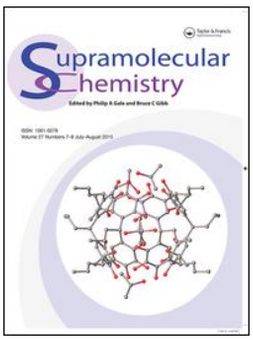

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ARTICLE

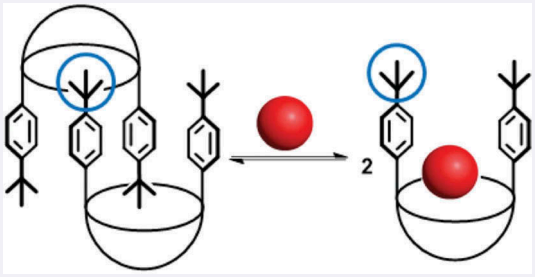
A clip-like host that undergoes self-assembly and competitive guest-induced disassembly in water

Meagan A. Beatty, Jil A. Busmann, Noah G. Fagen, Graham A. E. Garnett and Fraser Hof

Department of Chemistry, University of Victoria, Victoria, Canada

ABSTRACT

Certain calix[4]arenes that are anionic and appended with a single hydrophobic substituent can self-assemble into homodimers in water. The unusual behaviours of these assemblies in water solutions are largely attributed to them being formed from like-charged building blocks. We report here a new entry into this series – a difunctionalized analog with two hydrophobic arms, a net – 3 charge on each monomer, and an overall U-shaped 'clip' topology. We use ^{1}H NMR, 1-D DOSY and ITC to show that the new compound dimerizes in water but remains monomeric in organic solvents. Various cationic ammonium ion guests are able to drive dimer disassembly in favor of 1:1 host-guest complexes. The extent of competition is proportional to overall guest hydrophobicity.



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Calixarene; host-guest chemistry; hydrophobic effect; self-assembly

Introduction

Molecular clips and tweezers are important motifs for molecular recognition in water. The combination of a rigid, U-shaped hydrophobic surface with peripheral solubilizing groups creates a poorly hydrated concave surface that is predisposed to strong binding of hydrophobic partners in water. In the most prominent examples, a U-turn topology has been achieved using glycoluril-derived structures, as pioneered by Issacs (1–3), or norbornene-derived structures as introduced by Klärner and Schrader (4, 5). Some of these examples have involved self-complementary clips that form homo- or heterodimers (6–8), while other clips are shaped to minimize self-assembly so they can bind guests within their hydrophobic clefts (1, 9, 10). In some interesting examples, these two binding modes compete with each other – individual clips form homodimers that can be disrupted in favor of guest binding in pure, unbuffered water (11, 12).

We previously reported on a series of mono-functionalized calix[4]arenes that assemble into 1:1 homodimers in water. These molecules have the self-complementary topology of a yin-yang symbol – the hydrophobic pendant arm of each monomer binds as a guest into the other monomer's binding pocket (1, Figure 1(a)). Unlike most self-assembling species, the monomers in this family bear an overall –4 charge in neutral water that is mutually repulsive. The assemblies' responses to added co-solutes is largely explained by considering their unique combination of like charges, hydrophobic binding elements, and complementary shapes (13). The dimers remain assembled in salty water, mock serum and human urine. The strongest of the series used a hydrophobic *t*-butylphenyl group as the pendant arm.

We converted this motif into a new structure that has both clip-like character and symmetry. Adding a second *t*-butylphenyl group to the upper rim of **1** produces a monomer with a net charge of – 3 at neutral

CONTACT Fraser Hof fhof@uvic.ca Department of Chemistry, University of Victoria, Victoria, Canada
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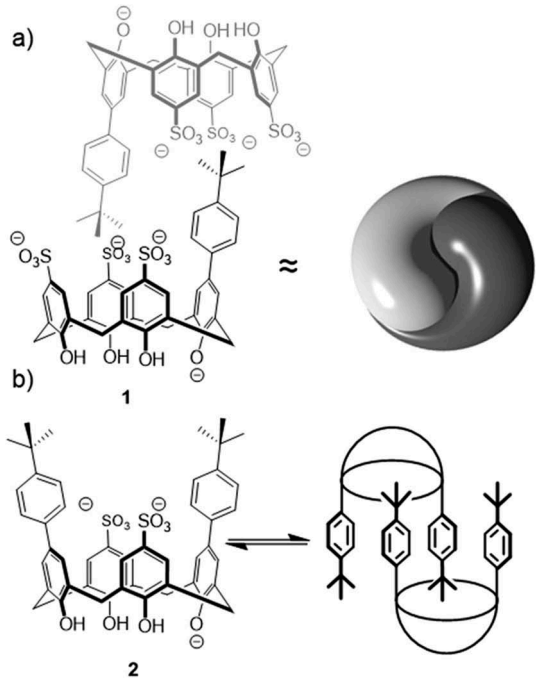


Figure 1. (a) Previously reported dimeric monofunctionalized calix[4]arene, **1**, and (b) the new difunctionalized clip-like calix[4]arene, **2**.

pH, two similar arms, overall C_{2v} symmetry, and an increased hydrophobic surface area in the form of an extended cleft (**2**, Figure 1(b)). Unlike prior water-soluble clips and tweezers, the clip incorporates a sulfonated calix[4]arene binding pocket of a type that is generally useful for binding biological guests (14–18). We report here our studies of the new molecule's homodimerization in water. We expect that molecules like this should be promiscuous binders of hydrophobic ammonium ions, and we were unsure of how homodimerization would compete with guest binding. Unlike our previous studies of **1**, in the current work we report the response of this homodimer to a variety of guests in competitive buffered water solutions.

Materials and methods

General methods

^{1}H, ^{13}C, 1-D DOSY and variable temperature experiments were recorded on a Bruker Avance 500 MHz spectrometer unless otherwise indicated and processed with MestReNova by Mestrelab Research S.L. 1-D DOSY protocol is outlined in Supplementary Information (Section 2c). All reported chemical shifts were reported in ppm with respect to an internal standard: cis-butenedioic acid at 6.2 ppm. Deuterated solvents were purchased from Sigma Aldrich and NaH_2PO_4/Na_2HPO_4 (50 mM, pD 8.5) in D_2O were prepared in lab and the pD was adjusted with 5% NaOD/DCl solutions. Isothermal calorimetery experiments were conducted at 303 K in buffered water on a MicroCal VP-ITC and fitted to include heat of dilution outlined in Supplementary Information (Section 2a). Mass spectra of novel compounds were collected on an Thermo Scientific Ultimate 3000 ESI-Orbitrap Exactive. All UV-Vis and fluorescence spectra were collected on a Molecular Device Spectra M5 spectrometer in NUNC 96-well black walled plate. Infrared (IR) spectra were obtained using a Perkin Elmer 1000 FT-IR spectrometer. Data are represented as follows: frequency of absorption (cm^{-1}), intensity of absorption (s = strong, m = medium, w = weak, br = broad). Melting points were collected on a Gallenkamp Melting Point apparatus. Compound **2** was purified using a Shimadzu Prominence HPLC system on a 9.4 mm × 250 mm semi-preparative Agilent Eclipse XDB-C18 5 µm with UV detection at 280 nm.

Synthesis

5,17-dibromo-26,28-dibenzoyloxy-calix[4]arene (4).

Compound **3** (0.9 g, 1.5 mmol) was dissolved in $CHCl_3$ (30 mL) and Br_2 (0.4 mL, 15 mmol) was added. The mixture was stirred at room temperature for 4 h. The mixture was then diluted with CH_2Cl_2, the organic layer was washed with $Na_2S_2O_3$ (5 g/100 mL) aqueous solution, followed by water, brine and dried over Na_2SO_4. The solution was decanted, reduced under pressure to a pure yellow solid (0.99 g, 83%). Mp: decomposed > 230 °C. FT-IR (cm^{-1}): 3537 (br.), 1733 (m), 1706 (m), 1451 (m), 1265 (s), 1173 (s), 1023 (m), 1056 (m), 861 (w), 754 (m), 709 (s). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.24 (dd, J = 8.5 Hz, 1.5 Hz, 4H), 7.77 (tt, J = 7.4 Hz 1.4 Hz, 2H), 7.62 (t, 7.9 Hz, 4H), 7.11 (s, 4H), (m, 6H), 5.22 (s, 2H), 3.85 (d, J = 14.6 Hz, 4H), 3.53 (d, J = 14.6 Hz, 4H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.6, 152.0, 146.1, 134.0, 132.0, 131.7, 130.5, 129.8, 129.7, 129.3, 126.8, 111.8, 33.1. HR-ESI-MS ($[M–H]^-$, m/z): Calculated for $C_{42}H_{29}Br_2O_6^-$ 787.03364, Found 787.03363

5,17-dibromo-25,26,27,28-tetrahydroxy-calix[4]arene (5).

Compound **4** (1 g, 1.25 mmol) was dissolved in MeOH (50 mL) and NaOH (0.7 g, 18 mmol) was added. The mixture was heated to reflux for 4 h. The product was precipitated out of solution by the addition of 1 M HCl. The solid was vacuum filtered and washed with hexanes to afford a light yellow solid (0.53 g, 72%). Mp: decomposed > 230 °C. FT-IR (cm^{-1}): 3141 (br), 1448 (s), 1209 (s), 856 (w), 828 (w), 750 (s), 665 (m) 514 (m). ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.06 (s, 4H), 7.17 (s, 4H), 7.08 (d, J = 7 Hz, 4H), 6.80 (t, J = 6.8 Hz, 2H), 4.20 (br. s, 4H),

3.52 (br. s., 4H). ^{13}C (125 MHz, $CDCl_3$): δ 148.8, 147.9, 131.6, 130.2, 129.3, 127.5, 122.52, 114.0, 31.5. HR-ESI-MS ($[M-H]^-$, *m/z*): Calculated for $C_{28}H_{21}Br_2O_4^-$ 578.98121, Found 578.98106.

5,17-dibromo-25,26,27,28-tetrahydroxy-11,23-disulfonatocalix[4]arene (6).

Compound **5** (50 mg, 86 µmol) was dissolved in CH_2Cl_2 (2 mL) followed with conc. H_2SO_4 (100 µL). The reaction was heated to reflux for 24 h to afford a residue. The CH_2Cl_2 was decanted, and the residue was rinsed with fresh CH_2Cl_2. The solid was suspended in EtOAc, transferred into a conical tube and diluted with cold Et_2O. The suspension was centrifuged to a pellet, the supernatant was decanted and the resuspension/centrifugation/decanting process was repeated three times. The pellet was left to air dry overnight to afford a gray solid (51 mg, 81%). Mp: decomposed > 178 °C. IR (KBr disc) (cm^{-1}): 2408 (br), 3221 (br), 2961 (w), 1651 (m), 1204 (m), 1163 (m), 1036 (m), 625 (m). ^{1}H NMR (500 MHz, d_6-DMSO): δ 9.62 (br. s, 7H), 7.43 (s, 4H), 7.28 (s, 4H), 3.89 (br. s, 8H). ^{13}C NMR (125 MHz, d_6-DMSO): δ 151.6, 149.8, 140.0, 131.5, 131.3, 127.6, 127.0, 112.3, 41.0, 30.6. HR-ESI-MS ($[M-2H]^{2-}$, *m/z*): Calculated for $C_{28}H_{20}Br_2O_{10}S_2^{2-}$ 368.94378, Found 368.94376.

5,17-di(4-t-butylphenyl)-25,26,27,28-tetrahydroxy-11,23-disulfonatocalix[4]arene (2).

Compound **6** (50 mg, 67 µmol), *t*-butyl-phenylboronic acid (26 mg, 148 µmol), K_2CO_3 (74 mg, 0.51 mmol) and $Pd(OAc)_2$ (5 mg, 22 µmol) were dissolved in a microwave vial with 3 mL of 1:1 EtOH:deionized water. The reaction was irradiated to a temperature of 150 °C for 5 minutes with cooling air and stirring on (Biotage Initiator Microwave Reactor). After, thiourea (1 M, 0.5 mL) was added and the reaction stirred at 90°C for 1 h. The solution was filtered through a PDVF syringe filer (0.45 µm), and concentrated until solution became cloudy. The slurry was re-dissolved with small amounts of CH_3CN and purified by HPLC purification with a gradient running from 90% H_2O (+ 0.1% TFA)/10% CH_3CN (+ 0.1% TFA) to 40% H_2O (+ 0.1% TFA)/60% CH_3CN (+ 0.1% TFA) over 18 minutes. Lyophilization of collected fractions afforded a white powder in 40% yield (22 mg). Mp: decomposed > 190 °C. FT-IR (cm^{-1}): 3398 (br), 2962 (m), 2874 (w), 1456 (m), 1268 (m), 1153 (m), 1038 (s), 822/32 (m), 750 (s), 629 (s), 542 (s). ^{1}H NMR (500 MHz, CD_3OD): δ 7.69 (s, 4H), 7.44 (s, *J* = 7.4 Hz, 4H), 7.41 (s, 4H), 7.39 (d, *J* = 7.4 Hz, 4H), 4.07 (br. s, 8H), 1.31 (s, 18H). ^{13}C NMR (125 MHz, d_6-DMSO): δ 151.0, 149.52, 149.50, 140.2, 137.5, 133.7, 129.2, 128.3, 127.5, 126.9, 126.4, 125.9, 34.6, 31.6, 31.1. HR-MS ($[M-2H]^{2-}$, *m/z*): Calculated for $C_{48}H_{46}O_{10}S_2^{2-}$423.12717, Found 423.12712.

Results and discussion

The synthesis of **2** is achieved through selective upper-rim functionalization reactions. It starts with the selective dibromination of 1,3-dibenzoyl calix[4]arene **(3)** (19) which occurs selectively at the two positions *para* to unprotected phenols to give **4**. The benzoyl groups are removed **(5)** and the newly exposed phenols are *para* sulfonated along the upper rim upon treatment with H_2SO_4 to yield the key precursor **6**. The final compound, **2**, is obtained by a double Suzuki coupling with *t*-butylphenyl boronic acid and obtained in 40% yield after HPLC purification. Compound **2** is most soluble in slightly basic water, at which it is expected to have a net charge of – 3 due to the low pKa for the first phenol deprotonation in calix[4]arenes (20).

Dimerization of **2** was apparent when comparing the ^{1}H NMR spectra in CD_3OD and D_2O. In CD_3OD, the pendant phenyl and *t*-butyl resonances were found as sharp peaks at 7.45 ppm, 7.40 ppm and 1.32 ppm, as expected for an unaggregated monomeric state. In D_2O both the phenyl and *t*-butyl resonances broadened and shifted upfield to 7.25 ppm, 7.0 ppm and 0.33 ppm, respectively (Figure S2). This pattern of upfield shifts is diagnostic for encapsulation of a *t*-butylphenyl substituent within a calix[4]arene's electron-rich pocket (13). The fact that this is not observed in pure CD_3OD (or even upon addition of small amounts of CD_3OD) indicates that the dimerization is primarily driven by the hydrophobic effect.

The existence of dimer in solution was confirmed by DOSY. The diffusion coefficient of monomeric **2** was obtained by 1-D DOSY in 20% CD_3OD in Na_2HPO_4/NaH_2PO_4 (50 mM, pD 8.5) buffer – conditions under which 1D chemical shifts show that pure monomer is present. The Stokes-Einstein equation was used to determine the monomer's hydrodynamic radius as 8.51 Å (Table 1). This value is similar to the value determined in the same CD_3OD/buffer conditions for

Table 1. Diffusion coefficients obtained by 1-D DOSY and corresponding hydrodynamic radii.

	D (m^2/s)	r_H (Å)
PSC[(a)] (monomer control)	2.2×10^{-10}	7.3
2[(a)] (monomer)	1.9×10^{-10}	8.51 ± 0.03
2[(b)] (dimer)	2.2×10^{-10}	11.31 ± 0.04
1[(c)] (dimer)	1.5×10^{-10}	11.3

(a) 20% CD_3OD in Na_2HPO_4/NaH_2PO_4 (50 mM, pD 8.5) buffer, [PSC] or [**2**] = 1 mM.
(b) Na_2HPO_4/NaH_2PO_4 (50 mM, pD 8.5) buffer
(c)previously reported data (13), in D_2O.

Table 2. ITC-derived thermodynamic parameters for homodimerization of **1** and **2**[(a)].

	K_d (mM)	ΔG (kcal/mol)	ΔH (kcal/mol)	−TΔS (kcal/mol)
1[(b)]	1.0	−4.2	−11	6.9
2	0.57	−4.5	5.5	−10

(a) ITC dilution titrations in H_2O containing Na_2HPO_4/NaH_2PO_4 (100 mM, pH 7.4.)
(b) Data taken from reference (13).

the control compound *para*-sulfonatocalix[4]arene (PSC), which can't undergo dimerization. In 100% aqueous Na_2HPO_4/NaH_2PO_4 (50 mM, pD 8.5) solution the hydrodynamic radius of the dimer was found to be 11.31 Å consistent with the value observed for its thoroughly characterized dimeric analog **1** (Table 1) (13).

ITC dilution titrations show that compound **2** forms stronger dimers than **1**, with different enthalpic and entropic contributions. The K_d decreased from 1.0 mM **(1)** to 0.54 mM **(2)** with the addition of a second *t*-butylphenyl group (Table 2). In spite of the relatively small structural change, the association of **1** was enthalpically favoured while **2** was entropically driven. NMR data suggest dimers of similar structures, so we attribute the differences mainly to the swap of a sulfonate for a second *t*-butylphenyl group. Additional hydrophobic surface area must be de-solvated upon dimerization of **2**. The increase in entropic driving force follows the classical view of the hydrophobic effect, which is appropriate considering that the new appended hydrophobic group is not in a confined space and is largely exposed to solvent in the free state.

We were interested in testing **2** as a host with select guests known to form strong inclusion complexes with calix[4]arenes (Figure 2(a)). As this was not previously tested with **1** and others in its series, we were interested seeing if hydrophobic cationic guests could perturb the dimer and form traditional 1:1 host-guest complexes (Figure 2(b)).

^{1}H NMR proved the formation of host-guest complexes with concomitant dimer dissociation upon guest addition. All resonances for these simple guest binding studies were in fast exchange. Under these conditions, the position of the chemical shift between the extremes expected for free and bound states qualitatively indicate the relative amounts of each state (Table 3). The diagnostic position and shape of the *t*-butyl singlet was used to track monomer-dimer equilibrium: a strong hydrophobic guest like *N*-ethyl-4-methyl-pyridinium caused the *t*-butyl singlet of **2** to sharpen and shift downfield (blue diamonds, Δδ + 0.76 ppm) to the position expected for monomeric **2** (Figure 3). The guest's CH_3 triplet broadened and shifted upfield (red circle, Δδ–0.25 ppm). This indicated that while the dimer

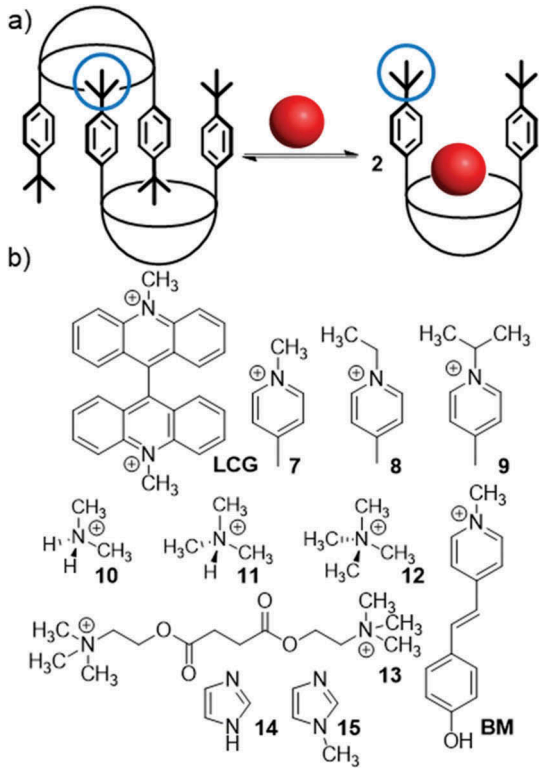


Figure 2. (a) cartoon depiction of dimeric **2** dissociating to form new host-guest complex which can be observed by the change in chemical shift of the *t*-butyl singlet (blue circle) with (b) various guests.

dissociated, the guest was encapsulated to form a host-guest complex of the type that is well known for sulfonated calix[4]arenes (21, 22). Similar observations were made with other *N*-alkylated pyridinium guests (Figure S4, S5 and Table 3). Complete NMR titrations showing the evolving response upon addition of selected guests are shown in the Supp. Info.

Comparison of simple methylammonium ion guests showed that more hydrophobic guests are more

Table 3. Guest-induced chemical shift perturbation of resonances for **2** away from the positions observed in the pure **2•2** homodimer.

	Δδ of resonances from **2** (ppm)[(a)]	
Guest	*t*-butyl	*ortho*-aryl
7	0.71	0.18
8	0.79	0.24
9	0.80	0.26
10	0.01	0.01
11	0.39	0.24
12	0.67	0.40
13[(b)]	0.83	0.48
14	0.03	0.06
15	0.15	0.06

(a) All solutions are 1:1 mixtures of **2** and guest at 1 mM in Na_2HPO_4/NaH_2PO_4 (50 mM, pD 8.5)-buffered D_2O. See Supporting Information for full titrations and spectra for selected guests.
(b) This ditopic guest was studied at a ratio of 0.5:1 guest:**2** ([**2**] = 1 mM).

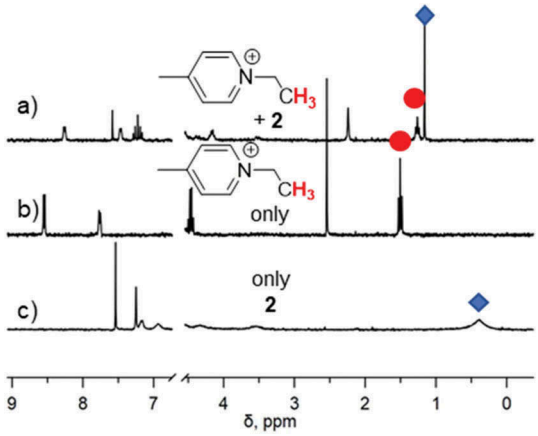


Figure 3. NMR spectra demonstrate competition between **2•2** homodimerization and host-guest binding. (a) 1:1 (1 mM) host-guest complex formed with *N*-ethyl-4-methyl-pyridinium (**8**) indicated with an upfield shift of guest protons (red dot) from its (b) unbound resonance and the downfield shift of **2** (blue diamond) (c) from its dimeric state. Buffer = Na_2HPO_4/NaH_2PO_4 (50 mM, pD 8.5) in D_2O.

effective at disrupting dimerization. The *t*-butyl singlet of **2** travels from 0.4 ppm (Figure 4(c)) to 0.79 ppm (4b) and to finally 1.06 ppm (4a) when treated with similar concentrations of di-, tri- and tetramethylammonium, respectively (blue diamonds). Similarly, the guest methyl resonances show upfield shifts from their free chemical shifts that are proportional to the expected strength of guest binding: 2.39 ppm (dimethylammonium), 1.88 ppm (trimethylammonium), and 1.49 ppm (tetramethylammonium). Weaker guests like imidazole did not perturb the dimer at all (Figure 5(c), S6 and Table 3). Yet its methylated counterpart, *N*-CH_3-

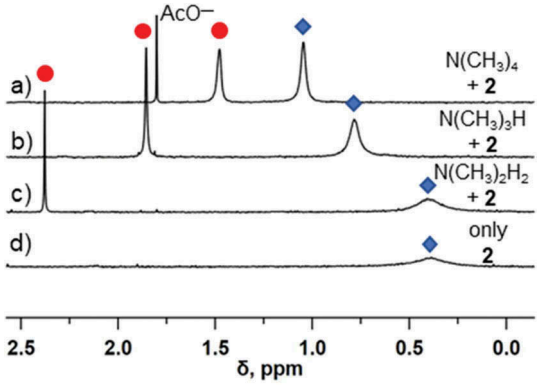


Figure 4. NMR spectra show that more hydrophobic guests disrupt **2•2** homodimer more effectively. Each spectrum contains a 1:1 (1 mM) mixture of **2** and guest. The downfield shift of **2** (blue diamonds) upon dimer disruption, and the upfield shift (red dot) of guests upon host-guest complex formation are apparent. (a) $N(CH_3)_4^+$ (**12**); (b) $HN(CH_3)_3^+$ (**11**); (c) $H_2N(CH_3)_2^+$ (**10**); (d) no guest added. Buffer = Na_2HPO_4/NaH_2PO_4 (50 mM, pD 8.5) in D_2O.

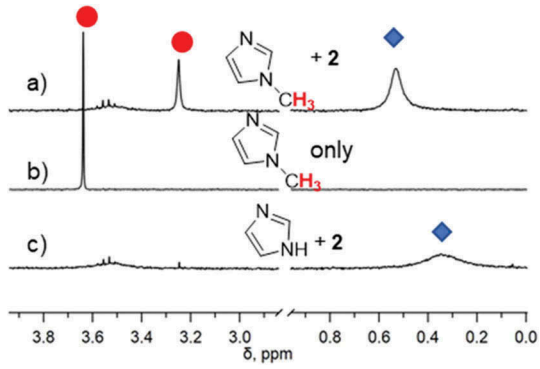


Figure 5. NMR spectra show that more hydrophobic guests disrupt **2•2** homodimer more effectively. Host and guests in each sample are as shown; each is at 1 mM. The downfield shift of **2** (blue diamonds) upon dimer disruption, and the upfield shift (red dot) of guests upon host-guest complex formation are apparent. *N*-methyl imidazole (**13**) disrupts dimer formation but imidazole (**14**) does not. Buffer = Na_2HPO_4/NaH_2PO_4 (50 mM, pD 8.5) in D_2O.

imidazole, partially formed a complex, as supported by the methyl singlet shifting Δδ– 0.37 ppm and the *t*-butyl shifting Δδ + 0.2 ppm (Figure 5(a) and Table 3). A bis-(trimethylammonium) guest, suxamethonium (**9**), also induced dissociation of **2** by Δδ 0.83 and the guest protons upfield shifted and broadened by Δδ– 1.44 ppm at 0.4 eq. (Figure S7 and Table 3).

Lucigenin (LCG), a quinaldinium dye, showed unusual host-guest behaviour with **2** and allowed fluorescence characterization of the complexes. LCG is quenched upon binding sulfonated calixarenes (23). A 1:1 mixture of LCG and **2**, each at 1 mM, yielded a perfectly flat ^{1}H NMR spectrum– all host and guest resonances are in intermediate exchange, and the solution is clear and homogeneous, suggesting the formation of an undefined soluble aggregate (Figure 6(b)). The LCG emission is mostly quenched in a 1:1 solution prepared at lower concentrations that are appropriate for fluorescence measurements ([LCG] = [**2**] = 1 μM), as ≤ 10% of the intensity of free LCG emission is observed in this sample. Upon changing the stoichiometry only ≥ 20% away from 1:1 ratio in either direction, resonances for whichever species is in excess start to appear alongside an otherwise flat NMR spectrum. This indicates that the soluble aggregate, although not structurally understood, is something that requires a 1:1 ratio of **2** and LCG. When **2** is in excess the resonances for homodimeric **2•2** are observed in the NMR spectrum (Figure 6(a)). Fluorescence is completely quenched under these conditions, showing that the LCG is fully engaged in an aggregate with **2** (Figure 6(a)). Conversely, when LCG is in excess, the NMR resonances for the uncomplexed dye are observed emerging from the flat NMR spectrum that arises from the **2**•LCG

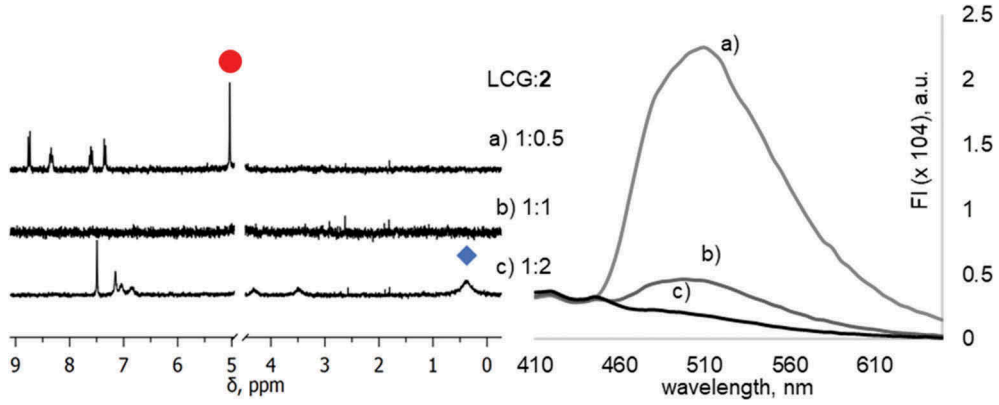
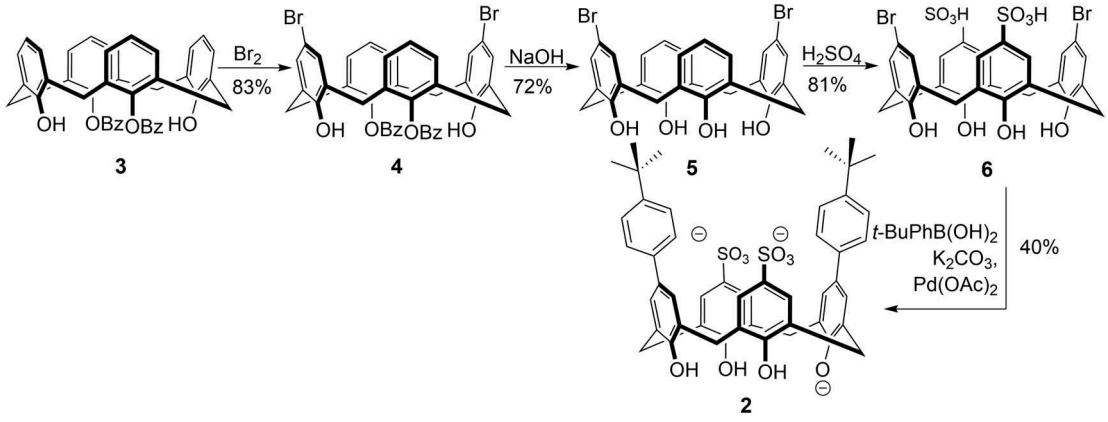


Figure 6. Aggregation behaviour of LCG and **2** monitored by ^{1}H NMR (left, [LCG] = 1 mM) and fluorescence spectroscopy (right, [LCG] = 1 μM). (a) At LCG:**2** ratio of 1:0.5 – free LCG resonances observed in the NMR, with significant fluorescence emission observed to arise from free LCG. (b) At LCG:**2** ratio of 1:1, no NMR resonances are observed which indicates a soluble aggregate undergoing intermediate exchange with complete line broadening. Low fluorescence emission is observed, showing that most LCG is bound to calixarene under these conditions. (c) At LCG:**2** ratio of 1:2, homodimer **2•2** is observed by NMR and no free LCG emission is seen by fluorescence spectroscopy. Buffer = Na_2HPO_4/NaH_2PO_4 (10 mM, pD 8.5) in D_2O (for NMR) or H_2O (for fluorescence).



Scheme 1. The synthesis of the calix[4]arene clip, **2**.

aggregate. Partial (30%) fluorescence intensity is observed, as expected for a solution containing a small amount of free LCG. Upon heating the solution we observed at 80°C by NMR the appearance of resonances of monomeric **2** and free LCG, as both the soluble aggregate and the homodimer dissociate (Figure S10). Data suggesting this sort of hetero-aggregate behaviour were also observed for mixtures of **2** and Brooker's merocyanine– another cationic pyridinium dye (Figure S3 and S9).

In this report, we have synthesized a new calix[4]arene clip, **2**, characterized its homodimeric self-assembly in buffered water, and studied the effects of competing guests. Multiple research groups have used trisulfonated calixarenes with a single upper-rim functionalization to modulate guest binding, and/or to impart many different functionalities to the host structure (15, 17, 24–28). The synthesis of difunctionalized **2** involves a Suzuki coupling that could be applied to several other binding arms in order to introduce a similarly diverse set of functions (29). This work opens the door to a wide variety of clip-like hosts that combine self-assembly and guest responsiveness in competitive aqueous solutions.

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Disclosure statement

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