

## Supporting Information

### Enhancing Binding Affinity and Selectivity through Preorganization and Cooperative Enhancement of the Receptor

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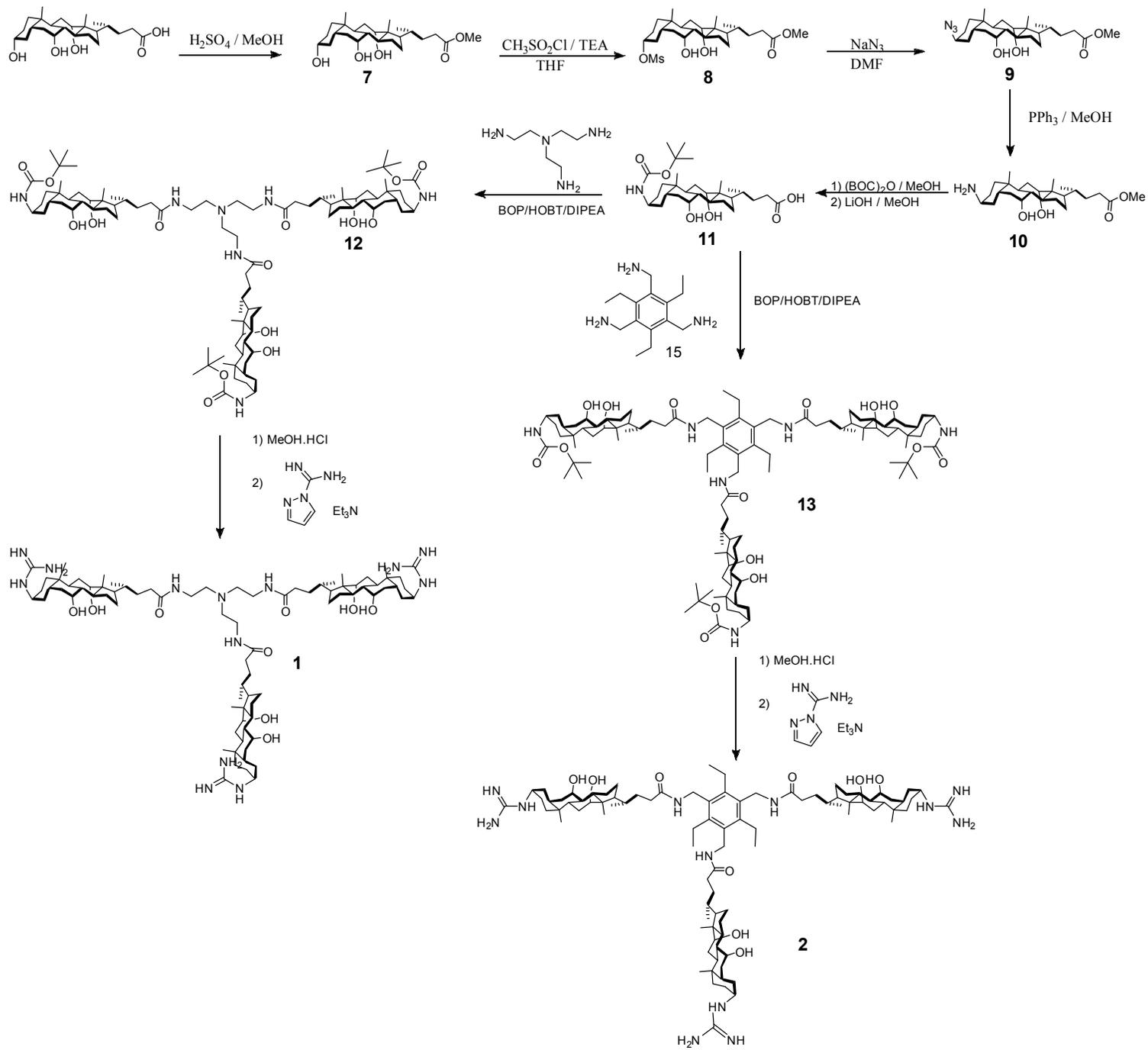
#### *Table of Contents*

<i>General Method</i> .....	S2
<i>Scheme S1</i> .....	S3
<i>Scheme S2</i> .....	S4
<i>Scheme S3</i> .....	S4
<i>Syntheses</i> .....	S4
Figure S1 .....	S9
Figure S2 .....	S10
Figure S3 .....	S11
Figure S4 .....	S12
Figure S5 .....	S13
Figure S6 .....	S14
Figure S7 .....	S15
Figure S8 .....	S16
Figure S9 .....	S17
Figure S10 .....	S18
<i><sup>1</sup>H and <sup>13</sup>C NMR spectra</i> .....	S19

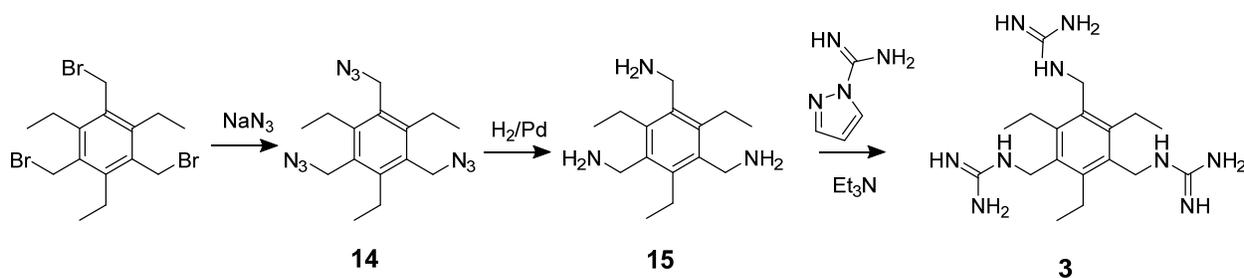
## ***General Method***

For spectroscopic purpose, methanol, tetrahydrofuran, hexane, and ethyl acetate were of HPLC grade. All other reagents and solvents were of ACS-certified grade or higher, and were used as received from commercial suppliers. Routine  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker DRX-400, on a Bruker AV II 600 or on a Varian VXR-400 spectrometer. MALDI-TOF mass was recorded on a Thermobioanalysis Dynamo mass spectrometer. ITC was performed using a MicroCal VP-ITC Microcalorimeter with Origin 7 software and VPViewer2000 (GE Healthcare, Northampton, MA). Fluorescence spectra were recorded at ambient temperature on a Varian Cary Eclipse Fluorescence spectrophotometer.

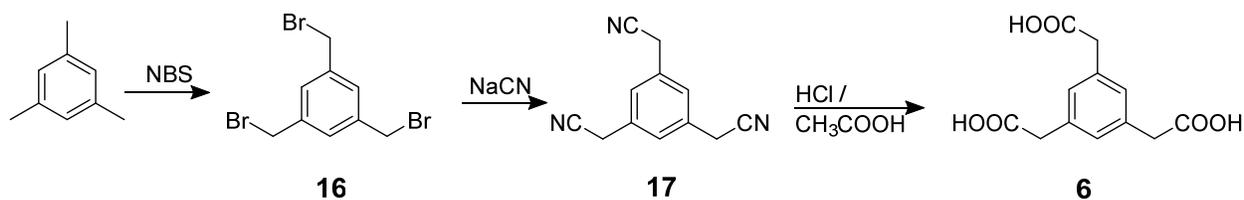
### Scheme S1



## Scheme S2



## Scheme S3



## Syntheses

Syntheses of compounds **6**,<sup>1</sup> **7**,<sup>2</sup> **8**,<sup>2</sup> **9**,<sup>2</sup> **10**,<sup>2</sup> **14**,<sup>3</sup> **15**,<sup>3</sup> **16**,<sup>4</sup> and **17**<sup>5</sup> were previously reported.

<sup>1</sup> David, G., *e-EROS Encyclopedia of Reagents for Organic Synthesis*, **2001**,1.

<sup>2</sup> Ryu, E.H.; Ellern, A.; Zhao, Y., *Tetrahedron Lett.* **2006**, 62, 6808.

<sup>3</sup> Saha, S.; Moorthy, J. N., *Eur. J. Org. Chem.* **2010**, 33, 6359.

<sup>4</sup> Hermann, K.; Turner, D. A.; Hadad, C. M.; Badjić, J. D., *Chem. Eur. J.* **2012**, 18, 8301.

<sup>5</sup> Aakeroy, C. B.; Smith, M. M.; Desper, J., *Cryst. Eng.* **2012**, 14, 71.

**Compound 11.** Compound **10** (0.9 g, 2.13 mmol) was dissolved in methanol (25.00 mL) and sodium bicarbonate (0.35g, 4.2 mmol) was added to it. Di-tert-butyl dicarbonate (0.511g, 2.34 mmol) was added to the reaction content and stirred at room temperature under nitrogen gas. The reaction was monitored by TLC and completed in 12 h. 2 M lithium hydroxide (11 mL, 21.30 mmol) was then added to it. The mixture was stirred at room temperature for 4 h. The reaction was monitored by TLC. The organic solvent was removed by rotary evaporation. After a dilute HCl solution (0.05 M, 30 mL) was added to the reaction mixture, the precipitate formed was collected by suction filtration, washed with cold water, and dried *in vacuo* to get a white powder (1.00 g, 99%). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD/CDCl<sub>3</sub>, 1:1, δ): 5.44(s, 1H), 3.93 (s, 1H), 3.78 (s, 1H), 3.73 (s, 1H), 2.60-0.92 (series of m), 0.66 (s, 3H). ESI-MS (*m/z*): [M+H]<sup>+</sup> calcd for C<sub>29</sub>H<sub>50</sub>NO<sub>6</sub>, 508.3633; found, 508.3631.

**Compound 12.** Compound **11** (0.91 g, 1.8 mmol), tris(2-aminoethyl)amine (0.087 mL, 0.58 mmol), (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP, 1.53 g, 3.48 mmol), 1-hydroxybenzotriazole hydrate (HOBt, 0.47 g, 3.48 mmol), and N,N-diisopropylethylamine (DIPEA, 1.21 mL, 6.96 mmol) were dissolved in dimethyl formamide (6 mL). The reaction was stirred for 1 h in a microwave reactor at 65 °C (150 W), cooled down to room temperature, and poured into a dilute HCl aqueous solution (0.05 M, 20 mL). The precipitate formed was collected by suction filtration, washed with water, dried in air, and purified by column chromatography over silica gel with 6:1 dichloromethane/methanol as the eluent to give an off-white powder (0.655 g, 70%). <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD/CDCl<sub>3</sub>, 1:1, δ): 6.21 (s, 3H), 3.94 (s, 3H), 3.79 (s, 3H), 3.72 (s, 3H), 3.22 (t, 6H), 2.60-0.92 (series of m), 0.70 (s, 9H). <sup>13</sup>C NMR (150 MHz, CD<sub>3</sub>OD/CDCl<sub>3</sub>, 1:1, δ): 175.5, 156.4, 77.6, 72.7, 67.8, 53.8, 47.7,

46.6, 43.2, 41.6, 39.5, 37.5, 36.8, 35.6, 34.9, 34.2, 34.0, 33.7, 33.0, 32.0, 30.5, 28.4, 27.6, 27.4, 25.9, 24.7, 23.0, 22.3, 20.6, 16.7, 11.9. ESI-MS ( $m/z$ ):  $[M+H]^+$  cacl'd for  $C_{93}H_{160}N_7O_{15}$ , 1615.1894; found, 1615.1950.

**Compound 1.** Compound **12** (0.45 g, 0.28 mmol) was stirred with methanolic hydrochloric acid (6 mL, pH = 1) at room temperature for 6 h. The reaction was monitored by TLC. The solvent was removed by rotary evaporation (bath temperature  $<40$  °C), to give an off-white powder (0.37 g, 100%). The off-white powder (0.370 g, 0.28 mmol), 1H-Pyrazole-1-carboxamide hydrochloride (0.132 g, 0.90 mmol) and triethyl amine (4 mL) in DMF (2 mL) was stirred at 60 °C under nitrogen. After 17 h, ether was added to the reaction mixture. The precipitate formed was collected by suction filtration, washed with ether, and dried in air. The crude product was crystallized from 1:1:1 ether/acetonitrile/ethanol (15 mL) to give yellow color powder. (0.360 g, 89%).  $^1H$  NMR (600 MHz,  $CD_3OD/CDCl_3$ , 1:1,  $\delta$ ): 3.96 (s, 3H), 3.81 (s, 3H), 3.50 (s, 6H), 3.17 (q,  $J = 7.4$  Hz, 6H), 2.78 (t,  $J = 14.6$  Hz, 3H), 2.60-0.92 (series of m), 0.70 (s, 9H).  $^{13}C$  NMR (150 MHz,  $CD_3OD/CDCl_3$ , 1:1,  $\delta$ ): 175.5, 156.3, 78.1, 77.7, 77.4, 72.6, 72.5, 67.9, 67.6, 67.4, 56.5, 55.6, 53.8, 48.6, 48.4, 48.2, 48.0, 47.9, 47.8, 47.7, 47.5, 47.4, 47.3, 46.8, 46.6, 46.4, 46.2, 41.6, 39.4, 37.5, 37.0, 36.7, 35.7, 35.7, 34.9, 33.9, 33.6, 33.1, 32.0, 31.5, 30.6, 30.2, 29.1, 28.4, 27.5, 25.9, 24.3, 23.0, 22.5, 22.3, 21.9, 21.8, 16.7, 12.0, 8.4, 6.9. ESI-MS ( $m/z$ ):  $[M+H]^+$  cacl'd for  $C_{81}H_{142}N_{13}O_9$ , 1441.0975; found, 1441.1048.

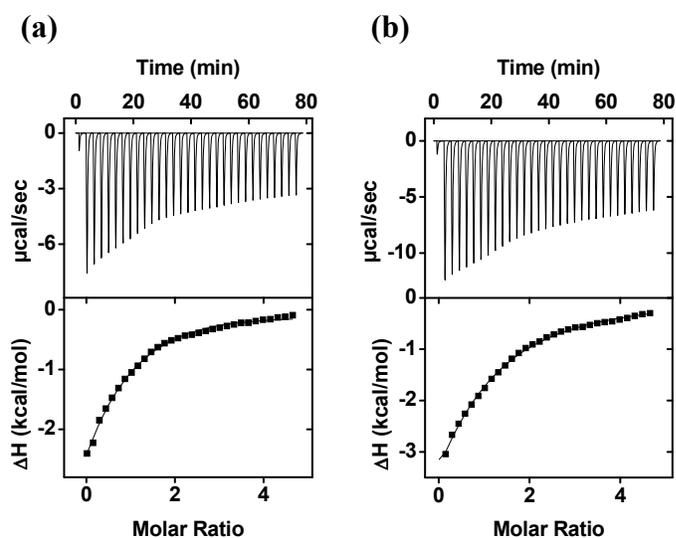
**Compound 13.** Compound **11** (0.46 g, 0.89 mmol), compound **15** (0.070 g, 0.28 mmol), (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP, 0.495 g, 1.12 mmol), 1-hydroxybenzotriazole hydrate (HOBT, 0.15 g, 1.12 mmol), and N,N-

diisopropylethylamine (DIPEA, 0.585 mL, 3.36 mmol) were dissolved in dimethyl sulfoxide (4 mL). The reaction was stirred for 3 h in a microwave reactor at 65 °C (150 W), cooled down to room temperature, and poured into a dilute HCl aqueous solution (0.05 M, 20 mL). The precipitate formed was collected by suction filtration, washed with water, dried in air, and purified by column chromatography over silica gel with 10:1 dichloromethane/methanol as the eluent to give an off-white powder (0.320 g, 67%). <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD/CDCl<sub>3</sub>, 1:1, δ): 4.39 (s, 6H), 3.92 (t, *J* = 2.8 Hz, 3H), 3.79 (m, 3H), 3.72 (s, 3H), 2.68 (m, 6H), 2.60-0.92 (series of m), 0.68 (s, 9H). <sup>13</sup>C NMR (150 MHz, CD<sub>3</sub>OD/CDCl<sub>3</sub>, 1:1, δ): 175.0, 143.8, 131.6, 126.8, 77.9, 77.8, 77.6, 77.2, 72.8, 67.9, 48.1, 47.9, 47.7, 47.5, 46.7, 46.2, 41.6, 39.2, 37.8, 36.9, 35.5, 35.0, 34.1, 32.6, 31.9, 28.3, 27.9, 27.5, 25.8, 23.0, 22.5, 17.8, 16.7, 15.7, 12.03. ESI-MS (*m/z*): [M+H]<sup>+</sup> caclcd for C<sub>102</sub>H<sub>169</sub>N<sub>6</sub>O<sub>15</sub>, 1618.2568; found, 1618.2640.

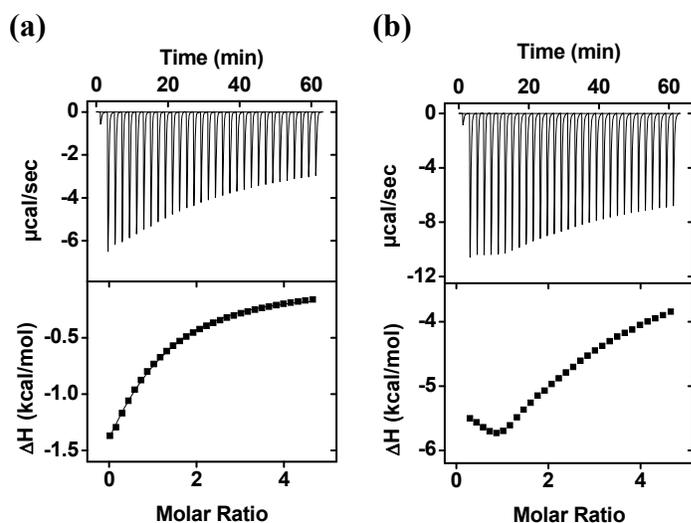
**Compound 2.** Compound **13** (0.32 g, 0.18 mmol) was stirred with methanolic hydrochloric acid (5 mL, pH = 1) at room temperature for 6 h. The solvent was removed by rotary evaporation (bath temperature <40 °C) to give an off-white powder (0.37 g, 100%). The material obtained (0.31 g, 0.22 mmol) was combined with 1H-Pyrazole-1-carboxamide hydrochloride (0.101 g, 0.69 mmol) and triethyl amine (3 mL) in DMF (2 mL) and the mixture was stirred at 60 °C under nitrogen. The reaction was monitored by TLC. After 17 h, ether was added to the reaction. The precipitate formed was collected by suction filtration, washed with ether, and dried in air. The crude product was crystallized from 1:1:1 ether/acetonitrile/ethanol (10 mL) to give a white color powder (0.29 g, 86%). <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD/CDCl<sub>3</sub>, 1:1, δ): 7.63 (s, 3H), 4.39 (s, 6H), 3.95 (s, 3H), 3.80 (s, 3H), 3.71 (s, 3H), 2.65 (d, *J* = 8.6 Hz, 6H), 2.68 (m, 6H), 2.60-0.92 (series of m), 0.69 (s, 9H). <sup>13</sup>C NMR (150 MHz, CD<sub>3</sub>OD/CDCl<sub>3</sub>, 1:1, δ): 175.0, 156.3, 143.9, 131.4,

72.7, 67.7, 65.7, 57.2, 48.4, 47.8, 47.7, 47.7, 47.5, 46.8, 46.6, 46.3, 46.3, 41.6, 39.5, 39.4, 37.9, 36.8, 35.6, 35.0, 33.9, 33.2, 32.7, 31.9, 30.7, 30.2, 28.3, 27.5, 25.9, 24.3, 23.0, 22.8, 22.5, 22.0, 17.4, 16.7, 15.7, 15.7, 14.5, 12.1. ESI-MS ( $m/z$ ):  $[M+3H]^{3+}$  caclcd for  $C_{90}H_{151}N_{12}O_9$ , 515.7299; found, 515.7310.

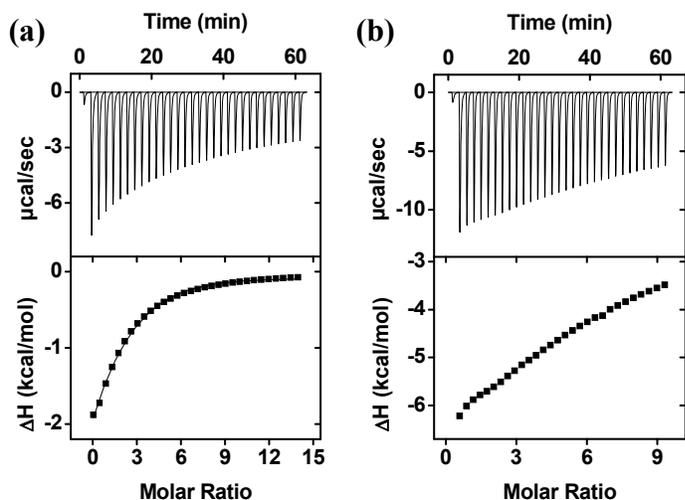
**Compound 3.** Compound **15** (0.10 g, 0.40 mmol), 1H-Pyrazole-1-carboxamide hydrochloride (0.19 g, 0.69 mmol), and triethyl amine (0.5 mL) in in DMF (2 mL) was stirred at 60 °C under nitrogen. After 17 h, ether was added to the reaction mixture. The precipitate formed was collected by suction filtration, washed with ether, and dried in air. The crude product was crystallized from 1:1:1 ether/acetonitrile/ethanol (5 mL) to give white color powder (0.13 g, 89%).  $^1H$  NMR (400 MHz,  $CD_3OD/CDCl_3$ , 1:1,  $\delta$ ): 4.42 (s, 2H), 2.71 (d,  $J = 7.4$ , 2H), 2.05 (s, 1H), 1.18 (t,  $J = 8.1$  Hz, 3H).  $^{13}C$  NMR (100 MHz,  $CD_3OD/CDCl_3$ , 1:1,  $\delta$ ): 156.3, 145.3, 129.6, 39.6, 22.4, 15.2. ESI-MS ( $m/z$ ):  $[M+H]^+$  caclcd for  $C_{18}H_{34}N_9$ , 376.2859; found, 376.2932.



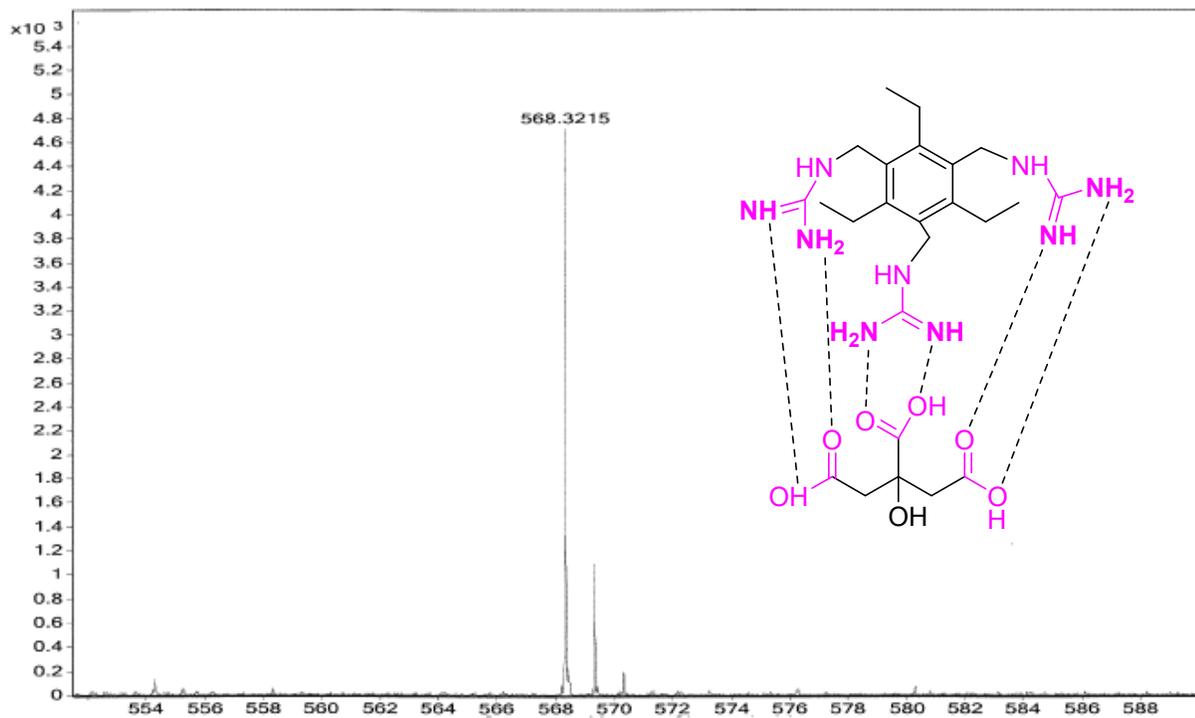
**Figure S1.** ITC titration curves obtained at 298 K for the binding of (a) **5** (4 mM) by CER **1** (0.2 mM) in D.I. water (pH = 7.0) and (b) **6** (4 mM) by CER **1** (0.2 mM) in D.I. water (pH = 7.0). The data correspond to entries 2 and 3 respectively, in Table 1. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of CER to the substrate. The solid line is the best fit of the experimental data to the sequential binding of N equal and independent binding sites on the CER. The heat of dilution for the substrate, obtained by adding the substrate to D.I. water (pH = 7.0), was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.



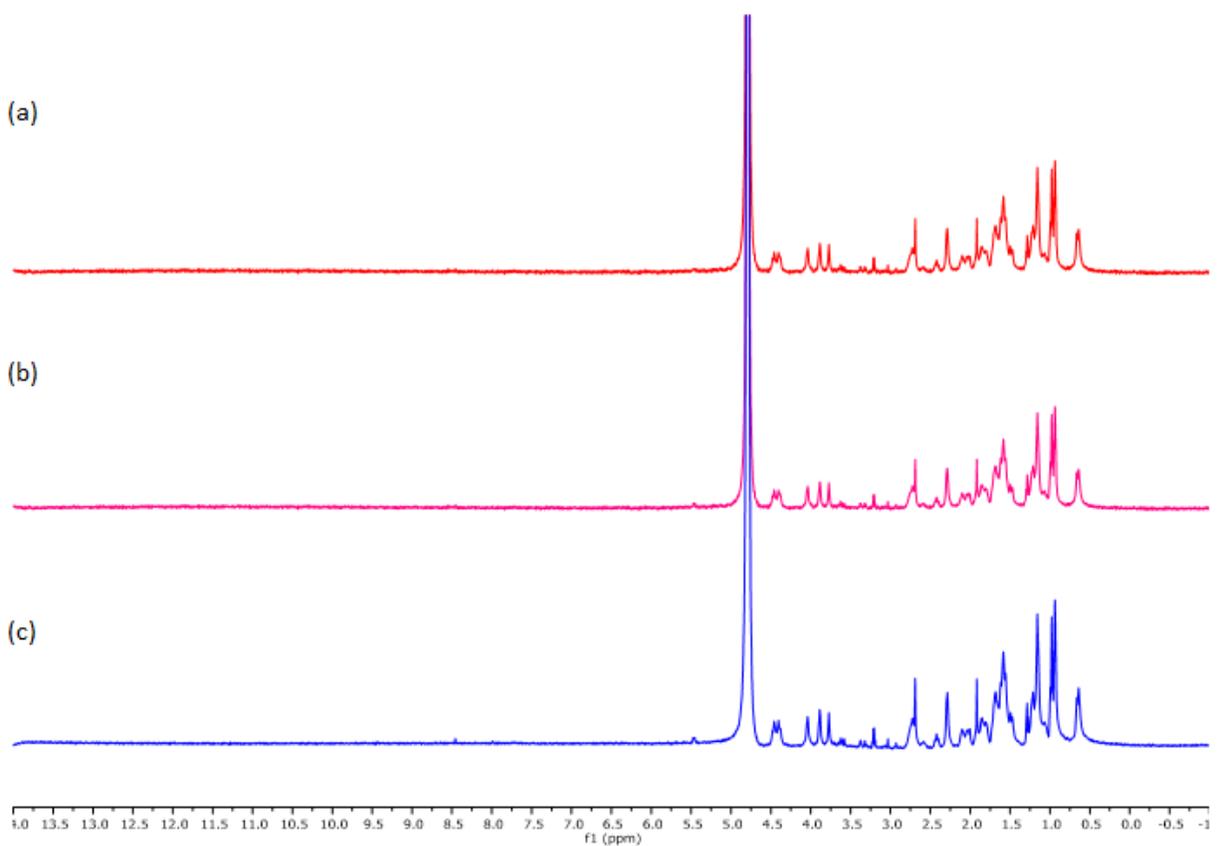
**Figure S2.** ITC titration curves obtained at 298 K for the binding of (a) **5** (4 mM) by CER **2** (0.2 mM) in D.I. water (pH = 7.0) and (b) **6** (4 mM) by CER **2** (0.2 mM) in D.I. water (pH = 7.0). The data correspond to entries 5 and 6 respectively, in Table 1. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of CER to the substrate. The solid line is the best fit of the experimental data to the sequential binding of  $N$  equal and independent binding sites on the CER. The heat of dilution for the substrate, obtained by adding the substrate to D.I. water (pH = 7.0), was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.



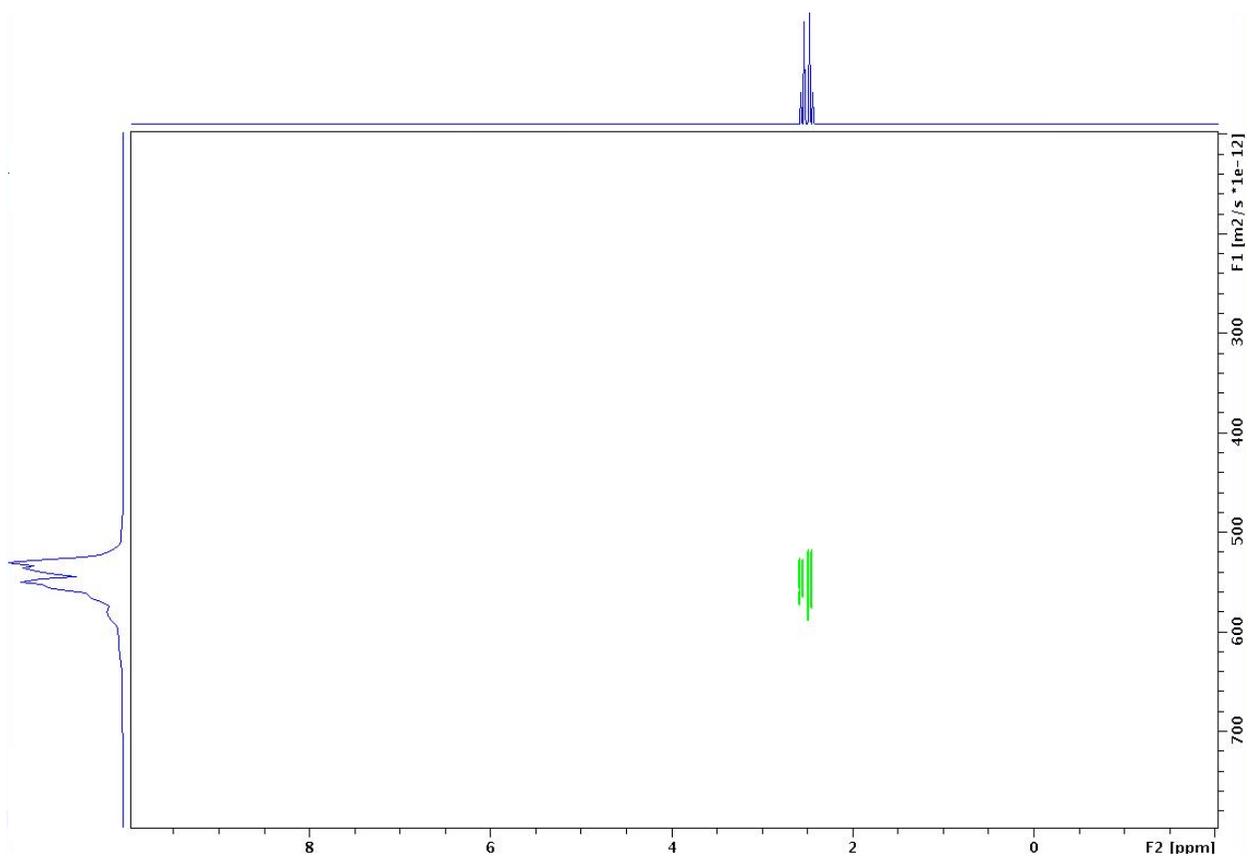
**Figure S3.** ITC titration curves obtained at 298 K for the binding of (a) **5** (6 mM) by CER **3** (0.1 mM) in D.I. water (pH = 7.0) and (b) **6** (4 mM) by CER **3** (0.1 mM) in D.I. water (pH = 7.0). The data correspond to entries 8 and 9 respectively, in Table 1. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of CER to the substrate. The solid line is the best fit of the experimental data to the sequential binding of N equal and independent binding sites on the CER. The heat of dilution for the substrate, obtained by adding the substrate to D.I. water (pH = 7.0), was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.



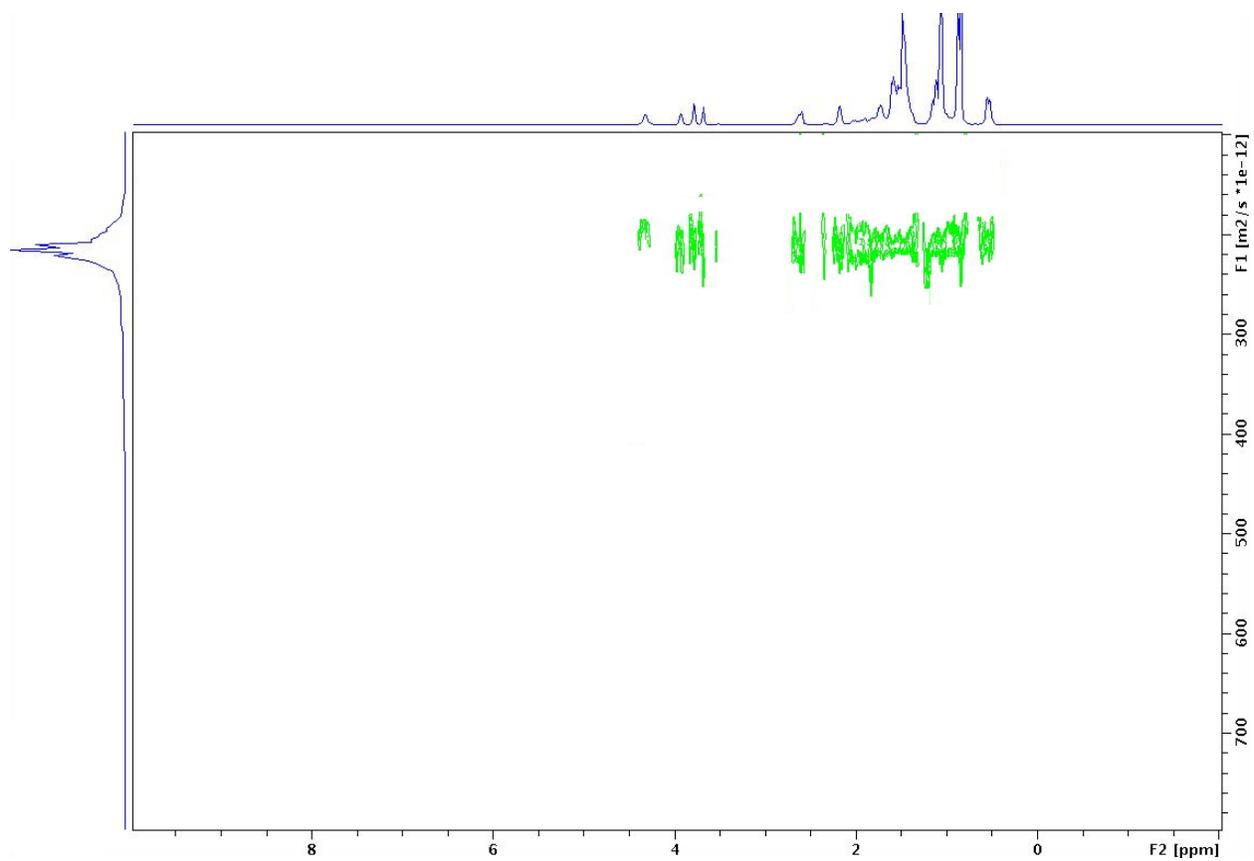
**Figure S4.** ESI-MS ( $m/z$ ) for CER 3 with compound 4:  $[M+H]^+$  caclcd for  $C_{24}H_{42}N_9O_7$ , 568.3142; found, 568.3215.



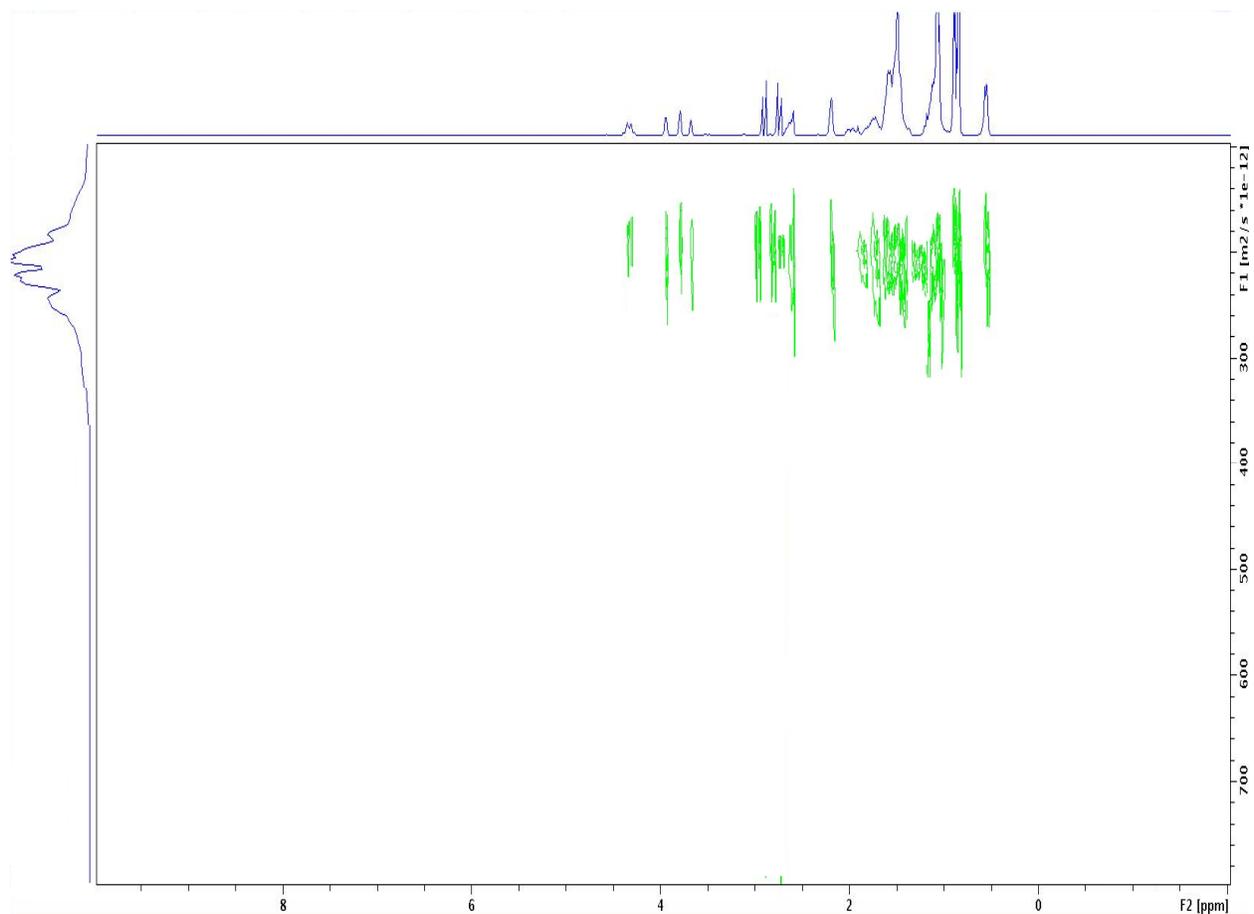
**Figure S5.** NMR dilution experiment (a) 0.2 mM, (b) 0.4 mM, and (c) 0.6 mM CER 2 concentration at 298 K.



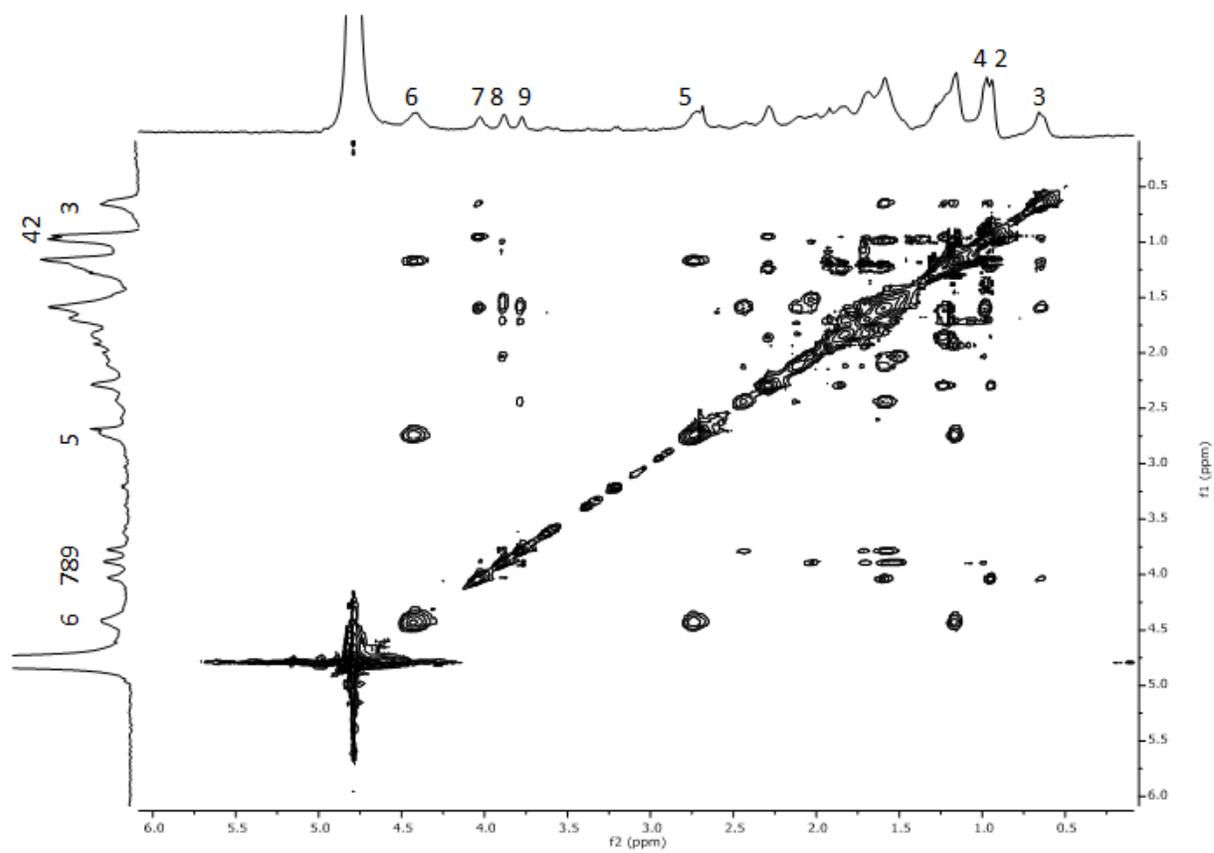
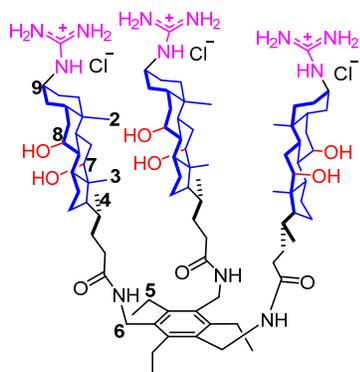
**Figure S6.** 400 MHz 2D DOSY NMR spectra obtained at 298 K in D<sub>2</sub>O solution of **4**. ( $D_4 = 5.570 \times 10^{-10} \text{ m}^2\text{S}^{-1}$ , 1.5 mM)



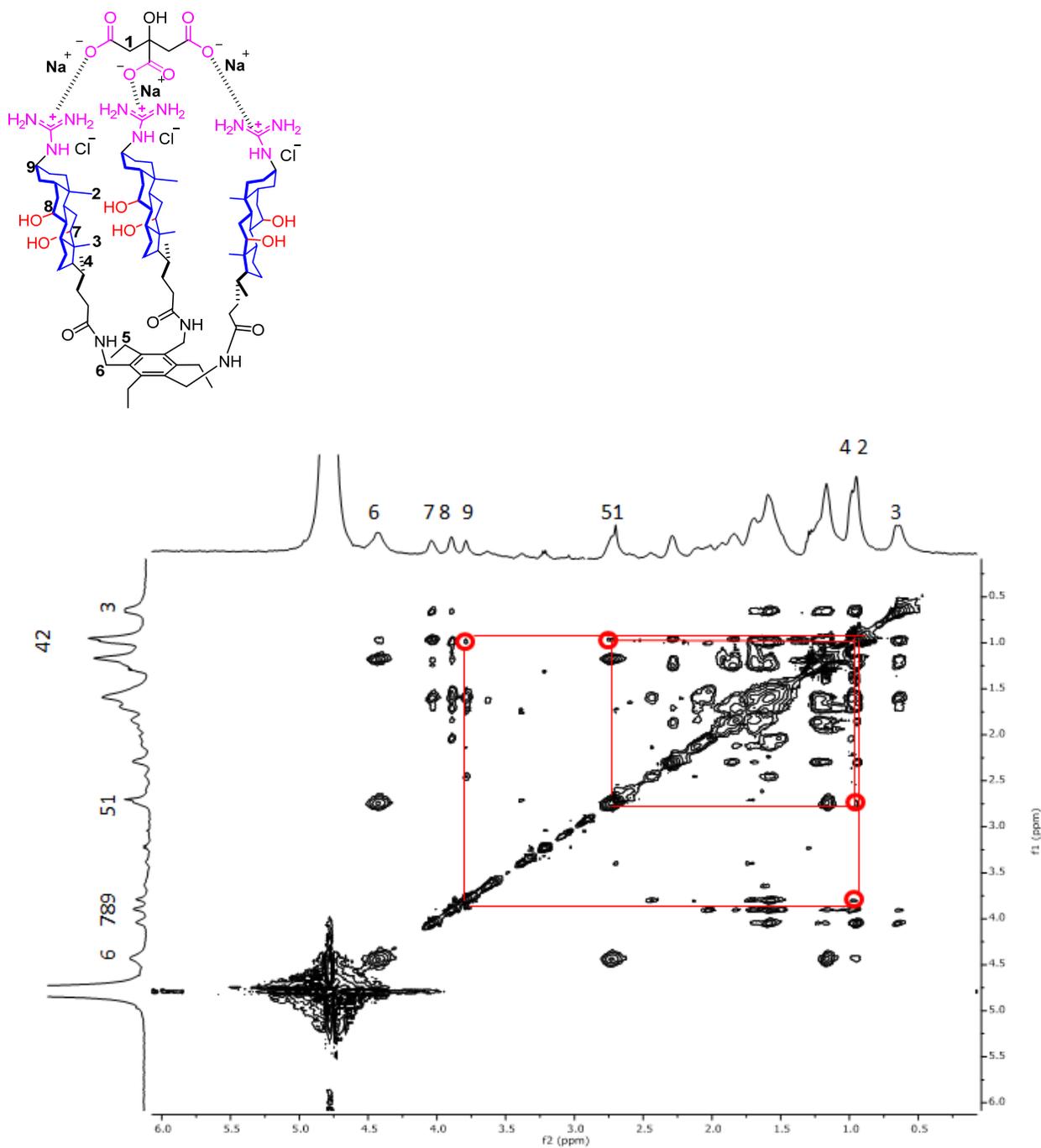
**Figure S7.** 400 MHz 2D DOSY NMR spectra obtained at 298 K in D<sub>2</sub>O solution of CER 2. ( $D_{\text{CER 2}} = 2.434 \times 10^{-10} \text{ m}^2\text{S}^{-1}$ , 1.5 mM)



**Figure S8.** 400 MHz 2D DOSY NMR spectra obtained at 298 K in  $\text{D}_2\text{O}$  solution of 1:1 mixture of CER **2** and Compound **4**. ( $D_{\text{Complex}} = 2.164 \times 10^{-10} \text{ m}^2\text{S}^{-1}$ , 1.5 mM)

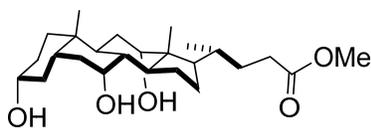


**Figure S9.** The 2D NOESY spectrum of CER 2 in D<sub>2</sub>O at 298 K.

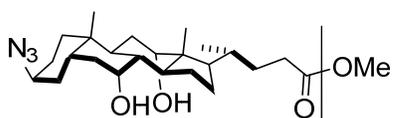
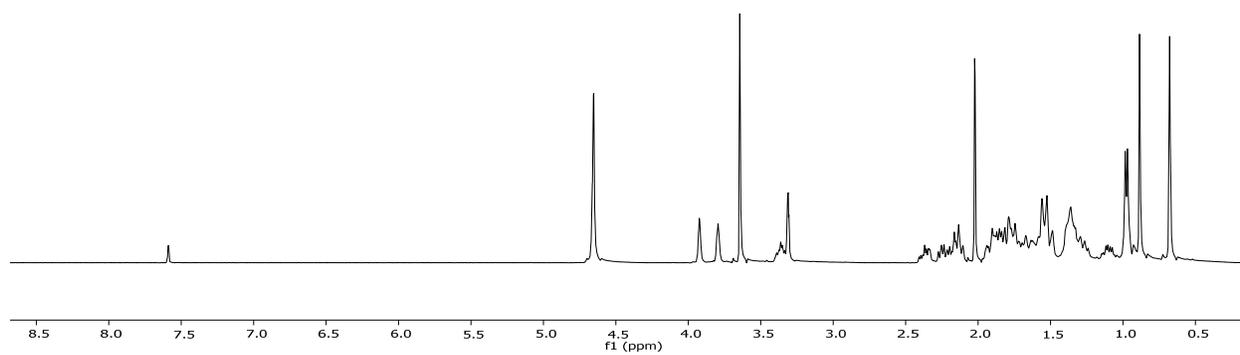


**Figure S10.** The 2D NOESY spectrum of 1:1 mixture of CER **2** and Compound **4** in D<sub>2</sub>O at 298 K. The cross-peaks circled in red were absent in receptor **2** (Figure S9) and indicated the close contact between the cholate β faces and between citrate and the receptor.

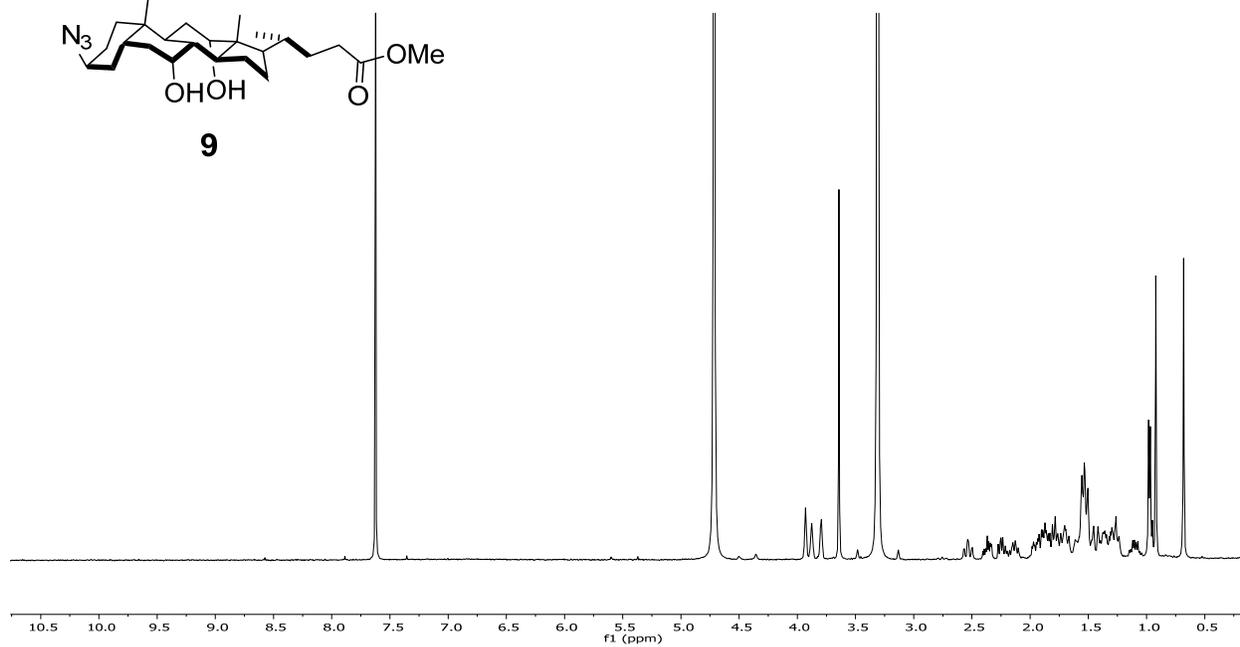
# *<sup>1</sup>H and <sup>13</sup>C NMR spectra*

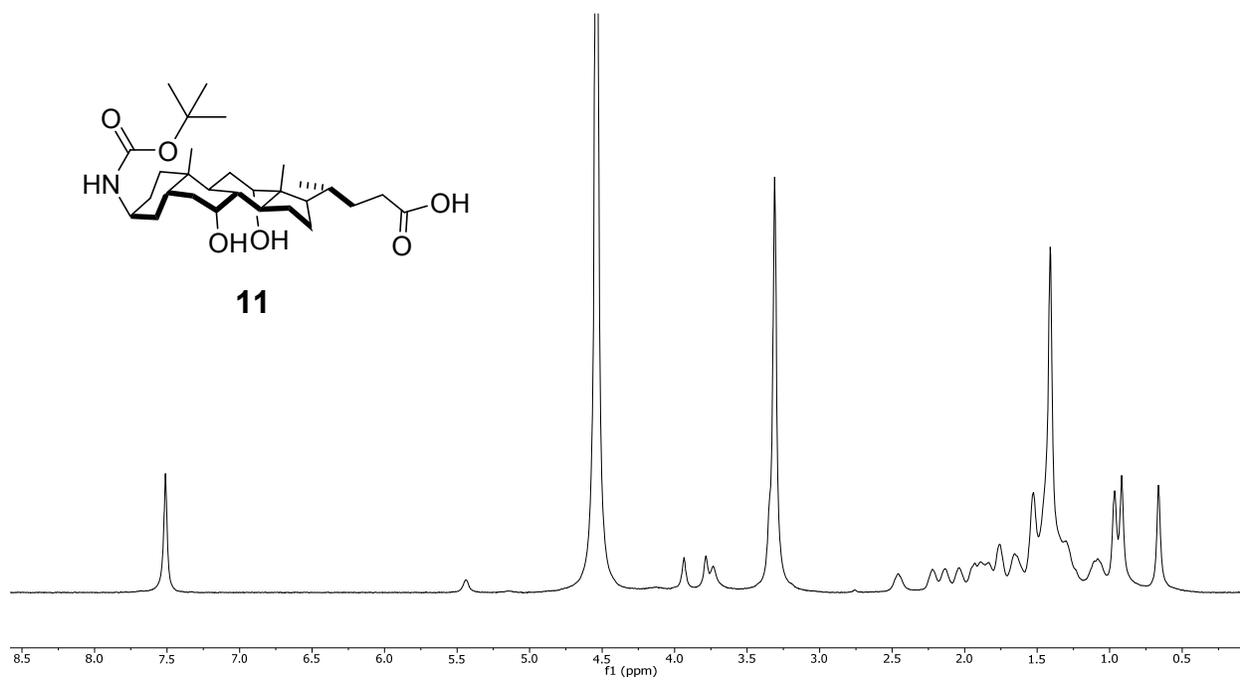
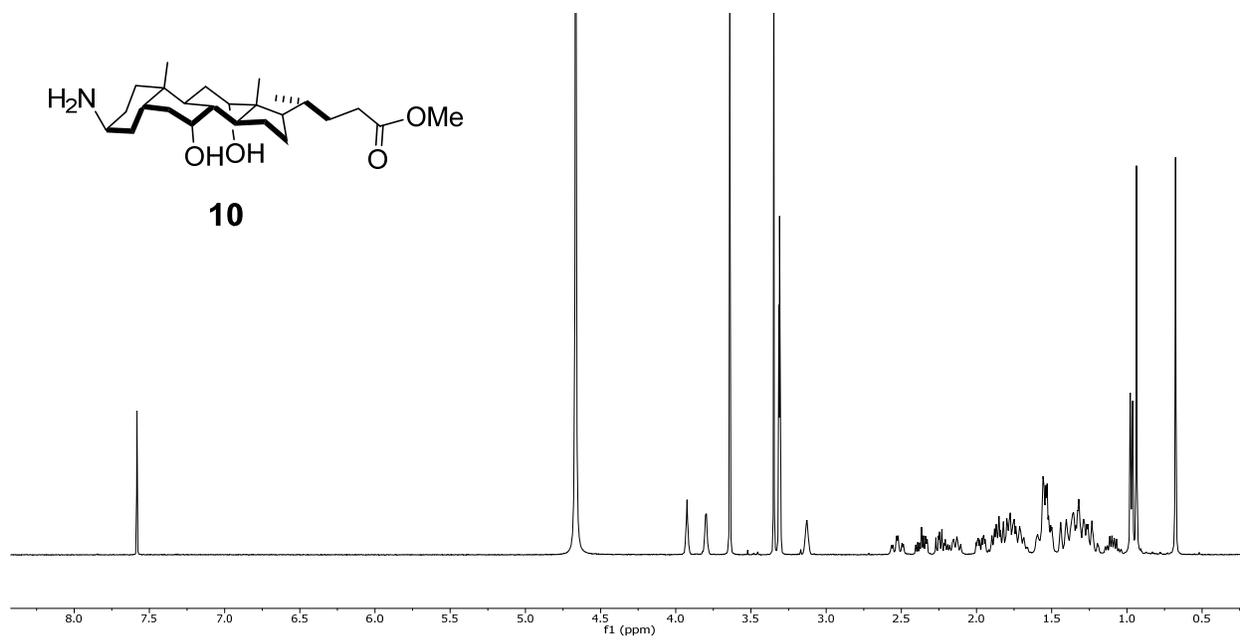


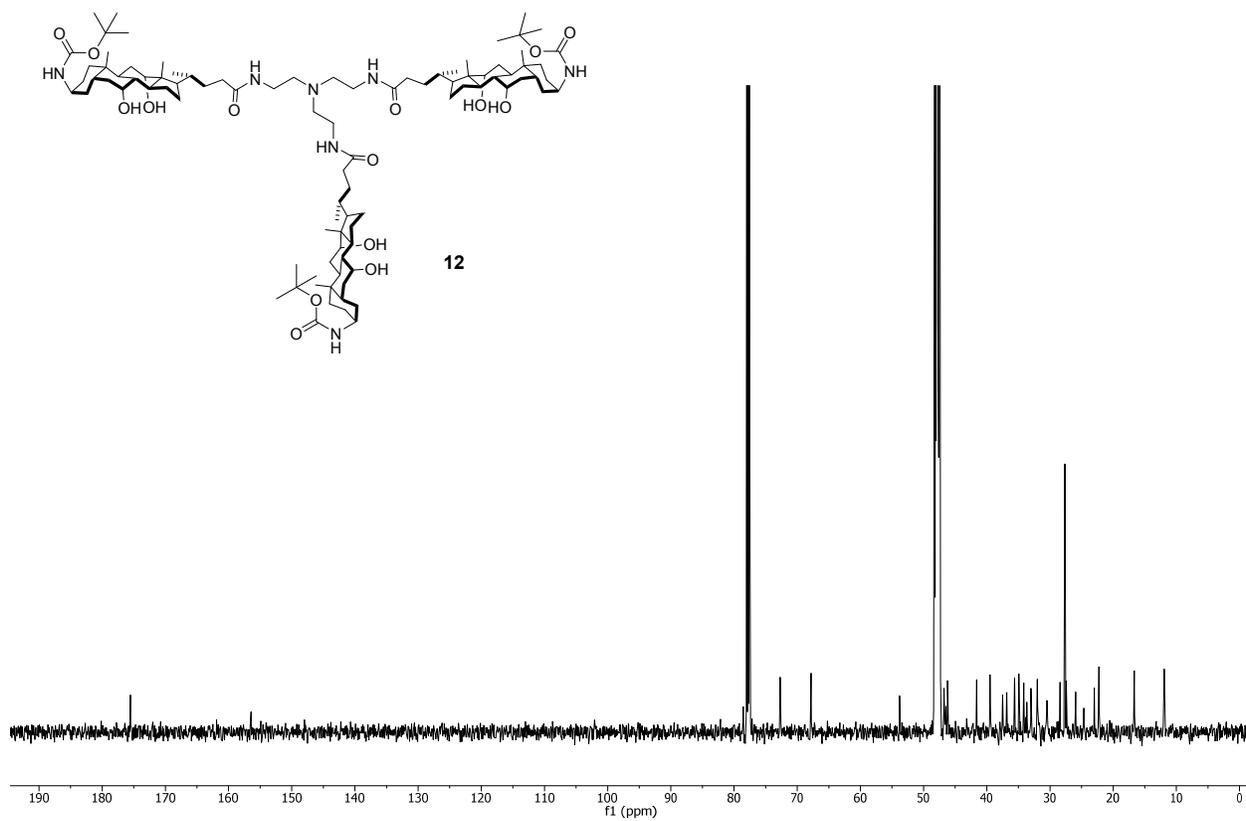
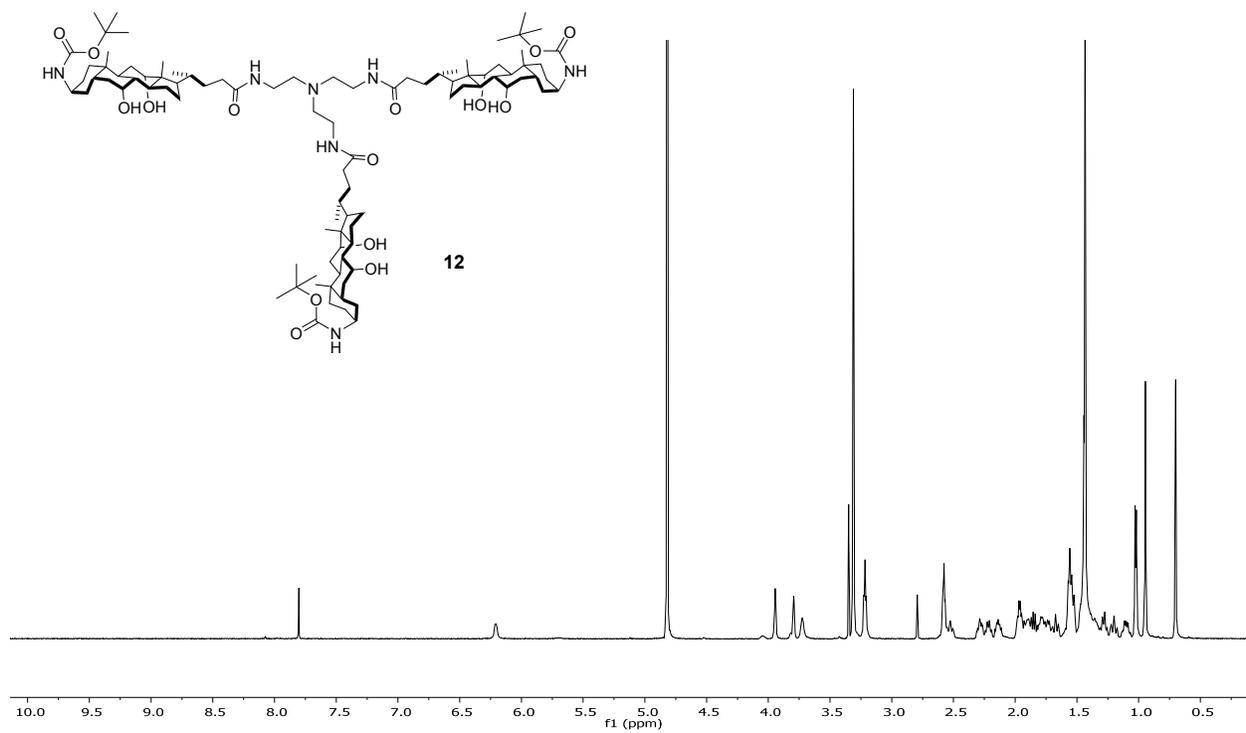
**7**

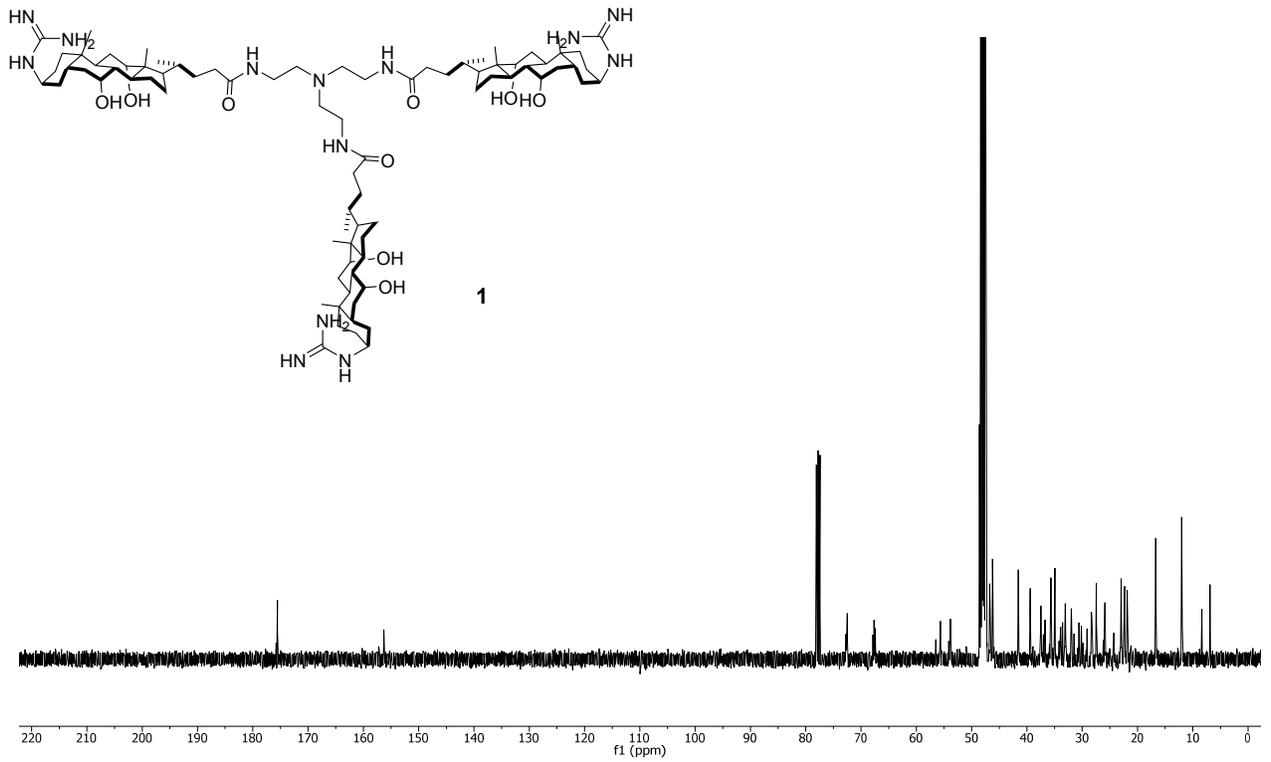
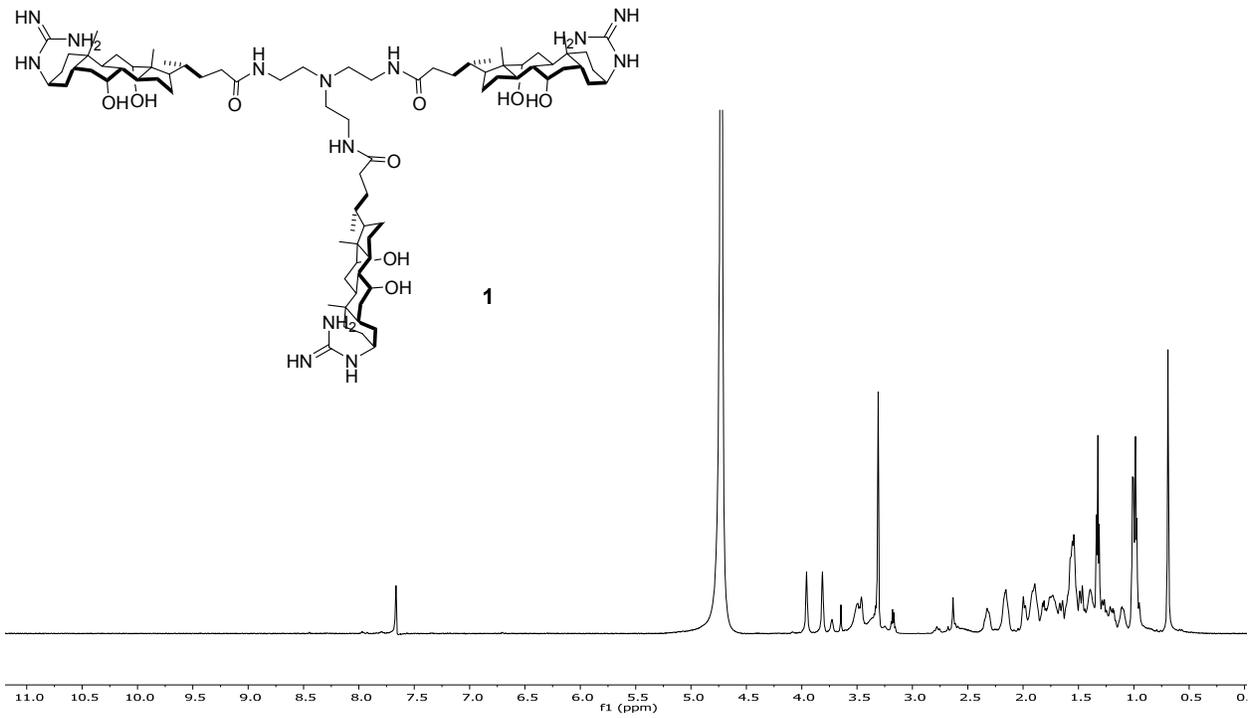


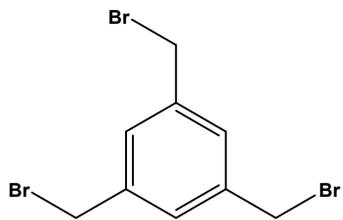
**9**



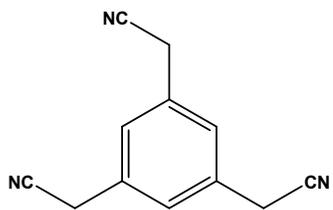
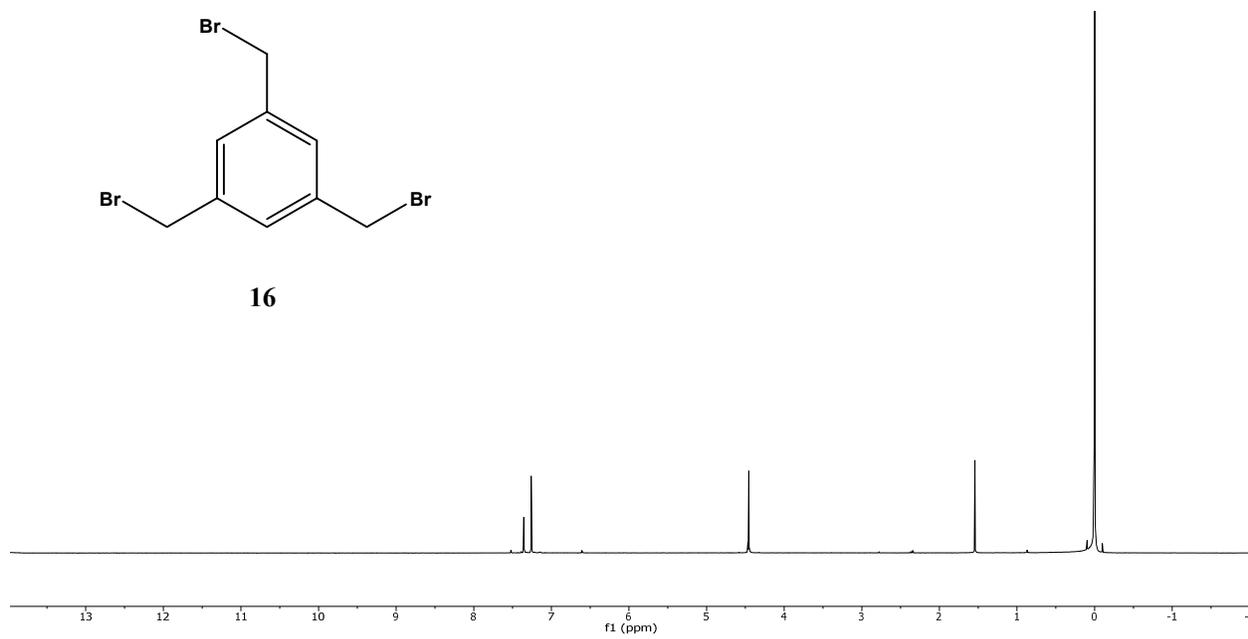








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