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Well-Defined, Organic Nanoenvironments in Water: The Hydrophobic Effect Drives a Capsular Assembly

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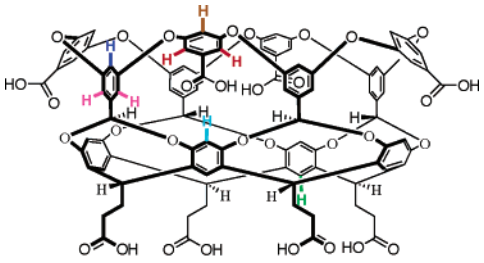
Container compounds engender the isolation of guest molecules in distinct, localized environments. Containers constructed using only covalent bonding provide for long residency times and a means to isolate highly reactive guests that normally have only a fleeting existence.[1,2] In contrast, a combination of covalent bonds and kinetically labile noncovalent interactions leads to dynamic vessels whose assembly and disassembly control the exposure of the guest to the external environment. In organic solvents, hydrogen bonding[3−11] and metal coordination[12−14] have proven to be the most successful approaches to adding dynamism to container molecules, while large capsule formation in water has been limited to metal coordination processes.[15−18] Here we demonstrate that shape complementarity and the hydrophobic effect drive deep-cavity cavitand **1** to form a capsular complex with an internal volume large enough to store steroids.[19,20]

Host **1** was synthesized (Supporting Information) using chemistry analogous to the organic-soluble hosts we have previously reported on.[21−24] It possesses an external coat of carboxylic acid groups, an internal hydrophobic pocket approximately 1 nm in width and depth, and importantly, a wide hydrophobic rim around the entrance to the cavity.

The distinctiveness of each aromatic hydrogen atom in the host leads to a relatively simple aromatic region of the ^{1}H NMR spectrum (Figure 1a). These signals confirm that at 1 mM the cavitand exists as a well-defined species, although they do not differentiate between a monomeric host (C_{4v}) or a well-defined dimeric structure (D_{4h}).



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Addition of half an equivalent of estradiol **2** leads to a more complex picture (Figure 1b). The changes in the spectrum are consistent with the formation of a C_1 symmetric capsular complex that is kinetically stable on the (500 MHz) NMR time scale. Four of the six signals (green, blue, brown, and turquoise) split into two. In host **1**, these signals correspond to different sets of homotopic protons. In the capsule, however, one "hemisphere" (arbitrarily defined as the northern) hosts the aliphatic D-ring of **2**, while the southern hemisphere binds the aromatic A-ring. Thus, in the capsule each set of protons in the northern hemisphere differs from its counterpart in the south. The remaining two signals (red and purple), each integrating for eight protons, are seen to split into four. The responsible protons are enantiotopic in the free host. That is to say,

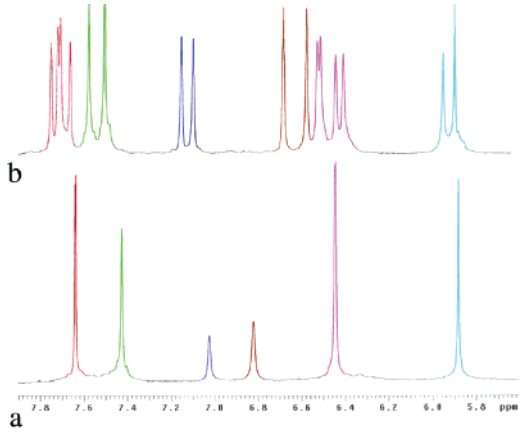


Figure 1. (a) Aromatic region of the ^{1}H NMR spectra (500 MHz, D_2O, sodium borate, pD = 8.9) of host **1**. (b) 2:1 mixture of **1** and **2**. Host concentration 1 mM. Color-coding as per structure **1**.

they are indistinguishable by NMR unless in a chiral environment. Binding of the steroid provides such a chiral environment. In the complex, the eight protons of each set are split into two diastereomeric (nonequivalent) subsets of four protons. In addition, as the southern and northern hemispheres of the capsule differ, the signals are doubled not once, but twice. The NMR signals from the guest also confirm encapsulation. For example, in the shielded, electron-rich environment of the ca. 1 × 2 nm cavity, the signal for the C-18 methyl group is shifted upfield to −1.0 ppm (Supporting Information).

NOESY ^{1}H NMR confirms the spatial proximity of the different components of the complex. In the host, two sets of hydrogens (the blue and brown in structure **1**) are located near the rim of the cavity and are ideal reporters for interhemisphere interactions. The off-diagonal peak between the blue sets of the northern ($Blue_N$) and southern ($Blue_S$) hemispheres confirms their spatial proximity (Figure 2). Also evident are the other anticipated off-diagonal peaks corresponding to the spatial proximity of: $Brown_N$−$Brown_S$, $Blue_S$−$Brown_N$, and $Brown_S$−$Blue_N$. In addition to these host−host contacts, NOESY NMR also demonstrates through-space interactions between capsule and guest. Thus, the inward pointing benzal hydrogens (black in **1**) of the northern hemisphere are seen to interact with, among other atoms, the C-17 methine and C-18 methyl protons of **2**, while the benzal hydrogens in the opposing hemisphere interact with the aromatic protons H-1, H-2, and H-4 of the guest (Supporting Information).

NMR also provided a lower limit for the association constant of encapsulating **2**. A dilution experiment revealed no loss of capsule integrity at 5 μM (Supporting Information). Assuming there is less than 5% free host at this concentration, a *minimum* association constant K_{app} for the occupation of an empty capsule of 1 × 10^8 M^{-1} can be calculated. The structures of the host and guest infer that the hydrophobic effect lies at the heart of this strong binding;

10.1021/ja0475611 CCC: $27.50

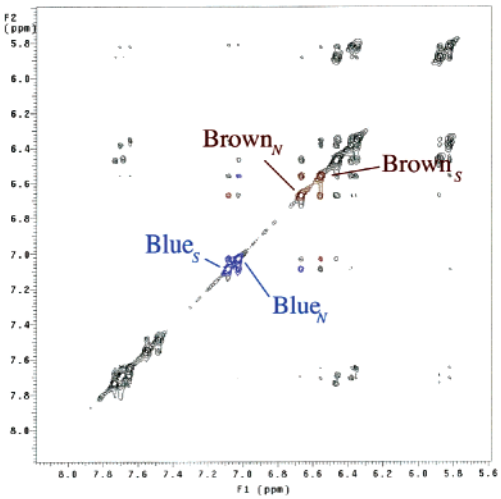
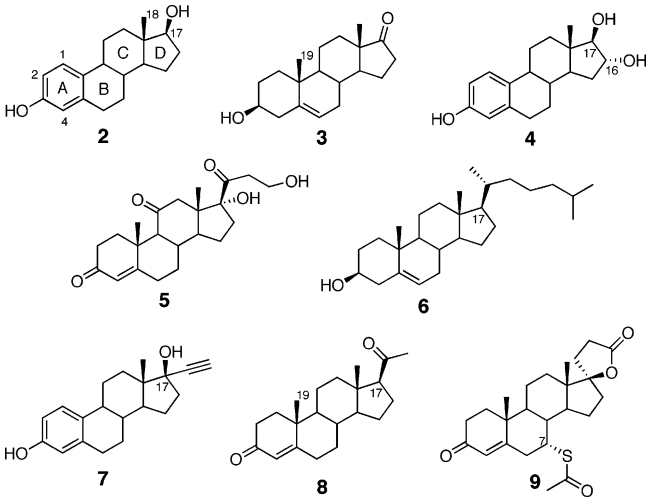


Figure 2. Selected NOESY ^{1}H NMR interactions in the **1·2** complex (500 MHz, D_2O, sodium borate, pD = 8.9, 5 mM). Highlighted are the signals from the blue and brown hydrogens located on the rim of the cavity. The signals Blue$_N$ and Blue$_S$ correspond to those sets in the hemispheres hosting the D- and A-rings of **2**, respectively. The corresponding off-diagonal (shown in blue) confirms their proximity. A similar off-diagonal interaction (shown in brown) arises through the proximity of Brown$_N$ and Brown$_S$. In addition, highlighted in blue (brown) are the off-diagonal interactions arising through the proximity of Blue$_N$ and Brown$_S$ (and Blue$_S$ and Brown$_N$).

a conclusion supported by the observation that addition of methanol to an aqueous solution of the capsule led to its breakdown at ca. 20% MeOH.

To probe the shape and size of the cavity of the capsule, a number of other steroids (**3**−**9**) were examined as guests.



In aqueous solutions of **1**, the solubility of all of these guests was greatly enhanced relative to pure buffer solution. NMR analysis of 2:1 mixtures of host and steroid revealed that the guests **2**−**4**, **7**, and **8** formed well-defined, kinetically stable complexes. In contrast, the relatively large and water-soluble adrenocorticoid cortisone **5** formed a weaker complex whose assembly/disassembly rate was on the NMR time scale. Likewise, cholesterol **6** and spironolactone **9** did not form well-defined complexes. An examination of CPK models reveals that the long, C-17 chain of **6** prevents the two hemispheres of the host from clamping down on each other. The midsection of the capsule is therefore open and solvent-exposed. Similarly, both the length of **9** and its C-7 thioacetate functionality prevent the capsule from fully closing. Competition experiments revealed the following order of preference for the capsule: (+)-dehydroisoandrosterone **3** > progesterone **8** > estradiol **2** > 17α-ethynylestradiol **7** > estriol **4** > cortisone **5**, cholesterol **6,** spironolactone **9**. The shape and polarity of the capsule interior is such that guests **5**, **6**, and **9** form less stable complexes. DHEA **3** is the best guest; its length is near ideal, and its aliphatic A-ring and C-19 methyl group fill the cavity better than the aromatic ring of **2**. Progesterone **8** lies midway between these two guests; it has the advantages of a voluminous A-ring and a C-19 methyl group, but the C-17 acetyl group reduces binding somewhat relative to **3**. Similarly, the capsule can accommodate ethynyl groups at C-17 (**7**) or hydroxy groups at C-17 and C-16 (**4**), but such guests bind more weakly than their counterparts with smaller or fewer substituents on the D-ring. The strength of binding, allied to the size of the internal volume of the capsule, suggests that many molecules can be encapsulated with the confines of $\mathbf{1}_2$. We are currently investigating some of these possibilities.

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Supporting Information Available: Synthesis and characterization of **1** and NMR characterization of complex **1·2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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