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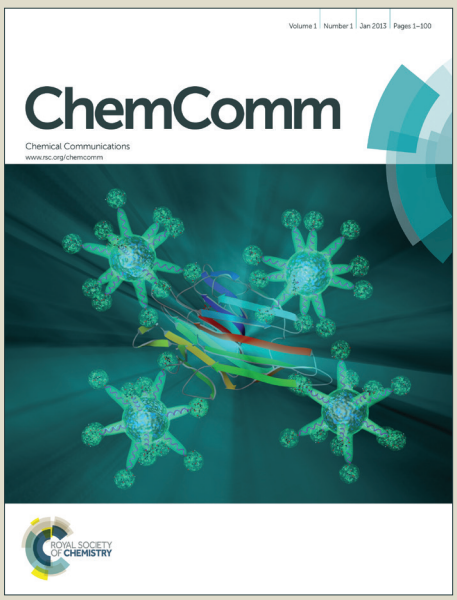

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ARTICLE TYPE

Complexation of alkyl groups and ghrelin in a deep, water-soluble cavitand

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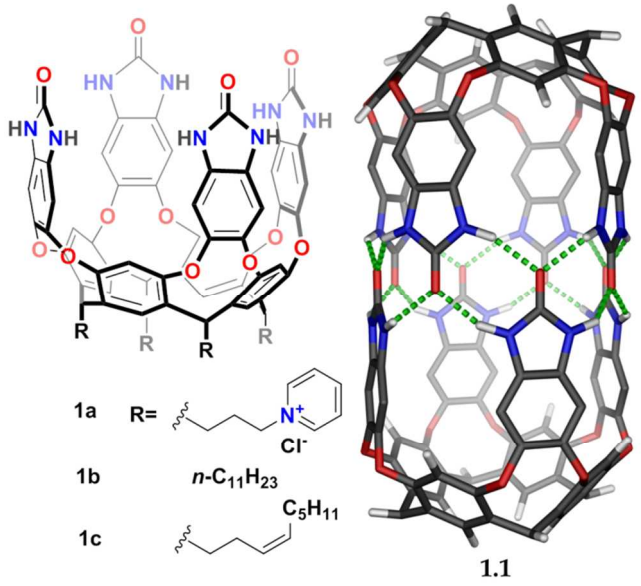
A cavitand with ionic, but nonionizable "feet" folds around hydrophobic guests in D_2O. Short alkanes and ibuprofen are included and exchange rates are slow on the NMR timescale. Normal octanoyl groups show good affinity for the cavitand and the gastric peptide ghrelin is bound at low pH and physiological temperature.

We recently described a deep cavitand **1a** with pyridinium "feet" that shows good solubility in aqueous media.[1] The self-complementary array of hydrogen bond donors and acceptors on the "rim" was introduced a decade ago by de Mendoza,[2] with cavitands **1b** and **1c**. The array leads to dimerization in organic solvents and results in encapsulation complexes when suitable guests are present. Surprisingly, **1a** also dimerized through hydrogen bonding in water (D_2O) with lengthy, hydrophobic guests despite the competitive nature of the solvent. We find now that even the monomeric cavitand **1a** forms complexes in water



and describe here its binding behavior.[3]

Figure 1. Cavitands and their dimeric capsule **1.1**. The hydrogen bonds are highlighted in **1.1**; the "feet" are not shown.

Normal alkanes are effective probes of container shape,[4] capacity[5] and chemical surface,[6] and brief sonication of **1a** in D_2O with a series of these guests gave stoichiometric complexes. They showed a new set of NMR signals (Figure 2), shifted characteristically upfield by the magnetic shielding of the 8 aromatic panels that line the host structure. Separate signals are seen for free and bound guest. The broadening of the signals for the bound guests is due to their exchange rates with free alkanes, which occurs on the NMR timescale. The shorter alkane guests show symmetrized spectra: only 4 peaks appear for C8 and 5 peaks appear for C9. These alkanes flip rapidly (on the NMR timescale) in the space, and their spectra are time-averaged by the exchange of magnetic environments of the two ends of the alkane.[#] Similar signal patterns have been observed in other cavitands where alkanes tumble rapidly in the space.[7]

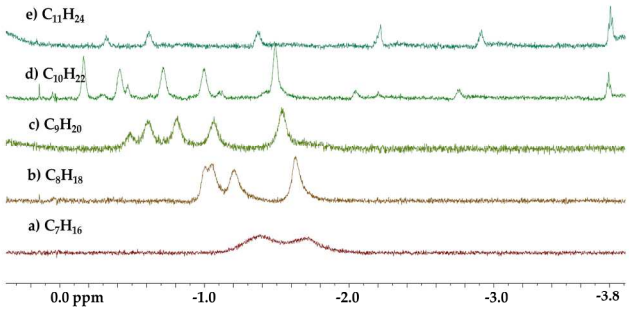


Figure 2. Partial ^{1}H NMR (600MHz, D_2O, 298K) spectra of the complexes formed between host **1a** (0.5mM) and a) *n*-heptane; b) *n*-octane; c) *n*-nonane; d) *n*-decane; e) *n*-undecane;

A qualitative change occurs in the spectrum of *n*-decane, which shows a second set of signals (Figure 2d and SI). This guest is present in two different environments: In one its 5 H-signals are condensed between 0 and -1.5ppm; in the other the signals are spread out between 0 and -4ppm (see SI). The narrower set reflects a complex similar to that of the shorter alkanes, but probably with C10 in a compressed conformation. This can be accomplished through *gauche* interactions that make it short enough to tumble freely in the space. The other set with the larger spread of signals is characteristic of an extended alkane in a symmetrical environment, specifically in capsules such as **1.1**.[1,8]

Since it is rare for a guest to find two containers that compete simultaneously as hosts,[9] we examined this case by 2D NOESY experiments (Figure 3, bottom). The spectrum showed the appropriate cross-peaks indicating the exchange of *n*-decane between the two environments on the NMR timescale at room temperature. The preferred environment of the guest proved temperature-dependent (Figure 3, top). At elevated temperature the compressed form disappears and at 340 K the extended form predominates (Fig 3c). The present case adds an example to the recent description of monomeric cavitand - multimeric capsule equilibria.[4b]

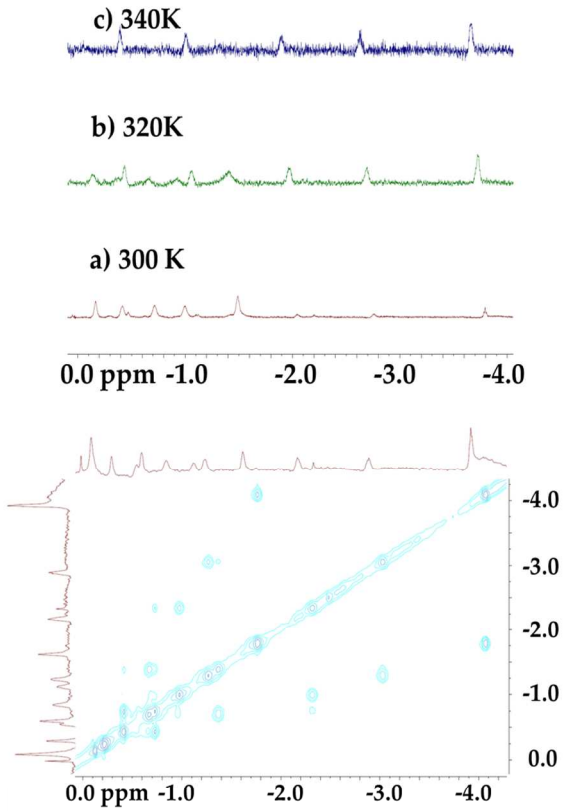


Figure 3. Top) The temperature-dependent ^{1}H NMR spectrum (600 MHz, D_2O) of the complex formed between host **1** (0.5 mM) and *n*-decane at a) 300K; b) 320K; c) 340K. Bottom) The upfield region of the 2D NOESY spectrum (600 MHz, 300K in D_2O).

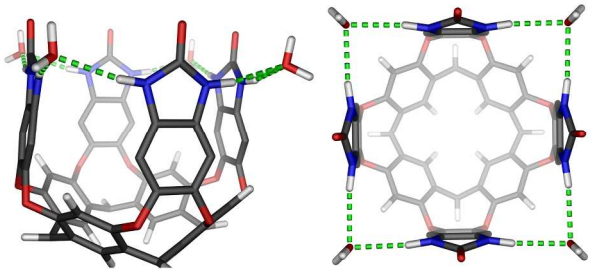


Figure 4. A semi-empirical (AM1) minimized structure of the water-stabilized cavitand: side view (left) & top view (right).

The overall vase conformation of **1a** is doubtless stabilized by water molecules which bridge adjacent benzimidazolones through hydrogen bonds, and one or more water molecules at each corner could be involved. Figure 4 shows a structure for the minimal arrangement, but additional waters in the bridges could effect the cavitand's volume, shape and guest exchange rates.

On exposure to octanoic acid in D_2O, the new cavitand formed a 1:1 complex (Figure. 5a). The upfield shifts In the NMR spectrum of the guest indicate that its alkyl chain is entirely in the hydrophobic cavity. The lipopeptide ghrelin,[10] (Figure 5) a 28 amino acid peptide which bears an octanoyl ester on serine 3, gave a similar spectrum in the cavitand (Figure 5c). The sharp signals for the included heptyl group speak for slower in/out exchange rates. Apparently, the complex can be stabilized by other contacts between guest and the rim of the cavitand host. However, no binding to the neighboring phenylalanine was observed. The shape of the octanoyl groups in the cavitand were explored with O-octanoyl serine (See S.I.), a compound showing identical chemical shifts as those of other included octanoyl groups. The 2D NMR spectrum revealed a *gauche* conformation about the C_6-C_7 bond but extended conformations elsewhere along the chain. Longer alkyl chains in related cavitands show exaggerated upfield shifts and helically coiled conformations.[3g]

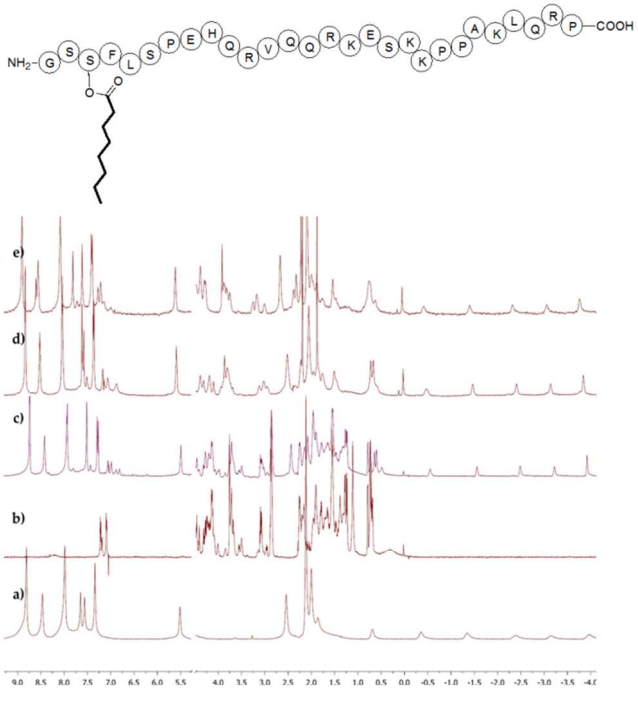


Figure 5. Top) Sequence of ghrelin, an appetite stimulant. Bottom) 1H NMR spectra (600MHz, D2O, 298K) of **1a** (0.5 mM) with a) octanoic acid (0.50 mM); c) ghrelin 1-28 (0.50 mM); d) ghrelin 1-10 (0.50 mM); e) ghrelin 1-10 (0.50 mM) at pH=2 and 37°C; Ghrelin 1-28 alone (0.50 mM) is shown in trace b).

The binding constant of **1** to ghrelin or truncated rat-ghrelin (residues 1-10) was too large to determine by NMR methods, but ITC titrations (see S.I.) with truncated rat-ghrelin gave good agreement for a 1:1 stoichiometry with K_a = 2.5 (± .29) x 10^5 M^{-1}.

Complex formation is both enthalpically and entropically favored in accordance with nonclassical hydrophobic binding.[11] Ghrelin is expressed primarily in the stomach and has been shown to decrease insulin levels and increase appetite, body weight and fat accumulation. No changes in the NMR spectra of the ghrelin complexes were observed when the pH was lowered to 2 or when the temperature was raised to 37° C (see SI).

Among other guests (see S.I.) ibuprofen was bound in **1a**. The spectrum (Figure 6) is consistent with the carceroisomer[12] shown, with the carboxyl exposed to the solvent. Integration indicates a 1:1 complex and ibuprofen is too long for a capsular (2:2) complex.[13] The antiparasitic albendazole[14] was also bound in the cavitand (see S.I.). Unlike organic media, where guests compete with solvent molecules for the container's space, water is reluctant to enter the cavity of **1**. Accordingly, its 55 M concentration is not an advantage when hydrocarbon guests are present.

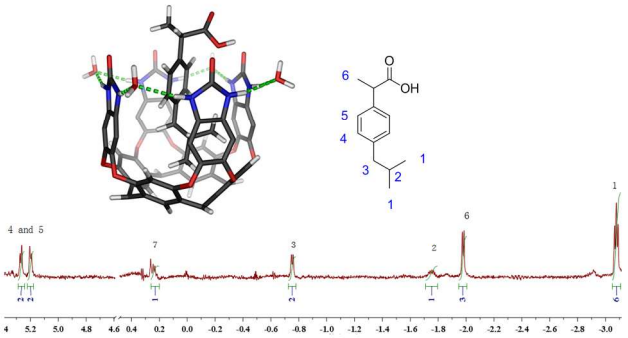


Figure 6. Partial ^{1}H NMR spectrum (600MHz, D_2O, 298K) of the major complex of ibuprofen with **1a**. A semi-empirical (AM1) minimized structure of the complex is shown; the positions of the 4 bridging water molecules are assumed.

Conclusions

Cavitands provide small spaces that reveal molecular behavior that is inaccessible in bulk solution. Coiled conformations, reactive intermediates and chemical catalysis have all been seen in deep cavitands derived from resorcinarenes.[3-5] The spaces can be deepened by the addition of aromatic walls, a modification that inevitably decreases their solubility in aqueous systems. The water-soluble cavitand described here now provides a vehicle for small molecule payloads in biocompatible settings.

Notes and references

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For the octane case, one methyl is deep in the resorcinarene (Δδ = -4.6ppm) and the other end is near the rim of the cavitand in an environment where Δδ = -0.4ppm. The observed methyl signal at -1.7ppm (Δδ = -2.5ppm) is the average: (-4.6 and -0.4)/2.

† Electronic Supplementary Information (ESI) available: [Details of NMR spctra and ITC experiments]. See DOI: 10.1039/b000000x/

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