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Self-assembly of Crown Ether-based Amphiphiles for Constructing Synthetic Ion Channels: The Relationship between Structure and Transport Activity

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Supplementary figures

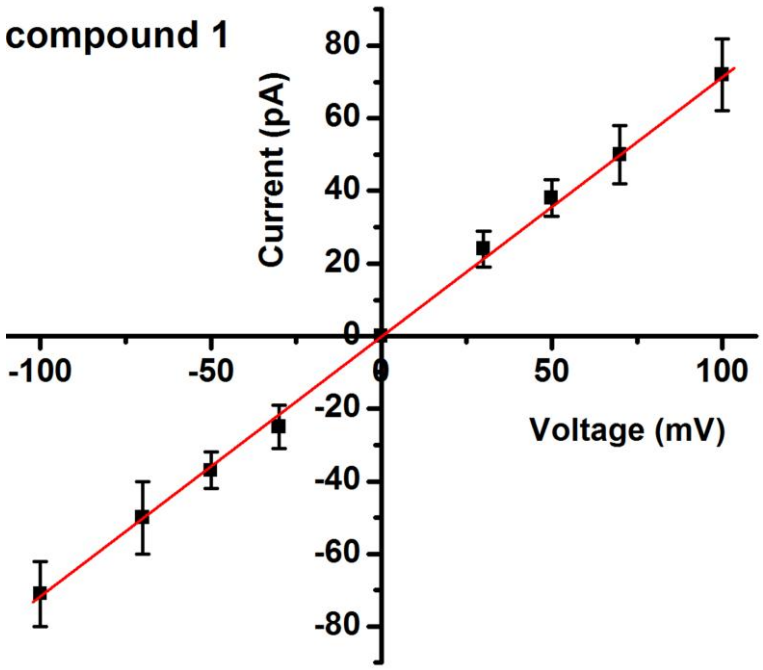

Figure S1. The linear current–voltage curve corresponding to the compound **1** (0.8 μM), the conductance was calculated to be 0.8 ± 0.1 nS.

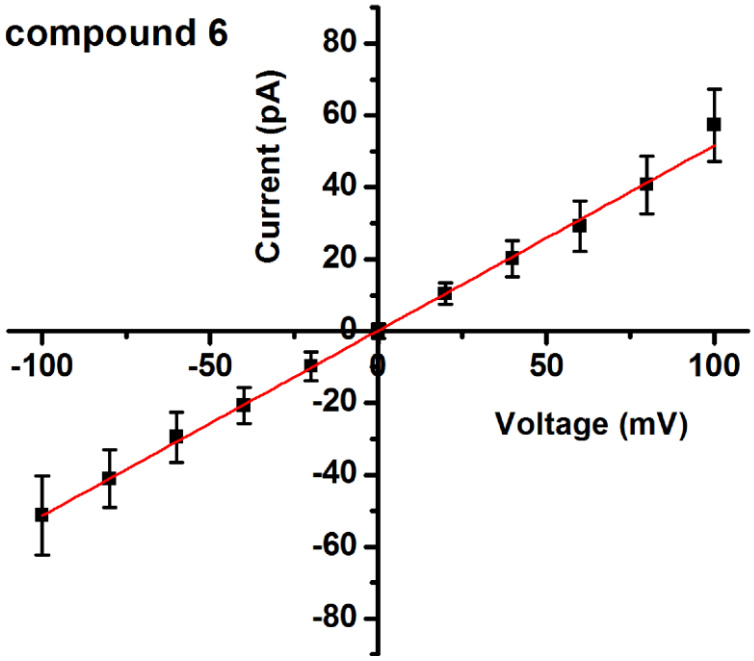

Figure S2. The linear current–voltage curve corresponding to the compound **6** (2.5 μM), the conductance was calculated to be 0.48 ± 0.1 nS.

General materials

All starting materials were obtained from commercial suppliers and were used without further purification unless otherwise stated. All air- or moisture-sensitive reactions were performed using oven-dried or flame-dried glassware under an inert atmosphere of dry argon. Air- or moisture-sensitive liquids and solutions were transferred via syringe. Tetrahydrofuran (THF) was distilled from sodium benzophenone; dichloromethane was distilled from calcium hydride; triethylamine (TEA) was redistilled and stored over KOH pellets prior to use. Egg yolk phosphatidylcholine (EYPC) was obtained from Avanti Polar lipids as a solution in chloroform (25 mg/mL). 8-hydroxy-1, 3, 6-pyrenetrisulfonate (HPTS) and Trixon-100 were obtained from Sigma-Aldrich and used without further purification.

Characterizations

Proton and carbon magnetic resonance spectra (1H, 13C NMR) were recorded on a Bruker Avance 500 (400 MHz) spectrometer. Chemical shifts were reported in parts per million (ppm) downfield from the Me4Si resonance which was used as the internal standard when recording 1H NMR spectra. Mass spectra were recorded on a Micromass GCTTM and a Micromass LCTTM. Fluorescence measurements were performed with a Varian Cary Eclipses fluorescence spectrometer equipped with a stirrer and a temperature controller (measurements at 25 ℃ unless otherwise noted). Transmission electron microscopy (TEM) was measured on a JEOL JEM-1400 at 120 kV. The TEM

sample of the self-assembly in water was prepared by dropping the trans-sample THF solution into 10 mM HEPES solution and freeze-drying in vacuum overnight. The cis-sample was obtained by irradiating the trans-sample in HEPES solution with 365 nm light (8 mW/cm2) for 10 min, which was frozen immediately in liquid nitrogen and submitted to freeze-drying in vacuum overnight. Scanning electron micrograph (SEM) was measured on a JSM-6360LV. A Mini-Extruder used for the preparation of large unilamellar vesicles (LUVs) was purchased from Avanti Polar lipids. The size of EYPC vesicles was determined using a Delsa™ Nano Submicron Particle Size and Zeta Potential Particle Analyzer (Beckman Coulter Inc., USA).

Preparation of large unilamellar vesicles (LUVs) and determination the transport activity of compounds with the HPTS assay were as same as the description in our previous report.38 Here, the mixture of EYPC and cholesterol (10:1, wt:wt) was used for the membrane of LUVs, the final concentration of the lipids in the experiments was 33 μM (assuming 100% of lipids were incorporated into liposomes), and the size of the vesicles was around 150 nm. In the time-dependent change in fluorescence intensity, 30 μL 0.5 M KOH was added at t = 50 s, 30 μL transporter in THF with different concentrations was added at t = 100 s, and 60 μL of 5% Triton X-100 aqueous solution was added at t = 350 s for final completed balance. The transmembrane activity of the compounds were determined by the change in ratiometric HPTS fluorescence intensity at 510 nm ($I_{ex,\,450}/I_{ex,\,405}$).

Patch-clamp measurements were performed with the Axon patch clamp workstation using DPhPC as the lipid memembrane38 and data analysis was performed using the Clampfit suite software (version 9.2; Axon Instruments, Foster City, CA) and OriginLab 8.0 (OriginLab Corporation, Northampton, MA, USA).

Synthesis of compounds 1-6

Compounds 23-20, 18 and 1 were synthesized as reported in our previous report.[1] Compounds 19,[2] 17,[3] 16-15,[4] 13,[5] 10[6] was synthesized by the same method as literature.

Compound 12

Compound 13 (0.49 g, 1 mmol) was dissolved in 15 mL dry dichloromethane under an atmosphere of dry argon, then 1-Chloro-N,N,2-trimethylpropenylamine (0.5 mL, 3.75 mmol) was added dropwise via syringe, the reaction mixture was stirred for 4 h at room temperature, and then the solvent and the excess 1-Chloro-N,N,2-trimethylpropenylamine were evaporated under reduced pressure to obtain the corresponding acid chloride. A solution of 21 (0.24 g, 1 mmol) and triethylamine (0.36 mL, 2.5 mmol) in 20 mL dry dichloromethane under an atmosphere of dry argon was placed in another 50 mL round bottomed flask. The flask was cooled with an ice–water bath, the corresponding acid chloride which was dissolved in 10 mL of dry dichloromethane was added dropwise via syringe, and the reaction mixture was

stirred overnight at room temperature. After the reaction was completed, the solvent was removed under vacuum, and then recrystallized from ethanol with chloroform to obtain 0.3 g bright orange solid. Yield: 42%. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.26 (d, J =8.8 Hz, 2H), 8.01 (q, J =8.8 Hz, 4H), 7.30 (d, J =8.8 Hz, 2H), 5.38 (d, J =4 Hz, 1H), 4.68 (m, 1H), 2.93 (t, J =6.8 Hz, 2H), 2.76 (t, J =6.8 Hz, 2H), 2.35 (d, J =7.6 Hz, 2H), 2.02-0.67 (m, 42H). The ^{13}C NMR spectrum was hard to obtain due to the bad solubility in most deuterium solvents. MS(EI): m/z: Calcd. For $C_{44}H_{58}N_2O_6^+$ $[M]^+$: 709.9. Found: 709.5.

Compound 9

Compound 10 (2 g, 7.3 mmol), N,N-Diisopropylethylamine (3 mL, 18.2 mmol) were dissolved in 50 mL dry dichloromethane, 4-toluene sulfonyl chloride (2.1 g, 11 mmol) was dissolved in 20 mL dry dichloromethane and added dropwise to above solution at 0 ˚C for 0.5 h. The reaction mixture was stirred for 12 h at room temperature, after the reaction was completed, the mixture was washed several times with saline solution and water, dried over $MgSO_4$ and concentrated in vacuum. The product was purified by chromatography (SiO_2) eluting with ethyl acetate/ petroleum ether (1/6) to obtain compound 9 as a faint yellow oil (2.1g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.80 (d, J = 8.3 Hz, 2H), 7.34 (d, J = 8.1 Hz, 2H), 4.17 (t, J = 6.8 Hz, 2H), 3.69 (t, J = 6.8 Hz, 2H), 3.60-3.55 (m, 2H), 3.53-3.49 (m, 2H), 3.41 (t, J = 6.8 Hz, 2H), 1.60-1.51 (m, 2H), 1.36-1.20 (m, 16H), 0.88 (t, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 144.8, 133.1, 129.8, 128.0, 71.6, 70.8, 70.0, 69.3, 68.7, 31.9, 29.7, 29.6, 29.5,

29.4, 26.1, 22.7, 21.7, 14.1. MS(ESI): m/z: Calcd. For $C_{23}H_{40}O_5SNa^+$ $[M+Na]^+$: 451.2. Found: 451.2.

Compound 8

Compound 9 (1.1 g, 2.7 mmol), Methyl 4-hydroxybenzoate (0.45 g, 3 mmol) and Cesium carbonate (1.1 g, 3.3 mmol) was mixed with 40 mL dry dimethyl formamide (DMF), then the mixture was reacted at 110 ˚C for 12 h. After cooling to room temperature, the mixture was added to 200 mL ice water, filtered and the filter cake was air-dried. The crude product was purified by chromatography (SiO_2) eluting with ethyl acetate/ petroleum ether (1/4) to obtain compound 8 as a colorless oil (0.9 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.98 (d, J = 8.9 Hz, 2H), 6.93 (d, J = 8.9 Hz, 2H), 4.19 (t, J = 6.7 Hz, 2H), 3.92-3.85 (m, 5H), 3.72 (t, J = 6.8 Hz, 2H), 3.61(t, J = 6.8 Hz, 2H), 3.46 (t, J = 6.8 Hz, 2H), 1.65-1.52 (m, 2H), 1.34-1.21 (m, 16H), 0.88 (t, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 166.9, 162.6, 131.7, 131.6, 122.7, 115.8, 114.2, 71.6, 71.0, 70.1, 69.6, 67.6, 51.9, 31.9, 29.7, 29.6, 29.5, 29.4, 26.1, 22.7, 14.1. MS(ESI): m/z: Calcd. For $C_{24}H_{40}O_5Na^+$ $[M+Na]^+$: 431.3. Found: 431.3.

Compound 7

Compound 8 (0.7 g, 1.7 mmol) was dissolved in 30 mL methanol/THF (1/1), NaOH (0.17 g, 4.3 mmol) was dissolved in 3 mL water and dropwise added to the solution, the reaction was stirred at 50 ˚C for 5 h. After reaction completed, removing the solvent under vacuum, the obtained soild was dissolved in 20 mL ice water and

adjusted pH to 2 using hydrochloric acid, filtered and washed with 100 mL water, and finally dried in a vacuum. The compound 7 (0.56 g, 84%) was obtained as white solid. ^{1}H NMR (400 MHz, DMSO), δ (ppm): 12.57 (s, 1H), 7.88 (d, J = 8.8 Hz, 2H), 7.02 (d, J = 8.8 Hz, 2H), 4.16 (t, J = 6.7 Hz, 2H), 3.76 (t, J = 6.8 Hz, 2H), 3.58 (t, J = 6.8 Hz, 2H), 3.48 (t, J = 6.8 Hz, 2H), 1.49-1.40 (m, 2H), 1.30-1.18 (m, 16H), 0.85 (t, J = 6.7 Hz, 4H). ^{13}C NMR (100 MHz, DMSO), δ (ppm): 166.9, 162.1, 131.3, 123.0, 114.2, 70.3, 69.9, 69. 5, 68.7, 67.4, 31.3, 29.2, 29.0, 28.8, 28.7, 25.6, 22.1, 13.9. MS(ESI): m/z: Calcd. For $C_{23}H_{38}O_5Na^+$ $[M+Na]^+$: 417.3. Found: 417.2.

Compound 2

Compound 2 was synthesized from compound 20 and aniline by the same method as for compound 1.[1] ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.04 (d, J = 2.4 Hz, 1H), 8.00 (q, J = 8.6 Hz, 4H), 7.90 (dd, J = 8.7, 2.4 Hz, 1H), 7.85 (s, 1H), 7.67 (d, J = 7.9 Hz, 2H), 7.40 (t, J = 7.9 Hz, 2H), 7.18 (t, J = 7.4 Hz, 1H), 7.06 (d, J = 8.8 Hz, 1H), 4.14 (t, J = 6.5 Hz, 2H), 1.94-1.85 (m, 2H), 1.53-1.48 (m, 2H), 1.44-1.21 (m, 16H), 0.88 (t, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 165.0, 157.5, 154.4, 146.5, 137.8, 136.4, 129.2, 128.1, 125.5, 124.8, 124.1, 123.3, 123.0, 120.3, 112.5, 69.6, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.0, 25.9, 22.7, 14.1. MS (HR-ESI): m/z: Calcd. For $C_{31}H_{40}N_3O_2^+$ $[M+H]^+$: 486.3115. Found: 486.3120.

Compound 3a

Compound 17 (0.18 g, 0.41 mmol) was disolved and refluxed in dry toluene (10 mL) under the protection of Ar gas. After refluxing for 2 h, compound 22 (0.135 g, 0.41

mmol) in 10 mL toluene was added in above mixture and kept refluxing overnight. Finally, the obtained mixture was evaroprated in vaccum and purified by flash chromatography (SiO_2) eluting with MeOH/DCM (10%) to obtain compound 3a as a yellowish solid (150 mg, 50% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 9.13 (s, 1H), 8.73 (s, 1H), 7.83 (t, J = 8.8 Hz, 4H), 7.65 (d, J = 8.8 Hz, 2H), 7.22 (s, 1H), 7.10 (d, J = 9.2 Hz, 2H), 6.88 (s, 2H), 4.06 (m, 6H), 3.79 (m, 4H), 3.61 (s, 12H), 1.75 (m, 2H), 1.43 (m, 2H), 1.24 (s, 16H), 0.86 (t, J = 6.4 Hz, 3H). ^{13}C NMR (100MHz, $CDCl_3$), δ (ppm): 161.2, 153.6, 147.7, 147.5, 146.9, 142.9, 142.2, 133.6, 124.4, 123.6, 118.7, 114.7, 112.9, 112.8, 111.9, 69.7, 69.6, 69.5, 69.0, 68.8, 68.4, 67.6, 67.0, 31.9, 29.7, 29.6, 29.4, 29.3, 26.1, 22.7, 14.2. MS (HR-ESI): m/z: Calcd. For $C_{41}H_{58}N_4ONa^+$ $[M+Na]^+$: 757.4152; $C_{41}H_{58}N_4OK^+$ $[M+K]^+$: 773.3892. Found: 757.4153; 773.3934.

Compound 3b

To a solution of 15 (52 mg, 0.135 mmol), 4'-carboxybenzo-18-crown-6 (40 mg, 0.11 mmol) and 4-dimethylaminopyridine (21 mg, 0.168 mmol) in dried CH_2Cl_2 (10 mL), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (33 mg, 0.168 mmol) was added in portions at 0 ℃ under argon atmosphere. The mixture was stirred for 2h at room temperature. After evaporation of the solvent under reduced pressure, the mixture was purified by flash chromatography on silica gel, eluting with CH_2Cl_2 /MeOH = 100/2. Compound 3b was obtained as a yellow solid (56 mg, 71% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.94 (dd, J = 17.1, 8.2 Hz, 4H), 7.85 (d, J = 8.4 Hz, 1H), 7.68 (s, 1H), 7.34 (d, J = 8.1 Hz, 2H), 7.01 (d, J = 8.3 Hz, 2H), 6.94 (d, J = 8.4 Hz, 1H),

4.25 (s, 4H), 4.05 (t, J = 6.5 Hz, 2H), 4.01-3.92 (m, 4H), 3.79 (s, 4H), 3.72 (d, J = 12.9 Hz, 8H), 1.88-1.79 (m, 2H), 1.53-1.43 (m, 2H), 1.42-1.20 (m, 16H), 0.88 (t, J = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 164.7, 161.8, 153.6, 152.6, 150.3, 148.4, 146.8, 124.8, 124.8, 123.7, 122.4, 121.7, 114.7, 114.5, 112.0, 70.9, 70.9, 70.8, 70.7, 70.6, 70.5, 69.4, 69.3, 69.0, 68.8, 68.4, 31.9, 29.7, 29.6, 29.4, 29.20, 26.0, 22.7, 14.2. MS (HR-ESI): m/z: Calcd. For $C_{41}H_{56}N_2O_9Na^+$ $[M+Na]^+$: 743.3878; $C_{41}H_{56}N_2O_9K^+$ $[M+K]^+$: 759.3617. Found: 743.3881; 759.3675.

Compound 4a

Compound 4a was synthesized from compound 22 and 19 by the same method as for compound 1.[1] ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.13 (s, 1H), 8.02 (d, J = 8.1 Hz, 2H), 7.95 (d, J = 8.2 Hz, 4H), 7.48 (s, 1H), 7.07 (d, J = 8.6 Hz, 1H), 7.03 (d, J = 8.9 Hz, 2H), 6.84 (d, J = 8.7 Hz, 1H), 4.15 (dd, J = 12.7, 8.7 Hz, 4H), 3.91 (s, 7H), 3.81-3.65 (m, 13H), 1.25 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 161.6, 148.0, 146.0, 144.5, 134.9, 130.9, 127.8, 127.1, 127.0, 124.2, 121.8, 113.3, 113.2, 111.9, 106.0, 69.7, 68.6, 68.5, 68.2, 67.8, 54.6. MS (HR-ESI): m/z: Calcd. For $C_{30}H_{35}N_3O_8Na^+$ $[M+Na]^+$: 588.2316; $C_{30}H_{35}N_3O_8K^+$ $[M+K]^+$: 604.2056. Found: 588.2301; 604.2043.

Compound 4b

Compound 4b was synthesized from compound 22 and 18 by the same method as for compound 1.[1] ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):8.05 – 7.90 (m, 6H), 7.82 (s, 1H), 7.49 (s, 1H), 7.02 (d, *J* = 8.8 Hz,3H), 6.88 (d, *J* = 8.6 Hz, 1H), 4.19 (m, 4H), 4.05 (t, *J*

= 6.5 Hz, 2H), 3.94 (s, 4H), 3.83 – 3.65 (m, 12H), 1.90 – 1.76 (m, 2H), 1.54 – 1.43 (m, 2H), 1.40 – 1.18 (m, 24H), 0.88 (t, *J* = 6.6 Hz,3H). The ^{13}C NMR can't gained as the limited solubility in $CDCl_3$. MS (HR-ESI): m/z: Calcd. For $C_{45}H_{65}N_3O_8Na^+$ $[M+Na]^+$: 798.4669; $C_{45}H_{65}N_3O_8K^+$ $[M+K]^+$: 814.4409. Found: 798.4637; 814.4446.

Compound 4c

Compound 4c was synthesized from compound 22 and 12 by the same method as for compound 1.[1] ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.10 (m, 7H), 7.49 (s, 1H), 7.29 (d, J =8.8 Hz, 2H), 7.07 (d, J =8 Hz, 1H), 6.85 (d, J =8.4 Hz, 1H), 5.38 (d, J =3.6 Hz, 1H), 4.66 (m, 1H), 4.18 (d, J = 16 Hz, 4H), 3.91 (s, 4H), 3.75 (m, 12H), 2.93 (t, J = 6 Hz, 2H), 2.76 (t, J = 6.4 Hz, 2H), 2.35 (d, J =8 Hz, 2H), 2.02-0.67 (m, 42H). ^{13}C NMR (100MHz, $CDCl_3$), δ (ppm): 171.5, 170.7, 165.0, 154.1, 153.1, 150.1, 148.6, 145.4, 139.5, 136.7, 132.2, 128.4, 124.4, 123.0, 122.8, 122.3, 113.8, 113.2, 107.3, 74.7, 70.5, 69.5, 69.4, 68.9, 68.5, 56.7, 56.1, 50.0, 42.3, 39.7, 39.5, 38.0, 37.0, 36.6, 36.2, 35.8, 31.9, 31.9, 29.7, 29.5, 29.4, 28.2, 28.0, 27.8, 24.3, 23.8, 22.8, 22.6, 21.0, 19.3, 18.7, 11.9. MS(HR-ESI): m/z: Calcd. for $C_{60}H_{81}N_3O_{11}Na^+$ $[M+Na]^+$: 1042.5871. Found: 1042.5768.

Compound 5

Compound 5 was synthesized from compound 22 and tridecylic acid by the same method as for compound 1.[1] ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.70 (s, 1H), 7.37 (s, 1H), 6.89 (dd, J = 8.6, 2.2 Hz, 1H), 6.76 (dd, J = 8.5, 2.2 Hz, 1H), 4.11 (d, J = 3.1 Hz, 4H), 3.95-3.82 (m, 4H), 3.79-3.63 (m, 12H), 2.32 (t, J = 7.5 Hz, 2H), 1.74- 1.64

(m, 2H), 1.39-1.18 (m, 16H), 0.88 (t, J = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 171.6, 148.9, 145.2, 132.5, 114.5, 112.2, 106.9, 70.7, 69.6, 69.5, 69.4, 68.7, 37.6, 31.9, 29.6, 29.5, 29.4, 29.3, 29.2, 25.7, 22.7, 14.1. MS (HR-ESI): m/z: Calcd. For $C_{28}H_{47}NO_7Na^+$ $[M+Na]^+$: 546.3407; $C_{28}H_{47}NO_7K^+$ $[M+K]^+$: 562.3146. Found: 546.3448; 562.3090.

Compound 6

Compound 6 was synthesized from compound 22 and 7 by the same method as for compound 1.[1] ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.81 (d, J = 8.2 Hz, 2H), 7.71 (s, 1H), 7.46 (s, 1H), 6.99-6.95 (m, 3H), 6.86 (d, J = 8.6 Hz, 1H), 4.19 - 4.14 (m, 6H), 3.95 – 3.83 (m, 6H), 3.82 – 3.67 (m, 14H), 3.66 – 3.59 (m, 2H), 3.48-3.44 (m, 2H), 1.76 (s, 2H), 1.62-1.55 (m, 2H), 1.34 - 1.25(m, 16H), 0.87 (t, J = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 161.7, 149.4, 145.7, 132.3, 128.8, 127.3, 114.7, 114.6, 112.6, 107.3, 71.7, 71.0, 70.9, 70.8, 70.7, 70.1, 69.7, 69.6, 69.0, 67.6, 32.0, 29.7, 29.6, 29.50, 29.4, 26.1, 22.7, 14.1. MS (HR-ESI): m/z: Calcd. For $C_{39}H_{61}NO_{10}Na^+$ $[M+Na]^+$: 726.4193; $C_{39}H_{61}NO_{10}K^+$ $[M+K]^+$: 742.3933. Found: 726.4171; 742.3924.

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