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Solvent-Tunable Binding of Hydrophilic and Hydrophobic Guests by Amphiphilic Molecular Baskets

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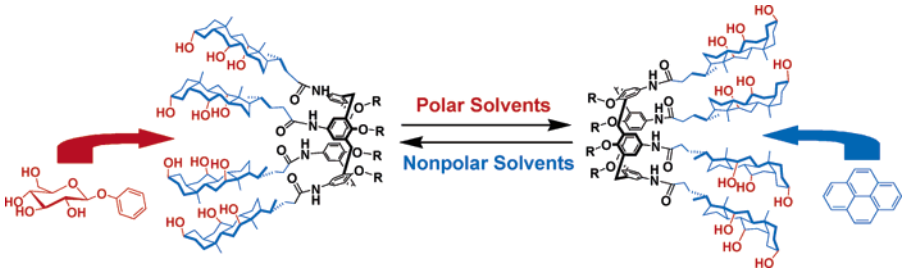

Responsive amphiphilic molecular baskets were obtained by attaching four facially amphiphilic cholate groups to a tetraaminocalixarene scaffold. Their binding properties can be switched by solvent changes. In nonpolar solvents, the molecules utilize the hydrophilic faces of the cholates to bind hydrophilic molecules such as glucose derivatives. In polar solvents, the molecules employ the hydrophobic faces of the cholates to bind hydrophobic guests. A water-soluble basket can bind polycyclic aromatic hydrocarbons including anthracene, pyrene, and perylene. The binding free energy ($-\Delta G$) ranges from 5 to 8 kcal/mol and is directly proportional to the surface area of the aromatic hosts. Binding of both hydrophilic and hydrophobic guests is 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,[1] the substrate of an enzyme can cause necessary conformational change of the active site (to bring the catalytic groups into proper alignment), but nonsubstrates cannot. Allosteric proteins change their conformations, and in turn their binding or catalytic functions, upon binding with effectors or inhibitors.[2] 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 profound influence on the conformations of biomolecules, as hydrophobic interaction[3] (or, more generally, solvophobic interaction) is a major driving force for the folding of polypeptide chains. One class of biomolecules that adopts dramatically different conformations with the change of environmental polarity is α-helical antimicrobial peptides.[4] 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

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(1) (a) Koshland, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1958**, *44*, 98−104. (b) Koshland, D. E. *Nat. Med.* **1998**, *4*, 1112−1114, and references therein.

(2) (a) *Regulation of Enzyme Activity and Allosteric Interactions*; Kvamme, E., Pihl, A., Eds.; Academic Press: New York, 1968. (b) *Allosteric Regulation*; Tokushige, M., Ed.; University Park Press: Baltimore, MD, 1971. (c) *Allosteric Enzymes*; Hervé, G., Ed.; CRC Press: Boca Raton, FL, 1989. (d) Perutz, M. F. *Mechanisms of Cooperativity and Allosteric Regulation in Proteins*; Cambridge University Press: Cambridge, U.K., 1990.

(3) (a) Tanford, C. *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, 2nd ed.; Wiley: New York, 1980. (b) Ben-Mailm, A. *Hydrophobic Interactions*; Plenum Press: New York, 1980. (c) Dill, K. A. *Biochemistry* **1990**, *29*, 7133−7155. (e) Blokzijl, W.; Engberts, J. B. F. N. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1545−1579.

(4) (a) Hwang, P. M.; Vogel, H. J. *Biochem. Cell Biol.* **1998**, *76*, 235−246. (c) Hancock, R. E. W.; Chapple, D. S. *Atimicrob. Agents Chemother.* **1999**, *43*, 1317−1323. (d) Epand, R. M.; Vogel, H. J. *Biochim. Biophys. Acta* **1999**, *1462*, 12−28. (e) Tossi, A.; Sandri, L.; Giangaspero, A. *Biopolymers* **2000**, *55*, 4−30. (f) van't Hof, W.; Veerman, E. C.; Helmerhorst, E. J.; Amerongen, A. V. *Biol. Chem.* **2001**, *382*, 597−619.

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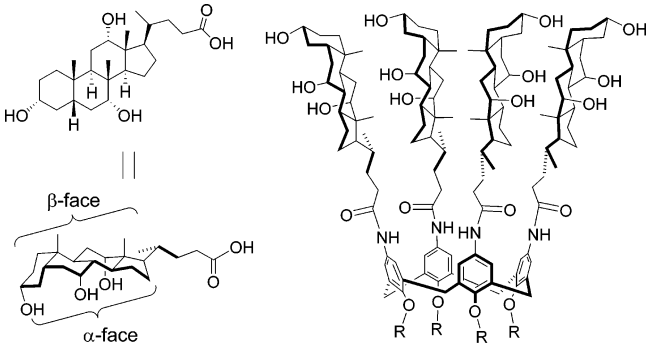
much less polar environment. In fact, polarity-induced conformational change is important to many biological processes including the translocation of proteins across membranes.[5]

Design of synthetic molecules with controllable conformations has received much attention in recent years and is highlighted in foldamer research.[6] 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 as difficult challenges.[6]

We previously reported an amphiphilic molecular basket **1a** constructed from cholic acid.[7] Cholic acid[8] is an example of facial amphiphiles.[9] 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.[10,11] We hypothesize that the internal cavity of **1a** is sufficiently large to bind guest molecules and that 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,[12] 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.[13] The binding stoichiometry was studied by the Job plots (Figure 1). Even though a few data points (at $\chi = 0.1$ and 0.9) are missing because of 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 $\pi-\pi$ interaction between the phenyl and the calixarene and solvophobic interaction between the sugar unit and the cholate groups.

(5) Cserhåti, T.; Szögyi, M. *Int. J. Biochem.* **1994**, *26*, 1−18, and references therein.

(6) For several recent reviews, see: (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173−180. (b) Kirschenbaum, K.; Zuckerman, R. N.; Dill, D. A. *Curr. Opin. Struct. Biol.* **1999**, *9*, 530−535. (c) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893−4012. (d) Cubberley, M. S.; Iverson, B. L. *Curr. Opin. Chem. Biol.* **2001**, *5*, 650−653. (e) Sanford, A. R.; Gong, B. *Curr. Org. Chem.* **2003**, *7*, 1649−1659.

(7) Ryu, E.-H.; Zhao, Y. *Org. Lett.* **2004**, *6*, 3187−3189.

(8) For some examples of supramolecular systems constructed from cholic acid, see: (a) Davis, A. P.; Bonar-Law, R. P.; Sanders, J. K. M. In *Comprehensive Supramolecular Chemistry*; Atwood, J. L., Davis, J. E. D., MacNicol, D. D., Vögtle, F., Eds.; Elsevier: Oxford, U.K., 1996; Vol. 4, Chapter 7, and references therein. (b) Li, Y.; Dias, J. R. *Chem. Rev.* **1997**, *97*, 283−304, and references therein. (c) Maitra, U. *Curr. Sci.* **1996**, *71*, 617−624. (d) Smith, B. D.; Lambert, T. N. *Chem. Commun.* **2003**, 2261−2268, and references therein. (e) Davis, A. P.; Joos, J.-B. *Coord. Chem. Rev.* **2003**, *240*, 143−156, and references therein. (f) Burrows, C. J.; Sauter, R. A. *J. Inclusion Phenom.* **1987**, *5*, 117−121. (g) Janout, V.; Lanier, M.; Regen, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 1573−1574. (h) Ariga, K.; Terasaka, Y.; Sakai, D.; Tsuji, H.; Kikuchi, J.-I. *J. Am. Chem. Soc.* **2000**, *122*, 7835−7836. (i) Werner, F.; Schneider, H.-J. *J. Inclusion Phenom. Macrocyclic Chem.* **2001**, *41*, 37−40. (j) Yoshino, N.; Satake, A.; Kobuke, Y. *Angew. Chem., Int. Ed.* **2001**, *40*, 457−459.

(9) For other examples of facial amphiphiles, see: (a) Stein, T. M.; Gellman, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 3943−3950. (b) Cheng, Y.; Ho, D. M.; Gottlieb, C. R.; Kahne, D.; Bruck M. A. *J. Am. Chem. Soc.* **1992**, *114*, 7319−7320. (c) Venkatesan, P.; Cheng, Y.; Kahne, D. *J. Am. Chem. Soc.* **1994**, *116*, 6955−6956. (d) McQuade, D. T.; Barrett, D. G.; Desper, J. M.; Hayashi, R. K.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 4862−4869. (e) Broderick, S.; Davis, A. P.; Williams, R. P. *Tetrahedron Lett.* **1998**, *39*, 6083−6086. (f) Isaacs, L.; Witt, D.; Fettinger, J. C. *Chem. Commun.* **1999**, 2549−2550. (g) Taotafa, U.; McMullin, D. B.; Lee, S. C.; Hansen, L. D.; Savage, P. B. *Org. Lett.* **2000**, *2*, 4117−4120. (h) Arnt, L.; Tew, G. N. *J. Am. Chem. Soc.* **2002**, *124*, 7664−7665.

(10) A linear and a dendritic polymer have recently been shown to interchange between micellar and reversed-micellar forms. (a) Basu, S.; Vutukuri, D. R.; Shyamroy, S.; Sandanaraj, B. S.; Thayumanavan, S. *J. Am. Chem. Soc.* **2004**, *126*, 9890−9891. (b) Vutukuri, D. R.; Basu, S.; Thayumanavan, S. *J. Am. Chem. Soc.* **2004**, *126*, 15636−15637.

(11) Molecules that respond to solvent changes have been frequently reported, but supramolecular hosts that change the properties of their internal cavities are rare. For some related examples, see: (a) Ikeda, H.; Nakamura, M.; Ise, N.; Oguma, N.; Nakamura, A.; Ikeda, T.; Toda, F.; Ueno, A. *J. Am. Chem. Soc.* **1996**, *118*, 10980−10988. (b) Lane, A. S.; Leigh, D. A.; Murphy, A. *J. Am. Chem. Soc.* **1997**, *119*, 11092−11093 (c) Leigh, D. A.; M. Pérez, E. M. *Chem. Commun.* **2004**, 2262−2263.

(12) Fendler, J. H. *Membrane Mimetic Chemistry*; Wiley: New York, 1982; Chapter 3.

(13) The signals on the sugar residue either stay unchanged or overlap with the signals on the host.

TABLE 1. Association Constants (K_a) between 1 and Several Hydrophilic Guests at 20 °C

entry	guest	host	solvent mixture	K_a (M^{-1})	$-\Delta G$ (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 low solubility of **1a** in the solvent mixture. [b] Nearly no change in chemical shifts occurred during NMR titration, suggesting negligible binding.

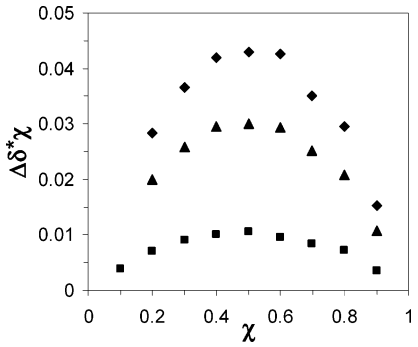


FIGURE 1. The Job plots for the binding between **1a** and β-D-glucopyranoside, in which χ is the molar fraction of **1a**. The chemical shift changes (Δδ) are for the para (◆), meta (▲), and ortho (■) protons of phenyl β-D-glucopyranoside.

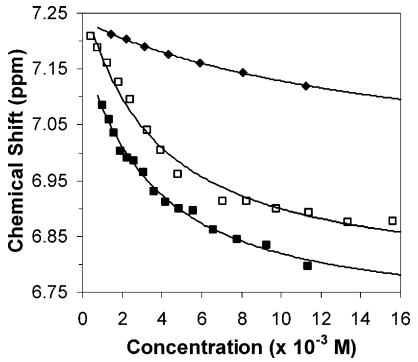


FIGURE 2. Plot of the chemical shift of the ortho protons in phenyl β-D-glucopyranoside as a function of concentration of **1a** in 85/15 (◆), 90/10 (□), and **1b** in 95/5 (■) of CCl_4/CD_3OD (vol/vol). Theoretical curves are nonlinear least-squares fitting to a 1:1 binding isotherm.

In Figure 2, the chemical shift of ortho protons on 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** 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 ■).

Aggregation of the host is negligible under the binding conditions because 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-squares 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 titration (entry 4). Binding properties of **1a** and the more soluble **1b** are quite similar in the reversed-micelle-like 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 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 decisive roles. This is because π−π interaction is expected to decrease in a solvent with higher polarizability.[14] Thus, a π−π-based binding should increase in strength when methanol (a less polarizable solvent) increases and carbon tetrachloride (a more polarizable solvent) decreases in the solvent mixture. We also performed a similar titration of 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[15] found that, in several solvophobically driven host−guest complexations, the binding free energies correlate linearly with the solvophobicity parameters[16] of the solvents. Because solvophobicity parameters of binary mixtures are almost linearly related to the volume percentages, binding energies ($-\Delta G$) were found to vary linearly as a function of solvent volume

(14) For two reviews, see: (a) Schneider, H.-J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1417−1436. (b) Schneider, H.-J.; Yatsimirsky, A. *Principles and Methods in Supramolecular Chemistry*; Wiley: Chichester, U.K., 2000; pp 96−101.

(15) Schneider, H.-J.; Kramer, R.; Simova, S.; Schneider, U. *J. Am. Chem. Soc.* **1988**, *110*, 6442−6448.

(16) (a) Abraham, M. H. *J. Am. Chem. Soc.* **1982**, *104*, 2085−2094. (b) Abraham, M. H.; Grellier, P. L.; McGill, R. A. *J. Chem. Soc., Perkin Trans. 2* **1988**, 339−345.

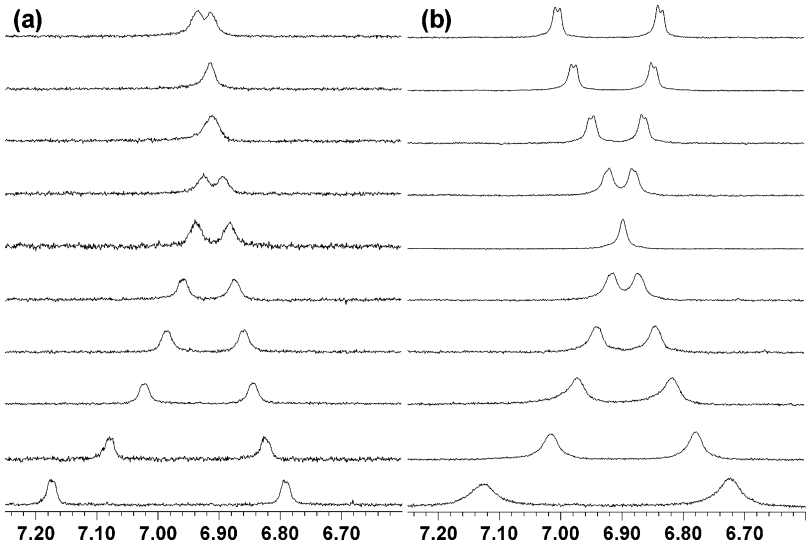


FIGURE 3. (a) The aromatic regions of the ^{1}H NMR (300 MHz) spectra of **1b** in different ratios of DMSO-d_6/CCl_4 at ambient temperature. (b) The aromatic regions of the ^{1}H NMR (300 MHz) spectra of **1a** in different ratios of CD_3OD/CCl_4 at ambient temperature. The solvents in both cases are 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90% CCl_4 from top to bottom.

percentages.[14,15] 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 important roles in stabilizing the reversed-micelle-like conformer, we are interested in its effect on binding affinities. Another polar solvent, DMSO, is also miscible with carbon tetrachloride. Figure 3a shows ^{1}H NMR spectra of **1b** in different ratios of DMSO/carbon tetrachloride. The aromatic protons ortho to the amido group show distinct changes according to 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.[7] 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 be a good indicator for the stability of a particular (micelle-like or reversed-micelle-like) conformer.[7] Therefore, DMSO in carbon tetrachloride seems to be an especially good solvent mixture for reversed-micelle-like conformer.

However, binding between **1b** and phenyl β-D-glucopyranoside is extremely weak in carbon tetrachloride/DMSO (90/10) and is almost undetectable by NMR 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 need 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, that is, 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 has 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 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 Micellelike Conformer in Polar Solvents. In a polar environment, **1a** is expected to bind hydrophobic guests by its micellelike conformer. We use a mixture of deuterated methanol/water (80/20) as the solvent, in which **1a** has solubility in the millimolar range. Addition of pyrene causes upfield changes of the methyl protons on the hydrophobic β face of the cholates. Hence, the guest is bound through favorable hydrophobic contact with the host. 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 titration with 1-aminopyrene, which is more soluble than pyrene in aqueous methanol. The binding constant was 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})	$-\Delta G$ (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]	**2**	water	7.8×10^3	5.3
4	pyrene[b]	**2**	water	5.0×10^4	6.4
5	perylene[b]	**2**	water	6.8×10^5	8.0

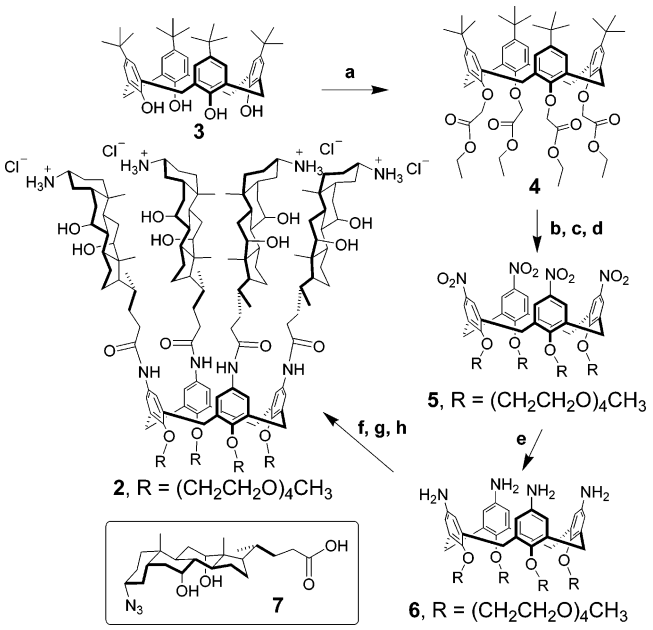
[a] Determined by ^1H NMR titration. [b] Determined by a dye solubilization method with linear fitting of the experimental data (see text). The R value is 0.982, 0.994, and 0.982 for anthracene, pyrene, and perylene, respectively. [c] Nearly no change in chemical shifts occurred during NMR titration,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

Such a low binding affinity ($-\Delta G$ = 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 from 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.

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.[17] 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 water solubility of the calixarene, we attach 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).[18] However, the cone conformer was one of four products formed. In our synthesis, we avoid this problem by using ester **4** as the key intermediate. Ester **4** is prepared in a high yield according to literature procedures, and, most importantly, is already in the cone conformation.[19] It is 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

SCHEME 1. Synthesis of Water-Soluble Amphiphilic Basket 2[a]



[a] 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.

6, and the resulting product is reduced and protonated to afford the final water-soluble basket **2**.

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.[21] 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).[22]

Solubilization of the aromatic compounds by basket **2** 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 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 is truly qualified as a unimolecular micelle. Our experiments indicate that basket **2** is most efficient at solubilizing pyrene, followed by anthracene and perylene. More efficient solubilization, nonetheless, does not mean

(17) It is often difficult to determine the relative contributions of classical solvophobic interaction and van der Waals interaction to the association of hydrophobic molecules in polar solvents. The issue is often controversial, see: (a) Blokzijl, W.; Engberts, J. B. F. N. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1545−1579. (b) Schneider, H.-J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1417−1436.

(18) Conner, M.; Kudelka, I.; Regen, S. L. *Langmuir* **1991**, *7*, 982−987.

(19) Arnaud-Neu, F.; Collins, E. M.; Deasy, M.; Ferguson, G.; Harris, S. J.; Kaitner, B.; Lough, A. J.; McKervey, M. A.; Marques, E.; Ruhl, B. L.; Schwing-Weill, M. J.; Seward, E. M. *J. Am. Chem. Soc.* **1989**, *111*, 8681−8691.

(20) Acid **7** was synthesized according to a literature procedure: Davis, A. P.; Dresen, S.; Lawless, L. J. *Tetrahedron Lett.* **1997**, *38*, 4305−4308. See the Supporting Information for experimental details.

(21) Schott, H. *J. Phys. Chem.* **1966**, *70*, 2966−2973.

(22) Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* **1977**, *99*, 2039−2044.

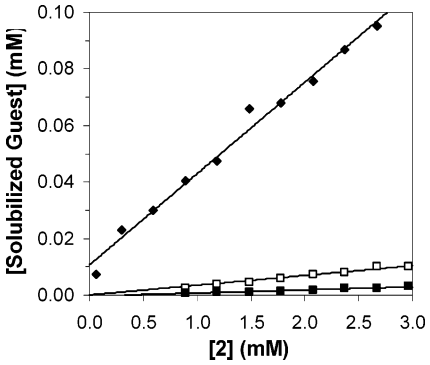


FIGURE 4. Solubilization of anthracene (□), pyrene (◆), and perylene (■) in water by **2**. Theoretical lines are line fitting of the experimental data.

stronger binding, because the amount of the solubilized guest also depends on the solubility of the guest by itself. For 1:1 complexations,[23] the binding constant can be calculated from these dye-solubilization experiments according to the following equation:[24]

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

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 as the intercept, we used the literature values instead ($s_0 = 0.45$, 0.67, and 0.0016 μM for anthracene, pyrene, and perylene, respectively).[25] 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 solvophobic interaction is directly proportional to the area of solvophobic surface removed from solvent contract during complexation.[3]

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 Method. See the Supporting Information.
Compound 1a. See the Supporting Information.
Compound 1b. See the Supporting Information.
Compound 4. See the Supporting Information.

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 via 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 was quenched by 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 was cooled to 0 °C. Nitric acid (90%, 10 mL) was added slowly. The solution was stirred at room temperature for 3 h and was 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 eluents to give an orange oil. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.42 (s, 8H), 4.57 (d, 4H, J = 14.0 Hz), 4.16 (br, 8H), 3.72 (br, 8H), 3.55−3.40 (m, 48H), 3.30 (d, 4H, J = 14.0 Hz), 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, 4H, J = 13.2 Hz), 3.90 (t, 8H, J = 5.6 Hz), 3.75 (t, 8H, J = 5.6 Hz), 3.65−3.46 (m, 48H), 3.30 (s, 12H), 2.82 (d, 4H, J = 13.2 Hz). ^{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.

(23) We were not able to obtain Job plots for complexation between **1a** and 1-aminopyrene because of the low binding affinity. A 1:1 binding stoichiometry was assumed for all three aromatic guests because **1a** and phenyl-β-D-glucopyranoside (which is similar to anthracene in size and smaller than pyrene and perylene) formed a 1:1 complex.

(24) Schneider, H.-J.; Yatsimirsky, A. *Principles and Methods in Supramolecular Chemistry*; Wiley: Chichester, U.K., 2000; p 199.

(25) (a) Abraham, M. H.; Le, J. *J. Pharm. Sci.* **1999**, *88*, 868−880. (b) Futoma, D. J.; Smith, S. R.; Smith, T. E.; Tanaka, J. *Polycyclic Aromatic Hydrocarbons in Water Systems*; CRC Press: Boca Raton, FL, 1981; pp 14−22.

(26) Schmidt, M.; Amstutz, R.; Crass, G.; Seebach, D. *Chem. Ber.* **1980**, *113*, 1691−1707.

Compound 7. See the Supporting Information.

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 eluents 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, 4H, J = 12.8 Hz), 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, 4H, J = 12.8 Hz), 2.77 (t, 4H, J = 10.4 Hz), 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)[27] was added. After 1 h, solvent and HCl was 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 11 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. ^{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. Maximum at 0.5 molar fraction indicated a 1:1 binding stoichiometry.

(27) Nudelman, N.; Bechor, Y.; Falb, E.; Fischer, B.; Wexler, B. A.; Nudelman, A. *Synth. Commun.* **1998**, *28*, 471−474.

^{1}H NMR 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**. 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 were transferred to 16 separate NMR tubes. ^{1}H NMR spectrum was taken for each sample and the chemical shifts of phenyl protons of guest were measured. The binding constants (K_a) were obtained by least-squares 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 11 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 3 days. The excess pyrene was removed by filtration through syringe filters [Millipore 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. Fluorescence intensity of each sample was measured in a quartz cuvette. 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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Supporting Information Available: The general method of the experiments, synthetic procedures (for **1a**, **1b**, **4**, and **7**), and NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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