

Electronic Supplementary Information

Selective recognition of the di/trimethylammonium motif by an artificial carboxycalixarene receptor

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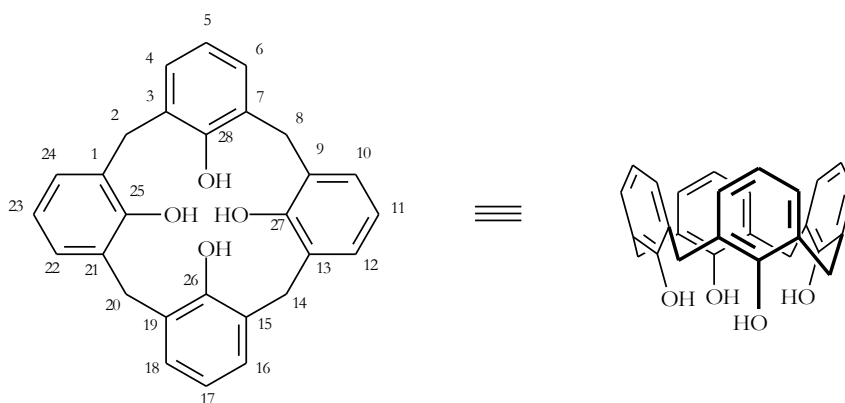
Experimental

Synthesis of 1 and 2

General Considerations:

Melting points have been determined on a microscope heating stage PHMK Rapido (VEB Dresden Analytik) and are uncorrected. ^1H and ^{13}C NMR spectra were measured with a Bruker Avance 500 at 500.1 (^1H) and 125.8 MHz (^{13}C) using TMS as internal standard. The chemical shifts for proton and carbon resonances are given in ppm (δ). Signal multiplicity is characterized by s (singlet), d (doublet), dd (double doublet) and t (triplet). Mass spectra were recorded on a Hewlett Packard 5890 Series II/MS 5971 A. Elemental analyses have been performed on a Heraeus CHN-Rapid Analyzer. IR spectra were measured on a Nicolet ATR-FT-IR 510 as KBr pellets and with the ATR method. Analytical TLC was performed on precoated silica gel plates (60 F₂₅₄, Merck). The reagents and solvents were used as purchased from the chemical suppliers.

We applied the following number system for the calixarenes:



5-Formyl-25,26,27,28-tetrahydroxycalix[4]arene (**2**) has been synthesized by treating the underivatized calixarene with 1,1-dichlorodimethylether and tin tetrachloride (Gross formylation) as described in the literature.¹

5-Carboxy-25,26,27,28-tetrahydroxycalix[4]arene (**1**)

260 mg (0.575 mmol) of the formylated calixarene **2** were dissolved in a mixture of 15 ml of chloroform and 15 ml of acetone. To the resulting solution 0.18 g (1.84 mmol) sulfamic acid and 0.14 g (1.61 mmol) sodium chlorite dissolved in 4.2 ml water were added. After stirring for 3 h the solvents have been removed and the remaining residue was suspended in 2M hydrochloric acid (25 ml). The insoluble solid is collected via filtration and dried carefully. Yield: 262 mg (97 %) of a orange powder. Mp. >310 °C (decomp.). ¹H-NMR: (DMSO-d₆) δ = 3.88 (br s, 8H, Ar-CH₂-Ar); 6.64 (m, 3H, Ar-H); 7.11 (m, 7H, Ar-H); 7.70 (s, 2H, Ar-H); 9.81 (br s, 4H, OH). ¹³C-NMR: (DMSO-d₆) δ = 31.0, 31.1 (Ar-CH₂-Ar); 121.5, 121.7 (11-, 17-, 23-ArC); 128.7, 129.0, 129.1, 129.2, 129.2 (1-, 4-, 6-, 9-, 10-, 12-, 13-, 15-, 16-, 18-, 19-, 21-, 22-, 24-ArC); 130.7 (3-, 7-ArC); 149.8, 150.2 (25-, 26-, 27-ArC); 155.6 (28-ArC); 167.4 (COOH). IR: (KBr disc) 3239 ν(O-H); 3094 ν(C-H [phenyl]); 2944, 2877 ν(C-H) [alkyl]; 1688 ν(C=O); 1637, 1605 ν(C=C); 1465, 1451; 1259; 1243; 1206; 753. MS (ESI) calc. [M] = 468.3, found m/e = 467.3 [M-H⁺]. Anal. Calcd for C₂₉H₂₄O₆ · ½ H₂O: C, 72.94; H, 5.28 %. Found: C, 72.86 %; H, 5.22 %.

Peptide Synthesis: Histone fragment peptides corresponding to residues 30-41 of histone H3, and containing either *N*^ε-dimethyl-L-lysine or *N*^ε-monomethyl-L-lysine at residue 36 (sequence = PATGGV-K(Me_{1/2})-KPHRY), were synthesised using standard Fmoc-mediated solid-phase

¹ C. N. Baki and E. U. Akkaya, *J. Org. Chem.*, 2001, **66**, 1512.

peptide synthesis using a Liberty Blue Microwave Peptide Synthesiser (CEM, USA). The peptides were purified to >95 % purity (as assessed by ^1H NMR) via high-performance liquid chromatography using a Vydac C18 column. N^α -Fmoc- N^ϵ -dimethyl-L-lysine and N^α -Fmoc- N^ϵ -monomethyl-L-lysine were purchased from GL Biochem (China). All other reagents were purchased from either Merck, Sigma-Aldrich or AGTC Bioproducts.

Histone fragment peptides corresponding to residues 30-41 of histone H3 containing N^ϵ -trimethyl-L-lysine at residue 36 (sequence = PATGGV-K(Me $_{1/2}$)-KPHRY) and residues 7-14 of histone H3 containing either N^ϵ -trimethyl-L-lysine, N^ϵ -dimethyl-L-lysine or N^ϵ -methyl-L-lysine at position 9 were synthesised using standard Fmoc-mediated solid-phase peptide synthesis using a PS3 Peptide Synthesiser (Protein Technologies, Inc, USA). The peptides were purified to >95 % purity (as assessed by analytical RP-HPLC) via reversed-phase high-performance liquid chromatography using a Phenomenex Luna 5 micron C18 100 Å column. N^α -Fmoc- N^ϵ -dimethyl-L-lysine and N^α -Fmoc- N^ϵ -monomethyl-L-lysine were purchased from AK Scientific (USA).

NMR experiments: All NMR experiments were conducted with a Bruker Avance III HD 500 MHz instrument equipped with a BBFO probe. Standard 5 mm tube (Wilmad) were used. Unless otherwise stated, all experiments were conducted at 300 K. Samples were buffered with 50 mM sodium phosphate buffer pH 7.5 in 90% H $_2$ O and 10% D $_2$ O. Standard ^1H experiments were used. Water suppression was achieved using the excitation sculpting method. 6 μM DSS was added to all samples as chemical shift reference.

Single concentration screening: Experiments were conducted with 500 μM small molecule and 250 μM carboxycalixarene **1**. Stock solution of carboxycalixarene **1** was dissolved in DMSO- d_6 . Control experiments with 500 μM small molecule and DMSO- d_6 was also

conducted to ensure any changes in chemical shifts are due entirely to binding of the small molecule to the calixarene.

Titration experiment: Experiments were conducted with 100 μM small molecule (or 50 μM peptide) in the presence of varying concentrations of carboxycalixarene **1**. Control experiments titrating DMSO- d_6 was also conducted. The corrected chemical shift changes were then fitted by SigmaPlot 12.5 with the following equation to obtain binding constants:

$$\Delta\delta = \Delta\delta_{\text{max}} \cdot \frac{[\text{carboxycalixarene}]}{K_D + [\text{carboxycalixarene}]}$$

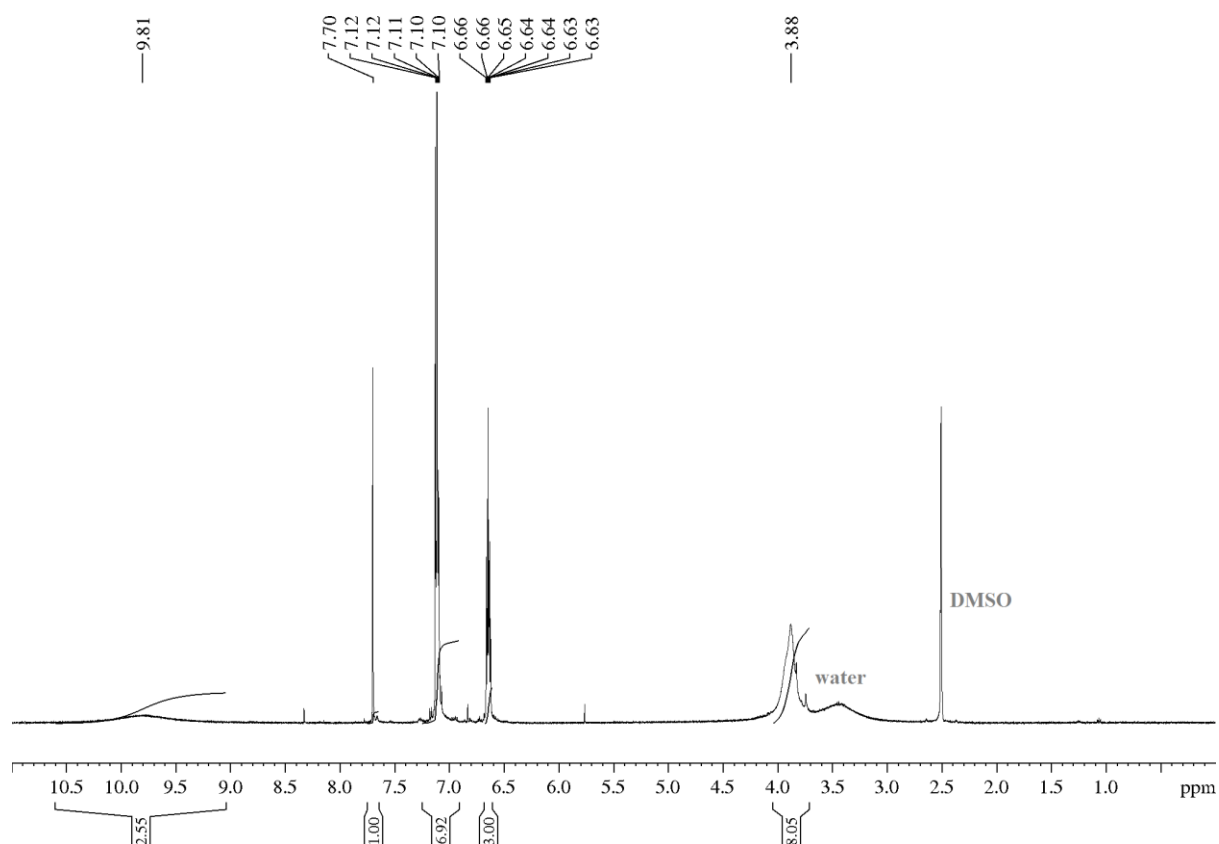
The equation is based on a 1:1 binding isotherm as described by Thordarson,² in which $\Delta\delta$ is the observed chemical shift change and [carboxycalixarene] is the titrated concentration of **1**. Non-linear curve fitting was done with 1000 iterations with no pre-defined constraints for K_D or $\Delta\delta_{\text{max}}$.

Cell lysate experiment: An overnight culture of *Escherichia coli* BL21(DE3) was incubated at 37 °C. The cells were harvested by centrifugation, freeze-thawed and re-suspended in 50 mM sodium phosphate buffer (pH 7.5) at a concentration of 200 mg/mL. Cells were lysed using sonication, and cell lysate was recovered by centrifugation to remove cell debris. After filtration, the cell lysate was supplemented with 450 μM of dimethylammonium and trimethylammonium-containing small molecules for NMR experiments.

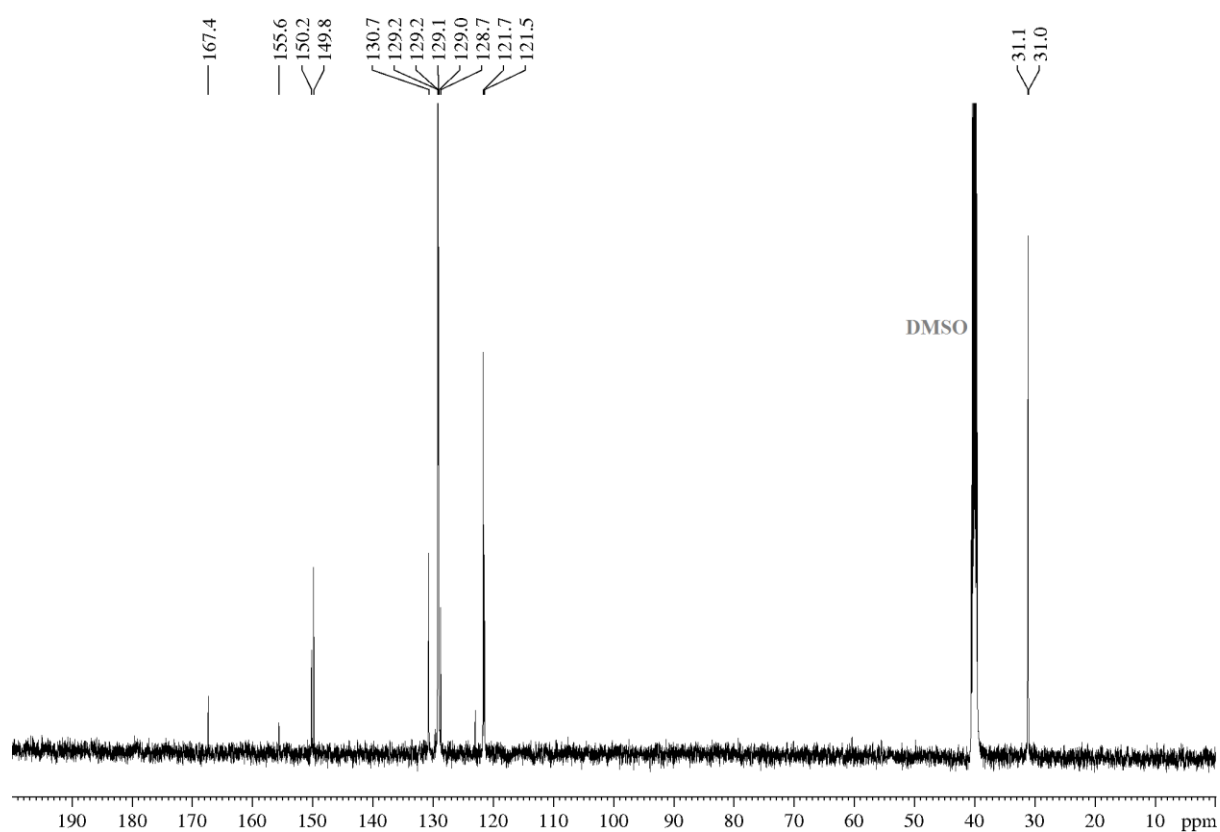
Molecular Modelling: For the energy minimizations, we used the program MacroModel V.9.8 (OPLS_2001 force field, MCMM, solvent: water, 20,000 steps). The calixarene host was introduced as an anion, the guests as zwitterions with the N^+ as an ammonium ion.

² P. Thordarson, *Chem. Soc. Rev.*, 2011, **40**, 1305.

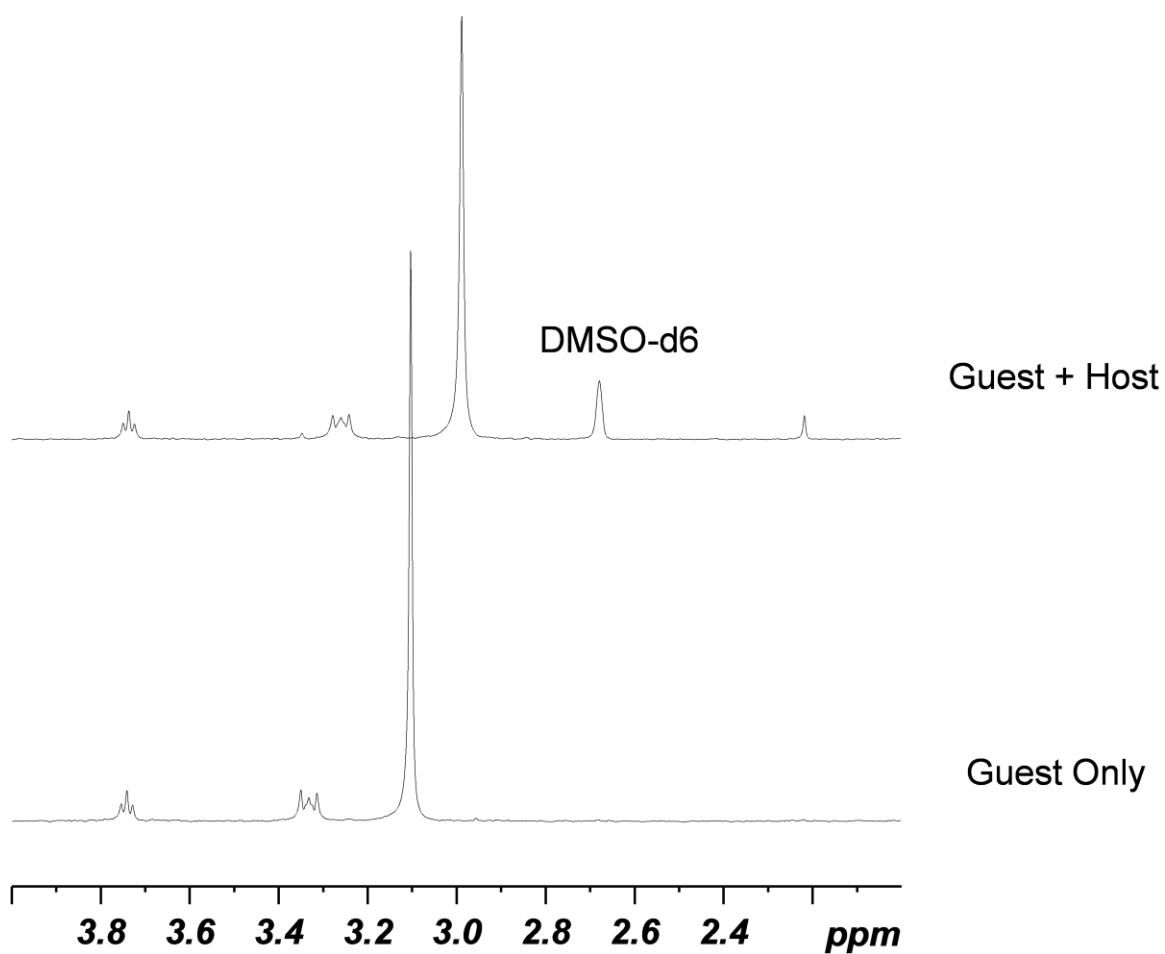
NMR spectra and related material



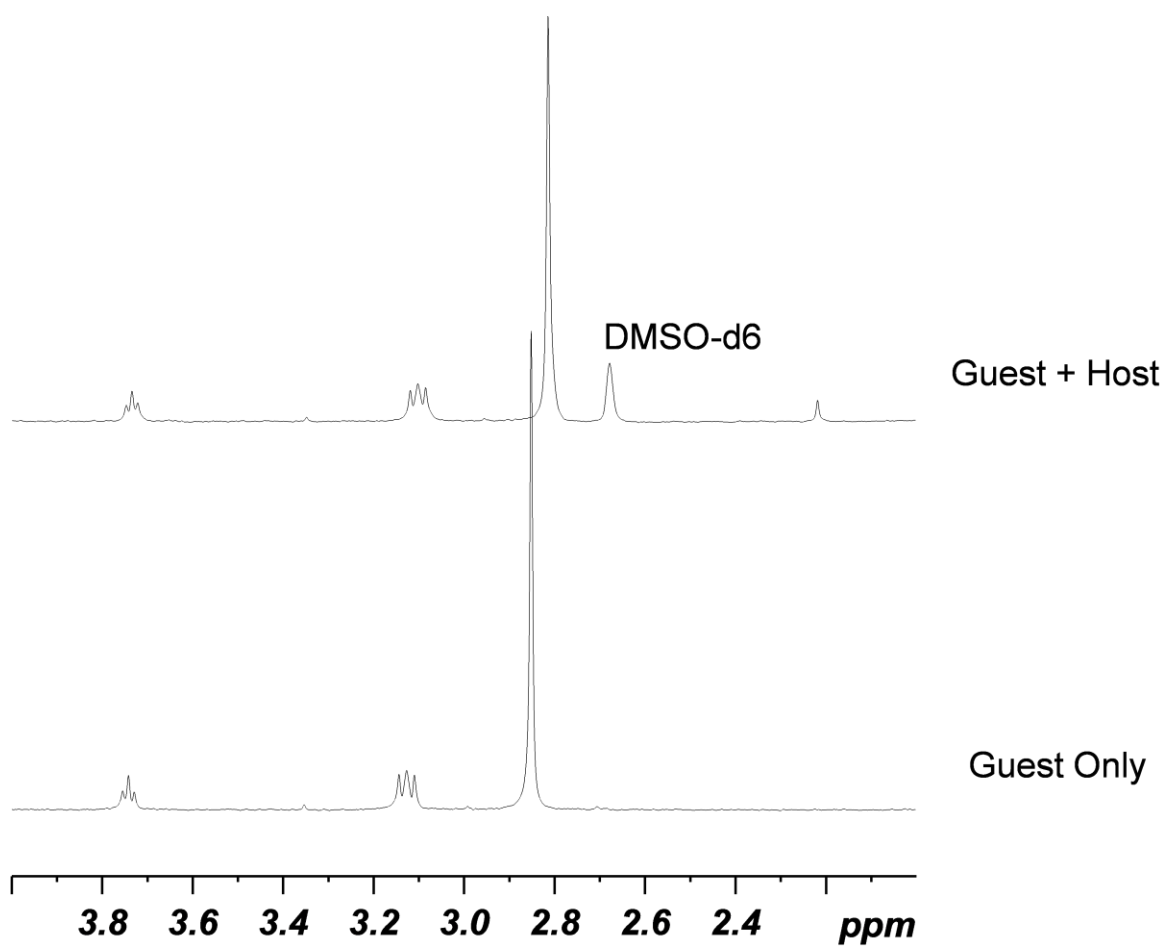
Supplementary Figure S1: ^1H NMR spectrum of carboxycalixarene **1** (500 MHz, DMSO). The signal of the four phenolic hydroxyl groups is very broad and the respective integral is smaller than expected. We attribute this to fast exchange processes involving the hydroxyl groups, the carboxylic acid and the solvent DMSO.



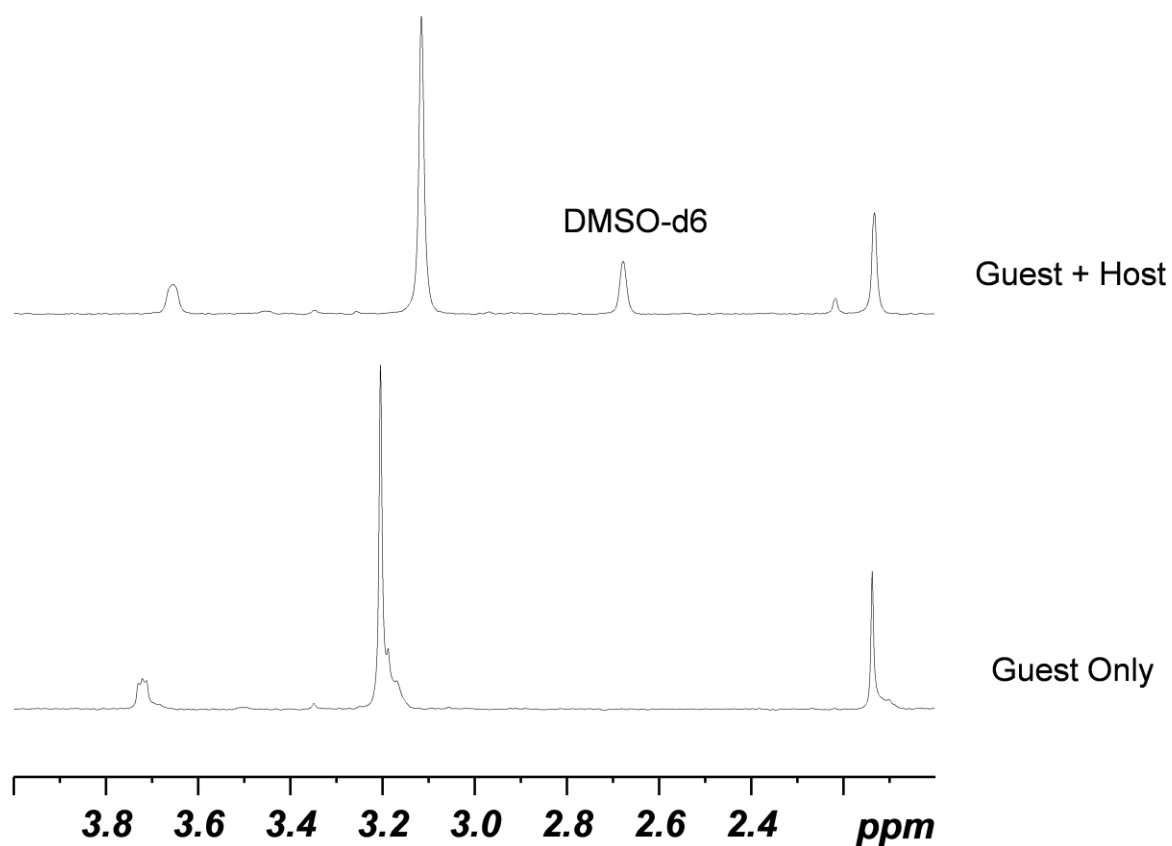
Supplementary Figure S2: ¹³C NMR spectrum of carboxycalixarene **1** (125 MHz, DMSO).



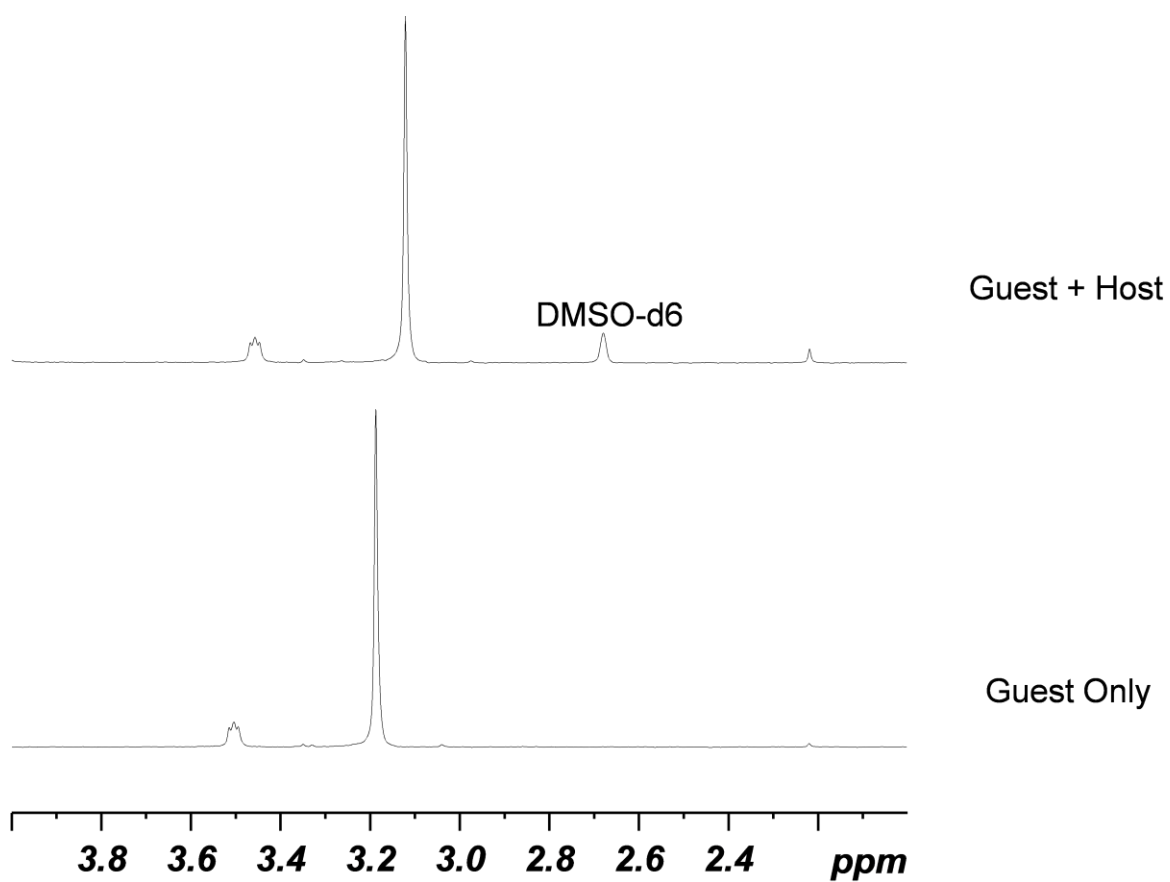
Supplementary Figure S3: ^1H NMR showing the binding of **3** to carboxycalixarene **1**, as indicated by the changes in chemical shifts of **3**. The concentrations of **3** and **1** were 500 μM and 250 μM respectively. Experiments were conducted in 50 mM sodium phosphate buffer pH 7.5 at 300 K.



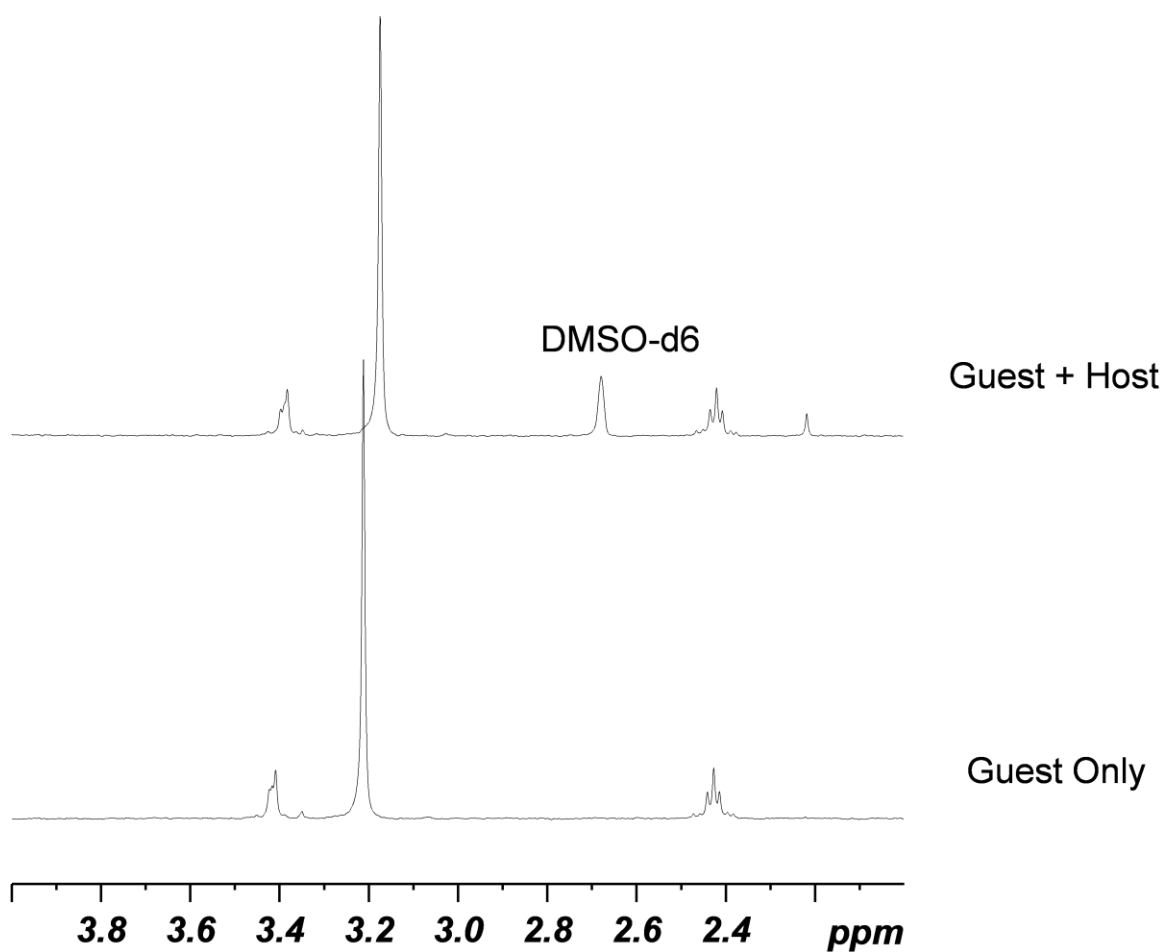
Supplementary Figure S4: ^1H NMR showing the binding of **4** to carboxycalixarene **1**, as indicated by the changes in chemical shifts of **4**. The concentrations of **4** and **1** were 500 μM and 250 μM respectively. Experiments were conducted in 50 mM sodium phosphate buffer pH 7.5 at 300 K.



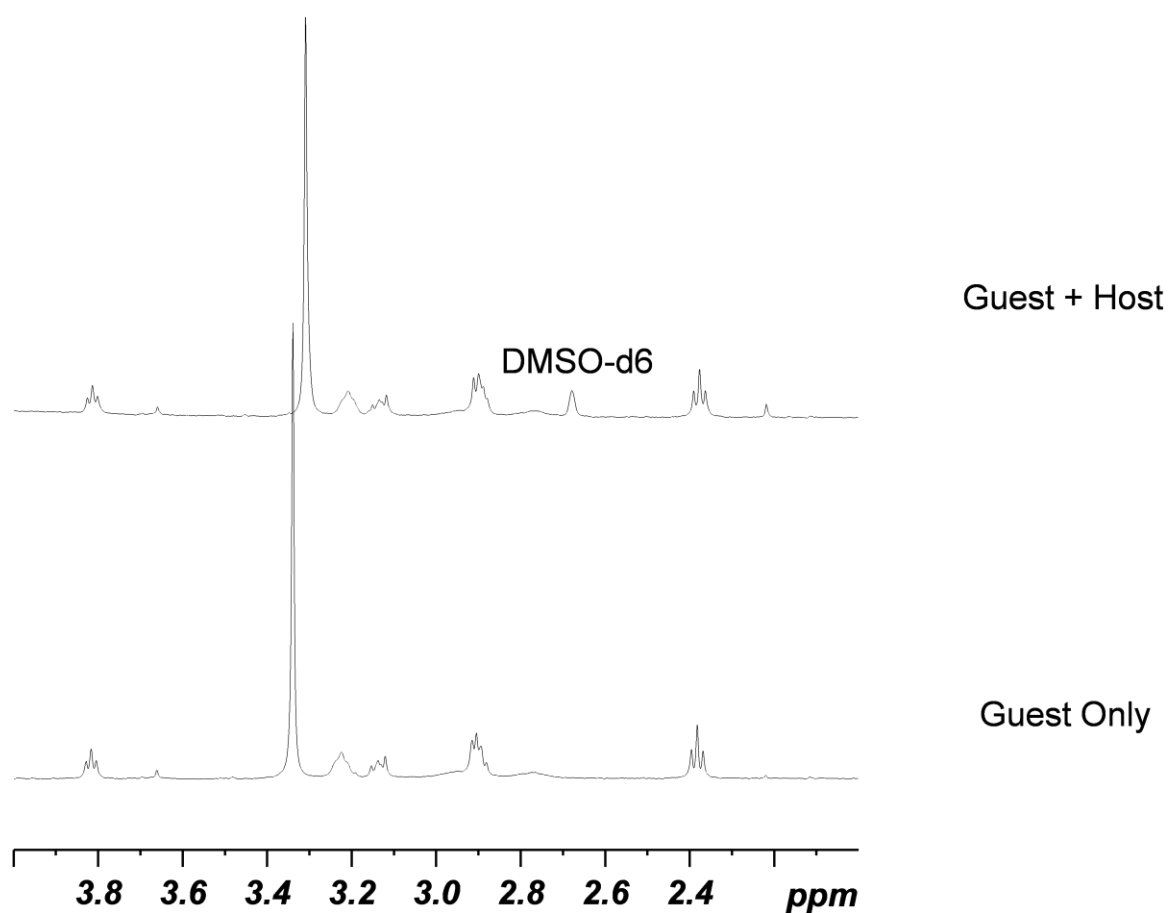
Supplementary Figure S5: ^1H NMR showing the binding of **5** to carboxycalixarene **1**, as indicated by the changes in chemical shifts of **5**. The concentrations of **5** and **1** were 500 μM and 250 μM respectively. Experiments were conducted in 50 mM sodium phosphate buffer pH 7.5 at 300 K.



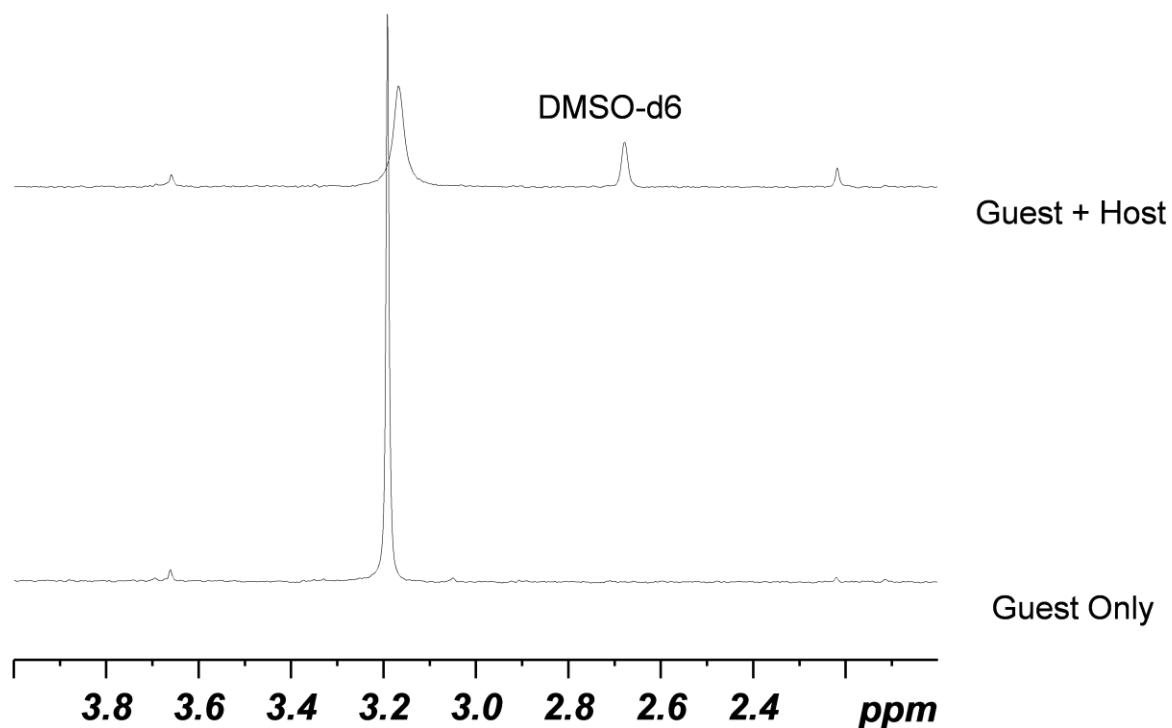
Supplementary Figure S6: ^1H NMR showing the binding of **6** to carboxycalixarene **1**, as indicated by the changes in chemical shifts of **6**. The concentrations of **6** and **1** were 500 μM and 250 μM respectively. Experiments were conducted in 50 mM sodium phosphate buffer pH 7.5 at 300 K.



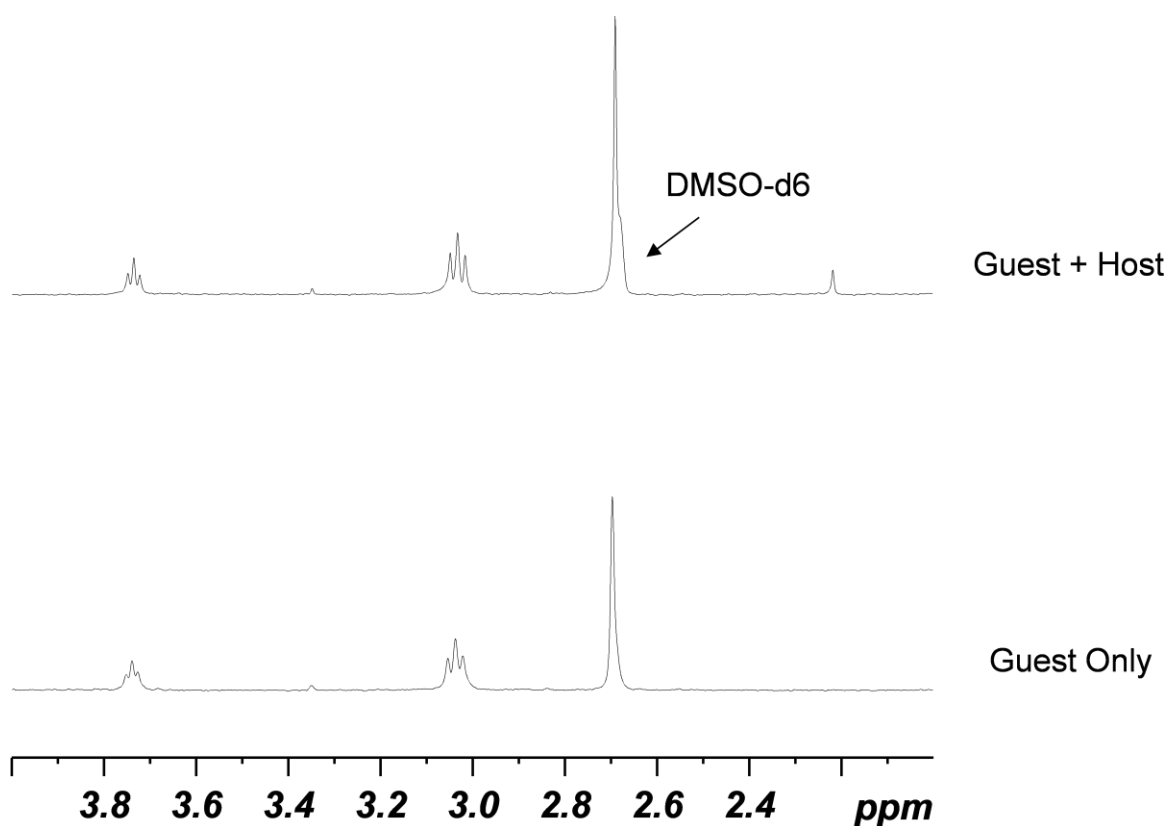
Supplementary Figure S7: ^1H NMR showing the binding of **7** to carboxycalixarene **1**, as indicated by the changes in chemical shifts of **7**. The concentrations of **7** and **1** were 500 μM and 250 μM respectively. Experiments were conducted in 50 mM sodium phosphate buffer pH 7.5 at 300 K.



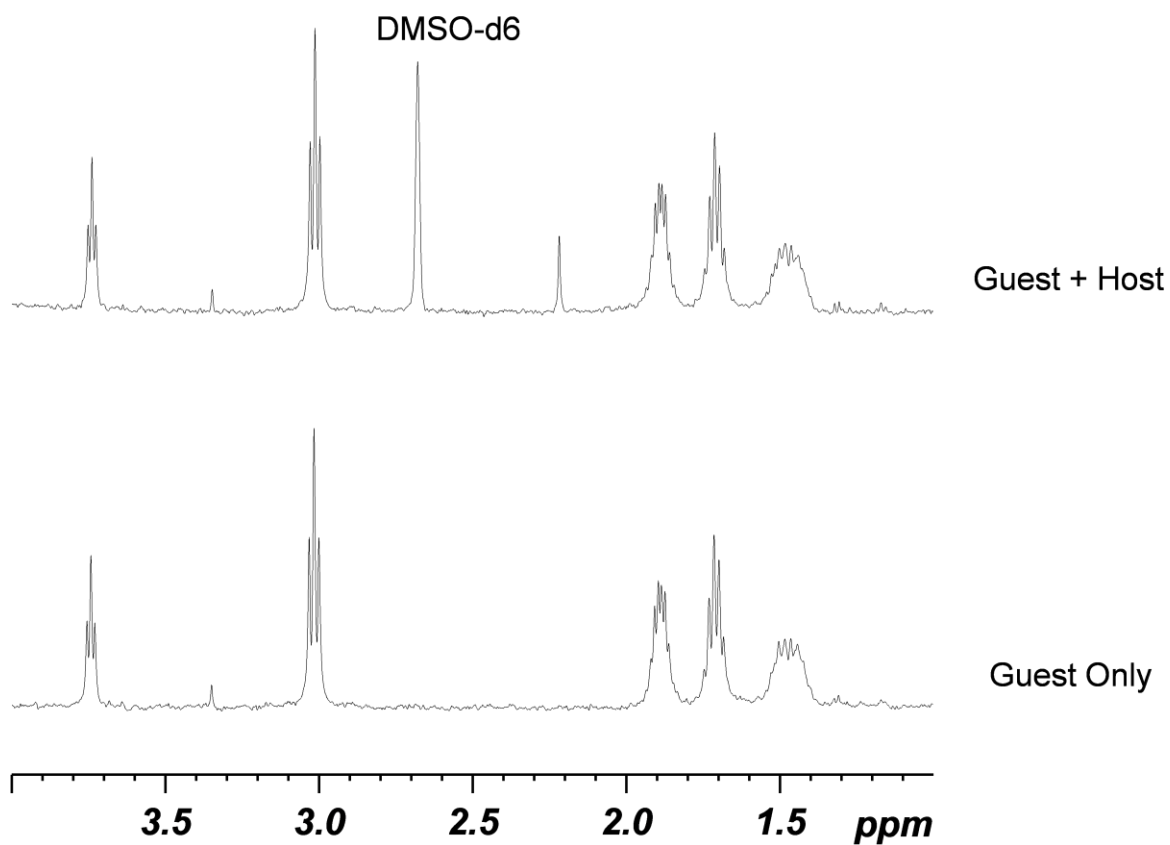
Supplementary Figure S8: ^1H NMR showing the binding of **8** to carboxycalixarene **1**, as indicated by the changes in chemical shifts of **8**. The concentrations of **8** and **1** were 500 μM and 250 μM respectively. Experiments were conducted in 50 mM sodium phosphate buffer pH 7.5 at 300 K.



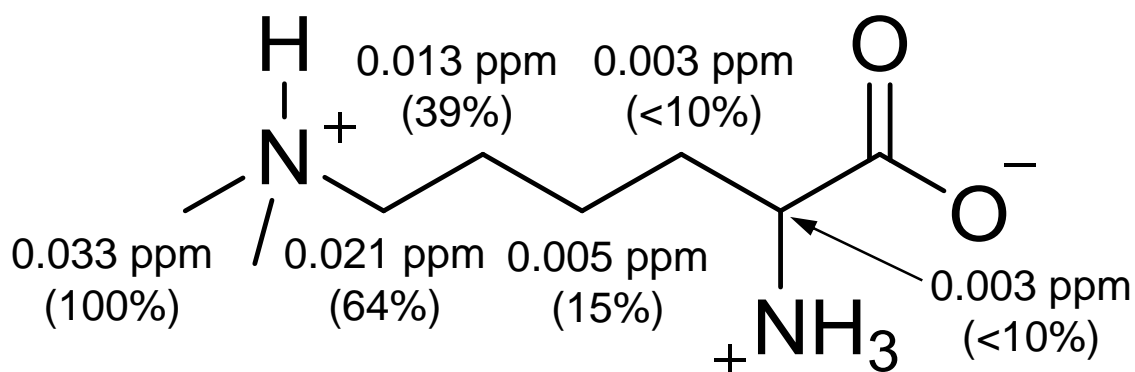
Supplementary Figure S9: ^1H NMR showing the binding of **9** to carboxycalixarene **1**, as indicated by the changes in chemical shifts of **9**. The concentrations of **9** and **1** were 500 μM and 250 μM respectively. Experiments were conducted in 50 mM sodium phosphate buffer pH 7.5 at 300 K.



Supplementary Figure S10: ^1H NMR showing **10** is a non-binder/weak binder to carboxycalixarene **1**, as indicated by lack of chemical shift changes of **10** in the presence of **1**. The concentrations of **10** and **1** were 500 μM and 250 μM respectively. Experiments were conducted in 50 mM sodium phosphate buffer pH 7.5 at 300 K.

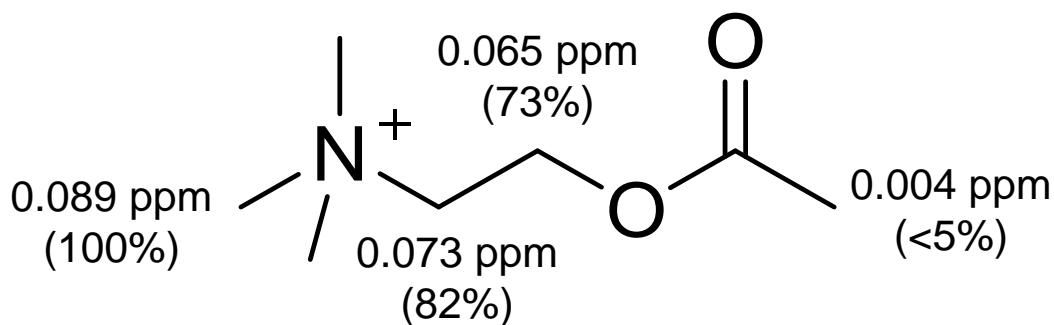


Supplementary Figure S11: ^1H NMR showing **11** is a non-binder/weak binder to carboxycalixarene **1**, as indicated by lack of chemical shift changes of **11** in the presence of **1**. The concentrations of **11** and **1** were 500 μM and 250 μM respectively. Experiments were conducted in 50 mM sodium phosphate buffer pH 7.5 at 300 K.



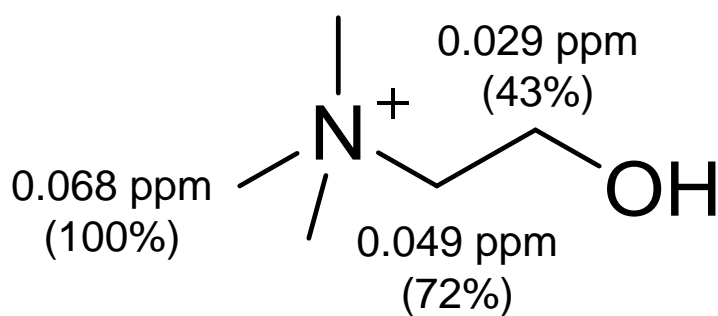
Changes in chemical shift / ppm
(Relative changes / %)

Supplementary Figure S12: Binding epitope mapping of **4** showing changes in chemical shifts (and relative percentage) upon binding to carboxycalixarene **1**.



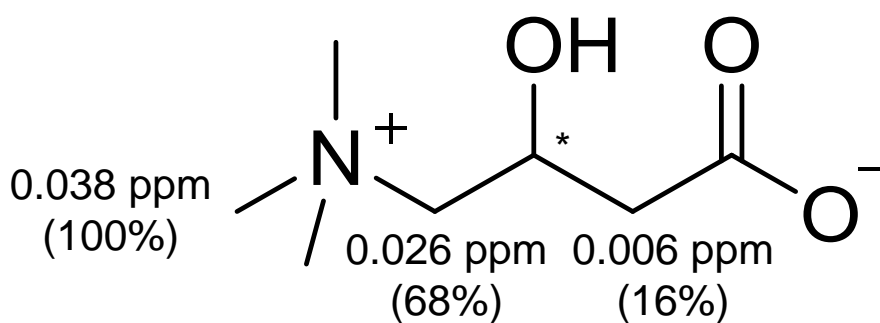
Changes in chemical shift / ppm
(Relative changes / %)

Supplementary Figure S13: Binding epitope mapping of **5** showing changes in chemical shifts (and relative percentage) upon binding to carboxycalixarene **1**.



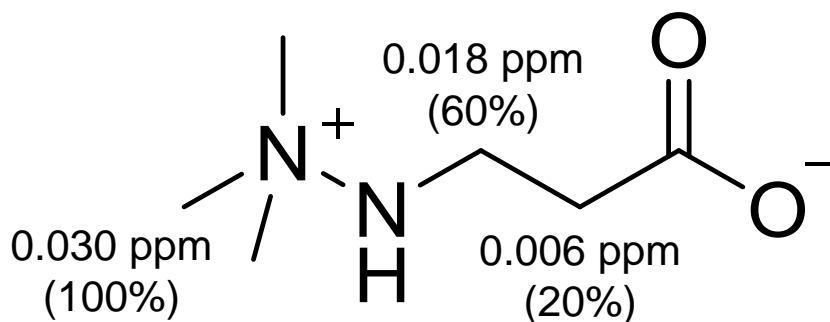
Changes in chemical shift / ppm
(Relative changes / %)

Supplementary Figure S14: Binding epitope mapping of **6** showing changes in chemical shifts (and relative percentage) upon binding to carboxycalixarene **1**.



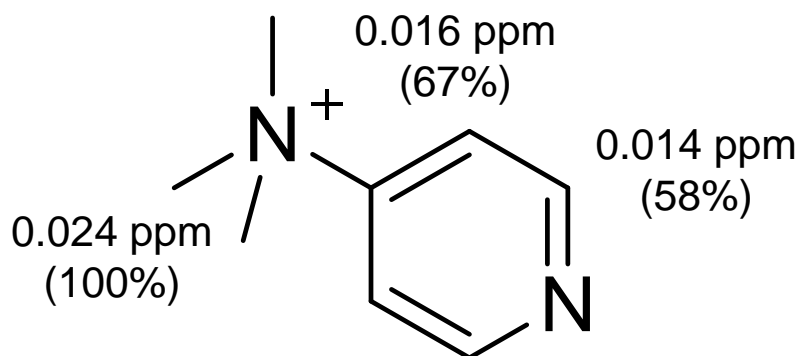
Changes in chemical shift / ppm
(Relative changes / %)

Supplementary Figure S15: Binding epitope mapping of **7** showing changes in chemical shifts (and relative percentage) upon binding to carboxycalixarene **1**. Asterisk (*): resonance overlap with water peak.



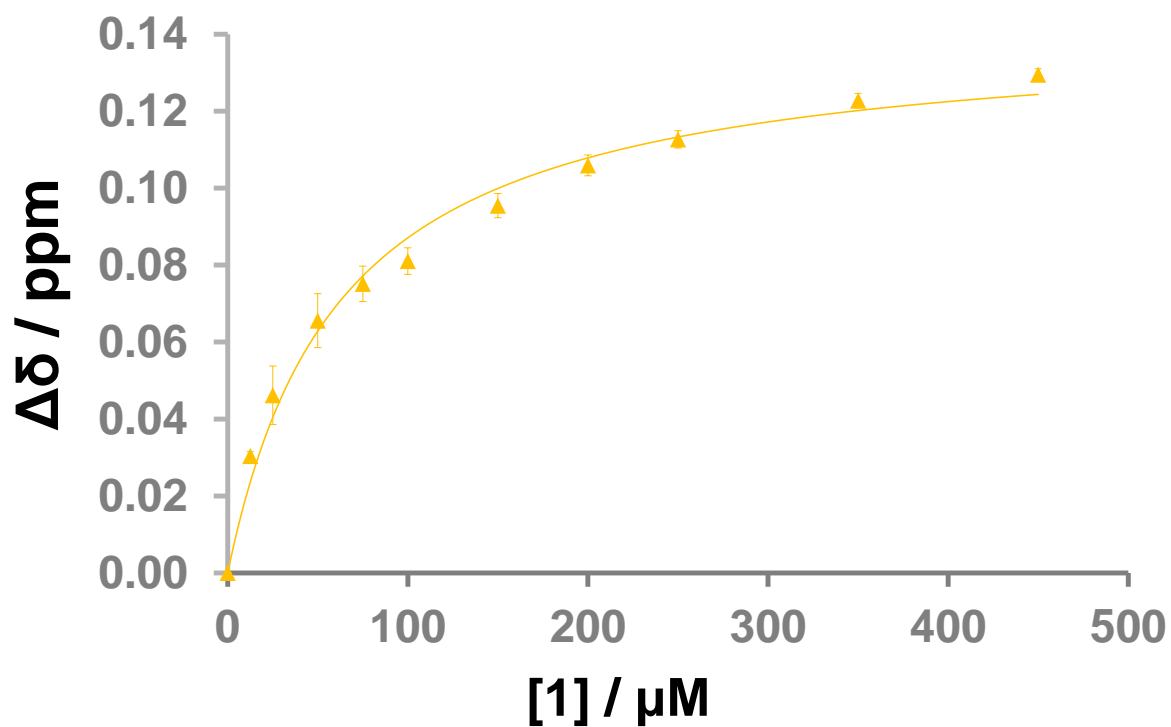
Changes in chemical shift / ppm
(Relative changes / %)

Supplementary Figure S16: Binding epitope mapping of **8** showing changes in chemical shifts (and relative percentage) upon binding to carboxycalixarene **1**.

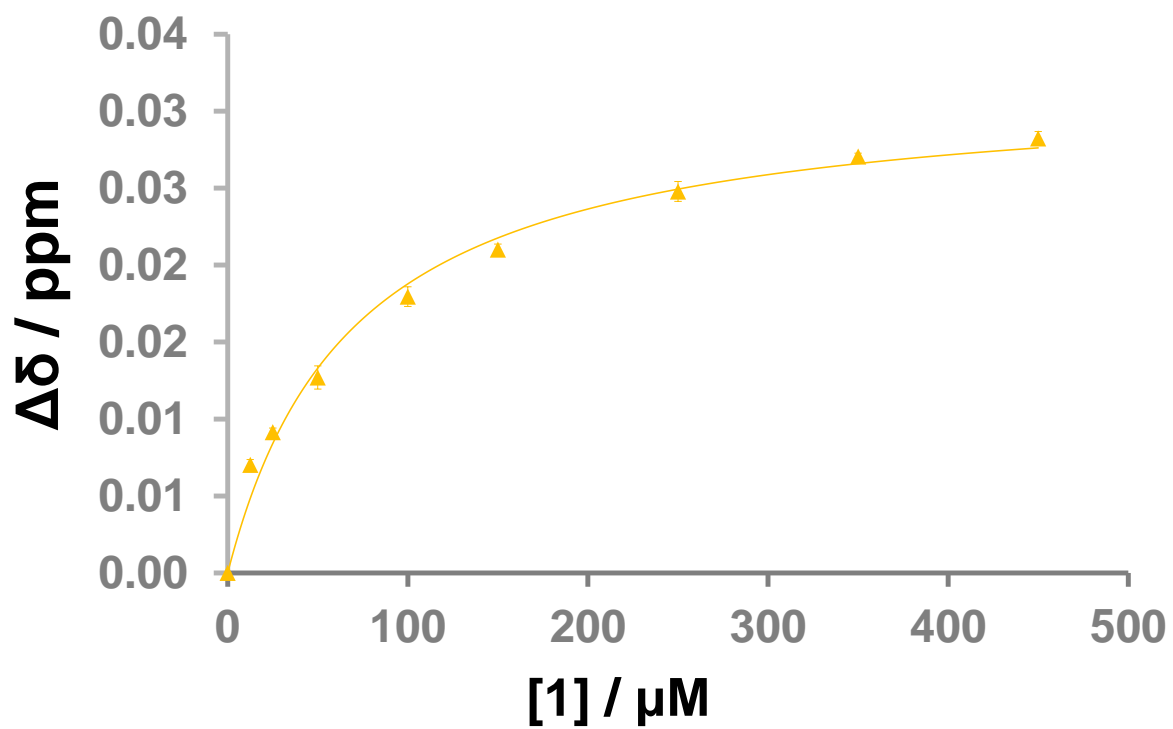


Changes in chemical shift / ppm
(Relative changes / %)

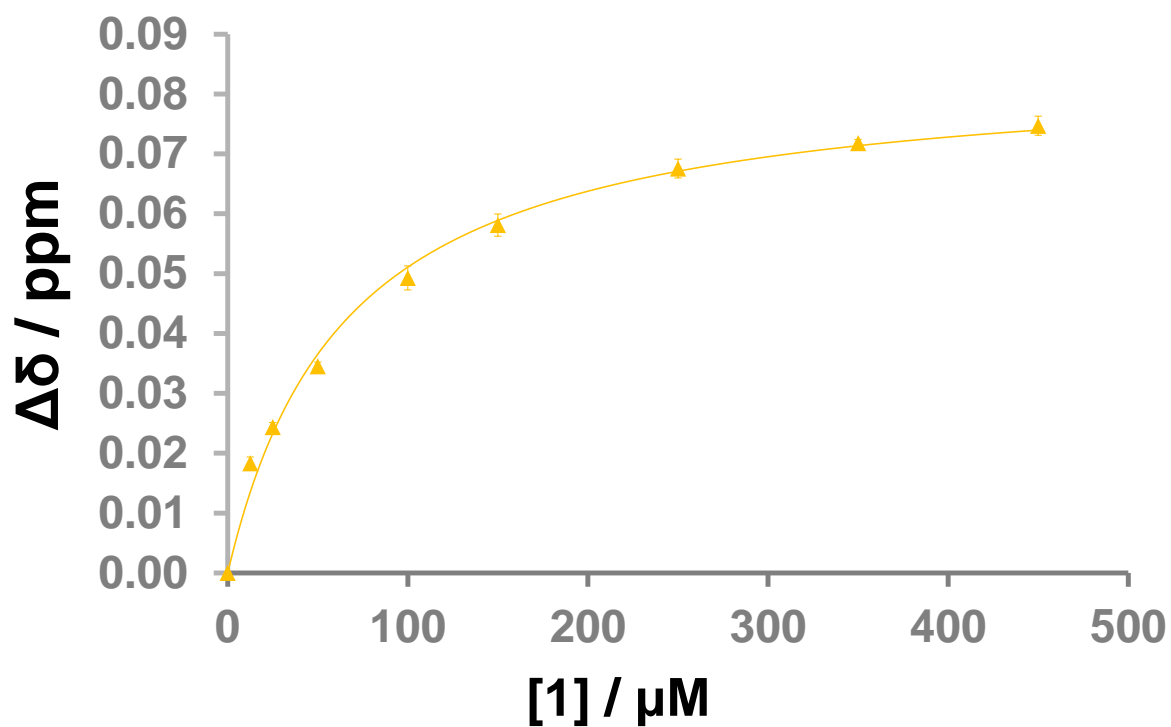
Supplementary Figure S17: Binding epitope mapping of **9** showing changes in chemical shifts (and relative percentage) upon binding to carboxycalixarene **1**.



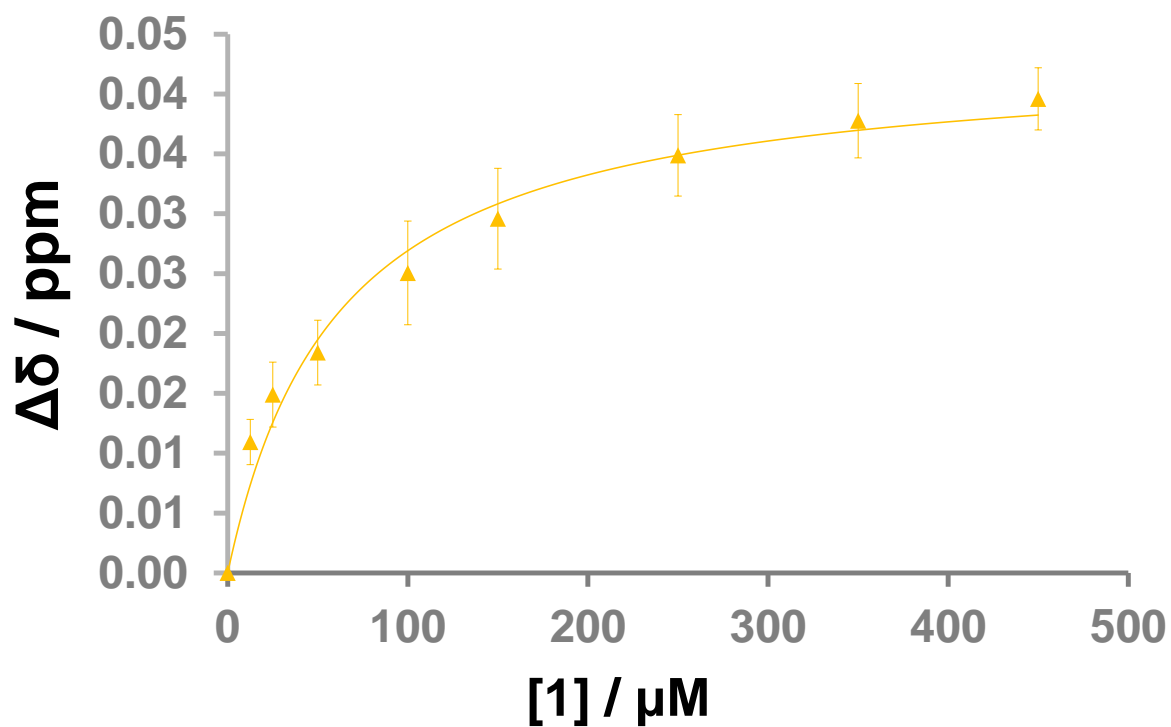
Supplementary Figure S18: Titration of **1** to 100 μM of **3** as monitored by changes in the N-Me resonance chemical shift. The K_D was $60 \pm 10 \mu\text{M}$. Experiments were conducted in triplicate and errors were shown as standard derivation.



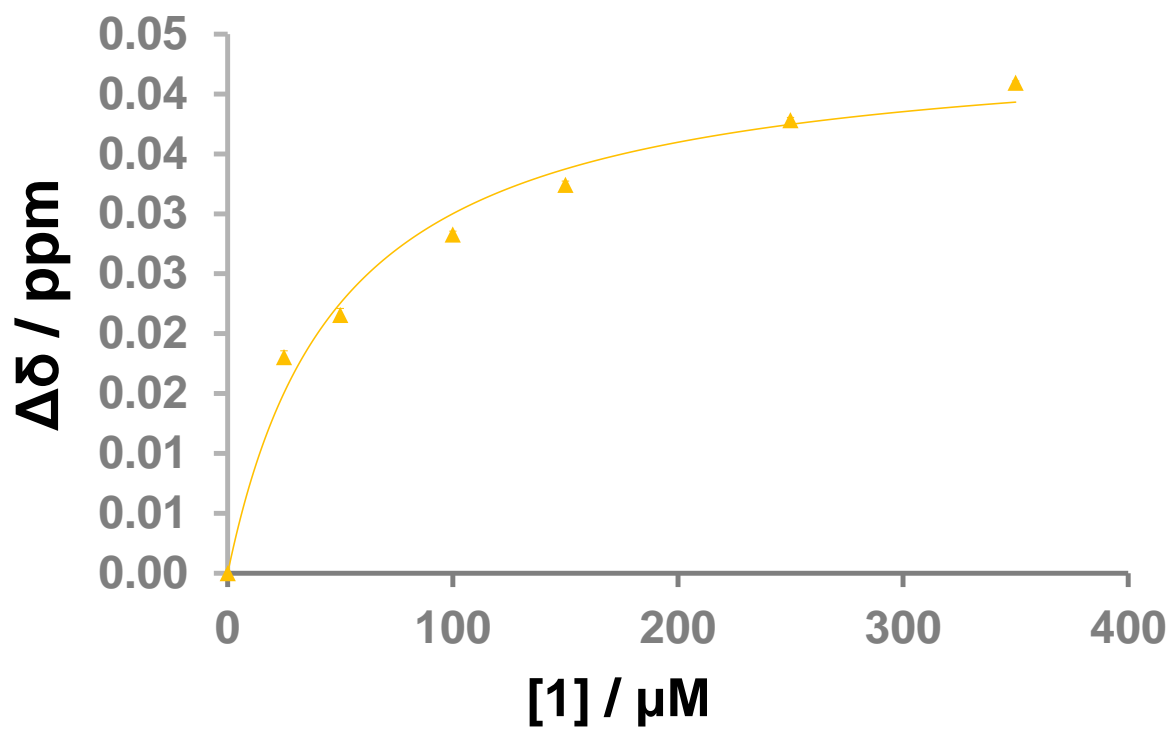
Supplementary Figure S19: Titration of **1** to 100 μM of **4** as monitored by changes in the N-Me resonance chemical shift. The K_D was $70 \pm 5 \mu\text{M}$. Experiments were conducted in triplicate and errors were shown as standard derivation.



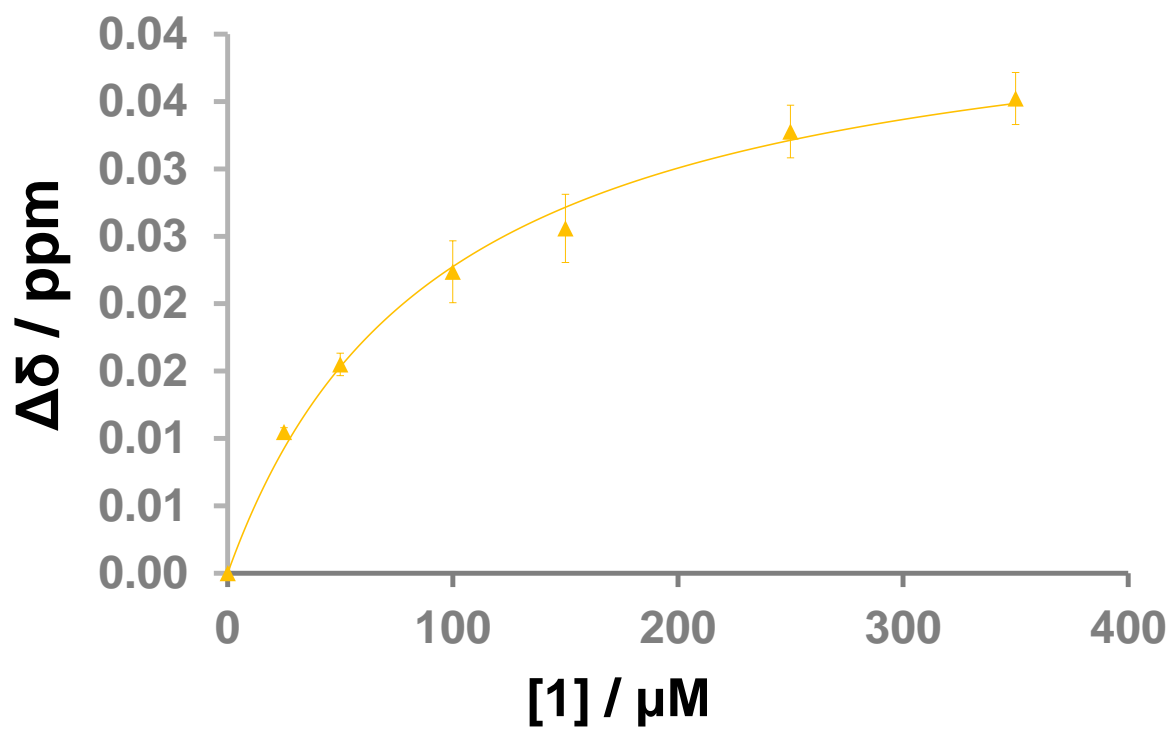
Supplementary Figure S20: Titration of **1** to 100 μM of **6** as monitored by changes in the N-Me resonance chemical shift. The K_D was $65 \pm 5 \mu\text{M}$. Experiments were conducted in triplicate and errors were shown as standard derivation.



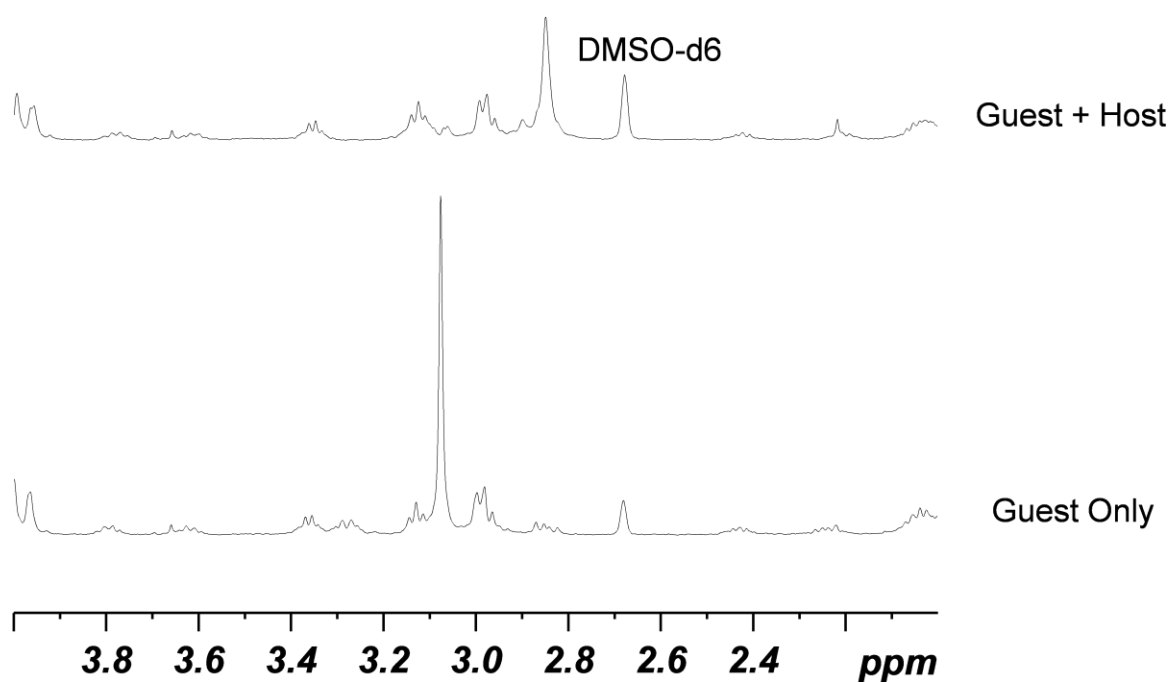
Supplementary Figure S21: Titration of **1** to 100 μM of **7** as monitored by changes in the N-Me resonance chemical shift. The K_D was $60 \pm 15 \mu\text{M}$. Experiments were conducted in triplicate and errors were shown as standard derivation.



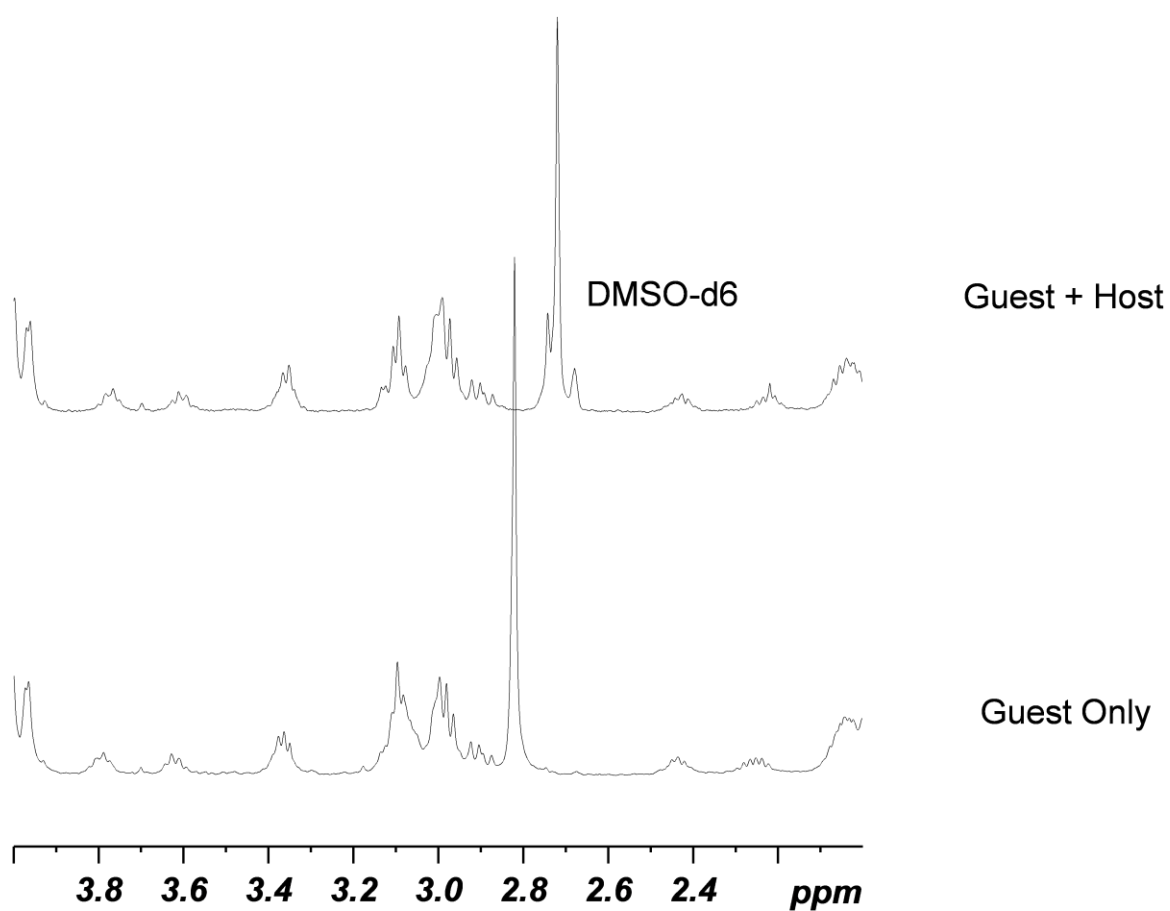
Supplementary Figure S22: Titration of **1** to 100 μM of **8** as monitored by changes in the N-Me resonance chemical shift. The K_D was $50 \pm 1 \mu\text{M}$. Experiments were conducted in triplicate and errors were shown as standard derivation.



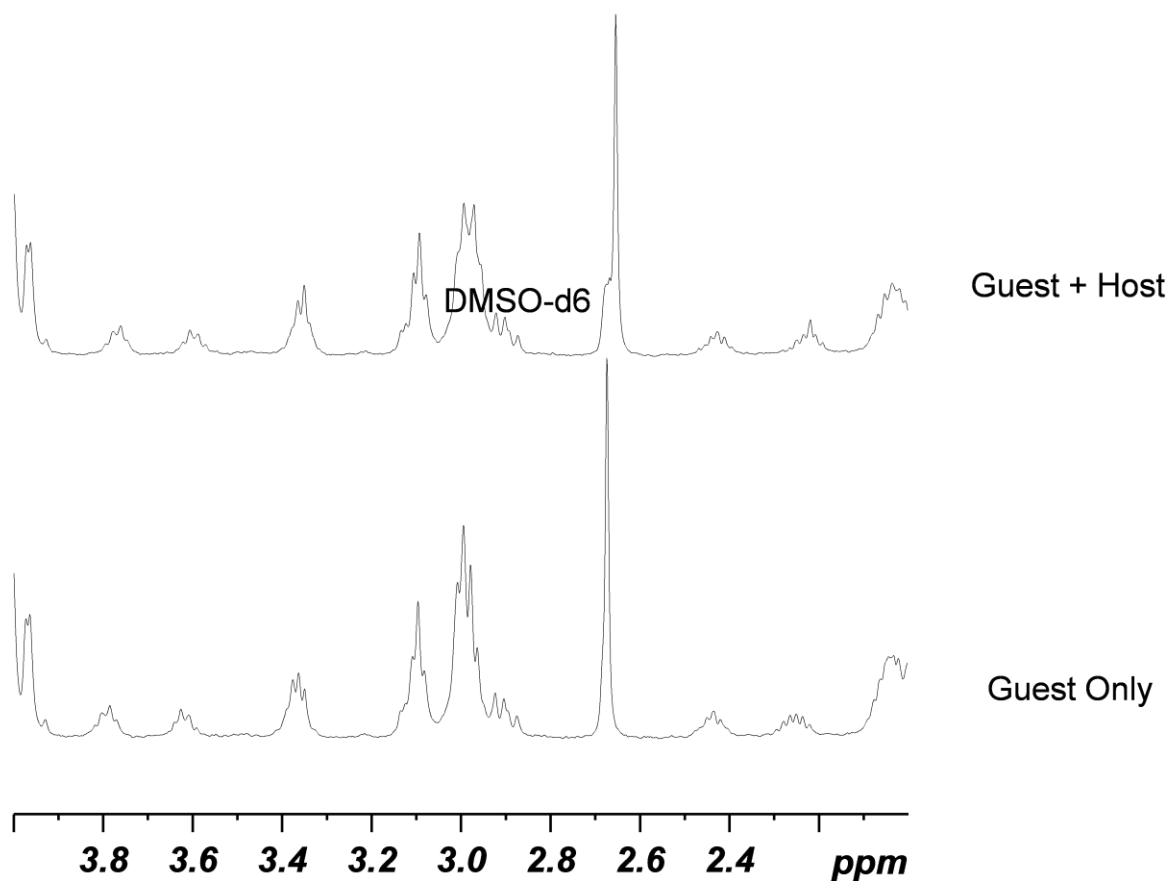
Supplementary Figure S23: Titration of **1** to 100 μM of **9** as monitored by changes in the N-Me resonance chemical shift. The K_D was $95 \pm 10 \mu\text{M}$. Experiments were conducted in triplicate and errors were shown as standard derivation.



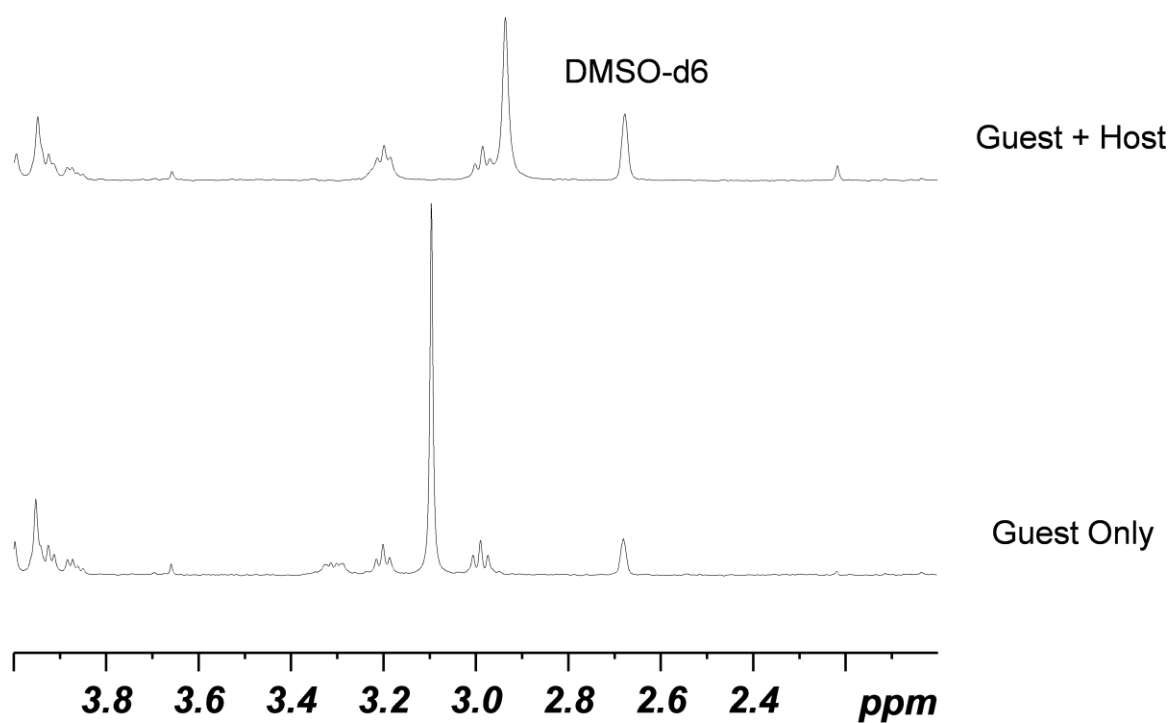
Supplementary Figure S24: ¹H NMR showing the binding of **PATGGV(Kme3)KPHRY** to carboxycalixarene **1**, as indicated by the changes in N-methyl chemical shift of the peptide. The concentrations of the peptide and **1** were 500 μM and 250 μM respectively. Experiments were conducted in 50 mM sodium phosphate buffer pH 7.5 at 300 K.



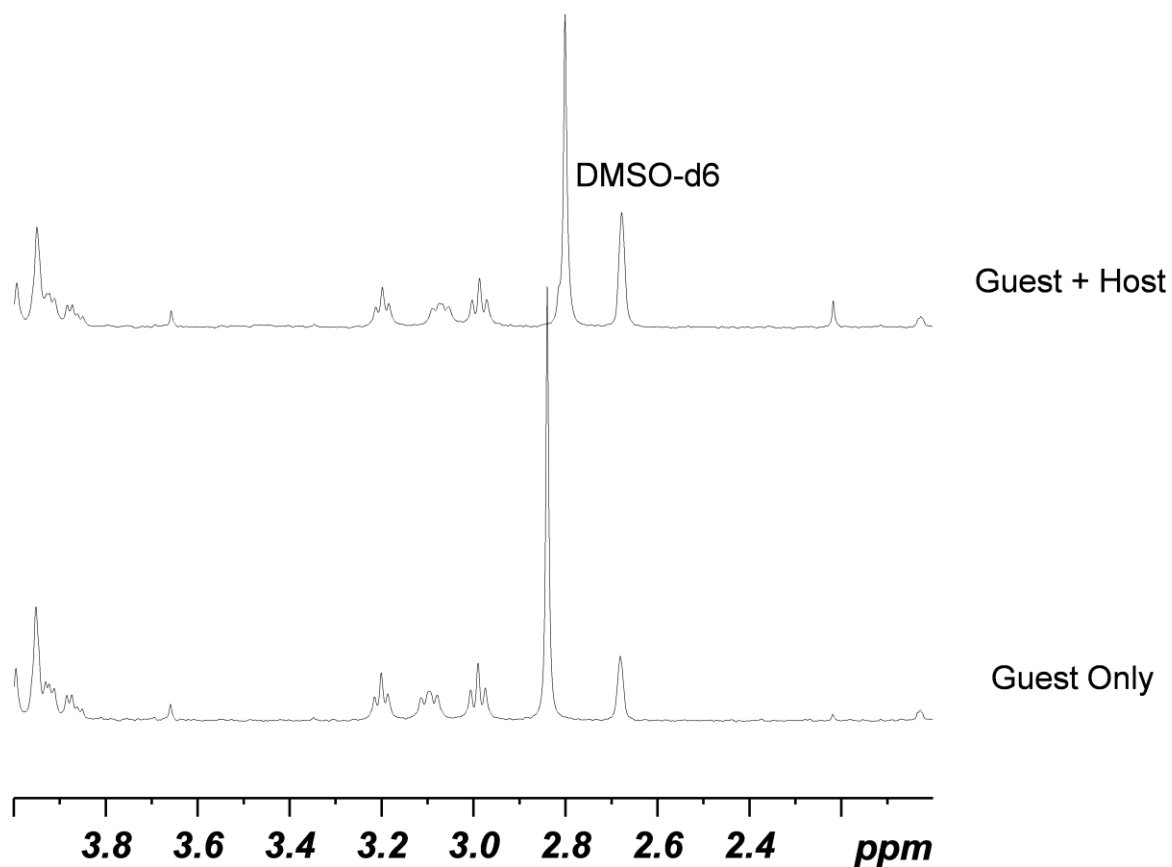
Supplementary Figure S25: ¹H NMR showing the binding of **PATGGV(Kme2)KPHRY** to carboxycalixarene **1**, as indicated by the changes in N-methyl chemical shift of the peptide. The concentrations of the peptide and **1** were 500 μM and 250 μM respectively. Experiments were conducted in 50 mM sodium phosphate buffer pH 7.5 at 300 K.



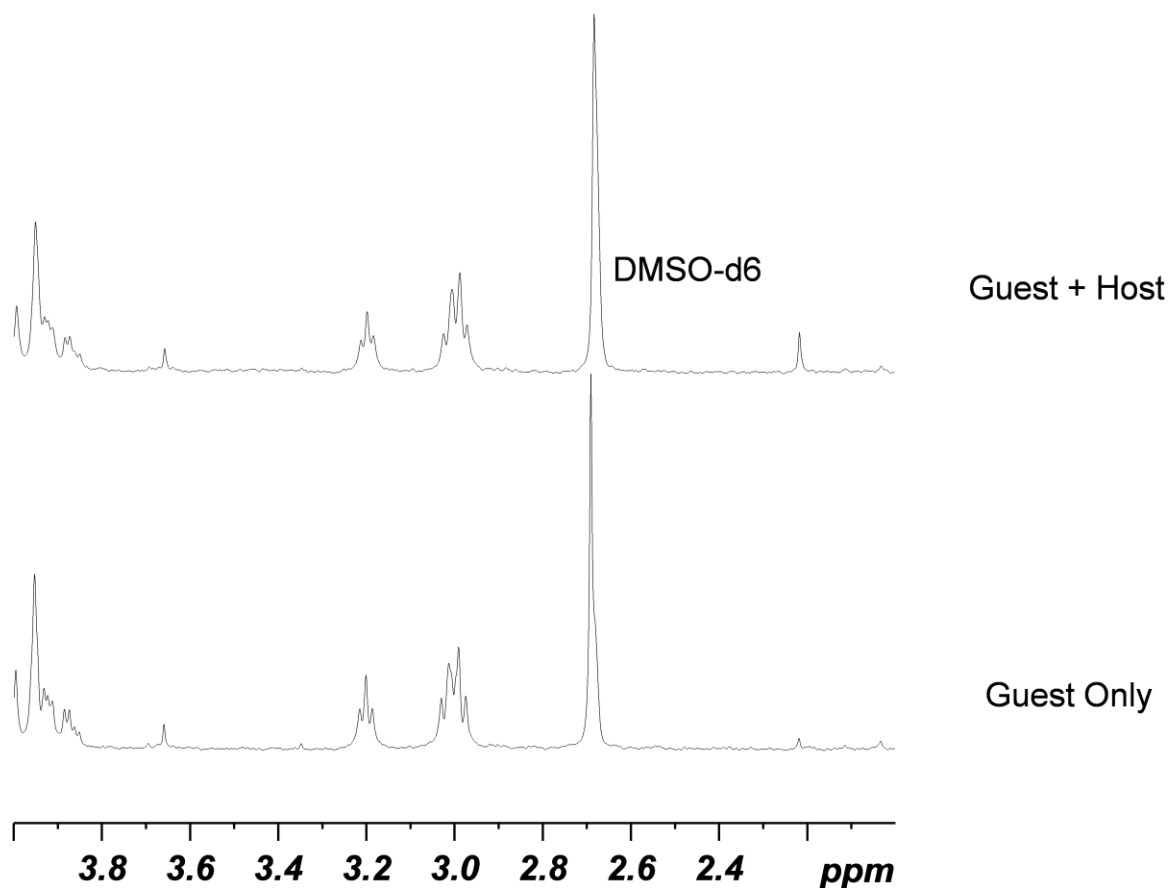
Supplementary Figure S26: ¹H NMR showing **PATGGV(Kme1)KPHRY** is a non-binder or weak binder to carboxycalixarene **1**, as indicated by the small change in N-methyl chemical shift of the peptide. The concentrations of the peptide and **1** were 500 μM and 250 μM respectively. Experiments were conducted in 50 mM sodium phosphate buffer pH 7.5 at 300 K.



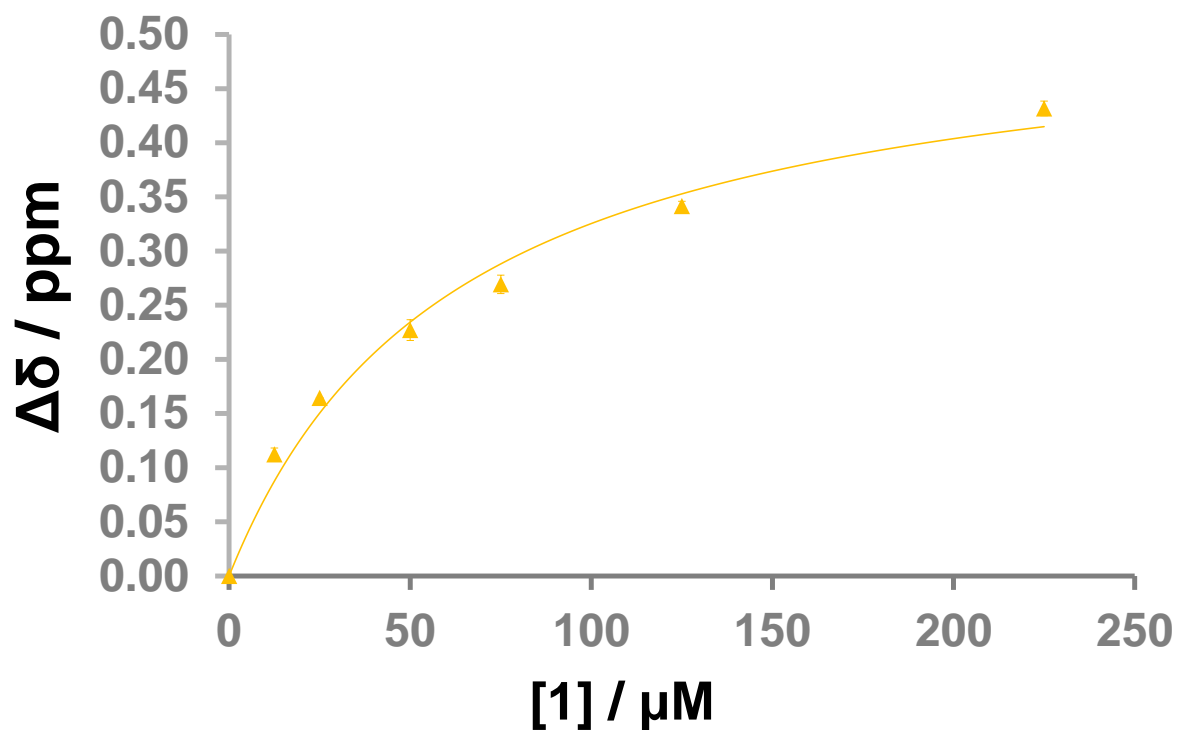
Supplementary Figure S27: ^1H NMR showing the binding of **AR(Kme3)STGGK** to carboxycalixarene **1**, as indicated by the changes in N-methyl chemical shift of the peptide. The concentrations of the peptide and **1** were 500 μM and 250 μM respectively. Experiments were conducted in 50 mM sodium phosphate buffer pH 7.5 at 300 K.



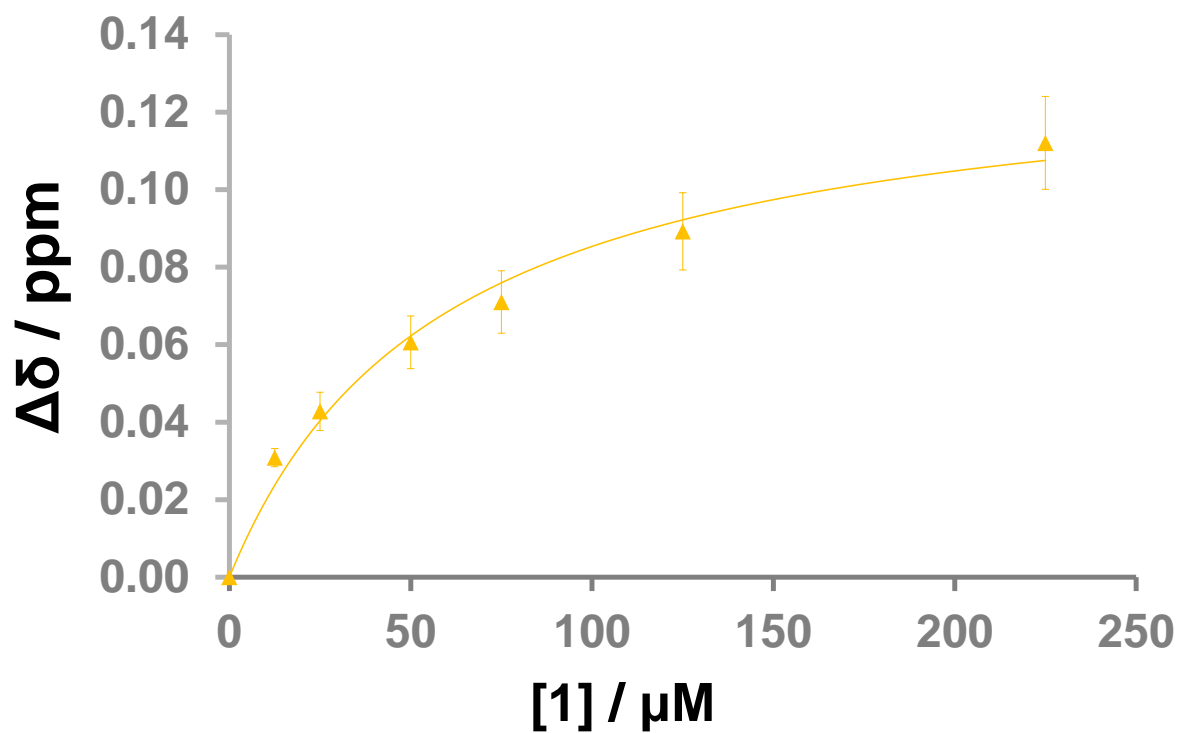
Supplementary Figure S28: ^1H NMR showing the binding of **AR(Kme2)STGGK** to carboxycalixarene **1**, as indicated by the changes in N-methyl chemical shift of the peptide. The concentrations of the peptide and **1** were 500 μM and 250 μM respectively. Experiments were conducted in 50 mM sodium phosphate buffer pH 7.5 at 300 K.



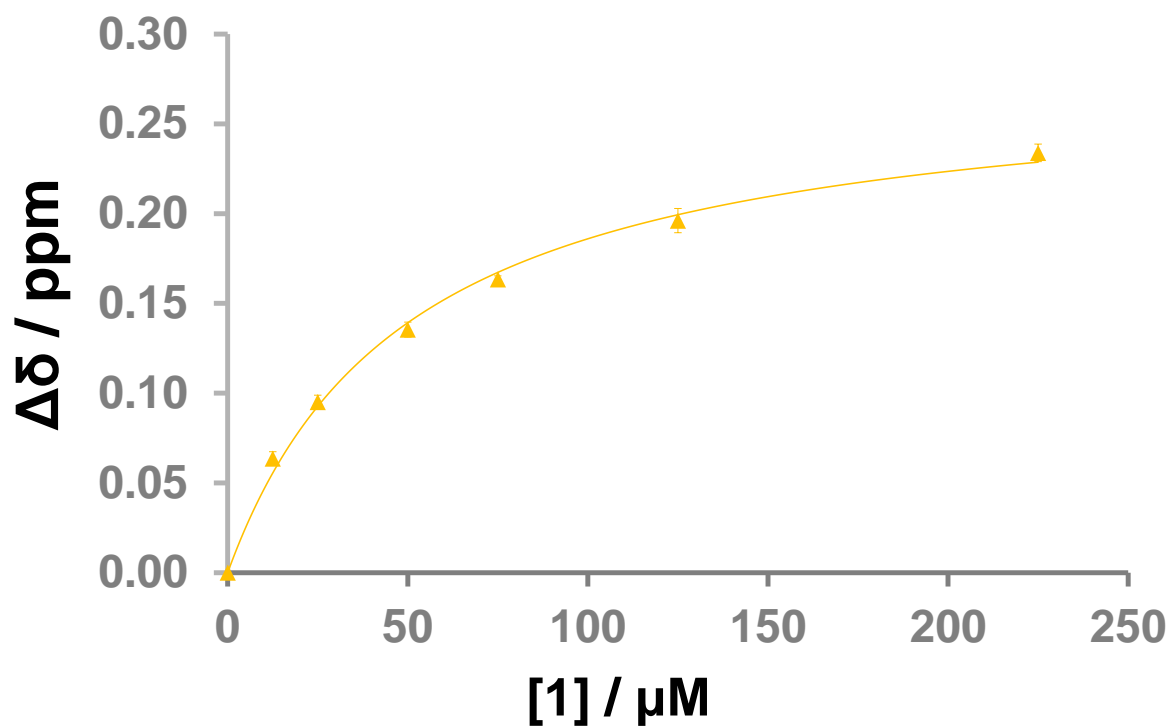
Supplementary Figure S29: ¹H NMR showing **AR(Kme1)STGGK** is a non-binder or weak binder to carboxycalixarene **1**, as indicated by the small change in N-methyl chemical shift of the peptide. The concentrations of the peptide and **1** were 500 μM and 250 μM respectively. Experiments were conducted in 50 mM sodium phosphate buffer pH 7.5 at 300 K.



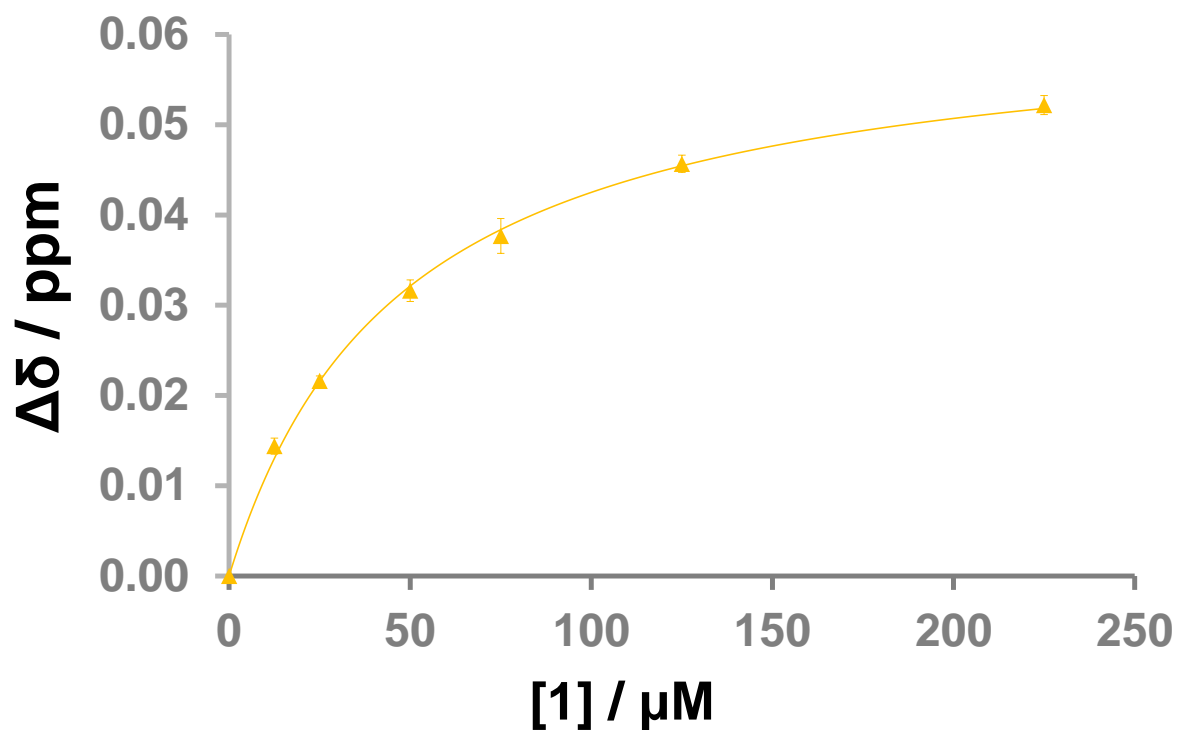
Supplementary Figure S30: Titration of **1** to 50 μM of **PATGGV(Kme3)KPHRY** as monitored by changes in the N-Me resonance chemical shift. The K_D was $65 \pm 5 \mu\text{M}$. Experiments were conducted in triplicate and errors were shown as standard derivation.



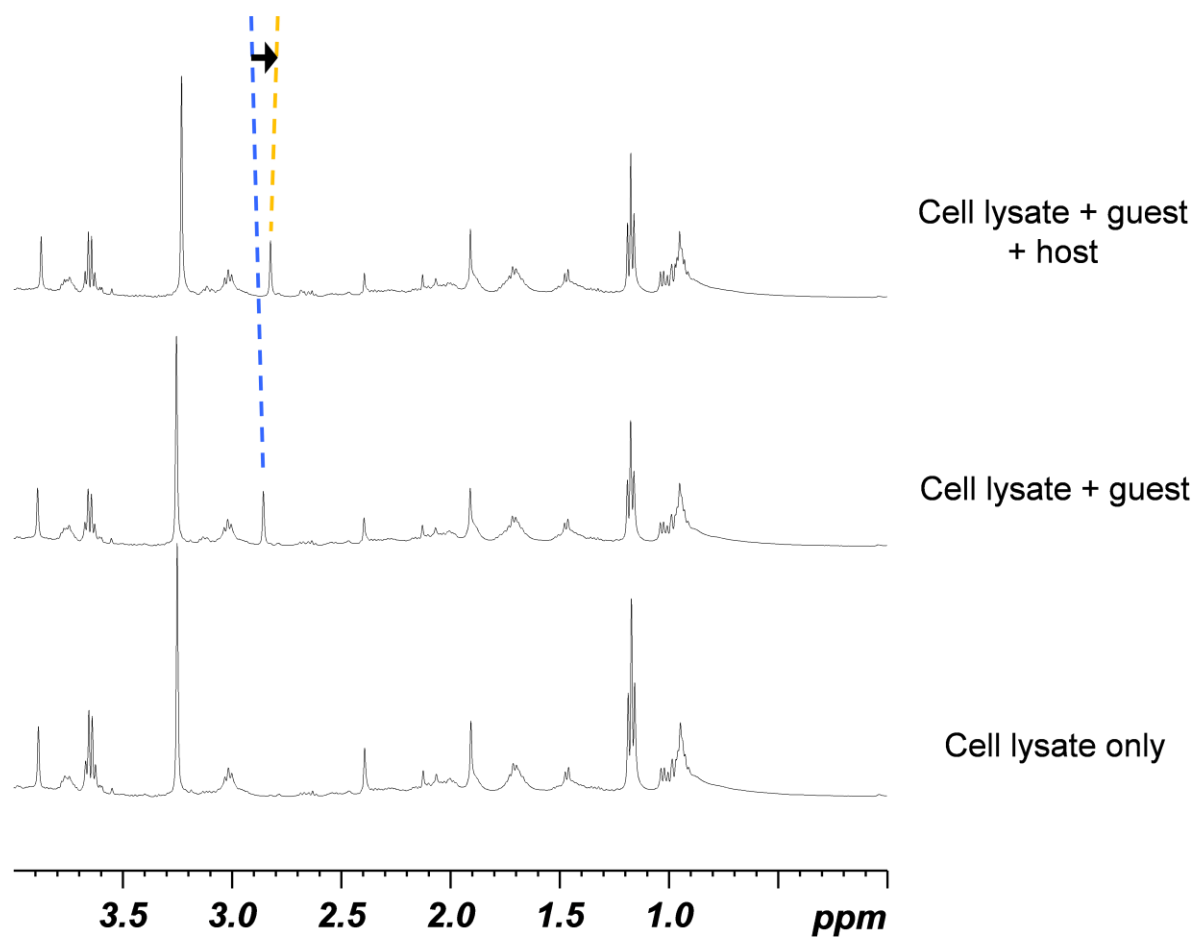
Supplementary Figure S31: Titration of **1** to 50 μM of **PATGGV(Kme2)KPHRY** as monitored by changes in the N-Me resonance chemical shift. The K_D was $60 \pm 1 \mu\text{M}$. Experiments were conducted in triplicate and errors were shown as standard derivation.



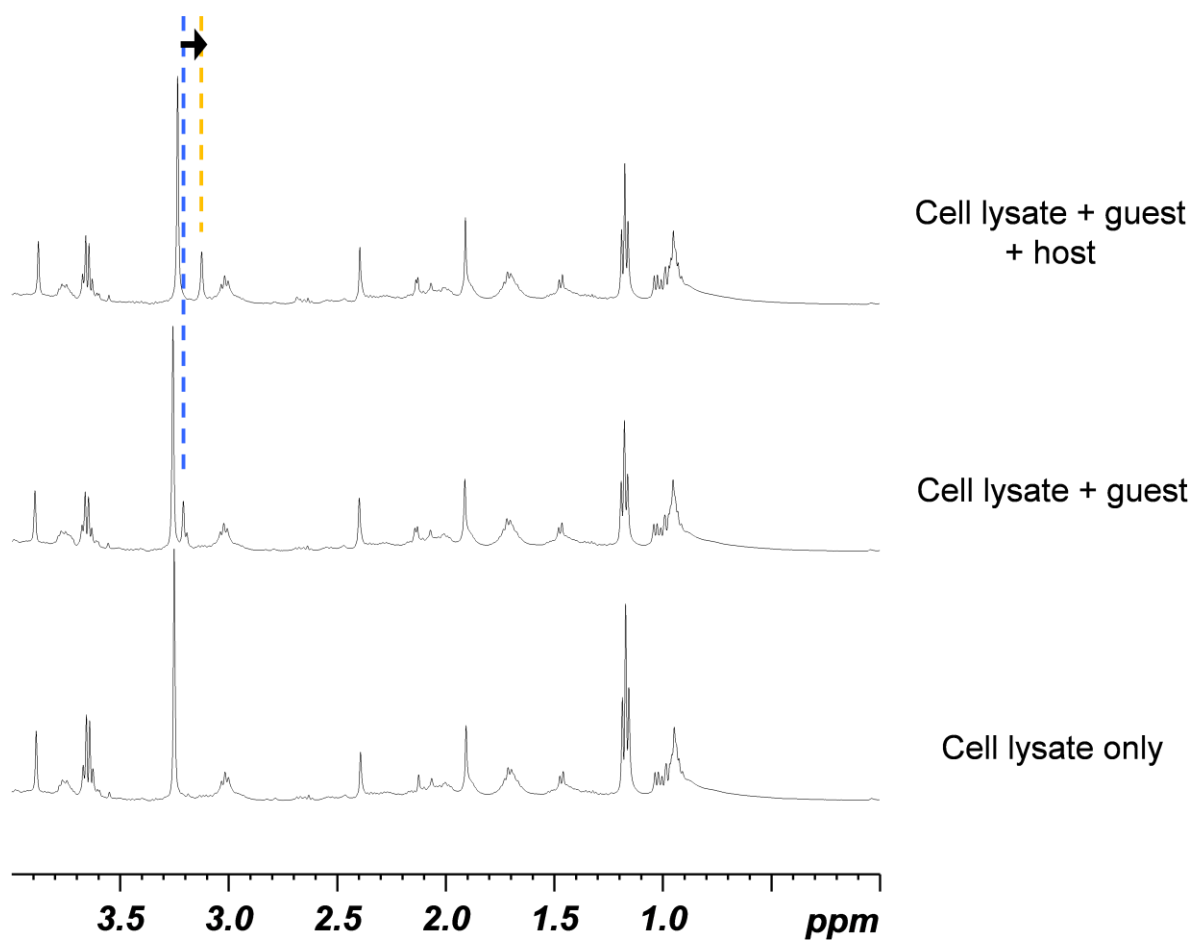
Supplementary Figure S32: Titration of **1** to 50 μM of **AR(Kme3)STGGK** as monitored by changes in the N-Me resonance chemical shift. The K_D was $50 \pm 2 \mu\text{M}$. Experiments were conducted in triplicate and errors were shown as standard derivation.



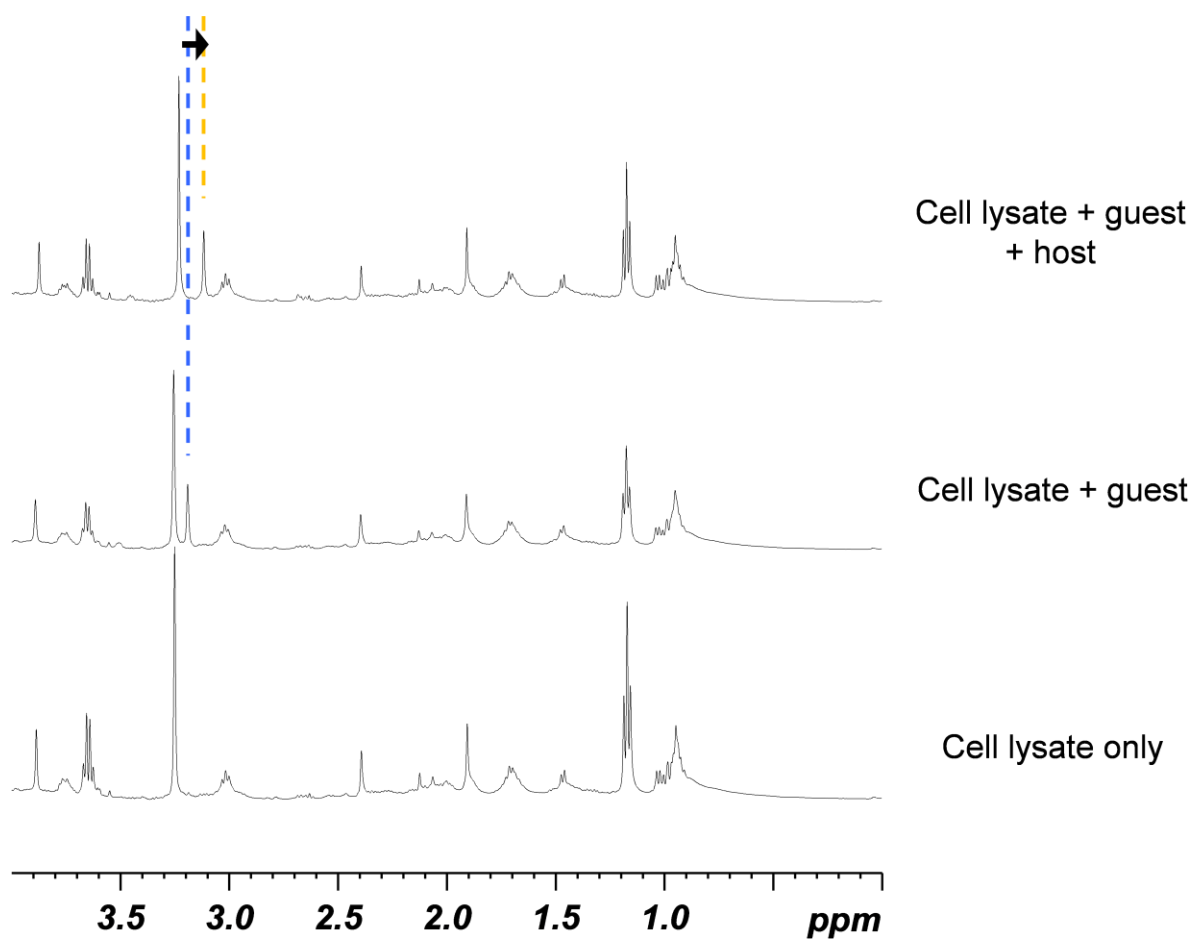
Supplementary Figure S33: Titration of **1** to 50 μM of **AR(Kme2)STGGK** as monitored by changes in the N-Me resonance chemical shift. The K_D was $50 \pm 5 \mu\text{M}$. Experiments were conducted in triplicate and errors were shown as standard derivation.



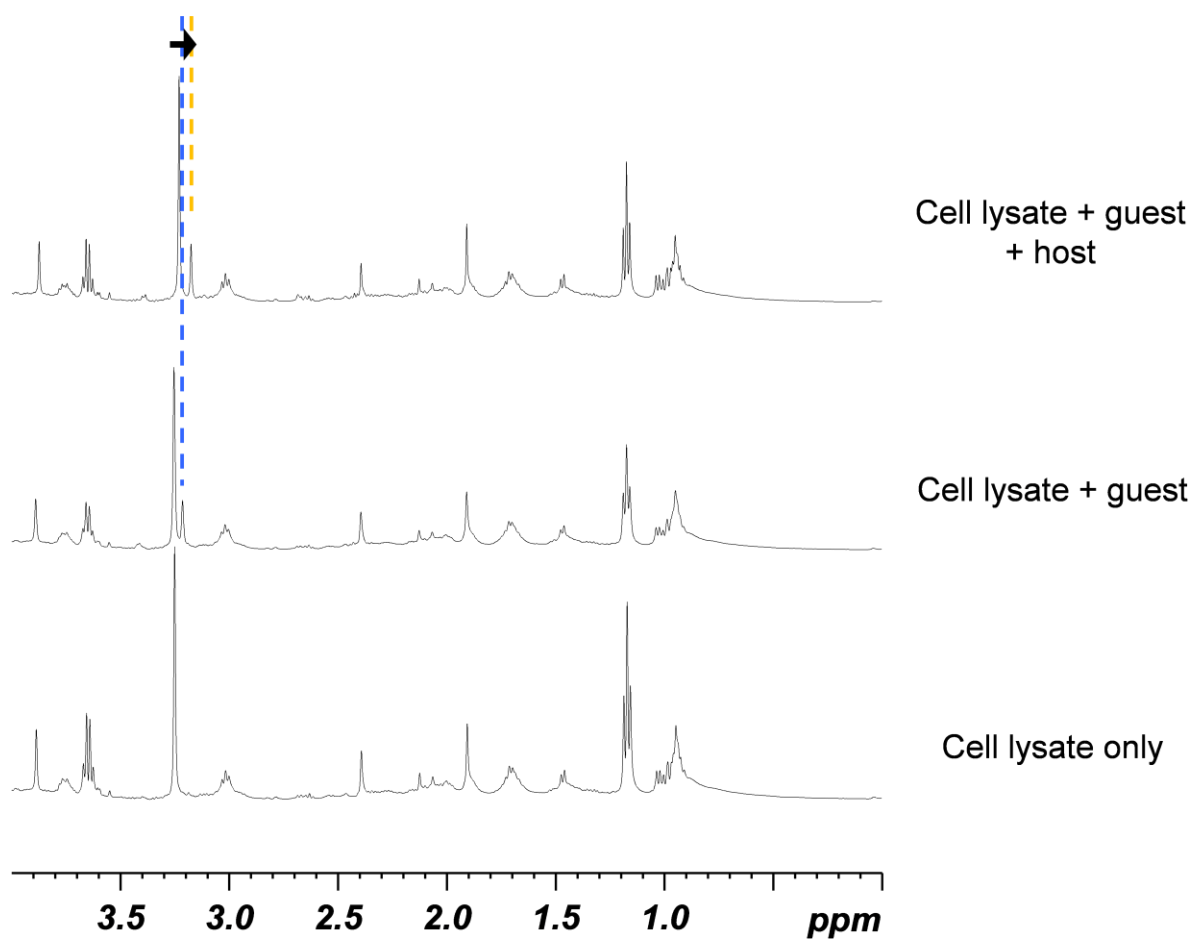
Supplementary Figure S34: Carboxycalixarene **1** recognised and bound **4** in cell lysate mixture, as indicated by changes in the N-Me resonance chemical shift of **4** (blue to orange).



Supplementary Figure S35: Carboxycalixarene **1** recognised and bound **5** in cell lysate mixture, as indicated by changes in the N-Me resonance chemical shift of **5** (blue to orange).



Supplementary Figure S36: Carboxycalixarene **1** recognised and bound **6** in cell lysate mixture, as indicated by changes in the N-Me resonance chemical shift of **6** (blue to orange).



Supplementary Figure S37: Carboxycalixarene **1** recognised and bound **7** in cell lysate mixture, as indicated by changes in the N-Me resonance chemical shift of **7** (blue to orange).

Supplementary Table 1: Intermolecular interactions found in the energy-minimised complexes of calixarene **1** with differently methylated lysines (MacroModel V.9.8, OPLS_2001 force field, MCMM, solvent: water, 20,000 steps)

interactions	guests			
	lysine (11)	methyl- lysine (10)	dimethyl- lysine (4)	trimethyl- lysine (3)
cation $\cdots\pi$	-	-	+	+
N-H \cdots O bond between guest and host (Å)	1.597	1.543 1.634	1.592	1.596
C-H $\cdots\pi$ -interactions between CH ₂ / CH ₃ groups and aromatic rings/double bonds (Å)	2.613 2.828 2.835	-	2.654 2.870 2.900 3.023	2.778 2.779 2.817 2.851 2.944
intramolecular N-H \cdots O-hydrogen bond in guest (Å)	1.752	-	-	-