

Kinetically Stable Complexes in Water: The Role of Hydration and Hydrophobicity

Shannon M. Biros, Elke C. Ullrich, Fraser Hof, Laurent Trembleau, and Julius Rebek, Jr.*

Contribution from The Skaggs Institute for Chemical Biology and the Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

Received October 1, 2003; E-mail: jrebek@scripps.edu

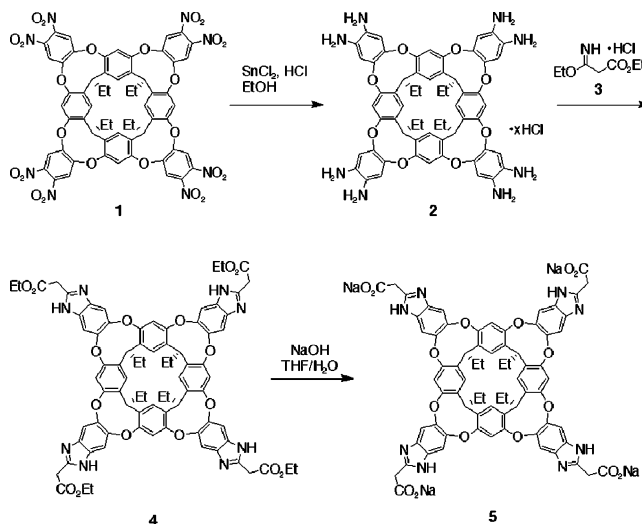
Abstract: We describe here the synthesis and characterization of a molecular receptor that forms kinetically and thermodynamically stable host–guest complexes in water. This cavitand-based host is composed of a preorganized aromatic pocket whose rim is decorated with four negatively charged carboxylate groups. ¹H NMR and isothermal titration calorimetry have been used to characterize the behavior of the resulting complexes in response to changes in pH, buffer identity, and salt concentration and in the presence of sodium dodecyl sulfate micelles.

I. Introduction

Synthetic receptors are attractive targets that provide fundamental understanding of molecular recognition. When this recognition occurs in water, the noncovalent forces on offer include electrostatic, cation– π , CH– π , and van der Waals interactions as well as the hydrophobic effect. Previous artificial receptors have been based on cyclophanes,^{1–5} clefts,^{6–10} calixarenes,^{11–17} porphyrins,^{18–20} and metal–ligand clusters.²¹ These constructs have provided host–guest complexes with varying degrees of thermodynamic stability, but it has proven

- (1) Webb, T. H.; Suh, H.; Wilcox, C. S. *J. Am. Chem. Soc.* **1991**, *113*, 8554–8555.
- (2) Méric, R.; Vigneron, J.-P.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.* **1993**, 129–131.
- (3) Ngola, S. M.; Dougherty, D. A. *J. Org. Chem.* **1998**, *63*, 4566–4567.
- (4) Ngola, S. M.; Kearney, P. C.; Mecozzi, S.; Russell, K.; Dougherty, D. A. *J. Am. Chem. Soc.* **1999**, *121*, 1192–1201.
- (5) Kano, K.; Kitae, T.; Shimofuri, Y.; Tanaka, N.; Mineta, Y. *Chem.–Eur. J.* **2000**, *6*, 2705–2713.
- (6) Metzger, A.; Lynch, V. M.; Anslyn, E. V. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 862.
- (7) Metzger, A.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **1998**, *37*, 649–652.
- (8) Grawe, T.; Schrader, T.; Zadnarm, R.; Kraft, A. *J. Org. Chem.* **2002**, *67*, 3755–3763.
- (9) Niiikura, K.; Metzger, A.; Anslyn, E. V. *J. Am. Chem. Soc.* **1998**, *120*, 8533–8534.
- (10) Rekharsky, M.; Inoue, Y.; Tobey, S.; Metzger, A.; Anslyn, E. V. *J. Am. Chem. Soc.* **2002**, *124*, 14959–14967.
- (11) Atwood, J. L.; Barbour, L. J.; Junk, P. C.; Orr, G. W. *Supramol. Chem.* **1995**, *5*, 105–108.
- (12) Lehn, J.-M.; Méric, R.; Vigneron, J.-P.; Cesario, M.; Guilhem, J.; Pascard, C.; Asfari, Z.; Vicens, J. *Supramol. Chem.* **1995**, *5*, 97–103.
- (13) Sansone, F.; Barbosa, S.; Casnati, A.; Sciotto, D.; Ungaro, R. *Tetrahedron Lett.* **1999**, *40*, 4741–4744.
- (14) Arena, G.; Contino, A.; Fujimoto, T.; Sciotto, D.; Aoyama, Y. *Supramol. Chem.* **2000**, *11*, 279–288.
- (15) Park, S. J.; Hong, J.-I. *Tetrahedron Lett.* **2000**, *41*, 8311–8315.
- (16) Lazzarotto, M.; Sansone, F.; Baldini, L.; Casnati, A.; Cozzini, P.; Ungaro, R. *Eur. J. Org. Chem.* **2001**, 595–602.
- (17) Corbellini, F.; Costanzo, L. D.; Crego-Calama, M.; Geremia, S.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **2003**, *125*, 9946–9947.
- (18) Sirish, M.; Schneider, H.-J. *Chem. Commun.* **1999**, 907–908.
- (19) Jain, R. K.; Hamilton, A. D. *Org. Lett.* **2000**, *2*, 1721–1723.
- (20) Mizutani, T.; Wada, K.; Kitagawa, S. *J. Am. Chem. Soc.* **2001**, *123*, 6459–6460.
- (21) Caulder, D. L.; Raymond, K. N. *Acc. Chem. Res.* **1999**, *32*, 975.

Scheme 1. Synthesis of Water-Soluble Cavitand 5



more difficult to develop kinetically stable complexes in water.²² To date this goal has been achieved only with the aid of metals.²¹ We recently communicated the synthesis and initial binding studies of a water-soluble, cavitand-based host bearing four carboxylates along its upper rim.²³ This host forms kinetically stable complexes in water with guests of the appropriate size, shape, and charge.

II. Synthesis

Known octanitro cavitand **1**²⁴ was reduced with tin chloride in the presence of hydrochloric acid to afford octaamine cavitand

- (22) See, for example, Zadnarm, R.; Junkers, M.; Schrader, T.; Grawe, T.; Kraft, A. *J. Org. Chem.* **2003**, *68*, 6511–6521.
- (23) Hof, F.; Trembleau, L.; Ullrich, E. C.; Rebek, J. J. *Angew. Chem., Int. Ed.* **2003**, *42*, 3150–3153.
- (24) Amrhein, P.; Shivanyuk, A.; Johnson, D. W.; Rebek, J. J. *J. Am. Chem. Soc.* **2002**, *124*, 10349.

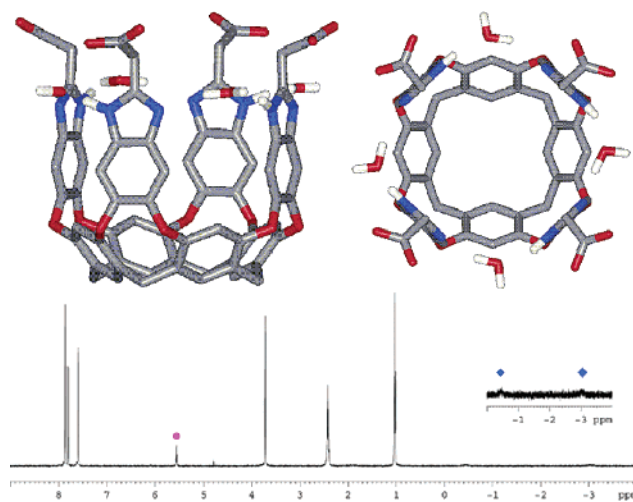


Figure 1. Top: side and top views of **5** in the C_{4v} vase conformation displaying the water-containing hydrogen-bonding seam along the cavity's upper rim. Bottom: ^1H NMR spectrum of a 1 mM solution of host **5** in D_2O at 25 $^\circ\text{C}$: \blacklozenge , resonances of encapsulated THF; \bullet , resorcinarene methine proton resonating at ~ 5.5 ppm. Structures were minimized using Maestro (AMBER force field). Some hydrogens and the pendant ethyl groups have been omitted for clarity.

2 (Scheme 1). Condensation with the imidate of ethylcyanoacetate (**3**)²⁵ provided tetrabenzimidazole ester **4**, which was subsequently saponified under standard conditions to give the target compound **5** as the tetrasodium salt.

III. Host Properties in Water

Compound **5** is soluble in water up to concentrations of ~ 10 mM. The ^1H NMR spectrum of a 1 mM solution of host **5** in D_2O reveals that the flexible walls of the host exist in a well-defined "vase" conformation. The diagnostic methine protons resonate at 5.5 ppm, and sharp signals for host protons indicate that the aggregating "kite" conformation observed for other cavitands^{26–28} is not present in significant amounts (Figure 1). It is likely that host **5** is held in this conformation by a hydrogen-bonding seam composed of four water molecules bridging the nitrogens of the benzimidazole walls. Investigations into this proposed hydrogen-bonding seam are precluded as the host is insoluble in other polar solvents (MeOH, THF, DMF, DMSO) in the absence of water. However, a similar motif has been shown to stabilize a related cavitand in wet chloroform.²⁹ The benzimidazole walls tautomerize rapidly on the NMR time scale at temperatures as low as 4 $^\circ\text{C}$, giving the host overall C_{4v} symmetry.

Additional resonances at -0.6 and -3.2 ppm indicate the presence of one molecule of THF bound within the cavity ($\Delta\delta = 4.6$ ppm). Attempts to remove this adventitious solvent under high vacuum (<1 Torr) and high temperature (ca. 100 $^\circ\text{C}$) proved futile. Molecular modeling shows that a single THF molecule is of insufficient size to fill the cavity of **5**, so it is

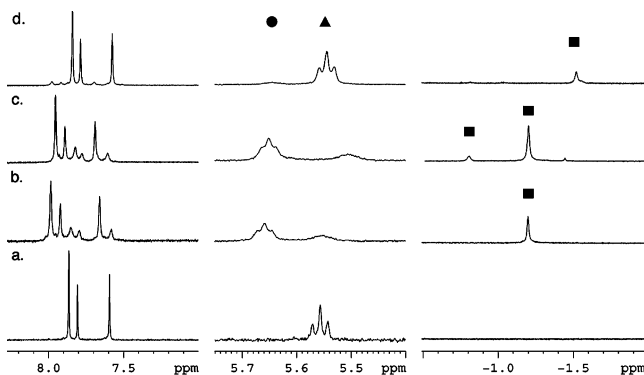


Figure 2. ^1H NMR spectra of 1 mM host **5** in D_2O before addition of guest (a) and in the presence of 1 equiv of tetramethylammonium bromide (**6**) (b), acetylcholine chloride (**12**) (c), and L-carnitine (**13**) (d). Marked resonances indicate free host (\blacktriangle), bound host (\bullet), and bound guest (\blacksquare).

Table 1. Association Constants and Thermodynamic Data Determined by ^1H NMR for Complexation of Host **5** with a Variety of Tetraalkylammonium Salts

Guest	K (M^{-1})
Me_4NBr (6)	4300
Et_4NBr (7)	$> 10^3$
EtMe_3NBr (10)	$> 10^4$
choline chloride (11)	$> 10^4$
acetylcholine chloride (12)	$> 10^4$
L-carnitine (13)	140

likely that the remainder of the cavity is occupied by several water molecules. Collectively, these data suggest the conformation for host **5** shown in Figure 1: that of a preorganized binding pocket occupied by solvent molecules, prepared to bind guests of the appropriate size and shape.

IV. Guest Binding and Selectivity in Water

1. Tetraalkylammonium Salts. The addition of a suitable guest to a solution of host **5** results in the displacement of bound THF, and new signals for both free and bound guest and host appear in the ^1H NMR spectrum (Figure 2). The host–guest complex is kinetically stable on the ^1H NMR time scale. The upfield shift of guest signals ($\Delta\delta$ 4.4–4.9 ppm) is characteristic of binding deep within the shielding aromatic cavity of the host, as opposed to association with the solvent-exposed tetracarboxylate upper rim. Cationic guests such as tetramethylammonium (**6**⁺) and tetraethylammonium (**7**⁺) are of suitable size and shape to fill the cavity and thus form stable complexes (Table 1, Figure 3). Tetrapropylammonium (**8**⁺) and tetrabutylammonium (**9**⁺) fail to form such complexes simply because they are too large to fit inside the preorganized cavity.

Isothermal titration calorimetry (ITC) data reveal that complex formation is both enthalpically and entropically favorable for most guests (Table 1, Supporting Information). In addition to electrostatic attraction between the cationic guest and the host's anionic upper rim, cation– π and CH– π interactions between these tetraalkylammonium guests and the interior aromatic surfaces of the host likely contribute favorable enthalpy for complex formation.³⁰ Displacement of solvent molecules surrounding the guest and interior surfaces of the cavity upon

(25) Church, T. J.; Cutshall, N. S.; Gangloff, A. R.; Jenkins, T. E.; Linsell, M. S.; Litvak, J.; Rice, K. D.; Spencer, J. R.; Wang, V. R. (Axys Pharmaceuticals Corp.). PCT Int. Appl. WO 97-US21849, 1998; p 108.

(26) Cram, D. J.; Choi, H.-J.; Bryant, J. A.; Knobler, C. B. *J. Am. Chem. Soc.* **1992**, *114*, 7748–7765.

(27) Haino, T.; Rudkevich, D. M.; Rebek, J. J. *J. Am. Chem. Soc.* **1999**, *121*, 11253–11254.

(28) Haino, T.; Rudkevich, D. M.; Shivanyuk, A.; Rissanen, K.; Rebek, J. J. *Chem.–Eur. J.* **2000**, *6*, 3797–3805.

(29) Far, A. R.; Shivanyuk, A.; Rebek, J. J. *J. Am. Chem. Soc.* **2002**, *124*, 2854–2855.

(30) Diederich, F. *Cyclophanes: Monographs in Supramolecular Chemistry*; The Royal Society of Chemistry: Cambridge, U.K., 1991.

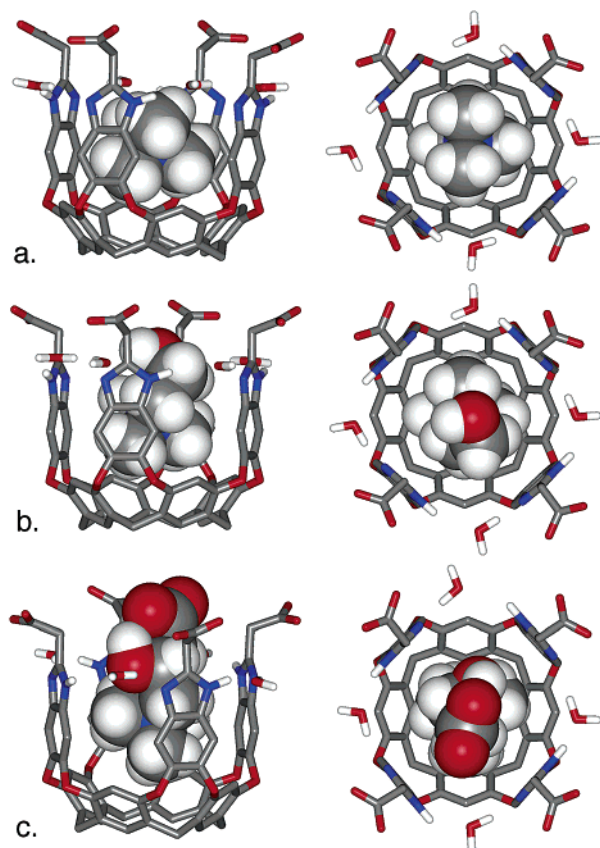


Figure 3. Minimized structures (viewed from the top and side) of host–guest complexes involving compound **5** and tetraalkylammonium salts (a) Me₄N, (b) choline, and (c) L-carnitine. Structures were minimized using Maestro (AMBER force field). Some hydrogens and the pendant ethyl groups have been omitted for clarity.

binding (the hydrophobic effect) is likely responsible for this favorable entropic term.³¹

We investigated a variety of trimethylammonium-containing guests capable of presenting different functionalities to the tetraanionic upper rim. Neutral functional groups such as ethyl (ethyltrimethylammonium, **10**⁺) hydroxyethyl (choline, **11**⁺), and acetoxyethyl (acetylcholine, **12**⁺) attached to this anchor all produce kinetically stable complexes on the NMR time scale with relatively high association constants (Table 1). Presentation of a negatively charged carboxylate moiety to this upper rim (L-carnitine, **13**), however, resulted in the formation of a kinetically stable complex—but with a 100-fold diminished association constant relative to those of the other tetraalkylammonium derivatives. In the case of L-carnitine, the carboxylate moiety of the guest is in proximity to the negative upper rim of the host, resulting in electrostatic repulsion between host and guest (Figure 4). A portion of the difference in binding may also be attributed to the more favorable solvated state of zwitterionic L-carnitine than the singly charged counterparts **10**⁺–**12**⁺, resulting in a lower thermodynamic gain upon complexation within the hydrophobic cavity of the host.

Compounds possessing a positively charged moiety attached to a tetramethylammonium anchor were also examined as

(31) Two guests, choline chloride (**11**) and tetraethylammonium bromide (**10**), generated negative entropic values, with choline's being strongly negative at -10.0 cal/(mol K). As this guest also gave an n^* value of 0.6, it is possible there may be another association process occurring in solution which is not detectable by NMR. Please see the Supporting Information for raw ITC data and other thermodynamic values.

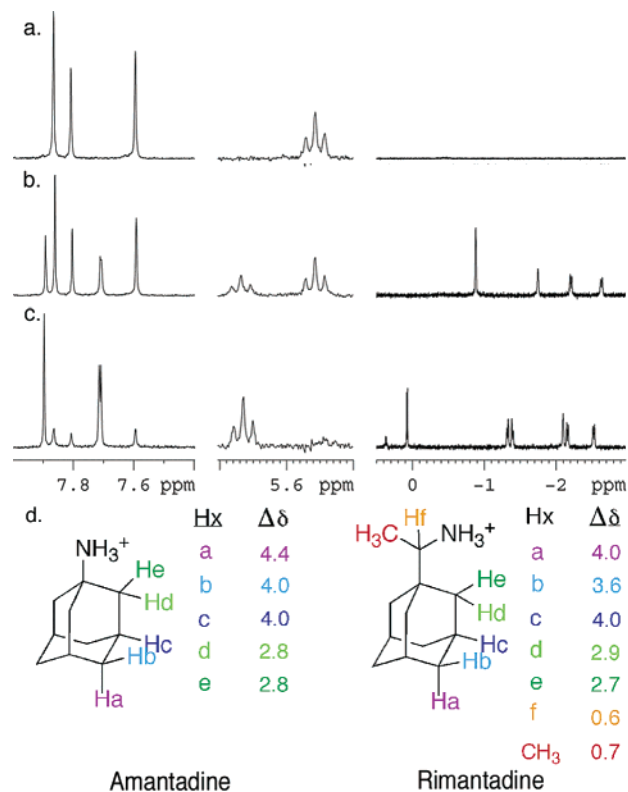
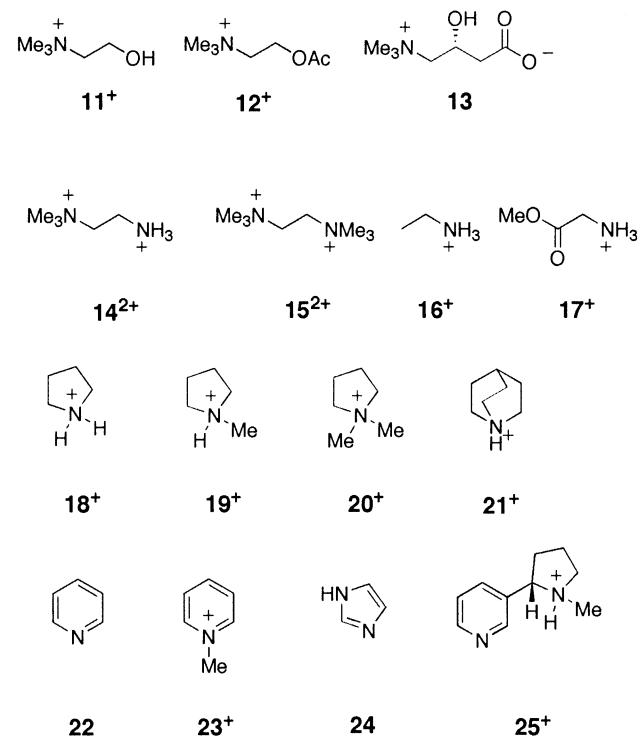


Figure 4. ¹H NMR spectra of host **5** at 1 mM in D₂O: (a) before addition of guest, and in the presence of ca. 1 equiv of (b) amantadine hydrochloride (**26**) and (c) rimantadine hydrochloride (**27**); (d) changes in amantadine (left) and rimantadine (right) proton resonances observed upon encapsulation within host **5**, indicating the guest's orientation in the cavity.

potential guests for this system. Much to our surprise, *N,N,N*-trimethylethylenediamine (**14**²⁺) and its hexamethyl counterpart (**15**²⁺; Chart 1) failed to produce kinetically stable complexes with the host on the NMR time scale. Molecular modeling of the hypothetical complexes (with the trimethylammonium moiety deep in the pocket) places the second cation of the guest among the anionic carboxylates of the host, poised to provide additional electrostatic attraction. The highly solvated nature of these compact dications in water may override their desire to seek encapsulation within the cavity of the host. These compounds could also be engaged in strong electrostatic interactions with the host's tetraanionic upper rim and suspended above the cavity.

2. Other Cationic Amines. Primary amines ethylamine (**16**) and glycine methyl ester (**17**) show no indication of kinetically stable complex formation in their respective ¹H NMR spectra (Chart 1). Neither pyrrolidine (**18**) nor *N*-methylpyrrolidine (**19**) can be enticed to enter the cavity of host **5** until the nitrogen center is exhaustively methylated. *N,N*-Dimethylpyrrolidine (**20**) and the more hydrophobic quinuclidinium hydrochloride (**21**⁺) both form kinetically stable complexes with host **5** (Table 2). These results demonstrate that complementarity of size, shape, and charge is not sufficient to drive guest binding. Primary and secondary amines (presumably protonated at neutral pH) are rejected by the hydrophobic host—possibly due to their reluctance to give up their waters of hydration. Aromatic amines such as pyridine (**22**), *N*-methylpyridinium (**23**⁺), and imidazole (**24**) also fail to form kinetically stable complexes, although they are approximately the correct size and shape to fit inside the cavity.

Chart 1. Cationic Amines Tested for Complexation with Host **5**^a

^a The protonation states shown are the major species present in aqueous solution under experimental conditions (pD 7.8).

Table 2. Association Constants of Amines in D₂O^a

guest	K_a in D ₂ O (M ⁻¹)
<i>N,N</i> -dimethylpyrrolidine (20 ⁺)	5×10^3
quinuclidinium (21 ⁺)	$> 10^4$
<i>S</i> -nicotine (25 ⁺)	$> 10^4$

^a All association constants were determined by ¹H NMR of solutions containing 0.5–1.0 mM host **5** and 1 equiv of guest.

Interestingly, *S*-nicotine (**25**⁺) and host **5** form a kinetically stable complex with an association constant of $> 10^4$ M⁻¹. This guest is composed of the structural elements *N*-methylpyrrolidine and pyridine—neither of which forms a complex with host **5**. Raymond and co-workers have also observed this binding trend with metal–ligand clusters in aqueous solutions.³² One explanation for this anomalous behavior may be found in the experimental log *P* values for each component. In the familiar hydrophobicity model using 1-octanol/water partition, nicotine (experimental log *P* = 1.17) is more hydrophobic than its structural constituents pyridine and *N*-methylpyrrolidine (log *P* values of 0.65 and 0.92, respectively).³³ The magnitude of these partition values cannot fully account for the differences observed for binding by host **5**. Nevertheless, it seems likely that the host's discrimination between guests with similar charges and shapes is not to be derived from specific host–guest contacts, but from differences in guest hydration and hydrophobicity.

3. Adamantanes. Sonication of a solution of **5** in water in the presence of solid adamantane results in the extraction of the insoluble hydrocarbon into water. ¹H NMR analysis shows

formation of a 1:1 kinetically stable complex between adamantane and host **5**, much like those described above. Since this guest does not offer cation– π or electrostatic interactions, the binding is likely driven by a large hydrophobic component as well as CH– π interactions.

The amino-substituted adamantanes amantadine hydrochloride (**26**) and rimantadine hydrochloride (**27**) also form stable 1:1 complexes with **5** in water with binding constants of 1.1×10^3 and $> 10^4$ M⁻¹, respectively. The ¹H NMR spectra of these complexes show that the hydrophobic adamantane base is bound deep within the cavity, while the primary amines are directed toward the tetracarboxylate rim and solvent (Figure 4). The addition of a negatively charged phosphonate, as in the case of adamantan-1-yl phosphonate, results in no observable binding on the NMR time scale. Within this series of monoionic substituted adamantanes the binding orientation, hydration, and hydrophobicity of each guest are expected to be similar. The rejection of the phosphonate-containing guest by the host demonstrates that charge complementarity between the host's carboxylates and the guest *can* be a determining factor in guest selectivity.

V. pH-Dependent Behavior of the Host

Throughout the course of our experiments we noticed that addition of more than ca. 1.5 equivalents of ammonium chloride salts resulted in the precipitation of host **5**. The distance between the carboxylates of this host resembles those of pyromellitic acid (1,2,4,5-tetrabenzoic acid) and 1,2,4,6-tetracarboxylcyclooctatetraenes. In both cases, the third and fourth p*K*_a values of these carboxyl groups are ~ 4.5 and ~ 5.7 , respectively.^{34,35} As ammonium chloride salts are added to a 1 mM solution of host **5** in D₂O (pD³⁶ 8.2), initial protonation events most likely occur at the tetracarboxylate upper rim. Schrader and co-workers have shown that calixarenes bearing four anilinium groups on their upper rim precipitate from methanol/water solutions upon deprotonation of two or more acidic sites.³⁷ It is reasonable that protonation of one or two of the carboxylates decorating host **5** would have a substantial effect on its solubility.

In an attempt to solve this problem we carried out binding experiments in the presence of two buffers (Tris and sodium phosphate), with pD values ranging from 6.2 to 11.2. At neutral or slightly basic pD, the presence of the buffers in 10-fold excess relative to the host results in precipitation of the host (and consequently loss of guest binding). Adjusting each buffer system to pD ≥ 9 restores host solubility at the concentrations required for NMR measurements (1 mM). Unexpectedly, the guest-binding behavior of the host is not only dependent on pD, but also dependent on the identity of the buffer. A solution of host **5** with guest EtMe₃N⁺ (1 mM each) at pD 10 shows good binding in the presence of Tris (10 mM), while an equivalent sample in phosphate buffer (10 mM) provides no evidence of complex formation.

In general, binding constants measured in the presence of Tris buffer are of the same order of magnitude as those measured in unbuffered D₂O. However, competition experiments between

(32) Johnson, Darren W. Personal communication. For nicotine binding, please see: Terpin, A. J.; Ziegler, M.; Johnson, D. W.; Raymond, K. N. *Angew. Chem., Int. Ed.* **2001**, *40*, 157–160.

(33) Hansch, C.; Leo, A. *Exploring QSAR: Fundamentals and Applications in Chemistry and Biology*; American Chemical Society: Washington, DC, 1995.

(34) Leto, J. R.; Leto, M. F. *J. Am. Chem. Soc.* **1961**, *83*, 2944–2951.

(35) Imai, H.; Yaehashi, Y.; Misuno, Y. *Nippon Kagaku Zasshi* **1969**, *90*, 1048–1050.

(36) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188.

(37) Zadnadar, R.; Junkers, M.; Schrader, T.; Grawe, T.; Kraft, A. *J. Org. Chem.* **2003**, *68*, 6511–6521.

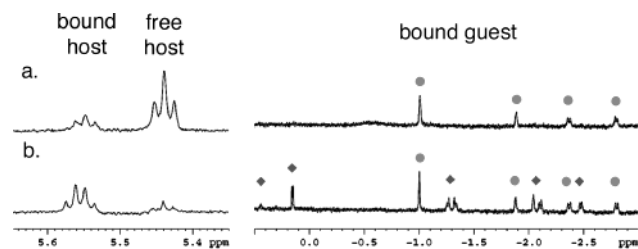


Figure 5. ^1H NMR spectra of host **5** (1 mM) with equivalent amounts (1 mM each) of amantadine hydrochloride (●) and rimantadine hydrochloride (◆) in (a) D_2O and (b) 10 mM Tris buffer at pD 11.2.

rimantadine hydrochloride (**27**) and amantadine hydrochloride (**26**) reveal a dramatic change in the selectivity of host **5** for these guests under buffered and unbuffered conditions (Figure 5). In unbuffered D_2O , host **5** (1 mM, pD 7.8) binds only amantadine when given a choice between equal amounts of amantadine and rimantadine (0.5 mM each).³⁸ The addition of basic Tris changes the pD of the solution to 11.2 and alters the selectivity of the host; both amantadine and rimantadine are bound with equal magnitude. The $\text{p}K_{\text{a}}$ values of each of these primary amines are similar, and each should be in the same protonation state under buffered conditions. As such, the reason for the host's altered guest-binding selectivity is not readily understood.

VI. Salt-Dependent Host Behavior

The addition of various salts to a 1 mM solution of **5** in D_2O causes precipitation of the host. The minimum threshold concentration depends on the identity of the salt: tetraalkylammonium chlorides trigger host precipitation at ca. 5 mM, while NaCl produces a similar result at 1 M. CsCl must be present at 12 M to generate the same effect. This trend follows the Hofmeister series of kosmotropic and chaotropic ions, derived over a century ago from the effects of different salts on protein solubility.^{39,40} This suggests that the host precipitation is driven by the salts' effect on bulk water structure and interfacial phenomena; a kosmotropic ion (such as tetramethylammonium cation) creates order in water, and subsequently increases hydrophobic aggregation and precipitation. These observations may also shed light on the sensitivity of host **5** to the presence of 10 mM sodium phosphate buffer. Although the sodium cation is a relatively benign agent for inducing host precipitation, phosphate anions are strongly kosmotropic, and may be responsible for the observed precipitation of host **5** at pD 7.2.

VII. Guest Binding under Physiological Conditions

Despite the foreknowledge that buffers and salts can have deleterious effects on the guest-binding behavior of host **5**, we attempted to study its ability to bind guests under near physiological conditions. A standard phosphate-buffered saline was used to approximate the pH and salt concentrations present in human blood (pH 7.4, $[\text{NaCl}] = 120$ mM, $[\text{KCl}] = 2.7$ mM). Not surprisingly, under these conditions the host is completely

insoluble and, consequently, no guest binding is observed. The addition of sodium dodecyl sulfate (SDS) above its critical micellar concentration (8 mM) solubilizes host **5**. Diffusion-ordered NMR spectroscopy (DOSY) reveals that the host associates with the micelles,⁴¹ and the ^1H NMR shows that within this lipid environment the preorganized vesi-like structure is recovered (Figure 6). Given the amphiphilic nature of the host, we envision a supramolecular structure in which the aromatic walls and aliphatic feet of **5** are buried in the micelle's core, while the carboxylate-lined mouth is located at the surface of the micelle, open to bulk solvent and able to bind guests. Table 3 shows the binding constants under these conditions for a selection of guests from each series. Solutions containing only SDS and guest reveal no kinetically stable complexes.

VIII. Conclusions and Outlook

Within each structurally related family of guests the host forms kinetically stable complexes with a few of its favorite members. Host-guest systems that operate in organic solvents demonstrate guest selectivity based on size, shape, and the positioning of complementary chemical functionality. The present host also exhibits these basic aspects of molecular recognition in guest selection. Within families of related compounds having similar size, shape, and charge, an extra level of discrimination is observed. Subtle changes in chemical functionality give rise to examples of unanticipated selection. The common thread that connects these instances is the relative hydrophilicity of the related guests. Even when size, shape, and charge are complementary, a guest that interacts strongly with water or buffers will not forsake its solvation shell in favor of the host's hydrophobic cavity.

The structure of this cavitand-based host is reminiscent of cation-binding proteins such as acetylcholinesterase: the guest is attracted by anionic carboxylates that line the mouth of the binding cavity, but is bound exclusively through contacts with aromatic residues.²³ As such, this cavitand-based host offers an entrance into a class of receptors that are soluble in water and mimic the behavior of proteins. The host is able to bind important biological targets such as neurotransmitters (the cholines) and anti-influenza drugs (the aminoadamantanes),^{42,43} and can function under physiological conditions within lipid superstructures. We are hopeful that this host will prove useful for applications in biologically relevant settings.

IX. Experimental Section

1. General Considerations. Deuterated solvents were used as purchased from Cambridge Isotope Laboratories. All other chemicals were obtained from Sigma-Aldrich or Acros Chemicals and used without further purification unless otherwise stated. ^1H and ^{13}C NMR spectral data were recorded on a Bruker 600-DRX spectrophotometer. Chemical shifts are expressed as parts per million (δ) relative to the peak for SiMe_4 (TMS; $\delta = 0$), and referenced internally with respect to that for the protio solvent impurity. NMR structural studies in D_2O were conducted with water suppression using a 3–9–19 pulse sequence with gradients.^{44,45} For samples requiring accurate integration, no solvent

(38) It is surprising that the host binds *only* amantadine in the presence of both adamantane derivatives when the association constant for rimantadine is at least an order of magnitude greater, and that this binding does not saturate the host.

(39) Hofmeister, F. *Arch. Exp. Pathol. Pharmacol.* **1888**, *24*, 247.

(40) Cacace, M. G.; Landau, E. M.; Ramsden, J. J. *Q. Rev. Biophys.* **1997**, *30*, 241–277.

(41) Trembleau, L.; Rebek, J. J. *Chem. Commun.* **2004**, 58–59.

(42) Kornhuber, J.; Schoppmeyer, K.; Riederer, P. *Neurosci. Lett.* **1993**, *163*, 129–131.

(43) McGahen, J. W.; Neumayer, E. M.; Grunert, R. R.; Hoffmann, C. E. *Ann. N. Y. Acad. Sci.* **1970**, *173* (Art. 1), 557–567.

(44) Sklenar, V.; Piotto, M.; Leppik, R.; Saudek, V. *J. Magn. Reson., Ser. A* **1993**, *102*, 241–245.

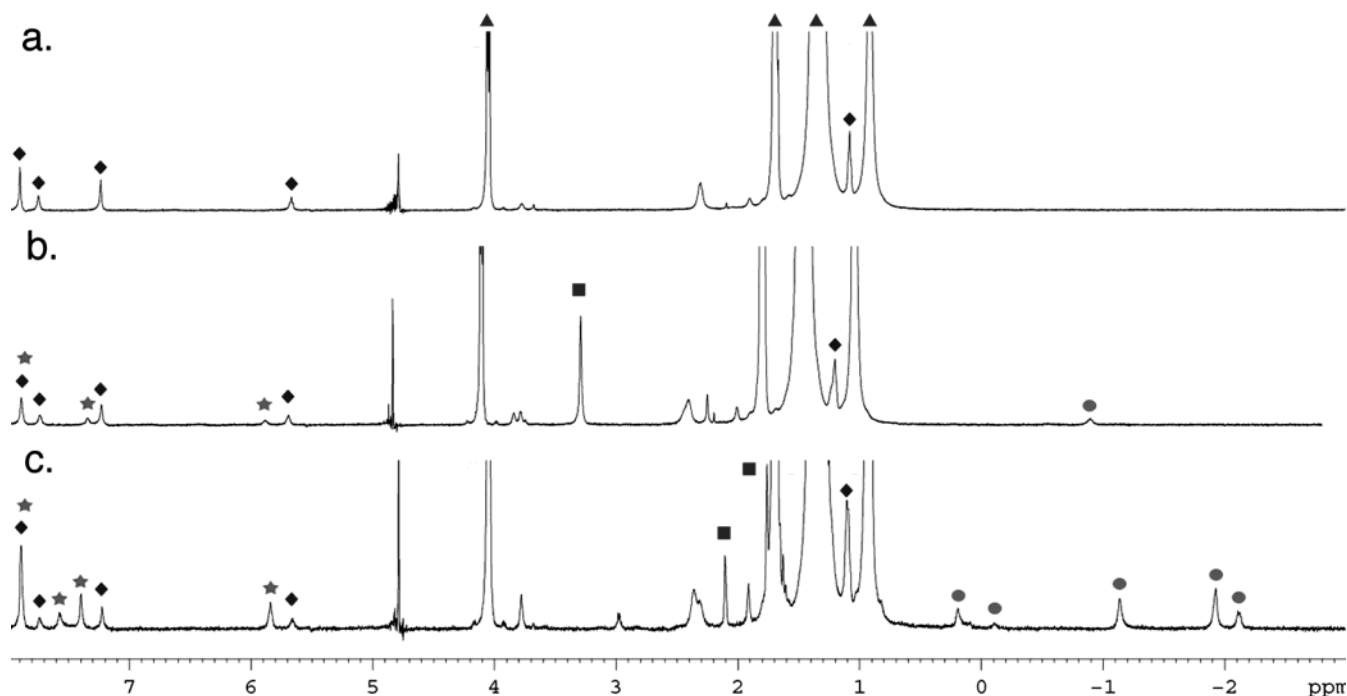


Figure 6. ^1H NMR spectra of 1 mM host **5** in phosphate-buffered saline ([phosphate] = 10 mM, pH 7.4 (pD 7.8) at 25 °C, [NaCl] = 120 mM, [KCl] = 2.7 mM) in the presence of 40 mM SDS before the addition of guest (a) and after addition of 1 equiv of acetylcholine (b) and after addition of 1 equiv of rimantadine hydrochloride (c). Key: ▲, SDS; ◆, free host; ★, bound host; ■, free guest; ●, bound guest.

Table 3. Association Constants for Select Guests under Physiological Conditions^a

guest	K_a (M^{-1})
tetramethylammonium bromide	700
acetylcholine chloride	650
rimantadine hydrochloride	7800

^a Binding constants for host **5** determined in phosphate-buffered saline (Sigma) with 40 mM SDS in D_2O . Solutions contain 1 mM host and 1 mM guest.

suppression pulse was used and the relaxation time (d1) was set to 10 s (5 times the maximum relaxation time for host protons). Matrix-assisted laser desorption/ionization (MALDI) FTMS spectra were determined on an IonSpec FTMS mass spectrometer. Molecular modeling (molecular mechanics calculations) was carried out using the AMBER force field⁴⁶ with the solvation (dielectric) setting for water as implemented by MacroModel or Maestro (Schroedinger, Inc.) on a Silicon Graphics Octane workstation.

2. ITC Studies. ITC data were obtained on a VP-ITC MicroCalorimeter, MicroCal, LLC (Northampton, MA). Titrations were performed at 25 °C with a host concentration of approximately 1 mM in the cell (1.4348 mL), and a guest concentration of approximately 20 mM in the syringe (250 μL). All solutions were prepared with distilled water. Injection volumes varied from 5 to 10 μL , with a 400 s spacing between injections. All titrations were performed in triplicate. After the reference titration was subtracted, the revised data were fitted to a theoretical titration curve using the One Set of Sites model of the Origin 7.0 software provided by MicroCal, LLC.

3. Synthesis. a. Cavitand Octaamine Hydrochloride (2). Octanitro cavitand **1**²⁴ (2.50 g, 2.0 mmol) and tin(II) chloride (17.54 g, 46.5 mmol) were combined in ethanol (180 mL) and 37% HCl (50 mL). After being

heated at 65 °C overnight, the reaction was cooled, and most of the ethanol was removed using a rotary evaporator. Water (50 mL) was added, and the resulting precipitate was filtered, washed with water, and dried under high vacuum to give the product as the hydrochloride salt (1.45 g, 70%), which was used without further purification.

b. Cavitand Tetraester (3). The hydrochloride salt of the cavitand octaamine (380 mg, 0.36 mmol) and the imidate **4**²⁵ (570 mg, 2.91 mmol) were combined in anhydrous EtOH (8 mL, stored over 4 Å molecular sieves) and heated at reflux overnight. The mixture was cooled to room temperature, concentrated to dryness on a rotary evaporator, and taken up in CH_2Cl_2 (20 mL). The suspension was filtered and washed with copious CH_2Cl_2 followed by copious water. The solid was dried under high vacuum for 48 h to yield the product as a buff-colored solid (312 mg, 62%). ^1H NMR ($\text{THF}-d_8$: D_2O = 2:1, 600 MHz): δ 1.36 (t, 12H, J = 7.2 Hz), 1.60 (t, 12H, J = 7.2 Hz), 2.71 (dqr, 8H, J = 8.2, 7.2 Hz), 4.57 (qr, 8H, J = 7.2 Hz), 6.00 (t, 4H, J = 8.2 Hz), 7.80 (s, 4H), 8.15 (s, 4H), 8.40 (s, 8H). ^{13}C NMR ($\text{THF}-d_8$: D_2O = 2:1, 151 MHz): δ 12.8, 14.1, 25.8, 36.2, 62.7, 67.9, 110.0, 117.4, 124.4, 132.0, 136.1, 149.3, 151.4, 156.8, 168.4. MALDI FTMS (MH^+ , m/z): calcd for $\text{C}_{80}\text{H}_{73}\text{N}_8\text{O}_{16}$ 1401.5139, found 1401.5174.

c. Cavitand Tetrasodium Salt (5). The cavitand tetraester (102 mg, 0.073 mmol) was dissolved in a mixture of THF (9 mL) and water (6 mL), and NaOH (96 mg, 2.4 mmol) was added as a solid. After the mixture was stirred for 1 h, a precipitate had formed. Additional water (1 mL) gave a clear solution, and stirring was continued at room temperature for 2 d. The THF was slowly removed from the solution by rotary evaporation over the course of 1 h, allowing a cohesive precipitate to form (faster evaporation gives material that is too fine to allow filtration). The solid was collected by filtration and dried under high vacuum to give the product as a buff-colored solid (79 mg, 79%). ^1H NMR (D_2O : $\text{THF}-d_8$ = 3:1, 600 MHz): δ 1.17 (t, 12H, J = 7.0 Hz), 2.47 (dqr, 8H, J = 8.2, 7.0 Hz), 3.84 (s, 8H), 5.82 (t, 4H, J = 8.2 Hz), 7.51 (s, 4H), 7.83 (s, 4H), 7.92 (s, 8H). ^{13}C NMR (D_2O : $\text{THF}-d_8$ = 3:1, 151 MHz): δ 12.5, 25.6, 35.8, 38.3, 117.3, 123.4, 135.4, 148.7, 154.0, 156.6, 175.4. MALDI FTMS (acidified sample, $[\text{M} - 4\text{Na} + 5\text{H}]^+$, m/z): calcd for $\text{C}_{72}\text{H}_{57}\text{N}_8\text{O}_{16}$ 1289.3887, found 1289.3836.

(45) Piotto, M.; Saudek, V.; Sklenar, V. *J. Biomol. NMR* **1992**, *2*, 661–666.

(46) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.

Acknowledgment. We thank The Skaggs Institute for Chemical Biology (TSRI), the NIH (Grant GM 27932), and the Deutsche Forschungsgemeinschaft (E.C.U.) for support. We are also grateful to Prof. Pablo Ballester (Universitat de les Illes Balears, Spain) for helpful discussions and Laura Pasternak (TSRI) for NMR assistance.

Supporting Information Available: Raw ITC data and all thermodynamic values for titrations performed on the guests listed in Table 1 (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

JA038823M