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Unveiling the Formation 1:2 Supramolecular Complexes Between Cucurbit[7]uril and a Cationic Calix[4]arene Derivative.

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1. Experimental Section

ESI-MS studies

ESI-MS spectra were obtained using a Bruker Daltonics HCT *ultra* mass spectrometer under positive polarity. The ions were continuously generated by infusing aqueous solutions of complexes in MilliQ water at 4 μL min^{-1} into the mass spectrometer ion source, with the help of a syringe pump (KdScientific, model 781100, USA). The spray and ion optics conditions were optimized to detect **1**-CB7 complexes under different solution stoichiometries. Typical conditions for detection of 2:1 complexes were the following: capillary voltage, -2.8 kV; capillary exit voltage, 35.5 V; skimmer voltage, 100 V; Lens 1, -2 V; Lens 2, -25 V; Octopole 1, 9,61 V; Octopole 2, 0.0 V; Octopole RF frequency, 295.8 Vpp; Trap drive, 75.0; drying gas, 300 ºC at 10 L min^{-1}; nebulizer gas pressure, 20 psi. Signal assignments were based on the m/z values, isotope distributions and fragmentation patterns (see SI file).

NMR

All stock solutions were prepared in D_2O (99.9%). The **1**-CB7 systems for NMR measurements were prepared by mixing appropriate volumes of stock solutions of CB7 and **1**. In all cases, the concentration of **1** was kept constant, below the critical micellar concentration (cmc), while CB7 concentration was changed.

The DOSY spectra were acquired with the standard stimulated echo pulse sequence using LED and bipolar gradients pulses.[1] Rectangular-shaped pulse field gradients of 2 ms duration were applied with a power level linearly incremented from 2.1 to 64.3 G cm^{-1} in 20 steps. The gradient power was previously calibrated with the same DOSY experiment with a reference sample of 99 % D_2O at 25 °C (D = 1.872 × 10^{-9} m^2 s^{-1}). To obtain reliable results of the diffusion coefficient, the

diffusion time Δ of the experiment was optimized for each sample in order to capture smoothly the attenuation of the signal intensity, while affording the maximum difference in intensity between the traces with the maximum and minimum gradient power.

ITC

ITC experiments were carried out on a VP-ITC instrument at 25 °C under stirring at 459 rpm. The ITC experiments were performed by placing **1** or CB7 solution in the reaction cell and adding respectively a concentrated CB7 or **1** solution using a microsyringue. The concentration of **1** was always kept below the cmc to avoid heat effects arising from the dissociation of the micelles.

2. Critical micelle concentration of calixarene 1.

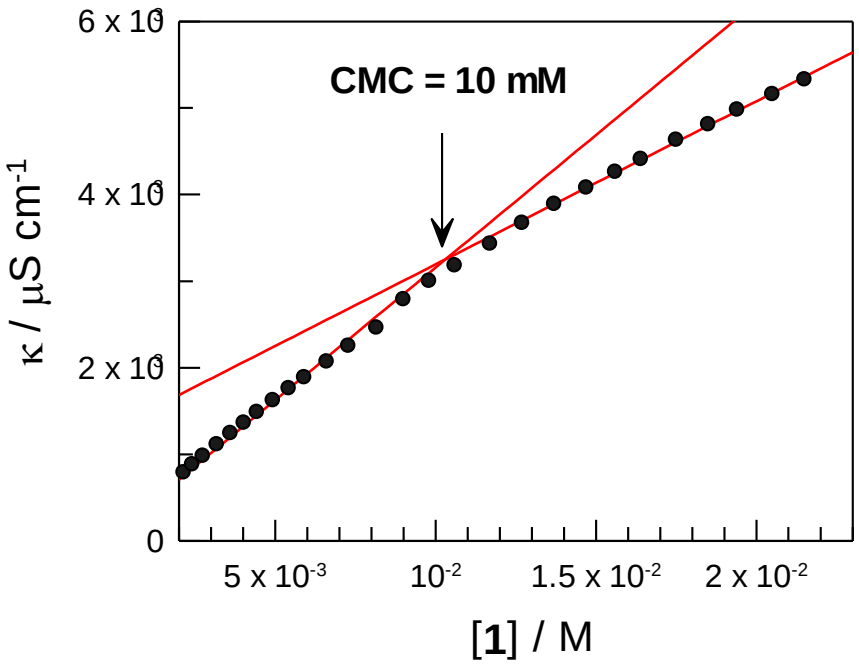


Figure S1 – Conductivity versus concentration plot for compound **1** in aqueous solution at 25 ^{0}C.

3. NMR titration of 1 by CB7.

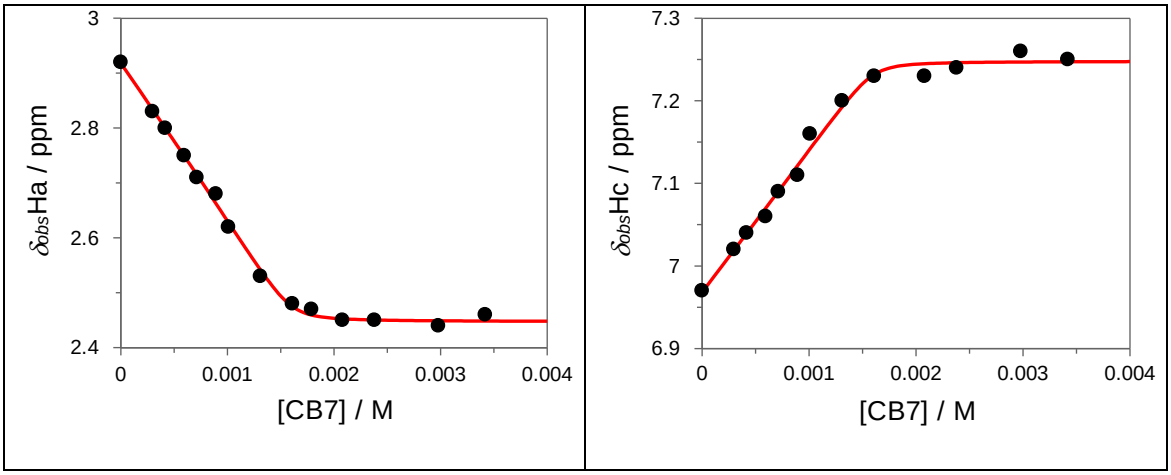


Figure S2. Observed ^{1}H NNMR chemical shifts for protons a and c of **1** plotted against the concentration of CB7 at fixed concentration of **1** (0.8 mM). All experiments were carried out in D_2O at 25 ^{o}C. The data was fitted to a 2:1, 1:1 and 1:2 (CB7:**1**) three species binding model.

4. ESI-MS fragmentation spectrum of the 1:2 (CB7:1) complex.

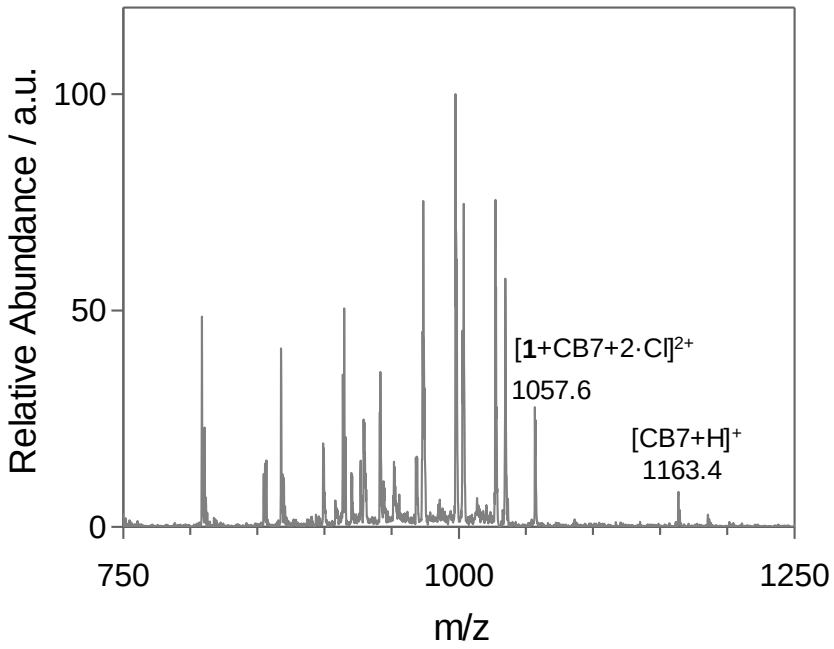
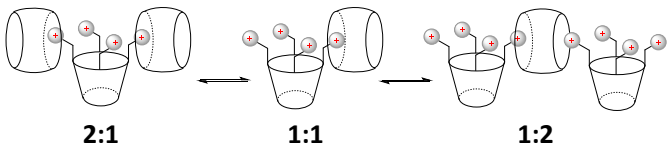


Figure S3. Fragmentation spectrum (ESI-MS) of the 2:1 (**1**:CB7) complex (1034.6).

5. Data Analysis



Scheme S1. Different host:guest complexes formed between **1** and CB7.

The formation of the 3 different types of host:guest complexes (Scheme S1) observed in mixtures of **1** and CB7 can be accounted by the following set of equilibrium and mass balance equations:

$$K_{11} = \frac{[\mathbf{1}:CB7]}{[\mathbf{1}][CB7]};\ K_{12} = \frac{[2\mathbf{1}:CB7]}{[\mathbf{1}][\mathbf{1}:CB7]};\ K_{21} = \frac{[\mathbf{1}:2CB7]}{[CB7][\mathbf{1}:CB7]} \qquad S1-S3$$

$$[\mathbf{1}]_{\mathbf{0}} = [\mathbf{1}] + [\mathbf{1}:CB7] + 2[2\mathbf{1}:CB7] + [\mathbf{1}:2CB7] \qquad S4$$

$$[CB7]_{\mathbf{0}} = [CB7] + [\mathbf{1}:CB7] + [2\mathbf{1}:CB7] + \mathbf{2}[\mathbf{1}:2CB7] \qquad S5$$

Combining the above equations:

$$[\mathbf{1}] + K_{11}[\mathbf{1}][CB7] + 2K_{11}K_{12}[\mathbf{1}]^{\mathbf{2}}[CB7] + K_{11}K_{21}[\mathbf{1}][CB7]^{\mathbf{2}} - [\mathbf{1}]_{\mathbf{0}} = 0 \qquad S6$$

$$[CB7] + K_{11}[\mathbf{1}][CB7] + K_{11}K_{12}[\mathbf{1}]^{\mathbf{2}}[CB7] + \mathbf{2}K_{11}K_{21}[\mathbf{1}][CB7]^{\mathbf{2}} - [CB7]_{\mathbf{0}} = 0 \qquad S7$$

The above system of equations can be numerically solved using the Newton-Raphson algorithm implemented in an excel spreadsheet to obtain the equilibrium concentrations of **1** and CB7 for a given set of binding constants.[2] Then, the equilibrium concentrations of the 3 complexes are obtained from equations S1-S3.

ITC

For the ITC experiments the cumulative heat (Q) involved in the mixing of **1** with CB7 is given by:[2-4]

$$Q = V(\Delta H_{1:1}[\mathbf{1}:CB7] + \Delta H_{1:2}[2\mathbf{1}:CB7] + \Delta H_{2:1}[\mathbf{1}:2CB7]) \qquad S8$$

where V is effective volume of the calorimeter cell and $\Delta H_{1:1}$, $\Delta H_{1:2}$ and $\Delta H_{2:1}$ are the apparent enthalpy associated with the formation of the respective complex.

The experimentally observed stepwise molar heat change after an injection (i), counting the corrections due to the constant cell volume of the employed VP-ITC microcalorimeter, is given by:

$$\Delta Q_i = Q_i - Q_{i-1} + \frac{\Delta V_i}{2V}(Q_i + Q_{i-1}) \qquad S9$$

The theoretical values obtained from equation S9 are then fitted to the experimentally observed calorimetric data and the K and ΔH parameters optimized using the solver tool from Microsoft Excel.

^{1}H NMR

When the exchange between two or more states is fast on the chemical shift time scale, the observed chemical shifts are given by sum to the chemical shifts of all species averaged by their respective mole fractions.[5] For the present system, the observed chemical shifts (δ_{obs}) for **1** can be expressed by the following equation:

$$\delta^{\mathbf{1}}_{obs} = \delta^{\mathbf{1}}\chi^{\mathbf{1}} + \delta^{\mathbf{1}:CB7}\chi^{\mathbf{1}:CB7} + 2\delta^{\mathbf{21}:CB7}\chi^{\mathbf{21}:CB7} + \delta^{\mathbf{1}:2CB7}\chi^{\mathbf{1}:2CB7} \qquad S10$$

As in the case of ITC, equation S10 is coupled with equations S1-S3 and S6-S7, to fit the experimental NMR data and optimize the binding constants together with the chemical shifts of free and bound species ($\delta^{\mathbf{1}}$, $\delta^{\mathbf{1}:\mathrm{CB7}}$, $\delta^{2}\mathbf{1}:\mathrm{CB7}$, $\delta^{\mathbf{1}:2\mathrm{CB7}}$)

DOSY

Similarly, the diffusion coefficients, in case of fast exchange, are given by is a mole fraction weighted average of the free and bound diffusion coefficients.[6] Equations S11 and S12 can be globally fitted to the experimental diffusion data to obtain the binding constants and the diffusion coefficients of all species.

$$D_{obs}^{\mathbf{1}} = D^{\mathbf{1}}\chi^{\mathbf{1}} + D^{\mathbf{1}:CB7}\chi^{\mathbf{1}:CB7} + 2D^{\mathbf{21}:CB7}\chi^{\mathbf{21}:CB7} + D^{\mathbf{1}:2CB7}\chi^{\mathbf{1}:2CB7} \qquad S11$$

$$D_{obs}^{CB7} = D^{CB7}\chi^{\mathbf{1}CB7} + D^{\mathbf{1}:CB7}\chi^{\mathbf{1}:CB7} + D^{\mathbf{21}:CB7}\chi^{\mathbf{21}:CB7} + 2D^{\mathbf{1}:2CB7}\chi^{\mathbf{1}:2CB7} \qquad S12$$

6. References

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