Metalloenzymes mimics

Enzymes are amongst the most selective catalysts available. Their active sites provide a variety of non-covalent interactions with substrates that control the recognition process and, therefore, the selectivity of the enzyme. These include hydrogen bonding, charge/charge, CH/π and cation/π interactions. Many enzymes contain metal ions that play a key role in the structural organization of the peptide, in the recognition processes of substrates or as the catalytic centers in the active site.
In the latter case, the enzyme does not only act as a very large ligand with preorganized metal binding sites, but it also provides a cavity and a corridor to control the access of the substrate and, therefore, selectivity and reactivity of the metal ion site.

Metalloenzyme mimics

- Phopshatase-catalyzed reaction 2-hydroxypropyl-p-nitrophenyl phosphate (HPNP) is used as RNA model substrate, the intramolecular cyclization reaction, and the release of p-nitrophenolate being the driving force of the reaction.

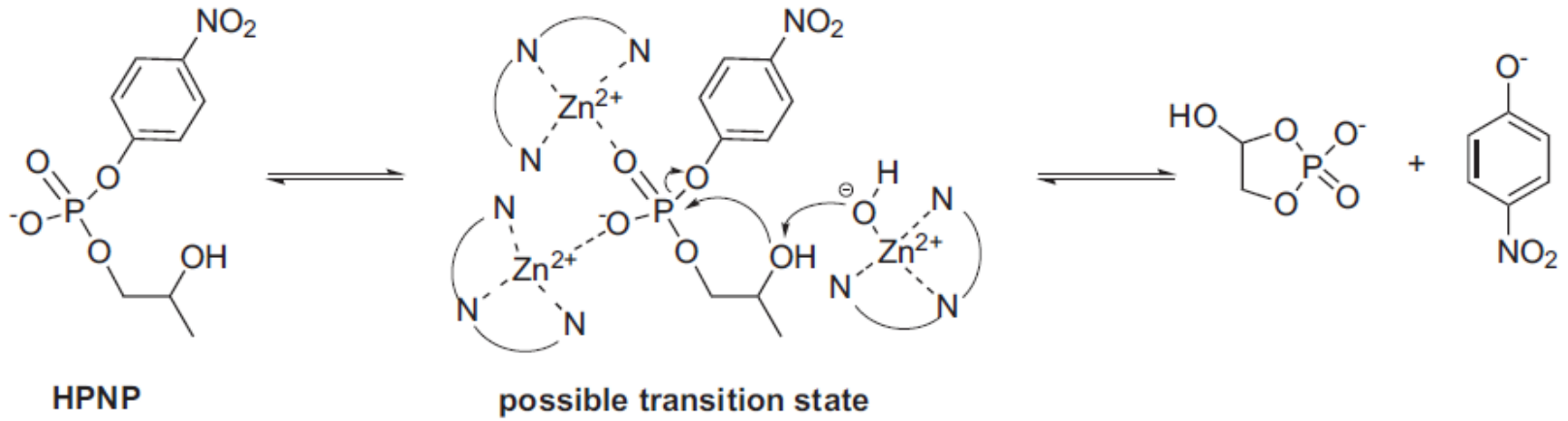


Fig. 10. Possible mechanism for HPNP cleavage by trinuclear phosphatase models [35].

Many metal containing phosphodiesterases use one, two or three divalent metal centers such as Zn^{2+} for the activation of the substrate.
The prearrangement of metal centers using synthetic spacers is crucial in mimics of such enzymes since tetrahedrally coordinated Zn^{2+} centers are assumed to activate the phosphate group and a nucleophilic water molecule, to stabilize the pentacoordinate phosphorus transition state and possibly the leaving group by cooperative action

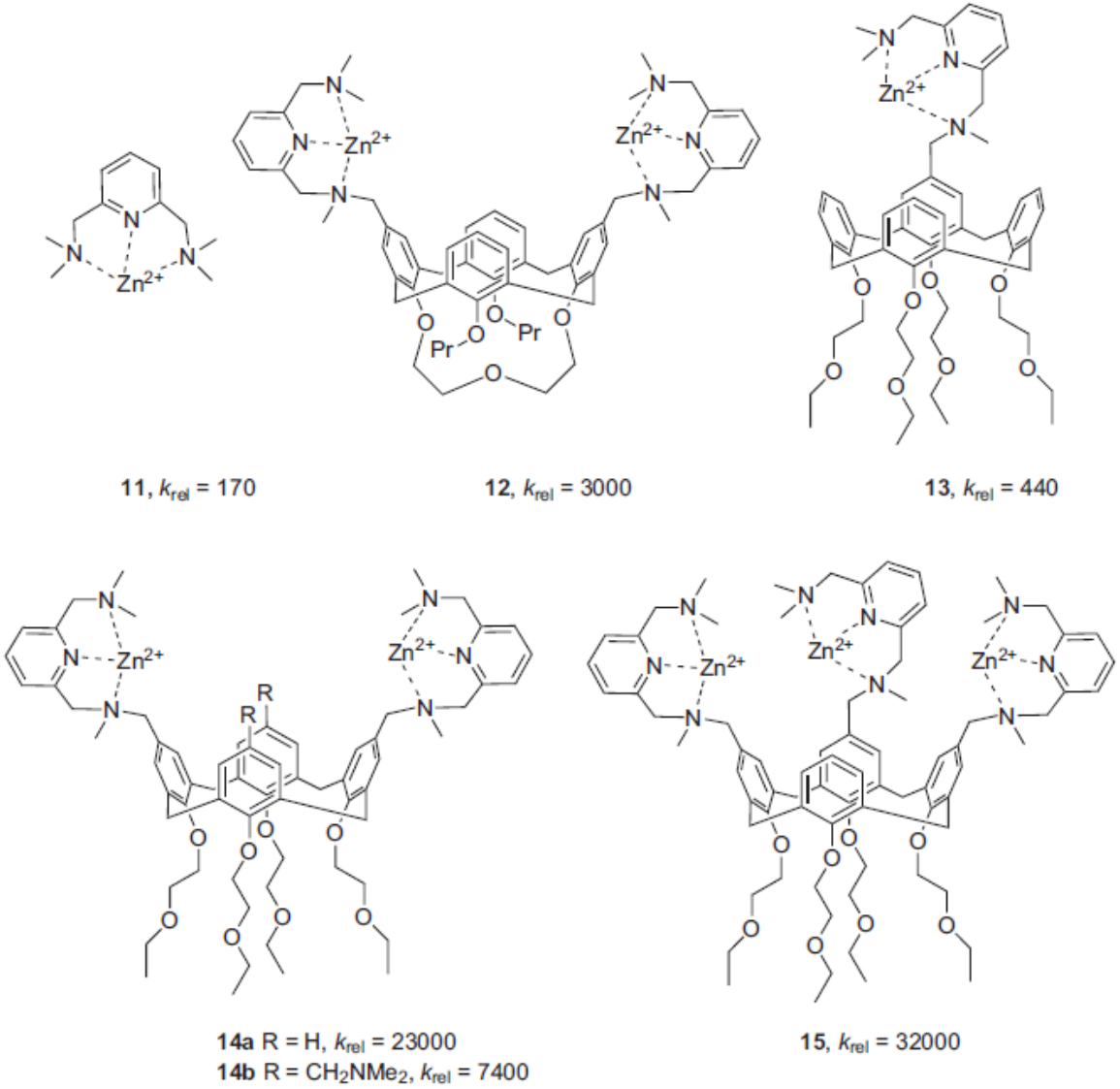
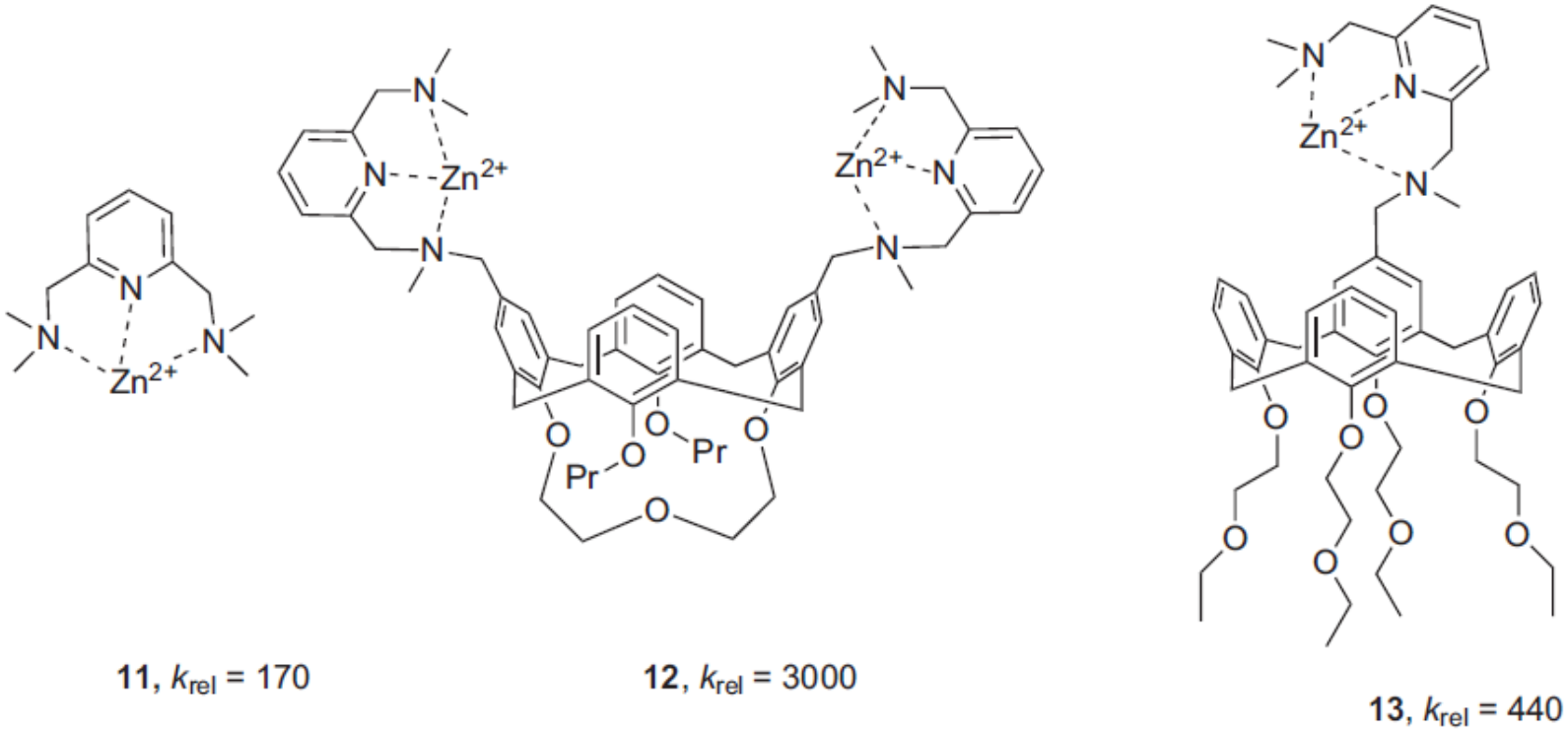


Fig. 11. Mono-, di- and trinuclear Zn(II) phosphatase mimics and relative rate acceleration factors k_{rel} [33–35].

Relative rate acceleration factors k_{rel}, defined as the ratio of the observed rate constant of the reaction in the presence of the catalyst and the rate constant of the non-catalyzed reaction, can be used to directly compare the activity of catalysts.

Reinhoudt and co-workers investigated the performance of calix[4]arenes 12–15 as enzyme mimics.

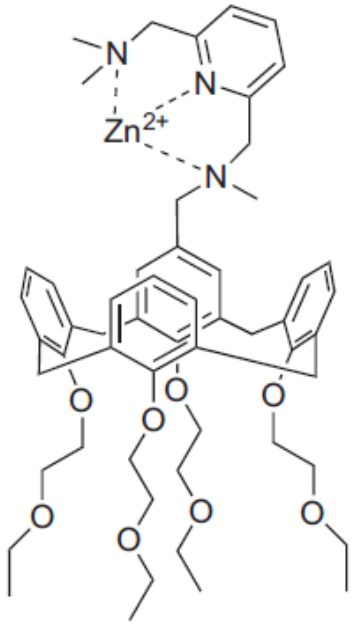


11, k_{rel} = 170

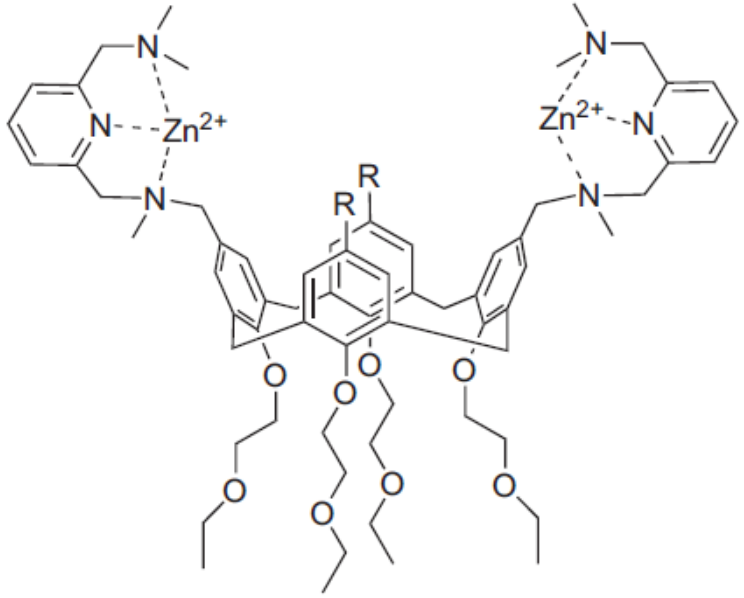
12, k_{rel} = 3000

13, k_{rel} = 440

The parent system 11 has a much lower catalytic activity than its mononuclear calixarene based conjugate 13.
This indicates that the calixarene backbone is involved in the catalysis, probably by binding the substrate inside the hydrophobic pocket [35].



13, k_{rel} = 440

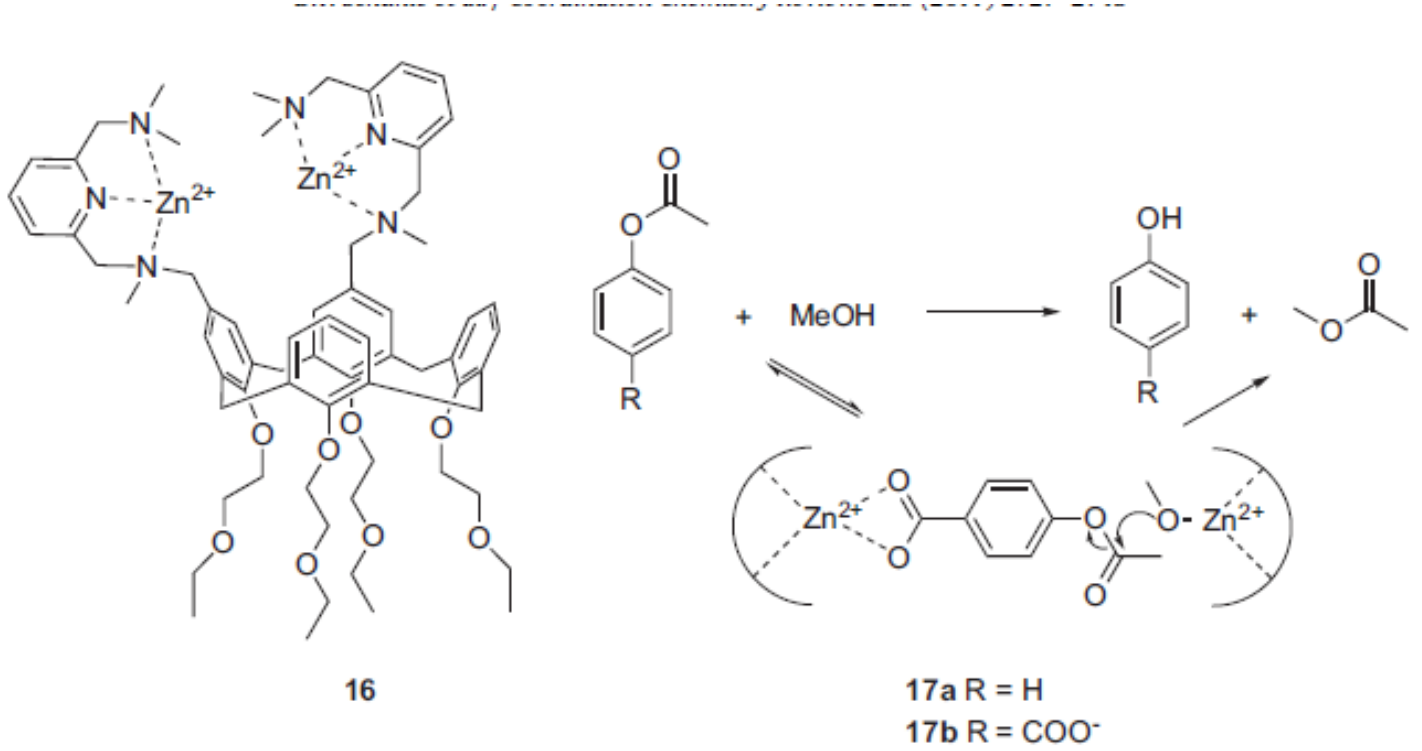


14a R = H, k_{rel} = 23000
14b R = CH_2NMe_2, k_{rel} = 7400

The dinuclear complex 14a shows a rate acceleration of 23,000 in the transesterification of HPNP over the non-catalyzed reaction, whereas the mononuclear 13 analogue is 50 times less active.

This suggests that the metal centers have cooperative action and that the hydrophobic cavity is involved in the binding of the substrate.

The ability of complexes 11, 14a, 15 and 16 to cleave carboxylic acid esters (esterase mimics) was investigated by solvolysis experiments in methanol.



16

17a R = H
17b R = COO^-

Fig. 12. Vicinal Zn^{2+} complex **16** and solvolysis reaction tested [36].

Acylase mimics

Many enzyme catalyzed transacylation reactions proceed via a double displacement mechanism.
First, an acylated enzyme intermediate is generated, which then acts as acylation agent for the nucleophilic substrate [46]. This principle is well-known in organic chemistry, for example from the pyridine catalyzed esterification of activated carboxylic acids. In such reactions, first pyridine reacts with the acylation agent creating a highly active species that subsequently transfers the acyl group to an attacking nucleophile.

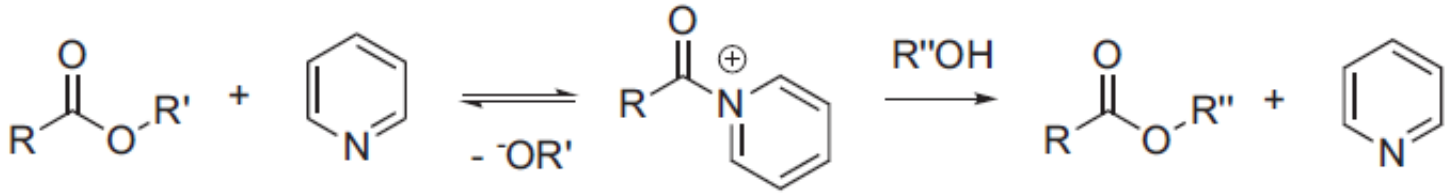


Fig. 15. Double displacement mechanism using pyridine as a catalyst.

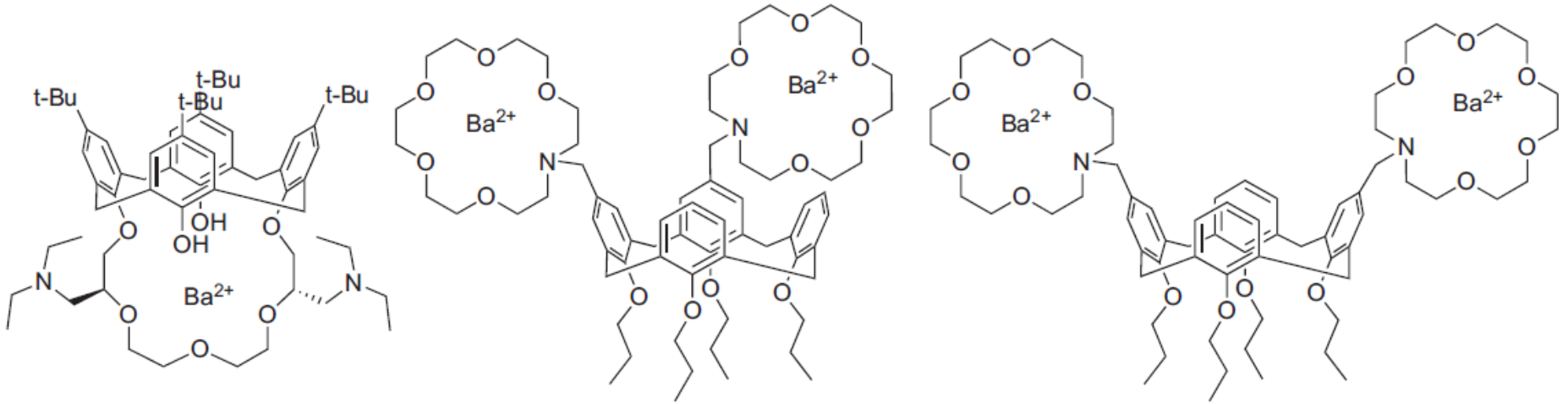
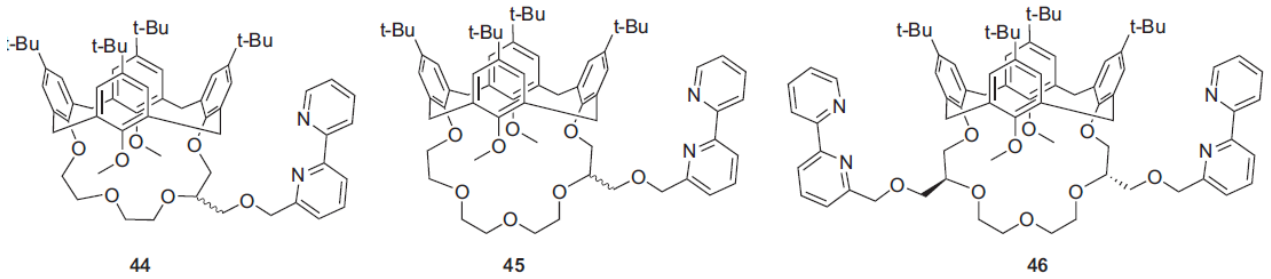


Fig. 16. Ba^{2+} containing artificial acyl-transferase **25** and acylases **26–27** [46,47].

In mimics, the challenge is to achieve efficient transfer of the activated acyl to the nucleophile.

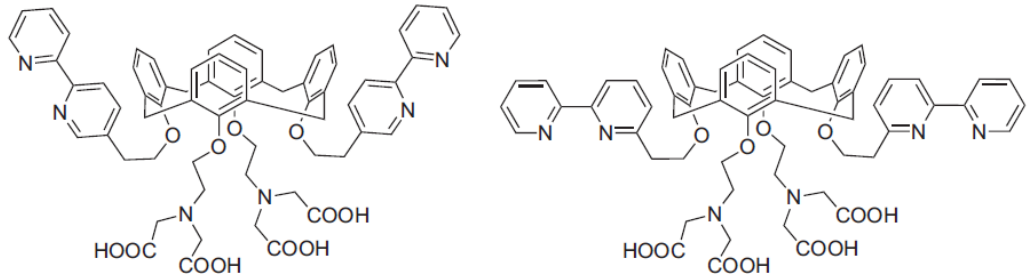
This can be realized by using phenolic alcoholates, which are better leaving groups than alkoxides. In calixarene 25, the phenolic groups can thus act as acyl-acceptors/acyl-donors, whereas the complexed Ba^{2+} acts as activating Lewis acid.

Calixarenes as luminescence agents for in vivo use



44 45 46

Fig. 27. Lanthanide chelators for luminescence studies[93].



47 48

Fig. 28. Strong chelators for luminescent lanthanide ions [94].

The lanthanides Eu^{3+} and Tb^{3+} are widely used as luminescent probes. Because of their toxicity, they need to be complexed by strong chelators. The complexation is also necessary to shield the metal ions from coordination of water, which would quench the luminescence. Still not enough water soluble for in vivo studies

MRI contrast agents

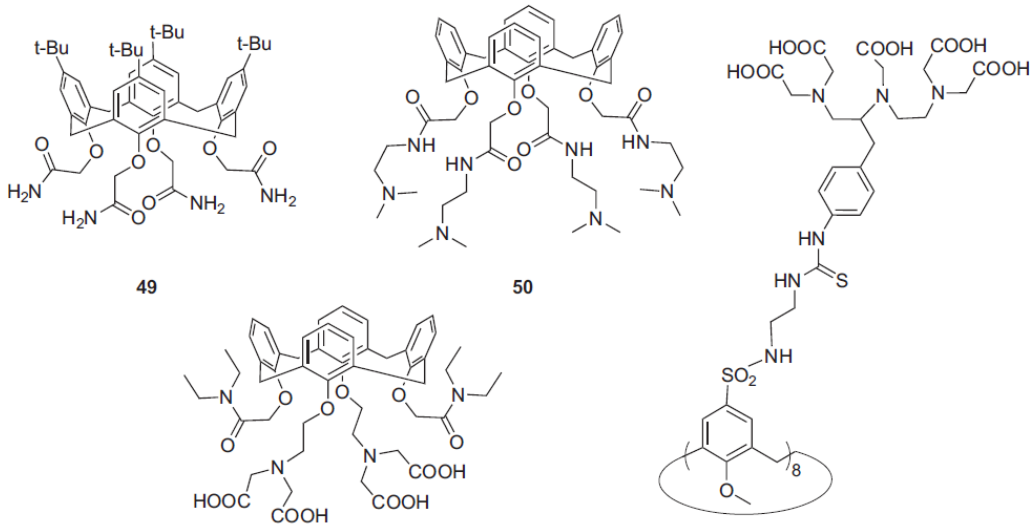


Fig. 29. Calixarene based chelators for MRI contrast agents [96,97].

In most clinical magnetic resonance imaging (MRI) scans, the ^{1}H signal of water protons is observed and the image reflects either the proton density or differences in the longitudinal (T1) or transversal (T2) relaxation time of water protons in certain compartments of the body.
The majority of MRI contrast agents contain Gd^{3+}-ions that are able to shorten the T1 relaxation times of surrounding water molecules very efficiently and lead to a brightening in MR images of areas that contain the agent.

Self-aggregating MRI contrast agents

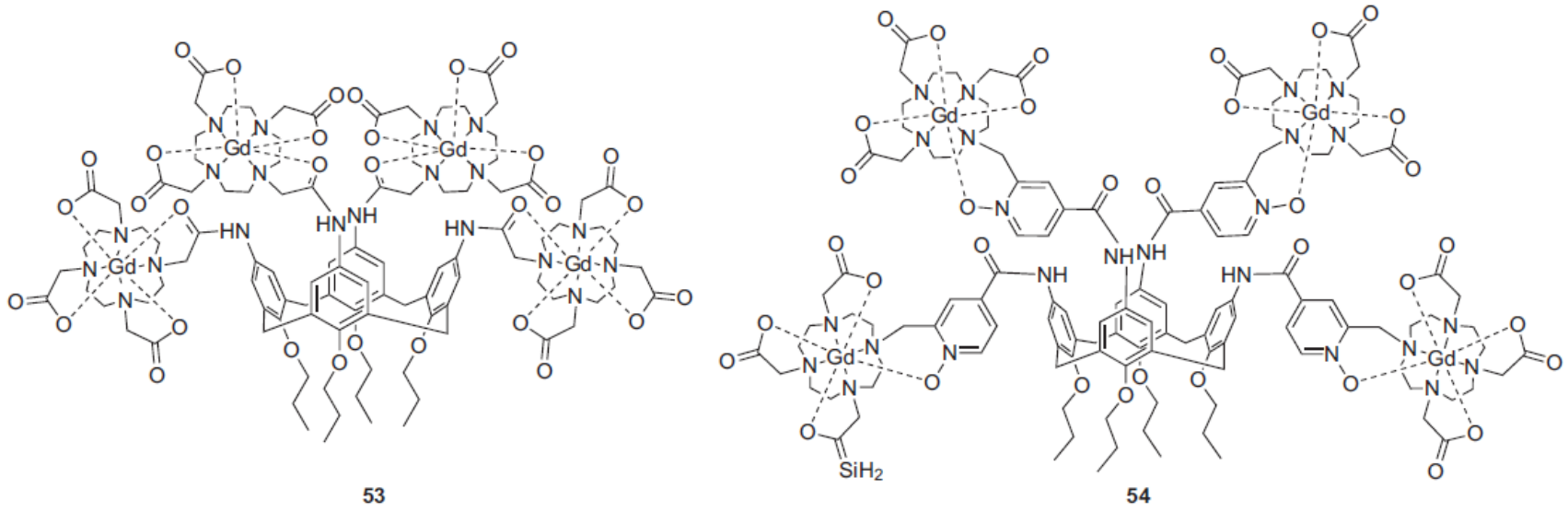


Fig. 30. Self-aggregating MRI contrast agents [100,101].

ChemComm
FEATURE ARTICLE

Host–guest chemistry of calixarene capsules

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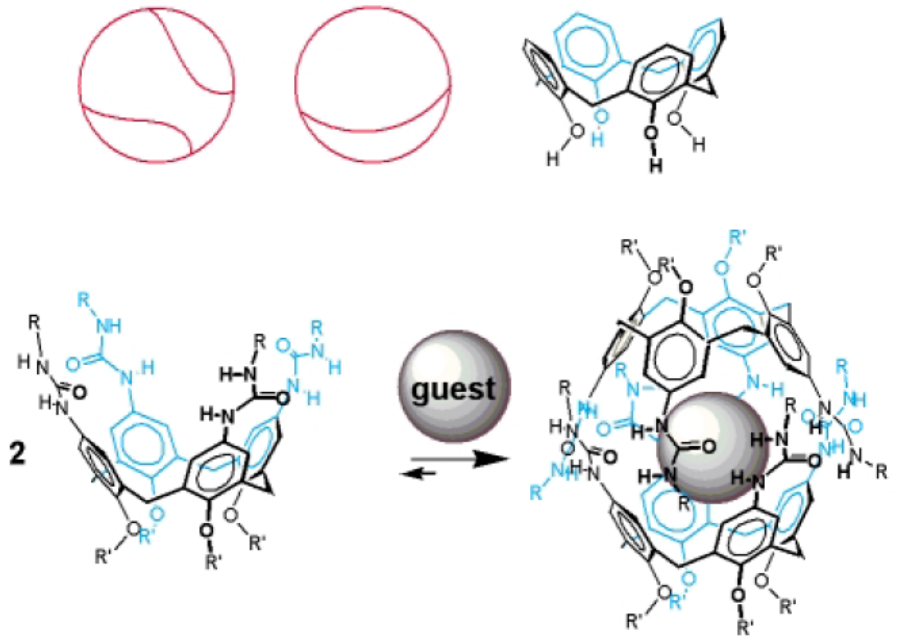
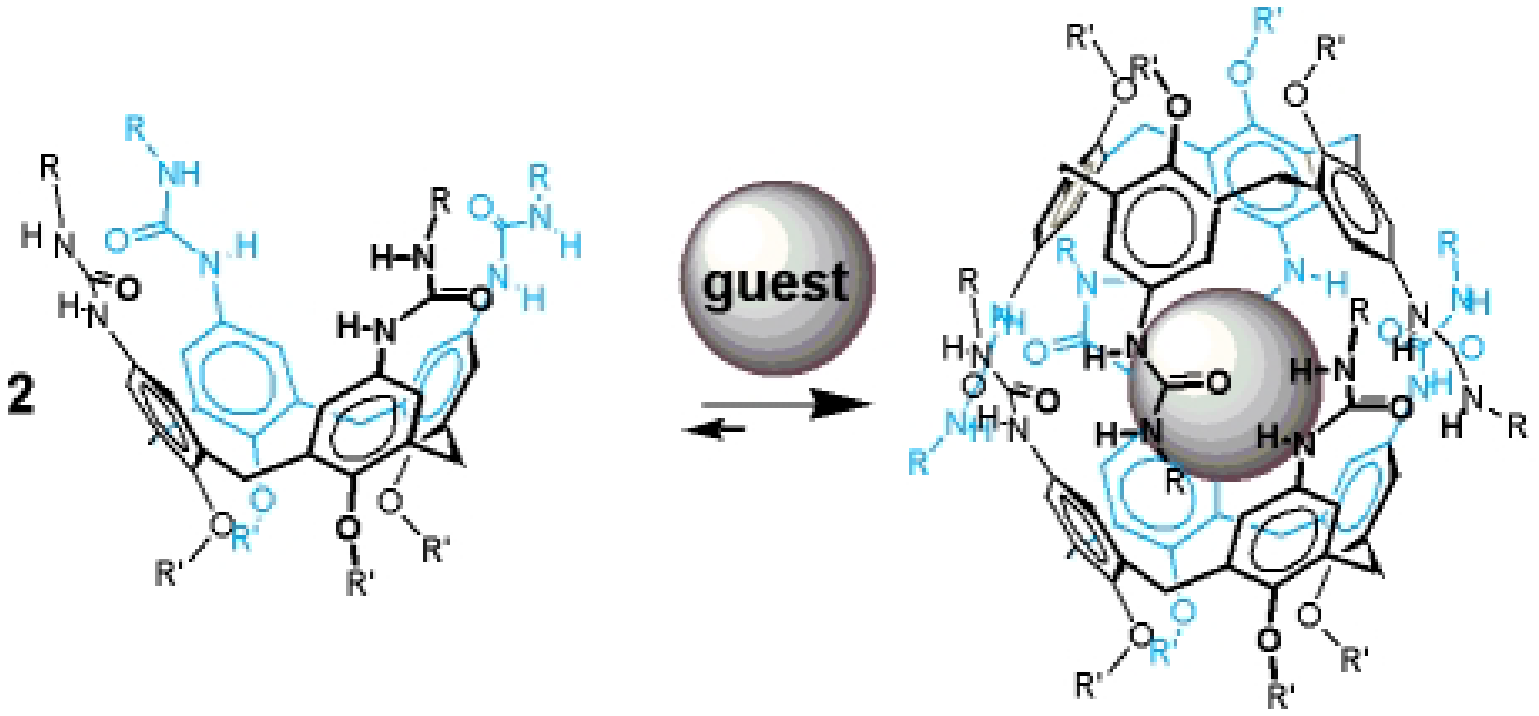


Fig. 1 *Top*. Ways of dividing a spherical surface and the curvature of a calix[4]arene in a cone conformation. *Bottom*. The calix[4]arene bearing ureas on the upper rim forms a dimeric capsule when an appropriate guest is present.



Shimizu's idea had been to bring two of these calixarenes together, rim-to-rim, and the enduring fashion in the group was to use hydrogen bonding patterns on self-complementary molecules to accomplish this. The moderate directionality and reversible formation of hydrogen bonds had been successful in other ongoing projects and Ken used the nature of ureas to nurture a seam of hydrogen bonds between the hemispheres. A circle of eight ureas, four from each hemisphere, assembled head-to-tail as shown in Fig. 1.

Calixarenes capsules and their guests

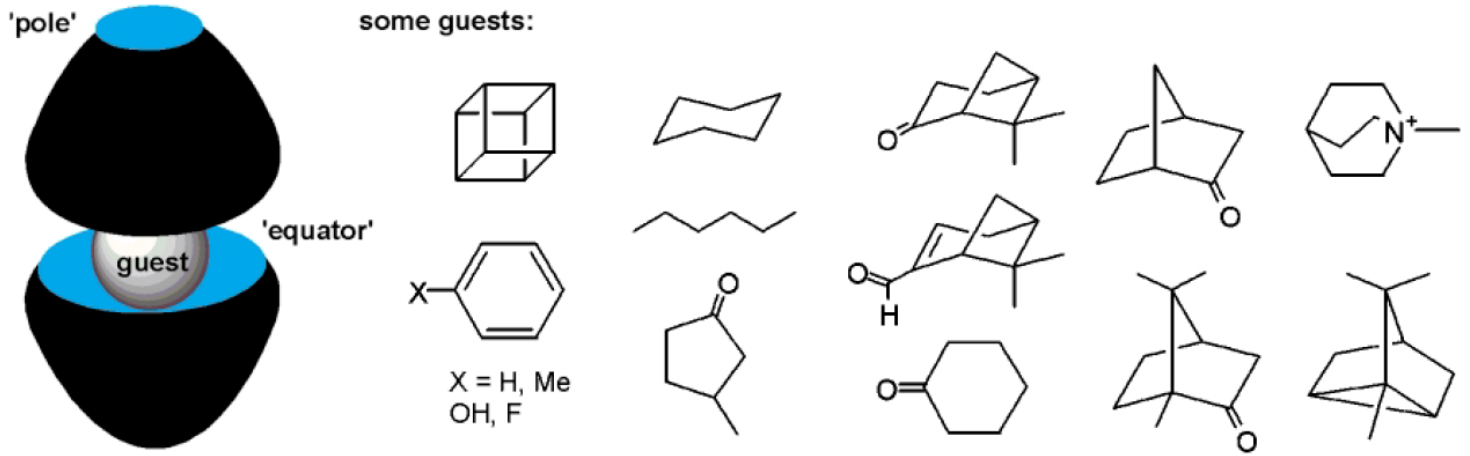


Fig. 2 *Left*. Cartoon representation of the calixarene capsules used elsewhere in this review. *Right*. Some of the many guests encapsulated by these dimers.

Aromatic guests

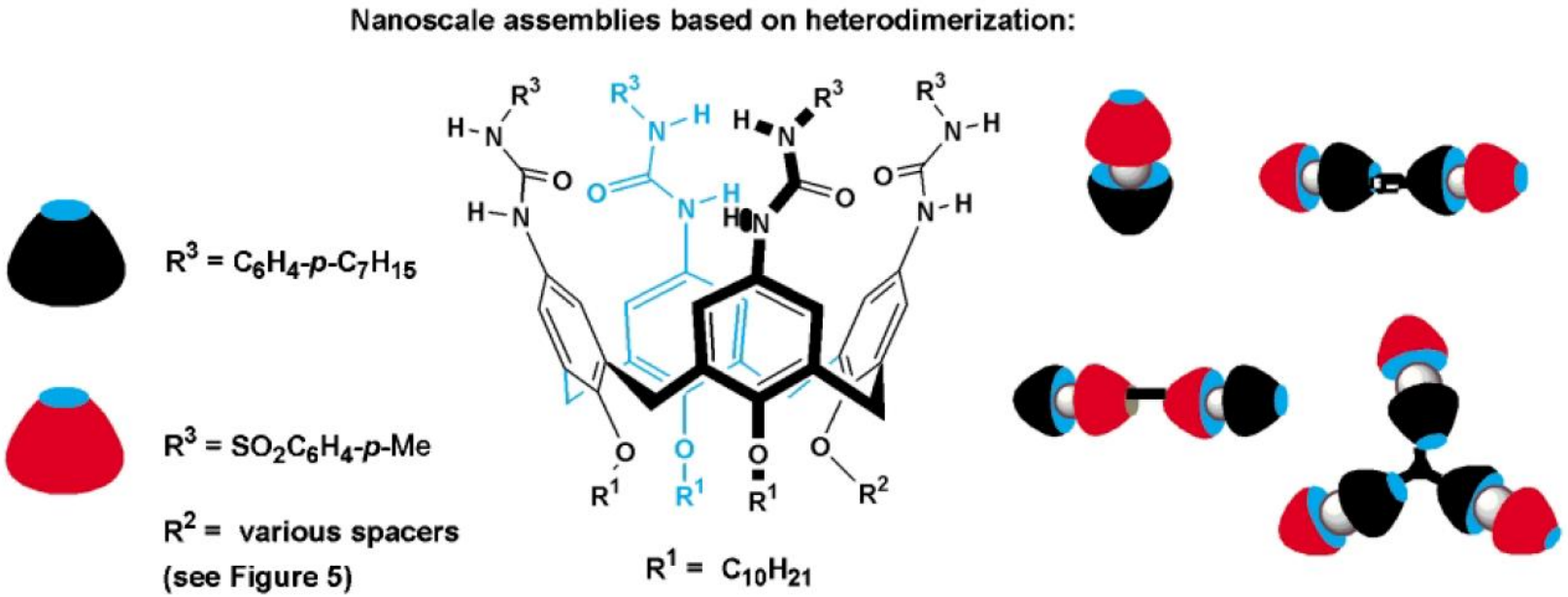
Halobenzenes, especially fluorobenzenes were also readily encapsulated and gave us some information about their orientation when trapped within. For example, in fluorobenzene the chemical shifts of the ortho, meta and para protons suggest a positioning in which the C–F bond and the para proton are along the equator (a polar microenvironment), directed at the seam of hydrogen bonds.
The resonance of the para proton of C6H5F was shifted only moderately upfield in the NMR spectrum, while the ortho and meta protons, directed at the eight aromatic faces in the poles of the cavity, experience the largest upfield shifts.

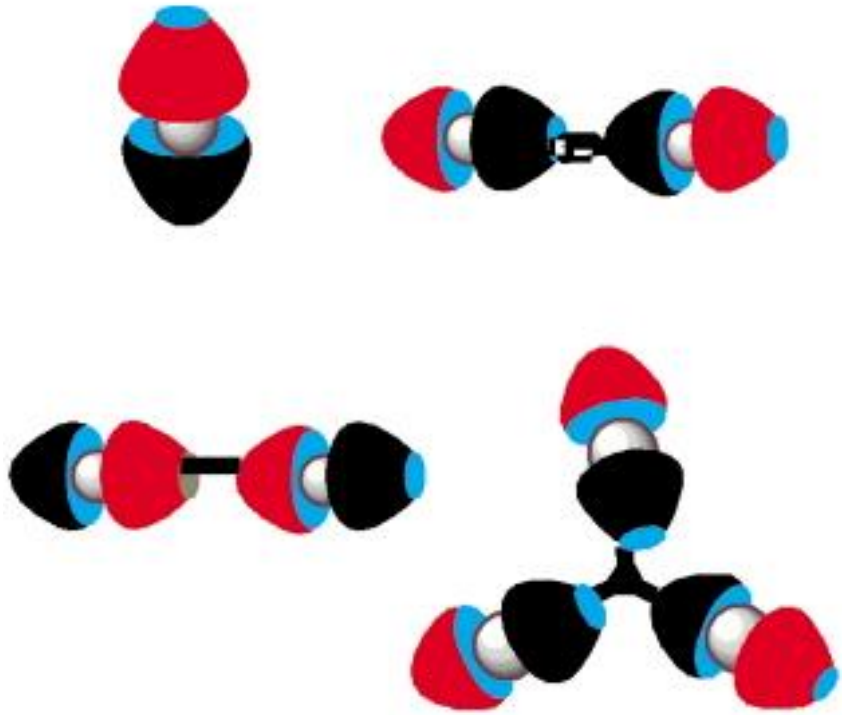
The observation

A number of other functional groups were attached to the upper rims of the calixarenes then screened for capsule formation.
One of these led to a discovery that had far-reaching consequences for our program. Ron Castellano and Professor Byeang Hyean Kim made a sulfonyl urea derivative, similar to one earlier reported by Reinhoudt.

It was characterized as a capsule, but in the presence of a aryl urea capsule, disproportionation took place in an exclusive manner: only the heterodimeric system was observed by NMR!
Probably the superior acidity of the sulfonyl urea finds its counterpart in the basicity of the aryl urea, but there must be other intermolecular forces involved since simple alkyl ureas do not show the same tendency to heterodimerize.

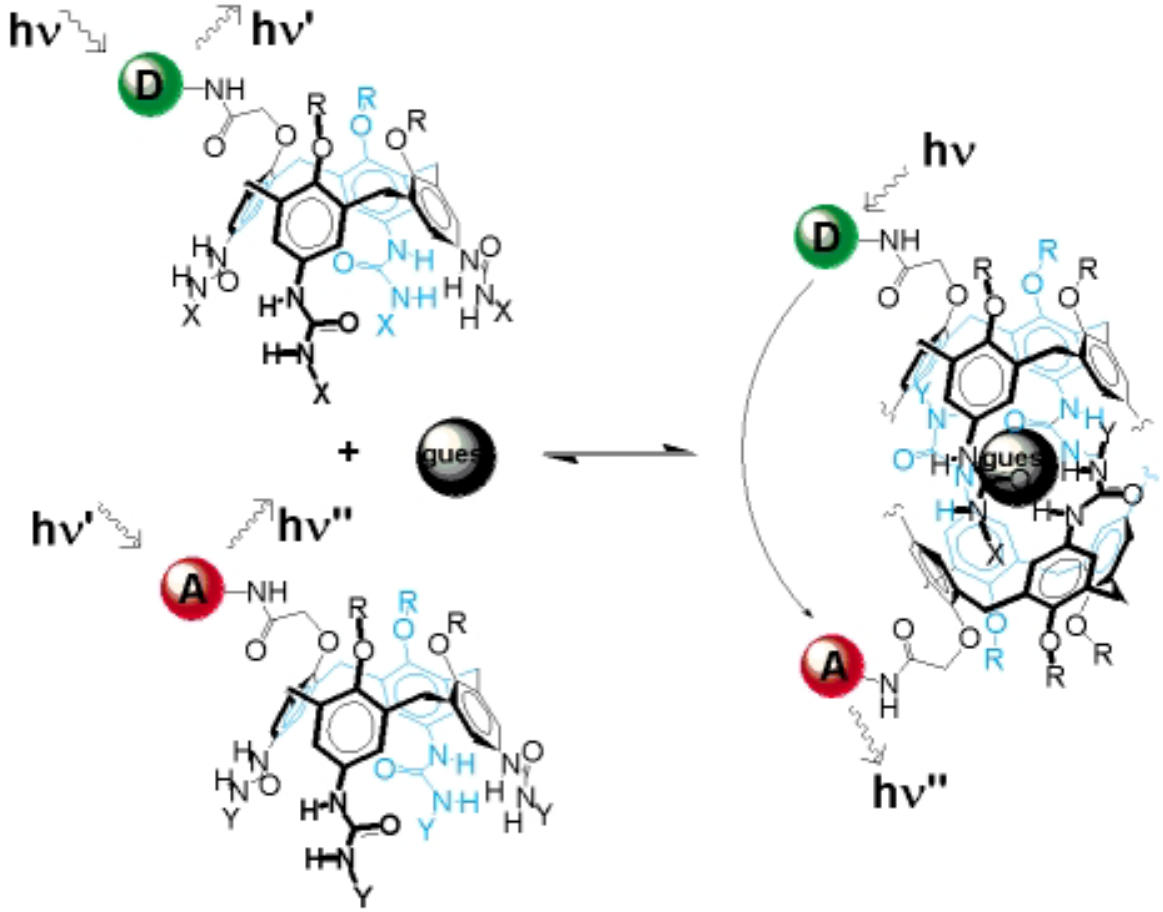
Polycaps: sulfonyl and aryl ureas calixarenes form only heterodimers





Whatever the cause, this phenomenon helped us characterize a number of systems of increasing complexity (Fig. 3). For example, using a 1,3,5-trisubstituted aromatic spacer, we were able to observe an assembly in solution that was capped by three sulfonyl ureas.21 This is one of the most complicated assemblies we have prepared to date; it consists of seven molecules – the centerpiece, three caps and three guests. It maintains its structure in solution and in the gas phase when quinuclidinium is the guest.

Monitoring the self-assembly by fluorescence resonance energy transfer, or FRET



1D X = SO_2-*p*-C_6H_4-Me
2A Y = *p*-C_6H_4-Me
R = $C_{10}H_{21}$

hν = 340 nm
hν' = 430 nm
hν" = 470 nm

Two different dyes are placed on each of the lower rims of two different calixarenes and only when they are held in a heterodimeric capsule are the dyes close enough together to permit energy transfer.

Excitation of the donor (hν) results in two colors of emitted light: one fluorescence band at the donor emission wavelength (hνA), and a second at the acceptor emission wavelength (hνB) that signals the noncovalent union of three species—donor, acceptor and guest. By monitoring these wavelengths, assembly and dissociation processes can be observed in real time.

The heterodimeric capsules are chiral

The capsule exists as a pair of enantiomers but we expected very little in the way of enantioselective recognition from such a system.
After all, the chirality exists in the lining, in the seam of the hydrogen bonds, as a clockwise or counter-clockwise arrangement with respect to the tether.

Molecular replicators

When the recognition surfaces are arranged in a way that all sites find their complements in a dimer, then additional possibilities arise: these self-complementary structures can give rise to the simplest molecular replicators.

Experiments in the Ghadiri lab have recently shown that trimeric peptide helix bundles are also capable of replication, and there is no reason to exclude tetramers or higher order aggregates, even if no specific cases exist at this writing

Linking through the lower rim two calixarenes

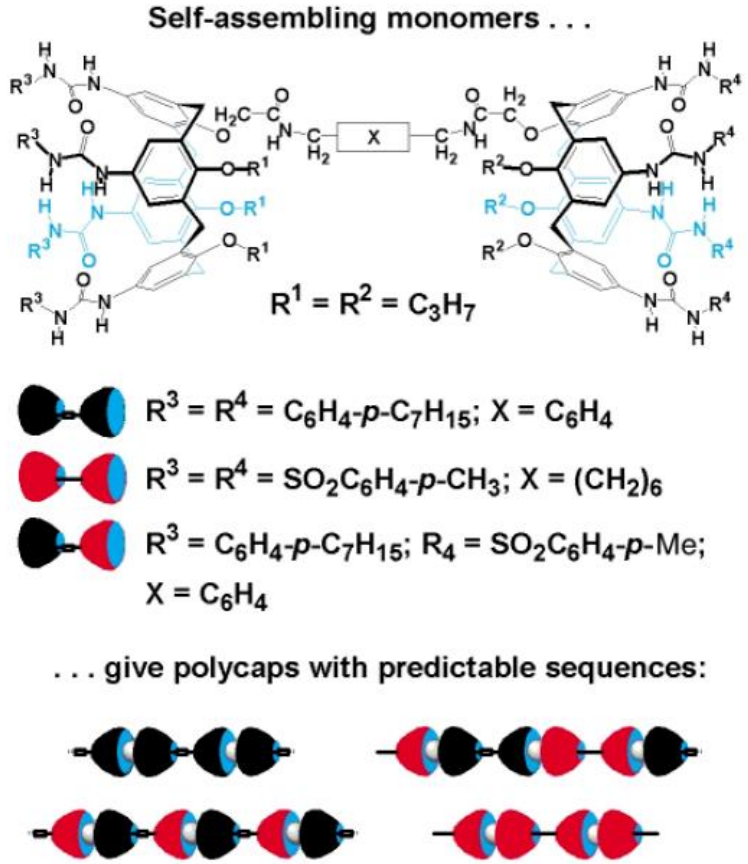
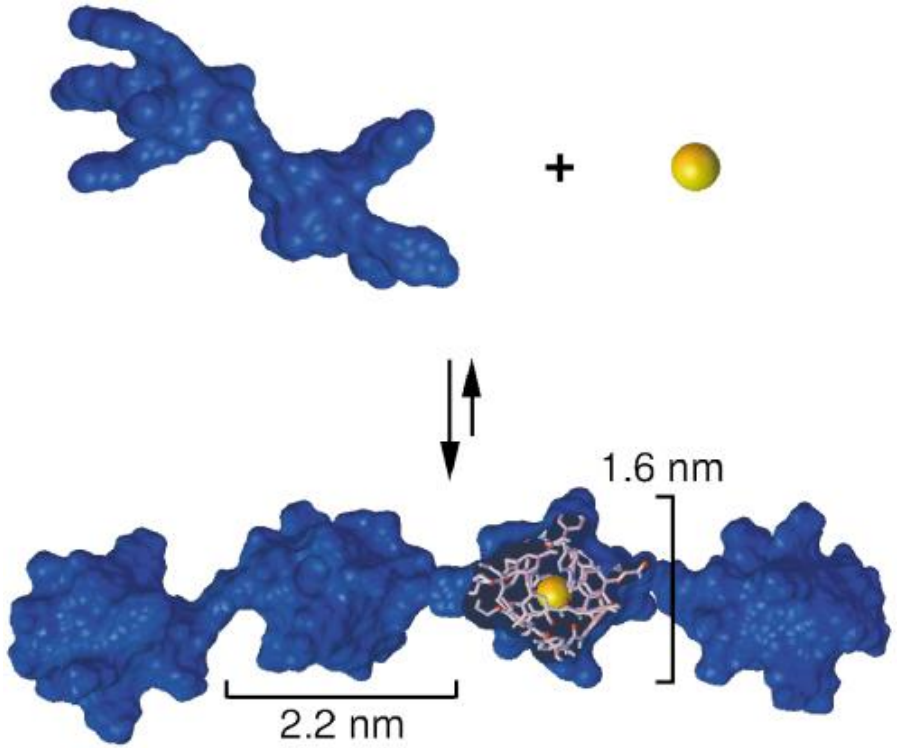


Fig. 5 Heterodimerization preferences lead to predictable polymer sequences from either complementary or self-complementary subunits.

Polycaps polymers

Consider, now, the assembly of such a unit in a linear polymeric array. Capsules appear like beads on a string, and each site is at a characteristic distance from the end of the polymer. At first glance, one might think there is symmetry about the center of the supermolecule, but the two halves of each capsule are different, each cavity is chiral, that is, the head-to-tail arrangement of ureas is either clockwise or counterclockwise at each capsule.



Cucurbiturils

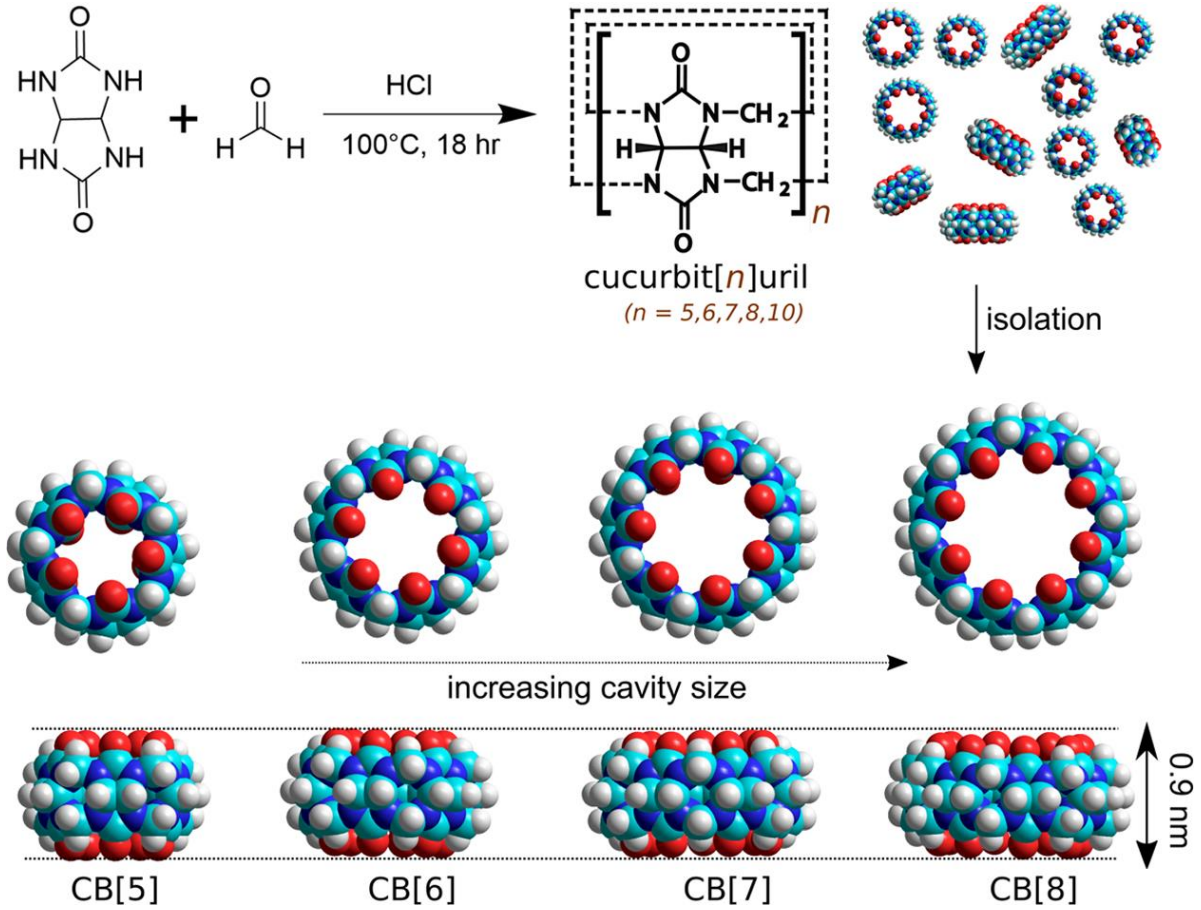
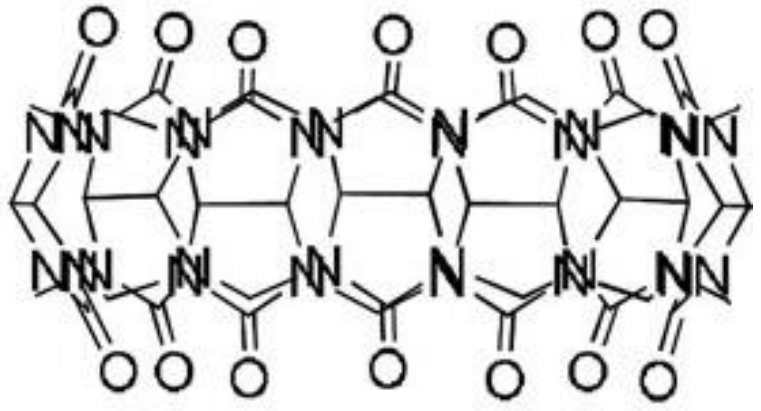


Table 1. Structural Parameters for Uncomplexed CB[5]–CB[8]

	CB[5][a]	CB[6][a]	CB[7][a]	CB[8][a]	CB[10][b]
portal diameter (Å)	2.4	3.9	5.4	6.9	9.5–10.6
cavity diameter (Å)	4.4	5.8	7.3	8.8	11.3–12.4
cavity volume (Å^3)	82	164	279	479	870
outer diameter (Å)	13.1	14.4	16.0	17.5	20.0
height (Å)	9.1	9.1	9.1	9.1	9.1

[a]Adapted with permission from ref 4. Copyright 2000 American Chemical Society. [b]Reproduced with permission from ref 39. Copyright 2009 The Royal Society of Chemistry.

reaction between glycoluril and formaldehyde was first reported in a thesis by Eberhard Meyer in 19041 and then published by Behrend and co-workers
in 1905;2 however, it was not until 1981 that a product was successfully crystallized from the reaction: a macrocycle consisting of 6 glycoluril units bound together by 12 methylene bridges



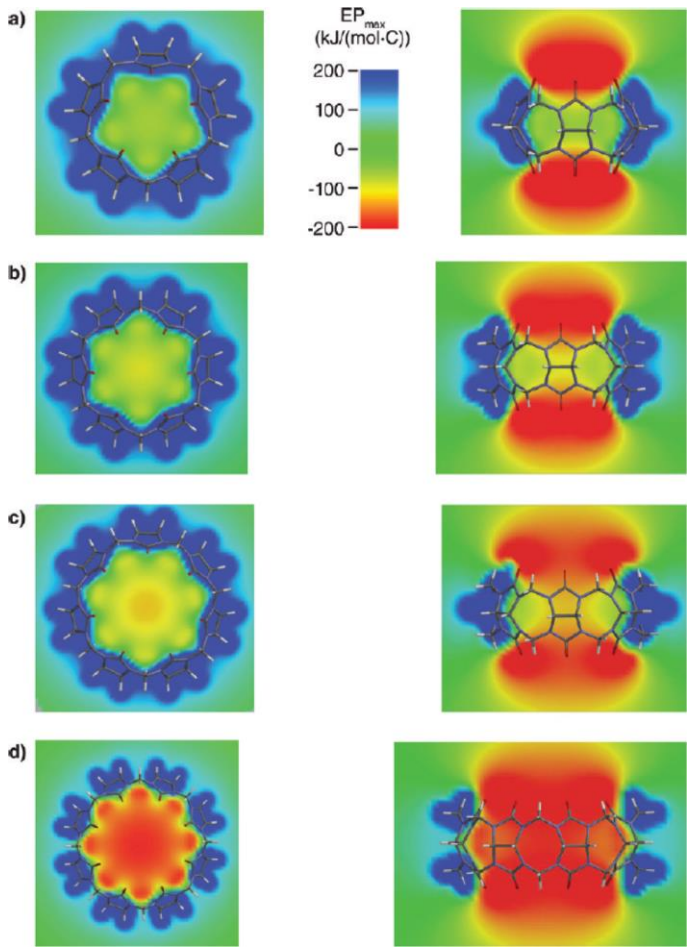
The dipolar nature of the carbonyl-fringed portals of CB[n]s make the portals highly attractive for cation binding through the ion−dipole effect

During the 1980s and 1990s, interest in crystal engineering and noncovalent interactions saw the popularity of cucurbituril increase due to its ability to facilitate noncovalent binding.

This is made possible via the formation of complexes with cations through interactions with the carbonyl-lined portals of CB[6], along with its ability to internalize alkyl chains within its hydrophobic cavity.

The new additions to the cucurbituril family were named cucurbit[5]uril, cucurbit[7]uril, cucurbit[8]uril, and cucurbit[10]uril in light of them containing 5, 7, 8, or 10 glycoluril units, respectively.

The cucurbit[n]urils (CB[n], n = 5-8, 10, 13-15) are a family of host molecules (Figure 1) which form stable host/guest complexes with organic cations in aqueous solution.



Cucurbit[n]urils consist of n glycoluril molecules that are bound in a ring-like arrangement via methylene bridges. This arrangement of the glycoluril subunits is key to understanding the binding and encapsulation properties of CBs, as the number of repeat units defines the portal size and cavity volume and the alignment of the glycoluril units results in a hydrophobic cavity with carbonyl-lined portals.

In the figure the electrostatic potential for CB[5]− CB[8], which highlights the strength of the negative charge associated with the portals of CB.

Calculated electrostatic potential (EP) for (a) CB[5], (b) CB[6], (c) CB[7], and (d) CB[8].

While the portals of CB[n]s are highly electronegative, the cavities of the macrocycle are encompassed by the fused rings of the glycoluril subunits, which leave no functional groups or electron pairs accessible to the inner cavity.

Thus, the inner cavity of CB[n]s are remarkably hydrophobic and show a preference toward the encapsulation of hydrophobic compounds.

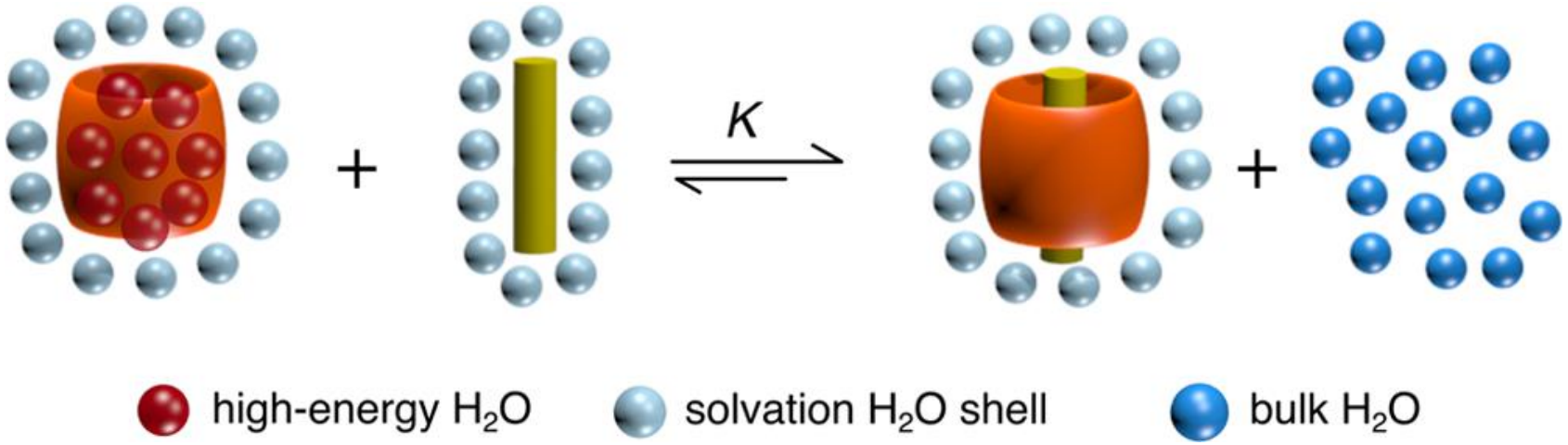
Hence, alkylated ammonium and imidazolium ions bind to CB[n]s such that the cation remains at the portal region, while the alkyl chains become encapsulated within the CB cavity given its inherent hydrophobicity.

It should not be surprising then to discover that compounds with high binding constants toward CB[n]s tend to be dicationic species where the cations are separated by an organic/hydrophobic region. Examples of such strong binders for CB[6], CB[7], and CB[8] include cadaverine, methylviologen, and aminoadamantane, respectively, with the size of the hydrophobic region being tailored for the specific volume of each CB cavity.

High-energy water molecules in CB cavity

Typically, aqueous environments cause a drop in host– guest affinity, compared to organic solvents, as water can compete strongly for hydrogen bonds and efficiently solvate charged species.

CB[n]s overcome this issue via the presence of high-energy water, which is present in the CB[n] cavity and supplies a driving force for guest complexation.



A good predictor of CB[n] complex binding comes from the assessment of whether the inner cavity of the host and the shape of the guest display good complementarity.

The cavity volumes of CB[5]−CB[8] are shown in Table 1 and range from 82 $Å^3$ for CB[5] to 479 $Å^3$ for CB[8].24 As a result of its small size, CB[5] is mostly suitable for the encapsulation of gases (discussed in section 7), whereas CB[6] can bind alkyl chains, CB[7] can accommodate small aromatic compounds, and CB[8] can simultaneously complex with two molecules,

Table 1. Structural Parameters for Uncomplexed CB[5]–CB[8]

	CB[5][a]	CB[6][a]	CB[7][a]	CB[8][a]	CB[10][b]
portal diameter (Å)	2.4	3.9	5.4	6.9	9.5–10.6
cavity diameter (Å)	4.4	5.8	7.3	8.8	11.3–12.4
cavity volume ($Å^3$)	82	164	279	479	870
outer diameter (Å)	13.1	14.4	16.0	17.5	20.0
height (Å)	9.1	9.1	9.1	9.1	9.1

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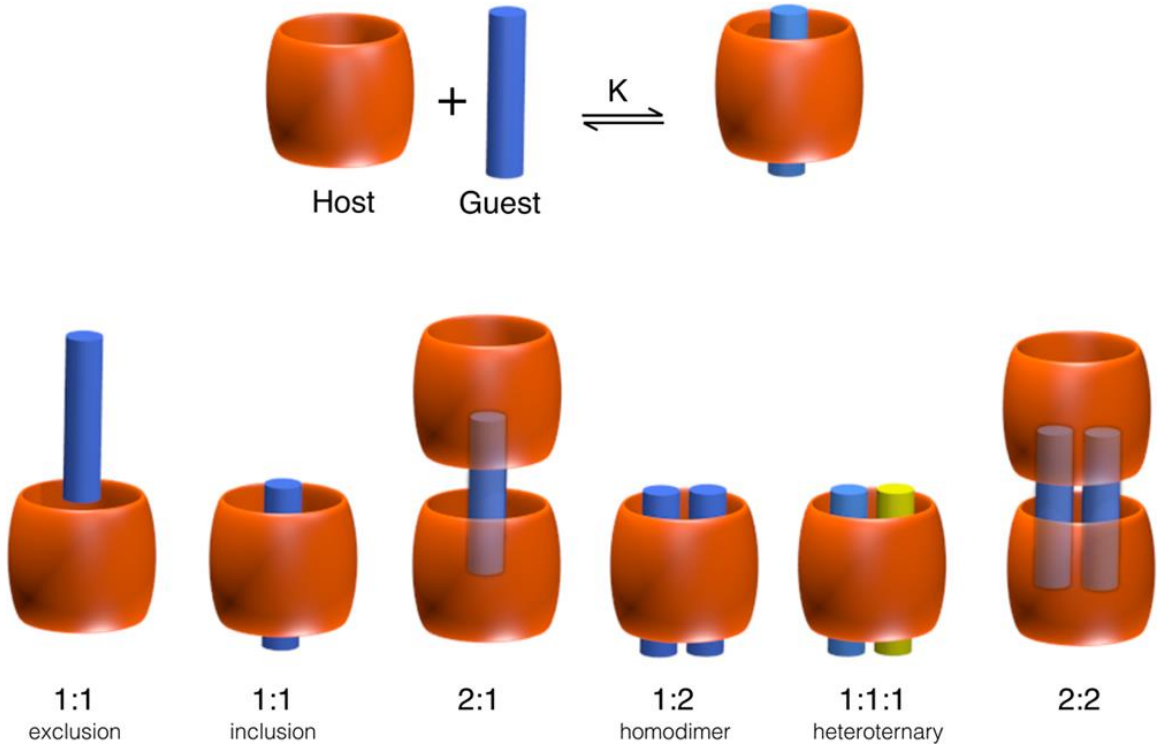
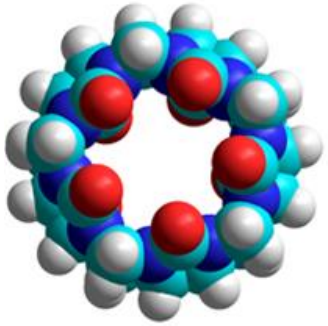
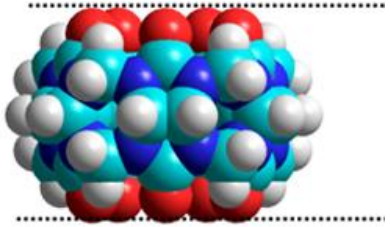
Cucurbituril complexes



CB[5]





CB[5]

CB[5] and decamethyl– CB[5] have also been shown to form portal complexes with alkali, alkaline earth, and ammonium cations.

Even though CB[5] is not big enough to incorporate large molecules into its cavity, its small size gives rise to its niche: the encapsulation of gaseous molecules. Decamethyl– CB[5] has been demonstrated to encapsulate small gaseous species in both aqueous environments and in solid form.

Gases such as N_2 , O_2 , Ar, N_2O, CO, and CO_2 have been shown to be encapsulated in both powdered and aqueous decamethyl–CB[5], while He, Ne, H_2 , Kr, Xe, and CH_4 could only be encapsulated in an aqueous environment, with Kr, Xe, and CH_4 requiring heating of the sample to assist encapsulation

It has been shown that protons can bind to the portals of CB[n]s, and indeed, this is the mechanism by which the more insoluble CBs can be dissolved through the use of aqueous acidic media.

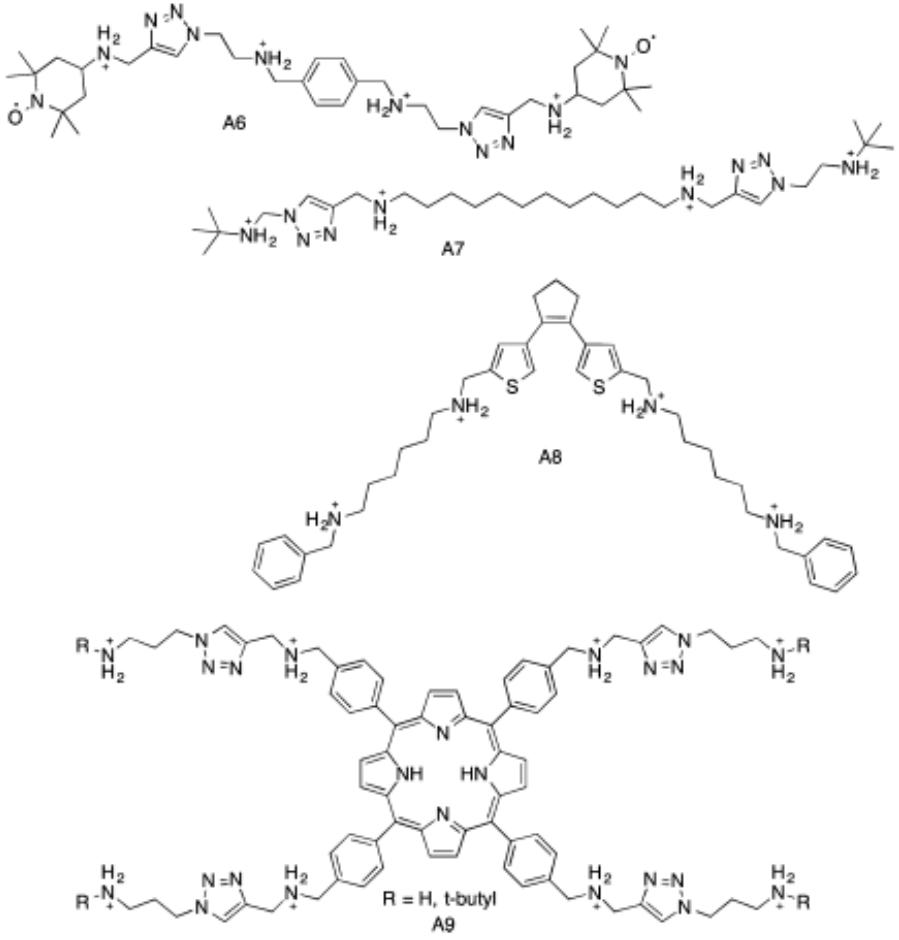
CB[6]

CB[6] is the most abundant homologue, and with a **portal diameter of 3.9 A** it is commonly known to form stable complexes with aliphatic amines.

The insolubility of CB[6] in organic solvents and sparing solubility in water has, however, had a limiting impact on its applications in its unmodified form.

Mock and co-workers first studied the complexation of a variety of alkyl- and aryl-substituted alkylammonium and alkyldiammonium ions in aqueous formic acid.

The results highlighted the size and shape complementarity exhibited by CB[6] contributing to its selective binding properties. It is well established that the stability of these complexes is a result of strong interactions between the positively charged protonated amines and the six electronegative carbonyl moieties at the portals.



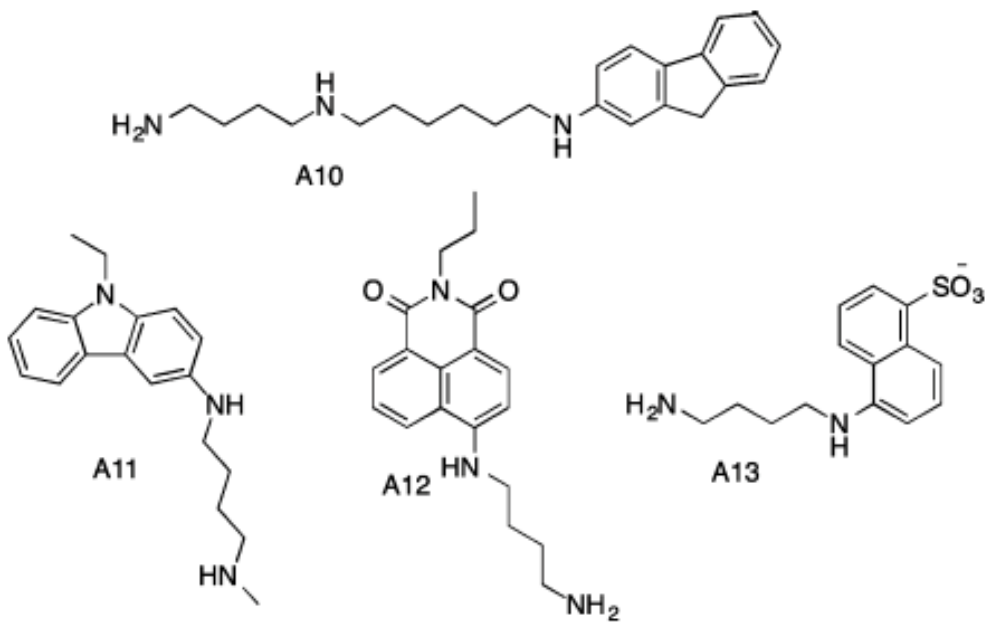
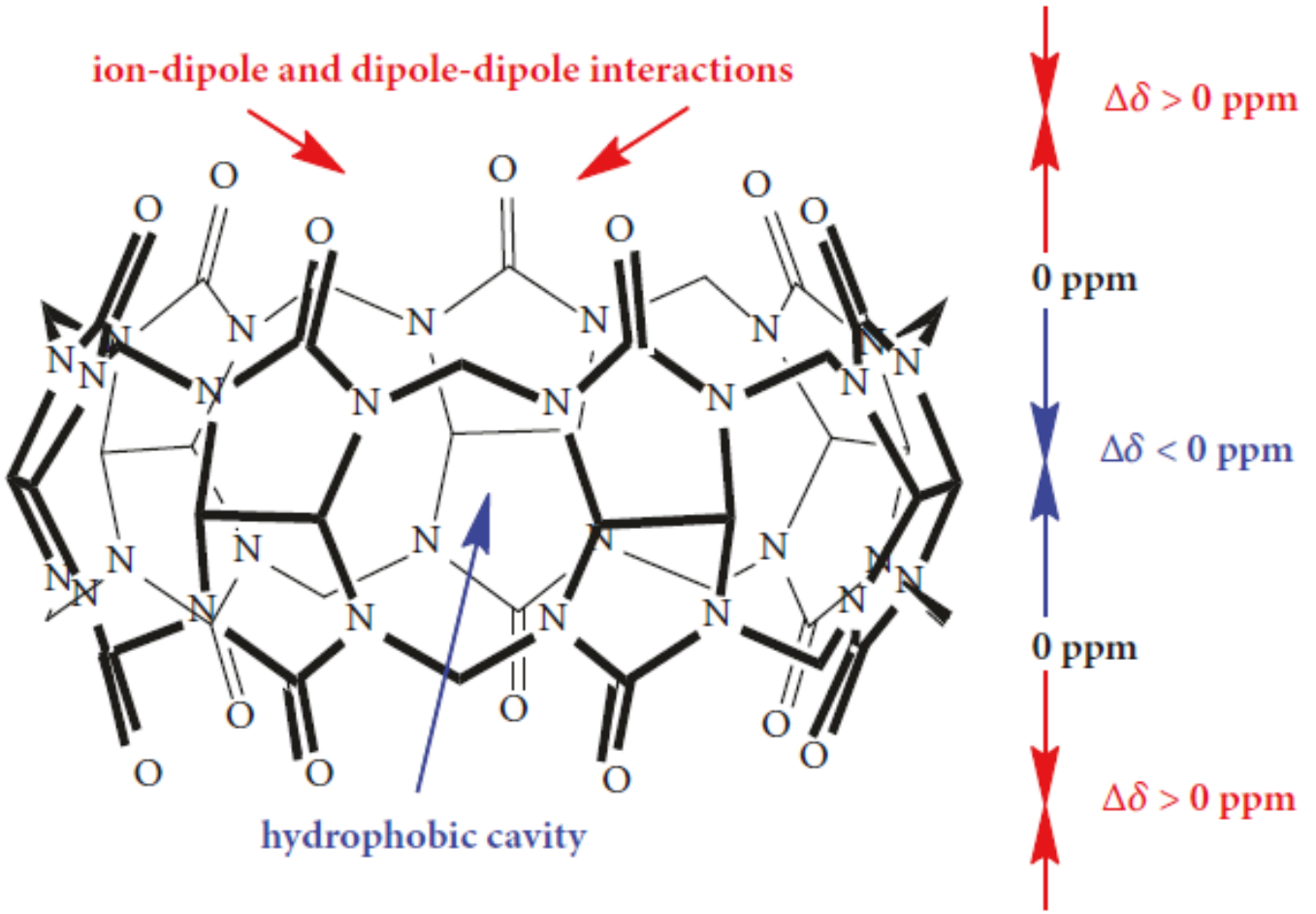


Figure 7. Alkyldiammonium–dye conjugates used for fluorescence sensor designs with CB[6].[67–70]

These selective recognition properties of CB[6] were first utilized by Kim and co-workers in order to construct a fluorescent molecular switch based on a pseudorotaxane structure.

NMR cucurbituryl/guest titrations



Design of a Fluorescent Dye for Indicator Displacement from Cucurbiturils: A Macrocycle-Responsive Fluorescent Switch Operating through a pK_a Shift

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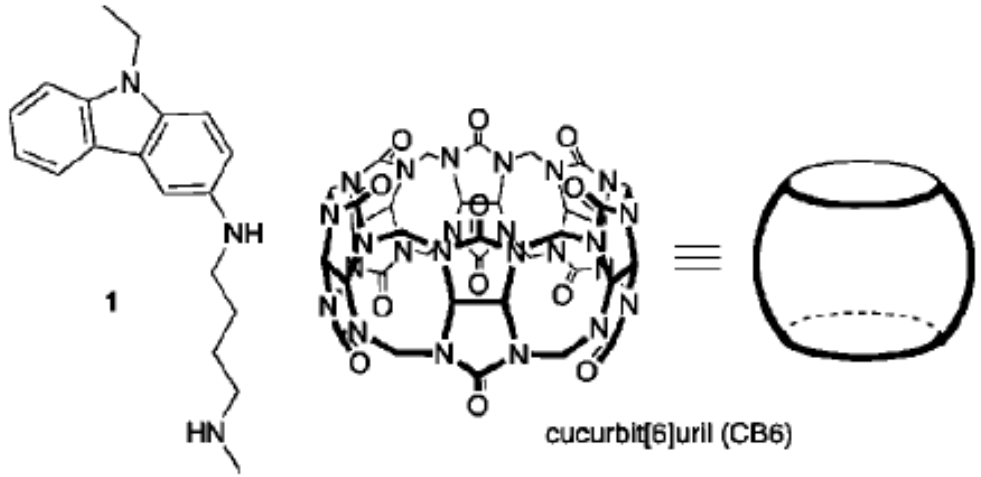


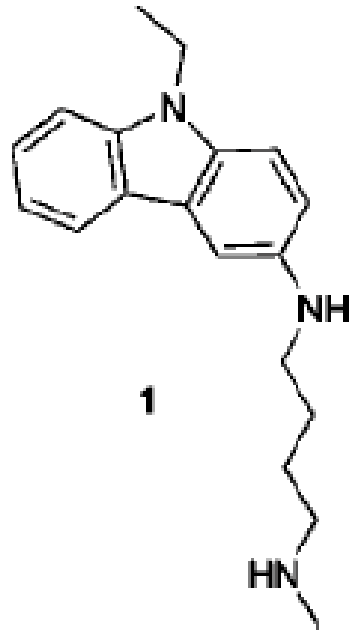
We aimed at developing an improved system with a reliable relay mechanism through which the fluorescence of the dye undergoes a large and predictable change upon complexation of the anchor.

In particular, complexation-induced pKa shifts, which have recently been conceptualized, appeared to be attractive.

Design of the host-guest system

Accordingly, a host- dye pair can be selected such that the dye remains unprotonated in its uncomplexed form but becomes protonated, accompanied by pronounced photophysical effects, upon complexation (host-assisted guest protonation).

Such supramolecular pKa shifts range from 1- 5 units. Thus, when working in a pH region between the pKa values of the uncomplexed and complexed dye, large fluorescence changes are expected upon addition of host or upon addition of a competitive binder to the preformed host- guest complex.



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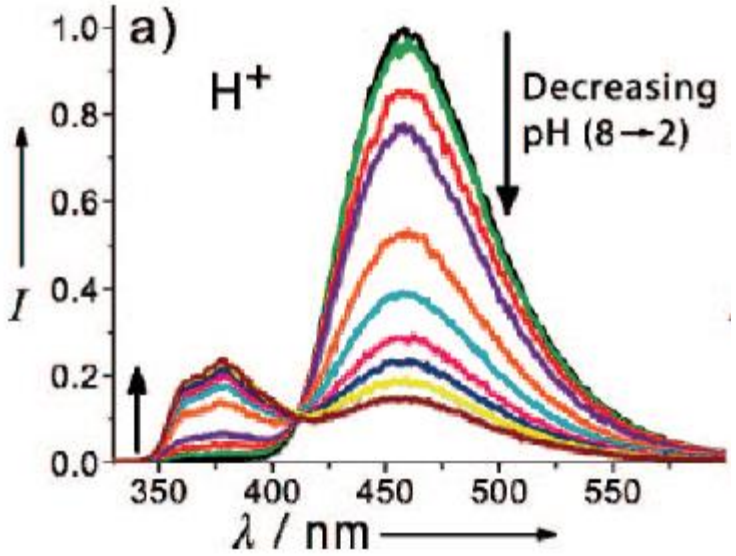
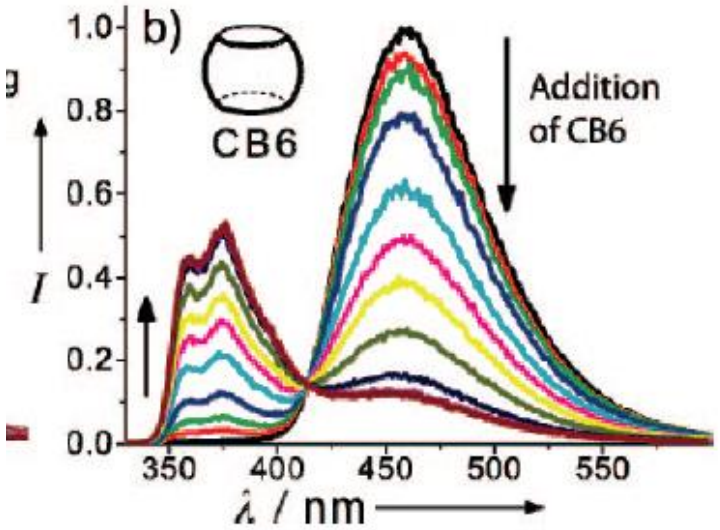
the actual chromophore does not have to be included in the host cavity, this strategy allows for a large variablity in the choice of the fluorescent dye with respect to size, charge, and binding affinity.

To document our idea, we present here a carbazole dye derivatized with a diamino-anchor (1) with a high affinity to cucurbiturils

Expectedly, the fluorescence of 1 is pH-sensitive. Above neutral pH (e.g. 7), a strong fluorescence band with a maximum near 458 nm is observed which is attributed to the charge transfer type electronic transition of the unprotonated anilino nitrogen form. At acidic pH (e.g., 3), the weaker locally excited band (λmax 375 nm) predominates





We anticipated that the complexation by a cation-receptor macrocycle would promote the protonation of the same nitrogen.

Indeed, upon successive addition of CB6 (up to 50 μ M), the long-wavelength fluorescence band showed a systematic decrease (Figure 1b). In contrast, the short wavelength band increased by a factor of 100 to reach a plateau value at high cucurbituril concentration.

The acidic microenvironment of CB

The variations in the fluorescence spectra are fully consistent with the idea that the complexation of the macrocycle causes a concomitant protonation of the anilino nitrogen of 1 ; that is, the fluorescence of the protonated form increases at the expense of the fluorescence characteristics of the unprotonated form.

Strikingly, the net effect of addition of CB6 resembles the effect of addition of acid because complexation exposes the dye to an apparently more acidic microenvironment.

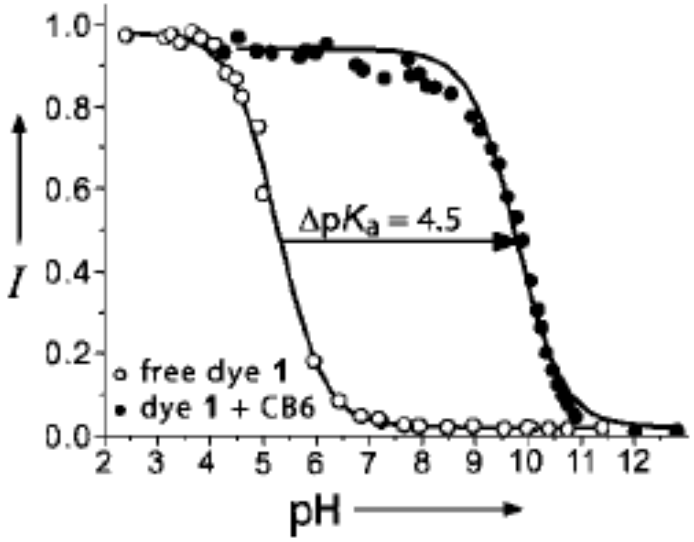


Figure 2. pK_a shift of **1** upon complexation by cucurbit[6]uril monitored through the fluorescence change upon pH variation for the free dye **1** (open circles) and the **1**·CB6 complex (solid circles) (λ_{exc} = 311 nm, λ_{em} = 375 nm).

A typical displacement titration is depicted in Figure 3 where the addition of 1,5-diamino pentane (cadaverine), a well-known high-affinity guest toward several cucurbiturils, fully reverts the fluorescence changes originally caused by the addition of the macrocycle. This fluorescence response, namely, a decrease of the short-wavelength band with a parallel increase of the long-wavelength fluorescence band, is fully consistent with the notion that the added diamine, present in its diammonium form at pH 7, displaces efficiently the protonated dye from the complex, such that the original fluorescence properties of the unprotonated dye are restored

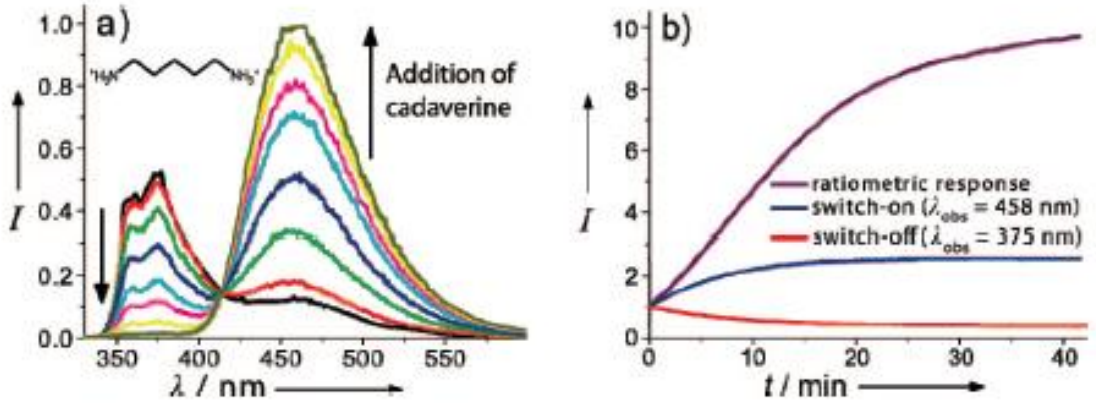


Figure 3. (a) Fluorescence titration for the competitive displacement of **1** (47 μM) from CB6 (50 μM) by cadaverine (up to 50 μM) in 10 mM NH_4OAc buffer at pH 7. (b) Supramolecular tandem assay

In conclusion, we have introduced a general supramolecular concept for designing high-affinity dyes which show a large and predictable fluorescence on- off as well as off- on switching upon complexation with cation-receptor macrocycles.

Not only can the affinity of the dye be tuned through the structure of a suitable anchor but also the charge, size, and hydrophobicity of the actual chromophore can be varied over a large range because it remains positioned outside the cavity.

The fluorescence response is solely ensured through a host-assisted protonation of an aryl amino group, which mimics a pH jump by 4 units or more.

CB-based fluorescent sensors

Supramolecular Chemistry

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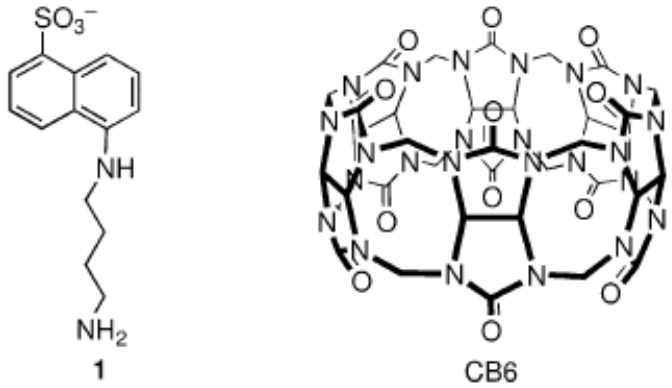
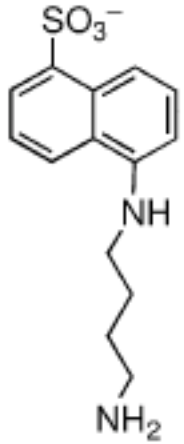
Strong Binding of Hydrocarbons to Cucurbituril Probed by Fluorescent Dye Displacement: A Supramolecular Gas-Sensing Ensemble**

*Mara Florea and Werner M. Nau**



The inclusion of small hydrocarbons into molecular container compounds in solution has received considerable attention.[1–9] It allows for a puristic understanding of the solvophobic driving force for the formation of discrete host–guest complexes[10] and has additional potential for gas storage, uptake, and separation, thus complementing solid-state applications of porous materials[11, 12] or surface-immobilized macrocycles



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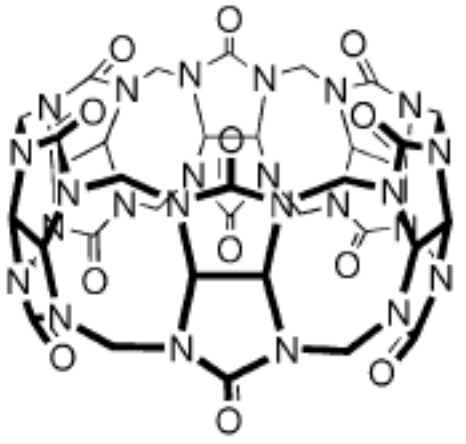


CB6

In the quest for a convenient method to monitor volatile hydrocarbon binding to CB6, we selected an indicator displacement strategy based on our recently developed anchor dye approach.

In detail, compound 1 possesses a putrescine anchor for strong binding with CB6, and a microenvironmentally highly sensitive 1-naphtylamine-5-sulfonic acid chromophore to ensure a robust fluorescence response upon binding.

Complexation of 1 by CB6 increases its locally excited fluorescence band (λexc=283 nm, λobs= 334 nm) by a factor of 50–1000, depending on pH.

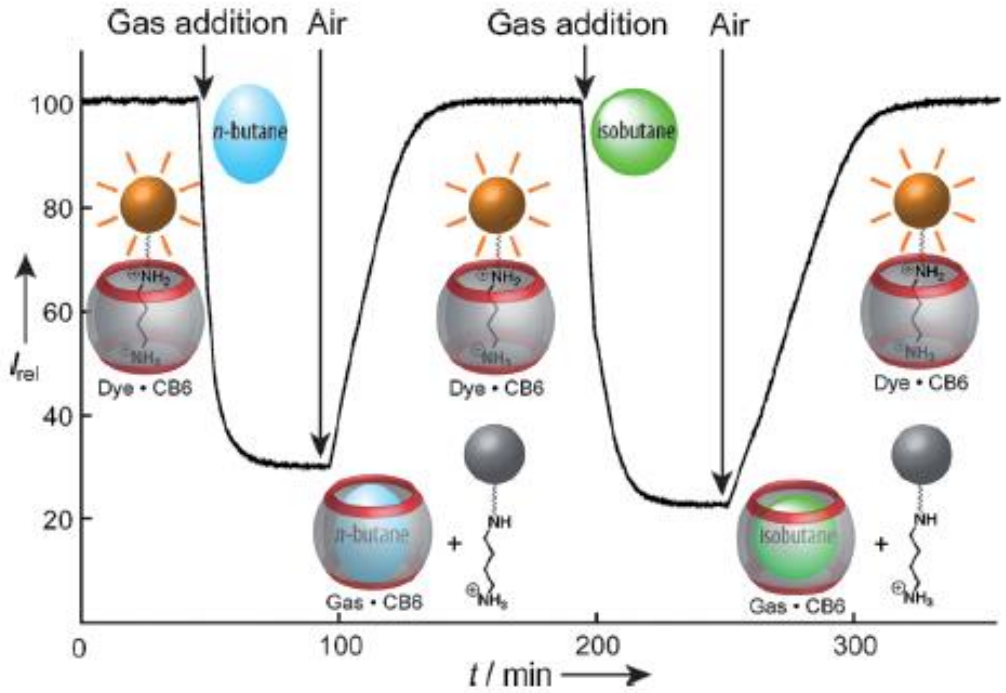
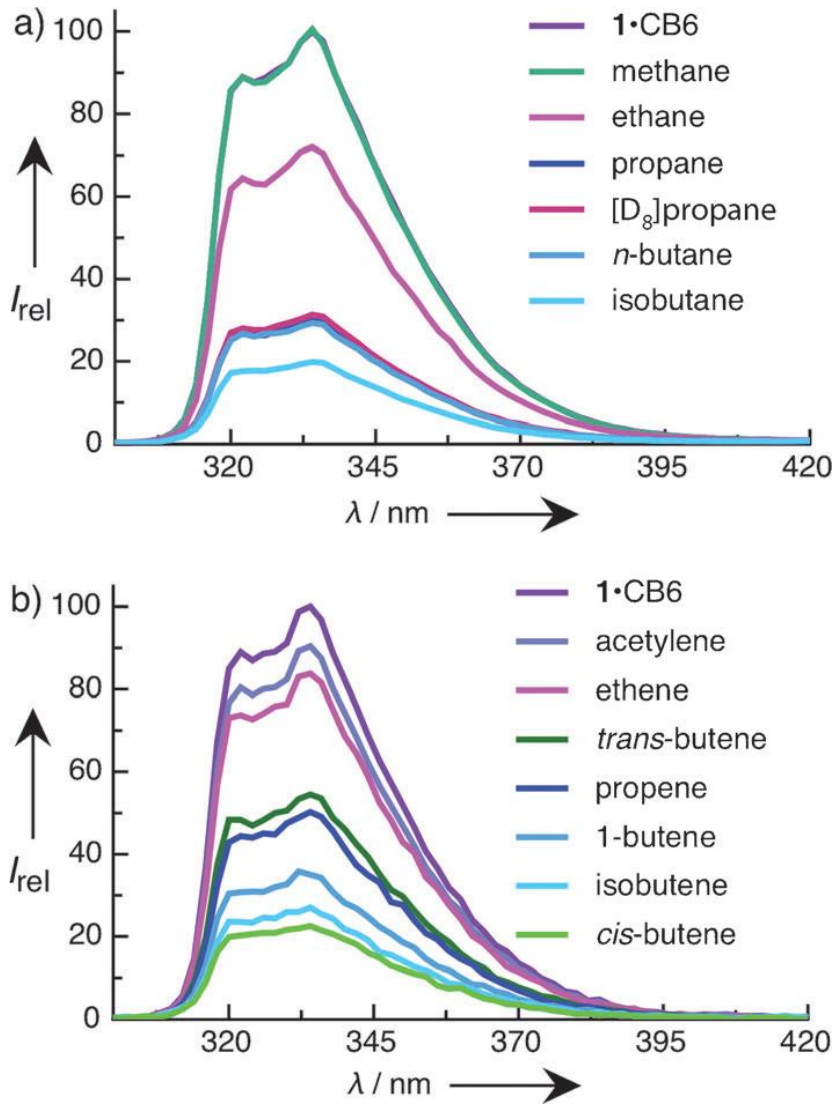


Figure 1. Fluorescence-based approach for gas sensing in aqueous solution. The trace refers to actual experiments with sequential uptake and release of *n*-butane and isobutane.

Starting from the pre-assembled highly fluorescent host–dye complex (4 mm CB6 and 1), the addition of gas results (with an immediate onset signaling fast exchange on the time scale of the experiment) in a continuous displacement of the dye until the saturation limit has been achieved.



Displacement of dye 1 (4 mm) from CB6 (4 mm) monitored by fluorescence after saturation of aqueous solutions with different gaseous a) alkanes and b) alkenes and acetylene.

Measurements in water at pH 3.0, λexc=283 nm. Note that hydrocarbons showing similar displacements may exhibit different binding constants because of varying solubilities (see the Supporting Information).

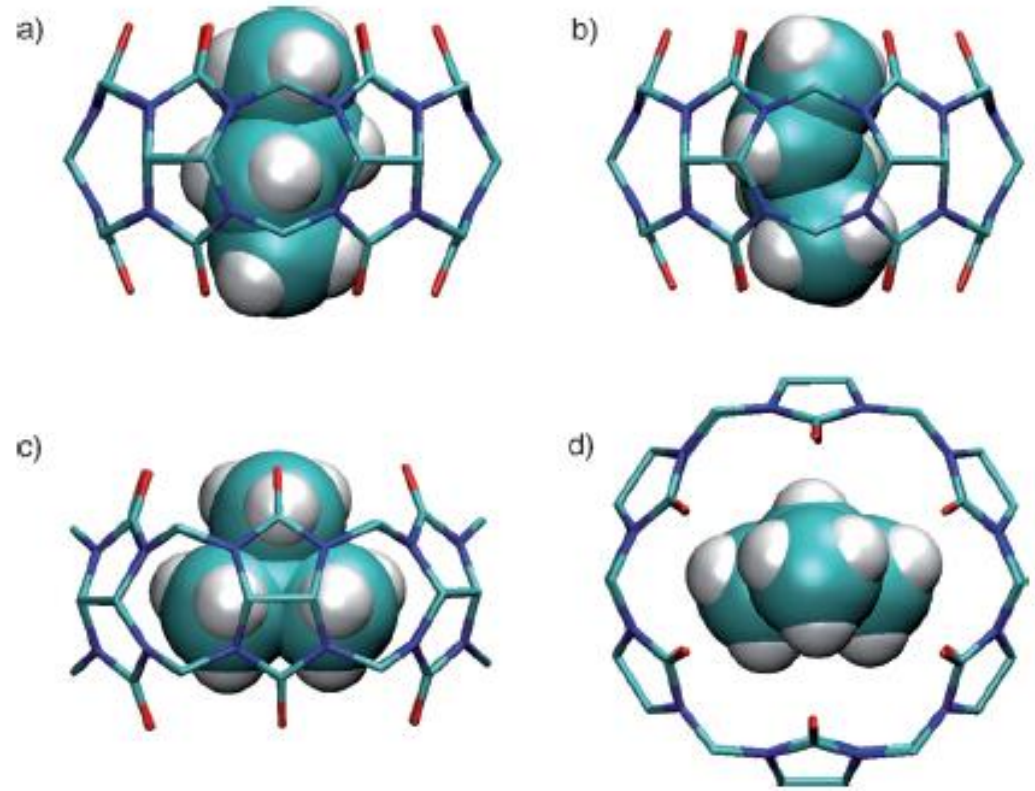


Figure 3. Optimized geometries of the CB6 complexes with a) *n*-butane, b) 1-butene, c) isobutane (side view), and d) isobutane (top view) at the B3LYP/6-31G** level of theory (gas phase).

Recently, the fluorescence emission of pyrene was reported to get enhanced in the presence of CB[6] as a result of external supramolecular interactions between pyrene and CB[6].

CB[6] has also been used for the detection of 3,4 methylenedioxymethamphetamine, the active constituent in ecstasy samples, using cyclic voltammetry.

Catecholamines such as adrenaline and isoprenaline show distinct binding behavior between CB[6].

The high binding affinities of CB[6] toward aliphatic amines compared to aromatic groups have led to various rotaxane and pseudorotaxane constructs, where the alkylamines act as an axle and the CB[6] the wheel such as with 1,8-diaminooctane