Electronic Supplementary Material (ESI) for ChemComm.

Hydrazide macrocycles as effective transmembrane channels for ammonium

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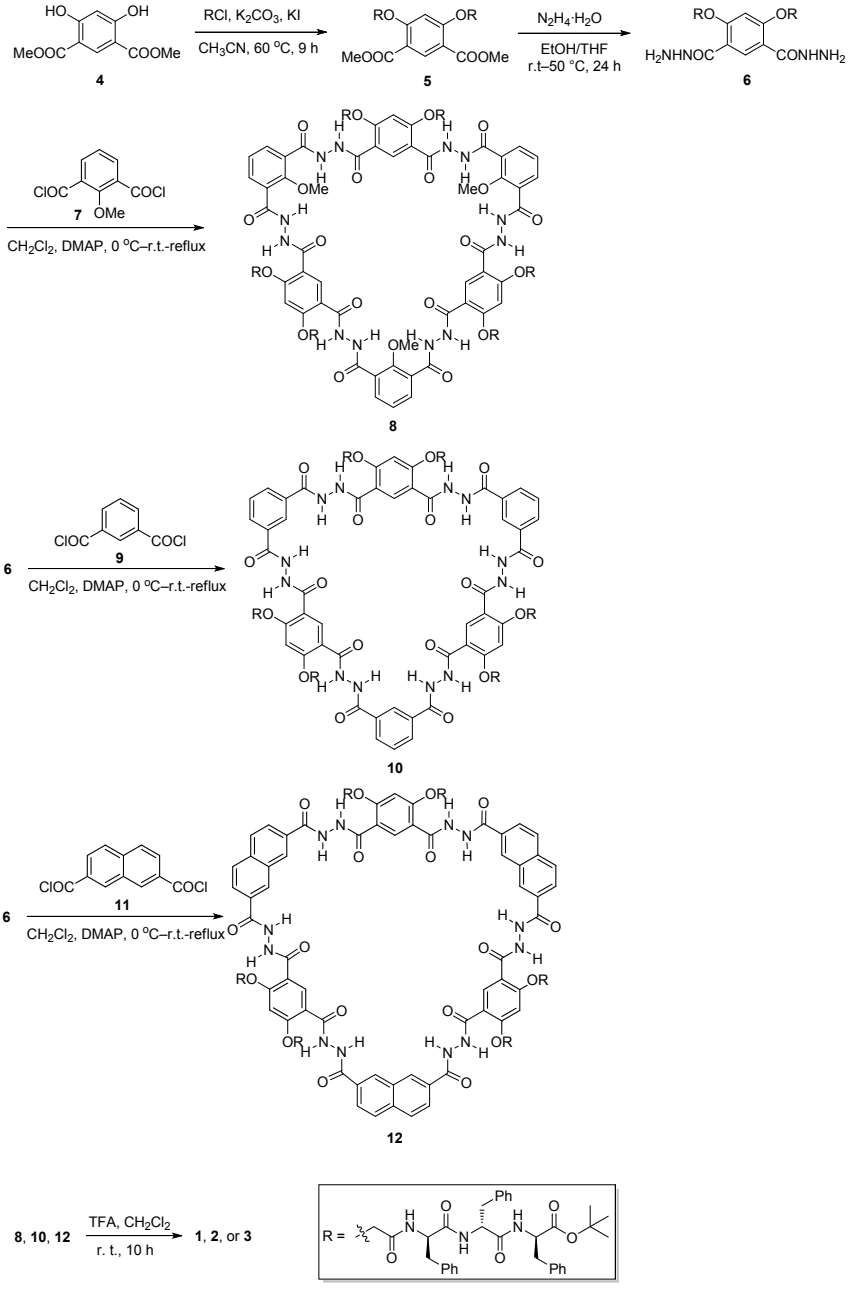
Contents:

1. General:

Egg yolk L-α-phosphatidylcholine was obtained from Sigma-Aldrich as chloroform solution (100 mg/mL). 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (diPhyPC) was obtained from Avanti Polar Lipids as chloroform solution (10 mg/mL). ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz with Mercury plus 400 spectrometer at 298 K. Chemical shifts were referenced to solvent residue. Mass spectra were recorded with Bruker MicroTOF II spectrometer by using positive or negative mode. The peptides were synthesized according to the classical liquid phase synthesis method by employing EDCI as condensation reagent.

2. Synthetic procedures and characterization data for 1-3:



Compound 5. To a solution of compound **4**[1] (0.68 g, 3.00 mmol) in CH_3CN (100 mL) was added the chloride (5.33 g, 9.0 mmol), K_2CO_3 (2.49 g, 18 mmol), and KI (0.25 g, 1.5 mmol). The mixture was stirred at 60 °C for 12 h and then concentrated under reduced pressure. The residue was dissolved in ethyl acetate (200 mL). The organic solution was then washed with water (100 mL) and brine (100 mL), and dried over anhydrous Na_2SO_4. After removing of the solvent under reduced pressure, the resulting crude product was purified by column chromatography on silica gel to yield **5** as a white solid (3.87 g, 95%). ^{1}H NMR ($CDCl_3$): δ 8.69 (s, 1 H), 8.43 (d, J = 8 Hz, 2 H), 7.24-6.99 (m, 30 H), 6.86 (s, 1 H), 6.60 (d, J = 8 Hz, 1 H), 6.37 (s, 1 H), 6.31(d, J = 8 Hz, 2 H), 4.8 (m, 2 H), 4.66-4.58 (m, 4 H), 4.36 (m, 4 H), 3.95 (s, 6 H), 3.21 (d, J = 4 Hz, 1 H), 3.18 (d, J = 4 Hz, 1 H), 3.10-2.92 (m, 10 H), 1.34 (s, 18 H). ^{13}C NMR ($CDCl_3$): δ 171.0, 169.9, 168.0, 164.6, 162.0, 137.6, 136.8, 136.4, 135.9, 129.5, 129.3, 129.0, 128.4, 128.3, 126.9, 126.7, 126.6, 111.5, 99.2, 82.2, 67.6, 54.1, 53.7, 52.2, 38.4, 38.0, 37.5, 27.8. HR-MS (ESI-TOF): Calcd. For $C_{76}H_{84}N_6O_{16}Na$ $[M+Na]^+$: 1359.5842. Found: 1359.5836.

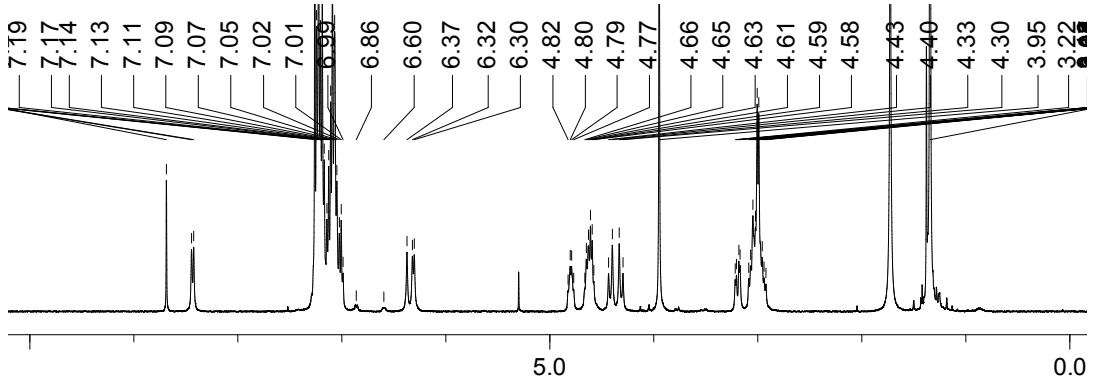


Fig. S1 ^{1}H NMR spectrum (400 MHz) of compound **5** in $CDCl_3$ at 25 °C.

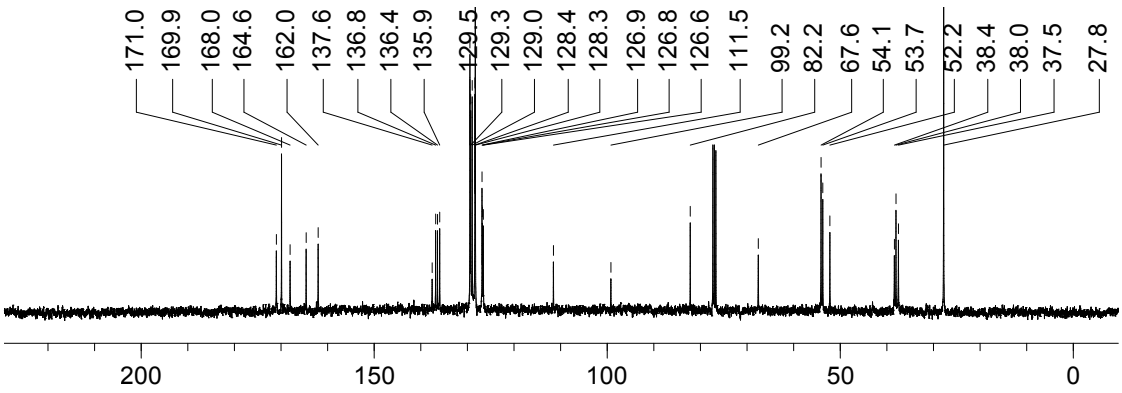


Fig. S2 ^{13}C NMR spectrum (100 MHz) of compound **5** in $CDCl_3$ at 25 °C.

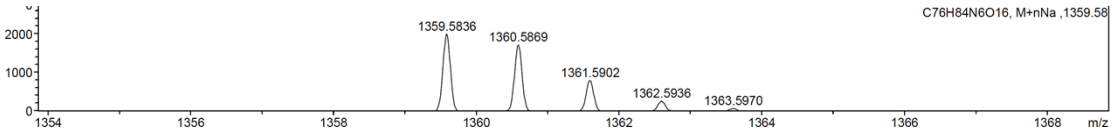


Fig. S3 HR-MS of compound **5**.

Compound 6. To a solution of compound **5** (1.34 g, 1.00 mmol) in EtOH/THF (20/30 mL) was added hydrazine hydrate (85%, 9.7 mL, 0.20 mol). The mixture was stirred at 50 °C for 24 h. The precipitation formed was collected by filtration, washed with water and then dried in vacuo to yield compound **6** as white solid (0.60 g, 45%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.61 (s, 2 H), 8.51-8.48 (m, 4 H), 8.32 (d, J = 8 Hz, 2H), 8.16 (s, 1 H), 7.28-7.03 (m, 30 H), 6.26 (s, 1 H), 4.61-4.56(m, 6 H), 4.42-4.37 (m, 8 H), 3.04-2.96 (m, 8 H), 2.82-2.65 (m, 4 H), 1.30 (s, 18 H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.5, 171.3, 170.9, 167.5, 164.4, 158.7, 138.1, 138.0, 137.6, 129.8, 129.759, 129.6, 128.8, 128.6, 128.4, 127.1, 126.8, 126.7, 116.1, 81.2, 68.0, 54.8, 54.1, 38.3, 37.4, 28.1. HR-MS (ESI-TOF): Calcd. for $C_{74}H_{85}N_{10}O_{14}$ $[M+H]^+$: 1337.6247. Found: 1337.6229.

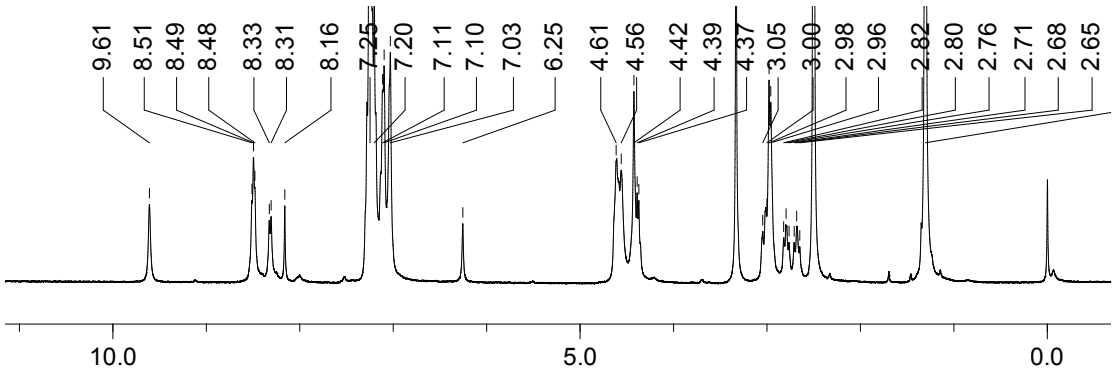


Fig. S4 ^{1}H NMR spectrum (400 MHz) of compound **6** in DMSO-d_6 at 25 °C.

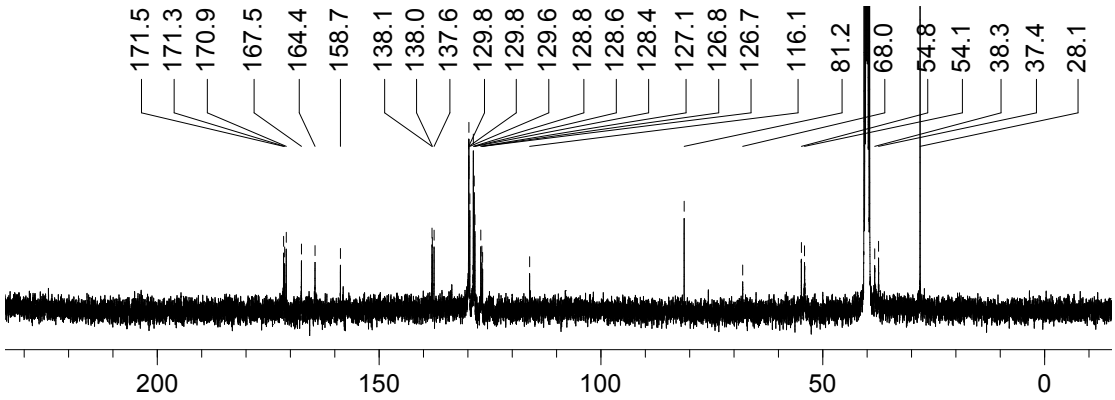


Fig. S5 ^{13}C NMR spectrum (100 MHz) of compound **6** in DMSO-d_6 at 25 °C.

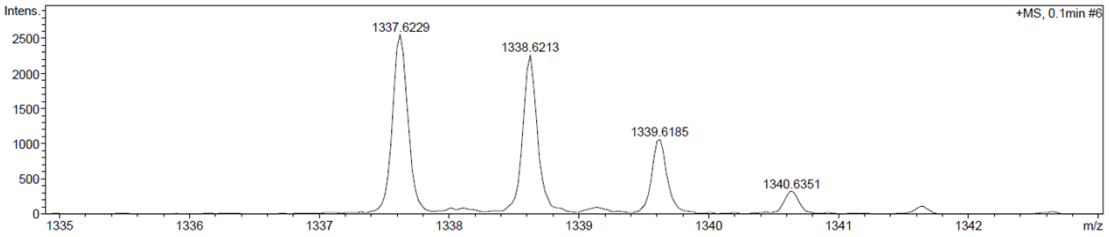


Fig. S6 HR-MS of compound **6**.

Compound 8. The solution of compound **7** (0.12 g, 0.50 mmol) in anhydrous dichloromethane (10 mL) was added dropwise to the mixture of compound **6** (0.67 g, 0.50 mmol) and DMAP (122mg, 1.0 mmol) in anhydrous dichloromethane (20 mL) at 0 °C. The mixture was stirred at that temperature for 2 h. The reaction mixture was then stirred under reflux for 24 h. The mixture was concentrated under reduced pressure. The residue was then dissolved in dichloromethane (50 mL). The organic solution was washed with dilute HCl (0.5 M, 50 mL), water (50 mL) and brine (50 mL), and dried over anhydrous Na_2SO_4. After removing of the solvent with a rotavapor, the resulting crude product was purified by column chromatography on silica gel to yield compound **8** as a white solid (0.47 g, 63%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.45-10.39 (m, 10H), 8.51-8.33 (m, 20H), 7.74-7.70 (m, 6H), 7.22-7.07 (m, 96H), 6.33-6.29 (m, 3H), 4.68-4.34(m, 30H), 4.01 (s, 6H), 3.9 (s, 3H), 3.04-2.96 (m, 24H), 2.80-2.67 (m, 12H), 1.28 (s, 54H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.3, 170.8, 167.3, 165.4, 163.9, 159.6, 137.9, 137.5, 129.7, 129.5, 128.6, 128.5, 127.0, 126.7, 115.3, 81.2, 68.1, 63.5, 54.7, 54.1, 38.2, 38.0, 37.4, 28.0. HRMS: Calcd for $C_{249}H_{264}N_{30}Na_2O_{51}$ $[M+Na]^{2+}$: 2268.9425. Found: 2268.9414.

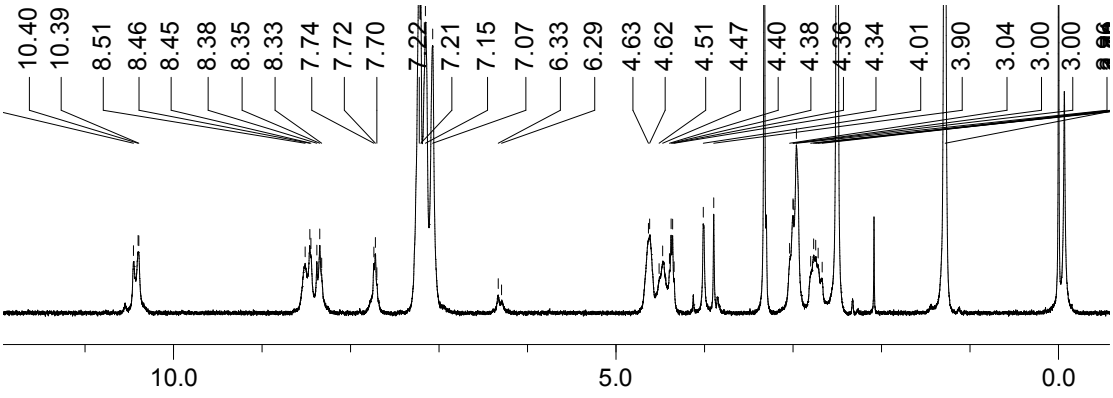


Fig. S7 ^{1}H NMR spectrum (400 MHz) of compound **8** in DMSO-d_6 at 25 °C.

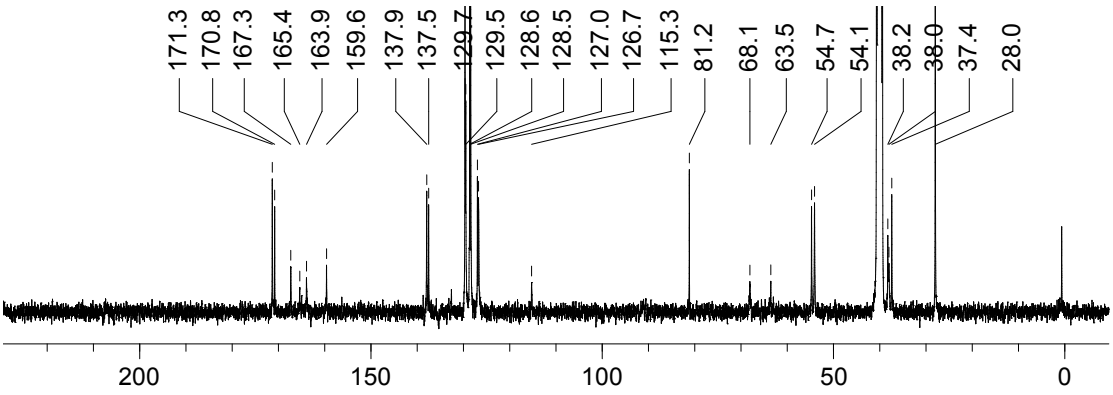


Fig. S8 ^{13}C NMR spectrum (100 MHz) of compound **8** in DMSO-d_6 at 25 °C.

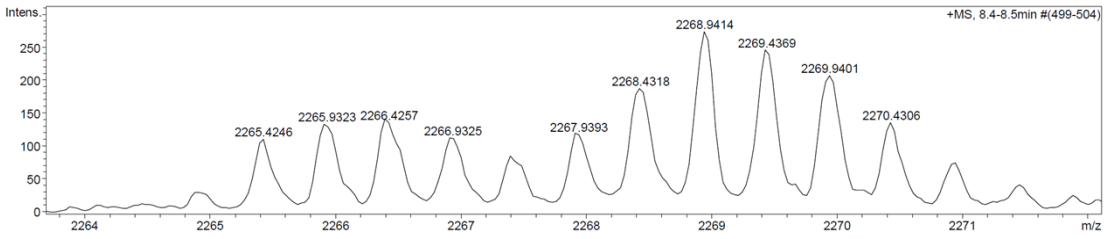


Fig. S9 HR-MS of compound **8**.

Compound 10. This compound was synthesized as a white solid in 51% yield from the reaction of compounds **6** and **9** according to the procedure described for compound **8**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.80 (s, 5H), 10.38 (s, 5H), 8.55-8.14 (m, 29H), 7.66-7.64 (m, 3H), 7.22-7.07 (m, 93H), 6.25 (br, 3H), 4.65-4.33(m, 30H), 3.02-2.94 (m, 24H), 2.75-2.67 (m, 12H), 1.27 (s, 54H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.3, 170.8, 167.2, 165.4, 164.1, 159.5, 137.8, 137.5, 133.3, 129.6, 129.4, 128.6, 128.4, 128.3, 126.9, 126.7, 115.3, 81.1, 67.9, 54.7, 54.0, 38.1, 38.0, 37.3, 27.9. HRMS: Calcd for $C_{246}H_{258}N_{30}Na_2O_{48}$ $[M+Na]^{2+}$: 2223.9266. Found: 2223.9249.

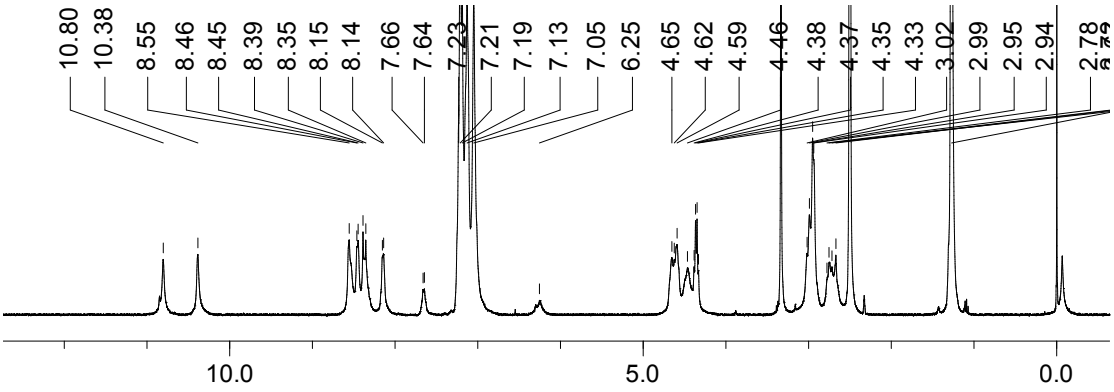


Fig. S10 ^{1}H NMR spectrum (400 MHz) of compound **10** in DMSO-d_6 at 25 °C.

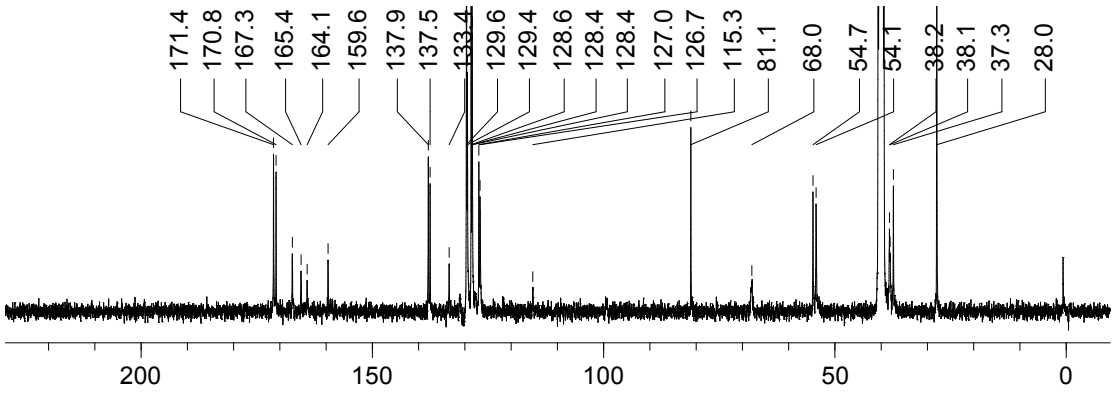


Fig. S11 ^{13}C NMR spectrum (100 MHz) of compound **10** in DMSO-d_6 at 25 °C.

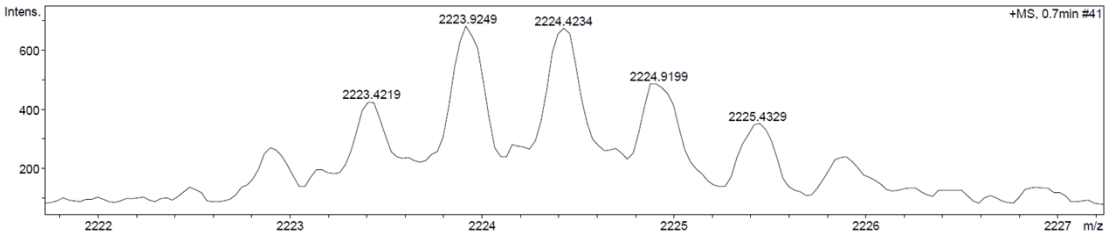


Fig. S12 HR-MS of compound **10**.

Compound 12. This compound was synthesized as a whit solid in 59% yield from the reaction of **6** and **11** according to the procedure described for **8**. ^{1}H NMR (400 MHz, DMSO) δ 10.93 (s, 5H), 10.44 (s, 5H), 8.68-8.14 (m, 41H), 7.20-7.07 (m, 90H), 6.35 (br, 3H), 4.66-4.34(m, 30H), 2.99-2.94 (br, 24H), 2.74 (br, 12H), 1.26 (s, 54H). ^{13}C NMR (100 MHz, DMSO) δ 171.3, 170.8, 167.3, 165.7, 164.2, 159.5, 137.9, 137.9, 137.5, 131.9, 131.7, 131.1, 129.6, 129.4, 128.6, 128.4, 126.9, 126.6, 115.5, 81.1, 68.0, 54.7, 54.0, 38.1, 37.9, 37.3, 27.9. HRMS: calcd for $C_{258}H_{264}N_{30}Na_2O_{48}$ $[M+Na]^{2+}$ 2298.9501, found 2298.9488.

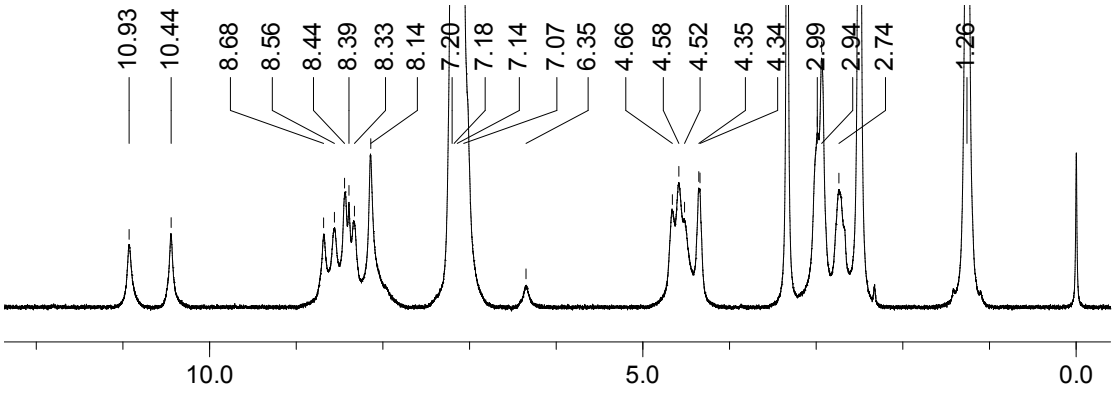


Fig. S13 ^{1}H NMR spectrum (400 MHz) of compound **12** in DMSO-d_6 at 25 °C.

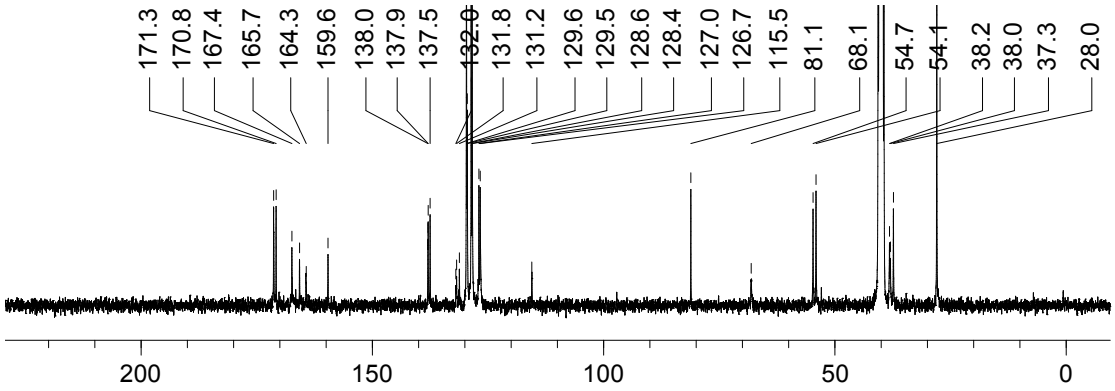


Fig. S14 ^{13}C NMR spectrum (100 MHz) of compound **12** in DMSO-d_6 at 25 °C.

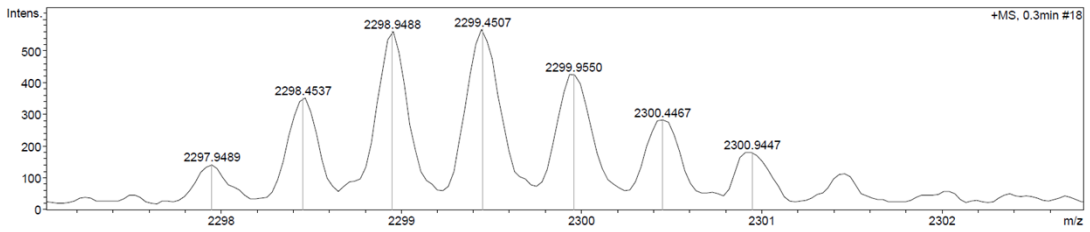


Fig. S15 HR-MS of compound **12**.

Compound 1. To a solution of compound **8** (90 mg, 0.020 mmol) in dichloromethane (10 mL) was added trifluoroacetic acid (1.0 mL). The mixture was stirred at room temperature for 24 h and then concentrated under reduced pressure. The crude product was washed with diethyl ether and then recrystallized from ethanol to yield compound **1** as a aple yellow solid (73 mg, 88%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.81 (s, 6H), 10.46-10.41 (m, 10H), 8.54-8.33 (m, 20H), 7.73-7.67 (m, 6H), 7.22-7.07 (m, 96H), 6.31-6.27 (br, 3H), 4.65-4.31(m, 30H), 4.01 (s, 6H), 3.90 (s, 3H), 3.08-2.89 (m, 24H), 2.77-2.67 (m, 12H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.1, 171.3, 167.3, 165.4, 163.9, 159.6, 137.9, 129.6, 129.6, 129.5, 128.7, 128.5, 126.9, 126.7, 115.3, 68.0, 63.6, 54.3, 54.0, 38.2, 38.0, 37.2. HRMS: Calcd for $C_{225}H_{220}N_{31}NaO_{51}$ $[M+Na+NH_4]^{2+}$: 2098.2770. Found: 2098.2769.

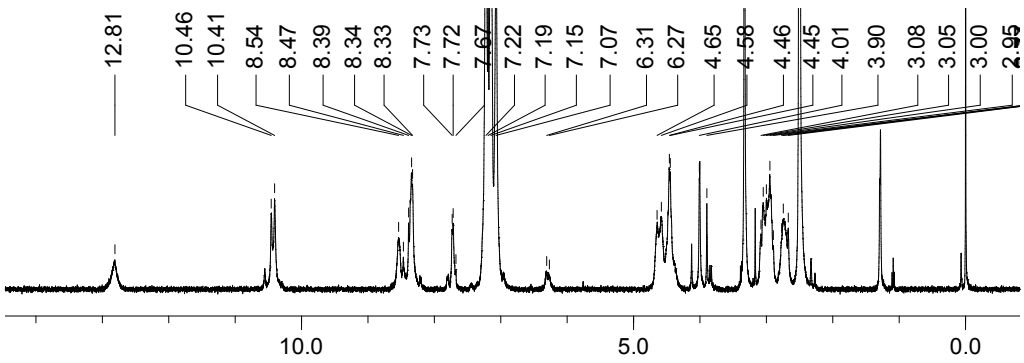


Fig. S16 ^{1}H NMR spectrum (400 MHz) of compound **1** in DMSO-d_6 at 25 °C.

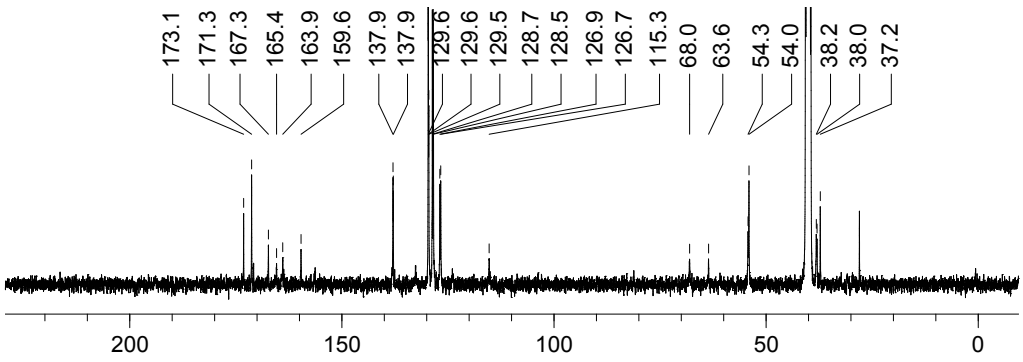


Fig. S17 ^{13}C NMR spectrum (100 MHz) of compound **1** in DMSO-d_6 at 25 °C.

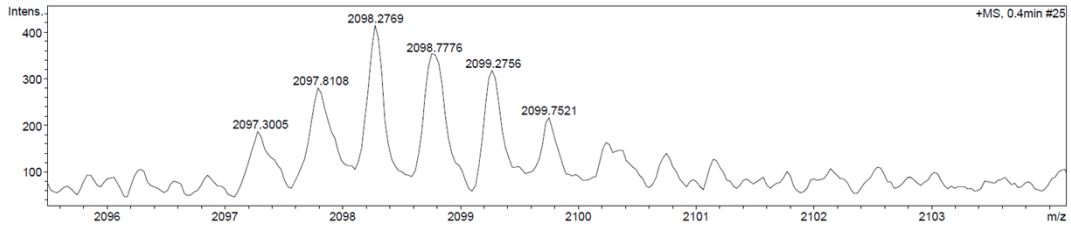


Fig. S18 HR-MS of compound **1**.

Compound 2. This compound was synthesized in 84% yield as a pale yellow solid from compound **10** according to the procedure described for compound **1**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.80 (br, 6H), 10.80 (s, 5H), 10.38 (s, 5H), 8.56-8.14 (m, 29H), 7.66-7.65 (m, 3H), 7.21-7.05 (m, 93H), 6.25 (br, 3H), 4.65-4.33(m, 30H), 3.07-2.91 (m, 24H), 2.76-2.71 (m, 12H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.1, 171.3, 168.3, 167.2, 165.4, 159.5, 137.8, 137.8, 133.4, 129.5, 129.4, 128.6, 128.4, 128.3, 126.9, 126.6, 54.1, 53.9, 38.1, 38.0, 37.1. HRMS: calcd for $C_{222}H_{210}N_{30}Na_2O_{48}$ $[M+Na]^{2+}$: 2055.7388. Found: 2055.7381.

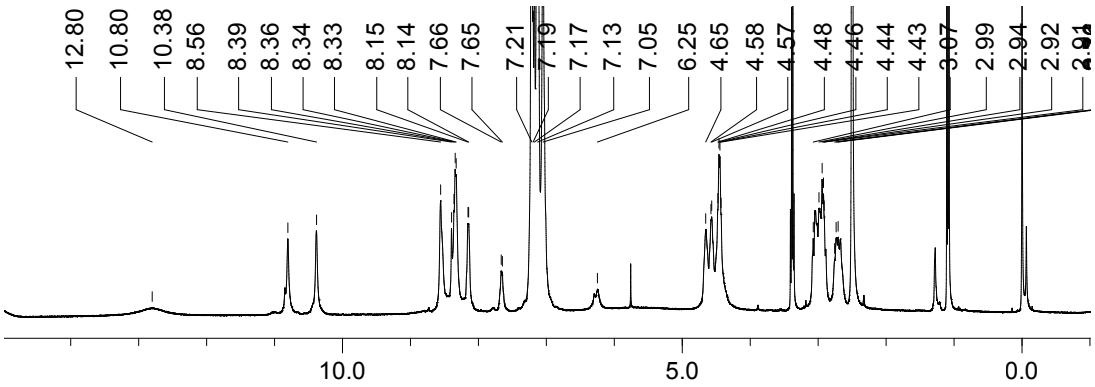


Fig. S19 ^{1}H NMR spectrum (400 MHz) of compound **2** in DMSO-d_6 at 25 °C.

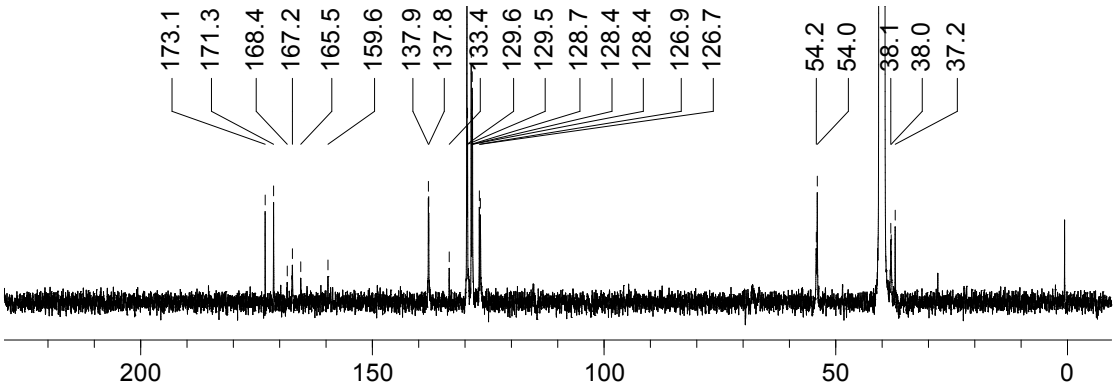


Fig. S20 ^{13}C NMR spectrum (100 MHz) of compound **2** in DMSO-d_6 at 25 °C.

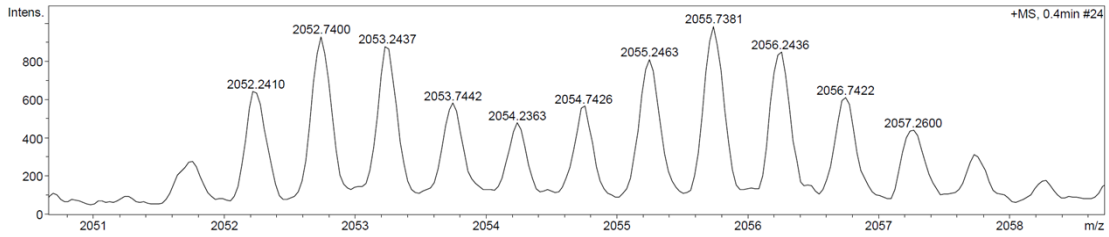


Fig. S21 HR-MS of compound **2**.

Compound 3. This compound was synthesized in 91% yield as a pale yellow solid from compound **12** according to the procedure described for compound **1**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.78 (br, 6H), 10.91 (s, 5H), 10.44 (s, 5H), 8.68-8.15 (m, 41H), 7.21-7.07 (m, 90H), 6.35 (br, 3H), 4.66-4.36(m, 30H), 3.03-2.92 (m, 24H), 2.74 (br, 12H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.1, 171.2, 167.3, 165.7, 164.3, 159.6, 159.5, 137.9, 137.8, 131.7, 131.1, 129.6, 129.5, 129.4, 128.6, 128.4, 126.8, 126.6, 115.4, 81.1, 68.1, 54.2, 54.0, 53.9, 38.0, 37.1. HRMS: calcd for $C_{234}H_{218}N_{30}O_{48}$ $[M+H]^{2+}$ 2108.7804, found 2108.7778.

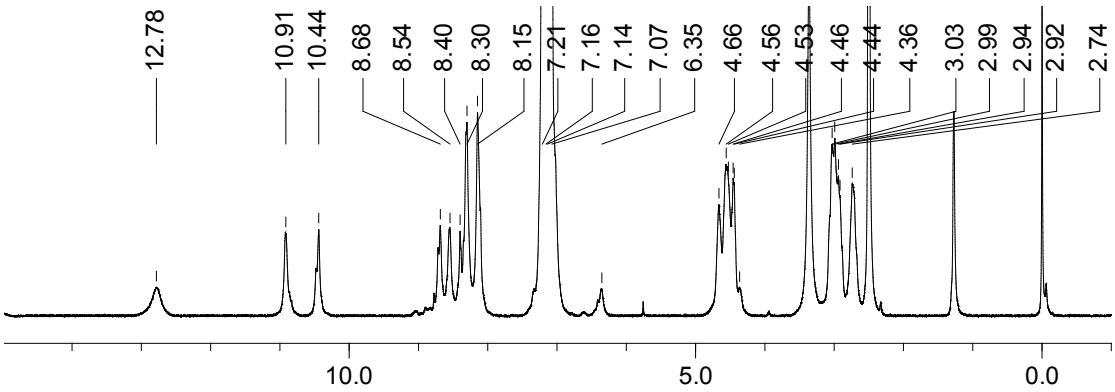


Fig. S22 ^{1}H NMR spectrum (400 MHz) of compound **3** in DMSO-d_6 at 25 °C.

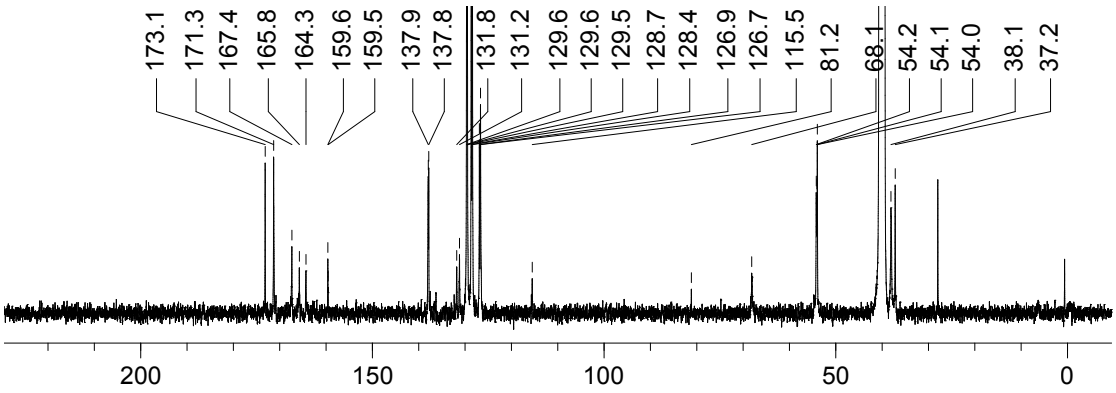


Fig. S23 ^{13}C NMR spectrum (100 MHz) of compound **3** in DMSO-d_6 at 25 °C.

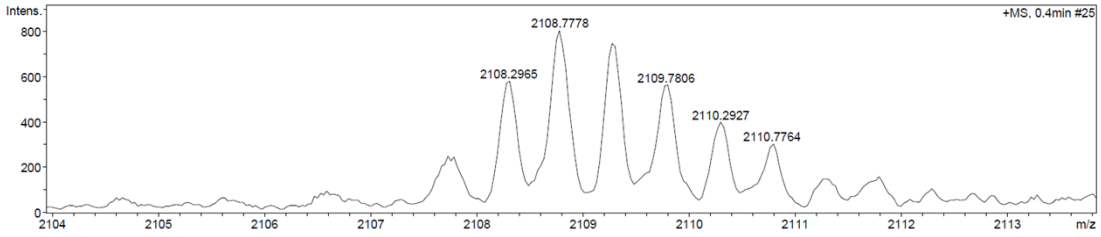


Fig. S24 HR-MS of compound **3**.

3. Procedures for proton transport experiments:[2]

Preparation of HPTS containing large unilamellar vesicles (LUVs): EYPC (15 mg, 20 μmol) in $CHCl_3$ (0.15 mL) was diluted with $CHCl_3$ (5.0 mL). The solution was evaporated under reduced pressure, and the resulting thin film was dried under high vacuum for 3 h. The lipid film was hydrated with HEPES buffer solution (1.5 mL, HEPES (10 mM), KCl (100 mM), pH = 7.2) containing HPTS (0.1 mM) at 40 °C for 2 h to give a milky suspension. The resulting suspension was subjected to ten freeze-thaw cycles by using liquid nitrogen to freeze and warm water bath to thaw. The suspension was dialyzed with membrane tube (MWCO = 14000) against the same HEPES buffer solution (200 mL, without HPTS) for six times to remove un-entrapped HPTS and to produce vesicle suspension ([lipid] = 13.3 mM).

Fluorescent experiments: HEPES buffer solution (2.0 mL, HEPES (10 mM), KCl (100 mM), pH = 6.0) and the prepared vesicle suspension (13.3 mM, 100 μL) were placed in a fluorimetric cuvette. To the cuvette, the solution of compounds **1**-**3** in DMSO (5 μL) was added to reach a required channel concentration (molar ratio relative to lipid, represented by x) with gentle stirring. Fluorescent intensity (I_t) was continuously monitored at 510 nm (excitation at 460 nm) in 10 min. Then, Triton aqueous solution (50%, 10 μL) was added with gentle stirring. The intensity was monitored until the fluorescent intensity (I_∞) did not change. The collected data were

then normalized into the fractional change in fluorescence given by $(I_t-I_0)/(I_\infty-I_0)$, where I_0 is the initial intensity.

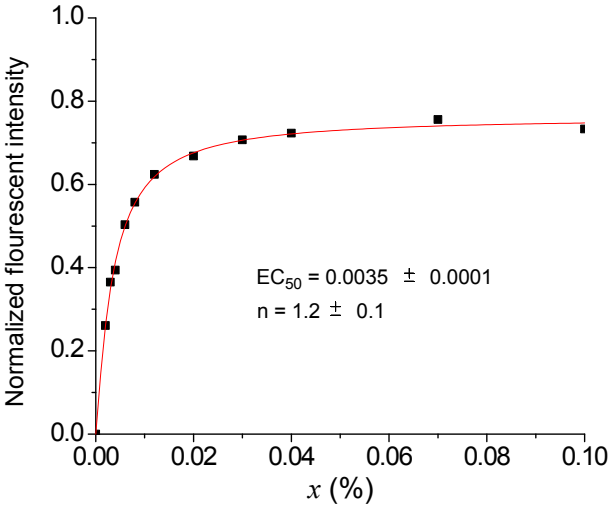


Fig. S25 Changes in the normalized fluorescent intensity of HPTS (λ_{ex} = 460 nm, λ_{em} = 510 nm) in vesicles with the concentration of compound **1** (molar ratio relative to lipid, represented by *x*). By fitting the plot with the Hill equation, the effective concentration needed for 50% activity (EC_{50}) for **1** was determined to be 0.0035(±0. 0001)% and the *n* value was determined to be 1.2±0. 1.

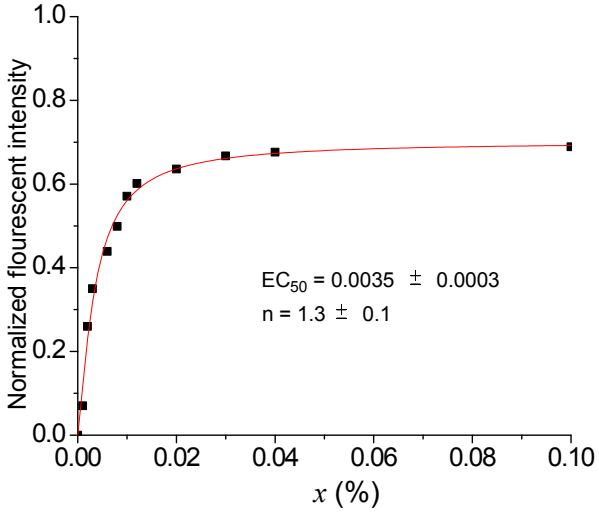


Fig. S26 Changes in the normalized fluorescent intensity of HPTS (λ_{ex} = 460 nm, $\lambda_{emission}$ = 510 nm) in vesicles with the concentration of compound **2** (molar ratio relative to lipid, represented by *x*). By fitting the plot with the Hill equation, the effective concentration needed for 50% activity (EC_{50}) for **2** was determined to be 0.0035(±0. 0003)% and the *n* value was determined to be 1.3±0. 1.

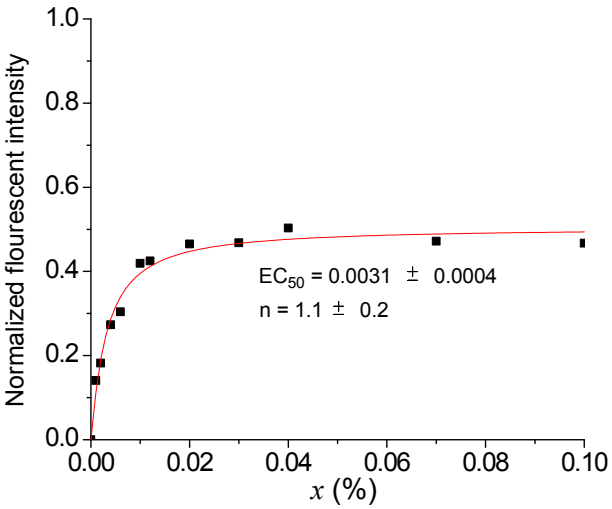


Fig. S27 Changes in the normalized fluorescent intensity of HPTS (λ_{ex} = 460 nm, $\lambda_{emission}$ = 510 nm) in vesicles with the concentration of compound **3** (molar ratio relative to lipid, represented by *x*). By fitting the plot with the Hill equation, the effective concentration needed for 50% activity (EC_{50}) for **3** was determined to be 0.0031(±0. 0004)% and the *n* value was determined to be 1.1±0. 2.

4. Procedures for patch clamp experiments:[3]

The solution of diPhyPC in chloroform (10 mg/ml, 20 μL) was evaporated with nitrogen gas to form a thin film and re-dissolved in n-decane (5 μL). The lipid solution (0.5 μL) was injected on to the aperture (diameter = 200 μm) of the Delrin® cup (Warner Instruments, Hamden, CT) and then evaporated with nitrogen gas. In a typical experiment for measurement of the channel conductance for an ion, the chamber (*cis* side) and the Delrin cup (*trans* side) were filled with aqueous MCl solution (1.0 M, 1.0 mL, M = NH_4^+, Cs^+, Rb^+, K^+, Na^+ or $AcCh^+$). Ag-AgCl electrodes were applied directly to the two solutions and the *cis* one was grounded. Planar lipid bilayer was formed by painting the lipids solution (1.0 μL) around the pretreated aperture and by judgment of capacitance (80-120 pF). The solution of the test channel **1-3** DMSO (1 mM, 0.5 μL) was added to the *cis* chamber and the solution was stirred for 5 min. Membrane currents were measured using a Warner BC-535D bilayer clamp amplifier and were collected by PatchMaster (HEKA) with sample interval at 5 kHz and then filtered with a 8-pole Bessel filter at 1 kHz (HEKA). The data were analyzed by FitMaster (HEKA) with a digital filter at 100 Hz.

For the measurement of the transport selectivity of M^+ (M = NH_4^+, Cs^+, Rb^+, K^+, Na^+ or ACh^+) over K^+, the *cis* chamber was charged with MCl (1.0 M) and the *trans* one was charged with KCl (1.0 M). For the measurement of the transport selectivity of Cl^- over K^+, the *cis* and *trans* chambers were charged with KCl of 0.3 M and 1.0 M,

respectively.

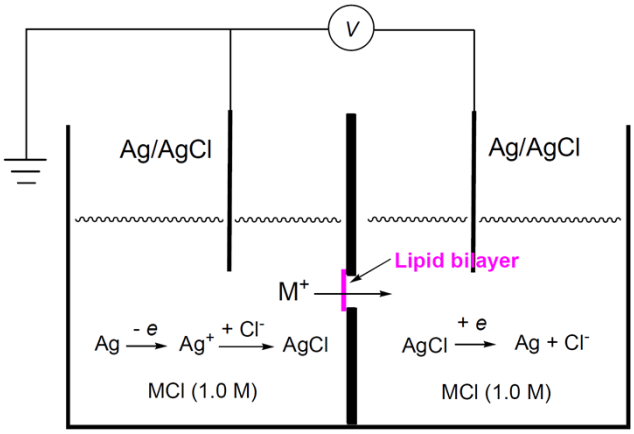


Fig. S28 Schematic representation for the patch clamp experiments with planar lipid bilayer. The redox reactions on both Ag/AgCl electrodes were inserted to illustrate the nature of charge balance during M^+ transmembrane transport.

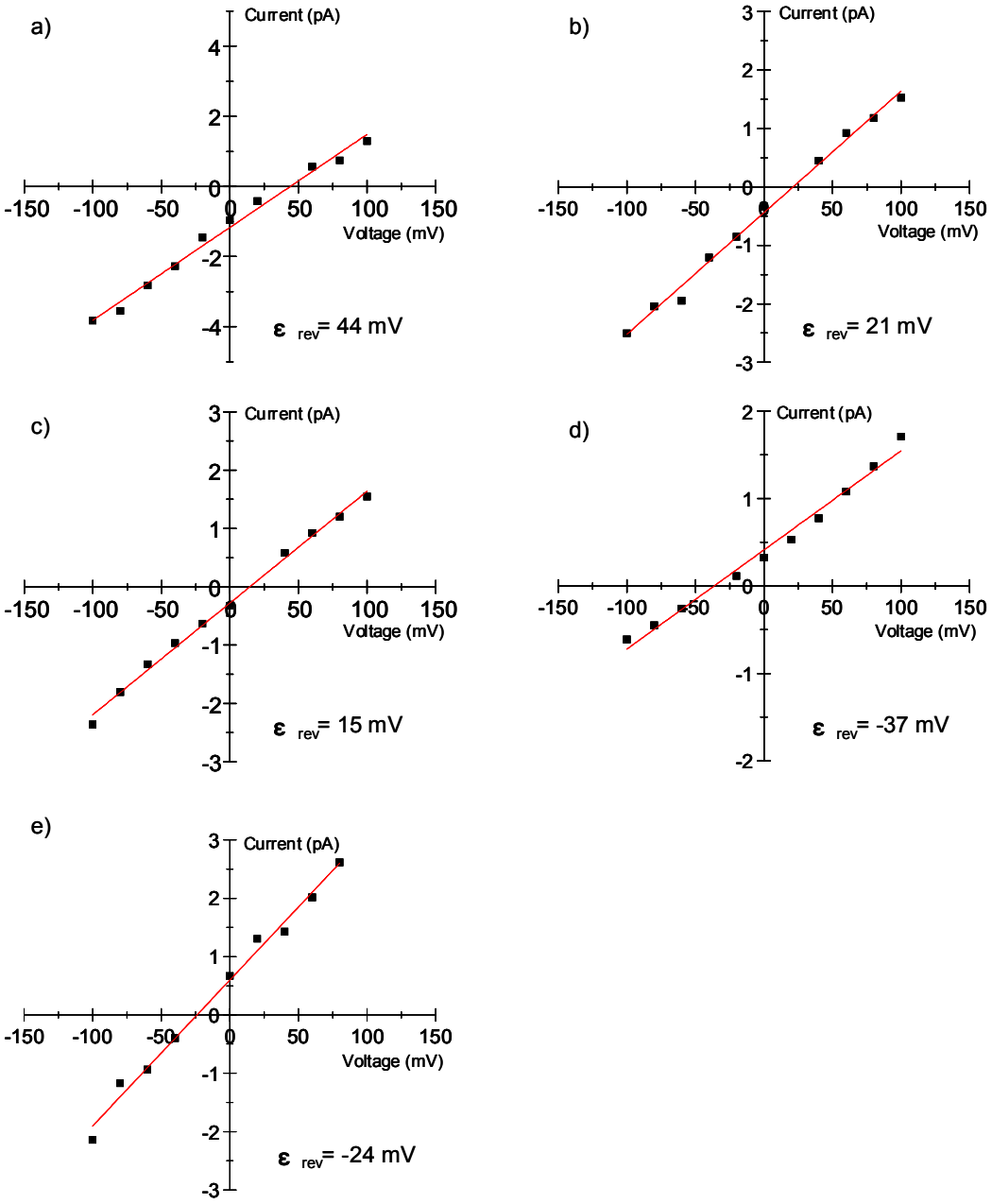


Fig. S29 Current-voltage relationship of channel **1** by using unsymmetrical solution at both side of the bilayer. *trans* chamber: KCl (1.0 M), cis chamber: (a) NH_4Cl (1.0 M), (b) CsCl (1.0 M), (c) RbCl (1.0 M), (d) NaCl (1.0 M), (e) KCl (0.3 M).

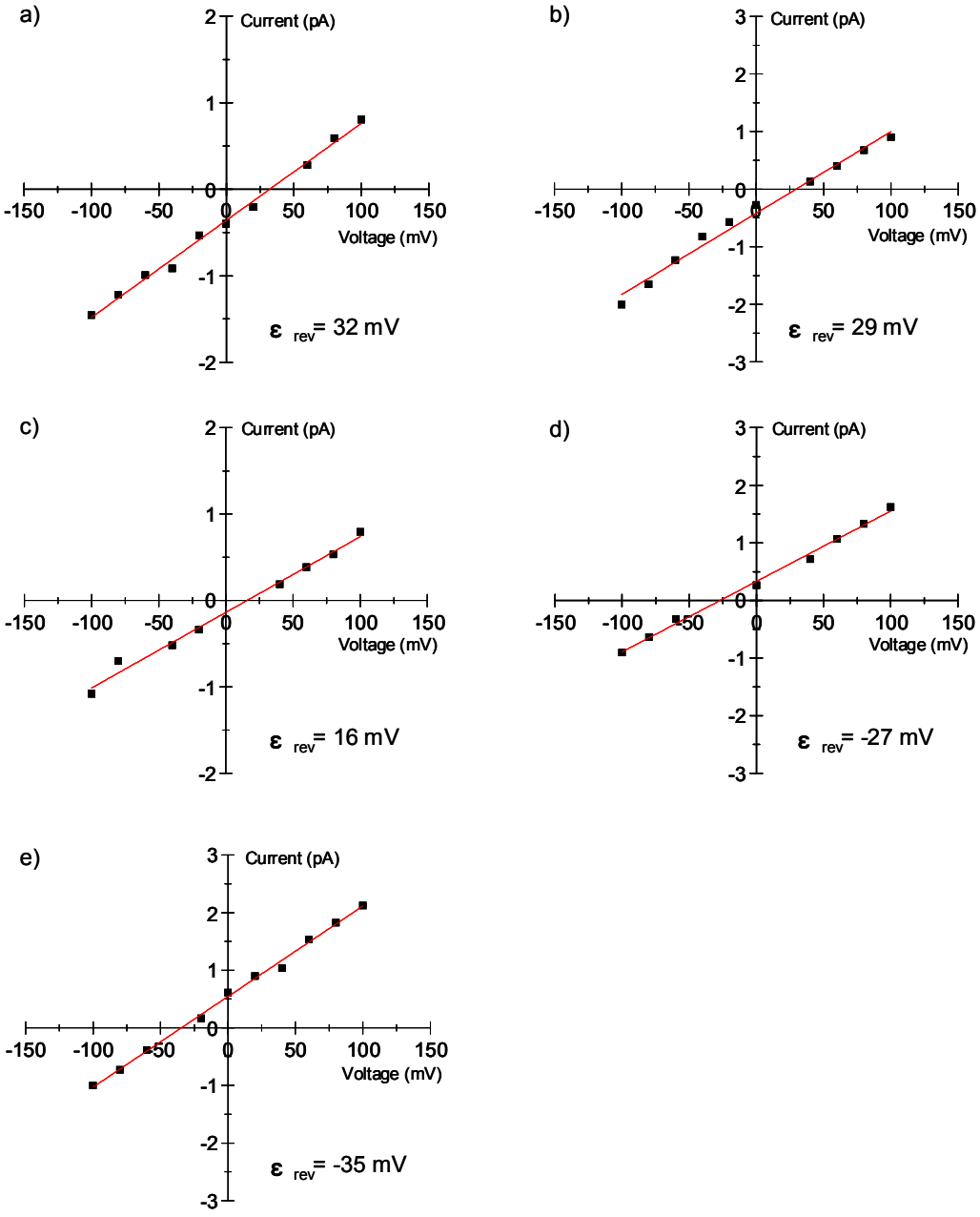


Fig. S30 Current-voltage relationship of channel **2** by using unsymmetrical solution at both side of the bilayer. *trans* chamber: KCl (1.0 M), cis chamber: (a) NH_4Cl (1.0 M), (b) CsCl (1.0 M), (c) RbCl (1.0 M), (d) NaCl (1.0 M), (e) KCl (0.3 M).

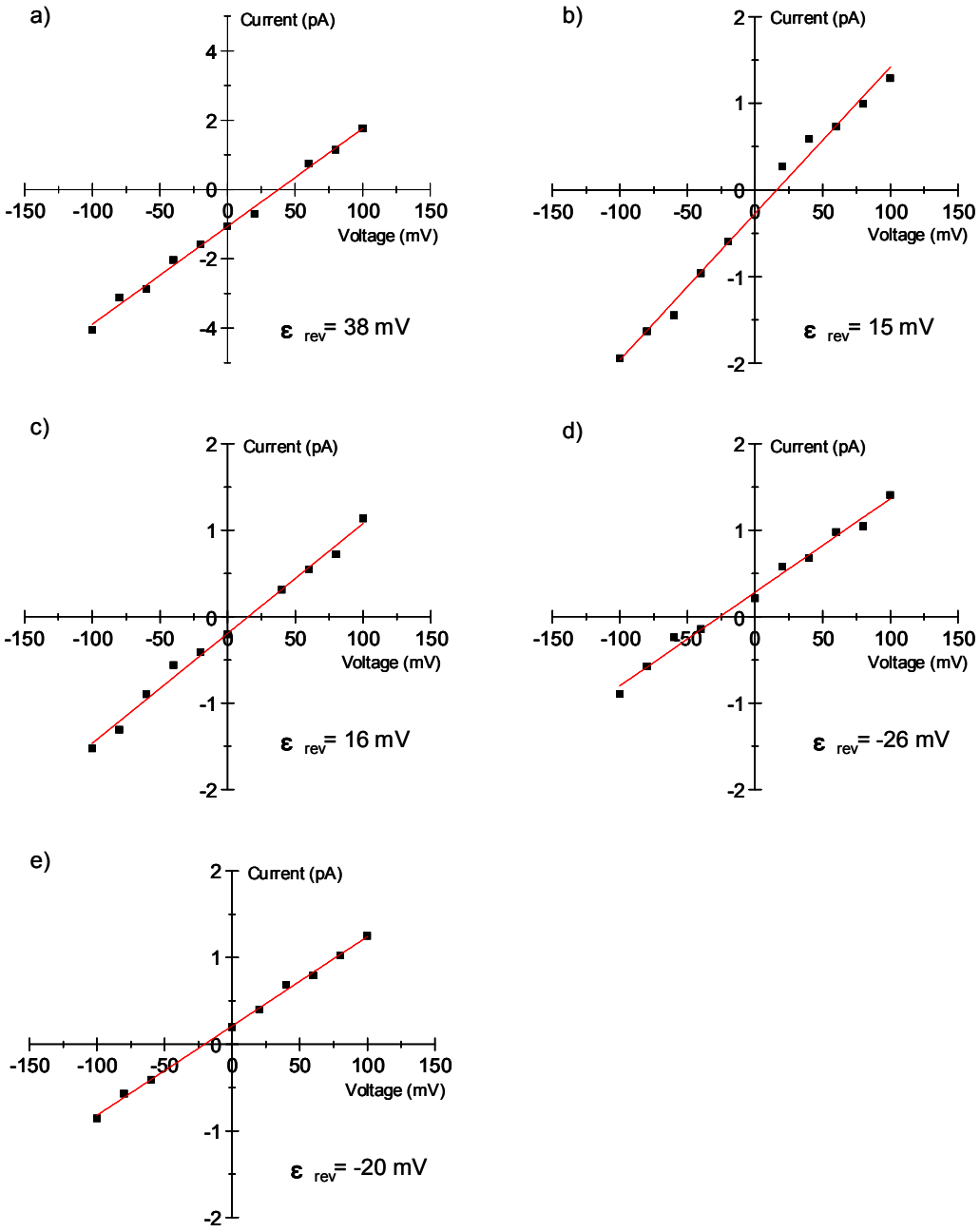


Fig. S31 Current-voltage relationship of channel **3** by using unsymmetrical solution at both side of the bilayer. *trans* chamber: KCl (1.0 M), cis chamber: (a) NH_4Cl (1.0 M), (b) CsCl (1.0 M), (c) RbCl (1.0 M), (d) NaCl (1.0 M), (e) KCl (0.3 M).

5. References:

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2 X.-B. Hu, Z. Chen, G. Tang, J.-L. Hou, Z.-T. Li, *J. Am. Chem. Soc.* 2012, **134**, 8384.

3 R. H. Ashley, *Ion Channels: A Practical Approach*, Oxford University Press, Oxford, 1995.