

Water-Soluble Acylhydrazone Macrocycles as Potent Reversal Agents for Cisatracurium-Induced Neuromuscular Blockade

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1. Materials and Methods:

Materials: All reagents were purchased commercially and used without further purification unless otherwise noted. Minimum Eagle's medium (MEM) and Dulbecco's modified Eagle medium (DMEM) were purchased from Gibco (Thermo Fisher Scientific). Fetal bovine serum (FBS), penicillin-streptomycin and PBS were purchased from Invitrogen (Carlsbad, CA, USA).

Methods: UV-vis spectra were performed on a Pu xi TU-1900 spectrophotometer with 1 cm quartz cells. ^1H NMR spectra, and ^{13}C NMR spectra were collected on a Bruker AVANCEIII 600 MHz instrument at 298 K. Chemical shifts are expressed in parts per million (δ) using residual solvent protons as internal standard. The couple constants values (J) are in Hertz (Hz). The following abbreviations were used for signal multiplicities: s, singlet; d, doublet; t, triplet; m, multiplet; and br, broad. Mass spectra were measured on a Bruker Daltonics Autoflex Speed Series: High-Performance MALDI-TOF Systems. DLS data were obtained on a Malvern Zetasizer Nano ZEN 3690. Transmission electron microscopy (TEM) images were recorded using field emission TEM (FE-TEM, F200). Ground-state geometries were derived using density functional theory (DFT). All computations were performed using the Gaussian 09 program on a personal computer with B3LYP in conjunction with 6-31 G (d, p). All analyses and drawing of various types of maps were completed using Multiwfn 3.7 code. In addition to the energy-optimised structure, the electron densities of the target compounds were investigated using electrostatic potentials (ESP) and noncovalent interactions (NCIs).

anhydrous Na₂SO₄. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 3:1 → 1:1, v/v) to afford compound **2** as a yellow oil (1.96 g, 53%). ¹H NMR (600 MHz, CDCl₃, 298 K): δ 9.91 (s, 1H), 8.32 (d, *J* = 2.2 Hz, 1H), 7.99 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.12 (d, *J* = 8.7 Hz, 1H), 4.32-4.27 (m, 2H), 3.95-3.92 (m, 2H), 3.90 (s, 3H), 3.79-3.74 (m, 2H), 3.69-3.61 (m, 8H), 3.56-3.51 (m, 2H), 3.37 (s, 3H). ¹³C NMR (100 MHz, CDCl₃, 298 K): δ 190.3, 163.1, 134.6, 134.5, 129.3, 121.0, 113.6, 72.0, 71.2, 70.8, 70.7, 70.6, 69.4, 69.3, 59.2, 52.4. HRMS (ESI): *m/z* Calcd for C₁₈H₂₇O₈: calcd. 371.1700 [M+H]⁺, found: 371.1699.

Compound 3. To a 100 mL round-bottom flask were added compound **2** (1.85 g, 5.00 mmol), tetrahydrofuran (8.0 mL), methanol (4.0 mL), water (2.0 mL), and lithium hydroxide (479 mg, 20.0 mmol). The mixture was stirred at room temperature for 4.0 h, and the reaction progress was monitored by TLC until completion. Most of the solvent was removed under reduced pressure, and the pH of the residue was adjusted to approximately 2.0 with hydrochloric acid (2.00 M). The mixture was extracted with ethyl acetate (25 mL), and the organic layer was retained. The organic phase was washed successively with water and saturated brine, dried over anhydrous sodium sulfate, and filtered. The solvent was removed under reduced pressure to give compound **3** as a yellow oily liquid (1.62 g, 91%). ¹H NMR (600 MHz, CDCl₃, 298 K): δ 9.97 (s, 1H), 8.60 (d, *J* = 2.2 Hz, 1H), 8.10 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.17 (d, *J* = 8.6 Hz, 1H), 4.45-4.42 (m, 2H), 3.98-3.95 (m, 2H), 3.77-3.75 (m, 2H), 3.70-3.64 (m, 8H), 3.57-3.55 (m, 2H), 3.37 (s, 3H). ¹³C NMR (150 MHz, CDCl₃, 298 K): δ 190.1, 164.9, 161.8, 137.1, 134.3, 130.9, 119.9, 114.0, 72.1, 71.1, 70.8, 70.7, 70.7, 70.5, 69.7, 68.6, 59.1. HRMS (ESI): *m/z* Calcd for C₁₇H₂₅O₈: 357.1543 [M+H]⁺, found: 357.1543.

Compound 5. To a 100 mL round-bottom flask were added compound **4** (2.13 g, 10.0 mmol), anhydrous potassium carbonate (4.15 g, 30.0 mmol), potassium iodide (166 mg, 1.00 mmol), and N, N-dimethylformamide (25 mL). The mixture was stirred at room temperature for 30 min, after which PEG-OTs (10.7 g, 30.0 mmol) was added. The reaction mixture was refluxed at 90 °C for 48 h, and the progress was monitored by TLC until completion. The reaction was then stopped and allowed to cool to room temperature, followed by the addition of ethyl acetate (100 mL). The organic layer was collected by filtration, washed successively with water and saturated brine, and then dried over anhydrous sodium sulfate. After filtration, the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography

using dichloromethane/methanol (100:1–50:1, v/v) as the eluent to afford compound **5** as a yellow oily liquid (4.27 g, 72%). ¹H NMR (600 MHz, CDCl₃, 298 K): δ 8.57 (s, 1H), 6.68 (s, 1H), 4.30–4.28 (m, 2H), 4.28–4.25 (m, 2H), 3.95–3.91 (m, 4H), 3.85 (s, 3H), 3.75 (m, 4H), 3.67–3.60 (m, 18H), 3.54–3.51 (m, 4H), 3.35 (s, 6H). ¹³C NMR (150 MHz, CDCl₃, 298 K): δ 164.1, 163.8, 157.5, 132.3, 131.3, 112.3, 99.4, 72.0, 71.3, 71.2, 70.7, 70.7, 70.6, 70.6, 70.59, 70.1, 69.8, 69.5, 69.3, 59.1, 52.2. HRMS (ESI): m/z Calcd for C₂₆H₄₄NO₁₄: 594.2756 [M+H]⁺, found: 594.2751.

Compound 6. To a 25 mL round-bottom flask were added compound **5** (2.97 g, 5.00 mmol), tetrahydrofuran (8.0 mL), methanol (4.0 mL), water (2.0 mL), and potassium hydroxide (1.12 g, 20.0 mmol). The mixture was stirred at room temperature for 4.0 h, and the progress of the reaction was monitored by TLC until completion. Most of the solvent was removed under reduced pressure, and the pH of the residue was adjusted to approximately 2.0 using hydrochloric acid (2.00 M). The mixture was extracted with ethyl acetate (25 mL), washed with saturated brine, dried over anhydrous sodium sulfate, and filtered. The solvent was then removed under reduced pressure to afford compound **6** as a white solid (2.58 g, 89%). ¹H NMR (600 MHz, CDCl₃, 298 K): δ 8.69 (d, *J* = 7.7 Hz, 1H), 6.74 (s, 1H), 4.38 (m, 2H), 4.33 (m, 2H), 3.95–3.92 (m, 4H), 3.74 (m, 4H), 3.68–3.60 (m, 16H), 3.56–3.52 (m, 4H), 3.37–3.35 (m, 6H). ¹³C NMR (150 MHz, CDCl₃, 298 K): δ 164.1, 164.0, 161.91, 161.9, 157.7, 134.0, 134.0, 132.1, 132.0, 111.7, 99.7, 72.0, 71.2, 71.0, 71.0, 70.7, 70.7, 70.7, 70.6, 70.5, 70.4, 70.0, 69.4, 68.6, 68.6, 59.1, 59.1. HRMS (ESI): m/z Calcd for C₂₅H₄₂NO₁₄: 580.2583 [M+H]⁺, found: 580.2600.

Compound 7. To a 25 mL round-bottom flask, compound **6** (2.89 g, 5.00 mmol), NH₂NHBoc (2.64 g, 20.0 mmol), and 4-dimethylaminopyridine (244 mg, 2.00 mmol) were added in an ice bath, followed by anhydrous tetrahydrofuran (10 mL). A solution of N, N'-dicyclohexylcarbodiimide (1.13 g, 5.50 mmol) in anhydrous tetrahydrofuran (10 mL) was then added dropwise to the reaction mixture. The mixture was stirred at room temperature for 12 h, and the reaction progress was monitored by TLC until completion. The resulting insoluble white solid was filtered off, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/methanol (50:1, v/v) as the eluent to afford compound **7** as a yellow oily liquid (2.81 g, 81%). ¹H NMR (600 MHz, CDCl₃, 298 K): δ 8.78 (s, 1H), 6.65 (s, 1H), 4.35–4.33 (m, 2H), 4.30 (t, *J* = 4.8 Hz, 2H), 3.97–3.94 (m, 2H), 3.94–3.91 (m, 2H), 3.74 (m, 4H), 3.67–3.62 (m, 16H), 3.54 (m, 4H), 3.37 (m, 6H),

1.49 (s, 9H). ¹³C NMR (150 MHz, CDCl₃, 298 K): δ 161.1, 156.9, 155.5, 134.0, 131.3, 99.3, 81.4, 72.0, 72.0, 71.2, 70.9, 70.8, 70.7, 70.7, 70.6, 70.3, 69.4, 69.4, 68.7, 59.2, 59.2, 28.4. HRMS (ESI): m/z Calcd for C₃₀H₅₂N₃O₁₅: 694.3393 [M+H]⁺, found: 694.3396.

Compound 8. Under a hydrogen atmosphere, compound **7** (693 mg, 1.00 mmol) and 10% Pd/C (50.0 mg) were added to a 25 mL Schlenk tube and subjected to hydrogenation at atmospheric pressure for 6 h. After completion, the catalyst (Pd/C) was removed by filtration, and the filtrate was concentrated under reduced pressure. compound **3** (264 mg, 2.00 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI, 186 mg, 1.20 mmol), and dichloromethane (10 mL) were then added to the flask. The reaction mixture was stirred at room temperature for 24 h, and the progress was monitored by TLC until completion. The reaction mixture was washed successively with saturated sodium bicarbonate solution, water, and saturated brine. The crude product was concentrated under reduced pressure and purified by silica gel column chromatography using dichloromethane/methanol (40:1, v/v) as the eluent to afford compound **8** as a yellow oily liquid (610 mg, 61%). ¹H NMR (400 MHz, CDCl₃, 298 K): δ 9.99 (s, 1H), 9.79 (s, 1H), 8.88 (s, 1H), 8.73 (d, *J* = 2.2 Hz, 1H), 8.03 (d, *J* = 10.7 Hz, 1H), 7.21 (s, 1H), 6.63 (s, 1H), 4.51 (t, *J* = 4.8 Hz, 2H), 4.29 (s, 4H), 3.97-3.91 (m, 4H), 3.86 (m, 2H), 3.73 (m, 2H), 3.65 (m, 16H), 3.60-3.56 (m, 12H), 3.53-3.50 (m, 4H), 3.38 (s, 3H), 3.35 (s, 3H), 3.35 (s, 3H), 1.49 (s, 9H). ¹³C NMR (150 MHz, CDCl₃, 298 K): δ 190.9, 162.1, 161.2, 155.0, 153.5, 136.8, 132.4, 130.5, 126.8, 123.3, 122.1, 114.1, 98.8, 72.0, 70.9, 70.8, 70.8, 70.7, 70.7, 70.6, 70.6, 70.6, 70.6, 70.5, 70.5, 69.6, 69.4, 69.3, 69.2, 69.0, 59.2, 59.1, 59.1, 28.4. HRMS (ESI): m/z Calcd for C₄₇H₇₆N₃O₂₀: 1002.5017 [M+H]⁺, found: 1002.5011.

Compound PEG-MC. To a 25 mL round-bottom flask were added compound **3-8** (100 mg, 0.100 mmol), trifluoroacetic acid (766 μL, 10.0 mmol), and chloroform (10 mL). The mixture was stirred at room temperature for 6.0 h, and the progress of the reaction was monitored by TLC until completion. The reaction mixture was washed successively with saturated sodium bicarbonate solution, water, and saturated brine. The crude product was concentrated under reduced pressure and purified by gel column chromatography using dichloromethane/methanol (1:1, v/v) as the eluent to afford compound **PEG-MC** as a pale yellow oily liquid (244 mg, 92%). ¹H NMR (600 MHz, CDCl₃, 298 K, with a small amount of TFA): δ 9.88 (s, 3H), 9.71 (s, 3H), 8.65 (d, *J* = 2.2 Hz, 3H), 8.45 (s, 3H), 8.19 (dd, *J* = 8.8, 2.2 Hz, 3H), 7.28 (d, *J* = 8.8 Hz, 3H), 6.73

(s, 3H), 4.53-4.49 (m, 8H), 4.34 (m, 12H), 4.04-4.00 (m, 8H), 3.95 (m, 12H), 3.88-3.83 (m, 18H), 3.80 (m, 20H), 3.74-3.70 (m, 54H), 3.47 (m, 18H), 1.58 (m, 27H). ¹³C NMR (150 MHz, CDCl₃, 298 K, with a small amount of TFA): δ 190.8, 162.7, 161.8, 161.3, 158.1, 154.3, 153.4, 146.7, 133.8, 128.4, 127.3, 126.7, 123.3, 122.6, 122.5, 114.4, 98.5, 72.0, 71.9, 70.9, 70.8, 70.7, 70.6, 70.5, 70.4, 70.3, 70.0, 69.7, 69.6, 69.5, 69.3, 69.2, 69.1, 68.6, 59.1, 59.0, 29.8. HRMS (ESI): m/z Calcd for C₁₂₆H₁₉₇Br₉O₅₁: 884.7731 [M+2H]²⁺ m/z, found m/z 884.7761.

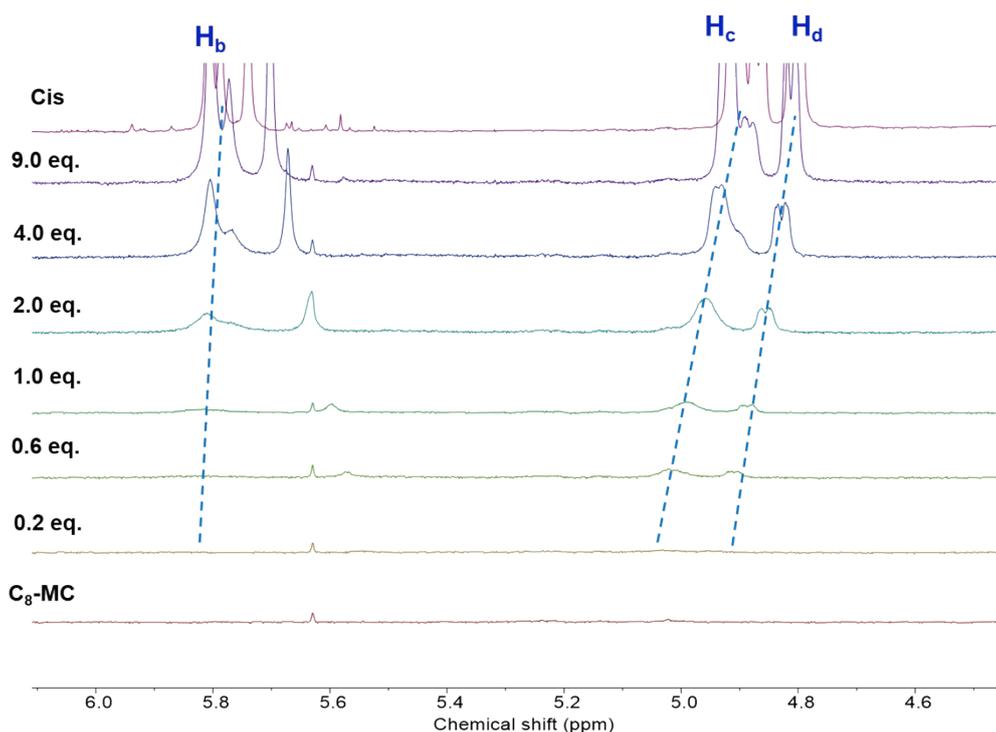


Figure S1. Partial ^1H NMR spectra (600 MHz, 298 K) of **C8-MC** (1.00 mM in CDCl_3 : $\text{DMSO-}d_6 = 4:1$, v/v) upon incremental addition of **Cis** (0-9 equiv).

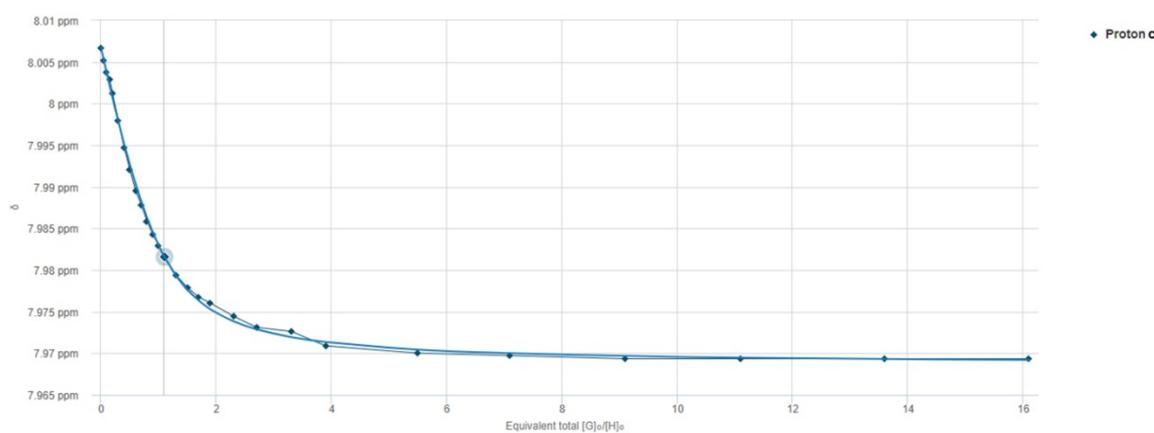


Figure S2. Nonlinear least-squares analysis of the ^1H NMR binding data (Figure 1a and S1) corresponding to the formation of **Cis** \subset **C8-MC** complexes. The data were fitted to a 1:1 (host: guest) binding model to give $K_a = 4.40 \times 10^3 \text{ M}^{-1}$. The residual distribution is shown below the binding isotherm. All solid lines were obtained from non-linear curve-fitting with the Nelder-Mead method to a 1: 1 binding model using the <http://supramolecular.org/> web applet.

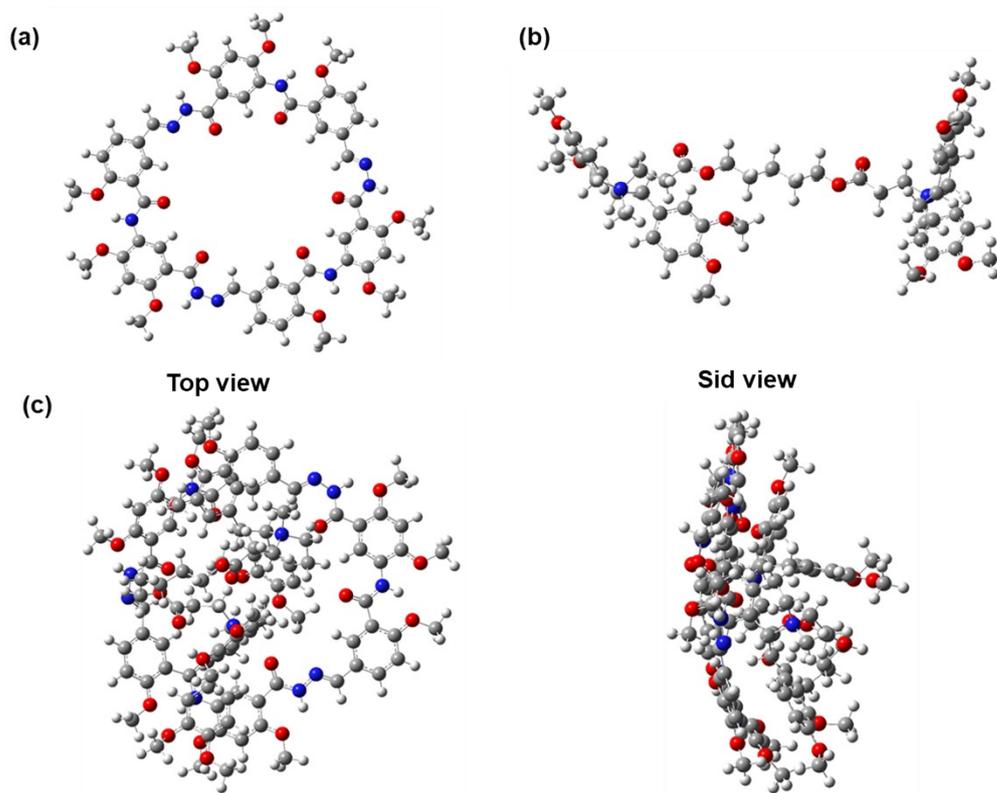


Figure S3. Optimized geometry of MC, Cis, and host-guest complex (Cis \subset MC) formed by MC and Cis, calculated in CHCl₃.

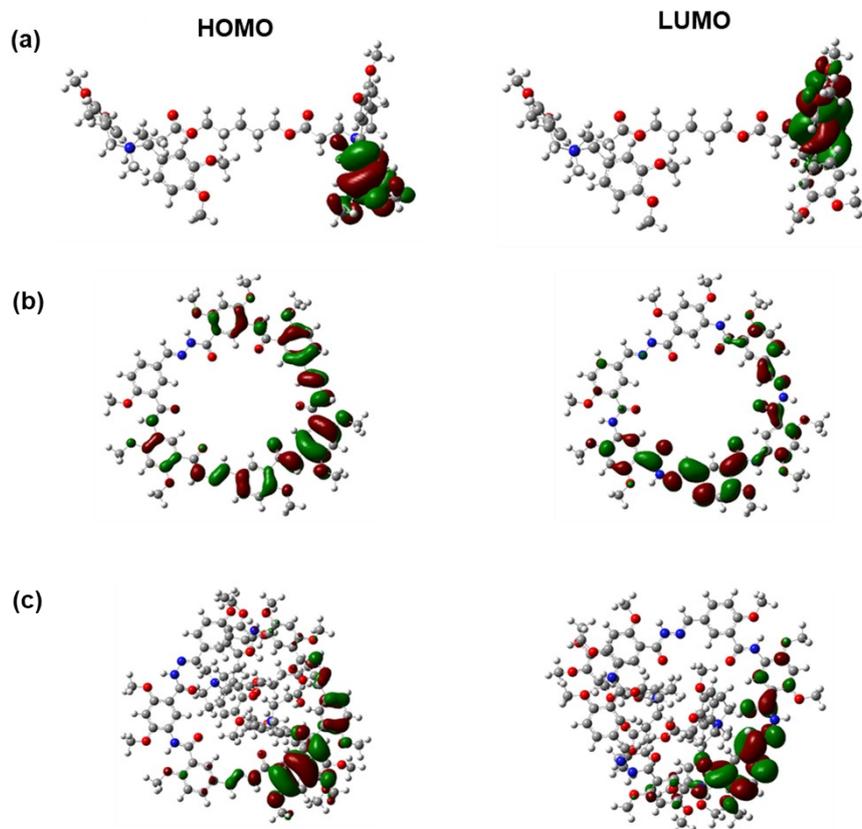


Figure S4. The electronic structures of Cis, MC and Cis \subset MC, calculated in CHCl₃.

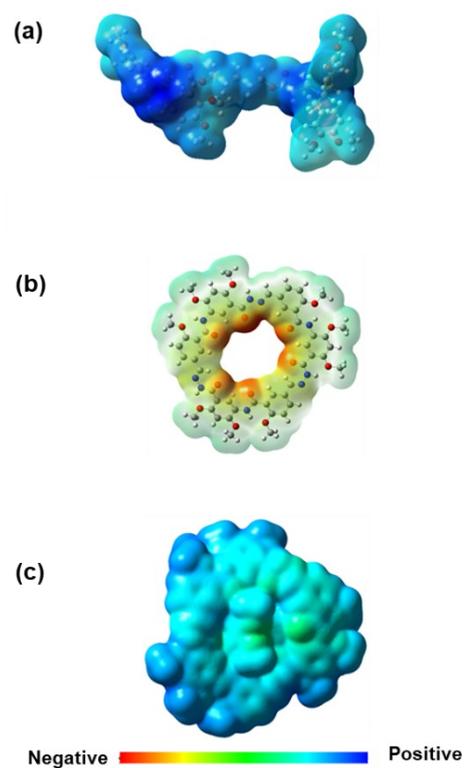


Figure S5. The molecular electrostatic surface potential (ESP) of (a) **Cis**, (b) **MC**, and (c) **Cis** \subset **MC**, calculated in CHCl_3 . The red part represents negative and the blue part represents positive.

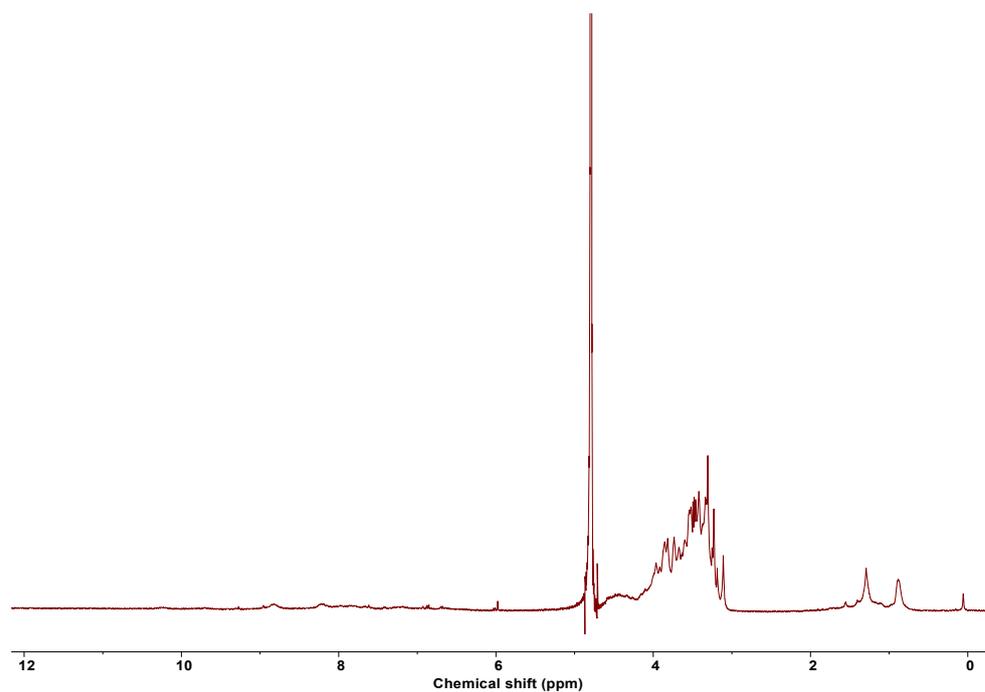


Figure S6. ^1H NMR spectrum (D_2O , 298 K, 600 MHz) of **PEG-MC**.

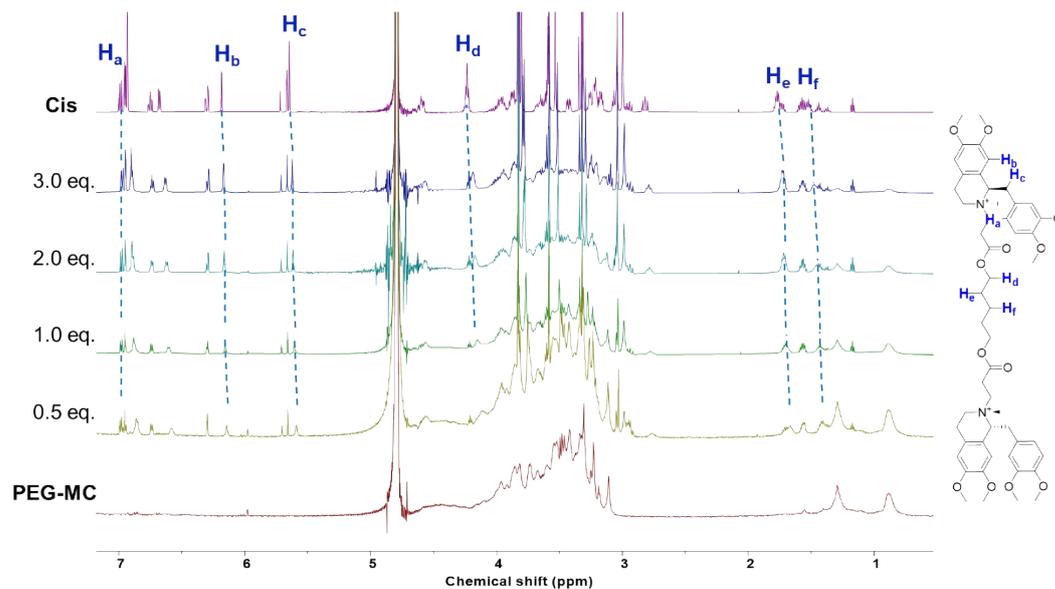


Figure S7. ^1H NMR spectra (D₂O, 298 K, 600 MHz) of PEG-MC upon incremental addition of Cis (0-3.0 equiv).

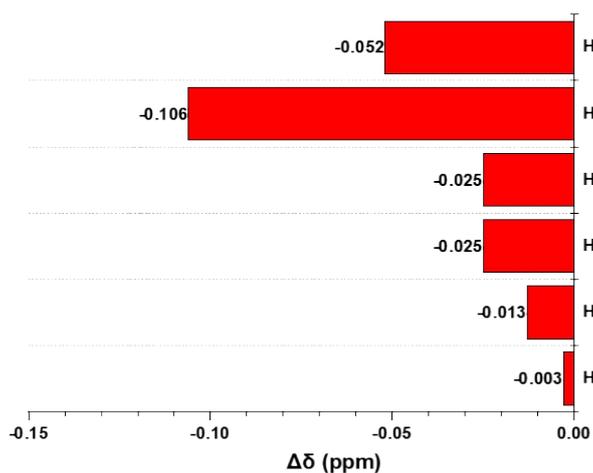


Figure S8. Normalized ^1H NMR chemical shift changes ($\Delta\delta = \delta_{\text{obsd}} - \delta_{\text{bound}}$) of Cis protons as a function of the guest concentration titrated into a 1.0 mM solution of PEG-MC.

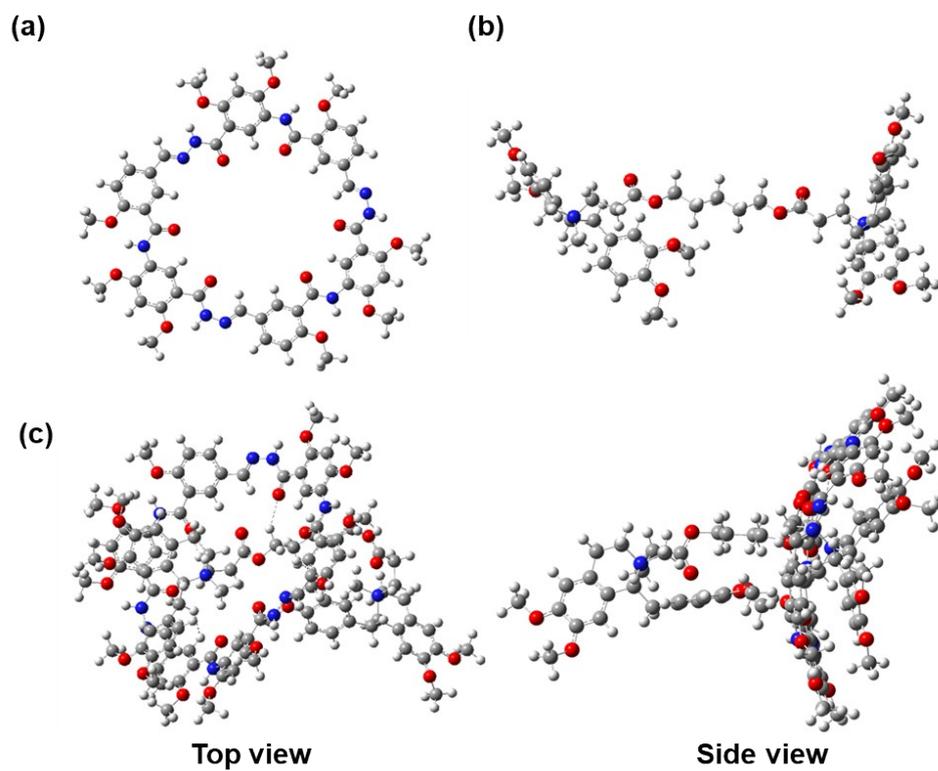


Figure S9. Optimized geometry of MC, Cis, and host-guest complex (Cis \subset MC) formed by MC and Cis, calculated in water.

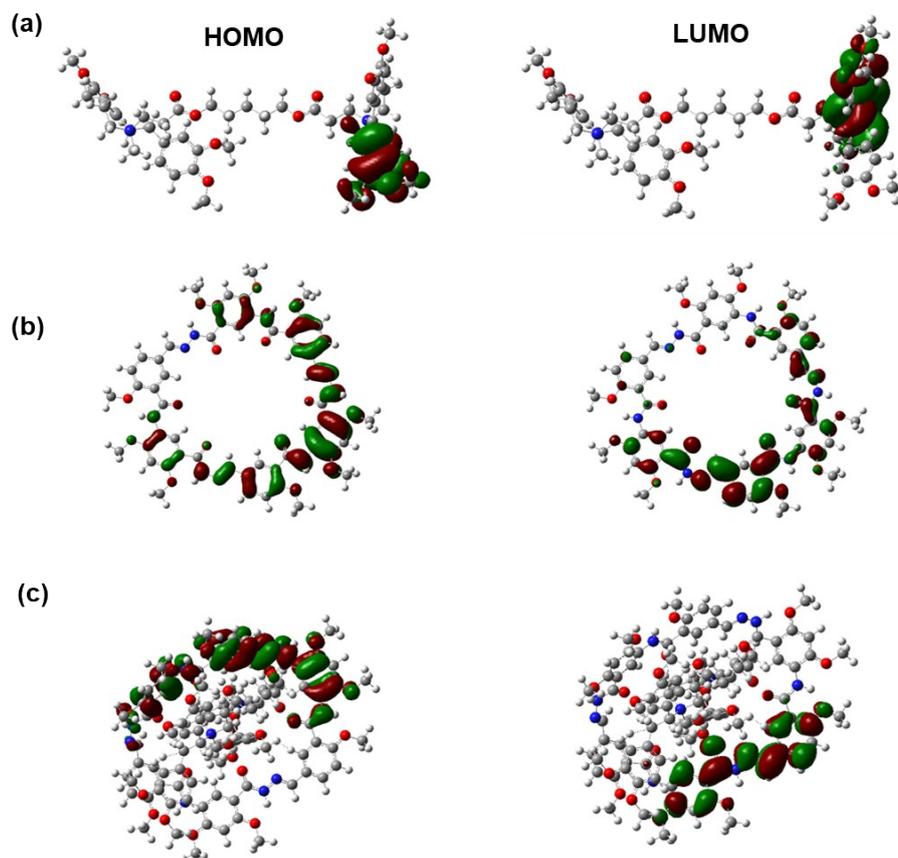


Figure S10. The electronic structures of Cis, MC and Cis \subset MC, calculated in water.

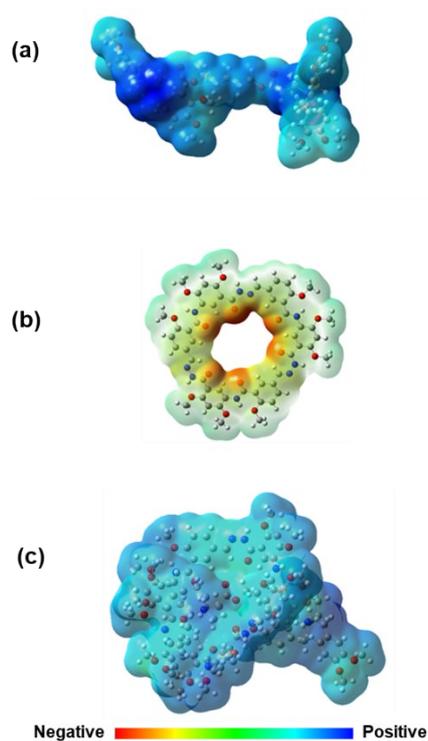


Figure S11. The molecular electrostatic surface potential (ESP) of (a) **Cis**, (b) **MC**, and (c) **Cis** \subset **MC**, calculated in water. The red part represents negative and the blue part represents positive.

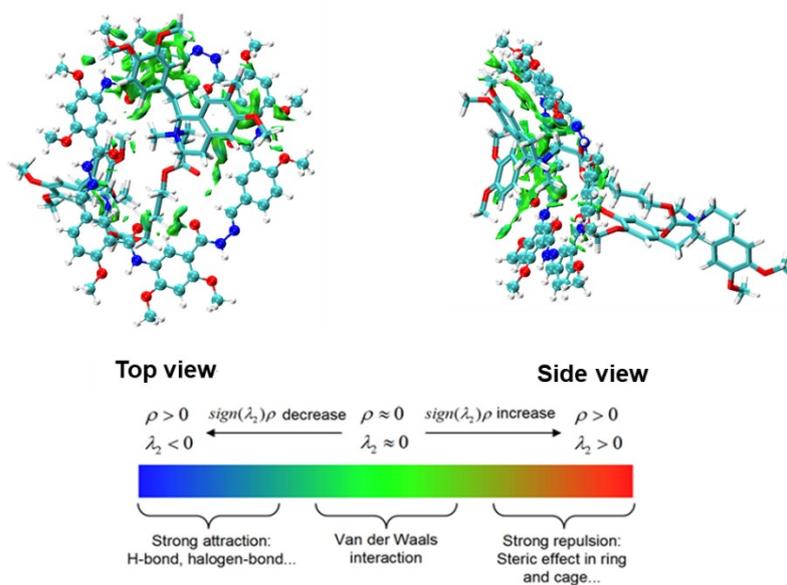


Figure S12. The noncovalent binding surfaces of **Cis** \subset **MC**, calculated in water.

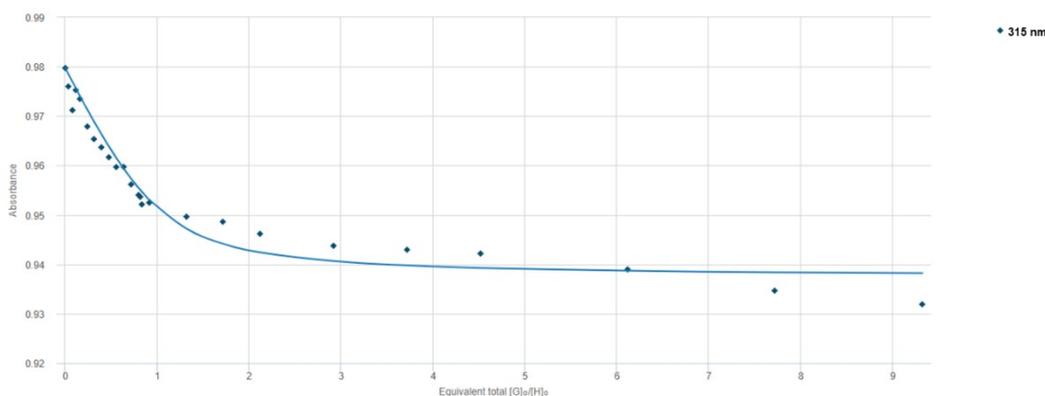


Figure S13. Nonlinear least-squares fitting of the absorbance changes of **PEG-MC** at 315 nm during titration experiments of **PEG-MC** with model guest **Cis**. The data were fitted to a 1:1 (host: guest) binding model to give $K_a = 5.98 \times 10^5 \text{ M}^{-1}$. All solid lines were obtained from non-linear curve-fitting with the L-BFGS-B method to a 1:1 binding model using the <http://supramolecular.org/> web applet.

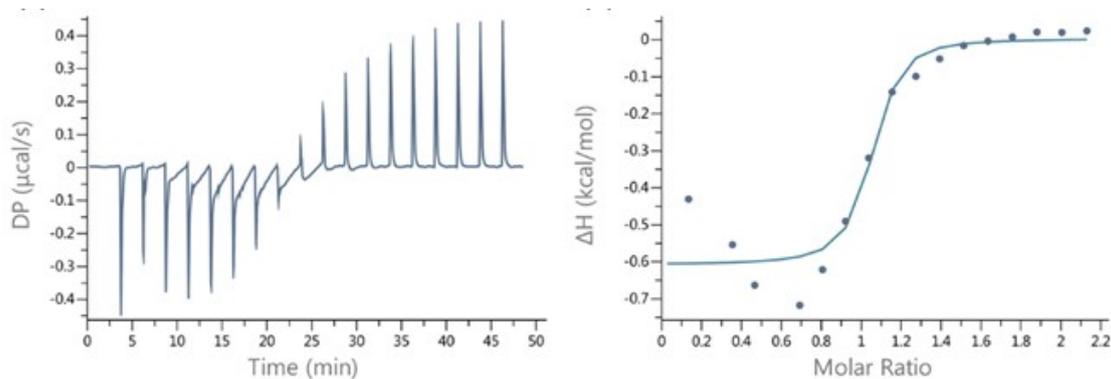


Figure S14. (a) Plot of DP vs time from the titration of **PEG-MC** (0.10 mM) and with **Cis** (0.01 mM) in H_2O ; (b) plot of ΔH as a function of molar ratio. The solid line represents the best non-linear fit of the data to a 1:1 binding model ($K_a = 5.58 \times 10^5 \text{ M}^{-1}$, $\Delta H = -0.530 \text{ kcal/mol}$, $\Delta G = -7.87 \text{ kcal/mol}$, $\Delta S = -2.46 \times 10^{-2} \text{ kcal mol}^{-1} \text{ K}^{-1}$).

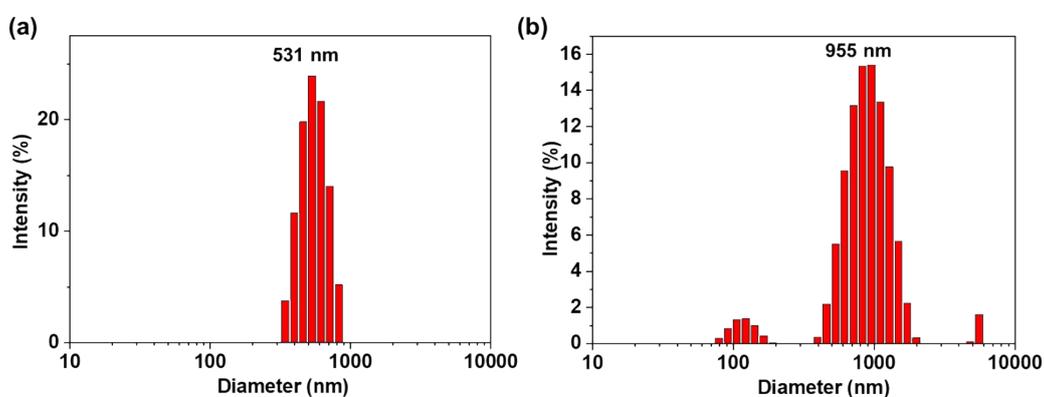


Figure S15. DLS size distribution of (a) **PEG-MC** and (b) **Cis** \subset **PEG-MC** ($[\text{PEG-MC}] = 1.0 \times 10^{-5} \text{ M}$).

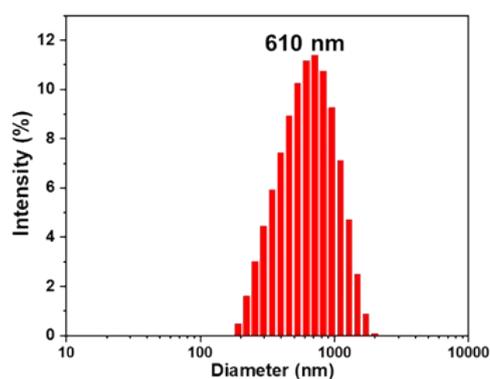


Figure S16. DLS size distribution of freshly prepared **PEG-MC** dispersed in H₂O after storage at room temperature for 10 days([**PEG-MC**] = 1.0×10^{-5} M).

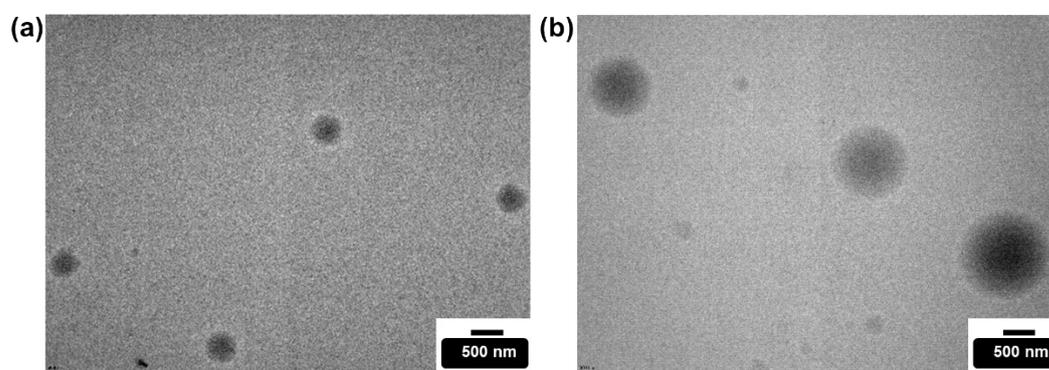


Figure S17. TEM images of (a) **PEG-MC** and (b) **Cis c PEG-MC**.

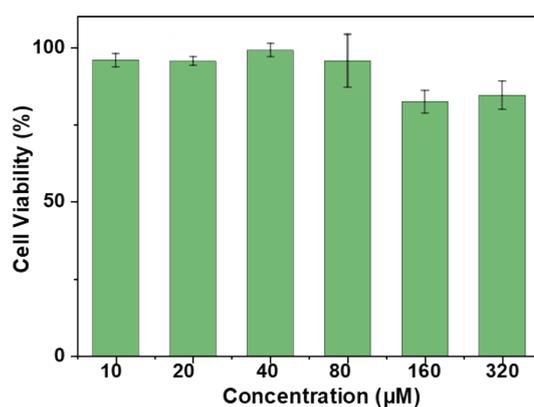


Figure S18. *In vitro* cell viability of 293T cells after 24 h treatment with **PEG-MC**. Data points represent mean \pm SD (n = 6).

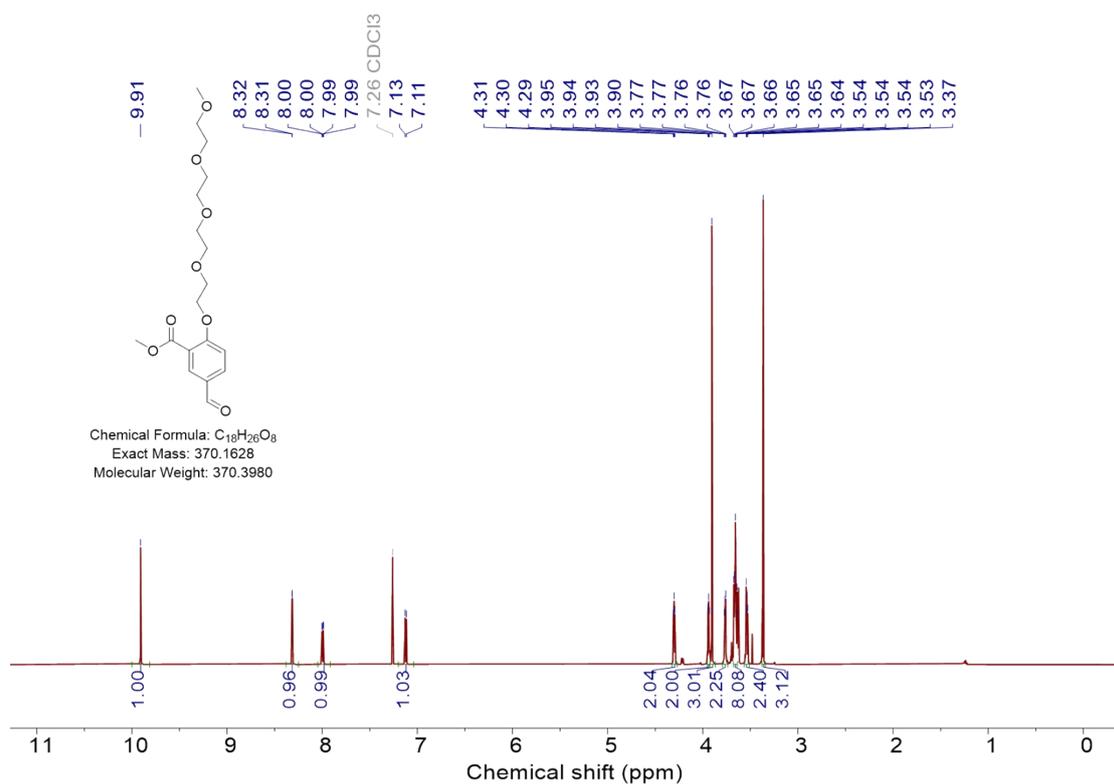


Figure S19. ¹H NMR spectrum (600 MHz, CDCl₃, 298 K) of compound **2**.

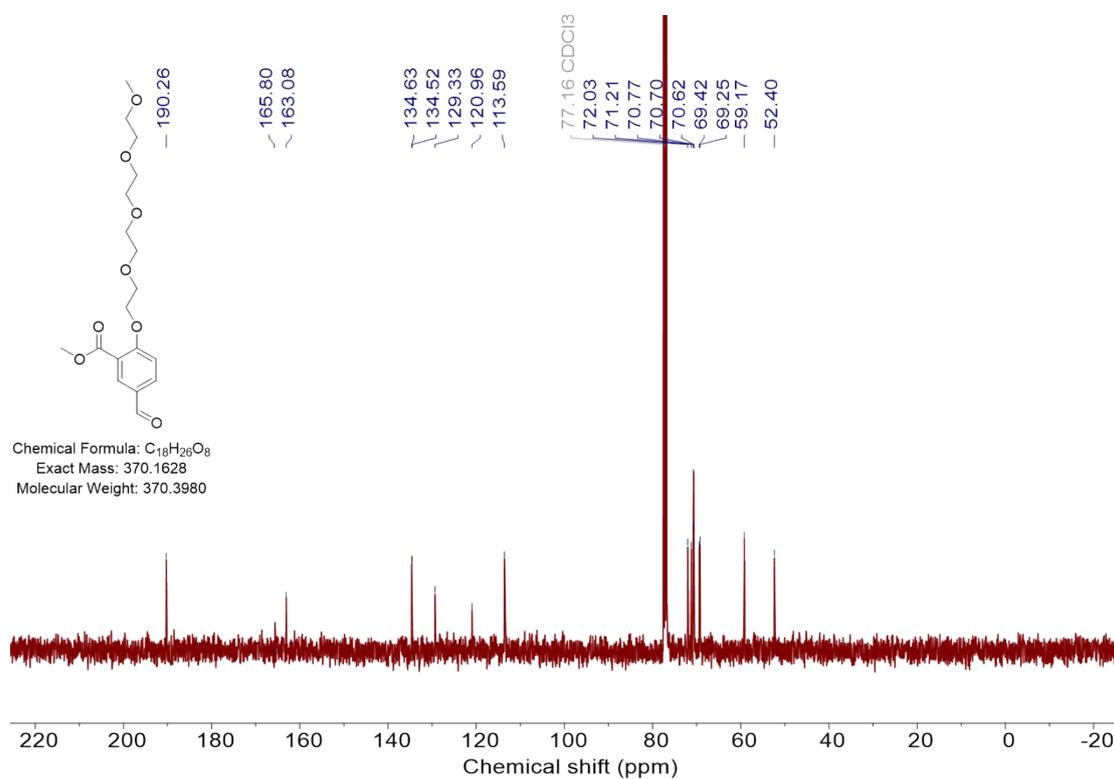


Figure S20. ¹³C NMR spectrum (150 MHz, CDCl₃, 298 K) of compound **2**.

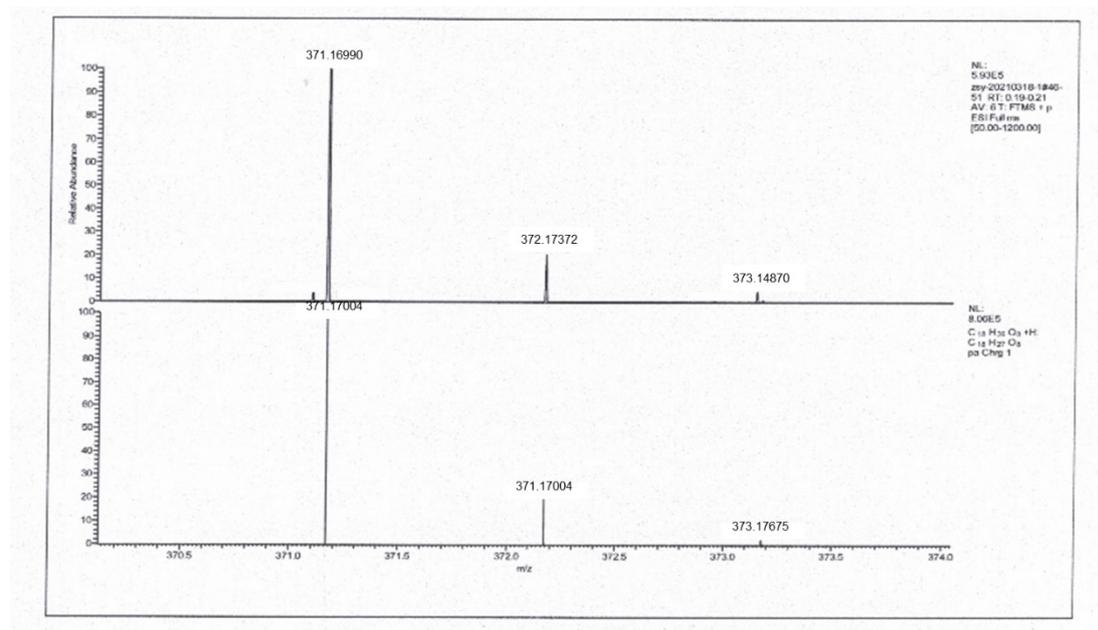


Figure S21. Mass spectrum of compound 2.

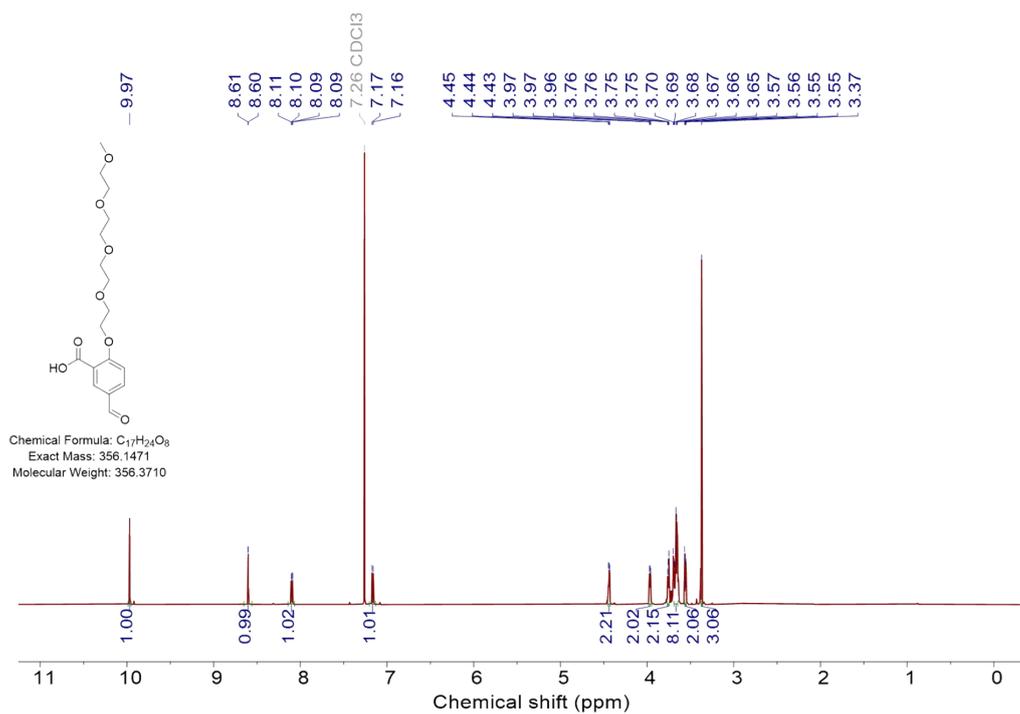


Figure S22. ¹H NMR spectrum (600 MHz, CDCl₃, 298 K) of compound 3.

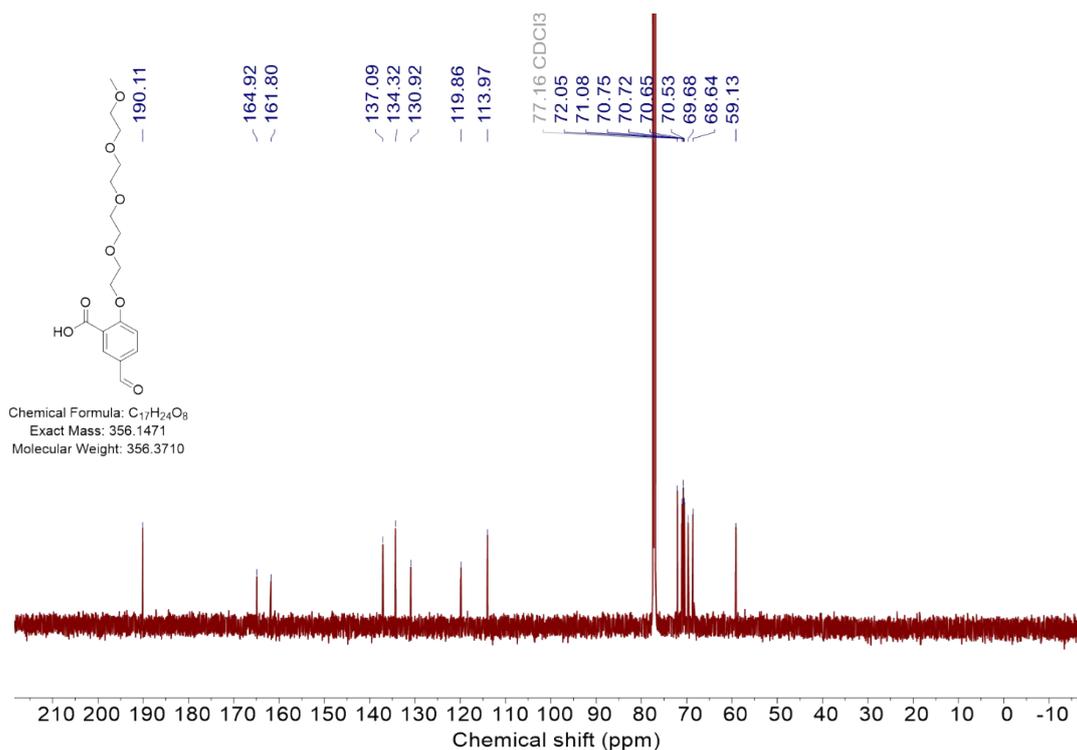


Figure S23. ¹³C NMR spectrum (150 MHz, CDCl₃, 298 K) of compound **3**.

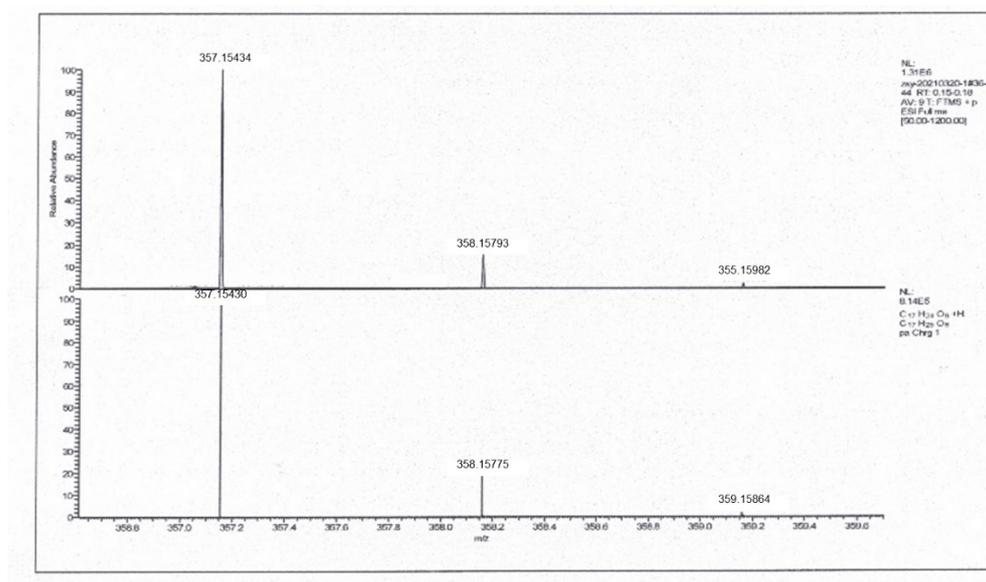


Figure S24. Mass spectrum of compound **3**.

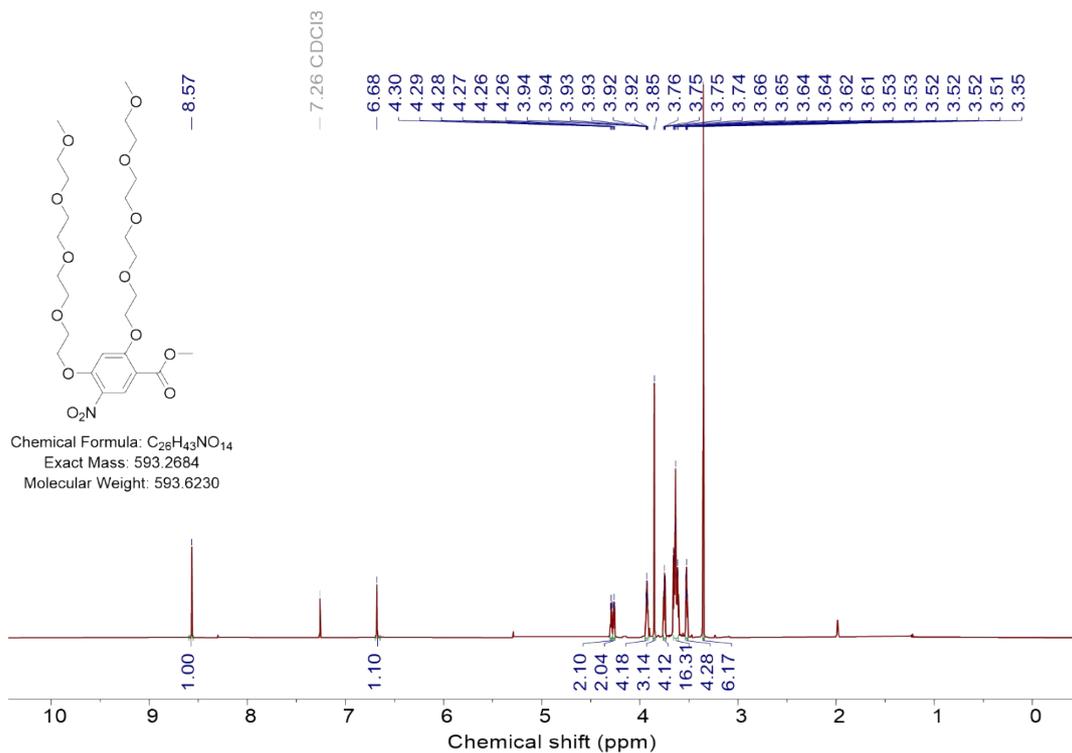


Figure S25. ^1H NMR spectrum (600 MHz, CDCl_3 , 298 K) of compound **5**.

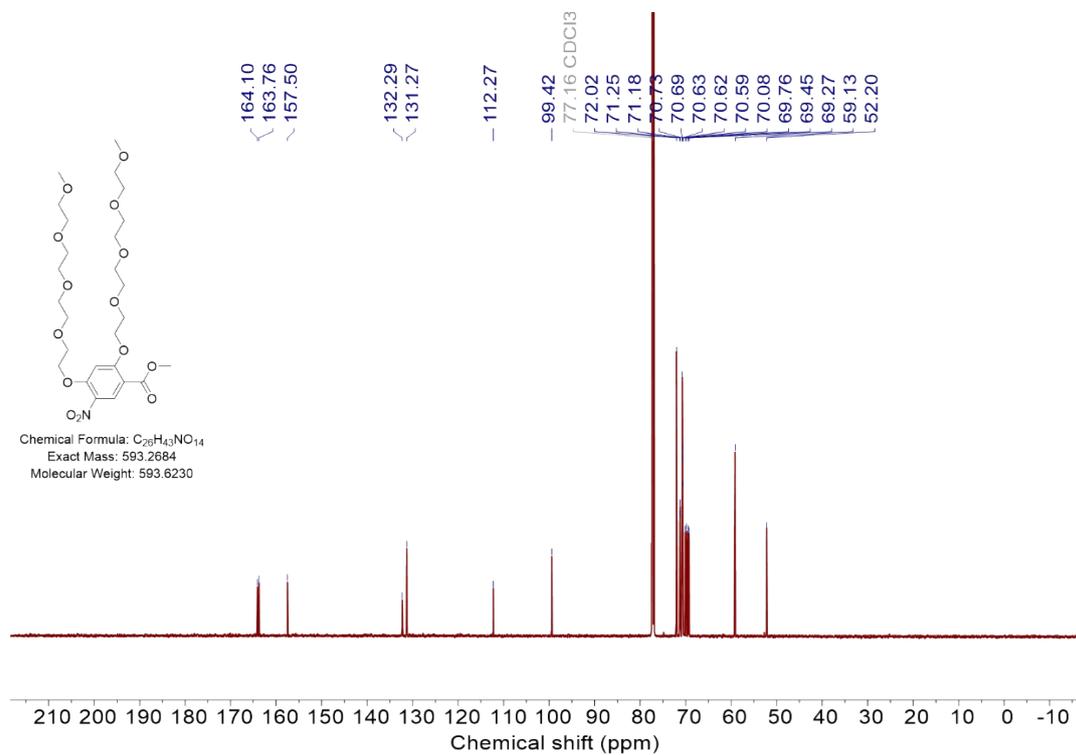


Figure S25. ^{13}C NMR spectrum (150 MHz, CDCl_3 , 298 K) of compound **5**.

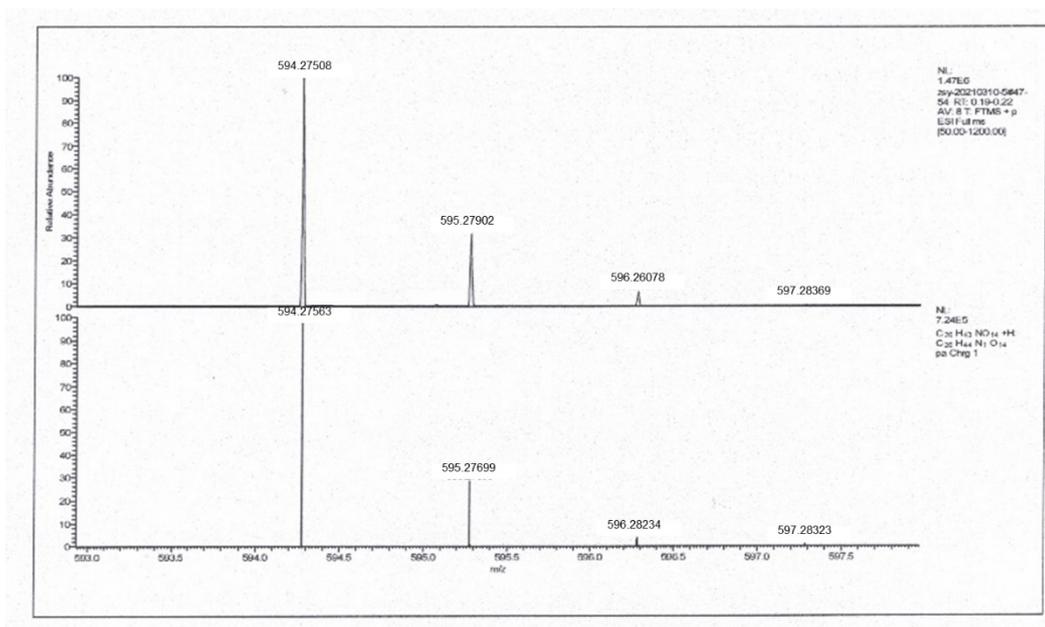


Figure S26. Mass spectrum of compound 5.

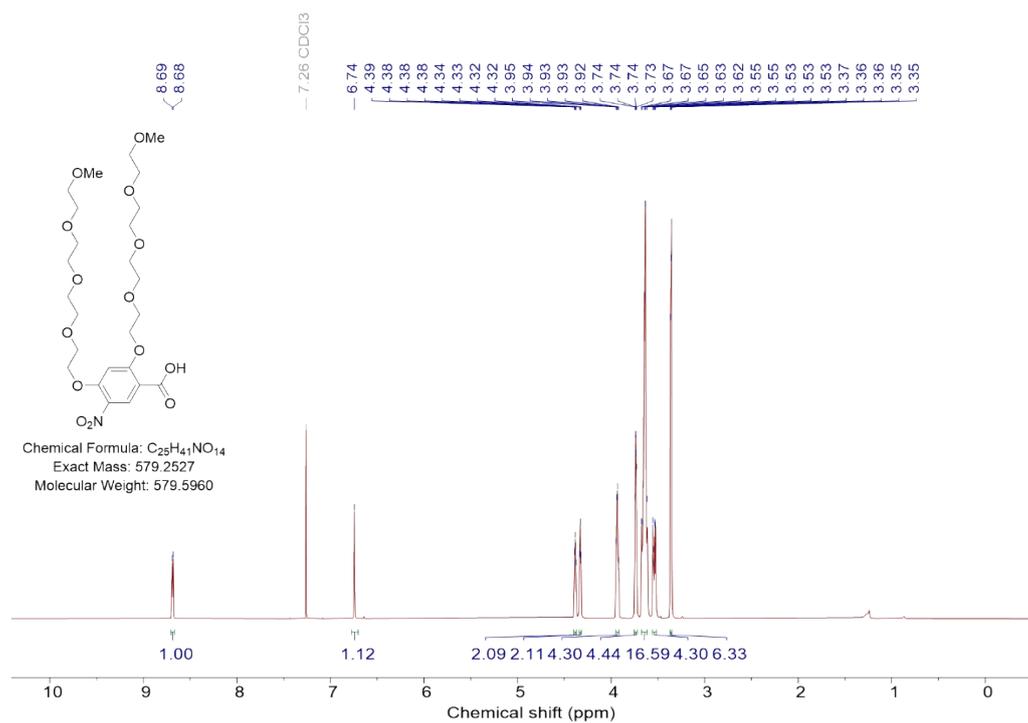


Figure S27. 1H NMR spectrum (600 MHz, $CDCl_3$, 298 K) of compound 6.

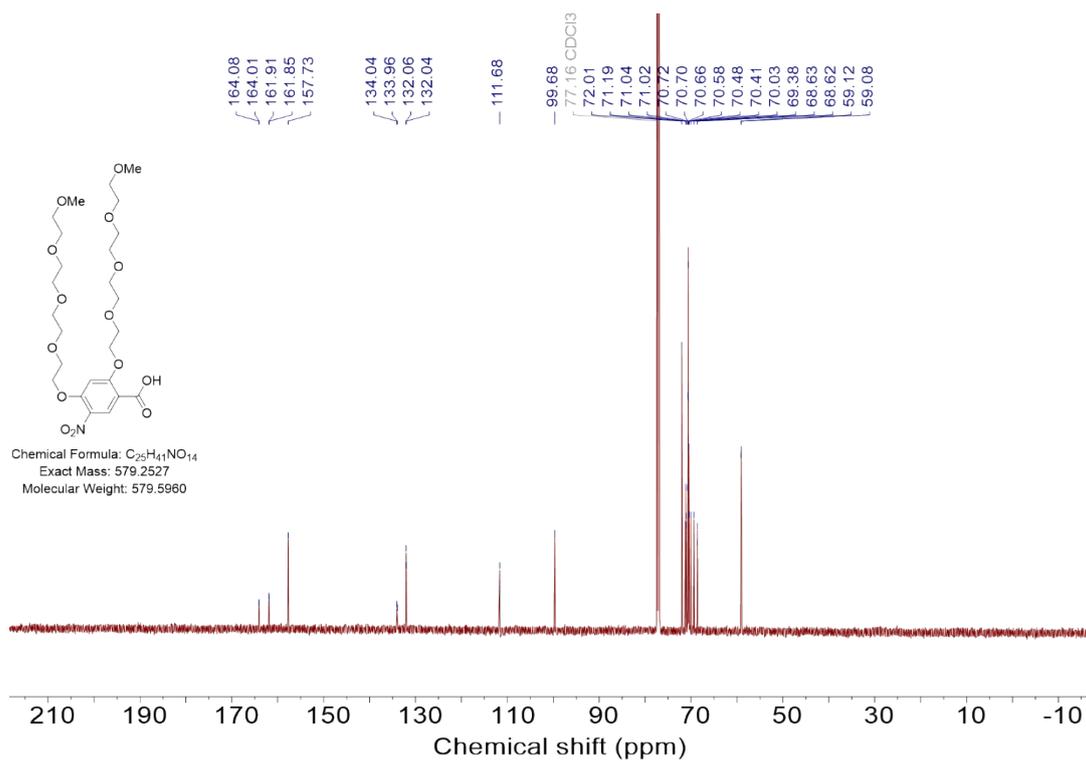


Figure S28. ¹³C NMR spectrum (150 MHz, CDCl₃, 298 K) of compound 6.

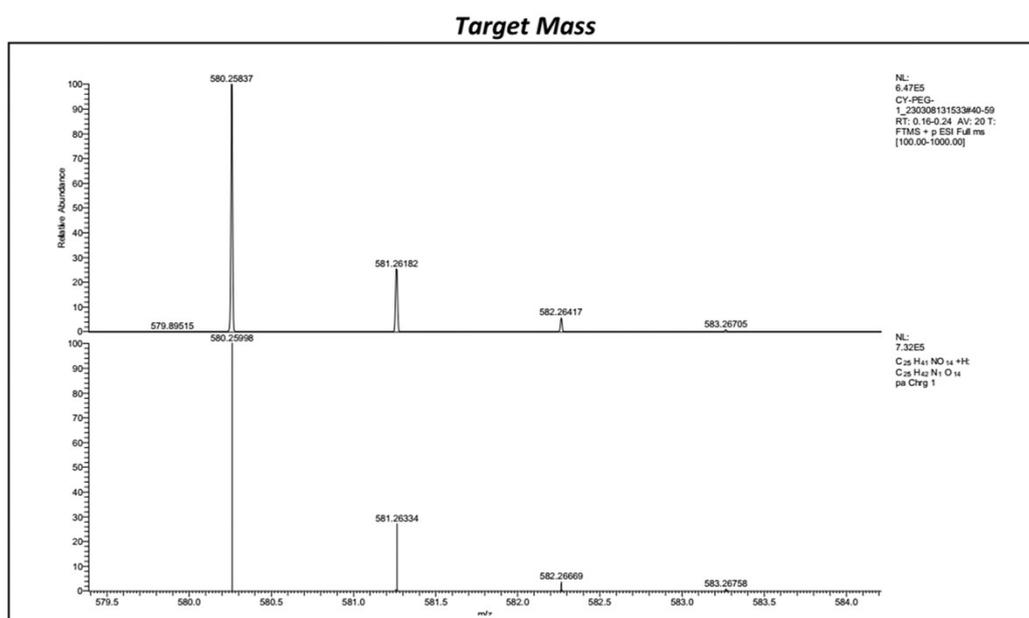


Figure S29. Mass spectrum of compound 6.

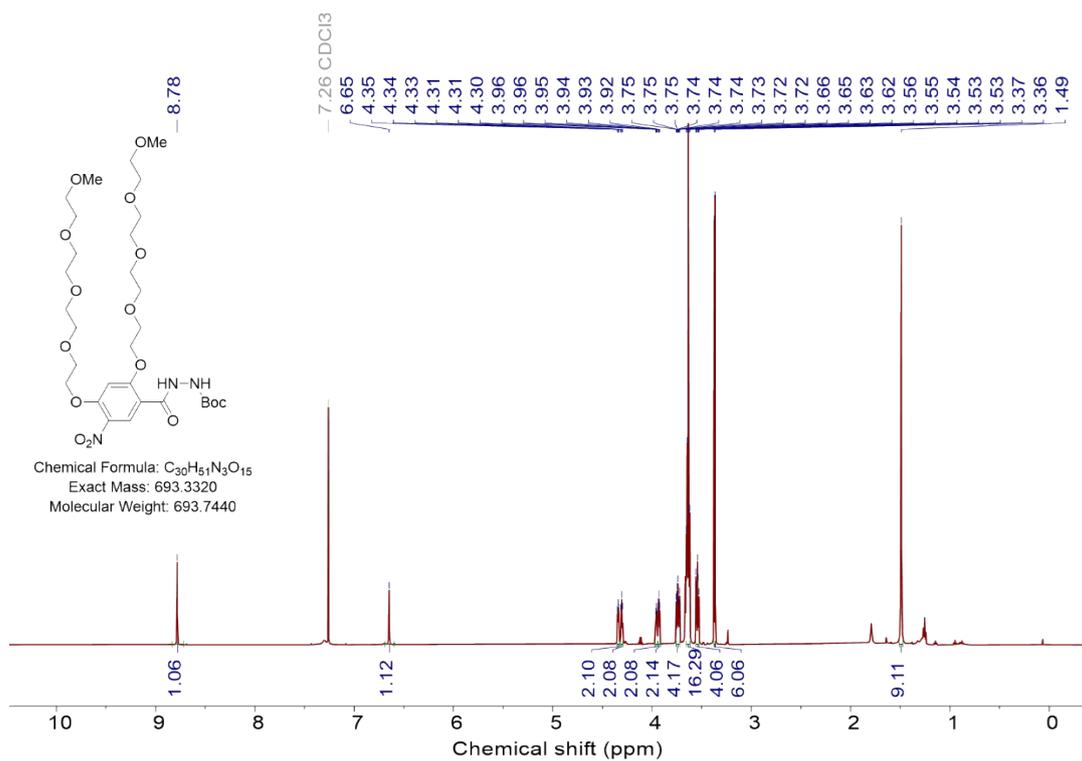


Figure S30. ^1H NMR spectrum (600 MHz, CDCl_3 , 298 K) of compound 7.

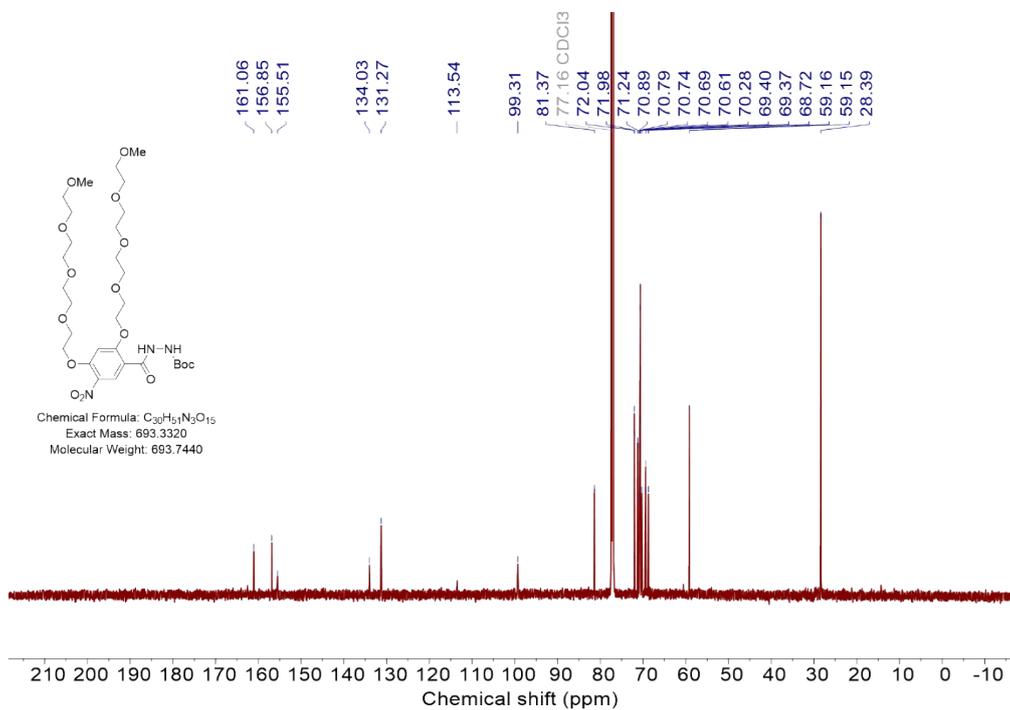


Figure S31. ^{13}C NMR spectrum (150 MHz, CDCl_3 , 298 K) of compound 7.

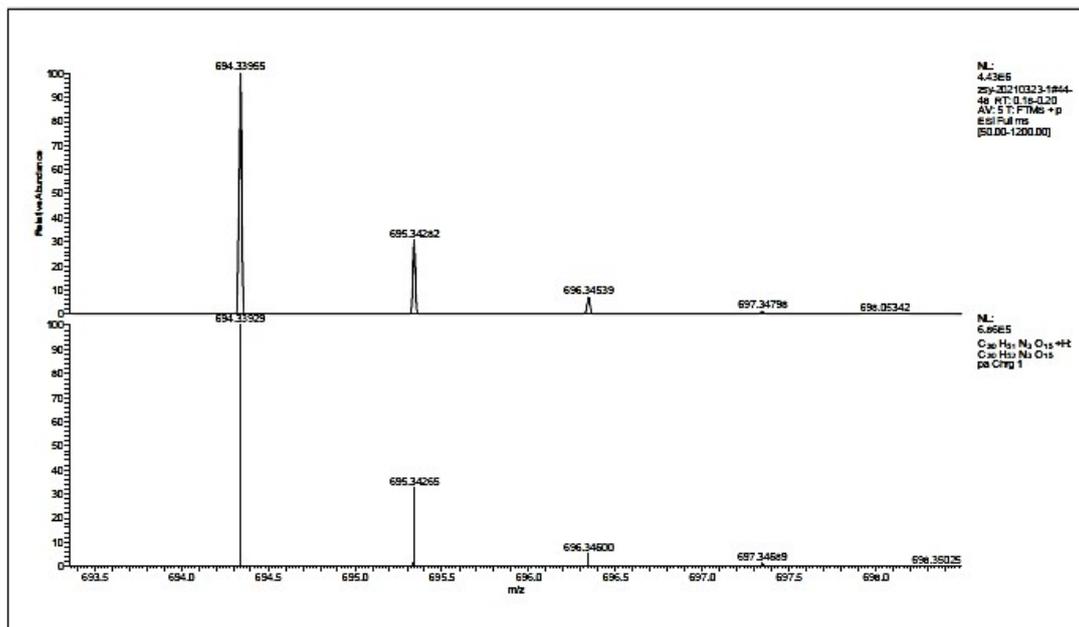


Figure S32. Mass spectrum of compound 7.

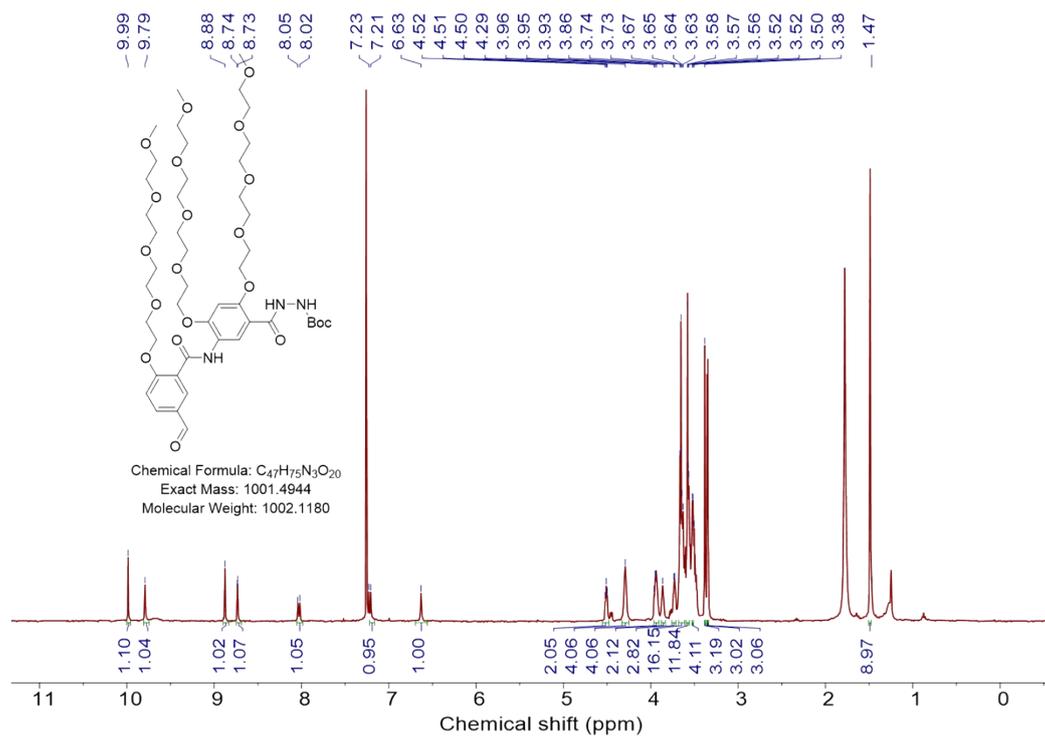


Figure S33. ¹H NMR spectrum (600 MHz, CDCl₃, 298 K) of compound 8.

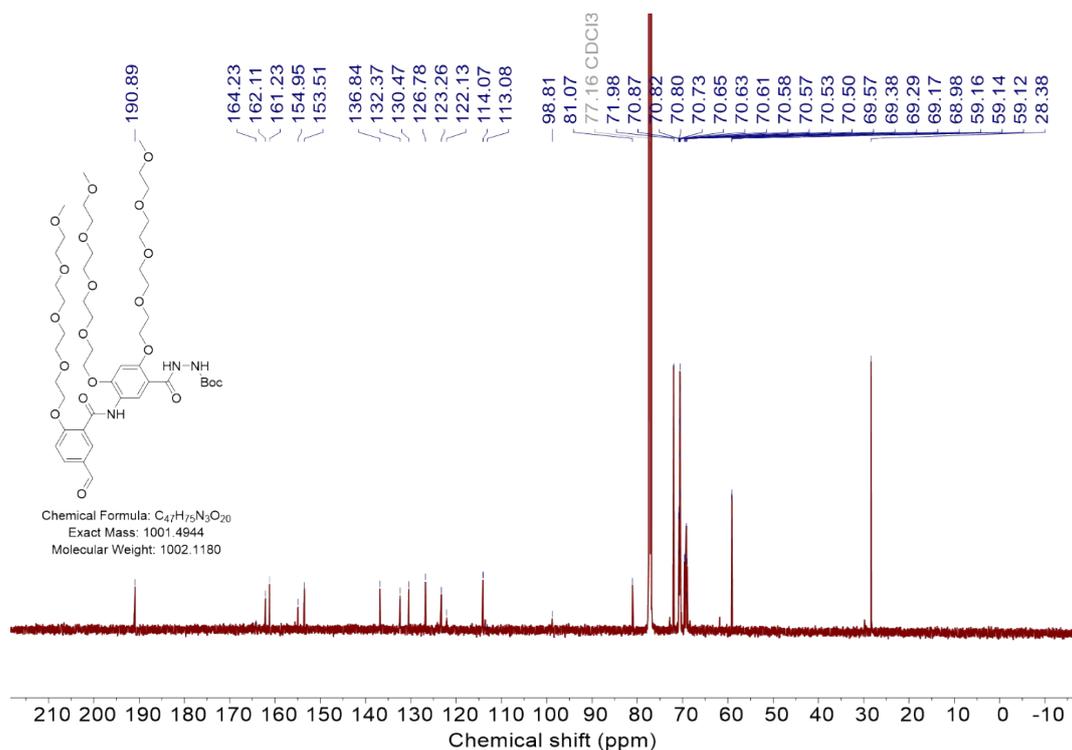


Figure S34. ¹³C NMR spectrum (150 MHz, CDCl₃, 298 K) of compound 8.

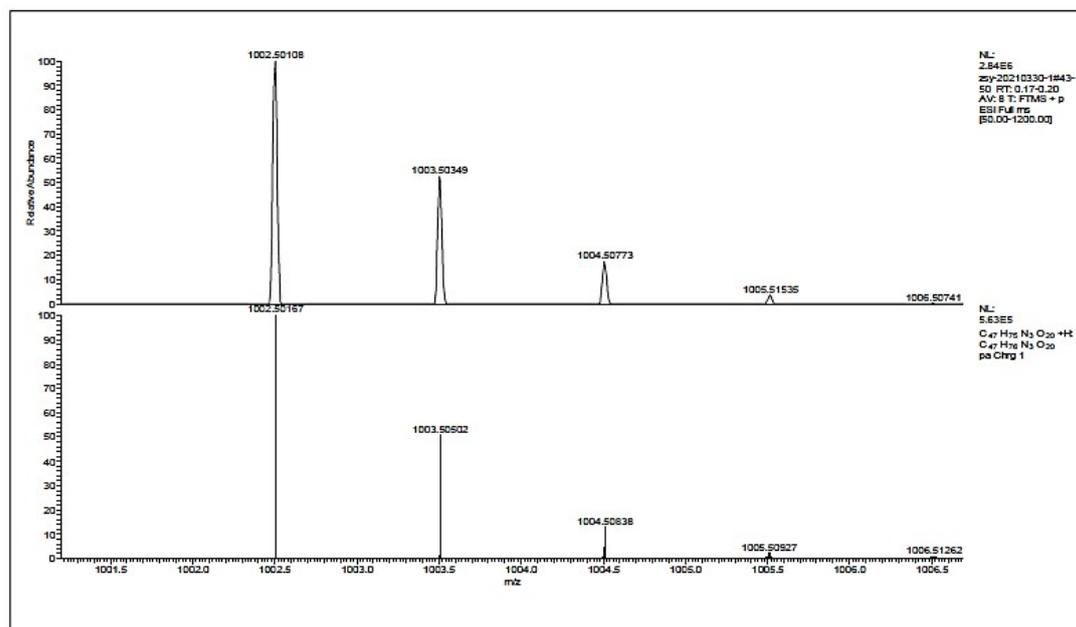


Figure S35. Mass spectrum of compound 8.

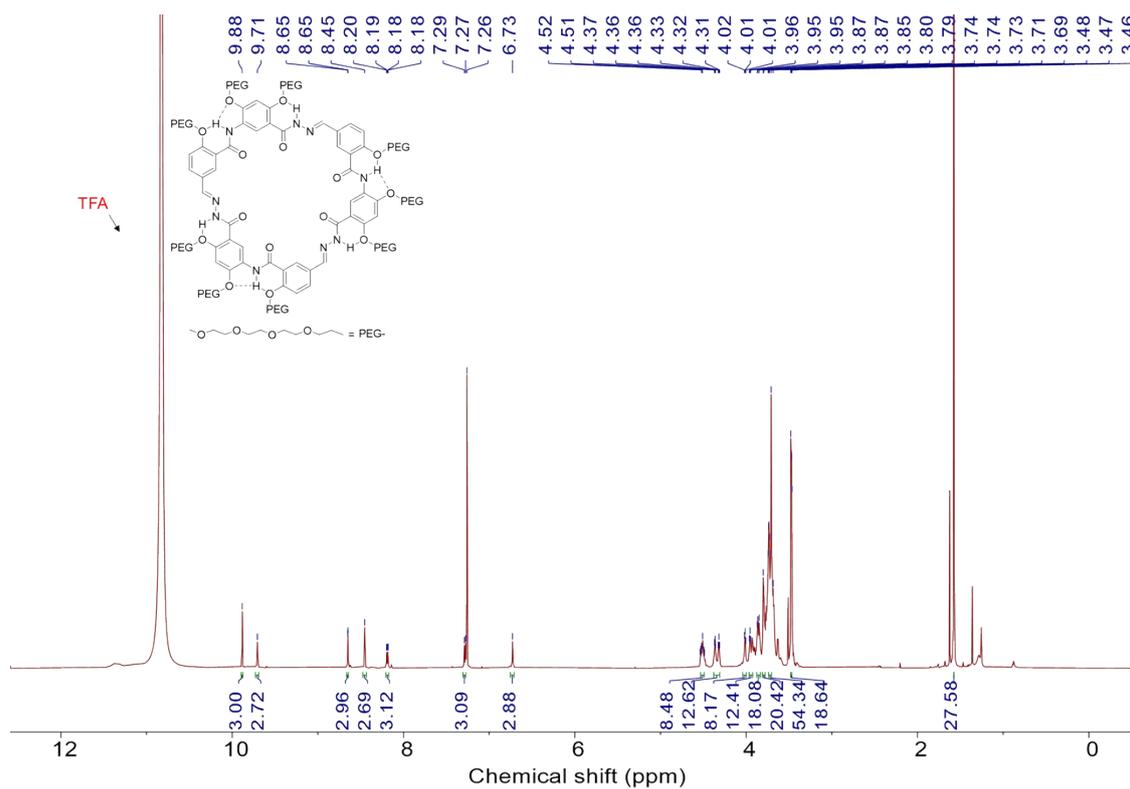


Figure S36. ^1H NMR spectrum (600 MHz, 298 K, CDCl_3 with a small amount of TFA) of compound PEG-MC.

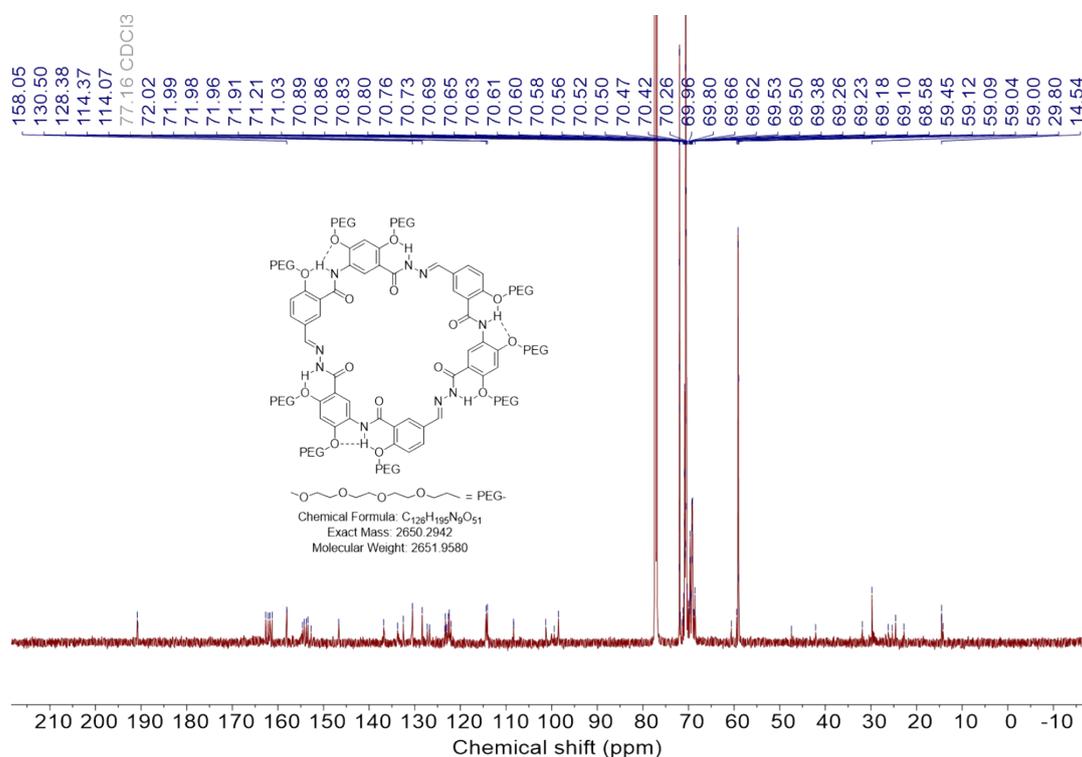


Figure S37. ^{13}C NMR spectrum (600 MHz, 298 K, CDCl_3 with a small amount of TFA) of compound PEG-MC.

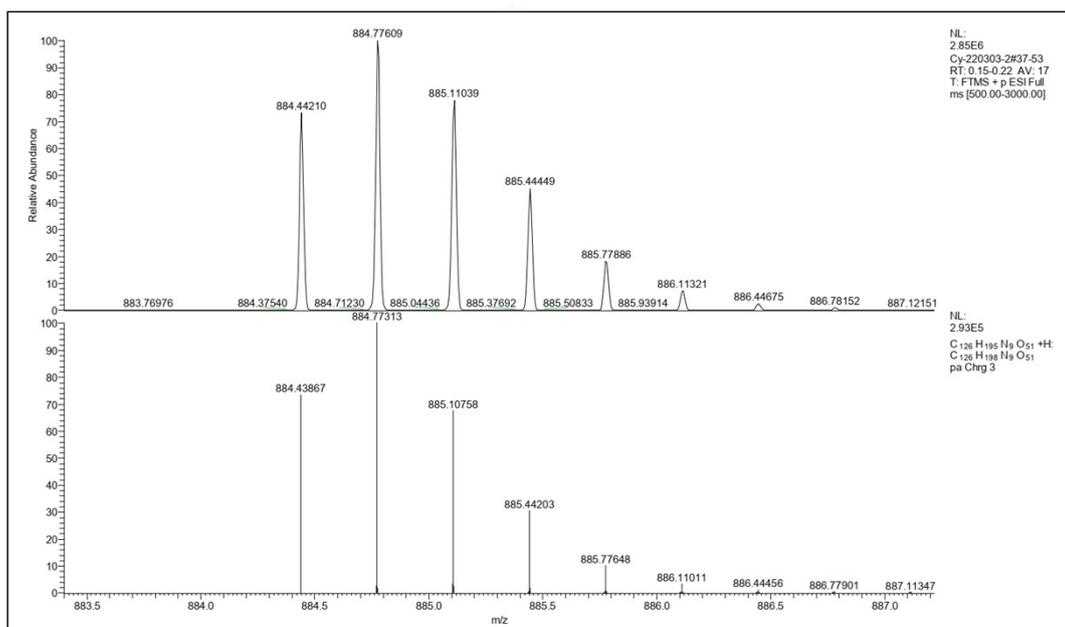


Figure S38. Mass spectrum of compound PEG-MC.

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