Supporting Information

Enhancing K^+ transport activity and selectivity of synthetic K^+ channels via electron-donating effects

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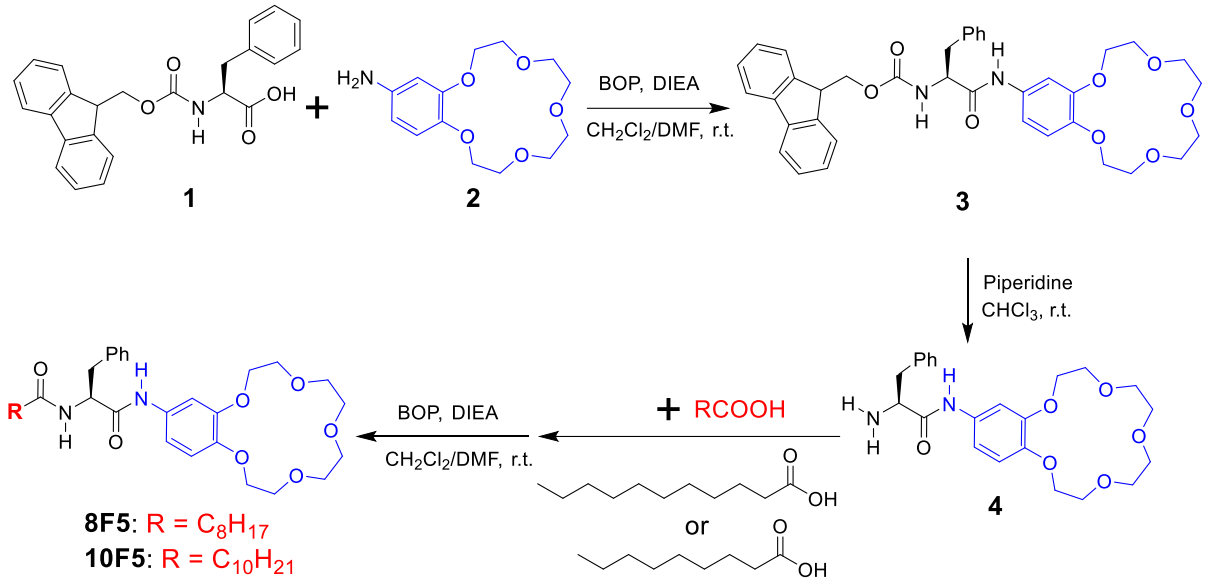
General Remarks

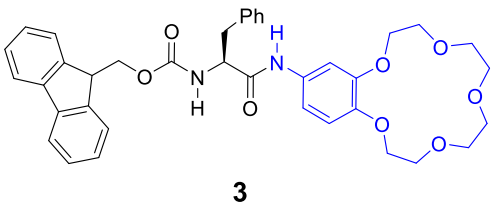
All the reagents were obtained from commercial suppliers and used as received unless otherwise noted. Aqueous solutions were prepared from MilliQ water. Flash column chromatography was performed using pre-coated 0.2 mm silica plates from Selecto Scientific. Chemical yield refers to pure isolated substances. ^{1}H and ^{13}C NMR spectra were recorded on either a Bruker ACF-400 (400 MHz). The solvent signal of $CDCl_3$ was referenced at δ = 7.26 ppm. The solvent signal of DMSO-d_6 was referenced at δ = 2.50 ppm. Coupling constants (J values) are reported in 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 ACF-400 (400 MHz). The solvent, $CDCl_3$, was referenced at δ = 77 ppm. DMSO-*d6* was referenced at δ = 39.5 ppm. $CDCl_3$ (99.8%-Deuterated) and DMSO-*d6* (99.5%-Deuterated) were purchased from Aldrich and used without further purification. Mass spectra were acquired with Waters 3100 mass spectrometer.

Synthetic Scheme and Chemical Structures of 8F5 and 10F5



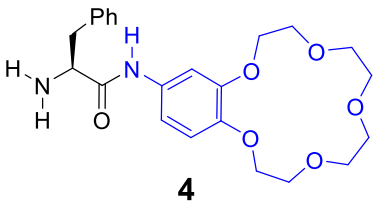
Experimental Procedures and Compound Characterizations

Compounds **5F8** and **5F10** were prepared according to the procedures reported (C. L. Ren, J. Shen and H. Q. Zeng, *J. Am. Chem. Soc.*, 2017, **139**, 12338). The corresponding ^{1}H NMR spectra and data are consistent with the published ones. BOP = benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluorophosphate



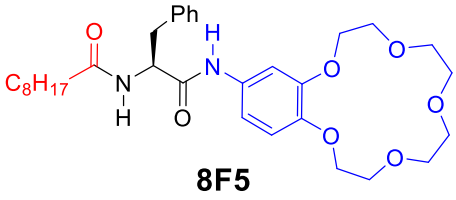
Fmoc-Phe-OH (compound **1**, 271 mg, 0.70 mmol), 4'-aminobenzo-15-crown-5 (compound **2**, 198 mg, 0.70 mmol) and BOP (371 mg, 0.84 mmol) were dissolved in CH_2Cl_2/DMF (8 mL:2 mL) to which DIEA (*N*,*N*-diisopropylethylamine, 0.78 ml, 4.40 mmol) was added. The reaction mixture was stirred for 18 h at room temperature. Removal of the solvent in *vacuo* gave the crude product which was washed with acetonitrile (10 mL) to yield the pure product **3** as a pale pink solid. Yield: 340 mg, 75%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 7.5 Hz, 2H), 7.55 (t, *J* = 6.6 Hz, 2H), 7.51 (s, 1H), 7.42 (t, *J* = 7.4 Hz, 2H), 7.35 – 7.29 (m, 5H), 7.26 (s, 2H), 7.12 (s, 1H), 6.77 (d, *J* = 8.6 Hz, 1H), 6.70 (d, *J* = 7.8 Hz, 1H), 5.54 (d, *J* = 5.9 Hz, 1H), 4.58 – 4.32 (m, 3H), 4.22 (t, *J* = 6.8 Hz, 1H), 4.11 (dd, *J* = 5.3, 3.1 Hz,

4H), 3.91 (dd, *J* = 8.3, 3.9 Hz, 4H), 3.77 (s, 8H), 3.32 – 3.00 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 156.2, 149.2, 146.0, 143.6, 141.3, 136.4, 131.0, 129.4, 128.9, 127.8, 127.3, 127.2, 125.0, 120.1, 114.3, 112.7, 106.9, 71.0, 70.9, 70.5, 70.4, 69.6, 69.4, 68.7, 67.2, 57.0, 47.1, 38.6. MS-ESI: calculated for $[M+Na]^+$ ($C_{38}H_{40}N_2O_8Na$): *m/z* 675.74, found: *m/z* 675.28.



4

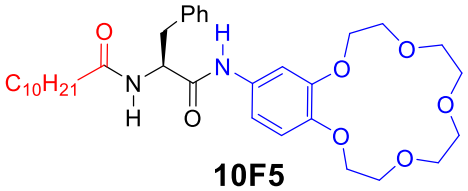
To a solution of **3** (261 mg, 0.40 mmol) in $CHCl_3$ (5 mL) was added piperidine (0.5 mL), and reaction was allowed to stir at room temperature for 12 h. The solvent was then removed in *vacuo* and the crude product was purified by flash column chromatography (MeOH:CH_2Cl_2 = 1:20, v:v) to afford the target compound **4** as a white solid. Yield: 102 mg, 60%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.39 (s, 1H), 7.43 (d, *J* = 2.4 Hz, 1H), 7.38 – 7.32 (m, 2H), 7.31 – 7.25 (m, 3H), 6.96 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.83 (d, *J* = 8.6 Hz, 1H), 4.19 – 4.10 (m, 4H), 3.95 – 3.85 (m, 4H), 3.79 – 3.71 (m, 9H), 3.39 (dd, *J* = 13.8, 3.8 Hz, 1H), 2.78 (dd, *J* = 13.8, 9.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 149.2, 145.4, 137.7, 132.0, 129.3, 128.9, 127.0, 114.4, 111.8, 106.071, 70.9, 70.8, 70.4, 70.3, 69.6, 69.4, 68.6, 56.9, 40.6. MS-ESI: calculated for $[M+Na]^+$ ($C_{23}H_{30}N_2O_6$ Na)： *m/z* 453.21, found: *m/z 453.20*.



8F5

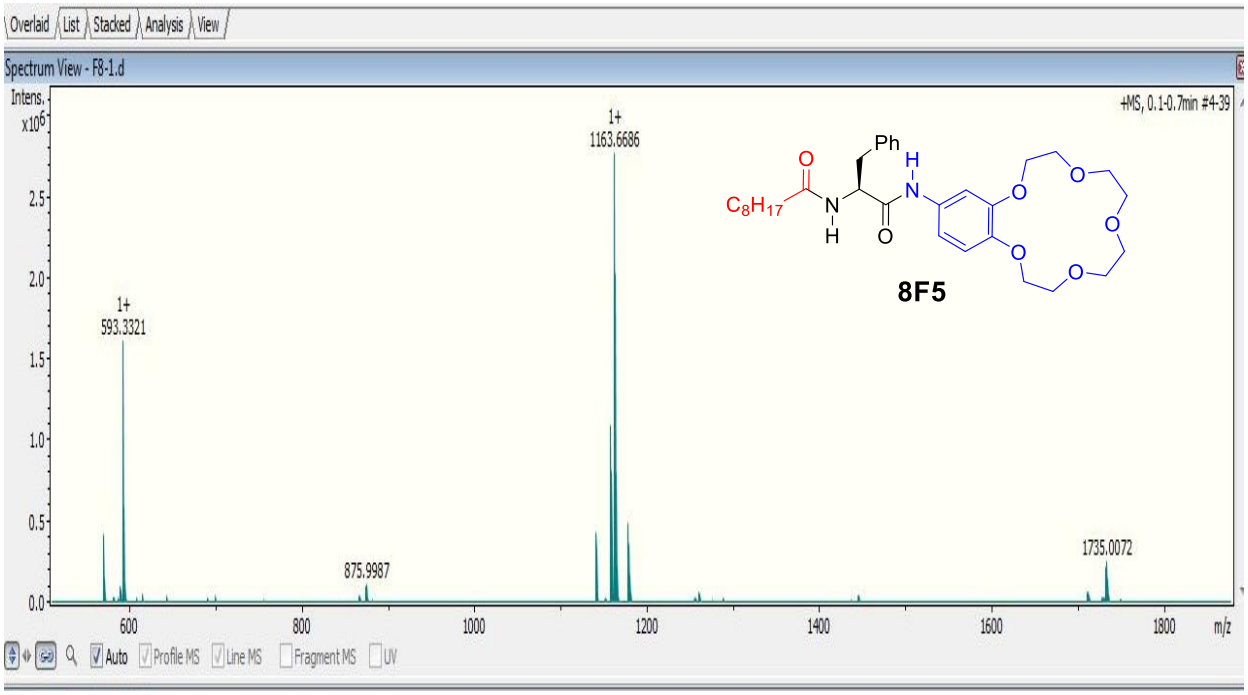
Compound **4** (43 mg, 0.10 mmol), nonanoic acid (15.8 mg, 0.10 mmol) and BOP (66 mg, 0.15 mmol) were dissolved in CH_2Cl_2/DMF (4 mL:1 mL) to which DIEA (53 μl, 0.30 mmol) was added. The reaction mixture was stirred for 20 h at room temperature. Solvents were removed in *vacuo* and the crude product was dissolved in CH_2Cl_2 (30 mL), and washed with water (2 x 40 mL), which was purified by flash column chromatography (MeOH:CH_2Cl_2 = 3:100, v:v) to afford the target compound **8F5** as a white solid. Yield: 30 mg, 55%. ^{1}H NMR (400 MHz, DMS-d_6) δ 9.98 (s, 1H), 8.20 (d, *J* = 8.3 Hz, 1H), 7.33 – 7.22 (m, 5H), 7.22 – 7.16 (m, 1H), 7.10 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.89 (d, *J* = 8.7 Hz, 1H), 4.63 (td, *J* = 9.6, 4.9 Hz, 1H), 4.03 – 3.90 (m, 4H), 3.80 – 3.69 (m, 4H), 3.61 (d, *J* = 2.0 Hz, 8H), 3.01 (dd, *J* = 13.7, 4.8 Hz, 1H),

2.82 (dd, *J* = 13.6, 9.9 Hz, 1H), 2.05 (t, *J* = 7.3 Hz, 2H), 1.36 (dd, *J* = 14.5, 7.3 Hz, 2H), 1.31 – 1.06 (m, 10H), 0.86 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 170.4, 148.8, 145.0, 138.3, 133.2, 129.6, 128.5, 126.7, 114.7, 112.2, 106.5, 70.9, 70.3, 70.2, 69.4, 69.3, 68.7, 55.1, 38.2, 35.6, 31.7, 29.2, 29.1, 28.9, 25.7, 22.6, 14.5. MS-ESI: calculated for $[M+Na]^+$ ($C_{32}H_{46}N_2O_7$ Na)： *m/z* 593.73, found: *m/z* 593.33.



10F5

Compound **4** (43 mg, 0.1 mmol), undecanoic acid (18.6 mg, 0.1 mmol) and BOP (66 mg, 0.15 mmol) were dissolved in CH_2Cl_2/DMF (4 mL:1 mL) to which DIEA (53 μl, 0.3 mmol) was added. The reaction mixture was stirred for 20 h at room temperature. Solvents were 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 purified by flash column chromatography (MeOH:CH_2Cl_2 = 3:100, v:v) to afford the target compound **10F5** as a white solid. Yield: 35 mg, 59%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.98 (s, 1H), 8.20 (d, *J* = 8.3 Hz, 1H), 7.33 – 7.23 (m, 5H), 7.22 – 7.16 (m, 1H), 7.10 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.89 (d, *J* = 8.7 Hz, 1H), 4.63 (td, *J* = 9.6, 4.9 Hz, 1H), 4.06 – 3.93 (m, 4H), 3.82 – 3.71 (m, 4H), 3.61 (d, *J* = 2.0 Hz, 8H), 3.01 (dd, *J* = 13.7, 4.8 Hz, 1H), 2.82 (dd, *J* = 13.7, 4.8 Hz, 1H), 2.05 (t, *J* = 7.3 Hz, 2H), 1.36 (dd, *J* = 14.5, 7.3 Hz, 2H), 1.28 – 1.08 (m, 14H), 0.86 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 170.4, 148.8, 145.0, 138.3, 133.2, 129.6, 128.5, 126.7, 114.7, 112.2, 106.5, 70.9, 70.3, 70.2, 69.4, 69.3, 69.3, 68.7, 55.1, 38.2, 35.6, 31.8, 29.5, 29.4, 29.3 29.2, 28.9, 25.7, 22.6, 14.5. MS-ESI: calculated for $[M+H]^+$ ($C_{32}H_{47}N_2O_7$)： *m/z* 599.78, found: *m/z* 599.38.



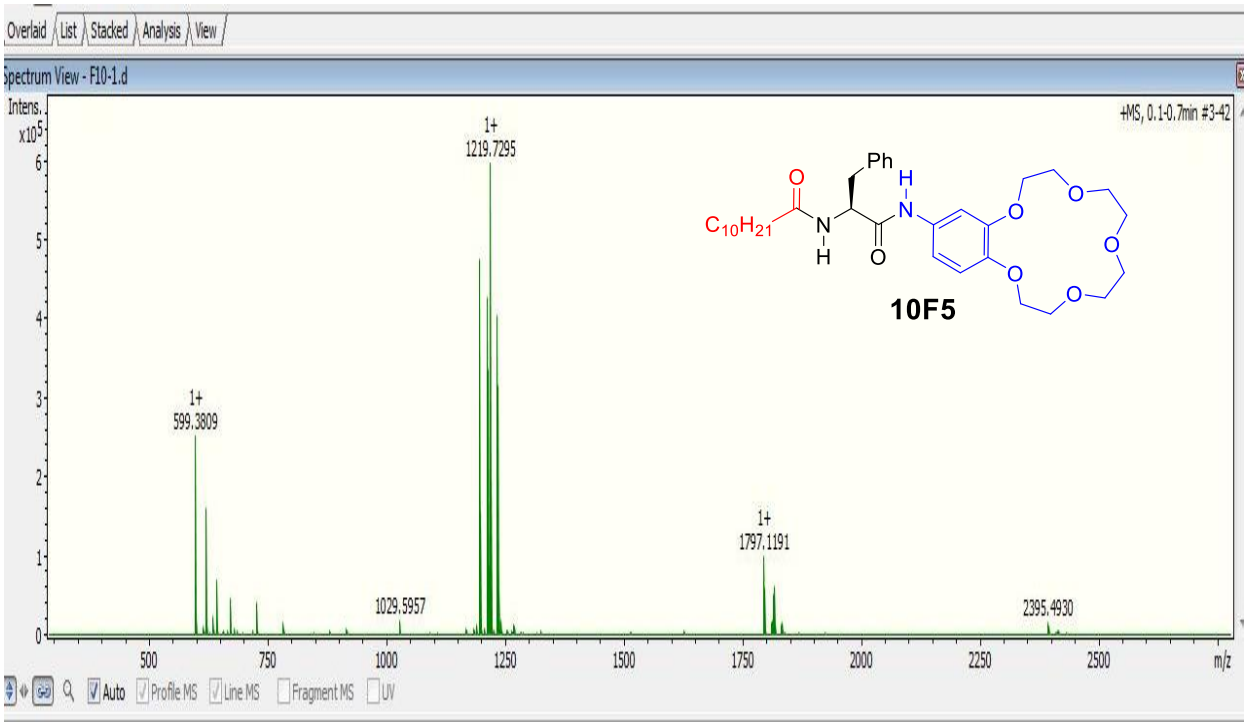


Figure S1. EIS-MS specta of **8F5** and **10F5**, revealing the presence of doubly and triply H-bonded ensembles, i.e., $(\mathbf{8F5})_2$, $(\mathbf{8F5})_3$, $(\mathbf{10F5})_2$ and $(\mathbf{10F5})_3$.

Protocol for Ion Transport Study and EC_{50} Measurements

Egg yolk L-α-phosphatidylcholine (EYPC, 0.3 ml, 25 mg/mL in $CHCl_3$, Avanti Polar Lipids, USA) was dissolved in $CHCl_3$ (10 mL). The mixed solvents were removed under reduced pressure at room temperature. After drying the resulting film under high vacuum overnight at room temperature, the film was hydrated with 4-(2-hydroxyethyl)-1-piperazine-ethane sulfonic acid (HEPES) buffer solution (1.5 mL, 10 mM HEPES, 100 mM NaCl, pH = 7.0) containing a pH sensitive dye 8-hydrox-ypyrene-1,3,6-trisulfonic acid (HPTS, 0.1 mM) in thermostatic shaker-incubator at 37 ºC for 2 hours to give a milky suspension. The mixture was then subjected to 9 freeze-thaw cycles: freezing in liquid N2 for 30 seconds and heating at 37 ºC for 1.5 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 suspension of LUVs was dialyzed for 16 hours with gentle stirring (300 r/min, 4 ºC) using membrane tube (MWCO = 10,000) against the same HEPES buffer solution (300 mL, without HPTS) for 8 times to remove the unencapsulated HPTS and further diluted to yield LUVs with lipids at a concentration of 6.5 mM.

The HPTS-containing LUV suspension (30 μL, ~~13~~ 6.5 mM in 10 mM HEPES buffer containing 100 mM NaCl at pH = 7.0) was added to a HEPES buffer solution (1.75 mL, 10 mM HEPES, 100 mM NaCl at pH = 8.0) 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 samples, 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 (20 μL, 20% v/v) was immediately added to achieve the maximum change in fluorescence dye emission. The final transport trace was obtained as a ratiometric value of I460/I403 and normalized based on the ratiometric value of I460/I403 after addition of triton using the following equation E1.

$$I_f = [(I_t - I_0)/(I_1 - I_0)] \qquad \text{(E1)}$$

where, I_f = Fractional emission intensity, It = Fluorescence intensity at time t, I_1 = Fluorescence intensity after addition of Triton X-100, and I_0 = Initial fluorescence intensity.

The fractional change R_M^+ was calculated for each curve using the normalized value of I_{460}/I_{403} at 300 s before the addition of triton, with the ratio of blank that contains only DMSO in the absence of channel molecules set as 0 and that of triton as 1. Fitting the fractional transmembrane activity R_M^+ vs channel concentration using the Hill equation: $Y=1/(1+ (EC_{50}/[C])^n)$ gave the Hill coefficient n and EC_{50} values.

Protocol for Membrane Integrity using Self-Quenching CF dye

EYPC (0.3 ml, 25 mg/mL in $CHCl_3$, Avanti Polar Lipids, USA) was dissolved in $CHCl_3$ (10 mL). The mixed solvents were removed under reduced pressure at room temperature. After drying the resulting film under high vacuum overnight at room temperature, the film was hydrated with a HEPES buffer solution (1.5 mL, 10 mM HEPES, 100 mM NaCl, pH = 7.5) containing a 5(6)-fluorescein (CF, 50 mM) in thermostatic shaker-incubator at 37 ^oC for 2 h to give a milky suspension. The mixture was then subjected to 9 freeze-thaw cycles: freezing in liquid N_2 for 30 s and heating at 37 ^oC for 1.5 minutes. The vesicle suspension was extruded through polycarbonate membrane (0.1 μm) to produce a homogeneous suspension of LUVs of about 120 nm in diameter with CF dye encapsulated inside. The suspension of LUVs was dialyzed for 16 h with gentle stirring (300 r/min, 4 ^oC) using membrane tube (MWCO = 10,000) against the same HEPES buffer solution (300 mL, without CF dye) for 8 times to remove the unencapsulated HPTS to yield LUVs with lipids at a concentration of ~~13~~ 6.5 mM.

The CF-containing LUV suspension (30 μL, ~~13~~ 6.5 mM in 10 mM HEPES buffer containing 100 mM NaCl at pH = 7.5) was added to a HEPES buffer solution (1.75 mL, 10 mM HEPES, 100 mM NaCl at pH = 7.5) to create a concentration gradient for CF dye transport study. A solution of **8F5** and **10F5** (5 μM) or natural poreforming peptide **Melittin** in DMSO at different concentrations was then injected into the suspension under gentle stirring. Upon the addition of molecular swing or pore-forming peptide molecules, the emission of CF was immediately monitored at 517 nm with excitations at 492 nm for 300 s using fluorescence spectrophotometer (Hitachi, Model F-7100, Japan). At t = 300 s, an aqueous solution of Triton X-100 (20 μL, 20% v/v) was immediately added to completely dissipate the chloride gradient. The final transport trace was obtained by normalizing the fluorescence intensity using equation E1.

EC$_{50}$ Determination using the HPTS assay and Hill Analysis

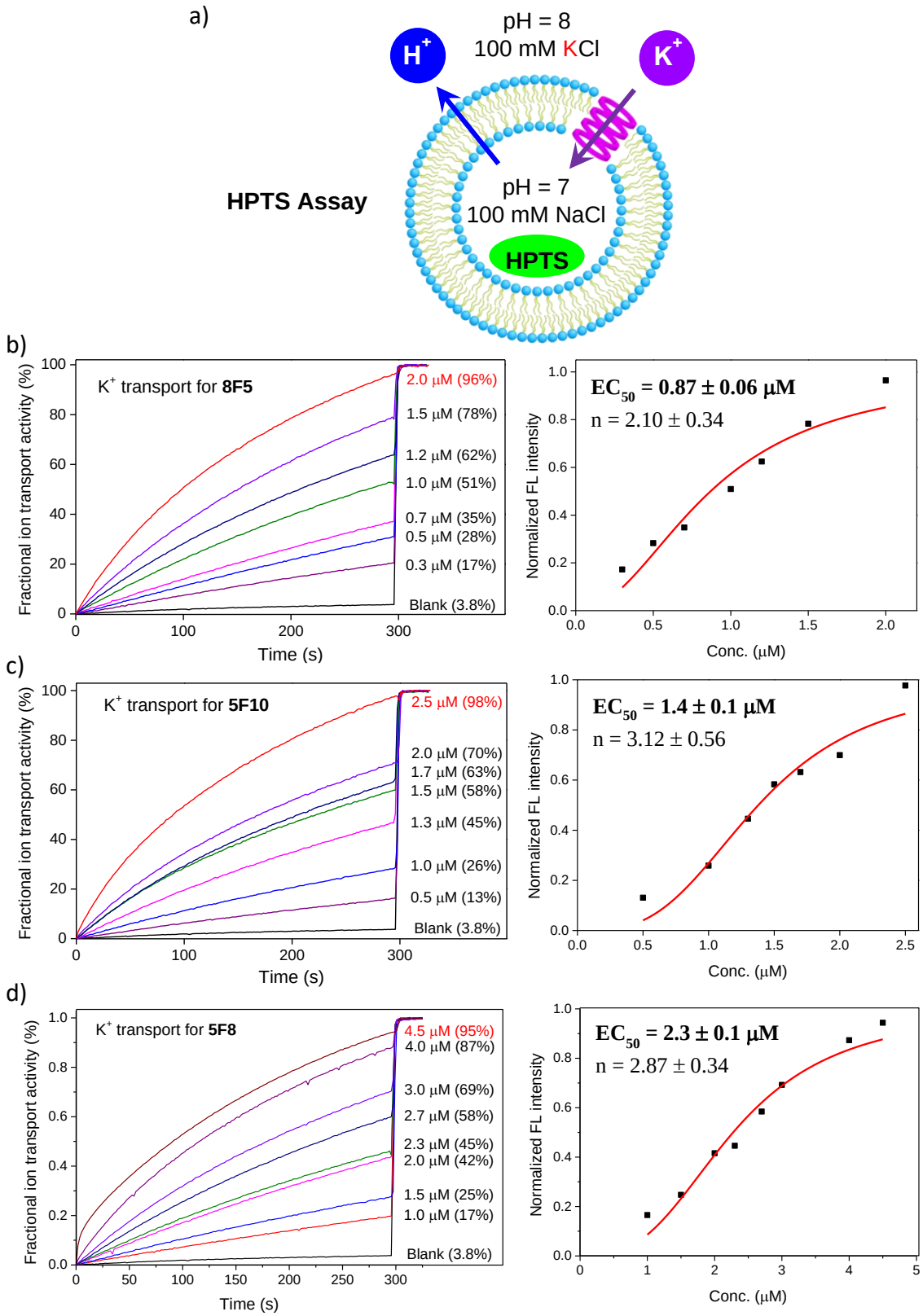
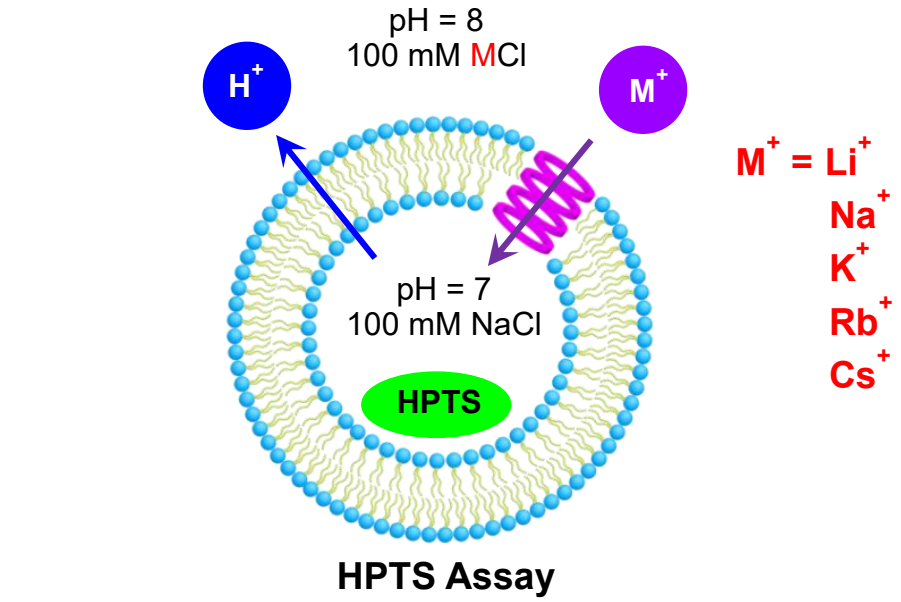


Figure S2. Determination of *EC*$_{50}$ values for K^+ using the ratiometric values of I_{460}/I_{403} at different concentrations as a function of time for **8F5**, **5F8** and **5F10**. All blanks contain DMSO.

Background signals for determining ion transport selectivity using the HPTS assay



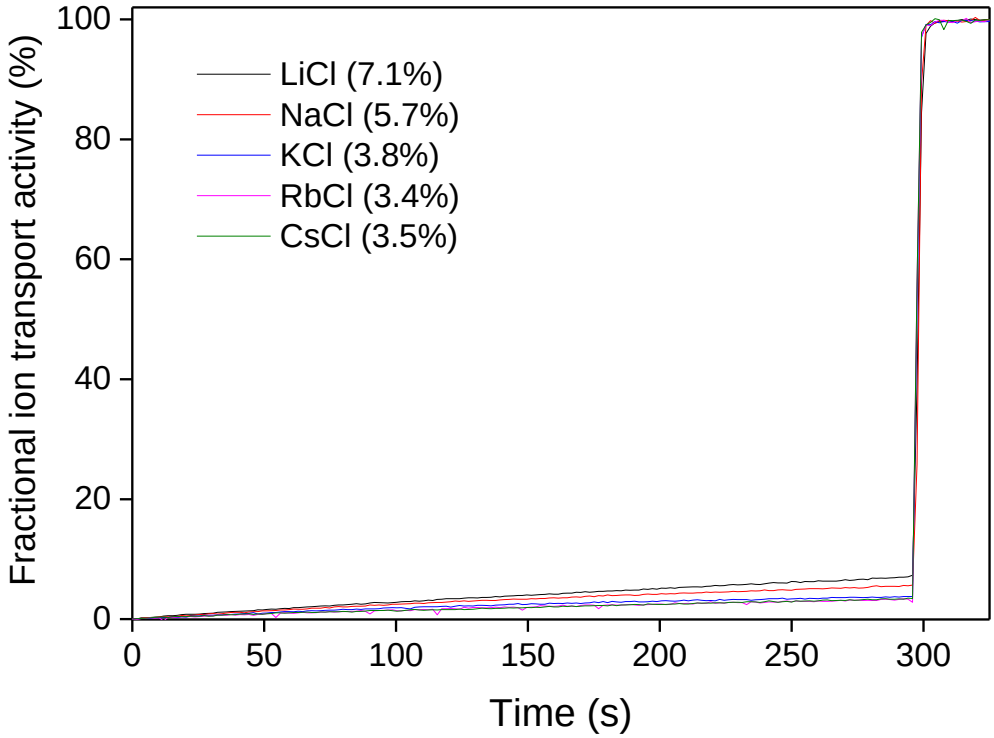
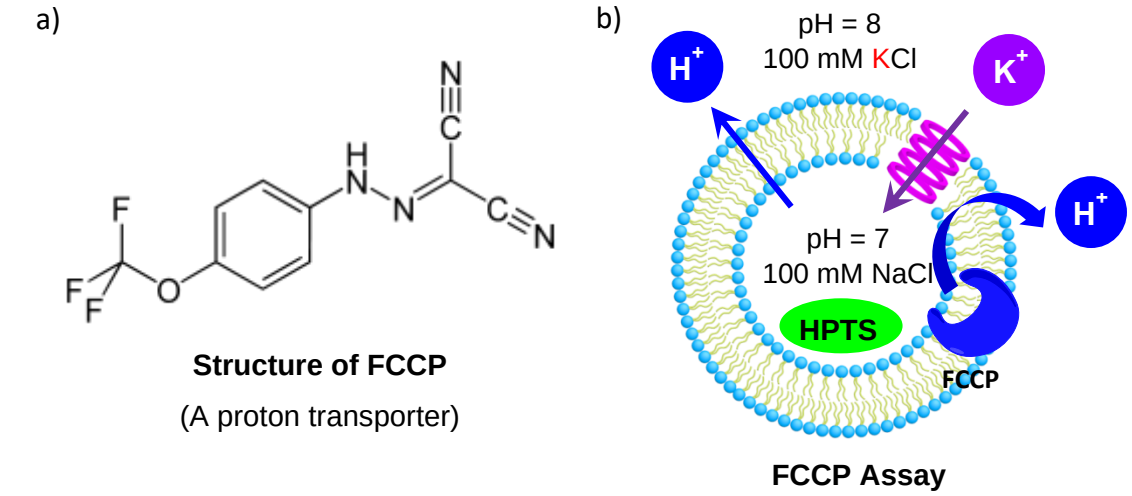


Figure S3. Backgrounk signals for various extravesicular salts (100 mM MCl, M = Li^+, Na^+, K^+, Rb^+, and Cs^+) for the LUVs prepared using the membrane dialysis methods. All blanks contain DMSO.



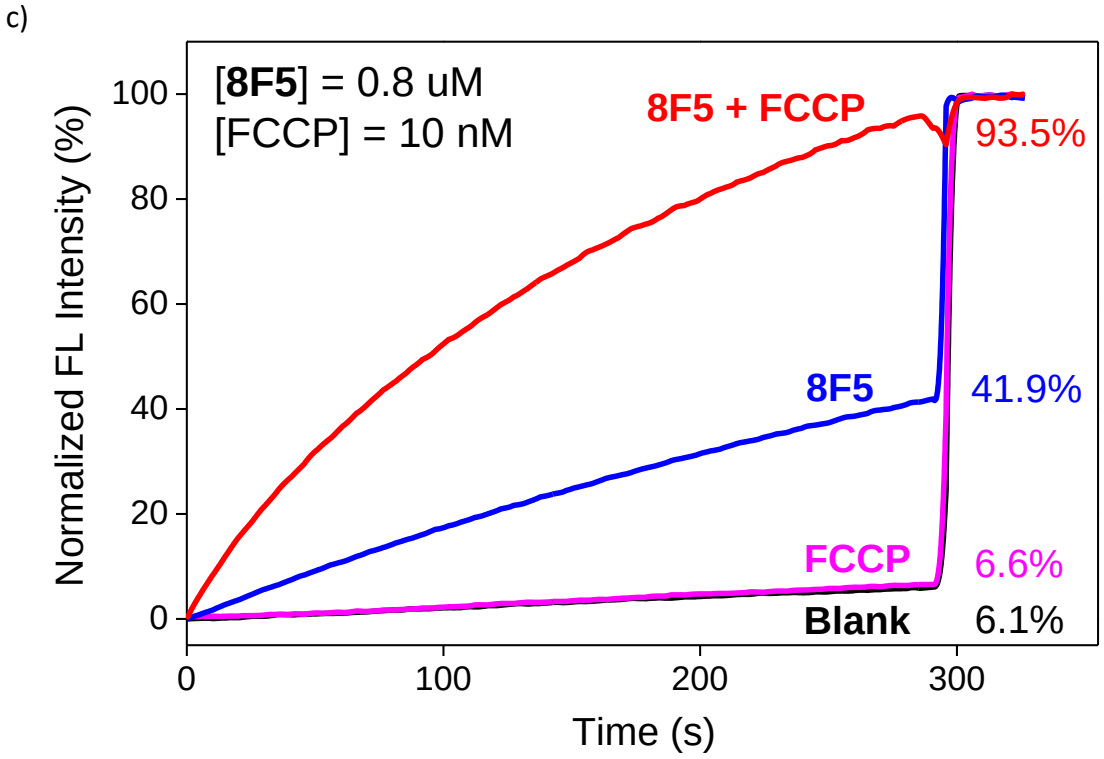


Figure S4. FCCP assay to confirm that **8F5**-mediated K^+ transport is much faster than that of H^+. The blank contains DMSO.

Single channel current measurement using planar lipid bilayer workstation (Warner Instruments, Hamden, CT):

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 of the planar lipid bilayer workstation (Scheme S1), followed by removing the n-decane using nitrogen gas. This step to pretreat the aperture with lipidcontaining n-decane is necessary in order to increase the chance of channel insertion into the lipid bilayer painted across the apterture in the subsequent step.

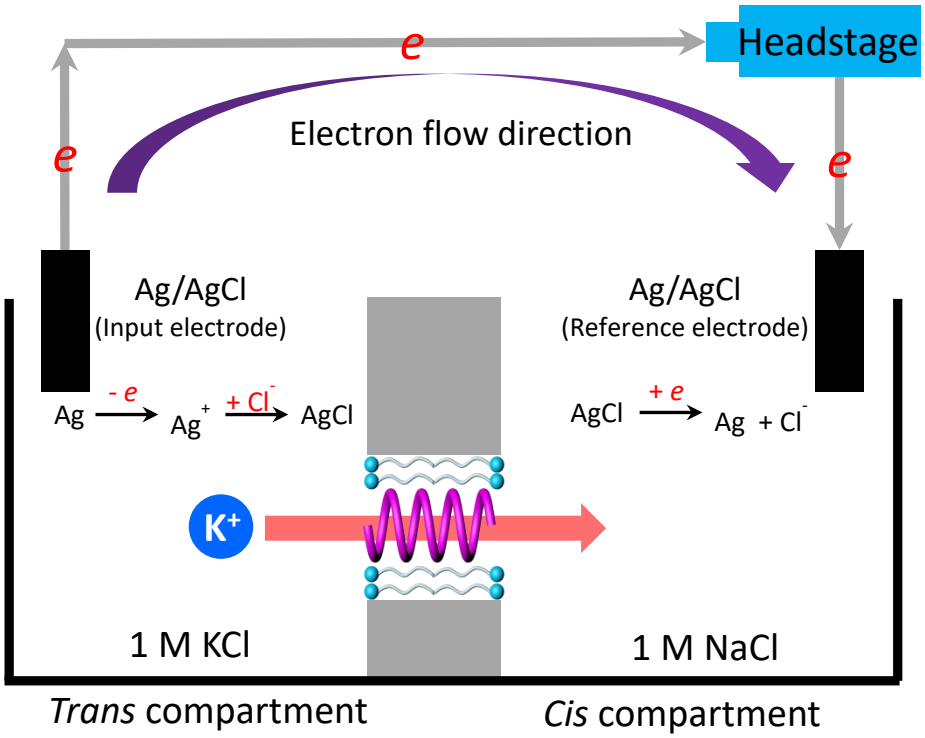
Next, the *cis* chamber was filled with 1 mL of 1M NaCl and the *trans* chamber with 1 mL of 1M KCl were filled with an aqueous KCl solution (Scheme S1). Ag-AgCl electrodes were then inserted into the two salt-containing chambers with the cis chamber grounded to have zero voltage. Planar lipid bilayer was formed by painting 0.3 uL of the lipid-containing n-decane solution around the n-decane-pretreated aperture as described above. Successful formation of non-leaking planar lipid bilayers in the aperture can be established with a capacitance value ranging from 80-150 pF without applying any voltage. After forming a stable lipid bilayer made up of diPhyPC in the aperture, a solution of channel-containing THF (0.1-1 mM) was added with increment of 0.1 uL to the cis compartment near the lipid bilayer in the aperture to reach a final concentration of around 1-100 nM. Upon each incremental addition of 0.1 uL of channel molecules in THF into the cis chamber, stirring solution in cis chamber was carried out to allow the channel molecule to insert into lipid bilayer, and the stirring was stopped after 20 s in order not to rupture the lipid bilayer formed in the aperture by lengthened stirring. This was followed by applying a voltage of 100 or -100 mV, and observation time to check if single channel current traces evolved or not was within 3 min at each voltage. If no single channel currents were observed, this stirring-observation process will be repeated four times, leading to a total observation time of 20 mins per addition of 0.1 uL sample. If single channel current traces still were not observed, more samples in THF at increment of 0.1 uL will be added. A typical experiment might involve 5-10 incremental additions and takes up to three hours. Please note that Lipid bilayer may also break at any time point during such screening. If this happens, the aperture will be washed extensively using water for 2.5 hours after which time a new set of experiment will start over from the very first step of pretreating the aperture with lipid-containing n-decane.

In the fortunate scenario where single current traces were observed at either 100 or -100 mV voltage at some points, they will be measured using a Warner BC-535D bilayer clamp amplifier, collected by PatchMaster (HEKA) with a sample interval at 5 kHz and filtered with an 8-pole Bessel filter at 1 kHz (HEKA). Subsequently, screening a full voltage range from 200 to -200 mV will be conducted at increment of every 20 - 40 mV. At each voltage, observation time is kept around 2-5 min. Thus, it takes another 20 - 100 min to screen a full range of 200 to -200 mV to collect sufficient points for data fitting. In general, one single experiment, which takes one full day, often is not enough to

generate sufficient data points that cover 200 to -200 mV. Multiple such experiments over a week or longer are often needed. The obtained current-voltage (I-V) curve was then analysed by FitMaster (HEKA) with a digital filter at 100 Hz and fitted using the simplified Goldman−Hodgkin−Katz equation:

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

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.



Scheme S1. Schematic illustration of the experimental setup for determining potassium transport selectivity (P_{K+}/P_{Na+}). Redox reaction schemes on both Ag/AgCl electrodes were used to highlight the charge balance during K^+ transport.

Preparation of Alkali metal Picrates

The alkali metal picrates (Li^+, Na^+, K^+, Rb^+ and Cs^+) 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 N_2 protection. The anhydrous metal picrates were stored in a desiccator.

Procedure for Picrate Extraction Experiment for Determining Association Constant between Pentamers and Alkali Metal Ions

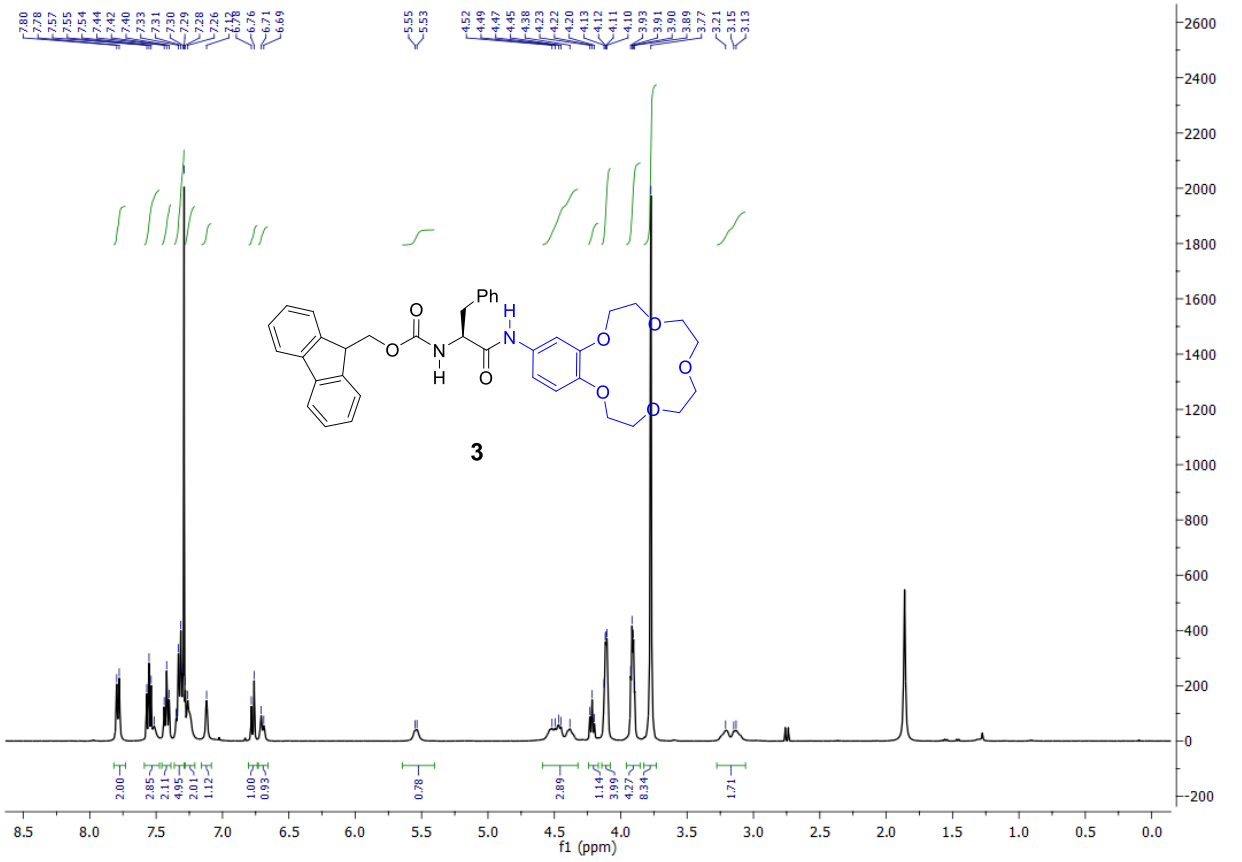
Extractions of alkali metal picrates (Li^+, Na^+, K^+, Rb^+ and Cs^+) with hosts were performed by placing 1.0 mL of a 10 mM solution of the metal picrate in deionized water and 1.0 mL of a 10 mM solution of the hosts in chloroform into a 4-mL sample vial and mixing the solutions on a vortex mixer for 60 seconds. The sample was then allowed to stand for 20 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 250 nm to 500 nm. The extraction constants (K_{ex})[1] and association constants (K_a)[2] were calculated according to the method previously described (S. S. Moore, T. L. Tarnowski, M. Newcomb, D. J. Cram, *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 K_{ex} and K_a values.

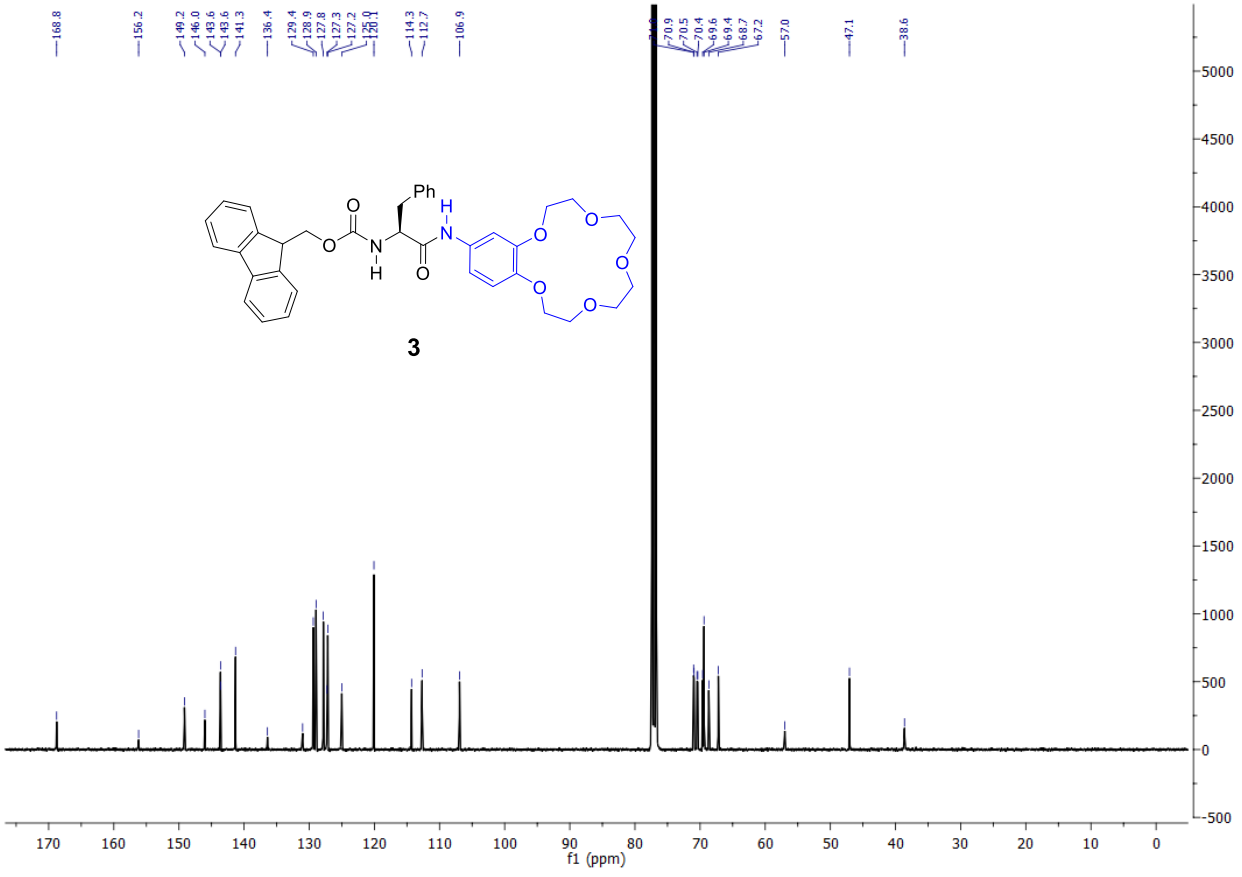
Table S1. K_a and R for channel molecules complexing alkali picrate salts from Li^+ to Cs^+ in $H_2O/CHCl_3$ at 25 ˚C obtained by using the Cram's method.[a]

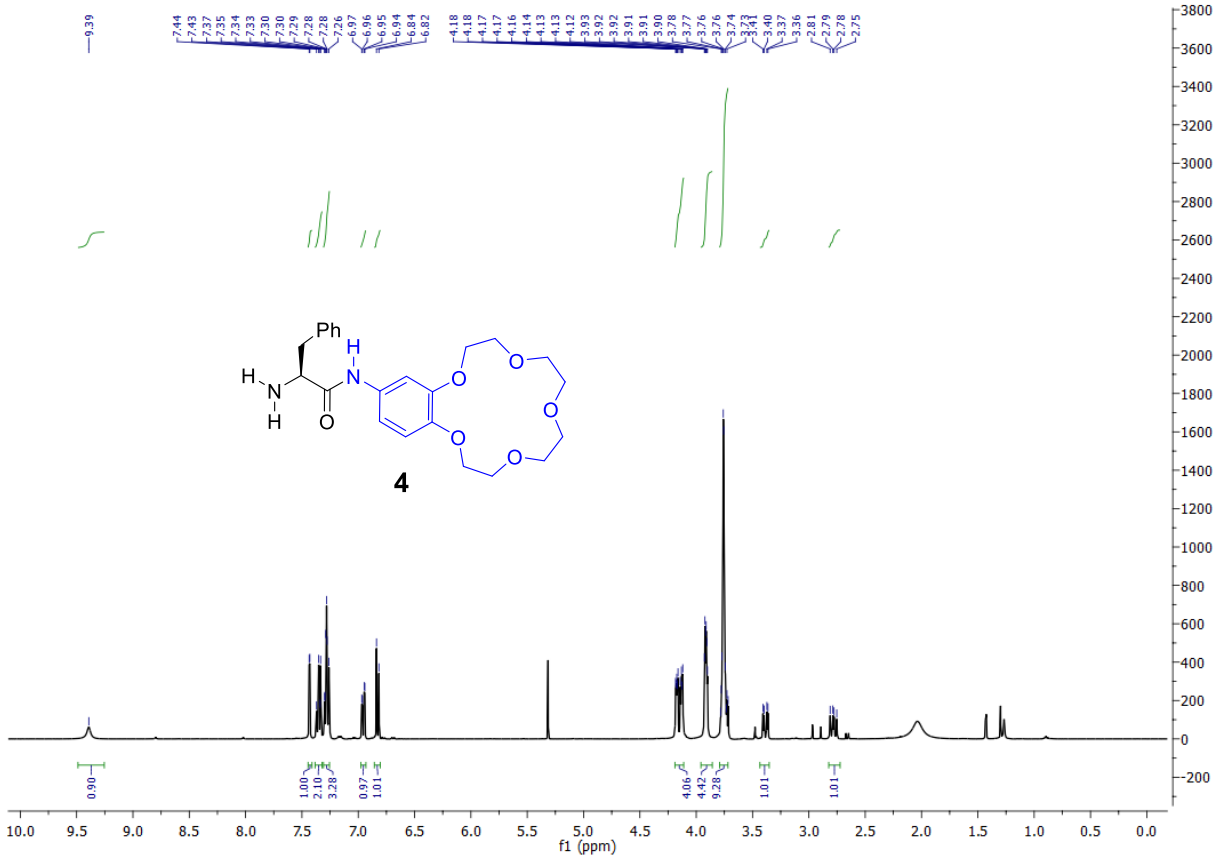
	Na^+		**K^+**		$K_a(K^+)/K_a(Na^+)$	P_{K+}/P_{Na+}[d]
	R[b]	K_a (M^{-1})[c]	R[b]	K_a (M^{-1})[c]		
5F8	0.409	2.92×10^3	0.300	0.35×10^3	0.120	9.8
5F10	0.422	3.22×10^3	0.294	0.34×10^3	0.106	< 9
8F5	0.420	3.16×10^3	0.380	0.65×10^3	0.206	10.7
10F5	0.409	2.89×10^3	0.384	0.66×10^3	0.228	14.0

[a] See S. S. Moore, T. L. Tarnowski, M. Newcomb, D. J. Cram, *J. Am. Chem. Soc.* 1977, **99**, 6398-6405. [b] [Guest]/[Host] ratio in $CHCl_3$ layer at equilibrium. [c] Binding constants averaged over three runs with the assumption of 1:1 guest:host binding stoichiometry. [d] K^+/Na^+ Ion selectivity taken from Table 1 in the manuscript for easy comparison.

^{1}H NMR and ^{13}C NMR



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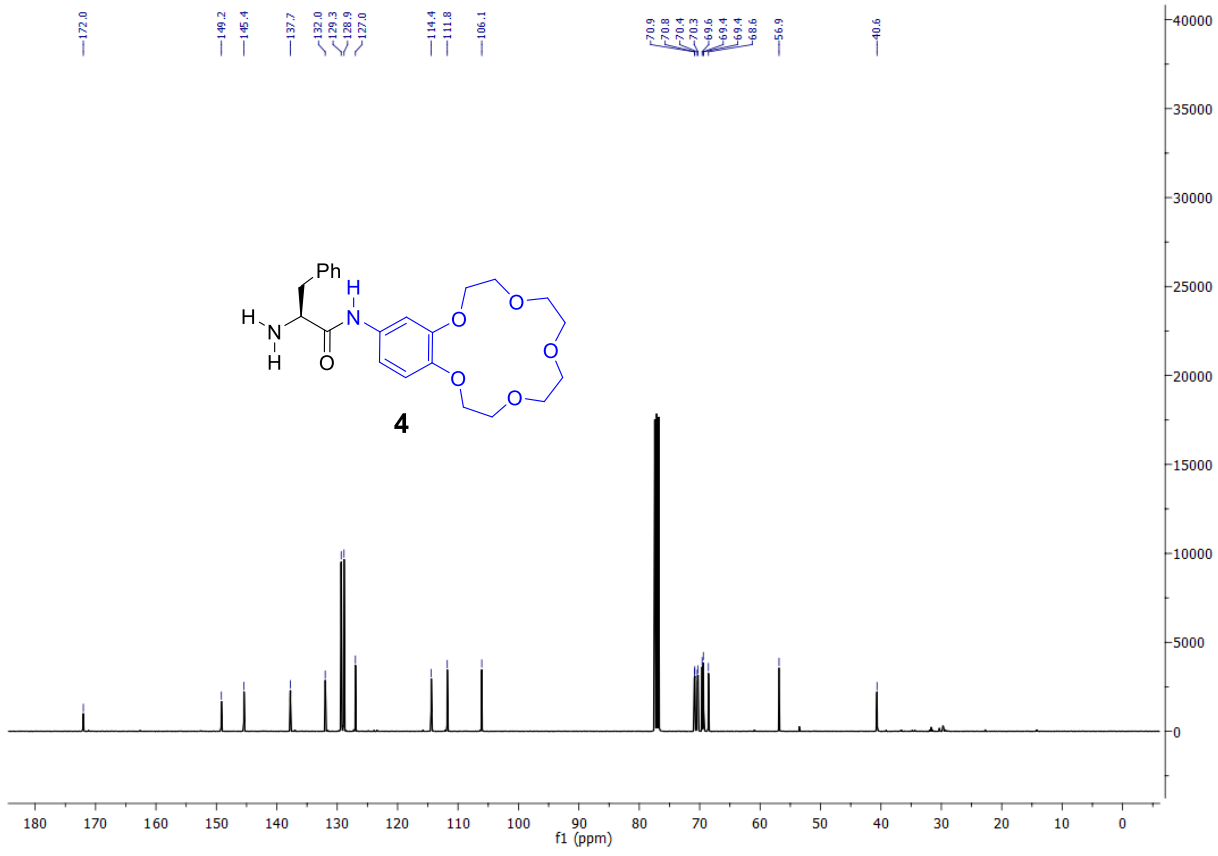
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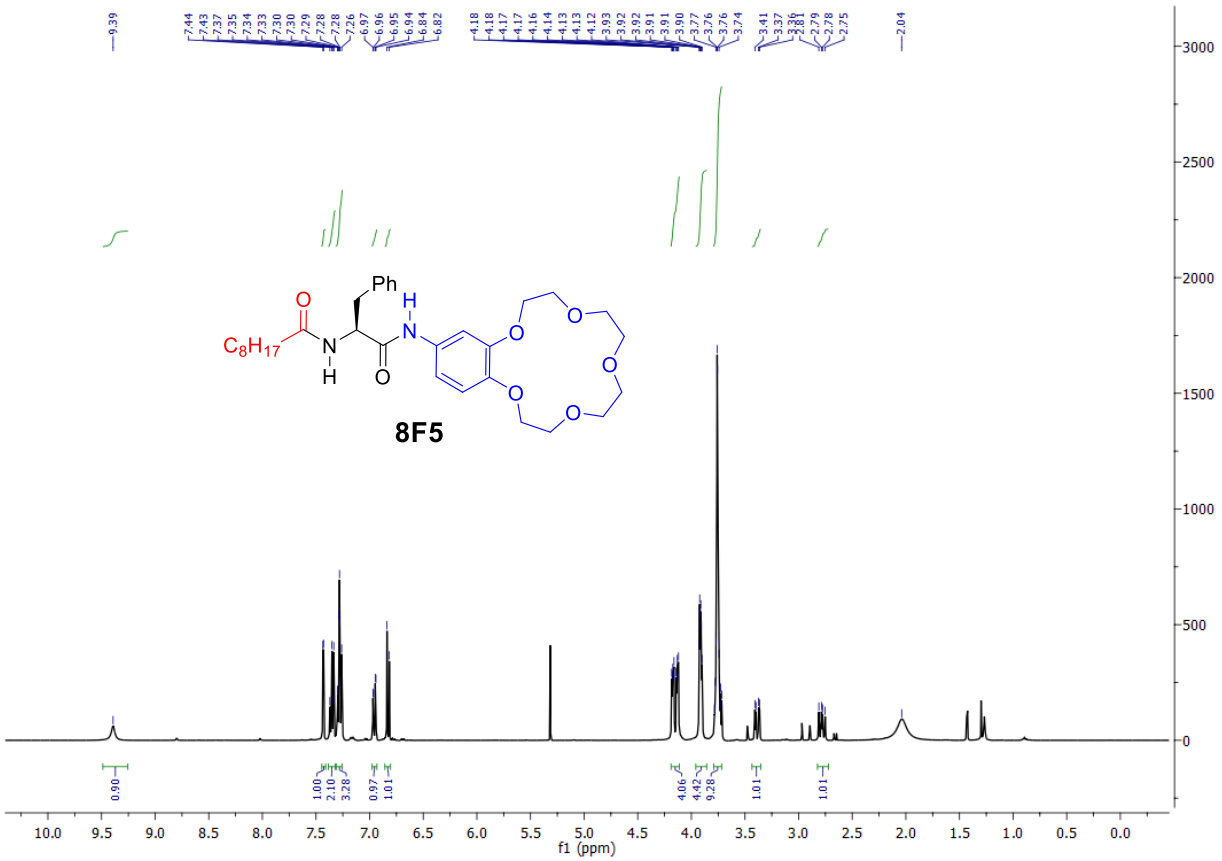
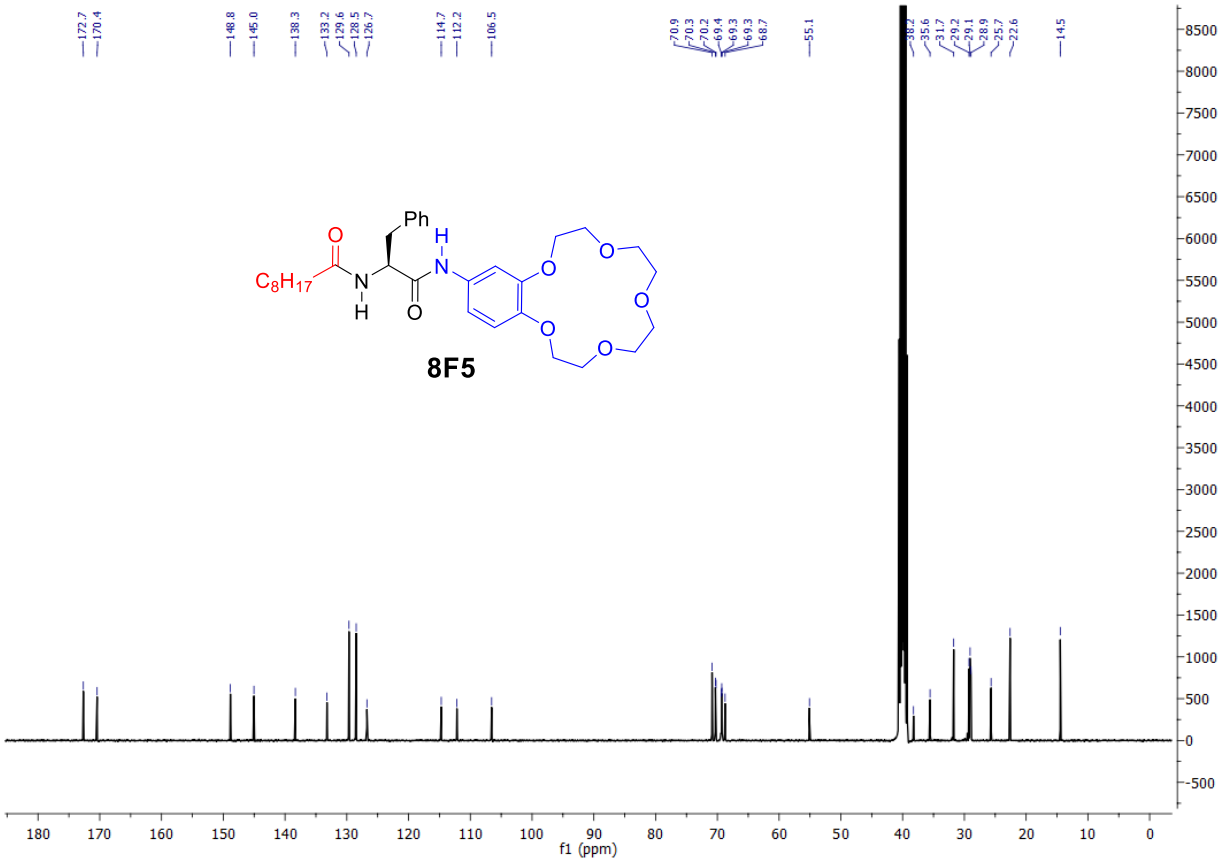
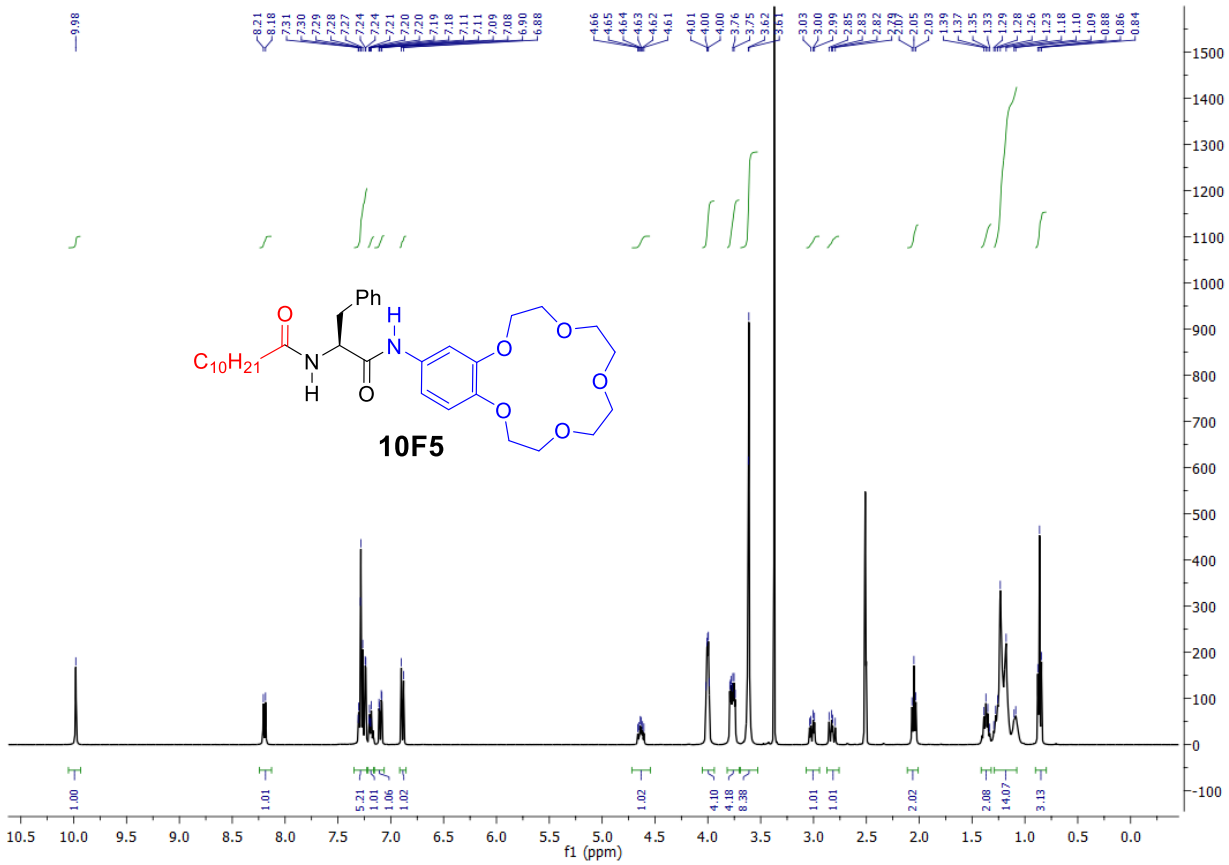

8F5
f1 (ppm)


8F5
f1 (ppm)


Ph
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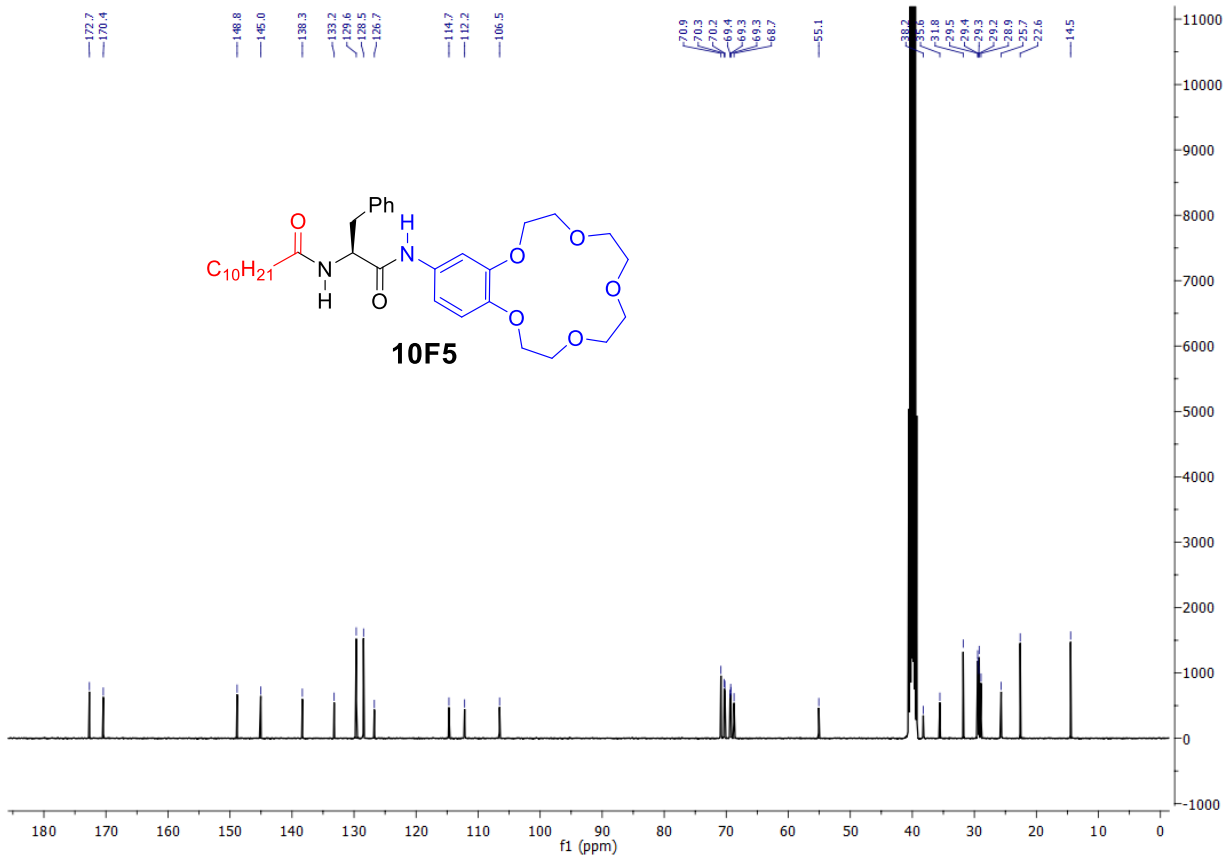
N
O
N
H
O
$C_{10}H_{21}$
O
O
O
O
10F5
f1 (ppm)


Ph
H
N
O
N
H
O
$C_{10}H_{21}$
O
O
O
O
10F5
f1 (ppm)