One-pot formation of hydrazide macrocycles with modified cavities: An example of pH-sensitive unimolecular cation channels

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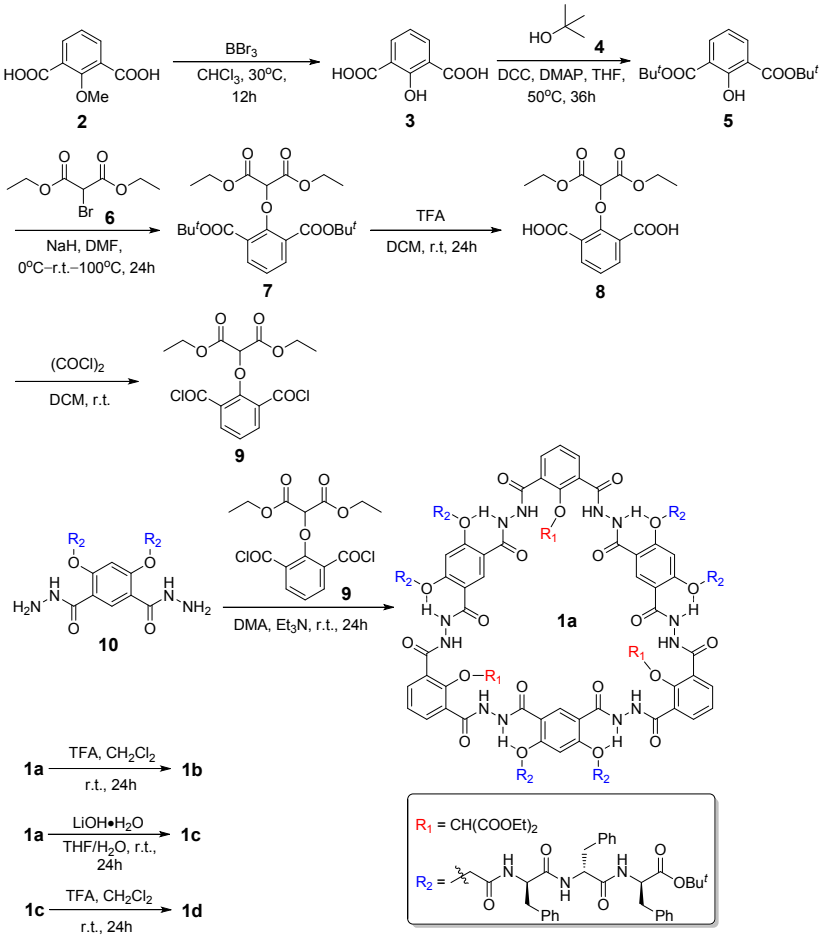
Contents

1. General:

Egg yolk L-α-phosphatidylcholine was obtained from Sigma-Aldrich as ethanol 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 on commercial instruments (400 MHz) 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:



Compound 3. To a solution of **2** (2.0 g, 10.2 mmol) in dry dichloromethane (DCM) (50 mL) was added BBr_3 (15.9 g, 63.5 mmol) dropwise under nitrogen at 0 °C. After addition, the mixture was stirred at that temperature for several minutes and then warm to 30 °C for additional 12h. Then the mixture was added dropwise to the cold water until no gas was liberated. The reaction mixture was then filtrated. The precipitation was collected and washed with water to yield **3** as a white solid.

Yield: 50%. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 12.60 (br, 3H), 7.95 (d, J = 8 Hz, 2H), 6.92 (t, J = 8Hz, 1H). HRMS: Calcd for $C_8H_5O_5$ $[M-H]^-$: 181.0137. Found:

181.0135.

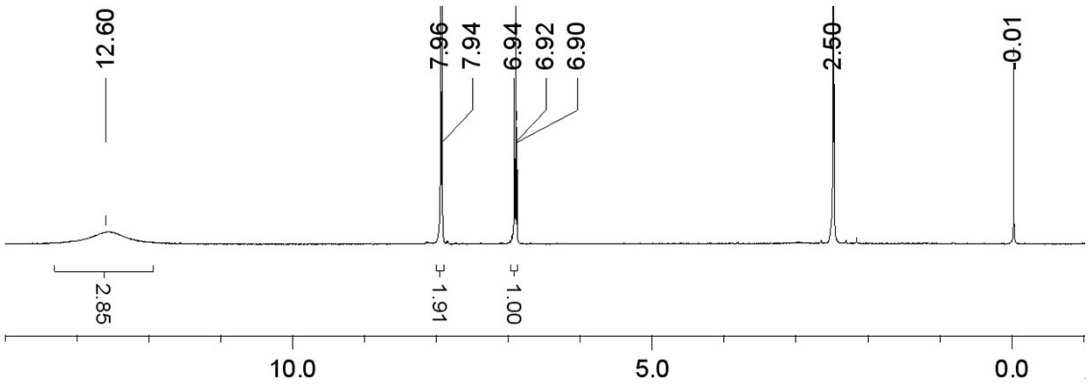


Figure S1. [1]H NMR spectrum of **3** in DMSO-*d6*.

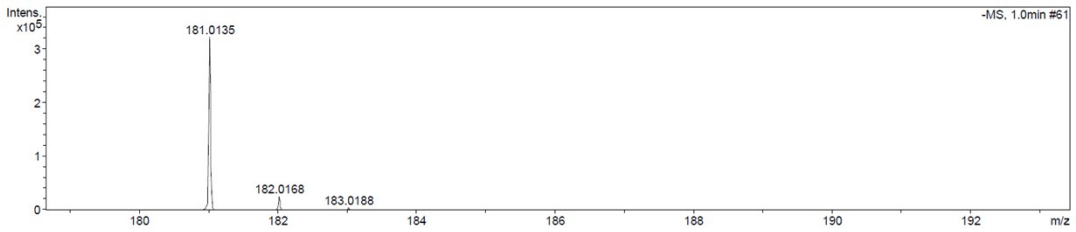


Figure S2. HR-MS of **3**.

Compound 5. To a solution of **3** (1.5 g, 8.2 mmol), DMAP (40 mg, 0.33 mmol) in tBu-OH/THF (50/40 mL) was added DCC (4.25g, 20 mmol). After addition, the mixture was stirred at 50 ^{o}C for 36 h under nitrogen. Then the reaction mixture was filtrated and the residue was washed with DCM. The organic solution was dried over anhydrous Na_2SO_4. After removing of the solvent, the crude product was purified by column chromatography on silica gel to yield **5** as colorless oil.

Yield: 48%. [1]H NMR (400 MHz, $CDCl_3$) δ 12.95 (s, 1H), 7.92 (d, *J* = 8 Hz, 2H), 6.85 (t, *J* = 8 Hz,1H), 1.6 (s, 18H). HRMS: Calcd for $C_{16}H_{23}O_5$ $[M+H]^+$: 295.1545. Found: 295.1539.

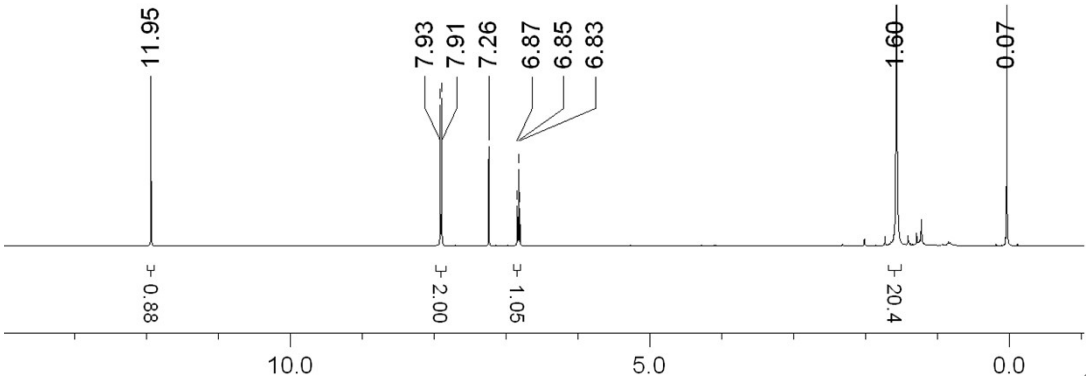


Figure S3. [1]H NMR spectrum of **5** in $CDCl_3$.

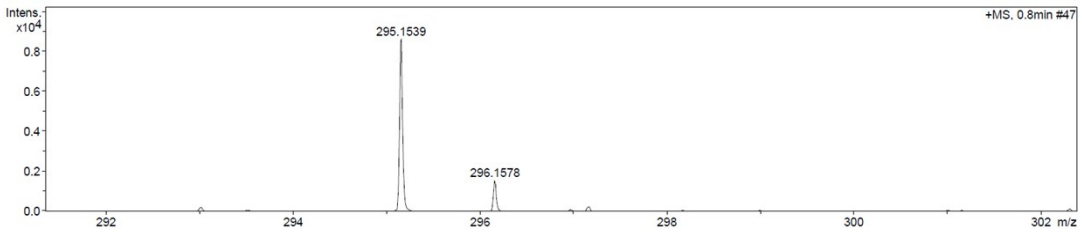


Figure S4. HR-MS of **5**.

Compound 7. The solution of **5** (0.2 g, 0.7 mmol) in anhydrous DMF (20 mL) was stirred at 0°C. Then the NaH (60%, 0.22 g, 0.83 mmol) was added to the mixture. After stirred at 0°C for 15 min, the reaction mixture was warmed to room temperature. The **6** (125 μL, 0.83 mmol) was added to the mixture. The mixture was then stirred at room temperature for 1h and then heated to 60 °C for additional 24h. The mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate. The organic solution was then washed with water, dried over anhydrous Na_2SO_4. After removing of the solvent, the crude product was purified by column chromatography on silica gel to yield **7** as light yellow oil.

Yield: 15%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, *J* = 8 Hz, 2H), 7.17 (t, *J* = 8 Hz, 1H), 5.14 (s, 1H), 4.27 (q, *J* = 8 Hz, 4H), 1.56 (s, 18H), 1.25 (t, *J* = 8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.5, 164.6, 134.3, 127.9, 123.8, 82.1, 61.9, 27.8, 13.9. HRMS: Calcd for $C_{23}H_{36}NO_9$ $[M+NH_4]^+$: 470.2390. Found: 470.2388.

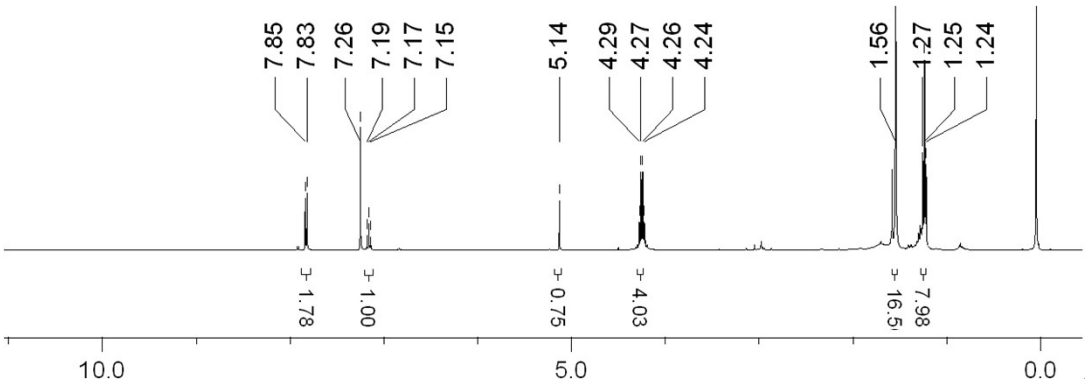


Figure S5. ^{1}H NMR spectrum of **7** in $CDCl_3$.

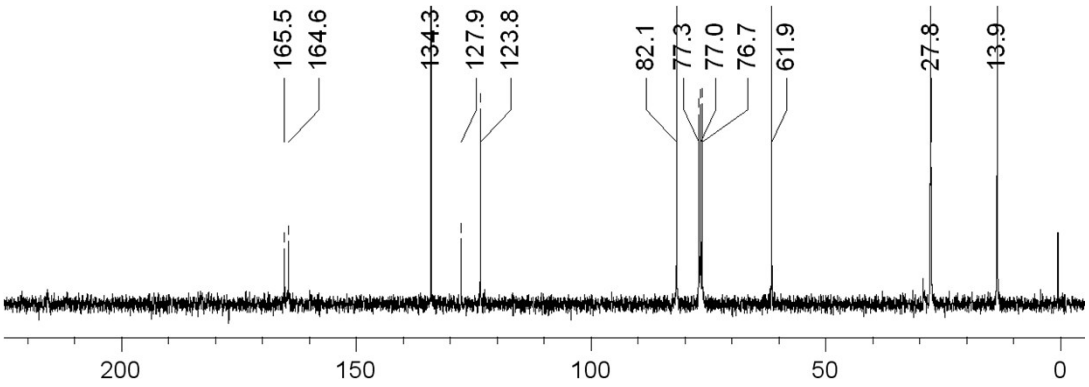


Figure S6. ^{13}C NMR spectrum of **7** in $CDCl_3$.

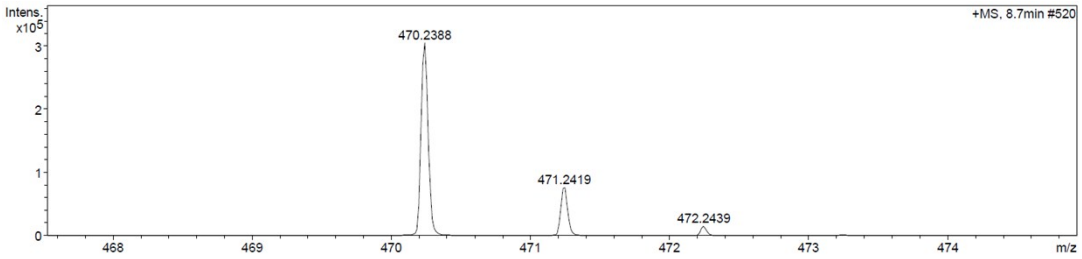


Figure S7. HR-MS of **7**.

Compound 8. To a solution of **7** (0.2 g, 0.44 mmol) in DCM (15 mL) was added TFA (1.5 mL). The mixture was stirred at room temperature for 24 h and then the solvent was removed under reduced pressure. The residue was washed with diethyl ether. After removing of the solvent, the product was obtained as a pale yellow solid. Yield: 89%. ^1H NMR (400 MHz, DMSO-*d6*) δ 13.2 (br, 2H), 7.92 (d, *J* = 8 Hz, 2H), 7.33 (t, *J* = 8 Hz, 1H), 5.04 (s, 1H), 4.21-4.10 (m, 4H), 1.18 (t, *J* = 8 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 170.0, 164.1, 161.4, 140.8, 129.8, 125.8, 123.9, 63.4, 13.7. HRMS: Calcd for $C_{15}H_{17}O_9$ $[M+H]^+$: 341.0873. Found: 341.0867.

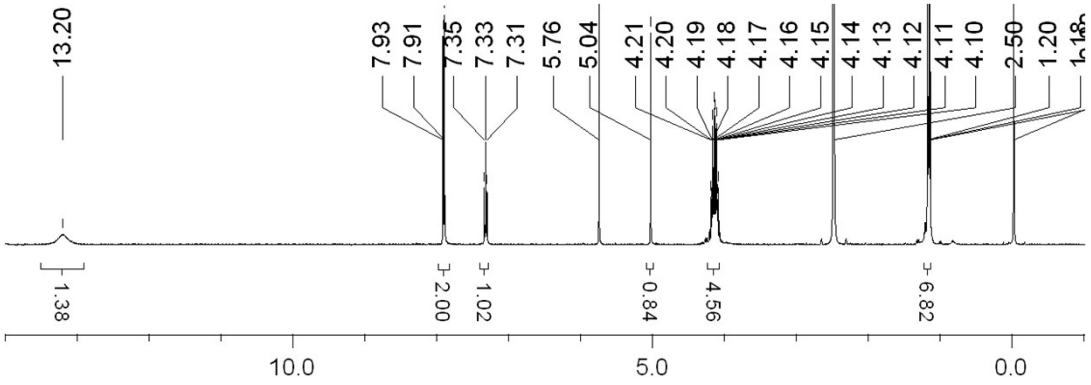


Figure S8. ^1H NMR spectrum of **8** in DMSO-*d6*.

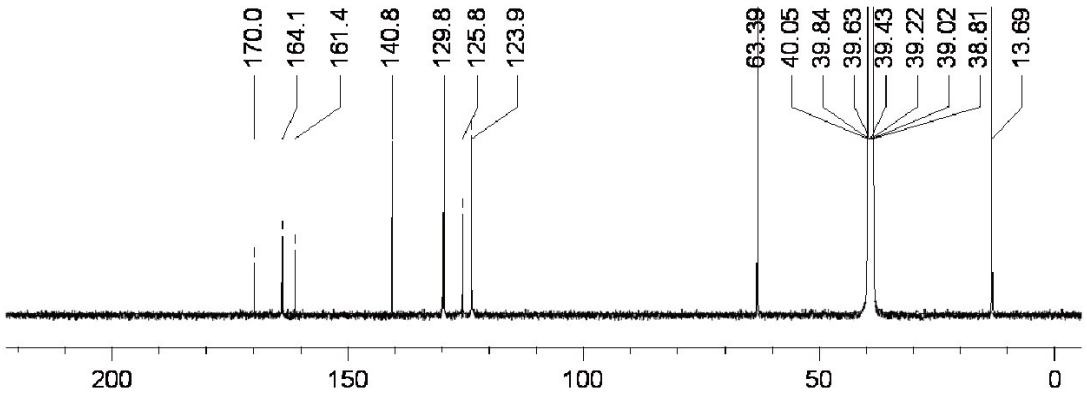


Figure S9. ^{13}C NMR spectrum of **8** in DMSO-*d6*.

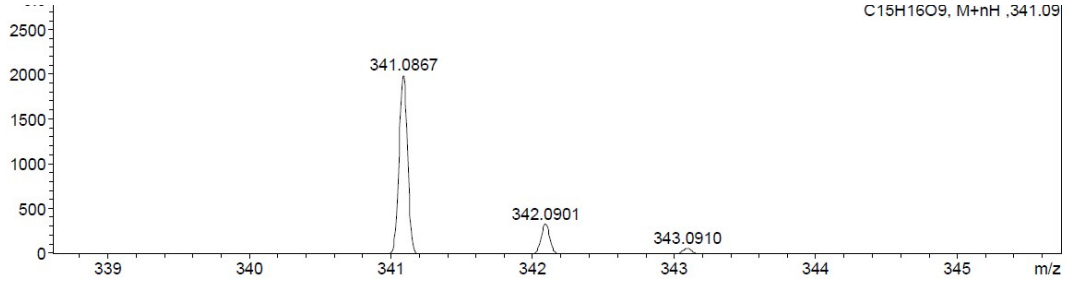


Figure S10. HR-MS of **8**.

Compound 10. This compound was synthesized from the procedure we have reported.[1]

Compound 1a. To a solution of **8** (0.06 g, 0.15 mmol) in anhydrous dichloromethane (10 mL) was added oxalyl chloride (0.2 mL, 2.4 mmol). The mixture was stirred at room temperature for 3 h. The solvent was removed under reduced pressure to give the corresponding chloride (**9**) which was then re-dissolved in anhydrous DMA (15 mL). To this mixture was added **10** (0.2 g, 0.15 mmol) and triethylamine (0.08 mL, 0.45 mmol). The mixture was then stirred at room temperature for 24 h and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel to yield **1a** as a pale yellow solid.

Yield: 64%. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.70-10.30 (m, 10H), 8.56-8.37 (m, 20H), 7.83 (br, 6H), 7.23-7.08 (m, 96H), 6.29-6.14 (m, 3H), 5.86-5.79 (m, 3H), 4.67-4.11 (m, 42H), 3.11-2.97 (m, 24H), 2.78-2.67 (m, 12H), 1.29 (s, 54H), 1.17 (t, J = 6 Hz, 18H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 171.3, 171.2, 171.1, 170.7, 137.8, 137.4, 129.5, 129.4, 128.5, 128.3, 126.9, 126.6, 119.8, 81.1, 67.8, 63.7, 62.4, 54.6, 54.0, 46.0, 41.8, 38.2, 38.1, 37.3, 27.9, 14.1, 14.0, 11.5. HRMS: calcd for $C_{267}H_{29}0N_{30}O_{63}$ $[M+2H]^{2+}$ 2463.0239, found 2463.0271.

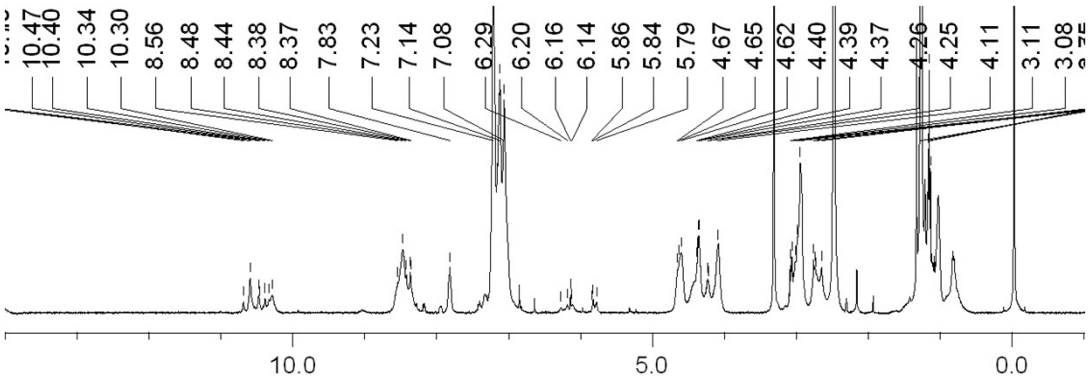


Figure S11. ^{1}H NMR spectrum of **1a** in DMSO-*d6*.

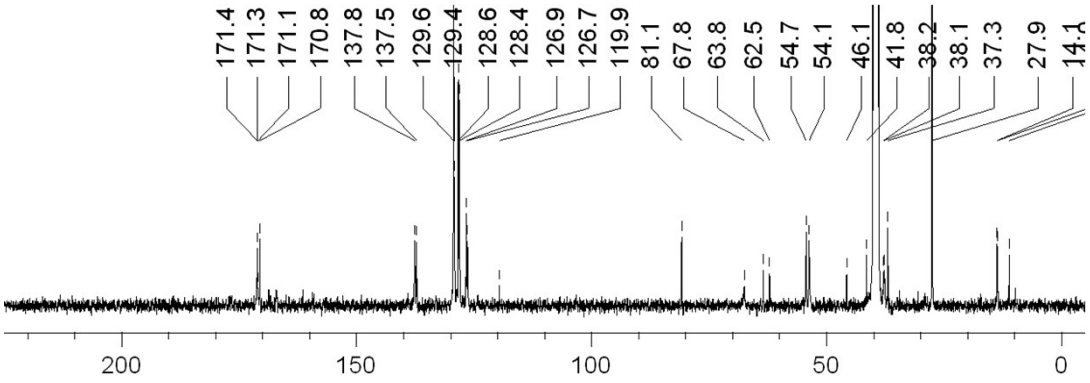


Figure S12. ^{13}C NMR spectrum of **1a** in DMSO-*d6*.

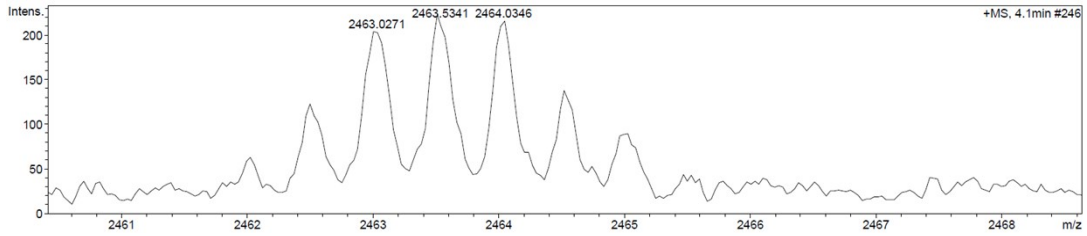


Figure S13. HR-MS of **1a**.

Compound 1b. To a solution of **1a** (0.08 g, 0.016 mmol) in dichloromethane (10 mL) was added trifluoroacetic acid (1 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 **1b** as a yellow solid.

Yield: 84%. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 12.79 (s, 6H), 10.70-10.28 (m, 10H), 8.55-8.37 (m, 20H), 7.83 (br, 6H), 7.23-7.07 (m, 96H), 6.27-6.11 (m, 3H), 5.84-5.78 (m, 3H), 4.67-4.12 (m, 42H), 3.10-2.93 (m, 24H), 2.75-2.67 (m, 12H), 1.09 (t, J = 8 Hz, 18H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 173.1, 171.1, 167.2, 161.6, 159.5, 137.8, 129.5, 129.4, 128.5, 128.3, 126.8, 126.6, 63.7, 54.2, 54.0, 46.1, 38.0, 37.1, 27.9, 14.1, 9.1. HRMS: calcd for $C_{243}H_{238}N_{30}O_{63}$ $[M\text{-}2H]^{2-}$ 2292.8205, found 2292.8194.

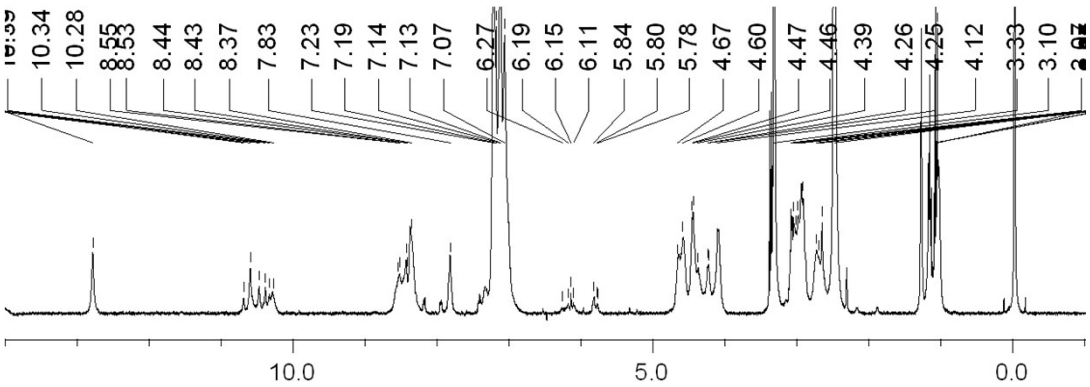


Figure S14. ^{1}H NMR spectrum of **1b** in DMSO-*d6*.

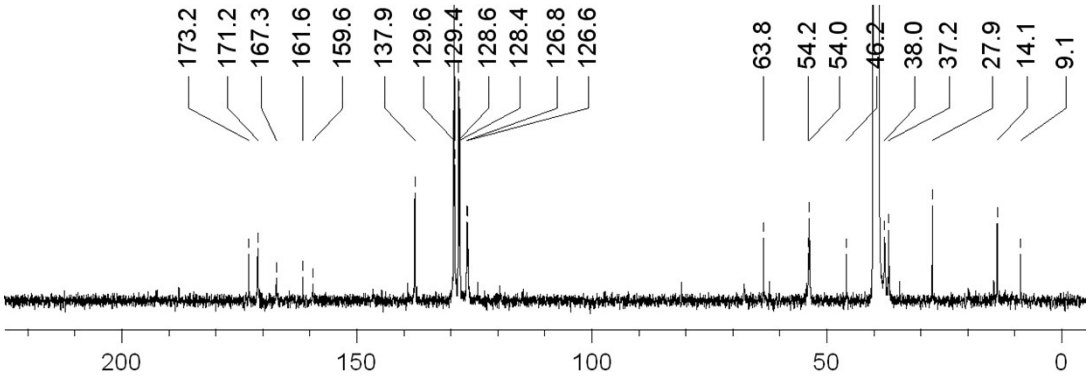


Figure S15. ^{13}C NMR spectrum of **1b** in DMSO-*d6*.

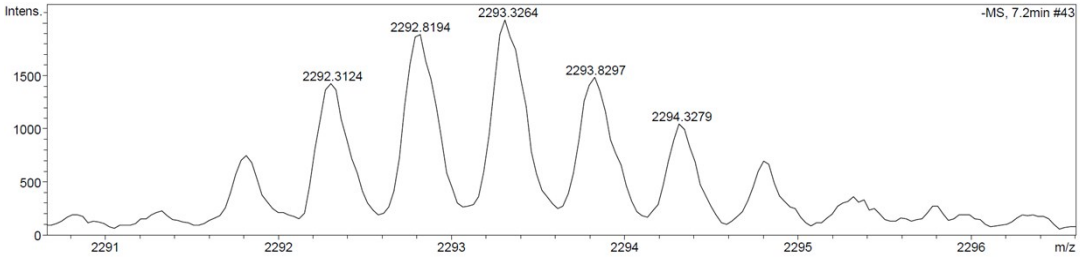


Figure S16. HR-MS of **1b**.

Compound 1c. To a solution of **1a** (0.12 g, 0.025 mmol) in THF/H_2O (15/5 mL) was added LiOH•H_2O (0.025 g, 0.6 mmol). The mixture was stirred at room temperature for 24 h and then the THF was removed under reduced pressure. The residual solution was diluted with water (10 mL) and then acidified with aqueous HCl solution (2%). Then the mixture was filtrated and the residue was washed with water. The product was obtained as a white solid.

Yield: 77%. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 13.09 (br, 2H), 12.20 (s, 4H), 11.04-10.08 (m, 10H), 8.52-8.35 (m, 20H), 7.85 (br, 6H), 7.23-7.09 (m, 96H), 6.21 (br, 3H), 5.90 (br, 3H), 4.61-4.37 (m, 30H), 2.96 (br, 24H), 2.76 (br, 12H), 1.29 (s, 54H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 171.2, 170.7, 167.3, 137.8, 137.4, 129.5, 129.3, 128.5, 128.3, 126.9, 126.6, 81.1, 67.8, 54.6, 54.0, 38.1, 37.3, 27.9, 14.9, 14.3, 11.5. HRMS: calcd for $C_{255}H_{262}N_{30}O_{63}$ $[M-2H]^{2-}$ 2376.9144, found 2376.9202.

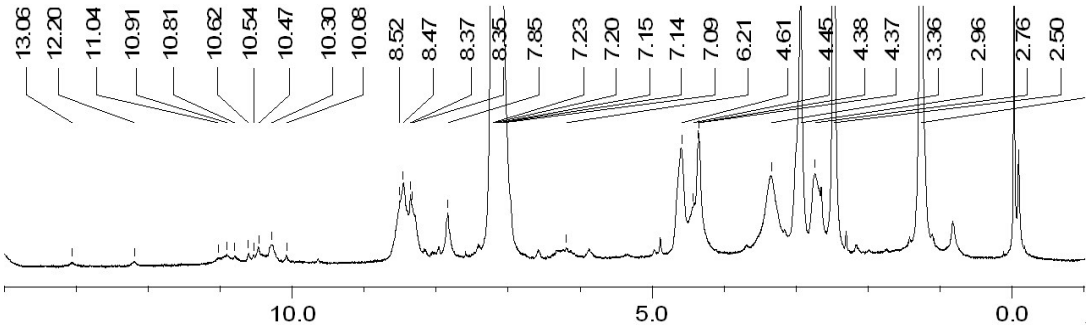


Figure S17. ^{1}H NMR spectrum of **1c** in DMSO-*d6*.

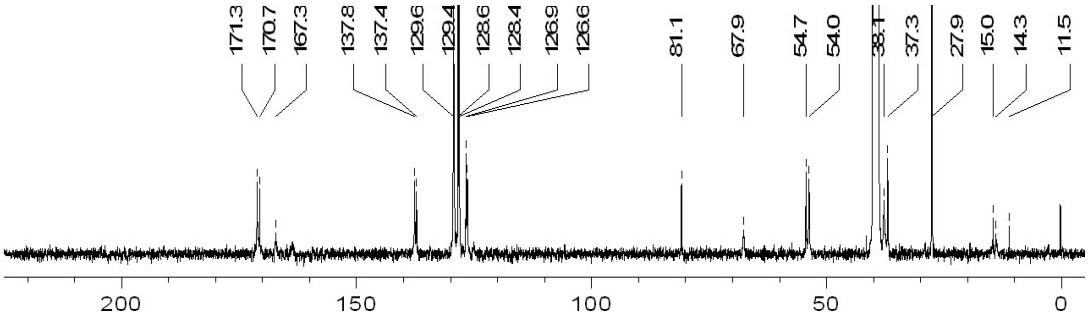


Figure S18. ^{13}C NMR spectrum of **1c** in DMSO-*d6*.

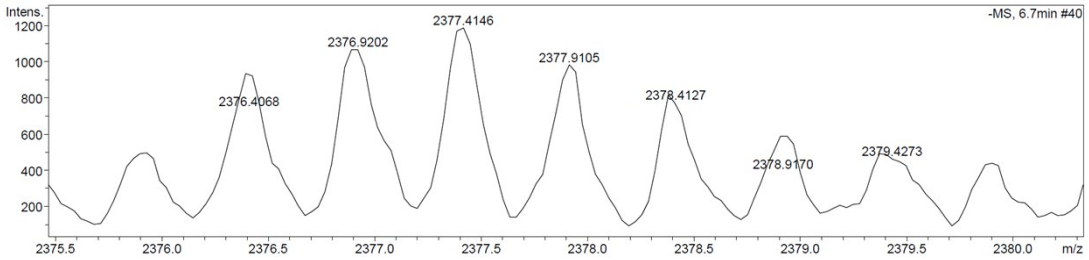


Figure S19. HR-MS of **1c**.

Compound 1d. To a solution of **1c** (0.06 g, 0.014 mmol) in dichloromethane (10 mL) was added trifluoroacetic acid (1 mL). The mixture was stirred at room temperature for 24 h and then the solvent was removed under reduced pressure. The residue was washed with diethyl ether and then recrystallized from ethanol to yield **1d** as a yellow solid.

Yield: 79%. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 12.76 (br, 6H), 12.26 (br, 2H), 12.68-11.57 (m, 4H), 11.78-10.09 (m, 10H), 8.50-8.16 (m, 20H), 7.86 (d, *J* = 8Hz 6H), 7.24-7.07 (m, 96H), 6.58-6.30 (m, 6H), 4.62-4.44 (m, 30H), 3.06-2.94 (m, 24H), 2.74-2.67 (m, 12H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 199.0, 173.1, 171.2, 167.3, 137.8, 129.5, 128.6, 128.3, 126.8, 126.6, 122.6, 81.1, 75.7, 68.0, 54.0, 41.8, 37.9, 37.1, 34.8, 27.9, 11.4. HRMS: calcd for $C_{231}H_{214}N_{30}O_{63}$ $[M\text{-}2H]^{2-}$ 2208.7266, found 2208.7291.

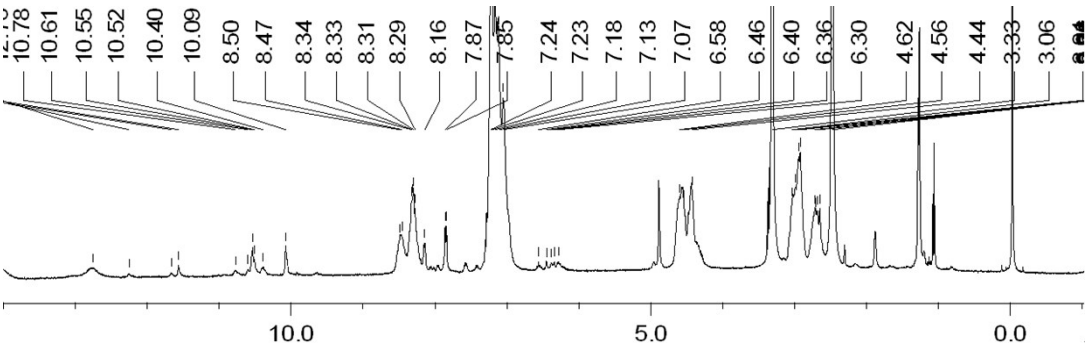


Figure S20. [1]H NMR spectrum of **1d** in DMSO-*d6*.

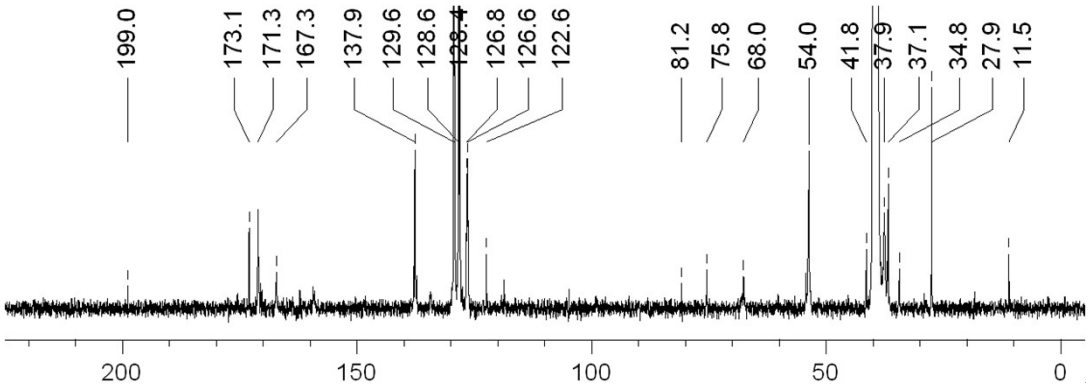


Figure S21. [13]C NMR spectrum of **1d** in DMSO-*d6*.

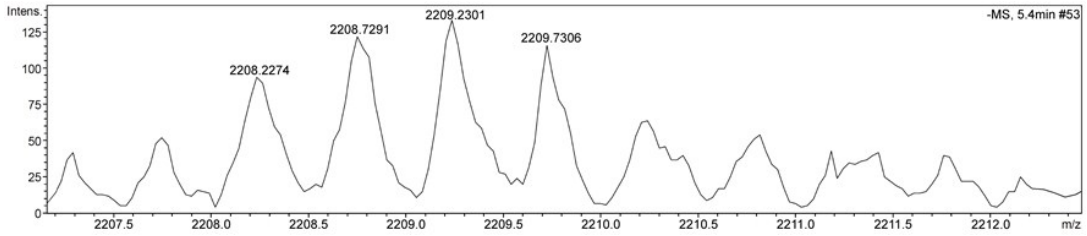


Figure S22. HR-MS of **1d**.

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 N_2 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 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 compound **1a-1d** 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 $(I_t\text{-}I_0)/(I_\infty\text{-}I_0)$,

where I_0 is the initial intensity.

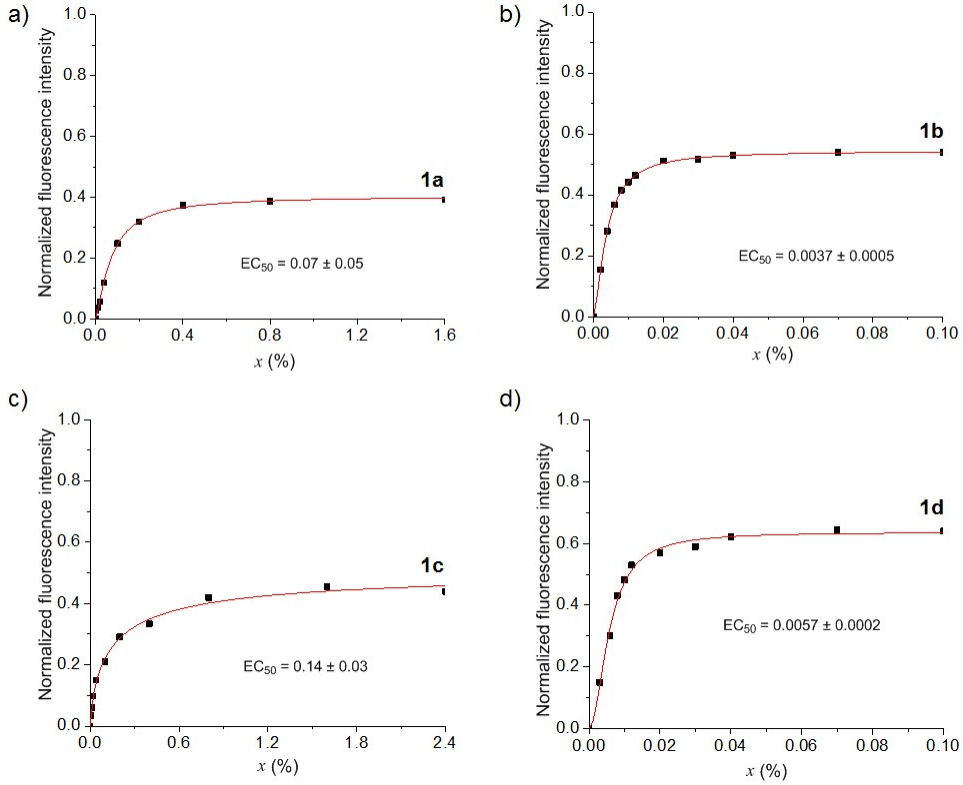


Figure S23. Changes in normalized fluorescent intensity of HPTS (λ_{ex} = 460 nm, λ_{em} = 510 nm) in vesicles with the concentration of **1a-1d** (molar ratio relative to lipid, represented by *x*). By fitting the plot with Hill equation, the effective concentration needed for 50% activity (EC_{50}) for **1a-1d** was determined to be 0.07% (**1a**), 0.0037% (**1b**), 0.14% (**1c**) and 0.0057% (**1d**), respectively.

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 = Cs^+, Rb^+, K^+ or Na^+). 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). 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.

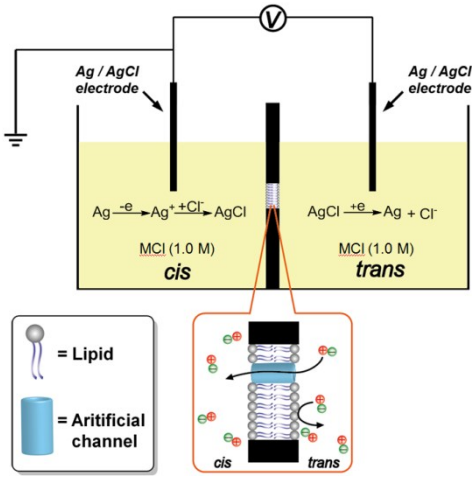


Figure S24. Schematic representation for the patch clamp experiments with planar lipid bilayer. The redox reactions on both Ag/AgCl electrodes are inserted to illustrate the nature of charge balance during M^+ transmembrane transport.

For the single-channel conductance measurement, two chambers were charged with NH_4Cl (1 M, 1 mL). And the solution of compound **1a-1d** in DMSO (1 mM, 0.5 μL) was added to the *cis* compartment and the solution was stirred for 5 min.

For the measurement of the transport selectivity of M^+ (M = NH_4^+, Cs^+, Rb^+ or Na^+) over K^+, the *cis* chamber was charged with MCl (1.0 M) and the *trans* one was charged with KCl (1.0 M). The solution of compound **1a-1d** in DMSO (1 mM, 0.5 μL) was added to the *cis* compartment and the solution was stirred for 5 min.

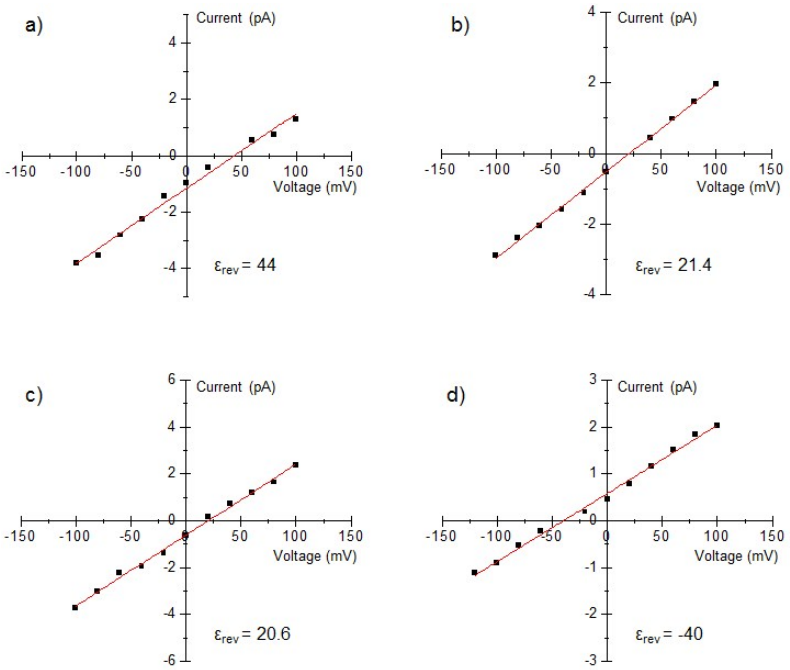


Figure S25. Current–voltage relationship of channel **1a** by using unsymmetrical

solution at both side of the bilayer. *trans* chamber: KCl (1.0 M), *cis* chamber: (a) NH_4Cl, (b) CsCl (1.0 M), (c) RbCl (1.0 M), (d) NaCl (1.0 M).

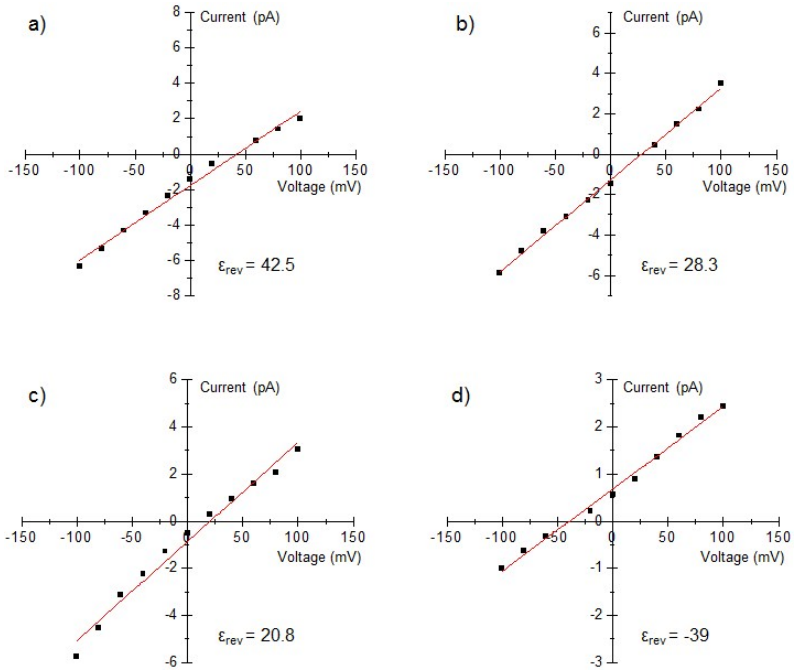


Figure S26. Current–voltage relationship of channel **1b** by using unsymmetrical solution at both side of the bilayer. *trans* chamber: KCl (1.0 M), *cis* chamber: (a) NH_4Cl, (b) CsCl (1.0 M), (c) RbCl (1.0 M), (d) NaCl (1.0 M).

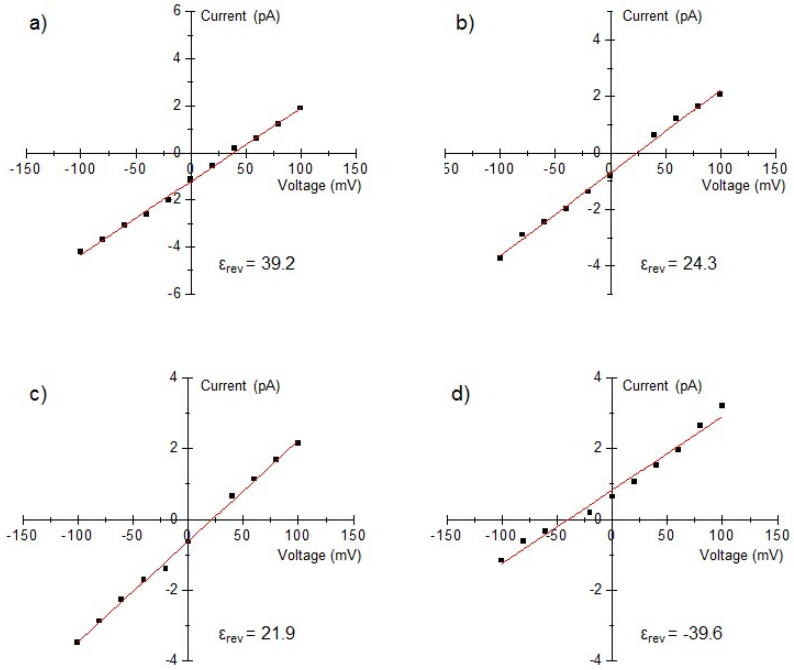


Figure S27. Current–voltage relationship of channel **1c** by using unsymmetrical solution at both side of the bilayer. *trans* chamber: KCl (1.0 M), *cis* chamber: (a) NH_4Cl, (b) CsCl (1.0 M), (c) RbCl (1.0 M), (d) NaCl (1.0 M).

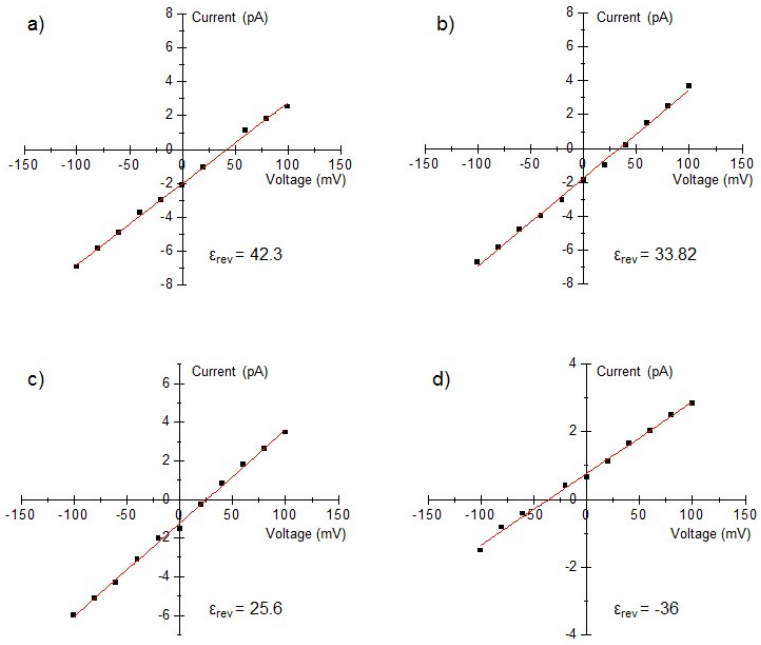


Figure S28. Current–voltage relationship of channel **1d** by using unsymmetrical solution at both side of the bilayer. *trans* chamber: KCl (1.0 M), *cis* chamber: (a) NH_4Cl, (b) CsCl (1.0 M), (c) RbCl (1.0 M), (d) NaCl (1.0 M).

For the measurement of the transport selectivity of K^+ over Cl^-, the KCl solutions (0.2M and 1M) were adjusted to pH 4.0, 7.0, or 10.0 by using HCl or KOH. Then, the KCl solutions were added to the both side of the bilayer (diPhyPC), *trans* chamber: KCl (1.0 M), *cis* chamber: KCl (0.2 M). The solution of compound **1b-1d** in DMSO (1 mM, 0.5 μL) was added to the *cis* compartment and the solution was stirred for 5 min.

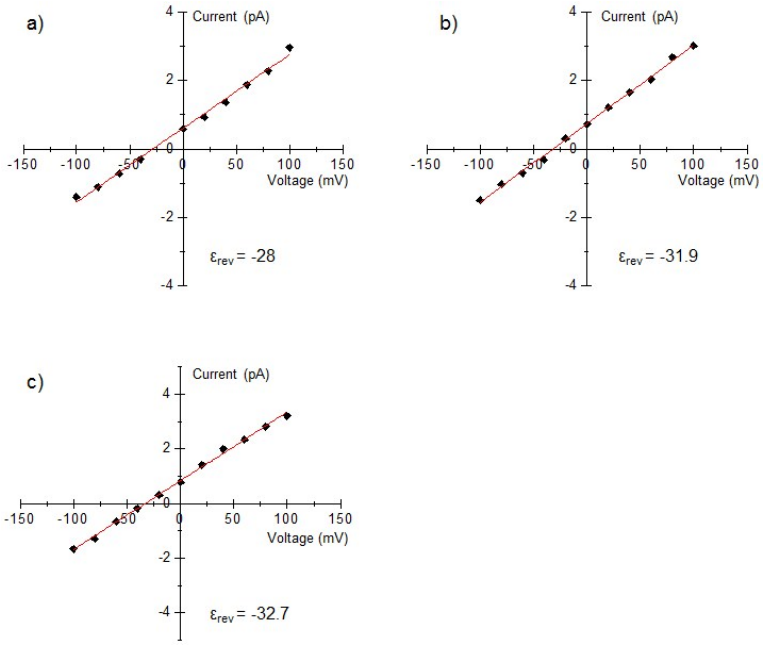


Figure S29. Current–voltage relationship of channel **1b** by using unsymmetrical

solution at both side of the bilayer. *trans* chamber: KCl (1.0 M), *cis* chamber: KCl (0.2 M). (a) pH = 4.0; (b) pH = 7.0; (c) pH = 10.0.

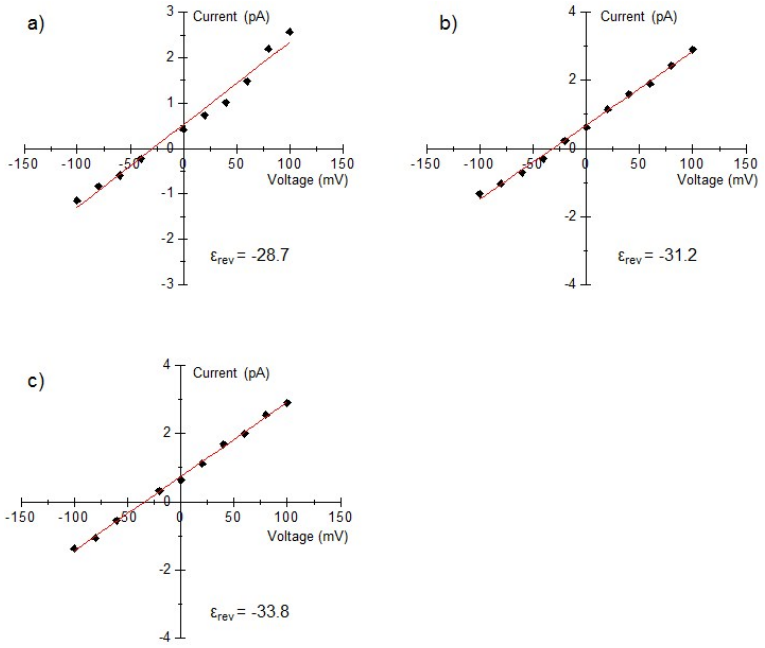


Figure S30. Current–voltage relationship of channel **1c** by using unsymmetrical solution at both side of the bilayer. *trans* chamber: KCl (1.0 M), *cis* chamber: KCl (0.2 M). (a) pH = 4.0; (b) pH = 7.0; (c) pH = 10.0.

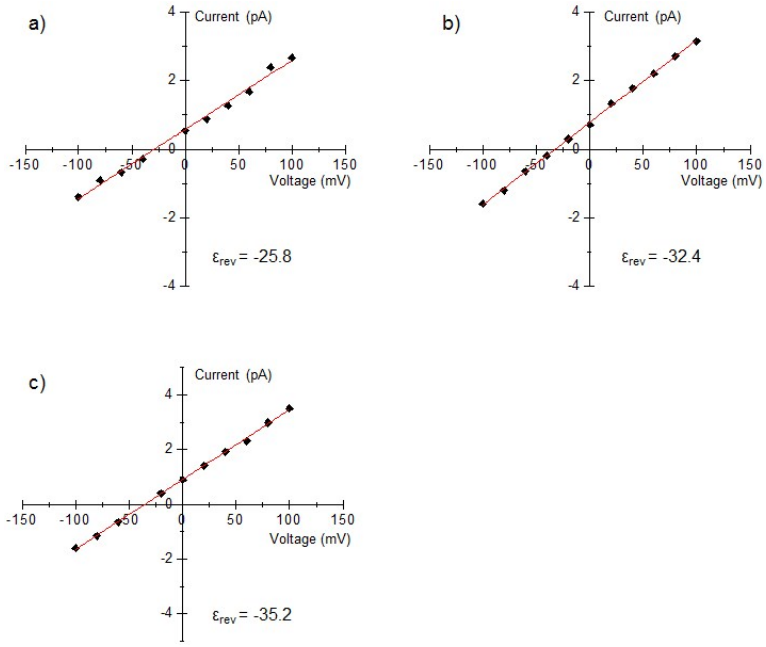


Figure S31. Current–voltage relationship of channel **1d** by using unsymmetrical solution at both side of the bilayer. *trans* chamber: KCl (1.0 M), *cis* chamber: KCl (0.2 M). (a) pH = 4.0; (b) pH = 7.0; (c) pH = 10.0.

5. References:

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