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Conformationally controllable amphiphiles as tunable supramolecular hosts and catalysts

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Conformationally controllable amphiphiles as tunable supramolecular hosts and catalysts

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LIST OF ABBREVIATIONS

BOP	Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate
*m*CPBA	3-Chloroperbenzoic acid
MPE	*m*-phenylene ethylene
CMC	Critical micelle concentration
DCC	Dicyclohexylcarbodiimide
DDQ	2,2-Dichloro-5,6-dicyano-*p*-benzoquinone
DIPEA	Diisopropylethylamine
DMAP	*N*,*N*'-Dimethylaminopyridine
DMF	*N*,*N'*-Dimetylformamide
DMPU	1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone, or *N*,*N*'-Dimethylpropyleneurea
DMSO	Dimethylsulfoxide
EI	Electron ionization
EDCI	*N*-Ethyl-*N*'-(3-dimethylaminoprophyl)carbodiimide
ESI-MS	Electrospray ionization mass spectrometry
FT-IR	Fourier transform-infrared
HBTU	*O*-Benzotrioazol-1-yl-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate
H2CFTPP	Octacholate-functionalized tetraphenylporphyrin
H_2TPP	Tetraphenylporphyrin
MALDI-TOF-MS	matrix assisted laser desorption time-of-flight mass spectrometry
m/z	mass/charge ratio
NBS	*N*-Bromosuccinimide
NMR	Nuclear magnetic resonance
TEA	Triethylamine
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TsCl	Tosylchloride
UV	Ultra-violet

ABSTRACT

The objective of this project is to use solvophobic interactions to guide conformational changes of molecular containers constructed with cholic acid as a building block. This dissertation describes 1) conformationally controllable amphiphilic molecules with solvent-responsiveness and/or photo-responsiveness, and their applications as supramolecular hosts and catalysts, 2) inclusion compounds of β-aminocholic acid with high guest/host (=4/1) ratio, and 3) an efficient synthetic method to make multivalent water-soluble calixarenes with click chemistry.

Amphiphilic "molecular baskets" with multiple facially amphiphilic cholates could aggregate intramolecularly to form a micelle-like conformer in polar solvents and a reversed-micelle-like one in nonpolar solvents. The stability of the reversed micelle-like conformers were influenced by the preorganization of the scaffold, the difference in solvophobicity between the α and the β faces of the cholate, and the spacers between the cholates and the scaffold. Microphase-separation of solvents was found to occur within the baskets. The environmentally responsive baskets could act as a novel supramolecular host to bind hydrophilic guests in nonpolar solvent mixtures and hydrophobic guests in polar solvents. Solvent-responsiveness could be easily coupled with photo-responsiveness by the introduction of azobenzene-spacers. A porphyrin could be used as the scaffold as well. Binding studies of the basket with the Zn-porphyrin scaffold indicated that the conformational change could be utilized to tune the substrate-selectivity of the metalloporphyrin. The basket with a Fe-porphyrin scaffold could act as a solvent-tunable supramolecular catalyst.

Bile acids such as cholic acid are well known to include a wide variety of organic compounds in their crystal lattices. 3β-Amino cholic acid was found to include with a high guest/host (=4/1) ratio into the solid state, because of the charge-assisted hydrogen bonds between the amino and the carboxyl group.

Despite broad interest in water-soluble calixarenes as multivalent ligands, their synthesis represents a challenge because many reactions to introduce water-soluble groups have poor functional-group tolerance. Via the high-yielding [3+2] cycloaddition between an azide and an alkyne (i.e., a click reaction), water-soluble calixarenes carrying cationic, anionic, and nonionic groups were synthesized. Cationic and anionic calixarenes were fully soluble in water and their aggregation was investigated by ^{1}H NMR spectroscopy.

CHAPTER 1. General introduction

Dissertation organization

This dissertation is divided into eight chapters. The current chapter is a review of environmentally responsive molecules, including naturally occurring antimicrobial peptides and synthetic analogues, such as foldamers. Chapter 2 was published in *Organic Letters* in 2004.[1] Four facially amphiphilic cholate units were assembled on a cone-shaped calix[4]arene scaffold. The resulting "molecular baskets" could turn either the hydrophilic faces or the hydrophobic faces of the cholates outward, depending on the solvent polarity. The two conformers resemble unimolecular micelles and reversed micelles, respectively. Chapter 3 was taken from a paper published in *The Journal of Organic Chemistry* in 2006.[2,3] Several amphiphilic baskets were synthesized and their formation of the reversed micelle-like conformer was studied with ^{1}H NMR spectroscopy. In a solvent mixture consisting of mostly a nonpolar solvent and a small amount of polar solvent, the microphase-separation of solvents occurred within the baskets, causing the polar solvent to be concentrated from the bulk to the interior of the baskets. Chapter 4 was published in *The Journal of Organic Chemistry* in 2005.[4] Molecular baskets have been demonstrated to act as tunable supramolecular baskets, binding hydrophilic guests in nonpolar solvent mixtures and hydrophobic guests in polar solvents. Chapter 5 was accepted by *The Journal of Organic Chemistry* in 2006.[5] A cholate-derived conformational change have been combined with the *trans-cis* isomerization of azobenzene to create a molecular basket sensitive to both solvent polarity and UV irradiation. Chapter 6 was taken from a paper accepted by

Organometallics in 2006.[6] Eight cholate units were attached to a tetraphenylporphyrin. With the solvent-dependent intramolecular aggregation of cholates, local microenvironments were generated above and below the surface of the metal center. Because these microenvironments could be formed and destroyed by solvent changes, the resulting metal porphyrin could change its selectivity toward hydrophilic or hydrophobic substrates, depending on the solvent polarity. Chapter 7 was published in *Tetraheron* in 2006.[7] 3β-Aminocholic acid was found to include a large number of methanol molecules in the solid state. The guest/host (= 4/1) ratio was higher than all previously prepared inclusion compounds of bile acids. Dr. Allen helped solve the single crystal structure by X-ray single crystal crystallography. Chapter 8 was published in *Organic Letters* in 2005.[8] Despite broad interest in water-soluble calixarenes as multivalent ligands, their synthesis represents a challenge because introduction of water-soluble groups (e.g., via sulfonation) often has poor functional-group tolerance. Calixarenes carrying cationic, anionic, and nonionic groups were synthesized through a high-yielding [3+2] cycoladdition between an azide and an alkyne (i.e., a click reaction).

Environmentally responsive amphiphilic molecules

Environmentally responsive molecules are ubiquitous in nature. Many biomolecules, including proteins and DNA, can respond to environmental stimuli by changing their conformations. Antimicrobial peptides are one class of such environmentally responsive amphiphilic biomolecules.[9] These relatively short peptides (6-50 residues) are generated in living organisms. They can kill bacteria by destroying their membranes, but show low toxicity towards mammalian cells. They tend to have

both cationic and hydrophobic amino acid residues in their backbones. Their ability to adopt different conformations in aqueous solution and on membranes is critical to their biological activity. Several mechanisms have been proposed to explain their antimicrobial properties. In the "barrel-stave" model, the peptides first bind with the membrane in a parallel orientation, interacting with the anionic hydrophilic face of the bilayer. The peptides then change from a random conformation to ordered conformations, such as α-helices or β-sheets. Because their interactions with bacterial membranes do not involve any specific receptors, antimicrobial peptides are not subject to bacterial resistence, a feature that has attracted great interest by many researchers.

These environmentally dependent conformational changes have inspired chemists to develop synthetic analogues with similar properties. Foldamers are synthetic mimics of responsive biomolecules. They are linear oligomers that can adopt compact, ordered conformations.[10–12] The folded conformations can be stabilized by a variety of noncovalent forces, such as hydrogen bonds, metal-ligand complexation, π–π interactions, van der Waals forces, and/or solvophobic interactions. Many researchers choose to fold the foldamer chain by directional forces (e.g. hydrogen bonds). The β-peptides (**1**, **2**) reported by Gellman and co-workers are good examples.[13] Their folded conformations are stabilized by amide hydrogen bonds, in a way similar to the α-helices in natural peptides.

It is challenging to use nondirectional forces, such as solvophobic interactions to control the conformations of foldamers. Moore and colleagues reported *m*-phenylene ethylene (*m*PE) foldamers that successfully overcame this difficulty.[10] In a solvent (e.g. chloroform) that strongly solvates the aromatic backbone, the foldamer assumed

extended, random conformations. In a solvent (e.g. acetonitrile) that is a poor solvent for the *m*PE backbone, the foldamer collapsed into a helical conformation, with the aromatic units stacking over one another with the tri(ethylene glycol) units on the periphery of the aromatic core. In their design, a critical feature is the *meta*-substituted benzene. The semirigid monomer unit, by its fixed 120° angle, restricts the movement of the chain. It not only simplies the conformational control problem, but also preorganizes the chain to fold in the designed fashion. Rigid linkers, however, do not always need to be used to fold solvophobic foldamers. Iverson and co-workers reported foldamers (i.e. aedamers) with alternating electron-rich (donor) and electron-deficient (acceptor) aromatic units.[14] The donor-acceptor interactions are preferred over either the donor-donor or the acceptor-acceptor interactions. This "polarization" of the aromatic solvophobes was used to guide the folding of the aedamers.

It is difficult to use aliphatic solvophobes to construct foldamers. Unlike aromatic groups, aliphatic solvophobes (e.g. a hydrocarbon chain) are flexible. As a result, their aggregation generally does not have a preferred orientation as aromatic solvophobes do. Because of the flexibility, it is also difficult to arrange solvophobic and solvophilic groups in precise directions on an aliphatic solvophobes. Zhao and co-workers recently overcame these challenges and synthesized amphiphilic foldamers based on aliphatic cholates.[3,15] The foldamers adopted helical structures in nonpolar solvents with a small amount of polar solvent. A highly unusual feature of the folded helix was the "hollow" hydrophilic interior, about 1 nm in diameter. The interior can be used as a binding pocket useful for molecular recognition or supramolecular catalysis.

Despite the great interest in creating linear, oligomeric mimics (i.e. foldamers) of responsive biomolecules, some researchers have also designed nonfoldamers that showed responsiveness to external stimuli. Regan's molecular umbrella was constructed with two (or more) facially amphiphilic units and a "stem" that could be a drug molecule.[16] If the environment has polarity similar to that of the stem, the stem will be exposed. On the other hand, if the environment and the stem have opposite polarities, the facial amphiphiles will sandwich the stem and shield it from the environment. The molecules have been demonstrated to be effective at transporting hydrophilic agents across hydrophobic lipid bilayers. [16d,16e]

Chemists have just begun to design molecules that can respond to environmental stimuli in predictable manners. Conformational control is the underlying strategy in many of the reported responsive molecules. As chemists develop better ways to control the conformations of synthetic molecules, they will be able to not only develop a better understanding of how biomolecules fold and function, but also create "smart" materials with biomolecule-like properties.

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CHAPTER 2. Environmentally responsive molecular baskets: unimolecular mimics of both micelles and reversed micelles

A paper published in *Organic Letters* **2004**, *6*, 3187-3189.[1]

Abstracts

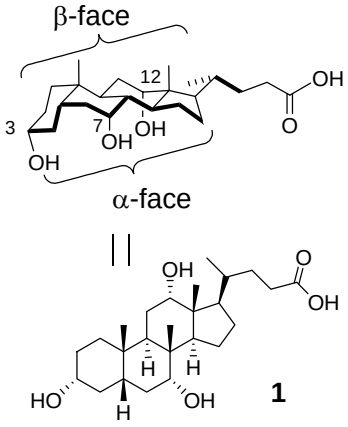
When four facially amphiphilic cholate derivatives were attached to a tetraaminocalixarene scaffold, the resulting molecule responded to environmental changes by rotation of the cholate units. In polar solvents, the molecule adopted a micelle-like conformation with the hydrophilic α faces of the cholates pointing outward. In nonpolar solvents, it turned inside out, assuming a reversed micelle-like conformation with the hydrophobic β-faces pointing outward. Switching between the two conformations was driven by solvophobic interactions and was fully reversible.

Introduction

Many peptides and proteins have distinct water-soluble and membrane-bound states.[2] Their ability to adopt radically different conformations in different environments is critical to their functions. Despite much attention to novel amphiphiles in recent years,[3] very few amphiphilic molecules have been reported to display well-defined conformational changes according to environmental stimuli.[4] We now describe amphiphiles that adopt conformations mimicking normal micelles in polar solvents and reversed micelles in nonpolar ones. Previously reported unimolecular micelles (and reversed micelles) are mostly dendrimers with a hydrophilic exterior and a hydrophobic core (and *vice versa* for reversed micelles).[7]

Interchange between the two states is usually prohibited by the fixed arrangement of hydrophilic and hydrophobic moieties.

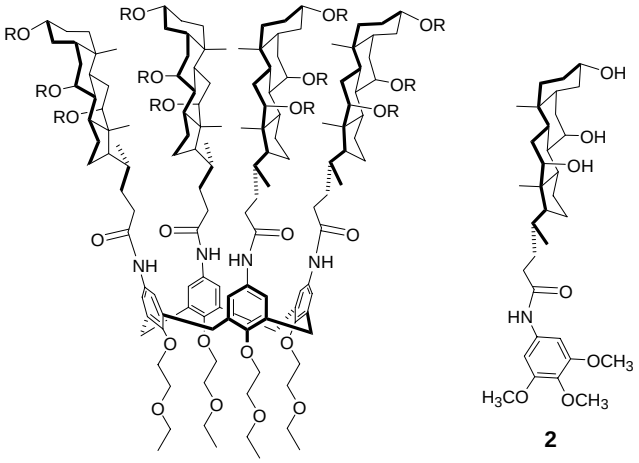
Cholic acid (**1**) has an unusual distribution of functional groups: the α face is hydrophilic with three hydroxyl groups and the β face is hydrophobic consisting of only hydrocarbons. Because of its unique structure and commercial availability, cholic acid is a popular building block in supramolecular chemistry.[8] In recent years, it has been used to construct environmentally responsive molecules.[9–11] Taking advantage of the facial amphiphilicity of cholates,[12] we prepared molecular baskets that undergo transitions between micelle-like and reversed-micelle-like conformations induced by solvent changes[13] and cholate foldamers with nanometer-sized hydrophilic cavities.[14]



Results and discussion

For the basic design, we use cholic acid derivatives as the "walls" and a cone-shaped tetraaminocalixarene as the scaffold.[15] The ethoxylethyl groups on the lower rim of calixarene are used for compatibility with both hydrophilic and hydrophobic solvents. Cholic acid is an example of so-called facial amphiphiles.[16] Its α-face is hydrophilic with three hydroxyl groups,

whereas the β-face is completely hydrophobic, being all hydrocarbon. Bearing four cholic acid units, molecule **1a** has a total of 12 hydroxyl groups on the hydrophilic faces, and **1b** has 24.



1a, R = H
1b, R = $CH_2CH(OH)CH_2OH$

2

We have studied the conformational behavior of **1a** and **1b** in a mixture of (deuterated) chloroform and methanol. We postulated that the nonpolar chloroform would favor the α-faces of the amphiphiles and the polar methanol would prefer the β-faces. Miscibility of the two solvents allows us to vary the solvent ratios continuously. In the ^{1}H NMR spectra of **1a** in different solvent mixtures (Figure 1), the most noticeable change occurs in the aromatic region. The aromatic protons are equivalent in 60% methanol. However, with either a higher or lower percentage of methanol, the two aromatic protons *ortho* to the amido groups split into two peaks. The two peaks have the same intensity and are coupled by a small coupling constant of 2.4 Hz, which is in the typical range of coupling constants for two *meta* protons on a phenyl ring.[17] Because secondary aromatic amides are known to adopt a *trans* conformation,[18] we assume that the splitting is a result of hindered rotation of the nitrogen-aryl bonds (*vide infra*). In contrast, the ^{1}H NMR spectrum of the control compound **2** is completely unchanged in different solvents (Figure 2).

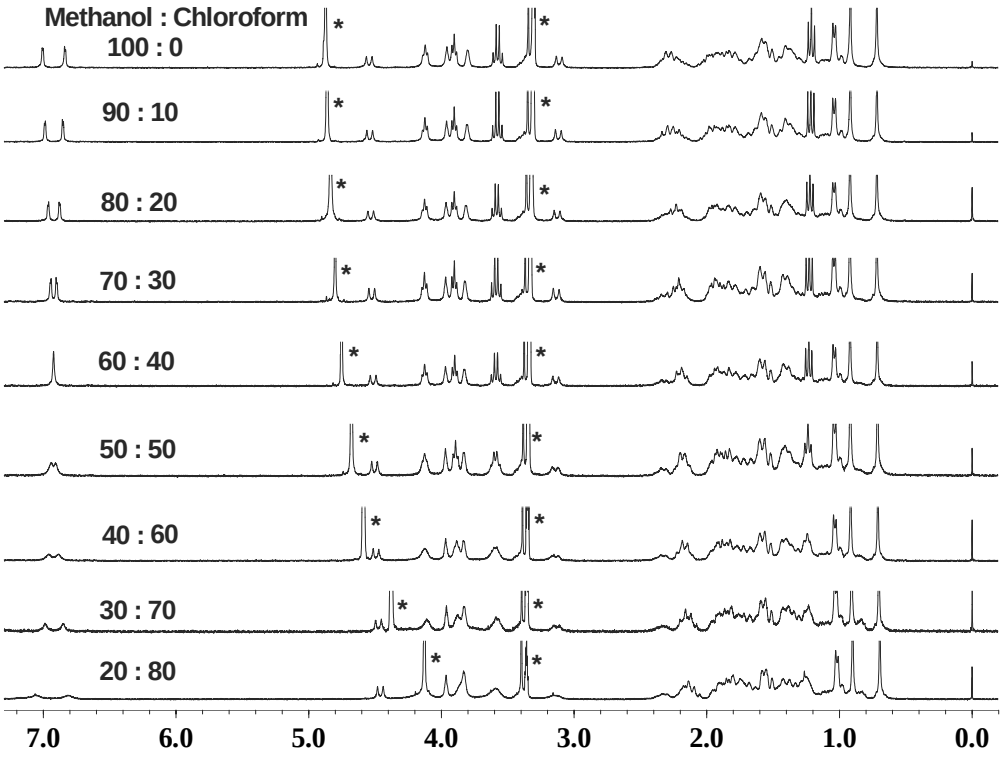


Figure 1. ^{1}H NMR spectra (300 MHz) of **1a** in different ratios of $CD_3OD/CDCl_3$ (v/v) at ambient temperature. The 20%/80% mixture also contains an additional 1% of D_2O. Solvent peaks (CD_3OH and CD_2HOD) are marked with * on the right. The signal at 0 ppm is from added tetramethylsilane (TMS).

The two aromatic peaks coalesce at higher temperatures. In 65% methanol, the coalescence temperature (t_c) is 50 °C. The rotation barrier is calculated[12] to be $\Delta G^{\ddagger}$ = 17.0 kcal/mol with a $\Delta\nu$ = 8.8 Hz. The barrier increases to $\Delta G^{\ddagger}$ = 17.7 kcal/mol (t_c = 70 °C, $\Delta\nu$ = 15.6 Hz) in 70% methanol, and further to $\Delta G^{\ddagger}$ > 17.9 kcal/mol (t_c > 80 °C, $\Delta\nu$ = 28.0 Hz) in 75% methanol.[20] Upon cooling, the singlet in 60% methanol splits into two peaks. The rotational barrier is $\Delta G^{\ddagger}$ = 13.8 kcal/mol (t_c = 0 °C, $\Delta\nu$ = 15.6 Hz at -40 °C). Clearly, the distance between the two aromatic peaks at ambient temperature is a measure of the rotational barrier around the nitrogen-aryl bonds (Figure 3).

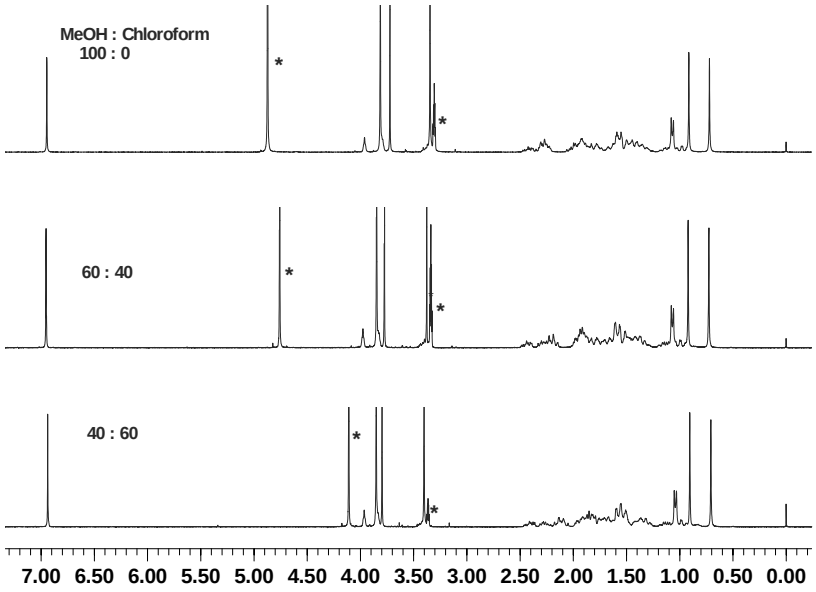
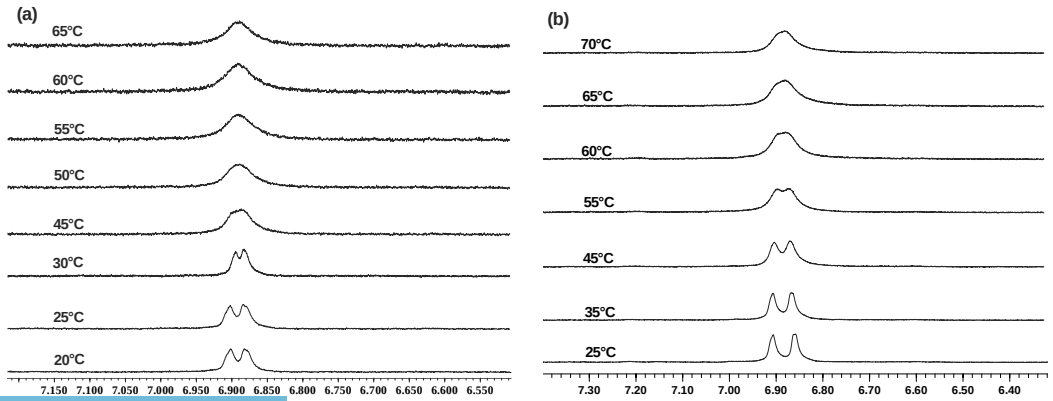


Figure 2. ^{1}H NMR spectra (300 MHz) of **2** in different ratios of $CD_3OD/CDCl_3$. Solvent peaks (CD_3OH and CD_2HOD) are marked with * on the right. The signal at 0 ppm is from added tetramethylsilane (TMS).

The splitting of the *ortho* aromatic protons has been found in other amido calixarenes and typically caused by hydrogen bonds that hindered rotation of the nitrogen-aryl bonds.[21] Hydrogen bonds, however, are unlikely to be responsible in the current system. This is because rotation is most hindered when the percentage of methanol (which is a competitive hydrogen bonding solvent) is either high or low, but least hindered in the intermediate range.



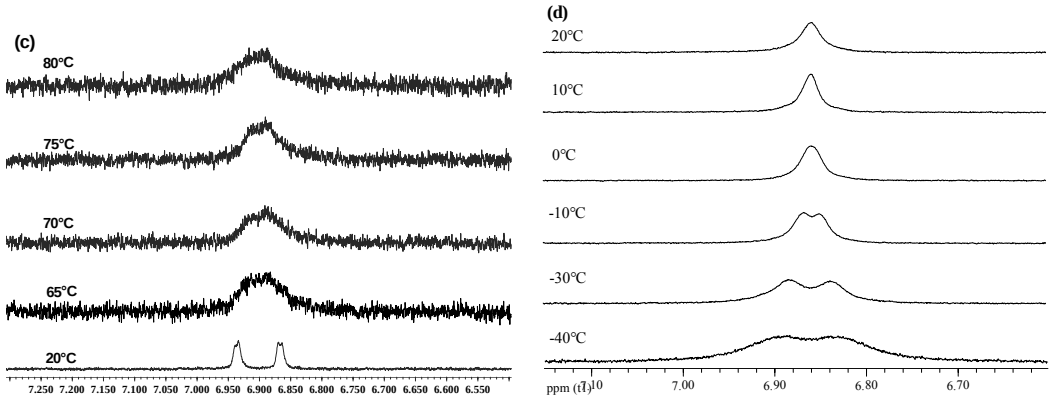


Figure 3. Variable temperature NMR spectra (400 MHz) of **1a** in different ratios of $CD_3OD/CDCl_3$: (a) 65% CD_3OD/45% $CDCl_3$; (b) 70% CD_3OD/30% $CDCl_3$; (c) 75% CD_3OD/25% $CDCl_3$ (d) 60% CD_3OD/40% $CDCl_3$.

We propose that **1a** adopts a normal micelle conformation in polar solvents (>60% methanol) and a reversed micelle conformation in nonpolar solvents (<60% methanol). It seems that there is no preference for either face of the cholic acid in 60% methanol and rotation of the nitrogen-aryl bonds is thus the least restricted. In a methanol-rich environment, the solvent prefers the α-faces, causing the hydrophobic β-faces to aggregate intramolecularly.[22] Solvophobic interactions probably constrain the cholate units and result in hindered rotation. As the ratio of methanol increases, the micelle-resembling conformer becomes more favorable compared to other conformers with exposed hydrophobic β-faces. Indeed, a progressively larger splitting is seen as methanol is increased from 60 to 100%. Note that our variable temperature NMR spectra data also suggest that a larger splitting at ambient temperature corresponds to a higher rotational barrier.

The exact opposite trend is observed when the percentage of methanol drops below 60%. This can be explained by solvophobic interactions in the context of reversed micelles. Molecule **1a** is not soluble in chloroform with less than 20% methanol. A small amount of

water (ca. 1%), however, can significantly increase the solubility.[23] Such behavior is typical for reversed micelles formed by regular surfactants, which require a small amount of water for stability.[24]

Changes in other areas of the spectra in general are relatively small. Toward the low-polarity end, signals from the calixarene protons, including those from the ethoxyethyl groups (i.e. a triplet at 1.2 ppm and a quartet at 3.6 ppm) become quite broad. The peak broadening is likely caused by intermolecular aggregation. However, if carbon tetrachloride, instead of chloroform, is used in the solvent mixtures, the signals become much sharper (Figure 4). This behavior is consistent with a reversed-micelle-like conformer, which should be more stable in carbon tetrachloride than the more polar chloroform. A more stable conformer has its solvophobic faces better shielded from the solvent and thus has a lower tendency for aggregation. Besides the sharpness of the signals, two other pieces of evidence support the hyprthesis that carbon tetrachloride is a better solvent than chloroform for the reversed-micelle conformer. First, at the low-polarity end, splitting between the aromatic protons is larger in methanol/carbon tetrachloride mixtures than in methanol/chloroform mixtures: 0.098 ppm in 40/60 mixture of CD_3OD/CCl_4 vs. 0.074 in $CD_3OD/CDCl_3$; 0.156 ppm in 30/70 mixture of CD_3OD/CCl_4 vs. 0.135 ppm in $CD_3OD/CDCl_3$. Second, molecule **1a** has greater solubility in methanol/carbon tetrachloride than in methanol/chloroform. Only 5% methanol is needed in the former mixture to solubilize **1a**, whereas > 20% methanol is required in the latter.

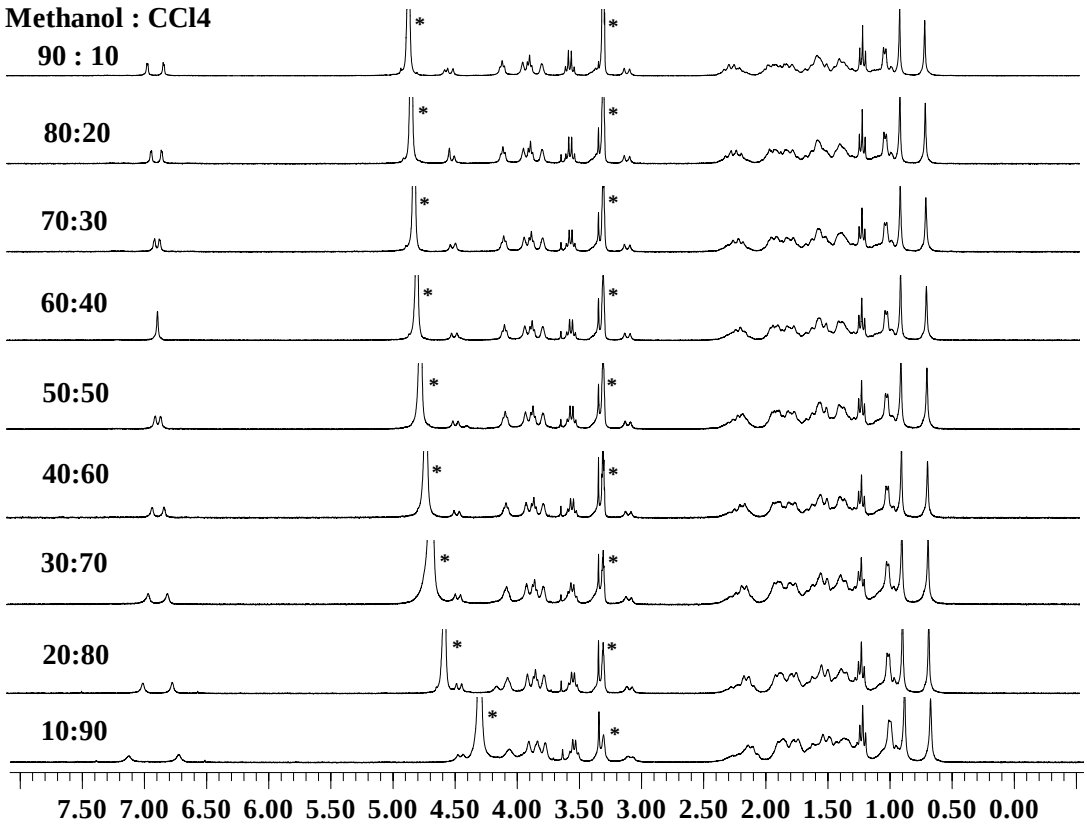


Figure 4. ^{1}H NMR spectra (300 MHz) of **1a** in different ratios of CD_3OD/CCl_4. Solvent peaks (CD_3OH and CD_2HOD) are marked with * on the right.

A similar splitting of aromatic protons is found for **1b** in methanol/chloroform mixtures (Figure 5). Importantly, **1b** shows consistently higher sensitivity toward solvent changes than **1a**. When the difference in the chemical shifts of the *ortho* aromatic protons is plotted as a function of solvent ratios (Figure 6a), **1b** gives a similar, but steeper, curve than **1a**. This is probably due to the larger difference between the solvophobicities of the α- and the β-faces in **1b** than in **1a**. Quite interestingly, the sensitivity enhancement is largest toward the ends of the polarity scales, but smallest in the middle, which is again in agreement with the solvophobic mechanism.

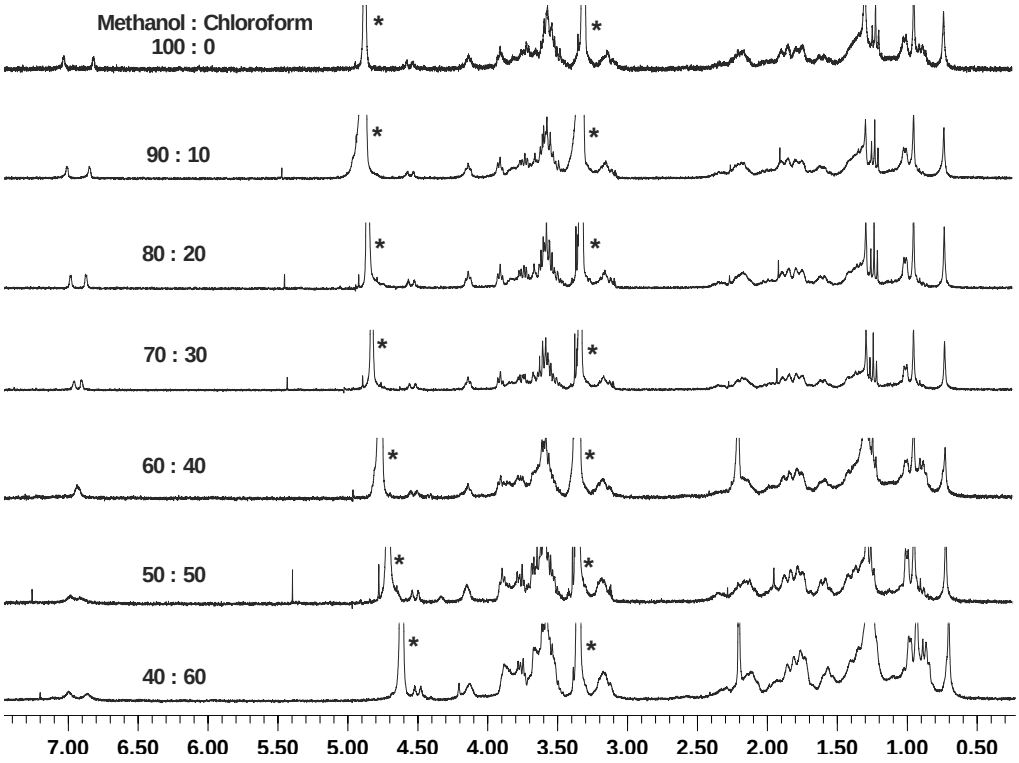


Figure 5. ^{1}H NMR spectra (300 MHz) of **1b** in different ratios of $CD_3OD/CDCl_3$. Solvent peaks (CD_3OH and CD_2HOD) are marked with * on the right.

Further evidence for the solvophobically driven conformational change comes from the effect of water in the solvent mixture. The amphiphiles are assumed to adopt normal micelle structures in methanol (*vide supra*). The addition of water increases the polarity of the environment and is anticipated to further stabilize the micelle conformation. In fact, the distance between the *ortho* aromatic protons continues to enlarge with a higher percentage of water (Figure 6b). Molecule **1a** reaches solubility limits after the addition of 20% water. With increased hydrophilicity, **1b** stays soluble in a nearly 1:1 mixture of CD_3OD and D_2O with a splitting of 0.56 ppm between the two aromatic protons.

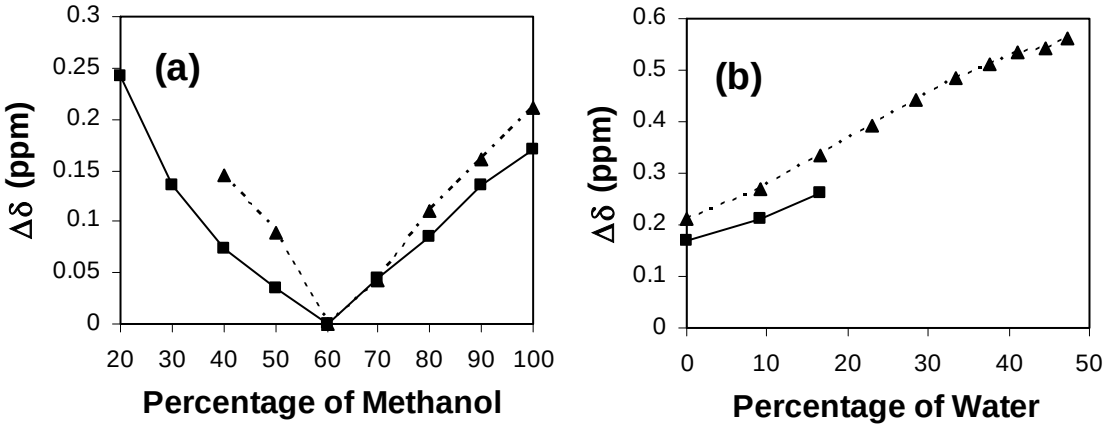


Figure 6. Chemical shift difference (Δδ) of the *ortho* aromatic protons as a function of solvent composition for **1a** (■) and **1b** (▲) (a) in a mixture of (deuterated) methanol and chloroform and (b) in a mixture of (deuterated) water and methanol.

Conclusions

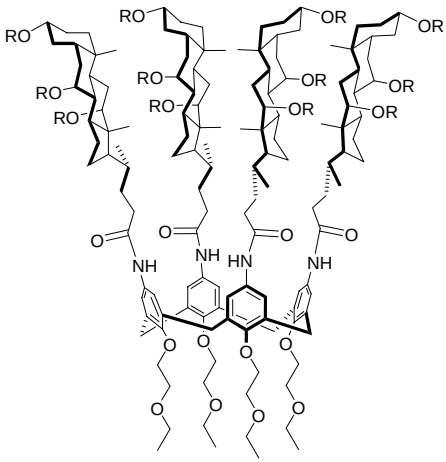
In summary, we have designed and synthesized amphiphiles that have basketlike structures. The amphiphiles respond to solvent changes to act like unimolecular micelles in polar environments and unimolecular reversed micelles in nonpolar environments. Switching between the two conformations is driven by solvophobic interactions and is fully reversible. Potential applications of these novel amphiphiles include colloid stabilization, catalysis, and solubilization and transport of agents through incompatible phases.

Experimental Section

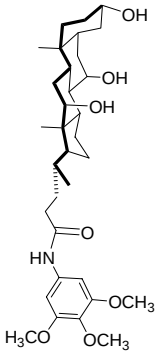
General methods

Anhydrous tetrahydrofuran (THF) and methylene chloride were dried by passage through a column of activated alumina under compressed nitrogen. Cholic acid was

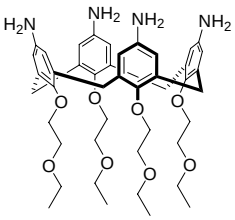
crystallized from 95% ethanol. All other reagents and solvents were of A. C. S. certified grade or higher, and were used as received from commercial suppliers. All glassware and syringes were dried in an oven at least overnight prior to use. Routine ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-300, VXR-400, or Bruker-400 spectrometer. Elemental analyses are obtained on a Perkin-Elmer model 2400 series 2 CHN/S elemental analyzer. MALDI-TOF masses are recorded on a Thermobioanalysis Dynamo mass spectrometer.



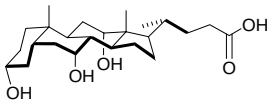
1a, R = H
1b, R = $CH_2CH(OH)CH_2OH$



2



3

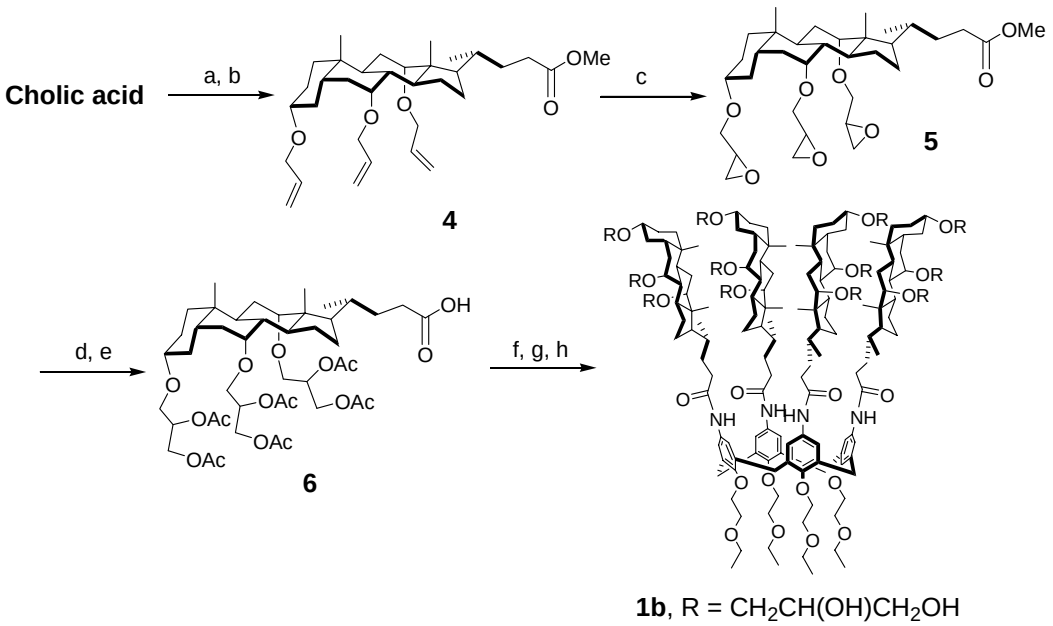


Cholic acid

Synthesis

Compound 3. The aminocalixarene **3** was synthesized according to a literature procedure.[26] ^{1}H NMR (300 MHz, $CDCl_3$,δ) 6.08 (s, 8H), 4.34 (d, J = 13.2 Hz, 4H), 3.98 (t, J = 6.0 Hz, 8H), 3.79 (t, J = 6.0 Hz, 8H), 3.79 (t, J = 6.0 Hz, 8H), 3.53 (q, J = 6.9 Hz, 8H), 2.91 (d, J = 13.2 Hz, 4H), 2.34 (b, 8H), 1.19 (t, J = 6.9 Hz, 12H).

Scheme 1. Preparation of compound **1b**



Reaction conditions: (a) Allyl iodide, Bu_4NI, NaH; (b) MeOH, H_2SO_4; (c) *m*CPBA; (d) H_2O, KOH; (e) Ac_2O, pyridine, DMAP; (f) oxalyl chloride, pyridine; (g) **3**, NEt_3; h) NaOMe, MeOH.

Compound 1a. A mixture of cholic acid (195 mg, 0.48 mmol), **3** (74 mg, 0.10 mmol), and *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDCI) hydrogen chloride salt (109 mg, 0.57 mmol) were stirred in anhydrous CH_2Cl_2 (5 mL) at room temperature under N_2 for 24 h. Solvent was evaporated *in vacuo*. The residue was dissolved in methanol (0.5 mL) and precipitated in water (5 mL). The product was purified by column chromatography over silica gel using MeOH/CH_2Cl_2/acetone(1/5/1) as the eluent to give a light yellow powder (155 mg, 70 % yield). ^{1}H NMR (MeOH-d4, 300 MHz, δ) 7.00 (d, J = 2.4 Hz, 4H), 6.84 (d, J = 2.4 Hz, 4H), 4.54 (d, J = 12.6 Hz, 4H), 4.12 (t, J = 5.7 Hz, 8H), 3.96 (s, 4H), 3.90 (t, J = 5.4 Hz, 8H), 3.80 (s, 4H), 2.40-0.90 (m, 148H), 0.72 (s, 12H); ^{13}C NMR (MeOH-d4, 75 MHz, δ) 173.38, 152.81, 134.98, 132.97, 120.86, 73.78, 73.69, 73.57, 72.90, 72.80, 71.75, 71.67, 69.83, 67.93, 66.32, 46.41, 44.38, 42.02, 41.83, 39.87, 39.30, 38.09, 36.25, 35.99, 35.60, 35.44, 35.36, 34.82, 33.97, 32.03, 31.11, 30.75, 30.07, 28.50, 27.94, 27.69, 27.61, 26.70, 23.23, 23.18, 22.30, 16.95, 16.61,

14.80, 12.23, 12.06. MALDI-TOFMS: calcd. for $C_{140}H_{212}N_4O_{24}Na$ $[M+Na]^+$: 2358.2; found: 2358.3.

Compound 4. Cholic acid (2.1 g, 5.16 mmol) was dissolved in anhydrous THF (25 mL). Under N_2, allyl iodide (3.8 mL, 41.60 mmol) was added by a syringe. The first batch of NaH (0.43 g, 11.20 mmol) was added under a N_2 flush and the mixture was stirred at 40 °C for 12 h. The second batch of NaH (0.44 g, 11.40 mmol) was added and the reaction was continued for 12 h. The third (0.44 g, 11.40 mmol) and the fourth (0.44g, 11.40 mmol) were added similarly. Reaction was quenched by slow addition of water (2 mL) and then acidified with 1M H_2SO_4 until pH = 3. It was extracted with ethyl ether (2 x 30 mL). The organic phase was washed with brine (2 x 20 mL), dried with $MgSO_4$, and concentrated *in vacuo*. The residue was combined with methanol (40 mL) and 5 drops of conc. H_2SO_4, and was heated to reflux for 24 h. Water (40 mL) was added and the mixture was extracted with hexane (50, 30, and 30 mL). The combined hexane solution was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by column chromatography using EtOAc/hexane (1/5) as the eluent to give colorless oil (1.96 g, 70% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, δ) 5.90 (m, 3H), 5.25 (m, 3H), 5.08 (m, 3H), 4.07 (m, 2H), 3.98 (d of t, 2H), 3.75 (m, 2H), 3.64 (s, 3H), 3.52 (br s, 1H), 3.324 (broad s, 1H), 3.12 (m, 1H), 2.32-0.92 (m, 30H), 0.70 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz, δ) 174.18, 174.16, 135.48, 135.44, 115.69, 115.09, 115.05, 80.28, 78.67, 74.44, 68.88, 68.82, 68.31, 45.95, 45.66, 42.20, 41.61, 35.01, 34.81, 34.64, 34.59, 30.63, 30.47, 28.47, 27.60, 27.08, 22.80, 22.60, 17.06, 12.16; MS (EI) m/z 525 (M^+-vinyl, 3), 442 (15), 441 (81), 440 (54), 439 (22), 437 (11), 384 (18), 383 (100), 381 (73), 311 (22), 310 (10), 254 (22), 253 (55), 252 (27), 226 (17), 225 (11), 211 (15), 160 (10), 158 (11), 157 (10), 147 (16), 145 (33), 131 (16).

Compound 5. Compound **4** (2.0 g, 3.76 mmol) was dissolved in anhydrous CH_2Cl_2 (30 mL). *m*-Chloroperbenzoic acid (3.2 g, 18.80 mmol) was added and the solution was stirred at room temperature overnight. The solvent was removed *in vacuo*. Water (30 mL) was added and the mixture was extracted with ether (2 x 30 mL). The combined organic solution was washed with brine (20 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel using CH_2Cl_2/acetone (10/1) as the eluent to afford viscous oil (1.80 g, 75 % yield). ^{1}H NMR ($CDCl_3$, 300 MHz, δ) 3.69-3.24 (m, 6H), 3.18-3.05 (m, 9H), 2.78-2.51 (m, 6H), 2.49-0.84 (m, 30H), 0.63-0.60 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz, δ) 81.85, 81.80, 80.30, 76.81, 76.10, 69.86, 69.81, 68.98, 68.93, 68.91, 68.76, 68.65, 53.73, 51.67, 51.50, 51.46, 51.45, 51.36, 51.28, 46.58, 46.22, 46.20, 45.35, 45.15, 45.10, 45.06, 44.92, 44.85, 42.87, 42.78, 41.96, 39.81, 39.78, 35.35, 35.31, 35.24, 35.09, 35.06, 35.05, 34.86, 31.17, 31.08, 31.01, 30.97, 28.18, 27.66, 23.67, 23.34, 23.09, 17.56, 12.66, 12.64. Anal. Calcd for $C_{34}H_{54}O_8$: C, 69.12; H, 9.21. Found: C, 69.18; H, 9.32.

Compound 6. Coumpound **5** (1.0 g, 1.69 mmol), KOH (0.19 g, 3.38 mmol), and water (10 mL) were heated to 90 °C with rapid stirring for 4 h. The brown solution was acidified with H_2SO_4 (2N) until pH = 3. The solution was lyophilized to give an off-white powder. The powder was combined with pyridine (5 mL). 4-(Dimethylamino)pyridine (10 mg) and acetic anhydride (6 mL) were added to the cooled (0 °C) suspension. The mixture was stirred at room temperature overnight. The solvent was removed *in vacuo*. Water (40 mL) and CH_2Cl_2 (20 mL) were added. The aqueous layer was extracted with CH_2Cl_2 (2 x 40 mL). The combined organic solution was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel using EtOAc/hexane (3/1) as the eluent to give yellow oil (0.45 g, 30 % yield). ^{1}H NMR ($CDCl_3$, 300 MHz, δ) 5.11-5.07 (m, 3H), 4.48-

4.41 (m, 6H), 3.67-3.46 (m, 5H), 3.27-3.24 (m, 3H), 3.02 (s, 1H), 2.48-0.83 (m, 48H), 0.61 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz, δ) 174.48, 170.46, 170.41, 170.37, 170.36, 170.31, 170.24, 170.23, 170.19, 170.16, 80.41, 80.06, 76.56, 76.46, 70.83, 70.73, 70.62, 67.01, 66.81, 66.21, 66.11, 66.02, 65.88, 63.03, 53.77, 51.37, 46.36, 36.35, 46.21, 46.19, 42.45, 42.39, 42.30, 41.81, 41.64, 39.57, 35.12, 34.97, 34.87, 34.82, 34.80, 31.01, 30.79, 28.92, 28.74, 27.66, 27.41, 27.03, 23.21, 22.83, 22.55, 21.01, 20.95, 20.92, 20.88, 20.76, 20.69, 20.68, 20.60, 17.50, 17.41, 17.38, 12.31. Anal. Calcd for $C_{45}H_{70}O_{17}$: C, 61.21; H, 7.99. Found: C, 60.99; H, 7.35.

Compound 1b. Compound **6** (350 mg, 0.40 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL). Under N_2, oxalyl chloride (67 mg, 0.53 mmol) was added by a syringe, followed by three drops of pyridine. The mixture was refluxed for 6 h. The solvent was evaporated *in vacuo*. The residue was redissolved in anhydrous CH_2Cl_2 (10 mL). A solution of **3** (66 mg, 0.09 mmol) and triethylamine (44 mg, 0.44 mmol) in anhydrous CH_2Cl_2 (5 ml) was added slowly via a syringe. The mixture was stirred for 6 h at room temperature. The mixture was acidified with 2N HCl and was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic solution was washed with water (10 mL) and brine (10 mL), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified with column chromatography over silica gel using CH_2Cl_2/acetone (4/1) as an eluent to give a yellow powder. The intermediate was hydrolyzed by NaOMe (170 mg) in methanol (5 mL) for 12 h at room temperature. The product was purified by preparative silica gel TLC (CH_2Cl_2/MeOH/H_2O = 3/1/0.1 as the solvents, R_f = 0.40) to give a white powder (67 mg, 30 % overall yield). ^{1}H NMR (MeOH-d4, 300 MHz, δ) 7.03 (d, J = 2.1 Hz, 4H), 6.82 (d, J = 2.1 Hz, 4H), 4.55 (d, J = 12.6 Hz, 4H), 4.13 (t, J = 5.7 Hz, 8H), 3.90 (t, J = 5.4 Hz, 8H), 3.89-3.00 (m, 76H), 3.15-3.00 (m, 8H), 2.33-0.91 (m, 132H), 0.72 (s, 12H); ^{13}C NMR (MeOH-d4, 75 MHz, δ) 174.02, 163.01, 135.10, 132.77,

121.08, 81.15, 80.15, 76.89, 73.40, 71.55, 71.50, 71.40, 71.29, 70.05, 69.81, 69.54, 69.32, 69.06, 66.44, 64.35, 64.17, 64.07, 63.88, 63.38, 47.21, 46.37, 46.28, 42.74, 41.83, 39.77, 35.68, 35.40, 35.04, 34.78, 34.74, 33.66, 32.94, 31.99, 30.71, 28.67, 27.88, 27.44, 27.32, 24.77, 23.42, 22.84, 22.70, 22.34, 22.22, 17.54, 17.19, 14.76, 12.19, 11.89. MALDI-TOFMS: calcd for $C_{176}H_{284}N_4O_{48}Na$ $[M+Na]^+$, 3245.0; found, 3244.7. Anal. Calcd for $C_{224}H_{332}N_4O_{72}$ (the acetate intermediate): C, 63.56; H, 7.91; N, 1.32. Found: C, 63.31; H, 7.99; N, 1.27.

Compound 2. Cholic acid (200 mg, 0.32 mmol) and 3,4,5-trimethoxyaniline (116 mg, 0.63 mmol) were dissolved in anhydrous MeOH (10 mL). *N*-Ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDCI) hydrogen chloride salt (121 mg, 0.63 mmol) was added. The mixture was stirred under N_2 at room temperature for 12 h. A solvent was removed *in vacuo*. The residue was dissolved in methanol (0.5 mL) and precipitated in water (5 mL). The solid was collected by suction filtration and purified by column chromatography over silica gel using CH_2Cl_2/MeOH (1/4) as the eluent to give a white powder (150 mg, 70 % yield). mp = 125-130 °C; ^{1}H NMR (MeOH-d4, 300 MHz, δ) 6.95 (s, 2H), 3.96 (br s, 1H), 3.81 (m, 7H), 3.71 (s, 3H), 3.31 (m, 1H), 2.40-2.25 (m, 4H), 2.00-0.91 (m, 27H), 0.72 (s, 3H); ^{13}C NMR (MeOH-d4, 75 MHz, δ) 173.82, 153.22, 135.34, 134.11, 97.61, 97.51, 72.87, 71.69, 67.87, 60.13, 55.38, 46.84, 46.34, 41.99, 41.82, 39.83, 39.30, 35.83, 35.37, 34.76, 33.95, 32.01, 30.05, 28.46, 27.63, 26.70, 23.16, 22.15, 16.77, 12.02. MALDI-TOFMS: calcd for $C_{33}H_{51}N_1O_{10}Na$ $[M+Na]^+$, 596.8; found, 597.6.

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CHAPTER 3. Solvnet-induced amphiphilic molecular baskets: unimolecular reversed micelles with different size, shape, and flexibility

Taken from a paper published in *The Journal of Organic Chemistry* **2006**, *71*, 7205-7213.[1]

Abstracts

Amphiphilic molecular baskets were obtained by attaching facially amphiphilic cholate groups to a covalent scaffold, calix[4]arene. In a solvent mixture consisting of mostly a nonpolar solvent (i.e., CCl_4) and a polar solvent (i.e., DMSO), the hydrophilic faces of cholates turn inward to form a reversed-micelle-like conformer whose stability is strongly influenced by preorganization of the scaffold and the length and flexibility of the linker between cholates and the scaffold. Preferential solvation of the hydrophilic faces of the cholates within the molecule by the polar solvent is cooperative and gives the fundamental driving force to the conformational change. The reversed-micelle-like conformer is most stable in structures that allow multiple cholates to form a microenvironment that can efficiently enrich the polar solvent molecules from the bulk solvent mixture.

Introduction

Conformations represent different 3D arrangements of atoms in a molecule as a result of rotations around single bonds. As a molecule adopts different conformations, its size, shape, and distribution of functional groups change simultaneously. Since these properties are intimately related to the physical and chemical behavior of the molecule, conformational control could serve as a rational way to design environmentally responsive materials. This

strategy is utilized elegantly by biomolecules, such as proteins, whose binding and catalytic functions are frequently regulated through controlled conformational changes.[2] In recent years, foldamers have attracted a great deal of attention of chemists in different fields.[3] As mimics of biomolecules with specific, compact conformations, foldamers may not only shed new light on the folding and functions of biomolecules, but also enable chemists to prepare biomolecule-like, stimuli-responsive materials from a bottom-up approach.

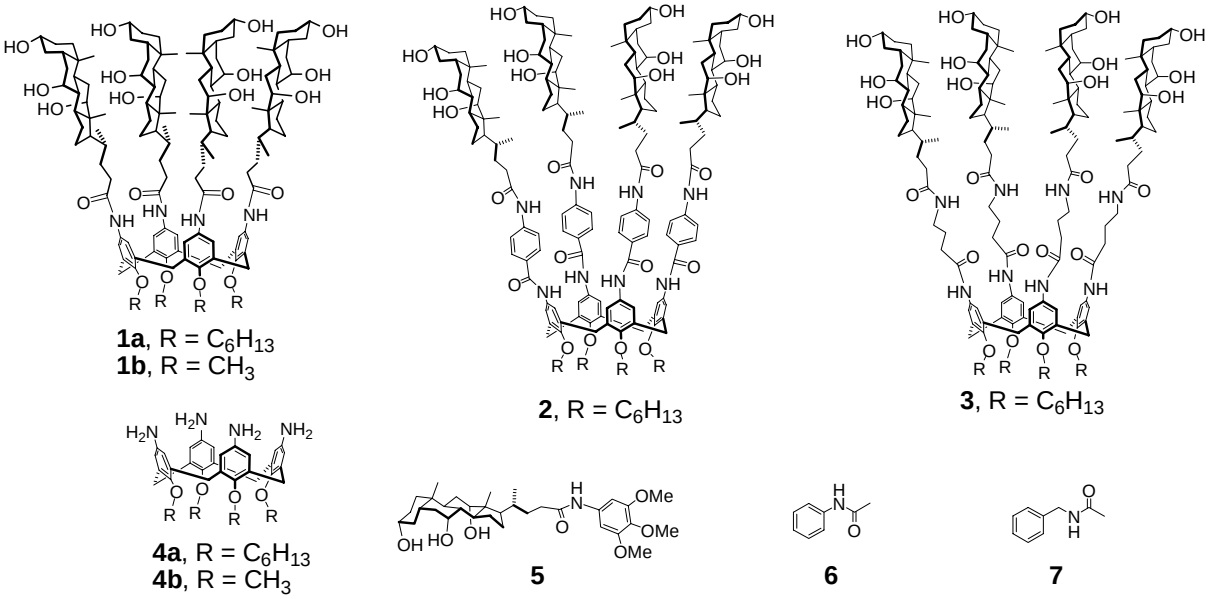
Conformational changes in biomolecules may be induced by specific molecules, such as an enzyme substrate or an allosteric effector,[2] or by general changes in environmental conditions, including temperature, pH, and solvent polarity. Response to solvent polarity is not a surprise, as hydrophobic interactions[4] represent a major driving force for the folding of polypeptide chains. An interesting class of biomolecules that display polarity-induced conformational changes is α-helical antimicrobial peptides.[5] These small peptides typically assume random conformations in water, but change to amphipathic α-helical structures (which are surface active and can destabilize the membrane) in contact with bacterial membranes, a much less polar environment. In fact, polarity-induced conformational change is important to many biological processes, including the translocation of proteins across membranes.[6]

We have been interested in using cholic acid[7] as a building block to construct both foldamers[8] and nonfoldamers,[9–10] whose conformations and properties can be reversibly switched. With its large steroid backbone and oppositely facing hydrophilic and hydrophobic groups,[11] cholic acid is uniquely suited for solvophobically driven conformational changes. Previously, we synthesized an amphiphilic molecular basket by coupling cholates to a cone-shaped, aminocalix[4]arene scaffold.[9] The molecule adopts micelle-like conformations in polar solvents with the hydrophilic α faces turned outward and reversed-micelle-like conformations

in nonpolar ones with the β faces inward. In this article, we extend the concept to prepare a series of cholate baskets with different size, shape, and flexibility. We were able to experimentally verify preferential solvation, which had been speculated to drive the conformational changes in the molecular basket. We also have found an interesting correlation between the stability of the reversed-micelle-like conformer and the ability of the cholates to form a microenvironment to enrich polar solvents.

Results and discussion

Design and synthesis of amphiphilic molecular baskets. The geometry of an amphiphile dictates the possible aggregates it can form. For a head/tail amphiphile, spherical micelles (or reversed micelles) are the most common aggregates obtained in water (or nonpolar solvents). With a contrafacial topology, cholate amphiphiles tend to associate through the solvophobic faces into oligomers that resemble micelles and reversed micelles in polar and nonpolar environments, respectively.[12] If several cholates are linked covalently, intramolecular aggregation should happen readily. The difference between inter- and intra-molecular aggregation is that cholates can freely approach one another to minimize solvophobic exposure in the former, but are restricted by the covalent linkers and the topological scaffold employed in the latter. Therefore, other than concentration-independency, "unimolecular" micelles and reversed micelles[13] from intramolecular aggregation of cholates have the additional advantage of being tuned systematically through structural modification.



1a, R = C_6H_{13}
1b, R = CH_3

2, R = C_6H_{13}

3, R = C_6H_{13}

4a, R = C_6H_{13}
4b, R = CH_3

5

6

7

The previously reported **1a** can encapsulate hydrophobic guests in polar solvents and hydrophilic guests in nonpolar solvents.[10] We were interested in creating larger baskets by insertion of a spacer between the cholates and the calixarene. This is represented by compound **2** with a rigid *para*-aminobenzoyl spacer and **3** with a flexible 4-aminobutyroyl spacer. Ring inversion in calix[4]arene happens readily when the alkyl substituents at the lower rim are smaller than propyl.[14] Compound **1b**, therefore, has a less preorganized scaffold and is used to test whether solvophobic interactions among the cholates are strong enough to fix the calixarene into one particular conformation. Compounds **5**, **6**, and **7** are control molecules used in our studies. Most of these compounds were synthesized in a straightforward fashion by amide coupling between the acids and the corresponding amines. Compounds **2** and **3** were prepared by coupling cholic acid to the spacer first and then the resulting extended acid to calixarene amine **4a**.

Conformational changes in calixarene-based molecular baskets. There were two main lines of evidence for the micelle- and reversed-micelle-like conformations of **1a**. It could bind hydrophobic guests, such as pyrene, in polar solvent mixtures (e.g., methanol/water =

80/20) and hydrophilic guests, such as phenyl β-D-gluocopyranoside, in nonpolar mixtures (e.g., methanol/CCl_4 = 5/95).[10] Pyrene caused upfield shifts of the methyl protons on the β faces of the cholates, consistent with the micelle-like conformation with inwardly facing β faces. The other line of evidence for the proposed conformations was from ^{1}H NMR spectral data in the absence of guests. The aromatic protons ortho to the amido group appear as a single peak in solvents with intermediate polarity, but as two peaks in either polar or nonpolar solvents.[9] The magnitude of splitting was found to correlate with not only solvent polarity but also the difference of solvophobicities of the α and β faces of the cholates.[9] Such a splitting is also observed for **1b** (Figure 1, ArH), which is based on a conformationally mobile scaffold. Splitting by itself does not prove the two proposed conformers, but does support transition between two ordered conformations as the solvents go from mostly polar to mostly nonpolar. Given the binding properties mentioned above, it is reasonable to assume the conformer in polar solvents is micelle-like and the one in the nonpolar solvents is reversed-micelle-like.

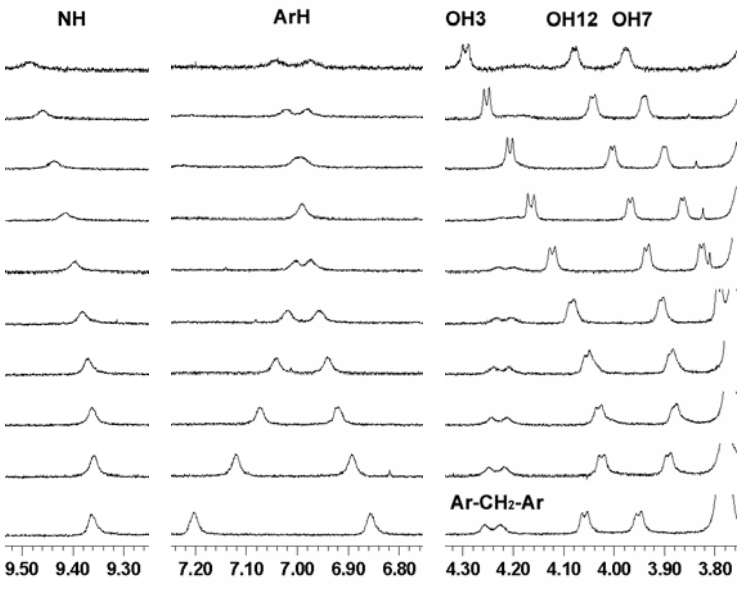


Figure 1. Portions of the ^{1}H NMR (400 MHz) spectra of **1b** in different ratios of DMSO-d_6/CCl_4 at ambient temperature. The solvents are 100, 90, 80, 70, 60, 50, 40, 30, 20, and 10% DMSO from top to bottom. See the structure of cholic acid for the OH labeling.

Cholate-substitution on the calixarene clearly had a dramatic effect on the calixarene, because the parent calixarene **4b** contained extremely broad peaks in the ^{1}H NMR spectrum. Since the distance between the two aromatic proton peaks correlate with the stability of the ordered, micelle-like or reversed-micelle-like conformers based in **1a**,[9,10] we expected a smaller splitting in the less preorganized **1b**, as part of the solvophobic interactions among the cholates need to compensate for the loss of entropy during formation of an ordered conformation. This is indeed the case. For example, the two peaks are separated by 158 Hz for **1a,** but 140 Hz for **1b** in 10% DMSO.

Change of conformation is also evident from the methylene bridge protons (Ar-CH_2-Ar), which are diagnostic of calix[4]arene conformations.[14] The "axial" protons at ~ 4.2 ppm start to appear as (part of) an AB quartet with DMSO ≈ 60% and become better formed with lower DMSO, consistent with the higher stability of the cone conformer. Conversion from a conformationally random calixarene to the cone-conformer in **1b** indicates the presence of intramolecular attractions among the cholates, in agreement with the proposed reversed-micelle-like conformations. Interestingly, appearance of the AB quartet concurs with the splitting of the aromatic peaks, implying both changes are of the same origin. This result confirms our previous conclusion that splitting is caused by the adoption of ordered conformations (i.e., a micelle-like conformation in polar solvents and a revered-micelle-like conformation in nonpolar solvents).

The possibility of intermolecular aggregation of **1a** was ruled out previously, because its ^{1}H NMR spectrum was nearly unchanged over 0.2–15 mM of concentration.[10] All the NMR experiments in the current study were performed at the lower end of the concentration range, typically about 1 mM. Aggregation should not be a problem.[16] Additional evidence against intermolecular aggregation comes from the appearance of the proton signals. The broadening of peaks typically associated with intermolecular aggregation was essentially absent in all compounds studied in this paper.

Splitting of the aromatic protons occurs in **1b** at the polar end (e.g., in 100% DMSO) as well, suggesting formation of another ordered structure, most likely the micelle-like conformer. The methylene bridge protons in this case, however, do not appear as an AB quartet characteristic of the cone, but are broad and nearly invisible. The original basket **1a** did not show such a difference in the polar end, because calix[4]arene is already fixed as the cone by the long hexyl groups.[9] It seems that solvophobic interactions among the cholates are different for the normal and reversed micelle-like conformers. This is quite likely because solvophobic interactions occur through direct contact of the cholate β faces in polar solvents for the former conformer, but is probably mediated by polar solvent molecules for the latter. In other words, in the reversed-micelle-like conformer, DMSO is enriched in the interior of the molecule from the mostly nonpolar environment and serves to "bridge" the gap between the α faces of the cholates. Direct contact of the α faces to simultaneously satisfy all hydrogen bonds in four cholates seems impossible, especially because the cholate backbone is bent toward the α face. In fact, mediation by polar solvents is also required in surfactant reversed micelles, which typically need to be stabilized by a small amount of water.[17] The cone conformation allows the α faces of all four cholates to be simultaneously solvated by entrapped DMSO molecules and

thus should be the best for the reversed-micelle-like conformer. On the other hand, direct contact of the β faces, which is preferred by the normal-micelle-like conformer, may not be best in the cone-shaped calixarene. Given that sodium cholate frequently forms dimers in aqueous solutions,[12] it is quite possible that other conformations (such as 1,3-alternate) are equally good for solvophobic interactions; thus, no particular conformation of calixarene may be favored by the normal micelle-like conformer.

In aprotic solvents, such as DMSO/CCl_4, both the NH and the OH protons are clearly visible. As shown in Figure 1, these protons generally move to high field with a decrease in the DMSO percentage. Note that the doublets of OH protons are clearly visible in all solvent mixtures (as also in Figure 4). In nonpolar solvents, aggregation would occur through hydrogen bonds and undoubtedly would complicate the OH signals. Clear OH signals thus once again provide evidence against intermolecular aggregation. Interestingly, the upfield shift seems to slow down below 50–60% DMSO and even reverses for the OH protons in < 20% DMSO. The trend is more obvious when changes in the chemical shifts are plotted against the DMSO percentages (Figure 2). The chemical shifts of the NH and OH directly reflect the extent of hydrogen-bonding interactions involved by these protons.[18–23] In our mixed solvents, only DMSO can participate in hydrogen bonding; thus the chemical shifts of the NH and OH groups are good indicators of the "local concentration" of DMSO near these groups. Figure 2a shows the relationship between $-\Delta\delta_{NH}$ and the DMSO percentage for several compounds. The curves are nearly identical for monomer **5** and phenyl acetamide **6**, indicating similar solvation of amides in both compounds. Apparently, a single cholate group does not have any DMSO-enriching effect in comparison to a simple amide. Under such conditions, $-\Delta\delta_{NH}$–DMSO% simply reflects the concentration of DMSO in the bulk mixture. When multiple cholates are

assembled on a calixarene, a completely different situation is observed. The $-\Delta\delta_{NH}$–DMSO% curves for **1a** and **1b** (◆ and □) initially trace those of control compounds **5** and **6** (△ and ×), suggesting that the NH groups are sensing the DMSO concentration in the bulk in high DMSO solvents just as **5** and **6**. When DMSO in the bulk drops below 50–60%, however, the curves bend downward substantially. In fact, the NH protons of **1a** and **1b** experience the same degree of hydrogen bonding interactions (as indicated by the magnitude of $-\Delta\delta$ in 10% as in 40–50% DMSO). In other words, it seems as if 40–50% DMSO is still present near the amide protons even when the bulk solvent only contains 10% DMSO.

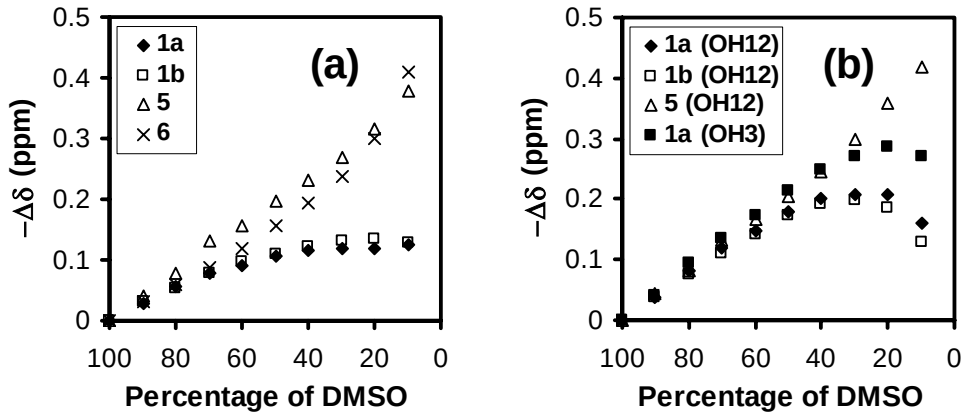


Figure 2. Changes in the ^{1}H NMR chemical shifts of (a) NH and (b) OH as a function of solvent composition in mixtures of DMSO-d_6/CCl_4 for compounds **1a**, **1b**, **5**, and **6**. Data for OH7 sometimes cannot be obtained because of overlapp with the solvent signals (as shown by Figure 1). Assignment of the OH groups is based on a 2D COSY spectrum (Figure 3).

The OH12 group on the cholate shows a similar downward deviation from the control curve (Figure 2b). In fact, the "DMSO-enriching" effect is so strong toward the low polarity end that, as far as this hydroxyl is concerned, the local DMSO concentration actually increases

when the bulk DMSO is decreased from 20 to 10%. Most interestingly, the OH3 proton, located at the periphery of the basket, shows less pronounced DMSO-enrichment (■ in Figure 2b) than OH12 (◆ in Figure 2b), which is in the interior. Such a trend is more or less maintained in all of our cholate-derived compounds. This suggests that cooperativity exists between the polar groups to enrich DMSO. Among a cluster of polar groups, the ones in the center are more strongly solvated by DMSO than the ones near the edge.

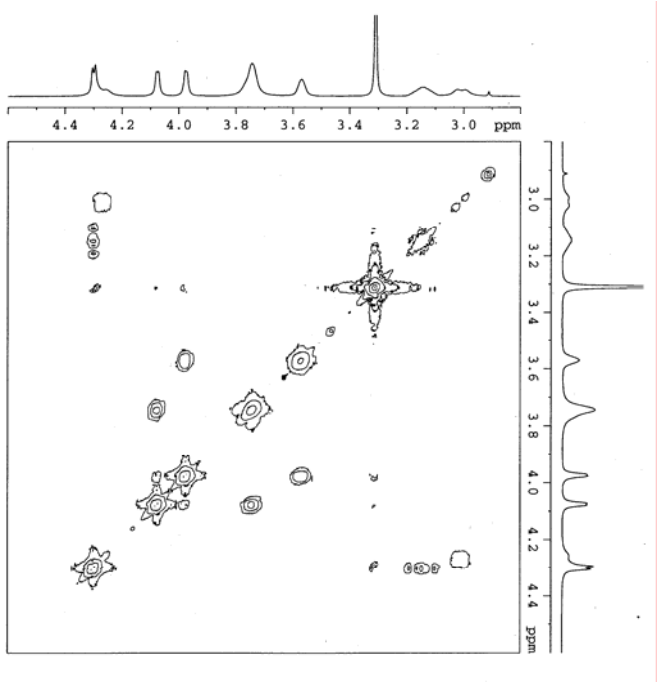


Figure 3. The COSY NMR spectrum (400 MHz) of **1a** in DMSO-d_6.

Conformational changes in our amphiphilic baskets were previously hypothesized to occur as a result of preferential solvation of the hydrophilic faces of the cholates by the polar solvent molecules.[9] Similar conclusions were also inferred from their guest-binding properties in response to polar solvents.[10] The –Δδ–DMSO% curves give an estimate of the average DMSO concentration near the polar groups and provide further evidence for the formation of the reversed-micelle-like conformers. Such conformers by definition have inwardly facing

polar groups and, similar to surfactant reversed micelles, should enrich polar solvents from a nonpolar environment to its interior. According to Figures 2a and 2b, preferential solvation (shown by deviation from control curves) seems to become important below 50–60% DMSO and is most pronounced when the solvent contains 10–20% DMSO. This effect should be even stronger below 10% DMSO. However, solubility often becomes a problem in such mixtures, precluding measurement.

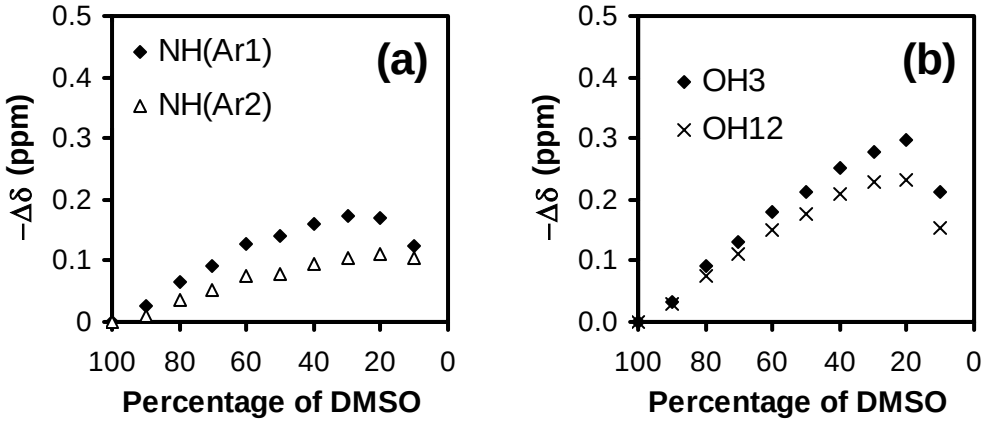


Figure 4. Changes in the ^{1}H NMR chemical shifts of (a) NH and (b) OH as a function of the solvent composition in mixtures of DMSO-d_6/CCl_4 for compound **2**.

For compounds **2** and **3**, splitting of the aromatic protons is hardly observable even under the most polar or nonpolar conditions. Thus, direct connection between cholate and the calixarene is necessary for the splitting and conformational insight cannot be obtained in this way. The –Δδ–DMSO% curves for NH/OH, however, show similar DMSO-enrichment as in **1a** and **1b**, suggesting adoption of a reversed-micelle-like conformation in low-polarity solvents (Figures 4 and 5). Apparently, insertion of short spacers between the cholates and calixarene does not affect the conformational changes significantly, at least for the reversed-micelle-like conformer.

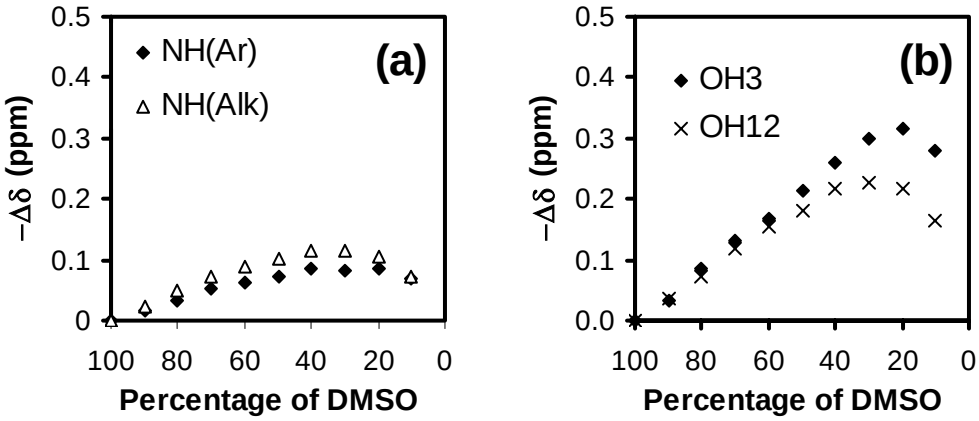


Figure 5. Changes in ^{1}H NMR chemical shifts of (a) NH and (b) OH as a function of solvent composition in mixtures of DMSO-d_6/CCl_4 for compound **3**.

Conclusions

Multiple cholates attached to a covalent scaffold can readily aggregate intramolecularly to form unimolecular micelles and reversed micelles. The micelle-like conformer prefers direct contact of the hydrophobic β faces of cholates and seem to be best formed in "tight" structures. The reversed-micelle-like conformer is mediated by polar solvent molecules entrapped in the interior of the molecule and can tolerate significant modification of the structures. The calix[4]arene-derived baskets can also tolerate spacers. Both rigid 4-aminobenzoyl and flexible 4-aminobutyroyl spacers afford stable reversed-micelle-like conformers in calix[4]arene-based baskets.

Experimental Section

General Methods.

Anhydrous tetrahydrofuran (THF) and methylene chloride were dried by passage through a column of activated alumina under compressed nitrogen. Cholic acid was

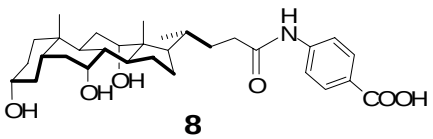
crystallized from 95% ethanol and dried at 90 °C under vacuum. All other reagents and solvents were of A.C.S. certified grade or higher, and were used as received from commercial suppliers. All glassware and syringes were dried in an oven at least overnight prior to use.

Synthesis

Compound 4a. This aminocalixarene was synthesized according to a literature procedure.[25] ^{1}H NMR (300 MHz, $CDCl_3$, δ) 6.07 (s, 8H), 4.29 (d, J = 13.2 Hz, 4H), 3.74 (t, J = 7.6 Hz, 8H), 2.90 (d, J = 13.2 Hz, 4H), 1.90–1.80 (m, 8H), 1.45–1.13 (m, 24H), 0.89 (t, J = 7.2 Hz, 12H).

Compound 4b. This aminocalixarene was synthesized according to a literature procedure.[26] ^{1}H NMR (DMSO-d_6, 300 MHz, δ) 6.08 (br s, 8H), 4.31 (br s, 8H), 3.30 (m, 12H).

Compound 1a. The synthesis was reported previously.[27] ^{1}H NMR (CD_3OD, 300 MHz, δ) 6.97 (d, J = 2.4 Hz, 4H), 6.82 (d, J = 2.4 Hz, 4H), 4.44 (d, J = 12.0 Hz, 4H), 4.96 (s, 4H), 3.88 (t, J = 6.6 Hz, 8H), 3.80 (s, 4H), 3.37 (m, 4H), 3.11 (d, J = 12.0 Hz, 4H), 2.40–0.90 (m, 120H), 0.72 (s, 12H); ^{13}C NMR (CD_3OD, 75 MHz, δ): 173.6, 153.3, 135.1, 132.3, 121.0, 75.6, 73.1, 71.7, 68.2, 47.1, 46.5, 41.8, 41.8, 39.73, 39.72, 39.3, 35.9, 35.4, 34.9, 34.0, 32.3, 32.0, 30.4, 30.1, 28.4, 27.7, 26.6, 26.2, 23.34, 23.32, 23.0, 22.4, 17.1, 13.8, 12.4. MALDI-TOFMS: $[M+Na]^+$ calcd. for $C_{148}H_{228}N_4NaO_{20}$: 2405.7; found: 2405.7.



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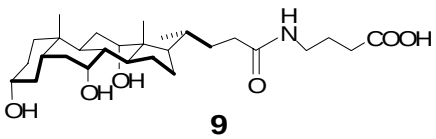

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Compound 8.[28] Cholic acid triformate[29] (1.92 g, 3.90 mmol) was dissolved in dry CH_2Cl_2 (40 mL). Oxalyl chloride (0.60 mL, 6.88 mmol) was added by a syringe, followed by 3

drops of dry DMF. The mixture was stirred at rt under a N_2 flush for 40 min. The solvent was removed *in vacuo*. Dry CH_2Cl_2 (10 mL) was added and evaporated again *in vacuo*. The residue was dissolved in dry CH_2Cl_2 (30 mL), to which a solution of ethyl 4-aminobenzoate (0.664 g, 4.02 mmol), and Et_3N (0.7 mL, 5.0 mmol) in CH_2Cl_2 (50 mL) was added. The mixture was stirred at rt under N_2 for 46 h, diluted with CH_2Cl_2 (20 mL), washed with 2N HCl, dried ($MgSO_4$), and concentrated *in vacuo* to give a yellow foam. The foam was dissolved in methanol (100 mL), THF (20 mL), and 1 M LiOH (45 mL). The mixture was allowed to sit at rt for 17 h. Most of the solvent was evaporated *in vacuo*. The residue was acidified with 1M H_2SO_4 untill pH = 2. The solid was collected by suction filtration and washed with CH_3CN (10 mL) to afford a white powder (1.779 g, 86% yield). ^{1}H NMR (DMSO-d_6, 300 MHz, δ) 10.17 (s, 1H), 7.85 (d, J = 8.7 Hz, 2H), 7.68 (d, J = 8.7 Hz, 2H), 4.30 (br s, 1H), 4.11 (d, J = 2.9 Hz, 1H), 4.00 (d, J = 2.9 Hz, 1H), 3.78 (br s, 1H), 3.60 (br s, 1H), 3.16 (br s, 1H), 2.42–0.76 (m, 30H), 0.58 (s, 3H).

***N*-Hydroxysuccinimide ester of cholic acid.**[30] Dicyclohexylcarbodiimide (DCC, 1.339 g, 6.49 mmol) was added to a solution of cholic acid (2.447 g, 5.99 mmol) and *N*-hydroxysuccinimide (0.733 g, 6.37 mmol) in CH_3CN/THF (10 mL/50 mL). After 17 h at rt, the solid was removed by filtration. The filtrate was concentrated *in vacuo* to give a white foam. The material was used without further purification.

Compound 9.[31] The *N*-hydroxysuccinimide ester of cholic acid prepared above was dissolved in DMF (20 mL) and H_2O (3 mL). 4-Aminobutyric acid (0.642 g, 6.23 mmol) and DIPEA (1.562 g, 12.1 mmol) were added. The mixture was stirred at 75 °C for 23 h. The solvent was removed in vacuo. The residue was purified by column chromatography over silica gel using $CHCl_3$/MeOH/AcOH (6/1/0.1 to 5/1/0.1) as the eluent to give a white foam (1.463 g, 49.5% yield). ^{1}H NMR (CD_3OD, 300 MHz, δ) 3.95 (br s, 1H), 3.79 (br s, 1H), 3.37

(m, 1H), 3.42–3.33 (m, 1H), 3.21–3.18 (m, 2H), 2.32 (t, J = 7.5 Hz, 2H), 2.26–0.91 (m, 32H), 0.70 (s, 3H).

Compound 5. Compound **5** was synthesized according to a literature procedure.[32] ^{1}H NMR (CD_3OD, 300 MHz, δ) 6.95 (s, 2H), 3.96 (broad s, 1H), 3.81 (m, 7H), 3.71 (s, 3H), 3.31 (m, 1H), 2.40–2.25 (m, 4H), 2.00–0.91 (m, 27H), 0.72 (s, 3H); ^{13}C NMR (CD_3OD, 75 MHz, δ) 173.8, 153.2, 135.3, 134.1, 97.6, 97.5, 72.9, 71.7, 67.9, 60.1, 55.4, 46.8, 46.3, 42.0, 41.8, 39.8, 39.3, 35.8, 35.4, 34.8, 34.0, 32.0, 30.1, 28.5, 27.6, 26.7, 23.2, 22.2, 16.8, 12.0. MALDI-TOFMS (*m/z*): $[M + Na]^+$ calcd for $C_{33}H_{51}N_1O_{10}Na$, 596.8; found, 597.6.

Compound 1b. Cholic acid (349.3 mg, 0.855 mmol), **4b** (105.0 mg, 0.194 mmol), and benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP, 412.4 mg, 0.931 mmol) were dissolved in anhydrous DMF (15 mL) in N_2. Diisopropylethylamine (DIPEA, 238.0 mg, 1.707 mmol) was added by a syringe. The reaction mixture was stirred at 50 °C for 18 h. The solution was poured into brine (50 mL). The precipitate was filtered, washed with water (2 × 10 mL) and CH_3CN (5 mL), and purified by preparative TLC (SiO_2, $CHCl_3/CH_3OH$ = 8/1) to give a white powder (301.9 mg, 0.144 mmol, 74 % yield). ^{1}H NMR (DMSO-d_6/CCl_4 = 1/1, 400 MHz, δ) 9.36 (s, 4H), 7.20 (s, 4H), 6.86 (s, 4H), 4.24 (d, J = 11.6 Hz, 4H), 4.06 (d, J = 4.4 Hz, 4H), 3.95 (d, J = 4.0 Hz, 4H), 3.78 (m, 16H), 3.62 (s, 4H), 3.18 (s, 4H), 3.10 (d, J = 12.4 Hz, 4H), 2.19–0.75 (m, 124H), 0.61 (s, 12H); ^{13}C NMR (75 MHz, CD_3OD, δ) 171.6, 153.9, 134.2, 134.1, 119.9, 71.5, 70.9, 66.8, 46.7, 46.2, 42.0, 41.8, 35.8, 35.4, 34.9, 33.9, 32.0, 30.9, 29.1, 27.8, 26.7, 23.3, 23.1, 17.6, 12.9. MALDI-TOFMS (*m/z*): $[M + Na]^+$ calcd for $C_{128}H_{187}N_4O_{20}Na$: 2125.9; found: 2128.6.

Compound 2. Acid **8** (450 mg, 0.854 mmol, see the Supporting Information for its synthesis), **4a** (159 mg, 0.194 mmol), and BOP (378 mg, 0.854 mmol) were dissolved in

anhydrous DMF (10 mL). DIPEA (220 mg, 1.708 mmol) was added by a syringe. The reaction mixture was stirred at 50 °C for 20 h, and solid was precipitated in acetone (100 mL). The precipitate was filtered, washed with acetone (2 × 10 mL), and purified by preparative TLC (SiO_2, $CHCl_3/CH_3OH$ = 4/1) to give a white powder (250.4 mg, 45 % yield). ^{1}H NMR (DMSO-d_6/CCl_4 = 1/1, 300 MHz, δ) 10.04 (s, 4H), 9.77 (s, 4H), 7.79 (d, *J* = 9.0 Hz, 8H), 7.59 (d, *J* = 8.4 Hz, 8H), 4.32 (s, 8H), 4.11 (s, 4H), 4.00 (d, *J* = 2.1 Hz, 4H), 3.80 (m, 8H), 3.60 (m, 4H), 3.15 (m, 8H), 2.30–0.79 (m, 168H), 0.57 (s, 12H); ^{13}C NMR (75 MHz, DMSO-d_6/CCl_4 = 1/1, δ) 174.1, 166.6, 153.6, 141.9, 135.2, 132.2, 129.7, 128.4, 121.8, 119. 7, 77.8, 75.7, 73.2, 71.7, 68.3, 47.0, 46.5, 35.4, 34.9, 34.7, 34.2, 32.3, 32.0, 31.27, 31.25, 30.4, 30.1, 28.3, 27.74, 26.5, 26.2, 23.3, 23.0, 22.5, 17.3, 14.1, 12.5. MALDI-TOFMS (*m/z*): $[M + Na]^+$ calcd for $C_{176}H_{247}N_8O_{24}Na$: 2881.9; found: 2881.8.

Compound 3. Acid **9** (264.6 mg, 0.536 mmol, see the Supporting Information for its synthesis), **4a** (100.1 mg, 0.122 mmol), and BOP (237.4 mg, 0.536 mmol) were dissolved in anhydrous DMF (10 mL) in N_2. DIPEA (138 mg, 1.072 mmol) was added by a syringe. The reaction mixture was stirred at 50 °C for 20 h, and solid was precipitated in acetone (50 mL). The precipitate was filtered, washed with acetone (2 × 10 mL), and purified by preparative TLC (SiO_2, $CHCl_3/CH_3OH$ = 4/1) to give a white powder (120.2 mg, 36 % yield). ^{1}H NMR (DMSO-d_6/CCl_4 = 1/1, 300 MHz, δ) 9.45 (s, 4H), 7.76 (s, 4H), 6.89 (d, *J* = 4.0 Hz, 8H), 4.32 (m, 8H), 4.09 (s, 4H), 4.00 (s, 4H), 3.75 (s, 8H), 3.57 (s, 4H), 3.14 (m, 8H), 2.99 (m, 12H), 2.24–0.77 (m, 168H), 0.54 (s, 12H); ^{13}C NMR (75 MHz, DMSO-d_6/CCl_4 = 1/1, δ) 173.2, 170.8, 152.4, 134.7, 133.8, 120.2, 75.6, 71.7, 71.1, 66.9, 46.8, 46.4, 42.2, 42.0, 38.8, 36.0, 35.9, 35.6, 35.1, 34.3, 33.4, 32.4, 32.4, 31.1, 30.4, 29.2, 28.0, 26.9, 26.2, 26.0, 23.5, 23.3, 23.1, 17.8, 14.6, 13.0. MALDI-TOFMS (*m/z*): $[M + H]^+$ calcd for $C_{164}H_{255}N_8O_{24}$: 2723.8; found: 2725.9.

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CHAPTER 4. Solvent-tunable binding of hydrophilic and hydrophobic guests by amphiphilic molecular baskets

A paper published in *The Journal of Organic Chemistry* **2005**, *70*, 7585-7591.[1]

Abstracts

Responsive amphiphilic molecular baskets were obtained by attaching four facially amphiphilic cholate groups to a tetraaminocalixarene scaffold. Their binding properties could be switched by solvent changes. In nonpolar solvents, the molecules utilized the hydrophilic faces of the cholates to bind hydrophilic molecules, such as glucose derivatives. In polar solvents, the molecules employed the hydrophobic faces of the cholates to bind hydrophobic guests. A water-soluble basket could bind polycyclic aromatic hydrocarbons, including anthracene, pyrene, and perylene. The binding free energy (–ΔG) ranged from 5 to 8 kcal/mol and was directly proportional to the surface area of the aromatic hosts. Binding of both hydrophilic and hydrophobic guests was driven by solvophobic interactions.

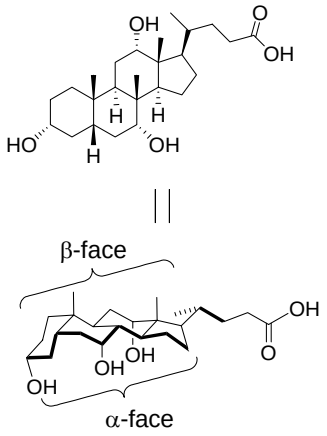
Introduction

Rigid supramolecular hosts with minimal conformational flexibility have traditionally been favored by chemists because of their perceived benefits in binding affinities. Most biomolecules, on the other hand, can respond to environmental stimuli by changing their conformations. As suggested by the induced-fit model,[2] the substrate of an enzyme can cause a necessary conformational change of the active site (to bring the catalytic groups into proper alignment), but non-substrates cannot. Allosteric proteins change their conformations—and in turn their binding or catalytic functions—upon binding with effectors or inhibitors.[3]

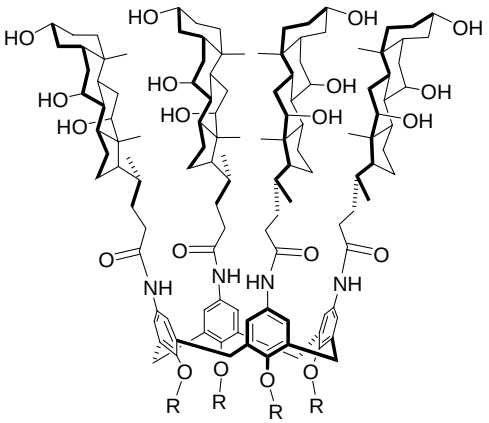
Conformational responses may result from changes of general environmental properties as well. Proteins may denature, or undergo drastic unfolding of the peptide chains, when pH, ionic strength, temperature, or other environmental properties are altered.

In addition, solvent polarity also has a profound influence on the conformations of biomolecules, as hydrophobic interaction[4] (or, more generally, solvophobic interaction) is a major driving force for the folding of polypeptide chains. One class of biomolecules that adopt dramatically different conformations with the change of environmental polarity is α-helical antimicrobial peptides.[5] These peptides tend to assume random conformations in water, but change to amphipathic α-helical structures when they come in contact with bacterial membranes, a much less polar environment. In fact, polarity-induced conformational change is important to many biological processes, including the translocation of proteins across membranes.[6]

The design of synthetic molecules with controllable conformations has received much attention in recent years and is highlighted in foldamer research.[7] Foldamers are synthetic oligomers with biomolecule-like, ordered conformations. Because their conformational flexibility allows their folding and unfolding (and in turn their properties) to be controlled by physical or chemical stimuli, they are very attractive as responsive materials. However, using weak, noncovalent forces to stabilize desired conformations in foldamers (and in synthetic molecules in general) remains a difficult challenge.[7]



Cholic acid



1a, R = $CH_2CH_2OCH_2CH_3$
1b, R = $(CH_2)_5CH_3$

We previously reported an amphiphilic molecular basket **1a** constructed from cholic acid.[8] Cholic acid[9] is an example of a facial amphiphile.[10] The cone-shaped aminocalix[4]arene is used as a scaffold to promote intramolecular aggregation among the cholates. In polar solvents, the hydrophilic α faces of the cholates point outward and the molecule resembles a unimolecular micelle. In nonpolar solvents, the hydrophobic β faces turn outward, giving a reversed-micelle-like conformation.[11,12] We hypothesize that the internal cavity of **1a** is sufficiently large to bind guest molecules and its conformational flexibility will allow it to bind either hydrophilic or hydrophobic guests in a solvent-dependent fashion. In this paper, we report the dual binding properties of **1** in different solvents. We also find that a water-soluble version of **1** indeed acts as a unimolecular micelle to solubilize hydrophobic molecules in aqueous solutions.

Results and discussion

Binding Properties of the Reversed-Micelle-like Conformer in Nonpolar Solvents. Similar to surfactant reversed micelles,[13] the reversed-micelle-like conformer of **1a** requires a small amount of a polar solvent for stability. A typical solvent mixture is carbon

tetrachloride/methanol (90/10). Carbon tetrachloride is a better solvent than chloroform for the reversed-micelle-like conformer, which has a nonpolar exterior. In the reversed-micelle-like conformer, all the hydroxyl groups turn inward to create a binding pocket, which should be mostly filled with the polar solvent. We expect that **1a** should bind a hydrophilic guest of appropriate size. Because cholate groups are totally aliphatic, we choose hydrophilic guests with an aromatic substituent, hoping to monitor the binding event by complexation-induced ^{1}H NMR chemical shifts. Also, during NMR titrations, both the host and the guest need to be sufficiently soluble in the solvents; a totally hydrophilic guest may not have good enough solubility for the titration experiments.

Indeed, when **1a** is mixed with phenyl β-D-glucopyranoside in carbon tetrachloride/methanol (90/10), the proton signals on the phenyl of the guest shift upfield.[14] The binding stoichiometry was studied by using Job plots (Figure 1). Even though a few data points (at χ = 0.1 and 0.9) are missing due to signal overlap, the maximum at 0.5 molar fraction clearly indicates a 1:1 binding stoichiometry. The changes in chemical shifts are most significant for the para protons, followed by the meta and the ortho protons. It seems that the guest resides in the binding site with its phenyl pointing down to the calixarene, possibly as a result of favorable π–π interaction between the phenyl and the calixarene and solvophobic interaction between the sugar unit and the cholate groups.

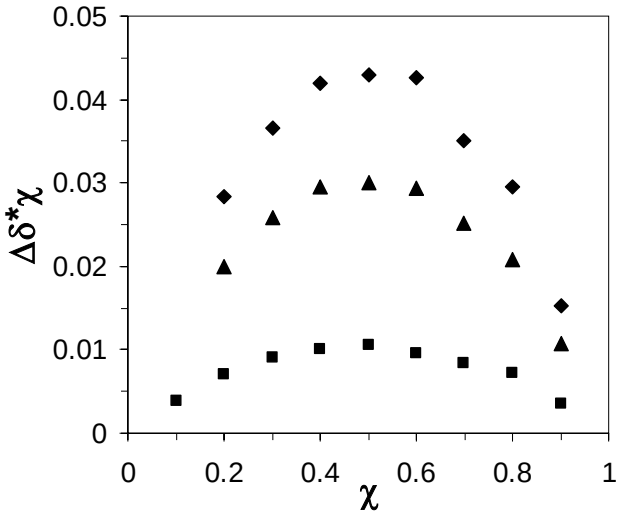


Figure 1. The Job plots for the binding between **1a** and phenyl β-D-glucopyranoside, in which is the molar fraction of **1**. The chemical shift changes (Δδ) are for the para (◆), meta (▲), and ortho (■) protons of phenyl β-D-glucopyranoside.

In Figure 2, the chemical shift of the ortho protons of the guest is plotted as a function of **1a** in different solvents. The binding strength clearly decreases as the percentage of methanol increases from 10 to 15% (data shown as □ and ◆, respectively). Binding is even weaker in 20% methanol, as the chemical shifts of the guest protons are nearly unchanged at different concentrations of host **1a** (data not shown). Because **1a** has limited solubility in 5% methanol, we synthesized **1b** in order to determine the association constant (K_a) more accurately. Host **1b** has guest-binding substructures identical to **1a**, but has four hexyl groups at the lower rim of the calixarene and thus is more soluble in nonpolar solvents than **1a**. As expected, the chemical shift changes of the β-D-glucopyranoside guest are most pronounced in 5% methanol with the addition of **1b** (data shown as ■).

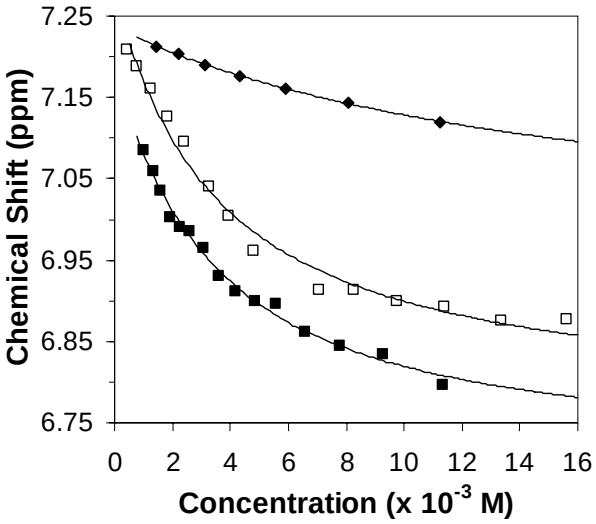


Figure 2. Plot of the chemical shift of the ortho protons in phenyl β-D-glucopyranoside as a function of the concentration of **1a** in 85/15 (◆), and 90/10 (□), and **1b** in 95/5 (■) CCl_4/CD_3OD (vol/vol). Theoretical curves are nonlinear least-square fitting to a 1:1 binding isotherm.

Aggregation of the host is negligible under the binding conditions, as indicated by the fact that the ^{1}H NMR spectrum of **1a** or **1b** is essentially the same when its concentration is varied from 0.2 to 15 mM. The binding constants are obtained by nonlinear least-square fitting and are summarized in Table 1. According to the binding data, host-guest interaction between **1a** and phenyl β-D-glucopyranoside becomes weaker as the percentage of methanol increases in the solvent mixture: $-\Delta G$ = 3.4, 3.3, and 2.5 kcal/mol in 5, 10, and 15% methanol, respectively (Entries 1-3 of Table 1). In 20% methanol, no binding can be detected by ^{1}H NMR spectroscopy titration (entry 4). The binding properties of **1a** and the more soluble **1b** are quite similar in the reversed-micelle-like

Table 1. Association constants (K_a) between **1** and several hydrophilic guests at 20 °C.

Entry	Guest	Host	Solvent Mixture	K_a (M^{-1})	–ΔG (kcal/mol)
1	phenyl-β-D-glucopyranoside	**1a**	CCl_4/CD_3OD = 95/5	330 ± 180[a]	3.4
2	phenyl-β-D-glucopyranoside	**1a**	CCl_4/CD_3OD = 90/10	290 ± 60	3.3
3	phenyl-β-D-glucopyranoside	**1a**	CCl_4/CD_3OD = 85/15	70 ± 10	2.5
4	phenyl-β-D-glucopyranoside	**1a**	CCl_4/CD_3OD = 80/20	--[b]	--[b]
5	phenyl-β-D-glucopyranoside	**1b**	CCl_4/CD_3OD = 95/5	340 ± 60	3.4
6	phenyl-β-D-glucopyranoside	**1b**	CCl_4/DMSO = 90/10	--[b]	--[b]
7	phenyl-α-D-glucopyranoside	**1a**	CCl_4/CD_3OD = 90/10	140 ± 30	2.9

[a] The error is larger than usual because of the low solubility of **1a** in the solvent mixture.
[b] Almost no change in the chemical shifts occurrs during ^{1}H NMR spectroscopy titration, suggesting negligible binding.

conformation: K_a is 330 M^{-1} (Entry 1) with **1a** and 340 M^{-1} with **1b** (Entry 5) for the binding of phenyl β-D-glucopyranoside in 5% methanol.

These data rule out the a π–π interaction between the calixarene and the phenyl group of the guest as the major driving force for the binding. Instead, solvophobic interaction plays a decisive role. This is because π–π interaction is expected to decrease in a solvent with higher polarizability.[15] Thus, a π–π-based binding should increase in strength when the amount of methanol (a less polarizable solvent) increases and the carbon tetrachloride (a more polarizable

solvent) decreases in the solvent mixture. We also performed a similar titration for phenol in CCl_4/CD_3OD (90/10) and found no shifts in the proton signals of either the guest or the host. This result again suggests that the contribution of π–π interaction to the overall binding energy is minor at most.

Interestingly, the initial 5% increase in methanol reduces the binding affinity only slightly (~0.1 kcal/mol), but a further increase by the same magnitude (i.e., from 10 to 15%) causes a much larger reduction (~0.8 kcal/mol). Such a solvent response is different from what have been observed in conventional solvophobically driven associations in rigid supramolecular hosts. For example, Schneider and co-workers[16] found that, in several solvophobically driven host-guest complexations, the binding free energies correlate linearly with the solvophobicity parameters[17] of the solvents. Because solvophobicity parameters of binary mixtures are almost linearly related to the volume percentages, the binding energies ($-\Delta G$) were found to vary linearly as a function of the solvent volume percentages.[15,16] The nonlinear solvent effect in our system probably is a result of the conformational flexibility of the host. As the percentage of methanol increases, two solvent effects are conceivable: (a) the guest and the guest-binding surface of the host become better solvated; (b) the reversed-micelle-like conformer of host **1a** becomes less stable. The first solvent effect is universal and causes a general reduction in binding affinities as the host or the guest is better solvated. The second effect makes **1a** an inferior host and is unique for conformationally mobile hosts.

Because the polar solvent plays an important role in stabilizing the reversed-micelle-like conformer, we were interested in the effect of a polar solvent on the binding affinities. The polar solvent, DMSO, is also miscible with carbon tetrachloride. Figure 3a shows the ^1H NMR spectra of **1b** in different ratios of DMSO/carbon tetrachloride. The aromatic protons ortho to

the amido group show distinct changes according to the solvent composition as a single peak at an intermediate ratio (90% DMSO in this case) but as two peaks above or below this ratio. Such nonequivalence of the aromatic protons also happens with **1a** (Figure 3b) and has been attributed to the formation of ordered (micelle- or reversed-micelle-like) conformations.[8] Unlike **1a**, however, the reversed-micelle-like conformer of **1b** gives rather sharp proton signals, especially in solvents with less than 20% DMSO. Also, the splitting between the two peaks for **1b** in carbon tetrachloride/DMSO is consistently larger than those for **1a** in carbon tetrachloride/methanol. Previously, the splitting between the two peaks was found to a good indicator of the stability of a particular (micelle-like or reversed-micelle-like) conformer.[8] Therefore, DMSO in carbon tetrachloride seems to be an especially good solvent mixture for studying reversed-micelle-like conformers.

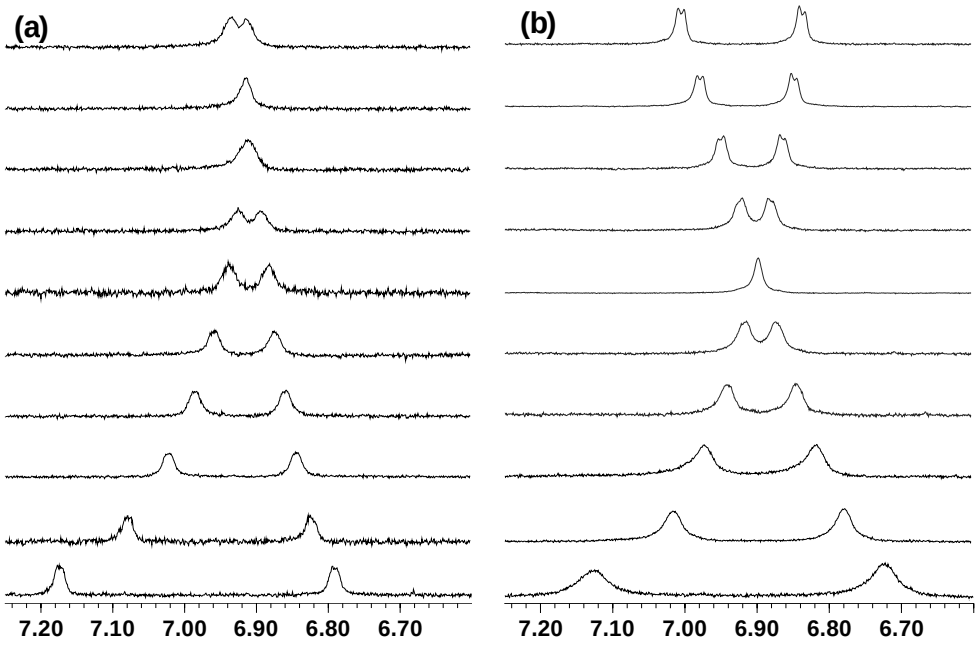


Figure 3. (a) The aromatic regions of the ^{1}H NMR (300 MHz) spectra of **1b** in different ratios of DMSO-d_6/CCl_4 at ambient temperature. (b) The aromatic regions of the ^{1}H NMR (300

MHz) spectra of **1a** in different ratios of CD_3OD/CCl_4 at ambient temperature. The solvents in both cases are 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90% CCl_4 from top to bottom.

However, binding between **1b** and phenyl-β-D-glucopyranoside is extremely weak in carbon tetrachloride/DMSO (90/10) and almost undetectable by ^1H NMR spectroscopic titration. This result was quite a surprise to us initially. We then realized that weak binding is only unexpected if one assumes that a more stable conformer is a better host. Strong binding, however, requires more than a suitable host structure. This is because the polar solvents entrapped by the host needs to be displaced by the guest during binding. It is more difficult to displace strongly solvating solvent molecules than weakly solvating ones. Therefore, the same interaction that stabilizes the reversed-micelle-like conformer (i.e., preferential solvation of the hydrophilic α faces of cholates by DMSO or methanol) actually works against the host in the guest binding. Apparently, selection of solvents in solvophobically driven molecular recognition is even more important in conformationally mobile systems than in rigid ones. The amphiphilic baskets described in this paper in fact only have limited conformational mobility, which mostly comes from the few bonds between the fused steroidal rings and the calixarene. Even for such a molecule, a small change in solvent composition has a very large effect on its conformational and binding properties.

Host **1a** also can bind the α-anomer of phenyl α-D-glucopyranoside, albeit with a reduced association constant of 140 M^{-1} (entry 7 of Table 1) in carbon tetrachloride/methanol (90/10). This moderate selectivity is probably due to the shape of the binding pocket, which prefers the straighter β-anomer because of the upright arrangement of the cholate units.

Binding Properties of the Micelle-like Conformer in Polar Solvents. In a polar environment, **1a** is expected to bind hydrophobic guests by its micelle-like conformer. We have used a mixture of deuterated methanol/water (80/20) as the solvent, in which **1a** has solubility in the millimolar range. The addition of pyrene causes an upfield shift of the methyl protons on the hydrophobic β face of the cholates. Hence, the guest is bound through favorable hydrophobic contact with the host. An accurate determination of the association constant is difficult, because neither the host nor the guest has good solubility in the solvent. We then performed ^{1}H NMR spectroscopic titration with 1-aminopyrene, which is more soluble than pyrene in aqueous methanol. The binding constant was found to be about 10 M^{-1} (entry 1 of Table 2).

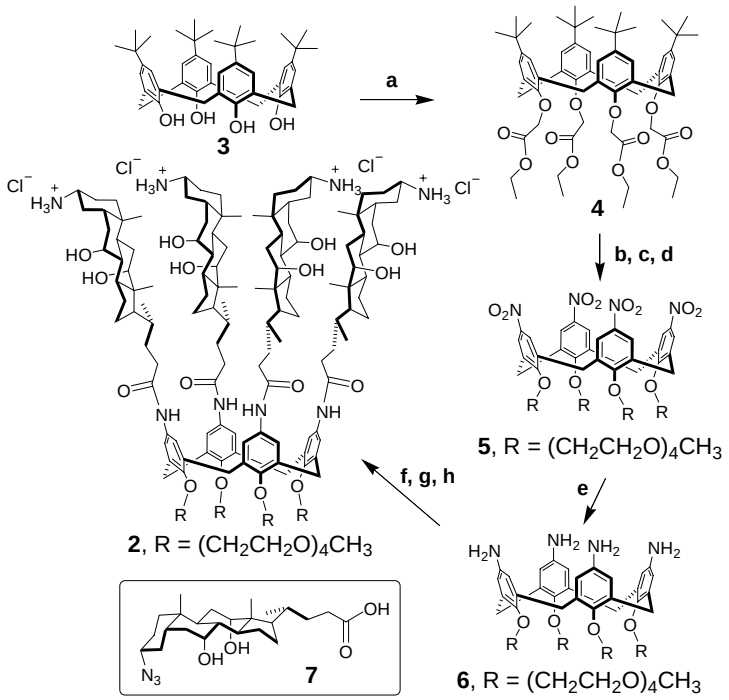
Table 2. Association constants (K_a) between **1** or **2** and several hydrophobic guests at 20 °C.

Entry	Guest	Host	Solvent Mixture	K_a (M^{-1})	–ΔG (kcal/mol)
1	1-aminopyrene [a]	**1a**	CD_3OD/D_2O = 80/20	10 ± 5	1.3
2	1-aminopyrene	**1a**	CD_3OD	--[c]	--[c]
3	anthracene[b]	**1b**	water	7.8×10^3	5.3
4	pyrene[b]	**1b**	water	5.0×10^4	6.4
5	perylene[b]	**1a**	water	6.8×10^5	8.0

[a] Determined by ^{1}H NMR titration.
[b] Determined by a dye solubilization method with linear fitting of the experimental data (see text). The R value is 0.982, 0.994, and 0.982 for anthracene, pyrene, and perylene, respectively.
[c] Nearly no change in chemical shifts occurred during ^{1}H NMR spectroscopic titration, suggesting negligible binding.

Such a low binding affinity (–ΔG = 1.3 kcal/mol) is entirely unsatisfactory. Weak binding may have resulted from tight intramolecular aggregation among the cholate units of **1a**. This is quite possible, because the cholate groups are very close to one another. Intramolecular aggregation, nevertheless, does not seem to cause any problems in the reversed-micelle-like conformer, as the hydrophilic guests are bound with reasonable strength. This contrast is likely due to the curvature of the cholate backbone, which is bent toward the hydrophilic α face and is expected to prevent tight aggregation of the α faces in the reversed-micelle-like conformer.

Scheme 1. Synthesis of the water-soluble amphiphilic basket **3**.



Reaction conditions: (a) K_2CO_3, ethyl bromoacetate, refluxing acetone; (b) $LiAlH_4$, THF; (c) NaH, $MsO(CH_2CH_2O)_3CH_3$, DMF; (d) HNO_3, HOAc, CH_2Cl_2; (e) $SnCl_2$, refluxing MeOH; (f) BOP, DIPEA, **7**, DMF; (g) PPh_3, THF, H_2O; (h) HCl, MeOH.

When the solvent is changed from methanol/water (80/20) to pure methanol, the methyl proton signals on the cholates no longer experience any shifts with the addition of 1-aminopyrene, suggesting negligible binding (entry 2 of Table 2). Hence, solvophobic interaction is also the main driving force in this conformer.[18] Encouraged by this fact, we decided to prepare a water-soluble version of the amphiphilic basket.

The cationic host **2** is prepared according to Scheme 1. To increase the water-solubility of the calixarene, we attached oligomeric ethylene glycol chains to its lower rim. A literature procedure describes direct attachment of triethylene glycol monomethyl ether to *tert*-butylcalix[4]arene **3** under standard alkylation conditions (i.e., NaH, RBr).[19] However, the cone-conformer was one of four products formed. In our synthesis, we have avoided this problem by using ester **4** as the key intermediate. Ester **4** is prepared in a high yield according to a literature procedure, and, most importantly, is already in the cone-conformation.[10] It was reduced by lithium aluminum hydride, alkylated by the mesylate of triethylene glycol monomethyl ether, and nitrated to afford **5** in an overall 54% yield. The azidocholic acid (**7**)[20] is coupled to amine **6**, and the resulting product is reduced and protonated to afford the final water-soluble basket **2**.

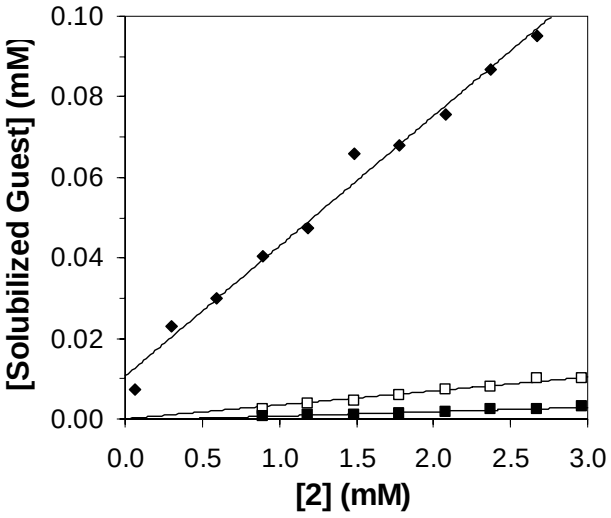


Figure 4. Solubilization of anthracene (□), pyrene (◆), and perylene (■) in water by **2**. Theoretical lines are line fitting of the experimental data.

With the water-soluble basket **2** in hand, we performed solubilization of anthracene and perylene, in addition to pyrene. These polycyclic aromatic hydrocarbons have extremely low solubility in water; thus, their binding can be monitored by enhanced solubilization. The experiment is similar to the dye-solubilization test used in the characterization of the critical micelle concentration (CMC) of surfactants.[22] In these experiments, a hydrophobic dye, which has nearly zero solubility in water below the CMC, is solubilized by surfactant micelles above the CMC. When the concentration of the solubilized dye is plotted against the concentration of the surfactant, a kinked curve is therefore obtained, with the inflection point corresponding to the CMC. In fact, pyrene has been frequently used to determine the CMC of surfactants because of its low water-solubility and fluorescence (which allows for its sensitive detection).[23]

Solubilization of the aromatic compounds by basket **2** clearly does not follow the pattern of typical surfactants. Instead of a kinked curve, the concentration of the solubilized polycyclic aromatics is linearly related to the concentration of **2** (Figure 4). The absence of a

concentration dependence in the solubilizing power suggests that aggregation is not necessary for **2** to solubilize hydrophobic guests. In other words, **2** does not have a CMC and truly qualifies as a unimolecular micelle. Our experiments indicate that basket **2** is most efficient in solubilizing pyrene, followed by anthracene and perylene. More efficient solubilization, nonetheless, does not mean stronger binding, because the amount of the solubilized guest also depends on the solubility of the guest by itself. For 1:1 complexation,[24] the binding constant can be calculated from these dye-solubilization experiments according to the following equation:[25]

$$s = s_0 + \{K_a s_0 / (1 + K_a s_0)\}[\text{host}]$$

in which s_0 is the solubility of the guest in the absence of any host, s is the solubility of the guest at a given host concentration [host], and K_a is the binding constant. Because s_0 has an extremely large effect on the calculation of K_a, but cannot be determined accurately at the intercept, we used the literature values instead (s_0 = 0.45, 0.67, and 0.0016 μM) for these anthracene, pyrene, and perylene, respectively.[26] The binding constants (K_a) obtained for three aromatic compounds are extremely large: 7.8×10^3, 5.0×10^4, and 6.8×10^5 M^{-1} for anthracene, pyrene, and perylene (entries 3, 4, and 5 of Table 2). Strong binding is probably a result of much higher solvophobic driving force in water as compared to aqueous methanol. It may also be due to poor intramolecular aggregation among the cholates, which are now positively charged. These binding constants correspond to $-\Delta G$ of 5.3, 6.4, and 8.0 kcal/mol, respectively. Therefore, the binding affinity increases linearly with the size of the aromatic guests. Such a trend is consistent with the solvophobic binding mechanism, because the strength of the solvophobic interaction is directly proportional to the area of the solvophobic surface removed from solvent contract during complexation.[4]

Conclusions

In summary, we have shown that judicious introduction of conformational flexibility converts an otherwise simple host into a novel environmentally responsive molecule. The binding properties respond to solvent changes as the host undergoes conformational changes. The reversed-micelle-like conformer prefers hydrophilic guests in solvent mixtures consisting of mostly a nonpolar solvent with a small amount of a polar solvent. Preferential solvation of the hydrophilic faces of the cholate groups by the polar solvent is important to the stability of the reversed-micelle-like conformer. Too strong solvation, however, leads to weak binding, because the polar solvent molecules entrapped by the host cannot be easily displaced by the guest. The micelle-like conformer binds hydrophobic guests in polar solvents. Binding is weak for 1-aminopyrene ($-\Delta G < 1.5$ kcal/mol) in a methanol/water (80/20) mixture. In pure water, however, very strong binding ($-\Delta G = 5–8$ kcal/mol) is observed for anthracene, pyrene, and perylene.

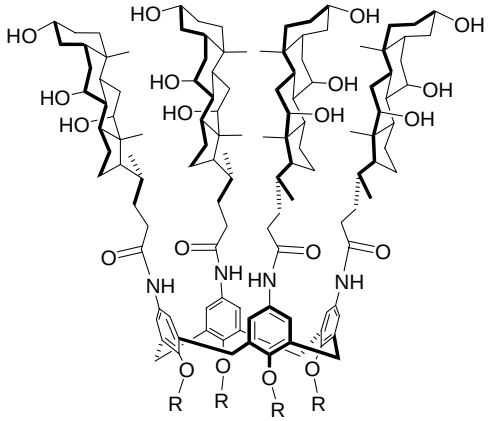
Experimental Section

General Methods

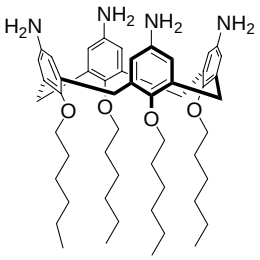
Anhydrous tetrahydrofuran (THF) and methylene chloride were dried by passage through a column of activated alumina under compressed nitrogen. Cholic acid was crystallized from 95% ethanol and dried at 90 °C under vacuum. All other reagents and solvents were of A.C.S. certified grade or higher, and were used as received from commercial suppliers. All glassware and syringes were dried in an oven at least overnight prior to use. All aqueous solutions for the dye solubilization measurements were prepared using Millipore water.

Synthesis

Compound 1a. Compound **1a** was synthesized according to a literature procedure.[30] ^{1}H NMR (CD_3OD, 300 MHz, δ) 7.00 (d, J = 2.4 Hz, 4H), 6.84 (d, J = 2.4 Hz, 4H), 4.54 (d, J = 12.6 Hz, 4H), 4.12 (t, J = 5.7 Hz, 8H), 3.96 (s, 4H), 3.90 (t, J = 5.4 Hz, 8H), 3.80 (s, 4H), 2.40–0.90 (series of m, 120H), 0.72 (s, 12H); ^{13}C NMR (CD_3OD, 75 MHz, δ) 173.4, 152.8, 135.0, 133.0, 120.9, 73. 8, 73.7, 73.57, 72.9, 72.8, 71.8, 71.7, 69.8, 67.9, 66.3, 46.4, 44.4, 42.0, 41.8, 39.9, 39.3, 38.1, 36.3, 36.0, 35.6, 35.4 (two peaks), 34.8, 34.0, 32.0, 31.1, 30.8, 30.1, 28.5, 27.9, 27.7, 27.6, 26.7, 23.23, 23.18, 22.3, 17.0, 16.6, 14.8, 12.2, 12.1. MALDI-TOFMS: $[M+Na]^+$calcd. for $C_{140}H_{212}N_4\,NaO_{24}$: 2358.2; found: 2358.3.



1a, R = $CH_2CH_2OCH_2CH_3$
1b, R = $(CH_2)_5CH_3$



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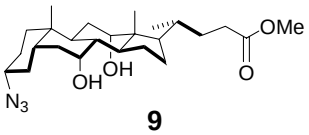

9

Compound 8. This aminocalixarene was synthesized according to a literature procedure.[31] ^{1}H NMR ($CDCl_3$, 300 MHz, δ) 6.07 (s, 8H), 4.29 (d, J = 13.2 Hz, 4H), 3.74 (t, J = 7.6 Hz, 8H), 2.90 (d, J = 13.2 Hz, 4H), 1.90–1.80 (m, 8H), 1.45–1.13 (m, 24H), 0.89 (t, J = 7.2 Hz, 12H).

Compound 1b. A mixture of cholic acid (195 mg, 0.48 mmol), **8** (74 mg, 0.10 mmol), and *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDCI) hydrogen chloride salt (109 mg, 0.57 mmol) were stirred in anhydrous CH_2Cl_2 (5 mL) at room temperature under N_2 for 24 h. Solvent was evaporated *in vacuo*. The residue was dissolved in methanol (0.5 mL) and precipitated in water (5 mL). The product was purified by column chromatography over silica gel using MeOH/CH_2Cl_2 (1/10) as eluents to give a light yellow powder (155 mg, 70 % yield). ^{1}H NMR (CD_3OD, 300 MHz, δ) 6.97 (d, J = 2.4 Hz, 4H), 6.82 (d, J = 2.4 Hz, 4H), 4.44 (d, J = 12.0 Hz, 4H), 4.96 (s, 4H), 3.88 (t, J = 6.6 Hz, 8H), 3.80 (s, 4H), 3.37 (m, 4H), 3.11 (d, J = 12.0 Hz, 4H), 2.40–0.90 (m, 120H), 0.72 (s, 12H); ^{13}C NMR (CD_3OD, 75 MHz, δ) 173.6, 153.3, 135.1, 132.3, 121.0, 75.6, 73.1, 71.7, 68.2, 47.1, 46.5, 41.8, 41.8, 39.73, 39.72, 39.3, 35.9, 35.4, 34.9, 34.0, 32.3, 32.0, 30.4, 30.1, 28.4, 27.7, 26.6, 26.2, 23.34, 23.32, 23.0, 22.4, 17.1, 13.8, 12.4; MALDI-TOFMS: $[M+Na]^+$ calcd. for $C_{148}H_{228}N_4NaO_{20}$: 2405.7; found: 2405.7.

Compound 4.[32] *t*-Butylcalix[4]arene **3** (5.02 g, 7.7 mmol), ethyl bromoacetate (12.8 g, 77 mmol), and K_2CO_3 (10.6 g, 77 mmol) were combined with dry acetone (100 mL). The reaction mixture was heated to reflux for 5 days. After the mixture was cooled to room temperature, the solid was filtered and washed with acetone (10 mL). The combined organic solution was concentrated *in vacuo*. The oily residue was crystallized from ethanol to give a white powder (5.84 g, 76% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, δ) 6.77 (s, 8H), 4.85 (d, J = 12.6 Hz, 4H), 4.80 (s, 8H), 4.21 (q, J = 7.2 Hz, 8H), 3.19 (d, J = 12.6 Hz, 4H), 1.29 (t, J = 7.2 Hz, 12H), 1.07 (s, 36H).

Compound 9.[33] Methanesulfonic acid (3.2 mL, 7.81 mmol) was added by a syringe to a solution of methyl cholate (10.0 g, 24 mmol) and PPh_3 (18.8 g, 72 mmol) in anhydrous THF (120 mL). The reaction mixture was warmed to 40–50 °C. Diisopropyl azodicarboxylate (14.0

mL, 72 mmol) was added dropwise by a syringe over a 15 minute period. The mixture was stirred for 24 h at 40–50 °C under N_2. The mixture was cooled to room temperature and the white solid (mostly triphenylphosphine oxide) was removed by filtration. The filtrate was concentrated *in vacuo* and purified by flash chromatography over silica gel using EtOAc/hexane (3:1) as the eluent to afford a viscous oil (slightly impure). The oil was dissolved in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU, 50 mL). Sodium azide (4.7 g, 72 mmol) was added. The reaction mixture was stirred at 50 °C for 9 h. The mixture was poured into water (100 mL) and extracted with ethyl acetate (2 × 50 mL). The combined organic phase was washed with water, dried over $MgSO_4$, filtered, concentrated by rotary evaporation, and purified by column chromatography over silica gel using EtOAc/hexane (1:4) as the eluent to afford a viscous oil (4.59 g, 45% yield). ^{1}H NMR (300 MHz, $CDCl_3$, TMS, δ) 3.99 (s, 1H), 3.86 (s 1H), 3.67 (s, CO_2CH_3, 3H), 3.09–3.22 (m, 1H), 1.34–2.36 (series of m, 27H), 0.96–0.98 (d, J = 8.7 Hz, 3H), 0.91(s, 3H), 0.69 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, δ) 175.0, 73.2, 68.5, 61.5, 51.7, 47.4, 46.8, 42.1, 42.0, 39.6, 35.7, 35.6, 35.5, 35.0, 34.8, 31.2, 31.0, 28.4, 27.7, 27.0, 26.8, 23.4, 22.8, 17.5, 12.7.

Compound 5. Compound **4** (2.194 g, 2.21 mmol) was dissolved in anhydrous THF (20 mL). Lithium aluminum hydride (1.0 M in ether, 11.2 mL, 11.2 mmol) was added by a syringe. The mixture was stirred at room temperature under N_2 for 3.5 h. The reaction was quenched by slow addition of EtOAc (5 mL), followed by 6 N HCl (20 mL) and brine (20 mL). The aqueous layer was extracted with ether (40 mL). The combined organic phase was dried ($MgSO_4/K_2CO_3$), concentrated *in vacuo*, and pumped dry at 70 °C. The alcohol intermediate (1.765 g) was combined with $MsO(CH_2CH_2O)_3CH_3$[26] (5.400 g, 22.3 mmol), and Bu_4NI (0.077 g, 0.21 mmol) in anhydrous THF (50 mL). NaH (60%, 0.912 g, 22.8 mmol) was added in one

portion. The mixture was heated to reflux under N_2 for 23 h. Another batch of the mesylate (1.07 g, 2.42 mmol) and NaH (0.205 g, 5.13 mmol) was added. After another 4.5 h, the reaction was cooled to room temperature and quenched by the careful addition of water (10 mL). The mixture was extracted with ether (40 mL). The combined organic phase was dried ($MgSO_4$) and concentrated *in vacuo*. The residual oil was dissolved in CH_2Cl_2/HOAc (20 mL/20 mL) and cooled to 0 °C. Nitric acid (90%, 10 mL) was added slowly. The solution was stirred at room temperature for 3 h, diluted with chloroform (30 mL) and water (60 mL). The organic phase was evaporated *in vacuo*. The residue was purified by column chromatography over silica gel using chloroform/acetone (1/1) as the eluent to give an orange oil. ^{1}H NMR (400 MHz, $CDCl_3$, δ) 7.42 (s, 8H), 4.57 (d, J = 14.0 Hz, 4H), 4.16 (br s, 8H), 3.72 (br s, 8H), 3.55–3.40 (m, 48H), 3.30 (d, J = 14.0 Hz, 4H), 3.24 (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, δ) 162.0, 142.9, 135.9, 124.0, 77.7, 74.6, 72.0, 70.72, 70.68, 70.6, 70.5, 59.1, 31.2. ESI-MS (*m/z*): $[M + K + H]^{2+}$ calcd for $C_{64}H_{93}N_4KO_{28}$, 702.5; found, 702.0.

Compound 6. A solution of compound **5** (412 mg, 0.302 mmol) and $SnCl_2 \cdot 2H_2O$ (857 mg, 2.80 mmol) in MeOH (15 mL) was heated to reflux for 24 h. NaOH (2 N, 30 mL) was added. The aqueous layer was extracted with chloroform (3 × 40 mL). The combined organic phase was washed with brine (20 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give a brown oil (337 mg, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$, δ) 5.97 (s, 8H), 4.23 (d, J = 13.2 Hz, 4H), 3.90 (t, J = 5.6 Hz, 8H), 3.75 (t, J = 5.6 Hz, 8H), 3.65–3.46 (m, 48H), 3.30 (s, 12H), 2.82 (d, J = 13.2 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$, δ) 149.7, 140.8, 135.6, 115.8, 73.0, 72.1, 70.8, 70.7, 70.5, 59.2, 31.3. ESI-MS (*m/z*): $[M + H]^+$ calcd for $C_{64}H_{101}N_4O_{20}$, 1245.5; found, 1246.0; $[M + 4K]^{4+}$ calcd for $C_{64}H_{100}N_4K_4O_{20}$, 350.3; found, 350.0.

Compound 7. LiOH (2M, 45 ml, 90 mmol) was added to the solution of compound **9** (4.0 g, 8.95 mmol) in methanol (50 mL). The mixture was stirred at room temperature for 21 h. HCl (2N) was added until pH = 4–5. The mixture was extracted ethyl acetate (2 × 80 mL). The combined organic phase was washed with water, dried over $MgSO_4$, and concentrated *in vacuo* to afford a white powder (3.73 g, 96% yield). ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ = 1:1, δ) 4.01 (br s, 1H), 3.87 (br s, 1H), 3.28–3.12 (m, 1H), 2.49–1.06 (series of m, 27H), 1.05 (d, 3H), 0.92 (s, 3H), 0.69 (s, 3H).

Compound 2. Compound **7** (110.3 mg, 0.254 mmol), compound **6** (63.3 mg, 0.0508 mmol), and O-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU, 97.6 mg, 0.257 mmol) were dissolved in anhydrous THF (3 mL). Diisopropylethylamine (91.6 mg, 0.709 mmol) was added. The mixture was heated to reflux under N_2 for 24 h. Solvent was evaporated in vacuo. The residue was purified by column chromatography over silica gel and preparative TLC using chloroform/methanol (15/1) as the eluent to afford a brown glass. The tetraazide intermediate and triphenylphosphine (41.0 mg, 0.156 mmol) was dissolved in THF/water (80/20, 2 mL). The mixture was heated to reflux for 14 h. Another batch of triphenylphosphine (39.5 mg) was added. The reaction was continued for another 6 h. Solvent was removed *in vacuo*. The residue was purified by preparative TLC using chloroform/methanol/ammonium hydroxide (5/3/1) as the developing solvents to afford a light brown glass (24.3 mg, 19%). ^{1}H NMR (400 MHz, CD_3OD, δ) 6.99 (s, 4H), 6.87 (s, 4H), 4.57 (d, J = 12.8 Hz, 4H), 4.15 (b, 8H), 4.02–3.91 (m, 12H), 3.80 (s, 4H), 3.71-3.46 (m, 48H), 3.34 (s, 12H), 3.12 (d, J = 12.8 Hz, 4H), 2.77 (t, J = 10.4 Hz, 4H), 2.44–1.01 (series of m, 108H), 0.95 (s, 12H), 0.72 (s, 12H); ^{13}C NMR (75 MHz, CD_3OD, δ) 173.4, 153.0, 135.1, 135.0, 132.8, 120.8, 120.7, 73.5, 72.6, 71.8, 70.6, 70.4, 70.2, 67.5, 58.0, 51.5, 47.1, 46.4, 41.9, 41.8, 39.9,

36.0, 34.9, 34.6, 34.5, 34.3, 33.9, 31.9, 31.1, 29.6, 28.5, 27.7, 26.8, 25.7, 23.1, 22.0, 16.7, 12.1. MALDI-TOFMS (*m/z*): $[M + H]^+$ calcd for $C_{160}H_{257}N_8O_{32}$, 2803.78; found, 2811.15. The glass was dissolved in MeOH (2 mL). An excess of HCl in MeOH (prepared by addition of acetyl chloride to MeOH)[28] was added. After 1 h, the solvent and HCl were evaporated by a gentle N_2 flow. The white solid was pumped under high vacuum to afford a light brown power.

Job Plot. Stock solutions (1.43 mM) of **1a** and phenyl β-D-glucopyranoside in carbon tetrachloride/deuterated methanol (90/10 = v/v) were prepared. In eleven separate NMR tubes, portions of the two solutions were added such that their ratios changed from 0 to 1, while maintaining a total volume of 0.6 ml. A ^{1}H NMR spectrum was taken for each sample. The changes in the chemical shifts of the *ortho*, *meta*, and *para*-protons of the phenyl in the guest were monitored. The maximum at 0.5 molar fraction indicated a 1:1 binding stoichiometry.

^{1}H NMR Spectroscopic Titrations. For the binding of hydrophilic guests, the guest was titrated with different amounts of the host, and the chemical shifts of the phenyl protons in the guest were monitored. For binding of the hydrophobic guests, the host was titrated with different amounts of the guest, and the chemical shifts of the methyl protons in the host were monitored. A typical procedure is as follows. Stock solutions of **1a** (0.050 M) and phenyl β-D-glucopyranoside (0.010 M) in CH_3OH were prepared. To 16 separate vials, 60 μL of the phenyl β-D-glucopyranoside solution was added, followed by 12, 16, 19, 23, 27, 31, 37, 43, 50, 58, 67, 79, 93, 111, 136 and 170 μL of **1a**. The solvent in each vial was removed in vacuo. Then 600 μL of CCl_4/CD_3OD (90/10) was added to each vial. The samples were gently shaken for 1 h and then transferred to 16 separate NMR tubes. A ^{1}H NMR spectrum was taken for each sample and the chemical shifts of the phenyl protons of the guest were measured. The

binding constants (K_a) were obtained by least-square nonlinear curving fitting of the titration data.

Dye solubilization. A typical procedure is as follows. A stock solution (2.96 mM) of **2** was prepared in Millipore water. To eleven separate vials, 500, 450, 400, 350, 300, 250, 200, 150, 100, 50, and 10 μL of stock solution were added. Millipore water was added to make the total volume of each sample 500 μL. These solutions were gently rocked in the presence of excess solid pyrene for three days. The excess pyrene was removed by filtration through syringe filters [Milipore Millex hydrophilic poly(tetragluoroethylene) filters, 0.45 μm]. An aliquot of 100 μL of each sample was diluted with 2.5 mL of absolute ethanol. The fluorescence intensity of each sample was measured in a quartz cuvet. Each experiment was repeated three times with separately prepared solutions. The concentration of the solubilized pyrene was determined by a calibration curve. The excitation wavelength was 340, 320, and 400 nm for anthracene, pyrene, and perylene, respectively.

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CHAPTER 5. An amphiphilic molecular basket sensitive to both solvent changes and UV irradiation

A paper accepted in *The Journal of Organic Chemistry* **2006**[1]

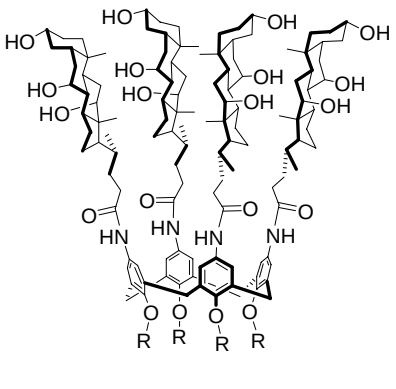
Abstracts

A molecular basket was obtained by linking four cholate units to a cone-shaped calix[4]arene scaffold through azobenzene spacers. The molecule turned its polar faces inward in nonpolar solvents to bind polar molecules, such as sugar derivatives. In polar solvents, the nonpolar faces turned inward, allowing the binding of hydrophobic guests, such as pyrene. The molecule could also respond to UV irradiation by *trans-cis* isomerization of the azobenzene spacers. Response toward both solvents and UV light was fully reversible.

Introduction

Conformational control is a powerful approach to environmentally responsive materials, because the conformation of a molecule dictates many of its properties including size, shape, and distribution of functional groups, and yet may be altered easily by environmental stimuli. The interest in conformationally controlable molecules is highlighted in foldamer research, which aims at creating synthetic analogues of biopolymers that can adopt well-defined, compact conformations.[2] A benefit in creating responsive materials based on conformational changes is the possibility to integrate conformational responsiveness with other responsive mechanisms, so that materials sensitive to multiple stimuli may be rationally designed. We have been interested in using cholic acid as a building block to construct conformationally

controllable foldamers[3] and nonfoldamers.[4] We reported a "molecular basket" **1** that can reversibly switch between a micelle-like conformation (with the hydrophilic faces of cholates point outward) in polar environments and a reversed micelle-like conformation in nonpolar environments.[4a,5] As a result of the conformational change, the molecule can act as a tunable supramolecular host to bind polar guests in nonpolar solvents and nonpolar ones in polar solvents.[4b] As the ordered, micelle- or reversed-micelle-like conformations originate from intramolecular aggregation of the cholates, we reasoned that insertion of azobenzene[6,7] linkers would create a molecular basket **2** sensitive to both solvents and photoirradiation. The idea is that aggregation should be promoted by the straight *trans* azobenzene spacers but deteriorated by the kinked *cis* isomers. Complete *cis*-isomerization is probably unnecessary, as mixed *trans/cis* spacers, may be even worse for the alignment of the cholates than all *cis* ones. A similar concept also has been employed recently by the groups of Hecht[8] and Parquette[9] to prepare foldamers sensitive to both solvents and UV irradiation.



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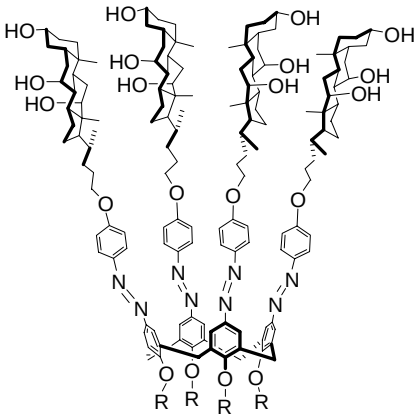

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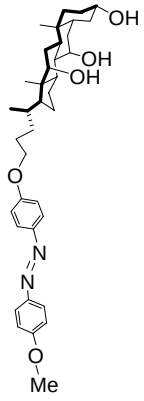

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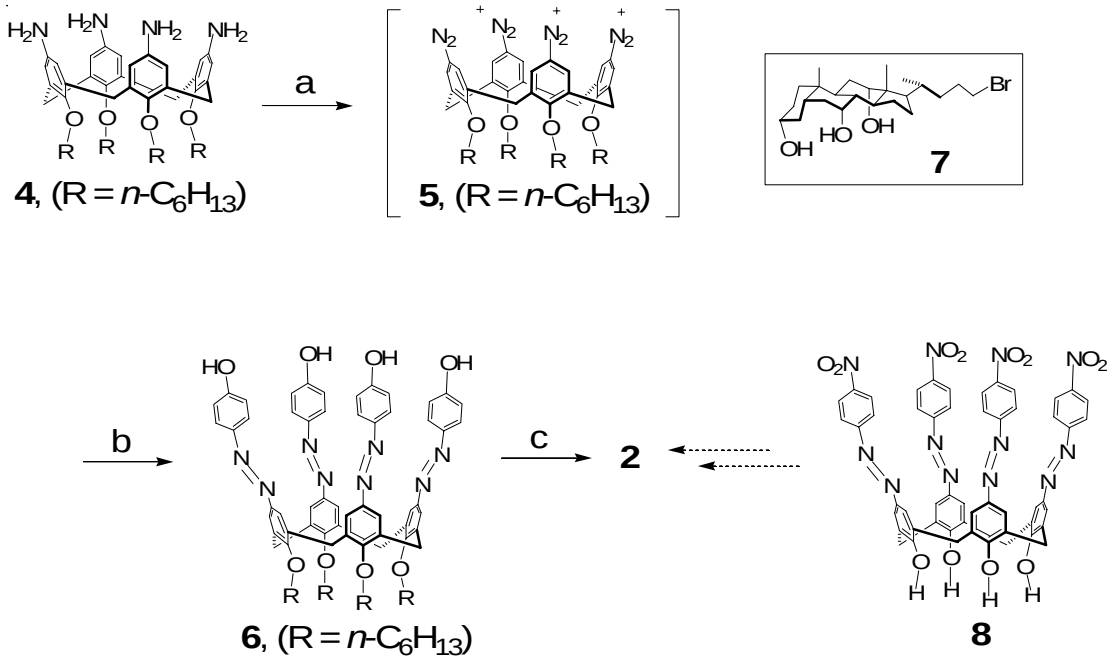
Azobenzene-derived calixarenes have attracted considerable interest from supramolecular chemists,[10] ever since Shinkai and co-workers discovered the autoaccelerative

diazo coupling of calix[4]arene.[11] Many calixarenes with azobenzene at the upper rim have been prepared according to Shinkai's method.[12] To ensure a basket-like conformation, we prefer to have the calix[4]arene scaffold preorganized into the cone conformation by alkyl substitution at the lower rim.[4a] However, with one exception,[12e] nearly all of the previously reported azocalixarenes have unsubstituted hydroxyl groups *para* to the azobenzene groups.

Results and discussion

The synthesis of **2** is illustrated in Scheme 1. Tetraaminocalix[4]arene (**4)** was prepared according to a literature procedure.[13] It was diazotized by nitrous acid at 0 °C in aqueous THF to afford the tetradiazonium intermediate **5**, which was reacted directly with an excess of phenol in THF and pyridine. Considering the instability of the highly crowded tetradiazonium salt, the yield of this reaction was remarkably high, over 70% if the reaction conditions were properly controlled. Precooling of all solutions (i.e., both **4**/$NaNO_2$ in aqueous THF and the aqueous HCl solution added to the first mixture) was extremely important. A slight increase in reaction temperature during diazotization could reduce the yield from >70% to <10%. The tetraphenol intermediate **6** was generally used in the next step without much purification. Alkylation with brominated cholate derivative **7** occurred smoothly in about 60% yield. In addition, compound **3** with a single cholate unit was prepared as a control. We did attempt an alternative route and prepared compound **8**.[14] Its alkylation to cone-shaped calixarene, however, was unsuccessful.

Scheme 1. Synthesis of compound **2**.



Reaction conditions: (a) $NaNO_2$, HCl, H_2O, THF; (b) phenol, pyridine, THF; (c) **7**, K_2CO_3, Bu_4NI, DMF.

The aromatic protons of **1** *ortho* to the amido groups appeared as a single peak when the molecule adopted a random conformation, but split into two peaks as the molecule assumed either the micelle- or reversed-micelle-like conformation.[4a,4c] Such a change was not observed in **2,** but the result was expected. Splitting of the aromatic peaks probably originated from hindered rotation of the N-Ar bonds[15] during intramolecular aggregation of the cholates,[4a] and was previously found to be absent whenever spacers were inserted in between the cholates and the calixarene or noncalixarene scaffold.[4c]

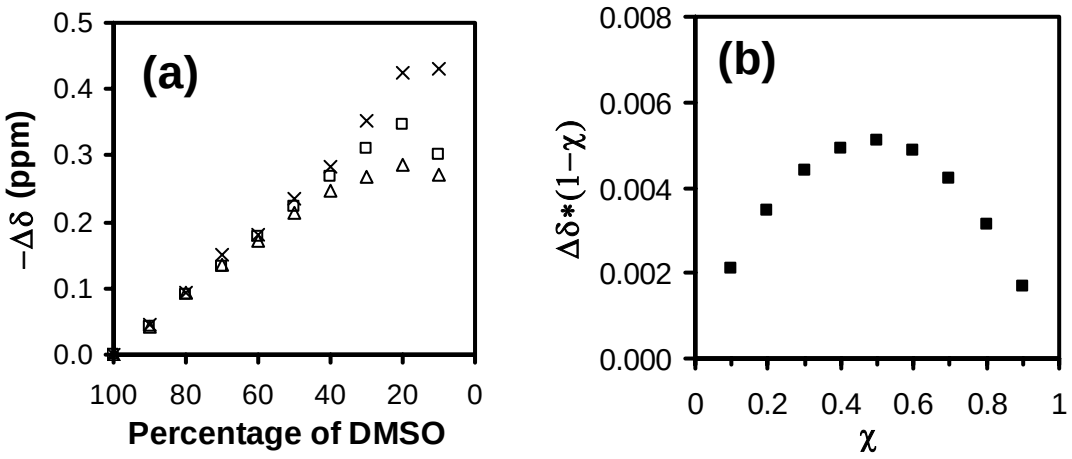
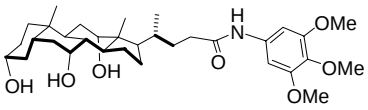


Figure 1. (a) Changes in the ^{1}H NMR chemical shifts of OH3 as a function of the solvent composition in mixtures of DMSO-d_6/CCl_4 for compounds **1** (△), **2** (□), and **9** (×). OH3 is the hydroxyl group on the A-ring of the steroid backbone. (b) The Job plots for the binding between **2** and phenyl β-D-glucopyranoside (**10**), in which χ is the molar fraction of the guest. The chemical shift changes (Δδ) are for the *para* proton of **10**.

Another way of studying the conformation of these baskets is to monitor the chemical shifts of the OH (or NH for **1**) protons during solvent titration. When the changes in chemical shift of the OH protons are plotted as a function of the DMSO percentage in CCl_4, the curves for the compounds capable of adopting the reversed-micelle-like conformation show distinct downward deviation from the control curve for a monomeric cholate, such as **9**.[4c] Such a deviation indicates a higher local concentration of DMSO near the OH protons than that in the bulk, and is a consequence of the reversed micelle-like conformer, which enriches DMSO from the solvent mixture by its inwardly facing polar groups. Although not as significant as in compound **1** (△, Figure 1a), a downward deviation was clearly visible for **2** below 20% DMSO (□).



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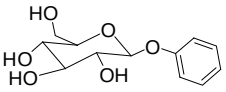

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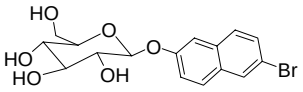

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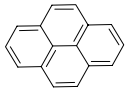

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We also studied the conformation of **2** through its guest-binding properties. In 5% CD_3OD/CCl_4, a mostly nonpolar mixture, **2** binds phenyl β-D-gluocopyranoside (10) with an association constant (K_a) of 380 ± 130 M^{-1}. Over the range of concentrations used for the ^1H NMR spectroscopic titration, the proton signals of the host showed no sign of broadening, indicating a lack of self-association. Binding was confirmed to be 1:1 by the Job plot (Figure 1b). This binding constant was essentially the same as that between **1** and **10** (K_a = 340 ± 60 M^{-1}).[2d] With extensive aromatic components (i.e., azobenzene) in the structure, binding by **2** should benefit from additional π–π interactions. Indeed, the binding constant (K_a = 700 ± 150 M^{-1}) almost doubled for guest **11**, which had a larger aromatic group than **10,** but otherwise shared the same hydrophilic substructure. The binding of polar molecules in a nonpolar mixture indicated that, similar to the parent basket **1**, azobasket **2** adopts a reversed micelle-like conformation.

Pyrene (**12**) was found to be a suitable guest for the micelle-like conformer.[4b] Its binding by **2** was evidenced by upfield shifts of the methyl protons on the hydrophobic faces of the cholates. Even though K_a was quite low, only about 5–10 M^{-1} in methanol for both **2** and **1**, binding with pyrene through the hydrophobic faces of the cholates did support the formation of micelle-like conformations in polar solvents. Previously, it was shown that much stronger binding could be obtained once the basket was made water-soluble to create a higher hydrophobic driving force.[4b]

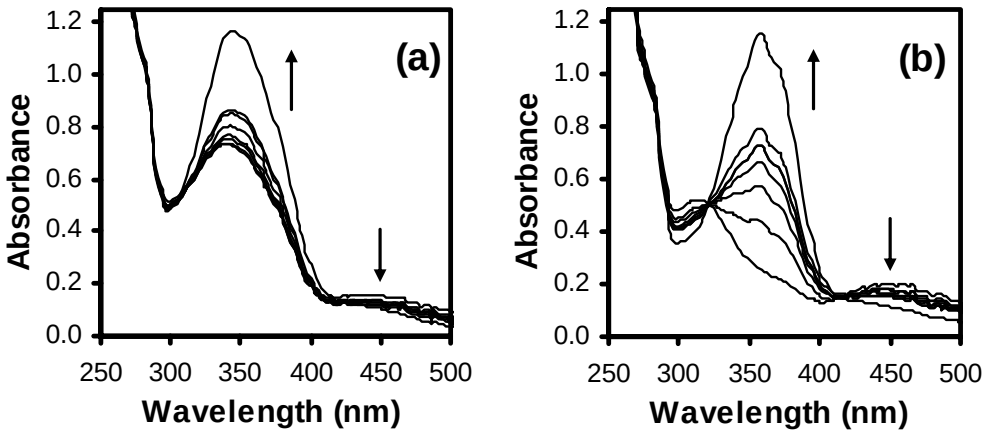


Figure 2. UV spectra of compounds (a) **2** and (b) **3** recorded at 0, 10, 20, 30, 40, 50, and 60 min, and then at 24 h after UV irradiation. The spectra at 24 h were nearly identical to those before irradiation. [Azobenzene] = 0.15 mM. Solvents = 5% MeOH/CCl_4 in both cases.

Azobasket **2** displayed a π–π* transition near 350 nm and a very weak n–π* band at 450 nm in the UV spectrum. With **2** irradiated by 360 nm UV light for five minutes, the π–π* band lost about 40% of the initial intensity, while the n–π* band grew stronger (Figure 2a), indicative of *trans–cis* isomerization.[6,7] Similar changes could be observed for the control **3**, but the extent of isomerization was much higher in this compound, as its π–π* band almost completely disappeared and was replaced by a peak near 310 nm assigned to the *cis*-isomer (Figure 2b).[6,7] The lower conversion in **2** probably did not come from its conformational preference, because similar situations occurred in other solvents (i.e., 50/50 or 95/5 methanol/CCl_4) that favored the random and micelle-like conformations. It seems that solvophobic forces were not strong enough to significantly influence the much higher energy photochemical process. Both compounds completely revert back to all *trans* configurations after 24 h in the dark. Recovery is again slower in **2** than in the monomeric control. Since

solvophobic interactions should help the all *trans* structure of **2**, its slower kinetics must be caused by factors (e.g., steric crowdedness) other than its conformational properties.

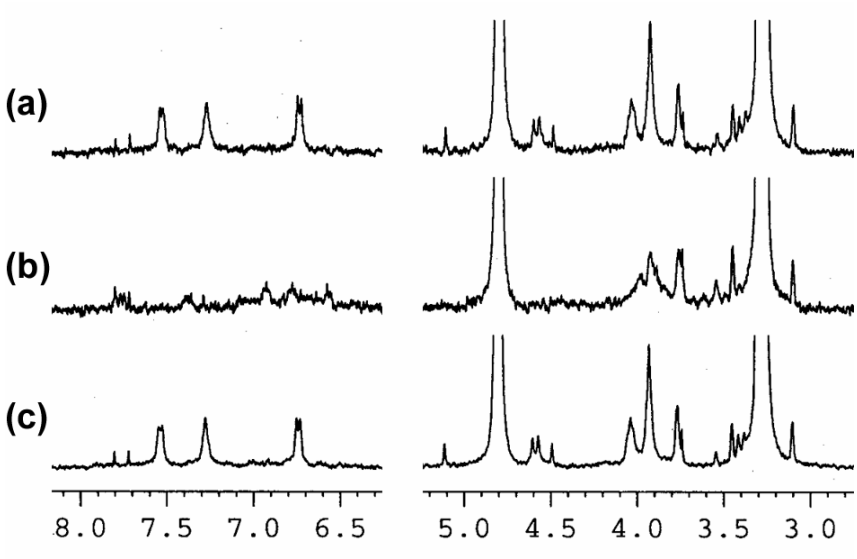


Figure 6. Portions of the ^{1}H NMR spectra of compound **2** (a) before, (b) immediately after, and (c) at 24 h in the dark after irradiation. The peaks between 3.3–4.1 ppm are from protons adjacent to the OH and O in **2**. The large peaks at 3.3 and 4.8 ppm come from undeuterated solvents. Solvent = 5% CD_3OD/CCl_4.

We also monitored the photoisomerization by ^{1}H NMR spectroscopy. Portions of the spectra for **2** are shown in Figure 3. Before irradiation, the aromatic region showed two doublets at ca. 6.7 & 7.5 ppm for the protons on the top aromatic rings and a single peak at 7.3 ppm for the bottom calixarene aromatic protons. The aromatic protons became extremely complex after UV irradiation (Figure 3b). At the same time, (part of) the AB quartet for the calixarene methylene bridge ($ArCH_2Ar$) at 4.6 ppm disappeared completely. It is unlikely that UV irradiation will change the preorganized cone-conformation of the calix[4]arene.

Disappearance of the $ArCH_2Ar$ signals probably happened as numerous configurational isomers were generated by partial conversion of the *trans* to the *cis* azobenzene. Importantly, the original spectrum recovered completely after 24 h in the dark, demonstrating the reversibility of the process.

Conclusions

In summary, we have combined the photoisomerization of azobenzene with solvent-induced conformational change, and synthesized a molecular basket (**2**) that showed dual responsive properties. Much improvement is still needed before it can be used as a smart delivery vehicle. The result, nonetheless, demonstrates that it is feasible to integrate conformational control with other switching mechanisms and rationally design materials responsive to multiple signals.

Experimental Section

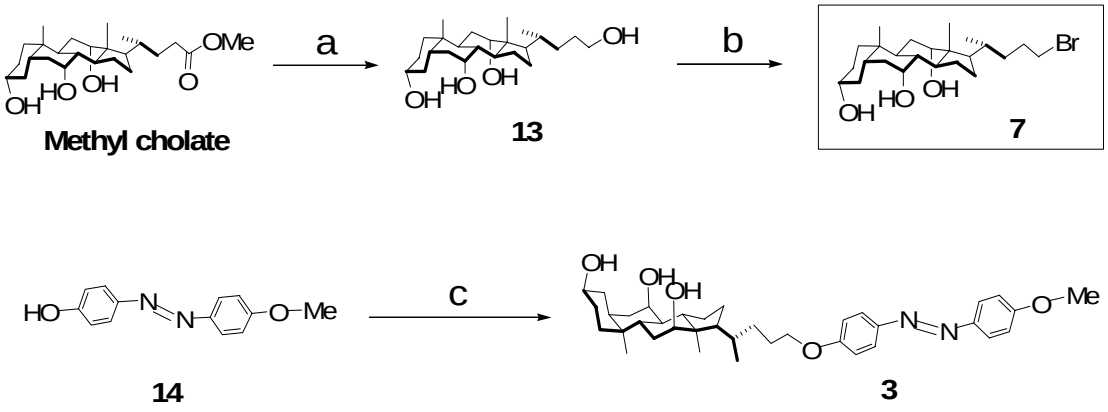
General Methods

Anhydrous tetrahydrofuran (THF) and methylene chloride were dried by passage through a column of activated alumina under compressed nitrogen. Cholic acid was crystallized from 95% ethanol and dried at 90 °C under a vacuum. All other reagents and solvents were of A.C.S. certified grade or higher, and were used as received from commercial suppliers. All glassware and syringes were dried in an oven at least overnight prior to use.

Synthesis

Compound 1. The synthesis of compound **1** was reported previously.[16]

Scheme 1. Synthesis of compound **3.**



Reaction conditions: (a) $LiAlH_4$, THF; (b) CBr_4, PPh_3, THF; (c) **7**, K_2CO_3, DMF.

Compound 13. Compound **13** was synthesized according to a literature procedure.[17] mp 221-223 °C; ^{1}H NMR (300 MHz, CD_3OD, δ) 3.96 (s, 1H), 3.80 (m, 1H), 3.51 (t, J =6.3 Hz, 2H), 3.37 (m, 1H), 2.34-0.91 (m, 30H), 0.72 (s, 3H).

Compound 7. Compound **7** was synthesized according to a modified literature procedure.[17] Compound **13** (1.10 g, 2.79 mmol) and Ph_3P (0.89 g, 3.38 mmol) were dissolved in anhydrous DMF (15 mL). CBr_4 (1.12 g, 3.38 mmol) was added slowly under a N_2 flush. After 6 h at rt, the reaction mixture was poured into H_2O (100 mL). The precipitate formed was collected by suction filtration and washed with water (2 × 5 mL). The final product was purified by column chromatography over silica gel using CH_2Cl_2/acetone as the eluent to give a white powder (511 mg, 40% yield). mp 120-122 °C; ^{1}H NMR (300 MHz, CD_3OD, δ) 3.95 (s, 1H), 3.79 (m, 1H), 3.41 (m, 3H), 2.23-0.91 (m, 30H), 0.72 (s, 3H).

Compound 4. Compound **4** was synthesized according to a literature procedure.[18] ^{1}H NMR (300 MHz, $CDCl_3$, δ) 6.07 (s, 8H), 4.29 (d, J = 13.2 Hz, 4H), 3.74 (t, J = 7.6 Hz, 8H), 2.90 (d, J = 13.2 Hz, 4H), 1.90-1.80 (m, 8H), 1.45-1.13 (m, 24H), 0.89 (t, J = 7.2 Hz, 12H).

Compound 6. An aqueous HCl solution (3%, 1 mL) was added to a solution of **4** (101 mg, 0.12 mmol) in THF (4 mL) at 0 °C. A solution of $NaNO_2$ (43 mg, 0.61 mmol) in H_2O (3 mL) precooled to 0 °C was added slowly by a syringe. The reaction mixture was stirred at rt for 1 h. A solution of phenol (115 mg, 1.22 mmol) in pyridine (2 mL) and THF (4 mL) was added slowly by a syringe at 0 °C. After 12 h at rt, the reaction mixture was poured slowly into H_2O (100 mL). The precipitate formed was collected by suction filtration and washed with water (2 × 10 mL). The product was dried *in vacuo* and used in the next step without further purification (108 mg, 0.087 mmol, 73 % yield). ^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.48 (d, J = 8.7 Hz, 8H), 7.28 (s, 8H), 6.68 (d, J = 8.7 Hz, 8H), 4.56 (d, J = 13.2 Hz, 4H), 4.00 (t, J = 7.2 Hz, 8H), 3.33 (d, J = 9.3 Hz, 4H), 1.98 (m, 8H), 1.48-1.39 (m, 24H), 0.96 (t, J = 6.6 Hz, 12H).

Compound 2. Compound **6** (108 mg, 0.087 mmol), **7** (199 mg, 0.44 mmol), K_2CO_3 (122 mg, 0.88 mmol), and Bu_4NI (10 mg, 0.028 mmol) were mixed with anhydrous DMF (5 mL). After 6 h at 50 °C, the reaction mixture was poured slowly into H_2O (100 mL). The precipitate was collected by suction filtration and washed with water (2 × 10 mL). The product was purified by column chromatography over silica gel using CHCl3/methanol as the eluent to give a yellow powder (148 mg, 62% yield). ^{1}H NMR (300 MHz, CD_3OD/CCl_4, δ) 7.49 (d, J = 8.7 Hz, 8H), 7.28 (s, 8H), 6.69 (d, J = 9.0 Hz, 8H), 4.58 (d, J = 6.9 Hz, 4H), 4.02 (t, J = 7.2 Hz, 8H), 3.94 (s, 4H), 3.78 (m, 4H), 3.39 (m, 16H), 2.22–0.90 (m, 120H), 0.70 (s, 12H); ^{13}C NMR (75 MHz, CD_3OD/CCl_4, δ) 161.2, 159.2, 148.3, 146.9, 135.6, 124.3, 123.1, 114.6, 75.8, 73.1, 71.7, 68.8, 68.3, 58.7, 47.4, 46.5, 41.8, 39.6, 39.3, 35.8, 35.4, 34.9, 34.7, 32.2, 30.5,

30.0, 28.3, 27.8, 26.5, 26.2, 23.8, 23.3, 23.0, 22.5, 19.7, 17.5, 14.0, 13.4, 12.5. MALDI-TOFMS (m/z): calcd. for $C_{172}H_{249}N_8O_{20}$ $[M+H]^+$: 2748.9; found: 2744.5.

Compound 3. Compound **14** (50 mg, 0.22 mmol), **7** (100 mg, 0.22 mmol), and K_2CO_3 (151 mg, 1.10 mmol) were dissolved in anhydrous THF (10 mL). The reaction mixture was heated to reflux for 12 h. Solvent was evaporated *in vacuo*. The product was purified by column chromatography over silica gel using $CHCl_3$/methanol as the eluent to give a yellow powder (82 mg, 0.14 mmol, 62% yield). ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$, δ) 7.85 (d, J = 3.0 Hz, 2H), 7.82 (d, J = 3.0 Hz, 2H), 7.06 (d, J = 5.7 Hz, 2H), 7.03 (d, J = 5.7 Hz, 2H), 4.04 (t, J = 6.3 Hz, 2H), 3.97 (s, 1H), 3.88 (s, 3H), 3.80 (m, 1H), 3.37 (m, 1H), 2.21 (m, 3H), 1.97-0.92 (m, 30H), 0.73 (s, 3H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$, δ) 161.6, 161.3, 146.9, 146.7, 124.3, 124.3, 114.7, 114.2, 73.0, 71.5, 68.8, 68.3, 55.5, 47.2, 46.3, 41.6, 41.3, 39.3, 39.2, 35.5, 35.2, 34.7, 34.4, 32.0, 29.8, 28.0, 27.6, 26.3, 25.9, 23.2, 22.4, 17.5, 12.4. MALDI-TOFMS (m/z): $[M + H]^+$ calcd for $C_{37}H_{52}N_2O_5$: 604.8; found: 605.8.

Job Plot. Two stock solutions (1.43 mM) of **2** and phenyl-β-D-glucopyranoside (**10**) in CCl_4/CD_3OD (v/v = 90/10) were prepared separately. In eleven separate NMR tubes, portions of the two solutions were added such that their ratios changed from 0 to 1 while maintaining a total volume of 0.6 mL. A ^{1}H NMR spectrum was recorded for each sample. The changes in the chemical shifts of the *ortho*, *meta*, and *para*-protons of the phenyl in phenyl β-D-glucopyranoside were monitored. The maximum at 0.5 molar fraction indicated a 1:1 binding stoichiometry.

^{1}H NMR Spectroscopic Titrations. For the binding of **10** and **11**, the guest was titrated with different amounts of the host, and the chemical shifts of the aromatic protons in the guest were monitored. A typical procedure is as follows. Stock solutions of **2** (0.050 M)

and phenyl β-D-glucopyranoside (**10**) (0.010 M) in CH_3OH were prepared. To 12 separate vials, 60 μL of the phenyl β-D-glucopyranoside solution was added, followed by 11, 14, 17, 20, 24, 29, 34, 39, 46, 54, 63, 74, 88, 106, and 130 μL of **2**. The solvent in each vial was completely evaporated. Then 600 μL of CCl_4/CD_3OD (v/v = 90/10) was added to each vial. The samples were gently shaken for 1 h in dark and then transferred to 12 separate NMR tubes. A ^1H NMR spectrum was recorded for each sample and the chemical shifts of the phenyl protons of phenyl β-D-glucopyranoside were monitored. The binding constants (K_a) were obtained by nonlinear least-squares curve fitting of the titration data.

Acknowledgments

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CHAPTER 6. Solvent-responsive metalloporphyrins: binding and catalysis

Taken from a paper accepted by *Organometallics* **2006**[1]

Abstracts

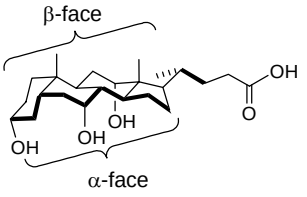
A cholate-functionalized tetraphenylporphyrin (H_2CFTPP) was obtained by attaching eight cholate units at the *meta* positions of the phenyl rings. Zn(CFTPP) favored binding a hydrophilic pyridyl ligand over a hydrophobic analogue in nonpolar solvents such as 20% MeOH/CCl_4, but had the reverse selectivity in 95% MeOH/CCl_4. Tunability of the ligand binding resulted from the cholates that aggregated intramolecularly to form either unimolecular micelle- or reversed micelle-like structures depending on solvent polarity. The micelle-like structures appear less well organized than the reversed-micelle-like conformations, and might be induced by hydrophobic guests. The solvent-dependent intramolecular aggregation of cholates can be used to tune the catalytic activity of an iron porphyrin derivative.

Introduction

Conformational control is a strategy employed by nature to achieve selectivity and regulate activity in enzymes. According to the induced-fit model,[2] the substrate of an enzyme can "turn on" catalysis by bringing the catalytic groups into proper alignment, whereas a nonsubstrate, even having the same reactive group, remains untransformed because it cannot induce the necessary conformational change in the enzyme. Signal molecules—referred to as effectors and inhibitors, depending on whether the molecule activates or deactivates the catalyst—can alter the conformations of allosteric enzymes and, in consequence, serve to

regulate their properties.[3] Chemists have long been intrigued by these features of biological catalysts but, until now, have not been able to develop a general approach toward conformationally controllable catalysts.[4] In recent years, there has been great interest in developing synthetic oligomers (i.e., foldamers) that can adopt biomolecule-like, folded conformations.[5] Advancements in conformational control in synthetic molecules will not only shed light on how biomolecules fold and function, but also enable the development of synthetic counterparts with similar responsive and tunable properties.

We have been interested in using cholic acid as a building block to construct both foldamers[6,7] and nonfoldamers[8–10] whose conformations and properties can be reversibly switched. With a large steroid backbone that positions hydrophilic and hydrophobic groups on opposing faces, cholic acid is uniquely suited for solvophobically driven conformational changes. Previously, we synthesized an amphiphilic "molecular basket" by coupling four cholates to a cone-shaped, 4-aminocalix[4]arene scaffold.[8] The molecule adopts micelle-like conformations in polar solvents with the hydrophilic α faces turned outward and reversed micelle-like conformations in nonpolar ones with the α faces inward.[11] In this article, we extend the concept to construction of a solvent-responsive metalloporphyrin. Both its binding and catalytic properties can be altered using solvent polarity as the stimulant. Through this strategy, substrates that differ by only one or two hydroxyl groups remote from the reactive site (i.e., C=C bond) can be clearly distinguished by the metalloporphyrin.



Cholic acid

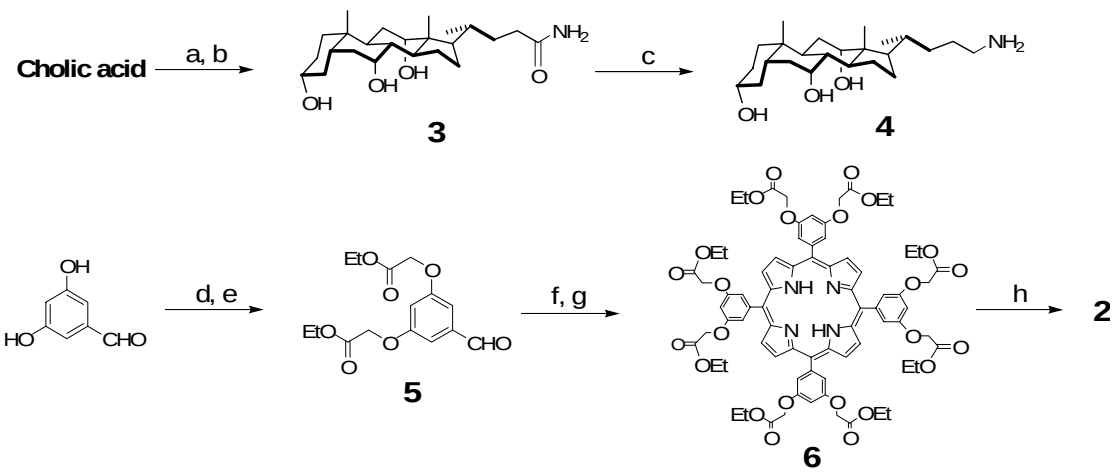
1 (H_2TPP)

2 (H_2CFTPP)

Results and discussion

Design and Synthesis. Metalloporphyrins were chosen as the catalytic platform for several reasons. First, they are important catalysts in both biological and synthetic transformations such as olefin epoxidation, alkane hydroxylation, cyclopropanation, and a range of other reactions.[12] Second, they can tolerate many functional groups and solvents. Common polar groups such as hydroxyl, amides, and ethers do not interfere with their catalysis. Third, the phenyl groups in tetraphenylporphyrin (H_2TPP) **1** are nearly perpendicular to the porphyrin plane[13] and introduction of functional groups with predictable spatial orientation is possible on the phenyl rings. Hence, for an octacholate-functionalized tetraphenylporphyrin (H_2CFTPP) **2**,[14] it is reasonable to expect that the four cholate units can interact intramolecularly to create a microenvironment above and below the catalytic site (i.e., metalloporphyrin) that can be used to regulate the activity/selectivity of the catalyst.[15]

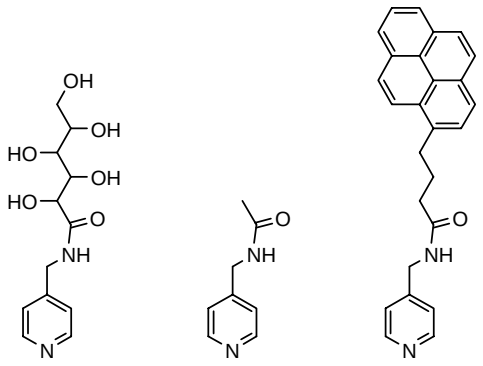
Scheme 1. Synthesis of H_2CFTPP, **2**.



Reaction conditions: (a) N-HO-Su, DCC, CH_2Cl_2; (b) NH_4OH, DMF; (c) $LiAlH_4$, THF; (d) $BrCH_2CO_2Et$, K_2CO_3, acetone; (e) pyrrole, BF_3-OEt; (f) DDQ; (g) NaOH; (h) **4**, BOP, DIPEA, DMF.

H_2CFTPP (**2**) was synthesized using the route shown in Scheme 1. Cholic acid was converted to the N-hydroxysuccinimide ester using dicyclohexylcarbodiimide (DCC) as the coupling reagent. The activated ester was transformed into cholate amide **3**, which was reduced by $LiAlH_4$ to afford amino cholate **4**. To obtain the octaester porphyrin **6**, 3,5-dihydroxybenzaldehyde was first alkylated with ethyl 2-bromoacetate. The resulting ester-substituted benzaldehyde **5** was condensed with pyrrole in the presence of a Lewis acid, BF_3•OEt_2, to afford the desired product **6** in 41% yield.[16] After basic hydrolysis of **6**, the resulting octacarboxylic acid was coupled to amine **4** using benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) to afford H_2CFTPP (**2**). (The synthesis of **6** and its hydrolysis were performed by Dr. Yibo Zhou in Prof. Keith Woo's group at Iowa State University.)[1]

Binding Properties. The scaffolds used in all the previously synthesized cholate-derived molecular baskets were "compact".[8–10] For example, the distance between the two opposite amino groups in 4-aminocalix[4]arene is about 6–8 Å according to CPK models. In these structures, close proximity of the cholates allows efficient intramolecular association. The TPP scaffold, however, is larger (the distance between the two *meta*-hydrogens on the phenyl rings across the porphyrin is about 15 Å). Therefore, a potential concern for H_2CFTPP (**2**) was whether the cholates could interact intramolecularly to create a microenvironment over the two faces of the porphyrin. Without intramolecular micellization or reversed micellization, the cholates would have little effect on the catalytic behavior of the metalloporphyrin. Another possible problem was that a cholate might prefer to interact with the other cholate on the same phenyl ring instead of with the other three cholates on the same side of porphyrin face. In this case, intramolecular association of cholates occurs, but in four pairs that probably would not be able to significantly influence the catalytic activity.



7 **8** **9**

In order to address these questions, we synthesized three derivatives of 4-aminopyridine by acylating the amino group with a hydrophilic (**7**), a "neutral" (**8**), and a hydrophobic (**9**) group. If the proposed intramolecular micellization or reversed micellization does happen, the

hydrophilic ligand **7** should be preferred by Zn(CFTPP) in nonpolar solvents and the hydrophobic analogue **9** in polar media. Compound **8** is a control used to investigate the general solvent-effect for Zn-pyridine complexation. Because the different functional groups in **7**–**9** are remote and "insulated" from the pyridyl nitrogen by the saturated methylene bridge, electronic effects should be negligible in the comparison of the binding of **7**–**9**.

Table 1. Association constants (K_a, in M^{-1})[a] between Zn(CFTPP) and several pyridine guests at 20 °C in different solvents.

Guest	Solvent Composition						
	CCl_4/MeOH = 5/95	CCl_4/MeOH = 20/80	CCl_4/MeOH = 40/60	CCl_4/MeOH = 60/40	CCl_4/MeOH = 80/20	CCl_4/MeOH = 90/10	CCl_4/DMSO = 90/10
7	50 ± 10	100 ± 10	100 ± 10	760 ± 340	3100 ± 1800[b]	---[c]	---[d]
8	5 ± 2	12 ± 1	12 ± 2	15 ± 2	50 ± 2	70 ± 2	130 ± 20
9	170 ± 50	90 ± 20 (80 ± 20)	60 ± 40	90 ± 10	90 ± 20 (120 ± 10)	210 ± 10 (230 ± 30)	< 1[e]

[a] Determined by UV titrations. Numbers in parentheses were obtained from ^{1}H NMR titrations. The errors are from nonlinear least-squares curve fitting.
[b] Determined by NMR dilutions.
[c] The guest is not soluble in this solvent mixture.
[d] Not determined.
[e] Binding was too weak to be measurable.

A pyridyl ligand normally complexes with zinc porphyrin in a 1:1 ratio.[17] We chose pyridyl ligands instead of previously used, generic hydrophilic or hydrophobic guests such as phenyl β-D-gluocopyranoside or pyrene[9] because the functionalized pyridines can probe the environment above/below the metal center. Generic hydrophilic and hydrophobic guests may be bound, but not necessarily near the metal center. Because minimal amounts of materials

could be used in UV spectroscopic studies due to the intense absorption of porphyrin derivatives, the majority of data were obtained from UV titrations. All titration experiments were performed at concentrations where intermolecular aggregation was negligible.[18] The association constants (K_a) between Zn(CFTPP) and **7**–**9** are summarized in Table 1. A ^{1}H NMR dilution test was used for **7** in 20% MeOH, as the guest was not sufficiently soluble to titrate the host. In some cases (e.g., ligand **7** in 40% MeOH), solubility problems prevented coverage of a broad range of guest concentrations, and the errors from nonlinear least-squares curve fittings were larger than in other cases. In selected cases, both ^{1}H NMR and UV titrations were performed, and the results from the two methods generally showed good agreements.

For the control compound **8**, there is a *gradual* increase of K_a with a decrease of methanol in the solvent mixture. This result is not surprising because alcohol is also a known ligand for zinc porphyrin.[17] Because CCl_4 is a much weaker ligand, an increase in methanol makes the solvent better able to compete with **8** for the metal center and reduce its apparent binding affinity. For the hydrophilic ligand **7**, changes in K_a are more dramatic, especially with more nonpolar solvent mixtures. For example, K_a is hardly changed in 95, 80, and 60% methanol—this is the similar trend observed for **8**—but increases by 30-fold over 60–20% methanol; yet, over the same range of solvent polarity, the increase is less than 4-fold for **8**. For the hydrophobic ligand **9**, on the other hand, K_a displays an unusual increase toward the *polar* end of solvent composition. The association constant is 60 M^{-1} in 60% methanol, but increases to 90 M^{-1} in 80% methanol, and further to 170 M^{-1} in 95% methanol. Although these changes are not large, they clearly go in *opposite* trends as compared to those for **7** and **8**.

The general trends in K_a are clear in Figure 1, in which the binding constants are plotted on a logarithmic scale against the percentage of methanol in the solvents. The overall shapes of the curves for the hydrophilic ligand **7** and the control **8** are similar, except that the increase in

K_a toward the low-MeOH end is more pronounced for the former. The curve for the hydrophobic ligand **9** is quite different (binding is stronger in both the high- and low-MeOH solvents, but is weaker in solvents with intermediate polarity).

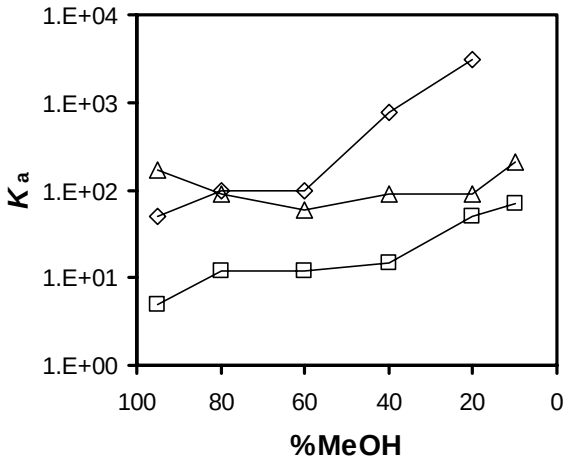


Figure 1. Plots of K_a between Zn(CFTPP) and **7** (◇), **8** (□), or **9** (△) as the function of %MeOH in MeOH/CCl_4. The data points are connected to guide the eye.

These binding constants generally seem to be consistent with the predicted conformational changes. As expected, the nonpolar ligand **9** is preferred by Zn(CFTPP) in methanol-rich solvents but is less preferred than the hydrophilic ligand **7** in CCl_4-rich cases. In addition, the preference in binding is more noticeable at the polar/nonpolar extremes than in the intermediate region of the polarity scale. All these observations suggest that the local environment above/below the metalloporphyrin binding site is influenced by the cholates. Collective (tetrameric) aggregation of cholates over the two faces of porphyrin is probably more reasonable than aggregation in four pairs, as localized aggregation around the peripheral phenyl groups is unlikely to significantly influence ligand binding to the zinc center.

However, some results are not consistent with our initial predictions. For example, we had predicted that binding of **8** would be stronger than **7** but weaker than **9** in polar, methanol-rich solvents. This is because, if indeed a micelle-like conformer is formed, it should repel **7** from its nonpolar interior. Instead, **8** is found to be a weaker ligand than **7** in methanol-rich solvents. Is the micelle-like conformer still formed in Zn(CFTPP)? If not, why is **9** bound more strongly than **7** or **8** in polar solvents? Our previous work[9,10] suggests that direct contact of the β faces is required for the normal micelle-like conformation—this is similar to micelles of surfactants formed through direct contact of the hydrophobic tails. Such a direct contact, however, is less likely to occur over the large face of the porphyrin. Therefore, the most likely possibility is that the micelle-like conformer is not formed in the absence of **9** but is *induced* by its presence. Hydrophobic binding between **9** and Zn(CFTPP) reduces solvophobic exposure of both the guest and the cholate β faces of the host, and thus may have promoted intramolecular micellization. If this is the case, stronger binding of **7** than **8** is understandable. With multiple OH/NH groups on both the host and **7**, it is easy to imagine that some intermolecular hydrogen-bonding interactions, albeit not very strong in solvents such as 95% MeOH/CCl_4, can make **7** a better ligand.

With inwardly facing hydrophilic α faces of the cholates, the reversed-micelle-like conformer can enrich MeOH solvents within its interior from a mostly nonpolar solvent mixture such as 10% MeOH/CCl_4.[8–10] This is not surprising because reversed micelles formed by surfactants often also need to be stabilized by a pool of water molecules in the center.[19] For this reason, we initially thought, as Zn(CFTPP) adopts the reversed-micelle-like conformation with decreasing polarity, the hydrophobic ligand **9** would be "repelled" by the entrapped polar methanol. Yet, its K_a increases from 90 to 210 M^{-1} when methanol is decreased from 20 to

10% (Table 1). One factor clearly contributing to this unpredicted increase is methanol being a competitive ligand for Zn (lower methanol always strengthens binding, as seen in the binding of **8** in different MeOH/CCl_4 mixtures. This factor, however, cannot explain why the increase in K_a for **9** over 20–10% methanol is even higher than that for **8**.

Although the behavior of Zn(CFTPP) supports the proposed conformational responses, the comparison between **7**/**9** and the control **8** was unexpected. Is there another important factor not considered? Since **8** and **7**/**9** also differ greatly in their size, is it possible that a larger guest is inherently preferred over a smaller one? Note that **8** (□) is a weaker ligand than **7**/**9** (◇/△) in every MeOH/CCl_4 composition (Figure 1). Polar solvents are known to be enriched from the nonpolar environment into the basket during reversed micellization.[8–10] During binding, some of these polar molecules (methanol in this case) will be displaced by the guest. Undoubtedly, larger guests such as **7** and **9** will "release" more solvent molecules than small ones (e.g. **8**). This desolvation is favorable on one hand because the solvents are no longer constrained locally, but is unfavorable on the other hand because the hydrogen bonds between the polar groups (NH/OH) of the cholates and methanol will be broken. It is entirely possible that such release of solvent is overall a favorable process for methanol, especially if methanol is only loosely associated with the α faces of the cholates.

According to our previous studies, DMSO solvates the α faces of cholates more strongly than MeOH.[6–10] For example, to stabilize the reversed-micelle-like conformer in a mixture of polar/nonpolar solvents, DMSO was more effective than methanol as the polar component.[6,9] The irony is that the same preferential solvation that helps stabilize this conformer also makes it an inferior host at the same time, because DMSO is much more difficult to be displaced than methanol by the guest. This contrast between DMSO and

methanol once again is found in the CFTPP case. Whereas replacement of 10% methanol by DMSO in CCl_4 enhances the binding of the control ligand **8** by slightly less than 2-fold, it *weakens* the binding of **9** to the point of nondetection (Table 1). Apparently, once the reversed-micelle-like conformer is filled with the strongly associating polar DSMO solvent, the hydrophobic guest is indeed "repelled" and becomes a much weaker ligand than the control. Therefore, the size of guest can be quite important, especially when significant desolvation occurs during the binding process.[20]

Overall, these binding studies indicate that the preference for different guests by Zn(CFTPP) can be tuned. Nonpolar guests are preferred in polar solvents and polar guests in nonpolar ones. The reversed micelle-like conformer seems to be better formed than the normal micelle-like one. Because the possibility of a guest-induced conformational change always exists, the actual conformation of Zn(CFTPP) is not very clear in the absence of guests. Nonetheless, it will be interesting to see if the solvent-dependent intramolecular aggregation of cholates can be used to regulate catalysis. Solvent-tunable catalysis indeed was demonstrated by Dr. Yibo Zhou in Prof. Woo's group. Substrates different by one or two hydroxyl groups were by distinguished by Fe(CFTPP)Cl in catalytic epoxidation.[1]

Conclusions

The conformational behavior of the cholate-functionalized porphyrin complex is not as well-defined as previously synthesized amphiphilic baskets constructed on "compact" scaffolds such as calix[4]arene. With a large scaffold, solvophobic interactions are less effective at controlling intramolecular aggregation of the cholates. However, even with these more diffuse structures, conformational changes can still have significant impact on the binding of the

metalloporphyrin derivative. In a mostly polar mixture, a hydrophobic ligand (**9**) is preferred by Zn(CFTPP), whereas a hydrophilic one (**7**) is favored in a mostly nonpolar mixture.

Experimental Section

General Methods

Chloroform was distilled from anhydrous K_2CO_3. Anhydrous tetrahydrofuran (THF) and methylene chloride were dried by passage through a column of activated alumina under nitrogen. Pyrrole was distilled over CaH_2 at atmospheric pressure. Stock solutions of $BF_3.Et_2O$ were prepared by diluting $BF_3.Et_2O$ (Aldrich, 8.1 M) to 2.5 M in $CHCl_3$ and were used within 2 weeks. Cholic acid was crystallized from 95% ethanol and dried at 90 °C under vacuum. All other reagents and solvents were of A.C.S. certified grade or higher and were used as received from commercial suppliers. Details of these syntheses can be found in Supporting Information. All glassware and syringes were dried in an oven at least overnight prior to use. Routine ^1H and ^{13}C NMR spectra were recorded on a Varian VXR-300 and VXR-400 spectrometer. MALDI-TOF mass was recorded on a Thermobioanalysis Dynamo mass spectrometer. UV-vis spectra were recorded at ambient temperature on an HP 8452 Spectrometer.

Synthesis

Compound 3.[23] Cholic acid (1.07 g, 2.62 mmol), DCC (590 mg, 2.86 mmol), and *N*-hydroxysuccinimide (430 mg, 3.78 mmol) were dissolved in anhydrous THF (50 mL) and CH_3CN (5 mL). After 8 h at room temperature, the white solid formed was filtered out and the filtrate was concentrated in vacuo to give a white foam (1.19 g, 91% yield). A portion of this solid (350 mg, 0.700 mmol) was dissolved in anhydrous DMF (5 mL). NH_4OH (42 mg, 27%

aqueous solution) was added. After 12 h at 50 °C, the mixture was poured into brine (50 mL). The precipitate was collected by suction filtration, washed with water (2 × 10 mL), and purified with column chromatography over silica gel using CH_2Cl_2/CH_3OH (8/1) as the eluent to give a white powder (215 mg, 78% yield). ^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.21 (s, 1H), 6.61 (s, 1H), 4.31 (d, J = 4.2 Hz, 1H), 4.09 (d, J = 3.6 Hz, 1H), 4.00 (d, J = 3.3 Hz, 1H), 3.76 (s, 1H), 3.59 (s, 1H), 3.16 (m, 1H), 2.20–0.79 (m, 28H), 0.56 (s, 3H).

Compound 4.[23] Compound **3** (305 mg, 0.763 mmol) was dissolved in anhydrous THF (20 mL) under N_2. $LiAlH_4$ (15.2 mL, 0.5 M in diglyme, 7.60 mmol) was added slowly by a syringe. The reaction mixture was heated to reflux for 12 h. A small amount of ethyl acetate was added slowly and the solvent was concentrated *in vacuo*. The residue was purified by column chromatography over silica gel using CH_2Cl_2/CH_3OH (10/1) and CH_3OH/Et_3N (50/1) as the eluent to give a white solid (168 mg, 56% yield). ^{1}H NMR (300 MHz, CD_3OD, δ) 4.30 (d, J = 4.2 Hz, 1H), 4.08 (d, J = 3.3 Hz, 1H), 3.99 (d, J = 3.0 Hz, 1H), 3.59 (s, 1H), 3.38 (s, 1H), 3.15 (m, 2H), 3.05 (m, 1H), 2.21–0.76 (m, 30H), 0.57 (s, 3H).

Compound 5. To a solution of 3,5-dihydroxybenzaldehyde (438 mg, 3.17 mmol) in acetone (10 mL) was added anhydrous K_2CO_3 (2.28 g, 16.5), ethyl bromoacetate (0.92 mL, 8.3 mmol) and a catalytic amount of NaI. The mixture was stirred at room temperature overnight. It was diluted with water (20 mL) after acetone was removed *in vacuo*. The mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated by rotary evaporation. The residue was purified by column chromatography over silica gel using hexane/ethyl acetate (2/1) as the eluent to give **5** as a white solid (869 mg, 88% yield). ^{1}H NMR (300 MHz, $CDCl_3$, δ) 9.88 (s, 1H), 7.03 (d, J = 2.4 Hz, 2H), 6.78 (t, J = 2.4 Hz, 1H), 4.66 (s, 4H), 4.28 (q, J = 7.2 Hz, 4H),

1.31 (t, J = 7.2 Hz, 6H); ^{13}CNMR (75 MHz, $CDCl_3$, δ) 14.3, 61.8, 65.7, 108.7, 108.8, 138.7, 159.7, 168.4, 191.5. Anal. Calcd for $C_{15}H_{18}O_7$: C, 58.15; H, 5.94. Found: C, 58.06; H, 5.85. EIMS m/z: M^+ 310.

Compound 6. An oven-dried, three-necked, 3 liter, round-bottomed flask equipped with a magnetic stirring bar and a gas-dispersion tube was charged with **5** (2.53g, 8.18 mmol). Chloroform (820 mL) distilled over K_2CO_3 was transferred to the flask through a cannula. Pyrrole (0.57 mL, 8.18 mmol) was added by a syringe. The solution was purged with nitrogen for 20 minutes. Boron trifluoride diethyl etherate (2.5 M solution in CH_3Cl, 0.49 mL, 1.23 mmol, 0.15 equiv.) was added by a syringe and the flask was wrapped with aluminum foil to shield it from light. The solution was stirred under nitrogen at room temperature for 24 h. DDQ (1.39 g, 6.12 mmol) was added in one portion. The mixture was then heated to 65 ˚C for another 4 h. After the mixture was cooled to room temperature, and triethylamine (6.5 mL) was added. The reaction mixture was concentrated in vacuo. The residue was purified with column chromatography over silica gel using CH_2Cl_2/ethyl acetate (20/1) as the eluent to give **6** as a purple solid (1.19 g, 41% yield). ^{1}H NMR (300 MHz, $CDCl_3$, δ) 8.88 (s, 8H), 7.73 (d, J = 2.1 Hz, 8H), 6.97 (t, J = 7.2 Hz, 4H), 4.92 (s, 16H), 4.27 (q, J = 7.2 Hz, 16H), –2.95 (s, 2H) ppm. Anal. Calcd for $C_{76}H_{78}N_4O_{24}$: C, 63.31; H, 5.83; N, 3.89. Found: C, 63.77; H, 5.49; N, 3.91. EIMS m/z: M^+ 1432. UV (CH_2Cl_2) λ_{max}, nm (ε): 421 (543000), 455 (17200), 514 (20600), 549 (6000), 588 (6300), 645 (3300).

H_2CFTPP, 2. To a solution of **6** (180 mg, 0.13 mmol) in THF (40 mL) and MeOH (10 mL) was added aqueous 1M NaOH (10 mL). The mixture was stirred at room temperature for 2 h. The bottom purple aqueous layer was separated from the top, light yellow, organic layer, and was acidified with 1M HCl to pH ≈ 2 while the solution was kept at 0 ˚C. The green

precipitate was collected by centrifugation, washed with water (twice) and MeOH (3 times), and dried *in vacuo* (122 mg, 81%)**.** A portion of this acid (88 mg, 0.224 mmol), **4** (30 mg, 0.025 mmol), and benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP, 99 mg, 0.225 mmol) were dissolved in anhydrous DMF (6 mL). Diisopropylethylamine (62 mg, 0.448 mmol) was added by a syringe. The reaction mixture was stirred at 60 °C for 24 h under N_2 and was poured into brine (50 mL). The solid was collected by suction filtration, washed with water (2 × 10 mL), and purified by preparative TLC (SiO_2, $CHCl_3/CH_3OH$ = 4/1) to give a red powder (51 mg, 48% yield). ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$, δ) 8.88 (s, 16H), 8.14 (s, 16H), 7.36 (s, 16H), 7.06 (s, 8H), 4.63 (s, 32H), 4.28 (d, *J* = 4.2 Hz, 16H), 3.95 (d, *J* = 1.8 Hz, 16H), 3.82 (d, *J* = 0.9 Hz, 16H), 3.56 (br s, 16H), 3.07 (br s, 40H), 2.10 (q, J = 9.6 Hz, 16H), 1.97 (m, 16H), 1.95–0.55 (m, 232H), 0.37 (m, 16H), 0.05 (s, 24H); ^{13}C NMR (75 MHz, $CDCl_3/CD_3OD$, δ) 169.2, 157.4, 144.3, 119.5, 115.3, 102.3, 72.7, 71.6, 67.6, 46.6, 45.9, 41.8, 41.3, 39.6, 39.4, 39.2, 35.4, 35.3, 34.6, 34.5, 33.0, 29.9, 28.2, 27.4, 3.3, 25.9, 22.6, 22.2, 17.0, 11.6. MALDI-TOFMS: calcd. for $C_{252}H_{375}N_{12}O_{40}$ $[M+H]^+$: 4212.72; found: 4206.2.[23] UV (CH_2Cl_2/CH_3OH) λ_{max}, nm (ε): 422 (129500), 514 (13200), 548 (6700), 590 (6200), 646 (4700).

Zn(CFTPP). $Zn(OAc)_2$•$2H_2O$ (6 mg, 0.024 mmol) and **2** (35 mg, 0.0083 mmol) were mixed in CH_3OH (5 mL). The mixture was stirred at room temperature for 3 h. Brine (50 mL) was added to solution. The precipitate collected by suction filtration and washed with water and CH_3CN to give a dark red powder (34 mg, 97 % yield). ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$, δ) 8.85 (s, 32H), 8.15 (br s 32H), 7.37 (s, 32H), 7.04 (s, 16H), 4.63 (s, 32H), 4.28 (d, *J* = 4.2 Hz, 16H), 4.01 (d, *J* = 2.1 Hz, 16H), 3.89 (d, *J* = 1.8 Hz, 16H), 3.69 (s, 16H), 3.49 (s, 16H), 3.09 (m, 40H), 2.32–0.74 (m, 232H), 0.38 (s, 24H); ^{13}C NMR (75 MHz,

$CDCl_3/CD_3OD$, δ) 169.1, 156.8, 149.9, 132.7, 126.8, 117.0, 72.9, 72.9, 71.9, 71.6, 70.3, 68.0, 67.6, 57.1, 47.0, 46.4, 46.2, 41.8, 41.7, 41.6, 39.7, 39.7, 39.4, 39.2, 35.6, 35.4, 34.9, 34.9, 34.9, 34.8, 34.5, 33.1, 31.6, 31.5, 30.0, 29.8, 29.7, 28.2, 27.7, 26.6, 26.4, 26.0, 23.1, 23.0, 22.4, 22.3, 21.1, 21.0, 17.3, 12.2. MALDI-TOFMS: calcd. for $C_{252}H_{372}N_{12}O_{40}Zn$ $[M+H]^+$: 4276.1; found: 4276.5. UV (CH_2Cl_2/ CH_3OH) λ_{max}, nm (ε): 427 (356100), 557 (26500), 597 (16100), 633 (16000).

Compound 7. Compound **7** was synthesized according to a literature procedure.[25] 4-Aminopyridine (520 mg, 4.81 mmol) and δ-gluconolactone (850 mg, 4.80 mmol) were dissolved in pyridine (10 mL). The reaction mixture was heated to reflux for 12 h and was poured into CH_2Cl_2 (100 mL). The solid was collected by suction filtration and was washed with CH_2Cl_2 (2 × 20 mL) to give a white powder (1.090 g, 75% yield). ^{1}H NMR (300 MHz, CD_3OD, δ) 8.44 (dd, *J* = 4.5 Hz , *J* = 1.8 Hz, 2H), 8.33 (t, *J* = 6.3 Hz, 1H), 7.26 (dd, *J* = 4.5 Hz , *J* = 1.5 Hz, 2H), 4.59-4.50 (m, 3H), 4.40–4.22 (m, 3H), 4.09 (dd, *J* = 5.1 Hz, *J* = 3.6 Hz, 1H), 3.96 (m, 1H), 3.60–3.32 (m, 4H).

Compound 9. 4-Aminopyridine (100 mg, 0.93 mmol), 1-pyrenebutyric acid (266 mg, 0.92 mmol), and BOP (452 mg, 1.11 mmol) were dissolved in anhydrous DMF (10 mL). Diisopropylethylamine (320 mg, 2.30 mmol) was added by a syringe. The reaction mixture was stirred at 50 °C for 12 h and was poured into brine (100 mL). The solid was collected by suction filtration, washed with water (2 × 20 mL), and purified by column chromatography over silica gel using $CHCl_3/CH_3OH$ (4/1) as the eluent to give a white powder (218 mg, 75% yield). ^{1}H NMR (400 MHz, CDCl3, δ) 8.44 (dd, *J* = 3.3 Hz, *J* = 1.2 Hz, 2H), 8.25 (d, *J* = 6.9 Hz, 1H), 8.14 (d, *J* = 6.0 Hz, 2H), 8.06 (dd, *J* = 5.7 Hz, *J* = 3.3 Hz, 2H), 8.00 (s, 2H), 7.97 (dd, *J* = 6.0 Hz, *J* = 5.4 Hz, 1H), 7.81 (d, *J* = 6.0 Hz, 1H), 7.05 (dd, *J* = 3.3 Hz, *J* = 1.2 Hz, 2H),

5.84 (br s, 1H), 4.36 (d, J = 4.8 Hz, 2H), 3.39 (t, J = 5.4 Hz, 2H), 2.29 (m, 2H), 2.22 (m, 2H). ^{13}C NMR (75 MHz, CDCl3, δ) 172.7, 149.8, 149.3, 136.9, 131.4, 130.9, 129.8, 128.7, 127.9, 127.9, 127.7, 127.0, 126.5, 125.4, 125.2, 124.8, 124.7, 123.9, 122.6, 96.0, 41.7, 35.4, 32.8, 28.0. MALDI-TOFMS: calcd. for $C_{26}H_{23}N_2O$ $[M+H]^+$: 379.5; found: 380.7.

UV Titrations. The host was titrated with different amounts of the guest, and absorption at the Soret band of complex was monitored. A typical procedure is as follows. Stock solutions of Zn(CFTPP) (0.10 M) and **7** (0.14 M) in $CH_3OH/CHCl_3$ (50/50) were prepared. CCl_4/MeOH (20/80, 3.0 mL) was added to a cuvet, to which an aliquot (2.0 μL) of the stock solution of **7** was added via a microsyringe. The sample was vortexed for 1 min. UV absorbance at 433 nm was measured. The binding constant was determined by nonlinear least-squares curve fitting of the titration data.

^{1}H NMR titrations. A ^{1}H NMR dilution experiment was performed with equimolar amounts of Zn(CFTPP) and **7**, and the chemical shifts of the pyridyl protons in the guest were monitored. For the binding of **9**, Zn(CFTPP) was titrated with different amounts of the guest. A typical procedure is as follows. Stock solutions of Zn(CFTPP) (0.010 M) and **9** (0.10 M) in $CH_3OH/CHCl_3$ (50/50) were prepared. To 14 separate vials, 12.0 μL of the Zn(CFTPP) stock solution was added, followed by 5.0, 7.0, 9.0, 11.0, 14.0, 17.0, 21.0, 25.0, 31.0, 38.0, 48.0, 61.0, 81.0, and 114.0 μL of the stock solution of **9**. The solvents in each vial were removed in vacuo. Then 600 μL of CCl_4/CD_3OD (20/80) was added to each vial. The samples were gently shaken for 1 h and then transferred to 14 separate NMR tubes. ^{1}H NMR spectra were recorded for each sample and the chemical shifts of pyridyl protons of the guest were measured. Binding constants were determined by nonlinear least-squares curve fitting of the titration data.

Acknowledgments

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(21) The yields given are the averages from three separate experiments. The errors are the standard deviations. See Table 2S in Supporting Information for the actual yields.

(22) Fe(CFTPP)Cl was noticeably more soluble than Zn(CFTPP), which was shown to aggregate intermolecularly with <20% MeOH in CCl_4. Due to its paramagnetic nature, the extent of aggregation for Fe(CFTPP)Cl could not be determined by ^{1}H NMR spectroscopy. It should be mentioned that any potential intermolecular aggregation of Fe(CFTPP)Cl in low polarity solvents would only reduce the contribution of the reversed micelle-like conformer and decrease the substrate selectivity. Therefore, the observed preference for polar substrates should represent the lower limit achievable by the reversed micelle-like conformer.

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CHAPTER 7. High guest inclusion by 3β-amino-7, 12α-dihydroxycholan-24-oic acid made possible by charge-assisted hydrogen bonds

A paper published in *Tetrahedron* **2006**, *62*, 6808-6813.[1]

Abstracts

3β-Amino-7α,12α-dihydroxycholan-24-oic acid (**2**) formed inclusion compounds with high ratio (host/guest = 1/4) of guest methanol. Both hydrogen bonds and hydrophobic interactions were important to the solid structure. The cholates assembled in a head-to-tail fashion to form infinite hydrogen-bonded chains. The chains were interconnected between cholates and also through the guests. Large channels were formed along the crystallographic *a* axis where most of the methanol molecules were located. Presence of a dominant hydrogen bonding motif (i.e., ammonium-carboxylate ion pairing) was probably responsible for high guest incorporation.

Introduction

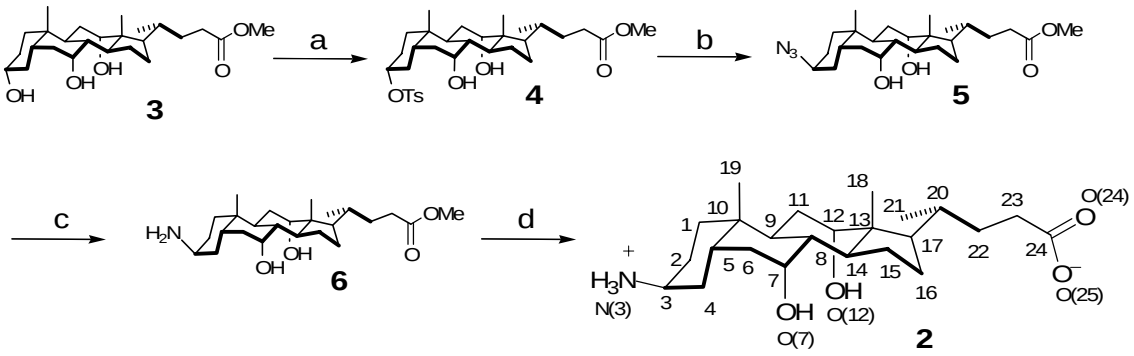
Interesting feature of cholic acid (and bile acids in general) is their ability to form inclusion compounds with various organic compounds.[2] This is an attractive application because bile acids are chiral and can be used for enantiomeric and diastereomeric separation of guest molecules.[3,4] The number and the orientation of hydrogen bonds greatly influence the solid state structures of the bile acids as well as the inclusion compounds that can be formed. For example, deoxycholic acid, only different from cholic acid (**1**) by missing one hydroxyl group at C-7, is known for over a hundred years to form inclusion compounds with a wide

variety of organic molecules including hydrocarbons, alcohols, ethers, ketones, acids, esters, and nitriles.[2,5] The ability of cholic acid to form inclusion compounds, however, was discovered much later, but received increased attention in recent years.[2] Its crystal lattice is quite stable and can survive reversible incorporation and removal of guest molecules in some cases,[6,7] making it potentially useful as "organic zeolite" for separation and chemical reactions.

In our recent study of cholate derivatives, we synthesized 3β-Amino-7α,12α-dihydroxycholan-24-oic acid (**2**) and found it could include guest molecules such as methanol. Most interestingly, large void volumes can be formed in the solid structure so that four solvent molecules can be incorporated per host molecule. In contrast, the number of guest molecules in preciously reported bile acid inclusion compounds almost never goes above two.

Results and discussion

Scheme1. Synthesis of compound **2.**



Reaction conditions: (a) TsCl, pyridine; (b) NaN_3, DMPU; (c) PPh_3, THF, H_2O; (d) LiOH.

Synthesis of **2** was adapted from literature procedures (Scheme 1).[8] Cholic acid was treated with catalytic amount of sulfuric acid in refluxing methanol to give methyl ester **3**. Among the three hydroxyl groups, the one at 3α position is most reactive and was selectively

tosylated in 84% yield. Tosylate **4** was replaced by azide through nucleophilic substitution with sodium azide in 74% yield. The azide intermediate **5** was then reduced by triphenylphosphine in aqueous THF and was hydrolyzed to give the final product **2** in good yields.

Compound **2** has low solubility in many organic solvents including chloroform, tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF), and even dimethyl sulfoxide (DMSO)—the latter two typically dissolve cholate derivatives very easily. Apparently, charges from the ammonium and carboxylate interact more strongly than neutral hydrogen-bonding donors and acceptors in most bile acids and give exceptionally high stability to the solid. It is insoluble in water at neutral pH but is soluble under both acidic and basic conditions, presumably due to formation of micellar aggregates. The compound is soluble in hot methanol and easily forms large transparent needlelike crystals upon cooling.

According to single crystal X-ray structure determination one independent molecule of **2** and four methanol solvent molecules were found in asymmetric unit of orthorhombic cell (space group $P2_12_12_1$). The molecule assembles in a head-to-tail fashion with the amine and the carboxyl group hydrogen bond to each other (Figure 1). The α faces of the cholates tilt up and down alternately along the chain. In fact, every other molecule along the chain is equivalent and can be converted to one other by translational operation. Similar to other bile acids, each repeating unit propagates along the crystallographic *c* axis in a helical fashion,[9] possibly as a result of the bent backbone caused by the *cis*-fused A/B rings. Along the *a* axis, the chains are completely parallel. These chains are bridged by methanols to give pleated sheets in this direction. The chains are zigzagged and antiparallel between neighboring layers. Along the crystallographic *b* axis, the chains are connected by hydrogen bonds between the carbonyl oxygen O(24) of one cholate and the hydroxyl group O(7) of another.

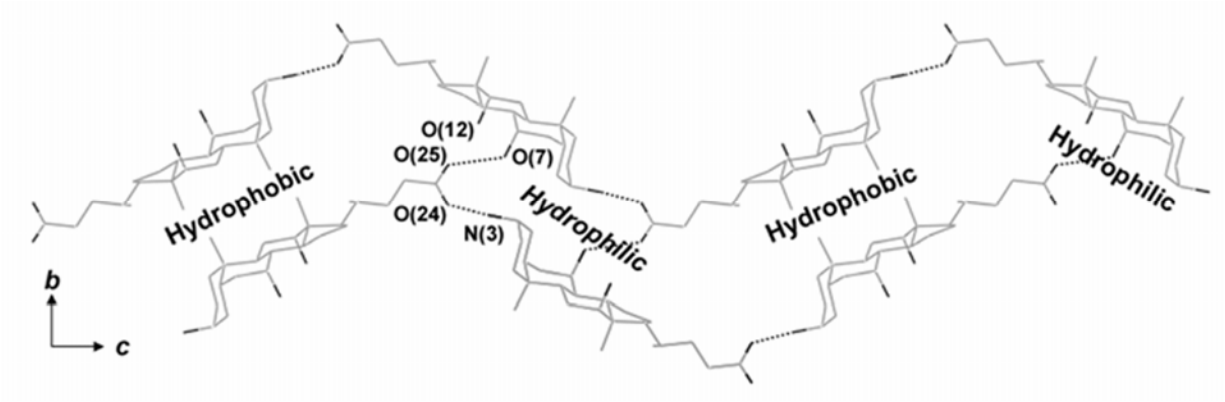


Figure 1. Two hydrogen bonded chains of **2** (with methanol molecules) along the crystallographic *a* axis. Hydrogen bonds are shown in dotted lines. Hydrogen atoms are omitted for clarity.

Amphiphilicity is important in the structure as both hydrophilic and hydrophobic portions of the molecules are clearly segregated (Figure 1). Hydrophobic contact is maintained by closely packed methyl groups on the β faces of cholates between neighboring chains. Unlike most bile acids,[9] however, the hydrophobic layers are discontinuous along the *c* axis. This is the direct result of alternating α and β faces along the chains (which is likely caused by strong interactions between the amine and the carboxyl group and the β orientation of the amine). The hydrophobic contact is continuous along the *a* axis, forming multiple hydrophobic "belts" in this direction. Hydrophilic region is located around the amine/carboxyl pair and the two hydroxyl groups O(7) and O(12) of another cholate molecule.

There are four cholates and 16 methanol molecules in one unit cell. This guest/host ratio (4/1) is unusually high for bile acid inclusion compounds. For example, cholic acid (1) only incorporates one or two methanol in its crystal.[10–12] Deoxycholic acid does not form

inclusion compounds with simple alcohols. In fact, the guest/host ratio in the majority of bile acid inclusion compounds is 1:1 or lower.[2] Figure 2 shows the hydrogen-bonding network

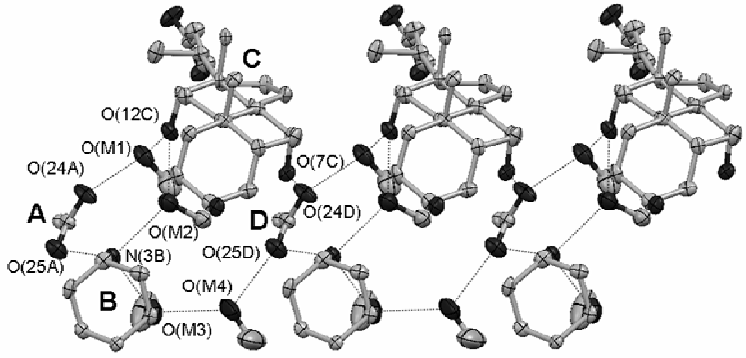


Figure 2. Hydrogen bonding network within the crystal lattice of **2**. Hydrogen atoms and parts of cholates A, B, and D are omitted for clarity. O(M1), O(M2), O(M3), and O(M4) are the oxygen atoms on the four methanol molecules.

formed by the cholates and methanol. Not surprisingly, all the polar atoms (i.e., oxygen and nitrogen) from both the hosts and the guests are involved in hydrogen bonding. Each cholate is hydrogen bonded to six methanol molecules. The carboxylate of cholate A is bonded to the amine of cholate B and to hydroxyl O(12) of cholate 3 through methanol M1. The hydroxyl O(12) of cholate C is then reconnected back to the amine of cholate 2 through methanol M2. Interestingly, two additional methanol molecules (M3 and M4) sit between closely bonded amine/carboxylate pairs from cholates A and B.

Typical hydrogen-bonded O···O distances range from 2.36 to 3.69 Å, with the latter being the van der Waals cutoff value.[13] Table 1 summarizes the hydrogen bond distances and bond angles in the crystal structure. The O···O distance in our structure ranges from 2.65 to 2.79 Å, representing medium-strengthed (2.65 to 2.80 Å) hydrogen bonds according to literature

Table 1. Hydrogen bond distances (with H···A distances < 2.5 Å) and angles in the solid structure of **2.**

Entry	Hydrogen Bond[a]	D-H (Å)	H···A (Å)	D···A (Å)	D-H-A Bond angle (°)
1	O(7C)-H···O(24D)	0.84	2.14	2.785(6)	133.4
2	O(12C)-H···O(M1)	0.84	1.99	2.755(7)	150.9
3	O(M3)-H···O(M4)	0.84	1.88	2.655(11)	153.6
4	O(M1)-H···O(24A)	0.84	1.86	2.660(8)	158.5
5	O(M2)-H···O(12C)	0.84	1.82	2.653(8)	171.0
6	O(M4)-H···O(25D)	0.84	1.83	2.660(10)	168.2
7	N(3B)-H···O(M2)	0.91	1.82	2.700(9)	162.5
8	N(3B)-H···O(25A)	0.91	2.02	2.831(8)	147.6
9	N(3B)-H···O(M3)	0.91	2.06	2.855(10)	145.5

[a] See Figure 2 for atom numbering. A, B, C, and D are the four labeled cholates. M1, M2, M3, and M4 are the four labeled methanol molecules.

classification.[13,14] Strong hydrogen bonds tend to have linear geometry. Many of the D-H-A bond angles, however, are smaller than 160°, possibly because the shape of 2 prevents optimal alignment of the donor and the acceptor atoms. Among all the polar atoms, O(7) is the only one that hydrogen-bonds strongly to just one other polar atom—the next closest distance between O(7) and another polar atom is 3.30 Å. The O···N distance ranges from 2.70 to 2.86 Å (entries 7–9), similar to the values (2.66–3.12 Å with an average of 2.84 Å) found in amino acids and peptides.[15]

As in most supramolecular systems, the final product formed (crystal structure in this case) represents a minimum in either the global or local energy landscape (corresponding to the thermodynamically controlled or kinetically trapped structures). Multiple intermolecular forces

have to work together and balance among themselves to reach the best compromise in a crystal structure. In typical bile acid inclusion compounds, the most important interactions are hydrogen bonds and hydrophobic interactions.[2] Since all hydrogen bonds (O-H···O) are of similar nature, no one can dominate in an bile acid that are functionalized only with hydroxy and carboxylic acid groups. Under such a circumstance, the molecules have many ways of optimization and can form tightly packed structures fairly easily. This probably explains why bile acid inclusion compounds rarely incorporate more than one or two guest per host even for small guests like methanol.

In the current structure, however, the ammonium-carboxylate is the dominant force. In fact, charge-assisted hydrogen bonds are well know to be stronger than neutral ones[14,16–17] and are, therefore, generally maintained in the solid state. Görbitz surveyed 749 amino acid and peptides and found the ammonium carboxyl is always maintained despite the presence of many other hydrogen-bond donors and acceptors in the structures.[15] Aakeröy and co-workers had the same observation in a series of substituted benzylammonium benzoate derivatives.[18] Presence of a dominant force puts a severe constraint on the number of possible ways to optimize the structures. The price of maintaining a particular interaction is to sacrifice other hydrogen bonds and/or close packing of the molecules. Therefore, it should be much easier to incorporate a larger number of guests in such a system.

The crystal structure has channels along the *a* axis (Figure 3). These channels are fairly hydrophobic except at the corners where the polar atoms are clustered. They are nearly triangular in shape and are fairly large in size: the shorter edge is about 5Å and the longer ones roughly 7Å in length. Three (M1, M3, and M4) of the methanol molecules are located within the channels and are connected to the "wall" through hydrogen bonds. All three of them have their methyl groups point to the hydrophobic side of the wall. M1 has lower mobility because it

is bonded to the wall via two connections (see also Figure 2). M3 and M4, on the other hand, are interconnected to each other and are hydrogen-bonded to the wall only through a one-point contact. As a result, they have the largest thermal motions among all the atoms, presumably because they can move up and down easily without significantly changing the hydrogen-bonding network. The fourth methanol (M2) is located in the hydrophilic region in between the carboxyl, amine, and hydroxyl O(7). It is tightly held in a narrow space, which explains the smallest thermal motion observed for this methanol among all the solvents.

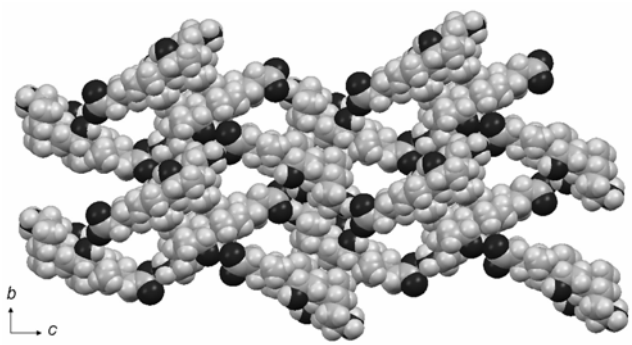


Figure 3. Space filling models of crystal structure of **2** viewed along the crystallographic *a* axis (carbon and hydrogen shown in light gray; oxygen and nitrogen shown in black). Methanol molecules are omitted to show the channels.

Conclusions

Ammonium-carboxylate interaction is maintained in the crystal structure of 3β-amino-7α,12α-dihydroxycholan-24-oic acid (**2**). Combination of a dominant hydrogen-bonding interaction with shape awkwardness of the steroid backbone is probably responsible for incorporation of an unusually large number of guest molecules in the inclusion compound. Such a feature can be very useful in preparing inclusion compounds with high loading

capacities. Another potentially beneficial feature of **2** as a supramolecular host is its low solubility in a range of polar and nonpolar solvents. This could be useful in reversible incorporation and release of guest molecules for separation and chemical reactions.[3,4]

Bile acid inclusion compounds occupy a unique position in the field of crystal engineering. They have multiple polar groups to stabilize the crystal lattice, facial amphiphilicity allowing incorporation of both hydrophilic and hydrophobic guests, chirality for enantiomeric and/or diastereomeric selectivity, and awkward shapes to avoid close packing. Many systematic modifications on the basic structures have been performed including variation on the number and the orientation of hydroxyl groups, on the type of functionality (e.g., acid, ester, amide, alcohol) at the C24 carbon, and the length of the carboxy tail.[2] In contrast, amino-derived cholates have received little or no attention in their inclusion abilities. Since charge-assisted hydrogen bonds are commonly used to rationally design molecular solids,[16–17] amino-derived bile acids as a group may become highly valuable host compounds for crystal engineering.

Experimental Section

General methods

Anhydrous tetrahydrofuran (THF) and methylene chloride were dried by passage through a column of activated alumina under compressed nitrogen. Cholic acid was crystallized from 95% ethanol and dried at 90 °C under vacuum. All other reagents and solvents were of A.C.S. certified grade or higher, and were used as received from commercial suppliers. All glassware and syringes were dried in an oven at least overnight prior to use.

Routine ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-300 and VXR-400 spectrometer.

Synthesis

Compound 4. Methyl cholate **3** (3.03 g, 7.17 mmol) was dissolved in anhydrous pyridine (20 mL). Toluenesulfonyl chloride (1.95 g, 10.79 mmol) was added under N_2. The reaction mixture was stirred for 4 hr at 50 °C. Solvent was removed in *in vacuo*. The residue was dissolved in ethyl acetate (50 mL), washed with 2N HCl (50 mL) and water (2 × 50 mL), dried with $MgSO_4$, and concentrated *in vacuo* to give a white powder (3.58 g, 6.21 mmol, 87 % yield). This material was generally used in the next step without further purification. ^{1}H NMR (DMSO-d_6, 400 MHz, δ) 7.74 (d, *J*=8.4 Hz, 2H), 7.42 (d, *J* =8.4 Hz, 2H), 4.21 (m, 1H), 3.71 (s, 1H), 3.52 (s, 3H), 2.58-0.78 (m, 33H), 0.54 (s, 3H).

Compound 5. Tosylate **4** (3.58 g, 6.21 mmol) and NaN_3 (2.16 g, 33.22 mmol) were dissolved in *N*,*N*′-dimethylpropyleneurea (DMPU, 20 mL). The reaction mixture was stirred for 12 hr at 60 °C. Water (100 mL) was added. The precipitate was collected by filtration and washed with water (2 × 50 mL). The residue was purified with column chromatography over silica gel using ethyl acetate/hexane (1/4) as the eluent to give a white powder (2.05 g, 4.59 mmol, 74 % yield). ^{1}H NMR (DMSO-d_6, 400 MHz, δ) 3.95 (br s, 1H), 3.73 (br s, 1H), 3.57 (br s, 1H), 3.53(s, 3H) 2.58(m, 1H), 2.32-0.73(m, 29H), 0.54 (s, 3H).

Compound 6. Azide ester **5** (203 mg, 0.459 mmol) and PPh_3 (168 mg, 0.641 mmol) were dissolved in THF (5 mL) and water (0.3 mL). The reaction mixture was heated to reflux for 12 h. Solvent was removed *in vacuo*. The residue was purified by column chromatography over silica gel using first ethyl acetate/hexane (4/1) and then methanol/triethylamine (50/1) as

the eluent to give a white solid (135 mg, 0.321 mmol, 70 % yield). mp 225-230 °C dec; ^{1}H NMR (CD_3OD, 400 MHz, δ) 3.94 (br s, 1H), 3.80 (m, 1H), 3.64 (s, 3H), 3.09 (s, 1H), 2.57 (m, 1H), 2.42-2.11 (m, 3H), 1.96-0.91 (m, 26H), 0.71 (s, 3H).

Compound 2. LiOH (2M, 5 ml) was added to the solution of **5** (135 mg, 0.321 mmol) in methanol (10 mL). The mixture was stirred at room temperature for 21 h. HCl (2N) was added until pH = 7-8. Solvent was removed in *in vacuo*. Residue was purified by column chromatography using MeOH/triethylamine (50/1) as the eluent to give a white solid (121 mg, 0.298 mmol, 93 % yield).[25] mp 240-245 °C dec; ^{1}H NMR (CD_3OD/D_2O = 1:1, 400 MHz, δ) 3.59 (s, 1H), 3.26 (s, 1H), 2.00-0.75 (m, 30H), 0.52 (s, 3H).

X-ray crystallography

A colorless small solvent dependent crystal (0.25 × 0.18 × 0.13 mm^3) was covered with epoxy glue and immediately mounted and centered in the stream of cold nitrogen. The crystal evaluation and data collection were performed on a Bruker CCD-1000 diffractometer at 193 K, Mo K_α (λ = 0.71073 Å) radiation, detector to crystal distance of 5.03 cm. The data were collected using the full sphere routine (0.3º scans in ω, 30 sec per frame). This dataset was corrected for Lorentz and polarization effects. The absorption correction was based on fitting a function to the empirical transmission surface as sampled by multiple equivalent measurements[19] using SADABS software.[20] The structure was solved using direct methods was refined in full-matrix anisotropic approximation for all non-hydrogen atoms. All hydrogen atoms were placed in the structure factor calculation at idealized positions and were allowed to ride on the neighboring atoms with relative isotropic displacement coefficients.

The crystals of **2** are orthorhombic, $C_{24}H_{41}NO_4 \times 4(CH_4O)$, space group $P2_12_12_1$; at 193(2) K, a = 7.606(2), b = 13.516(4), c = 29.156(8) Å, V = 2997.2(14) Å3, Z = 4, M = 535.75, D_{calc} = 1.187 Mg/m3, μ = 0.085 mm-1, F(000) = 1184.ion, R1 = 0.0814, wR2 = 0.2189 (data/parameters = 2819/348), GOF = 1.085.

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 600390 Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44-(0)1223-336033 or e-mail:deposit@ccdc.cam.ac.uk].

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CHAPTER 8. Efficient synthesis of water-soluble calixarenes using click chemistry

A paper published in *Organic Letters* **2005,** *7*, 1035-1037.[1]

Abstracts

Several water-soluble calix[4]arenes were synthesized via Huisgen 1,3-dipolar cycloaddition between azides and alkynes. Cationic, anionic, and nonionic calixarenes were prepared from a common azidocalixarene intermediate. Azidocalixarenes performed better than alkynylcalixarenes as precursors. The aggregation behavior of the water-soluble calixarenes was studied by ^{1}H NMR spectroscopy.

Introduction

Calixarenes are among the most versatile and useful building-blocks in supramolecular chemistry.[2] Water-soluble calixarenes have attracted a considerable attention very early on because their well formed hydrophobic cavities make it possible to study molecular recognition in water. Water-soluble groups such as sulfonates,[3] carboxylic acids,[4] amines,[5] and phosphonates[6] have been introduced through various reactions. More recently, calixarenes become attractive multivalent scaffolds for making amphiphiles useful in both biological[7,8] and chemical applications.[9]

However, synthesis of multivalent water-soluble calixarenes represents a considerable challenge.[10] Certain reaction conditions (e.g., sulfonation) have a poor functional group compatibility. If the reaction does not give a high conversion, the separation of the (highly polar) persubstituted products from incompletely substituted ones is difficult. Because many of

the biological and chemical applications mentioned above are influenced by the charge characteristics of water-soluble calixarenes, it is highly desirable to have a modular synthesis that can introduce a variety of water-soluble groups without using protective/deprotective chemistry.

"Click chemistry"[11] seems to be particularly suitable for attaching water-soluble groups. Click reactions are modular, tolerant of wide range of solvents and functional groups, simple to perform, and very high yielding. Click reactions have already been used successfully to prepare enzyme inhibitors in situ,[14] to functionalize surfaces,[15] and to synthesize dendritic polymers.[16] In this communication, we report the preparation of water-soluble calixarenes using the Huisgen 1,3-dipolar cycloaddition of an azide and an alkyne to form a triazole,[15] one of the most efficient click reactions to date.[16]

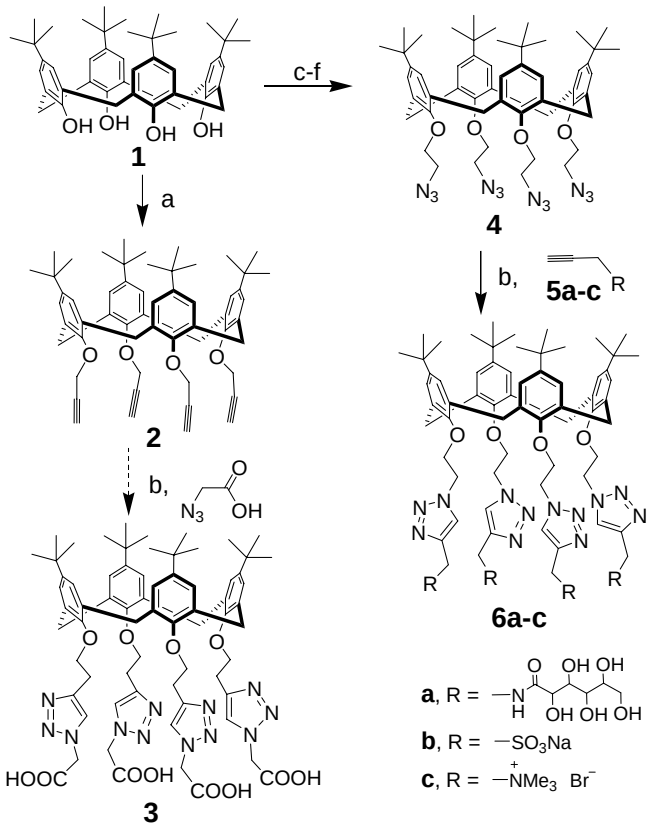
Results and discussion

To attach water-soluble groups via the cycloaddition, we can potentially employ calixarenes functionalized with either alkynes or azido groups (Scheme 1). We first attempted the synthesis of **3** because its precursor **2** could be prepared in one step from commercially available *tert*-butylcalix[4]arene **1**. However, no reaction occurred at room temperature but complex mixtures formed at 60 °C.[17]

We then explored the second route using azidocalixarene **4** and water-soluble alkynes (**5a-c**). Reactions proceeded very smoothly under similar conditions. One distinctive advantage of this route is that the alkyne-coupling side reaction[18] at most would consume some of **5** but otherwise cause no harm to the calixarene precursor **4**. Another advantage is in the preparation of the water-soluble alkynes **5a-c**, which could be synthesized from readily

available starting materials in high yields and stored in a freezer indefinitely.[18] High stability is particularly important from the standpoint of safety, because potentially explosive, small organic azides have to be used in the other route involving alkynylcalixarenes.[19]

Scheme 1. Preparation of water-soluble calix[4]arenes



Reagent condition; (a) propargyl bromide, NaH (b) $CuSO_4$, sodium ascorbate (c) ethyl bromoacetate, K_2CO_3 (d) $LiAlH_4$ (e) MsCl, Et_3N (f) NaN_3

In general, the coupling reaction between **4** and **5** was complete within 24 h at 60 °C in THF/EtOH/H_2O (1/2/2). Calixarene **6a** was purified by simple precipitation into acetone and **6b**/**6c** purified by reverse-phase column chromatography with aqueous methanol as eluent. The

isolated yield in general was about 80%. We also performed the reactions using copper (I) iodide as the catalyst in the presence of organic bases such as triisopropylethylamine, but the reactions were not as clean.

Solubility of the resulting calixarene (**6a-c**) varied greatly. The nonionic **6a**, to our surprise, was not soluble at all in water.[20] Anionic calixarene **6b** was soluble in water but insoluble in methanol, acetone, acetonitrile, and tetrahydrofuran. Cationic **6c** had solubility properties quite similar to **6b** in most solvents except methanol, in which it was quite soluble.

Calixarenes **6b** and **6c** were soluble in water probably because of micelle formation. To study their aggregation behavior, we recorded their ^{1}H NMR spectra at different concentrations in D_2O (figure 2 and 3). This method requires minimal amount of material and has been used previously in the characterization of similar water-soluble calixarenes.[21]

When the concentration of anionic **6b** was increased from 0.5 mM to 4.5 mM, the chemical shifts of several hydrogens changed significantly. The largest change in the chemical shift was observed for the *endo* methylene bridge ($ArCH_2Ar$) hydrogens. Significant changes were observed above 1 mM (Figure 4a). The signals also became substantially broader above this concentration. Analysis of the line widths (Figure 4b) gave the same critical micelle concentration (CMC) of 1 mM. The CMC of the cationic calixarene **6c** was also about 1 mM (see Figure 4a and b, data shown in ■). This is not a surprise because, other than carrying opposite charges, the trimethylammonium and sulfonate head groups are quite similar.

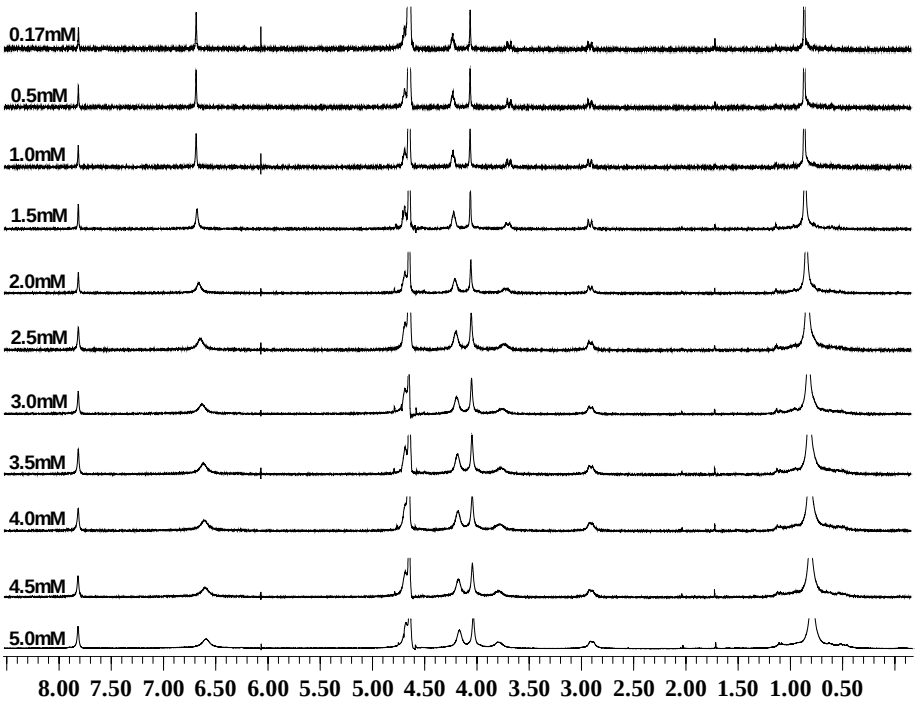


Figure 2. ^{1}H NMR spectra of **6b** at different concentrations in D_2O at 20°C.

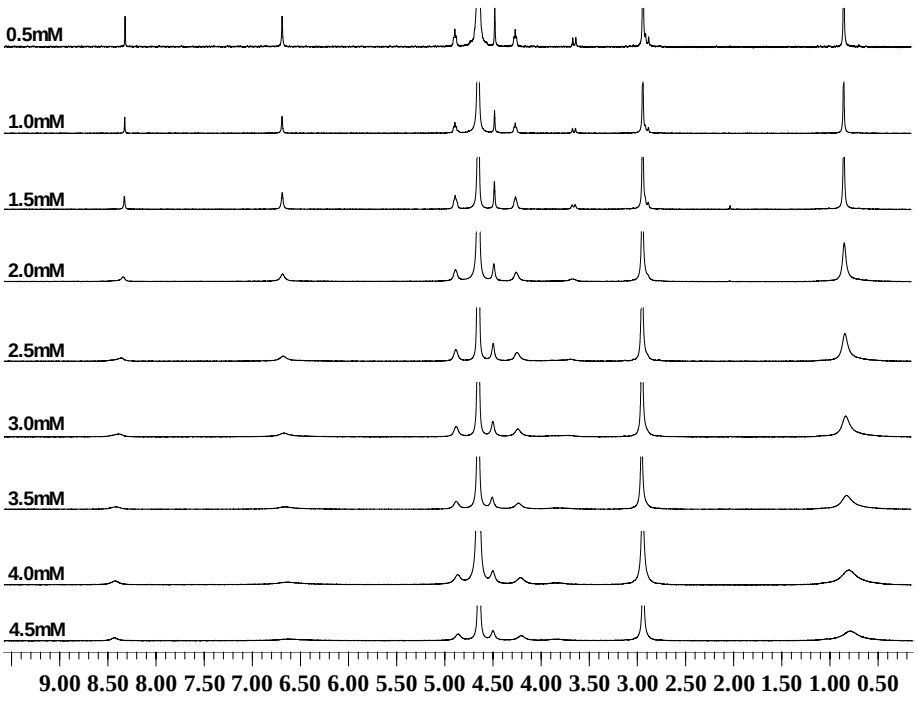


Figure 3. ^{1}H NMR spectra of **6c** at different concentrations in D_2O at 20°C.

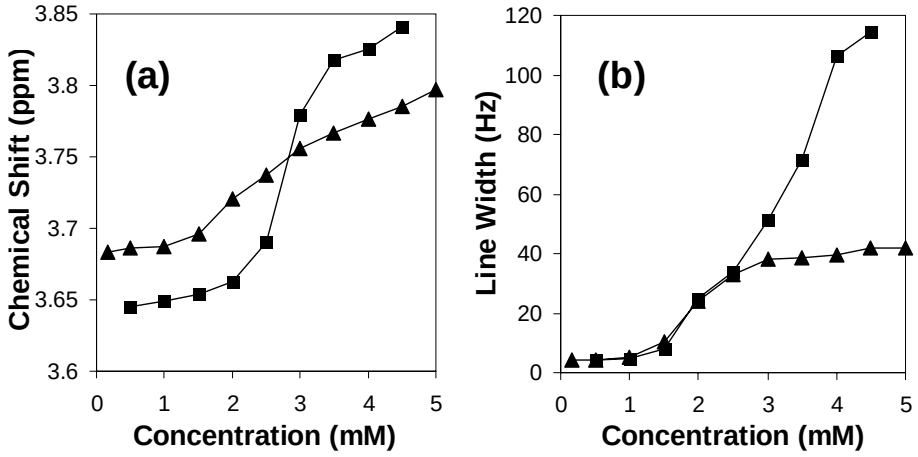


Figure 4. ^{1}H NMR data for **6b** (▲) and **6c** (■) as a function of concentration of the calixarene in D_2O. (a) Chemical shift of the *endo* $ArCH_2Ar$ hydrogens, and (b) line width of the phenyl hydrogen signal vs. concentration.

Conclusions

In summary, we have applied click chemistry to the synthesis of water-soluble calixarenes. Because of possible side reactions between the alkynes, couplings between nonpolar azides and water-soluble alkynes gave much better results than those between nonpolar alkynes and water-soluble azides. The highly selective nature of the alkyne-azide cycloaddition should make this click reaction a general way to introduce polar groups without protective/deprotective chemistry.

Experimental Section

General methods

Anhydrous tetrahydrofuan (THF), CH_2Cl_2, and ethyl ether were dried by passage through a column of activated alumina under compressed nitrogen. All other reagents and

solvents were of A. C. S. certified grade or higher, and were used as received from commercial suppliers. All glassware and syringes were dried in an oven at least overnight prior to use. Routine ^{1}H and ^{13}C NMR spectra were recorded on Varian VXR-300, VXR-400, and Bruker DRX-400 spectrometer. MALDI-TOF mass was recorded on a Thermobioanalysis Dynamo mass spectrometer.

Synthesis

Compound 2.[22] *t*-Butylcalix[4]arene (5.01 g, 7.7 mmmol) was dissolved in anhydrous THF (400 mL) and DMF (20 mL). Under N_2, propagyl bromide (5.95 mg, 50.1 mmol) was added by a syringe. NaH (1.6 g, 39.3 mmol) was added under a N_2 flush and the mixture was stirred at 60 °C for 12 h. Solvent was evaporated *in vacuo*. $CHCl_3$ (100 mL) was added and 2N HCl solution was slowly added to reaction mixture until the pH = 3. It was extracted with $CHCl_3$ (2 x 50 mL). The organic phase was washed with brine (2 x 20 mL), dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography using benzene/hexane (1/1) as the eluent to give a white solid (4.04 g, 65% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, δ) 6.78 (s, 8H), 4.79 (s, 8H), 4.60 (d, J = 12.8 Hz, 4H), 3.16 (d, J = 12.8 Hz, 4H), 2.48 (s, 4H), 1.08 (s, 36H).

Compound 7.[23] *t*-Butylcalix[4]arene (5.02 g, 7.7 mmol), ethyl bromoacetate (12.8 g, 77 mmol), and K_2CO_3 (10.6 g, 77 mmol) were combined with dry acetone (100 mL). The reaction mixture was heated to reflux for 5 days. After the mixture was cooled to room temperature, the solid was filtered and washed with acetone (10 mL). The combined organic solution was concentrated *in vacuo*. The oily residue was crystallized from ethanol to give a white powder (5.84 g, 76% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, δ) 6.77 (s, 8H), 4.85 (d, J =

12.6 Hz, 4H), 4.80 (s, 8H), 4.21 (q, *J* = 7.2 Hz, 8H), 3.19 (d, *J* = 12.6 Hz, 4H), 1.29 (t, *J* = 7.2 Hz, 12H), 1.07 (s, 36H).

Compound 8.[24] Compound **7** (3.01 g, 3.02 mmol) was dissolved in anhydrous ethyl ether (100 mL) and cooled with an ice bath. Lithium aluminum hydride (1.00 g, 26.35 mmol) was added slowly over 5 min. The reaction mixture was heated to reflux for 6 h. Hydrochloric acid (2N) was added slowly until pH = 3 and the organic layer was separated. The organic layer was washed with 2*N* HCl (20 mL), brine (100 mL), and then dried with $MgSO_4$. Solvent was evaporated *in vacuo*. The residue was triturated with hot hexane to give a white powder (1.74 g, 70% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, δ) 6.85 (s, 8H), 4.35 (d, *J* = 12.8 Hz, 4H), 4.00 (t, *J* = 1.6 Hz, 8H), 3.48 (t, *J* = 1.6 Hz, 8H), 3.23 (d, *J* = 12.8 Hz, 4H), 1.08 (s, 36H).

Compound 9.[5] Compound **8** (2.50 g, 3.00 mmol) and triethylamine (1.50 g, 15.15 mmol) were dissolved in anhydrous CH_2Cl_2 (50 mL). The mixture was cooled with an ice bath. Methanesulfonyl chloride (1.76 g, 15.15 mmol) was added by a syringe. The mixture was stirred for 6 h at room temperature. The organic layer (diluted with 50 mL of CH_2Cl_2) was washed with brine (50 mL), dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography using CH_2Cl_2/methanol (8/1) as the eluent to give a white solid (1.36 g, 40% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, δ) 6.80 (s, 8H), 4.71 (t, *J* = 5.4 Hz, 8H), 4.35 (d, *J* = 12.6 Hz, 4H), 3.21 (d, *J* = 12.6 Hz, 4H), 3.21 (s, 12H), 1.07 (s, 36H).

Compound 4.[25] A mixture of compound **9** (298 mg, 0.264 mmol) and NaN_3 (687 mg, 10.56 mmol) in *N, N'*-dimethylpropyleneurea (DMPU, 10 mL) was stirred at 60 ^{o}C for 12 h. Water (20 mL) was added. The precipitate was collected by filtration and washed with water (2 x 2 mL). The product was purified by column chromatography using $CHCl_3$/acetone (15/1) as the eluent to give a white powder (171 mg, 70 % yield). ^{1}H NMR ($CDCl_3$, 300 MHz,

δ) 6.80 (s, 8H), 4.35 (d, J = 12.6 Hz, 4H), 4.06 (t, J = 6.0 Hz, 8H), 3.87 (t, J = 6.0 Hz, 8H), 3.20 (d, J = 12.6 Hz, 4H), 1.08 (s, 36H).

Compound 5a.[26] Compound **5a** was synthesized according to a modified literature procedure.[27] δ-Gluconolactone (2.339 g, 13.1 mmol), propargylamine (4.0 mL, 62.5 mmol) were dissolved in pyridine (5 mL). The mixture was stirred at room temperature for 18 h. The mixture was poured into ether (80 mL). The white solid was collected by suction filtration, washed with ether (10 mL), and pumped dry (2.958 g, 97% yield). ^{1}H NMR (300 MHz, DMSO-d6, δ) 8.00 (t, J = 5.7 Hz, 1H), 5.40 (d, J = 5.2 Hz, 1H), 4.54-3.36 (m, 12H), 3.02 (t, J = 2.5 Hz, 1H); ^{13}C NMR (DMSO-d6, 75 MHz, δ): 173.07, 82.02, 74.21, 73.26, 72.97, 72.18, 70.74, 64.00, 28.441.

Compound 5b. Propargyl bromide (2.0 mL in toluene, 80%, 18.0 mmol) and sodium sulfite (2.86 g, 22.7 mmol) were dissolved in a mixture of water (7 mL) and methanol (7 mL). The mixture was stirred at 65 °C for 7 h. MeOH (120 mL) was added and the precipitate was filtered off. The filtrate was concentrated *in vacuo* to about 5 mL. It was diluted with acetone (100 mL) to give a white precipitate. (2.357 g, 92%). ^{1}H NMR (300 MHz, D_2O, δ) 3.69 (d, J = 2.7 Hz, 2H), 2.56 (t, J =2.7 Hz, 1H).

Compound 5c. Propargyl bromide (0.8 mL in toluene, 80%, 7.2 mmol) was dissolved in acetone (5 mL). Trimethylamine (2.0 mL, 50% aqueous solution, 14.5 mmol) was added. A brown aqueous layer quickly separated. The top acetone layer was removed. The bottom layer was washed with acetone (2 x 5 mL). The aqueous solution was mixed in acetonitrile (15 mL). The mixture was added to acetone (100 mL) to give a white powdery precipitate. (1.027 g, 80%). ^{1}H NMR (300 MHz, D_2O, δ) 4.11 (s, 2H), 3.07 (s, 9H).

Compound 6a. A mixture of **4** (50.8 mg, 0.055 mmol), **5a** (64.4 mg, 0.276 mmol), $CuSO_4 \cdot 5H_2O$ (2.3 mg, 0.0092 mmol), and sodium ascorbate (13.7 mg, 0.069 mmol) were mixed with 2 mL of THF/EtOH/H_2O (1/2/2). The mixture was stirred at 60 ^oC for 24 h under N_2. The mixture was combined with hot EtOH/water (10 mL), filtered to remove insoluble impurities, and precipitated into acetone (60 mL) to give a greenish powder. (51.9 mg, 51%). ^1H NMR (300 MHz, DMSO-d6, δ) 8.10 (t, J = 5.8 Hz, 4H), 7.98 (s, 4H), 6.77 (s, 8H), 5.47 (d, J = 4.4 Hz, 4H), 4.90-3.25 (m, 68H), 3.04 (d, J = 12.3 Hz, 4H), 1.01 (s, 36 H); ^{13}C NMR (75 MHz, DMSO-d6, δ) 173.34, 152.77, 145.87, 145.09, 133.66, 125.66, 123.73, 74.37, 73.02, 72.16, 70.77, 63.95, 50.00, 34.802, 34.23, 31.78. ESI-MS: calcd. for $C_{88}H_{128}N_{16}O_{28}$ [M + 2 Na^+] m/z = 952.0; found, 952.

Compound 6b. A mixture of **4** (50.2 mg, 0.054 mmol), **5b** (35.7 mg, 0.226 mmol), $CuSO_4 \cdot 5H_2O$ (5.0 mg, 0.02 mmol), and sodium ascorbate (48.1 mg, 0.243 mmol) were mixed with 3 mL of THF/EtOH/H_2O (1/2/2). The mixture was stirred at 60 ^oC for 24 h under N_2. Acetone (10 mL) was added to the cooled reaction mixture. The yellow precipitate was collected by filtration and washed with acetone (2 x 5 mL). The solid was dissolved in water (5 mL) and the insoluble impurities were filtered off. The filtrate was lyophilized. The product was purified by reverse-phase column chromatography over C18 silica gel using H_2O/CH_3OH (80/20 to 50/50) as the eluent to give a light yellow powder (64.0 mg, 79 % yield). ^1H NMR (300 MHz, D_2O, δ) 7.82 (s, 4H), 6.68 (s, 4H), 4.69 (t, J = 5.2 Hz, 8H), 4.23 (t, J = 5.2 Hz, 8H), 4.06 (s, 8H), 3.68 (d, J = 12.8 Hz, 4H), 2.91 (d, J = 12.8 Hz, 4H), 0.86 (s, 36H); ^{13}C NMR (75 MHz, D_2O/MeOH-d4, δ) 152.13, 145.45, 139.70, 133.37, 125.23, 125.08, 71.82, 50.53, 33.49, 30.72, 30.25; ESI-MS: calcd. for $C_{64}H_{80}N_{12}O_{16}Na_4S_4$ [M^{4-}+2H^+] m/z = 701.8; found, 702; calcd. for [M^{4-}+H^+] m/z = 467.5; found, 467; calcd. for [M^{4-}] m/z = 350.4; found, 350.

Compound 6c. A mixture of **4** (50.1 mg, 0.054 mmol), **5c** (40.2 mg, 0.226 mmol), $CuSO_4 \cdot 5H_2O$ (5.0 mg, 0.02 mmol), and sodium ascorbate (48.1 mg, 0.243 mmol) were mixed with 3 mL of THF/EtOH/H_2O (1/2/2). The mixture was stirred at 60 ^oC for 24 h under N_2. Acetone (10 mL) was added to the cooled reaction mixture. The yellow precipitate was collected by filtration and washed with acetone (2 x 5 mL). The solid was dissolved in water (5 mL) and the insoluble impurities were filtered off. The filtrate was lyophilized. The product was purified by reverse-phase column chromatography over C18 silica gel using H_2O/CH_3OH (80/20 to 50/50) as the eluent to give a light yellow powder (69.0 mg, 78 % yield). ^1H NMR (300 MHz, D_2O, δ) 8.32 (s, 4H), 6.68 (s, 8H), 4.89 (t, J = 5.2 Hz, 8H), 4.47 (s, 8H), 4.26 (t, J = 5.2 Hz, 8H), 3.65 (d, J = 12.8 Hz, 4H), 2.93 (s, 36H), 2.89 (d, J = 12.8 Hz, 4H), 0.84 (s, 36H); ^{13}C NMR (75 MHz, MeOH-d4, δ): 152.53, 145.79, 136.00, 133.32, 128.78, 125.56, 72.45, 60.03, 52.38, 47.83, 50.64, 33.69, 30.74, 30.72. ESI-MS: calcd. for $C_{76}H_{116}N_{16}O_4Br_4$ $[M^{4+}+2Br^-]$ m/z = 738.8; found, 739; calcd. for $[M^{4+}+Br^-]$ m/z = 465.9; found, 466; calcd. for $[M^{4+}]$ m/z = 329.5; found, 329.

CMC measurement

A typical procedure for the CMC measurement is as follows.[28] A stock solution (1.95 mM) of **6c** was prepared in D_2O. In 11 separate vials, 490, 440, 390, 340, 290, 250, 200, 150, 100, and 50 μL of the stock solution were added. The total volume of the solution was increased to 600 μL by adding appropriate amounts of D_2O. The vials were swirled gently to avoid foam formation. The samples were carefully transferred to 11 NMR tubes and the ^1H NMR spectra were recorded. The CMC was obtained by plotting the change of chemical shift of protons on the calixarene as a function of concentration.

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