From Cholapod to Cholaphane transmembrane anion carriers: Accelerated transport through binding site enclosure.

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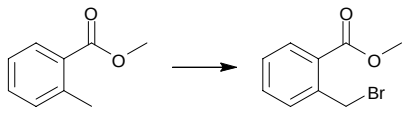
Supplementary Information

Synthesis of receptors 3 and 4.

General: ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded at 399.78 MHz on a Jeol Delta/GX 400 spectrometer, or 400.18 MHz on a Jeol Eclipse 400 spectrometer. Chemical shifts are reported in ppm downfield from tetramethylsilane, for ^{1}H and ^{13}C. Mass spectra (electron impact and chemical ionisation) were recorded on a VG Analytical Autospec. Infrared spectra were recorded on a Perkin Elmer Spectrum One FT-IR. Fluorescence decay was followed with a Perkin Elmer LS45 spectrometer. Elemental analysis was carried out by the microanalysis department at the School of Chemistry, University of Bristol. All commercially available compounds were used without further purification except where stated. Lipids used in the transport studies were purchased from Avanti Polar Lipids Inc. The solvents were dried by passage through a modified Grubbs system[1] employing alumina columns and manufactured by Anhydrous Engineering. Routine monitoring of reactions was performed using precoated silica gel TLC plates (Merck silica gel 60 F_{254}), with visualisation by UV light, ethanolic phosphomolybdic acid or ninhydrin. R_f values are given under these conditions. Flash column chromatography[2] was performed using silica gel (Fisher brand silica 60 Å particle size 35-70 micron) as the absorbent.

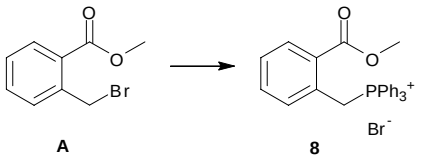


7 **A**

Methyl 2-(bromomethyl)benzoate A.

To a solution of methyl 2-methylbenzoate **7** (16.8 mL, 120 mmol) in methyl acetate (600 mL) was added *N*-bromosuccinimide (23.5 g, 132 mmol). The solution heated to reflux by irradiation of the flask with a 100 W lamp overnight, after which the solvent was removed *in vacuo*. The mixture was diluted with hexane (400 mL) and filtered. Evaporation of the filtrate *in vacuo* yielded the target compound **A** as a colourless oil (27.5 g, 120 mmol, 100%). R_f 0.4 (10% EtOAc in hexane); ^{1}H NMR (399.78 MHz, $CDCl_3$): δ = 3.95 (3 H, s, OCH_3), 4.96 (2 H, s, CH_2Br), 7.36-7.40 (1 H, m, Ar-*H*), 7.45-7.52 (2 H, m, Ar-*H*), 7.97 (1 H, dd, *J* = 0.7, 7.8 Hz, Ar-*H*); ^{13}C (100.53 MHz, $CDCl_3$): δ = 31.5

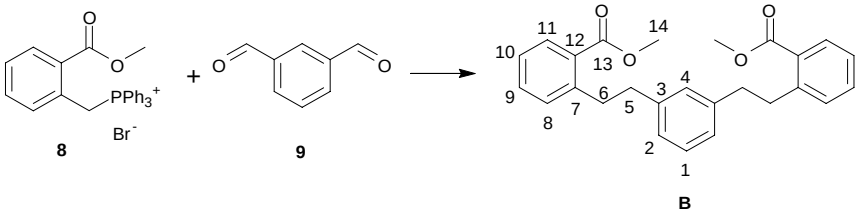
(*C*H_2Br), 52.3 (O*C*H_3), 128.5 (Ar-*C*H), 129.0 (Ar-*C*), 131.3 (Ar-*C*H), 131.7 (Ar-*C*H), 132.5 (Ar-*C*H), 139.2 (Ar-*C*), 167.0 (*C*=O); IR (Neat): ν = 3059, 2946, 1748, 1715, 1596, 1439, 1368, 1288, 1219, 1051, 1003, 740 cm^{-1}; HRMS (ES+): m/z calculated for $[(^{81}Br)M + Na]^+$ = 252.9658, found 252.9651, m/z calculated for $[(^{79}Br)M + Na]^+$ = 250.9678, found 250.9671. The ^1H NMR spectrum of the product was consistent with literature data.[3]



A **8**

2-(Methoxycarbonyl)benzyltriphenylphosphonium bromide 8.

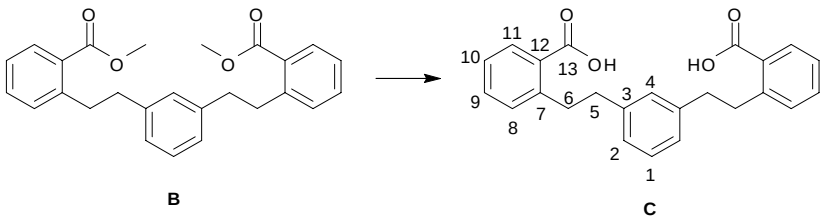
Methyl 2-(bromomethyl)benzoate **A** (27.5 g, 120 mmol) was stirred in acetone (250 mL) for 15 min at room temperature. Triphenylphosphine (35.8 g, 144 mmol) was added and the reaction mixture was stirred at 60 °C overnight. After cooling to room temperature the precipitate was removed by filtration, washed with acetone and dried overnight to give compound **8** as a white solid (42.0 g, 86 mmol, 72%). R_f 0.1 (15% MeOH in CH_2Cl_2); mp 244-246 °C (lit 250 °C[4]); ^1H NMR (399.78 MHz, MeOD): δ =3.54 (3 H, s, O*C*H_3), 5.45 (2 H, d, *J* = 15.2 Hz, *C*H_2P), 7.34-7.37 (1 H, m, Ar-*H*), 7.51-7.61 (8 H, m, Ar-*H*), 7.68-7.73 (6 H, m, Ar-*H*), 7.78-7.98 (4 H, m, Ar-*H*); ^{13}C (100.53 MHz, MeOD): δ = 29.8 (d, *J* = 50.0 Hz, *C*H_2P), 52.9 (*C*H_3), 119.1 (d, *J* = 86.1 Hz, Ar-*C*), 130.7 (d, *J* = 3.8 Hz, Ar-*C*H), 131.4 (Ar-*C*H), 131.5 (Ar-*C*H), 131.7 (Ar-*C*), 131.8 (Ar-*C*), 135.6 (Ar-*C*H), 135.7 (Ar-*C*H), 133.9 (d, *J* = 5.4 Hz, Ar-*C*H), 136.6 (d, *J* = 3.1 Hz, Ar-*C*H), (*C*=O not observed); IR (Neat): ν = 2993, 2863, 1698, 1431, 1265, 1110, 1075, 759, 703 cm^{-1}; HRMS (ES+): m/z calculated for $[M + Na]^+$ = 411.1508, found 411.1513.



1,3-Bis[2-(2-methoxycarbonylphenyl)ethyl]benzene B.

Sodium methoxide (2.64 g, 48.8 mmol) was dissolved in dry methanol (200 mL) and stirred at room temperature for 15 min. Compound **8** (20.0 g, 40.7 mmol) was then added and the reaction mixture was stirred for a further 30 min at room temperature and then warmed to 50 °C. Isophthalaldehyde **9** (2.73 g, 20.4 mmol) was added and the reaction mixture was stirred and heated under reflux overnight. The solvent was removed *in vacuo* and the residue redissolved in ethyl acetate (200 mL) and washed with water (2 × 100 mL) and brine (100 mL). The organic layer was dried (Na_2SO_4) and evaporated. The crude product was purified by column chromatography (5% - 30% EtOAc in hexane) yielding a mixture of dienes **10** (5.94 g, 14.9 mmol, 73%). R_f 0.3 (20% EtOAc in hexane); HRMS (ES+): m/z calculated for [M + Na]+ = 421.1410, found 421.1420.

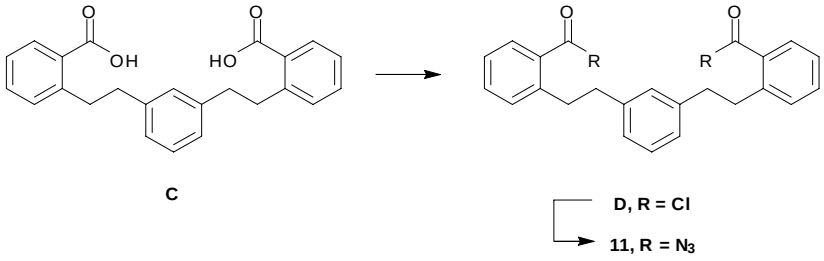
Palladium on carbon (0.5 g, 10 % wt. loading) was added to a stirred solution of **10** (5.00 g, 12.5 mmol) in ethanol (60 mL). This mixture was then hydrogenated at 4 atm. (60 psi) for 48 h. The palladium on carbon was removed by filtration through celite and the filtrate was evaporated, yielding compound **B** as a colourless oil (5.00 g, 12.4 mmol, 99%). R_f 0.4 (10% EtOAc in hexane); ^{1}H NMR (400.18 MHz, $CDCl_3$): δ = 2.87 (4 H, AA'XX' system, H_5), 3.23 (4 H, AA'XX' system, H_6), 3.92 (6 H, s, H_{14}), 7.04-7.08 (3 H, m, H_2, H_4), 7.20-7.23 (3 H, m, H_1, H_8), 7.26 (2 H, dt, J = 7.6, 1.2 Hz, H_{10}), 7.41 (2 H, dt, J = 7.6, 1.5, H_9), 7.91 (2 H, dd, J = 7.8, 1.5, H_{11}); ^{13}C (100.63 MHz, $CDCl_3$): δ = 36.9 (*C6*), 38.1 (*C5*), 52.0 (*C14*), 126.0 (*C10*), 126.1 (*C2*), 128.3 (*C1*), 128.8 (*C4*), 129.5 (*C12*), 130.8 (*C11*), 131.2 (*C8*), 131.9 (*C9*), 141.9 (*C3*), 143.7 (*C7*), 168.0 (*C13*); IR (Neat): ν = 3024, 2950, 1717, 1433, 1250, 1125, 1076, 735, 705 cm^{-1}; HRMS (ES+): m/z calculated for $[M + Na]^+$ = 425.1723, found 425.1740.



B **C**

1,3-Bis[2-(2-carboxyphenyl)ethyl]benzene C.

Sodium hydroxide (3.45 g, 85.7 mmol) was added to a solution of diester **B** (4.79 g, 11.9 mmol) in methanol (150 mL) and water (50 mL). The mixture was stirred with heating under reflux for 1 h. The mixture was then reduced to a volume of 50 mL and 6 M aqueous HCl (14.3 mL, 85.8 mmol) was added. The resulting white precipitate was removed by filtration, washed with water and dried overnight, yielding compound **C** as a white solid (3.76 g, 10.0 mmol, 84%). R_f 0.2 (15% MeOH in CH_2Cl_2); mp 166-167 °C; ^{1}H NMR (400.18 MHz, MeOD): δ = 2.83 (4 H, AA'XX' system, *H*$_5$), 3.22 (4 H, AA'XX' system, *H*$_6$), 7.00-7.03 (3 H, m, *H*$_2$, *H*$_4$), 7.12-7.15 (1 H, m, *H*$_1$), 7.20 (2 H, dd, *J* = 7.5, 1 Hz, *H*$_8$), 7.27 (2 H, dt, *J* = 7.6, 1.3 Hz, *H*$_{10}$), 7.41 (2 H, dt, *J* = 7.6, 1.5 Hz, *H*$_9$), 7.89 (2 H, dd, *J* = 7.8, 1.2 Hz, *H*$_{11}$); ^{13}C (100.63 MHz, MeOD): δ = 38.2 (*C5*), 39.4 (*C6*), 127.2 (*C2*), 127.2 (*C10*), 129.3 (*C4*), 130.0 (*C1*), 131.5 (*C12*), 132.0 (*C11*), 132.5 (*C8*), 133.1 (*C9*), 143.4 (*C3*), 145.0 (*C7*), 171.4 (*C13*); IR (Neat): ν = 3031, 3002, 2944, 2859, 2649, 1677, 1301, 1283, 936738, 703, 658 cm^{-1}; HRMS (ES-): m/z calculated for $[M - H]^-$ = 373.1445, found 373.1430.



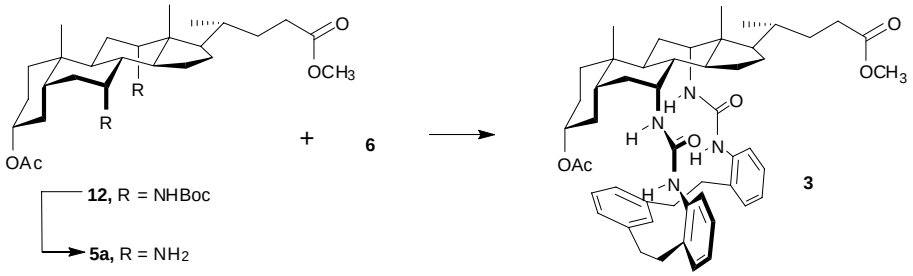
C

1,3-Bis[2-(2-azidocarbonylphenyl)ethyl]benzene 11.

Thionyl chloride (630 μL, 8.64 mmol) and Et_3N (125 μL, 0.90 mmol) were added to a solution of the diacid **C** (250 mg, 0.67 mmol) in CH_2Cl_2 (30 mL). The reaction mixture was stirred at reflux for 2 h. On completion the solution was evaporated to give crude product **D**, which was used in the next step without further purification.

A solution of the bis-acid chloride **D** in dry THF (10 mL) was added dropwise to a solution of sodium azide (305 mg, 4.69 mmol) in water (4 mL) while cooling in an ice bath. The mixture was left to stir for 2 h, after which toluene (10 mL) and saturated sodium bicarbonate (10 mL) were added and the aqueous phase was extracted with toluene (2 × 10 mL). The combined organic phases were washed with saturated sodium bicarbonate (10 mL) and brine (10 mL), then dried ($MgSO_4$) and filtered. A further portion of $MgSO_4$ was added and the suspension was stirred overnight. Evaporation to a volume of ~10 mL *in vacuo* gave a solution of **11** in toluene which was used in the next step without further purification.

Caution: 1,3-Bis[2-(azidocarboxylphenyl)ethyl]benzene **11** is potentially explosive in concentrated form under heat or pressure. The risk can be minimised by keeping the compound in solution and avoiding evaporation to dryness, as in the above procedure.

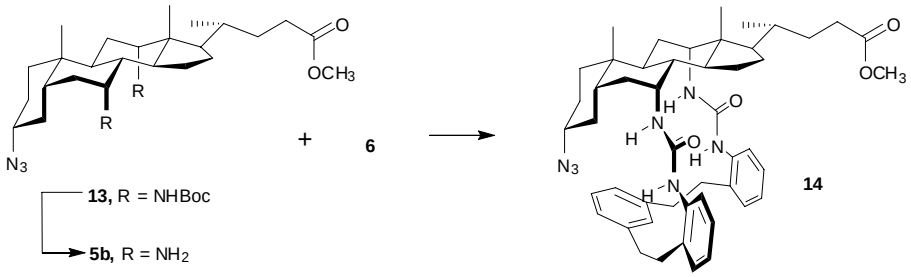


Cholaphane 3.

Trifluoroacetic acid (3 mL) was added dropwise over 2 min to a stirred solution of the di-Boc amine **12** (433 mg, 0.67 mmol) in CH_2Cl_2 (7 mL). The reaction mixture was stirred at room temperature under N_2 overnight, after which the solvent was removed *in vacuo*. The residue was redissolved in CH_2Cl_2 (20 mL), and the solution was washed with saturated sodium bicarbonate (2 × 10 mL), dried (Na_2SO_4) and evaporated to give the crude diamine **5a**.

The above-described solution of bis-acyl azide **11** in toluene (~0.67 mmol, 10 mL) was slowly heated from 60 °C to reflux and then stirred at reflux for 2 h. The solution of di-amine **5a** in toluene (~0.67 mmol, 10 mL) was then added and the mixture was left to stir at reflux overnight. The solvent was removed *in vacuo* and the residue was redissolved in CH_2Cl_2 (20 mL), and then washed with HCl (0.5 M, 10 mL), saturated sodium bicarbonate (10 mL) and dried (Na_2SO_4). Evaporation

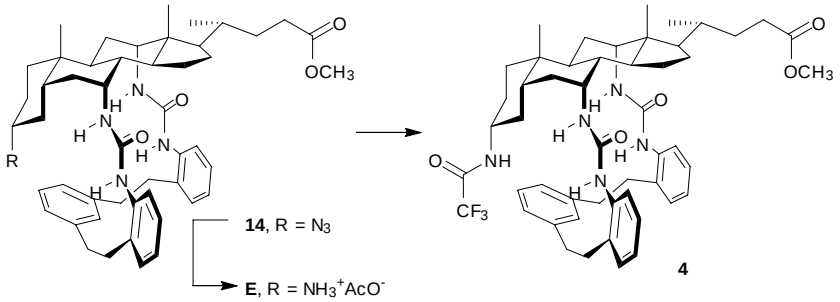
followed by flash chromatography (EtOAc in CH_2Cl_2 20% to 50%) yielded the cholaphane **3** as a white solid (123 mg, 0.15 mmol, 22% over two steps). R_f 0.2 (50% EtOAc in hexane); mp 131-133 °C; ^{1}H NMR (400.18 MHz, $(CD_3)_2CO$); δ = 0.93 (3 H, s, 18-*H*$_3$), 0.97 (3 H, d, *J* = 6.6 Hz, 21-*H*$_3$), 1.11 (3 H, s, 19-*H*$_3$), 1.93 (3 H, s, C*H*$_3$CO$_2$), 2.98-3.12 (8 H, m, C*H*$_2$), 3.63 (3 H, s, CO$_2$C*H*$_3$), 4.01 (1 H, br s, 7β-*H*), 4.26-4.30 (1 H, m, 12β-*H*), 4.58-4.65 (1 H, m, 3β-*H*), 5.62 (1 H, br d, *J* = 8.5 Hz, 12α-N*H*), 5.68 (1 H, br d, *J* = 8.1 Hz, 7α-N*H*), 6.78 (2 H, br s, Ar-N*H*), 7.11-7.16 (2 H, m, Ar-*H*), 7.28-7.34 (4 H, m, Ar-*H*), 7.37 (1 H, q, *J* = 7.6 Hz, Ar-*H*), 7.45-7.48 (2 H, m, Ar-*H*), 7.70 (1 H, m, Ar-*H*), 8.13 (1H, dd, *J* = 1.1, 8.2 Hz, Ar-*H*), 8.25 (1 H, dd, *J* = 1.3, 8.3 Hz, Ar-*H*); ^{13}C (100.63 MHz, $(CD_3)CO$); δ =14.1 (18-*C*H$_3$), 17.9 (21-*C*H$_3$), 21.3 (*C*H$_3$CO$_2$), 23.2 (19-*C*H$_3$), 24.3, 27.7, 27.9, 28.2, 31.6, 31.7, 33.5, 34.6, 34.9, 35.6 (*C*H$_2$), 35.8 (*C*H$_2$), 36.1, 36.2, 36.6, 37.1, 38.3, 42.3, 45.3 (7-*C*H), 45.8, 47.4, 49.4, 51.5 (CO$_2$*C*H$_3$), 53.4 (12-*C*H), 74.8 (3-*C*H), 122.2 (Ar-*C*H), 122.6 (Ar-*C*H), 123.2 (Ar-*C*H), 123.5 (Ar-*C*H), 127.4 (Ar-*C*H), 127.5 (Ar-*C*H), 127.6 (Ar-*C*H), 127.7 (Ar-*C*H), 128.4 (Ar-*C*H), 129.6 (Ar-*C*H), 129.7 (Ar-*C*H), 138.6, 138.7, 142.9, 143.0, 155.5 (NH*C*ONH), 170.3 (CH$_3$*C*O$_2$), 174.4 (*C*O$_2$CH$_3$); IR (Neat): ν = 3393, 2947, 2869, 1692, 1587, 1531, 1450, 1294, 1246, 1027, 753, 703 cm^{-1}; HRMS (ES+): m/z calculated for $[M + Na]^+$ =853.4875, found 853.4860, m/z calculated for $[M + H]^+$ = 831.5055, found 831.5058; elemental analysis calculated (%) for $C_{51}H_{66}N_4O_6 + H_2O$, C 72.14, H 8.07, N 6.60, found, C 72.28, H 8.05, N 6.08.



3α-azidocholaphane 14.

Bis-carbamate **13** was converted to azidocholaphane **14** using the method described above for the preparation of **3**. Purification of the crude product by column chromatography (5%-40% EtOAc in CH_2Cl_2), yielded compound **14** as a white solid (130 mg, 0.16 mmol, 24%). R_f 0.2 (20% EtOAc in CH_2Cl_2); ^{1}H NMR (400.18 MHz, ($CDCl_3$); δ = 0.77 (3 H, s, 18-*H*$_3$), 0.88 (3 H, d, *J* = 5.4 Hz, 21-*H*$_3$), 0.97 (3 H, s, 19-*H*$_3$), 2.66-2.74 (5 H, m, C*H*$_2$), 2.89-3.14 (3 H, m, C*H*$_2$), 3.32 (1H, br s, 3β-*H*), 3.61

(3 H, s, CO_2CH_3), 4.05 (1 H, m, 7β-*H*), 4.20-4.22 (1 H, m, 12β-*H*), 4.42 (1 H, br d, *J* = 8.4 Hz, 7α-N*H*), 4.73 (1 H, br d, *J* = 7.6 Hz, 12α-N*H*), 5.78 (1 H, br s, Ar-N*H*), 6.00 (1 H, br s, Ar-N*H*), 6.42 (1 H, br s, Ar-*H*), 6.96-7.00 (3 H, m, Ar-*H*), 7.08-7.17 (3 H, m, Ar-*H*), 7.23-7.31 (2 H, m, Ar-*H*), 7.41 (1 H, br t, *J* = 7.6 Hz, Ar-*H*), 7.50-7.55 (2 H, m, Ar-*H*); ^{13}C (100.63 MHz, ($CDCl_3$); δ =13.7 (18-*C*H_3), 17.3 (21-*C*H_3), 22.9, 23.3 (19-*C*H_3), 26.6, 27.0, 29.6, 30.4, 31.1, 32.6, 34.6, 34.9 (*C*H_2), 37.0 (*C*H_2), 41.5, 44.7, 46.5 (7-*C*H), 48.5, 51.4 (CO_2CH_3), 52.4 (12-*C*H), 58.5, 61.3, 126.8 (*C*H), 127.0 (*C*H), 127.4 (*C*H), 128.4 (*C*H), 128.9 (*C*H), 136.3, 141.2, 141.4, 155.4 (NH*C*ONH), 174.3 (*C*O_2CH_3); IR (Neat): ν = 3394, 2936, 2866, 2090, 1735, 1669, 1513, 1447, 1203, 751, 703 cm^{-1}; HRMS (ES+): m/z calculated for $[M + Na]^+$ = 836.4834, found 836.4839.



Cholaphane 4.

Zinc dust (77.9 mg, 70 % by weight) was added to a solution of 3α-azide **14** (111 mg, 136 μmol) in glacial acetic acid (10 mL) and the reaction was stirred under N_2 for 16 h. On completion the zinc was removed by filtration and washed with acetic acid (2 × 10 mL). The filtrate and washings were evaporated, and residual acetic acid was removed by addition-evaporation of toluene (2 × 5 mL) followed by CH_2Cl_2 (2 × 5 mL), to give ammonium salt **E** as a white solid (115 mg, 0.14 mmol).

To a solution of **E** (115 mg, 0.14 mmol) in dry CH_2Cl_2 (10 mL) was added Et_3N (45 μL, 0.32 mmol) followed by trifluoroacetic anhydride (45 μL, 0.32 mmol). The reaction mixture was stirred for 3 h. The solvent was removed *in vacuo* and the residue was redissolved in CH_2Cl_2 (10 mL), washed with HCl (1 M, 2 × 10 mL), and dried (Na_2SO_4). Evaporation followed by flash chromatography (5% MeOH in CH_2Cl_2) yielded compound **4** as a white solid (65.4 mg, 0.07 mmol, 54%). R_f 0.4 (20% MeOH in CH_2Cl_2); mp 172-174 °C; ^1H NMR (399.78 MHz, $(CD_3)_2CO$); δ = 0.87 (3 H, s, 18-*H*$_3$),

0.88 (3 H, d, J = 6.4 Hz, 21-H_3), 1.09 (3 H, s, 19-H_3), 2.69-3.02 (8 H, m, CH_2), 3.41 (1 H, m, 3β-*H*), 3.53 (3 H, s, CO_2CH_3), 4.01 (1 H, br s, 7β-*H*), 4.26-4.28 (1 H, m, 12β-*H*), 5.89 (1 H, br d, J = 8.6 Hz, 12α-N*H*), 5.91 (1 H, br d, J = 8.1 Hz, 7α-N*H*), 6.97-7.03 (2 H, m, Ar-*H*), 7.05 (1 H, br s, N*H*), 7.10 (1 H, br s, N*H*), 7.16-7.22 (4 H, m, Ar-*H*), 7.27-7.31 (4 H, m, Ar-*H*), 8.06-8.11 (2 H, m, Ar-*H*); ^{13}C (100.63 MHz, $(CD_3)_2CO$); δ =14.0 18-CH_3), 17.9 (21-CH_3), 23.6 (19-CH_3), 24.3, 27.0, 27.7, 28.2, 31.7, 33.5, 34.7 (CH_2), 35.9, 36.1, 36.4, 36.8, 36.9, 38.3, 43.0, 45.3, 45.8, 47.7 (7-*C*H), 49.5, 51.5 (CO_2CH_3), 53.4 (12-*C*H), 118.0 (CF_3, q, J = 238.3 Hz), 122.5, 122.7, 123.4 (Ar-*C*H), 123.6 (Ar-*C*H), 127.4(Ar-*C*H), 127.5(Ar-*C*H), 127.6(Ar-*C*H), 127.7 (Ar-*C*H), 128.4 (Ar-*C*H), 129.7 (Ar-*C*H), 129.8 (Ar-*C*H), 138.6, 138.7, 142.9, 143.1, 155.5 (NH*C*ONH), 155.6 (NH*C*ONH), 158.1 (*C*OCF_3, q, J = 43.8 Hz), 174.4 (*C*O_2CH_3); IR (Neat): ν = 3376, 2924, 1703, 1529, 1449, 1156, 1049, 749, 701 cm^{-1}; HRMS (ES+): m/z calculated for $[M + Na]^+$ = 906.4752, found 906.4765.33; elemental analysis calculated (%) for $C_{51}H_{64}F_3N_5O_5$ + 2.5 H_2O C 65.93, H 7.49, N 7.54, found C 66.12, H 7.51, N 7.53.

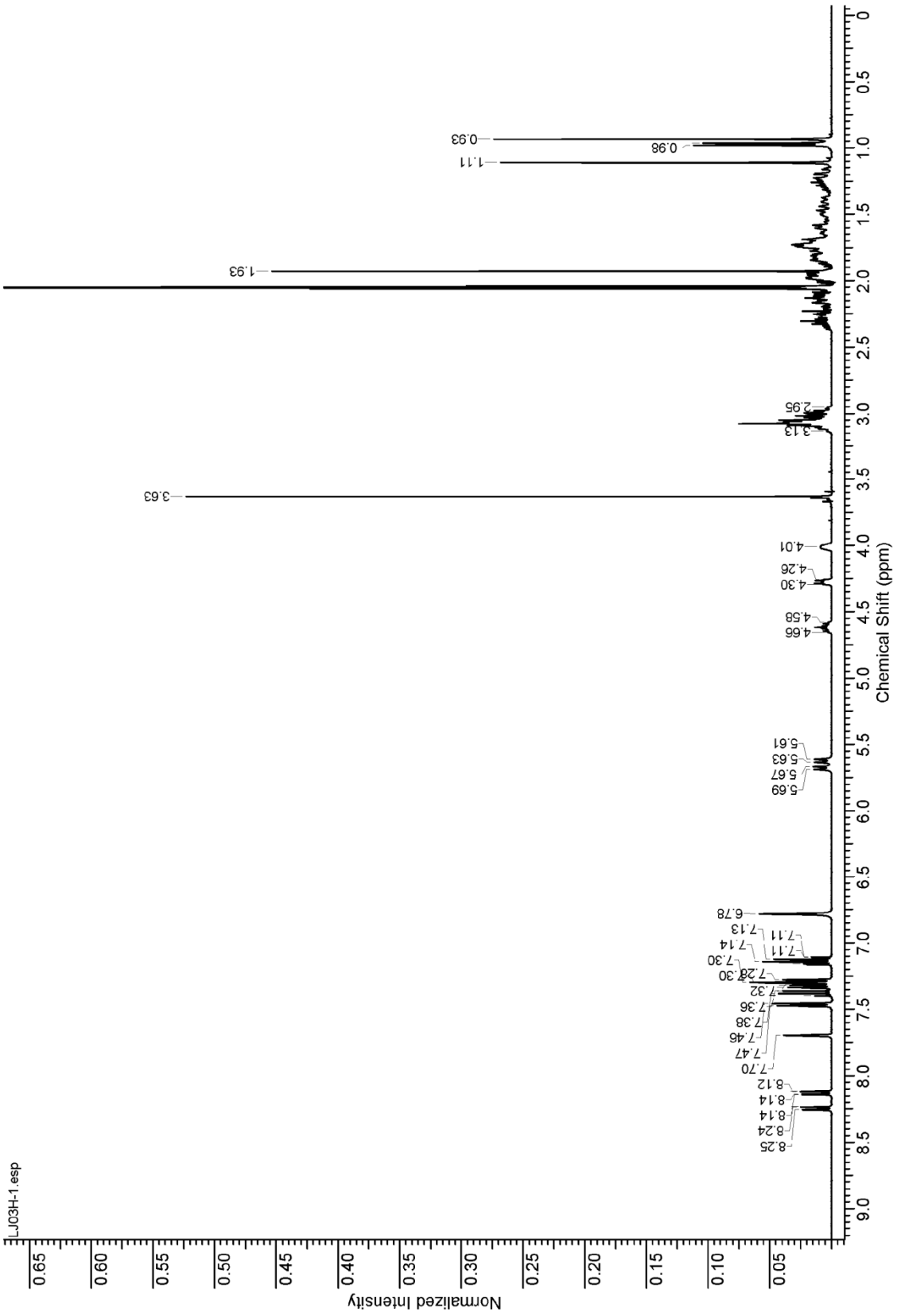


Figure S1. ^{1}H NMR of receptor **3** in acetone-d_6

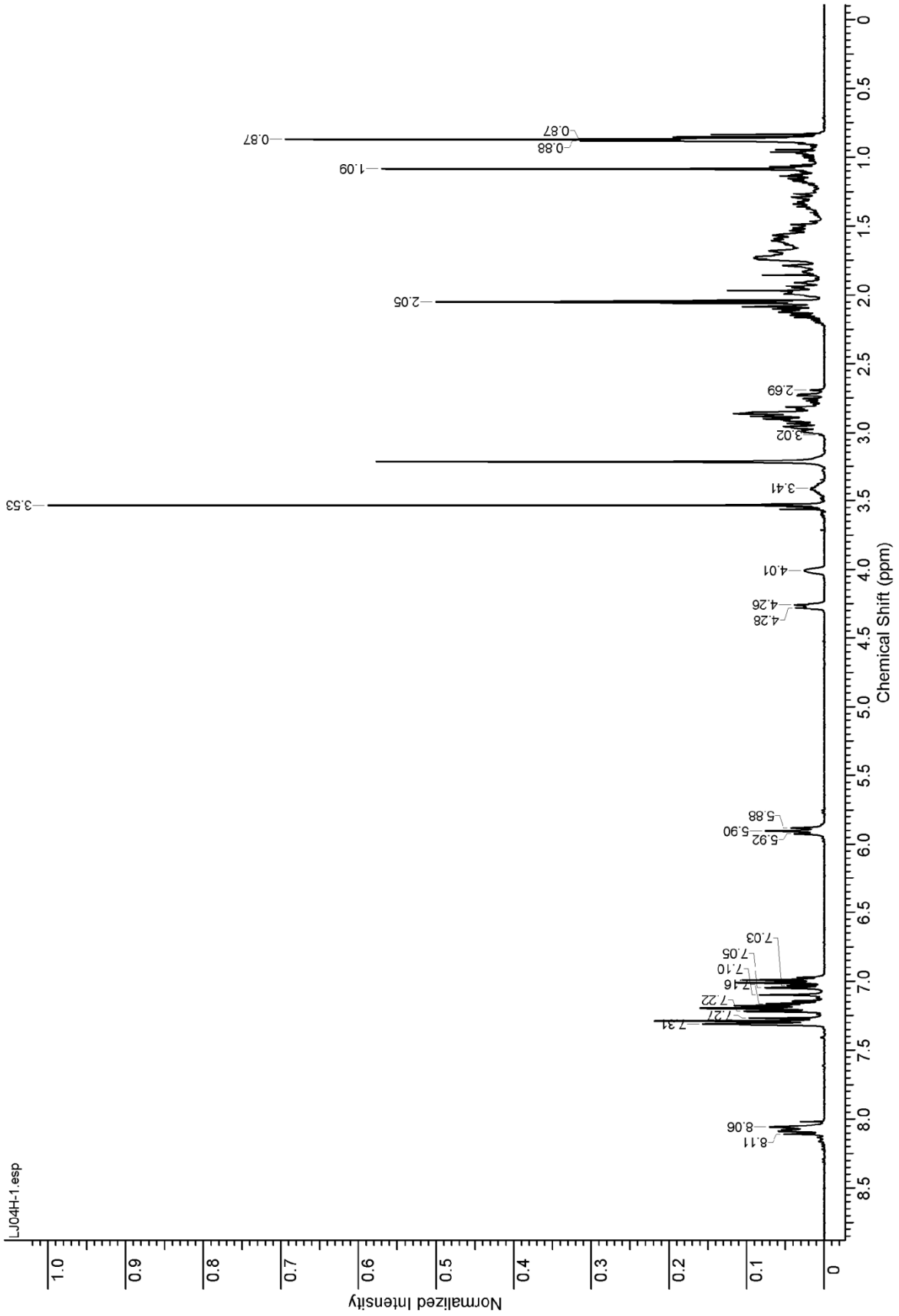


Figure S2. ^{1}H NMR of receptor **4** in acetone-d_6

Molecular Modelling:

Molecular modelling was carried out using Macromodel 9.1, accessed via Maestro 7.5 running in a Redhat Enterprise Linux Workstation 4 OS environment. The structure shown in Figure 1 is the global minimum from a Monte Carlo Molecular Mechanics search, employing the MMFFs force field, in which the non-steroidal bonds linking the two urea groups were allowed to rotate. The programme yields NH···Cl^- and CH···Cl^- contact distances which conform to those found in crystallographic surveys.[5]

Measurement and calculation of transport rates:

Chloride transport was measured using large unilamellar vesicles (LUVs, 200 nm average diameter) composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and cholesterol at a ratio of 7:3. The receptor (4.00 μL, 1.00 mM in deacidified $CHCl_3$) was added to a solution of POPC (543 μL, 12.9 mM in deacidified $CHCl_3$) and cholesterol (175 μL, 17.1 mM in deacidified $CHCl_3$) to give a receptor to lipid ratio of 1:2500. The lipid/receptor mixture was evaporated under a stream of N_2 and dried under high vacuum for 1 h. The residue was hydrated in 500 μL of a solution of $NaNO_3$ (225 mM) and lucigenin (1 mM) and was then sonicated for 30 s and stirred for 1 h to give heterogeneous LUVs. The heterogeneous LUVs were disrupted by 10 freeze-thaw cycles and then the solution was carefully extruded (25 times) through a polycarbonate membrane (200 nm pore size) to give a uniform distribution of LUVs. The external lucigenin was removed by passing the solution though a size exclusion column (sephadex 50G, eluted in $NaNO_3$ 225 mM) and the collected vesicles were made up to a volume of 25 mL (0.4 mM) with $NaNO_3$ solution (225 mM). After the addition of NaCl (75 μL, 1 M) to a cuvette containing 3 mL of the vesicle solution the fluorescence decay was monitored over 900 s.

The initial rate of fluorescence decay was converted to initial rate of chloride influx using equation 1 from Pope *et al*,[6] which was first used in the lucigenin assay by Wissing *et al*:[7]

$$\frac{d[Cl^-]_{in}}{dt_{(t=0)}} = \frac{-1}{K_{sv} \cdot F_{in}} \cdot \frac{dF}{dt_{(t=0)}} \qquad (1)$$

$[Cl^-]_{in}$ is the internal chloride concentration, K_{sv} is the Stern-Volmer constant (106.2 M^{-1}, see Figure S3), F_{in} is the internal fluorescence in the absence of internal chloride and dF/dt (t=0) is the initial rate of change in fluorescence, which was obtained by fitting a double exponential to

the initial plot. F_{in} can be calculated from the initial drop in fluorescence on addition of chloride as shown in equation 2.

$$F_{in} = F_0 - (F_0 - F) \cdot \frac{1 + K_{sv} \cdot [Cl^-]_{ex}}{K_{sv} \cdot [Cl^-]_{ex}} \quad (2)$$

F_0 is the normalised total fluorescence ($F_0=1$) in the absence of external chloride $[Cl^-]_{ex}$ at $t=0$ and F is the normalised fluorescence at the addition of NaCl.

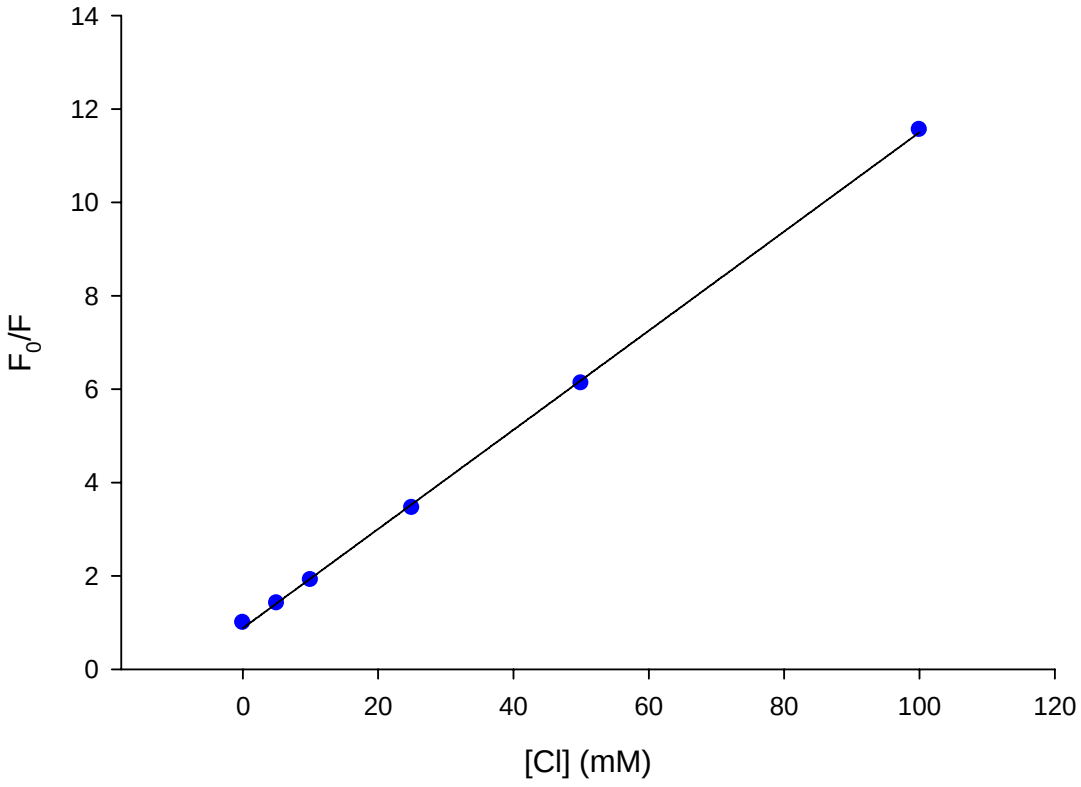


Figure S3. Stern Volmer plot. The Stern Volmer constant (K_{sv}) of lucigenin (0.01 mM) external to liposomes (200 nM) consisting of POPC:cholesterol (7:3, 0.4 mM) in $NaNO_3$ (225 mM) was calculated from the trend line to be 106.2 M^{-1} (the graph shown is an average of 3 data sets).

Measurement of binding constants to tetraethylammonium salts in $CHCl_3$.[8]

Et_4N^+ salts were obtained from chemical suppliers. All host solutions were prepared using chloroform that had been deacidified by passage through a flash chromatography column containing

basic alumina, and pre-saturated with deionised water that had been passed through a Millipore filtration system. Hygroscopic salts were dried and stored under dry nitrogen in a desiccator prior to solution preparation. Guest solutions were prepared using deionised water that had been passed though a Millipore filtration system.

The following is a typical example of an extraction experiment involving receptor **4** and substrate $Et_4N^+Cl^-$.

A solution of cholaphane **4** (0.7 mM) in water-saturated chloroform was prepared and portion (3 mL) was added to a sample tube (30 mL). To this organic solution was added an aqueous solution of $Et_4N^+Cl^-$ (10 mL, 10 mM). A small magnetic stirring bar was then added to the tube and the lower half of the tube was immersed in a water bath that was heated to 303 K. The tube was stoppered after 30 seconds and the contents were stirred vigorously. After 30 minutes, stirring was stopped and the two phases were allowed to separate. The majority of the aqueous phase was then removed using a pipette and the remainder was removed by filtration through Whatman 1PS hydrophobic filter paper. The filtrate was evaporated and the resulting solid was dissolved in deuterated chloroform containing $Ph_4P^+Br^-$ (7.5 mM).* The ^{1}H NMR spectrum was recorded and analysed as described in ref. 8.

* Addition of excess salt sharpens the ^{1}H NMR spectrum, presumably by ensuring that all receptor molecules are bound.

References and Notes

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