Supporting Information

Fast-Switching pH-Responsive Biomimetic Ion Channels with Bidirectional Gating Control

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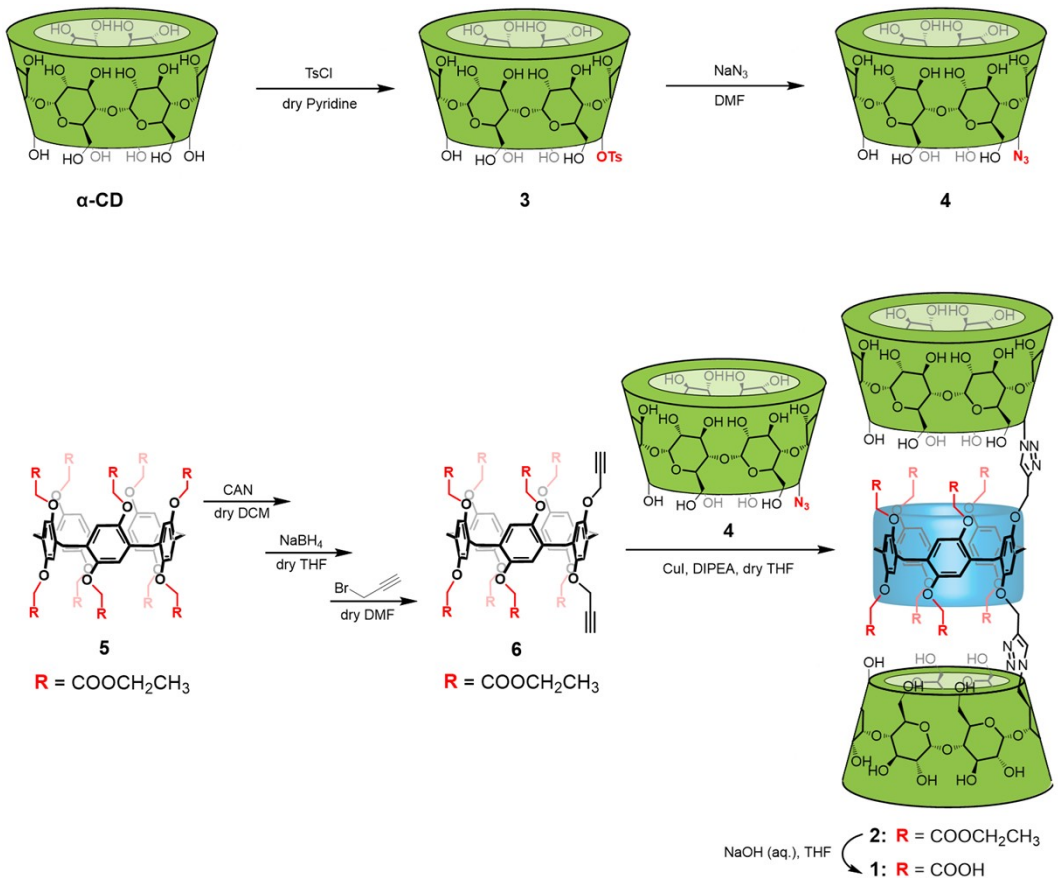
1. General:

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). The ^{1}H and ^{13}C NMR spectra were recorded on commercial instruments (Bruker AVANCE III HD 600 MHz, Bruker AVANCE NEO 400 MHz) at 298 K. Chemical shifts were referenced to solvent residue. Mass spectra were recorded with Bruker MicroTOF II spectrometer and Thermo Fisher LC-HRMS spectrometer. The fluorescent experiments on vesicles were performed on Varian Cary Eclipse fluorescence spectrophotometer. The conductance measurement on planar lipid bilayer was performed on Warner BC-535D Planar Lipid Bilayer Workstation.

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



Compound 3. Compound **3** was synthesized according to the reported procedure.[1]

3: Yield: 20%.^{1}H NMR (400MHz, DMSO-d_6) δ 7.77 (d, J = 8.0 Hz, 2H), 7.45 (d, J = 8.0 Hz, 2H), 5.54-5.40 (m, 12H), 4.79 (s, 4H), 4.72 (s, 1H), 4.66 (s, 1H), 4.50 (s, 3H), 4.32-4.28 (m, 3H), 3.87-3.55 (m, 23H), 3.47-3.38 (m, 6H), 3.28-3.16 (m, 6H), 2.41 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 144.9, 132.5, 130.0, 127.7, 102.1, 102.0, 101.6, 82.1, 82.1, 81.8, 73.3, 73.1, 72.9, 72.1, 71.9, 71.7, 69.7, 68.9, 59.9, 40.1, 39.9, 39.7, 39.5, 39.3, 39.1, 39.0, 38.9, 21.2. HRMS**:** calcd for $C_{43}H_{66}NaO_{32}S$ $[M+Na]^+$: 1149.3150, found 1149.3134.

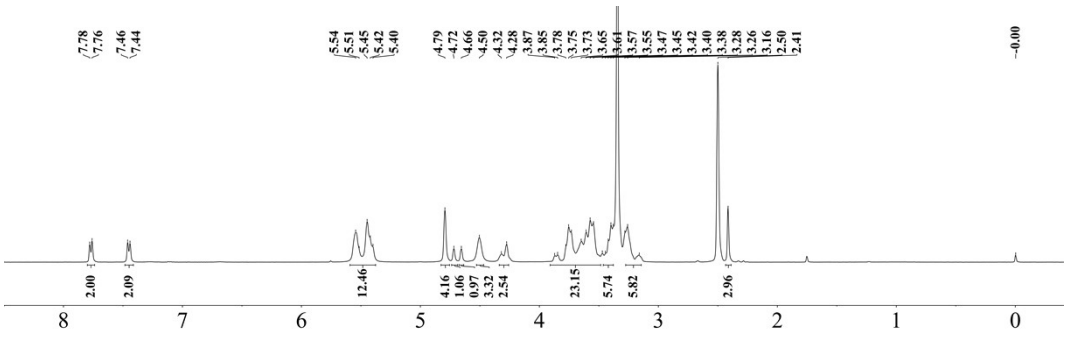


Figure S1. ^{1}H NMR spectrum of **3** in DMSO-d_6.

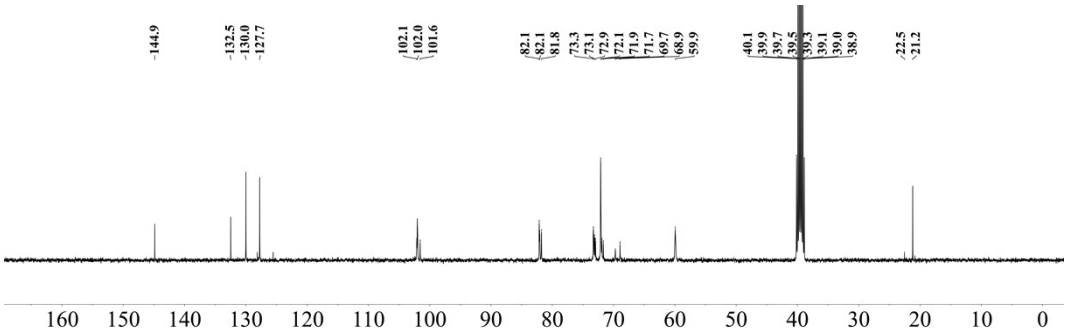


Figure S2. ^{13}C NMR spectrum of **3** in DMSO-d_6.

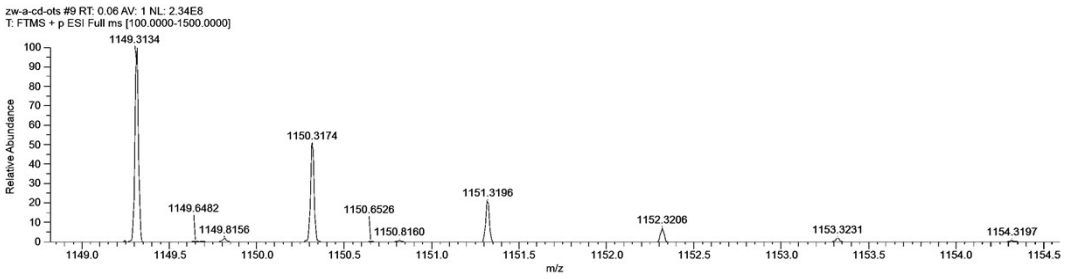


Figure S3. HR-MS spectrum of **3**.

Compound 4. Compound **3** (3.0 g, 2.6 mmol) was dissolved in anhydrous N,N-dimethylformamide (DMF, 60 mL), then sodium azide (NaN_3, 3.46 g, 53.2 mmol) was added. The reaction mixture was stirred at 80 °C for 12 h. After the reaction was complete, the reaction mixture was cooled to ambient temperature and reprecipitated in mixed solvent of acetone : water (200 mL: 10 mL). The precipitate was filtrated and dried to obtain 1.2 g of compound **4** as a white solid.

4: Yield: 45%.^{1}H NMR (400MHz, DMSO-d_6) δ 5.55-5.38 (m, 12H), 4.86-4.77 (m, 6H), 4.58-4.49 (m, 5H), 3.79-3.76 (m, 6H), 3.64-3.57 (m, 17H), 3.43-3.39 (m, 6H), 3.31-3.26 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 102.3, 102.1, 101.9, 83.3, 82.5, 82.3, 82.2, 82.2, 73.4, 73.3, 73.0, 72.5, 72.2, 72.0, 70.5, 60.4, 60.1, 51.4, 40.1, 39.9, 39.7, 39.5, 39.3, 39.1, 38.9. HRMS**:** calcd for $C_{23}H_{59}NaN_3O_{29}$ $[M+Na]^+$: 1020.3126, found 1020.3181.

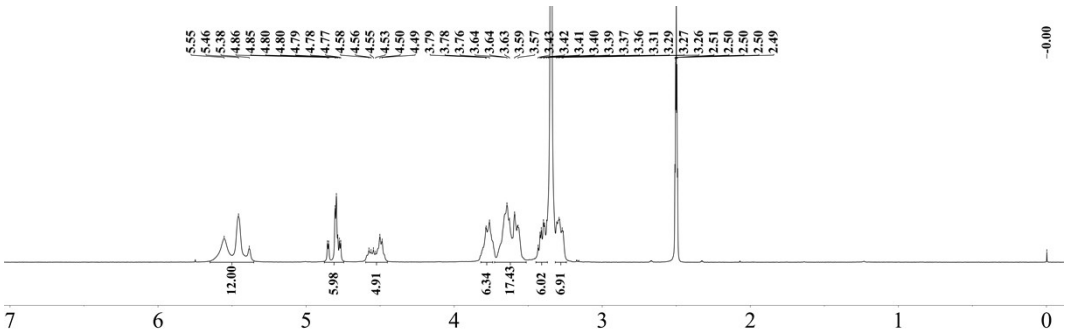


Figure S4. ^{1}H NMR spectrum of **4** in DMSO-d_6.

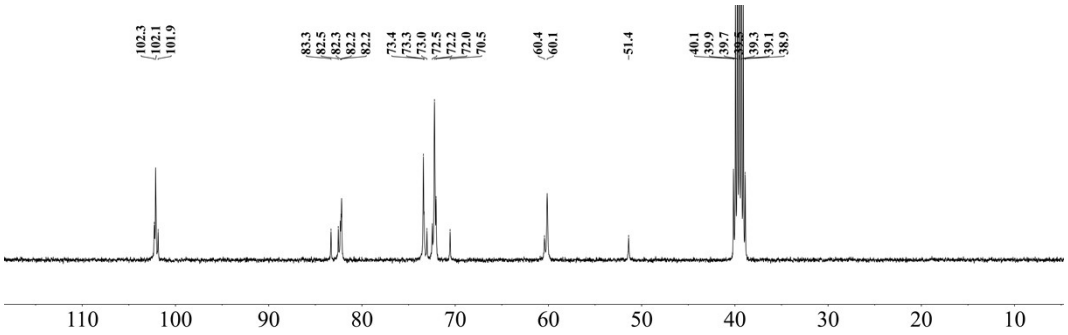


Figure S5. ^{13}C NMR spectrum of **4** in DMSO-d_6.

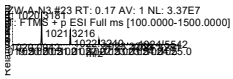


Figure S6. HR-MS spectrum of **4**.

Compound 5. Compound **5** was synthesized according to the reported procedure.[2]

5: Yield: 60%. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.04 (s, 10H), 4.59-4.49 (m, 20H), 4.01-4.12 (m, 20H), 3.85 (s, 10H), 0.96 (t, J = 7.2 HZ, 30H). ^{13}C NMR ($CDCl_3$, 100 MHz,) δ 169.5, 149.1, 128.8, 114.6, 77.5, 77.2, 76.8, 65.8, 61.0, 29.3, 14.0. HRMS: calcd for $C_{75}H_{90}NaO_{30}$ $[M+Na]^+$ 1493.5409, found 1493.5369.

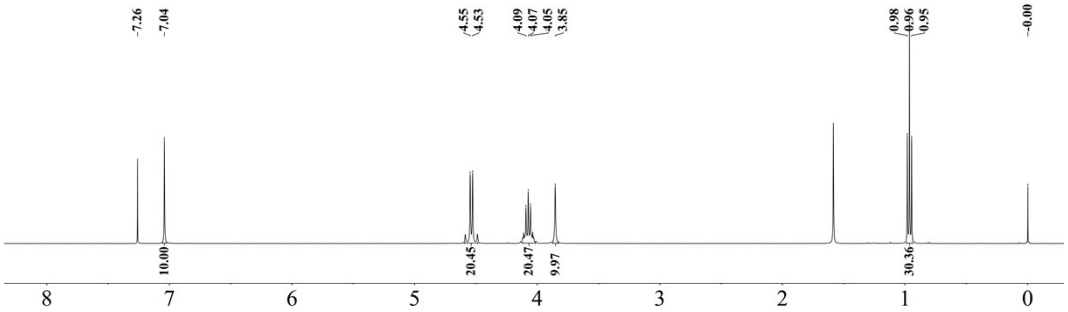


Figure S7. ^{1}H NMR spectrum of **5** in $CDCl_3$.

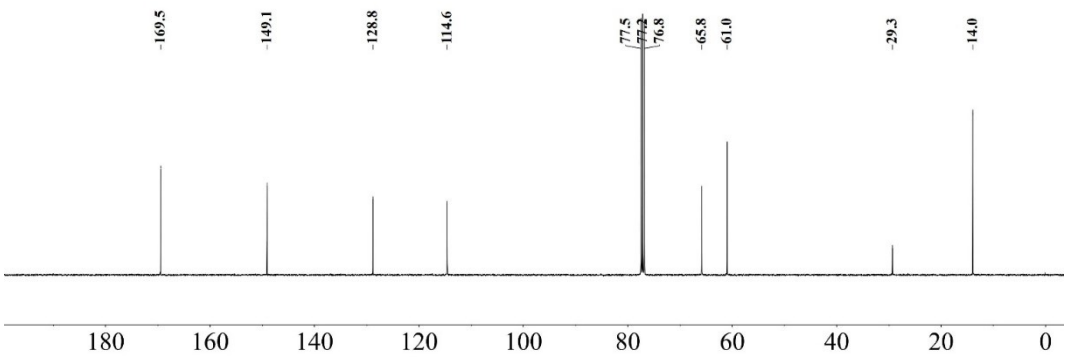


Figure S8. ^{13}C NMR spectrum of **5** in $CDCl_3$.

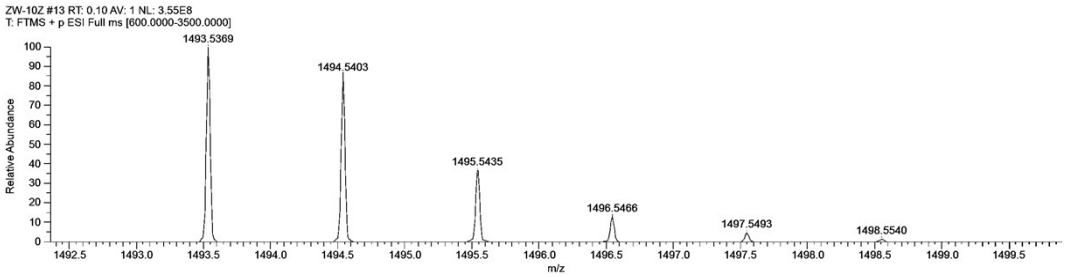


Figure S9. HR-MS of **5**.

Compound 6. Compound **5** (776 mg, 0.53 mmol) was dissolved in dry DCM (20 mL). Then, a solution of the ceric ammonium nitrate ($Ce(NH_4)_2(NO_3)_6$, 578.3mg, 1 mmol) in water (1 mL) was added dropwise. The reaction mixture was stirredat 25 ℃ for 30 min, washed with water (15 mL × 2) and brine (15 mL) and dried with anhydrous Na_2SO_4. After removing of the solvent, the obtained oxidation products was dissolved in dry THF (30 mL), then sodium borohydride ($NaBH_4$, 90.8 mg, 2.4 mmol), cerium chloride ($CeCl_3$, 591.8 mg, 2.4 mmol) and EtOH (8 mL) were added. The reaction mixture was stirred under argon at 25 ℃ for 20 min, HCl (1 M) was added into the mixture (adjusting the pH to 7), Then, the reaction mixture was extracted with DCM (25 mL), and the organic solution was washed with brine (15 mL) and dried with anhydrous Na_2SO_4. After removing of the solvent, the obtained reduction products was dissolved in dry DMF (30 mL), K_2CO_3 (255 mg, 1.84 mmol) and propargyl bromide (0.15 mL, 1.84 mmol) was added, the reaction mixture was stirred under argon at 80 ℃ for 12 h. After removal of the solvent, the resulting solid was dissolved in DCM (50 mL), and the organic solution was washed with water (35 mL) and brine (35 mL) and dried with anhydrous Na_2SO_4. The mixture was concentrated under reduced pressure, and purified by column chromatography on silica gel using a mixture of DCM : EtOAc (30 : 1) as eluent to give the desired compound **6**.

6: Yield: 10%. ^1H NMR (600MHz, $CDCl_3$) δ 7.07 (s, 2H), 7.02-7.01 (m, 6H), 6.84 (s, 2H), 4.63-4.49 (m, 20H), 4.17-4.10 (m, 4H), 4.06-3.90 (m, 10H), 3.87-3.77 (m, 12H), 2.48 (t, J = 2.4 Hz, 2H), 1.10 (t, J = 6.0 Hz, 6H), 0.90-0.85 (m, 12H), 0.70 (t, J = 7.2 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.5, 169.5, 169.3, 149.1, 149.1, 149.1, 149.0, 149.0, 128.9, 128.8, 128.8, 128.7, 128.6, 114.9, 114.7, 114.6, 114.4, 113.9, 79.8, 77.4, 77.2, 76.9, 74.9, 65.9, 65.8, 65.7, 61.1, 61.0, 61.0, 56.2, 29.4, 29.3, 14.1, 13.9, 13.8, 13.6. HRMS: calcd for $C_{73}H_{82}NaO_{26}$ $[M+Na]^+$ 1397.4986, found 1397.4943.

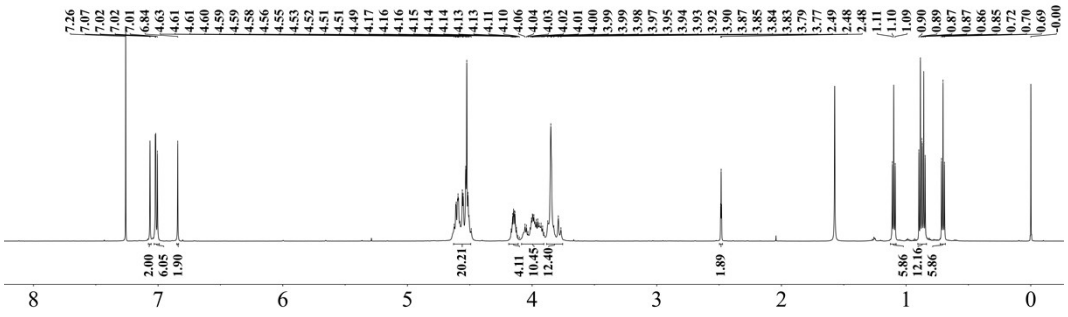


Figure S10. ^{1}H NMR spectrum of **6** in $CDCl_3$.

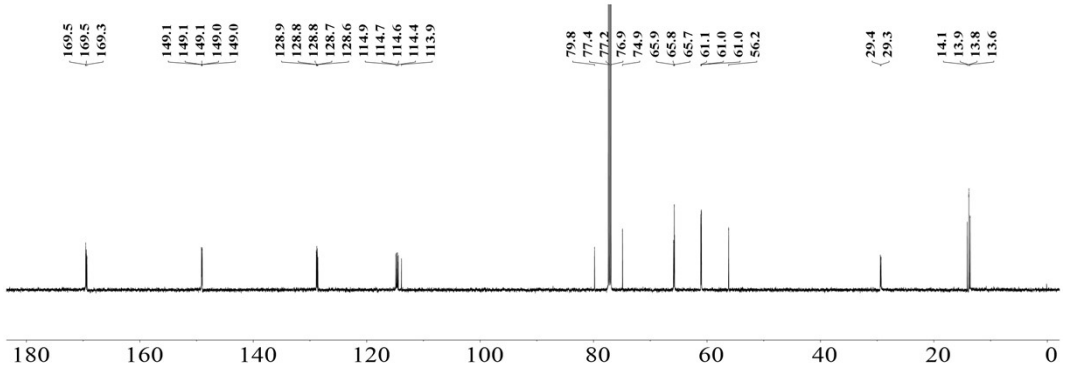


Figure S11. ^{13}C NMR spectrum of **6** in $CDCl_3$.

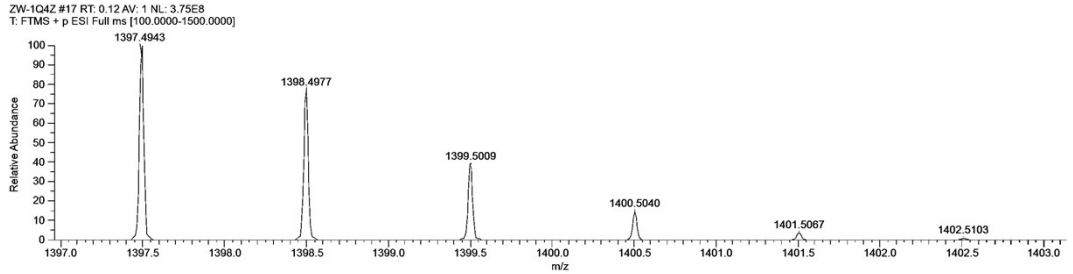


Figure S12. HR-MS of **6**.

Compound 2. Compounds **4** (102 mg, 0.10 mmol) and **6** (40 mg, 0.029 mmol) were dissolved in dry DMF (7 mL). After complete dissolution, CuI (15 mg, 0.079 mmol) and DIPEA (15 μL, 0.086 mmol) were added, and the mixture was stirred at 100 °C for 48 h. The reaction mixture was concentrated under reduced pressure, and the crude product was purified by HPLC to yield **2** as white solid.

The synthesized compound was purified via preparative reverse-phase HPLC on an Agilent 1260 system, utilizing an SB-C18 column (21.2 × 250 mm, 7 μm). The purification process employed two eluents: A (100% water) and B (100% acetonitrile). The purification progress was monitored by UV detection at 215 nm. The linear gradient from 95% A and 5% B to 5% A and 95% B over 15 min was used in the purification processes.

2: Yield: 30%. ^{1}H NMR (400MHz, DMSO-d_6) δ 8.16 (d, J = 12.0, 2H), 7.18 (s, 2H), 7.03-6.98 (m, 6H), 6.77 (d, J = 28.0, 2H), 5.81 (d, J=8.0, 1H), 5.72-5.63 (m, 5H), 5.61-5.53 (m, 10H), 5.49-5.39 (m, 10H), 5.08 (d, J = 8.0, 2H), 4.96-4.92 (m, 4H), 4.85-4.77 (m, 12H), 4.71-4.51 (m, 20H), 4.47-4.41 (m, 6H), 4.15-3.94 (m, 20H), 3.80-3.72 (m, 26H), 3.63-3.58 (m, 16H), 3.50-3.38 (m, 16H), 3.30-3.12 (m, 16H), 1.12-1.01 (m, 12H), 0.99-0.91 (m, 6H), 0.89-0.81 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.1, 169.0, 169.0, 168.9, 168.8, 148.9, 148.6, 148.4, 148.3, 142.8, 142.6, 128.1, 127.9, 127.7, 113.8, 101.9,

82.0, 79.2, 73.3, 72.1, 65.0, 63.1, 60.5, 60.4, 60.4, 59.9, 54.9, 40.1, 39.9, 39.7, 39.5, 39.3, 39.1, 38.9 28.7, 13.8, 13.8, 13.7, 13.7, 13.5. HRMS: calcd for $C_{145}H_{200}O_{84}N_6Na_2$ $[M+2Na]^{2+}$: 1708.0690, found 1708.0690.

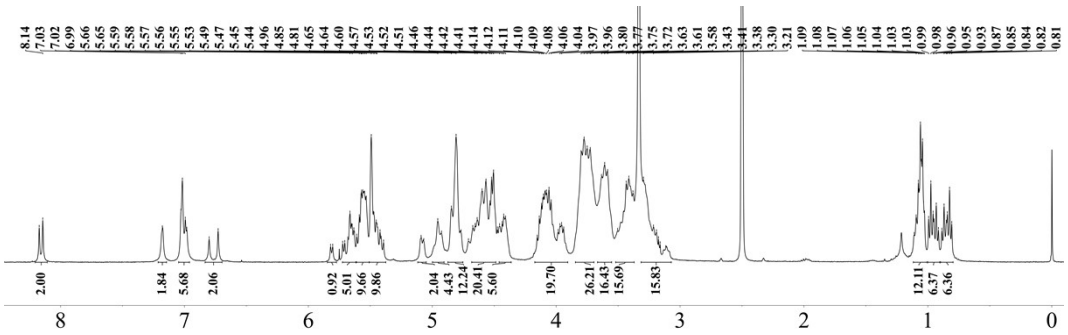


Figure S13. ^{1}H NMR spectrum of **2** in DMSO-d_6.

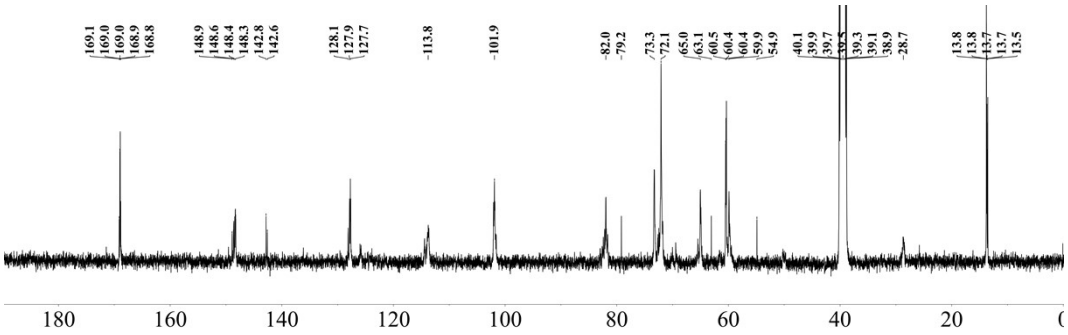


Figure S14. ^{13}C NMR spectrum of **2** in DMSO-d_6.

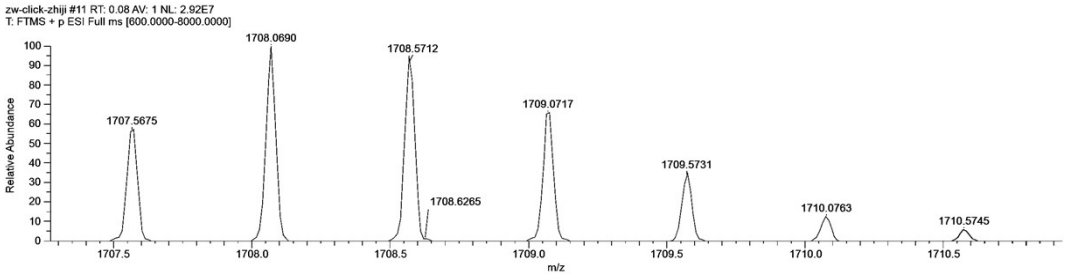


Figure S15. HR-MS of **2**.

Compound 1. Compound **2** (80 mg, 0.024 mmol) was dissolved in THF (10 mL), an aqueous solution of NaOH (20%, 5 mL) was added. The mixture was then refluxed at 65 °C for 24 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure, and the residue was diluted with water (20 mL) and HCl (1 M) was added to acidify the mixture and afford the white solid. The product was collected by centrifugation and washed by water to give the desired compound **1** as white solid.

1: Yield: 39%. ^{1}H NMR (600MHz, DMSO-d_6) δ 12.95 (br, 8H), 8.16 (d, J = 12.0 Hz, 2H), 7.29 (d, J = 6.0 Hz, 2H), 7.12 (s, 6H), 6.71 (d, J = 24.0 Hz, 2H), 5.64-5.40 (m, 20H), 5.16-5.11 (m, 2H), 5.06-5.01 (m, 2H), 4.92-4.79 (m, 12H), 4.72-4.53 (m, 12H), 4.46-4.25 (m, 10H), 4.09-4.04 (m, 2H), 3.85-3.77 (m, 18H), 3.73 (s, 6H), 3.68-3.56 (m, 24H), 3.46-3.37 (m, 20H), 3.32-3.06 (m, 20H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.5, 148.9, 148.5, 142.8, 128.3, 128.0, 114.4, 102.1, 101.9, 82.2, 82.1, 73.3, 72.7, 72.4,

72.1, 65.2, 60.0, 49.7, 40.1, 39.9, 39.8, 39.7, 39.5, 39.4, 39.2, 39.1, 28.7. HRMS: calcd for $C_{129}H_{165}O_{84}N_6[M\text{-}3H]^{3-}$: 1047.6820, found 1047.6283.

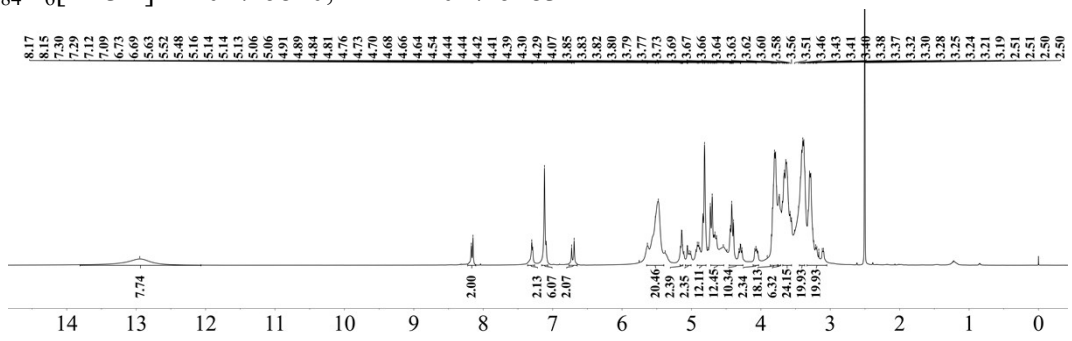


Figure S16. ^{1}H NMR spectrum of **1** in DMSO-d_6.

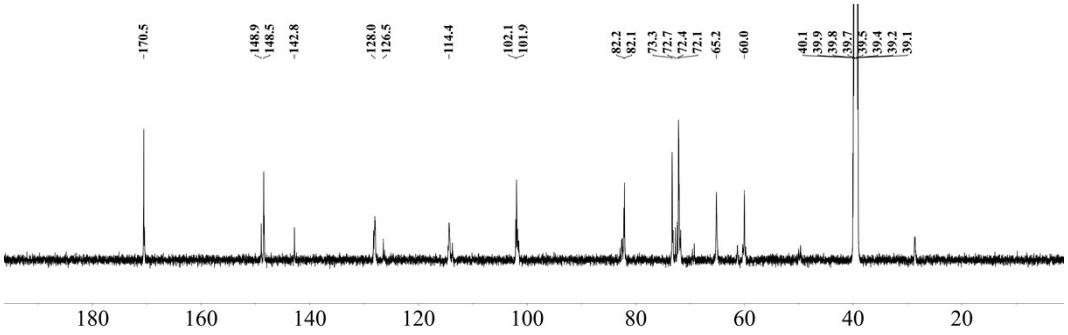


Figure S17. ^{13}C NMR spectrum of **1** in DMSO-d_6.

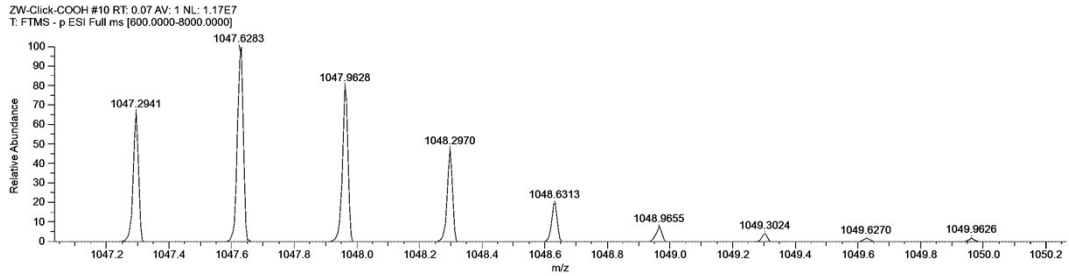


Figure S18. HR-MS of **1**.

3. Ionophoric experiment with HPTS assay:

(a) Preparation of HPTS containing large unilamellar vesicles (LUVs): EYPC (Egg yolk L-α-phosphatidylcholine, 30 mg, 20 μmol) in EtOH (0.3 mL) was diluted with EtOH (15.0 mL), the solution was transferred to a round-bottomed flask and then 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 (3.0 mL, HEPES (10 mM), NaCl (100 mM), pH = 7.0) containing HPTS (8-hydrox-ypyrene-1,3,6-trisulfonic acid, 0.1 mM) at 40 ℃ 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 extruded via polycarbonate membrane (0.2 μm) suspension nine times and then 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).

(b) Fluorescent experiments: A pH gradient for ion transport study was established by adding prepared vesicle suspension (13.3 mM, 100 μL) to a HEPES buffer solution (2 mL, HEPES (10 mM), NaCl (100 mM), pH = 6.0). A required channel concentration was achieved by adding of compound **1** or **2** in DMSO

with gentle stirring. Fluorescent intensity (I_t) was continuously monitored at 510 nm (excitation at 460 nm) and the vesicles were lysed with Triton aqueous solution (20%, 10 μL) at the 5-minute mark. The fluorescent intensity was monitored continuously until it reached a stable value (I_∞). Subsequently, the gathered data were normalized to reflect the fractional fluorescence change, calculated as $(I_t\text{-}I_0)/(I_\infty\text{-}I_0)$, where I_0 represents the initial intensity.

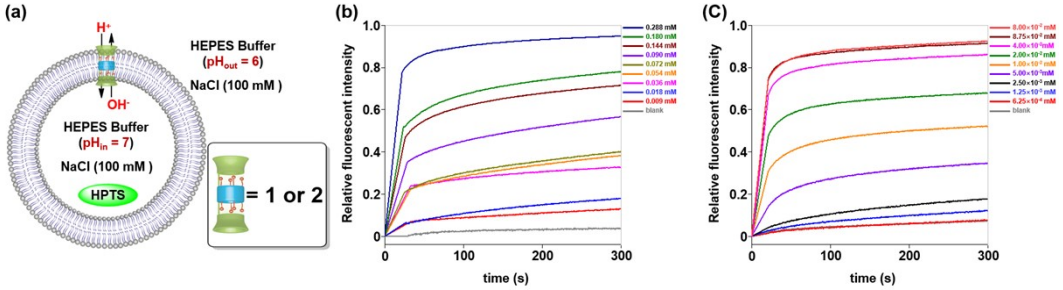


Figure S19 (a) Schematic representation of the HPTS assay. Changes in the relative fluorescence intensity of HPTS (λ_{ex} = 460 nm, λ_{em} = 510 nm) invesicles with time in the presence of different concentrations of **1** (b) and **2** (c).

(c) Preparation of large unilamellar vesicles (LUVs) containing both channel molecules and HPTS: Egg yolk L-α-phosphatidylcholine (EYPC, 10 mg) and the corresponding equivalent of **1** or **2** was diluted with EtOH (5 mL), the solution was transferred to a round-bottomed flask and then 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 (2 mL, HEPES (10 mM), NaCl (100 mM), pH 7.0) containing 8-hydrox-ypyrene-1,3,6-trisulfonic acid (HPTS, 0.1 mM) at 40 °C for 2 h to give a milky suspension (gently vortexing after every 0.5 h to ensure the lipid film complete hydrated under nitrogen protection). 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 extruded nine times through polycarbonate membrane (0.2 μm) and then 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).

(d) Fluorescent experiments: HEPES buffer solution (2.0 mL, HEPES (10 mM), NaCl (100 mM), pH 7.0) and the prepared LUVs suspension (13.3 mM, 100 μL) containing corresponding equivalent of **1** or **2** were placed in a fluorometric cuvette. A pH gradient was introduced across the membranes by addition of HCl (1 M, 4.2 μL, ΔpH = 1.0). Fluorescent intensity (I_t) was continuously monitored at 510 nm (excitation at 460 nm) in 5 min. Then, Triton aqueous solution (20%, 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.

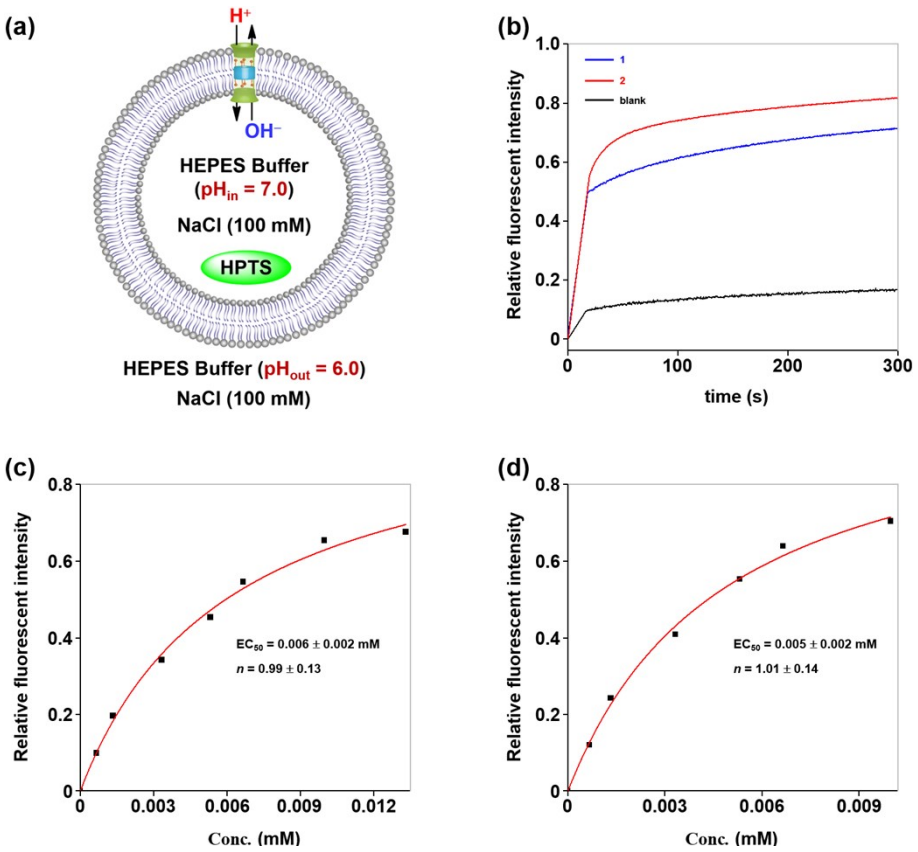


Figure S20 (a) Schematic presentation for the HPTS assay with pre-incorporated channels. (b) Changes in the fluorescence intensity of HPTS (λ_{ex} = 460 nm, λ_{em} = 510 nm) in vesicles with time in the presence of **1** and **2** (0.0063 mM). Changes in normalized fluorescent intensity of HPTS in vesicles with the concentration of (c) **1** and (d) **2**.

4. Membrane Integrity by CF Assay:

(a) Preparation of CF containing large unilamellar vesicles (LUVs): The solution of EYPC (20 mg) in $CHCl_3$ (10.0 mL) was transferred to a round-bottomed flask and then slowly evaporated under reduced pressure, and the resulting thin film was dried under high vacuum for 3 h. The lipid film was hydrated at 40 °C for 2 h with HEPES buffer solution (1 mL, HEPES (10 mM), KCl (100 mM), pH 7.5) containing 5(6)-Carboxyfluorescein (CF, 50 mM), with gentle vortexing after every 0.5 h to ensure complete hydration of the lipid film, resulting in 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 extruded nine times through polycarbonate membrane (0.2 μm) and then dialyzed with membrane tube (MWCO = 14000) against the same HEPES buffer solution (200 mL, without CF) for nine times to remove un-entrapped CF and diluted with the HEPES buffer solution to yield 4 mL lipid stock solution ([lipid] = 6.7 mM).

(b) Fluorescent experiments: HEPES buffer solution (2.0 mL, HEPES (10 mM), KCl (100 mM), pH = 7.5) and the prepared LUVs suspension (6.7 mM, 100 μL) were placed in a fluorometric cuvette. Then, a solution of compound **1**, **2** or natural pore forming peptide Melittin in DMSO at different concentrations was then injected into the suspension under gentle stirring. Fluorescent intensity (I_t) was continuously monitored at 517 nm (excitation at 492 nm) and the vesicles were lysed with Triton aqueous solution (20%, 10 μL) at the 8-minute mark. The fluorescent intensity was monitored continuously until it reached

a stable value (I_∞). Subsequently, the gathered data were normalized to reflect the fractional fluorescence change, calculated as $(I_t\text{-}I_0)/(I_\infty\text{-}I_0)$, where I_0 represents the initial intensity.

5. Ion transport mechanism studies with SPQ assay:

(a) Preparation of HPTS containing large unilamellar vesicles (LUVs): EYPC (Egg yolk L-α-phosphatidylcholine, 30 mg, 20 μmol) in EtOH (0.3 mL) was diluted with EtOH (15.0 mL), the solution was transferred to a round-bottomed flask and then 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 (3.0 mL, $NaNO_3$ (200 mM)) containing SPQ (6-Methoxy-N- (3-Sulfopropyl) Quinolinium, 0.5 mM) at 40 ℃ for 2 h to give a milky suspension (gently vortexing after every 0.5 h to ensure the lipid film complete hydrated). 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 extruded via polycarbonate membrane (0.2 μm) suspension nine times and then dialyzed with membrane tube (MWCO = 14000) against the same buffer solution (200 mL, without SPQ) for six times to remove un-entrapped SPQ and produce vesicle suspension ([lipid] = 13.3 mM).

(b) Fluorescent experiments: HEPES buffer solution (2.0 mL, NaCl (200 mM)) and the prepared vesicle suspension (13.3 mM, 100 μL) were placed in a fluorometric cuvette. To the cuvette, the solution of compound **1** (14 mM, 9 μL) in DMF was added with gentle stirring. Fluorescent intensity (I_t) was continuously monitored at 440 nm (excitation at 380 nm) in 10 min. Then, Triton aqueous solution (20%, 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.

6. Procedures for planar lipid bilayer conductance experiments:

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 (8 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 KCl solution (1.0 M, 1.0 mL). 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 (100-120 pF). Membrane currents were measured using a Warner BC-535D bilayer clamp amplifier and were collected by PatchMaster (HEKA) with sample interval at 10 kHz and then filtered with an 8-pole Bessel filter at 1 kHz (HEKA). The data were analyzed by FitMaster (HEKA) with a digital filter at 210 Hz. The conductance (γ) was obtained by further analyzing the data using the Clampfit software.

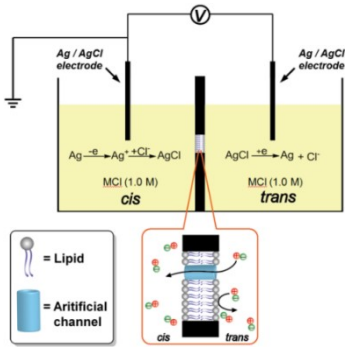


Figure S21. Schematic representation for the patch clamp experiments with planar lipid bilayer. The redox reactions on both Ag/AgCl electrodes are inserted to illustrate the nature of charge balance during M^+ transmembrane transport.

For the single-channel conductance measurement, two chambers were charged with HEPES buffer (5 mM HEPES, 1 M KCl, pH = 5.5, 1 mL). And the solution of compound **1** or **2** (0.1 mM, 1 μL) in DMSO was added to the *cis* compartment and the solution was stirred for 5 min.

For the measurement of the transport selectivity of K^+ over Cl^-, the KCl solutions (0.5 M and 1 M) were added to the both side of the bilayer (diPhyPC), *cis* chamber: HEPES buffer (5 mM HEPES, 1 M KCl, pH = 6.0, 1 mL), *trans* chamber: HEPES buffer (5 mM HEPES, 0.5 M KCl, pH = 6.0, 1 mL). The solution of compound **1** or **2** (0.1 mM, 1 μL) in DMSO was added to the *cis* compartment and the solution was stirred for 5 min. The measured reversal potentials obtained from the *I-V* plots needed adjustment, accounting for the redox potential produced by disparate voltage drops at the electrode-solution interface in different electrolyte concentrations.[3] The redox potential (E_{redox}) was calculated from the equation:

$$E_{redox} = \frac{RT}{zF}\ln\frac{\gamma_{C_H} c_H}{\gamma_{C_L} c_L} \quad (1)$$

where the R, T, z, F, and γ are the gas constant, temperature, charge number, Faraday constant, and mean activity coefficient. When *cis/trans* = 1.0 M/0.5 M KCl, $^{\gamma_{C_H}} = 0.604$, $^{\gamma_{C_L}} = 0.649$, $^{c_H} = 1.0$, $^{c_L} = 0.5$.

Then, the corrected reversal voltage (V_r) was calculated from the equation:

$$V_r = V_{measured} - E_{redox} \quad (2)$$

where the $V_{measured}$ is the measured reversal voltage.

The P_{K^+}/P_{Cl^-} values were calculated from the equation derived from Goldman-Hodgin-Katz equation:

$$P_{K^+}/P_{Cl^-} = \left[a_{K,cis} - a_{K,trans}\exp(\frac{-V_r F}{RT})\right] / \left[a_{Cl,cis}\exp(\frac{-V_r F}{RT}) - a_{Cl,trans}\right] \quad (3)$$

where $a_{K,\,cis}$ and $a_{K,\,trans}$ are the activities of K^+ in the *cis* and the *trans* chambers, $a_{Cl,\,cis}$ and $a_{Cl,\,trans}$ the same for Cl^-.

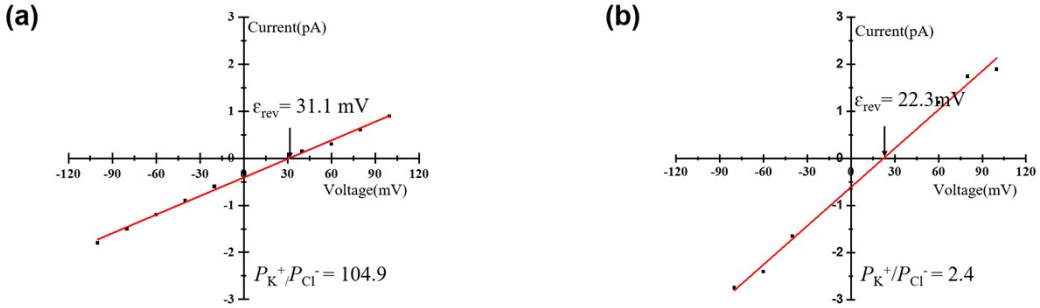


Figure S22 *I-V* plots of **1** (a) and **2** (b) by using unsymmetrical solution at both side of the bilayer. *cis* chamber: KCl (1.0 M), *trans* chamber: KCl (0.5 M).

7. Ion transport in different pH values studies with HPTS assay:

(a) Preparation of HPTS containing large unilamellar vesicles (LUVs): The vesicles (2 mL, HEPES (10 mM), NaCl (100 mM), pH = 7.0) were prepared as described in Section 3

(b) Fluorescent experiments: The prepared vesicle suspension (13.3 mM, 30 μL) were suspended in the external medium consisting of HEPES buffer solution (2 mL, HEPES (10 mM), NaCl (100 mM), pH = 4.0, 5.0, 6.0, 8.0, 9.0) to create a pH gradient for ion transport study. A required channel concentration was achieved by adding of compound **1** (28 mM, 7 μL) or **2** (3 mM, 7 μL) in DMSO with gentle stirring. Fluorescent intensity (I_t) was continuously monitored at 510 nm (excitation at 460 nm) and the vesicles were lysed with Triton aqueous solution (20%, 10 μL) at the 5-minute mark. The fluorescent intensity was monitored continuously until it reached a stable value (I_∞). Subsequently, the gathered data were normalized to reflect the fractional fluorescence change, calculated as $(I_t\text{-}I_0)/(I_\infty\text{-}I_0)$, where I_0 represents the initial intensity.

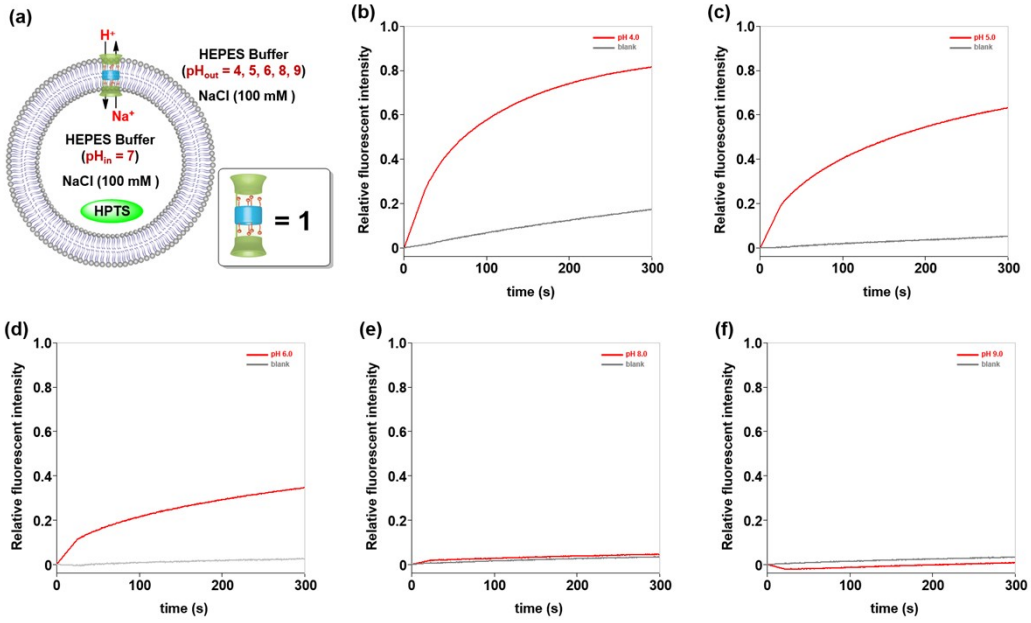


Figure S23. (a) Schematic representation of the HPTS assay; Changes in the fluorescence intensity of HPTS (λ_{ex} = 460 nm, λ_{em} = 510 nm) in vesicles with time after the addition of compound **1** at varying external pH (b) pH 4.0, (c) pH 5.0, (d) pH 6.0, (e) pH 8.0, (f) pH 9.0.

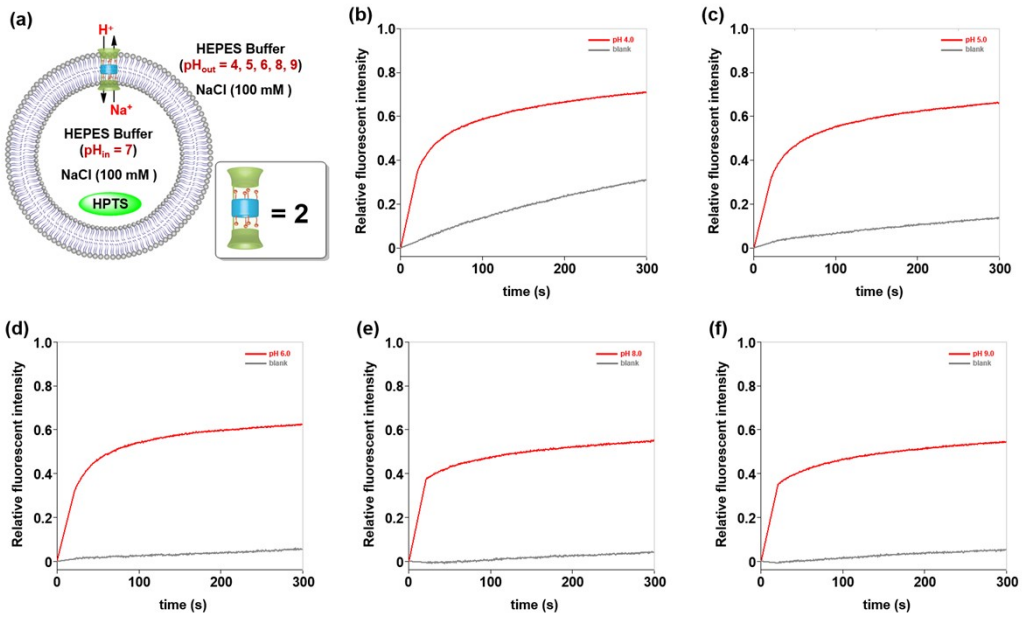


Figure S24. (a) Schematic representation of the HPTS assay; Changes in the fluorescence intensity of HPTS (λ_{ex} = 460 nm, λ_{em} = 510 nm) in vesicles with time after the addition of compound **2** at varying external pH: (b) pH 4.0, (c) pH 5.0, (d) pH 6.0, (e) pH 8.0, (f) pH 9.0.

8. Ion transport states switch studies with HPTS assay:

(a) Preparation of HPTS containing large unilamellar vesicles (LUVs): The vesicles (2 mL, HEPES (10 mM), NaCl (100 mM), pH = 7.0) were prepared as described in Section 3.

(b) Fluorescent experiments: The prepared vesicle suspension (13.3 mM, 30 μL) were suspended in the external medium consisting of HEPES buffer solution (2 mL, HEPES (10 mM), NaCl (100 mM), pH = 8.0) to create a pH gradient for ion transport study. A required channel concentration was achieved by adding of compound **1** (64 mM, 5 μL), **2** (16 mM, 5 μL) or gA (gramicidin A, 1 mM, 5 μL) in DMSO with gentle stirring at the 0.5-minute mark. The pH value of the external medium was adjusted to switch between 6 and 8 by the controlled addition of HCl or NaOH solution at 5-minute intervals. Fluorescent intensity (I_t) was continuously monitored at 510 nm (excitation at 460 nm) and the vesicles were lysed with Triton aqueous solution (20%, 10 μL) at the 30.5-minute mark. The fluorescent intensity was monitored continuously until it reached a stable value (I_∞). Subsequently, the gathered data were normalized to reflect the fractional fluorescence change, calculated as $(I_t\text{-}I_0)/(I_\infty\text{-}I_0)$, where I_0 represents the initial intensity.

9. Procedures for the confocal laser scanning microscopy (CLSM) experiment:[4]

(a) Cell culture: Chinese Hamster Ovary (CHO) cells were grown at 5% CO_2 and 37 °C in the complete DMEM/F-12 medium supplemented with 10% (v/v) of fetal bovine serum.

(b) Procedures for labeling of channel 2: To a solution of **1** (10 mg, 0.003 mmol) in dry DMSO (3 mL) was added 6-((2-aminoethyl) amino)-1-oxo-1H-phenalene-2,3-dicarbonitrile (4.6 mg, 0.015 mmol), 4-dimethylaminopyridine (DMAP, 7.8 mg, 0.06 mmol), and 1-(3-dimethylaminopropyl)-3 ethylcarbodiimide hydrochloride (EDCI, 2 mg, 0.006 mmol). The mixture was stirred at room temperature for 24 h. 10 portions of water was added to the reaction mixture, and the obtained mixture

was freeze-dried. The residues washed with aqueous HCl solution (2 %, 5 mL) and saturated aqueous NaCl to give the crude product. Then the crude product was prufied by HPLC to yield OPD-labeled compound **1** as red solid. The HR-MS of this compound demonstrated that one OPD groups were labeled onto compound **1**. HRMS: calcdfor $C_{148}H_{183}N_{11}O_{84}$ $[M+CH_3CN+2H]^{2+}$: 1729.5325, found 1729.6061.

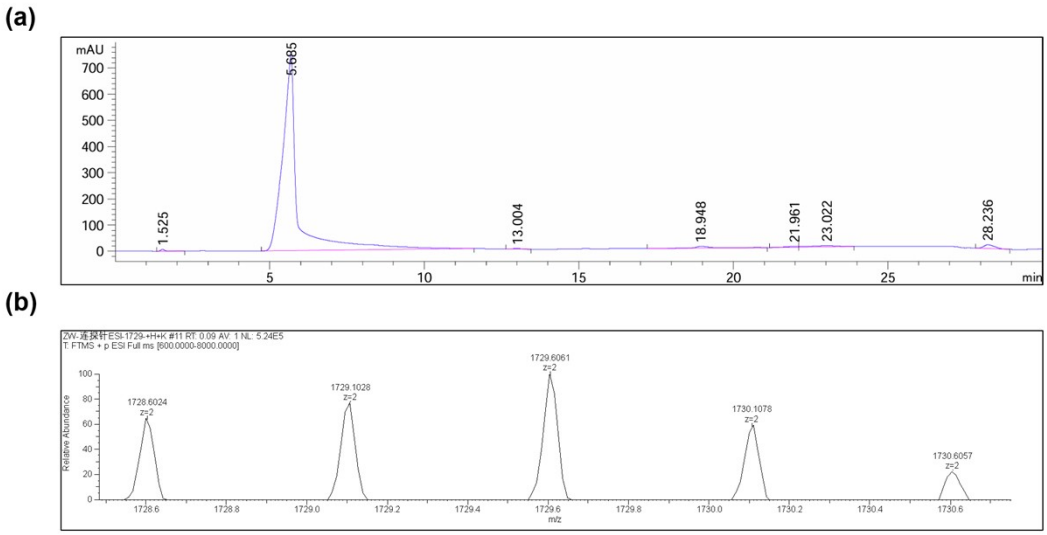


Figure S25. (a) HPLC analytic trace of OPD-labeled compound **1**, (b) HR-MS of OPD-labeled compound **1**.

(c) Confocal laser scanning microscopy (CLSM) imaging experiments: CHO cells were seeded in 24-well plate with a cover slip on top. Following adhesion, cells were incubated with probe-labeled compounds in serum-free DMEM/F12 medium (pH-adjusted to 5.8 or 7.2) for 1 hour at 37°C. After washed with PBS buffer for three times to remove free OPD-labeled channel **1**, the CHO cells were subjected to CLSM imaging (FV1200MOE, Olympus, Japan) and the fluorescence was excited by a 559 nm laser and the emission was collected 570-662 nm.

10. Molecular dynamics simulations:

(a) Model Preparation: The initial conformation of the protonated and deprotonated channel molecule was derived from our previous study, with appropriate modifications made to the structure accordingly.[5] The restrained electrostatic potential (RESP) charges were computed at the B3LYP(D3)/def2-TZVP level using the ORCA[6,7] software package (version 6.0). The complete ion channel-lipid membrane system was built using the PACKMOL-Memgen software,[8] where the lipid bilayer consisted of 1,2-dioleoyl-sn-glycero-phosphocholine (DOPC) molecules, and the ionic strength was adjusted to 1.0 mol/L KCl. The TLEaP program was used to assign force field parameters: GAFF[9] was applied to the ion channel, the TIP3P was used for water molecules,[10] and Lipid21was employed for DOPC lipids.[11] To avoid boundary effects in the molecular dynamics (MD) simulations, periodic boundary conditions (PBC) were implemented.

(b) Molecular dynamics simulations: Molecular dynamics (MD) simulations were performed using the GPU-accelerated PMEMD module in the AMBER24 software package.[12,13] Both systems followed a standardized simulation protocol. Initially, energy minimization was conducted in four sequential steps:

(1) Minimization of water molecules with the membrane atoms and ion channel atoms restrained by a force constant of 25 kcal/mol^{-1}·Å^{-2}; (2) Further water minimization with restraints of 5 kcal/mol^{-1}·Å^{-2} on the membrane atoms and ion channel atoms; (3) Minimization of the membrane and ion channel atoms with restrained of 5 kcal/mol^{-1}·Å^{-2} force constant; (4) Unrestrained minimization of the entire system. Subsequently, the system was heated in two stages: (1) A short (5 ps) NVT heating from 0 K to 100 K with restraints on the protein and the membrane, to initialize the system velocities; (2) A longer (200 ps) NPT heating from 100 K to 300 K with restraints on the protein, allowing the simulation box dimensions to equilibrate to the system shape. After heating, a 5 ns MD simulation was conducted under the NPT ensemble to equilibrate system density. Finally, under conditions of 300K and 1.0 atm, MD simulations were carried out in the NVT ensemble with a time step of 1fs, extending over 100 ns. Temperature was controlled using a Langevin thermostat at 300 K,[14] and pressure was regulated with a Berendsen borastat at 1.0 atm.[15] All bonds involving hydrogen atoms were constrained using the SHAKE algorithm.[16] The RMSD analysis indicates that all systems have reached equilibrium.

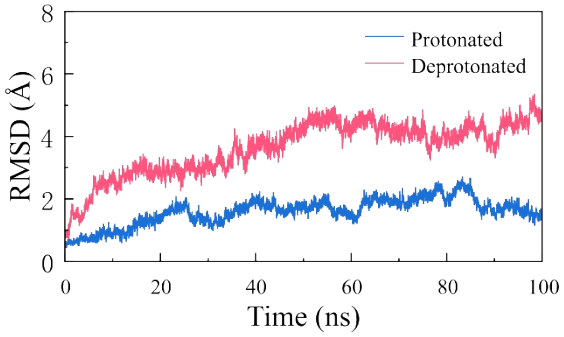


Figure S26. The RMSDs of molecular dynamics trajectories for all the systems.

(c) Potentials of mean force (PMF) simulation: The equilibrated conformation was extracted from the production trajectory of the classical molecular dynamics (MD) simulations and used as the initial structure for the constant-velocity steered molecular dynamics (SMD) simulations. In this step, the translocation coordinate (Z) was defined as the axis perpendicular to the bilayer surface. During the SMD simulations, K^+ and Cl^- ions were independently pulled along the channel axis at a constant velocity of 2 Å/ns. The spring constant connecting the K^+ ion and the virtual atom was set to 5 kcal·mol^{-1}·Å^{-2}. To prevent overall drift of the system, the backbone atoms of α-CD were restrained along the Z direction (i.e., the pulling direction) with the same force constant. The potentials of mean force (PMF) along the channel axis were calculated using the umbrella sampling method. The simulations were divided into 60 windows covering the entire length of the channel, and each window was subjected to a 5-ns production run, yielding a total simulation time of 300 ns. The same simulation protocol was applied for the Cl^- ion.

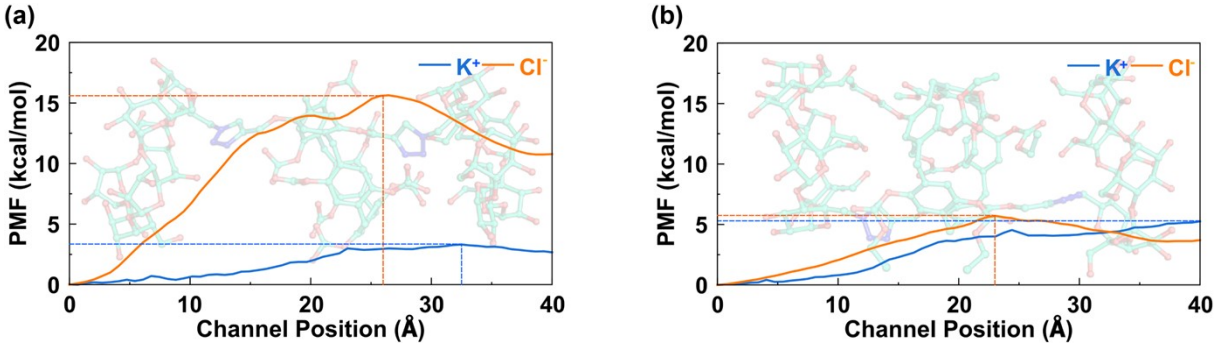


Figure S27. Potential of the mean force (PMF) for K^+ and Cl^- as a function of the channel position of the ions (a) **1** and (b) **2**.

11. Procedures for stop-flow experiments:[17]

(a) Preparation of HPTS containing LUVs: The solution of EYPC (20 mg) in $CHCl_3$ (10.0 mL) was transferred to a round-bottomed flask and then slowly evaporated under reduced pressure, and the resulting thin film was dried under high vacuum for 3 h. The lipid film was hydrated at 40 °C for 2 h with HEPES buffer solution (2 mL, HEPES (10 mM), NaCl (100 mM), pH = 6.0 or 8.0) containing 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS, 0.1 mM), with gentle vortexing after every 0.5 h to ensure complete hydration of the lipid film, resulting in 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 extruded nine times through polycarbonate membrane (0.2 μm) and then dialyzed with membrane tube (MWCO = 14000) against the same HEPES buffer solution (200 mL, without HPTS) for nine times to remove un-entrapped HPTS and diluted with the HEPES buffer solution to yield 20 mL lipid stock solution ([lipid] = 1.33 mM).

(b) Stop-flow experiments: The solution of the channel to be tested in DMSO (32 mM, 14 μL) was added to the vesicle solution (2.0 mL, pH = 6.0 or 8.0) and the mixture was incubated for 10 min. Subsequently, this mixture was transferred to the reservoir of the instrument and place acidic or alkaline buffer solutions (2.0 mL, HEPES (10 mM), NaCl (100 mM), pH = 6.0 or 8.0) in another reservoirs. The fluorescence intensity of HPTS was monitored when the vesicle solution (80 μL) was mixed with the buffer solution (80 μL) within the instrument, using an excitation wavelength of 460 nm and collecting emission at 510 nm through a 480 nm cutoff filter. The experiment was repeated five times, and the signals were averaged. A blank experiment was conducted by using pure DMSO. The data were acquired with an interval of 12.5 μs and dead time of 1.2 ms. For each measurement, data were collected by averaging 3 individual runs.

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