Supporting Information For:

Chloride Transport Across Lipid Bilayers and Transmembrane Potential Induction by an Oligo-phenoxyacetamide

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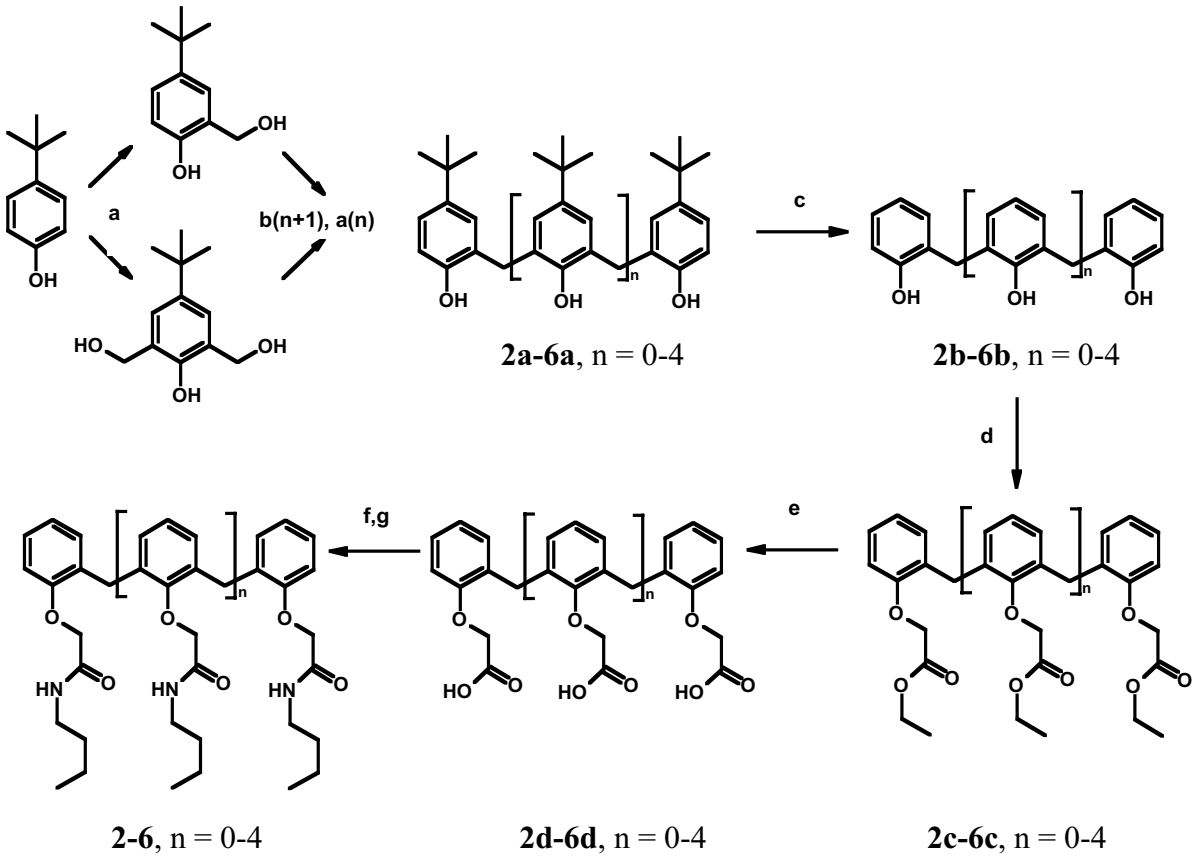
1. General Experimental
2. Synthetic Details for Compounds **2-6**
3. Figures and Experimentals

General. The ^{1}H NMR spectra were recorded on a Bruker DRX400 instrument operating at 400.130 MHz. Chemical shifts are reported in ppm relative to the residual protonated solvent peak. The ^{13}C NMR spectra were recorded on the same instrument at 100.613 MHz and chemical shift values are reported in ppm relative to the solvent peak. Both ^{1}H and ^{13}C spectra were taken in the same solvent for each sample. Mass spectra were recorded on a JEOL SX-102A magnetic sector mass spectrometer using the fast atom bombardment (FAB) technique. All fluorimetric experiments were carried out on an SLM Aminco® (Aminco Bowman® Series 2) Luminescence Spectrometer. Vapor pressure osmometry (VPO) was performed on a Wescor 5520, Vapro™ instrument. The pH of solutions was monitored with an Orion pH-meter, model 420A, with a Ag/AgCl pH-sensitive electrode. Chromatography was performed using 60-200 mesh silica purchased from Baker and 40-120 μ Sephadex G-10 purchased from Pharmacia Fine Chemicals. Thin layer chromatography was performed on Kieselgel 60 F254 and Uniplate™ Silica Gel GF silica-coated glass plates and visualized by UV and I_2. High-pressure extrusion was performed on the Avanti™ mini-extruder with a 0.1 μm polycarbonate membrane. All chemicals and solvents were purchased from Sigma, Fluka, Aldrich or Acros. EYPC was purchased from Avanti Polar Lipids.

Synthesis. The general synthetic scheme for the compounds **2**-**6** is shown in Scheme S1. The synthesis of calix[4]arene **C1**, calix[4]arene **C2** and N-butyl-2-phenoxyacetamide **1** was reported previously[1].

[1] (a) Sidorov, V.; Kotch, F.W.; El-Kouedi, M.; Davis, J.T. *Chem. Commun*., **2000**, 2369. (b) Sidorov, V.; Kotch, F.W.; Abdrakhmanova, G.; Mizzani, R; Fettinger, J.C.; Davis, J.T. *J. Am. Chem. Soc.*, **2002**, *124*, 2267.



Scheme S1. Synthesis of butylamides **2-6**. a), b) and c) literature procedures,[2] d) $BrCH_2COOEt$, acetone, Cs_2CO_3, reflux, e) KOH aq, THF, MeOH, rt, f) $SOCl_2$, benzene, g) $BuNH_2$, Et_3N, CH_2Cl_2, rt.

Bis-(ethyl phenoxyacetate) 2c. Diphenol **2b** (382 mg, 1.91mmol), ethyl bromoacetate (0.87 g, 5.2 mmol) and Cs_2CO_3 (1.24 g, 3.82 mmol) were suspended in 50 mL of acetone, and stirred under reflux overnight. The reaction mixture was cooled to the room temperature, the solvent evaporated under reduced pressure. The residual solid was partitioned in chloroform-water, and the organic layer washed with 0.1 N HCl. The organic layer was separated and solvent removed under reduced pressure. The crude solid was purified by the column chromatography (silica gel, methylene chloride), and 537 mg of bis-(ethyl phenoxyacetate) **2c** was obtained (1.44 mmol, 75%). ^{1}H NMR (DMSO-d6, 25 ^{o}C) δ: 7.15-7.10 (m, 4H, Ar-H), 6.86-6.83 (m, 4H, Ar-H), 4.78 (s, 4H, Ar-O-*CH_2*-CO),

[2] Sone, T.; Ohba, Y.; Yamazaki, H. *Bull. Chem. Soc. Jpn.,* **1989**, *62*, 1111.

4.17 (q, 4H, J = 7.1 Hz, Ar-O-CH_2-CO-*CH_2*-CH_3), 3.96 (s, 2H, Ar-*CH_2*-Ar), 1.21 (t, 6H, J = 7.1 Hz, Ar-O-CH_2-CO-CH_2-*CH_3*). ^{13}C δ: 169.3, 155.9, 130.8, 129.1, 127.6, 121.4, 112.0, 65.4, 61.0, 29.6, 14.5. MS (FAB) ($[M+H]^+$): 373.1, calcd for $C_{21}H_{24}O_6$ 372.16.

Diphenoxyacetic acid 2d. Bis-(ethyl phenoxyacetate) **2c** (476 mg, 1.28 mmol) was dissolved in 3 mL MeOH and 5 mL THF, and 1 mL of 45% aqueous KOH was added dropwise. The reaction mixture was stirred overnight at room temperature under nitrogen, evaporated to dryness, dissolved in 10 mL of water and acidified with 6 N HCl to pH 1. The precipitate formed was filtered and washed with water. The aqueous layer was extracted with chloroform 4 times, the organic layer separated and solvent removed under reduced pressure. The solid formed was recombined with the filtrate and dried for 3 hrs under high vacuum to give 434 mg of diphenoxyacetic acid **2d** (1.37 mmol, 107%). ^{1}H NMR (DMSO-d6, 25 ^{o}C) δ: 7.12-7.06 (m, 4H, Ar-H), 6.82-6.78 (m, 4H, Ar-H), 4.56 (s, 4H, Ar-O-*CH_2*-CO), 3.95 (s, 2H, Ar-*CH_2*-Ar). ^{13}C δ: 170.8, 156.4, 130.7,129.2, 127.4, 120.9, 112.1, 66.1, 29.5. MS (FAB) ($[M+H]^+$): 317.1, calcd for $C_{17}H_{16}O_6$ 316.09.

Bis-(N-butyl-2-phenoxyacetamide) 2. Diphenoxyacetic acid **2d** (418 mg, 1.32 mmol) was suspended in benzene (25 mL), and $SOCl_2$ (2 mL, 27.4 mmol) was added. The reaction mixture was stirred under reflux for 2.5 hours. Over the course of the reaction, the turbid suspension changed into a yellow transparent solution. The reaction mixture was cooled to room temperature, the solvent and excess of thionyl chloride was removed under reduced pressure, and the remaining traces of thionyl chloride were removed by subsequent co-evaporation with benzene. Diphenoxyacetyl chloride was reacted without further purification. The total amount obtained was dissolved in 25 mL of dry CH_2Cl_2 and Et_3N (556 μL, 3.99 mmol) and $BuNH_2$ (652 μL, 6.6 mmol) were added. The reaction mixture was stirred overnight at room temperature, the solvent was evaporated under reduced pressure, and the resulting oil was dried under high vacuum, dissolved in chloroform and washed with water. The organic layer was separated, dried over sodium sulfate, and the solvent removed under reduced pressure. Purification by column chromatography (silica gel, MeOH:CH_2Cl_2 1:99) gave 448 mg of bis-(N-butyl-2-phenoxyacetamide) **2** (1.05 mmol, 85% from **2c**). ^{1}H NMR (DMSO-d6, 25 ^{o}C) δ: 7.62 (t,

2H, J = 6 Hz, CONH), 7.16 (dt, 2H, J = 1.6 Hz, 7.8 Hz, Ar-H), 7.03 (dd, 2H, J = 1.2 Hz, 7.6 Hz, Ar-H), 6.90-6.85 (m, 4H, Ar-H), 4.45 (s, 4H, Ar-O-*CH_2*-CO), 4.04 (s, 2H, Ar-*CH_2*-Ar), 3.08 (app q, 4H, J = 6.8 Hz, NH-*CH_2*-CH_2-CH_2-CH_3), 1.35 (m, 4H, J = 7.2 Hz, NH-CH_2-*CH_2*-CH_2-CH_3), 1.21 (m, 4H, J = 7.2 Hz, NH-CH_2-CH_2-*CH_2*-CH_3), 0.83 (t, 6H, J = 7.2 Hz, NH-CH_2-CH_2-CH_2-*CH_3*). ^{13}C δ: 167.8, 156.0, 130.6, 129.2, 127.7, 121.5, 112.3, 67.7, 38.4, 31.6, 30.0, 19.9, 14.1. MS (FAB) ($[M+H]^+$): 427.31, calcd for $C_{25}H_{34}N_2O_4$ 426.25.

Tris-(ethyl phenoxyacetate) 3c. Triphenol **3b** (53 mg, 0.17mmol), ethyl bromoacetate (117 mg, 0.70 mmol) and Cs_2CO_3 (166 mg, 0.51 mmol) were suspended in 25 mL of acetone, and stirred under reflux overnight. The reaction mixture was cooled to room temperature, the solvent evaporated under reduced pressure, the residual solid was suspended in chloroform-water, and the organic layer washed with 0.1 N HCl. The organic layer was separated and solvent removed under reduced pressure. The crude solid was purified by column chromatography (silica gel, methylene chloride), and 68 mg of tris-(ethyl phenoxyacetate) **3c** was obtained (0.14 mmol, 83%). ^{1}H NMR ($CDCl_3$, 25 ^{o}C) δ: 7.15-6.72 (m, 11H, Ar-H), 4.60 (s, 4H, Ar-O-*CH_2*-CO), 4.39 (s, 2H, Ar-O-*CH_2*-CO), 4.25-4.18 (m, 6H, Ar-O-CH_2-CO-*CH_2*-CH_3), 4.12 (s, 4H, Ar-*CH_2*-Ar), 1.25(app t, 9H, J = 7.2 Hz, Ar-O-CH_2-CO-CH_2-*CH_3*). ^{13}C δ: 169.1, 169.0, 155.9, 155.0, 133.6, 131.0, 129.7, 129.0, 127.3, 124.5, 121.6, 111.4, 70.1, 65.7, 61.2, 61.0, 29.7, 14.1. MS (FAB) ($[M+H]^+$): 565.19, calcd for $C_{32}H_{36}O_9$ 564.24.

Triphenoxyacetic acid 3d. Tris-(ethyl phenoxyacetate) **3c** (59 mg, 0.104 mmol) was dissolved in 2 mL MeOH and 2 mL THF, and 0.7 mL of 45% aqueous KOH was added dropwise. The reaction mixture was stirred overnight at room temperature under nitrogen, evaporated to dryness, dissolved in 2 mL of water, acidified with 6 N HCl to pH 1, and the precipitate formed was filtered and washed with water. The aqueous layer was extracted with chloroform 4 times, the organic layer separated and solvent removed under reduced pressure. The solid formed was recombined with the filtrate and dried for 6 hrs under high vacuum to give 57 mg of triphenoxyacetic acid **3c**. ^{1}H NMR (acetone-d6, 25 ^{o}C) δ: 7.2-6.85 (m, 11H, Ar-H), 4.70 (s, 4H, Ar-O-*CH_2*-CO), 4.49 (s, 2H, Ar-O-*CH_2*-

CO), 4.13 (s, 4H, Ar-*CH*$_2$-Ar). ^{13}C δ: 170.4, 156.8, 155.8, 134.7, 131.5, 130.4, 129.7, 128.1, 125.1, 122.0, 117.7, 112.5, 70.4, 65.6, 30.1.

Tris-(N-butyl-2-phenoxyacetamide) 3. Triphenoxyacetic acid **3d** (48 mg, 0.1 mmol) was activated with $SOCl_2$ (1 mL in 3 mL of benzene, 13.7 mmol) by the same procedure as described for the compound **3**. The triphenoxyacetyl chloride was reacted without purification. The total amount obtained was dissolved in 10 mL of dry CH_2Cl_2, and Et_3N (125 μL, 0.9 mmol, 3-fold excess) and $BuNH_2$ (150 μL, 1.5 mmol, 5-fold excess) were added. The reaction mixture was stirred overnight at room temperature, the solvent was evaporated under reduced pressure, and the resulting oil was dried under high vacuum, dissolved in chloroform and washed with water. The organic layer was separated, and solvent removed under reduced pressure. Purification by column chromatography (silica gel, MeOH:CH_2Cl_2 2:98) gave 51 mg of tris-(N-butyl-2-phenoxyacetamide) **3** (0.079 mmol, 89% from **3c**) as white crystals. ^{1}H NMR (DMSO-d6, 25 ^{o}C) δ: 7.99 (t, 1H, J = 5.6 Hz, CONH), 7.66 (t, 2H, J = 5.6 Hz, CONH), 7.18 (dt, 2H, J = 1.6Hz, 8Hz, Ar-H), 7.04 (dd, 2H, J = 1.6 Hz, 8 Hz, Ar-H), 6.96-6.87 (m, 7H, Ar-H), 4.43 (s, 4H, Ar-O-*CH*$_2$-CO), 4.19 (s, 2H, Ar-O-*CH*$_2$-CO), 4.06 (s, 4H, Ar-*CH*$_2$-Ar), 3.13-3.05 (m, 6H, NH-*CH*$_2$-CH$_2$-CH$_2$-CH$_3$), 1.43-1.31 (m, 6H, NH-CH$_2$-*CH*$_2$-CH$_2$-CH$_3$), 1.28-1.16 (m, 6H, NH-CH$_2$-CH$_2$-*CH*$_2$-CH$_3$), 0.82 (t, 9H, J = 7.6Hz, NH-CH$_2$-CH$_2$-CH$_2$-*CH*$_3$). ^{13}C δ: 167.3, 155.4, 154.4, 133.4, 130.3, 128.8, 128.5, 127.5, 124.3, 121.0, 111.8, 38.0, 31.2, 29.3, 19.5, 13.6; MS (FAB) ([M+H]$^+$): 646.42, calcd for $C_{38}H_{51}N_3O_6$ 645.38.

Tetrakis-(ethyl phenoxyacetate) 4c. Tetraphenol **4b** (571 mg, 1.38 mmol), ethyl bromoacetate (1.38 g, 8.3 mmol) and Cs_2CO_3 (1.8 g, 5.5 mmol) were suspended in 25 mL of acetone, and stirred under reflux overnight. The reaction mixture was cooled down to the room temperature, the solvent evaporated under reduced pressure, the residual solid was partitioned in chloroform-water, and the organic layer washed with 0.1 N HCl. The organic layer was separated and solvent removed under reduced pressure. The crude solid was purified by the column chromatography (silica gel, ethyl acetate:hexanes 1:2), and 800 mg of the tetrakis-(ethyl phenoxyacetate) **4c** was obtained (1.06 mmol, 76.6%). ^{1}H NMR (DMSO-d6, 25 ^{o}C) δ: 7.16 (dt, 2H, J = 1.6 Hz, 8.0 Hz, Ar-H), 7.06 (dd, 2H, J = 1.6

Hz, 8.0 Hz, Ar-H), 6.97-6.83 (m, 10H, Ar-H), 4.77 (s, 4H, Ar-O-*CH*$_2$-CO), 4.43 (s, 4H, Ar-O-*CH*$_2$-CO), 4.17-4.08 (m, 8H, Ar-O-CH$_2$-CO-*CH*$_2$-CH$_3$), 4.05 (s, 2H, Ar-*CH*$_2$-Ar), 4.00 (s, 4H, Ar-*CH*$_2$-Ar), 1.20-1.14 (m, 12H, Ar-O-CH$_2$-CO-CH$_2$-*CH*$_3$), ^{13}C δ: 169.2, 169.0, 155.8, 155.1, 134.0, 130.9, 129.2, 129.0, 127.9, 124.4, 121.5, 112.2, 70.1, 65.3, 61.0, 61.0, 29.5, 14.4, 14.4. MS (FAB) ([M+H]$^+$): 757.18, calcd for $C_{43}H_{48}O_{12}$ 756.31.

Tetraphenoxyacetic acid 4d. Tetrakis-(ethyl phenoxyacetate) **4c** (765 mg, 1.01 mmol) was dissolved in 3 mL MeOH and 3 mL THF, and 1 mL of 45% aqueous KOH was added dropwise. The reaction mixture was stirred overnight at room temperature under nitrogen, evaporated to dryness, dissolved in 10 mL of water, acidified with 6 N HCl to pH 1, the precipitate formed was filtered and washed with water. The aqueous layer was extracted with chloroform 4 times, the organic layer separated and solvent removed under reduced pressure. The solid formed was recombined with the filtrate and dried for 4 hrs under high vacuum to give 677 mg of tetraphenoxyacetic acid **4d**. ^{1}H NMR (DMSO-d6, 25 ^{o}C) δ: 13.80 (s, 4H, COOH), 7.55 (dt, 2H, J = 1.6 Hz, 7.8 Hz, Ar-H), 7.44 (dd, J = 1.6 Hz, 7.8 Hz, Ar-H), 7.32-7.17 (m, 10H, Ar-H), 4.86 (s, 4H, Ar-O-*CH*$_2$-CO), 4.51 (s, 4H, Ar-O-*CH*$_2$-CO), 4.20 (s, 2H, Ar-*CH*$_2$-Ar), 4.13 (s, 4H, Ar-*CH*$_2$-Ar), ^{13}C δ: 170.3, 170.1, 155.6, 154.8, 133.6, 130.4, 128.8, 128.6, 127.4, 124.4, 120.9, 111.7, 69.7, 64.8, 29.1, 29.0. (FAB) ([M+H]$^+$): 645.03, calcd for $C_{35}H_{32}O_{12}$ 644.19.

Tetrakis-(N-butyl-2-phenoxyacetamide) 4. Tetraphenoxyacetic acid **4d** (649 mg, 1.05 mmol) was activated with $SOCl_2$ (5 mL in 15 mL of benzene, 68.5 mmol) by the same procedure as described for the compound **2**. The tetraphenoxyacetyl chloride was reacted without purification. The total amount obtained was dissolved in 25 mL of dry CH_2Cl_2 and 3.4 mL of Et_3N (24.4 mmol, 6-fold excess) and 4.1 mL of $BuNH_2$ (41.4 mmol, 10-fold excess) were added subsequently. The reaction mixture was stirred overnight at room temperature, the solvent was evaporated to dryness under reduced pressure, and the resulting oil was dried under high vacuum, dissolved in chloroform and washed with water. The organic layer was separated, and solvent removed under vacuum. Purification by column chromatography (silica gel, MeOH:CH_2Cl_2 2:98 vv) gave 577 mg of tetrakis-(N-butyl-2-phenoxyacetamide) **4** (0.67 mmol, 66 % from **4c**). ^{1}H NMR (DMSO-d6, 25

^{o}C) δ: 7.98 (t, 2H, J = 5.8 Hz, CO-NH), 7.68 (t, 2H, J = 5.8 Hz, CO-NH), 7.18 (dt, 2H, J = 1.2 Hz, 7.6 Hz, Ar-H), 7.03 (dd, 2H, J = 1.2 Hz, 7.6 Hz, Ar-H), 6.98-6.84 (m, 10H, Ar-H), 4.43 (s, 4H, Ar-O-*CH$_2$*-CO), 4.14 (s, 4H, Ar-O-*CH$_2$*-CO), 4.08 (s, 2H, Ar-*CH$_2$*-Ar), 4.05 (s, 4H, Ar-*CH$_2$*-Ar), 3.12-3.05 (m, 8H, NH-*CH$_2$*-CH$_2$-CH$_2$-CH$_3$), 1.39-1.33 (m, 8H, NH-CH$_2$-CH$_2$-*CH$_2$*-CH$_3$), 1.25-1.17 (m, 8H, NH-CH$_2$-CH$_2$-*CH$_2$*-CH$_3$), 0.82 (t, 12H, J = 7.4 Hz, NH-CH$_2$-CH$_2$-CH$_2$-*CH$_3$*). ^{13}C δ: 167.3, 155.4, 154.4, 133.5, 133.4, 130.2, 128.7, 127.5, 124.4, 121.1, 111.8, 71.8, 67.1, 37.9, 31.2, 31.1, 29.3, 19.5, 19.5, 13.6. (FAB) ([M + H]$^{+}$): 865.4, calcd for $C_{51}H_{68}N_4O_8$ 864.51.

Pentakis-(ethyl phenoxyacetate) 5c. Pentaphenol **5b** (508 mg, 0.98 mmol), ethyl bromoacetate (1.23 g, 7.4 mmol, 1.5-fold excess) and Cs_2CO_3 (1.6 g, 4.9 mmol) were suspended in 25 mL of acetone, and stirred under reflux overnight. The reaction mixture was cooled down to the room temperature, the solvent evaporated under vacuum, the residual solid was partitioned in chloroform-water, and the organic layer washed with 0.1 N HCl. The organic layer was separated and solvent removed under vacuum. The crude solid was submitted to the column chromatography (silica gel, ethyl acetate:hexanes 1:2), and 540 mg of the product was obtained (0.57 mmol, 58%). ^{1}H NMR (DMSO-d6, 25 ^{o}C) 7.16 (dt, 2H, j1 = 1.6 Hz, j2 = 7.8 Hz, Ar-H), 7.06 (dd, j1 = 1.6 Hz, j2 = 7.6 Hz, Ar-H), 7.00-6.82 (m, 13H, Ar-H), 4.77 (s, 4H, Ar-O-*CH$_2$*-CO), 4.43 (s, 4H, Ar-O-*CH$_2$*-CO), 4.40 (s, 2H, Ar-O-*CH$_2$*-CO), 4.17-4.08 (m, 10H, Ar-O-CH$_2$-CO-*CH$_2$*-CH$_3$), 4.06 (s, 4H, Ar-*CH$_2$*-Ar), 4.01 (s, 4H, Ar-*CH$_2$*-Ar), 1.20-1.13 (m, 15H, Ar-O-CH$_2$-CO-CH$_2$-*CH$_3$*). ^{13}C δ: 169.2, 169.0, 155.8, 155.3, 155.1, 134.0, 134.0, 133.9, 130.9, 129.2, 129.0, 127.9, 125.0, 124.9, 121.5, 112.2, 70.1, 65.3, 61.0, 61.0, 29.7, 29.5, 14.4, 14.4. (FAB) ([M + H]$^{+}$): 949.1, calcd for $C_{54}H_{60}O_{15}$ 948.39.

Pentaphenoxyacetic acid 5d. Pentakis-(ethyl phenoxyacetate) (515 mg, 0.54 mmol) was dissolved in 3 mL MeOH and 2 mL THF, and 1 mL of 45% aqueous KOH was added dropwise. The reaction mixture was stirred overnight at room temperature under nitrogen, co-evaporated with acetone to dryness, acidified with 6 N HCl to pH 1, and co-evaporated with acetone to dryness. The solid residue was triturated with acetone, the precipitate filtered out, and the organic solution was evaporated to dryness to give 509

mg of the product. ^{1}H NMR (DMSO-d6, 25 ^{o}C) δ: 12.92 (s, 5H, COOH), 7.16 (dt, j1 = Hz, j2 = Hz, Ar-H), 7.07 (dd, 2H, j1 = Hz, j2 = Hz, Ar-H), 6.96-6.80 (m, 13H, Ar-H), 4.67 (s, 4H, Ar-O-*CH*$_2$-CO), 4.36 (s, 4H, Ar-O-*CH*$_2$-CO), 4.34 (s, 2H, Ar-O-*CH*$_2$-CO), 4.08 (s, 4H, Ar-*CH*$_2$-Ar), 4.01 (s, 4H, Ar-*CH*$_2$-Ar). ^{13}C δ: (FAB) ([M + H]$^{+}$): 809.0, calcd for $C_{44}H_{40}O_{15}$ 808.2.

Pentakis-(N-butyl-2-phenoxyacetamide) 5. Pentaphenoxyacetic acid (515 mg, 0.64 mmol) was activated with $SOCl_2$ (3 mL in 10 mL of dry benzene, 41.1 mmol, 13-fold excess.) by the same procedure as described for the compound **3**. The pentaphenoxyacetyl chloride was reacted without purification. The total amount obtained was dissolved in 25 mL of dry CH_2Cl_2 and 3 mL of Et_3N (21.5 mmol, 6.7-fold excess) and 3 mL of $BuNH_2$ (30.3 mmol, 9.5-fold excess) were added subsequently. The reaction mixture was stirred overnight at room temperature, the solvent was evaporated to dryness under reduced pressure, and the resulting oil was dried under high vacuum, dissolved in chloroform and washed with water. The organic layer was separated, and solvent removed under vacuum. Purification by column chromatography (silica gel, MeOH:CH_2Cl_2 3:97) gave 480 mg (0.44 mmol, 80 % from **5c**) of pentakis-(N-butyl-2-phenoxyacetamide) **5**. ^{1}H NMR (DMSO-d6, 25 ^{o}C) δ: 7.97-7.95 (m, 3H, CO-NH), 7.66 (t, 2H, j = 5.6 Hz, CO-NH), 7.18 (app t, 2H, J = 7.8 Hz, Ar-H), 7.03 (app d, 2H, J = 7.2 Hz, Ar-H), 6.99-6.84 (m, 13H, Ar-H), 4.43 (s, 4H, Ar-O-*CH*$_2$-CO), 4.14 (s, 4H, Ar-O-*CH*$_2$-CO), 4.11 (s, 2H, Ar-O-*CH*$_2$-CO), 4.08 (s, 4H, Ar-*CH*$_2$-Ar), 4.05 (s, 4H, Ar-*CH*$_2$-Ar), 3.12-3.05 (m, 10H, NH-*CH*$_2$-CH$_2$-CH$_2$-CH$_3$), 1.41-1.31 (m, 10H, NH-CH$_2$-CH$_2$-*CH*$_2$-CH$_3$), 1.27-1.17 (m, 10H, NH-CH$_2$-CH$_2$-*CH*$_2$-CH$_3$), 0.83-0.80 (m, 15H, NH-CH$_2$-CH$_2$-CH$_2$-*CH*$_3$). ^{13}C δ: 167.8, 167.8, 167.7, 155.9, 154.9, 154.8, 134.0, 133.9, 133.9, 130.7, 129.3, 129.2, 129.2, 129.0, 128.0, 124.9, 121.5, 112.3, 72.2, 72.1, 67.6, 31.6, 31.6, 29.7, 29.5, 19.9, 19.9, 14.1. (FAB) ([M + H]$^{+}$): 1084.30, calcd for $C_{64}H_{85}N_5O_{10}$ 1083.63.

Hexakis-(ethyl phenoxyacetate) 6c. Hexaphenol **6b** (253 mg, 0.41 mmol), ethyl bromoacetate (0.616 g, 3.7 mmol) and Cs_2CO_3 (0.802 g, 2.5 mmol) were suspended in 40 mL of acetone, and stirred under reflux overnight. The reaction mixture was cooled to room temperature, the solvent evaporated under reduced pressure, the residual solid was

partitioned in chloroform-water, and the organic layer washed with 0.1 N HCl. The organic layer was separated and solvent removed under reduced pressure. The crude solid was submitted to the column chromatography (silica gel, ethyl acetate:hexanes 1:2), and 211 mg of the hexakis-(ethyl phenoxyacetate) **6c** was obtained (0.19 mmol, 46%). ^{1}H NMR ($CDCl_3$, 25 ^{o}C) 7.15 (dt, 2H, J=1.6 Hz, 7.9 Hz, Ar-H), 7.08 (dd, 2H, J = 1.6 Hz, 7.2 Hz, Ar-H), 6.98-6.84 (m, 16H, Ar-H), 4.6 (s, 4H, Ar-O-*CH_2*-CO), 4.37 (s, 4H, Ar-O-*CH_2*-CO), 4.34 (s, 4H, Ar-O-*CH_2*-CO), 4.24-4.07 (m, 22H, Ar-O-CH_2-CO-*CH_2*-CH_3 and Ar-*CH_2*-Ar), 1.27-1.13 (m, 18H, Ar-O-CH_2-CO-CH_2-*CH_3*). ^{13}C δ: (FAB) ([M + Na]$^{+}$): 1163.04, calcd for $C_{65}H_{72}O_{18}$ 1140.47.

Hexaphenoxyacetic acid 6d. Hexakis-(ethyl phenoxyacetate) **6c** (202 mg, 0.18 mmol) was dissolved in 1 mL MeOH and 2 mL THF, and 0.3 mL of 45% aqueous KOH was added dropwise. The reaction mixture was stirred overnight at room temperature under nitrogen, co-evaporated with acetone to dryness, acidified with 6 N HCl to pH 1, and co-evaporated with acetone. The solid residue was triturated with acetone, the precipitate filtered out, and the organic solution was evaporated under reduced pressure and dried under high vacuum to give 201 mg of the product. ^{1}H NMR (DMSO-d6, 25 ^{o}C) δ: 12.86 (s, 6H, COOH), 7.13 (dt, J = 1.6 Hz, 7.9 Hz, Ar-H), 7.03 (dd, 2H, J = 1.6 Hz, 7.2 Hz, Ar-H), 6.96-6.79 (m, 16H, Ar-H), 4.65 (s, 4H, Ar-O-*CH_2*-CO), 4.33 (s, 4H, Ar-O-*CH_2*-CO), 4.32 (s, 4H, Ar-O-*CH_2*-CO), 4.06 (br s, 6H, Ar-*CH_2*-Ar), 3.98 (s, 4H, Ar-*CH_2*-Ar).

Hexakis-(N-butyl-2-phenoxyacetamide) 6. Hexaphenoxyacetic acid **7d** (201 mg, 0.20 mmol) was activated with $SOCl_2$ (1.9 mL in 10 mL of dry benzene, 26.0 mmol) by the same procedure as described for the compound **3**. The hexaphenoxyacetyl chloride was reacted without purification. The total amount obtained was dissolved in 10 mL of dry CH_2Cl_2 and Et_3N (0.5 mL, 3.6 mmol) and $BuNH_2$ (0.5 mL, 5.5 mmol) were added. The reaction mixture was stirred overnight at room temperature, the solvent was evaporated under reduced pressure, and the resulting oil was dried under high vacuum, dissolved in chloroform and washed with water. The organic layer was separated, and solvent removed under reduced pressure. Purification by column chromatography (silica gel, MeOH:CH_2Cl_2 3:97) gave 160 mg (0.12 mmol, 68 % from **6c**) of hexakis-(N-butyl-2-

phenoxyacetamide) **6**. ^{1}H NMR (DMSO-d6, 25 ^{o}C) δ: 7.97-7.93 (m, 4H, CO-NH), 7.65 (t, 2H, J = 6.0 Hz, CO-NH), 7.16 (dt, 2H, J = 1.6 Hz, 7.9Hz, Ar-H), 7.03 (dd, 2H, J = 1.6 Hz, 7.2 Hz, Ar-H), 7.00-6.84 (m, 16H, Ar-H), 4.43 (s, 4H, Ar-O-*CH$_2$*-CO), 4.13 (s, 4H, Ar-O-*CH$_2$*-CO), 4.10 (s, 4H, Ar-O-*CH$_2$*-CO), 4.07 (s, 6H, Ar-*CH$_2$*-Ar), 4.04 (s, 4H, Ar-*CH$_2$*-Ar), 3.12-3.04 (m, 12H, NH-*CH$_2$*-CH$_2$-CH$_2$-CH$_3$), 1.41-1.31 (m, 12H, NH-CH$_2$-CH$_2$-*CH$_2$*-CH$_3$), 1.27-1.17 (m, 12H, NH-CH$_2$-CH$_2$-*CH$_2$*-CH$_3$), 0.83-0.79 (m, 18H, NH-CH$_2$-CH$_2$-CH$_2$-*CH$_3$*). ^{13}C δ: 167.3, 167.2, 155.4, 154.4, 133.5, 133.4, 130.2, 128.8, 128.7, 128.6, 127.5, 124.5, 124.4, 121.0, 111.8, 71.8, 71.7, 67.1, 37.9, 31.1, 29.3, 29.1, 19.5, 19.4, 13.6. (FAB) ([M+H]$^{+}$): 1303.37, calcd for $C_{77}H_{102}N_6O_{12}Cs$ 1302.76.

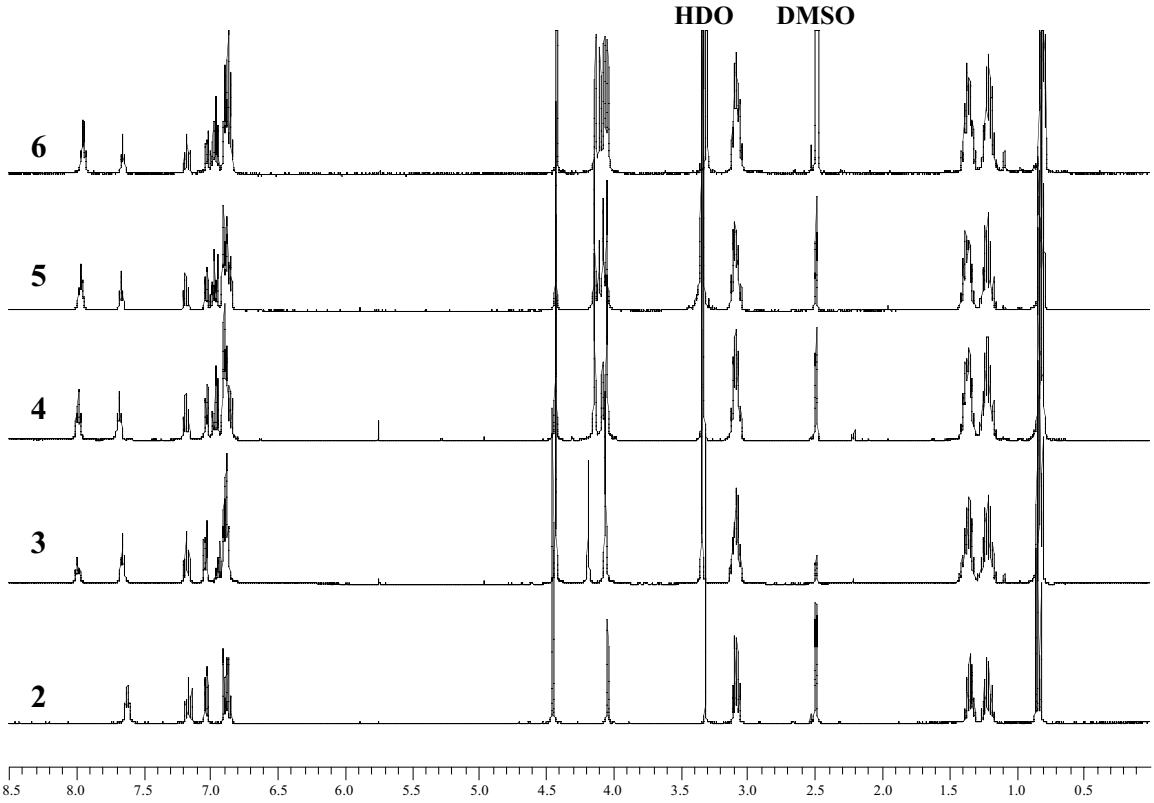


Figure S1. A series of 400 MHz ^{1}H NMR spectra for oligophenoxyacetamides **2-6**.

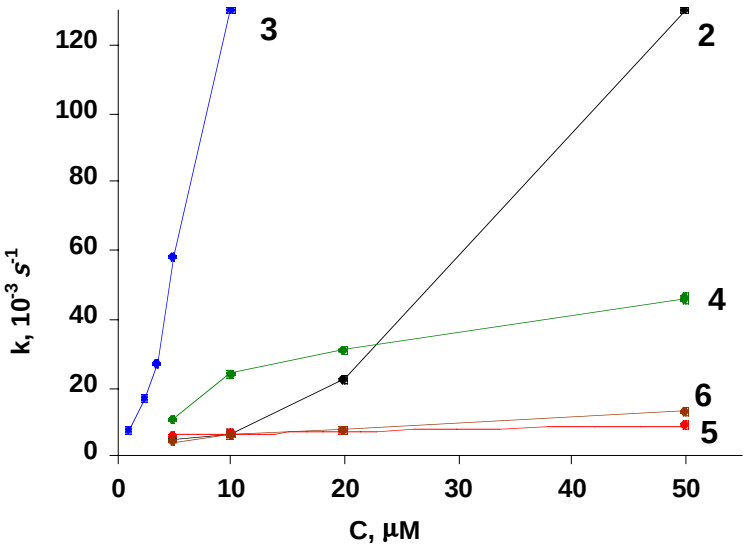


Figure S2. Dependence of the pseudo-first order initial rate constants on the concentration of the ionophore **2-6**. Each point on the plot represents a pH-stat fluorimetric experiment (HPTS emission is monitored at 510 nm with excitation at 403 and 460 nm, EYPC LUV's (1.9 mL of LUV's suspension, 500 µM of EYPC, 10 mM sodium phosphate, pH = 6.4, 100 mM NaCl inside, and outside) with following time events: 20 *s* – 20 µL of DMSO solution of the compound of interest at the appropriate concentration is added (compounds are denoted by the colored legend), 60 *s* – 21 mL of 0.5 M NaOH is injected, 500 *s*- 40 µL of 5% aqueous Triton X100 is injected). Color code for traces denotes application of: **2; 3; 4; 5; 6**. The rate constants values calculated as described in the *Fluorimetric Transport Assays* section below.

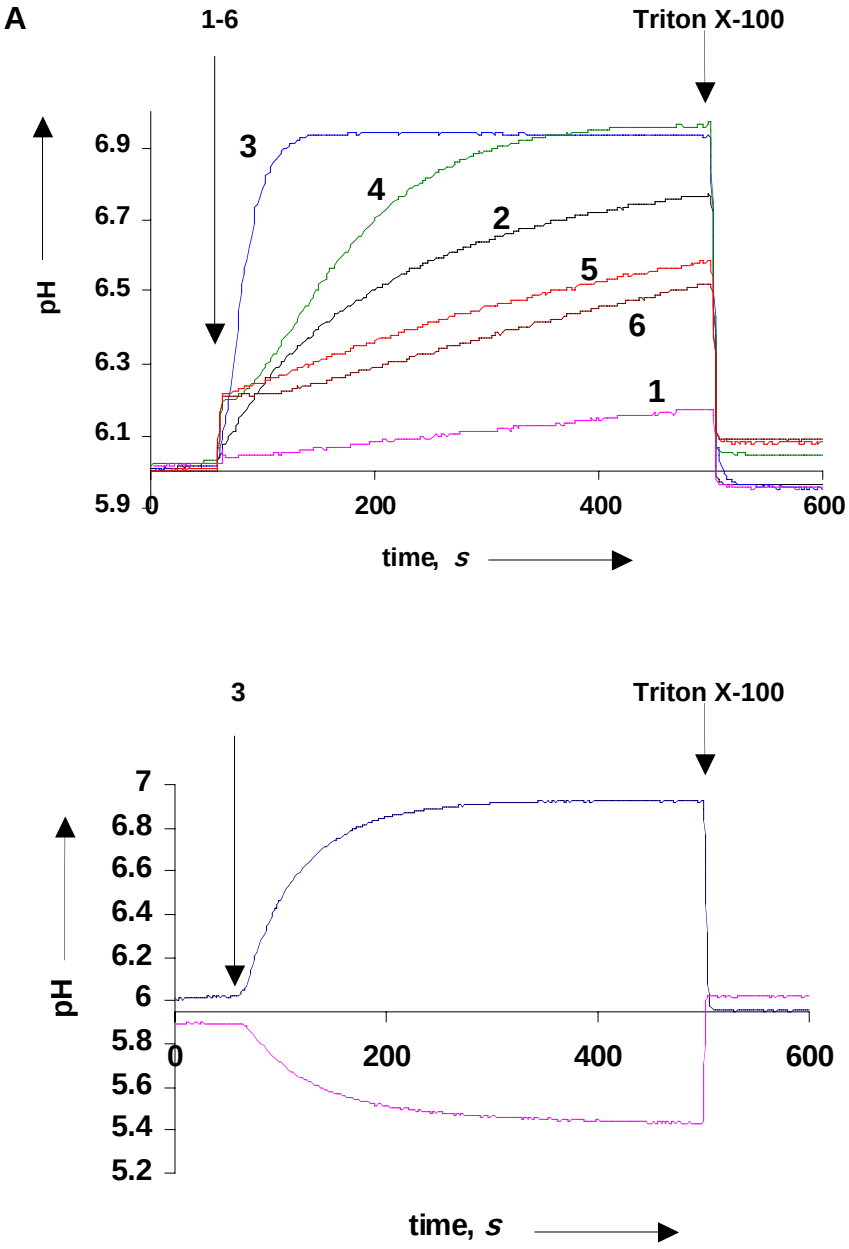


Figure S3. (**A**) Alkalinization of the vesicular aqueous compartment upon application of **1**-**6** to a suspension of NaCl-containing vesicles in isoosmomolar Na_2SO_4 buffer. Concentration of **1**-**6** in the vesicular suspension was 20 μM, or 4:100 ligand to lipid ratio. Color code for traces denotes application of: –**1**; –**2**; –**3**; –**4**; –**5**; –**6**.
(**B**) Alkalinization (upper trace) and acidification (lower trace) of the vesicular aqueous compartment upon injection of **3** (final concentration 5 μM or 1:100 ligand to lipid ratio) to a suspension of NaCl-containing vesicles in isoosmomolar Na_2SO_4 buffer (upper trace) and to a suspension of Na_2SO_4-containing vesicles in isoosmomolar NaCl buffer (lower trace). Liposomes were lysed with Triton X-100 at the end of each experiment.

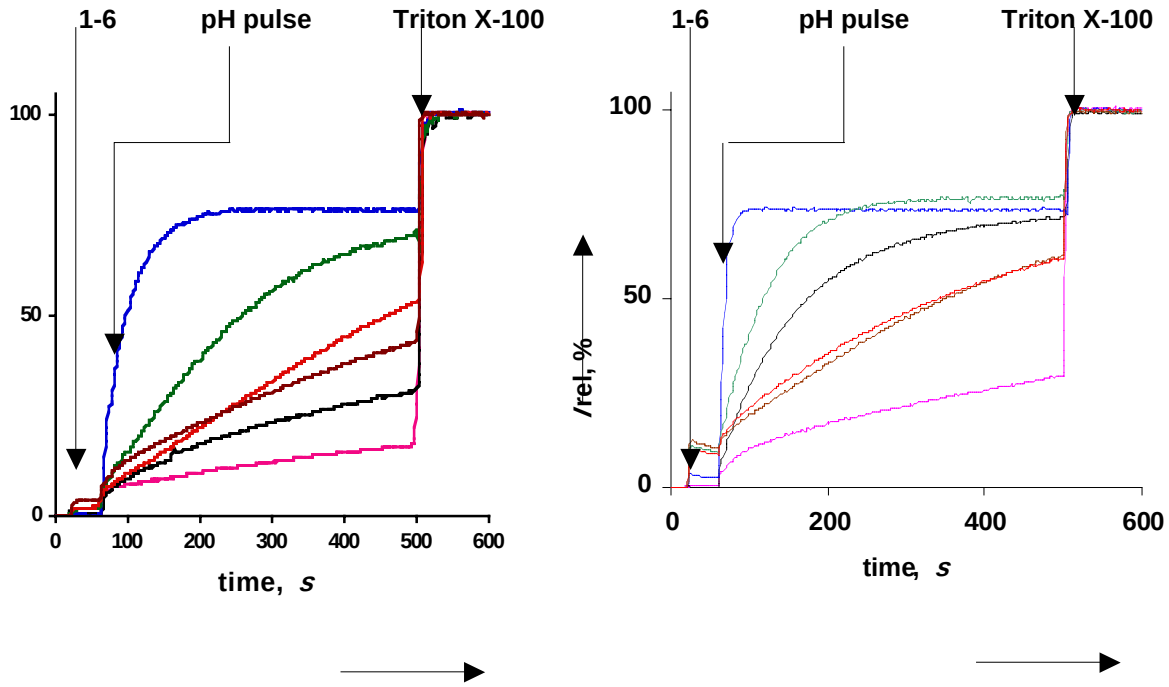


Figure S4. Liposome pH-stat transport assays with **1-6**. Suspension of EYPC LUVs (500 μM of lipid) containing the pH-sensitive dye pyranine (ex 405 and 460 nm, em 510 nm) in a phosphate buffer was used (10 mM $Na_nH_{3-n}PO_4$, n=1,2, pH 6.4, 100 mM NaCl inside and outside). Time events: *20 s:* 20 μL of (A) 0.5 mM or (B) 2.0 mM solution of **2-6** in DMSO added to give 1:100 or 4:100 transporter:lipid molar ratios respectively, *60 s:* 21 μL of 0.5 M NaOH is added, *500 sec:* 40 μL of 5% Triton X-100 is added. Colors in the plots denote application of: _ **1**, _ **2**, _ **3**, _ **4**, _ **5**, _ **6**.

Fluorimetric transport assays

Liposome Preparation for Fluorimetric Assays. Egg yolk L-α-phosphatidylcholine (EYPC ethanol solution, 60 μL, 79 μmol) was dissolved in a $CHCl_3$/MeOH mixture, the solution was evaporated under reduced pressure and the resulting thin film was dried under high vacuum for 2 h. The lipid film was hydrated in 1.2 mL of phosphate buffer (10 mM sodium phosphate, pH = 6.4, 75-100 mM M_nX, M=Na^+, K^+, X=Cl^-, SO_4^{2-}, n=1,2) containing 10 μM HPTS (pyranine, 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt) for 40 min. During hydration, the suspension was submitted to 5 freeze-thaw cycles (liquid nitrogen, water at rt). The large multilamellar liposome suspension (1

mL) was submitted to high-pressure extrusion at rt (21 extrusions through a 0.1 μm polycarbonate membrane afforded a suspension of LUVs with an average diameter of 100 nm). The LUV suspension was separated from extravesicular dye by size exclusion chromatography (SEC) (stationary phase: Sephadex G-10, mobile phase: phosphate buffer) and diluted with the same phosphate buffer to give a stock solution with a lipid concentration of 11 mM (assuming 100% of lipid was incorporated into liposomes).

pH-stat transport assays. Typically, 100 μL of HPTS-loaded vesicles (stock solution) was suspended in 1.9 mL of the corresponding buffer and placed into a fluorimetric cell. The emission of HPTS at 510 nm was monitored with excitation wavelengths at 403 and 460 nm simultaneously. During the experiment, 20 μL of a 0-10 mM DMSO solution of the compound of interest was added (through an injection port), followed by injection of 21 μL of 0.5 M aqueous NaOH. Addition of the NaOH resulted in a pH increase of approximately 1 pH unit in the extravesicular buffer. Maximal possible changes in dye emission were obtained at the end of each experiment by lysis of the liposomes with detergent (40 μL of 5% aqueous Triton X100). The final transport trace was obtained as a ratio of the emission intensities monitored at 460 and 403 nm and normalized to 100% of transport. Pseudo-first order rate constants were calculated from slopes of the plot of $\ln([H^+_{ins}]-[H^+_{out}])$ versus time, where $[H^+_{ins}]$ and $[H^+_{out}]$ are the intravesicular and extravesicular proton concentrations, respectively. The $[H^+_{out}]$ was assumed to remain constant over the course of the experiment, while $[H^+_{ins}]$ values were calculated for each point from the HPTS emission intensities according to the calibration equation $pH = 1.1684 \cdot \log(I_o/I_1)+6.9807$, where I_o is the emission intensity with excitation at 460 nm and I_1 is emission intensity with excitation at 403 nm. The calibration was performed by measuring the HPTS emission intensities and the pH values of a 470 pM HPTS solution in 10 mM phosphate buffer containing 100 mM NaCl (see figure s3). The absolute values for rate constants varied depending on the age of the vesicular solution and the actual stock solution of liposomes used. The ratios between absolute values of rate constants obtained from experiments on the same batch of liposomes, however, did not vary significantly.

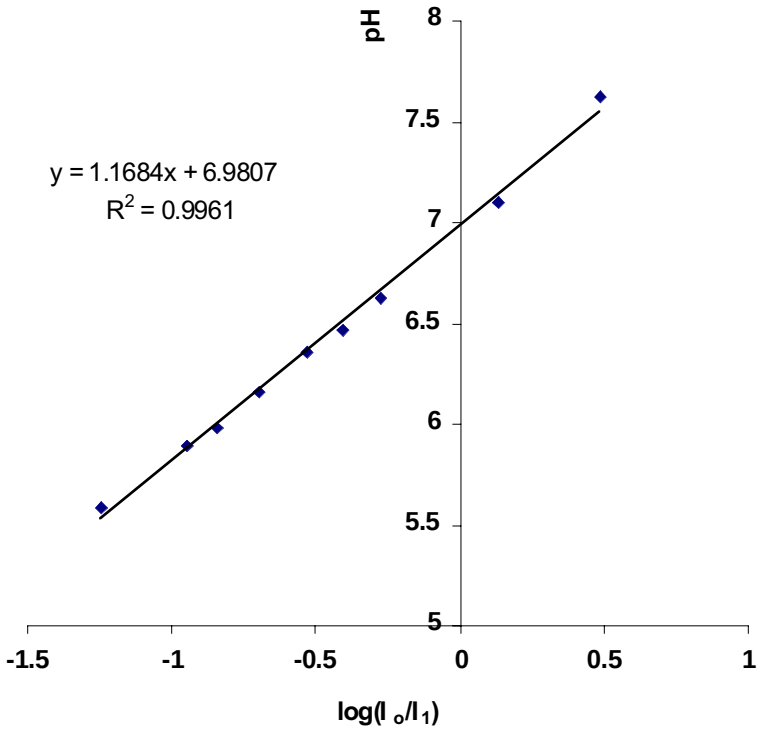


Figure S5. Calibration plot relating the HPTS emission intensity to a pH of the solution.

Analysis of pH changes in the liposomes experiencing a Cl^- gradient. a) Outwardly directed gradient of Cl^-: HPTS-loaded vesicles (100 μL of the stock solution), filled with a saline phosphate buffer (10 mM sodium phosphate, pH 6.4, 100 mM NaCl) were suspended in 1.9 mL of an isoosmotic phosphate sulfate buffer (10 mM sodium phosphate, pH 6.0, 75 mM Na_2SO_4) and placed into a fluorimetric cell. The emission of HPTS at 510 nm was monitored with excitation wavelengths at 403 and 460 nm simultaneously. During the experiment, 20 μL of a 0-2 mM DMSO solution of the compound of interest was added through an injection port. Intravesicular pH values were obtained as described for pH-stat transport assays. At the end of experiment, the aqueous compartment of liposomes was equilibrated with extravesicular solution by lysis of liposomes with detergent (40 μL of 5% aqueous Triton X100). b) Inwardly directed gradient of Cl^-: HPTS-loaded vesicles (100 μL of the stock solution), filled with a isoosmomolar sulfate phosphate buffer (10 mM sodium phosphate, pH 6.0, 75 mM Na_2SO_4) were suspended in 1.9 mL of an isoosmotic phosphate saline buffer (10 mM

sodium phosphate, pH 6.4, 100 mM NaCl) and placed into a fluorimetric cell. Further analysis was conducted as described for the case of an outwardly directed gradient of Cl^-.

Analysis of the electrostatic potential on the surface of liposomes. LUVs (100 μL of stock solution, 100 mM KCl inside and outside or 75 mM Na_2SO_4 inside and outside) were suspended in 1.9 mL of 100 mM NaCl buffer containing 60 nM safranin O (a potential-sensitive dye). Safranin O emission was monitored at 580 nm with excitation at 520 nm. During the course of the experiment, 20 μL of a 0-0.12 mM DMSO solution of valinomycin or 0-2 mM of **3**-**8** DMSO solution was added. Experiments were completed by injection of 20 μL of 1 mM aqueous solution of the defect-inductive peptide melittin.[3]

^{35}Cl NMR Transport Assays

Liposome Preparation for ^{35}Cl NMR Assays. Egg yolk L-α-phosphatidylcholine (1.0 mg/mL EYPC ethanol solution, 250 μL, 329 μmol) was dissolved in a $CHCl_3$/MeOH mixture, the solution was evaporated under reduced pressure and the resulting thin film was dried under high vacuum for 2.5 h. The lipid film was hydrated in 1.0 mL of phosphate buffer (9:1 H_2O:D_2O, 10 mM sodium phosphate, pH = 5.4, 100 mM NaCl) containing 10 mM $CoCl_2$ (^{35}Cl shift reagent) for 40 min. During hydration, the suspension was submitted to 5 freeze-thaw cycles (liquid nitrogen, water at rt). The giant liposome suspension (1 mL) was submitted to high-pressure extrusion at rt (41 passes through a 5.0 μm polycarbonate membrane). The giant vesicle suspension was partially separated from extravesicular $CoCl_2$ by size exclusion chromatography (SEC) (stationary phase: Sephadex G-25, mobile phase: 9:1 H_2O:D_2O, 10 mM sodium phosphate, pH = 6.4, 75 mM Na_2SO_4) to give 12 mL of the giant vesicle suspension (NaCl/$CoCl_2$ inside, Na_2SO_4 outside). The suspension was concentrated to approximately 1.5 mL by centrifugation at 14,000 rpm for 30 *sec* followed by removal of the non-vesicle containing buffer (note, this step further decreased the $CoCl_2$ extravesicular concentration). The 1.5 mL suspension was diluted to 3 mL with the Na_2SO_4 buffer (9:1 H_2O:D_2O, 10 mM sodium phosphate, pH = 6.4, 75 mM Na_2SO_4) to give a suspension that was 88 mM in EYPC (assuming 100% of lipid was incorporated into liposomes and

estimating 20% was lost in purification/concentration). This suspension was used directly in ^{35}Cl NMR transport assays.

^{35}Cl NMR Measurements. All ^{35}Cl NMR spectra were recorded on a Bruker DRX500 instrument operating at 49.0023 MHz for the ^{35}Cl resonance and chemical shift values are reported in ppm relative to 25 mM NaCl in D_2O at 0 ppm (external standard). A 5 mm broad band probe was used. A 90^o pulse width (22 μs), acquisition time of 200 ms with no delay between pulses, and a sweep width of 8.2 kHz was used in all experiments. The number of transients ranged from 3,000 to 20,000. For each run, 400 μL of the giant vesicle suspension was placed in a 5 mm NMR tube and the instrument was locked on D_2O. Details of each spectrum are given in the Figure legends.

[3] Hider, R. C.; Khader, F.; Tatham, A. S. *Biochim. Biophys. Acta*. **1983**, *728*, 206.