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Hydrogen-Bonded Encapsulation Complexes in Protic Solvents

Toru Amaya and Julius Rebek, Jr.*

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

Received June 1, 2004; E-mail: jrebek@scripps.edu

Abstract: We describe here the behavior of the hydrogen-bonded capsule **1·1** and its complexes in protic solvents. The kinetics and thermodynamics of the encapsulation process were determined through conventional ^{1}H NMR methods. The enthalpies and entropies of encapsulation are both positive, indicating a process that liberates solvent molecules. The rates of dissociation−association of the capsule were comparable to the rates for the in−out exchange of large guests, which suggests that guest exchange occurs by complete dissociation of the capsule in protic solvents. The stability of the hydrogen-bonded capsule **1·1** toward protic solvents depends strongly on the guests, with the best guest being dimethylstilbene **8**. The results establish guidelines for the properties of capsules that could be accessed in water.

Introduction

Molecular recognition, self-assembly, and nanoscale organic chemistry are all features of reversible encapsulation complexes. Whether held together by hydrogen bonds[1] or metal−ligand interactions,[2] these complexes of molecules inside molecules provide a means to explore intimate molecular relationships. The metal−ligand capsules are formed in water, but the hydrogen-bonded systems are stable in solvents that do not compete well for donors and acceptors. Most of these assemblies dissociate after the addition of small amounts of highly polar solvents, but there are exceptions. We reported the encapsulation behavior of the "tennis ball" in DMF[3] and a dimeric pyrogallol[4]-arene in methanol.[4] Atwood and co-workers used a hexameric pyrogallol[4]arene as a host in protic media,[5] while Böhmer showed high kinetic stability of hydrogen-bonded calixarene capsules outfitted with bulky residues in DMSO.[6] There are recent reports by Reinhoudt of salt-bridged capsules that persist in methanol[7] and even in water.[8] The present research was undertaken to examine the behavior of the hydrogen-bonded cylindrical capsule **1·1** (Figure 1) in the presence of competitive protic solvents, with an eye to finding what properties are needed for hydrogen-bonded capsules that could persist in water.

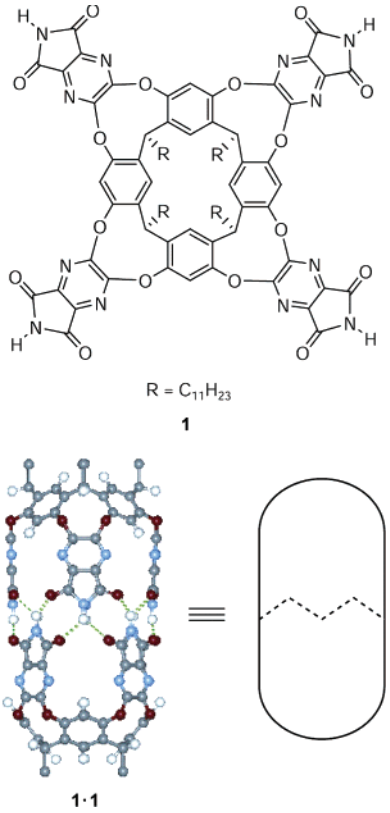

Figure 1. Line drawing of the tetraimide subunit (top) and the ball-and-stick model of the cylindrical capsule and cartoon representation used elsewhere in this work (bottom).

Cavitand **1** forms a cylindrical capsule **1·1** (Figure 1) in apolar organic solvents,[9] such as benzene, toluene, chloroform, and dichloromethane, but does not form in polar solvents, such as

(1) For reviews, see: (a) Conn, M. M.; Rebek, J., Jr. *Chem. Rev.* **1997**, *97*, 1647−1668. (b) Rebek, J., Jr. *Acc. Chem. Res.* **1999**, *32*, 278−286. (c) Rebek, J., Jr. *Chem. Commun.* **2000**, 637−643. (d) Reinhoudt, D. N.; Timmerman, P. *Angew. Chem., Int. Ed.* **2001**, *40*, 2382−2426. (e) Craig, S. L.; Nuckolls, C.; Rebek, J., Jr. *Angew. Chem., Int. Ed.* **2002**, *41*, 1488−1508.
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(3) Branda, N.; Grotzfeld, R. M.; Valdés, C.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1995**, *117*, 85−88.
(4) Shivanyuk, A.; Rebek, J., Jr. *Chem. Commun.* **2001**, 2374−2375.
(5) Atwood, J. L.; Barbour, L. J.; Jerga, A. *Chem. Commun.* **2001**, 2376−2377.
(6) Vysotsky, M. O.; Thondorf, I.; Böhmer, V. *Chem. Commun.* **2001**, 1890−1891.
(7) Corbellini, F.; Fiammengo, R.; Timmerman, P.; Crego-Calama, M.; Versluis, K.; Heck, A. J. R.; Luyten, I.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **2002**, *124*, 6569−6575.
(8) Corbellini, F.; Di Costanzo, L.; Crego-Calama, M.; Geremia, S.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **2003**, *125*, 9946−9947.

DMF and DMSO.[10] While mesitylene is too large to be accommodated, encapsulation complexes form in this solvent *when suitable guests are present*. The capsule offers space for one large (dodecane, **2**), two medium (toluene), or three small (isopropyl chloride, **3**) guests. In addition, two different guests can be coencapsulated when together they fill about one-half of the cavity (e.g., **3** and *p*-xylene (**4**) or **3** and naphthalene (**5**)). The lifetime of the occupied capsule in mesitylene is ~0.5 s, and the exchange rates of guest species in and out of the capsule are typically slow on the NMR time scale (at ambient temperature and 600 MHz). This allows the observation of guests inside by conventional NMR techniques, and separate signals are observed for free and bound guests.

Results and Discussion

The Effects of Methanol. The stability of the cylindrical capsules against protic solvents was investigated by dilution experiments with methanol-d_4. The number of guests and their positions in the capsule were varied: a single molecule each of dodecane (**2**), (*E*)-4,4′-dimethylbiphenyl (**6**), stilbene (**7**), and 4,4′dimethylstilbene (**8**) or coencapsulation complexes of isopropyl chloride and *p*-xylene (**9**) or naphthalene (**10**). Encapsulation phenomena are governed by a good fit of the guest inside the host. For many guests, the optimal fit corresponds to filling ~55% of the cavity.[11] The volumes and packing coefficients of these guests are shown in Figure 2. Capsules **9** and **10** are at about 45% occupancy; dodecane[12] encapsulation **11** offers a snug fit at 48%, and complexes **12** and **13** have lower PCs but allow some sliding of guests **6** and **7** within the capsule. The best guest among these is **8**, which fills about 48% of the space in complex **14**.

Dilution experiments were carried out in mesitylene-d_{12} (600 μL) in the presence of excess guest, with methanol-d_4 added in 5 μL increments. The degree of capsule dissociation could be followed by ^{1}H NMR. Figure 3 shows the spectra of capsule **12** and the new set of signals for monomeric **1** (blue arrows) that appear and grow with the addition of methanol-d_4. After addition of 40 μL of methanol-d_4, capsule **12** was completely dissociated. The resonances for monomeric **1** were identified in a separate NMR experiment using the same amount of methanol with no guest. The results for several guests are summarized in Figure 4. Coencapsulation complexes **9** and **10** dissociated after the addition of only 25 μL of methanol-d_4; capsules **12** and **13** survived up to the addition of ~40 μL of methanol-d_4, and capsule **11** required 60 μL. However, capsule **14** was stable even in the presence of ~2500 equiv of methanol. This stability is not a kinetic effect since no difference was observed in the NMR spectrum of the sample with 60 μL methanol-d_4 after 1 month. These results show that a capsule with multiple guests, even one with a high PC, is not as stable as a well-filled capsule with a single guest. This behavior prompted us to probe the stability of complex **14** when exposed to other alcohols.

The Stability of Capsule 14 in Protic Solvents. To follow the dissociation of capsule **14** in methanol, the ratio of methanol-d_4 (v/v in mesitylene-d_{12}) was varied from 0 to 60%. Figure 5 shows the ^{1}H NMR spectra and its assignments. In the presence of excess guest **8** (25 mM), capsule **14** was formed quantitatively (Figure 5a). In the spectrum with 10% methanol (Figure 5b), a set of small signals of monomer **1** appeared. Like those of the experiments with other capsules, these signals gradually grew with increased addition of methanol-d_4. Surprisingly, approximately 12% of capsule **14** remained even in 50% methanol-d_4 (Figure 5f). The upper limit for detection of **14** by NMR was approximately 60% methanol-d_4 (Figure 5g), although some precipitation of **8** occurred at this level. Ethanol-d_6 and 2-propanol-d_8 were also used as protic solvents (Figure 6). Capsule **14** showed the highest stability in the presence of 2-propanol-d_8 and decreased in ethanol-d_6 and further in methanol-d_4. Binding constants, K (M^{-2}), and binding free energies, ΔG (kcal/mol), at 300 K in this dissociation−recombination process (Figure 7) were calculated (Table 1). The binding constant increases by approximately 1 order of magnitude when changing the solvent from methanol-d_4 to ethanol-d_6 and again from ethanol-d_6 to 2-propanol-d_8.

The stability of **14** results from a combination of host−guest interactions, such as solvophobic effects,[13] CH−π interactions,[14]

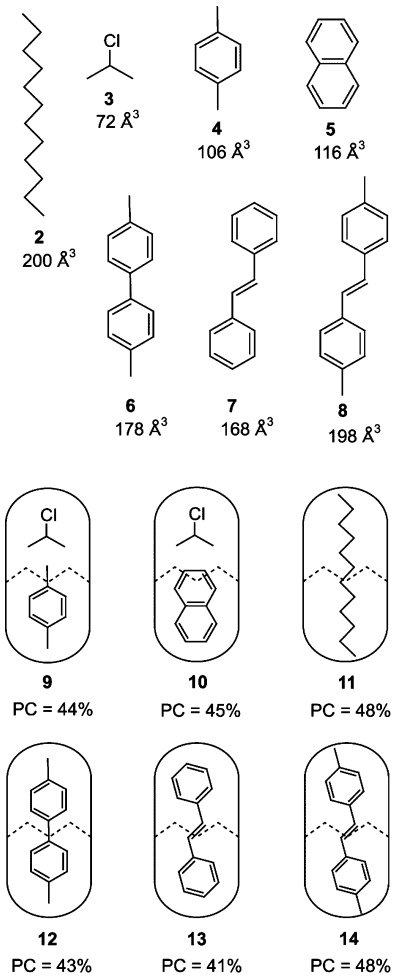

Figure 2. Guests and encapsulation complexes. The packing coefficients (PCs) were obtained from the guest volumes as energy-minimized with the Hyperchem 7.0 program (Hypercube Inc., 2002), at the semiempirical PM3 level, and calculated with WebLab Viewer Pro 4.0 (Molecular Simulation Inc.).

(9) Heinz, T.; Rudkevich, D. M.; Rebek, J., Jr. *Nature* **1998**, *394*, 764−766.
(10) (a) Körner, S. K.; Tucci, F. C.; Rudkevich, D. M.; Heinz, T.; Rebek, J., Jr. *Chem.—Eur. J.* **2000**, *6*, 187−195. (b) Shivanyuk, A.; Rebek, J., Jr. *Chem. Commun.* **2002**, 2326−2327.
(11) Mecozzi, S.; Rebek, J., Jr. *Chem.—Eur. J.* **1998**, *4*, 1016−1022.
(12) Dodecane **2** shows the highest affinity among the hydrocarbons C_{10} to C_{14}: Scarso, A.; Trembleau, L.; Rebek, J., Jr. *Angew. Chem., Int. Ed.* **2003**, *42*, 5499−5502.

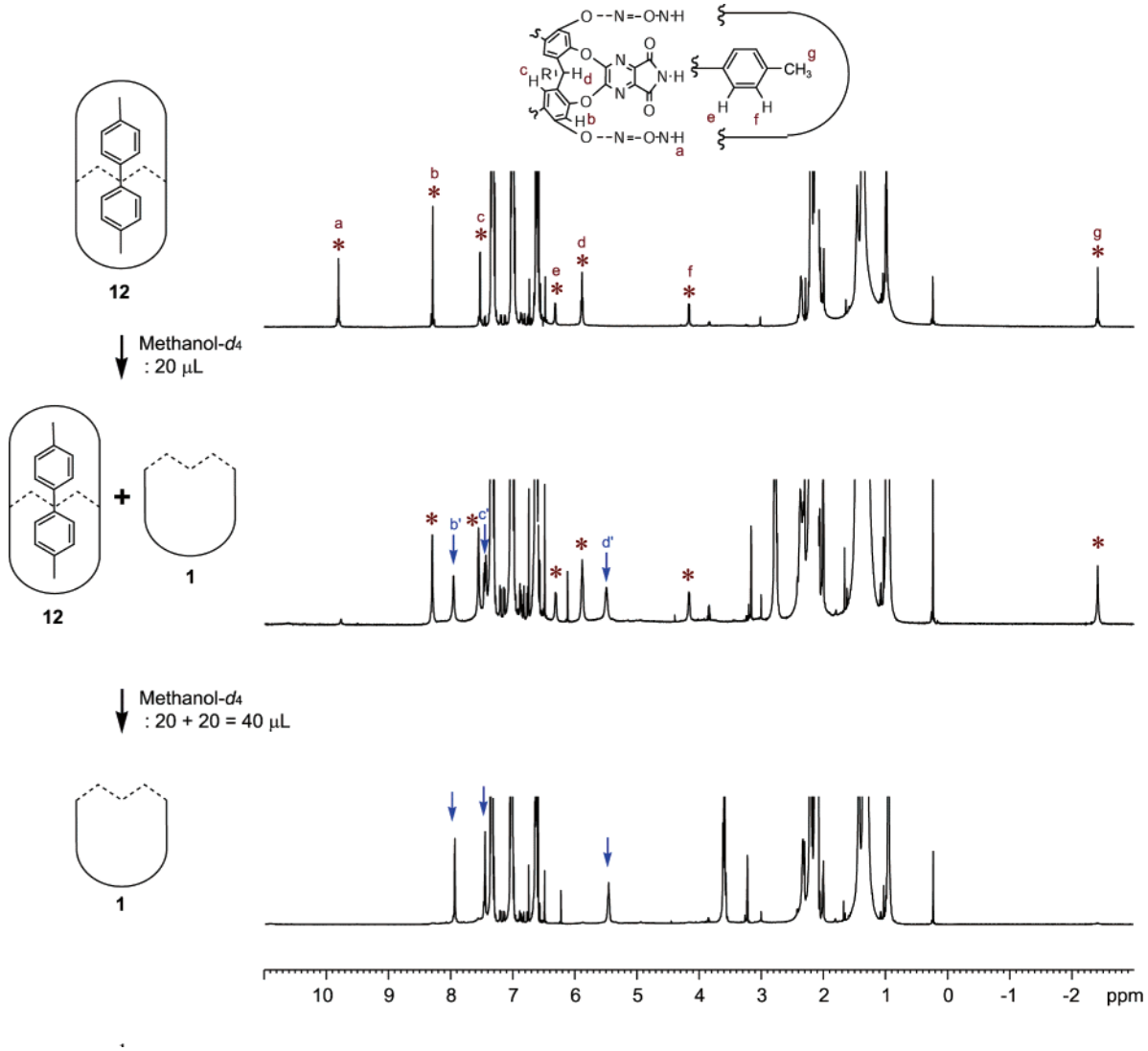

Figure 3. ^{1}H NMR spectra of dilution experiments with capsule **12** (600 MHz, 300 K, $[\mathbf{12}]_0 = 1.0$ mM, $[\mathbf{6}]_0 = 50$ mM, mesitylene-d_{12} = 600 μL): (∗) capsule **12**; (↓) dissociated capsule **1**.

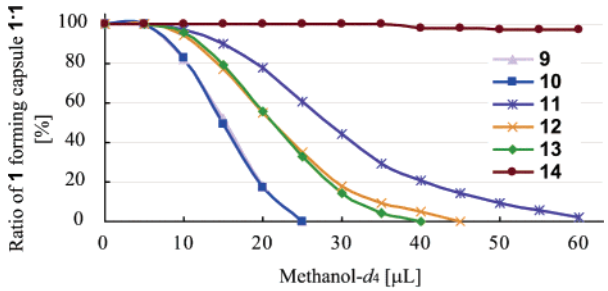

Figure 4. Dilution experiments with methanol-d_4 (300 K, $[\mathbf{12}]_0 = 1.0$ mM, $[\mathbf{guest}]_0 = 50$ mM, mesitylene-d_{12} = 600 μL).

$\pi-\pi$ stacking,[15] and hydrogen bonds. The inner space in the cylindrical capsule **1·1** is lined with eight aromatic rings at each end and the eight pyrazine imides of the central seam. The energy-minimized structure of **14** (Figure 9) shows that the methyl groups of **8** are nicely positioned to interact with the host aromatic rings at each end of the capsule. The benzene rings of **8** are properly positioned to interact with the capsule's walls.[16] The capsule tends to dissociate to a greater extent in the presence of less sterically crowded protic solvents. Guest **8** is insoluble in pure methanol, ethanol, and 2-propanol, and it is likely that solvophobic effects are also involved in the encapsulation process in these solvents.

Dependence of the Dissociation−Recombination Process on Temperature. We investigated the thermodynamic behavior of the capsule in the dissociation−recombination process (Figure 6). The temperature was varied from 285 to 320 K, and in each case, the concentration of capsule **14** increased with an increase in temperature. Linear van't Hoff plots (Figure 9 and Supporting Information) reveal that encapsulation of **8** in the presence of methanol is *endothermic* ($\Delta H > 0$) (Table 2), that is, this association process (Figure 6) is entropically driven (positive ΔS). Isothermal titration calorimetry (ITC) data (for 10% methanol in mesitylene; Supporting Information)[17] also showed that the encapsulation process is *endothermic*.

Most processes involving host−guest associations are entropically unfavorable but enthalpically favorable.[18] For encap-

(13) Cubberley, M. S.; Iverson, B. L. *J. Am. Chem. Soc.* **2001**, *123*, 7560−7563.
(14) Meyer, E. A.; Castellano, M. R.; Diederich, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 1210−1250.
(15) Waters, M. L. *Curr. Opin. Chem. Biol.* **2002**, *6*, 736−741.
(16) For example, see: Colquhoun, H. M.; Zhu, Z.; Williams, D. J. *Org. Lett.* **2003**, *5*, 4353−4356.

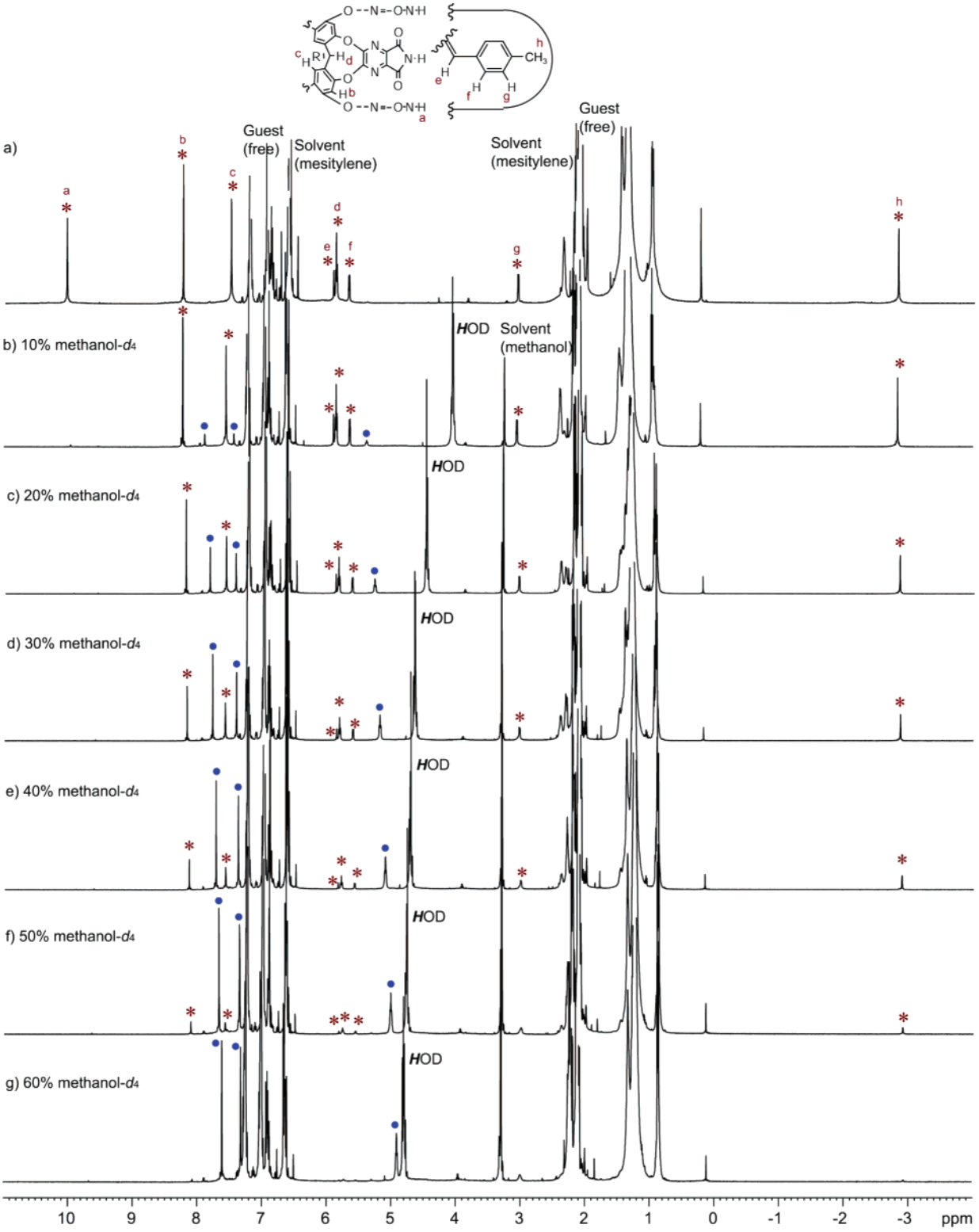

Figure 5. ^{1}H NMR spectra at 300 K (600 MHz, $[\mathbf{1}]_{total}$ = 2.0 mM, $[\mathbf{8}]_{total}$ = 25 mM, methanol-d_4 + mesitylene-d_{12} = 600 μL): (∗) capsule **14**; (●) dissociated capsule **1**.

sulation, the entropically favorable displacement of two solvent molecules *inside* a capsule by a single large guest was encountered earlier.[19−21] For the case at hand, the two completely dissociated monomers combine to encapsulate a large

(17) The ITC experiments were carried out as follows. The solution of **1** (0.40 mM) in 10% methanol (v/v in mesitylene) was titrated with guest **8** (4.0 mM in the same solvents). The curve obtained was smooth and showed the process was endothermic (Supporting Information). However, we could not access a proper curve-fitting program to calculate thermodynamic parameters for the case of a 2:1 (host−guest) complex with the available software (Origin 7.0 software, MicroCal, LLC).

(18) (a) Izatt, R. M.; Bradshaw, J. S.; Pawlak, K.; Bruening, R. L.; Tarbet, B. J. *Chem. Rev.* **1992**, *92*, 1261−1354. (b) Houk, K. N.; Leach, A. G.; Kim, S. P.; Zhang, X. *Angew. Chem., Int. Ed.* **2003**, *42*, 4872−4897.

(19) Kang, J.; Rebek, J., Jr. *Nature* **1996**, *382*, 239−241.

(20) An entropically favorable case of guest displacement of a solvent-filled capsule is known: Yamanaka, M.; Shivanyuk, A.; Rebek, J., Jr. *J. Am. Chem. Soc.* **2004**, *126*, 2939−2943.

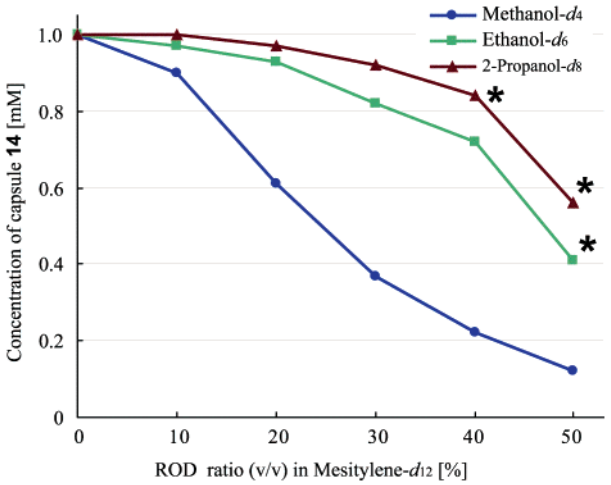

Figure 6. Stability of capsule **14** in the presence of protic solvents (300 K, $[\mathbf{1}]_{total} = 2.0$ mM, $[\mathbf{8}]_{total} = 25$ mM, protic solvent + mesitylene-d_{12} = 600 μL): (*) precipitation occurred.

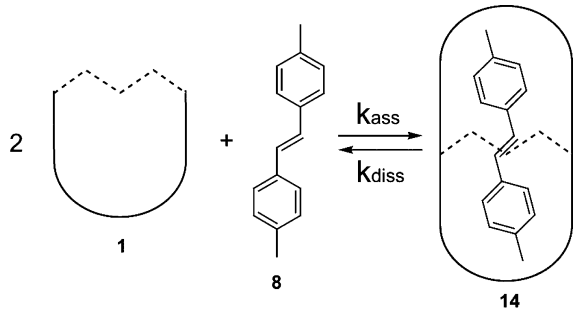

Figure 7. Equilibrium of encapsulation of **8**.

guest in a process that is also entropically driven, even though the two halves of the capsule must come together (must associate).

The dissociated cavitand **1** adopts a predominantly vase-shaped conformation, as shown by the diagnostic chemical shifts of its methine protons[22] (5.0−5.4 ppm). It is likely that the solvent (mesitylene-d_{12} or methanol-d_4) resides in the cavity of **1** to avoid an empty volume (vacuum), but all attempts to observe bound methyl groups failed to give the appropriate upfield-shifted signals. It is likely (but unproven) that the exchange of these guests in and out of the half-capsule is rapid. A signal for the imide NH of **1** appeared in the downfield region (around 11 ppm) of the ^{1}H NMR spectrum when methanol was used in place of methanol-d_4. This shift suggests that the oxygen of methanol is hydrogen bonded to imide NHs. The positive enthalpy observed is ascribed to the energy needed to assemble in these solvents (two mesitylenes and eight methanols), assuming that one methanol oxygen (the best acceptor) hydrogen bonds to each NH (the best donor) of **1**. The positive entropy apparently results from the liberation of these bound solvents (Figure 10). This overrides the negative enthalpic contribution involved in the formation of the encapsulation complex **14**.

(21) Cram and co-workers reported the entropy-driven dimerization of velcrands in apolar solvents; those systems did not involve guest encapsulation: (a) Bryant, J. A.; Ericson, J. L.; Cram, D. J. *J. Am. Chem. Soc.* **1990**, *112*, 1254−1255. (b) Bryant, J. A.; Ericson, J. L.; Cram, D. J. *J. Am. Chem. Soc.* **1990**, *112*, 1255−1256. (c) Cram, D. J.; Choi, H.-J.; Bryant, J. A.; Knobler, C. B. *J. Am. Chem. Soc.* **1992**, *114*, 7748−7765.

(22) Moran, J. R.; Ericson, J. L.; Dalcanale, E.; Bryant, J. A.; Knobler, C. B.; Cram, D. J. *J. Am. Chem. Soc.* **1991**, *113*, 5707−5714.

Table 1. Binding Constants K (M^{-2}) and Binding Free Energies ΔG (kcal/mol) at 300 K in the Association−Dissociation Equilibria ($[\mathbf{1}]_{total} = 2.0$ mM, $[\mathbf{8}]_{total} = 25$ mM, Methanol-d_4 + Mesitylene-d_{12} = 600 μL)

	K^a	ΔG^b
Methanol-d_4 (v/v in mesitylene-d_{12})		
10%	9.0×10^5	−8.2
20%	4.0×10^4	−6.3
30%	9.3×10^3	−5.5
40%	3.6×10^3	−4.9
50%	1.6×10^3	−4.4
Ethanol-d_6 (v/v in mesitylene-d_{12})		
10%	1.1×10^7	−9.7
20%	1.9×10^6	−8.6
30%	2.5×10^5	−7.4
40%	9.2×10^4	−6.8
50%	*c*	*c*
2-Propanol-d_8 (v/v in mesitylene-d_{12})		
10%	$>1.0 \times 10^8$	
20%	1.1×10^7	−9.7
30%	1.4×10^6	−8.5
40%	*c*	*c*
50%	*c*	*c*

[a] $K = [HGH]/([H]^2[G])$, $[G] \approx [G]_0$. [b] $\Delta G = -RT \ln K$. [c] Precipitation occurred.

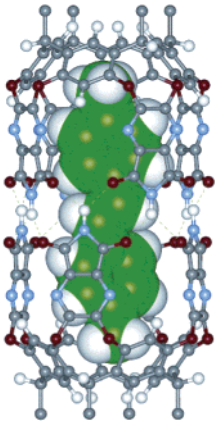

Figure 8. Energy-optimized structure (MM^+ force field) of capsule **14**.

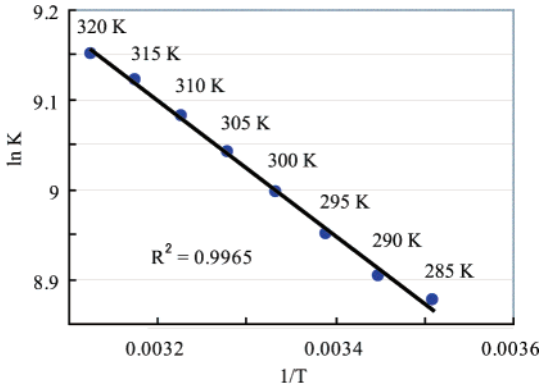

Figure 9. van't Hoff plot for 30% methanol-d_4 (v/v in mesitylene-d_{12}) ($[\mathbf{1}]_{total} = 2.0$ mM, $[\mathbf{8}]_{total} = 25$ mM, methanol-d_4 + mesitylene-d_{12} = 600 μL).

Kinetics of the Encapsulation Process. The rates of dissociation and recombination were determined using EXSY NMR spectroscopy.[23] The resonances of capsule **14** in each concentration (10−50%) of methanol gave intense EXSY cross-peaks with the signals of the dissociated capsule **1** at 300 K. Figure

(23) Perrin, C. L.; Dwyer, T. J. *Chem. Rev.* **1990**, *90*, 935−967.

Table 2. Thermodynamic Parameters in the Association−Dissociation Equilibrium ([**1**]$_{total}$ = 2.0 mM, [**8**]$_{total}$ = 25 mM, Methanol-d_4 + Mesitylene-d_{12} = 600 μL), ΔH (kcal/mol) and ΔS (cal/mol)]

methanol-d_4 (v/v in mesitylene-d_{12})	ΔH^a	ΔS^b
10%	6.4	49
20%	2.7	30
30%	1.5	23
40%	*c*	*c*
50%	*c*	*c*

[a] ΔH values were obtained by van't Hoff plots. [b] $\Delta G = \Delta H - T\Delta S$. [c] The changes in concentrations of **1** and **14**, due to temperature, were too small to obtain accurate values by ^{1}H NMR.

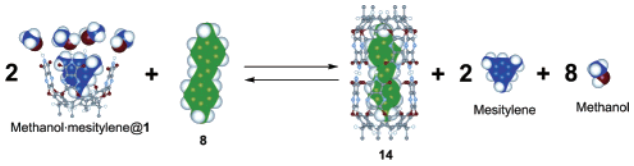

Figure 10. Proposed liberation of solvents from cavitand **1**, assuming that one methanol acceptor coordinates to each imide N−H donor of **1**.

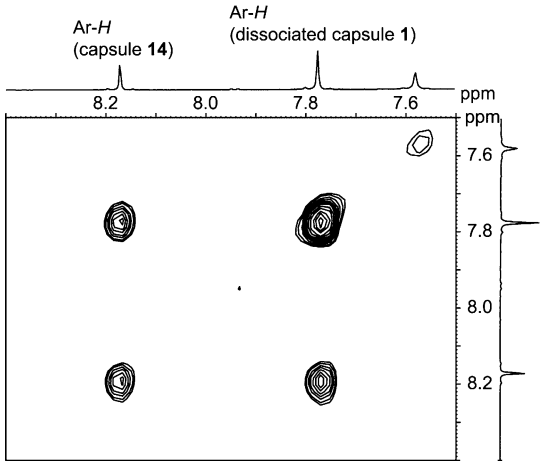

Figure 11. Downfield regions of EXSY spectra (600 MHz, 300 K, [**1**]$_{total}$ = 2.0 mM, [**8**]$_{total}$ = 25 mM, 30% methanol-d_4 in mesitylene-d_{12}, mixing time 1.0 s).

11 shows the downfield region of the EXSY spectrum for **14** in 30% methanol-d_4 (v/v in mesitylene-d_{12}). The rate constants and $\Delta G^{\ddagger}$ values are summarized in Table 3, which were determined from integration of the EXSY cross-peaks between the aromatic protons of **14** and **1** (Figure 11).[24] The rate constant, k_{diss} (s^{-1}), approximately doubles when the amount of methanol-d_4 is changed from 20 to 30% and again when changed from 30 to 40%. Concomitantly, there are decreases in the free energy of activation for dissociation, $\Delta G_{diss}^{\ddagger}$ (kcal/mol).

Earlier, we reported the mechanism by which guests enter and exit host capsules[25] and proposed the mechanism of guest exchange for the same cylindrical capsule **1·1** in apolar

Table 3. Rate Constants for the Dissociation of Capsule **14** (k_{diss}, s^{-1}) and Association of Cavitand **1** (k_{ass}, M^{-2} s^{-1}), and Free Activation Enthalpy of Dissociation ($\Delta G^{\ddagger}_{diss}$, kcal/mol) and Association ($\Delta G^{\ddagger}_{ass}$, kcal/mol) (600 MHz, 300 K, [**1**]$_{total}$ = 2.0 mM, [**8**]$_{total}$ = 25 mM, Methanol-d_4 + Mesitylene-d_{12} = 600 μL, Mixing Time 1.0 s)

methanol-d_4 (v/v in mesitylene-d_{12})	$k_{diss}{}^a$	$k_{ass}{}^b$	$\Delta G^{\ddagger}_{diss}{}^c$	$\Delta G^{\ddagger}_{ass}{}^d$
10%	*e*	*e*	*e*	*e*
20%	0.41	1.6×10^4	18.1	11.8
30%	0.95	8.8×10^3	17.6	12.2
40%	2.0	7.1×10^3	17.2	12.3
50%	*f*	*f*	*f*	*f*

[a] Determined by EXSY spectra. [b] $d[HGH]/dt = -k_{diss}[HGH] + k_{ass}[H]^2[G]$. In steady state, $k_{ass} = k_{diss}[HGH]/([H]^2[G])$; $[G] \approx [G]_0$. [c] $\Delta G^{\ddagger}_{diss} = -RT \ln(hk_{diss}/k_BT)$. [d] $\Delta G^{\ddagger}_{ass} = -RT \ln(hk_{diss}/k_BT)$. [e] The peak of dissociated cavitand **1** was too small to integrate accurately in the 2D EXSY experiment. [f] The peak of capsule **14** was too small to integrate accurately in the 2D EXSY experiment.

solvents.[26] The exchange rate of benzene in *p*-xylene was much faster than the dissociation−recombination rate of the two halves of the host capsule. Guest exchange was interpreted to result from partial disruption of the hydrogen-bonded seam by conformational changes leading to a partial cone (partial kite shape) rather than from complete dissociation of the capsule. This mechanism is analogous to that proposed for the exchange in the dimeric "tennis balls" and "softballs"[21] (in contrast, the guest release from the hydrogen-bonded calix[4]arene tetraurea dimer requires the complete dissociation of the capsule).[27]

In the presence of protic solvents, the mechanism for guest exchange in the capsule at hand is different. The exchange rates of the guest (k_{in} (M^{-2} s^{-1}) and k_{out} (s^{-1})) and the exchange rates of the host with cavitand **1** (k_{diss} (s^{-1}) and k_{ass} (M^{-2} s^{-1})) were revealed by EXSY spectroscopy. In this experiment, a small excess of guest **8** (1.8 equiv of **14**$_{total}$) was used in the presence of 12% methanol-d_4 (v/v in mesitylene-d_{12}) at 300 K. The resonances of capsule **14** gave intense EXSY cross-peaks with the signals of the dissociated cavitand **1**; intense cross-peaks were also observed between guest **8** inside and outside the capsule. Figure 12 shows the downfield region of the EXSY spectrum and its assignments for the cross-peaks. The rate constants are summarized in Table 4, which were determined by integration of the EXSY cross-peaks, b−b′ for host exchange and f−f′ for guest exchange (Figure 12). There is no significant difference between k_{out} and k_{diss} (k_{out} = 0.17 s^{-1}; k_{diss} = 0.16 s^{-1}) or between k_{in} and k_{ass} ($k_{in} = 4.8 \times 10^4$ M^{-2} s^{-1}; $k_{ass} = 4.6 \times 10^4$ M^{-2} s^{-1}). These results indicate that complete dissociation of the capsule is required to exchange the guest in the presence of protic solvents. With these competitive solvents, solvophobic effects, CH−π interactions, and π−π stacking, rather than hydrogen bonding, are the dominant factors for the encapsulation phenomenon.

Conclusions

The dilution experiments revealed that the stability of the hydrogen-bonded capsule toward protic solvents depends strongly on the guests, with the best guest in this series being dimethylstilbene **8**. Only slight dissociation was found in the presence of ~2500 equiv of methanol, and some assembly was seen even

(24) The rate constant, k_{diss}, was calculated using the 2D EXSY program: Abel, E. W.; Coston, T. P. J.; Orrel, K. G.; Sik, V.; Stephenson, D. *J. Magn. Reson.* **1986**, *70*, 34−53.

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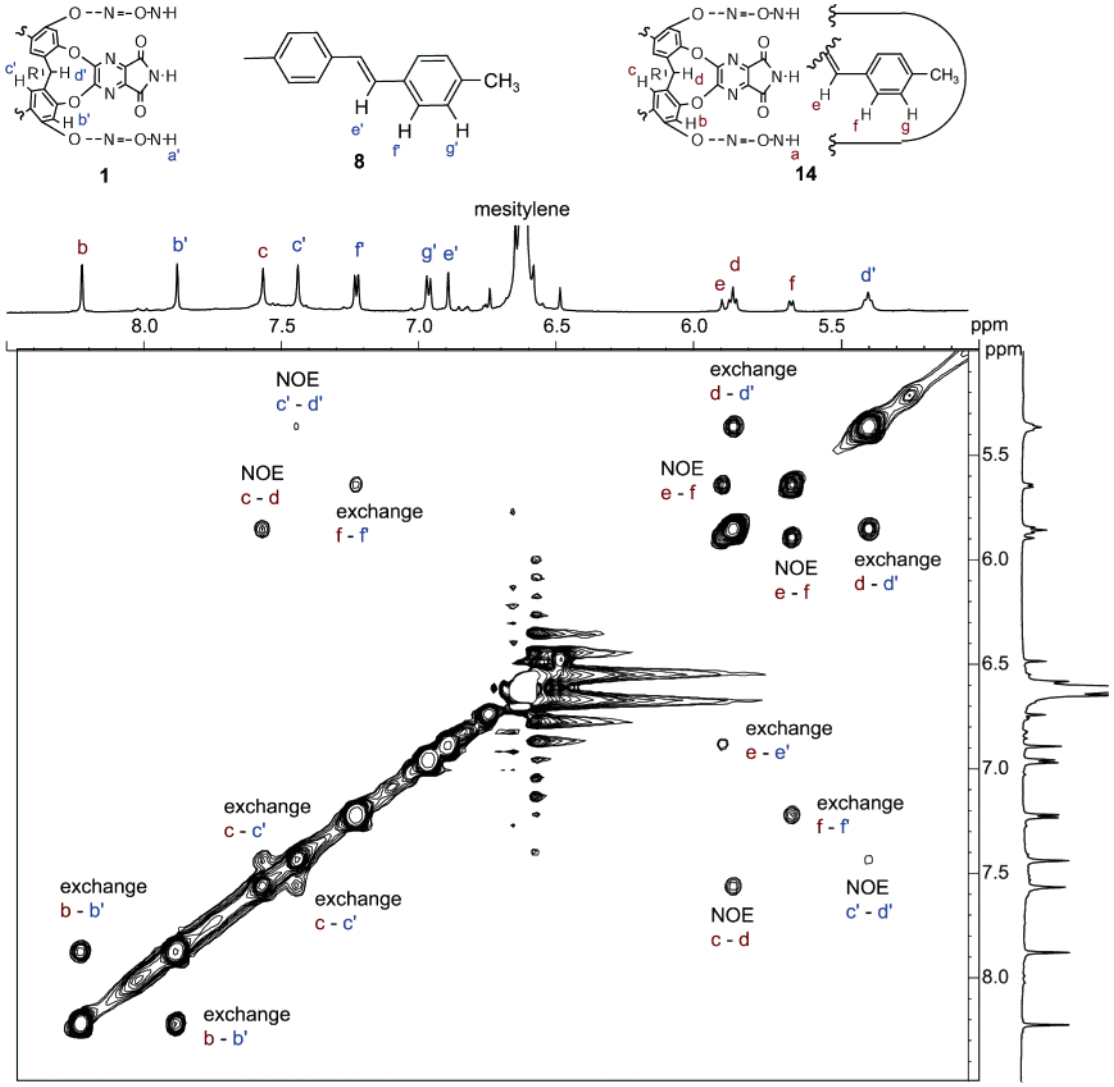

Figure 12. Downfield region of the EXSY spectrum (600 MHz, 300 K, $[\mathbf{1}]_{total} = 2.2$ mM, $[\mathbf{8}]_{total} = 2.0$ mM, 12% methanol-d_4 in mesitylene-d_{12}, mixing time 1.0 s) and their assignments.

Table 4. Rate Constants for the Dissociation of Capsule **14** (k_{diss}, s^{-1}), the Association of Cavitand **1** (k_{ass}, $M^{-2}\ s^{-1}$), the Release of Guest **8** (k_{out}, s^{-1}), and the Uptake of Guest **8** (k_{in}, $M^{-2}\ s^{-1}$) (600 MHz, 300 K, $[\mathbf{1}]_{total} = 2.2$ mM, $[\mathbf{8}]_{total} = 2.0$ mM, 12% Methanol-d_4 in Mesitylene-d_{12}, Mixing Time 1.0 s)

k_{diss}[a]	k_{ass}[a]
0.16	4.6×10^4
k_{out}[a]	k_{in}[a]
0.17	4.8×10^4

[a] These values were calculated as shown in Table 3.

in a 50% methanol solution (v/v in mesitylene). The encapsulation process is entropy-driven, and guest exchange occurs by complete dissociation of the capsule in the presence of protic solvents. Since hydrogen bonds in biological systems are also stable in water, it is likely that hydrogen-bonded encapsulation phenomena can be observed in the most biorelevant solvent if capsules with the appropriate solubility can be made. We are directing our efforts at this goal.

Experimental Section

General. ^{1}H NMR spectra were recorded on a Bruker DRX-600 (600 MHz) spectrometer. Guests were purchased from Aldrich and used without further purification. Compound **1** was prepared by following the published procedure.[28]

Encapsulation Studies. ^{1}H NMR experiments were carried out at 300 K using a 600 MHz spectrometer. Mesitylene-d_{12}, methanol-d_4, ethanol-d_6, and 2-propanol-d_8 were used as purchased from Cambridge Isotope Laboratories, Inc. In dilution experiments, the suspension in mesitylene-d_{12} (600 μL) included capsule (1.0 mM_{total}) and guest (50 mM_{total}) and was sonicated for several hours. Methanol-d_4 was added to a solution of capsule in 5 μL increments followed by equilibration for ~30 min. This titration was continued until the capsules were completely dissociated.

In the encapsulation studies (1D ^{1}H NMR and 2D EXSY NMR) for **14** in the presence of protic solvents, the solution (capsule (1.0 mM_{total}), guest (25 mM_{total}), methanol-d_4, ethanol-d_6, or 2-propanol-d_8 + mesitylene-d_{12} = 600 μL) was sonicated for several hours to reach an equilibrium state before the NMR spectra were taken. 2D EXSY (2D NOESY) experiments were acquired with a spectrum width of 18 ppm and a relaxation delay (d_1) between each scan of 2.0 s, using 1024 data points in the t_2 dimension and 512 in t_1, with subsequent weighting with the sinebell function with 32 scans for each t_1 increment, and the mixing time (d_8) was 1.0 s. Exchange cross-peaks were integrated using

(28) Hayashida, O.; Rebek, J., Jr. *J. Org. Chem.* **2002**, *67*, 8291−8298.

XWINNMR Bruker software. The estimated integrations were then processed using the 2D EXSY program[24] to give the chemical exchange (k). In the steady state, the association rate constant (k_{ass}) is given in eq 1. The rate constant (k) was further substituted to the Eyring equation (eq 2) to derive the free energy of activation ($\Delta G^{\ddagger}$)

$$k_{ass} = k_{diss}[HGH]/([H]^2[G]) \quad (1)$$

$$k = (k_B T/h) \exp(-\Delta G^{\ddagger}/RT) \quad (2)$$

where T is temperature, k_B is the Boltzmann constant, and h is Planck's constant.

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Supporting Information Available: van't Hoff plots and isothermal titration calorimetry (ITC) data. This material is available free of charge via the Internet at http://pubs.acs.org.

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