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Supporting Information

Allosteric Binding of Sodiumdeoxycholate by a Bis(β-cyclodextrin)-2,2´-bipyridine Receptor

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Table of contents

1. NMR and mass spectra

3^A-Amino-3^A-deoxy-monoaltro-β-cyclodextrin (11)

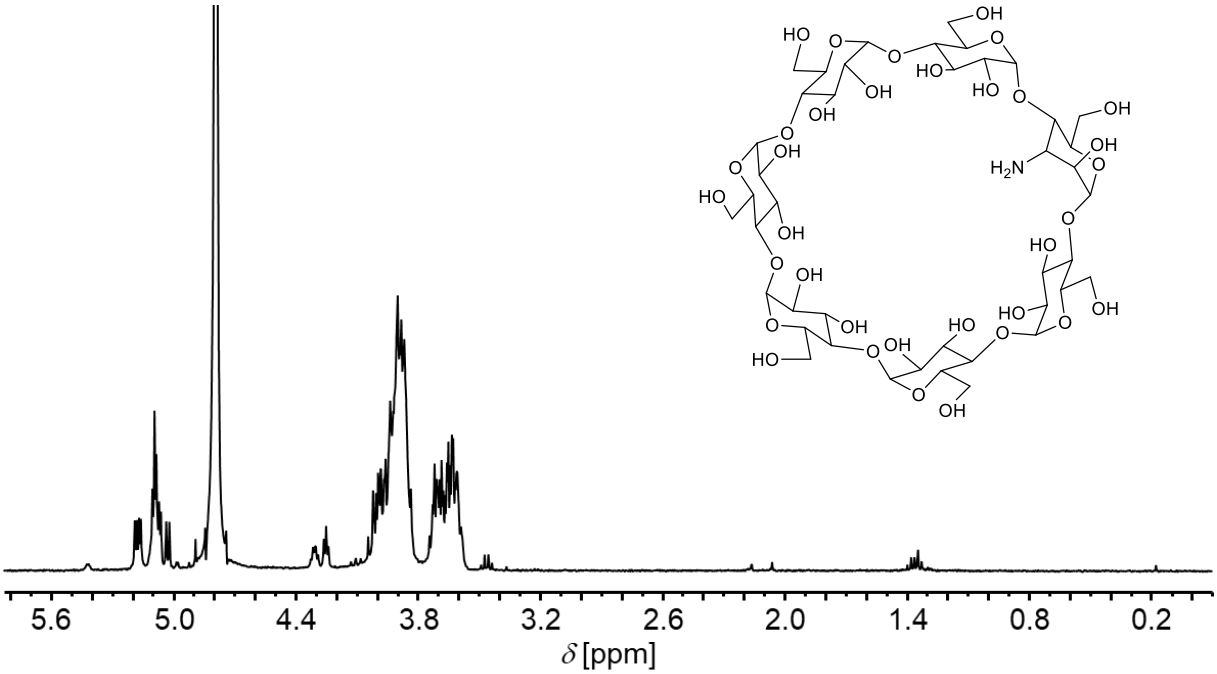


Figure S1: ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectrum of **11**.

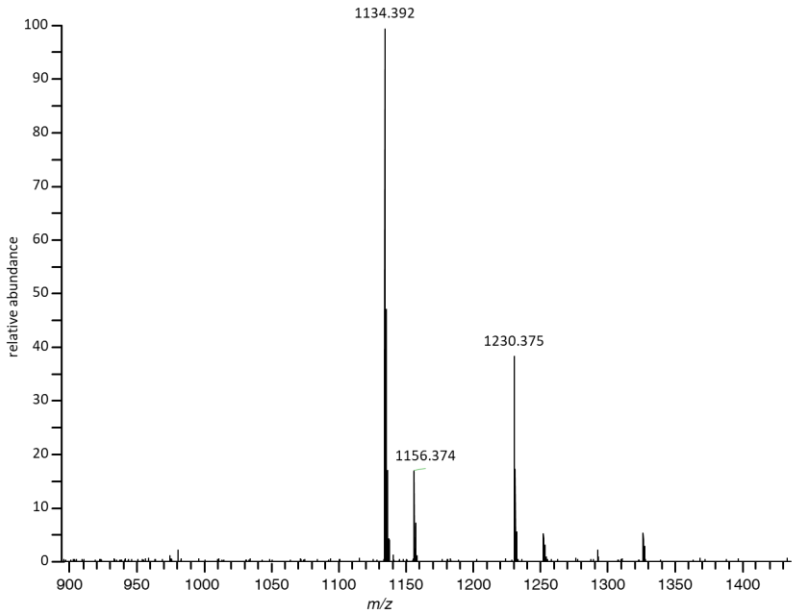


Figure S2: ESI positive mass spectrum of **11.**

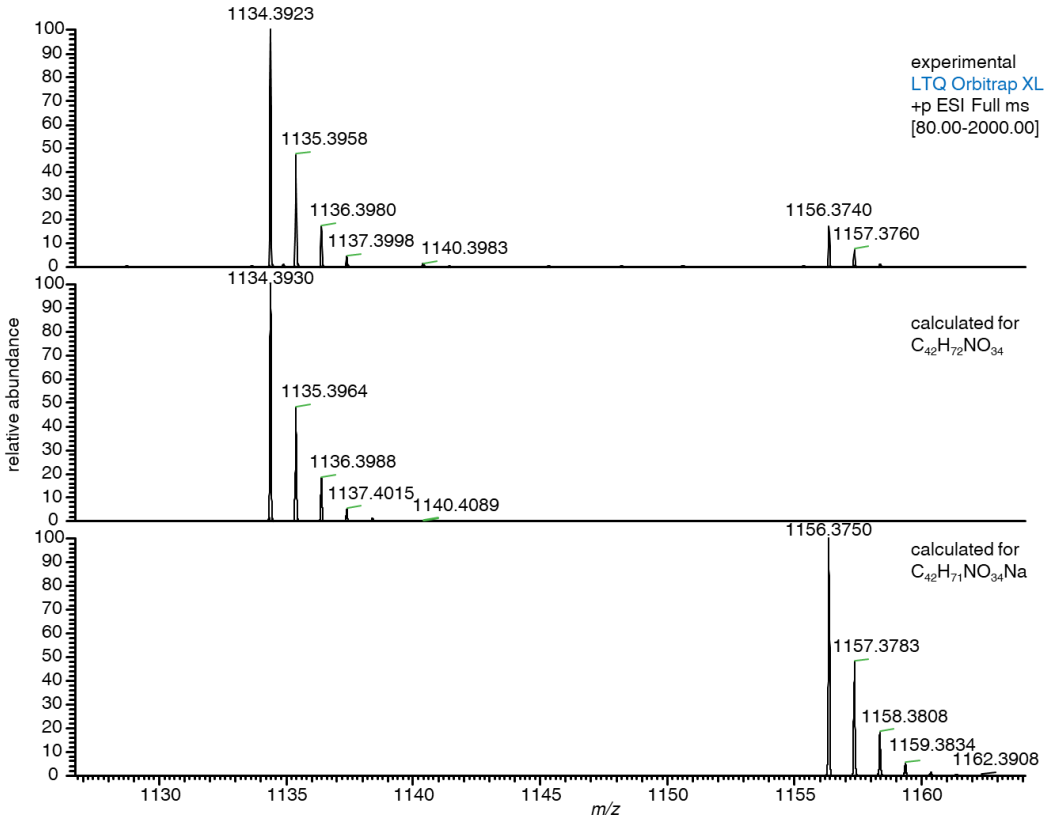


Figure S3: Calculated and experimental isotope patterns of ESI HR mass spectrum (pos. mode) of **11.**

2^A,2^B,2^C,2^D,2^E,2^F,2^G,3^B,3^C,3^D,3^E,3^F,3^G,6^A,6^B,6^C,6^D,6^E,6^F,6^G-Icosa-(*O*-trimethylsilyl)-3^A-amino-deoxy-β-cyclodextrine 7

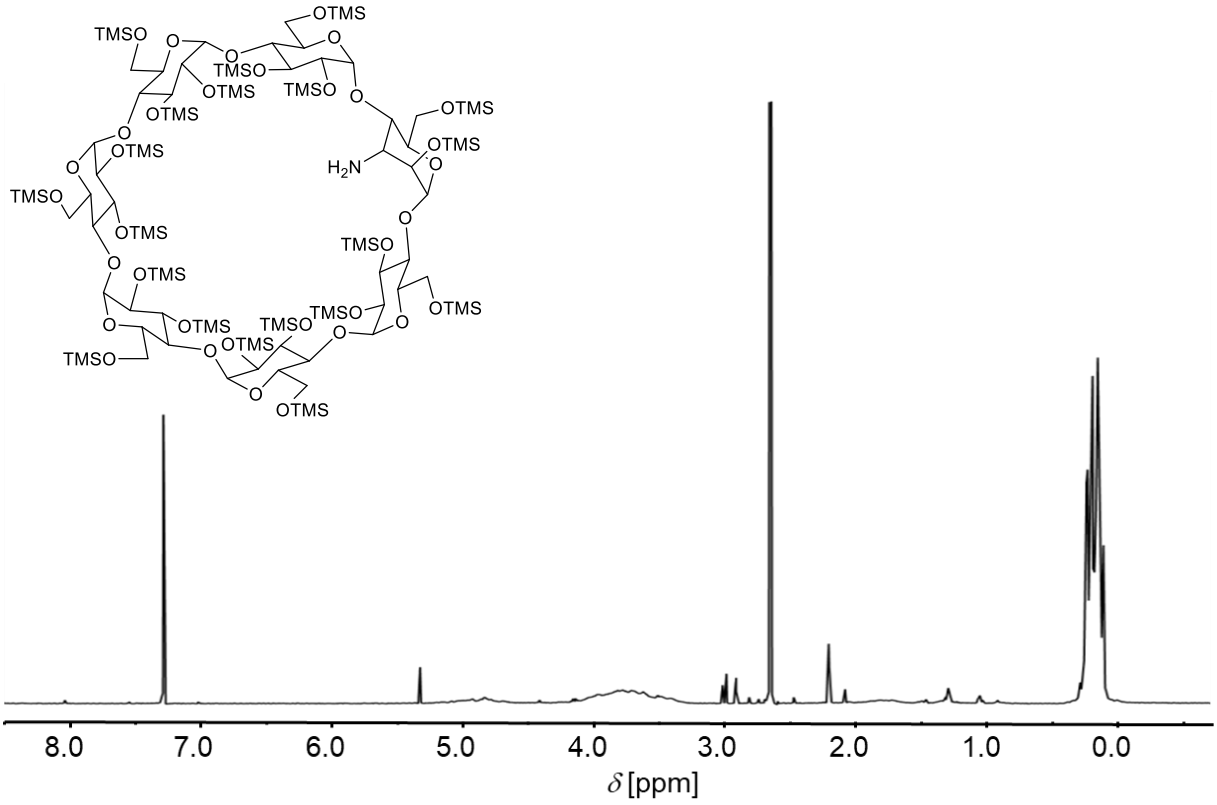
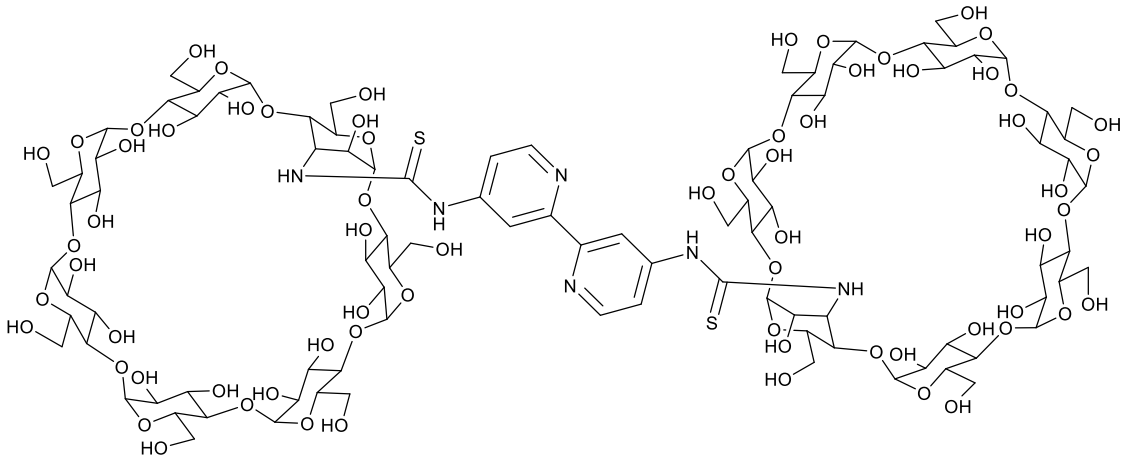


Figure S4: ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectrum of **7**.

1,1'-([2,2'-Bipyridine]-4,4'-diyl)bis(3-[3^{A}-deoxy-mono-altro-β-cyclo-dextrin-3^{A}-yl]thiourea) 1



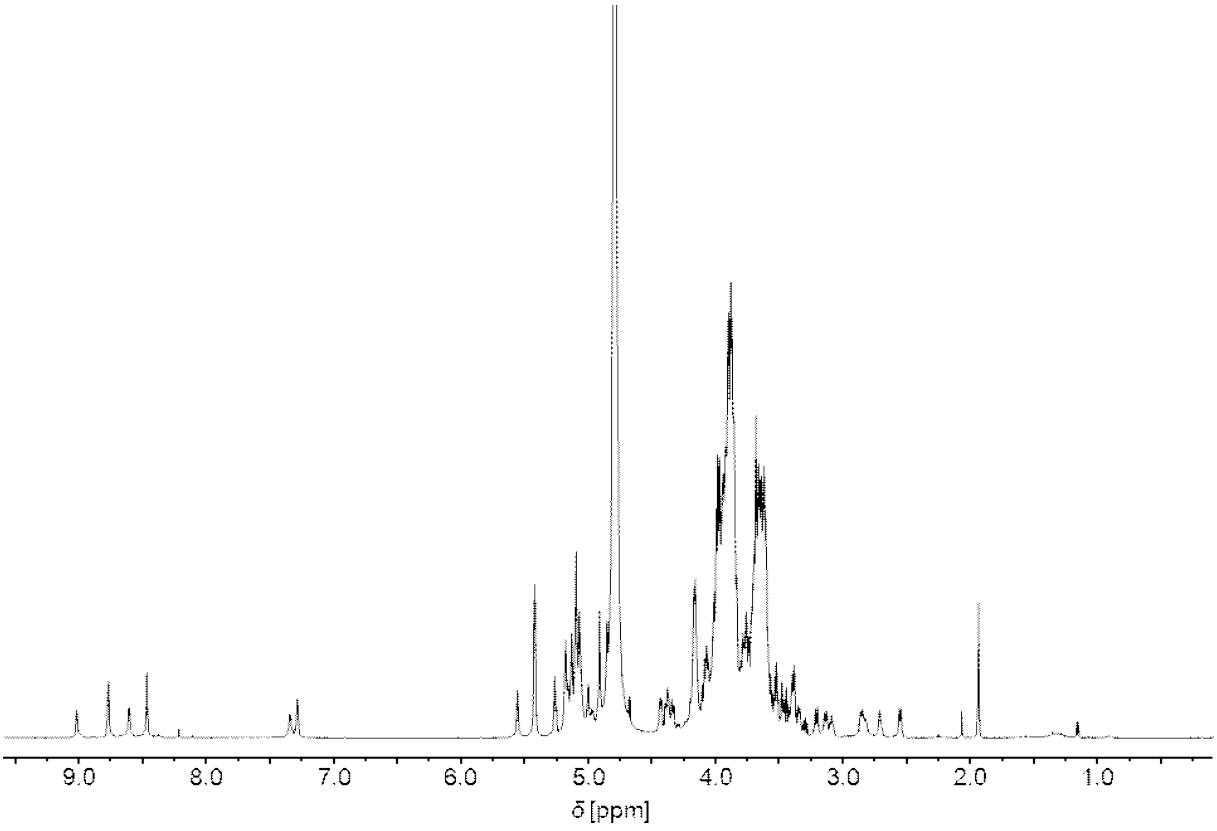


Figure S5: ^{1}H NMR spectrum (700 MHz, D_2O, 298 K) of **1**.

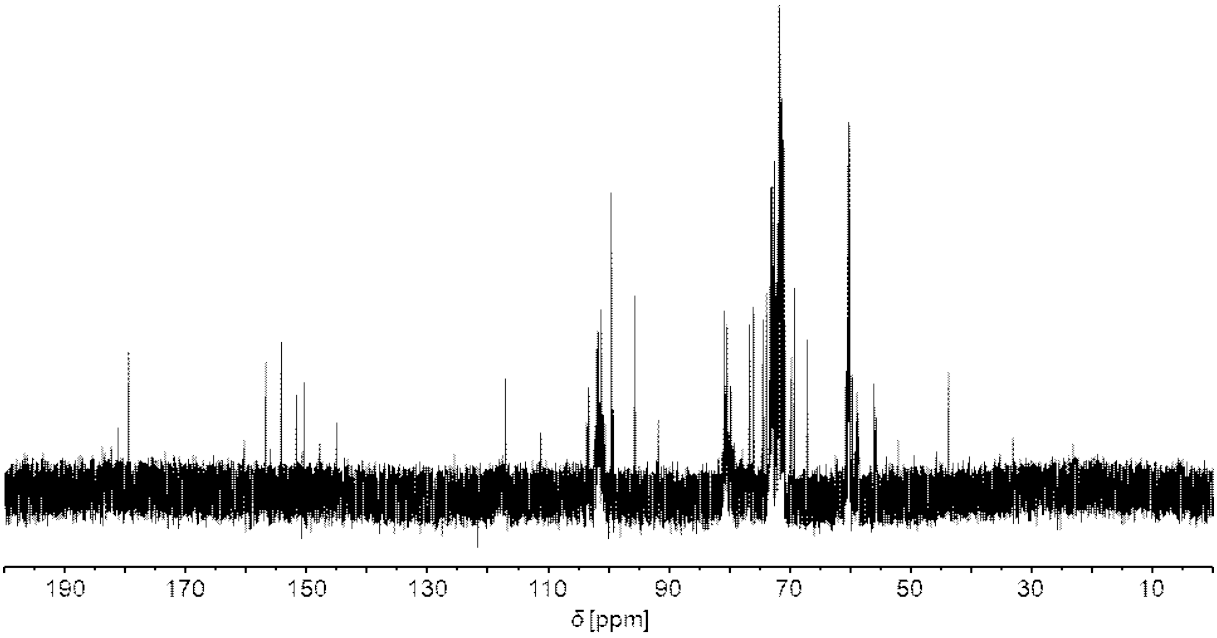


Figure S6: ^{13}C NMR spectrum (176.1 MHz, D_2O, 298 K) spectrum of **1**.

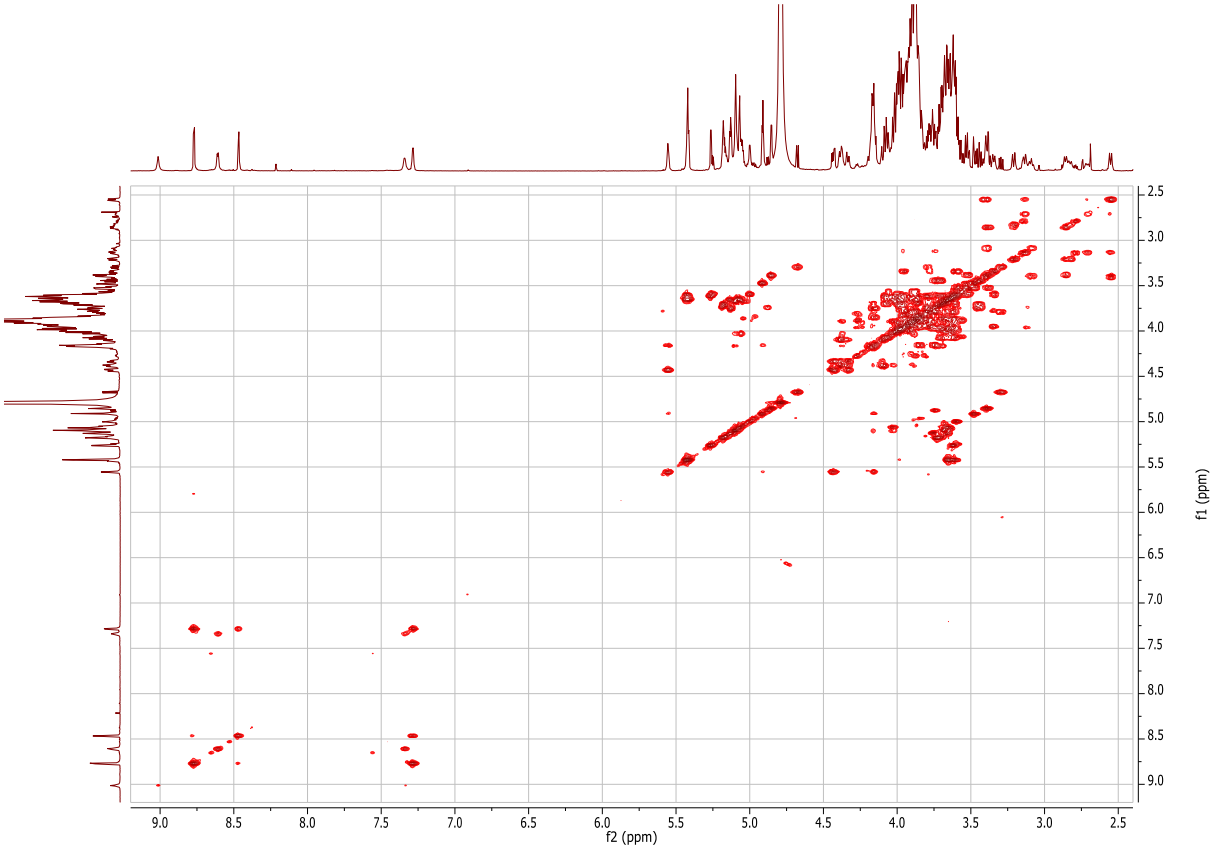


Figure S7: $^{1}H,^{1}H$ 2D-COSY NMR spectrum (700 MHz, D_2O, 298 K) of **1**.

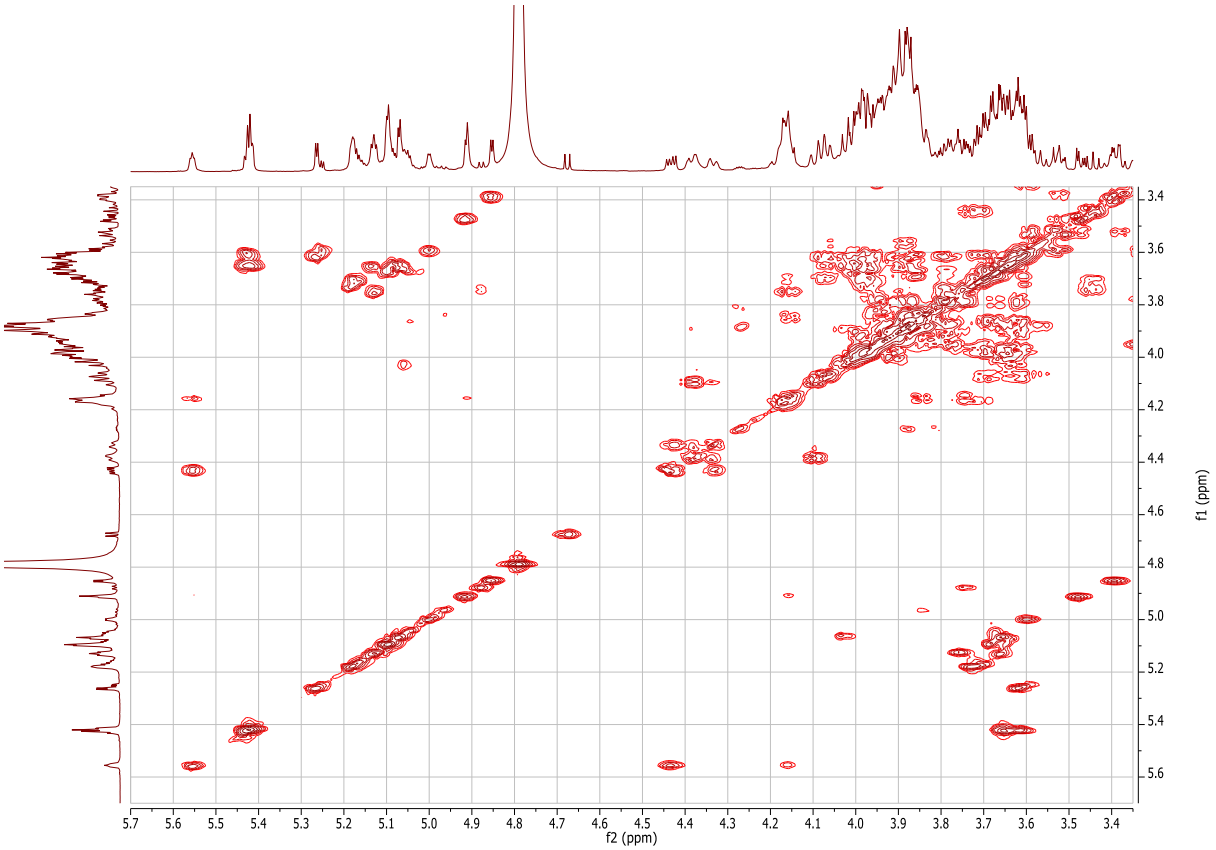


Figure S8: Upfield region of $^{1}H,^{1}H$ 2D-COSY NMR spectrum (700 MHz, D_2O, 298 K) of **1**.

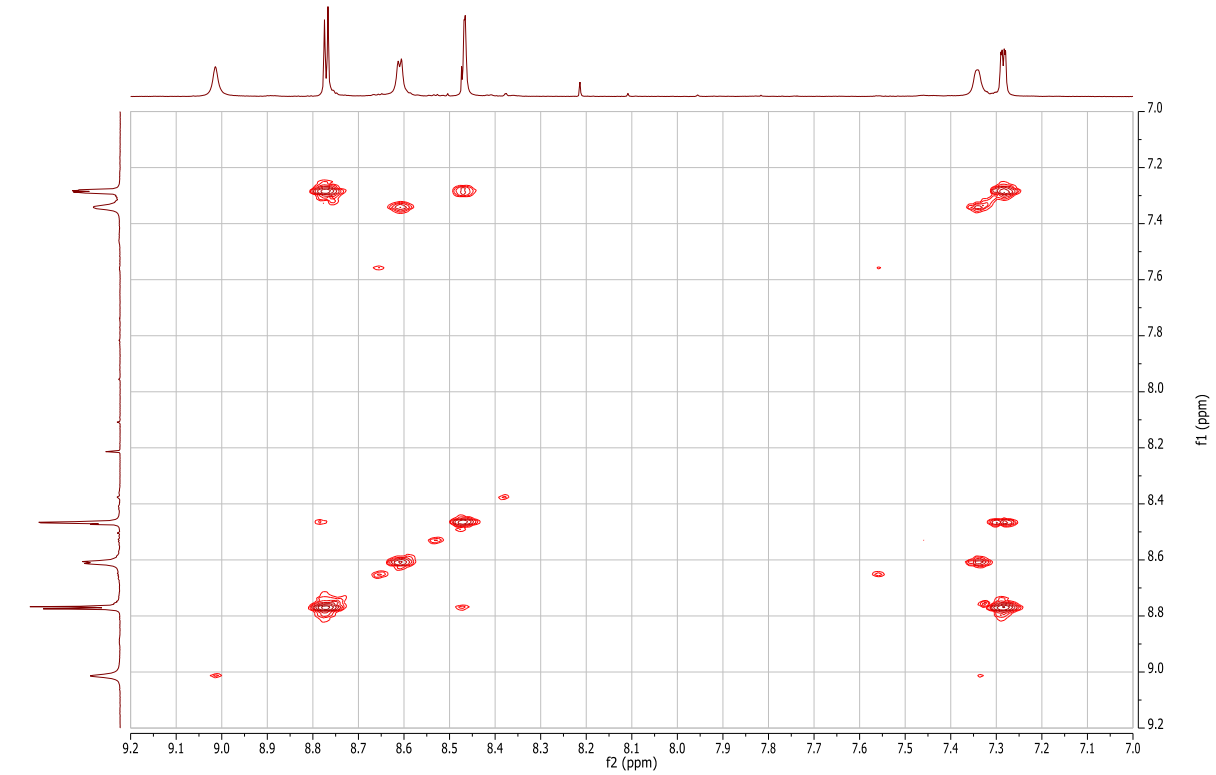


Figure S9: Downfield region of $^{1}H,^{1}H$ 2D-COSY NMR spectrum (700 MHz, D_2O, 298 K) of **1**.

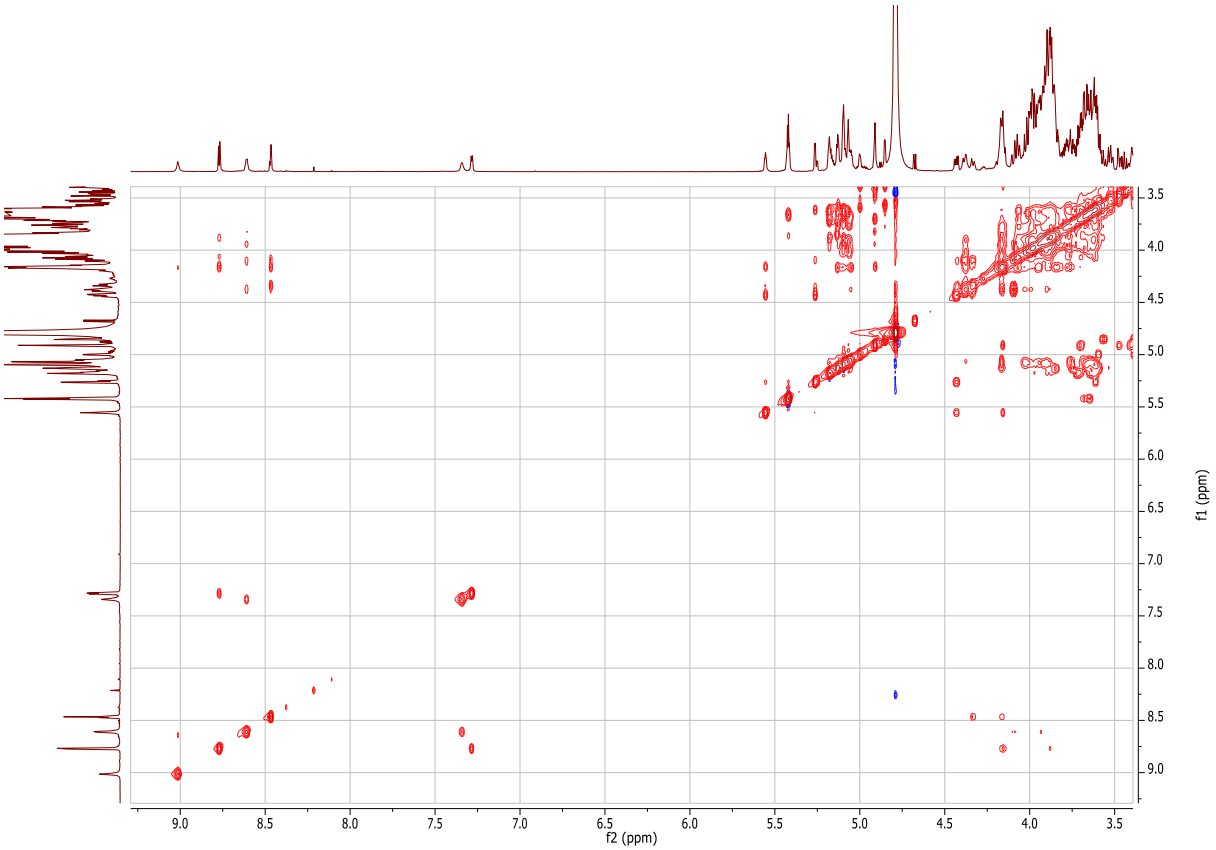


Figure S10: ^{1}H 2D-NOESY NMR spectrum (700 MHz, D_2O, 298 K) of **1**.

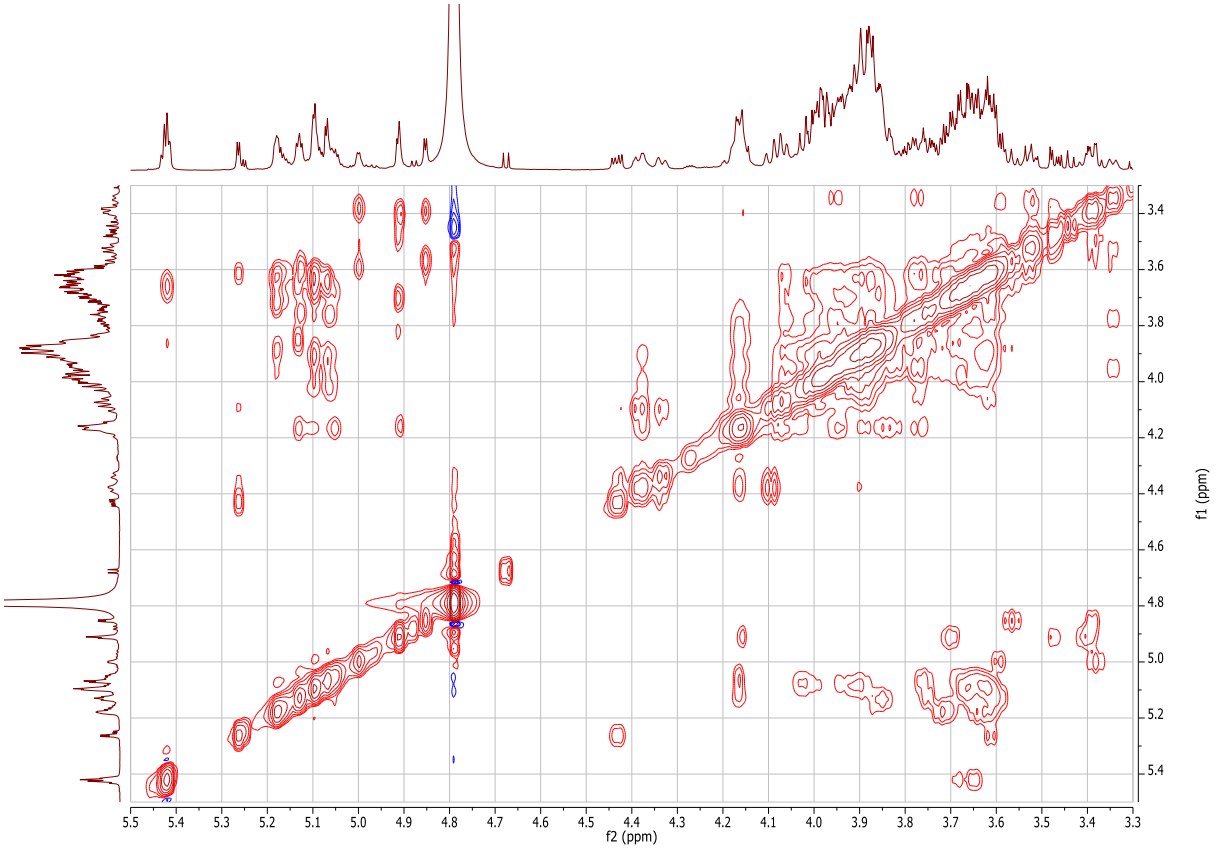


Figure S11: Upfield region of ^{1}H 2D-NOESY NMR spectrum (700 MHz, D_2O, 298 K) of **1**.

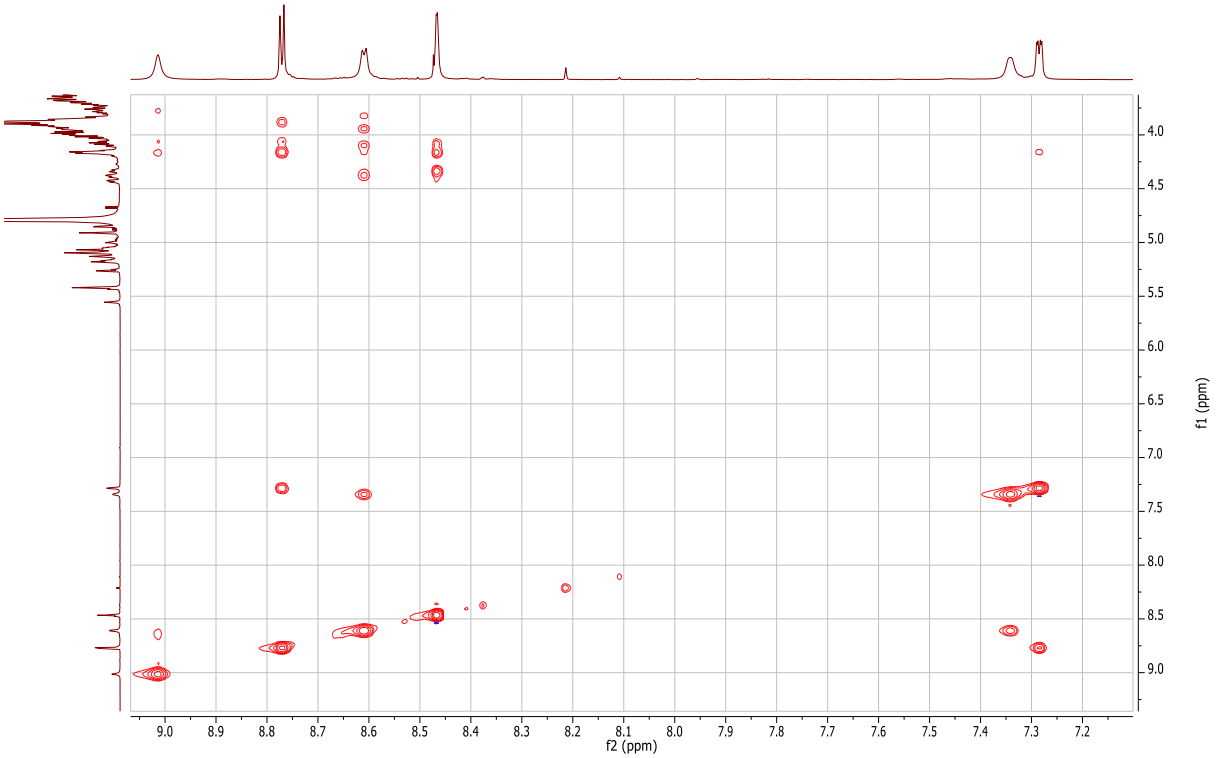


Figure S12: Downfield region of ^{1}H 2D-NOESY NMR spectrum (700 MHz, D_2O, 298 K) of **1**.

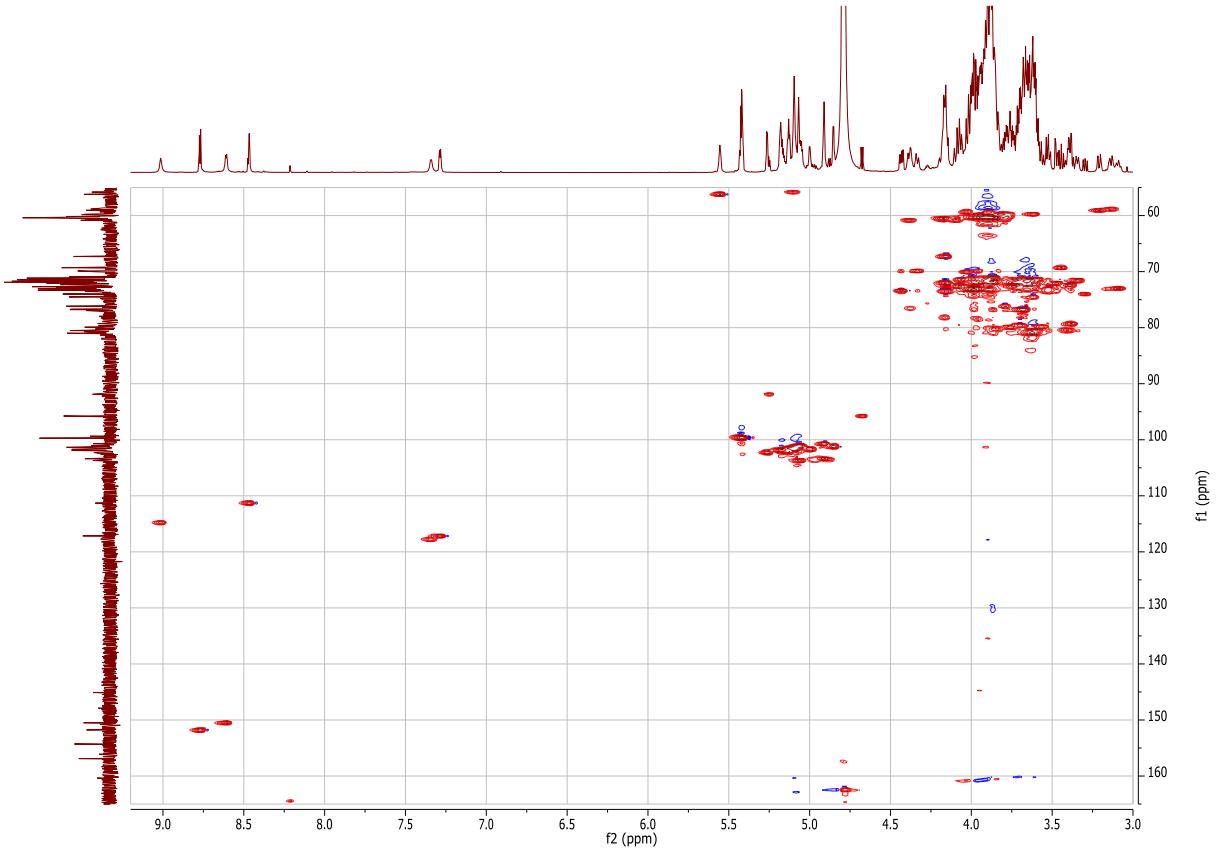


Figure S13: $^{1}H,^{13}C$ HSQC NMR spectrum (700 MHz, D_2O, 298 K) of **1**.

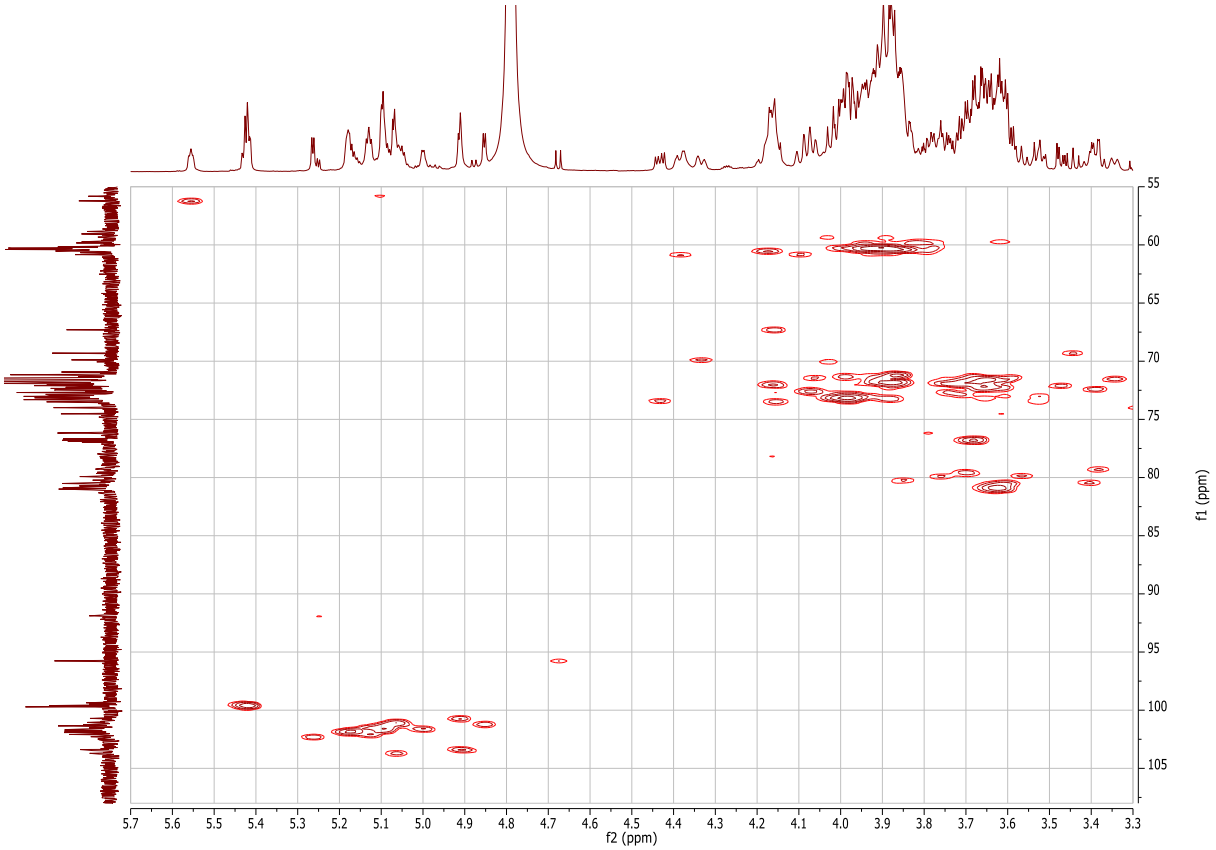


Figure S14: Region of cyclodextrin signals of $^{1}H,^{13}C$ HSQC NMR spectrum (700 MHz, D_2O, 298 K) of **1**.

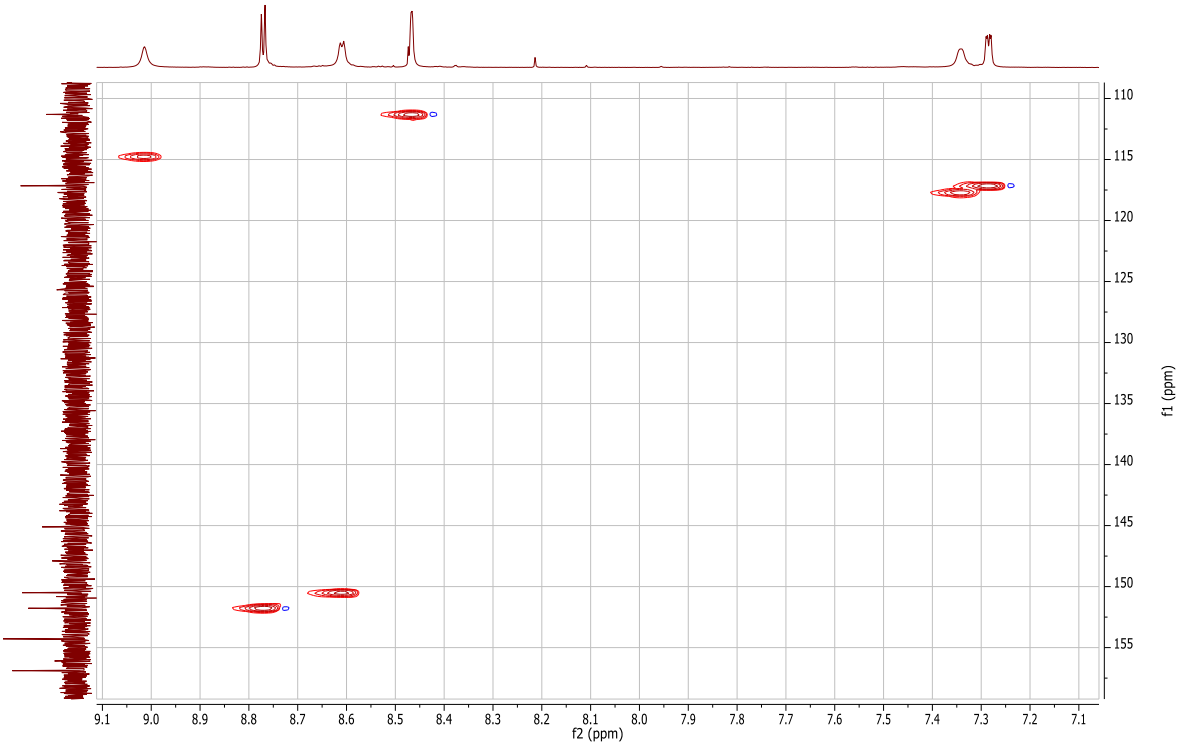


Figure S15: Aromatic region of $^{1}H,^{13}C$ HSQC NMR spectrum (700 MHz, D_2O, 298 K) of **1**.

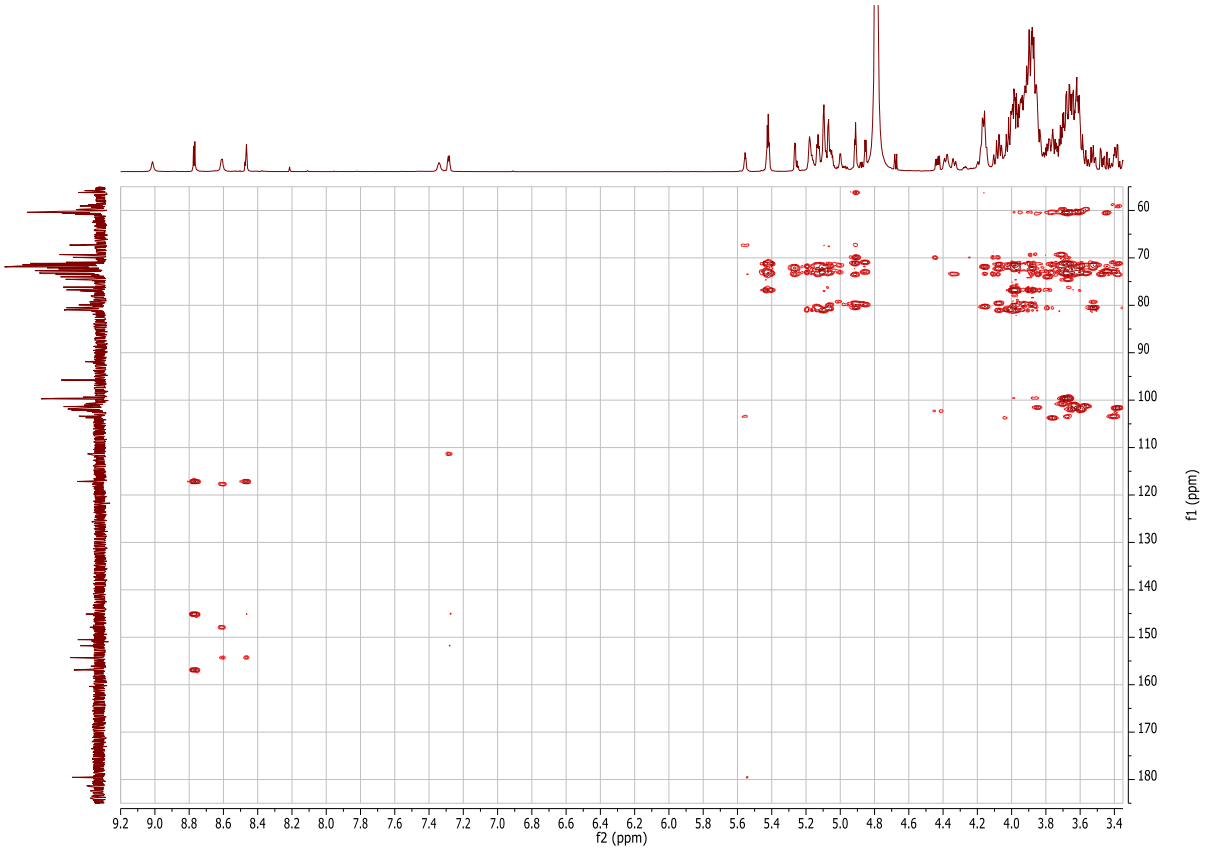


Figure S16: $^{1}H,^{13}C$ HMBC NMR spectrum (700 MHz, D_2O, 298 K) of **1**.

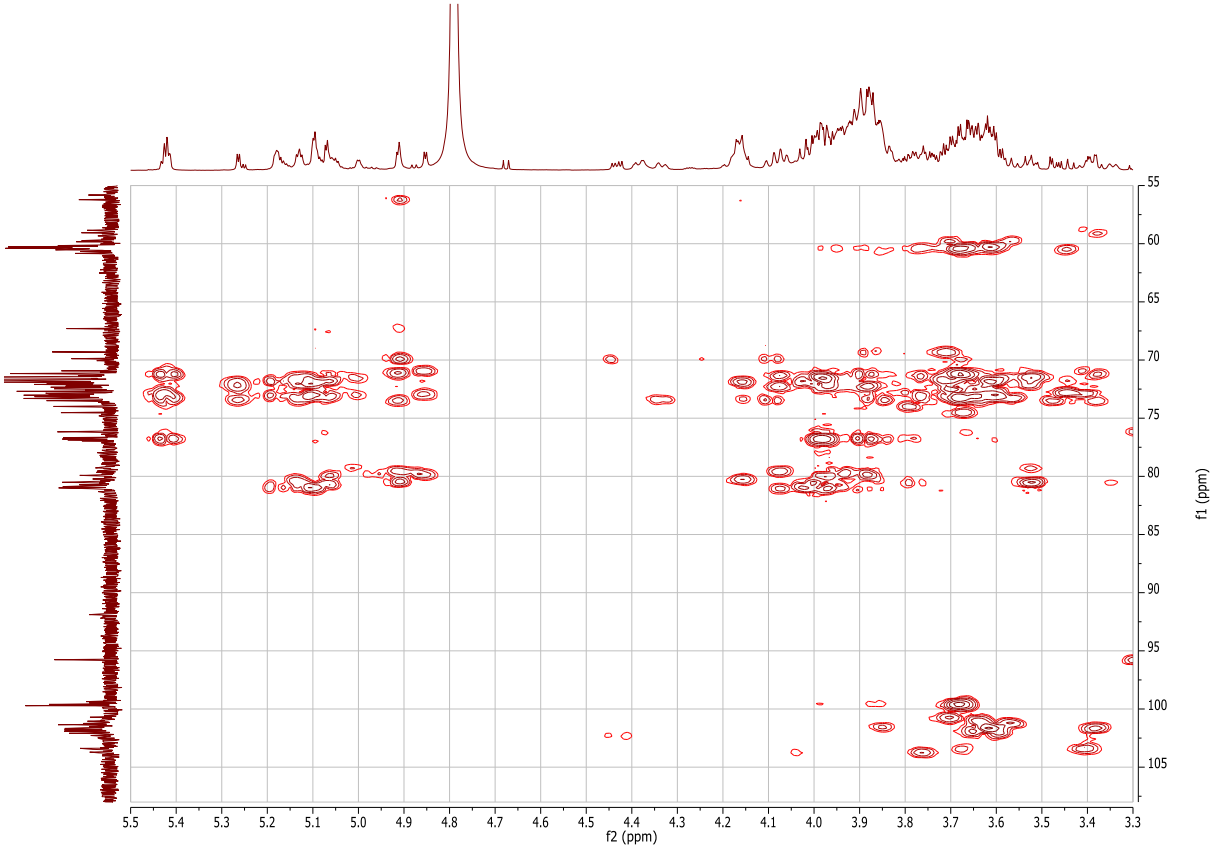


Figure S17: Region of cyclodextrin signals of $^{1}H,^{13}C$ HMBC NMR spectrum (700 MHz, D_2O, 298 K) of **1**.

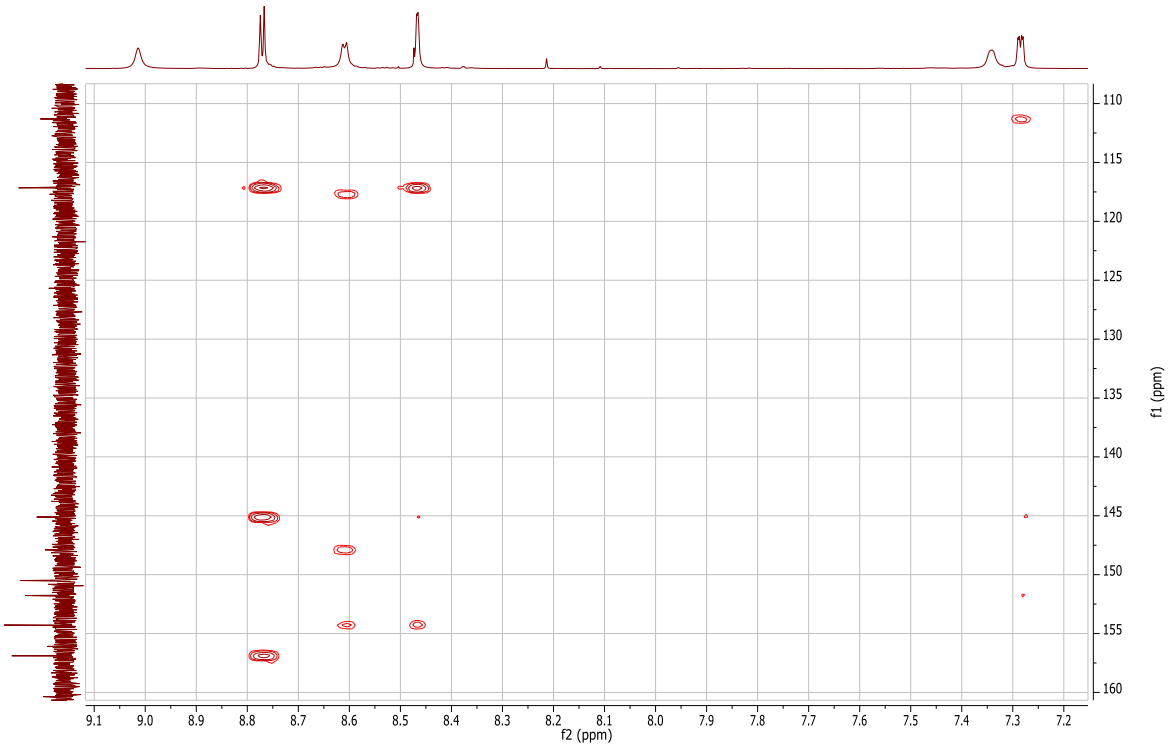


Figure S18: Aromatic region of ^{1}H,^{13}C HMBC NMR spectrum (700 MHz, D_2O, 298 K) of **1**.

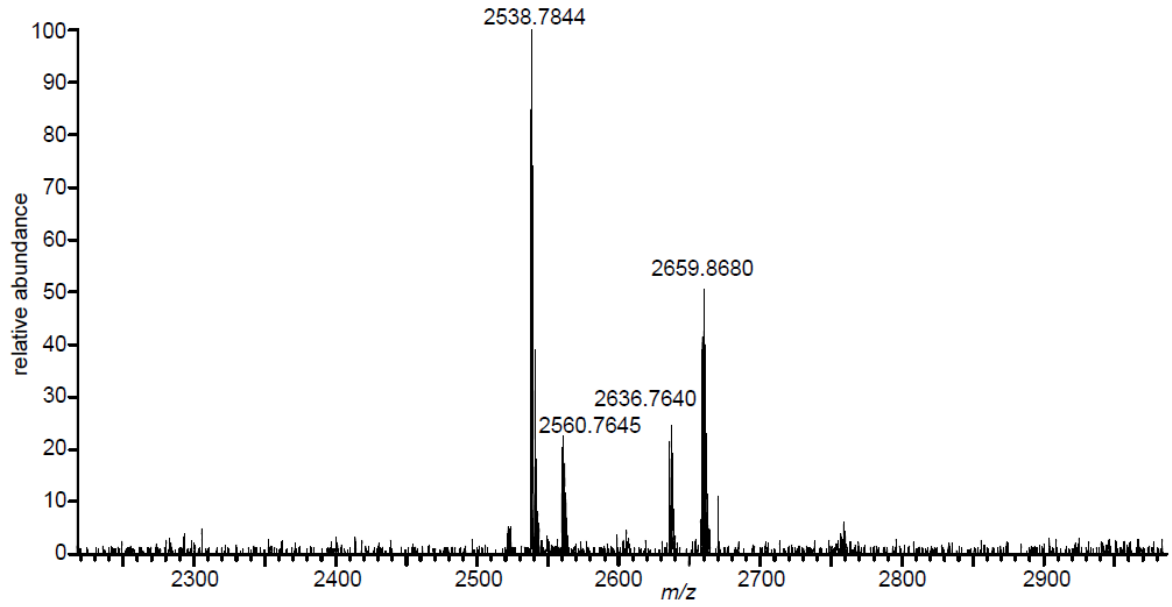


Figure S19: ESI positive mass spectrum of **1.**

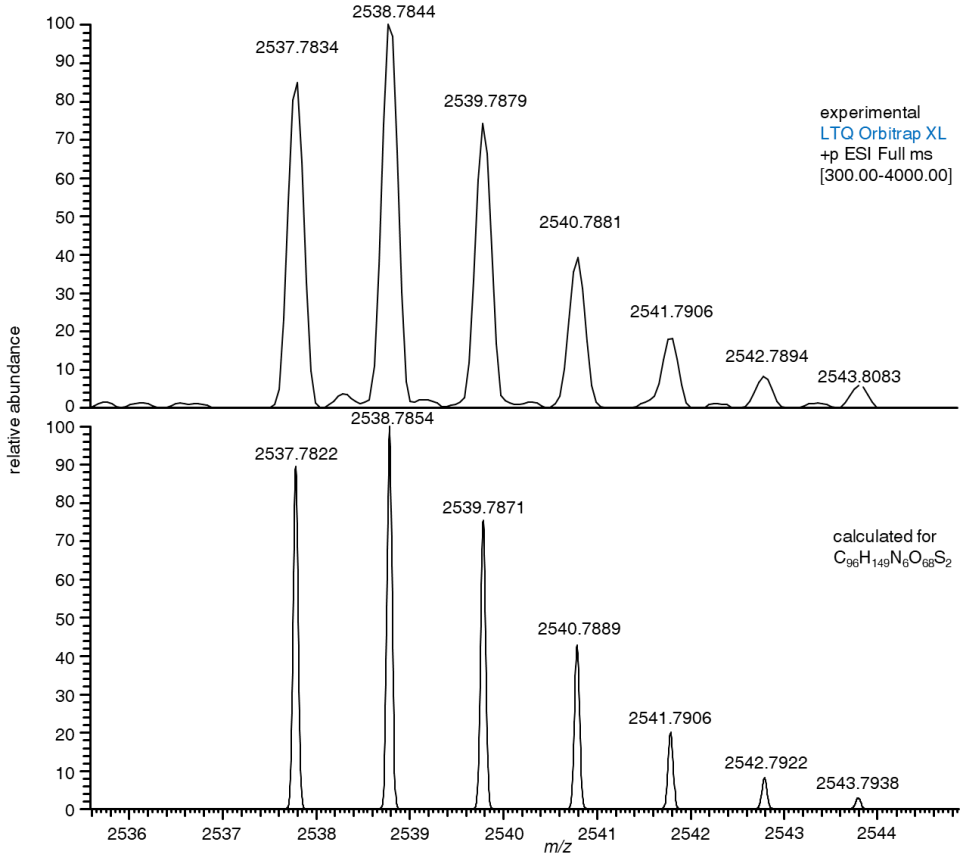


Figure S20: Calculated and experimental isotope patterns of ESI HR mass spectrum (pos. mode) of **1**.

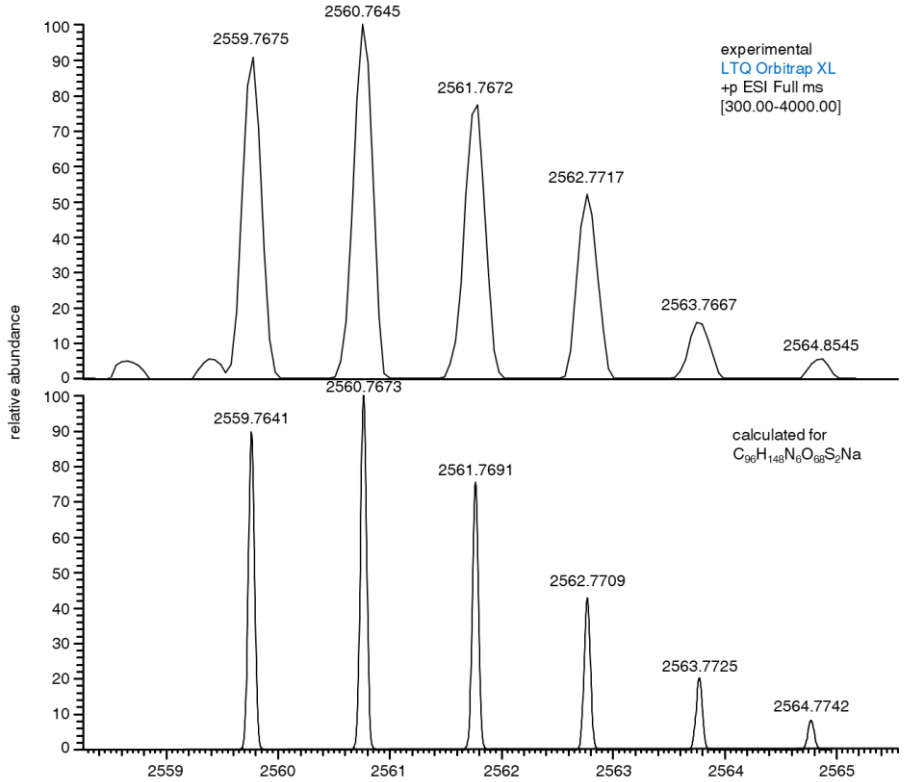


Figure S21: Calculated and experimental isotope patterns of ESI HR mass spectrum (pos. mode) of **1**.

1,1'-([2,2'-Bipyridine]-4,4'-diyl)bis(3-[3^{A}-deoxy-mono-altro-β-cyclo-dextrin-3^{A}-yl]thiourea) (2,6-bis(1,4,7,10-tetraoxadodecyl)-1,10-phenanthroline) zinc(II)-tetrafluoroborate complex [Zn(1)(13)](BF$_4$)$_2$

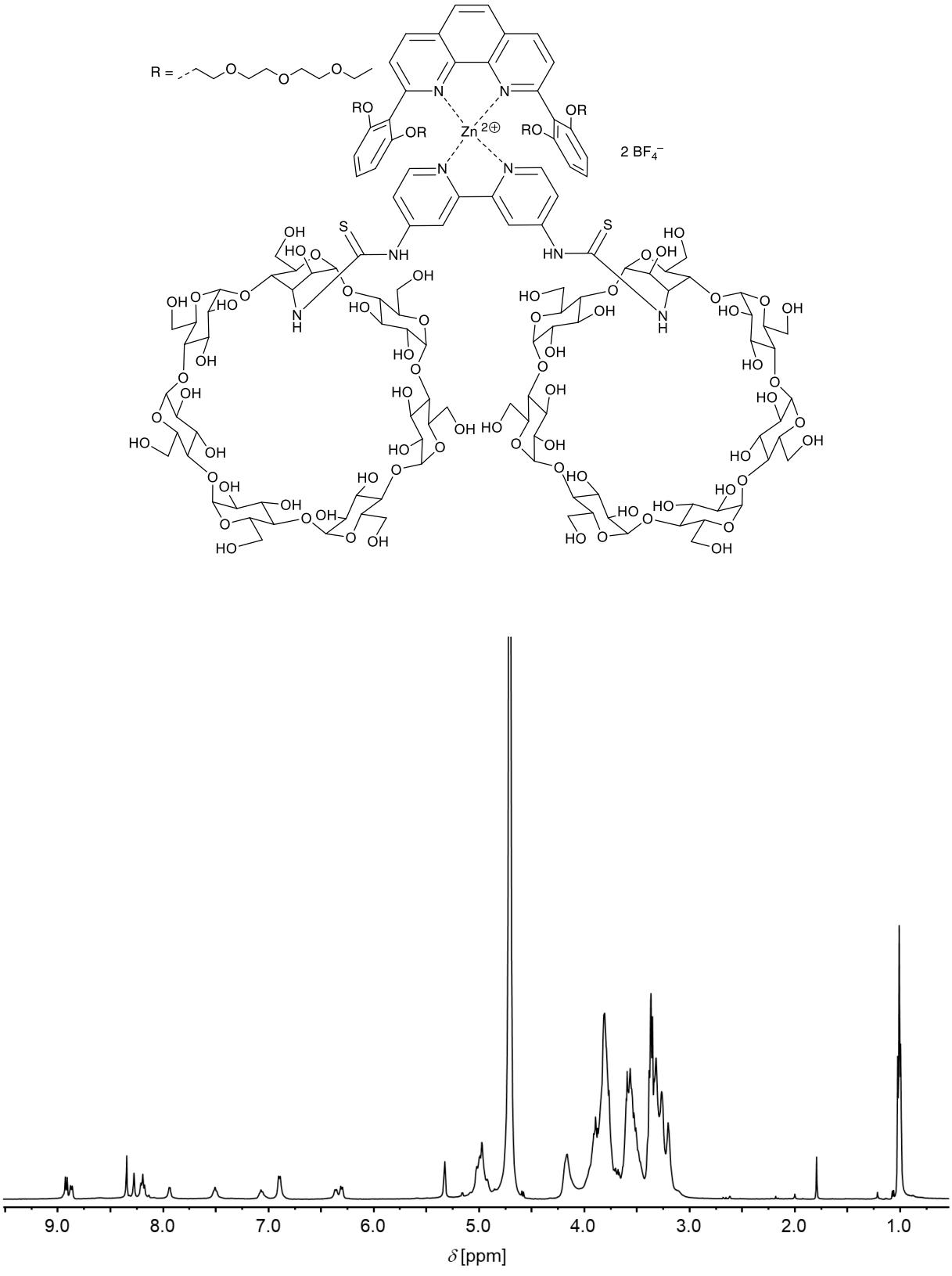


Figure S22: ^{1}H NMR spectrum (500 MHz, D_2O, 298 K) of [Zn(**1**)(**13**)](BF$_4$)$_2$.

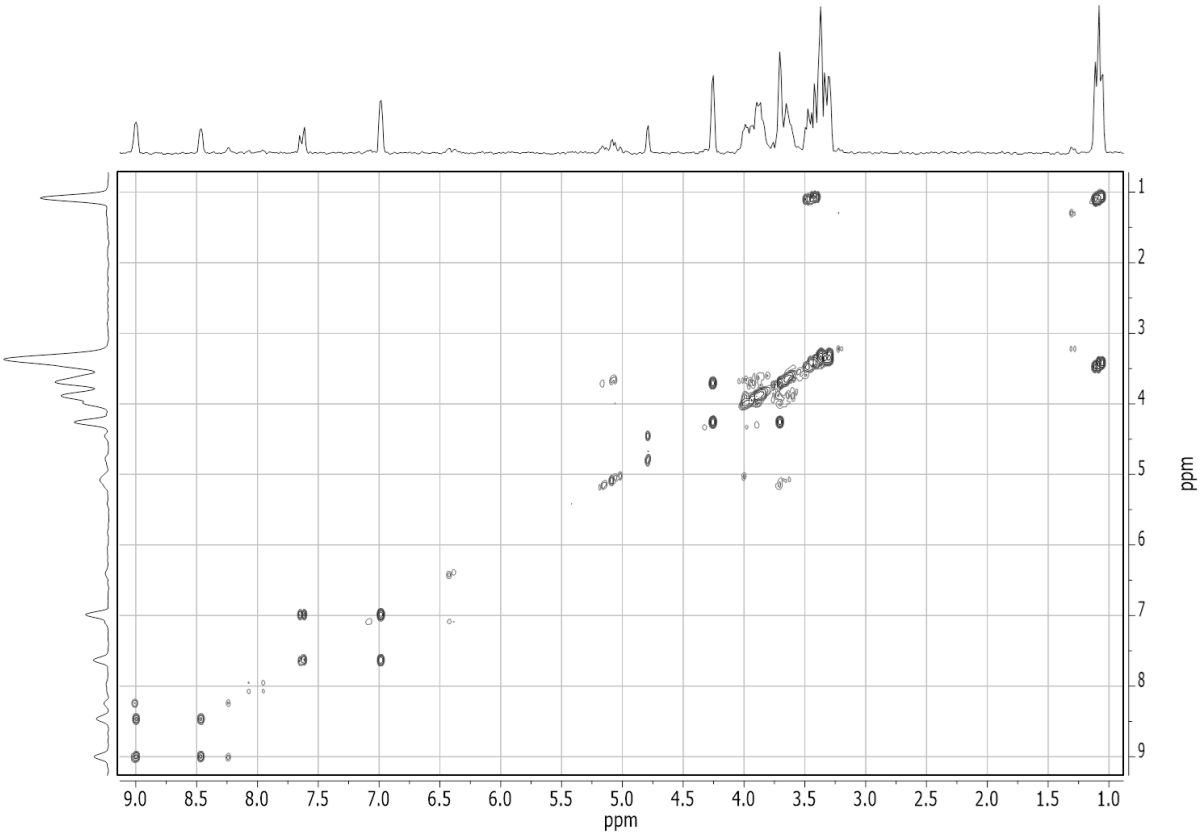


Figure S23: $^{1}H,^{1}H$ 2D-COSY NMR spectrum (700 MHz, D_2O, 298 K) of [Zn(**1**)(**13**)]$(BF_4)_2$.

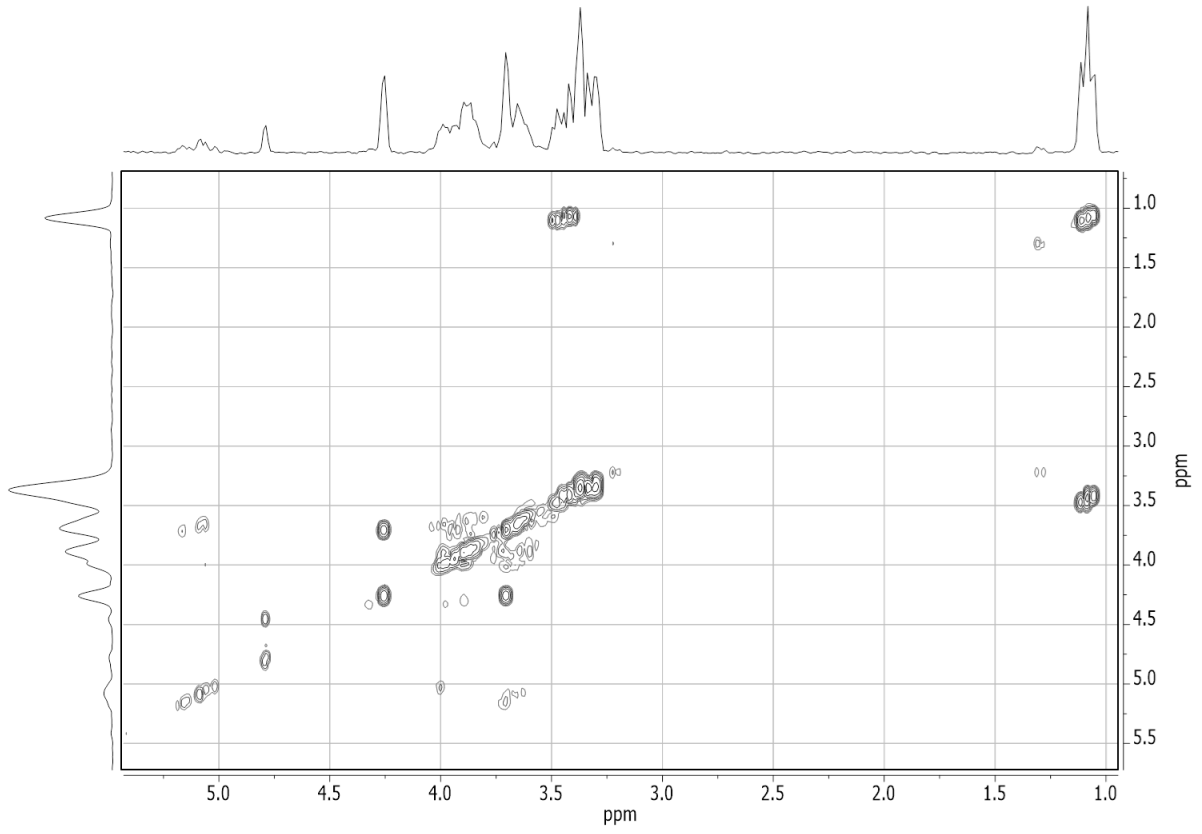


Figure S24: Upfield region of $^{1}H,^{1}H$ 2D-COSY NMR spectrum (700 MHz, D_2O, 298 K) of [Zn(**1**)(**13**)]$(BF_4)_2$.

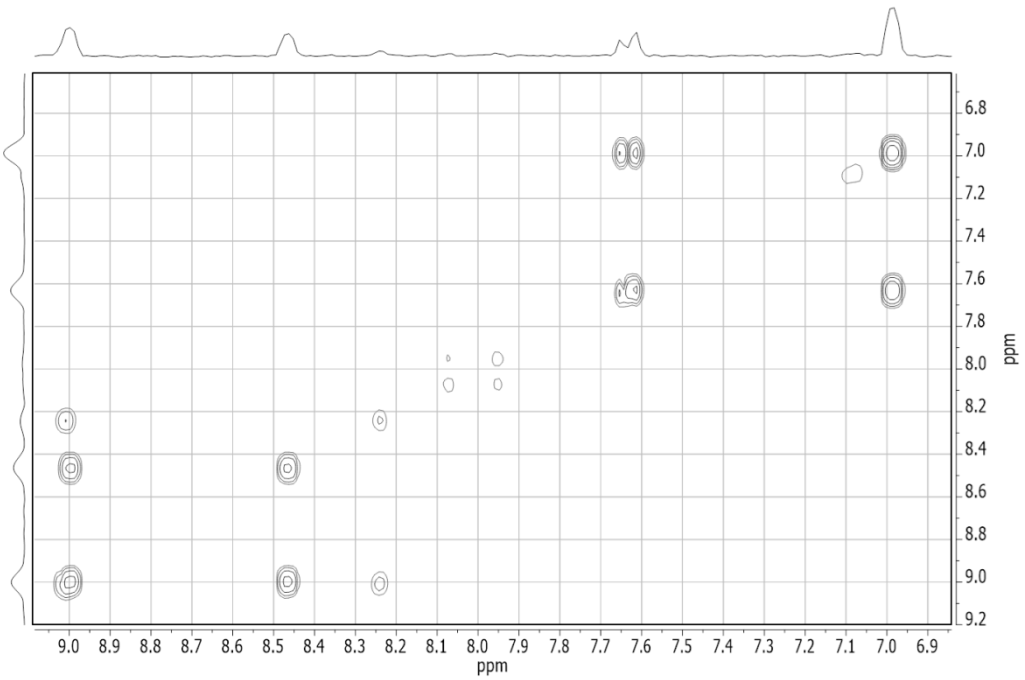


Figure S25: Downfield region of $^{1}H,^{1}H$ 2D-COSY NMR spectrum (700 MHz, D_2O, 298 K) of [Zn(**1**)(**13**)]$(BF_4)_2$.

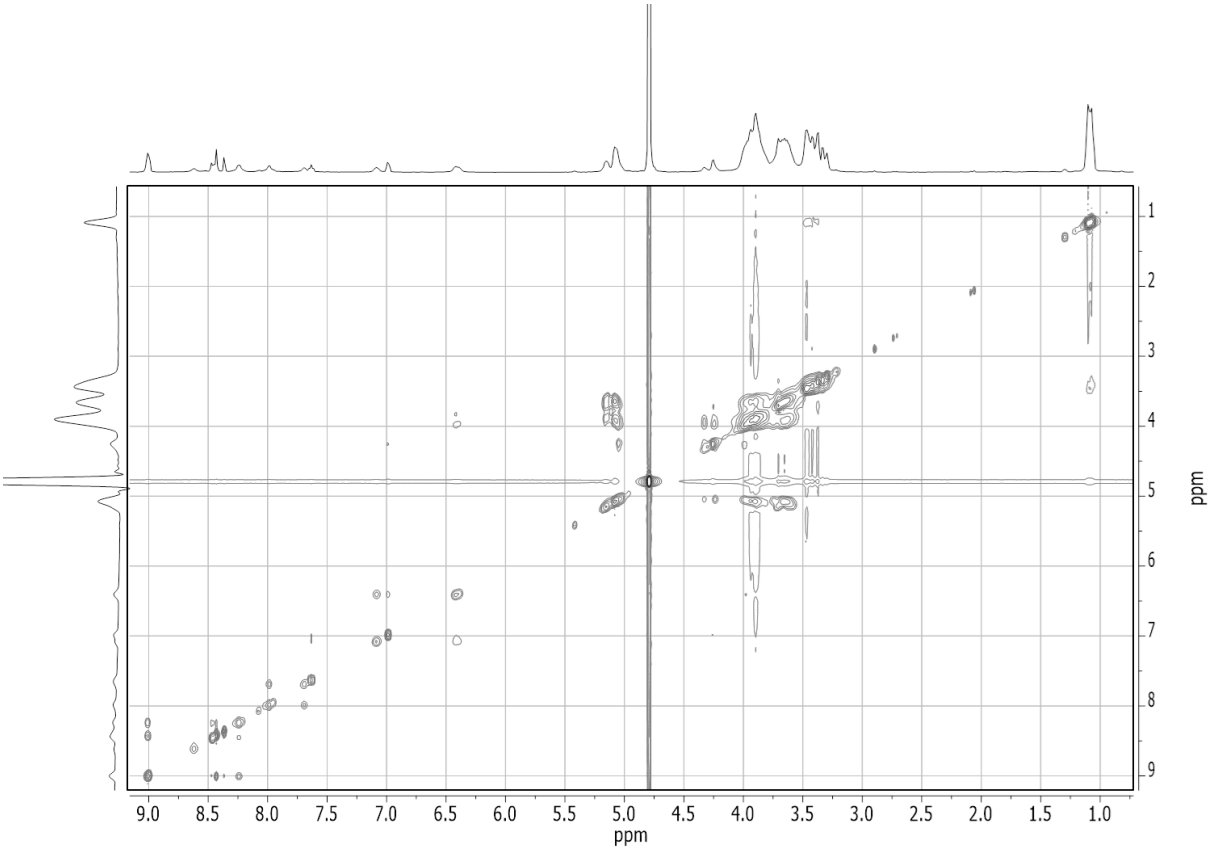


Figure S26: ^{1}H 2D-NOESY NMR spectrum (700 MHz, D_2O, 298 K) of [Zn(**1**)(**13**)]$(BF_4)_2$.

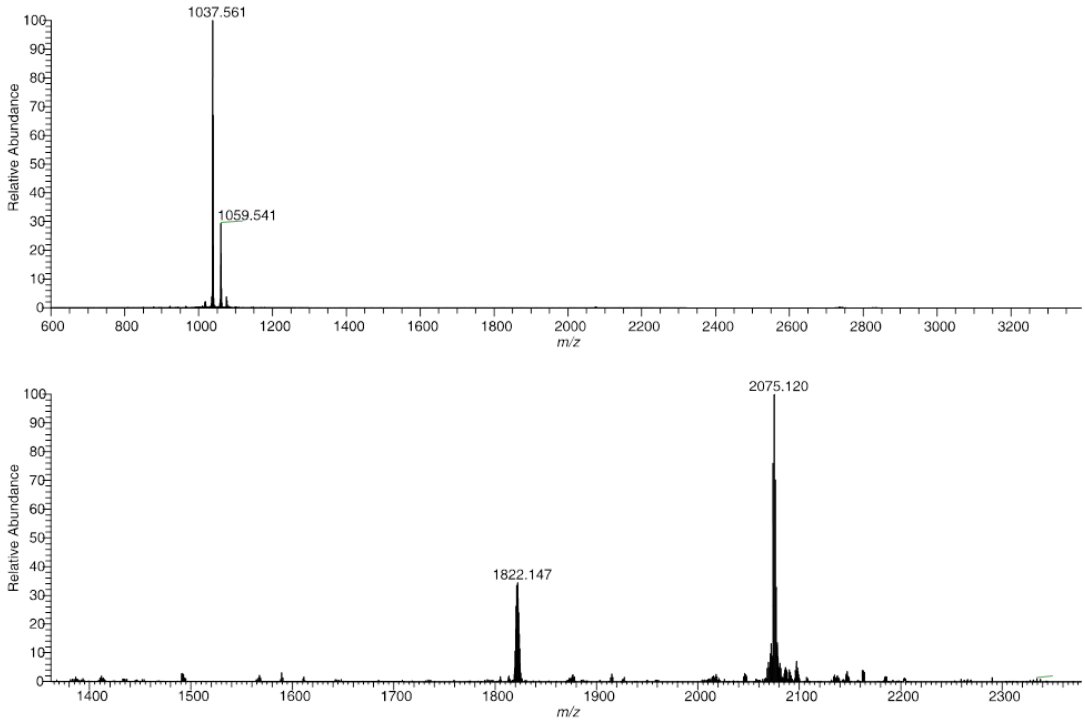


Figure S27: ESI mass spectrum (pos. mode) of [Zn(**1**)(**13**)](BF$_4$)$_2$.

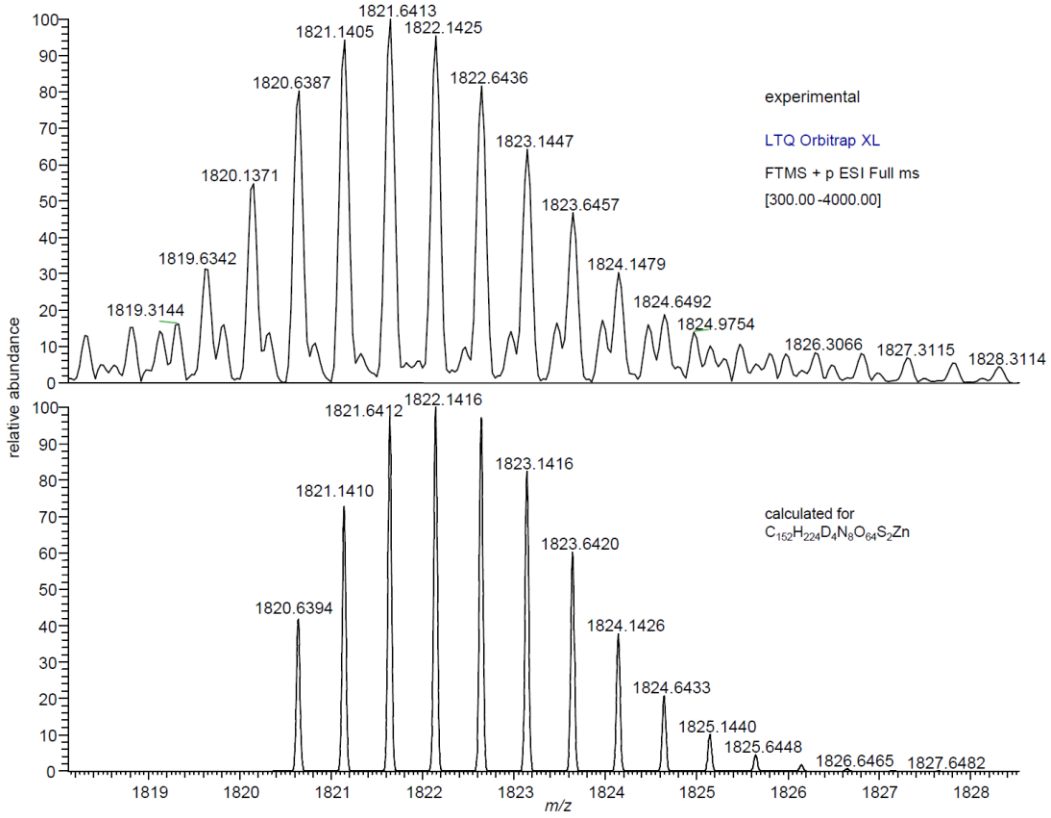


Figure S28: Calculated and experimental isotope patterns of ESI HR mass spectrum (pos. mode) of [Zn(**1**)(**13**)](BF$_4$)$_2$.

2. Binding studies

Please note, that ITC experiments proofed to be superior because NMR spectroscopy has some drawbacks in our specific case: a) neither the cyclodextrin units of **1** nor the deoxycholate contain any functional groups that would cause larger inductive or magnetic anisotropy effects upon binding, and hence, would induce large an easy to follow signal shifts in the NMR spectra. b) an expected binding constant of $K_a > 10^4$ M^{-1} with regard to the findings of Vargas-Berenguel[251] for receptor **1** in its "*on*"-state would be at the upper limit of values that can be determined accurately by NMR spectroscopic means.

Hence, all ITC experiments as the better suited experiments to determine information on the stoichiometry of the complexes as well as quantitative thermodynamic data on the complex formation were repeated two times so that the data reported here are the average of three titration experiments.

Because of the above reasons, NMR experiments are generally less suitable for determining the stoichiometry and thermodynamic data of complex formations very accurately. Therefore, they were performed more to confirm the results of the ITC experiment with an independent analytical method. All titrations were therefore performed only once.

[1] a) J. M. Casas-Solvas, I. Quesada-Soriano, D. Carreño-Gázquez, J. J. Giménez-Martínez, L. García-Fuentes and A. Vargas-Berenguel, β-Cyclodextrin Dimers Linked through Their Secondary Faces with Rigid Spacer Arms as Hosts for Bile Salts, *Langmuir* 2011, **27**, 9729; b) M. C. Martos-Maldonado, I. Quesada-Soriano, J. M. Casas-Solvas, L. García-Fuentes and A. Vargas-Berenguel, Secondary Face-to-Face 2-2' β-Cyclodextrin Dimers Linked with Fluorescent Rigid Spacer Arms: A Cyclodextrin-Based Ratiometric Sensor for Bile Salts, *Eur. J. Org. Chem.* 2012, 2560.

2.1. Job-Plot analysis

The stock solution of host **1** and guest were prepared with concentrations of 0.5 mmol/L in pure D_2O. A total of six samples were prepared mixing solutions in guest/host ratios of 0 to 1, 0.1 to 0.9, 0.3 to 0.7, 0.5 to 0.5, 0.7 to 0.3 and 0.9 to 0.1, respectively. After heating the samples to 40 °C for an hour ^{1}H-NMR spectra were recorded. The observed shifts of the primary methyl groups signals of sodium deoxycholate were plotted according to Job´s method.

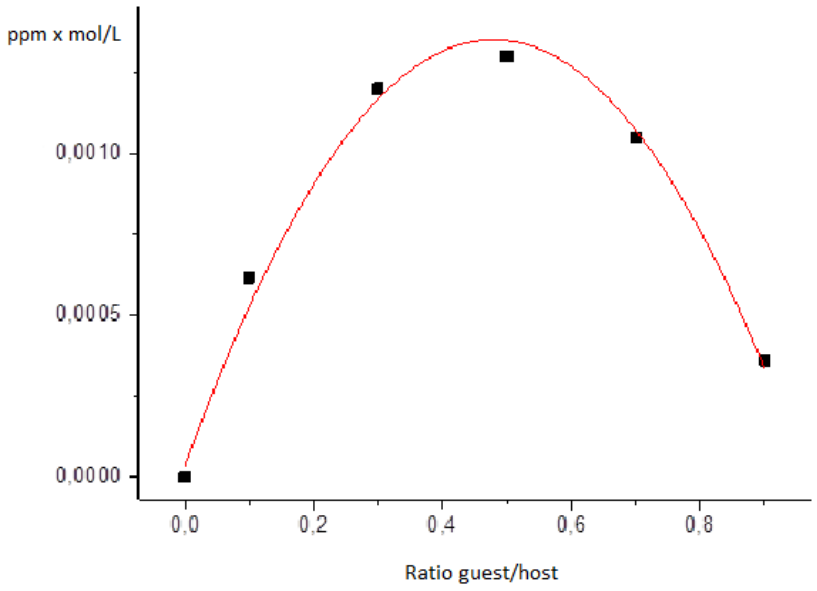


Figure S29: Job-Plot of the binding of sodium deoxycholate by receptor **1**.

2.2. ITC Experiments

ITC experiments were performed on a TAM III Calorimeter with the TAM Assistant Software. Titrations were carried out by adding 25 times 10 µL aliquots of stock solutions of of either a 20.7 mM solution of sodium deoxycholate in water (10 µL) from a computer controlled syringe to the 0.91 mM solution of host **1** in the same solvent or a 13.5 mM solution of sodium deoxycholate in water (10 µL) from a computer controlled syringe to the 1.26 mM solution of host-effector-complex [Zn(**1**)(**13**)]$(BF_4)_2$ in the same solvent, respectively. The heats of dilution were substracted manually. The binding isotherms obtained from these titrations were fitted to different binding models with the TAM Assistant software. In all cases the best fit was observed for a 1:1 binding model which was used for calculating the thermodynamic data set for both reactions with the TAM Assistant software.

The results of the different experiments listed in Table S1 are averaged values obtained from three titrations. Figures S22 and S23 show typical results for one of each titrations with host 1 in its "*off*"- and "*on*"-state ([Zn(**1**)(**13**)]$(BF_4)_2$).

Table S1: Results of the ITC experiments to quantify the binding affinity of **1** and [Zn(**1**)(**13**)]$(BF_4)_2$ towards sodium deoxychelate, respectively.

	binding of deoxycholate to **1**	binding of deoxycholate to [Zn(**1**)(**13**)]$(BF_4)_2$
K_a [M^{-1}]	$1.73\times10^3 \pm 80$	$3.15\times10^4 \pm 1.8\times10^3$
ΔG [kJ mol^{-1}]	–18.5 ± 0.1	–25.7 ± 0.1
ΔH [kJ mol^{-1}]	–3.9 ± 0.1	–20.4 ± 0.2
ΔS [J K^{-1} mol^{-1}]	49.0 ± 2.3	17.6 ± 1.8

ITC titration experiment of 1 with sodium deoxycholate

Model

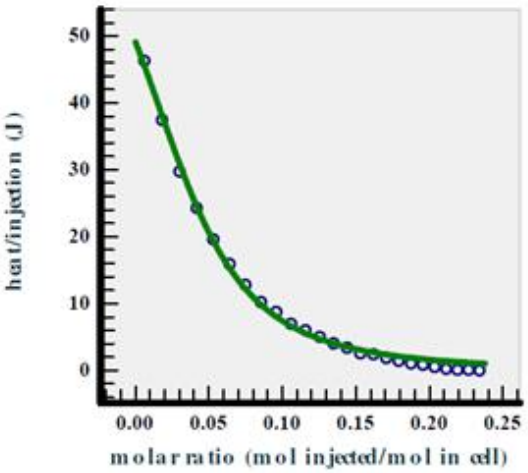
$M + L \leftrightarrow ML$ $\beta_1, \Delta H_1$

Thermodynamics Parameters

k_1	$1.74 \cdot 10^3 \pm$	$8.7 \cdot 10^1$
ΔH_1	-3.86kJ/mol	±69J/mol
ΔG_1	-18.492kJ/mol	
ΔS_1	49.084J/(K*mol)	

Statistics

Degrees of freedom	23
ξ^2	$1.26 \cdot 10^{-9}$
Reduced ξ^2	$5.49 \cdot 10^{-11}$
Standard error of point	$7.41 \cdot 10^{-6}$



Input Data

GH8_NaDCh4

Volume in vessel	800µl
Number of datapoints	25
Concentration of [M] in vessel	908.505µM
Concentration of [L] in syringe	20.821mM

No	Volume (µl)	Q_{exp} (µJ)	Q_{calc} (µJ)	Residual (µJ)
1	10.0	461.76	453.82	7.94
2	10.0	373.69	379.07	-5.37
3	10.0	296.73	307.38	-10.65
4	10.0	242.27	244.46	-2.19
5	10.0	195.57	192.91	2.66
6	10.0	158.77	152.50	6.27
7	10.0	127.60	121.55	6.06
8	10.0	101.93	98.00	3.94
9	10.0	87.28	80.03	7.25
10	10.0	69.35	66.21	3.14
11	10.0	59.95	55.46	4.50
12	10.0	50.06	46.99	3.07
13	10.0	40.42	40.23	0.19
14	10.0	33.81	34.78	-0.97
15	10.0	25.47	30.32	-4.86
16	10.0	23.92	26.65	-2.73
17	10.0	18.57	23.59	-5.02
18	10.0	14.00	21.01	-7.01
19	10.0	10.34	18.83	-8.49
20	10.0	8.49	16.96	-8.47
21	10.0	5.39	15.35	-9.96
22	10.0	2.35	13.96	-11.61
23	10.0	0.77	12.75	-11.98
24	10.0	0.72	11.68	-10.96
25	10.0	-0.00	10.74	-10.74

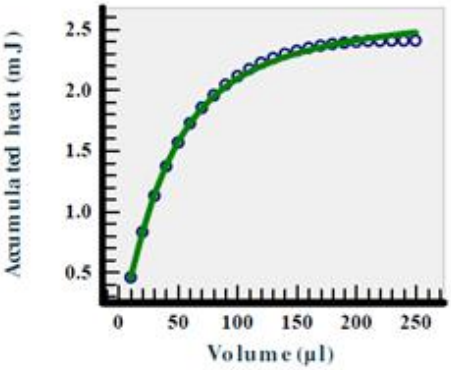


Figure S30: Exemplary binding report obtained for the titration of receptor **1** in its "o*ff*"-state and sodium deoxycholate.

ITC titration experiment of $[Zn(1)(13)](BF_4)_2$ with sodium deoxycholate

Model

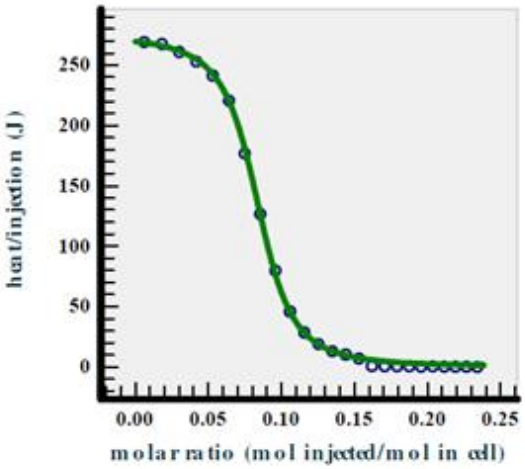
$M + L \leftrightarrow ML$ $\quad \beta_1, \Delta H_1$

Thermodynamics Parameters

k_1	$3.25 \cdot 10^4 \pm$	$1.3 \cdot 10^3$
ΔH_1	-20.54kJ/mol	±85J/mol
ΔG_1	-25.751kJ/mol	
ΔS_1	17.472J/(K*mol)	

Statistics

Degrees of freedom	23
ξ^2	$1.02 \cdot 10^{-8}$
Reduced ξ^2	$4.44 \cdot 10^{-10}$
Standard error of point	$2.11 \cdot 10^{-5}$



Input Data

GH8_Zn_NaDCh5

Volume in vessel	800µl
Number of datapoints	25
Concentration of [M] in vessel	1.26mM
Concentration of [L] in syringe	13.447mM

No	Volume (µl)	Q_{exp} (mJ)	Q_{calc} (mJ)	Residual (mJ)
1	10.0	2.69	2.68	0.01
2	10.0	2.67	2.65	0.02
3	10.0	2.60	2.61	-0.01
4	10.0	2.52	2.54	-0.01
5	10.0	2.41	2.41	-0.00
6	10.0	2.20	2.19	0.02
7	10.0	1.76	1.79	-0.03
8	10.0	1.26	1.26	0.01
9	10.0	0.80	0.77	0.02
10	10.0	0.46	0.46	-0.00
11	10.0	0.28	0.28	-0.00
12	10.0	0.19	0.19	0.00
13	10.0	0.13	0.13	-0.00
14	10.0	0.10	0.10	0.01
15	10.0	0.07	0.07	-0.00
16	10.0	0.01	0.06	-0.05
17	10.0	0.01	0.05	-0.04
18	10.0	0.00	0.04	-0.03
19	10.0	0.00	0.03	-0.03
20	10.0	0.00	0.03	-0.02
21	10.0	0.00	0.02	-0.02
22	10.0	0.00	0.02	-0.02
23	10.0	0.00	0.02	-0.02
24	10.0	0.00	0.02	-0.01
25	10.0	0.00	0.01	-0.01

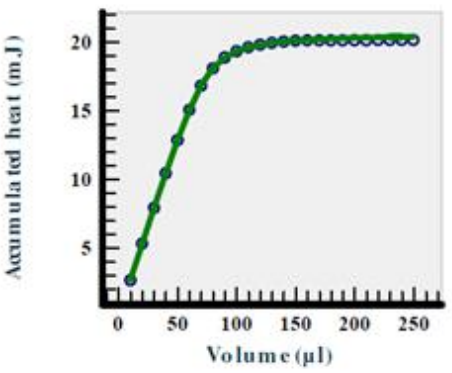


Figure S31: Exemplary binding report obtained for the titration of receptor **1** in its "o*n*"-state as the $[Zn(\mathbf{1})(\mathbf{13})](BF_4)_2$ complex and sodium deoxycholate.

2.3. Determination of Binding constants by ^{1}H NMR spectroscopic titration

To determine the binding constants of the receptor **1** and sodium deoxycholate stock solutions of host and guest in D_2O were prepared (host: 0.007 mol/L, guest: 0.001 mol/L). For the receptor in the "*on*"-state a complex of receptor **1** and Ligand **13** and $Zn(BF_4)_2$ in D_2O was prepared with a concentration of 0.007 mol/L. Now samples with a volume of 0.6 mL each were prepared. The ratio of guest to host was varied from 0.33 to 7 in both experiments. After heating the samples to 40 °C for an hour ^{1}H-NMR spectra were recorded and analyzed. The ^{1}H-NMR shifts of the sodium deoxycholate´s primary methyl groups were plotted according to the following theoretical background.

The formation of a complex host-guest complex with a 1:1 stoichiometry is described by the following equation:

(1) $$K_a = \frac{[GH]}{[G][H]}$$

[G], *[H]* and *[GH]* are the concentrations of the free guest and host and the host-guest complex in equilibrium. K_a is the binding constant. These concentrations are connected to the total concentrations $[G]_t$ and $[H]_t$:

(2) $$[G] = [G]_t - [GH]$$

(3) $$[H] = [H]_t - [GH]$$

The combination of equations (1), (2) and (3) yields:

(4) $$K = \frac{[GH]}{([G]_t - [GH])([H]_t - [GH])}$$

This equation is solved for the complex concentration:

(5) $$[GHn] = \frac{1}{2}\left([H]_t + [G]_t + \frac{1}{K_a}\right) - \sqrt{\left([H]_t + [G]_t + \frac{1}{K_a}\right) - 4[H]_t[G]_t}$$

The observed shift is related to the complex concentration *[GH]* according to the following equation:

(6) $$[GH] = \frac{\Delta\delta_{obs}\,[G]_t}{\Delta\delta_c}$$

Now equations (5) and (6) are combined to get an equation independent of complex concentration [GH]:

(7) $$\Delta\delta_{obs} = \frac{\Delta\delta_c}{[G]_t}\left(\frac{1}{2}\left([G]_t + [H]_t + \frac{1}{K_a}\right) - \sqrt{\frac{1}{4}\left([G]_t + [H]_t + \frac{1}{K_a}\right)^2 - [G]_t[H]_t}\right)$$

This equation is solved for K_a:

(8) $$K_a = \frac{\Delta\delta_c * \Delta\delta_{obs}}{(\Delta\delta_{obs} - \Delta\delta_c)([G]_t\Delta\delta_{obs} - [H]_t\Delta\delta_c)}$$

After plotting $\Delta\delta_{obs}$ against $[H]_t$ and nonlinear regression to the equation (9) the saturation shift $\Delta\delta_c$ (a = $(\Delta\delta_c)^{-1}$) is obtained

(9) $$y = \frac{x}{ax+b}$$

The saturation shift $\Delta\delta_c$ is used in equation (7) and the binding constant K_a can be determined.

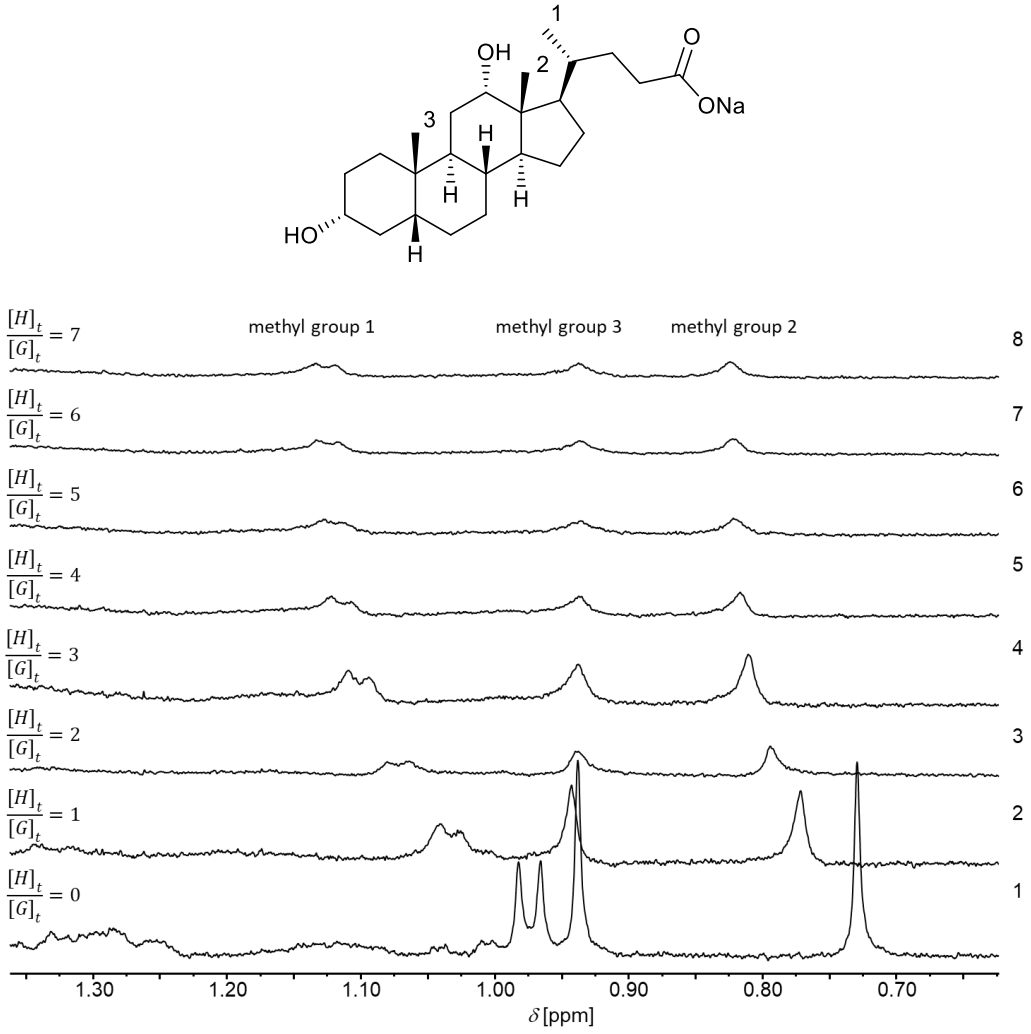


Figure S32: Observed shifts in the binding titration of receptor **1** in its "*off*"-state and sodium deoxycholate.

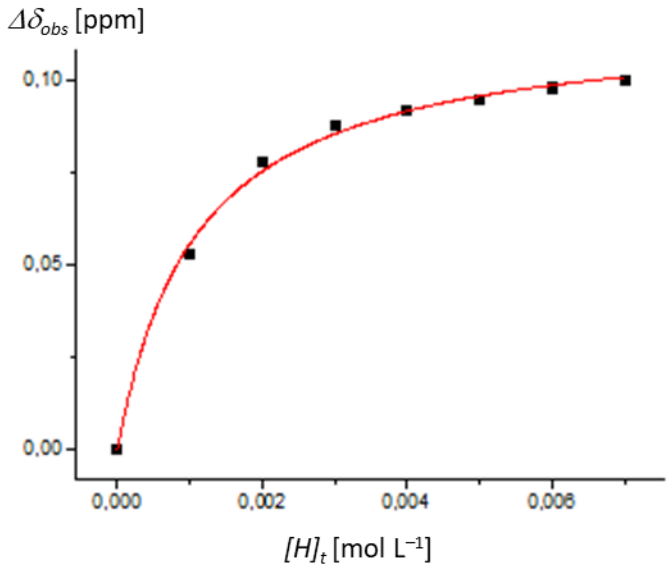


Figure S33: Observed shifts (black squares) of methyl group 2 of sodium deoxycholate upon binding to receptor **1** in its "*off*"-state and the corresponding nonlinear calculated fit (red).

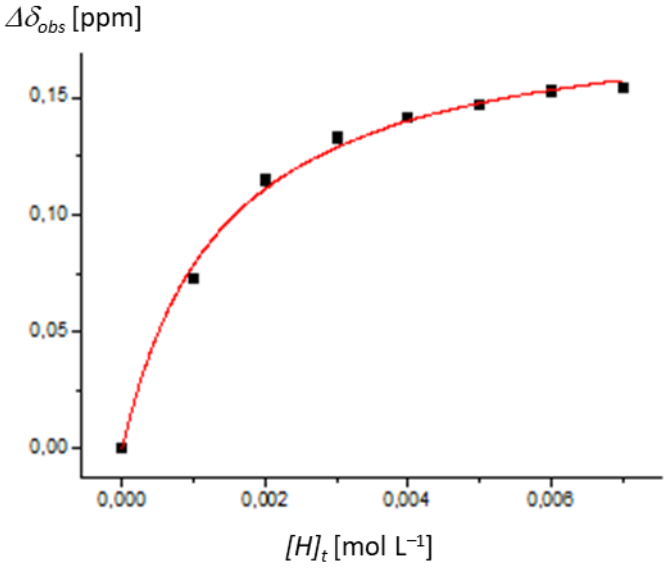


Figure S34: Observed shifts (black squares) of methyl group 1 of sodium deoxycholate upon binding to receptor **1** in its "*off*"-state and the corresponding nonlinear calculated fit (red).

The observed shifts $\Delta\delta_{obs}$ for methyl groups 1 and 2 of deoxycholate were plotted against the total host concentration $[H]_t$. Based on the nonlinear fits of both binding isotherms a binding constant of receptor **1** ($K_{a,}$*off*) of $K_{a,off}$ = 1430 ± 220 L/mol was calculated with regard to the shifts observed for methyl group 1 and of $K_{a,off}$ = 990 ± 130 L/mol with regard to the shifts observed for methyl group 2, respectively.

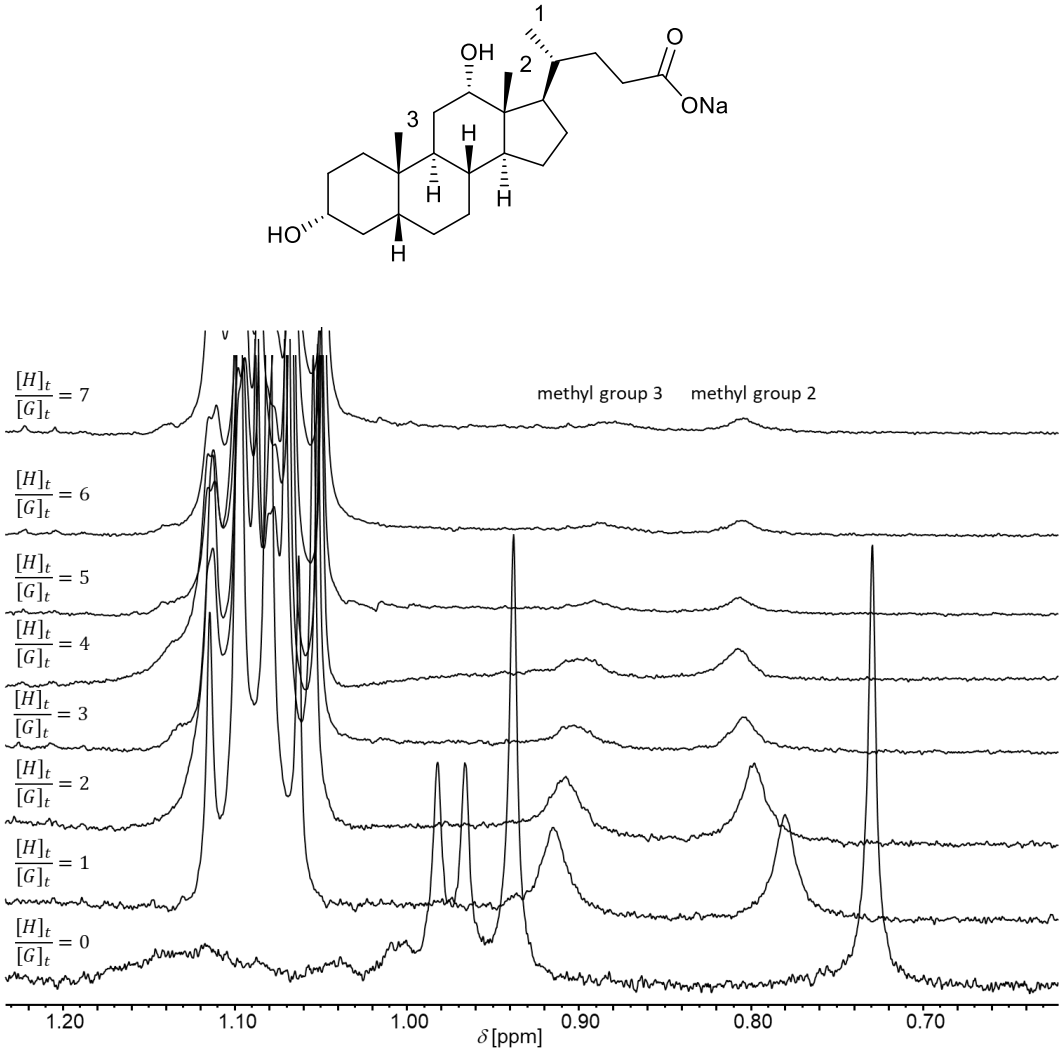


Figure S35: Observed shifts in the binding titration of receptor **1** in its "*on*"-state as the [Zn(**1**)(**13**)]$(BF_4)_2$ complex and sodium deoxycholate.

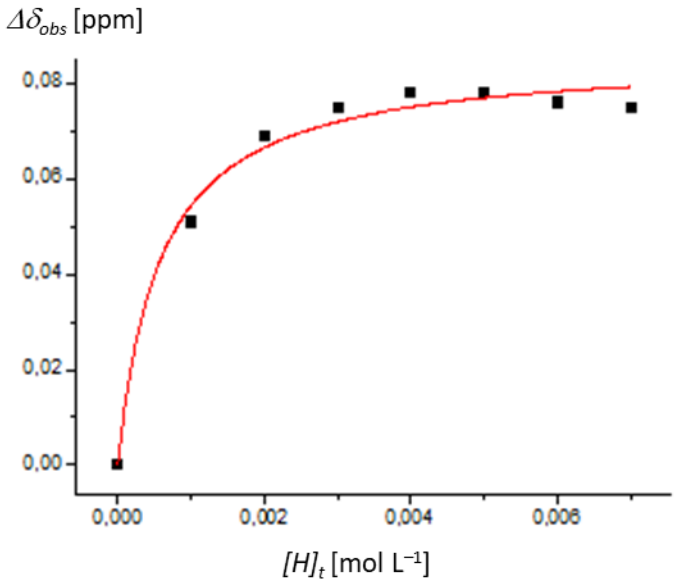


Figure S36: Observed shifts (black squares) of methyl group 2 of sodium deoxycholate upon binding to receptor **1** in its *"on"*-state as the [Zn(**1**)(**13**)]$(BF_4)_2$ complex and the corresponding nonlinear calculated fit (red).

For receptor **1** in its "on"-state as the [Zn(**1**)(**13**)]$(BF_4)_2$ complex the binding constant $K_{a,on}$ with regard to binding of sodium deoxycholate exceeds the limits of the NMR titration experiment. Hence, we could not determine an accurate value but conclude that $K_{a,on}$ > 10.000 L/mol.