Supporting Information

Thiourea-Based Rotaxanes: Anion Transport Across Synthetic Lipid Bilayers and Antibacterial Activity Against *Staphylococcus Aureus.*

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Affiliations

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List of Abbreviations

AA	Arachidonic acid
Chol	Cholesterol
CFU	Colony forming units
DLS	Dynamic Light Scattering
DPPC	Dipalmitoylphosphatidylcholine
DPX	*p*-Xylene-bis(N-pyridinium bromide)
ESI	Electrospray Ionization
EYPC	Egg Yolk Phosphatidylcholine
HPLC	High-performance Liquid Chromatography
HPTS	8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt
HRMS	High-resolution Mass Spectrometry
Lucigenin	*N*,*N*′-Dimethyl-9,9′-biacridinium dinitrate
MALDI	Matrix Assisted Laser Desorption/Ionization
MS	Mass Spectrometry
NMR	Nuclear Magnetic Resonance
PEG	Polyethylene Glycol
TLC	Thin Layer Chromatography
TSA	Tryptic soy agar
TSB	Tryptic soy broth
w/w	Weight by weight

S1. Materials and General Experimental Methods

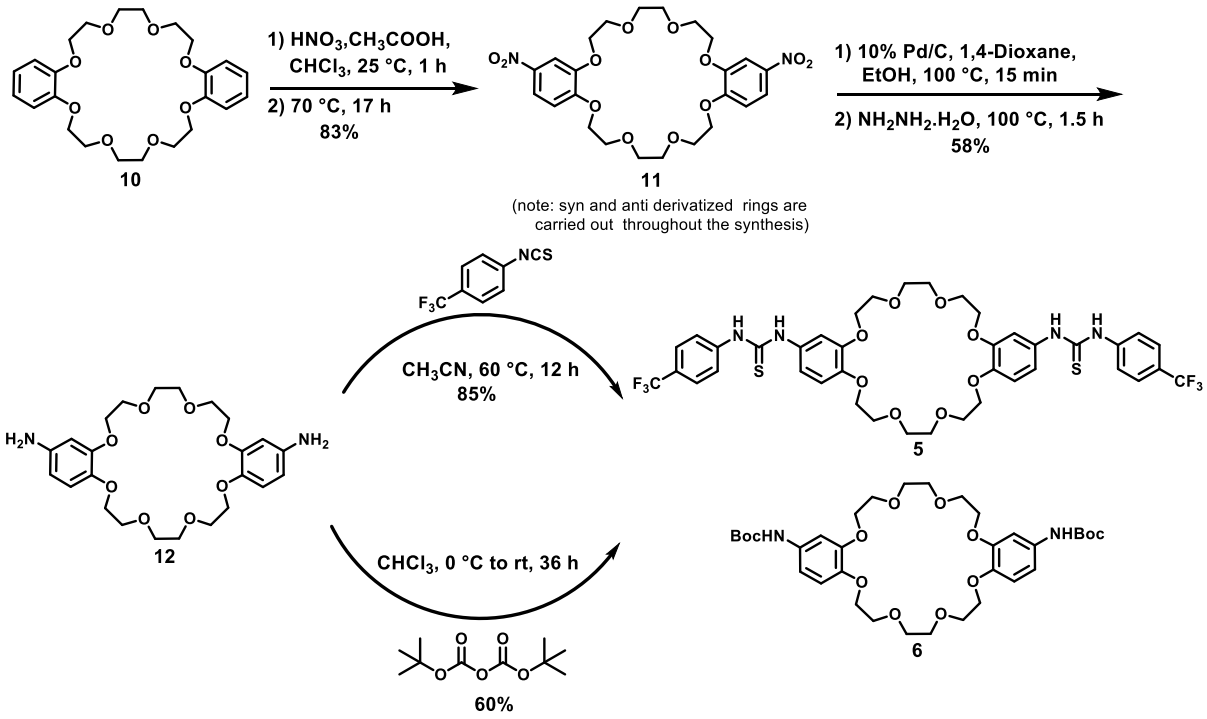
Glassware for synthesis was dried overnight in an oven before use. All the reactions were carried out under an inert atmosphere unless otherwise stated in the individual protocols. All the required chemicals, solvents, and lipids were purchased from different commercial sources and were used directly without purification. Solvents

such as tetrahydrofuran, dimethylformamide, acetonitrile, dichloromethane, and toluene were dried in a solvent purification system (Pure Process Technology) under the argon atmosphere. ACS-grade solvents were used for liquid extractions as well as for column chromatography. Flash column chromatography (FC) was performed using a Buchi Pure FlashPrep C-850 Chromatography System using silica as the stationary phase (60 Å, 230-400 mesh, silicycle) at 21 °C. The reaction progress was monitored by thin layer chromatography (TLC) using aluminum sheets coated with silica gel 60 F254 (Supelco, Sigma Aldrich), and visualization was done with UV light (254/365 nm). ^{1}H nuclear magnetic resonance (NMR) spectra were recorded using Bruker AV III 400 and Bruker AV III 500 spectrometers. All the ^{13}C NMR spectra were recorded on a Bruker AV III 500 spectrometer. Chemical shifts (δ) values are reported in ppm using the residual non-deuterated solvent signals as an internal reference (chloroform-d: δ_H = 7.26 ppm, δ_C = 77.16 ppm; acetone-d_6: δ_H = 2.05 ppm, δ_C = 29.84 ppm; acetonitrile-d_3: δ_H = 1.94 ppm, δ_C = 1.32 ppm; methanol: δ_H = 3.31 ppm, δ_C = 49.00 ppm, dimethyl sulfoxide-d_6: δ_H = 2.50 ppm, δ_C = 39.52 ppm. Coupling constant (*J*) values are recorded in hertz (Hz), and resonance multiplicity of peaks are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiple), and br (broad). High-resolution mass spectrometry (HR-MS) was performed by the Mass Spectrometry Facility at Louisiana State University using an Agilent 6230 ESI TOF and a Bruker UltrafleXreme MALDI TOF/TOF. All the standard buffer salts and the dye molecules were purchased from Sigma Aldrich for biophysical assays. Fluorescence studies were carried out using an FS5 spectrofluorometer from Edinburgh Instruments. Dynamic light scattering experiments were performed using the Litesizer DLS 100 instrument. All the ion-selective electrodes were purchased from Orion Instruments-Thermo Fisher Scientific with the following catalog numbers: 9617BNWP (chloride electrode), 9635BNWP (bromide electrode), 9653BNWP (iodide electrode), and 9707BNWP (nitrate electrode). All these electrodes were connected to the ORION STAR A214 meter. Egg Yolk Phosphatidylcholine (EYPC), 1,2-dihexadecanoyl-sn-glycero-3-phosphocholine (DPPC) and cholesterol were purchased from Sigma-Aldrich or Avanti Lipids to prepare the LUVs. Milli-Q Water was obtained through a Barnstead™ Water Purification System and used to prepare all the buffer solutions. Molecular biology grade dimethyl sulfoxide was used to prepare the stock solutions of the compounds, while chloroform (molecular biology grade) was used to prepare the stock solutions of

the lipids.

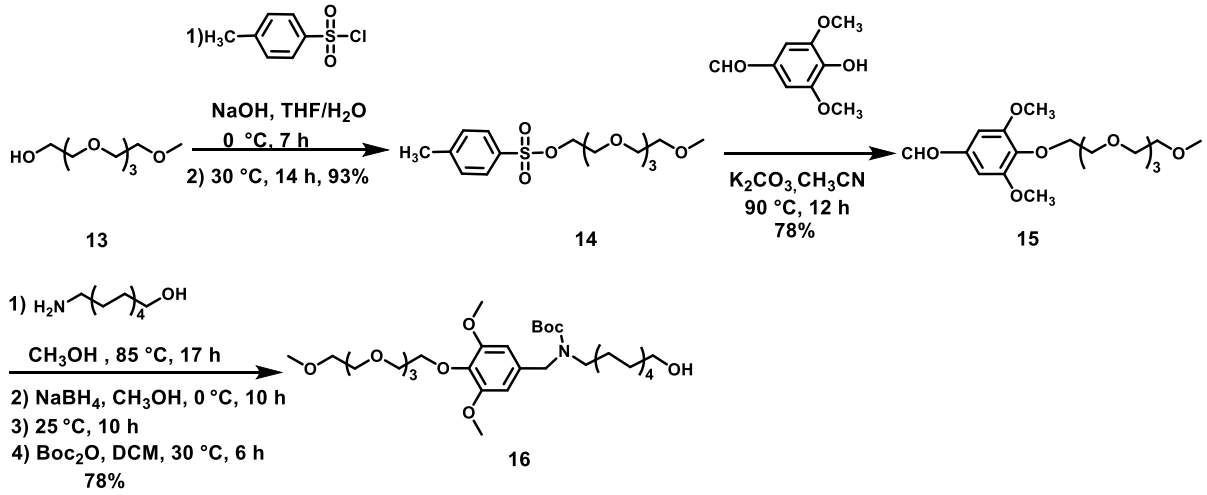
S2. Synthetic Schemes

S2.1. Synthetic schemes for the preparation of 5 and 6



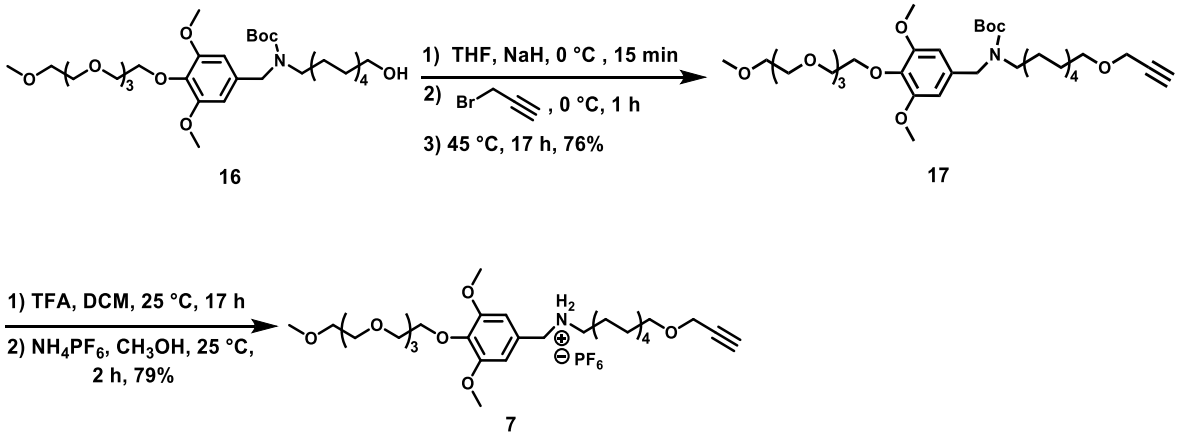
Scheme S1. Synthesis of rings **5** and **6**.

S2.2. Synthetic schemes for the preparations of common intermediate 16



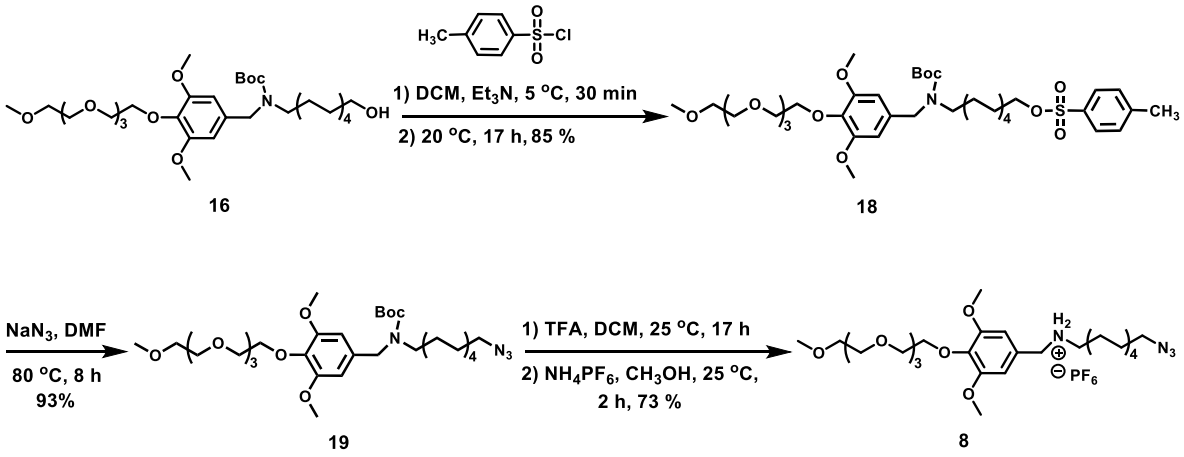
Scheme S2. Synthesis of common intermediate **16**.

S2.3. Synthetic schemes for the preparation of 7



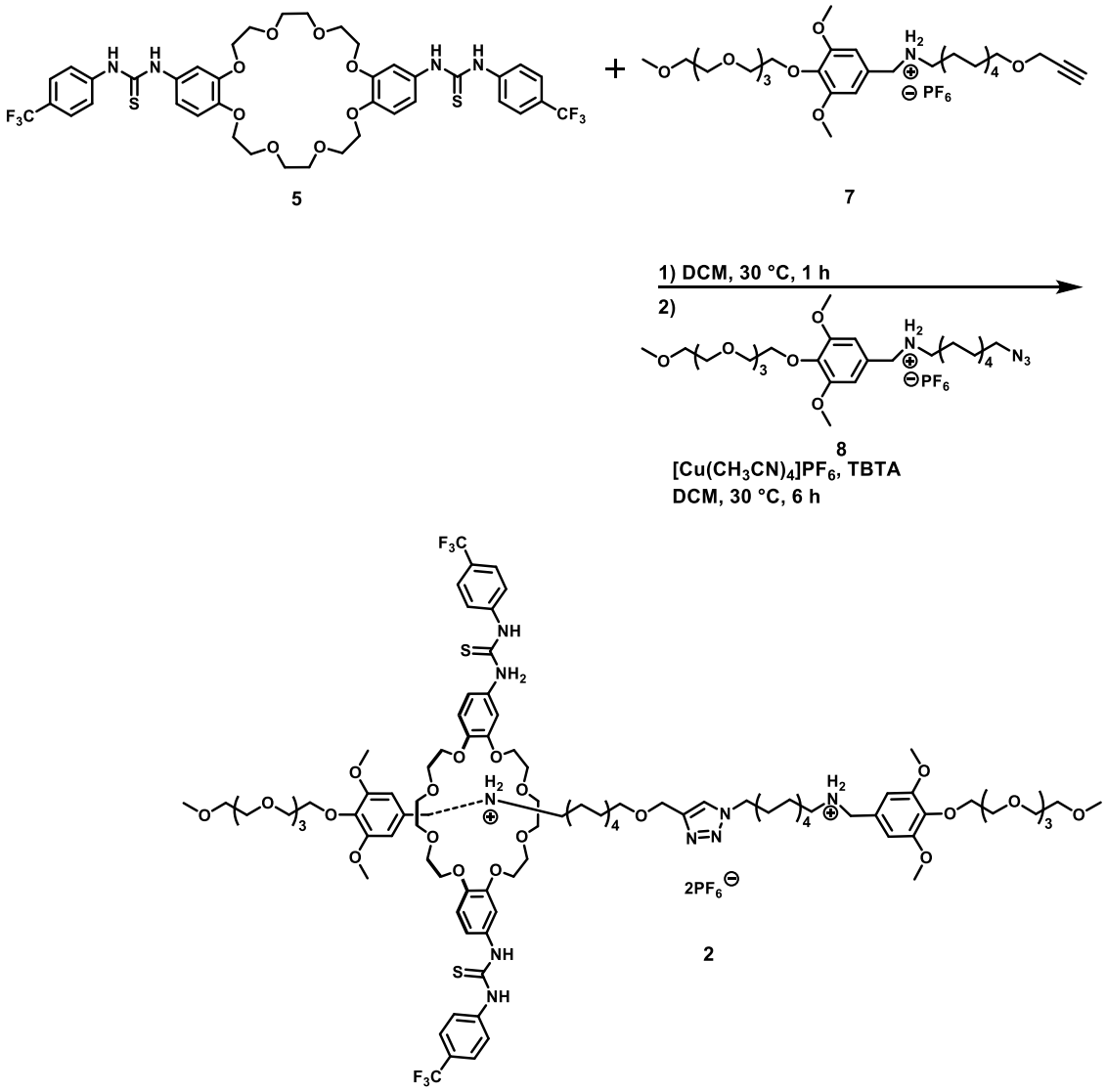
Scheme S3. Synthesis of **7.**

S2.4. Synthetic scheme for the preparation of 8



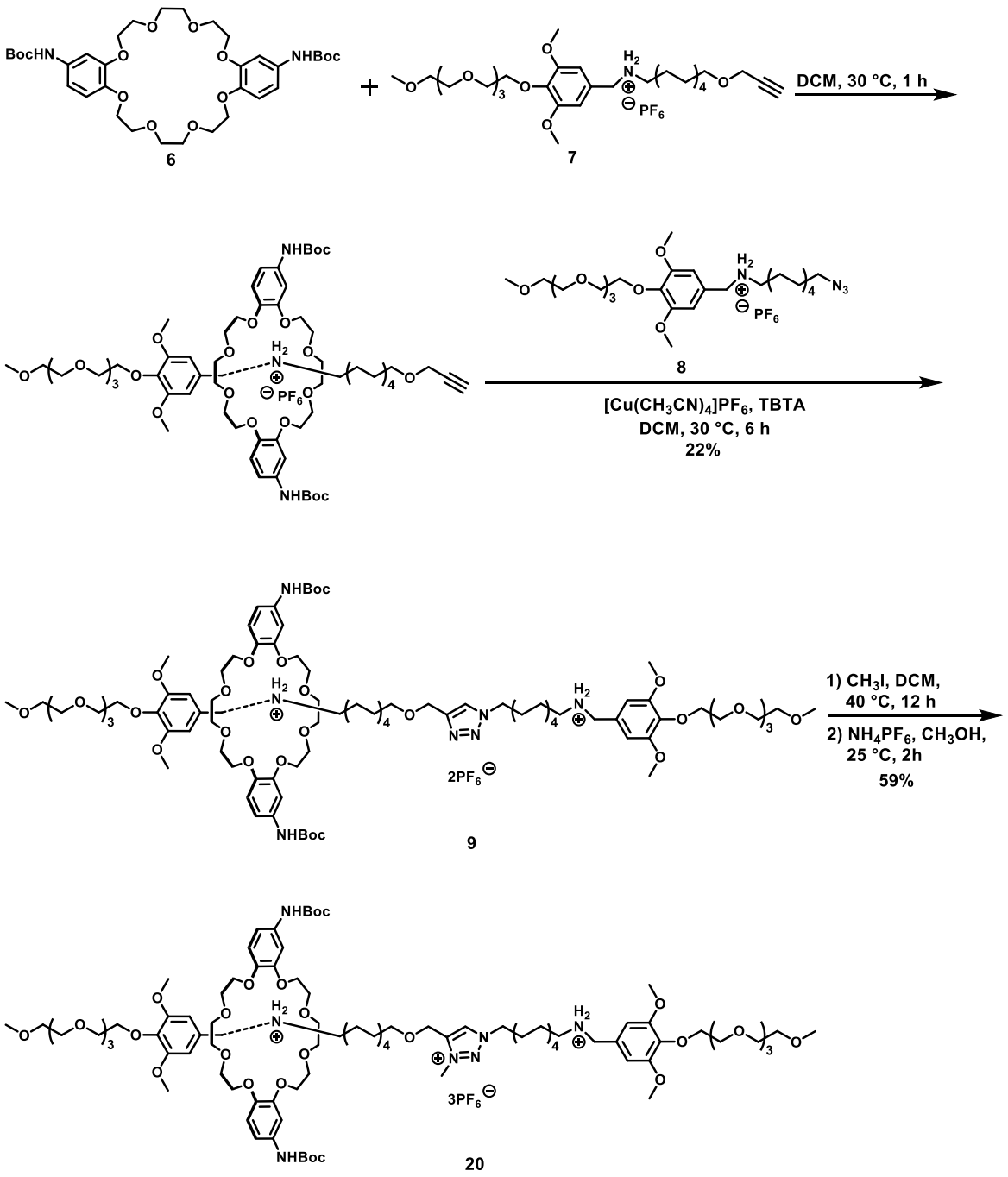
Scheme S4. Synthesis of **8**.

S2.5. Alternative synthetic approach for the synthesis of rotaxane 2



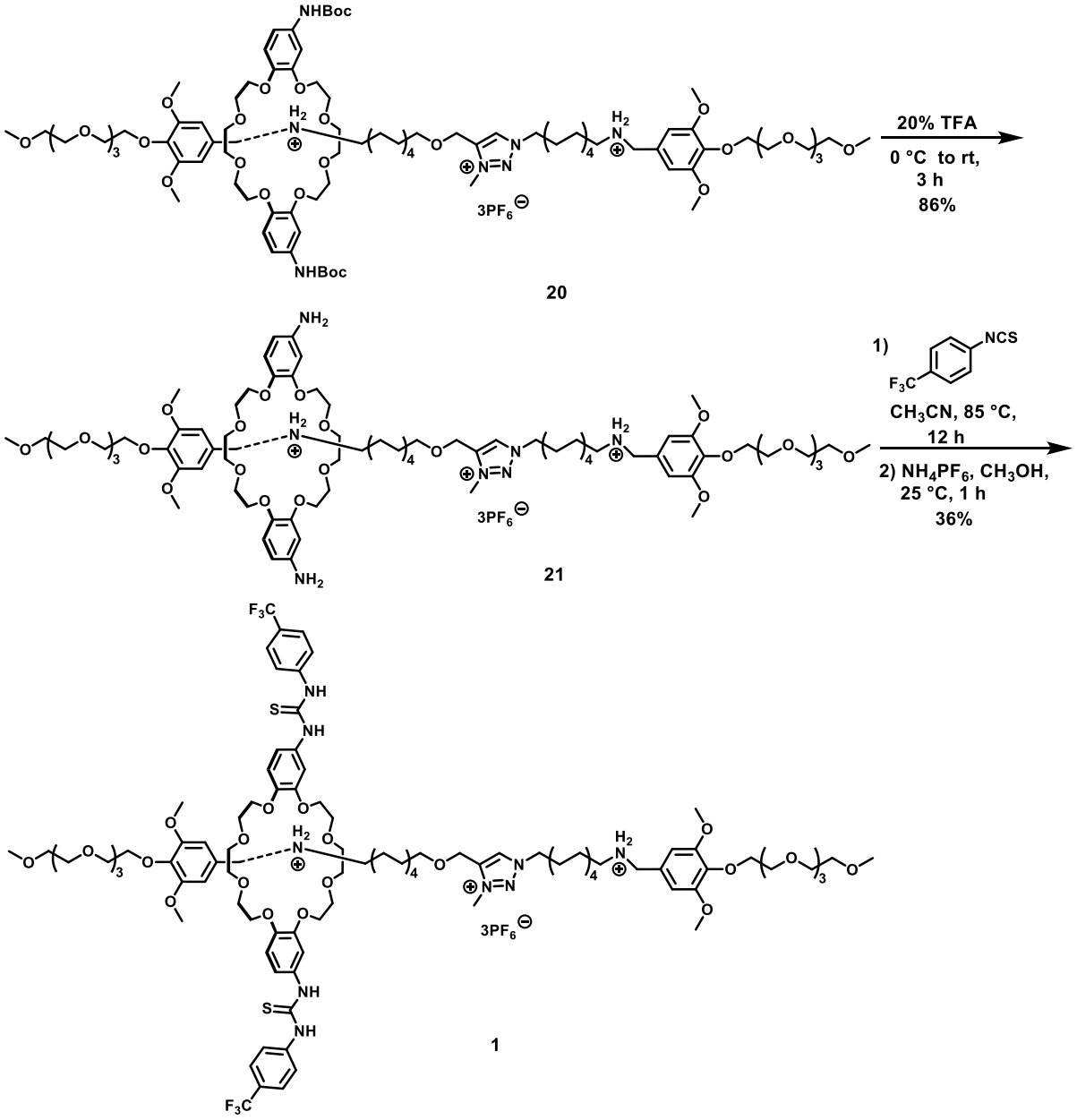
Scheme S5. An unsuccessful attempt to synthesize rotaxane **2** through direct threading of ring **5**.

S2.6. Synthetic scheme for the preparation of 9 and 20



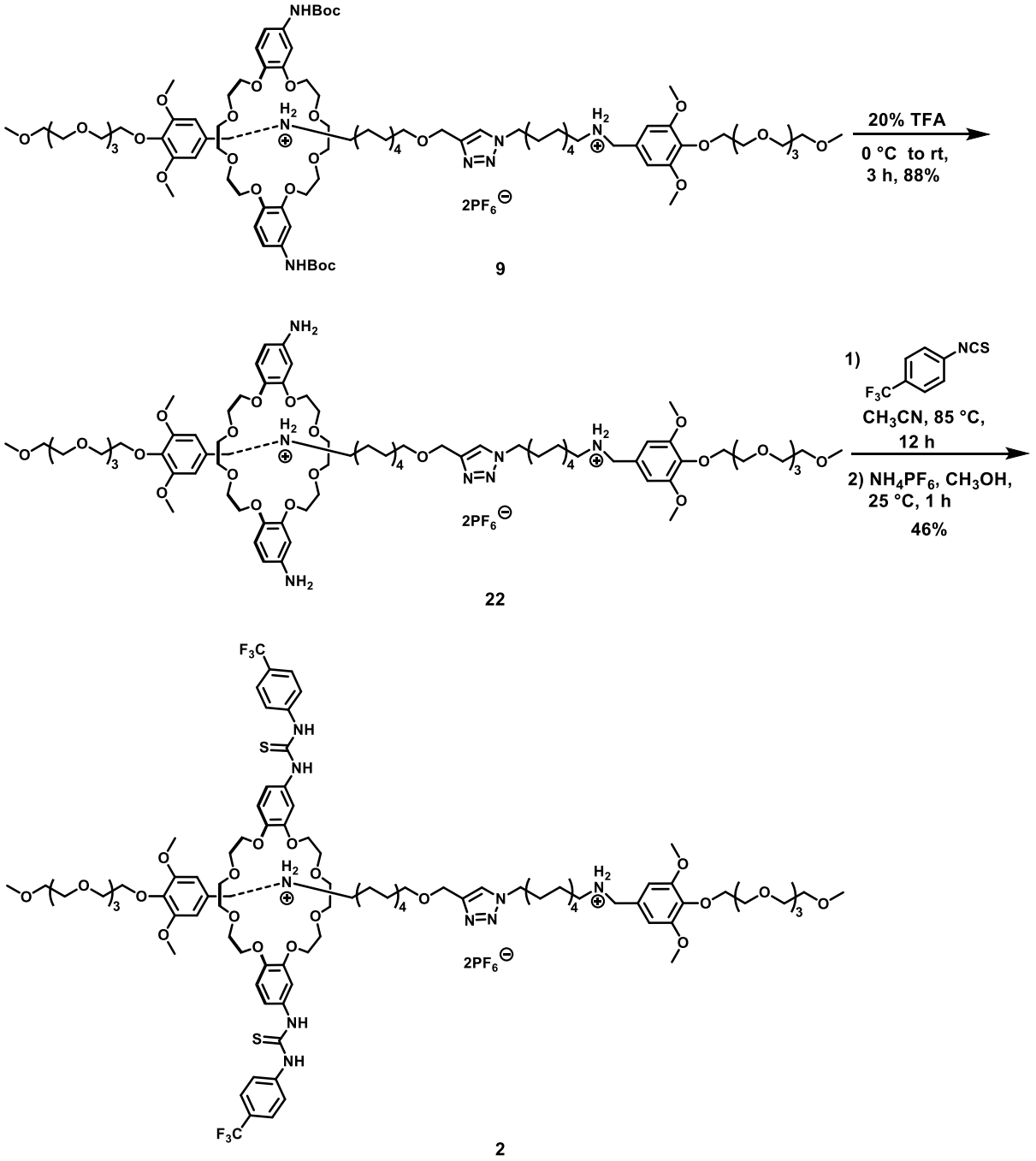
Scheme S6. Synthesis of **9** and **20**.

S2.7. Synthetic scheme for the preparation of rotaxane 1



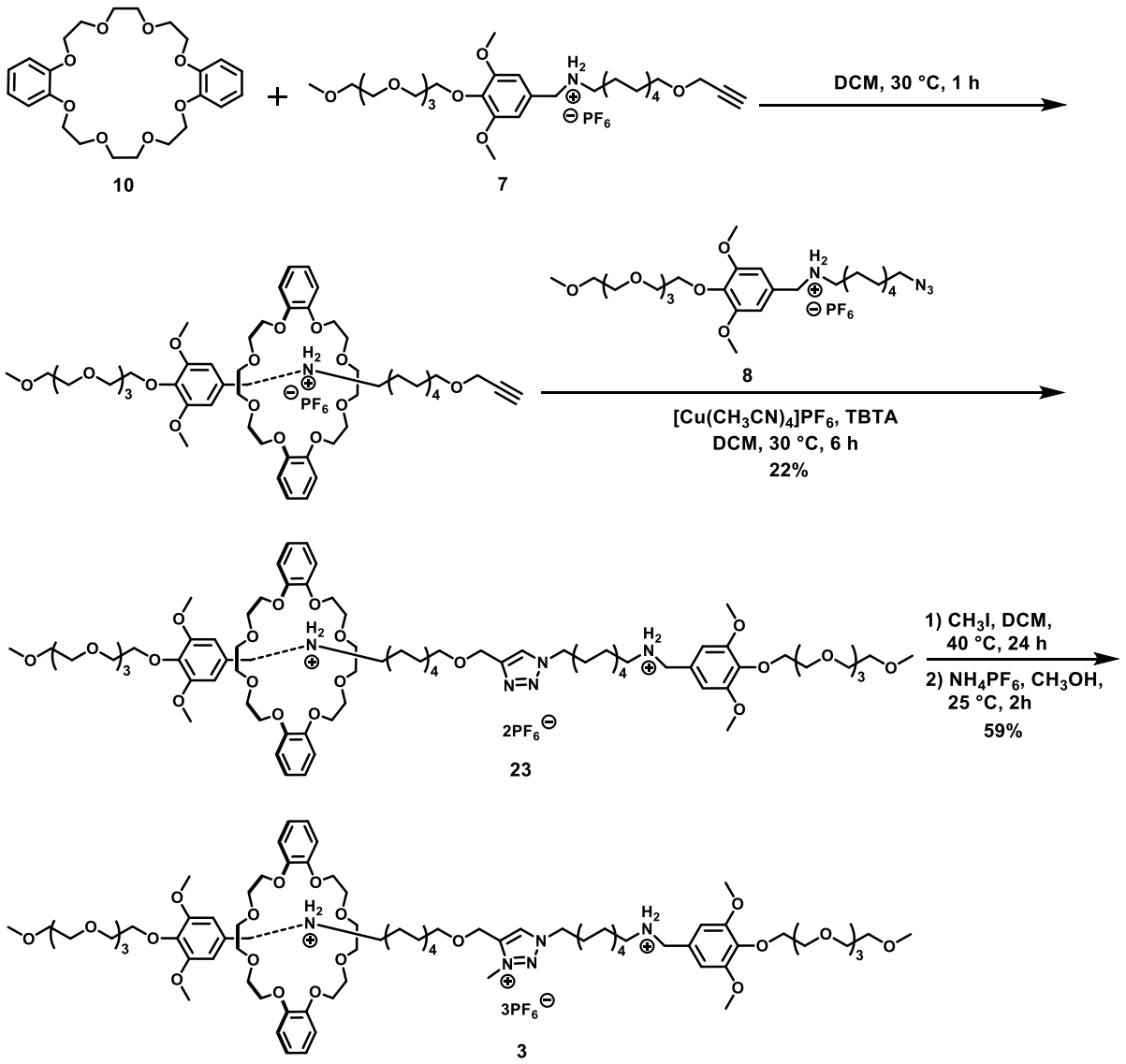
Scheme S7. Synthesis of methylated rotaxane **1.**

S2.8. Synthesis of the non-methylated rotaxane 2



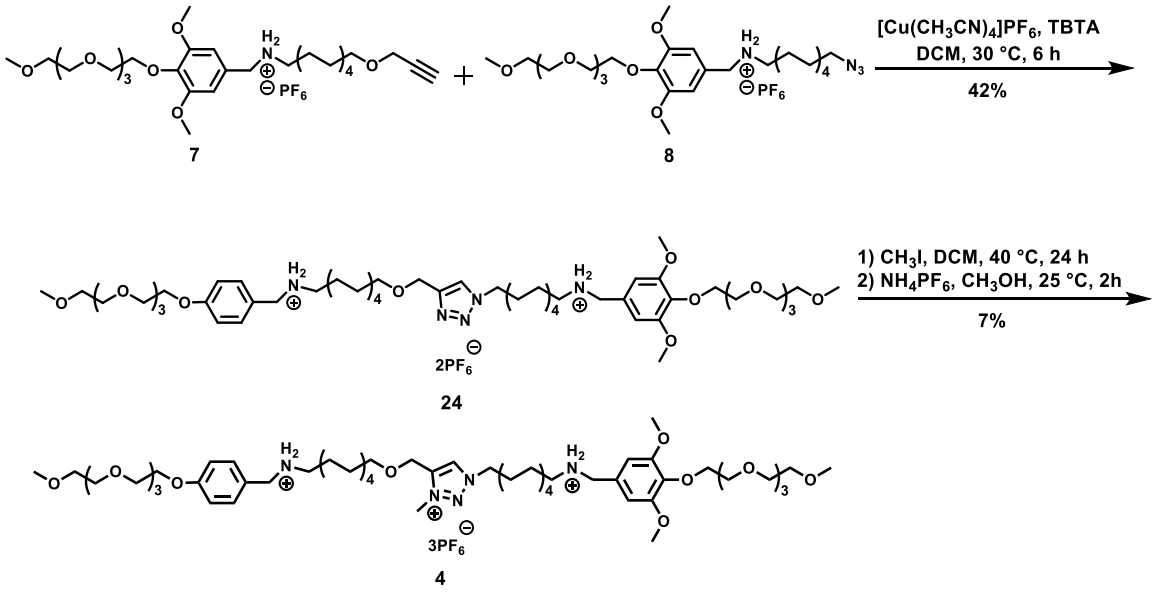
Scheme S8. Synthesis of non-methylated rotaxane **2.**

S2.9. Synthetic scheme for the preparation of control rotaxane 3



Scheme S9. Synthesis of control rotaxane **3**.

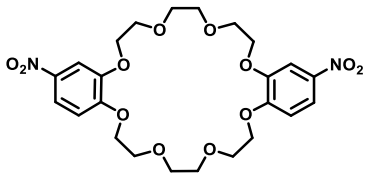
S2.10. Synthetic schemes for the preparation of control axel 4



Scheme S10. Synthesis of control axel **4**.

S3. Synthetic Procedures and Characterization

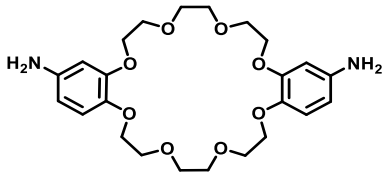
S3.1. Compound 11



Adapted from a reported procedure.[1] A solution of **10** (500 mg, 1.11 mmol) in chloroform (3 mL) was placed in a 100 mL two-neck round-bottom flask at 0 °C. Then, a mixture of 70% (w/w) nitric acid (3 mL) and acetic acid (10 mL) was added dropwise to the solution. The reaction mixture was stirred for 2 h at 25 °C and then refluxed at 70 °C for 2 h. Then the reaction mixture was cooled down to room temperature and ice water was added to it. Then the organic solvent was evaporated under vacuo, and the precipitate was filtered out. The precipitate was washed with cold water to obtain **11** (500 mg, 83%) as a white solid. ^{1}H NMR (400 MHz, chloroform-*d*): δ_{ppm} = 7.87 – 7.83 (m, 2H), 7.70 (t, *J* = 2.8 Hz, 2H), 6.85 (d, *J* = 8.9 Hz, 2H), 4.24 – 4.21 (m, 8H), 3.96 – 3.95 (m, 8H), 3.84 – 3.83 (m, 8H); ^{13}C NMR (126 MHz, chloroform-d): δ_{ppm} = 154.49, 154.47, 148.61, 148.57, 141.71, 141.67, 118.22, 118.18, 111.46, 111.43,

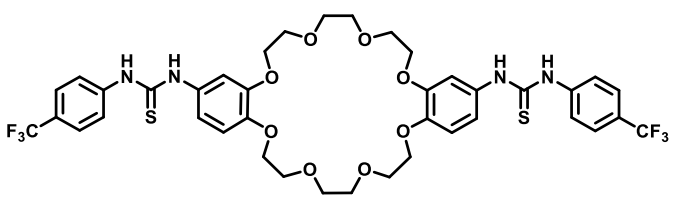
108.46, 108.43, 71.79, 72.72, 71.70, 69.91, 69.90, 69.86, 69.67; HRMS (ESI) (m/z): Calcd for $C_{24}H_{30}N_2O_{12}NH_4^+$ $[M+NH_4]^+$ 556.2137, found: 556.2129; $C_{24}H_{30}N_2O_{12}Na^+$ $[M+Na]^+$ 561.1691, found: 561.1683; $C_{24}H_{30}N_2O_{12}K^+$ $[M+K]^+$ 577.1431, found: 577.1428. **Note:** The final product was obtained as a mixture of *syn* and *anti*-isomers that could not be separated, so the mixture was used for the following reactions. We draw only the *syn* isomer for simplicity.

S3.2. Compound 12



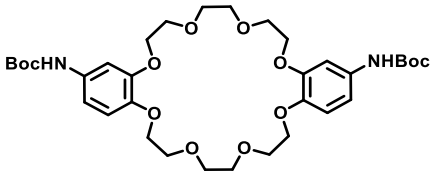
Adapted from a reported procedure.[1] Absolute ethanol (20 mL) was added to dissolve compound **11** (500 mg, 0.92 mmol) in a 100 mL two-neck round-bottom flask. Then 10% (w/w) Pd/C (10 mg) was added at once, and the resulting mixture was stirred for 30 min at 100 °C. Hydrazine monohydrate (3.6 mL, 32 mM) was added dropwise over a period of 10 minutes, and the mixture was stirred for another 2 h at 100 °C. After completion, the reaction was passed through celite to remove the Pd/C. The liquid layer was collected and evaporated under reduced pressure. Finally, recrystallization in cold ethanol provided crown ether **12** (260 mg, 58%) as a white solid. ^{1}H NMR (400 MHz, chloroform-*d*): δ_{ppm} = 6.71 (d, *J* = 8.4 Hz, 2H), 6.26 (d, *J* = 2.6 Hz, 2H), 6.20 – 6.18 (m, 2H), 4.09 – 4.05 (m, 8H), 3.89 – 3.83 (m, 8H), 3.80 – 378 (m, 8H), 3.45 (brs, 4H); ^{13}C NMR (126 MHz, chloroform-*d*): δ_{ppm} = 150.46, 141.97, 141.64, 117.54, 107.54, 107.52, 102.93, 102.91, 71.39, 71.19, 71.16, 70.84, 70.80, 70.39, 70.07, 69.31, 69.23; HRMS (ESI) (m/z): Calcd for $C_{24}H_{34}N_2O_8NH_4^+$ $[M + NH_4]^+$ 496.2654, found: 496.2657; $C_{24}H_{34}N_2O_8Na^+$ $[M+Na]^+$: 501.2208, found: 501.2204; $C_{24}H_{34}N_2O_8K^+$ [$M+K]^+$ 517.1947, found: 517.1950.

S3.3. Compound 5



In a 15 mL pressure vessel, compound **12** (50 mg, 0.104 mmol) and 4-(trifluoromethyl)phenyl isothiocyanate (85 mg, 0.418mmol) were dissolved in dry acetonitrile (5 mL) under nitrogen atmosphere. Then the solution was stirred for 10 h at 60 °C. After that, the solvent was evaporated under vacuo. Finally, the concentrated solid was washed with the diethyl ether to get compound **5** as a yellow solid (78.5 mg, 85%). ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6): δ_{ppm} = 9.91 (d, J = 4.6 Hz, 4H), 7.73 (d, J = 8.5 Hz, 4H), 7.66 (d, J = 8.7 Hz, 4H), 7.11 (s, 2H), 6.95–6.89 (m, 4H), 4.08 – 4.04 (m, 8H), 3.77 – 3.75 (m, 8H), 3.66 – 3.65 (m, 8H); ^{13}C NMR (126 MHz, dimethyl sulfoxide-d_6): δ_{ppm} = 179.67, 152.31, 148.15, 148.04, 146.13, 145.86, 143.58, 132.78, 126.59, 126.35 (q, $^{3}J_{C\text{-}F}$ = 3.8 Hz), 125.84, 125.61, 124.45, 123.24, 117.13, 117.05, 115.38, 115.13, 113.93, 113.13, 111.44, 111.23, 70.51, 69.26, 69.13, 68.91, 68.85; HRMS (ESI) (m/z): Calcd for $C_{40}H_{42}F_6N_4O_8S_2$ $[M+H]^+$ 885.2421, found:885.2420.

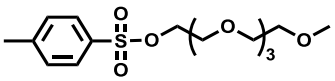
S3.4. Compound 6



Compound **12** (300 mg, 0.45 mmol) was dissolved in chloroform (15 mL) using a 50 mL single-neck round-bottom flask. Then, triethylamine (0.4 ml) was added into the flask, and the reaction mixture was stirred for 15 min. After that, di-tert-butyl dicarbonate (515 mg, 4.5 mmol) dissolved in chloroform (3 mL) was added dropwise during 20 min. The reaction mixture was cooled down at 0 °C and stirred for 3 h. After that, the mixture was stirred for another 48 h at 25 °C. To remove triethylamine, the mixture was evaporated under vacuum and redissolved in chloroform (40 mL) again. Then, an extraction was done with water (50 mL), and the organic layer was collected and dried over magnesium sulfate. Finally, the solution was concentrated, evaporated under vacuum, and purified by FC (silica, dichloromethane:methanol = 90:10) to afford **6** as a light yellow solid (255 mg, 60%). ^{1}H NMR (500 MHz, chloroform-d): δ_{ppm} = 7.11 (brs, 2H), 6.79 – 6.76 (m, 2H), 6.71 – 6.68 (m, 2H), 6.37 (s, 2H), 4.15 – 4.09 (m, 8H), 3.90 – 3.86 (m, 8H), 3.81 – 3.79 (m, 8H), 1.50 (s, 18H); ^{13}C NMR (126 MHz, chloroform-d) δ_{ppm} = 153.12, 149.60, 149.58, 144.89, 132.85, 132.82, 115.4, 115.3, 111.21,

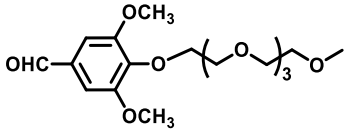
105.99, 80.51, 71.45, 71.42, 71.37, 71.34, 70.21, 70.19, 70.01, 69.98, 69.47, 28.58; HRMS (ESI) (m/z): calcd. for $C_{34}H_{50}N_2O_{12}Na^+$ $[M+Na]^+$ 701.3256, found: 701.3257.

S3.5 Compound 14



Following a reported procedure,[2] a solution of tetraethylene glycol monomethyl ether **13** (3.0 g, 4.40 mmol) in tetrahydrofuran (5 mL) was prepared in a 100 mL two-neck round-bottom flask. Sodium hydroxide (528 mg, 13.2 mmol) dissolved in water (10 mL) was added to the reaction mixture at room temperature. The mixture was then cooled down to 0 °C while being stirred for 15 minutes. Then, a solution of *p*-toluenesulfonyl chloride (3.29 g, 17.28 mmol) in tetrahydrofuran (6 mL) was added dropwise over 1 h. The reaction mixture was stirred for 7 h at 0 °C and then for 14 h at 25 °C. The tetrahydrofuran layer was separated, and the aqueous layer was washed with diethyl ether (2 × 25 mL). The tetrahydrofuran and diethyl ether fractions were combined and washed with 10% (w/w) sodium hydroxide (150 mL), water (150 mL), and brine (150 mL) separately. The collected organic layer was dried over magnesium sulfate and evaporated under reduced pressure to obtain product **14** as a pale-yellow oil (4.85 g, 93%). ^{1}H NMR (500 MHz, acetone-d_6); δ_{ppm} = 7.82 (d, J = 8.4 Hz, 2H), 7.48 (d, J = 8.8 Hz, 2H), 4.17 – 4.15 (m, 2H), 3.67 – 3.66 (m, 2H), 3.58–3.54 (m, 6H), 3.52 (s, 4H), 3.47 – 3.45 (m, 2H), 3.28 (s, 3H), 2.46 (s, 3H); ^{13}C NMR (126 MHz, acetone-d_6): δ_{ppm} = 145.72, 134.32, 130.79, 128.69, 72.62, 71.21, 71.17 71.15, 71.13, 71.02, 70.65, 69.24, 58.76, 21.51; HRMS (ESI) (m/z): calcd for $C_{16}H_{27}O_7S^+$ [M + H] 363.1472, found: 363.1477; $C_{16}H_{27}O_7SNH_4^+$ $[M + NH_4]^+$ 380.1738, found: 380.1736; $C_{16}H_{26}O_7SNa^+$ $[M+Na]^+$ 385.1292, found: 385.1293; $C_{16}H_{26}O_7SK^+$ [$M+K]^+$ 401.1031, found: 401.1034.

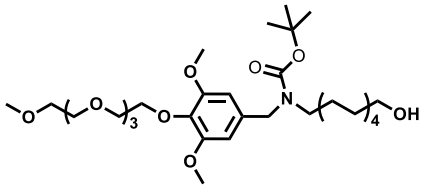
S3.6. Compound 15



Adapted from a reported procedure,[3] compound **14** (3.00 g, 8.27 mmol) and 4-hydroxy-3,5-dimethoxybenzaldehyde (2.26 g, 12.40 mmol) were dissolved in dry

acetonitrile (25.0 mL) in a 100 mL two-neck round-bottom flask under a nitrogen atmosphere. Potassium carbonate (4.04 g, 12.40 mmol) was then added, and the mixture was heated at 90 °C for 17 h. The mixture was then cooled down to room temperature, and the solvent was evaporated under reduced pressure. The crude was then redissolved in ethyl acetate (50 mL) and filtered. The filtrate was extracted with water (2 × 50 mL) and brine (500 mL). Then, the collected organic layer dried over anhydrous magnesium sulfate. The solvent was then evaporated under reduced pressure to obtain the crude, which was then purified by FC (silica, dichloromethane: methanol = 95:5).to obtain compound **15** as a pale-yellow oil (2.40 g, 78%). ^{1}H NMR (500 MHz, chloroform-*d*): δ_{ppm} = 9.74 (s, 1H), 7.00 (s, 2H), 4.15 – 4.10 (m, 2H), 3.79 (s, 6H), 3.72 – 3.69 (m, 2H), 3.60 – 3.58 (m, 2H), 3.55 – 3.49 (m, 8H), 3.43 – 3.40 (m, 2H), 3.25 (s, 3H); ^{13}C NMR (126 MHz, chloroform-*d*): δ_{ppm} = 190.89, 153.82, 142.79, 131.81, 106.48, 72.18, 71.70, 70.41, 70.37, 70.35, 70.26, 70.24, 58.75, 56.03; MALDI-MS (m/z): Calcd. for $C_{18}H_{28}O_8Na^+$ $[M+Na]^+$: 395.1677, found: 395.1683.

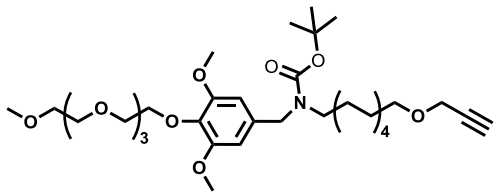
S3.7. Compound 16



Adapted from a reported procedure,[4] compound **15** (1.46 g, 3.92 mmol) and 10-amino-1-decanol (0.77 g, 4.31 mmol) were dissolved in dry methanol (40 mL) in a 100 mL two-neck round bottom flask. The reaction mixture was heated under reflux at 85 °C for 17 h under a nitrogen atmosphere. Then, the reaction mixture was cooled to 0 °C, and sodium borohydride (1.48 g, 39.2 mmol) was added and stirred for 7 h. Afterward, the temperature was increased to 25 °C, and the mixture was stirred for 17 h. The solution was then poured into water (20 mL) and extracted with dichloromethane (3 × 50 mL). The organic layer was dried over anhydrous magnesium sulfate. The mixture was filtered, and the solvent was collected and evaporated to give the amine derivative, which was dissolved in dry dichloromethane (25 mL). Di-tert-butyl dicarbonate (3.60 mL, 15.68 mmol) was added to this mixture, which was then stirred at 25°C for 10 h. The solvent was removed under reduced pressure, and the crude product was purified via FC (silica, dichloromethane:methanol = 94:6) to give

compound **16** as a yellow oil (1.93 g, 3.06 mmol, 78%). ^{1}H NMR (400 MHz, chloroform-*d*): δ_{ppm} = 6.41 (s, 2H), 4.31 (s, 2H), 4.08 (t, *J* = 12.6, 5.1 Hz, 2H), 3.80 – 3.75 (m, 8H), 3.71 – 3.68 (m, 2H), 3.66 – 3.57 (m, 10H), 3.53 – 3.50 (m, 2H), 3.34 (s, 3H), 3.17 – 3.04 (m, 2H), 1.60 (s, 1H), 1.56 – 1.49 (m, 4H), 1.46 – 1.41 (m, 9H), 1.29 – 1.19 (m, 12H); ^{13}C NMR (126 MHz, chloroform-*d*): δ_{ppm} = 153.76, 153.37, 146.76, 137.38, 136.01, 130.10, 106.40, 104.61, 104.05, 85.19, 79.52, 72.29, 72.22, 71.94, 70.66, 70.62, 70.59, 70.57, 70.50, 70.39, 70.35, 62.83, 60.31, 59.01, 56.28, 56.07, 53.36, 50.71, 50.16, 46.72, 32.73, 31.22, 29.48, 29.39, 29.32, 29.24, 29.02, 28.48, 27.42, 26.85, 26.65, 26.24, 25.75; HRMS (ESI) (m/z): calcd. for $C_{33}H_{59}NO_{10}NH_4^+$ $[M + NH_4]^+$ 647.4478, found: 647.4485; $C_{33}H_{59}NO_{10}Na^+$ $[M+Na]^+$: 652.4032, found 652.4029; $C_{33}H_{59}NO_{10}K^+$ $[M+K]^+$ 668.3771, found: 668.3780.

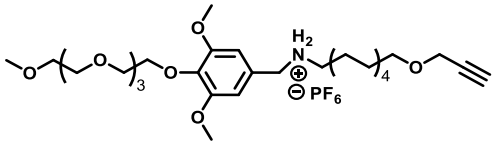
S3.8. Compound 17



Adapted from a reported procedure,[4] Compound **16** (1.38 g, 2.19 mmol) was dissolved in dry tetrahydrofuran (15 mL) in a 100 mL two-neck round bottom flask and cooled to 0 °C. Then, sodium hydride (0.53 g, 21.91 mmol) was added at once, and the mixture was stirred at 0 °C for 1 h under a nitrogen atmosphere. Afterward, the temperature was increased to 40 °C. Then, propargyl bromide (1.34 mL, 10.95 mmol) was added dropwise over 1 h. The reaction was stirred at 30 °C for 17 h. Upon completion, sodium hydride excess was removed by filtration and washed with tetrahydrofuran (20 mL). The collected organic filtrate was then evaporated under reduced pressure. The residue was redissolved in dichloromethane (50 mL) and was washed with brine (50 mL). The organic layer was then dried over anhydrous magnesium sulfate. The crude was purified by FC (silica, dichloromethane:methanol = 94:6) to obtain compound **17** as a yellow oil (0.70 g, 76%). ^{1}H NMR (400 MHz, chloroform-*d*): δ_{ppm} = 6.42 (s, 2H), 4.33 (s, 2H), 4.12 – 4.09 (m, 4H), 3.80 (s, 6H), 3.79 – 3.77 (m, 2H), 3.73 – 3.70 (m, 2H), 3.68 – 3.62 (m, 8H), 3.55 – 3.53 (m, 2H), 3.49 (t, *J* = 6.6 Hz, 2H), 3.37 (s, 3H), 3.19–3.12 (m, 2H), 2.40 (t, *J* = 2.4 Hz, 1H), 1.61 – 1.52 (m, 2H), 1.48 – 1.43 (m, 11H), 1.30 – 1.25 (m, 12H); ^{13}C NMR (126 MHz, chloroform-

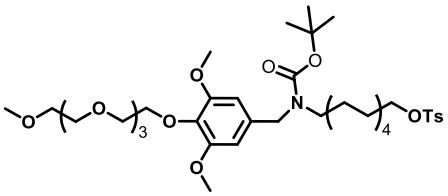
d): δ_{ppm} = 156.19, 155.52, 153.33, 135.83, 134.67, 134.42, 104.41, 103.86, 80.03, 79.48, 74.15, 74.15, 74.13, 72.18, 71.91, 70.64, 70.60, 70.57, 70.56, 70.50, 70.33, 70.26, 59.04, 58.00, 56.03, 50.62, 50.05, 46.59, 29.54, 29.49, 29.38, 29.34, 29.26, 28.46, 28.16, 27.93, 27.81, 26.87, 26.07; HRMS (ESI) (m/z): Calcd. for $C_{36}H_{61}NO_{10}Na^+$ $[M+Na]^+$ 690.4188, found: 690.4189.

S3.9. Compound 7



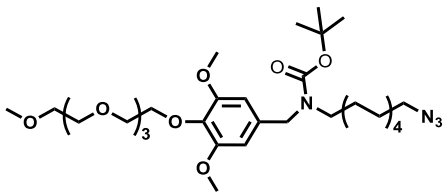
Adapted from a reported procedure.[4] Compound **17** (856 mg, 1.28 mmol) was dissolved in dry dichloromethane (15 mL) in a 50 mL two-neck round-bottom flask. Then, trifluoroacetic acid (3.92 mL, 18.74 mmol) was added, and the mixture was stirred at 25 °C for 17 h. The solvent was removed under reduced pressure, and a solution of ammonium hexafluorophosphate (3.5 g, 21.47 mmol) in methanol (10 mL) was added. The mixture was stirred for 4 h at 30 °C. Methanol was evaporated under reduced pressure, the residue was redissolved in dichloromethane (5 mL) and then washed with water (50 mL). The organic layer was collected, dried over anhydrous magnesium sulfate, and concentrated to get a yellow oil. This oil crude was purified by FC (silica, dichloromethane:methanol = 93:7) to obtain compound **7** as an orange oil (715 mg, 79%). ^{1}H NMR (400 MHz, chloroform-*d*): δ_{ppm} = 7.00 (s, 2H), 6.79 (s, 2H), 4.15 – 4.13 (m, 2H), 4.13 – 4.11 (m, 2H), 4.09 (s, 2H), 3.82 (s, 6H), 3.78 – 3.75 (m, 2H), 3.66 – 3.64 (m, 2H), 3.58 – 3.48 (m, 12H), 3.32 (s, 3H), 3.11 (t, *J* = 8.1 Hz, 2H), 2.42 (t, *J* = 2.4 Hz, 1H), 1.74 – 1.67 (m, 2H), 1.61 – 1.54 (m, 2H), 1.35 – 1.28 (m, 12H) ; ^{13}C NMR (126 MHz, chloroform-*d*): δ_{ppm} = 153.15, 136.41, 127.96, 107.16, 80.04, 74.16, 72.14, 71.51, 70.61, 70.33, 70.26, 70.20, 69.39, 58.99, 57.99, 56.11, 52.75, 49.17, 29.47, 29.40, 29.34, 29.14, 27.55, 26.51, 26.48, 26.37, 26.05; HRMS (ESI) (m/z): Calcd. for $C_{31}H_{54}NO_8^+$ $[M+H]^+$ 568.3844, found: 568.3867; $C_{31}H_{53}NO_8Na^+$ $[M+Na]^+$: 590.3644, found: 590.3688.

S3.10. Compound 18



Adapted from a reported procedure,[4] triethylamine (0.72 mL, 5.17 mmol) was added to a solution of compound **16** (2.5 g, 23.98 mmol) in dry dichloromethane (12 mL) in a 100 mL two-neck round-bottom flask at 0 °C. A solution of *p*-toluenesulfonyl chloride (0.83 g, 4.37 mmol) in dry dichloromethane (5 mL) was then added dropwise at 0 °C over 1 h. The reaction mixture was stirred for 7 h and then at 25 °C for 17 h. Once the reaction was complete, the mixture was washed with saturated sodium bicarbonate (100 mL), water (3 × 50 mL) and brine (100 mL) separately. The organic layer was dried over anhydrous magnesium sulfate and concentrated. FC (silica, dichloromethane:methanol = 95:5) afforded compound **18** as a yellow oil (2.21 g, 85%). ^{1}H NMR (400 MHz, chloroform-*d*): δ_{ppm} = 7.74 (d, *J* = 8.3 Hz, 2H), 7.30 (d, *J* = 8.1 Hz, 2H), 6.40 (s, 2H), 4.32 (s, 2H), 4.07 (t, *J* = 5.2 Hz, 2H), 3.97 (t, *J* = 6.5 Hz, 2H), 3.80 – 3.74 (m, 8H), 3.70 – 3.67 (m, 2H), 3.65 – 3.59 (m, 8H), 3.51 – 3.48 (m, 2H), 3.33 (s, 3H), 3.15 – 3.05 (m, 2H), 2.40 (s, 3H), 1.62 – 1.55 (m, 2H), 1.44 (s, 10H), 1.28 – 1.14 (m, 13H); ^{13}C NMR (101 MHz, chloroform-*d*): δ_{ppm} = 153.49, 144.72, 136.17, 134.64, 133.40, 129.91, 127.97, 104.70, 104.44, 79.61, 72.33, 72.05, 70.79, 70.77, 70.73, 70.70, 70.69, 70.62, 70.47, 59.11, 56.19, 50.23, 46.81, 29.54, 29.47, 29.42, 29.40, 29.00, 28.93, 28.58, 26.97, 25.43, 21.73; HRMS (ESI) (m/z): calcd. for $C_{40}H_{65}NO_{12}SNH_4^+$ $[M+NH_4]^+$ 801.4566, found: 801.4562; $C_{40}H_{65}NO_{12}SNa^+$ $[M+Na]^+$: 806.4120, found 806.4112; $C_{40}H_{65}NO_{12}SK^+$ $[M+K]^+$ 822.3860, found: 822.3856.

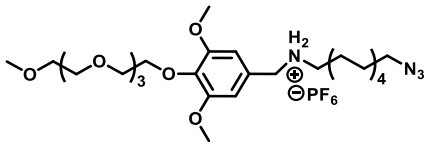
S3.11. Compound 19



Adapted from a reported procedure.[4] Compound **18** (1.57 g, 2.00 mmol) was dissolved in dry dimethylformamide (10 mL) in a two-neck round bottom flask. After that, sodium azide (0.39 g, 6.00 mmol) was added and stirred at 80 °C for 8 h. Upon

completion, the reaction mixture was poured into water (500 mL) and extracted with ethyl acetate (3 × 50 mL). The organic phase was then washed with water (4 × 200 mL) and brine (300 mL) separately, then dried over anhydrous magnesium sulfate. The crude was purified using FC (silica, dichloromethane:methanol = 93:7) to obtain compound **19** as a yellow oil (1.23 g, 93%). ^{1}H NMR (500 MHz, chloroform-*d*): δ_{ppm} = 6.41 (s, 2H), 4.32 (s, 2H), 4.09 (t, *J* = 5.Hz, 2H), 3.79 − 3.74 (m, 8H), 3.71 – 3.69 (m, 2H), 3.66 – 3.61 (m, 8H), 3.53 – 3.51 (m, 2H), 3.35 (s, 3H), 3.22 (t, *J* = 7.0 Hz, 2H), 3.18 − 3.06 (m, 2H), 1.59 – 1.53 (m, 2H), 1.46 – 1.41 (m, 11H), 1.36 − 1.30 (m, 2H), 1.28 − 1.20 (m, 10H); ^{13}C NMR (126 MHz, chloroform-*d*): δ_{ppm} = 156.22, 153.43, 136.07, 134.49, 129.75, 127.21, 106.42, 104.64, 104.08, 79.54, 72.27, 72.00, 70.71, 70.67, 70.65, 70.63, 70.56, 70.40, 59.07, 56.12, 51.52, 50.70, 50.17, 46.74, 29.52, 29.44, 29.38, 29.16, 28.88, 28.52, 26.92, 26.75; HRMS (ESI) (m/z): calcd. for $C_{33}H_{58}N_4O_9NH_4^+$ [M + Na]$^+$ 672.4543, found: 672.4534; $C_{33}H_{58}N_4O_9Na^+$ [M+Na]$^+$ 677.4096, found: 677.4087; $C_{33}H_{58}N_4O_9K^+$ [M+K]$^+$ 693.3869, found:693.3836.

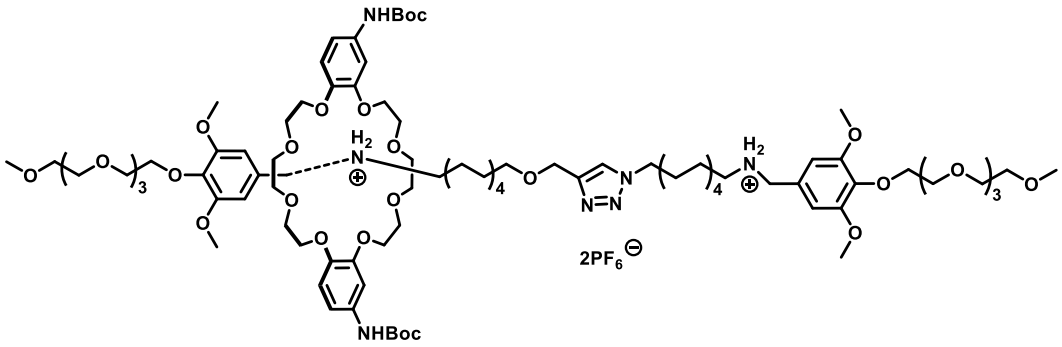
S3.12. Compound 8



Adapted from a reported procedure.[4] Compound **18** (950 mg, 1.45 mmol) was dissolved in dry dichloromethane (10 mL) in a 100 mL two-neck round-bottom flask. Then, trifluoroacetic acid (4.4 mL, 58 mmol) was added to the reaction mixture and stirred at 30 °C for 17 h. After that, the solvent was evaporated under reduced pressure, and a solution of ammonium hexafluorophosphate (2.40 g, 14.5 mmol) in dry methanol (10 mL) was added. The resulting mixture was stirred at 30 °C for 4 h. The organic layer was evaporated under reduced pressure, the residue was redissolved in dichloromethane (15 mL) and washed with water (50 mL). The collected organic layer was dried over anhydrous magnesium sulfate and concentrated. The crude was purified by FC (silica, dichloromethane:methanol = 93:7) to obtain **8** as a yellow oil (742 mg, 73%). ^{1}H NMR (400 MHz, chloroform-*d*): δ_{ppm} = 7.01 (s, 2H), 6.81 (s, 2H), 4.15 − 4.13 (m,2H),4.09 (s,2H), 3.82 (s, 6H), 3.77 – 3.74 (m, 2H), 3.66 – 3.63 (m, 2H), 3.58 − 3.46 (m, 10H), 3.30 (s, 3H), 3.24 (t, *J* = 6.9 Hz, 2H), 3.10 (t, *J* = 8.1 Hz, 2H), 1.76 − 1.66 (m, 2H), 1.57 (q, *J* = 7.1 Hz, 2H), 1.37 – 1.28 (m, 12H); ^{13}C NMR

(126 MHz, chloroform-*d*): δ_{ppm} = 153.27, 136.69, 127.29, 107.39, 72.20, 71.61, 70.78, 70.51, 70.34, 70.30, 70.27, 68.48, 67.18, 59.03, 56.18, 53.57, 52.79, 51.54, 49.27, 29.40, 29.37, 29.20, 29.15, 28.89, 26.75, 26.51, 26.41; HRMS (ESI) (m/z): Calcd. for $C_{28}H_{51}N_4O_7^+$ $[M + H]^+$ 555.3753, found: 555.3762; $C_{28}H_{50}N_4O_7Na^+$ $[M+Na]^+$ 577.3572, found: 577.3585; $C_{28}H_{50}N_4O_7K^+PF_6^-$ $[M+H+K+PF_6]^+$ 723.3292, found: 723.3311.

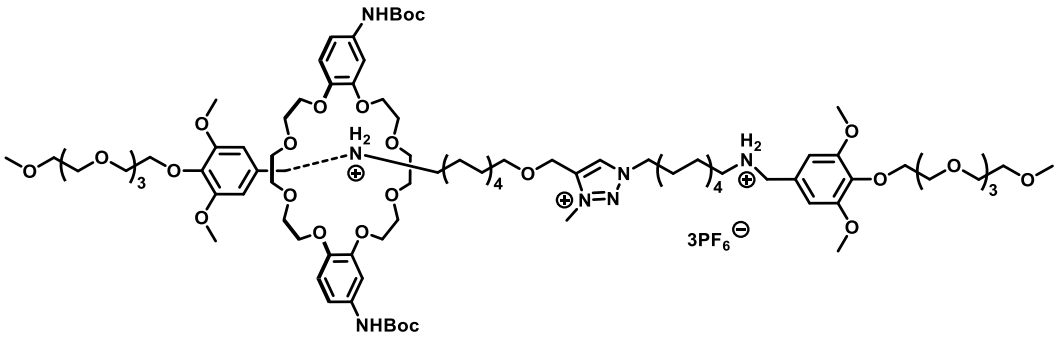
S3*.13. Compound 9.*



Compound **7** (100 mg, 0.143 mmol) and ring **6** (62.80 mg, 0.14 mmol) were dissolved in deoxygenated dry dichloromethane (1.5 mL) in a 20 mL vial and then stirred at 30 °C for 1 h under nitrogen atmosphere. Then, the mixture was transferred to another 20 mL reaction vial pre-loaded with compound **5** (100.19 mg, 0.143 mmol), tetrakis(acetonitrile)copper (I) hexafluorophosphate (10.66 mg, 0.286 mmol) and tris(benzyltriazolylmethyl)amine (15.17 mg, 0.286 mmol). Then the reaction mixture was deoxygenated again for 15 min and stirred at 30 °C for 6 h. The reaction mixture was poured into water (20 mL) and the crude compound was extracted with dichloromethane (3 × 25 mL). The organic layer was dried over anhydrous magnesium sulfate, and then evaporated under vacuo. Finally, the crude was purified by FC (dichloromethane:methanol = 93:7) to obtain compound **9** (58 mg, 22 %) as pale-yellow solid. ^{1}H NMR (500 MHz, acetonitrile-d_3): δ_{ppm} = 7.71 (s, 1H), 7.45 (d, J = 9.7 Hz, 2H), 7.24 (s, 2H), 7.13 (s, 2H), 6.84 – 6.77 (m, 8H), 6.68 (s, 2H), 4.64 – 4.60 (m, 2H), 4.52 – 4.47 (m, 2H), 4.35 – 4.31 (m, 2H), 4.14 – 4.03 (m, 12H), 3.96 (t, J = 4.2 Hz, 2H), 3.90 – 3.82 (m, 12H), 3.81 – 3.74 (m, 6H), 3.69 – 3.55 (m, 24H), 3.55 – 3.52 (m, 2H), 3.49 – 3.43 (m, 4H), 3.42 – 3.40 (m, 2H), 3.26 – 3.21 (m, 2H), 3.07 (s, 3H), 2.98 (s, 2H), 2.96 (t, J = 3.4 Hz, 2H), 1.88 – 1.80 (m, 2H), 1.63 (s, 4H), 1.56 – 1.49 (m, 4H), 1.46 (s, 18H), 1.35 – 1.20 (m, 20H), 1.18 – 1.13 (m, 4H), 1.12 – 1.05 (m, 4H); ^{13}C NMR (126 MHz, acetonitrile-d_3): δ_{ppm} = 154.28, 154.04, 153.95, 153.77, 148.34, 145.94,

143.92, 136.89, 136.07, 134.32, 130.82, 130.38, 124.17, 113.55, 111.59, 108.08, 107.67, 104.98, 80.49, 73.17, 73.14, 72.11, 72.02, 71.63, 71.57, 71.21, 71.16, 71.11, 71.01, 70.68, 70.64, 70.61, 70.56, 70.52, 70.46, 70.19, 70.14, 69.21, 69.18, 68.95, 68.92, 64.57, 64.49, 58.98, 57.00, 56.75, 53.17, 52.68, 50.79, 49.91, 48.90, 30.92, 30.80, 30.47, 30.42, 30.39, 30.10, 30.06, 29.97, 29.88, 29.80, 29.77, 29.73, 29.70, 29.61, 29.55, 29.52, 29.49, 29.47, 29.41, 28.56, 28.53, 27.79, 27.24, 27.02, 27.00, 26.96, 26.90, 26.88, 26.80; HRMS (ESI) (m/z): calcd. for $C_{93}H_{155}N_7O_{27}Na^{3+}$ $[M+2H+Na]^{3+}$ is 608.6962, found 608.6958; $C_{93}H_{154}N_7O_{27}Na_2{}^{3+}[M+H+2Na]^{3+}$ 616.0235, found: 616.0232.

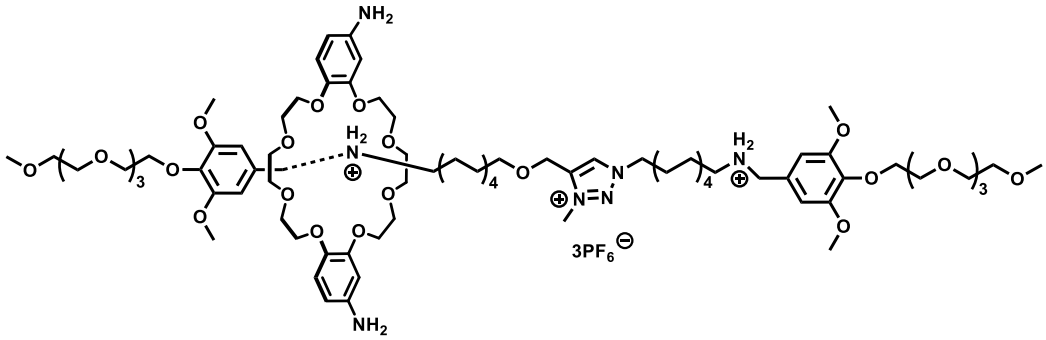
S3.14. Compound 20



Methyl iodide (2 mL, 32 mmol) was added to rotaxane **9** (58 mg, 0.03 mmol) in dry dichloromethane (1.5 mL) in a 15 mL pressure vessel. After sealing the vessel with a screw cap, the mixture was stirred at 40 °C for 24 h. Then, the solvent was evaporated under vacuo. A saturated solution of ammonium hexafluorophosphate (50 mg, 3.07 mmol) in methanol (2 mL) was added to the crude and stirred at 25 °C for 1 h. The solvent was evaporated, and the residue was extracted with dichloromethane (3 ×10 mL) and washed with water (20 mL). The organic layer was collected and dried over anhydrous magnesium sulfate and evaporated under vacuo. The crude product was purified by FC (dichloromethane:methanol = 93:7) to afford **20** (36 mg, 59%) as a pale-yellow solid. ^{1}H NMR (500 MHz, acetonitirile-d_3): δ_{ppm} = 8.26 (d, J = 3.1 Hz, 1H), 7.37 (s, 2H), 7.23 (s, 2H), 7.11 (s, 2H), 6.82 – 6.77 (m, 8H), 6.66 (d, J = 2.0 Hz, 2H), 4.67 (d, J = 1.5 Hz, 3H), 4.65 – 4.61 (m, 2H), 4.52 – 4.47 (m, 6H), 4.16 (d, J = 1.3 Hz, 4H), 4.13 (dd, J = 4.4, 2.3 Hz, 2H), 4.07 (d, J = 6.4 Hz, 3H), 3.86 (d, J = 1.1 Hz, 12H), 3.68 – 3.60 (m, 24H), 3.55 – 3.51 (m, 4H), 3.46 – 3.43 (m, 6H), 3.25 (s, 2H), 3.07 (d, J = 0.7 Hz, 3H), 3.05 (s, 3H), 2.92 (s, 3H), 1.65 (d, J = 8.1 Hz, 4H), 1.58 (td, J = 6.0, 2.9

Hz, 4H), 1.46 (s, 18H), 1.34 – 1.26 (m, 20H), 1.22 – 1.12 (m, 8H); ^{13}C NMR (126 MHz, acetonitirile-d_3): δ_{ppm} = 154.25, 153.97, 153.65, 148.28, 143.87, 136.68, 134.21, 129.71, 113.43, 108.04, 107.99, 107.58, 73.11, 72.22, 71.98, 71.57, 71.52, 71.17, 70.97, 70.62, 70.51, 70.48, 70.44, 70.39, 70.07, 69.96, 68.97, 68.80, 60.87, 58.96, 58.94, 58.90, 56.98, 56.68, 55.27, 54.73, 53.35, 52.51, 49.81, 48.80, 48.73, 47.97, 39.02, 30.14, 30.06, 29.90, 29.88, 29.60, 29.45, 28.49, 27.29, 27.02, 26.69, 26.64; HRMS (ESI) (m/z): calcd. for $C_{94}H_{157}N_7O_{27}Na^{3+}$ $[M+H+Na]^{3+}$ is 613.3681, found 613.3655; $C_{94}H_{157}N_7O_{27}NaPF_6^{2+}$ $[M+H+Na+PF_6]^{2+}$ 992.5345, found: 992.5326.

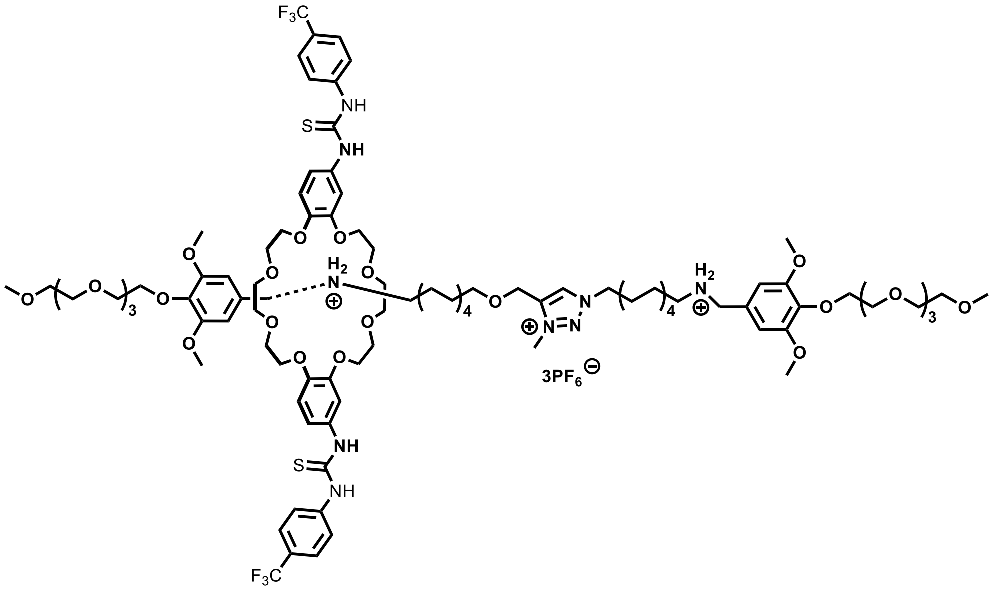
S3.15. Compound 21



Rotaxane **20** (50 mg, 0.022 mmol) was dissolved in dichloromethane (4 mL) using a 50 mL one-neck round-bottom flask. Then, an excess amount of trifluoroacetic acid (1 mL) was added dropwise to the reaction mixture under nitrogen atmosphere and stirred the mixture at 0 °C for 1.5 h. Further, the reaction mixture was stirred at 25 °C for another additional 1.5 h. After the completion of the reaction, the solvent was evaporated under vacuo. To remove excess trifluoroacetic acid and residual isobutylene (generated from Boc deprotection reaction), multiple evaporation cycles of the reaction mixture were carried out using methanol and purged it with nitrogen over the 1 h. Finally, the compound was dried under vacuo to afford the **21** as light-yellow solid (39.0 mg, 86%). ^{1}H NMR (400 MHz, acetonitrile-d_3): δ_{ppm} = 9.11 (brs, 2H), 8.48 (brs, 1H), 7.14 (brs, 2H), 6.93 – 6.77 (m, 6H), 6.66 – 6.61(m, 2H), 5.61 (brs, 4H), 4.73 – 4.53 (m, 6H), 4.23 – 4.12 (m, 15H), 3.94 – 3.84 (m, 15H), 3.70 – 3.59 (m, 35H), 3.49 – 3.33(m, 6H), 3.15 – 3.09 (m, 6H), 2.95 (s, 2H), 1.73 – 1.61 (m, 6H), 1.35 – 1.30 (m, 26H); ^{13}C NMR (126 MHz, acetonitrile-d_3): δ_{ppm} = 161.37, 161.10, 154.26, 154.01, 149.17, 142.11, 136.73, 130.12, 130.06, 129.20, 108.33, 107.90, 73.15,73.10, 72.24, 72.19, 71.62, 71.45, 71.07, 70.79, 70.72, 70.68,70.65, 70.63, 70.28, 61.01, 59.03,

56.9, 56.88, 56.74, 54.82, 52.08, 48.14, 47.06, 39.13, 30.44, 30.14, 30.10, 29.99, 29.94, 29.88, 29.82, 29.76, 29.71, 29.67,29.63, 29.40, 27.22, 27.17, 27.12, 26.67, 26.64, 26.61, 26.50, 9.03.

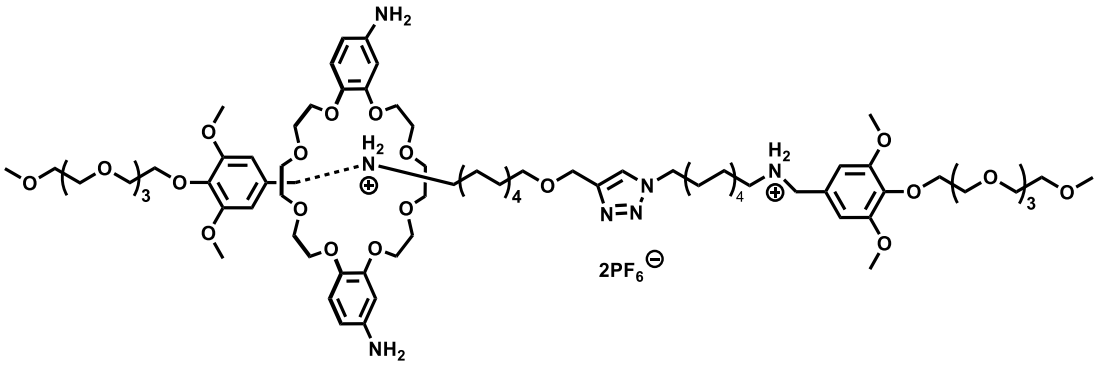
S3.16. Rotaxane 1



Rotaxane **21** (50 mg, 0.024 mmol) was dissolved in dry acetonitrile (2.5 mL) in a 15 mL pressure vessel. A solution of 4-(trifluoromethyl)phenyl isothiocyanate (20 mg, 0.1 mmol) and triethylamine (14 µL, 0.097 mmol) in dry acetonitrile (2.5 mL) was added under nitrogen atmosphere. Then after sealing the vessel with a screw cap, the resulting mixture was stirred at 85 °C for 12 h. Upon completion, the solvent was removed under vacuo. Finally, the purification of the desired product was carried out through several washings with the diethyl ether to get the pure product as light-yellow solid, which was further treated with a saturated solution of ammonium hexafluorophosphate in dry methanol (2 mL) and stirred at 25 °C for 1 h. Finally, the methanol was evaporated under vacuo, and the residue was extracted with dichloromethane (3 ×10 mL) and washed with water (20 mL). The organic layer was collected and dried over anhydrous magnesium sulfate and evaporated under vacuo to afford the rotaxane **1** as light-yellow solid (22 mg, 36%). ^{1}H NMR (500 MHz, methanol-d_4): δ_{ppm} = 8.72 (s, 1H), 7.75 – 7.73 (m, 2H), 7.65 – 7.63 (m, 2H), 7.60 – 7.58 (m, 4H), 7.54 – 7.52 (m, 4H), 6.91 – 6.79 (m, 2H), 6.75 – 6.67 (m, 4H), 5.11 (s, 3H), 4.92 – 4.91 (m, 1H), 4.83 – 4.82 (m, 1H),4.77 – 4.75 (m, 1H), 4.62 – 4.56 (m, 2H), 4.29 – 4.28 (m, 2H), 4.12 – 4.00 (m, 8H), 3.87 – 3.84 (m, 13H), 3.82 – 3.73 (m, 7H),

3.69 – 3.64 (m, 21H), 3.63 – 3.55 (m, 7H), 3.52 – 3.48 (m, 7H), 3.43 – 3.36 (m, 2H), 3.29 – 3.25 (m, 1H), 3.22 – 3.17 (m, 6H), 2.01 – 1.98 (m, 1H), 1.73 – 1.60 (m, 5H), 1.37 – 1.27 (m, 22H), 1.20 – 1.17 (m, 4H); ^{13}C NMR (126 MHz, methanol-d_4): δ_{ppm} = 183.84, 155.02, 146.12, 144.47, 142.69, 139.77,136.59, 128.26, 127.95, 127.69, 127.68, 127.08, 126.61, 126.58, 124.94, 124.71, 123.17, 122.99, 122.39, 121.51, 119.96, 115.60, 105.80, 73.63, 73.11, 72.90, 71.37, 71.26, 67.21, 61.58, 59.31, 57.01, 56.96, 55.29, 39.01, 30.99, 30.86, 30.78, 30.70, 30.62, 30.61, 30.23, 28.57, 28.52, 28.05, 27.99, 27.43, 15.74; HRMS (ESI) (m/z): Calcd. for $C_{100}H_{165}F_6N_{13}O_{23}S_2^{6+}$ [M+H +4NH_4]$^{6+}$ 349.1915, found: 349.1939.

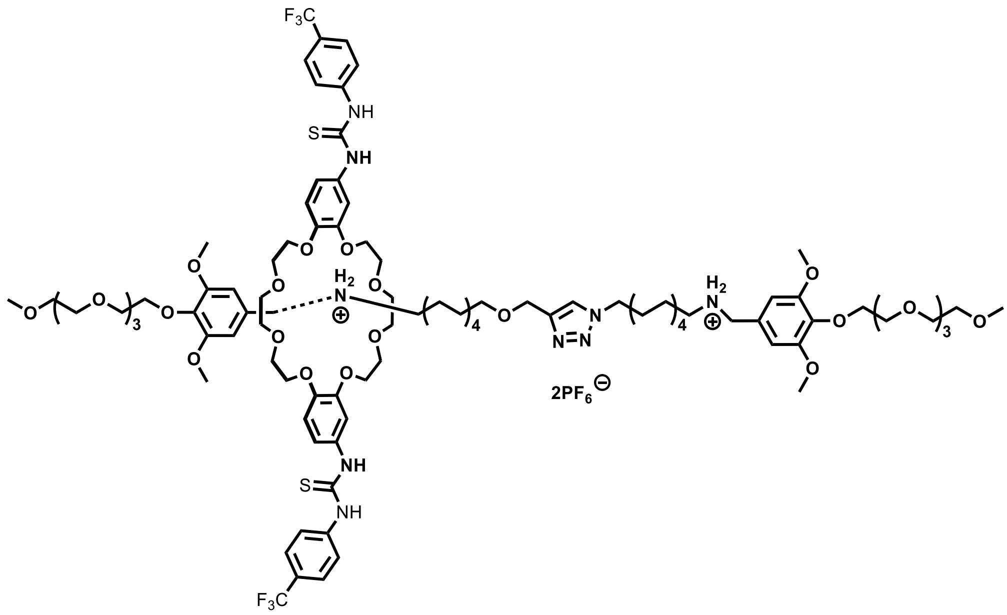
S3.17. Compound 22



Rotaxane **9** (50 mg, 0.02 mmol) was dissolved in dichloromethane (4 mL) using a 50 mL one neck round bottom flask. Then, an excess amount of trifluoroacetic acid (1 mL) was added dropwise to the reaction mixture under nitrogen atmosphere and stirred the mixture at 0 °C for 1.5 h. Further, the reaction mixture was stirred at 25 °C for additional 1.5 h. After the completion of the reaction, the solvent was evaporated under vacuo. To remove excess trifluoroacetic acid and residual isobutylene (generated from Boc deprotection reaction), multiple evaporation cycles of the reaction mixture were carried out using methanol and purged it with nitrogen over the 1 h. Finally, the compound was dried under high vacuo to afford the pure **22** as light yellow viscous liquid (40 mg, 88%).^{1}H NMR (500 MHz, acetonitirile-d_3): δ_{ppm} = 8.67– 8.59 (m, 2H), 7.77 – 7.73 (m, 1H), 6.81 (brs, 6H), 6.48 (brs, 1H), 5.16 (brs, 4H), 4.54 – 4.48 (m, 3H), 4.34 – 4.30 (m, 2H), 4.22 – 4.10 (m, 2H), 4.04 – 4.00 (m, 7H), 3.97 – 3.92 (m, 3H), 3.90 – 3.88 (m, 2H), 3.80 – 3.77 (m, 14H), 3.65 – 3.63 (m, 10H), 3.60 – 3.58 (m, 5H), 3.57 – 3.53 (m, 14H), 3.65 – 3.63 (m, 10H), 3.60 – 3.58 (m, 5H), 3.57 – 3.53 (m, 14H), 3.52 – 3.48 (m, 2H), 3.46 – 3.41 (m, 6H), 3.35 – 3.32 (m, 2H), 3.22 – 3.20 (m, 5H), 3.08

(s, 2H), 2.91 (s, 3H), 1.85 – 1.80 (m, 2H), 1.67 – 1.64 (m, 4H), 1.52 – 1.48 (m, 2H), 1.27 – 1.23 (m, 24H); ^{13}C NMR (126 MHz, acetonitirile-d_3) δ_{ppm} = 154.74, 154.72, 153.77, 138.22, 128.59, 108.63, 107.78, 73.38, 73.36, 72.84, 72.78, 72.76, 72.30, 71.52, 71.46, 71.43, 71.41, 71.37, 71.35, 71.33, 71.32, 71.28, 71.23, 71.20,71.17, 71.13, 71.11, 69.44, 69.33, 59.29, 59.28, 57.13, 56.89, 52.57, 51.23, 48.56, 31.14, 31.12, 30.73, 30.45, 30.42, 30.37, 30.34, 30.33, 30.26, 30.23, 30.20, 30.18, 30.15, 30.09, 30.07, 30.02, 29.8, 29.93, 29.87, 29.82, 27.78, 27.44, 27.41, 27.38, 27.36, 27.32, 27.26, 27.20, 26.96, 26.94.

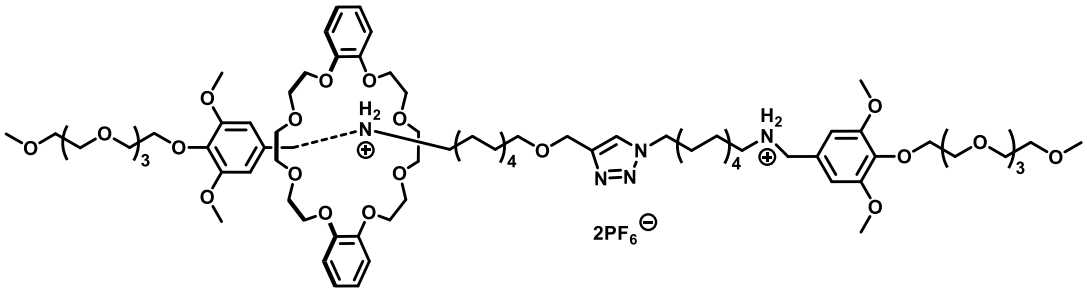
S3.18. Rotaxane 2



Rotaxane **22** (50 mg, 0.026 mmol) was dissolved in dry acetonitrile (2.5 mL) using a 15 mL pressure vessel. A solution of 4-(trifluoromethyl)phenyl isothiocyanate (21 mg, 0.105 mmol) and triethyl amine (15μL, 0.105 mmol) in dry acetonitrile (2.5) was added under nitrogen atmosphere. Then after sealing the vessel with a screw cap, the resulting mixture was stirred at 85 °C for 12 h. Finally, the solvent was evaporated under vacuo and purification of the desired product was carried out through the several washing with the diethyl ether to obtain rotaxane **2** as light-yellow solid, which was further treated with a saturated solution of ammonium hexafluorophosphate in methanol (2 mL) and stirred at 25 °C for 1 h. The solvent was evaporated, and the residue was extracted with dichloromethane (3 × 10 mL) and washed with water (20 mL). The organic layer was collected, dried over anhydrous

magnesium sulfate, and evaporated under vacuo to afford the pure rotaxane **2** as light yellow solid (28 mg, 46%). ^{1}H NMR (500 MHz, acetonitrile-d_3): δ_{ppm} = 10.46 (brs, 2H), 7.92 – 7.90 (m, 2H), 7.68 – 7.59 (m, 2H), 7.54 – 7.45 (m, 4H), 7.12 – 7.07 (m, 2H), 6.88 – 6.74 (m, 3H), 6.64 – 6.50 (m, 3H), 5.04 (brs, 2H), 4.50 – 4.48 (m, 3H), 4.31 – 4.28 (m, 2H), 4.09 – 4.03 (m, 4H), 4.0 – 3.98 (m, 3H), 3.89 – 3.86 (m, 2H), 3.78 – 3.75 (m, 12H), 3.68 – 3.65 (m, 8H), 3.62 – 3.53 (m, 28H), 3.45 – 3.40 (m, 6H), 3.34 – 3.31 (m, 1H), 3.28 – 3.25 (m, 6H), 3.06 – 3.04 (m ,6H), 1.85 – 1.79 (m, 2H), 1.66 – 1.64 (m, 2H), 1.56 – 1.48 (m, 4H), 1.27 – 1.22 (m, 20H), 1.14 – 1.08 (m, 4H); ^{13}C NMR (126 MHz, acetonitrile-d_3): δ_{ppm} = 183.18, 154.90, 149.19, 146.16, 145.82,137.58, 137.45, 134.24, 130.24, 129.63, 129.22, 127.22, 126.98, 126.63, 126.60, 126.49, 126.46, 126.43, 125.22, 124.77, 124.34, 124.06, 120.71, 119.47, 119.37, 113.42, 111.30, 105.90, 73.38, 73.35, 72.97, 72.12, 71.59, 71.57, 71.55, 71.52, 71.36, 71.29, 70.69, 70.17, 64.94, 62.96, 59.25, 57.13, 57.08, 57.04, 55.83, 54.70, 53.87, 51.11, 48.52, 47.55, 33.98, 31.23, 30.75, 30.54, 30.50, 30.42, 30.36, 30.33, 30.25, 29.89, 28.16, 27.74, 27.71, 27.38, 27.23, 9.37; HRMS (ESI) (m/z): Calcd. for $C_{99}H_{147}F_6N_9O_{23}S_2^{2+}$ $[M+2H]^{2+}$ 1004.4989, found: 1004.4989.

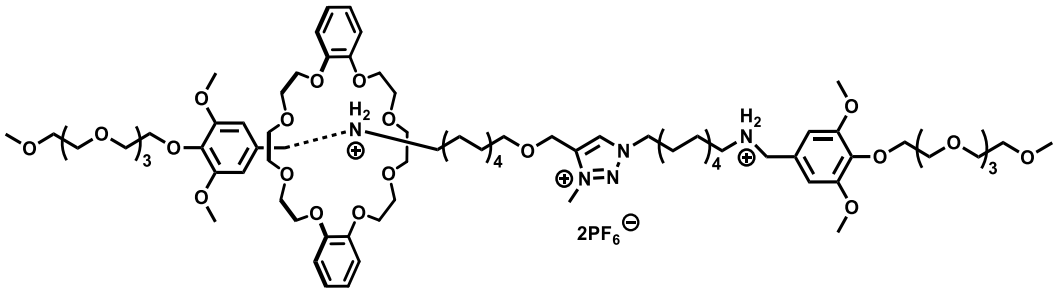
S3.19. Compound 23



Compound **7** (100 mg, 0.143 mmol) and ring **10** (62.80 mg, 0.14 mmol) were placed in a 5 mL vial and deoxygenated dry dichloromethane (1.5 mL) was added. The resulting mixture was stirred at 30 °C for 1 h under nitrogen atmosphere. Then, the reaction mixture was transferred to a reaction vial pre-loaded with compound **8** (100.19 mg, 0.143 mmol), tetrakis(acetonitrile)copper(I) hexafluorophosphate (10.66 mg, 0.286 mmol) and tris(benzyltriazolylmethyl)amine (15.17 mg, 0.286 mmol). Then the reaction mixture was deoxygenated again and stirred at 30 °C for 6 h. The mixture was poured into water (20 mL) and extracted with dichloromethane (3×25 mL). Combined dichloromethane layers were dried over magnesium sulfate. Then the

organic layer was evaporated under vacuo to obtain the crude product. FC (silica, dichloromethane:methanol = 93:7) gave compound **23** (58 mg, 22%) as pale-yellow solid. ^{1}H NMR (500 MHz, acetonitrile-d_3): δ_{ppm} = 7.70 (s, 1H), 7.43 – 7.29 (m, 2H), 7.22 (s, 2H), 6.90 – 6.87 (m, 8H), 6.80 – 6.77 (M, 2H), 6.65 – 6.63 (m, 2H), 4.61 (s, 2H), 4.52 – 4.48 (m, 2H), 4.34 – 4.30 (m, 2H), 4.16 – 4.10 (m, 12H), 4.07 – 4.04 (m, 4H), 3.89 – 3.85 (m, 6H), 3.84 – 3.77 (m, 14H), 3.69 – 3.65 (m, 4H), 3.62 – 3.54 (m, 24H), 3.48 – 3.44 (m, 4H), 3.28 – 3.23 (m, 8H), 2.99 (s, 2H), 1.83 (s, 2H), 1.65 (s, 2H), 1.58 – 1.43 (m, 4H), 1.30 − 1.22 (m, 20H), 1.15 − 1.14 (m, 4H), 1.09 – 1.07 (m, 4H); ^{13}C NMR (126 MHz, acetonitrile-d_3): δ_{ppm} = 154.36, 153.88, 153.85, 149.51, 148.38, 138.51, 137.68, 137.63, 129.45, 129.39, 127.85, 122.56, 122.27, 115.34, 113.28, 108.39, 107.58, 73.02, 72.45, 71.50, 71.30, 71.25, 71.20, 71.09, 71.05, 70.99, 70.96, 70.21, 69.60, 68.88, 58.95, 58.92, 56.80, 56.44, 53.16, 52.86, 50.92, 49.71, 49.17, 30.76, 30.68, 30.63, 30.36, 30.30, 30.15, 30.03, 29.95, 29.80, 29.76, 29.69, 29.67, 29.61, 29.47, 29.43, 29.37, 29.34, 27.16, 27.13, 27.07, 26.91, 26.87, 26.83, 26.80, 26.77, 26.55; HRMS (ESI) (m/z): Calcd. for $C_{83}H_{137}N_5O_{23}^{2+}$ $[M + 2H]^{2+}$ 785.9847, found: 785.9857; $C_{83}H_{137}N_5O_{23}^{2+}PF_6^-Na^+$ $[M+2H+PF_6+Na]^{2+}$ 869.9617, found: 869.9632; $C_{83}H_{136}N_5O_{23}^+$ $[M+H]^+$ 1570.9621; found: 1570.9616; $C_{83}H_{136}N_5O_{23}PF_6$ [M + H + PF_6] 1716.9301, found: 1716.9320.

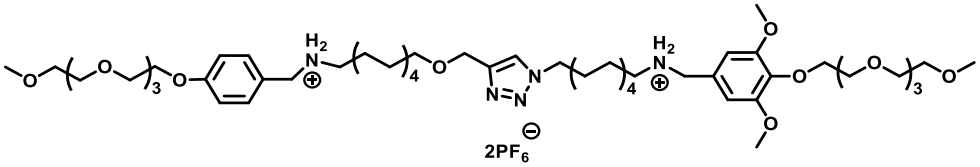
S3.20. Rotaxane 3



Methyl iodide (2 mL, 32 mmol) was added to a solution of rotaxane **23** (58 mg, 0.03 mmol) in dry dichloromethane (1.5 mL) which was pre-loaded in a 15 mL pressure vessel and sealed with a screw cap. Then, the mixture was stirred at 40 °C for 24 h. After that, the solvent was evaporated under vacuo. A saturated solution of ammonium hexafluorophosphate (50 mg, 3.07 mmol) in dry methanol (2 mL) was added to the crude and stirred at 25 °C for 1 h. The solvent was evaporated under vacuo, and the residue was extracted with dichloromethane (3 × 10 mL) and ×washed with water (20

mL). The organic layer was collected and dried over anhydrous magnesium sulfate and evaporated under vacuo. FC (silica, dichloromethane:methanol = 93:7) gave compound **3** (36 mg, 59%) as a pale-yellow solid. ^{1}H NMR (400 MHz, acetonitrile-d_3) δ_{ppm} = 8.26 (s, 1H), 7.25 (s, 2H), 6.92 – 6.87 (m, 8H), 6.80 – 6.80 (m, 2H), 6.68 (s, 2H), 4.68 – 4.64 (m, 4H), 4.52 – 4.48 (m, 2H), 4.17 (s, 4H), 4.15 – 4.11 (m, 10H), 4.08 (s, 3H), 3.97 (t, J = 4.4 Hz, 2H), 3.86 (s, 12H), 3.84 – 3.77 (m, 4H), 3.70 – 3.65 (m, 4H), 3.64 – 3.60 (m, 24H), 3.49 – 3.41 (m, 9H), 3.27 – 3.21 (m, 2H), 3.11 (s, 2H), 3.09 (s, 3H), 1.68 – 1.64 (m, 3H), 1.61 – 1.57 (m, 3H), 1.55 – 1.50 (m, 2H), 1.34 – 1.29 (m, 20H), 1.20 – 1.10 (m, 8H); ^{13}C NMR (126 MHz, acetonitrile-d_3): δ_{ppm} = 154.40, 154.37, 154.02, 153.87, 153.85, 142.00, 141.90, 138.01, 137.97, 137.02, 135.32, 129.76, 127.68, 127.60, 122.34, 113.37, 108.87, 108.39, 107.63, 105.23, 79.93, 73.05, 73.01, 72.34, 72.30, 72.28, 72.21, 72.16, 71.53, 71.08, 70.97, 70.92, 70.89, 70.86, 70.83, 70.80, 70.78, 70.76, 70.73, 70.69, 70.63, 70.52, 62.56, 60.90, 60.88, 58.92, 58.89, 56.91, 56.65, 56.54, 54.73, 53.18, 52.61, 52.52, 49.77, 48.83, 48.70, 39.01, 33.55, 30.24, 30.18, 30.13, 30.10, 30.07, 30.06, 30.01, 29.98, 29.92, 29.90, 29.86, 29.83, 29.79, 29.65, 29.63, 29.57, 29.54, 29.51, 29.47, 29.42, 28.59, 27.45, 27.24, 27.02, 27.00, 26.64, 26.60, 26.57, 26.53, 26.50; HR-ESI-TOFMS: m/z (%) of $[M+H+Na]^{3+}$ calculated for $C_{84}H_{140}N_5O_{23}Na^{2+}$ is 536.3248, found 536.3250. HRMS (ESI) (m/z): Calcd. for $C_{84}H_{139}N_5O_{23}Na^{3+}$ $[M+H+Na]^{3+}$ 536.3248, found:536.3250; $C_{84}H_{139}N_5O_{23}NaPF_6^{2+}$ $[M+H+Na+PF_6]^{2+}$ 876.9695, found: 876.9710; $C_{84}H_{139}N_5O_{23}Na_2P_2F_{12}^{2+}$ $[M+H+2Na+2PF6]^{2+}$ 960.9465, found: 960.9474.

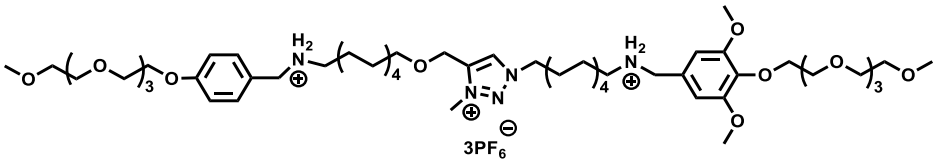
S3.21. Compound 24



Compound **7** (50 mg, 0.071 mmol), compound **8** (49 mg, 0.071 mmol), tetrakis(acetonitrile)copper(I) hexafluorophosphate (5.29 mg, 0.0142 mmol), and tris(benzyltriazolylmethyl)amine (7.53 mg, 0.0142 mmol) were added to a 5 mL vial and deoxygenated. Then dry deoxygenated dichloromethane (1 mL) was added, and mixture was stirred at 30 °C for 6 h under a nitrogen atmosphere. The reaction mixture was poured into water (20 mL) and extracted with dichloromethane (3×25 mL).

Combined dichloromethane layers were washed with brine (30 mL), dried over anhydrous magnesium sulfate, and evaporated under vacuo. FC (silica, dichloromethane:methanol = 93:7= 93:7) gave axle **24** (42 mg, 42 %) as pale-yellow oil. ^{1}H NMR (400 MHz, acetonitrile-d_3): δ_{ppm} = 7.73 (s, 1H), 6.81 (s, 4H), 4.48 (s, 2H), 4.33 (t, J = 6.9 Hz, 2H), 4.13 – 4.08 (m, 8H), 3.84 (d, J = 2.4 Hz, 12H), 3.65 – 3.55 (m, 28H), 3.48 – 3.44 (m, 6H), 3.15 – 3.11 (m, 6H), 2.99 (s, 4H), 1.89 – 1.81 (m, 2H), 1.65 (s, 4H), 1.55 – 1.49 (m, 2H), 1.32 – 1.22 (m, 20H); ^{13}C NMR (126 MHz, acetonitrile-d_3): δ_{ppm} = 154.38, 137.24, 108.26, 73.22, 72.20, 71.05, 70.83, 70.76, 70.73, 70.71, 70.69, 70.55, 70.51, 64.47, 59.08, 57.05, 52.67, 50.89, 48.91, 30.71, 30.36, 30.03, 29.99, 29.89, 29.68, 29.56, 29.40, 29.31, 27.00, 26.90, 26.84, 26.80, 1.94, 1.73, 1.53, 1.32, 1.11, 0.91, 0.70; HRMS (ESI) (m/z): Calcd. for $C_{59}H_{104}N_5O_{15}Na^{2+}$ $[M+H+Na]^{2+}$ 572.8708, found: 572.8725; $C_{59}H_{103}N_5O_{15}Na^{+}$ $[M+Na]^{+}$ 1144.7343, found: 1144.7369.

S3.22. Compound 4



Following a reported procedure,[4] methyl iodide (2 mL, 32 mmol) was added to **23** (42 mg, 29.7 mmol) in dichloromethane (1.5 mL) in a 15 mL pressure vessel and sealed with a screw cap. Then the mixture was stirred at 40 °C for 24 h. After that, the solvent was evaporated under vacuo. Then the saturated solution of ammonium hexafluorophosphate (100 mL) in dry methanol (2 mL) was added to the crude and stirred at 25 °C for 2 h. The solvent was evaporated, and the residue was extracted with dichloromethane (3 × 10 mL) and washed with water (20 mL). The organic layer was collected and dried over anhydrous magnesium sulphate and evaporated under vacuo. The crude product was purified by CF (silica, dichloromethane:methanol = 93:7) to afford axle **4** (7 mg, 7%) as a pale-yellow solid. ^{1}H NMR (400 MHz, acetonitrile-d_3): δ_{ppm} = 8.26 (s, 1H), 6.81 (s, 4H), 4.67 (s, 2H), 4.50 (t, J = 7.3 Hz, 2H), 4.17 (s, 3H), 4.15 – 4.13 (m, 4H), 4.07 (m, 4H), 3.86 (s, 12H), 3.64 – 3.63 (m, 13H), 3.59 – 3.57 (m, 8H), 3.56 – 3.44 (m, 4H), 3.07 – 3.05 (m, 6H), 2.99 – 2.96 (m, 4H), 1.70 – 1.56 (m, 8H), 1.33 – 1.29 (m, 29H); ^{13}C NMR (126 MHz, acetonitrile-d_3) δ_{ppm} = 154.21, 141.93,

136.54, 129.69, 128.90, 108.36, 107.99, 73.12, 72.22, 70.57, 70.48, 70.44, 70.39, 70.35, 70.04, 69.95, 62.54, 60.86, 58.96, 57.05, 54.71, 52.66, 48.85, 39.00, 30.12, 30.04, 30.01, 29.91, 29.89, 29.85, 29.79, 29.67, 29.65, 29.59, 29.43, 29.32, 29.25, 27.03, 27.02, 26.81, 26.62, 26.51; HRMS (ESI) (m/z): Calcd. for $C_{60}H_{106}N_5O_{15}Na_2^{3+}$ $[M+2Na]^{3+}$ 394.2488, found: 394.2479.

S4. Binding Studies in Solution

^{1}H NMR titrations were carried out to determine the binding constant of ring **5** towards Cl^- anions. Stock solutions of ring **5** (5 mM) and tetrabutyl ammonium chloride (TBACl, 500 mM) were prepared in acetonitrile-d_3. Ring **5** (0.5 mL, 5 mM) was placed in an NMR tube, and the ^{1}H NMR spectrum was collected upon the addition of different amounts of the TBACl solution. Chemical shifts of interacting N-H protons were plotted against equivalent total ($[G]_0/[H]_0$) using the 1:1 model of BindFit v0.5 program to calculate the binding constant values.[5, 6] Three sets of repetitions were carried out to ensure the robustness and reliability of the results. Moreover, fresh stock solutions were used for each repetition.^{1}H NMR titration of compound ring **5** with other anions (Br^-, I^-, NO_3^-) was also carried out using a similar experimental protocol. The TBABr, TBAI, and $TBANO_3$ salts were used as the source of Br^-, I^-, NO_3^-, respectively. For all these studies, MestReNova software was used to process the NMR spectra.

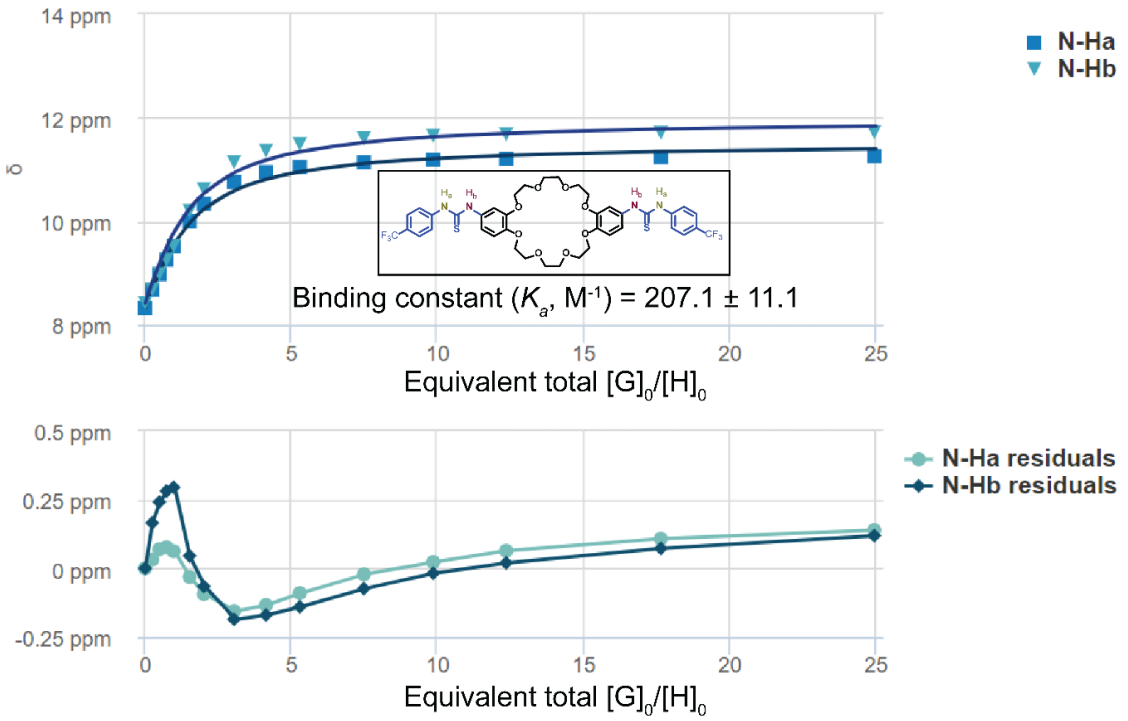


Figure S1. ^{1}H NMR titrations to determine the binding of Cl^- anions. Chemical shift (δ) of N-H_a and N-H_b protons vs. equivalent total ($[G]_0/[H]_0$) were plotted, fitted to 1:1 binding model using BindFit v0.5 program. H = host (**5**) and G = guest (TBACl). This is a representative BindFit spectrum obtained from one of the triplicate experiments.

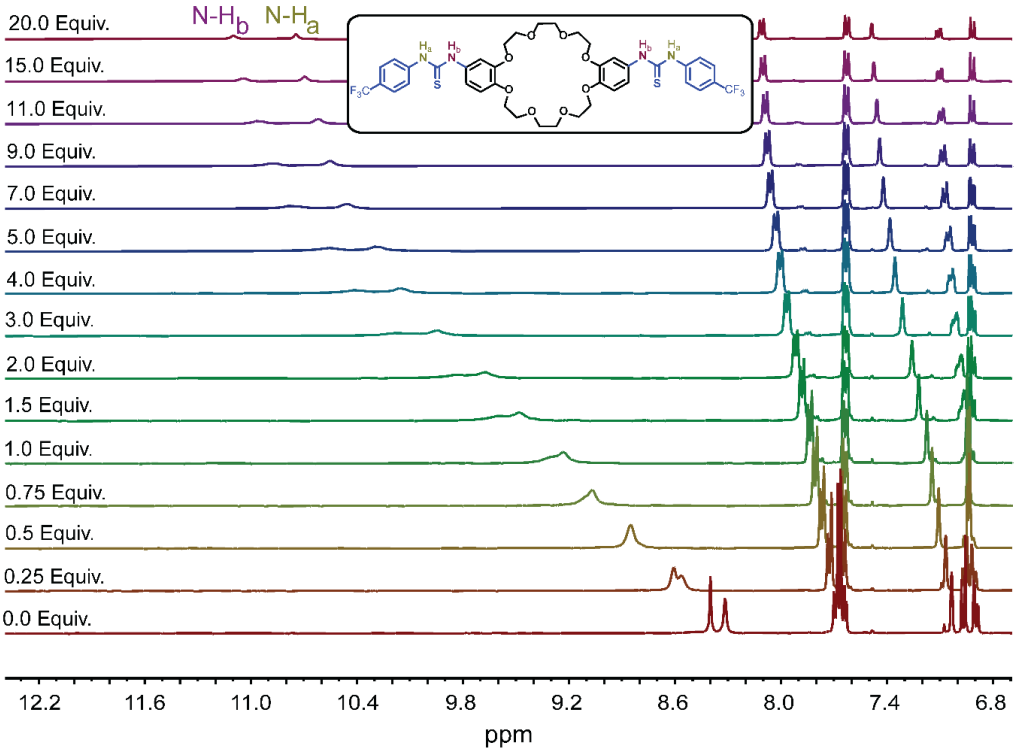


Figure S2. Recognition and binding of Br^- anions by thiourea units on ring **5**. ^{1}H NMR spectra showing the chemical shift of the N-H peaks during the titration of ring **5** with different equivalents of TBABr in acetonitrile-d_3. This is a representative titration spectrum obtained from one of the triplicate experiments.

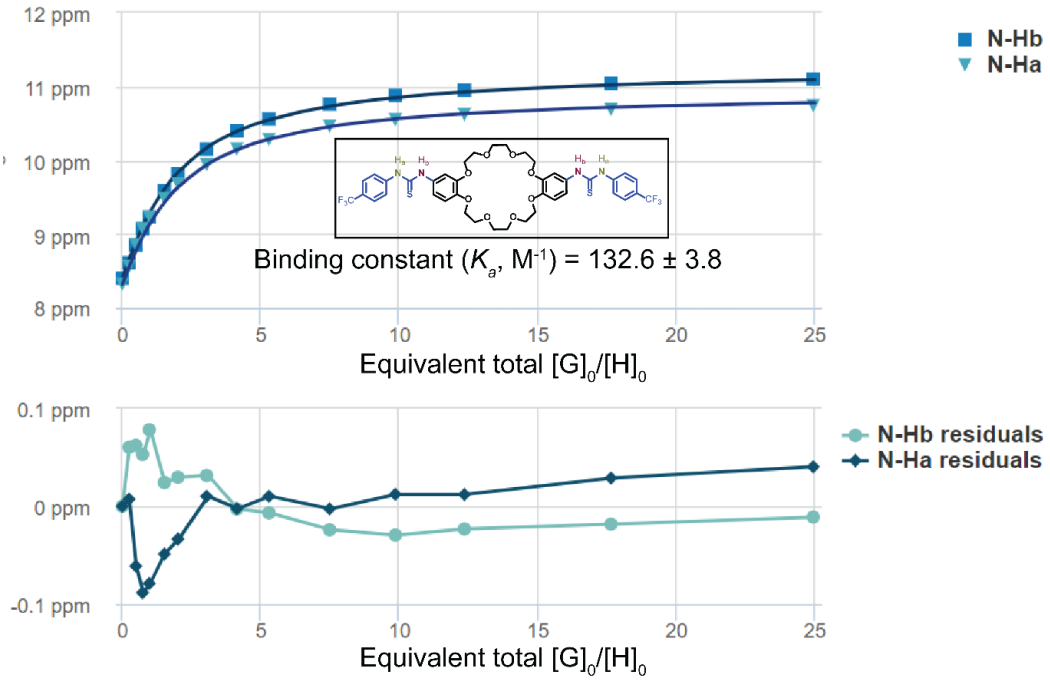


Figure S3. ^{1}H NMR titrations to determine the binding of Br^- anions. Chemical shift (δ) of N-H_a and N-H_b protons vs. equivalent total ($[G]_0/[H]_0$) were plotted, fitted to 1:1 binding model using BindFit v0.5 program. H = host (**5**) and G = guest (TBABr). This is a representative BindFit spectrum obtained from one of the triplicate experiments.

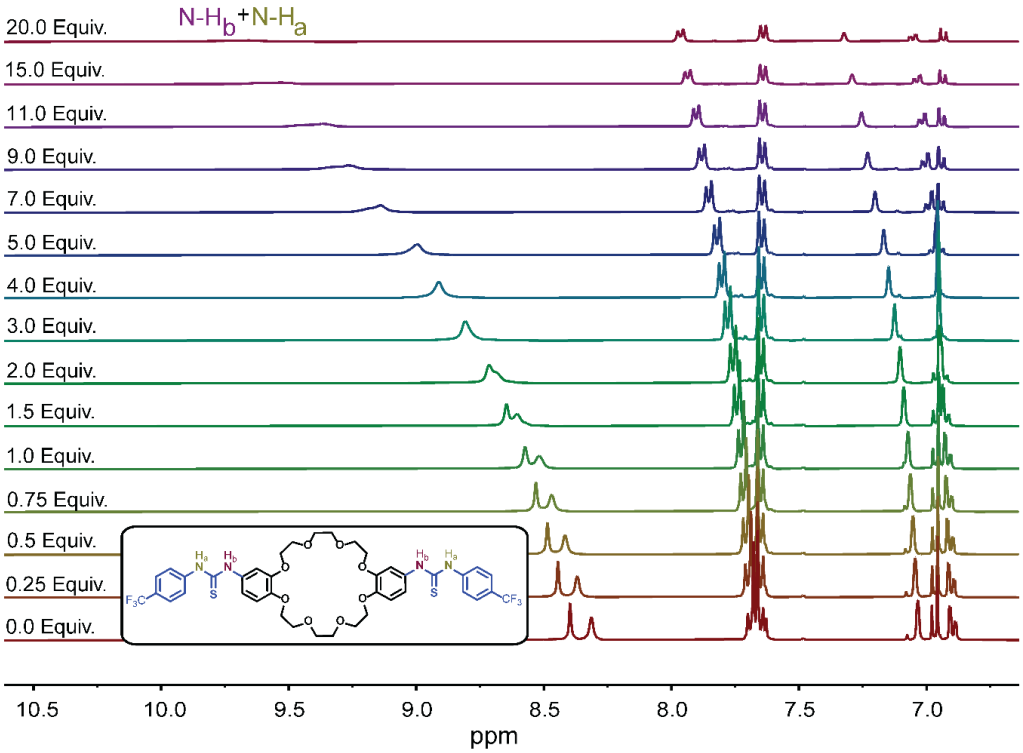


Figure S4. Recognition and binding of I^- anions by thiourea units on ring **5**. ^{1}H-NMR spectra showing the chemical shift of the N-H peaks during the titration of ring **5** with different equivalents of TBAI in acetonitrile-d_3. This is a representative titration spectrum obtained from one of the triplicate experiments.

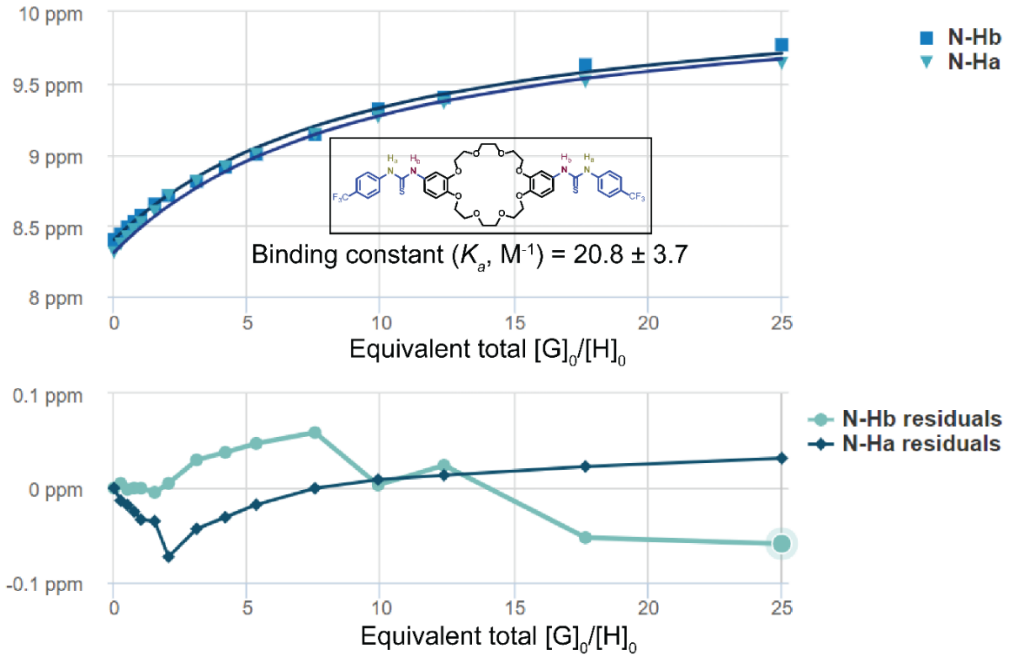


Figure S5. ^{1}H NMR titrations to determine the binding of I^- anions. Chemical shift (δ) of N-H_a and N-H_b protons vs. equivalent total ($[G]_0/[H]_0$) were plotted, fitted to 1:1 binding model using BindFit v0.5 program. H = host (**5**) and G = guest (TBAI). This is a representative BindFit spectrum obtained from one of the triplicate experiments.

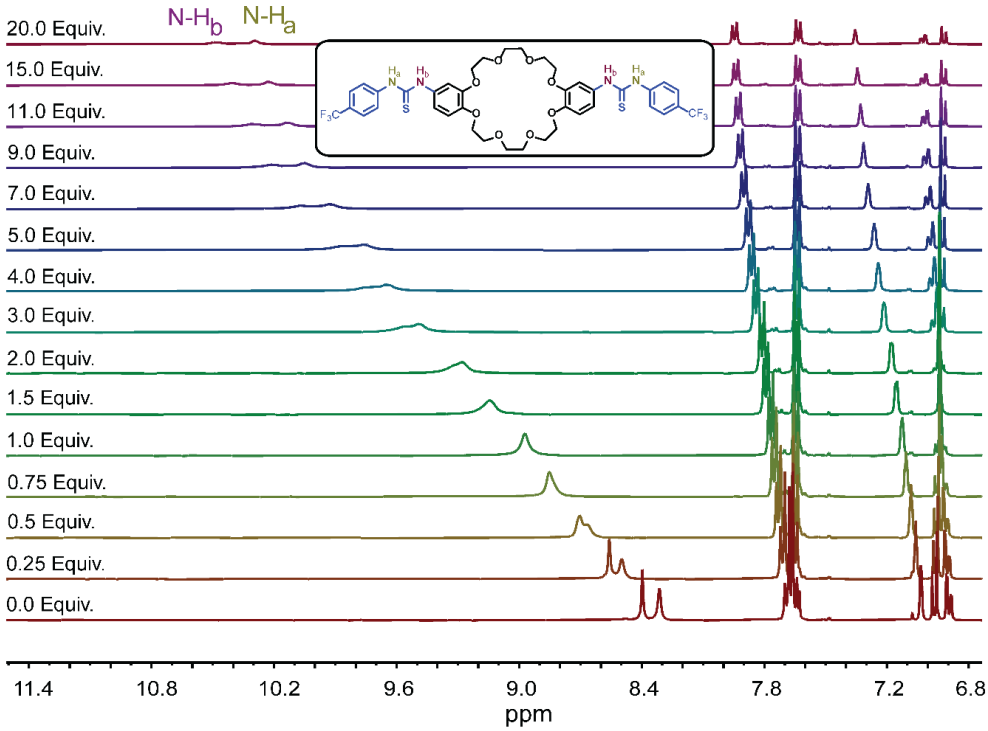


Figure S6. Recognition and binding of NO_3^- anions by thiourea units on ring **5**. ^{1}H-NMR spectra showing the chemical shift of the N-H peaks during the titration of ring **5** with different equivalents of $TBANO_3$ in acetonitrile-d_3. This is a representative titration spectrum obtained from one of the triplicate experiments.

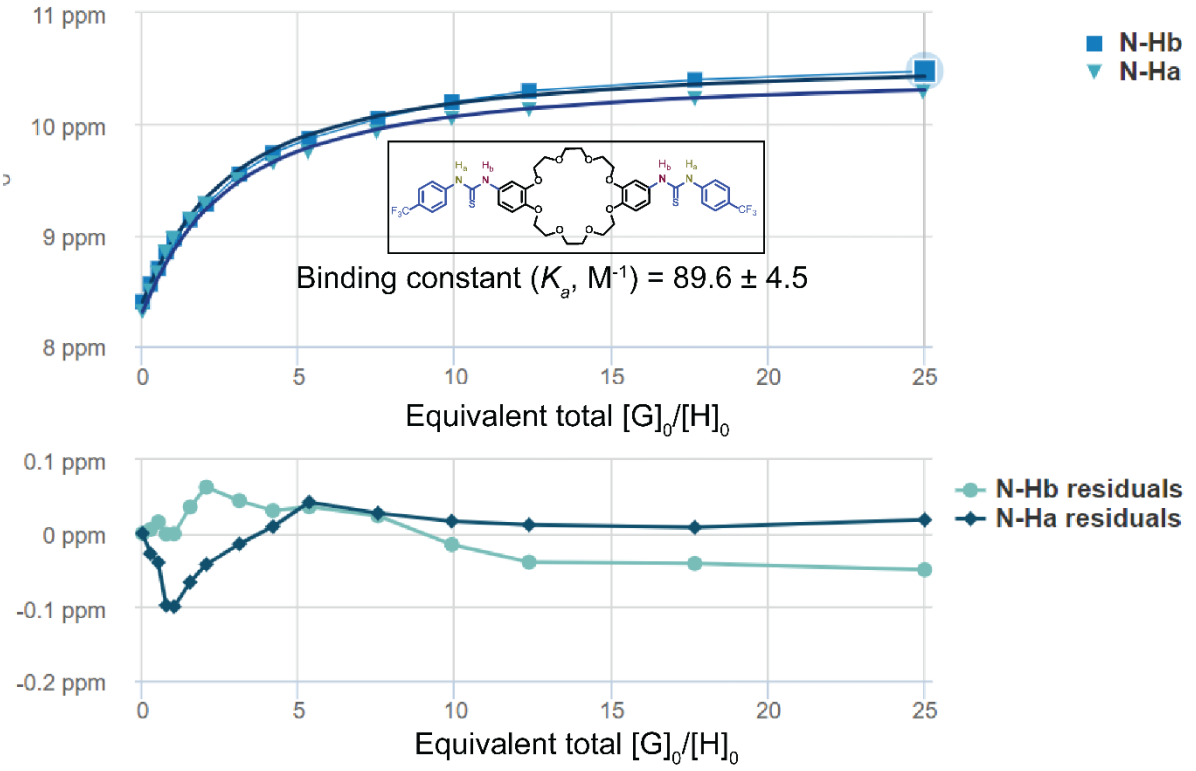


Figure S7. ^{1}H NMR titrations to determine the binding of NO_3^- anions. Chemical shift (δ) of N-H_a and N-H_b protons vs. equivalent total ($[G]_0/[H]_0$) were plotted, fitted to 1:1 binding model using BindFit v0.5 program. H = host (**5**) and G = guest ($TBANO_3$). This is a representative BindFit spectrum obtained from one of the triplicate experiments.

Table S1. Binding constant (K_a; M^{-1}) values of ring **5** upon addition of TBAX (X = Cl^-, Br^-, I^- and NO_3^-) using the 1: 1 model of bindfit.

Anion	K_a from Set 1	K_a from Set 2	K_a from Set 3	Average K_a
TBACl	207.1	190.7	197.6	198.5 ± 8.0
TBABr	132.6	124.9	134.9	130.8 ± 5.0
TBAI	20.8	21.0	21.0	20.9 ± 0.1
$TBANO_3$	89.6	90.1	90.9	90.2 ± 1.0

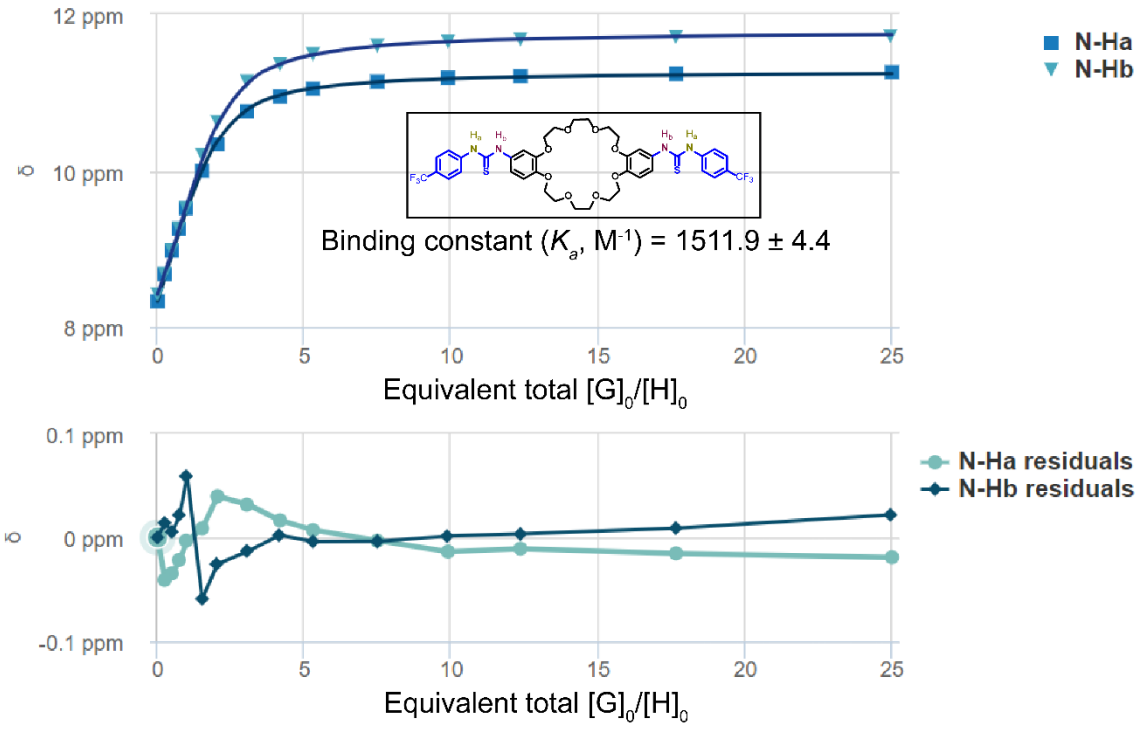


Figure S8. Example of one ^{1}H NMR titration to determine the binding of Cl^- anions. The chemical shift (δ) of N-H_a and N-H_b protons vs. equivalent total ($[G]_0/[H]_0$) were plotted, fitted to 1: 2 model under a non-cooperative model of BindFit program. H = host (**5**) and G = guest (TBACl). This is a representative BindFit spectrum obtained from one of the triplicate experiments.

Table S2. Binding constant (K_a; M^{-1}) values of ring **5** upon addition of TBAX (X = Cl^-, Br^-, I^- and NO_3^-) using the 1: 2 non-cooperative model of BindFit program.

Anion	K_a from Set 1	K_a from Set 2	K_a from Set 3	Average K_a
TBACl	1511.9	1115.6	987.2	1204.9 ± 273.5
TBABr	Fitting Failed	Fitting Failed	Fitting Failed	-
TBAI	Fitting Failed	Fitting Failed	Fitting Failed	-
$TBANO_3$	Fitting Failed	Fitting Failed	Fitting Failed	-

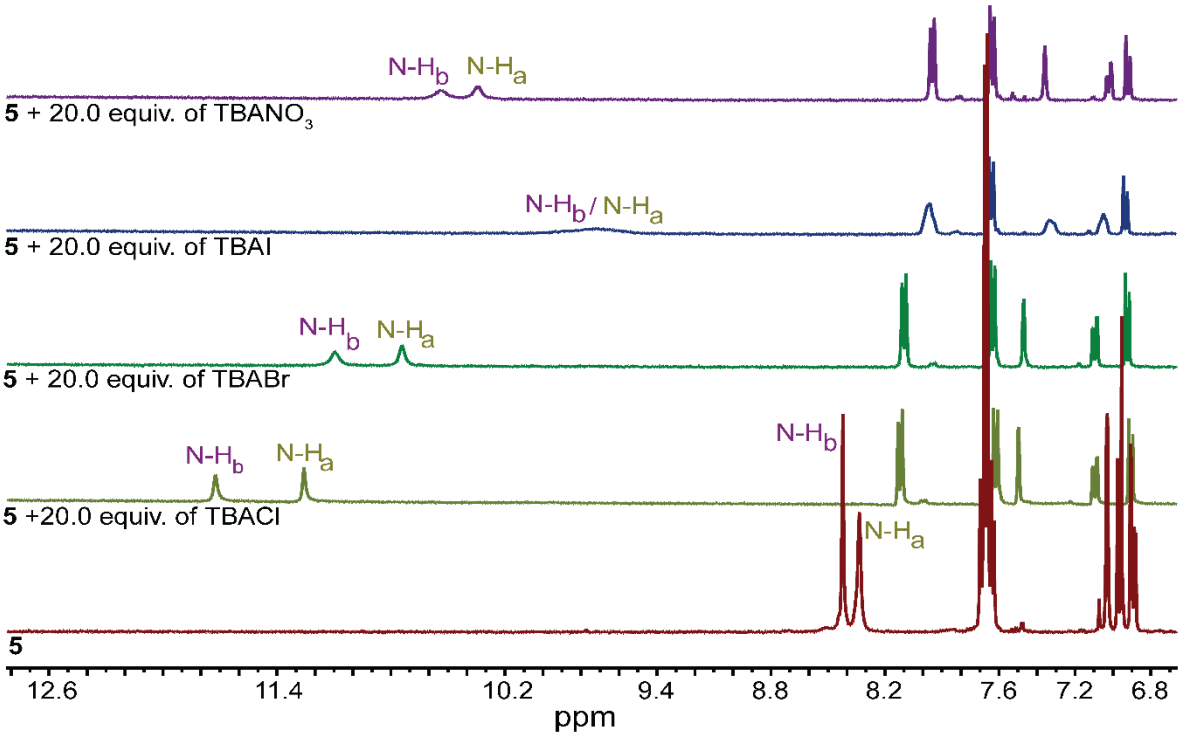


Figure S9. ^{1}H NMR spectrum of ring **5** (5 mM) after addition of 20 equivalents of different salts of anions in CD_3CN.

5. Mass Spectrometry Study

Stock solutions of ring **5** (5 mM) and TBACl (500 mM) were prepared using spectroscopy-grade acetonitrile. Afterward, these solutions were mixed so that TBACl concentration was approximately 10 equivalent with respect to ring **5** and further diluted using spectroscopy-grade acetonitrile. The resulting diluted sample was used to carry out the mass analysis in negative ion mode, where it was electro-sprayed at a flow rate of 400 µL/min with a capillary voltage of 1.0 kV.

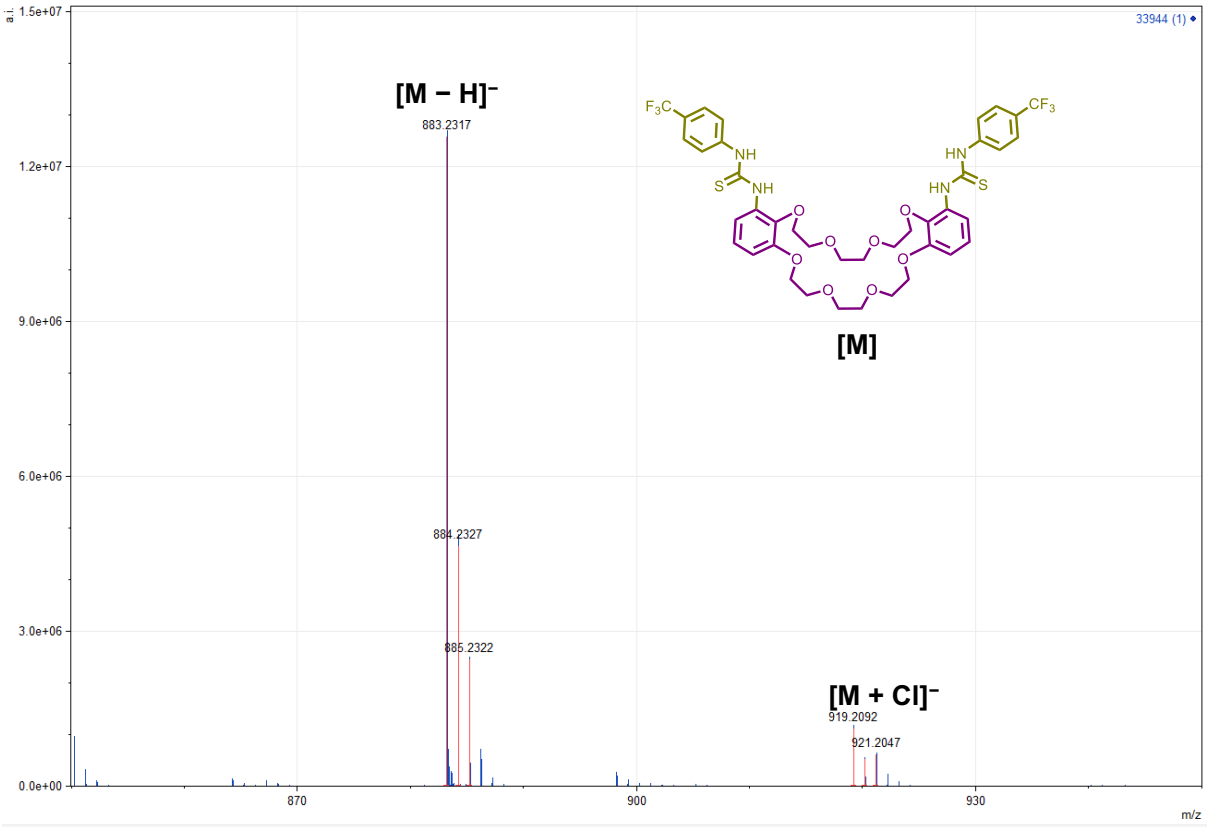


Figure S10. Negative mood ESI-MS spectrum of a mixture of ring **5** and TBACl in acetonitrile.

6. Shuttling Rate Studies of Rotaxanes

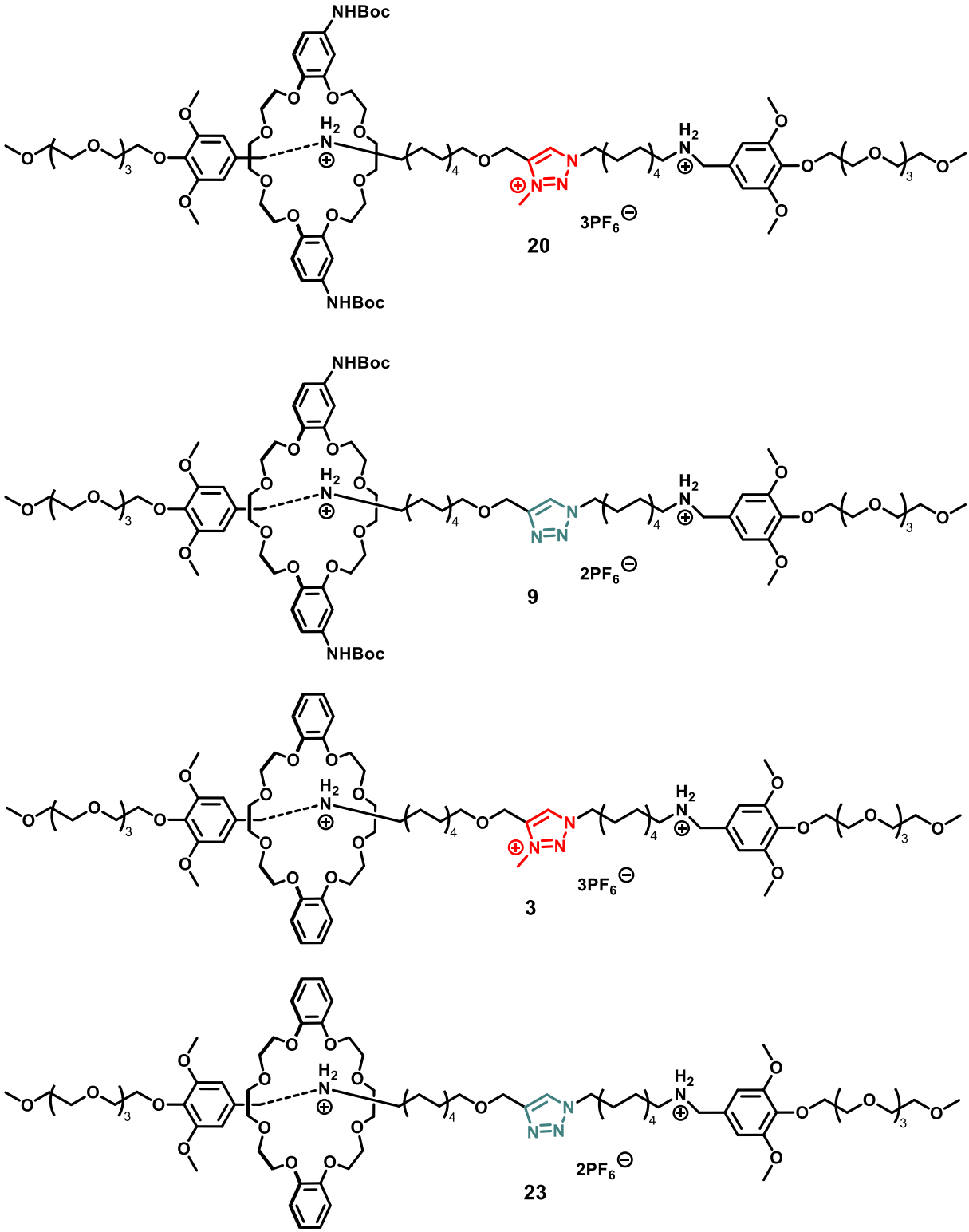


Figure S11. Rotaxanes for the 2D EXSY.

6.1. Shuttling Rate of Rotaxane 20

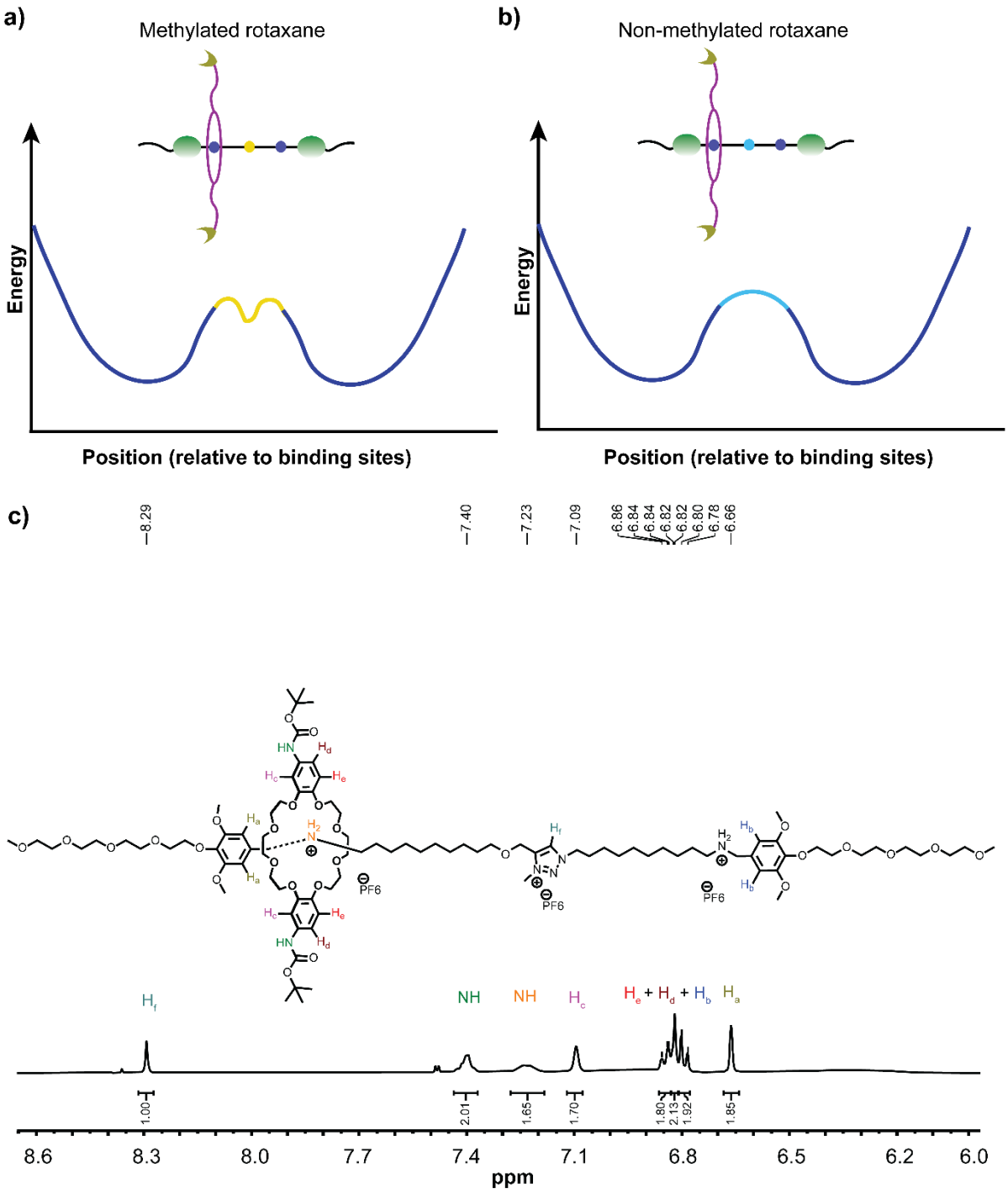


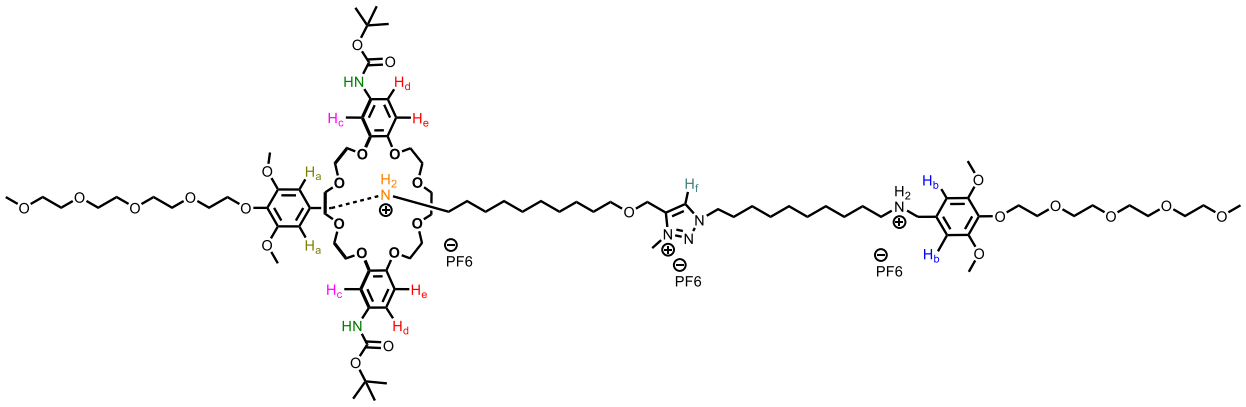
Figure S12. (**a**,**b**) Schematic representation of energy diagram of a methylated and non-methylated rotaxane. (**c**) Detailed view of the chemical shifts of the aromatic region of rotaxane **20.**

The shuttling rate of rotaxane **20** was determined through 2D-EXSY analysis, which comprises a series of 2D-NOESY experiments using various mixing times (τ_m, 400 ms, 600 ms, 700 ms).[4] In this study, acetonitrile-d_3 was used as a solvent, and

cross peaks of H_a and H_b were monitored at different mixing times. The signal for the H_b was initially difficult to assign, but it was confirmed by a series of 2D experiments (HSQC, HMBC, and COSY) that it overlaps with the signals corresponding to H_d and H_e protons of the dibenzyl crown ether. **Figure S12c** shows each proton assignment. Further, the protons of H_a and H_b are considered as **A** and **B** protons for the shuttling rate calculation study.

The exchange rate (k) can be calculated by using the equations S1-S2, where I_{AA} and I_{BB} are the diagonal peak intensities and I_{AB} and I_{BA} are the cross-peak intensities. The average exchange rate (k) is the sum of forward (k_1) and backward (k_{-1}) and can be calculated using Eq. S3.

Windows software EXSYCalc was used to calculate forward (k_1) and (k_{-1}), shuttling rate. The value of k was calculated by taking the average of the rates obtained at different mixing times. For rotaxane **20**, the average calculated values of k_1 and k_{-1} were both found to be 0.028 Hz because of its symmetry. Additionally, the average value of k was determined to be 0.056 Hz, according to the calculations presented in Table S2.



$$r = \frac{I_{[AA]} + I_{[BB]}}{I_{[AB]} + I_{[BA]}} \quad \text{(S1)}$$

$$k = \frac{1}{\tau_m} ln \frac{r + 1}{r - 1} \quad \text{(S2)}$$

$$k = k_1 + k_{-1} \quad \text{(S3)}$$

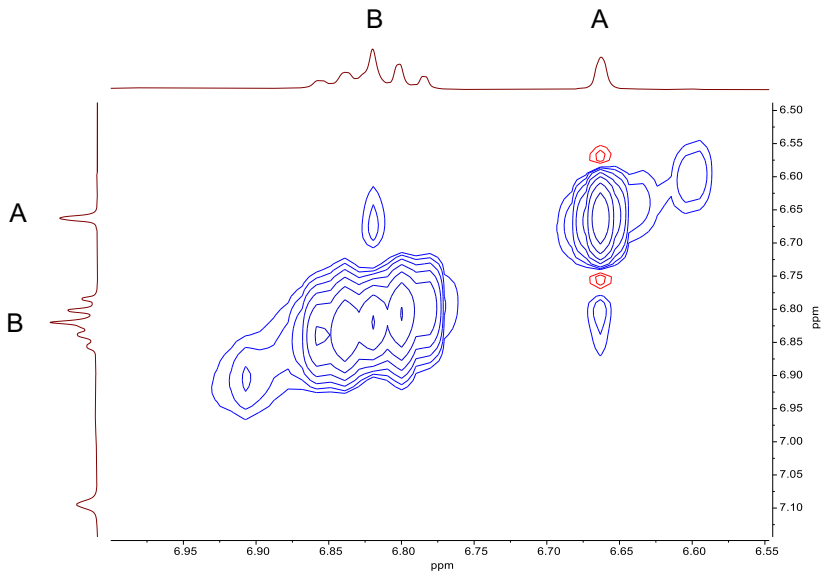


Figure S13. Expanded 2D EXSY spectrum of rotaxane **20** (500 MHz, 298 K) at 400 ms mixing time**.**

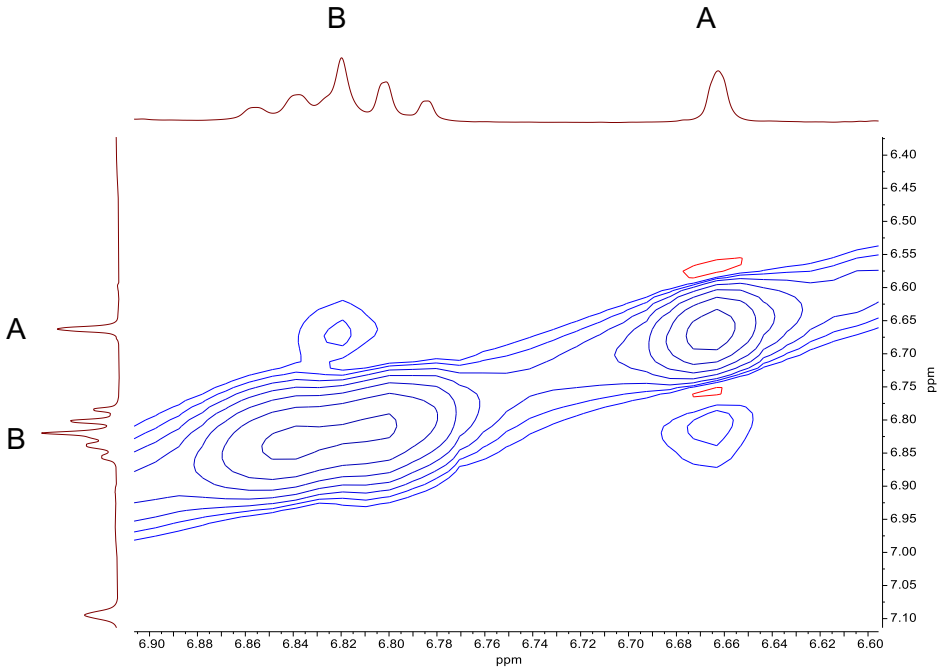


Figure S14. Expanded 2D EXSY spectrum of rotaxane **20** (500 MHz, 298 K) at 600 ms mixing time.

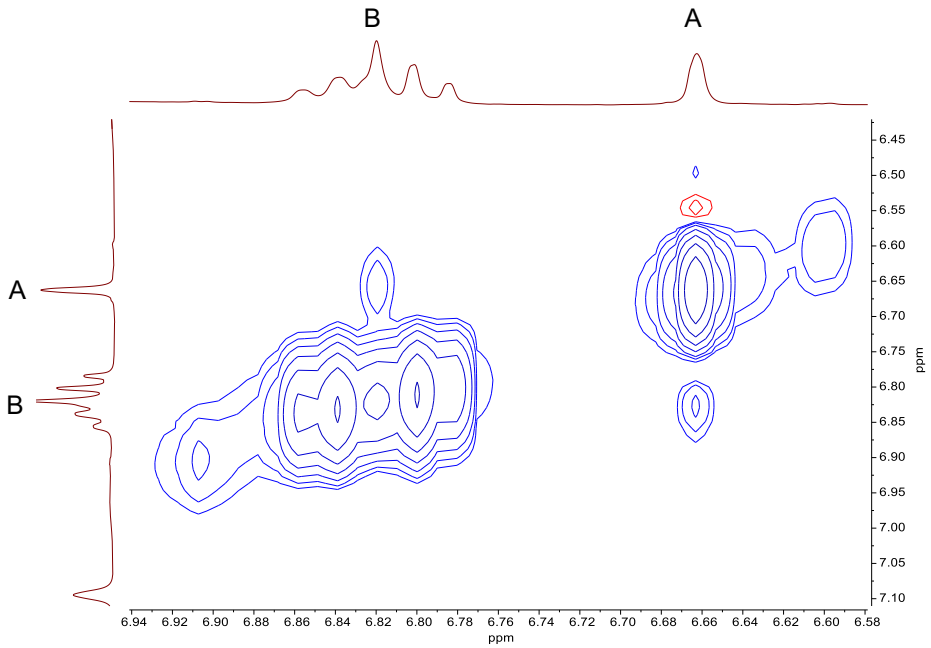


Figure S15. Expanded 2D EXSY spectrum of rotaxane **20** (500 MHz, 298 K) at 700 ms mixing time.

Table S3. Integrations (I) of diagonal peaks $[AA]$, $[BB]$ and cross-peaks $[AB]$, $[BA]$ in rotaxane **20** under different τ_m and the exchange rates of forward and reverse shuttling motions calculated by the EXSYCalc program.

τ_m / ms	$I_{[AA]}$	$I_{[AB]}$	$I_{[BB]}$	$I_{[BA]}$	k_1 (Hz)	k_{-1} (Hz)	k (Hz)
400	1	0.01	1.01	0.01	0.025	0.025	0.05
600	1	0.02	1.07	0.02	0.032	0.032	0.064
700	1	0.02	1.09	0.02	0.027	0.027	0.054

6.2. Shuttling Rate Studies of Control Rotaxane 3

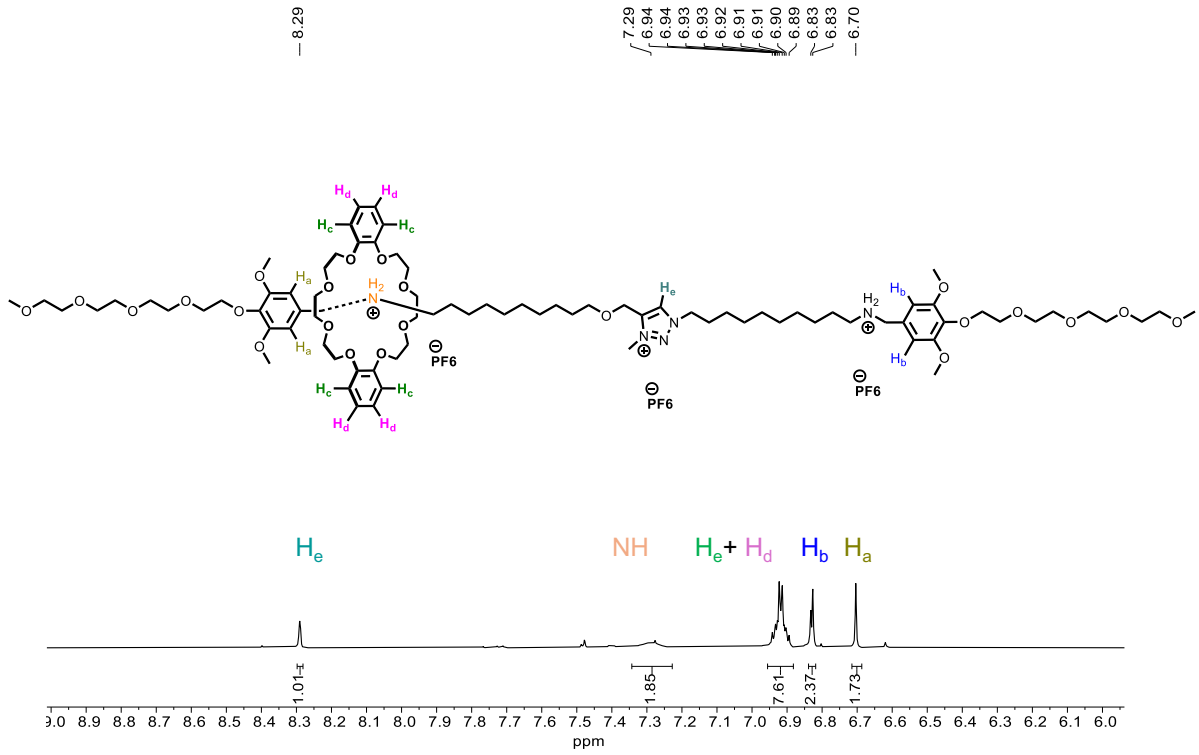


Figure S16. Detailed view of the aromatic region of rotaxane **3**.

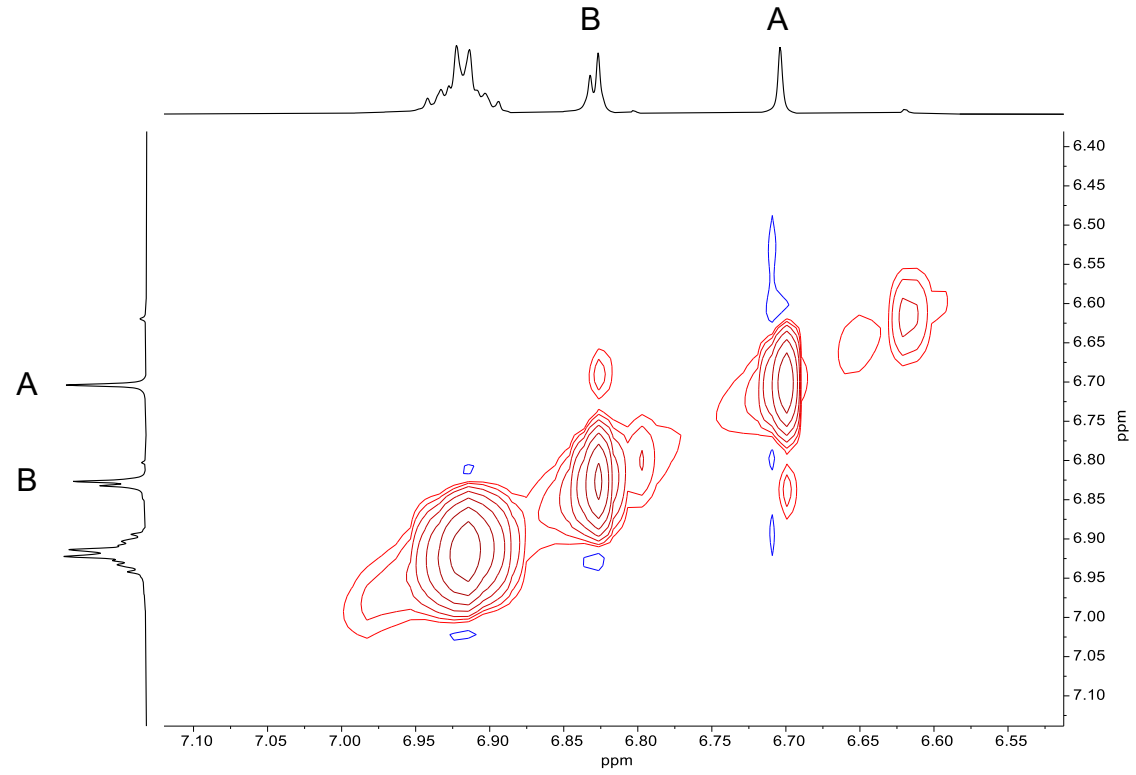


Figure S17. Expanded 2D EXSY spectrum of rotaxane **3** (500 MHz, 298 K) at 500 ms mixing time.

Table S4. The integrations (I) of diagonal peaks $[AA]$, $[BB]$ and cross-peaks $[AB]$, $[BA]$ in rotaxane **3** under different τ_m and the exchange rates of forward and reverse shuttling motions calculated by the EXSYCalc program.

τ_m / ms	$I_{[AA]}$	$I_{[AB]}$	$I_{[BB]}$	$I_{[BA]}$	k_1 (Hz)	k_{-1} (Hz)	k (Hz)
100	1	0	0.8	0	0	0	0
200	1	0	1.07	0	0	0	0
300	1	0	1.1	0	0	0	0
400	1	0	0.96	0	0	0	0
500	1	0.02	1.3	0.03	0.035	0.052	0.087
600	1	0	1.34	0	0	0	0
700	1	0	1.19	0	0	0	0
900	1	0	1.1	0	0	0	0

S7. Investigation of Anion Transport Activity Using a Chloride Selective Electrode

LUVs were prepared following a reported procedure.[7] 154 μL of Egg Yolk Phosphatidylcholine (EYPC, 50 mg/mL in chloroform) and 39 μL of cholesterol (25 mg/mL in chloroform) were mixed in a 10 mL glass vial to make a molar ratio of 8:2. This chloroform mixture was dried under vacuum with continuous rotation of the vial to make a transparent thin film of lipids at the wall the glass vial. The transparent thin film was kept under vacuum for an additional 5 h to remove all the traces of the chloroform. Next, the dry thin film was hydrated with 500 μL buffer (5 mM phosphate buffer, 100 mM NaCl, pH 7.2) for 2 h with 6–7 times occasional vortexing (10 min for each time). During this step, a suspension was formed, which was passed through 12–13 cycles of freeze-thaw (freezing with liquid nitrogen and melting with hot water at 80°C) to break up potential multilamellar vesicles. After this step, the lipid suspension was extruded 19–21 times (must be an odd number) using a mini extruder with a polycarbonate membrane (200 nm pore size) from Avanti Polar Lipids. The formed LUVs composed of (EYPC/Chol*)* were dialyzed using 5 mM phosphate buffer containing 100 mM $NaNO_3$ (pH 7.2) to remove the unencapsulated NaCl. After dialysis, the liposomal solutions were collected for the efflux studies. The final lipid concentration was 25 mM (assuming 100 % lipid regeneration).

The chloride electrode was calibrated before the measurements using the 1 ppm, 10 ppm, and 100 ppm standard Cl^- anion solutions with an ionic strength adjuster solution. Further, the recommended filling solution was also dispensed into the electrode for accurate results during the experiment and calibration process. After the calibration, we wash the electrodes with millipore water before using it for transport studies.

Having prepared the LUVs encapsulating the Cl^- anions, we determined the efficiency of rotaxanes to transport Cl^- anions by measuring the Cl^- anions efflux using the chloride selective electrode (Orion Instruments-Thermo Fisher Scientific). According to the procedure, 3940 µL of phosphate buffer (5 mM, pH 7.2) containing $NaNO_3$ (100 mM) and 50 µL of the LUVs (EYPC/Chol, 8:2 molar ratio) were added into a 20 mL glass vial. The mixture was stirred for 3 minutes to make the solution homogenous. Next, the chloride selective electrode was introduced into the mixture, which corresponds to t = 0 s. The respective anion transporter **1**-**5** (10 µL of stock solution) was added into the stirring solution at t = 50 s, and the concentration (ppm) of the effluxed Cl^- anions was monitored over time. At t = 600 s, the vesicles were lysed by adding 50 µL of 20% aqueous solution of Triton X-100 (a surfactant to lyse the vesicles). The final efflux reading was taken at 900 s. The percentage of Cl^- anions efflux across the membrane was calculated using the Cl^- anions efflux at 900 s as 100%.

The chloride transport efficiency of all the transporters (**1**–**5**) were normalized and the concentration of Cl^- anions efflux appearing at t = 50 s and t = 900 s were considered as 0 and 100 units respectively. The normalized chloride efflux efficiency (EE) at t = 600 s (prior to the addition of Triton X-100) was considered for the measurement of Cl^- anions transport efficiency of the transporters.

S8. Quantitative Measurement of Cl^- Anions Transport Activity.

The transport data at various concentrations were recorded, and the Cl^- anion efflux (%) data during t = 600 s was plotted as a function of the transporter concentration (µM) using the modified Hill equation from the OriginLab program.

$$y = a + (b - a) * \left(\frac{x^n}{k^n + x^n}\right) = Vmax\frac{x^n}{k^n + x^n} = 100 * \left(\frac{x^n}{EC_{50}^n + x^n}\right) \qquad (S4)$$

where, a = START = control value (dimethyl sulfoxide), b = END = 100, to get a common window for accurate transport comparison. y is the chloride efflux values at 600 s (%) and x is the concentration of the compounds being used for the efflux assay (μM). The V_{max}, k and n are the parameters to be fitted where V_{max} is the maximum efflux possible (usually fixed to 100 % as this is the maximum chloride efflux possible), n is the Hill coefficient, and k is the carrier concentration required to reach $V_{max}/2$ (when V_{max} is fixed to 100% then *k* is the EC_{50}). EC_{50} values at 600 s can be obtained directly from the Hill plot.

To get the effective concentration (EC_{50}) values, the Cl^- anions efflux efficiency values of all the tested transporters (**1**-**5**) were plotted against concentration and fitted using Hill equation (Eq. S4). The EC_{50} calculation for the ring **5** was not possible because of their low transport and solubility issues at higher concentration.

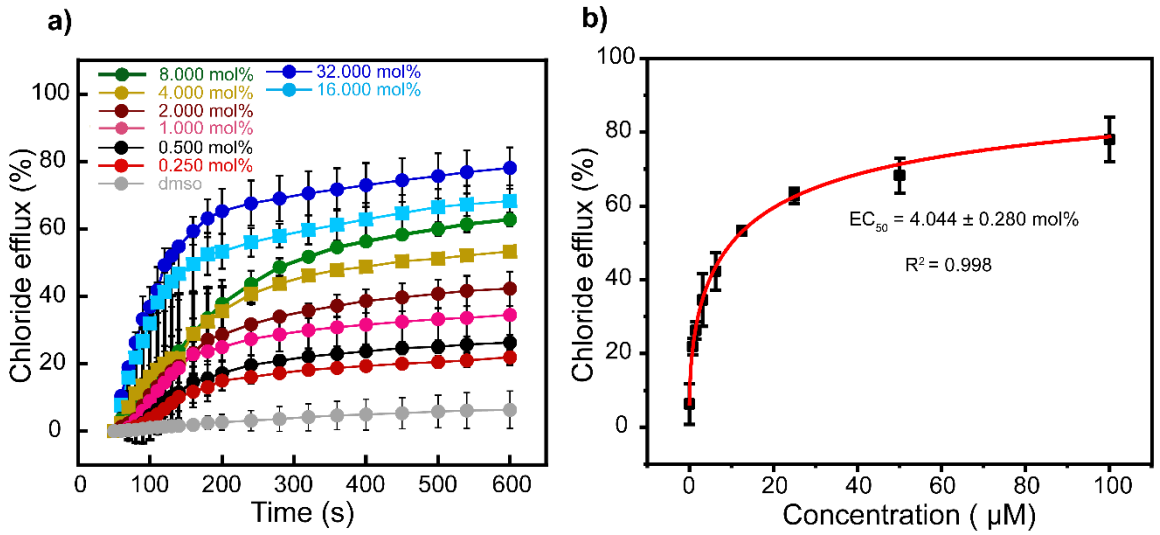


Figure S18. Transport of Cl^- anions across the bilayer of LUVs (EYPC/Chol, 8:2 molar ratio) by compound **3** at pH 7.2. (**a**) Transport of Cl^- anions over time by different mol% of compound **3.** (**b**) Fitting curve of Hill analysis to determine the EC_{50} value for compound **3**. Error bars represent standard deviations from two runs.

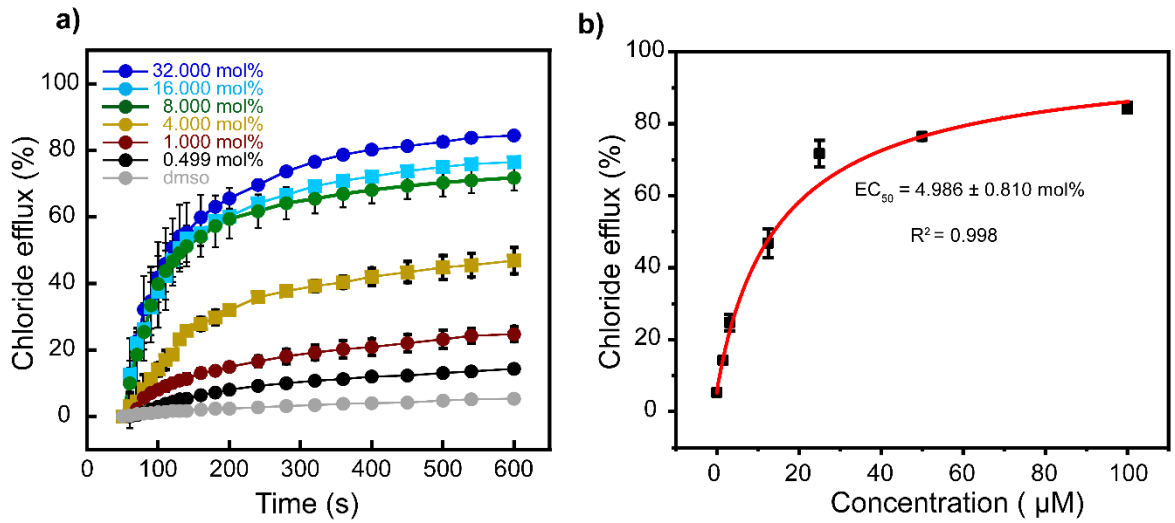


Figure S19. Transport of Cl^- anions across the bilayer of LUVs (EYPC/Chol, 8:2 molar ratio) by compound **4** at pH 7.2. (**a**) Transport of Cl^- anion over time by different mol% of compound **4.** (**b**) Fitting curve of Hill analysis to determine the EC_{50} value for compound **4**. Error bars represent standard deviations from two runs.

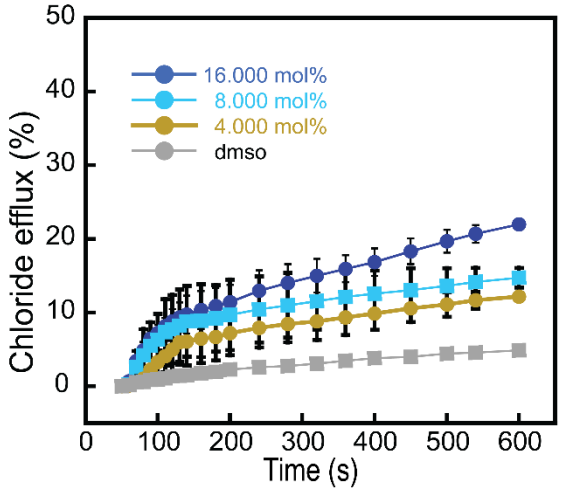


Figure S20: Transport of Cl^- anions across the bilayer of LUVs (EYPC/Chol, 8:2 molar ratio) by different mol% of ring **5** at pH 7.2. Error bars represent standard deviations from two runs.

S9. Anion Selectivity of Rotaxane 1

S9.1. Anion Selectivity using the different ion-selective electrodes.

We prepared LUVs composed of EYPC/Chol with a molar ratio of 8:2, encapsulating Cl^-, Br^-, I^- and NO_3^- using the procedure mentioned in **section S7**. NaCl, NaBr, NaI, and $NaNO_3$ were used as the source of the anions. The resulting unilamellar vesicles were dialyzed using 5 mM phosphate buffer containing 100 mM $NaNO_3$ (pH 7.2) to remove the unencapsulated NaX (X = Cl^-, Br^- or I^-) except for NO_3^- encapsulated liposome. For this case, we dialyzed the unilamellar vesicles using

the 5 mM phosphate buffer containing 100 mM NaCl to remove the unencapsulated NO_3^- anions. After dialysis, the liposomal solutions were collected and were ready for the efflux studies. The final lipid concentration for all the individual lipids was 25 mM (assuming 100 % lipid regeneration).

Having prepared the LUVs encapsulating either Cl^-, Br^-, I^-, or NO_3^-, we determined the efficiency of rotaxane **1** to transport each anion separately by measuring the anion efflux using the specific anion-selective electrode (Orion Instruments-Thermo Fisher Scientific) according to the procedure described in **section S7.** Importantly, each electrode was calibrated before the measurements using the 1 ppm, 10 ppm, and 100 ppm standard solutions with an ionic strength adjuster solution. Further, the recommended filling solutions of each electrode were also dispensed into the electrode before starting the calibration and the experiment. After the calibration, we washed the electrodes with Millipore water before using them for transport studies.

The measurements of the efflux of Cl^-, Br^-, and I^- were carried out without any complication. However, we observed a relatively sluggish response of the electrode when measuring the NO_3^- anions efflux with the corresponding nitrate-selective electrode. According to the electrode's user manual, this may be due to the presence of phosphate ions that behave as interfering ions.

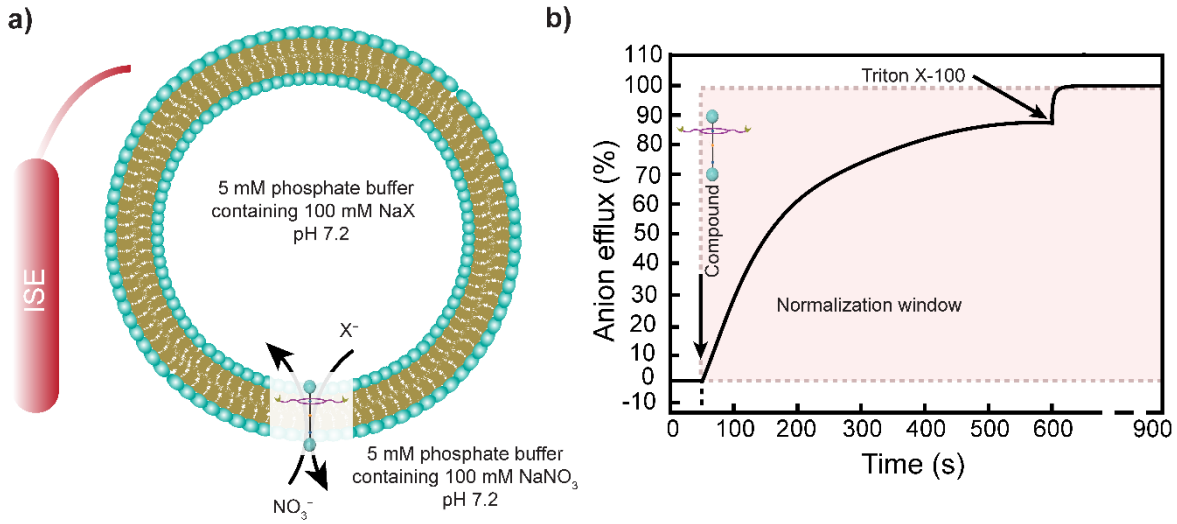


Figure S21. Transport of X^- anions across different NaX (X = Cl^-, Br^-, and I^-) encapsulated LUVs (EYPC/Chol, 8:2 molar ratio) at pH 7.2 by rotaxane **1**. (**a**) Representation of X^- anions transport monitored by an individual ion selective electrode for each anions. (**b**) X^- anions efflux kinetics across the LUVs.

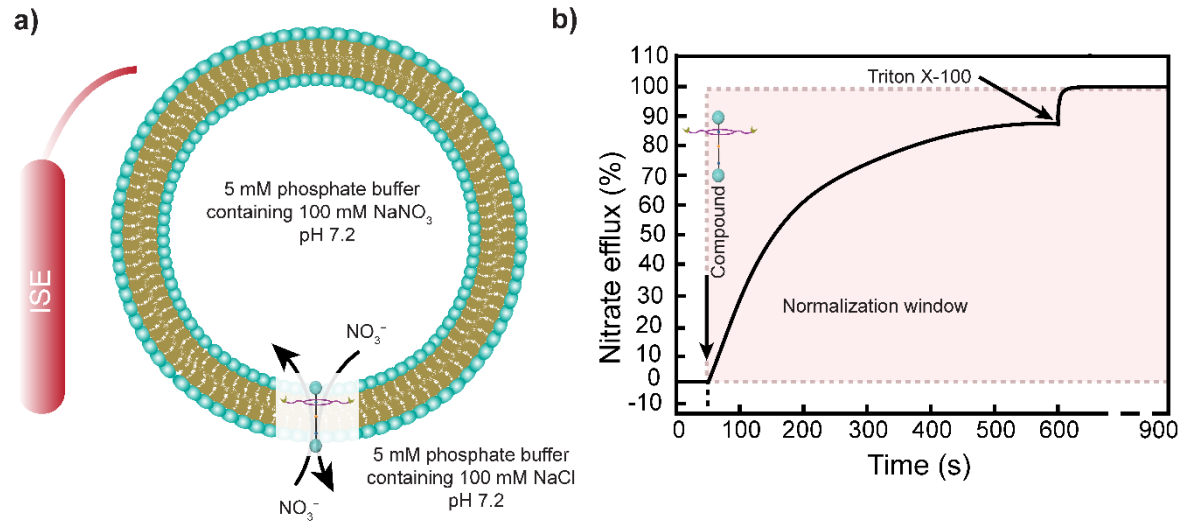


Figure S22. Transport of NO_3^- anions across $NaNO_3$ encapsulated LUVs (EYPC/Chol, 8:2 molar ratio) at pH 7.2 by rotaxane **1**. (**a**) Representation of NO_3^- anions transport monitored by a Nitrate selective electrode. (**b**) NO_3^- anions efflux kinetics across the LUVs.

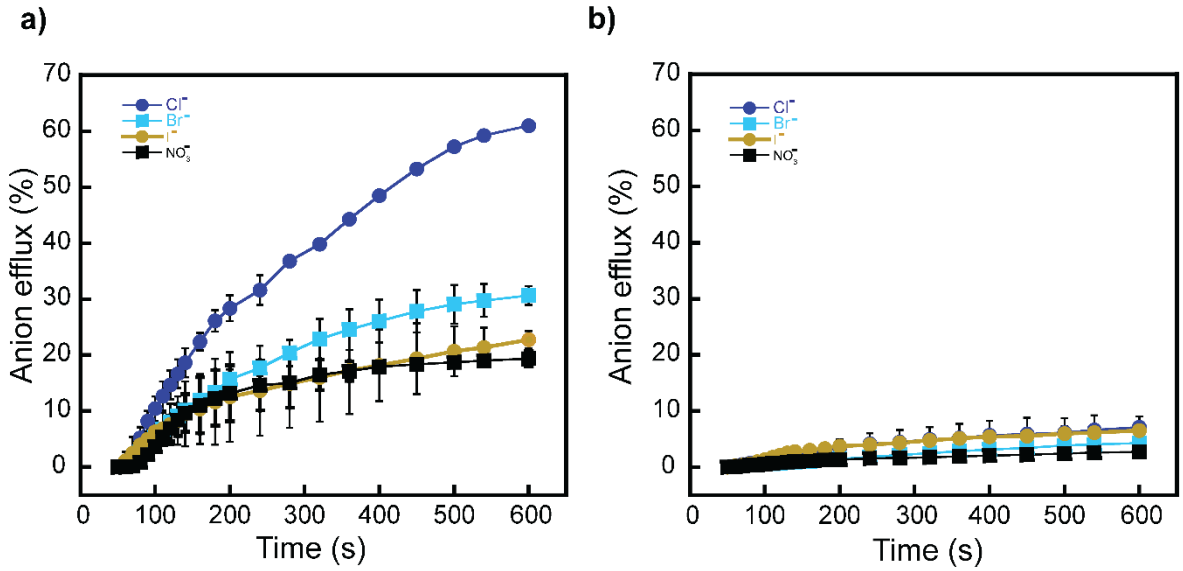


Figure S23. Anions efflux across LUVs (EYPC/Chol, 8:2 molar ratio) encapsulating different NaX (X = Cl^-, Br^-, I^- and NO_3^-) by rotaxane **1.** (**a**) [**1**] = 0.499 mol%. (**b**) Without rotaxane **1**. Error bars represent standard deviations from two runs.

S9.2. Anion Selectivity using the fluorescence-based assay

S9.2.1. Preparation of the Vesicles

The HPTS assay was carried out using EYPC/Cholesterol (ratio 8:2) encapsulating HPTS dye (1 mM). These LUVs were prepared following a reported procedure.[7] 154 µL of Egg Yolk Phosphatidylcholine (EYPC, 50 mg/mL in chloroform) and 39 µL of

cholesterol (25 mg/mL in chloroform) were mixed in a 10 mL glass vial to make a molar ratio of 8:2. This chloroform mixture was dried under vacuum with continuous rotation of the vial to make a transparent thin film of lipids at the wall the glass vial. The transparent thin film was kept in a high vacuum for 5 h to remove all the traces of the chloroform.

Afterward, the dry thin film was hydrated with 500 µL buffer (10 mM HEPES buffer,100 mM NaCl, pH 7.0, 1 mM HPTS dye) for 2 h with 6-7 times occasional vortexing. During this step, a suspension was formed, which was passed through 10-12 cycles of freeze-thaw (freezing with liquid nitrogen and melting with hot water at 80 ºC) to break up potential multilamellar vesicles. After this step, the lipid suspension was extruded 19-21 times (must be an odd number) using a mini extruder with a polycarbonate membrane (200 nm pore size) from Avanti Polar Lipids. The unencapsulated HPTS dye was removed by an 8.3 mL Sephadex G-25 resin column using an external solution of NaX (X = Cl^-, Br^-, NO_3^-, and I^-) buffered at pH 7.0 with 10 mM HEPES to get $NaCl^{in}/NaX^{out}$ Vesicle suspension. The final lipid concentration was 12.5 mM (assuming 100 % lipid regeneration).

S9.2.2. Calibration of HPTS

HPTS calibration was carried out using NaCl encapsulated (represented as $NaCl^{in}$) LUVs, and external buffer solutions were prepared from pH 5.3 - 9.2 using 1 M NaOH and 1 M HCl to adjust the pH using a pH electrode. The vesicles were prepared as mentioned above. (50 µL of the $NaCl^{in}$ vesicle, 2.95 mL of NaCl external solutions). Further, the pH of intravesicles was equilibrated by adding monensin (7.5 µL of 0.5 Mm solution). Measurement of the ratiometric fluorescence response(I_{460}/I_{403}) (λ_{ex} = 460 nm, λ_{em} = 510 nm, base form divided by λ_{ex} = 403 nm, λ_{em} = 510 nm, acid form) was carried out at different pH. The pH was plotted against the ratiometric values, and the curve was fitted using the Henderson-Hasselbalch equation to obtain the calibration plot.[8, 9]

Equation: $y = \log\frac{ax-b}{c-x}$ ……(S5)

where x = I_{460}/I_{403}, y = pH

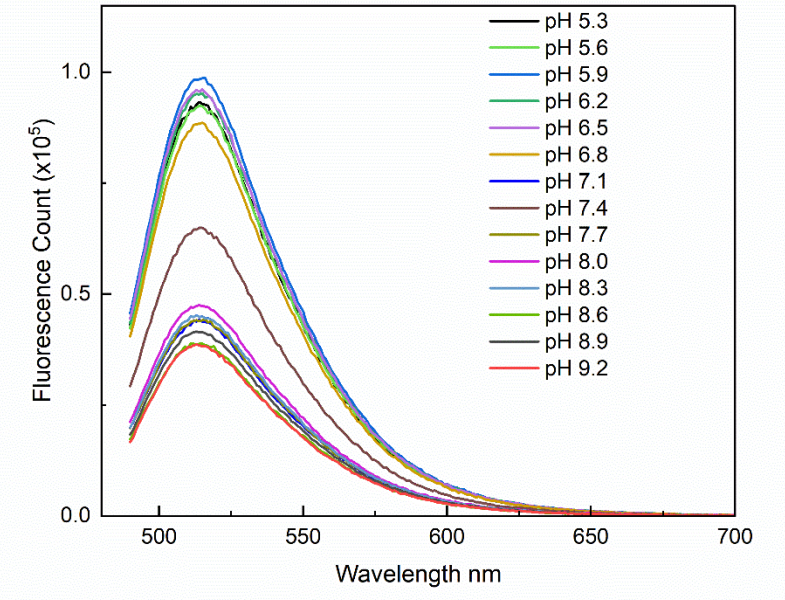


Figure S24. Fluorescence measurement with pH buffer (5.3 – 9.2) at 403 excitation wavelengths and 510 nm emission wavelengths

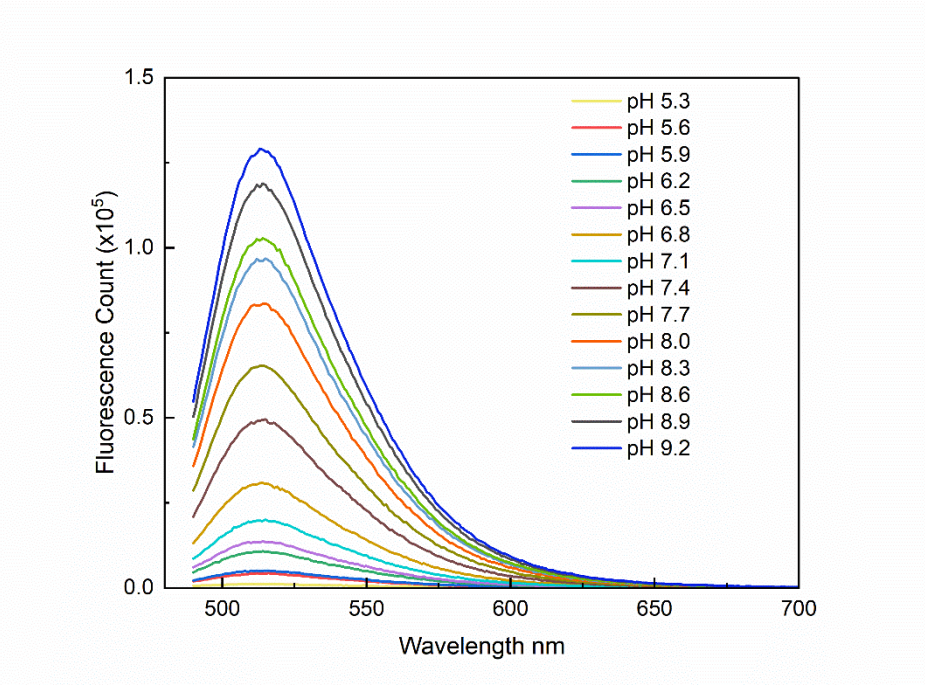


Figure S25. Fluorescence measurement with pH buffer (5.3 – 9.2) at 460 nm excitation wavelengths and 510 nm emission wavelengths

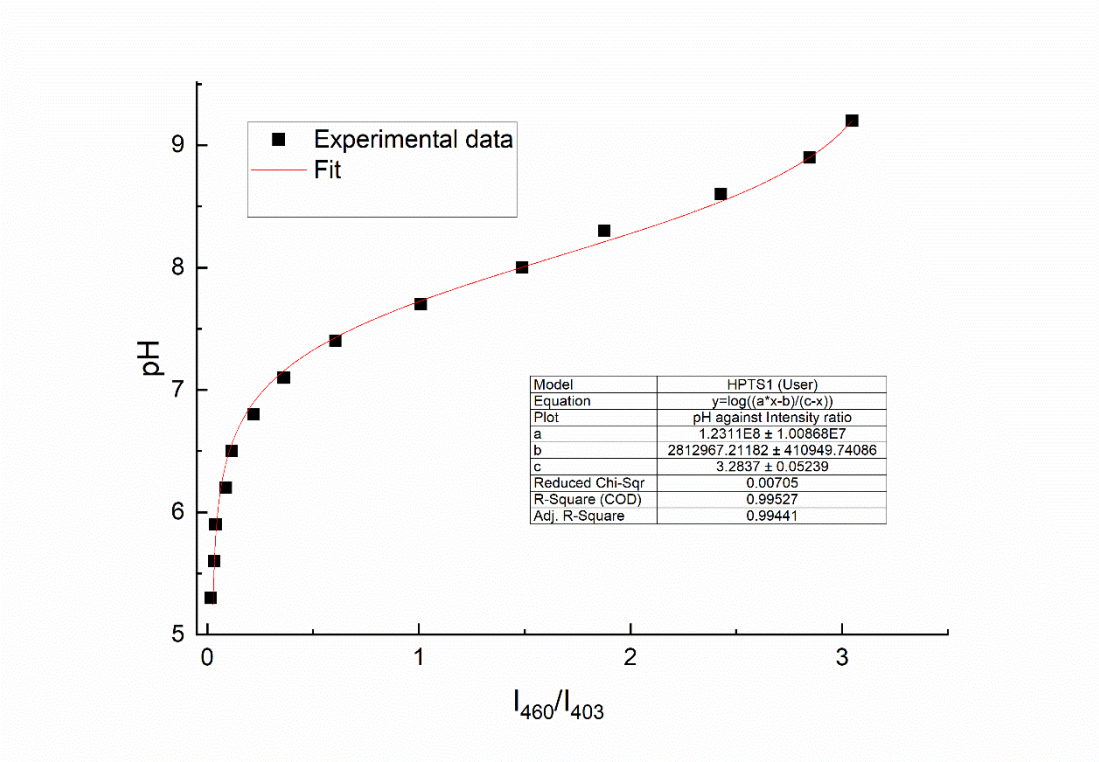


Figure S26. HPTS assay calibration using $NaCl^{in}$ vesicles.

S9.1.3. Anion Gradient Assay

Preparation of the vesicle was done following the reported procedure.[7] The LUVs were diluted using NaX (X = Cl^-, Br^-, NO_3^- or I^-) to get $NaCl^{in}/NaX^{out}$ vesicles suspended in 2.95 mL samples containing 0.15 mM of the EYPC/Chol. The rotaxane (10 µL from compound's stock in dmso) was added when time was at 0 to effect pH changes. Measurement of the ratiometric fluorescence response(I_{460}/I_{403}) (λ_{ex} = 460 nm, λ_{em} = 510 nm, base form divided by λ_{ex} = 403 nm, λ_{em} = 510 nm, acid form) was carried and the I_{460}/I_{403} values were converted to pH using the calibration curve.[9]

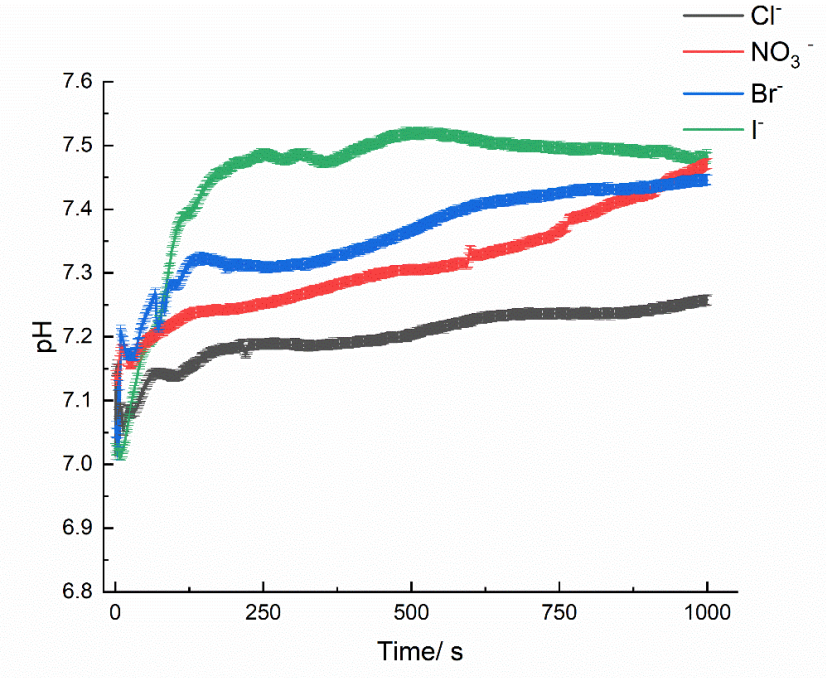


Figure S27. Anion selectivity of rotaxane **1** in LUVs (EYPC/Chol, 8:2 molar ratio) at pH 7.2. [**1**] = 0.499 mol% This plot corresponds to a duplicate experiment.

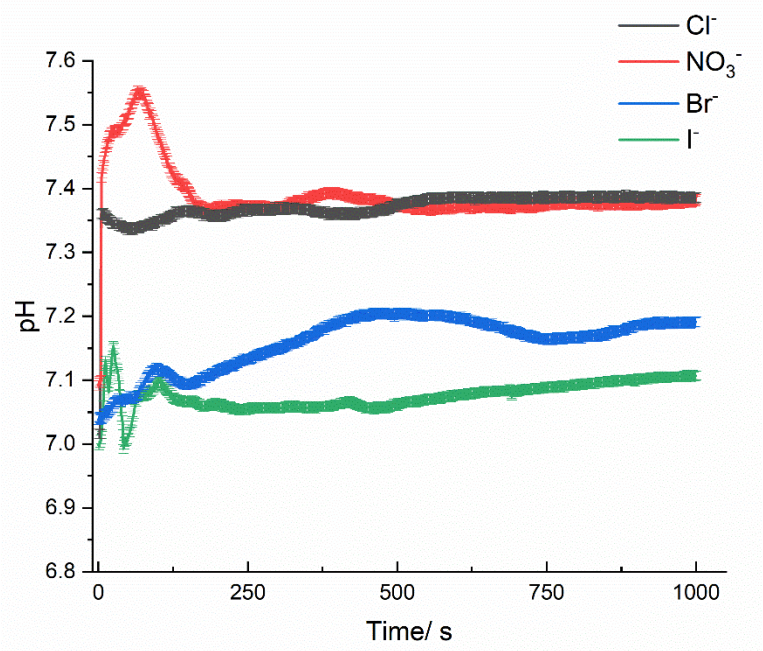


Figure S28. Anion selectivity of DMSO in LUVs (EYPC/Chol, 8:2 molar ratio) at pH 7.2. This plot corresponds to a duplicate experiment.

S10. Cation Selectivity of Rotaxane 1

Cation selectivity studies were also carried out by the ISE-based assay as described in **section S7**. We prepared LUVs composed of EYPC/Chol with a molar

ration of 8:2, where 5 mM phosphate buffer containing the corresponding MCl salt (100 mM, M = Li^+ ,Na^+ , K^+ , Rb^+ , Cs^+) was used as the intravesicular medium. The extravesicular medium consisted of 5 mM phosphate buffer containing 100 mM $NaNO_3$. The pH was fixed at 7.2 in the intra- and extravesicular media. Cl^- anions transport studies across these LUVs were carried out as described in **section S7**.

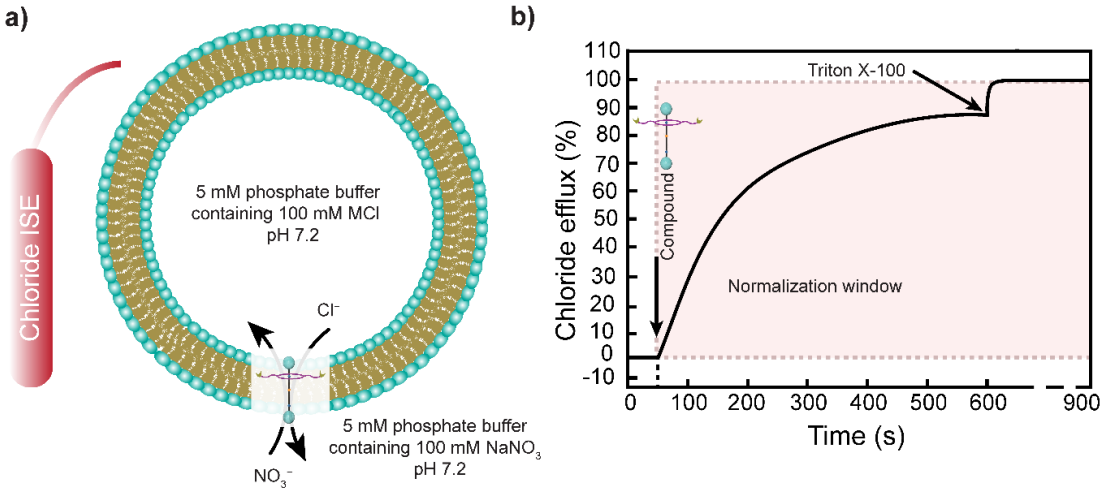


Figure S29. Representation of experimental setup to determine cation selectivity. (**a**) Representation of Cl^- anions efflux across LUVs encapsulating different MCl (M = Li^+, Na^+, K^+, Rb^+, and Cs^+) monitored by a chloride selective electrode. (**b**) Cl^- anions efflux kinetics across the LUVs.

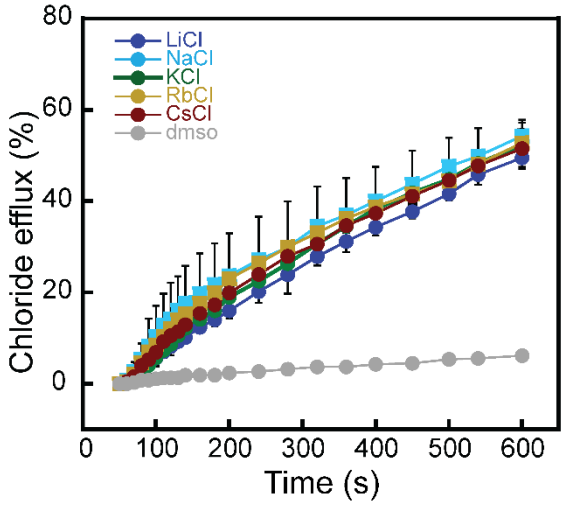


Figure S30. Cl^- anions efflux across LUVs (EYPC/Chol, 8:2 molar ratio) encapsulating different MCl (M = Li^+, Na^+, K^+, Rb^+, and Cs^+) at pH 7.2 by rotaxane **1**. [**1**] = 0.499 mol%. Error bars represent standard deviations from two runs.

S11. Effect of Rotaxanes 1 and 2 on the Size and Permeability of LUVs

11.1. Preparation of LUVs (EYPC/Chol, 8:2 molar ratio) encapsulating HPTS

Large unilamellar vesicles (LUVs) were prepared according to the procedure discussed in **section S7** with a modification: the dry thin film was hydrated with 500 µL of HEPES buffer (20 mM HEPES, 100 mM NaCl, pH = 7.2) containing 1 mM HPTS dye. Further, we used size exclusion chromatography to remove the unencapsulated HPTS dye from the liposome mixture. We used a PD-10 column (prepacked with Sephadex^{TM} G-25 M) and HEPES (20 mM HEPES, 100 mM NaCl, pH = 7.2) as an eluting buffer to get the final liposome with a 25 mM concentration (assuming 100 % lipid regeneration). Further, the liposome solution was diluted to make the 1 mM stock for the DLS and DPX-based assay.[10]

S11.2. Dynamic Light Scattering (DLS) measurements

The average hydrodynamic radius (R_H) of liposomes was measured in the presence and absence of the rotaxanes (**1**-**3**). Briefly, 2890 µL of HEPES buffer (20 mM HEPES, 100 mM NaCl, pH = 7.2) was transferred to a quartz cuvette, followed by the addition of 100 µL of HPTS encapsulated LUVs (EYPC/Chol, 8:2 molar ratio) from 1 mM liposome stock. The samples without or with **1, 2,** and **3** (10 µL from each stock solution in dimethyl sulfoxide, 5.0 µM final concentration) were analysed by DLS. Further, 40 µL of Triton X-100 (5% aqueous solution) was added to check the average hydrodynamic radius (R_H) of disrupted vesicles which served as crucial control for the study.

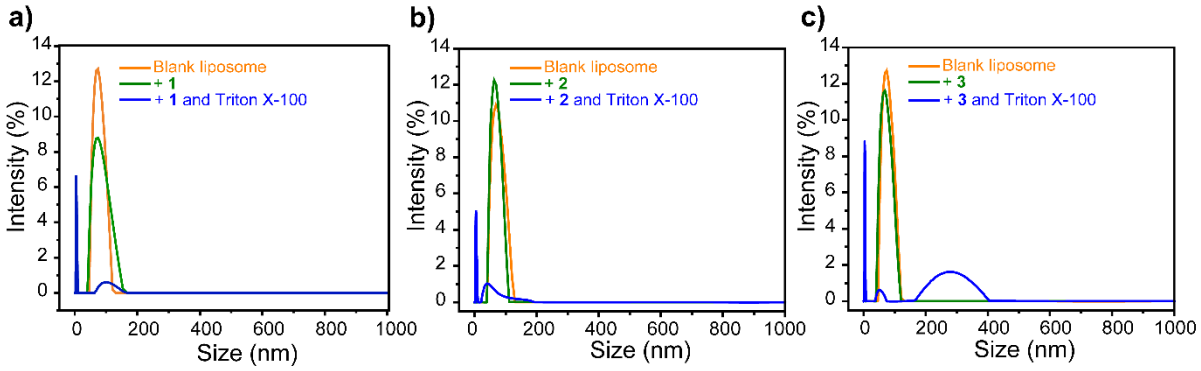


Figure S31. DLS analysis of HPTS encapsulated LUVs (EYPC/Chol, 8:2 molar ratio) after the addition of rotaxane**1** (**a**), rotaxane **2** (**b**), and rotaxane **3** (**c**) in the absence and presence of surfactant Triton X-100 (5% aqueous solution).

S11.3. Vesicle leakage studies by the DPX-based quenching experiments

Potential changes in the LUVs permeability due to rotaxanes **1** and **2** were determined by monitoring the changes in the excitation spectra of HPTS encapsulated in LUVs and in the presence of *p*-xylene-bis-pyridinium bromide (DPX) acting as a quencher. It is anticipated that if leakage occurs, HPTS interacts with the quencher located outside the vesicles, significantly reducing the excitation spectrum, whereas the excitation spectrum would remain the same if the rotaxanes (**1**–**2**) do not induce any leakage. Based on this premise, we carried out the experiment where 2890 μL of HEPES buffer (20 mM HEPES, 100 mM NaCl, pH = 7.2) and 100 μL of LUVs (EYPC/Chol, 8:2 molar ratio) encapsulating HPTS from 1 mM liposome stock were mixed in a quartz cuvette. Then, the respective rotaxanes (10 μL stock solutions in dimethyl sulfoxide, the final concentrations are 5.0 μM) were added into the cuvette and stirred for 10 min for its proper incorporation into the lipid membrane. Subsequently, *p*-xylene-bis-pyridinium bromide (DPX) quencher (1.0 mM) was added and the fluorescent excitation spectra of HPTS was recorded (emission at λ_{em} = 510 nm). Finally, to get 100% quenching, 40 μL of Triton X-100 (5% aqueous solution) was used as a surfactant to completely lyse the vesicles.

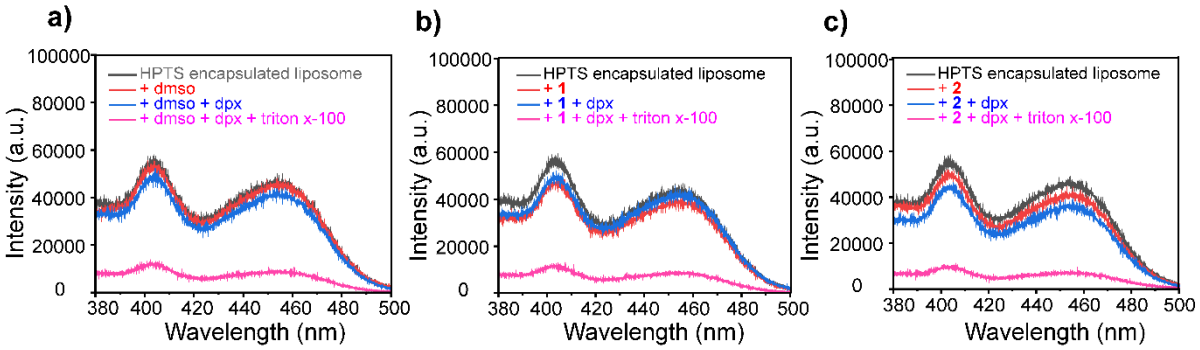


Figure S32. Leakage studies in LUVs (EYPC/Chol, 8: 2 molar ratio) encapsulating HPTS in the presence of (**a**) dimethyl sulfoxide, (**b**) rotaxane **1,** and (**c**) rotaxane **2**. The concentration of rotaxanes (**1**–**2**) was fixed at 5 μM, and the emission wavelength is λ_{em} = 510 nm.

S12. Test for Leaching of Rotaxanes from the Membranes

This study was carried out by adapting previously reported protocols.[7, 11] LUVs (EYPC/Chol, 8:2 molar ratio) encapsulating NaCl were prepared according to the procedure described in **section S7**. 3940 μL of 5 mM phosphate buffer (100 mM $NaNO_3$, pH 7.2) and 50 μL of the LUVs solution (final concentration of liposomes are

100 μM, 200 μM, 300 μM or 400 μM) were added to a 20 mL glass vial and stirred. The chloride selective-selective electrode was then dipped into the solution and stirred for a few minutes until we observed a stable reading. After that, 10 μL of rotaxane **1** from the respective stock solution (in all cases lipid/transporter ratio was fixed at around 200) was added at t = 50 s and the chloride efflux kinetics was monitored until 600 s. Then, the vesicles were lysed using 50 μL of 20% Triton X-100 solution. The Cl^- transport efficiency of rotaxane **1** was calculated using the method described in **section S7**.

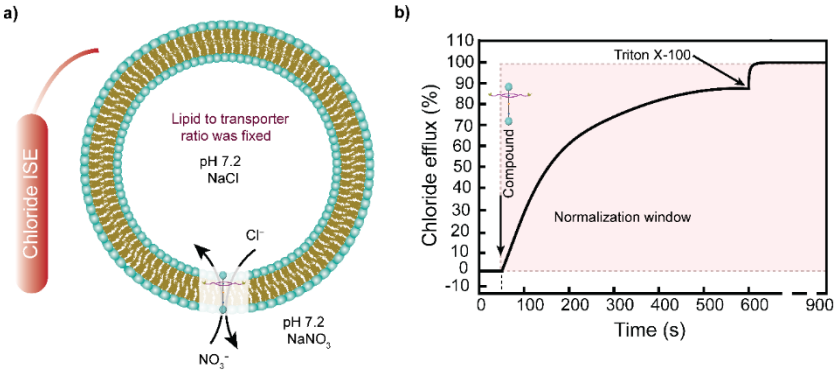


Figure S33. Transport of Cl^- anions across the bilayer of LUVs (EYPC/Chol, 8:2 molar ratio) at pH 7.2 by rotaxane **1**. (**a**) Representation of Cl^- anions transport monitored by a chloride selective electrode in the presence of a different compound to lipid ratio (**b**) Cl^- anions efflux kinetics across the LUVs.

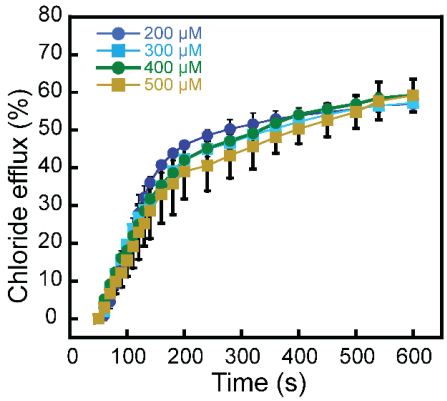


Figure S34. Cl^- anions efflux facilitated by rotaxane **1** in solutions of different LUVs concentrations (200, 300, 400, and 500 μM). The lipid-to-transporter ratio was fixed at 200. Error bars represent standard deviations from two runs.

S13. Mechanistic Studies of the Cl^- Anion Transport Across Lipid Bilayers

S13.1. Cl^- anion transport efficiency of rotaxane 1 in the presence of valinomycin

This assay was carried out using EYPC/Cholesterol (ratio 8:2) encapsulating lucigenin dye (1 mM). These LUVs were prepared following the procedure from the literature.[12] 154 µL of Egg Yolk Phosphatidylcholine (EYPC, 50 mg/mL in chloroform) and 39 µL of cholesterol (25 mg/mL in chloroform) were mixed in a 10 mL glass vial to make a molar ratio of 8:2. This chloroform mixture was dried under vacuum with continuous rotation of the vial to make a transparent thin film of lipids at the wall the glass vial. The transparent thin film was kept in a high vacuum for 5 h to remove all the traces of the chloroform.

Afterward, the dry thin film was hydrated with 500 µL buffer (10 mM HEPES buffer, 200 mM $NaNO_3$, pH 7.0, 1 mM lucigenin dye) for 2 h with 6-7 times occasional vortexing. During this step, a suspension was formed, which was passed through 10-12 cycles of freeze-thaw (freezing with liquid nitrogen and melting with hot water at 80 ºC) to break up potential multilamellar vesicles. After this step, the lipid suspension was extruded 29 times (must be an odd number) using a mini extruder with a polycarbonate membrane (200 nm pore size) from Avanti Polar Lipids. The unencapsulated lucigenin dye was removed by an 8.3 mL Sephadex G-25 resin column using an external solution of $NaNO_3$ buffered at pH 7.0 with 10 mM HEPES. The final lipid concentration was 12.5 mM (assuming 100 % lipid regeneration).

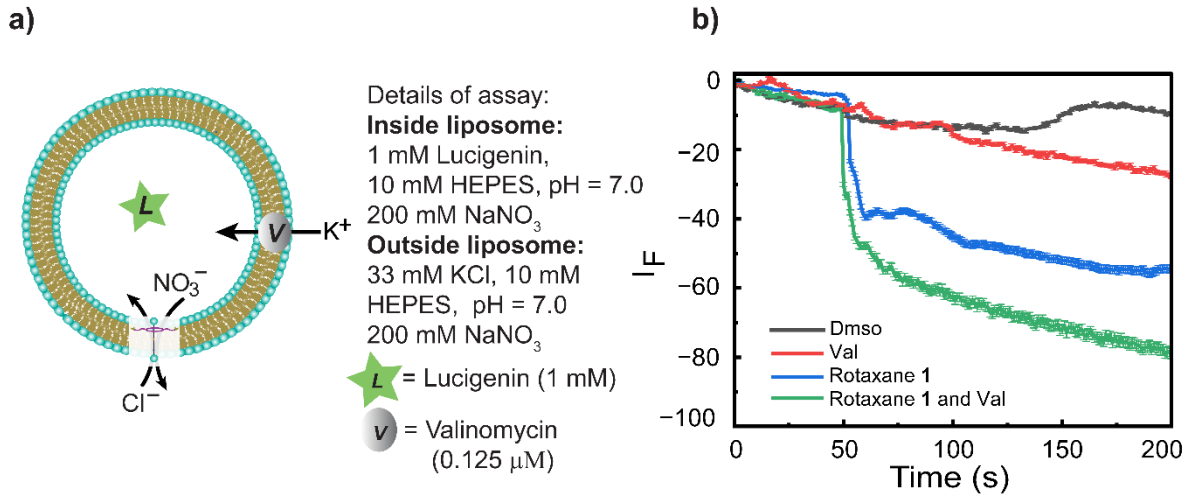


Figure 35. (a) Schematic representation of fluorescence kinetics assay for checking antiport mechanism in the presence of Valinomycin across lucigenin encapsulated LUVs. (b) Cl^- transport activity of rotaxane **1** ([**1**] = 0.499 mol%) in the presence and absence of valinomycin (0.125 µM). Error bars represent standard deviations from two runs.

36 µL of the prepared liposome and 2949 µL of external solution (containing 200 mM $NaNO_3$, 33 mM of KCl, and 10 mM HEPES, pH 7) were added to a cuvette and stirred inside the fluorometer (t = 0 s). The rotaxane **1** (0.499 mol%) and/or Valinomycin (0.125 µM) were added at t = 50 s. 20 µL of 20% Triton - X were added at t = 300 s to lyse the vesicles for 100% transport. Fluorescence intensities (F_t) were observed at λ_{em} = 535 nm (λ_{ex} = 450 nm) and normalized to the fractional emission intensity I_F using the equation below:[12]

Normalized FI Intensity (I_F) = $[(F_t - F_0)/ (F_\infty - F_0)] \times (-100)$……..(S6)

F_0 = Fluorescence intensity before the compound addition (t = 0 s).

F_∞ = Fluorescence intensity after complete leakage (t = 350 s).

F_t = Fluorescence intensity at time t

S13.2. U-tube experiment to monitor H^+ transport by rotaxane 1

This study was carried out by adapting previously reported protocols.[7, 13] The capability of rotaxane **1** to transport H^+ ions across an organic phase was evaluated by a classical U-tube experiment (**Figure 6b**). Thus, rotaxane **1** (0.2 mM) in chloroform was placed at the bottom of the U-tube and stirred. The left arm of the tube was filled with 0.1 N aqueous HCl solution (15 mL), and the right arm was filled with 0.1 N aqueous $NaNO_3$ solution (15 mL). The pH of the receiver end (right arm) was monitored using a pH meter over 100 h to detect H^+ transport by rotaxane **1**.

S13.3. U-tube experiment to monitor Cl^- transport by rotaxane 1

This study was carried out by adapting a previously reported protocol.[13] We used a similar U-tube experiment (**Figure 6a**) to assess whether rotaxane **1** has the capability to transport Cl^- anions through an organic phase. Rotaxane **1** (0.2 mM) in chloroform was placed at the bottom of the U-tube and stirred. The left arm of the tube was filled with 0.1 N aqueous NaCl solution (15 mL), and the right arm was filled with 0.1 N aqueous $NaNO_3$ solution (15 mL). The Cl^- anion concentration (ppm) of the receiver end (right arm) was monitored using a chloride ion selective electrode over 100 h.

S14. Effect of Membrane Fluidity on Anion Transport

S14.1. Preparation of LUVs with different cholesterol levels

We prepared LUVs with different EYPC/Chol ratios (10:0, 8:2, and 6:4) following the procedure described in **section S7**. The Cl^- anion efflux by rotaxanes **1** and **2** was monitored as described in **section S7.**

S14.2. Transport studies in LUVs composed of DPPC at different temperatures

We prepared LUVs composed of only DPPC lipids by adapting the procedure described in **section S7.** For this, we used 184 µL of DPPC stock solution (50 mg/mL in $CHCl_3$). Moreover, the extrusion of the LUVs was carried out at 50 °C.

Having the LUVs encapsulating NaCl, we monitored the Cl^- anion transport ability of rotaxane **1** at different temperatures (25 °C and 45 °C) using the chloride selective electrode. The sample for the measurement was prepared by mixing 50 µL of LUVs stock solution and 3940 µL of 5 mM phosphate buffer containing 100 mM $NaNO_3$ (pH = 7.2) in a 20 mL glass vial under continuous stirring. The chloride selective electrode was then immersed into the solution under mild stirring. 10 µL of the respective rotaxane (from DMSO stock solution) was added at t =50 s and monitored the Cl^- anion efflux kinetics until 600 s. After 600 s, the vesicles were completely lysed using 50 µL of 20% Triton X-100 solution and monitored its Cl^- anion concentration until 900 s.

S15. Antibacterial Activity Against *Staphylococcus aureus*

Bacterial growth. *Staphylococcus aureus* USA300, a community-acquired methicillin-resistant strain, was a generous gift from Dr. Paul Dunman.[14] USA300 was streaked to tryptic soy agar (TSA, Becton-Dickinson, Franklin Lakes, NJ) and incubated 18 h at 37 °C. Single colonies were picked, inoculated into 5 mL of tryptic soy broth (TSB, Becton-Dickinson) in aeration tubes, and incubated at 37 °C and a 45° angle with orbital shaking at 180 rpm for 16 h in an Innova42 shaking incubator (Eppendorf, Hauppauge, NY).

Kinetic growth curves. *S. aureus* overnights were diluted 100-fold into TSB. TSB, TSB + 1 M NaCl (VWR International, Radnor, PA), or TSB + 20 µM arachidonic acid

(AA, TCI America, Portland, OR) +/- rotaxane **1** was added to the wells of an untreated round bottom 96 well plate (Corning, Corning, NY). Bacteria was added to each well to give a final bacterial dilution of 1000-fold. All growth curves were performed at 37 °C with linear shaking (567 cpm, 3 mm) for 24 h while taking optical density readings at 600 nm every 30 min on an EPOCH2 plate reader (Agilent, Santa Clara, CA). Data were visualized in Prism 10 (GraphPad, La Jolla, CA).

Dilution plating for colony forming units. *S. aureus* overnights were diluted 100-fold into TSB. TSB, TSB + 1 M NaCl, or TSB + 20 μM arachidonic acid (AA) +/- rotaxane **1** was added to the wells of an untreated round bottom 96 well plate (Corning, Corning, NY). Bacteria was added to each well to give a final bacterial dilution of 1000-fold. Each plate was incubated shaking at 37 °C with orbital shaking at 180 rpm for 4 h in an Innova42 shaking incubator. Each sample was diluted from 10^0 through 10^{-6} in 10-fold intervals in PBS. 10 μL of each dilution was plated to TSA and the plates were incubated overnight at 37 °C, followed by CFU enumeration. Data were visualized and statistically analyses were performed in Prism 10.

S16. NMR Spectra of Synthesized Compounds

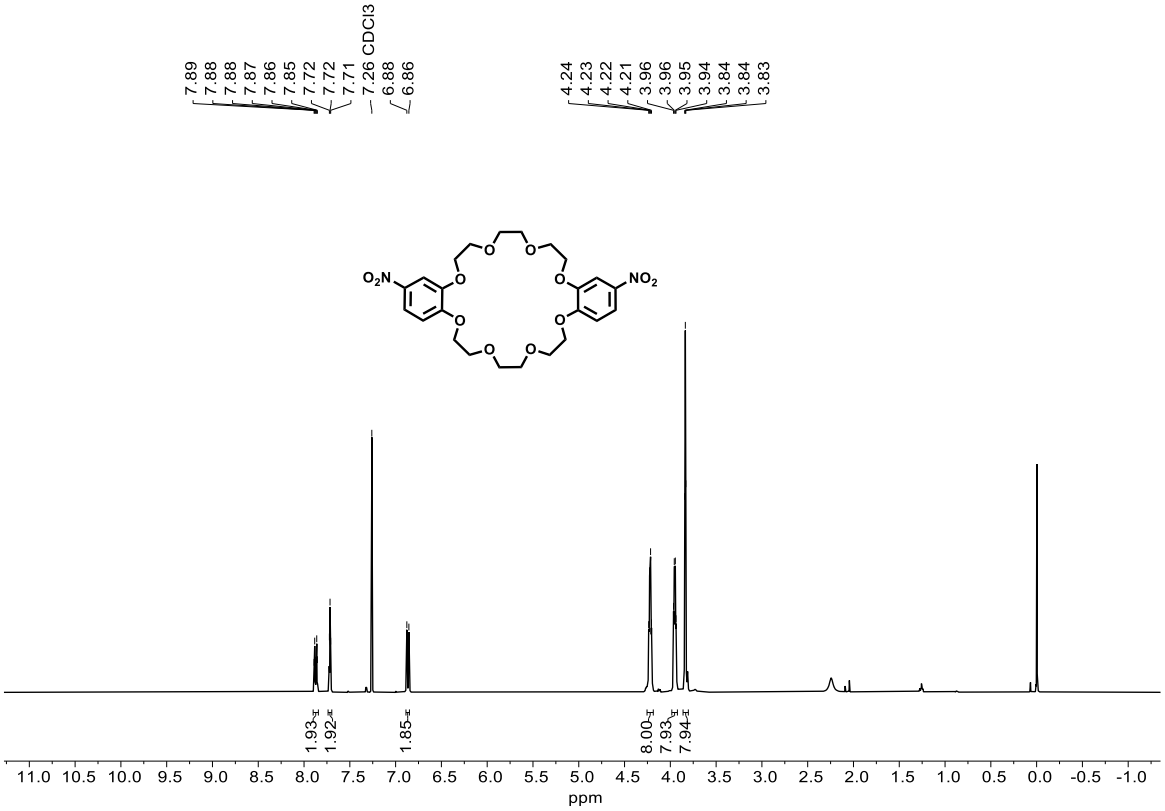


Figure S36. ^{1}H NMR spectrum (400 MHz, *T* = 298 K) of **11** in $CDCl_3$.

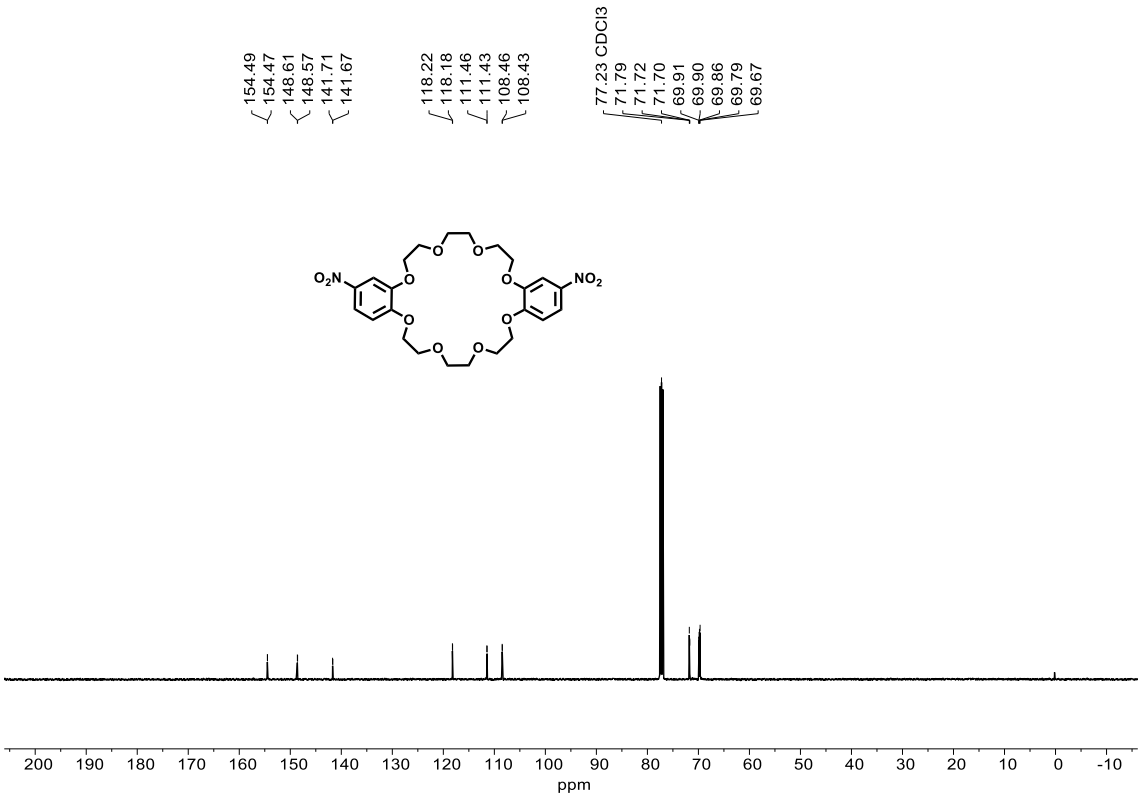


Figure S37. ^{13}C NMR spectrum (126 MHz, *T* = 298 K) of **11** in $CDCl_3$.

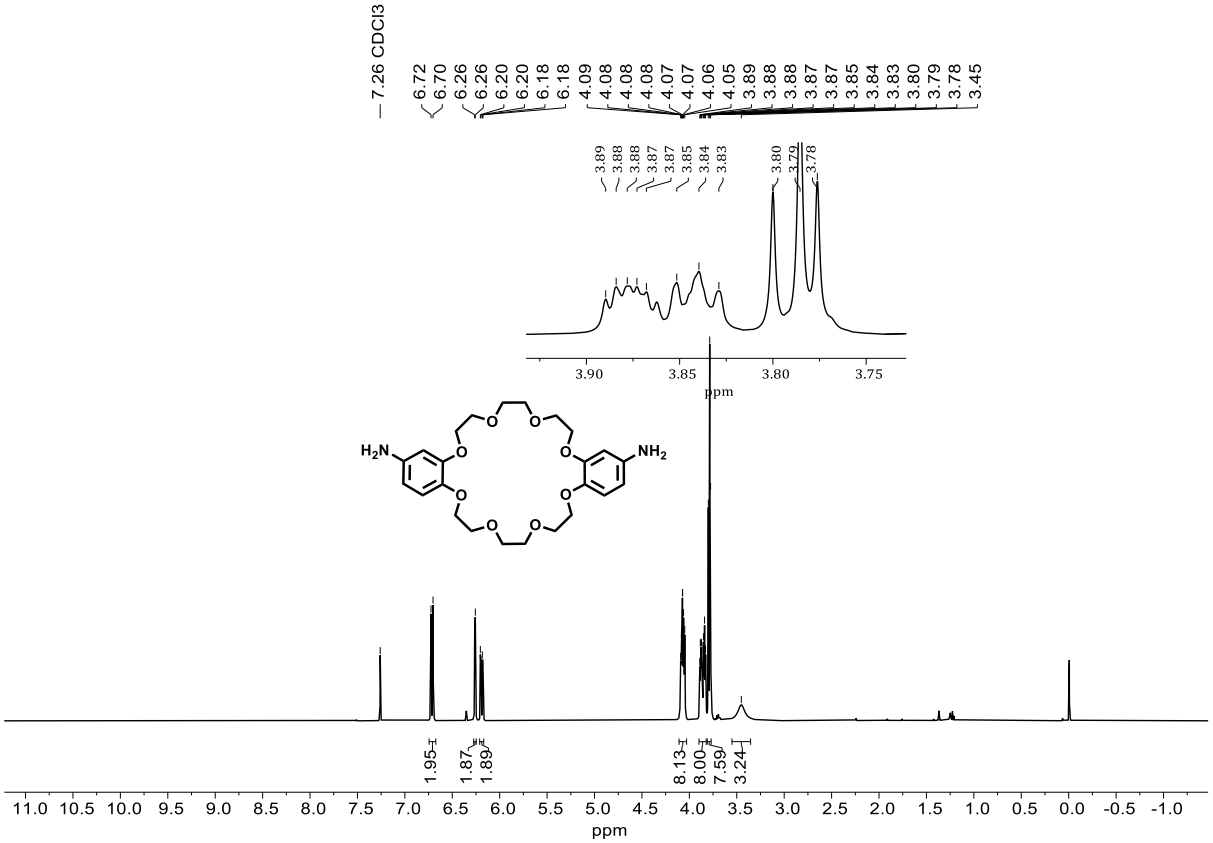


Figure S38. ^{1}H NMR spectrum (400 MHz, *T* = 298 K) of **12** in $CDCl_3$.

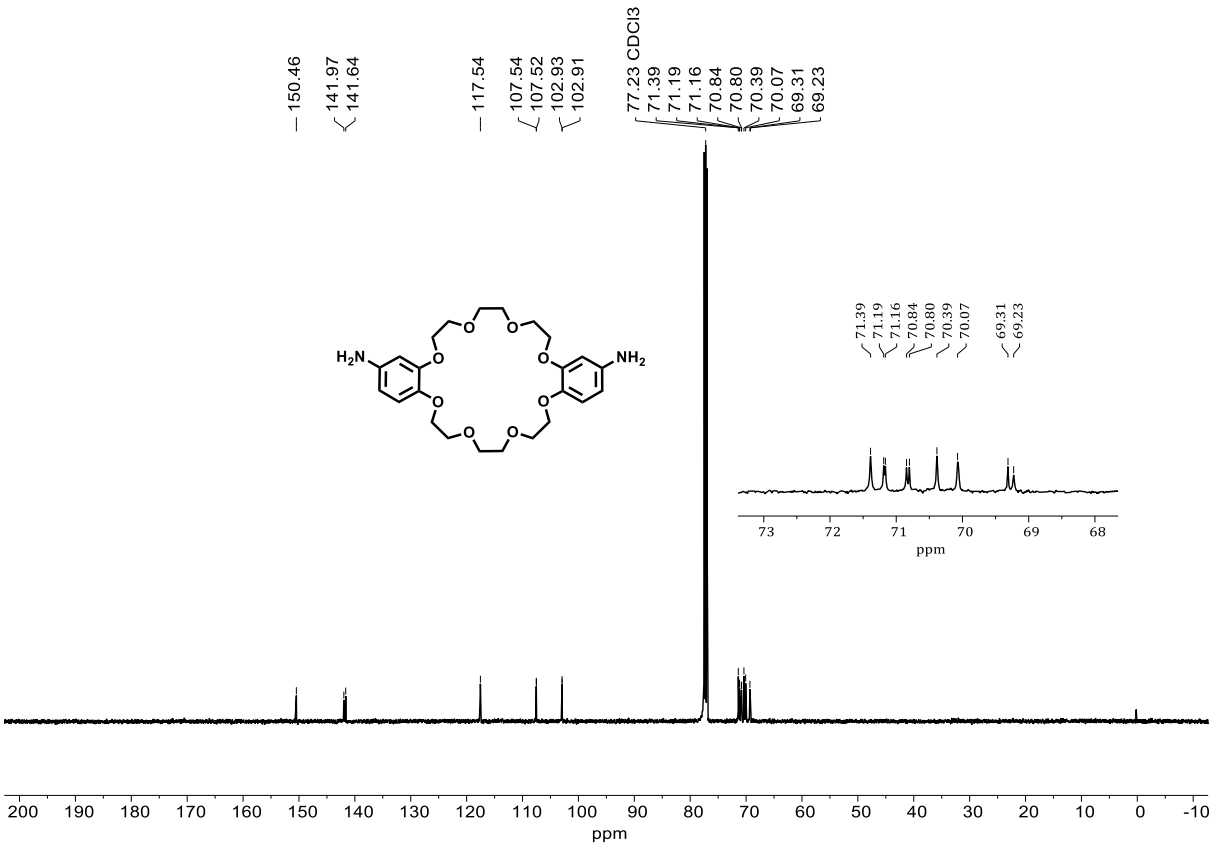


Figure S39. ^{13}C NMR spectrum (126 MHz, *T* = 298 K) of **12** in $CDCl_3$.

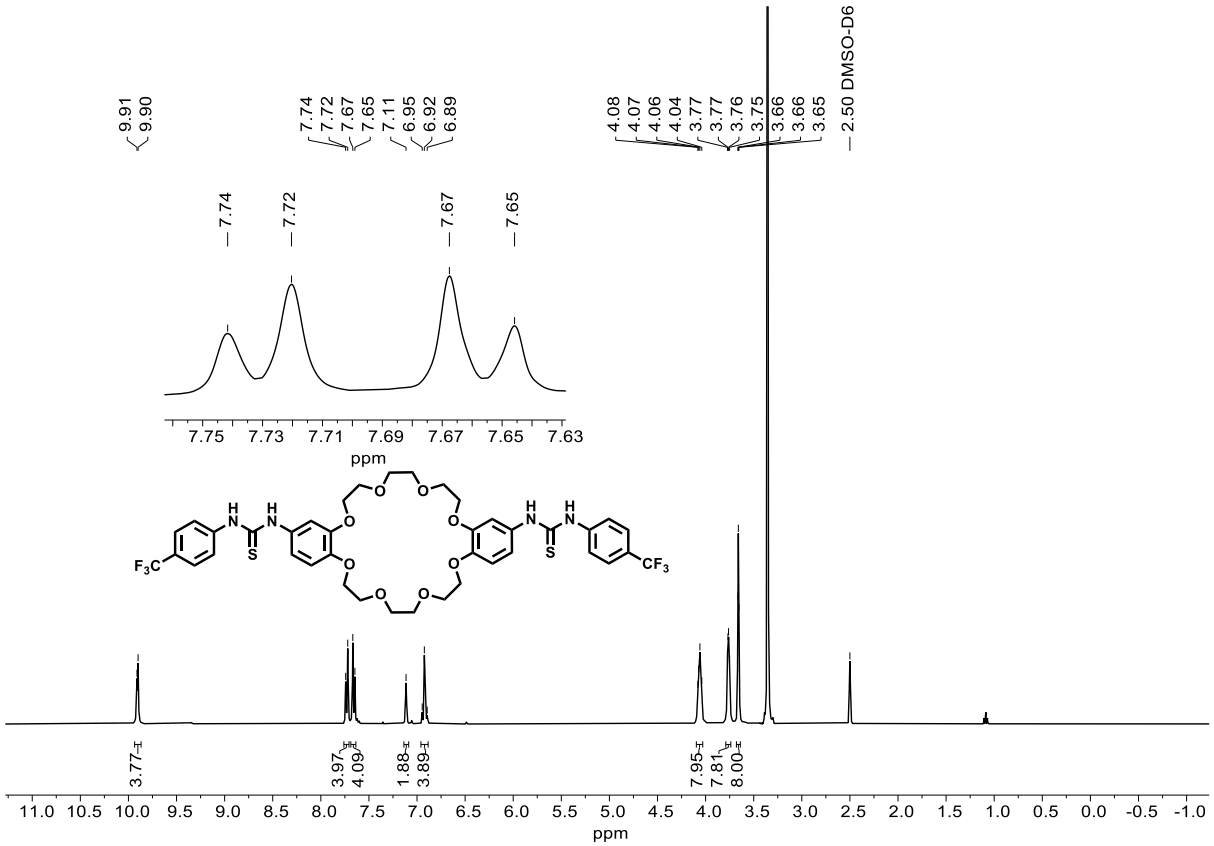


Figure S40. ^{1}H NMR spectrum (400 MHz, T = 298 K) of **5** in DMSO-d_6.

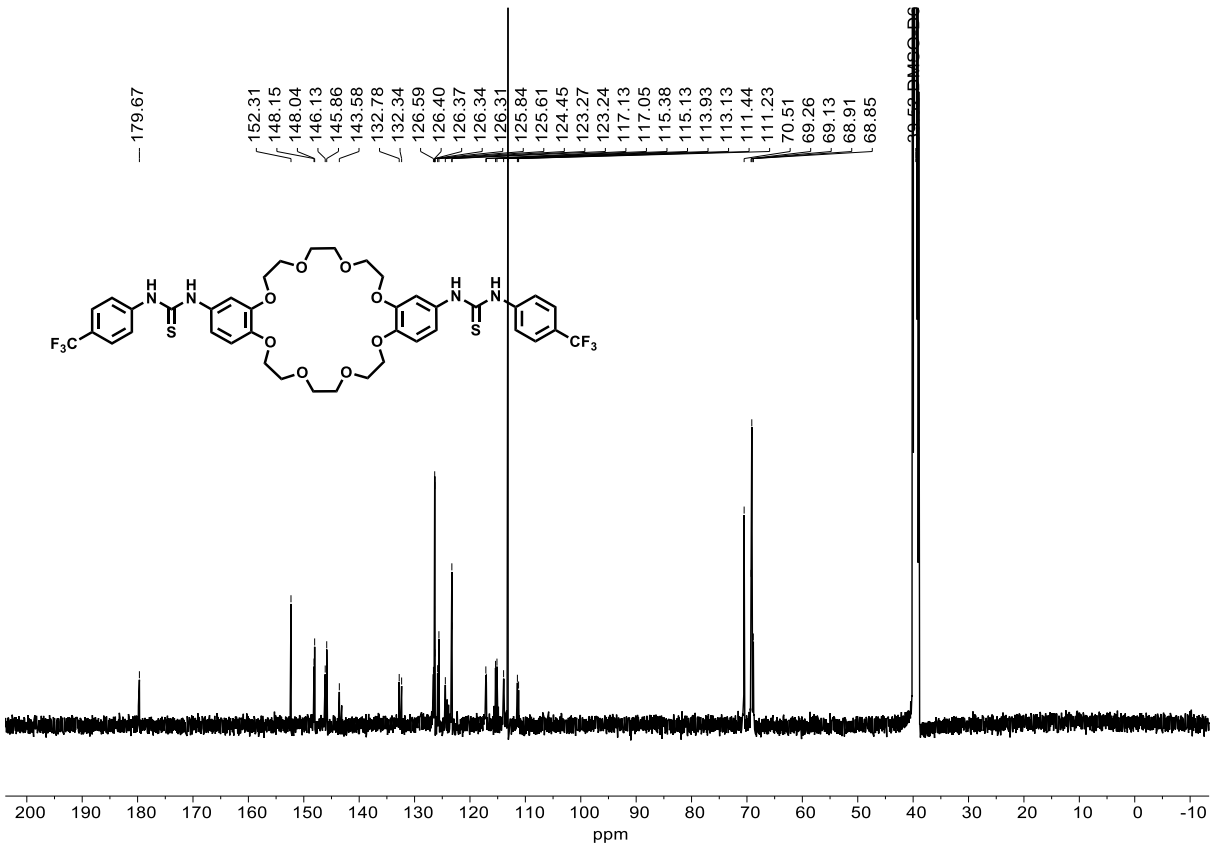


Figure S41. ^{13}C NMR spectrum (126 MHz, T = 298 K) of **5** in DMSO-d_6.

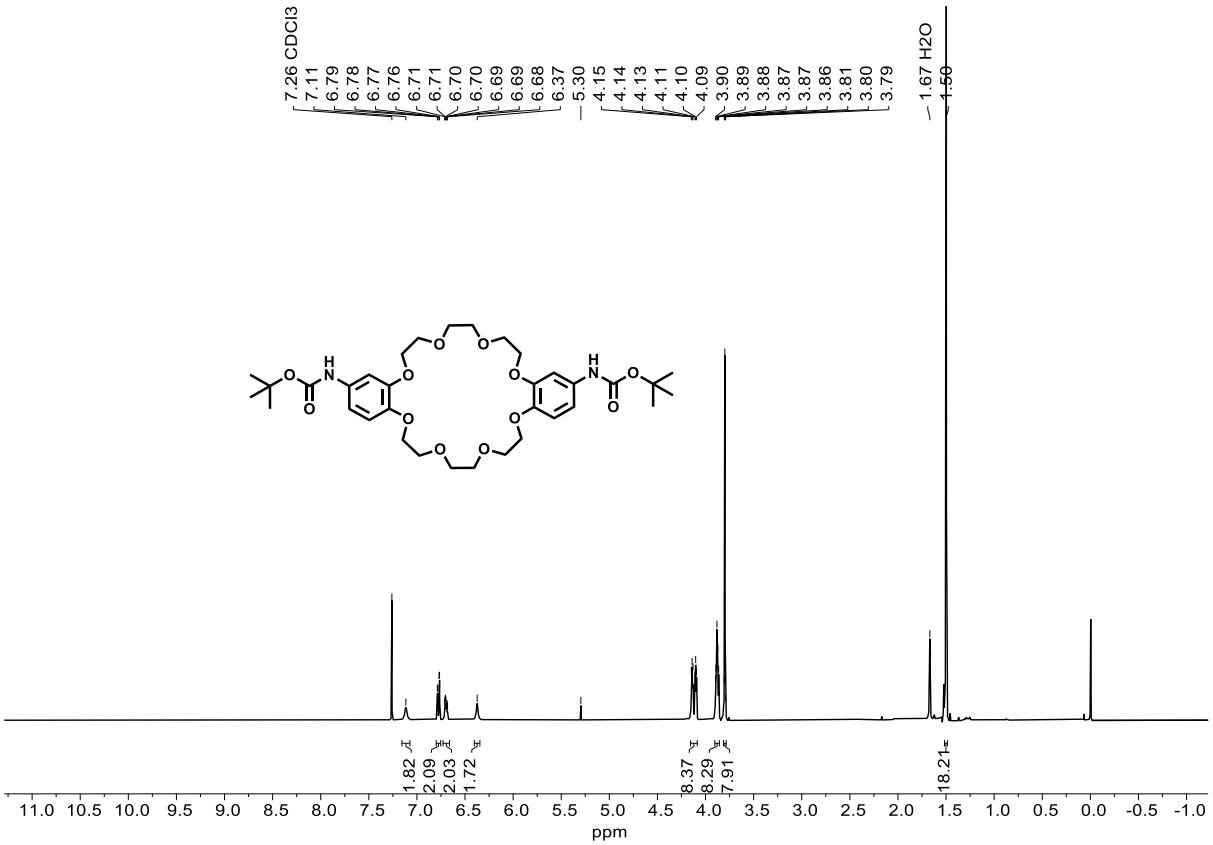


Figure S42. ^{1}H NMR spectrum (500 MHz, *T* = 298 K) of **6** in $CDCl_3$.

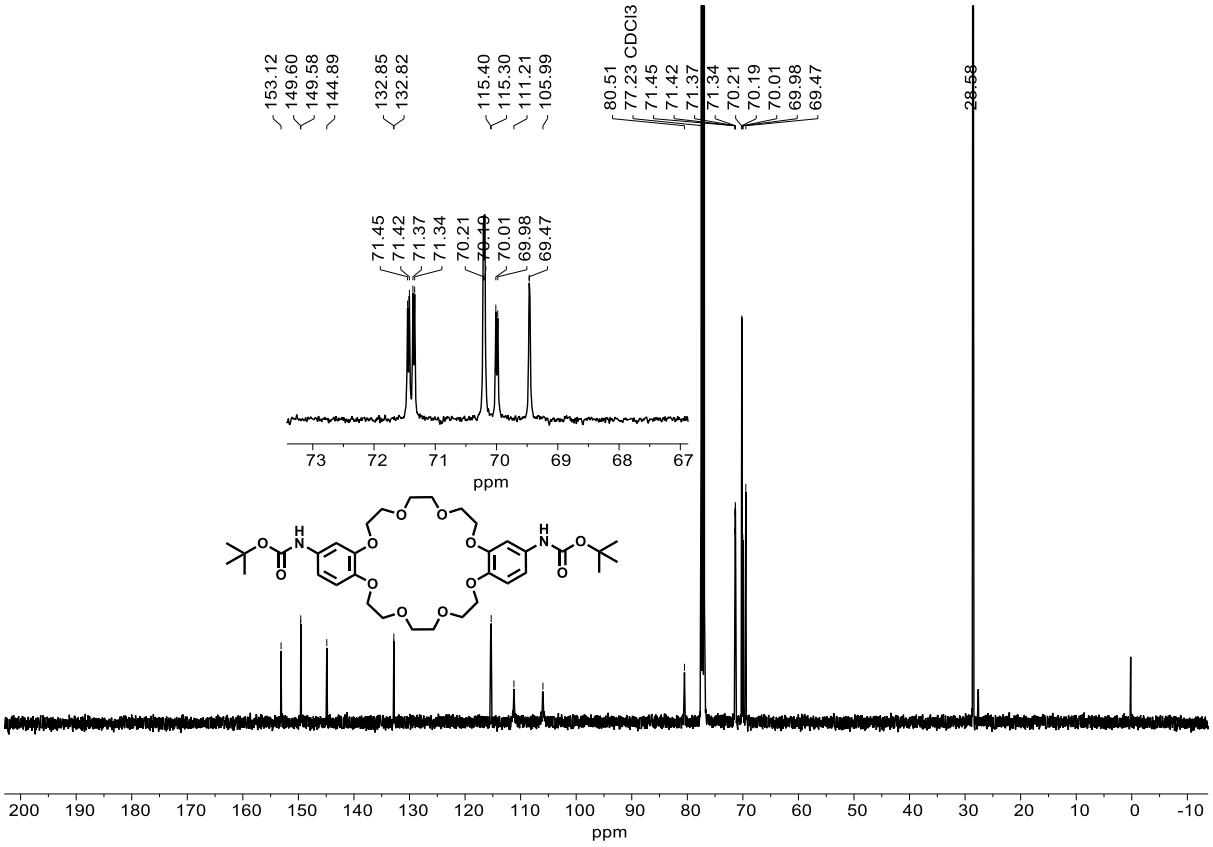


Figure S43. ^{13}C NMR spectrum (400 MHz, *T* = 298 K) of **6** in $CDCl_3$.

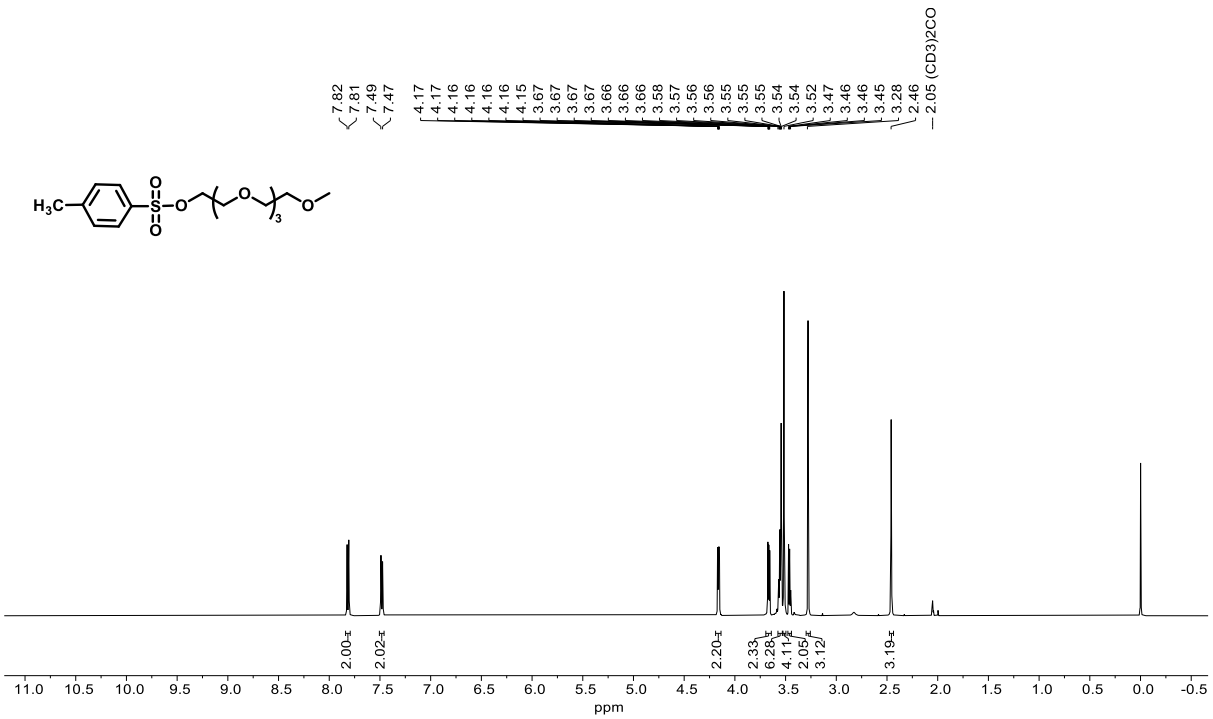


Figure 44. ^{1}H NMR spectrum (500 MHz, T = 298 K) of **14** in $(CD_3)_2CO$.

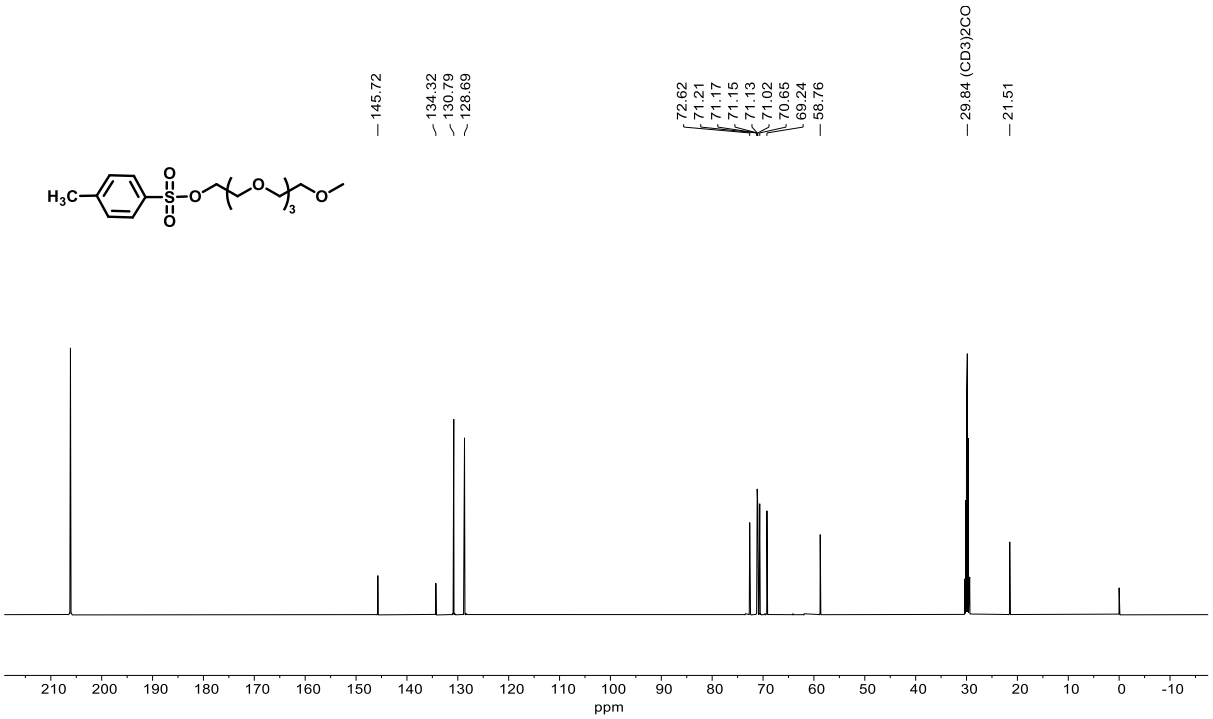


Figure S45. ^{13}C NMR spectrum (126 MHz, T = 298 K) of **14** in $(CD_3)_2CO$.

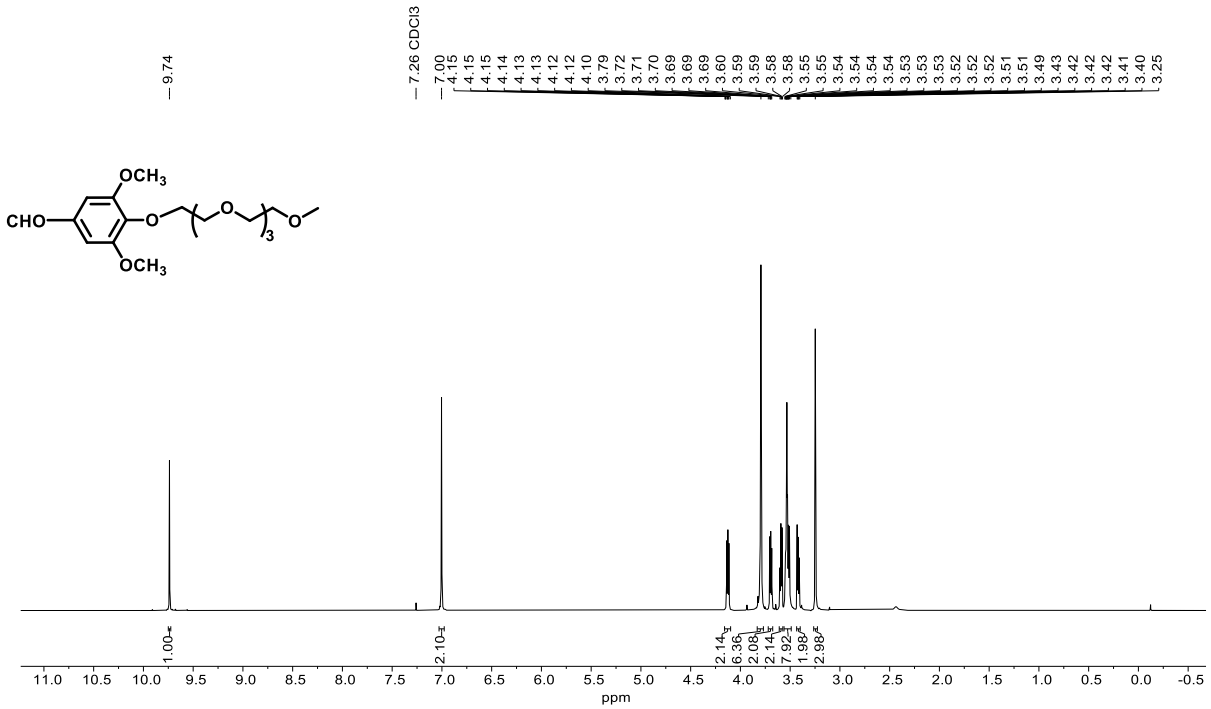


Figure S46. ^{1}H NMR spectrum (500 MHz, *T* = 298 K) of **15** in $CDCl_3$.

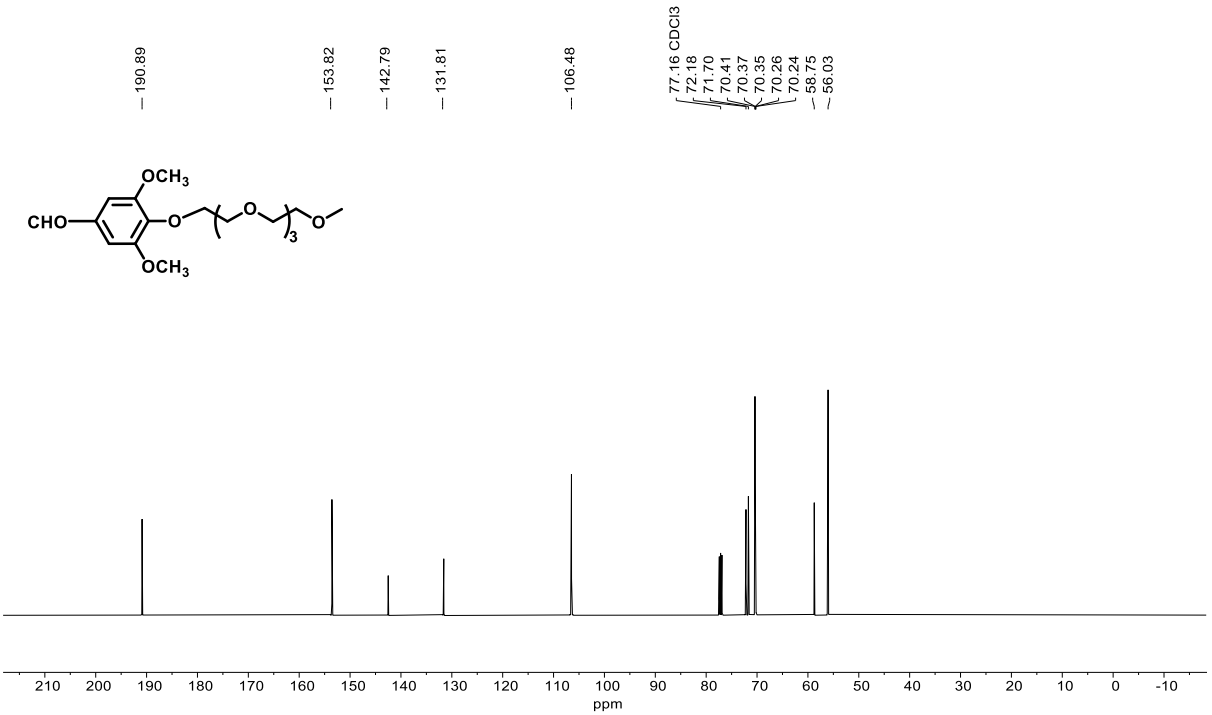


Figure S47. ^{13}C NMR spectrum (126 MHz, *T* = 298 K) of **15** in $CDCl_3$.

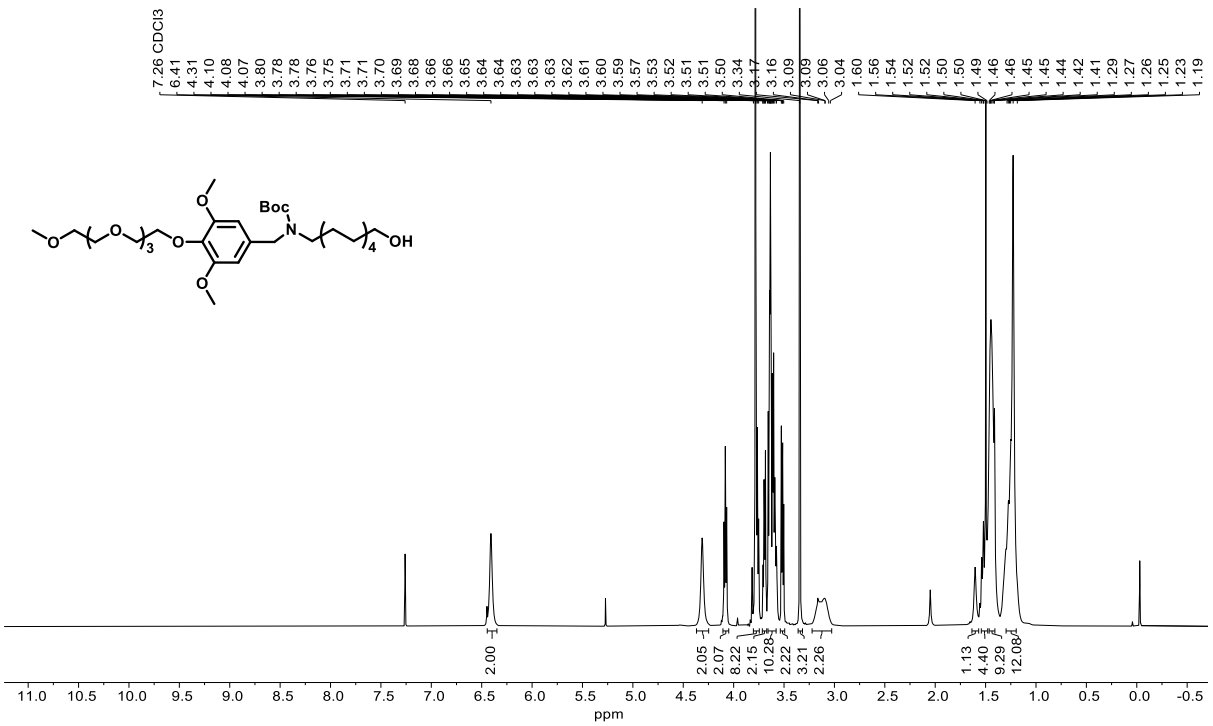


Figure S48. ^{1}H NMR spectrum (400 MHz, *T* = 298 K) of **16** in $CDCl_3$.

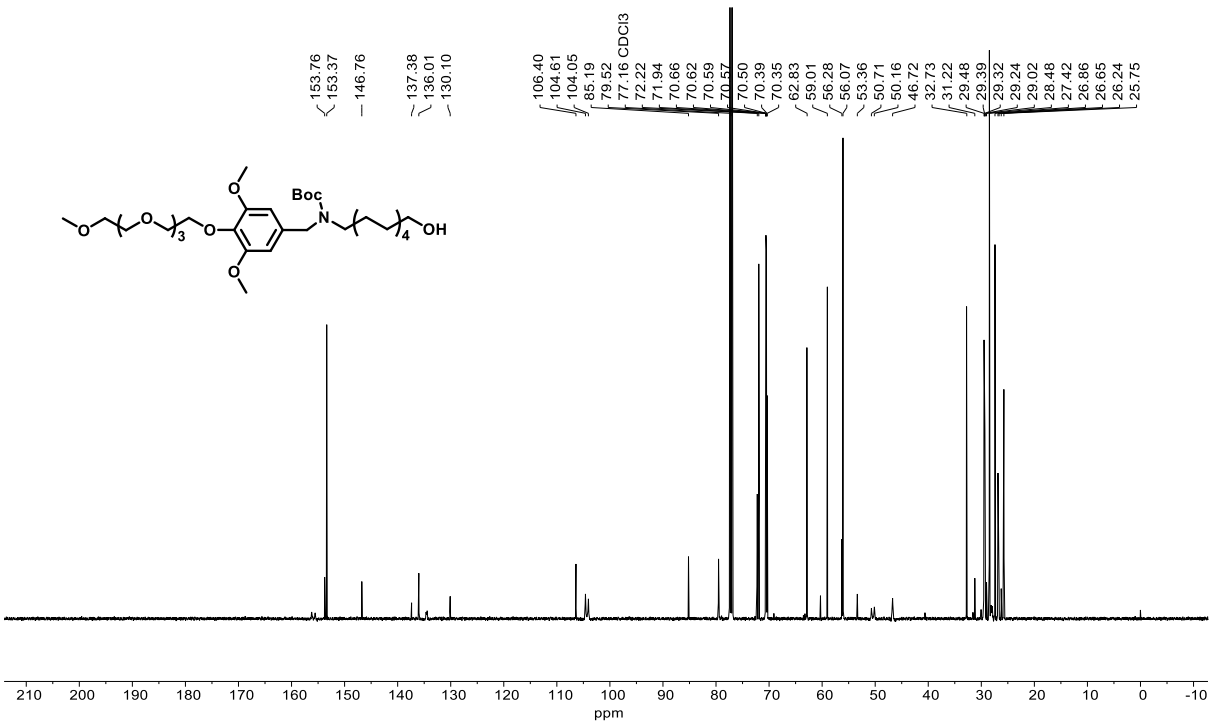


Figure S49. ^{13}C NMR spectrum (126 MHz, *T* = 298 K) of **16** in $CDCl_3$.

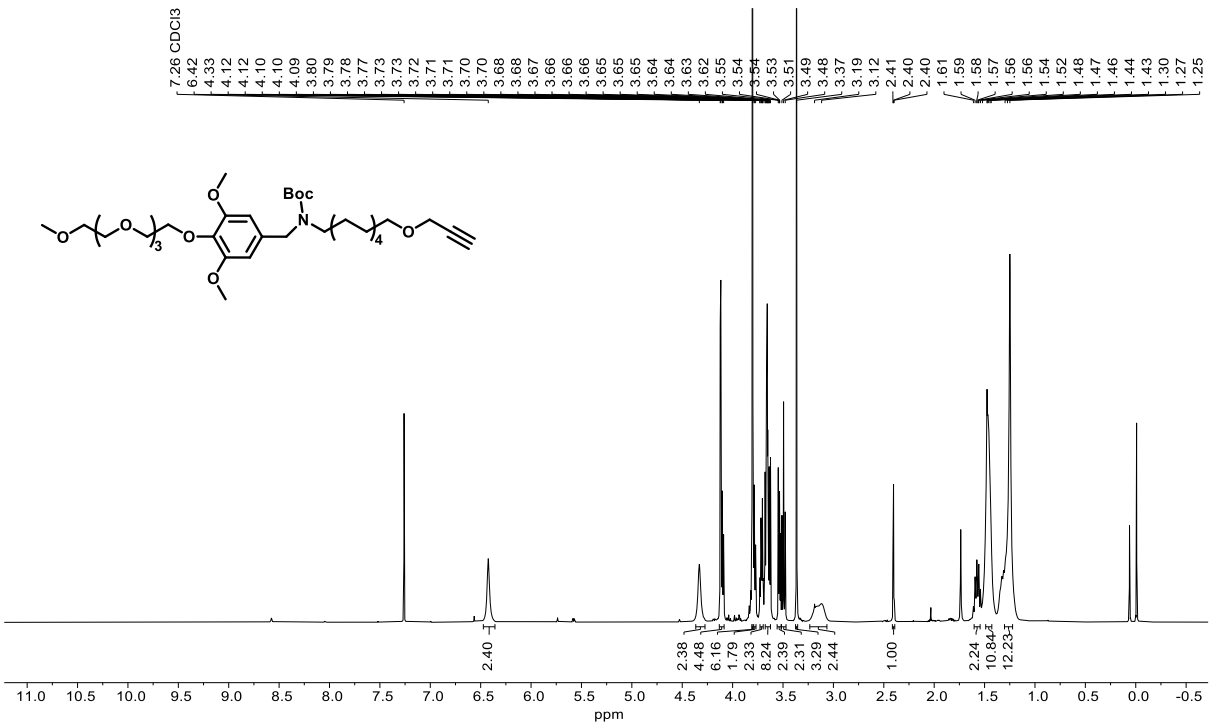


Figure S50. ^{1}H NMR spectrum (400 MHz, *T* = 298 K) of **17** in $CDCl_3$.

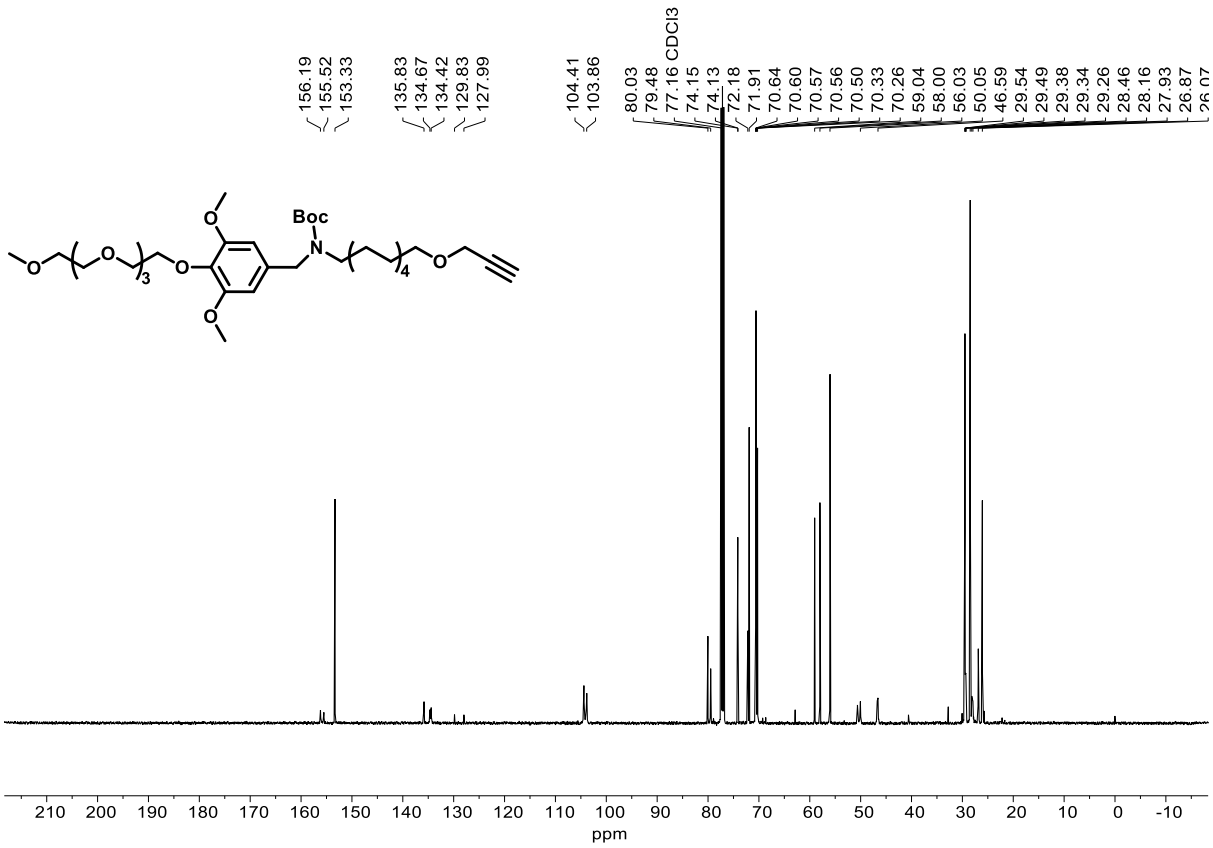


Figure S51. ^{13}C NMR spectrum (126 MHz, *T* = 298 K) of **17** in $CDCl_3$.

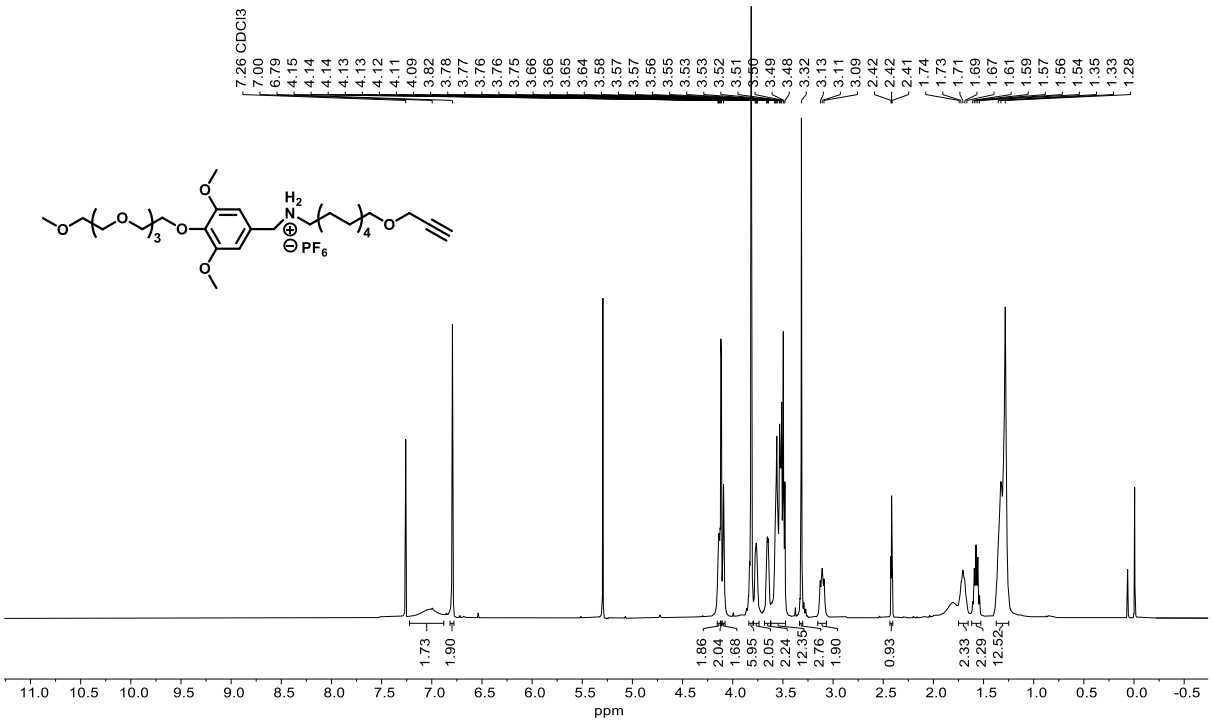


Figure S52. ^{1}H NMR spectrum (400 MHz, T = 298 K) of **7** in $CDCl_3$.

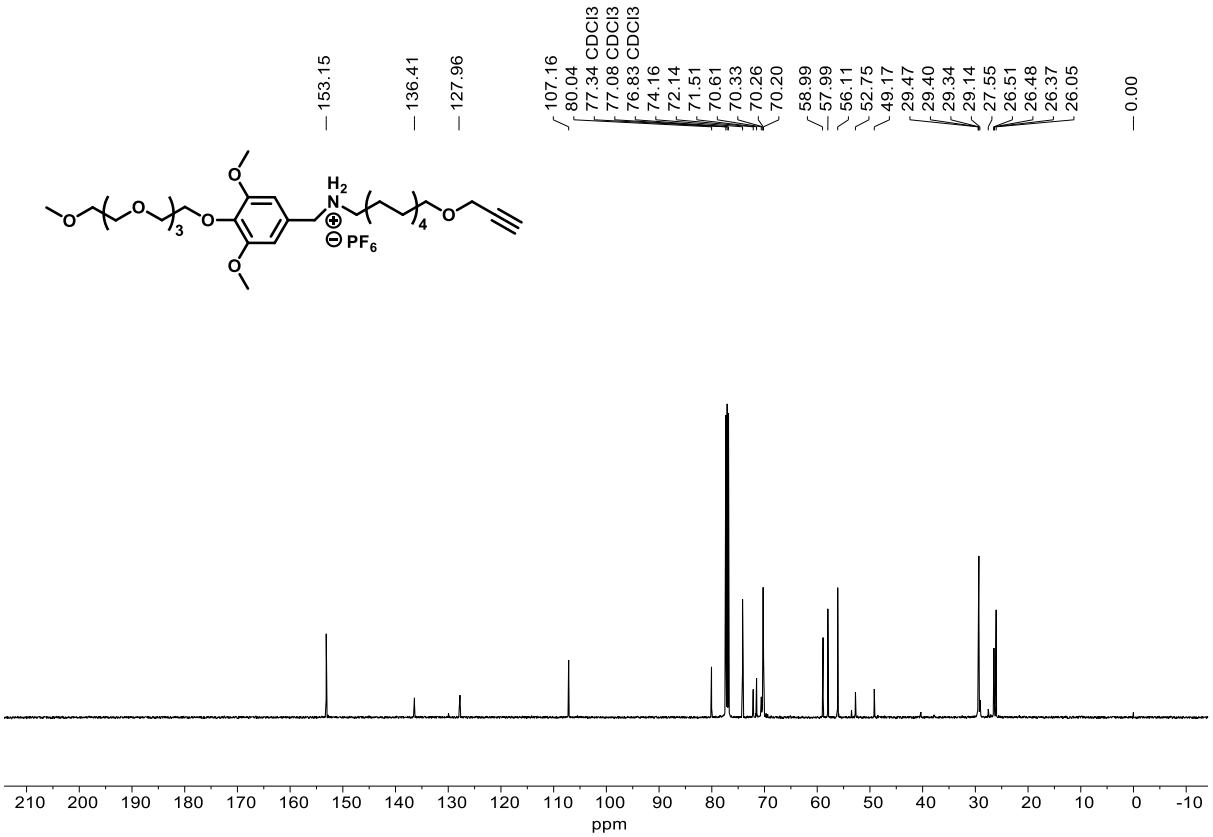


Figure S53. ^{13}C NMR spectrum (126 MHz, T = 298 K) of **7** in $CDCl_3$.

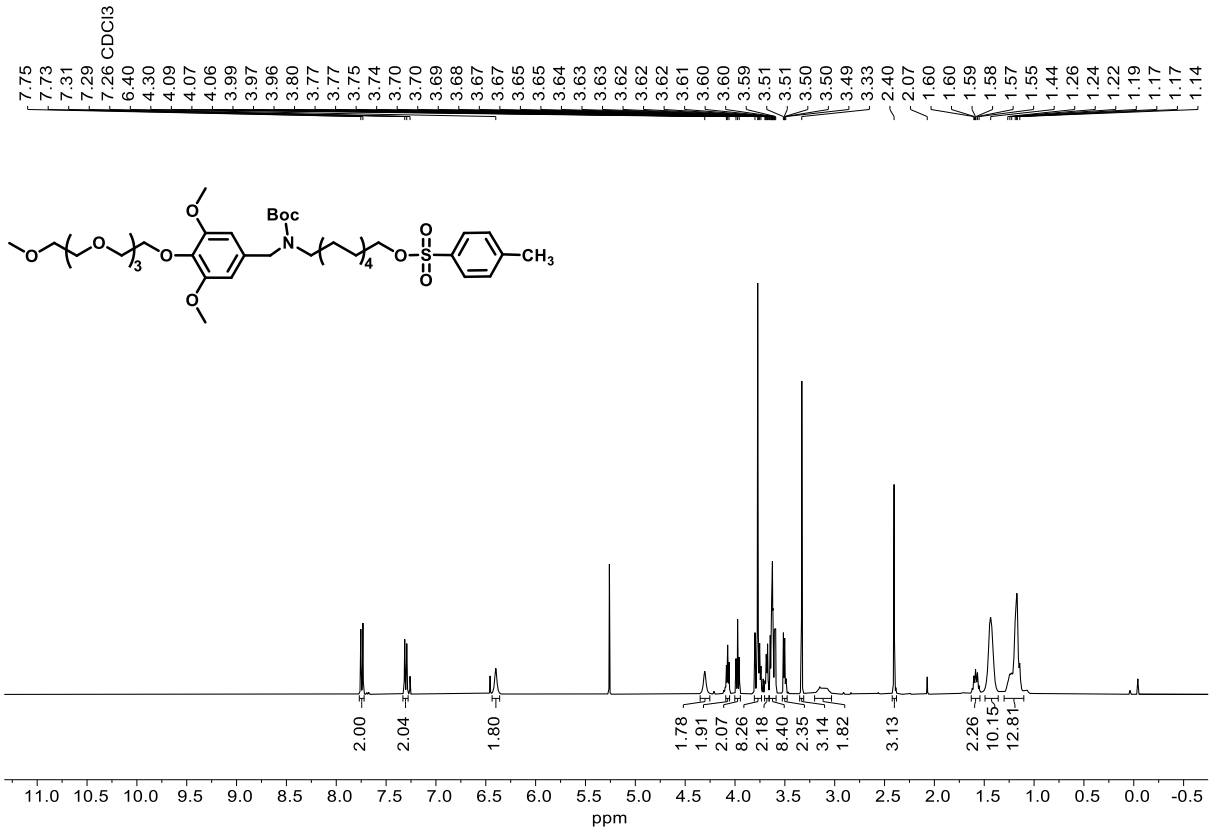


Figure S54. ^{1}H NMR spectrum (400 MHz, *T* = 298 K) of **18** in $CDCl_3$.

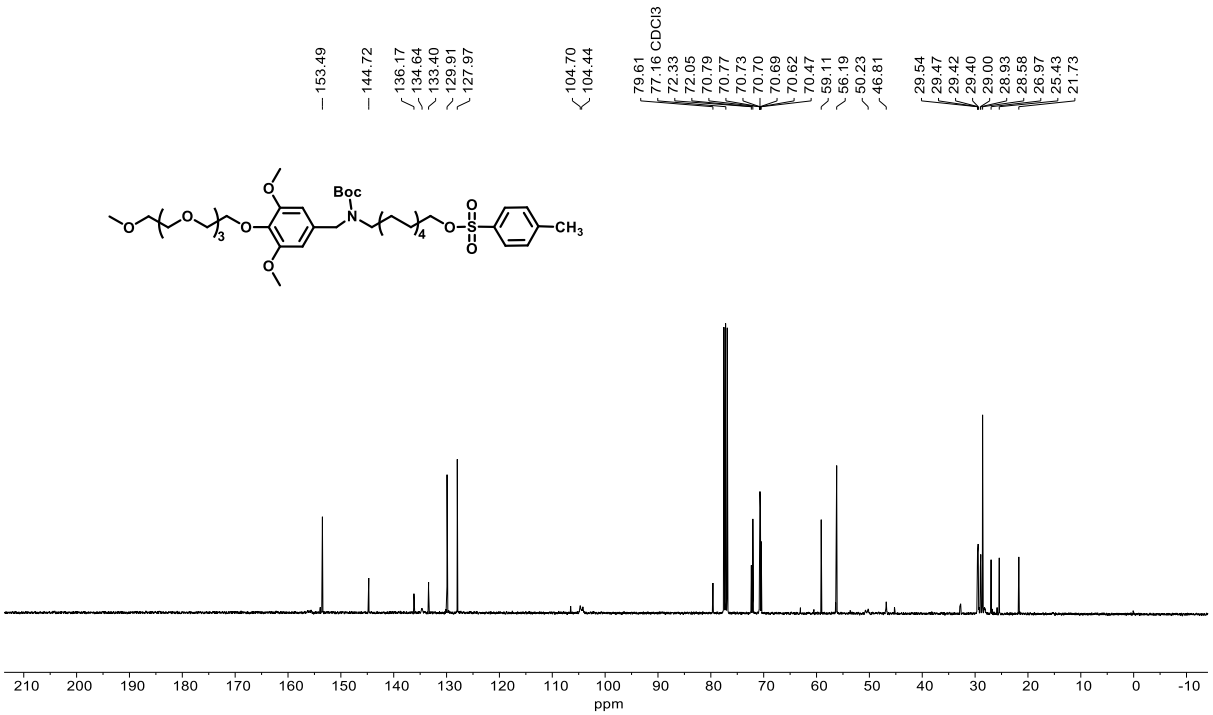


Figure S55. ^{13}C NMR spectrum (101 MHz, *T* = 298 K) of **18** in $CDCl_3$.

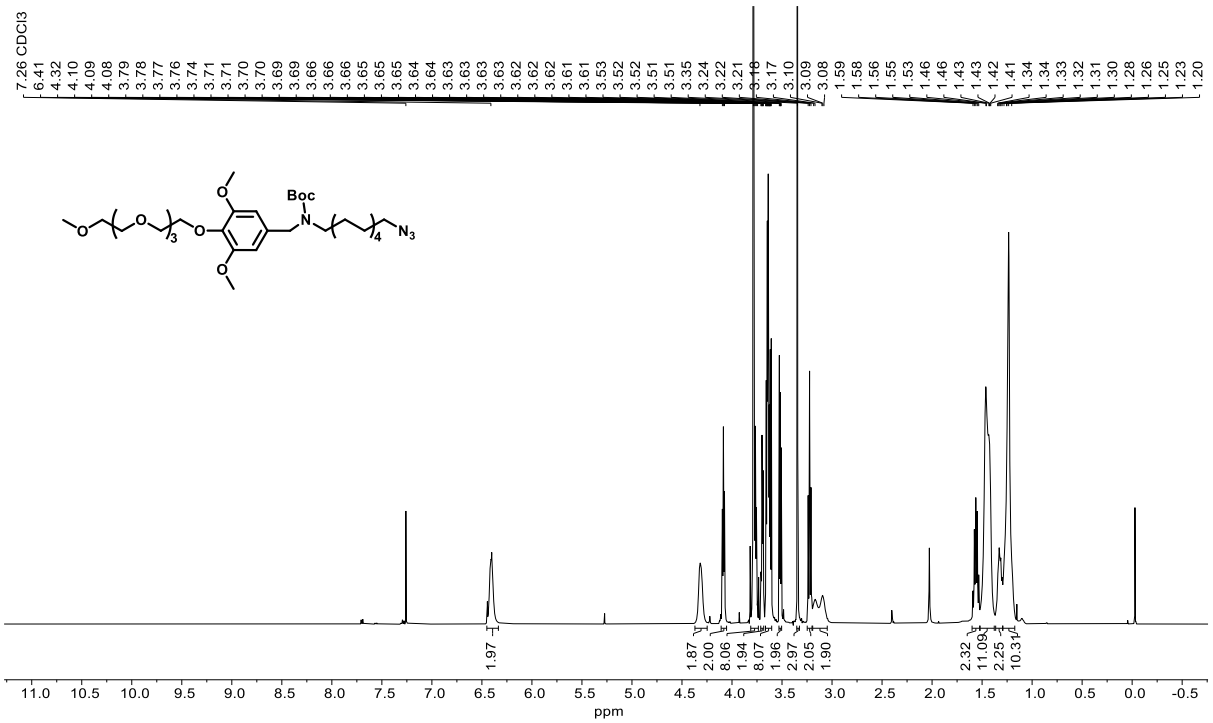


Figure S56. ^{1}H NMR spectrum (500 MHz, T = 298 K) of **19** in $CDCl_3$.

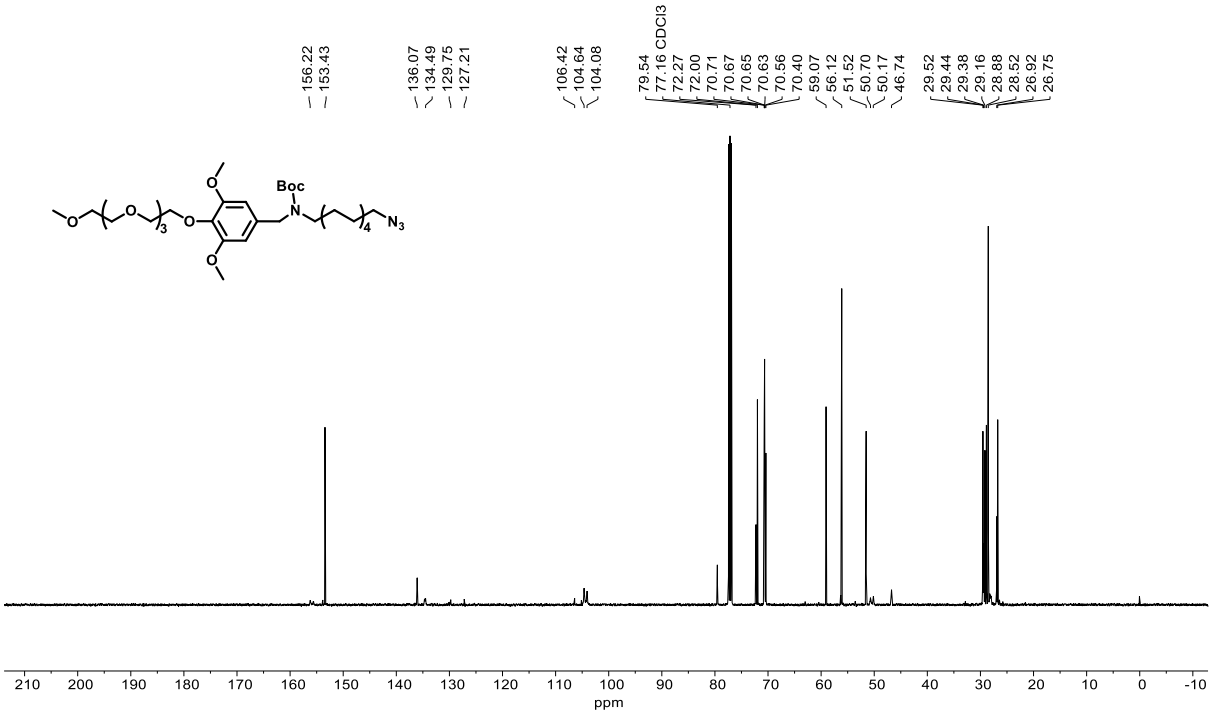


Figure S57. ^{13}C NMR spectrum (126 MHz, T = 298 K) of **19** in $CDCl_3$.

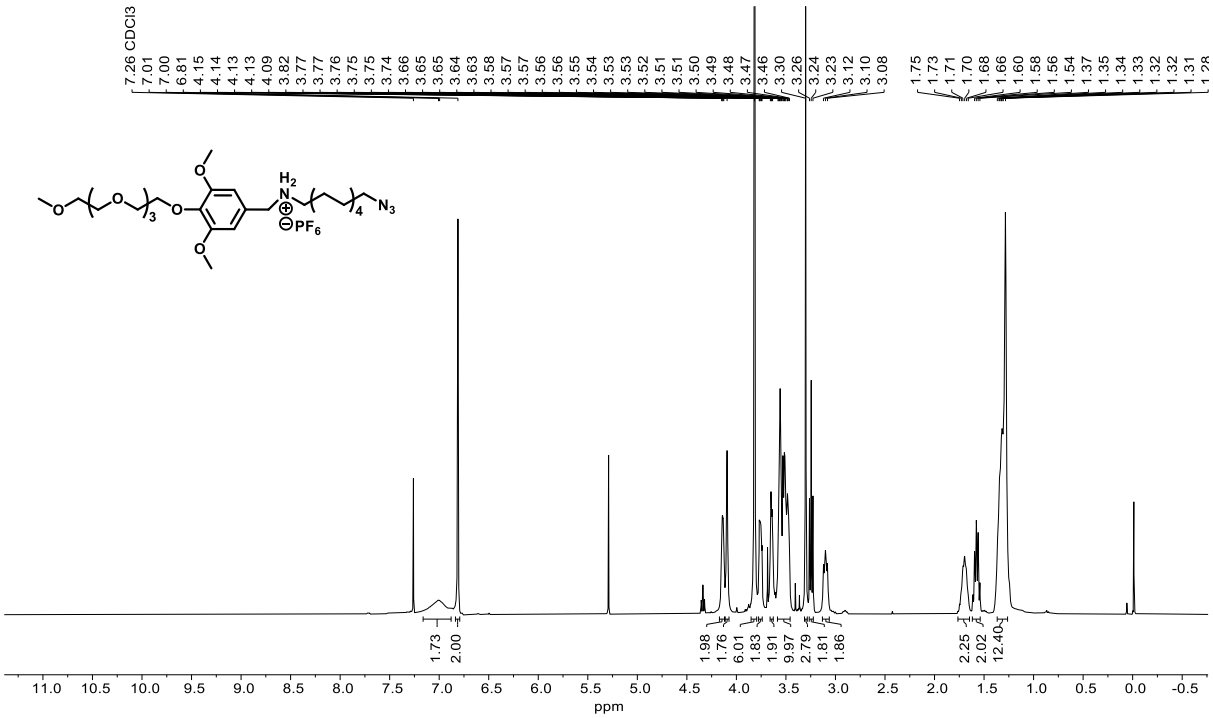


Figure S58. ^{1}H NMR spectrum (400 MHz, *T* = 298 K) of **8** in $CDCl_3$.

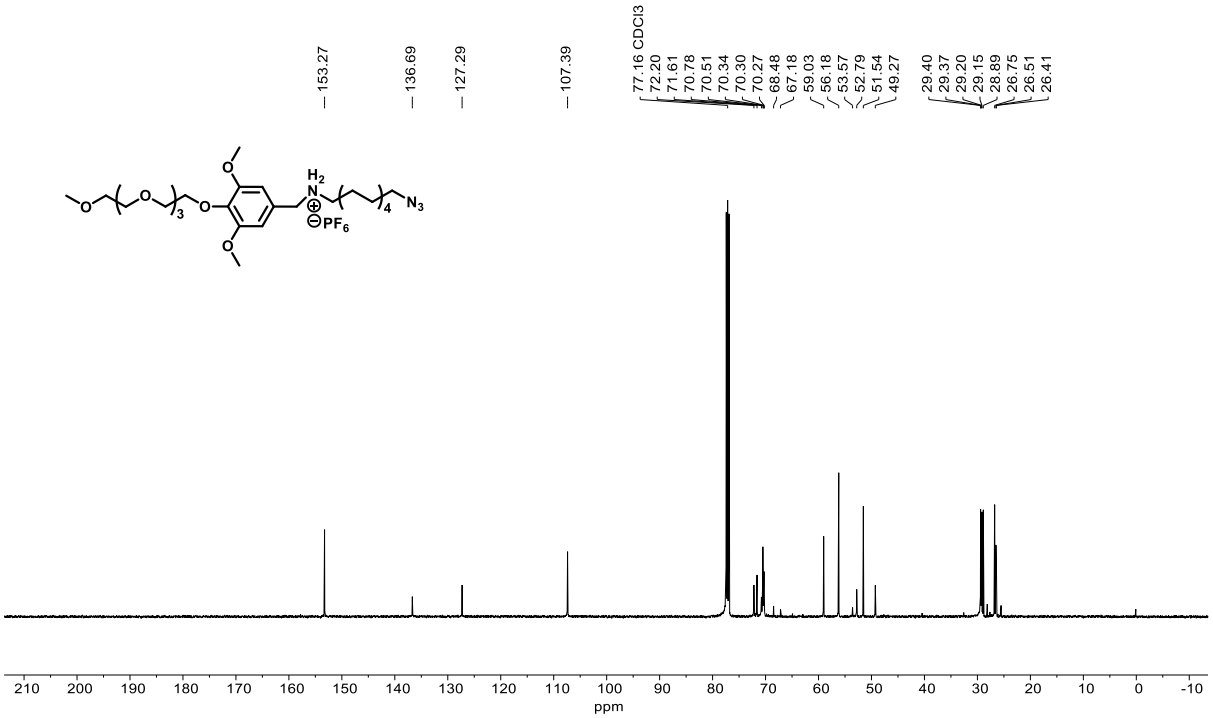


Figure S59. ^{13}C NMR spectrum (126 MHz, *T* = 298 K) of **8** in $CDCl_3$.

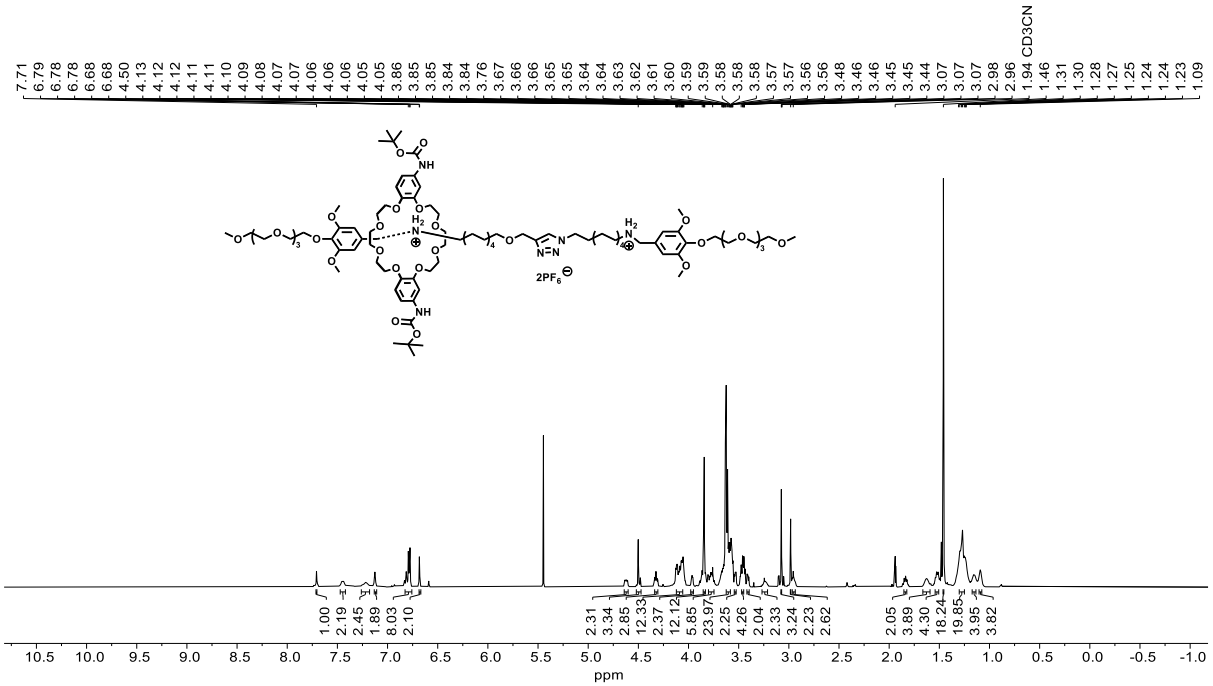


Figure S60. ^{1}H NMR spectrum (500 MHz, T = 298 K) of **9** in CD_3CN.

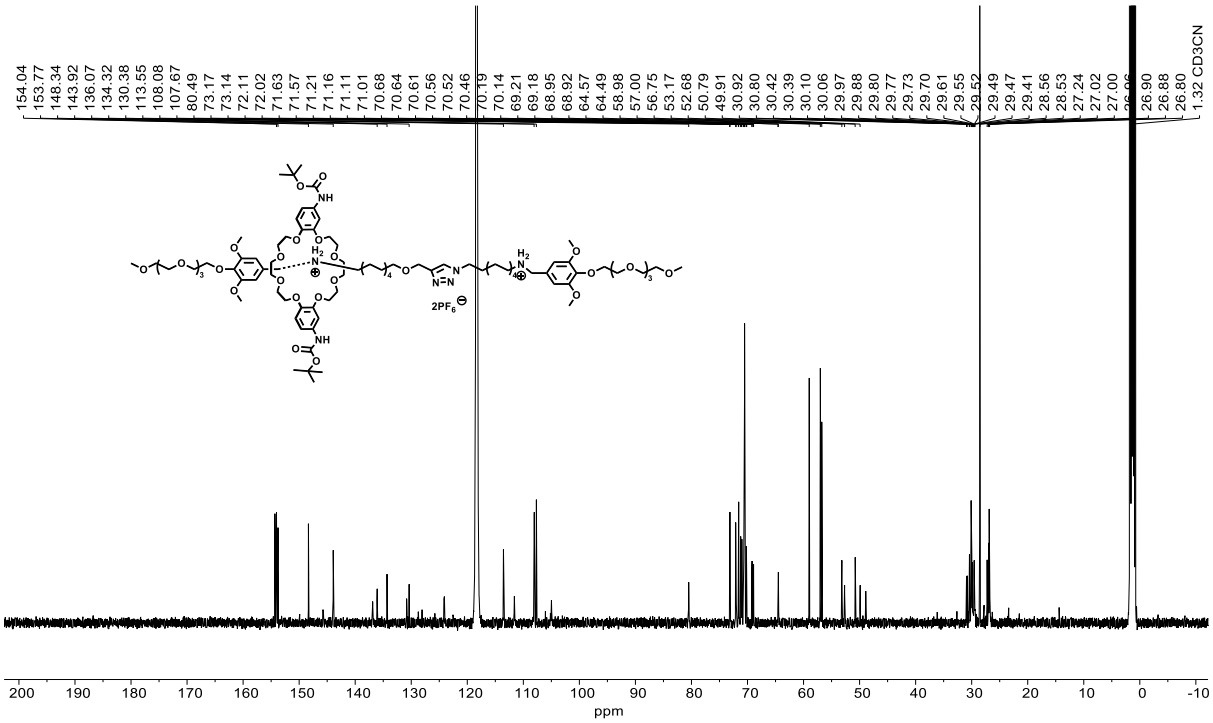


Figure S61. ^{13}C NMR spectrum (126 MHz, T = 298 K) of **9** in CD_3CN.

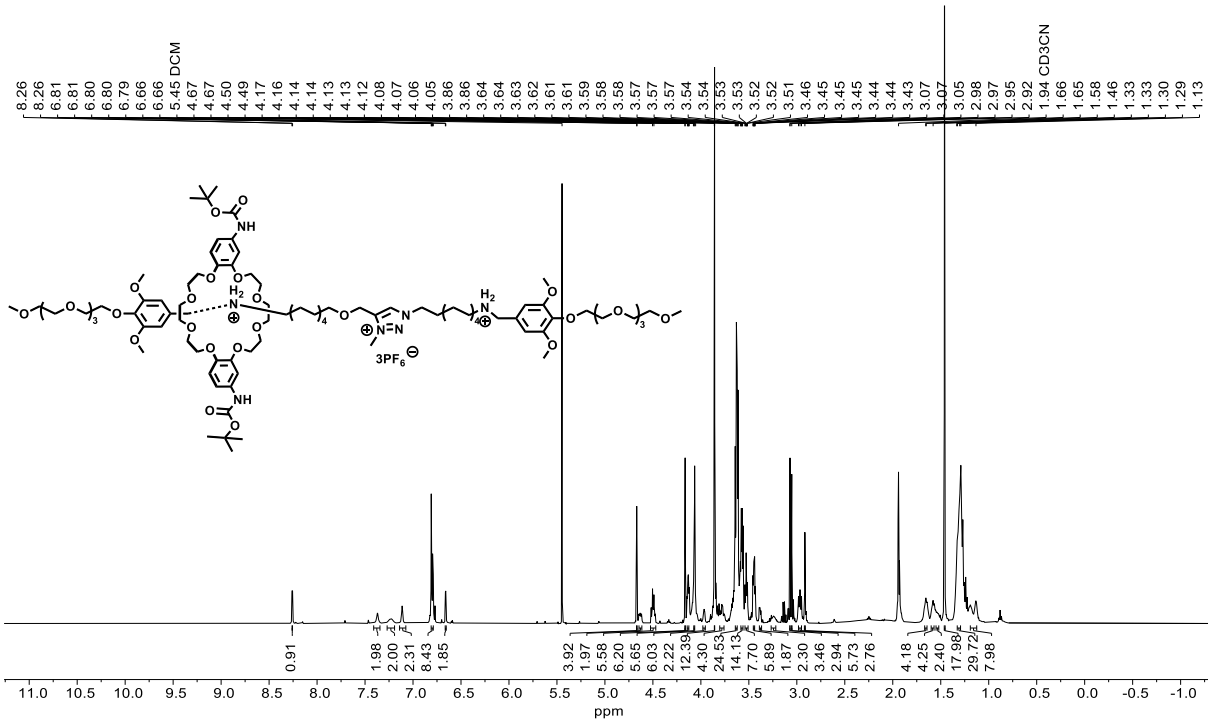


Figure S62. ^{1}H NMR spectrum (500 MHz, *T* = 298 K) of **20** in CD_3CN.

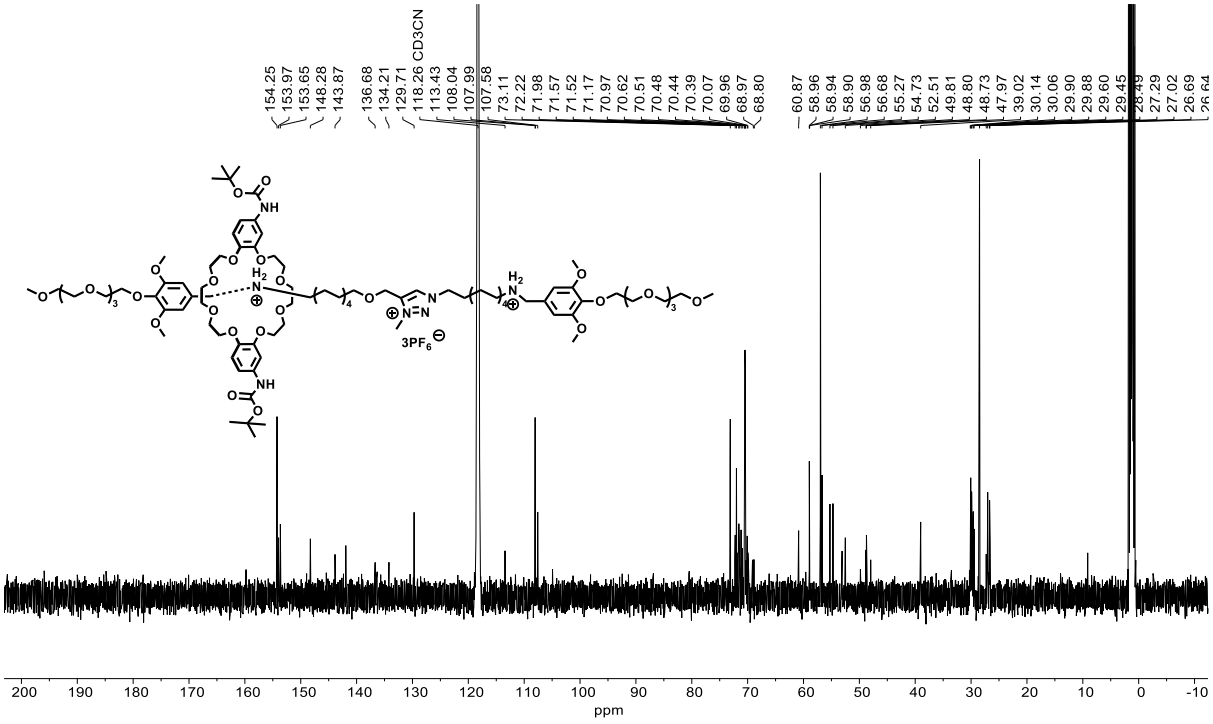


Figure S63. ^{13}C NMR spectrum (126 MHz, *T* = 298 K) of **20** in CD_3CN.

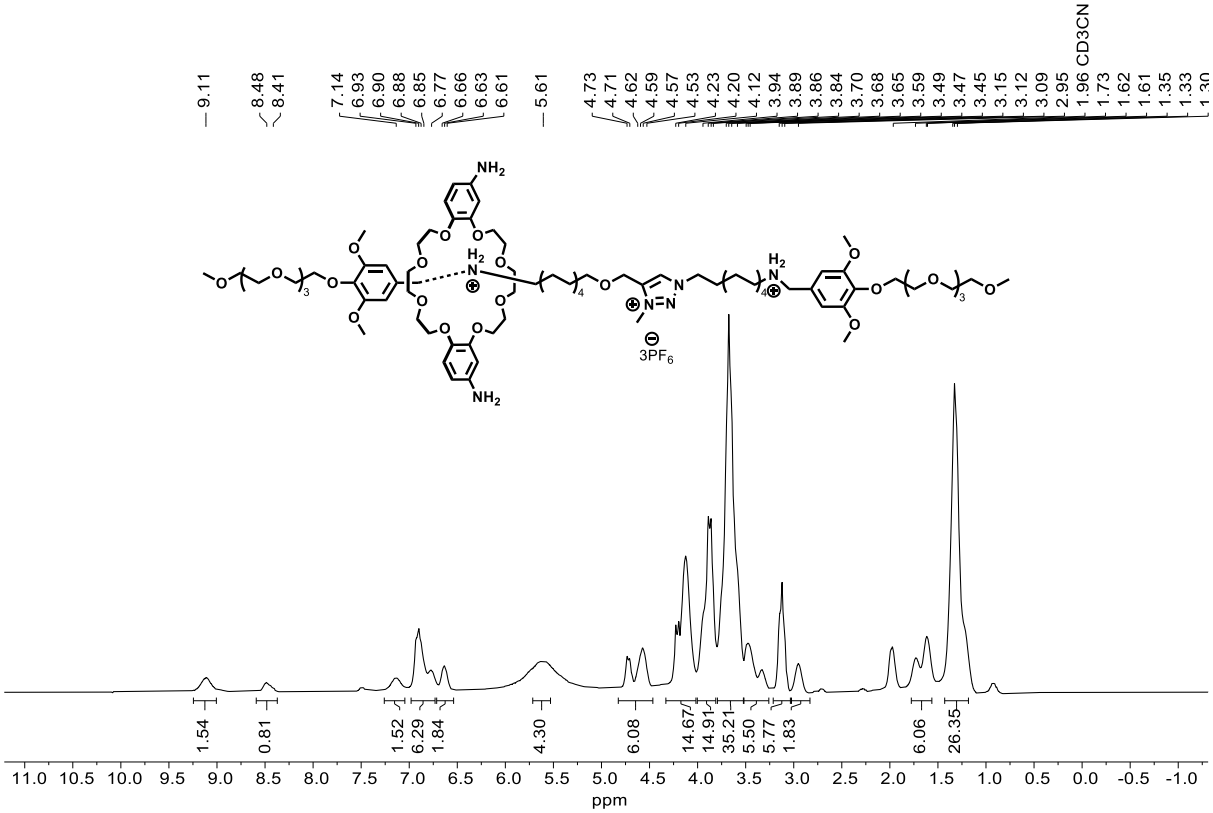


Figure S64. ^{1}H NMR spectrum (400 MHz, *T* = 298 K) of **21** in CD_3CN.

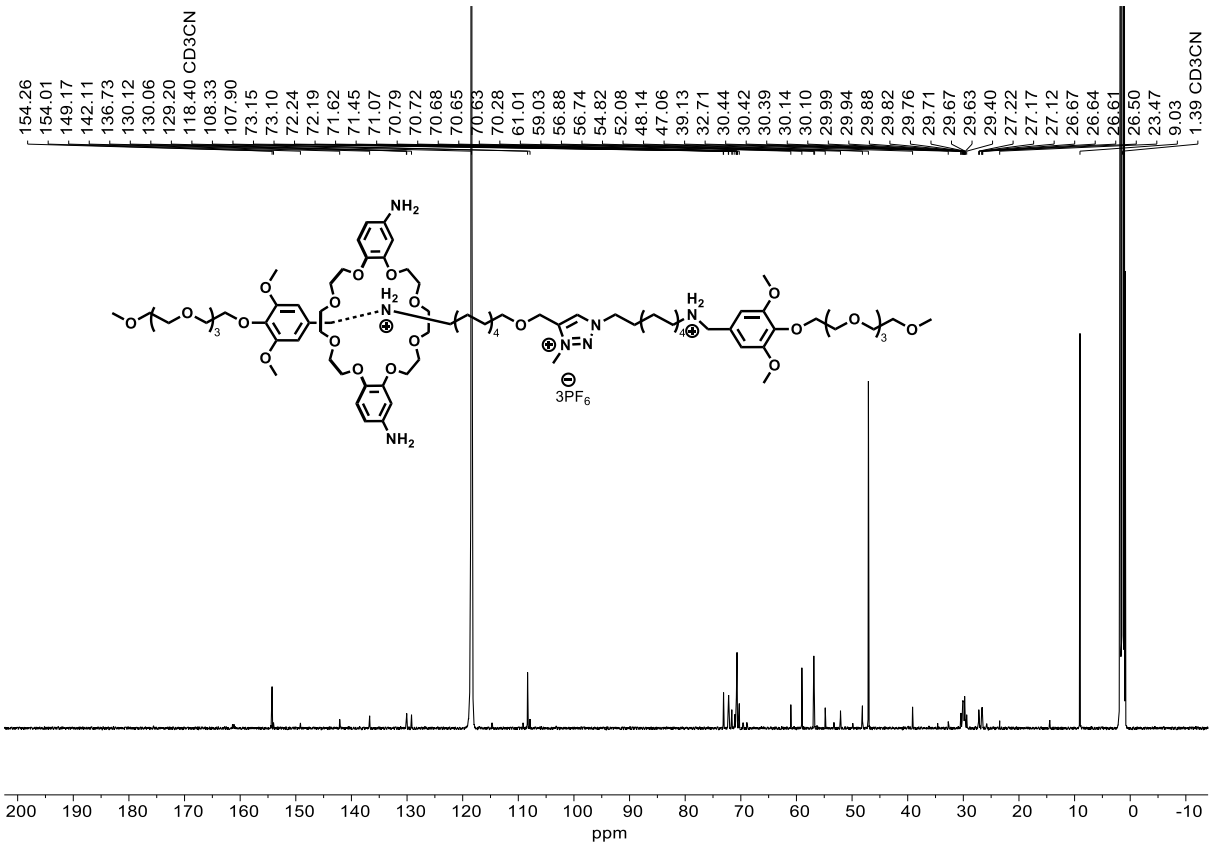


Figure S65. ^{13}C NMR spectrum (126 MHz, *T* = 298 K) of **21** in CD_3CN.

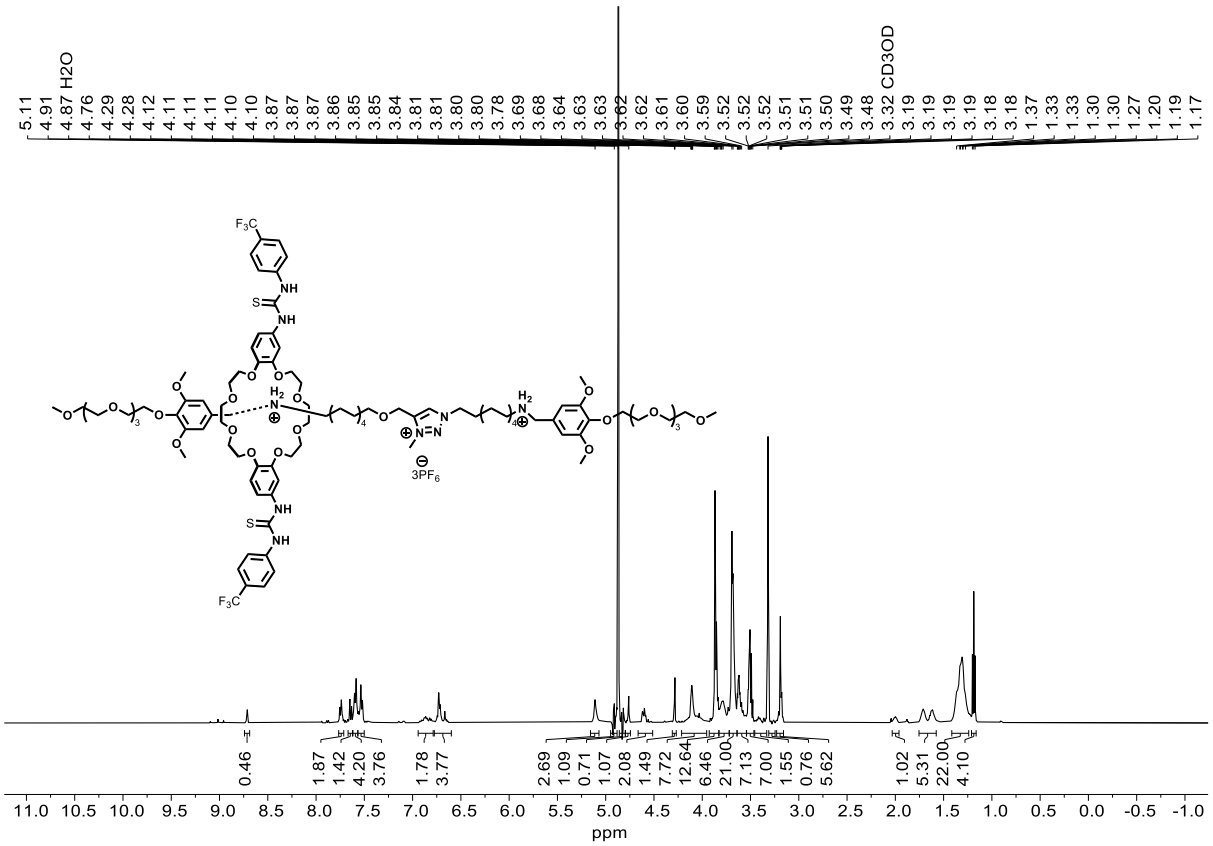


Figure S66. ^{1}H NMR spectrum (500 MHz, *T* = 298 K) of **1** in CD_3OD.

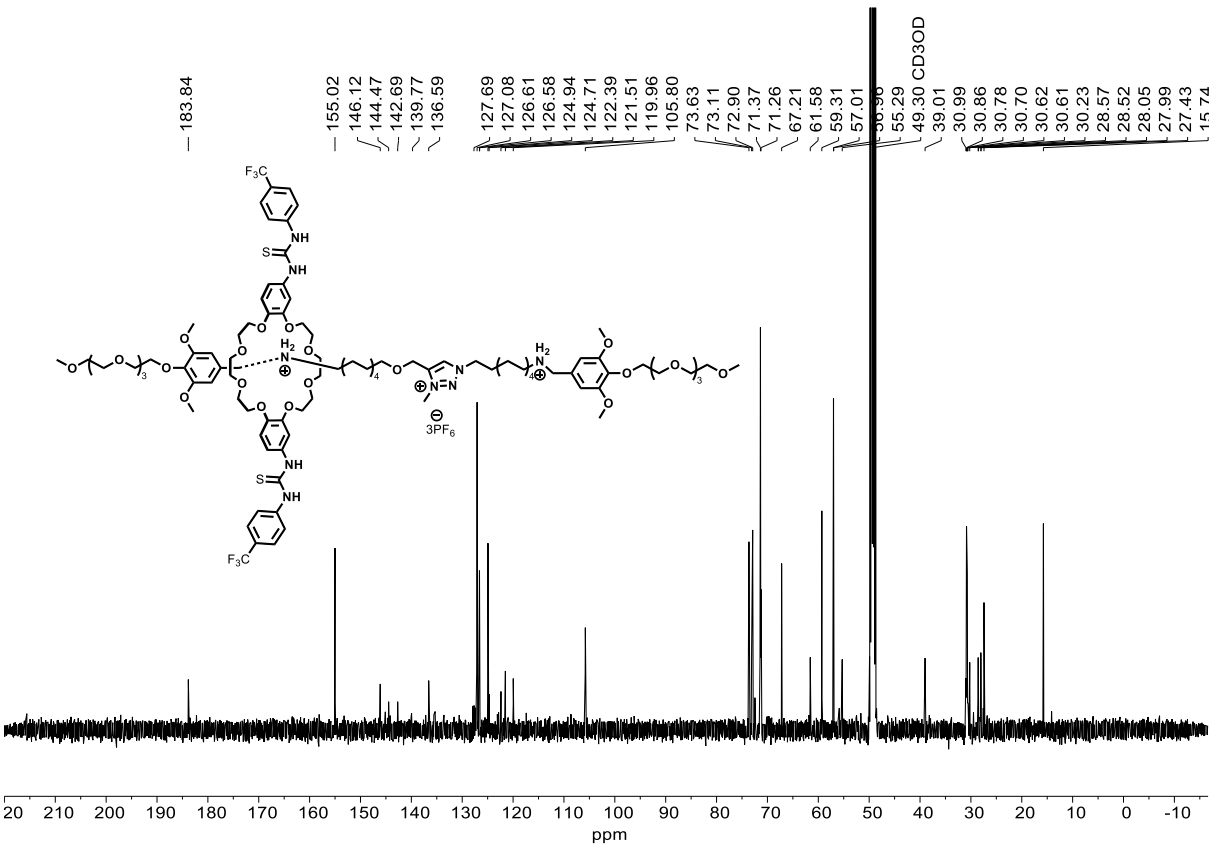


Figure S67. ^{13}C NMR spectrum (126 MHz, *T* = 298 K) of **1** in CD_3OD.

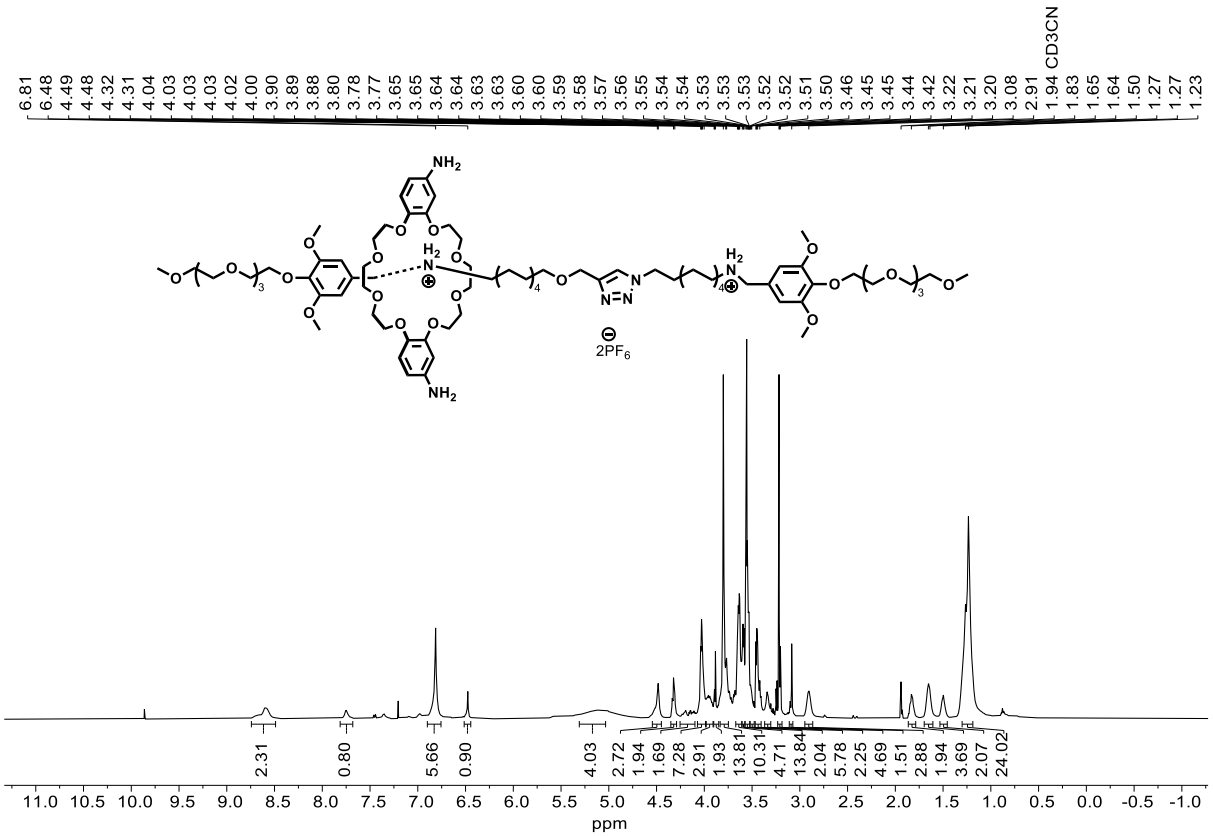


Figure S68. ^{1}H NMR spectrum (500 MHz, *T* = 298 K) of **22** in CD_3CN.

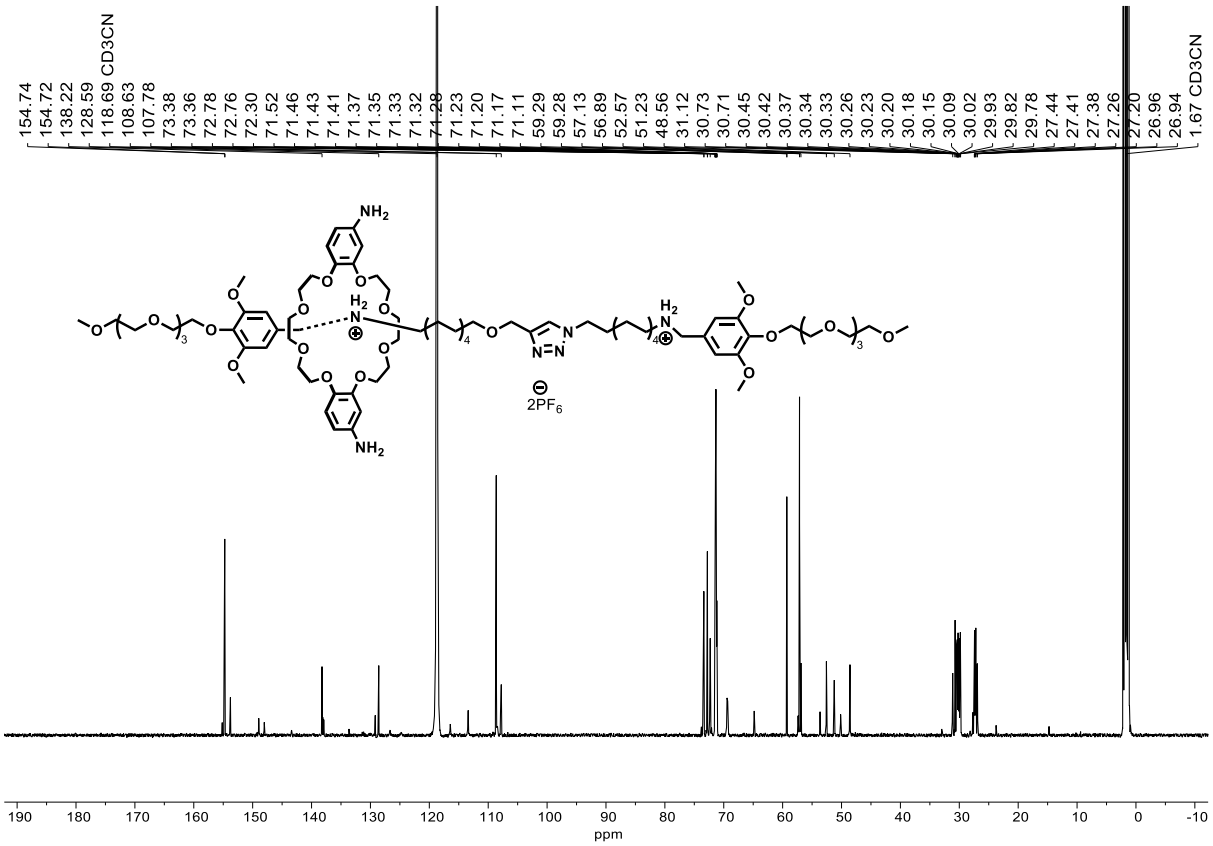


Figure S69. ^{13}C NMR spectrum (126 MHz, *T* = 298 K) of **22** in CD_3CN.

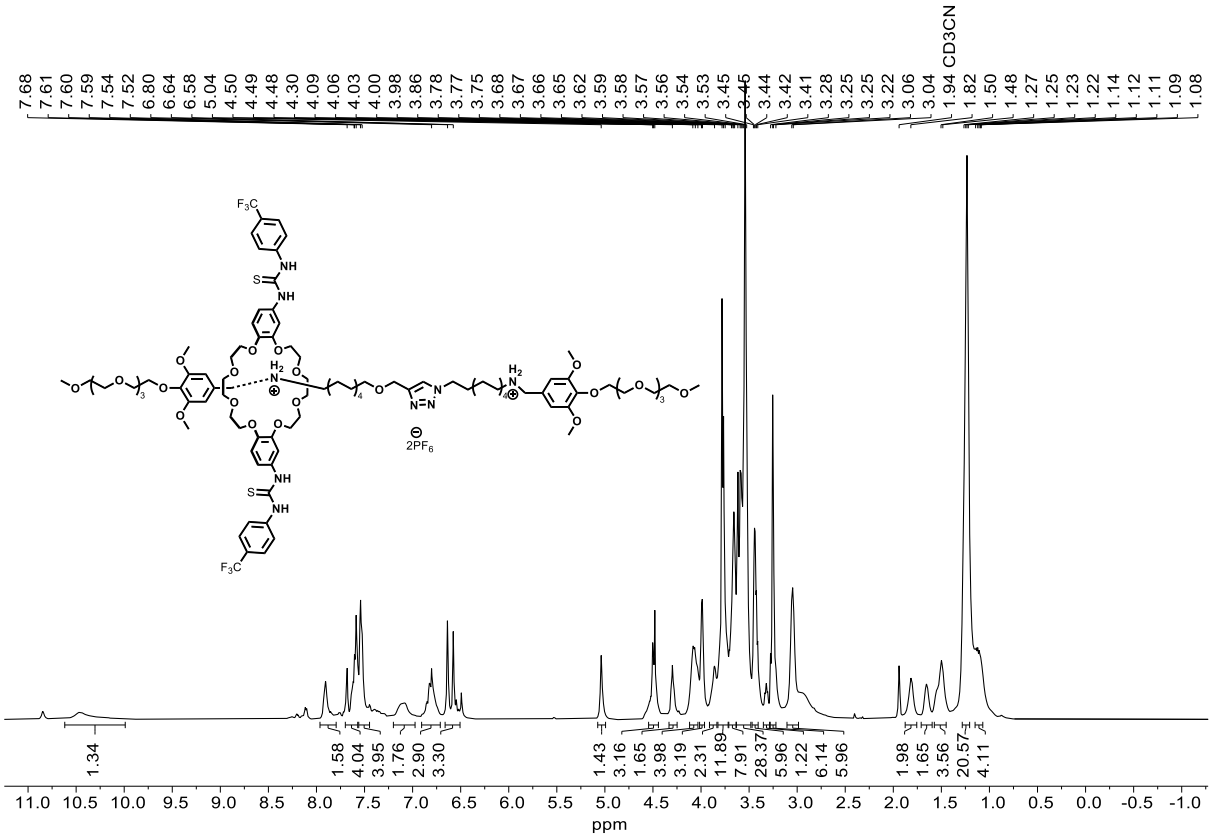


Figure S70. ^{1}H NMR spectrum (500 MHz, T = 298 K) of **2** in CD_3CN.

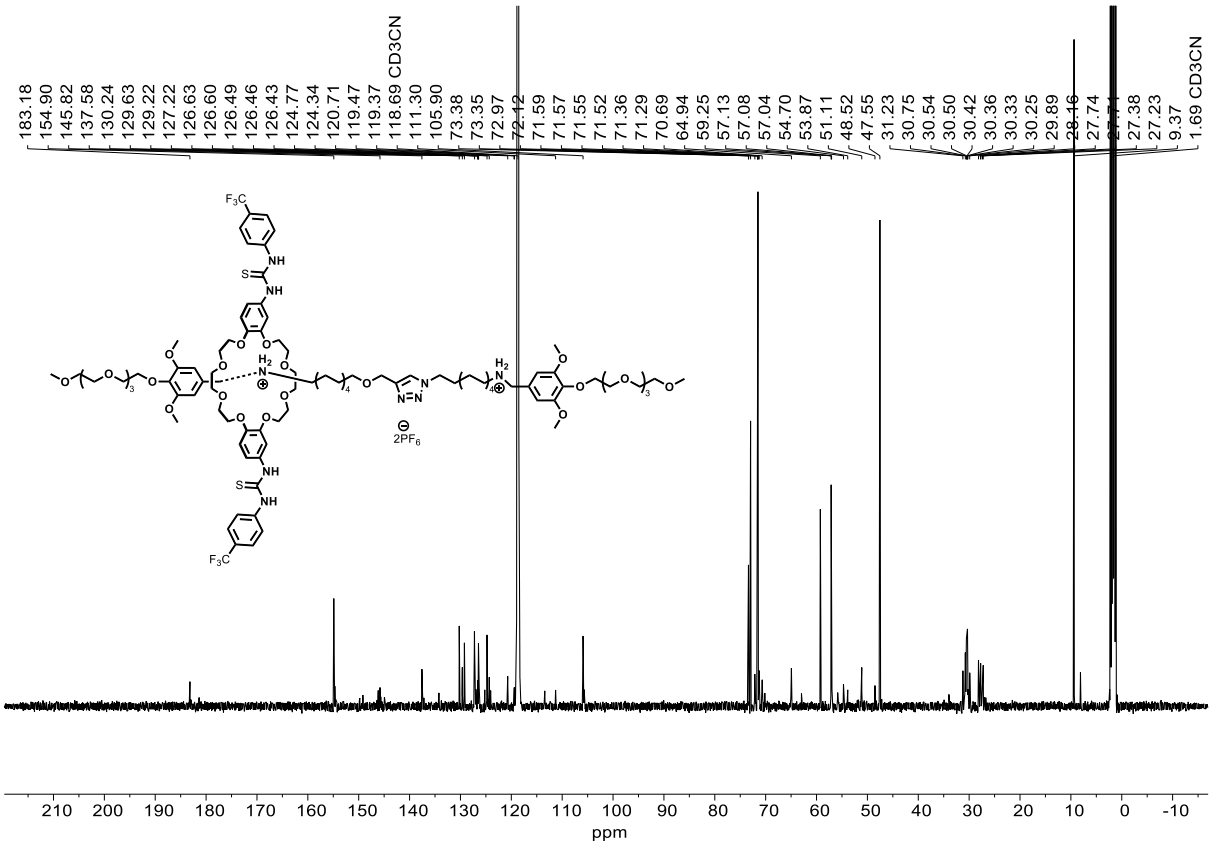


Figure S71. ^{13}C NMR spectrum (126 MHz, T = 298 K) of **2** in CD_3CN.

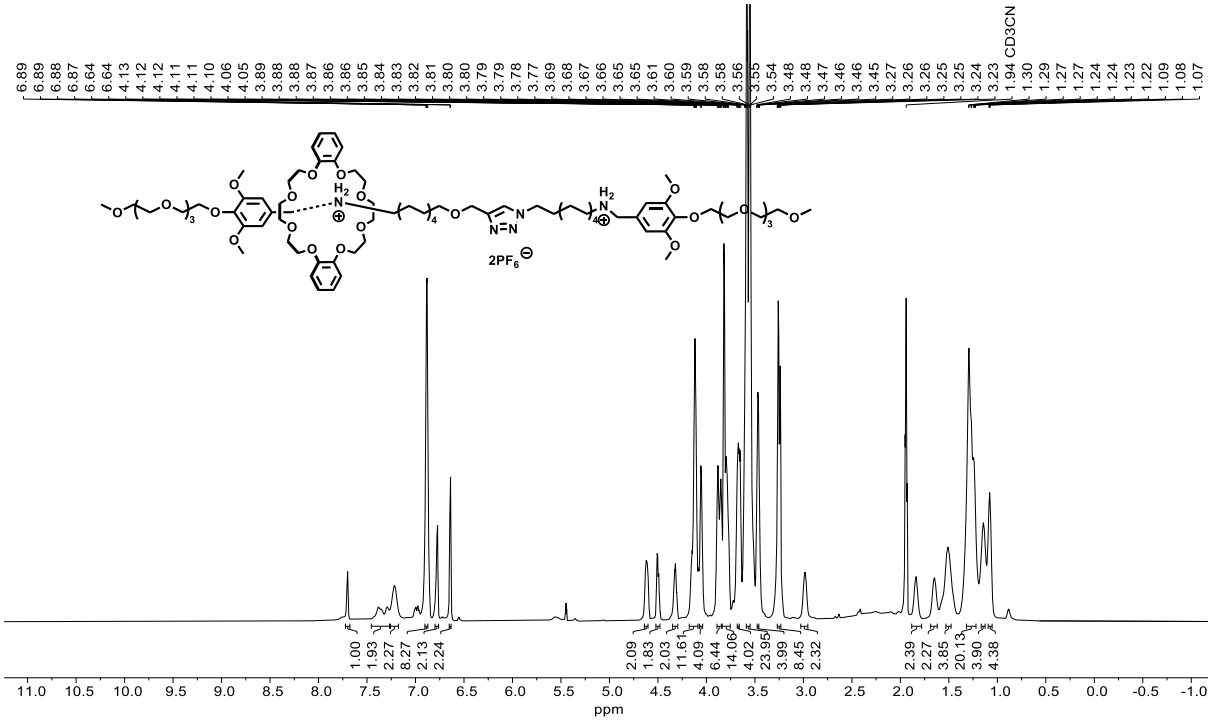


Figure S72. ^{1}H NMR spectrum (500 MHz, *T* = 298 K) of **23** in CD_3CN.

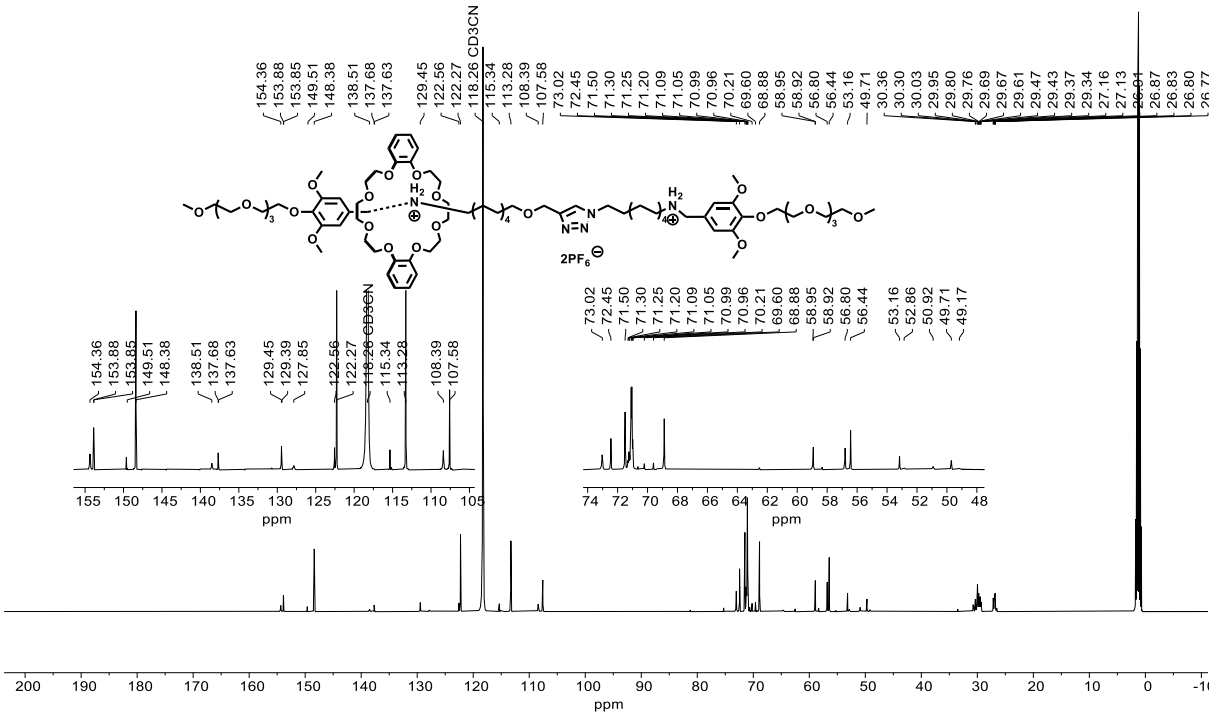


Figure S73. ^{13}C NMR spectrum (126 MHz, *T* = 298 K) of **23** in CD_3CN.

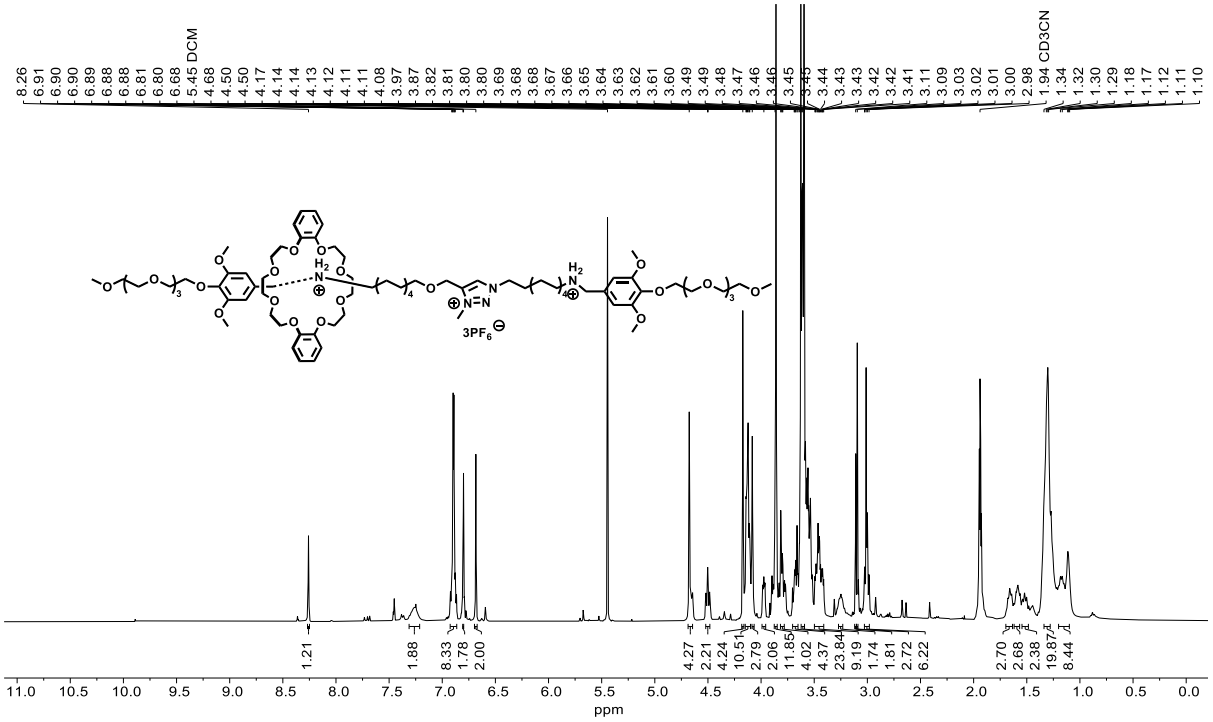


Figure S74. ^{1}H NMR spectrum (400 MHz, *T* = 298 K) of **3** in CD_3CN.

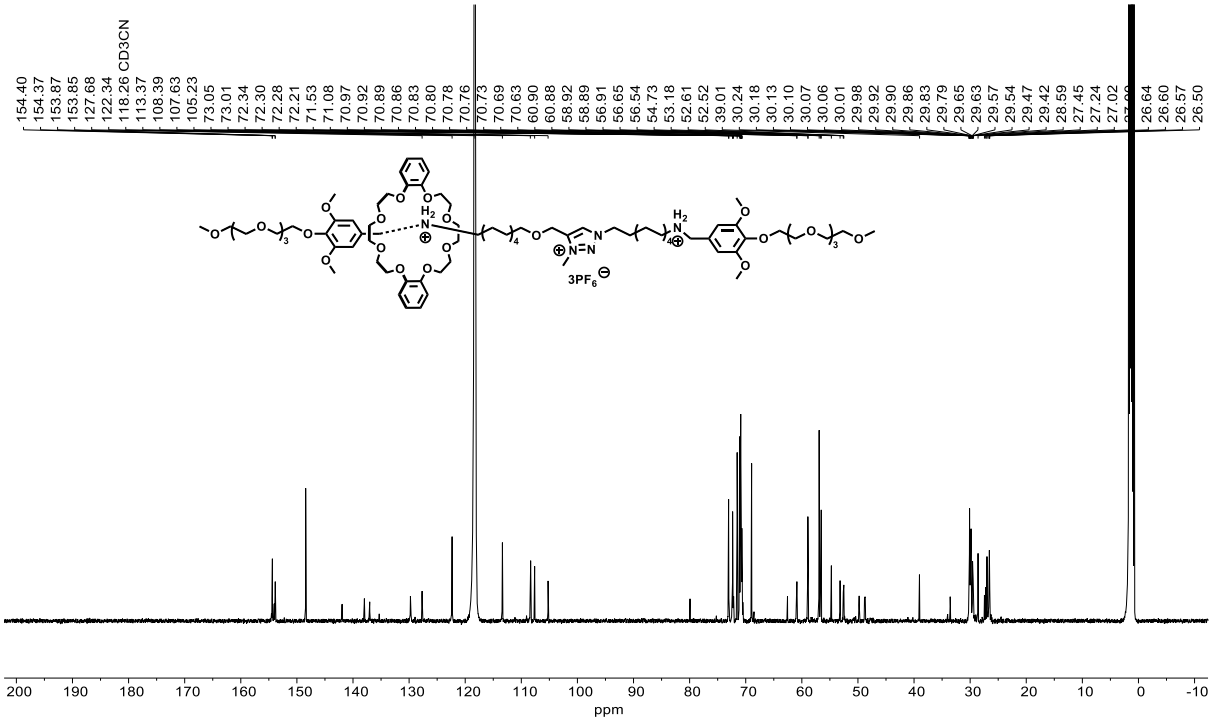


Figure S75. ^{13}C NMR spectrum (126 MHz, *T* = 298 K) of **3** in CD_3CN.

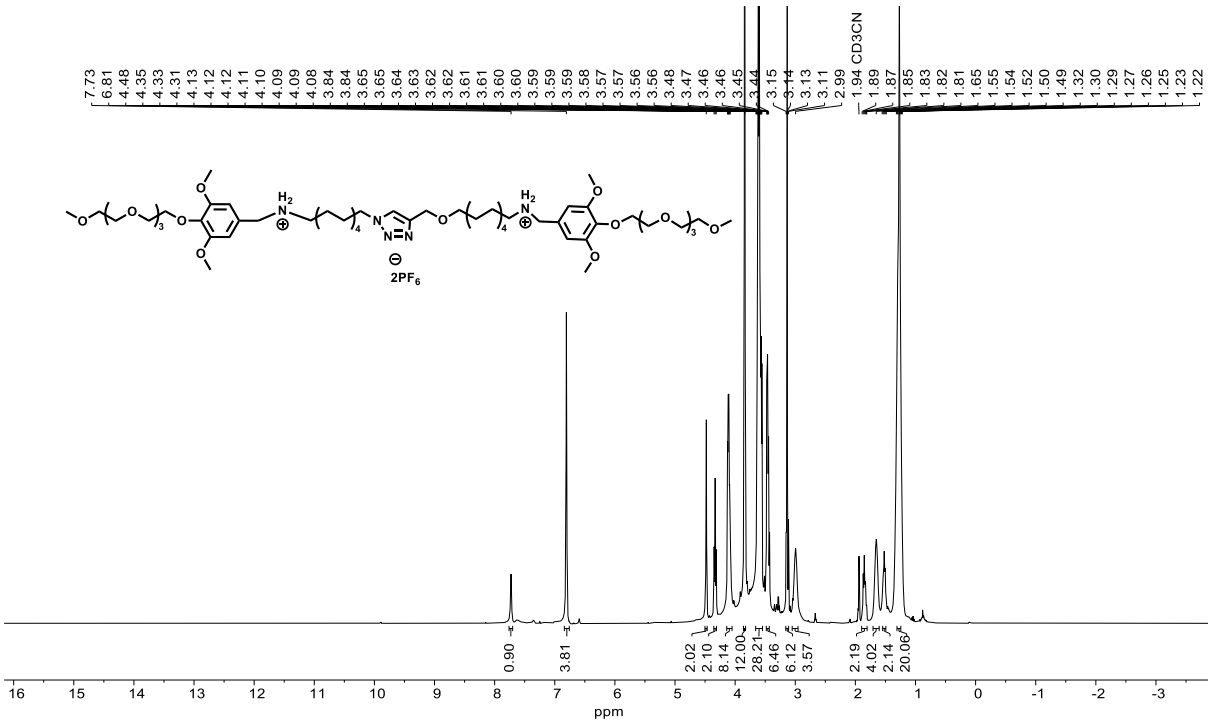


Figure S76. ^{1}H NMR spectrum (400 MHz, *T* = 298 K) of **24** in CD_3CN.

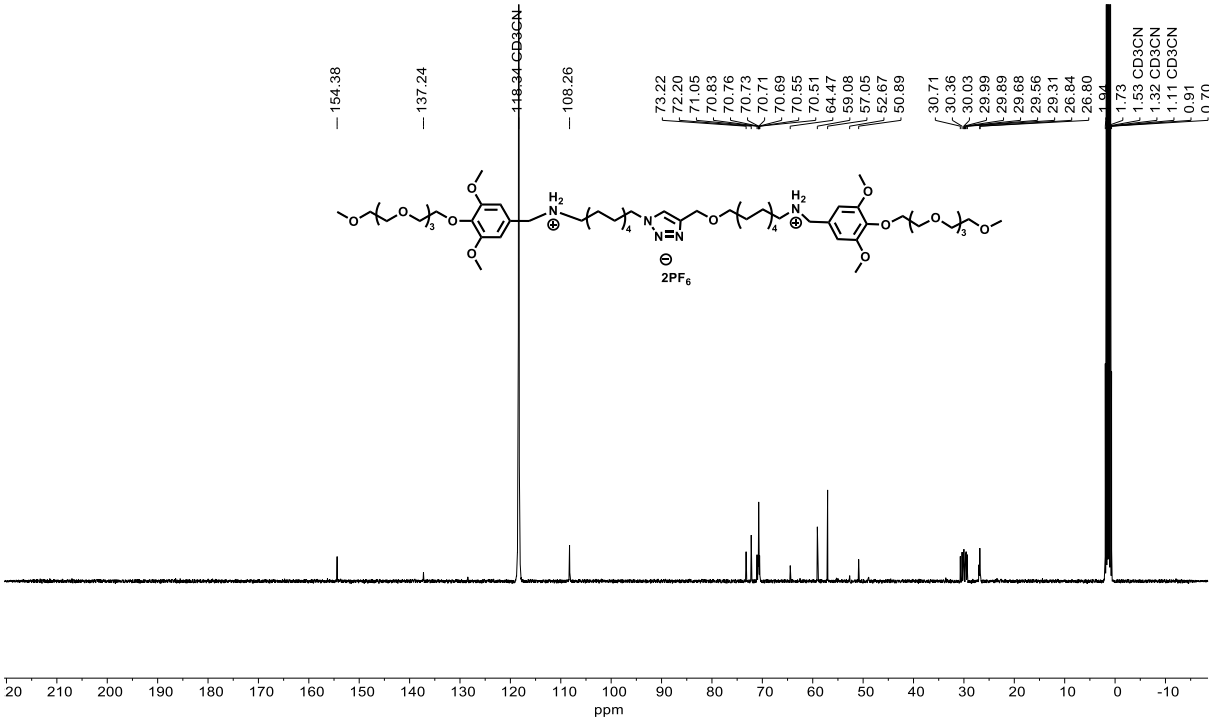


Figure S77. ^{13}C NMR spectrum (126 MHz, *T* = 298 K) of **24** in CD_3CN.

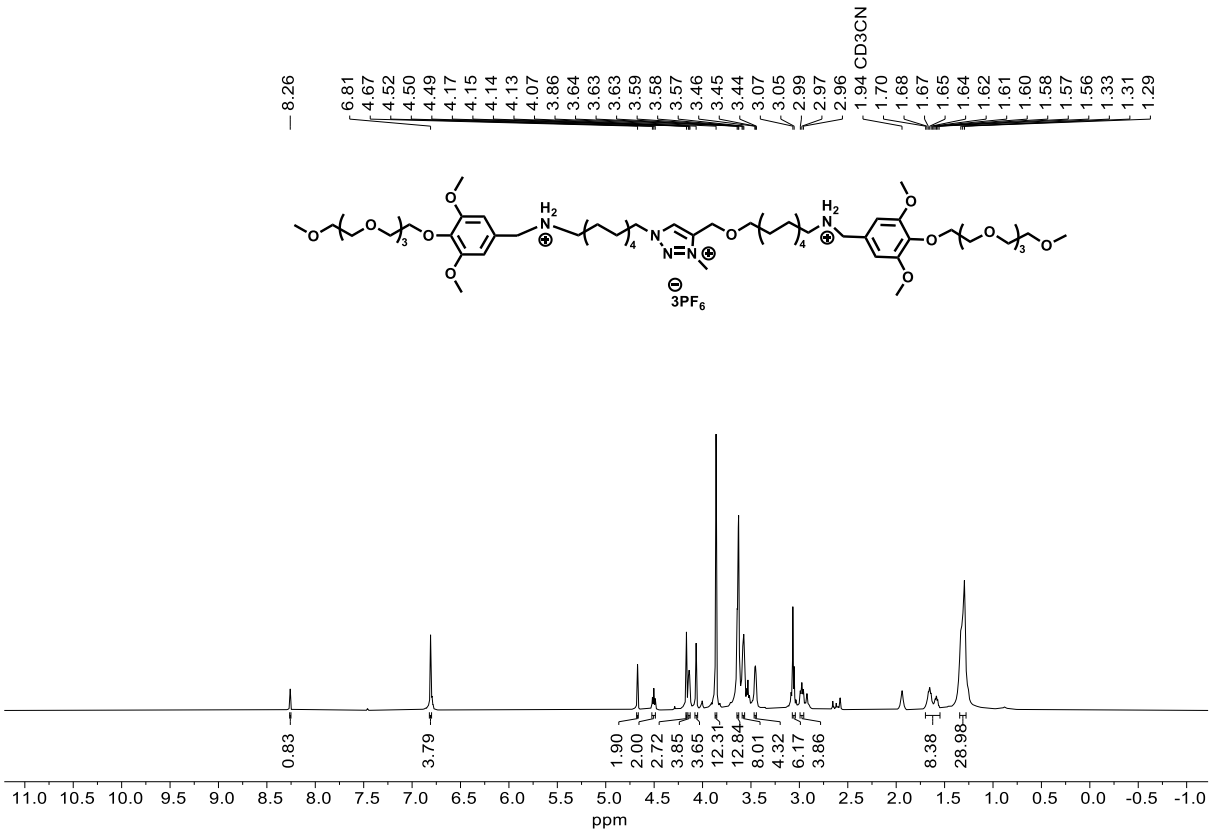


Figure S78. ^{1}H NMR spectrum (400 MHz, *T* = 298 K) of **4** in CD_3CN.

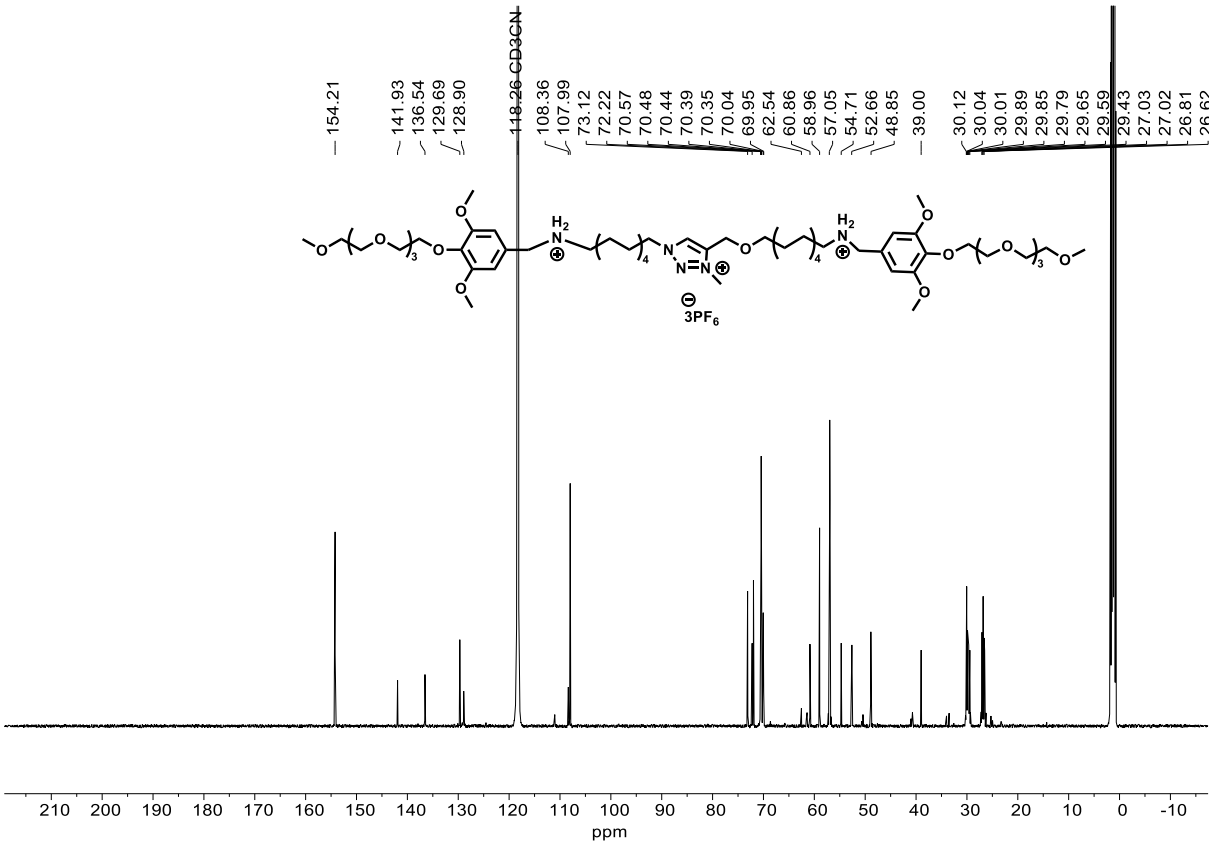


Figure S79. ^{13}C NMR spectrum (126 MHz, *T* = 298 K) of **4** in CD_3CN.

S17.0. Mass Spectra of Synthesized Compounds

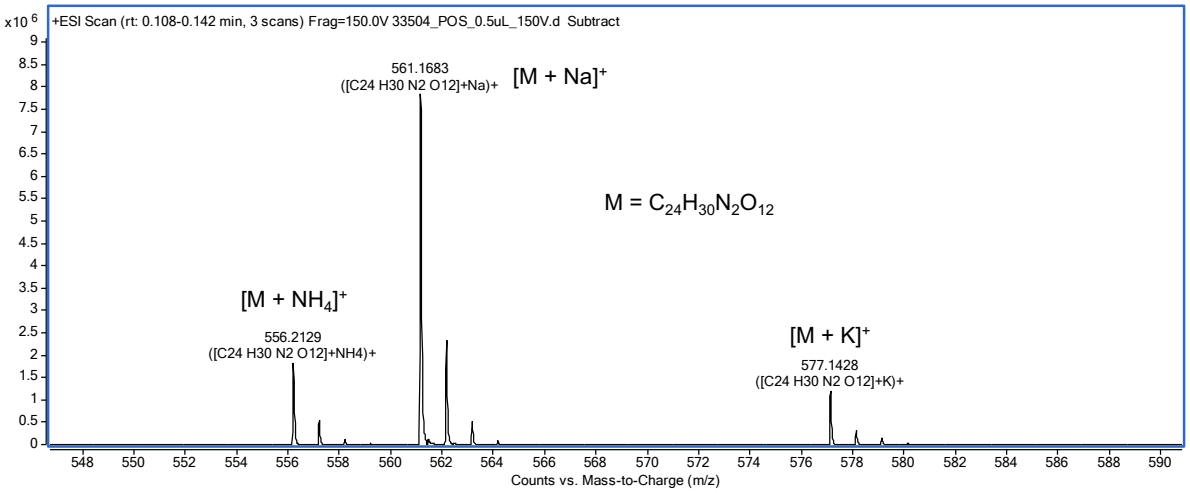


Figure S80. Mass spectrum of compound **11**.

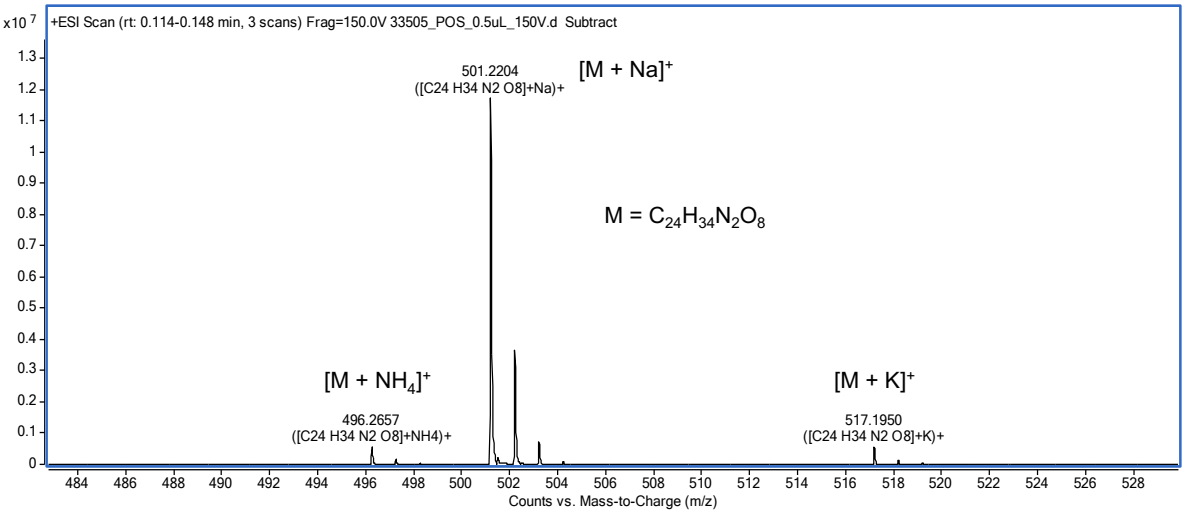


Figure S81. Mass spectrum of compound **12.**

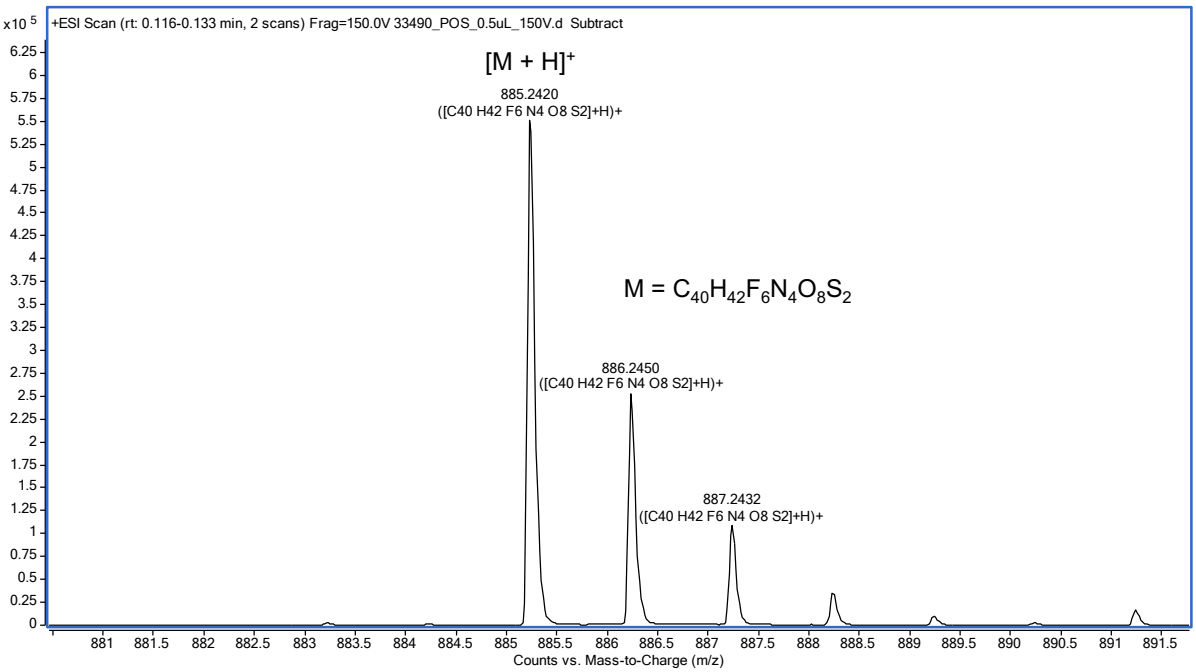


Figure S82. Mass spectrum of compound **5**.

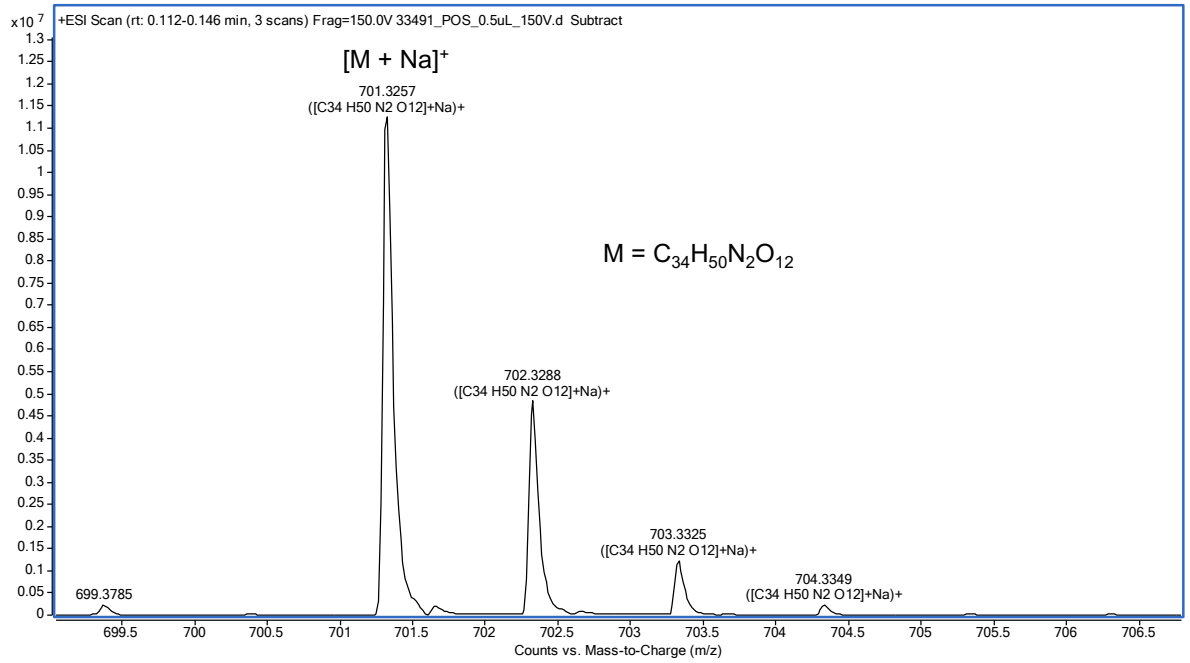


Figure S83. Mass spectrum of compound **6**.

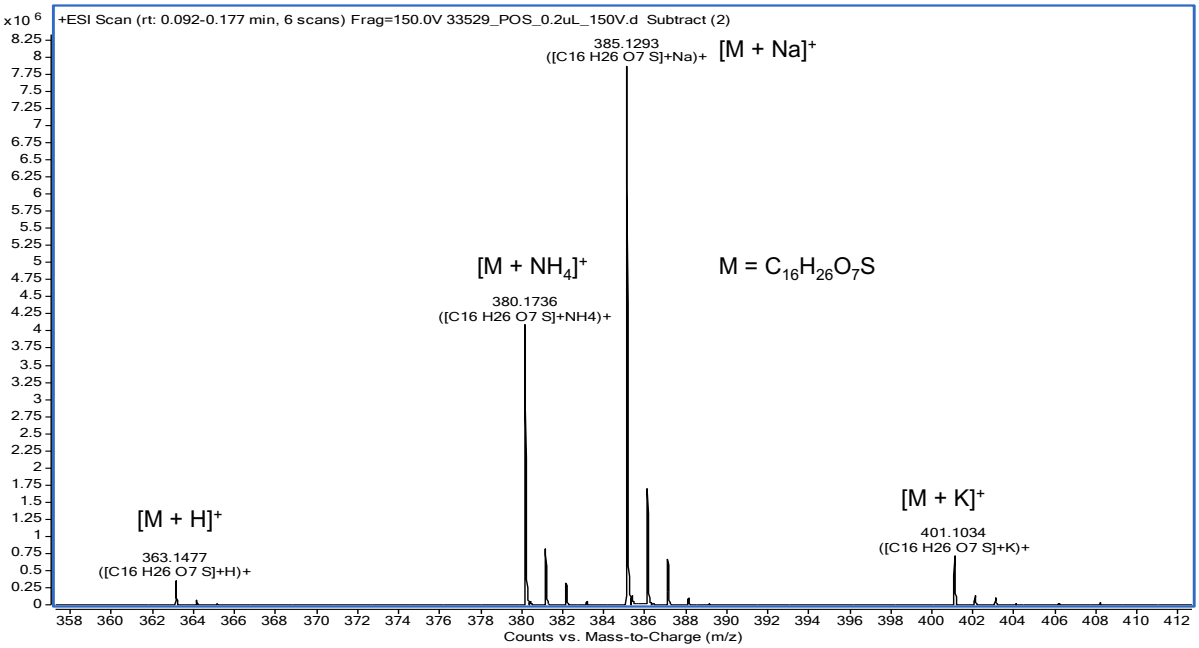


Figure S84. Mass spectrum of compound **14**.

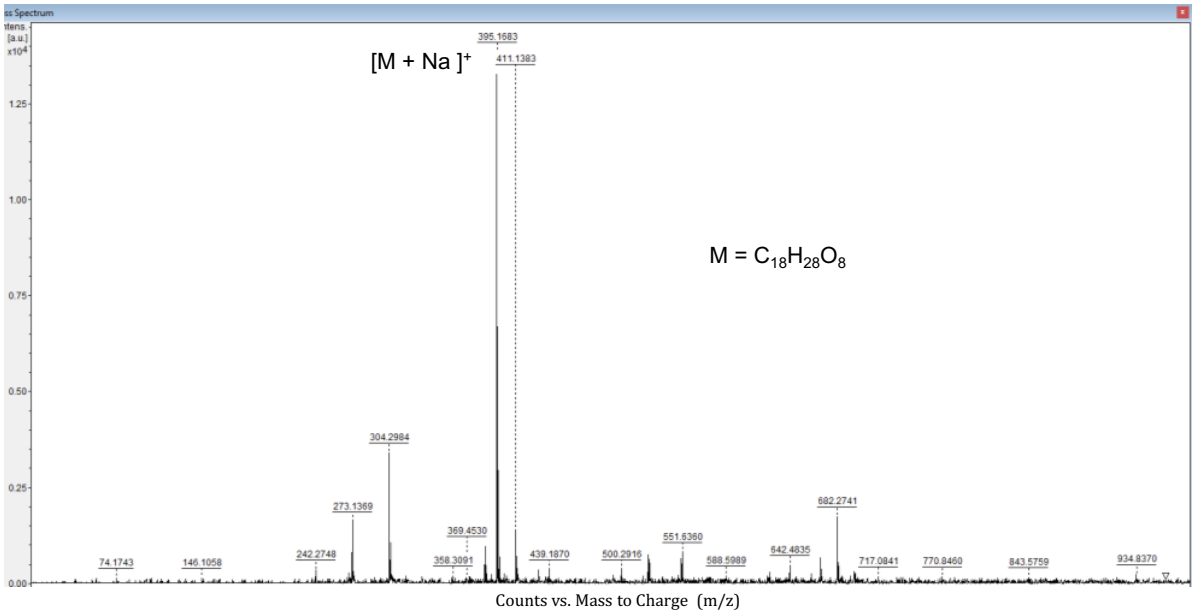


Figure S85. Mass spectrum of compound **15**.

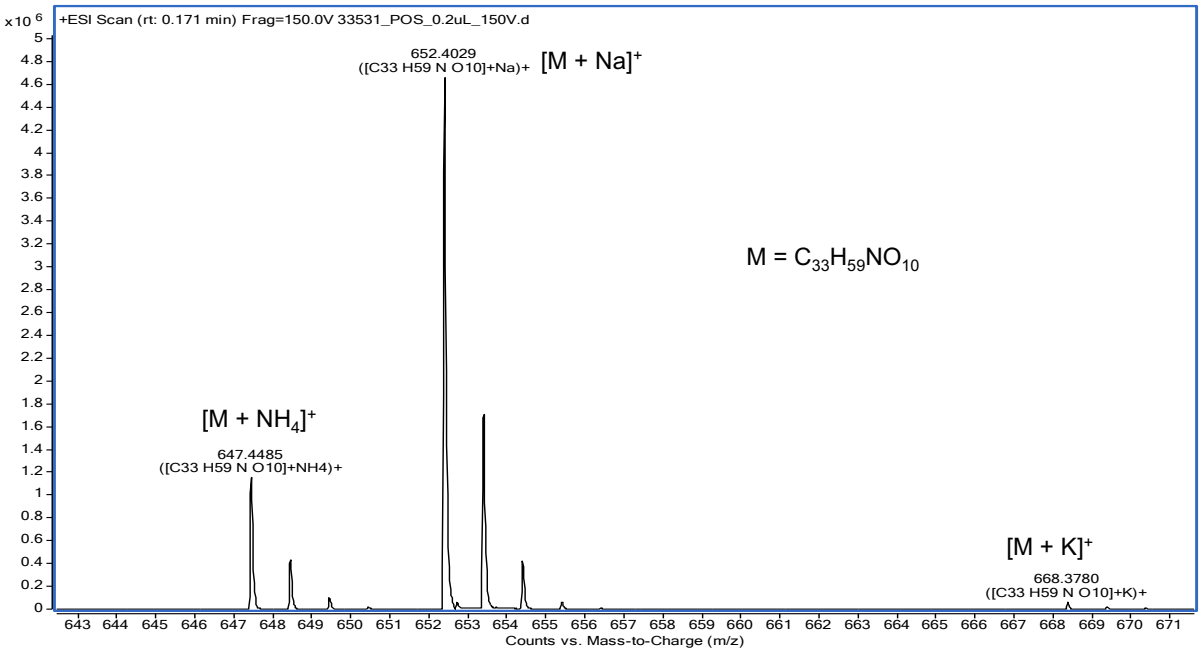


Figure S86. Mass spectrum of compound **16**.

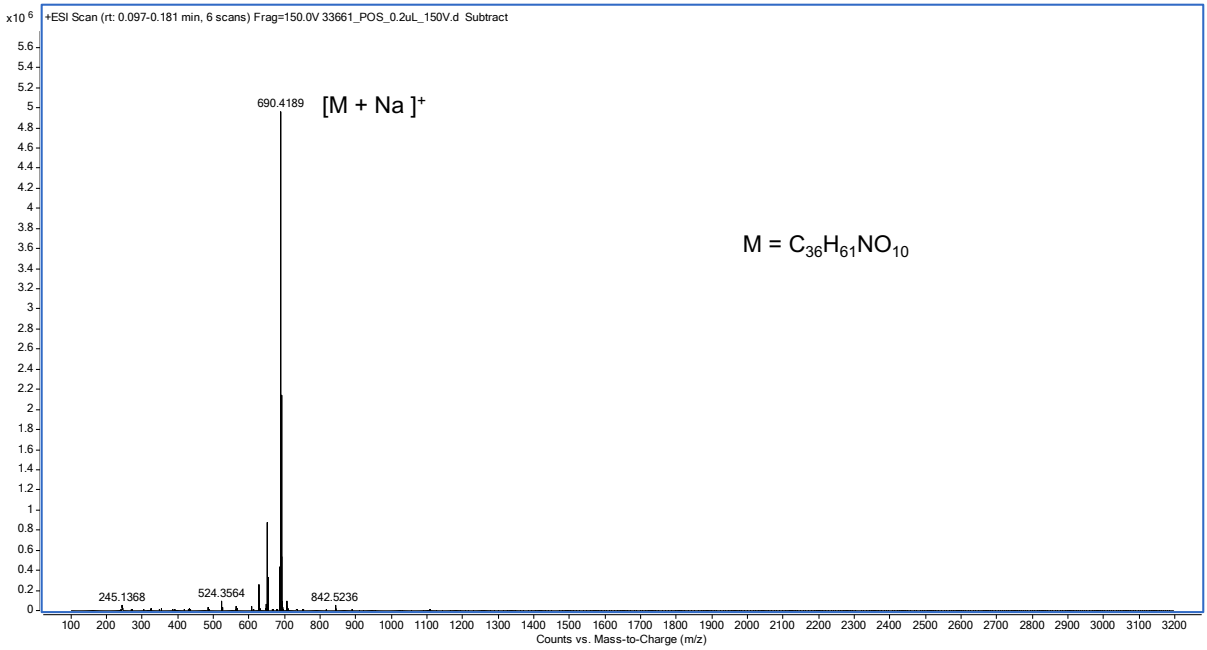


Figure S87. Mass spectrum of compound **17**.

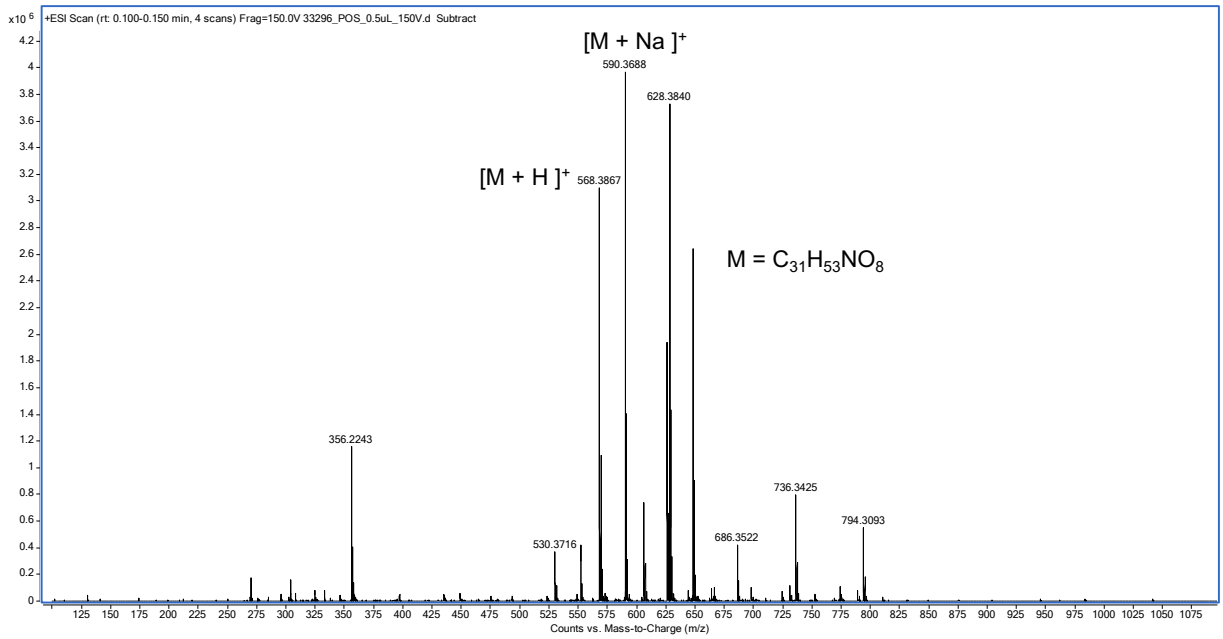


Figure S88. Mass spectrum of compound **7**.

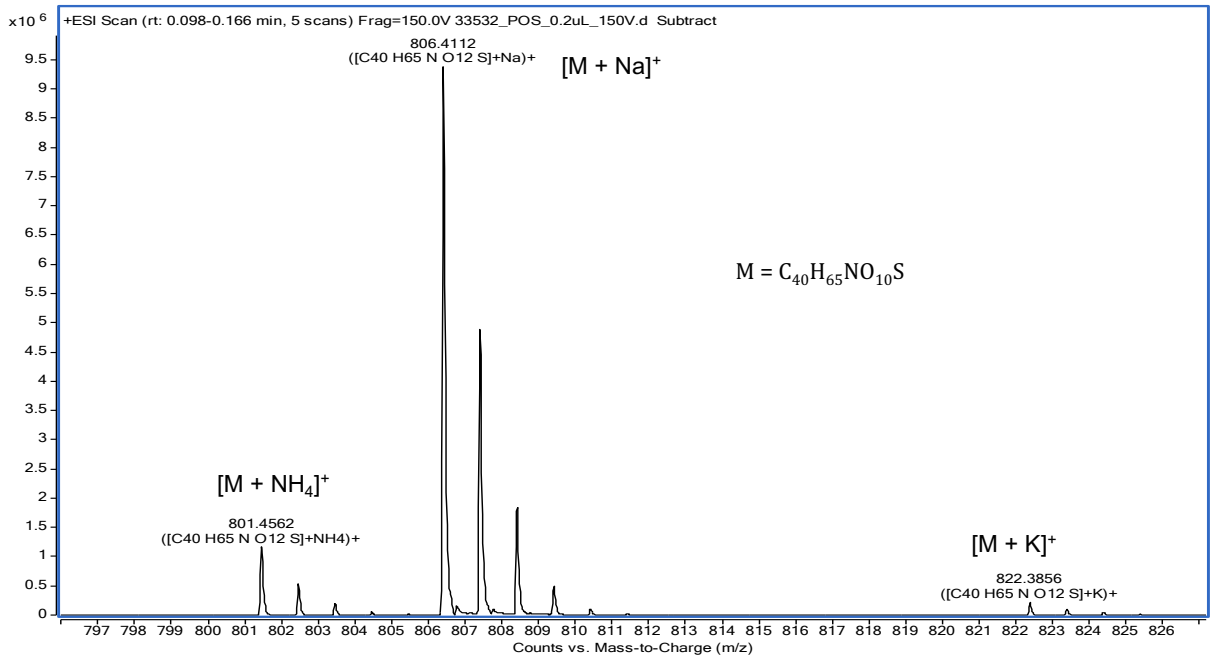


Figure S89. Mass spectrum of compound **18**.

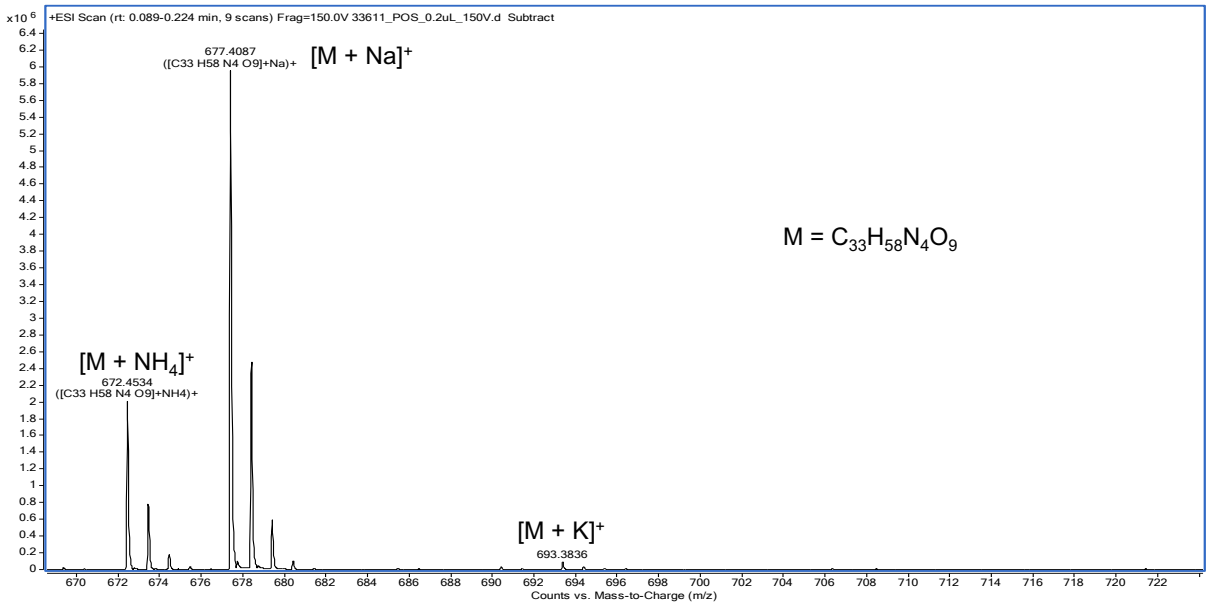


Figure S90. Mass spectrum of compound **19**.

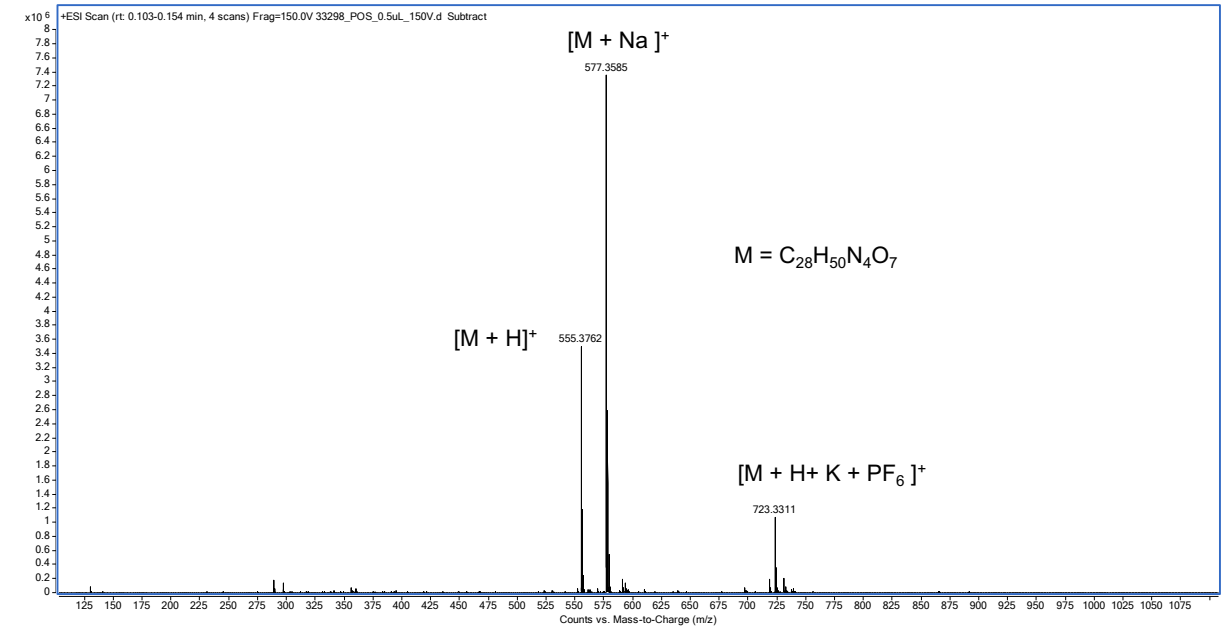


Figure S91. Mass spectrum of compound **8**.

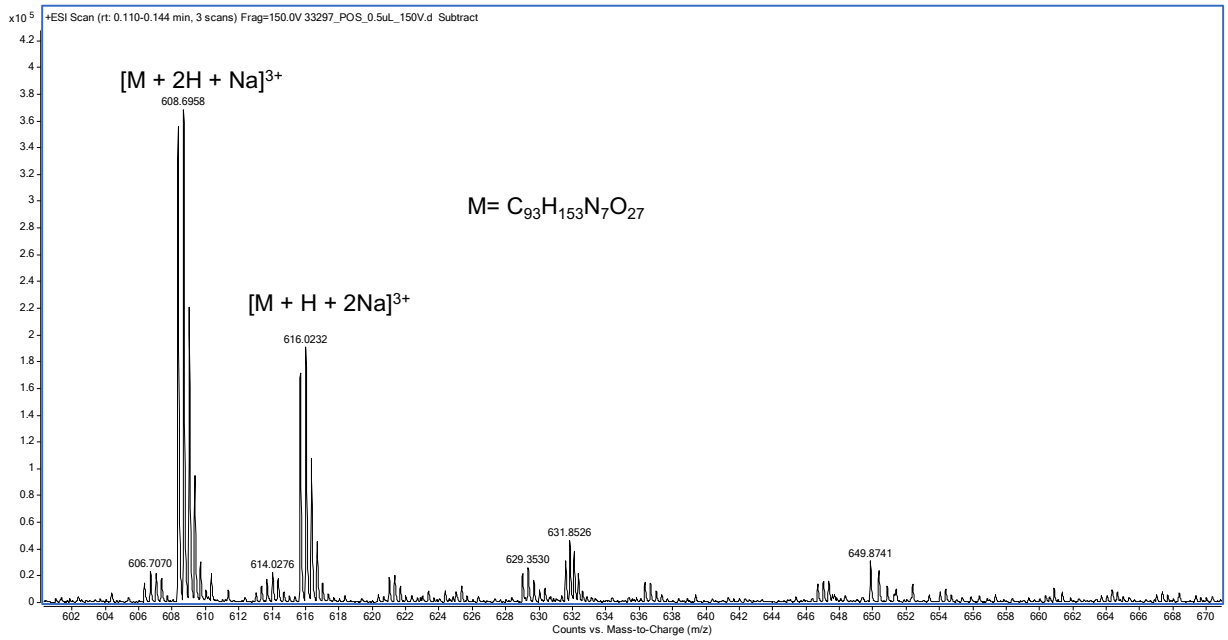


Figure S92. Mass spectrum of compound **9**.

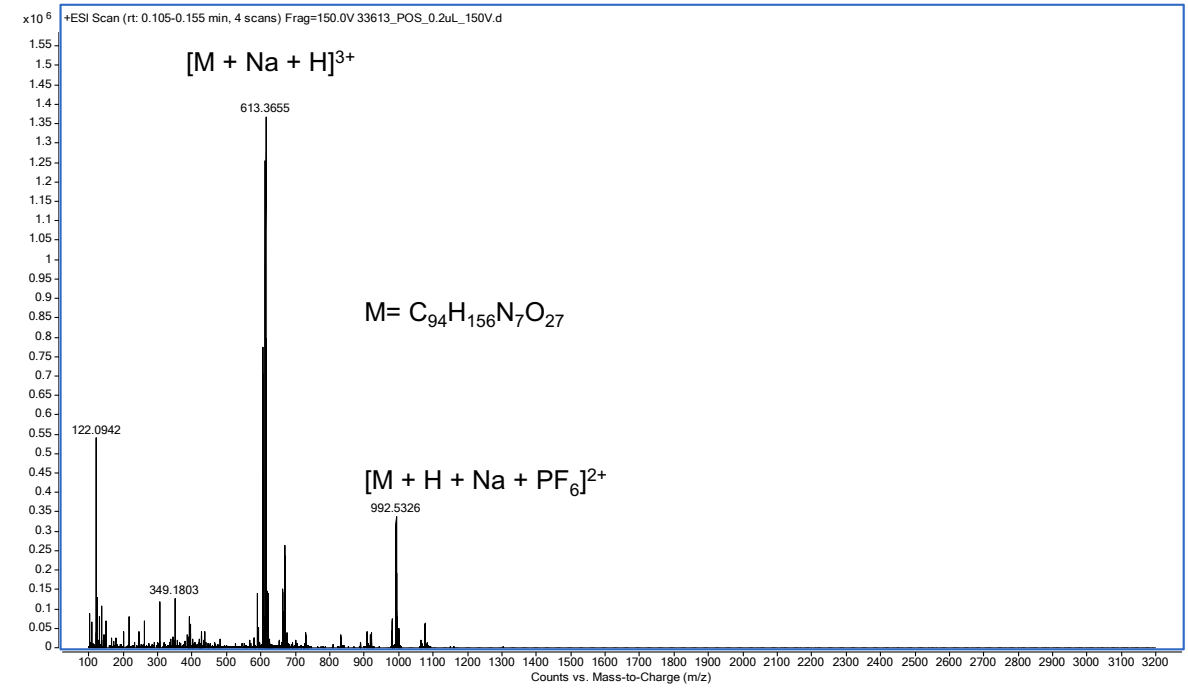


Figure S93. Mass spectrum of compound **20**.

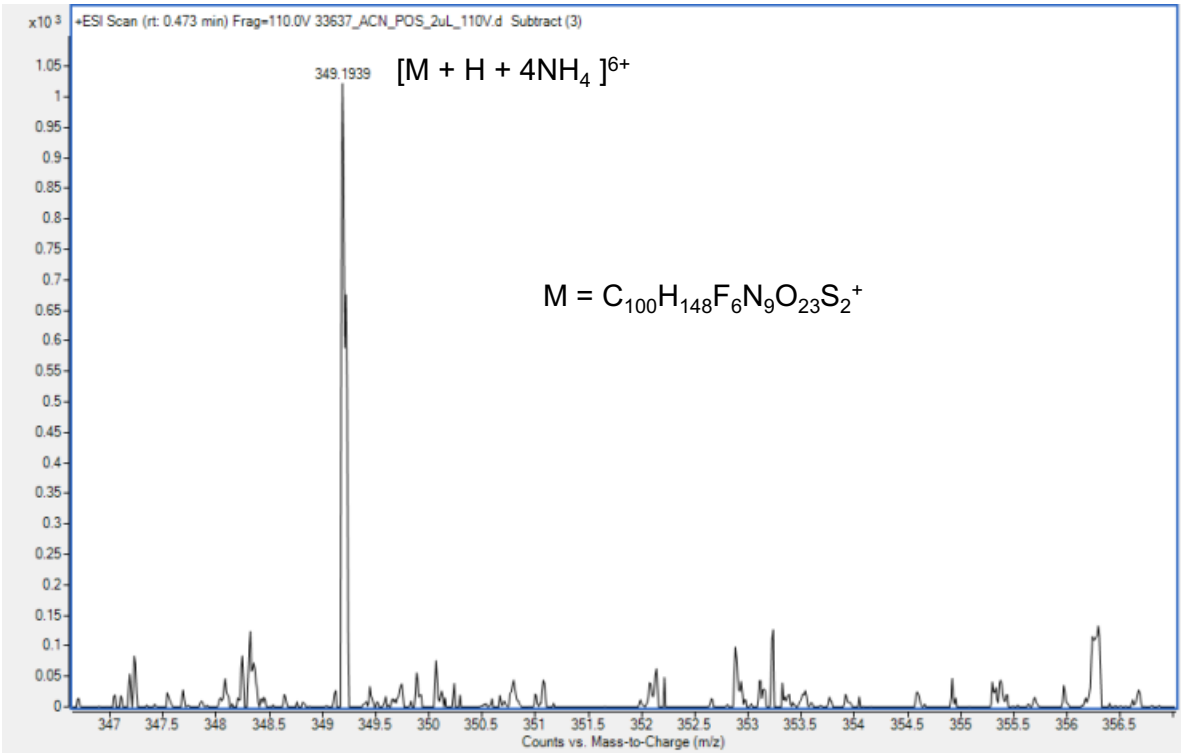


Figure S94. Mass spectrum of Rotaxane **1**.

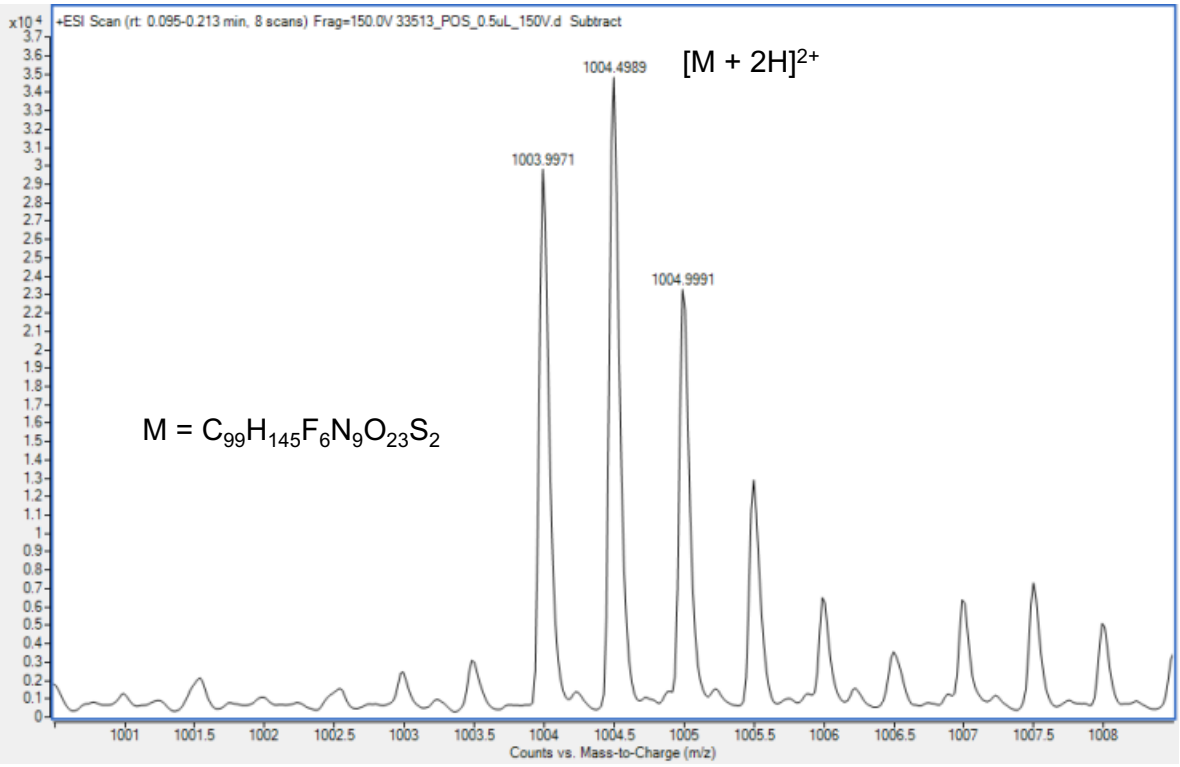


Figure S95. Mass spectrum of Rotaxane **2**.

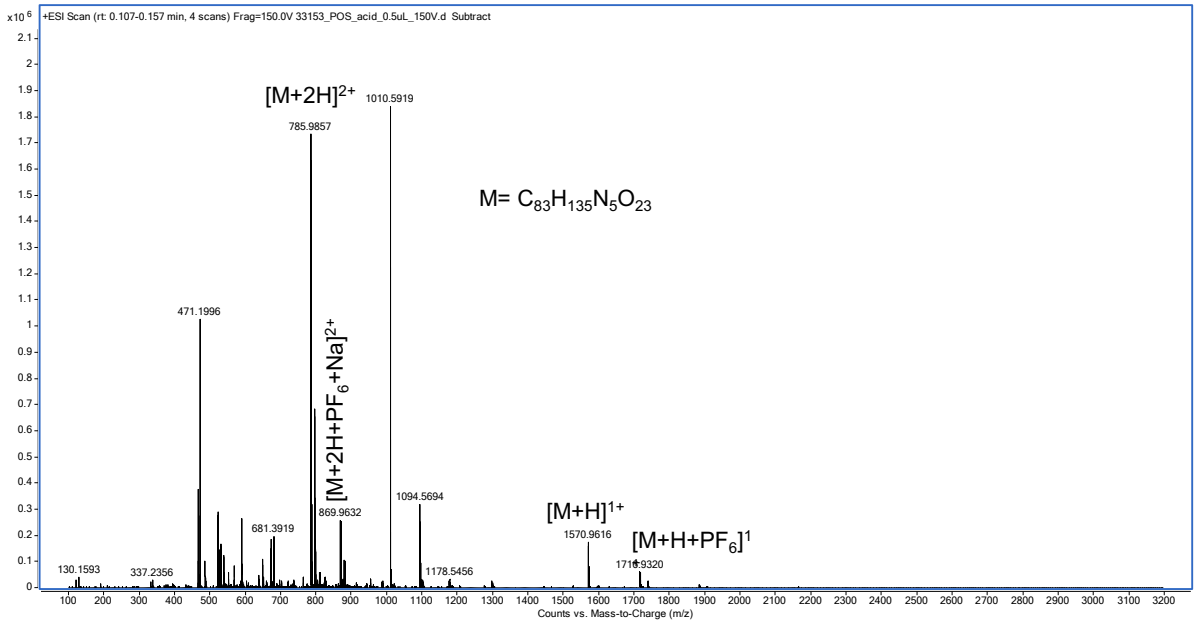


Figure S96. Mass spectrum of compound **23**.

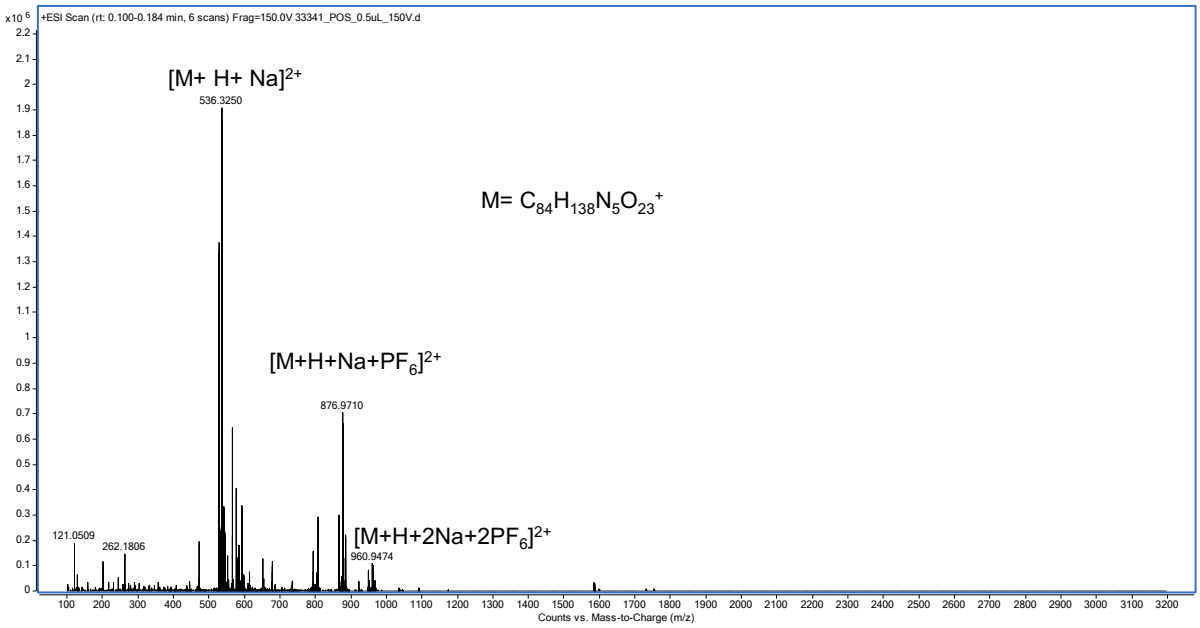


Figure S97. Mass spectrum of Rotaxane **3**.

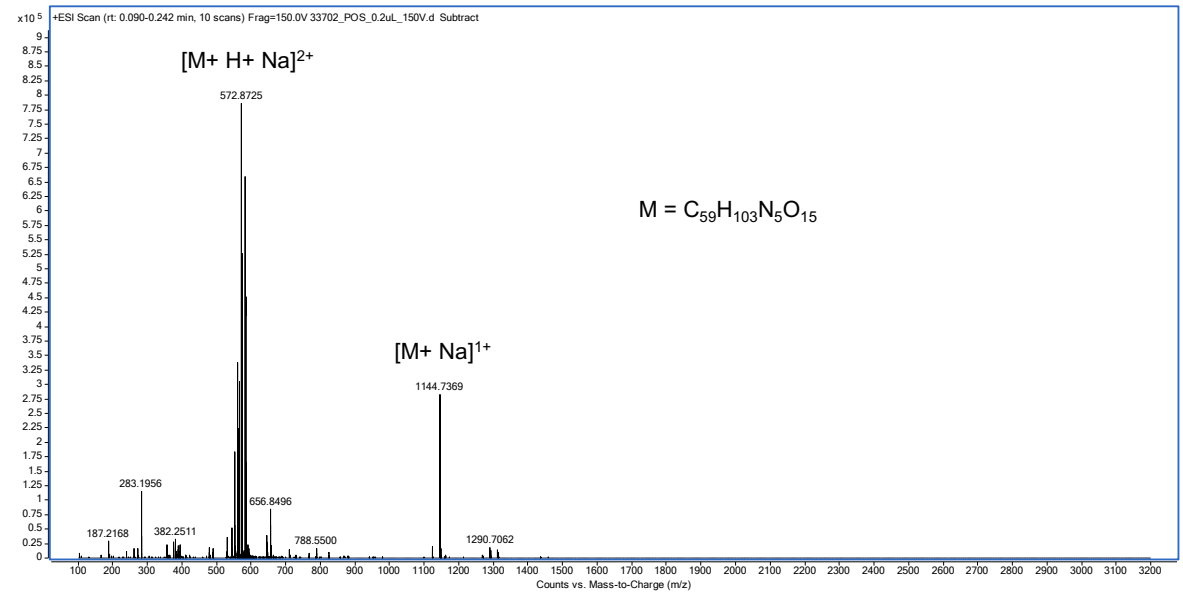


Figure S98. Mass spectrum of compound **24**.

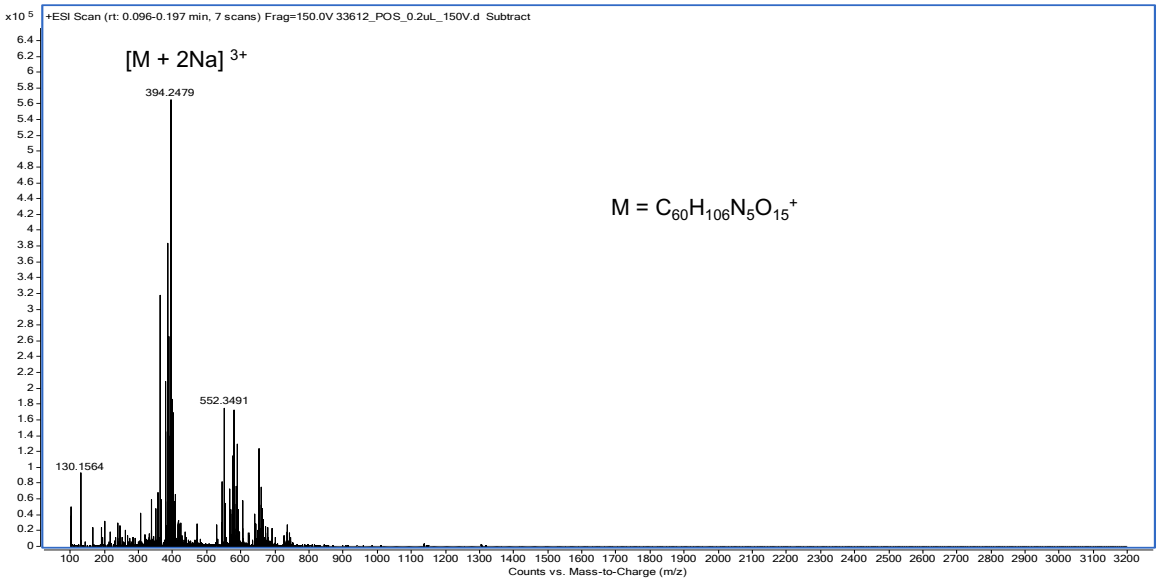


Figure S99. Mass spectrum of compound **4**.

S18. References

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