

Cationic Cyclocholamides; Toroidal Facial Amphiphiles with Potential for Anion Transport

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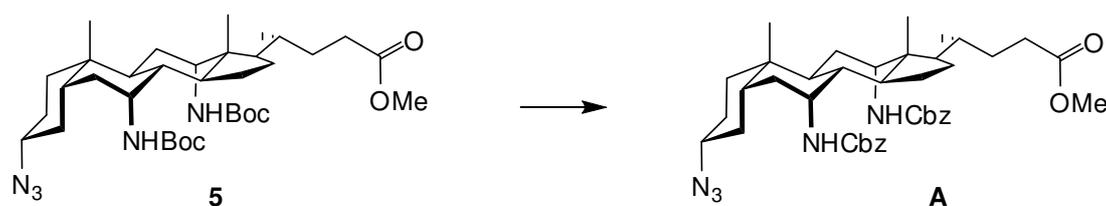
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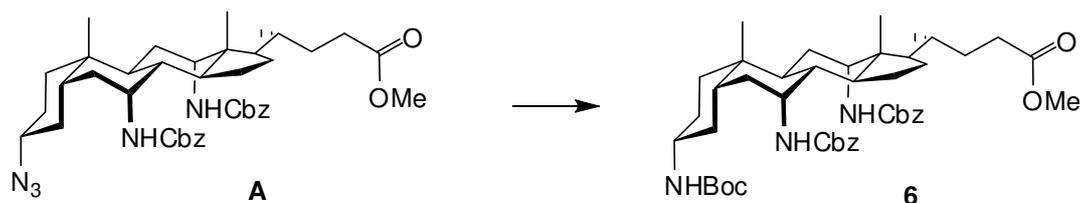
Supplementary Information

Synthesis of Macrocycles 3

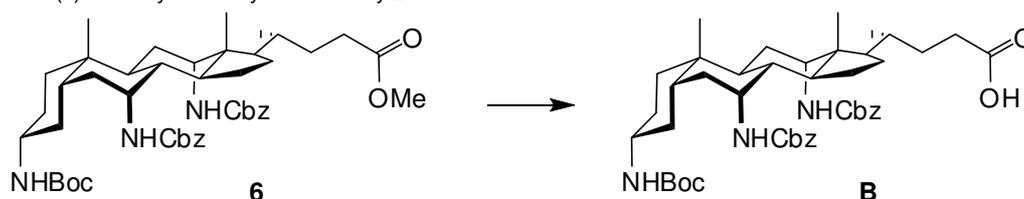
General: Reagents were obtained from commercial suppliers and used without further purification unless otherwise stated. When quoted, dry solvents were obtained from an Anhydrous Engineering Solvent Purification System, or by reflux over a standard drying agent and then distillation as described by Perrin.¹ ¹H and ¹³C NMR spectra were recorded using Jeol Delta GX400 MHz, Jeol Eclipse 400 MHz or Jeol Alpha 500 MHz spectrometers. Chemical shifts (δ) are reported in ppm relative to the residual signal of CHCl₃ (δ_{H} 7.26, δ_{C} 77.0), MeOH (δ_{H} 3.31, δ_{C} 49.0), or TMS (δ_{H} 0.00, δ_{C} 0.0). Coupling constants are expressed in Hz. IR spectra were measured on a Perkin-Elmer Spectrum One spectrometer using ATR-IR. Mass spectrometry was carried out using either an Applied Biosystems QStar XL QQQTOF spectrometer equipped with an Advion Biosciences nanomate nanospray inlet device or a Quattro spectrometer manufactured by VG equipped with an ESI inlet device. Accurate masses were obtained using Bruker Daltonics Apex 4e 7.0T FT-MS, FTICR-MS equipped with an ESI inlet device. HPLC was carried out using Gilson manufactured 321 pump stage, 156 UV/Vis detection stage, 402 syringe pump and 231XL sample injector. Melting points were measured on a Gallenkamp melting-point apparatus and are quoted as uncorrected values. Analytical TLC was performed using Merck DC Alufolien Kieselgel 60 F₂₅₄ 0.2 mm aluminium backed sheets pre-coated with silica gel. Ratios of mixed solvent systems are quoted as v/v unless otherwise stated. UV chromophoric compounds were visualised under 254 nm UV light, high molecular weight compounds were visualised by staining with phosphomolybdic acid (5 % w/v in ethanol) and subsequent charring. Amino compounds were visualised by staining with ninhydrin (5 % w/v in ethanol). Flash chromatography refers to chromatography carried out using DAVISIL 60 silica gel manufactured by Fisher Scientific (35-70 micron) according to the method of Still *et al.*² All reactions were performed under nitrogen and at room temperature unless otherwise indicated.



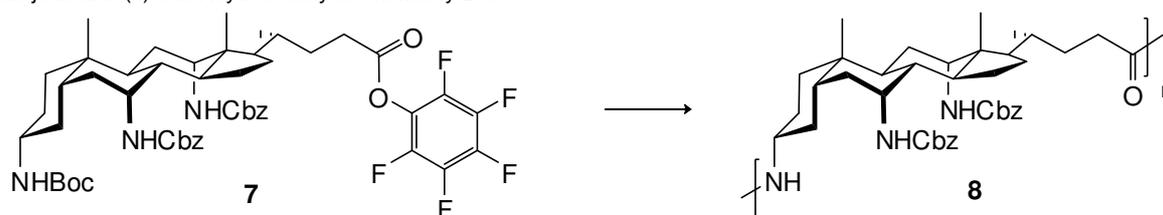
Methyl 3 α -azido-7 α ,12 α -di[N-(benzyloxycarbonyl)amino]-5 β -cholan-24-oate A. Methyl 3 α -azido-7 α ,12 α -di[N-(*t*-butyloxycarbonyl)amino]-5 β -cholan-24-oate **5**³ (1.0 g, 1.55 mmol) was dissolved in DCM (20 ml). To the stirred solution was added TFA (10 ml) in a dropwise fashion. The clear colourless solution was then sealed under air and allowed to stir overnight. The resulting pale yellow solution was concentrated in vacuo to produce a yellow oil. Repeated addition-evaporation of DCM (5 \times 50 ml) then chloroform (2 \times 50 ml) gave a cream/white foam (1.12 g). The foam was dissolved in DCM (20 ml) and washed with sat. aq. NaHCO₃ (2 \times 10 ml) whereupon some effervescence was observed. The organic phase was then dried over anhydrous sodium sulphate and concentrated in vacuo to form a white powder (690 mg). This was dissolved in THF (20 ml) and Cbz-Cl (660 μ l, 4.5 mmol) was added. Sat. aq. NaHCO₃ (10 ml) solution was then added and the mixture was stirred for 48 h. The phases were separated and the aqueous layer extracted with DCM (2 \times 20 ml) and combined with the THF layer. The organic phases were then concentrated in vacuo before being redissolved in DCM (20 ml) and washed sequentially with dilute aq. HCl (20 ml), water (2 \times 20 ml) and finally brine (10 ml). Drying over anhydrous sodium sulphate, evaporation and purification by flash chromatography (1:4 ethyl acetate/hexane) gave the product as a white powder (1.04 g, 1.47 mmol, 95 %); R_f = 0.59 (1:1 EtOAc/Hex); m.p. 117-120 $^{\circ}$ C; ¹H NMR (400 MHz, CDCl₃, 25 $^{\circ}$ C, TMS): δ = 0.70 (d, ³ J (H,H) = 5.8 Hz, 3 H; 21-CH₃), 0.74 (s, 3 H; 18-CH₃), 0.95 (s, 3 H; 19-CH₃), 3.21-3.33 (m, 1 H; 3 β -H), 3.57 (s, 3 H; OCH₃), 3.71 (br s, 1 H; 7 β -H), 4.02-4.04 (m, 1 H; 12 β -H), 4.89-5.15 (m, 4 H; Ph-CH₂) 5.67 (br d, ³ J (H,H) = 6.8 Hz, 1 H; NH) 5.82 (br d, ³ J (H,H) = 9.8 Hz NH) 7.19-7.40 (m, 10 H; CH₂Ph); ¹³C NMR (100 MHz, CDCl₃, 25 $^{\circ}$ C, TMS): δ = 13.5 (C-18), 17.3 (C-21), 22.9 (C-19), 23.1, 27.1, 27.2, 27.3 (CH₂), 28.4 (CH), 30.3, 31.1, 31.8, 31.9 (CH₂), 34.8 (C-10), 35.3 (CH₂), 37.1, 41.8, 44.0 (CH), 44.7 (C-13), 47.9 (C-7), 49.6 (C-12) 51.6, 52.4 (CH), 53.8 (CH₃), 62.0 (C-3), 66.5 (CH₂ \times 2), (128.0, 128.2, 128.3, 128.5, 128.6), (CH \times 10), 136.8 (C \times 2), 156.1 (COCONH \times 2), 177.3 (C-24); IR (solid state): $\tilde{\nu}$ = 3377 cm⁻¹ (N-H), 2941, 2868 cm⁻¹ (C-H), 2089 cm⁻¹ (N₃), 1716, 1683 cm⁻¹ (C=O), 1519 cm⁻¹ (CONH), 1230 cm⁻¹ (C-O); MS (ESI+): m/z (%): 736 (100) [M + Na]⁺, HRMS (FTICR ESI+): m/z mass calculated for [M + Na]⁺ = 736.4044, found 736.4055.



Methyl 3 α -[N-(t-butyloxycarbonyl)amino]-7 α ,12 α -di[N-(benzyloxycarbonyl)amino]-5 β -cholan-24-oate 6. Azide A (1.0 g, 1.40 mmol) was dissolved in dry THF (10 ml). A solution of PMe_3 in THF (1 M, 2.4 ml) was added with stirring under a dry inert nitrogen atmosphere. The mixture was heated to 50 °C and left stirring for 1 d. Water (2 ml) was then added and the clear pale yellow solution allowed to stir for a further hour at 50 °C. The mixture was then allowed to cool to room temperature under air. $(\text{Boc})_2\text{O}$ (480 mg, 2.20 mmol) was added using 5 ml of THF to aid the addition. Sat. aq. NaHCO_3 (10 ml) was then added and the biphasic mixture allowed to stir vigorously for 2 days at room temperature. The aqueous layer was separated and washed three times with DCM (3 \times 10 ml). The organic layers were combined and concentrated in vacuo. The pale yellow residue was redissolved in DCM (20 ml) before washing sequentially with dilute aq. HCl (10 ml), water (10 ml) and brine (10 ml). The organic layer was then dried using anhydrous sodium sulphate before the solvent was removed yielding a white foam. Purification by flash chromatography (1:4 EtOAc/Hex) gave the product as a white powder (0.85 g, 1.10 mmol, 77 %); $R_f = 0.70$ (1:1 EtOAc/Hex); m.p. 103-105 °C; $^1\text{H NMR}$ (400 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$, 25 °C, TMS): δ 0.78 (s, 3 H; 18- CH_3), 0.83 (m, 3 H; 21- CH_3), 0.93 (s, 3 H; 19- CH_3), 1.45 (s, 9 H; $(\text{CH}_3)_3\text{C}$), 2.27 (m, 1 H; 3 β -H), 3.23 (br s, 1 H; 12 β -H), 3.62 (s, 3 H; OCH_3), 3.74 (br s, 1 H; 7 β -H), 4.93-5.42 (m, 4 H; Ph- CH_2), 7.25-7.33 m, 10 H; CH_2Ph); $^{13}\text{C NMR}$ (100 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$, 25 °C, TMS): $\delta = 13.4$ (C-18), 17.5 (C-21), 22.7 (C-19), 23.1, 27.1, 27.4, (CH_2), 28.2 (CH), 28.5 ($\text{C}(\text{CH}_3)_3$), 29.7, 30.9, 31.2, 31.6, 32.4 (CH_2), 34.4 (C-10), 35.0 (CH), 35.7, 35.9, 36.3 (CH_2), 36.7, 42.1, 44.1 (CH), 44.9 (C-13), 48.1 (C-7), 48.5 (C-12) 50.6, 51.6 (CH), 53.8 (CH_3), 66.4, 66.9 (CH_2), 79.2 ($(\text{CH}_3)_3\text{C}$), (127.7, 127.8, 128.0, 128.1, 128.5), ($\text{CH} \times 10$), 137.1 (C $\times 2$), 156.3 ($\text{COCONH} \times 2$), 156.4 ($(\text{CH}_3)_3\text{COCONH}$), 175.2 (C-24); IR (solid state): $\tilde{\nu} = 3380\text{cm}^{-1}$ (N-H), 2938, 2866 cm^{-1} (C-H), 1713, 1683 cm^{-1} (C=O), 1498 cm^{-1} (CONH), 1229 cm^{-1} (C-O); MS (ESI+): m/z (%): 810 (100) $[M + \text{Na}]^+$, HRMS (FTICR ESI+): m/z mass calculated for $[M + \text{Na}]^+ = 810.4668$, found 810.4676.



3 α -[N-(t-butyloxycarbonyl)amino]-7 α ,12 α -di[N-(benzyloxycarbonyl)amino]-5 β -cholan-24-oic acid B. Ester **6** (850 mg, 1.10 mmol) was dissolved in methanol (9 ml) and set to stir. To the clear colourless solution was added NaOH (260 mg) followed by water (1 ml). Upon addition of water a white precipitate was observed to form. The milky mixture was warmed to 40 °C and left to stir for 8 hours, after which the precipitate had redissolved to form a clear colourless solution. After a further 11 hours stirring NH₄Cl (250 mg) was added along with enough water to allow full dissolution. The solvents were then removed in vacuo to produce a white powder. The solid was redissolved in DCM/H₂O 2:1 (20 ml), the aqueous layer was extracted twice with DCM (10 ml), and the organic layers were combined and washed sequentially with acid water (pH 4) then brine. After drying over anhydrous sodium sulphate and removing the solvent in vacuo the product was produced as a white powder (816 mg, 1.05 mmol, 98 %); *R_f* = 0.23 (1:1 EtOAc/Hex); m.p. 144-146 °C; ¹H NMR (400 MHz, CDCl₃, 25 °C, TMS): δ 0.75 (s, 3 H; 18-CH₃), 0.81 (m 3 H; 21-CH₃), 0.88 (s, 3 H; 19-CH₃), 1.49 (s, 9 H; (CH₃)₃C), 2.50 (m, 1 H; 3 β -H), 3.26 (br s, 1 H; 7 β -H), 3.54 (br s, 1 H; 12 β -H), 4.08 (d, ³*J*(H,H) = 7.3 Hz, 1 H; NH), 4.32 (d, ³*J*(H,H) = 6.8 Hz, 1 H; NH) 4.77-5.55 (m, 4 H; Ph-CH₂), 6.72 (d, ³*J*(H,H) = 10.7 Hz, 1 H; NH), (7.25-7.33 m, 10 H; CH₂Ph); ¹³C NMR (100 MHz, CDCl₃, 25 °C, TMS): δ = 13.2 (C-18), 17.1 (C-21), 22.9 (C-19), 23.0, 26.7, 27.3, 27.4 (CH₂), 28.1 (CH), 28.3 (CH₂), 28.5 (C(CH₃)₃), 31.7, 33.2, (CH₂), 34.4 (C-10), 35.7, 36.7 (CH₂), 36.9, 41.9, 43.7 (CH), 44.6 (C-13), 47.9, 48.6 (CH), 49.2 (C-7), 50.8 (C-12), 54.2 (C-3), 66.0, 67.5 (CH₂), 79.2 ((CH₃)₃C), (127.4, 127.8, 128.3, 128.5, 128.7), (CH \times 10), 136.0 (C \times 2), 156.4 (COCONH \times 2), 158.4 ((CH₃)₃COCONH) 180.9 (C-24); IR (solid state): $\tilde{\nu}$ = 3355 cm⁻¹ (N-H), 2936, 2868 cm⁻¹ (C-H), 1684 cm⁻¹ (C=O), 1514 cm⁻¹ (CONH), 1232 cm⁻¹ (C-O); MS (ESI+): *m/z* (%): 796 (100) [*M* + Na]⁺, HRMS (FTICR ESI+): *m/z* mass calculated for [*M* + Na]⁺ = 796.4507, found 796.4500



N-Cbz-protected Macrocycles 8. Pentafluorophenyl ester **7** (600 mg, 0.64 mmol) was dissolved in DCM (20 ml), set to stir and cooled with ice/salt to $-6\text{ }^{\circ}\text{C}$. Trifluoroacetic acid (10 ml) was added dropwise over 5 minutes and the pale yellow solution was allowed to warm slowly to room temperature. After 2 h TLC confirmed that all the starting material had been consumed. The pale yellow solution was concentrated to a yellow oil. Repeated addition-evaporation of dry DCM (5×30 ml) gave a white foam (620 mg) which was then subjected to 5 cycles of addition-evaporation of dry toluene. After further drying overnight at $40\text{ }^{\circ}\text{C}$ under vacuum, the resulting deprotected TFA salt (605 mg) was dissolved in freshly distilled dry THF (50 ml) under a dry nitrogen atmosphere in carefully dried glassware.* The clear colourless solution was then slowly added over 30 hours using a syringe pump to a stirred solution of DMAP (1.2 g) in freshly distilled dry THF (750 ml) (final concentration 0.8 mM). The resulting colourless solution was left to stir for 10 days with samples taken each day. The solution became pale yellow from day 7. The reaction mixture was concentrated in vacuo yielding a cream foam solid. This was redissolved in DCM (50 ml) and successively washed with dilute aq. HCl (2×100 ml), water (100 ml) and brine (100 ml). It was observed that most of the yellow colour from the organic phase had moved to the aqueous layers during the washing process. After drying over anhydrous sodium sulphate the organic phase was concentrated in vacuo yielding a pale yellow foam. This crude material was purified by flash chromatography (DCM/Methanol 2 to 10 %). The crude material contained three different compounds by TLC which could not be separated by flash chromatography. Final purification was achieved using HPLC (see below) which yielded:

(a) **8b** ($n = 3$) as a white powder (142 mg, 0.072 mmol, 34 %); $R_f = 0.29$ (1:9 MeOH/DCM); m.p. $214\text{--}216\text{ }^{\circ}\text{C}$; $^1\text{H NMR}$ (400 MHz, $\text{CDCl}_3/\text{CD}_3\text{CN}$ (3:1), $25\text{ }^{\circ}\text{C}$, TMS): δ 0.79 (s, 3 H; 18- CH_3), 0.86 (m, 3 H; 21- CH_3), 0.91 (s, 3 H; 19- CH_3), 3.51 (m, H; 3 β -H), 3.68 (m, 1 H; 7 β -H), 4.00 (m, 1 H; 12 β -H), 4.68-5.97 (m, 7 H; Ph- CH_2 and NH), (7.15-7.41 m, 10 H; CH_2 Ph); $^{13}\text{C NMR}$ (100 MHz, CDCl_3 , $25\text{ }^{\circ}\text{C}$, TMS): (100 MHz, $\text{CDCl}_3/\text{CD}_3\text{CN}$ (3:1), $25\text{ }^{\circ}\text{C}$, TMS): $\delta = 13.4$ (C-18), 17.2 (C-21), 22.8 (C-19), 23.2, 27.3, 27.7 (CH_2), 29.4, 30.3, 30.7 (CH), 31.0, 31.6, 31.8, 32.5 (CH_2), 34.4 (C-10), 35.6, 36.0 (CH_2), 36.6, 41.7 (CH), 44.8 (C-13), 45.3, 48.1, 49.6, 54.3 (CH), 65.8, 66.7 (CH_2),

* All glassware and cannulars were carefully cleaned and dried before drying in an oven $130\text{ }^{\circ}\text{C}$ for 24 hours before use. On removal from the oven all glassware was allowed to cool to room temperature under vacuum before a dry nitrogen atmosphere was established.

127.5, 127.9, 128.1, 128.7 (CH × 10), 137.7 (C × 2), 156.1 (COCONH × 2), 172.8 (C-24); IR (solid state): $\tilde{\nu} = 3320\text{cm}^{-1}$ (N–H), 2959 cm^{-1} (C–H), 1695 cm^{-1} (C=O), 1531, 1512 cm^{-1} (CONH); MS (ESI+): m/z (%): 1989 (80) $[M + \text{Na}]^+$, HRMS (FTICR ESI+): m/z mass calculated for $[M + \text{Na}]^+ = 1989.1848$, found 1989.1890.

(b) **8c** (n = 4) as a white powder (67 mg, 0.025 mmol, 16 %); $R_f = 0.24$ (1:9 MeOH/DCM); ^1H NMR (400 MHz, $\text{CDCl}_3/\text{CD}_3\text{CN}$ (3:1), 25 °C, TMS): δ 0.78 (s, 3 H; 18-CH₃), 0.86 (br s, 3 H; 21-CH₃), 0.92 (s, 3 H; 19-CH₃), 3.47 (br s, 1 H; 3 β -H), 3.66 (br s, 1 H; 7 β -H), 3.93-4.08 (m, 1 H; 12 β -H), 4.77-5.23 (m, 4; Ph-CH₂), 5.72-6.02 (m, 3 H; NH), (7.17-7.37 m, 10 H; CH₂Ph); ^{13}C NMR (100 MHz, $\text{CDCl}_3/\text{CD}_3\text{CN}$ (3:1), 25 °C, TMS): (100 MHz, $\text{CDCl}_3/\text{CD}_3\text{CN}$, 25 °C, TMS): $\delta = 13.3$ (C-18), 17.4 (C-21), 22.7 (C-19), 23.1, 27.1, 27.9 (CH₂), 30.5 (CH), 31.6, 31.9, 32.3, 33.7 (CH₂), 34.4 (C-10), 34.9 (CH), 35.6, 36.1 (CH₂), 36.6, 41.6 (CH), 44.5 (C-13), 47.7, 49.7, 53.6 (CH), 65.7 (CH₂ × 2), 127.0, 127.9, 128.3 (CH × 10), 137.2 (C × 2), 155.6 (COCONH × 2), 172.8 (C-24); IR (solid state): $\tilde{\nu} = 3334\text{ cm}^{-1}$ (N–H), 2937 cm^{-1} (C–H), 1686 cm^{-1} (C=O), 1514, 1498 cm^{-1} (CONH); MS (ESI+): m/z (%): 2622 (87) $[M + \text{H}]^+$, HRMS (FTICR ESI+): m/z mass calculated for $[M + \text{Na}]^+ = 2622.6013$, found 2622.6008.

(c) **8d** (n = 5) as a white powder (10 mg, 0.003 mmol, 2 %); $R_f = 0.2$ (1:9 MeOH/DCM); ^1H NMR (400 MHz, $\text{CDCl}_3/\text{CD}_3\text{CN}$ 3:1, 25 °C, TMS): δ 0.78 (s, 3 H; 18-CH₃), 0.86 (br s, 3 H; 21-CH₃), 0.92 (s, 3 H; 19-CH₃), 3.47 (br s, 1 H; 3 β -H), 3.66 (br s, 1 H; 7 β -H), 4.02 (br s, 1 H; 12 β -H), 4.87-5.14 (m, 4; Ph-CH₂), 5.72-6.01 (m, 3 H; NH), (7.17-7.37 m, 10 H; CH₂Ph); MS (ESI+): m/z (%): 1661 (100) $[M + 2\text{Na}]^{2+}$, HRMS (FTICR ESI+): m/z mass calculated for $[M + 2\text{H}]^{2+} = 1639.5004$, found 1639.5035.

HPLC Separations of Macrocycles 8. Analytical HPLC was performed on a Thermo C18 Hypersil column, dimensions 150 × 4.6 mm, manufactured using spherical porous silica of particle size 5 μm . A typical trace is shown in Figure S1 below. Solvents used were (a) water containing 0.1 % by volume of trifluoroacetic acid, and (b) methanol. The flow rate was 0.8 ml min⁻¹. Solvent programme: 0 - 2 min, isocratic 75 % methanol; 2 - 4 min, gradient to 95 % methanol; 4 - 15 min isocratic 95 % methanol; 15 - 23 min gradient to 99 % methanol; 23 - 28 min isocratic 99 % methanol. Preparative separations were performed on a HICHROM C18 spherical porous silica preparative column, dimensions 150 × 21.2 mm, particle size 5 μm , with a similar solvent programme but a flow rate of 17 ml min⁻¹. Injections of 40-50 mg were feasible. A typical trace is shown in Figure S2 below.

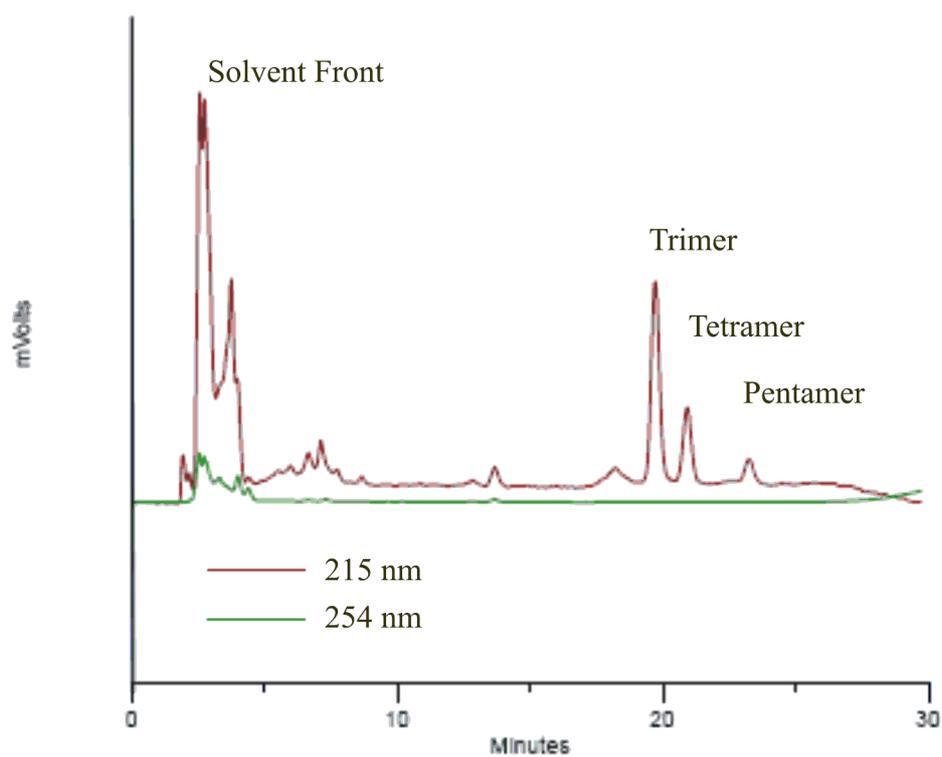


Figure S1. Analytical HPLC separation of macrocycles **8**.

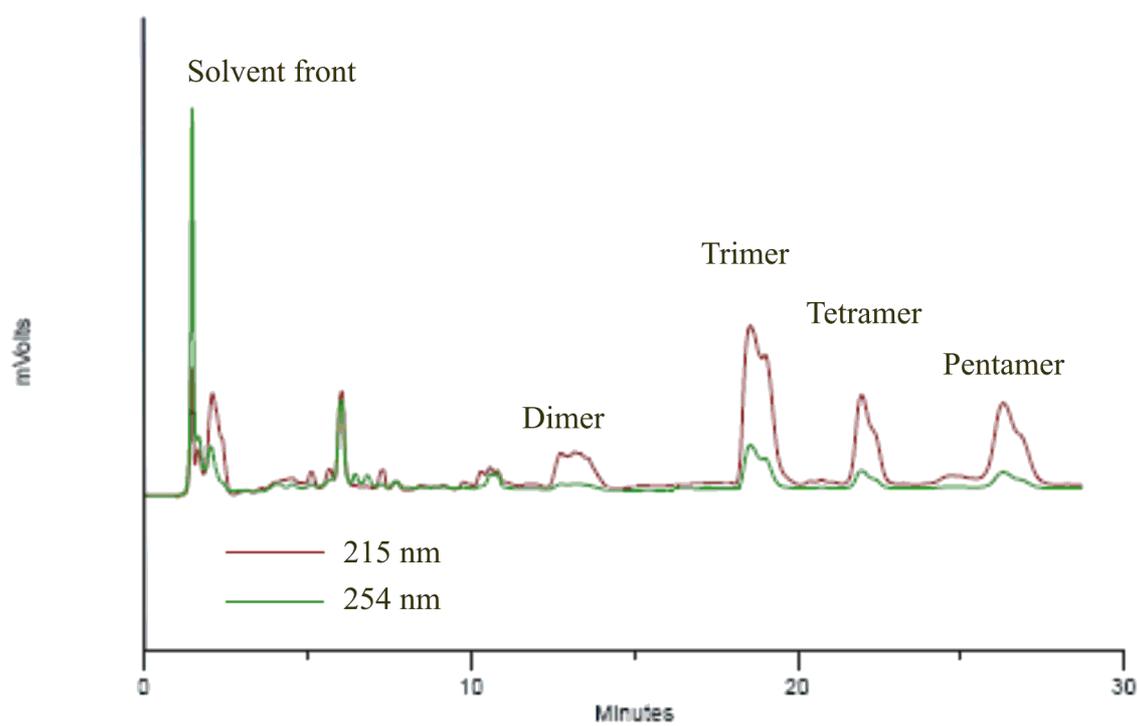
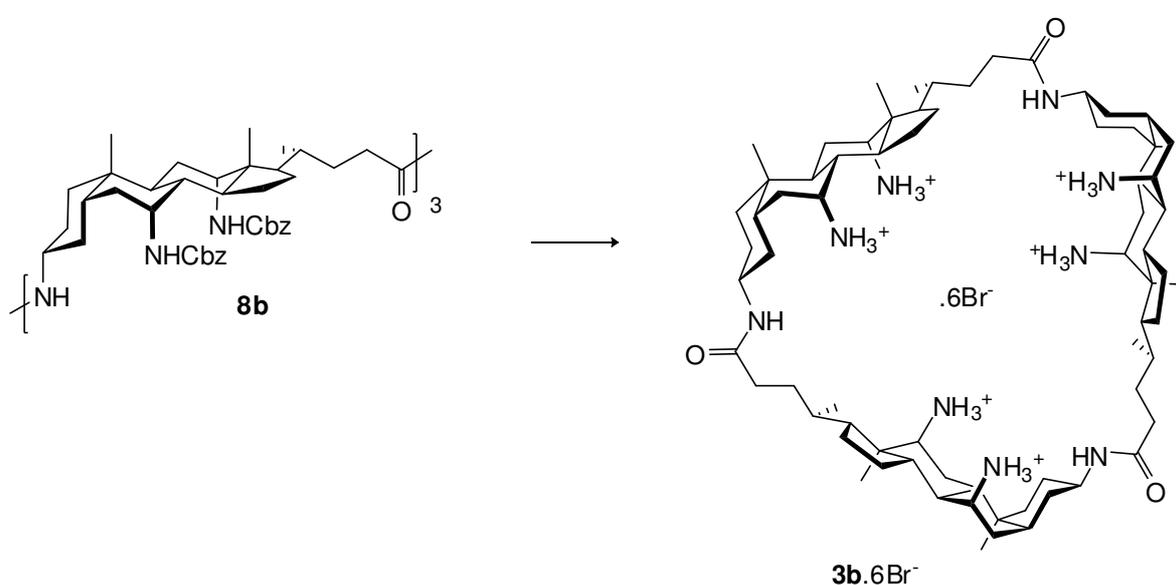


Figure S2. Preparative HPLC separation of macrocycles **8**.



Cyclotrimeric Toroidal Facial Amphiphile 3b. N-CBz-protected cyclotrimer **8b** (10 mg, 5 μmol) was dissolved in 45 % HBr/AcOH (5ml) and left to stir for 4 hours, after which deprotection was complete by TLC. Evaporation of the orange solution in vacuo gave a pale orange powder. Repeated addition-evaporation of toluene (3×10 ml) then DCM (3×10 ml) followed by drying overnight under high vacuum gave **3b.6Br⁻** as a pale orange powder (8.5 mg, 4.8 μmol , 96 %) $R_f = 0.00$ (2:8 MeOH/EtOAc); m.p. (decomposition) 275 $^{\circ}\text{C}$; ^1H NMR (400 MHz, CD_3OD , 25 $^{\circ}\text{C}$): δ 0.95 (s, 3 H; 18- CH_3), 1.02-1.07 (m, 6 H; 19- CH_3 and 21- CH_3), 2.31-2.48 (m, 2 H), 2.64-2.75 (m, 1 H), 3.50-3.56 (m, 1 H; 3 β -H), 3.69-3.70 (m, 1 H; 7 β -H), 3.76-3.81 (m, 1 H; 12 β -H); ^{13}C NMR (100 MHz, CD_3OD , 25 $^{\circ}\text{C}$): $\delta = 13.1$ (C-18), 16.9 (C-21), 21.7 (C-19), 23.7, 24.9, 27.3, 28.8 (CH_2), 29.1 (CH), 31.2, 32.9, 35.1, 35.2, 36.7 (CH_2), 35.8 (C-10), 37.6, 37.7, 41.6, 43.7 (CH), 46.1 (C-13), 48.8, 49.7, 50.9, 57.2 (CH) 175.4 (C-24); IR (solid state): $\tilde{\nu} = 3380\text{cm}^{-1}$ (N-H), 2923cm^{-1} (C-H), 1622cm^{-1} (C=O), $1543, 1510\text{cm}^{-1}$ (CONH); MS (ESI+): m/z (%): 581 (100) $[M + 2\text{H}]^{2+}$, 1162 (15) $[M + \text{H}]^+$, HRMS (FTICR ESI+): m/z mass calculated for $[M + 2\text{H}]^{2+} = 581.9947$, found 581.9966.

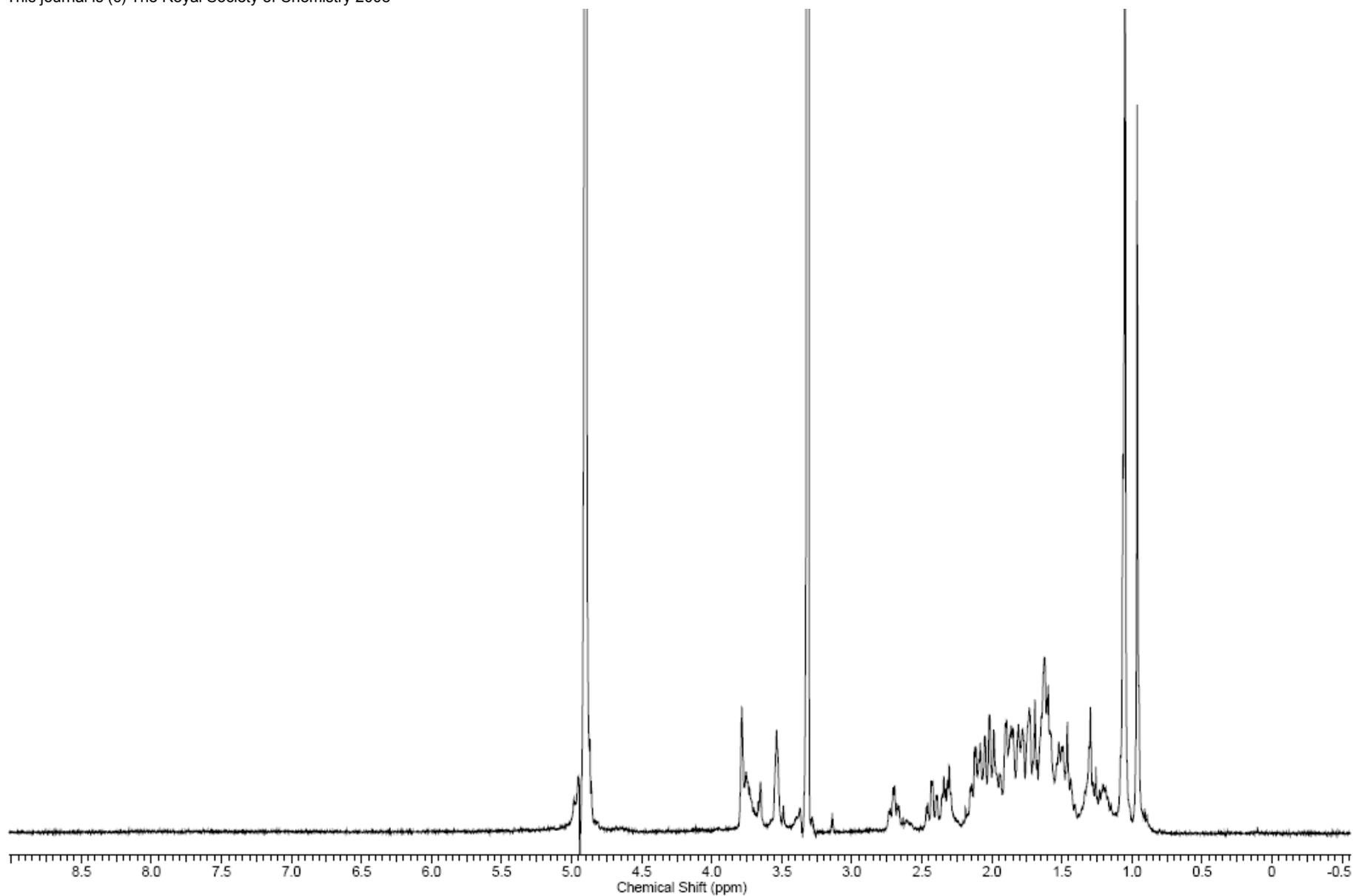
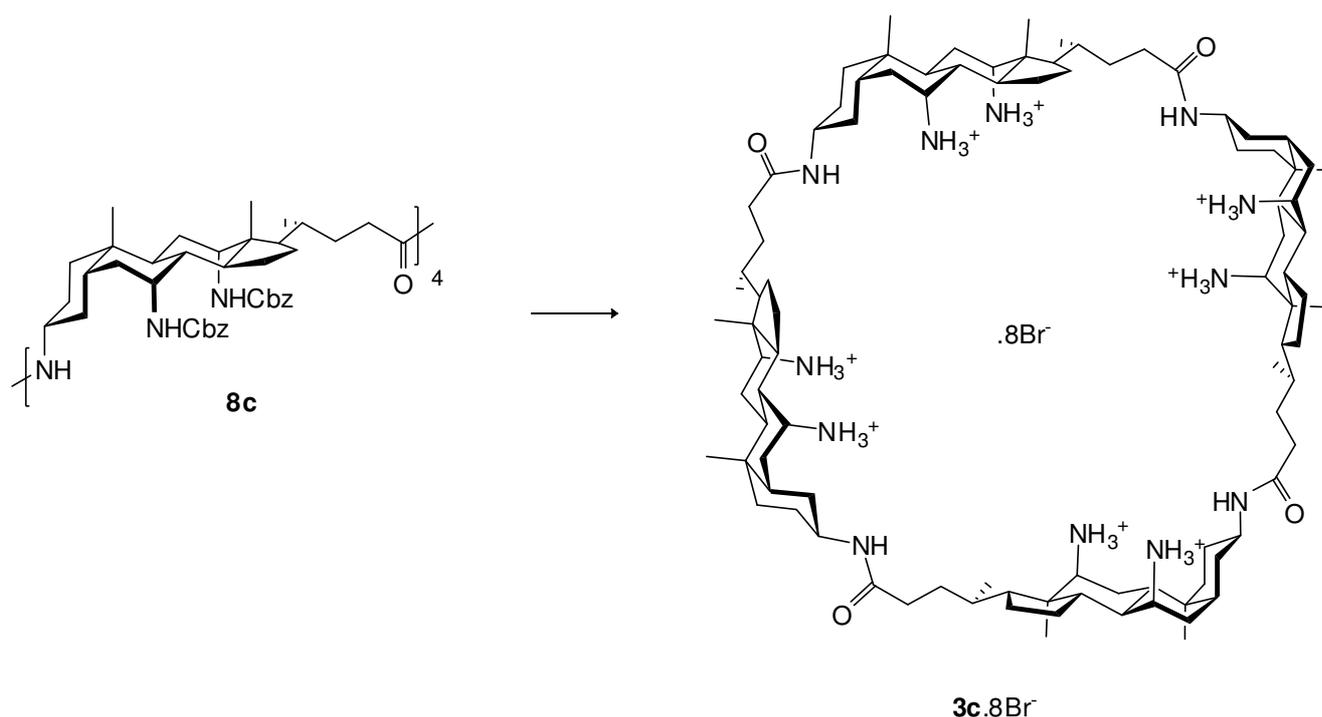


Figure S3. ¹H NMR spectrum of cyclotrimer **3b**.6Br⁻ in CD₃OD. Singlets at ~3.3 and ~4.9 p.p.m. are due to solvent and HOD respectively.



Cyclotetrameric Toroidal Facial Amphiphile 3c. N-CBz-protected cyclotetramer **8c** (10 mg, 3.8 μmol) was dissolved in 45 % HBr/AcOH (5ml) and left to stir for 4 hours, after which deprotection was complete by TLC. Evaporation of the orange solution in vacuo gave a pale orange powder. Repeated addition-evaporation of toluene (3×10 ml) then DCM (3×10 ml) followed by drying overnight under high vacuum gave **3c.8Br⁻** as a pale orange powder (8 mg, 3.7 μmol , 97 %); $R_f = 0.00$ (2:8 MeOH/EtOAc); $^1\text{H NMR}$ (400 MHz, CD_3OD , 25 $^\circ\text{C}$): δ 0.95 (s, 3 H; 18- CH_3), 1.04 (s, 3 H; 19- CH_3), 1.07 (d, $^3J(\text{H,H}) = 5.7$ Hz, 3 H; 21- CH_3), 2.05-2.18 (m, 1 H), 2.25-2.38 (m, 1 H), 2.55-2.65 (m, 1 H), 3.50-3.56 (m, 1 H; 3 β -H), 3.64-3.72 (m, 1 H; 7 β -H), 3.73-3.78 (m, 1 H; 12 β -H); $^{13}\text{C NMR}$ (100 MHz, CD_3OD , 25 $^\circ\text{C}$): $\delta = 13.3$ (C-18), 17.2 (C-21), 22.1 (C-19), 23.7, 25.3, 27.7, 28.7 (CH_2), 29.3 (CH), 31.5, 33.1, 34.1, 35.9, 36.2 (CH_2), 36.6 (C-10), 37.0, 37.9, 42.1, 43.6 (CH), 46.2 (C-13), 49.0, 50.6, 51.0, 57.1 (CH) 175.4 (C-24); IR (solid state): $\tilde{\nu} = 3379$ cm^{-1} (N-H), 2925 cm^{-1} (C-H), 1619 cm^{-1} (C=O), 1537, 1511 cm^{-1} (CONH); MS (ESI+): m/z (%): 776 (100) [$M + 2\text{H}$] $^{2+}$, HRMS (FTICR ESI+): m/z mass calculated for [$M + 2\text{H}$] $^{2+} = 775.6572$, found 775.6592.

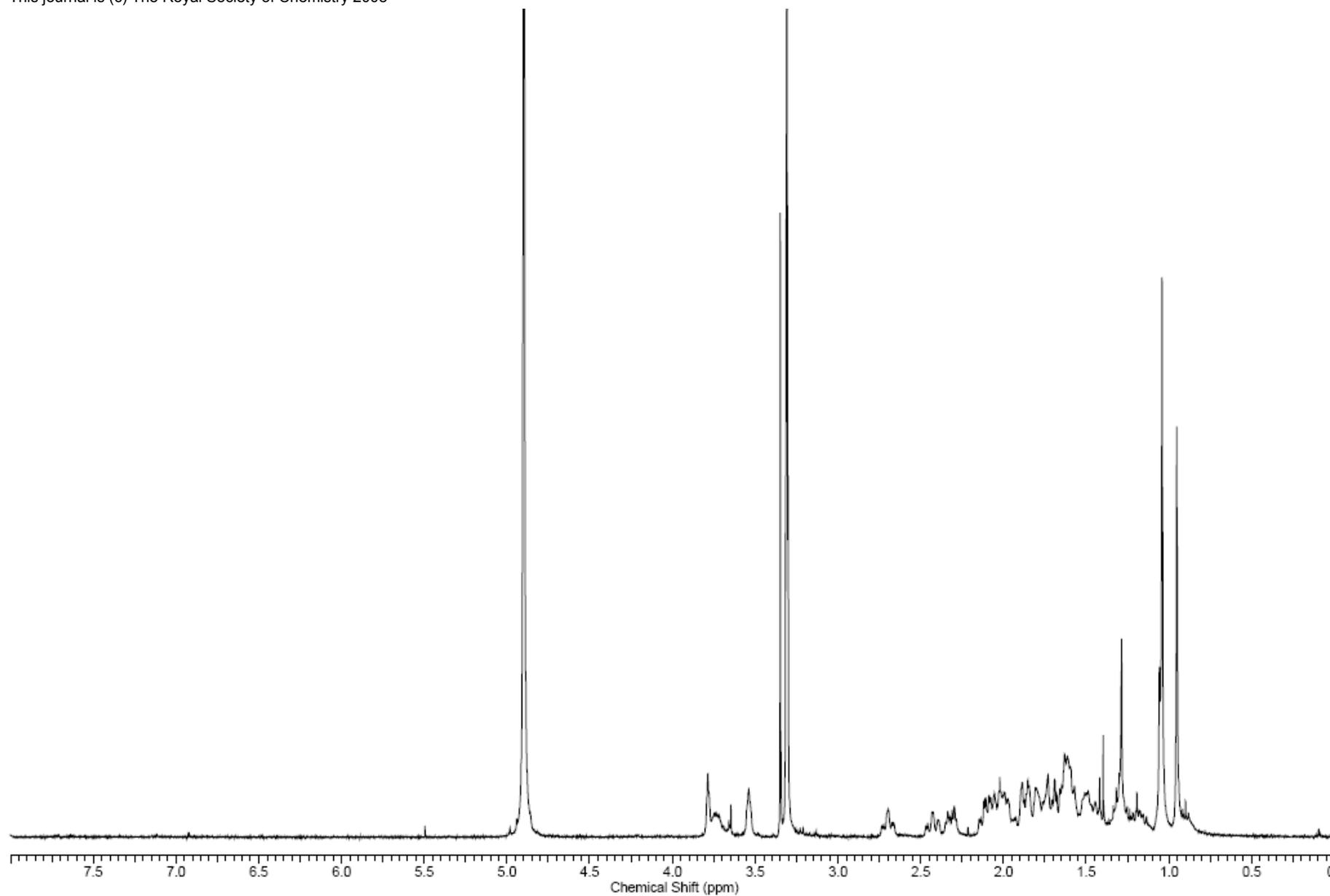


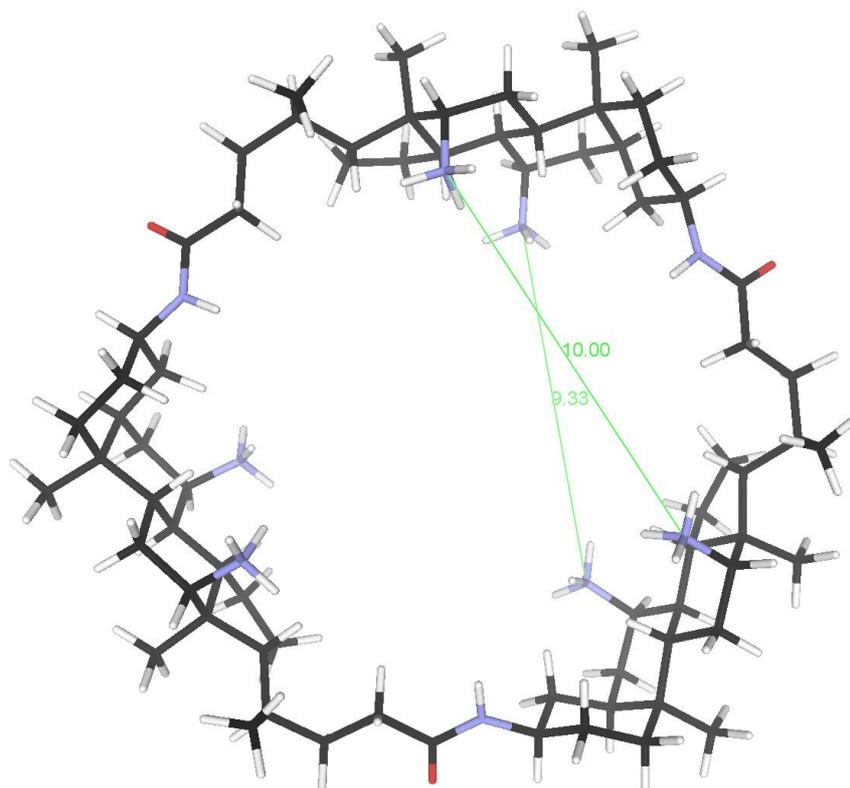
Figure S4. ^1H NMR spectrum of cyclotetramer **3c**. 8Br^- in CD_3OD . Singlets at ~ 3.3 and ~ 4.9 p.p.m. are due to solvent and HOD respectively.

Molecular Modelling

Monte Carlo molecular mechanics calculations on **3b** and **3c** were performed using Macromodel 9.1 with the Maestro 7.5 interface, the MMFFs force field and GB/SA (water) continuum solvation. Rotation was allowed about bonds C20-C22, C22-C23, C23-C24, C24-N and N-C3 (for numbering, see **4**). 1000 Monte Carlo steps were performed with 1000 steps minimisation for each, saving structures within 50 kJ mol⁻¹ of the global minimum. The resulting structures were then further minimised to convergence. The global minima are depicted in space-filling mode in Figure 1.

In the case of cyclotrimer **3b**, conformations with inward-directed NH₃⁺ groups were strongly favoured. A major reason is that, perhaps counterintuitively, structures with externally-directed NH₃⁺ possess shorter NH₃⁺...NH₃⁺ distances. This is illustrated in Figure S5, where the global minimum (a) is compared to a conformation (b) with outward-pointing NH₃⁺ groups. To redirect the ammonium groups a steroidal unit must be folded inwards. The rigid, curved geometry of the 5β-cholanoyl framework means that the attached NH₃⁺ are brought closer to their transannular counterparts.

(a)



(b)

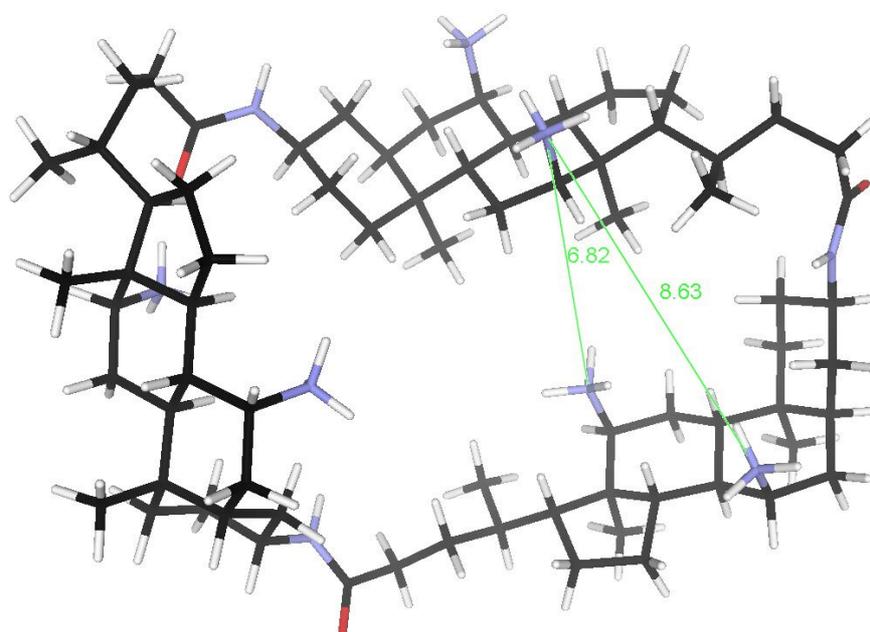


Figure S5. (a) Global minimum conformation of **3b** showing transannular $\text{NH}_3^+\cdots\text{NH}_3^+$ distances (9.33 and 10 Å). (b) A conformation of **3b** with outward-directed NH_3^+ and consequently shorter transannular $\text{NH}_3^+\cdots\text{NH}_3^+$ distances (6.82 and 8.63 Å). This structure was found to be 31 kJ mol⁻¹ above the global minimum.

Electron Microscopy

The morphology of the aggregate structures was examined with a Jeol 1200EX transmission electron microscope (TEM) operated at an accelerating voltage of 120 keV. TEM samples were prepared by applying a drop of the turbid suspension onto carbon coated copper grids. Excess solvent was immediately removed using filter paper to minimize drying down artefacts. Images are shown in Fig. 2 (main paper) and Fig. S6 below.

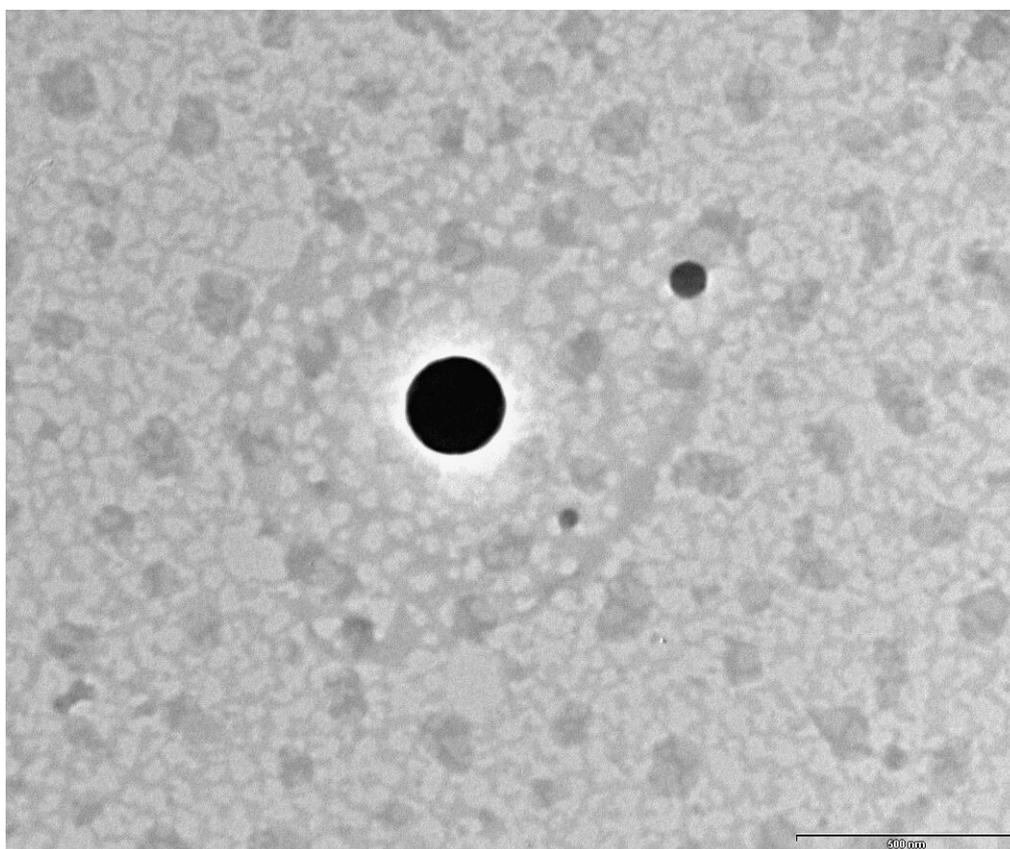


Figure S6. TEM image of spheroids formed by cyclotetramer **3c.8Br⁻**.

Transport Experiments - General Procedures

Vesicle preparation. Vesicles were prepared following a procedure based on those reported by Mayer et al.⁴ and Koulov et al.⁵ Aliquots of lipids (0.03 mmol total) as solutions in chloroform were placed within a round-bottomed flask and the solvent carefully removed in vacuum, leaving a thin film of lipids on the surface of the glassware. This was then hydrated using an aqueous solution of salts as required for the experiment (1 mL, typically NaCl or KCl, 500 mM, with added buffer in some cases). The lipids were suspended in solution by short bursts from an ultrasonic bath to form a milky white mixture, which was stirred gently for 30 minutes. The milky white suspension was then subjected to a freeze-thaw routine, by placing alternately in liquid nitrogen until the mixture was completely solid followed by placing in a warm water bath until the solid melted. The lipid mixture was then passed through a nylon membrane with 200 nm pores 15 times using the LiposofastBasic™ vesicle extrusion apparatus. The liposome suspension was then dialyzed (Bio Design Dialysis Tubing™) against aqueous NaNO₃ (500 mM) buffered if appropriate, using in total 2 L of solution. After dialysis, only minor traces of chloride could be detected on the outside of the vesicles. The lipid solution was diluted to a final volume of 30 mL using the same aqueous solution used for the dialysis. Assuming that there is no loss of lipid during the extrusion, the final concentration of the vesicles solution is 1 mM.

Efflux measurements using ion-selective electrodes. The efflux of Cl⁻, Br⁻ or K⁺ from the vesicles was monitored using ion-selective electrodes (ISEs) from Nico2000 Limited. Each ISE was used with an appropriate external reference electrode, connected via an ELIT Electrode Head (catalogue number: 201). The chloride electrode (ELIT 8261) and bromide electrode (ELIT 8721), were paired with the Double Junction potassium nitrate (002n), the potassium electrode (ELIT 8031) with the Double Junction lithium acetate (003n). The electrodes were interfaced to a pc with Nico2000 ELIT Ion Analysers. The “2 channel” model (catalogue number: 9801) was used for monitoring a single type of ion, the “4 channel” model (9804) for monitoring K⁺ and Cl⁻ simultaneously. The chloride combined electrode was calibrated using a standard solution of NaCl in NaNO₃ (500mM). The bromide combined electrode was calibrated using a standard solution of KBr in NaNO₃. The potassium combined electrode was calibrated using KCl in NaNO₃ (500mM), confirming that the electrode could be used in the presence of a large excess of Na⁺. Before an experiment, the relevant electrode was immersed in the 500 mM NaNO₃ solution for 20 minute to equilibrate, then carefully rinsed with deionised water and dried with paper tissue. The electrode was then immersed in the gently stirred vesicle solution and an initial reading was taken,

corresponding to 0% ion release. After 60-120 sec macrocycle **3b**.(CF₃CO₂⁻)₆ was added, dissolved in a small volume of methanol (in a typical example, 7.5 μL of a 0.01 M solution of **3b**.(CF₃CO₂⁻)₆ was added to 7.5 ml of vesicles solution, giving a 10 μM final concentration of **3b**). At the end of the experiment the non-ionic detergent octaethylene glycol monododecyl ether was added, disrupting the vesicles and releasing the remaining entrapped ions (100% ion release).

Examples of traces are shown in Figs. S7 - S9 below. The “stepping” of data points in Figs. S7 and S8 was due to a limitation of the Ion Analyser software. This issue was resolved for later experiments, e.g. Fig. S9 and Fig. 3, through an updated version of the software.

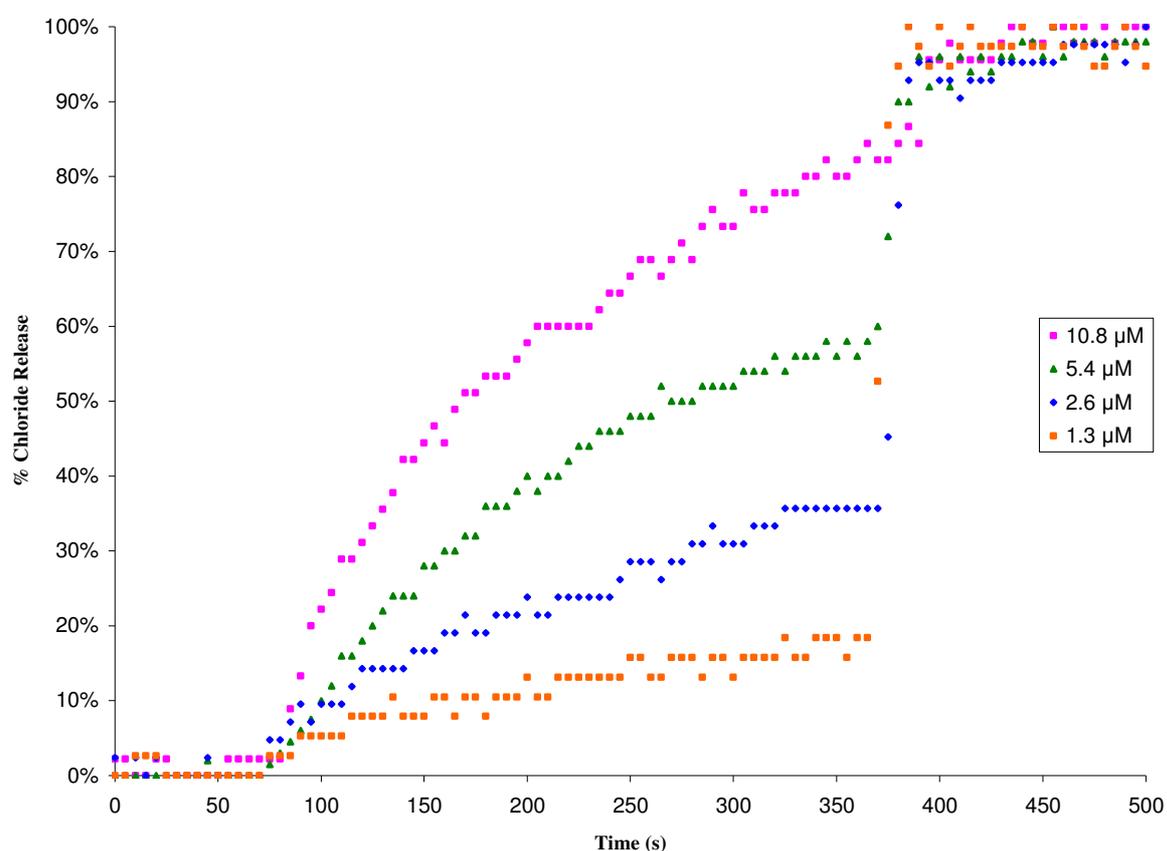


Figure S7. Chloride release from EYPC vesicles on addition of **3b**.(CF₃CO₂⁻)₆. At time 60 s, 1.3, 2.6, 5.4, or 10.8 μM (final concentration) **3b**.(CF₃CO₂⁻)₆ was added to 7.5 ml of a 1 mM dispersion of unilamellar egg yolk phosphatidylcholine (EYPC) vesicles (200 nm mean diameter) containing NaCl aq. (500 mM). The vesicles were dispersed in NaNO₃ aq. (500 mM). At time 360 s, detergent (octaethyleneglycol monododecyl ether) was added.

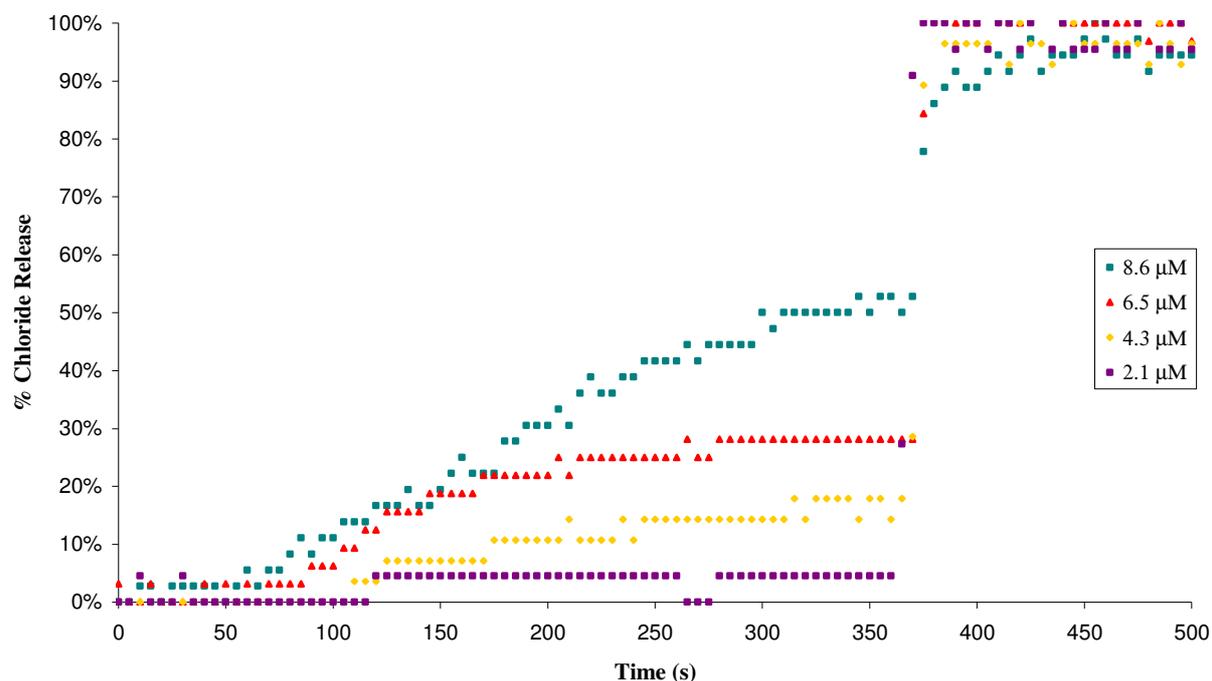


Figure S8. Chloride release from POPC/cholesterol (7:3) vesicles on addition of **3b**.(CF_3CO_2^-)₆. At time 60 s, 2.1, 4.3, 6.5 or 8.6 μM (final concentration) **3b**.(CF_3CO_2^-)₆ was added to 7.5 ml of a 1 mM dispersion of unilamellar 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)/cholesterol (7:3) vesicles (200 nm mean diameter) containing NaCl aq. (500 mM). The vesicles were dispersed in NaNO₃ aq. (500 mM). At time 360 s, detergent (octaethyleneglycol monododecyl ether) was added.

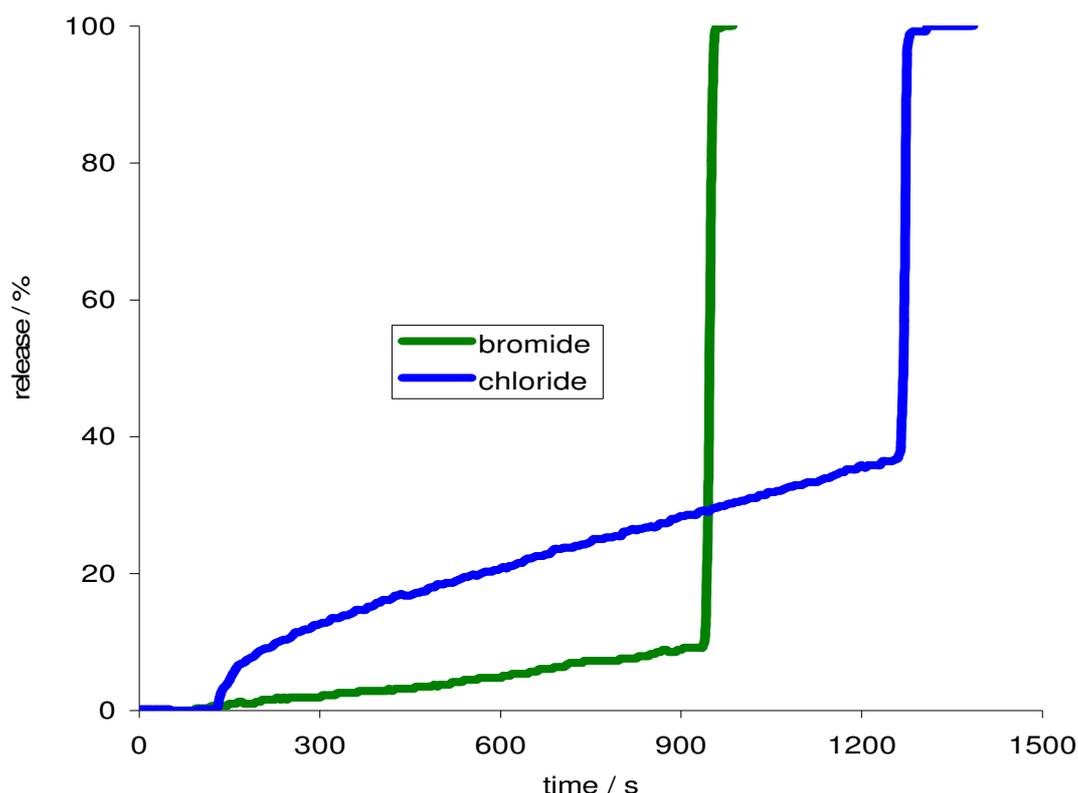


Figure S9. Chloride vs. bromide selectivity of **3b**.(CF_3CO_2^-)₆. **Green trace - bromide transport:** At time 125 s, 20 μM (final concentration) **3b**.(CF_3CO_2^-)₆ was added to 7.5 ml of a 1 mM dispersion of unilamellar egg yolk phosphatidylcholine (EYPC)/cholesterol (7:3) vesicles (200 nm mean diameter) containing NaBr (500 mM) in aqueous phosphate buffer (pH = 7, 10 mM). The vesicles were dispersed in NaNO_3 (500 mM) in the same buffer. At time 950 s, detergent (octaethyleneglycol monododecyl ether) was added. **Blue trace - chloride transport:** The experiment was performed in the same way except that NaCl was used instead of NaBr, and the detergent was added somewhat later. These data were also shown in Fig 3 (main paper).

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