Supramolecular Chemistry

Recognition in water of bioactive substrates by a sulfonato *p*-*tert*-butylcalix[5]arene

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Supplementary Material

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Experimental

Sodium 5,11,17,23,29-pentakis(1,1-dimethylethyl)-31,32,33,34,35-penta(4-butoxysulfonato) calix[5]arene (**1**) was synthesized as reported in literature (1). Deuterium oxide 99.9% and 2-phenylethylamine hydrochloride (Pea·HCl, **2**), 4-(2-aminoethyl)phenol hydrochloride (Tyrm·HCl, **3**), 4-(2-aminoethyl)-1,2-benzenediol hydrochloride (Dopa·HCl, **4**), 3-(2-aminoethyl)-5-hydroxyindole hydrochloride (Sert·HCl, **5**), 2-(4-imidazolyl)ethylamine dihydrochloride (Hist·2HCl, **6**), 4-[(1*R*)-2-amino-1-hydroxyethyl]benzene-1,2-diol (Nore·HCl, **7**) and 4-aminobutanoic acid (Gaba·HCl, **8**) were purchased from Sigma-Aldrich.

¹H NMR Binding Experiments

All spectra were recorded at 298 K in D₂O on a Varian 500 MHz instrument, using solvent suppression pulse sequences (either PRESAT or WET). Determination of the effective concentration of calixarene **1** present in solution in the non-aggregated form was carried out according to the quantification protocol described by Henderson (2). To this end, a 0.4 mM solution of **1** in D₂O was left to equilibrate at room temperature for 1 h, filtered through a 0.1 μm Millipore membrane filter and then examined by ¹H NMR spectroscopy. Specifically, 530 μL aliquots of this solution were transferred into a 5 mm WILMAD NMR tube into which a stem coaxial tube (WILMAD, WGS-5BL, 50 mm L × 2 mm stem o.d.), loaded with 60 μL of a 20 mM D₂O solution of maleic acid, was subsequently inserted. Accurate integration of the CH resonance of maleic acid ($\delta = 6.28$ ppm) vs that of the ArH peaks of **1** provided, over a 48 h time span, a constant and reproducible value for effective receptor concentration (0.22 mM). Routinely, *K_a* determinations were carried out on 1:1 host-guest solutions (0.20 mM in each) obtained by mixing aliquots (60 μL) of a given guest solution (2.2 mM, in D₂O) with 600 μL of the stock solution of **1** (0.22 mM). Samples of the resulting host-guest solution (530 μL) were then analyzed by ¹H NMR in the presence of maleic acid as a quantitative internal

(1) Gattuso, G.; Notti, A.; Pappalardo, A.; Pappalardo, S.; Parisi, M.F.; Puntoriero, F. *Tetrahedron Lett.* **2013**, 54, 188–191.

(2) Henderson, T.J. *Anal. Chem.* **2002**, 74, 191–198.

reference (see above). Effective concentrations of the free and *endo*-cavity complexed forms of host and guests, needed for K_a determination, were then quantitatively calculated (2) by accurate integration of convenient peaks belonging to maleic acid (CH), **1** (ArH) and guest (α - or β -CH₂).

Figure S1. ^1H NMR spectra (500 MHz, D_2O , 298 K) of: a) $[\mathbf{1}] = 0.22$ mM (effective concentration of the monomeric species), b) $[\mathbf{1}] = [\text{Pea}\cdot\text{HCl}] = 0.20$ mM and c) $[\text{Pea}\cdot\text{HCl}] = 0.20$ mM. In trace (b), the following symbols designate peaks belonging to: free host ($\circ$), free guest (∇) and *endo*-cavity complexed host and guest species ($\bullet$ and $\blacktriangledown$, respectively). In trace (a) the peak designated with a diamond ($\blacklozenge$) refers to maleic acid, added as an internal standard in a coaxial NMR tube. The residual HDO signal is marked with an asterisk.

Figure S2. ^1H NMR spectra (500 MHz, D_2O , 298 K) of: a) $[\mathbf{1}] = 0.22$ mM (effective concentration of the monomeric species), b) $[\mathbf{1}] = [\text{Dopa}\cdot\text{HCl}] = 0.20$ mM and c) $[\text{Dopa}\cdot\text{HCl}] = 0.20$ mM. In trace (b), the following symbols designate peaks belonging to: free host ($\circ$), free guest (∇) and *endo*-cavity complexed host and guest species ($\bullet$ and $\blacktriangledown$, respectively). In trace (a) the peak designated with a diamond ($\blacklozenge$) refers to maleic acid, added as an internal standard in a coaxial NMR tube. The residual HDO signal is marked with an asterisk.

Figure S3. ^1H NMR spectra (500 MHz, D_2O , 298 K) of: a) $[\mathbf{1}] = 0.22$ mM (effective concentration of the monomeric species), b) $[\mathbf{1}] = [\text{Dopa}\cdot\text{HCl}] = 0.20$ mM and c) $[\text{Dopa}\cdot\text{HCl}] = 0.20$ mM. In trace (b), the following symbols designate peaks belonging to: free host ($\circ$), free guest (∇) and *endo*-cavity complexed host and guest species ($\bullet$ and $\blacktriangledown$, respectively). In trace (a) the peak designated with a diamond ($\blacklozenge$) refers to maleic acid, added as an internal standard in a coaxial NMR tube. The residual HDO signal is marked with an asterisk.

Figure S4. ^1H NMR spectra (500 MHz, D_2O , 298 K) of: a) $[\mathbf{1}] = 0.22$ mM, b) $[\mathbf{1}] = [\text{Hist}\cdot 2\text{HCl}] = 0.20$ mM and c) $[\text{Hist}\cdot 2\text{HCl}] = 0.20$ mM. The residual HDO signal is marked with an asterisk.

Figure S5. ^1H NMR spectra (500 MHz, D_2O , 298 K) of: a) $[1] = 0.22 \text{ mM}$, b) $[1] = [\text{Nore}\cdot\text{HCl}] = 0.20 \text{ mM}$ and c) $[\text{Nore}\cdot\text{HCl}] = 0.20 \text{ mM}$. The residual HDO signal is marked with an asterisk.

Figure S6. ^1H NMR spectra (500 MHz, D_2O , 298 K) of: a) $[1] = 0.22 \text{ mM}$, b) $[1] = [\text{Gaba}\cdot\text{HCl}] = 0.20 \text{ mM}$ and c) $[\text{Gaba}\cdot\text{HCl}] = 0.20 \text{ mM}$. The residual HDO signal is marked with an asterisk.