A Light-Regulated, Exceptionally Active and Selective Artificial Potassium Channel

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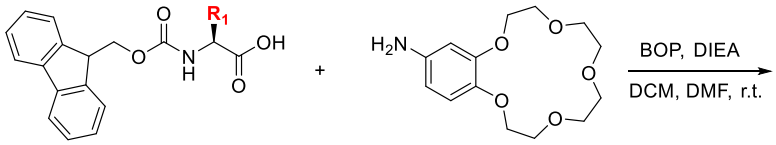
Supporting Information

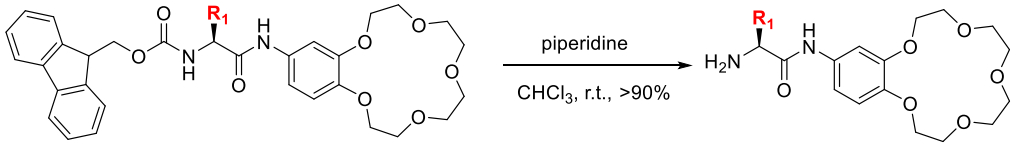
General Remarks

All 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 Qingdao Haiyang. 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 $CDCl_3$ and DMSO-d_6 were referenced at δ = 7.26 ppm and 2.50 ppm, respectively. Coupling constants (*J* values) are reported in Hertz (Hz). ^{1}H NMR data were 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, $CDCl_3$ and DMSO-d_6, were referenced at δ = 77.2 ppm and 39.5 ppm, respectively. $CDCl_3$ (99.8%-deuterated) and DMSO-d_6 (99.8%-deuterated) were purchased from Adamas and used without further purification. Mass spectra were acquired with Shimadzu LCMS-8030. Fluorescence experiments were carried out on a Fluorescence Spectrometer (Shimadzu, Model RF-6000, Japan). UV-visible spectra were obtained on a UV-vis Absorption Spectrometer (Shimadzu, Model UV-1800, Japan).

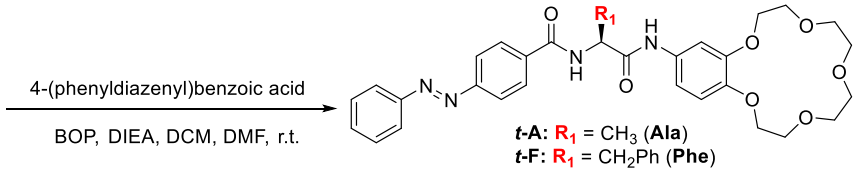
Synthetic routes that afford target compounds



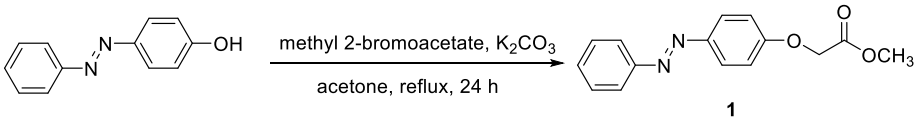
Fmoc-AA-OH



Fmoc-AA **NH_2-AA**



***t*-A:** R_1 = CH_3 (**Ala**)
***t*-F:** R_1 = CH_2Ph (**Phe**)
***t*-L:** R_1 = $CH_2CH(CH_3)_2$ (**Leu**)



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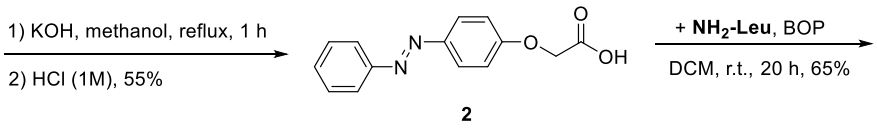

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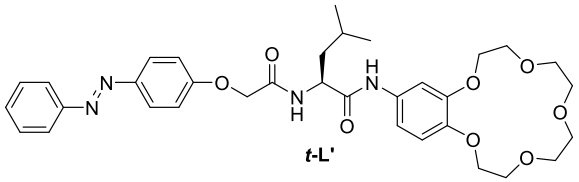
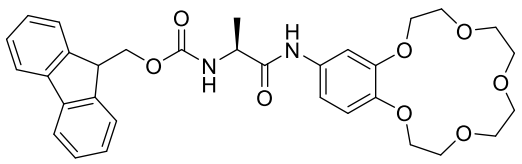


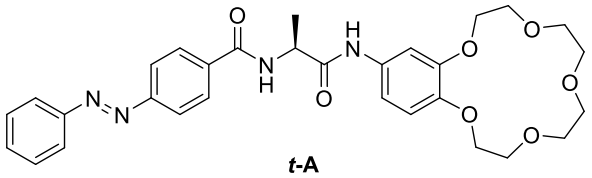
***t*-L'**

Experimental Procedures and Compound Characterizations



Fmoc-Ala

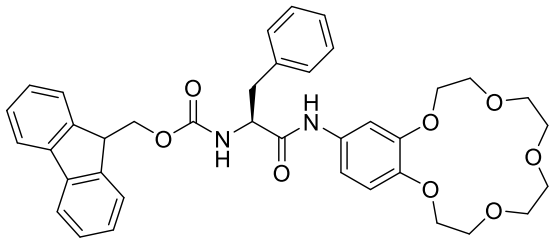
Fmoc-Ala-OH (622 mg, 2.00 mmol), Benzo-15-crown-5 (534 mg, 2 mmol), and BOP (972 mg, 2.20 mmol) were dissolved in CH_2Cl_2 (15 mL), then DIEA (0.78 mL, 4.40 mmol) was added. The reaction mixture was stirred for 20 h at room temperature. The reaction mixture was washed by water (2×20 mL), dried with Na_2SO_4 and filtered. After removing the solvent, the crude product was purified by flash column chromatography (CH_2Cl_2: MeOH = 30: 1, v: v) to afford **Fomc-Ala** as a white solid. Yield: 850 mg, 74%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.76 (d, *J* = 7.6 Hz, 2H), 7.58 (d, *J* = 7.5 Hz, 2H), 7.39 (t, *J* = 7.6 Hz, 2H), 7.34 – 7.26 (m, 3H), 6.89 – 6.76 (m, 2H), 5.30 (s, 1H), 4.46 (d, *J* = 6.9 Hz, 2H), 4.33 (s, 1H), 4.22 (t, *J* = 6.8 Hz, 1H), 4.12 (q, *J* = 4.6 Hz, 4H), 3.89 (q, *J* = 4.7 Hz, 4H), 3.75 (d, *J* = 2.4 Hz, 8H), 1.46 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.35, 141.32, 127.82, 127.13, 124.96, 120.05, 105.90, 99.99, 71.05, 70.97, 70.40, 69.44, 47.12. MS-ESI: calculated for $[M+Na]^+$ ($C_{32}H_{36}N_2O_8Na$)： m/z 599.24, found: m/z 599.26.



***t*-A**

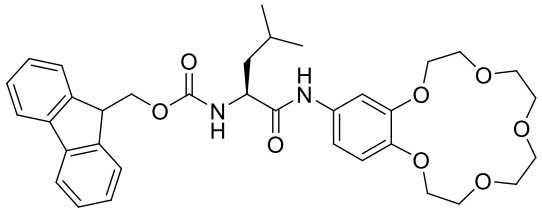
To a solution of **Fmoc-Ala** (576 mg) in $CHCl_3$ (5 mL) was added piperidine (0.50 mL), and the reaction was allowed to stir at room temperature for 12 h. The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography (MeOH:CH_2Cl_2 = 1: 20, v: v) to afford compound **NH_2-Ala** as a pale-yellow oil. Yield: 318 mg, 90%. In the second step, **NH_2-Ala** (318 mg, 0.90 mmol), 4-(phenyldiazenyl)benzoic acid (203 mg, 0.90 mmol), and BOP (438 mg, 0.99 mmol) were dissolved in in CH_2Cl_2 (10 mL), then DIEA (0.36 mL, 2.00 mmol) was added. The reaction mixture was stirred for 20 h at room temperature. The reaction mixture was washed by water (2×10 mL), dried with Na_2SO_4, filtered and the solvent was removed. The crude product was purified by flash column chromatography (CH_2Cl_2: MeOH = 20: 1, v: v) to afford ***t*-A** as a white solid. Yield: 393 mg, 70%. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.96 (s, 1H), 8.83 (d, *J* = 7.0 Hz, 1H), 8.15 (d, *J* = 8.2 Hz, 2H), 7.96 (dd, *J* = 12.5, 7.7 Hz, 4H), 7.63 (d, *J* = 6.5 Hz, 3H), 7.34 (d, *J* = 2.3 Hz, 1H), 7.12 (d, *J* = 8.7 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 1H), 4.62 (p, *J* = 7.1 Hz, 1H), 4.01 (t, *J* = 4.4 Hz, 4H), 3.76 (dt, *J* = 9.1, 4.0 Hz, 4H), 3.61 (s, 8H), 1.45 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 171.31, 165.90, 153.80, 152.39, 148.93, 144.95, 136.60, 133.53, 130.03, 129.39, 123.21, 122.74, 114.89, 112.08, 106.60, 70.83, 70.32, 70.22, 69.40, 69.26, 68.81, 50.42, 36.94, 36.91, 18.31. HRMS: calculated for $[M+Na]^+$ ($C_{30}H_{34}N_4O_7Na$)： m/z 585.2325, found: m/z 585.2308.

Preparation of Fmoc-Phe and Fmoc-Leu follows the same synthetic procedure as Fmoc-Ala.



Fmoc-Phe

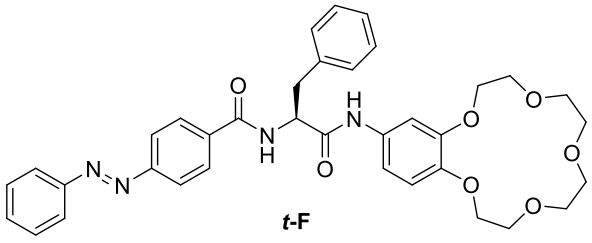
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 7.88 (d, J = 7.6 Hz, 2H), 7.81 (dd, J = 20.8, 7.7 Hz, 1H), 7.66 (t, J = 8.5 Hz, 2H), 7.45 – 7.16 (m, 10H), 7.11 (dd, J = 8.7, 2.4 Hz, 1H), 6.90 (d, J = 8.7 Hz, 1H), 4.38 (td, J = 9.4, 4.5 Hz, 1H), 4.23 – 4.09 (m, 3H), 4.01 (t, J = 4.2 Hz, 4H), 3.76 (dt, J = 11.6, 4.4 Hz, 4H), 3.61 (s, 8H), 3.04 (dd, J = 13.7, 4.7 Hz, 1H), 2.89 (dd, J = 13.6, 10.2 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.50, 156.35, 148.92, 145.11, 144.21, 141.12, 138.43, 129.72, 128.54, 128.08, 127.51, 126.80, 125.83, 125.74, 120.55, 114.87, 112.28, 106.74, 70.84, 70.33, 70.24, 69.39, 69.27, 68.85, 66.14, 57.34, 47.03, 38.01. MS-ESI: calculated for $[M+Na]^+$ ($C_{38}H_{40}N_2O_8Na$): m/z 675.27, found: m/z 675.28.



Fmoc-Leu

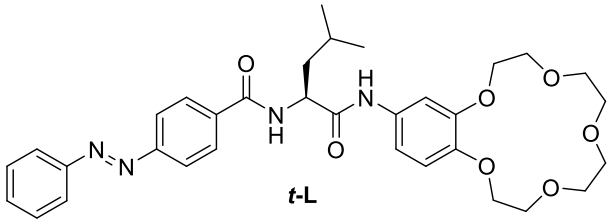
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.75 (d, J = 7.6 Hz, 2H), 7.57 (d, J = 7.3 Hz, 2H), 7.38 (t, J = 7.5 Hz, 2H), 7.32 – 7.22 (m, 2H), 6.89 (s, 1H), 6.76 (d, J = 8.6 Hz, 1H), 5.49 (s, 1H), 4.44 (s, 1H), 4.40 – 4.29 (m, 2H), 4.20 (t, J = 7.0 Hz, 1H), 4.08 (s, 4H), 3.87 (d, J = 8.4 Hz, 4H), 3.73 (d, J = 6.5 Hz, 8H), 1.49 – 1.35 (m, 2H), 1.32 – 1.21 (m, 2H), 0.96 (t, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.68, 143.58, 141.30, 127.80, 127.15, 124.99, 120.01, 47.09, 24.76, 22.96. MS-ESI: calculated for $[M+Na]^+$ ($C_{35}H_{42}N_2O_8Na$): m/z 641.28, found: m/z 641.29.

Preparation of *t*-F and *t*-L follows the same synthetic procedure as *t*-A.



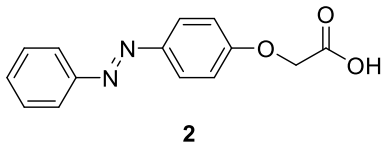
***t*-F**

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.11 (s, 1H), 8.94 (d, J = 8.1 Hz, 1H), 8.05 (d, J = 8.3 Hz, 2H), 7.94 (t, J = 6.9 Hz, 4H), 7.62 (d, J = 6.3 Hz, 3H), 7.43 (d, J = 7.4 Hz, 2H), 7.30 (t, J = 6.2 Hz, 3H), 7.17 (dd, J = 24.8, 8.1 Hz, 2H), 6.91 (d, J = 8.6 Hz, 1H), 4.87 (q, J = 8.0, 7.0 Hz, 1H), 4.02 (t, J = 4.3 Hz, 4H), 3.77 (dt, J = 10.0, 4.1 Hz, 4H), 3.61 (s, 8H), 3.23 – 3.06 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.26, 166.11, 152.38, 145.14, 138.62, 130.03, 129.68, 129.29, 128.59, 123.22, 122.74, 114.88, 112.34, 106.80, 70.84, 70.33, 70.24, 69.39, 69.28, 68.85, 56.33, 38.01. HRMS: calculated for $[M+Na]^+$ ($C_{36}H_{38}N_4O_7Na$): m/z 661.2638, found: m/z 661.2617.



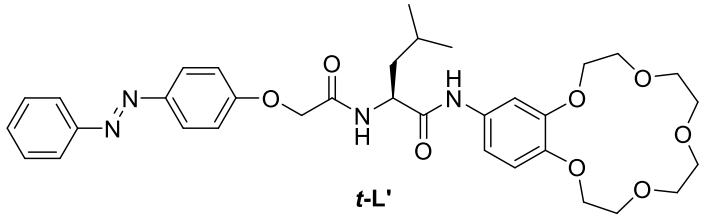
***t*-L**

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.01 (s, 1H), 8.78 (d, *J* = 7.8 Hz, 1H), 8.14 (d, *J* = 8.2 Hz, 2H), 8.01 – 7.91 (m, 4H), 7.63 (d, *J* = 6.6 Hz, 3H), 7.32 (d, *J* = 2.3 Hz, 1H), 7.15 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 1H), 4.67 (s, 1H), 4.01 (t, *J* = 4.3 Hz, 4H), 3.76 (dt, *J* = 9.6, 4.2 Hz, 4H), 3.61 (s, 8H), 1.89 – 1.72 (m, 2H), 1.60 (ddd, *J* = 13.2, 8.7, 4.9 Hz, 1H), 0.95 (dd, *J* = 9.9, 6.4 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.17, 153.80, 130.04, 129.41, 123.22, 122.74, 114.88, 70.84, 70.33, 70.22, 69.40, 69.27, 68.80, 25.02, 23.56, 21.93. HRMS: calculated for $[M+Na]^+$ ($C_{33}H_{40}N_4O_7Na$): m/z 627.2795, found: m/z 627.2777.



2

p-Hydroxyazobenzene (396 mg, 2.00 mmol) was dissolved in acetone (20 mL) to which anhydrous K_2CO_3 (1.382 g, 10.0 mmol) and methyl 2-bromoacetate (0.284 mL, 3 mmol) were added. The mixture was heated under reflux for 24 h. The reaction mixture was then filtered and the solvent was removed in *vacuo*. The residue was dissolved in CH_2Cl_2 (20 mL), washed with water (2×10 mL), and dried over anhydrous Na_2SO_4. Removal of CH_2Cl_2 in *vacuo* gave the crude product **1**, which was directly used in the next step without further purification. **1** was dissolved in methanol (10 mL), followed by adding 1 M NaOH (10 mL). The mixture was heated under reflux for 2 h and then the reaction solvent was removed *in vacuo* to yield a yellow solid, which was dissolved in water and neutralized with 1 M HCl (15 mL) to yield compound **2** as a yellow solid. Yield: 141 mg, 55%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.21 (s, 1H), 7.87 (dd, *J* = 16.2, 8.0 Hz, 4H), 7.57 (dt, *J* = 13.7, 7.1 Hz, 3H), 7.12 (d, *J* = 8.6 Hz, 2H), 4.81 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.29, 160.97, 152.45, 146.88, 131.36, 129.86, 124.92, 122.74, 115.62, 65.26. MS-ESI: calculated for $[M+Na]^+$ ($C_{14}H_{12}N_2O_3Na$): m/z 279.07, found: m/z 279.09.



***t*-L'**

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (s, 1H), 7.99 – 7.79 (m, 4H), 7.49 (dt, *J* = 14.8, 7.1 Hz, 3H), 7.26 (s, 2H), 7.17 (d, *J* = 7.9 Hz, 1H), 7.06 (d, *J* = 8.5 Hz, 2H), 6.99 (d, *J* = 8.5 Hz, 1H), 6.72 (d, *J* = 8.7 Hz, 1H), 4.67 (q, *J* = 7.8 Hz, 1H), 4.62 (s, 2H), 4.08 (s, 4H), 3.88 – 3.77 (m, 3H), 3.70 (d, *J* = 14.1 Hz, 10H), 1.84 – 1.61 (m, 1H), 1.40 (dd, *J* = 19.0, 6.7 Hz, 2H), 0.96 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.09, 168.68, 159.21, 147.86, 130.73, 129.09, 124.87, 122.69, 115.08, 105.90, 68.97, 67.21, 52.66, 40.43, 36.69, 24.85, 22.97, 21.80. HRMS: calculated for $[M+Na]^+$ ($C_{34}H_{42}N_4O_8Na$): m/z 657.2900, found: m/z 657.2882.

Computational Methods

Construction of the hexamer: The initial monomer structure of the ***t*-L** and ***c*-L** were optimized at M06-2x/6-31+G(d) level of theory using periodic boundary conditions. Using the optimized structure, we created the structure of the hexamer $(t\text{-L})_6$ and $(c\text{-L})_6$ by applying the geometrical transformations.

Membrane System Building. The initial membrane system was generated using CHARM-GUI platform[1]. In this system, $(t\text{-L})_6$ and $(c\text{-L})_6$ were positioned at the center of lipid bilayer containing 100 molecules of POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine), respectively. The membrane system was subsequently placed in the center of a cubic box with dimensions of 60 Å × 60 Å × 70 Å, ensuring a 10 Å thick layer of water molecules on each side. To achieve physiological ion concentration, a 0.15 M ion of KCl was added.

Molecular Dynamics (MD) Simulations. The MD simulations in an isothermal isobaric ensemble (300 K, 1 bar) were conducted for 200 ns by using the GROMACS 2018 package[2], with a time step of 2 fs. The CHARMM36 force field[3] was utilized for the POPC molecules and the channel of $(t\text{-L})_6$ and $(c\text{-L})_6$, while the TIP3P model[4] was employed for the water molecules. Electrostatic interactions were calculated using the particle mesh Ewald (PME) algorithm[5], with a cut-off of 10 Å for both van der Waals and electrostatic interactions. All chemical bonds were constrained with the SHAKE algorithm[6]. Temperature and pressure were maintained using the Nosé-Hoover thermostat[7] and the Berendsen barostat[8], respectively.

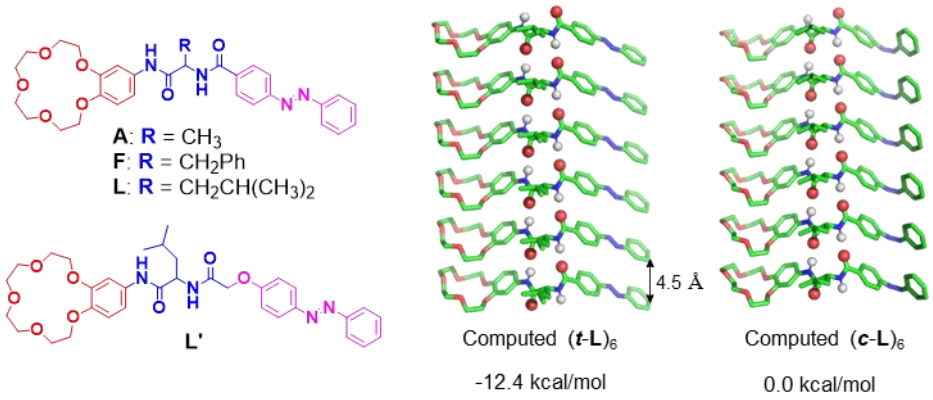


Figure S1. The hexamer ensembles $(t\text{-L})_6$ and $(t\text{-L})_6$ were computationally optimized at the level of M06-2X/6-31+G(d) using periodic boundary conditions.

[1] S. Jo, X. Cheng, J. Lee, S. Kim, S. J. Park, D. S. Patel, A. H. Beaven, K.I. Lee, H. Rui, S. Park, H. S. Lee, B. Roux, A. D. MacKerell, Jr, J. B. Klauda, Y. Qi, W. Im, *J. Comput. Chem.* **2017**, 38, 1114-1124.

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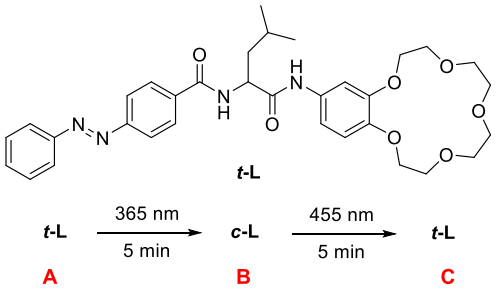
HPLC analysis of isomerization in solution and lipids

The HPLC analysis was performed on Agilent 1260 Infinity II.

L in MeOH: A 100 μM ***t*-L** methanol solution was prepared, which is taken as solution **A**. Irradiating **A** with 365 nm UV light for 5 min to induce a conformational change, which gives solution **B**. A portion of **B** is further irradiated at 455 nm light for 5 min to revert it to its original state, which gives solution **C**. HPLC experiments were then performed to analyze the ratio of ***t*-L** and ***c*-L** in these three solutions **A**-**C**. The results are shown in **Table S1** and **Fig. S2**.

L in LUV (lipids): A 2.0 mL buffer solution (100 mM NaCl, 10 mM HEPES, pH = 8) containing 1 mM LUV and 34 μM ***t*-L** was prepared, which is taken as solution **A**. Irradiating 1 mL of **A** with 365 nm UV light for 5 min gives solution **B**, which is further irradiated at 455 nm light for 5 min to give **C**. HPLC experiments were then performed to analyze the ratio of ***t*-L** and ***c*-L** in these three solutions **A**-**C**. The results are shown in **Table S1** and **Fig. S3**.

Table S1. Relative percentages of ***t*-L** and ***c*-L** after alternative light irradiation at 365 nm and 455 nm for 5 min in MeOH and lipids using the HPLC analysis and in $CDCl_3$ using ^1H NMR analysis.



	L in $CDCl_3$ by ^1H NMR			L in methanol by HPLC			L in LUV (lipid) by HPLC		
	A	B	C	A	B	C	A	B	C
***t*-L%**	99%	34%	88%	92%	38%	80%	89%	62%	75%

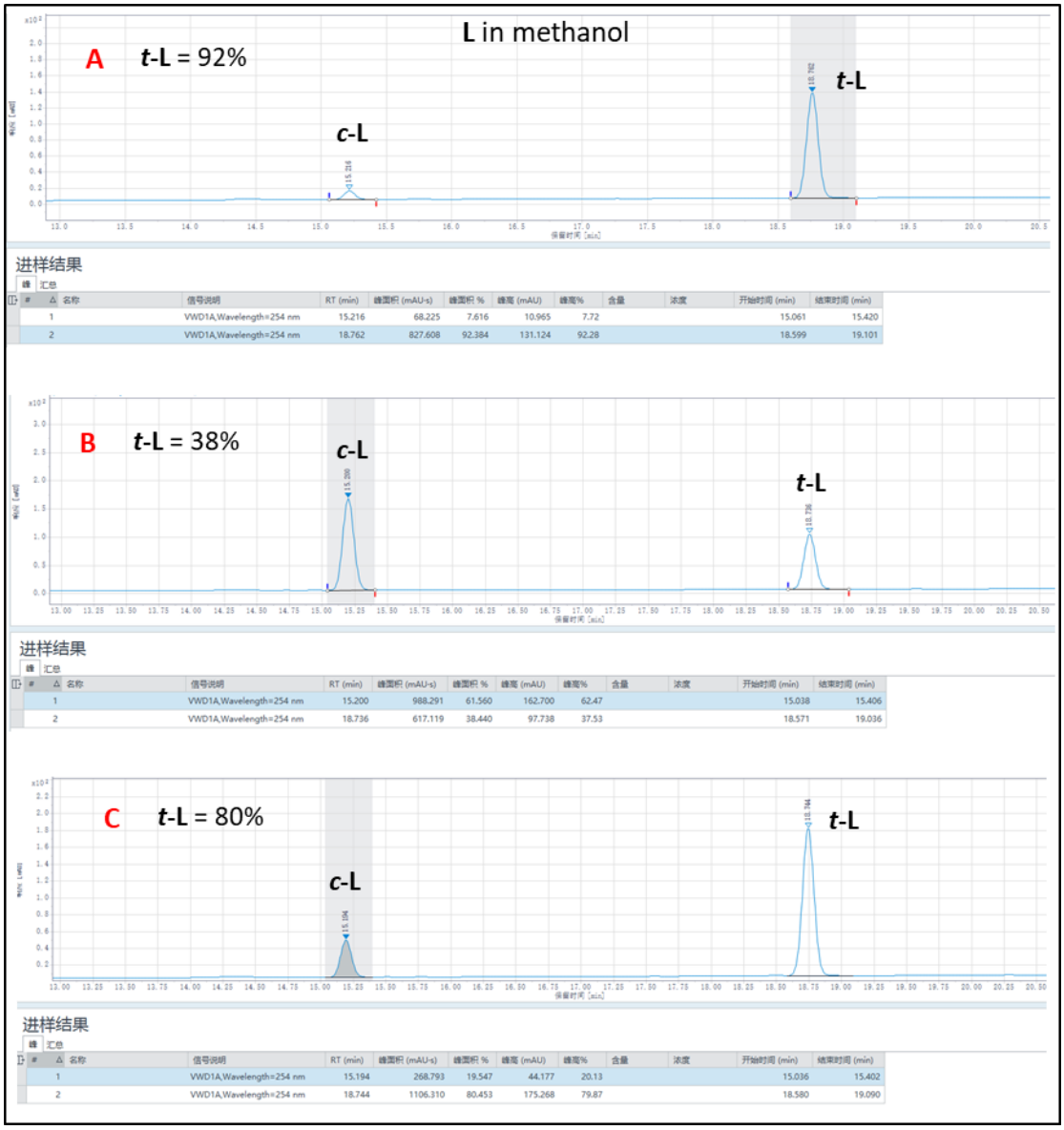


Figure S2. The *trans*/*cis* ratio of **L** in MeOH-based solutions **A**-**C**.

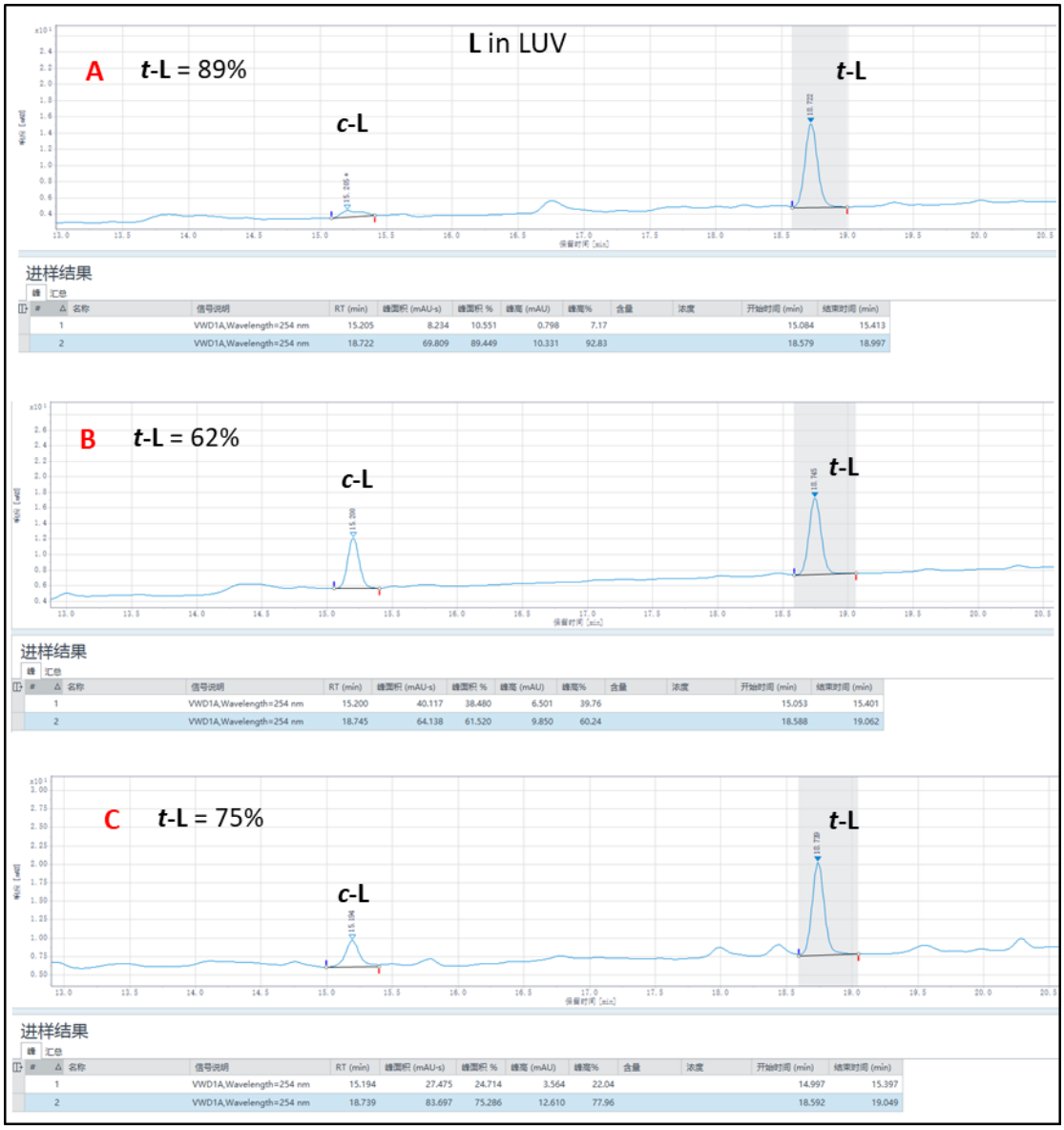


Figure S3. The *trans*/*cis* ratio of **L** in LUV-containing solutions **A**-**C**.

Experimental methods for ion transport study

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 °C. After drying the resulting film under high vacuum overnight, the film was hydrated with the HEPES (4-(2-hydroxyethyl)-1-piperazine-ethane sulfonic acid) buffer solution (1 mL, 10 mM HEPES, 100 mM NaCl, pH = 7.0) containing pH sensitive dye HPTS (8-hydrox-ypyrene-1,3,6-trisulfonic acid, 1 mM) at room temperature for 60 min to give a milky suspension. The mixture was then subjected to 10 freeze-thaw cycles: freezing in liquid N_2 for 1 min and heating in 55 °C water bath for 2 min. 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 the 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 alternate excitations at 460 and 403 nm for 300 s using fluorescence spectrophotometer (Shimadzu, Model RF-6000, 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}, 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 transporter concentration using the Hill equation: $Y = 1/(1 + (EC_{50}/[C])^n)$ gave the Hill coefficient n and EC_{50} values.

Table S2. Fractional ion transport activity values for ***t*-L** and ***c*-L** determined over three runs.

Samples	Ions	No.1	No.2	No.3
[***t*-L**] = 1.2 μM	K^+	97%	96%	98%
	Na^+	10%	10%	10%
[***c*-L**] = 2.8 μM	K^+	100%	98%	99%
	Na^+	21%	20%	20%

The HPTS assay in the presence of FCCP

The HPTS-containing LUV suspension (25 μL, 2.5 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 NaCl) to create a pH gradient for ion transport study. A solution of FCCP (carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone) and compound **L** in DMSO was then injected into the suspension under gentle stirring at 20 s and 70 s, respectively. Upon the addition of carrier molecules, the emission of HPTS was immediately monitored at 510 nm with excitations at both 460 and 403 nm recorded simultaneously for 300 s using fluorescence spectrophotometer (Shimadzu, Model RF-6000, Japan). 300 s later, aqueous solution of Triton X-100 (20 μL, 20% v/v) was immediately added to achieve the maximum change in dye fluorescence emission. The final transport trace was obtained 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 SPQ assay for anion selectivity

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 °C. After drying the resulting film under high vacuum overnight, the film was hydrated with the $NaNO_3$ solution (1 mL, 200 mM) containing Cl^--sensitive dye SPQ (6-methoxy-N-(3-sulfopropyl)quinolinium, 0.5 mM) at room temperature for 60 min to give a milky suspension. The mixture was then subjected to 10 freeze-thaw cycles: freezing in liquid N_2 for 1 min and heating at 55 °C for 2 min. 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 SPQ encapsulated inside. The free unencapsulated SPQ dye was separated

from the LUVs by using size exclusion chromatography (stationary phase: Sephadex G-50, GE Healthcare, USA, mobile phase: HEPES buffer with 200 mM $NaNO_3$) and diluted with the mobile phase to yield 5 mL of 6.5 mM lipid stock solution.

The SPQ-containing LUV suspension (25 μL, 6.5 mM in 200 mM $NaNO_3$) was added to a NaCl solution (1.95 mL, 200 mM) to create an extravesicular chloride gradient. A solution of compound **L** in DMSO was then injected into the suspension under gentle stirring. Upon the addition of transporter molecules, the emission of SPQ was immediately monitored at 430 nm with excitations at 360 nm for 300 seconds using fluorescence spectrophotometer (Shimadzu, Model RF-6000, 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 following equation.

$$I_f = (I_t - I_1) / (I_0 - I_1)$$

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.

Membrane leaking and pore size determination using CF dye

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 °C. After drying the resulting film under high vacuum overnight at room temperature, the film was hydrated with the HEPES buffer solution (1 mL, 10 mM HEPES, 100 mM NaCl, pH = 7.5) containing 5(6)-fluorescein molecules (CF, 50 mM) at room temperature for 60 min to give a milky suspension. The mixture was then subjected to 10 freeze-thaw cycles: freezing in liquid N_2 for 1 min and heating at 55 °C in water bath for 2 min. 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 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 transporter molecules or pore-forming melittin in DMSO at different concentrations was then injected into the suspension under gentle stirring. Upon the addition of transporter molecules, the emission of CF was immediately monitored at 517 nm with excitation at 492 nm for 300 s using fluorescence spectrophotometer (Shimadzu, Model RF-6000, 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 .

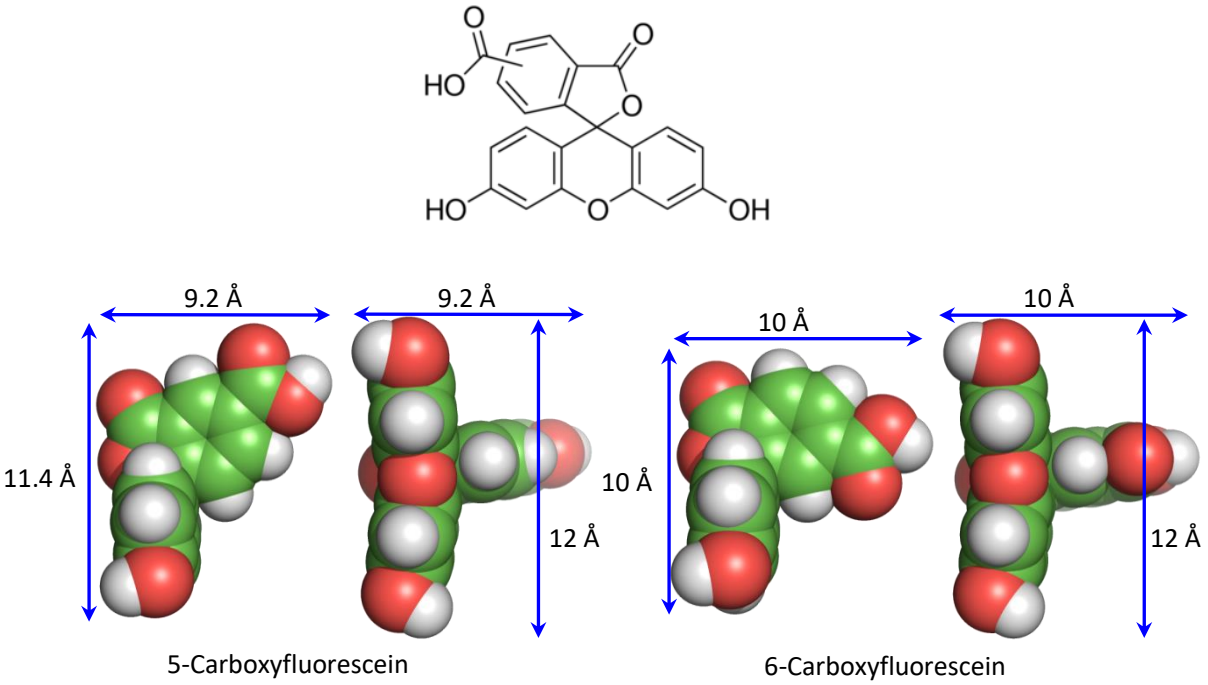


Figure S4. Molecular dimensionality of CF dye molecules consisting of 5-and 6-carboxyfluorescein. These molecules measure 9.2 - 12 Å in three dimensions.

Single Channel Current Measurement in Planar Lipid Bilayers

The chloroform solution of 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 typical experiment for conductance measurement, both the chamber (cis side) and Delrin cup (trans side) were filled with an aqueous KCl solution (1.0 M, 1.0 mL). Ag-AgCl electrodes were inserted into the two solutions with the cis chamber grounded. Planar lipid bilayer was formed by painting 0.3 uL 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. Then at each time, 0.1 μL of sample in THF was added to the cis compartment and the solution was stirred for 20 s. After 5 to 10 times, a single current trace appeared. And the final concentration of the sample was around 10-8 M. These single channel currents were then 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). The data were analysed by FitMaster (HEKA) with a digital filter at 100 Hz.

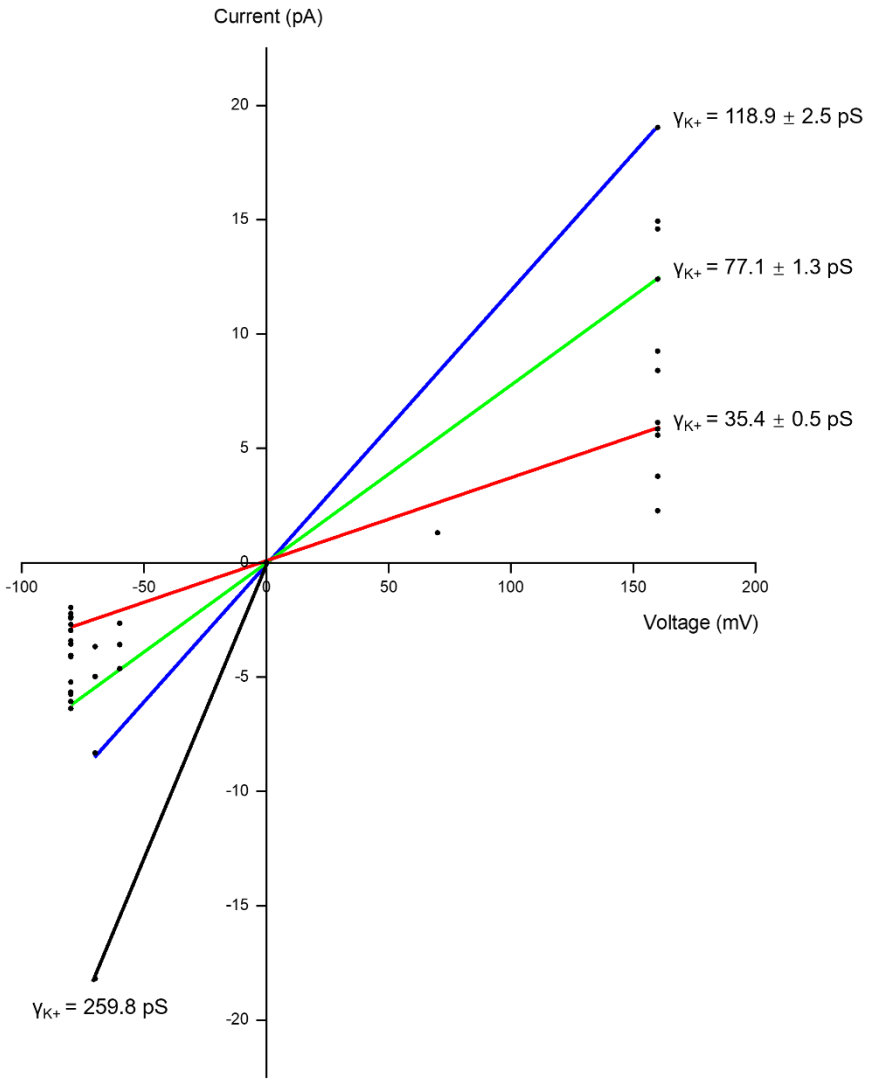


Figure S5. Current vs voltage plots for *t*-L that illustrate a complex channel-forming process. During this process, many channel ensembles likely form that generate currents of varying magnitudes. For how these currents were obtained, please see **Figs. S11-S18**.

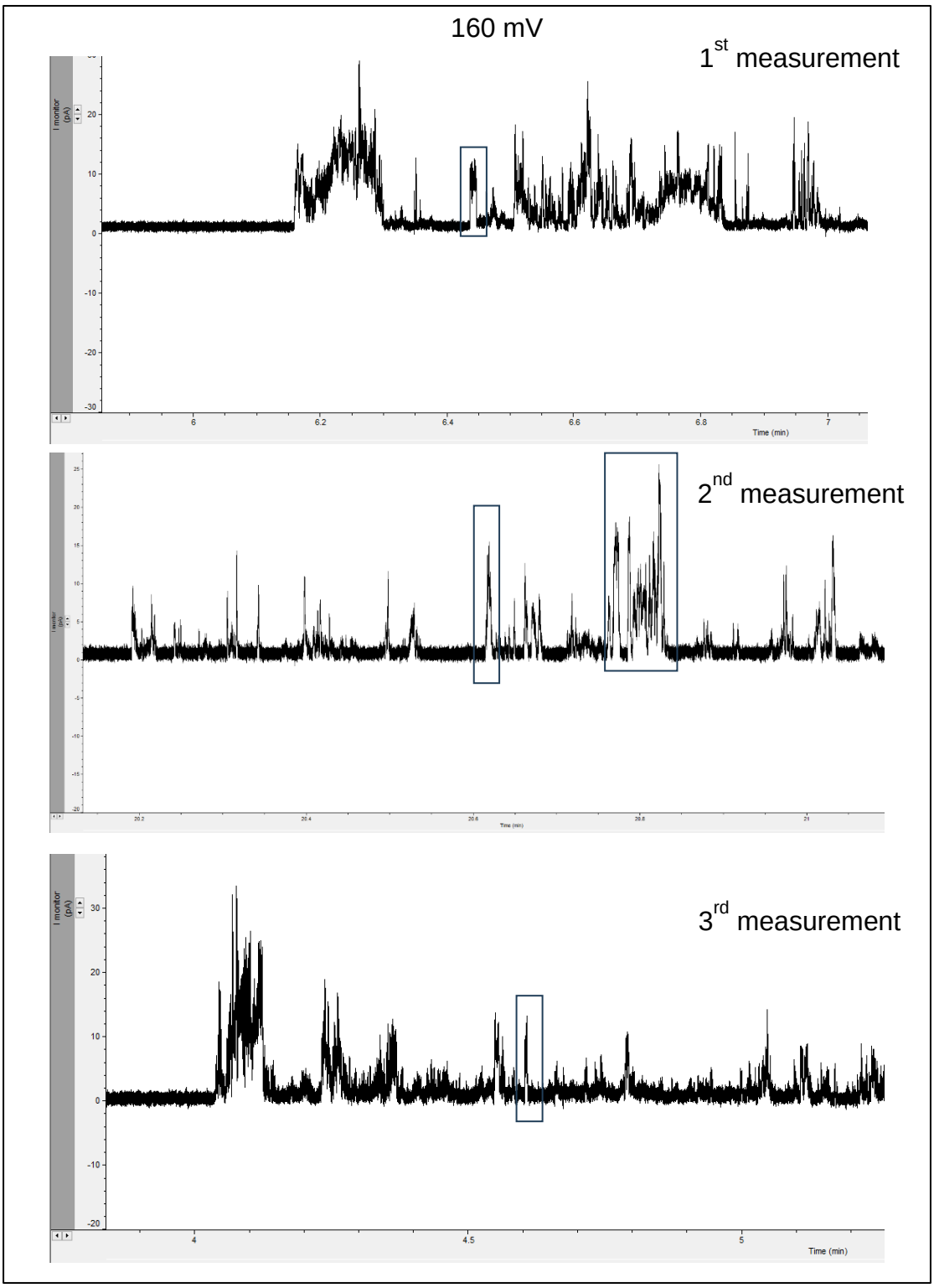


Figure S6. Recorded single channel current traces at 160 mV for ***t*-L**.

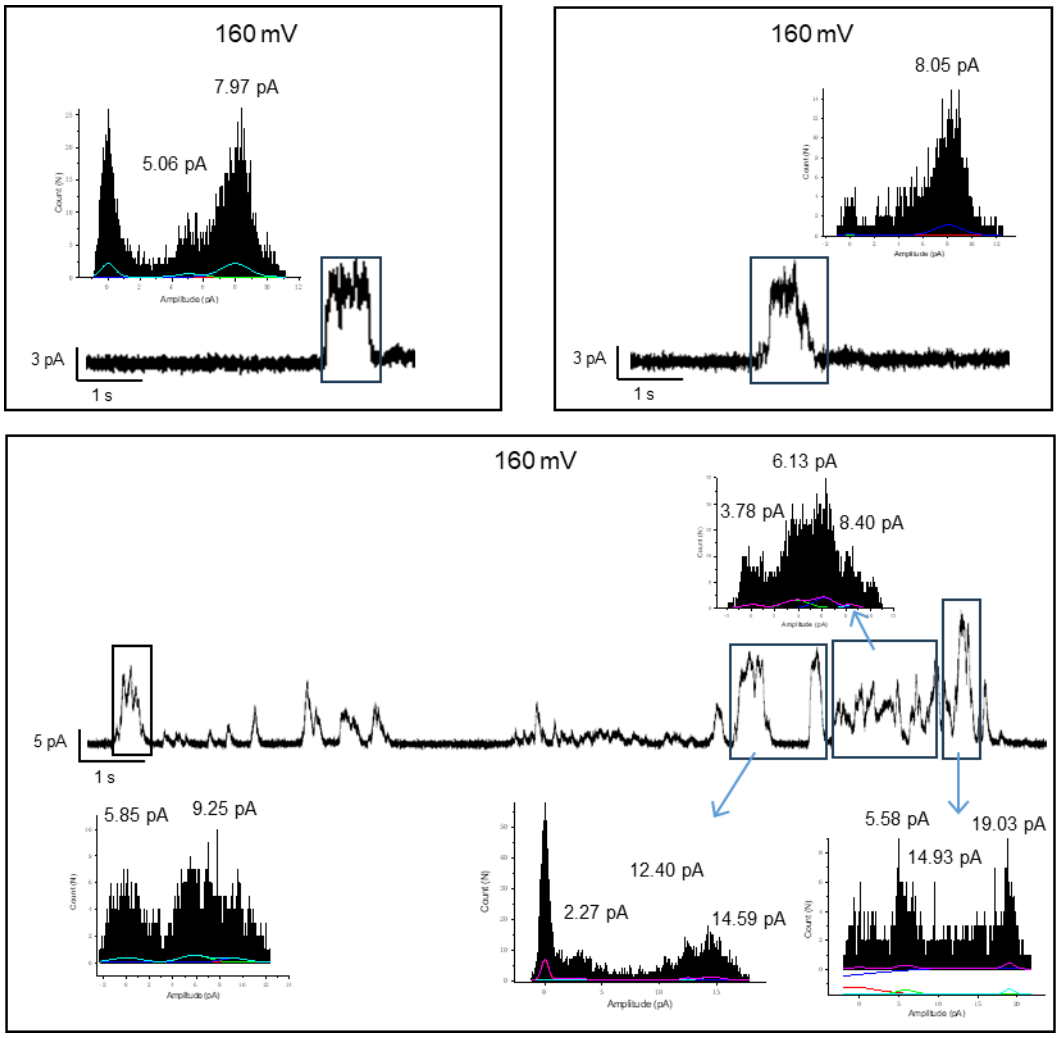


Figure S7. Histograms generated at voltage of 160 mV for ***t*-L**. Simulating these histograms yield average channel currents.

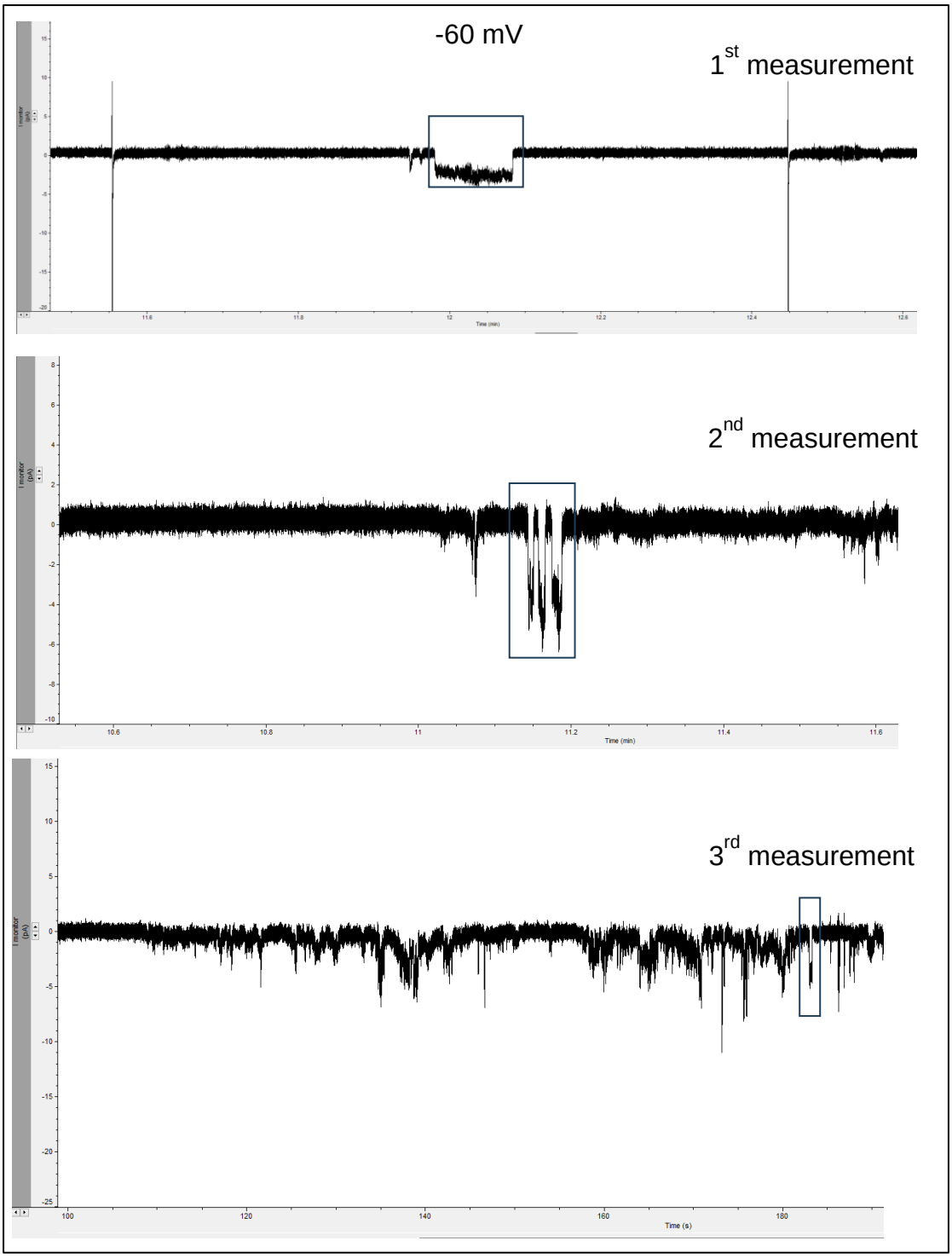


Figure S8. Recorded single channel current traces at -60 mV for ***t*-L**

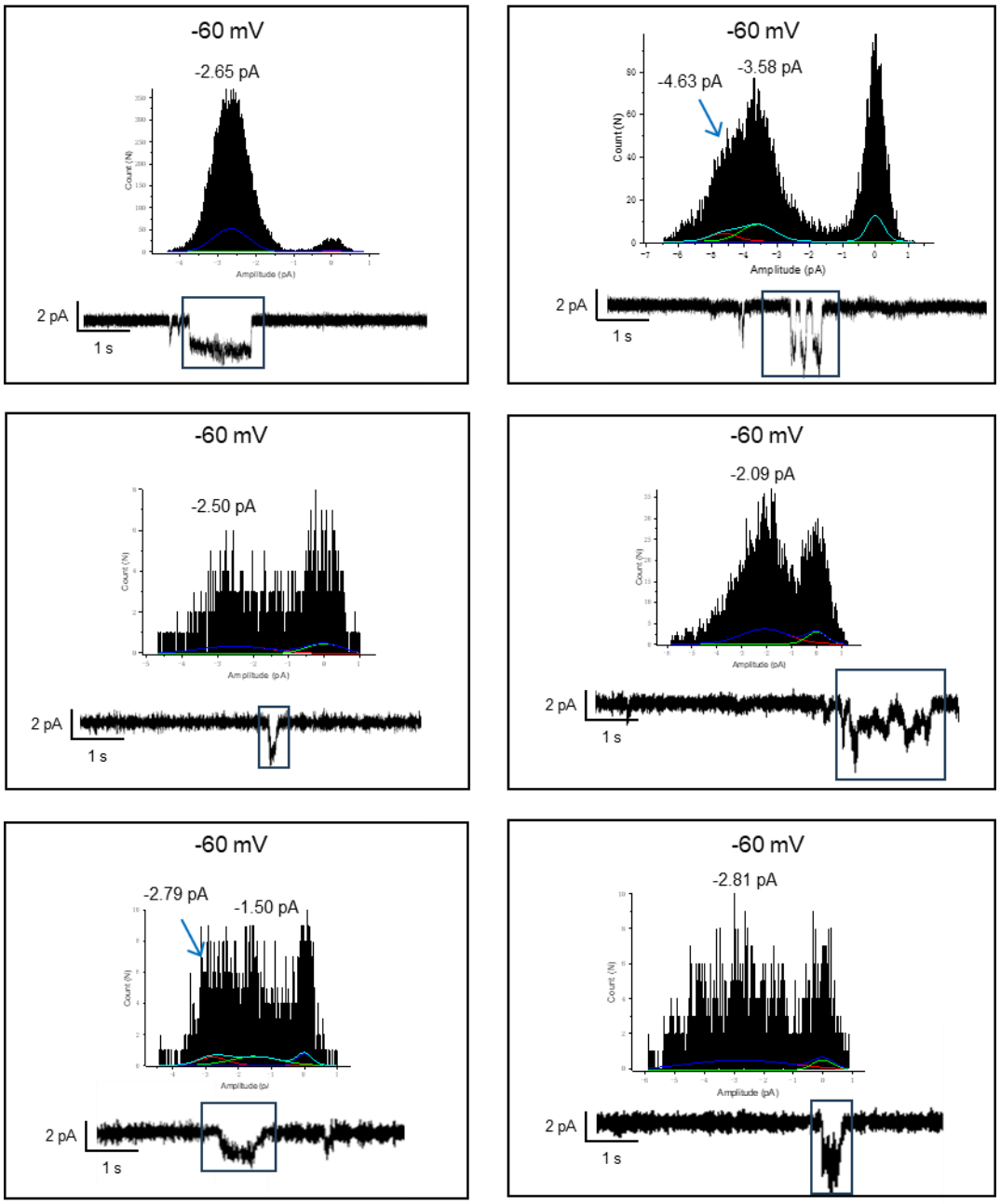


Figure S9. Histograms generated at voltage of -60 mV for ***t*-L**. Simulating these histograms yield average channel currents.

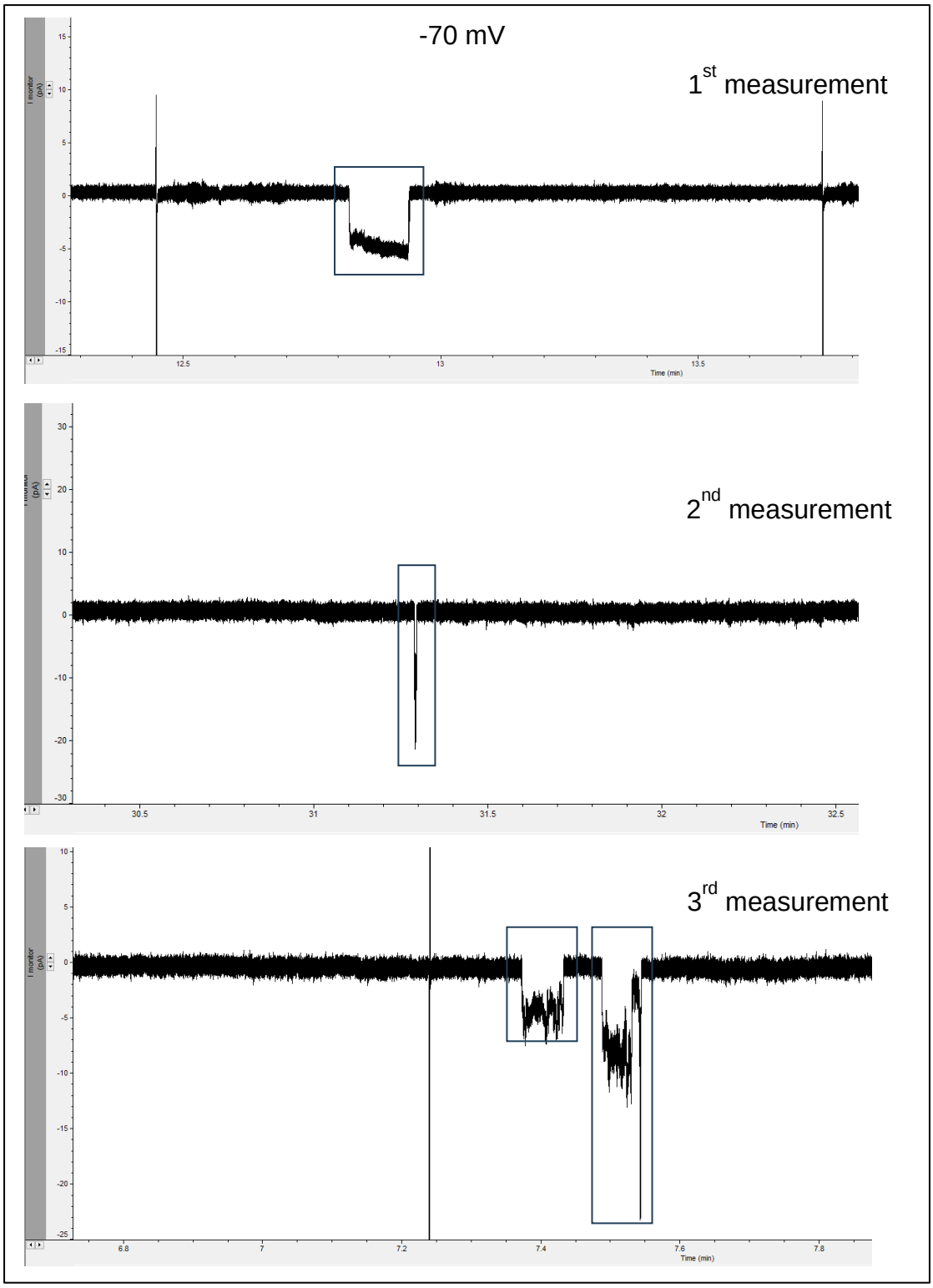


Figure S10. Recorded single channel current traces at -70 mV for ***t*-L**

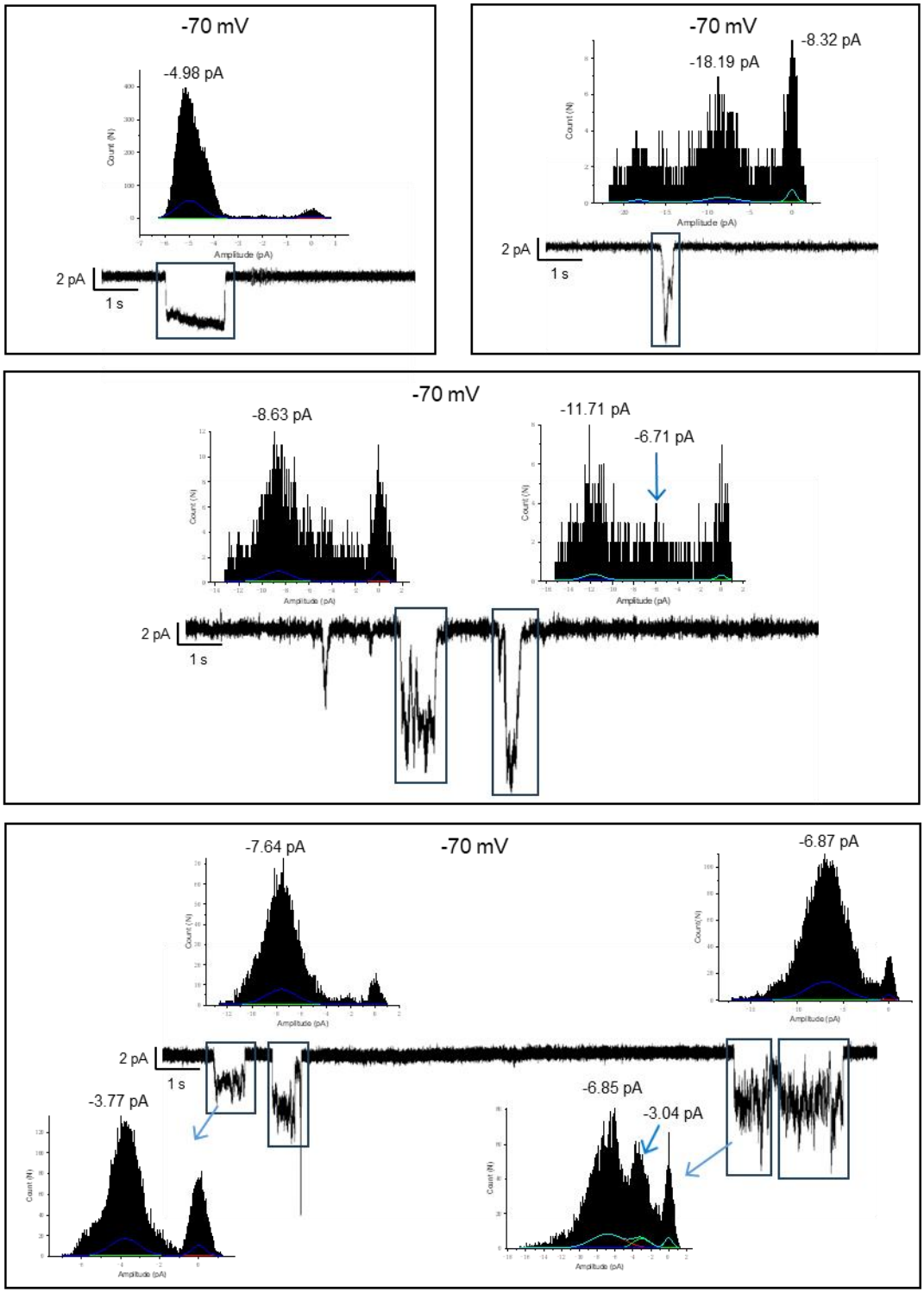


Figure S11. Histograms generated at voltage of -70 mV for ***t*-L**. Simulating these histograms yield average channel currents.

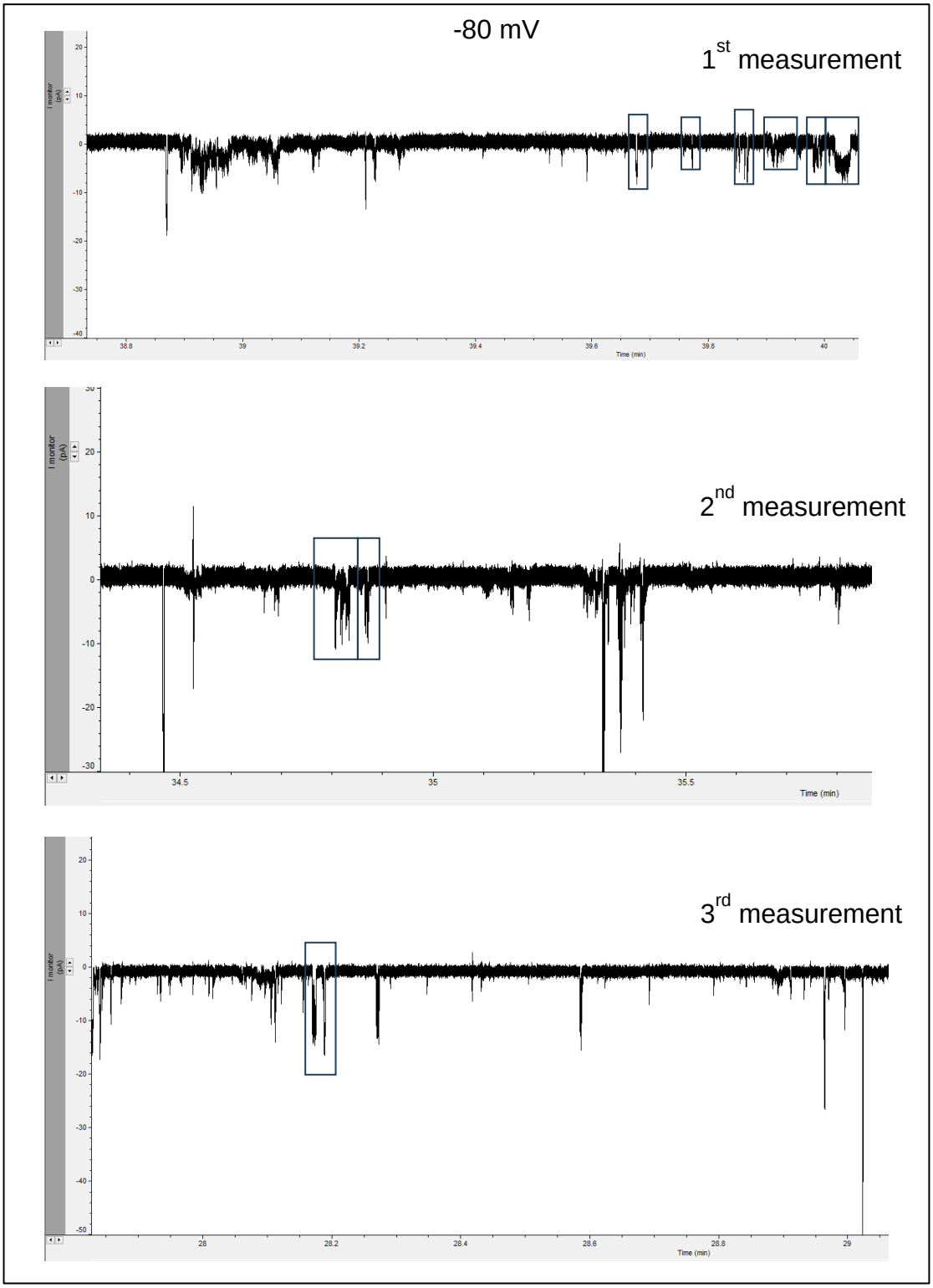


Figure S12. Recorded single channel current traces at -80 mV for ***t*-L**

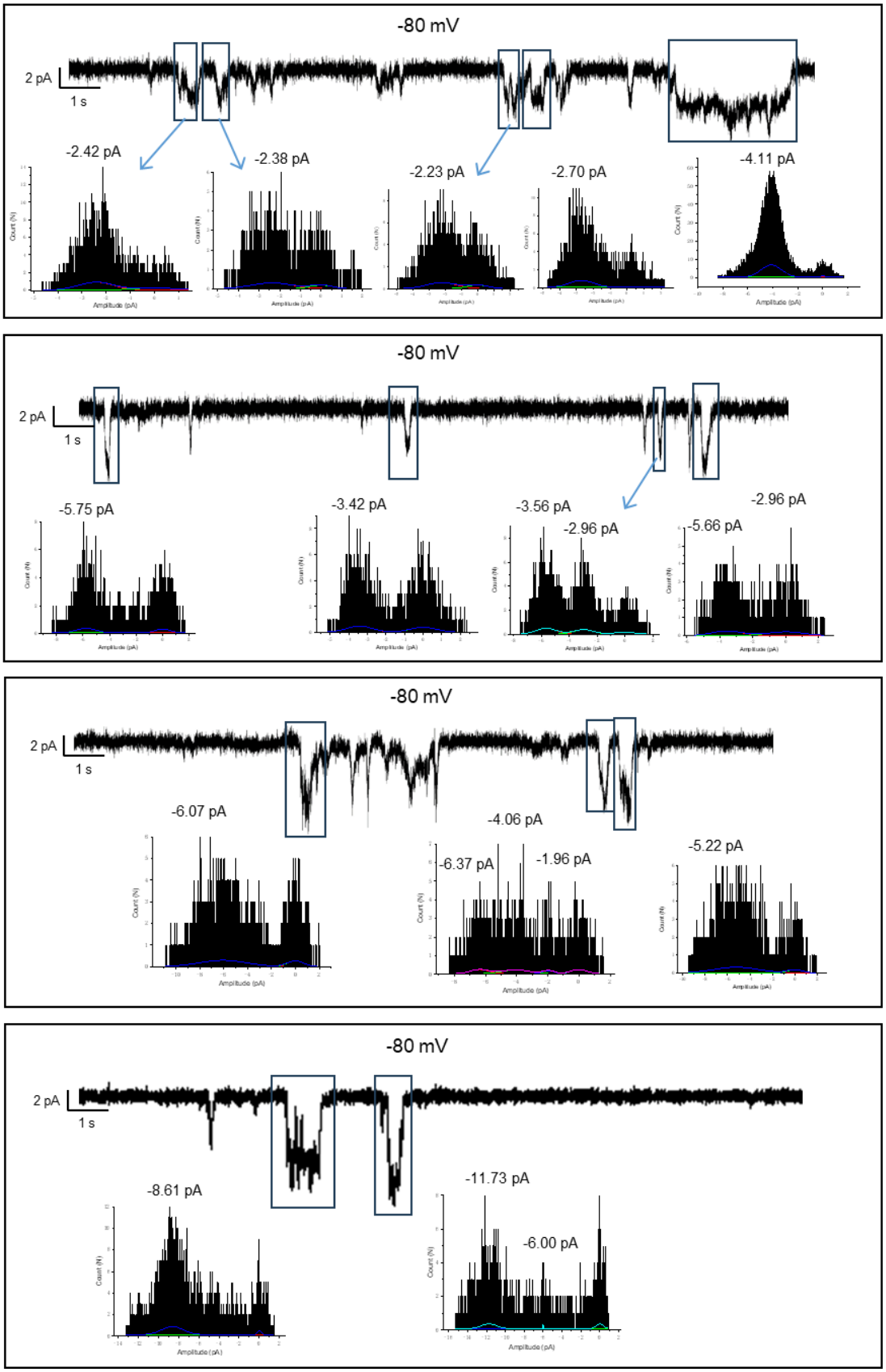
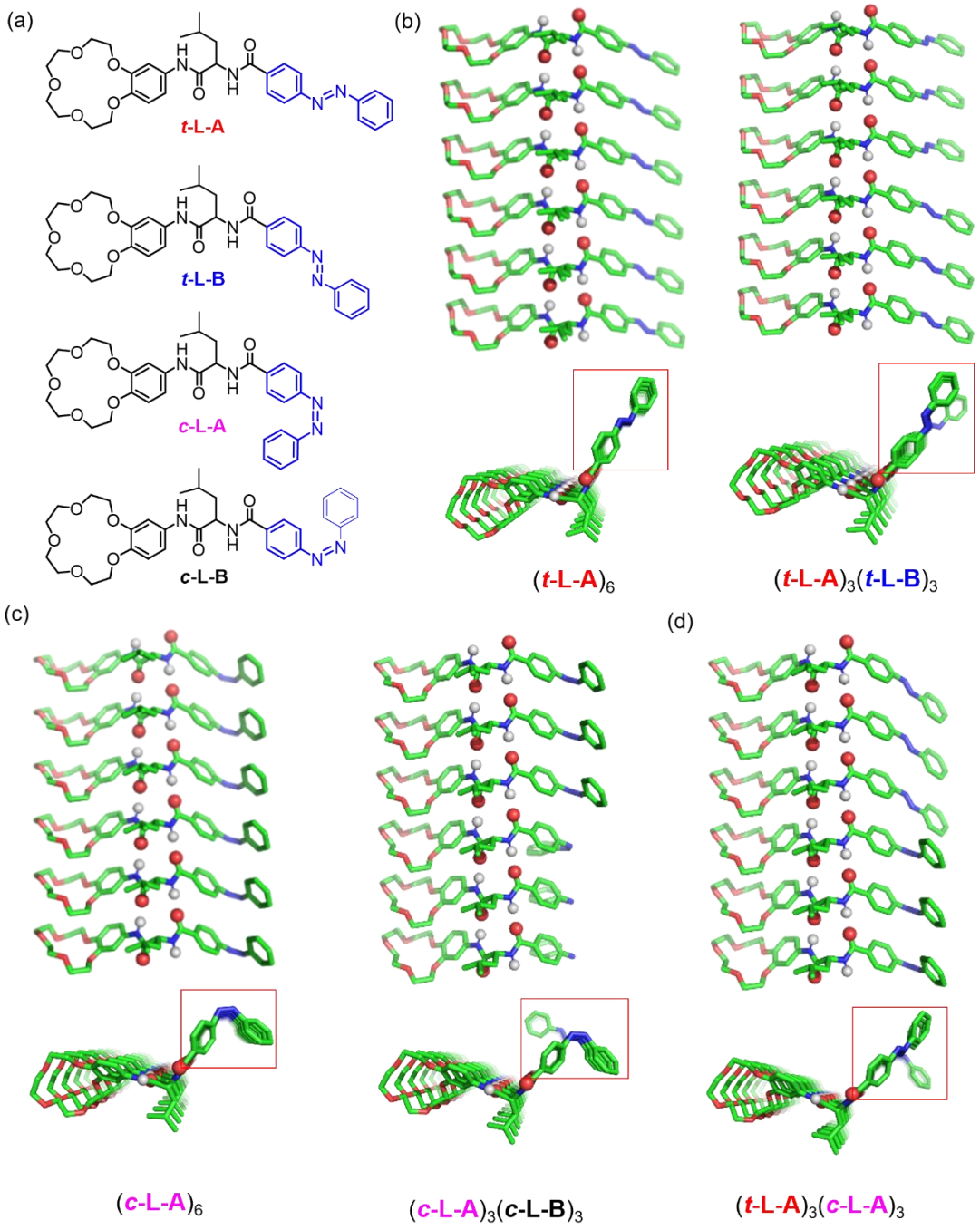
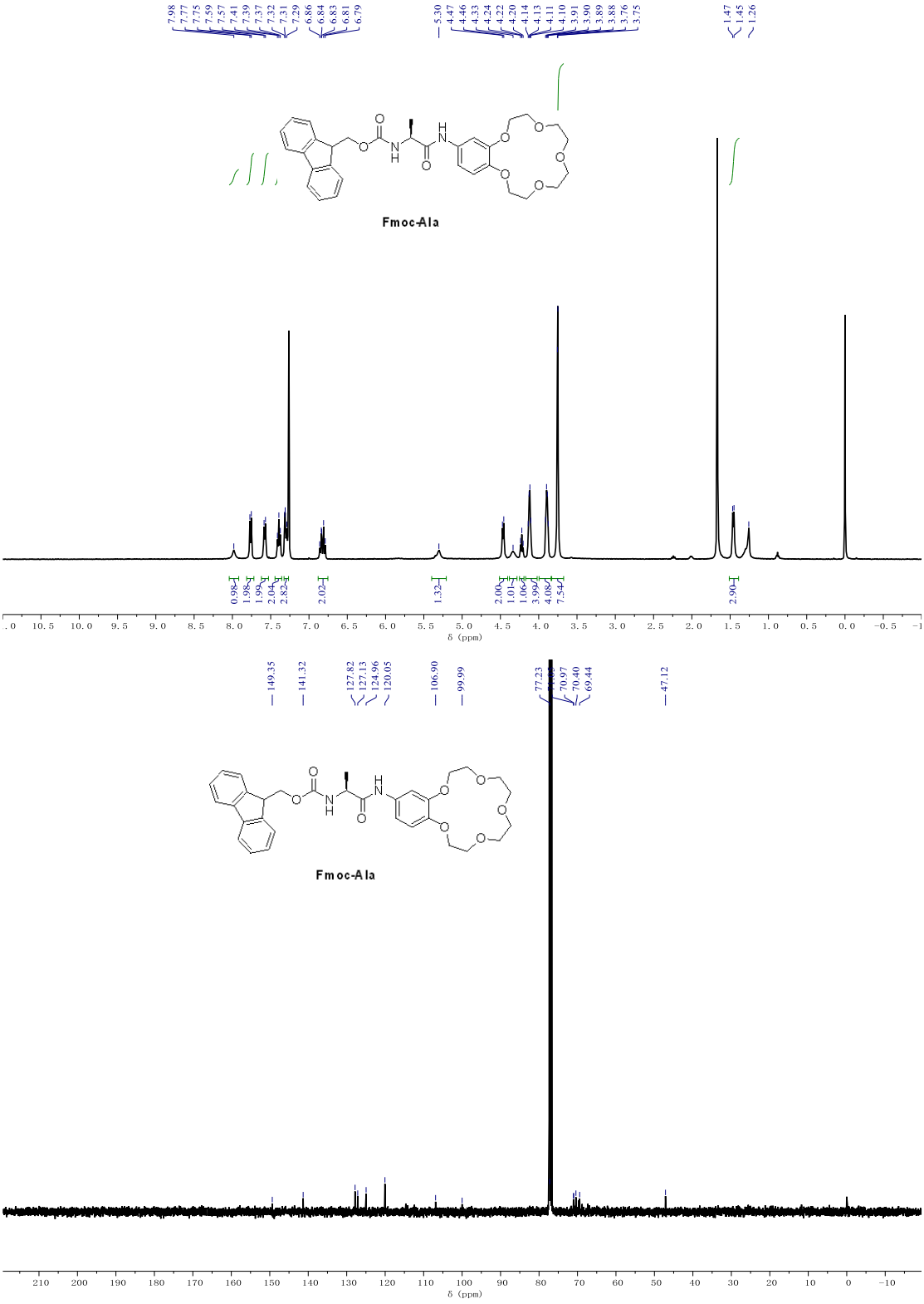


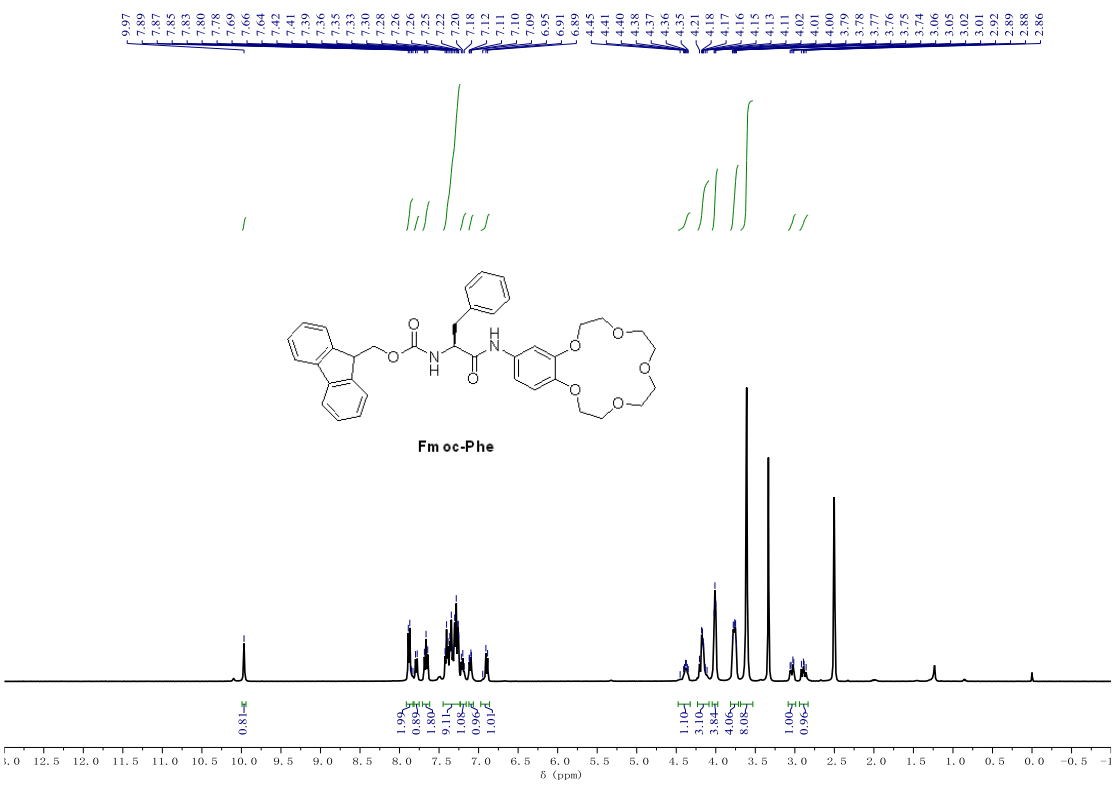
Figure S13. Histograms generated at voltage of -80 mV for ***t*-L**. Simulating these histograms yield average channel currents.



Figures S14. Schematic illustration of five representative self-assembled structures of (**L**)$_6$, consisting of (a) ***t*-L** and those having a different orientation of the azobenzene group, (b) ***c*-L** and those having a different orientation of the azobenzene group and (c) ***t*-L** and ***c*-L** in different ratios that may further come with a different orientation of the azobenzene group.

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

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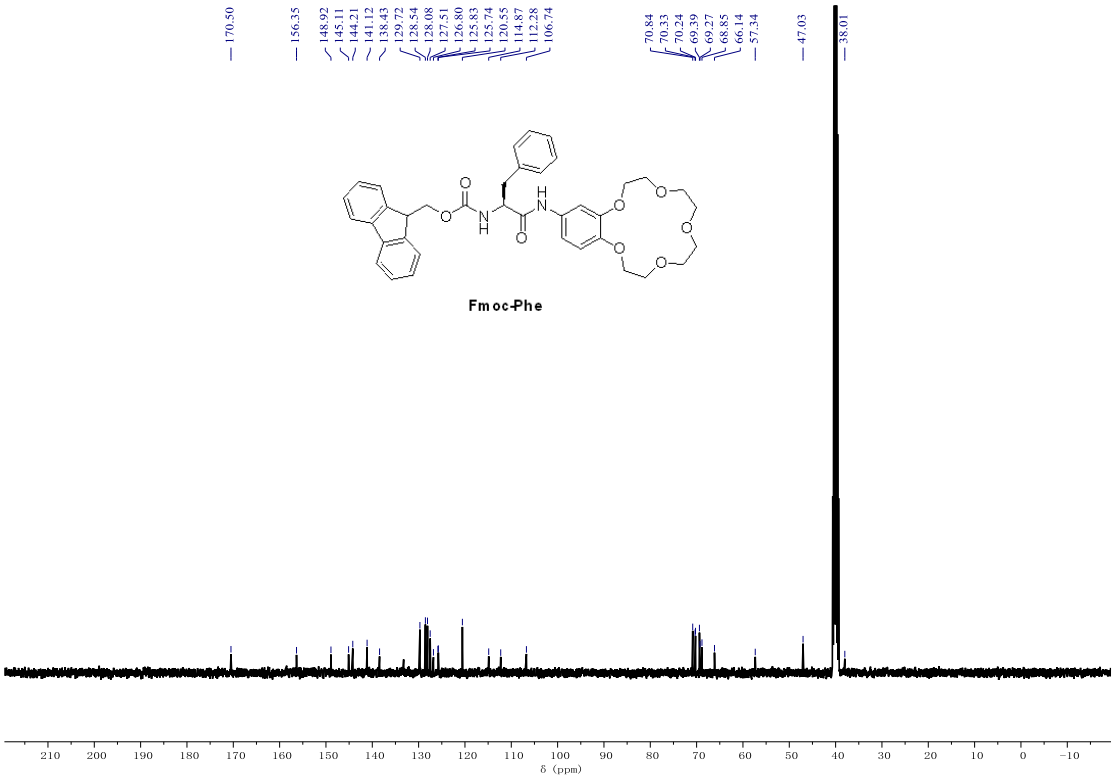
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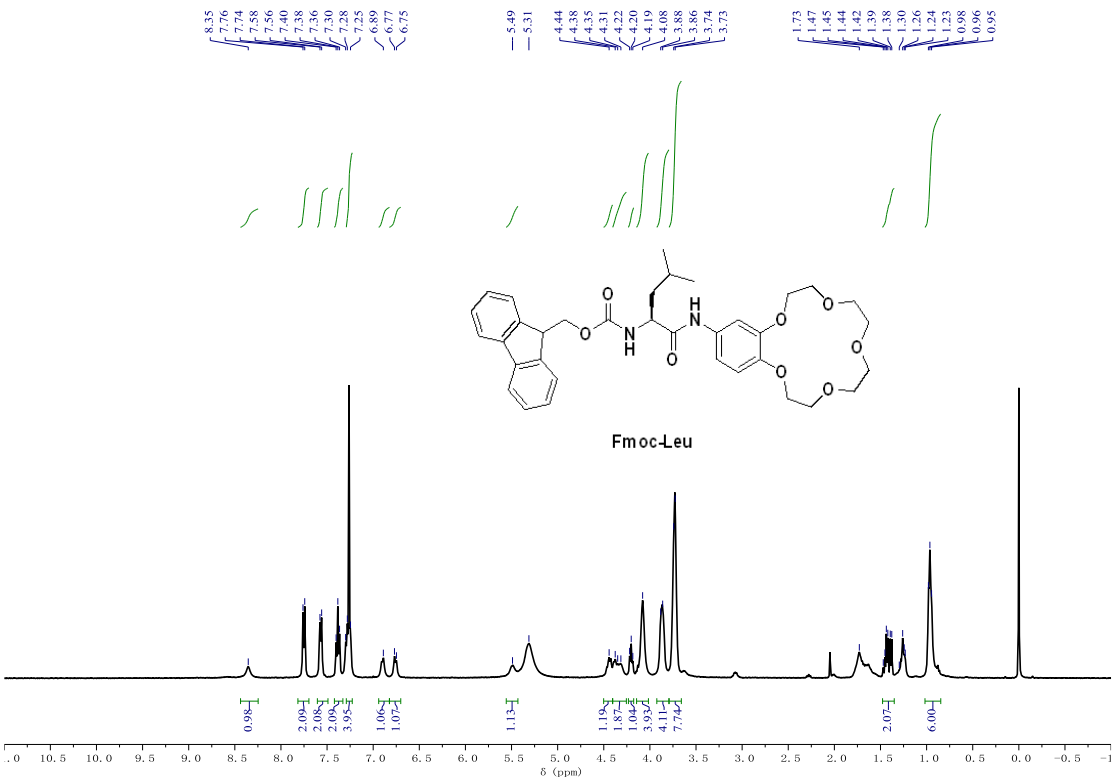
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6.75
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5.31
4.44
4.38
4.35
4.31
4.22
4.20
4.19
4.08
3.88
3.86
3.74
3.73
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1.44
1.42
1.39
1.38
1.30
1.26
1.24
1.23
0.98
0.96
0.95
Fmoc-Leu
0.98
2.09
2.08
2.09
3.95
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4.11
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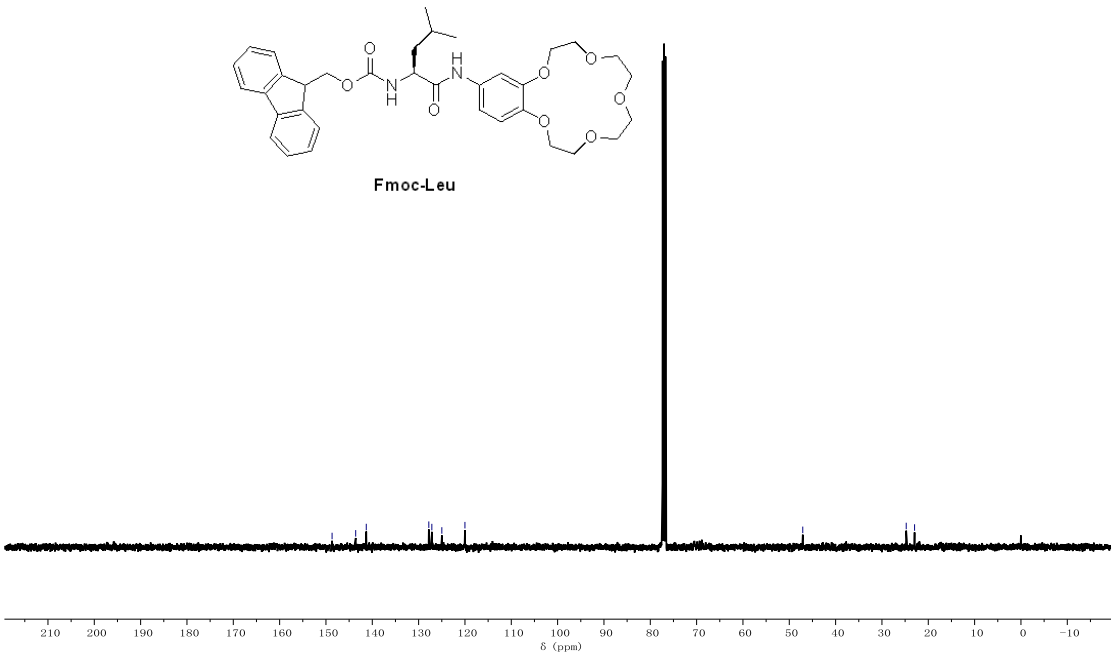
6.00
δ (ppm)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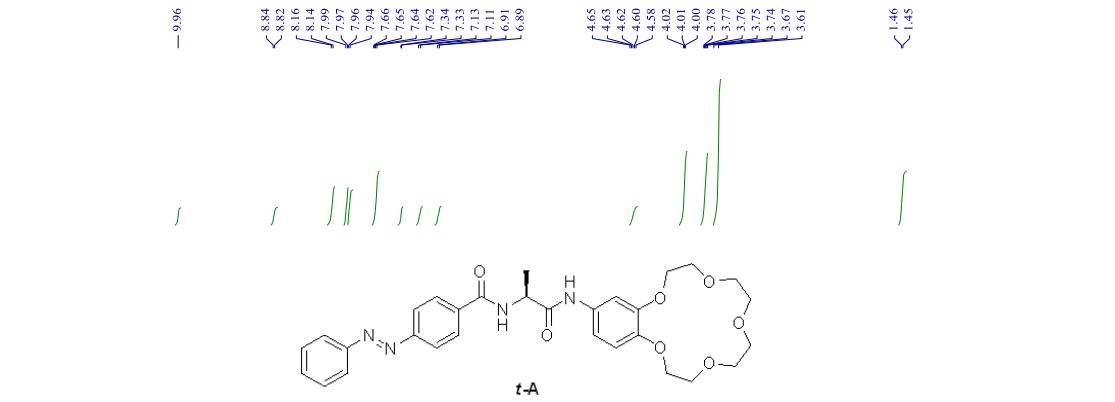
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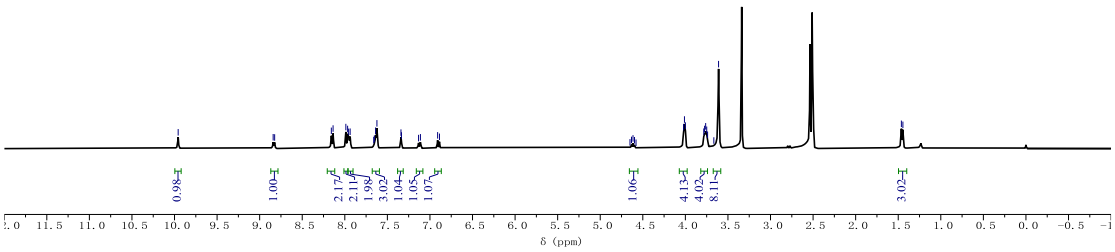
1.45
t-A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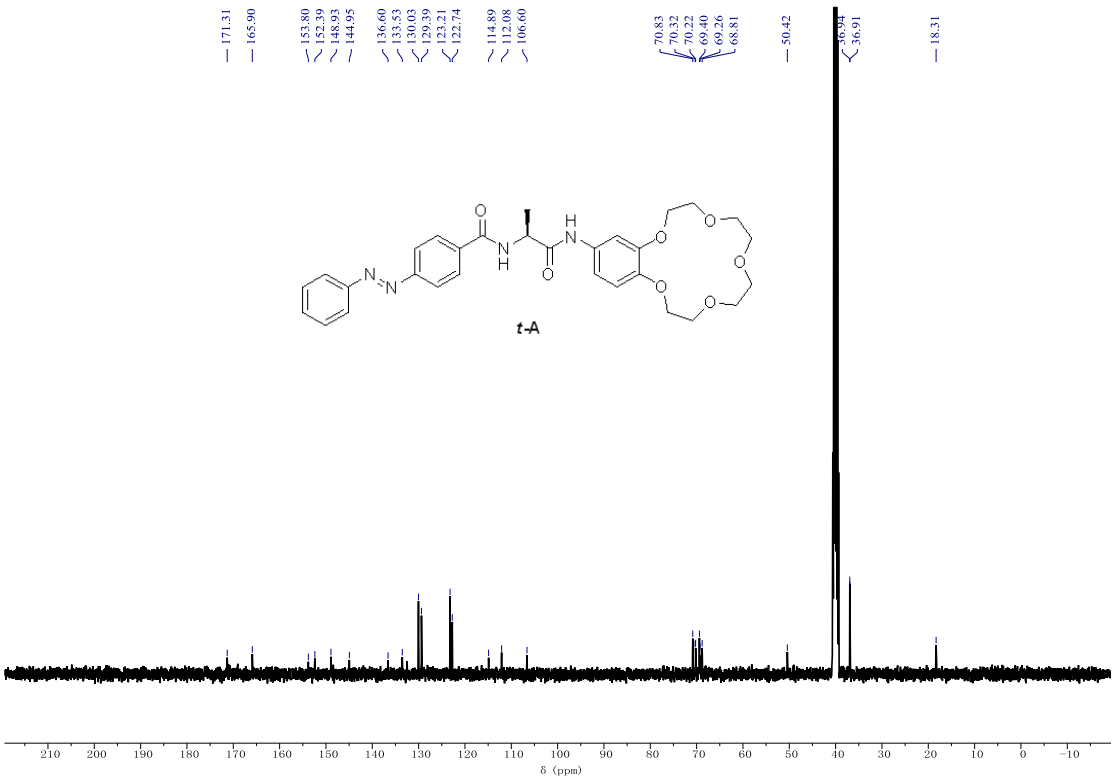
δ (ppm)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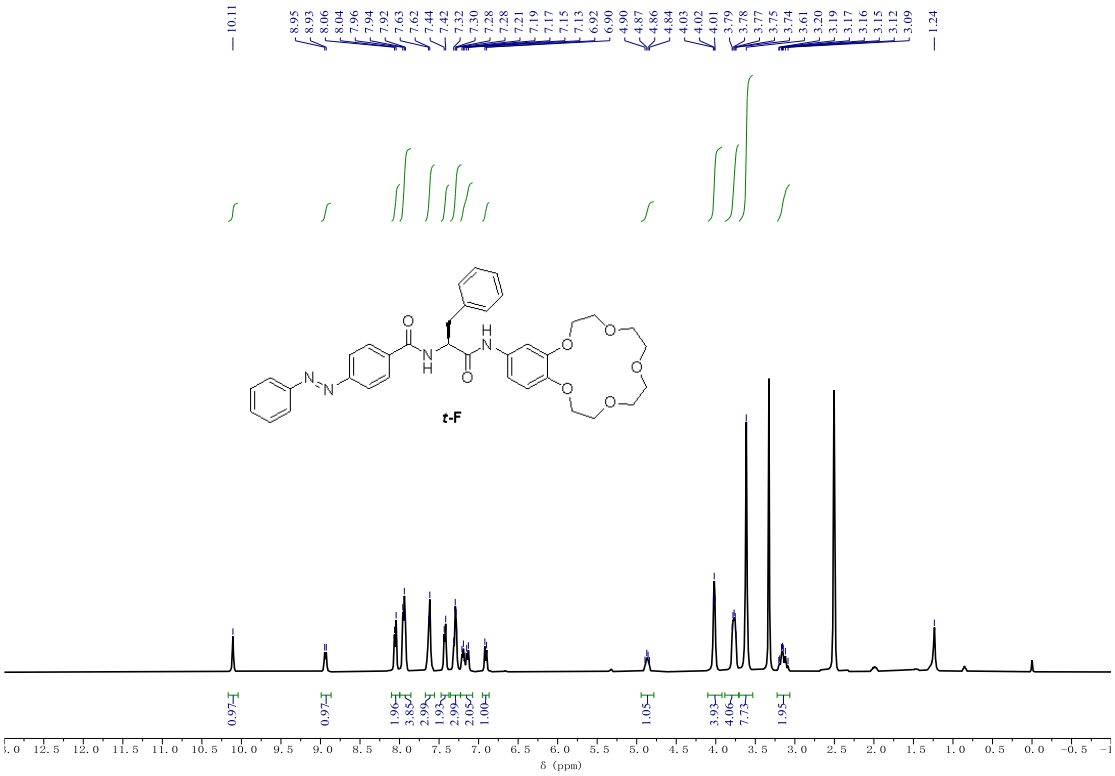
t-A
δ (ppm)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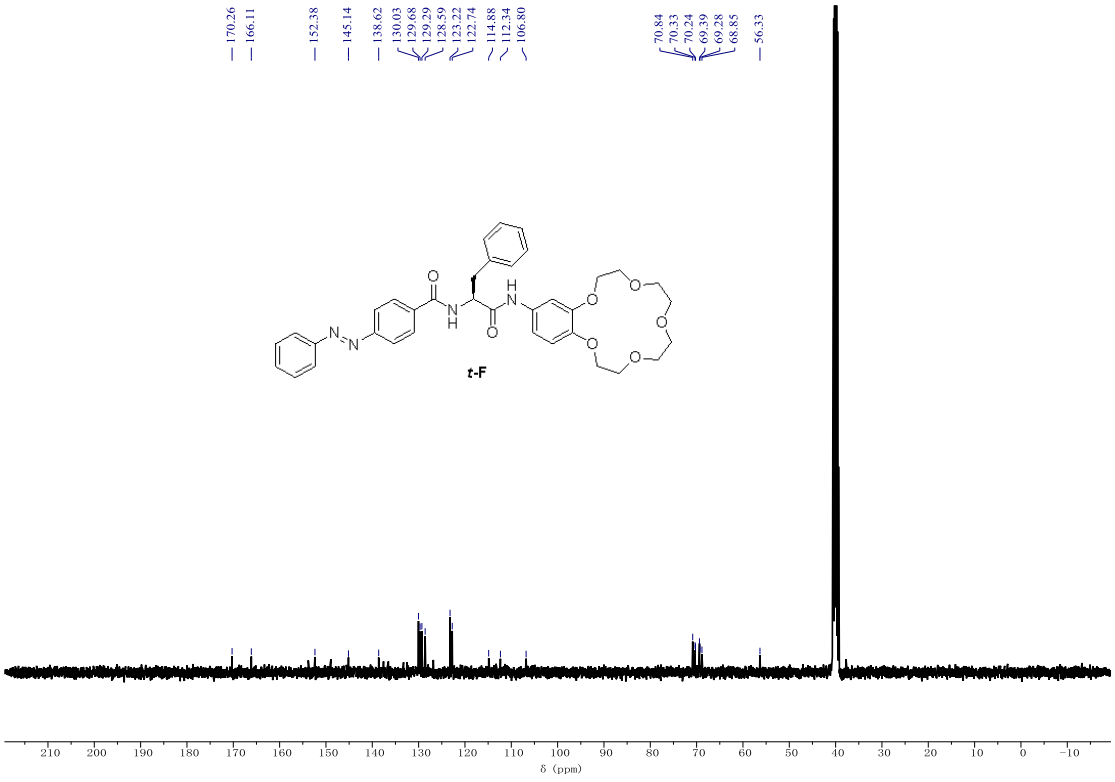
δ (ppm)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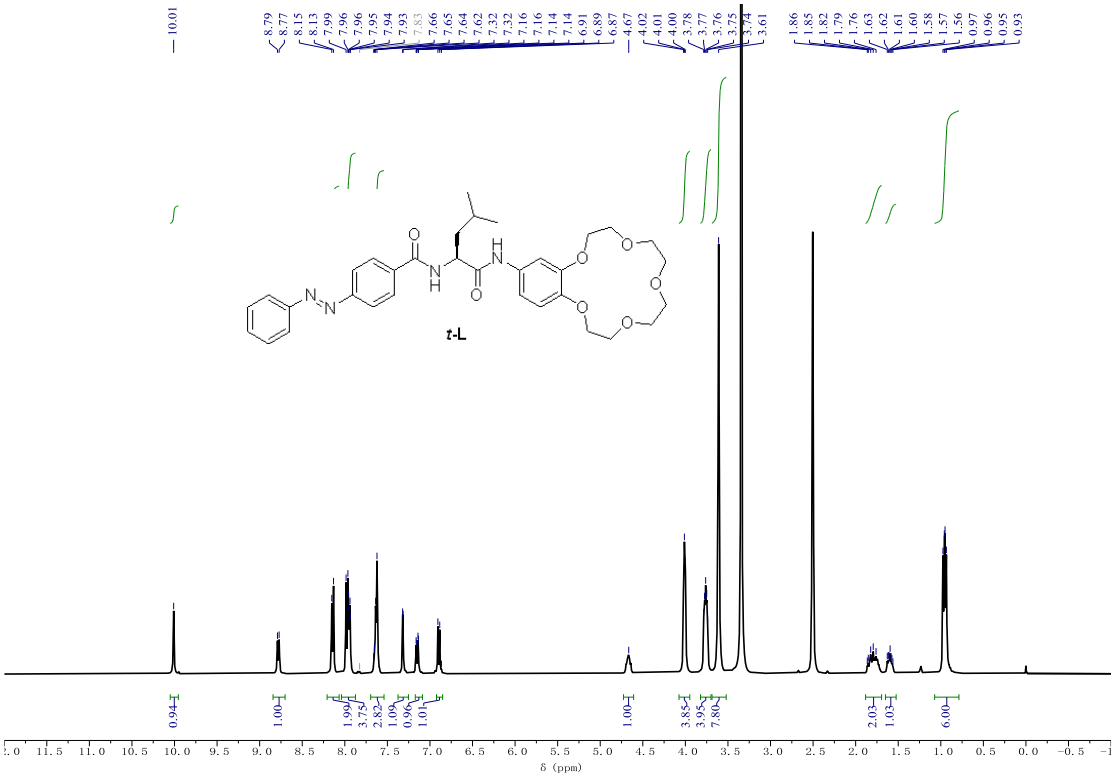
t-F
δ (ppm)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1.03
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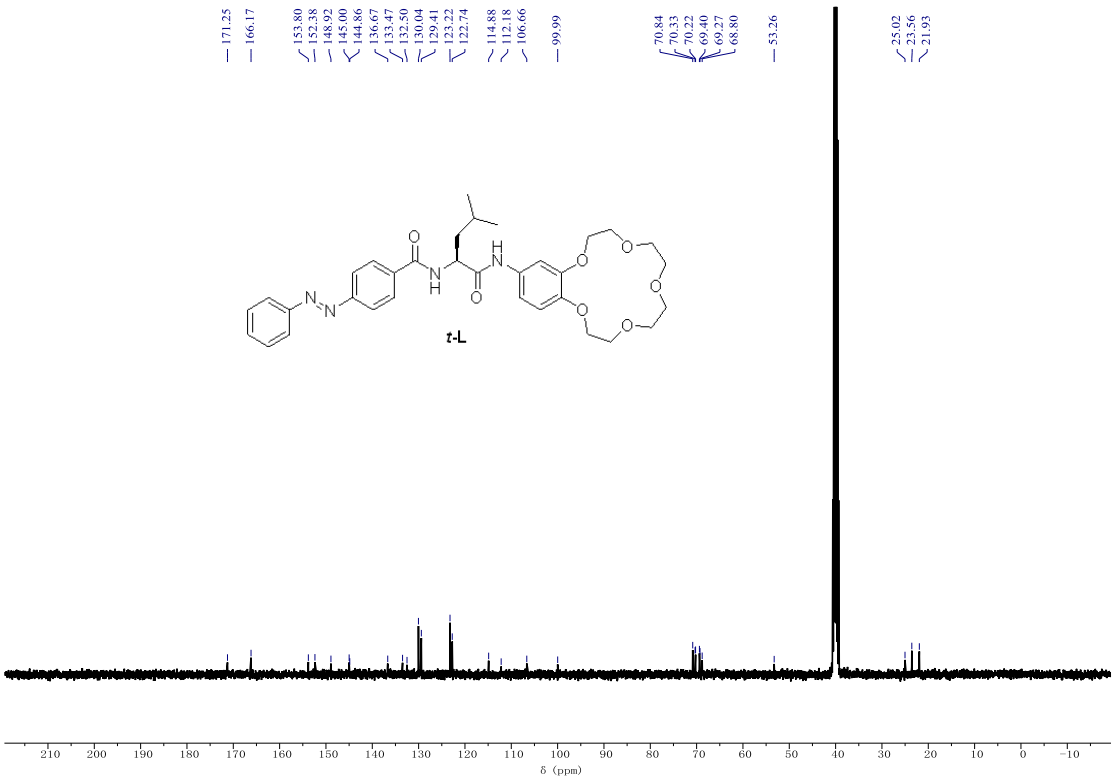
t-L
δ (ppm)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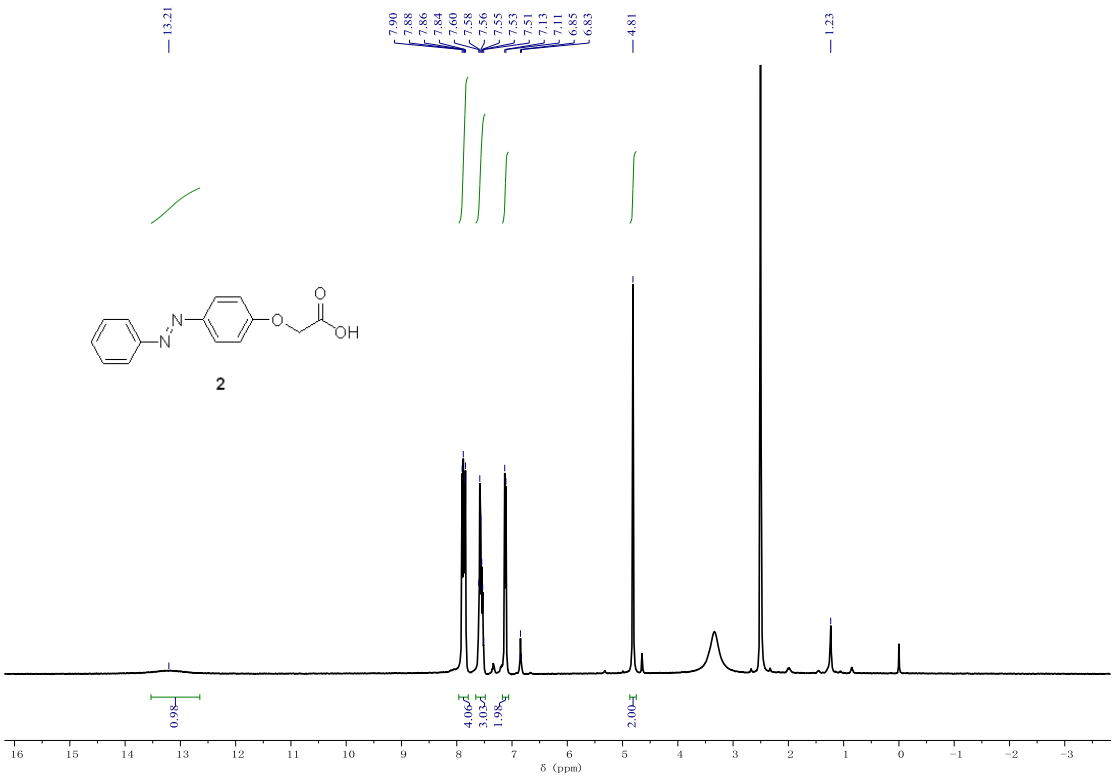
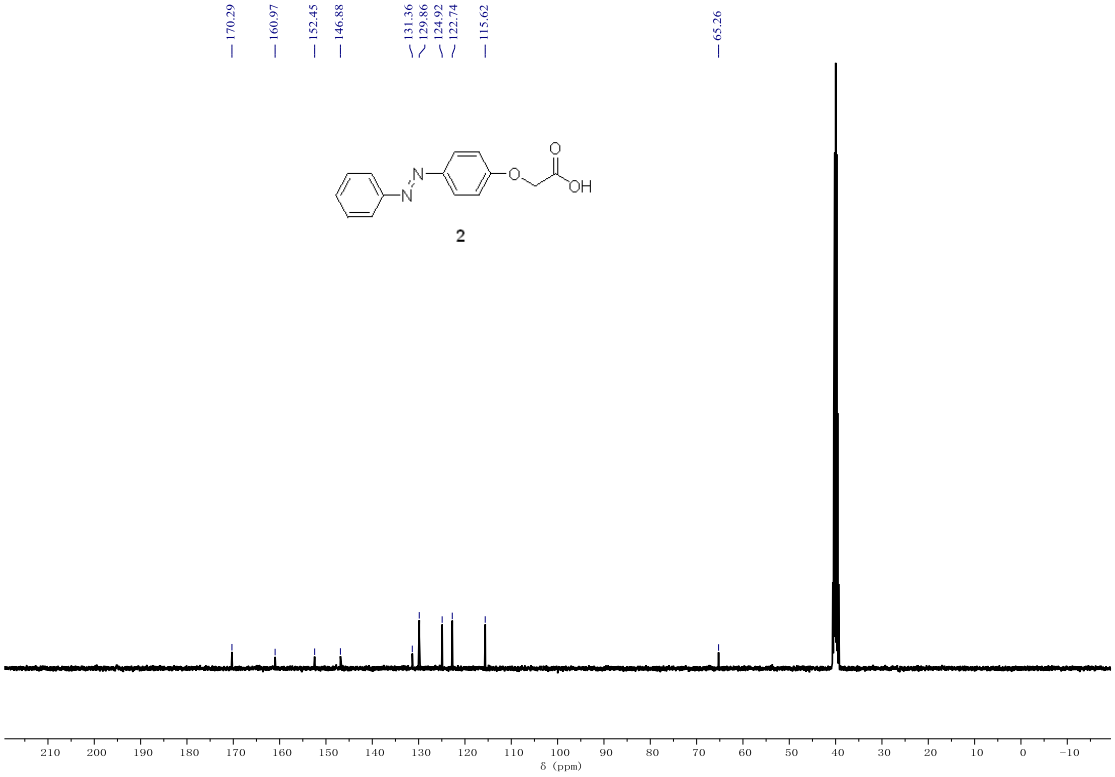
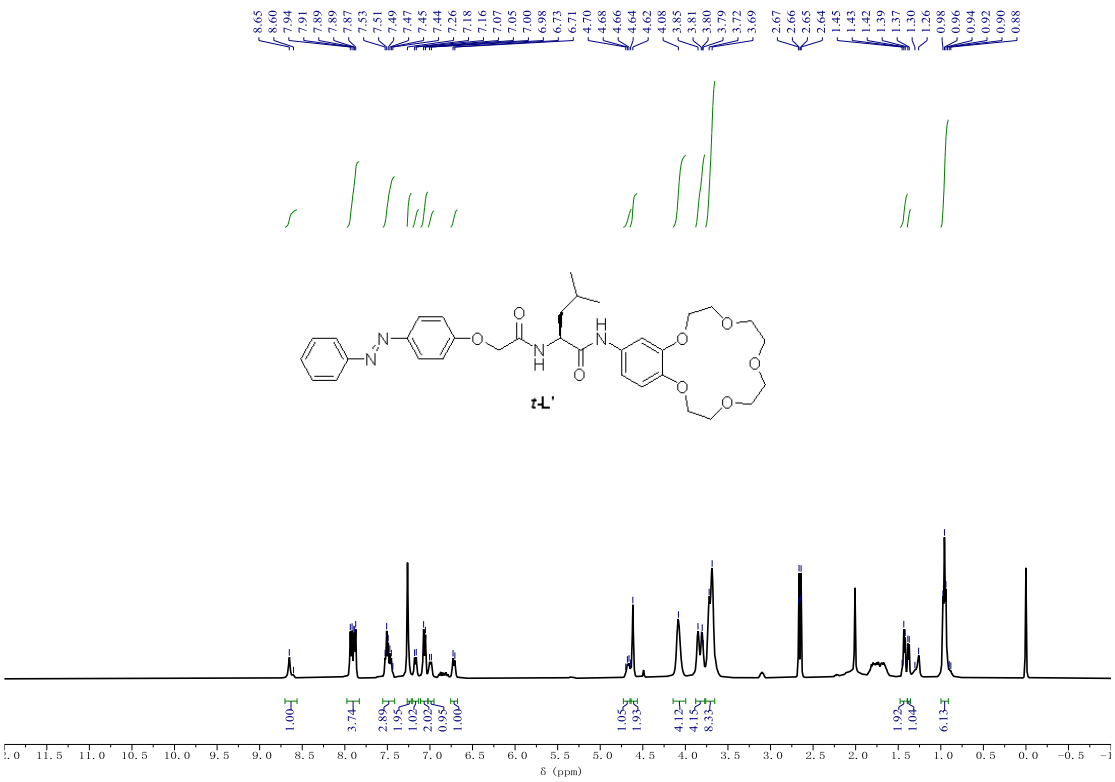
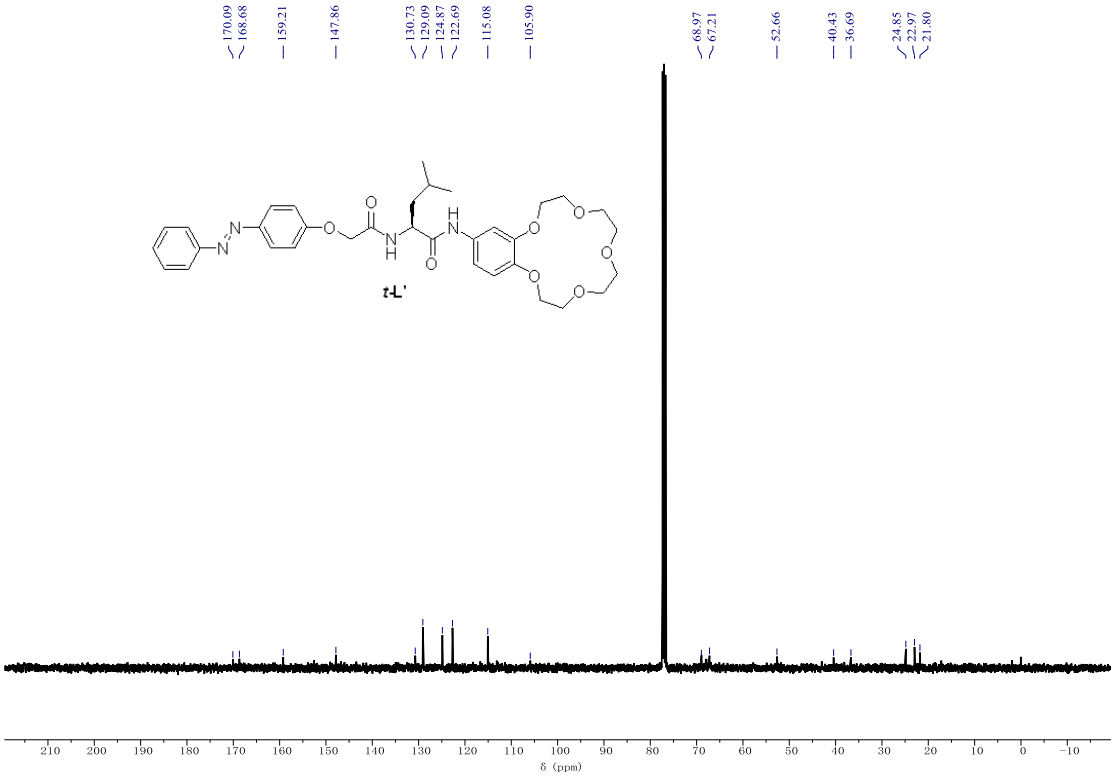
130.04
129.41
123.22
122.74
114.88
112.18
106.66
99.99
70.84
70.33
70.22
69.40
69.27
68.80
53.26
25.02
23.56
21.93
t-L
δ (ppm)


13.21
7.90
7.88
7.86
7.84
7.60
7.58
7.56
7.55
7.53
7.51
7.13
7.11
6.85
6.83
4.81
1.23
O
N
N
O
OH
2
0.98
4.06
3.03
1.98
2.00
δ (ppm)


170.29
160.97
152.45
146.88
131.36
129.86
124.92
122.74
115.62
65.26
O
N
N
O
OH
2
δ (ppm)


8.65
8.60
7.94
7.91
7.89
7.89
7.87
7.53
7.51
7.49
7.47
7.45
7.44
7.26
7.18
7.16
7.07
7.05
7.00
6.98
6.73
6.71
4.70
4.68
4.66
4.64
4.62
4.08
3.85
3.81
3.80
3.79
3.72
3.69
2.67
2.66
2.65
2.64
1.45
1.43
1.42
1.39
1.37
1.30
1.26
0.98
0.96
0.94
0.92
0.90
0.88
1.00
3.74
2.89
1.95
1.02
2.02
0.95
1.00
1.05
1.93
4.12
4.15
8.33
1.92
1.04
6.13
δ (ppm)


170.09
168.68
159.21
147.86
130.73
129.09
124.87
122.69
115.08
105.90
68.97
67.21
52.66
40.43
36.69
24.85
22.97
21.80
δ (ppm)