Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Transport of Macrocyclic Compounds Across Phopholipid Bilayers by Umbrella-Rotaxanes

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Electronic Supporting Information

Experimental Section

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. All the ACS or HPLC grade solvents were purchased from Fisher Scientific, Anachemia or EMD. Anhydrous solvents were dried and deoxygenated by Glasscontour solvents system. Synthesis of compounds **1**, **5** and **7** were previously described.[12] L-α-phosphatidylcholine was purchased from Avanti Polar Lipids. The extrusion of liposomes was performed with a Avestin LiposoFast-Basic extruder. Liposome fluorimetric assays were recorded using a Varian Cary Eclipse Fluorescence spectrophotometer. NMR experiments were recorded on Advance 300 or 400 Bruker spectrometers. 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).

3,5-Di-tert-butylbenzaldehyde ***2*** To a solution of *N*-bromosuccinimide (8.9707 g, 50.4 mmol, 1.03 eq) in CCl_4 (85 mL) were added 3,5-di-*tert*-butyltoluene (12.2 mL, 48.93 mmol, 1 eq) and benzoyl peroxide 70% (1.1901 g, 4.893 mmol, 0.1 eq). The media was heated to 80°C for 1 h then filtered off. The filtrate was concentrated under vacuum and dissolved in MeOH/H_2O 1/1 (80 mL). Hexamethylenetetramine (28.8099 g, 0.2055 mol, 4.2 eq) was added and the solution was heated to reflux for 2 h followed by addition of HCl 37% (20 mL). The mixture was reheated to reflux for 2 h then cooled to room temperature and extracted with CH_2Cl_2 (3x80 mL). The organic layer was dried over $MgSO_4$ and evaporated under vaccum. After purification by flash chromatography (SiO_2, AcOEt/Hex: 2/98), a white solid (4.2478 g, 40%) was isolated. ^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm) 10.10 (s, 1H); 7.72 (m, 3H); 1.37 (s, 18H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ(ppm) 193.3; 151.9; 136.3; 129.0; 124.3; 35.1; 31.4; HR-MS ESI $[M+H]^+_{calc}$= 219.1743, $[M+H]^+_{found}$= 219. 1742; $[M+Na]^+_{calc}$= 241.1563, $[M+Na]^+_{found}$= 241.1563.

Compound ***3*** In a dry flask under N_2 were dissolved ethyl 4-aminobenzoate **1** (1.2256 g, 6.838 mmol, 1 eq) and 3,5-di-*tert*-butylbenzaldehyde **2** (1.493 g, 6.838 mmol, 1 eq) in anhydrous toluene (20 mL). Sodium sulfate (1.1656 g, 8.2056 mmol, 1.2 eq) was added and the suspension heated to reflux under N_2 for 21 h. The white solid was filtered off and the yellow solution was evaporated in vacuo. The yellow solid was recrystallized from hot EtOH giving white crystals (1.8322 g, 71%).^{1}H NMR ($CDCl_3$, 400 MHz) δ(ppm) 8.41 (s, 1H); 8.02 (d, ^{3}J=8.0Hz, 2H); 7.63 (d, ^{4}J=2.0 Hz, 2H); 7.53 (t, ^{4}J=2.0 Hz, 1H); 7.41 (d, ^{3}J=8.0 Hz, 2H); 4.88 (s, 2H); 4.37 (q, ^{3}J=7.2 Hz, 2H); 1.39 (t, ^{3}J=7.2 Hz, 3H); 1.35 (s, 18H) ; ^{13}C NMR ($CDCl_3$, 75 MHz) δ(ppm) 166.7; 164.0; 151.4; 144.8; 135.5;129.8; 129.2; 127.9; 125.5; 122.8; 64.8; 61.0; 35.1; 31.6; 14.5 ; HR-MS ESI $[M+H]^+_{calc}$=380.25851, $[M+H]^+_{found}$=380.25858

Compound ***4*** To a solution of compound **3** (1.6146 g, 4.254 mmol, 1 eq) in MeOH/THF 1/1 (60 ml) was added sodium borohydride (0.2656 g, 7.02 mmol, 1.65 eq). The yellow solution was stirred at room temperature for 2 h. Then a second portion of sodium borohydride (0.2656 g, 7.02 mmol, 1.65 eq) was added and the medium stirred for 18 h at room temperature. The mixture was quenched with a solution of HCl 1 M until pH 2 was reached and the solvent was removed under vaccum. The residue was dissolved in a saturated solution of K_2CO_3 (185 mL) then extracted with CH_2Cl_2 (3x175 mL). The organic layers were dried over $MgSO_4$ and evaporated under vaccum. A light yellow oil (1.5095 g, 93%) was obtained from purification by flash chromatography (SiO_2, AcOEt/Hex: 3/7). ^{1}H NMR ($CDCl_3$, 400 MHz) δ(ppm) 8.07 (d, ^{3}J=8.4 Hz, 2H); 7.48 (d, 3J=8.4 Hz, 2H); 7.39 (t, ^{4}J=2.0 Hz, 1H); 7.23 (d, ^{4}J=2.0 Hz, 2H); 4.41 (q, ^{3}J=7.2 Hz, 2H); 3.93 (s, 2H);

3.84 (s, 2H); 1.43 (t, ^{3}J=7.2Hz, 3H); 1.39 (s, 18H) ; ^{13}C NMR ($CDCl_3$, 75 MHz) δ(ppm) 166.7; 150.9; 145.9; 139.2; 129.8; 129.3; 128.1; 122.5; 121.2; 60.97; 53.9; 53.0; 34.9; 31.6; 14.5 ; HR-MS ESI $[M+H]^+_{calc}$= 382.27406, $[M+H]^+_{found}$= 382.27498; $[M+Na]^+_{calc}$= 404.256, $[M+Na]^+_{found}$= 404.25529. To a solution of the previous compound (0.1308 g, 0.3428 mmol, 1 eq) in CH_2Cl_2 (6 ml) cooled to 0°C was added dropwise a solution of di-*tert*-butyle dicarbonate (0.0786 g, 0.3599 mmol, 1.05 eq) in CH_2Cl_2 (5 ml). The mixture was then stirred at room temperature for 24 h. The colorless solution was evaporated under vacuum to give a white oil which directly used for the next step without further purification. To a solution of the ester (1.6575 g, 3.441 mmol, 1eq) in MeOH (70 mL) was added potassium hydroxide (1.9308 g, 34.412 mmol, 10 eq). The white mixture was heated to reflux for 5 h then cooled to room temperature. The solution was acidified with a solution of HCl 10% until pH 1. The aqueous layer was extracted with AcOEt (4x50 mL) then the combined organic layers were washed with water (2x40 mL), dried over $MgSO_4$ and evaporated under vacuum. A viscous yellow oil (1.5159 g, 97%) was obtained from purification by flash chromatography (SiO_2, AcOEt/Hex: 3/7). ^{1}H NMR ($CDCl_3$, 400 MHz) δ(ppm) 8.04 (d, ^{3}J=8.0 Hz, 2H); 7.32 (s, 2H); 7.23 (s, 1H); 7.03 (m, 2H); 4.49-4.34 (m, 4H); 1.50 (m, 9H); 1.30 (s, 18H) ; HR-MS ESI $[M+Na]^+_{calc}$= 476.27866, $[M+Na]^+_{found}$= 476.27861.

Compound ***6*** To a solution of umbrella **5**[12] (1.0155 g, 1.1131 mmol, 2.25 eq) in dry DMF (25 mL) were added triethylamine (0.34 mL, 2.4735 mmol, 15 eq) and a solution of compound **4** (0.296 g, 0.4947 mmol, 1 eq) in dry DMF (10 mL). The yellow solution was stirred at room temperature under N_2 for 20 h, then evaporated under vacuum. A white solid (0.2306 g, 35%) was obtained from purification by flash chromatography (SiO_2, CH_2Cl_2/MeOH: 85/15). ^{1}H NMR (MeOD, 400 MHz) δ(ppm) 7.31 (m, 5H); 7.08 (m, 2H); 4.43 (m, 4H); 3.94 (m, 2H); 3.79 (br s, 2H); 3.55 (m, 2H); 3.37 (m, 2H); 3.28 (m,2H); 3.01 (m, 2H); 2.37-1.52 (m, 39H); 1.49 (s, 9H); 1.31 (s, 18H); 1.29-0.93 (m, 12H); 0.91 (s, 6H); 0.69 (m, 6H) ; ^{13}C NMR (MeOD, 75 MHz) δ(ppm) 176.8; 176.6; 176.3; 169.6; 157.6; 152.1; 128.8; 128.6; 123.0; 122.2; 81.6; 73.9; 72.8; 68.9; 47.9; 47.4; 43.1; 42.9; 40.9; 40.4; 37.8; 37.2; 36.9; 36.8; 36.5; 35.8; 35.6; 34.2; 34.1; 33.3; 33.0; 31.9; 31.1; 29.6; 28.8; 28.6; 27.8; 24.2; 23.2; 18.0; 17.9; 17.8; 13.1; HR-MS ESI $[M+H]^+_{calc}$=1347.98091, $[M+H]^+_{found}$=1347.97890; $[M+Na]^+_{calc}$=1369.96288, $[M+Na]^+_{found}$= 1369.96131.

Compound ***UT*** To a solution of the compound **6** (0.1323 g, 0.0982 mmol, 1 eq) in EtOH cooled to 0°C was added a solution of HCl 4 N (1.84 mL, 7.3612 mmol, 75 eq) dropwise. The medium was stirred at 0°C for 20 min then at room temperature for 48 h. The yellow solution was evaporated under vacuum and recrystallized in $CHCl_3$/Et_2O to give a light yellow solid (88.7 mg, 70%). NMR ^{1}H (MeOD, 400 MHz) δ(ppm) 7.63 (d, ^{3}J=7.6 Hz, 2H); 7.53 (s, 1H); 7.48 (d, ^{3}J=7.6 Hz, 2H); 7.41 (s, 2H); 4.34 (s, 2H); 4.28 (s, 2H); 3.95 (br s, 2H); 3.80 (br s, 2H); 3.56 (m, 2H); 3.37 (m, 2H); 3.27 (m, 2H); 2.97 (m, 2H); 2.37-1.37 (m, 48H); 1.35 (s, 18H); 1.32-0.93 (m, 12H); 0.92 (s, 6H); 0.70 (m, 6H) ; NMR ^{13}C (MeOD, 75 MHz) δ(ppm) 176.8; 176.7; 173.3; 153.1; 138.9; 133.9; 131.6; 128.3; 125.3; 124.6; 74.0; 72.8; 69.0; 52.9; 51.7; 48.0; 47.5; 44.1; 43.1; 43.0; 41.0; 40.4; 38.0; 37.6; 36.9; 36.5; 35.8; 34.2; 34.1; 33.3; 31.8; 31.2; 29.6; 28.7; 28.5; 27.8; 24.2; 23.2; 17.8; 13.1 ; HR-MS ESI $[M]^+_{calc}$= 1247.92846, $[M]^+_{found}$= 1247.93045. To a solution of the previous compound (0.2579 g, 0.2008 mmol, 1 eq) in MeOH (4 mL) was added a saturated solution of ammonium hexafluorophosphate (0.3273 g, 2.0081 mmol, 10 eq) in water (2 mL). The mixture was stirred at room temperature for 24 h then evaporated in vacuo. The white solid was washed with water and filtered to give a white powder (0.2678 g, 98%). ^{1}H NMR (MeOD, 400 MHz) δ(ppm) 7.57 (d, ^{3}J=8.4 Hz, 2H); 7.54 (t, ^{4}J=1.6 Hz, 1H); 7.48 (d, ^{3}J=8.4 Hz, 2H); 7.35 (d, ^{4}J=1.6 Hz, 2H); 4.29 (s, 2H); 4.23 (s, 2H); 3.95 (br s, 2H); 3.80 (br s, 2H); 3.56 (m, 2H); 3.38 (m, 2H); 3.27 (m, 2H); 2.96 (m, 2H); 2.35-1.36 (m, 48H); 1.35 (s, 18H); 1.31-0.95 (m, 12H); 0.92 (s, 6H); 0.70 (m, 6H) ; ^{13}C NMR (MeOD, 75 MHz) δ(ppm) 176.8; 173.4; 153.2; 139.0; 133.9; 131.7; 131.6; 128.3; 125.2; 124.7; 74.0; 72.8; 69.0; 53.0; 51.8; 48.0; 47.5; 44.0; 43.2; 43.0; 37.9;

37.7; 36.9; 36.5; 35.8; 34.3; 34.1; 33.3; 31.8; 31.2; 29.6; 28.7; 28.5; 27.7; 24.2; 23.2; 17.8; 13.0 ; HR-MS ESI $[M]^{+}_{calc}$= 1247.92840, $[M]^{+}_{found}$= 1247.93077.

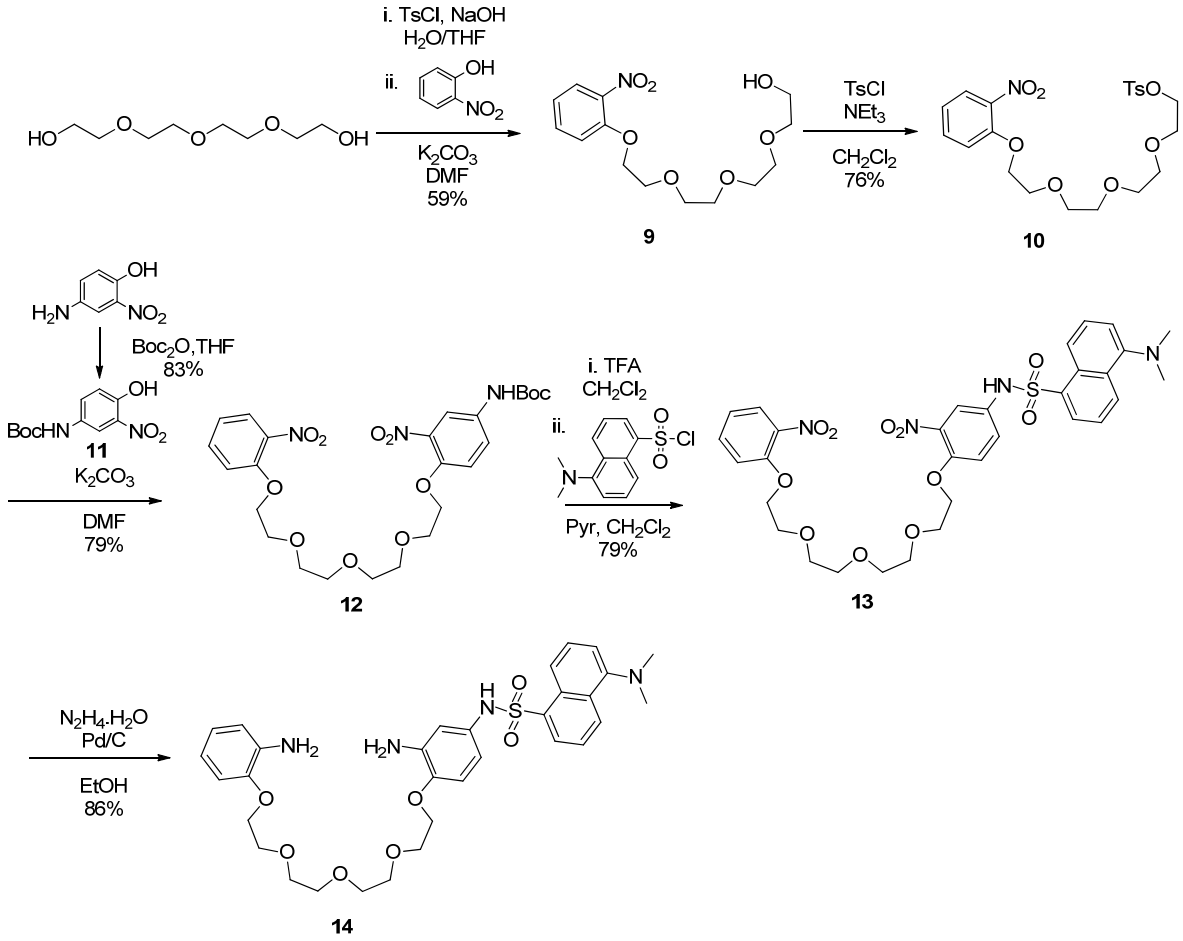
*Compound **14*** To a suspension of compound **13** (0.5883 g, 0.8592 mmol, 1 eq) and palladium on activated charcoal 10% (0.091 g, 0.0859 mmol, 0.1 eq) in EtOH (11 mL) at 50°C was added dropwise a solution of hydrazine hydrate 50% (1.17 mL, 12.029 mmol, 14 eq) in EtOH (4 mL). The black mixture was heated to reflux for 2 h then cooled to room temperature and filtered on celite. The yellow solution was evaporated in vacuo. A yellow oil (0.464 g, 86%) was obtained from purification by flash chromatography (SiO_2, AcOEt). ^{1}H NMR ($CDCl_3$, 400 MHz) δ(ppm) 8.47 (d, ^{3}J=8.8 Hz, 1H); 8.33 (d, ^{3}J=8.8 Hz, 1H); 8.08 (d, ^{3}J=7.6 Hz, 1H); 7.53 (t, ^{3}J=8.0 Hz, 1H); 7.39 (t, ^{3}J=8.0 Hz, 1H); 7.17 (d, ^{3}J=7.6 Hz, 1H); 6.74 (m, 3H); 6.45 (d, ^{3}J=8.8 Hz, 1H); 6.31 (d, ^{3}J=2.4 Hz, 1H); 6.18 (dd, ^{3}J=8.8 Hz, ^{4}J=2.4 Hz, 1H); 4.11 (m, 2H); 3.97 (m, 2H); 3.80 (m, 2H); 3.75-3.59 (m, 12H); 2.87 (s, 6H) ; ^{13}C NMR ($CDCl_3$, 75 MHz) δ(ppm) 146.9; 145.9; 137.5; 136.5; 135.7; 134.6; 130.7; 130.6; 130.6; 130.3; 129.8; 128.5; 123.3; 122.1; 119.6; 119.0; 116.4; 115.3; 114.0; 113.2; 112.7; 119.0; 116.4; 115.3; 114.0; 113.2; 112.7; 110.5; 70.8; 70.7; 70.6; 69.9; 69.6; 69.1. 68.5; 45.6; 18.2 ; HR-MS ESI $[M+H]^{+}_{calc}$= 625.26900, $[M+H]^{+}_{found}$= 625.269412; $[M+Na]^{+}_{calc}$= 647.25101, $[M+Na]^{+}_{found}$= 647.25178.

*Assembly of **UR1*** A solution of umbrella thread hexafluorophosphate **UT** (24.8 mg, 0.0178 mmol, 1 eq), 2,6-pyridinedicarboxaldehyde (2.4 mg, 0.0178 mmol, 1 eq) and clip **7Error! Bookmark not defined.** (6.7 mg, 0.0178 mmol, 1 eq) in acetonitrile (0.6 mL) was stirred at room temperature for 5 h. Borane tetrahydrofuran 1M (0.09 mL, 0.089 mmol, 5 eq) was added and the mixture was stirred for 24 h 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=7.6 Hz, 2H), 7.28 (m, 2H), 6.97 (m, 4H); 6.79-6.64 (m, 6H); 6.42 (d, ^{3}J=8.0 Hz, 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) ; ^{13}C NMR (MeOD, 75 MHz) δ(ppm) 160.4; 153.6; 148.2; 139.7; 139.2; 138.2; 135.4; 133.3; 129.1; 128.9; 128.6; 128.5; 125.6; 124.4; 123.3; 122.8; 121.2; 120.2; 114.0; 113.9; 111.4; 74.0; 72.8; 72.5; 72.4; 71.7; 69.0; 68.6; 65.5; 62.8; 54.5; 53.1; 50.6; 47.5; 43.2; 42.9; 41.0; 40.5; 36.5; 35.9; 35.7; 33.1; 31.7; 31.2; 30.7; 30.1; 29.6; 28.5; 27.8; 24.2; 23.7; 23.2; 14.4; 13.1; 12.9; HR-MS ESI $[M+2H]^{2+}_{calc}$= 864.59315, $[M+2H]^{2+}_{found}$= 864.59211; $[M+H]^{+}_{calc}$= 1728.17837, $[M+H]^{+}_{found}$= 1728.17620.

*Assembly of **UR$_2$*** A solution of umbrella thread hexafluorophosphate **UT** (7.6 mg, 0.055 mmol, 1 eq), 2,6-pyridinedicarboxaldehyde (3 mg, 0.0219 mmol, 1 eq) and compound **14** (13.7 mg, 0.0219 mmol, 1 eq) in acetonitrile (1 mL) was stirred at room temperature for 5 h. Borane tetrahydrofuran 1M (0.11 mL, 0.09 mmol, 20 eq) was added and the mixture was stirred for 21 h at room temperature then fitered off. The solution was evaporated under vacuum. The yellow solid was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH: 85/15) then preparative TLC (SiO_2, CH_2Cl_2/MeOH: 85/15) to afford a yellow solid (6mg, 51%). ^{1}H NMR (MeOD, 400 MHz) δ(ppm) 8.51 (m, 1H); 8.34 (d, ^{3}J=8.8 Hz, 1H); 7.93 (m, 1H); 7.59 (m, 2H); 7.48-7.12 (m, 8H); 6.94 (br s, 2H); 6.92-6.58 (m, 7H); 6.40 (m, 1H); 4.57 (m, 1H); 4.12-3.33 (m, 31H); 3.18 (m, 2H); 2.88 (br s, 6H); 2.76 (m, 2H); 2.59-1.23 (m, 48H); 1.13-1.00 (m, 24H); 1.01-0.80 (m, 12H); 0.77-0.57 (m, 6H) ; ^{13}C NMR (MeOD, 75 MHz) δ(ppm) 156.3; 153.5; 145.5; 139.7; 138.6; 138.2; 136.7; 133.2; 132.2; 131.4; 131.2; 131.1; 131.1; 129.3; 128.9; 128.8; 128.5; 128.3; 125.7; 125.6; 124.4; 124.2; 123.5; 123.2; 122.8; 120.7; 116.5; 111.3; 74.0; 72.9; 72.5; 72.3; 72.2; 71.7; 71.5; 69.0; 68.7; 68.6; 54.4; 50.9; 47.5; 45.9; 43.2; 43.0; 41.0; 40.5; 38.1; 37.9; 37.1; 37.0; 36.9; 36.5; 35.9; 35.7; 35.5; 34.3; 33.3; 33.1; 33.1; 32.0; 31.9, 31.8; 31.7; 31.6; 31.2; 30.8; 30.4; 30.1; 29.6;

28.7; 28.6; 27.8; 24.2; 23.7; 23.2; 18.0; 17.8; 14.4; 13.1 ; HR-MS ESI $[M+2H]^{2+}_{calc}$= 988.62372, $[M+2H]^{2+}_{found}$= 988.62585; $[M+H]^{+}_{calc}$= 1976.24029, $[M+H]^{+}_{found}$= 1976.23631.

Synthesis of 14



1-Hydroxy-11-(2'-nitrophenoxy)-3,6,9-trioxaundecane ***9*** To a solution of tetra (ethyleneglycol) (8.89 mL, 51.48 mmol, 10 eq) in THF (2 mL) was added a solution of sodium hydroxide (0.3120 g, 7.8 mmol, 1.5 eq). The mixture was cooled to 0°C and a solution of *p*-toluenesulfonyl chloride (0.9489 g, 4.98 mmol, 1 eq) in THF (5 mL) was added dropwise. The light yellow mixture was stirred at 0°C for 2 h then extracted with CH_2Cl_2 (3x50 mL). The organic layer was washed with H_2O (2x50 mL) then dried over $MgSO_4$, filtered off and evaporated in vacuo affording a colorless oil (1.5416 g, 89%) who was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm) 7.80 (d, ^{3}J=8.4 Hz, 2H); 7.34 (d, ^{3}J=8.4 Hz, 2H); 4.16 (t, ^{3}J=4.2 Hz, 2H); 3.76-3.55 (m, 14H); 2.44 (s, 3H) ; ^{13}C NMR ($CDCl_3$, 75 MHz) δ(ppm) 144.97; 133.09; 129.99; 128.15; 72.60; 70.88; 70.79; 70.60; 70.46; 69.37; 68.84; 61.88; 21.79 ; HR-MS ESI $[M+H]^{+}_{calc}$=

349.1316, $[M+H]^+_{found}$= 349.133; $[M+NH_4]^+_{calc}$= 366.1581, $[M+NH_4]^+_{found}$= 366.159. To a solution of 2-nitrophenol (0.024 g, 0.1724 mmol, 1 eq) and potassium carbonate (0.0238 g, 0.1724 mmol, 1 eq) in DMF (0.6 mL) was added a solution of the previous compound (0.06g, 0.1724mmol, 1eq) in DMF (0.4 mL). The orange mixture was heated to reflux for 16 h then cooled down to room temperature. The solvent was concentrated in vacuo. The residue was quenched with H_2O (15 mL) then extracted with AcOEt (3x15 mL). The organic layer was washed with H_2O (3x15 mL) then dried over $MgSO_4$, filtered off and evaporated under vacuum. Purification by flash chromatography (SiO_2, CH_2Cl_2/MeOH: 95/5) afforded a brown oil (0.0361 g, 66%).^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm) 7.82 (d, ^{3}J=8.1 Hz, 1H); 7.52 (t, ^{3}J=8.0 Hz, 1H); 7.11 (d, ^{3}J=8.7 Hz, 1H); 7.03 (t, ^{3}J=7.8 Hz, 1H); 4.27 (t, ^{3}J=4.5 Hz; 2H); 3.91 (t, ^{3}J=4.8 Hz, 2H); 3.81-3.57 (m, 12H) ; ^{13}C NMR ($CDCl_3$, 75 MHz) δ(ppm) 152.39; 140.19; 134.22; 125.74; 120.76; 115.04; 72.58; 71.24; 70.72; 70.44; 69.73; 69.39; 61.87 ; HR-MS ESI $[M+H]^+_{calc}$= 316.1390, $[M+H]^+_{found}$= 316.1405.

*Compound **10*** To a solution of compound **9** (2.1230 g, 6.72 mmol, 1 eq) and triethylamine (1.41 mL, 10.095 mmol, 1.5 eq) in CH_2Cl_2 (5 mL) was added a solution of *p*-toluenesulfonyl chloride (1.5404 g, 8.08 mmol, 1.2 eq) in CH_2Cl_2 (20 mL). The yellow suspension was stirred at room temperature for 24 h then washed with a saturated solution of $NaHCO_3$ (100 mL). The aqueous layer was extracted with CH_2Cl_2 (3x50 mL). The combined organic layers were dried over $MgSO_4$, filtered off and evaporated in vacuo. Purification by flash chromatography (SiO_2, CH_2Cl_2/MeOH: 98/2) gave a yellow oil (2.4070 g, 76%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm) 7.80 (m, 3H); 7.51 (t, ^{3}J=8.0 Hz, 1H); 7.33 (d, ^{3}J=8.1 Hz, 2H); 7.10 (d, ^{3}J=8.4 Hz, 1H); 7.03 (t, ^{3}J=7.8 Hz, 1H); 4.26 (t, ^{3}J=4.5 Hz, 2H); 4.15 (t, ^{3}J=4.8 Hz, 2H); 3.90 (t, ^{3}J=4.8 Hz, 2H0; 3.73 (m, 2H); 3.67 (t, ^{3}J=5.1 Hz, 2H); 3.62 (m, 2H); 3.58 (s, 4H); 2.43 (s, 3H) ; ^{13}C NMR ($CDCl_3$, 75 MHz) δ(ppm) 152.39; 144.93; 140.20; 134.23; 133.08; 129.95; 128.12; 125.70; 120.71; 115.07; 71.20; 70.85; 70.81; 70.63; 69.72; 69.40; 68.78; 21.77 ; HR-MS ESI $[M+H]^+_{calc}$= 470.1479, $[M+H]^+_{found}$= 470.1472.

*2-Nitro-4-(1,1-dimethylethoxycarbonylamino)phenol **11*** To a solution of 4-amino-2-nitrophenol (0.25 g, 1.6221 mmol, 1 eq) in THF (3 mL) was added a solution of di-*tert*-butyl dicarbonate (0.3894 g, 1.7843 mmol, 1.1 eq). The dark red media was heated to reflux for 24 h then cooled to room temperature. It was diluted with AcOEt (10 mL) and washed with a solution of HCl 1% (5 mL), H_2O (5 mL), a saturated solution of $NaHCO_3$ (5 mL) and brine (5 mL). The organic layer was dried over $MgSO_4$, filtered off and evaporated in vacuo. A yellow solid (0.3415 g, 83%) was obtained from purification by flash chromatography (SiO_2, AcOEt/Hex: 2/8). ^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm) 10.36 (s, 1H); 8.18 (br s, 1H); 7.57 (d, ^{3}J=7.8 Hz, 1H); 7.10 (d, ^{3}J=9.0 Hz, 1H); 6.46 (s, 1H); 1.52 (s, 9H) ; ^{13}C NMR ($CDCl_3$, 75 MHz) δ(ppm) 152.80; 151.16; 133.24; 131.39; 129.33; 120.38; 114.08; 81.51; 28.40 ; HR-MS ESI $[M+Na]^+_{calc}$= 277.0795, $[M+Na]^+_{found}$= 277.0800.

*Compound **12*** To a solution of compound **10** (1.5649 g, 3.33 mmol, 1.3 eq) and potassium carbonate (1.1 g, 4.61 mmol, 1.8 eq) in DMF (20 mL) was added 2-nitro-4-(1,1-dimethylethoxycarbonyl amino)phenol **11** (0.66 g, 2.56 mmol, 1 eq). The brown solution was heated to 85°C for 18 h and quenched with H_2O (100 mL) then extracted with AcOEt (3x50 mL). The organic layer was washed with brine (3x30 mL), dried over $MgSO_4$, filtered off and evaporated under vacuum. Purification by flash chromatography (SiO_2, AcOEt/Hex: 6/4) gave a yellow solid (1.1131 g, 79%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm) 7.92 (s, 1H); 7.81 (d, ^{3}J=9.0 Hz, 1H); 7.49 (m, 2H); 7.09 (d, ^{3}J=6.0 Hz, 1H); 7.04 (m, 2H); 6.65 (s, 1H); 4.21 (m, 4H); 3.87 (m, 4H); 3.72 (m, 4H); 3.66 (m, 4H); 1.50 (s, 9H) ; ^{13}C NMR ($CDCl_3$, 75 MHz) δ(ppm) 153.79; 141.50; 140.38; 134.24; 125.70; 120.69; 116.41; 112.30; 106.41; 106.22; 100.61; 96.48; 92.67;

79.44; 74.53; 71.22; 71.21; 71.19; 70.78; 70.77; 70.70; 70.33; 69.72; 69.55; 69.41; 68.55; 28.41 ; HR-MS ESI $[M+Na]^+_{calc}$= 574.2007, $[M+Na]^+_{found}$= 574.2026.

*Compound **13*** Compound **12** (0.5206 g, 0.944 mmol, 1 eq) was dissolved in a solution of trifluoroacetic acid (4.7 mL, 63.23 mmol, 67 eq) in CH_2Cl_2 (20 mL). The brown solution was stirred at room temperature for 4 h then diluted with CH_2Cl_2 (50 mL) and washed with a saturated solution of K_2CO_3 (30 mL) and brine (30 mL). The organic layer was dried over $MgSO_4$, filtered off and evaporated in vacuo affording a viscous orange oil (0.4116 g, 97%) directly used in the next step. To a solution of the previous compound (0.481 g, 1.065 mmol, 1 eq) in CH_2Cl_2 (3 mL) cooled to 0°C was added pyridine (0.65 mL, 7.9875 mmol, 7.5 eq). A solution of dansyl chloride (0.3555 g, 63.23 mmol, 1.2 eq) in CH_2Cl_2 (6 mL) was added dropwise and the ice bath was removed. The orange mixture was stirred at room temperature for 2 h then diluted with CH_2Cl_2 (20 mL) and washed with a saturated solution of $NaHCO_3$ (30 mL). The aqueous layer was extracted with CH_2Cl_2 (3x10 mL) and the combined organic layers were washed with a solution of HCl 1 M (30 mL), dried over $MgSO_4$, filtered off and evaporated in vacuo. Purification by flash chromatography (SiO_2, AcOEt/Hex: 7/3) afforded a yellow oil (0.5883 g, 81%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ(ppm) 8.62 (br s, 1H); 8.34 (d, ^{3}J=8.8 Hz, 1H); 8.08 (d, ^{3}J=7.2 Hz, 1H); 7.81 (dd, ^{3}J=8.0 Hz, ^{4}J=1.6 Hz, 1H); 7.61 (t, ^{3}J=8.0 Hz, 1H); 7.48 (m, 2H); 7.18 (dd, ^{3}J=8.8 Hz, ^{4}J=2.8 Hz, 1H); 7.09 (d, ^{3}J=8.0 Hz, 1H); 7.01 (t, ^{3}J=8.4 Hz, 1H); 6.91 (d, ^{3}J=9.2 Hz, 1H); 4.24 (t, ^{3}J=5.2 Hz,2H); 4.14 (m, 2H); 3.87 (t, ^{3}J=4.8 Hz, 2H); 3.81 (t, ^{3}J=4.4 Hz, 2H); 3.69 (m, 4H); 3.61 (m, 4H); 2.96 (br s, 6H) ; ^{13}C NMR ($CDCl_3$, 75 MHz) δ(ppm) 152.36; 150.10; 140.06; 139.57; 134.29; 133.74; 131.12; 130.97; 130.59; 129.57; 129.44; 128.91; 128.75; 125.67; 123.51; 120.67; 118.80; 115.93; 115.72; 115.04; 106.88; 104.40; 71.14; 71.09; 70.67; 69.92; 69.64; 69.32; 45.62; 29.80 ; HR-MS ESI $[M+H]^+_{calc}$= 685.21741, $[M+H]^+_{found}$= 685.21824; $[M+Na]^+_{calc}$= 707.1994, $[M+Na]^+_{found}$= 707.2000.

Preparation of liposomes for lucigenin-based assays. A stock solution of egg-yolk phosphatidylcholine (EYPC) in $CHCl_3$ (50 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 100 mM $NaNO_3$ and 10 mM phosphate buffer (pH=6.4) to obtain a 0.2-0.25 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 50 µL aliquot of a solution of **UR1** or **UR2** in MeOH at different concentration was injected after 30 s. The temperature was set to 35 °C. Experiments were repeated in triplicate and all traces reported are the average of the three trials.

Determination of EC_{50}. For each compound, the transport was followed by fluorescence with different rotaxane concentrations. The dose-response curves at 200 s were drawn.

α-Chymotrypsin digestion.[i] A solution of α-chymotrypsin from bovin pancreas 60 units/mL in pure water was added to a mixture of solution of **UR2** 50 mM in 2,92 mL of 1/1MeOH/80 mM TrisHCl buffer (pH 7.6) and $CaCl_2$ 2 M (80 µL). The mixture was monitored at 37°C by UV at 280 nm and then analyzed by LC-MS.

Preparation of liposomes containing α-chymotrypsin and in situ release of the macrocycle.

A stock solution of egg-yolk phosphatidylcholine (EYPC) in $CHCl_3$ (50 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 a solution of 355 µL 80 mM TrisHCl buffer (pH 7.8) containing 100 mM NaCl, 20 µL 2 M $CaCl_2$, 350 µL 2 mM lucigenin and 25 µL of a solution of α-chymotrypsin (1000 units/mL) in 1 mM HCl. 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 α-chymotrypsin and lucigenin. The stock solution was used right immediately. A 250 µL aliquot of the stock solution of EYPC liposomes was added to a solution containing 250 µL of a solution of 100mM $NaNO_3$ and 10 mM phosphate buffer (pH 6.4) and a solution of **UR2** or unthreaded macrocycle 1 mM in MeOH. The mixture was frequently stirred at room temperature for 4 h. The mixture was then heated to 60 °C for 45 minutes and then analyzed by LC-MS.

Dose-response curves at 200 nm and EC50 determination

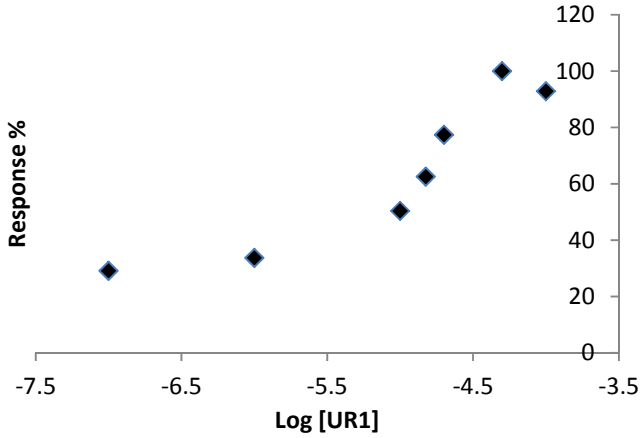


Figure S1. Dose-response curve at 200 s for the determination of EC_{50} for **UR1**.

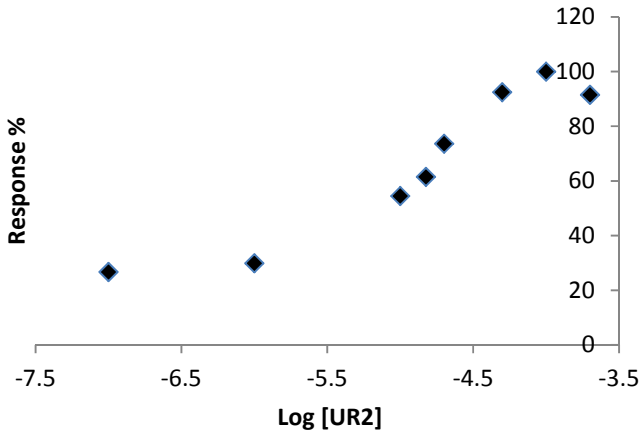


Figure S2. Dose-response curve at 200 s for the determination of EC_{50} for **UR2**.

We noticed a lower plateau maximum when the concentration of umbrella-rotaxane solution was higher (0.1 mM for **UR1** and 0.2 mM for **UR2**), this phenomenon being due to the precipitation of the compound in the UV cuvette. Thus the dose-response curve maximum was reached at 0.05 mM and 0.1 mM .

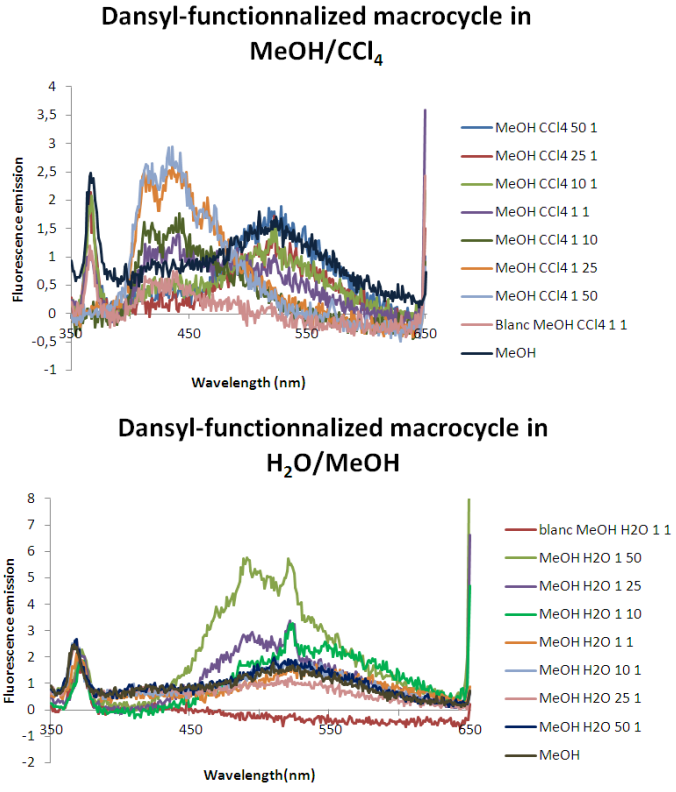


Figure S3. Fluorescence spectra of dansyl-functionnalized macrocycle in different media.

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[i] R.Wirnt, H.U. Bergmeyer, (1974) Chymotrypsin, *Methods of Enzymatic Analysis, New York*, 1009-1012.