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A Pseudorotaxane Umbrella Thread With Chloride Transmembrane Transport Properties

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Electronic Supporting information

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GENERAL EXPERIMENTAL

Cholic acid, diisopropylethylamine and selenium oxide were purchased from Alfa Aesar. (Benzotriazol-1-yloxy)tris(dimethylamino) phosphonium hexafluorophosphate was purchased from NovaChem and acetyl chloride from EMD. All the other reagents were purchased from Aldrich. They were all used without further purification. L-α-phosphatidylcholine was purchased from Avanti Polar Lipids. Liposome fluorimetric assays were recorded using a Varian Cary Eclipse Fluorescence spectrophotometer. NMR experiments were recorded on Advance 300 or 400 Bruker. Chemical shifts are given in ppm (δ) and measured relative to residual solvent. High-resolution mass spectra (HRMS) were recorded on a TSQ Quantum Ultra (Thermo Scientific) with accurate mass options instrument (Université de Montréal Mass Spectrometry Facility).

SYNTHESIS

*4-Benzyloxybenzaldehyde **1***
To a solution of 4-hydroxybenzaldehyde (2.5 g, 0.0205 mol, 1eq) and potassium carbonate (3.678 g, 0.0266 mol, 1.3 eq) in DMF (10 mL) was added benzyl bromide (2.68 mL, 0.225 mol, 1.1 eq). The brown mixture was stirred at 60°C for 3h then cooled to room temperature and quenched with water (40 mL). After extraction with diethyl ether (3 x 30 mL), the organic layer was washed with brine (15 mL), dried over $MgSO_4$ and evaporated in vacuo. The yellow solid was washed with hexane and filtered to give a light yellow solid (3,555 g, 82%). ^{1}H NMR ($CDCl_3$; 300 MHz) δ(ppm) 9,89 (s, 1H); 7,84 (d, ^{3}J=8.8Hz, 2H); 7.32-7.47 (m, 5H); 7.08 (d, ^{3}J=8.8Hz, 2H); 5.15 (s, 2H); ^{13}C NMR ($CDCl_3$; 75Hz) δ(ppm) 191.66; 164.57; 136.77; 132.85; 130.95; 129.59; 129.19; 128.34; 115.99; 71.11; HR-MS ESI $[M+H]^+_{calc}$=213.09101, $[M+H]^+_{found}$= 213.09094; $[M+Na]^+_{calc}$= 235.07295, $[M+Na]^+_{found}$= 235.07263.

*Ethyl 4-aminobenzoate **2***
To a suspension of 4-aminomethylbenzoic acid (1 g, 6.62 mmol, 1eq) in EtOH (50 mL) was added acetyl chloride (2.8 mL, 39.38 mmol, 6 eq) dropwise. The white suspension was heated to reflux for 24h. The solution was cooled to room temperature. The white crystals formed were filtered, washed with hexane then dissolved in a saturated solution of K_2CO_3 (50 mL). After extraction with AcOEt (3 x 40 mL), the organic layer was dried over $MgSO_4$ and evaporated in vacuo affording a light yellow oil (0.6845 g, 58%). ^{1}H NMR ($CDCl_3$; 300 MHz) δ(ppm) 8.01 (d, ^{3}J=8.4 Hz, 2H); 7.38 (d, ^{3}J=8.1Hz, 2H); 4.37 (q, ^{3}J=7.2 Hz, 2H); 3.93 (s, 2H); 1.56 (s, 2H); 1.39 (t, ^{3}J= 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$; 75Hz) δ(ppm) 166.03; 148.09; 129.34; 128.55; 126.50; 60.41; 45.71; 13.93; HR-MS ESI $[M+H]^+_{calc}$= 180.10191, $[M+H]^+_{found}$= 180. 10196.

*Compound **3***
In a dry flask under N_2 were dissolved compound **2** (4.957 g, 0.0234 mol, 1 eq) and compound **1** (5.3549 g, 0.0234 mol, 1 eq) in anhydrous toluene (120 mL). Sodium sulfate (9.953 g, 0.0701 mol, 3 eq) was added and the yellow suspension heated to reflux under N_2 for 23h. The white solid was filtered off and the yellow solution was evaporated in vacuo. The yellow solid was recrystallized from hot EtOH giving a light yellow solid (6.2582 g, 72%). ^{1}H NMR ($CDCl_3$; 400 MHz) δ(ppm) 8.34 (s, 1H); 8.01 (d, ^{3}J=8.4 Hz, 2H); 7.73 (d, ^{3}J=8.8Hz, 2H); 7.45-7.30 (m, 7H); 7.02 (d, ^{3}J=8.4 Hz, 2H); 5.12 (s, 2H); 4.83(s, 2H); 4.37 (q, ^{3}J=7.2 Hz, 2H); 1.39 (t, ^{3}J=7.2 Hz, 3H) ; ^{13}C NMR ($CDCl_3$, 75MHz) δ(ppm) 162.12; 136.61; 130.16; 129.90; 129.28; 128.79; 128.48; 128.26; 127.86; 127.62; 115.11; 82.33; 75.75; 70.20; 61.20; 28.17; 14.48; 10.27; HR-MS ESI $[M+H]^+_{calc}$= 374.17507, $[M+H]^+_{found}$= 374.17485.

*Compound **4***
To a yellow solution of compound **3** (0.1304 g, 0.3492 mmol, 1 eq) in THF/MeOH 1/1 (5 mL) was added a first portion of sodium borohydride (0.0223 g, 0.5762 mmol, 1.65 eq) and the mixture was stirred at room temperature for 2h. The second portion of sodium borohydride (0.0223 g,

0.5762 mmol, 1.65 eq) was then added and the mixture was stirred at room temperature for 21h. The solution was concentrated in vacuo then quenched with a solution of 10% HCl (5 mL). The white precipitate was filtered off and washed with water. It was dissolved in a saturated solution of K_2CO_3 (15mL) and CH_2Cl_2 (15 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL). The organic was dried over $MgSO_4$ and evaporated in vacuo to give a colorless solid (0.1016 g, 81%) without further purification. ^{1}H NMR ($CDCl_3$; 400 MHz) δ(ppm) 8.00 (d, ^{3}J =8 Hz, 2H); 7.44-7.30 (m, 7H); 7.25 (d, ^{3}J= 8 Hz, 2H); 6.95 (d, ^{3}J=12 Hz, 2H); 5.06 (s, 2H); 4.37 (q, ^{3}J = 8 Hz, 2H); 3.85 (s, 2H); 3.74 (s, 2H) 1.39 (t, ^{3}J= 8 Hz, 3H) ; ^{13}C NMR ($CDCl_3$, 75MHz) δ(ppm) 167.16; 166.68; 158.13; 145.48; 137.16; 132.16; 129.87; 129.58; 128.70; 128.21; 128.07; 127.09; 114.95; 70.16; 61.01; 52.62; 52.18; 14.47; HR-MS ESI $[M+H]^+_{calc}$= 376.19072, $[M+H]^+_{found}$= 376.19016; $[M+Na]^+_{calc}$= 398.17266, $[M+Na]^+_{found}$= 398.17288.

*Thread **I***
To a solution of compound **4** (14.1 mg, 0.0376 mmol, 1 eq) in MeOH (2 mL) was added a solution of 10% HCl (1 mL). The white mixture was stirred at room temperature for 1h then evaporated under vacuum. The white residue was washed with H_2O and Et_2O and filtered off. The white solid was dissolved in MeOH (2 mL) and a solution of ammonium hexafluorophosphate (64.4 mg, 0.3755 mmol, 10 eq) in H_2O (1 mL) was added. The white mixture was stirred at room temperature for 24h, then evaporated in vacuo. The white solid was washed with H_2O and filtered off affording a light yellow solid (10.7mg, 55%). ^{1}H NMR (MeOD, 300 MHz) δ(ppm) 8.09 (d, ^{3}J = 8,2 Hz, 2H); 7.58 (d, ^{3}J = 8,2 Hz, 2H); 7.44-7.27 (m, 7H); 7.08 (d, ^{3}J = 8,6 Hz, 2H); 5.12 (s, 2H); 4.39 (q, ^{3}J = 7,1Hz, 2H); 4.29 (s, 2H); 4.20 (s,2H); 1.39 (t, ^{3}J = 7,1 Hz, 3H) ; ^{13}C NMR (MeOD, 75 MHz) δ(ppm) 166.04; 159.52; 136.59; 135.88; 130.92; 130.74; 129.50; 129.39; 127.81; 127.26; 126.84; 122.63; 114.93; 69.29; 51.11; 50.28; 49.26; 4.88; HR-MS ESI $[M+H]^+_{calc}$= 376.19072, $[M+H]^+_{found}$=376.19048.

*N-Succinimidyl ester of cholic acid **5***
In a dry flask under N_2, to a solution of cholic acid (5 g, 12.24 mmol, 1 eq) in dry THF (90 mL), was added *N*-hydroxysuccinimide (1.4507 g, 12.6 mmol, 1.03 eq) and the solution stirred for 5 min. A solution of dicyclohexylcarbodiimide (2.601 g, 12.6 mmol, 1.03 eq) was added dropwise and the mixture was stirred at room temperature under N_2 for 48h. The white mixture was filtered off and washed with AcOEt. The solution was evaporated under vacuum. A white solid (6,0394g, 98%) was obtained from purification by flash chromatography (SiO_2, CH_2Cl_2/MeOH: 9/1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ(ppm) 3.98 (m, 1H); 3.85 (m, 1H); 3.45 (m, 1H); 2.83 (br s, 4H); 2.77-2.50 (m, 2H); 2.29-2.15 (m, 2H); 2.00-1.10 (m, 22 H); 1.04 (d, ^{3}J = 6 Hz, 3H); 0.89 (s, 3H); 0.70 (s, 3H) ; ^{13}C NMR ($CDCl_3$, 75 MHz) δ(ppm) 169.45; 169.30; 73.09; 72.04; 68.49; 46.83; 46.54; 41.67; 39.55; 39.41; 35.35; 35.15; 34.84; 34.71; 30.63; 30.36; 28.25; 28.10; 27.53; HR-MS ESI $[M+NH_4]^+_{calc}$= 523.33778, $[M+NH_4]^+_{found}$= 523.33856; $[M+Na]^+_{calc}$= 528.29317, $[M+Na]^+_{found}$= 528.29324.

*Umbrella **II***
To a solution of *N*-succinimidyl ester of cholic acid **5** (1,255 g, 2,482 mmol, 1,82e q) in DMF (2,5 mL) heated to 50°C was added *N*-(3-aminopropyl)-1,3-propane diamine (0,162 mL, 0,0376 mmol, 1 eq). The mixture was heated to 70°C for 6h then cooled to room temperature and evaporated in vacuo. A white solid (0,9439 g, 76%) was obtained from purification by flash chromatography (SiO_2, gradient CH_2Cl_2/MeOH: 8/2 to 7/3). ^{1}H NMR (MeOD, 400 MHz) δ(ppm) 3.95 (m, 2H); 3.80 (m, 2H); 3.38 (m, 2H); 3.27 (t, ^{3}J= 6.4 Hz, 2H); 2.83 (t, ^{3}J=7.2 Hz, 2H); 2.36-1.07 (m, 54H); 1.03 (d, ^{3}J=6 Hz, 6H); 0.98 (m, 2H); 0.92 (s, 6H); 0.71 (s, 6H) ; ^{13}C NMR (MeOD, 75 MHz) δ(ppm) 74.03; 72.87; 69.04; 47.98; 47.49; 43.19; 41.00; 40.46; 37.34; 36.96; 36.48; 35.90; 33.99; 33.32; 31.19; 29.61; 28.74; 28.58; 27.89; 26.21; 24.24; 23.17; 19.67; 17.74; 13.01; HR-MS ESI $[M+H]^+_{calc}$= 912.70354, $[M+H]^+_{found}$= 912.70514; $[M+Na]^+_{calc}$= 934.68549, $[M+Na]^+_{found}$= 934.68684.

*Boc-protected thread **6***
To a solution of compound **4** (3.59 g, 9.5613 mmol, 1 eq) in CH_2Cl_2 (100 mL) cooled to 0°C was

added dropwise a solution of di-*tert*-butyl dicarbonate (2.1911 g, 10.039 mmol, 1.05 eq) in CH_2Cl_2 (50 mL). The mixture was stirred at room temperature for 18h. The colorless solution was concentrated under vacuum to give a white oil (5.3853 g, quantitative) directly used in the next step. To a solution of the ester previously obtained (1.3186 g, 2.7726 mmol, 1 eq) in MeOH (50 mL) was added potassium hydroxide (1.7285 g, 27.726 mmol, 10 eq). The white mixture was heated to reflux for 3h then cooled to room temperature and acidified with a solution of HCl 10% (20 mL). The aqueous layer was extracted with AcOEt (3 x 80 mL) and the organic layer was washed with water (100 mL) and dried over $MgSO_4$ and evaporated in vacuo. A colorless oil (1,0523 g, 85%) was obtained from purification by flash chromatography (SiO_2, AcOEt/Hex: 4/6). ^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm) 8.08 (d, ^{3}J=8.1 Hz, 2H); 7.46-7.25 (m, 7H); 7.15 (br s, 2H); 6.94 (d, ^{3}J=8.4 Hz, 2H); 5.07 (s, 2H); 4.55-4.25 (m, 4H); 1.50 (br s, 9H). ^{13}C NMR ($CDCl_3$, 75MHz) δ(ppm) 171.86; 158.32; 156.05; 137.05; 130.61; 130.04; 129.60; 129.02; 128.73; 128.37; 128.12; 127.90; 127.60; 127.30; 115.06; 80.58; 70.18; 49.27; 28.56. HR-MS ESI $[M+Na]^+_{calc}$= 470.19379, $[M+Na]^+_{found}$= 470.19441.

Umbrella thread ***III***

To a solution of *Boc*-protected thread **6** (10.2 mg, 0.0228 mmol, 1eq) in dry CH_2Cl_2 (2 mL) were added 3-hydroxy-1,2,3-benzotriazin-4(*3H*)-one (4.2 mg, 0.0251 mmol, 1,1 eq) and *N,N'*-dicyclohexylcarbodiimide (5.2 mg, 0.0251 mmol, 1.1 eq). The slightly white mixture was stirred at room temperature under N_2 for 24h. The white solid was filtered off and washed with CH_2Cl_2 (3 x 5 mL). The colorless solution was evaporated in vacuo. Purification by flash chromatography (SiO_2, AcOEt/Hex: 3/7) afforded a colorless oil (13.5 mg, quant.). ^{1}H NMR ($CDCl_3$, 400 MHz) δ(ppm) 8.42 (dd, ^{3}J = 8 Hz, ^{4}J = 1.6 Hz, 1H); 8.27 (d, ^{3}J= 8 Hz, 1H); 8.21 (d, ^{3}J= 8.4 Hz, 1H); 8.04 (td, ^{3}J= 7.6 Hz, ^{4}J= 1.6 Hz, 1H); 7.87 (t, ^{3}J= 8.4 Hz, 1H); 7.46-7.30 (m, 7H); 7.15 (m, 2H); 6.95 (d, ^{3}J= 8.8 Hz, 2H); 5.07 (s, 2H); 4.54-4.29 (m, 4H); 1.50 (br s, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ(ppm) 167.27; 162.72; 158.68; 158.41; 150.61; 144.56; 135.56; 132.89; 131.63; 131.23; 131.12; 130.15; 129.96; 129.19; 128.73; 128.12; 127.61; 125.98; 124.33; 115.14; 80.66; 70.20; 49.48; 28.56. HR-MS ESI $[M+H]^+_{calc}$= 593.23946, $[M+H]^+_{found}$= 593.23860; $[M+Na]^+_{calc}$= 615.22141, $[M+Na]^+_{found}$= 615.22146.

To a solution of umbrella **II** (54.4 mg, 0.0467 mmol, 2.25 eq) in dry DMF (1 mL) were added triethylamine (0.04 mL, 0.3131 mmol, 15 eq) and a solution of the previous oil (12.3 mg, 0.0208 mmol, 1 eq) in dry DMF (1,5 mL). The yellow solution was stirred at room temperature under N_2 for 18h, then evaporated under vacuum. The *Boc*-protected umbrella thread was obtained as a white solid (24.6 mg, 88%) by flash chromatography (SiO_2, CH_2Cl_2, MeOH: 8/2). ^{1}H NMR (MeOD, 300 MHz) δ(ppm) 7.44-7.09 (m, 11H); 6.96 (d, ^{3}J= 8.8 Hz, 2H); 5.07 (s, 2H); 4.39 (m, 4H); 3.93 (m, 2H); 3.79 (m, 2H); 3.54 (m, 2H); 3.37 (m, 3H); 3.00 (m, 2H); 2.36-1.31 (m, 60H); 1.17-0.94 (m, 10H); 0.91 (s, 6H); 0.69 (br d, ^{3}J= 16 Hz, 6H). ^{13}C NMR (MeOD, 75 MHz) δ(ppm) 176.88; 173.98; 161.73; 159.65; 157.78; 157.58; 155.25; 148.74; 138.66; 136.63; 130.31; 130.26; 129.26; 129.5; 128.86; 128.53; 127.79; 116.07; 81.73; 79.46; 73.98; 72.85; 71.01; 69.00; 58.30; 50.63; 50.46; 48.02; 47.48; 43.16; 42.96; 40.99; 40.44; 36.90; 36.48; 35.88; 34.19; 33.25; 31.17; 29.59; 28.74; 27.85; 24.24; 23.20; 17.79; 13.07; HR-MS ESI $[M+H]^+_{calc}$= 1341.89755, $[M+H]^+_{found}$= 1341.89381; $[M+Na]^+_{calc}$= 1363.8795, $[M+Na]^+_{found}$= 1363.87604. A solution of 4N HCl (0.35 mL, 1.375 mmol, 75 eq) was added dropwise to a solution of the *Boc*-protected umbrella thread (24.6 mg, 0.0183 mmol, 1 eq) in EtOH (2 mL) at 0°C. The mixture was stirred for 20min at 0°C then the ice bath was removed and the solution was stirred at room temperature for 41h. The solution was evaporated under vacuum. The white residue was washed with $CHCl_3$ and filtered off affording the chloride salt of the umbrella thread as a white solid (23.4 mg, quant.). ^{1}H NMR (MeOD, 300MHz) δ(ppm) 7.63 (br s, 2H); 7.53-7.26 (m, 9H); 7.08 (d, ^{3}J= 7.5 Hz, 2H); 5.12 (s, 2H); 4.31 (s, 2H); 4.24 (s, 2H); 3.95 (s, 2H); 3.80 (s, 2H); 3.58 (m, 2H); 3.34 (m, 4H); 3.03 (m, 2H); 2.52-1.11 (m, 54H); 1.03 (m, 8H); 0.91 (s, 6H); 0.71 (s, 6H). ^{13}C NMR (MeOD, 75MHz) δ(ppm) 170.88; 161.16; 142.91; 138.74; 138.31; 134.06; 132.82; 131.70; 131.62; 129.52; 128.95; 128.54; 128.32; 124.38; 116.60; 107.42; 95.35; 81.66; 73.96; 72.88; 71.02; 68.99; 51.87; 51.43; 47.89; 47.46; 43.13; 43.02; 40.96; 40.39; 36.97; 36.44; 35.86; 33.75; 33.40; 31.14; 29.60; 28.71; 27.88; 24.22; 23.17; 17.74; 13.01. HR-MS ESI $[M+H]^+_{calc}$= 1241.84512, $[M+H]^+_{found}$= 1241.84304.

To a solution of the chloride salt previously obtained (28.3 mg, 0.0221 mmol, 1 eq) in MeOH (0.6 mL) was added a solution of ammonium hexafluorophosphate (3.8 mg, 0.2214 mmol, 10 eq) in

H_2O (0.2 mL). The mixture was stirred at room temperature for 24h then evaporated in vacuo. The white solid was washed with H_2O then $CHCl_3$ and filtered off affording a light yellow solid (24,9mg, 81%). ^{1}H NMR ($MeOD_3$, 300 MHz) δ(ppm) 7.57 (d, ^{3}J= 8,2 Hz, 2H); 7.47 (d, ^{3}J= 8 Hz, 2H); 7.42 (m, 4H); 7.36 (t, ^{3}J= 7.6 Hz, 2H); 7,30 (m, 1H); 7.08 (d, ^{3}J= 8,8 Hz, 2H); 5.13 (s, 2H); 4.29 (br s, 2H); 4.22 (s, 2H); 3.95 (br s, 2H); 3.80 (br s, 2H); 3.56 (m, 2H); 3.38 (m, 2H); 3.26 (m, 4H); 2.97 (m, 2H); 2.35-1.07 (m, 54H); 1.08-0.93 (m, 8H); 0.92 (s, 6H); 0.71 (br s, 6H). ^{13}C NMR (MeOD, 75 MHz) δ(ppm) 176.84; 173.33; 165.39; 161.25; 139.04; 138.31; 133.93; 132.70; 131.47; 129.55; 129.00; 128.57; 128.36; 124.36; 116.69; 74.01; 72.85; 71.03; 69.04; 51.91; 51.48; 48.00; 47.46; 43.16; 43.03; 40.98; 40.46; 37.92; 37.66; 36.93; 36.47; 35.89; 34.22; 34.06; 33.37; 31.18; 29.66; 28.71; 27.91; 24.23; 23.18; 17.76; 13.02; HR-MS ESI $[M]^+_{calc}$= 1241.84512, $[M+H]^+_{found}$= 1241.8429; $[M+2H]^{2+}_{calc}$= 621.93011; $[M+2H]^{2+}_{found}$= 621.92933.

1,11-Bis(2-nitrophenoxy)-3,6,9-trioxaundecane ***7***

To a solution of tetra(ethylene glycol) (1.124 g, 5.787 mmol, 1 eq) and *p*-toluenesulfonyl chloride (2.2066 g, 11.574 mmol, 2 eq) in CH_2Cl_2 (20 mL) cooled to 0°C was slowly added potassium hydroxide (2.5976 g, 46.296 mmol, 8 eq). The white mixture was stirred at 0°C for 3h then quenched with water (60 mL) and CH_2Cl_2 was added (30 mL). The organic layer was extracted with CH_2Cl_2 (3 x 30 mL). The organic layer was washed with water (50mL), dried over $MgSO_4$ and evaporated under vacuum affording a colorless liquid (2.5373 g, 87%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm) 7.76 (d, ^{3}J= 8.3 Hz, 4H); 7.31 (d, ^{3}J= 8.3 Hz, 4H); 4.12 (dd, ^{3}J= 4.7 Hz, ^{3}J= 4,9 Hz, 4H); 3.64 (dd, ^{3}J= 4.7 Hz, ^{3}J= 4.9 Hz, 4H); 3.53 (s, 8H); 3.41 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ(ppm) 144.89; 132.92; 129.88; 127.96; 70.70; 70.53; 69.33; 68.66; 21.65. HR-MS ESI $[M+H]^+_{calc}$= 503.1404, $[M+H]^+_{found}$= 503.14097; $[M+NH_4]^+_{calc}$= 520.166951, $[M+NH_4]^+_{found}$= 520.16744; $[M+Na]^+_{calc}$= 525.12235 , $[M+Na]^+_{found}$= 525.1225.

To a solution of 2-nitrophenol (0.6608 g, 4.6554 mmol, 2 eq) and potassium carbonate (0.6434 g, 4.6554 mmol, 2 eq) in DMF (15mL) was added a solution of tetra(ethylene glycol) di-*p*-tosylate previously obtained (1.1699 g, 2.3277 mmol, 1eq) in DMF (10 mL). The orange mixture was heated to reflux for 21h then cooled to room temperature and concentrated in vacuo. The dark orange residue was dissolved in water (50mL) and extracted with AcOEt (4 x 25mL). The organic layer was washed with water (3 x 30mL), dried over $MgSO_4$ and evaporated under vacuum. A viscous yellow oil (0.6417 g, 63%) was obtained from purification by flash chromatography (SiO_2, Hex/AcOEt : 3/7). ^{1}H NMR ($CDCl_3$, 400 MHz) δ(ppm) 7.80 (dd, ^{3}J= 8.0 Hz, ^{4}J= 1.6 Hz, 2H); 7.50 (td, ^{3}J= 7.9 Hz, ^{4}J= 1.7 Hz, 2H); 7.08 (dd, ^{3}J= 7.9 Hz, ^{4}J= 0.7 Hz, 2H); 7.01 (td, ^{3}J= 7.8 Hz, ^{4}J= 1.1 Hz, 2H); 4.25 (t, ^{3}J= 4.6 Hz, 4H); 3.89 (t, ^{3}J= 4.9 Hz, 4H); 3.77-3.57 (m, 8H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ(ppm) 152.39; 140.20; 134.18; 125.64; 120.65; 115.10; 71.19; 70.75; 69.68; 69.37. HR-MS ESI $[M+NH_4]^+_{calc}$= 454.18201, $[M+NH_4]^+_{found}$= 454.18246; $[M+Na]^+_{calc}$= 459.1374, $[M+Na]^+_{found}$= 459.13839.

1,11-Bis(2-aminophenoxy)-3,6,9-trioxaundecane ***8***

To a solution of 1,11-bis(2-nitrophenoxy)-3,6,9-trioxaundecane **7** (0.6293 g, 1.442 mmol, 1 eq) in EtOH (30 mL) was added palladium on activated charcoal 10% (0.1535 g, 0.1442 mmol, 0.1 eq) and the solution was heated to 50°C. A solution of hydrazine hydrate 50-60% (1.98 mL, 20.188 mmol, 14 eq) in EtOH (5 mL) was added dropwise at 50°C then the mixture was heated to reflux for 3h. The black mixture was filtered over celite and the yellow filtrate was evaporated under vacuum giving a white viscous oil (0.4385 g, 81%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm) 6.87-6.65 (m, 8H); 4.33 (ls, 2H); 4.17-4.07 (m, 4H); 3.89- 3.79 (m, 4H); 3.77-3.62 (m, 10H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ(ppm) 146.75; 136.23; 122.00; 119.07; 116.03; 113.33; 70.83; 70.72; 69.86; 68.63. HR-MS ESI $[M+H]^+_{calc}$= 377.2071, $[M+H]^+_{found}$= 377.20767; $[M+Na]^+_{calc}$= 399.18904, $[M+Na]^+_{found}$= 399.1902.

2,6-Pyridinedicarboxaldehyde ***9***

To a solution of 2,6-pyridine dimethanol (1 g, 7.1865 mmol, 1 eq) in dioxane (18 mL) was added selenium oxide (0.8772 g, 7.9051 mmol, 1.1 eq). The mixture was heated to reflux for 3h then cooled to room temperature and filtered off on celite. The yellow solution was evaporated in vacuo. The white solid was dissolved in CH_2Cl_2 and passed on a silica pad. The solution was evaporated in vacuo and the white solid was recrystallized from hot Et_2O affording white

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crystals (0.2806 g, 29%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ(ppm) 10.18 (s, 2H); 8.19 (d, ^{3}J= 7.6 Hz, 2H); 8.09 (d, ^{3}J= 7.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ(ppm) 192.48; 153.14; 138.52; 125.47 ; HR-MS ESI $[M+H]^+_{calc}$= 136.0393, $[M+H]^+_{found}$= 136.03894; $[M+Na]^+_{calc}$= 158.02125, $[M+Na]^+_{found}$= 158.02059.

*Macrocycle **IV***

A solution of 5-(3,5-di-*tert*-butylbenzylamino)pentanoic acid hexafluorophosphate (0.0776 g, 0.1668 mmol, 1 eq), 1,11-bis(2-aminophenoxy)-3,6,9-trioxaundecane ***8*** (0.0628 g, 0.1668 mmol, 1eq) and 2,6-pyridinedicarboxaldehyde ***9*** (0.0225 g, 0.1668 mmol, 1eq) in MeCN (1 mL) was stirred at room temperature for 5h. Borane tetrahydrofuran 1M (0.83 mL, 0.834mmol, 5 eq) was added and the mixture was stirred for 18h at room temperature and was filtered off. The yellow solution was evaporated in vacuo. A white solid (0.0644 g, 81%) was obtained from purification by flash chromatography (SiO_2, CH_2Cl_2/MeOH : 9/1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm) 7.59 (t,^{3}J= 7.7 Hz, 1H); 7.21 (d, ^{3}J= 7.7 Hz, 2H); 6.94-6.85 (m, 4H); 6.67-6.61 (m, 4H); 4.51 (s, 4H); 4.20-4.15 (m, 4H); 3.89-3.81 (m, 4H); 3.77-3.68 (m, 4H); 3.60-3.52 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ(ppm) 158.41; 146.29; 140.12; 137.27; 123.03; 119.94; 116.59; 114.53; 110.67; 70.98; 70.84; 70.46; 70.14; 49.22. HR-MS ESI $[M+2H]^{2+}_{calc}$= 240.62829, $[M+2H]^{2+}_{found}$= 240.62848; $[M+H]^+_{calc}$= 480.2493, $[M+H]^+_{found}$= 480.24905.

Umbrella-rotaxane synthesis

A solution of umbrella axle hexafluorophosphate (24.8mg, 0.0178mmol, 1eq), 2,6-pyridinedicarboxaldehyde (2.4mg, 0.0178mmol, 1eq) and tetraethyleneglycol bis(2-aminophenyl)ether (6.7mg, 0.0178mmol, 1eq) in acetonitrile (0.6mL) was stirred at room temperature for 5h. Borane tetrahydrofuran 1M (0.09 mL, 0.089mmol, 5 eq) was added and the mixture was stirred for 24h at room temperature. The yellow solution was evaporated under vacuum. The yellow solid was purified by flash chromatography (SiO_2, $CHCl_3$/MeOH: 85/15) to afford a white solid (7mg, 21%). ^{1}H NMR (MeOD, 400 MHz) δ(ppm) 7.80 (t, ^{3}J=7.6Hz, 1H); 7.47 (s, 1H); 7.37 (d, ^{3}J=7.6Hz, 2H), 7.28 (m, 2H), 6.97 (m, 4H); 6.79-6.64 (m, 6H); 6.42 (d, ^{3}J=8Hz, 2H), 4.59 (m, 4H); 4.27 (m, 2H); 4.16-3.63 (m, 20H); 3.51-3.35 (m, 8H); 3.28-3.10 (m, 4H); 2.38-1.31 (m, 50H); 1.09 (s, 18H); 1.07-0.93 (m, 9H); 0.90 (m, 6H); 0.68 (m, 6H). HR-MS ESI $[M+2H]^{2+}_{calc}$= 864.59307, $[M+2H]^{2+}_{found}$= 864.59207; $[M+H]^+_{calc}$= 1728.17830, $[M+H]^+_{found}$= 1728.17622.

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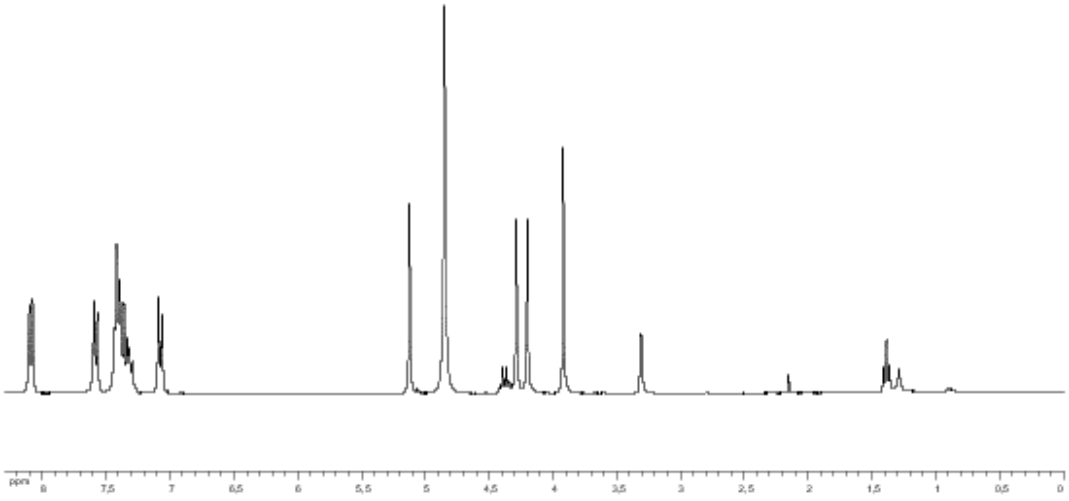
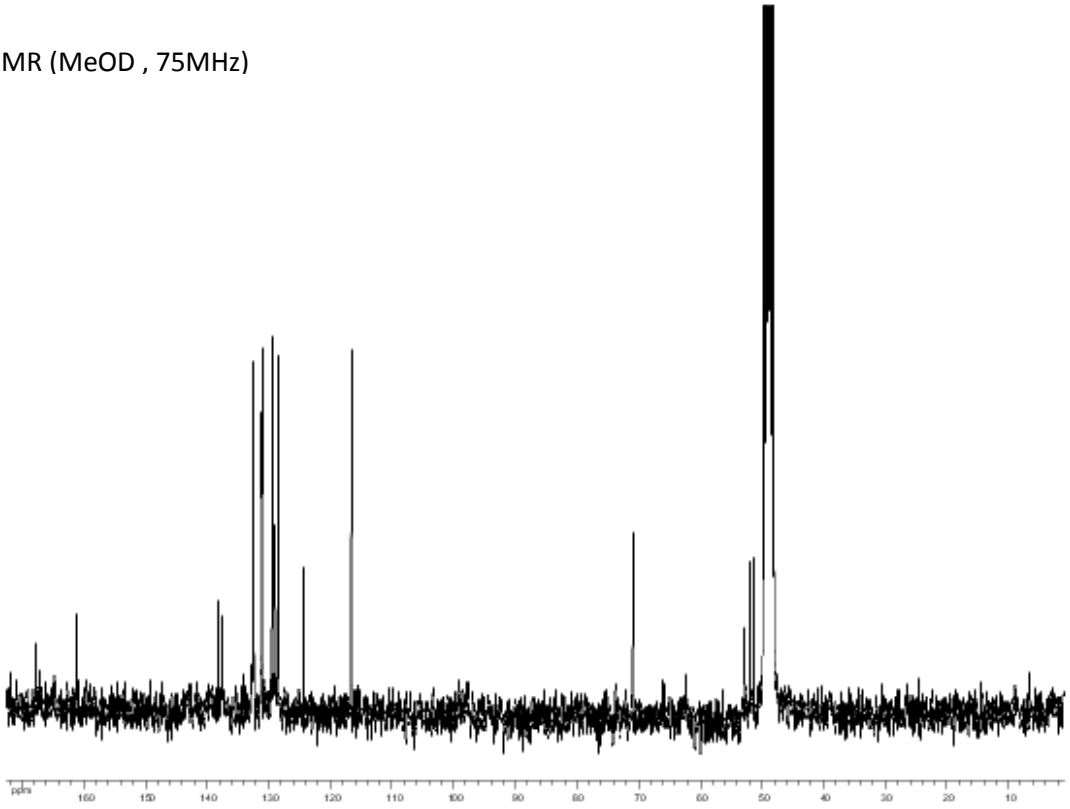
NMR CHARACTERIZATION OF I-IV

^{1}H NMR (MeOD , 300MHz)



^{13}C NMR (MeOD , 75MHz)



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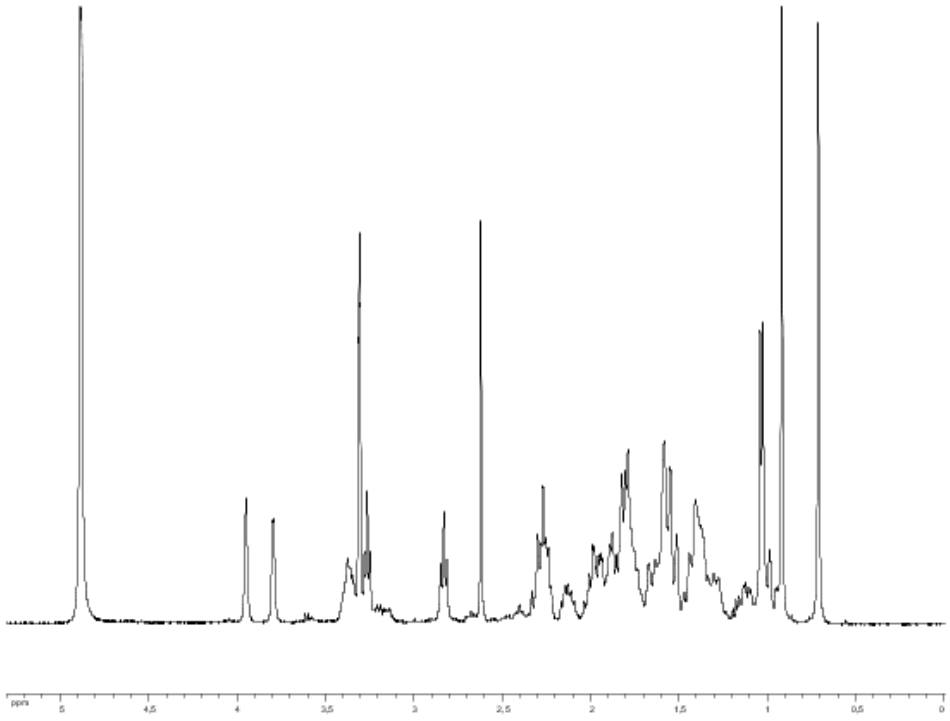
^{1}H NMR (MeOD , 400MHz)



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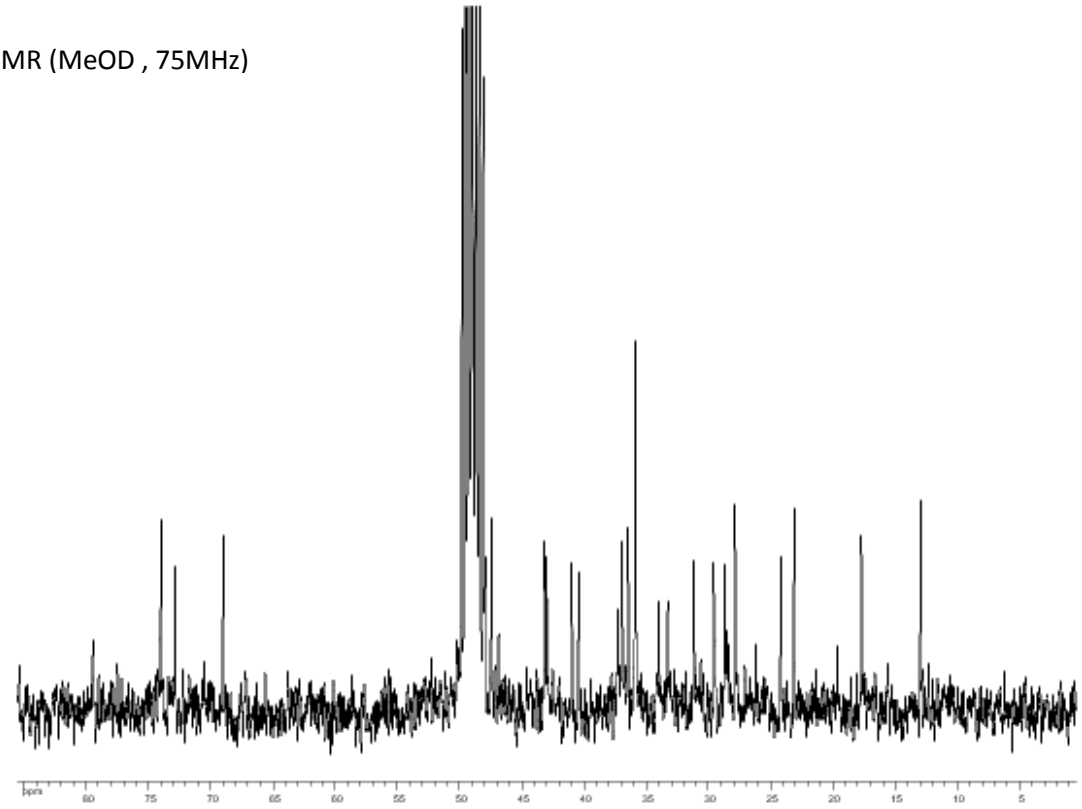
^{13}C NMR (MeOD , 75MHz)



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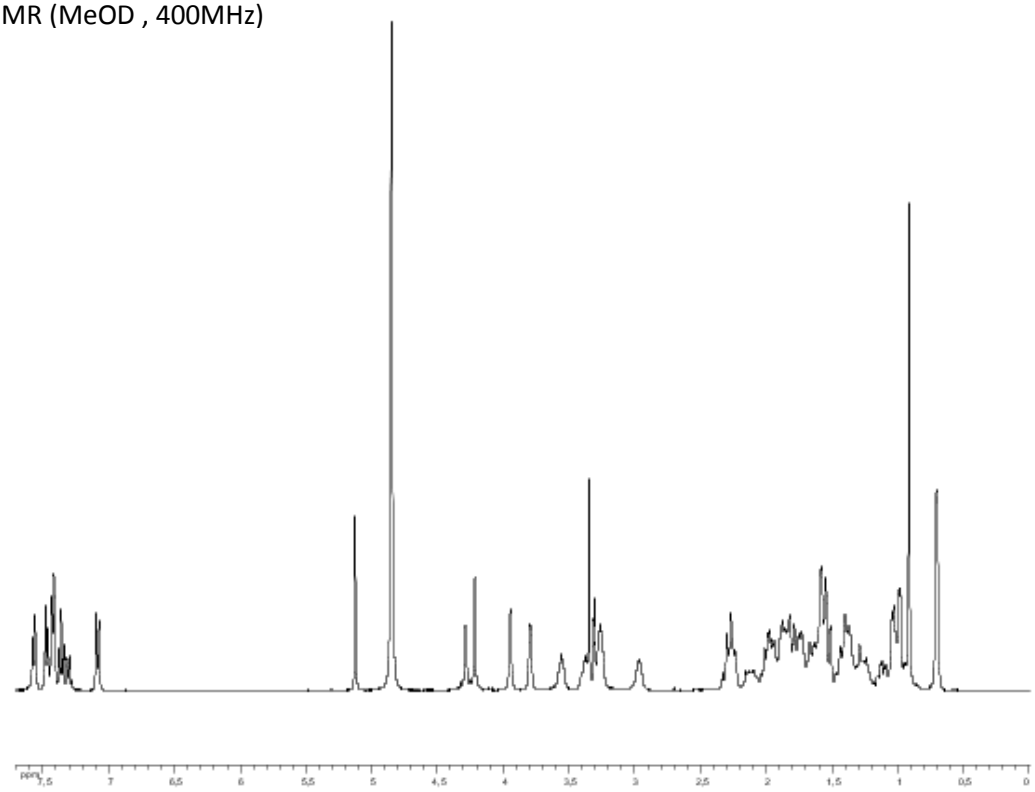
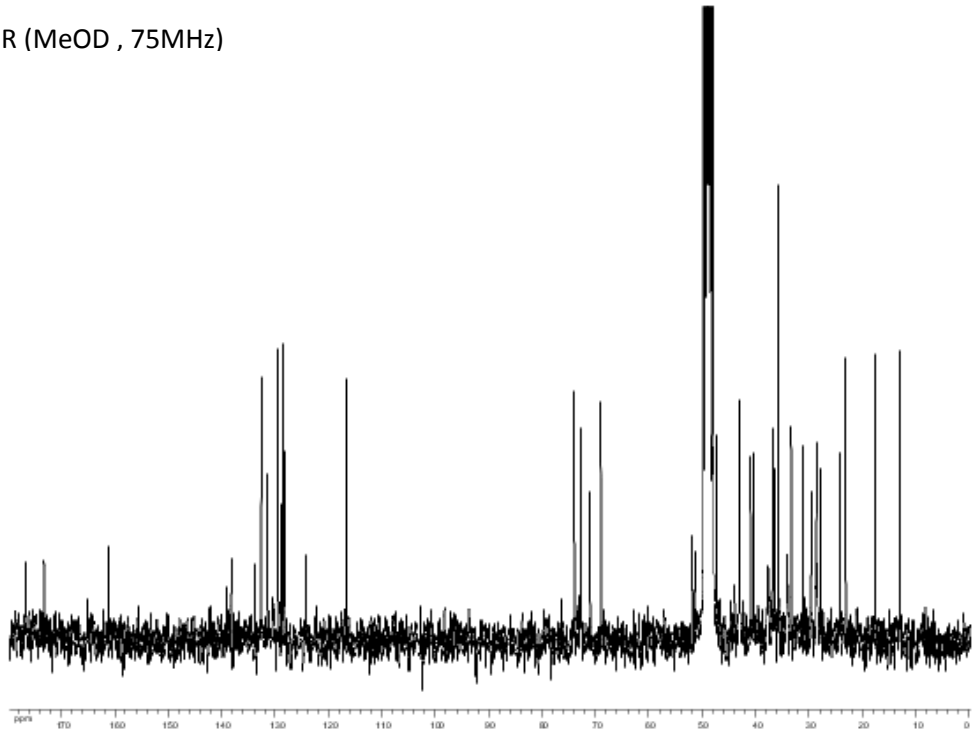
^{1}H NMR (MeOD , 400MHz)



^{13}C NMR (MeOD , 75MHz)



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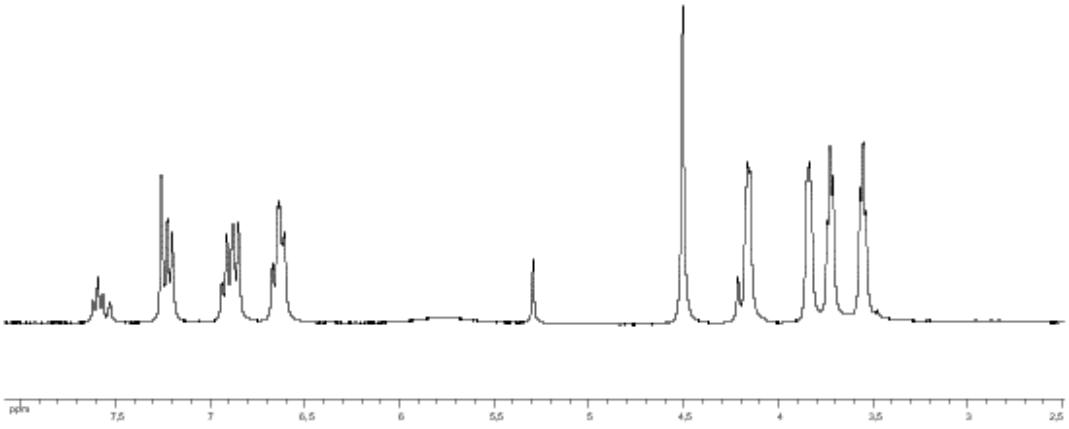
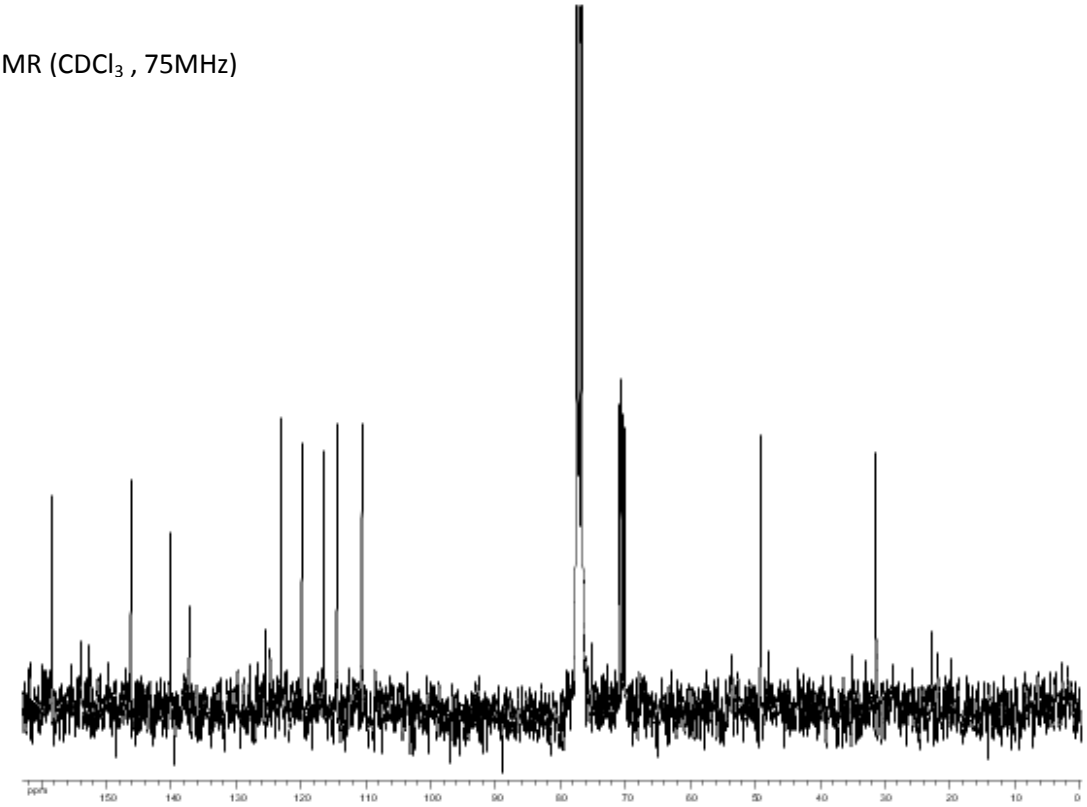
^{1}H NMR ($CDCl_3$, 300MHz)



^{13}C NMR ($CDCl_3$, 75MHz)



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TRANSPORT STUDIES

Preparation of liposomes for lucigenin-based assays

A stock solution of egg-yolk phosphatidylcholine (EYPC) in $CHCl_3$ (200 mg in 2mL) was evaporated under reduced pressure over a water bath at r.t. to produce a thin film that was dried in vacuo for 2 h. The lipid film was hydrated with 1 mL of 10 mM sodium phosphate containing 100 mM NaCl and 2 mM lucigenin. Freeze/thaw cycles were repeated at least 30 times until no solid particles were visible. The solution was frozen at -78°C then warmed to 35°C. The mixture was placed on a vortex 6 to 8 times for 1 min to facilitate hydration. The yellow solution was extruded with an Avanti High Pressure Mini-Extruder through a 100 nm polycarbonate membrane at least 20 times until the solution became transparent. A Sephadex G-25 column (18 cm x 1 cm) was used to remove the extravesicular lucigenin. Each stock solution of liposomes was stored under 4°C and used during the two following days.

Lucigenin-based ion transport assays

A 20 µL aliquot of the stock solution of EYPC liposomes was added to a cuvette containing 2 mL of a solution of 100mM $NaNO_3$ and 10 mM phosphate buffer (pH=6.4) to obtain a 0.25-0.3 mM solution of phospholipid. The fluorescence of intravesicular dye was monitored by excitation at 369 nm and the emission was recorded at 503 nm. A 500 µL aliquot of a 0,1mM solution of **I**, **II**, **III** and **IV** in MeOH was injected after 50s. At the end of the experiment (typically after 500 s), 10% aqueous Triton X-100 was injected to lyse the liposomes. The temperature was set to 35 °C. Experiments were repeated in triplicate and all traces reported are the average of the three trials.

Preparation of liposomes for HPTS-based assays

A stock solution of egg-yolk phosphatidylcholine (EYPC) in $CHCl_3$ (200 mg in 2mL) was evaporated under reduced pressure over a water bath at r.t. to produce a thin film that was dried in vacuo for 2 h. The lipid film was hydrated with 1 mL of 10 mM sodium phosphate containing 100 mM NaCl and 0.1 mM HPTS. Freeze/thaw cycles were repeated at least 30 times until no solid particles were visible. The solution was frozen at -78°C then warmed to 35°C. The mixture was placed on a vortex 6 to 8 times for 1 min to facilitate hydration. The white solution was extruded with an Avanti High Pressure Mini-Extruder through a 100 nm polycarbonate membrane at least 20 times until the solution became transparent. A Sephadex G-25 column (18 cm x 1 cm) was used to remove the extravesicular HPTS. Each stock solution of liposomes was stored under 4°C and used during the two following days.

HPTS-based ion transport assays

A 20 µL aliquot of the stock solution of EYPC liposomes was added to a cuvette containing 2 mL of a solution of 100mM $NaNO_3$ and 10 mM phosphate buffer (pH=6.4) to obtain a 0.25-0.3 mM solution of phospholipid. The fluorescence of intravesicular HPTS was monitored by excitation at both 403 nm and 460 and the emission was recorded at 510 nm. A 500 µL aliquot of a 0.1mM

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solution of **I**, **II**, **III** and **IV** in MeOH was injected after 50s. At the end of the experiment (typically after 500 s), 10% aqueous Triton X-100 was injected to lyse the liposomes. The temperature was set to 35 °C. Experiments were repeated in triplicate and all traces reported are the average of the three trials.

NMR STUDY: FORMATION OF THE [2]-PSEUDOROTAXANE

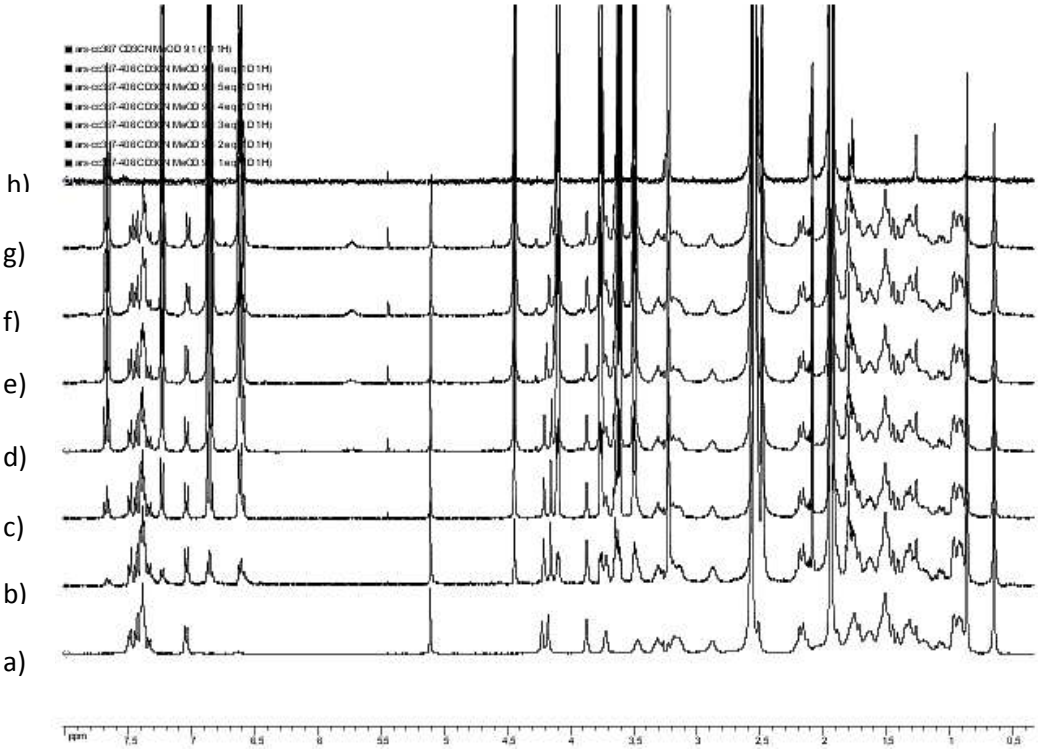

Figure S1. 400 MHz NMR spectra of **III** at 1mM and **IV** at a) 1/0, b) 1/1, c) 1/2, d) 1/3, e) 1/4, f) 1/5, g) 1/6 and h) 0/1 ratios in 9/1 CD_3CN/CD_3OD at room temperature.

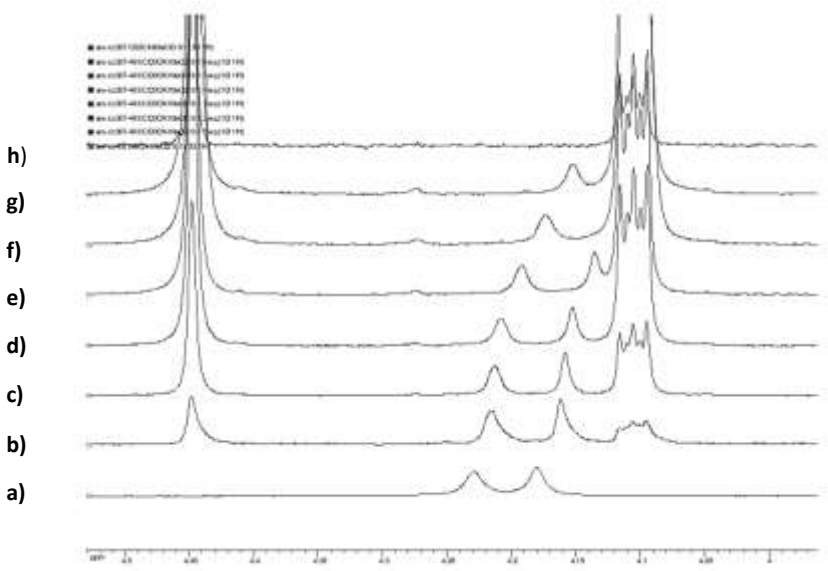

Figure S2. Zoom-in to the two CH_2 next to the ammonium site on III. The two singlets shift downfield, showing complexation on the ammonium site as IV is added.

MOLECULAR MODELING

Calculations were performed on a Windows® XP workstation with HyperChem 7.5 software. The initial configuration of **III** was obtained from PM6/SCF-MO semi-empirical calculations. A model of the EYPC bilayer was constructed using 200 molecules of phosphatidylcholine, with different conformations and 15 Å layers of water molecules on each side, after a 500 ps molecular dynamics (MD) simulation at 1000 K, as previously described.[1] To investigate the conformational flexibility of **III**, we performed a 200 ps MD simulation with periodic boundary conditions at 300 K. The cut-off for non-bonded interactions was taken to be 12 Å throughout all simulations. At the beginning, we carried out high temperature annealed MD simulations starting at 1000 K (2 ps) annealing to 0 K (10 ps). Heating to 1000 K is necessary to enable the molecules to overcome energy barriers between different conformations and to prevent the system from getting stuck in a particular region of the conformational space. Simulations at lower temperatures yielded

[1] Heller, M Schaefer, & K Schulte, J. Phys. Chem. **1993**, 97, 8343

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very similar conformations. The simulations in aqueous solution were relaxed using the steepest descent method until a gradient difference of 0.01 kcal/mol was reached. After energy minimization of the system at 0 K, the MD simulation was initialized using a time step of 1 fs for 200 ps. The temperature was kept constant at 300 K yielding a canonical ensemble (NVT).

FLUORESCENCE STUDY

A 10 µL aliquot of the stock solution of 0.2 mM lucigenin was added to a cuvette containing 1 mL 10 mM phosphate buffer (pH=6.4) and 10 mM NaCl. An additional 250 µL aliquot of additive (MeOH or different aliquots of a solution 0.1 mM of **IV** in MeOH) was added and the fluorescence of lucigenin was scanned. The only observed effect on the lucigenin's fluorescence is due to dilution.