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Supplementary Material to Accompany

Synthetic, cation-conducting channels obey the Hammett relationship in a phospholipid bilayer membrane

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Experimental section

^{1}H-NMR were recorded at 300, 500, or 600 MHz in $CDCl_3$ solvent and are reported in ppm (δ) downfield from internal $(CH_3)_4Si$ unless otherwise specified. ^{13}C-NMR were recorded at proportional frequencies as noted above. Infrared spectra were recorded on a Perkin-Elmer 1710 Fourier Transfrorm Infrared Spectrophotometer and were calibrated against the 1601 cm^{-1} band of polystyrene. Melting points were determined on a Thomas Hoover apparatus in open capillaries and are uncorrected. Thin layer chromatographic (TLC) analyses were performed on aluminum oxide 60 F-254 neutral (Type E) with a 0.2 mm layer thickness or on silica gel 60 F-254 with a 0.2 mm layer thickness. Preparative chromatography columns were packed with activated aluminum oxide (MCB 80-325 mesh, chromatographic grade, AX 611) or with Kieselgel 60 (70-230 mesh). Chromatotron chromatography was performed on a Harrison Research Model 7924 Chromatotron with 2 mm thick circular plates prepared from Kieselgel 60 PF-254.

All reactions were conducted under dry N_2 unless otherwise stated. All reagents were the best (non-LC) grade commercially available and were distilled, recrystallized, or used without further purification, as appropriate. Molecular distillation temperatures refer to the oven temperature of a Kugelrohr apparatus. Combustion analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and are reported as percents.

Reagents. Phophatidylcholine ($CHCl_3$:CH_3OH solution), phosphatidylglycerol ($CHCl_3$:CH_3OH solution), Na_2HPO_4, NaH_2PO_4, K_2HPO_4, NaCl, gramicidin D (85% gramicidin A, 15% gramicidin B and C) and, sodium tripolyphosphate (98%) were purchased from Sigma, St. Louis, MO and used without further purification. Diethyl ether (anhydrous) was purchased from Mallinckrodt and distilled over sodium/benzophenone prior to use. The water used to prepare the buffer solution was distilled and deionized. 2,2,2- Trifluoroethanol (TFE), NMR grade, $DyCl_3 \bullet 6H_2O$ (99.99%), D_2O, KCl, $CHCl_3$ (HPLC grade), picric acid, NaOH volumetric solution (0.0975M), and 1,4-dinitrobenzene (98%) were purchased from Aldrich and used without further purification. Chloroform-d (99.8%) was purchased from Isotec, Ohio. Polycarbonate membranes (0.4 μm, 2.5 cm diameter) and membrane holders were obtained from Poretics Corp., Livermore, CA. The sonication was done in a water sonication bath, Branson, model 1200, or in a Branson, Bransonic 12. The mean diameters of the vesicles were determined in a Coulter, submicron particle analyzer, model N4 ND.

Compounds studied. Gramicidin and valinomycin were purchased from Sigma Aldrich and used as received.

***N,N'*-bis(Benzyl)-4,13-diaza-18-crown-6,1**, was prepared as previously reported.[1]

***N,N'*-bis(Dodecyl)-4,13-diaza-18-crown-6, 2**, was prepared as previously reported.[2]

***N,N'*-bis(11-Carboxyundecyl)-4,13-diaza-18-crown-6, 3.** *Benzyl 12-bromodedecanoate (3A):* To a mixture of 12-bromododecanoic acid (4.75 g, 17.0 mmol), DCC (3.86 g, 18.7 mmol), and DMAP (0.23 g, 1.87 mmol) in ether (50 mL) was added benzyl alcohol (2.02 g, 18.7 mmol) at room temperature. After 2 h, the precipitate that formed was removed by filtration and the filtrate was washed successively with 5% $NaHCO_3$ (2×15 mL), water (2×15 mL), 10% citric acid (2×15 mL), water (15 mL), and brine (15 mL). The ether layer was dried ($MgSO_4$) and concentrated. SiO_2 column chromatography for the remaining oil gave **3A** (4.03 g, 64 %) as a colorless oil, bp 197-200 °C (0.5 torr). ^{1}H-NMR: 1.20-1.35 (12H, m, alkyl), 1.35-1.50 (2H, m, $BrCH_2CH_2CH_2CH_2$), 1.57-1.70 (2H, m, $COCH_2CH_2CH_2CH_2$), 1.851 (2H, quintet, J = 7.3 Hz, $BrCH_2CH_2CH_2CH_2$), 2.354 (2H, t, J = 7.5 Hz, $COCH_2CH_2CH_2$), 3.408 (2H, t, J = 6.9 Hz, $BrCH_2CH_2$), 5.116 (2H, s, $ArCH_2O$), 7.32-7.38 (5H, m, aromatics): IR (neat film): 3066, 3034, 2927, 2854, 1737, 1498, 1456, 1383, 1353, 1256, 1214, 1164, 1120, 1003, 908, 736, 698 cm^{-1}. Anal. Calcd for $C_{19}H_{29}BrO_2$; C, 61.79; H, 7.91; Br, 21.63%. Found C, 61.93; H, 7.91; Br, 21.48%.

N,N-bis(11-Benzyloxycarbonylundecyl)-4,13-diaza-18-crown-6, **3B**. A mixture of 4,13-diaza-18-crown-6 (1.20 g, 4.57 mmol), **3A** (3.38 g, 9.15 mmol), Na_2CO_3 (9.69 g,

91.4 mmol), and KI (30 mg, 0.18 mmol) in *n*-PrCN (50 mL) was heated at reflux for 24 h. After cooling, the insoluble materials were removed by filtration and the resulting solution was concentrated to an oil which was re-dissolved in CH_2Cl_2 (40 mL), washed with water (4×15 mL), dried ($MgSO_4$), and concentrated. Toluene was added and evaporated to assure complete removal of *n*-PrCN. The crude, oily product was purified by chromatography (alumina, 3% *i*-PrOH-CH_2Cl_2), afforded **3B** (3.04.g, 79%) as colorless oil, mp < 25 °C, $bp_{0.5}$ > 300°C: ^{1}H-NMR: 1.20-1.36 (28H, m, alkyl), 1.36-1.48 (4H, br, $NCH_2CH_2CH_2$), 1.638 (4H, quintet, J = 7.4 Hz, $COCH_2CH_2CH_2$), 2.352 (4H, t, J = 7.6 Hz, $COCH_2CH_2$), 2.475 (4H, t, J = 7.8 Hz, $NCH_2CH_2CH_2$), 2.771 (8H, t, J = 6.0 Hz, NCH_2CH_2O), 3.56-3.64 (16H, m, CH_2OCH_2), 5.114 (4H, s, $ArCH_2O$), 7.31-7.37 (10H, m, Aromatics): IR (neat film): 3065, 3034, 2927, 2854, 1737, 1456, 1352, 1255, 1163, 1127, 994, 751, 698 cm^{-1}. Anal. Calcd for $C_{50}H_{82}N_2O_8$; C, 71.56; H, 9.85; N, 3.34%. Found, C, 71.60; H, 9.88; N, 3.25%.

N,N'-Bis(11-carboxyundecyl)-4,13-diaza-18-crown-6, 3. A pressure bottle was charged with **3B** (2.00 g, 2.38 mmol) dissolved with 70 mL of *i*-PrOH. To this solution, water (20 mL) was carefully added to keep the solution clear. The vessel was purged with N_2, 10% Pd on carbon was added, and the resulting suspension was shaken on a Parr hydrogenator under H_2 (2 atm) at room temperature. The catalyst was removed (pad of Celite), the mixture was concentrated to dryness, leaving **3** (1.54 g, 97%) as a colorless solid, mp 77-79 °C: ^{1}H-NMR: 1.20-1.38 (28H, m, alkyl), 1.48-1.66 (8H, br, $NCH_2CH_2CH_2$, $COCH_2CH_2CH_2$), 2.269 (4H, t, J = 6.8 Hz, $COCH_2CH_2$), 2.746 (4H, t, J = 8.0 Hz, $NCH_2CH_2CH_2$), 3.066 (8H, t, J = 5.3 Hz, NCH_2CH_2O), 3.592 (8H, s, NCH_2CH_2O), 3.726 (8H, t, J = 5.4 Hz, OCH_2CH_2O): IR (KBr disk): 3837, 2914, 2850, 1698, 1472, 1358, 1343, 1266, 1127, 1062, 1016, 974, 866, 832, 805, 720, 600, 562, 513 cm^{-1}. Anal. Calcd for $C_{36}H_{70}N_2O_8$: C, 65.62; H, 10.71; N, 4.25%. Found: C, 65.36; H, 10.66; N, 4.25%.

***N,N*-bis[*N*-(4,13-diaza-18-crown-6)dodecyl]-4,13-diaza-18-crown-6, 4**, was prepared as previously reported.

5, 6, and **7** were prepared as previously described.[3]

4,13-bis[12-{13-(4-Methoxybenzyl)-4,13-diaza-18-crown-6-4yl}dodecyl]-4,13-diaza-18-crown-6, 8. *N-(4-Methoxybenzyl)-4,13-diaza-18-crown-6, 8A.* A mixture of 4,13-diaza-18-crown-6 (3.54 g, 13.5 mmol), 4-methoxybenzyl chloride (1.90 g 12.2 mmol), Na_2CO_3 (14.3 g, 135 mmol), and KI (48 mg, 0.3 mmol) in *n*-PrCN (300 mL) was heated at reflux for 2 h. After cooling, the mixture was filtered and the filtrate was concentrated to leave a slightly yellow oil. Toluene was added and then evaporated (3×) to remove any residual *n*-PrCN. The resulting oil was chromatographed over alumina and eluted with 2% MeOH-CH_2Cl_2. **8A** was eluted next to the disubstituted one. After removal of the solvent, **8A** was obtained (2.38 g, 46% based on 4,13-diaza-18-

crown-6) as a slightly yellow oil which gradually gelled. ^{1}H-NMR: 2.699 (4H, t, J = 4.8 Hz, NCH_2CH$_2$O), 3.123 (4H, t, J = 4.5 Hz, NHCH_2CH$_2$O), 3.50-3.96 (21H, m, NH, CH_2OCH_2CH_2OCH_2, PhCH_2N, CH_3OPh), 6.910 (2H, d, J = 8.4 Hz), 7.236 (2H, d, J = 8.4 Hz): IR (KBr disk) cm^{-1}, 3467, 3241, 3056, 3025, 2876, 1612, 1582, 1513, 1474, 1458, 1367, 1352, 1301, 1268, 1242, 1173, 1113, 1033, 954, 836, 793, 756, 574, 524. Compound **8A** was used in the next step without additional purification.

4,13-bis[12-{13-(4-Methoxybenzyl)-4,13-diaza-18-crown-6-4yl}dodecyl]-4,13-diaza-18-crown-6 (8): A mixture of **8A** (1.04g, 2.71 mmol), 4,13-bis(12-bromododecyl)-4,13-diaza-18-crown-6 (1.00 g, 1.32 mmol), Na_2CO_3 (2.875 g, 27.1 mmol), and KI (20 mg, 0.12 mmol) in *n*-PrCN (20 mL) was heated under reflux for 7.5 h. After cooling, the mixture was filtered and the filtrate was concentrated to a yellow oil. Toluene was added and then evaporated (2×) to assure removal of *n*-PrCN. The resulting oil was chromatographed over alumina (*i*-PrOH-Hexanes-CH_2Cl_2 (2:40:10)). Evaporation of the solvent gave the crude product which solidified on standing. Repeated crystallization (95% EtOH) gave **8** (0.158 g, 9% based on 4,13-bis(12-bromododecyl)-4,13-diaza-18-crown-6) as a slightly yellow powder, mp 68.5-69 °C. ^{1}H-NMR: 1.22-1.28 (32H, pseudo-s, alkyl), 1.42-1.54 (8H, br, NCH$_2$CH_2CH$_2$), 2.482 (8H, t, J = 6.0 Hz, NCH_2CH$_2$CH$_2$), 2.74-2.82 (24H, m, NCH_2CH$_2$O) 3.58-3.64 (52H, m, CH_2OCH_2CH_2OCH_2, PhCH_2O), 3.795 (6H, s, CH_3OPh), 6.834 (4H, d, J = 8.7 Hz), 7.234 (4H, d, J = 8.7 Hz): IR (KBr disk): 2918, 2871, 1619, 1518, 1536, 1333, 1297, 1265, 1240, 1172, 1125, 1072, 1039, 966, 942, 881, 844, 831, 817, 803, 718, 605, 573 cm^{-1}. Anal. Calcd for $C_{76}H_{138}N_6O_{14}$; C, 67.12; H, 10.23; N, 6.18; found C, 67.02; H, 10.21; N, 6.12%.

4,13-bis[12-{13-(4-nitrobenzyl)-4,13-diaza-18-crown-6-4-yl}dodecyl]-4,13-diaza-18-crown-6, 9. ***N-(4-Nitrobenzyl)-4,13-diaza-18-crown-6,*** **9A.** A mixture of 4,13-diaza-18-crown-6 (3.54 g, 13.5 mmol), 4-nitrobenzyl bromide (2.62 g 12.2 mmol), Na_2CO_3 (14.3 g, 135 mmol), and KI (48 mg, 0.3 mmol) in *n*-PrCN (300 mL) was heated at reflux for 4.5 h. After cooling, the mixture was filtered and the filtrate was concentrated to a yellow oil. Toluene was added and then evaporated (2×) to assure removal of *n*-PrCN. The resulting oil was chromatographed over alumina (3% MeOH-CH_2Cl_2). Compound **9A** eluted second; solvent was removed, and the yellow oil thus obtained (2.36 g, 44% based on 4,13-diaza-18-crown-6) solidified on standing. ^{1}H-NMR: 2.802 (4H, t, J = 4.7 Hz, NCH_2CH$_2$O), 3.168 (4H, t, J = 3.9 Hz, NHCH_2CH$_2$O), 3.52-3.66 (13H, m, NH, CH_2OCH_2CH_2O), 3.756 (2H, s, PhCH_2N), 3.875 (4H, t, J = 4.2 Hz, HNCH$_2$CH_2O), 7.584 (2H, d, J = 8.1 Hz), 8.185 (2H, d, J = 7.8 Hz): IR (KBr disk) cm^{-1}, 3467, 2884, 2499, 1625, 1605, 1515, 1459, 1346, 1278, 1244, 1106, 1084, 974, 937, 855, 829, 802, 744, 737, 708, 648, 523, 442. Compound **9A** was used in the next step without further purification.

4,13-bis[12-{13-(4-nitrobenzyl)-4,13-diaza-18-crown-6-4-yl}dodecyl]-4,13-diaza-18-crown-6, 9. The mixture of **9A** (1.077 g, 2.71 mmol), 4,13-bis(12-bromododecyl)-4,13-diaza-18-crown-6 (1.00 g, 1.32 mmol),), Na_2CO_3 (2.875 g, 27.1 mmol), and KI (20 mg, 0.12 mmol) in *n*-PrCN (20 mL) was heated under reflux for 7.5 h. After cooling, the mixture was filtered and the filtrate was concentrated to a yellow oil. Toluene was added and evaporated (2×) to assure removal of *n*-PrCN. The resulting yellow oil was chromatographed (alumina, 10% *i*-PrOH-hexanes, then *i*-PrOH-hexanes-CH_2Cl_2 (2:40:10)). Evaporation of the solvent gave a yellow oil which solidified on standing. Crystallization (95% EtOH) gave **9** (0.72 g, 39% based on 4,13-bis(12-bromododecyl)-4,13-diaza-18-crown-6) as a yellow solid, mp. 61.5-62 °C. ^{1}H-NMR: 1.22-1.28 (32H, pseudo-s, alkyl), 1.42-1.56 (8H, br, $NCH_2CH_2CH_2$), 2.50-2.60 (8H, br, $NCH_2CH_2CH_2$), 3.58-3.70 (48H, m, $CH_2OCH_2CH_2OCH_2$), 3.794 (4H, s, $PhCH_2C$), 7.553 (4H, d, J = 8.7 Hz), 8.162 (4H, d, J = 8.7 Hz): IR (KBr disk) cm^{-1}, 2918, 2870, 1607, 1518, 1473, 1356, 1296, 1239, 1125, 1072, 1045, 965, 843, 740, 718, 694, 605, 422: Anal. Calcd for $C_{74}H_{132}N_8O_{16}$; C, 63.95; H, 9.57; N, 8.06%. Found C, 63.85; H, 9.56; N, 8.07%.

NMR studies of tranport in bilayers. The procedure is as reported in reference 3.

Bulk membrane transport experiments. UV-vis spectra were recorded on a Beckman DU-8 spectrophotometer. To obtain the transport rate of sodium picrate, the procedure described by Gokel and co-workers has been followed with the following modifications: 20 ml Beakers were used instead of the 18 mm vials. The internal diameter of the concentric tube was 12 mm. Sodium picrate transport was followed by measurement of the %T in the receiving phase at a wavelength of 354 nm. A layer of 6.0 mL of $CHCl_3$ in a 20 mL beaker was stirred (synchronous stirrer, power 1) using a 7 mm Teflon-coated magnetic bar. The source and the receiving phases were separated by a glass tube (12 mm id) suspended 5 mm above the bottom of the beaker, but below the surface of the $CHCl_3$ layer. The source phase was placed inside the concentric tube. The receiving phase was in the external compartment. 1.0 mL of the sodium picrate solution (1.0 mM in 0.100 M NaOH) (source phase) and 6.0 mL of distilled and deionized water (receiving phase) were placed on top of the chloroform layer. The concentration of all the compounds studied was 1.0 mM. The transport rate is reported as mol transported/24 h. Ion transport was determined by following the change in absorbance (%T) of the receiving phase at a wavelength of 354 nm. The concentration of sodium picrate was determined from the calibration curve.[4]

Log P_{oct} Determination for *N,N'*-dibenzyldiaza-18-crown-6. 1-Octanol (200 mL, A.C.S. spectroscopic grade) was saturated with deionized water. To saturate the octanol, 200 mL of alcohol were placed in a 1.0 L bottle with 400 mL of deionized

water. The two-phase system was gently shaken for 3 min and then placed in a thermostatic water bath at 21.0 °C overnight. Double deionized water was similarly saturated with 1-octanol.

A solution of *N,N'*-dibenzyldiaza-18-crown-6 (10^{-2} M, 3.00 mL) in water-saturated 1-octanol was placed in a separatory funnel and 100 mL of the water saturated octanol were added. The funnel was gently shaken for 3 min. The aqueous layer was drained and the octanol layer was removed with the aid of a Pasteur pipette. A 0.2 mL-sample of this octanol layer was placed in a spectroscopic cuvette and diluted with 1-octanol (1.8 mL). The partition coefficient was determined by comparison of the absorbance of this solution with that of a freshly prepared 10^{-3} M solution of *N,N'*-dibenzyldiaza-18-crown-6 in 1-octanol.

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