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Guest Packing Motifs within a Supramolecular Nanocapsule and a Covalent Analogue

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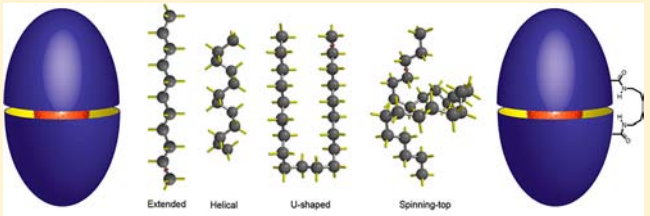
Supporting Information

ABSTRACT: Two hosts that utilize the hydrophobic effect to assemble and/or encapsulate guest molecules were studied. The hosts, octa-acid (OA) and hexalene diamine-linked octa-acid (HOA), were shown to complex a broad range of *n*-alkanes up to *n*-hexacosane ($C_{26}H_{54}$). A combination of ^{1}H NMR, NMR diffusion, COSY, and NOESY experiments revealed four different guest packing motifs, depending on the size of the guest and the nature of the host. As a function of guest size, smooth transitions from one motif to the next were observed and allowed qualification of their relative stabilities. Furthermore, although the two hosts engender ostensibly identical encapsulation environments, their different assembly properties lead to quite distinct packing-motif profiles, i.e., how the motifs change as a function of guest size.



Filling space within molecular and supramolecular capsules

INTRODUCTION

Nature routinely utilizes a confining nanospace to bring about phenomena such as storage and protection, transport, and catalysis. Thus vaults,[1] the GroEL-GroES chaperonin complex,[2] two-partner secretion B (TpsB) transporters such as FhaC,[3] orthogonal β-barrel intestinal Fatty Acid Binding proteins,[4] and of course enzymes in general all utilize compartmentalization to bring about, respectively, RNA transport (suspected), polypeptides folding, FHA protein transport, and catalysis. It is well established that the precise control of substrate and transition state conformation is essential in enzyme catalysis, and it is most certainly the case that the guest-packing motif[5] within these other structures is also key to their properties.

One approach to understanding the fundamentals of how molecules pack within a confined nanospace is to use relatively simple synthetic hosts and guests. An appreciation of how and what kind of packing motifs are templated by a host not only serves in moving these hosts toward applications such as artificial nanoreactors[6] or separators[7] but also yields useful information about packing motifs within systems found in Nature. One general finding from studies of synthetic hosts is that even in cases where the inner walls of the host are devoid of groups capable of making strong and directional interactions with the guest, the host can act as an external template to promote highly specific guest conformations rarely found in solution. One prominent example from the Rebek group is the templation of alkyl chains of amphiphiles into helical conformations upon binding to a water-soluble cavitand.[8] The hydrophobic effect was in large part responsible for this complexation, but the generalization of helix templation was demonstrated in a subsequent study of an organic-solvent soluble cavitand that dimerized around *n*-alkane guests to form capsular 2:1 host−guest complexes.[9] In our own studies on the ability of a water-soluble supramolecular capsule to complex *n*-alkane guests, helical guest conformations were also observed.[10] However, in these cases the range of guests that formed helices was much narrower, and the degree of helicity, as judged by ^{1}H NMR NOESY experiments, was much lower, suggesting that the differences in the gross cavity shapes defined by the two families of hosts play an important role in guest packing. This idea has been further supported by reports on guest packing within a resorcinarene hexameric host,[11] and the observed 'J-shaped' guest conformations within a new kind of dimeric cavitand host.[12] A recent review[11] highlights these findings as well as potential applications[13] for guest packing control. In related work, studies with cucurbiturils have revealed that the complete envelopment of the guest is not essential for controlling guest packing. Thus, the Kim group has revealed that amphiphiles and bolaamphiphiles respectively adopt 'J-shaped' and 'U-shaped' conformations when bound within the hydrophobic pocket of cucurbiturils.[14] In totality, these studies reveal that complexation can lead to well-defined packing motifs that in free solution are sufficiently high in energy that they represent but a small fraction of the different conformations normally adopted.

In this report the bindings of *n*-alkanes up to *n*-hexacosane ($C_{26}H_{54}$) to dimeric assemblies of octa-acid (OA) host **1** and a covalent dimer, hexalene diamine-linked octa-acid (HOA), **2** (Figure 1), are reported. These studies reveal that for both hosts the guest-packing motifs change as a function of packing coefficient (the ratio of the volume of space available to the

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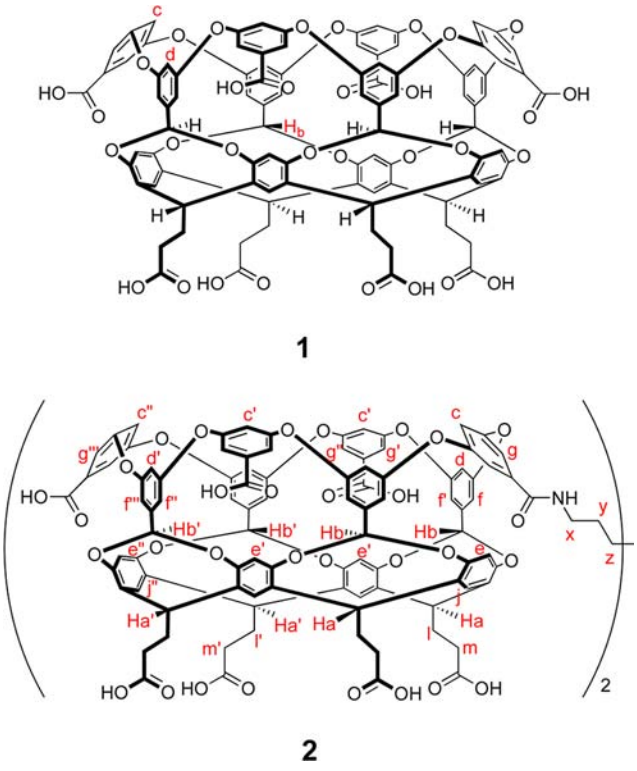

Figure 1. Structures of hosts OA **1** and HOA **2**. Proton designations discussed in the text are highlighted in red.

volume of guest); i.e., in a series of complexes with homologous guests, transitions from one packing motif to another are observed when the capacity of the host for a particular motif is reached. Additionally, although these hosts provide very similar binding environments, because their assembly properties differ, the types of guest-packing motifs displayed by each host are quite distinct.

Synthesis of Hosts 1 and 2. The synthesis of OA **1** followed an improved procedure[15] based on that initially reported.[16] The synthesis of HOA **2** is shown in Scheme 1. The key to the synthesis of **2** is ready access to hepta-ester **3**. This compound was initially identified as a minor product during the synthesis of **1**, in which crude **1** was converted to the corresponding octa-ester (3:2 EtOH/$CHCl_3$ and HCl).[15] However, subsequent studies revealed that hepta-ester **3** could be isolated in 65% yield by esterification of pure **1** using EtOH/HCl. Omitting the cosolvent $CHCl_3$ led to the bulk of the formed hepta-ester **3** precipitating from solution. Two copies of monoacid **3** were subsequently covalently linked using 1,6-hexanediamine and HBTU as a coupling agent to give the tetradeca-ester **4** in 75% yield. Subsequently, this was hydrolyzed to the corresponding tetradeca-acid **2** in 95% yield using LiOH in aqueous DMA. As expected, HOA **2** is freely soluble in basic aqueous solution but possesses low solubility at neutral pH.

RESULTS AND DISCUSSION

These studies center around the complexation and encapsulation on *n*-alkanes. There were three reasons for this type of guest. First, all of the homologues from C1 (methane) to C50 (pentacontane) are commercially available in high purity. These guests are therefore ideal for systematically probing the carrying capacity of hosts. Second, their hydrophobicity and conformational flexibility mean that they form relatively strong complexes with **1** and **2** and that a range of binding motifs is theoretically possible. Third, the high symmetry of these guests (C_{2v} for odd numbers of C-atoms, C_{2h} for even) results in encapsulation complexes that are relatively easy to interpret by ^{1}H NMR.

Guest Binding to OA (1). In an earlier study *n*-heptadecane ($C_{17}H_{36}$) had been identified as the largest *n*-alkane that could be encapsulated within host **1**. However, subsequent studies revealed that the limiting factor in these encapsulation experiments was not the actual capacity of the host, but the dissolution of the guest in the aqueous solution and/or the rate of uptake. Thus, a combination of heating and sonication in the presence of a slight excess of guest allowed the complexation and encapsulation of all the *n*-alkanes from *n*-heptadecane ($C_{17}H_{36}$) to *n*-hexacosane ($C_{26}H_{54}$). At equilibrium the percentage of complex formed by each guest (Supporting Information) was found to decrease from 100% in the case of *n*-hexadecane ($C_{16}H_{34}$) to ~10% in the case of *n*-hexacosane ($C_{26}H_{54}$). The ^{1}H NMR spectra of each complex revealed the characteristic high-field signals corresponding to the encapsulated guest (Figure 2). Signal integration, combined with NMR diffusion studies,[17] revealed that each guest formed a ternary 2:1 host−guest complex. Furthermore, the observance of both free and bound signals for the host and (where possible) the guest confirmed that even in the weakest of complexes exchange between the free and bound states was slow on the (500 MHz) NMR time scale.

For a comparison of the NMR spectra shown in Figure 2, consider the previously reported[10] *n*-tetradecane ($C_{14}H_{30}$) complex (Figure 2a). In this complex the methyl groups of the bound guest generate a signal at ca. −3.22 ppm ($\Delta\delta \approx 4$ ppm), while the signals for the intervening chain of methylene groups are spread out between −0.05 and −1.42 ppm. For this size of *n*-alkane guest, the NMR shifts arise because they adopt a packing motif in which the methyl groups are anchored into the 'poles' of each hemisphere via C−H$\cdots\pi$ interactions, and the intervening methylene chain fills the bulk of the cavity. In this arrangement the methyl groups reside more deeply in the tapering pocket and so experience the greater degree of shielding. This general model, that the deeper the point of residency the greater the upfield shift, holds true for many types of guests. However, for guests with 'sharper' termini such as terminal alkynes, the methyne C−H can probe even more deeply into the binding pocket. In such cases both experiment and nucleus-independent chemical shift calculations at the B3LYP/6-31G* level clearly show that the expected upfield shift with increasing guest size reverses as the termini are forced into the deepest part of the cavity.[18]

With the methyl groups as anchors in each 'polar region' of a hemisphere, an important question concerns the conformation of the intervening chain. The literature suggests two possible guest motifs.[9,10] The first is that the guest adopts a more or less fully extended conformation in which the intervening methylene chain is defined by a contiguous series of *anti* dihedral angles. Note however that any guest longer than *n*-undecane ($C_{11}H_{24}$), ~15.5 Å, cannot adopt a fully extended conformation within the nanospace defined by the dimer of OA **1**. Guests longer than this must deviate from a fully extended conformation. Neither previous studies nor those reported here can differentiate between an extended motif and a compressed equivalent. ^{1}H NMR shift data imply that such guests adopt a multitude of different conformations. This type of pole-to-pole

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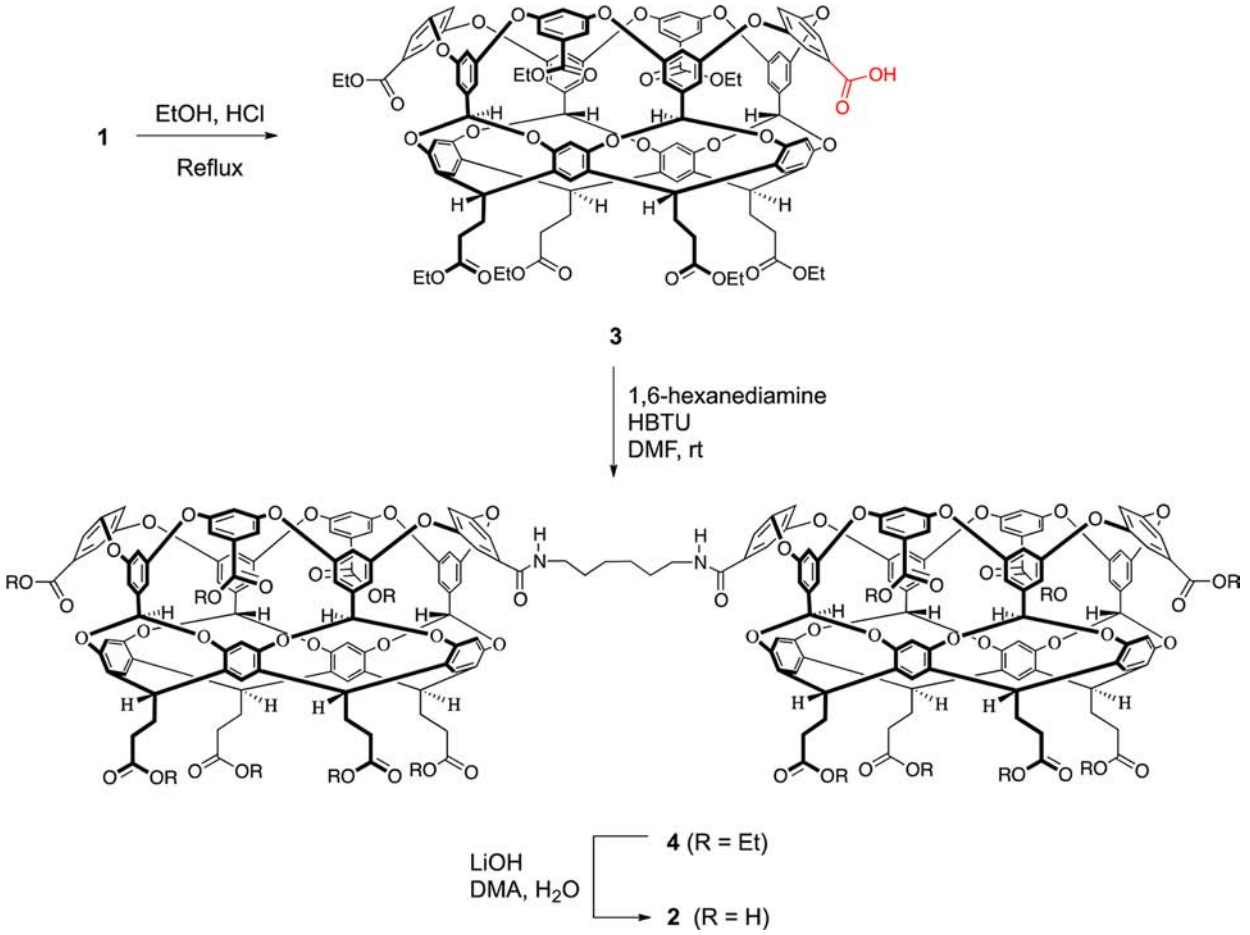
Scheme 1. Synthesis of HOA 2



motif is therefore labeled as E/C (extended or compressed, Figure 3a). The second packing motif for guests too long to assume an extended conformation is one that is helical (Figure 3b). Helices possess a contiguous series of gauche interactions down the length of the chain, and as the *gauche* conformation of butane is ~0.55 kcal mol^{-1} higher in energy than the *anti*-conformation,[19] a helical conformation in a long *n*-alkane represents a relatively high energy conformation in free solution. However, within a host a helical motif allows the guest to efficiently pack the cavity and maximize its C−H···π interactions with the walls of the cavity. The guests *n*-dodecane ($C_{12}H_{26}$) and *n*-tetradecane ($C_{14}H_{30}$, Figure 2a) bind to the dimer of **1** with helical motifs characterized by long-range NOESY interactions between methylene groups (e.g., $i \rightarrow i + 4$ interactions).[10] As discussed above, this ability of a host to act as an external template is apparently quite general in cavitands,[8] and related cavitand-based dimeric capsules with a slightly narrower equatorial diameter than that of the dimer of **1** (⌀ ~6.6 Å verses ~8.5 Å) are able to template helix formation in the guests *n*-undecane ($C_{11}H_{24}$) through *n*-tetradecane ($C_{14}H_{30}$).[9]

The ^{1}H NMR spectrum of the complex formed by *n*-heptadecane ($C_{17}H_{36}$) is quite different from that of the *n*-tetradecane ($C_{14}H_{30}$) complex (Figure 2). In combination with COSY studies (Supporting Information), it is apparent that the methyl groups of the former complex lead to a relatively downfield signal at −2.32 ppm, while the signals for the intervening chain of methylene groups appear over a relatively narrow region between −0.41 and −0.78 ppm. With a maximal length of 25.5 Å, *n*-heptadecane ($C_{17}H_{36}$) cannot adopt a fully extended conformation within the host. However, an examination of the NOESY NMR spectrum of the complex revealed no long-range interactions along the length of the guest indicative of a helix. Hence this guest adopts a compressed E/C motif, a conclusion supported by a comparison of the complexes **1** formed with the guests *n*-tetradecane ($C_{14}H_{30}$) to *n*-hexadecane ($C_{16}H_{34}$)[10] which show a transition away from a helical guest motif to an E/C motif displaying little magnetic anisotropy for the different methylenes. As a result of both the anchoring of the methyl groups and the overall length of *n*-heptadecane ($C_{17}H_{36}$), the guest cannot undergo a flipping process whereby its two termini exchange hemispheres. Hence movement must be restricted to that around the long axis of the capsule. Inside the capsule formed by **1**, this movement must lead to a number of fast-exchanging compressed motifs that result in the methylene groups experiencing little anisotropy and hence a relatively narrow spread of signals in the high-field region.

COSY experiments were used to assign the guest signals for all the other guests from *n*-octadecane ($C_{18}H_{38}$) to *n*-hexacosane ($C_{26}H_{54}$). These results identified two new packing motifs inside host **1**. For the six guests *n*-octadecane ($C_{18}H_{38}$) through *n*-tricosane ($C_{23}H_{48}$), the high-field region of the ^{1}H NMR of their complexes (Figure 2) revealed a return to considerable magnetic anisotropy for the methylene signals. In these cases however, COSY NMR spectra identify U-shaped

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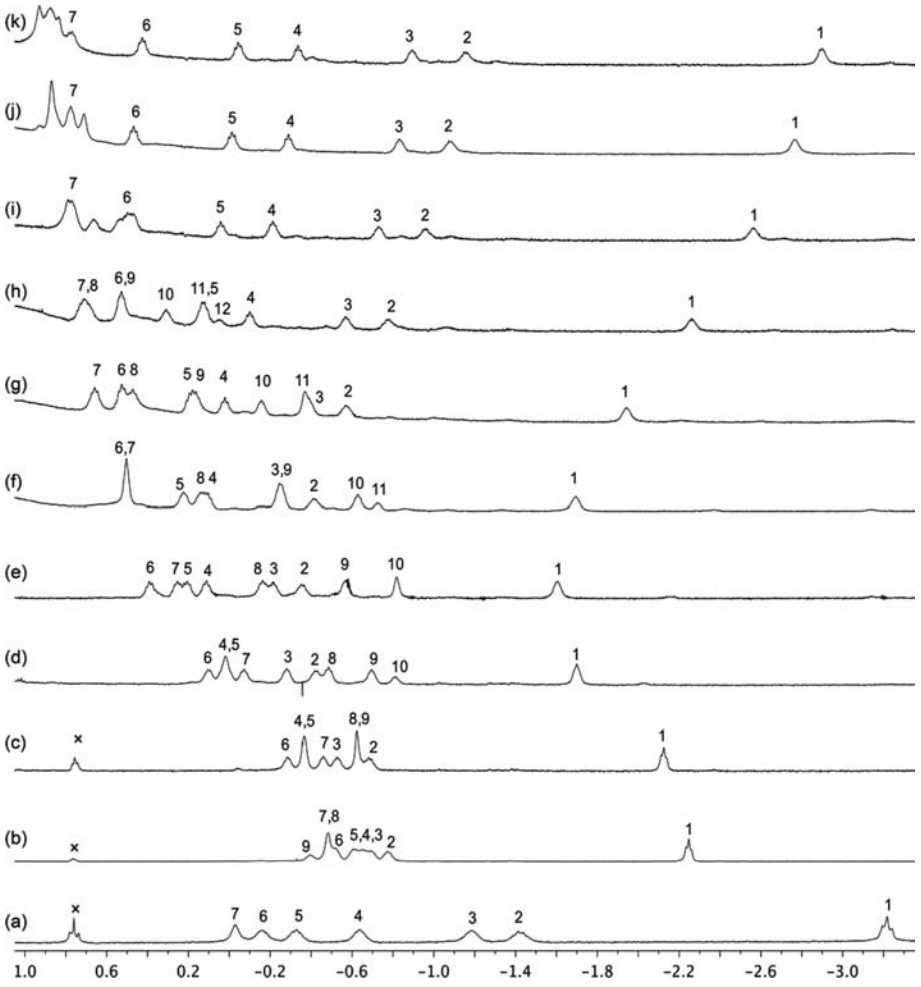

Figure 2. High-field regions of the ^{1}H NMR spectra of the complexes formed between host OA **1** and (a) *n*-tetradecane ($C_{14}H_{30}$); (b) *n*-heptadecane ($C_{17}H_{36}$); (c) *n*-octadecane ($C_{18}H_{38}$); (d) *n*-nonadecane ($C_{19}H_{40}$); (e) *n*-eicosane ($C_{20}H_{42}$); (f) *n*-heneicosane ($C_{21}H_{44}$); (g) *n*-docosane ($C_{22}H_{46}$); (h) *n*-tricosane ($C_{23}H_{48}$); (i) *n*-tetracosane ($C_{24}H_{50}$); (j) *n*-pentacosane ($C_{25}H_{52}$); (k) *n*-hexacosane ($C_{26}H_{54}$). In each case a 1 mM solution of the host in 10 mM LiOH was treated with excess guest. Numbering is from the terminal methyl signal. The signals from the methyl groups of free guest are indicated with an "×".

motifs (Figure 3c). The COSY spectrum of the *n*-eicosane ($C_{20}H_{42}$) complex (Figure 4a) is illustrative. The U-shaped motif results in the H-atoms on C-9(12) and C-10(11) being shifted more than the penultimate C2(19) methylenes, demonstrating that they are located deep in one of the hemispheres of the capsular complex. This is in contrast to the E/C or helical packing motifs which result in a straightforward trend in which the nearer the atom is to a terminus the greater the shift in the signal from the free state. The U-shaped motif necessitates both methyl groups sharing one 'polar' region of the binding cavity, and the methylene groups in the mid section of the guest forming a turn residing in the opposite 'polar' region (Figure 3c). Confirming this, NOESY NMR (Figure 4b) revealed that the C-10(11) methylene groups are located deep in the binding pocket adjacent to the benzal (H_b, see structure **1**) protons on the inner wall of the host.

With previous results having demonstrated that relatively narrow cavitands template E/C or helical motifs,[9] and wider hosts[11,12] and more open cucurbit[8]urils[14] template either J- or U-shaped motifs, the results here point to the possibility of general rules for engineering hosts to template specific guest-packing motifs. Namely, a narrow bore such as the Rebek capsule (⌀ = ∼6.6 Å) is a strong promoter of E/C and helical motifs, whereas a wider bore, such as the capsules described here (⌀ = ∼8.8 Å), can promote a turn in a bound guest.

The sharpest turn that an alkane can make is one involving five bonds. However, CPK models demonstrate that a 9.0 Å diameter cavity can allow a turn over seven bonds: a less strained turn, and one that in the case of **1** would leave some void space in the opposing hemisphere for *n*-heneicosane ($C_{21}H_{44}$) and smaller guests. The bound guest region of the NOESY NMR of the *n*-eicosane ($C_{20}H_{42}$) complex was examined to identify the nature of the turn. However, the low concentration of the sample combined with the limited amount of complex formed meant that only $i \rightarrow i + 2$ interactions between the C-9(10) and C-7(8) methylene groups were apparent. None of the other complexes from this series of guests provided additional information about the turn of the guest. Nevertheless, these results demonstrate that *n*-heptadecane ($C_{17}H_{36}$) represents the size limit for *n*-alkanes binding in an E/C motif; for the guests *n*-octadecane ($C_{18}H_{38}$) to *n*-tricosane ($C_{23}H_{48}$) this is energetically not an option and these guests bind by literally folding in two. As *n*-docosane ($C_{22}H_{46}$) folded in two is approximately the length of the cavity defined by the host, this change in packing motif can afford only a limited amount of extra space for guest encapsulation.

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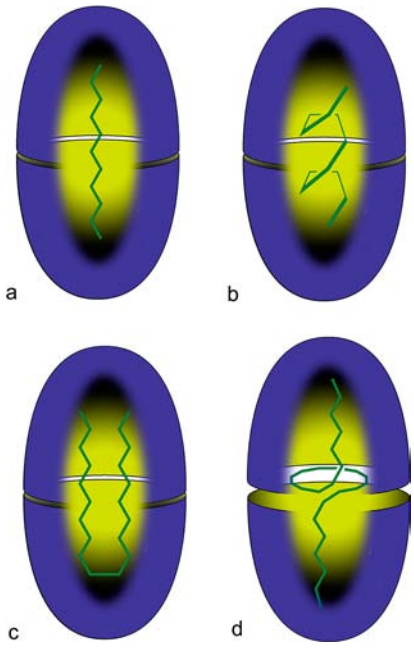

Figure 3. Important packing motifs within cylindrical nanospace defined by $\mathbf{1}_2$: (a) The extended or compressed (E/C). Shown is a motif of *n*-undecane ($C_{11}H_{24}$) which can adopt an essentially fully extended motif; (b) the helical motif of *n*-dodecane ($C_{12}H_{26}$);[10] (c) the U-shaped motif of *n*-eicosane ($C_{20}H_{42}$); (d) the 'spinning-top' motif of *n*-hexacosane ($C_{26}H_{54}$).

Consequently, longer guests must not only fold in two, but their dihedral angles down the two lengths of chain must deviate from the extended or antiperiplanar conformation in order for the guest to fit within the capsule. This is straightforward for 'single strand' guests such as *n*-dodecane ($C_{12}H_{26}$) to *n*-heptadecane ($C_{17}H_{36}$), but in a 'double stranded' U-shaped guest, CPK models demonstrate that there is very little available space. Unsurprisingly, *n*-tricosane ($C_{23}H_{48}$) is the largest guest that provides unequivocal evidence of a U-shaped packing motif. For the largest guests seen to bind to host **1**, *n*-tetracosane ($C_{24}H_{50}$), *n*-pentacosane ($C_{25}H_{52}$), and *n*-hexacosane ($C_{26}H_{54}$), there is little or no evidence of a U-shaped guest conformation. For example, the high-field region of the NMR (Figure 2k) of the *n*-hexacosane ($C_{26}H_{54}$) complex (Figure 2k) shows the characteristic methyl signal and a series of methylene signals in a 2.00 ppm wide window, a 'fingerprint' similar to that observed for much smaller guests possessing E/C and helical motifs. In total however these bound guest signals account for less than half of the guest. The remaining methylene signals are coincident at ~0.80 ppm, having undergone a very modest shift from the free state of $\delta\Delta$ ~0.40 ppm. Thus, the central 12 methylenes in *n*-hexacosane ($C_{26}H_{54}$) experience little host-induced shielding or anisotropy. A combination of weak signal strength from the low concentration of the host (to avoid aggregation) and the relatively small amounts of this complex formed prevented NOESY NMR analysis. Consequently, it was not possible to garner any information pertaining to through space guest−guest or host−guest interactions. This point not withstanding, the ^{1}H NMR shift data and an examination of CPK models strongly suggest that these largest of guests possess a packing motif akin to a spinning top, with the midsection of the guest lying in a disc at the equator of the host and the two termini extending above and below it to fill the remaining voids (Figure 3d). These guests are too large to allow two hemispheres of the host to clamp down on one another, and so the outer edge of the disk of the spinning top must literally be sandwiched between the two rims of the cavitands. As a result, their NMR signals undergo only a modest shift.

Guest Binding to HOA (2). The 10-atom linker of HOA **2** is long enough such that closure of the capsule is possible, but short enough such that when closed the methylene linker chain must adopt a fully extended conformation and the two rings it is attached to must tilt toward the 'horizontal' (Figure 5). We hypothesized not only that HOA **2** would show similar binding properties to **1** but also that because it would form binary rather than ternary complexes with *n*-nonane (C_9H_{20}) and longer guests, the entropy of change of complexation would be more favorable while the enthalpy of complexation would be similar. In other words, **2** was expected to show stronger complexation abilities and hence bind a wider series of guests.

In addition to simple capsular complexes, HOA **2** can theoretically form a number of other assemblies (Scheme 2). Thus, in the presence of a suitable amphiphile, 1:2 host−guest complexes are possible in which the two hemispheres are essentially independent of each other. In addition, it is also possible for two hosts to associate to form a bis-capsule complex possessing two separate cavities (D_{2h} symmetry, 2:2 host−guest complex shown) or a capsular complex possessing a singular large cavity (D_{2d} symmetry, 2:2 host−guest complex shown). Finally, it is also possible to form acyclic (or cyclic) supramolecular polymers.[20]

In the absence of guests, a ^{1}H NMR spectrum of the host in 10 mM sodium tetraborate ($Na_2B_4O_7$) buffer showed a multitude of signals indicative of aggregation (Supporting Information), and this was the case even at a concentration of 0.1 mM. However, with 10 mM lithium hydroxide (LiOH) as buffer a host concentration of 0.5 mM or less gave a well-resolved spectrum. This buffer was therefore the buffer of choice for the studies of **2**.

Toward understanding the binding properties of HOA **2**, a comparison of the NMR signal shifts that occurred upon formation of an open 1:2 complex and a closed 1:1 capsular complex was made. Two guests were chosen: adamantane carboxylic acid ($C_{10}H_{15}CO_2H$) and *n*-dodecane ($C_{12}H_{26}$). Under the basic conditions used the first of these is deprotonated, and it has been previously shown that this carboxylate forms an orientation-specific 1:1 complex with **1** in which the hydrophobic adamantane group occupies the binding site and the carboxylate is exposed to bulk solution.[21] Hence this guest was expected to from an open 1:2 host−guest complex (Scheme 2). In contrast, *n*-dodecane ($C_{12}H_{26}$) was expected to from a tight 1:1 capsular complex analogous to the ternary (2:1) capsular complex formed with **1**. The ^{1}H NMR spectra for free host **2** and the complexes it forms with these guests are shown in Figure 6.

COSY and NOESY NMR experiments of the free host and the complexes gave the assignments in structure **2** and Figure 6. Figure 7 shows the host region of the COSY and NOESY NMR spectra of the *n*-dodecane ($C_{12}H_{26}$) complex. The triplet signal at 6.15 ppm (Figure 6a) is assigned to the H_c proton, the aryl-H proton *para* to the linker group of HOA **2**. This assignment is based on the fact that the chemical shift is typical for such protons and because it undergoes one of the largest shifts as a result of capsule formation. Overall, there are four 'c' type protons, H_c, two $H_{c'}$, and $H_{c''}$, leading to three signals integrating in a 1:2:1 ratio (Figure 6a and b). Based on the

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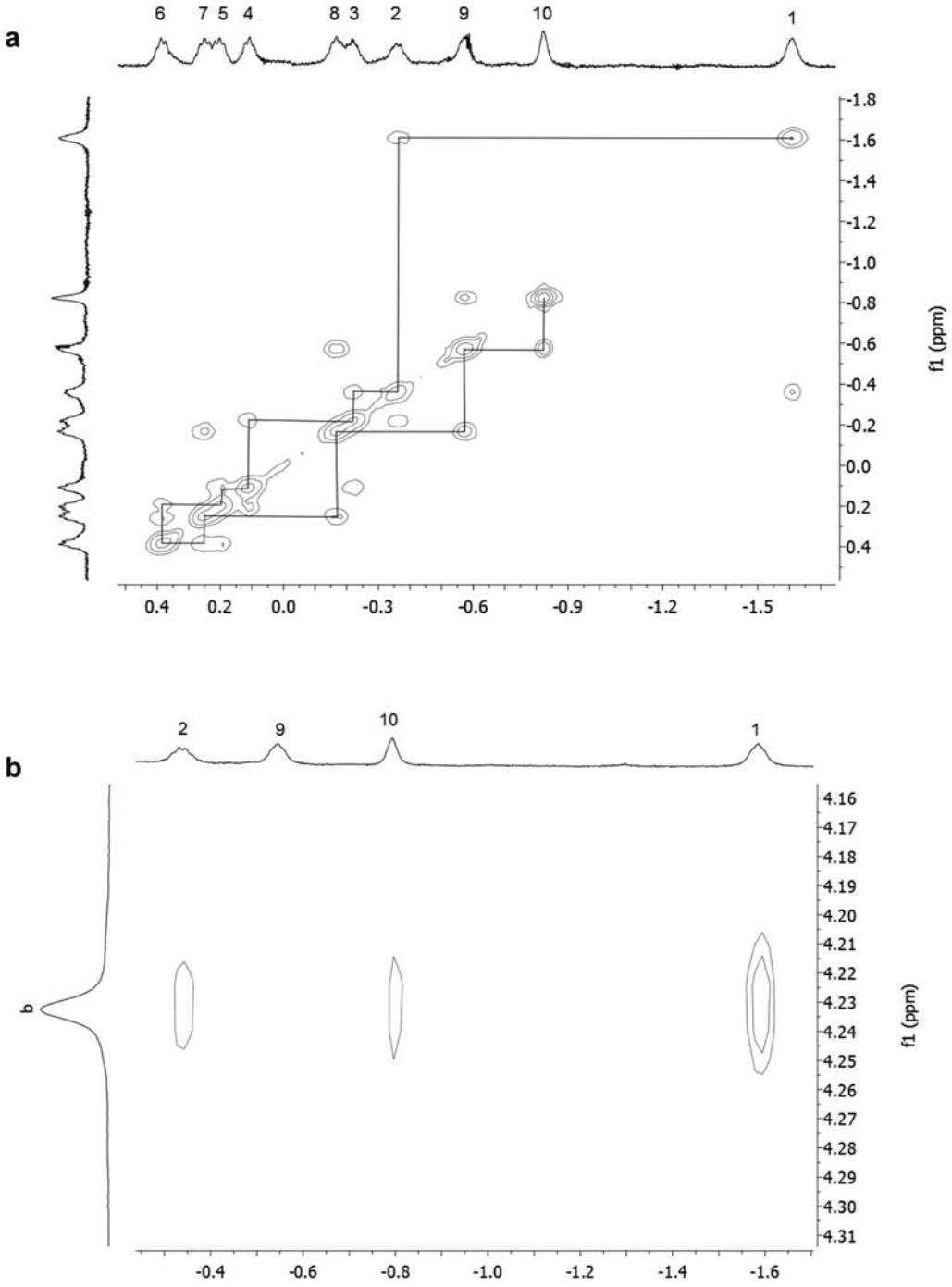

Figure 4. (a) High-field region of the COSY NMR of the 2:1 complex formed between host OA **1** and *n*-eicosane ($C_{20}H_{42}$). [Complex] = 0.5 mM, [Buffer] = 10 mM LiOH. (b) Partial NOESY NMR spectrum of the 2:1 complex formed between host **1** and *n*-eicosane ($C_{20}H_{42}$). [Complex] = 0.5 mM, [Buffer] = 10 mM LiOH.

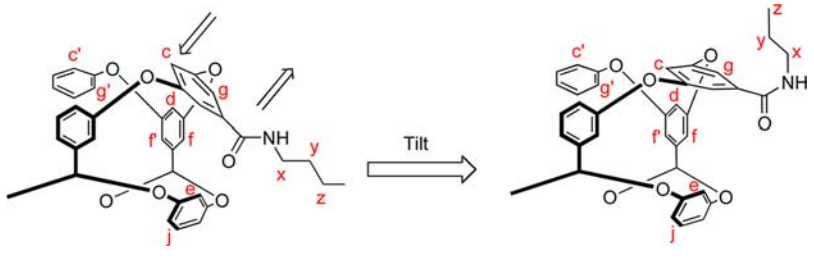

Figure 5. Tilting of the linker-substituted third-row ring in response to capsule closure.

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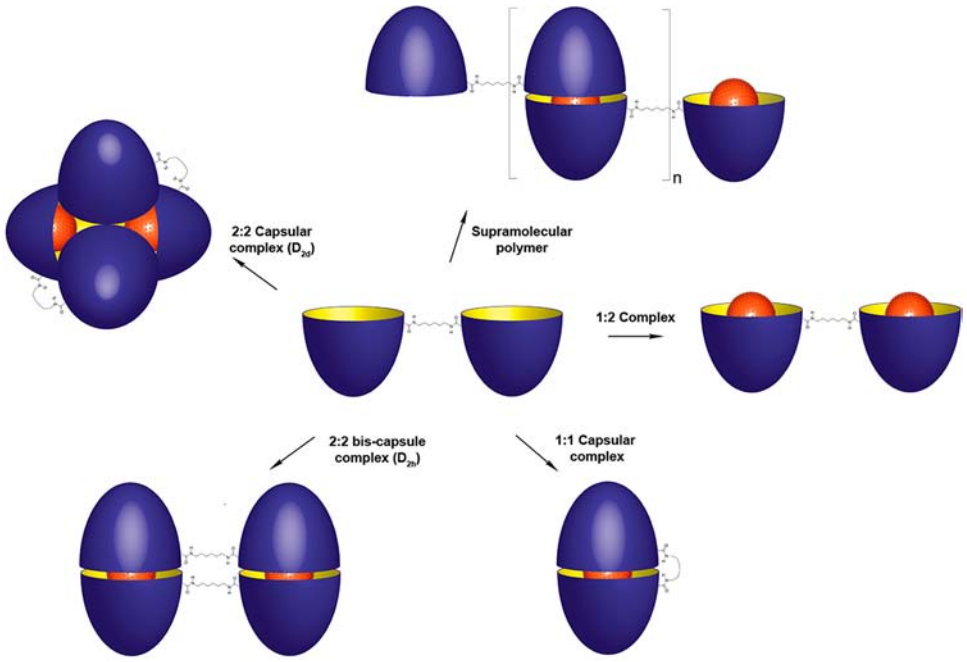
Scheme 2. Selected Assembly Options for HOA 2[a]



[a]Clockwise from top: supramolecular polymer; 1:2 host–guest complex; 1:1 capsular complex; 2:2 *bis*-capsule complex (D_{2h}); 2:2 capsular complex (D_{2d}).

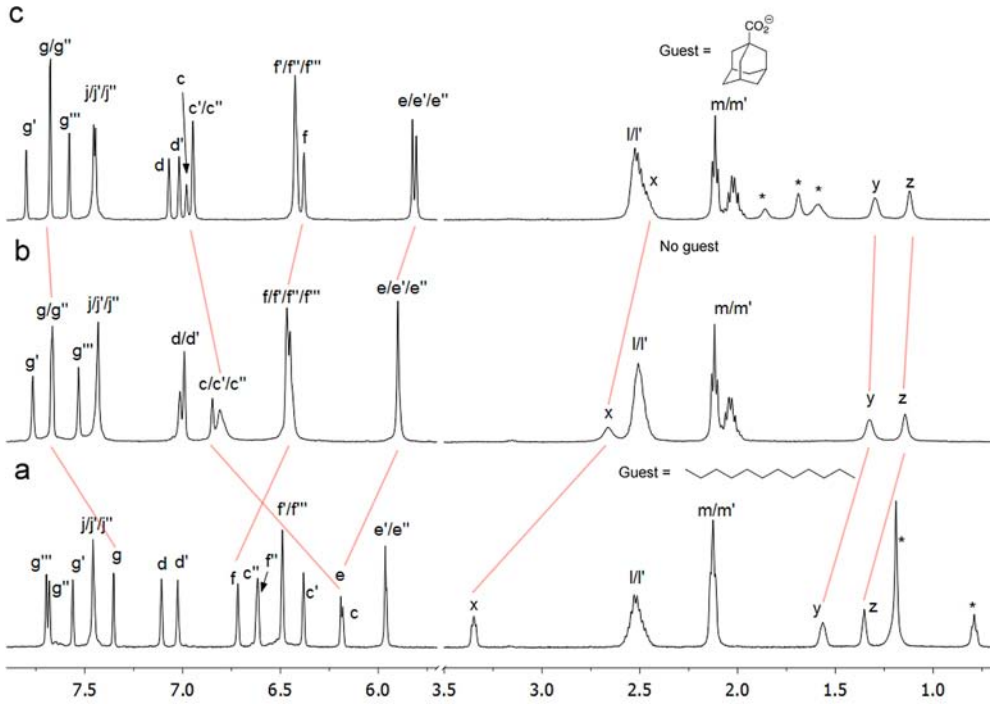


Figure 6. Host signal region of the ^{1}H NMR spectra of (a) host **2** and 1 equiv of *n*-dodecane ($C_{12}H_{26}$); (b) Free host **2**; (c) host **2** and 2 equiv of adamantane carboxylate ($C_{10}H_{15}CO_2^-$). In each, [host] = 0.5 mM, [buffer] = 10 mM LiOH. Proton designations are defined in structure **2**. Protons from excess (free) guest are indicated as '*'.

initial assignment of H_c, the $H_{c'}$ and $H_{c''}$ signals are unequivocally identified as respectively those at 6.36 and 6.60 ppm. The cross-peaks of the different H_c signals in the COSY spectrum (Figure 7a) identify the coupled protons that share what is termed the third row aromatic rings, i.e., H_c and H_g. Hence, the correlations with the different H_c proton signals identify the signals from the four H_g protons. As expected, the H_g and $H_{g''}$ signals appear as doublets, while the nonequivalent $H_{g'}$ and $H_{g''}$ appear as doublets of doublets. The COSY NMR spectrum also identifies the coupling between the pairs of protons H_d and H_f in the second row aromatic rings, but it does not unequivocally identify each set.

However, the NOESY NMR identifies the through space interactions between protons on the first and second rows of

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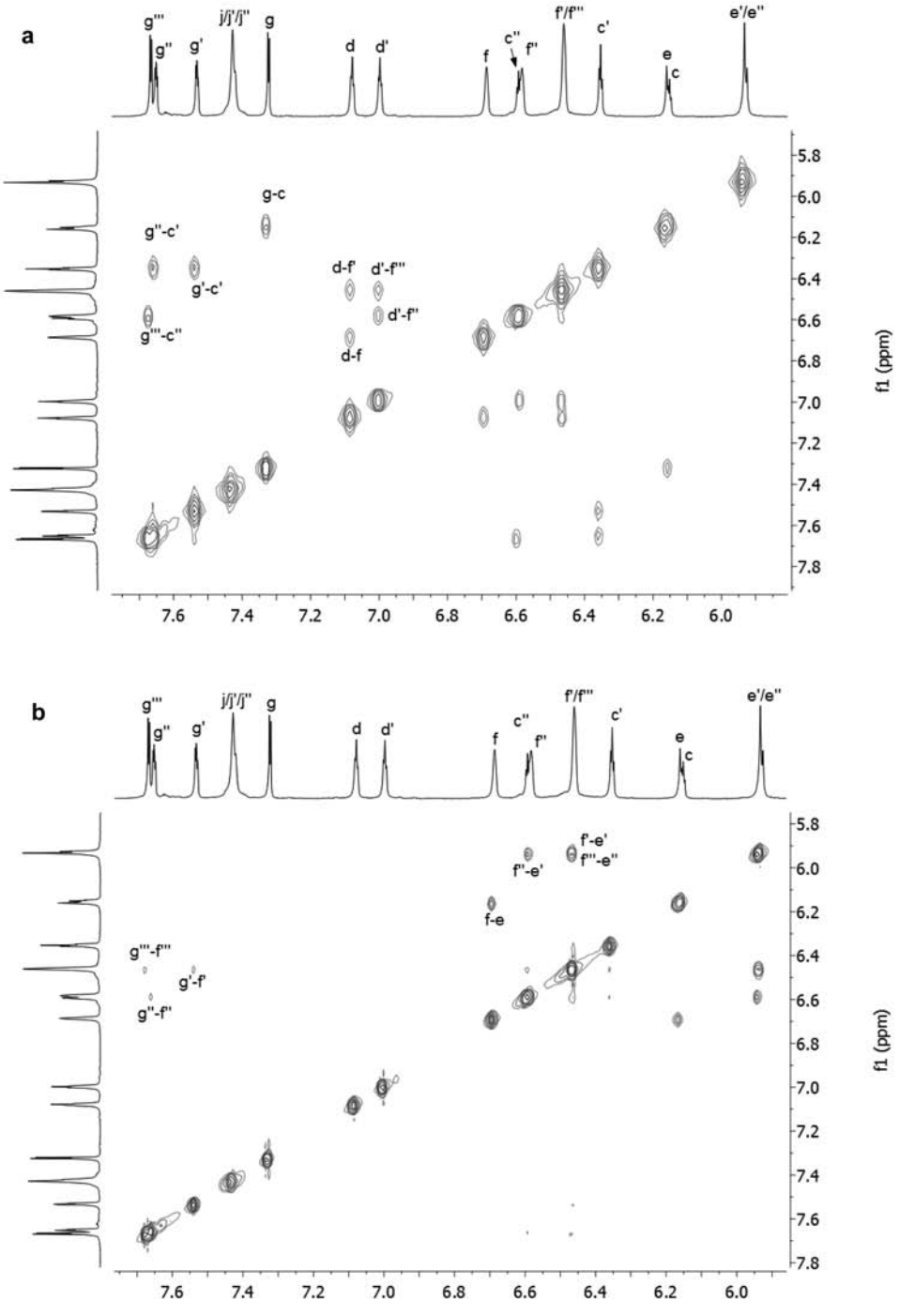

Figure 7. (a) Low-field region of the COSY NMR of the 1:1 complex formed between HOA **2** and *n*-dodecane ($C_{12}H_{26}$). [Complex] = 0.5 mM, [Buffer] = 10 mM LiOH. (b) Partial NOESY NMR spectrum of the same complex.

aromatic rings (H_e and H_f) and those between protons on the second and third row rings (H_g and H_f). Consequently, the assigned H_g protons identify each of the H_f signals in the NOESY spectrum, which, returning to the COSY spectrum, identifies each of the H_d signals. Finally, the H_j signals were assigned based on their typical position for these types of hosts and their lack of NOE or COSY interactions with other aromatic protons. Similar analyses were used for the free host and the host complex with adamantane carboxylic acid.

The signal shifts that occur upon the formation of the bis-adamantane carboxylate complex parallels those seen when OA **1** binds the same guest. Specifically, the H_b and the H_e proton signals are shifted upfield (former not shown in Figure 6c), while the H_c proton signals, one of which is broad in the free host, are downfield shifted and appear as sharp signals. While these differences are noteworthy, it is closure of the capsule that leads to the most significant spectral changes in the host (Figure 6a compared to 6b and 6c). Thus, formation of a capsular 1:1 complex with *n*-dodecane ($C_{12}H_{26}$) leads to greater proton anisotropy, principally through large shifts in the signals from the linker methylenes, the protons of the ring that the linker is attached to (H_c and H_g), as well as their nearest-neighbor protons (H_e and H_f). The largest shift observed is for the H_x protons ($\Delta\delta \approx 0.69$ ppm), with the signals from the

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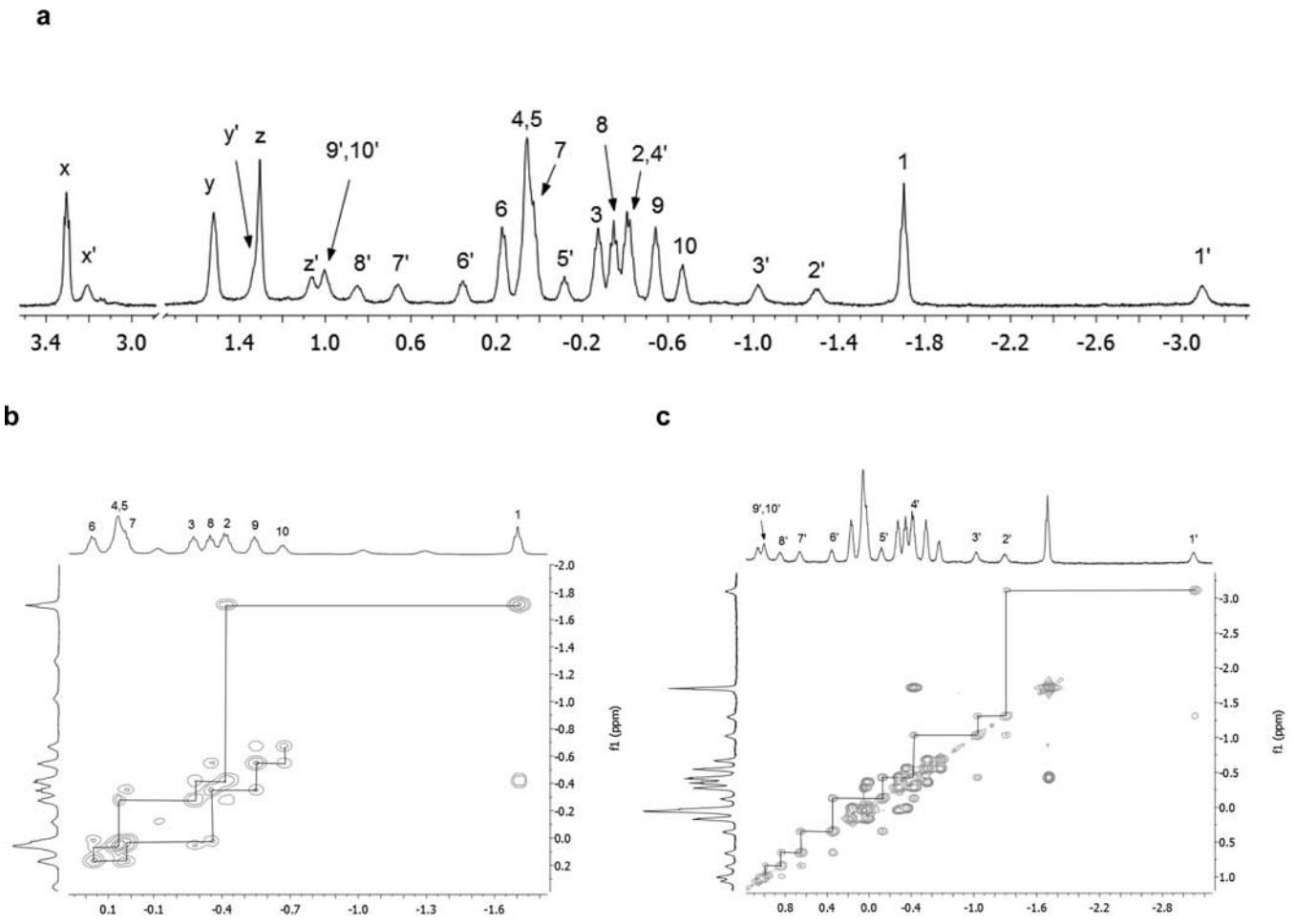

Figure 8. (a) Partial ^{1}H NMR spectrum of the complexes formed between host **2** and *n*-nonadecane ($C_{19}H_{40}$). (b) COSY NMR spectrum of the bound guest region of the major complex. (c) COSY NMR spectrum of the bound guest region of the minor complex. [$\mathbf{2}_{total}$] = 0.5 mM, [Buffer] = 10 mM LiOH.

other linker methylene groups, H_y and H_z, also undergoing significant shifts. Additionally, all of the signals from the 'c' protons (H_c, $H_{c'}$, and $H_{c''}$) are observed to move upfield, with the H_c signal undergoing the largest shift ($\Delta\delta \approx 0.63$ ppm). In part, these upfield shifts arise from the mutual shielding of the two rims of the cavitands as they clamp down on one another, but the large shift in the H_c signal is envisioned to also arise from the necessary tilting of the ring in response to the mechanical forces created by capsule closing (Figure 5). CPK models demonstrate that such tilting moves the H_c proton into a more shielded zone of the inner wall of the cavity. Additionally, the shifts of the signals for the protons in the vicinity of this tilting ring, specifically H_e, H_f, H_g, and $H_{g'}$ are also considerable. An inspection of the other capsular complexes formed by **2** (*vide infra*) confirms that the signal shifts for H_c and H_x are diagnostic of HOA **2** folding into a capsular form.

Capsule formation with host **2** was also observed with the smaller guests *n*-hexane (C_6H_{14}), *n*-octane (C_8H_{18}), and *n*-decane ($C_{10}H_{22}$), as well as the larger *n*-hexadecane ($C_{16}H_{34}$) (Supporting Information). Similarly to OA **1**, HOA **2** formed a capsular complex containing two copies of *n*-hexane, formed 1:1 and 1:2 host−guest complexes with *n*-octane, and formed only 1:1 complexes with the larger guests. As discussed below, with guests larger than *n*-hexadecane ($C_{16}H_{34}$) the binding properties of HOA **2** are quite different from those of **1**.

An examination of the complexes formed by HOA **2** with the guests from *n*-heptadecane ($C_{17}H_{36}$) through *n*-hexacosane ($C_{26}H_{54}$) revealed several key differences to host **1**: (1) The largest guest observed to bind to **2** was *n*-tricosane ($C_{23}H_{48}$). This is attributed to the fact that the linker prevents sufficient separation of the two hemispheres to accommodate larger guests in a spinning-top motif. (2) In contrast to OA **1**, the complexes of HOA **2** were all formed in 100% yield. In part this is attributed to binary rather than ternary nature of these complexes. (3) Host **2** formed (*vide infra*) assemblies inaccessible to **1** and, as a consequence of this, demonstrated a different binding motif profile.

The *n*-heptadecane ($C_{17}H_{36}$) complex with **2** was similar to that of **1** and possessed an E/C motif with no evidence of helicity. In contrast, the complexation of the guests from *n*-octadecane ($C_{18}H_{38}$) to *n*-tricosane ($C_{23}H_{48}$) led to two complexes with different packing motifs. The guest *n*-nonadecane ($C_{19}H_{40}$) is illustrative. Figure 8a shows the ^{1}H NMR of the guest-binding region of the mixture and the two bound methyl signals at −1.70 (∼65%) and −3.09 (∼35%) ppm respectively. COSY NMR (Figure 8b) analysis of the mixture revealed that the guest in the major complex had a 'U-shaped" packing motif and that the guest of the minor complex existed as an overall E/C motif. Additionally, PGSE NMR experiments revealed that the diffusion constant for the 'U-shaped' packed complex was identical to that of the 1:1

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complex between HOA **2** and *n*-dodecane (D = 1.31 × 10^{-6} cm^2 s^{-1}), but the diffusion constant of the E/C complex was much smaller (D = 0.95 × 10^{-6} cm^2 s^{-1}). Assuming the assemblies to approximate to spheres, the Stokes−Einstein equation gives hydrodynamic volumes for the major and minor assemblies of 19.4 and 50.8 nm^3 respectively. As it is geometrically impossible to form an assembly of three copies of HOA **2** that is smaller than three times the size of the monomer,[22] the diffusion data identify the minor E/C motif complex as a relatively large 2:2 complex.

The higher stoichiometry of the minor complex was confirmed by an analysis of the NMR spectrum as a function of concentration. Thus, as the initial concentration of the host was raised from 0.5 to 1.5 mM, the ratio of the 1:1 and 2:2 complexes changed from ∼7:3 to ∼1:9. More importantly, negative mode ESI analysis of the 0.5 mM sample led to a mass spectrum with signals for the free host and both the 1:1 and 2:2 complexes (Supporting Information). Interestingly, isotope patterns corresponding to the 2:2 complex were only observed when sodium ions were also part of the ion cluster.[23]

There are two possible dimeric assemblies, a D_{2h} assembly with two separate binding sites and a D_{2d} assembly with one large binding pocket (Scheme 2). Three lines of evidence point to the latter. First, each complex defines a different nanospace. A D_{2h} assembly possesses two cavities of essentially the same volume as that of the dimer of **1**, i.e. 2 × 650 or 1300 $Å^3$. In contrast, the volume of the D_{2d} assembly is the sum of the volume of the four cavitands plus the central (pseudo) tetrahedral volume defined by the assembly, a total of >1500 $Å^3$. As the guests in the dimeric assembly do not possess the U-shaped motif observed in the 1:1 complex (or the corresponding complex with **1**) but the more relaxed E/C packing motif, the assembly must be the more capacious D_{2d} assembly. There is not enough space inside the cavities of the D_{2h} complex for *n*-octadecane ($C_{18}H_{38}$) to bind in an E/C motif. Second, the diffusion data confirm a host that is larger than twice the volume of HOA **2**. Third, the ^{1}H NMR signal from the H_x protons is observed to undergo a large shift to a position almost as far downfield as that seen in the 1:1 capsular complexes with **2**. Considering the ∼180° bite angle between two cavitands in open 1:2 complexes, a D_{2h} assembly would be expected to lead to a relatively small upfield shift in the H_x signal. Only in a D_{2d} assembly with a relatively narrow bite angle between the two cavitand moieties of each subunit would such a large shift be expected.[24]

As the size of the guest was increased, the amount of the D_{2d} 2:2 complex with the E/C motif increased at the expense of the 1:1 complex (Supporting Information). For example, in the case of *n*-tricosane ($C_{23}H_{48}$), the ratio of the 1:1 and 2:2 complex was 35:65. In each of the D_{2d} complexes COSY and NOESY NMR studies did not reveal any well-defined packing of the guest. This is perhaps not surprising since in these 2:2 complexes there are also interguest packing possibilities, such as those where the two guests adopt an X-motif, a double-U motif (with each cavitand anchoring a methyl group rather than both methyls in one cavitand), and a similar double-U motif where the U's are 'catenated'. Furthermore, models suggest that U-shaped motifs are also incongruous to the two guests adopting helicity. In short, the increased number of possible motifs in the D_{2d} dimer, combined with the lack of directional noncovalent interactions between hosts and guests, preclude any well-defined packing. The contents of these capsular assemblies are more akin to the core of a micelle. An overall view of guest binding motifs of HOA **2** is given in the Supporting Information.

CONCLUSIONS

Although hosts OA **1** and HOA **2** engender ostensibly identical encapsulation environments, their encapsulation profiles in the 2:1 and 1:1 capsular complexes that they form are quite distinct. For guests smaller than *n*-heptadecane ($C_{17}H_{36}$) both the supramolecular capsule of OA **1** and the capsule of HOA **2** bind *n*-alkane guests in an E/C motif. The only apparent difference for guests in this range is that host **1** is seen to promote helical guest motifs, whereas **2** does not. For guests larger than *n*-heptadecane ($C_{17}H_{36}$) both hosts accommodate the guest in a higher energy U-shaped motif, but with *n*-octadecane ($C_{18}H_{38}$) the binding profiles of the hosts diverge. For this and larger guests OA **1** continues to engender a U-shaped packing, but in the case of HOA **2** it is more energetically preferable to switch to a higher energy D_{2d} dimeric assembly because the intrinsically less stable host shell is compensated for by guests that can once again adopt low energy, relaxed, E/C motifs. For the largest guests seen to bind to OA **1** a third binding motif, the bend of last resort, is observed. This spinning-top motif is not observed with HOA **2**, because the linker prevents sufficient separation of the host hemispheres. Nevertheless, HOA **2** is the most capacious host to date whose assembly is driven by the hydrophobic effect; by the metric on *n*-alkanes, it can accommodate 46 non-hydrogen atoms worth of guest(s).

In totality these studies reveal two points. First, hosts can encapsulate guests in multiple packing motifs, and smooth transitions from one packing motif to another are observed as a function of guest size. In the hosts studied here, the energetics of the guest motif follows the order E/C motif ∼ helical motif < U-shaped motif < spinning-top motif. Second, hosts that can change their assembly state add a layer of complication to their encapsulation profile. In the case of HOA **2**, the more capacious D_{2d} assembly is engendered because it can release the packing strain of large guests. We are continuing to study the properties of OA **1** and a variety of covalent dimers and will report on these in due course.

ASSOCIATED CONTENT

Supporting Information

For host **1**: ^{1}H NMR spectra for the capsular complexes with **C17−C26**, ^{1}H NMR shifts data, NMR diffusion coefficient data, percentage complexations, and overall view of guest binding motifs. For **2**, details of synthesis of host **2**, ^{1}H NMR of free **2** and selected complexes, NMR diffusion coefficient data, mass analysis, ^{1}H NMR concentration dependence study, and overall view of guest binding motifs. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Tanaka, H.; Kato, K.; Yamashita, E.; Sumizawa, T.; Zhou, Y.; Yao, M.; Iwasaki, K.; Yoshimura, M.; Tsukihara, T. *Science* **2009**, *323*, 384−388.

(2) (a) Xu, Z.; Horwich, A. L.; Sigler, P. B. *Nature* **1997**, *388*, 741−750. (b) Horwich, A. L.; Farr, G. W.; Fenton, W. A. *Chem. Rev.* **2006**, *106*, 1917−1930.

(3) Clantin, B.; Delattre, A. S.; Rucktooa, P.; Saint, N.; Meli, A. C.; Locht, C.; Dubuisson, F. J.; Villeret, V. *Science* **2007**, *317*, 957−961.

(4) Sacchettini, J. C.; Gordon, J. I.; Banaszak, L. J. *J. Mol. Biol.* **1989**, *208* (2), 327−39.

(5) We use this term synonymously with protein tertiary and quaternary structure. It is useful to recall that whereas for small molecules dihedral angles can be used to define structure, this is not the case with larger molecules. Thus, in proteins one must consider not only local conformations defined by the dihedral angles Φ and Ψ, and the *cis*-/*trans*-stereochemistry of the peptide bond of the polypeptide chain, but also how these are repeated to generate defined secondary structure, and how combinations of these lead to tertiary and quaternary structure. Whereas the repeating polypeptide structure of proteins facilitates their qualitative description, it seems unlikely considering the vast range of structures in the organic realm that a highly specific nomenclature can be formulated for guest packing motifs. Consequently, general qualifications such as "J-shaped" or "U-shaped" are routinely used to define the packing of, for example, long-chain fatty acids within intestinal Fatty Acid Binding proteins (ref 4).

(6) (a) Brown, C. J.; Miller, G. M.; Johnson, M. W.; Bergman, R. G.; Raymond, K. N. *J. Am. Chem. Soc.* **2011**, *133* (31), 11964−6. (b) Murase, T.; Horiuchi, S.; Fujita, M. *J. Am. Chem. Soc.* **2010**, *132*, 2866−2867. (c) Crisostomo, F. R. P.; Lkedo, A.; Shenoy, S. R.; Iwasawa, T.; Rebek, J., Jr. *J. Am. Chem. Soc.* **2009**, *131*, 7402−7410. (d) Shenoy, S. R.; Crisostomo, F. R. P.; Iwasawa, T.; Rebek, J., Jr. *J. Am. Chem. Soc.* **2008**, *130*, 5658−5659. (e) Pluth, M. D.; Bergman, R. G.; Raymond, K. N. *J. Am. Chem. Soc.* **2008**, *130*, 11423−11429. (f) Yamaguchi, T.; Fujita, M. *Angew. Chem., Int. Ed.* **2008**, *47*, 2067−2069. (g) Nishioka, Y.; Yamaguchi, T.; Kawano, M.; Fujita, M. *J. Am. Chem. Soc.* **2008**, *130*, 8160−8161. (h) Pluth, M. D.; Bergman, R. G.; Raymond, K. N. *Science* **2007**, *316*, 85−88. (i) Pluth, M. D.; Bergman, R. G.; Raymond, K. N. *Angew. Chem., Int. Ed.* **2007**, *46*, 8587−8589. (j) Nishioka, Y.; Yamaguchi, T.; Yoshizawa, M.; Fujita, M. *J. Am. Chem. Soc.* **2007**, *129*, 7000−7001. (k) Fiedler, D.; van Halbeek, H.; Bergman, R. G.; Raymond, K. N. *J. Am. Chem. Soc.* **2006**, *128*, 10240−10252. (l) Yoshizawa, M.; Tamura, M.; Fujita, M. *Science* **2006**, *312*, 251−254. (m) Hooley, R. J.; Rebek, J., Jr. *J. Am. Chem. Soc.* **2005**, *127*, 11904−11905. (n) Chen, J.; Rebek, J., Jr. *Org. Lett.* **2002**, *4*, 327−329. (o) Kang, J.; Santamaría, J.; Hilmersson, G.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1998**, *120*, 7389−7390. (p) Kang, J.; Rebek, J., Jr. *Nature* **1997**, *385*, 50−52.

(7) (a) Gibb, C. L. D.; Gibb, B. C. *J. Am. Chem. Soc.* **2006**, *128*, 16498−16499. (b) Liu, S.; Gan, H.; Hermann, A. T.; Rick, S. W.; Gibb, B. C. *Nat. Chem.* **2010**, *2*, 847−852.

(8) Trembleau, L.; Rebek, J., Jr. *Science* **2003**, *301*, 1219−1220.

(9) (a) Scarso, A.; Trembleau, L.; Rebek, J., Jr. *Angew. Chem., Int. Ed.* **2003**, *42*, 5499−5502. (b) Scarso, A.; Trembleau, L.; Rebek, J., Jr. *J. Am. Chem. Soc.* **2004**, *126*, 13512−13518.

(10) Gibb, C. L. D.; Gibb, B. C. *Chem. Commun.* **2007**, 1635−1637.

(11) Rebek, J., Jr. *Chem. Commun.* **2007**, 2777−2789.

(12) Asadi, A.; Ajami, D.; Rebek, J., Jr. *J. Am. Chem. Soc.* **2011**, *133* (28), 10682−4.

(13) Ajami, D.; Rebek, J., Jr. *J. Am. Chem. Soc.* **2006**, *128*, 15038−15039.

(14) (a) Ko, Y. H.; Kim, H.; Kim, Y.; Kim, K. *Angew. Chem., Int. Ed.* **2008**, *47*, 4106−4109. (b) Baek, K.; Kim, Y.; Kim, H.; Yoon, M.; Hwang, I.; Ko, Y. H.; Kim, K. *Chem. Commun.* **2010**, 4091−4093. (c) Ko, Y. H.; Kim, Y.; Kim, H.; Kim, K. *Chem.—Asian J.* **2011**, *6* (2), 652−657.

(15) Liu, S.; Whisenhunt-Ioup, S. E.; Gibb, C. L. D.; Gibb, B. C. *Supramol. Chem.* **2011**, *24*, 480−485.

(16) Gibb, C. L. D.; Gibb, B. C. *J. Am. Chem. Soc.* **2004**, *126*, 11408−11409.

(17) Cohen, Y.; Avram, L.; Frish, L. *Angew. Chem., Int. Ed.* **2005**, *44*, 520−554.

(18) Ajami, D.; Iwasawa, T.; Rebek, J., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103* (24), 8934−6.

(19) Eliel, E. L.; Wilen, S. H. *Stereochemistry of Organic Compounds*; John Wiley and Sons: New York, 1994.

(20) (a) Kuad, P.; Miyawaki, A.; Takashima, Y.; Yamaguchi, H.; Harada, A. *J. Am. Chem. Soc.* **2007**, *129*, 12630−12631. (b) Liu, Y.; Chen, Y. *Acc. Chem. Res.* **2006**, *39*, 681−691. (c) Castellano, R. K.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1998**, *120*, 3657−3663. (d) Rowan, S. J.; Cantrill, S. J.; Cousins, G. R. L.; Sanders, J. K. M.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **2002**, *41*, 898−952.

(21) Sun, H.; Gibb, C. L. D.; Gibb, B. C. *Supramol. Chem.* **2008**, *20*, 141−147.

(22) The smallest volume trimer would be either of the following: (1) A triangular array of capsules in which each apex is a capsule constructed from two different copies of host 2. Such a complex would possess a Möbius strip topology and define a large, poorly solvated, central region; (2) An octahedral (O_h) assembly with an inner space defined by six cavitands and a large central cubic void. Both of these assemblies would possess volumes larger than three times the total volume of **2**.

(23) Three points suggest that MS analysis of these complexes warrants further study: (1) The relative stability of these and related complexes (see for example: Tang, H.; de Oliveira, C.; Sonntag, G.; Gibb, C. L. D.; Gibb, B. C; Bohne, C. *J. Am. Chem. Soc.* **2012**, *134*, 5544−5547) suggests that the decrease in the hydrophobic effect as a result of droplet-size reduction during the ionization process may play a role in the observance of a preponderance of free host; (2) The apparent necessity for sodium ions in the 2:2 complex suggests a possible role of metal ion templation in the larger assemblies; (3) Variations in cluster distributions as a function in sample suggest other yet unappreciated important factors in the MS analysis. Further experiments on these and other complexes will be reported in future communications.

(24) The complicated aromatic region of this mixture (Supporting Information) precluded the determination of the shifts of other pertinent signals such as H_c, H_e, H_f, H_g, and $H_{g'}$.