Supporting Information

Well-Defined Organic Nano-Environments in Water: Hydrophobic Forces Drive Capsular Assemblies

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1) Synthesis of 1

a) Synthetic Route

The synthesis of 1 is shown in Scheme S1. Known1 dodecanol 10 (R = CH₂OH) was treated with a slight excess of 3,5-dibromobenzal bromide.2-4 The resulting octabromide 11 (R = CH₂OH) possessed limited solubility, and therefore for routine synthesis was not purified but directly alkylated with benzyl bromide to give 11 (R = CH₂OBn). This intermediate was then subjected to an eight-fold Ullman ether reaction with 3,5-dihydroxybenzyl alcohol, to yield cavitand 12 (R = CH₂OBn, R¹ = CH₂OH). Removal of the benzyl groups gave the octol 12 (R = R¹ = CH₂OH). Finally, per-oxidation gave octacid 1.

![Scheme S1: The synthesis of host 1.](image)

b) Characterization of intermediates and host 1

All reagents were purchased from Aldrich Chemical Company, and were used as received without further purification. Melting points were determined using a hot-stage apparatus and are uncorrected. ¹H NMR was performed at 500 MHz. Mass spectra were obtained with the MALDI and ESI techniques. Elemental analyses were conducted by
Atlantic Microlab. Column chromatography was performed using Natland® International 200 – 400 mesh silica gel. THF was distilled over sodium benzophenone ketyl.

**Synthesis of Dodecanol 10**

The synthesis of dodecanol 10, 3,5 dihydroxybenzyl alcohol, and 3,5-dibromobenzal bromide have been previously reported.

**Synthesis of cavitand 11 (R = CH₂OH)**

To a dry flask containing 250 mL of degassed dimethylacetamide (DMA) was added 5.00 g (6.95 mmol) of 10 and 16.9 g (41.4 mmol) of 3,5 dibromobenzal bromide. To the resulting solution was added 8.3 mL (55.5 mmol) 1,8-diazabicycloundec-7-ene (DBU), and the reaction stirred for 24 h at 60 °C. The DMA was removed under reduced pressure and the resulting mixture partitioned three times with CHCl₃ and water. The organic layers were combined, dried with anhydrous Na₂SO₄ and filtered. Removal of the solvent under reduced pressure gave a crude solid that was dissolved in a minimum of CHCl₃. An excess of hexanes was then added to precipitate the crude product, which after filtration, was dried at 120 °C under reduced pressure. The 11.9 g of light brown solid of crude 11 (R = CH₂OH) was protected without further purification.

**Synthesis of cavitand 11 (R = CH₂OBn)**

The 11.90 g of crude 11 (R = CH₂OH) was added to a flask containing 240 mL THF and 3.36 g NaH (84 mmol, 60% suspension in oil, previously washed three times with pentane). To this flask was added 6.6 mL of benzyl bromide (55.5 mmol), and the
reaction refluxed for 2 d. After cooling to rt, the reaction was quenched with MeOH and the solvent removed under reduced pressure. The crude mixture was partitioned three times with CHCl₃ and water, and the organic layers combined, dried with anhydrous Na₂SO₄ and filtered. Removal of the solvent under reduced pressure gave the crude solid that was purified by column chromatography (8:2 CHCl₃:hexane mobile phase). The product was crystallized from 100% ethyl acetate; 40% for two steps. m.p, > 125 °C. ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 1.73 (m, 8H), 2.40 (m, 8H), 3.57 (m, 8H), 4.53 (s, 8H), 4.91 (t, J = 8.2 Hz, 4H), 5.35 (s, 4H), 6.62 (s, 4H), 7.30 (m, 24H), 7.72 (m, 12H). MS (MALDI): Calcd. 2173 [M + Ag]⁺, Found: 2173 [M+Ag]⁺. Anal. Calcd. for C₉₆O₂₁H₈₀Br₈: C, 55.84; H, 3.91. Found: C, 55.56; H 3.82.

**Synthesis of cavitand 12 (R = CH₂OBn, R¹ = CH₂OH)**

Nitrogen gas was bubbled through a suspension of 2.00 g (0.97 mmol) of 11 (R = CH₂OBn), 804 mg (5.83 mmol) of 3,5-dihydroxybenzyl alcohol, 1.60 g (11.6 mmol) K₂CO₃ in 75 mL pyridine. Subsequently, 924 mg (11.6 mmol) of CuO nano-powder was added and the solution vigorously refluxed for 8 d. After this time, the reaction was cooled and the solvent removed under reduced pressure. The remaining solid was dissolved in a minimal amount of CHCl₃ and run through a short silica gel plug with 20% acetone in CHCl₃. Removal of the solvent under reduced pressure gave the crude solid. Purification with column chromatography (5% then 8% acetone in CHCl₃ as mobile phase) gave the product as a white solid in 40% yield. m.p, > 250 °C. ¹H NMR (DMSO-d₆, 400 MHz) δ (ppm) 1.50 (m, 8H), 2.38 (m, 8H), 3.46 (t, J = 6.2 Hz, 8H), 4.41 (s, 4H), 4.43 (s, 8H), 4.54 (t, J = 8.0 Hz, 4H), 4.61 (d, J = 6.0 Hz, 8H), 5.46 (t, J = 5.8 Hz, 4H),
5.79 (s, 4H), 6.41 (s, 8H), 6.49 (s, 4H), 7.07 (s, 4H), 7.26 (m, 28H), 7.64 (s, 4H). MS (MALDI): Calcd. 2085 [M + Ag]^+, Found: 2085 [M+Ag]^+. Anal. Calcd. for C_{124}O_{24}H_{104}: C, 75.29; H, 5.30 Found: C, 75.00; H 5.25.

**Synthesis of cavatand 12 (R = R^1 = CH_2OH)**

For 5 min., H_2 was bubbled into a flask containing 100 mL THF, 500 mg (0.253 mmol) of 12 (R = CH_2OBn, R^1 = CH_2OH), and 500 mg of 10% Pd on carbon. The reaction was stirred for 2 d at rt, using a balloon to maintain a hydrogen atmosphere. After this time, the resulting suspension was filtered and the solvent removed under reduced pressure to give the product as a white solid in quantitative yield. m.p, > 250 °C. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm) 1.39 (m, 8H), 2.38 (m, 8H), 3.47 (m, 8H), 4.41 (s, 8H), 4.47 (t, J = 4.6 Hz, 4H), 4.53 (t, J = 8.0 Hz, 4H), 4.61 (d, J = 5.6 Hz, 8H), 5.47 (t, J = 5.6 Hz, 4H), 5.78 (s, 4H), 6.40 (s, 8H), 6.50 (s, 4H), 7.08 (s, 4H), 7.24 (s, 8H), 7.70 (s, 4H). MS (MALDI): Calcd. 1725 [M + Ag]^+, Found: 1725 [M+Ag]^+. Anal. Calcd. for C_{90}O_{24}H_{80}-2H_2O: C, 69.72; H, 5.12. Found: C, 69.94; H 5.12.

**Synthesis of Octa-acid 1**

To a flask containing 16 mL each of DMA and t-BuOH, was added 316 mg (0.195 mmol) of 12 (R = R^1 = CH_2OH), and 988 mg (6.25 mmol) of KMnO_4. The resulting mixture was stirred for 24 h at rt. The reaction was then quenched with excess MeOH and stirred at rt for 1 h. The mixture was then filtered, and the solid MnO_2 washed with H_2O. The solvent of the resulting solution was removed under reduced pressure. The resulting solid was then suspended in 20% HCl and filtered to give the
product as a white solid in 92% yield. m.p, > 250 °C. ¹H NMR (DMSO-d₆, 500 MHz) δ (ppm) 2.20 (m, 8H), 2.60 (m, 8H), 4.16 (s, 4H), 4.57 (t, J = 7.8 Hz, 4H), 5.78 (s, 4H), 6.41 (s, 8H), 6.98 (s, 4H), 7.17 (s, 4H), 7.70 (s, 4H), 7.78 (s, 8H), 12.20 (broad s, 4H), 13.61 (broad s, 4H). MS (ES): Calcd. 863.7 [M-2H]²⁺, Found: 863.9 [M-2H]²⁺. Anal. Calcd. for C₉₆O₃₂H₆₄·4H₂O: C, 64.00; H, 4.03. Found: C, 63.81; H 3.92.
2) Binding studies, NMR Data, and competition experiments

a) General Protocol for Binding Studies and NMR Characterization

Six hundred µL of a D$_2$O stock solution of host 1 (1 mM) and sodium borate buffer (10 mM) was added to a NMR tube. The resulting NMR 1 is shown in Figure S1. To this was added 0.5 equivalents of the steroid as a solution in DMSO-$d_6$. The concentration of the DMSO solution (30 mM) was such that 10 µL was added to the solution of the host. A purely aqueous solution of the complex could be made by stirring the solid steroid in the host solution, but the kinetics of uptake was usually very slow. Hence, with the exception of cholesterol, addition as a DMSO solution was the preferred route for complex formation. As cholesterol is not soluble in DMSO, the solid-uptake approach to complex formation was chosen for this guest.

![Figure S1: 1H NMR (500 MHz) of host 1 (1 mM in 10 mM sodium borate buffer, D$_2$O). The suppressed water signal is indicated thus*.](image)

The complete $^1$H NMR (500 MHz, 10 mM sodium borate buffer, D$_2$O) of a 2:1 mixture of host 1 and estradiol 2 is shown in Figure S2. Each signal from the guest is spread out over a range of ca. 6.00 pm. Most distinctive of these is the C-18 methyl group signal at –1.00 ppm. That the assembly is indeed a 2:1 complex can be directly
determined by integration of selected host and guest signals. Thus, the C-18 methyl signal at –1.00 ppm corresponds to three protons, while the signal at 7.05 ppm corresponds to eight protons.

**Figure S2:** $^1$H NMR (500 MHz) of the 2:1 complex between 1 and guest estradiol 2 (1 mM in 10 mM sodium borate, D$_2$O). The suppressed water signal is indicated thus*.

The aromatic region of the $^1$H NMR (500 MHz) of a 5 µM solution of the 2:1 complex in 10 µM sodium borate/D$_2$O is shown in Figure S3. At this concentration, there is no evidence of decomplexation (for example, free host gives a signal at 6.8 ppm). Assuming that there is no more than 5% free host, a minimum association constant ($K_{app}$) for the occupation of an empty capsule of $1.0 \times 10^8$ M$^{-1}$ can be calculated.
The complete 2D NOESY $^1$H NMR (500 MHz) of a 2:1 mixture of host 1 and estradiol 2 (5 mM, in 50 mM sodium borate/D$_2$O) is shown in Figure S4. A selection of the off-diagonal peaks that confirm the proximity of selected host and guest atoms are highlighted. The benza hydrogens are deep within the cavity of each hemisphere and interact with either the D-ring and C-18 methyl hydrogen atoms (benzal-H/H-17 and benzal-H/H-18 highlighted), or the aromatic A ring of the steroid (benzal-H/H-1, benzal-H/H-2 and benzal-H/H-4 highlighted)
Figure S4: 2D NOESY $^1$H NMR (500 MHz) of a 5 mM of the 2:1 complex between 1 and 2 in 50 mM sodium borate/D$_2$O.

**b) Competition Experiments**

For the competition studies, 600 µL of a 1 mM stock solution of host 1 in 10 mM sodium borate/D$_2$O was added to a NMR tube. To this was added 0.5 equivalents of guest I as a solution in DMSO-$d_6$. The concentration of the DMSO solution (30 mM) was such that only 10 µL was added to the solution of the host. After confirmation of
complex formation, guest II was added, again as a DMSO solution. After 5 minutes a NMR spectrum of the sample was again recorded to determine if guest exchange had occurred. If no exchange was evident, the same sample was examined 24 h. later to examine for a slow exchange process. No exchange process appeared to occur this slowly. Regardless of whether or not exchanged occurred, the reverse experiment in which the ability of guest I to displace guest II was also carried out. From these studies, the following order of complexation was determined: (+)-Dehydroisoandrosterone (DHEA) 3 > progesterone 8 > estradiol 2 > 17α-ethynylestradiol 7 > estriol 4 > cortisone 5, cholesterol 6, spironolactone 9.

**References**