SUPPORTING INFORMATION

A traceless linker for aliphatic amines that rapidly and quantitatively fragments after reduction

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Experimental Procedures

All commercially available compounds were purchased from Sigma Aldrich unless otherwise stated.All solvents were purchased dry from Sigma Aldrich with a Sure/Seal system. NMR spectra were recorded on a Bruker Avance 400 (400 MHz for ^{1}H; 101 MHz for ^{13}C) or Bruker's 900 MHz US2 (900 MHz for ^{1}H; 226 MHz for ^{13}C) spectrometer. The chemical shifts (δ) are given in parts per million relative to $CDCl_3$ (7.26 ppm for ^{1}H) $CDCl_3$ (77.16 ppm for ^{13}C). Flash column chromatography was performed on silica gel (particle size 200-300 mesh, purchased from SiliCycle Inc. Canada). CPP-azide (FITC-R10K1 azide) was purchased from GenScript (860 Centennial Ave. Piscataway, NJ 08854 USA). Inductively Coupled Plasma (ICP) was recorded on Perkin Elmer 5300 DV optical emission ICP with auto sampler. High-performance liquid chromatography (HPLC) analysis were carried out on Shimadzu LC-6AD with Solvent A: Solvent B gradients between 5:95 (Solvent A: H_2O with 0.1 % TFA; Solvent B: CH_3CN with 0.1 % TFA). HEK293T eGFP cells were a generous gift from GenEdit.

Release mechanism for the reduction sensitive linkers DEC, DCB and DTB

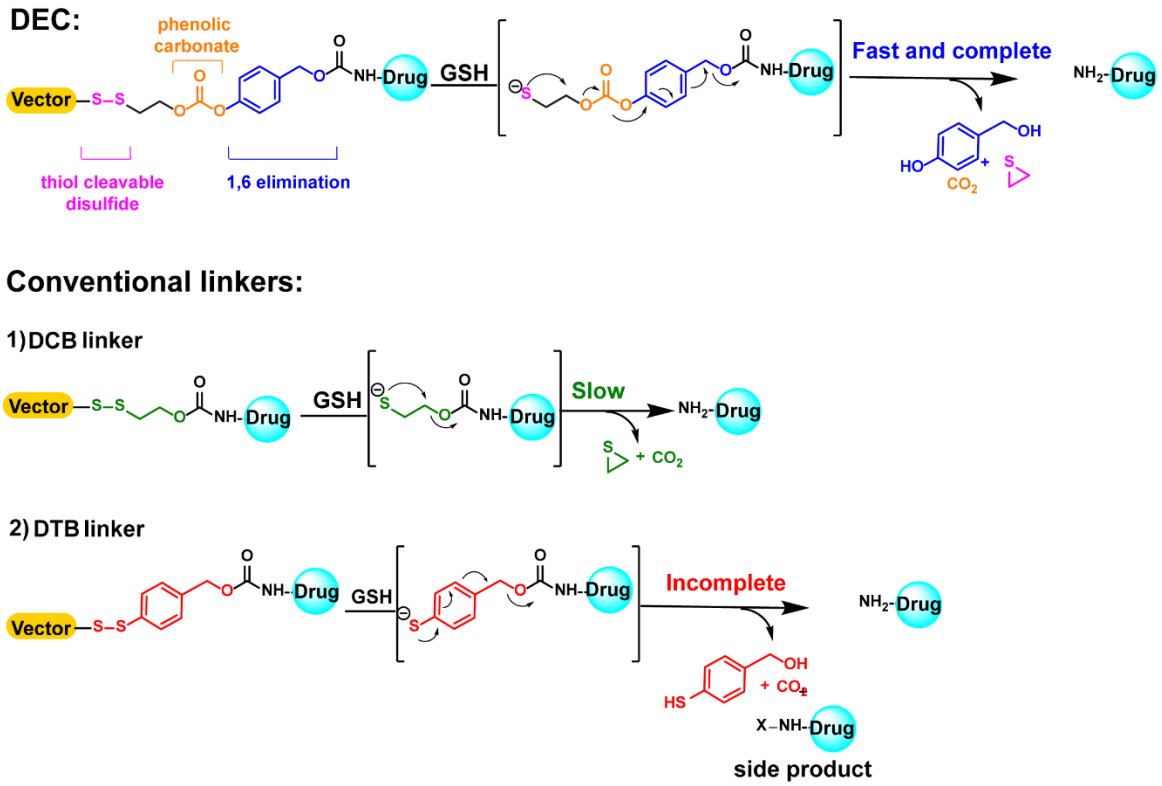


Fig. S1 DEC is composed of a disulfide linkage, a phenolic carbonate and a 1,6-elimination linker. Reduction of DEC generates a free thiol, which cyclizes and generates a phenol, which then releases the amine via a 1,6 elimination. DEC releases amines rapidly and quantitatively after reduction. In contrast, existing DTB and DCB linkers are either too slow, or undergo side reactions that results in incomplete release of the amine.

Synthesis of DEC and PEG-DEC-Cipro (Fig. S2)

Synthesis of Compound 2 (DCB).

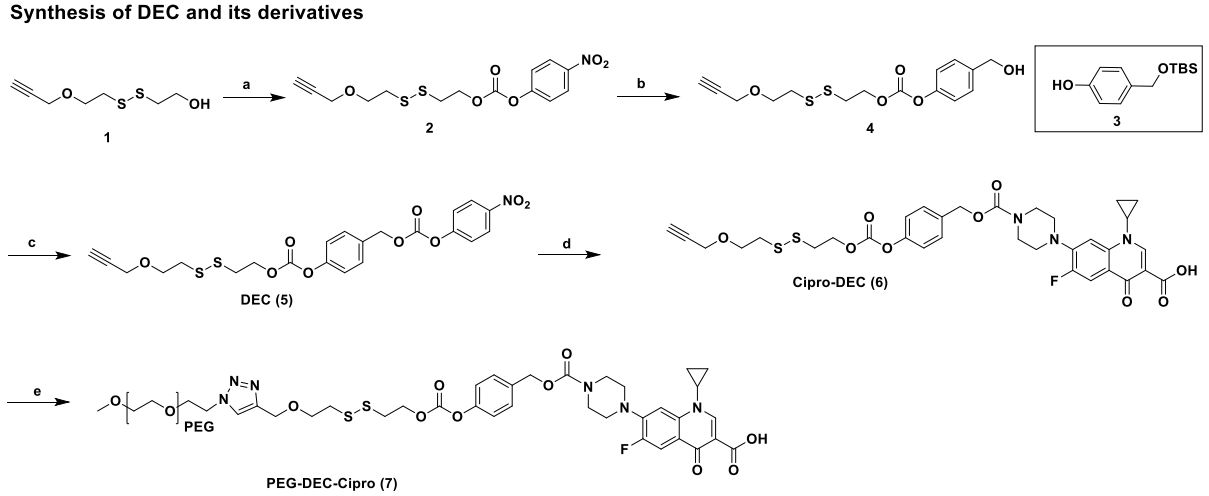


Fig. S2 Synthesis of DEC and PEG-DEC-Cipro. a) 4-Nitrophenyl chloroformate, pyridine, CH_3CN, 0 °C to rt, 18 h, 78.6%. b) I. Compound **3**, DMAP, Et_3N, THF, rt, 18 h, 70.1%; II. 1.2 M HCl, rt, 2 h, 80.0% for 2 steps. c) 4-Nitrophenyl chloroformate, pyridine, CH_2Cl_2, rt, 3 h, 90.0%. d) Ciprofloxacin, Et_3N, DMF, rt, 48 h, 80.1%. e) PEG2000 azide, CuI, DIPEA, DMF, N_2, rt, 12 h; 76.3%.

To a solution of compound **1**[1] (513.41 mg, 2.67 mmol) in dry CH_2Cl_2 (25 mL) was added pyridine (0.32 mL, 4.0 mmol), the solution was cooled to 0 °C, 4-Nitrophenyl chloroformate (644.99 mg, 3.2 mmol) was added, the mixture was stirred at room temperature overnight, then the solvent was evaporated and the residue was purified by flash chromatography (hexane: ethyl acetate 5:1) to afford compound **2** as a colorless oil. m= 750.1 mg, yield 78.6%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.32 – 8.25 (m, 2H), 7.47 – 7.34 (m, 2H), 4.61 – 4.51 (m, 2H), 4.21 (t, *J* = 2.1 Hz, 2H), 3.90 – 3.73 (m, 2H), 3.14 – 3.00 (m, 2H), 3.01 – 2.90 (m, 2H), 2.47 (q, *J* = 2.2 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 155.4, 152.3, 145.5, 125.4, 121.8, 79.3, 74.9, 68.0, 66.9, 58.3, 38.7, 36.7. ESI m/z calcd. for $C_{14}H_{16}NO_6S_2^+$ $[M + H]^+$: 358.0419, found 358.0432.

Synthesis of Compound 4.

Step 1: Compound **2** (357.2 mg, 1.0 mmol) was dissolved in 10 mL of anhydrous THF, to this solution was added DMAP (122.2 mg, 1.0 mmol) and Et_3N (0.7 mL, 5.0 mmol), the solution was brought to 0 °C and compound **3**[2] (250.32 mg, 1.05 mmol) was added. The reaction mixture was stirred at 0 °C for 30 min then warmed to room temperature and stirred overnight, the solvent was then removed by vacuum. The residue was purified by column chromatography (hexane: ethyl acetate 10:1) to afford the product as a colorless oil. m= 320.1 mg, yield 70.1%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.34 (d, *J* = 8.1 Hz, 2H), 7.15 (d, *J* = 8.7 Hz, 2H), 4.74 (d, *J* = 2.9 Hz, 2H), 4.59 – 4.44 (m, 2H), 4.29 – 4.15 (m, 2H), 3.81 (td, *J* = 6.4, 5.6, 2.3 Hz, 2H), 3.09 – 3.02 (m, 2H), 3.01 – 2.88 (m, 2H), 2.47 (dt, *J* = 3.2, 1.7 Hz, 1H), 0.94 (s, 9H), 0.10 (s, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 153.6, 149.9, 139.4, 127.1, 120.7, 79.4, 76.8, 76.7, 74.9, 68.0, 66.4, 64.5, 58.3, 38.7, 36.9, 26.0, 18.4, -5.2.

Step 2: to the above product (200.82 mg, 0.44 mmol) was added 1.2 M HCl in EtOH (2 mL, purchased from Sigma) at 0 °C, the reaction mixture was stirred at room temperature for 2 h, the solvent was removed under vacuum. The residue was purified by column chromatography (hexane: ethyl acetate 5:1 to 1:1) to afford compound **4** as a colorless oil. m = 120.5 mg, yield 80.0%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.40 (d, *J* = 8.3 Hz, 2H), 7.19 (d, *J* = 8.7 Hz, 2H), 4.71 (s, 2H), 4.52 (t, *J* = 6.6 Hz, 2H), 4.21 (d, *J* = 2.4 Hz, 2H), 3.81 (t, *J* = 6.4 Hz, 2H), 3.05 (t, *J* = 6.7 Hz, 2H), 2.96 (t, *J* = 6.4 Hz, 2H), 2.47 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-d) δ 153.5, 150.4, 138.8, 128.1, 121.1, 79.3, 74.9, 68.0, 66.4, 64.6, 58.2, 38.6, 36.9. ESI m/z calcd. for $C_{15}H_{19}O_5S_2^+$ $[M + H]^+$: 343.0674, found 343.0669.

Synthesis of compound 5 (DEC).

Compound **4** (787.6 mg, 2.3 mmol) was dissolved in 20 mL of anhydrous CH_2Cl_2, to this solution was added pyridine (0.28 mL, 3.5 mmol), the solution was cooled to 0° C, then 4-Nitrophenyl chloroformate (876.8 mg, 2.45 mmol) was added, the mixture was stirred at room temperature overnight, the solvent was evaporated and the residue was purified by flash chromatography (hexane: ethyl acetate 3:1) to afford compound **5** as a colorless oil. m= 1.05 g, yield 90.0 %. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.37 – 8.26 (m, 2H), 7.54 – 7.46 (m, 2H), 7.44 – 7.37 (m, 2H), 7.32 – 7.23 (m, 2H), 5.30 (s, 2H), 4.63 – 4.46 (m, 2H), 4.21 (dd, *J* = 2.4, 0.7 Hz, 2H), 3.88 – 3.77 (m, 2H), 3.05 (t, *J* = 6.6 Hz, 2H), 2.97 (t, *J* = 6.4 Hz, 2H), 2.47 (td, *J* = 2.4, 0.7 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-d) δ 155.4, 153.2, 152.4, 151.4, 145.3, 132.1, 130.1, 125.3, 121.8, 121.4, 79.3, 74.9, 70.1, 68.0, 66.5, 58.2, 38.6, 36.8. ESI m/z calcd. for $C_{22}H_{21}KNO_9S_2^+$ $[M+K]^+$: 546.0289, found 546.0288.

Synthesis of compound 6 (Cipro-DEC).

DEC (507.5 mg, 1.0 mmol) was dissolved in 10 mL of anhydrous CH_2Cl_2, to this solution was added ciprofloxacin (331.3 mg, 1.0 mmol) and Et_3N (0.7 mL, 5.0 mmol). The mixture was stirred at room temperature for 24 h, then the solvent was removed under vacuum, and the resulting residue was purified by column chromatography (CH_2Cl_2: MeOH 15:1) to afford the desired product as a pale yellow solid (560.3 mg), yield 80.1 %. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 14.94 (s, 1H), 8.78 (s, 1H), 8.05 (d, *J* = 12.9 Hz, 1H), 7.41 (d, *J* = 8.8 Hz, 2H), 7.37 (d, *J* = 7.1 Hz, 1H), 7.19 (d, *J* = 8.7 Hz, 2H), 5.16 (s, 2H), 4.52 (t, *J* = 6.6 Hz, 2H), 4.20 (d, *J* = 2.4 Hz, 2H), 3.81 (t, *J* = 6.4 Hz, 2H), 3.75 (t, *J* = 5.1 Hz, 4H), 3.53 (tt, *J* = 7.2, 4.0 Hz, 1H), 3.30 (s, 4H), 3.04 (t, *J* = 6.6 Hz, 2H), 2.95 (t, *J* = 6.3 Hz, 2H), 2.46 (t, *J* = 2.4 Hz, 1H), 1.39 (q, *J* = 7.0 Hz, 2H), 1.22 – 1.16 (m, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 184.1, 177.1, 166.9, 154.9, 153.4, 150.8, 147.6, 145.6, 139.0, 134.3, 129.5, 121.2, 112.8, 112.6, 108.3, 105.1, 74.9, 68.0, 66.8, 66.5, 58.2, 49.7, 38.6, 36.8, 35.3, 8.3. HRMS (ESI) m/z $C_{33}H_{34}FN_3O_9S_2$ calculated 700.1799 $[M+H]^+$ found 700.1787.

Synthesis of compound 7 (PEG-DEC-Cipro).

To a flame-dried 10 mL round-bottom flask was added Cipro-DEC (35.0 mg, 0.05 mmol), PEG2000 azide (106.0 mg, 0.053 mmol, purchased from Sigma-Aldrich), a catalytic amount of CuI (1.2 mg), and anhydrous DMF (2 mL). The mixture was degassed for 10 min, and DIPEA (10 μL) was added and stirred under N_2 for 12 h. The solvent was removed by vacuum and the residue was purified by column chromatography (CH_2Cl_2:MeOH 15:1) to afford PEG-DEC-Cipro as a pale yellow solid (95.6 mg), yield 76.3 %. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 14.91 (s, 1H), 8.71 (s, 1H), 7.97 (d, *J* = 12.4 Hz, 1H), 7.74 (s, 1H), 7.36 (d, *J* = 8.5 Hz, 3H), 7.14 (d, *J* = 8.6 Hz, 2H), 5.11 (s, 2H), 4.62 (s, 2H), 4.49 (t, *J* = 5.1 Hz, 2H), 4.44 (t, *J* = 6.6 Hz, 2H), 3.82 (t, *J* = 5.1 Hz, 2H), 3.76 (d, *J* = 5.9 Hz, 4H), 3.73 – 3.68 (m, 4H), 3.64 – 3.54 (m, 262H), 3.50 (dd, *J* = 6.3, 3.3 Hz, 3H), 3.42 (t, *J* = 4.9 Hz, 1H), 3.33 (d, *J* = 1.0 Hz, 4H), 2.96 (t, *J* = 6.6 Hz, 2H), 2.89 (t, *J* = 6.1 Hz, 2H), 1.36 (s, 2H), 1.15 (s, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 177.0, 166.8, 154.9, 154.8, 153.3, 152.3, 150.8, 147.5, 145.6, 145.5, 139.0, 134.3, 129.4, 121.1, 112.5, 112.3, 108.1, 105.2, 71.8, 70.6, 70.6, 70.5, 70.5, 70.5, 70.4, 70.4, 69.9, 66.6, 66.4, 58.9, 50.6, 49.6, 46.0, 36.8, 35.4, 15.2, 8.7, 8.2.

Synthesis of DCB and PEG-DCB-Cipro (Fig. S3)

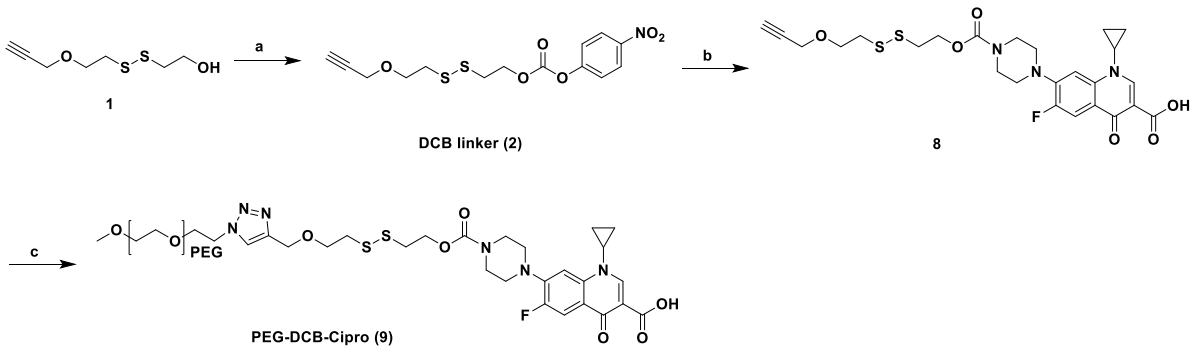


Fig. S3 Synthesis of DCB linker and its derivatives. a) 4-Nitrophenyl chloroformate, pyridine, CH_3CN, 0 °C to rt, 18 h, 78.6%. b) Ciprofloxacin, Et_3N, DMF, rt, 12 h, 85.1%. c) PEG2000 azide, CuI, DIPEA, DMF, N_2, 12 h, 69.3%.

Synthesis of compound 8 (Cipro-DCB).

To a solution of DCB (synthesis procedure see page S4) (107.2 mg, 0.3 mmol) in anhydrous DMF (5 mL), was added ciprofloxacin (106.0 mg, 0.32 mmol) and TEA (0.42 mL, 3.0 mmol). The reaction solution was stirred at rt for 12 h, and the solvent was removed under vacuum. The residue was purified by short column chromatography (DCM: MeOH 15:1) to afford the desired product as a white solid. m= 140.2 mg, yield 85.1%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 14.99 (s, 1H), 8.82 (s, 1H), 8.08 (d, *J* = 12.8 Hz, 1H), 7.42 (d, *J* = 7.1 Hz, 1H), 4.45 (t, *J* = 6.3 Hz, 2H), 4.24 (d, *J* = 2.4 Hz, 2H), 3.84 (t, *J* = 6.4 Hz, 2H), 3.80 – 3.74 (m, 4H), 3.59 (dq, *J* = 7.1, 3.6 Hz, 1H), 3.35 (t, *J* = 5.1 Hz, 4H), 3.03 (t, *J* = 6.3 Hz, 2H), 2.98 (t, *J* = 6.4 Hz, 2H), 2.50 (t, *J* = 2.4 Hz, 1H), 1.50 – 1.41 (m, 2H), 1.28 – 1.20 (m, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 13C NMR (101 MHz, cdcl3) δ 177.1, 166.9, 154.8, 147.6, 139.0, 112.8, 112.5, 108.3, 105.1, 79.3, 74.8, 68.0, 63.5, 58.2, 49.7, 43.6, 38.6, 37.8, 35.3, 8.3. ESI m/z calcd. for $C_{25}H_{29}FN_3O_6S_2^+$ $[M + H]^+$: 550.1482, found 550.1473.

Synthesis of compound 9 (PEG-DCB-Cipro).

To a flame-dried 10 mL round-bottom flask was added compound **8** (26.4 mg, 0.048 mmol), PEG2000 azide (100.0 mg, 0.05 mmol), a catalytic amount of CuI (1.3 mg), and anhydrous DMF (2 mL). The mixture was degassed for 10 min, and DIPEA (10 μL) was added and stirred under N_2 for 12 h. The solvent was removed by vacuum and the residue was purified by column chromatography (CH_2Cl_2:MeOH 15:1) to afford **PEG-DCB-Cipro** as a pale yellow

solid (84.8 mg), yield 69.3%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 14.95 (s, 1H), 8.85 – 8.72 (m, 1H), 8.04 (dd, *J* = 12.8, 1.2 Hz, 1H), 7.39 (d, *J* = 6.2 Hz, 1H), 4.59 (s, 3H), 4.40 (s, 4H), 3.91 (s, 4H), 3.83 – 3.78 (m, 1H), 3.72 (s, 4H), 3.63 (s, 187H), 3.54 (dd, *J* = 6.1, 3.3 Hz, 3H), 3.45 (dd, *J* = 5.9, 4.0 Hz, 1H), 3.36 (d, *J* = 0.6 Hz, 3H), 3.31 (s, 4H), 1.48 – 1.33 (m, 2H), 1.24 – 1.17 (m, 2H).

Synthesis of DCB_{Me} and PEG-DCB_{Me}-Cipro (Fig. S4)

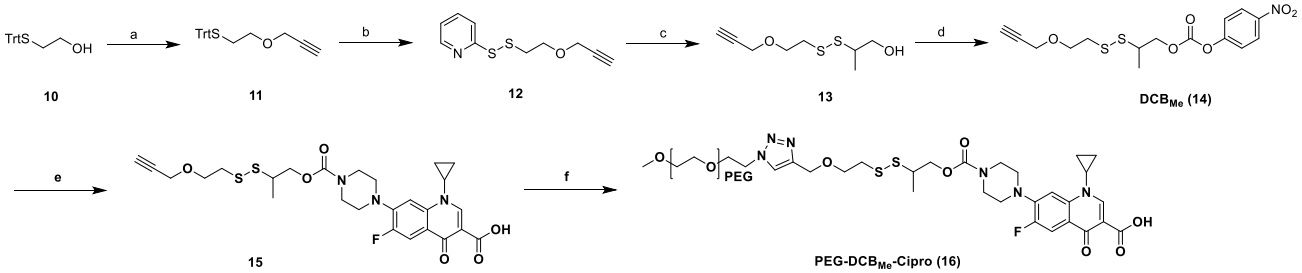


Fig. S4 Synthesis of the DCB_{Me} linker and its derivatives. a) Propargyl bromide, NaH, THF, 0 °C to rt, overnight, 91.9%. b) i. TFA, Et_3SiH, CH_2Cl_2, 20 min, 0 °C; ii. 2,2'-Dithiodipyridine, CH_2Cl_2, 0 °C to rt, 2 h, 87.2% for 2 steps. c) 2-mercaptopropan-1-ol, N_2, 12 h, 71.5%. d) 4-Nitrophenyl chloroformate, pyridine, CH_2Cl_2, rt, 2 h, 80.2%. e) Ciprofloxacin, Et_3N, DMF, rt, 12 h, 81.1%. f) PEG2000 azide, CuI, DIPEA, DMF, N_2, rt, 12 h, 71.7%.

Synthesis of compound 11.

Compound **10**[3] (2.0 g, 6.24 mmol) was dissolved in 10 mL of THF, the mixture was cooled to 0 °C, to this solution was added NaH (300.0 mg, 7.5 mmol, 60% in mineral oil), and was stirred at 0 °C for 30 min before adding propargyl bromide (224.64 mg, 9.36 mmol). The reaction solution was strried under Ar overnight, then quenched with H_2O, and extracted with ethyl acetate. The combined organic phase was washed with brine and dried over Na_2SO_4, the solvent was evaporated under vacuum and the residue was purified by column chromatography (hexane:ethyl acetate 10:1) to afford the desired product as a yellow oil. m= 2.05 g, yield 91.9 %. This compound was used in the next step without further characterization.

Synthesis of compound 12.

To a solution of compound **11** (1.0 g, 2.8 mmol) in CH_2Cl_2 (25 mL), was added TFA (2.5 mL, 10% v/v) at 0 °C, the reaction mixture turned dark brown immediately. Then Et_3SiH (0.67 mL, 4.2 mmol) was added dropwise, the solution turned yellow and the mixture was kept 0 °C for 20 min. Then 2,2'-Dithiodipyridine (740.3 mg, 3.4 mmol) was added, the reaction mixture was stirred at 0 °C for 0.5 h then warmed to room temperature and keep stirring for 2 h. The solvent was removed under vacuum. The residue was purified by column chromatography (hexane:ethyl acetate 10:1) to afford the desired product **13** as a light yellow oil. m= 550.4 mg, yield 87.2 % for two steps. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.50 (d, *J* = 4.8 Hz, 1H), 7.81 (d, *J* = 8.1 Hz, 1H), 7.70 (td, *J* = 7.8, 1.9 Hz, 1H), 7.22 – 7.05 (m, 1H), 4.17 (d, *J* = 2.4 Hz, 2H), 3.82 (t, *J* = 6.2 Hz, 2H), 3.06 (t, *J* = 6.2 Hz, 2H), 2.46 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.2, 149.4, 137.2, 120.7, 119.9, 79.3, 74.8, 67.6, 58.3, 38.4. ESI m/z calcd. for $C_{10}H_{12}NOS_2^+$ $[M + H]^+$: 226.0360, found 226.0371.

Synthesis of compound 13.

To a solution of compound **12** (500.0 mg, 2.22 mmol) in CH_2Cl_2 (20 mL), was added 2-mercaptopropan-1-ol (245.5 mg, 2.66 mmol), the reaction mixture was stirred at rt for 12 h, then the solvent was removed under vacuum. The residue was purified by column chromatography (hexane:ethyl acetate 4:1) to afford the desired product as a light yellow oil. m= 327.5 mg, yield 71.5%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.23 (d, *J* = 2.4 Hz, 2H), 3.83 (t, *J* = 6.4 Hz, 2H), 3.78 – 3.64 (m, 2H), 3.03 (dd, *J* = 11.9, 6.8 Hz, 1H), 2.95 (t, *J* = 6.4 Hz, 2H), 2.50 (t, *J* = 2.4 Hz, 1H), 1.34 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 79.3, 74.9, 68.3, 65.2, 58.3, 48.5, 39.2, 16.8. ESI m/z calcd. for $C_8H_{14}O_2S_2Na^+$ $[M + Na]^+$: 229.0333, found 229.0327.

Synthesis of compound 14 (DCB_{Me}).

To a solution of compound **13** (206.3 mg, 1.0 mmol) in CH_2Cl_2 (10 mL), was added pyridine (120.7 μL, 1.5 mmol). The reaction solution was cooled to 0 °C and 4-Nitrophenyl chloroformate (339.5 mg, 0.95 mmol) was added. The mixture was stirred at rt for 2 h, and the solvent was removed under vacuum. The residue was purified by column chromatography (hexane:ethyl acetate 20:1) to afford the desired product as a colorless oil. m= 298.5 mg, yield 80.4%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.33 (d, *J* = 9.2 Hz, 2H), 7.44 (d, *J* = 9.2 Hz, 2H), 4.53 (dd, *J* = 10.9, 5.7 Hz, 1H), 4.35 (dd, *J* = 11.0, 7.2 Hz, 1H), 4.24 (d, *J* = 2.4 Hz, 2H), 3.84 (td, *J* = 6.4, 2.0 Hz, 2H), 3.35 – 3.21 (m, 1H), 2.99 (t, *J* = 6.8 Hz, 2H), 2.50 (t, *J* = 2.4 Hz, 1H), 1.43 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ

155.5, 152.3, 145.5, 125.4, 121.8, 79.3, 74.9, 71.5, 68.1, 58.3, 43.9, 39.4, 17.0. ESI m/z calcd. for $C_{15}H_{17}NO_6S_2Na^+$ $[M + Na]^+$: 394.0395, found 394.0385.

Synthesis of compound 15 (Cipro-DCB$_{Me}$).
To a solution of compound **14** (100.3 mg, 0.27 mmol) in anhydrous DMF (2 mL), was added ciprofloxacin (89.2 mg, 0.27 mmol) and TEA (75.8 μL, 0.54 mmol). The reaction solution was stirred at rt for 12 h, and the solvent was removed under vacuum. The residue was purified by short column chromatography (CH_2Cl_2 to CH_2Cl_2: MeOH 20:1) to afford the desired product as a white solid. m= 123.5 mg, yield 81.1%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 14.96 (s, 1H), 8.75 (s, 1H), 8.01 (dd, *J* = 12.9, 1.5 Hz, 1H), 7.40 (d, *J* = 7.1 Hz, 1H), 4.36 – 4.24 (m, 2H), 4.22 (d, *J* = 2.4 Hz, 2H), 3.82 (td, *J* = 6.5, 1.1 Hz, 2H), 3.77 (d, *J* = 5.6 Hz, 4H), 3.60 (tt, *J* = 6.9, 4.0 Hz, 1H), 3.36 (t, *J* = 5.2 Hz, 4H), 3.20 (q, *J* = 6.4 Hz, 1H), 2.95 (t, *J* = 6.6 Hz, 2H), 2.49 (t, *J* = 2.3 Hz, 1H), 1.43 (t, *J* = 6.7 Hz, 2H), 1.38 (d, *J* = 6.9 Hz, 3H), 1.27 – 1.22 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.0, 166.9, 154.9, 152.4, 147.5, 145.6, 139.0, 112.6, 112.4, 108.1, 105.2, 79.4, 76.7, 74.9, 68.2, 68.0, 58.2, 49.7, 44.8, 39.3, 35.4, 17.2, 8.3. $C_{26}H_{29}FN_3O_6S_2^-$ $[M - H]^-$: 562.1487, found 562.1480.

Synthesis of compound 16 (PEG-DCB$_{Me}$-Cipro).
To a flame-dried 10 mL round-bottom flask was added compound **15** (28.2 mg, 0.05 mmol), PEG2000 azide (100.0 mg, 0.05 mmol), a catalytic amount of CuI (0.5 mg), and anhydrous DMF (2 mL). The mixture was degassed for 10 min, and DIPEA (10 μL) was added and stirred under N_2 for 12 h. The solvent was removed by vacuum and the residue was purified by column chromatography (CH_2Cl_2:MeOH 20:1) to afford **PEG-DCB$_{Me}$-Cipro** as a white solid (91.9 mg), yield 71.7%. ^{1}H NMR (900 MHz, Chloroform-*d*) δ 8.78 (s, 1H), 8.05 (d, *J* = 12.3 Hz, 1H), 7.77 (s, 1H), 7.41 (d, *J* = 6.7 Hz, 1H), 4.66 (s, 2H), 4.53 (t, *J* = 5.1 Hz, 2H), 4.22 (ddd, *J* = 37.3, 11.2, 6.3 Hz, 2H), 3.86 (t, *J* = 5.1 Hz, 2H), 3.78 (t, *J* = 6.6 Hz, 2H), 3.64 (s, 202H), 3.55 (dt, *J* = 9.5, 4.8 Hz, 4H), 3.37 (s, 3H), 3.32 (s, 4H), 3.13 (q, *J* = 6.7 Hz, 1H), 2.90 (q, *J* = 6.3 Hz, 2H), 1.42 (d, *J* = 6.9 Hz, 2H), 1.33 (d, *J* = 7.0 Hz, 3H), 1.20 (d, *J* = 4.3 Hz, 2H). ^{13}C NMR (226 MHz, $CDCl_3$) δ 177.1, 166.9, 154.8, 154.2, 153.1, 147.5, 145.6, 145.6, 144.5, 139.0, 123.9, 120.3, 112.6, 112.5, 108.2, 105.3, 77.2, 71.9, 70.6, 70.6, 70.6, 70.5, 70.5, 70.4, 70.4, 69.4, 68.5, 67.9, 64.3, 59.0, 50.2, 49.7, 44.7, 39.2, 35.4, 17.2, 8.3.

Synthesis of DTB and PEG-DTB-Cipro (Fig. S5)

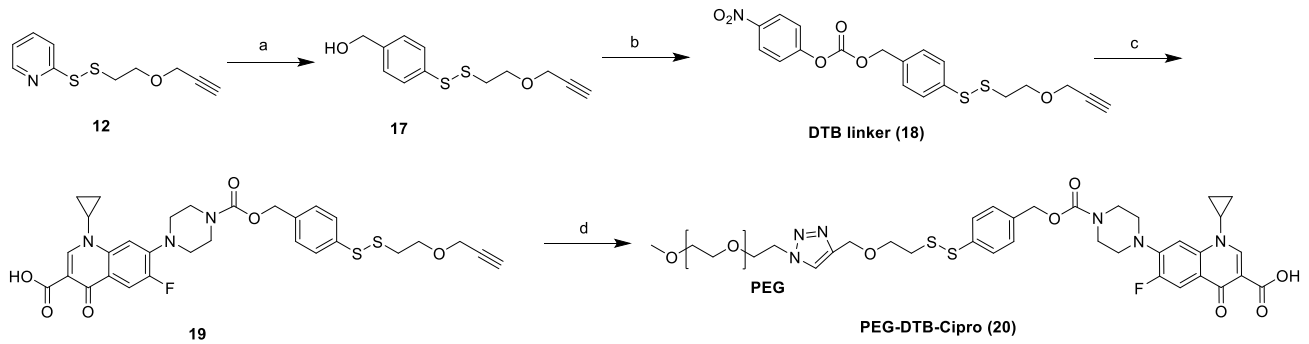


Fig. S5 Synthesis of the DTB linker and its derivatives. a) 4-Mercaptobenzyl alcohol, N_2, 12 h, 95.4%. b) 4-Nitrophenyl chloroformate, pyridine, CH_2Cl_2, rt, 2 h, 98.5%. c) Ciprofloxacin, Et_3N, DMF, rt, 12 h, 45.5%. d) PEG2000 azide, CuI, DIPEA, DMF, N_2, rt, 12 h, 52.3%.

Synthesis of compound 17.
To a solution of compound **12** (163.1 mg, 0.724 mmol) in CH_2Cl_2 (2 mL), was added 4-Mercaptobenzyl alcohol[4] (103.5 mg, 0.738 mmol), the reaction mixture was stirred at rt for 12 h, then the solvent was removed under vacuum. The residue was purified by column chromatography (hexane:ethyl acetate 10:1 to 2:1) to afford the desired product as a light yellow oil. m= 175.7 mg, yield 95.4%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.54 (d, *J* = 8.3 Hz, 1H), 7.33 (d, *J* = 8.3 Hz, 2H), 4.68 (s, 2H), 4.10 (d, *J* = 2.4 Hz, 1H), 3.75 (t, *J* = 6.4 Hz, 2H), 2.94 (t, *J* = 6.4 Hz, 2H), 2.43 (t, *J* = 2.5 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 139.9, 136.7, 128.0, 127.7, 79.4, 74.8, 67.7, 64.8, 58.2, 38.3. ESI m/z calcd. for $C_{12}H_{15}O_2S_2^+$ $[M + H]^+$: 255.0513, found 255.0511.

Synthesis of compound 18 (DTB).
To a solution of compound **17** (160.2 mg, 0.63 mmol) in anhydrous CH_2Cl_2 (5 mL), was added pyridine (80 μL, 0.95 mmol). The reaction solution was cooled to 0 °C and 4-Nitrophenyl chloroformate (141.1 mg, 0.7 mmol) was added. The mixture was stirred at rt for 2 h, and the solvent was removed under vacuum. The residue was purified by

column chromatography (hexane: CH_2Cl_2 1:1) to afford the desired product as a light yellow oil. m= 260.3 mg, yield 98.5%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.27 (d, *J* = 9.3 Hz, 2H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.39 (dd, *J* = 11.9, 8.8 Hz, 4H), 5.27 (s, 2H), 4.10 (d, *J* = 2.3 Hz, 2H), 3.75 (t, *J* = 6.3 Hz, 2H), 2.95 (t, *J* = 6.3 Hz, 2H), 2.43 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 155.5, 152.4, 145.5, 138.7, 132.8, 129.4, 127.4, 125.3, 121.8, 79.3, 74.9, 70.4, 67.6, 58.2, 38.4. EI m/z calcd. for $C_{19}H_{18}NO_6S_2$ $[M]^+$: 419.0497, found 419.0499.

Synthesis of compound 19 (Cipro-DTB).

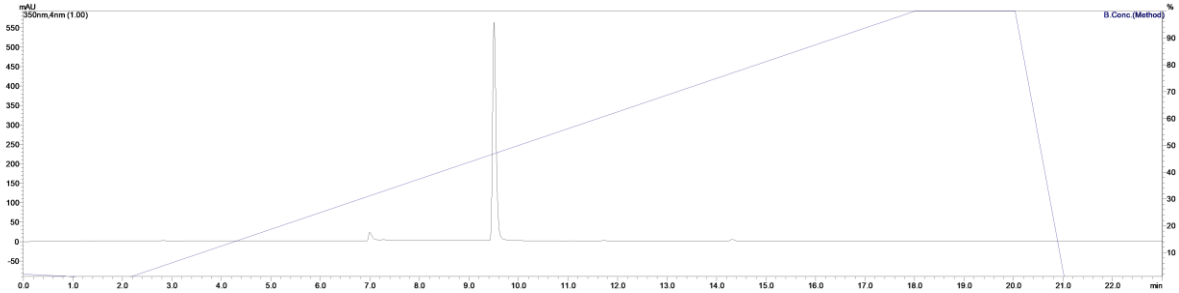
To a solution of compound **18** (75.4 mg, 0.18 mmol) in anhydrous DMF (2 mL), was added ciprofloxacin (66.3 mg, 0.2 mmol) and TEA (66.9 μL, 0.48 mmol). The reaction solution was stirred at rt for 12 h, and the solvent was removed under vacuum. The residue was purified by short column chromatography (DCM to DCM: MeOH 30:1) to afford the desired product as a white solid. m= 50.1 mg, yield 45.5%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 14.99 (s, 1H), 8.81 (s, 1H), 8.08 (d, *J* = 12.9 Hz, 1H), 7.59 (d, *J* = 8.3 Hz, 2H), 7.40 (t, *J* = 7.3 Hz, 3H), 5.19 (s, 2H), 4.14 (d, *J* = 2.4 Hz, 2H), 3.79 (d, *J* = 6.4 Hz, 6H), 3.58 (tt, *J* = 7.2, 4.0 Hz, 1H), 3.34 (s, 2H), 3.11 – 2.87 (m, 4H), 2.48 (t, *J* = 2.4 Hz, 1H), 1.44 (d, *J* = 6.8 Hz, 2H), 1.24 (t, *J* = 5.5 Hz, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 177.1, 166.9, 155.0, 152.4, 145.5, 139.0, 137.6, 135.2, 128.9, 127.6, 112.8, 112.6, 108.3, 105.2, 79.3, 74.8, 67.7, 67.0, 58.2, 49.8, 38.3, 35.3, 8.3. ESI m/z calcd. for $C_{30}H_{31}FN_3O_6S_2^+$ $[M + H]^+$: 612.1638, found 612.1631.

Synthesis of compound 20 (PEG-DTB-Cipro).

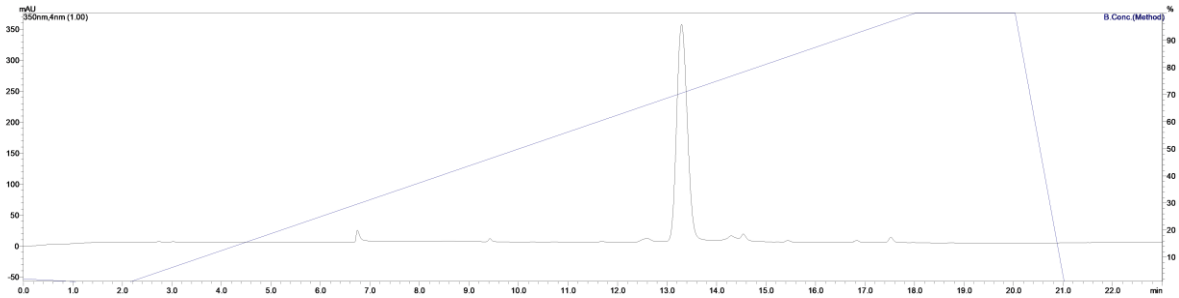
To a flame-dried 10 mL round-bottom flask was added compound **19** (24.5 mg, 0.04 mmol), PEG2000 azide (86.0 mg, 0.043 mmol), a catalytic amount of CuI (1.2 mg), and anhydrous DMF (2 mL). The mixture was degassed for 10 min, and DIPEA (10 μL) was added and stirred under N_2 for 12 h. The solvent was removed by vacuum and the residue was purified by column chromatography (CH_2Cl_2:MeOH 15:1) to afford **PEG-DTB-Cipro** as a pale yellow solid (54.6 mg), yield 52.3%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 14.98 (s, 1H), 8.80 (s, 1H), 8.06 (s, 1H), 7.80 (s, 1H), 7.56 (d, *J* = 8.1 Hz, 2H), 7.36 (d, *J* = 8.0 Hz, 2H), 5.17 (s, 2H), 4.57 (s, 4H), 3.89 (s, 2H), 3.83 (d, *J* = 5.3 Hz, 2H), 3.77 (s, 6H), 3.66 (s, 313H), 3.48 (s, 3H), 3.40 (s, 5H), 3.03 – 2.89 (m, 2H), 1.43 (s, 2H), 1.24 (d, *J* = 7.3 Hz, 2H).

HPLC of the synthesized PEG2k-disulfide-Cipro

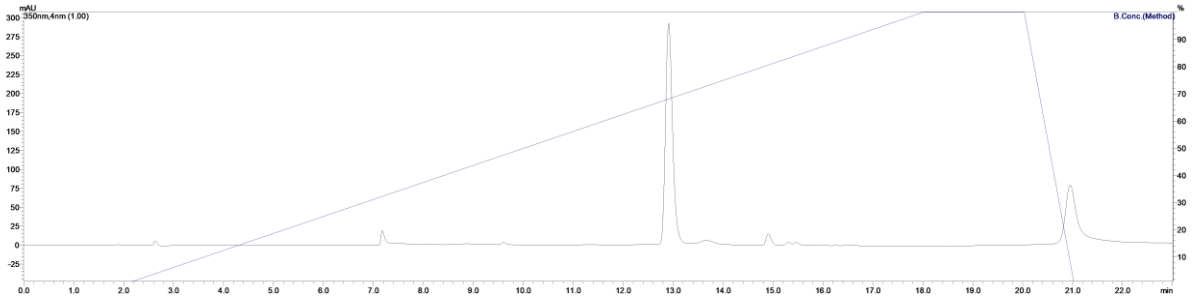
Ciprofloxacin Standard



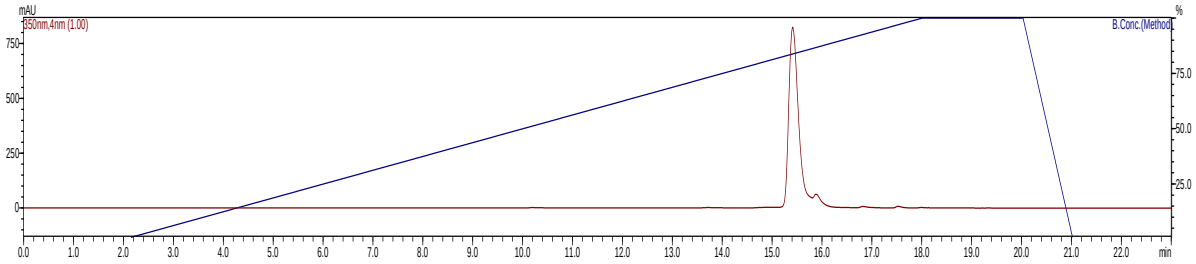
PEG2k-DEC-Cipro



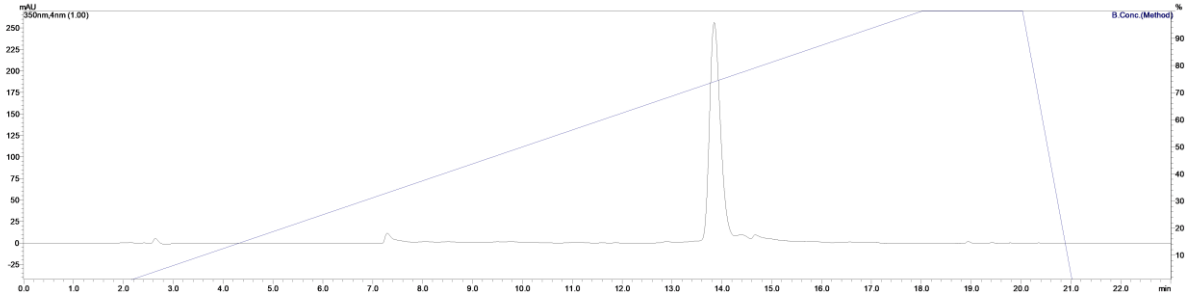
PEG2k-DCB-Cipro



PEG2k-DCB$_{Me}$-Cipro



PEG2k-DTB-Cipro



Determination of the release kinetics of four PEG-Cipro disulfides at pH 7.4

PEG-Cipro disulfide (500 µM) was dissolved in PBS buffer (1X, pH 7.4) containing 10% Acetonitrile and 10mM of GSH (final concentration) to reach a final volumn of 100 µL. The solution was incubated at 37 °C under argon. At different time points, 50 µL of the reaction solution was analyzed by HPLC (350 nm wavelength).

	Stock concentration	Final concentration	Volume
PEG2k-linker-Cipro	5 mM	500 µM	10 µL
GSH (pH 7.4)	100 mM	10 mM	10 µL
PBS 1X			80 µL
Total			100 µL

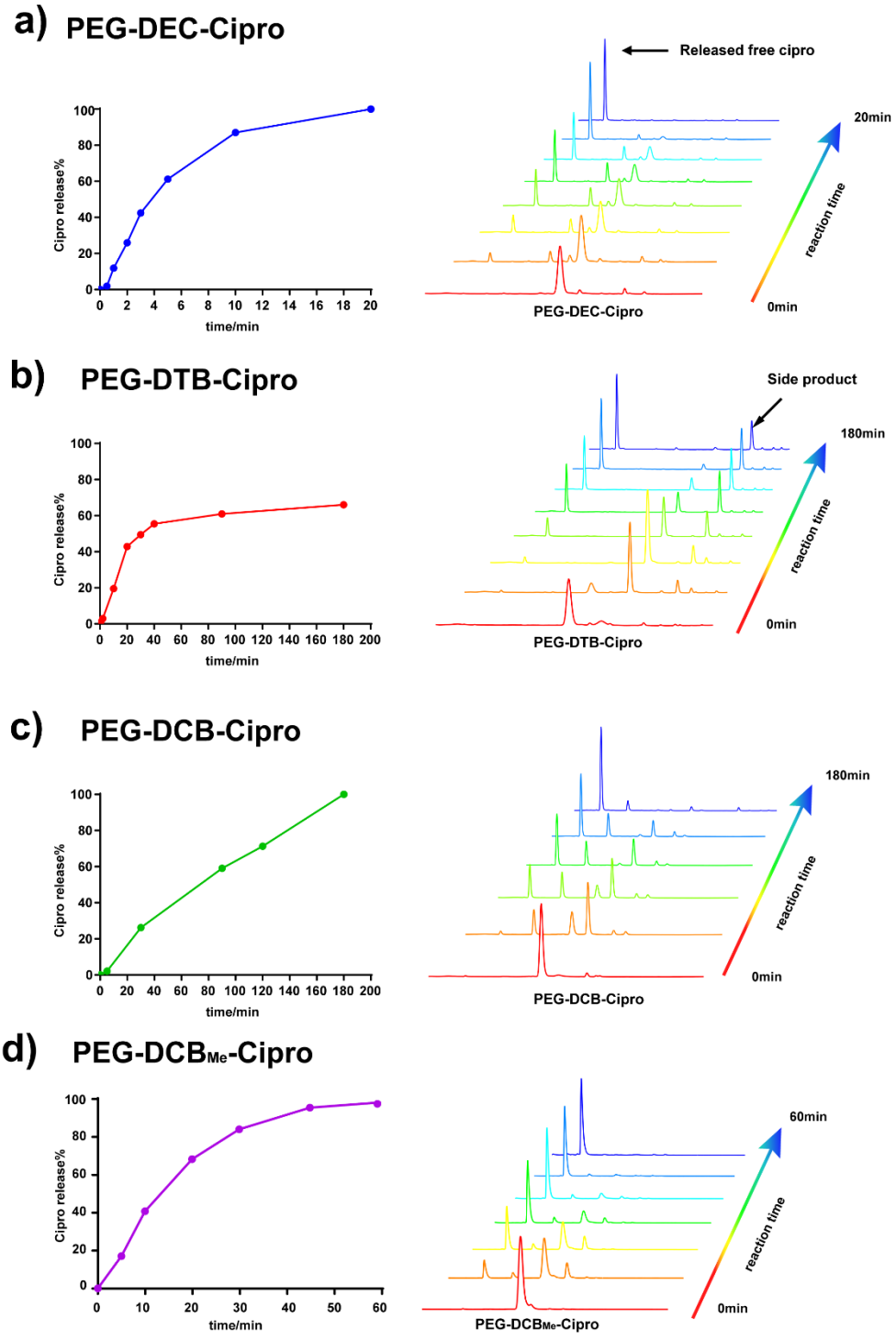


Fig S6 Release kinetics and HPLC monitoring of four PEG-Cipro disulfides in PBS (1 X, pH 7.4, 10% CH_3CN) in the presence of GSH (10 mM). a) Cipro released from PEG-DEC-Cipro as monitored by HPLC (350 nm wavelength). b) Cipro released from PEG-DTB-Cipro as monitored by HPLC (350 nm wavelength). c) Cipro release kinetics of PEG-DCB-Cipro as monitored by HPLC (350 nm wavelength). d) Cipro release kinetics of PEG-DCB_{Me}-Cipro as monitored by HPLC (350 nm wavelength).

Determination of the release kinetics of the three PEG-Cipro disulfides at pH 5

PEG-Cipro disulfide (500 µM) was dissolved in sodium acetate-acetic acid buffer (1X, pH 5) containing 10% acetonitrile and 10mM of GSH (final concentration) to reach a final volumn of 100 µL. The solution was incubated at 37 °C under argon. At different time points, 50 µL of the reaction solution was analyzed by HPLC (350nm wavelength).

	Stock concentration	Final concentration	Volume
PEG2k-linker-Cipro	5 mM	500 µM	10 µL
GSH (pH 7.4)	100 mM	10 mM	10 µL
NaOAc/AcOH 1X (pH 5)			80 µL
Total			100 µL

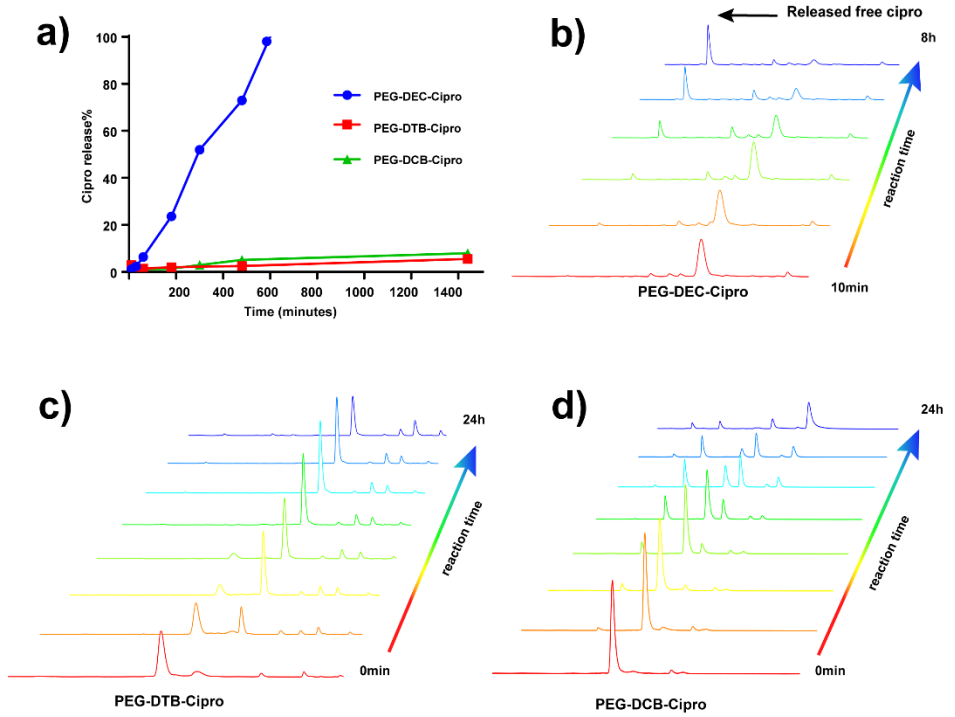


Fig S7 a) Release kinetics of the three PEG-Cipro disulfides at pH 5 (contains 10% CH_3CN) in the presence of GSH (10mM). b) Release kinetics of Cipro released from PEG-DEC-Cipro as monitored by HPLC (350 nm wavelength). c) Cipro released from PEG-DTB-Cipro as monitored by HPLC (350 nm wavelength). d) Cipro release kinetics of PEG-DCB-Cipro as monitored by HPLC (350 nm wavelength).

In vitro antibacterial activity

The minimum inhibitory concentration (MIC) of PEG-DEC-Cipro and free Cipro were determined in a 96-well plate format.[5] Each well contained a 100 µL of Mueller-Hinton II (with or without 10 mM GSH), a 100 µL of *E. coli* SF 207 (isolated from a patient with bloodstream infection in the San Francisco General Hospital) (OD600 = 0.1) and a 100 µL of stock drug solution (free ciprofloxacin and PEG-DEC-Cipro). The plate was incubated at 37°C for 24 h, MIC results were obtained by testing different concentrations of the drugs on bacteria, in particular: 8 µg/mL, 4 µg/mL, 2 µg/mL, 1 µg/mL, 0.5 µg/mL, 0.25 µg/mL, and 0.125 µg/mL. The MIC was defined as the minimum concentration of drug that causes complete inhibition of bacterial growth. Bacterial growth was measured by absorption spectroscopy (OD600) using a Tecan Infinite 200 plate reader. The results are shown in **Table S1**. PEG-DEC-Cipro with GSH had identical activity to free ciprofloxacin on the *E. coli* strain (SF207), both had an MIC of 0.25 µg/mL, while PEG-DEC-Cipro without GSH had a MIC higher than 1 µg/mL. The addition of 10 mM GSH

increased the efficacy of the PEG-DEC-Cipro, and the MIC of this compound with GSH was the same as the MIC of free ciprofloxacin.

Table S1: MIC of free ciprofloxacin and PEG-DEC-Cipro with/without GSH (10 mM)

	Free Cipro		PEG-DEC-Cipro	
	+GSH	-GSH	+GSH	-GSH
MIC (μg/mL)	0.25	0.25	0.25	>1

Synthesis of PEG5k-disulfide linker for protein PEGylation

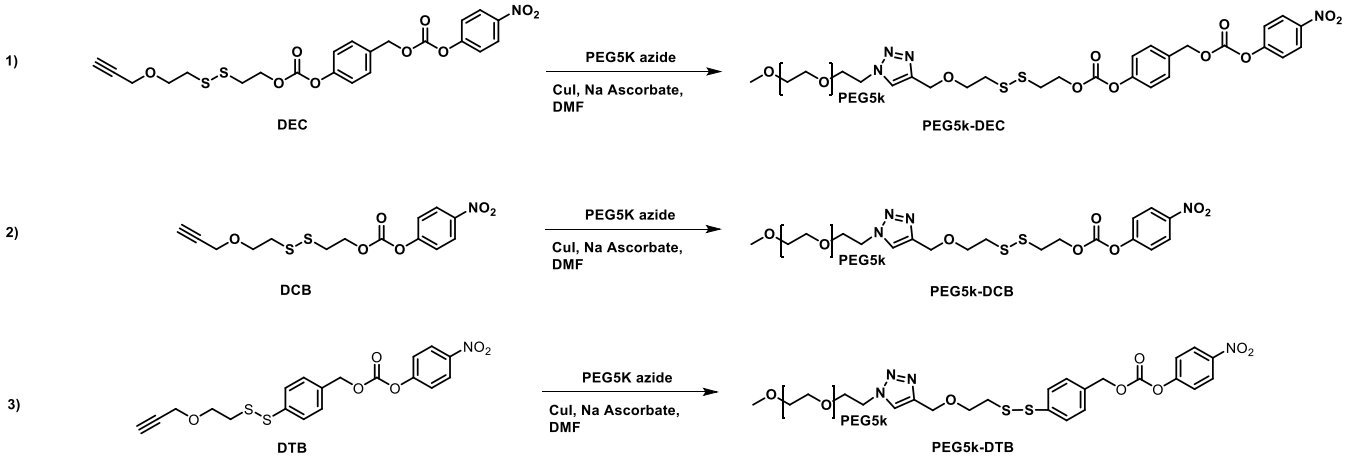


Fig S8 synthesis of PEG-DEC, PEG-DCB and PEG-DTB. Conditions: PEG5000 azide, CuI, Na Ascorbate, DMF, N_2, rt, 12 h.

1) **Synthesis of PEG5k-DEC**: PEG5000 azide (200.0 mg, 0.04mmol) was dissolved in 3 mL of dry DMF, to this solution was added DEC (30.45mg, 0.06mmol, 1.5 equiv), then CuI (0.4 mg, 0.002 mmol) and sodium ascorbate (4.2 mg, 0.02 mmol) were added and the mixture was degassed for 10 min before stirring under N_2 overnight. The reaction was monitored by TLC, after completion the solvent was removed by vacuum, The residue was purified by short column chromatography (hexane:EtOAc 1:1 to DCM: MeOH 10:1) to afford the desired product as a light yellow solid. m= 100.2 mg, yield 45.5%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.32 – 8.15 (m, 2H), 7.68 (s, 1H), 7.47 – 7.42 (m, 2H), 7.39 – 7.34 (m, 2H), 7.23 – 7.18 (m, 2H), 5.26 (s, 2H), 4.65 (s, 2H), 4.53 (d, *J* = 5.1 Hz, 2H), 4.47 (t, *J* = 6.6 Hz, 2H), 3.85 (t, *J* = 5.1 Hz, 2H), 3.81 – 3.77 (m, 4H), 3.61 (s, 460H), 3.53 – 3.51 (m, 2H), 3.46 – 3.42 (m, 2H), 3.35 (s, 3H), 2.98 (t, *J* = 6.6 Hz, 2H). ^{13}C NMR (226 MHz, $CDCl_3$) δ 13C NMR (226 MHz, CDCl3) δ 155.6, 153.4, 152.6, 151.6, 145.6, 132.3, 131.1, 130.3, 129.0, 125.5, 122.0, 121.6, 72.1, 70.7, 70.7, 70.6, 70.5, 70.5, 70.4, 70.3, 69.6, 68.6, 66.7, 64.4, 59.2, 50.5, 38.8, 36.9, 29.8.

2) **Synthesis of PEG5k-DCB**: PEG5000 azide (150.0 mg, 0.03 mmol) was dissolved in 3 mL of dry DMF, to this solution was added DCB (12.9 mg, 0.036 mmol, 1.2 equiv), then CuI (0.3 mg, 0.0015 mmol) and sodium ascorbate (3.0 mg, 0.015 mmol) were added and the mixture was degassed for 10 min before stirring under N_2 overnight. The reaction was monitored by TLC, after completion the solvent was removed by vacuum, The residue was purified by short column chromatography (hexane:EtOAc 1:1 to DCM: MeOH 10:1) to afford the desired product as a light yellow solid. m= 86.0 mg, yield 53.5%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.29 (dd, *J* = 9.2, 1.3 Hz, 2H), 7.77 (s, 1H), 7.59 (d, *J* = 7.0 Hz, 2H), 7.41 (t, *J* = 7.9 Hz, 2H), 5.29 (s, 2H), 4.62 (s, 2H), 4.57 (t, *J* = 5.1 Hz, 2H), 3.89 (t, *J* = 5.1 Hz, 2H), 3.83 (d, *J* = 4.9 Hz, 4H), 3.78 (dd, *J* = 7.5, 6.3 Hz, 2H), 3.66 (d, *J* = 1.3 Hz, 625H), 3.57 (d, *J* = 6.1 Hz, 4H), 3.48 (t, *J* = 4.9 Hz, 4H), 3.39 (d, *J* = 1.3 Hz, 3H), 2.96 (t, *J* = 6.3 Hz, 2H). ^{13}C NMR (226 MHz, $CDCl_3$) δ 13C NMR (226 MHz, CDCl3) δ 155.6, 152.5, 145.6, 144.7, 125.5, 124.1, 122.0, 77.4, 77.2, 77.1, 72.1, 70.7, 70.6, 69.6, 68.6, 67.1, 64.5, 59.2, 50.4, 38.9, 36.8.

3) **Synthesis of PEG5k-DTB**: PEG5000 azide (150.0 mg, 0.03 mmol) was dissolved in 3 mL of dry DMF, to this solution was added DTB (18.9 mg, 0.045 mmol, 1.5 equiv), then CuI (0.3 mg, 0.0015 mmol) and sodium ascorbate (3.0 mg, 0.015 mmol) were added and the mixture was degassed for 10 min before stirring under N_2 overnight. The reaction was monitored by TLC, after completion the solvent was removed by vacuum, The residue was purified by short column chromatography (hexane:EtOAc 1:1 to DCM: MeOH 10:1) to afford the desired product as a white solid. m= 75.8 mg, yield 46.6%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.37 – 8.26 (m, 2H), 7.79 (s, 1H), 7.49 – 7.38 (m, 2H), 4.69 (s, 2H), 4.60 – 4.51 (m, 4H), 3.91 – 3.87 (m, 2H), 3.85 – 3.80 (m, 4H), 3.66 (s, 475H), 3.58 – 3.54 (m, 4H), 3.52 – 3.45 (m, 4H), 3.39 (s, 3H), 3.03 (t, *J* = 6.5 Hz, 2H), 2.96 (t, *J* = 6.3 Hz, 2H). ^{13}C NMR (226 MHz, $CDCl_3$)

δ 155.6, 152.6, 145.6, 144.7, 138.8, 132.9, 129.6, 127.5, 125.5, 124.0, 122.0, 72.1, 70.7, 70.6, 70.6, 69.6, 68.2, 64.5, 59.2, 50.4, 38.6.

Cas9 PEGylation and De-PEGylation with PEG5k-disulfide linkers

20 μL of Cas9 stock solution (40 μM, purchased from qb3. Berkeley Macrolab) was diluted with 72 μL of PBS buffer (1X) (final Cas9 concentration 8 μM), and 8 μL of PEG5k-disulfide linker stock solution (Stock concentration: 10 mM) was added to reach a final concentration of 800 μM. The mixture was incubated at room temperature for 3 h, then the reaction solution was diluted with 100 μL PBS (1X), and spin filtrated through a spin filtration column (Amicon™ Ultra 50k cutoff) 3 times (10 min, 20 °C, 10000 rcf). The final concentration of Cas9-linker-PEG5k was determined by the BCA assay.

	Stock	Final	Volume
mCherryCas9	40 μM	8 μM	20 μL
PEG5k-disulfide linker	10 mM	800 μM	8 μL
PBS 1X			72 μL
Total			100 μL

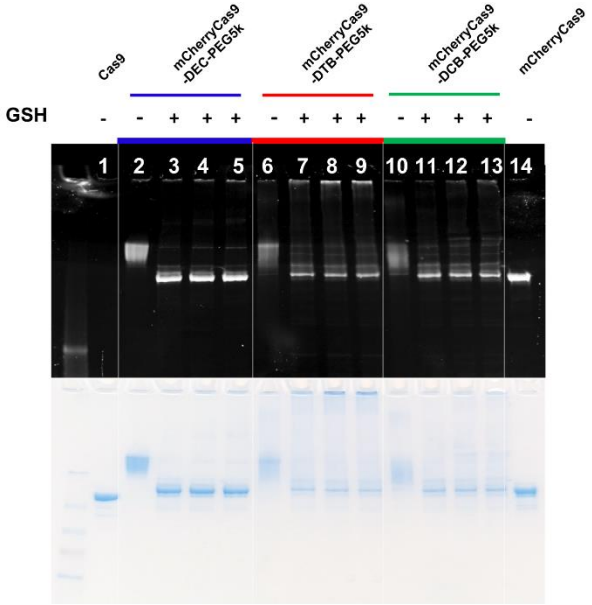


Fig S9 SDS-PAGE gel of mCherryCas9-PEG conjugated via three disulfide linkers (lane 2, 6, 10), and their release efficiency under 10mM GSH at the 1h time point. Lane 3, 4, 5; 7, 8, 9; and 10, 11, 12 represents three replicates. The gel was imaged with ChemiDoc MP using ImageLab software, v6.0 (http://www.bio-rad.com/en-cn/product/image-lab-software; Bio-Rad). Lane 1: Cas9; lane 2: mCherryCas9-DEC-PEG; lane 3-5: mCherryCas9-DEC-PEG + GSH; lane 6: mCherryCas9-DTB-PEG; lane 7-9: mCherryCas9-DTB + GSH; lane 10: mCherryCas9-DCB-PEG; lane 11-13: mCherryCas9-DCB-PEG + GSH; lane 14: mCherryCas9.

Nuclease Activity of Cas9-linker-PEG5k with or without GSH: mCherryCas9 or mCherryCas9-linker-PEG5k (6.25 μM * 2 μL) was mixed with gRNA (10 μM *2 μL) in 5 μL duplex buffer, and incubated on ice for 15 min, CutSmart® Buffer (1 X, 10 μL, purchased from BioLabs) was added, 50 ng of template DNA (1 μL) was then added followed by GSH (2 μL of 100 mM) or PBS. The mixture was incubated at 37 °C for 4 h and analyzed by SDS-PAGE gel. Nucleic acid staining was conducted with SYBR Green, and the gel was imaged with ChemiDoc MP using ImageLab software, v6.0 (http://www.bio-rad.com/en-cn/product/image-lab-software; Bio-Rad).

crRNA 20nt recognition sequence: GCTGAAGCACTGCACGCCGT.
Template DNA sequence:
ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAA
ACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTT
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TGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGT
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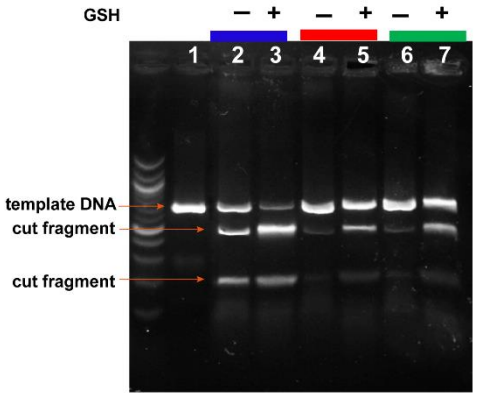


Fig S10 mCherryCas9-PEG *in vitro* nuclease activity in the presence and absence of GSH (10mM). Lane 1: template DNA; lane 2: template DNA + mCherryCas9-DEC-PEG; lane 3: template DNA + mCherryCas9-DEC-PEG + GSH; lane 4: template DNA + mCherryCas9-DTB-PEG; lane 5: template DNA + mCherryCas9-DTB-PEG + GSH; lane 6: template DNA + mCherryCas9-DCB-PEG; lane 7: template DNA + mCherryCas9-DCB-PEG + GSH.

mCherryCas9-DEC-PEG stability in mouse plasma

Whole mouse blood (mouse strain: FVB/NJ, 100 weeks old) was freshly collected with anticoagulant-treated tubes. Cells were removed from plasma by centrifugation for 10 minutes at 1000 rcf at 4 °C. The resulting supernatant (plasma) was immediately transferred into a clean polypropylene tube and used for the next step.

a) Analysis of mCherryCas9-DEC-PEG stability in mouse plasma (without additional GSH): To 5 μL of freshly prepared mouse plasma was added 1 μL of mCherryCas9-DEC-PEG (1μg/μL). The mixture was incubated at 37 °C for various times; and was then analyzed by SDS-PAGE gel.

b) Analysis of mCherryCas9-DEC-PEG stability in mouse plasma with GSH (10mM): To 5 μL of freshly prepared mouse plasma was added 1 μL of mCherryCas9-DEC-PEG (1μg/μL). The mixture was incubated at 37 °C for various times, then GSH (10mM final) was added and further incubated for 30min, and analyzed by SDS-PAGE gel.

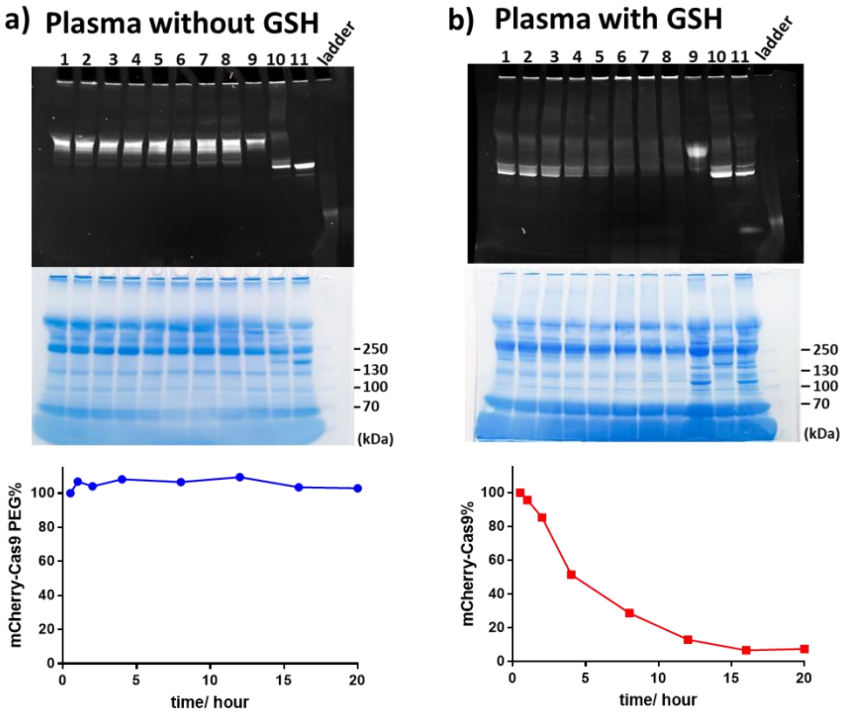


Fig. S11 mCherryCas9-PEG *in vitro* stability in plasma in the absence (a) and presence (b) of GSH (10mM). Lane 1: 0.5 h; lane 2: 1 h; lane 3: 2 h; lane 4: 4 h; lane 5: 8 h; lane 6: 12 h; lane 7: 16 h; lane 8: 20 h; lane 9: mCherryCas9-PEG standard; lane 10: mCherryCas9-PEG standard + GSH; lane 11: mCherryCas9 standard. Data was quantified by fluorescence densitometry.

In vivo brain distribution of Alexa$_{488}$Cas9 RNP and Alexa$_{488}$Cas9 RNP-DEC-PEG5k

Preparation of Alexa$_{488}$Cas9

A 10 mM solution of Alexa Fluor™ 488-NHS ester (Succinimidyl Ester, ThermoFisher, cat. #A20000) in DMSO was diluted into 24.25 µL of gel filtration (GF) buffer (20 mM HEPES, 150 mM NaCl, 10% v/v glycerol, pH 7.5) and gently mixed with Cas9-1NLS in GF buffer, purified as previously described[6], at a molar ratio of 1.5:1. The covered sample were incubated on a rotation wheel for 2 hours at room temperature and dialyzed overnight, with stirring at 200 rpm at 4 °C in GF buffer followed by a second dialysis in fresh GF buffer for 8 hours, to remove excess free fluorophore. The concentration of the conjugated protein was measured with a Nanodrop spectrophotometer (9 µM), flash-frozen in liquid nitrogen, and stored at -80 °C.

Preparation of Alexa$_{488}$Cas9 RNP and Alexa$_{488}$Cas9 RNP-DEC-PEG5k

a. Alexa$_{488}$Cas9 RNP assembly: Cas9 protein and sgRNA were mixed in 1/1 volume and at molar ratio of 1: 1.2, then Alexa$_{488}$Cas9/sgRNA was incubated at 37 ^{o}C for 15 minutes. The concentration was adjusted to 1.0 µg/µL as quantified by the BCA assay. The RNP was then flash-frozen in liquid nitrogen, and stored at -80 °C before use.

b. Alexa$_{488}$Cas9 RNP-DEC-PEG5k assembly: Cas9 protein and sgRNA were mixed in 1/1 volume at a molar ratio of 1: 1.2, then Alexa$_{488}$Cas9/sgRNA was incubated at 37 ^{o}C for 15 minutes. PEG5k-DEC was added at a 100:1 molar ratio, and the mixture was incubated on a rotation wheel for 3 hours at room temperature, the excess PEG5k-DEC was removed by spin filtration (Amicon™ Ultra 50k cutoff) 3 times (10 min, 20 ^{o}C, 10000 rcf). The concentration was ajusted to 1.0 µg/µL as quantified by the BCA assay. The RNP was flash-frozen in liquid nitrogen, and stored at -80 °C before use.

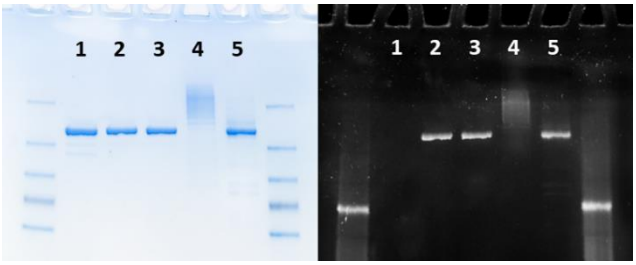


Fig S12 SDS-PAGE gel of Alexa$_{488}$Cas9 RNP and Alexa$_{488}$Cas9 RNP-DEC-PEG5k with or without GSH (10mM). Lane 1, Cas9; lane 2: Alexa$_{488}$Cas9 RNP; lane 3 Alexa$_{488}$Cas9 RNP + GSH; lane 4: Alexa$_{488}$Cas9 RNP-DEC-PEG5k; lane 5: Alexa$_{488}$Cas9 RNP-DEC-PEG5k + GSH. Incubation time: 30min at 37 ^{o}C.

Stereotactic surgery

C57BL/6J female mice (12-week old) were anesthetized with 2% v/v isoflurane (Baxter; Deerfield, IL) in 3 L/min oxygen, and were placed in a small-animal stereotactic frame (David Kopf Instruments; Tujunga, CA). A sagittal incision was made in the skin, and burr-holes were made in the skull by a dental drill, 0.5 mm anterior to the bregma and 2 mm to the right and left of the midline. All infusions were performed by Convection-enhanced Delivery (CED).[7] To minimize trauma and reflux, a fused silica with a 1-mm stepped cannulae (O.D. 235 mm; I.D. 100 mm) were used (Polymicro Technologies, Phoenix, AZ). The cannulae were attached directly to Nanofill-100 syringes placed in the pumps controlled by a Micro4 MicroSyringe Pump Controller (World Precision Instruments Inc., Sarasota, FL). Alexa$_{488}$Cas9-RNP and Alexa$_{488}$Cas9 RNP-DEC-PEG5k were resuspended in PBS (pH 7.4) at a concentration of 1.0 µg/µL and were infused bilaterally into the left and right striatum accordingly (AP: 0.5 mm; ML: ± 2.0 mm; DV: -2.5 mm). The coordinates were taken from the mouse brain atlas.[8] Seven microliters were infused into each striatum at a rate 1µL/min.

Brain processing and immunohistochemical staining

The mice were euthanized immediately after infusion (T_0) by CO_2 narcosis. After euthanasia, mice were transcardially perfused with cold PBS and then 10% buffered formalin. The brains were removed and post-fixed in formalin overnight. Brains were washed briefly in PBS, transferred to a 30% w/w sucrose solution in 0.01 M PBS for cryopreservation and then cut into 40-µm serial coronal sections on a cryostat. Frozen sections were collected in a series in antifreeze solution and stored at -80 ^{0}C. Every tenth section was mounted on a glass slide,

coverslipped, and photographed under the Zeiss Imager Z.1 fluorescence microscope (green channel: 488 nm). To validate the distribution of the injected nanoparticles based on the fluorescent signal, we also employed immunohistological staining (IHC) against Cas9 protein. Briefly, after 3 washes (3 x 5 minutes) in PBS + 0.01 Tween-20 (PBST), the sections were incubated in 1% v/v H_2O_2/PBS for 20 min. After three more washes in PBST, sections were incubated in 1x animal-free blocker (Vector Laboratories, Burlingame, CA) for 30 min, followed by overnight incubation in primary antibody solution (a rabbit polyclonal Guide-it™ Cas9 antibody in 1x animal-free blocker at 1:1000 dilution; room temperature). After three washes in PBST, sections were incubated in ImmPress horse anti-rabbit IgG peroxidase polymer (Vector Laboratories, Burlingame, CA) for 1 hour. After three washes in PBST (3 x 5 minutes), immunoreactivity was visualized with DAB Peroxidase Substrate Kit (Vector Laboratories, Burlingame, CA). Sections were mounted onto slides, dried and covered with glass coverslips.

Image analysis

The volume of distribution (V_d) of Cas9-RNPs in the mouse brain was calculated from the Cas9-immunohistochemically stained sections. The Macintosh-based image analysis system (NIH ImageJ 1.52q; Bethesda, MD, USA) was used. Images of IHC-stained brain sections were captured by a CCD camera and Adobe Photoshop software (Adobe Systems, Inc., San Jose, CA, USA). With the NIH Image analysis software, the area of distribution of Cas9 signal in each tissue section was determined automatically at a 50% threshold of the maximum stained optical density. The sum of the areas of infusion was used to determine the V_d in each striatum and/or hemisphere. Quantification was performed on every 10th serial section (a distance of 400 μm between analyzed sections) by the Cavalieri method.[9]

Mouse	**Left striatum** $Alexa_{488}$Cas9-RNP	**Right striatum** $Alexa_{488}$Cas9 RNP-DEC-PEG5K	Fold difference
V_d ST	3.00 mm^3	11.09 mm^3	3.7
ST coverage	26.4%	85.8%	3.2
V_d HEM	3.55 mm^3	16.02 mm^3	4.5

Table S2. Volumetric analysis of the injected nanoparticles in the mouse brain. V_d ST – volume of distribution within the striatum (target structure); ST coverage – percentage of striatal coverage; V_d HEM – volume of distribution within the entire hemisphere (striatum + other adjacent brain structures). All the quantifications and analysis were performed by the Cavalieri method.[9]

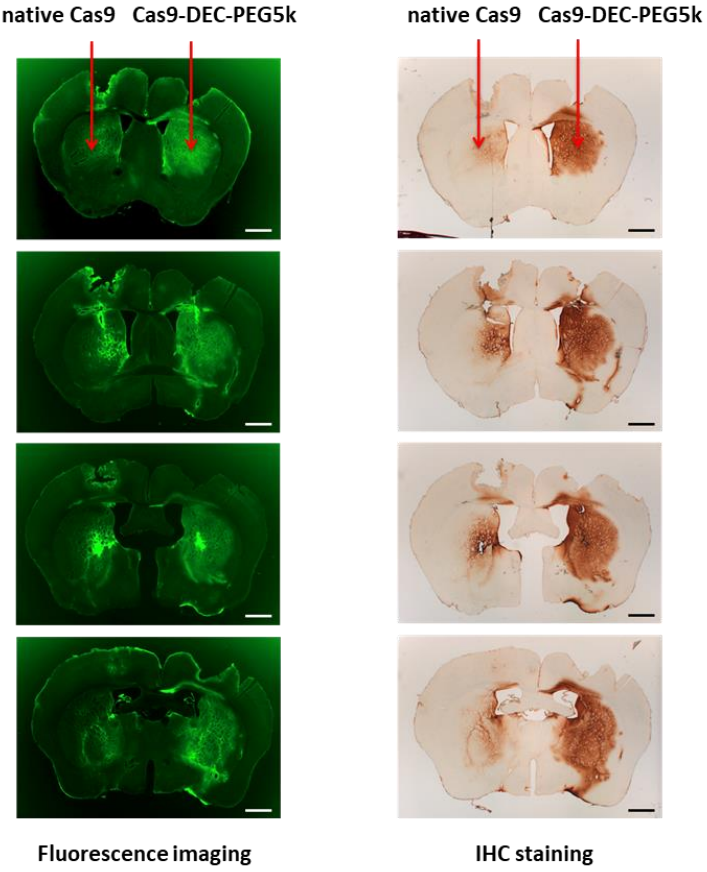


Fig S13 Striatum distribution in mice brain after injection with $Alexa_{488}$Cas9 RNP (left striatum) or $Alexa_{488}$Cas9 RNP-DEC-PEG5k (right striatum). Fluorescence microscope (green channel: 488 nm). Scale bar: 1mm.

Synthesis of FITC-R10K1-DEC (CPP-DEC)

CPP azide (FITC-R10K1-N_3) (7.5 mg, 3.4 μmol) (purchased from GenScript) was dissolved in 0.5 mL of DMF, to this solution were added DEC (8.5 mg, 16.8 μmol, 5 equiv), CuI (0.1 mg) and sodium ascorbate (0.4 mg, 2 μmol. The mixture was degassed for 10 min and stirred under N_2 for 4 h. After 4 h, HPLC showed that the FITC-R10K1-N_3 was fully consumed and the reaction solution was filtered through a 0.45 mm filter, and added dropwise to 30 mL of cold ether. The yellow prepitate was collected and redissolved in 0.2 mL of DMF, and precipitated with cold ether 3 times, and dried under vacuum, the yellow solid was further purified by HPLC to afford CPP-DEC as a yellow powder after lyophilization. m= 8.0 mg, yield 86.0 %. ^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.20 (d, *J* = 9.3 Hz, 1H), 8.10 (d, *J* = 7.8 Hz, 2H), 7.94 (s, 1H), 7.79 (s, 1H), 7.50 (s, 1H), 7.30 (s, 1H), 7.20 (d, *J* = 6.8 Hz, 2H), 7.01 (d, *J* = 8.3 Hz, 3H), 6.89 (s, 1H), 6.79 (s, 2H), 6.62 (s, 2H), 5.14 (s, 2H), 4.48 (s, 3H), 4.32 (s, 2H), 4.16 (d, *J* = 11.9 Hz, 13H), 3.64 (s, 2H), 3.00 (d, *J* = 18.4 Hz, 18H), 2.79 (d, *J* = 28.9 Hz, 4H), 1.56 (d, *J* = 84.1 Hz, 44H), 1.23 (s, 2H), 1.14 (s, 2H). $C_{115}H_{175}N_{47}O_{27}S_3$ calculated 2742.2928 $[M+H]^+$ found 915.4399 ($[M+H]^{3+}$); 1178.1387 ($[M+H]^{2+}$); 785.7613 $C_{98}H_{161}N_{46}O_{19}S_2$ ($[M+H]^{3+}$) (disulfide bond cleaved fragment).

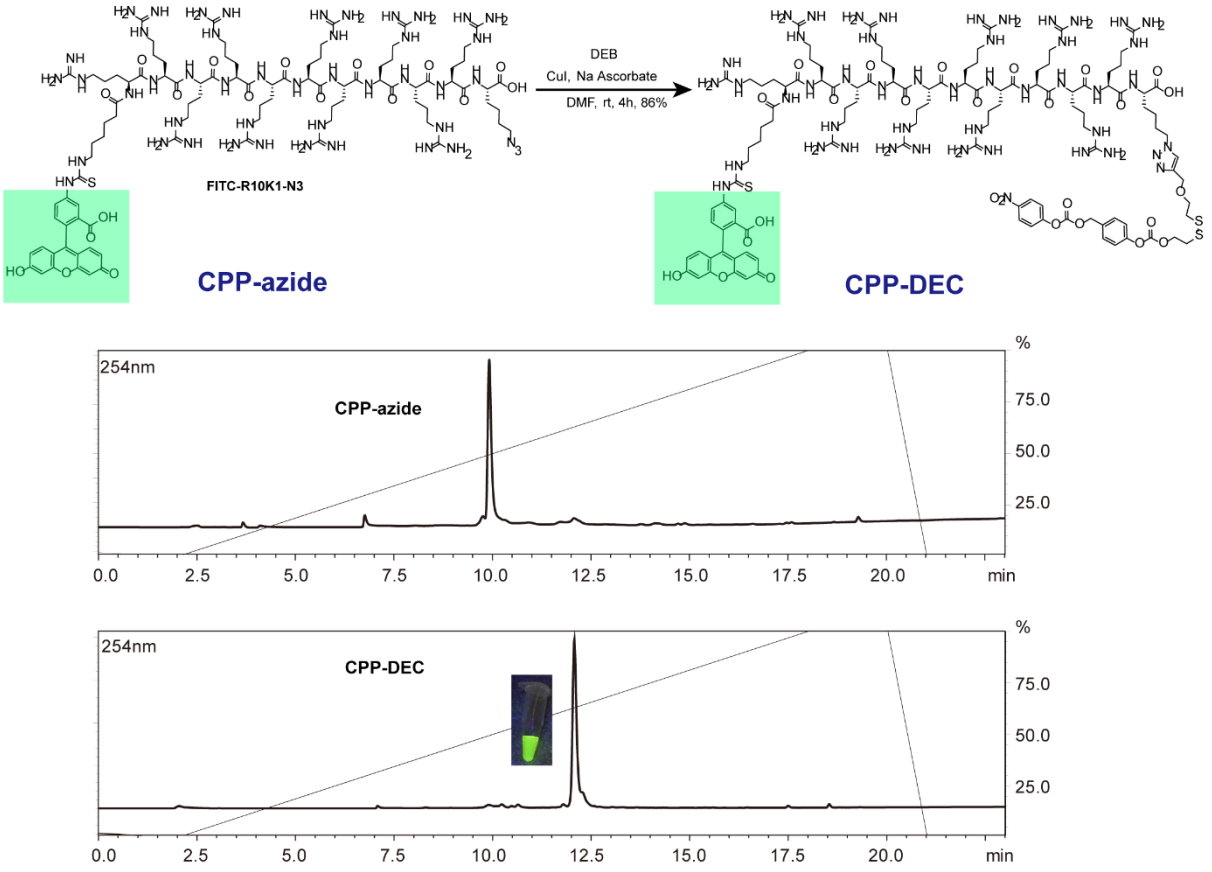


Fig S14 Synthesis of CPP-DEC (up) and HPLC analysis of CPP azide and CPP-DEC (down).

Preparation of Cas9-CPP or mCherryCas9-CPP

1) Cas9-CPP: 70 μL of Cas9 stock solution (40 μM stock solution purchased from qb3. Berkeley Macrolab) was diluted with 123.4 μL of PBS buffer (0.2 X) (final Cas9 concentration 0.53 μg/μL, 3.3 μM), and 6.6 μL of CPP-DEC stock solution (Stock concentration: 10 mM) was added to reach a final concentration of 333 μM. The mixture was incubated at room temperature for 4 h, then the reaction solution was diluted to 500 μL with dialysis buffer (20 mM HEPES-KOH, 1% glycerol. pH 7.5), and run through a spin filtration column (Amicon™ Ultra 50k filter) 3 times (10 min, 20 °C, 10000 g). SDS-PAGE gel electrophoresis and absorption measurements confirmed that the free peptide was removed from the conjugate. The final concentration of Cas9-CPP was 240 μg/mL, as determined by the BCA assay. See **Fig. S15a** for SDS-PAGE gel analysis.

2) mCherryCas9-CPP: 90 μL of mCherryCas9 (final 0.55 μg/uL, 3 μM, purchased from qb3. Berkeley Macrolab) was diluted with 104 μL of PBS buffer (0.2 X), and 6 μL of CPP-DEC (stock concentration: 10mM) was added to reach a final concentration of 300 μM. The mixture was incubated at room temperature for 4 h, then the reaction

solution was diluted with Dialysis buffer (20 mM HEPES-KOH, 1% glycerol. pH 7.5) to 500 μL, and run through a spin filtration column (Amicon™ Ultra 50k filter) 3 times (10 min, 20 °C, 10000 g), to remove the free peptide. The final concentration of mCherryCas9-CPP was measured by the BCA assay. Final concentration: 768 μg/mL. See **Fig. S15b** for SDS-PAGE gel analysis.

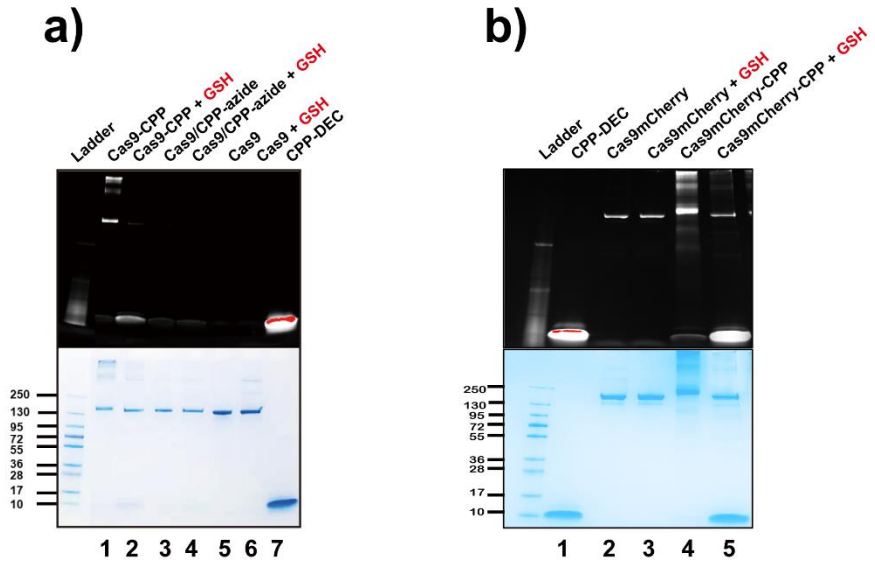


Fig. S15 a) SDS-PAGE gel of Cas9-CPP conjugate (lane 1), Cas9-CPP conjugate + GSH (10 mM) (lane 2), Cas9 mixed with CPP azide and purified by spin filtration (control for non-covalent binding) (lane 3), Cas9 mixed with CPP azide + GSH (10 mM) (lane 4), unmodified Cas9 (lane 5), unmodified Cas9 + GSH (lane 6), CPP-DEC (lane 7). b) SDS-PAGE gel of CPP-DEC (lane 1), unmodified mCherryCas9 (lane 2), unmodified mCherryCas9 + GSH (lane 3), mCherryCas9-CPP conjugate (lane 4), mCherryCas9-CPP conjugate + GSH (10 mM) (lane 5).

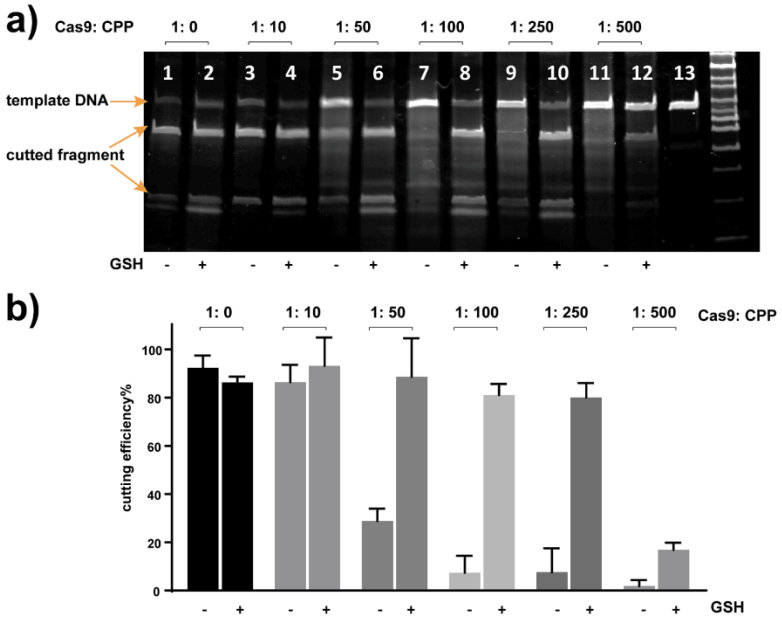


Fig. S16 Cas9 and Cas9-CPP (prepared at different ratios) *in vitro* nuclease activity in the presence and absence of GSH. a) Unmodified Cas9 *in vitro* cutting efficiency was used as a positive control. Lanes 1-2: Cas9 RNP (+/- GSH); Lane 3-4: Cas9 RNP-CPP 1:10 molar ratio (+/- GSH); Lanes 5-6: Cas9 RNP-CPP 1:50 molar ratio (+/- GSH); Lanes 7-8: Cas9 RNP-CPP 1:100 molar ratio (+/- GSH); Lanes 9-10: Cas9 RNP-CPP 1:250 molar ratio (+/- GSH); Lanes 11-12: Cas9 RNP-CPP 1:500 molar ratio (+/- GSH); Lane 13: template DNA standard. b) Bar graph quantification of data from **Fig S16a** by fluorescence densitometry. Error bars represent s.d.. (n = 3).

Transfection of HEK293T eGFP cells with Cas9-CPP and GFP knockdown

HEK293T eGFP cells[10] were seeded in 48 well plates at a density of 20K per well in DMEM medium supplemented with 10% FBS, 1% penicillin-streptomycin. After 24 hours, the medium was removed and replaced with a 150 μL Opti-MEM (Gibco) containing Cas9-CPP RNP (5 μg Cas9 equiv.). The cells were transfected for 48 h at 37 °C in a humidified incubator containing 5% CO_2 and the medium was replaced with fresh DMEM medium supplemented with 10% FBS, 1% penicillin-streptomycin and 1 mg/mL doxycycline. RT HEK cells were detached by Accutase (Invitrogen) and the cell pellets were collected by centrifugation at 500g for 5 min. Genomic DNA was extracted with a genomic extraction kit (Qiagen) and amplified by PCR following the PCR product purification guidelines provided by Qiagen. Sanger sequencing was conducted at the UC Berkeley DNA sequencing facility and gene editing efficiency was quantified by TIDE analysis https://tide.deskgen.com/. (**Fig. 5a**). In addition, in a separated experiment, HEK293T eGFP cells were treated as described above, and were analyzed with an Attune NxT flow cytometer for GFP knockdown (ThermoFisher Invitrogen) (see **Fig. S17**).

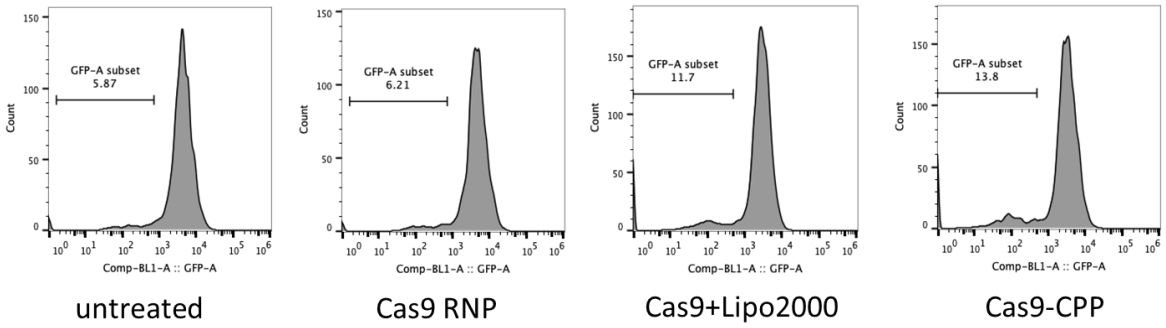


Fig. S17 Flow cytometry data for GFP knockdown with Cas9-CPP.

Cell internalization studies analyzed by flow cytometry and fluorescent microscopy

1) HEK293T eGFP cells were seeded in 48 well plates at a density of 20K per well in DMEM medium supplemented with 10% FBS, 1% penicillin-streptomycin. After 24 hours, the medium was removed and replaced with a 150 μL Opti-MEM (Gibco) containing mCherryCas9-CPP (5 μg Cas9 equiv.). The cells were transfected for 48 h at 37 °C in a humidified incubator containing 5% CO_2 and the medium was replaced with fresh DMEM medium supplemented with 10% FBS, 1% penicillin-streptomycin and 1 μg/mL doxycycline. HEK293T eGFP cells were detached by Accutase (Invitrogen) and the FITC and mCherry signals were analyzed with a Attune NxT flow cytometer (ThermoFisher Invitrogen) (**Fig. 5b**).

The intracellular delivery of mCherryCas9-CPP was also investigated using fluorescence microscopy. HeLa cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. Glass cover slides were placed on the bottom of the 6 well plates and the cells (50,000 cells/well) were seeded the day before the addition of mCherryCas9-CPP. mCherryCas9-CPP (5 μg/mL) was added to the cell cultures and incubated for 2h. The cells were washed three times with PBS (1X), stained with DAPI for nucleus staining and visualized with a fluorescent microscope (Zeiss Axiovert 200M Inverted Fluorescence Microscope) (**Fig. S18**).

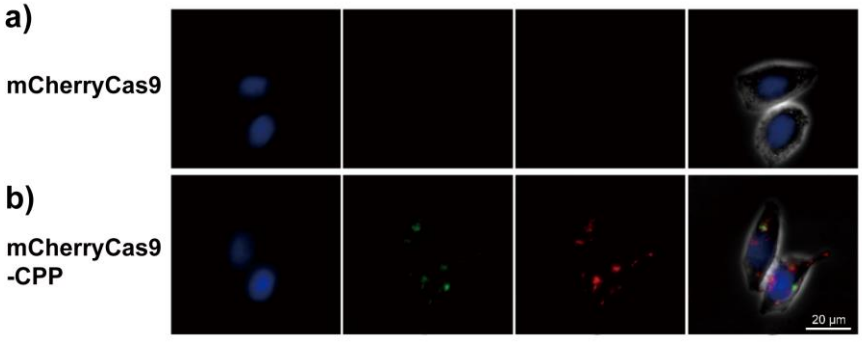


Fig. S18 Detection of mCherryCas9 in live HeLa cells. Cells were incubated with mCherryCas9 (5 μg/mL) (a) or mCherryCas9-CPP (5 μg/mL) (b) for 2h. The cells were washed 3 times with PBS. Imaging was performed on a Zeiss Axiovert 200M Inverted Fluorescence Microscope. Scale bar: 20 μm.

2) **Uptake efficiency of mCherryCas9-CPP in cardiomyocytes**. Human stem cell derived cardiomyocytes were incubated overnight with 80 µL of RPMI 1640 Medium (11875093, Thermo Fisher Scientific) with 30 to 120 µg/mL mCherryCas9-CPP or mCherryCas9 (control) to evaluate their uptake efficiency. Then, they were rinsed three times in PBS and fixed in a 4% solution of paraformaldehyde (Santa Cruz Biotechnology) for 15 min and then permeabilized with 0.1% Triton-X-100 (Fisher Scientific) for 15 min. After blocking with 3% BSA for 1 hour, cells were incubated overnight at 4 °C with a primary antibody for Cardiac Troponin T (ab8295, Abcam). Subsequently, cardiomyocytes were stained with Alexa Fluor-conjugated secondary antibodies (A11008, Life Technologies) for 1 hour at room temperature and with a nuclei-stain applied (DAPI, 4,6- diamidino- 2-phenylindole; 15500, Sigma-Aldrich). In between each step, cells were rinsed three times in PBS with 0.1% Tween-20. Microscopy images were acquired using the ImageXpress Micro XLS in wells (Molecular Devices). Samples were imaged with a 20 X objective.

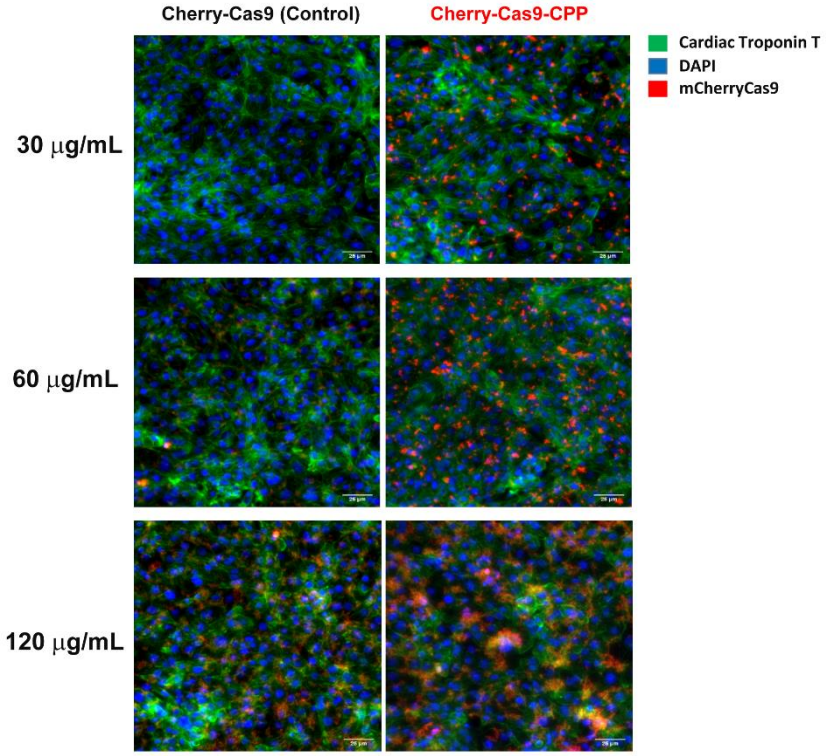


Fig S19 Uptake efficiency of mCherryCas9-CPP in cardiomyocytes. mCherryCas9-CPP or mCherryCas9 was added at different concentrations (30-120 µg/mL). Microscopy images were acquired using the ImageXpress Micro XLS in wells (Molecular Devices). Samples were imaged with a 20X objective. Scale bar: 25 µm.

Synthesis of PEG2k-DEC-N_3

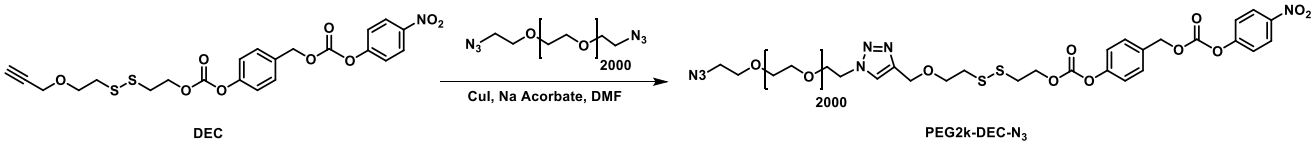


Fig. S20 Synthesis of PEG2k-DEC-N_3. Condition and reagents: azide-PEG2000-azide, CuI, Na Ascorbate, DMF, N_2, rt, 12h. 44%

Procedure: Azide-PEG2000-Azide (Purchased from Creative PEGWorks) (110.0 mg, 0.055mmol, 1.1 equiv) was dissolved in 2 mL of dry DMF, to this solution was added DEC (25.35 mg, 0.05 mmol, 1 equiv), then CuI (0.5 mg, 0.0025 mmol) and sodium ascorbate (5.0 mg, 0.025 mmol) were added and the mixture was degassed for 10 min before stirring under N_2 overnight. The reaction was monitored by TLC, after completion the solvent was removed by vacuum, The residue was purified by short column chromatography (hexane:EtOAc 1:1 to DCM: MeOH 10:1) to afford the desired product as a white solid. m= 55.5 mg, yield 44.3%. ^{1}H NMR (900 MHz, Chloroform-*d*) δ 8.26 (d, *J* = 8.6 Hz, 2H), 7.77 (s, 1H), 7.46 (d, *J* = 8.2 Hz, 2H), 7.37 (d, *J* = 8.7 Hz, 2H), 7.22 (d, *J* = 8.1 Hz, 2H), 5.27 (s, 2H), 4.66 (s, 2H), 4.52 (t, *J* = 5.2 Hz, 2H), 4.48 (t, *J* = 6.6 Hz, 2H), 3.85 (t, *J* = 5.2 Hz, 2H), 3.79 (t, *J* = 6.3 Hz, 2H), 3.76 – 3.43 (m, 163H), 3.37 (t, *J* = 5.2 Hz, 1H), 2.99 (t, *J* = 6.6 Hz, 2H), 2.93 (t, *J* = 6.4 Hz, 2H). ^{13}C NMR (226 MHz,

$CDCl_3$) δ 155.6, 153.4, 152.5, 151.6, 145.6, 132.3, 130.2, 125.5, 122.0, 121.6, 70.8, 70.8, 70.8, 70.8, 70.8, 70.8, 70.8, 70.7, 70.7, 70.7, 70.7, 70.6, 70.6, 70.6, 70.6, 70.6, 70.6, 70.6, 70.6, 70.3, 70.2, 69.6, 68.6, 66.7, 64.5, 50.8, 50.5, 38.8, 36.9.

Cas9 RNP-DEC-N_3 preparation and click reaction

1). **Cas9 RNP-DEC-N_3 preparation**: 64 μL of pre-assembled Cas9 RNP solution (6.25 μM, gRNA targeting the BFP gene, see sequence at page S24) was diluted with 34 μL of PBS buffer (1X) (final Cas9 concentration 4 μM), and 2 μL of PEG2k-DEC-N_3 solution (Stock concentration: 10 mM) was added to reach a final concentration of 200 μM (50-fold molar excess). The mixture was incubated at room temperature for 3 h, then the reaction solution was diluted with 100 μL PBS (1X), and filtrated through a spin filtration column (Amicon™ Ultra 50k cutoff) 3 times (10 min, 20 °C, 10000 rcf). The final concentration of Cas9 RNP-DEC-N_3 was determined by the BCA assay.

	Stock	Final	Volume
Cas9 RNP	6.25 μM	4 μM	64 μL
PEG2k-DEC-N_3	10 mM	200 μM	2 μL
PBS 1X			34 μL
Total			100 μL

2). **Cas9 RNP-DEC-N_3 reaction with AF488-DBCO dye**: 1 μL Cas9 RNP-DEC-N3 (stock:12.5μM) was diluted with 8 μL of PBS buffer (1X) (final Cas9 concentration 1.25 μM), and 1 μL of AF488-DBCO solution (Stock concentration: 62.5 μM) was added to reach a final concentration of 6.25 μM (5-fold molar excess). The mixture was incubated at room temperature for 1 h, then the reaction solution was analysized by SDS-PAGE gel (**Fig. S21b**).

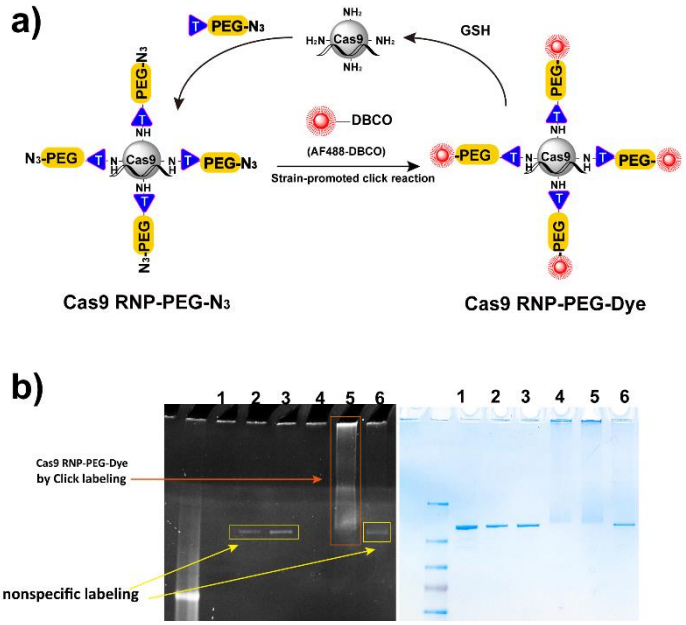


Fig. S21 Preparation of Cas9 RNP-DEC-N3. a) Carton shows the synthesis of Cas9 RNP-DEC-N_3 and its click reaction with AF488-DBCO. b) SDS-PAGE gel shows Cas9 RNP-DEC-N3 was labeled with AF488 by the SPAAC click reaction (lane 5), and released under GSH (lane 6). As the control, native Cas9 was incubated with AF488-DBCO under the same conditions, and it shows AF488-DBCO non-specifically labeled Cas9 protein with very low efficiency (Lanes 2 and 3). Lane 1: Cas9; lane 2: Cas9 + AF488-DBCO; lane 3: Cas9 + AF488-DBCO + GSH; lane 4: Cas9 RNP-DEC-N_3; lane 5: Cas9 RNP-DEC-N_3 + AF488-DBCO; lane 6: Cas9 RNP-DEC-N_3 + AF488-DBCO + GSH.

DBCO-DNA preparation and click reaction

1). **DBCO-DNA preparation**: 50 μL Donor DNA-amine (purchased from IDT, for sequence see page S24) (stock: 100 μM) was diluted with 45 μL of PBS buffer (1X) (final DNA concentration 50 μM), and 5 μL of DBCO-NHS ester (Stock concentration: 10 mM) was added to reach a final concentration of 500 μM (100 fold molar excess) (**Fig. S22a**). The mixture was incubated at room temperature for 12 h, then passed through a Zeba™ Spin Desalting

Column, 40K MWCO (Thermo Scientific) 2 times, the final concnetration of DBCO-DNA was quanatified by nanodrop 2000 (Thermo Scientific).

2). **DBCO-DNA reaction with AF488-N3 dye**: 1 μL Cas9 RNP-DEC-N3 (stock:15 μM) was diluted with 3 μL of PBS buffer (1X) (final DBCO-DNA 3 μM), and 1 μL of AF488-Azide solution (Stock concentration: 75 μM) was added to reach a final concentration of 15 μM (5 fold molar excess). The mixture was incubated at room temperature for 1 h, then the reaction solution was analysized by agarose gel (**Fig. S22b**).

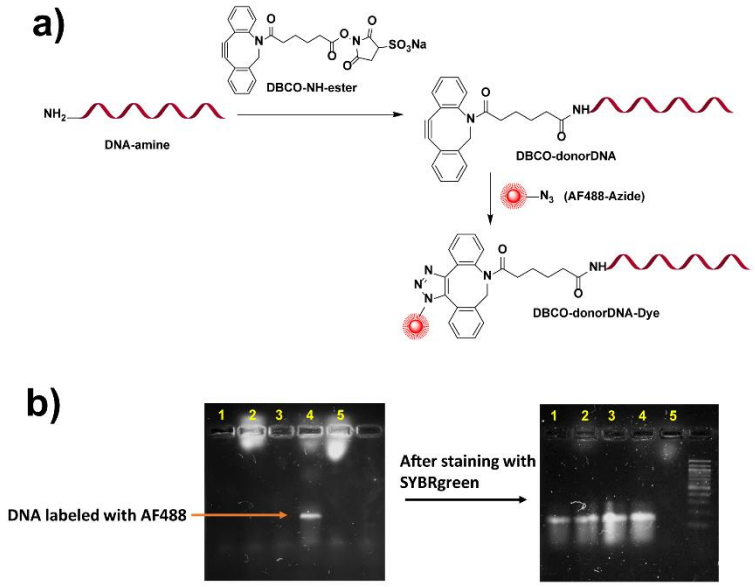


Fig. S22 Preparation of DBCO-donor DNA. a) Carton shows the preparation of DBCO-donor DNA from DNA amine and its click reaction with AF488-azide. b) Agarose gel shows the synthesized DBCO-donor DNA was able to conjugate with AF488-azide by click reaction (lane 4). As the control, Donor DNA amine was incubated with AF488-azide at same condition, and no labeling was observed (lane 2). Lane 1: Donor-DNA amine; lane 2: Donor DNA + AF488 azide; lane 3: DBCO-donor DNA; lane 4: DBCO-donor DNA + AF488 azide; lane 5: AF488 azide.

Preparation of Cas9 RNP-DEC-DNA by click conjugation of DBCO-DNA with Cas9 RNP-DEC-N_3

1).**Cas9 RNP-DEC-DNA preparation**: 10 μL of Cas9 RNP-DEC-N_3 solution (stock 12.5 μM, gRNA targeting BFP gene, sequence see page S24) was mixed with 40 μL of DBCO-DNA (stock 15 μM), The mixture was incubated at room temperature for 2 h, then the mixed solution was used for cell work without further purification.

	Stock	Final	Volume
Cas9 RNP-DEC-N_3	12.5 μM	2.5 μM	10 μL
DBCO-DNA	15 μM	12 μM	40 μL
Total			50 μL

2). ***In vitro* Cas9 RNP-DEC-DNA nuclease activity**: 2.5 μL Cas9 RNP-DEC-DNA (stock: 2.5 μM) was diluted with 6.5 μL of CutSmart® Buffer (1X), 20 ng of template DNA (1 μL) was then added followed GSH (final 10 mM) or PBS. The mixture was incubated at 37 °C for 4 h and analyzed by SDS-PAGE gel.

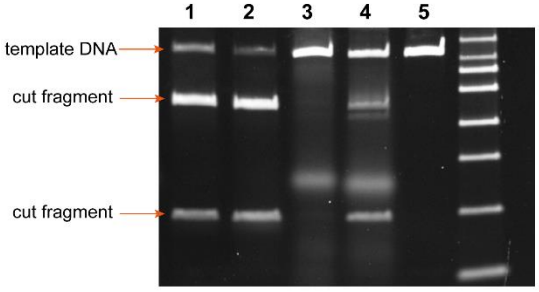


Fig. S23 Cas9 RNP-DEC-DNA regained its nuclease activity *in vitro* in the presence of GSH. SDS-PAGE gel shows Cas9 RNP-DEC-DNA recovered its nuclease activity *in vitro* in the presence of GSH (lane 4). Lane 1: template DNA + Cas9 RNP; Lane 2: template DNA + Cas9 RNP + GSH; lane 3: template DNA + Cas9 RNP-DEC-DNA; lane 4: template DNA + Cas9 RNP-DEC-DNA + GSH; lane 5: template DNA.

HDR test protocol

BFP-HEK293T cells were plated at a density of 10^5 cells per well in a 24-well plate, one day before editing experiments were performed. BFP-HEK cells were detached with Accutase solution (Stemcell Technologies), spun down at 600 g for 3 min, and washed with PBS. Nucleofection was conducted using SE Cell Line 4D-NucleofectorTM X Kit (Lonza) in the 4D-NucleofectorTM X Unit (100 µL format) following the manufacturer's protocol, and using Cas9 RNP (Cas9 – 5 µg, 31.25 pmole, sgRNA – 37.5 pmole) mixed with Donor DNA (187.5 pmole of Donor DNA) or equivalent amount of Cas9 RNP-DEC-DNA for each sample. After the nucleofection, BFP-HEK cells were allowed to sit in the cuvette for 10 min and spun down at 600 g for 3 min. The cell pellets were resuspended in 1 mL growth media, plated in a 24 well plate and incubated at 37℃ in a humidified incubator containing 5% CO_2 for another 72 h before flow cytometry analysis was performed. The HDR frequency was quantified by determining the GFP positive population. See **Fig S24** for flow cytometry data.

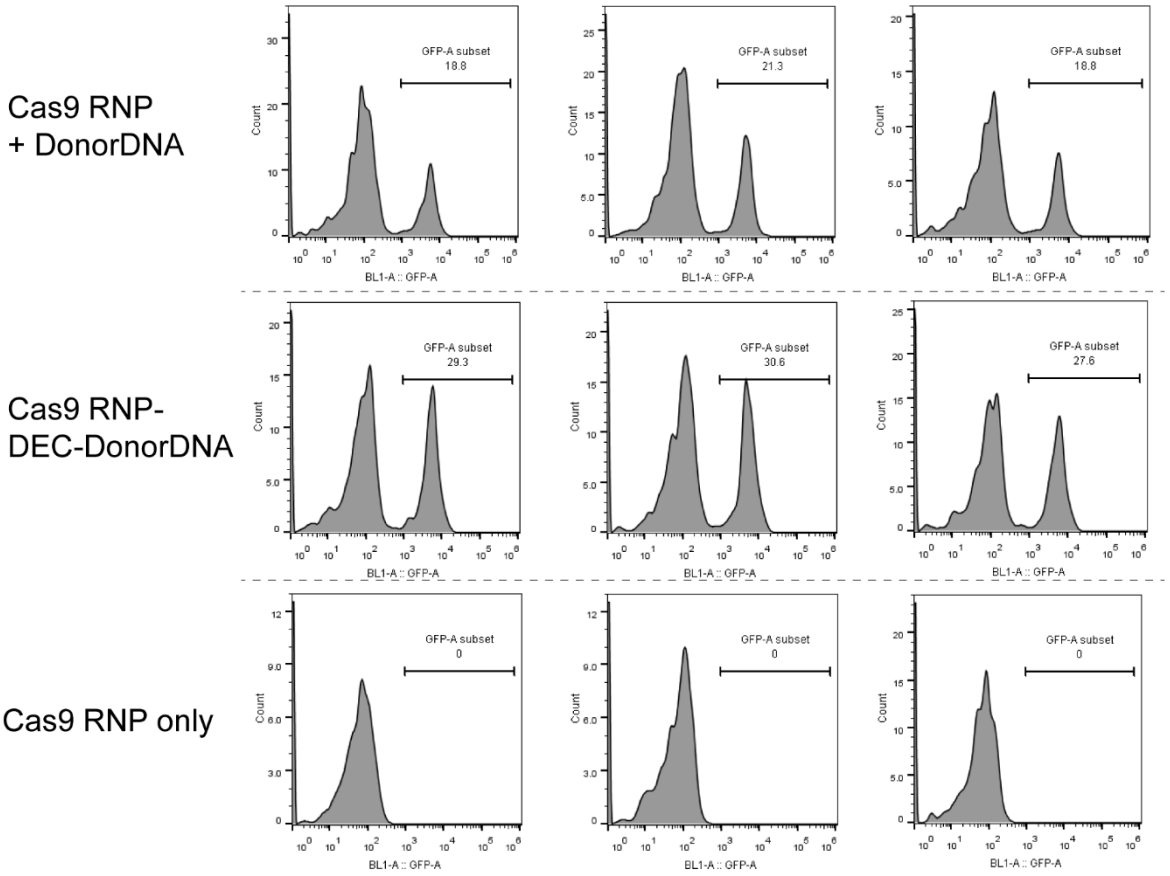


Fig. S24 Flow cytometry data measuring HDR efficiencey in BFP HEK cells (BFP to GFP).

Cell viability assay

A cell viability assay was performed to assess the cytotoxicity of mCherryCas9-CPP. HeLa cells (1×10^4 cells/well) were seeded in 96-well plates and allowed to grow in DMEM (Gibco) supplemented with 10% (v/v) FBS, 0.05 mg/mL penicillin G and 80 µg/mL streptomycin and incubated at 37 °C with 5% CO_2 for 24 h. Cells were treated with various concentrations of mCherryCas9-CPP. Control cells were added with equivalent volume of fresh media. After incubation for 24 h, a final concentration of 1 µg/ml Resazurin (sigma) was added to each well and incubated for 4 h under cell culture conditions. The absorption was measured with a plate reader (TECAN infinite m200) with Ex = 540 nm, Em = 590 nm. The cell growth inhibition was calculated by dividing the absorption of the test sample with the absorption of the control sample.

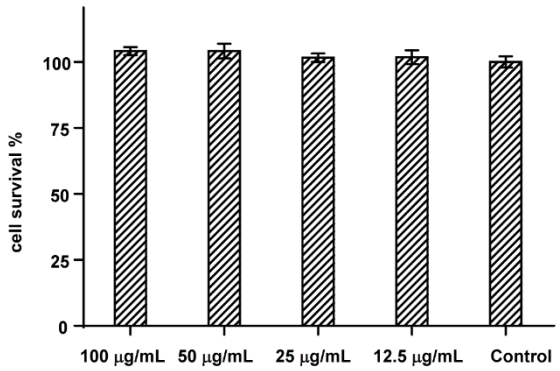
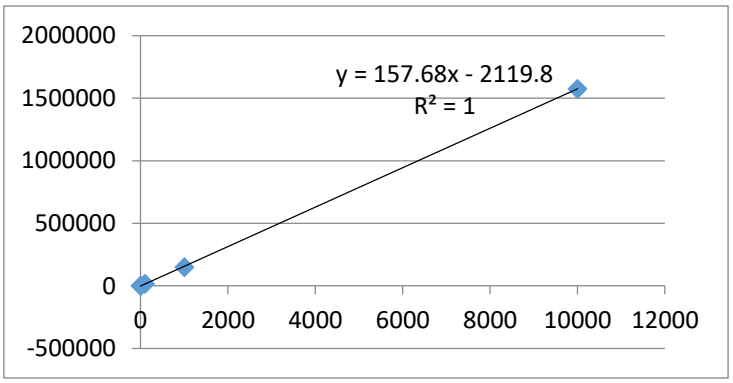


Fig. S25 Cytotoxicity of mCherryCas9-CPP against HeLa cell. mCherryCas9-CPP was incubated at different concentrations (12.5-100 μg/mL) with HeLa cells for 24 h and the toxicity was measured, no toxicity was observed at concentrations at or below 100 μg/mL.

ICP measurement

Cu residue measurement was done on a Perkin Elmer 5300 DV optical emission ICP. Copper standard for ICP was purchased from sigma (1000 mg/L Cu in 2% nitric acid). The instrument was calibrated using 0, 0.01, 1, 100, 1000, 10000 μg/L of Cu standard solution prepared in 2% (v/v) nitric acid. The concentrations of Cu was calculated as μg of metal per g of test compounds.



Cu standard calibration curve

Table S3 ICP measurement of Cu residue

Sample ID	Cu residue (ppm)
PEG-DEC-Cipro	166.2
PEG-DTB-Cipro	92.6
PEG-DCB-Cipro	110.8
CPP-DEC	14.7
PEG5000-DEC	122
PEG2000-DEC-N_3	105.6

gRNA and DNA sequence

GFP Sequence:
ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAA
ACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTT
CATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAG
TGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGT
CCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGC
GACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACA

AGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTG
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CCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGA
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GACGAGCTGTACAAGTAA

BFP sequence
ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAA
ACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTT
CATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCCATGGCGTGCAG
TGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGT
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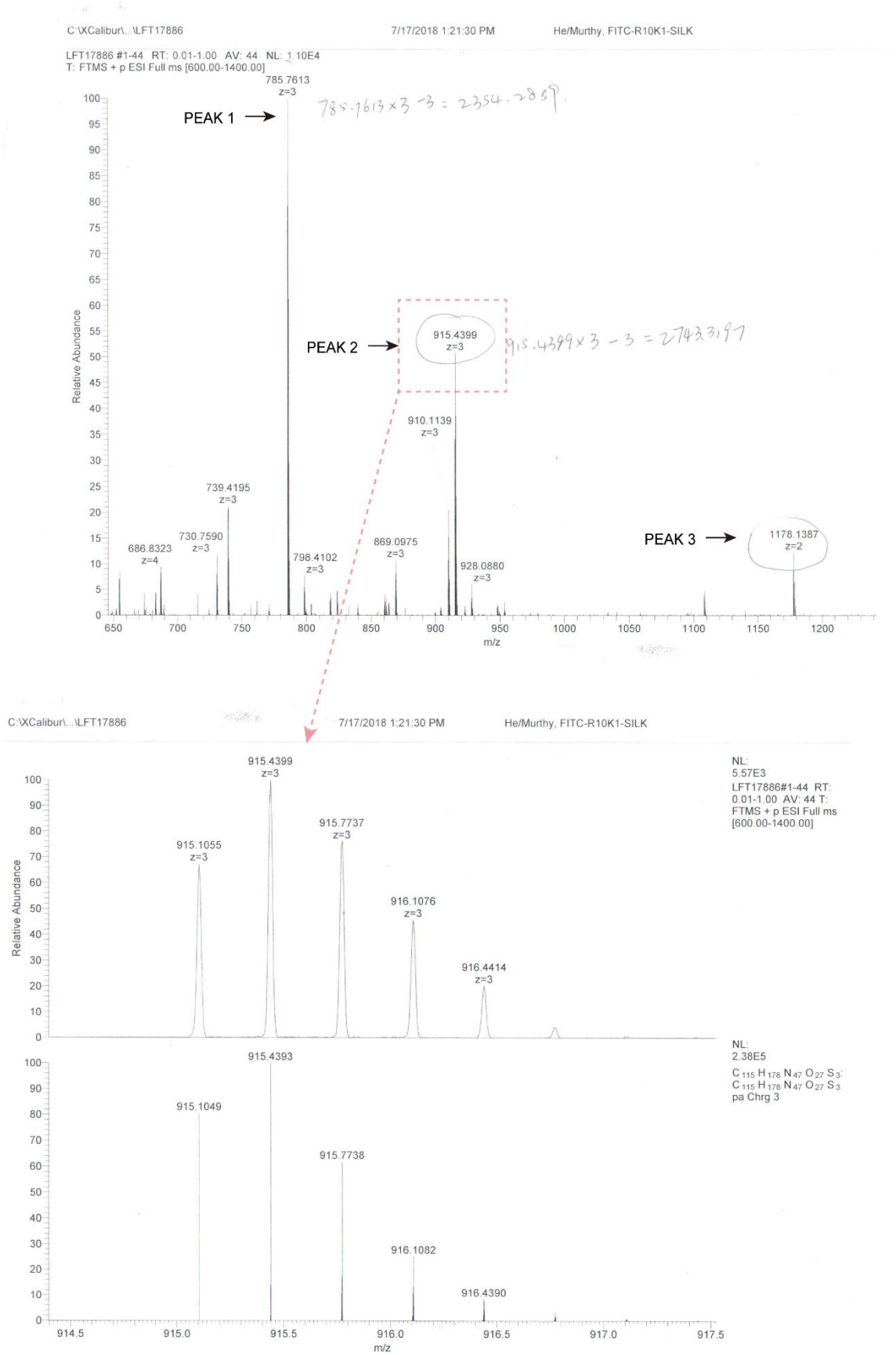
CCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATG
GACGAGCTGTACAAGTAA

GFP sgRNA targeting sequence:
GCTGAAGCACTGCACGCCGT

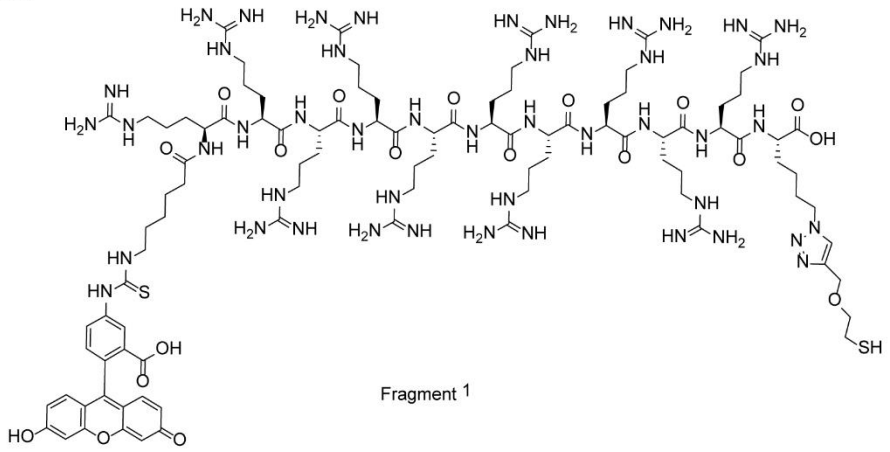
BFP sgRNA targeting sequence:
GCTGAAGCACTGCACGCCAT

Donor DNA sequence:
GCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCC
TCGTGACCACCCTGACGTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGA

Mass spectra for FITC-R10K1-DEC (CPP-DEC)



PEAK 1



Fragment [1]

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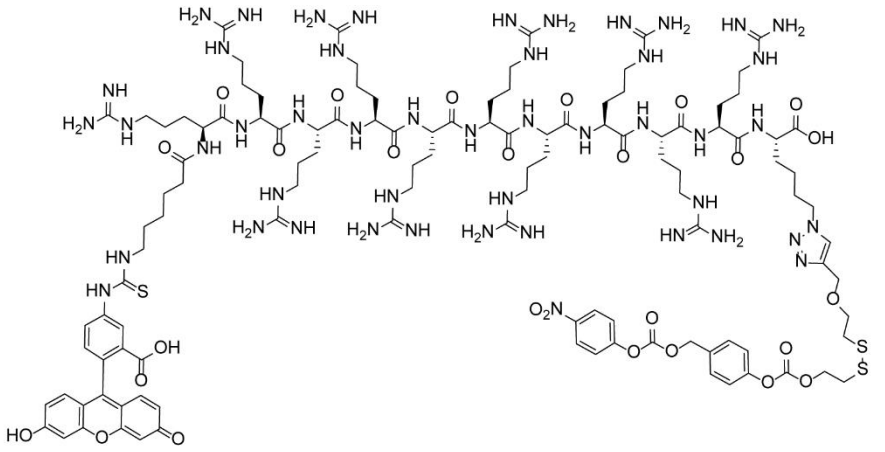
Molecular Weight: 2352.7970

PEAK 2 and PEAK 3



Chemical Formula: $C_{115}H_{175}N_{47}O_{27}S_3$
Exact Mass: 2742.2928
Molecular Weight: 2744.1470

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Elemental composition search on mass 915.11

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Isotope  Min    Max
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O-16       0     27
C-12       0    115
H-1        0    200
S-32       3      3
Charge 3
Mass tolerance 5.00 ppm
Nitrogen rule not used
RDB equiv -1.00-100.00
max results 10
   m/z      Theo.     Delta      RDB       Composition
             Mass     (ppm)    equiv.
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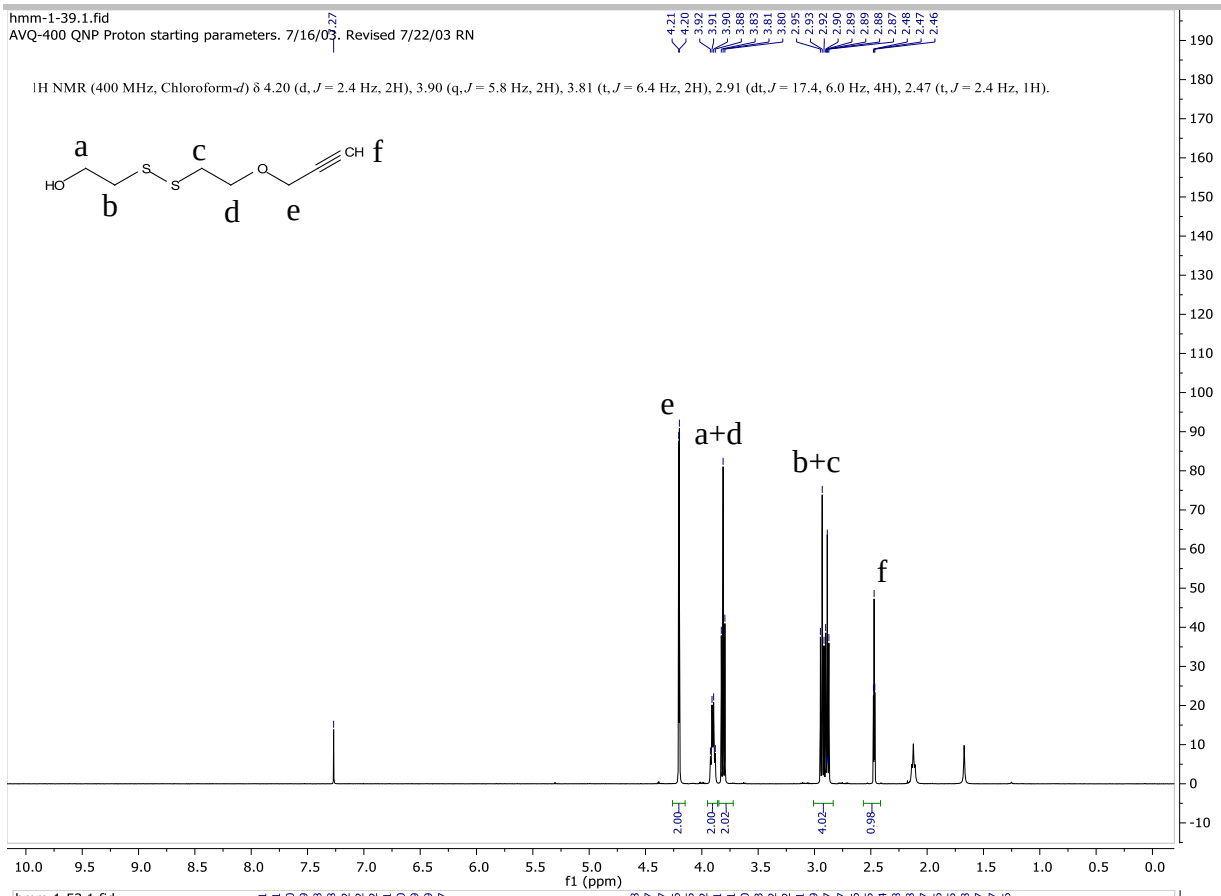
^{1}H NMR and ^{13}C NMR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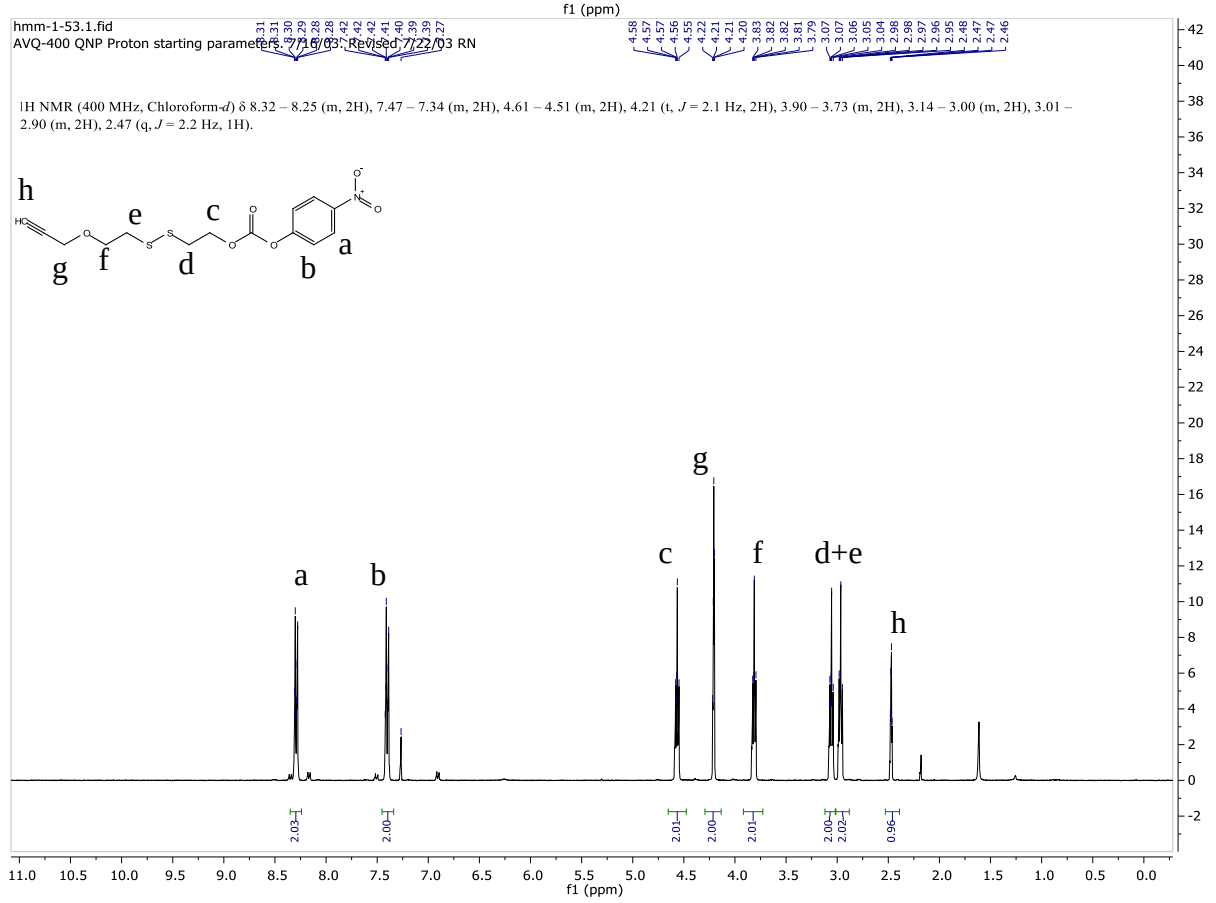
HO
S
S
O
CH
a
b
c
d
e
f
e
a+d
b+c
f
f1 (ppm)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h
e
c
g
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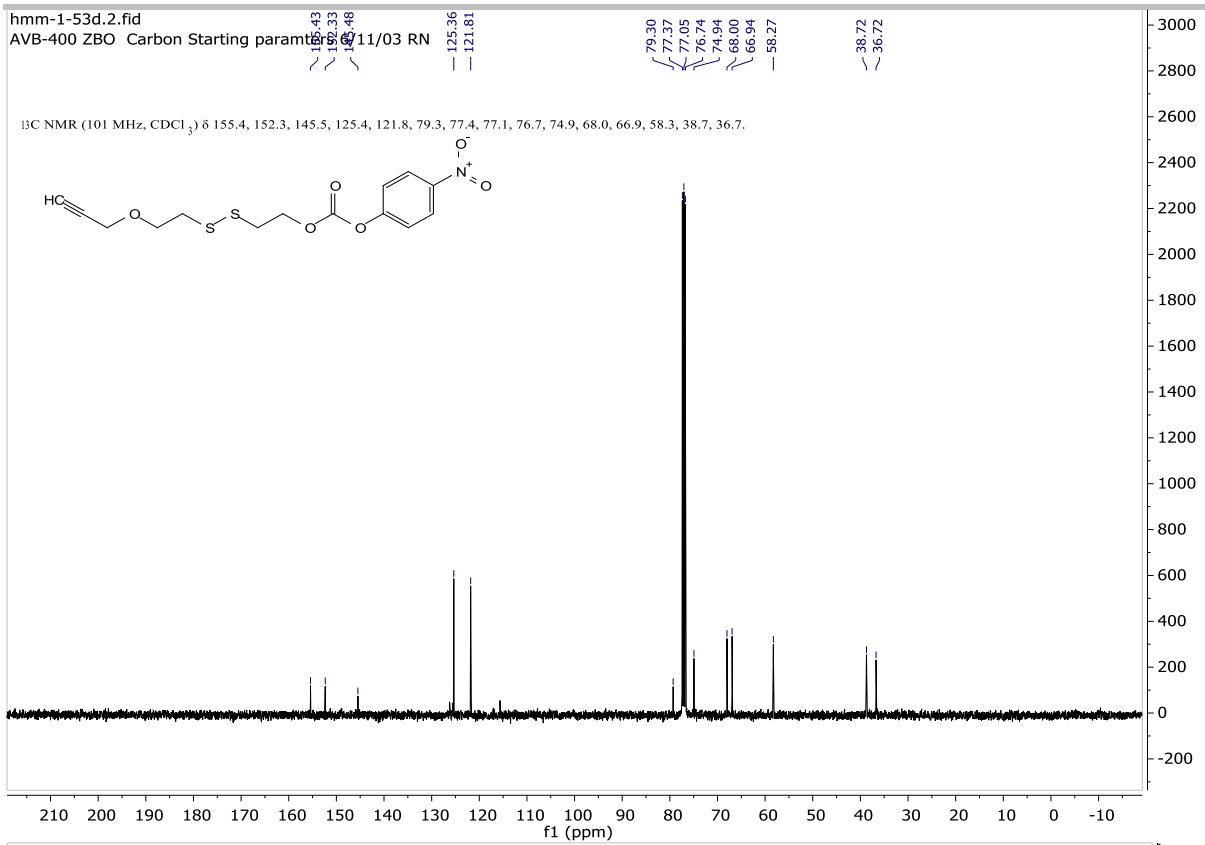
a
b
c
g
f
d+e
h
f1 (ppm)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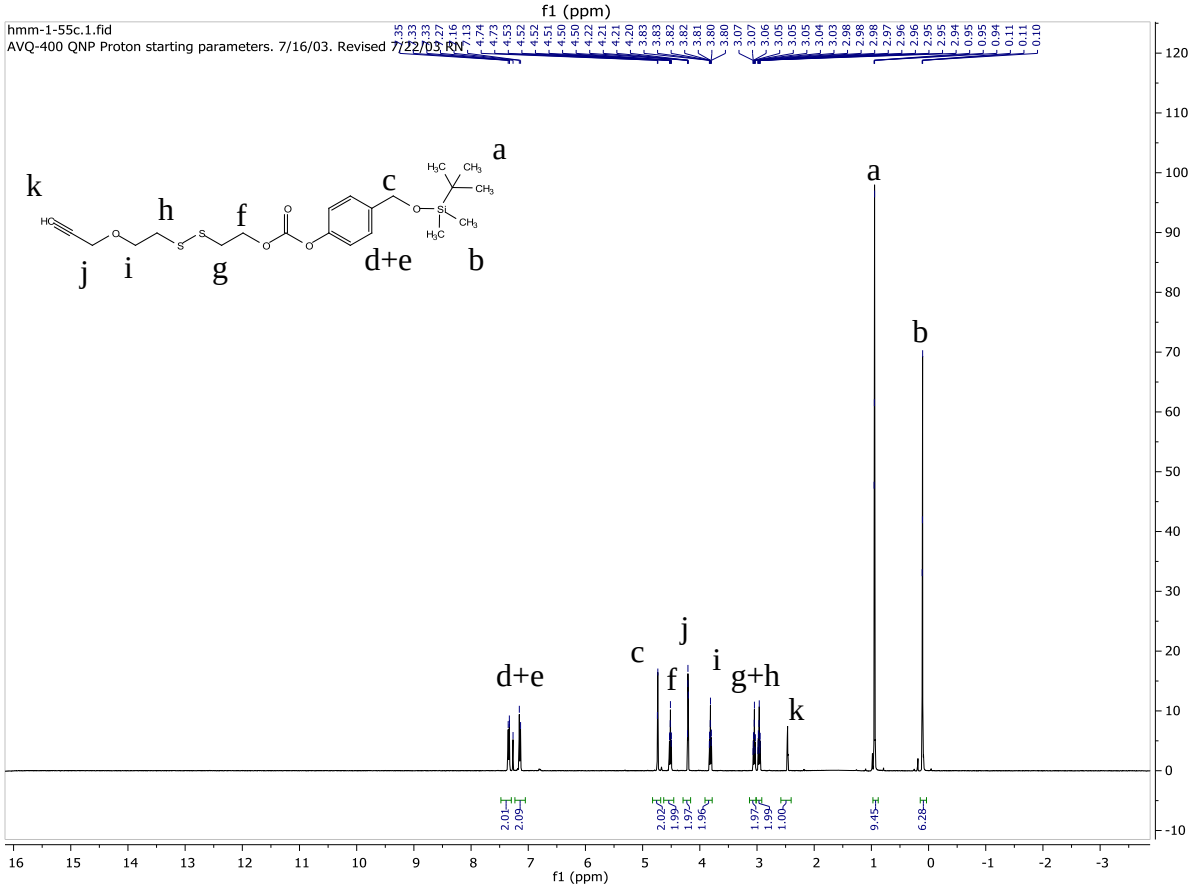
79.30
77.37
77.05
76.74
74.94
68.00
66.94
58.27
38.72
36.72
13C NMR (101 MHz, CDCl 3) δ 155.4, 152.3, 145.5, 125.4, 121.8, 79.3, 77.4, 77.1, 76.7, 74.9, 68.0, 66.9, 58.3, 38.7, 36.7.
f1 (ppm)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a
b
c
d+e
f
g+h
i
j
k
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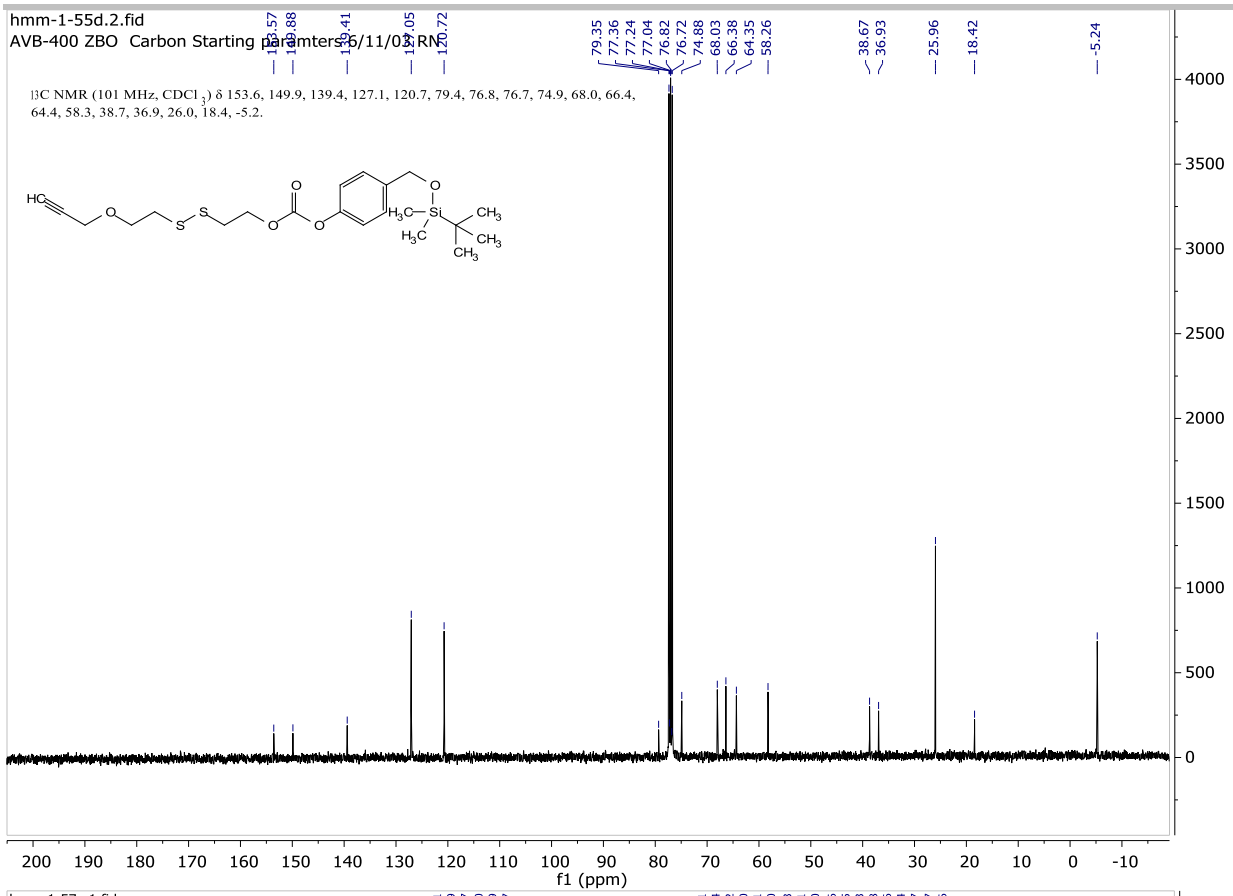
2.02
1.99
1.97
1.96
1.97
1.99
1.00
9.45
6.28
f1 (ppm)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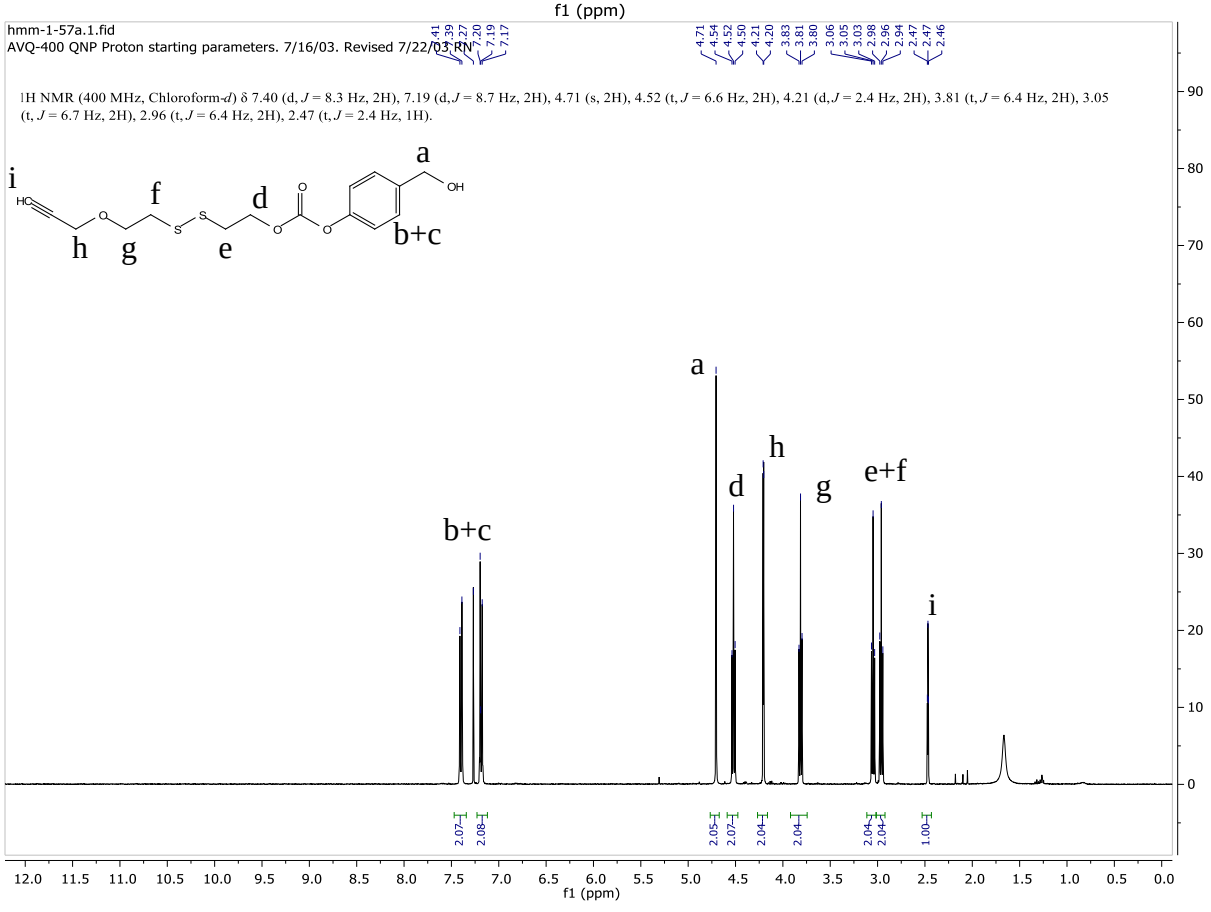
18.42
-5.24
f1 (ppm)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7.17
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4.54
4.52
4.50
4.21
4.20
3.83
3.81
3.80
3.06
3.05
3.03
2.98
2.96
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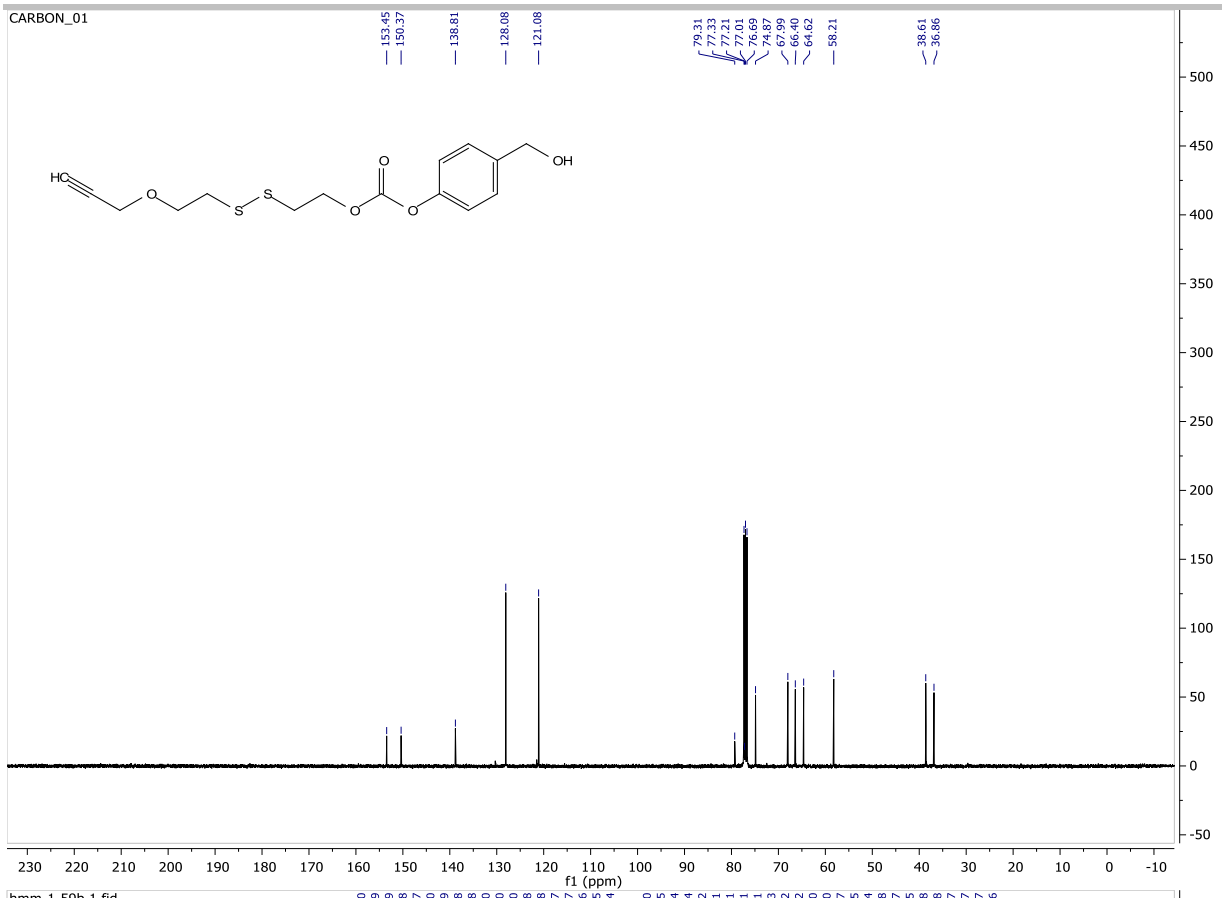
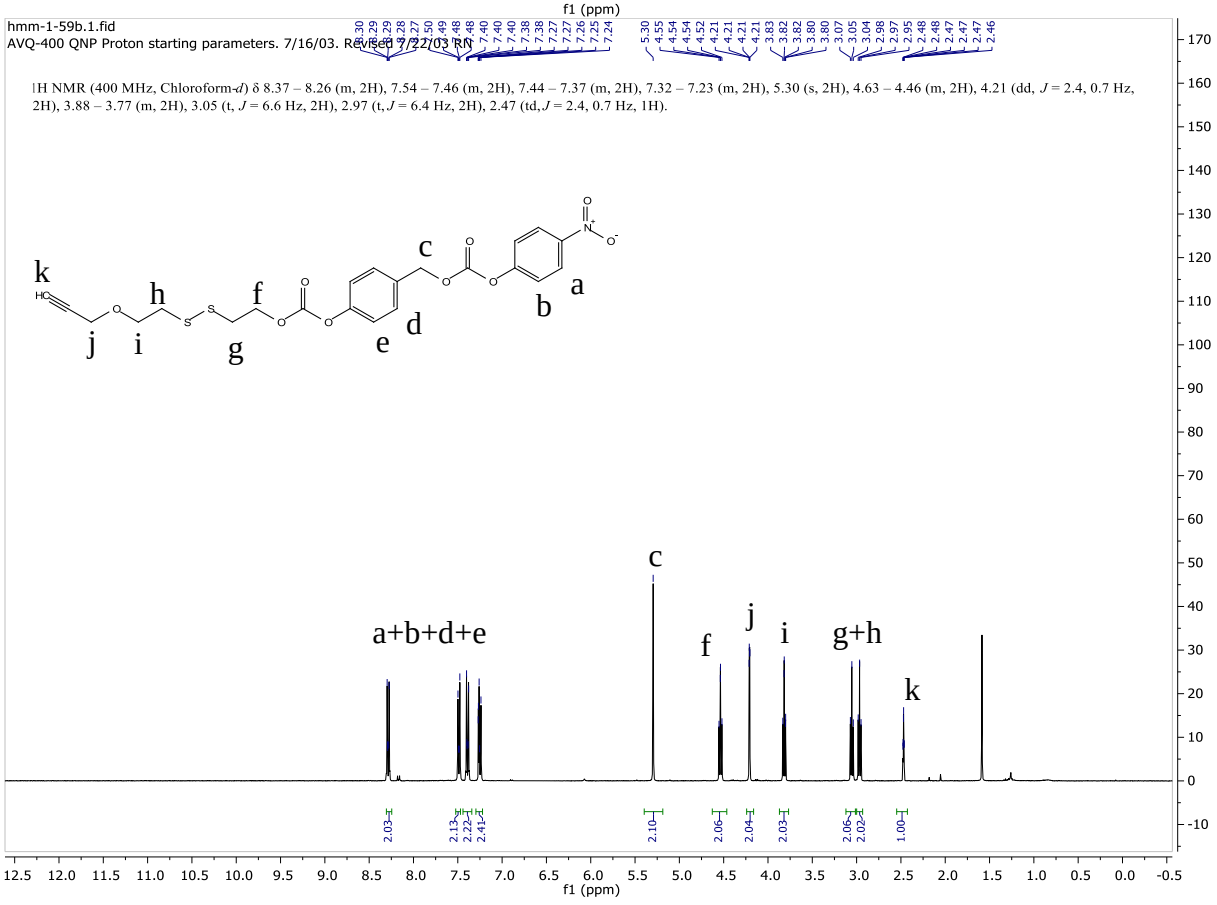
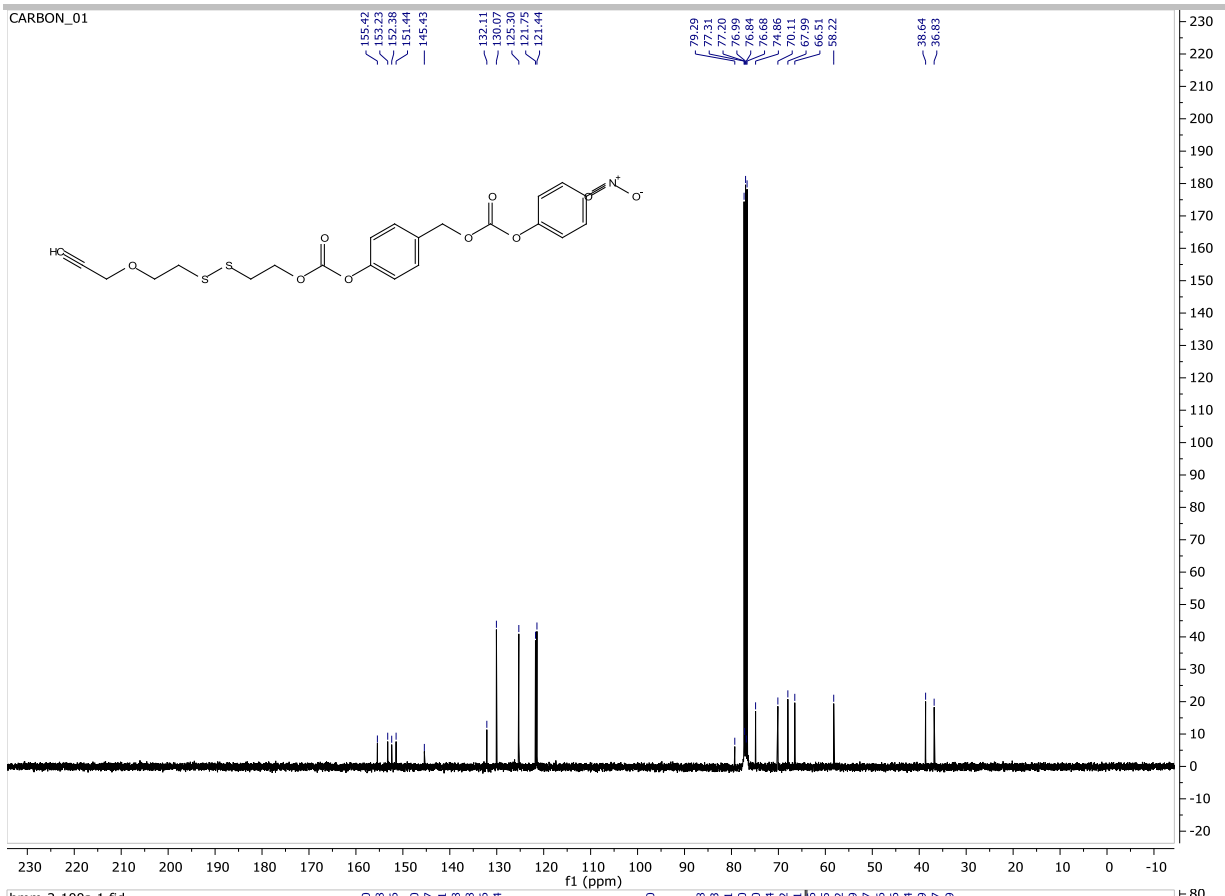
2.47
2.46
a
b+c
d
h
g
e+f
i
f1 (ppm)





^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.37 – 8.26 (m, 2H), 7.54 – 7.46 (m, 2H), 7.44 – 7.37 (m, 2H), 7.32 – 7.23 (m, 2H), 5.30 (s, 2H), 4.63 – 4.46 (m, 2H), 4.21 (dd, *J* = 2.4, 0.7 Hz, 2H), 3.88 – 3.77 (m, 2H), 3.05 (t, *J* = 6.6 Hz, 2H), 2.97 (t, *J* = 6.4 Hz, 2H), 2.47 (td, *J* = 2.4, 0.7 Hz, 1H).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153.23
152.38
151.44
145.43
132.11
130.07
125.30
121.75
121.44
79.29
77.31
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70.11
67.99
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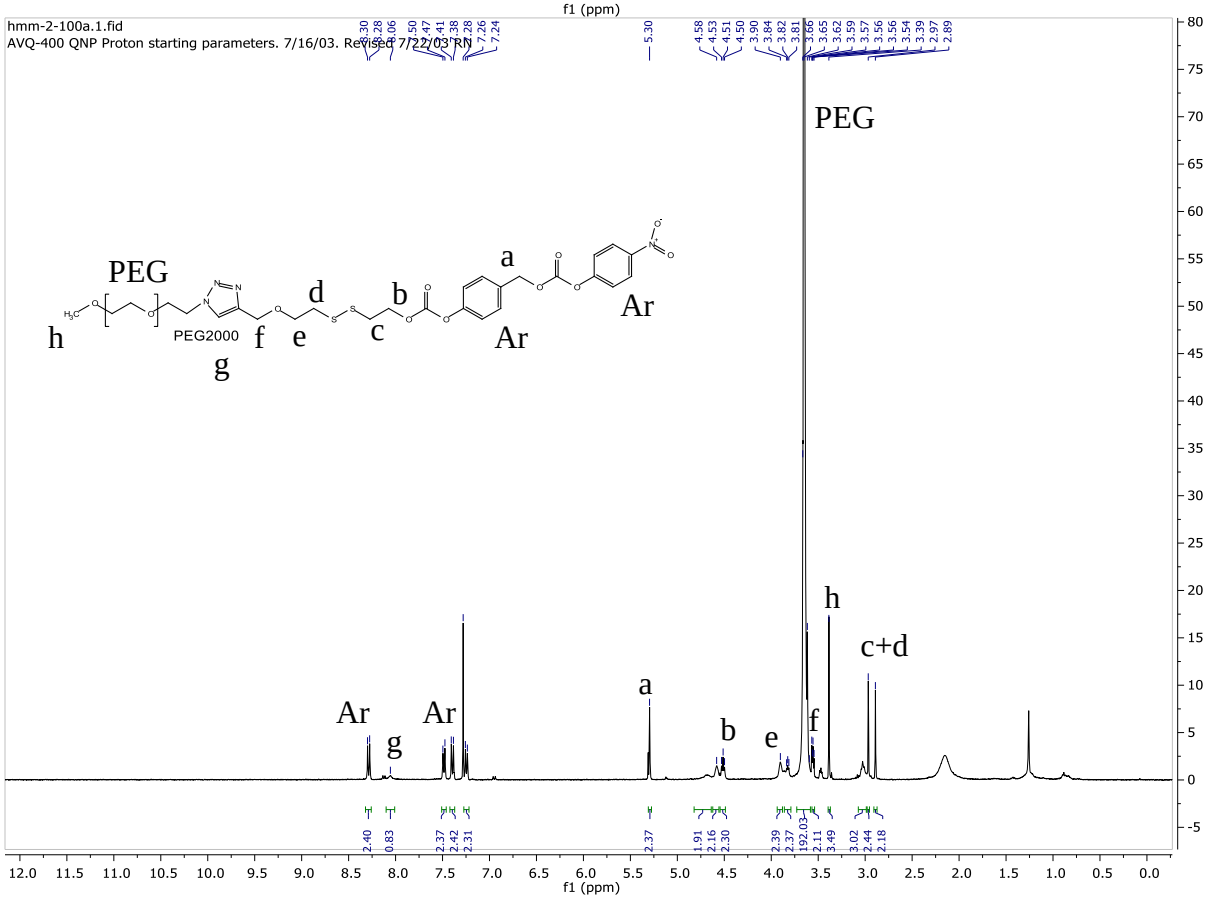
58.22
38.64
36.83
f1 (ppm)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PEG
PEG2000
h
g
f
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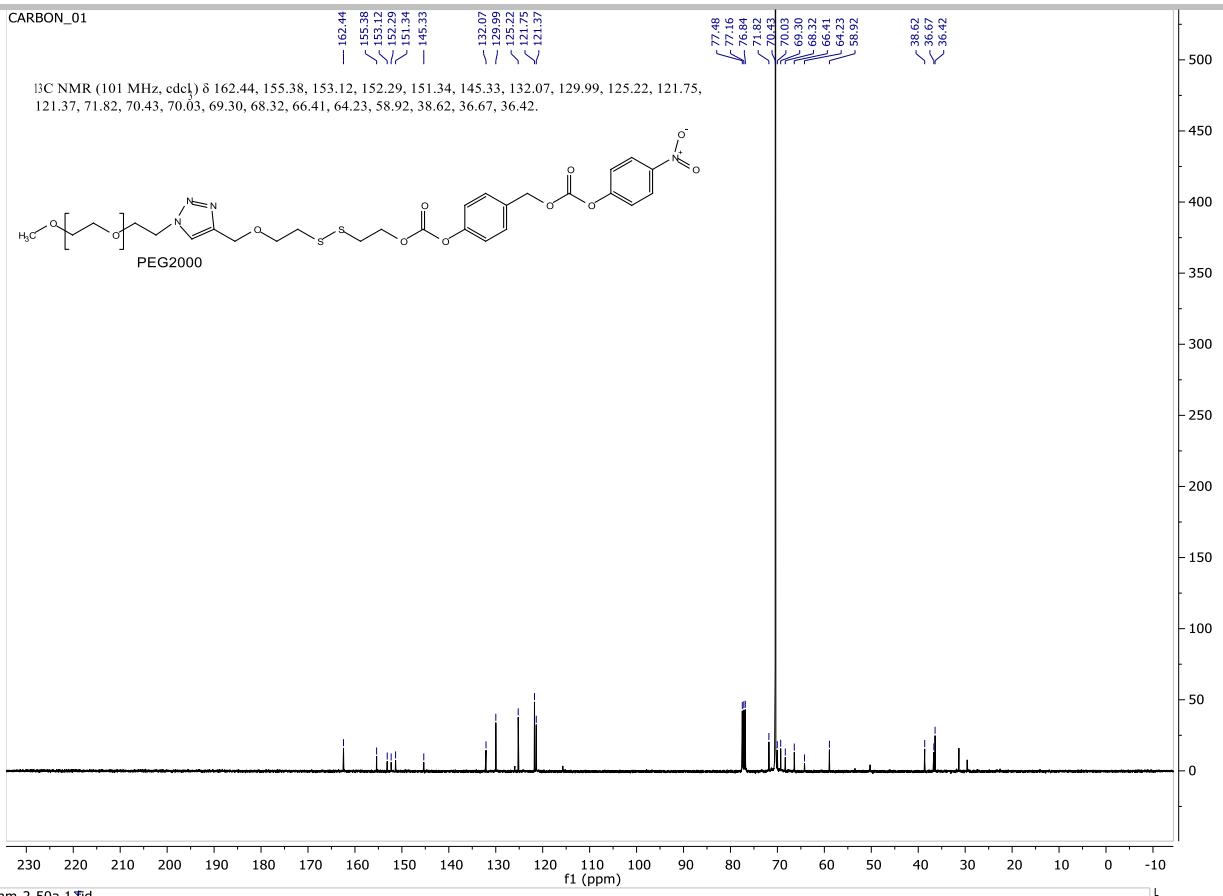
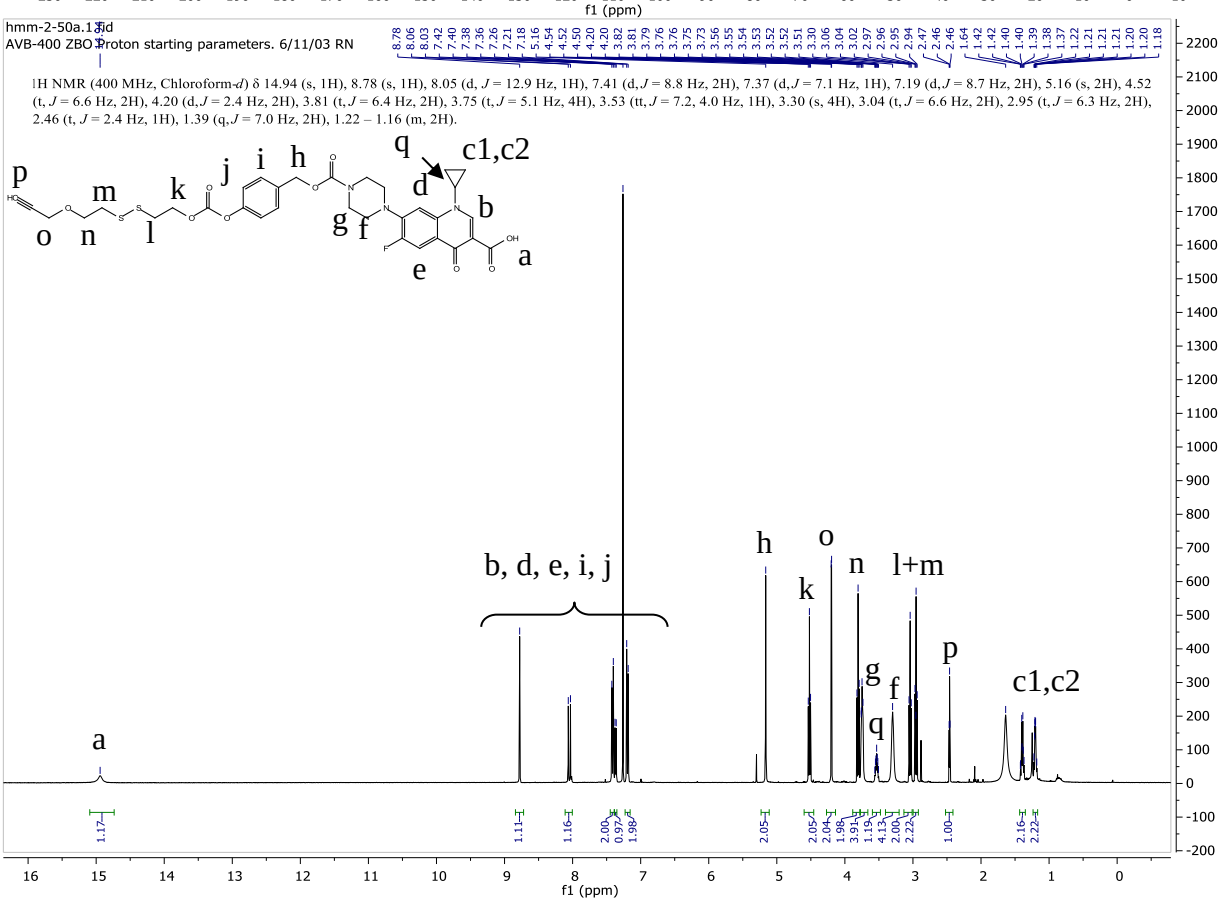
a
b
e
f
h
c+d
PEG
f1 (ppm)


CARBON_01
13C NMR (101 MHz, cdcl3) δ 162.44, 155.38, 153.12, 152.29, 151.34, 145.33, 132.07, 129.99, 125.22, 121.75, 121.37, 71.82, 70.43, 70.03, 69.30, 68.32, 66.41, 64.23, 58.92, 38.62, 36.67, 36.42.
PEG2000
f1 (ppm)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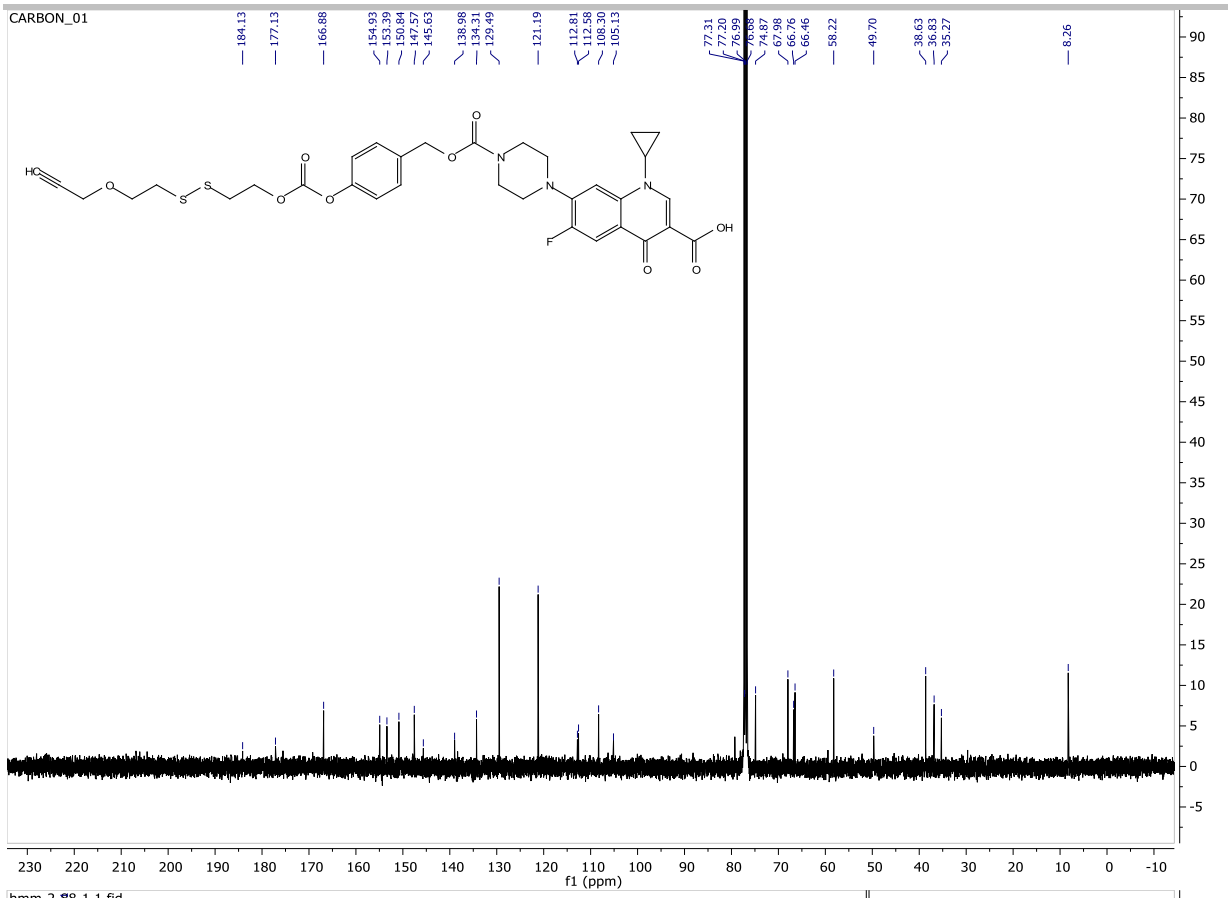
b, d, e, i, j
f1 (ppm)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154.93
153.39
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134.31
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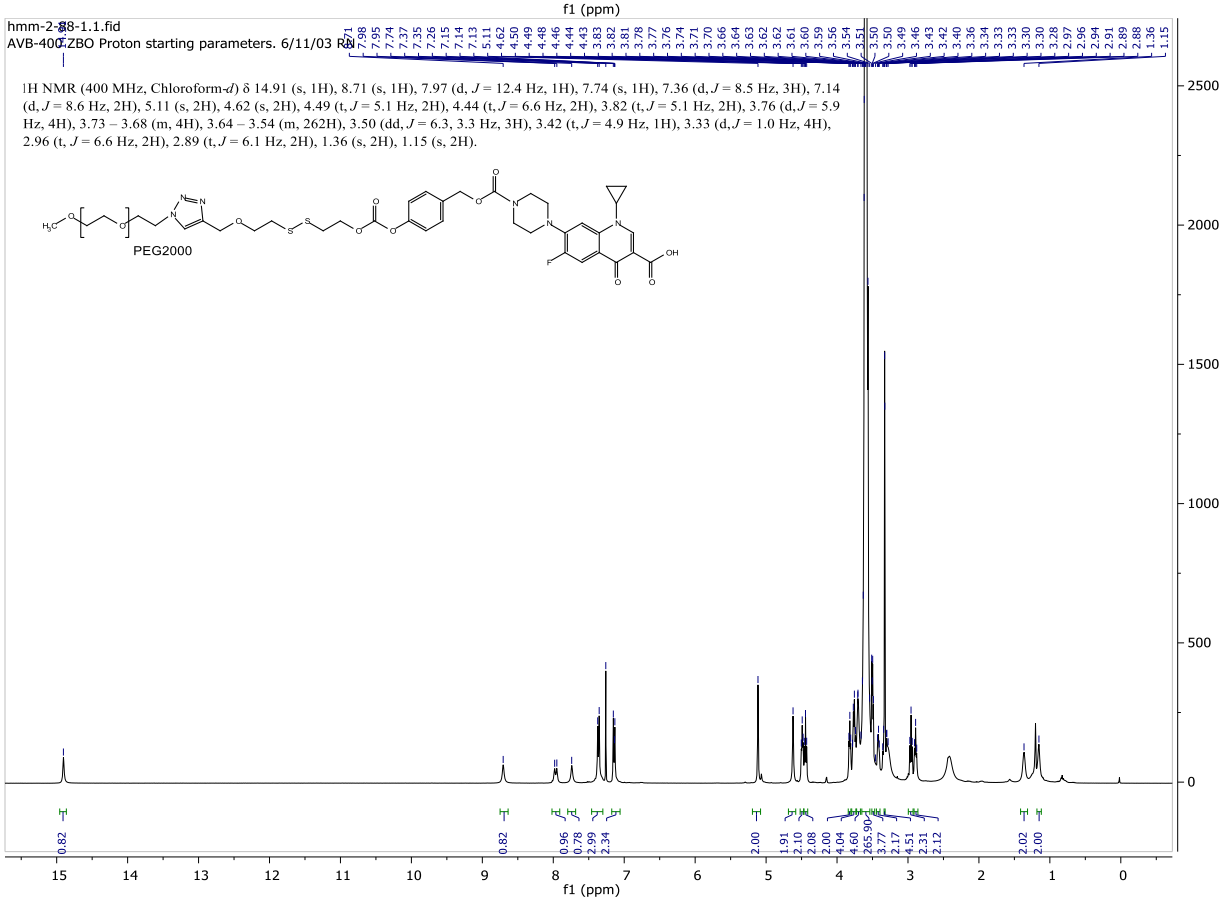
36.83
35.27
8.26
f1 (ppm)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AVB-400-ZBO Proton starting parameters. 6/11/03 RM
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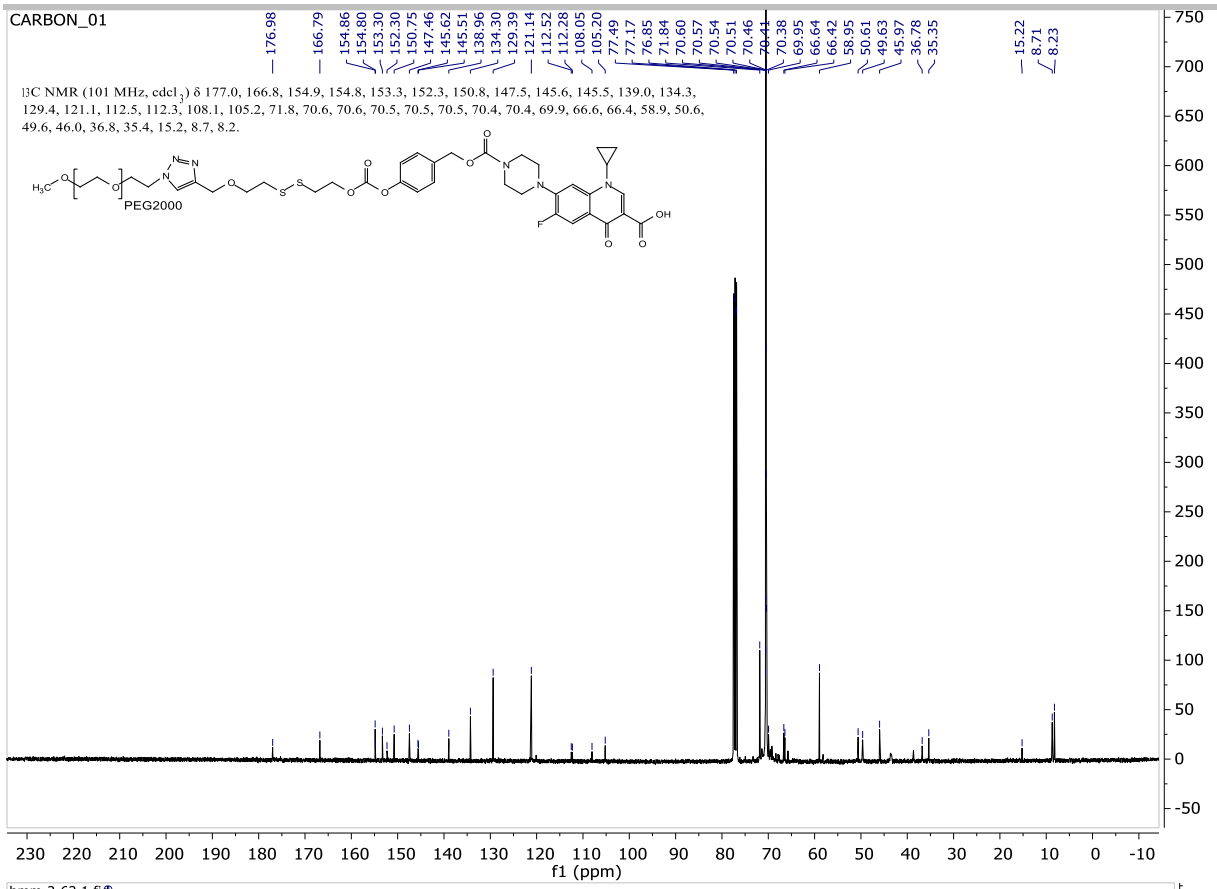
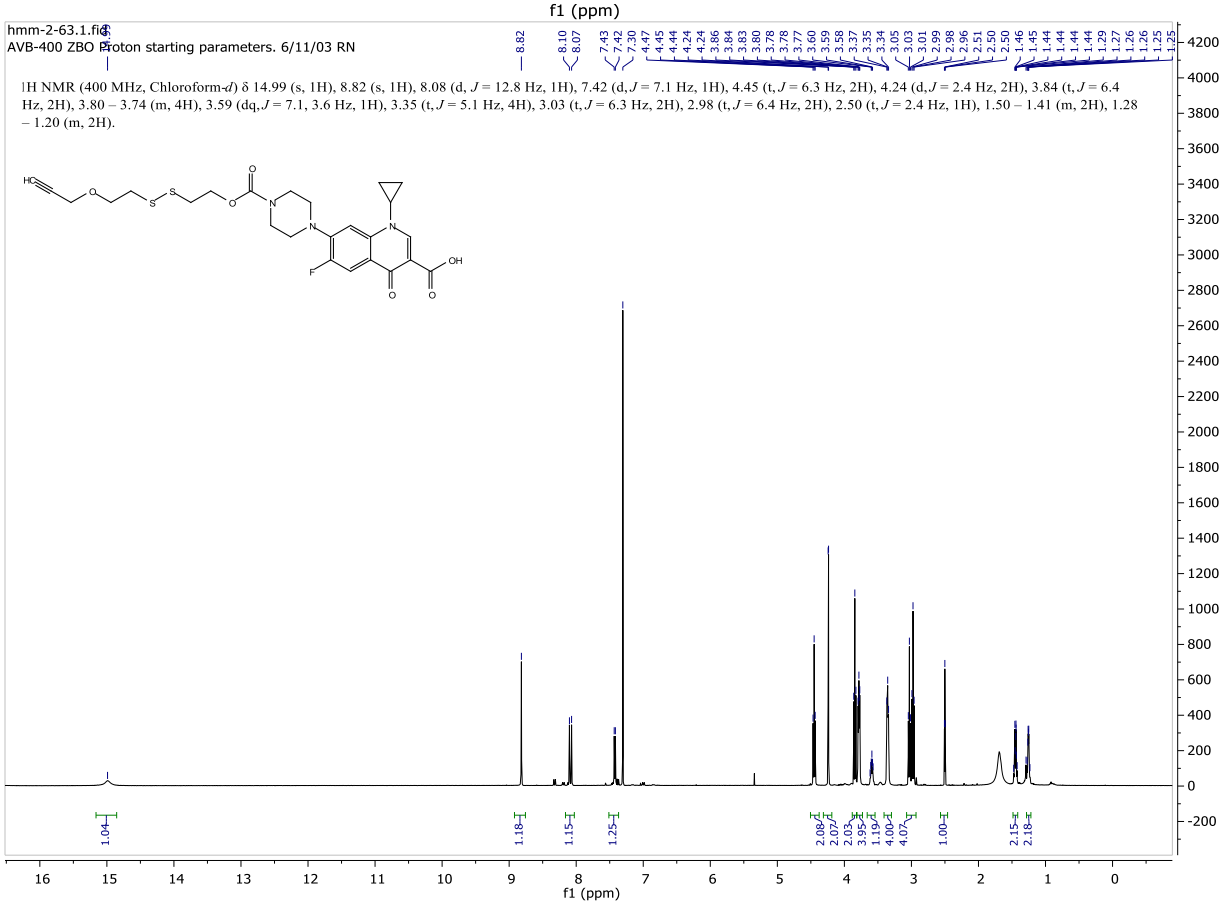
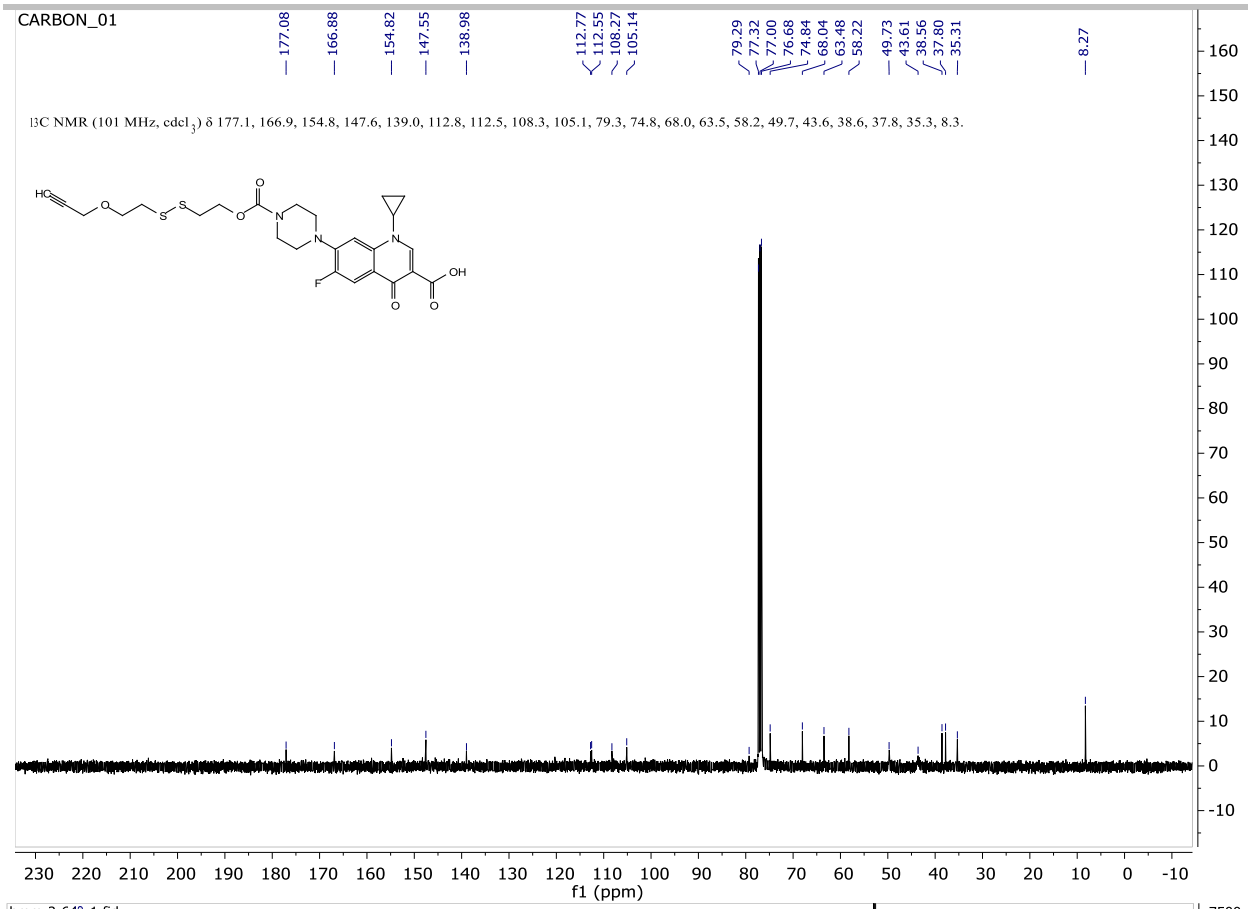
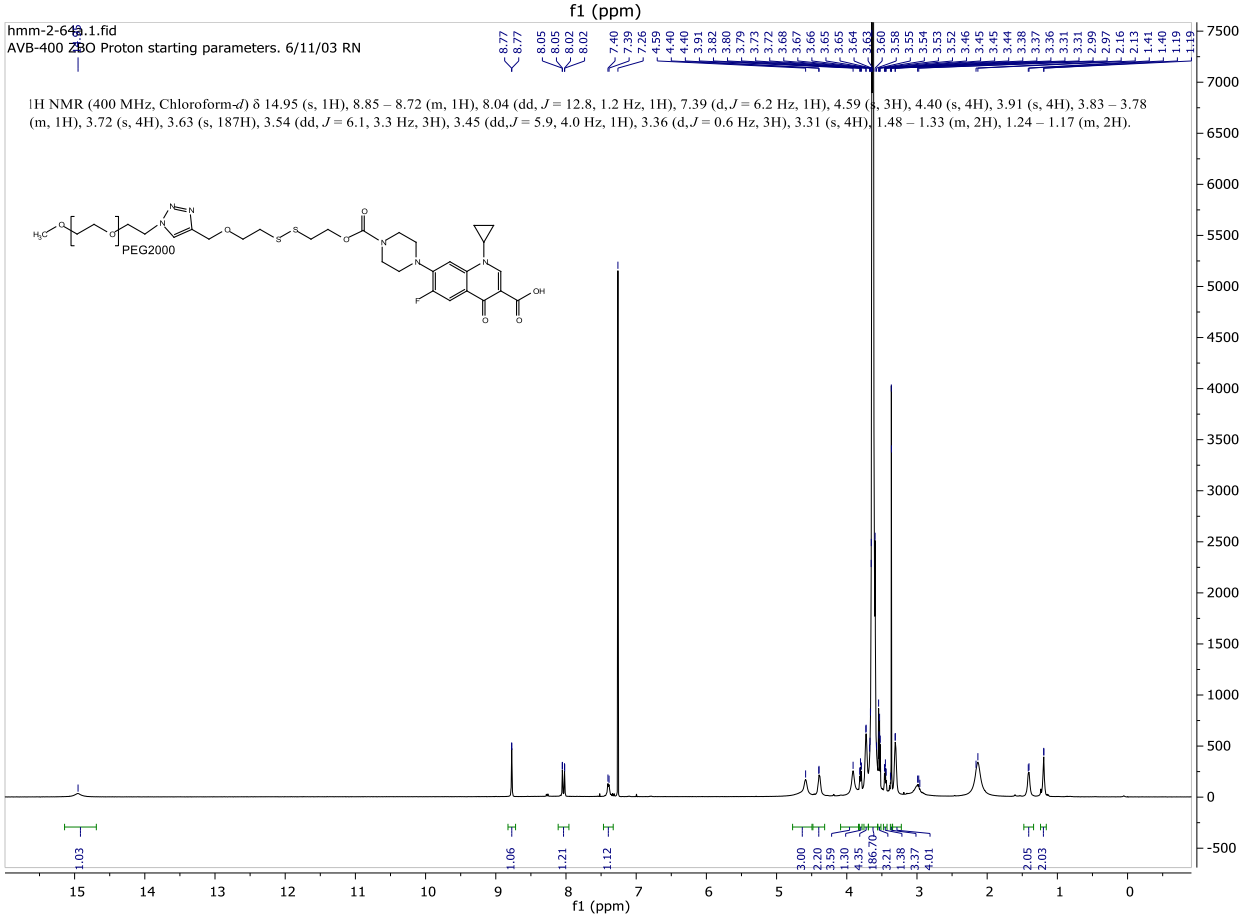
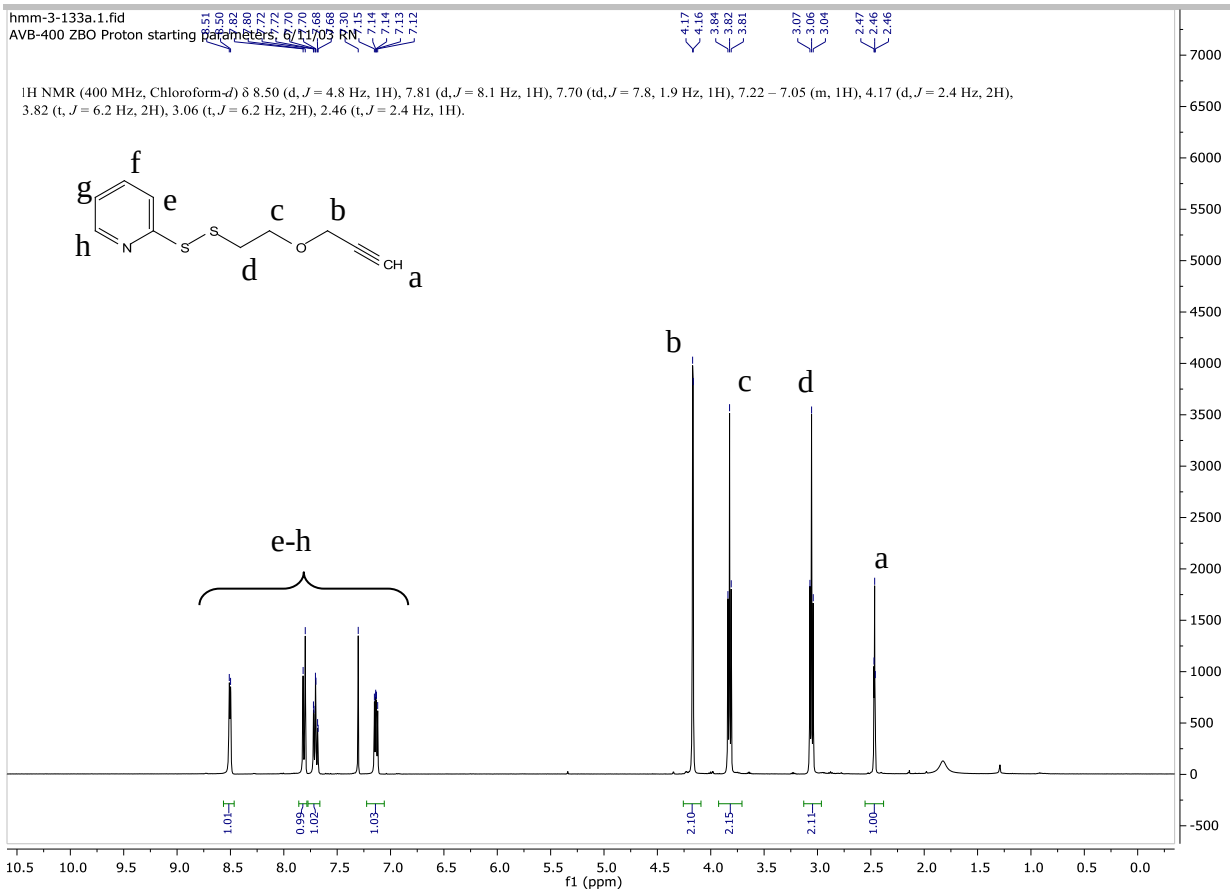
PEG2000
f1 (ppm)

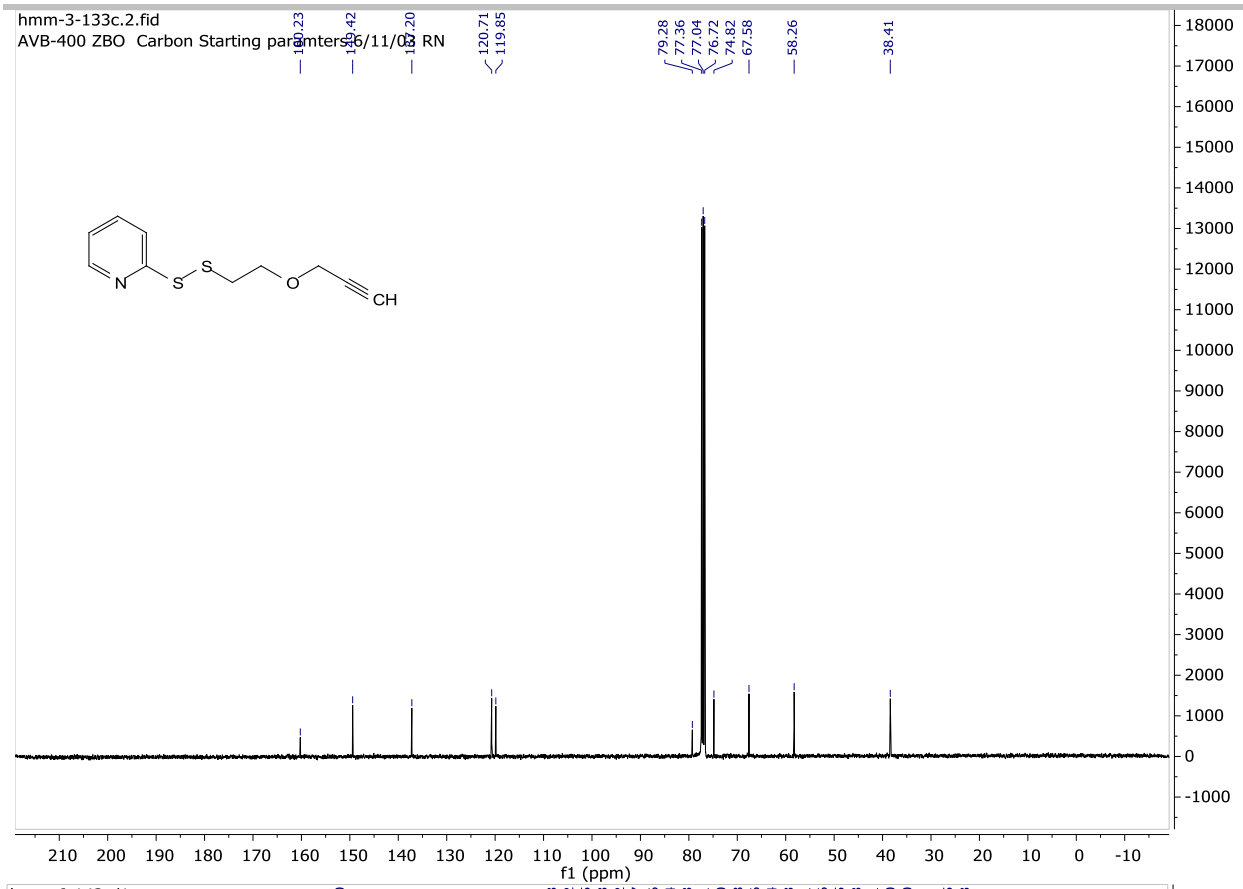

CARBON_01
13C NMR (101 MHz, cdcl3) δ 177.0, 166.8, 154.9, 154.8, 153.3, 152.3, 150.8, 147.5, 145.6, 145.5, 139.0, 134.3, 129.4, 121.1, 112.5, 112.3, 108.1, 105.2, 71.8, 70.6, 70.6, 70.5, 70.5, 70.5, 70.4, 70.4, 69.9, 66.6, 66.4, 58.9, 50.6, 49.6, 46.0, 36.8, 35.4, 15.2, 8.7, 8.2.
PEG2000
f1 (ppm)

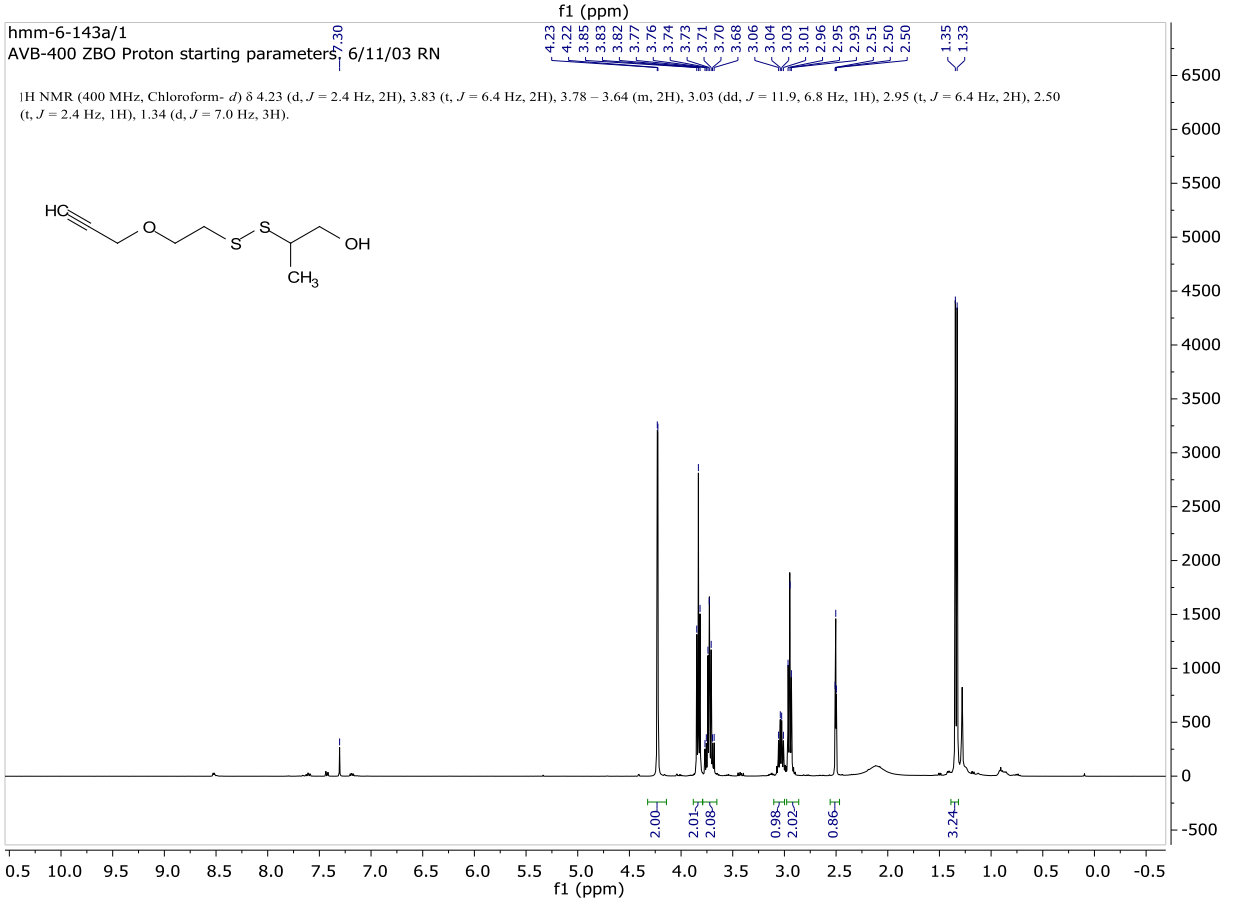

hmm-2-63.1.fid
AVB-400 ZBO Proton starting parameters. 6/11/03 RN
1H NMR (400 MHz, Chloroform-d) δ 14.99 (s, 1H), 8.82 (s, 1H), 8.08 (d, J = 12.8 Hz, 1H), 7.42 (d, J = 7.1 Hz, 1H), 4.45 (t, J = 6.3 Hz, 2H), 4.24 (d, J = 2.4 Hz, 2H), 3.84 (t, J = 6.4 Hz, 2H), 3.80 – 3.74 (m, 4H), 3.59 (dq, J = 7.1, 3.6 Hz, 1H), 3.35 (t, J = 5.1 Hz, 4H), 3.03 (t, J = 6.3 Hz, 2H), 2.98 (t, J = 6.4 Hz, 2H), 2.50 (t, J = 2.4 Hz, 1H), 1.50 – 1.41 (m, 2H), 1.28 – 1.20 (m, 2H).
f1 (ppm)

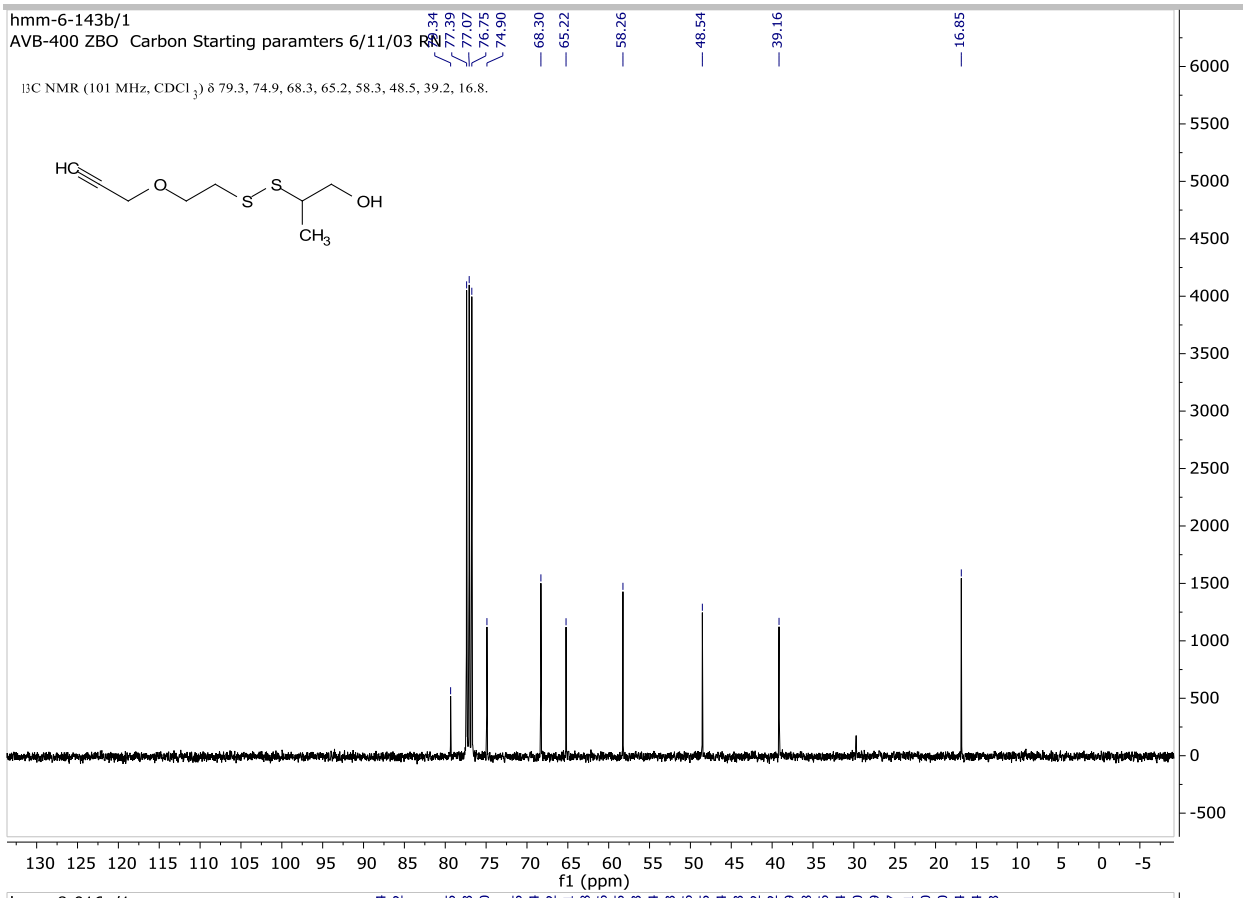

CARBON_01
177.08
166.88
154.82
147.55
138.98
112.77
112.55
108.27
105.14
79.29
77.32
77.00
76.68
74.84
68.04
63.48
58.22
49.73
43.61
38.56
37.80
35.31
8.27
13C NMR (101 MHz, cdcl3) δ 177.1, 166.9, 154.8, 147.6, 139.0, 112.8, 112.5, 108.3, 105.1, 79.3, 74.8, 68.0, 63.5, 58.2, 49.7, 43.6, 38.6, 37.8, 35.3, 8.3.
f1 (ppm)

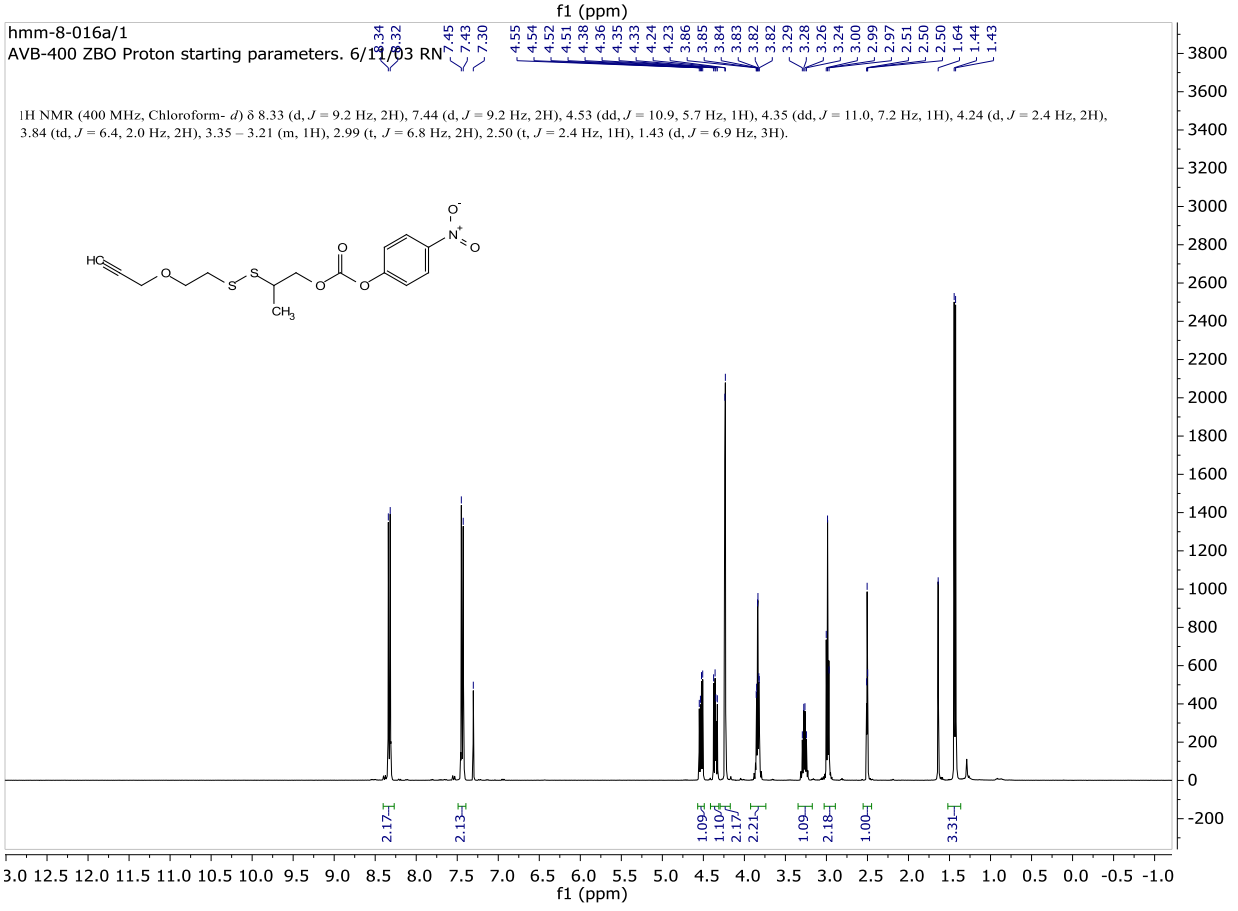

hmm-2-64a.1.fid
AVB-400 ZBO Proton starting parameters. 6/11/03 RN
1H NMR (400 MHz, Chloroform-d) δ 14.95 (s, 1H), 8.85 – 8.72 (m, 1H), 8.04 (dd, J = 12.8, 1.2 Hz, 1H), 7.39 (d, J = 6.2 Hz, 1H), 4.59 (s, 3H), 4.40 (s, 4H), 3.91 (s, 4H), 3.83 – 3.78 (m, 1H), 3.72 (s, 4H), 3.63 (s, 187H), 3.54 (dd, J = 6.1, 3.3 Hz, 3H), 3.45 (dd, J = 5.9, 4.0 Hz, 1H), 3.36 (d, J = 0.6 Hz, 3H), 3.31 (s, 4H), 1.48 – 1.33 (m, 2H), 1.24 – 1.17 (m, 2H).
PEG2000
f1 (ppm)

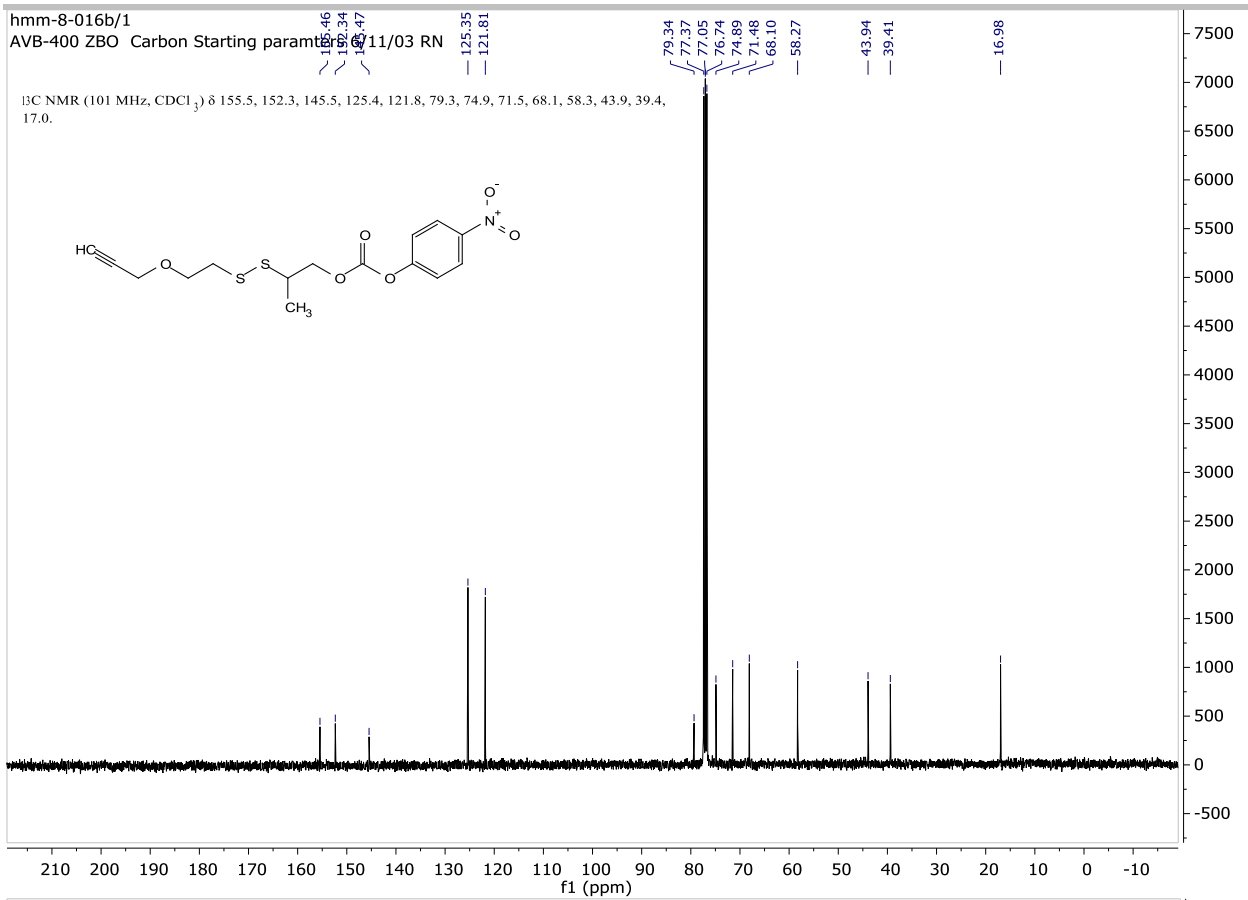

hmm-3-133a.1.fid
AVB-400 ZBO Proton starting parameters, 6/1/1703 RN
1H NMR (400 MHz, Chloroform-d) δ 8.50 (d, J = 4.8 Hz, 1H), 7.81 (d, J = 8.1 Hz, 1H), 7.70 (td, J = 7.8, 1.9 Hz, 1H), 7.22 – 7.05 (m, 1H), 4.17 (d, J = 2.4 Hz, 2H), 3.82 (t, J = 6.2 Hz, 2H), 3.06 (t, J = 6.2 Hz, 2H), 2.46 (t, J = 2.4 Hz, 1H).
f
g
e
h
N
S
S
c
b
d
O
CH
a
b
c
d
e-h
a
f1 (ppm)

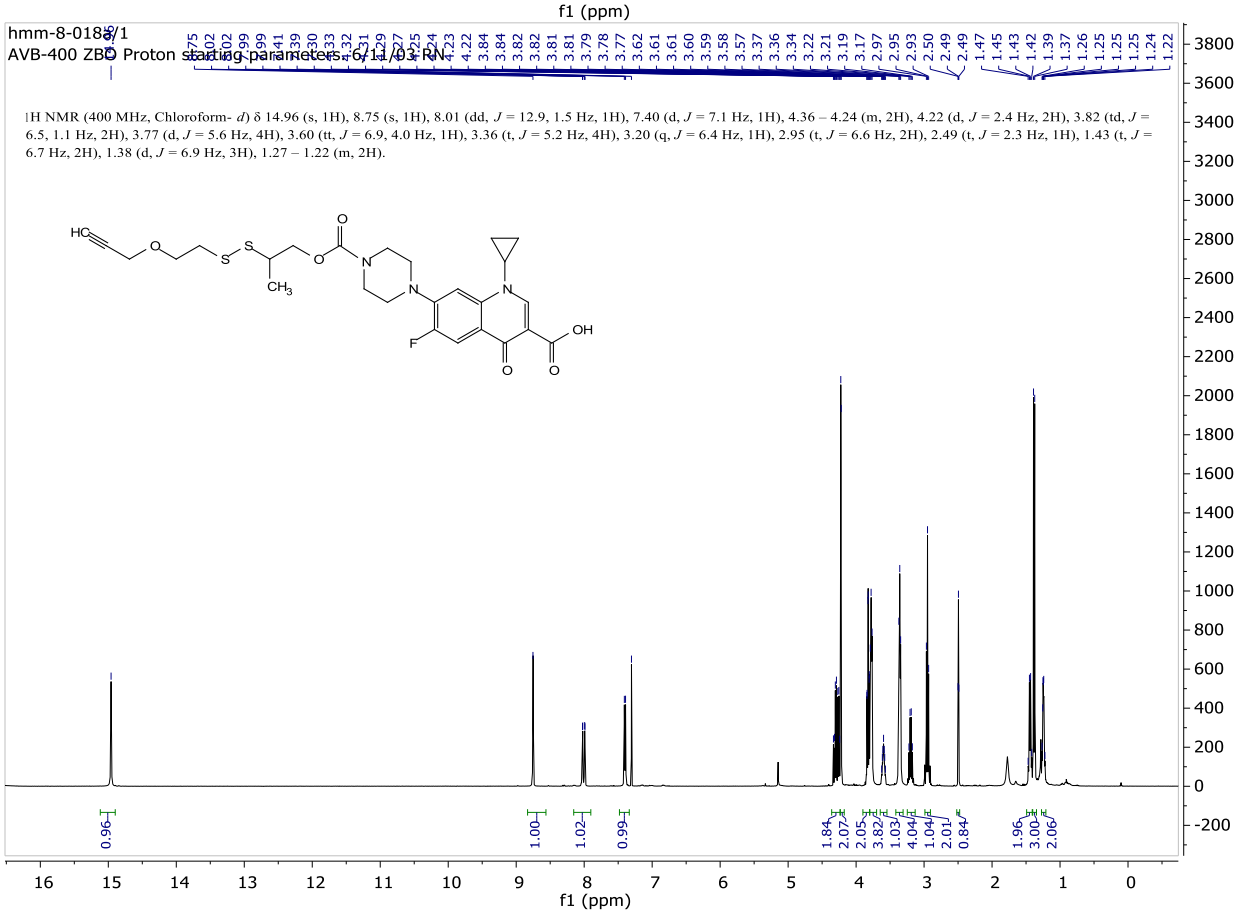

hmm-3-133c.2.fid
AVB-400 ZBO Carbon Starting parameters 6/11/03 RN
160.23
149.42
137.20
120.71
119.85
79.28
77.36
77.04
76.72
74.82
67.58
58.26
38.41
N
S
S
O
CH
f1 (ppm)


hmm-6-143a/1
AVB-400 ZBO Proton starting parameters 6/11/03 RN
7.30
4.23
4.22
3.85
3.83
3.82
3.77
3.76
3.74
3.73
3.71
3.70
3.68
3.06
3.04
3.03
3.01
2.96
2.95
2.93
2.51
2.50
2.50
1.35
1.33
¹H NMR (400 MHz, Chloroform- d) δ 4.23 (d, J = 2.4 Hz, 2H), 3.83 (t, J = 6.4 Hz, 2H), 3.78 – 3.64 (m, 2H), 3.03 (dd, J = 11.9, 6.8 Hz, 1H), 2.95 (t, J = 6.4 Hz, 2H), 2.50 (t, J = 2.4 Hz, 1H), 1.34 (d, J = 7.0 Hz, 3H).
HC
O
S
S
OH
CH3
2.00
2.01
2.08
0.98
2.02
0.86
3.24
f1 (ppm)

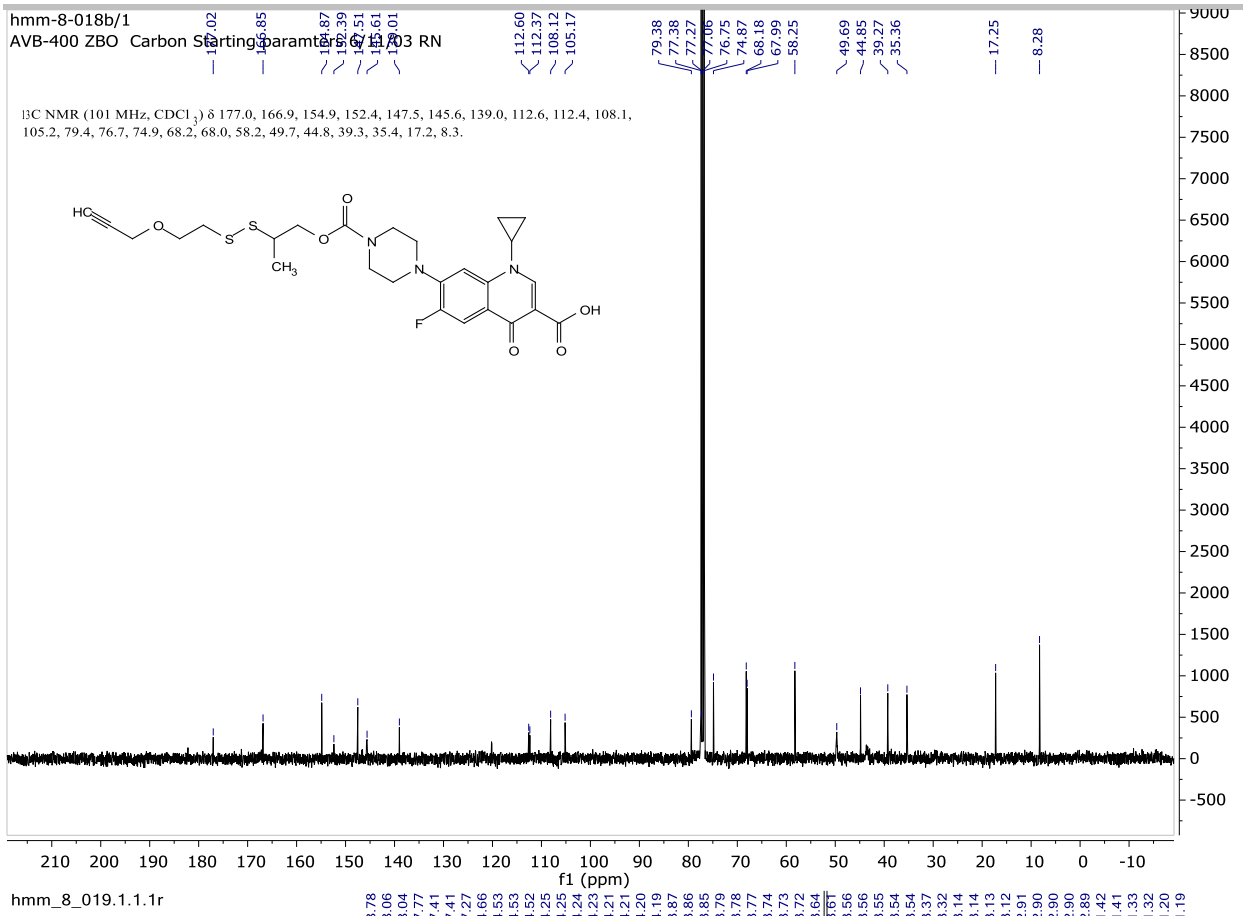

hmm-6-143b/1
AVB-400 ZBO Carbon Starting paramters 6/11/03 RN
13C NMR (101 MHz, CDCl3) δ 79.3, 74.9, 68.3, 65.2, 58.3, 48.5, 39.2, 16.8.
79.34
77.39
77.07
76.75
74.90
68.30
65.22
58.26
48.54
39.16
16.85
HC
O
S
S
OH
CH3
f1 (ppm)


hmm-8-016a/1
AVB-400 ZBO Proton starting parameters. 6/11/03 RN
1H NMR (400 MHz, Chloroform-d) δ 8.33 (d, J = 9.2 Hz, 2H), 7.44 (d, J = 9.2 Hz, 2H), 4.53 (dd, J = 10.9, 5.7 Hz, 1H), 4.35 (dd, J = 11.0, 7.2 Hz, 1H), 4.24 (d, J = 2.4 Hz, 2H), 3.84 (td, J = 6.4, 2.0 Hz, 2H), 3.35 – 3.21 (m, 1H), 2.99 (t, J = 6.8 Hz, 2H), 2.50 (t, J = 2.4 Hz, 1H), 1.43 (d, J = 6.9 Hz, 3H).
8.34
8.32
7.45
7.43
7.30
4.55
4.54
4.52
4.51
4.38
4.36
4.35
4.33
4.24
4.23
3.86
3.85
3.84
3.83
3.82
3.82
3.29
3.28
3.26
3.24
3.00
2.99
2.97
2.51
2.50
2.50
1.64
1.44
1.43
HC
O
S
S
O
O
O
O
N+
O
CH3
2.17
2.13
1.09
1.10
2.17
2.21
1.09
2.18
1.00
3.31
f1 (ppm)


hmm-8-016b/1
AVB-400 ZBO Carbon Starting parameters 6/11/03 RN
155.46
152.34
145.47
125.35
121.81
79.34
77.37
77.05
76.74
74.89
71.48
68.10
58.27
43.94
39.41
16.98
13C NMR (101 MHz, CDCl3) δ 155.5, 152.3, 145.5, 125.4, 121.8, 79.3, 74.9, 71.5, 68.1, 58.3, 43.9, 39.4, 17.0.
f1 (ppm)

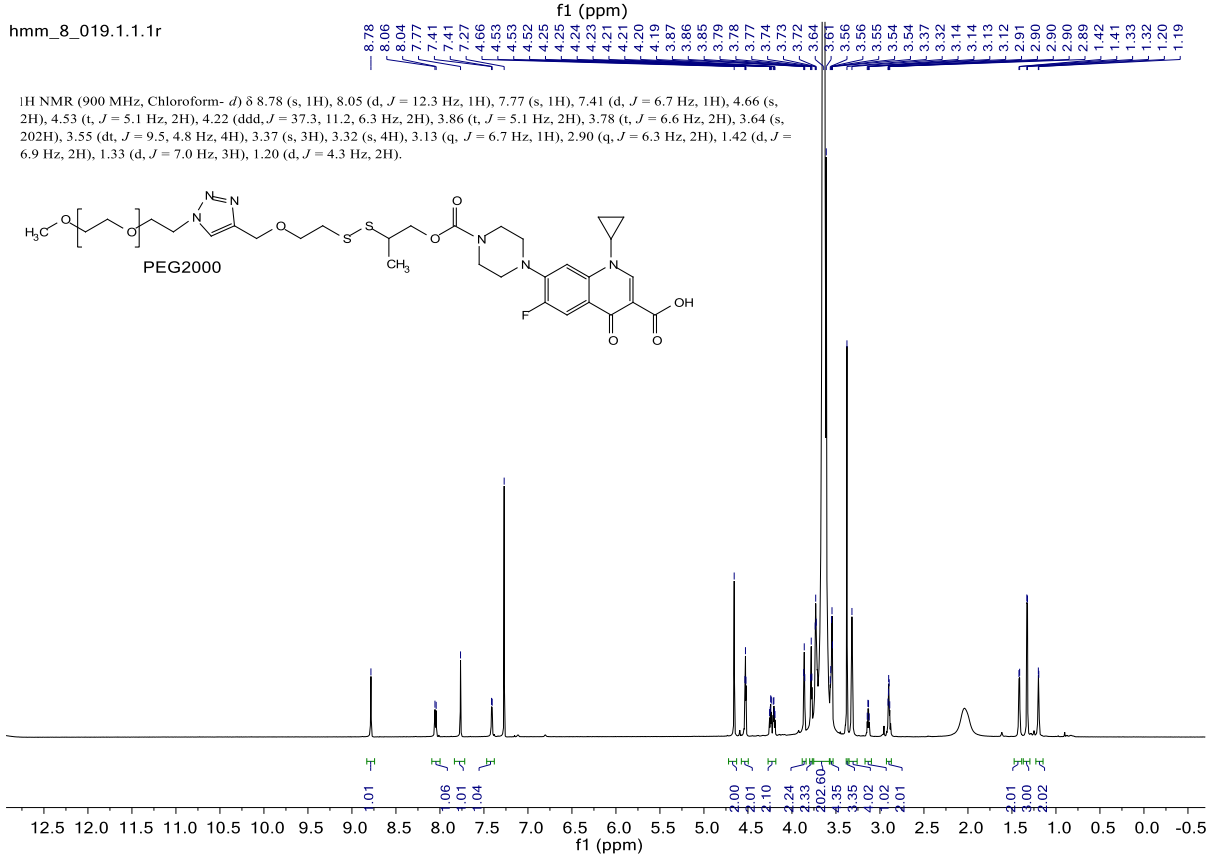

hmm-8-018a/1
AVB-400 ZBO Proton starting parameters 6/11/03 RN
1H NMR (400 MHz, Chloroform- d) δ 14.96 (s, 1H), 8.75 (s, 1H), 8.01 (dd, J = 12.9, 1.5 Hz, 1H), 7.40 (d, J = 7.1 Hz, 1H), 4.36 – 4.24 (m, 2H), 4.22 (d, J = 2.4 Hz, 2H), 3.82 (td, J = 6.5, 1.1 Hz, 2H), 3.77 (d, J = 5.6 Hz, 4H), 3.60 (tt, J = 6.9, 4.0 Hz, 1H), 3.36 (t, J = 5.2 Hz, 4H), 3.20 (q, J = 6.4 Hz, 1H), 2.95 (t, J = 6.6 Hz, 2H), 2.49 (t, J = 2.3 Hz, 1H), 1.43 (t, J = 6.7 Hz, 2H), 1.38 (d, J = 6.9 Hz, 3H), 1.27 – 1.22 (m, 2H).
f1 (ppm)

hmm-8-018b/1
AVB-400 ZBO Carbon Starting parameters 6/11/03 RN

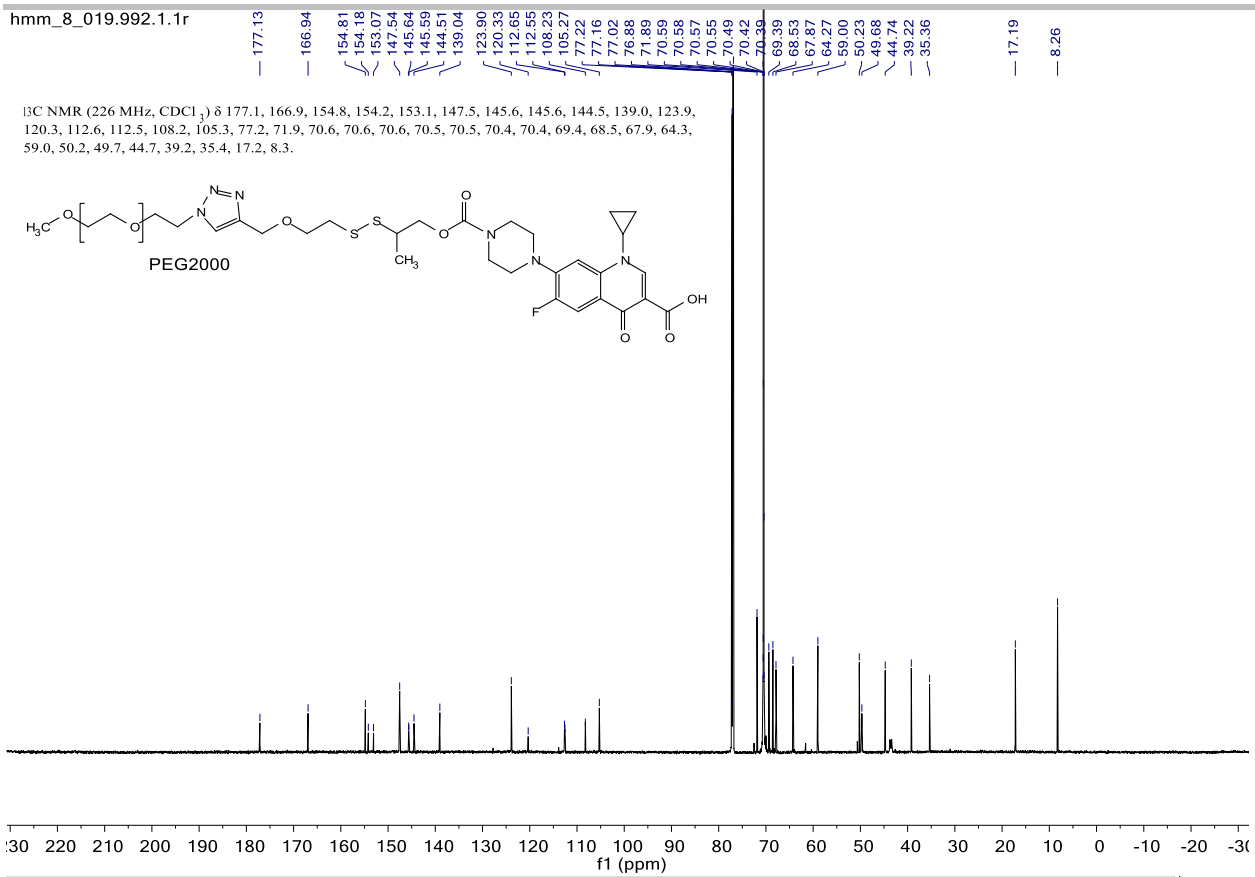


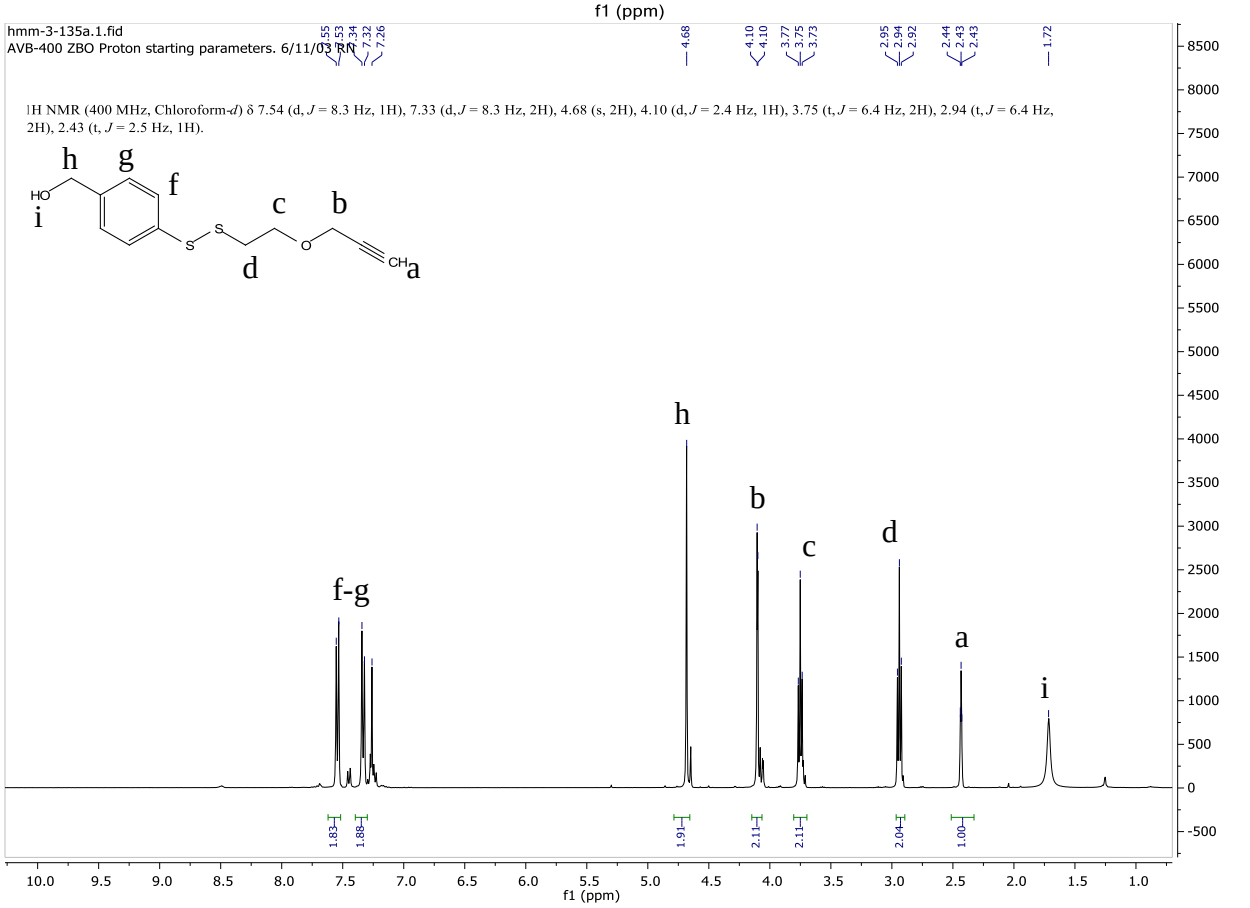
^{13}C NMR (101 MHz, $CDCl_3$) δ 177.0, 166.9, 154.9, 152.4, 147.5, 145.6, 139.0, 112.6, 112.4, 108.1, 105.2, 79.4, 76.7, 74.9, 68.2, 68.0, 58.2, 49.7, 44.8, 39.3, 35.4, 17.2, 8.3.

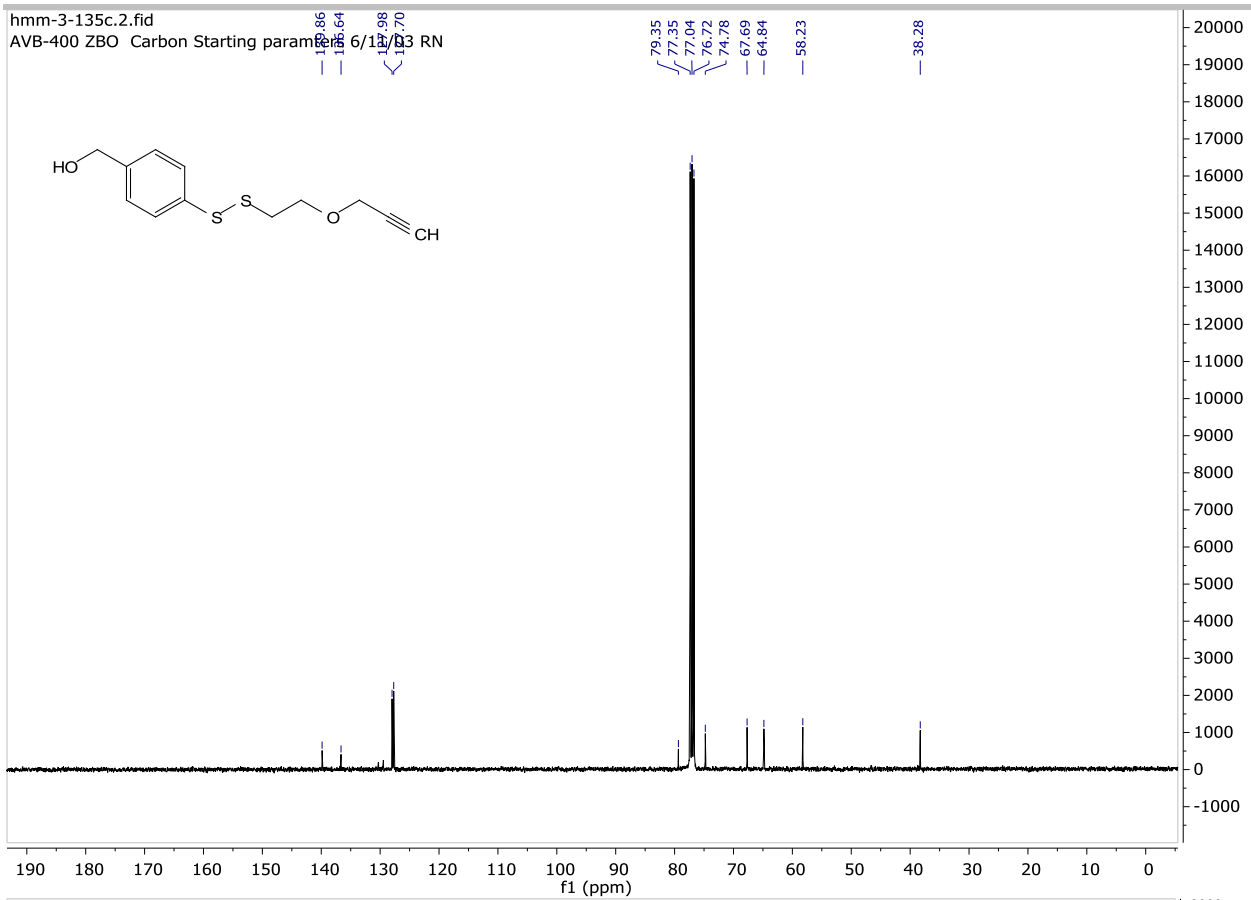
hmm_8_019.1.1.1r

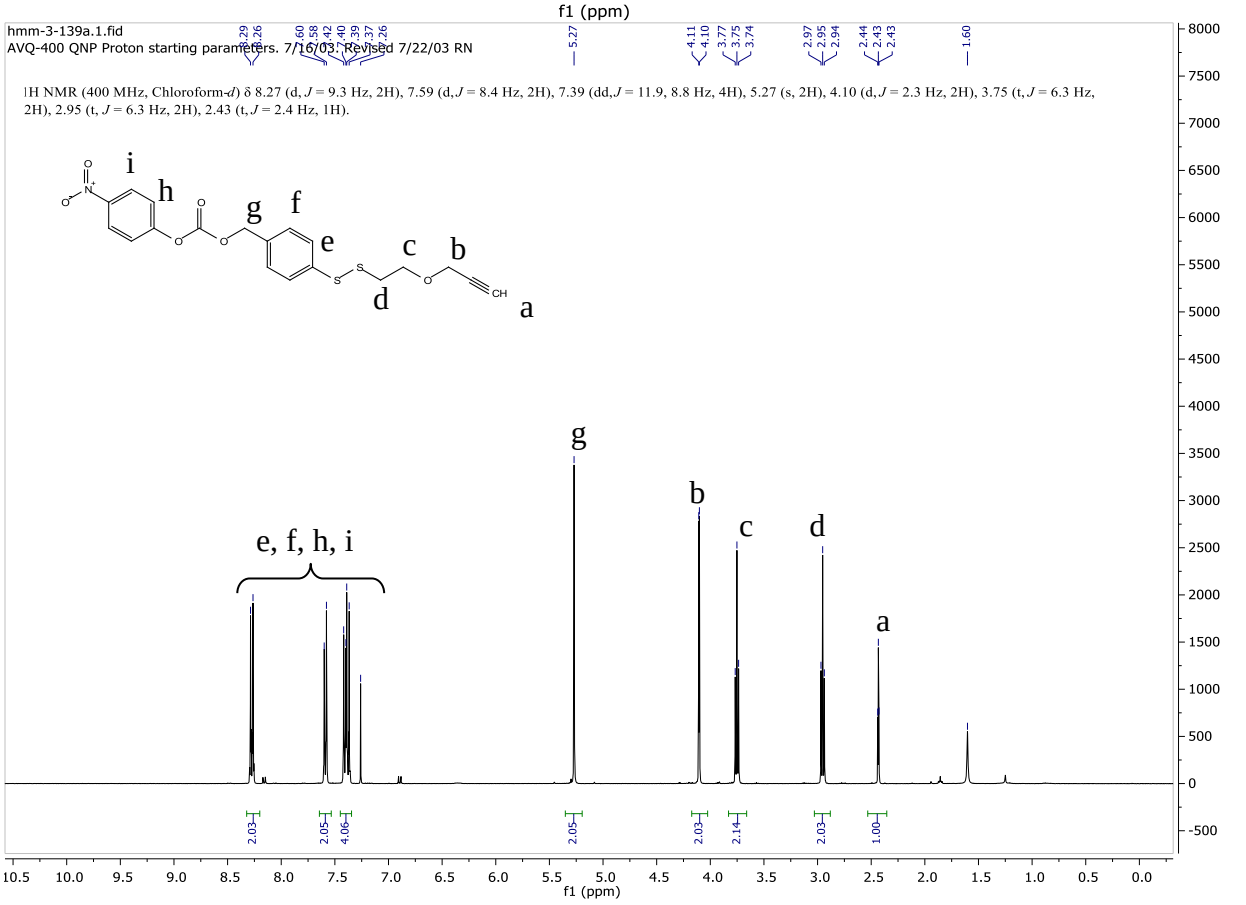


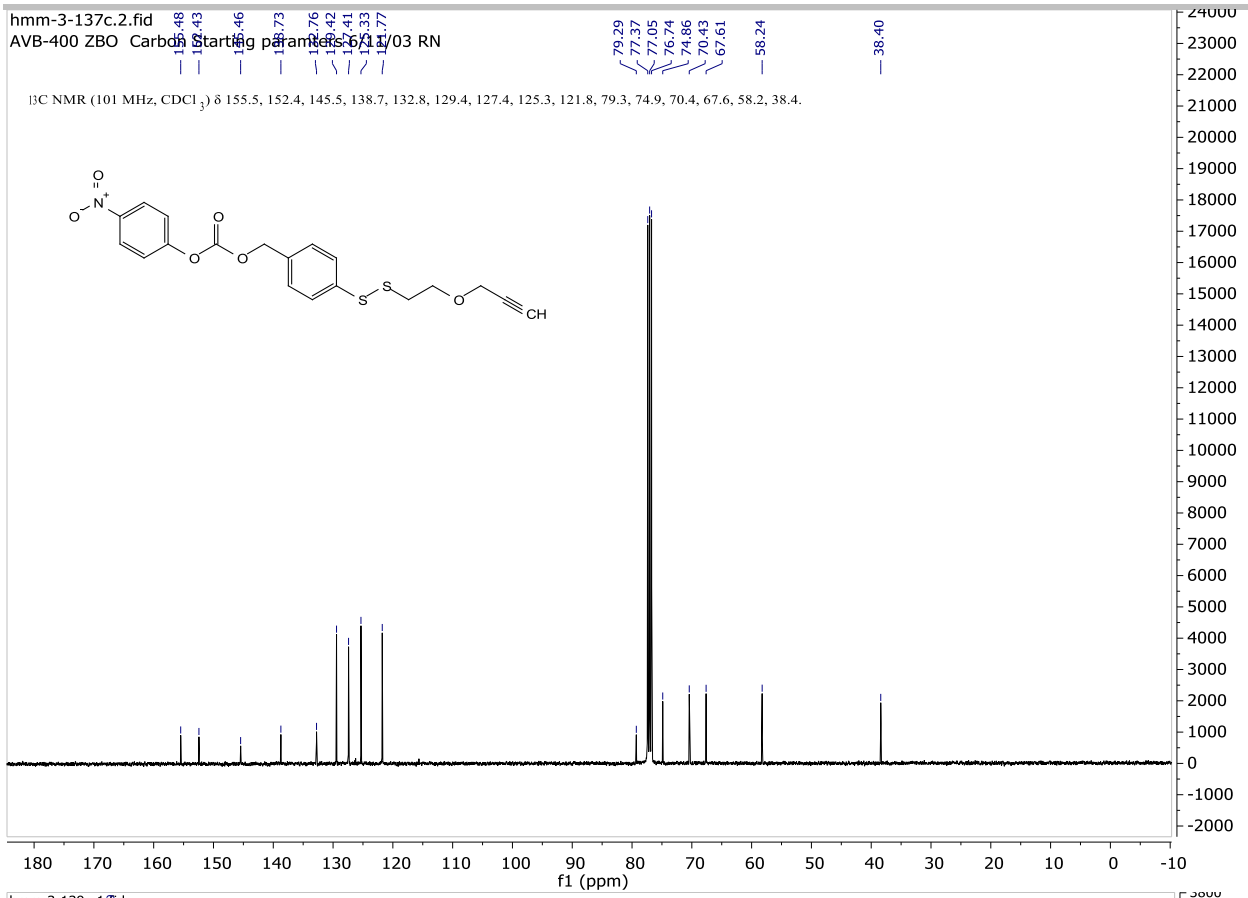
^{1}H NMR (900 MHz, Chloroform- *d*) δ 8.78 (s, 1H), 8.05 (d, *J* = 12.3 Hz, 1H), 7.77 (s, 1H), 7.41 (d, *J* = 6.7 Hz, 1H), 4.66 (s, 2H), 4.53 (t, *J* = 5.1 Hz, 2H), 4.22 (ddd, *J* = 37.3, 11.2, 6.3 Hz, 2H), 3.86 (t, *J* = 5.1 Hz, 2H), 3.78 (t, *J* = 6.6 Hz, 2H), 3.64 (s, 202H), 3.55 (dt, *J* = 9.5, 4.8 Hz, 4H), 3.37 (s, 3H), 3.32 (s, 4H), 3.13 (q, *J* = 6.7 Hz, 1H), 2.90 (q, *J* = 6.3 Hz, 2H), 1.42 (d, *J* = 6.9 Hz, 2H), 1.33 (d, *J* = 7.0 Hz, 3H), 1.20 (d, *J* = 4.3 Hz, 2H).

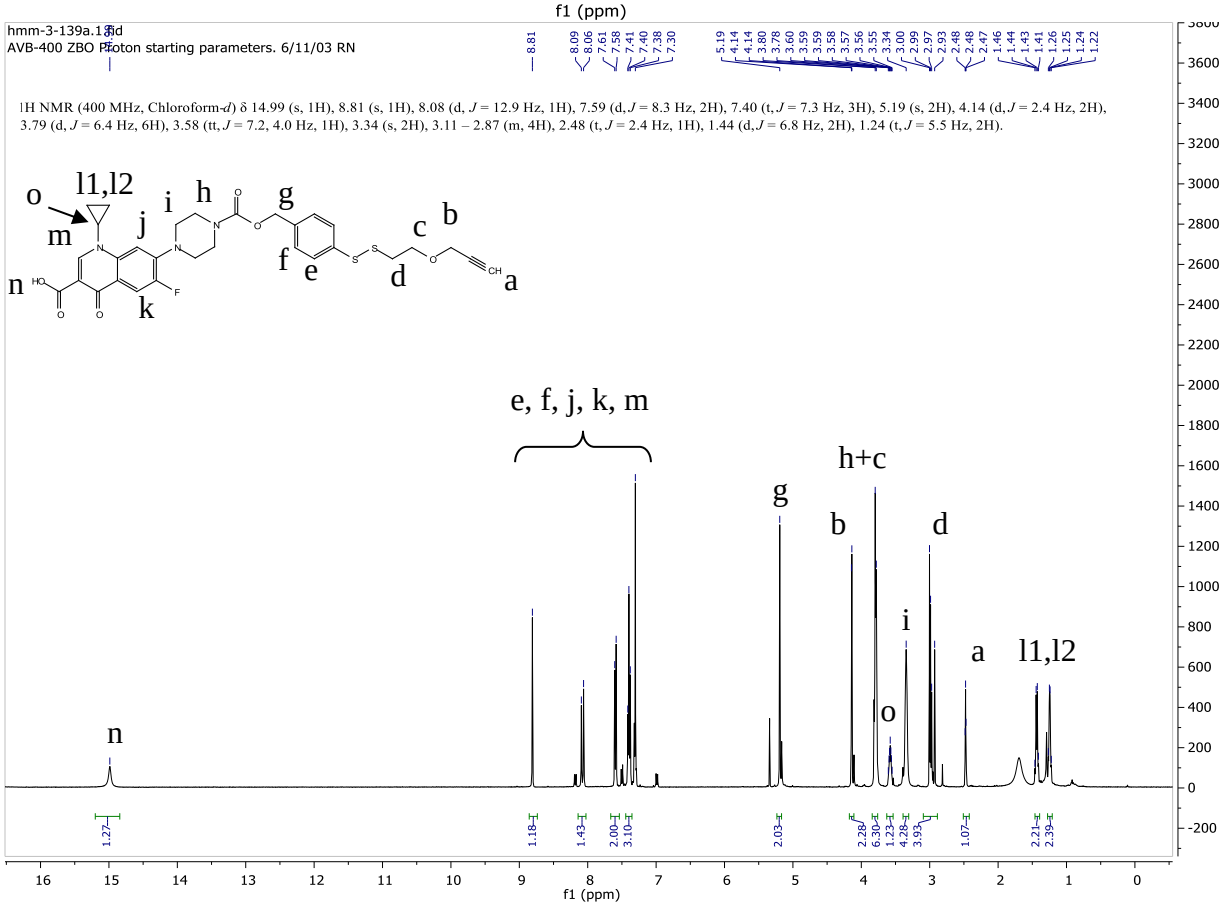

hmm_8_019.992.1.1r
177.13
166.94
154.81
154.18
153.07
147.54
145.64
145.59
144.51
139.04
123.90
120.33
112.65
112.55
108.23
105.27
77.22
77.16
77.02
76.88
71.89
70.59
70.58
70.57
70.55
70.49
70.42
70.39
69.39
68.53
67.87
64.27
59.00
50.23
49.68
44.74
39.22
35.36
17.19
8.26
13C NMR (226 MHz, CDCl3) δ 177.1, 166.9, 154.8, 154.2, 153.1, 147.5, 145.6, 145.6, 144.5, 139.0, 123.9, 120.3, 112.6, 112.5, 108.2, 105.3, 77.2, 71.9, 70.6, 70.6, 70.6, 70.5, 70.5, 70.4, 70.4, 69.4, 68.5, 67.9, 64.3, 59.0, 50.2, 49.7, 44.7, 39.2, 35.4, 17.2, 8.3.
PEG2000
f1 (ppm)

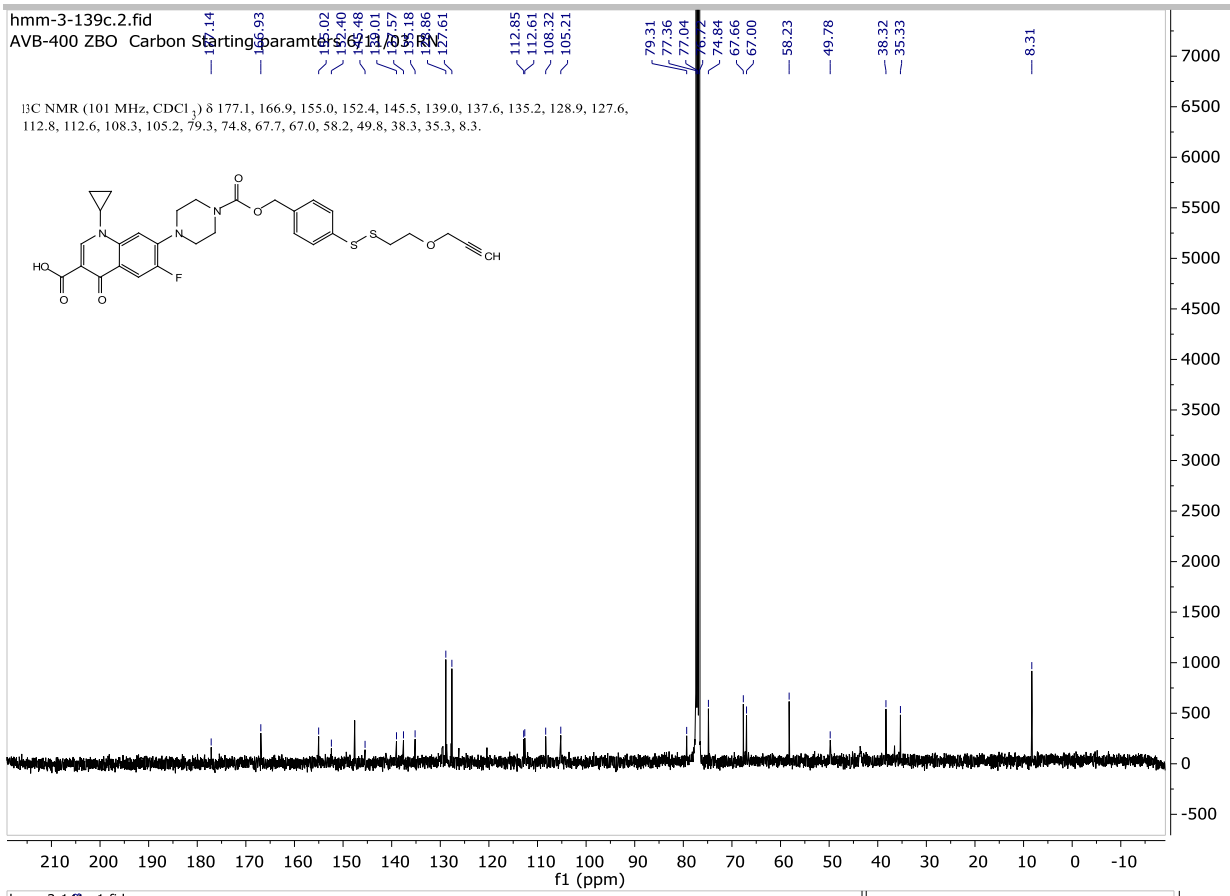

hmm-3-135a.1.fid
AVB-400 ZBO Proton starting parameters. 6/11/03 RM
7.55
7.53
7.34
7.32
7.26
4.68
4.10
4.10
3.77
3.75
3.73
2.95
2.94
2.92
2.44
2.43
2.43
1.72
1H NMR (400 MHz, Chloroform-d) δ 7.54 (d, J = 8.3 Hz, 1H), 7.33 (d, J = 8.3 Hz, 2H), 4.68 (s, 2H), 4.10 (d, J = 2.4 Hz, 1H), 3.75 (t, J = 6.4 Hz, 2H), 2.94 (t, J = 6.4 Hz, 2H), 2.43 (t, J = 2.5 Hz, 1H).
h
g
f
i
HO
c
b
d
a
CH
f-g
h
b
c
d
a
i
1.83
1.88
1.91
2.11
2.11
2.04
1.00
f1 (ppm)

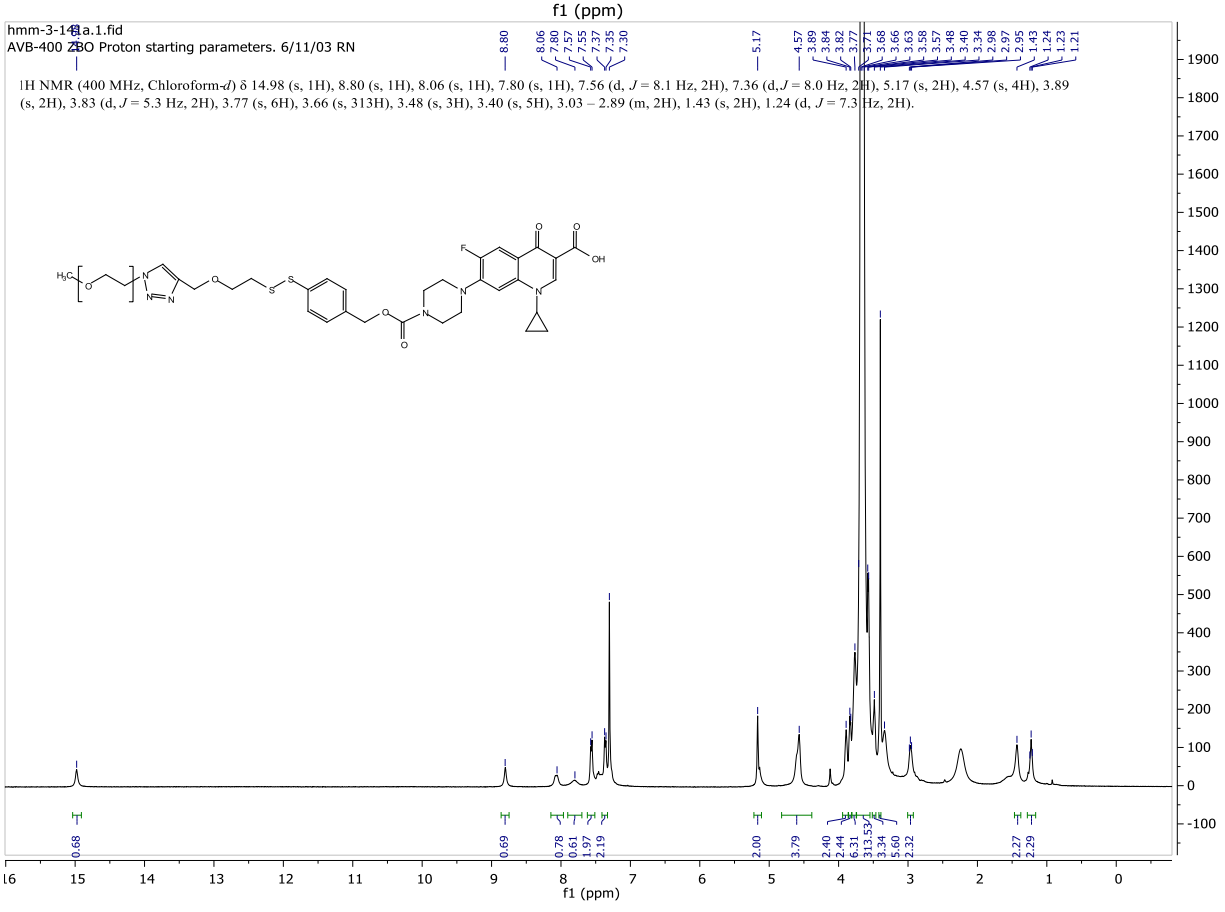

hmm-3-135c.2.fid
AVB-400 ZBO Carbon Starting parameters 6/11/03 RN
119.86
116.64
111.98
111.70
79.35
77.35
77.04
76.72
74.78
67.69
64.84
58.23
38.28
HO
S
S
O
CH
f1 (ppm)

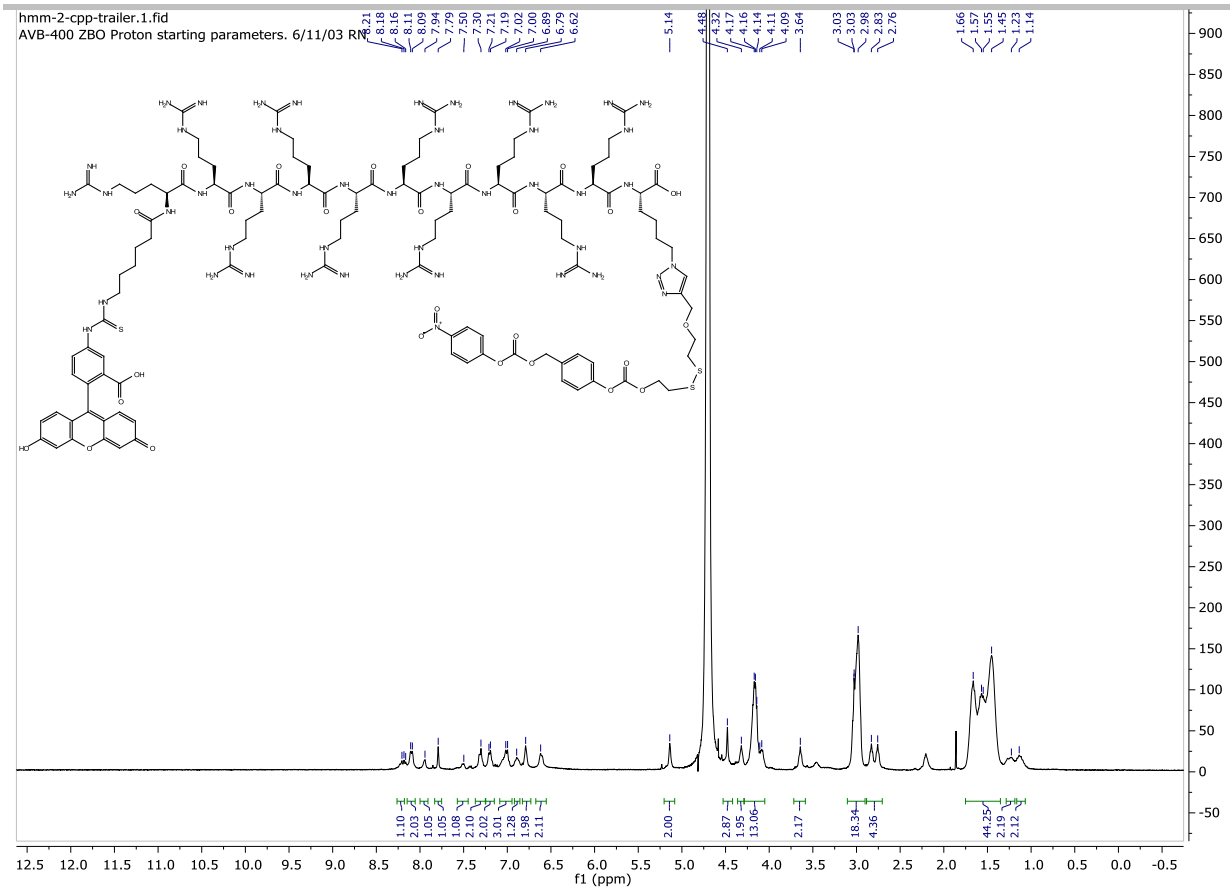

hmm-3-139a.1.fid
AVQ-400 QNP Proton starting parameters. 7/16/03. Revised 7/22/03 RN
8.29
8.26
7.60
7.58
7.42
7.40
7.39
7.37
7.26
5.27
4.11
4.10
3.77
3.75
3.74
2.97
2.95
2.94
2.44
2.43
2.43
1.60
1H NMR (400 MHz, Chloroform-d) δ 8.27 (d, J = 9.3 Hz, 2H), 7.59 (d, J = 8.4 Hz, 2H), 7.39 (dd, J = 11.9, 8.8 Hz, 4H), 5.27 (s, 2H), 4.10 (d, J = 2.3 Hz, 2H), 3.75 (t, J = 6.3 Hz, 2H), 2.95 (t, J = 6.3 Hz, 2H), 2.43 (t, J = 2.4 Hz, 1H).
i
h
g
f
e
c
b
d
a
CH
e, f, h, i
g
b
c
d
a
2.03
2.05
4.06
2.05
2.03
2.14
2.03
1.00
f1 (ppm)


hmm-3-137c.2.fid
AVB-400 ZBO Carbon starting parameters 6/11/03 RN
155.48
152.43
145.46
138.73
132.76
129.42
127.41
125.33
121.77
79.29
77.37
77.05
76.74
74.86
70.43
67.61
58.24
38.40
13C NMR (101 MHz, CDCl3) δ 155.5, 152.4, 145.5, 138.7, 132.8, 129.4, 127.4, 125.3, 121.8, 79.3, 74.9, 70.4, 67.6, 58.2, 38.4.
f1 (ppm)


hmm-3-139a.13.fid
AVB-400 ZBO Proton starting parameters. 6/11/03 RN
8.81
8.09
8.06
7.61
7.58
7.41
7.40
7.38
7.30
5.19
4.14
4.14
3.80
3.78
3.60
3.59
3.58
3.57
3.56
3.55
3.34
3.00
2.99
2.97
2.93
2.48
2.48
2.47
1.46
1.44
1.43
1.41
1.26
1.25
1.24
1.22
1H NMR (400 MHz, Chloroform-d) δ 14.99 (s, 1H), 8.81 (s, 1H), 8.08 (d, J = 12.9 Hz, 1H), 7.59 (d, J = 8.3 Hz, 2H), 7.40 (t, J = 7.3 Hz, 3H), 5.19 (s, 2H), 4.14 (d, J = 2.4 Hz, 2H), 3.79 (d, J = 6.4 Hz, 6H), 3.58 (tt, J = 7.2, 4.0 Hz, 1H), 3.34 (s, 2H), 3.11 – 2.87 (m, 4H), 2.48 (t, J = 2.4 Hz, 1H), 1.44 (d, J = 6.8 Hz, 2H), 1.24 (t, J = 5.5 Hz, 2H).
o
l1,l2
i
h
g
m
j
f
e
c
b
n
k
d
a
e, f, j, k, m
h+c
g
b
d
i
o
a
l1,l2
n
1.27
1.18
1.43
2.00
3.10
2.03
2.28
6.30
1.23
4.28
3.93
1.07
2.21
2.39
f1 (ppm)

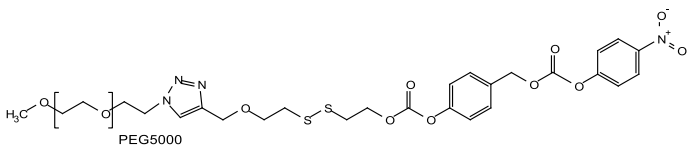

hmm-3-139c.2.fid
AVB-400 ZBO Carbon Starting paramters 6/11/03 RN
177.14
166.93
155.02
152.40
145.48
139.01
137.57
135.18
128.86
127.61
112.85
112.61
108.32
105.21
79.31
77.36
77.04
74.84
67.66
67.00
58.23
49.78
38.32
35.33
8.31
f1 (ppm)


hmm-3-141a.1.fid
AVB-400 ZBO Proton starting parameters. 6/11/03 RN
8.80
8.06
7.80
7.57
7.55
7.37
7.35
7.30
5.17
4.57
3.89
3.84
3.82
3.77
3.71
3.68
3.66
3.63
3.58
3.57
3.48
3.40
3.34
2.98
2.97
2.95
1.43
1.24
1.23
1.21
0.68
0.69
0.78
0.61
1.97
2.19
2.00
3.79
2.40
2.44
6.31
313.53
3.34
5.60
2.32
2.27
2.29
f1 (ppm)


hmm-2-cpp-trailer.1.fid
AVB-400 ZBO Proton starting parameters. 6/11/03 RM
f1 (ppm)

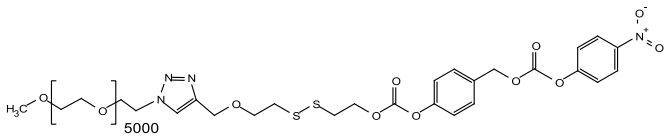
hmm-peg-trailer-1h/4
AVB-400 ZBO Proton starting parameters 6/11/03 RN

^{1}H NMR (400 MHz, Chloroform- *d*) δ 8.32 – 8.15 (m, 2H), 7.68 (s, 1H), 7.47 – 7.42 (m, 2H), 7.39 – 7.34 (m, 2H), 7.23 – 7.18 (m, 2H), 5.26 (s, 2H), 4.65 (s, 2H), 4.53 (d, J = 5.1 Hz, 2H), 4.47 (t, J = 6.6 Hz, 2H), 3.85 (t, J = 5.1 Hz, 2H), 3.81 – 3.77 (m, 4H), 3.61 (s, 460H), 3.53 – 3.51 (m, 2H), 3.46 – 3.42 (m, 2H), 3.35 (s, 3H), 2.98 (t, J = 6.6 Hz, 2H).



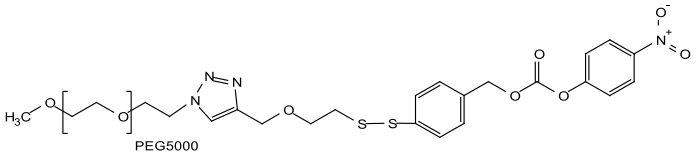
PEG5k_TRAILER.992.1.1r

^{13}C NMR (226 MHz, $CDCl_3$) δ 155.6, 153.4, 152.6, 151.6, 145.6, 132.3, 131.1, 130.3, 129.0, 125.5, 122.0, 121.6, 72.1, 70.7, 70.7, 70.6, 70.5, 70.5, 70.4, 70.3, 69.6, 68.6, 66.7, 64.4, 59.2, 50.5, 38.8, 36.9, 29.8.



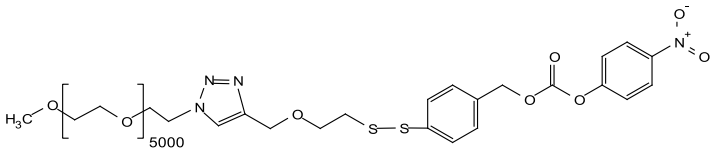
hmm-peg-dtb-1h/1
AVB-400 ZBO Proton starting parameters: 6/11/03 RN

^{1}H NMR (400 MHz, Chloroform- *d*) δ 8.29 (dd, *J* = 9.2, 1.3 Hz, 2H), 7.77 (s, 1H), 7.59 (d, *J* = 7.0 Hz, 2H), 7.41 (t, *J* = 7.9 Hz, 2H), 5.29 (s, 2H), 4.62 (s, 2H), 4.57 (t, *J* = 5.1 Hz, 2H), 3.89 (t, *J* = 5.1 Hz, 2H), 3.83 (d, *J* = 4.9 Hz, 4H), 3.78 (dd, *J* = 7.5, 6.3 Hz, 2H), 3.66 (d, *J* = 1.3 Hz, 625H), 3.57 (d, *J* = 6.1 Hz, 4H), 3.48 (t, *J* = 4.9 Hz, 4H), 3.39 (d, *J* = 1.3 Hz, 3H), 2.96 (t, *J* = 6.3 Hz, 2H).

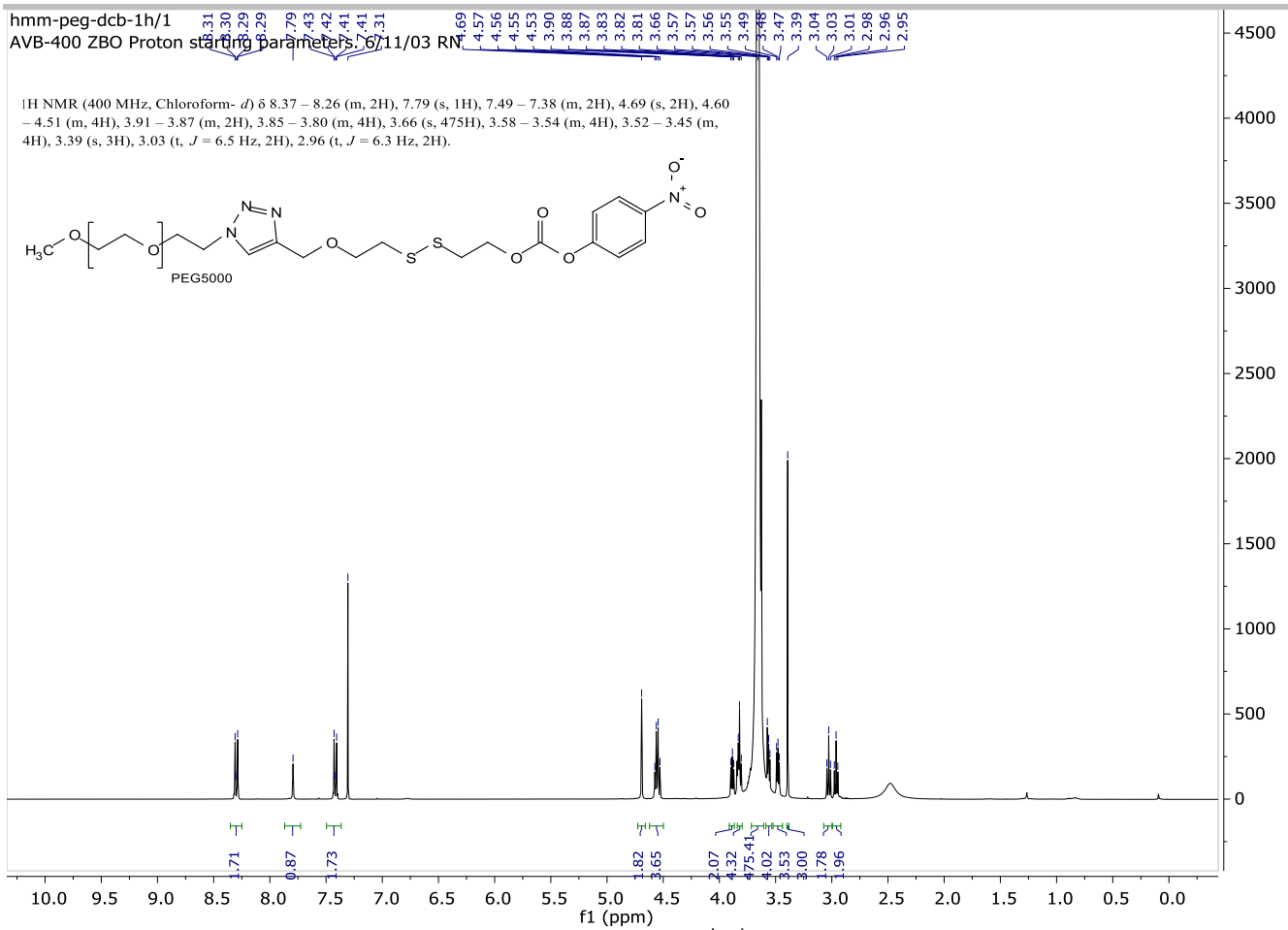


PEG5k_DTB.992.1.1r

^{13}C NMR (226 MHz, $CDCl_3$) δ 155.6, 152.6, 145.6, 144.7, 138.8, 132.9, 129.6, 127.5, 125.5, 124.0, 122.0, 72.1, 70.7, 70.6, 70.6, 69.6, 68.2, 64.5, 59.2, 50.4, 38.6.

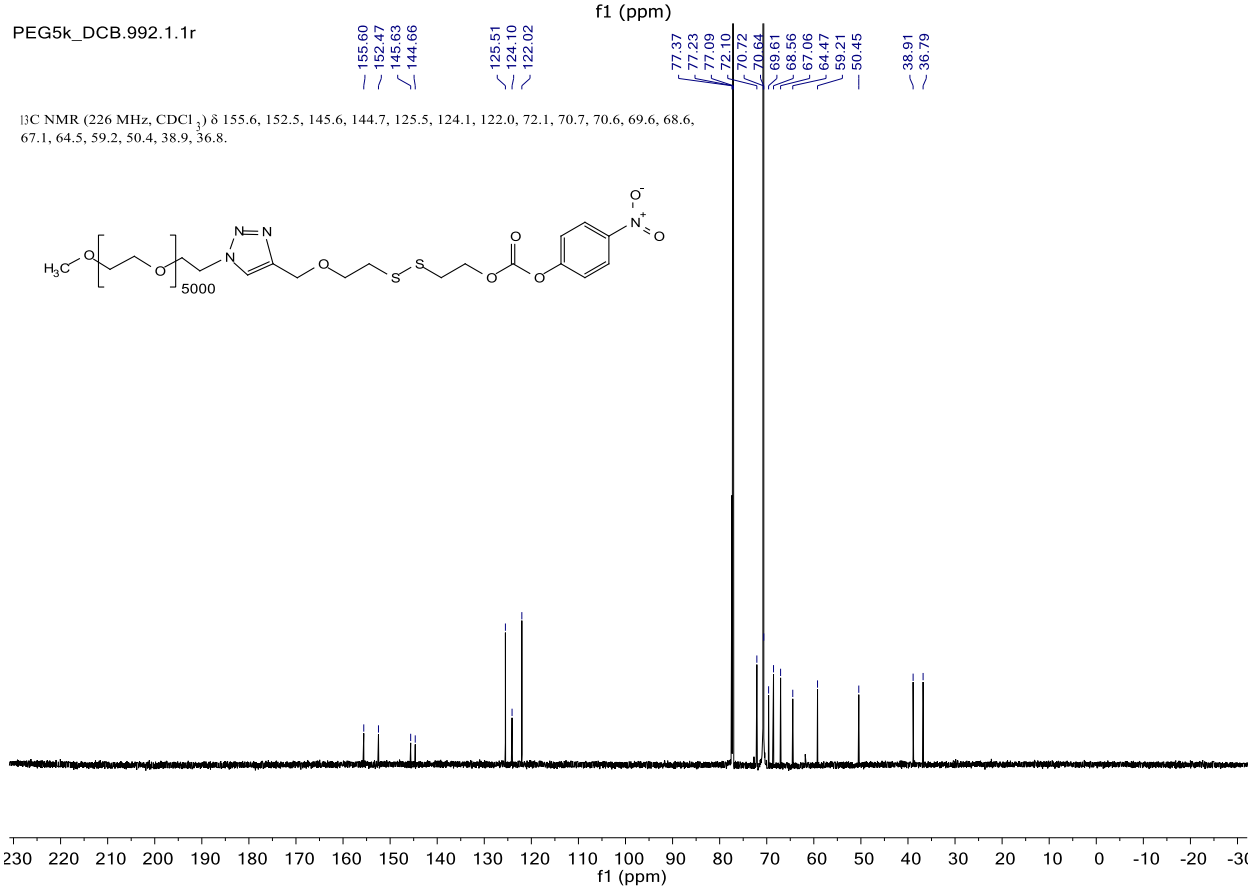


hmm-peg-dcb-1h/1
AVB-400 ZBO Proton starting parameters 6/11/03 RM



^{1}H NMR (400 MHz, Chloroform- *d*) δ 8.37 – 8.26 (m, 2H), 7.79 (s, 1H), 7.49 – 7.38 (m, 2H), 4.69 (s, 2H), 4.60 – 4.51 (m, 4H), 3.91 – 3.87 (m, 2H), 3.85 – 3.80 (m, 4H), 3.66 (s, 475H), 3.58 – 3.54 (m, 4H), 3.52 – 3.45 (m, 4H), 3.39 (s, 3H), 3.03 (t, *J* = 6.5 Hz, 2H), 2.96 (t, *J* = 6.3 Hz, 2H).

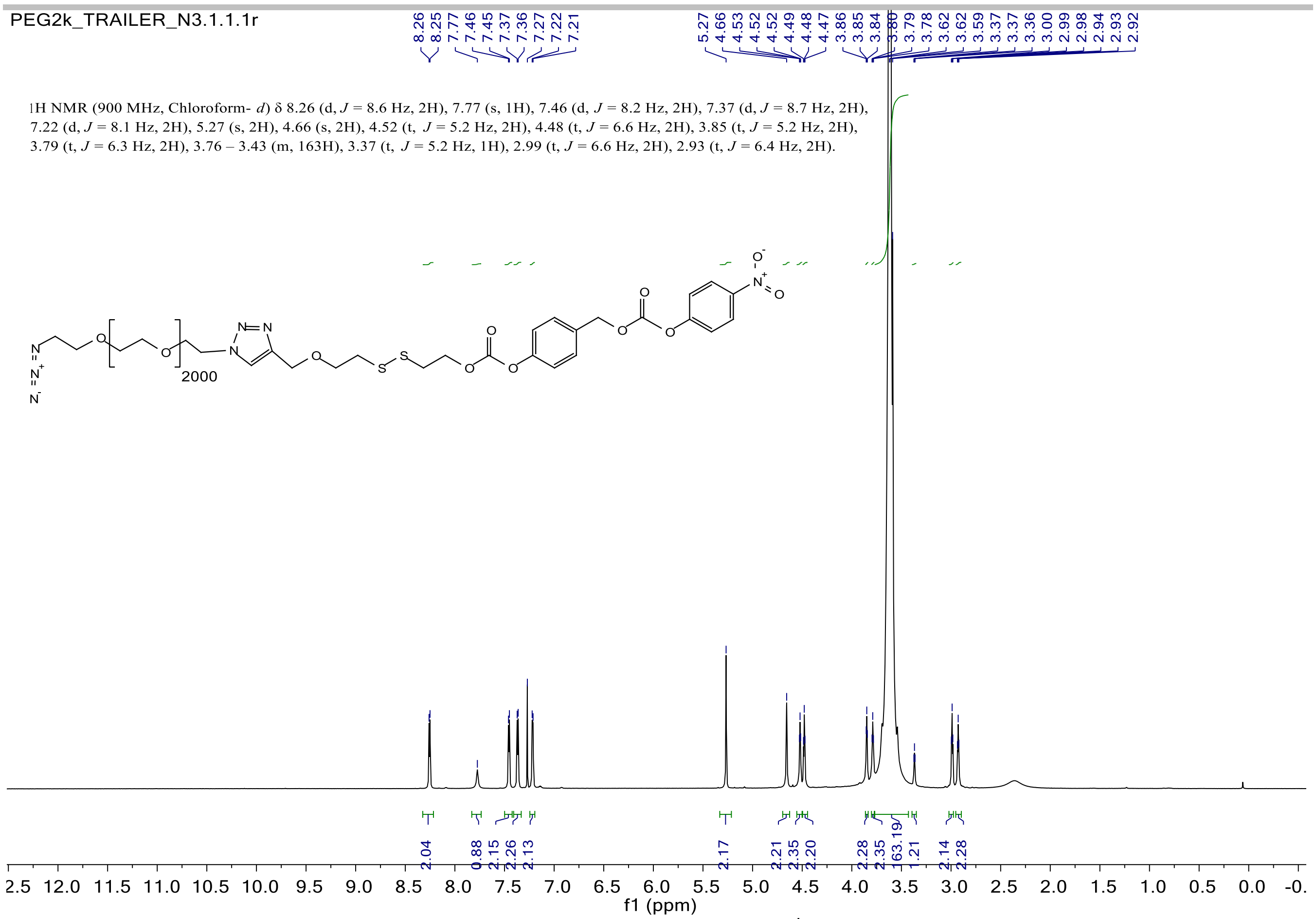
PEG5k_DCB.992.1.1r



^{13}C NMR (226 MHz, $CDCl_3$) δ 155.6, 152.5, 145.6, 144.7, 125.5, 124.1, 122.0, 72.1, 70.7, 70.6, 69.6, 68.6, 67.1, 64.5, 59.2, 50.4, 38.9, 36.8.

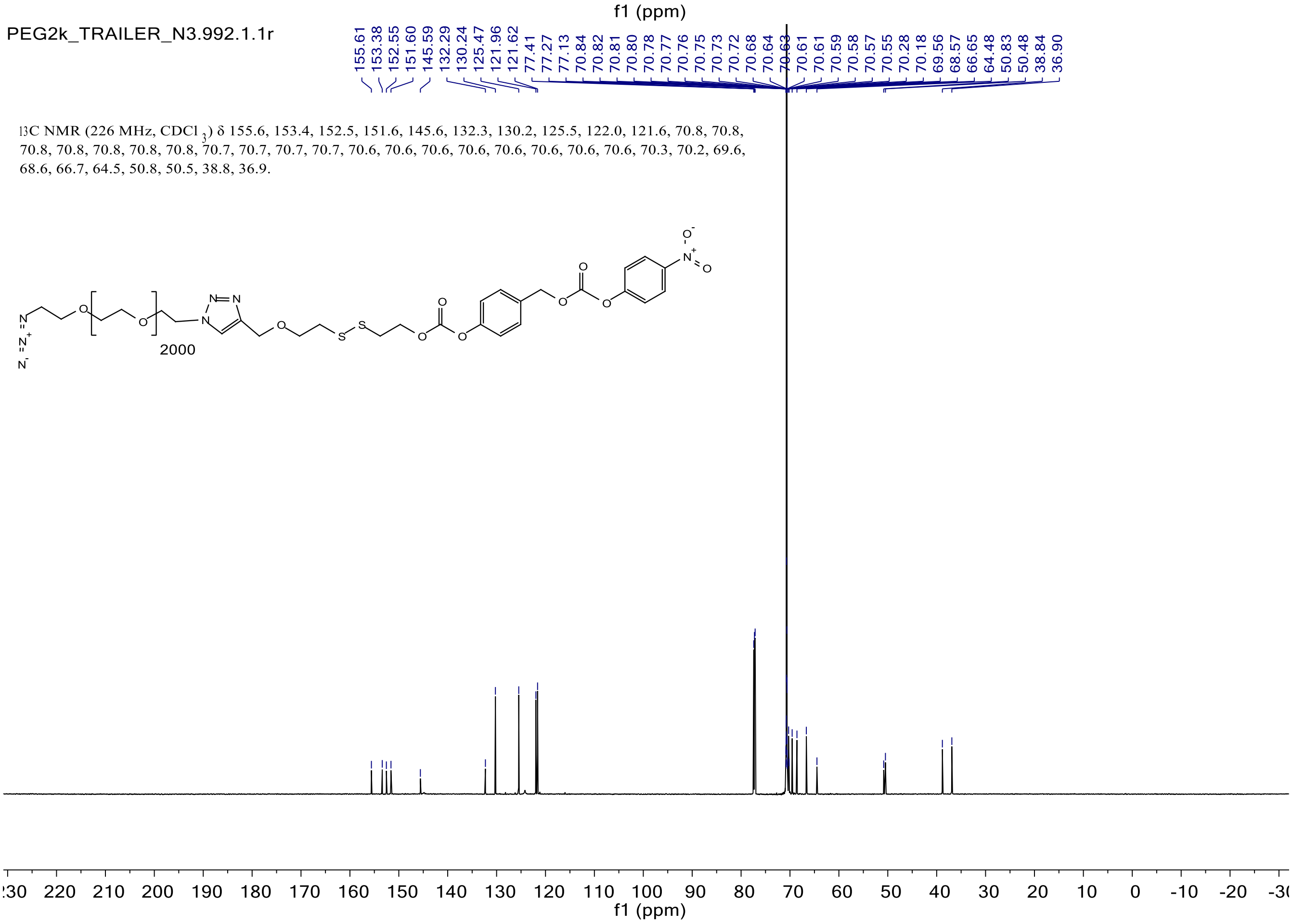
PEG2k_TRAILER_N3.1.1.1r

1H NMR (900 MHz, Chloroform- *d*) δ 8.26 (d, *J* = 8.6 Hz, 2H), 7.77 (s, 1H), 7.46 (d, *J* = 8.2 Hz, 2H), 7.37 (d, *J* = 8.7 Hz, 2H), 7.22 (d, *J* = 8.1 Hz, 2H), 5.27 (s, 2H), 4.66 (s, 2H), 4.52 (t, *J* = 5.2 Hz, 2H), 4.48 (t, *J* = 6.6 Hz, 2H), 3.85 (t, *J* = 5.2 Hz, 2H), 3.79 (t, *J* = 6.3 Hz, 2H), 3.76 – 3.43 (m, 163H), 3.37 (t, *J* = 5.2 Hz, 1H), 2.99 (t, *J* = 6.6 Hz, 2H), 2.93 (t, *J* = 6.4 Hz, 2H).



PEG2k_TRAILER_N3.992.1.1r

13C NMR (226 MHz, $CDCl_3$) δ 155.6, 153.4, 152.5, 151.6, 145.6, 132.3, 130.2, 125.5, 122.0, 121.6, 70.8, 70.8, 70.8, 70.8, 70.8, 70.8, 70.8, 70.7, 70.7, 70.7, 70.7, 70.6, 70.6, 70.6, 70.6, 70.6, 70.6, 70.6, 70.6, 70.3, 70.2, 69.6, 68.6, 66.7, 64.5, 50.8, 50.5, 38.8, 36.9.



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