

Supporting information

Membrane active cationic cholic acid-based molecular umbrellas

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General information

All chemicals were purchased from Aldrich Chemicals in their highest purity and used without further purification. CD₃OD and CDCl₃ were purchased from CDN Isotopes.

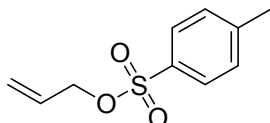
NMR spectra were recorded on Bruker Avance 400 or Bruker Avance 700 instruments. Coupling constants are given in Hertz (Hz) and chemical shifts are given in ppm (δ) measured relative to residual solvent. High-resolution mass spectra (HRMS) were recorded on a TSQ Quantum Ultra (Thermo Scientific) triple quadrupole instrument. MALDI-TOF spectra were obtained on a Bruker Ultraflex extreme Maldi TOF-TOF mass spectrometer using α -Cyano-4-hydroxycinnamic acid as matrix (Université de Montréal Mass Spectrometry Facility). L- α - Phosphatidylcholine was purchased from Avanti Polar Lipids. Fluorimetric studies were performed on a Varian Cary Eclipse Fluorescence spectrophotometer equipped with a temperature controller.

The antimicrobial assays were performed on a Fluostar Optima plate reader. The *Escherichia coli* (SK037) and *Bacillus thuringiensis* (HD73) strains were provided by prof. J. Pelletier (Chemistry Department, Université de Montréal).

Synthesis

The synthesis of the Umbrella part (“*Umbrella*” (*compound 6*)) used in *compound 7* procedure was already reported in the literature and was followed without modifications. Characterization data were in accordance with the literature.¹

(Allyl 4-methylbenzenesulfonate)



Allyl alcohol (1 eq, 10 mmol, 598 mg) was added to a solution of sodium hydride (3 eq, 31 mmol, 741 mg) in dry dichloromethane (30 ml). The white solution was stirred at 0° C. A suspension of 4-toluenesulfonyl chloride (1.5 eq, 15 mmol, 2.48 g) in dry dichloromethane (10 ml) was added dropwise to the solution and the mixture was stirred at room temperature for 24 h. After washing with NaHCO₃ (3 x 10ml), the organic layers were combined, dried over MgSO₄ and evaporated under vacuum, affording a light yellow oil. (1.87 g, **86 %**)

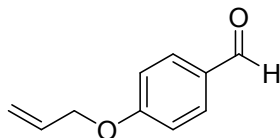
RMN ¹H (CDCl₃, 400 MHz) : δ(ppm) = 7.84 (d, *J*=8 Hz, 2 H), 7.38 (d, *J*=8 Hz, 2 H), 5.80 - 5.91 (m, 1 H), 5.26 - 5.39 (m, 2 H), 4.57 (dt, *J*₁=6.0, *J*₂=1.2 Hz, 2 H), 2.49 ppm (s, 3 H)

RMN ¹³C (CDCl₃, 100 MHz) δ(ppm) = 144.9, 133.4, 130.3, 129.9, 127.9, 120.3, 70.8, 21.6

HR-MS ESI [M+H]⁺_{calc} = 213.0579, ESI [M+H]⁺_{found} = 213.0570.

*Characterization data were in accordance with the literature.*²

Compound 1 (4-(Allyloxy)benzaldehyde)



A solution of allyl 4-methylbenzenesulfonate (1 eq, 8.82 mmol, 1.87 g) in CH₃CN (10 ml) was added dropwise to a suspension of 4-hydroxybenzaldehyde (1 eq, 8.82 mmol, 1.08 g) and K₂CO₃ (1 eq, 8.82 mmol, 1.22 g) in CH₃CN (20 ml). The pink mixture was heated to reflux for 4 h then cooled down to room temperature. The solvent was evaporated under vacuum and the crude product was solubilized in H₂O (80 ml). After extraction with EtOAc (3 x 30 ml), the organic layer was washed with H₂O (1 x 30 ml), dried over MgSO₄ and evaporated under vacuum. The yellow oil was purified by flash chromatography (SiO₂, 100 % CH₂Cl₂) to yield an yellow oil (1.36 g, **95 %**).

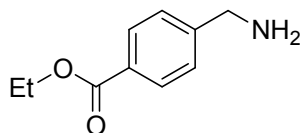
RMN ¹H (CDCl₃, 400 MHz) δ(ppm): 9.92 (s, 1 H), 7.87 (dt, *J*₁=8.0, *J*₂=2.0 Hz, 2 H), 7.05 (dt, *J*₁=8.0, *J*₂=4.0 Hz, 2 H), 6.01 - 6.16 (m, 1 H), 5.34 - 5.52 (m, 2 H), 4.66 (dt, *J*₁=5.3, *J*₂=1.5 Hz, 2 H)

RMN ¹³C (CDCl₃, 100 MHz) δ(ppm): 190.8, 163.6, 132.3, 132.0, 130.0, 118.4, 115.0, 69.0

HR-MS ESI [M+H]⁺_{calc} = 163.0754 ESI [M+H]⁺_{found} = 163.0749
[M+Na]⁺_{calc} = 185.0573 ESI [M+Na]⁺_{found} = 185.0567

*Characterization data were in accordance with the literature.*³

Compound 2 (Ethyl 4-(aminomethyl)benzoate)



Acetyl chloride (6 eq, 198 mmol, 14.2 ml) was added dropwise to a suspension of 4-aminomethylbenzoic acid (1 eq, 33 mmol, 5.00 g) in EtOH (125 ml). The white suspension was heated to reflux for 24 h and then the solution was cooled down to room temperature. The white crystals formed were filtered, washed with hexane then dissolved in a saturated solution of K₂CO₃ (200 ml). After extraction with CH₂Cl₂ (3 x 100 ml), the organic layer was dried over MgSO₄ and evaporated under vacuum, affording a light yellow oil (4.32 g, **73%**)

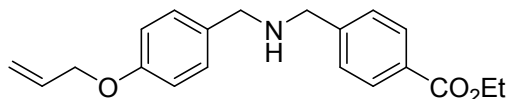
RMN ¹H (CDCl₃, 400 MHz) δ(ppm): 8.10 (d, *J*=8.4 Hz, 2 H), 7.38 (d, *J*=8.4 Hz, 2 H), 4.40 (q, *J*=7.2 Hz, 2 H), 3.97 (s, 2 H), 1.57 (s, 2 H), 1.42 (t, *J*=7.2 Hz, 3 H)

RMN ¹³C (CDCl₃, 75MHz) δ(ppm): 166.0, 148.1, 129.3, 128.5, 126.5, 60.4, 45.7, 13.9

HR-MS ESI [M+H]⁺_{calc} = 180.1019, ESI [M+H]⁺_{found} = 180.1017

*Characterization data were in accordance with the literature.*⁴

Compound 3 (Ethyl 4-((4-(allyloxy)benzylamino)methyl)benzoate)



A solution of 4-(allyloxy)benzaldehyde (1 eq, 7.13 mmol, 1.16 g), ethyl 4-(aminomethyl)benzoate (1 eq, 7.13 mmol, 1.28 g) and sodium sulfate (3 eq, 14.3 mmol, 2.03 g) in dichloromethane (30 ml) was heated to reflux for 24 h. After cooling down the mixture to room temperature, a first suspension of sodium borohydride (1.5 eq, 10.1 mmol, 0.41 g) in methanol (5 ml) was added. After 2h, a second

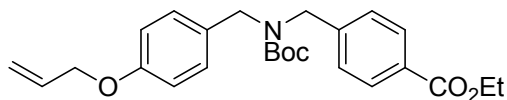
suspension of sodium borohydride (1.5 eq, 10.7 mmol, 0.41 g) in methanol (5 ml) was added and the mixture was stirred at room temperature for 24 h. The solution was acidified with 10 % HCl until pH= 2, evaporated under vacuum and the crude product was solubilized in a K₂CO₃ saturated solution. After extraction with CH₂Cl₂ (3 x 20 ml), the organic layer was dried over MgSO₄ and evaporated under vacuum. The colorless oil was purified by flash chromatography (SiO₂; Hexane:EtOAc 80:20) (1.51g, **68 %**)

RMN ¹H (CDCl₃, 400 MHz) δ(ppm) = 8.00 - 8.07 (m, 2 H), 7.45 (dt, *J*₁=8.8, *J*₂=0.7 Hz, 2 H), 7.27 (d, *J*=8.0 Hz, 2 H), 6.92 (dt, *J*₁=8.0, *J*₂=0.4 Hz, 2 H), 6.04 - 6.15 (m, 1 H), 5.45 (dd, *J*₁=17.2, *J*₂=1.7 Hz, 1 H), 5.32 (dd, *J*₁=10.5, *J*₂=1.5 Hz, 1 H), 4.57 (dt, *J*₁=5.3, *J*₂=1.6 Hz, 2 H), 4.41 (q, *J*=7.2 Hz, 2 H), 3.88 (s, 2 H), 3.77 (s, 2 H), 1.68 (br. s., 1 H), 1.43 (t, *J*=7.2 Hz, 3 H)

RMN ¹³C (CDCl₃, 125 MHz): 166.6, 157.7, 145.7, 133.4, 132.4, 129.8, 129.7, 129.3, 129.2, 128.8, 128.0, 127.9, 117.6, 114.7, 68.9, 60.9, 52.7, 52.6, 52.1, 14.4

HR-MS ESI [M+H]⁺_{calc} = 326.1751 ESI [M+H]⁺_{found} = 326.1760

(Ethyl 4-(((4-(allyloxy)benzyl)(tert-butoxycarbonyl)amino)methyl)benzoate)



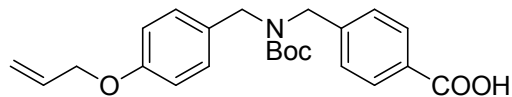
Compound 3 (1 eq, 4.6 mmol, 1.51 g) was added to a solution of di-*tert*-butyl dicarbonate (1 eq, 4.6 mmol, 1.00 g) and Amberlyst resin 15 (15 % weight, 0.23 g) in EtOH (10 ml) heated at 50°C. After 1 h, the mixture was cooled to room temperature, filtered and concentrated under vacuum to give an yellow oil. (1.87 g, **96 %**)

RMN ¹H (CDCl₃, 400 MHz) δ(ppm) = 8.03 (d, *J*=8.0 Hz, 2 H), 7.22 - 7.35 (m, 2 H), 7.15 (d, *J*=11.7 Hz, 2 H), 6.90 (d, *J*=7.7 Hz, 2 H), 6.02 - 6.15 (m, 1 H), 5.45 (d, *J*=17.2 Hz, 1 H), 5.32 (d, *J*=10.5 Hz, 1 H), 4.54 - 4.60 (m, 2 H), 4.25 - 4.51 (m, 6 H), 1.46 - 1.60 (m, 9 H), 1.43 (td, *J*₁=7.1, *J*₂=0.8 Hz, 3 H)

RMN ¹³C (CDCl₃, 125 MHz) δ(ppm) = 166.5, 158.0, 155.9, 143.4, 133.3, 129.9, 129.8, 129.7, 129.4, 129.1, 128.8, 128.7, 127.1, 117.7, 114.8, 80.3, 68.9, 60.9, 49.0, 48.7, 28.4, 27.4, 14.3

HR-MS ESI [M+Na]⁺_{calc} = 448.2094 ESI [M+Na]⁺_{found} = 448.2107

Compound 4 (4-(((4-(Allyloxy)benzyl)(tert-butoxycarbonyl)amino)methyl)benzoic acid)



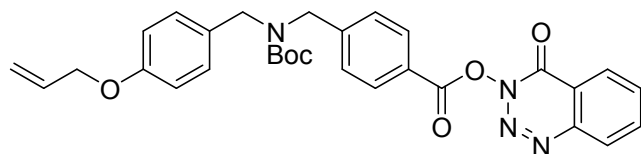
Potassium hydroxide (10 eq, 44 mmol, 2.47 g) was added to a solution of the previous Boc-compound (1 eq, 4.40 mmol, 1.87g) in MeOH (40 ml). The mixture was heated to reflux for 20 h then cooled to room temperature and acidified with HCl 10 %. The aqueous layer was extracted with EtOAc (3 x 80 ml) and the combined organic layers were washed with water (60 ml), dried over MgSO₄ and evaporated under vacuum to yield an yellow oil (1.68 g, **96%**).

RMN ¹H (CDCl₃, 400 MHz) δ(ppm): 8.09 (d, *J*=8.4 Hz, 2 H), 7.29 (m, 2 H), 7.16 (d, *J*=9.5 Hz, 2 H), 6.91 (d, *J*=8.0 Hz, 2 H), 6.01 - 6.18 (m, 1 H), 5.45 (dd, *J*=17.3, 1.6 Hz, 1 H), 5.33 (dd, *J*=10.5, 1.3 Hz, 1 H), 4.57 (d, *J*=5.3 Hz, 2 H), 4.27 - 4.51 (m, 4 H), 1.43 - 1.60 ppm (m, 9 H).

RMN ¹³C (CDCl₃, 125 MHz) δ(ppm) = 170.3, 158.0, 155.9, 133.2, 130.5, 129.8, 129.4, 128.9, 128.0, 127.8, 127.2, 117.7, 114.8, 80.4, 68.9, 49.2, 28.4

HR-MS ESI [M+Na]⁺_{calc} = 420.1781 ESI [M+Na]⁺_{found} = 420.1789.

Compound 5



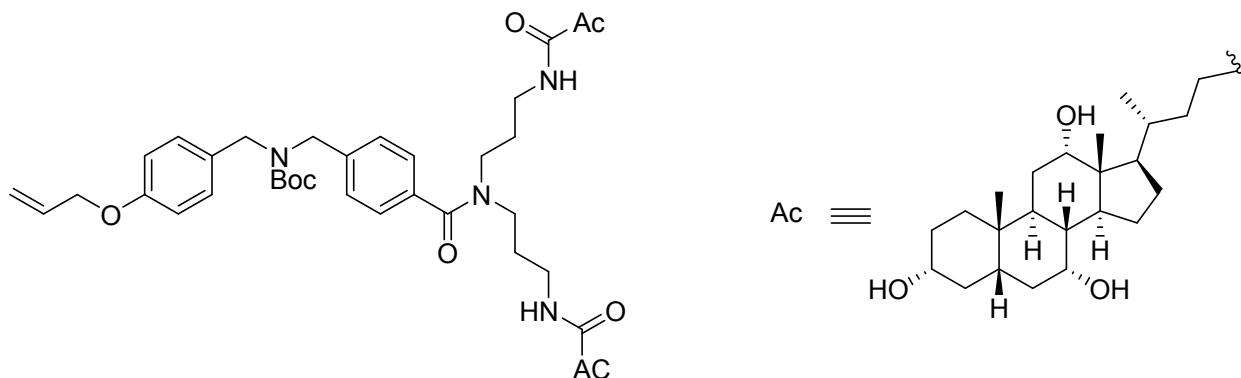
To a solution of **4** (1 eq, 4.23 mmol, 1.68 g) in dry dichloromethane (20 ml) were added hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine (1.1 eq, 4.66 mmol, 0.76 g) and N-N'-dicyclohexylcarbodiimide (1.1 eq, 4.66 mmol, 0.96 g). The mixture was stirred at room temperature for 24 h and the white product formed was filtered and washed with dichloromethane (20 ml). The colorless solution was concentrated under vacuum and the yellow oil obtained was purified by flash chromatography (SiO₂; CH₂Cl₂/MeOH 95/5) to give a white product (1.92 g, **84 %**)

RMN ¹H (CDCl₃, 400 MHz) δ(ppm) = 8.46 (dd, *J*₁=7.9, *J*₂=0.9 Hz, 1 H), 8.30 (d, *J*=8.0 Hz, 1 H), 8.25 (d, *J*=8.4 Hz, 2 H), 8.07 (td, *J*₁=7.7, *J*₂=1.4 Hz, 1 H), 7.88 - 7.94 (m, 1 H), 7.33 - 7.47 (m, 2 H), 7.10 - 7.25 (m, 2 H), 6.91 (d, *J*=7.9 Hz, 2 H), 6.03 - 6.16 (m, 1 H), 5.46 (dd, *J*₁=17.2, *J*₂=1.7 Hz, 1 H), 5.29 - 5.38 (m, 1 H), 4.58 (dt, *J*₁=5.3, *J*₂=1.5 Hz, 2 H), 4.46 (br. s., 4 H), 1.45 - 1.60 (m, 9 H)

RMN ¹³C (CDCl₃, 125 MHz) δ(ppm) = 162.6, 158.2, 155.9, 150.6, 144.5, 135.5, 133.3, 132.8, 131.0, 129.7, 129.5, 129.1, 128.8, 128.3, 127.5, 125.9, 124.2, 122.4, 117.8, 114.9, 80.5, 68.9, 49.4, 28.4

HR-MS ESI $[M+H]^+_{\text{calc}} = 543.2238$ ESI $[M+H]^+_{\text{found}} = 543.2242$
 $[M+Na]^+_{\text{calc}} = 565.2058$ ESI $[M+Na]^+_{\text{found}} = 565.2064$

Compound 7



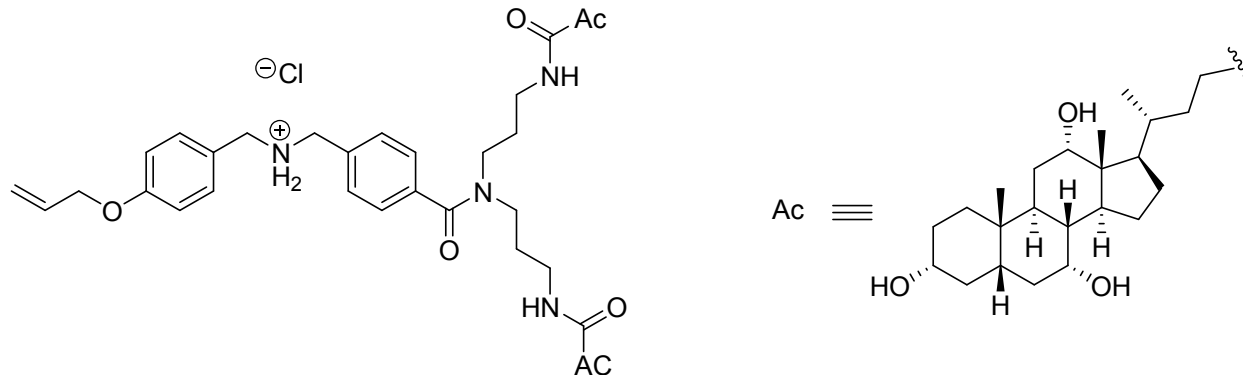
To a solution of *Umbrella* (2 eq, 0.550 mmol, 500 mg) in dry DMF (10 ml) heated to 50°C was added the triethylamine (15 eq, 3.64 mmol, 0.51 ml) and the **6** (1 eq, 0.243 mmol, 132 mg). The solution was heated to 70 °C for 24 h, cooled to room temperature and concentrated under vacuum. Purification by flash chromatography (SiO₂, CHCl₃/MeOH 9:1) yielded an orange solid (304 mg, 97%).

RMN ¹H (MeOD, 400 MHz) $\delta(\text{ppm}) = 7.26 - 7.41$ (m, 4 H), 7.10 - 7.22 (m, 2 H), 6.92 (dd, $J_1=8.6$, $J_2=2.0$ Hz, 2 H), 6.01 - 6.14 (m, 1 H), 5.44 (d, $J=20$ Hz, 1 H), 5.28 (d, $J=12$ Hz, 1 H), 4.53 - 4.58 (m, 2 H), 4.31 - 4.50 (m, 4 H), 3.96 (m, 2 H), 3.81 (br. s., 2 H), 3.51 - 3.62 (m, 2 H), 3.39 (br. s., 2 H), 2.97 - 3.08 (m, 2 H), 1.20 - 2.37 (m, 64 H), 0.96 - 1.09 (m, 9 H), 0.93 (d, $J=1.8$ Hz, 6 H), 0.71 ppm (d, $J=14.1$ Hz, 6 H)

RMN ¹³C (MeOD, 125 MHz) $\delta(\text{ppm}) = 175.5, 175.3, 172.6, 158.1, 156.2, 135.3, 133.5, 128.9, 128.5, 127.7, 127.3, 126.4, 116.1, 114.5, 80.3, 72.6, 71.4, 68.4, 67.7, 67.4, 46.6, 46.1, 42.6, 41.8, 41.6, 39.6, 39.0, 36.6, 36.3, 35.5, 35.1, 34.5, 32.8, 31.9, 29.8, 28.2, 27.4, 27.1, 26.5, 25.1, 22.9, 21.8, 16.4, 11.7, 7.8$

HR-MS ESI $[M+H]^+_{\text{calc}} = 1291.882$ ESI $[M+H]^+_{\text{found}} = 1291.874$
 $[M+Na]^+_{\text{calc}} = 1313.864$ ESI $[M+Na]^+_{\text{found}} = 1313.861$

Compound 8



A 4M HCl solution (75 eq, 11.4 mmol, 0.95 ml) was added dropwise to a solution of the **7** (1 eq, 0.15 mmol, 196 mg) in EtOH at 0°C. The mixture was stirred at room temperature for 24 h. The mixture was evaporated under vacuum the crude product was washed with CH₂Cl₂ (10 ml) and filtered to obtain the chloride salt of the umbrella thread. (179 mg, **quantitative yield**)

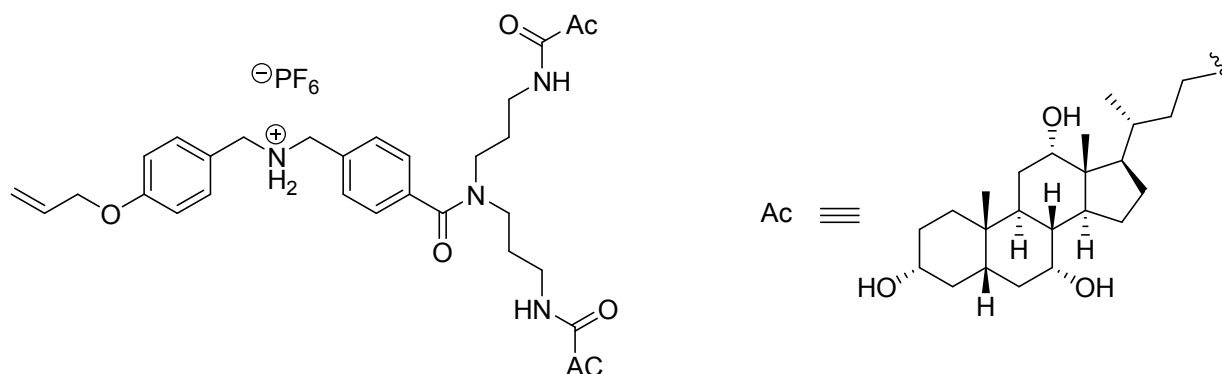
RMN ¹H (MeOD, 400 MHz) δ(ppm) = 7.67 (d, *J*=6.6 Hz, 2 H), 7.43 - 7.52 (m, 4 H), 7.04 (d, *J*=8.6 Hz, 2 H), 6.01 - 6.14 (m, 1 H), 5.42 (dd, *J*₁=17.2, *J*₂=1.3 Hz, 1 H), 5.27 (d, *J*=10.4 Hz, 1 H), 4.60 (d, *J*=5.3 Hz, 2 H), 4.33 (br. s., 2 H), 4.26 (s, 2 H), 3.97 (br. s., 2 H), 3.82 (br. s., 2 H), 3.58 (m, 2 H), 3.37 - 3.45 (m, 2 H), 3.24 - 3.31 (m, 4 H), 2.99 (br. s., 2 H), 1.23 - 2.54 (m, 54 H), 0.98 - 1.10 (m, 8 H), 0.93 (s, 6 H), 0.73 (br. s., 6 H).

RMN ¹³C (MeOD, 125 MHz) δ(ppm) = 175.6, 175.4, 171.9, 159.8, 137.6, 133.2, 132.5, 131.3, 130.2, 126.8, 122.8, 116.4, 114.8, 72.6, 71.4, 68.5, 67.7, 50.5, 50.1, 46.6, 46.1, 42.6, 41.8, 41.6, 39.6, 39.1, 36.6, 36.3, 35.6, 35.1, 34.5, 32.9, 32.6, 32.0, 29.9, 28.3, 27.4, 27.1, 26.5, 22.8, 21.8, 16.4, 11.7

HR-MS ESI [M*]⁺_{calc} = 1191.8295
[M+H]²⁺_{calc} = 596.9223

ESI [M*]⁺_{found} = 1191.8282
ESI [M+H]²⁺_{found} = 596.9213

Umbrella monomer (UM)



Potassium hexafluorophosphate (2 eq, 0.49 mmol, 91 mg) was added to a solution of **8** (1 eq, 0.25 mmol, 304 mg) in 1 ml of methanol. The yellow solution was stirred at room temperature for 24 h. The solution was evaporated under vacuum then the crude product was dissolved in water (5 ml) and filtered to obtain **UM**. (294 mg, **quantitative yield**)

RMN ^1H (MeOD, 400 MHz) $\delta(\text{ppm}) = 7.59$ (d, $J=8.0$ Hz, 2H), 7.50 (d, $J=8.0$ Hz, 2H), 7.44 (d, $J=8.0$ Hz, 2H), 7.05 (d, $J=8.0$ Hz, 2H), 6.08 (m, 1H), 5.42 (dq, $J=16.0, 4.0$ Hz, 1 H), 5.28 (dq, $J=12.0, 4.0$ Hz, 1 H), 4.60 (dt, $J=4.0$ Hz, 2 H), 4.31 (m, 2 H), 4.23 (br. s., 2 H), 3.97 (br. s., 2 H), 3.82 (br. s., 2 H), 3.59 (m, 2 H), 3.40 (m, 2 H), 3.27 (m, 4 H), 2.99 (m, 2 H), 1.20 - 2.37 (m, 54 H), 0.98 - 1.10 (m, 8 H), 0.94 (s, 6 H), 0.73 (s, 6 H)

RMN ^{13}C (MeOD, 125 MHz) $\delta(\text{ppm}) = 172.5, 172.0, 159.7, 137.7, 133.1, 132.6, 131.3, 130.1, 127.0, 122.9, 116.3, 115.1, 72.6, 71.4, 68.5, 67.6, 50.5, 50.0, 46.6, 46.1, 42.5, 41.6, 41.7, 39.6, 39.1, 36.5, 36.2, 35.6, 35.1, 34.5, 32.9, 32.6, 31.9, 29.8, 28.3, 27.3, 27.0, 26.4, 22.8, 21.7, 16.4, 11.6$

HR-MS ESI $[\text{M}^*]^+_{\text{calc}} = 1191.8295$ ESI $[\text{M}^*]^+_{\text{found}} = 1191.8272$
 $[\text{M}+\text{H}]^{2+}_{\text{calc}} = 596.9223$ ESI $[\text{M}+\text{H}]^{2+}_{\text{found}} = 596.9205$
 $[\text{M}^*]^-_{\text{calc}} = 144.9647$ ESI $[\text{M}^*]^-_{\text{found}} = 144.9654$

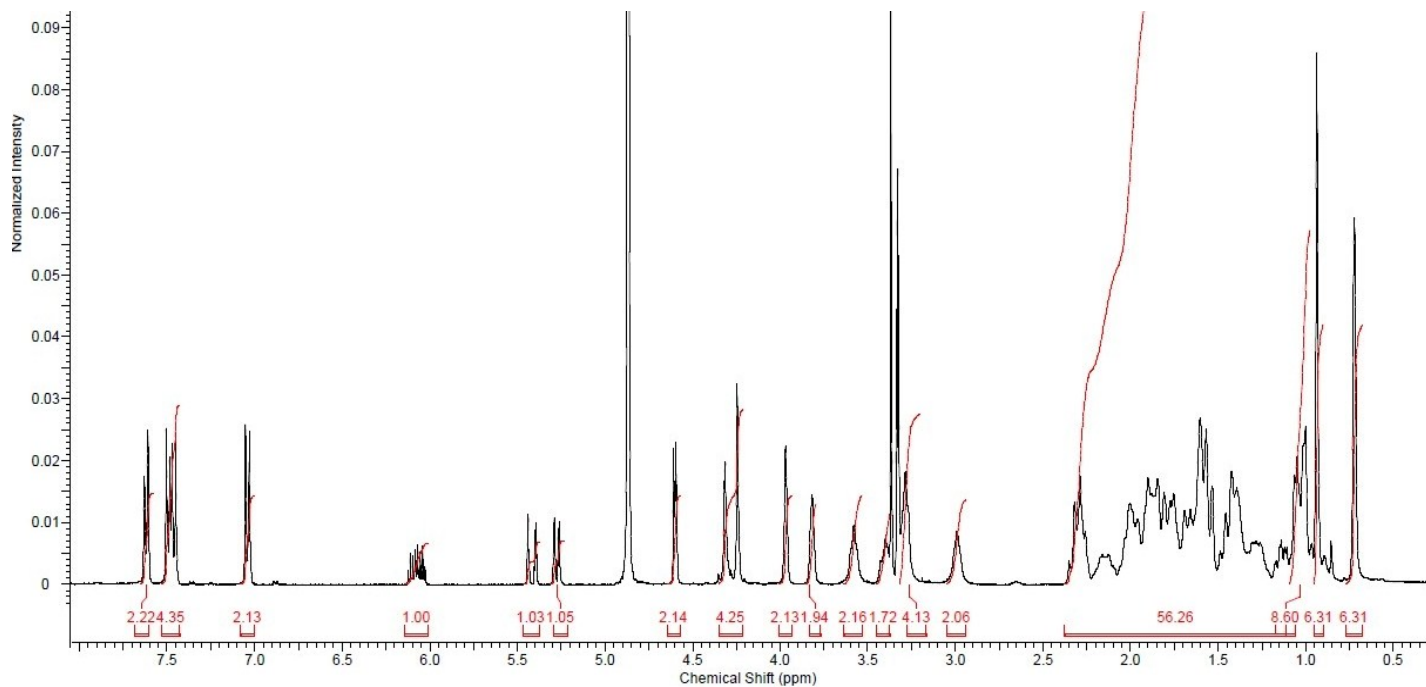


Figure S1 : ^1H NMR spectra of UM

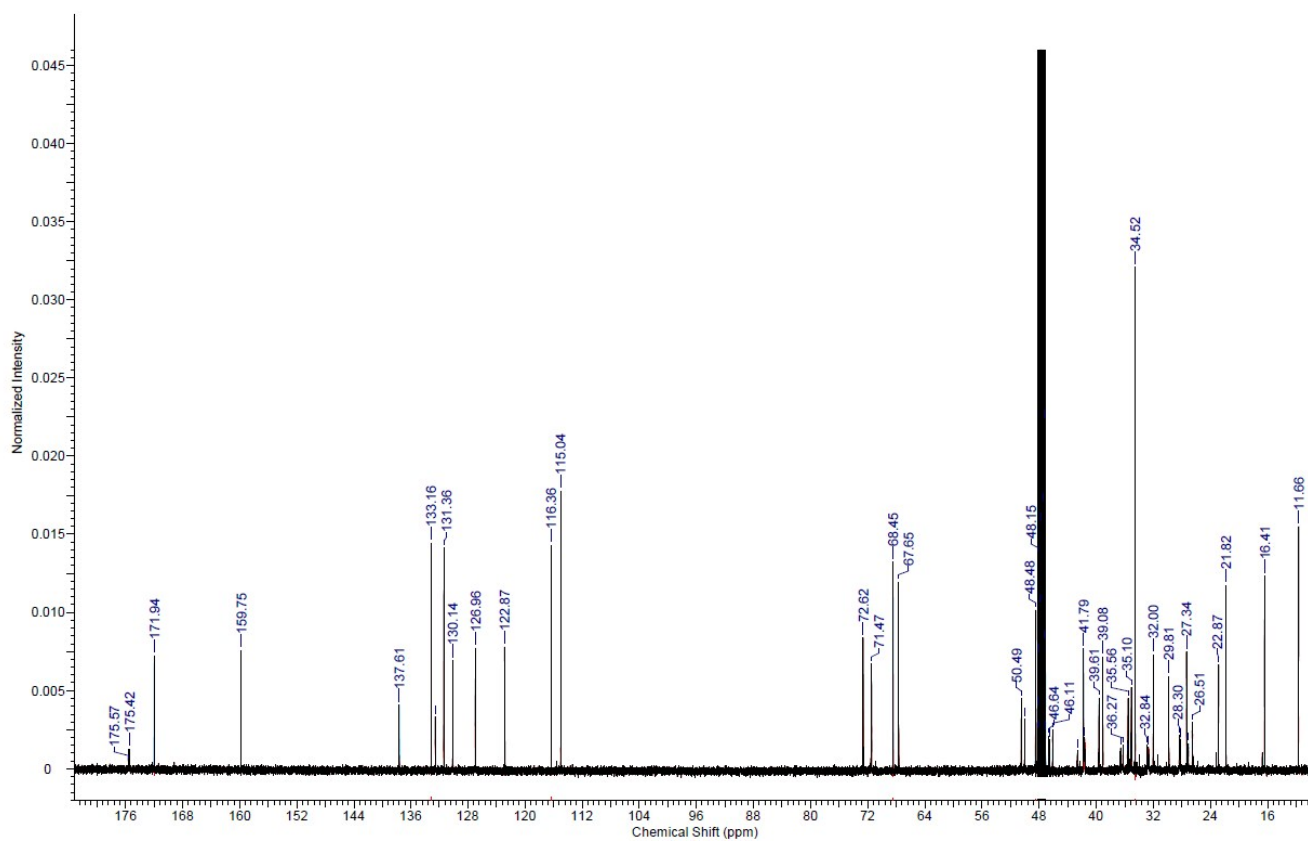
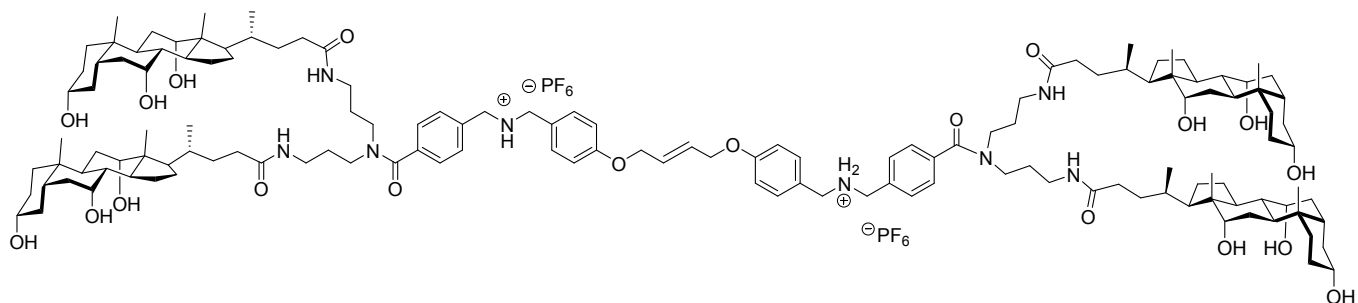


Figure S2 : ^{13}C NMR spectra of UM

Umbrella dimer (UD)



Grubb's second generation catalyst (10 mol%, $3.86 \cdot 10^{-3}$ mmol, 3.3 mg) and *p*-benzoquinone (10 mol%, $3.86 \cdot 10^{-3}$ mmol, ≈ 1 mg) were added to a solution of **UM** (1 eq, 0.04 mmol, 51.7 mg) in 3 ml CH₂Cl₂. The brown solution was stirred at 40°C for 24h. The mixture was cooled to room temperature and filtered. The brown solid was dissolved in methanol and evaporated under vacuum to give brown oil. The product was purified by preparative TLC (SiO₂, CH₂Cl₂/MeOH 9:1), to yield **UD** (90.9 mg, **quantitative yield**).

RMN ¹H : (MeOD, 700 MHz) δ (ppm) = 7.48 (m, 4 H), 7.39 (t, $J=7.4$ Hz, 4 H), 7.28 (m, 3 H), 7.20 (d, $J=9.0$ Hz, 2H), 6.94 (m, 3 H), 6.79 (d, $J=9.0$ Hz, 2 H), 4.61 (m, 2 H), 3.74 - 3.99 (m, 20 H), 3.57 (m, 5 H), 3.39 (m, 5 H), 3.29 (m, 8 H), 3.01(m, 5 H), 0.84 - 2.34 (m, 205 H), 0.71 ppm (m, 12 H).

RMN ¹³C : (MeOD, 175 MHz) δ (ppm) = 175.4, 175.3, 172.6, 129.8, 129.7, 128.7, 126.3, 115.0, 114.6, 114.5, 72.6, 71.5, 67.6, 51.5, 46.6, 46.1, 42.6, 41.8, 41.6, 39.6, 39.1, 36.6, 36.2, 35.5, 35.1, 34.5, 34.5, 32.8, 32.7, 31.9, 31.7, 29.8, 29.4, 29.3, 29.2, 29.1, 28.3, 28.2, 27.3, 27.1, 26.5, 26.2, 22.9, 22.3, 21.9, 21.8, 16.4, 13.0, 11.7

Maldi TOF-TOF [M-PF₆]⁺_{calc} = 2501.60 [M-PF₆]⁺_{found} = 2501.57

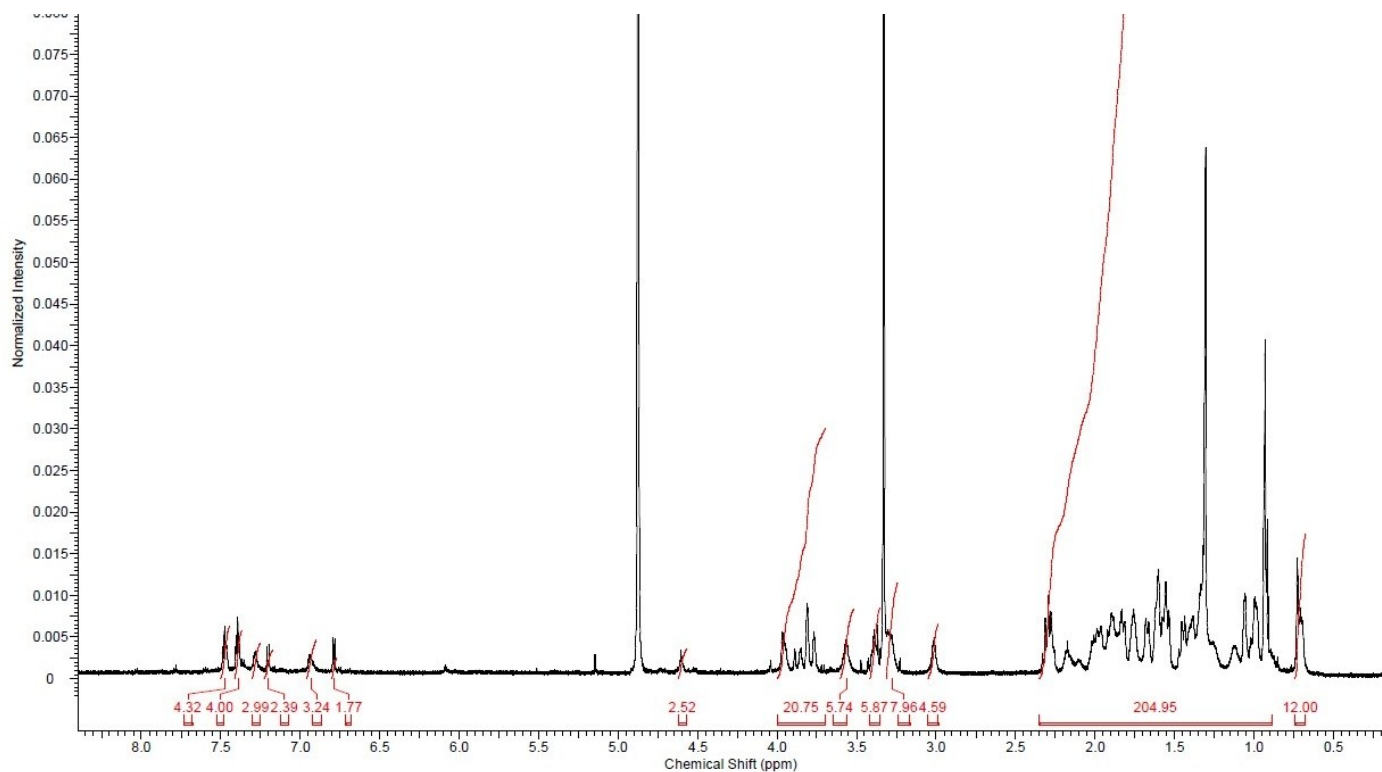


Figure S3 : ^1H NMR spectra of UD

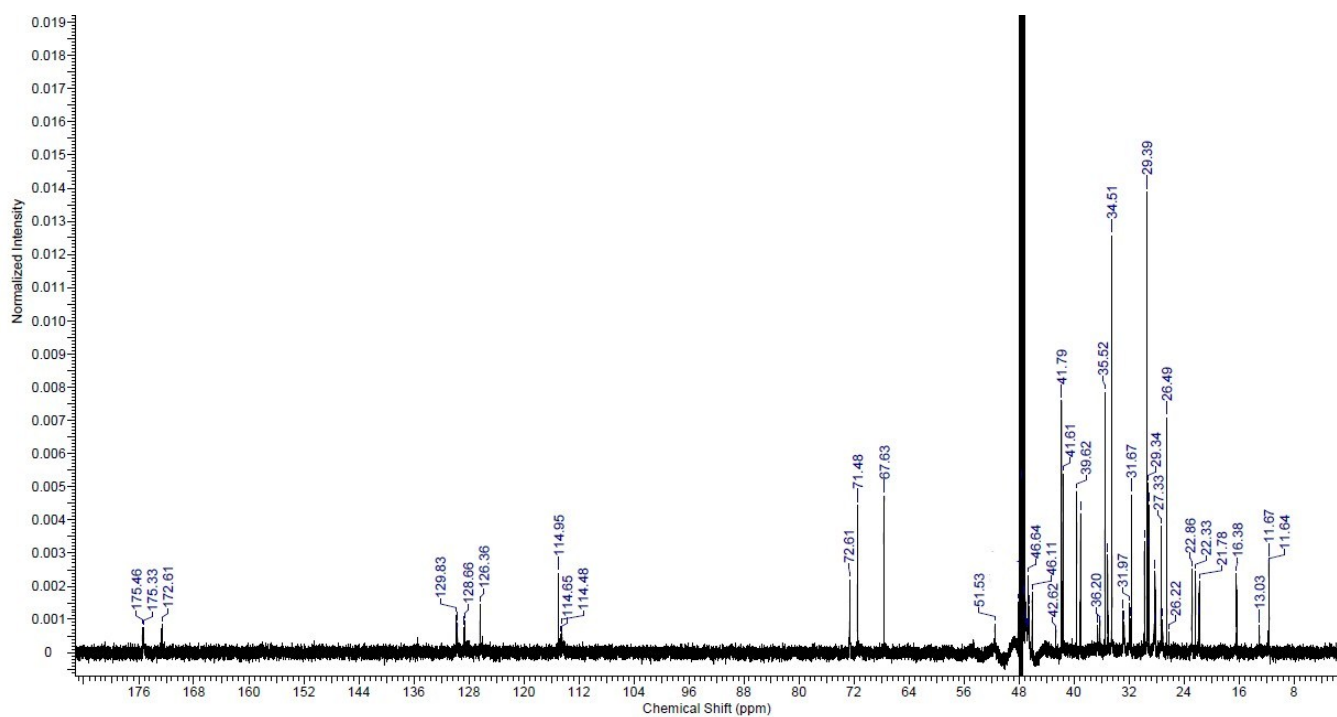


Figure S4 : ^{13}C NMR spectra of UD

Ion transport experiments

The procedures for EYPC liposomes formation and chloride transport assays are based on those previously reported in the literature.⁵

Preparation of EYPC large unilamellar vesicles (LUVs) for lucigenin based assays

A phospholipid film was formed by evaporating 1 ml chloroform solution containing 25 mg of EYPC, under vacuum at 25°C during 2 hours. The lipid film was then hydrated with 1 mL of a 2 mM lucigenin containing a NaCl (100 mM) and phosphate buffer solution (10 mM, pH = 6.2). The obtained suspension was subjected to at least 8 freeze/thaw/vortex cycles (1 cycle = 1 minute at -78°C followed by 1 minute at 35°C and 1 minute in vortex). The solution was then extruded through a 100 nm polycarbonate membrane 21 times until the solution was transparent and passed down a Sephadex G-25 column to remove extravesicular lucigenin dye. The liposomes were eluted with a solution containing 100 mM of NaCl and 10 mM of phosphate buffer (pH = 6.2). 5.3 mL of liposomes solution were isolated after separation. The stock solution was diluted to obtain a 5 mM lipid solution, assuming all EYPC was incorporated into the liposomes.

Chloride transport assays with EYPC LUVs

A 50 µL aliquot of the solution of EYPC LUVs (5 mM) were added to a 2.5 mL gently stirred thermostated to 25°C buffer solution containing 100 mM NaNO₃ and 10 mM phosphate salt (pH = 6.2). The lucigenin fluorescence was monitored by excitation at $\lambda_{\text{ex}} = 372$ nm and the emission was recorded at $\lambda_{\text{em}} = 503$ nm. At $t = 50$ s, 50µL of a solution of transporter at different concentrations in MeOH were added. At $t = 300$ s, 100 µL of a Triton-X 5% solution were added to lyse all liposomes to obtain the maximum of lucigenin fluorescence. The temperature was set to 25°C.

Experiments were repeated in triplicate and all the reported traces are the average of the three independent trials.

Conversion of fluorescence data into % of chloride efflux

The residual fluorescence of the transporter was subtracted in each transport assays, then the curves were normalized using the following equation:

$$\%Cl_{\text{efflux}} = 100 * \frac{I - I_0}{I_{\text{max}} - I_0}$$

With I = fluorescence intensity

I_0 = fluorescence intensity before addition of the transporter

I_{max} = fluorescence intensity at the end of the experiment.

Chloride transport assays with EYPC LUVs in different phosphate buffers: modification of external anions

A 100 μL aliquot of the solution of EYPC LUVs (5mM) were added to a 2.5 mL gently stirred thermostated to 25°C buffer solution containing 100 mM NaX ($\text{X} = \text{NO}_3^-$, HCO_3^- , ClO_4^-) or 50 mM Na_2X (SO_4^{2-}) and 10 mM phosphate salt (pH = 6.2). The lucigenin fluorescence was monitored by excitation at $\lambda_{\text{ex}} = 372$ nm and the emission was recorded at $\lambda_{\text{em}} = 503$ nm. At $t = 50$ s, 45 μL of solution of transporter in MeOH was added. At $t = 300$ s, 100 μL of a Triton-X 5% solution were added to lyse all liposomes and obtained the maximum of lucigenin fluorescence.

Experiments were repeated in triplicate and all traces reported are the average of the three trials.

Chloride transport assays and the conversion of fluorescence data for this experiment are identical of previous experiment (“Chloride transport assays with EYPC LUVs”)

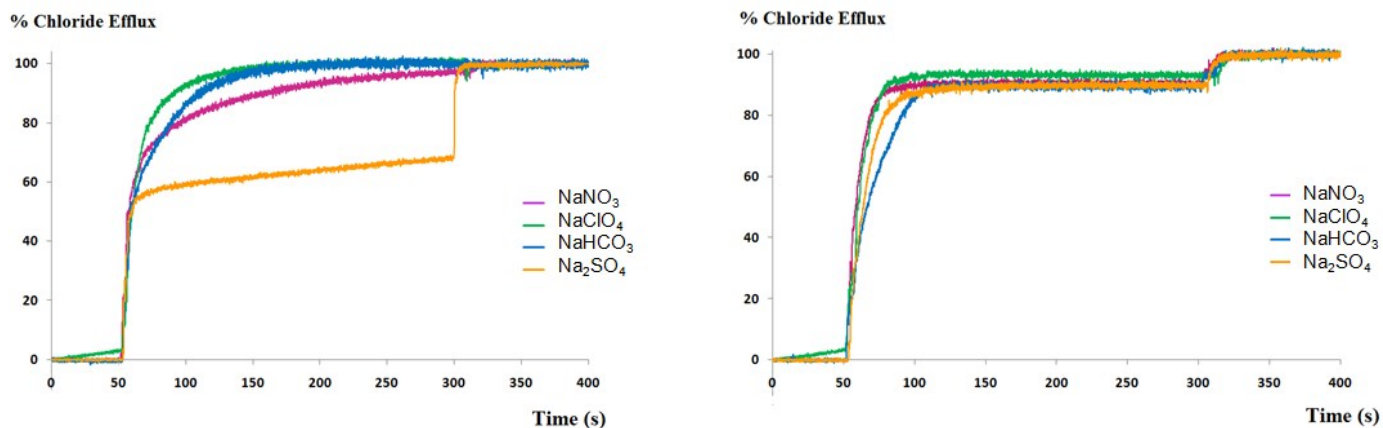


Figure S5: Relative chloride transport activity of **UM** (left) and **UD** (right) at 13.5 mol% (relative to the EYPC concentration) in liposomes buffered in different external solutions: NaNO_3 (pink), NaClO_4 (green), NaHCO_3 (blue) and Na_2SO_4 (orange).

Chloride transport assays with EYPC LUVs in different Phosphate buffer: modification of the external cations

A 100 μL aliquot of the solution of EYPC LUVs (5mM) were added to a 2,5 mL gently stirred thermostated to 25°C buffer solution containing 100 mM XNO_3 ($\text{X} = \text{Na}^+, \text{Cu}^+$) or 50 mM X_2NO_3 (Ca^{2+}) and 10 mM phosphate salt (pH = 6.2). The lucigenin fluorescence was monitored by excitation at $\lambda_{\text{ex}} = 372$ nm and the emission was recorded at $\lambda_{\text{em}} = 503$ nm. At $t = 50$ s, 45 μL of solution of transporter in MeOH was added. At $t = 300$ s, 100 μL of a Triton-X 5 % solution were added to lyse all liposomes and obtained the maximum of lucigenin fluorescence.

Experiments were repeated in triplicate and all traces reported are the average of the three trials.

Chloride transport assays and the conversion of fluorescence data for this experiment are identical of previous experiment (“Chloride transport assays with EYPC LUVs”).

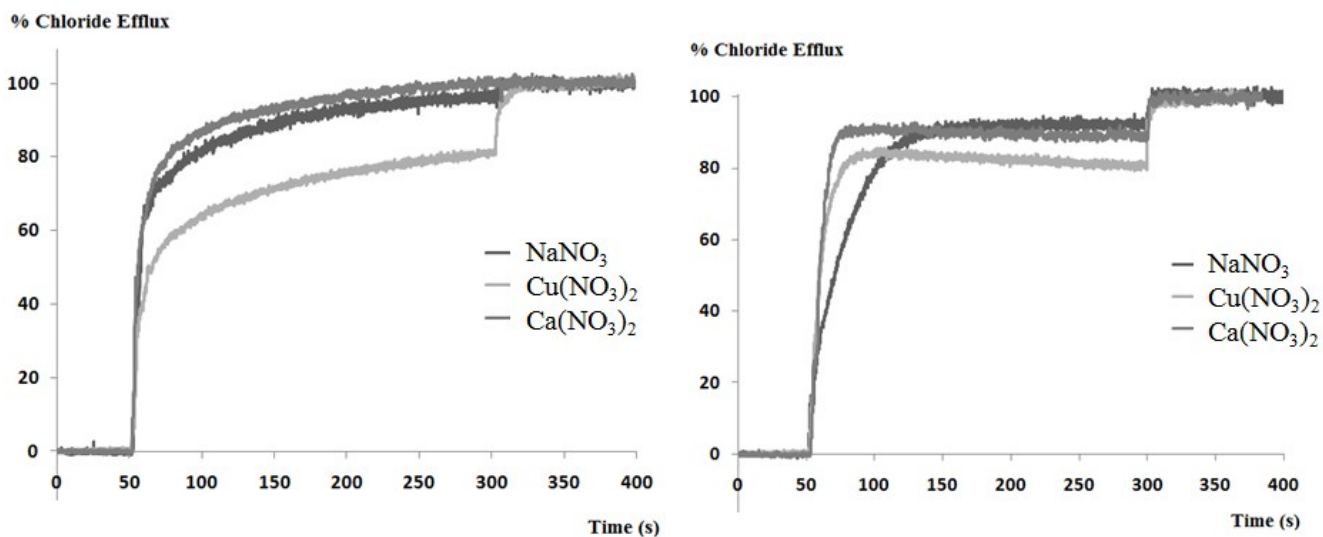


Figure S6: Relative chloride transport activity of **UM** (left) and **UD** (right) at 13.5 mol% (relative to EYPC concentration) in liposome buffered in different external solutions: NaNO_3 (dark grey), $\text{Cu}(\text{NO}_3)_2$ (light grey), $\text{Ca}(\text{NO}_3)_2$ (black).

Preparation of EYPC/Cholesterol 7/3 large unilamellar vesicles (LUVs) for lucigenin based assays

An EYPC/cholesterol liposomes (7/3, w/w, 30.4 mg) were formed by evaporating under vacuum at 25°C during 2 hours a solution of this lipids. The lipid film was then hydrated with 0.750 mL of a 2 mM lucigenin solution diluted in NaCl (100 mM) and phosphate buffer (10 mM, pH = 6.2) The obtained suspension was subjected to 8 freeze/thaw/vortex cycles (1 cycle = 1 minute at -78 °C followed by 1 minute at 37 °C and 1 minute in vortex). The solution was then extruded through a 100 nm polycarbonate membrane 21 times until the solution was transparent. Then, liposomes are passed down in a Sephadex G-25 column to remove extravesicular lucigenin dye. The eluant was a solution containing 100 mM of NaCl and 10 mM of phosphate buffer. 5.4 mL solution of liposomes were isolated after separation. The stock solution was diluted to obtain a concentration of 5 mM in lipids, assuming all EYPC and cholesterol were incorporated into the liposomes.

Chloride transport assays and the conversion of fluorescence data for this experiment are identical of previous experiment (“Chloride transport assays with EYPC LUVs”).

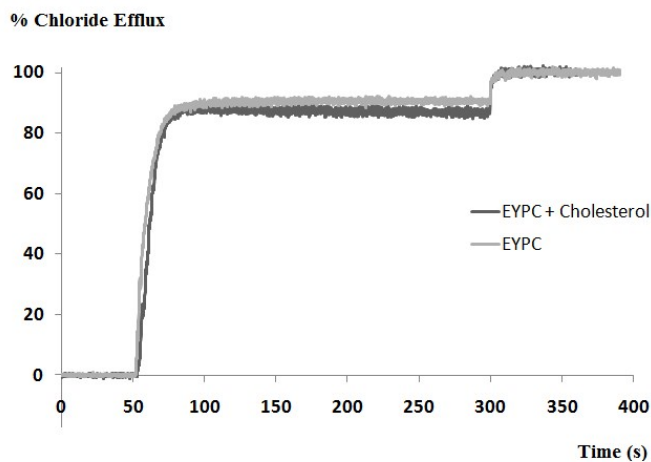
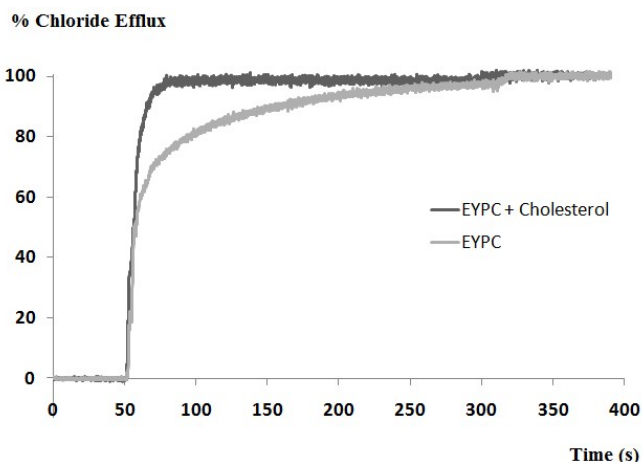


Figure S7: Relative chloride transport activity of **UM** (left) and **UD** (right) at 13.5 mol% (relative to EYPC concentration) in EYPC liposome (grey) and EYPC/Cholesterol (black).

Preparation of EYPC large unilamellar vesicles (LUVs) for carboxyfluorescein (CF) based assays

A phospholipid film was formed by evaporating 1 ml chloroform solution containing 25 mg of EYPC, under vacuum at 25°C during 2 hours. The lipid film was then hydrated with 0.750 mL of a 20 mM Carboxyfluorescein solution diluted in NaCl (100 mM) and phosphate buffer (20 mM, pH = 7.3). The obtained suspension was subjected to 10 freeze/thaw/vortex cycles (1 cycle = 1 minute at -78°C followed by 1 minute at 35°C and 1 minute in vortex). The solution was then extruded through a 100 nm polycarbonate membrane 21 times until the solution was transparent and passed down a Sephadex G-25 column to remove extravesicular carboxyfluorescein dye. The liposomes were eluted with a solution containing 100 mM of NaCl and 20 mM of phosphate buffer. 6.2 mL of liposomes solution were isolated after separation. The stock solution was diluted to obtain a 5 mM lipid solution, assuming all EYPC was incorporated into the liposomes.

Carboxyfluorescein transport assays with EYPC LUVs

A 10 μL aliquot of the solution of EYPC LUVs (5mM) were added to a 3 mL gently stirred thermostated to 25°C buffer solution containing 100 mM NaCl and 20 mM phosphate salt (pH = 7.3). The carboxyfluorescein fluorescence was monitored by excitation at $\lambda_{\text{ex}} = 497$ nm and the emission was recorded at $\lambda_{\text{em}} = 520$ nm. At $t = 50$ s, 5 μL of a solution of transporter in MeOH was added. At $t = 600$ s, 100 μL of a Triton-X 5% solution were added to lyse all liposomes and obtained the maximum of carboxyfluorescein fluorescence.

Experiments were repeated in triplicate and all traces reported are the average of the three trials.

Preparation of EYPC large unilamellar vesicles (LUVs) for HPTS based assays

A phospholipid film was formed by evaporating under vacuum at 25°C during 2 hours, a solution of chloroform containing 25 mg of EYPC. The lipid film was then hydrated with 0.750 mL of a 0.1 mM HPTS solution diluted in NaCl (100 mM) and phosphate buffer (10 mM, pH = 6.2). The obtained suspension was subjected to 8 freeze/thaw/vortex cycles (1 cycle = 1 minute at -78°C followed by 1 minute at 35°C and 1 minute in vortex). The solution was then extruded through a 100 nm polycarbonate membrane 21 times until the solution was transparent and passed down a Sephadex G-25 column to remove extravesicular HPTS dye. The eluent was a solution containing 100 mM of NaCl and 10 mM of phosphate buffer. 5,2 mL of liposome solution were isolated after separation. The stock solution was diluted with the buffer to obtain a concentration of 5mM in lipid, assuming all EYPC was incorporated into the liposomes.

HPTS assays with EYPC LUVs

A 100 μ L aliquot of the previous solution of EYPC SUVs (5 mM) were added to a 2.5 mL gently stirred thermostated to 25°C buffer solution containing 100 mM **NaX** ($X=NO_3^-,HCO_3^-, ClO_4^-$) or 50 mM **Na₂X** (SO_4^{2-} .) and 10 mM phosphate salt (pH = 6.2). The HPTS fluorescence was monitored by excitation at $\lambda_{ex} = 403$ nm and $\lambda_{ex} = 460$ nm and the emission was recorded at $\lambda_{em} = 510$ nm. At $t = 50$ s, 45 μ L of solution of transporter in MeOH were added. At $t = 300$ s, 100 μ L of a Triton-X 5% solution were added to lyse all liposomes.

Experiments were repeated in triplicate and all traces reported are the average of the three trials.

Determination of the minimal inhibitory concentration (MIC)

5 mL of lysogeny broth (LB) medium were inoculated with *Escherichia coli* (SK037 strain) or *Bacillus thuringiensis* (HD73 strain). The precultures were grown overnight at 37°C under stirring, and resuspended in 75 mL of a fresh LB medium before growing at 37°C during 2 more hours. After this time, the cultures were rediluted in fresh LB medium to obtain an optical density at 600 nm (OD₆₀₀) of 0.1-0.2.

Assays were performed in 96-well culture plates. Each well was filled with 185 µL bacterial cultures, 10 µL MiliQ water and 5 µL of DMSO or compounds in DMSO solution, as the final volume in each well was 200 µL and the concentration of DMSO was max 5%.

The plates were stirred in a thermostated incubator at 37°C and the OD₆₀₀ was monitored at t = 0 h and t = 24 h. Every experiment was repeated in triplicates on independent bacterial cultures. The MICs were determined as the minimal concentration at which no bacterial growth was detected.

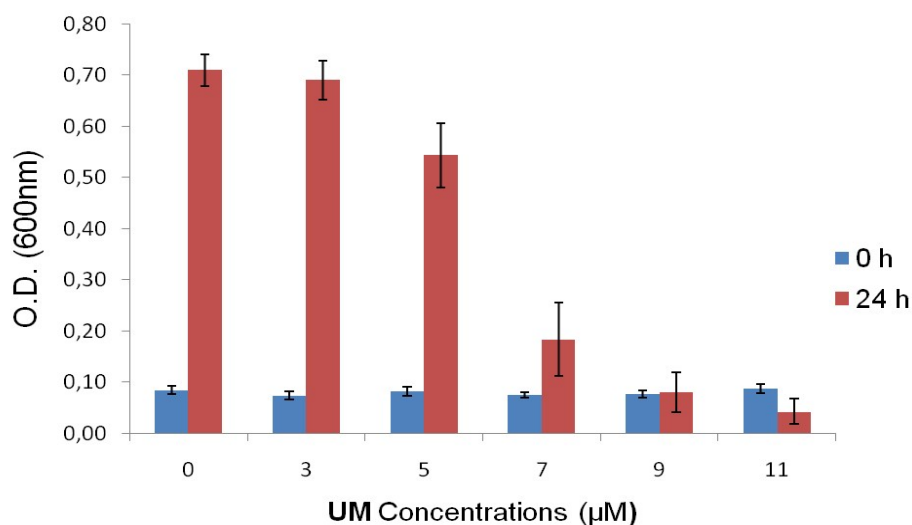


Figure S8: Dose-dependent growth inhibition of *Bacillus thuringiensis* by UM.

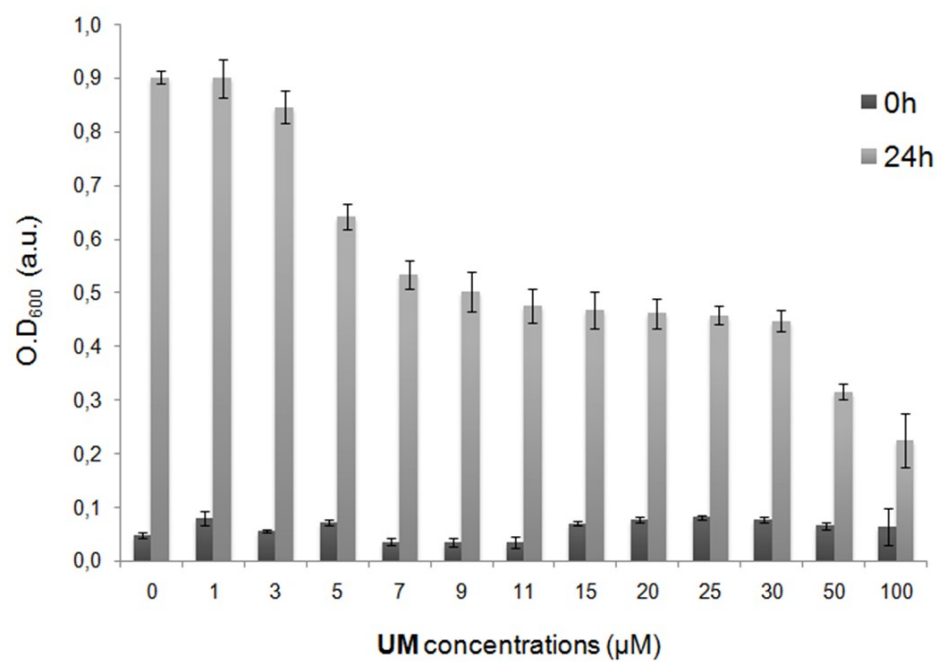


Figure S9 : Dose-dependent growth inhibition of *Escherichia coli* by UM.

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