ELECTRONIC SUPPLEMENTARY INFORMATION

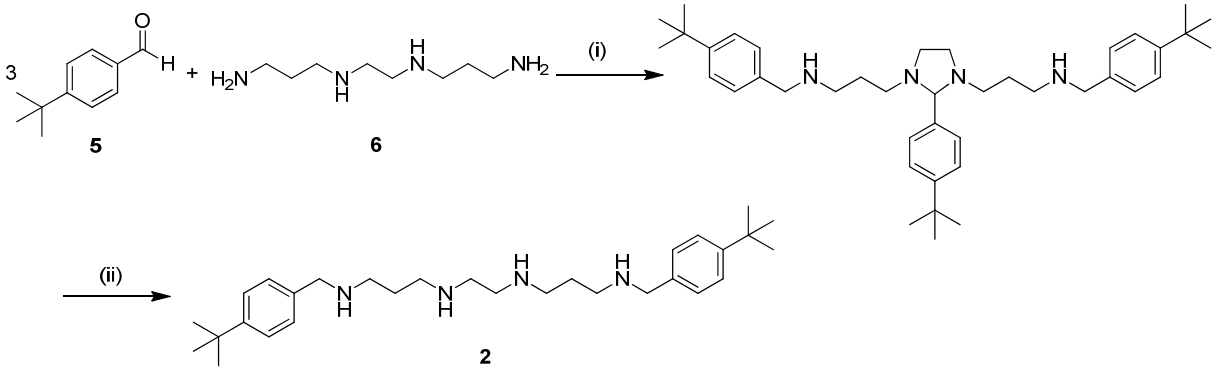
Metal Ion Responsive Adhesion of Vesicles by Conformational Switching of a Non-Covalent Linker

Siva Krishna Mohan Nalluri,[a] Jelle B. Bultema,[b] Egbert J. Boekema,[b] and Bart Jan Ravoo[a,*]

a) Organic Chemistry Institute and Graduate School of Chemistry, Westfälische Wilhelms-Universität Münster, Corrensstrasse 40, 48149 Münster, Germany. Email: b.j.ravoo@uni-muenster.de

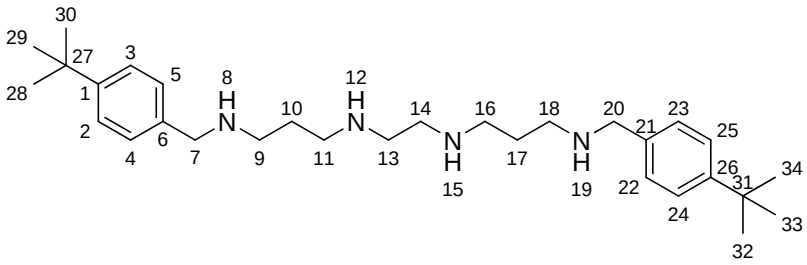
b) Department of Biophysical Chemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands

Synthesis of flexible linker 2:



Scheme S1. Reagents and conditions: (i) EtOH, reflux, 24 h, $NaBH_4$, overnight, rt, n.d.; (ii) 6M HBr, 35%.

N^1,$N^{1'}$-(ethane-1,2-diyl)bis(N^3-(4-(tert-butyl)benzyl)propane-1,3-diamine) 2:



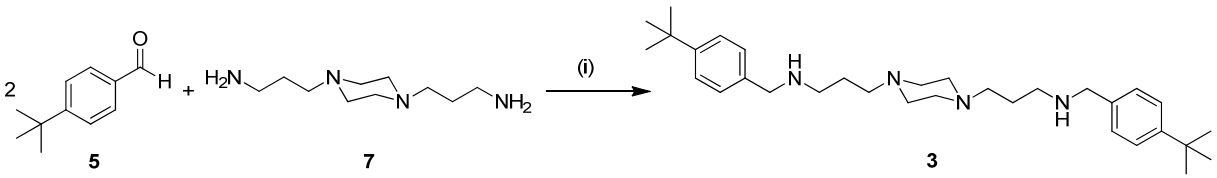
To a stirred solution of 0.38 g (2.2 mmol) of N^1,$N^{1'}$-(ethane-1,2-diyl)bis(propane-1,3-diamine) **6** in 10 ml of ethanol, 1.1 g (6.78 mmol) of 4-(tert-butyl)benzaldehyde **5** in 10 ml of ethanol was added dropwise and the reaction mixture was refluxed for 24 h under argon and then allowed to cool to room temperature. The solvent was evaporated to afford imine derivative as light yellow oil. To the imine residue which was taken up in 15 ml of MeOH, 0.34 g (8.8 mmol) of sodium borohydride was added and the reaction mixture was stirred at room temperature overnight. The reaction mixture was cooled to 0^o C and a 6M hydrogen bromide solution was added until a white color precipitate is formed. The precipitate formed was filtered, washed with cold ethanol and taken in the minimum amount of water. The solution was made basic with KOH (pH > 11) and extracted thrice with 20 ml of DCM. The organic phases were pooled, dried over $MgSO_4$ and concentrated. The crude product was purified by silica gel column chromatography (eluent, DCM / MeOH / NH_4OH 80:18:2) to afford 0.36 g (35% yield) of **2** as a light yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ = 7.30 (d, *J* = 8.3 Hz, 4H, 2,3,24,25-H), 7.21 (d, *J* = 8.3 Hz, 4H, 4,5,22,23-H), 4.02 (s, 4H, 7,20-H), 3.76 – 3.68 (m, 4H, 8,12,15,19-H), 2.84 – 2.62 (m, 12H, 9,11,13,14,16,18-H), 1.77 – 1.62 (m, 4H, 10,17-H), 1.24 (s, 18H, 28,29,30,32,33,34-H).

^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ = 149.96 (C_q, 1,26-C), 135.71(C_q, 6,21-C), 127.92 (CH, 4,5,22,23-C), 125.20 (CH, 2,3,24,25-C), 53.00 (CH_2, 7,20-C), 48.09 (CH_2, 13,14-C), 47.78 (CH_2, 9,18-C), 47.54 (CH_2, 11,16-C), 34.28 (C_q, 27,31-C), 31.21 (CH_3, 28,29,30,32,33,34-C), 28.14 (CH_3, 10,17-C).

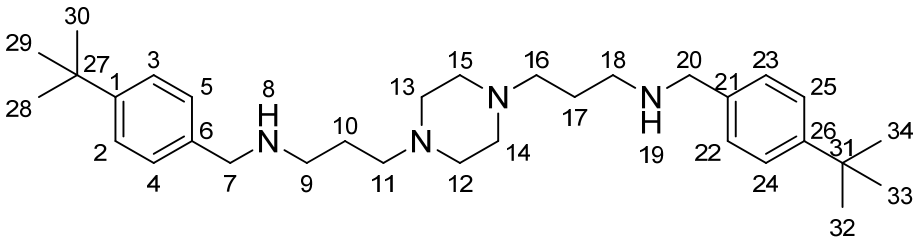
ESI-HRMS (m/z): Calculated for $[C_{30}H_{50}N_4H]^+$: 467.4108; Found: 467.4107.

Synthesis of rigid linker 3:



Scheme S2. Reagents and conditions: (i) EtOH, reflux, 12 h, $NaBH_4$, 5 h, rt, 53%.

3,3'-(piperazine-1,4-diyl)bis(N-(4-(tert-butyl)benzyl)propan-1-amine) 3:



To a stirred solution of 0.5 g (2.5 mmol) of 3,3'-(piperazine-1,4-diyl)bis(propan-1-amine) **7** in 25 ml of ethanol, 0.89 g (6.78 mmol) of 4-(tert-butyl)benzaldehyde **5** in 15 ml of ethanol was added dropwise and the reaction mixture was refluxed for 12 h under argon and then allowed to cool to room temperature. The solvent was evaporated to afford imine derivative as yellow oil. To the imine residue which was taken up in 10 ml of MeOH, 0.38 g (10.0 mmol) of sodium borohydride was added and the reaction mixture was stirred at room temperature for 5 hours. The solvent was

evaporated under vacuum. The crude residue was dissolved in the minimum amount of water and this was extracted twice with 20 ml of DCM. The organic phases were pooled, dried over $MgSO_4$ and concentrated. The crude product was purified by silica gel column chromatography (eluent, DCM / MeOH 80:20) to afford 0.65 g (53% yield) of **3** as pale yellow crystals.

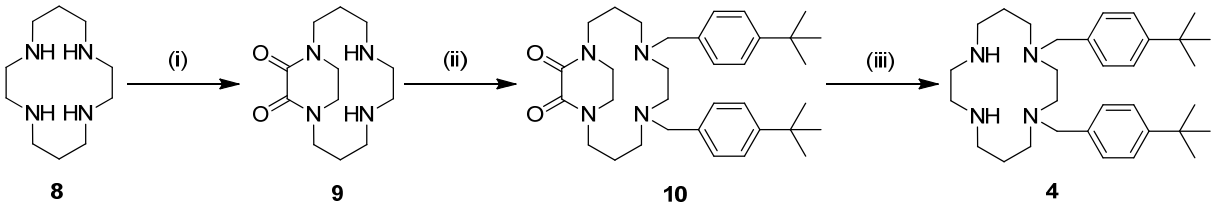
^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 7.29 – 7.24 (m, 4H, 2,3,24,25-H), 7.19 -7.14 (m, 4H, 4,5,22,23-H), 3.67 (s, 4H, 7,20-H), 2.60 (t, *J* = 6.9 Hz, 4H, 9,18-H), 2.5 – 2.2 (m, 12H, 11,12,13,14,15,16-H), 1.80 – 1.66 (m, 2H, 8,19-H), 1.65 – 1.55 (m, 4H, 10,17-H), 1.23 (s, 19H, 28,29,30,32,33,34-H).

^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 149.77 (C_q, 1,26-C), 137.51 (C_q, 6,21-C), 127.83 (CH, 4,5,22,23-C), 125.30 (CH, 2,3,24,25-C), 57.06 (CH_2, 12,13,14,15-C), 53.73 (CH_2, 7,20-C), 53.37 (CH_2, 11,16-C), 48.25 (CH_2, 9,18-C), 34.49 (C_q, 27,31-C), 31.46 (CH_3, 28,29,30,32,33,34-C), 27.10 (CH_2, 10,17-C).

ESI-HRMS (m/z): Calculated for $[C_{32}H_{52}N_4H]^+$: 493.4265; Found: 493.4264.

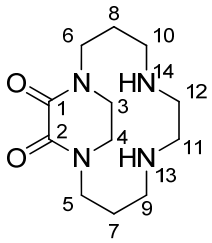
Elemental analysis: Calculated for $C_{32}H_{52}N_4$: C, 77.99; H, 10.64; N, 11.37. Found: C, 77.40, H 10.71, N, 11.21.

Synthesis of macrocyclic guest 4:



Scheme S3. Reagents and conditions: (i) diethyloxalate, EtOH, reflux, 12 h, 75%; (ii) 4-*tert*-butylbenzyl bromide, Na_2CO_3, DMF, 100^o C, 5 h, 69%; (iii) NaOH, EtOH, reflux, 20 h, 89%.

1,5,8,12-tetraazabicyclo[10.2.2]hexadecane-13,14-dione 9:



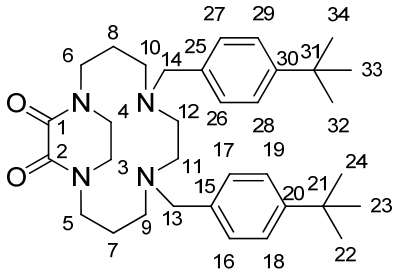
The synthesis was carried out as described in the literature.[S1] In short, to a stirred solution of 0.5 g (2.5 mmol) of cyclam **8** in 10 ml of dry ethanol, 0.34 ml (2.5 mmol) of diethyl oxalate was added and the reaction mixture was refluxed for 24 h under argon and then allowed to cool to room temperature. The solvent was evaporated under reduced pressure and the crude product was recrystallized in acetone-ethanol (20:1) to afford 0.48 g (75% yield) of **9** as white crystals.

^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ = 4.48 – 4.38 (m, 2H, 3,4-H), 3.87 – 3.76 (m, 2H, 5,6-H), 3.51 – 3.39 (m, 2H, 5,6-H), 2.90 – 2.81 (m, 2H, 9,10-H), 2.75 – 2.70 (m, 2H, 3,4-H), 2.66 – 2.61 (m, 2H, 9,10-H), 2.57 – 2.47 (m, 4H, 11,12-H), 1.92 – 1.80 (m, 2H, 7,8-H), 1.78 – 1.63 (m, 2H, 7,8-H), 1.52 – 1.21 (m, 2H, 13,14-H).

^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ = 158.64 (C_q, 1,2-C), 50.00 (CH_2, 11,12-C), 49.49 (CH_2, 9,10-C), 47.85 (CH_2, 5,6-C), 44.22 (CH_2, 3,4-C), 25.50 (CH_2, 7,8-C).

ESI-HRMS (m/z): Calculated for $[C_{12}H_{22}N_4O_2H]^+$: 255.1816; Found: 255.1820.

5,8-bis(4-(tert-butyl)benzyl)-1,5,8,12-tetraazabicyclo[10.2.2]hexadecane-13,14-dione 10:



[S1] F. Bellouard, F. Chubur, N. Kervarec, L. Toupet, S. Triki, Y. Le Mest, and H. Handel *J. Chem. Soc., Perkin Trans. 1*, **1999,** 3499−3505.

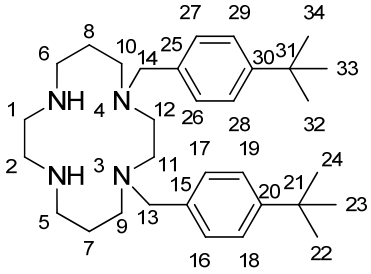
To a stirred solution of 0.3 g (1.18 mmol) of 5,8-bis(4-(tert-butyl)benzyl)-1,5,8,12-tetraazabicyclo[10.2.2]hexadecane-13,14-dione **9** in 10 ml of dry DMF, 0.28 g (2.61 mmol) of sodium carbonate and 0.48 ml (2.61 mmol) of 4-(tert-butyl)benzyl bromide were added and the reaction mixture was stirred at 100^{o} C for 5 hours. After removal of solvent under reduced pressure, the residue was dissolved in 15 ml of DCM, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluent, DCM / MeOH 98:2) to afford 0.45 g (69% yield) of **10** as white crystals.

^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ = 7.20 (d, *J* = 8.2 Hz, 4H, 18,19,28,29-H), 6.99 (d, *J* = 8.2 Hz, 4H, 16,17,26,27-H), 4.32 (m, 2H, 5,6-H), 4.10 – 3.95 (m, 2H, 3,4-H), 3.42 – 3.33 (m, 2H, 5,6-H), 3.31 (s, 4H, 13,14-H), 2.68 (m, 2H, 3,4-H), 2.46 – 2.28 (m, 8H, 9,10,11,12-H), 1.92 – 1.82 (m, 2H, 7,8-H), 1.70 – 1.52 (m, 2H, 7,8-H), 1.19 (s, 18H, 22,23,24,32,33,34-H).

^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ = 158.56 (C_q, 1,2-C), 149.92 (C_q, 20,30-C), 134.56 (C_q, 15,25-C), 129.54 (CH, 16,17,26,27-C), 125.01 (CH, 18,19,28,29-C), 56.88 (CH_2, 13,14-C), 52.27 (CH_2, 11,12-C), 52.00 (CH_2, 9,10-C), 46.54 (CH_2, 3,4-C), 44.70 (CH_2, 5,6-C), 34.50 (C_q, 21,31-C), 31.43 (CH_3, 22,23,24,32,33,34-C), 23.86 (CH_2, 7,8-C).

ESI-HRMS (m/z): Calculated for $[C_{34}H_{50}N_4O_2H]^+$: 547.4007; Found: 547.4007.

1,4-bis(4-(tert-butyl)benzyl)-1,4,8,11-tetraazacyclotetradecane 4:



A slurry of 220 mg (0.4 mmol) of 5,8-bis(4-(tert-butyl)benzyl)-1,5,8,12-tetraazabicyclo[10.2.2]hexadecane-13,14-dione **10** in a mixed solvent of 25 ml of 10 M NaOH and 10 ml of ethanol was refluxed for 20 hours. After removing the solvent under vacuum, 50 ml of water was added and the mixture was extracted by 4 × 20 ml of DCM. The organic layers were pooled, dried over $MgSO_4$ and concentrated. The crude product was purified by silica gel

column chromatography (eluent, DCM / MeOH / NH_4OH 80:18:2) to afford 0.18 g (89% yield) of **4** as pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ = 7.30 (d, *J* = 8.3 Hz, 4H, 18,19,28,29-H), 7.20 (d, *J* = 8.3 Hz, 4H, 16,17,26,27-H), 3.68 – 3.57 (m, 2H, 3,4-H), 3.43 (s, 4H, 13,14-H), 2.97 (s, 4H, 1,2-H), 2.85 – 2.74 (m, 4H, 5,6-H), 2.47 – 2.24 (m, 8H, 9,10,11,12-H), 1.79 (m, 4H, 7,8-H), 1.28 (s, 18H, 22,23,24,32,33,34-H).

^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ = 150.08 (C_q, 20,30-C), 134.93 (C_q, 15,25-C), 129.46 (CH, 16,17,26,27-C), 125.07 (CH, 18,19,28,29-C), 57.47 (CH_2, 13,14-C), 51.63 (CH_2, 11,12-C), 50.93 (CH_2, 9,10-C), 46.64 (CH_2, 5,6-C), 46.50 (CH_2, 1,2-C), 34.55 (C_q, 21,31-C), 31.47 (CH_3, 22,23,24,32,33,34-C), 25.02 (CH_2, 7,8-C).

ESI-HRMS (m/z): Calculated for $[C_{32}H_{52}N_4H]^+$: 493.4265; Found: 493.4267.

Isothermal Titration Calorimetry (ITC):

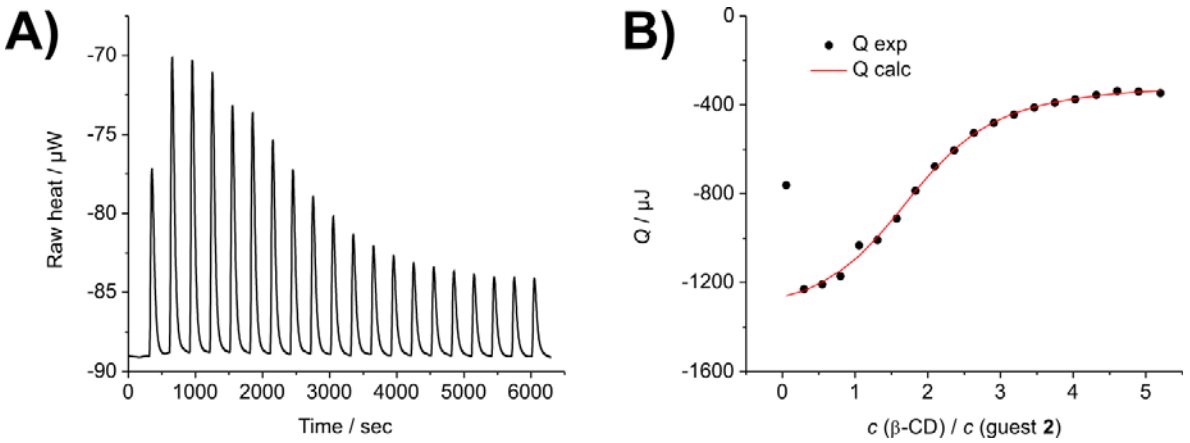


Figure S1: ITC data corresponding to the host-guest interaction of β-CD with guest **2**. A) Injection peaks (raw data vs. time). B) Integration of the injection peaks (heat vs. host/guest ratio). In the ITC fit, the first data point is omitted.

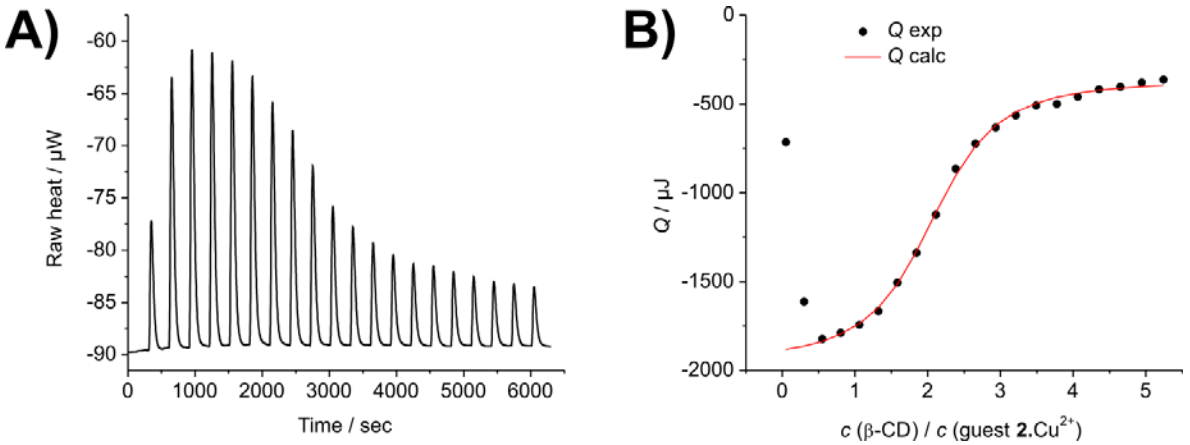


Figure S2: ITC data corresponding to the host-guest interaction of β-CD with guest **2** in the presence of $CuCl_2$. A) Injection peaks (raw data vs. time). B) Integration of the injection peaks (heat vs. host/guest ratio). In the ITC fit, the first two data points are omitted.

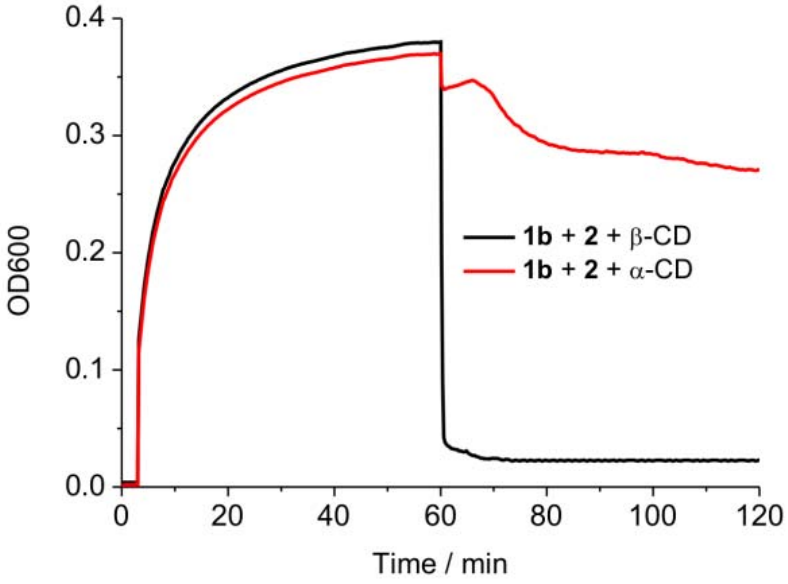


Figure S3: Effect of excess α-CD or β-CD on the aggregation of host vesicles of **1b** induced by guest **2:** time-dependent measurement of OD600. Conditions: [**1b**] = 30 μM and [**2**] = 8 μM and [α-CD] = [β-CD] = 750 μM in 10 mM carbonate-bicarbonate buffer (pH 9.0). Guest **2** was added at 3 min and excess α-CD or β-CD was added at 60 min.

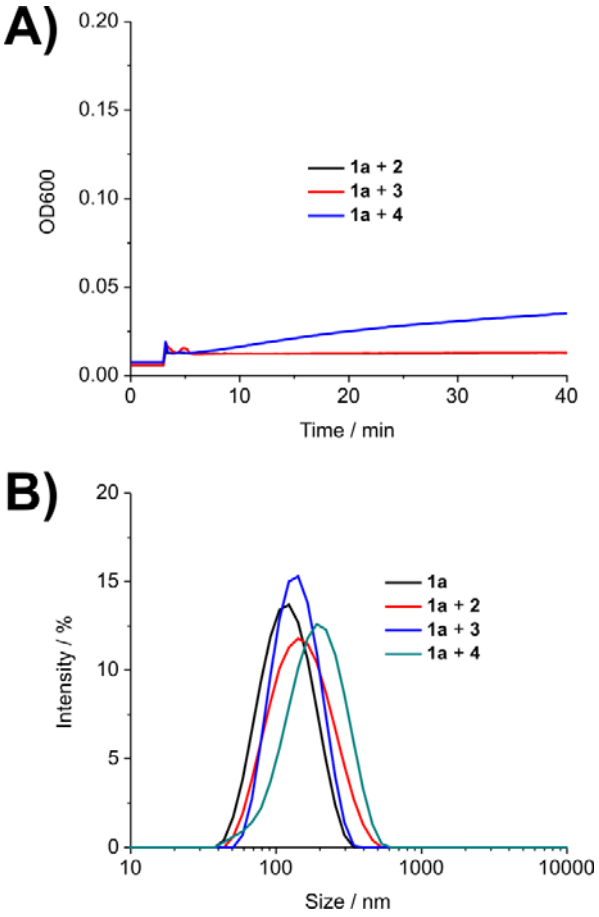


Figure S4: "Inert" host of vesicles of α-CD **1a** in the presence of guests **2, 3** and **4.** A) Time-dependent measurement of OD600. B) Size distribution of vesicles of **1a** according to DLS. Conditions: [**1a**] = 30 μM and [**2**] = [**3**] = [**4**] = 50 μM in 10 mM carbonate-bicarbonate buffer (pH 9.0). Guest **2** (or **3** or **4**) was added at 3 min.

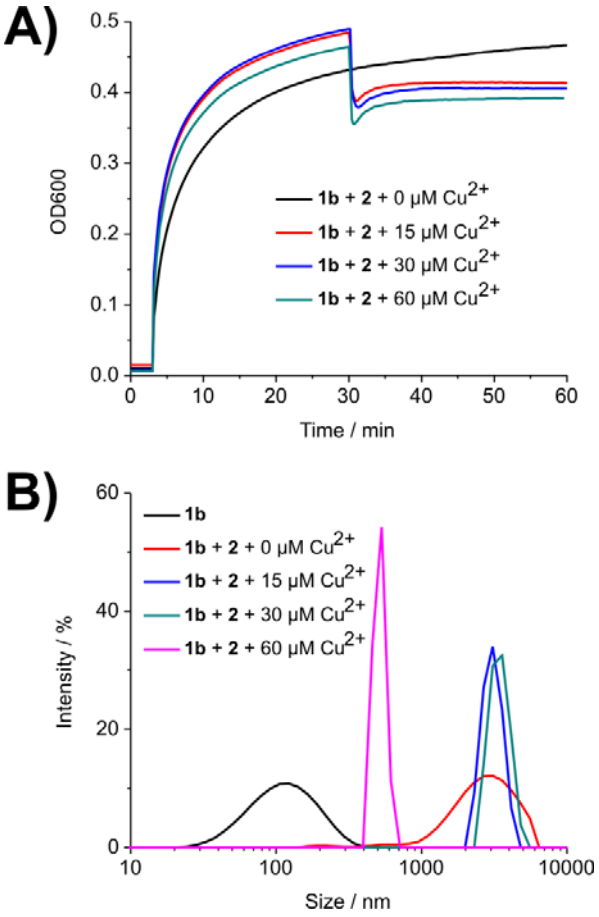


Figure S5: Effect of Cu^{2+} on the aggregation of host vesicles of **1b** by divalent guest **2.** A) Time-dependent measurement of OD600. B) Size distribution of vesicles of **1b** according to DLS. Conditions: [**1b**] = 30 μM and [**2**] = 15 μM in 10 mM carbonate-bicarbonate buffer (pH 9.0). Guest **2** was added at 3 min and Cu^{2+} was added at 30 min.

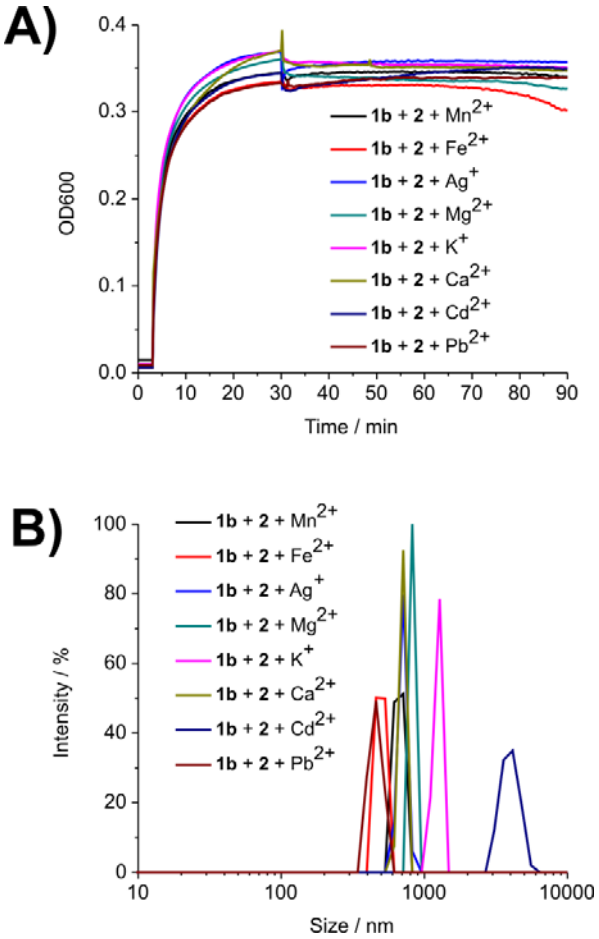


Figure S6: "Selectivity" of divalent metal ions on the aggregation of vesicles of **1b** in the presence of **2**. A) Time-dependent measurement of OD600. B) Size distribution of vesicles of **1b** according to DLS. Conditions: [**1b**] = 30 μM, [**2**] = 6 μM and [M^{2+}] = 14 μM in 10 mM carbonate-bicarbonate buffer (pH 9.0). Guest **2** was added at 3 min and M^{2+} was added at 30 min.

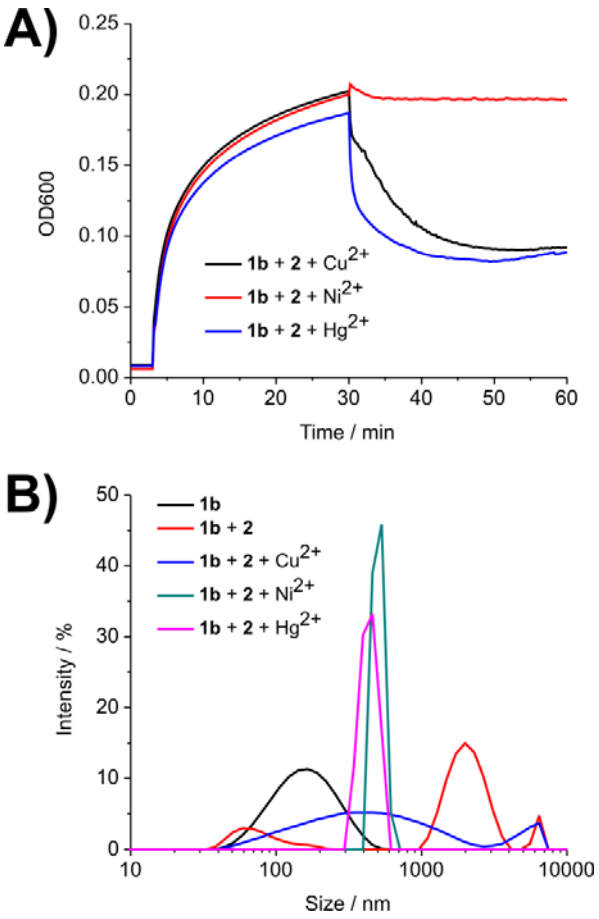


Figure S7: "Partially" metal ion-responsive aggregation of host vesicles of **1b** induced by guest **2** at pH 7.4. A) Time-dependent measurement of OD600. B) Size distribution of vesicles of **1b** according to DLS. Conditions: [**1b**] = 30 μM and [**2**] = 8 μM and [M^{2+}] = 16 μM in 10 mM phosphate buffer (pH 7.4). Guest **2** was added at 3 min and M^{2+} was added at 30 min.

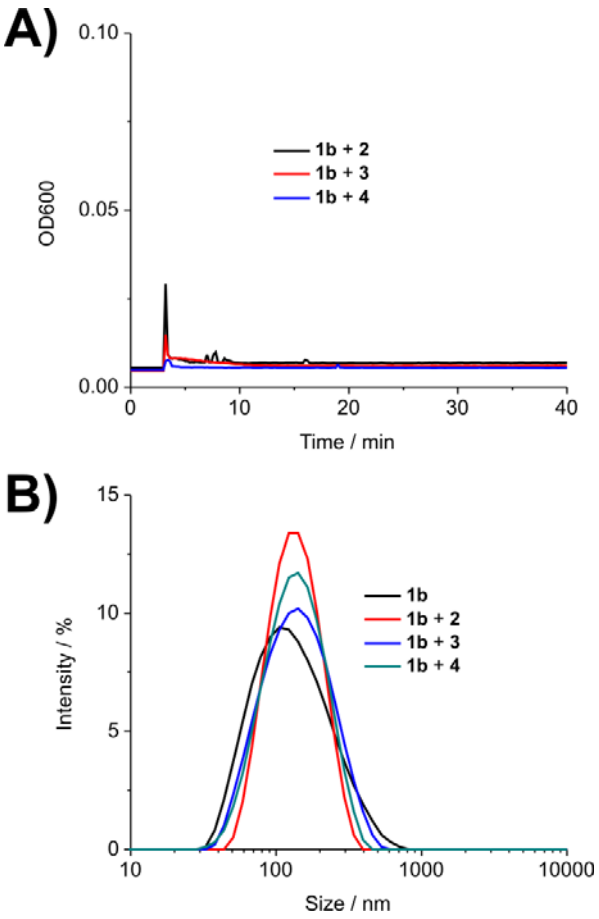


Figure S8: "Inert" host of vesicles of **1b** in the presence of guests **2, 3** and **4** at pH 4.0. A) Time-dependent measurement of OD600. B) Size distribution of vesicles of **1b** according to DLS. Conditions: [**1b**] = 30 μM and [**2**] = [**3**] = [**4**] = 40 μM in 10 mM acetate buffer (pH 4.0). Guest **2** (or **3** or **4**) was added at 3 min.

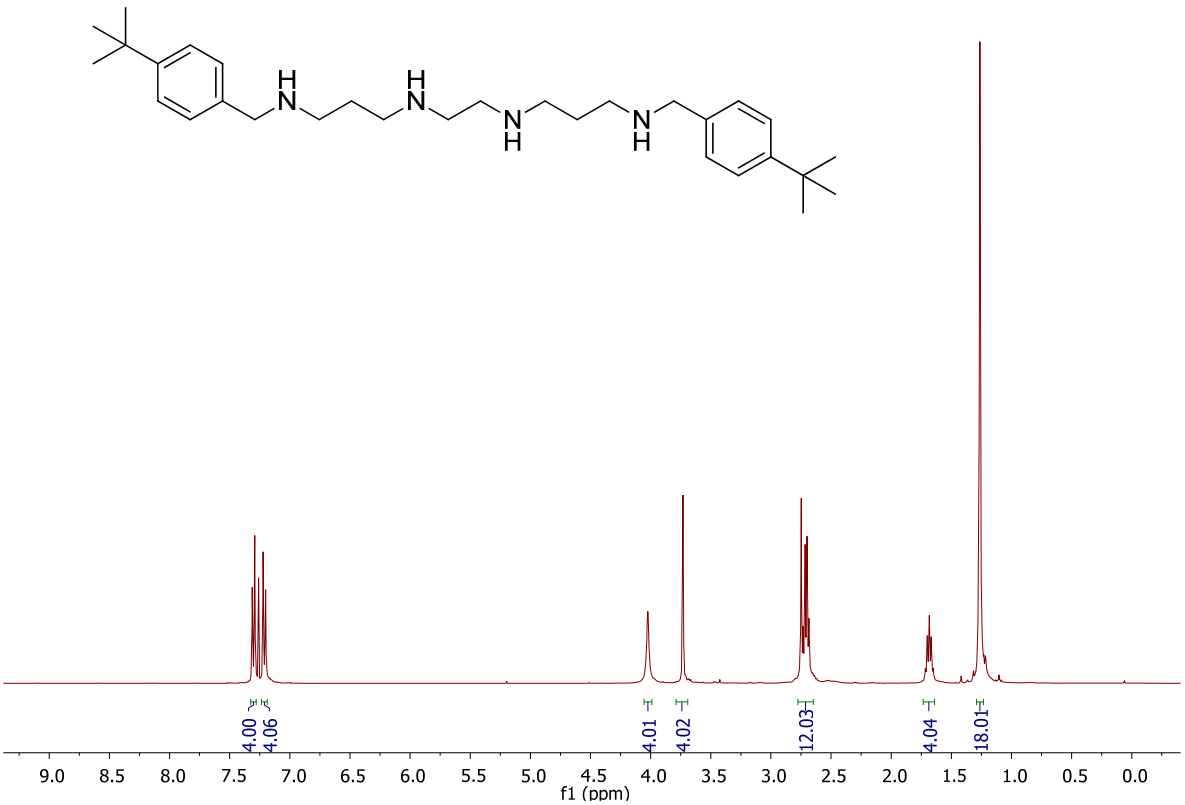


Figure S9: ^{1}H-NMR of guest **2** in $CDCl_3$ at 298 K.

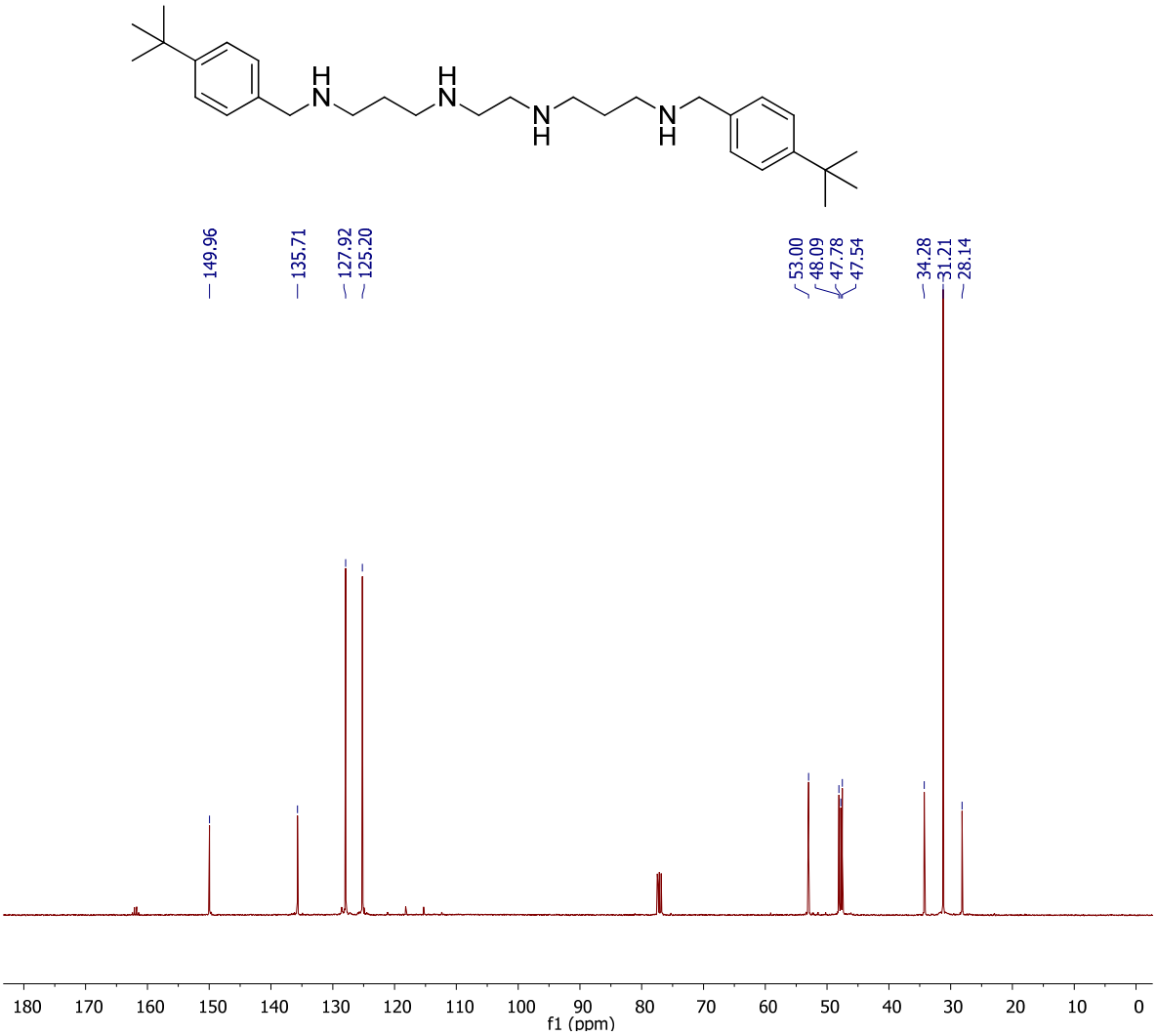


Figure S10: ^{13}C-NMR of compound guest **2** in $CDCl_3$ at 298 K.

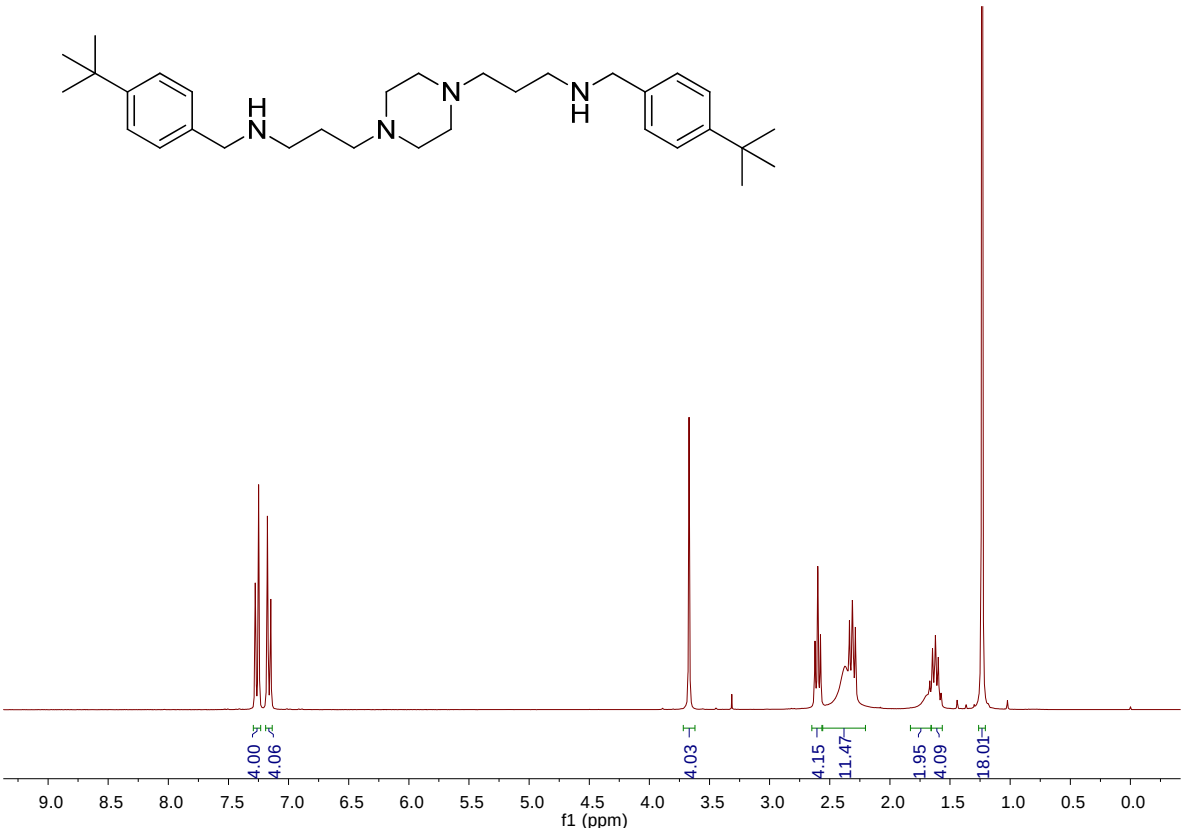


Figure S11: ^{1}H-NMR of guest **3** in $CDCl_3$ at 298 K.

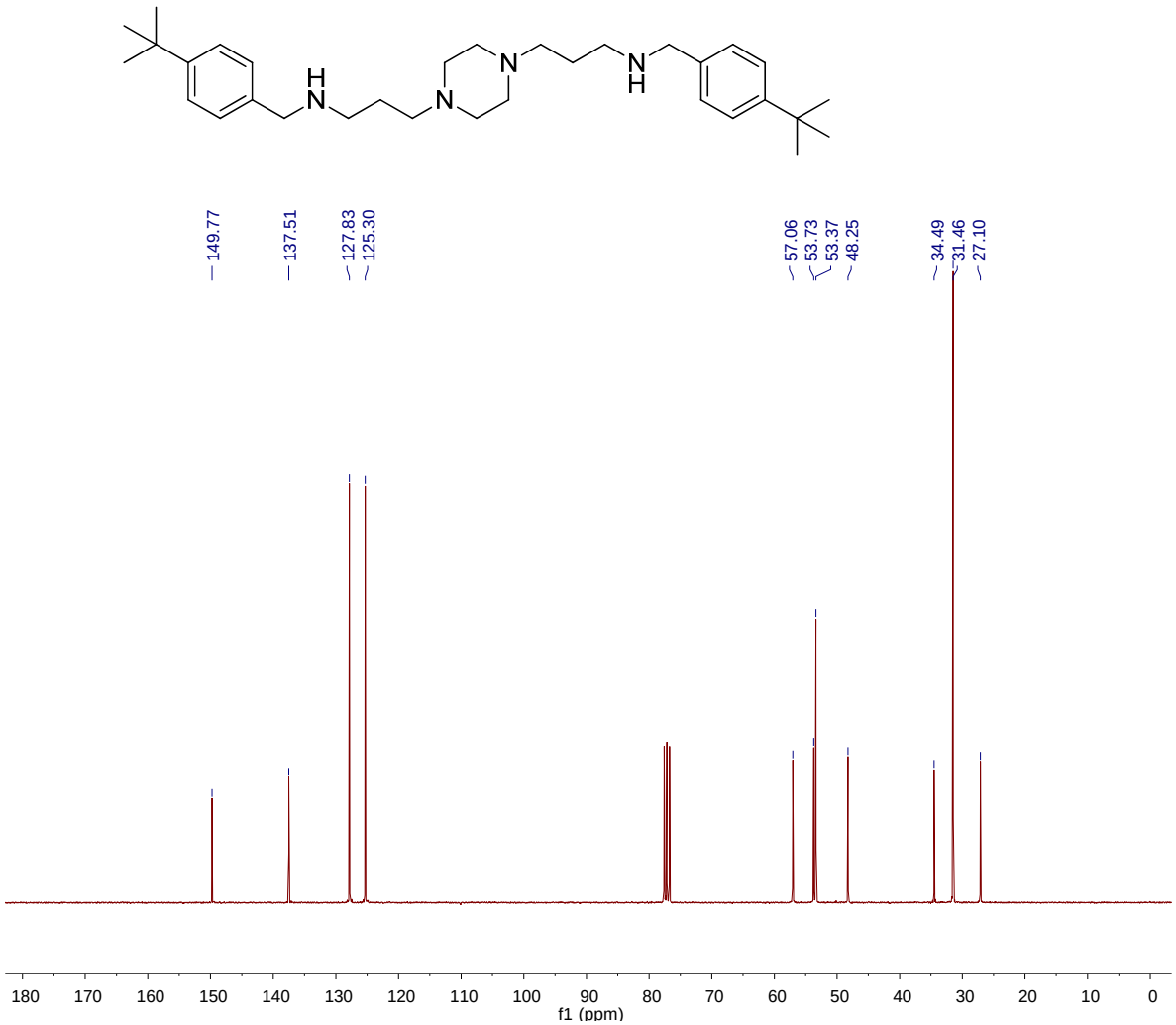


Figure S12: ^{13}C-NMR of guest **3** in $CDCl_3$ at 298 K.

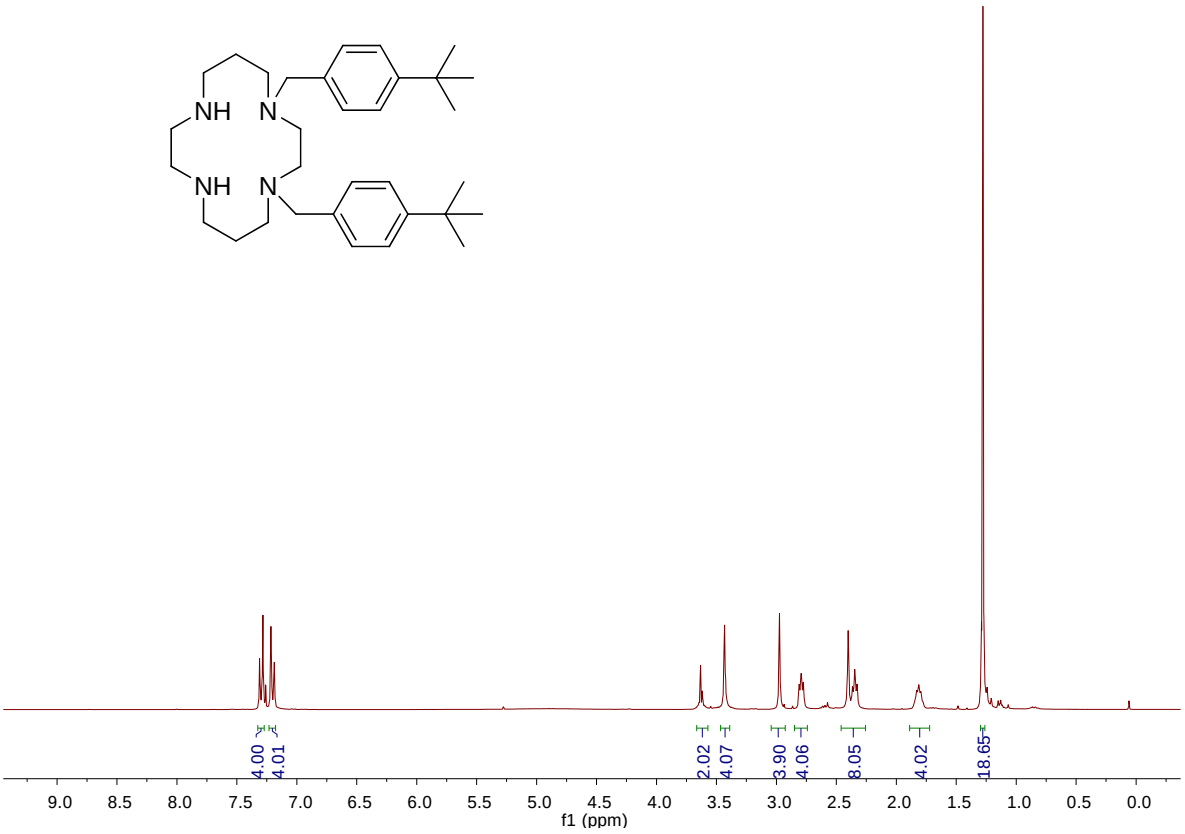


Figure S13: ^{1}H-NMR of guest **4** in $CDCl_3$ at 298 K.

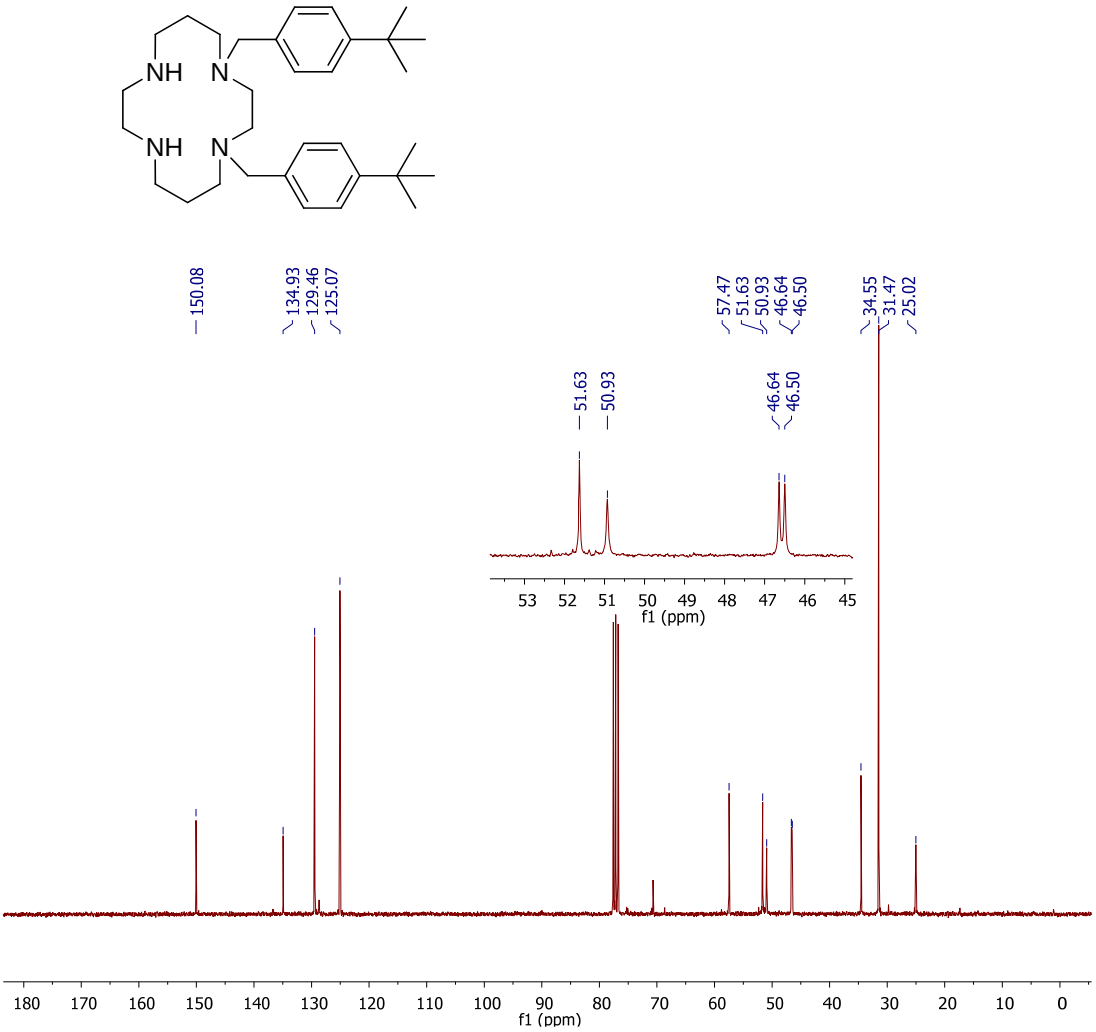


Figure S14: ^{13}C-NMR of guest **4** in $CDCl_3$ at 298 K.