Supplementary Material (ESI) for Organic & Biomolecular Chemistry

SUPPORTING INFORMATION

Remarkably stable inclusion complexes with Heptakis-[6-deoxy-6-(2-aminoethylsulfanyl)]-β-cyclodextrin

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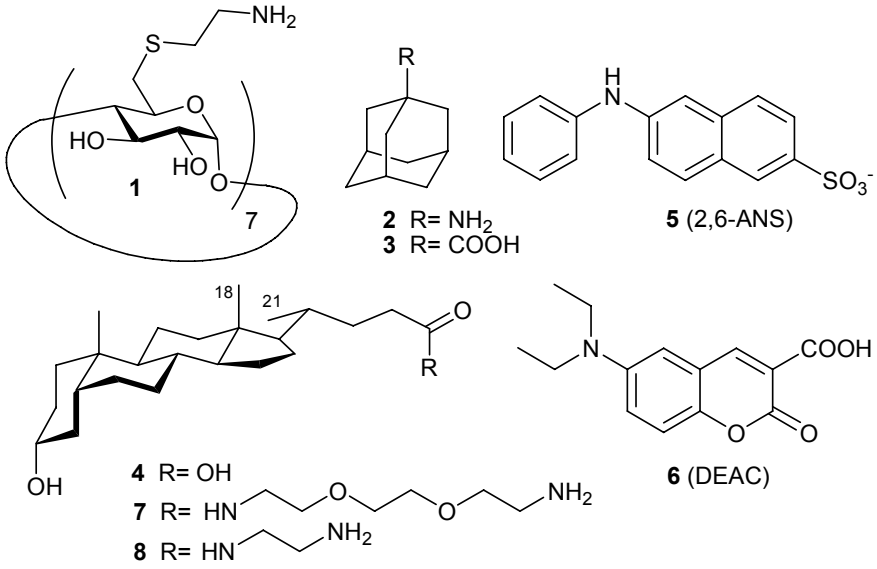
Contents

Instrumentation

^{1}H NMR spectra were recorded on a Varian 400 spectrometer at T = 297 K. ROESY NMR experiments were conducted on a Varian 500 spectrometer at T = 297 K. Absorption spectra were measured on a Schimadzu UV-2401PC spectrophotometer, whereas fluorescence spectroscopy was performed on a Fluorolog-3 spectrofluorometer. Isothermal Titration Calorimetry was performed on a Microcal calorimeter.



ROESY NMR experiments

ROESY NMR experiments were performed with 4 mM solutions of the 1:1 complexes of cyclodextrin (CD) **1** and the respective guests (G). A maleic acid buffer (25 mM) and an imidazole buffer (25 mM) were used to adjust the pH of the samples to pH 2.5 and 7.5 respectively. ^{1}H NMR spectra of the studied complexes and free **1** are supplied as reference material.

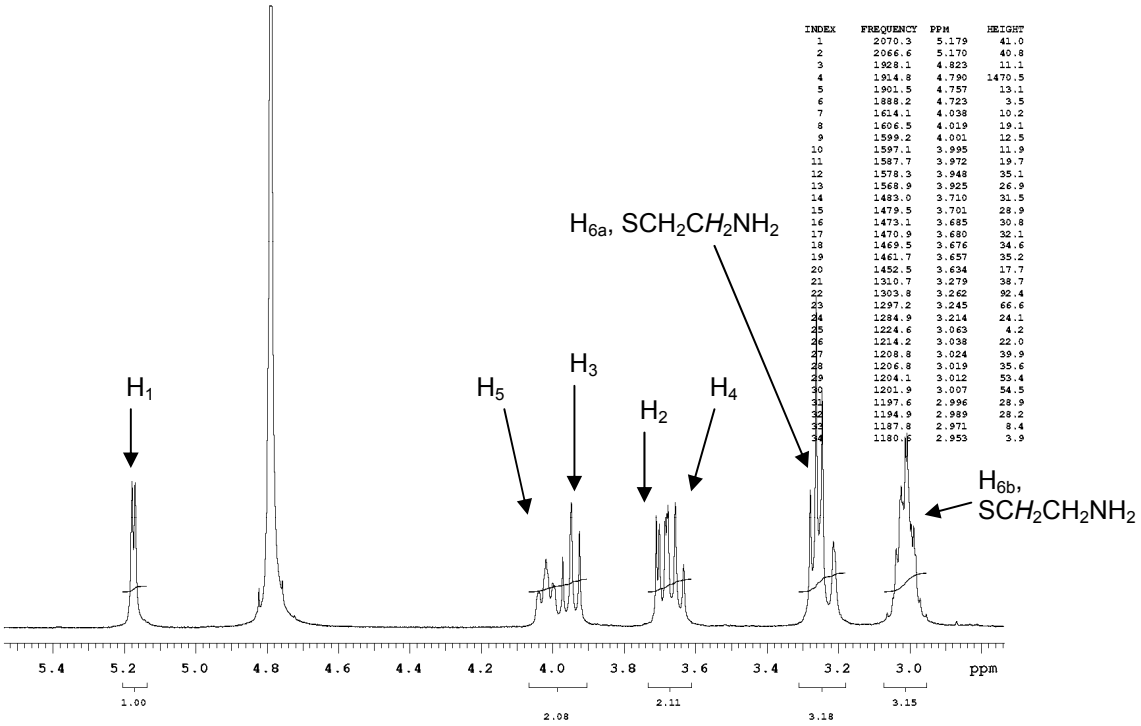


Figure S1a. ^{1}H NMR CD **1** at pH 7.5

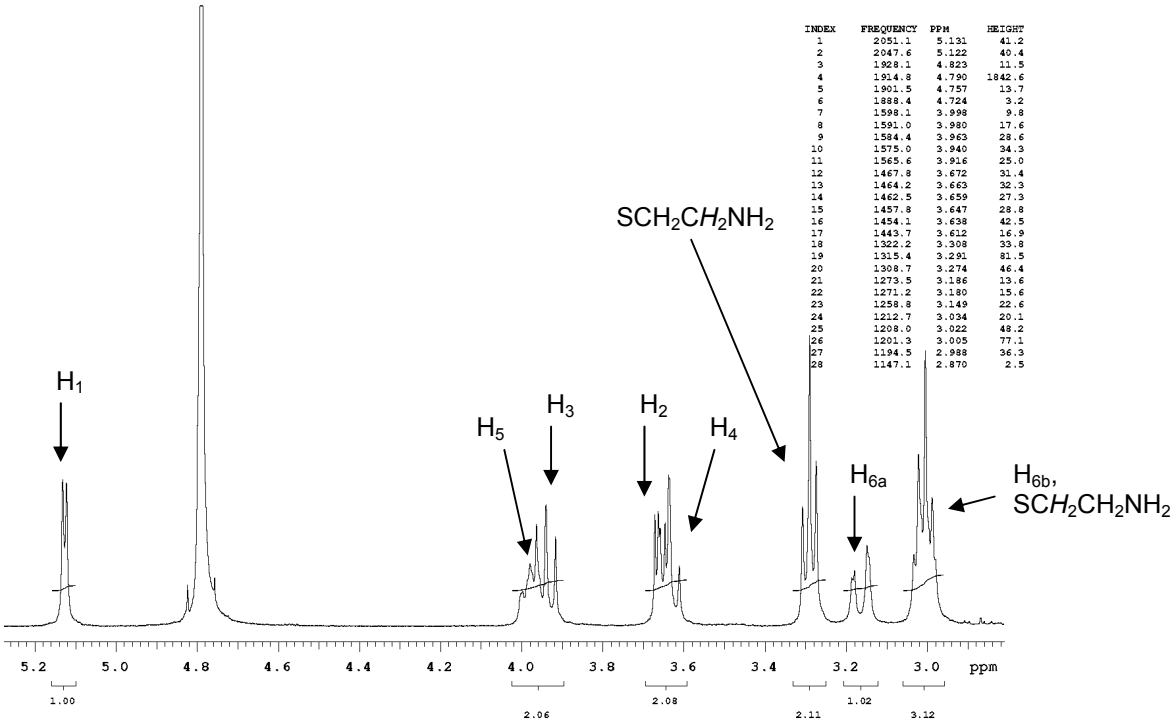
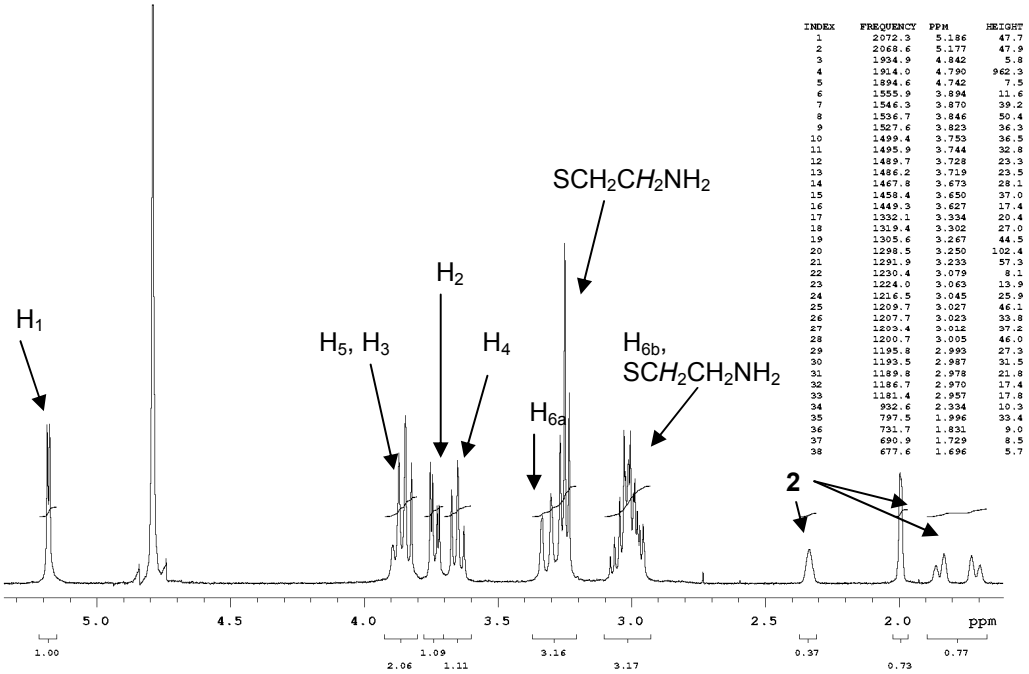


Figure S1b. ^{1}H NMR free **1** at pH 2.5



INDEX	FREQUENCY	PPM	HEIGHT
1	2072.3	5.186	47.7
2	2068.6	5.177	47.9
3	1934.9	4.842	5.8
4	1914.0	4.790	962.3
5	1894.6	4.742	7.5
6	1555.9	3.894	11.6
7	1546.3	3.870	39.2
8	1536.7	3.846	50.4
9	1527.6	3.823	36.3
10	1499.4	3.753	36.5
11	1495.9	3.744	32.8
12	1489.7	3.728	23.3
13	1486.2	3.719	23.5
14	1467.8	3.673	28.1
15	1458.4	3.650	37.0
16	1449.3	3.627	17.4
17	1332.1	3.334	20.4
18	1319.4	3.302	27.0
19	1305.6	3.267	44.5
20	1298.5	3.250	102.4
21	1291.9	3.233	57.3
22	1230.4	3.079	8.1
23	1224.0	3.063	13.9
24	1216.5	3.045	25.9
25	1209.7	3.027	46.1
26	1207.7	3.023	33.8
27	1203.4	3.012	37.2
28	1200.7	3.005	46.0
29	1195.8	2.993	27.3
30	1193.5	2.987	31.5
31	1189.8	2.978	21.8
32	1186.7	2.970	17.4
33	1181.4	2.957	17.8
34	932.6	2.334	10.3
35	797.5	1.996	33.4
36	731.7	1.831	9.0
37	690.9	1.729	8.5
38	677.6	1.696	5.7

Figure S2a. ^{1}H NMR (400 MHz) Complex **1•2** at D_2O pD 7.5

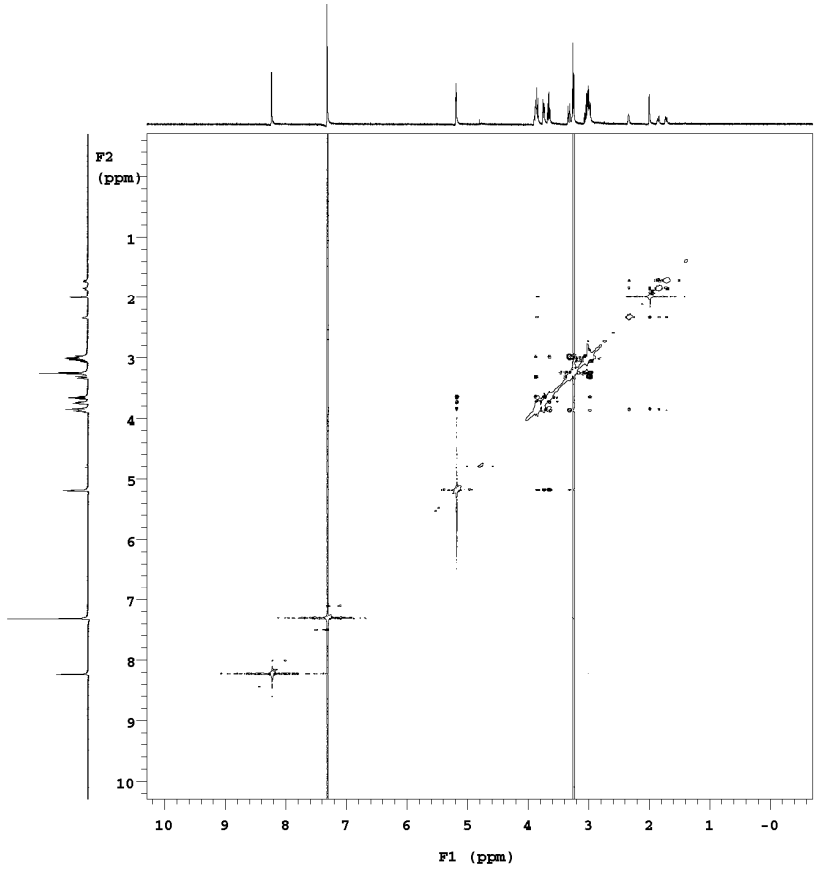


Figure S2b. ROESY (500 MHz)complex **1•2** at D_2O pD 7.5

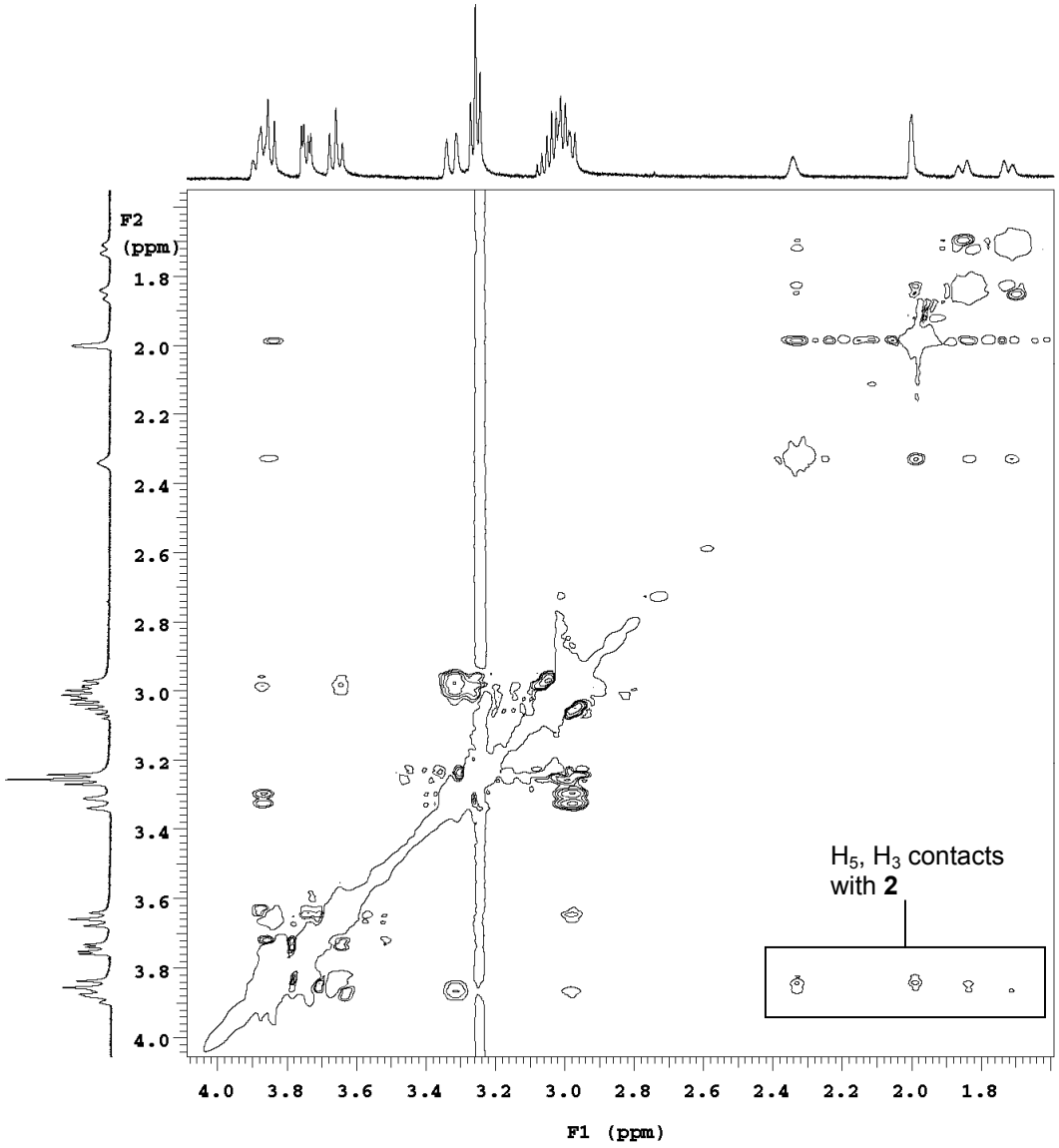
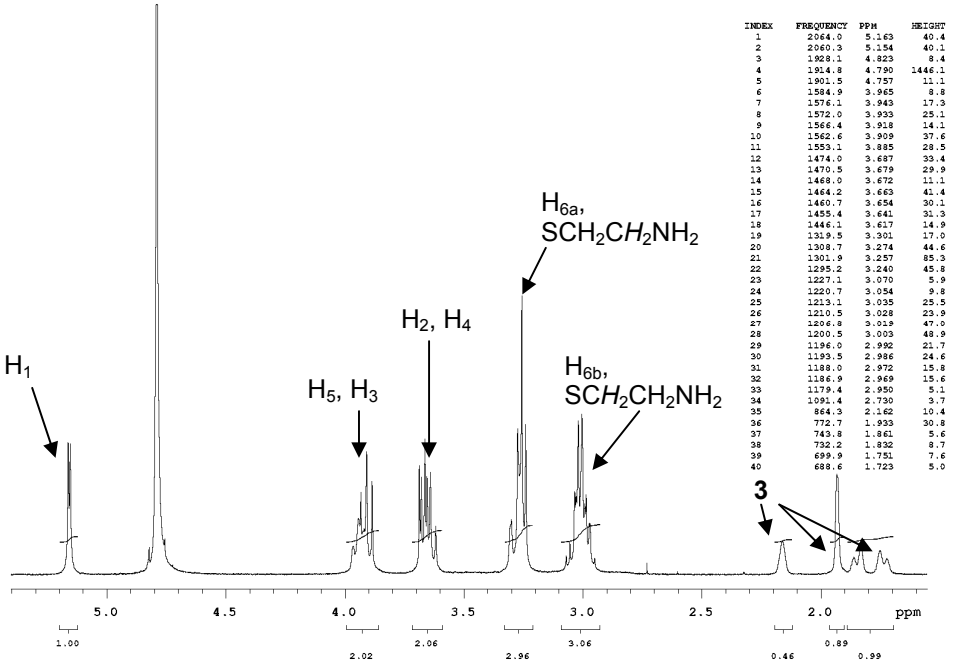


Figure S2C. Expansion of ROESY spectra complex **1•2** at pH 7.5



INDEX	FREQUENCY	PPM	HEIGHT
1	2064.0	5.163	40.4
2	2060.3	5.154	40.1
3	1928.1	4.823	8.4
4	1914.8	4.790	1446.1
5	1901.5	4.757	11.1
6	1584.9	3.965	8.8
7	1576.1	3.943	17.3
8	1572.0	3.933	25.1
9	1566.4	3.918	14.1
10	1562.6	3.909	37.6
11	1553.1	3.885	28.5
12	1474.0	3.687	33.4
13	1470.5	3.679	29.9
14	1468.0	3.672	11.1
15	1464.2	3.663	41.4
16	1460.7	3.654	30.1
17	1455.4	3.641	31.3
18	1446.1	3.617	14.9
19	1319.5	3.301	17.0
20	1308.7	3.274	44.6
21	1301.9	3.257	85.3
22	1295.2	3.240	45.8
23	1227.1	3.070	5.9
24	1220.7	3.054	9.8
25	1213.1	3.035	25.5
26	1210.5	3.028	23.9
27	1206.8	3.019	47.0
28	1200.5	3.003	48.9
29	1196.0	2.992	21.7
30	1193.5	2.986	24.6
31	1188.0	2.972	15.8
32	1186.9	2.969	15.6
33	1179.4	2.950	5.1
34	1091.4	2.730	3.7
35	864.3	2.162	10.4
36	772.7	1.933	30.8
37	749.8	1.861	5.6
38	732.2	1.832	8.7
39	699.9	1.751	7.6
40	688.6	1.723	5.0

Figure S3a. ^{1}H NMR (400 MHz) Complex **1•3** D_2O pD 7.5

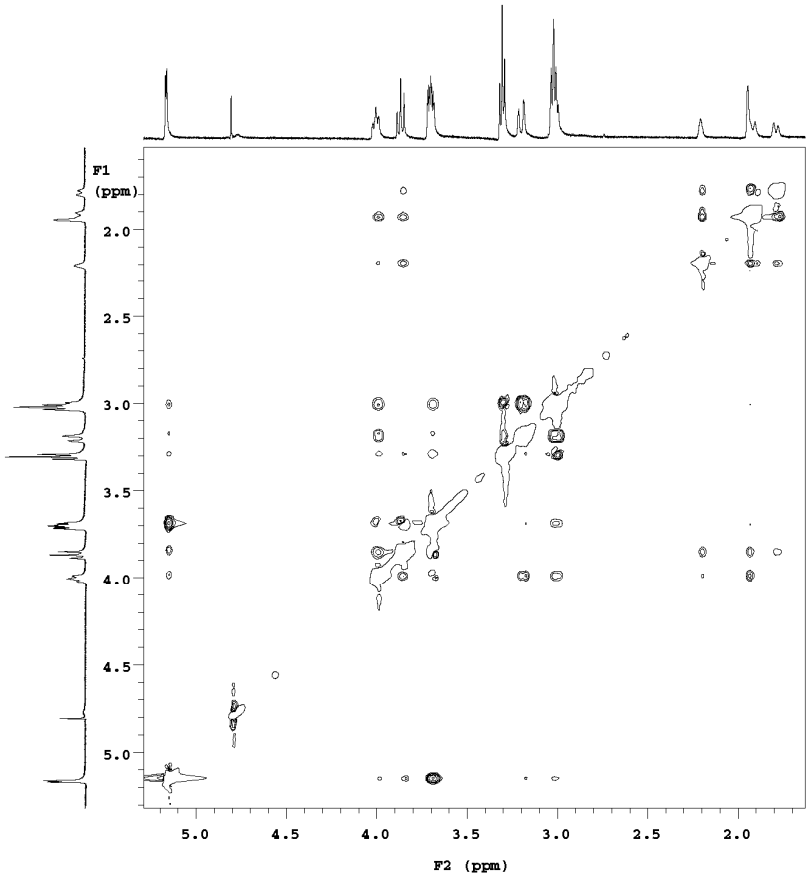


Figure S3b. ROESY NMR (500 MHz) Complex **1•3** D_2O pD 7.5

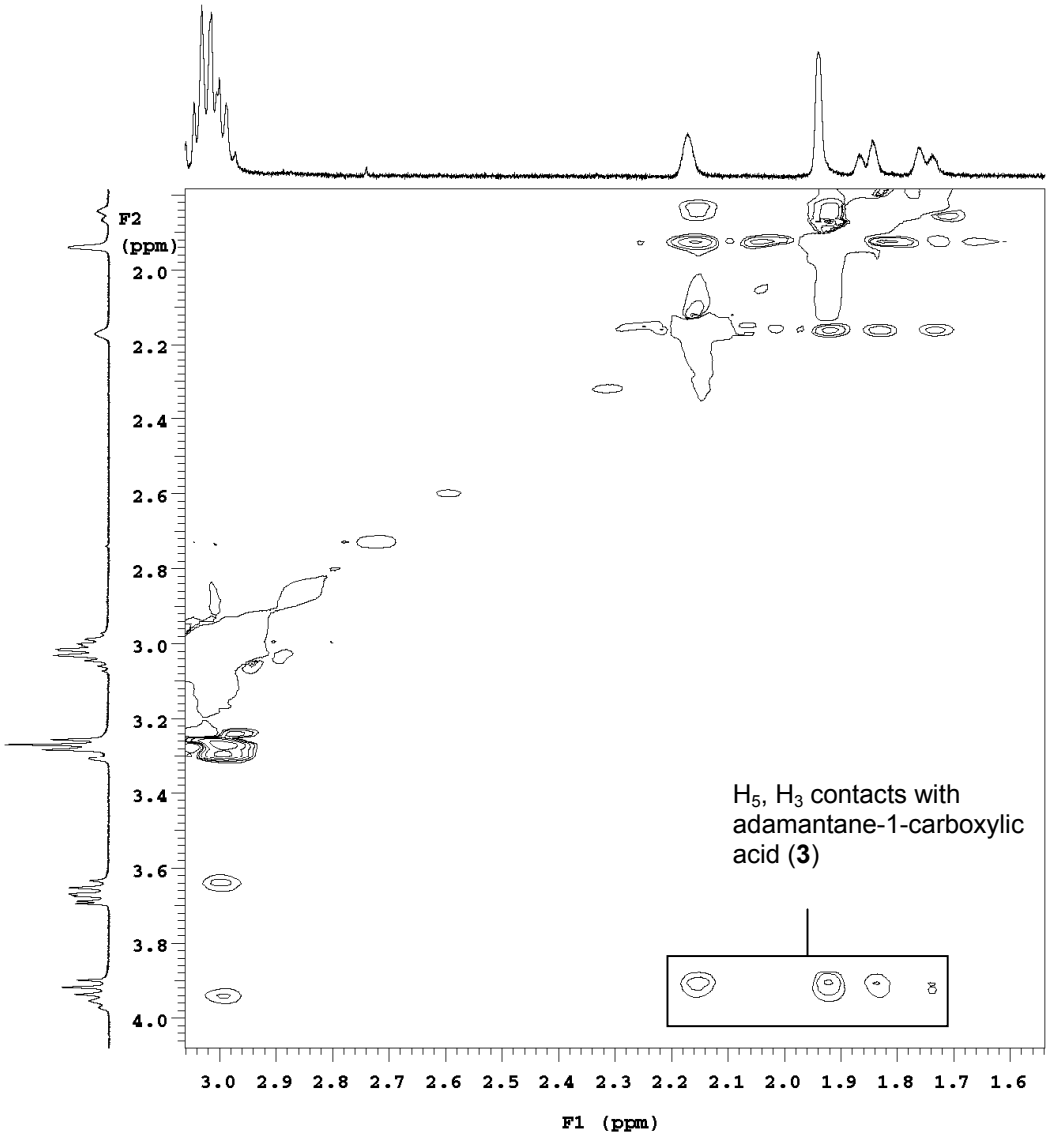


Figure S3c. Expansion of ROESY NMR Complex **1•3**

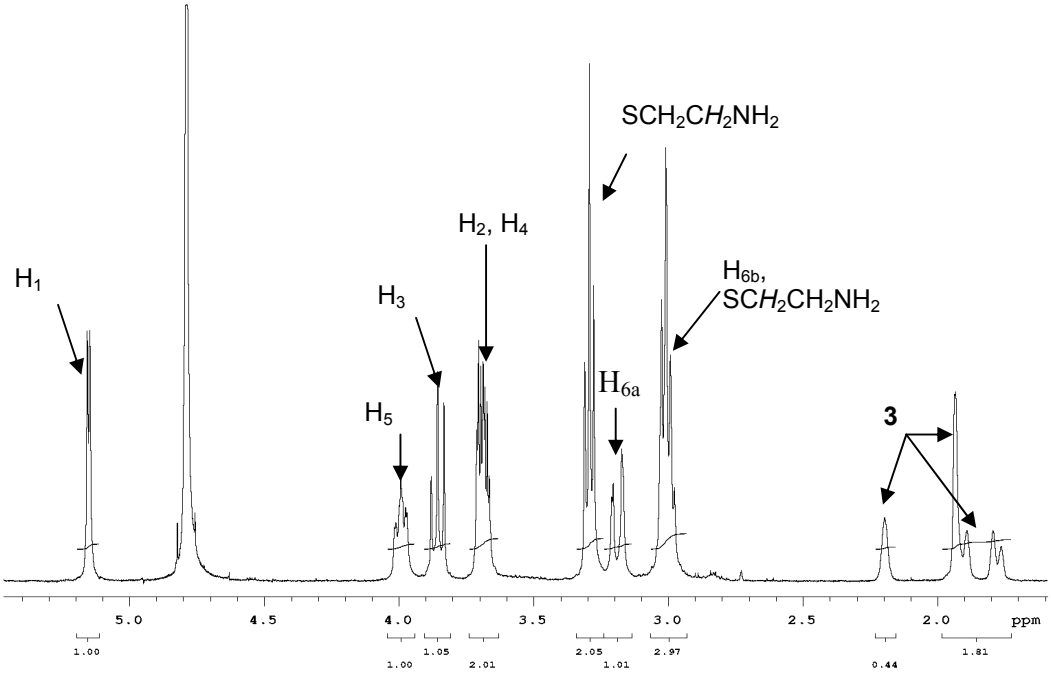


Figure S4a. ^{1}H NMR (400 MHz) Complex **1•3,** D_2O, pD 2.5

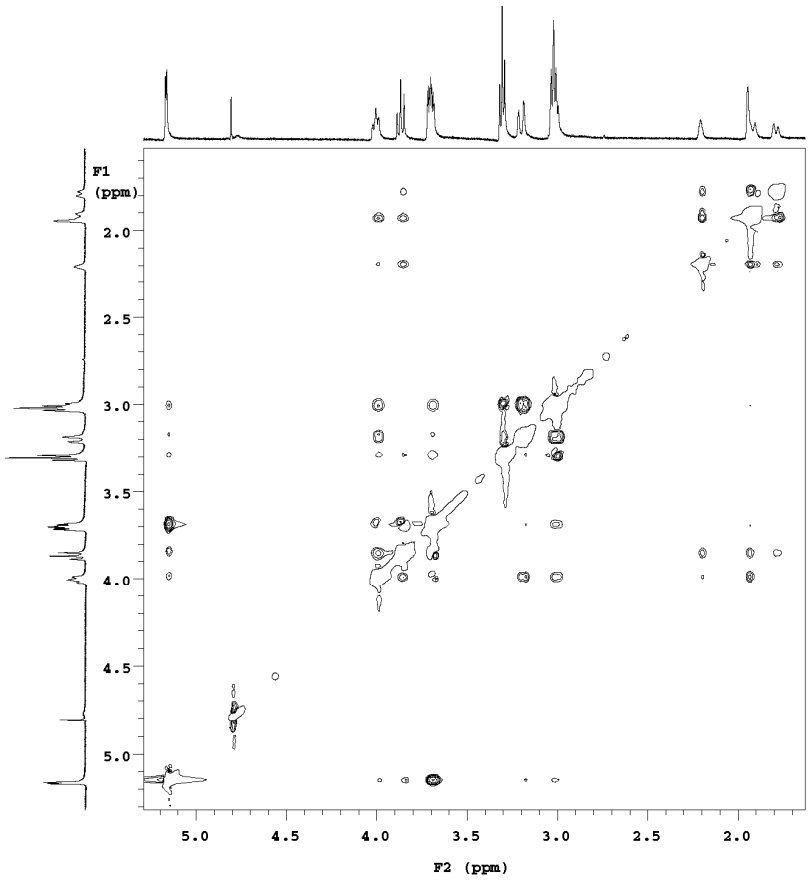


Figure S4b. ROESY NMR (500 MHz) Complex **1•3,** D_2O, pD 2.5

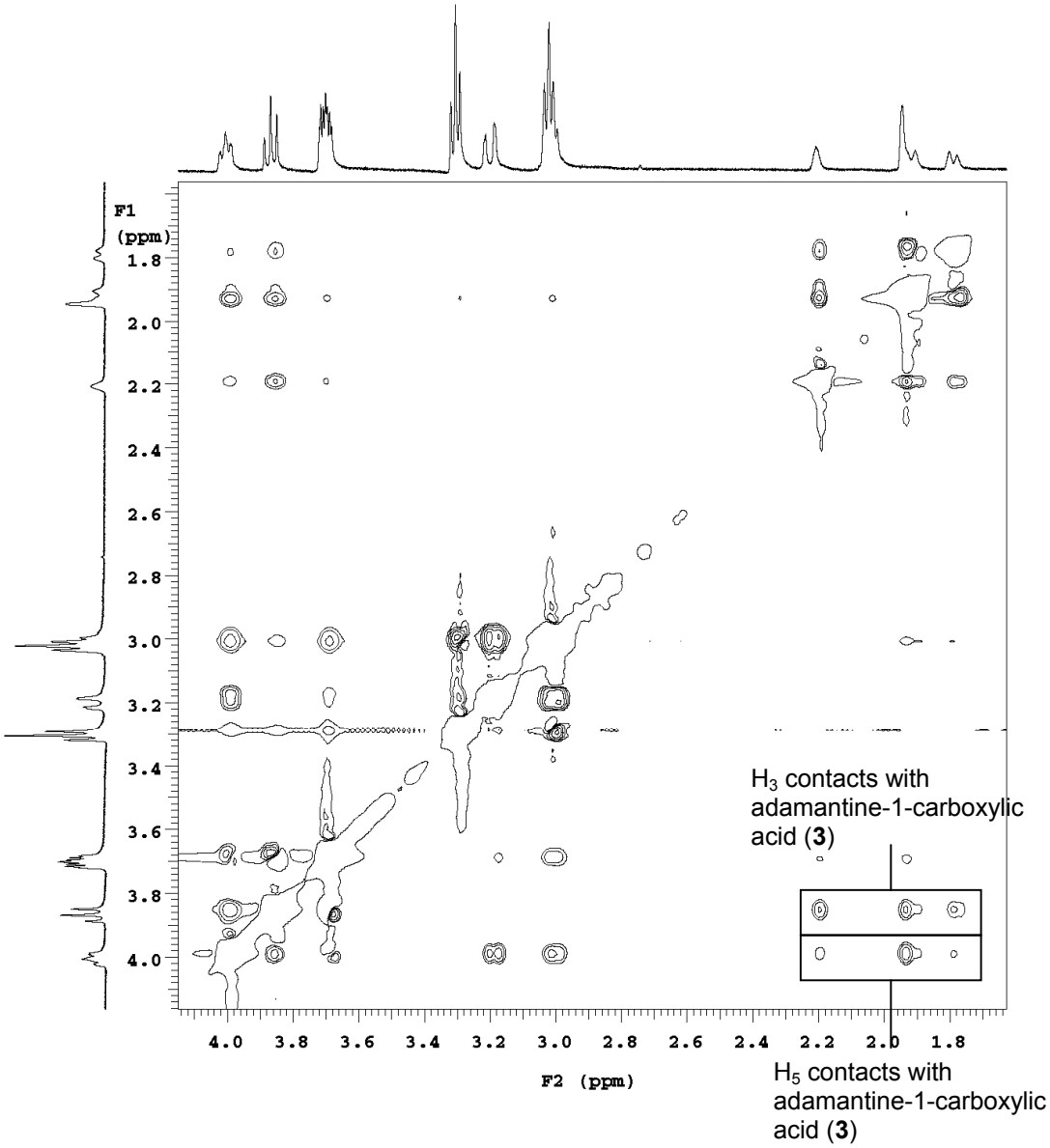


Figure S4c. Expansion of ROESY (500 MHz) of Complex **1•3,** D_2O, pD 2.5

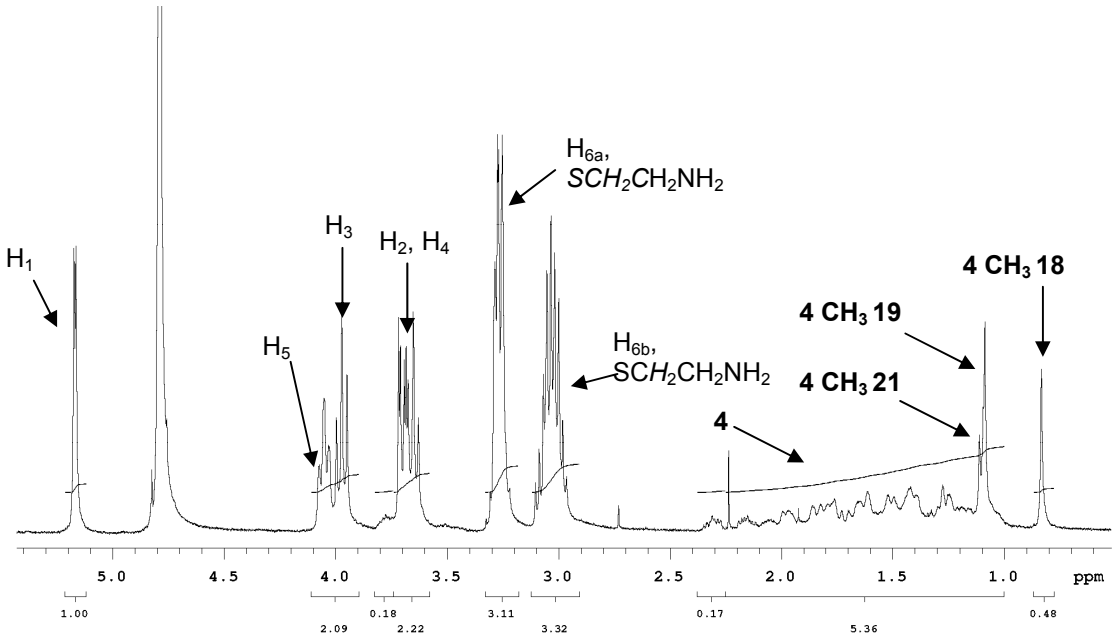


Figure S5a. ^{1}H NMR (400 MHz) Complex **1•4,** D_2O, pD 7.5

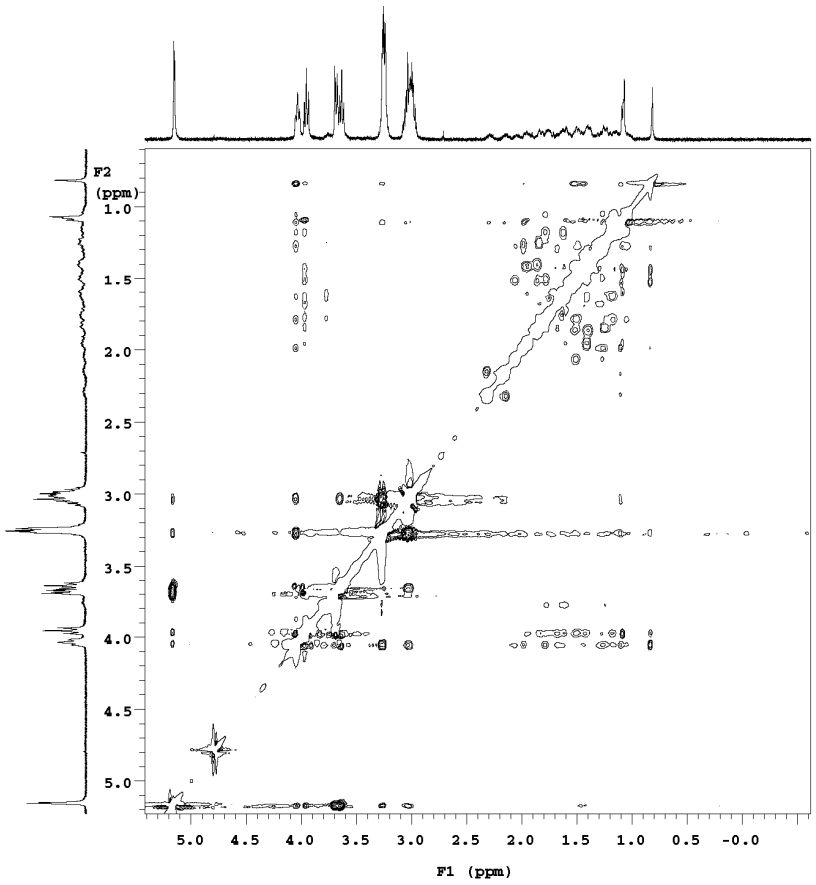


Figure S5b. ROESY NMR (500 MHz) Complex **1•4,** D_2O, pD 7.5

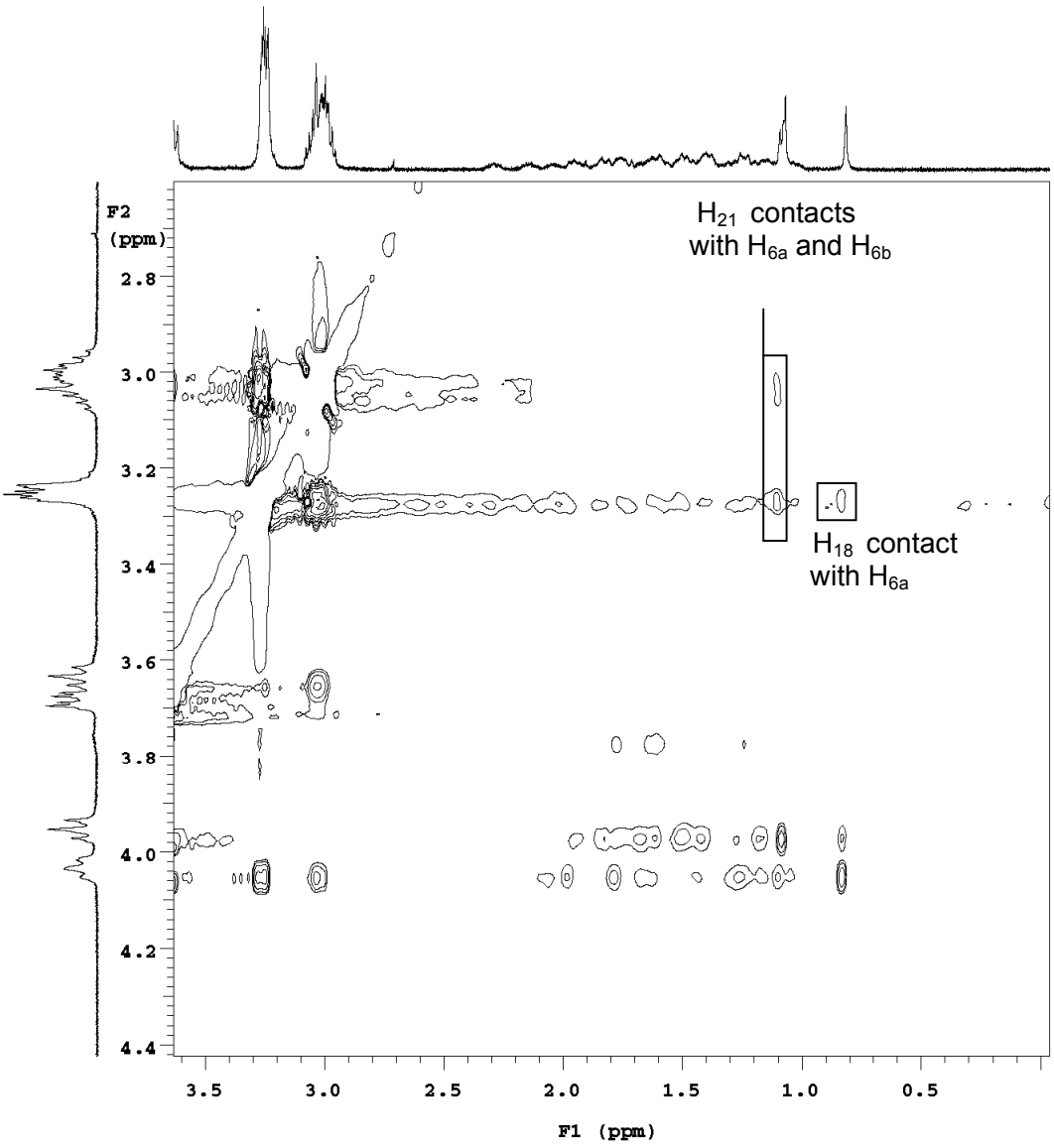


Figure S5c. Expansion of ROESY NMR (500 MHz) Complex **1•4,** D_2O, pD 7.5

Determination of association constants for host-guest complexes using fluorescence spectroscopy

*Direct titration of **1** to guests **5** and **6***

Due to the large increase in fluorescence emission observed upon complexation, the affinity of the fluorescent dyes **5** and **6** could be measured directly. Phosphate buffers (50 mM) were used to adjust the pH of the samples to pH 2.5 and 7.5. Plotting fluorescence versus the cyclodextrin concentration, the association constants (K_a) could be determined by fitting the data to equation (8) derived below. Association constants were calculated from the average of 3 independent titrations. The included graphs are a representative fit of the data.

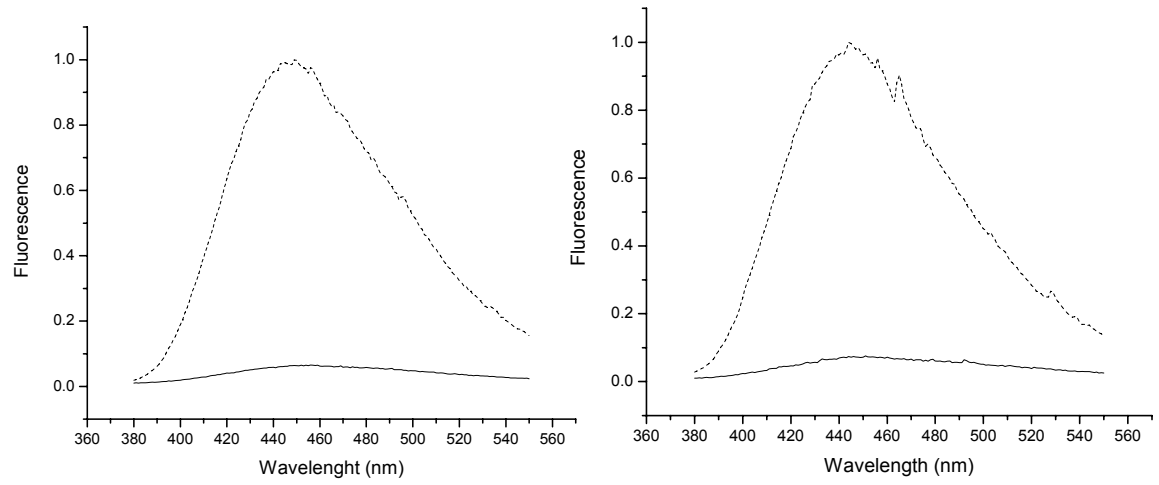


Figure S6. Fluorescence (λ_{ex} = 319 nm) of 0.27 μM 2,6-anilinonaphthalene sulfate (**5**, 2,6-ANS) in phosphate buffer 50 mM at pH 2.5 (left panel) and 7.5 (right panel) in the absence (solid line) and presence (dotted line) of 6 μM CD **1**.

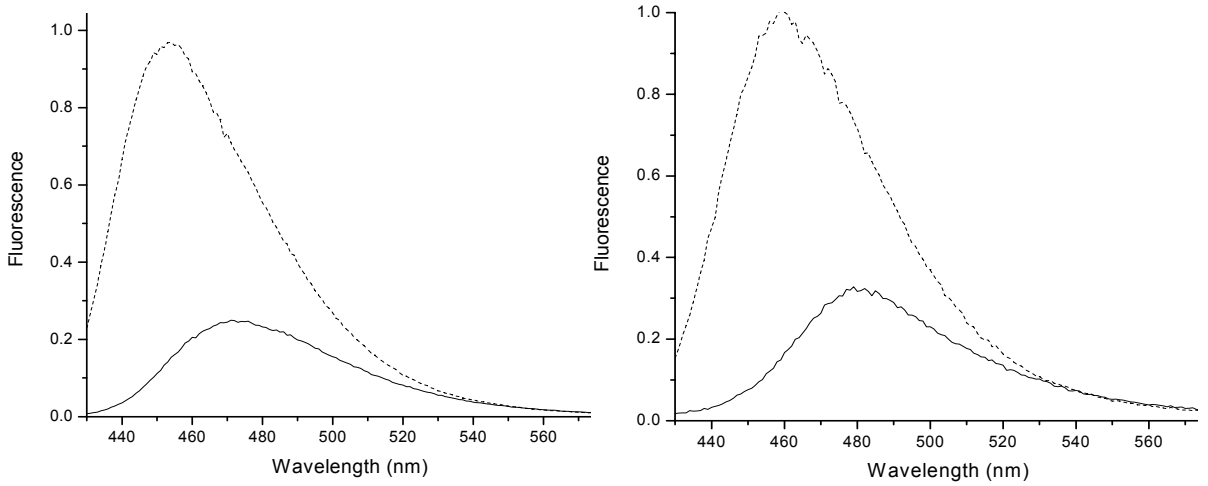
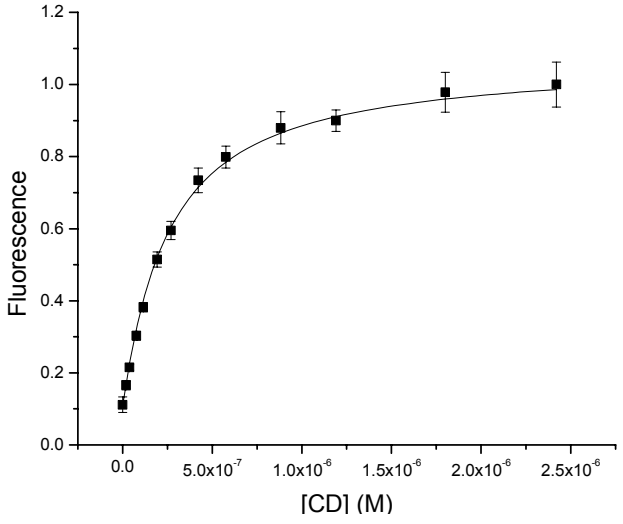


Figure S7. Fluorescence (λ_{ex} = 420 nm) of 10 μM 7-diethylaminocoumarin-3-carboxylic acid (**6**, DEAC) in phosphate buffer 50 mM at pH 2.5 (left panel) and 7.5 (right panel in the absence (solid line) and presence (dotted line) of 10 μM CD **1**.

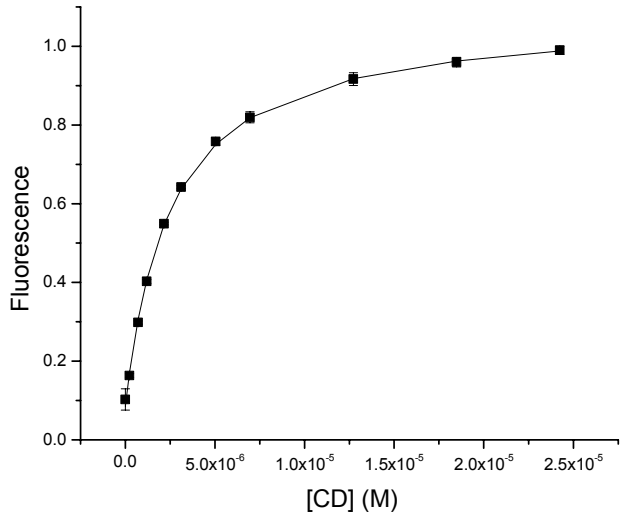


Guest (G): **5**
pH = 2.5
[G] = 54 nM
λ_{ex} = 319 nm
λ_{em} = 413 nm

K_a = 4.35 x 10^6 M^{-1}
± 0.18 x 10^6 M^{-1}

log(K_a) = 6.64

Figure S8. Fluorescence titration of **1** with guest **5** pH 2.5

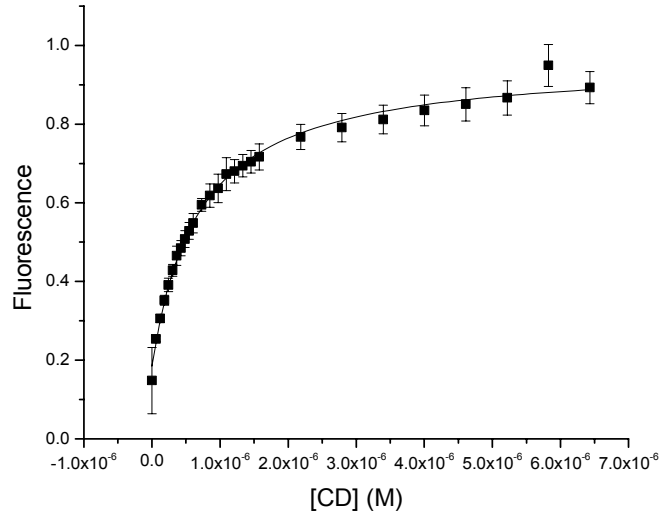


Guest (G): **5**
pH = 7.5
[G] = 54 nM
λ_{ex} = 319 nm
λ_{em} = 413 nm

K_a = 4.05 x 10^5 M^{-1}
± 0.049 x 10^5 M^{-1}

log(K_a) = 5.61

Figure S9. Fluorescence titration of **1** with guest **5** pH 7.5

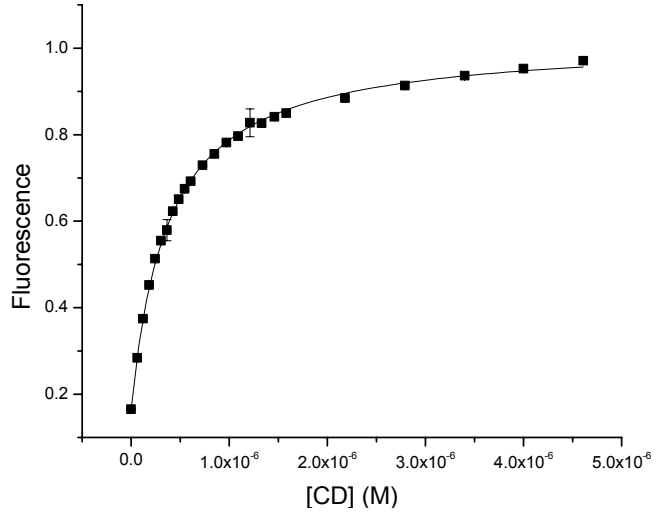


Guest (G): **6**
pH = 2.5
[G] = 6.3 nM
λ_{ex} = 420 nm
λ_{em} = 456 nm

K_a = 1.49 x 10^6 M^{-1}
± 0.035 x 10^6 M^{-1}

log(K_a) = 6.17

Figure S10. Fluorescence titration of **1** with guest **6** pH 2.5



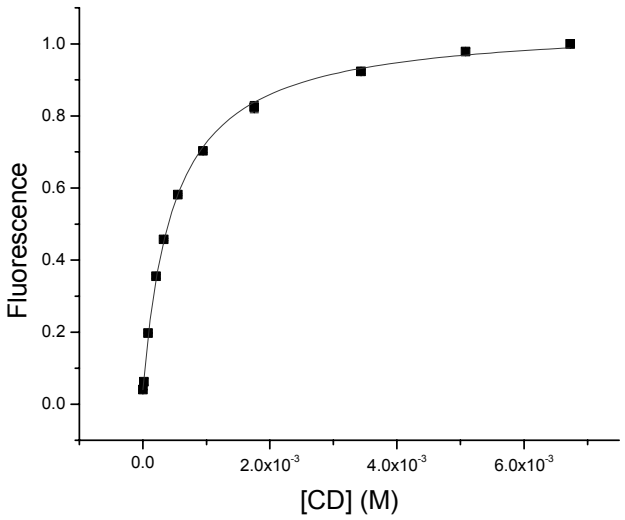
Guest (G): **6**
pH = 7.5
[G] = 5.0 nM
λ_{ex} = 420 nm
λ_{em} = 456 nm

K_a = 2.70 x 10^6 M^{-1}
± 0.20 x 10^6 M^{-1}

log(K_a) = 6.43

Figure S11. Fluorescence titration of **1** with guest **6** pH 7.5

*Direct titration of native β-cyclodextrin to guest **5***

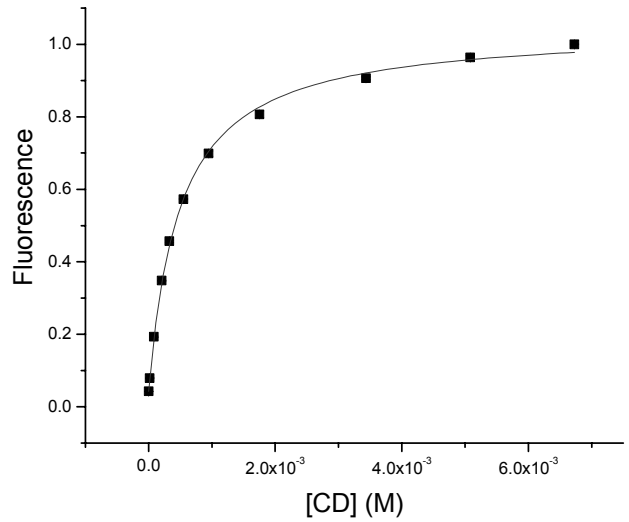


Guest (G): **5**
pH = 2.5
[G] = 4.68 μM
λ_{ex} = 319 nm
λ_{em} = 465 nm

K_a = 2.08 x 10^3 M^{-1}
± 0.38 x 10^2 M^{-1}

log(K_a) = 3.32

Figure S12. Fluorescence Titration of β-CD with guest **5**, pH 2.5



Guest (G): **5**
pH = 7.5
[G] = 4.68 μM
λ_{ex} = 319 nm
λ_{em} = 465 nm

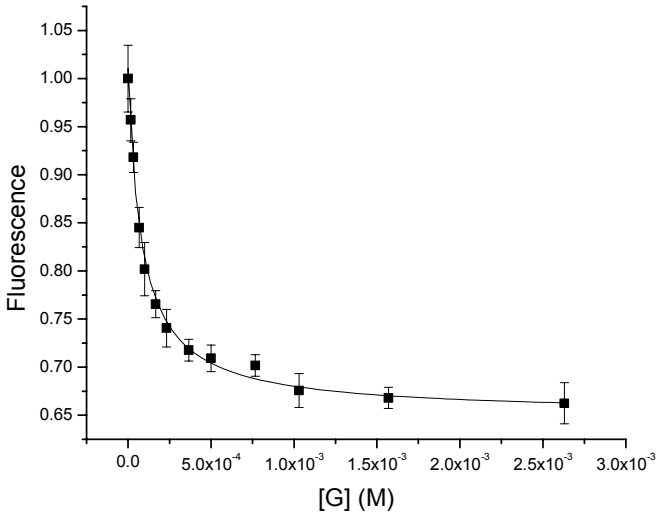
K_a = 2.06 x 10^3 M^{-1}
± 0.46 x 10^2 M^{-1}

log(K_a) = 3.31

Figure S13. Fluorescence Titration of β-CD with guest **5**, pH 7.5

*Competitive fluorescence experiments of **1** with guests **2**-**4**, **7** and **8***

Competitive fluorescence experiments were carried out with the non-fluorescent guests (**2**, **3**, **4** and **7**) using *N*,*N*-4-diethylaminocoumarin-3-carboxylic acid (**6**) as the fluorescent probe. Plotting fluorescence versus the guest concentration, the association constants (K_a) could be determined by fitting the data to equation (14) derived below. Association constants were calculated from the average of 4 independent titrations.

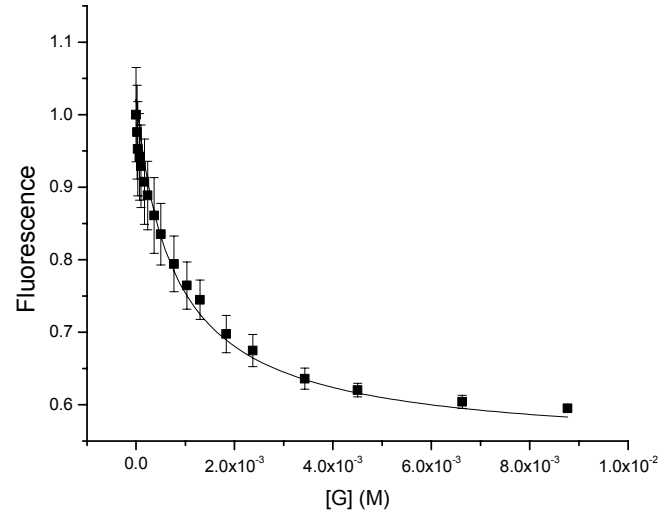


Guest (G): **2**
pH = 2.5
[CD] = 1.8 μM
[**6**] = 10 μM
λ_{ex} = 415 nm
λ_{em} = 456 nm

K_a = 1.69 x 10^5 M^{-1}
± 0.074 x 10^6 M^{-1}

log(K_a) = 5.23

Figure S14. Competitive fluorescence titration of **1** and **6** with guest **2** pH 2.5

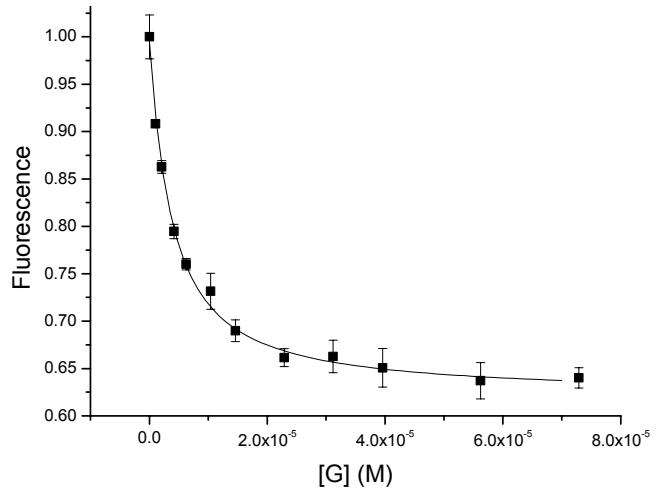


Guest (G): **2**
pH = 7.5
[CD] = 1.8 μM
[**6**] = 10 μM
λ_{ex} = 415 nm
λ_{em} = 456 nm

K_a = 3.11 x 10^4 M^{-1}
± 0.21 x 10^4 M^{-1}

log(K_a) = 4.49

Figure S15. Competitive fluorescence titration of **1** and **6** with guest **2** pH 7.5

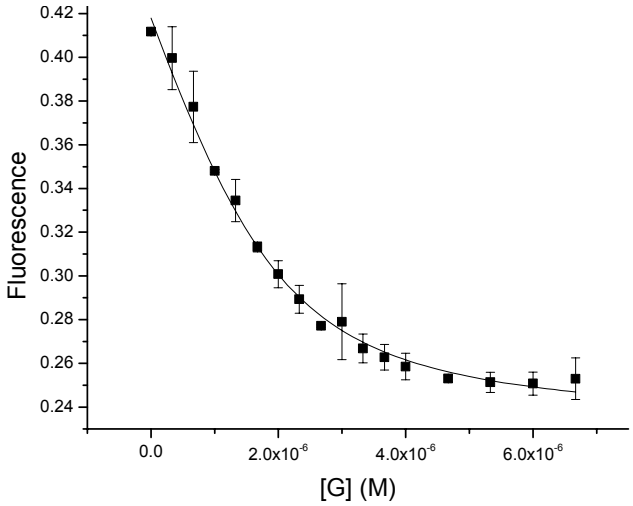


Guest (G): **3**
pH = 2.5
[CD] = 1.8 μM
[**6**] = 10 μM
λ_{ex} = 415 nm
λ_{em} = 456 nm

K_a = 4.65 x 10^6 M^{-1}
± 0.21 x 10^6 M^{-1}

log(K_a) = 6.67

Figure S16. Competitive fluorescence titration of **1** and **6** with guest **3** pH 2.5

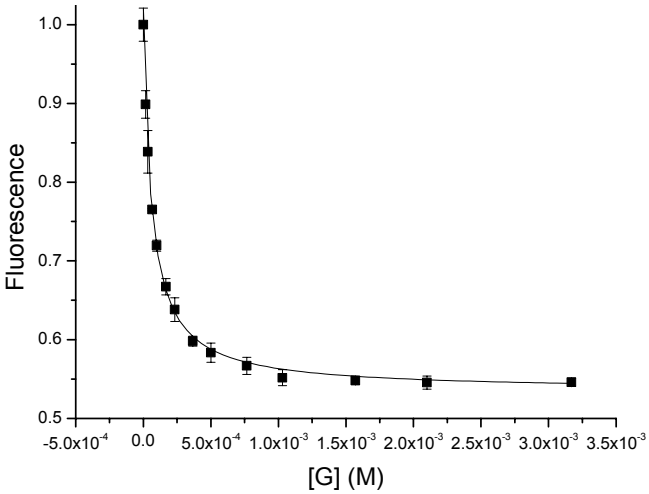


Guest (G): **3**
pH = 7.5
[CD] = 1.8 μM
[**6**] = 10 μM
λ_{ex} = 415 nm
λ_{em} = 456 nm

K_a = 4.35 x 10^5 M^{-1}
± 0.13 x 10^7 M^{-1}

log(K_a) = 5.64

Figure S17. Competitive fluorescence titration of **1** and **6** with guest **3** pH 7.5

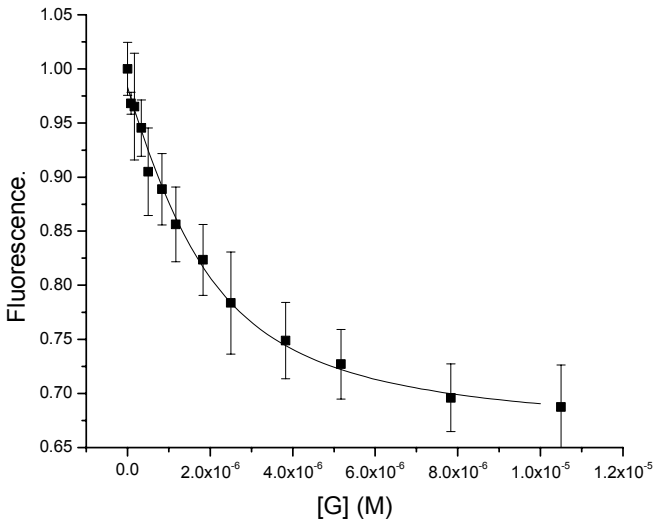


Guest (G): **4**
pH = 7.5
[CD] = 1.8 μM
[**6**] = 10 μM
λ_{ex} = 415 nm
λ_{em} = 456 nm

K_a = 5.52 x 10^7 M^{-1}
± 0.29 x 10^7 M^{-1}

log(K_a) = 7.74

Figure S18. Competitive fluorescence titration of **1** and **6** with guest **4** pH 7.5

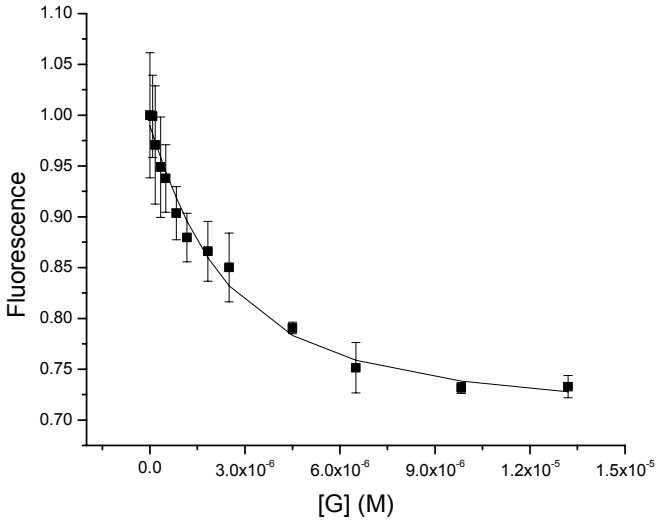


Guest (G): **7**
pH = 2.5
[CD] = 1.8 μM
[**6**] = 10 μM
λ_{ex} = 415 nm
λ_{em} = 456 nm

K_a = 1.57 x 10^7 M^{-1}
± 0.11 x 10^7 M^{-1}

log(K_a) = 7.20

Figure S19. Competitive fluorescence titration of **1** and **6** with guest **7** pH 2.5

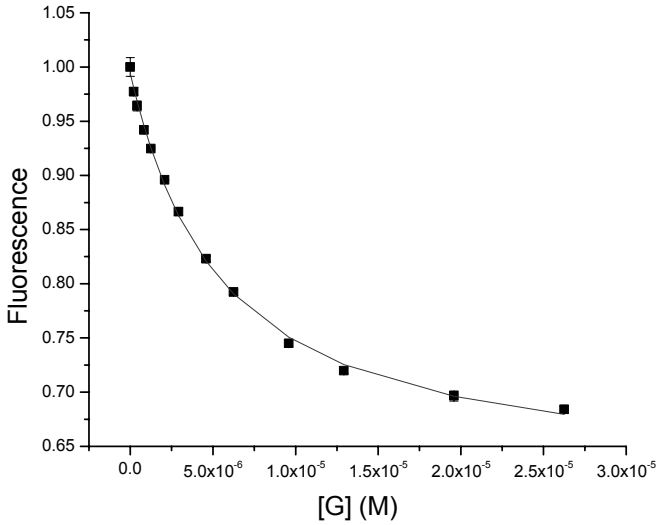


Guest (G): **7**
pH = 7.5
[CD] = 1.8 μM
[**6**] = 10 μM
λ_{ex} = 415 nm
λ_{em} = 456 nm

K_a = 1.85 x 10^7 M^{-1}
± 0.098 x 10^7 M^{-1}

log(K_a) = 7.27

Figure S20. Competitive fluorescence titration of **1** and **6** with guest **7** pH 7.5

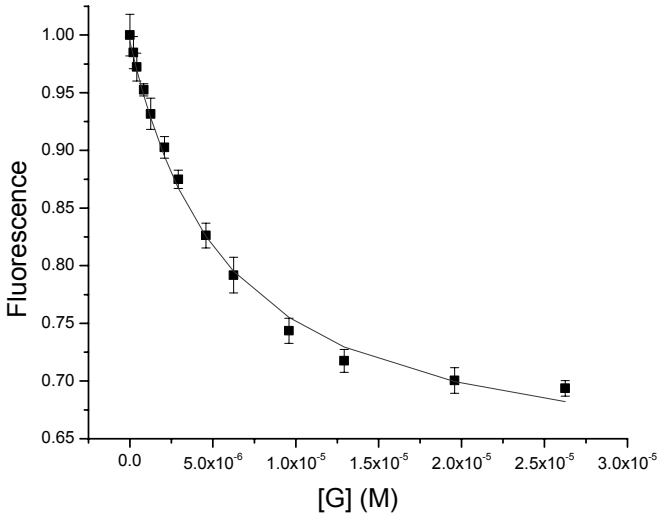


Guest (G): **8**
pH = 2.5
[CD] = 1.8 μM
[**6**] = 10 μM
λ_{ex} = 415 nm
λ_{em} = 456 nm

K_a = 3.09 x 10^6 M^{-1}
± 0.64 x 10^4 M^{-1}

log(K_a) = 6.48

Figure S21. Competitive fluorescence titration of **1** and **6** with guest **8** pH 2.5



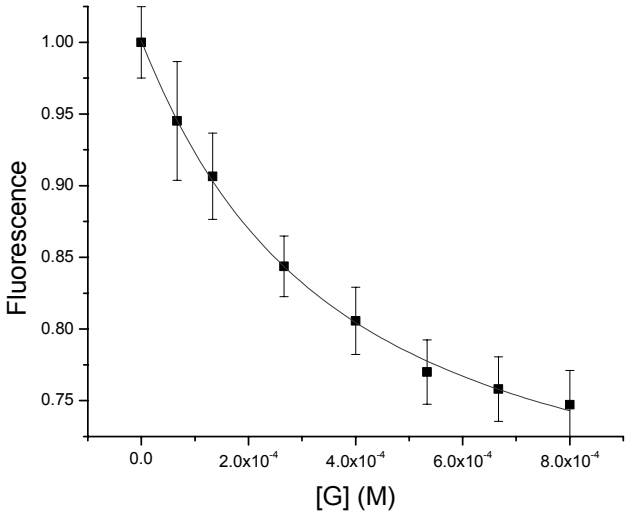
Guest (G): **8**
pH = 7.5
[CD] = 1.8 μM
[**6**] = 10 μM
λ_{ex} = 415 nm
λ_{em} = 456 nm

K_a = 5.31 x 10^6 M^{-1}
± 0.19 x 10^6 M^{-1}

log(K_a) = 6.72

Figure S22. Competitive fluorescence titration of **1** and **6** with guest **8** pH 7.5

*Competitive fluorescence experiments with native β-cyclodextrin for guests **2** and **3***

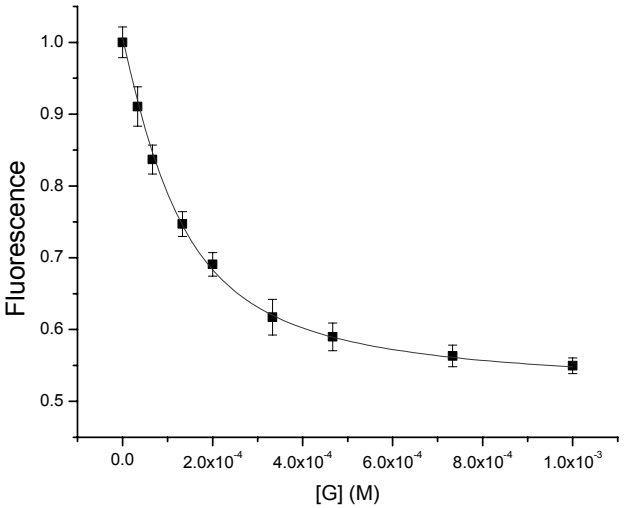


Guest (G): **2**
pH = 7.5
[CD] = 100 μM
[**5**] = 1000 μM
λ_{ex} = 319 nm
λ_{em} = 465 nm

K_a = 6.74 x 10^3 M^{-1}
± 0.131 x 10^3 M^{-1}

log(K_a) = 3.83

Figure S23. Competitive fluorescence titration of β-CD and **5** with guest **2** pH 7.5



Guest (G): **3**
pH = 7.5
[CD] = 100 μM
[**5**] = 1000 μM
λ_{ex} = 319 nm
λ_{em} = 465 nm

K_a = 2.92 x 10^4 M^{-1}
± 0.93 x 10^3 M^{-1}

log(K_a) = 4.46

Figure S24. Competitive fluorescence titration of β-CD and **5** with guest **3** pH 7.5

*Binding of the lithocholic acid guest **4** to β-cyclodextrin over time when added in DMSO (■) and phosphate buffer pH 7.5 (●)*

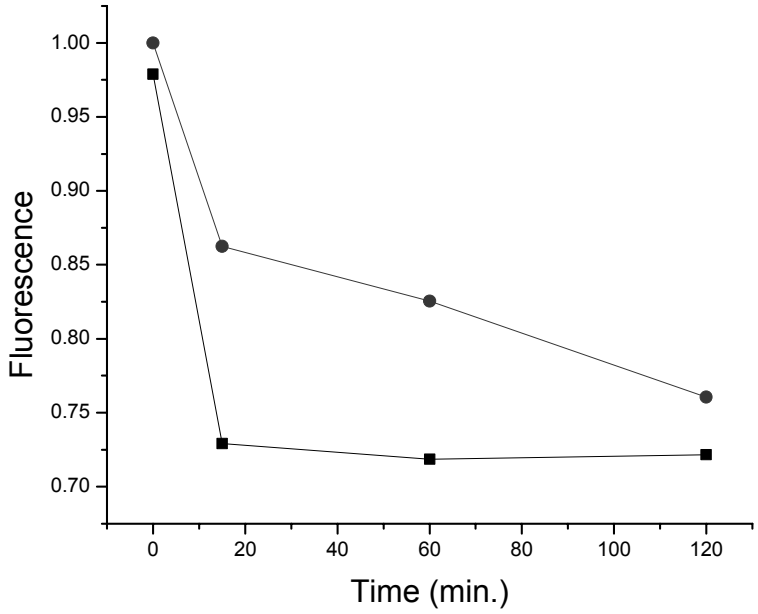


Figure S25. Time course of binding between lithocholic acid guest **4** to **1.** CD **1** (1.8 μM) was dissolved in phosphate buffer (50 mM, pH 7.5) and fluorescent guest **6** (10 μM) was added. The competitive guest **4** (1.8 μM) was added either as a solution in DMSO (●)(1.8% final DMSO concentration in solution) or as a colloidally stable suspension in phosphate buffer (50 mM pH 7.5) (●) at time 0.

Fitting of binding curves

A) Direct titrations:

$$CD \rightleftharpoons CD \bullet G$$

Where CD is cyclodextrin **1** and *G* is a fluorescent guest molecule.

$$K_G = \frac{[CD \bullet G]}{[CD_F][G_F]}, \quad (1)$$

Where $[CD_F]$ is the concentration of free **1**, $[G_F]$ is the concentration of free fluorescent guest, and K_G is the association constant of the guest and **1**.

$$\%bound\ \ fluorophore = \frac{(F - F_{\min})}{(F_{\max} - F_{\min})}, \quad (2)$$

Where *F* is the fluorescence emission detected and F_{min} and F_{max} are the minimum and maximum fluorescence signal that was detected (*i.e.* when $[CD]=0$ and $[CD] >> [G]$, respectively). Eqn. (3) can then be defined as follows:

$$\frac{[CD \bullet G]}{[G_T]} = \frac{(F - F_{\min})}{(F_{\max} - F_{\min})}, \quad (3)$$

And

$$[G_F] = [G_T] - [CD \bullet G] \quad (4)$$

$$[CD_F] = [CD_T] - [CD \bullet G] \quad (5)$$

Where $[G_T]$ is the total concentration of the guest molecule and $[CD_T]$ is the total concentration of **1**. Eqn. (6) was obtained by substituting (4) and (5) into (1):

$$K_G = \frac{[CD \bullet G]}{[CD_T][G_T] - [CD_T][CD \bullet G] - [G_T][CD \bullet G] + [CD \bullet G]^2} \quad (6)$$

And solved for $[CD \bullet G]$ as a quadratic function where the variable x = $[CD_T]$:

$$[CD \bullet G] = \frac{(K_G x + K_G[G_T] + 1) \pm \left((-K_G x - K_G[G_T] - 1)^2 - 4K_G{}^2 x[G_T]\right)^{\frac{1}{2}}}{2K_G} \quad (7)$$

We now define *q* as:

$$q = \frac{(K_G x + K_G[G_T] + 1) \pm \left((-K_G x - K_G[G_T] - 1)^2 - 4K_G{}^2 x[G_T]\right)^{\frac{1}{2}}}{2K_G},$$

By inserting (7) into (3) and solving for *F*, the final expression was obtained for the direct titration of a fluorescent guest and **1**,

$$F = \frac{q(F_{\max} - F_{\min}) + [G_T]F_{\min}}{[G_T]} \quad (8)$$

Plotting fluorescence versus $[CD_T]$, K_a of the fluorophore was determined by fitting the data to (8).

B) Competitive titrations

$$CD \bullet G \rightleftharpoons CD \rightleftharpoons CD \bullet DEAC$$

Competitive titration of coumarin **6** (*DEAC*) versus non fluorescent guest (*G*) with **1** were fit using the following equation:

$$[G_F] = [G_T] - [CD \bullet G] \quad (9)$$

Where $[G_F]$ is the concentration of free guest and $[G_T]$ is the total concentration of guest.

$$K_G = \frac{[CD \bullet G]}{[CD_F][G_F]}, \quad (10)$$

And,

$$[CD \bullet G] = [CD_T] - [CD \bullet DEAC] - [CD_F] \quad (11)$$

Where $[CD_F]$ is the concentration of free **1** and $[CD_T]$ is the total concentration of **1**, and K_G is the association constant of the guest and **1**. Assuming that $[CD_F]$ remains very small throughout the titration, the $[CD_F]$ term in equation (11) can be ignored. Expression (12) was obtained by substituting (9) into (10) and subsequently substituting (11) into their ensuing equation,

$$K_G = \frac{([CD_T] - [CD \bullet DEAC])}{[CD_F]([G_T] - [CD_T] + [CD \bullet DEAC])} \quad (12)$$

Rearranging (12) and (1) to equal $[CD_F]$ (and therefore to each other), by rearranging the resulting (13) was obtained:

$$\begin{aligned} 0 &= [CD \bullet DEAC^2](K_C - K_G) \\ &+ [CD \bullet DEAC](-[CD_T]K_C - [DEAC_T]K_C - [G_T]K_G + [CD_T]K_G) \\ &+ [CD_T]K_C[DEAC_T] \end{aligned} \quad (13)$$

Solving (13) as a quadratic function, where $y = [CD \bullet DEAC]$, and x = $[G_T]$, q_2 was defined as follows:

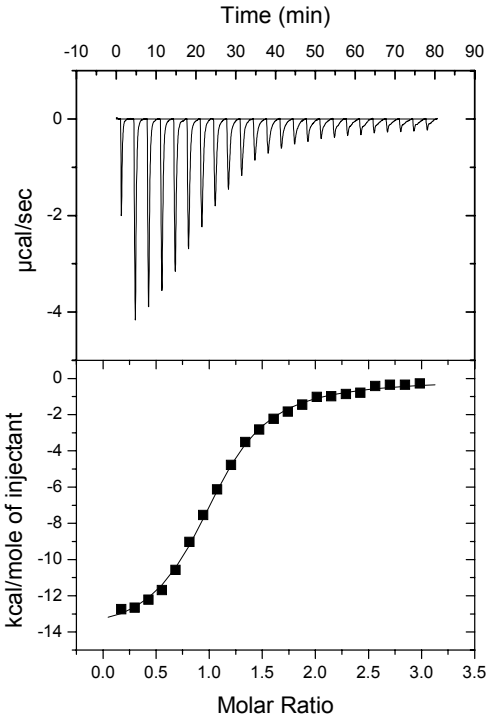
$$q_2 = \left(\begin{array}{c} -\left([CD_T]K_C - K_C[DEAC_T] - K_G x + K_G[CD_T]\right) \\ \pm\left(\left([CD_T]K_C - K_C[DEAC_T] - K_G x + K_G[CD_T]\right)^2 - 4\left(K_C - K_G\right)[CD_T]K_C[DEAC_T]\right)^{\frac{1}{2}} \\ \hline 2\left(K_C - K_G\right) \end{array} \right)$$

Inserting q_2 into (3) (where *G* = *DEAC*) and solving for *F* the final expression for the competitive titration of **6** versus guest and **1** is given,

$$F = \frac{q_2\left(F_{\max} - F_{\min}\right) + [DEAC_T]F_{\min}}{[DEAC_T]} \qquad (14)$$

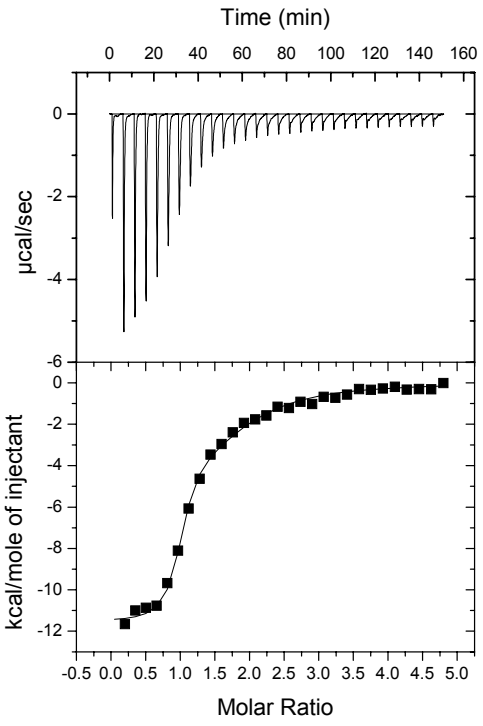
Isothermal titration calorimetry

A solution of **5** was titrated into a solution of **1** at 25° C with 300 rpm stirring. Both solutions were made in phosphate buffer (pH 2.5 and 7.5, 50mM) and dialyzed overnight using 100 MWCO dialysis tubing (spectrapore). The final concentration of **5** was determined using absorbance spectrometry (ε = 26,000 cm^{-1} M^{-1}, λ_{max} 319 nM). Heats of dilution were determined in separate experiments. The data was fit to a two sequential site binding model. During the curve fitting the association constant of the first binding event was held at the value determined by fluorescence titration. Representative curve fits from titration data are found below. Data reported are the average of two titrations.



ITC of **5** into **1** at pH 7.5.
[**1**] = 0.050 mM (Cell)
[**5**] = 1.1 mM (Titrant)
5 was titrated in 8 μL injections, with the exception of the first injection of 3 μL

K1	3.5 x 10^5 M^{-1}
ΔH1	-1.431 x 10^4
	$\pm$0.018 x 10^4 cal/mol
ΔS1	-22.55 cal/mol
K2	9293 $\pm$ 239 M^{-1}
ΔH2	-956.0
	$\pm$553.3 cal/mol
ΔS2	14.95 cal/mol



ITC of **5** into **1** at pH 2.5.

[**1**] = 0.060 mM (Cell)

[**5**] = 1.4 mM (Titrant)

5 was titrated in 9 μL injections, with the exception of the first injection of 3 μL

K1	4.2×10^{6} M^{-1}
ΔH1	-1.157×10^{4}
	$\pm 0.010 \times 10^{4}$ cal/mol
ΔS1	-8.486 cal/mol
K2	5.326×10^{4}
	$\pm 0.746 \times 10^{4}$ M^{-1}
ΔH2	-5151
	$\pm$307.3 cal/mol
ΔS2	4.347 cal/mol