Self-assembly via ionic interactions of calix[6]arene based receptors displaying remarkable host-guest properties toward neutral guests

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To a CDCl$_3$ solution (0.60 mL) containing calix[6]tris-amine $1$ (6 mg, 5.24 µmol) and CTV $3$ (3 mg, 5.15 µmol) were successively added DMF (> 1 equiv.) and a second guest ($G > 1$ equiv.) in such a ratio that a $^1$H NMR spectra recorded at rt showed the resonances of both endo-complexes $1^{3\text{H}+}\supset\supset DMF \cdot 3^{3\text{H}+}$ and $1^{3\text{H}+}\supset\supset G \cdot 3^{3\text{H}+}$ besides the signals corresponding to the free guests (DMF and $G$). Integration of the signals of the included guests, ie. DMF$_{in}$ and $G_{in}$, and of the free guests, ie. DMF$_{free}$ and $G_{free}$, allowed us to calculate the relative affinity $K_{G/DMF}$, defined as $[G_{in}]/[DMF_{in}] \times [DMF_{free}]/[G_{free}]$ (errors estimated ±10%).

**Determination of the association constant ($K$) for the self-assembly $1^{3\text{H}+}\supset\supset DMF \cdot 3^{3\text{H}+}$**

This constant was estimated to be at least $4.5 \times 10^6$ M$^{-2}$ based on the following equilibrium:

$$1 + 3 + DMF \rightleftharpoons 1^{3\text{H}+}\supset\supset DMF \cdot 3^{3\text{H}+}$$

The $K$ is thus defined by:

$$K = \frac{[1^{3\text{H}+}\supset\supset DMF \cdot 3^{3\text{H}+}]}{([1] \times [3] \times [DMF])}$$

$$[1]_{total} = [3]_{total} = C_0 = 9 \times 10^{-3} \text{ M}$$

As the $^1$H NMR spectrum at rt of a 1:1:2 mixture of $1$, $3$ and DMF revealed the formation of $1^{3\text{H}+}\supset\supset DMF \cdot 3^{3\text{H}+}$ as the only observable species, the concentrations at the equilibrium of $1$ and $3$ have been estimated to be $\leq 0.05 \times C_0$ and the concentration of DMF $\leq 1.05 \times C_0$. The concentration of $1^{3\text{H}+}\supset\supset G \cdot 3^{3\text{H}+}$ has been estimated to be $\geq 0.95 \times C_0$.

$$K \geq \frac{0.95 \times C_0}{(0.05 \times C_0)^2[1.05 \times C_0]}$$

Thus, $K \geq 4.5 \times 10^6$ M$^{-2}$

**Determination of the association constant ($K$) for the self-assembly $1^{3\text{H}+}\supset\supset DMSO \cdot 3^{3\text{H}+}$**

This constant was estimated to be *ca.* $10^5$ M$^{-2}$ based on the following equilibrium:

$$1 + 3 + DMSO \rightleftharpoons 1^{3\text{H}+}\supset\supset DMSO \cdot 3^{3\text{H}+}$$

The $K$ is thus defined by:

$$K = \frac{[1^{3\text{H}+}\supset\supset DMSO \cdot 3^{3\text{H}+}]}{([1] \times [3] \times [DMSO])}$$

$$[1]_{total} = [3]_{total} = C_0 = 0.14 \times 10^{-3} \text{ M}$$

Half of the self-assembly $1^{3\text{H}+}\supset\supset DMSO \cdot 3^{3\text{H}+}$ was present in 0.6 mL of a 99:1 CDCl$_3$/DMSO-d$_6$ mixture at 330 K. Thus, $K = \text{ca.} 10^5$ M$^{-2}$
Figure S1. $^{13}$C NMR spectrum (75 MHz, CDCl$_3$) of the self-assembly $^3$H$^+\supset\supset$M. 3$^3$H$^+$. Solvent has been labeled "S".
Figure S2. HMQC spectrum (CDCl$_3$) of the self-assembly 1$^{3}$H$^+$$\supset$$\supset$IMI . 3$^{3}$H$^+$.
Figure S3. COSY spectrum (CDCl₃) of the self-assembly $1^{3H^+}$-$\text{IMI} \cdot 3^{3H^+}$.
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Figure S5. NOESY spectrum (CDCl$_3$, 293 K) of the self-assembly $1^{3H+}$IMI$^{-3H+}$. 
Figure S6. NOESY spectrum (CDCl$_3$, 300 K) of the self-assembly $\text{1}^{3\text{H}^+}\text{·DMF} \cdot 3^{3\text{H}^+}$. 
Figure S7. $^1$H NMR spectra at 293 K of $\text{1}^{3\text{H}^+} \supset \text{G} . 7^{3\text{H}^+}$: a) CDCl$_3$, G = IMI; b) CD$_3$OD [*: G = IMI; ○: G = CD$_3$OD].▼: included IMI; ●: free IMI. Solvent has been labeled S. "cap" and "calix" stand for the triscarboxylate subunit and for the calixarene, respectively.

Figure S8. $^1$H NMR spectra (CDCl$_3$, 294 K) of: a) $\text{1}^{3\text{H}^+} \supset \text{IMI} . 9^{3\text{H}^+}$; b) $\text{1}^{3\text{H}^+} \supset \text{IMI} . 11^{3\text{H}^+}$.▼: included IMI; ●: free IMI. Solvents and reference have been labeled S and R respectively. "cap" and "calix" stand for the triscarboxylate subunit and for the calixarene, respectively.
NMR study of the inclusion of IMI by the self-assembly $1^{3\text{H}^+} \cdot 5^{3\text{H}^+}$

I General considerations on the NMR measurements.

Unless otherwise stated, the NMR spectra were recorded on a Varian VNMRS-600 NMR spectrometer, operating at 599.974 MHz for $^1\text{H}$ ($B_0 = 14.1$ T), equipped with an inverse $^1\text{H}$-$^{13}\text{C}$-$^{15}\text{N}$ triple resonance probe. The processing comprised correction of the initial decay of the FID (free induction decay) by backward linear prediction, exponential multiplication (lb=1.5 Hz), zero-filling (digital resolution of 0.07 Hz/point for line width measurements), Fourier transformation, phase correction, chemical shift calibration (residual solvent peak: CHCl$_3$ 7.26 ppm) and base line correction.

II Equilibrium NMR spectra

The spectrum shown in figure S9.a (and in the corresponding figures of the article) was recorded at 25°C using a 90° $^1\text{H}$ excitation pulse duration of 6.1 µs, a spectral width of 9.62 kHz, an acquisition time of 2 s, a relaxation delay of 18 s and 8 transients. As mentioned in the article, two sets of signals are observed for IMI (see figure S9.a); the methylene $^1\text{H}$ signals are observed around 3 ppm and at 0.27 ppm. In the following, the signal around 3 ppm is referred to as the methylene $^1\text{H}$ signal of IMI in site A and the signal at 0.27 ppm as the methylene $^1\text{H}$ signal of IMI in site B. The global exchange process between sites A and B can be written as:

$$
\text{IMI}_A \xrightleftharpoons[k_{AB}^{-1}\text{BA}]{k_{AB}^{-1}\text{BA}} \text{IMI}_B
$$

The IMI methylene signals (sites A and B), the CTV derivative aromatic signals observed at 6.84 ppm, $\text{ArH}_{\text{CTV}}$, and the calixarene derivative aromatic signal at 6.60 ppm, $\text{ArH}_{(\text{in})_{\text{CAL}}}$, were used for the determination of the molar ratio of these species (see figure S9.a). Spectra were also recorded using a 45° $^1\text{H}$ excitation pulse and a relaxation delay of 3 s. The molar ratios obtained from these spectra and from spectra recorded with a 90° $^1\text{H}$ excitation pulse and a relaxation delay of 18 s are not significantly different. The molar ratio data are given in Table S1.

<table>
<thead>
<tr>
<th>$\text{[IMI]}$ ([Calix])</th>
<th>$\text{[CTV]}$ ([Calix])</th>
<th>$\text{[IMI}_B]$ ([Calix])</th>
<th>$\text{[IMI}_A]$ ([Calix])</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.81 ± 0.02</td>
<td>1.018 ± 0.001</td>
<td>0.987 ± 0.004</td>
<td>0.349 ± 0.001</td>
<td>4</td>
</tr>
<tr>
<td>5.78 ± 0.03</td>
<td>1.005 ± 0.004</td>
<td>0.985 ± 0.003</td>
<td>0.205 ± 0.001</td>
<td>3</td>
</tr>
</tbody>
</table>

Table S1. Molar ratios determined by $^1\text{H}$ NMR. n is the number of spectra used in the statistics. The confidence intervals are given as ± the standard deviation; they do not account for possible systematic errors in the integral measurements.
Figure S9. Equilibrium spectrum (a), 1D-EXSY spectra (τ_m = 0.8 s) recorded with (b) selective excitation at the methylene ^1H signal of IMI in site B (δ_B = 0.27 ppm) and with (c) selective excitation at the signal of IMI in site A (δ_A = 3.16 ppm) 5.0 mM of 1H+. 5H+ in CDCl_3, 5.78 equivalents of IMI, 25°C.
III  Longitudinal magnetisation transfer experiments.

Longitudinal magnetisation transfers resulting from both nuclear Overhauser effects (NOE’s) and chemical exchange were observed via one-dimensional NMR experiments using the DPFGSE (double pulsed-field-gradient spin-echo) sequence and an iburp2 shaped pulses (47 ms, 100 Hz band-width) for selective excitation of the IMI methylene $^1$H. $^1$ The spectra were recorded at 25°C (acquisition time of 0.7 s, relaxation delay of 4.3 s, spectral width of 9.62 kHz). The mixing time, $\tau_m$, was increased from 25 ms to 250 ms in 25 ms steps (10 spectra), from 300 ms to 400 ms in 50 ms steps (3 spectra), from 500 ms to 800 ms in 100 ms steps (4 spectra) and from 1.0 s to 3.5 s in 0.5 s steps (6 spectra). For each value of the mixing time, 352 transients were recorded after 4 steady-state scans. The total measurement time was approximately 11.5 h for one series of 23 spectra. Examples of spectra are shown in figures S9.b and S9.c.

The intermolecular NOE’s arising from the IMI methylene $^1$H are discussed in the article. These data and chemical shift data provide evidences that site B corresponds to IMI within the cavity of the calixarene moiety of the supramolecular host while site A corresponds to IMI exchanging between the cavity of the CTV moiety and the solvent.

At 14.1 T and 25°C, the exchange of IMI between site B (i.e. the cavity of the calixarene derivative) and other environments is slow on the IMI $^1$H spectral time scale and also slow on the IMI $^1$H longitudinal relaxation time scale.

The evolution of the normalised integrated intensity of the IMI methylene $^1$H signals, $I_A(\tau_m)/I_A^\circ$ and $I_B(\tau_m)/I_B^\circ$, are shown in figure S10 for the sample containing 3.81 equivalents of IMI. The (pseudo) first-order rate constants $k_{AB}$ and $k_{BA}$ were determined as the initial slope of the signal intensity ratio $I_B(\tau_m)/I_A(\tau_m)$ measured in experiments with selective excitation at the methylene signal of IMI in site A, and as the initial slope of the ratio $I_A(\tau_m)/I_B(\tau_m)$ measured in experiments with selective excitation at the signal of IMI in site B, respectively. $^2$ Signal intensity ratios observed for $\tau_m$ ranging between 25 ms and 400 ms were fitted to the empirical function $\alpha \tau_m + \beta \tau_m^2$, with the coefficient $\alpha$ being taken as the initial slope (see figure S11). $^3$ The results of this analysis are presented in Table S2. It shows that the values of the ratio $k_{AB}/k_{BA}$ are in excellent agreement with the molar ratios of IMI in site B and A determined independently from the equilibrium spectra (see $[\text{IMI}_B]/[\text{IMI}_A]$ in Table S1). The data indicate that the residence time of IMI in the cavity of the calixarene moiety of the supramolecular host, $\tau_B$, is of the order of a few seconds. They suggest that it slightly decreases for increasing amount of IMI in solution. This might indicate that $\tau_B$ is intrinsically affected by the presence of IMI in the CTV derivative cavity or that the escape of IMI from the cavity of the calixarene moiety is more complex that a simple first-order dissociation process.

<table>
<thead>
<tr>
<th>[IMI]</th>
<th>$k_{AB}$</th>
<th>$k_{BA}$</th>
<th>$k_{AB}/k_{BA}$</th>
<th>$\tau_B = k_{BA}^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calix</td>
<td>(s$^{-1}$)</td>
<td>(s$^{-1}$)</td>
<td>(s)</td>
<td>(s)</td>
</tr>
<tr>
<td>3.81</td>
<td>(8.86 ± 0.03)×10$^{-2}$</td>
<td>(2.54 ± 0.01)×10$^{-1}$</td>
<td>0.349 ± 0.003</td>
<td>3.94 ± 0.02</td>
</tr>
<tr>
<td>5.78</td>
<td>(5.77 ± 0.02)×10$^{-2}$</td>
<td>(2.83 ± 0.01)×10$^{-1}$</td>
<td>0.204 ± 0.002</td>
<td>3.53 ± 0.02</td>
</tr>
</tbody>
</table>

**Table S2. (pseudo) first-order rate constants characterising the exchange of IMI between sites A and B measured at 25°C. The errors are fitting errors (for $k_{AB}$ and $k_{BA}$) or are calculated using these fitting errors.**

**Figure S10.** Evolution with mixing time $\tau_m$ of the normalised integrated intensity of the IMI methylene $^1$H signals observed in longitudinal magnetisation transfer experiments with (a-b) selective excitation at the signal of IMI in site A and (c-d) selective excitation at the signal of IMI in site B. The integrated intensities at equilibrium, $I_A^\circ$ and $I_B^\circ$, were measured in a spectrum obtained with a single 90° pulse recorded with identical gain (acquisition time of 2 s, relaxation delay of 18 s, 8 transients) 5.0 mM of $^{1}$H$^+$, $^{5}$H$^+$ in CDCl$_3$, 3.81 equivalents of IMI ($\delta_A = 2.98$ ppm, $\delta_B = 0.27$ ppm), 25°C.
Figure S11. Initial evolution with mixing time $\tau_m$ of the IMI methylene $^1$H signal intensity ratios observed in longitudinal magnetisation transfer experiments with (a) selective excitation at the signal of IMI in site A and (b) selective excitation at the signal of IMI in site B. The integrated intensities were determined as the product of the height of the signals and their line width measured at half-height. Experimental data are represented by the points. Solid lines are obtained using the fitting function described in the text. Dotted lines are the initial slopes provided by the fitting (not shown for 5.78 equivalents of IMI in graph b for clarity reasons).

5.0 mM of $^3$H$^+$, 5$^3$H$^+$ in CDCl$_3$, 3.81 equivalents of IMI ($\delta_A = 2.98$ ppm) or 5.78 equivalents of IMI ($\delta_A = 3.16$ ppm), 25°C.

IV DOSY experiments.

At 14.1 T and 25°C, the exchange of IMI between the cavity of the CTV moiety of the supramolecular host and the solvent is fast on the IMI $^1$H spectral time scale. Spectra recorded at temperatures lower than 25°C show significant broadening of the methylene $^1$H signal of IMI in site A (not shown). At 9.4 T (Varian VNMRS-400 spectrometer) and -55°C a single broaden line is still observed (not shown).

The partition of IMI between the solvent and the cavity of the CTV moiety of the supramolecular host was probed by Diffusion Ordered Spectroscopy (DOSY) NMR experiments. Experiments were conducted at 25°C with a diffusion delay of 65 ms using the GCSTESL (gradient compensated stimulated echo spin lock) pulse sequence. Experiments were also run using a version of this sequence that accounts for convection effects (GCSTESL_cc). The measurements were made using 32 values of the magnitude of the gradient pulses ranging between approximately 2 G cm$^{-1}$ and 60 G cm$^{-1}$ (acquisition time 2 s, relaxation delay 8 s, spectral width 9.62 kHz, 4 steady-state scans, 32 transients); ~95% decrease in the signal intensity of the host was achieved with the largest gradient magnitude.

For the determination of the diffusion coefficient of IMI dissolved in the solvent (CDCl$_3$), 19 values of the magnitude of the gradient pulses ranging between approximately 2 G cm$^{-1}$ and 30 G cm$^{-1}$ were used (acquisition time 2 s, relaxation delay 8 s or 23 s, 4 steady-state scans, 32 transients). Diffusion coefficients and DOSY display (see Figure S12 for an example) were obtained using the Varian VNMRJ diffusion processing package.
The chemical shift of the methylene $^1$H signal observed for IMI dissolved in the solvent ($\delta_S$) and for IMI in site A ($\delta_A$) are given in Table S3.

<table>
<thead>
<tr>
<th>[H] (mM)</th>
<th>[IMI]</th>
<th>$\delta_A$ (ppm)</th>
<th>$\delta_S$ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
<td>3.55</td>
</tr>
<tr>
<td>5.0</td>
<td>3.81</td>
<td>2.98</td>
<td>-</td>
</tr>
<tr>
<td>5.0</td>
<td>5.78</td>
<td>3.16</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table S3.** Chemical shift of the methylene $^1$H signal observed for IMI dissolved in the solvent ($\delta_S$) and for IMI in site A ($\delta_A$).

The standard deviation of the diffusion coefficient of CHCl$_3$ (residual solvent peak) calculated from a total of 11 measurements on 4 different samples is 4.2%. No systematic variation was found between data measured on the 4 different samples nor between data obtained using the two different pulse sequences. The diffusion coefficient of CHCl$_3$ was thus used as an internal reference value ($D_R$).

The diffusion coefficient of IMI dissolved in the solvent ($D_S$) was determined from the methylene $^1$H signal of IMI at a concentration of ~ 10 mM (5 experiments) and ~ 2.5 mM (1 experiment). The normalised diffusion coefficient, i.e. $D_S/D_R$ where $D_R$ is the diffusion coefficient of CHCl$_3$ measured in the same experiment as $D_S$, determined for these samples were found not significantly different. This suggests that IMI dissolved in CDCl$_3$ does not aggregate in this range of concentrations.

For each experiment conducted on the samples containing 3.81 or 5.78 equivalents of IMI (2 experiments for each sample), the average diffusion coefficient obtained from the various $^1$H signal of the calixarene derivative and from those of the CTV derivative are not significantly different (see Figure S12). This is expected since these two species form a discrete [1+1] supramolecular host. Moreover, the average diffusion coefficient determined from the two $^1$H signals of IMI in site B is not significantly different from the average value measured for the supramolecular host (see Figure S12). This is also expected since site B corresponds to IMI included in the calixarene moiety of the host and because the exchange of IMI between site B and other environments is slow on the IMI $^1$H spectral time scale. On the contrary, the average diffusion coefficient determined from the two $^1$H signals of IMI in site A is much higher (see Figure S12). The average values of the normalised diffusion coefficients provided by different experiments are quoted in Table S4. It shows that the value for IMI in site A, $D_A/D_R$, is significantly different in the two samples. They are higher than the value determined for the host ($D_H/D_R$) but smaller than the value determined for IMI dissolved in the solvent ($D_S/D_R$).

The normalised diffusion coefficient of IMI in site A can be written as:

$$ \frac{D_A}{D_R} = \frac{D_S}{D_R} + x(\frac{D_H}{D_R} - \frac{D_S}{D_R}) \quad (D1) $$

where $x$ is the mole fraction associated with the fast exchange between IMI included in the cavity of the CTV moiety and IMI free in solution. Equation (D1) permits the calculation of $x$ if the normalised diffusion coefficient of IMI free in solution is assumed identical to the value measured for IMI dissolved in the solvent. The results are quoted in Table S4.
Table S4. Average values of the experimental normalised diffusion coefficients and $x$ values calculated using equation (D1).

The errors on the normalised diffusion coefficients are standard deviation values calculated from 4 experimental $D_H/D_R$ ratios, 2 experimental $D_A/D_R$ ratios or 5 experimental $D_S/D_R$ ratios. The error on $x$ is twice the value provided by error propagation calculation.

![Figure S12](image_url)

**Figure S12.** DOSY display of the diffusion coefficients measured for the sample containing 5.78 equivalents of IMI.
V. Estimation of the apparent inclusion constants.

The self-assembly $1^{3H^+} \cdot 5^{3H^+}$ possesses two cavities: the cavity defined by the calixarene derivative moiety and the cavity defined by the CTV derivative moiety. Therefore, the following equilibriums should a priori be taken into account:

1. $\text{IMI}_F + H_{OO} \leftrightarrow H_{XO}$ with $K_{1X} = \frac{[H_{XO}]}{[\text{IMI}_F][H_{OO}]}$ (1)
2. $\text{IMI}_F + H_{OO} \leftrightarrow H_{OV}$ with $K_{1V} = \frac{[H_{OV}]}{[\text{IMI}_F][H_{OO}]}$ (2)
3. $\text{IMI}_F + H_{OV} \leftrightarrow H_{XV}$ with $K_{2X} = \frac{[H_{XV}]}{[\text{IMI}_F][H_{OV}]}$ (3)
4. $\text{IMI}_F + H_{XO} \leftrightarrow H_{XV}$ with $K_{2V} = \frac{[H_{XV}]}{[\text{IMI}_F][H_{XO}]}$ (4)

where $\text{IMI}_F$ stands for IMI free in the solvent.

$H_{OO}$ is the host that does not contain IMI molecules

$H_{XO}$ is the host that contains a single IMI molecule in the calixarene moiety

$H_{OV}$ is the host that contains a single IMI molecule in the CTV moiety

$H_{XV}$ is the host that contains an IMI molecule in both moieties

The corresponding mass balance equations are:

$$[\text{IMI}] = [\text{IMI}_F] + [H_{XO}] + [H_{OV}] + 2[H_{XV}] = [\text{IMI}_A] + [\text{IMI}_B]$$ (5)

$$[H] = [H_{OO}] + [H_{XO}] + [H_{OV}] + [H_{XV}]$$ (6)

where $[\text{IMI}]$ and $[H]$ stand for the total concentration of IMI and host, respectively.

Site A corresponds to IMI molecules exchanging between the cavity of the CTV moiety and the solvent. The concentration of IMI in site A is thus given by:

$$[\text{IMI}_A] = [\text{IMI}_F] + [H_{OV}] + [H_{XV}]$$ (7)

Site B is the cavity of the calixarene moiety; the concentration of IMI in site B is thus given by:

$$[\text{IMI}_B] = [H_{XO}] + [H_{XV}]$$ (8)

The molar ratios given in Table S1 can now be rewritten as:

$$\frac{[\text{IMI}]}{[\text{Calix}]} = \frac{[\text{IMI}]}{[H]}$$ (9)

$$\frac{[\text{IMI}_B]}{[\text{Calix}]} = \frac{[\text{IMI}_B]}{[H]} = \frac{[H_{XO}] + [H_{XV}]}{[H]}$$ (10)

$$\frac{[\text{IMI}_B]}{[\text{IMI}_A]} = \frac{[\text{IMI}_B]}{[\text{IMI}_B]} = \frac{[H_{XO}] + [H_{XV}]}{[\text{IMI}_F] + [H_{OV}] + [H_{XV}]}$$ (11)

V.1 Estimation of the apparent inclusion constant of IMI in the cavity of the CTV moiety.

The values of the ratio $[\text{IMI}]/[\text{Calix}]$ given in Table S1 indicate that, for both samples, the filling of the cavity of the calixarene moiety (i.e. site B) by IMI reaches 99%. This implies that the affinity of the calixarene moiety for IMI is high and, consequently, that the
concentration of the species $H_{OO}$ and $H_{OV}$ is almost zero in the samples under study. Therefore, $K_{1V}$ cannot be determined on the basis of the experimental data obtained in this work (see equation 2) but $K_{2V}$ can be estimated.

Neglecting the concentration of the species $H_{OV}$, equation (7) can be approximated by:

$$[\text{IMI}_A] = [\text{IMIF}] + [H_{XV}]$$

Therefore, the mole fraction governing the observed experimental data related to the fast exchange of IMI between the solvent and the cavity of the CTV moiety ($\delta_A$, $D_A$) is given by:

$$x = \frac{[H_{XV}]}{[\text{IMIF}] + [H_{XV}]}$$

Simple transformations of this equation lead to:

$$\frac{[\text{IMIF}]}{[H]} = 1 - x \frac{[H_{XV}]}{[H]}$$

Neglecting the concentration of the species $H_{OO}$ and $H_{OV}$ in equations (5) and (6) leads to:

$$\frac{[\text{IMIF}]}{[H]} = \frac{[\text{IMI}]}{[H]} - \frac{[H_{XO}]}{[H]} - 2\frac{[H_{XV}]}{[H]}$$

$$\frac{[H_{XO}]}{[H]} = 1 - \frac{[H_{XV}]}{[H]}$$

Equations (14-16) can be transformed to give:

$$\frac{[H_{XV}]}{[H]} = x \left( \frac{[\text{IMI}]}{[H]} - 1 \right)$$

Equations (14-17) and (4) with data of Table S4 ([IMI]/[H] ratio, see equation 9, and $x$ values as determined by DOSY experiments), provide the results quoted in the first two rows of Table S5.

<table>
<thead>
<tr>
<th>[IMI]</th>
<th>$x$</th>
<th>$[H_{XV}]$</th>
<th>$[H_{XO}]$</th>
<th>$[\text{IMIF}]$</th>
<th>$K_{2V}$ [H]</th>
<th>$K_{2V}$ (L mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.78</td>
<td>D</td>
<td>0.22 ± 0.02</td>
<td>1.05</td>
<td>0</td>
<td>3.8</td>
<td>-</td>
</tr>
<tr>
<td>3.81</td>
<td>D</td>
<td>0.27 ± 0.02</td>
<td>0.76</td>
<td>0.24</td>
<td>2.05</td>
<td>1.5</td>
</tr>
<tr>
<td>3.81</td>
<td>$\delta$</td>
<td>0.30</td>
<td>0.84</td>
<td>0.16</td>
<td>1.97</td>
<td>2.7</td>
</tr>
</tbody>
</table>

**Table S5.** Data related to the calculation of the inclusion constant $K_{2V}$ defined by equation (4) using $x$ values as determined by diffusion (D) or chemical shift ($\delta$) data at 25°C. [H] is 5.0 mM.

These results suggest that for the sample containing 5.78 equivalents of IMI, the filling of both cavities of the supramolecular host by IMI is essentially 100%. Therefore, for that sample, $[H_{OO}]=[H_{XO}]=[H_{OV}]=0$, $[H_{XV}]=[H]$ and $[\text{IMIF}]=[\text{IMI}]-2[H]$ (see equations 5-6). Equation (13) thus leads to:

$$x = \frac{[H]}{[\text{IMI}]-[H]} = \left( \frac{[\text{IMI}]}{[H]} - 1 \right)^{-1}$$

For the sample containing 5.78 equivalents of IMI, it gives $x = 0.209$. The chemical shift of the methylene $^1$H signal of IMI in site A ($\delta_A$) can be approximated by: 
\[
\delta_A = \delta_S + x (\delta_V - \delta_S)
\]  
(19)

where \(\delta_S\) is the chemical shift of the methylene \(^1\)H signal of IMI free in the solvent and \(\delta_V\) the corresponding value for IMI included in the cavity of the CTV moiety.

Using the chemical shift quoted in Table S3 (\(\delta_A = 3.16\) ppm and \(\delta_S = 3.55\) ppm) and \(x = 0.209\), equation (19) provides \((\delta_V - \delta_S) = -1.87\) ppm. Using this value and the \(\delta_A\) value observed for the sample containing 3.81 equivalents of IMI (2.98 ppm, see Table S3), equation (19) gives \(x = 0.30\), in agreement with the value determined via DOSY experiments (see Table S5). The corresponding \(K_{2V}\) value is given in Table S5.

V.2 Estimation of the apparent inclusion constant of IMI in the cavity of the Calixarene moiety.

The experimental data are not suitable for an accurate determination of the inclusion constants \(K_{1X}\) and \(K_{2X}\) since the concentration of the species \(H_{OO}\) and \(H_{OV}\) are close to zero (see equations 1 and 3). Assuming that the affinity of the calixarene moiety for IMI is not affected by the presence of an IMI molecule in the CTV moiety, a global inclusion constant can be defined as:

\[
K_X = \frac{[H_{XO}][H_{XV}]}{[H][IMI_F]([H_{OO}][H_{OV}])}
\]  
(20)

Using equation (6) and (8), it can be rewritten as equations (21) or (22):

\[
K_X = \frac{[IMI_B]}{[IMI_F]([H]-[IMI_B])}
\]  
(21)

\[
K_X [H]= \frac{[IMI_B]}{[H] \left(1- \frac{[IMI_B]}{[H]} \right)}
\]  
(22)

Considering that \([IMI_B]/[H] \geq 0.99\) (see Table S1 and equation 10) and using \([IMI_B]/[H] \approx 2\) (see Table S5 for the sample containing 3.81 equivalents of IMI), equation (22) leads to :

\[
K_X [H] \geq 50 , \text{ i.e. } K_X \geq 10^4 \text{ L mol}^{-1} \ (\text{[H]} = 5.0 \text{ mM})
\]