.^[4–7] Most were obtained by treatment of *meso*-di(4'-pyridyl)porphyrins, such as 5,10-bis(4'-pyridyl)-15,20-diphenylporphyrin (4'-*cis*DPyP) and 5,15-bis(4'-pyridyl)-10,20-diphenylporphyrin (4'-*trans*DPyP), with suitable *cis* and *trans* square-planar Pd^{II} and Pt^{II} complexes, which are precursors for angular (90°, **A_a**) or linear (180°, **L_a**) acidic building blocks.^[4, 5] There is current interest in the photophysical investigation of properly designed molecular squares of porphyrins which, unlike open-chain systems, may allow the locking of chromophores in a mutually coplanar geometry.^[8]

We report here a flexible, stepwise approach that, through the design and synthesis of novel chelating bis-porphyrin ruthenium intermediates, leads efficiently to unprecedented heterobimetallic molecular squares of porphyrins featuring different diagonally opposed metal ions. In particular, [2+2] molecular squares are described in which two 4'-*cis*DPyP units are linked through an octahedral, neutral Ru^{II} center and a square-planar, charged Pd^{II} center (see Scheme 1).

The Ru^{II}-sulfoxide complexes *trans*-[RuCl₂(Me₂SO)₄] (**1**, Me₂SO bound through S), *trans*-[RuCl₂(Me₂SO)₃(CO)] (**2**), and *trans*-[RuCl₂(Me₂SO)₂(CO)₂] (**3**, Me₂SO bound through O) easily undergo replacement of two *cis* Me₂SO ligands and are thus suitable precursors for octahedral **A_a** building blocks. Treatment of **1–3** with excess 4'-*cis*DPyP under mild conditions led to the isolation of *trans,cis,cis*-[RuCl₂(X)(Y)-(4'-*cis*DPyP)₂] compounds **4–6** in pure form (Scheme 1). The nature and geometry of the products were established by ¹H and ¹³C NMR spectroscopy,^[9] as described previously for the corresponding 5-(4'-pyridyl)-10,15,20-triphenylporphyrin (4'-MPyP) complexes.^[10] Integration of the signals for **4** and **5** revealed the adduct stoichiometry, whereas COSY spectra allowed unambiguous assignment of the signals.

In general, coordination of 4'-*cis*DPyP to the central *trans,cis*-RuCl₂(X)(Y) moiety (*t*Ru) induced downfield shifts for the H_{2,6} and H_{3,5} resonances of the 4'-N(py) ring (position 5, py = pyridyl) involved in the new bond. Conversely, the resonances of the six-membered rings at positions 10 and 20 experienced an upfield shift of about 0.2 ppm with respect to 4'-*cis*DPyP, which is characteristic of two mutually *cis* pyridylporphyrins displaying free rotation about the metal–pyridyl axis.^[10] Compounds **4** and **6** have C_{2v} symmetry,^[11] and thus the two porphyrins are equivalent. In **5** the two 4'-*cis*DPyP units are inequivalent; the resonances of the two *t*Ru-4'-N(py) rings are well resolved, whereas the other signals overlap.^[9]

Compounds **4–6**, in which the two *cis*-coordinated 4'-*cis*DPyP units have one residual unbound 4'-N(py) ring, are preprogrammed basic building blocks capable of chelating



Scheme 1. Synthesis of **8–10** and **10Zn**. For **4**, **5**, **8**, and **9** the Me₂SO molecule is bound through S.

suitable acidic modules displaying an angle of 90°. As an example, we investigated the reactivity of **4–6** towards [Pd^{II}(dppp)(OTf)₂] (**7**; dppp = 1,3-bis(diphenylphosphanyl)propane, OTf = trifluoromethanesulfonate = triflate), a widely used precursor for square-planar **A_a** units.^[4]

¹H NMR spectroscopy established that titration of **7** into solutions of **4–6** in chloroform led readily to the quantitative formation of the corresponding heterobimetallic [2+2] molecular squares of formula [Pd(dppp){*trans,cis,cis*-RuCl₂(X)(Y)(4'-*cis*DPyP)₂}(OTf)₂] (**8–10**, Scheme 1). Chelation of the Pd-bis-phosphane fragment by **4–6** induced characteristic shifts both in the ¹H NMR spectrum (which concerned mainly the resonances of the previously unbound 4'-N(py) ring at position 10, Figure 1),^[9] and in the proton-decoupled ³¹P NMR spectrum (upfield shift Δδ ≈ 10 compared to **7**, as is

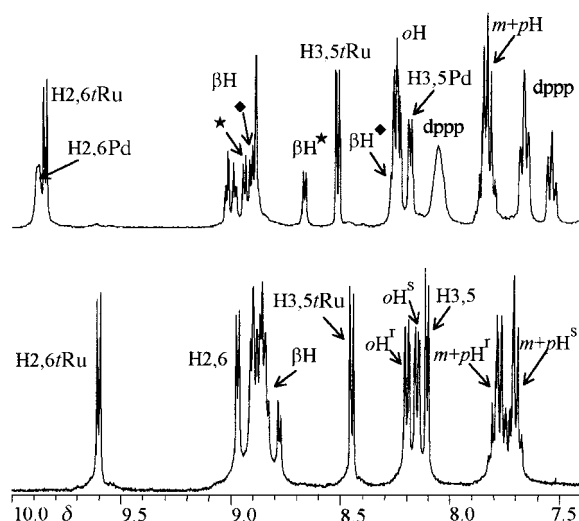


Figure 1. Bottom: Downfield region of the ^1H NMR spectrum of **6** in CDCl_3 (τ : position 15, δ : position 20). Top: The same spectral region after addition of one equivalent of **7**, leading to the quantitative formation of molecular square **10** (pyrrole (β) resonances labeled with $\star$ and $\blacklozenge$ are coupled to each other in the COSY spectrum).

the case for $[\text{Pd}(\text{dppp})(4'\text{-cisDPyP})_2(\text{OTf})_4]$ (**11**).^[4] The macrocycles were not affected by an accidental excess of **7**, and no evidence of homometallic molecular squares was found.

In solution squares **8** and **10** maintain the C_{2v} symmetry of the corresponding Ru precursor,^[12] and the two porphyrins at opposite corners are equivalent. The resonances of the Pd–4'N(py) rings are well resolved from those of the *t*Ru–4'N(py) rings. The most downfield H2,6 and the most upfield H3,5 signals (correlated in the COSY spectrum) are unaffected by the nature of X and Y, and were thus attributed to the Pd–4'N(py) rings. Two of the eight inequivalent pyrrole protons of **8** and **10** have remarkably upfield shifted resonances (one overlaps with *ortho*-H resonances) and are not correlated to each other in the COSY spectrum (Figure 1). We attributed these resonances to βH_8 and βH_{12} , that is, the pyrrole protons closest to Pd (Scheme 1).^[13] These assignments are also in good agreement with those reported by Stang et al. for **11**.^[4]

Macrocycle **9** lacks an orthogonal mirror plane containing the two metal atoms; hence the two porphyrin units are inequivalent. In the ^1H NMR spectrum of **9** the resonances of the two *t*Ru–4'N(py) rings are well resolved, whereas those of the Pd–4'N(py) rings overlap.^[9] The other porphyrin resonances, with the exception of those of the internal NH groups and of the pyrrole protons closest to Pd (upfield shifted signals), are not resolved. Also the $^{31}\text{P}\{^1\text{H}\}$ resonances for the two inequivalent phosphorous atoms of dppp overlap.

Some resonances in the ^1H NMR spectra of **8**–**10**—namely, H2,6 of Pd–4'N(py) rings as well as *ortho*-H and CH_2 moieties of the dppp ligand—are broadened. This is very likely due to conformational motion of the diphosphane bridge occurring at an intermediate rate on the NMR time scale at ambient temperature.

Treatment of **6** with excess zinc acetate led to the insertion of zinc into both porphyrin rings (**6Zn**); the ^1H NMR spectrum of **6Zn** in CDCl_3 is affected by the extensive

intermolecular coordination of the pyridyl groups, not bound to Ru, to the Zn atoms. Titration of **7** into a solution of **6Zn** in CDCl_3 quantitatively yielded the corresponding molecular square **10Zn** (Scheme 1), which represents the first example of a heterotrimetallic molecular square of porphyrins.^[15] The ^1H NMR spectrum of **10Zn** is very similar to that of **10**.^[9, 15]

The structure of **10**, in which two 4'-*cis*DPyP units alternate with the Pd^{II} and Ru^{II} angular units, was established by X-ray single crystal analysis (Figure 2).^[16] This represents the first case in which data on the solid-state structure of a molecular

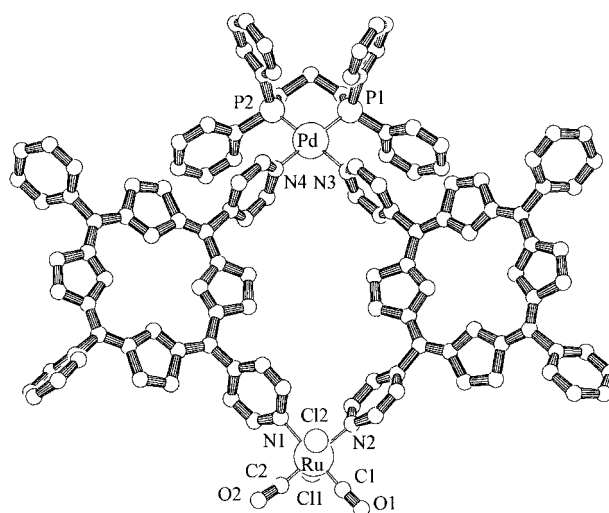


Figure 2. Molecular structure of the cation of molecular square **10**. Selected bond lengths [$\text{\AA}$] and angles [$^\circ$]: Pd–N4 2.117(12), Pd–N3 2.164(14), Pd–P2 2.255(6), Pd–P1 2.268(5), Ru–C2 1.85(2), Ru–C1 1.85(3), Ru–N2 2.132(13), Ru–N1 2.167(14), Ru–C1 2.379(7), Ru–C2 2.389(8); N3–Pd–P2 178.2(5), N4–Pd–P1 177.1(5), C2–Ru–N2 179.5(9), C1–Ru–N1 177.8(13), C1–Ru–C2 177.43(19).

square of porphyrins has become available.^[18] The distances from the metals to the porphyrin centers are about 9.83 $\text{\AA}$; the diagonal Pd $\cdots$ Ru distance of 14.015(3) $\text{\AA}$ is close to the value of 15 $\text{\AA}$ obtained from molecular modeling of similar square-shaped Pd^{II} adducts.^[4] Interestingly, in the solid state the macrocycle exhibits an arrangement in which the least-squares planes of the porphyrins form a dihedral angle of $41.7(1)^\circ$, approximating C_s symmetry. The *meso* pyridine and phenyl rings are tilted from the normal to the corresponding porphyrin plane by $64.2(4)^\circ$ – $79.2(6)^\circ$ and $43.4(6)^\circ$ – $60.6(4)^\circ$, respectively. The coordination bond lengths and angles are well in the range of values found in Ru^{II} and Pd^{II} compounds. An interesting feature is represented by the conformation of the diphosphane ligand, in which two phenyl rings display stacking interactions with the adjacent coordinating pyridines; the shortest distance between the *ipso*-C atom and the pyridine N atom is about 3.1 $\text{\AA}$.

In future, this stepwise synthetic approach might be efficiently applied to the construction of novel homo- and heterobimetallic discrete assemblies of porphyrins bearing metal ions with programmed features (such as geometry, charge, functionalities of the ancillary ligands) at spatially well-defined positions.

Experimental Section

Complexes **1–3** and **7** were prepared according to the literature procedures.^[4, 10] The ¹H NMR signals were referenced to signals from residual CHCl₃ ($\delta = 7.26$), and assignments were made with the aid of 2D COSY experiments as detailed in the text. The UV/Vis spectra were obtained on a Jasco V-550 spectrophotometer. All reagents were analytical grade. Column chromatography was performed on mesh silica gel (40–63 μ m; BDH Laboratory Supplies, UK).

4–6: Complexes **1–3** were treated at room temperature in CHCl₃ with a fourfold excess of 4'-cisDPyP (reaction time: 5 h (**4**), 24 h (**5**), 3 d (**6**)). Column chromatography of the reaction mixtures afforded pure products as noncrystalline purple solids which gave satisfactory elemental analyses. Pure **5** and **6** were obtained (third band) from columns eluted with CHCl₃/ethanol (99/1); pure **4** was obtained (fourth band) from a column eluted with CHCl₃/ethanol (99/1 → 98/2). Yields of isolated products: ca. 40%. As for the corresponding 4'-MPyP compounds,^[10] the electronic absorption spectra of **4–6** were very similar to that of the unbound porphyrin.

6Zn: Compound **6** (10 mg) dissolved in CHCl₃ (5 mL) was treated for 48 h with a fourfold excess of zinc acetate dissolved in methanol (2 mL). The product precipitated upon addition of *n*-hexane. It was washed thoroughly with water, methanol, and diethyl ether and then dried under vacuum (yield of isolated product: ca. 70%). The final product was purified by chromatography on a column eluted with CHCl₃ (first band).

Molecular squares **8–10** were obtained by addition of **7** (1 equiv) to solutions of the corresponding precursor in CDCl₃. The reactions were monitored by ¹H NMR spectroscopy, and the ratio of the reactants was adjusted accordingly. Formation of **8–10** also involved minor red shifts of the Soret and Q bands of **4–6** by about 4 nm (with clear isosbestic points), accompanied by a concomitant decrease in intensity (ca. 10%). Similar spectral variations were also observed in the formation of **11**.^[4] Analytically pure crystalline samples of the purple-red macrocycles were obtained by addition of *n*-hexane into the solutions. Yield of isolated products: ca. 80%.

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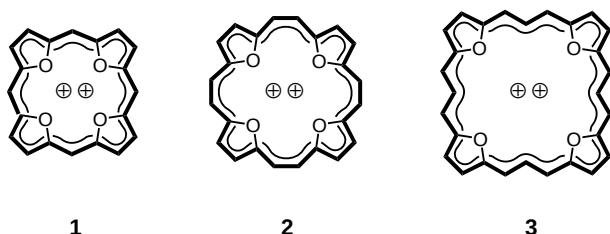
- [1] a) A. Harriman, J.-P. Sauvage, *Chem. Soc. Rev.* **1996**, 41–48; b) H. Kurreck, M. Huber, *Angew. Chem.* **1995**, *107*, 929–947; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 849–866; c) V. S.-Y. Lin, S. G. DiMaggio, M. J. Therien, *Science* **1994**, *264*, 1105–1111; d) M. R. Wasielewski, *Chem. Rev.* **1992**, *92*, 435–461.
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- [9] For the ¹H NMR spectra (400 MHz, CDCl₃) of **4–6**, **8–10**, and **10Zn**, see the Supporting Information.
- [10] E. Alessio, M. Macchi, S. L. Heath, L. G. Marzilli, *Inorg. Chem.* **1997**, *36*, 5614–5623.
- [11] A sharp singlet appears at $\delta = 3.66$ for the four equivalent Me₂SO methyl groups in the ¹H NMR spectrum of **4**. A singlet appears at $\delta = 195.0$ for the two equivalent CO groups in the ¹³C NMR spectrum of **6** (100.1 MHz, CDCl₃).
- [12] For **8** singlet appears at $\delta = 3.64$ for the four equivalent Me₂SO methyl groups in the ¹H NMR spectrum, and a singlet at $\delta = 5.8$ in the ³¹P{¹H} NMR spectrum (161.9 MHz, CDCl₃, H₃PO₄). For **10** a singlet appears at $\delta = 194.9$ for the two equivalent CO groups in the ¹³C NMR spectrum, and a singlet at $\delta = 5.7$ in the ³¹P{¹H} NMR spectrum. For **10Zn**: A singlet at $\delta = 194.9$ for the two equivalent CO groups in the ¹³C NMR spectrum, and a singlet at $\delta = 5.5$ in the ³¹P{¹H} NMR spectrum.
- [13] Interestingly, in **8–10** the H_{2,6} resonances of *t*Ru-4'N(py) and of Pd-4'N(py) are almost equally downfield shifted compared to 4'-cisDPyP ($\Delta\delta = 1.08/0.78$). In contrast, while the H_{3,5} resonance of *t*Ru-4'N(py) is shifted downfield ($\Delta\delta = 0.36/0.19$), that of Pd-4'N(py) is virtually unaffected by coordination. Thus the resonances of H_{3,5}-Pd and of the pyrrole protons closest to Pd are anomalously upfield. A similar situation is found in the corresponding homometallic Pd square **11**^[4] but not in the Ru squares.^[14] Therefore this effect seems to be attributable to the dppp ligand rather than to the metal. Examination of the solid-state structure of **10** suggests that these protons might fall into the shielding cone of the phenyl rings of dppp, whose conformation is not frozen in solution.
- [14] E. Iengo, E. Ciani, E. Zangrando, S. Geremia, E. Alessio, *Abstr. Pap. 7th Int. Conf. "The Chemistry of the Platinum Group Metals"* (Nottingham, UK) **1999**, p. 101.
- [15] Insertion of Zn was not performed for **4** and **5** because the corresponding zincated squares are expected to give auto-aggregation processes in solution, due to interactions between Zn and the oxygen atom of the Me₂SO groups.^[10]
- [16] Crystals of **10** were obtained by slow diffusion of *n*-hexane into a solution in CHCl₃. The crystals, when redissolved in CDCl₃, yielded the same spectrum as the raw material. Crystal data for **10** · *n*-hexane · CHCl₃ (C₁₂₂H₉₇Cl₃F₆N₁₂O₈P₂PdRuS₂): *M*_r = 2483.90, monoclinic, space group C2/c (no. 15), *a* = 40.315(6), *b* = 20.487(4) *c* = 35.125(6) Å, β = 104.70(2)°, *V* = 28061.57 Å³, *Z* = 8, ρ_{calcd} = 1.176 g cm⁻³, *F*(000) = 10 144, *T* = 100 K, μ = 3.767 mm⁻¹. Data collection performed at the X-ray diffraction beamline of Elettra Synchrotron, Trieste (Italy), on a 30 cm MAR2000 image plate, 32 frames collected with rotation of 3° about φ , fixed dose of radiation ($\lambda = 1.0527$ Å). Total reflections collected 14 472 (resolution 1.2 Å), unique 9281 (*R*_{int} = 0.0631). Data reduction and cell refinement carried out using the program MOSFLM.^[18] Two disordered CHCl₃ molecules (occupancy factor 0.5 each) and a molecule of *n*-hexane were detected on the ΔF map. Final *R*1 = 0.1171, *wR*2 = 0.2847, *S* (GOF) = 1.252 for 5149 observed reflections [*I* > 2 σ (*I*)] and 764 parameters. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-132681. 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).
- [17] W. Kabsch, *J. Appl. Crystallogr.* **1993**, *24*, 795–800.
- [18] **Note added in proof** (February 2, 2000): During publication of this work a paper containing the X-ray structure of **11** appeared: M. Schmitz, S. Leininger, T. Fan, A. M. Arif, P. J. Stang, *Organometallics* **1999**, *18*, 4817–4824.

Furan-Based Porphyrins: Tetraoxa[4*n*+2]porphyrin Dications with 18*π*-, 22*π*-, or 26*π*-Electron Systems**

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Georg Hohlneicher*

Dedicated to Professor Dieter Seebach

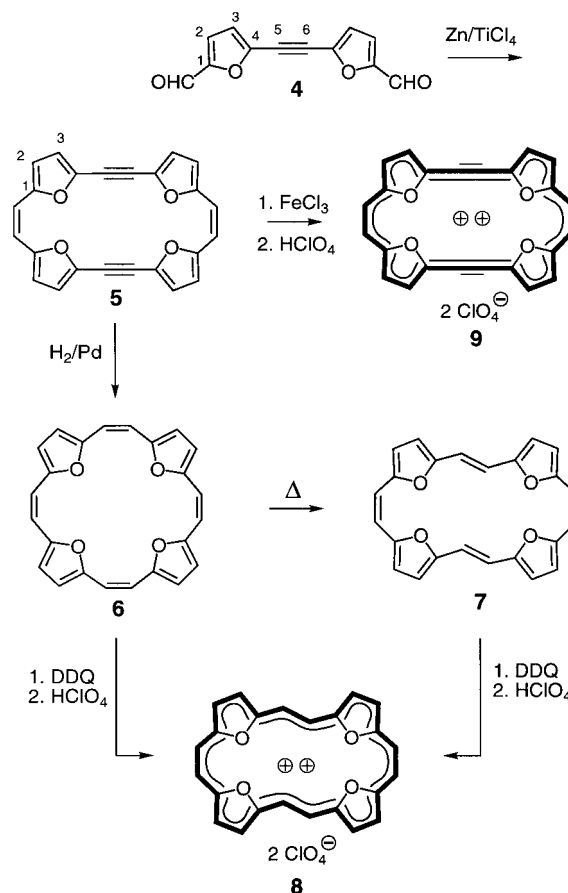
The D_{4h} -symmetric tetraoxa[18]porphyrin dication **1**,^[1] which is isoelectronic with diprotonated porphyrin, and the N,N',N'',N''' -tetramethyl[26]porphyrin-(3.3.3.3) dication (**3** with NCH_3 instead of O; as the octaethyl derivative) described by Franck et al.^[2] are prototypes of chalcogenaporphyrins^[3] and symmetrically expanded porphyrins, respectively.^[4] These dicationic porphyrin variants should be viewed as resonance hybrids (carbenium and onium ion structures) in which special importance is attributed to carbenium ion structures with $4n+2$ π electrons on the $4(n+1)$ carbon perimeter—in agreement with both experiment and theory.^[5]



A combination of N/O exchange and symmetrical expansion conceptually transforms porphyrin into homologues of **1**, the all-*cis*-tetraoxa[22]porphyrin-(2.2.2.2) and the tetraoxa[26]porphyrin-(3.3.3.3) dications **2** and **3**, respectively. Our interest in **2** and **3** was stimulated by the exceptional spectroscopic properties of the N,N',N'',N''' -tetramethyl[26]porphyrin dication from Franck et al., which prompted the authors to call the compound a super arene.^[2] If **2** and **3**—like **1**—were to exist as D_{4h} -symmetric molecules, the triad **1–3** would be ideally suited to provide meaningful spectroscopic correlations between the three members. However, the chance of obtaining a D_{4h} -symmetric all-*cis*-tetraoxa[22]porphyrin dication **2** seemed a priori fairly remote. According to calculations^[6] the compound is no less than about 90 kcal mol^{−1} higher in energy than the di-*trans* isomer **8** in

its preferred planar conformation. We describe here our attempts to prepare **2** and/or **8** as well as the synthesis of **3** (as the octaethyl derivative).^[7]

We considered tetraepoxytetradehydro[24]annulene **5** (red-violet prisms from dichloromethane; decomposition above 300 °C; yield 50 %), which was obtained by reductive carbonyl coupling of the dialdehyde **4**^[8] with Zn/CuCl and titanium tetrachloride in tetrahydrofuran, to be a key compound for preparation of tetraoxa[22]porphyrin-(2.2.2.2) dications (Scheme 1). Catalytic hydrogenation of **5** with Lindlar catalyst in tetrahydrofuran provided the all-*cis*-tetraepoxy[24]annulene **6** (violet crystals from tetrahydrofuran; decomposition above 270 °C; yield 35 %), a potential precursor of the all-*cis*-tetraoxa[22]porphyrin dication **2**.



Scheme 1. Synthesis of the tetraoxa[22]porphyrin-(2.2.2.2) dications **8** and **9** as the bisperchlorates.

The ¹H NMR spectrum (room temperature) of **6** does not show the expected simple form consisting of a singlet each for the furan protons and the olefinic protons. Instead singlets appeared at $\delta = 7.60$ and 6.30 for the furan protons and there was an AB system at $\delta = 5.96$ and 5.85 with $^3J_{AB} = 13.1$ Hz (*cis* double bond) for the olefinic protons. This result is understandable only under the assumption that **6** exists in a relatively stable, nonplanar conformation in which the furan proton pairs are arranged alternately inside and outside the C_{24} ring.^[9] As expected, the NMR spectrum is temperature dependent, but line broadening only occurs above 45 °C. From

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the coalescence temperature of the AB system (95 °C) it was possible to estimate that the free enthalpy of activation $\Delta G^\ddagger$ for the degenerate exchange of the furan rings is not less than 17 kcal mol⁻¹. The all-*cis*-tetraepoxy[24]annulene **6** is thermally labile, which became apparent from the occurrence of additional signals in the high-temperature ¹H NMR spectrum. A thermolysis study showed that **6** rearranges in boiling *o*-xylene (24 h) to form irreversibly the sterically uniform *cis,trans,cis,trans* isomer **7** (black-green crystals from ethyl acetate; decomposition above 300 °C; yield 65 %).^[10]

While our investigations on tetraoxa[22]porphyrin-(2.2.2.2) dications were still in progress,^[10] Märkl et al.^[11] reported an independent synthesis of **7** and its successful oxidation to the dication **8**. Since the results of the two groups are in satisfactory agreement mention is made to reference [10] regarding the physical data of **7** and **8** determined here.

Oxidation of the stereoisomeric tetraepoxy[24]annulenes **6** and **7**^[12] with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in tetrahydrofuran and treatment of the resulting amorphous products with 70 % perchloric acid furnished the same tetraoxa[22]porphyrin dication: the sterically uniform *cis,trans,cis,trans* isomer **8** as the bisperchlorate (blue platelets from perchloric acid; decomposition above 270 °C; yield in both cases 20–25 %).

The presence of **8** was confirmed by the ¹H NMR spectrum, which, clearly as the result of a restricted mobility of the molecule compared to **6** and **7**, no longer exhibits any significant temperature dependency. Owing to the marked low- and high-field position of the signals of the outer and inner protons, respectively, of the formal *trans* double bonds, **8** is characterized by a pronounced diamagnetic ring current. The NMR investigations, however, do not allow a distinction to be made as to whether **8** exists in a ruffled or in a planar conformation (C₂ versus C_{2h} symmetry).

A clarification of the conformation of **8**—which is of interest because of the spectroscopic comparison with **1** and **3**—by X-ray structure analysis has hitherto failed because of crystal disorder. In contrast, the *cis,trans,cis,trans*-octaethyltetraoxa[22]porphyrin dication **8a** as the bisperchlorate (Figure 1, Table 1),^[13] obtained analogously to our synthesis of **8**, was amenable to analysis. It emerged that the ring framework of **8a** is almost planar.

The usefulness of the tetraepoxytetrahydro[24]annulene **5** manifested itself not only in the *cis* hydrogenation to **6** but also in its two-electron oxidation with iron(III) chloride in acetonitrile. Treatment of the resulting solid with 70 % perchloric acid furnished the tetraoxatetrahydro[22]porphyrin-(2.2.2.2) dication **9** (as bisperchlorate), the oxygen analogue of diprotonated tetrahydro[22]porphyrin-(2.2.2.2) (blue needles from perchloric acid; decomposition above 280 °C; yield 24 %). The NMR and UV/Vis spectra of **9** correspond to those of the known octaethyltetrahydro[22]porphyrin-(2.2.2.2) in its doubly protonated form.^[14]

For the synthesis of the tetraoxa[26]porphyrin-(3.3.3.3) dication, as the octaethyl derivative **13** for reasons of solubility, we followed the route illustrated in Scheme 2, which was based on porphyrin chemistry methodology.^[2] The starting aldehyde **10** was obtained by vinylogous Vilsmeier formylation of 3,4-diethylfuran with 3-(*N,N*-dimethylami-

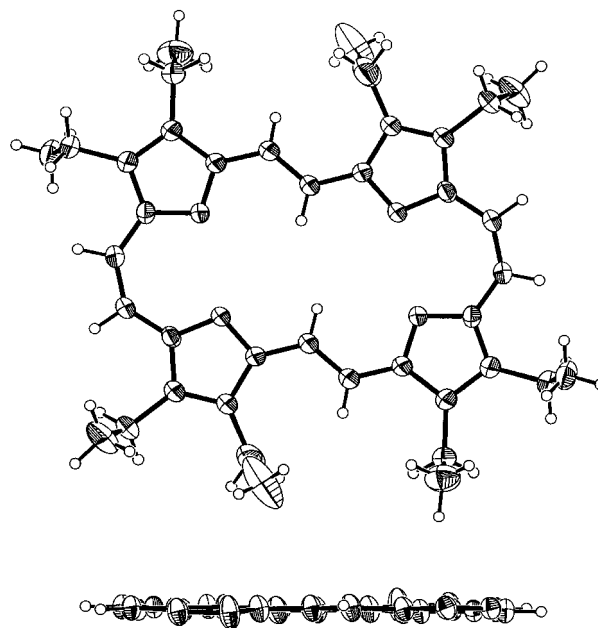


Figure 1. Crystal structure of the *cis,trans,cis,trans*-octaethyltetraoxa[22]porphyrin-(2.2.2.2) dication **8a** as the bisperchlorate. Top: plan view; bottom: side view without ethyl substituents.

no)acrolein and phosphorus oxychloride in dichloromethane at 0 °C (pale yellow crystals; m.p. 53–54 °C; yield 65 %). Reduction of **10** with sodium borohydride in tetrahydrofuran/water (25/1) gave the alcohol **11** (oily liquid) in almost quantitative yield. Because of its instability it was used immediately. Compound **11** unexpectedly showed only a slight tendency towards cyclotetracondensation under normal acidic conditions, but it was possible to obtain **12** by treating **11** with citric acid (equimolar amounts) in nitromethane at 70 °C (colorless crystals; m.p. 212 °C (decomposition); yield 3 % (based on **10**)). The method of choice for the oxidation step proved to be the reaction of **12** with bis(trifluoroacetoxy)-iodo(III)benzene in benzene (RT); the 26 π -electron dication precipitated out as the microcrystalline bis(trifluoroacetate). It was purified by chromatography on silica gel with acetonitrile (containing 2 % trifluoroacetic acid) and then converted into the bisperchlorate **13** with 70 % perchloric acid. Crystallization of **13** from formic acid resulted in green rhomboids with a metallic sheen which trapped solvent (suitable for X-ray structure analysis). This material gave the analytically pure compound (dark green powder; decomposition above 300 °C; yield 50 %) when heated at 140 °C (0.2 Torr; 48 h).

Within the limits of comparison the ¹H NMR spectrum of **13** agreed remarkably well with that of the octaethyl-*N,N',N'',N'''*-tetramethyl[26]porphyrin-(3.3.3.3) dication.^[2] As with its NCH₃ analogue, **13** is characterized by an unusually strong diamagnetic ring current which is manifested in the position of the signals of the outer- and inner-perimeter protons at extremely low and high field: δ = 14.61 (H_o), –10.92 (H_i). The presence of a D_{4h}-symmetric dication can be inferred from a combination of the ¹H and ¹³C NMR spectra (six signals) and the finding that the structure of **13** exhibits a planar ring framework (see the following communication^[19]).

Table 1. Selected physical data for **4–6**, **8a**, **9**, **10**, **12**, **13**, and **15** (^1H NMR: 300 MHz; ^{13}C NMR: 75.5 MHz).^[a]

4: ^1H NMR ($[\text{D}_6]\text{DMSO}$): δ = 9.64 (s, 2H; CHO), 7.66 (d, 2H; H-2,9; $^3J_{2,3}$ = 3.8 Hz), 7.37 (d, 2H; H-3,8); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$): δ = 178.4 (CHO), 153.1, 138.2, 123.0, 120.3, 84.7; MS (EI, 70 eV): m/z (%): 214 (78) [M^+], 157 (100), 129 (70); IR (KBr): $\tilde{\nu}$ = 3105, 1669, 1526, 1399, 1248, 1034, 962, 814, 781, 705 cm^{-1} ; UV/Vis (CH_2Cl_2): λ_{max} (ϵ) = 227 (10200), 343 (30800), 367 nm (27300)

5: ^1H NMR (CD_2Cl_2): δ = 6.30 (d, 4H; H-3,8,15,20; $^3J_{2,3}$ = 3.5 Hz), δ = 5.99 (d, 4H; H-2,9,14,21), 5.48 (s, 4H; H-11,12,23,24); ^{13}C NMR (CD_2Cl_2): δ = 154.4, 137.0, 119.1, 115.8, 113.9, 85.3; MS (EI, 70 eV): m/z (%): 364 (100) [M^+], 182 (9) [M^{2+}]; IR (CsI): $\tilde{\nu}$ = 3125, 2197, 1715, 1573, 1354, 1272, 1021, 946, 799 cm^{-1} ; UV/Vis (CH_2Cl_2): λ_{max} (ϵ) = 230 (20100), 312 (65900), 326 (106000), 332 (99000), 346 (72500) 430 nm (1800)

6: ^1H NMR ($[\text{D}_8]\text{THF}$, 295 K): δ = 7.60 (s, 4H; H-8,9,20,21, inward pointing furan protons), δ = 6.30 (s, 4H; H-2,3,14,15, outward pointing furan protons), 5.96 (d, 4H; H-6,11,18,23; $^3J_{5,6}$ = 13.1 Hz), 5.85 (d, 4H; H-5,12,17,24); ^{13}C NMR ($[\text{D}_8]\text{THF}$, 295 K): δ = 154.7, 151.7, 117.0, 115.2, 114.9, 114.3; MS (EI, 70 eV): m/z (%): 368 (100) [M^+], 184 (24) [M^{2+}]; IR (CsI): $\tilde{\nu}$ = 3125, 3030, 1708, 1623, 1598, 1551, 1240, 1168, 1023, 949, 794, 648 cm^{-1} ; UV/Vis (THF): λ_{max} (ϵ) = 337 (124000), 354 (179000), 505 nm (1200)

8a: ^1H NMR (CF_3COOD , CH_2Cl_2 int.): δ = 13.15 (d, 2H; H-5,17; $^3J_{5,6}$ = 14.9 Hz), -8.20 (d, 2H; H-6,18), 11.26 (d, 2H; H-11,23; $^3J_{11,12}$ = 12.3 Hz), 11.21 (d, 2H; H-12,24), 5.28 (q, 4H; H-8a,20a), 4.80 (q, 4H; H-3a,15a), 4.73 (q, 4H; H-9a,21a), 4.65 (q, 4H; H-2a,14a), 2.61 (t, 6H; H-8b,20b), 2.35 (t, 6H; H-3b,15b), 2.20 (t, 6H; H-9b,21b), 2.16 (t, 6H; 2b, 14b); ^{13}C NMR (CF_3COOD): δ = 161.3, 158.3, 156.9, 156.8, 156.4, 154.6, 151.4, 149.3, 119.1, 115.8, 112.0, 111.6, 24.2, 22.4, 22.3, 22.2, 18.7, 18.5, 18.4, 14.8; MS (EI, 70 eV): m/z (%): 592 (100) [$(M - 2\text{ClO}_4)^+$], 296 (16); IR (CsI): $\tilde{\nu}$ = 2967, 2933, 2871, 1550, 1449, 1205, 1127, 1083, 842, 624 cm^{-1} ; UV/Vis (HCOOH): λ_{max} (ϵ) = 270 nm (37300), 433 (63200) sh, 456 (518000), 474 (380000), 615 (4300), 665 (20000), 722 (36100), 730 (25800) sh

9: ^1H NMR (98% D_2SO_4 , CH_2Cl_2 int.): δ = 11.66 (d, 4H; H-2,9,14,21; $^3J_{2,3}$ = 3.7 Hz), 11.22 (d, 4H; H-3,8,15,20), 11.61 (s, 4H; H-11,12,23,24); ^{13}C NMR (98% D_2SO_4 , CH_2Cl_2 int.): δ = 157.6, 143.9, 139.3, 137.8, 117.9, 109.6; IR (CsI): $\tilde{\nu}$ = 3104, 1530, 1399, 1352, 1242, 1099, 1004, 958, 919, 776, 624 cm^{-1} ; UV/Vis (98% H_2SO_4): λ_{max} (ϵ) = 419 (200100), 436 sh (121900), 660 (6700), 675 (6900), 706 (41500), 721 nm (88600)

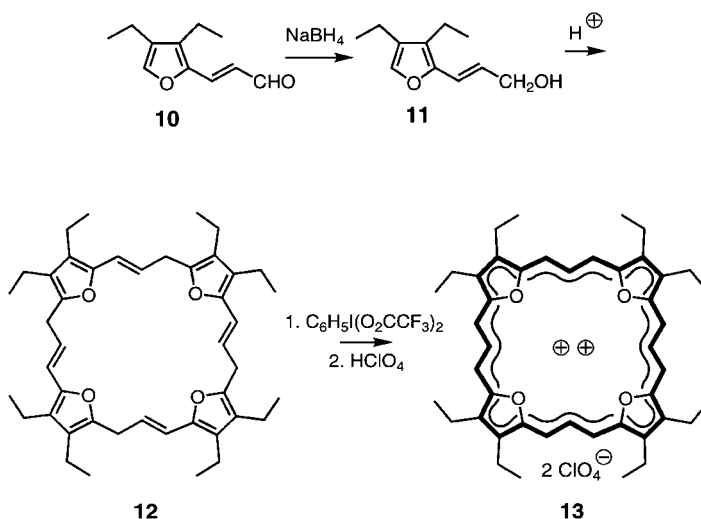
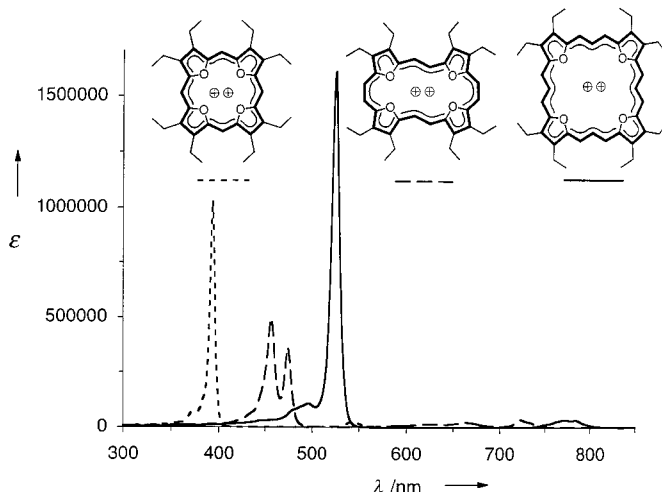
10: ^1H NMR (CDCl_3): δ = 9.58 (d, 1H; CHO), 7.26 (s, 1H, H-5), 7.19 (d, 1H; H-2a; $^3J_{2a,2b}$ = 15.4 Hz), 6.49 (dd, 1H; H-2b), 2.53 (q, 2H; H-3a), 2.40 (q, 2H; H-4a), 1.20 (t, 3H; H-4b), 1.15 (t, 3H; H-3b); ^{13}C NMR (CDCl_3): δ = 192.8, 146.6, 142.0, 136.0, 134.4, 129.8, 124.3, 17.1, 16.7, 15.1, 13.6; MS (EI, 70 eV): m/z (%): 178 (78) [M^+], 149 (100); IR (CsI): $\tilde{\nu}$ = 3129, 3007, 2970, 2877, 1657, 1581, 1520, 1462, 1398, 1376 cm^{-1} ; UV/Vis (CH_2Cl_2): λ_{max} (ϵ) = 259 (3100), 333 nm (32000)

12: ^1H NMR (CDCl_3): δ = 6.22 (d, 4H; H-5,12,19,26; $^3J_{5,6}$ = 15.6 Hz), 6.02 (m, 4H; H-6,13,20,27), 3.38 (d, 8H; H-7,14,21,28), 2.37 (q, 8H; H-3a,10a,17a,24a), 2.31 (q, 8H; H-2a,9a,16a,23a), 1.09 (t, 12H; H-3b,10b,17b,24b), 1.07 (t, 12H; H-2b,9b,16b,23b); ^{13}C NMR (CDCl_3): δ = 147.9, 146.3, 123.7, 122.7, 122.1, 118.5, 30.1, 16.8, 16.6, 15.7, 15.6; MS (EI, 70 eV): m/z (%): 648 (88) [M^+], 485 (100); IR (CsI): $\tilde{\nu}$ = 3038, 2970, 2826, 1655, 1549, 1465, 1322, 1292, 1227, 1101, 995, 953 cm^{-1} ; UV/Vis (CH_2Cl_2): λ_{max} (ϵ) = 285 (84800), 302 nm (41000)

13: ^1H NMR (CF_3COOD , CH_2Cl_2 int.): δ = 14.61 (d, 8H; H-5,7,12,14,19,21,26,28; $^3J_{5,6}$ = 13.4 Hz), -10.92 (t, 4H; H-6,13,20,27), 5.73 (q, 16H; H-2a,3a,9a,10a,16a,17a,23a,24a), 2.92 (t, 24H; H-2b,3b,9b,10b,16b,17b,23b,24b); ^{13}C NMR (CF_3COOD , CH_2Cl_2 int.): δ = 154.6, 150.9, 122.8, 121.9, 22.7, 18.9; MS (FAB, matrix: nitrobenzyl alcohol): m/z (%): 743 (9) [$(M - \text{ClO}_4)^+$], 644 (100) [$(M - 2\text{ClO}_4)^+$]; IR (CsI): $\tilde{\nu}$ = 2972, 2660, 1472, 1377, 1314, 1249, 1082, 1052, 974, 700, 671, 624 cm^{-1} ; UV/Vis (HCOOH): λ_{max} (ϵ) = 496 (117500), 525 (160000), 705 (2200), 772 (35100), 783 (34100), 846 nm (3500)

15: ^1H NMR (CDCl_3): δ = 7.43 (dd, 2H; H-1,10), 6.69 (dd, 2H; H-3,8), 6.41 (dd, 2H; H-2,9); $^3J_{1,2}$ = 1.8, $^3J_{2,3}$ = 3.4 Hz; ^{13}C NMR (CDCl_3): δ = 144.2, 136.4, 116.4, 111.0, 83.1; MS (EI, 70 eV): m/z (%): 158 (100) [M^+], 102 (31); IR (film): $\tilde{\nu}$ = 3132, 2205, 1494, 1376, 1282, 1075, 1015, 913, 816, 742 cm^{-1} ; UV/Vis (CH_2Cl_2): λ_{max} (ϵ) = 258 (12600), 295 (19900), 315 nm (13200)

[a] Correct elemental analyses are available for **4–10**, **8a**, **13**, and **15**.


 Scheme 2. Synthesis of octaethyltetraoxa[26]porphyrin-(3.3.3.3) dication **13** as the bisperchlorate.

 Figure 2. UV/Vis spectra (in HCOOH) of the octaethyltetraoxa[18]porphyrin-(1.1.1.1) dication **1a** (---), the *cis,trans,cis,trans*-octaethyltetraoxa[22]porphyrin-(2.2.2.2) dication **8a** (---), and the octaethyltetraoxa[26]porphyrin-(3.3.3.3) dication **13** (—) as the bisperchlorates.

A comparison of the UV/Vis spectrum of **13** with those of the octaethyl derivatives **1a**^[15] and **8a**^[13] of **1** and **8** is particularly informative. Characteristic of the spectra of **13** and **1a** is that each displays an extraordinarily sharp Soret band which exhibits almost no vibrational structure (half peak width ca. 600 cm^{-1} for **13** and 400 cm^{-1} for **1a**). The very narrow widths of these bands lead to record extinctions ϵ of 1.6×10^6 and $1 \times 10^6 \text{ M}^{-1} \text{ cm}^{-1}$ for **13** and **1a**, respectively, although the oscillator strengths for the two dications (3.8 and 2.9) are of the same order of magnitude as for diprotonated porphyrins (2.1). Two important conclusions may be drawn from the narrow widths of the Soret bands: 1) Compounds **13** and **1a** possess fourfold symmetry, which causes a degeneracy of the two Soret transitions (B transitions in the Platt nomenclature)^[16] and hence excludes π -bond fixation on the time scale of optical excitation ($< 10^{-13} \text{ s}$), and 2) when the weak vibrational progression is considered **13** and **1a** must be very rigid molecules whose geometry remains almost un-

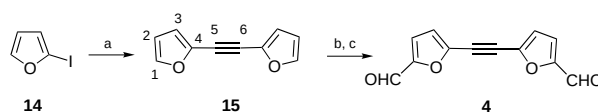
changed on excitation. In the case of **8a**, whose ring framework exhibits only C_{2h} symmetry, the two Soret transitions are no longer degenerate so that a splitting of the Soret band and a reduced maximum extinction is observed in comparison with **1a** and **13**. Based on the absence of significant vibrational structure, a rigid ring framework and thus extensive cyclic π -electron delocalization can also be inferred for **8a**. The oscillator strength of the Soret band region of **8a** (2.3) is smaller than that of **1a** rather than being intermediate between the values of **1a** (2.9) and **13** (3.8) as would have been expected for the all-*cis* compound **2**. The ratio between the transition moments M derived from the experimental oscillator strengths of the dications **1a**, **8a**, and **13** is reproduced quantitatively by INDO/S calculations.^[17] The fact that the squares of the transition moments of the three cations show the same ratios as the areas within the molecules suggests that the highest occupied and the lowest unoccupied orbitals are almost completely located on the carbon perimeter, in agreement with theoretical expectations.^[5, 18]

The meaningfulness of the UV/Vis spectroscopic comparison reported here is attributed to the existence of the tetraoxa[22]porphyrin-(2.2.2.2) dication as the planar *cis,trans*-, *cis,trans* isomer **8** or **8a**. Since the Soret bands in the spectra of **8a** and **1a/13** differ quite dramatically—the former having a split band with a relatively low extinction, and the others a sharp band with very high extinction—the outcome of the comparison may be regarded as a reference example of the significance of molecular symmetry on the shape of UV/Vis spectra.

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Scheme 3. a) Bubbling acetylene through a solution of **14**, $[(C_6H_5)_3P]_2Pd^{II}Cl_2$, and CuI in diethylamine, 6 h, RT. **15**: colorless oil, b.p. 70 °C/0.3 torr (yield 75 %), method of K. Sonogashira, Y. Tohda, N. Hagihara, *Tetrahedron Lett.* **1975**, 4467–4470; b) addition of *n*-butyllithium in *n*-hexane to a solution of **15** and *N,N,N',N'*-tetramethylethylenediamine (TMEDA) in *n*-hexane at 0 °C, then 3 h at RT; c) DMF, 0 °C then 1 h at RT. **4**: colorless crystals; m.p. 210–211 °C (from dichloromethane, then dichloromethane/*n*-hexane; yield 50 %).

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graphic 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-119094. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk). The synthesis of **8a**—analogous to that of **8**—is described in the dissertation of H.-S. Böhm, Universität Köln, 1993.

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Porphyrinoids with 26 π Electrons: Molecules with Exceptional Spectroscopic Properties**

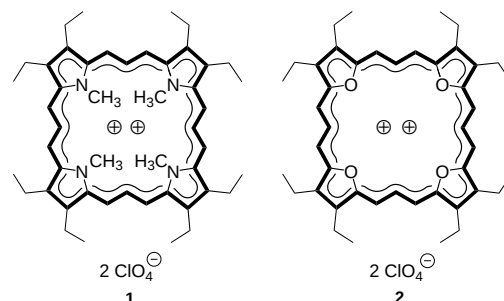
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Dedicated to Professor Burchard Franck

The interdisciplinary interest in porphyrins and their metal complexes has made the development of structural variants of the basic tetrapyrrole macrocycle one of the main lines of current porphyrin research. As a recently published monograph on this topic has demonstrated,^[1] the number of new porphyrinoid macrocycles which have emerged from the expansion, contraction, reorganization, or other modifications of the porphyrin ring system is now legion. From the outset, considerable attention has been paid to expanded porphyrins because it was anticipated that the enlargement of the π -electron system and hence of the cavity would give rise to unusual physical and chemical properties of the molecules involved.

Exceptional spectroscopic properties were indeed encountered with the N,N',N'',N''' -tetramethyl- and tetraoxa[26]por-

phyrin-(3.3.3.3) dications **1** (originally as the bis(trifluoroacetate)) and **2**, which were synthesized in complementary investigations by the groups of Franck^[2] and Vogel.^[3] Although the dications **1** and **2** are closely related, the two compounds surprisingly exhibit marked differences in part of their spectra. It therefore seemed attractive to us to perform a comparative study of the structures and spectra of **1** and **2**.



The octaethyltetraoxa[26]porphyrin dication as the bisperchlorate **2** gave crystals suitable for X-ray crystallographic analysis from anhydrous formic acid^[4] into which solvent molecules—necessary for maintenance of a stable crystal lattice—were incorporated. According to the analysis (Figure 1b) the alkyl groups are arranged in such a way that the

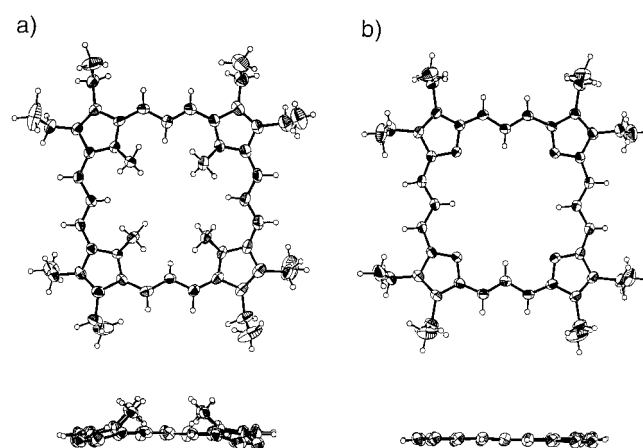


Figure 1. Molecular structures of the 26 π porphyrinoids **1** and **2** in the crystal. a) **1**; top: plan view; bottom: side view. b) **2**; top: plan view; bottom: side view (the ethyl groups have been omitted in the side views).

molecule as a whole becomes almost centrosymmetric. In agreement with this the ring system of **2** is nearly planar (a slight deviation of the framework from the implied D_{4h} symmetry is presumed to be caused by packing effects). The porphyrinoid, aromatic nature of **2** is reflected not only in an essential equalization of the C–C bond lengths in the C_3 segments, but is also inferred from the length relationship $C_{\alpha}-C_{\beta} > C_{\beta}-C_{\beta}$ in the five-membered heterocyclic rings.^[5] The two perchlorate ions, which are located at different distances above and below the ring system, are each associated with one formic acid molecule.

Crystals of the N,N',N'',N''' -tetramethyl[26]porphyrin dication as the bisperchlorate **1**, obtained by treatment of a

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solution of the bis(trifluoroacetate) in acetonitrile with 70% perchloric acid (green platelets with a metallic sheen with incorporation of acetonitrile in a ratio of 1:1), were also suitable for X-ray structure analysis.^[4] This analysis revealed the dication as an approximately C_4 -symmetric molecule whose ring skeleton assumes the shape of a flat bowl (Figure 1a). The slight deviation of the framework from planarity, which should not be too detrimental to the delocalization of the π -electron system in the C_{28} perimeter, arises because the four pyrrole rings are tilted by 16–19° out of the plane formed by the C_3 segments towards the same side of the molecule. As a result of pyramidalization at the nitrogen atoms the N-methyl groups protrude an additional 21–25° from the described reference plane (Figure 2).

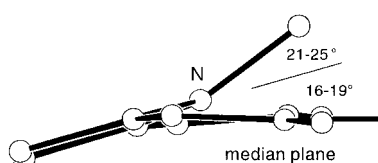


Figure 2. Partial structure of **1** (schematic) as viewed from the side. Illustration of the deviation of the pyrrole rings from the median plane and pyramidalization at the nitrogen atoms.

As determined from molecular models and calculations,^[6] the steric interaction of the N-methyl groups with the respective neighboring inner perimeter hydrogen atoms—and not a mutual hindrance—is responsible for their displacement from the interior of the molecule. With respect to the bond lengths in the C_3 perimeter segments and the bond-length relationship in the five-membered heterocyclic rings, **1** resembles the tetraoxa[26]porphyrin dication **2**. In anticipation of the NMR results, it should be mentioned here that **1** exhibits dynamics which are interpreted on the basis of an inversion process.

A comparison of the ^1H NMR spectra (room temperature) of dications **1** and **2** (Table 1) shows that the deviation of the ring framework from planarity detected in the structural analysis of **1** is too small to manifest itself in a weakening of

the diamagnetic ring current effect. This finding is in accordance with the observation that the lower homologue of **1**, the octaethyl- N,N',N'',N''' -tetramethyl[18]porphyrin dication (as the bisperchlorate),^[7a,b] in which the ring framework is markedly deformed, still exhibits a considerable ring current effect. As can be seen from Table 1, a conspicuously good agreement exists between the chemical shifts of the outer- and inner-perimeter protons H_o and H_i of **1** and **2**—and thus also for the $\Delta\delta$ value ($\delta(H_o) - \delta(H_i)$), which may be regarded as an indicator of the ring current effect. The low- and high-field shifts of H_o and H_i in **1** and **2**, which at a first glance appear abnormal (for comparison: the signal for H_o in **2** is shifted to lower field by about 2.8 ppm relative to that for the *meso* protons in the octaethyltetraoxa[18]porphyrin dication^[8]), may be rationalized in part if it is considered that the strength of the diamagnetic ring current effect increases with the number of π electrons and with the area of the molecule.^[9]

In light of the structural findings the ^1H NMR spectrum of **1** at room temperature, which suggests the presence of a D_{4h} -symmetric and thus planar molecule, can only be explained by the assumption that the NCH_3 groups oscillate through the ring framework very quickly on the NMR time scale. In agreement with this a splitting of the signals for the diastereotopic CH_2 ethyl protons occurs in the low-temperature spectrum (in $\text{CD}_2\text{Cl}_2/\text{CF}_2\text{Br}_2$ at -135°C) of the bis(trifluoroacetate) of the dication, which was used instead of the bisperchlorate because of improved solubility. By using the Eyring equation an activation barrier of $\Delta G^\ddagger = 8.6 \text{ kcal mol}^{-1}$ for the inversion process of the NCH_3 groups was derived from the coalescence temperature of the CH_2 signals (-93°C).

The comparison of the NMR spectra of **1** and **2** is appropriately complemented by inclusion of the spectra of the neutral [28]annulenes **3** and **4** ($4n\pi$ systems) and the 30π dianions **5** and **6** [$(4n+2)\pi$ systems] (Table 1), which can be generated from the corresponding dications by chemical two- or four-electron reduction with potassium in tetrahydrofuran.^[10] The tetraepoxy[28]annulene **4** (blue, microcrystalline solid; decomposition above 0°C) could be isolated with some effort by reoxidation of the readily formed dianion **6** (obtained at room temperature) with atmospheric oxygen at -78°C . In contrast **3**, which is formed without significant overreduction by reduction of **1** at -78°C , was characterized by NMR spectroscopy only. The final reduction products **5** and **6** are, as expected, completely stable in solution. According to Table 1 the transition from the diatropic dications **1** and **2** to the paratropic annulenes **3** and **4**, respectively, causes a spectacular reversal (kind of “umpolung”) of the chemical shifts of the inner- as well as the outer-perimeter protons. In the case of **1/3**, regardless of the slight deformation of the ring framework, this umpolung is similar in magnitude as that for **2/4**. The latter finding is remarkable in that the paratropy of $[4n]$ annulenes is relatively sensitive to deviations from planarity in the ring framework.^[7c] Apart from the observation that the increased electron density in **5** and **6** causes a moderate high-field shift of the signals for the perimeter protons, the spectra of the diatropic dianions **5** and **6** are analogous to those of the dications **1** and **2**.^[11]

Table 1. Characteristic ^1H NMR data of **1** and **2** (in CD_3CN) as well of **3–6** (in $[\text{D}_8]\text{THF}$).

	1, 2		3, 4	5, 6	
	1, 2		3, 4	5, 6	
X = NCH_3	1			3	5
$\delta(H_o)$	14.06			2.53	12.27
$\delta(H_i)$	−11.98	$\Delta\delta = 26.0$		16.48	−11.78
$\delta(\text{NCH}_3)$	−9.46			10.23	−8.89
X = O	2			4	6
$\delta(H_o)$	14.42			2.58	12.78
$\delta(H_i)$	−11.12	$\Delta\delta = 25.5$		15.94	−12.21

The UV/Vis spectra of **1** and **2** (Figure 3) show the expected similarity with respect to the position and appearance of the Soret and Q bands. Surprisingly, however, the spectra differ dramatically in terms of the molar extinction coefficients ϵ of the Soret band in that ϵ_{Soret} , which exhibits the unusually high value of almost $1 \times 10^6 \text{ M}^{-1} \text{ cm}^{-1}$ for **1**, is increased still further to the record value of $1.6 \times 10^6 \text{ M}^{-1} \text{ cm}^{-1}$ for **2**. This result is all

of tetraoxa[4n+2]porphyrin dications ($n = 4-6$).^[3c] While in that case the dependency of the habitus of the spectrum on the geometry of the respective planar C_{4n+2} perimeter was demonstrated, it could be shown here how a perturbation of the D_{4h} symmetry in [26]porphyrin dications affects the spectra of such molecules.

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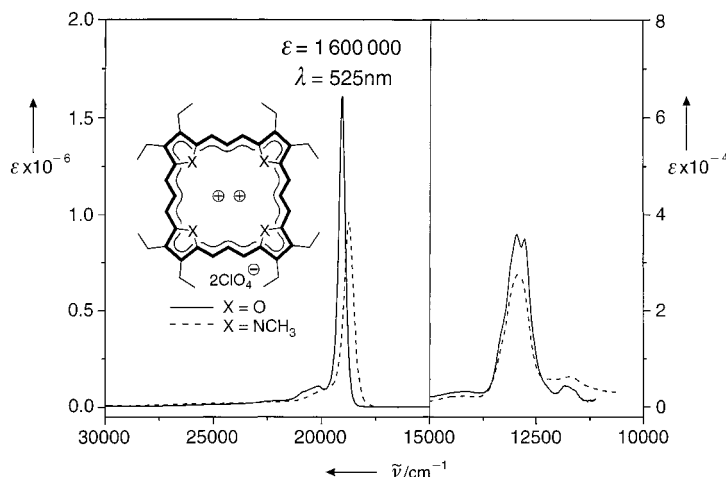


Figure 3. UV/Vis spectra (in HCOOH) of **1** (---) and **2** (—).

the more remarkable since the oscillator strength of the Soret band of **2** (3.8) is only marginally greater than that of **1** (3.6). The occurrence of only one Soret band in the spectra of **1** and **2** shows that the two Soret transitions are degenerate, as required by the presence of fourfold symmetry. For both **1** and **2** the Soret band exhibits only a weakly developed vibrational structure, which indicates that the molecular geometry in the electronic ground state S_0 and in the singlet state S_2 is almost identical. The extremely high ϵ_{Soret} value found for **2** corresponds with the considerable reduction in the half-peak width of the Soret band to 600 cm^{-1} compared to about 1000 cm^{-1} observed for **1**. The relatively large half-peak width of this band for **1** most likely results from a perturbation of the fourfold symmetry, which is caused by the different degree of twisting of the pyrrole rings and the associated scattering of the conformational alignment of the NCH_3 groups. The slight increase in the oscillator strength of **2** in comparison to **1** suggests that the frontier orbitals of **2** are more strongly located at the C_{28} perimeter than is the case for **1**.

Because of the fourfold molecular symmetry, the degenerate Q transitions^[12] are allowed for **1** and **2** in addition to the degenerate Soret transitions. However, the 0-0 transition of the Q band occurring at 11800 cm^{-1} for both molecules is relatively weak ($\epsilon \approx 4000$; Figure 3). The low intensity of this transition can be attributed to the S_1 state having negative pseudoparity.^[13] Since Soret and Q transitions in porphyrinoid systems with fourfold symmetry belong to the same irreducible representation a strong vibrational coupling results, which leads to a marked increase in the intensity at 12800 cm^{-1} .

In respect to the relationship between UV/Vis spectroscopy and molecular symmetry, the discussion of the UV/Vis spectra of **1** and **2** is closely associated with the previous investigation

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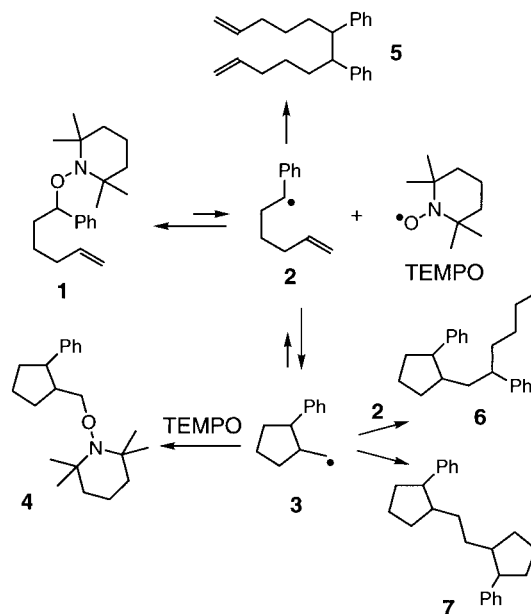
Tin-Free Radical Cyclization Reactions Using the Persistent Radical Effect**

Armido Studer*

Organotin compounds have found widespread application in conducting various types of radical reactions.^[1] Despite this important utility, there are drawbacks associated with tin-based radical chemistry, namely, toxicity of organostannanes that necessitate special handling of disposal, and in many instances problems with product purification. Therefore, various research groups have been looking for alternatives.^[2] Herein we report new tin-free radical cyclization reactions based on the persistent radical effect (PRE).

The PRE^[3,4] is a general principle that explains the highly specific formation of the cross-coupling product (R^1-R^2) between two radicals R^1 and R^2 when one species is persistent (long-lived) and the other transient; the two radicals must be formed at equal rates. The initial buildup in concentration of the persistent species, caused by self-termination of the transient radical, steers the reaction to follow a single pathway for the cross-reaction. The PRE has already been used in various chemical systems^[3,5] and is important in stable free radical polymerization (SFRP).^[6]

In Scheme 1, an application of the PRE for a 5-*exo* cyclization with the 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) radical as persistent species is suggested. It is known that α -phenyl-substituted alkoxyamines such as **1** have weak C–O bonds which are homolytically cleaved upon



Scheme 1. Radical 5-*exo*-cyclization with use of the persistent 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) radical.

heating.^[6,7] C–O bond homolysis in **1** will therefore lead to the persistent TEMPO radical and the transient radical **2**.^[6–10] Radical **2** can either be trapped by TEMPO to reform **1**, or it can undergo a 5-*exo* cyclization (the 6-*endo* cyclization is omitted in Scheme 1) to form a new radical **3**, which after trapping with TEMPO affords **4**. The trapping of **3** with TEMPO is irreversible, because the C–O bond in an alkoxyamine derived from TEMPO and a primary alkyl radical is too strong to be homolytically cleaved.^[7] Due to the low concentration of TEMPO during the isomerization,^[6b] **3** is long-lived and the cyclization from **2** to **3** is probably reversible.^[11] According to the PRE, the coupling products **5–7** from the transient radicals **2** and **3** should be formed in very low yields, and the isomerization of **1** should occur almost quantitatively.^[4] Furthermore, degenerate radical reactions (reversible initial C–O bond homolysis in our system) have been shown to suppress potential side reactions by reforming starting material when the desired cyclization does not proceed effectively.^[5b]

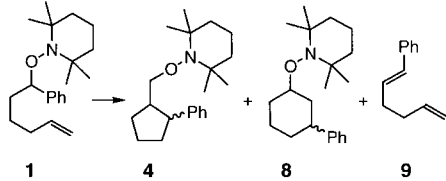
To test the concept of the PRE in cyclization reactions, alkoxyamine **1** was prepared from 1-bromo-1-phenyl-5-hexene and the Ca alkoxide (2 equiv) of 1-hydroxy-2,2,6,6-tetramethylpiperidine in refluxing THF (14 h, 80 %).^[12,13] The isomerization reaction depicted in Scheme 1 was studied under different conditions (Table 1). In *tert*-butylbenzene and *N,N'*-dimethyl-*N,N'*-propylene urea (DMPU), no isomerization was observed (entries 1 and 2). In DMF after 16 h, **4** was isolated in 56 % yield (*trans:cis* = 2.5:1)^[14] along with the 6-*endo* product **8** (10 %, *trans:cis* = 1:1; entry 3). In *t*BuOH (0.1 M) clean but slower isomerization occurred (entry 4) and lowering the concentration (0.1 M $\rightarrow$ 0.01 M) has no significant effect (entry 5). Interestingly, the reaction was accelerated by addition of camphorsulfonic acid (CSA, entry 6),^[15] with the best results obtained in *t*BuOH (0.02 M) with 10 % CSA (entry 7). The isomerization products were isolated in 83 % yield along with **9** (2 %).^[16]

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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. Isomerization of **1** under different conditions (130–132 °C, sealed tube).



Entry	Solvent	Conc. [M]	<i>t</i> [h]	4 [%]	8 [%]	9 [%]
1	<i>t</i> BuPh	0.1	22	< 2 ^[a]	< 2 ^[a]	20
2	DMPU	0.1	14	< 2 ^[a]	< 2 ^[a]	< 2 ^[a]
3	DMF	0.1	16	56	10	< 2 ^[a]
4	<i>t</i> BuOH	0.1	16	n.d. ^[b]	n.d. ^[b]	< 2 ^[a]
5	<i>t</i> BuOH	0.01	36	n.d. ^[c]	n.d. ^[c]	< 2 ^[a]
6	<i>t</i> BuOH	0.01	24 ^[d]	53	10	< 2 ^[a]
7	<i>t</i> BuOH	0.02	24 ^[d]	70	13	2

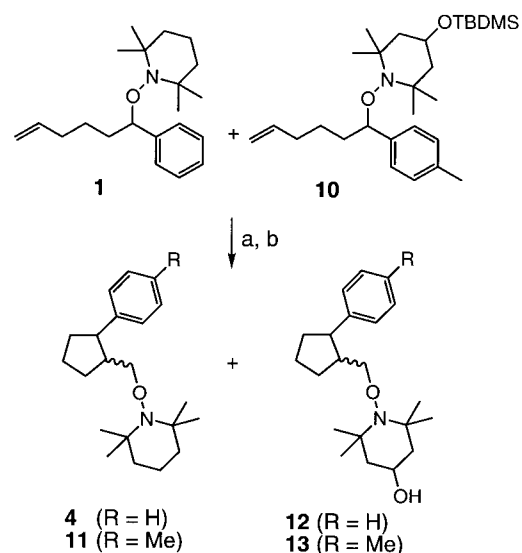
[a] In the 300-MHz ¹H NMR spectrum of the crude product, no signals of the corresponding compound were observed. [b] After 16 h, 62 % of the starting material remained. Products **4** and **8** were not isolated. [c] After 36 h, about 10 % of starting material remained. Products **4** and **8** were not isolated. [d] 10 % CSA was added.

It is known that the reaction of nitroxides with various C-centered radicals is dependent on solvent, with decreasing rates in polar protic solvents.^[17] Furthermore, the homolytic cleavage is faster in polar solvents.^[18] These synergistic effects^[6b] lead to the observed rate enhancement in the isomerization upon using polar solvents.

An ionic mechanism (C–O heterolysis) can be excluded since clean cationic isomerizations in *t*BuOH are unlikely. To further corroborate the radical nature of the process, we also conducted ESR experiments. A deoxygenated sample of **1** in *t*BuOH/*t*BuPh (1/1) containing an excess of the C-radical scavenger 2,2,10,10-tetraproterdeuteriomethyl-isoindolin-15N-oxyl (10 equiv) was heated to the reaction temperature, and the evolution of the ESR signal of the TEMPO radical was followed by continuous wave (CW) ESR spectroscopy.^[19] Almost 100 % of the TEMPO was generated, thus establishing initial C–O bond homolysis under these conditions.

To exclude a mechanism where isomerization occurs in a solvent cage, we also conducted a cross-over experiment. The alkoxyamines **1** and **10** (1/1 mixture) were isomerized under the optimized conditions. After desilylation, roughly equal amounts of the scrambled alkoxyamines **4** (19 %), **11** (19 %), **12** (16 %), and **13** (16 %) were isolated (Scheme 2).^[20]

To further examine the scope and the limitations of the reaction, we tested whether isomerization also occurs with alkoxyamines **14a–j** (variation of substituent R, Table 2).^[21–23] For bromide **14a**, a clean reaction occurred, and **15a** was isolated in 71 % (*trans*:*cis* = 2.7:1)^[24] with **16a** (8 %, entry 1). Isomerization of **14b** led to **17b** (10 %), and the products **15b** and **16b** were isolated in 54 % yield (**15b**:**16b** = 5.8:1, entry 2). Clean reactions were observed for heteroarenes **14c** and **14d** (entries 3 and 4). With **14e**, no 6-*endo* product was formed, and **15e** was isolated in 67 % yield (d.r. = 1:1, entry 5) along with 10 % of **17e**. The reaction with nitrile **14f** afforded 61 % of the 5-*exo* product and 7 % of **16f** (entry 6). No isomerization occurred with **14g** (R = H), **14h**



Scheme 2. Cross-over experiment with an equimolar mixture of the alkoxyamines **1** and **10**. a) *t*BuOH (0.02 M), 130 °C, 10 % CSA, 24 h; b) TBAF, THF. TBAF = tetrabutylammonium fluoride; TBDMS = *tert*-butyldimethylsilyl.

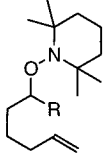
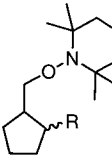
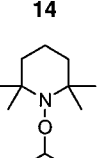
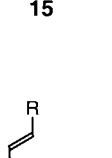
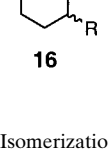
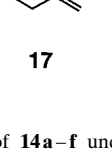
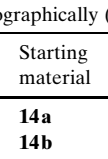
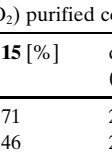
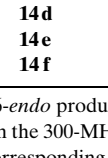
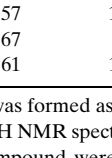
	R	Compound
	4-BrC ₆ H ₄	a
	4-CH ₃ OC ₆ H ₄	b
	2-thienyl	c
	2-pyridyl	d
	<i>t</i> BuO ₂ C	e
	NC	f
	H	g
	Me	h
	PhS	i
	PhSO ₂	j

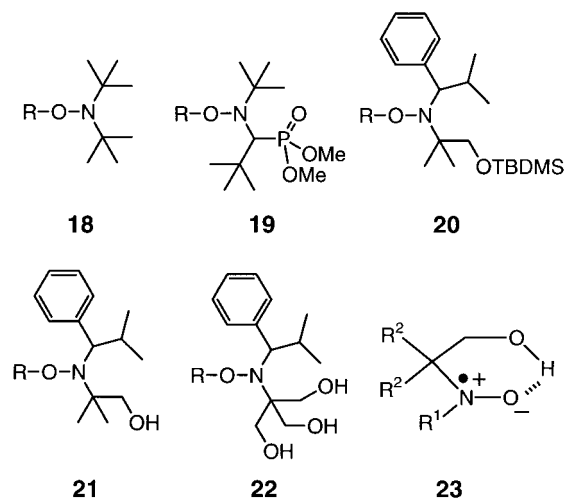
Table 2. Isomerization of **14a–f** under optimized conditions (*t*BuOH, 0.02 M, 24 h, 130–132 °C, 10 % CSA, sealed tube). Yields refer to chromatographically (SiO₂) purified compounds.

Entry	Starting material	15 [%]	d.r. (15) (<i>trans</i> : <i>cis</i>)	16 [%] ^[a]	17 [%]
1	14a	71	2.7:1	8	< 2 ^[b]
2	14b	46	2.8:1	8	10
3	14c	67	2.1:1	11	5
4	14d	57	1.6:1	12	5
5	14e	67	1:1	< 2 ^[b]	10
6	14f	61	1.1:1 ^[c]	7	< 2 ^[b]

[a] The 6-*endo* product was formed as a 1:1 mixture of the diastereoisomers. [b] In the 300-MHz ¹H NMR spectrum of the crude product, no signals of the corresponding compound were observed. [c] The relative configuration of the two isomers was not assigned.

(R = Me), and **14i** (R = SPh). It is likely that the C–O bond in **14g–i** is too strong to be effectively cleaved.^[7] As expected, sulfone **14j** was not stable at higher temperatures (elimination of PhSO₂H).

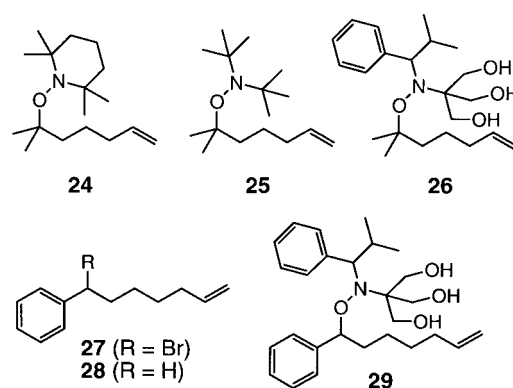
We then tested alkoxyamines **18–22** (R = 1-phenyl-5-hexenyl, variation of the nitroxide) in the isomerization.^[25–28] The reactions were conducted in *t*BuOH (0.02 M) with or



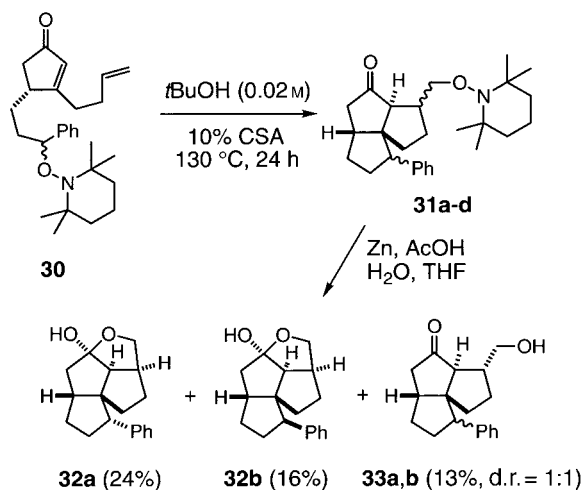
without added CSA (10%) at 130 °C, and the time necessary for complete consumption of the starting material was measured. The success of the isomerization is dependent on the bond dissociation energy (BDE) of the C–O bond (see above), which should correlate with the reaction time. The fastest isomerization (3 h) was observed for **18**. This acceleration, as compared to the cyclization of **1** (24 h with CSA), is not surprising, since it is known that alkoxyamines derived from di-*tert*-butyl nitroxide have very weak C–O bonds.^[18] Only 4.5 h were necessary to completely isomerize phosphonate **19**. Addition of CSA led to decomposition of the starting alkoxyamine (for **18** and **19**).

Based on results from Ingold and Beckwith on the stabilization of nitroxides by *intermolecular* hydrogen bonding,^[17] we decided to prepare new nitroxides capable of *intramolecular* hydrogen bonding (**23**).^[29] In addition to decreasing the trapping rate of the nitroxide with C-centered radicals, the hydrogen bonding should also influence the rate of C–O bond homolysis (late transition state). Indeed, **21** was observed to isomerize faster (7 h; 12 h without CSA) than its silylated congener **20** (16 h with CSA). With **22**, reaction was already completed after 4 h (9 h without CSA).

The advantage of our new alkoxyamines over derivatives derived from commercially available nitroxides is documented by the failure of **24** (no reaction) and **25** (decomposition) to isomerize and by the successful isomerization of triol **26** (52 %).^[30] We also conducted a slow 6-*exo* cyclization of a phenyl-stabilized radical. Reaction of **27** under Bu₃SnH conditions (1.2 equiv of hydride, syringe pump, 7 h, benzene, 0.1 M) afforded only 11 % of the cyclized product besides the dehalogenation product **28**. Alkoxyamine **29**, however, yielded the corresponding 6-*exo* product in 48 % yield. An additional advantage, besides higher yield and lower toxicity in the reaction of **29** as compared to the tin hydride mediated cyclization of **27**, is the fact that the cyclization product is functionalized (protected alcohol) in the former case.



To further illustrate the potential of the method, we conducted a radical cascade reaction (Scheme 3). Enone **30**^[31] was isomerized to give the angular triquinane **31** in 78 % yield as the expected mixture of stereoisomers.^[32] N–O bond cleavage with Zn in AcOH/THF/H₂O (3/1/1) afforded the hemiacetals **32a**, **b** (40 %) and the alcohols **33a**, **b** (13 %). The relative configuration of the major isomer **32a** was assigned by X-ray crystallographic analysis.^[33]



Scheme 3. Radical cascade reaction.

In conclusion, the new tin-free radical cyclization reactions afford products which can be considered as protected alcohol derivatives.

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Pseudoprolines: Targeting a *cis* Conformation in a Mimetic of the gp120 V3 Loop of HIV-1**

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Pseudoprolines (Ψ Pro) have been introduced recently as synthetic proline analogues readily obtained by cyclocondensation of the amino acids cysteine, threonine, or serine with aldehydes or ketones.^[1] Their application as structure-disrupting, solubilizing protecting groups in solid-phase peptide synthesis^[2, 3] was followed by conformational investigations concerning the Ψ Pro preceding peptide bond.^[4, 5] In fact, the propensity of the amino acid proline for forming a Xaa_{*i*-1}-Pro_{*i*}

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cis peptide bond can be strongly enhanced by the introduction of a pseudoproline residue, depending on its C(2) substituents. In particular, the 2,2-dimethylated thiazolidine derivative can induce up to 100 % *cis* conformation in the preceding peptide bond in model di- and tripeptides.^[4,5] We herein report the first example of this class of pseudoprolines as mimetics of biologically relevant *cis*-prolyl conformations.

An interesting target for the introduction of a *cis*-inducing pseudoproline unit is the V3 loop of the envelope protein gp120 of immunodeficiency virus type 1 (HIV-1), which has been the subject of numerous studies since it was shown to correspond to the major HIV-1 neutralizing epitope.^[6–8] Proteolytic cleavage of V3, after binding of gp120 to the cell surface receptor CD4, has been suggested as being a prerequisite for viral infection.^[9,10] A generally conserved tetrapeptide motif Gly-Pro-Gly-Arg is situated at the tip of the V3 loop, forming a type II β -turn.^[11] Johnson et al. proposed that a *trans/cis* isomerization towards a type VI β -turn with a *cis*-proline peptide bond is a necessary conformational change for cleavage and subsequent fusion.^[12] Other indications of the importance of conformational changes in gp120 preceding infection have recently appeared,^[13,14] and a type VI β -turn conformation has been observed in a peptide derived from the HIV-1_{IIIIB} V3 loop when bound to an anti-gp120 antibody.^[15] Here, we have targeted the proposed infection-active *cis* conformation by preparing pseudoproline-containing, *cis*-constrained V3 loop analogues as immunogens.

The eleven-residue cyclic loop structure cyclo(-Arg-His-Ile-Gly-Xaa-Gly-Arg-Ala-Phe-Cys-Tyr-) with a sequence based on the HIV-1_{MN} V3 variant and containing the tetrapeptide motif Gly-Xaa-Gly-Arg, was selected as a base for the mimetic (Figure 1). A cysteine was incorporated as a useful

cyclo(-Arg-His-Ile-Gly-Xaa-Gly-Arg-Ala-Phe-Cys-Tyr-) **I**^c, **II**^c, **III**^c
Ac-His-Ile-Gly-Xaa-Gly-Arg-Ala-Phe-Cys-NH₂ **I**^t, **II**^t

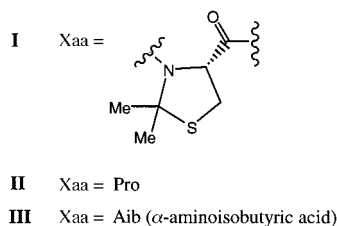


Figure 1. Sequences of cyclic (**I**^c, **II**^c, **III**^c) and linear (**I**^t, **II**^t) peptides.

linker site for immunological studies. Three cyclic V3-peptides were synthesized, a constrained pseudoproline-containing mimetic (Xaa = Cys($\psi^{Me,Me}$ pro), **I**^c), a proline-containing loop analogue (Xaa = Pro, **II**^c) similar to the native structure, and a peptide containing Aib (α -aminoisobutyric acid) replacing Pro (Xaa = Aib, **III**^c). In addition, the corresponding linear peptides **I**^t and **II**^t (Figure 1) were prepared for determining the impact of the cyclic structure upon the immunogenic properties of the peptides. In Figure 2, the Gly- ψ Pro and Gly-Pro regions of peptides **I**^c and **II**^c are represented, showing the *cis* and *trans* peptide bond.

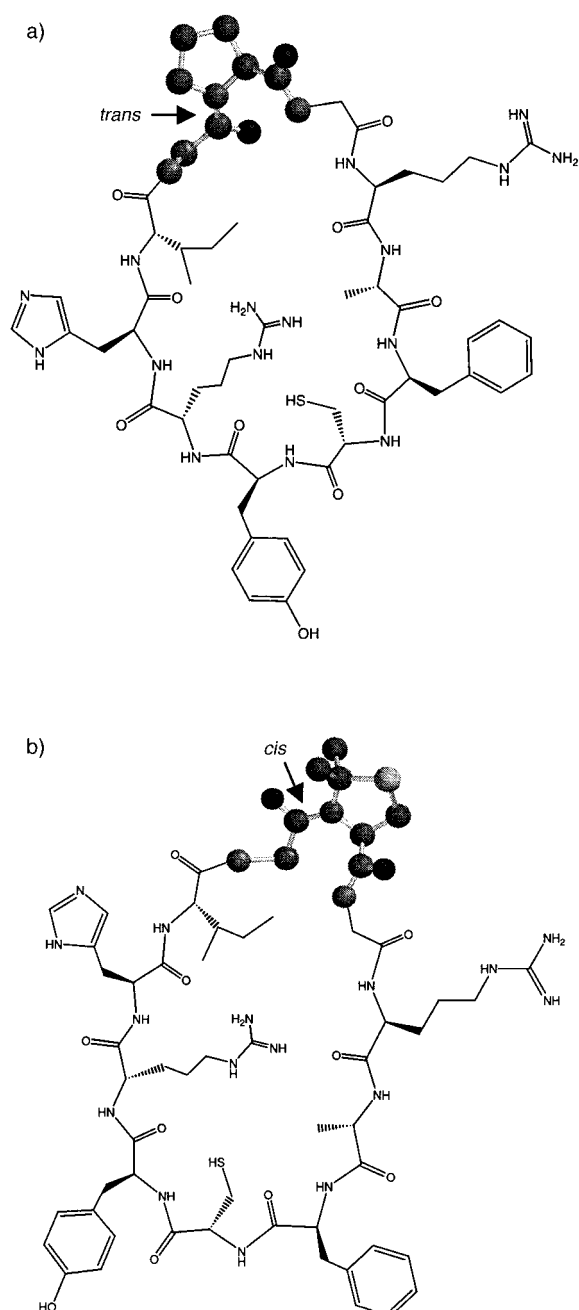


Figure 2. Model of the conformational change induced by the presence of the pseudoproline. a) Gly-Pro region of peptide **II**^c forming a *trans* peptide bond; b) Gly-Cys($\psi^{Me,Me}$ pro) region of peptide **I**^c forming a *cis* peptide bond.

Peptides **I**–**III**, obtained by solid-phase peptide synthesis,^[16] were characterized by mass spectrometry and by one- and two-dimensional NMR spectroscopy. 2D ¹H NMR TOCSY, COSY-DQF, and ROESY data allowed complete attribution of proton chemical shifts for peptides **I** and **II**. Three conformers were observed for the cyclic ψ Pro-containing peptide **I**^c. By integration of the β -methyl signals of Ile, their ratio was determined to be 80:10:10. In the ROESY experiment, the major conformer showed the typical pattern expected for a *cis* amide bond between Gly_{*i*-1} and Cys_{*i*} ($\psi^{Me,Me}$ pro), that is, αH_{i-1} – αH_i and $\alpha H'_{i-1}$ – αH_i cross peaks. The corresponding region is represented in Figure 3 a. One of

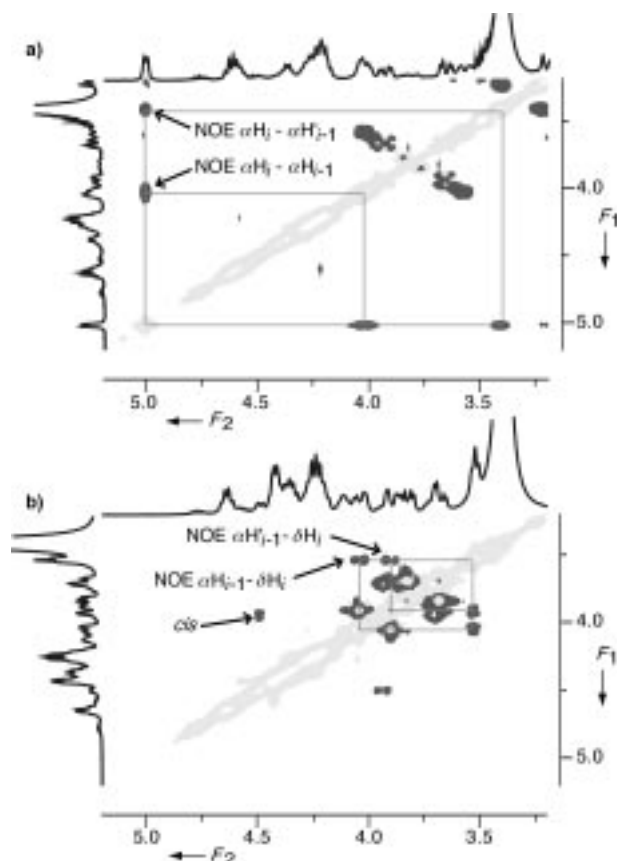


Figure 3. Expanded regions of the 2D ^1H -NMR ROESY spectra of the V3 loop analogues in DMSO- D_6 at 305 K (400 MHz, $\tau_m = 200$ ms). a) Ψ Pro-containing peptide **I^c** with NOE connectivities $\alpha\text{H}_i - \alpha\text{H}_{i-1}$ and $\alpha\text{H}_i - \alpha\text{H}'_{i-1}$ between Gly $_{i-1}$ and Cys($\Psi^{\text{Me,Me}}\text{pro}$); b) Pro-containing peptide **II^c** with NOE connectivities $\alpha\text{H}_{i-1} - \delta\text{H}_i$ and $\alpha\text{H}'_{i-1} - \delta\text{H}_i$ between Gly $_{i-1}$ and Pro $_i$.

the minor conformers was expected to be the corresponding *trans* form with a *trans* peptide bond between Gly $_{i-1}$ and Cys $_i$ ($\Psi^{\text{Me,Me}}\text{pro}$) but with characteristic NOE connectivities $\alpha\text{H}_{i-1} - \delta\text{H}_i$ and $\alpha\text{H}'_{i-1} - \delta\text{H}_i$ could not be observed because of the dimethylation of the δ -position. Spectra of the cyclic Pro-containing peptide **II^c** showed three conformers present at a ratio of 80:15:5. In the major conformer, the Gly-Pro peptide bond was shown to be *trans* as proved by the characteristic NOE connectivities $\alpha\text{H}_{i-1} - \delta\text{H}_i$ and $\alpha\text{H}'_{i-1} - \delta\text{H}_i$ between Gly $_{i-1}$ and Pro $_i$ (Figure 3b). A set of minor resonances could be attributed to the corresponding *cis* conformer with cross peaks $\alpha\text{H}_{i-1} - \alpha\text{H}_i$ and $\alpha\text{H}'_{i-1} - \alpha\text{H}_i$ between Gly $_{i-1}$ and Pro $_i$ (also shown in Figure 3b) which shows the inherent propensity of the amino acid proline for a Xaa $_{i-1}$ -Pro $_i$ *cis* peptide bond. The high conformational flexibility of Aib-containing peptide **III^c** resulted in very undefined spectra, which didn't allow for complete attribution of proton chemical shifts. NMR spectra of the linear peptide **I^f** interestingly showed a single conformation with a *cis* bond preceding the pseudoproline residue. For the linear peptide **II^f**, two conformations were observed at a ratio of 30:70, the minor conformer containing a *cis* Gly-Pro bond and the major conformer having a *trans* bond preceding proline.

Antibodies were raised against peptides **I^f** and **II^f**. In order to obtain an immunogenic preparation, both loop analogues were coupled to the carrier protein BSA (bovine serum

albumin) via linkage with 3-(*N*-maleimido)propionic acid hydroxysuccinimide ester. Balb/c mice were immunized with the two bioconjugates according to standard protocols.^[17] After one boost, the mice were bled and the obtained antisera were tested by ELISA (enzyme-linked immunosorbent assay) using covalent linkage of the cyclic peptide antigens to the plate surface, which allows sensitive testing conditions. In Figure 4, the immunological response of the antisera towards

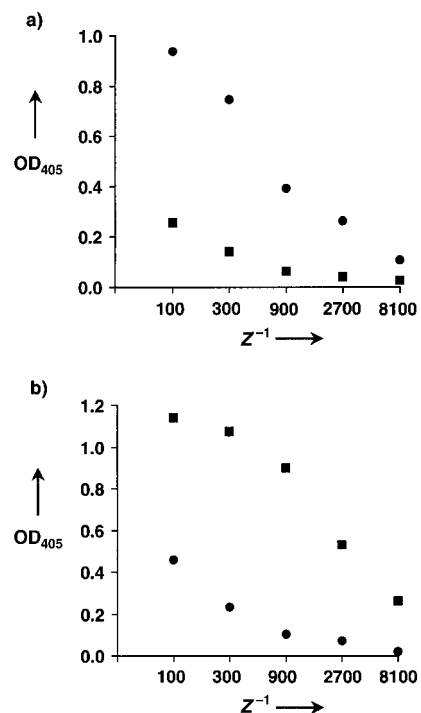


Figure 4. Specificity assays showing recognition of sera from mice immunized with Ψ Pro-containing peptide **I^c** (●) and Pro-containing peptide **II^c** (■). a) Coating peptide **I^c** ($c = 10 \mu\text{g mL}^{-1}$); b) Coating peptide **II^c** ($c = 10 \mu\text{g mL}^{-1}$). Z = serum dilution, OD₄₀₅ = optical density at 405 nm.

the haptens is represented. In both cases, the polyclonal antibodies show a high recognition for the corresponding immobilized hapten, but not for the foreign loop-peptide. In order to confirm this result, competition assays were carried out in which the sera were allowed to preincubate with peptides **I^f** or **II^f** for 30 minutes before addition to the immobilized hapten. The results with **I^f** as the plate antigen are shown in Figure 5. They demonstrate that the antigenic reaction is strongly inhibited with increasing concentration of **I^f**, compared to the much weaker influence of **II^f**, confirming the high selectivity between the *cis*-constrained and the unconstrained peptides.

Monoclonal antibodies (mAbs) were raised by fusion of spleen cells from the immunized animals with myeloma cells of an Ag8 strain.^[17, 18] Hybridoma cells which produced antibodies with the desired specificity were propagated and cloned, and the monoclonal antibodies purified. Monoclonal antibodies selected against **I^f** and **II^f** were tested for their recognition towards the cyclic and linear peptides. Both confirm the results obtained with polyclonal antibodies, specifically recognizing the corresponding cyclic antigen. As

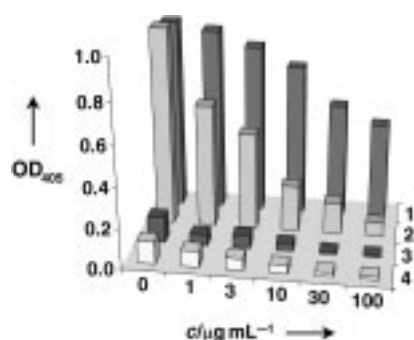


Figure 5. Competition assay showing inhibition of the antigenic reaction with increasing concentration of peptide I^c. Peptide II^c inhibits the antigen reaction considerably less. The plate antigen is I^c coated at a concentration of 10 $\mu\text{g mL}^{-1}$. 1: Serum anti-I^c + peptide II^c; 2: Serum anti-I^c + peptide I^c; 3: Serum anti-II^c + peptide II^c; 4: Serum anti-II^c + peptide I^c. c = concentration of competitive peptide.

shown in Figure 6, mAb Ψ Pro238.13 selected against the cyclic Ψ Pro-containing peptide I^c recognizes the Pro-containing peptide II^c to about 15%, in harmony with the degree of *cis* content in II^c determined by NMR. As a further indication of the presence of an mAb directed at *cis* amide bonds,

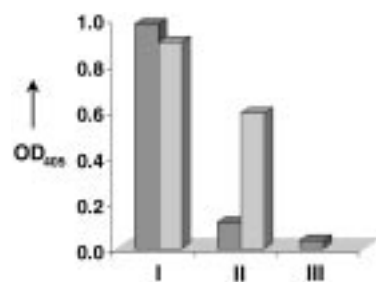


Figure 6. Recognition of monoclonal antibody mAb Ψ Pro238.13 selected against I^c towards cyclic (c, dark bars) and linear (l, light bars) peptides. I: Ψ Pro-containing peptides I^c and I^l; II: Pro-containing peptides II^c and II^l; III: Aib-containing peptide III^c.

no recognition was observed towards reference peptide III^c, which contained Aib as a structural mimetic of the C(2) dimethyl substituted Ψ Pro residue in peptide I (Figure 6). Most notably, the mAb directed towards the cyclic *cis*-containing peptide I^c shows also a high recognition for the corresponding linear peptide I^l. Due to the substantial differences of the cyclic and linear peptides with respect to their conformational flexibility and overall shape, the recognition of both species by mAb Ψ Pro238.13 points to the common *cis* amide bond as a recognition site. This observation is further validated by a partial recognition of the linear Pro-containing peptide II^l, which can be only rationalized by a *trans* to *cis* isomerization of the Gly–Pro bond of the flexible peptide II^l upon interaction with the *cis*-directed antibody. Most notably, this isomerization occurs to a much lower degree within the conformationally constrained cyclic peptide II^c, resulting in a substantially lower recognition by the *cis* bond directed antibody. This difference in recognition of monoclonal antibody anti-I^c towards the cyclic and linear peptides II^c and II^l which both contain the Gly–Pro chemical entity clearly indicates a conformational specificity.

In conclusion, a 2,2-dimethylated thiazolidine (Ψ Pro) derivative has been introduced into a biologically relevant cyclized peptide sequence from the V3 loop of HIV-1 which has induced a *cis* peptide bond and represents a conformation that is proposed to play a crucial role in the HIV infection process. Furthermore, immunological results demonstrate that antibodies have been raised which selectively distinguish between the *cis* and the *trans* conformation of Xaa-Pro imide bonds in linear and cyclic peptides, offering interesting perspectives for applying the Ψ -proline concept as a diagnostic tool for the detection of conformational changes during biological processes.

Experimental Section

Peptides were synthesized according to standard procedures of SPPS (solid-phase peptide synthesis)^[16] by the Fmoc (9-fluorenylmethoxycarbonyl) strategy on Sasrin (super acid sensitive resin)^[19] or Sieber amide resin.^[20] The pseudoproline, a cysteine derived 2,2-dimethyl-1,3-thiazolidine-4-carboxylic acid (Cys($\Psi^{\text{Me,Me}}$ Pro)), was introduced during SPPS as a preformed dipeptide building block^[3] (for experimental details, see Supporting Information). The products were characterized by ESI-MS (electrospray ionization mass spectrometry) and 1D and 2D ¹H NMR TOCSY, COSY-DQF and ROESY spectroscopy. Spectra were recorded at 400 MHz (τ_m = 200 ms) in DMSO- D_6 at 305 K (see Supporting Information).

The immunogenic construct was prepared by coupling the linker molecule 3-(*N*-maleimido)propionic acid hydroxysuccinimide ester to the carrier protein BSA in 0.1M phosphate buffer (pH 7.0)/dioxane (5/1; v/v). After dialysis against 0.1M phosphate buffer (pH 7.0) (molecular weight cut-off 2000), peptides I^c or II^c were added and left to incubate for 1 h before dialysis against water and lyophilization of the product. The number of V3-peptides coupled to each molecule of BSA was determined by SDS-PAGE (sodium dodecylsulfate polyacrylamide gel electrophoresis) to lie between 10 and 30.

For the elicitation of antibodies, antigen (15 μg in 25 μL of phosphate-buffered saline, PBS) was mixed with Titer Max Adjuvant (25 μL) and injected subcutaneously (base of tail) into eight week old Balb/c mice. Mice immunized with I^c were boosted after 5 weeks, and all mice were bled after 9 weeks.

ELISA-plates used for covalent linkage of thiol groups were AquaBind Combiplates from BioConcept. Coating was performed by incubating peptide solutions (10 $\mu\text{g mL}^{-1}$) in 0.2 mM carbonate buffer (pH 9.5) overnight at 4 °C. After washing the plates with PBS/0.05 % Tween 20, the surface was blocked with a 5 % solution of milk powder in PBS. Serum dilutions were prepared in PBS/0.05 % Tween 20 and incubated for two hours at room temperature. Plates were washed with PBS/0.05 % Tween 20 and incubation was effected with a conjugate of anti-mouse antibodies and alkaline phosphatase in PBS/0.05 % Tween 20 (1:1000, 1.5 h). After washing, a premixed substrate solution (*p*-nitrophenyl phosphate) was added to the plates and, after 30 min, the absorbance was read at 405 nm.

Myeloma cells used for fusion were of the x63Ag8.653 cell line.^[18] Fusion, propagation of hybridoma cells, cloning, and purification of the monoclonal antibodies was performed following standard protocols.^[17]

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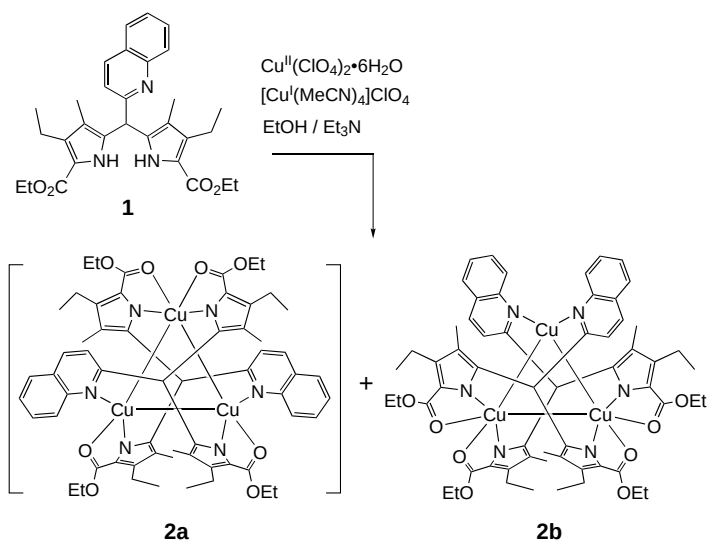
A Triangular Mixed-Valent Cu^{II}Cu^ICu^I Cluster Supported by the Tripod Ligand 2-Quinoly-2,2'-dipyrrolylmethane**

Jun-ichiro Setsune,* Tatsuo Yokoyama, Setsuo Muraoka, Hong-wei Huang, and Takeshi Sakurai

Trinuclear copper complexes are attracting increasing attention as models for the metal sites of multicopper

oxidases.^[1,2] While triangular Cu₃ complexes with C_{3v} symmetry are well known,^[3] those with lower symmetry are important because the metallosites of multicopper oxidases consist of two type-3 Cu sites and one type-2 Cu site.^[1] We report here that triangular Cu₃ cluster complexes with a Cu^{II}Cu^ICu^I mixed-valent state are obtained by using the mixed tripod ligand 2-quinoly-2,2'-dipyrrolylmethane. The X-ray structure and spectroscopic properties of the Cu₃ complex indicate that two Cu ions interact strongly with one another.

We have shown that 2-pyridyl-2,2'-[3,3'-dimethyl-4,4'-diethyl-5,5'-bis(ethoxycarbonyl)]dipyrrolylmethane occupies three coordination sites of the basal plane of a square pyramidal Cu^{II} center through a pyridine N atom, a pyrrole N atom, and the conjugated carbonyl O atom.^[4] This tridentate coordination mode would cause steric hindrance to the fourth coordination site of the Cu^{II} basal plane in the case of 2-quinoly-2,2'-[3,3'-dimethyl-4,4'-diethyl-5,5'-bis(ethoxycarbonyl)]dipyrrolylmethane (**1**). Hence, the three arms of the tripod ligand **1** would not converge to a single metal center. When a mixture of the ligand **1** (0.194 mmol), Cu(ClO₄)₂·6H₂O (0.194 mmol), Et₃N (0.73 mmol), and EtOH (6 mL) was stirred at room temperature for 1 h under argon, the dark green Cu complex **2a** and the light green Cu complex **2b** were obtained in 7% and 17% yield, respectively (Scheme 1). On



Scheme 1. Formation of Cu₃ cluster complexes.

the basis of FAB-MS (*m/z* = 1189) and analytical data, **2a** and **2b** are isomeric and composed of two doubly deprotonated ligands and three Cu atoms. Therefore, the total charge of the Cu₃ cluster seems to be +4. Indeed, the reaction of **1**, Cu^{II}(ClO₄)₂·6H₂O, and [Cu^I(MeCN)₄]ClO₄ in the molar ratio of 1.0/0.5/1.0 gave improved yields of **2a** and **2b**.^[5]

Single-crystal X-ray analysis of **2b** showed a triangular Cu₃ cluster with Cu–Cu distances of 2.596(2), 2.597(2), and 2.574(3) Å that is sandwiched between two tripod ligands (Figure 1). The Cu(1) and Cu(2) sites are ligated by two anionic pyrrole nitrogen atoms and two weakly coordinating carbonyl oxygen atoms. The Cu(3) site is ligated only by two

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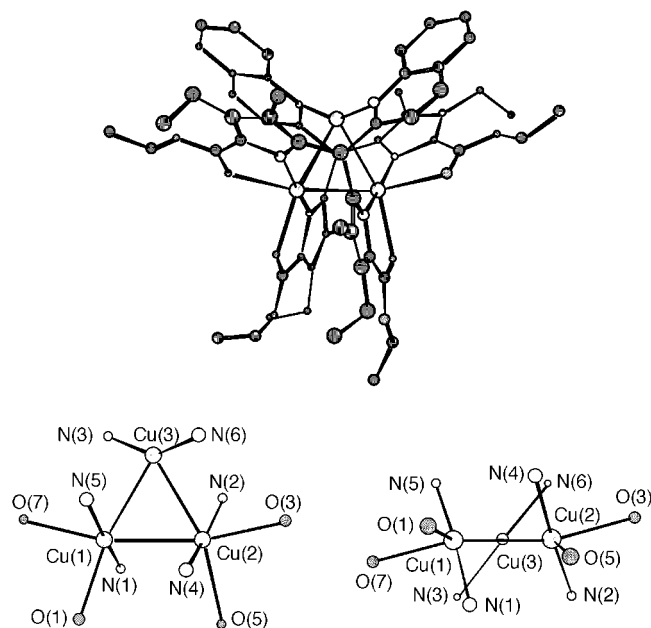


Figure 1. X-ray structure of **2b** (top) and top (bottom, left) and side views (bottom, right) of the Cu_3 core. Selected distances [Å] and angles [°]: Cu(1)–Cu(2) 2.597(2), Cu(1)–Cu(3) 2.574(3), Cu(2)–Cu(3) 2.596(2), Cu(1)–O(1) 2.223(8), Cu(1)–O(7) 2.289(8), Cu(2)–O(3) 2.331(8), Cu(2)–O(5) 2.328(8), Cu(1)–N(1) 1.888(8), Cu(1)–N(5) 1.897(9), Cu(2)–N(2) 1.907(8), Cu(2)–N(4) 1.888(8), Cu(3)–N(3) 1.987(9), Cu(3)–N(6) 1.988(9); N(1)–Cu(1)–N(5) 168.5(4), N(2)–Cu(2)–N(4) 169.2(4), N(3)–Cu(3)–N(6) 150.1(4), N(1)–Cu(1)–O(1) 81.0(3), N(2)–Cu(2)–O(3) 79.4(3), N(5)–Cu(1)–O(7) 80.4(3), N(4)–Cu(2)–O(5) 79.7(3), O(1)–Cu(1)–O(7) 88.0(3), O(3)–Cu(2)–O(5) 92.9(3), N(1)Cu(1)N(5)/Cu(1)Cu(2)–Cu(3) 85.5, N(2)Cu(2)N(4)/Cu(1)Cu(2)Cu(3) 84.5, N(3)Cu(3)N(6)/Cu(1)–Cu(2)Cu(3) 51.5.

quinoline nitrogen atoms, N(3) and N(6). The Cu–N_{quinoline} distances (1.987(9) and 1.988(9) Å) are about 0.1 Å longer than the Cu–N_{pyrrole} distances. The Cu(1) and Cu(2) sites are pseudooctahedral if the Cu–Cu bonds are taken into account. The Cu(3) site has a distorted tetrahedral geometry. The N(3)–Cu(3)–N(6) and Cu(1)–Cu(3)–Cu(2) angles are 150.1(4) and 60.30(6)°, respectively, and the dihedral angle between these two planes is 51.5°. The mixed tripod ligand **1** is well preorganized for mixed-valent cluster complexes in which metal sites with strong ligand fields coexist with sites having weak ligand fields.

The seven-line ESR spectrum observed for **2b** at room temperature ($g_0 = 2.141$, $A_{0(\text{Cu})} = 52.5$ G; Figure 2, right) shows that an $S = \frac{1}{2}$ spin is shared by two Cu ions. The spectrum at 77 K, however, is indicative of a spin-localized d_{z^2} -based Cu^{II} site. Therefore, the delocalization of the $S = \frac{1}{2}$ spin at room temperature is due to rapid ligand reorganization on the ESR time scale caused by intramolecular electron transfer.^[6] (Further discussion of the ESR spectra will be given at a later date.) An intervalence transfer band was observed for **2b** at 1194 nm ($\epsilon = 1700 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$; half-height width 2500 cm^{-1}) in CH_2Cl_2 solution.

The X-ray structure of a triangular $[\text{Cu}_3]^{3+}$ complex **3** with two hexadentate tris[3-(2-pyridyl)pyrazol-1-yl]hydroborate tripod ligands was recently reported, and its one-electron oxidation product, the $[\text{Cu}_3]^{4+}$ complex **4**, shows a seven-line ESR spectrum below 120 K.^[1c] Although the Cu–Cu distances

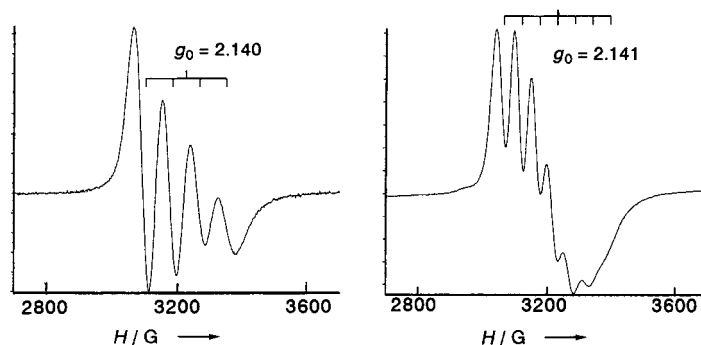


Figure 2. ESR spectra of **2a** (left) and **2b** (right) in toluene/ CH_2Cl_2 (10/1) solution at room temperature. Microwave frequencies were 9.631 GHz for **2a** and 9.632 GHz for **2b**.

(2.915, 3.500, 3.614 Å) of **3** are different from those of **2b**, there should be a close resemblance between **2b** and **4** in their structures and electronic properties. An electron-delocalized $\text{Cu}^{1.5+}\text{Cu}^{1.5+}$ pair is of major interest from the theoretical and bioinorganic viewpoints.^[6, 7] The Cu–Cu distances of **2b** are similar to those of fully delocalized mixed-valent complexes.

Complex **2a** shows a four-line ESR signal at $g_0 = 2.140$ with $A_{0(\text{Cu})} = 86.9$ G at room temperature (Figure 2, left). Hence, there should be a spin-localized Cu^{II} site in the $[\text{Cu}_3]^{4+}$ cluster of **2a**. A one-third turn of one tripod ligand of **2b** with respect to the other generates a single dianionic hexacoordinate site suitable for complexation with a Cu^{II} ion and two monoanionic pentacoordinate sites suitable for Cu^{I} ions, as illustrated for **2a** in Scheme 1.^[8]

Experimental Section

2a and **2b**: Triethylamine (0.1 mL, 7.2×10^{-4} mol) was added to a solution of **1** (97.1 mg; 1.94×10^{-4} mol), $\text{Cu}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ (36.0 mg; 9.72×10^{-5} mol), and $[\text{Cu}(\text{MeCN})_4]\text{ClO}_4$ (63.4 mg; 1.94×10^{-4} mol) in ethanol (6 mL) under argon. After stirring the mixture at room temperature for 1.5 h, the solvent was removed. The first dark green band and the second bright green band obtained by chromatography ($\text{SiO}_2/\text{CH}_2\text{Cl}_2$) were recrystallized from CH_2Cl_2 /pentane (or $\text{CH}_2\text{Cl}_2/\text{Et}_2\text{O}$) to give **2a** and **2b** in 25 and 14% yield, respectively.

2a: FAB-MS (dithranol): m/z 1189; magnetic moment (Guoy method at room temperature) $\mu = 1.96 \mu_B$; ESR (in a frozen glass of toluene/ CH_2Cl_2 (10/1) at 77 K): $g_1 = 2.004$, $A_{1(\text{Cu})} = 100.8$ G (quartet), $g_2 = 2.160$, $g_3 = 2.278$, $A_{3(\text{Cu})} = 84.4$ G (quartet); elemental analysis calcd for $\text{C}_{60}\text{H}_{66}\text{N}_6\text{O}_8\text{Cu}_3$: C 60.35, H 5.57, N 7.04; found: C 60.08, H 5.82, N 7.19.

2b: FAB-MS (dithranol): m/z 1189; magnetic moment (6–300 K; SQUID magnetometer) $\mu = 1.75 \mu_B$; ESR (in a frozen glass of toluene/ CH_2Cl_2 (10/1) at 77 K): $g_1 = 1.970$, $A_{1(\text{Cu})} = 114$ G (quartet), $g_2 = 2.146$, $g_3 = 2.211$, $A_{3(\text{Cu})} = 91$ G (quartet); UV/Vis/NIR (CH_2Cl_2): $\lambda_{\text{max}}(\lg \epsilon)$ 384 (3.77), 664 (2.81), 1194 nm (3.24); elemental analysis calcd for $\text{C}_{60}\text{H}_{66}\text{N}_6\text{O}_8\text{Cu}_3$: C 60.35, H 5.57, N 7.04; found: C 60.57, H 5.70, N 6.90.

X-ray crystal structure analysis of **2b** · CH_2Cl_2 (recrystallized from $\text{CH}_2\text{Cl}_2/\text{Et}_2\text{O}$): $\text{C}_{60}\text{H}_{66}\text{N}_6\text{O}_8\text{Cu}_3 \cdot \text{CH}_2\text{Cl}_2$, $M_r = 1274.79$, monoclinic, space group $P2_1/a$, $a = 12.679(3)$, $b = 23.590(2)$, $c = 20.164(3)$ Å, $\beta = 99.03(1)^\circ$, $V = 5956(1)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 1.421 \text{ g cm}^{-3}$, $\mu(\text{CuK}\alpha) = 25.44 \text{ cm}^{-1}$, $T = 296$ K, $R(F) = 0.062$, $R_w(F) = 0.044$, GOF = 1.59. 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-132711. 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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- [8] Cu₂ complexes analogous to **2a** and **2b** were obtained from 2-quinolyl-2,2'-[5,5'-bis(ethoxycarbonyl)]dipyrrolylmethane, and their X-ray structural analyses verified the structure of **2a**. Details will be reported elsewhere.

1,2,4,6-Cycloheptatetraene: Room-Temperature Stabilization inside a Hemicarcerand**

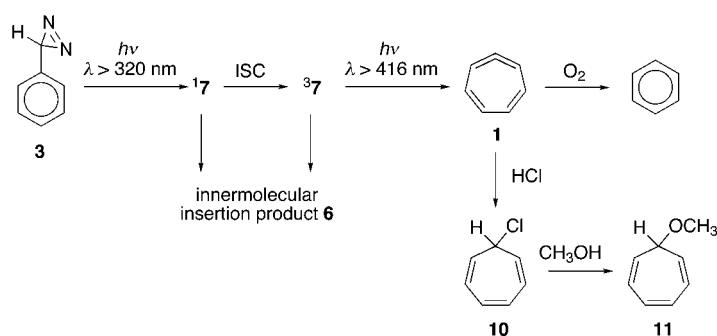
Ralf Warmuth* and Melissa A. Marvel

Dedicated to Professor Donald J. Cram on the occasion of his 80th birthday

The phenylcarbene rearrangement is one of the most important and fascinating carbene rearrangements. Despite intense experimental and theoretical studies neither is the exact mechanism known nor have the energetics of the

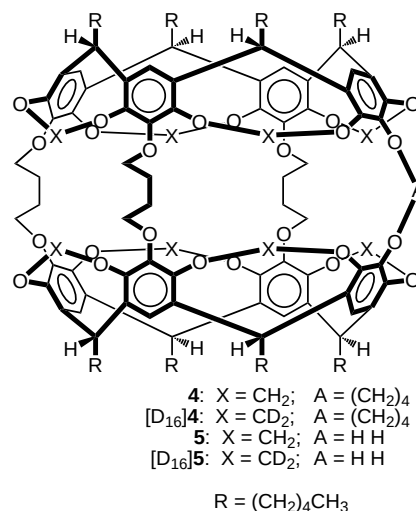
thermal interconversion between the involved intermediates been studied.^[1, 2] Chapman and co-workers photochemically generated 1,2,4,6-cycloheptatetraene **1** at 15 K in argon and provided spectroscopic evidence for this highly strained allene.^[3] Rapid dimerization at higher temperatures prevented a detailed study of the equilibration between **1** and cycloheptatrienyldiene (**2**), which plays an important role in the solution-phase chemistry of both species.^[4]

Incarceration in the inner phases of Cram's hemicarcerands is a novel, elegant, and extremely powerful way to stabilize reactive intermediates by preventing their dimerization.^[5] This method has previously been used to stabilize cyclobutadiene and *o*-benzyne (1,2-didehydrobenzene) ^[6, 7] Here, we report the room-temperature stabilization of **1**, which we generated from phenyldiazirine (**3**) by a photochemical phenylcarbene rearrangement inside hemicarcerand **4** (Scheme 1).^[8]



Scheme 1. Inner phase generation and chemistry of **1**. ISC = intersystem crossing.

We incarcerated **3** by reacting diol-host **5** with 1,4-butanediol dimethanesulfonate and Cs₂CO₃ in the presence of excess **3** in hexamethylphosphoramide.^[9, 10] This reaction provided the hemicarcerplex **4·3** in 80% yield (Figure 1a).^[11] Photolysis (λ > 320 nm) of a degassed solution of **4·3** in C₆D₅CD₃/CDCl₃ (9:1) at 77 K gave the innermolecular phenylcarbene insertion product **6**. In the hope that a small isotope effect would slow down this competing reaction, we prepared [D₁₆]**4·3** in



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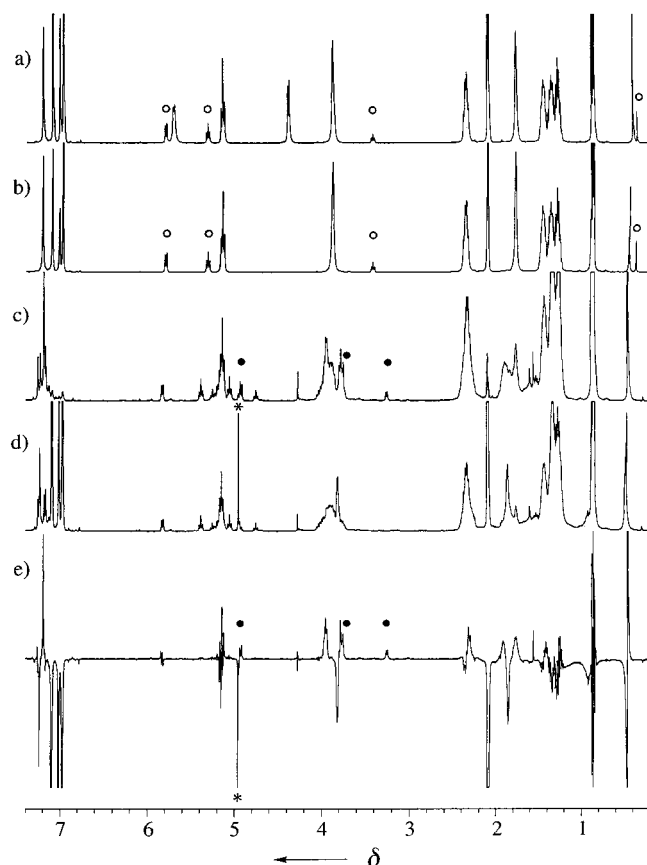
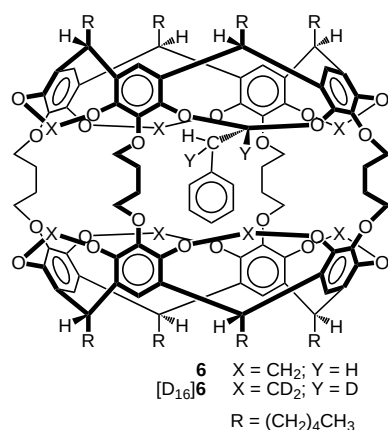
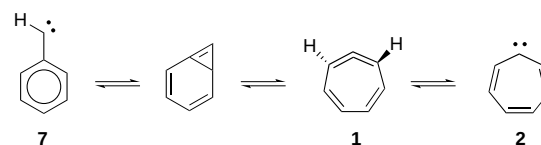


Figure 1. ^1H NMR spectra (400 MHz, $[\text{D}_8]\text{toluene}$, 20°C) of **4•3** (a) and $[\text{D}_{16}]\text{4•3}$ (b), as well as of a photolyzed solution of $[\text{D}_{16}]\text{4•3}$ under oxygen free conditions (c) and after 1 hour of exposure to atmospheric oxygen (d). e) Difference spectrum between c) and d). Proton signals of $[\text{D}_{16}]\text{4•1}$ point upwards, those of $[\text{D}_{16}]\text{4•benzene}$ point downwards. Peaks marked with a circle, a black dot, and an asterisk indicate the ^1H nuclei of incarcerated **3**, **1**, and benzene, respectively.

which all eight acetal carbons are fully deuterated (Figure 1b). Indeed, after UV irradiation ($\lambda > 320\text{ nm}$ at 77 K) of $[\text{D}_{16}]\text{4•3}$ in $[\text{D}_8]\text{toluene}$, the room-temperature ^1H NMR spectrum of this solution revealed the formation of a new hemicarceplex in 12% yield accompanied by mainly $[\text{D}_{16}]\text{5}$. Photolysis at 15.5 K improved the yield to 30% (Figure 1c). Addition of the triplet



sensitizer $[\text{D}_8]\text{acetophenone}$ to the bulk phase (15% (v/v)) further increased the yield (60%) and reduced the amount of $[\text{D}_{16}]\text{6}$ (10%) formed, consistent with the required excitation of triplet phenylcarbene $^3\text{7}$.^[3] The dramatic reduction in the amount of $[\text{D}_{16}]\text{6}$ formed under sensitized conditions suggests that **7** rather than $^3\text{7}$ undergoes the acetal C–H(D) insertion.



We assigned the newly formed hemicarceplexes to the cycloheptatetraene hemicarceplexes $[\text{D}_{16}]\text{4•1}$ and **4•1** based on the spectroscopic properties and inner phase reactivity of the incarcerated guest. The FT-IR spectrum of $[\text{D}_{16}]\text{4•1}$ in CDCl_3 shows a weak band at 1810 cm^{-1} , which is assigned to an allenic carbon–carbon stretching frequency of **1** which appeared at 1824 cm^{-1} and 1823 cm^{-1} in the argon-matrix isolation experiments.^[2, 3] The three ^1H multiplets in the ^1H NMR spectrum of the incarcerated guest are highlighted in Figure 2. From homonuclear ^1H – ^1H COSY and NOESY

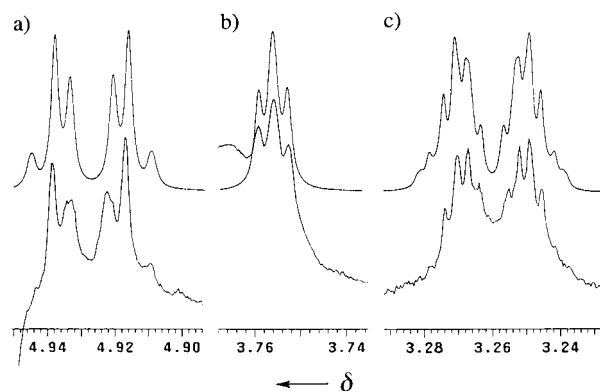
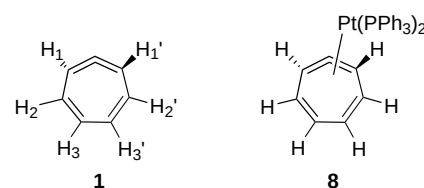


Figure 2. Part of a ^1H NMR spectrum (400 MHz, $[\text{D}_8]\text{toluene}$, 20°C) of $[\text{D}_{16}]\text{4•1}$. The multiplets are given with the best fit (upper trace): a) H_3/H_3' ; b) H_1/H_1' ; c) H_2/H_2' .

spectra, we conclusively assigned the multiplet at $\delta = 3.26$ to the protons H_2 and H_2' . The assignment of the remaining multiplets is based on the relative intensities of the cross peaks in the ^1H – ^1H COSY and NOESY spectra. The cross peaks between the protons H_1 and H_2 , and H_1' and H_2' are weaker than those between H_2 and H_3 and H_2' and H_3' , respectively. Similar observations were made by Jones and Lu for the cycloheptatetraene platinum complex **8**.^[12]



We estimated the chemical shifts of the free guest as described earlier for *o*-benzynes using free cycloheptatriene **9** and hemicarceplex **4·9** as model compounds (Table 1).^[7a] The predicted chemical shifts, the ¹H-¹H coupling constants (Table 2) obtained from a lineshape analysis,^[13] and the sharpness of the resonances of the hemicarceplex's acetal protons in the ¹H NMR spectrum of **4·1** leave little doubt that **1** is the guest, rather than **2** for which matrix ESR studies suggest a triplet ground state.^[14, 15]

Table 1. ¹H chemical shifts [δ_{exp}] of incarcerated guests in [D₁₆]**4·1** and **4·9**, and of **9**, as well as predicted chemical shifts [δ_{pred}] for free **1**.

	[D ₁₆] 4·1 δ_{exp}	4·9 δ_{exp}	9 δ_{exp} ($\Delta\delta$) ^[a]	1 δ_{pred} ^[b]
H1/H1'	3.76	3.19	5.39 (2.20)	5.84
H2/H2'	3.26	3.67	6.22 (2.55)	5.34
H3/H3'	4.93	4.61	6.62 (2.01)	7.01
H4		0.7	2.27 (1.57)	

[a] $\Delta\delta = \delta_{\text{exp}}(\mathbf{9}) - \delta_{\text{exp}}(\mathbf{4·9})$. [b] $\delta_{\text{pred}} = \delta_{\text{exp}}([\text{D}_{16}]\mathbf{4·1}) + \Sigma\Delta\delta(\text{Hi})/4$.

Table 2. ¹H-¹H coupling constants of **1**.^[a]

	H1,H1'	H1,H2/H1',H2'	H2,H3/H2',H3'	H3,H3'
$J_{\text{H,H}}$	−9.8 (0.5)	2.4 (0.5)	8.5 (0.2)	4.5 (0.2)

[a] The 95%-confidence limits are given in parentheses.

To confirm our conclusion, we subjected **4·1** to different bulk phase reactants that can pass through a portal of **4** and that would lead to isolatable hemicarceplexes. Saturating a solution of **4·1** with HCl gas afforded instantaneously **4·7**-chlorocyclohepta-1,3,5-triene (**10**). Subsequent addition of methanol gave **4·7**-methoxycyclohepta-1,3,5-triene (**11**) (Scheme 1), evident from the characteristic singlet of the guest methyl protons at $\delta = -0.85$. We confirmed the formation of the latter hemicarceplex by comparison with authentic **4·11** which conclusively proved the successful phenylcarbene rearrangement to yield incarcerated **1**.^[16] To our surprise, even after 3 h at 60 °C, the addition of methanol (5 M) to a solution of [D₁₆]**4·1** in [D₈]toluene did not result in the formation of [D₁₆]**4·11** although methanol is small enough to undergo through-shell reactions, for example with **10**. Kirmse et al. showed previously that free **1** and **2** instantaneously yield 7-ethoxycyclohepta-1,3,5-triene if generated in ethanolic solution through the protonation of **2**.^[4b] Hence, the inner phase reactivity of **1** differs strongly from its reactivity in the bulk phase. We explain our results with an extremely low reactivity of **1** towards methanol and a very unfavorable equilibrium between **1** and **2** in the nonpolar inner phase of **4**. As pointed out by Kirmse and Sluma,^[4c] a polar reaction phase strongly stabilizes the dipolar singlet state of **2**. For the same reason, the carbon–chlorine bond of **10** has covalent character in the inner phase rather than ionic.^[17]

Though unreactive towards alcohols, incarcerated **1** rapidly reacts with oxygen. Exposure of [D₁₆]**4·1** to atmospheric oxygen leads quantitatively to [D₁₆]**4·benzene** (Figure 1 d).^[8] We made use of this quantitative transformation to obtain a spectrum of [D₁₆]**4·1** by spectral subtraction (Figure 1 e).

We have demonstrated that phenylcarbene can be ring-expanded to cycloheptatetraene, which is stable for weeks at ambient temperature when protected from dimerization by incarceration. This clearly proves the superiority of inner phases for the investigation of highly strained reaction intermediates. We anticipate that this methodology will allow us to measure the kinetics of enantiomerization of **1** inside a chiral hemicarceplex and to draw mechanistic conclusions from the scrambling of isotopically labeled cycloheptatetraenes.

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Thioxylation as One-Atom-Substitution Generates a Photoswitchable Element within the Peptide Backbone**

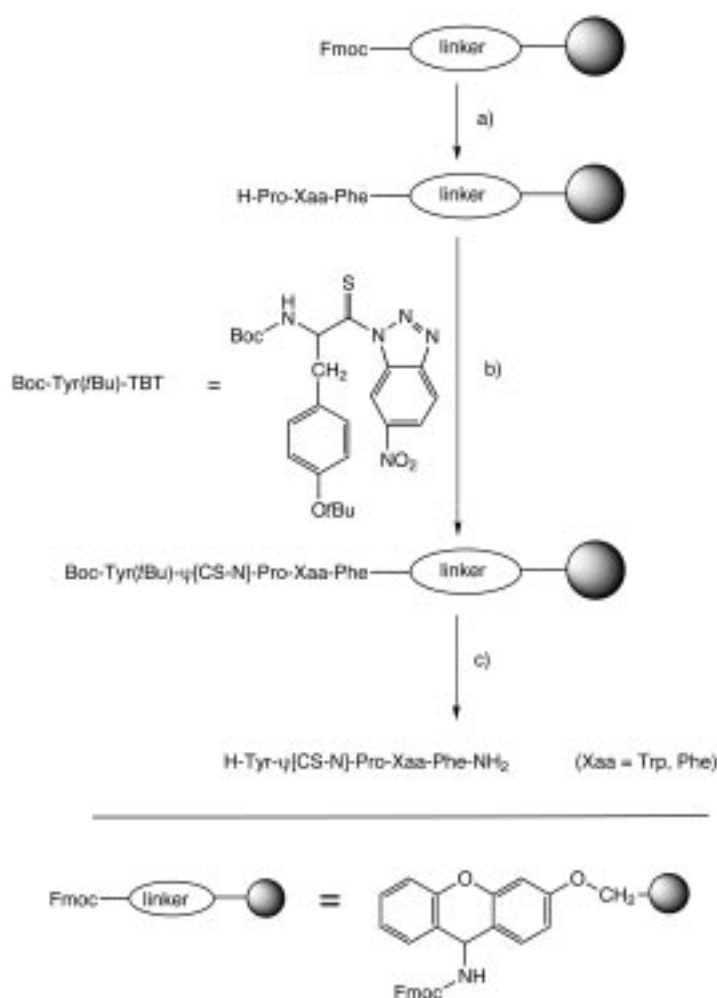
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The thio peptide bond $-\text{CS}-\text{NR}-$ ($\text{R}=\text{H}$, alkyl) represents an isosteric replacement of the peptide bond with only a slightly changed electron distribution in the ground state.^[1] This O/S substitution in biologically active oligopeptides is of considerable interest because of the enhanced proteolytic stability^[2] and the modulated activity and selectivity.^[3]

Proline, the only gene-coded cyclic amino acid, plays a unique role for the backbone conformation. In oligopeptides the peptidyl–prolyl bond causes a conformationally heterogeneous ensemble of molecules consisting of *cis* and *trans* peptide bond isomers. The *cis/trans* isomerization follows first-order kinetics, characterized by half-lives between 10 and 100 s at room temperature.^[4] Taken together these properties of the prolyl bond allow the detection of isomer-specific recognition in biological signaling.^[5] Evaluating the isomeric state of the peptidyl–prolyl bond in signaling complexes provides a sensitive probe for the biologically active conformation. Moreover, “freezing” the dynamics of the backbone can be achieved by peptide bond thioxylation.^[2a, 2c, 6] This further improves the analysis of isomer-specific interactions in biological recognition.

Here we report on the use of the thioxy prolyl bond as a probe of the isomeric state and on the ability to photoswitch between *cis* and *trans* isomers when this bond is present in the backbone of endomorphins.^[7] The thioxyated endomorphins $\text{Tyr}-\psi[\text{CS}-\text{N}]\text{-Pro-Trp-Phe-NH}_2$ (**1**) and $\text{Tyr}-\psi[\text{CS}-\text{N}]\text{-Pro-Phe-Phe-NH}_2$ (**2**) are effective μ -receptor agonists whose affinity for the μ -receptor is comparable to that of the natural endomorphins (unpublished results). The thioxy peptides **1** and **2** were synthesized by thioacylation of the resin-bound tripeptides Pro-Xaa-Phe using thioxyated tyrosyl-6-nitrobenzotriazolide (Boc-Tyr(*t*Bu)-TNB; Boc = *t*BuOCO).^[8] To circumvent the problems usually occurring during acidolytic deprotection of thioxy peptides,^[9] we used a newly developed method^[10] for the mild detachment of the thioxyated products from the resin. This procedure simultaneously deprotects the tyrosyl side chain in the presence of thioxy peptide bonds (Scheme 1).

UV/Vis and CD spectroscopy of peptides **1** and **2** in aqueous solution showed the characteristic absorption band



Scheme 1. Synthesis of peptides **1** and **2** on Sieber amide resin (linker): a) Fmoc amino acid (5 equiv), coupling conditions: TBTU/HOBt/DIEA (1/1/2) in NMP; Fmoc deprotection: 20% piperidine in NMP; b) Boc-Tyr(*t*Bu)-TBT (2 equiv), DIEA (6 equiv), 277 K, CH_2Cl_2 ; c) 2 M SnCl_4 in CH_3CN , CH_2Cl_2 , 1 h at 298 K, or 2.2 M $\text{ZnCl}_2 \cdot \text{Et}_2\text{O}$ complex, CH_2Cl_2 , 10 h at 298 K. Xaa = Trp (peptide **1**), Phe (peptide **2**); TBTU = *O*-(1-benzotriazolyl)tetra-*N*-methyluronium; HOBt = 1-hydroxy-1*H*-benzotriazole; DIEA = diisopropylethylamine; NMP = *N*-methylpyrrolidone.

for the $^1\pi-^1\pi^*$ transition of thioxy peptide bonds (Figure 1) and a negative Cotton effect (Table 1, Figure 2). Moreover, we found a positive Cotton effect for the $^1\text{n}-^3\pi^*$ transition (Table 1, Figure 2), which is indicative of an asymmetric center in close proximity to the chromophore.^[11]

The time dependence of the light-induced *cis/trans* photoisomerization of the $\text{Tyr}-\psi[\text{CS}-\text{N}]\text{-Pro}$ -bond (by excitation of the $^1\text{n}-^3\pi^*$ transition with a N_2 laser at 337 nm; Figure 3) demonstrates the existence of an isobestic point at 251 nm, as could be expected for a uniform transition between two conformers (shown in Figure 1 for **2**). In the case of thioxyated oligopeptides without aromatic side chains we were also able to demonstrate *cis/trans* photoisomerization by excitation of the $^1\pi-^1\pi^*$ transition. As depicted in Figure 1 this photoisomerization is reversible and occurs without photodecomposition. Even after four cycles of excitation/re-equilibration the UV/Vis spectrum of the thioxy peptide **1** remains unchanged. This is in contrast to the results obtained

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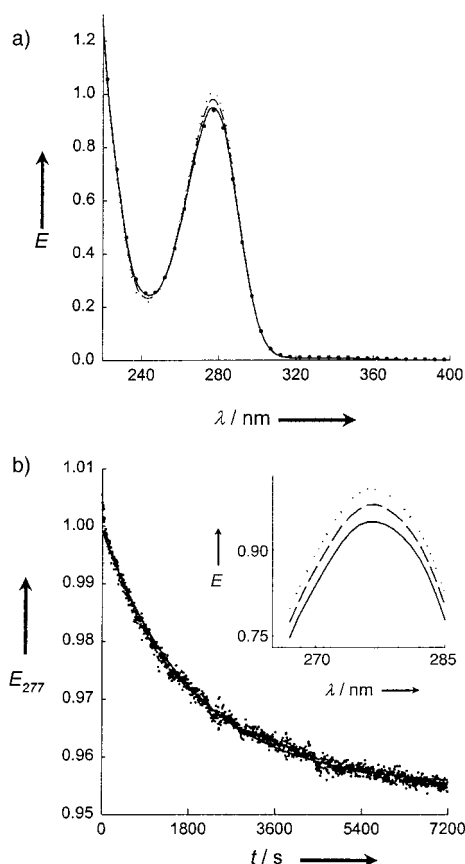


Figure 1. a) UV/Vis absorption spectra of peptide **2** in 0.01M sodium phosphate buffer (pH 7.4), 7×10^{-5} M, 313 K. Equilibrated peptide before irradiation at 337 nm by N_2 laserlight (—), peptide after 30 min of irradiation (····), peptide after 40 min of irradiation (---), peptide after four cycles of irradiation/reequilibration (····). By quantitative analysis of the ^1H NMR spectra under the same conditions a *cis* content of 21% for the peptide **2** was obtained. After irradiation a *cis* content of 58% was found. b) Time dependence of the *cis/trans* isomerization of peptide **2**: 0.01M sodium phosphate buffer (pH 7.4), 7×10^{-5} M, 313 K. The time dependence of the absorbance at 277 nm follows a first-order reaction characterized by a rate constant $k_{obs} = (5.23 \pm 0.03) \times 10^{-4} s^{-1}$. Inset: Enlargement of the UV spectra between 265 and 285 nm.

Table 1. UV/Vis and CD spectroscopic parameters of the thioxo prolyl bond of *cis-2* and *trans-2* dissolved in 0.01M sodium phosphate buffer (pH 7.4) at 277 K.

Conformer	UV/Vis		CD	
	λ [nm]	ϵ [$cm^2 mmol^{-1}$]	λ [nm]	θ [$cm^2 dmol^{-1}$]
	$^1\pi \rightarrow ^1\pi^*$	$^1n \rightarrow ^3\pi^*$	$^1\pi \rightarrow ^1\pi^*$	$^1n \rightarrow ^3\pi^*$
<i>trans-2</i>	277	335	277	341
	(13780)	(104)	(-7366)	(1962)
<i>cis-2</i>	276	334	277	340
	(15153)	(122)	(8988)	(1219)

by irradiation of *N*-methylthioacetamide with UV radiation or laser excitation at different wavelengths.^[12]

Using Raman and UV spectroscopy, these authors were able to demonstrate that the quantum yield for the *cis/trans* photoisomerization is comparable to that of the photodecomposition.^[12c] Thus, modification of the thioxo peptide bond does not provide a suitable probe for the detection of isomer-specific reactions of secondary amide bonds. However, the

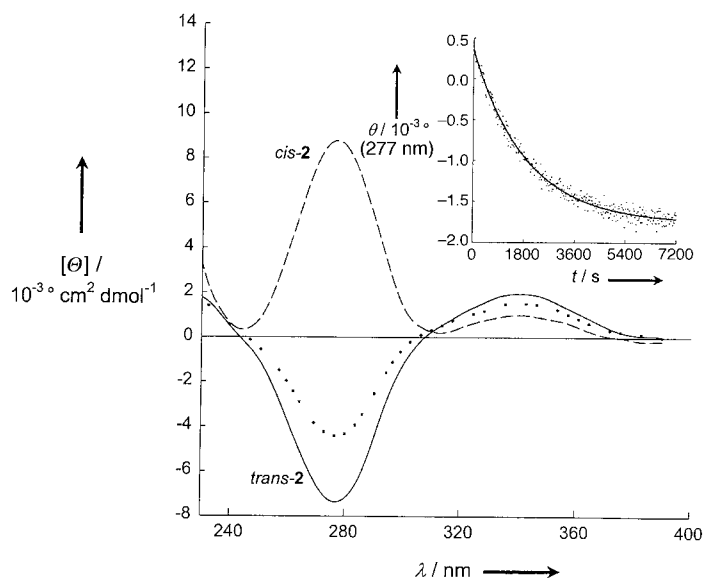


Figure 2. CD spectra of the pure conformers of peptide **2** and the equilibrated solution in 0.01M sodium phosphate buffer (pH 7.4), 2×10^{-4} M, 313 K; equilibrated peptide (····), *trans-2* (—), and *cis-2* (---). Inset: The time dependence of the *cis/trans* isomerization of peptide **2**: 0.01M sodium phosphate buffer (pH 7.4), 2×10^{-4} M, 313 K. The ellipticity at 277 nm follows a first-order reaction with a rate constant of $k_{obs} = (5.38 \pm 0.02) \times 10^{-4} s^{-1}$.

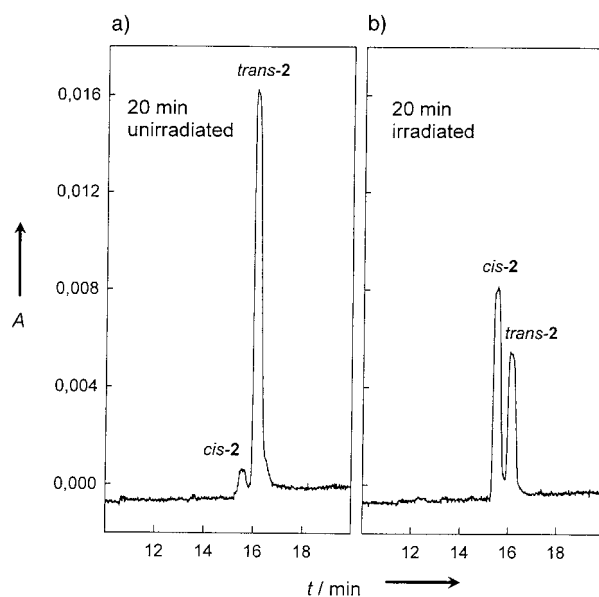


Figure 3. a) Electropherogram of *trans-2* 20 min after dissolution in 0.05 M sodium phosphate buffer (pH 7.4) at 298 K. b) Electropherogram of *trans-2* after 20 min of irradiation prior with a N_2 laser (wavelength 337 nm, pulse length 500 ps, pulse frequency 30 Hz, 400 μJ per pulse, 298 K). CE conditions: 0.05 M sodium phosphate buffer (pH 2.5), 285 K, quartz capillary: 60 cm $\times$ 50 μm , 30 kV, UV detection at 200 nm.

thioxylated tertiary amide bond of the thioxo prolyl moiety proved to be useful as a photoswitchable element within the oligopeptide backbone (Figure 3). The reequilibration after irradiation follows first-order kinetics with $k_{obs} = k_{cis \rightarrow trans} + k_{trans \rightarrow cis}$. Temperature- and time-dependent investigations of this reaction at 277 nm allow the determination of the rate constants of the *cis/trans* isomerization (Figure 1). The *cis/trans* isomerization of the Tyr- ψ [CS-N]-Pro bond in **2** is 245

times slower than isomerization of the Tyr–Pro bond in the oxo form of **1**. Thus, separation of *cis/trans* conformers of thioxo prolyl bonds using HPLC might become possible.

Up to now *cis/trans* conformers of thioxo prolyl bonds could be separated only in analytical amounts using capillary electrophoresis (CE).^[13] We now succeeded in separating *cis-1*, *trans-1*, *cis-2*, and *trans-2* on a preparative scale by reverse-phase HPLC. The lyophilized fractions are stable as pure conformers at 253 K for more than four weeks.

The dramatic isomer specificity of the Cotton effect for the $^1\pi-^1\pi^*$ transition (Figure 2) observed can be used for kinetic investigations of the *cis/trans* isomerization of thioxo peptides. We determined rate constants of $5.4 \times 10^{-4} \text{ s}^{-1}$ at 313 K for the sum of the isomerization in the forward and reverse directions (Figure 2), which is in good agreement with the values of 5.2×10^{-4} and $5.3 \times 10^{-4} \text{ s}^{-1}$ calculated from UV spectroscopic measurements (Figure 1) and ^1H NMR jump experiments, respectively. With CE measurements we calculated quantum yields of 0.05 for *cis-2* and 0.19 for *trans-2*. These quantum yields are smaller than those of the often used but sterically more demanding azobenzenes.^[14]

In summary, thioxo prolyl bonds represent sensitive probes for detecting isomer specificity in biological signaling, allowing the induction of well-defined changes of the backbone conformation by *cis/trans* photoisomerization. Kinetic and thermodynamic analysis of the *cis/trans* isomerization becomes possible because of the marked spectroscopic differences of the thioxo peptide bond isomers. In contrast to the oxo peptide bonds present in native proteins the bathochromic shift of the UV/Vis absorption of the thioxo peptide bond allows sensitive UV/Vis measurements of prolyl bond isomerization in a proteinaceous environment. Results concerning isomer specificity of the interaction of peptides **1** and **2** with the opioid μ -receptor from neuroglioma SH-SY5Y cells will be reported elsewhere.

Experimental Section

Peptides **1** and **2** were synthesized by 9-fluorenylmethoxycarbonyl (Fmoc) chemistry on Sieber amide resin (Calbiochem-Novabiochem, Bad Soden, Germany). Coupling of Boc-Tyr(tBu)- ψ [CS-N]-6-nitrobenzotriazole took place over 12 h and at 277 K in CH_2Cl_2 . The structures of the thioxylated compounds have been confirmed by ^1H NMR spectra.

Peptide 1: Simultaneous removal of the protecting groups and the peptide from resin: 2 M SnCl_4 in CH_3CN , 1 h, 298 K. Purification was performed on a HPLC gradient system (Sykam (LiChrospher, Nucleosil RP-18, 250 $\times$ 25 mm). We used 0.05 % trifluoroacetic acid (TFA) as mobile phase A and acetonitrile with 0.05 % TFA as mobile phase B. MS: $m/z = 627 [M^+]$, calcd for $\text{C}_{34}\text{H}_{38}\text{N}_6\text{O}_4\text{S}_1 = 626$ (monoisotopic).

Peptide 2: Simultaneous removal of the protecting groups and the peptide from resin: 2.2 M $\text{ZnCl}_2 \cdot \text{Et}_2\text{O}$ complex in CH_2Cl_2 , 10 h, 298 K. Purification: HPLC analogous to peptide **1**. MS: $m/z = 588 [M^+]$, calcd for $\text{C}_{32}\text{H}_{37}\text{N}_5\text{O}_4\text{S}_1 = 587$ (monoisotopic).

Separation of the conformers: To increase the amount of *cis* conformer in the thioxo peptide solution the sample was irradiated with a N_2 laser immediately before separation (wavelength 337 nm, pulse length 500 ps, pulse frequency 30 Hz, 400 μJ per pulse, 1 h, 277 K). Conditions of HPLC separation: LiChrospher, Nucleosil RP 18, 250 $\times$ 25 mm, 29 % acetonitrile, 0.05 % TFA; 277 K. The pure conformers ($t_{\text{R, trans-1}} = 12.3 \text{ min}$, $t_{\text{R, cis-1}} = 19.8 \text{ min}$, $t_{\text{R, trans-2}} = 10.4 \text{ min}$, $t_{\text{R, cis-2}} = 17.2 \text{ min}$) were collected in liquid nitrogen and lyophilized.

Determination of quantum yields by CE: the laser beam was fixed in a right angle 15 cm in front of the capillary. Using a light conductor cable we were able to excite the separated conformers inside the capillary. The *cis/trans* photoisomerization was measured at 251 nm (isosbestic point of the interconverting isomers). Correction of peak areas by migration time lead to reduced peak areas and allowed calculation of individual quantum yields.

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On the Unusual Electrochemical Stability of Nanofabricated Copper Clusters**

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Small metal clusters are known to have electronic and structural properties that often differ vastly from those of the respective bulk material. Particularly in the 1–10 nanometer range (i.e. several tens to a few hundred atoms), where the metal clusters are too large for a molecular description, yet too small for ignoring the reduced dimensionality, quantum confinement effects are expected to play a dominant role. While studies of the latter effects are frequent for thin metal layers on, for example, semiconductor substrates,^[1] fewer studies have been made for small metal particles.

Small clusters of copper can be arranged on gold surfaces by the tip of a scanning tunneling microscope (STM).^[2–4] Copper is first electrodeposited from solution onto the STM tip and then transferred from the tip to the substrate by an appropriate tip approach, during which a so-called “jump-to-contact”^[5] occurs. The resulting metal bridge between the tip and sample breaks upon retraction of the tip, leaving a copper cluster on the surface. Tip approach, as well as tip position, are externally controlled by a microprocessor. The height of the copper clusters can be controlled between two and approximately five atomic layers (i.e. 0.4 to 1.0 nm) by varying the tip approach.^[2, 3] The apparent width of the clusters is about 3.5 nm (measured as the full width at half maximum cluster height, FWHM) and since this measurement is subject to the limits of lateral resolution of the STM, the actual cluster width is obviously somewhat less. Under such premises one cluster should contain roughly 100 copper atoms. These nanofabricated particles revealed a surprisingly high stability against anodic dissolution.^[3, 4, 6] This unusual behavior prompted a more systematic investigation, as stable clusters are sought after as electrocatalysts.

In Figure 1, an STM image of an array of 25 tip-induced copper clusters on a gold(111) surface is shown. At $E_{\text{sample}} = +10$ mV versus Cu/Cu^{2+} the surface is covered with a monolayer of copper due to underpotential deposition (UPD);^[7] hence the copper clusters are sitting on this monolayer. The clusters have an average height of 0.6 nm and a width (FWHM) of 3.2 nm.

In order to avoid bulk copper deposition from solution onto the gold substrate, the electrode potential was usually set to +10 mV versus Cu/Cu^{2+} , that is, positive of the Nernst potential, where bulk copper deposition from solution is

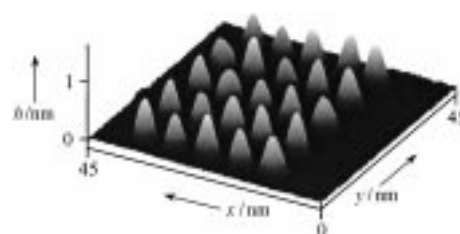


Figure 1. In situ STM image of an array of 25 tip-induced Cu clusters on Au(111) in 0.05 M H_2SO_4 + 0.05 mM CuSO_4 . $E_{\text{sample}} = +10$ mV, $E_{\text{Tip}} = -50$ mV vs. Cu/Cu^{2+} ; $I_{\text{Tunnel}} = 2$ nA.

thermodynamically impossible. On the other hand, bulk copper is unstable positive of the Nernst potential and copper clusters should be readily dissolved at +10 mV. Tip-induced copper clusters withstand anodic dissolution at +10 mV for a surprisingly long period: In Figure 2 the height of tip-induced

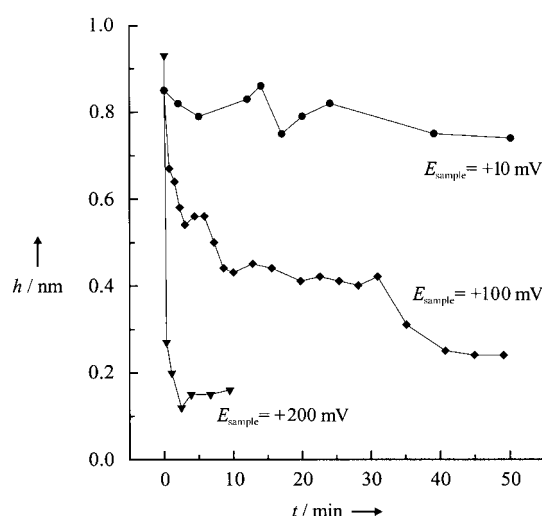


Figure 2. Heights (h) of three tip-induced Cu clusters on Au(111) in 0.05 M H_2SO_4 + 0.1 mM CuSO_4 as a function of time for three different sample potentials.

copper clusters on the gold substrate is shown as a function of time for three different electrode potentials. At +10 mV versus Cu/Cu^{2+} the cluster retains its height for more than 50 min, whereas at +200 mV versus Cu/Cu^{2+} the cluster disappears rapidly (except for a second layer on top of the UPD layer). At +100 mV versus Cu/Cu^{2+} the copper cluster dissolves layer-by-layer and the remainder becomes more stable with each dissolved layer.

In order to demonstrate the role of cluster size on the stability, considerably larger copper clusters were generated by normal copper deposition from solution. Such clusters were either obtained by nucleation and growth of copper at surface defects, such as monoatomic high steps,^[7] or by using tip-induced clusters as nucleation centers^[4] onto which copper was deposited from solution until the desired cluster size was reached. The stability of a 2.4 nm high copper cluster, grown by deposition from solution, against anodic dissolution at +10 mV versus Cu/Cu^{2+} is shown in Figure 3. The line scan across the cluster is shown as a function of time (x – t scan). The mesalike cluster was deposited at –310 mV versus

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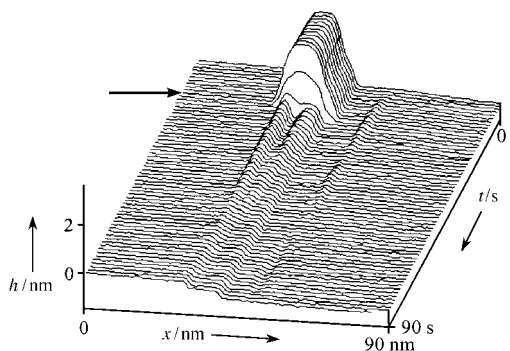


Figure 3. Scan ($x-t$) across an electrochemically grown Cu cluster on Au(111). The arrow at $t=15$ s indicates the sample potential jump from -310 mV to $+10$ mV vs. Cu/Cu^{2+} . Electrolyte: $0.05\text{ M H}_2\text{SO}_4 + 0.2\text{ mM CuSO}_4$.

Cu/Cu^{2+} and its height remained constant during the first 15 s of imaging. At $t=15$ s a potential step to $+10$ mV was applied (arrow in Figure 3) and the cluster immediately disappeared as expected for normal, bulk copper. Equally noteworthy is that large copper clusters, grown electrochemically on top of the tip-induced clusters, readily dissolved at $+10$ mV versus Cu/Cu^{2+} except for a remnant almost identical in shape and height to the original tip-induced cluster.

In order to determine the dissolution potential of the small, tip-induced copper clusters, their heights were monitored as a function of potential. Two different experimental approaches were chosen to ensure exclusion of artifacts. In the first experiment set, a single tip-induced cluster was imaged in an $x-t$ scan, while the electrode potential was increased at a constant rate. Figure 4 shows an $x-t$ scan for a tip-induced

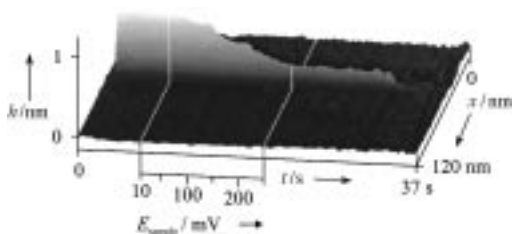


Figure 4. Scan ($x-t$) across a tip-induced Cu cluster on Au(111). The sample potential E_{sample} increased during imaging from $+10$ mV to $+250$ mV vs. Cu/Cu^{2+} at 20 mV s^{-1} . Electrolyte: $0.05\text{ M H}_2\text{SO}_4 + 1\text{ mM CuSO}_4$.

cluster, where the potential scan was started at $t=7$ s at $+10$ mV increasing at 20 mV s^{-1} up to $+250$ mV. Therefore, the time axis also represents a potential axis. It is clearly seen that cluster dissolution starts at about $E_{\text{sample}} = +40$ mV versus Cu/Cu^{2+} . Further steps in the cluster height can be seen at around 80 mV and 170 mV. A small remnant, about one monolayer high, remains stable at 250 mV versus Cu/Cu^{2+} for another 12 s before the cluster is completely dissolved. Since height variations for a single cluster in $x-t$ scans can be influenced by thermal drift of the STM, a second experiment set was employed: We produced an array of 27×10 copper clusters and took an STM image at $+10$ mV versus Cu/Cu^{2+} . The whole array was again imaged while the potential was

increased simultaneously from $+10$ mV up to $+250$ mV. This second image showed the decreasing cluster height with increasingly positive potential (Figure 5). Again we found the cluster height remains constant to about $50-60$ mV before dissolution began. It thus appears that copper clusters, three to four layers high, are stable up to an electrode potential

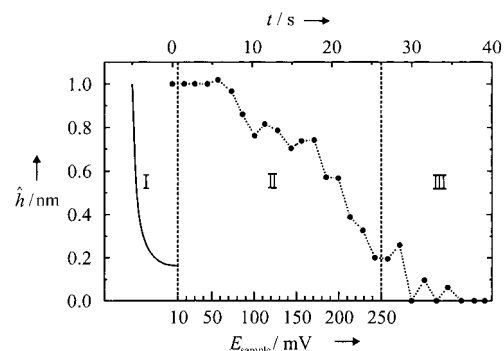


Figure 5. Normalized height ($\hat{h}$) of tip-induced Cu clusters as a function of time (normalized with respect to their initial height). Electrolyte: $0.05\text{ M H}_2\text{SO}_4 + 1\text{ mM CuSO}_4$. Region I: $E_{\text{sample}} = +10$ mV vs. Cu/Cu^{2+} . Region II: E_{sample} increased from $+10$ mV to $+250$ mV vs. Cu/Cu^{2+} at 10 mV s^{-1} . Region III: $E_{\text{sample}} = +250$ mV vs. Cu/Cu^{2+} . For comparison, in region I, the result for the electrochemically grown cluster dissolving at $+10$ mV vs. Cu/Cu^{2+} (see Figure 3) is shown again.

around 50 mV versus Cu/Cu^{2+} , smaller clusters are stable to around 180 mV, and the dissolution follows discrete steps. Figure 5, for comparison, shows also the stability range of large copper clusters, which presumably share properties of the bulk metal. The large clusters are almost nonexistent at the potential range used for small clusters.

We now turn to the question of how to explain the unusually high stability of small copper clusters on gold(111). For many in situ STM studies, the close proximity of the tip has been found to exert a noticeable influence on electrochemical reactions.^[8] Early explanations for these stability effects suggested tip shielding.^[2] Stability tests with tips that were retracted by about $3\text{ }\mu\text{m}$ during most of the time and only briefly engaged every few minutes for cluster height measurements, yielded the same result as studies with permanently engaged tips. Later explanations were based on kinetic or thermodynamic arguments. Assuming the small metal clusters to be more or less perfect single crystal units, a high overvoltage for dissolution was suggested due to lack of defects.^[3] Lorenz et al.^[9] coined the expression “zero-dimensional phase” for the small clusters. The stability of a metal increases in the order of three-, two- and one-dimensional forms, as bulk metal, UPD monolayers, and metal atoms at substrate steps, respectively. The “zero-dimensional phase” corresponds to a much more positive Nernst potential. None of the explanations were satisfactory.

The concept of a metal electrode in equilibrium with its ion in solution, which stands behind the Nernst equation, requires electronic states at the Fermi level. These states are plentiful in common metals. For semiconductor electrodes, metal deposition and dissolution are no longer determined by the Nernst potential of the metal but rather by the availability of electronic states in the electrode. If the dimensions of the

metal are reduced to those of the “electron in a box” problem (the nanometer range) the electronic states in the metal become discrete in their energy distribution. To a very simple approximation, the energy gap ΔE between the electron states in a small metal particle can be estimated by $\Delta E = E/N$, where E is the width of the energy band in the metal and N is the number of atoms per cluster.^[10] For $E = 10$ eV^[11] and $N = 100$ an energy separation of the order of 0.1 eV is obtained. This, of course, is a rather crude estimation, but it demonstrates that overpotentials (in this case in the thermodynamic sense) of 50 or 180 mV, as observed in the experiment, can result from quantum effects. Consider a redox potential, that is, the Fermi level of the redox system, lying within the energy gap of the metal cluster. Dissolution of the metal would only start after the energy states in the cluster have been shifted by a change in the electrode potential to match the redox potential. We thus propose that the stability range of our tip-induced copper clusters of at least 50 mV versus Cu/Cu²⁺ (which is quite significant in electrochemical terms) might be a consequence of quantum effects. An obstacle for such an interpretation is the fact that the copper clusters are on a metal rather than on an insulator surface, which raises the question of a quantum confinement normal to the surface. However, since the contact between copper cluster and the gold(111) substrate represents a discontinuity in structural as well as in electronic terms, this interface may well provide the necessary barrier for the electrons in the cluster.

Experimental Section

Both fabrication and imaging of the Cu clusters were performed with a PicoSPM (Molecular Imaging Corp., Tempe, Arizona). The electrolyte used in this study was 0.05 M H₂SO₄ with concentrations of Cu²⁺ between 0.05 and 1 mM, prepared from H₂SO₄ (Merck, 96 %) , CuSO₄ · 5 H₂O (Fluka, >99 %) and Milli-Q water (Millipore). Sample and tip potentials were controlled independently from each other by a bipotentiostat. As reference electrode, a Cu or a Pt wire was used, and the counter electrode was a Pt wire. The tip was made from a 0.25 mm diameter Pt/Ir (80/20) wire by electrochemical etching. All potentials are quoted against Cu/Cu²⁺ in the same solution. Other technical details are given in references [2, 3, 12].

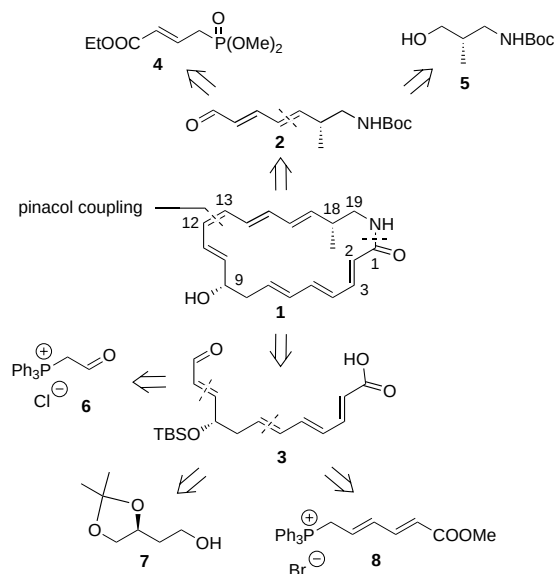
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Synthesis of the (9S,18R) Diastereomer of Cyclamenol A**

Marc Nazaré and Herbert Waldmann*

A key event in the humoral response to inflammation, tissue injury, and infection is the transport of leukocytes to the site of injury. It is facilitated by adhesion of the leukocytes to endothelial cells and their subsequent transmigration from the bloodstream through the wall of the blood vessel and the surrounding tissue.^[1] Over-recruitment of these blood cells can result in the establishment of various diseases and disorders ranging from chronic autoimmune diseases to acute inflammation. Intervention of leukocyte recruitment by inhibiting their adhesion to endothelial cells is considered to be a new strategy for the development of antiinflammatory agents. This approach may offer entirely new and alternative opportunities for the treatment of, for example, reperfusion injuries, strokes, asthma, and arthritis. Thus, the development of peptide and carbohydrate derivatives as inhibitors of the selectin/sialyl Lewis X interaction has attracted considerable interest.^[2] However, non-peptide and non-carbohydrate natural products have not been investigated so far.

Cyclamenol A (**1**) (Scheme 1), a macrocyclic polyene lactam isolated from *Streptomyces spec.* MHW 846 by chem-



Scheme 1. Retrosynthetic analysis of cyclamenol A (**1**). Boc = *tert*-butoxycarbonyl, TBS = *tert*-butyldimethylsilyl.

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ists at Bayer,^[3] is one of the very few natural products known to inhibit adhesion of leukocytes to endothelial cells.^[4] It blocks this process in an ex vivo model and completely inhibits adhesion of leukocytes to arterioles in hamsters (i.e. in vivo). Its mode of action and the structural parameters influencing its biological activity including the absolute configuration of the two stereogenic centers incorporated into cyclamenol A are unknown.

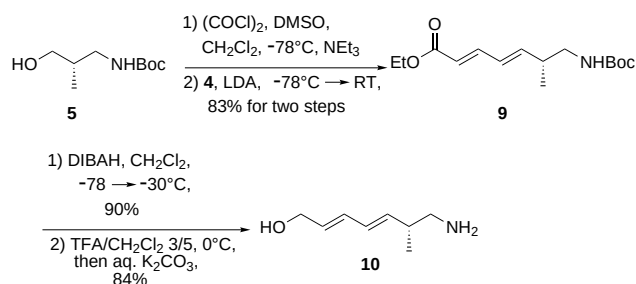
In the light of these important and unsolved medicinal and biological questions and as part of a study to develop tools for unravelling the biological activity of cyclamenol A we have embarked on syntheses of this natural product and analogues thereof with modified biological properties. Herein we report on the synthesis of (9*S*, 18*R*)-cyclamenol A.

In developing a strategy for the synthesis of (9*S*, 18*R*)-cyclamenol A particular attention was paid to the polyene system, which embodied one *Z* and six *E* double bonds. Thus, once the entire backbone was assembled it was feared it would react further to give a fully conjugated octaene system or that the *Z* double bond would isomerize. In addition, force field calculations indicated that a final ring closure by amide bond formation would be difficult to achieve since the triene and the tetraene system incorporated into cyclamenol A seco acid induce a conformation of the acyclic precursor in which the amine and the acid are not close to each other. Therefore, we planned to construct the sensitive polyene system in the final steps of the synthesis and to establish the entire backbone by a transformation that allows both ends of the acyclic precursor to be tied together under template control. The formation of pinacols from dialdehydes was chosen for this purpose.^[5] The pinacol generated thereby should be a reasonably stable intermediate and be amenable to generation of the required *Z* double bond.

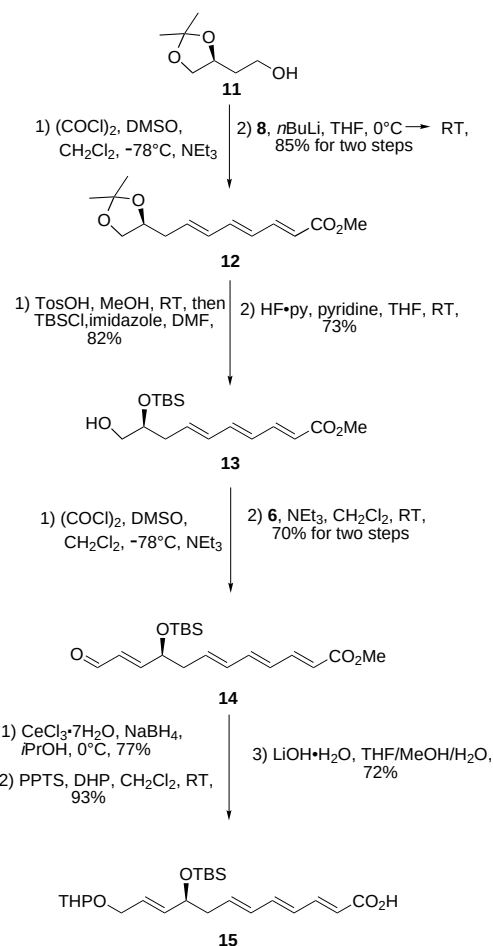
According to this plan the target compound was dissected between C12 and C13 and at the amide bond to yield aldehydes **2** and **3** as major intermediates (Scheme 1). Compound **2** was traced back to phosphonic acid ester **4** and protected amino alcohol **5**, which were to be coupled by means of a Horner olefination reaction after oxidation of **5**. Intermediate **3** was divided into building blocks **6**–**8** to be linked by Wittig olefination after subsequent oxidation of the primary alcohol functions of **7** to the respective aldehyde groups. Since the absolute configuration of cyclamenol A is not known the two stereocenters were arbitrarily introduced in the (9*S*) and the (18*R*) configuration, which are accessible from (*S*)-malic acid and (*R*)-hydroxyisobutyric acid, respectively.^[6]

The C13–C19 fragment **10** was built up in a short and efficient sequence from protected amino alcohol **5**^[7] (Scheme 2). Swern oxidation of the primary alcohol and chain elongation with crotonoyl-phosphonate **4** yielded doubly unsaturated ester **9**, which was reduced to the allyl alcohol. Removal of the Boc group and extractive workup with K₂CO₃ solution gave amino alcohol **10** in high yield. It was used directly for the subsequent steps.

The C1–C12 fragment was synthesized from selectively protected butanetriol **11**^[8] by two successive elongations of the carbon chain (Scheme 3). First the primary alcohol **11** was converted to the corresponding aldehyde by Swern oxida-



Scheme 2. Synthesis of C13–C19 intermediate **10**. DIBALH = diisobutylaluminum hydride.



Scheme 3. Synthesis of C1–C12 intermediate **15**. Tos = *p*-toluenesulfonyl, PPTS = pyridinium *p*-toluene sulfonate, DHP = dihydropyran, THP = tetrahydropyranyl.

tion; this was then treated with the ylide formed in situ from the phosphonium salt **8** (derived from sorbic acid) by deprotonation with butyllithium. The diol obtained after removal of the acetonide from **12** was rather unstable. Thus, it was immediately converted to the bis-silyl ether, and then the more labile *tert*-butyldimethylsilyl ether of the primary alcohol was cleaved selectively by treatment with a solution of pyridinium hydrofluoride in a mixture of pyridine and THF at room temperature. The selectively unmasked and highly unsaturated alcohol **13** was obtained in high overall yield. It was then subjected to a one-pot reaction sequence that included oxidation of the alcohol to the aldehyde and Wittig

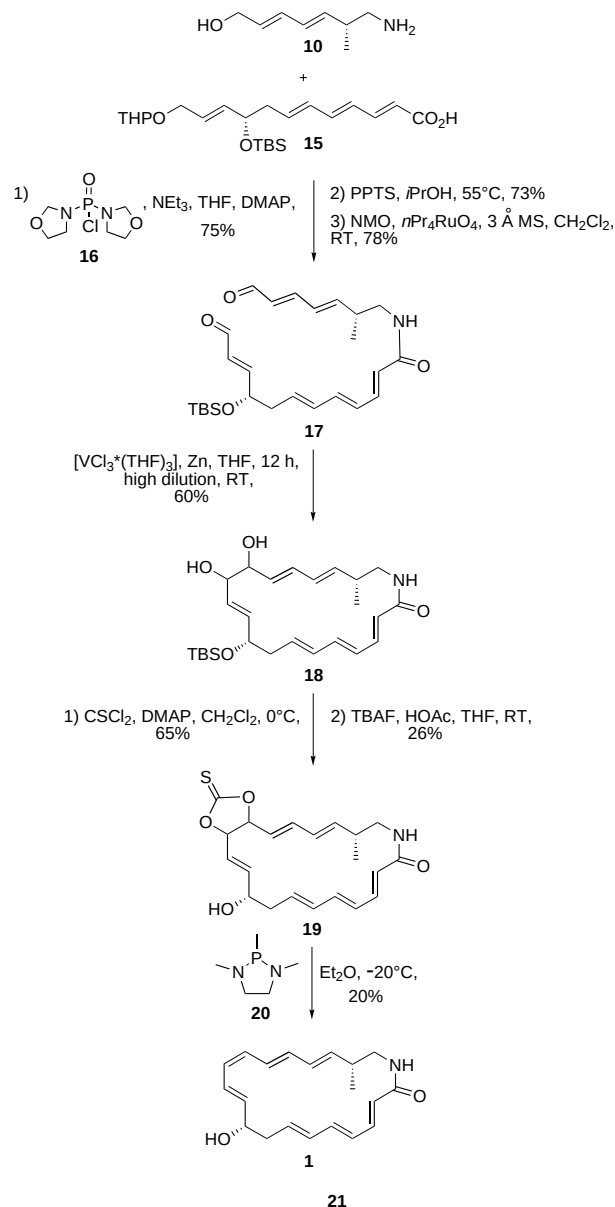
reaction with formylmethylenetriphenylphosphonium chloride **6** in the presence of NEt_3 . In this way the isolation of the very unstable α -alkoxyaldehyde intermediate was circumvented. The significantly more stable α,β -unsaturated aldehyde **14** could be isolated in high yield.

For the amide linkage of the C1–C12 and C13–C19 fragments, the aldehyde group in **14** had to be reduced and the resulting allyl alcohol was masked as a THP ether which is orthogonally stable to the TBS ether and the methyl ester in **14**. This protection proved to be necessary since the unmasked primary alcohol interfered with the subsequent formation of the amide bond. After saponification of the methyl ester the resulting carboxylic acid was preactivated by treatment with BOP-Cl (BOP = 1-benzotriazolyl-oxytris(dimethylamino)-phosphonium hexafluorophosphate). The activated intermediate reacted chemoselectively with amino alcohol **10** to yield the desired amide in high yield. Strong preactivation with BOP-Cl was necessary to achieve a high yield in this step since the tetraene carboxylic acid **15** is only moderately reactive. Other activation methods gave markedly lower yields. Next the THP ether was removed and the bis-allyl alcohol was oxidized to the moderately stable dialdehyde intermediate **17** (Scheme 4).

The formation of the carbon–carbon bond to generate the twenty-membered ring system turned out to be very difficult. Neither titanium- or samarium-mediated pinacol formation^[9] nor McMurry reaction employing various titanium reagents^[10] were successful. After substantial experimentation finally the use of the vanadium reagent $[\text{V}_2\text{Cl}_3 \cdot (\text{THF})_6][\text{Zn}_2\text{Cl}_6]$ ^[11] (prepared in situ) in THF as solvent and under high dilution conditions turned out to be the method of choice. It afforded the cyclic pinacol **18** in 60% yield.

After this crucial step had been accomplished attention was focussed on finding proper and gentle reaction conditions for generating the sensitive polyene system and for the deprotection of the secondary alcohol. In particular, the order in which these steps had to be carried out was crucial to the success of the synthesis. Thus, in a first approach, the final C12–C13 double bond was generated and then we attempted to cleave the silyl ether protecting group. However, once the heptaene is established protected cyclamenol A becomes very sensitive to both acid and base, and numerous attempts to cleave the silyl ether only led to decomposition. This final problem could, however, be solved by reversing the order of the synthetic transformations, that is the silyl ether was removed first and the sensitive polyene was generated in the final step. To this end, pinacol **18** was converted to the corresponding thionocarbonate **19** by reaction with thiophosgene, and then the TBS ether was cleaved to give the deprotected alcohol in moderate yield. Finally, the thionocarbonate **19** was fragmented by treatment with trimethyldiazaphospholidine **20**^[12] to yield (9*S*,18*R*)-cyclamenol A.

Comparison of the spectroscopic data recorded for the synthetic sample with the data obtained for the natural product^[3, 13] clearly proved that the desired cyclic polyene system had been formed. However, whereas naturally occurring cyclamenol A^[14] displayed a specific rotation of $[\alpha]_{\text{D}}^{20} = +1000^\circ$ ($c = 0.01$ in DMF), we measured a value of $[\alpha]_{\text{D}}^{20} = +60^\circ$ ($c = 0.01$ in DMF) for the synthetic compound. Thus the



Scheme 4. Coupling of fragments **10** and **15** and completion of the synthesis. NMO = *N*-methylmorpholine-*N*-oxide, DMAP = 4-dimethylaminopyridine, TBAF = tetrabutylammonium fluoride.

natural product does not have the (9*S*,18*R*)-configuration. We stress, however, that the enantiomers of chiral building blocks **5** and **7** are readily available, implying that the other diastereomers of the natural product are also accessible by the route detailed above.

We have developed a synthesis of the (9*S*,18*R*)-diastereomer of the leukocyte adhesion inhibitor cyclamenol A. The synthesis is highly convergent and proceeds with high efficiency, that is the longest linear sequence consists of ten steps and relays the stereogenic centers of the polyene macrolactam to two building blocks from the chiral pool that are readily available in both enantiomeric forms. The flexibility and convergent character of the synthetic route will give access to all diastereomers of the natural product as well as to various analogues with improved stability and

altered biological properties. This synthesis now provides the opportunity to develop a new class of non-carbohydrate and non-peptidic inhibitors of leukocyte adhesion to endothelial cells.

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- [13] **1**: $R_1 = 0.40$ ($\text{CHCl}_3/\text{MeOH}$ 9:1); $R_2 = 0.14$ (Et_2O); $^1\text{H NMR}$ (CDCl_3 , 500 MHz): $\delta = 7.20$ (dd, $^3J = 15$, $^3J = 10$ Hz, 1H), 6.88 (dd, $^3J = 15$, $^3J = 10$ Hz, 1H), 6.52 (dd, $^3J = 15$, $^3J = 10$ Hz, 1H), 6.20 (m, 5H), 6.06 (m, 1H), 6.02 (m, 1H), 5.80 (m, 2H), 5.52 (dd, $^3J = 15$, $^3J = 6$ Hz, 1H), 5.41 (d, $^3J = 15$ Hz, 1H), 4.20 (m, 1H), 3.31 (m, 1H), 2.96 (m, 1H), 2.40 (m, 1H), 2.32 (m, 1H), 2.24 (m, 1H), 1.02 (d, $^3J = 7$ Hz, 3H); MS (ESI, positive mode, $\text{MeOH}/\text{CHCl}_3$ 2:1): 312 [$\text{M}^+ + \text{H}$]; MS (ESI, negative mode, $\text{MeOH}/\text{CHCl}_3$ 2:1): 345 [$\text{M}^- + \text{Cl}$], 310 [$\text{M}^- - \text{H}$]. To further support the good agreement of the NMR data recorded for the olefin region of (9*S*,18*R*)-**1** with the values measured for the natural

stereoisomer of cyclamenol A, we have calculated the differences in total energy and total steric energy for the 12,13-*cis* and the 12,13-*trans* isomers of (9*S*,18*R*)-cyclamenol. The calculations were performed with the PM3 and the MM+ programs implemented in HyperChem 5.02. The semiempirical calculations at the PM3 level revealed a difference in total energy of $\Delta H_{\text{total}}(12,13\text{-cis}) - \Delta H_{\text{total}}(12,13\text{-trans}) = -37.2 \text{ kJ mol}^{-1}$ and the force field calculations at the MM+ level yielded a difference in total steric energy of $\Delta H_{\text{total}}(12,13\text{-cis}) - \Delta H_{\text{total}}(12,13\text{-trans}) = -49.7 \text{ kJ mol}^{-1}$. Thus, the 12,13-*cis* isomer is energetically much more favored than the corresponding *trans* isomer, and the formation of the *trans* isomer can be ruled out for energetic reasons. In addition, for related tetraene and polyene systems it is known that the energetically less favorable isomers spontaneously isomerize to the more stable analogues: a) K. C. Nicolaou, S. E. Webber, J. Ramphal, Y. Abe, *Angew. Chem.* **1987**, *99*, 1077–1079; *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 1019–1021; b) M. B. Andrus, S. D. Lepore, *J. Am. Chem. Soc.* **1997**, *119*, 2327–2328; c) B. Crousse, M. Alami, G. Linstrumenelle, *Tetrahedron Lett.* **1995**, *36*, 4245–4248. In addition, the $^1\text{H NMR}$ spectrum of the *O*-silylated thionocarbonate obtained from diol **18** displays a coupling constant of 7 Hz for the two CH(OCS) signals of the five-membered ring. This indicates that the two hydrogen atoms are *cis*-oriented (in related compounds coupling constants of 6.5–7.8 Hz are found: d) P. S. Manchand, P. S. Belica, M. J. Holman, T. Huang, H. Maehr, S. Y.-K. Tam, R. T. Yang, *J. Org. Chem.* **1992**, *57*, 3473–3478; e) I. Maeba, N. Morishita, P. Francom, M. J. Robins, *J. Org. Chem.* **1998**, *63*, 7539–7541; f) De Angelis, M. Marzi, P. Minetti, D. Misiti, S. Muck, *J. Org. Chem.* **1997**, *62*, 4159–4161; g) M. G. Lester, G. M. P. Giblin, G. G. A. Inglis, P. A. Procopiou, B. C. Ross, N. S. Watson, *Tetrahedron Lett.* **1993**, *34*, 4357–4360. Since the elimination of thionocarbonates to olefins is known to be a stereospecific *syn*-elimination the *cis*-olefin must have been formed.

[14] We are grateful to Dr. B. Fugmann, Bayer AG, for samples of natural cyclamenol A.

Crossed Condensation of Two Different Hydrosulfido-Bridged Dinuclear Complexes: Structures and Reactivities of TiRu_3S_4 Cubane-Type Sulfido Clusters**

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Rational construction of sulfido clusters has been a major objective in synthetic inorganic chemistry because these clusters have possible relevance to metalloenzymes and

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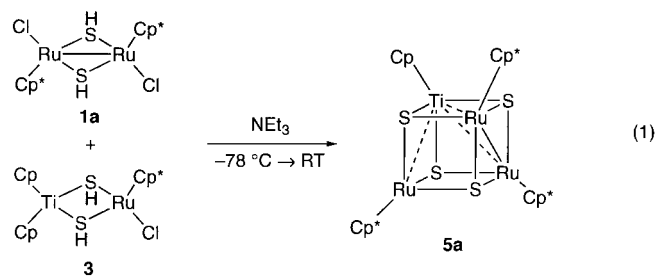
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nonmolecular metal sulfide materials including hydrosulfurization catalysts.^[1] We have recently demonstrated that the self-condensation of the hydrosulfido-bridged dinuclear complexes $[\text{Cp}^*\text{MCl}(\mu_2\text{-SH})_2\text{MClCp}^*]$ (**1a**: M = Ru; **1b**: M = Rh; **1c**: M = Ir; $\text{Cp}^* = \eta^5\text{-C}_5\text{Me}_5$) in the presence of a base affords a series of cubane-type sulfido clusters $[(\text{Cp}^*\text{M})_4(\mu_3\text{-S})_4]$ (**2a**: M = Ru;^[2] **2b**: M = Rh;^[3] **2c**: M = Ir^[3]). Interestingly, even cyclopentadiene is eliminated from the hydrosulfido-bridged heterobimetallic complex $[\text{Cp}_2\text{Ti}(\mu_2\text{-SH})_2\text{RuClCp}^*]$ (**3**; $\text{Cp} = \eta^5\text{-C}_5\text{H}_5$) to give the heterobimetallic cluster $[(\text{CpTi})_2(\text{Cp}^*\text{-Ru})_2(\mu_3\text{-S})_4]$ (**4**).^[4] As an extension of these studies, we describe here the crossed condensation of the two different hydrosulfido-bridged dinuclear complexes **1a** and **3**, which results in the formation of the novel TiRu_3S_4 cubane-type sulfido cluster $[(\text{CpTi})(\text{Cp}^*\text{Ru})_3(\mu_3\text{-S})_4]$ (**5a**).

When an excess of triethylamine was added to a 1:1 mixture of **1a** and **3** at -78°C , the dark reddish brown solution immediately changed to deep violet, which progressed further to dark brown as the solution was warmed to room temperature. The ^1H NMR spectrum of the reaction mixture indicated that the crossed condensation product **5a** was predominantly formed along with trace amounts of self-condensation products **2a** and **4**. Subsequent chromatographic workup and recrystallization afforded the cubane-type sulfido cluster **5a** in 71% isolated yield [Eq. (1)]. An X-ray



analysis confirmed the TiRu_3S_4 cubane-type structure of **5a** (Figure 1).^[5] The molecule has an approximate mirror plane bisecting the $\text{Ru}(1)\text{--Ru}(2)$ vector. As predicted by the

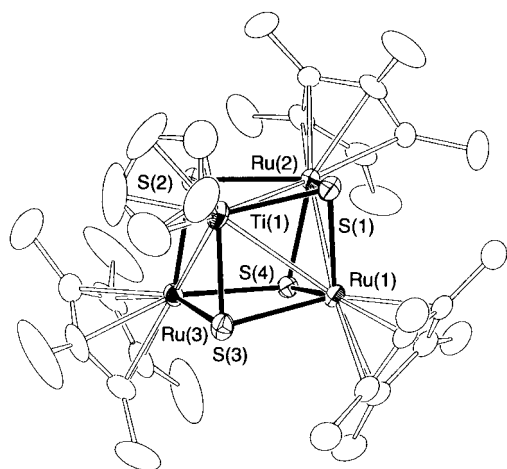
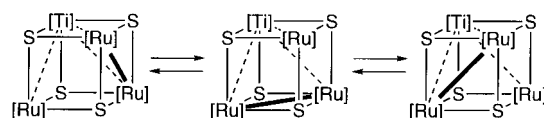


Figure 1. Molecular structure of **5a**. Hydrogen atoms are omitted for clarity. Selected interatomic distances [Å]: $\text{Ti}(1)\text{--Ru}(1)$ 2.9876(8), $\text{Ti}(1)\text{--Ru}(2)$ 3.0158(8), $\text{Ti}(1)\text{--Ru}(3)$ 2.9854(8), $\text{Ru}(1)\text{--Ru}(2)$ 2.8215(5), $\text{Ru}(1)\text{--Ru}(3)$ 3.612(1), $\text{Ru}(2)\text{--Ru}(3)$ 3.616(1).

effective atomic number (EAN) rule, the 64-electron cluster **5a** has one $\text{Ru}\text{--Ru}$ and three $\text{Ti}\text{--Ru}$ bonds. The $\text{Ti}\text{--Ru}$ distances (mean 2.996 Å) are comparable to those for the $\text{Ru}\rightarrow\text{Ti}$ dative bonds in the $\text{Ti}_2\text{Ru}_2\text{S}_4$ cluster **4** (mean 2.935 Å).^[4] The $\text{Ru}(1)\text{--Ru}(2)$ distance of 2.8215(5) Å is also consistent with the $\text{Ru}\text{--Ru}$ bond order of unity, whereas the other $\text{Ru}\text{--Ru}$ distances (mean 3.614 Å) preclude the presence of the direct bonding interaction between these Ru atoms. This metal-metal bonding scheme sharply contrasts with that in the electron-deficient, early-late heterobimetallic cluster **4**, which has 60 electrons and only four $\text{Ru}\rightarrow\text{Ti}$ dative bonds but no significant $\text{Ru}\text{--Ru}$ or $\text{Ti}\text{--Ti}$ interactions.^[4] Mixed-metal cubane-type^[7] or cuboidal clusters^[8] related to **5a** have been reported recently. Cluster **5a** also provides a still-limited example of cubane-type clusters with a MM_3S_4 core and four $\eta^5\text{-C}_5\text{R}_5$ ligands.^[9]

Variable-temperature ^1H NMR measurements indicated the mobility of the $\text{Ru}\text{--Ru}$ bond in **5a**, as shown in Scheme 1. In agreement with the solid-state structure of **5a**, the resonances for the Cp^* groups appear as two singlets in an

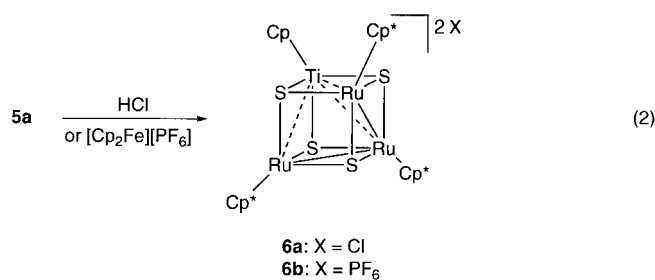


Scheme 1. Mobility of the intermetallic bond in **5a**. $[\text{Ti}] = \text{CpTi}$, $[\text{Ru}] = \text{Cp}^*\text{Ru}$.

intensity ratio of 2:1 at -50°C . These two signals coalesce at 0°C and appear as a sharp singlet at 80°C . The resonance for the Cp group appears as one sharp singlet throughout the measurements. The activation parameters, estimated by fitting the simulated spectra to the observed ones, are $\Delta H^\ddagger = 53 \pm 4 \text{ kJ mol}^{-1}$ and $\Delta S^\ddagger = -5 \pm 14 \text{ J mol}^{-1} \text{ K}^{-1}$ with $\Delta G^\ddagger = 55 \pm 6 \text{ kJ mol}^{-1}$ (0°C). Rauchfuss and co-workers have already reported similar fluxional behavior ascribed only to the arrangement of the metal-metal bond(s) for homometallic ruthenium^[10] or iridium^[11] clusters.

On the other hand, similar treatment of the rhodium or iridium analogue **1b** (or **1c**) with **3** afforded the mixture of **2b** (or **2c**), $[(\text{CpTi})(\text{Cp}^*\text{Ru})(\text{Cp}^*\text{M})_2(\mu_3\text{-S})_4]$ (**5b**: M = Rh; **5c**: M = Ir), and **4**. For example, the ratio of the clusters **2b**, **5b**, and **4** produced was approximately 5:12:7 as estimated from the ^1H NMR spectrum of the crude product. Attempts to separate these clusters by column chromatography or fractional recrystallization have failed.

We have already found that the electron-deficient $\text{Ti}_2\text{Ru}_2\text{S}_4$ cluster **4** is oxidized by HCl to afford the distorted cubane-type cluster $[(\text{CpTiCl}_2)(\text{CpTi})(\text{Cp}^*\text{Ru})_2(\mu_3\text{-S})_4]$, in which the chlorine atoms are coordinated to one of the titanium atoms to mitigate the electron deficiency of this atom.^[4b] The electron-precise TiRu_3 cluster **5a** was oxidized by HCl in a different manner. Thus, treatment of **5a** with an excess of HCl gas in toluene afforded the dicationic cluster $[(\text{CpTi})(\text{Cp}^*\text{Ru})_3(\mu_3\text{-S})_4]\text{Cl}_2$ (**6a**) as shown in Equation (2). Cluster **5a** was also oxidized with two equivalents of $[\text{Cp}_2\text{Fe}][\text{PF}_6]$ to give the PF_6^- salt **6b**. An X-ray analysis of $\text{6a} \cdot \text{HCl} \cdot \text{H}_2\text{O} \cdot \text{MeCN}$



clearly demonstrated the ionic nature of **6a** (Figure 2).^[5] The two-electron oxidation resulted in the formation of one more Ru–Ru bond than **5a**: the Ru(1)–Ru(2) and Ru(2)–Ru(3) distances (mean 2.845 Å) are congruent with the presence of Ru–Ru bonds, whereas the Ru(1)–Ru(3) distance (3.513(1) Å) is comparable to the nonbonding contacts in **5a**. The Ti–Ru

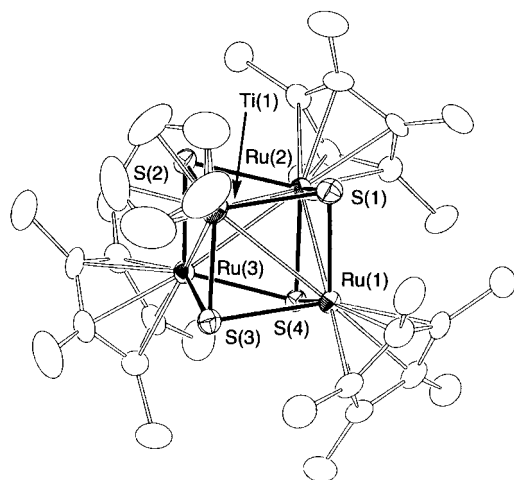


Figure 2. Structure of the cationic part of **6a**·HCl·H₂O·MeCN. Hydrogen atoms are omitted for clarity. Selected interatomic distances [Å]: Ti(1)–Ru(1) 3.012(2), Ti(1)–Ru(2) 2.934(2), Ti(1)–Ru(3) 3.007(2), Ru(1)–Ru(2) 2.8386(9), Ru(1)–Ru(3) 3.513(1), Ru(2)–Ru(3) 2.850(1).

distances (mean 2.984 Å) suggest the presence of three Ru→Ti dative bonds.^[12] These observations for the 62-electron clusters **6** are consistent with the EAN rule. Like **5a**, clusters **6a** and **b** exhibit fluxional behavior in solution; the simulations gave activation parameters of $\Delta H^\ddagger = 60 \pm 6 \text{ kJ mol}^{-1}$ and $\Delta S^\ddagger = 6 \pm 20 \text{ J mol}^{-1} \text{ K}^{-1}$ with $\Delta G^\ddagger = 58 \pm 8 \text{ kJ mol}^{-1}$ (–10 °C) for **6b**.

Condensation of preassembled metal-sulfur aggregates has attracted increasing attention because it provides a rational pathway to metal-sulfido clusters with higher nuclearity. Indeed, clusters with symmetrical structural motifs such as polycubanes,^[13] raft-type,^[14] and hexanuclear octahedral frameworks relating to Chevrel phases^[14a] have reasonably been prepared through this method. However, crossed condensation of different polynuclear units remains undeveloped. In the present study, this synthetic strategy has successfully been applied to the synthesis of cubane-type clusters with M₃M₃S₄ cores, which have been previously prepared only by using a self-assembly approach^[15] or incorporation of a heterometal into the preassembled M₃S₄ cores.^[16] Further investigation on syntheses of mixed-metal

cubane-type sulfido clusters through this synthetic pathway as well as the reactivities of the TiRu₃S₄ clusters **5a** and **6** is now under way.

Experimental Section

5a: To a mixture of **1a** (416.0 mg, 0.682 mmol) and **3** (352.6 mg, 0.683 mmol) in THF/CH₂Cl₂ (40 mL/40 mL) was added triethylamine (1.5 mL, 11 mmol) at –78 °C, and the mixture was slowly warmed to room temperature with stirring. After removal of the solvent in vacuo, the resultant red-brown oil was extracted with benzene. The extract was then subject to chromatography on alumina with benzene, followed by recrystallization from CH₂Cl₂/CH₃OH (10 mL/50 mL). The dark red-brown crystals that formed were filtered off and dried in vacuo (463.0 mg, 71 %). ¹H NMR (400 MHz, [D₈]toluene, –50 °C, TMS): $\delta = 5.94$ (s, 5H, C₅H₅), 1.91 (s, 15H, C₅Me₅), 1.50 (s, 30H, C₅Me₅); elemental analysis for C₃₅H₅₀Ru₃S₄Ti: calcd: C 44.24, H 5.30; found: C 43.88, H 5.36. For **5b**: ¹H NMR (400 MHz, [D₆]benzene, 18 °C, TMS): $\delta = 6.07$ (s, 5H, C₅H₅), 1.84 (s, 15H, RuC₅Me₅), 1.54 (s, 30H, RhC₅Me₅). For **5c**: ¹H NMR (400 MHz, [D₆]benzene, 18 °C, TMS): $\delta = 6.02$ (s, 5H, C₅H₅), 1.82 (s, 15H, RuC₅Me₅), 1.55 (s, 30H, IrC₅Me₅).

6a: Through a solution of **5a** (52.8 mg, 0.0556 mmol) in toluene (5 mL) was bubbled HCl gas for 5 min, and the mixture stirred for 2 h. The resultant brown suspension was evaporated to dryness in vacuo. Recrystallization of the residue from MeCN/Et₂O (3 mL/17 mL) afforded **6a**·HCl·H₂O·MeCN as dark red-brown crystals (50.5 mg, 81 %). ¹H NMR (400 MHz, [D₂]dichloromethane, –50 °C, TMS): $\delta = 6.01$ (s, 5H, C₅H₅), 1.91 (s, 15H, C₅Me₅), 1.80 (s, 30H, C₅Me₅); elemental analysis for C₃₇H₅₆Cl₃NORu₃S₄Ti: calcd: C 39.80, H 5.06, N 1.25, Cl 9.53; found: C 39.69, H 5.13, N 1.36, Cl 9.91.

6b: A mixture of **5a** (65.6 mg, 0.0690 mmol) and [Cp₂Fe][PF₆] (45.9 mg, 0.139 mmol) was dissolved in CH₂Cl₂ (10 mL) and stirred for 12 h. After removal of the solvent in vacuo, the resultant red-brown powder was washed with Et₂O. Recrystallization from MeCN/Et₂O (3 mL/17 mL) afforded **6b**·0.5MeCN·0.5Et₂O as red-brown crystals (78.6 mg, 88 %). ¹H NMR (400 MHz, [D₂]dichloromethane, –50 °C, TMS): $\delta = 6.01$ (s, 5H, C₅H₅), 1.81 (s, 15H, C₅Me₅), 1.78 (s, 30H, C₅Me₅); elemental analysis for C₃₈H_{56.5}F₁₂N_{0.5}O_{0.5}P₂Ru₃S₄Ti: calcd: C 35.17, H 4.39, N 0.54; found: C 35.25, H 4.39, N 0.78.

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ic, space group $P2_1/c$, $a = 14.627(2)$, $b = 14.762(2)$, $c = 20.654(2)$ Å, $\beta = 97.923(4)^\circ$, $V = 4417.1(8)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 1.679$ g cm⁻³, $2\theta_{\text{max}} = 55^\circ$, room temperature, $F(000) = 2248$, $\mu = 15.81$ cm⁻¹, Rigaku RAXIS-RAPID imaging plate area detector, MoK α radiation ($\lambda = 0.7107$ Å), graphite monochromator, 17753 measured reflections, Lorentz polarization and absorption corrections (transmission factors: 0.3044–0.7289), full-matrix least-squares refinement based on $|F|$, 461 parameters, H atoms except for those for HCl/H₂O included with fixed parameters, $R = 0.052$ and $R_w = 0.076$ ($w = 1/\sigma^2 F_o$) for 6248 observed reflections [$I > 3\sigma(I)$], max./min. residual electron density 0.78/–1.26 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-134168 (**5a**) and -134169 (**6a**·HCl·H₂O·MeCN). 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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Synthesis of Mixed-Metal High-Nuclearity Clusters by Fusion of Anionic Ruthenium Carbido Clusters through Palladium Units

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High-nuclearity cluster complexes, in particular heterometallic clusters, have attracted considerable interest because of their diverse properties.^[1, 2] One example is their application as precursors for metal dispersions with discrete metal atom composition, which may be useful as heterogeneous catalysts.^[3] The fusion of two smaller cluster units through different metal atoms should provide a straightforward method to increase the nuclearity of carbonyl clusters efficiently. A large number of fusions of transition metal clusters by treatment of the cluster anions with cations or halides of Cu,^[4] Ag,^[5, 6] Au,^[4e, 7] and Hg^[7d, 8] to give a “sandwiching” of these metal atoms between two cluster units have been reported. In contrast, the reactions of cluster anions with cation or halide of Group 10 transition metals have been known to give giant clusters comprising two different transition metals, for example, [Ni₃₈Pt₆(CO)₄₈(H)_{6–n}]^{n–},^[9] [Pd₃₃Ni₉(CO)₄₁(PPh₃)₆]^{4–},^[10] [Ni₃₆Pt₄(CO)₄₅]^{6–},^[11] and [Fe₆Pd₆(CO)₂₄H]^{3–}.^[12] In these examples, considerable fragmentation and recombination of the original cluster component appear to be the important factors. Herein we report on the coupling of ruthenium carbido carbonyl clusters through palladium species to give high-nuclearity Ru/Pd mixed-metal complexes of “sandwich cluster” type structure. This is similar to the many cases of Group 11 and 12 metals as linking agents, rather than the unpredictable cluster formation by fragmentation and spontaneous assembly pathways. Incorporation of a Group 10 transition metal as a linking agent in sandwiching products should greatly widen the scope of the fusion route as a method for the rational synthesis of large heterometallic clusters, particularly in terms of their application as potential precursors for tailored alloy catalysts. This investigation is significant in that ruthenium and palladium are utilized as heterogeneous catalysts in a wide variety of industrial reactions.^[13]

Treatment of the pentanuclear ruthenium carbido anion [Ru₅C(CO)₁₄]^{2–} (cation: PPN = N(PPh₃)₂⁺) with two equivalents of [Pd(C₃H₅)Cl]₂ in THF at room temperature for 12 h gave dark red crystals of a neutral cluster with the composition [Pd₈Ru₁₀C₂(CO)₂₇(C₃H₅)₄] (**1**) in 52% yield. X-ray structural analysis (Figure 1) revealed that the molecule has two pseudo mirror planes, one of which passes through Pd2, Pd5, Ru11, and Ru21 and the other through Pd7, Pd8, C20, O21, C8, and C11.

The Pd₈ core, which is flanked on each side by a square-pyramidal Ru₅ core, consists of two coplanar fused squares, Pd1–Pd2–Pd5–Pd4 and Pd2–Pd3–Pd6–Pd5, and two Pd atoms, Pd7 and Pd8, which cap each of the squares from the same

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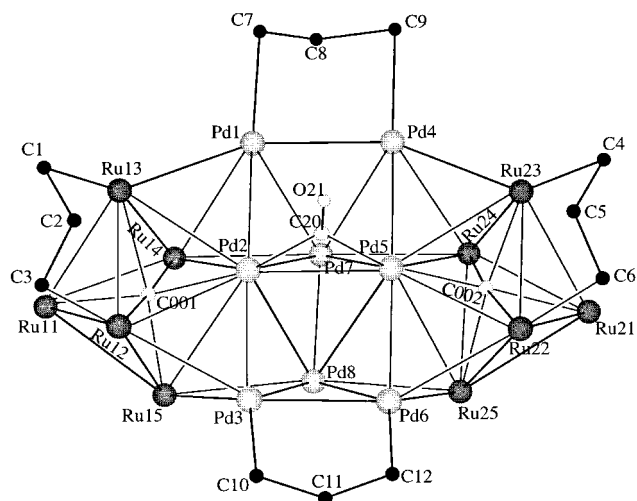


Figure 1. Structure of **1** in the crystal (26 CO ligands omitted for clarity). Four Pd–Ru bonds (Pd7–Ru14, Pd7–Ru24, Pd8–Ru15, Pd8–Ru25) and one Pd–Pd bond (Pd2–Pd5) have bridging CO, and four Pd–Ru bonds (Pd1–Ru13, Pd3–Ru12, Pd4–Ru23, Pd6–Ru22) have semibridging CO ligands, while all other CO ligands are terminal and bonded to Ru atoms. Selected interatomic distances [Å] and angles [°]: Pd1–Ru13 2.865(2), Pd1–Ru14 2.977(2), Pd2–Ru12 2.888(2), Pd2–Ru13 2.872(2), Pd2–Ru14 3.037(1), Pd2–Ru15 3.162(2), Pd3–Ru12 2.839(2), Pd3–Ru15 2.956(1), Pd4–Ru23 2.800(2), Pd4–Ru24 2.973(2), Pd5–Ru22 2.874(1), Pd5–Ru23 2.880(2), Pd5–Ru24 3.199(2), Pd5–Ru25 3.079(2), Pd6–Ru22 2.845(2), Pd6–Ru25 2.941(2), Pd7–Ru14 2.757(2), Pd7–Ru24 2.809(2), Pd8–Ru15 2.820(2), Pd8–Ru25 2.780(2), Pd1–C7 2.08(2), Pd4–C9 2.14(2), Ru12–C3 2.23(1), Ru13–C1 2.23(2); Pd2–C001–Ru11 179.8(8), Pd5–C002–Ru21 176.0(8).

side of the fused square plane and are in bonding distance (2.720(2) Å). There are 20 Pd–Ru bonds in the structure (Figure 1), and as a consequence of these Pd–Ru bonding interactions, the basal planes of the Ru pyramids tilt toward the Pd7–Pd8 bond. The dihedral angles between the basal planes and the fused Pd squares are 68.89(4) and 71.12(4)°, respectively. Four $\mu_2\text{-}\eta^3\text{-C}_3\text{H}_5$ groups are located on the Pd1–Pd4, Pd3–Pd6, Ru12–Ru13, and Ru22–Ru23 bonds. The ^1H NMR spectrum shows sets of signals for the Ru-allyl^[14] and Pd-allyl groups; these signals do not change even at 80 °C. The ^{13}C NMR spectrum exhibits two moderately broad peaks ($\delta = 198.2$ and 196.8; ratio 1:2) in the CO region. These can be assigned to CO ligands that interact with the Pd atoms and to those that are bonded solely to Ru atoms.

By using the hexanuclear ruthenium carbido anion $[\text{Ru}_6\text{C}(\text{CO})_{16}]^{2-}$ as the starting carbonyl cluster, the reactions with the palladium cation $[\text{Pd}(\text{CH}_3\text{CN})_4]^{2+}$ proceeded smoothly; however, the number of palladium atoms incorporated in the product complexes was found to be dependent on reaction conditions. Refluxing $[\text{PPN}]_2[\text{Ru}_6\text{C}(\text{CO})_{16}]$ in CH_2Cl_2 with about one equivalent of $[\text{Pd}(\text{CH}_3\text{CN})_4][\text{BF}_4]_2$ for 4 h under air afforded a dark red oil. Subsequent treatment of the latter with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ resulted in crystals of $[\text{PPN}]_2[\text{Pd}_2\text{Ru}_{12}\text{C}_2(\text{CO})_{30}]$ (**2**) in 33% yield. Reaction of the same reactants in the same feeding ratio in THF at room temperature for 12 h but under argon gave dark red crystals of the composition $[\text{PPN}]_2[\text{Pd}_4\text{Ru}_{12}\text{C}_2(\text{CO})_{32}]$ (**3**). The yield of this reaction was 34% based on the starting ruthenium complex and 68% based on the palladium cation used. The use of excess $[\text{Pd}(\text{CH}_3\text{CN})_4][\text{BF}_4]_2$ gives a much worse yield of

3. Refluxing a solution of **3** in CH_2Cl_2 under air did not give **2** but resulted in gradual decomposition of **3** to an intractable material.

The molecular structure of **2** determined by X-ray structure analysis (Figure 2) shows that the two Ru_6 octahedra are linked through two palladium atoms. The Ru_6 skeleton retains

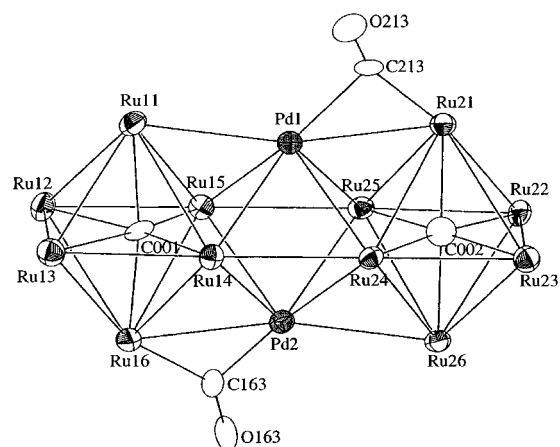


Figure 2. Structure of the anion of **2** in the crystal. The CO ligands, except those bridging the Pd–Ru bonds, are omitted for clarity; all other CO ligands are bonded solely to Ru atoms. Selected interatomic distances [Å] and angles [°]: Pd1–Ru11 2.836(1), Pd1–Ru14 2.754(1), Pd1–Ru15 2.788(1), Pd1–Ru21 2.786(1), Pd1–Ru24 2.735(1), Pd1–Ru25 2.767(1), Pd2–Ru14 2.759(1), Pd2–Ru15 2.722(1), Pd2–Ru16 2.762(1), Pd2–Ru24 2.797(1), Pd2–Ru25 2.762(1), Pd2–Ru26 2.848(1), Ru14–Ru15 3.215(2), Ru24–Ru25 3.212(2); Ru11–Pd1–Ru21 165.83(3), Ru16–Pd2–Ru26 165.87(3), Ru12–Ru13–Ru14 94.48(3), Ru13–Ru12–Ru15 93.31(3).

its original octahedral geometry except that the edge that interacts with the palladium atoms is broken; the Ru14–Ru15 and Ru24–Ru25 distances are 3.215(2) and 3.212(2) Å, respectively. Each palladium atom has six heterometallic bonds, four to the ruthenium atoms of the broken Ru–Ru edges and two to apical ruthenium atoms.

Complex **3** (Figure 3) contains two additional palladium atoms. The Pd_4 skeleton adopts the form of a bent square (angle 107.5°; nonbonded Pd2–Pd3 distance 3.485(2) Å). The two Ru_6 octahedra have local structures similar to those in **2** but their relative orientation is now twisted, in terms of the broken Ru–Ru edges, by almost 90° from that in **2**.

Apparently, formation of these heterometallic cluster complexes does not result from a simple combination reaction between cationic and anionic complexes but is accompanied by partial redox reactions. Formation of **1** may be viewed formally as the result of the reaction between two molecules of $[\text{Ru}_5\text{C}(\text{CO})_{14}]^{2-}$ and four molecules of the “ $\text{Pd}(\text{C}_3\text{H}_5)\text{Cl}$ ” species, in which four atoms of Pd^0 are further incorporated. In the synthesis of **2** and **3**, the neutral ruthenium cluster $[\text{Ru}_6\text{C}(\text{CO})_{17}]$ is always obtained as a minor product (ca. 20%). Cyclic voltammetric studies in CH_3CN with $[\text{Cp}_2\text{Fe}]/[\text{Cp}_2\text{Fe}^+]$ ($\text{Cp} = \text{C}_5\text{H}_5^-$) as reference gave $E_p(\text{reduction})$ of $[\text{Pd}(\text{CH}_3\text{CN})_4]^{2+}$ at -0.14 V, while $E_p(\text{oxidation})$ for $[\text{Ru}_6\text{C}(\text{CO})_{16}]^{2-}$ was -0.01 V. The oxidation peak of the cluster anion is, however, broad and the lower part of the voltammogram is extended over -0.14 V, suggesting that only gentle reduction of the palladium cation by the cluster anion is

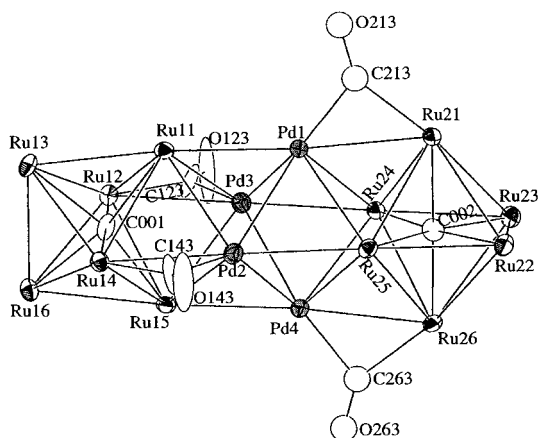


Figure 3. Structure of the anion of **3** in the crystal. The CO ligands, except those bridging the Pd–Ru bonds, are omitted for clarity; all other CO ligands are bonded solely to Ru atoms. Selected interatomic distances [Å]: Pd1–Pd2 2.825(4), Pd1–Pd3 2.720(2), Pd2–Pd3 3.485(2), Pd2–Pd4 2.744(2), Pd3–Pd4 2.824(3), Pd1–Ru11 2.774(2), Pd1–Ru21 2.740(2), Pd1–Ru24 2.902(2), Pd1–Ru25 2.840(3), Pd2–Ru11 2.833(3), Pd2–Ru14 2.750(2), Pd2–Ru15 2.905(2), Pd2–Ru25 2.763(2), Pd3–Ru11 2.916(2), Pd3–Ru12 2.748(2), Pd3–Ru15 2.833(3), Pd3–Ru24 2.764(2), Pd4–Ru15 2.771(2), Pd4–Ru24 2.820(4), Pd4–Ru25 2.909(3), Pd4–Ru26 2.744(2), Ru11–Ru15 3.458(4), Ru24–Ru25 3.454(2).

possible. By employing $[\text{Fe}(\text{C}_5\text{H}_5)_2]^+$ as an “innocent” oxidant that does not chemically react with the cluster anion or cluster species being oxidized, the reaction with one equivalent of $[\text{Ru}_5\text{C}(\text{CO})_{14}]^{2-}$ at 0 °C was found to give the self-condensation product $[\text{Ru}_{10}\text{C}_2(\text{CO})_{24}]^{2-}$ in a 83 % yield. The same complex was previously prepared by pyrolysis of $[\text{Ru}_6\text{C}(\text{CO})_{16}]^{2-}$ at 210 °C in 35 % yield.^[15] The investigation of the transformation of these high-nuclearity heterometallic clusters into supported fine metal particles of Ru/Pd alloy is now in progress.

Experimental Section

All reactions (except the synthesis of **2**) were carried out in dry solvents under purified argon. The products were purified by column chromatography on silica gel and crystallized from $\text{CH}_2\text{Cl}_2/\text{hexane}$ (**1**) or from $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (**2** and **3**). All data collections for X-ray analysis were made on a Rigaku RAXIS-IV imaging plate diffractometer with graphite-monochromated MoK_α ($\lambda = 0.71073$ Å) radiation. Structures were solved by direct methods, expanded using Fourier techniques, and refined by full-matrix least-squares. All calculations were performed by using the teXsan crystallographic software package (Molecular Structure Corporation 1992). 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-135943, CCDC-135944, and CCDC-135945. 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).

1: Yield 52 %; dark red plates; ^1H NMR (270 MHz, C_6D_6 , 25 °C): $\delta = 4.25$ (d, $J = 4.8$ Hz, 2H, *syn*-H, Pd-allyl), 4.17 (m, 1H, central-H, Pd-allyl), 3.39 (d, $J = 7.0$ Hz, 2H, *syn*-H, Ru-allyl), 2.89 (d, $J = 12.0$ Hz, 2H, *anti*-H, Pd-allyl), 1.99 (m, 1H, central-H, Ru-allyl), 0.37 (d, $J = 13.0$ Hz, 2H, *anti*-H, Ru-allyl); ^{13}C NMR (500 Hz, $[\text{D}_8]\text{toluene}$, 25 °C): $\delta = 198.2$ (CO), 196.8 (CO), 108.2 (allyl-terminal), 80.4 (allyl-terminal), 65.9 (allyl-central), 59.2 (allyl-central); IR (CH_2Cl_2): $\tilde{\nu}(\text{CO}) = 2072$ (m), 2036 (s), 2023 (s) cm^{-1} ; elemental analysis (%) calcd for $\text{C}_{41}\text{H}_{20}\text{O}_{27}\text{Pd}_8\text{Ru}_{10}$: C 17.54, H 0.72; found: 17.43, H 0.79. Crystal data for **1**·3 CH_2Cl_2 : $M_r = 3062.45$, monoclinic, space group $P2_1/n$ (no. 14), $a = 21.557(2)$, $b = 14.515(4)$, $c = 21.931(3)$ Å,

$\beta = 101.092(8)^\circ$, $V = 6733(2)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 3.019$ g cm^{−3}, $\mu = 45.61$ cm^{−1}, data collection at −100 °C, $R1 = 0.051$, $R2 = 0.049$ for 7401 data with $I > 4\sigma(I)$.

2: Yield 33 %; dark red plates; IR (CH_2Cl_2): $\tilde{\nu}(\text{CO}) = 2038$ (s), 2008 (s) cm^{-1} ; elemental analysis (%) calcd for $\text{C}_{105}\text{H}_{62}\text{Cl}_2\text{N}_2\text{O}_{30}\text{Pd}_4\text{Ru}_{12}$ (one CH_2Cl_2 molecule as solvent of crystallization): C 36.53, H 1.81, N 0.81; found: C 36.46, H 1.77, N 0.83. Crystal data for **2**·2 CH_2Cl_2 : $M_r = 3537.01$, monoclinic, space group Cc (no. 9), $a = 38.271(5)$, $b = 9.973(4)$, $c = 36.093(5)$ Å, $\beta = 123.592(8)^\circ$, $V = 11475(5)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 2.047$ g cm^{−3}, $\mu = 20.58$ cm^{−1}, data collection at −100 °C, $R1 = 0.043$, $R2 = 0.054$ for 9619 data with $I > 3\sigma(I)$.

3: Yield 34 % (based on Ru), 68 % (based on Pd); dark red plates; IR (CH_2Cl_2): $\tilde{\nu}(\text{CO}) = 2033$ (s), 2021 (s), 2002 (sh) cm^{-1} ; elemental analysis (%) calcd for $\text{C}_{106}\text{H}_{60}\text{N}_2\text{O}_{32}\text{Pd}_4\text{Ru}_{12}$: C 35.00, H 1.66, N 0.77; found: C 34.90, H 1.62, N 0.72. Crystal data for **3**: $M_r = 3635.97$, monoclinic, space group Cc (no. 9), $a = 23.979(3)$, $b = 31.343(8)$, $c = 19.177(2)$ Å, $\beta = 128.613(6)^\circ$, $V = 11262$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 2.144$ g cm^{−3}, $\mu = 23.13$ cm^{−1}, data collection at −100 °C, $R1 = 0.054$, $R2 = 0.058$ for 7430 data with $I > 4\sigma(I)$.

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