Supporting Information

A Hybrid Cavitand-Resorcin[4]arene Receptor for the Selective Binding of Choline and Related Compounds in Protic Media

Pablo Ballester and M. Angeles Sarmentero

ICREA and Institute of Chemical Research of Catalonia (ICIQ), Av. Països Catalans 16, 43007 Tarragona, Spain.

Contents for Electronic Supplementary Information

References for the preparation of the guests used in this study **S2**

Synthesis and characterization of receptor 6 **S2**

Titration Protocols

^{1}H- NMR Titrations **S5**

Fluorescence Titrations **S7**

^{1}H-^{1}H-NOESY spectrum of the 6·7a$^+$ complex **S12**

Changes of the ^{1}H NMR spectra acquired at 298 K during the titration of 6 with 7a$^+$ in 0.01 M KOH/DMSO-d_6 (1/1.5) **S13**

All guest compounds used in this study were commercially available except **2a**$^+$ and **2b**$^+$. These two were prepared by described literature procedures.[1, 2]

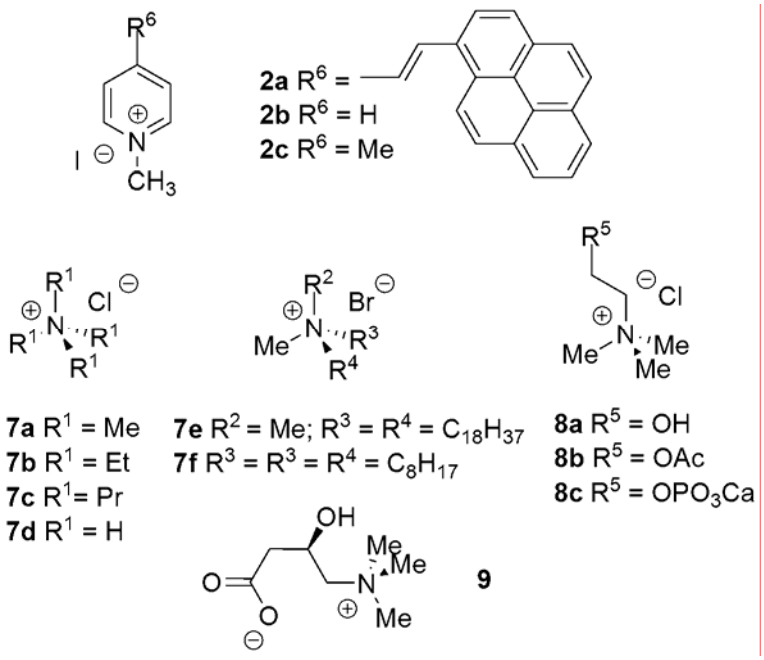
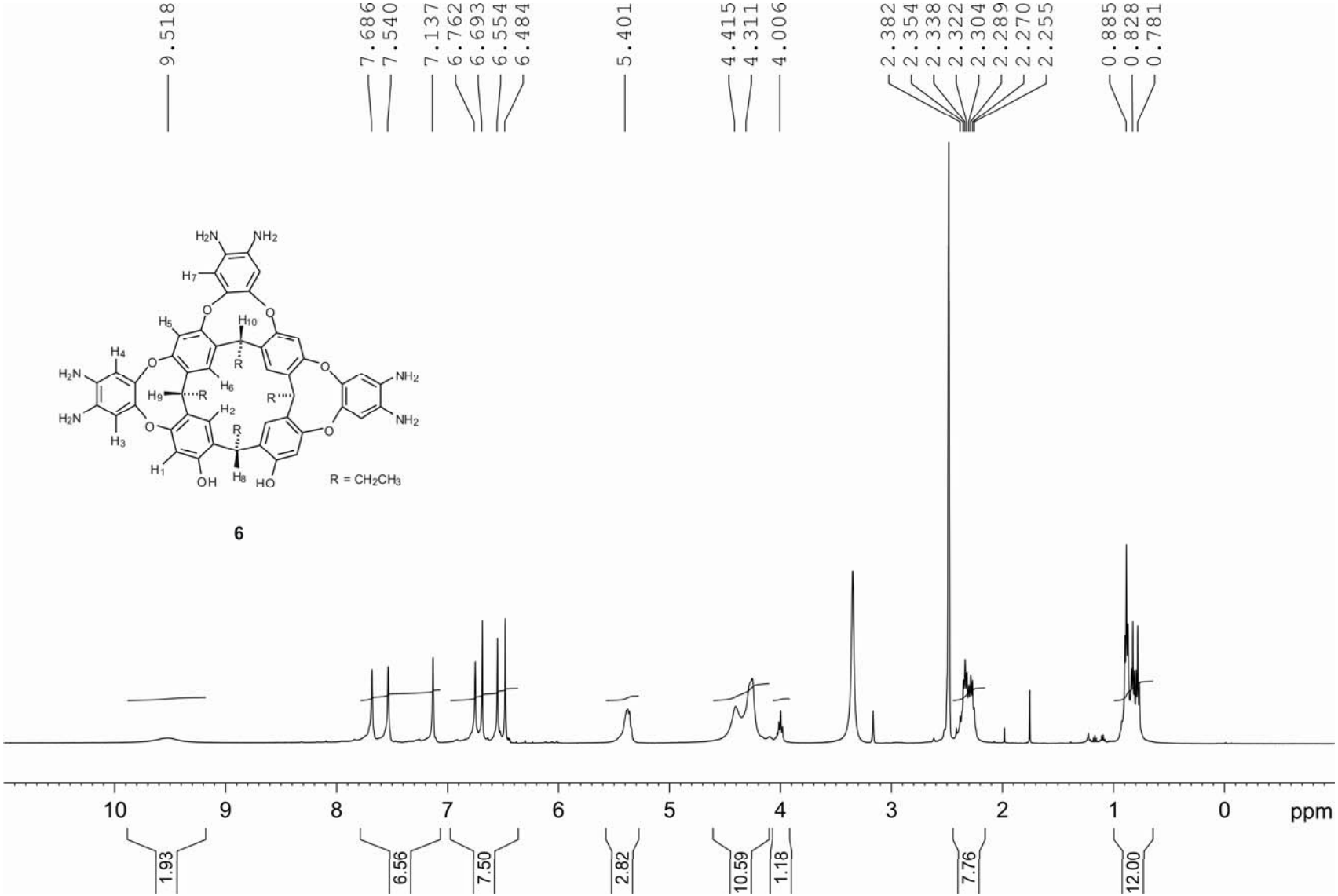


Figure S1: Structure of ammonium cations **2**, **7**, **8** and **9**.

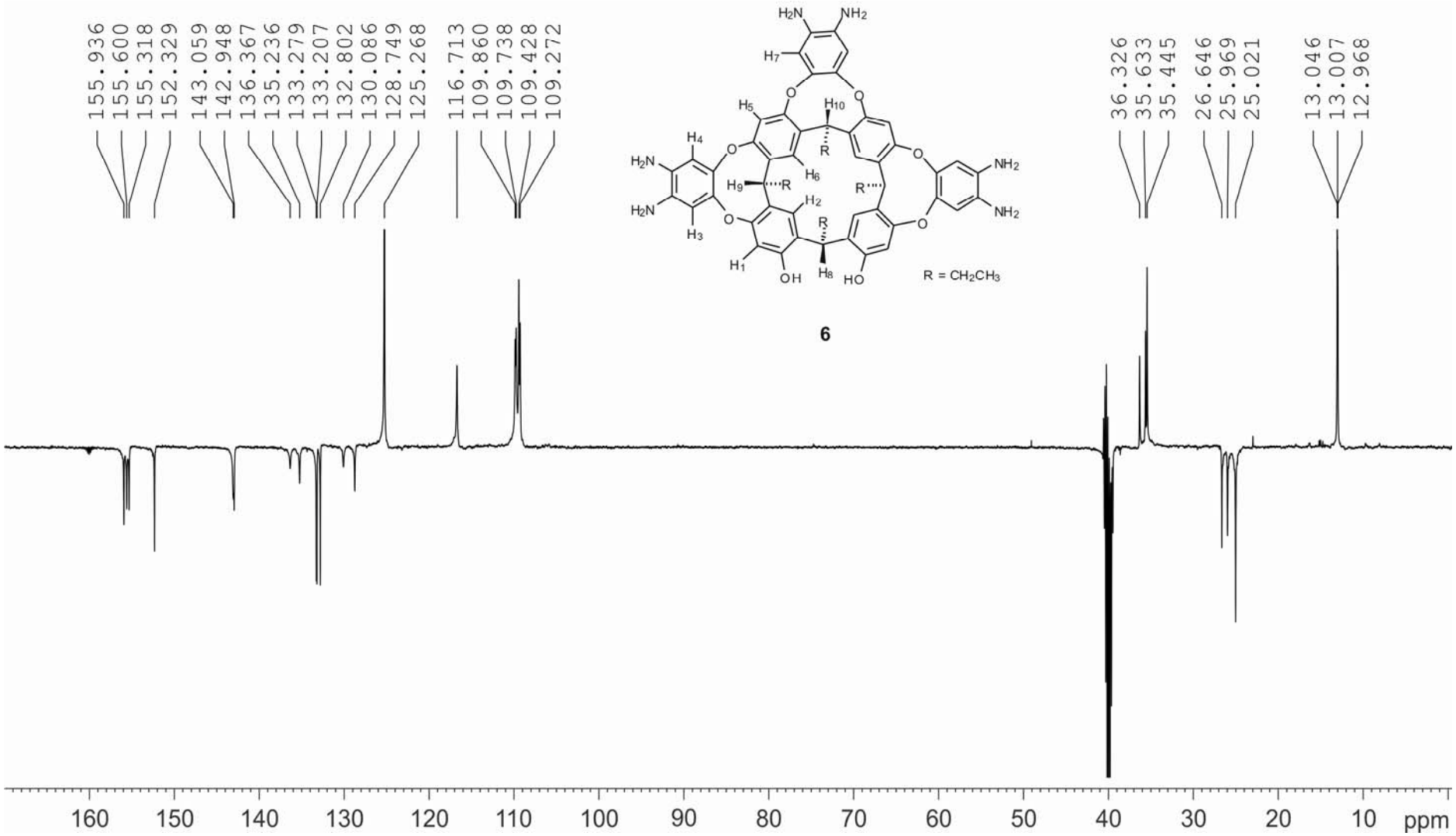
Synthesis and Characterization data of Receptor 6.

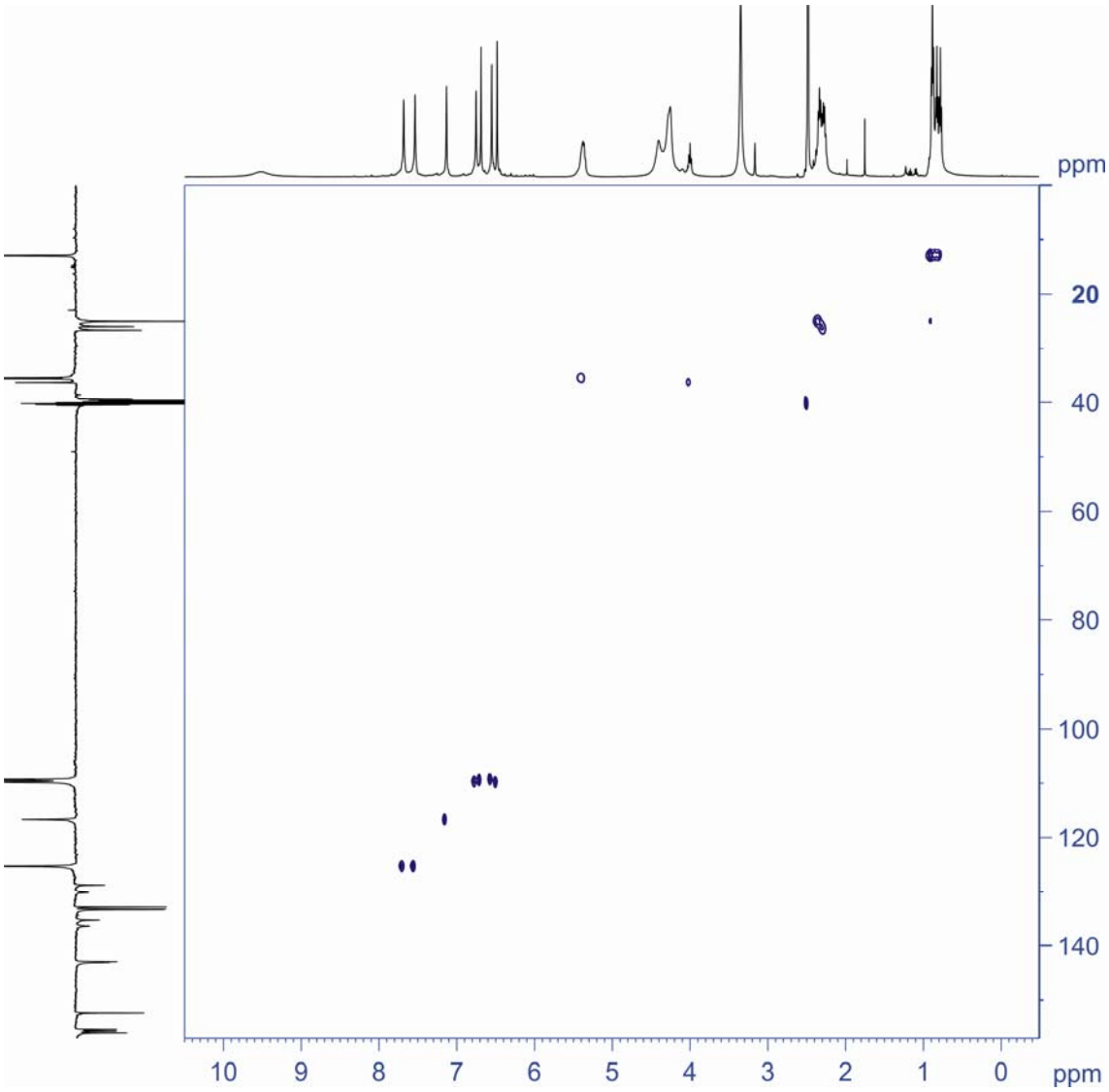
The preparation of receptor **6** was reported by Rebek[3] in 2002 but the compound was reacted "in situ". Consequently, **6** have not been previously isolated or characterized.
Following the methodology described by Rebek, the hydrochloride salt of **6** was isolated in a 70% yield as a white solid by filtration after EtOH evaporation from the reaction mixture. The isolated solid was treated for 30 min with a two layers mixture containing ethyl acetate and concentrated ammonia. The organic layer was separated, dried over Na_2SO_4, filtered and concentrated "in vacuo" yielding hexaamine **6** as a pale yellow solid (80% yield).
δ_H (500 MHz, DMSO-d_6): 0.78 (3H, t, J= 7.18Hz, CH_3), 0.83 (3H, t, J= 7.18Hz, CH_3), 0.89 (6H, t, J= 7.18Hz,2 x CH_3), 2.39-2.25 (8H, m, 4 × CH_2), 4.00 (1H, t, J= 7.18Hz, H_8), 4.31 (8H, s, NH_2), 4.42 (4H, s, NH_2), 5.40 (3H, m, H_9, H_{10}), 6.48 (2H, s, H_3), 6.55 (2H, s, H_1), 6.69 (2H, s, H_7), 6.76 (2H, s, H_4), 7.13 (2H, s, H_5), 7.54 (2H, s, H_2), 7.68 (2H, s, H_6), 9.51 (2H, bs, OH). δ_C (125 MHz, DMSO-d_6):155.94, 155.60, 155.32, 152.33, 143.06, 142.95, 136.37, 135.24, 133.28, 133.21, 132.81, 130.09, 128.75, 125.27, 116.71, 109.86 109.74, 109.43, 109.27, 36.33, 35.63, 35.44, 26.65, 25.97, 25.02, 13.05, 13.01, 12.97. HRMS *m/z* (MALDI) calcd for $C_{54}H_{53}N_6O_8$ $[M+H]^+$ 913.3925 found 913.3881.

(1)Koh, K. N.; Araki, K.; Ikeda, A.; Otsuka, H.; Shinkai, S. *J. Am. Chem. Soc.* **1996,** *118*, 755-758.
(2)Wen, X.; Qi, F.; Gang, X.; Wen-Tao, Y.; Guo-Qun, L.; Hong, L. *Huaxue Xuebao*, **2004,** *62*, 587.(Abstract from Scifinder)
(3)Amrhein, P.; Shivanyuk, A.; Johnson, D. W.; Rebek, J., Jr. *J. Am. Chem. Soc.* **2002,** *124*, 10349-10358.


9.518
7.686
7.540
7.137
6.762
6.693
6.554
6.484
5.401
4.415
4.311
4.006
2.382
2.354
2.338
2.322
2.304
2.289
2.270
2.255
0.885
0.828
0.781
R = CH2CH3
10
9
8
7
6
5
4
3
2
1
0
ppm
1.93
6.56
7.50
2.82
10.59
1.18
7.76
12.00

6


155.936
155.600
155.318
152.329
143.059
142.948
136.367
135.236
133.279
133.207
132.802
130.086
128.749
125.268
116.713
109.860
109.738
109.428
109.272
36.326
35.633
35.445
26.646
25.969
25.021
13.046
13.007
12.968
H2N
NH2
H7
H5
H10
H4
H6
H9
H2
H3
H1
OH
H8
HO
R = CH2CH3
6
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm



^{1}H-^{13}C HSQC

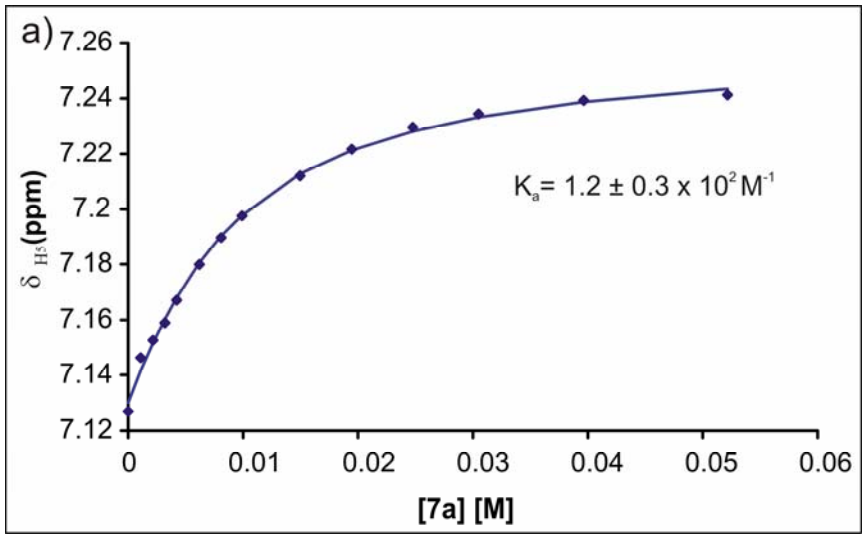
Titrations Protocols

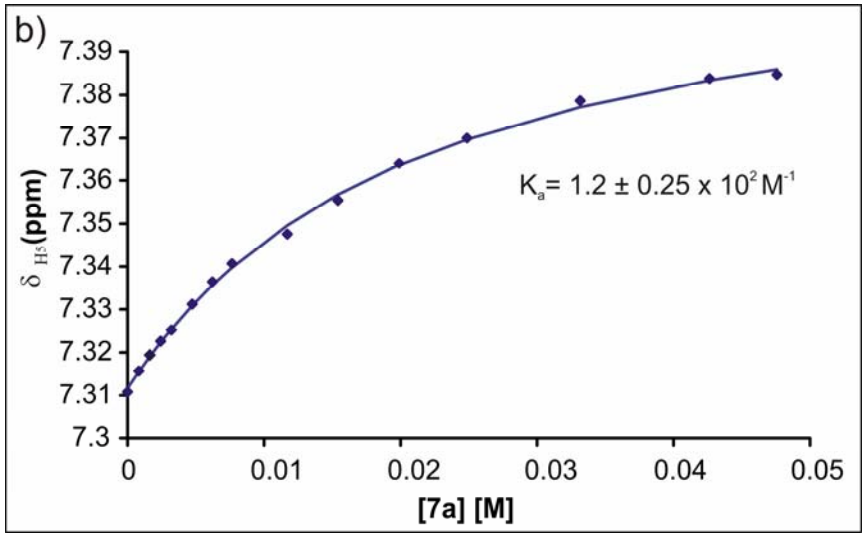
^{1}H- NMR Titrations

All titrations were carried out on a Bruker 500 MHz spectrometer, at 298 K, in different solvents DMSO-d_6, DMSO-d_6/H_2O-d_2 (1.5/1), DMSO-d_6/10 mM KOH H_2O-d_2 (1.5/1) and MeOH-d_4.

The association constants were determined using 3-10 mM solutions of hexaamine-diol **6** in the appropriate solvent at 298 K, and adding aliquots of a solution of the corresponding salt, approximately 10 times more concentrated, in the same solvent. The receptor **6** concentration was maintained constant all along the titration. The complexation process shows fast chemical exchange on the NMR timescale for the protons used in the fit. In all cases, the association constants were determined by following the chemical change of the aromatic proton H_5 in the NMR spectrum with different amount of the guest. Similar values are obtained using other aromatic protons.

The reported association constants were calculated using the software SPECFIT[4] which uses a global analysis system with expanded factor analysis and Marquardt least-squares minimization to obtain globally optimized parameters. The data were fitted to a simple 1:1 binding stoichiometry.





[4] SPECFIT, v 3.0.36, Spectrum Software Associates.

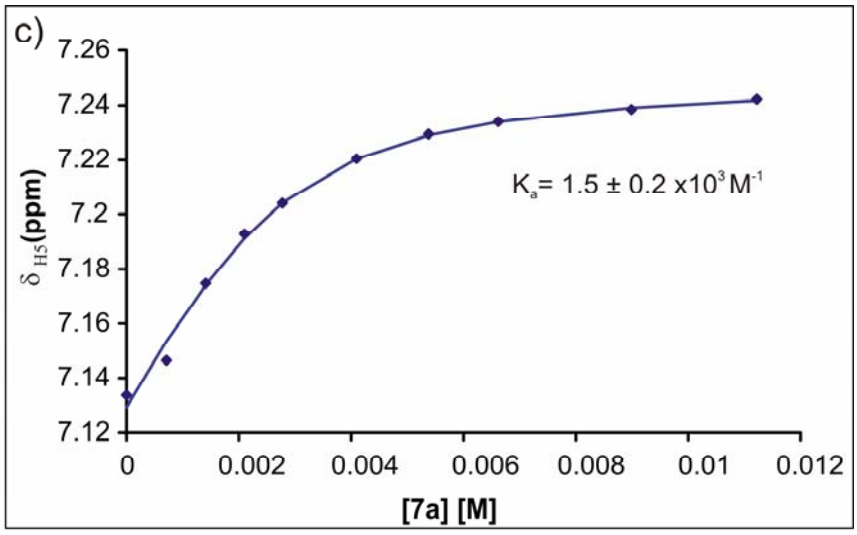


Figure S2: Plot of the chemical shift change of the aromatic proton H_5, in different solvents, during a titration of **6** with **7a**$^+$ fitted to a 1:1 binding isotherm (line). a) DMSO, b) D_2O/DMSO (1/1.5), c) 10 mM KOH/DMSO (1/1.5).

Fluorescence Titrations

Due to the intrinsic limitations of the ^{1}H NMR method for the accurate determination of association constants higher than 10^4 M^{-1} competitive fluorescence titrations were carried out using ammonium cation **2a**$^+$ as fluorescent indicator.

Fluorescence experiments were conducted on an Amicco Bowman Series 2, at 298 K, in MeOH. The sample volume was 2 mL.
The titration between hexaamine-diol **6** and the ammonium salt **2a**$^+$ in MeOH was carried out by adding small aliquots of a solution of **6**, 35-40 times more concentrated than **2a**$^+$ (8.25×10^{-6} M). The concentration of the fluorescent indicator **2a**$^+$ was maintained constant all along the titration. A spectrum was recorded after each addition and the resulting titration data were analysed using the SPECFIT computer program (1:1 binding model), as well as, by means of a simple linear relationship ($I_0/I = 1 + K_{ass}$ [**6**]) derived by assuming that the fluorescence is completely quenched on complex formation.[5] A similar value of binding constant was obtained using the two different mathematical treatments.

[5] Lakowicz, J. R. in Principles of Fluoresecence Spectroscopy, 2nd ed.; Kluwer Academic/Plenum Publishers,:New York, 1999, pag 242.

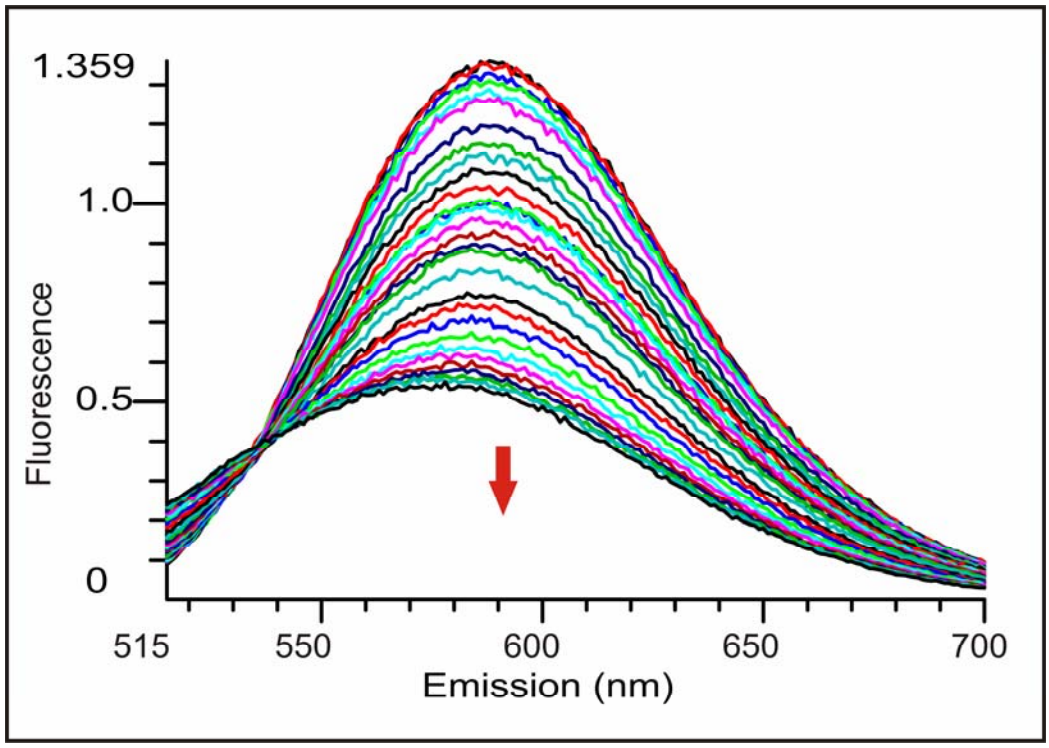


Figure S3: Fluorescence spectra of $\mathbf{2a}^{+}$ (8.25×10^{-6} M) in MeOH upon addition of incremental amounts of **6**.

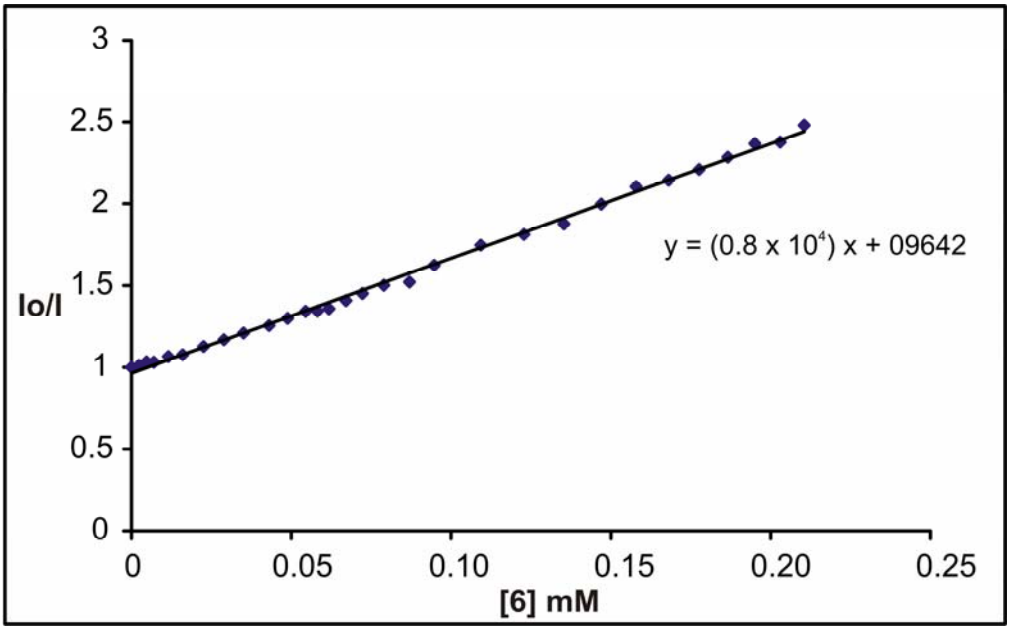


Figure S4. Linear plot of Io/I vs [**6**] fitted to the linear equation that assumes complete quenching of the fluorescence on complex formation

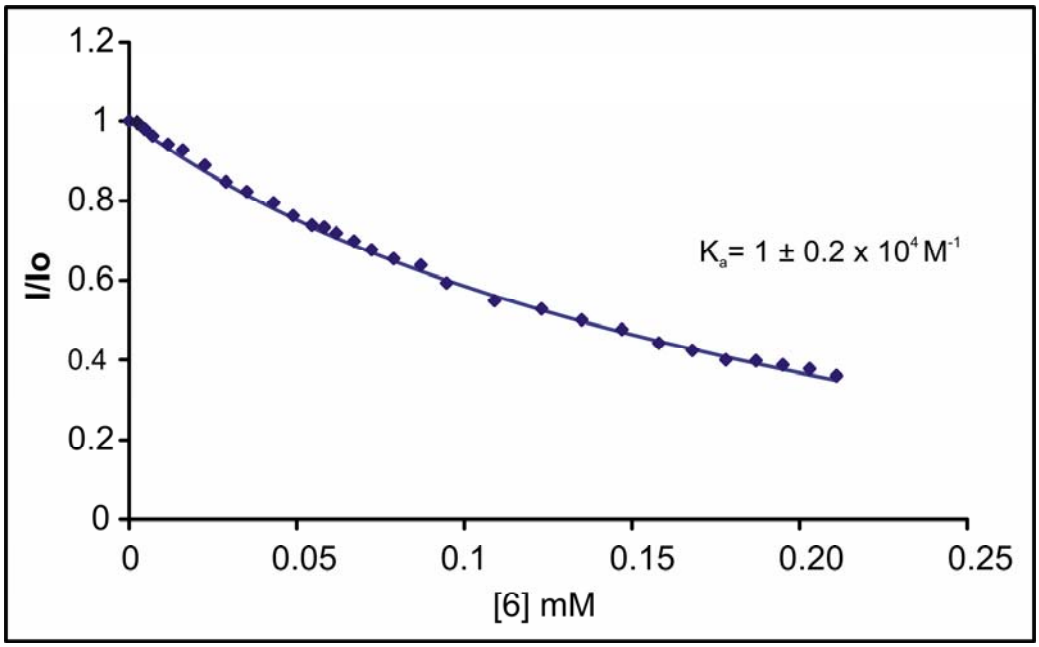


Figure S5. Plot of the relative fluorescence of **2a**$^+$ (8.25×10^{-6} M) vs the concentration of receptor **6** in MeOH fitted to a 1:1 binding model.

The competitive titrations between **6** and **2a**$^+$ and different ammonium salts **7**$^+$, **8**$^+$ **2b**$^+$, **2c**$^+$ and **9**$^+$ in MeOH were carried out adding small aliquots of the ammonium cation solution, about 30 times more concentrated, to a solution containing equimolecular amounts of **6** and **2a**$^+$ (0.1 - 0.2 mM). The addition was continued until a fluorescence plateau was achieved. The concentration of hexaamine-diol **6** and the fluorescent compound **2a**$^+$ was maintained constant all along the titration. A spectrum was recorded after each addition. The resulting titration data were analysed using the two components competitive binding model as implemented in the SPECFIT computer program affording the reported binding constant.

The fluorescence of **2a**$^+$ increased quickly with rising concentration of small cations like **7a**$^+$, **7b**$^+$, **8a**$^+$, **8b**$^+$, **8c**$^+$, **2b**$^+$ and **9**, indicating that these species can easily displace the **2a**$^+$ cation bound to **6**.

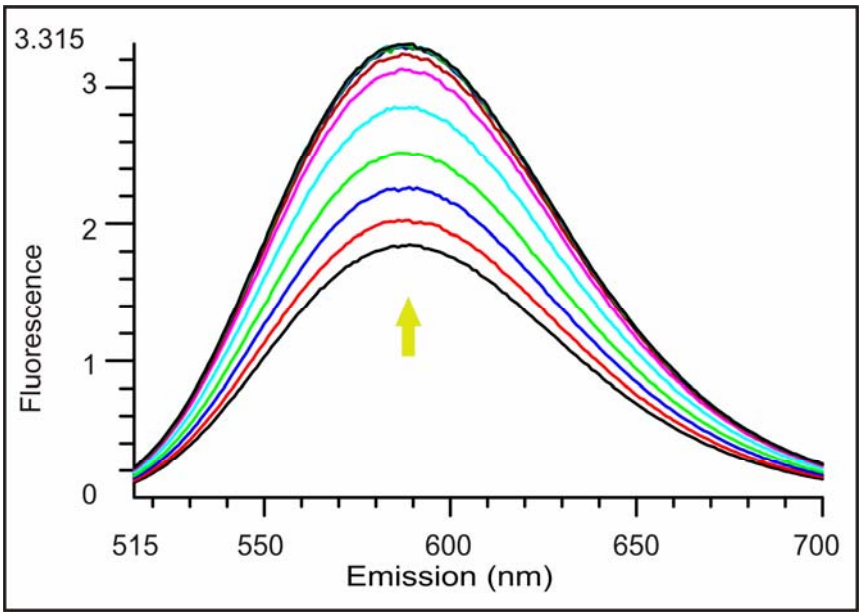


Figure S6: Fluorescence spectra of **2a**$^+$ (0 18 mM) in MeOH in the presence of **6** (0.15 mM) and upon addition of incremental amounts of **8c**$^+$

The fluorescence of **2a**$^+$ increases more slowly by the addition of cations that are slightly bigger than the previous ones i.e. **7e**$^+$. Consequently, more equivalents of the cation are necessary to reach saturation.

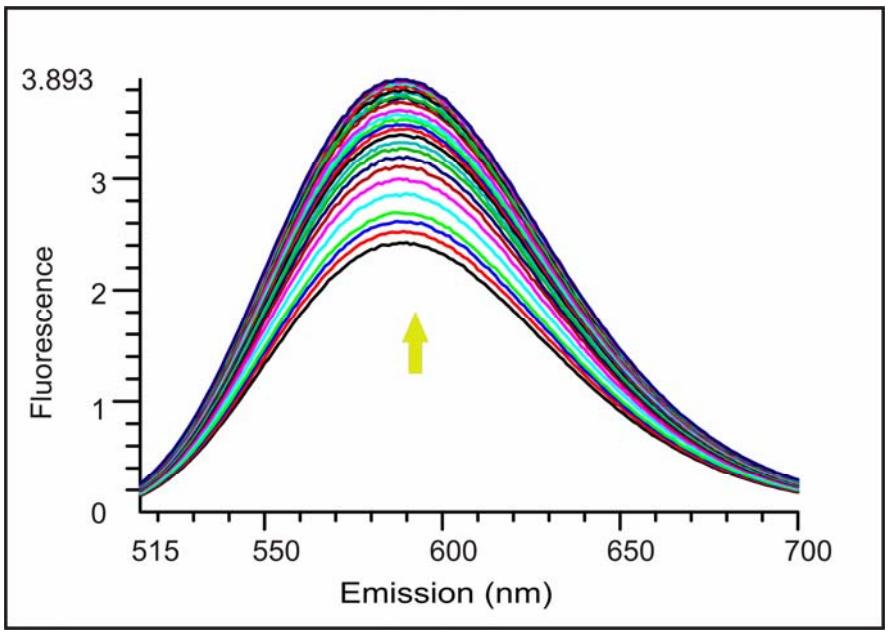


Figure S7: Fluorescence spectra of **2a**$^+$ (0.18 mM) in MeOH in the presence of **6** (0.17 mM) and upon addition of incremental amounts of **7e**$^+$.

For **7c**$^+$, **7d**$^+$ and **7f**$^+$ the fluorescence spectrum doesn't increase by incremental additions of the cation's solution indicating that these species cannot displace effectively the **2a**$^+$ cation bound to **6**.

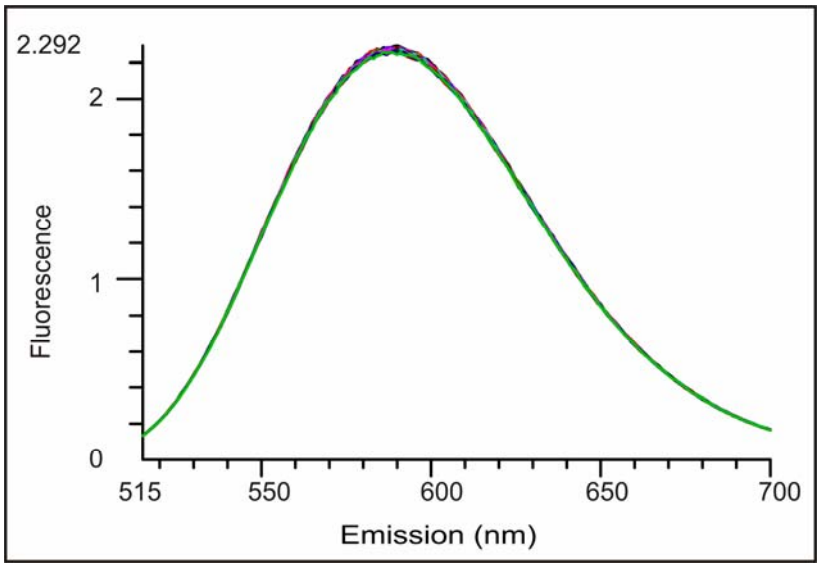


Figure S8: Fluorescence spectra of **2a**$^{+}$ (0.18 mM) in MeOH in the presence of **6** (0.17 mM) and upon addition of incremental amounts of **7f**$^{+}$.

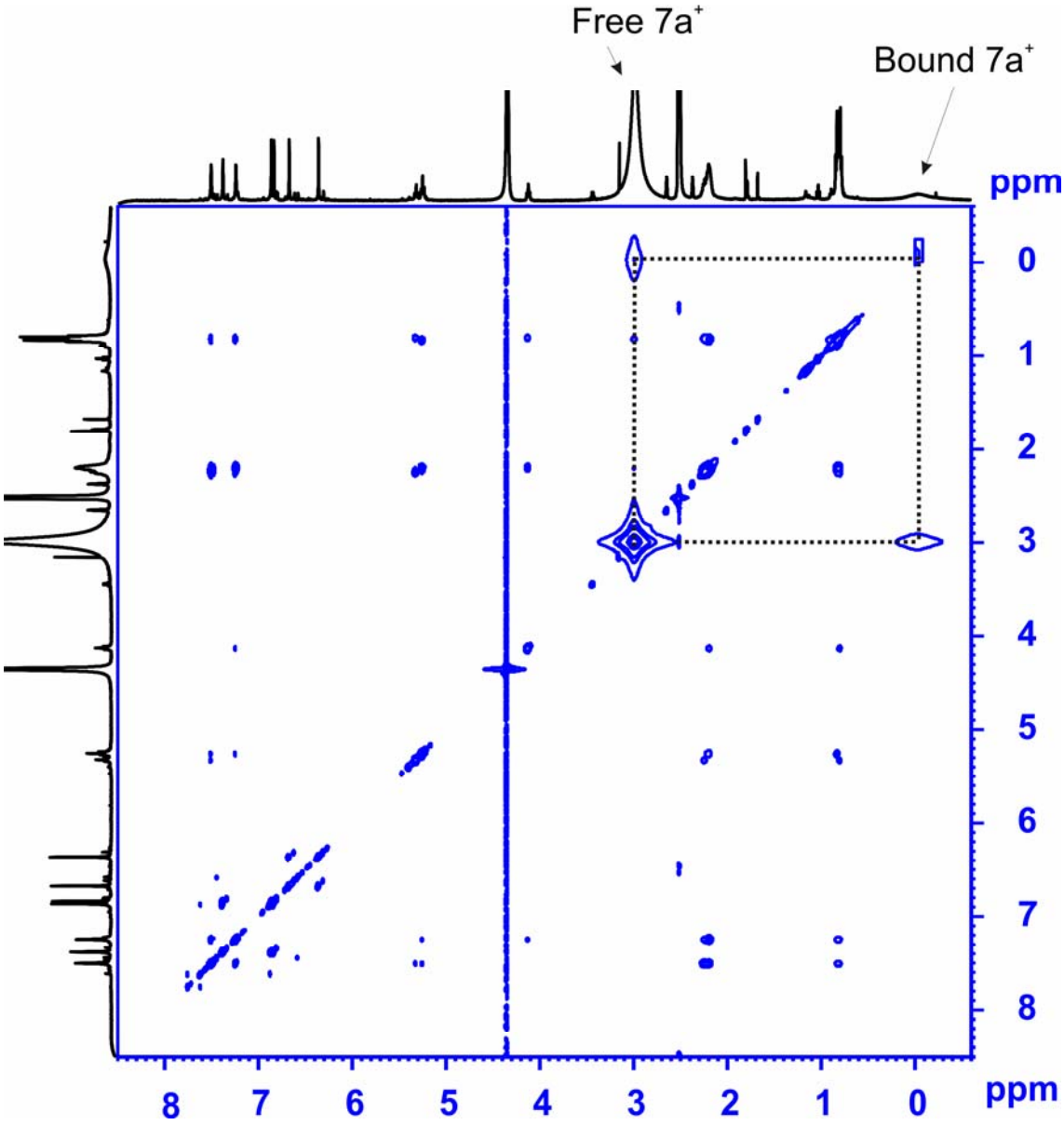


Figure S9: ^{1}H-^{1}H-NOESY spectrum of the **6·7a**$^{+}$ complex in 10 mM KOH D_2O/DMSO-d_6 (1/1.5) solution. The existence of slow chemical exchange on the NMR timescale for the methyl protons of free and bound **7a**$^{+}$ is indicated.

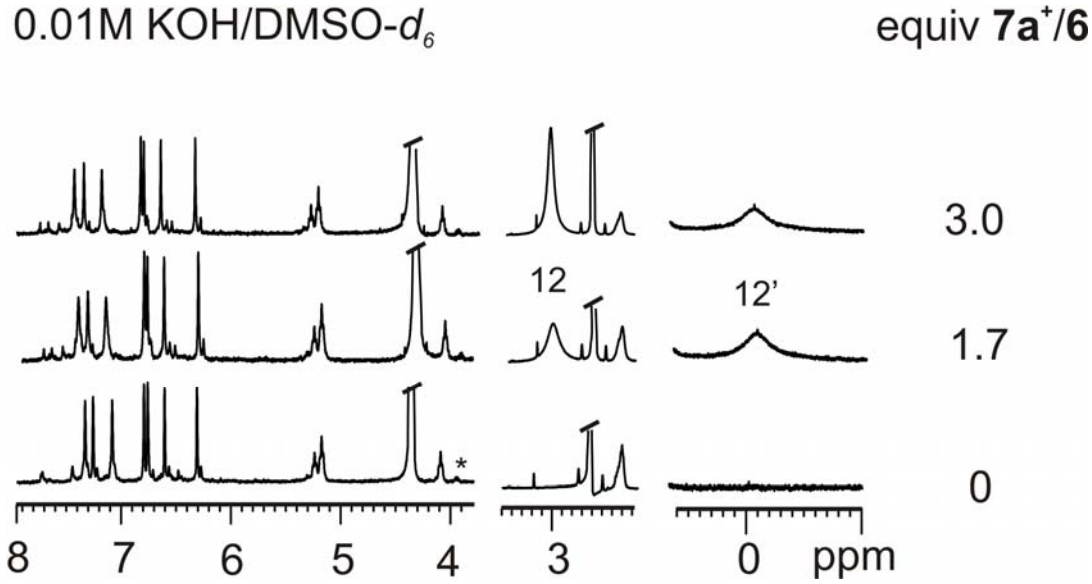


Figure S10: Changes of the ^{1}H NMR spectra acquired at 298 K during the titration of **6** with **7a**$^+$ in 0.01 M KOH/DMSO-d_6 (1/1.5), [**6**] = 3.3 mM. See Fig 1 in the text for proton assignements of **6**. 12 corresponds to the methyl protons of free **7a**$^+$ and 12' represents the same protons for the encapsulated guest. * Residual signal of ethyl acetate.