

## Supporting Information

### The Inverted Cucurbit[*n*]uril Family

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## Experimental Section.

**General.** The guests used in this study were purchased from commercial suppliers and were used without further purification. The crude cucurbit[*n*]uril (CB[*n*]) mixture was prepared according to the literature procedures.<sup>1,2</sup> Gel permeation chromatography was performed using Sephadex G-15 or Superdex 30. Melting points were measured on a Meltemp apparatus in open capillary tubes and are uncorrected. IR spectra were recorded on a Nicolet Magna or on a Perkin Elmer Spectrum GX FT-IR spectrophotometers as KBr pellets and are reported in  $\text{cm}^{-1}$ . NMR spectra were measured on a Bruker AM400 or on a Bruker DRX500 spectrometers operating at 400 or 500 MHz for  $^1\text{H}$  and 100 or 125 MHz for  $^{13}\text{C}$ . 2D NMR experiments were performed using the standard pulse sequences supplied by the manufacturer. Mass spectrometry was performed on a JEOL AccuTOF electrospray instrument, or on an ABI 4700 Proteomics Analyzer MALDI-TOF instrument. The formation constants for the inclusion of several guests in *i*CB[7] were determined by isothermal titration calorimetry using a VP-ITC instrument from MicroCal. All solutions were prepared in purified water (Milli-Q, Millipore). A solution (0.2 mM) of *i*CB[7] was placed in the sample cell. As 5 mM solution of guests was added in a series of fifty injections (4  $\mu\text{L}$ ), the heat evolved was recorded at 30 °C.

**Purification of *i*CB[6] Fractional Recrystallization.** A crude CB[*n*] reaction mixture prepared from 80 g of glycoluril by the literature method<sup>2</sup> was used in subsequent purification steps.

**Initial processing.** The reaction mixture which contains a large amount of solid was evaporated to a minimum volume. This slurry was poured into water (250 mL). The solid was collected by filtration to give the first crop (Crop 1) (contains: CB[6], CB[7], CB[8], and some *i*CB[6]). The filtrate was evaporated to about 60 mL and then slowly poured into a mixture of MeOH (300 mL) and water (20 mL) with vigorous stirring. After stirring overnight, the precipitate was obtained by filtration to give a second crop (Crop 2 contains CB[7], CB[6], and CB[5]).

**Subsequent purification.** The separation of each component (CB[5], CB[6], CB[7], CB[8], and *i*CB[6]) from Crop 1 and Crop 2 was enabled due to their differential solubility in HCl solutions.

- **CB[5] and CB[7].** CB[5] and CB[7] were isolated in pure form using the literature procedure<sup>1</sup> which relies on the solubility of both CB[5] and CB[7] in water and the moderate solubility of CB[5] in 50% aqueous MeOH (v/v).
- **CB[8].** CB[6] and *i*CB[6] have appreciable solubility in 3.5 M HCl solution whereas CB[8] is substantially less soluble. By washing the crude mixture of CB[6], CB[8], and *i*CB[6] with 3.5 M HCl it is possible to isolate CB[8] as an insoluble solid.
- ***i*CB[6] and CB[6].** CB[6] and *i*CB[6] were separated by fractional crystallization from different concentration HCl solutions. For example, the initial CB[6] / *i*CB[6] mixture was recrystallized from a minimum volume of conc. HCl. The filtrate is enriched in *i*CB[*n*]; adding the filtrate to MeOH gives the precipitate which is filtered and dried. The solid now enriched in *i*CB[6] is recrystallized from 17.5% HCl which gives CB[6] as a solid and filtrate further enriched in *i*CB[6]. In this manner, the ratio of *i*CB[6]:CB[6] is raised to  $\approx 80:20$ . At this point, the mixture is dissolved in a minimum of conc. HCl. To this solution is added enough

H<sub>2</sub>N(CH<sub>2</sub>)<sub>6</sub>NH<sub>2</sub> to complex all of the CB[6] (≈ 25%). The solution is then diluted 5-fold with H<sub>2</sub>O. The precipitate is isolated by centrifugation and washed several times with H<sub>2</sub>O which yields *i*CB[6] as a white solid in 2% overall yield.

***Purification of iCB[7] by Gel Permeation Chromatography.***

*Initial processing.* A partially purified CB[*n*] mixture (20 g) was stirred in aqueous 0.15 M NH<sub>4</sub>HCO<sub>3</sub> solution (500 mL) for 3 h. The insoluble solid (mostly CB[6] and *i*CB[6] (~86 %, 5:4), and some CB[8] (7 %)) was filtered off, and methanol (1 L) was added in small portions to the filtrate. The fine precipitate was collected by filtration to give the first crop (contains: CB[7], *i*CB[7] (~94%, 4:1), and some CB[6] and *i*CB[6] (~6%)). This procedure was repeated once to enrich the solid in *i*CB[7] (up to CB[7]:*i*CB[7] ≈ 3:2).

*Purification of iCB[7] by GPC.* A sample enriched in *i*CB[7] (~2 g) dissolved in 10 mL of 0.15 M NH<sub>4</sub>HCO<sub>3</sub> solution was injected on a Superdex™ 30 column (HiLoad™ Prep Grade, 26 x 600 mm). Elution with 0.15 M NH<sub>4</sub>HCO<sub>3</sub> solution in flow rate of 2 mL/min, while monitoring at 184 nm, provided baseline separation between CB[7] (retention volume, R<sub>v</sub> = 270~530 mL) and *i*CB[7] (R<sub>v</sub> = 660~890 mL). After evaluating the purity of selected fractions by <sup>1</sup>H NMR spectra, fractions were combined and lyophilized to obtain pure *i*CB[7] (69 mg).

***Characterization of iCB[6] and iCB[7].***

*i*CB[6]. White solid. M.p. > 300 °C. IR (KBr, cm<sup>-1</sup>): 3446s, 2994w, 2927w, 2850w, 1735s, 1478s, 1417m, 1377m, 1328m, 1238s, 1192m, 966m, 803s. <sup>1</sup>H NMR (400 MHz, 35% DCl / D<sub>2</sub>O): 5.60 (d, *J* = 8.5, 2H), 5.48 (d, *J* = 8.5, 2H), 4.50 – 4.45 (ABq, 4H), 5.42 (s, 2H), 5.32 (d, *J* = 15.7, 4H), 5.30 (d, *J* = 15.7, 4H), 5.13 (d, *J* = 14.0, 4H), 5.02 (s, 2H), 4.34 (d, *J* = 14.0, 4H), 4.32 (d, *J* = 15.7, 4H), 4.20 (d, *J* = 15.7, 4H). <sup>13</sup>C NMR (100 MHz, 35% DCl / D<sub>2</sub>O): 157.1, 156.9, 156.3, 156.0, 70.9, 70.6, 70.3, 70.1, 69.8, 62.8, 52.0, 51.7, 51.4. MS (ES): *m/z* 997 (100, [M + H]<sup>+</sup>). HR-MS (ES): *m/z* 997.3219 ([M + H]<sup>+</sup>, C<sub>36</sub>H<sub>37</sub>N<sub>24</sub>O<sub>12</sub>, calcd 997.3023). Anal. Calcd for C<sub>36</sub>H<sub>36</sub>N<sub>24</sub>O<sub>12</sub>•(H<sub>2</sub>O)<sub>6</sub>: C 39.13, H 4.37, N 30.42. Found: C 39.38, H 4.38, N 30.24.

*i*CB[7]. White solid. M.p. > 350 °C. IR (KBr, cm<sup>-1</sup>): 3445s, 2994w, 2928w, 1734s, 1474s, 1420m, 1377m, 1325m, 1233m, 1192m, 967m, 807s. <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O/NaCl, TSP): 5.82 (d, *J* = 15.2, 6H), 5.81 (d, *J* = 15.6, 4H), 5.71 (d, *J* = 8.5, 2H), 5.65 – 5.55 (m, 10H), 5.48 (d, *J* = 13.7, 4H), 5.39 (br, 2H), 4.49 (d, *J* = 13.7, 4H), 4.37 (d, *J* = 15.6, 4H), 4.30 (d, *J* = 15.2, 4H), 4.28 (d, *J* = 14.0, 2H). <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O): 157.5, 157.0, 156.9, 156.7, 71.9, 71.8, 71.7, 71.4, 71.0, 64.4, 53.3, 53.1, 52.7, 52.5. MS (MALDI-TOF): *m/z* 1163 ([M + H]<sup>+</sup>). HR-MS (MALDI-TOF): *m/z* 1163.3186 (100, [M + H]<sup>+</sup>, C<sub>42</sub>H<sub>43</sub>N<sub>28</sub>O<sub>14</sub>, calcd 1163.3508). Anal. Calcd for C<sub>42</sub>H<sub>42</sub>N<sub>28</sub>O<sub>14</sub>•(H<sub>2</sub>O)<sub>9</sub>: C 38.06, H 4.56, N 29.59. Found: C 38.22, H 4.82, N 29.69.

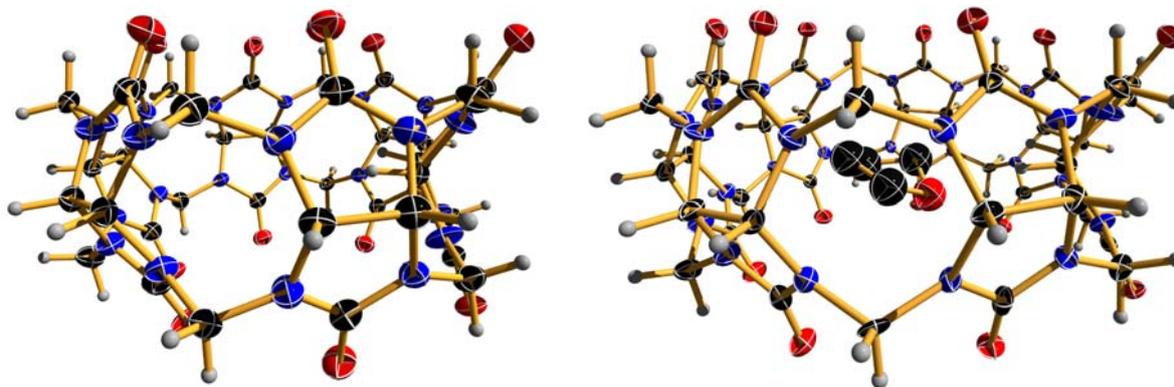
References:

- (1) Kim, J.; Jung, I. S.; Kim, S.-Y.; Lee, E.; Kang, J.-K.; Sakamoto, S; Yamaguchi, K.; Kim, K. *J. Am. Chem. Soc.* **2000**, *122*, 540-541.
- (2) Day, A. I.; Arnold, A. P.; Blanch, R. J.; Snushall, B. *J. Org. Chem.* **2001**, *66*, 8094-8100.

### ***X-ray crystallography.***

A colorless plate of *i*CB[6] with approximate dimensions  $0.022 \times 0.230 \times 0.380 \text{ mm}^3$ , was used for the X-ray crystallographic analysis. The X-ray intensity data were measured at 213(2) K on a three-circle diffractometer system equipped with a Bruker Smart1000 CCD area detector using a graphite monochromator and a MoK $\alpha$  fine-focus sealed tube ( $\lambda = 0.71073 \text{ \AA}$ ). Data were corrected for absorption effects with the semi-empirical method using SADABS. The structure was solved and refined using the SHELXS-97 and SHELXL-97 software. The final anisotropic full-matrix least-squares refinement on  $F^2$  converged to the R values listed in Table S1.

The diffraction data from a colorless block-shaped crystal of *i*CB[7] measuring  $0.21 \times 0.19 \times 0.06 \text{ mm}^3$  mounted on the loop were collected at 100 K on a ADSC Quantum 210 CCD diffractometer with synchrotron radiation ( $\lambda = 1.00000 \text{ \AA}$ ) at Macromolecular Crystallography Wiggler Beamline 4A, Pohang Accelerator Laboratory (PAL), Pohang, Korea. The crystal was rotated through a total of  $180^\circ$ . The autoindexing procedure performed with DENZO indicated that the crystals belong to a rhombohedral space group, with unit-cell parameters  $a = 32.200(5) \text{ \AA}$ ,  $c = 32.581(7) \text{ \AA}$ ,  $\gamma = 120^\circ$ . The raw data were processed and scaled using the program HKL2000. The space group was determined to be R-3. A total of 15222 measured reflections were merged into 6769 independent reflections. The structure was solved by directed methods and refined by full-matrix least-squares method implemented in SHELXTL program package. All the non-hydrogen atoms were refined anisotropically except included THF. Hydrogen atoms were added to their geometrically ideal positions. The crystallographic data are summarized in Table S2.



**Figure S1.** Another views of *i*CB[6] (left) and *i*CB[7] (right). Anisotropic atomic displacement ellipsoids for the non-hydrogen atoms are shown at the 50% probability level. A THF molecule is encapsulated in the cavity of *i*CB[7]. Water molecules and Cl<sup>-</sup> are omitted for clarity.

**Table S1.** X-ray crystal data for *i*CB[6]•H<sub>3</sub>O<sup>+</sup>•Cl<sup>-</sup>•8.7H<sub>2</sub>O

Empirical formula	C <sub>36</sub> H <sub>56.40</sub> Cl N <sub>24</sub> O <sub>21.70</sub>
Formula weight	1208.10
Temperature	213(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P 2 <sub>1</sub>
Unit cell dimensions	$a = 12.434(2)$ Å $\alpha = 90^\circ$ $b = 16.122(3)$ Å $\beta = 104.923(4)^\circ$ $c = 12.667(2)$ Å $\gamma = 90^\circ$
Volume	2453.6(8) Å <sup>3</sup>
Z	2
Density, $\rho_{\text{calc}}$	1.635 g/cm <sup>3</sup>
Absorption coefficient, $\mu$	0.188 mm <sup>-1</sup>
F(000)	1262
Crystal size	0.380 0.230 × 0.022 mm <sup>3</sup>
Index ranges	-14 ≤ <i>h</i> ≤ 10, -18 ≤ <i>k</i> ≤ 19, -14 ≤ <i>l</i> ≤ 14
Reflections collected	8493
Independent reflections	7318 [R(int) = 0.0193]
Observed reflection, I>2σ(I)	5794
Data / restraints / parameters	7318 / 34 / 850
Goodness-of-fit on F <sup>2</sup>	0.998
Final R indices [I>2σ(I)]	R <sub>1</sub> = 0.0431, wR <sub>2</sub> = 0.0998
R indices (all data)	R <sub>1</sub> = 0.0649, wR <sub>2</sub> = 0.1129
Largest diff. peak and hole	0.400 and -0.311 e <sup>-</sup> /Å <sup>3</sup>

$$R_1 = \frac{\sum ||F_o| - |F_c||}{\sum |F_o|}, \quad wR_2 = \left[ \frac{\sum w(F_o^2 - F_c^2)^2}{\sum w(F_o^2)^2} \right]^{1/2}$$

**Table S2.** X-ray crystal data for *i*CB[7]•THF•14H<sub>2</sub>O.

Empirical formula	C <sub>46</sub> H <sub>78</sub> N <sub>28</sub> O <sub>29</sub>	
Formula weight	1487.36	
Temperature	100(2) K	
Wavelength	1.00000 Å <sup>a</sup>	
Crystal system	Rhombohedral	
Space group	R-3	
Unit cell dimensions	$a = 32.200(5)$ Å	$\alpha = 90^\circ$
	$b = 32.200(5)$ Å	$\beta = 90^\circ$
	$c = 32.581(7)$ Å	$\gamma = 120^\circ$
Volume	$29255(8)$ Å <sup>3</sup>	
Z	18	
Density (calculated)	$1.520$ g/cm <sup>3</sup>	
Absorption coefficient	$0.301$ mm <sup>-1</sup>	
F(000)	14076	
Crystal size	$0.21 \times 0.19 \times 0.06$ mm <sup>3</sup>	
Index ranges	$-18 \leq h \leq 32, -32 \leq k \leq 21, -32 \leq l \leq 27$	
Reflections collected	15222	
Independent reflections	6769 [R(int) = 0.0992]	
Observed reflection, I>2σ(I)	5794	
Data / restraints / parameters	6769 / 217 / 1002	
Goodness-of-fit on F <sup>2</sup>	1.613	
Final R indices [I>2σ(I)]	R <sub>1</sub> = 0.0967, wR <sub>2</sub> = 0.3179	
R indices (all data)	R <sub>1</sub> = 0.0979, wR <sub>2</sub> = 0.3226	
Extinction coefficient	0.00046(9)	
Largest diff. peak and hole	1.173 and -0.797 e <sup>-</sup> /Å <sup>3</sup>	

$$R_1 = \frac{\sum ||F_o| - |F_c||}{\sum |F_o|}, \quad wR_2 = \left[ \frac{\sum w(F_o^2 - F_c^2)^2}{\sum w(F_o^2)^2} \right]^{1/2}$$

<sup>a</sup> The x-ray data were collected with synchrotron radiation at Macromolecular Crystallography Wiggler Beamline 4A, Pohang Accelerator Laboratory (PAL).

Acq. Data Name: SL new CB(6), 996, es  
Internal Sample Id:  
Ionization Mode: ESI+  
MS Calibration Name: Duo\_ESI\_pos  
Reduction History: Correct Base[5.0%];Average(MS[1]) 0.063..0.628  
Experiment Date/Time: 7/28/2005 2:22:59 PM

Orifice1 Volt Sweep: 91V  
Acquired m/z Range: 100.0..1500.0

Spec. Record Interval: 0.4[s]  
Ring Lens Volt: 7[V]  
Time of Maximum: 0.143[min]  
Operator Name: Administrator

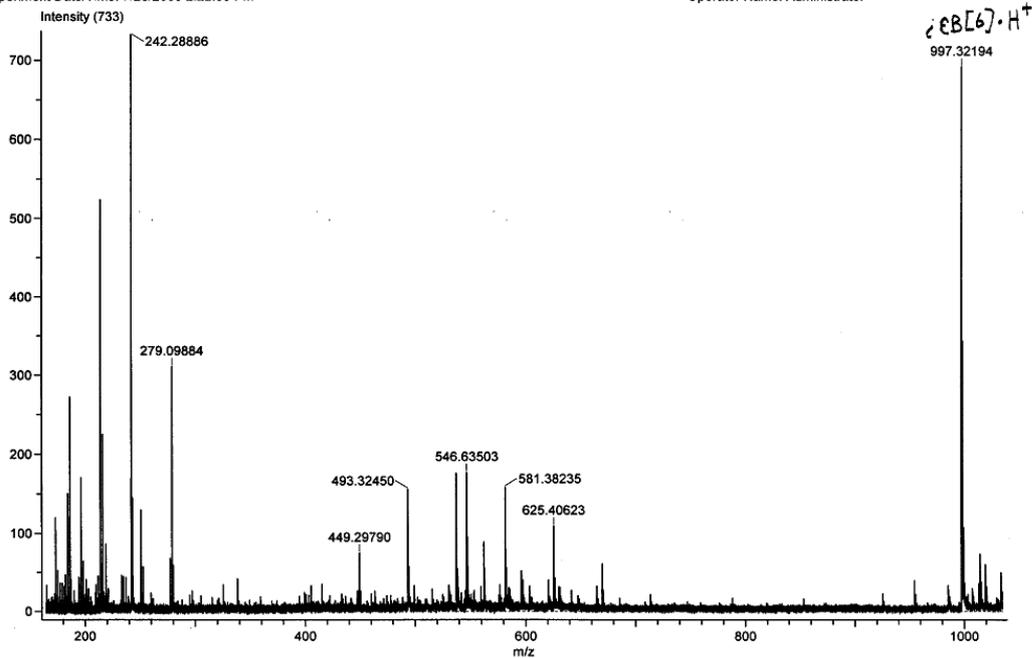


Figure S2. ES-MS spectrum of *iCB[6]*.

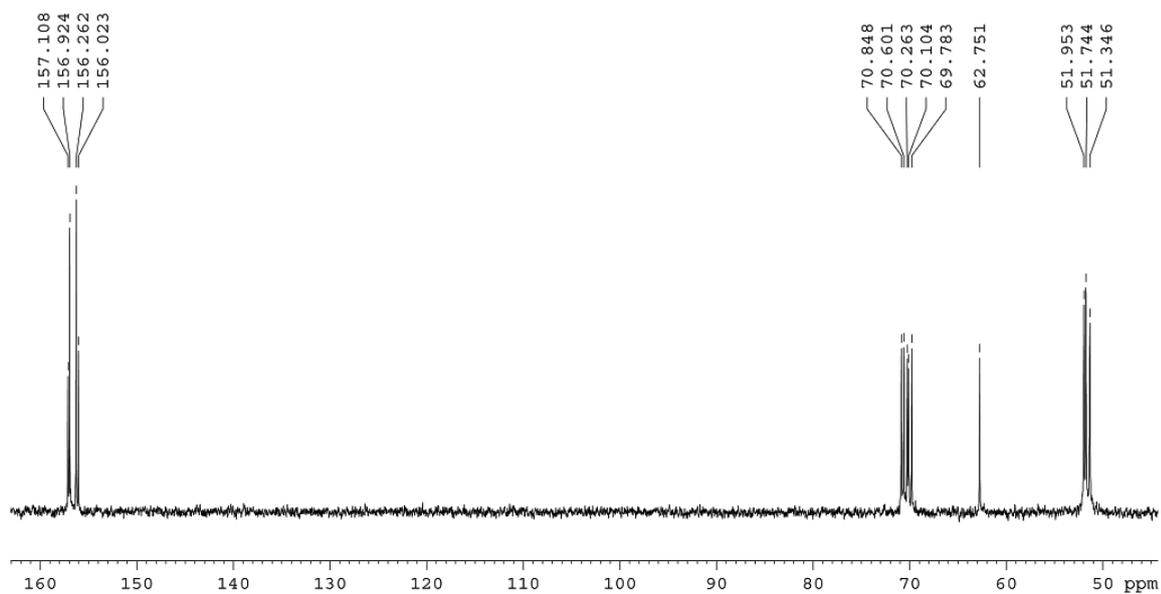


Figure S3.  $^{13}C$  NMR spectrum (100 MHz, RT, 35% DCl / D<sub>2</sub>O) for *iCB[6]*.

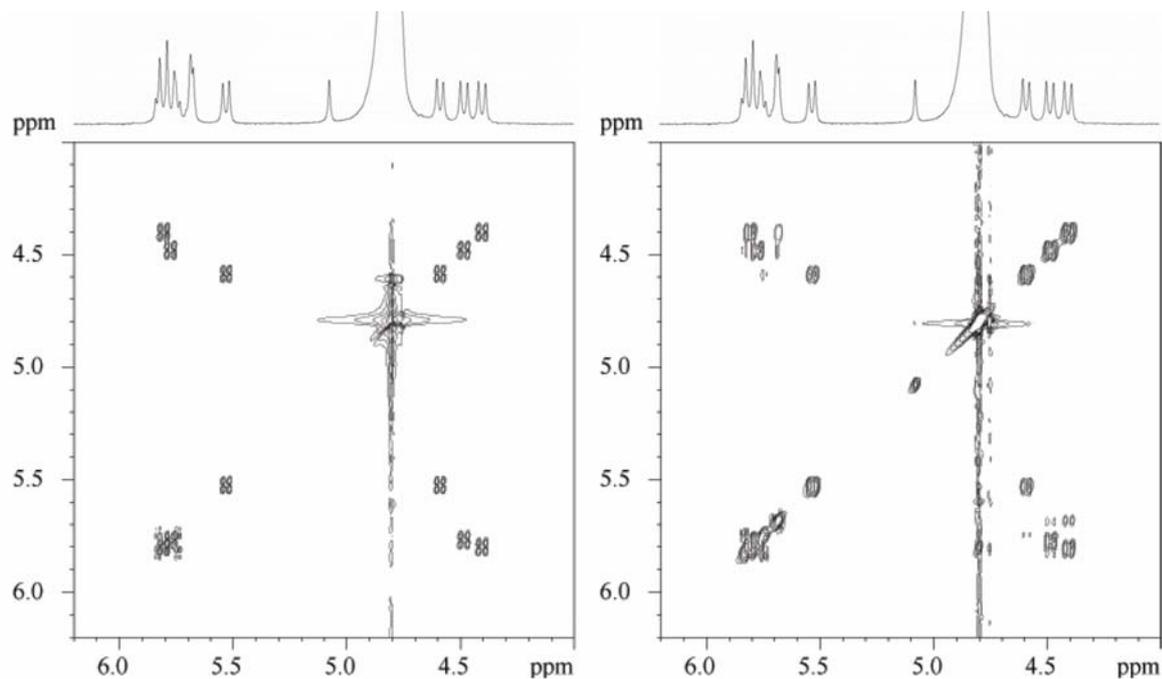


Figure S4. DQF-COSY and ROESY spectra of *i*CB[6].

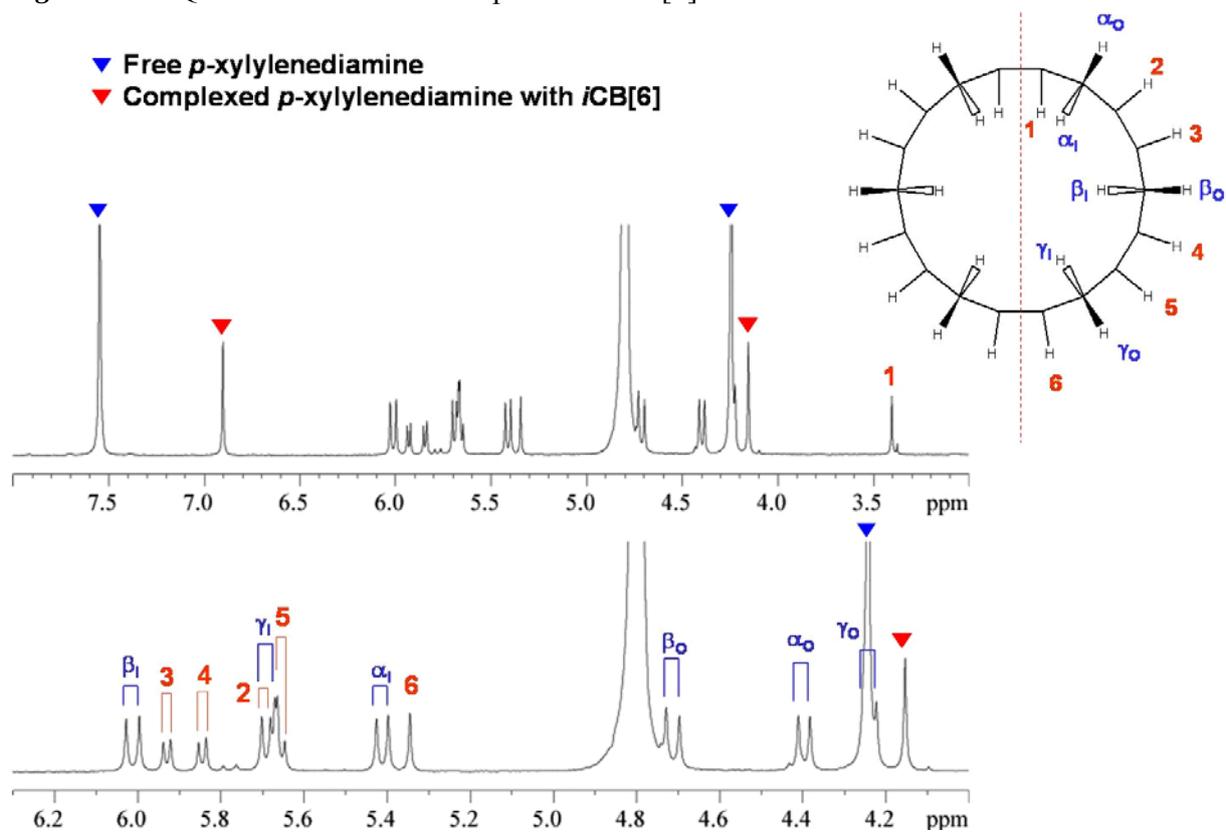
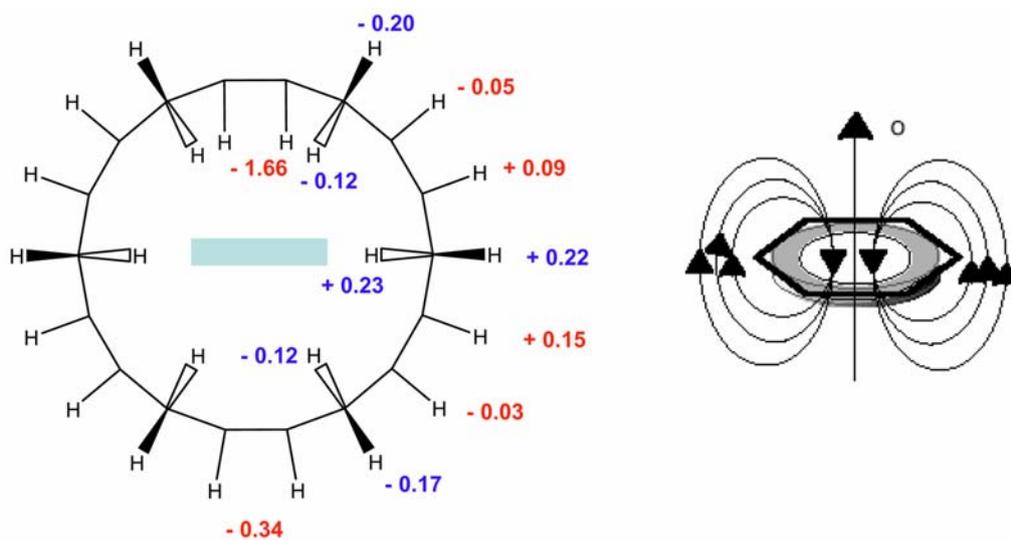
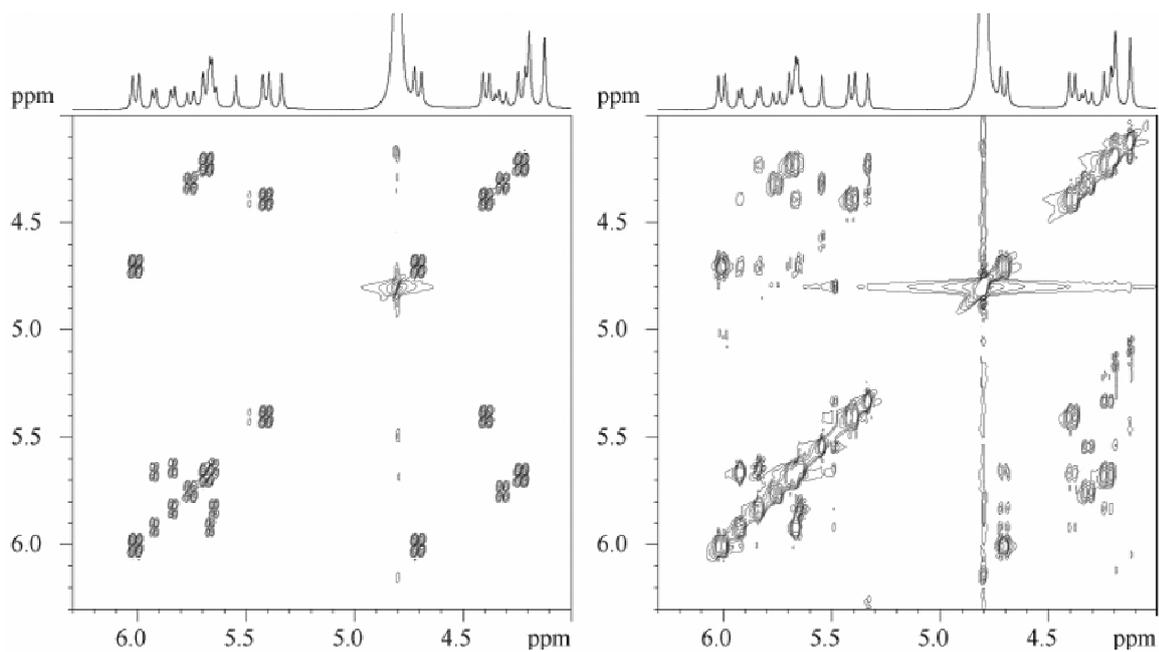


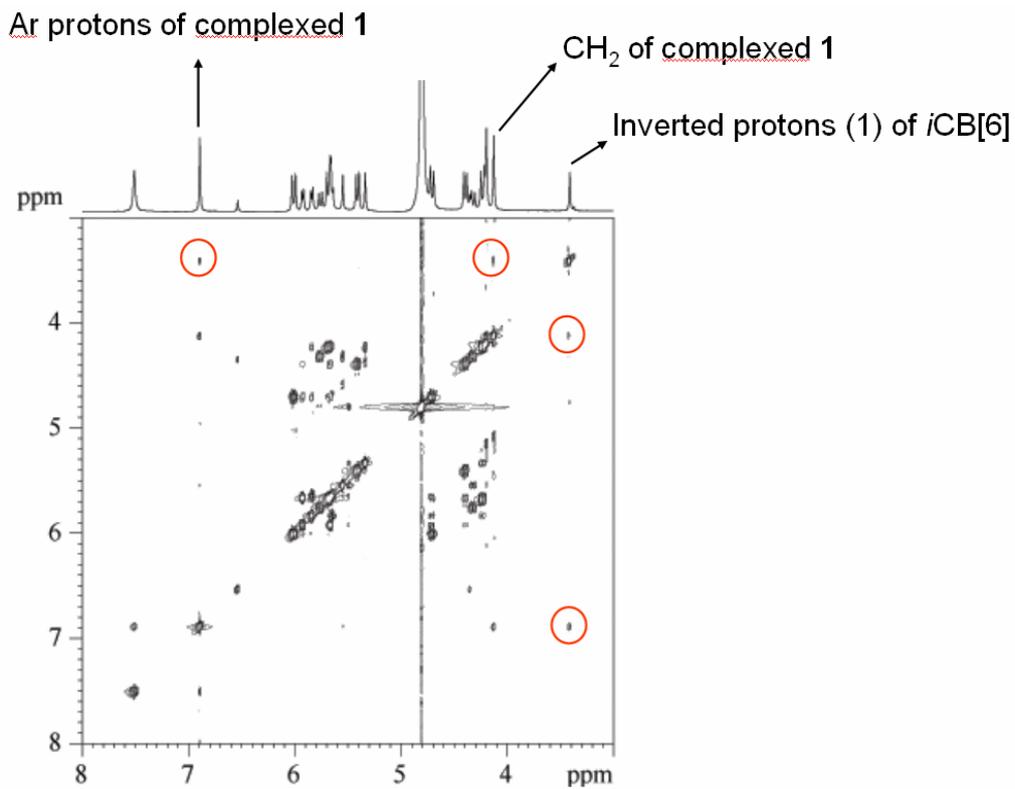
Figure S5.  $^1\text{H}$  NMR spectra of *i*CB[6] complexed with **1**.



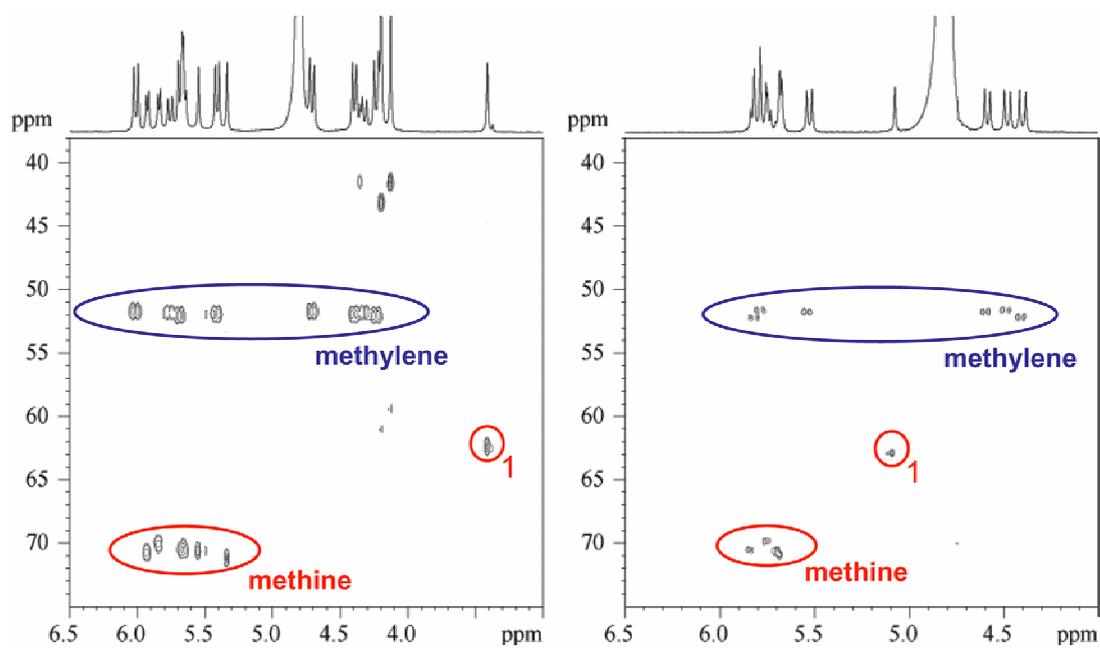
**Figure S6.** Complexation induced shifts of *i*CB[6] protons upon complexation with **1**.



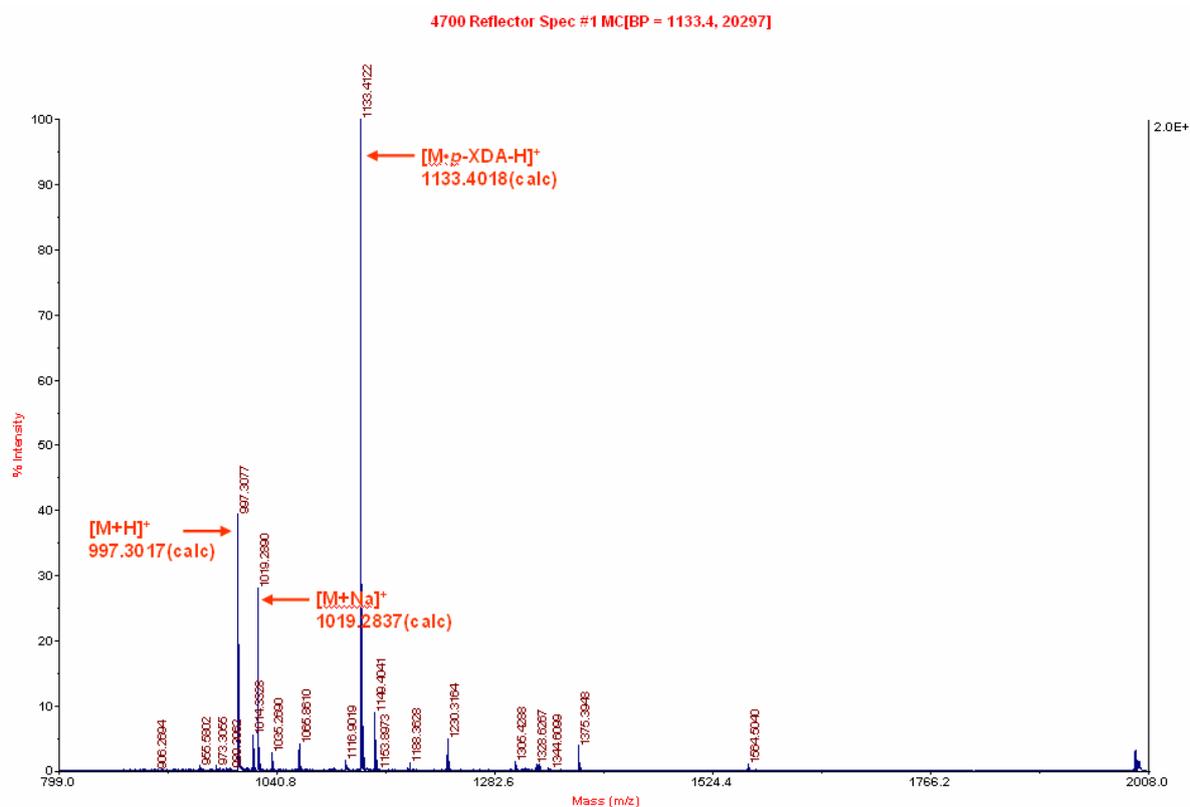
**Figure S7.** DQF-COSY and ROESY spectra of *i*CB[6]•**1**.



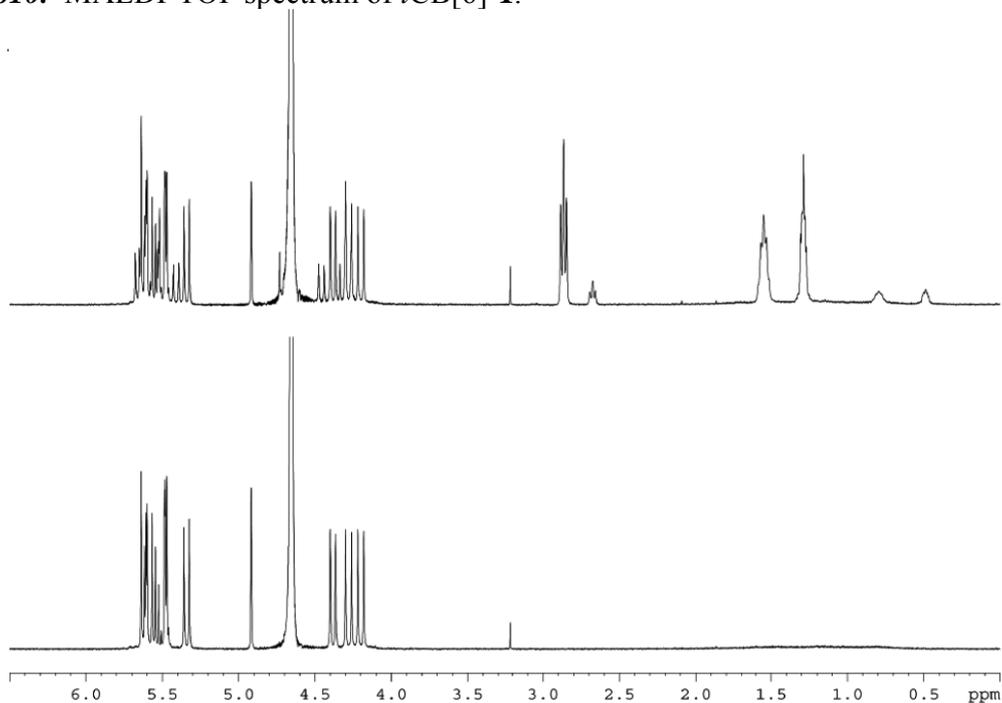
**Figure S8.** Intermolecular NOEs between *i*CB[6] and **1** in *i*CB[6]•**1**.



**Figure S9.** <sup>1</sup>H-<sup>13</sup>C HSQC spectra of *i*CB[6]•**1** and *i*CB[6].



**Figure S10.** MALDI-TOF spectrum of *i*CB[6]•**1**.



**Figure S11.** <sup>1</sup>H NMR spectra (0.1 M Na<sub>2</sub>SO<sub>4</sub> / D<sub>2</sub>O) for: *i*CB[6] alone (bottom) and with excess 1,6-hexanediamine (top).

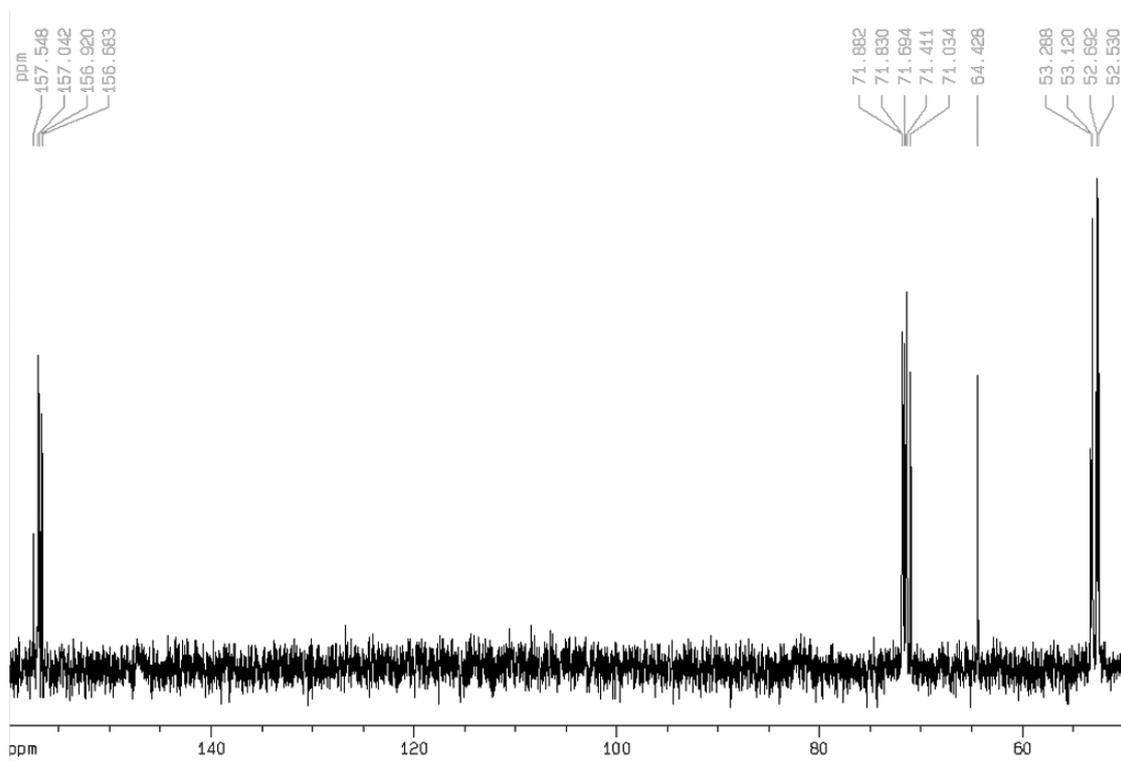


Figure S12.  $^{13}\text{C}$  NMR spectrum (125 MHz,  $\text{D}_2\text{O}$ ) for *i*CB[7].

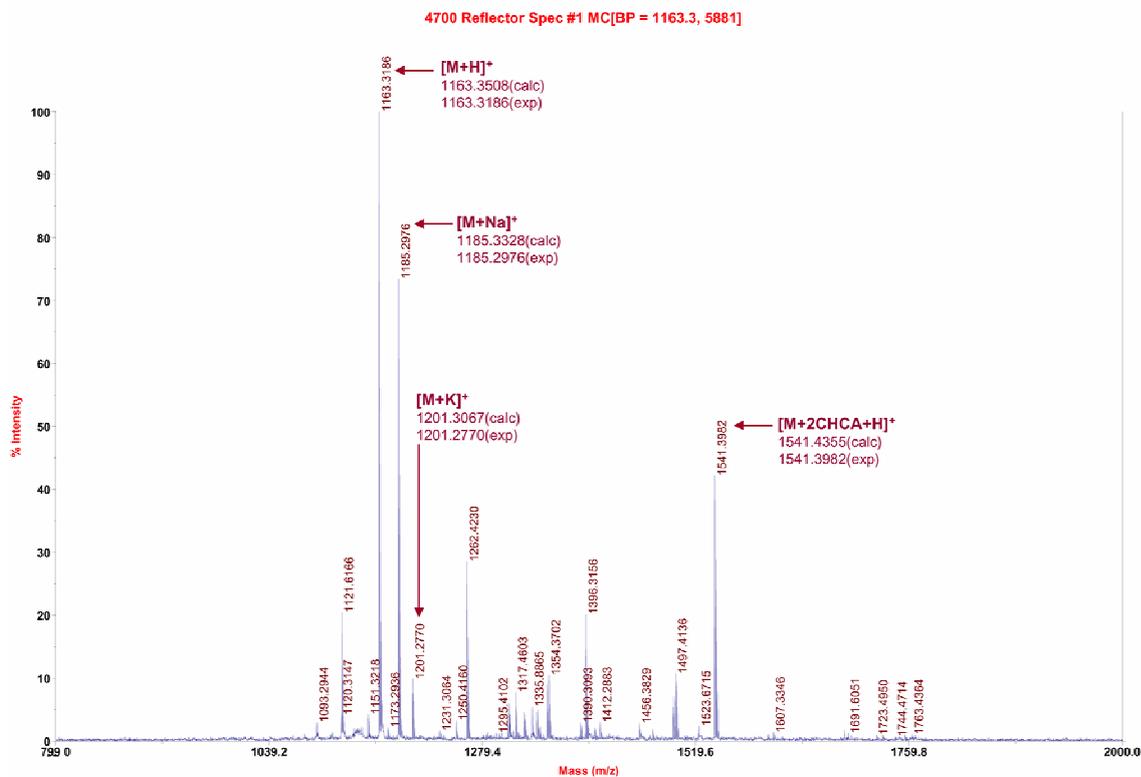
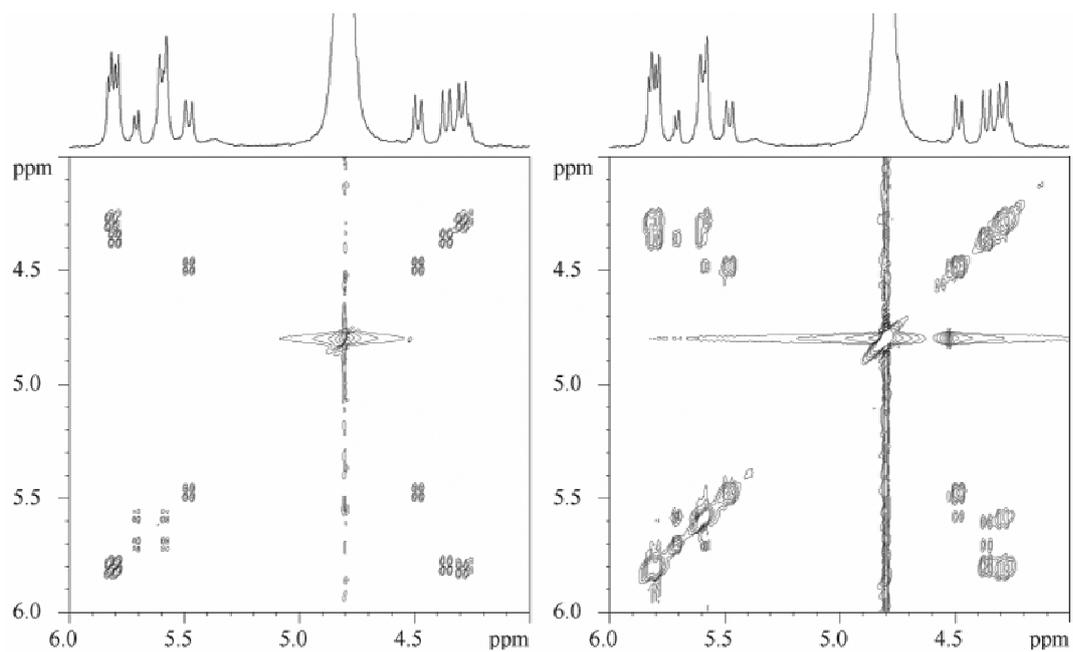
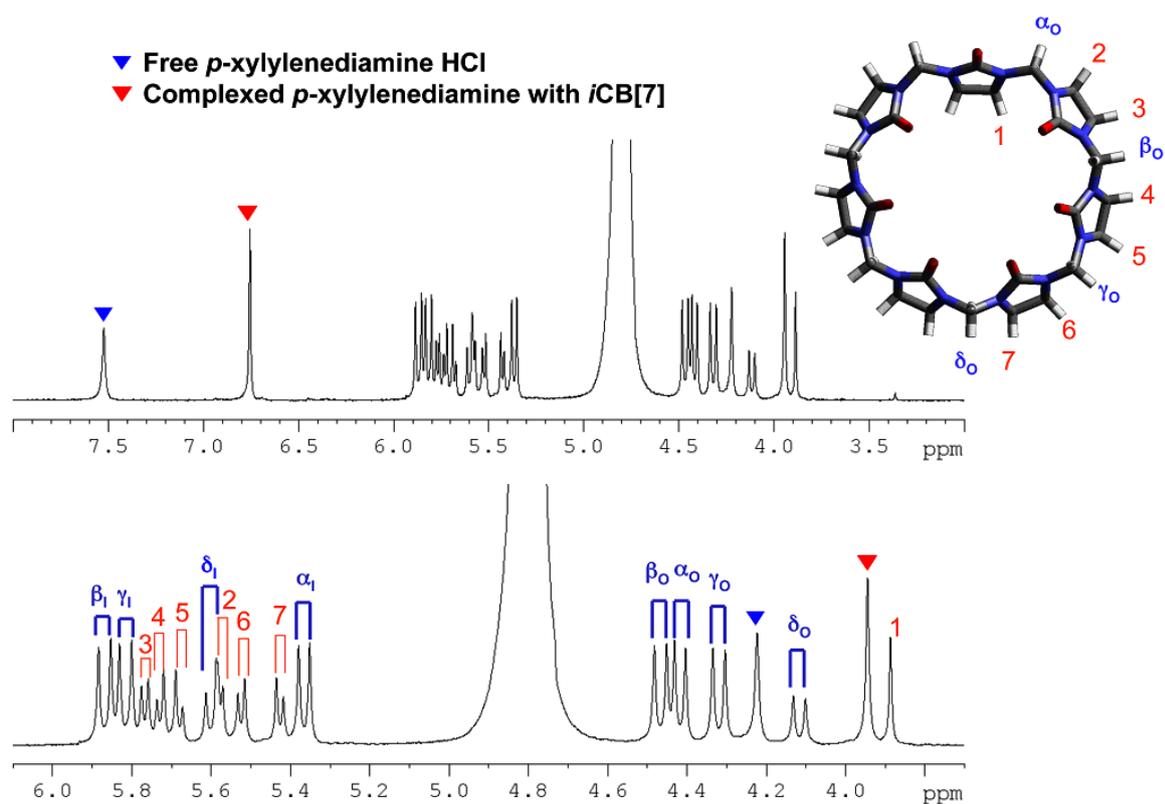


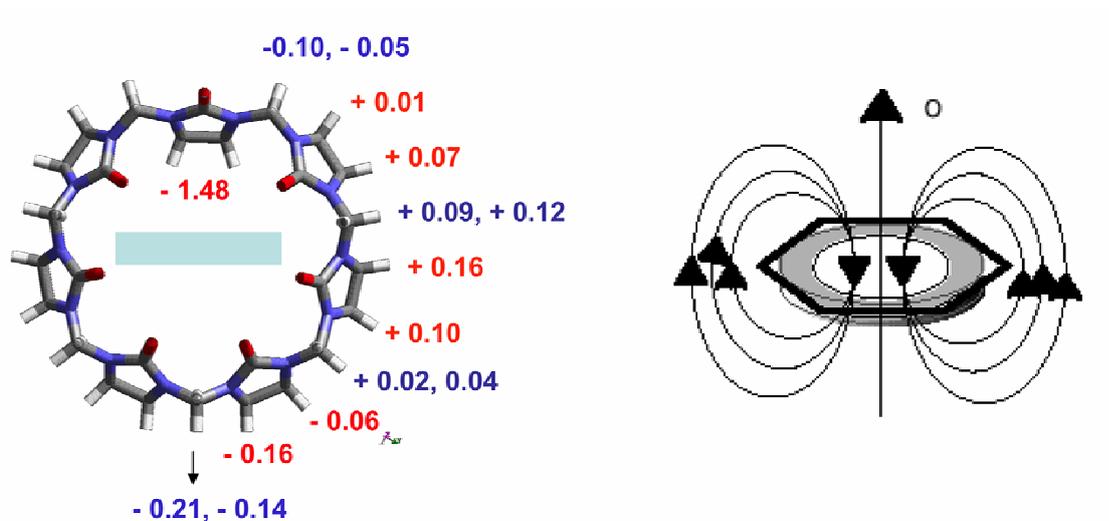
Figure S13. MALDI-TOF spectrum of *i*CB[7]. CHCA =  $\alpha$ -cyano-4-hydroxycinnamic acid.



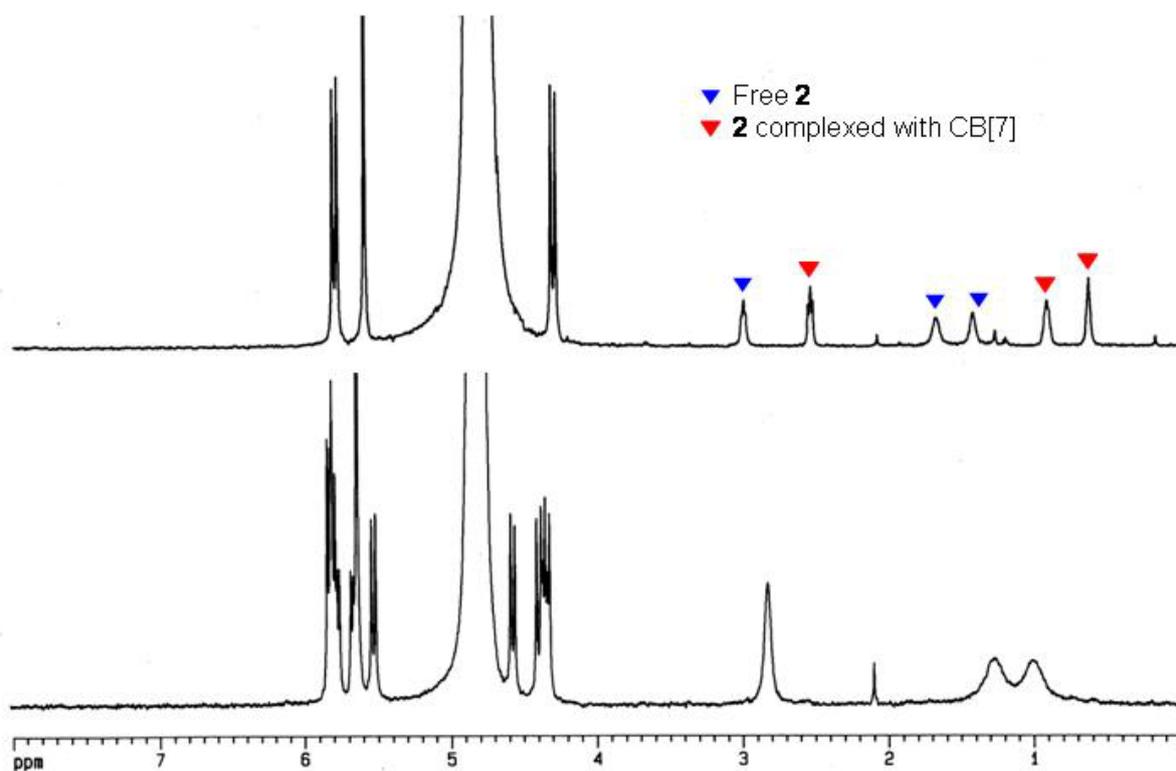
**Figure S14.** DQF-COSY and ROESY spectra of *i*CB[7].



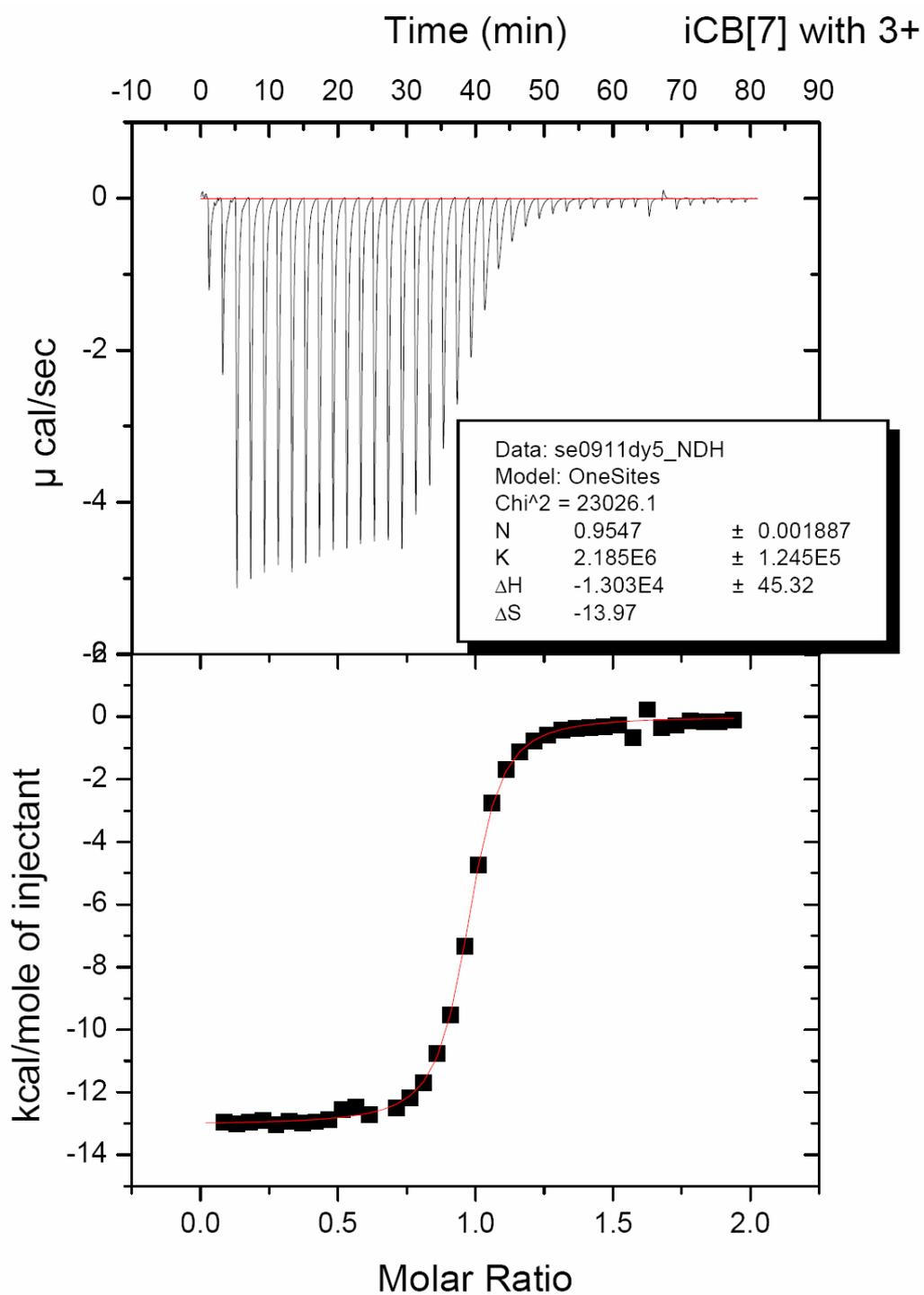
**Figure S15.**  $^1\text{H}$  NMR of *i*CB[7]•1.



**Figure S16.** Complexation induced shifts of *i*CB[7] protons upon complexation with **1**.



**Figure S17.** <sup>1</sup>H NMR spectra of CB[7] (top) and *i*CB[7] (bottom) with a slight excess of **2**. In the presence of CB[7] (top), there are two sets of NMR signals corresponding to free **2** and complexed **2** with CB[7], which indicates that the exchange of **2** in and out of CB[7] is slow on the NMR time scale whereas the spectrum of *i*CB[7] (bottom) with a slight excess of **2** shows only one set of NMR signals of **2**, indicating the fast exchange of **2** in and out of CB[7].



**Figure S18.** Thermogram (top) and binding isotherm (bottom) of (ferrocenemethyl)trimethylammonium ion complexing with *i*CB[7] at 303K.