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Supporting Information

A K^+-selective channel with high K^+/Na^+ selectivity of 20.1 achieved via side chain tuning†

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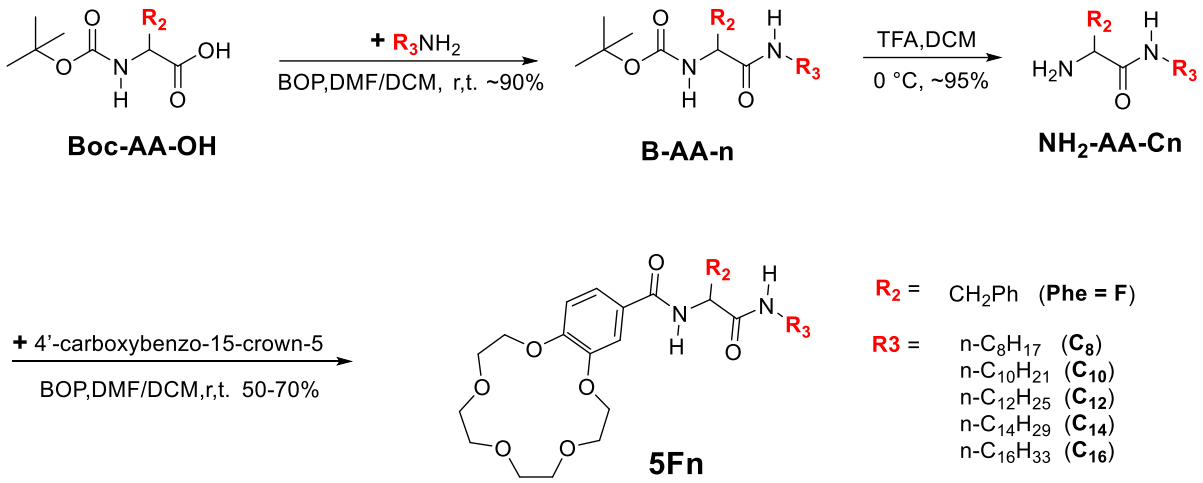
General remarks

All the reagents were obtained from commercial suppliers and used as received unless otherwise noted. Aqueous solutions were prepared from MilliQ water. The organic solutions from all liquid extractions were dried over anhydrous Na_2SO_4 for a minimum of 15 minutes before filtration. Flash column chromatography was performed using pre-coated 0.2 mm silica plates from Selecto Scientific. Chemical yield refers to pure isolated substances. ^{1}H and ^{13}C NMR spectra were recorded on either a Bruker ACF-400 spectrometer. The solvent signal of $CDCl_3$ was referenced at δ= 7.26 ppm. Coupling constants (J values) are reported in Hertz (Hz). ^{1}H NMR data are recorded in the order: chemical shift value, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), number of protons that gave rise to the signal and coupling constant, where applicable. ^{13}C spectra are proton-decoupled and recorded on Bruker ACF400 (400 MHz). The solvent, $CDCl_3$, was referenced at δ= 77 ppm. $CDCl_3$ (99.8%-Deuterated) was purchased from Aldrich and used without further purification. Mass spectra were acquired with Shimazu LCMS-2010EV. Single channel current measurements in planar lipid bilayers were carried out using Planar Lipid Bilayer Workstation (Warner Instruments, Hamden, CT).

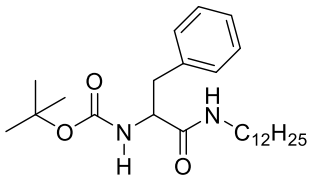
Scheme S1. Synthetic route that affords potassium channels.



Experimental procedures and compound characterizations

For synthesis of 5F8 and 5F10 see: Changliang Ren, Jie Shen, and Huaqiang Zeng. *J. Am. Chem. Soc.*, **2017,** *139,* 12338-12341.

BF12



BF12

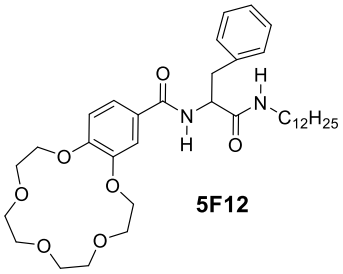
Boc-Phe-OH (2.65 g, 10 mmol), n-decylamine (2.3 ml, 10 mmol) and BOP (4.87 g, 11 mmol) were dissolved in CH_2Cl_2/DMF (25 mL: 5 mL) to which diisopropylamine (3.9 ml, 22 mmol) was added. The reaction mixture was stirred for 24 h at room temperature. Solvent was removed in vacuo and the crude product was dissolved in CH_2Cl_2 (30 mL), and washed with water (2 x 40 mL) the crude product was purified by flash column chromatography (MeOH:CH_2Cl_2 = 1:200, v:v) to afford the target compound **Boc-Phe-C12** as a pale white solid. Yield: 3.89 g, 90%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.26 (m, 2H), 7.25 – 7.18 (m, 3H), 5.69 (s, 1H), 5.11 (s, 1H), 4.26 (dd, *J* = 14.2, 7.1 Hz, 1H), 3.07 (dtd, *J* = 21.4, 13.3, 7.3 Hz, 4H), 1.41 (s, 9H), 1.33 – 1.14 (m, 20H), 0.87 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.10, 155.48, 136.87, 129.34, 128.63, 126.90, 80.10, 56.05, 39.54, 38.86, 31.95, 29.68, 29.66, 29.62, 29.53, 29.39, 29.32, 29.28, 28.30, 26.80, 22.72, 14.17. MS-ESI: calculated for $[M+Na]^+$ ($C_{26}H_{44}O_3N_2Na$)： *m/z* 455.30, found: *m/z* 455.21.

NH_2-Phe-C12

NH_2-Phe-C12

Compound **BF12** (1.5 g, 3.47 mmol) was dissolved in CH_2Cl_2 (15 mL), with an installation of N_2 balloon on top of the round bottom flask. This solution was cooled to 0 ºC using an ice bath. TFA (3.2 mL, 41.64 mmol) was slowly added to the solution. After that the reaction was allowed to stir at room temperature for 12 hours. Then the reaction mixture was neutralized using saturated aquous solution of $NaHCO_3$ in the 0 ºC ice bath. The product was extracted with CH_2Cl_2 (4 x 50 mL). Combination of the organic layer and dried over anhydrous Na_2SO_4 to give the pure product **NH_2-Phe-C12** as a light yellow solid, which was directly used in the next step without further purification. Yield: 1.11 g, 96%.

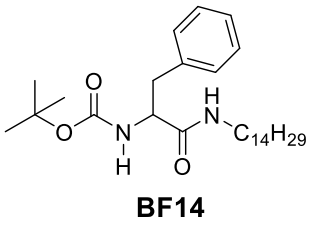
5F12



4’-carboxybenzo-15-crown-5 (312 mg, 1.0 mmol), **NH_2-Phe-C12** (332 mg, 1.0 mmol) and BOP (486 mg, 1.1 mmol) were dissolved in CH_2Cl_2/DMF (8 mL:2 mL) to which N,N-Diisopropylethylamine (0.39 ml, 2.2 mmol) was added. The reaction mixture was stirred for 24 h at room temperature. Solvent was removed in vacuo and the crude product was dissolved in CH_2Cl_2 (30 mL), and washed with water (2 x 40 mL), which was recrystallized from acetonitrile to to yield the pure product **5F12** as a white solid. Yield: 457 mg, 73%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.22 (m, 7H), 6.97 (d, *J* = 22.6 Hz, 1H), 6.81 (d, *J* = 8.3 Hz, 1H), 5.80 (s, 1H), 4.75 (d, *J* = 6.3 Hz, 1H), 4.15 (d, *J* = 3.7 Hz, 4H), 3.94 – 3.84 (m, 4H), 3.75 (s, 8H), 3.31 – 3.01 (m, 4H), 1.34 – 1.12 (m, 20H), 0.87 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.03, 166.75, 152.15, 148.74, 136.92, 129.40, 128.64, 126.98, 126.39, 120.52, 112.78, 112.24, 71.13, 70.36, 70.30, 69.36, 69.29, 68.92, 68.64, 55.30, 39.67, 38.79, 31.94, 29.69, 29.67, 29.64, 29.55, 29.39, 29.28, 26.84, 22.72, 14.18. MS-ESI: calculated for $[M+Na]^+$ ($C_{36}H_{54}O_7N_2Na$)： *m/z* 649.38, found: *m/z* 649.40.

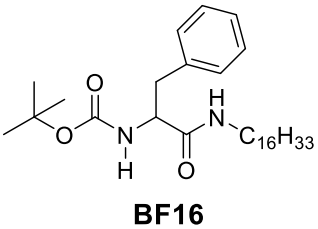
Preparation of BF14 and BF16 follows the same synthetic procedure as BF12.

BF14



^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (t, *J* = 7.2 Hz, 2H), 7.22 (dd, *J* = 14.3, 7.1 Hz, 3H), 5.60 (s, 1H), 5.08 (s, 1H), 4.33 – 4.18 (m, 1H), 3.21 – 2.95 (m, 4H), 1.41 (s, 9H), 1.32 – 1.14 (m, 24H), 0.88 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.96, 155.41, 136.86, 129.34, 128.69, 126.96, 80.19, 56.11, 39.53, 38.80, 31.96, 29.73, 29.70, 29.69, 29.62, 29.53, 29.40, 29.33, 29.27, 28.31, 26.79, 22.73, 14.18. MS-ESI: calculated for $[M+Na]^+$ ($C_{28}H_{48}O_3N_2Na$)： *m/z* 483.36, found: *m/z* 483.14.

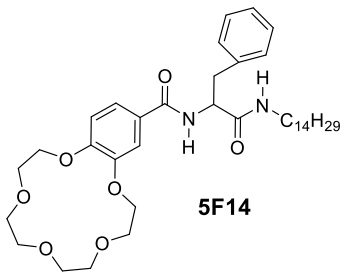
BF16



^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.27 (m, 2H), 7.25 – 7.18 (m, 3H), 5.61 (s, 1H), 5.08 (s, 1H), 4.25 (dd, *J* = 14.1, 7.1 Hz, 1H), 3.22 – 2.95 (m, 4H), 1.41 (s, 9H), 1.32 – 1.15 (m, 28H), 0.87 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.08, 155.47, 136.87, 129.35, 128.64, 126.91, 80.12, 56.06, 39.54, 38.86, 31.96, 29.74, 29.71, 29.69, 29.63, 29.54, 29.40, 29.32, 29.28, 28.30, 26.81, 22.73, 14.18. MS-ESI: calculated for $[M+Na]^+$ ($C_{30}H_{52}O_3N_2Na$)： *m/z* 511.39, found: *m/z* 511.3.

Preparation of 5F14 AND 5F16 follows the same synthetic procedure as 5F12.

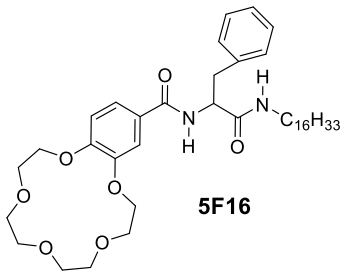
5F14



5F14

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.26 (m, 7H), 6.83 (d, *J* = 8.4 Hz, 2H), 5.59 (s, 1H), 4.72 (dd, *J* = 13.8, 8.0 Hz, 1H), 4.16 (dt, *J* = 4.5, 2.5 Hz, 4H), 3.91 (ddd, *J* = 8.7, 5.6, 3.5 Hz, 4H), 3.76 (d, *J* = 1.6 Hz, 8H), 3.29 – 3.00 (m, 4H), 1.32 – 1.14 (m, 24H), 0.88 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.14, 166.83, 152.23, 148.77, 136.79, 129.42, 128.69, 127.06, 126.26, 120.59, 112.80, 112.26, 71.14, 70.37, 70.31, 69.36, 69.30, 69.10, 68.94, 68.66, 55.37, 39.79, 38.78, 31.96, 29.74, 29.72, 29.70, 29.64, 29.55, 29.41, 29.27, 29.19, 26.83, 22.73, 14.18. MS-ESI: calculated for $[M+Na]^+$ ($C_{38}H_{58}O_7N_2Na$)： *m/z* 677.36, found: *m/z* 677.40.

5F16



5F16

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.20 (m, 7H), 7.01 (d, *J* = 10.3 Hz, 1H), 6.80 (d, *J* = 8.3 Hz, 1H), 5.98 (s, 1H), 4.86 – 4.72 (m, 1H), 4.14 (s, 4H), 3.93 – 3.86 (m, 4H), 3.75 (d, *J* = 2.5 Hz, 8H), 3.29 – 3.02 (m, 4H), 1.32 – 1.13 (m, 28H), 0.87 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.10, 166.78, 152.12, 148.71, 136.91, 129.41, 128.63, 126.97, 126.35, 120.57, 112.75, 112.21, 71.09, 70.34, 70.28, 69.33, 69.27, 68.88, 68.61, 55.33, 39.70, 38.77, 31.95, 29.74, 29.71, 29.69, 29.65, 29.56, 29.40, 29.29, 29.25, 26.85, 22.73, 14.18. MS-ESI: calculated for $[M+Na]^+$ ($C_{40}H_{62}O_7N_2Na$)： *m/z* 705.45, found: *m/z* 705.50.

^{1}H NMR dilution experiments for 5Fn (n = 10 - 16)

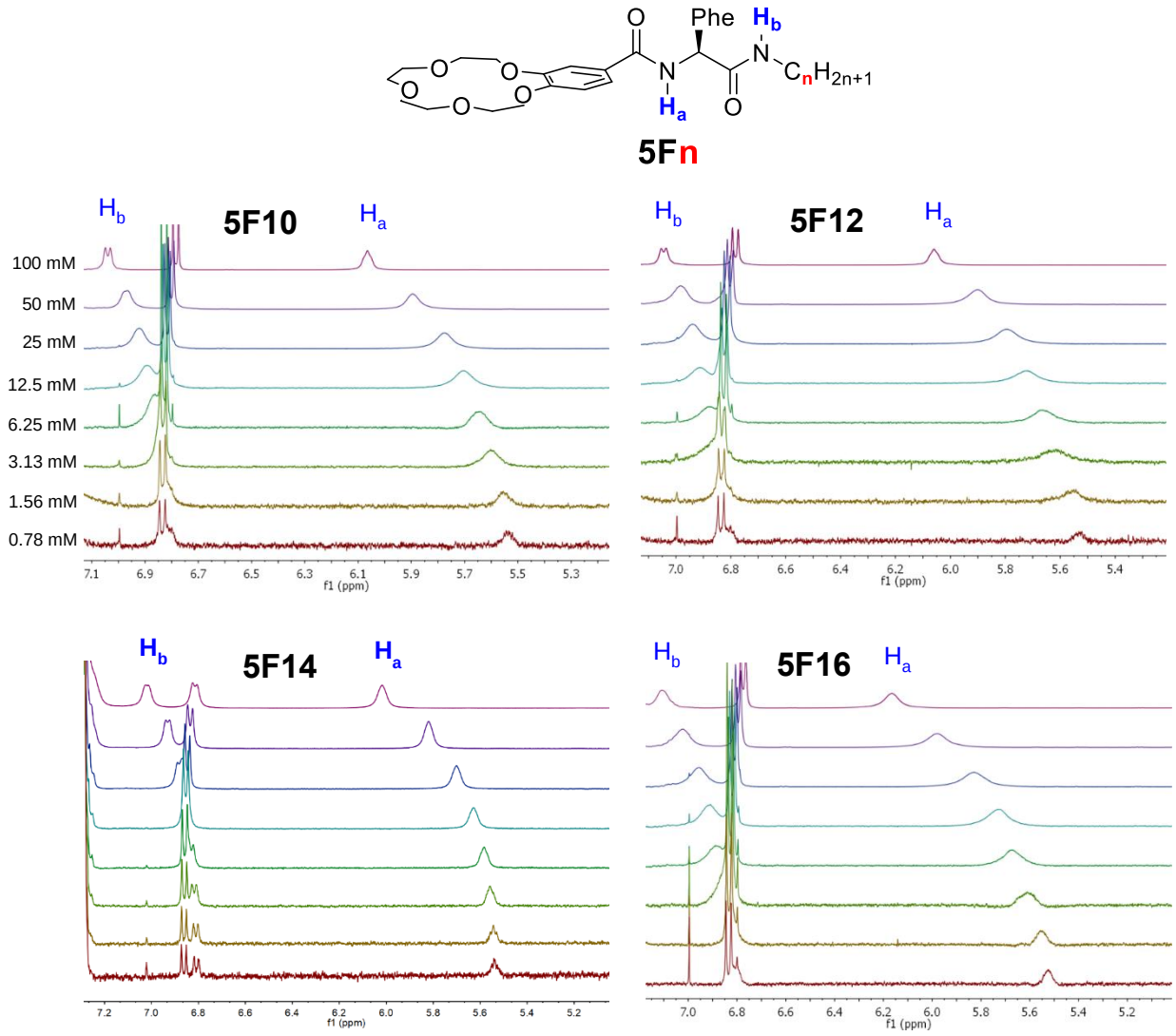


Figure S1. ^{1}H NMR dilution experiments were performed from 100 mM to 0.78 mM for channels **5F10**, **5F12**, **5F14** and **5F16**. Up-field shifts in H_a and H_b of 0.52 ppm and 0.21 ppm for **5F10**, of 0.54 ppm and 0.22 ppm for **5F12**, 0.48 ppm and 0.21 ppm for **5F14** and 0.75 ppm and 0.28 ppm for **5F16** were observed.

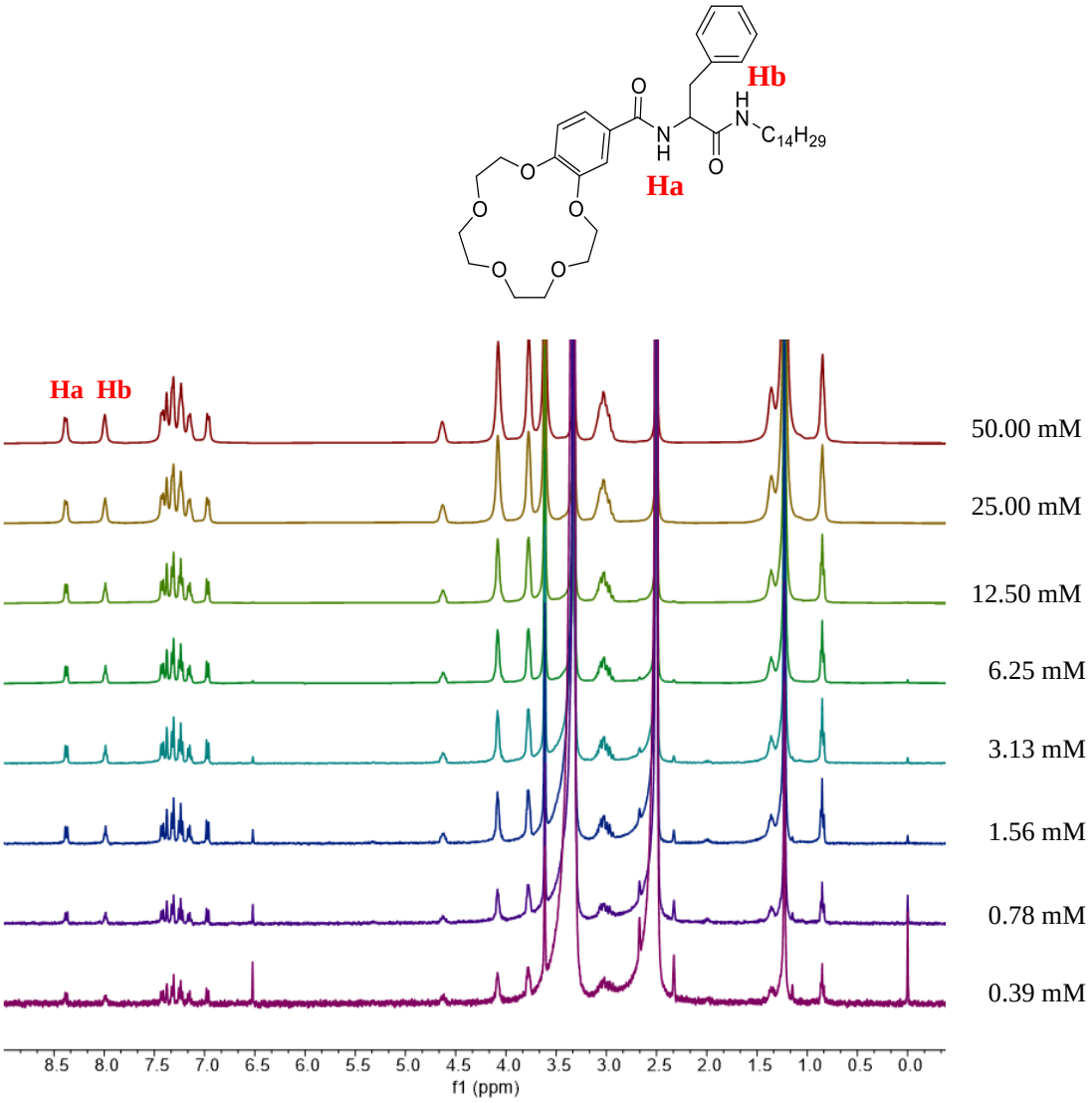


Figure S2. ^{1}H NMR dilution experiments were performed from 50 mM to 0.39 mM for channel **5F14** in DMSO-*d6*. No changes in H_a and H_b were observed, demonstrating that molecules **5Fn** lack the ability to generate H-bonded stacks in DMSO and in water.

Molecular dynamics (MD) simulation of $(5F12)_6$

To obtain its possible structures in lipid membrane, one-dimensionally aligned K^+-selective ion channel $(\mathbf{5F12})_n$ was first computationally optimized using the COMPASS force field.[1] The resultant structure was then placed in a bilayer of 128 POPC molecules (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) solvated on two sides by 2 x 2397 water molecules (Figure 5a) and subjected to molecular dynamics (MD) simulation using the CHARMM[1-9] program,[2-6] PME method[7] and SHAKE algorithm.[8] Given a hydrophobic thickness of 27-28 Å for POPC bilayer[10-12] and an inter-chain separation distance of 4.9 Å for $(\mathbf{5F12})_n$, n value was set to be 6 for MD simulation. After equilibration steps, the production run was carried out for 20 ns.

References

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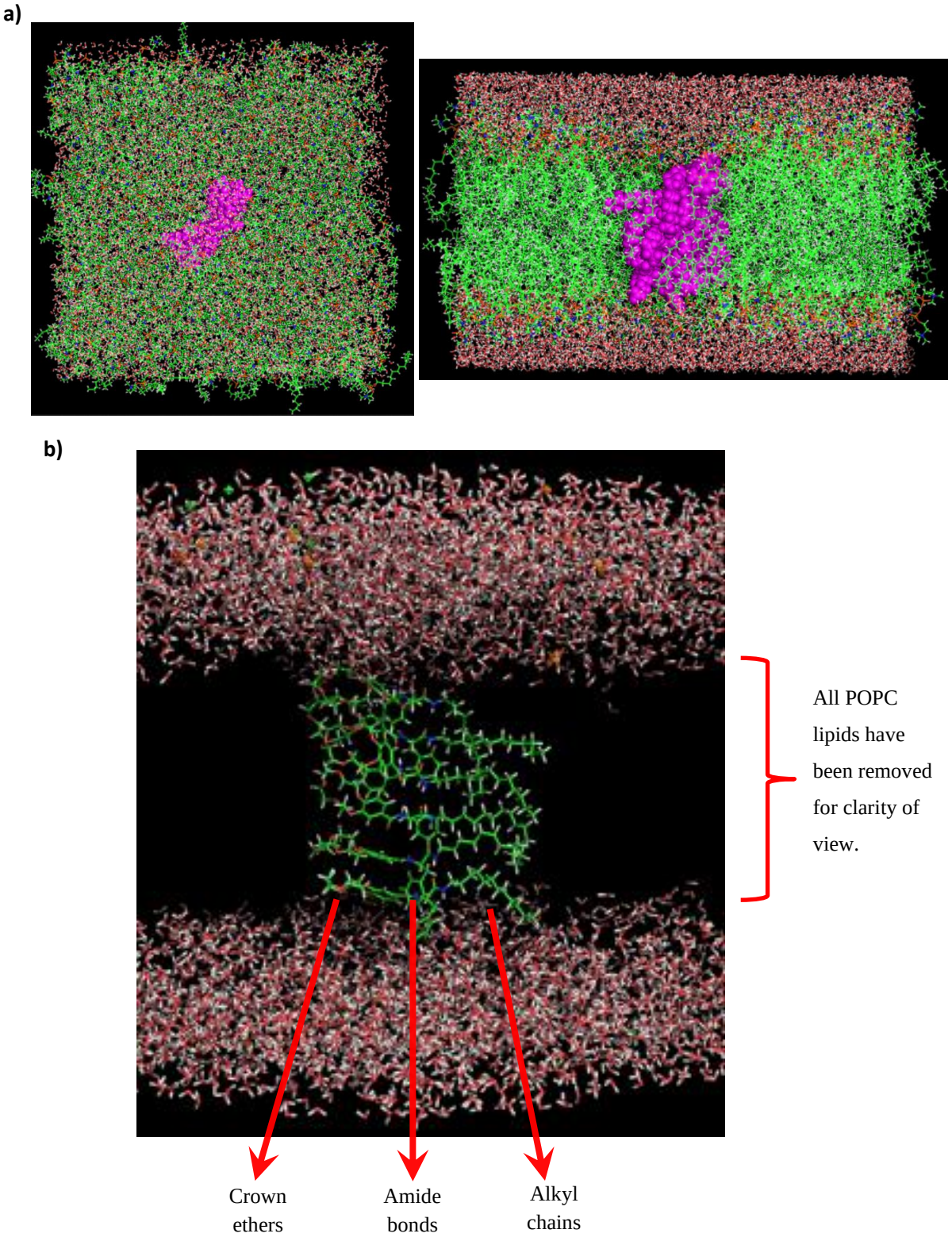


Figure S3. a) (**5F12**)$_6$ in pink ball representation was embedded in POPC membrane for 20 ns simulation. b) The highly populated and representative structure seen in the last 5 ns trajectories. From b), it can be seen that the alkyl chains of (**5F12**)$_6$ are mostly perpendicular to the lipid's hydrophobic tail. POPC= 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine.

UV absorbance spectra for 5F12 and 5F14 in the absence and presence of EYPC lipids

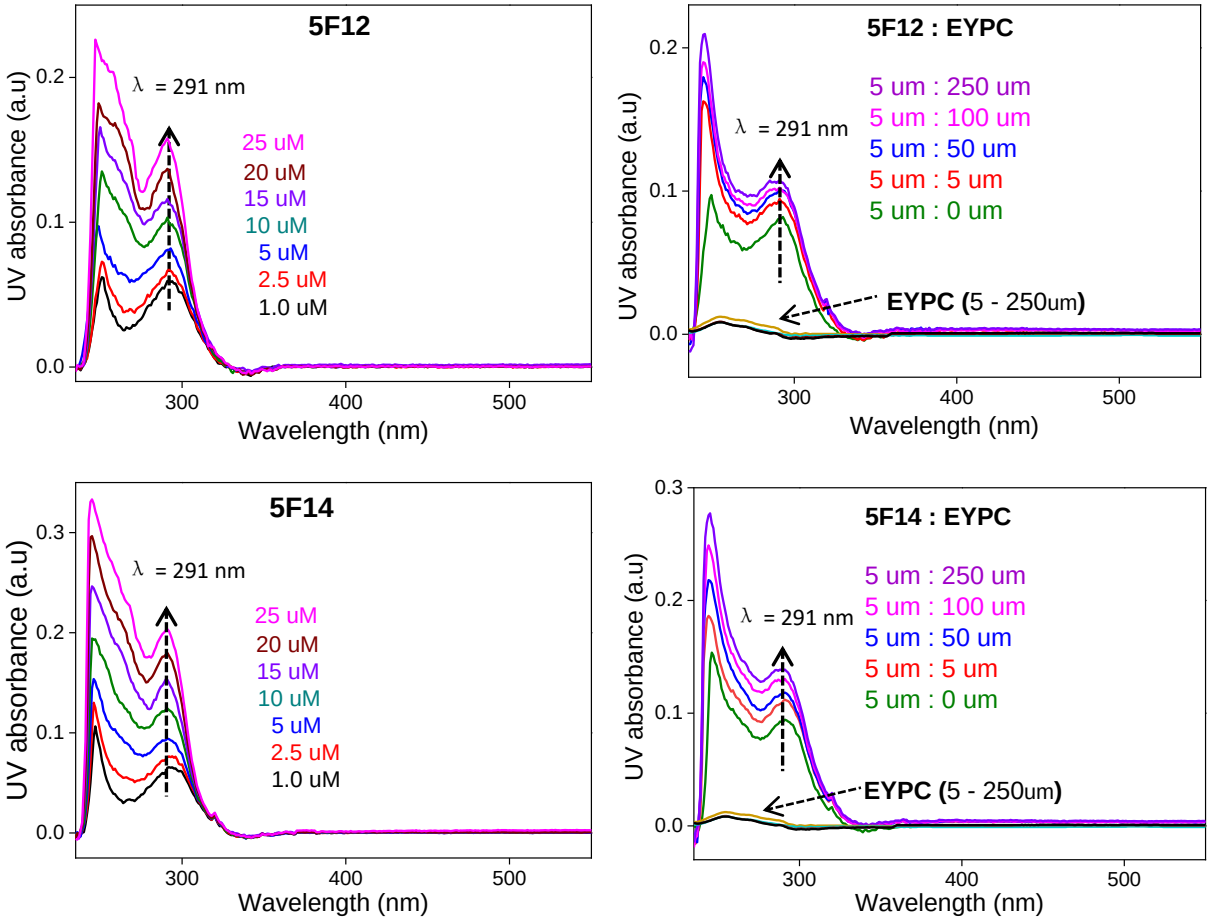


Figure S4. The concentration-dependent UV absorbances at 291 nm for **5Fn** alone from 1.0 - 25 μM and at 5 μM in the presence of 0 - 250 μM lipids.

Computed structures of crown-metal ion complexes

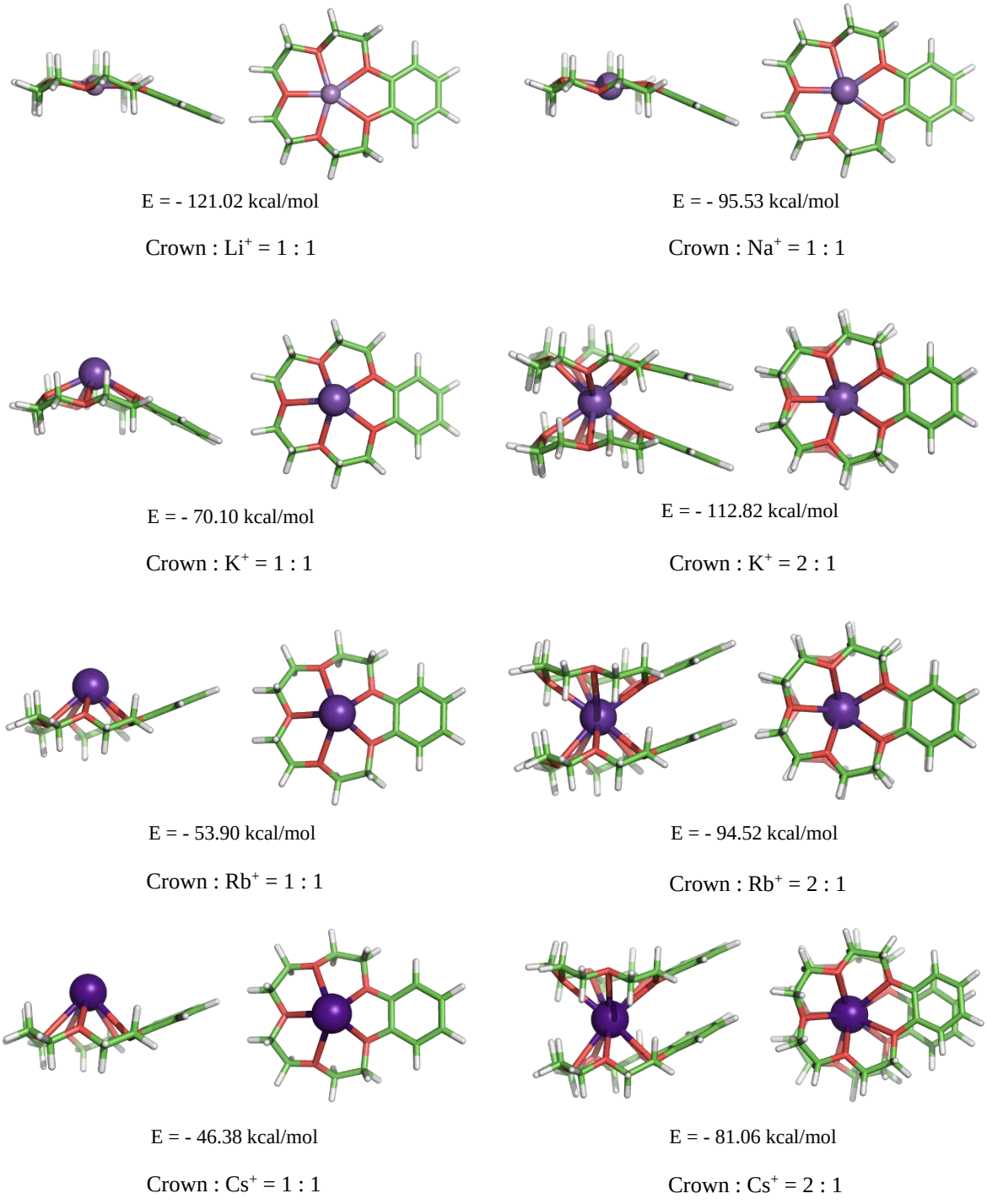


Figure S5. Computationally-derived binding energies (kcal/mol) between benzo-15-crown-5 and alkali metal ions (Li^+, Na^+, K^+, Rb^+, and Cs^+). All complexes (Li^+, Na^+, K^+ and Rb^+) were optimized at the level of M06-2x/6-31G in the gas phase, with energies calculated using M06-2x/6-311G**. M06-2x/Lanl2DZwas applied for structural optimization and energy calculation for Cs complexes in the gas phase. These data suggest 1:1 complexes to be energetically more favored than 1:2 complexes when host and guest molecules are mixed in a 1:1 molar ratio.

Picrate extraction experiment for determination of binding constants

Preparation of alkali metal picrates

The sodium and potassium picrates were prepared by dissolving picric acid in a minimum amount of distilled boiling water and slowly adding a stoichiometric amount of the alkali metal hydroxide. The alkali metal picrate solution was cooled to room temperature and placed in an ice bath to facilitate crystallization. The precipitate was filtered and recrystallized from distilled water. After filtration and extensive air drying, the salt was carefully heated to dryness in a vacuum oven at 75℃ for overnight and cooled to room temperature under N2 protection. The anhydrous metal picrates were stored in a desiccator.

Procedure for picrate extraction experiment for determining association constant between channel molecules and Na^+ or K^+

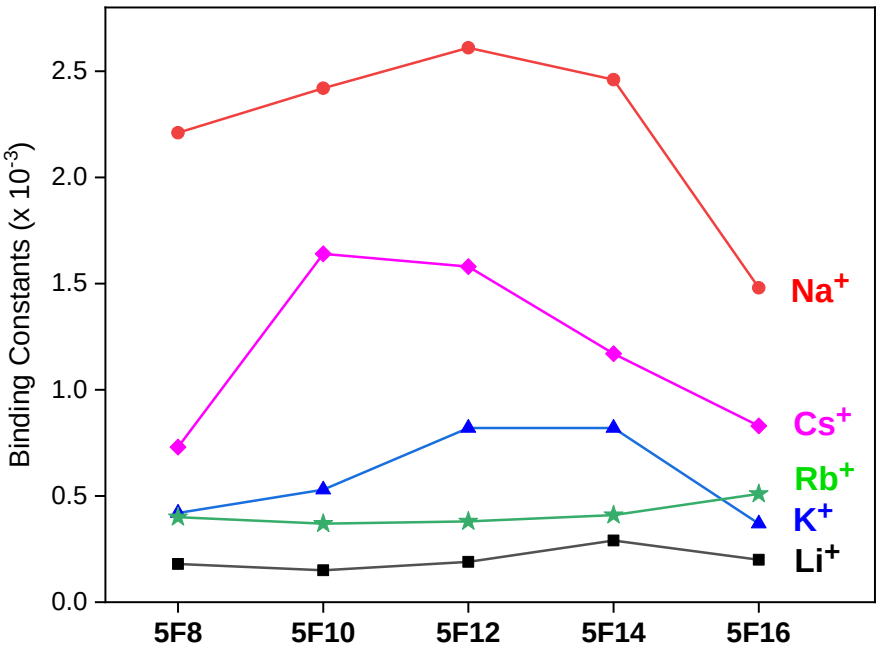
Extractions of sodium or potassium picrates with channels were performed by placing 1.0 mL of a 10 mM solution of the metal picrate in deionized water and 1.0 mL of a 10 mM solution of the channels in chloroform into a 4-mL sample vial and mixing the solutions on a vortex mixer for 60 seconds. The sample was then allowed to stand for 30 minutes to ensure a complete separation of the layers. Aliquot was taken from the aqueous phase of the sample, and the concentration of metal picrate in aqueous phase was determined by UV-Visible spectroscopy with a scanning from 300 nm to 500 nm. The association constants K_a were calculated according to method previously described (Moore, S. S.; Tarnowski, T. L.; Newcomb, M.; Cram, D. J. *J. Am. Chem. Soc.* **1977**, *99*, 6398-6405). Three samples were prepared for each picrate extraction experiment. Standard deviations from the analysis of the three samples were less than 10% in terms of the Ka values.

Table S2. Binding constants (K_a) between **5Fn** (n = 8 - 16) and alkali metal ions (Li, Na^+, K^+ , Rb^+ and Cs^+ ions) at 25 °C obtained by Cram's method.[a]

	Na^+		Li^+		$K_a(Na^+) / K_a(Li^+)$
	R_{Na+}	K_a (M^{-1})	R_{Li+}	K_a (M^{-1})	
5F8	0.399	$(2.11 \pm 0.27) \times 10^3$	0.057	$(0.18 \pm 0.03) \times 10^3$	11.7
5F10	0.417	$(2.42 \pm 0.21) \times 10^3$	0.047	$(0.15 \pm 0.01) \times 10^3$	16.1
5F12	0.427	$(2.61 \pm 0.19) \times 10^3$	0.036	$(0.19 \pm 0.02) \times 10^3$	13.7
5F14	0.419	$(2.46 \pm 0.22) \times 10^3$	0.083	$(0.29 \pm 0.02) \times 10^3$	8.48
5F16	0.352	$(1.48 \pm 0.15) \times 10^3$	0.062	$(0.20 \pm 0.03) \times 10^3$	7.40

	Na^+		K^+		$K_a(Na^+) / K_a(K^+)$
	R_{Na+}	K_a (M^{-1})	R_{K+}	K_a (M^{-1})	
5F8	0.399	$(2.11 \pm 0.27) \times 10^3$	0.56	$(0.42 \pm 0.03) \times 10^3$	5.02
5F10	0.417	$(2.42 \pm 0.21) \times 10^3$	0.585	$(0.53 \pm 0.05) \times 10^3$	4.57
5F12	0.427	$(2.61 \pm 0.19) \times 10^3$	0.633	$(0.82 \pm 0.08) \times 10^3$	3.18
5F14	0.419	$(2.46 \pm 0.22) \times 10^3$	0.632	$(0.82 \pm 0.06) \times 10^3$	3.00
5F16	0.352	$(1.48 \pm 0.15) \times 10^3$	0.546	$(0.37 \pm 0.03) \times 10^3$	4.00
	Na^+		Rb^+		$K_a(Na^+) / K_a(Rb^+)$
	R_{Na+}	K_a (M^{-1})	R_{Rb+}	K_a (M^{-1})	
5F8	0.399	$(2.11 \pm 0.27) \times 10^3$	0.167	$(0.40 \pm 0.02) \times 10^3$	5.28
5F10	0.417	$(2.42 \pm 0.21) \times 10^3$	0.158	$(0.37 \pm 0.01) \times 10^3$	6.54
5F12	0.427	$(2.61 \pm 0.19) \times 10^3$	0.161	$(0.38 \pm 0.02) \times 10^3$	6.87
5F14	0.419	$(2.46 \pm 0.22) \times 10^3$	0.169	$(0.41 \pm 0.03) \times 10^3$	6.00
5F16	0.352	$(1.48 \pm 0.15) \times 10^3$	0.193	$(0.51 \pm 0.06) \times 10^3$	2.90
	Na^+		Cs^+		$K_a(Na^+) / K_a(Cs^+)$
	R_{Na+}	K_a (M^{-1})	R_{Cs+}	K_a (M^{-1})	
5F8	0.399	$(2.11 \pm 0.27) \times 10^3$	0.112	$(0.73 \pm 0.05) \times 10^3$	2.89
5F10	0.417	$(2.42 \pm 0.21) \times 10^3$	0.191	$(1.64 \pm 0.09) \times 10^3$	1.48
5F12	0.427	$(2.61 \pm 0.19) \times 10^3$	0.187	$(1.58 \pm 0.11) \times 10^3$	1.65
5F14	0.419	$(2.46 \pm 0.22) \times 10^3$	0.155	$(1.17 \pm 0.06) \times 10^3$	2.10
5F16	0.352	$(1.48 \pm 0.15) \times 10^3$	0.123	$(0.83 \pm 0.07) \times 10^3$	1.78

[a] Determined based on the partition of 10 mM alkali picrate salts in 1 mL H_2O into 1 mL of $CHCl_3$ containing 10 mM **5Fn**.



In good accordance with the binding constant trends, **5Fn** transports ions in the preferred order of $K^+ > Rb^+ > Cs^+ > Na^+ \cong Li^+$.

$Ka(K^+)$ and $Ka(Rb^+)$ values are of intermediate, and so the most preferred ions to be transported.

Ion transport study using the HPTS assay and *EC*$_{50}$ measurements using the Hill analysis.

Egg yolk L-α-phosphatidylcholine (EYPC, 1 ml, 25 mg/mL in $CHCl_3$, Avanti Polar Lipids, USA) was added in a round-bottom flask. The solvent was removed under reduced pressure at 30 ^oC. After drying the resulting film under high vacuum overnight, the film was hydrated with 4-(2-hydroxyethyl)-1-piperazine-ethane sulfonic acid (HEPES) buffer solution (1 mL, 10 mM HEPES, 100 mM NaCl, pH = 7.0) containing a pH sensitive dye 8-hydrox-ypyrene-1,3,6-trisulfonic acid (HPTS, 1 mM) at room temperature for 60 minutes to give a milky suspension. The mixture was then subjected to 10 freeze-thaw cycles: freezing in liquid N_2 for 1 minute and heating 55 ^oC water bath for 2 minutes. The vesicle suspension was extruded through polycarbonate membrane (0.1 μm) to produce a homogeneous suspension of large unilamellar vesicles (LUVs) of about 120 nm in diameter with HPTS encapsulated inside. The unencapsulated HPTS dye was separated from the LUVs by using size exclusion chromatography (stationary phase: Sephadex G-50, GE Healthcare, USA, mobile phase: HEPES buffer with 100 mM NaCl) and diluted with the mobile phase to yield 5 mL of 6.5 mM lipid stock solution.

The HPTS-containing LUV suspension (25 μL, 6.5 mM in 10 mM HEPES buffer containing 100 mM NaCl at pH = 7.0) was added to a HEPES buffer solution (1.95 mL, 10 mM HEPES, 100 mM MCl at pH = 8.0, where M^+= Li^+, Na^+, K^+, Rb^+, and Cs^+) to create a pH gradient for ion transport study. A solution of channel molecules in DMSO was then injected into the suspension under gentle stirring. Upon the addition of channels, the emission of HPTS was immediately monitored at 510 nm with excitations at both 460 and 403 nm recorded simultaneously for 300 seconds using fluorescence spectrophotometer (Hitachi, Model F-7100, Japan) after which time an aqueous solution of Triton X-100 was immediately added to achieve the maximum change in dye fluorescence emission. The final transport trace was obtained, after subtracting background intensity at t = 0, as a ratiometric value of I_{460}/I_{403} and normalized based on the ratiometric value of I_{460}/I_{403} after addition of triton. The fractional changes $R_{K+}$was calculated for each curve using the normalized value of I_{460}/I_{403} at 300 seconds before the addition of triton, with triton with ratiometric value of I_{460}/I_{403} at t = 0 s as 0% and that of I_{460}/I_{403} at t = 300 s (obtained after addition of triton) as 100%. Fitting the fractional transmembrane activity R_{K+} vs channel concentration using the Hill equation: $Y=1/(1+(EC_{50}/[C])^n)$ gave the Hill coefficient *n* and EC_{50} values.

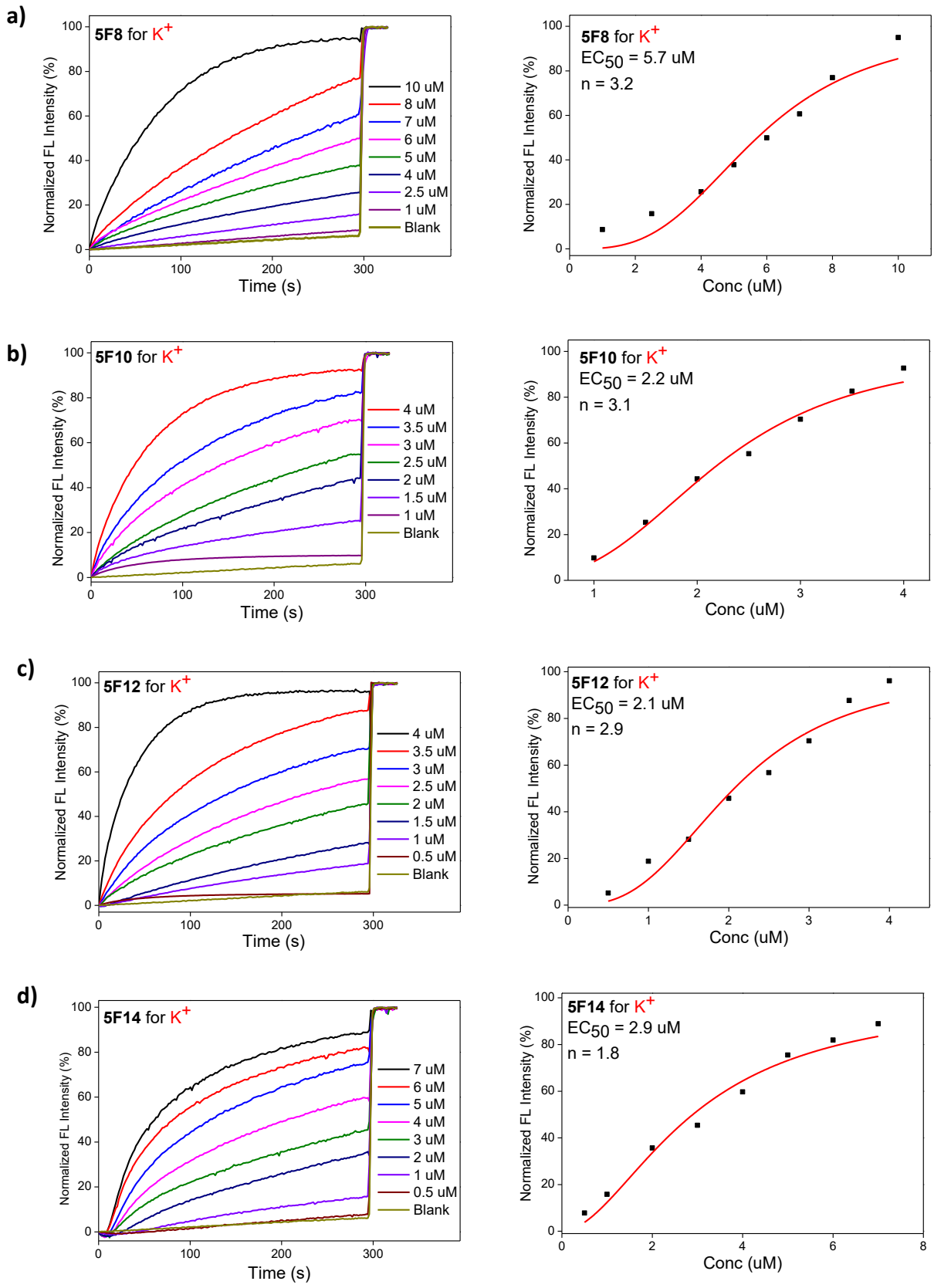


Figure S6. Determination of EC_{50} values for K^+ using the ratiometric values of I460/I403 at different concentrations as a function of time for (a) **5F8,** (b) **5F10**, (c) **5F12** and (d) **5F14**.

FCCP assay

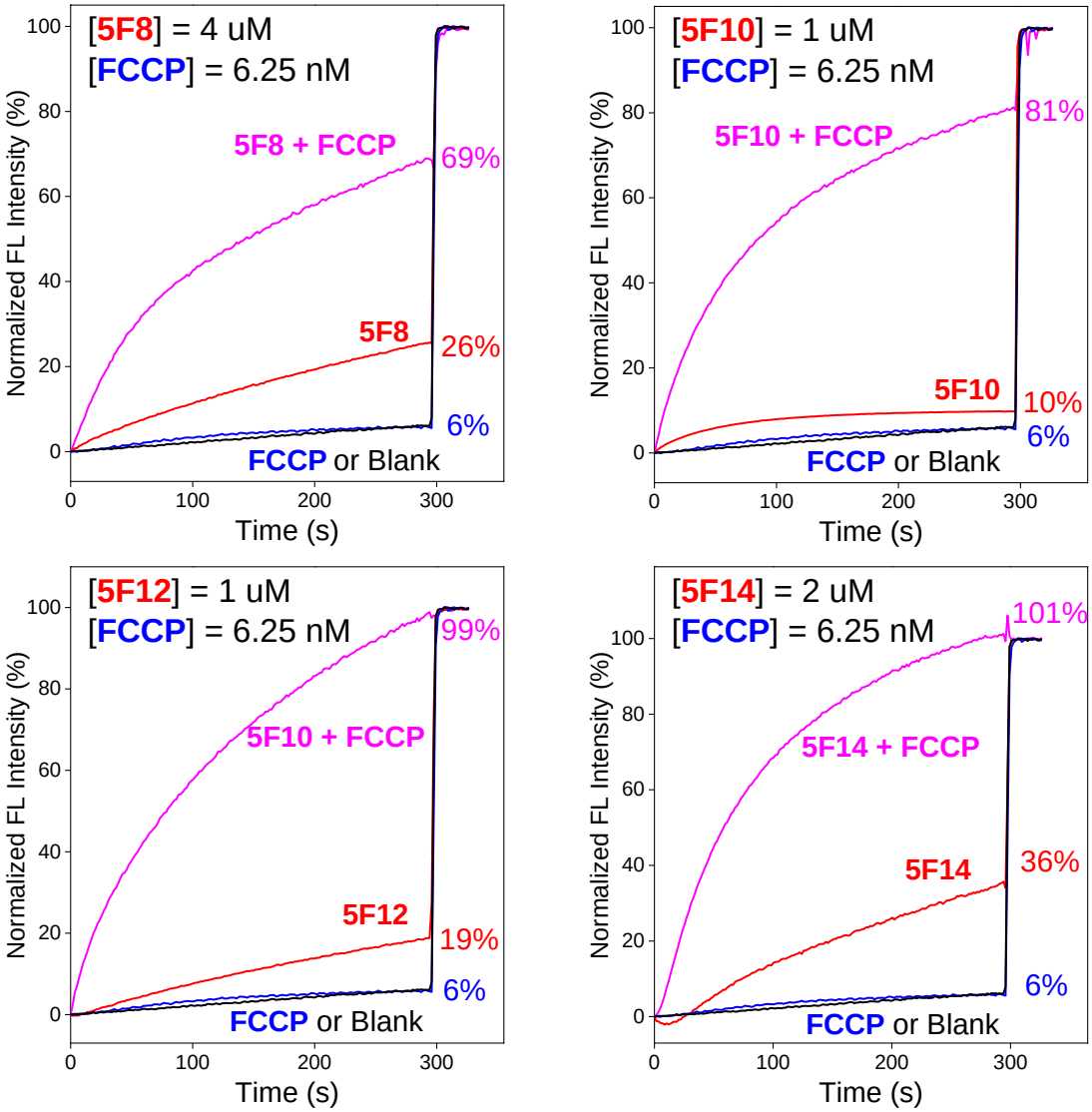


Figure S7. Applying the same HPTS scheme in Fig. 2a, the FCCP assay additionally employs a potent proton carrier FCCP (carbonylcyanide-p-trifluoromethoxyphenylhydrazone) to study the relative transport rates between K^+ and protons. Presence of FCCP at 6.25 nM elicits increases by 43% - 80% in fractional ion transport activity for **5F8 - 5F14** at 1 μM - 4 μM, unambiguously confirming the K^+ transport rate to be much faster than the H^+ transport rate.

Membrane leaking and pore size determination using CF dye.

Egg yolk L-α-phosphatidylcholine (EYPC, 1 ml, 25 mg/mL in $CHCl_3$, Avanti Polar Lipids, USA) was added into a round-bottom flask. The solvent was removed under reduced pressure at 30 ^oC. After drying the resulting film under high vacuum overnight at room temperature, the film was hydrated with HEPES buffer solution (1 mL, 10 mM HEPES, 100 mM NaCl, pH = 7.5) containing a 5(6)-fluorescein (CF, 50 mM) at room temperature for 60 minutes to give a milky suspension. The mixture was then subjected to 12 freeze-thaw cycles: freezing in liquid N_2 for 1 minute and heating at 55 ^oC in water bath for 2 minutes. The vesicle suspension was extruded through polycarbonate membrane (0.1 μm) to produce a homogeneous suspension of large unilamellar vesicles (LUVs) of about 120 nm in diameter with CF encapsulated inside. The free unencapsulated CF dye was separated from the LUVs by using size exclusion chromatography (stationary phase: Sephadex G-50, GE Healthcare, USA, mobile phase: HEPES buffer with 100 mM NaCl, pH = 7.5) and diluted with the mobile phase to yield 5 mL of 6.5 mM lipid stock solution.

The CF-containing LUV suspension (25 μL, 6.5 mM in 10 mM HEPES buffer containing 100 mM NaCl at pH = 7.5) was added to a HEPES buffer solution (1.95 mL, 10 mM HEPES, 100 mM NaCl at pH = 7.5) to create a concentration gradient for CF dye transport study. A solution of **our samples** or natural poreforming peptide Melittin in DMSO at different concentrations was then injected into the suspension under gentle stirring. Upon the addition of channel molecules, the emission of CF was immediately monitored at 517 nm with excitations at 492 nm for 300 seconds using fluorescence spectrophotometer (Hitachi, Model F-7100, Japan) after which time an aqueous solution of Triton X-100 (20 μL, 20% v/v) was immediately added to completely destruct the chloride gradient. The final transport trace was obtained by normalizing the fluorescence intensity using the equation of $I_f = [(I_t - I_0)/(I_1 - I_0)]$ where, I_f = Fractional emission intensity, I_t = Fluorescence intensity at time t, I_1 = Fluorescence intensity after addition of Triton X-100, and I_0 = Initial fluorescence intensity.

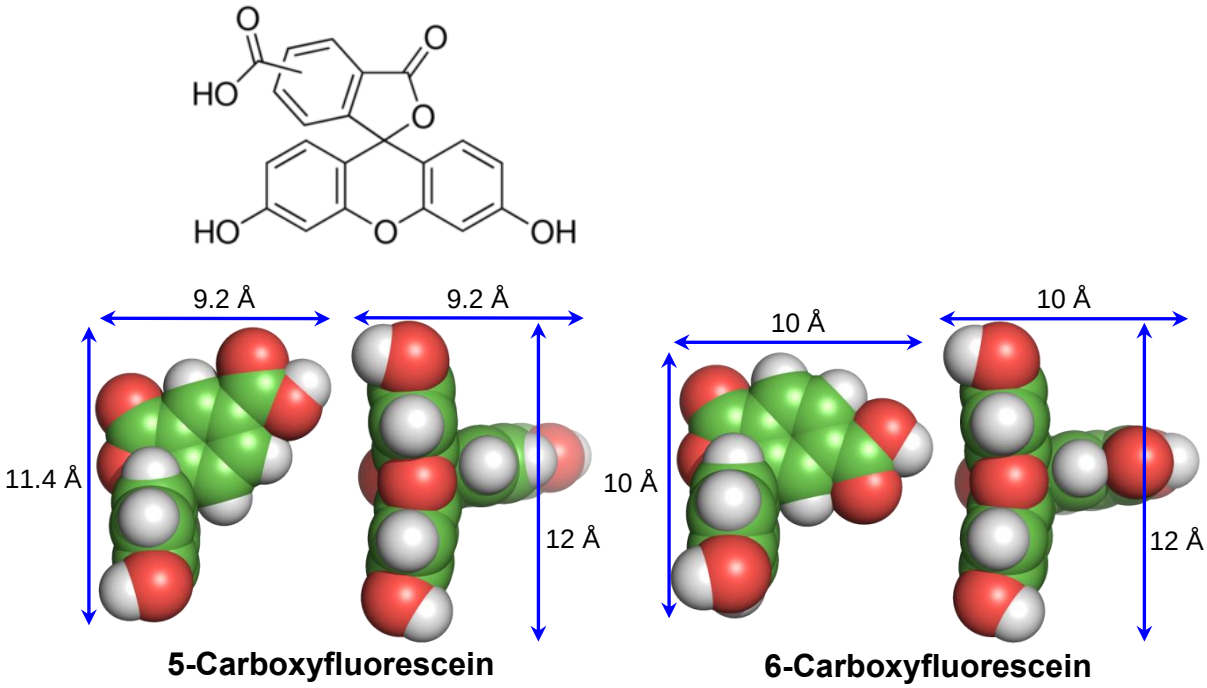


Figure S8. Molecular dimensionality of CF dye molecules consisting of a mixture of 5-carboxyfluorescein and 6-Carboxyfluorescein. These molecules measure 9.2 - 12 Å in diameter.

Single channel current measurement in planar lipid bilayers.

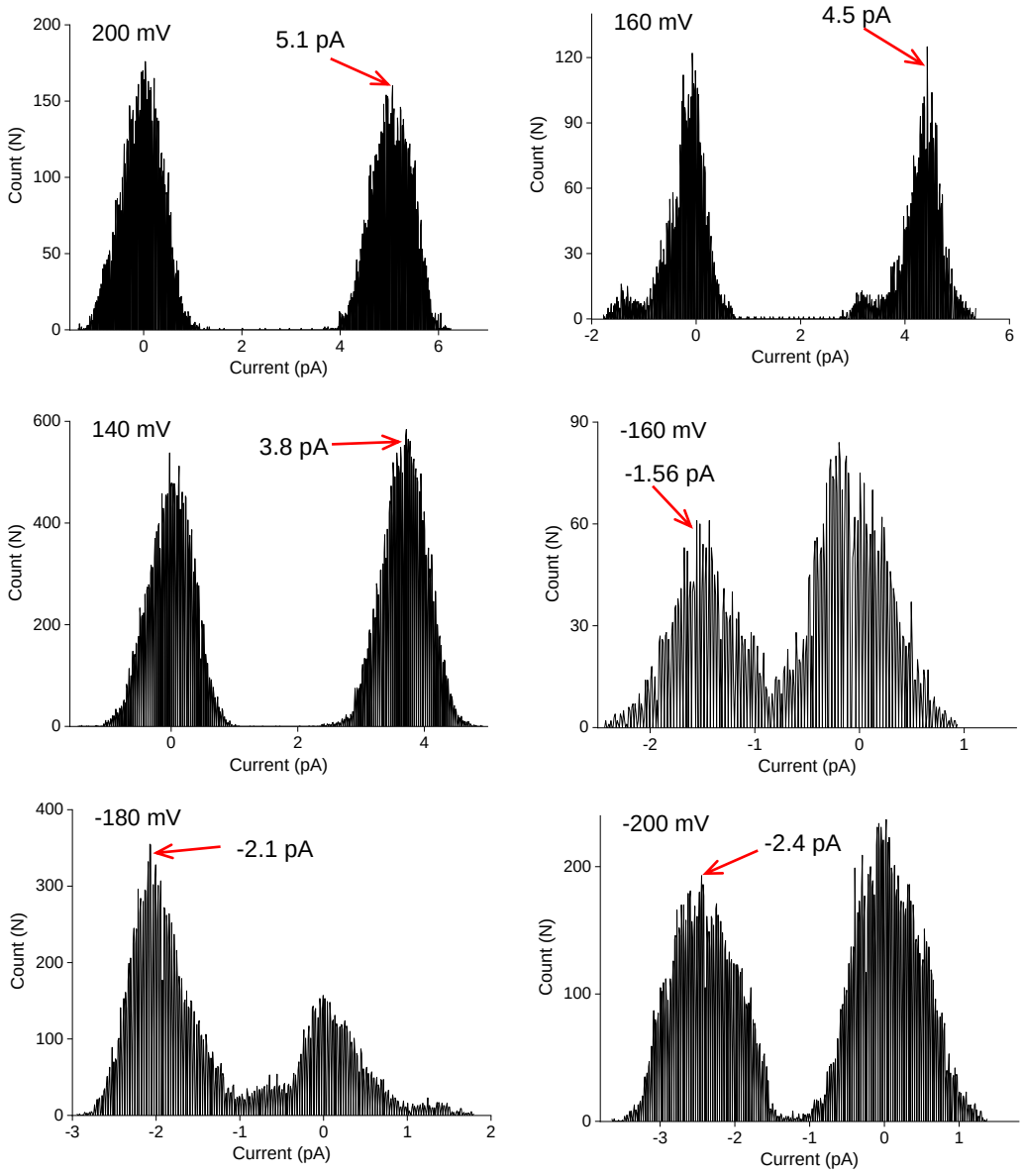
The chloroform solution of 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (diPhyPC, 10 mg/ml, 20 uL) was evaporated using nitrogen gas to form a thin film and re-dissolved in *n*-decane (8 uL). 0.2 μL of this *n*-decane solution was injected into the aperture (diameter = 250 um) of the Delrin® cup (Warner Instruments, Hamden, CT) with the *n*-decane removed using nitrogen gas. In a measurement of the transport selectivity of K^+ over Na^+, the *cis* chamber was charged with KCl (1.0 M) and the *trans* one was charged with MCl (1.0 M) where M^+= Na^+, Rb^+, and Cs^+, respectively. Ag/AgCl electrodes were used to impose voltages and record currents across the membrane. Agar (2%, 20 mg/mL) in 1 M KCl salt bridges were used to transfer the signal. Planar lipid bilayer was formed by painting 0.3 μL of the lipid-containing *n*-decane solution around the *n*-decane-pretreated aperture. Successful formation of planar lipid bilayers can be established with a capacitance value ranging from 80-120 pF. Samples in THF (0.5-1.0 μL) were added to the *cis* compartment to reach a final concentration of around 10^{-8} M and the solution was stirred for a few min until a single current trace appeared. The Warner Instruments planar lipid bilayer workstation was used for all experiments. Data were amplified (BC-535 Bilayer Amplifier; Warner Instruments, Hamden, CT), digitized (DigiData 1550; Axon Instruments, Low-Noise data Acquisition System), and stored on a HP PC using the Clampex program (version 10.4.0.36). Data Analysis was performed using the Clampfit software (version 10.4.0.36).

The selectivity of the channels for K^+ over M^+, defined as the permeability ratio of two ions, was calculated by using the simplified Goldman−Hodgkin−Katz equation:

$$\varepsilon_{rev} = RT/F \times \ln(P_{K^+} / P_{M^+})$$

where ε_{rev} is the reversal membrane potential; R is the universal gas constant (8.314 $J.K^{-1}.mol^{-1}$); T the temperature in Kelvin (298 K); F is the Faraday's constant (96485 $C.mol^{-1}$); *P* is the permeability of the channel for ions.

5F12 (P_{K^+}/P_{Na^+} = 16.0 ± 1.4)



Figures S9. Single channel conductance histograms at various voltages for **5F12** for determining its K^+/Na^+ selectivity.

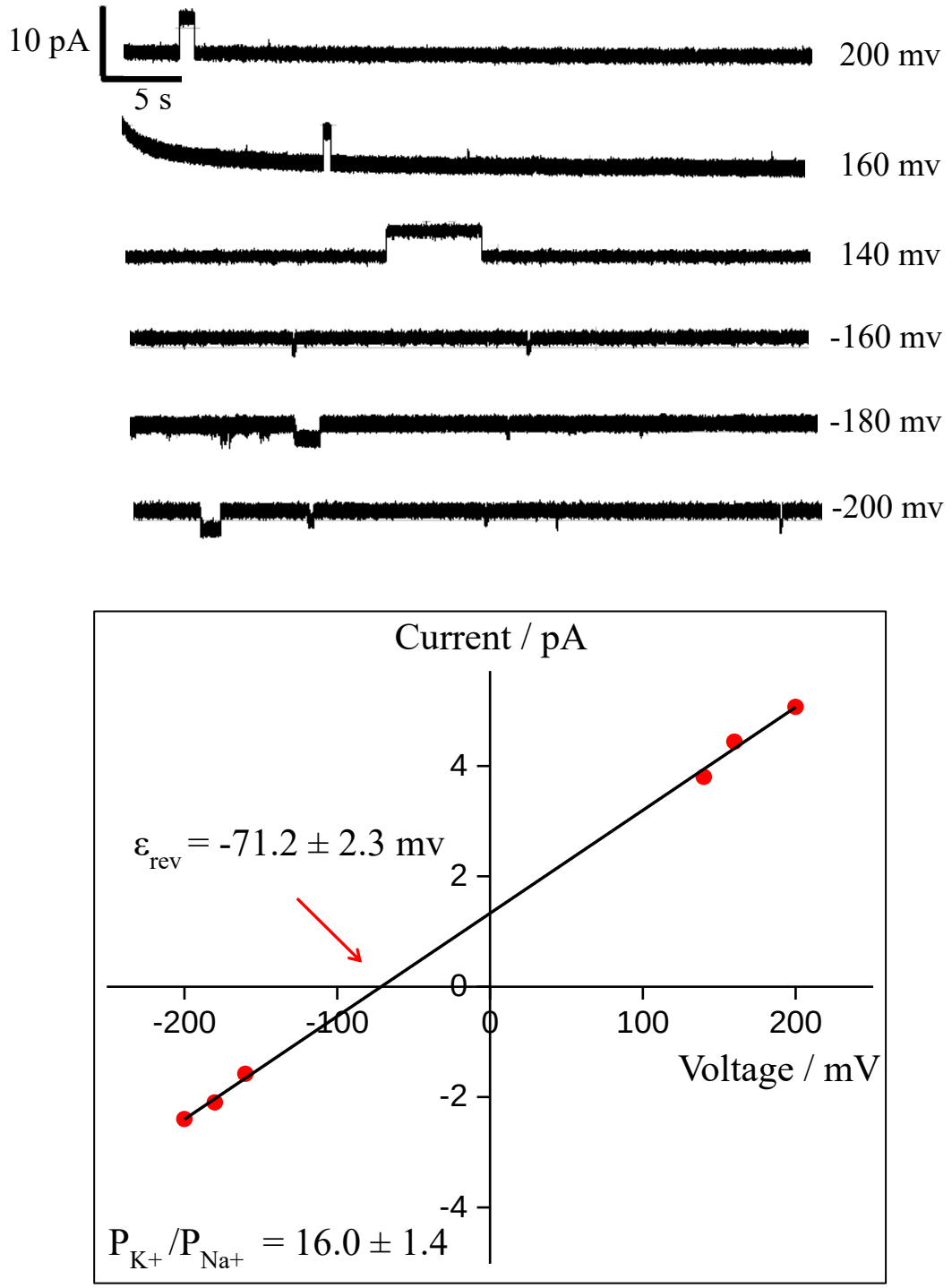
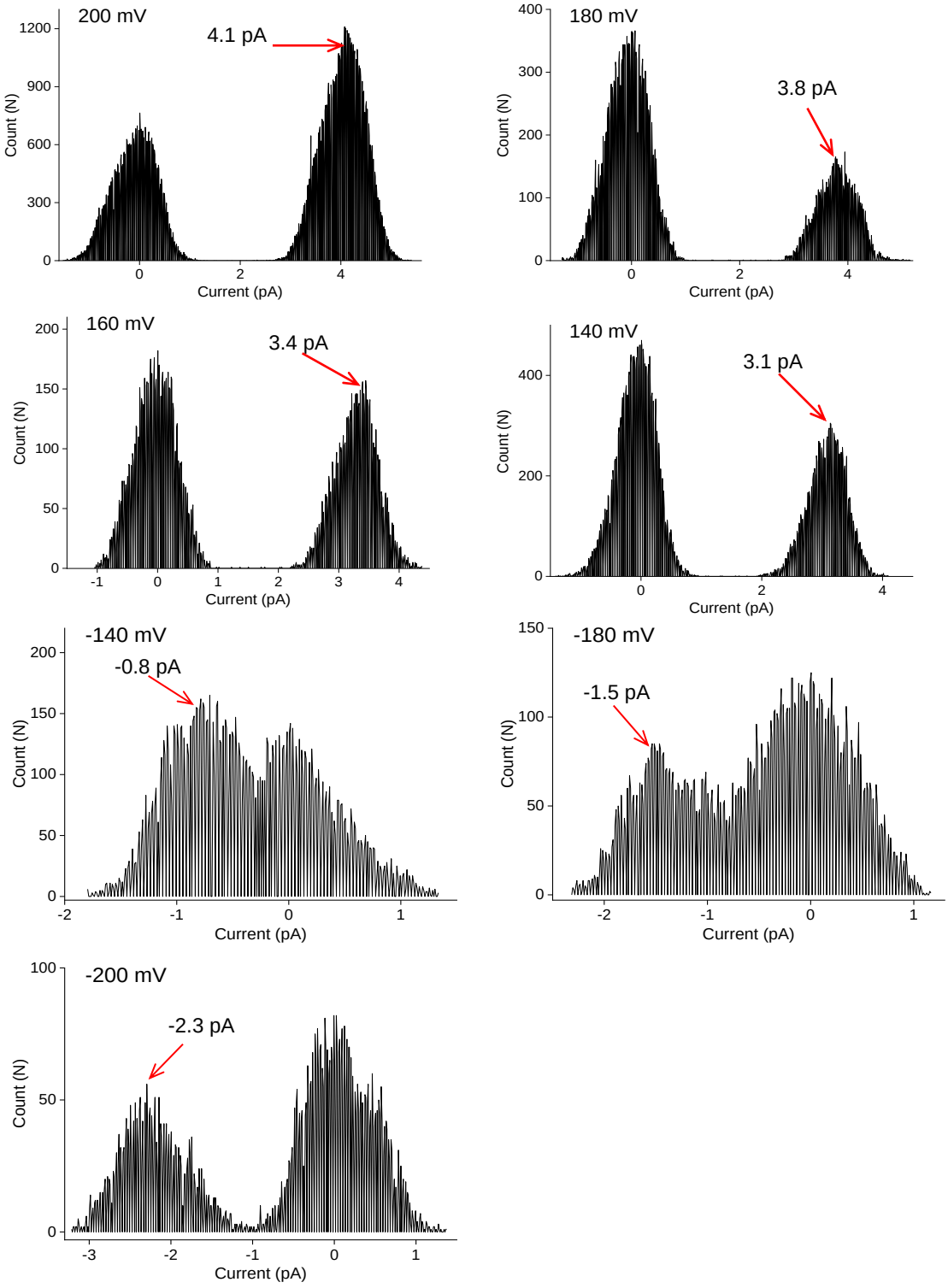
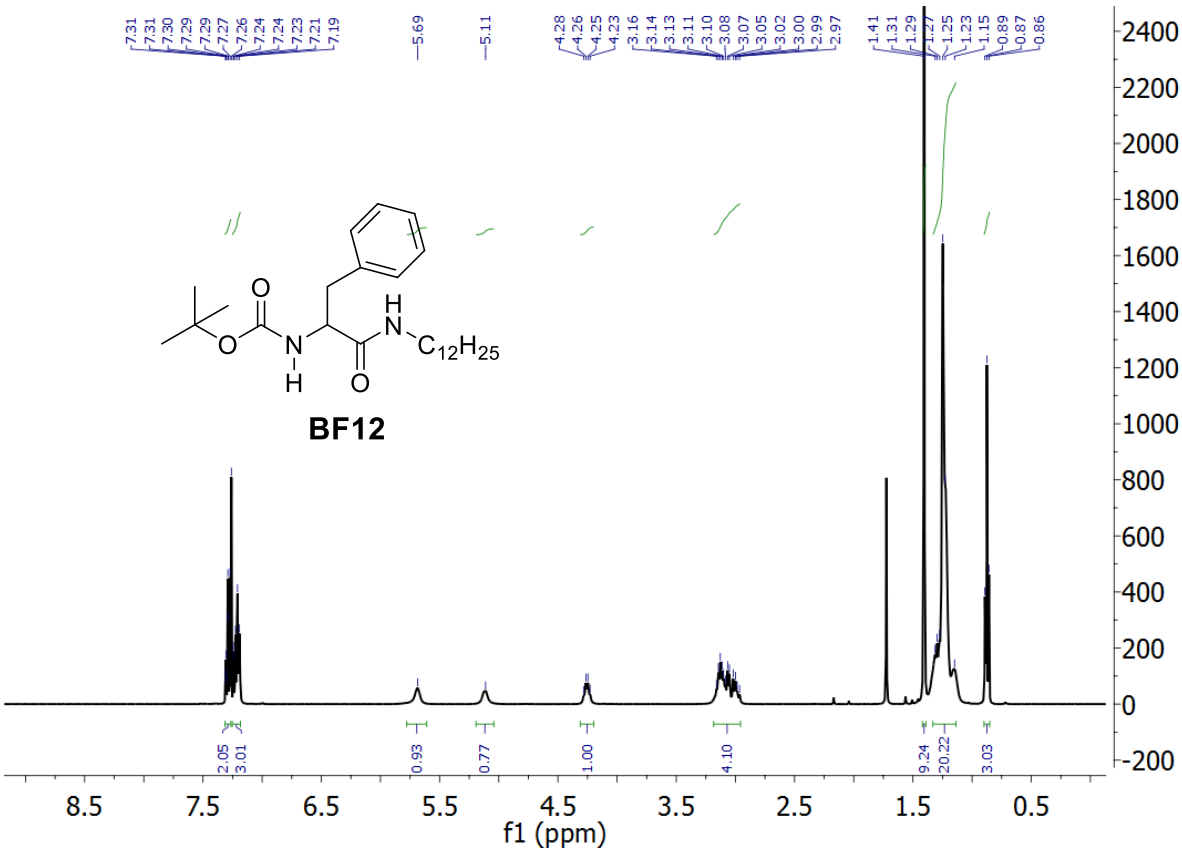


Figure S10. (a) Single channel currents recorded for **5F12**, with *cis* chamber having 1 M KCl and *trans* chamber having 1 M NaCl. (b) I-V curve for determining K+/Na+ selectivity.

5F14 (P_{K^+}/P_{Na^+} = 20.1 ± 1.5)



Figures S11. Single channel conductance histograms at various voltages for **5F14** for determining its K^+/Na^+ selectivity.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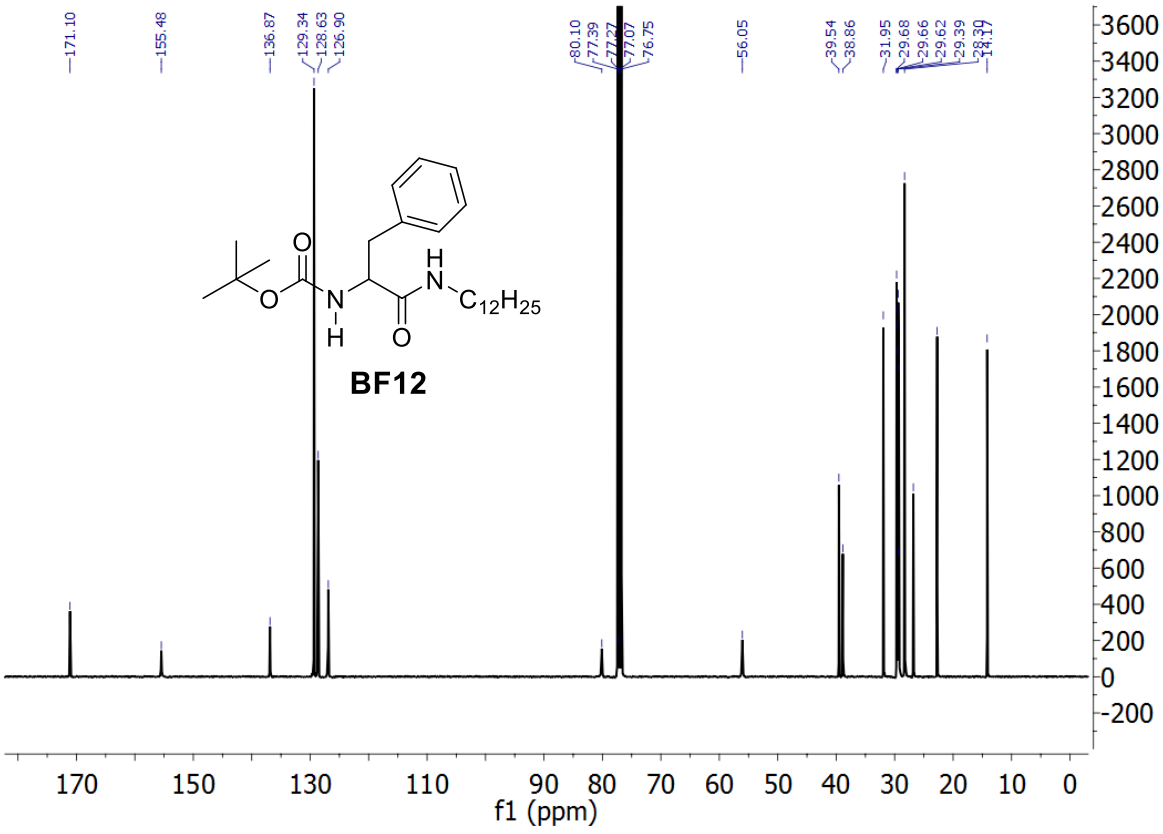
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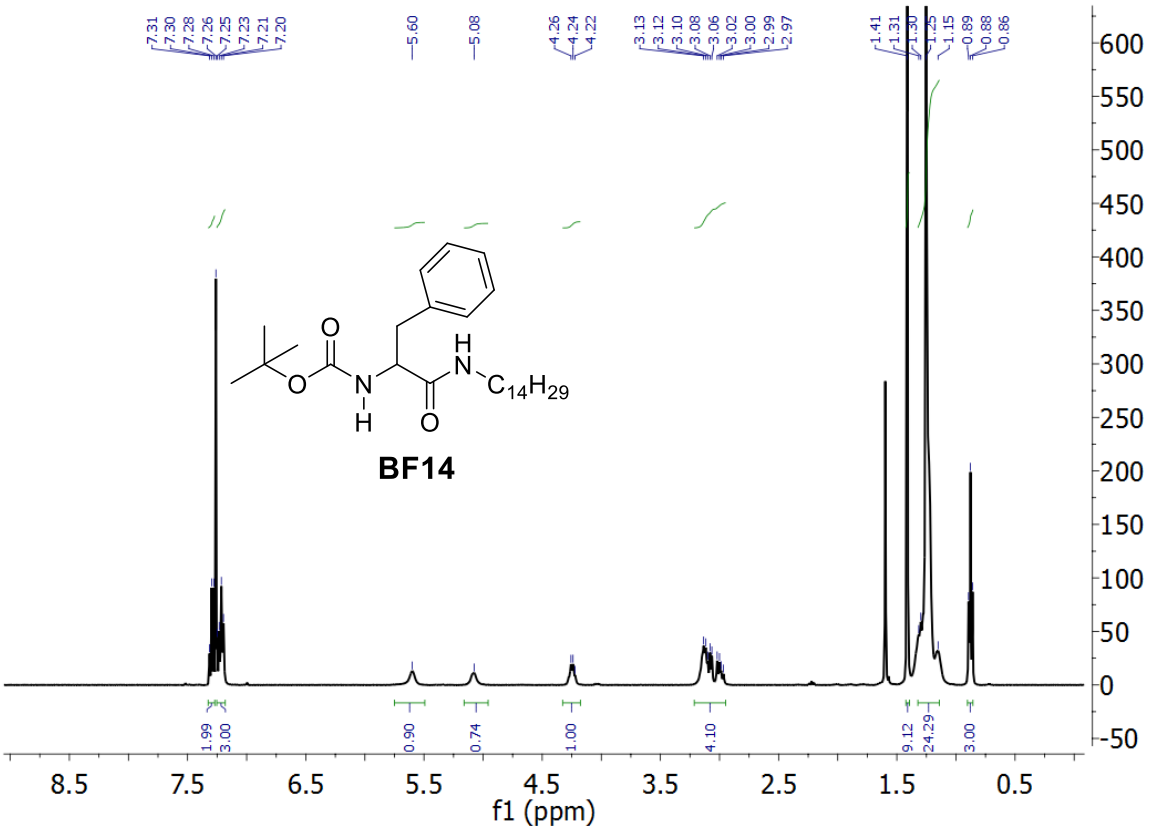
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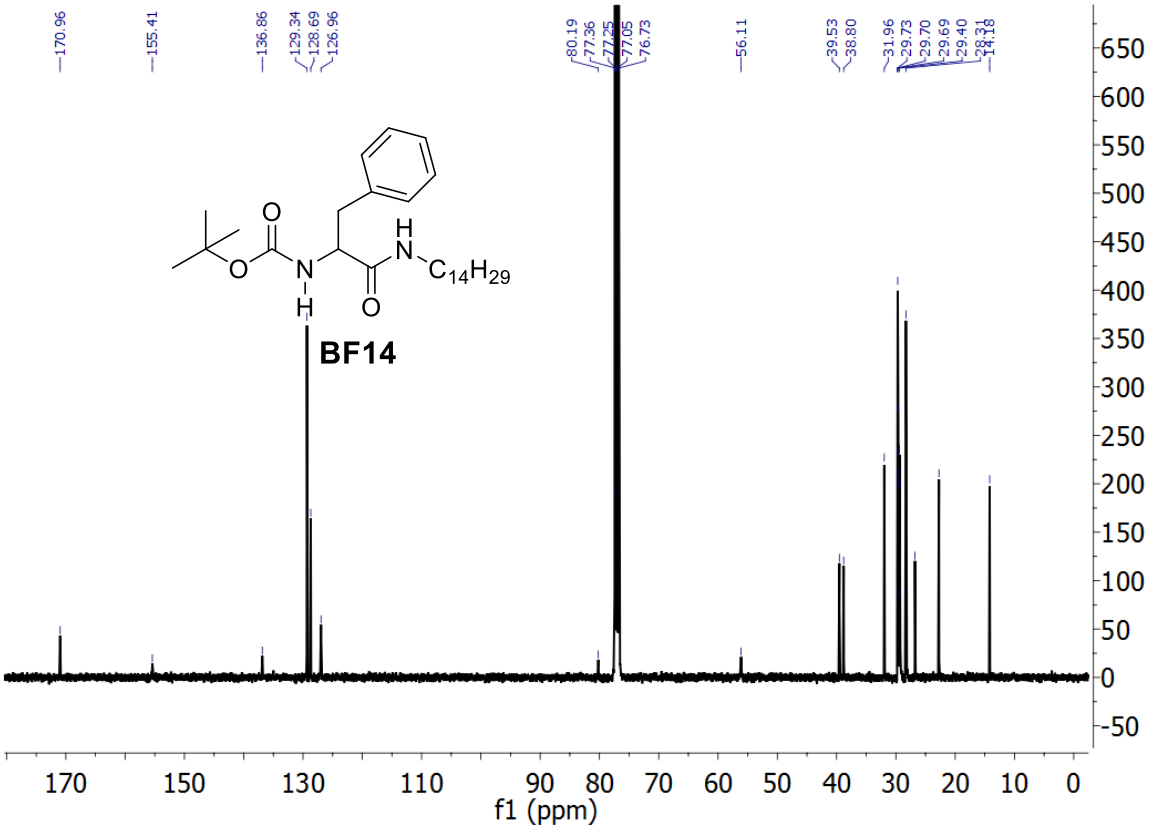
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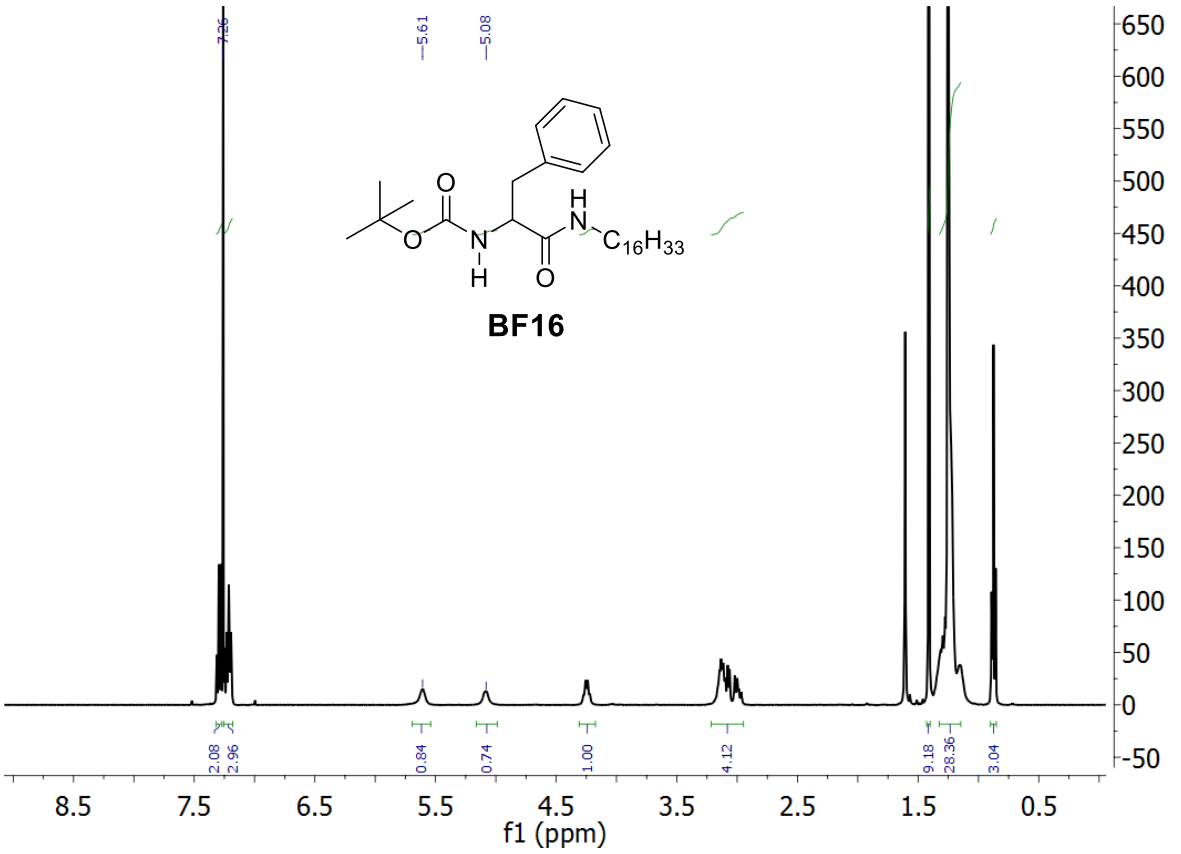
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O
N
H
H
N
C16H33
O
BF16
2.08
2.96
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0.74
1.00
4.12
9.18
28.36
3.04
650
600
550
500
450
400
350
300
250
200
150
100
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5.5
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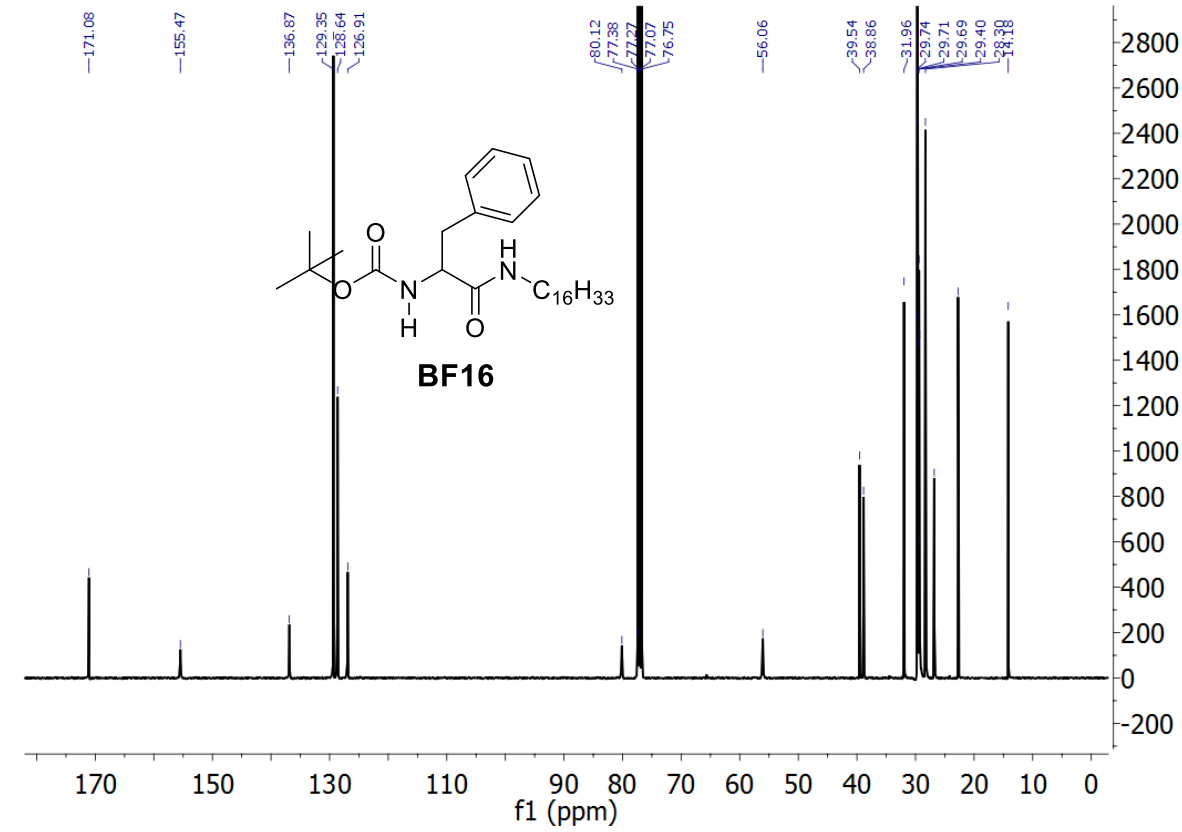
3.5
2.5
1.5
0.5
f1 (ppm)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38.86
31.96
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29.69
29.40
28.30
14.18
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O
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H
H
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C16H33
O
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2600
2400
2200
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1800
1600
1400
1200
1000
800
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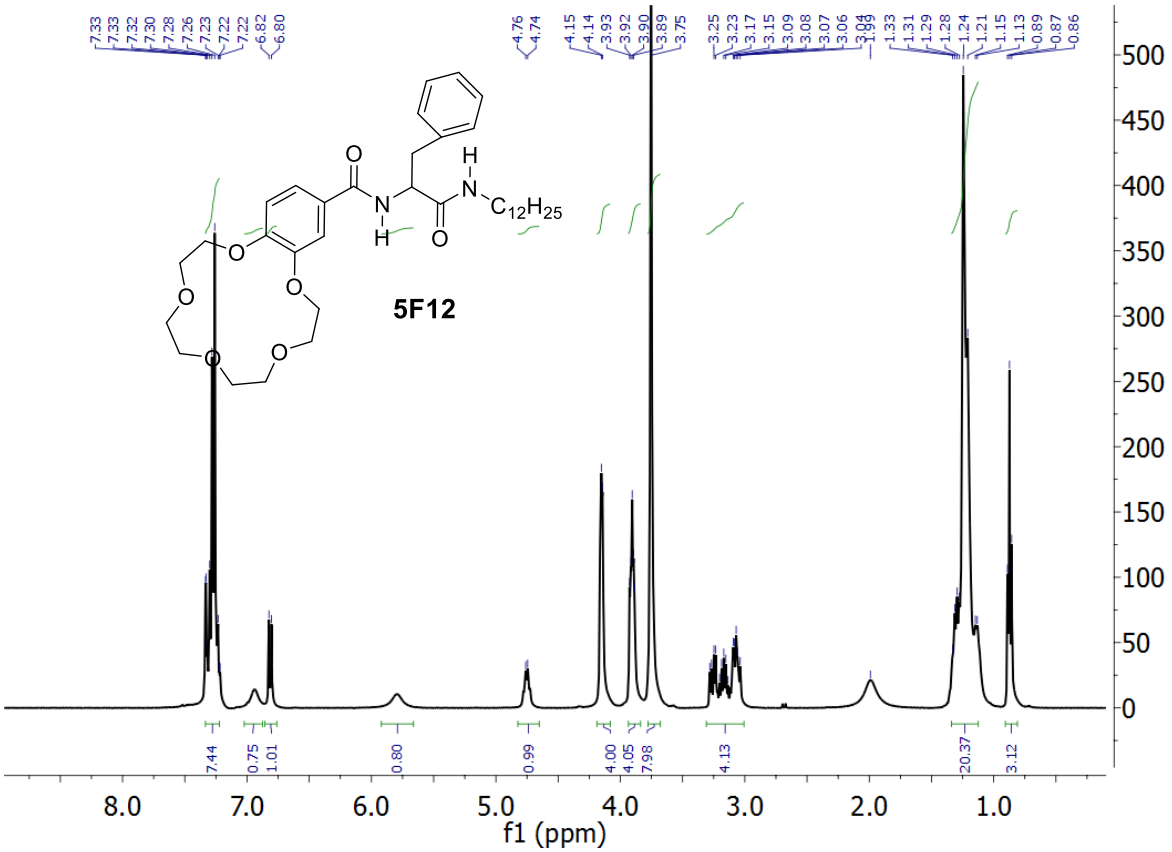
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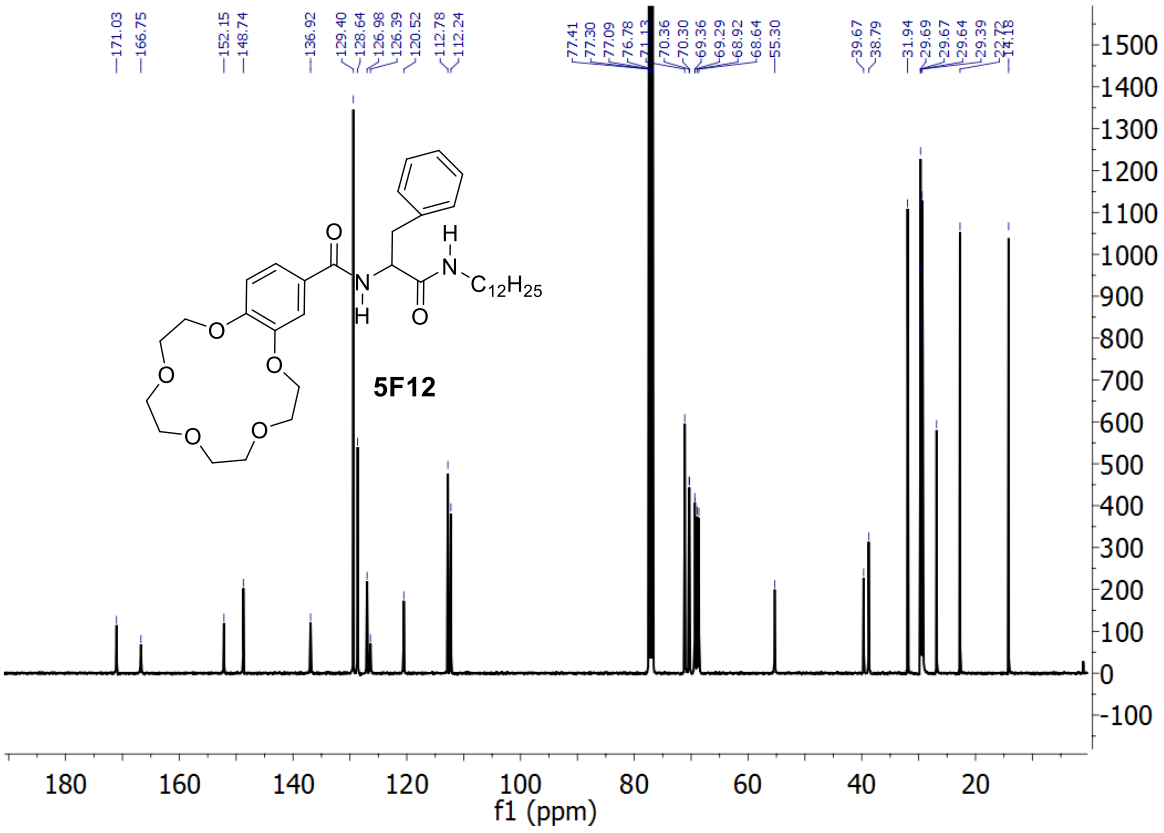
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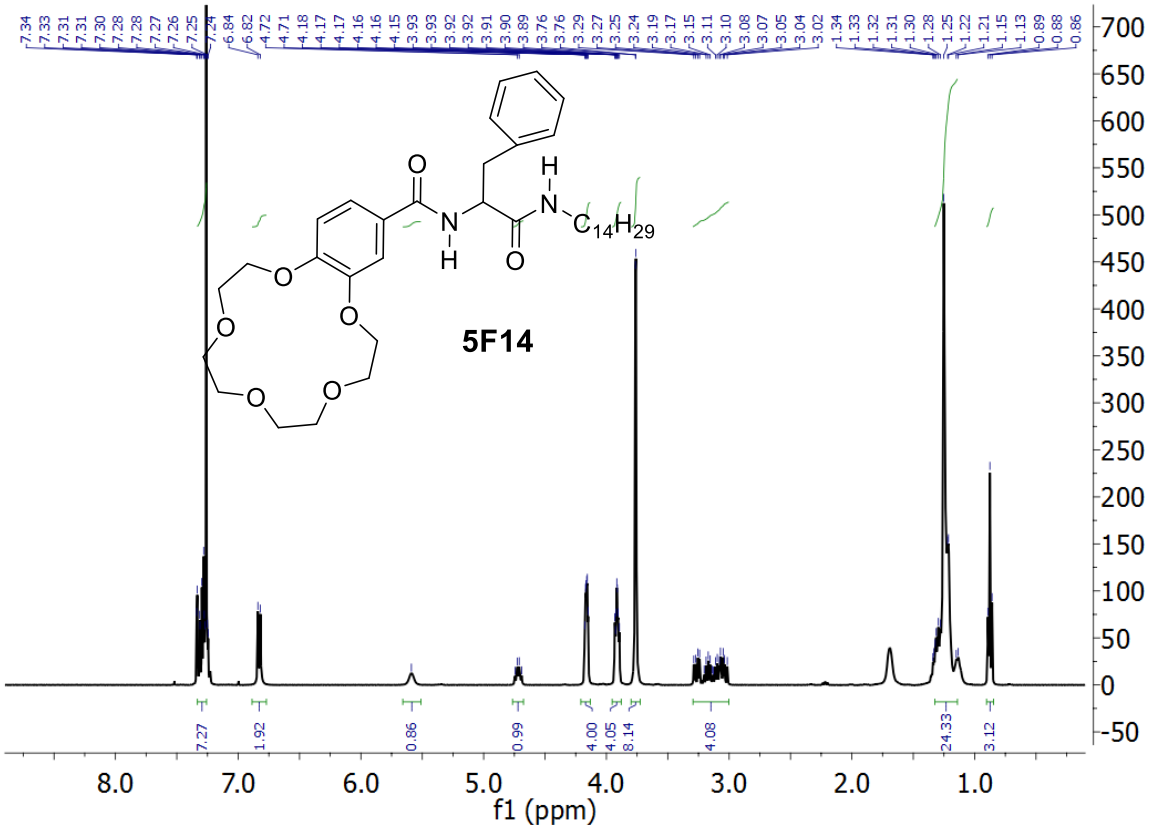
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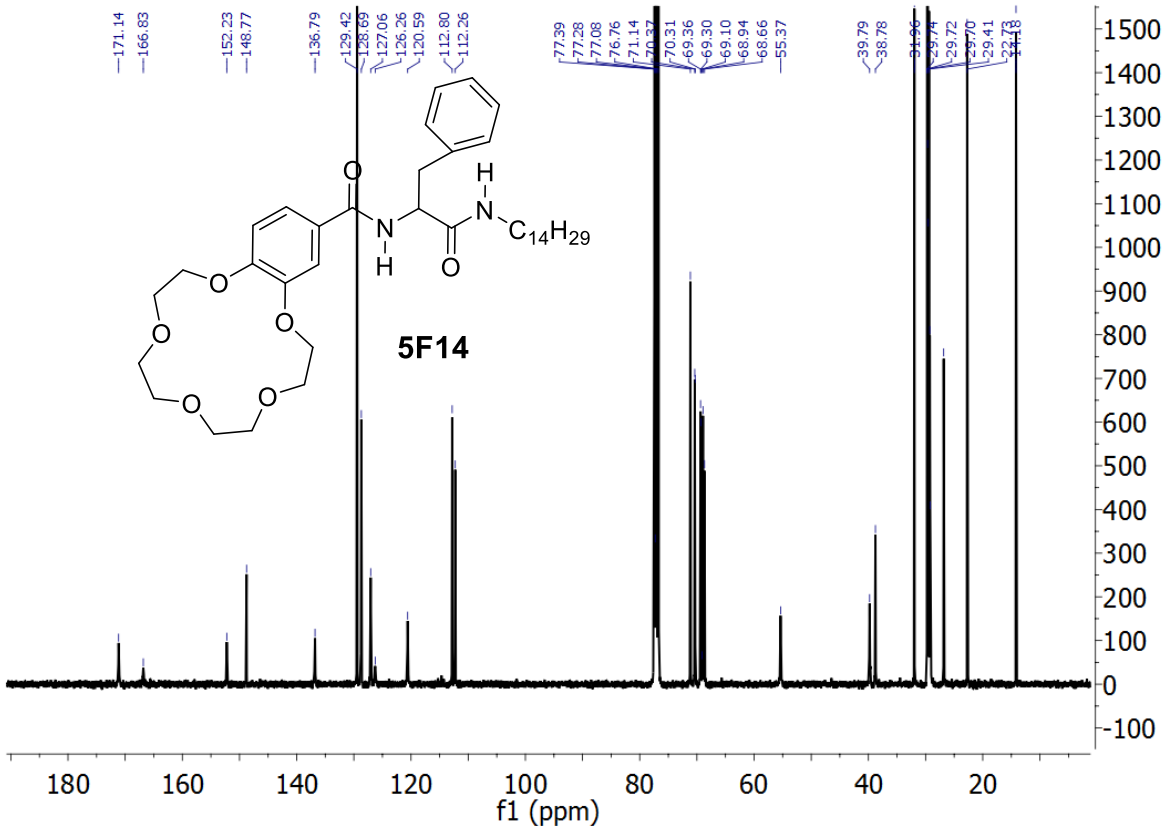
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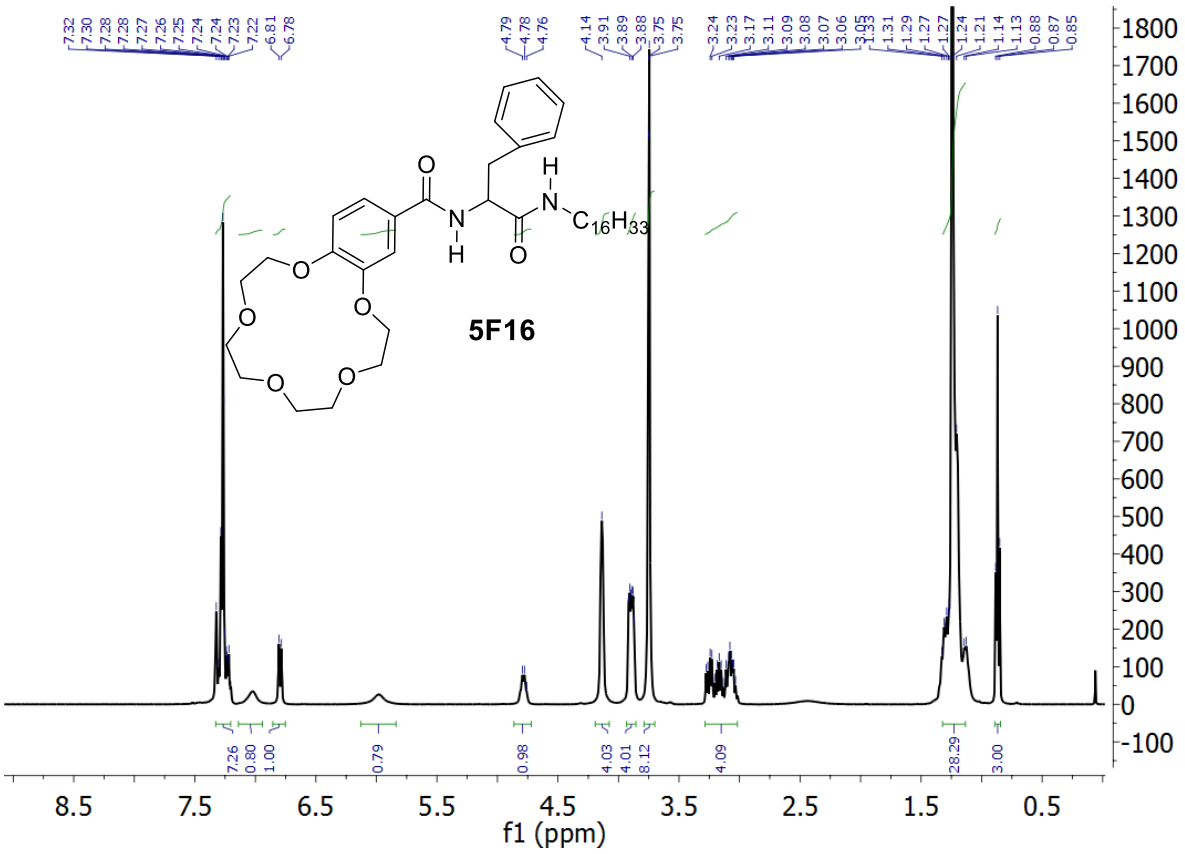
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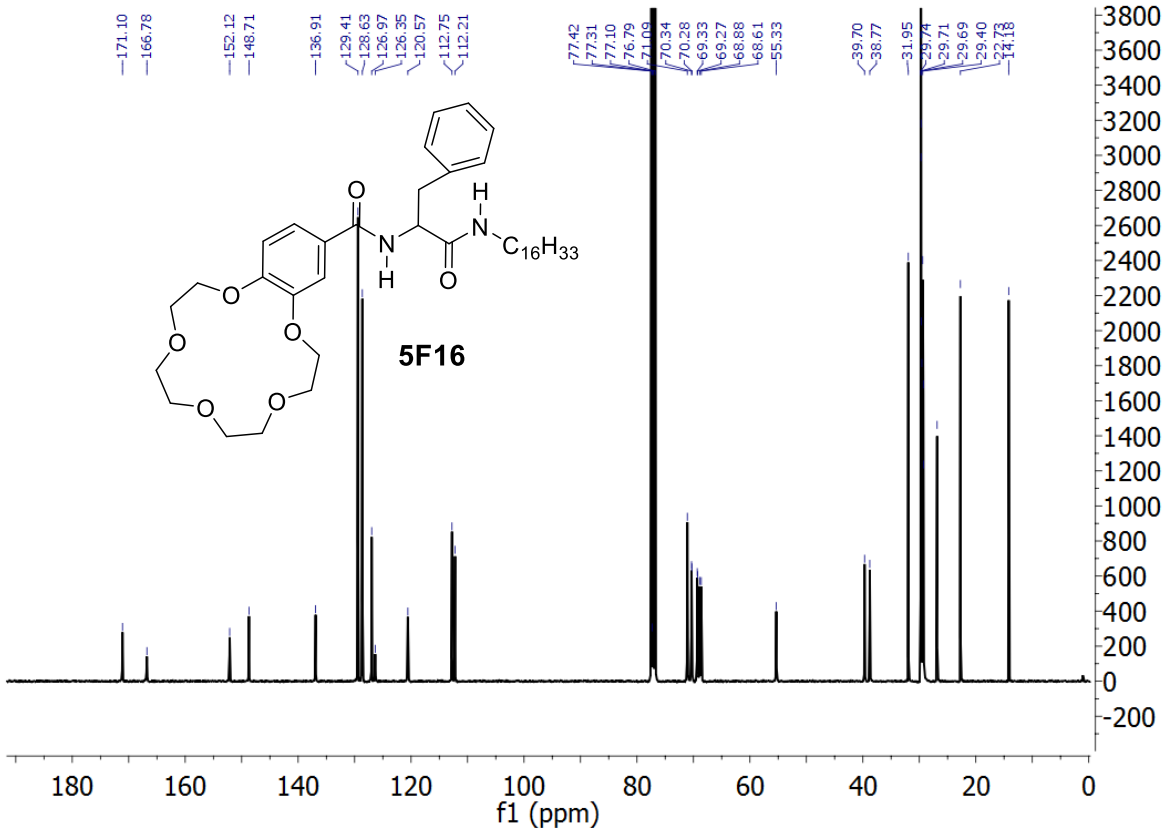
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