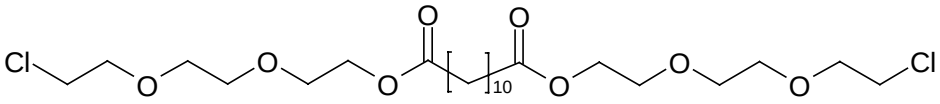
Supplemental materials to accompany: Transmembrane ion conductance by an acyclic bolaamphiphile, Thomas M. Fyles*, Chi-wei Hu, Ryan Knoy

1, 30-dichloro-10,21-dioxo-3, 6, 9, 22, 25, 28-hexaoxy-tricontane (**3)**



Dodecanedioyl dichloride (3.96 g, 14.8 mmol) and Et_3N were dissolved in THF (10 m L) separately and were simultaneously added to a stirred solution of 2-[2-(2-chloroethoxy)ethoxy]ethanol (5 g, 29.7 mmol) in THF (250 mL). The reaction was allowed to proceed overnight and the THF was removed under reduced pressure. The crude product was purified by column chromatography using Et_2O/hexanes (5:1) as eluant to give 4.29 g (56% yield) of **3** as clear oil. ^{1}H NMR ($CDCl_3$): δ 4.14 (t, 4H), 3.59 (m, 20H), 2.24 (t, 4H), 1.53 (t, 4H), 1.19 (m, 12H); ^{13}C NMR ($CDCl_3$): δ 24.8, 29.0, 29.2, 34.1, 42.6, 63.2, 69.2, 70.6, 71.3, 173.7. +LSIMS (mNBA) *m/z* (%): 531.3 (50). Exact mass (+LSIMS): Calculated for $C_{24}H_{45}O_8Cl_2^+$, 531.2491; Found. 531.2490.

Partial conversion to the diiodide: To a refluxed solution of NaI (5.8 g, 38.65 mmol) in acetone (30mL) was added compound **3** (2g, 3.86 mmol). The reaction mixture was refluxed for 6 hrs and the acetone was removed under reduced pressure. The dark yellow oil was extracted with CH_2Cl_2 and excess NaI was removed by filtering through an aluminum oxide column. ^{1}H NMR analysis indicated that this resultant product was a mixture of chloride and iodide in a ratio of 8:92. This mixture was used without further purification.

Figure S1: ^{1}H NMR spectrum of **3**

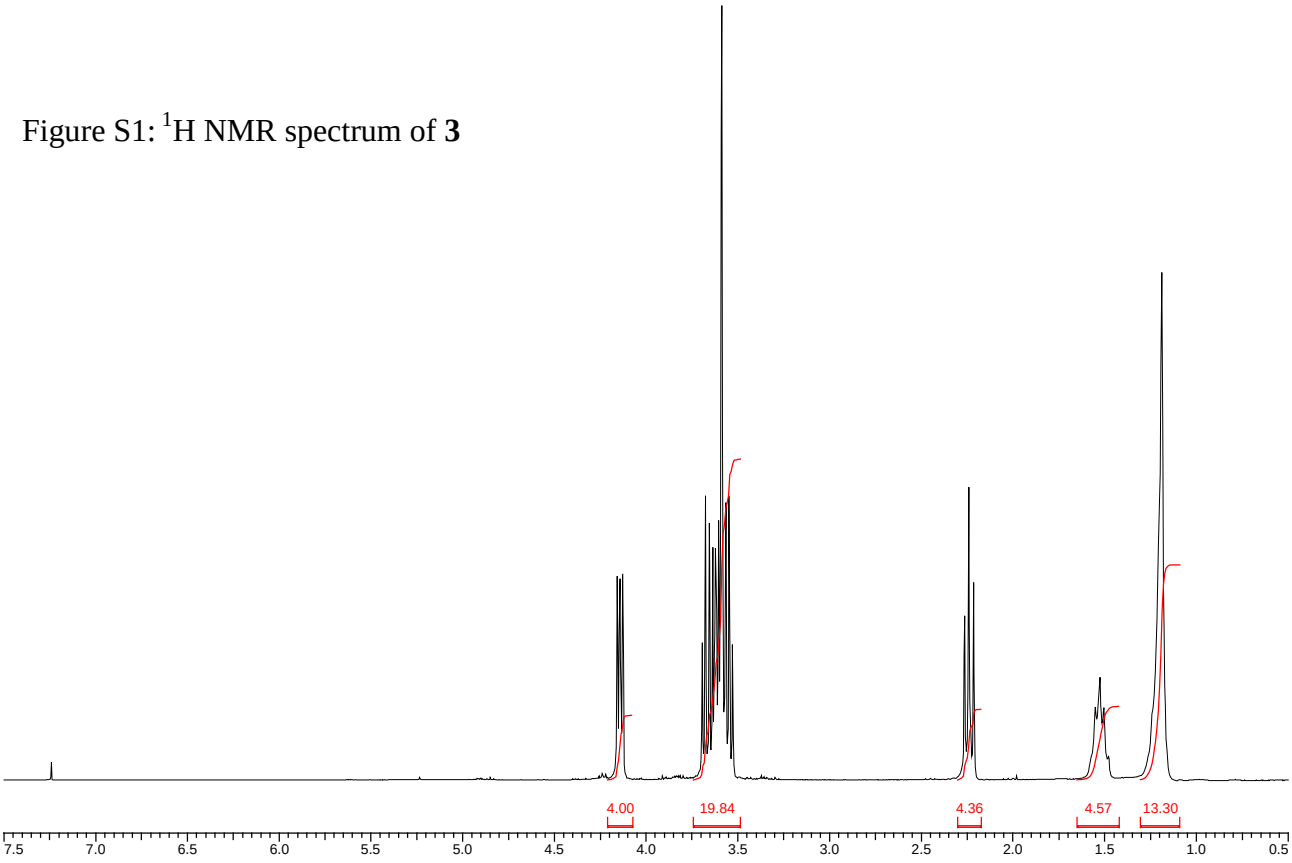
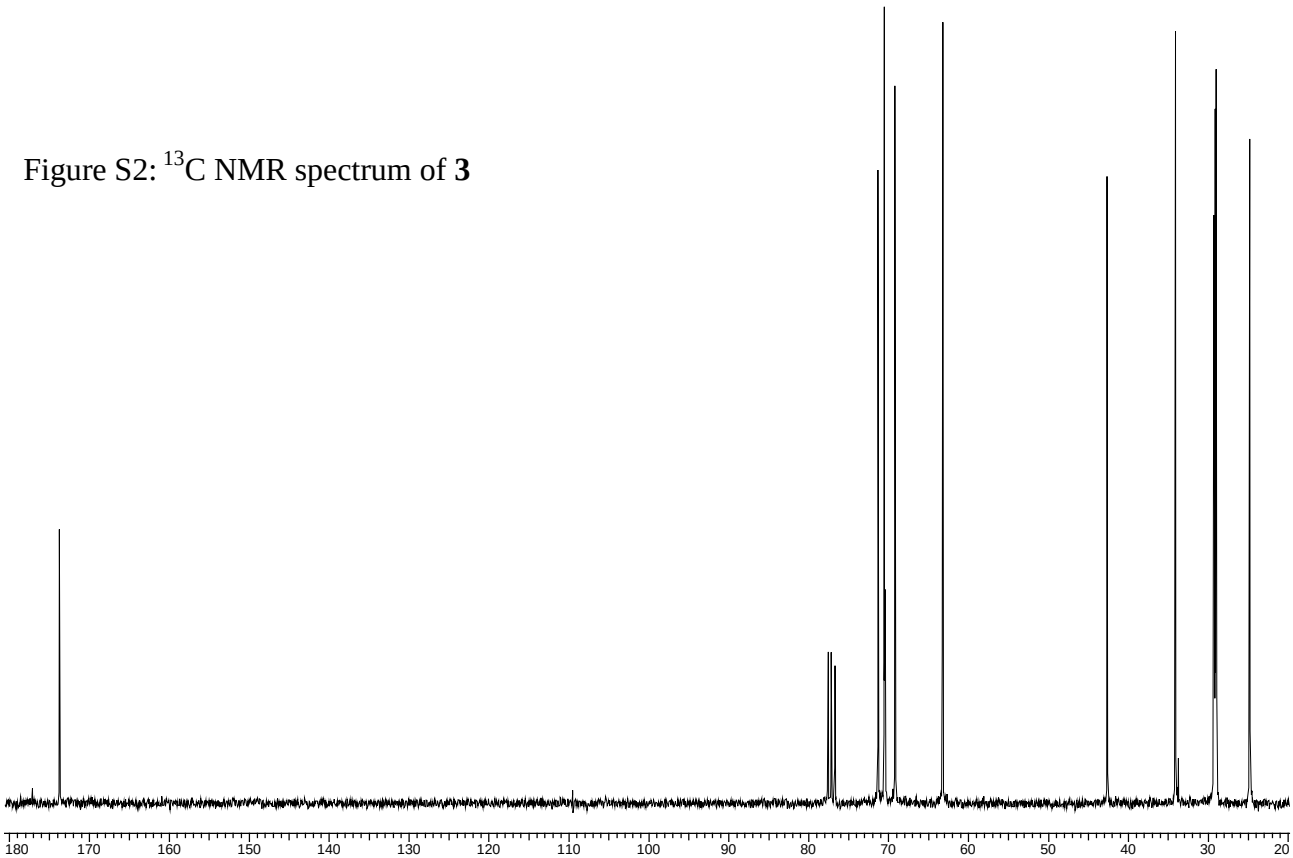
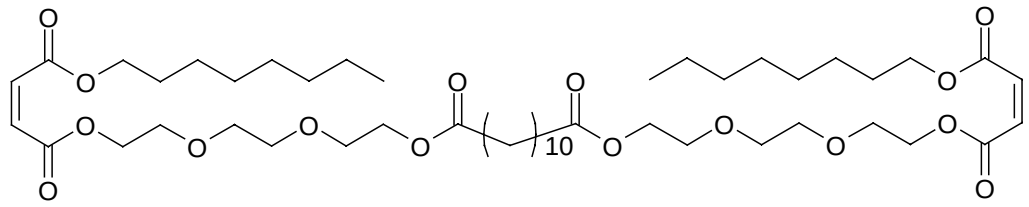


Figure S2: ^{13}C NMR spectrum of **3**



9,14,17,20,23,36,39,42,45,50-decaoxy-10,13,24,35,46,49-hexaoxo-octapentacontan -11,47-diene (**5**)



The iodo-chloro mixture derived from compound **3 (**1.69 mmol total) was added to a stirred solution of **4** (0.77 g, 3.37 mmol; prepared from maleic anhydride and 1-octanol) and tetramethyl ammonium hydroxide (Me_4NOH, 0.61 g, 3.37 mmol) in 30mL DMSO at 60°C under nitrogen. The reaction was allowed to proceed overnight and the DMSO was removed under reduced pressure. The solid product was triturated with THF, filtered, and the solvent removed to give **5** (1.37 g , 90%) as pale yellow oil. ^{1}H NMR δ: 6.21 (d, *J*=1.47 Hz, 4H), 4.29 (t, 4H), 4.20~4.10 (m, 8H), 3.71~3.61 (m, 16H), 2.27 (t, 4H), 1.70~1.50 (m, 8H), 1.40~1.15 (m, 32H), 0.83 (t, 6H); ^{13}C NMR δ 173.77, 165.23, 165.15, 130.18, 129.45, 70.54, 70.47, 69.22, 68.83, 65.45, 64.18, 63.28, 34.14, 31.73, 29.35, 29.21, 29.14, 29.08, 28.38, 25.81, 24.85, 22.59, 14.06. +LSIMS (mNBA) M+H (%): 915.6 (65). Exact mass (+LSIMS): Calculated for $C_{48}H_{83}O_{16}^{+}$, 915.568; Found, 915.571

Figure S3: ^{1}H NMR spectrum of **5**

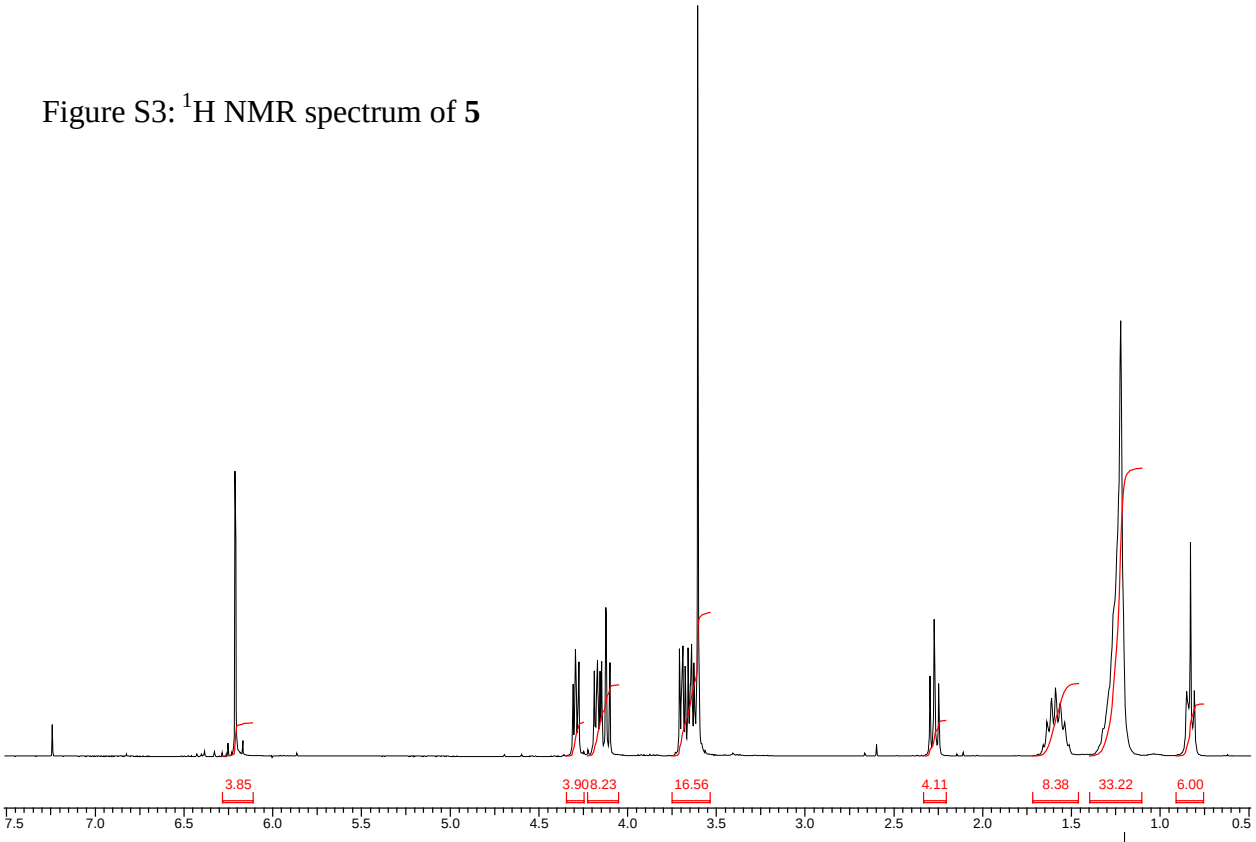
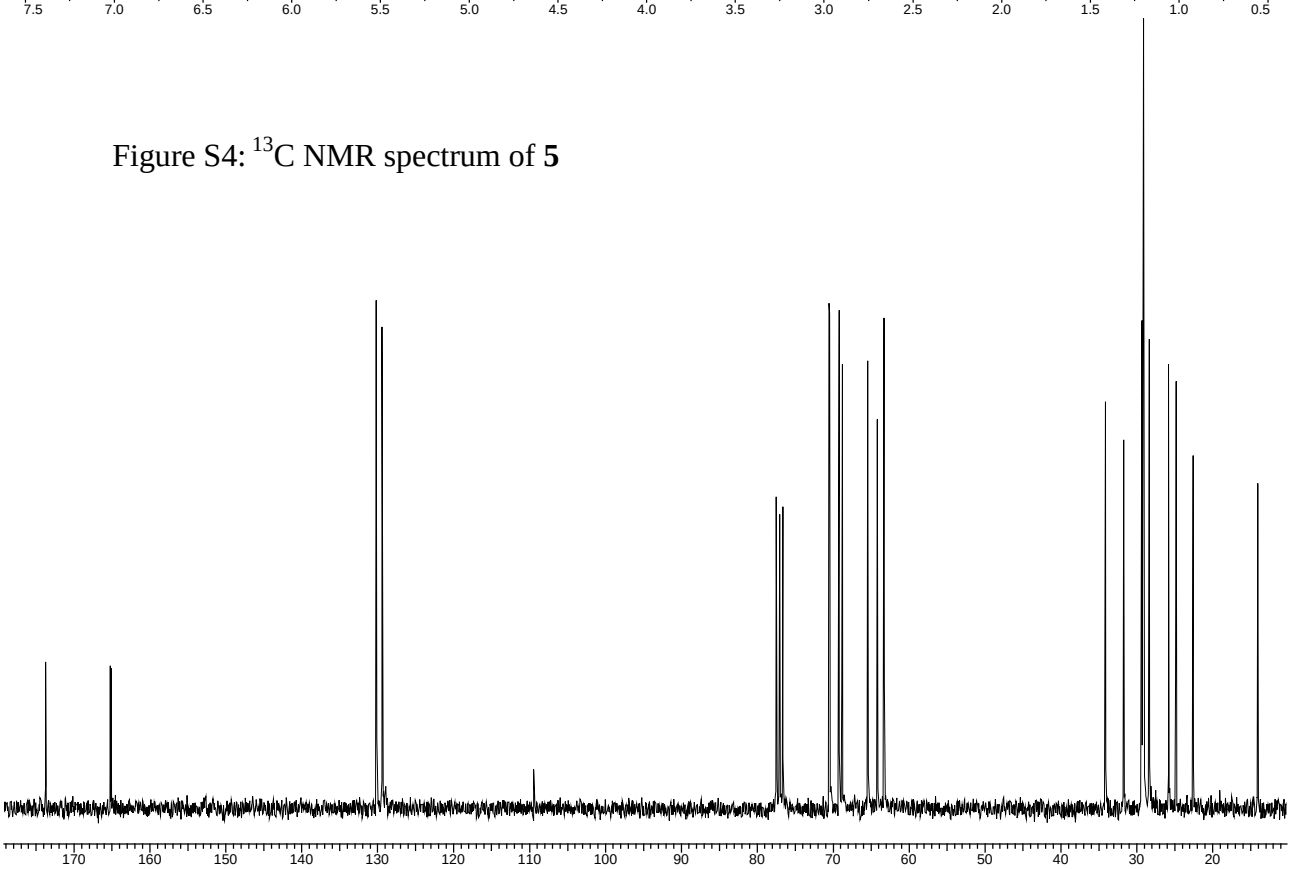
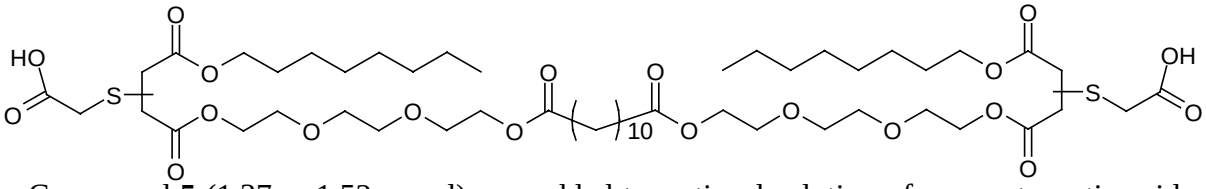


Figure S4: ^{13}C NMR spectrum of **5**



11 or 12 ,47 or 48-bis(carboxymethylmercaptyl)-9,14,17,20,23,36,39,42,45,50-decaoxy-10,13,24,35,46,49-hexaoxo-octapentacontane (**2**)



Compound **5** (1.37 g, 1.52 mmol) was added to a stirred solution of mercaptoacetic acid (0.56 g, 6.08mmol) and tetramethyl piperidine (0.86 g, 6.08 mmol) in THF (150mL) at 60EC. The reaction was allowed to proceed for 4 days. The THF was removed under reduced pressure and the resultant pale yellow oil was extracted with CH_2Cl_2 (30 mL) and washed with 1M HCl (2×100mL) and water (2×50 mL). The organic layer was dried over Na_2SO_3 and was identified as **2** (1.67 g, quantitative). ^{1}H NMR δ 4.30~4.38 (m, 1H), 4.18 (m, 7H), 4.13~4.00 (m, 4H), 3.84~3.78 (m, 2H), 3.71~3.58 (m, 16H), 3.55~3.48 (m, 2H), 3.41~3.30 (m, 2H), 3.00~2.89 (m, 2H), 2.76~2.63 (m, 2H), 2.27 (t, 4H), 1.50~1.60 (m, 8H), 1.12 (m, 32H), 0.82 (t, 6H); ^{13}C δ 174.0, 173.9, 173.2, 172.4, 171.4, 171.1, 170.4, 170.2, 70.5, 70.4, 70.1, 69.2, 69.0, 68.9, 65.9, 65.3, 64.7, 64.1, 63.2, 63.0, 42.0, 41.7, 36.1, 35.9, 34.2, 34.0, 31.7, 29.3, 29.2, 29.1, 29.1, 28.5, 25.7, 24.8, 22.6, 14.1.

-LSIMS (mNBA) M-H (%):1097.5. Calculated for $C_{52}H_{90}O_{20}S_2$: C, 56.81%; H, 8.25%; S, 5.83%. Found: C, 56.38%; H, 8.37%; S, 6.05%

Figure S5: ^{1}H NMR spectrum of **2**

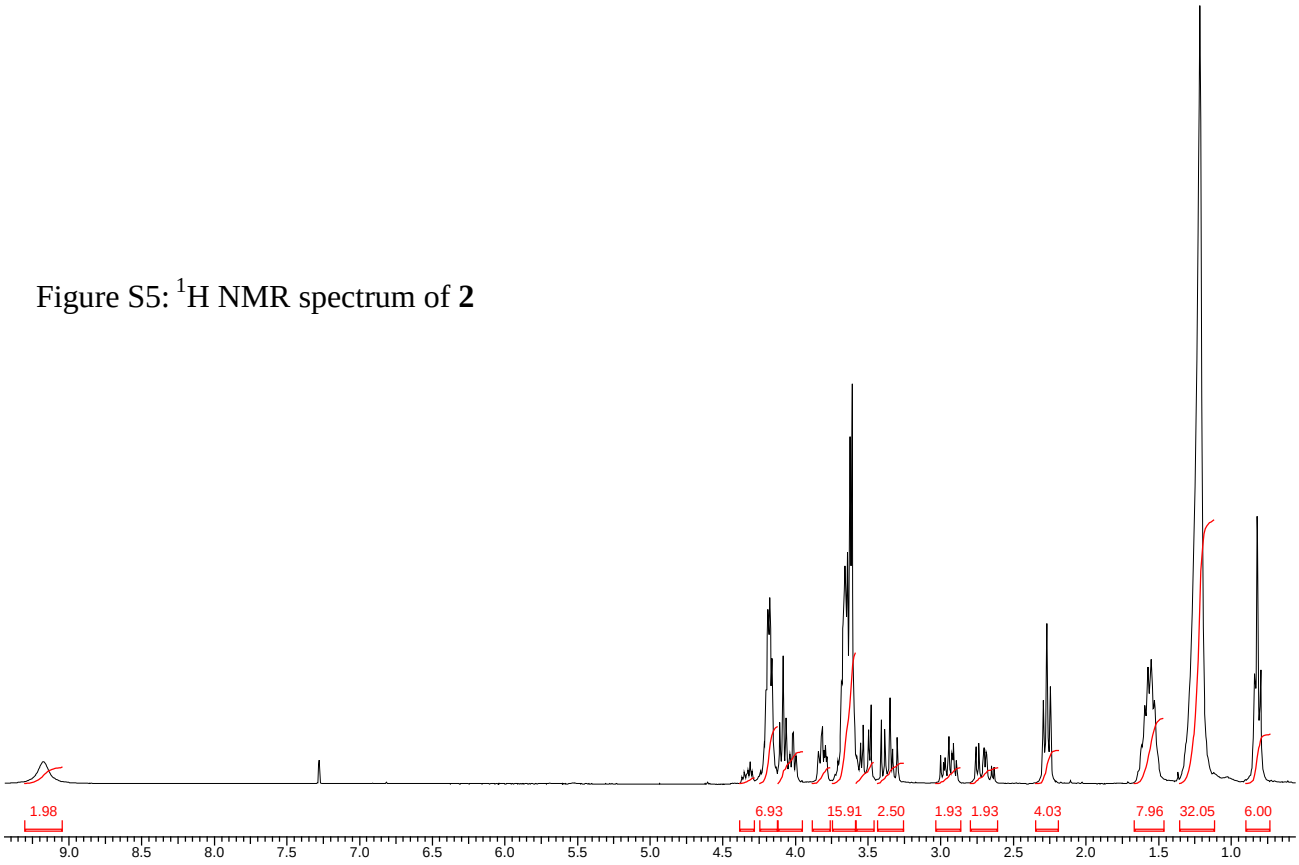
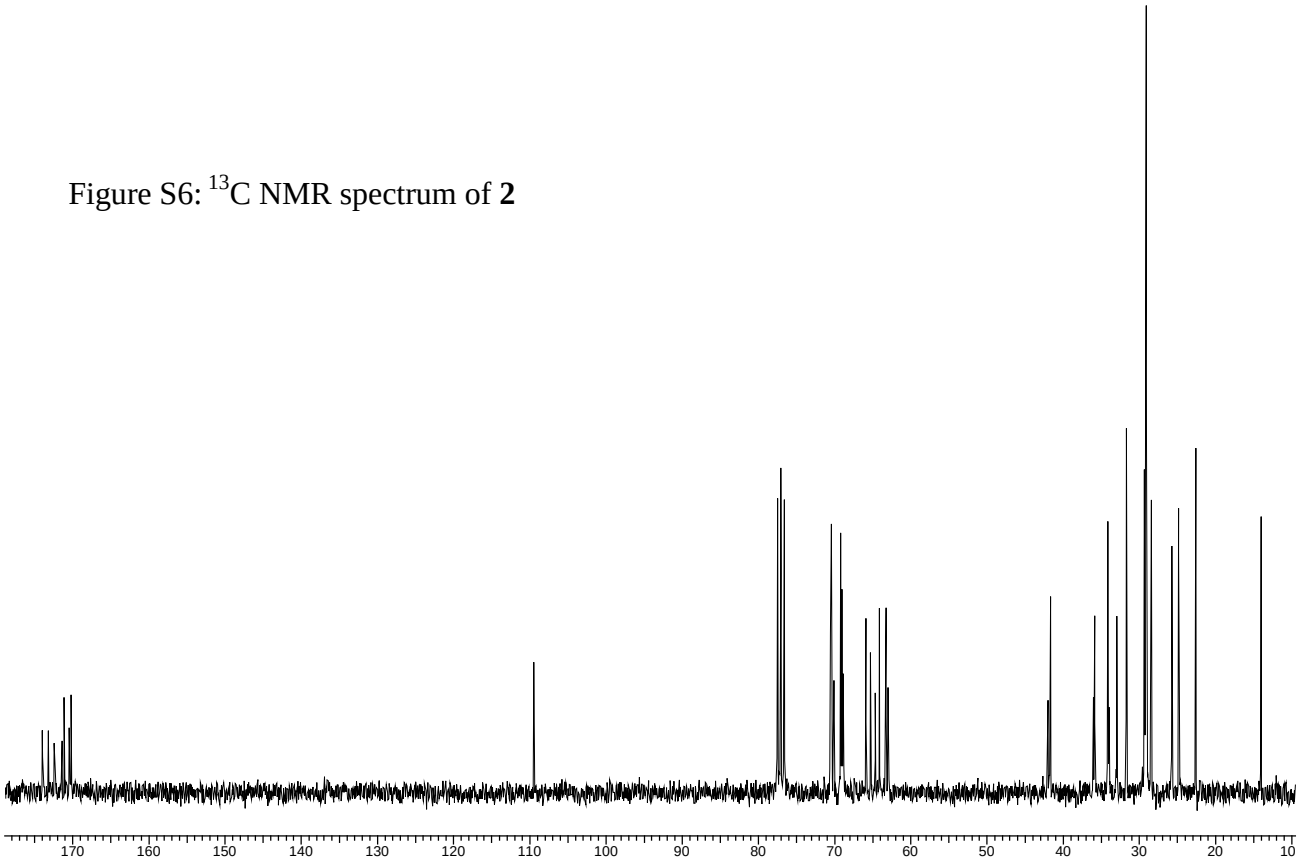


Figure S6: ^{13}C NMR spectrum of **2**



Transport measurements in vesicles:

The general procedures for transport measurements in vesicles have been previously reported.[1] Experiments followed these procedures exactly, with the modification that the alkali metal chloride concentration was 5 mM. Compound **2** was dissolved in methanol (5 mM), neutralized with choline hydroxide, and injected to the vesicle solution to give the final concentrations indicated in the table below. All experiments gave satisfactory fits to an expression for first-order consumption of titrant during the period when the pH-stat was fully controlling the set pH of 8.6. The initial rate was calculated from the apparent first-order rate constant (k in the table) by multiplication by the titrant concentration and the apparent volume change at infinite time. The extent of transport as a percentage of total entrapped acid is also given in the table. The numerical filenames indicate two different batches of vesicles. Reproducibility was assessed using gramicidin and indicated better than 10% agreement between the two batches.

File	Cation	Concentration μM	k s^{-1}	Initial rate $molH^+s^{-1}$	Extent %
22e	K	40.7	1.02E-02	8.5E-10	12
22f	K	61.1	1.25E-02	2.0E-09	22
22g	K	81.5	3.30E-02	7.4E-09	31
22h	K	30.6	2.60E-03	3.2E-10	16
22i	K	20.3	3.02E-03	1.5E-10	7
22k	K	51.0	5.70E-03	1.4E-09	31
25b	K	20.4	1.80E-03	3.5E-10	26
25c	K	30.6	3.90E-03	6.2E-10	20
25d	K	30.5	3.00E-03	7.1E-10	25
25e	K	20.4	2.10E-03	3.2E-10	19
25f	Na	20.5	1.90E-03	3.3E-10	22
25g	Cs	20.4	1.70E-03	3.7E-10	27

Transport measurements in bilayers:

The apparatus and general procedures for single-channel recording have been previously reported.[2] Agar salt bridges were used to stabilize junction potentials, and were

[1] Fyles, T. M.; James, T. D.; Kaye, K. C. J. Am. Chem. Soc. **1993**, *115*, 12315-12321.
[2] Fyles, T. M.; Loock, D.; van Straaten-Nijenhuis, W. F.; Zhou, X. *J. Org. Chem.* **1996**, *61*, 8866-8874.

employed between the electrolyte in each well of the cell and the Ag/AgCl electrode. New bridges were prepared for each isophthalic acid tested. Bilayers were formed by brushing as described previously and by dipping. After lipid (diphytanoyl phosphatidylcholine)in decane had been introduced by brushing, a lipid/decane film formed on the surface of the electrolyte, and bilayers could then be formed by the dipping method. The dipping method is usually the method used for forming bilayers in an experimental setup where an aperture in a thin sheet partition (PTFE) is clamped between pools of electrolyte, and the bilayer is formed by dipping through the air-water interface by withdrawl and replacement of the cuvette. In this apparatus, the entire cuvette was pulled vertically to expose one face of the aperture to the air-water interface held in the cell holder to oppose monolayers. Bilayers were also formed by initially dipping to form a bilayer that was brushed with downward strokes to sufficiently high capacitance. Both the painting and dipping methods have been used conjointly by other researchers.[3]

Once a bilayer of sufficient quality was formed, aliquots of **2** in methaonol solution (1.5 mM) were injected with a microlitre syringe as closely as possible to the bilayer in the free well of the cuvette holder (*cis* side). The measured voltage was applied with respect to the trans (cuvette) side of the bilayer, making the trans side the relative ground. Every 10 to 15 minutes, successive aliquots of compound were injected and the polarity was switched. Once activity was observed, data files of current as a function of time were acquired using the Fetchex program of the pClamp 6 suite. As noted in the text, incorporation failed on roughly half the attempts by the method above. Pre-mixed lipid-**2** mixtures failed to form stable bilayer membranes by either the brushing or dipping techniques. Recorded data files were imported into the spreadsheet Origin as DAT files and low pass filtering was performed at 100Hz using a Fast Fourier Transform.

[3] Benz, R.; Fröhlich, O.; Laüger, P.; Montal, M. *Biochim. Biophys. Acta* **1975**, *394*, 323-334