Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information for:

Understanding Functional Group and Assembly Dynamics in Temperature Responsive Systems Leads to Design Principles for Enzyme Responsive Assemblies

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Experiment Section

1. Materials and instruments

All the chemicals were commercially available and used without further purification, unless otherwise stated. ^{1}H and ^{13}C NMR were recorded on a Bruker Avance-400 MHz or Bruker Avance-500 MHz NMR spectrometer. Chemical shifts were reported in ppm with TMS and residual solvent as standard. ^{13}C NMR were reported with the internal chloroform signal at 77.20 ppm as standard. Mass spectrometric data were recorded by a Bruker ESI-TOF Mass Spectrometer (Micro mass). Dynamic light scattering (DLS) measurements were performed using a Malvern Zetasizer Nano ZS. Fluorescence spectra were recorded on a PerkinElmer LS 55 spectrofluorimeter.

2. Methods

2.1 Preparation of the supramolecular assemblies

200 nmol certain oligomer in 200 μL acetone was added dropwise into 2 mL deionized H_2O while stirring. The solution was stirred at room temperature in an uncapped vial for overnight, allowing the organic solvent to evaporate. The obtained solution was diluted to 25 μM and sonicated for 1 min before DLS and fluorescence measurement unless otherwise stated.

2.2 Dynamic Light Scattering

DLS was performed on a Malvern Zetasizer Nano ZS instrument with a 637 nm laser source with non-invasive backscattering technology detected at 173°. The sizes were reported as hydrodynamic diameter. The samples were equilibrated under certain temperature for 10 min before performing size measurements.

2.3 Fluorescence measurements

Fluorescence of assemblies were recorded using a PerkinElmer LS 55 spectrofluorimeter. All the sample were equilibrated for 15 min at certain temperature before performing fluorescence measurements. All the experiments were performed for three replicates.

2.4 Critical aggregation concentration (CAC) measurements

200 nmol certain oligomer in 150 μL acetone was added dropwise into 1 mL deionized H_2O while stirring. The solution was stirred at room temperature in an uncapped vial for overnight, allowing acetone to evaporate. Then a calculated 5 wt% loading of Nile Red solution in acetone (1 $mg \cdot mL^{-1}$) was added into the oligomer solution and stirred for another 6 h to allow Nile Red encapsulation and the evaporation of acetone. Next, the unencapsulated Nile Red was removed by passing through a cotton-plugged glass Pasteur pipette[1] and the final solution was adjusted to 1 mL using deionized water. The fluorescence of samples was recorded for every 1.5 times dilution with an excitation wavelength at 545 nm. Finally, the fluorescence emission intensity was plotted against the oligomer concentration and the concentration at the inflection point was reported as corresponding CAC.[2]

2.5 Enzyme triggered DTP shift

3 mL of the 100 μM particle solution of oligomer PEG7-hexyl-ES was prepared as the method introduced before. The particle solution was diluted to 50 μM in 4 mM Tris buffer (pH 7.4). Next, PLE was added into the particle solution to get 30 nM enzyme concentration. The solution was further stirred at room temperature for 15 h. The fluorescence and size of control group and PLE treated group were recorded at various temperature following the general method introduced above.

2.6 All-Atoms Molecular Dynamics (AA-MD) protocol

The All Atom (AA) model for each dendron was drawn with Avogadro[3]. Antechamber[4,5] was then used to obtain the topology of the system, while the charge distribution was estimated with Gaussian software[6].

The amber99sb[5] force field was applied to run the AA-MD simulations of the dendrons in water (tip3p) using GROMACS 2018.6 software[7]. After minimizing the energy of the system, we carried out the following equilibration steps:

- 100 ps in NVT ensamble at 300 K with v-rescale thermostat[8] (time step: 2fs);

- 100 ps in NPT ensamble at 1.013 bar, applying Berendsen barostat[9] (time step: 2fs);

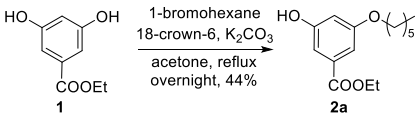
- 100 ns production run with Nose-Hoover[10] thermostat and Parrinello-Rahman[11] barostat to maintain T = 300 K, and p = 1.012 bar (time step 2fs).

2.7 Coarse-Grained Molecular Dynamics (CG-MD) protocol

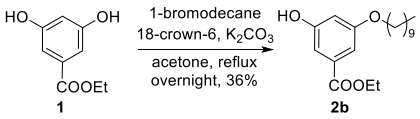
The coarse-grained (CG) models for the dendrons, built based on the MARINI force field[12], have been optimized for the best agreement with the AA models using the recently developed *Swarm-CG* software[13] In fact, the MARTINI forcefield is a widely used, flexible and reliable platform for studying inter-molecular self-assembly driven by hydrophobic effects (non-directional interactions), and therefore well-suited for the phenomena occurring in the systems studied herein. All the CG Molecular Dynamics (CG-MD) simulations have been carried out in GROMACS 2018.6 software[4] using a timestep of 20 fs in NPT conditions (constant N, number of particles, P, pressure, T, temperature), except for the simulations in vacuum (preliminary phase only: see below), where NVT ensemble has been used (constant N, number of particles, V, volume, T, temperature). To obtain a first aggregate in the most efficient way, 50 monomers were initially (randomly) placed in a 100 nm^3 simulation box, and the system was simulated in vacuum at 303 K of temperature. This preliminary phase produced the fast collapse of the dendrons into assembled particles. The obtained aggregates have been then immerged in explicit (W Martini) water molecules, and the assemblies have been equilibrated in water at 303 K. During these CG-MD the assemblies relaxed and equilibrated their structure in water. These pre-equilibrated assemblies have been then simulated at three different temperatures, namely 283 K, 303 K and 333 K. For the systems at 283 K, the 10% of the water beads have been replaced with anti-freezing Martini water particles to avoid water freezing. These CG-

MD simulations were conducted at 1 atm using the V-rescale thermostat[5] and Berendsen barostat[6] with isotropic pressure scaling, using a compressibility of $4.5 * 10^{-5}$ MPa^{-1} and coupling time constants of 1ps and 2 ps for V-rescale and Berendsen coupling schemes, respectively. For the CG-MD simulations in water, electrostatic and van der Waals cutoff were both fixed at 1.1 nm.

3. Molecular synthesis and characterization

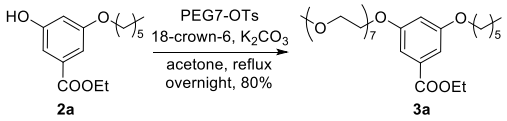


The mixture of compound **1** (3.92 g, 20 mmol), 18-crown-6 (2.11 g, 8 mmol) and K_2CO_3 (2.76 g, 20 mmol) in 120 mL acetone were heated to reflux for 30 min. Then 1-hexyl bromide (2.25 mL, 16 mmol) was added dropwise into the mixture and then the solution was refluxed for overnight. When the reaction was monitored to be completed, K_2CO_3 was removed by filtration and the residue was concentrated to remove acetone. Then ethyl acetate was added and the solution was washed with brine. Finally, the organic phase was dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel (hexane:EA = 5:1) to obtain the final product as light yellow solid with 44% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, 3H, J = 5.56 Hz), 1.30-1.40 (m, 7H), 1.41-1.48 (m, 2H), 1.73-1.80 (m, 2H), 3.96 (t, 2H, J = 5.22 Hz), 4.36 (q, 2H, J = 5.71 Hz), 6.60-6.62 (m, 1H), 7.14-7.19 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 14.4, 22.8, 25.9, 29.3, 31.7, 61.5, 68.6, 107.1, 108.1, 109.1, 132.4, 157.0, 160.6, 166.9; ESI-MS m/z calcd for $C_{15}H_{22}O_4$ [M + Na]+: 289.14; found: 289.15.

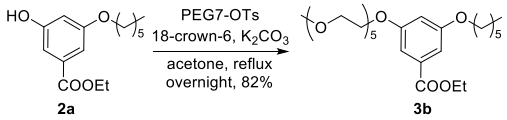


The mixture of compound **1** (6 g, 31 mmol), 18-crown-6 (1.63 g, 6.2 mmol) and K_2CO_3 (6.41 g, 46 mmol) in 130 mL acetone were heated to reflux for 30 min. Then 1-decyl bromide (5.48 g, 24.7 mmol) was added dropwise into the mixture and then the solution was refluxed for overnight. When the reaction was monitored to be completed, K_2CO_3 was removed by filtration and the residue was concentrated to remove acetone. Then ethyl acetate was added and the solution was washed with brine. Finally, the organic phase was dried with Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel (hexane:EA = 4:1) to obtain the final product as light yellow solid with 36% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t,

3H, *J* = 6.84 Hz), 1.21-1.47 (m, 17H), 1.72-1.81 (m, 2H), 3.95 (t, 2H, *J* = 6.56 Hz), 4.36 (q, 2H, *J* = 7.13 Hz), 5.87 (s, 1H), 6.62 (t, 1H, *J* = 2.28 Hz), 7.13-7.20 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 14.4, 22.9, 26.2, 29.3, 29.5, 29.6, 29.73, 29.75, 32.1, 61.5, 68.6, 107.1, 108.1, 109.1, 132.4, 157.0, 160.6, 166.9; ESI-MS m/z calcd for $C_{19}H_{30}O_4$ $[M + Na]^+$: 345.20; found: 345.18.

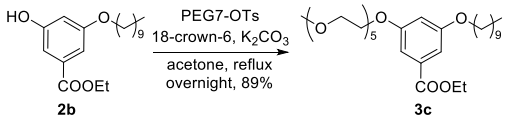


The mixture of compound **2a** (339 mg, 1.27 mmol), PEG7-OTs (567 mg, 1.14 mmol), 18-crown-6 (201 mg, 0.76 mmol) and K_2CO_3 (263 mg, 1.91 mmol) in 10 mL acetone was refluxed for overnight. When the reaction was monitored to be completed, K_2CO_3 was removed by filtration and the residue was concentrated to remove acetone. Then ethyl acetate was added and the solution was washed with brine. Finally, the organic phase was dried with Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel (DCM:MeOH = 30:1) to obtain the final product as colorless oil (542 mg) with 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (t, 3H, *J* = 7.02 Hz), 1.31-1.40 (m, 7H), 1.41-1.50 (m, 2H), 1.73-1.81 (m, 2H), 3.38 (s, 3H), 3.53-3.56 (m, 2H), 3.62-3.69 (m, 20H), 3.71-3.74 (m, 2H), 3.86 (t, 2H, *J* = 4.82 Hz), 3.96 (t, 2H, *J* = 6.56 Hz), 4.17 (t, 2H, *J* = 4.84 Hz), 4.35 (q, 2H, *J* = 7.12 Hz), 6.66 (t, 1H, *J* = 2.36 Hz), 7.16-7.19 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 14.5, 22.8, 25.9, 29.3, 31.7, 59.2, 61.3, 67.9, 68.5, 69.8, 70.7, 70.77, 70.80, 70.82, 71.0, 72.1, 106.7, 107.7, 108.3, 108.8, 132.4, 159.9, 160.3, 166.6; ESI-MS m/z calcd for $C_{30}H_{52}O_{11}$ $[M + Na]^+$: 611.34; found: 611.34.

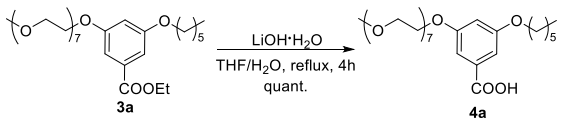


The mixture of compound **2a** (552 mg, 2.08 mmol), PEG5-OTs (804 mg, 1.98 mmol), 18-crown-6 (352 mg, 1.33 mmol) and K_2CO_3 (459 mg, 3.32 mmol) in 15 mL acetone was refluxed for overnight. When the reaction was monitored to be completed, K_2CO_3 was removed by filtration and the residue was

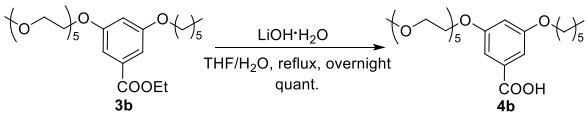
concentrated to remove acetone. Then ethyl acetate was added and the solution was washed with brine. Finally, the organic phase was dried with Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel (hexane:EA = 1:2) to obtain the final product as colorless oil (815 mg) with 82% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, 3H, *J* = 6.96 Hz), 1.30-1.40 (m, 7H), 1.40-1.49 (m, 2H), 1.73-1.81 (m, 2H), 3.37 (s, 3H), 3.52-3.55 (m, 2H), 3.61-3.69 (m, 12H), 3.70-3.74 (m, 2H), 3.85 (t, 2H, *J* = 4.80 Hz), 3.96 (t, 2H, *J* = 6.56 Hz), 4.14 (t, 2H, *J* = 4.82 Hz), 4.35 (q, 2H, *J* = 7.13 Hz), 6.65 (t, 1H, *J* = 2.32 Hz), 7.15-7.19 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 14.5, 22.8, 25.9, 29.3, 31.7, 59.2, 61.3, 67.9, 68.5, 69.8, 70.7, 70.75, 70.77, 70.78, 70.81, 71.0, 72.1, 106.7, 107.7, 108.3, 132.4, 159.9, 160.3, 166.6; ESI-MS m/z calcd for $C_{26}H_{44}O_9$ $[M + Na]^+$: 523.29; found: 523.28.



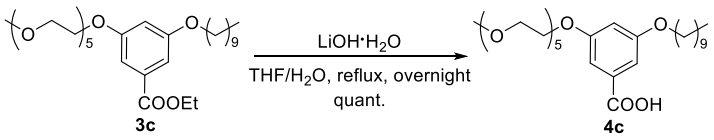
The mixture of compound **2b** (1.47 g, 4.56 mmol), PEG5-OTs (1.85 g, 4.56 mmol), 18-crown-6 (481 mg, 1.82 mmol) and K_2CO_3 (945 mg, 6.84 mmol) in 30 mL acetone was refluxed for overnight. When the reaction was monitored to be completed, K_2CO_3 was removed by filtration and the residue was concentrated to remove acetone. Then ethyl acetate was added and the solution was washed with brine. Finally, the organic phase was dried with Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel (hexane:EA = 1:2) to obtain the final product as colorless oil (2.19 g) with 89% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, 3H, *J* = 6.80 Hz), 1.23-1.40 (m, 15H), 1.41-1.48 (m, 2H), 1.73-1.82 (m, 2H), 3.37 (s, 3H), 3.52-3.56 (m, 2H), 3.62-3.70 (m, 12H), 3.71-3.74 (m, 2H), 3.86 (t, 2H, *J* = 4.78 Hz), 3.96 (t, 2H, *J* = 6.55 Hz), 4.15 (t, 2H, *J* = 4.78 Hz), 4.35 (q, 2H, *J* = 7.12 Hz), 6.66 (t, 1H, *J* = 2.10 Hz), 7.18 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3, 14.5, 22.9, 26.2, 29.4, 29.49, 29.54, 29.73, 29.74, 32.1, 59.2, 61.3, 67.9, 68.5, 69.8, 70.7, 70.75, 70.77, 70.79, 70.81, 71.0, 72.1, 106.7, 107.7, 108.3, 132.4, 159.9, 160.3, 166.6; ESI-MS m/z calcd for $C_{30}H_{52}O_9$ $[M + Na]^+$: 579.35; found: 579.34.



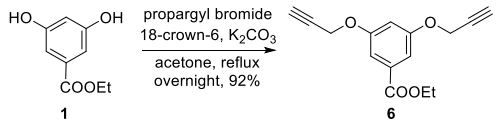
Compound **3a** (530 mg, 0.9 mmol) in 8 mL THF was mixed with $LiOH \cdot H_2O$ (113 mg, 2.7 mmol) in 4 mL H_2O and the solution was refluxed for 4h. After completion, THF was removed *in vacuo*, acidified with 1M HCl to pH 2 to 3 and extracted with EA. Then the organic phase was dried with Na_2SO_4, concentrated *in vacuo* to obtain the final product as pale yellow oil with quantitative conversion. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (t, 3H, J = 7.02 Hz), 1.30-1.38 (m, 4H), 1.40-1.50 (m, 2H), 1.73-1.82 (m, 2H), 3.40 (s, 3H), 3.56-3.61 (m, 2H), 3.63-3.71 (m, 20H), 3.72-3.76 (m, 2H), 3.87 (t, 2H, J = 4.56 Hz), 3.97 (t, 2H, J = 6.56 Hz), 4.17 (t, 2H, J = 4.56 Hz), 6.70 (t, 1H, J = 2.28 Hz), 7.19-7.24 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.8, 25.8, 29.3, 31.7, 59.0, 67.8, 68.6, 69.8, 70.3, 70.45, 70.48, 70.51, 70.58, 70.60, 70.9, 71.9, 107.6, 108.3, 108.8, 131.2, 159.9, 160.4, 170.6; ESI-MS m/z calcd for $C_{28}H_{48}O_{11}$ $[M + Na]^+$: 583.31; found: 583.29.



Compound **3b** (802 mg, 1.6 mmol) in 4 mL THF was mixed with $LiOH \cdot H_2O$ (202 mg, 4.8 mmol) in 2 mL H_2O and the solution was refluxed for overnight. After completion, THF was removed *in vacuo*, acidified with 1M HCl to pH 2 to 3 and extracted with EA. Then the organic phase was dried with anhydrous Na_2SO_4, concentrated *in vacuo* to obtain the final product as light yellow-brown oil with quantitative conversion. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (t, 3H, J = 7.00 Hz), 1.29-1.38 (m, 4H), 1.41-1.50 (m, 2H), 1.73-1.81 (m, 2H), 3.38 (s, 3H), 3.53-3.57 (m, 2H), 3.63-3.69 (m, 12H), 3.71-3.75 (m, 2H), 3.86 (t, 2H, J = 4.72 Hz), 3.97 (t, 2H, J = 6.56 Hz), 4.16 (t, 2H, J = 4.76 Hz), 6.70 (t, 1H, J = 2.30 Hz), 7.21-7.24 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.8, 25.8, 29.3, 31.7, 59.2, 67.9, 68.6, 69.8, 70.6, 70.7, 70.77, 70.79, 70.82, 71.1, 72.1, 107.7, 108.3, 108.7, 131.3, 160.0, 160.4, 171.0; ESI-MS m/z calcd for $C_{24}H_{40}O_9$ $[M + Na]^+$: 495.26; found: 495.26.

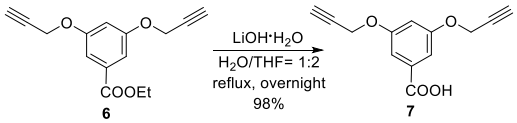


Compound **3c** (2.19 g, 4.04 mmol) in 30 mL THF was mixed with $LiOH \cdot H_2O$ (508 mg, 12.1 mmol) in 15 mL H_2O and the solution was refluxed for overnight. After completion, THF was removed *in vacuo*, acidified with 1M HCl to pH 2 to 3 and extracted with EA. Then the organic phase was dried with Na_2SO_4, concentrated *in vacuo* to obtain the final product as pale yellow oil with quantitative conversion. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, 3H, J = 6.90 Hz), 1.24-1.37 (m, 12H), 1.41-1.48 (m, 2H), 1.74-1.81 (m, 2H), 3.39 (s, 3H), 3.54-3.58 (m, 2H), 3.63-3.70 (m, 12H), 3.71-3.75 (m, 2H), 3.86 (t, 2H, J = 4.70 Hz), 3.97 (t, 2H, J = 6.58 Hz), 4.17 (t, 2H, J = 4.70 Hz), 6.70 (t, 1H, J = 2.23 Hz), 7.24 (d, 2H, J = 8.45 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3, 22.9, 26.2, 29.3, 29.5, 29.6, 29.7, 29.8, 32.1, 59.2, 68.0, 68.6, 69.8, 70.6, 70.7, 70.78, 70.79, 70.83, 71.1, 72.1, 107.7, 108.4, 108.7, 131.3, 160.0, 160.4, 170.4; ESI-MS m/z calcd for $C_{28}H_{48}O_9$ $[M + Na]^+$: 551.32; found: 551.31.

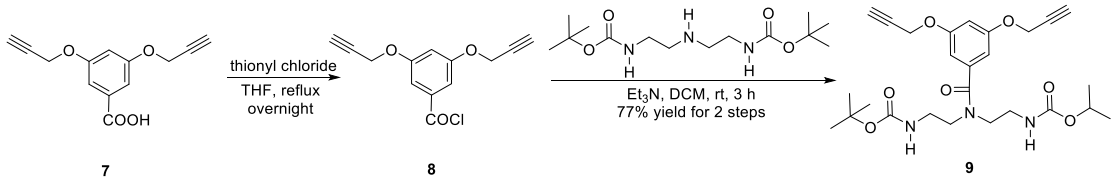


The mixture of compound **1** (1.96 g, 10 mmol), propargyl bromide (2.23 mL in 80% toluene, 20 mmol), 18-crown-6 (3.17 g, 12 mmol) and K_2CO_3 (4.15 g, 30 mmol) in 100 mL acetone were heated to reflux for overnight. When the reaction was monitored to be completed, K_2CO_3 was removed by filtration and the residue was concentrated to remove acetone. Then ethyl acetate was added and the solution was washed with brine. Finally, the organic phase was dried with Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel (hexane:EA = 10:1) to obtain the final product as light yellow solid (2.36 g) with 92% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.39 (t, 3H, J = 7.13 Hz), 2.55 (t, 2H, J = 2.38 Hz), 4.37 (q, 2H, J = 7.13 Hz), 4.72 (d, 4H, J = 2.35 Hz), 6.81 (t, 1H, J = 2.35 Hz), 7.30 (t, 2H, J = 2.40 Hz); ^{13}C NMR

(125 MHz, $CDCl_3$) δ 14.5, 56.3, 61.4, 76.1, 76.9, 77.2, 77.5, 78.1, 107.4, 109.1, 132.7, 158.7, 166.2; ESI-MS m/z calcd for $C_{15}H_{14}O_4$ $[M + Na]^+$: 281.08; found: 281.08.



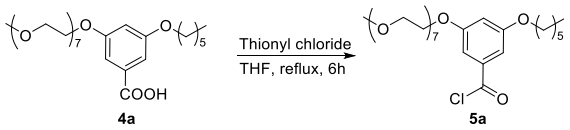
Compound **6** (2.34 g, 9.07 mmol) in 18 mL THF was mixed with $LiOH·H_2O$ (1.14 g, 27 mmol) in 8 mL H_2O and the solution was refluxed for 4h. After completion, THF was removed *in vacuo*, acidified with 1M HCl to pH 2 to 3 and extracted with EA. Then the organic phase was dried with Na_2SO_4, concentrated *in vacuo* to obtain the final product as pale yellow solid (2.04 g) with 98% yield. The obtained ^1H NMR is in accordance with the previous report.[14] ^1H NMR (400 MHz, DMSO-d_6) δ 3.60 (t, 2H, J = 2.34 Hz), 4.85 (d, 4H, J = 2.36 Hz), 6.85 (t, 1H, J = 2.36 Hz), 7.17 (d, 2H, J = 2.36 Hz), 13.10 (s, 1H).



Compound **7** (345 mg, 1.5 mmol) was dissolved into 5 mL THF, then thionyl chloride (1.09 mL, 15 mmol) was added and the mixture was heated to reflux for overnight. After completion, THF and excess amount of thionyl chloride was removed under reduced pressure then dried in *vacuo*. The compound **8** was obtained and used for the next step without further purification.

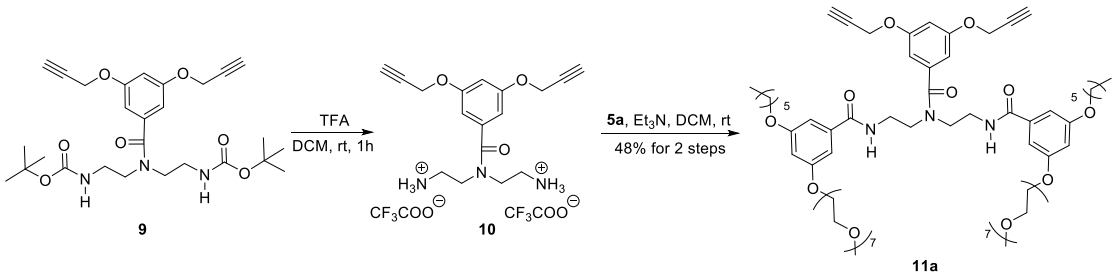
To the mixture of compound **8** and 1,7-Bis-Boc-1,4,7-triazaheptane (683 mg, 2.25 mmol) in 5 mL DCM was added triethylamine (936 μL, 6 mmol) dropwise under ice bath. After completion of the addition, the solution was warmed to room temperature and stirred for 3h. The final solution was extracted with EA and the organic phase was combined and dried with Na_2SO_4. Finally, it was concentrated *in vacuo* and purified by flash column on silica gel (DCM:MeOH = 30:1) to obtain the final product as yellow- brown

oil with 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 18H), 2.54 (t, 2H, *J* = 2.38 Hz), 3.10-3.75 (m, 8H), 4.69 (d, 4H, *J* = 2.36 Hz), 4.85 (s, 1H), 5.05 (s, 1H), 6.60 (d, 2H, *J* = 2.24 Hz), 6.65 (t, 1H, *J* = 2.28 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.6, 39.0, 39.3, 45.2, 49.7, 56.2, 76.2, 78.3, 79.7, 79.9, 103.6, 106.5, 138.5, 155.9, 156.6, 159.0, 172.3; ESI-MS m/z calcd for $C_{27}H_{37}N_3O_7$ $[M + Na]^+$: 538.25; found: 538.26.



4a **5a**

Thionyl chloride (1.5 mL, 20.6 mmol) and compound **4a** (654 mg, 1.24 mmol) was mixed into 3mL dry THF. The mixture was heated to reflux for 6 h. After completion of the reaction, the excess amount of THF and thionyl chloride were removed *in vacuo*. The crude product **5a** was used for next step without further purification.

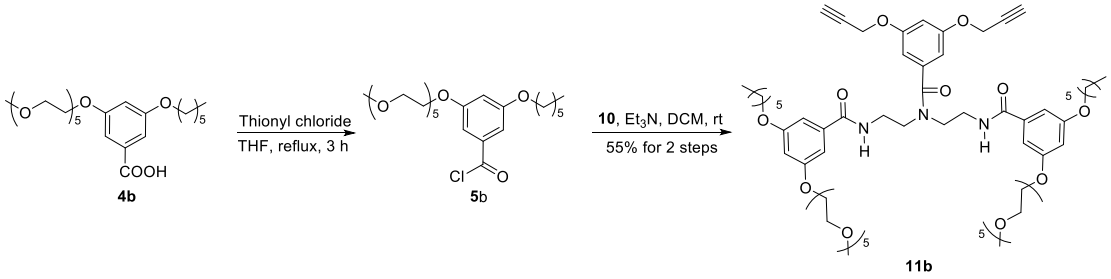


9 **10** **11a**

3.5 mL trifluoroacetic acid was mixed with compound **9** (335 mg, 0.65 mmol) in 9 mL DCM and the mixture was stirred at room temperature for 1 h. Then TFA and DCM were removed and the crude product **10** was directly used for next step without further purification.

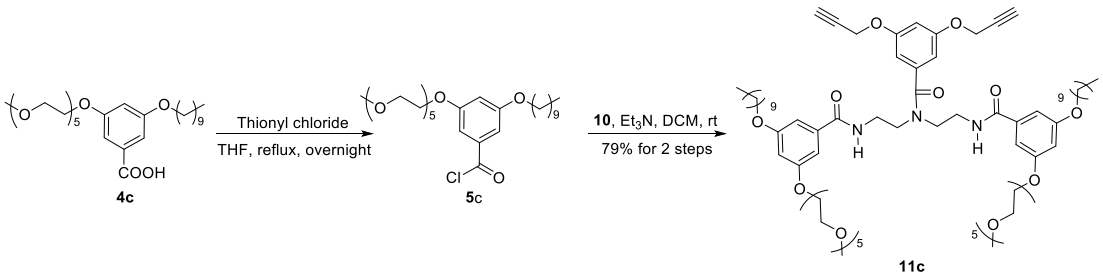
The mixture of compound **5** and **10** in dry DCM was cooled to 0 ℃ in ice bath for 5 min. Next, 0.72 mL Et_3N was added slowly into the mixture. Then the solution was warmed to room temperature to react for another 4 h. After completion, the mixture was extracted with brine, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel to obtain the final product as brown yellow oil (419 mg, 48% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, 6H, *J* = 6.74 Hz), 1.24-1.46 (m, 12H), 1.70-1.80 (m, 4H), 2.53 (t, 2H, *J* = 2.30 Hz), 3.37 (s, 6H), 3.51-3.71 (m, 52H), 3.77-3.99 (m, 12H),

4.04-4.19 (m, 4H), 4.47 (d, 4H, J = 2.32 Hz), 6.40 (d, 2H, J = 2.24 Hz), 6.58 (t, 3H, J = 2.16 Hz), 6.95 (s, 4H), 7.17 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.8, 25.8, 29.3, 31.7, 56.1, 59.2, 67.8, 67.9, 68.5, 69.8, 70.66, 70.72, 70.8, 70.9, 72.1, 76.3, 78.2, 104.0, 105.27, 105.30, 105.4, 105.5, 105.8, 106.0, 135.9, 136.2, 138.1, 158.9, 160.2, 160.6, 167.5, 168.1, 173.1; ESI-MS m/z calcd for $C_{73}H_{113}N_3O_{23}$ $[M + Na]^+$: 1422.77; found: 1422.46.



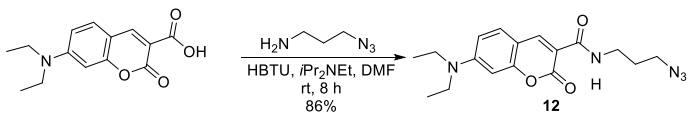
Thionyl chloride (0.36 mL, 4.9 mmol) and compound **4b** (231 mg, 0.49 mmol) was mixed into 2 mL dry THF. The mixture was heated to reflux for 3 h. After completion of the reaction, THF and excess amount of thionyl chloride were removed *in vacuo*. The crude product **5b** was used for next step without further purification.

The mixture of compound **5b** and **10** (0.95 equivalent) in 3 mL dry DCM was cooled to 0 ℃ in ice bath for 5 min. Next, 0.14 mL Et_3N was added slowly into the mixture. The solution was stirred at room temperature for another 8 h. After completion, the mixture was extracted with brine, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel to obtain the final product as brown yellow oil (174 mg, 55% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, 6H, J = 6.64 Hz), 1.25-1.46 (m, 12H), 1.68-1.76 (m, 4H), 2.50-2.55 (m, 2H), 3.36 (s, 6H), 3.51-3.71 (m, 36H), 3.76-3.99 (m, 12H), 4.05-4.18 (m, 4H), 4.46 (d, 4H, J = 2.16 Hz), 6.39 (d, 2H, J = 2.04 Hz), 6.54-6.62 (m, 3H), 6.95 (s, 4H), 7.06-7.20 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.8, 25.9, 29.3, 31.7, 56.1, 59.2, 67.8, 67.9, 68.5, 69.8, 70.67, 70.73, 70.8, 70.9, 72.1, 76.3, 78.2, 104.0, 105.31, 105.4, 105.6, 105.8, 106.0, 138.1, 158.9, 160.2, 160.6, 167.5, 168.1, 173.1; ESI-MS m/z calcd for $C_{65}H_{97}N_3O_{19}$ $[M + Na]^+$: 1246.66; found: 1246.40.



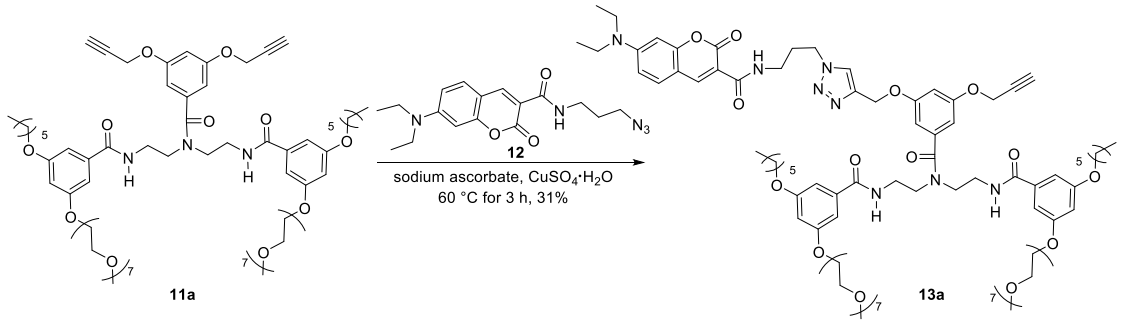
Thionyl chloride (0.73 mL, 10 mmol) and compound **4c** (548 mg, 1 mmol) was mixed into 3 mL dry THF. The mixture was heated to reflux for overnight. After completion of the reaction, THF and excess amount of thionyl chloride were removed *in vacuo*. The crude product **5c** was used for next step without further purification.

The mixture of compound **5c** and **10** (1 equivalent) in 10 mL dry DCM was cooled to 0 ℃ in ice bath for 5 min. Next, 0.84 mL Et_3N was added slowly into the mixture. Then the solution was stirred at room temperature for overnight. After completion, the mixture was extracted with brine, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel to obtain the final product as yellow-brown oil (573 mg) with 79% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 6H, J = 6.52 Hz), 1.23-1.45 (m, 28H), 1.64-1.80 (m, 4H), 2.54 (t, 2H, J = 2.22 Hz), 3.35 (s, 6H), 3.49-3.69 (m, 36H), 3.74-3.97 (m, 12H), 4.02-4.16 (m, 4H), 4.45 (s, 4H), 6.41 (d, 2H, J = 2.00 Hz), 6.52-6.60 (m, 3H), 6.95 (s, 4H), 7.32-7.46 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.7, 26.1, 29.2, 29.36, 29.44, 29.61, 29.63, 31.9, 55.9, 59.0, 67.6, 67.7, 68.3, 69.6, 70.5, 70.56, 70.59, 70.8, 71.9, 76.2, 76.9, 77.2, 77.4, 77.5, 78.1, 103.8, 105.0, 105.2, 105.5, 105.7, 106.0, 135.9, 136.2, 138.1, 158.8, 160.0, 160.4, 167.3, 168.0, 172.9; ESI-MS m/z calcd for $C_{73}H_{113}N_3O_{19}$ $[M + Na]^+$: 1358.79; found: 1358.50.



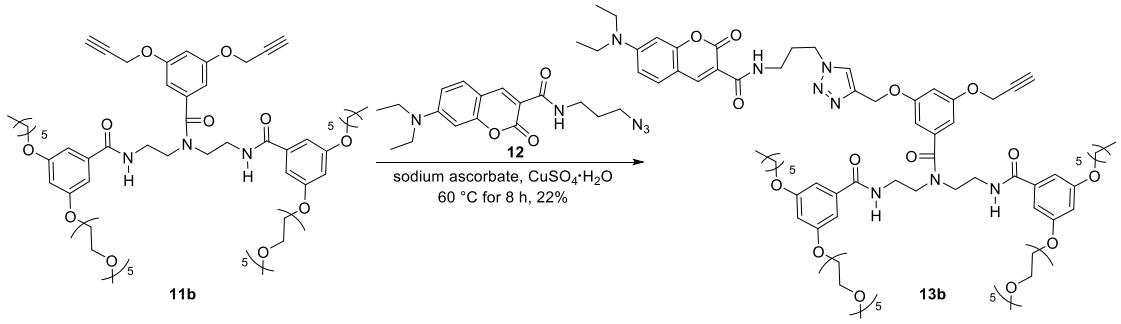
7-(Diethylamino)coumarin-3-carboxylic acid (523 mg, 2 mmol), 1-azidopropylamine (240 mg, 2.4 mmol) and HBTU (759 mg, 2 mmol) were dissolved into 20 mL DCM/DMF (4:1) mixed solvent. Then DIPEA (1.74 mL, 10 mmol) was added slowly into the mixture. After completion of the addition, the solution

was washed with 1M HCl and brine. Then the organic phase was dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel to obtain the final product **12** as yellow solid (587 mg, 86% yield). The obtained ^{1}H NMR is in accordance with the previous report.[15] ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24 (t, 6H, *J* = 7.12 Hz), 1.87-1.94 (m, 2H), 3.38-3.56 (m, 8H), 6.50 (d, 1H, *J* = 2.36 Hz), 6.65 (dd, 1H, J = 2.48, 9.00 Hz), 7.43 (d, 1H, *J* = 8.96 Hz), 8.68 (s, 1H), 8.92 (t, 1H, *J* = 5.12 Hz).

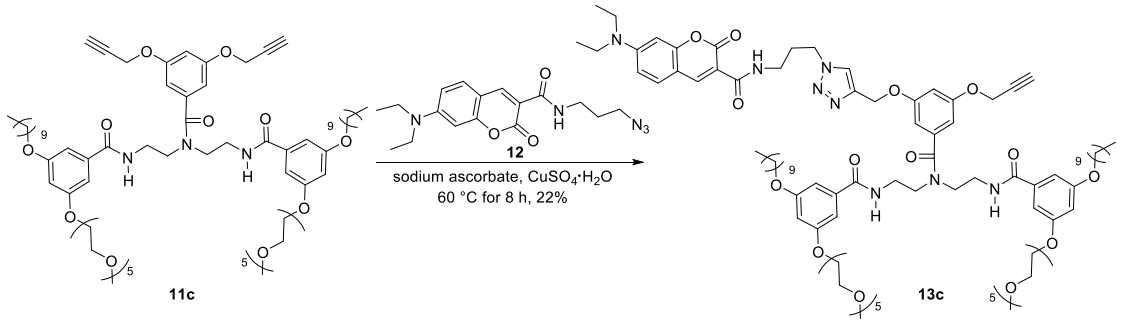


An aqueous solution (2 mL) of sodium ascorbate (11.3 mg, 0.057 mmol) and copper sulfate pentahydrate (14.2 mg, 0.057 mmol) was added into the THF solution (6 mL) of compound **11a** (80 mg, 0.057 mmol) and **12** (17.7 mg, 0.052 mmol). The mixture was heated to 60℃ for 3h and monitored to be completed. Then EA was added and the mixture was washed with brine. The organic phase was combined, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel to obtain the final product as pale yellow oil (30.9 mg, 31% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 6H, *J* = 6.62 Hz), 1.19-1.45 (m, 18H), 1.65-1.78 (m, 4H), 2.15-2.25 (m, 2H), 2.50 (t, 1H, *J* = 2.02 Hz), 3.36 (s, 6H), 3.42-3.49 (m, 6H), 3.50-3.57 (m, 6H), 3.59-3.70 (m, 48H), 3.80 (s, 6H), 3.86-3.96 (m, 6H), 4.10 (s, 4H), 4.38-4.50 (m, 4H), 5.07 (s, 2H), 6.37 (s, 1H), 6.49 (d, 1H, *J* = 1.84 Hz), 6.52-6.58 (m, 3H), 6.61 (s, 1H), 6.65 (dd, 1H, *J* = 2.12, 8.96 Hz), 6.97 (s, 4H), 7.27-7.34 (m, 1H), 7.42 (d, 1H, *J* = 8.96 Hz), 7.48-7.58 (m, 1H), 7.85 (s, 1H), 8.65 (s, 1H), 8.92 (t, 1H, *J* = 5.92 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.6, 14.2, 22.8, 25.8, 29.3, 29.9, 30.7, 31.7, 36.5, 45.3, 48.1, 56.0, 59.2, 61.7, 67.8, 68.5, 69.8, 70.65, 70.70, 70.74, 70.9, 72.1, 76.2, 78.2, 96.7, 104.6, 105.1, 105.2, 105.3, 105.6, 105.7, 106.0, 106.2, 108.5, 109.9, 110.3, 124.1, 131.4,

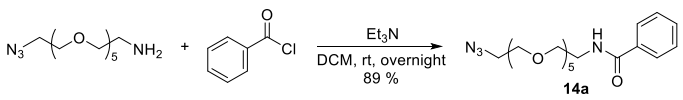
136.2, 136.3, 138.1, 143.5, 148.4, 152.9, 157.9, 159.0, 159.1, 160.05, 160.13, 160.5, 160.6, 162.9, 163.9, 167.7, 167.9, 173.2; ESI-MS m/z calcd for $C_{90}H_{134}N_8O_{26}$ $[M + Na]^+$: 1765.93; found: 1766.57.



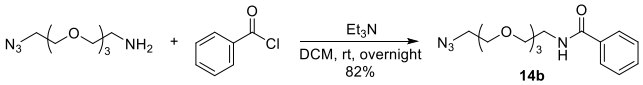
An aqueous solution (6 mL) of sodium ascorbate (28.6 mg, 0.14 mmol) and copper sulfate pentahydrate (36 mg, 0.14 mmol) was added into the THF solution (20 mL) of compound **11b** (160 mg, 0.13 mmol) and **12** (35.9 mg, 0.10 mmol). The mixture was heated to 60 ℃ for 8 h and monitored to be completed. Then EA was added and the mixture was washed with brine. The organic phase was combined, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel to obtain the final product as pale yellow oil (36 mg, 22% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 6H, *J* = 6.48 Hz), 1.21-1.44 (m, 18H), 1.66-1.77 (m, 4H), 2.15-2.26 (m, 2H), 2.48-2.52 (m, 1H), 3.36 (s, 6H), 3.40-3.49 (m, 6H), 3.50-3.56 (m, 6H), 3.58-3.71 (m, 32H), 3.80 (s, 6H), 3.86-3.96 (m, 6H), 4.10 (s, 4H), 4.40-4.49 (m, 4H), 5.07 (s, 2H), 6.37 (s, 1H), 6.47-6.51 (m, 1H), 6.52-6.58 (m, 3H), 6.61 (s, 1H), 6.65 (dd, 1H, *J* = 1.74, 8.94 Hz), 6.97 (s, 4H), 7.27-7.35 (m, 1H), 7.42 (d, 1H, *J* = 8.96 Hz), 7.48-7.60 (m, 1H), 7.85 (s, 1H), 8.65 (s, 1H), 8.92 (t, 1H, *J* = 5.90 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.6, 14.2, 22.8, 25.8, 29.3, 29.9, 30.8, 31.7, 36.5, 45.3, 48.1, 56.0, 59.2, 61.7, 67.8, 68.5, 69.8, 70.6, 70.7, 70.9, 72.1, 76.2, 78.2, 96.7, 104.6, 105.1, 105.25, 105.34, 105.7, 105.8, 106.0, 106.2, 108.5, 109.9, 110.3, 124.1, 131.4, 136.2, 136.3, 138.1, 143.5, 148.4, 152.9, 157.9, 159.0, 159.1, 160.1, 160.2, 160.5, 160.6, 163.0, 163.9, 167.7, 167.9, 173.2; ESI-MS m/z calcd for $C_{82}H_{118}N_8O_{22}$ $[M + Na]^+$: 1589.83; found: 1589.51.



An aqueous solution (3 mL) of sodium ascorbate (27 mg, 0.14 mmol) and copper sulfate pentahydrate (34 mg, 0.14 mmol) was added into the THF solution (8 mL) of compound **11c** (164 mg, 0.12 mmol) and **12** (42 mg, 0.12 mmol). The mixture was heated to 60 ℃ for 8 h and monitored to be completed. Then EA was added and the mixture was washed with brine. The organic phase was combined, dried with anhydrous Na_2SO_4, concentrated *in va cuo* and purified by flash column on silica gel to obtain the final product as pale yellow oil (45 mg, 22% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (t, 6H, *J* = 6.93 Hz), 1.22-1.32 (m, 30H), 1.35-1.42 (m, 4H), 1.66-1.76 (m, 4H), 2.16-2.25 (m, 2H), 2.50 (t, 1H, *J* = 2.28 Hz), 3.36 (s, 6H), 3.42-3.48 (m, 6H), 3.50-3.56 (m, 6H), 3.57-3.73 (m, 32H), 3.80 (s, 6H), 3.86-3.96 (m, 6H), 4.10 (s, 4H), 4.38-4.50 (m, 4H), 5.07 (s, 2H), 6.33-6.40 (m, 1H), 6.48-6.57 (m, 4H), 6.61 (s, 1H), 6.65 (dd, 1H, *J* = 2.35, 9.00 Hz), 6.90-7.02 (m, 4H), 7.42 (d, 1H, *J* = 8.95 Hz), 7.48-7.58 (m, 1H), 7.85 (s, 1H), 8.65 (s, 1H), 8.92 (t, 1H, *J* = 6.00 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.6, 14.3, 22.9, 26.2, 29.4, 29.5, 29.6, 29.75, 29.76, 30.8, 32.0, 36.5, 45.3, 48.1, 56.0, 59.2, 61.7, 67.8, 68.5, 69.8, 70.66, 70.71, 70.73, 70.8, 70.9, 72.1, 76.2, 78.2, 96.7, 104.6, 105.1, 105.25, 105.31, 105.66, 105.72, 106.0, 106.2, 108.5, 109.9, 110.3, 124.1, 131.4, 136.2, 136.3, 138.0, 143.5, 148.4, 152.9, 157.9, 159.0, 159.05, 159.08, 160.1, 160.2, 160.5, 160.6, 163.0, 164.0, 167.7, 167.9, 173.3; ESI-MS m/z calcd for $C_{90}H_{134}N_8O_{22}$ $[M + Na]^+$: 1701.95; found: 1702.61.



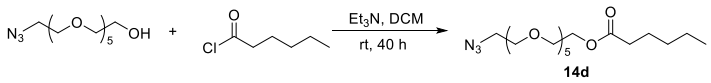
To a solution of azido-PEG5-amine (460 mg, 1.5 mmol) in 5 mL dry DCM, benzoyl chloride (174 μL, 1.5 mmol) and Et_3N (418 μL, 3 mmol) were dropped subsequently. The mixture was stirred at room temperature for overnight. When the reaction was completed, EA was added and the solution was washed with brine. Then the organic phase was combined, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel to obtain the final product as colorless oil (550 mg, 89% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 3.36 (t, 2H, J = 5.02 Hz), 3.60-3.69 (m, 22H), 7.03 (s, 1H), 7.40-7.52 (m, 3H), 7.80-7.85 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 39.9, 50.8, 70.1, 70.2, 70.4, 70.67, 70.69, 70.74, 70.79, 70.80, 127.8, 128.6, 131.5, 134.8, 167.7; ESI-MS m/z calcd for $C_{19}H_{30}N_4O_6$ $[M + Na]^+$: 433,21; found: 433.19.



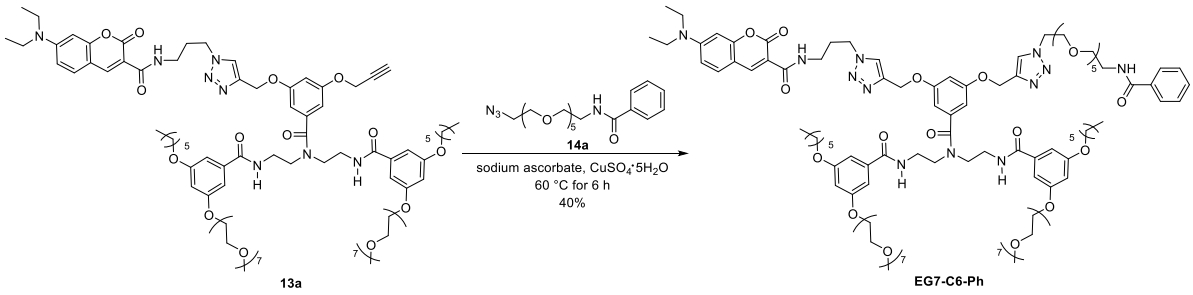
To a solution of azido-PEG3-amine (655 mg, 3 mmol) in 5 mL dry DCM, benzoyl chloride (348 μL, 3 mmol) and Et_3N (627 μL, 4.5 mmol) were dropped subsequently. The mixture was stirred at room temperature for overnight. When the reaction was completed, EA was added and the solution was washed with brine. Then the organic phase was combined, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel to obtain the final product as colorless oil (798 mg, 82% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 3.34 (t, 2H, J = 5.02 Hz), 3.60-3.70 (m, 14H), 6.76 (s, 1H), 7.40-7.46 (m, 2H), 7.47-7.52 (m, 1H), 7.78-7.82 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 39.9, 50.8, 70.0, 70.2, 70.4, 70.7, 70.77, 70.81, 127.2, 128.6, 131.5, 134.8, 167.7; ESI-MS m/z calcd for $C_{15}H_{22}N_4O_4$ $[M + Na]^+$: 345.15; found: 345.14.

To a solution of azido-PEG5-amine (184 mg, 0.6 mmol) in 5 mL dry DCM, 2-naphthoyl chloride (95 mg, 0.5 mmol) and Et_3N (140 μL, 1 mmol) were dropped subsequently. The mixture was stirred at room

temperature for 4 h. When the reaction was completed, EA was added and the solution was washed with brine. Then the organic phase was combined, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel to obtain the final product as colorless oil (180 mg, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.34 (t, 2H, *J* = 5.02 Hz), 3.57-3.74 (m, 22H), 7.05 (s, 1H), 7.50-7.58 (m, 2H), 7.85-7.95 (m, 4H), 8.35 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.1, 50.8, 70.1, 70.2, 70.5, 70.73, 70.74, 70.79, 70.80, 124.0, 126.8, 127.72, 127.73, 127.9, 128.5, 129.1, 132.0, 132.8, 134.9, 167.7; ESI-MS m/z calcd for $C_{23}H_{32}N_4O_6$ $[M + Na]^+$: 483.22; found: 483.20.

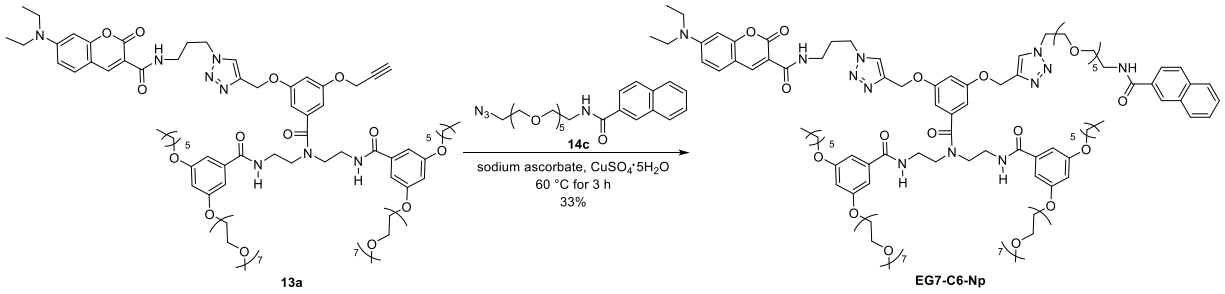


To a solution of 17-azido-3,6,9,12,15-pentaoxaheptadecan-1-ol (152 mg, 0.5 mmol) in 2.5 mL dry DCM, hexanoyl chloride (70 μL, 0.5 mmol) and Et_3N (140 μL, 1 mmol) were dropped subsequently. The mixture was stirred at room temperature for 40 h. When the reaction was completed, EA was added and the solution was washed with brine. Then the organic phase was combined, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel to obtain the final product as colorless oil (45 mg, 22% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, 3H, *J* = 6.96 Hz), 1.25-1.36 (m, 4H), 1.58-1.67 (m, 2H), 2.33 (t, 2H, *J* = 7.56 Hz), 3.39 (t, 2H, *J* = 5.08 Hz), 3.63-3.72 (m, 20H), 4.23 (t, 2H, *J* = 4.86 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.4, 24.7, 31.4, 34.3, 50.8, 63.5, 69.3, 70.2, 70.69, 70.71, 70.76, 70.80, 70.83, 174.0. ESI-MS m/z calcd for $C_{18}H_{35}N_3O_7$ $[M + Na]^+$: 428.24; found: 428.23.



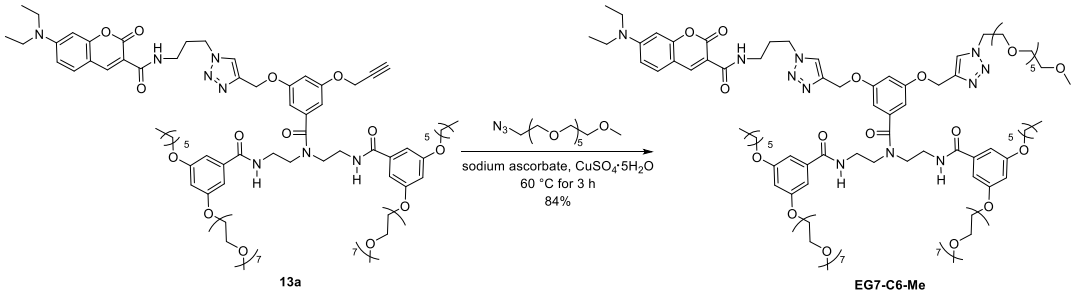
An aqueous solution (1 mL) of sodium ascorbate (5.7 mg, 0.029 mmol) and copper sulfate pentahydrate (7.2 mg, 0.029 mmol) was added into the THF solution (2 mL) of compound **13a** (25 mg, 0.014 mmol)

and **14a** (12 mg, 0.029 mmol). The mixture was heated to 60℃ for 6h and monitored to be completed. Then EA was added and the mixture was washed with brine. The organic phase was combined, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel to obtain the final product as pale yellow oil (12.4 mg, 40% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85-0.90 (m, 6H), 1.20-1.32 (m, 14H), 1.35-1.42 (m, 4H), 1.65-1.78 (m, 4H), 2.16-2.26 (m, 2H), 3.37 (s, 6H), 3.41-3.49 (m, 6H), 3.50-3.56 (m, 12H), 3.57-3.69 (m, 56H), 3.76-3.94 (m, 12H), 4.08 (s, 4H), 4.43 (t, 2H, *J* = 6.74 Hz), 4.50 (t, 2H, *J* = 4.84 Hz), 5.00-5.13 (m, 4H), 6.48-6.58 (m, 5H), 6.65 (dd, 1H, *J* = 2.08, 8.92 Hz), 6.94-7.04 (m, 4H), 7.07 (s, 1H), 7.37-7.49 (m, 4H), 7.73 (s, 1H), 7.78-7.84 (m, 3H), 8.66 (s, 1H), 8.92 (t, 1H, *J* = 5.86 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.6, 14.2, 22.8, 25.8, 29.3, 29.6, 29.9, 30.8, 31.7, 32.1, 36.6, 40.0, 45.3, 48.1, 50.5, 56.2, 59.2, 61.7, 67.8, 68.5, 70.0, 70.4, 70.6, 70.67, 70.72, 96.7, 104.7, 105.0, 105.3, 105.4, 105.7, 105.8, 106.0, 106.3, 108.5, 110.0, 110.3, 124.0, 124.7, 127.3, 128.6, 131.4, 131.5, 134.8, 136.3, 136.4, 138.1, 143.46, 143.48, 148.4, 152.9, 157.9, 159.4, 160.0, 160.2, 160.4, 160.6, 163.0, 163.9, 167.7, 167.8, 173.2; ESI-MS m/z calcd for $C_{109}H_{164}N_{12}O_{32}$ $[M + Na]^+$: 2176.15; found: 2176.79.



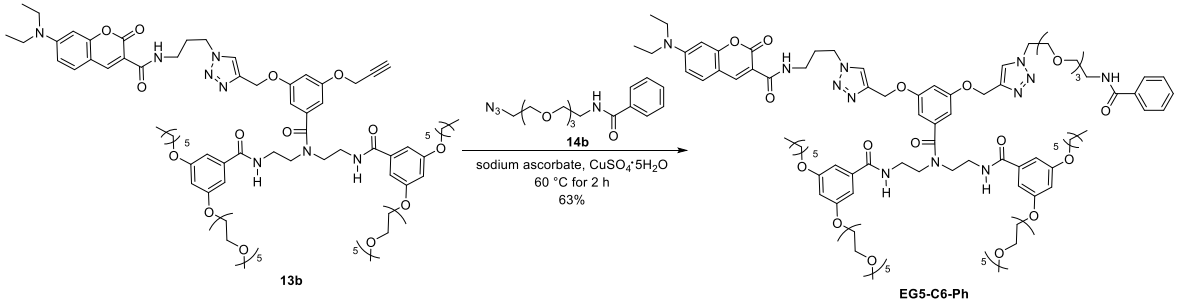
An aqueous solution (1 mL) of sodium ascorbate (3 mg, 0.015 mmol) and copper sulfate pentahydrate (3.8 mg, 0.015 mmol) was added into the THF solution (2 mL) of compound **13a** (13.2 mg, 0.0076 mmol) and **14c** (7 mg, 0.015 mmol). The mixture was heated to 60 ℃ for 3 h and monitored to be completed. Then EA was added and the mixture was washed with brine. The organic phase was combined, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel to obtain the final product as pale yellow oil (5.6 mg, 33% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85-0.90 (m, 6H), 1.20-1.32 (m, 14H), 1.34-1.42 (m, 4H), 1.65-1.73 (m, 4H), 2.14-2.25 (m, 2H), 3.36 (s, 6H), 3.41-3.58 (m, 22H),

3.59-3.70 (m, 58H), 3.74-3.82 (m, 8H), 3.84-3.96 (m, 6H), 4.08 (s, 4H), 4.37-4.52 (m, 4H), 5.01 (d, 4H, *J* = 7.20 Hz), 5.12 (s, 2H), 6.48-6.58 (m, 6H), 6.65 (dd, 1H, *J* = 2.32, 9.00 Hz), 6.94-7.04 (m, 4H), 7.33-7.45 (m, 3H), 7.47-7.57 (m, 2H), 7.68-7.92 (m, 7H), 8.36 (s, 1H), 8.65 (s, 1H), 8.92 (t, 1H, *J* = 5.94 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.6, 14.2, 22.8, 22.9, 25.8, 29.3, 29.5, 29.8, 29.9, 30.8, 31.7, 32.1, 36.6, 40.1, 45.3, 48.1, 50.4, 56.2, 59.2, 61.7, 67.8, 68.4, 69.4, 69.8, 70.1, 70.4, 70.5, 70.57, 70.62, 70.65, 70.70, 70.9, 72.1, 96.7, 104.7, 105.0, 105.2, 105.4, 105.7, 105.8, 106.0, 106.3, 108.5, 109.9, 110.3, 124.0, 124.1, 124.7, 126.8, 127.7, 127.8, 127.9, 128.4, 129.1, 131.4, 132.0, 132.8, 134.8, 136.3, 136.4, 138.1, 143.4, 148.4, 152.9, 157.9, 159.4, 160.0, 160.1, 160.4, 160.5, 162.9, 163.9, 167.0, 167.8, 173.2; ESI-MS m/z calcd for $C_{113}H_{116}N_{12}O_{32}$ $[M + Na]^+$: 2226.16; found: 2226.49.

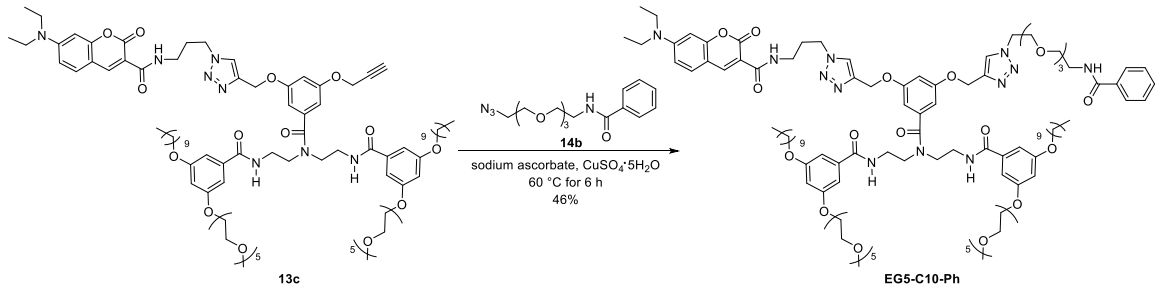


An aqueous solution (1 mL) of sodium ascorbate (4.5 mg, 0.023 mmol) and copper sulfate pentahydrate (5.7 mg, 0.023 mmol) was added into the THF solution (2 mL) of compound **13a** (20 mg, 0.012 mmol) and 19-azido-2,5,8,11,14,17-hexaoxanonadecane (7.4 mg, 0.023 mmol). The mixture was heated to 60 ℃ for 3 h and monitored to be completed. Then EA was added and the mixture was washed with brine. The organic phase was combined, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel to obtain the final product as pale yellow oil (20 mg, 84% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85-0.90 (m, 6H), 1.23-1.32 (m, 14H), 1.35-1.42 (m, 4H), 1.65-1.75 (m, 4H), 2.15-2.26 (m, 2H), 3.35 (s, 3H), 3.36 (s, 6H), 3.41-3.55 (m, 12H), 3.56-3.69 (m, 58H), 3.75-3.97 (m, 13H), 4.08 (s, 4H), 4.44 (t, 2H, *J* = 6.72 Hz), 4.50 (t, 2H, *J* = 4.88 Hz), 5.03 (d, 2H, J = 8.88 Hz), 5.11 (s, 2H), 6.47-6.59 (m, 5H), 6.65 (dd, 1H, *J* = 2.28, 8.96 Hz), 6.93-7.05 (m, 4H), 7.36 (s, 1H), 7.42 (d, 1H, *J* = 8.96 Hz), 7.69 (s, 1H), 7.82 (d, 2H, *J* = 2.40 Hz), 8.66 (s, 1H), 8.92 (t, 1H, *J* = 5.92 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ

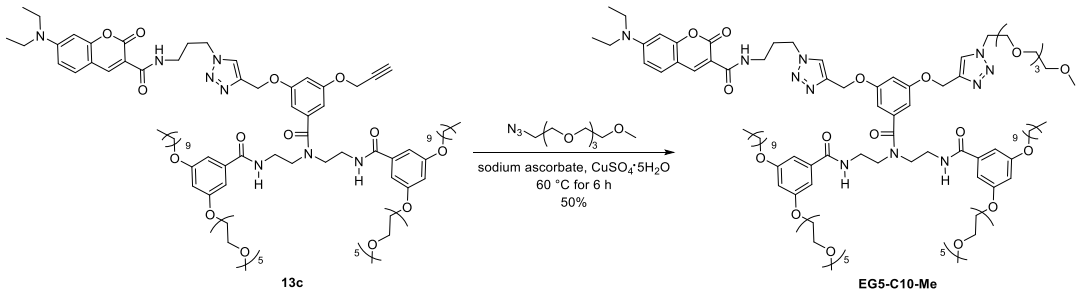
12.6, 14.2, 22.8, 22.9, 25.8, 29.8, 29.9, 31.7, 32.1, 36.6, 45.2, 48.1, 50.5, 56.2, 59.18, 59.20, 61.7, 67.8, 68.5, 69.5, 69.8, 70.6, 70.7, 70.9, 72.08, 72.10, 96.7, 104.8, 105.0, 105.3, 105.4, 105.7, 105.8, 105.9, 106.0, 106.3, 108.5, 110.0, 110.3, 124.0, 124.7, 131.4, 136.3, 136.4, 138.1, 143.5, 148.4, 152.9, 157.9, 159.36, 159.39, 160.0, 160.2, 160.4, 160.6, 163.0, 163.9, 167.0, 167.8, 173.3; ESI-MS m/z calcd for $C_{103}H_{161}N_{11}O_{32}$ $[M + Na]^+$: 2087.12; found: 2087.68.



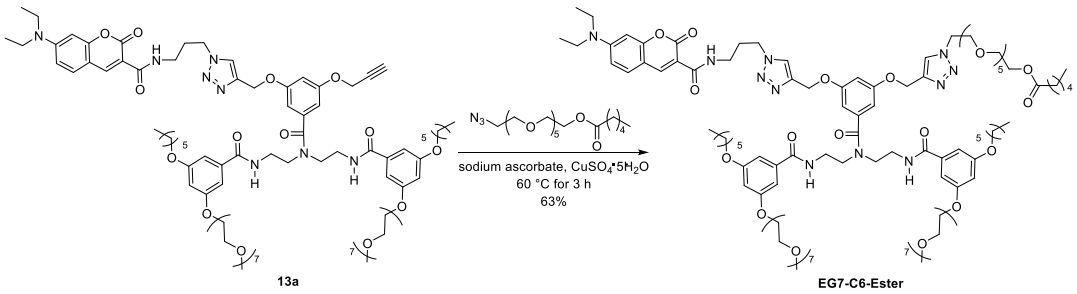
An aqueous solution (1 mL) of sodium ascorbate (6.1 mg, 0.031 mmol) and copper sulfate pentahydrate (7.6 mg, 0.031 mmol) was added into the THF solution (2 mL) of compound **13b** (24 mg, 0.015 mmol) and **14b** (10 mg, 0.031 mmol). The mixture was heated to 60°C for 2 h and monitored to be completed. Then EA was added and the mixture was washed with brine. The organic phase was combined, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel to obtain the final product as pale yellow oil (18.3 mg, 63% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85-0.90 (m, 6H), 1.21-1.33 (m, 14H), 1.35-1.43 (s, 4H), 1.65-1.73 (m, 4H), 2.15-2.24 (m, 2H), 3.36 (s, 6H), 3.42-3.69 (m, 58H), 3.75-3.83 (m, 8H), 3.85-3.96 (m, 6H), 4.08 (s, 4H), 4.40-4.48 (m, 4H), 5.00 (d, 4H, J = 3.08 Hz), 5.12 (s, 1H), 6.48-6.50 (m, 1H), 6.54 (s, 4H), 6.65 (dd, 1H, J = 2.04, 8.96 Hz), 6.95-7.03 (m, 5H), 7.33-7.49 (m, 5H), 7.73-7.80 (m, 4H), 7.82 (s, 1H), 8.66 (s, 1H), 8.93 (t, 1H, J = 5.92 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.6, 14.2, 22.8, 25.8, 29.3, 29.9, 30.8, 31.7, 36.6, 39.9, 45.3, 48.1, 50.4, 56.2, 59.2, 61.7, 61.8, 67.8, 68.5, 69.4, 69.8, 69.9, 70.3, 70.5, 70.60, 70.62, 70.64, 70.69, 70.71, 70.73, 70.9, 72.1, 96.7, 104.6, 105.0, 105.2, 105.4, 105.7, 105.78, 105.83, 106.0, 106.3, 108.5, 109.9, 110.3, 124.1, 127.2, 128.6, 131.4, 131.6, 134.7, 136.3, 136.4, 138.1, 143.4, 143.5, 148.4, 152.9, 157.9, 159.4, 160.0, 160.1, 160.4, 160.5, 163.0, 163.9, 167.7, 167.8, 173.2; ESI-MS m/z calcd for $C_{97}H_{140}N_{12}O_{26}$ $[M + Na]^+$: 1911.99; found: 1912.61.



An aqueous solution (1 mL) of sodium ascorbate (3.4 mg, 0.017 mmol) and copper sulfate pentahydrate (4.3 mg, 0.017 mmol) was added into the THF solution (2 mL) of compound **13c** (14.1 mg, 0.086 mmol) and **14b** (5.5 mg, 0.017 mmol). The mixture was heated to 60℃ for 6 h and monitored to be completed. Then EA was added and the mixture was washed with brine. The organic phase was combined, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel to obtain the final product as pale yellow oil (8 mg, 46% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86-0.90 (m, 6H), 1.21-1.31 (m, 30H), 1.34-1.43 (m, 4H), 1.65-1.73 (m, 4H), 2.15-2.24 (m, 2H), 3.36 (s, 6H), 3.42-3.70 (m, 56H), 3.74-3.95 (m, 16H), 4.07 (s, 4H), 4.40 -4.50 (m, 4H), 4.98-5.13 (m, 4H), 6.48-6.56 (m, 5H), 6.65 (dd, 1H, J = 2.10, 8.90 Hz), 6.95-7.04 (m, 5H), 7.32-7.48 (m, 5H), 7.72-7.84 (m, 4H), 8.66 (s, 1H), 8.93 (t, 1H, J = 5.80 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.6, 14.3, 22.9, 26.2, 29.4, 29.5, 29.6, 29.75, 29.77, 29.9, 30.8, 32.1, 36.6, 39.9, 45.3, 48.1, 50.4, 56.2, 59.2, 61.7, 61.8, 67.8, 68.5, 69.4, 69.8, 69.9, 70.3, 70.5, 70.6, 70.66, 70.71, 70.73, 70.8, 70.9, 72.1, 96.7, 104.7, 105.0, 105.2, 105.4, 105.7, 105.78, 105.84, 106.0, 106.3, 108.5, 109.9, 110.2, 124.1, 124.6, 127.2, 128.6, 131.4, 131.6, 134.7, 136.3, 136.4, 138.1, 143.4, 143.5, 148.4, 152.9, 157.9, 159.35, 159.36, 160.0, 160.2, 160.4, 160.6, 163.0, 163.9, 167.7, 167.8, 173.2; ESI-MS m/z calcd for $C_{105}H_{156}N_{12}O_{26}$ $[M + Na]^+$: 2024.11; found: 2024.79.



An aqueous solution (1 mL) of sodium ascorbate (3.6 mg, 0.018 mmol) and copper sulfate pentahydrate (4.6 mg, 0.018 mmol) was added into the THF solution (2 mL) of compound **13c** (16 mg, 0.0092 mmol) and 13-azido-2,5,8,11-tetraoxatridecane (4.3 mg, 0.018 mmol). The mixture was heated to 60℃ for 6 h and monitored to be completed. Then EA was added and the mixture was washed with brine. The organic phase was combined, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel to obtain the final product as pale yellow oil (9.2 mg, 50% yield). ^{1}H NMR (500 MHz, CDCl3) δ 0.86-0.90 (m, 6H), 1.21-1.32 (m, 30H), 1.34-1.42 (m, 4H), 1.66-1.74 (m, 4H), 2.17-2.24 (m, 2H), 3.33 (s, 3H), 3.36 (s, 6H), 3.43-3.70 (m, 56H), 3.75-3.97 (m, 16H), 4.09 (s, 4H), 4.44 (t, 2H, J = 6.80 Hz), 4.53 (t, 2H, J = 5.03 Hz), 5.03 (d, 4H, J = 11.6 Hz), 5.12 (s, 2H), 6.49 (d, 1H, J = 2.2 Hz), 6.54-6.59 (m, 4H), 6.65 (dd, 1H, J = 2.40, 9.00 Hz), 6.95-7.04 (m, 4H), 7.35 (s, 1H), 7.43 (d, 1H, J = 9.0 Hz), 7.70 (s, 1H), 7.82 (d, 2H, J = 4.5 Hz), 8.66 (s, 1H), 8.93 (t, 1H, J = 6.00 Hz); ^{13}C NMR (125 MHz, CDCl3) δ 12.6, 14.3, 22.9, 26.2, 29.36, 29.39, 29.5, 29.55, 29.59, 29.76, 29.77, 29.84, 29.9, 30.8, 32.1, 36.6, 39.9, 45.3, 48.1, 50.5, 56.2, 59.16, 59.20, 61.7, 67.8, 68.5, 69.5, 69.77, 69.80, 70.6, 70.66, 70.71, 70.8, 70.9, 72.07, 72.09, 96.7, 104.9, 105.0, 105.3, 105.4, 105.7, 105.8, 105.9, 106.0, 106.3, 108.5, 110.0, 110.3, 124.0, 124.7, 131.4, 136.3, 136.4, 138.1, 143.48, 143.51, 148.4, 152.9, 157.9, 159.35, 159.38, 160.0, 160.2, 160.4, 160.6, 163.0, 163.9, 167.0, 167.8, 173.3; ESI-MS m/z calcd for $C_{99}H_{153}N_{11}O_{26}$ $[M + Na]^+$: 1935.09; found: 1935.67.



An aqueous solution (1 mL) of sodium ascorbate (3.2 mg, 0.016 mmol) and copper sulfate pentahydrate (4 mg, 0.016 mmol) was added into the THF solution (2 mL) of compound **13c** (16 mg, 0.008 mmol) and **14d** (6.5 mg, 0.016 mmol). The mixture was heated to 60℃ for 3 h and monitored to be completed. Then EA was added and the mixture was washed with brine. The organic phase was combined, dried with

anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel to obtain the final product as pale yellow oil (10.8 mg, 63% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85-0.90 (m, 9H), 1.20-1.34 (m, 24H), 1.36-1.43 (m, 4H), 1.54-1.66 (m, 4H), 1.68-1.76 (m, 4H), 2.17-2.24 (m, 2H), 2.31 (t, 2H, *J* = 7.56 Hz), 3.37 (s, 6H), 3.41-3.56 (m, 12H), 3.57-3.72 (m, 62H), 3.75-3.95 (m, 14H), 4.06-4.12 (m, 4H), 4.20 (t, 2H, *J* = 4.86 Hz), 4.44 (t, 2H, *J* = 6.76 Hz), 4.53 (t, 2H, *J* = 5.02 Hz), 5.03 (d, 2H, *J* = 8.36 Hz), 5.12 (s, 1H), 6.48-6.59 (m, 5H), 6.65 (dd, 1H, *J* = 2.32, 9.00 Hz), 6.91-7.03 (m, 4H), 7.37-7.45 (m, 2H), 7.72 (s, 1H), 7.80-7.85 (m, 2H), 8.66 (s, 1H), 8.89-8.98 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.6, 14.1, 14.2, 14.3, 22.5, 22.8, 22.9, 24.8, 25.8, 29.3, 29.5, 29.9, 30.8, 31.5, 31.7, 32.1, 34.3, 36.6, 45.3, 48.1, 50.5, 56.0, 59.2, 61.7, 63.5, 67.8, 68.5, 69.4, 69.5, 69.8, 70.7, 70.9, 72.1, 96.7, 104.8, 105.0, 105.4, 105.7, 105.8, 105.9, 106.0, 106.3, 108.5, 109.9, 110.3, 124.0, 124.7, 131.4, 136.4, 138.1, 143.5, 148.4, 152.9, 157.9, 159.4, 160.0, 160.2, 160.6, 163.0, 164.0, 167.0, 167.8, 173.3, 174.0; ESI-MS m/z calcd for $C_{108}H_{169}N_{11}O_{33}$ $[M + Na]^+$: 2171.18; found: 2171.75.

4. Supporting figures and tables: Experimental data

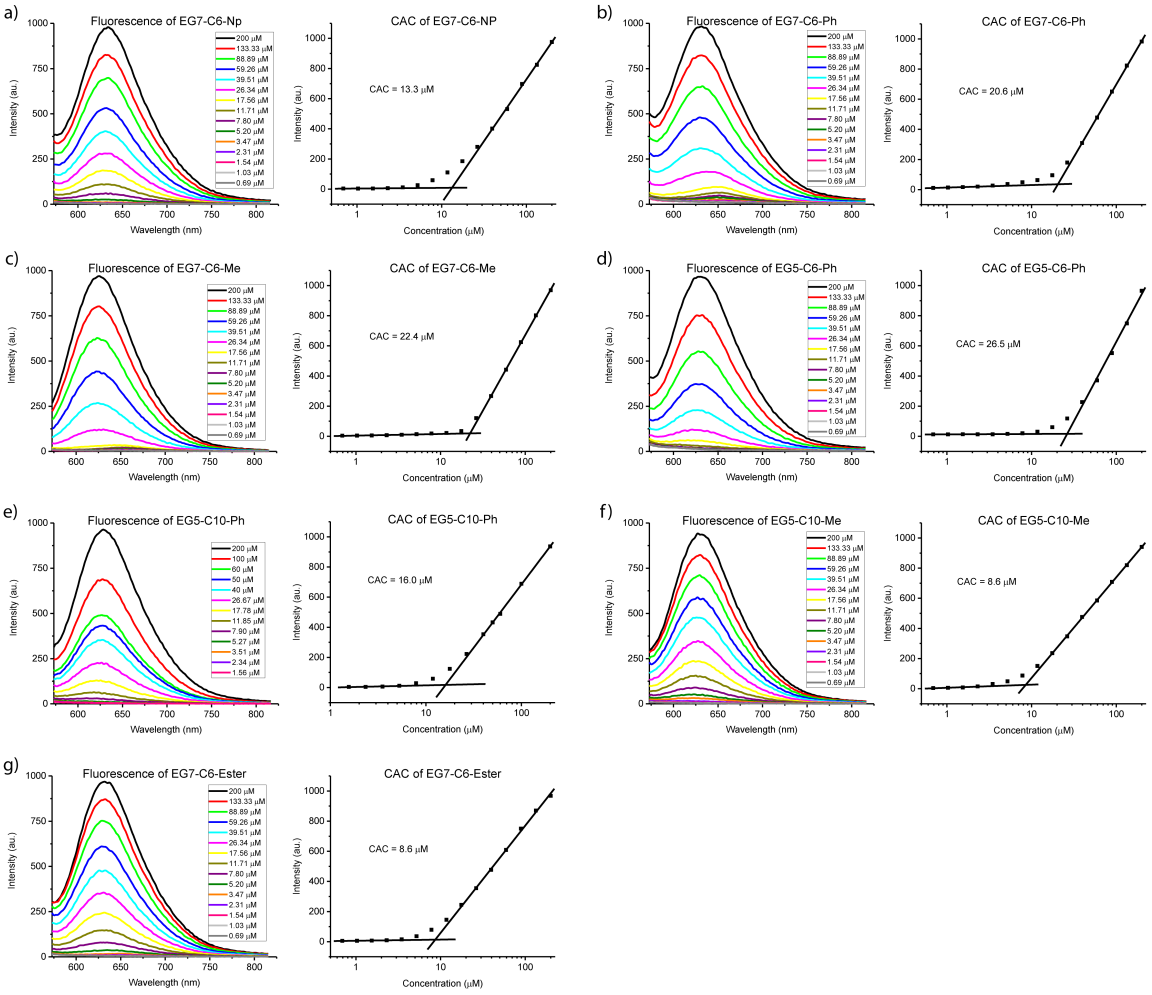


Figure S1. CAC of oligomers: (a) **EG7-C6-Np**, (b) **EG7-C6-Ph**, (c) **EG7-C6-Me**, (d) **EG5-C6-Ph**, (e) **EG5-C10-Ph**, (f) **EG5-C10-Me**, (g) **EG7-C6-Ester**.

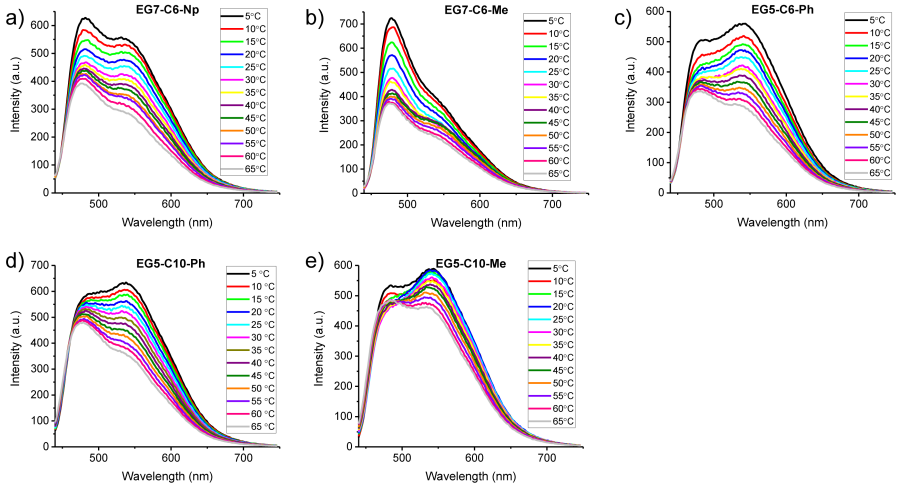


Figure S2. Temperature-dependent fluorescence of oligomers (25 μM): (a) **EG7-C6-Np**, (b) **EG7-C6-Me**, (c) **EG5-C6-Ph**, (d) **EG5-C10-Ph**, (e) **EG5-C10-Me**.

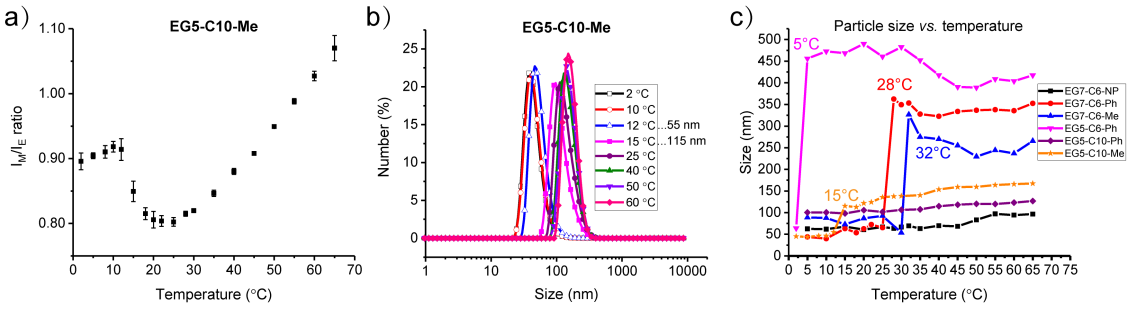


Figure S3. (a) I_M/I_E ratio of **EG5-C10-Me** at different temperature, (b) particle size of **EG5-C10-Me** at different temperature, (c) plots of particle sizes for assemblies at different temperature.

Table S1. Size and DTP of different oligomers

Oligomer	Sub-LCST	DTP
EG7-C6-NP	NA	22 °C
EG7-C6-Ph	28 °C	30 °C
EG7-C6-Me	32 °C	55 °C
EG5-C6-Ph	5 °C	15 °C
EG5-C10-Ph	NA	NA
EG5-C10-Me	15 °C	25 °C

5. Supporting figures and tables: Additional modelling data

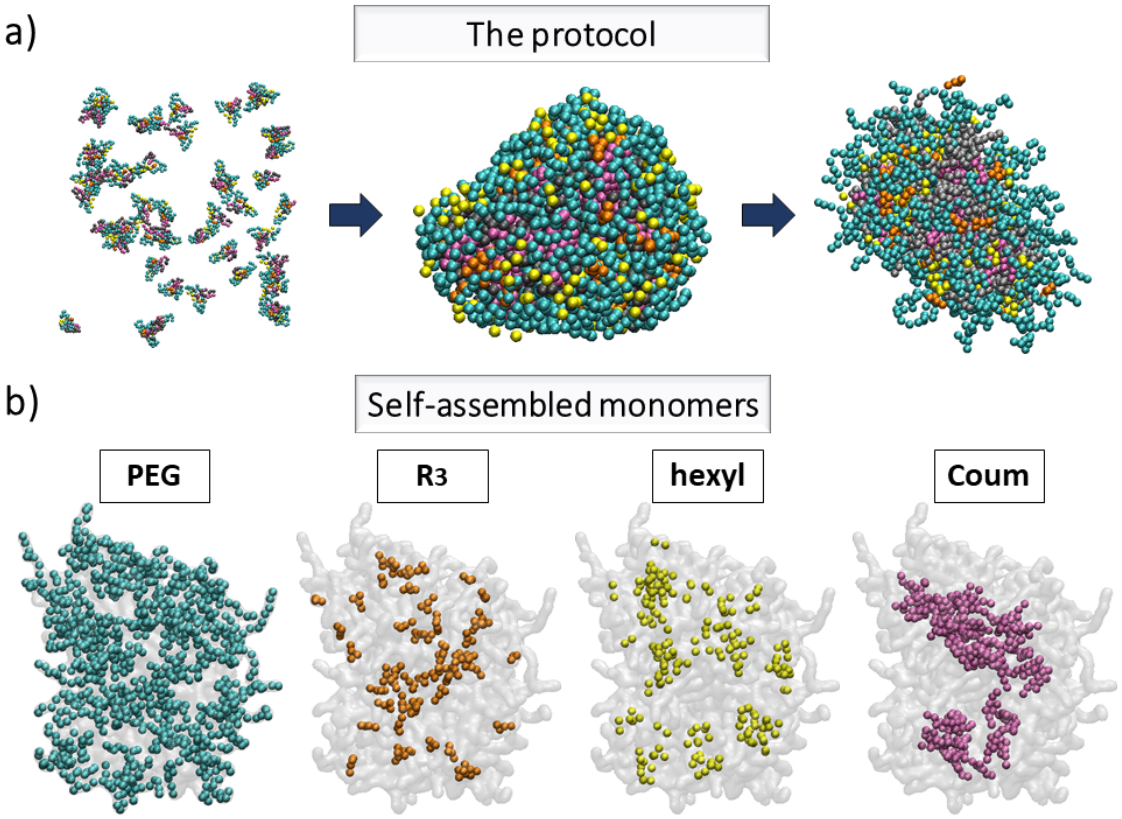


Figure S4. (a) CG-MD snapshots of 50 **EG7-C6-Ph** monomers (most left side) and the corresponding self-assembly in water (most right side). (b) CG-MD snapshot of the self-assembled EG7-C6-Ph monomers where the CG beads of the PEG, R_3 (Ph), hexyl, and Coumarin groups are colored in cyan, orange, yellow, and purple respectively.

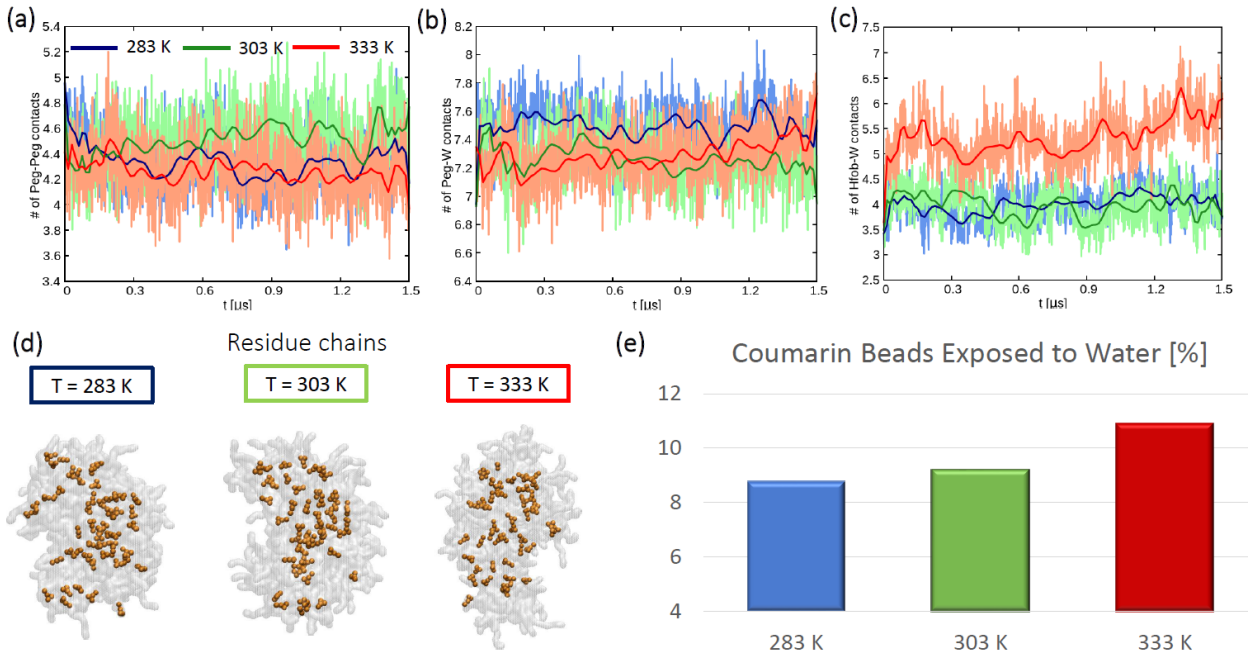


Figure S5. Number of contacts between (a) PEG-PEG beads, (b) PEG and water, (c) hydrophobic groups and water for **EG7-C6-Ph**. From 283 K to 303 K there is a slight increase in the number of PEG-PEG contacts and decrease in PEG-water contacts. This indicates that the 283 K assembly is more hydrated and hydrophilic than the 303 K assembly. From 303 K to 333 K there is a decrease in the number of PEG-PEG contacts because the structure vibrates more and therefore it is less tightly packed, while there is no significant difference in the number of PEG-water contacts. The increase of Hfob-water contacts and decrease of PEG-PEG contact from 303 K to 333 K indicate the swelling of assemblies. (d) CG-MD snapshots of assembly at 283 K, 303 K, and 333 K. The residual beads modelling the phenyl groups are highlighted in orange. (e) Percentage of Coumarin beads exposed to water over the total number of NP assembly beads. The enhancement of temperature leads to a higher water penetration inside the NP core, thereby increasing the percentage of Coumarin surface accessible to the solvent.

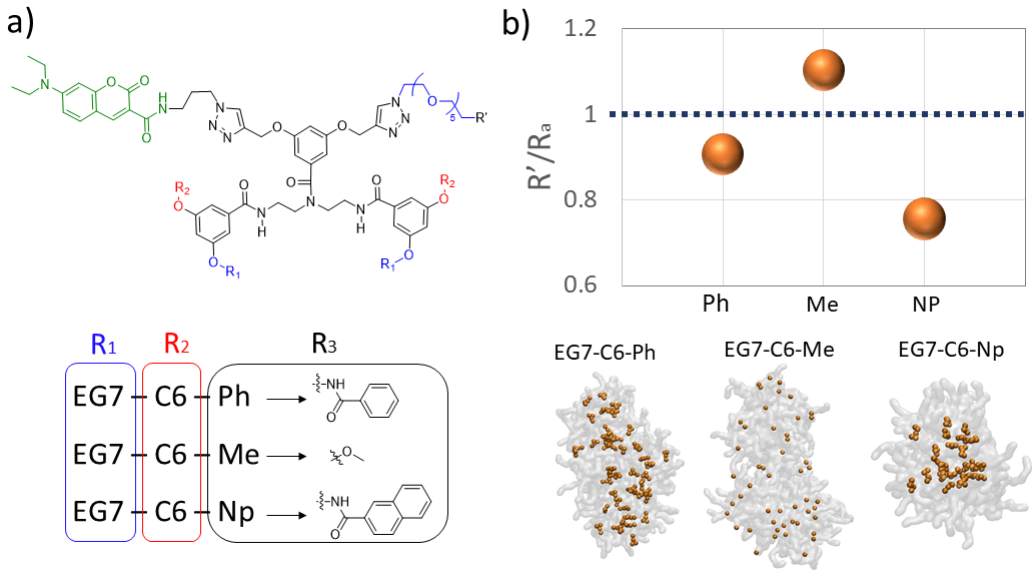


Figure S6. (a) Structures of amphiphilic oligomers investigated. (b) Ratio between the gyration radius of the R' (Ph/Me/NP) residual groups and that of the entire assembly (R_a).

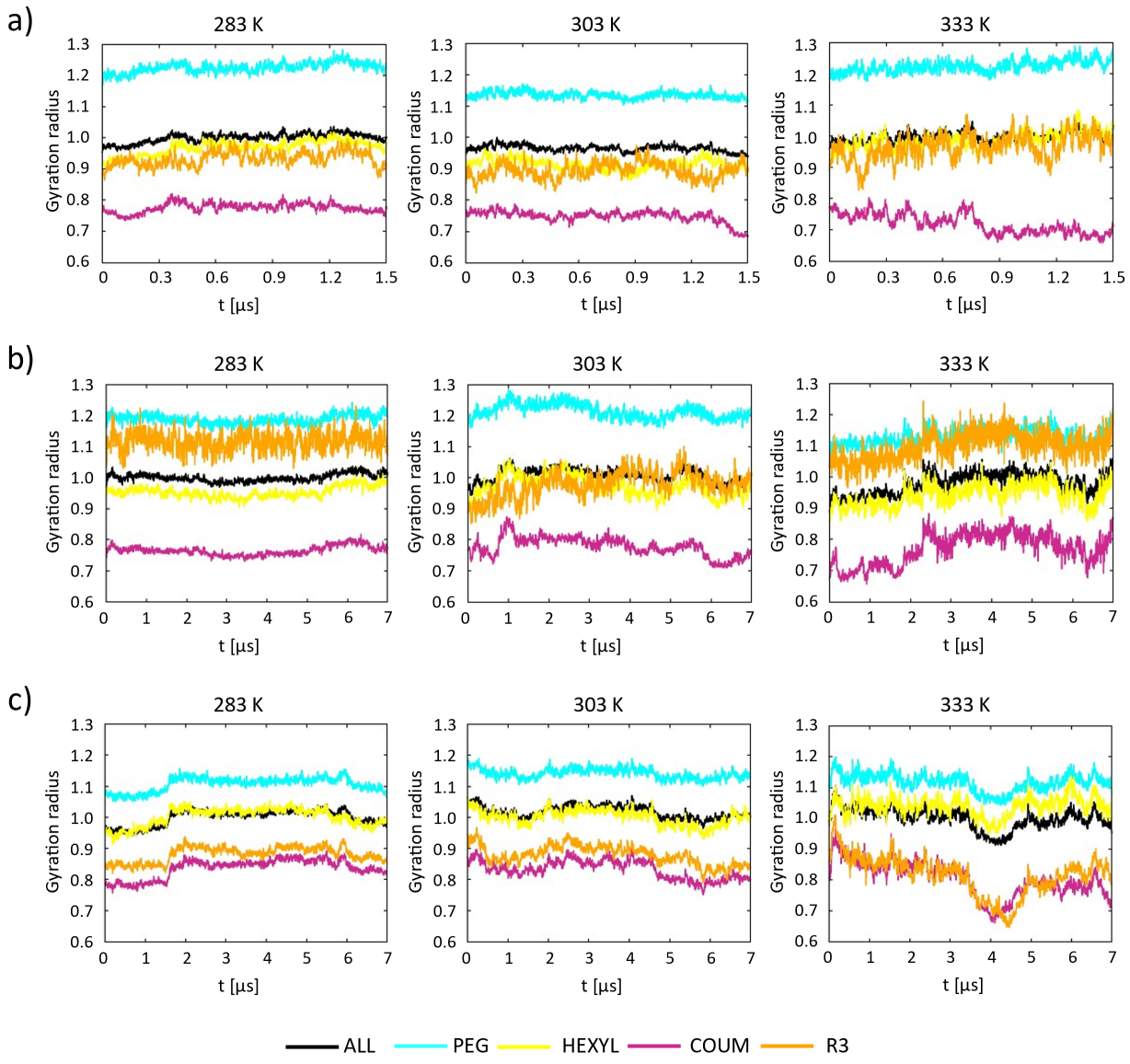


Figure S7. CG-MD time evolution of the normalized gyration radii at 283 K (left side panels), 303 K (central panel), and 333 K (right side panels) for a) **EG7-C6-Ph**, b) **EG7-C6-Me**, c) **EG7-C6-Np**. Note that the gyration radii of the single chains are normalized over the entire self-assembly radius, R_a.

6. References

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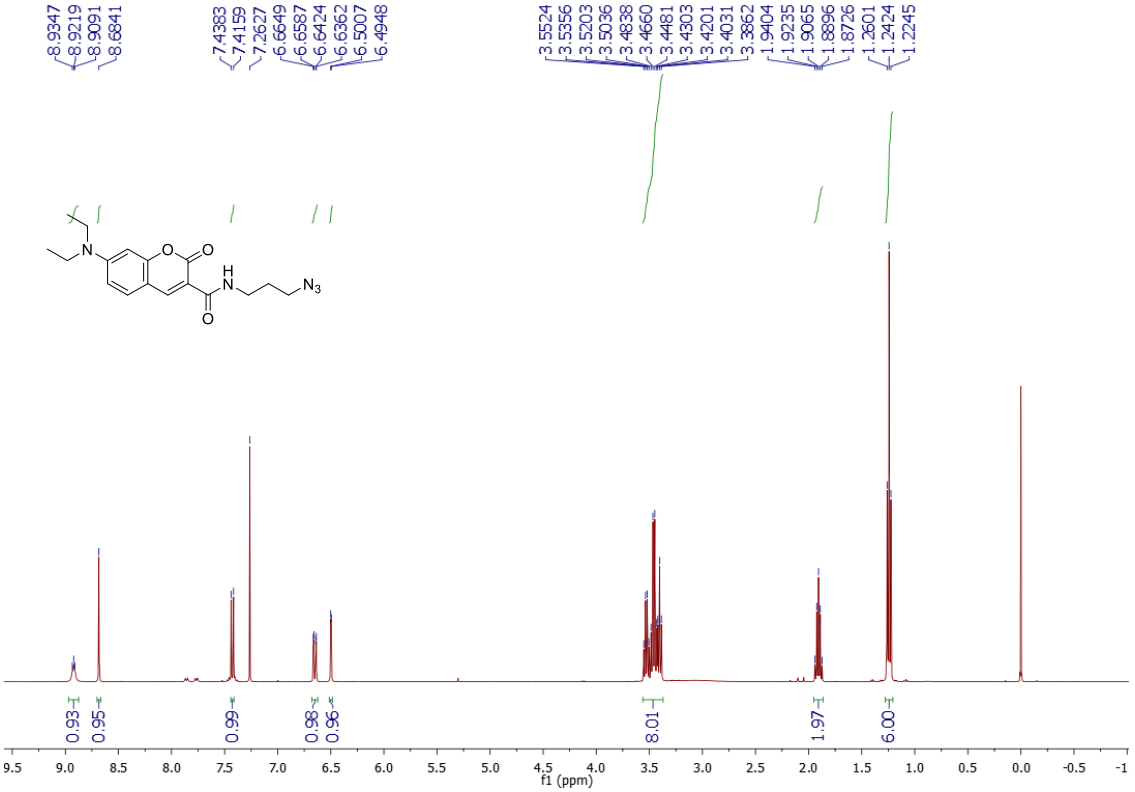
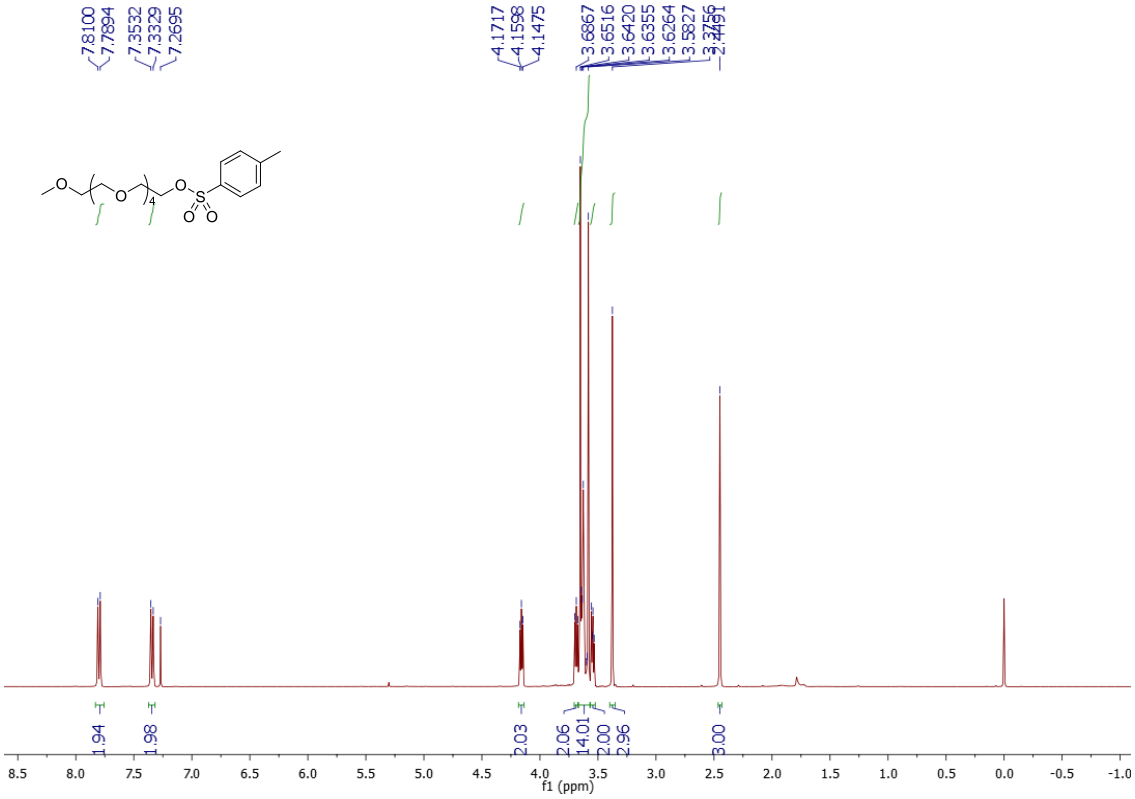
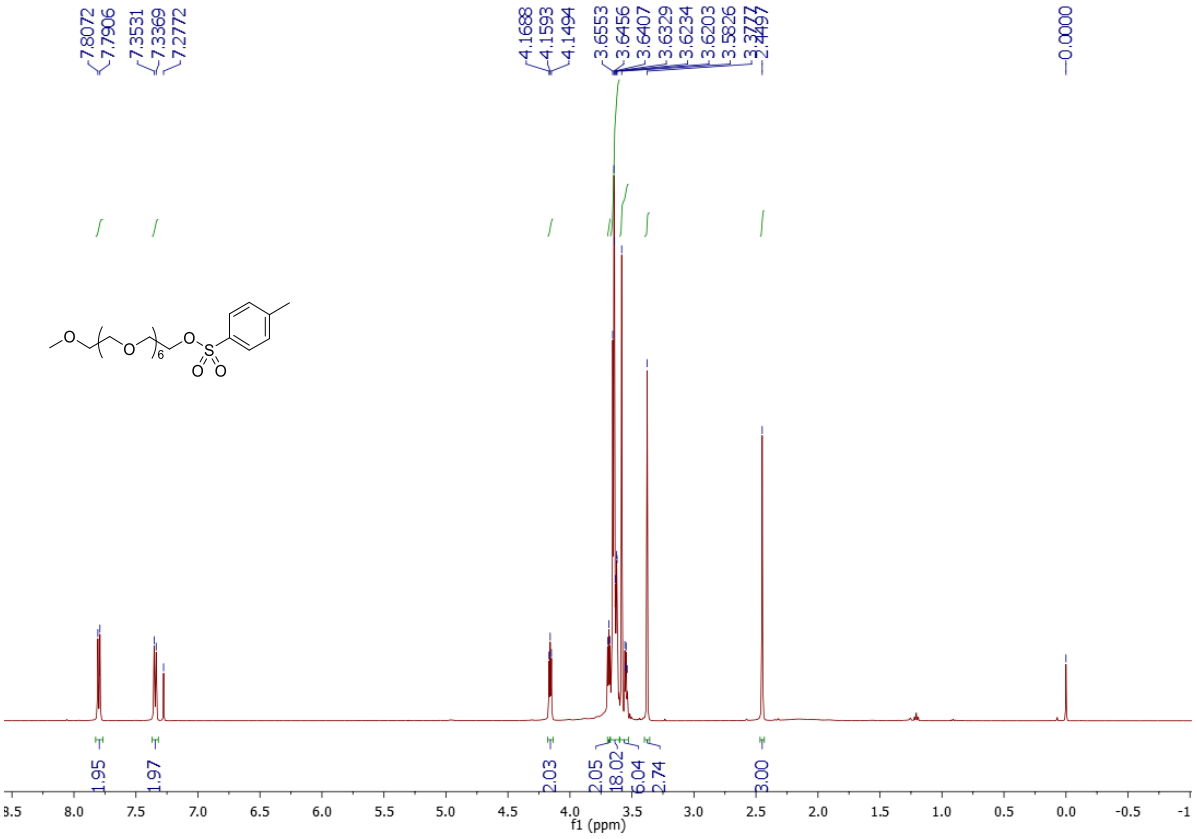
7. NMR spectra of synthesized molecules



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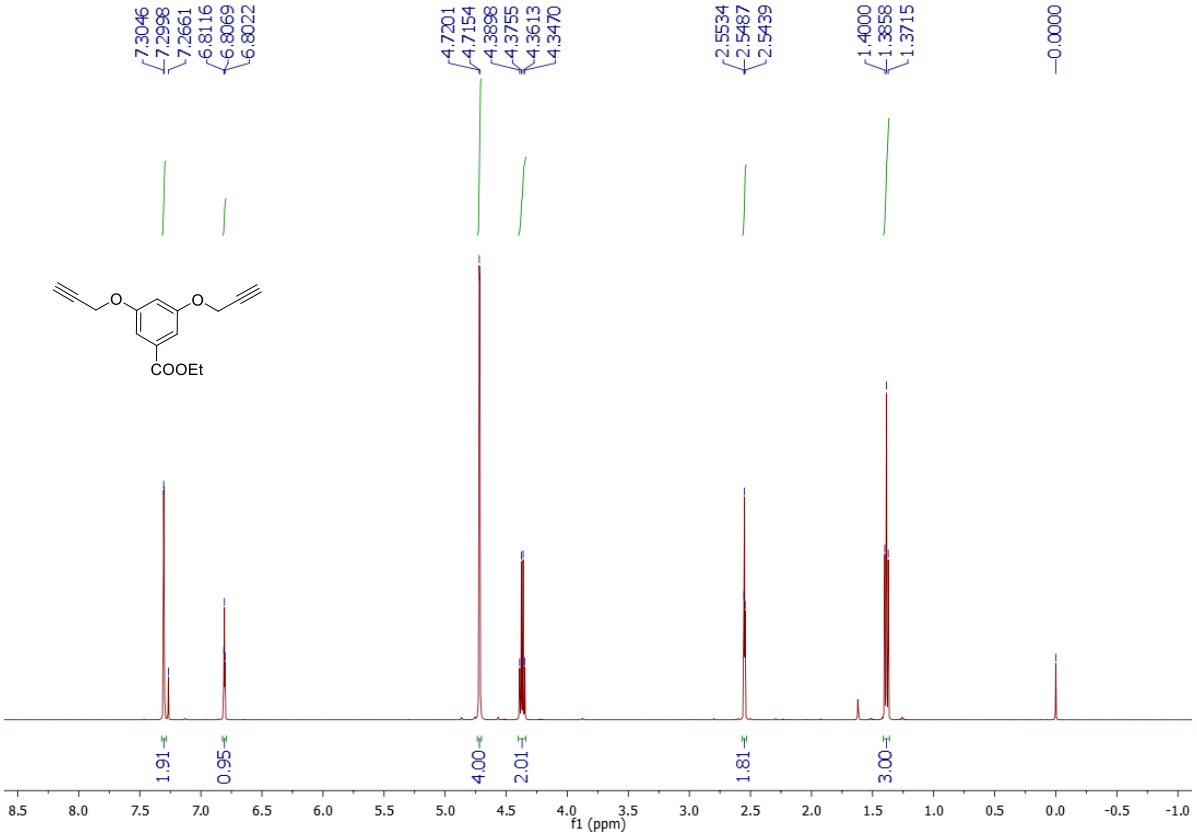
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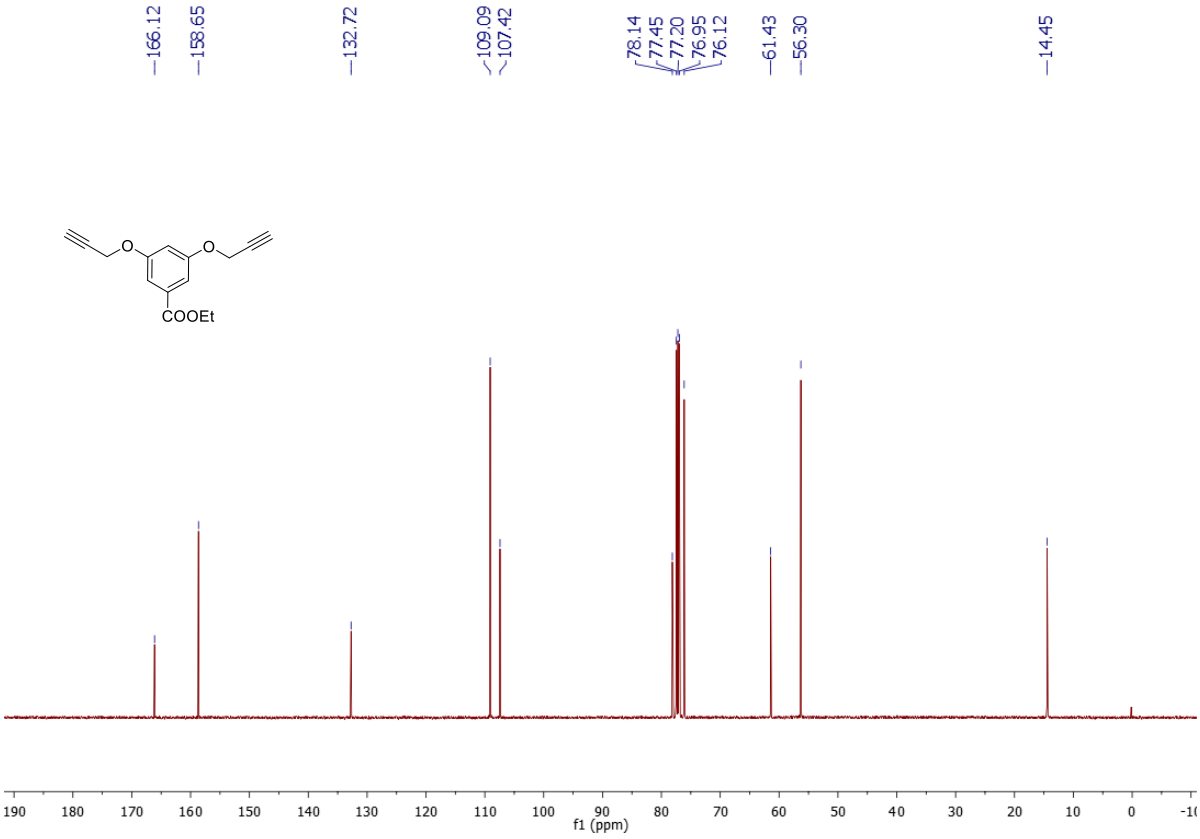
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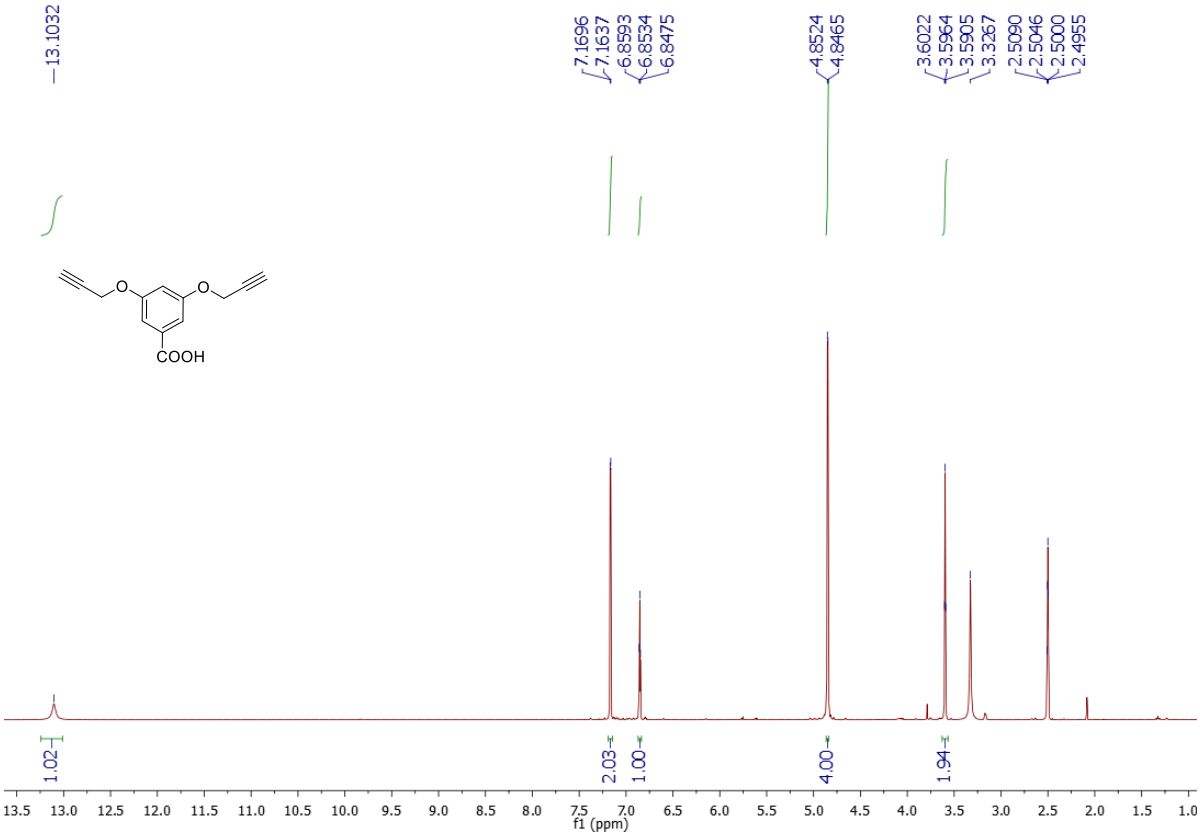
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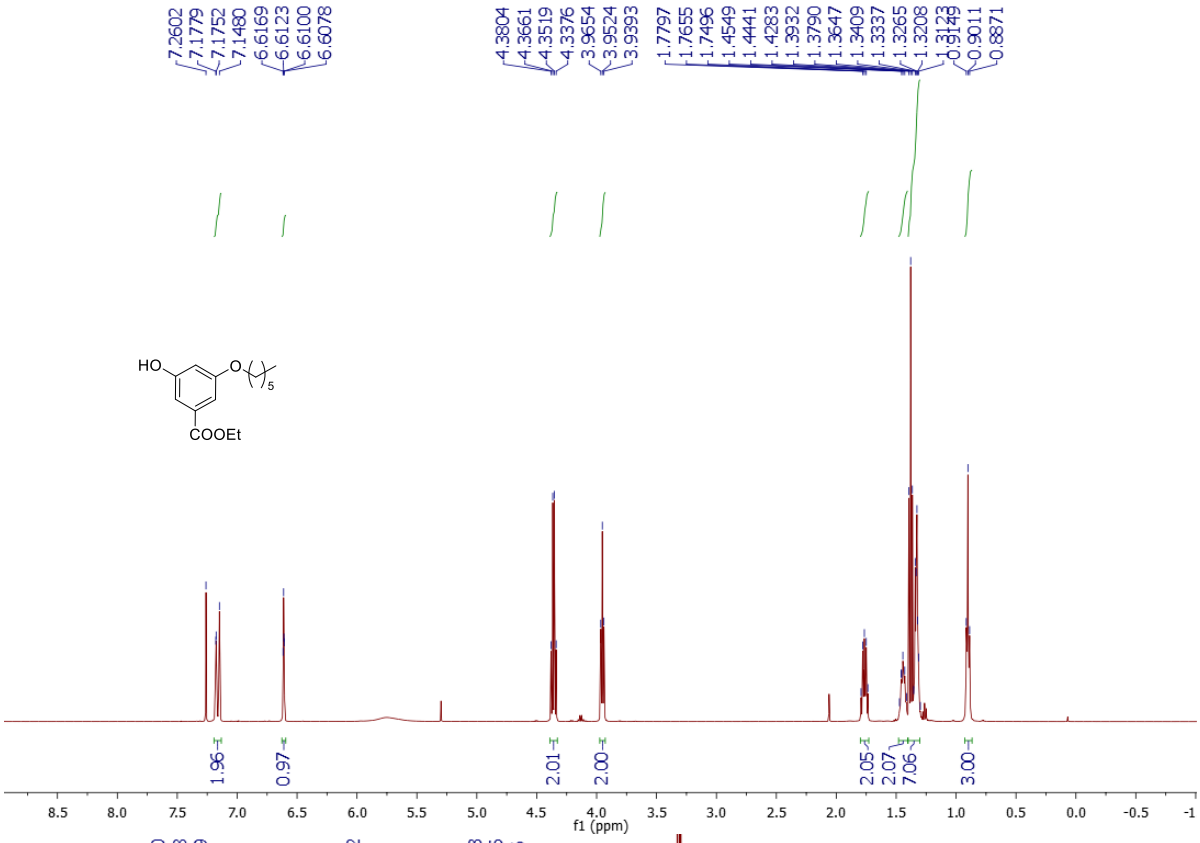
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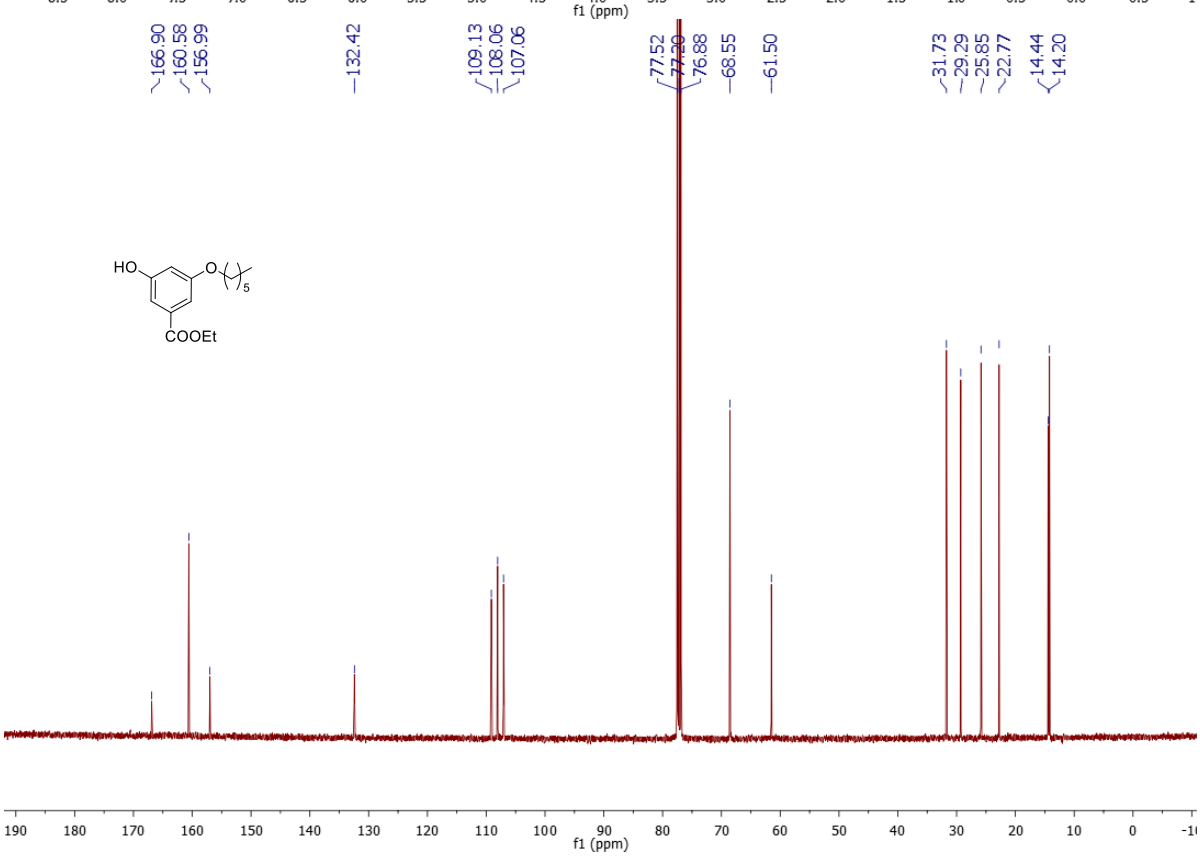
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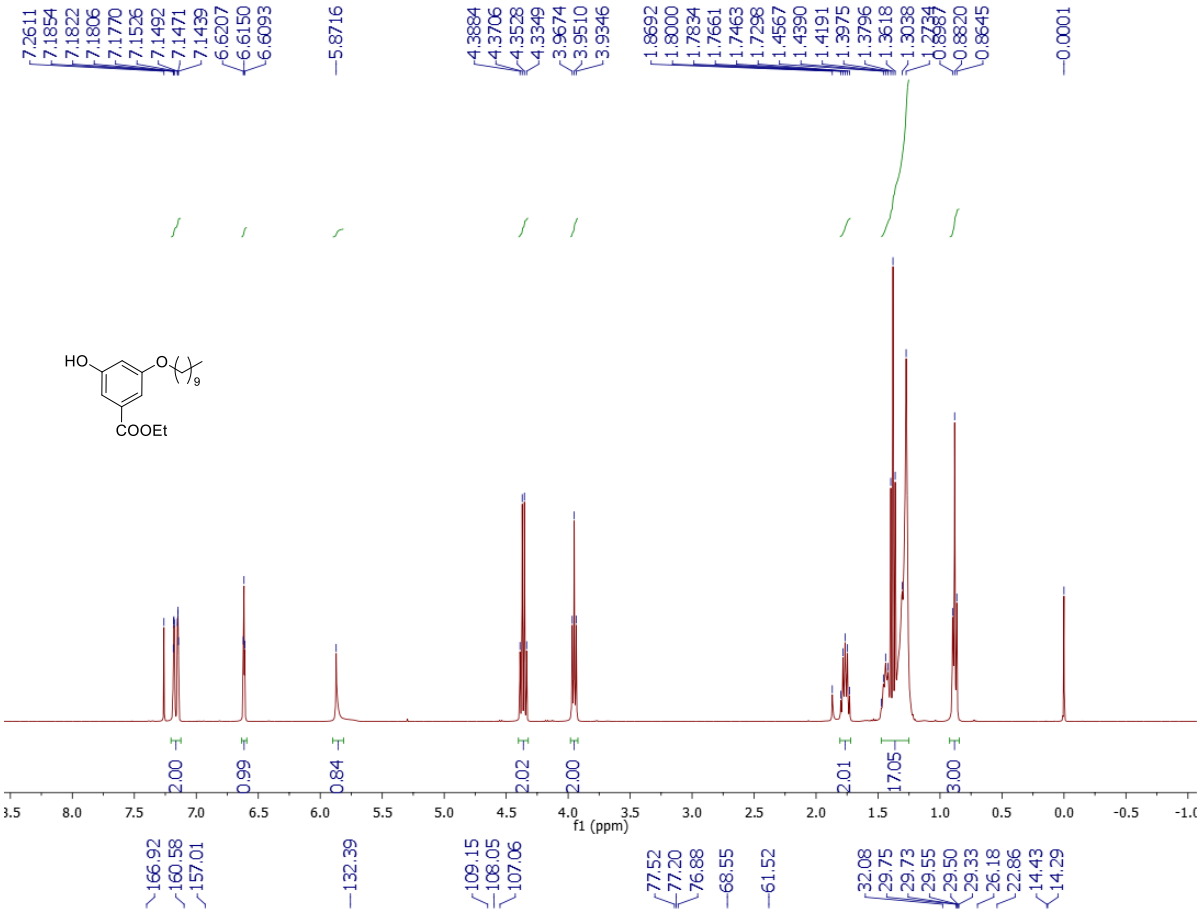
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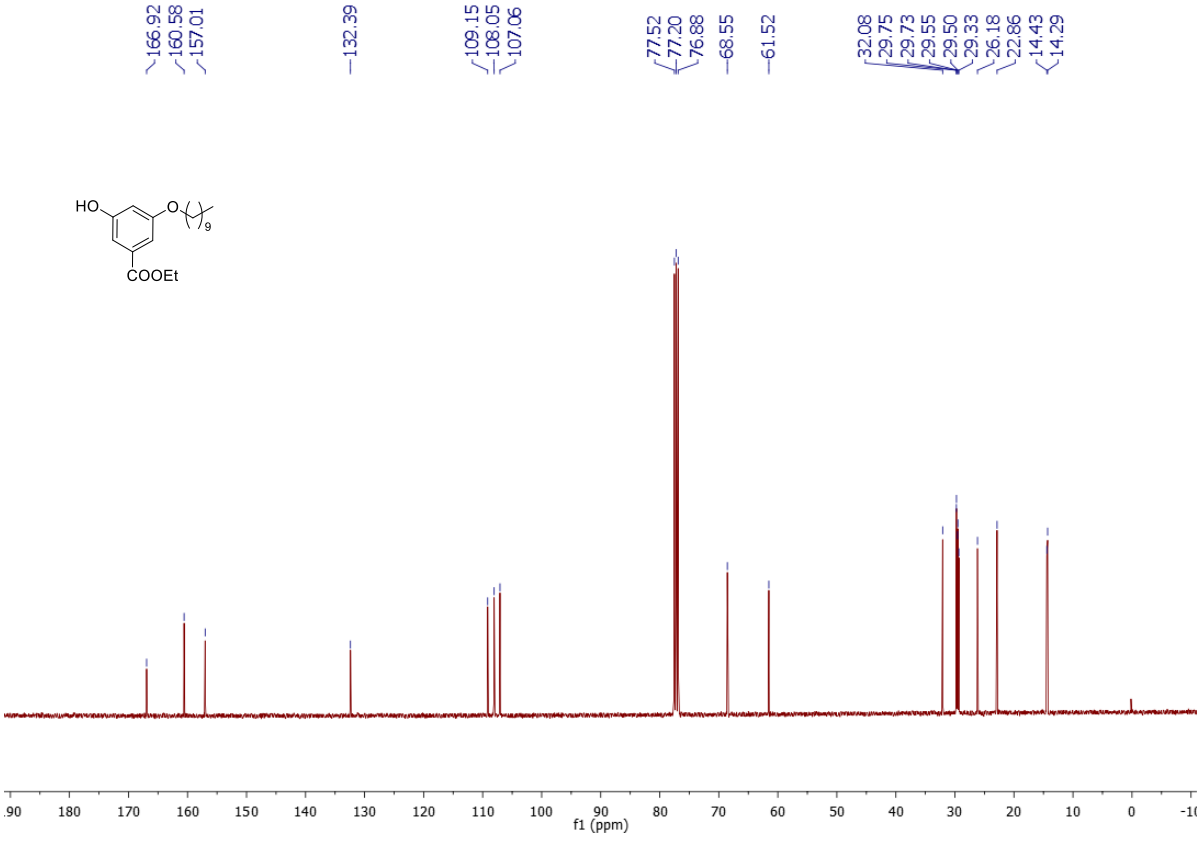
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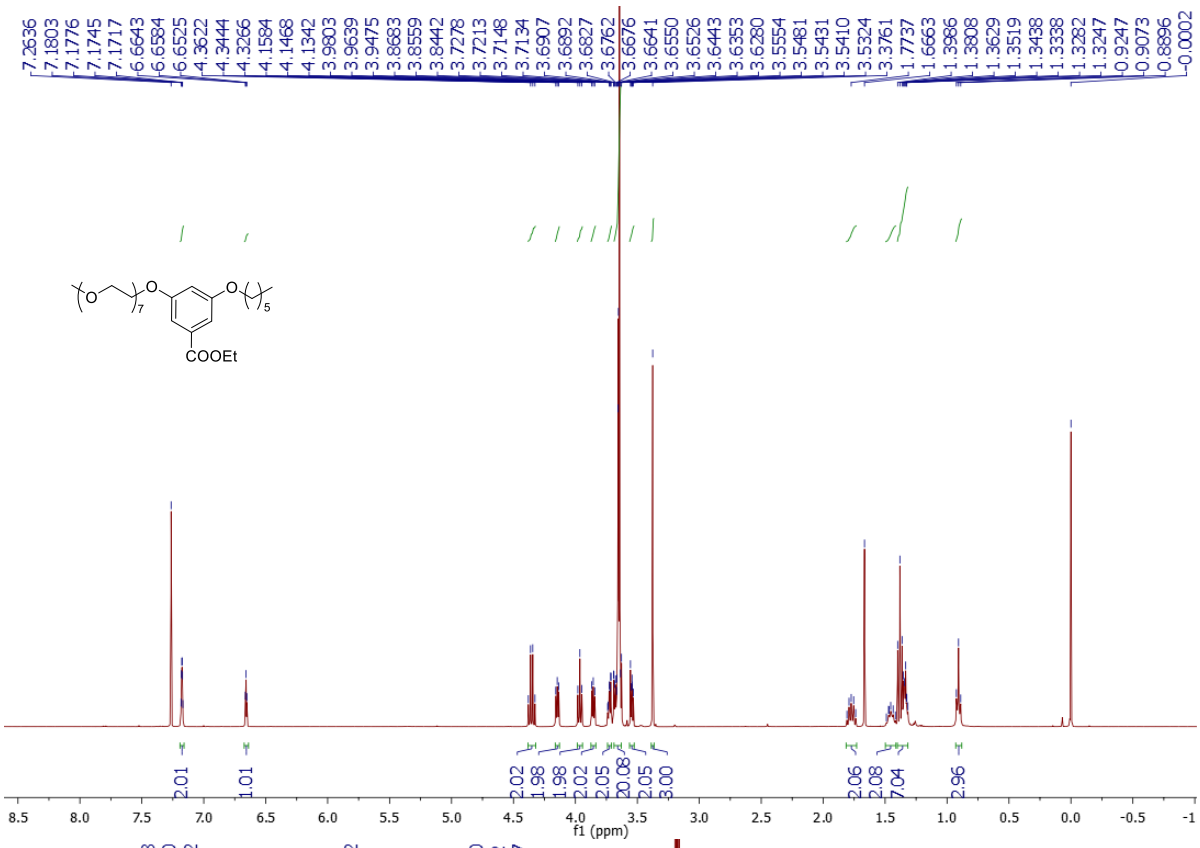
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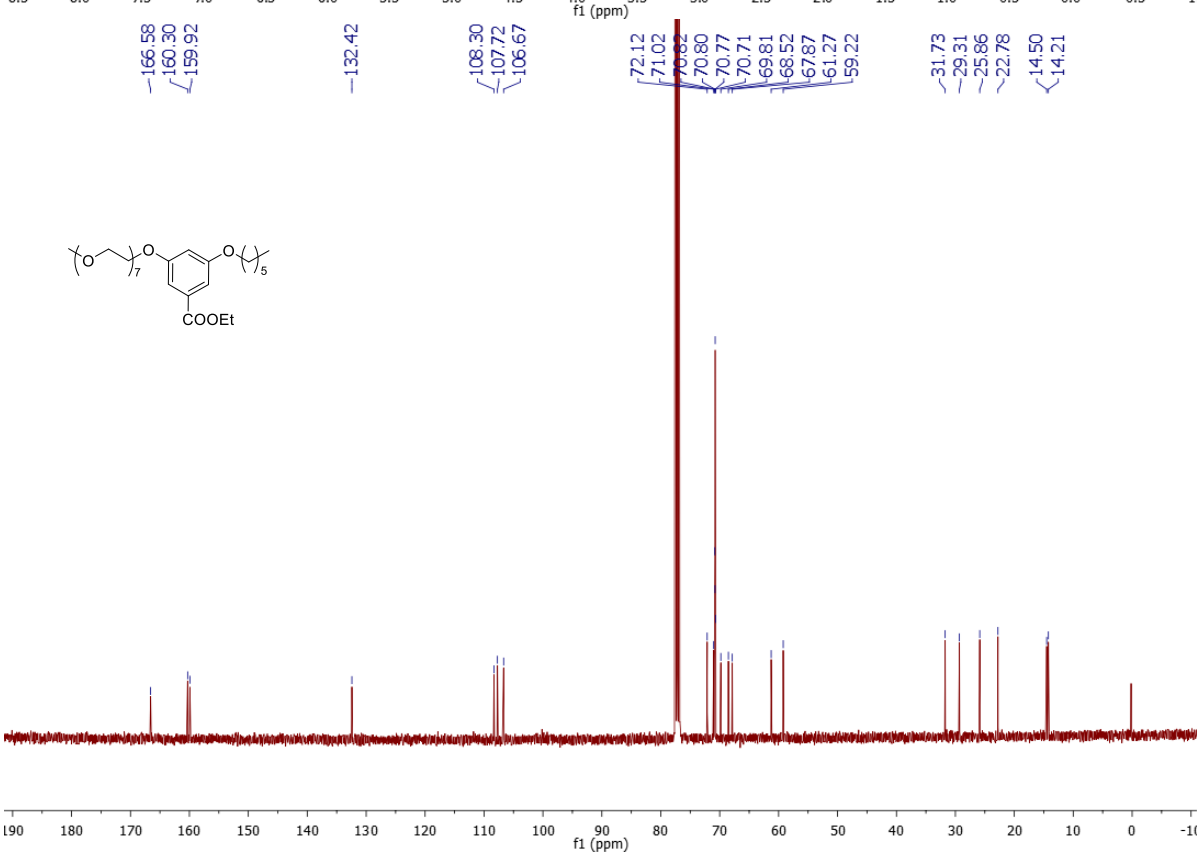
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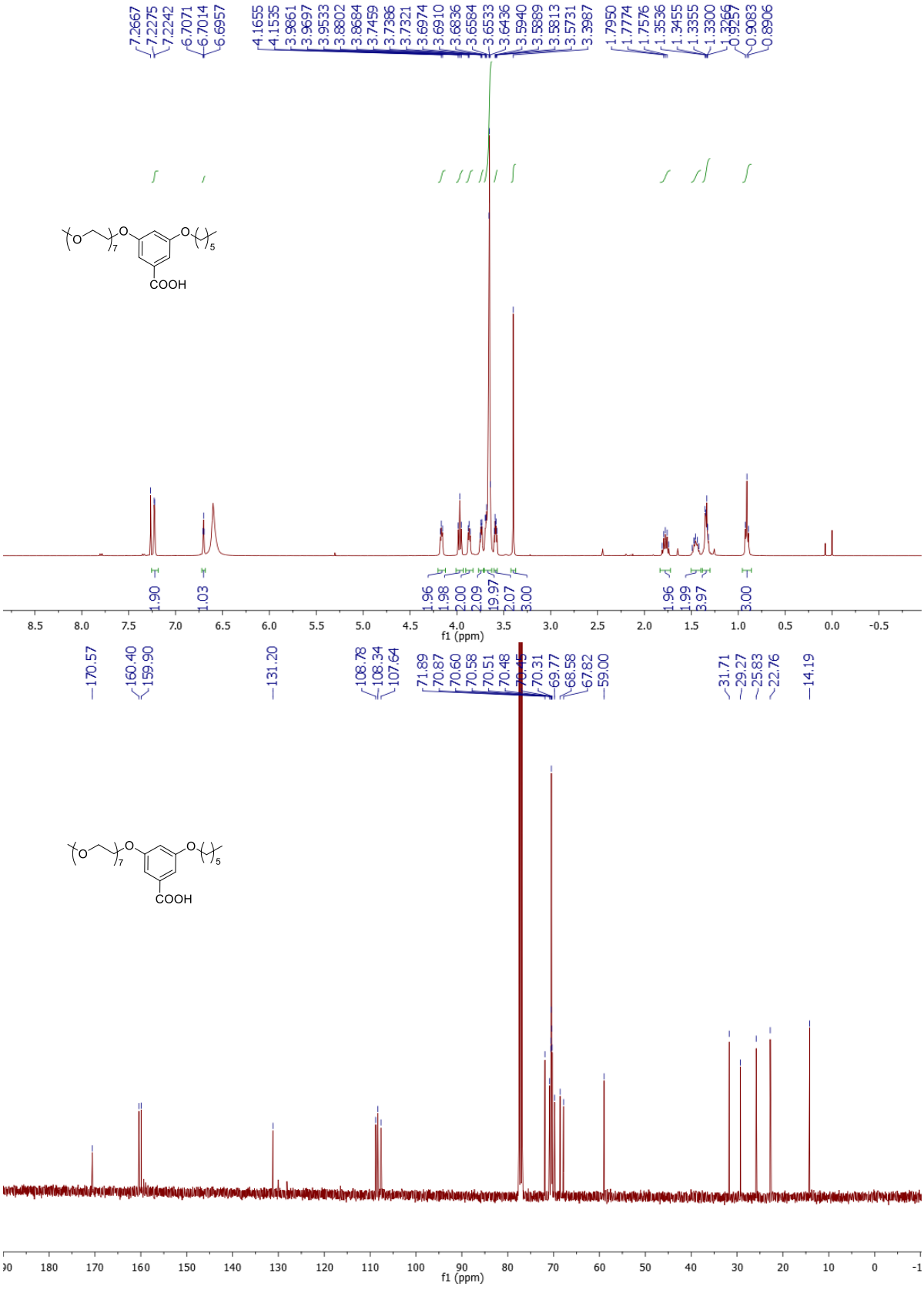
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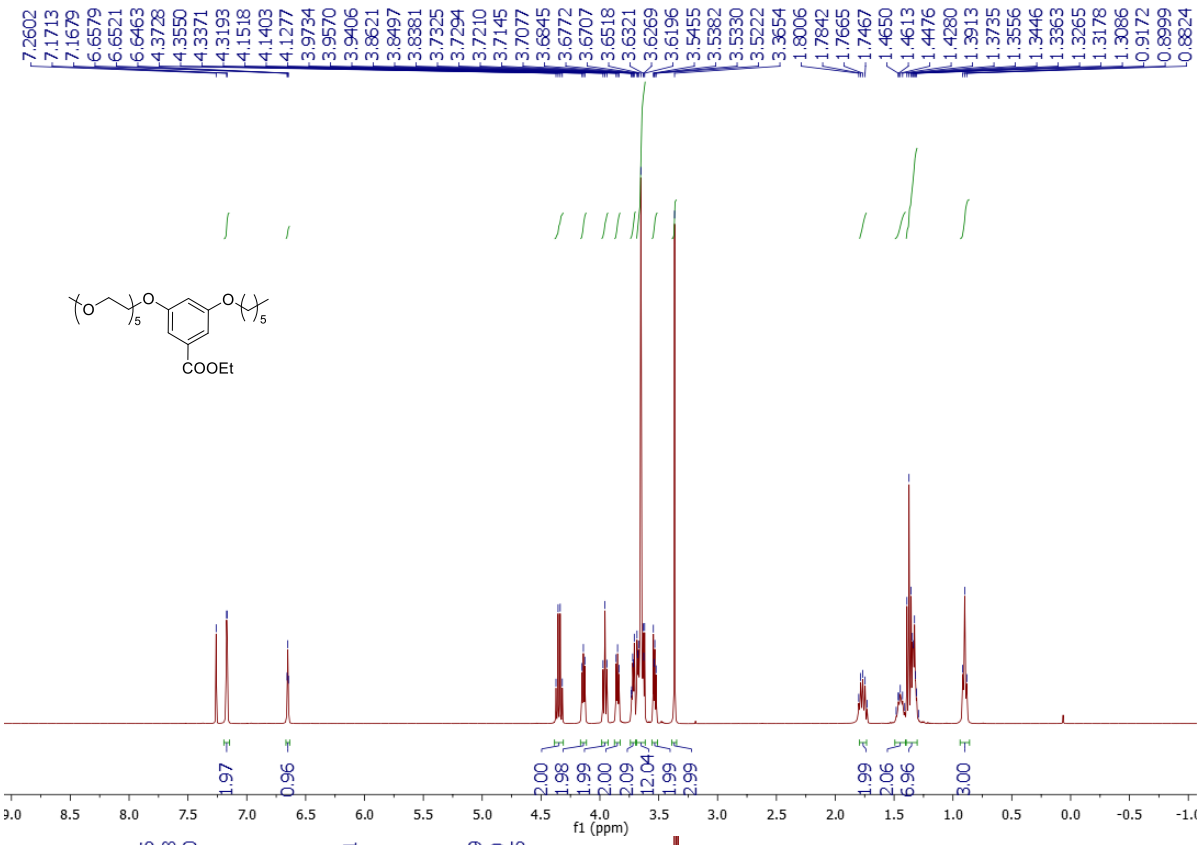
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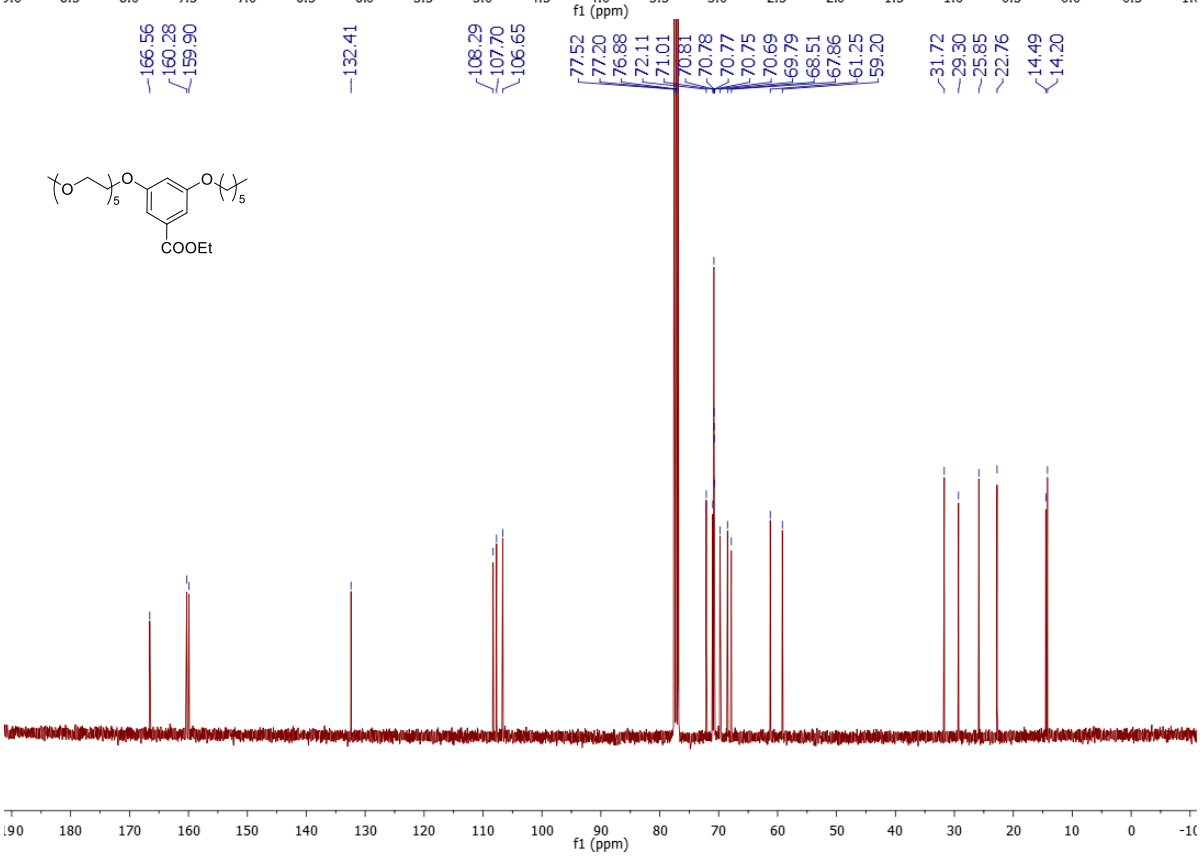
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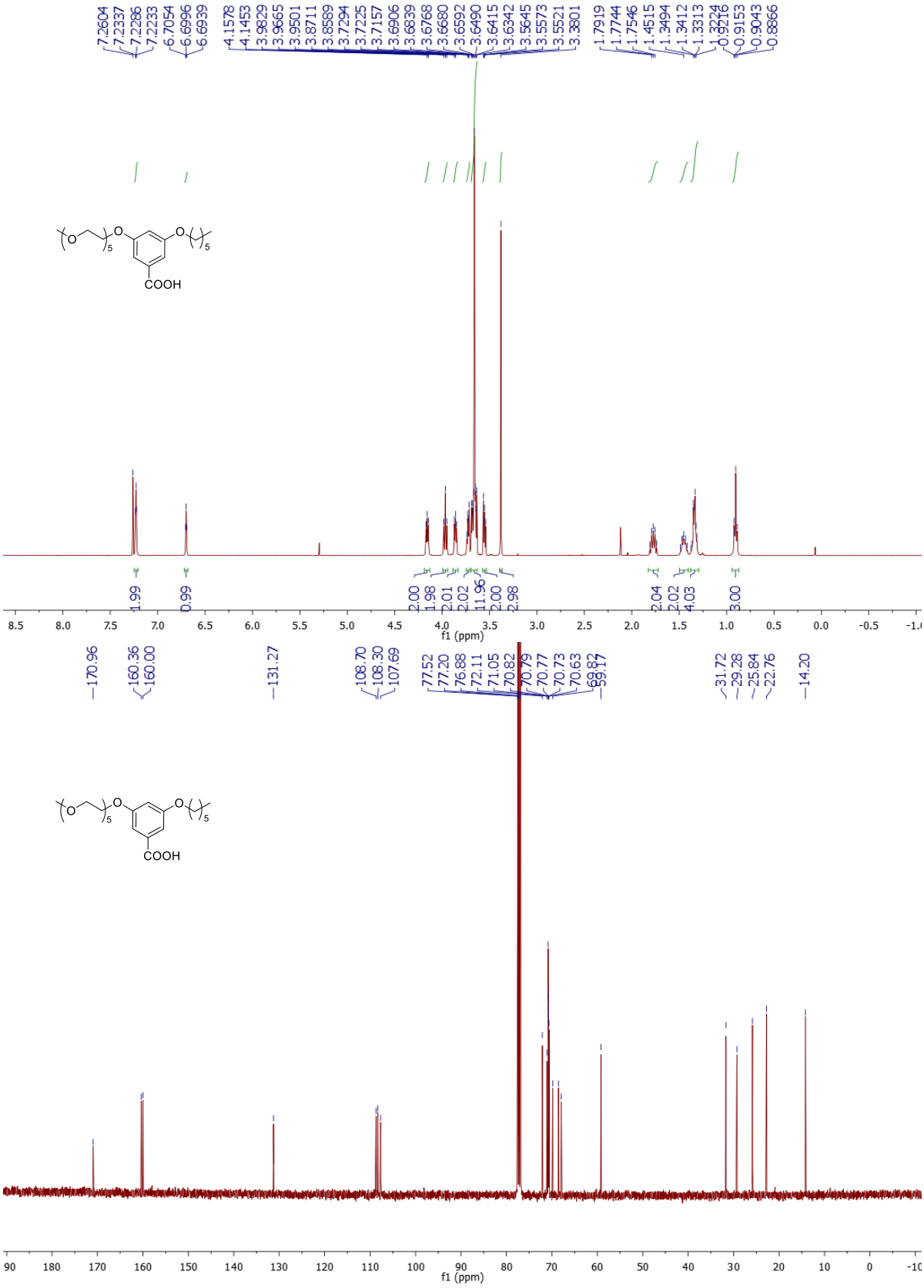
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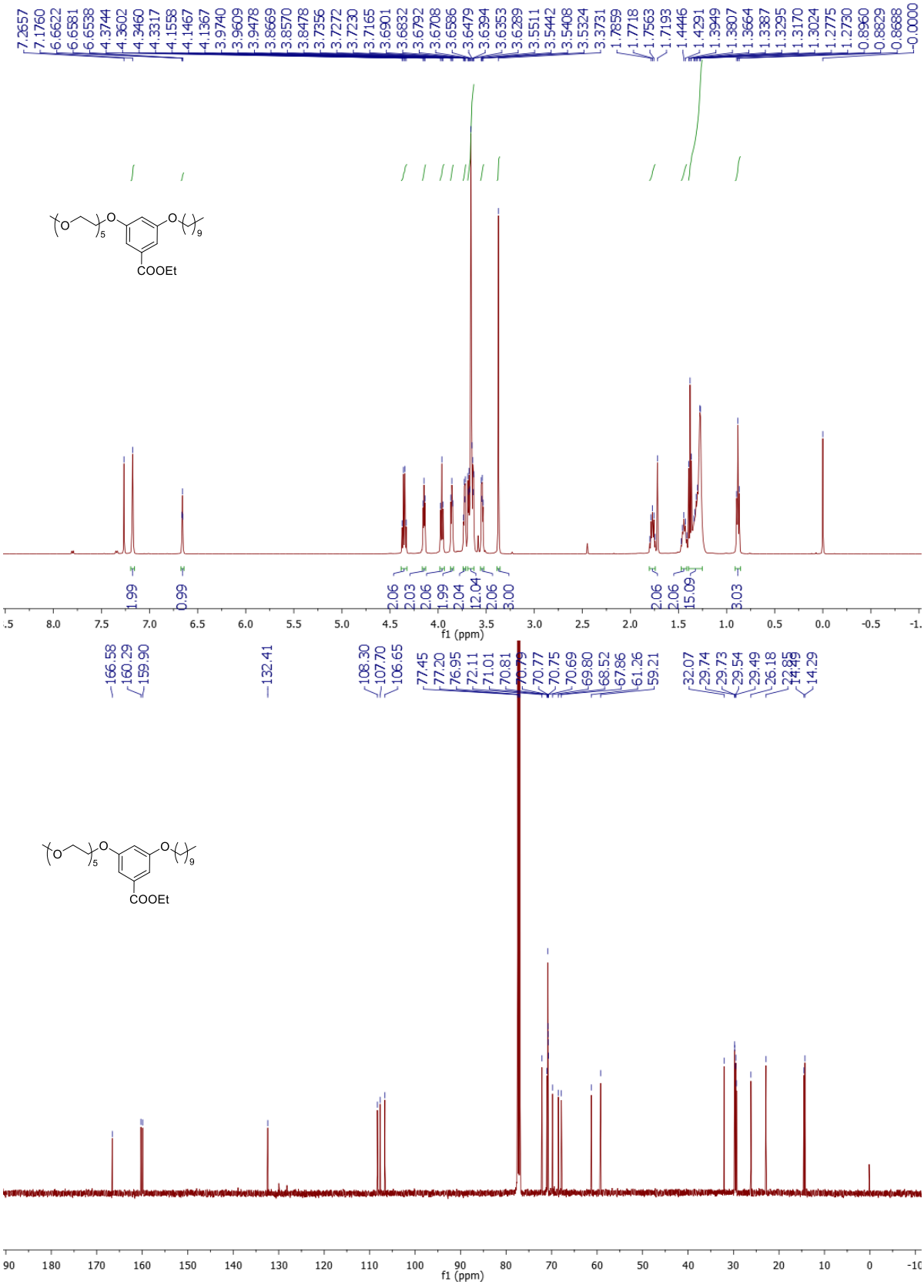
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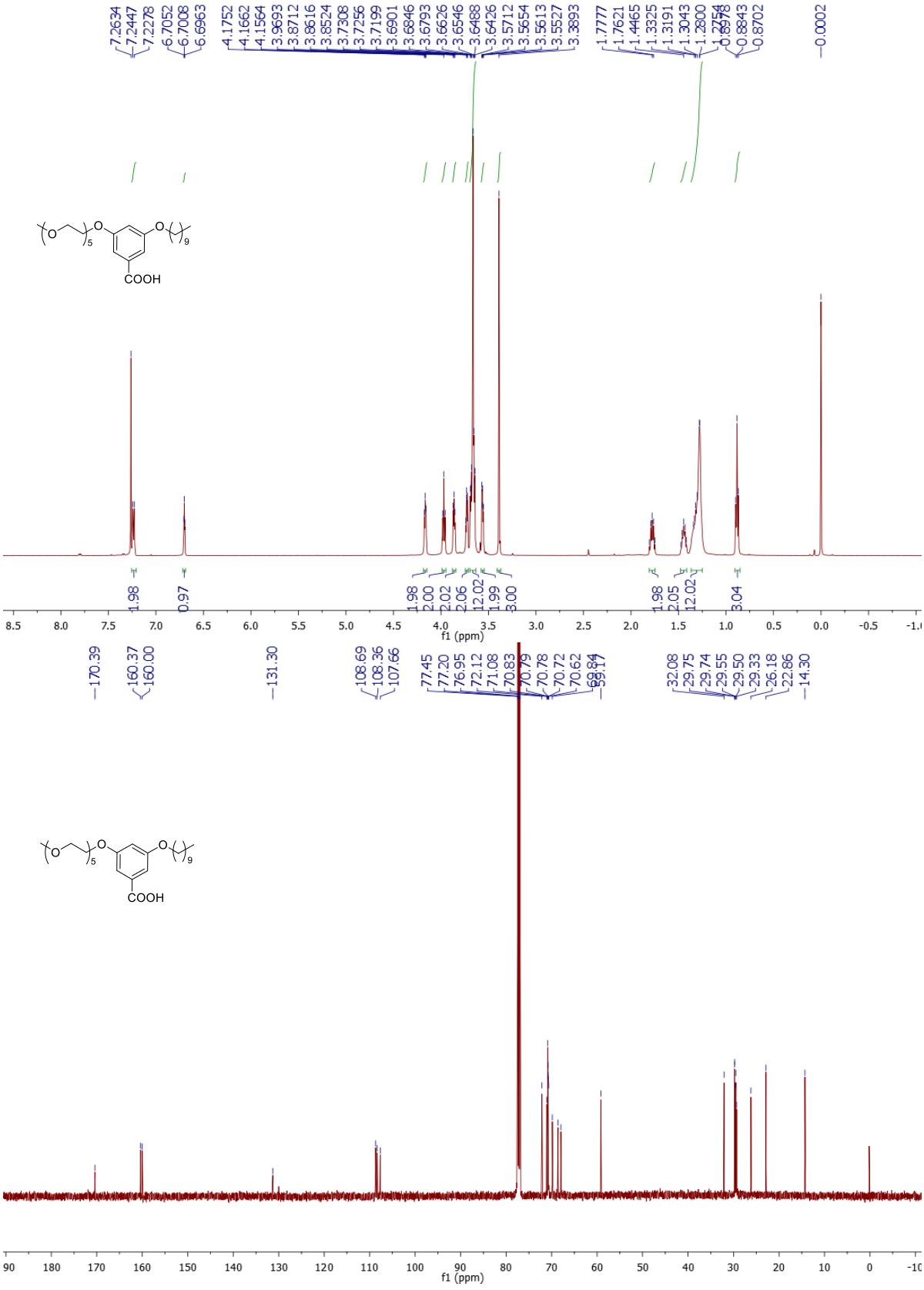
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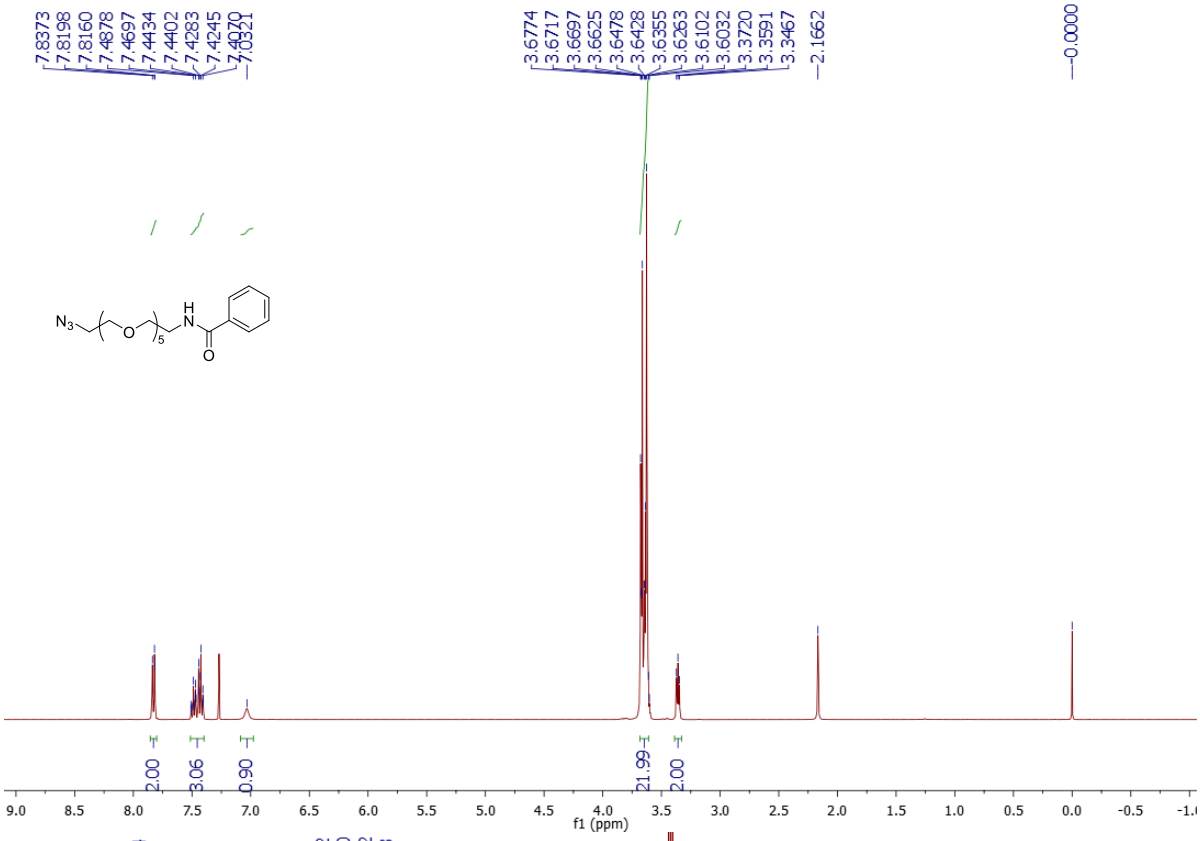
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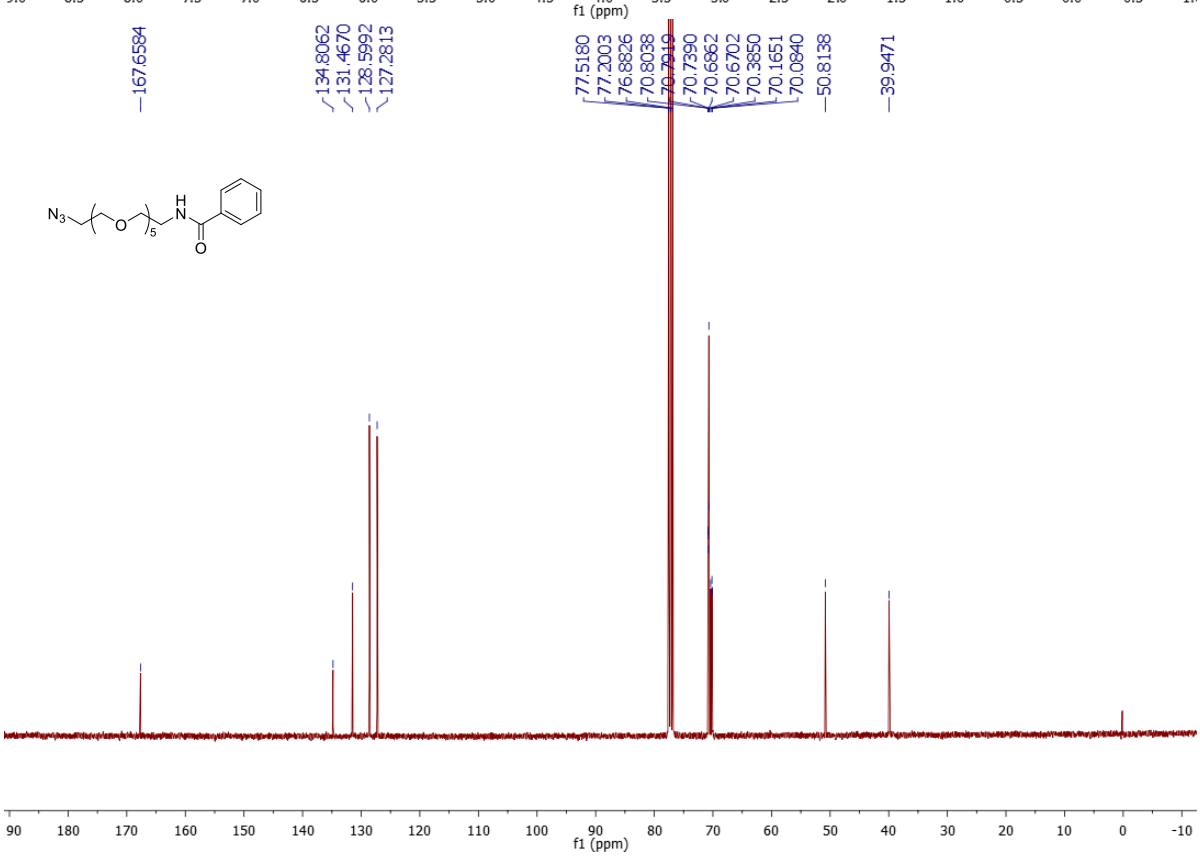
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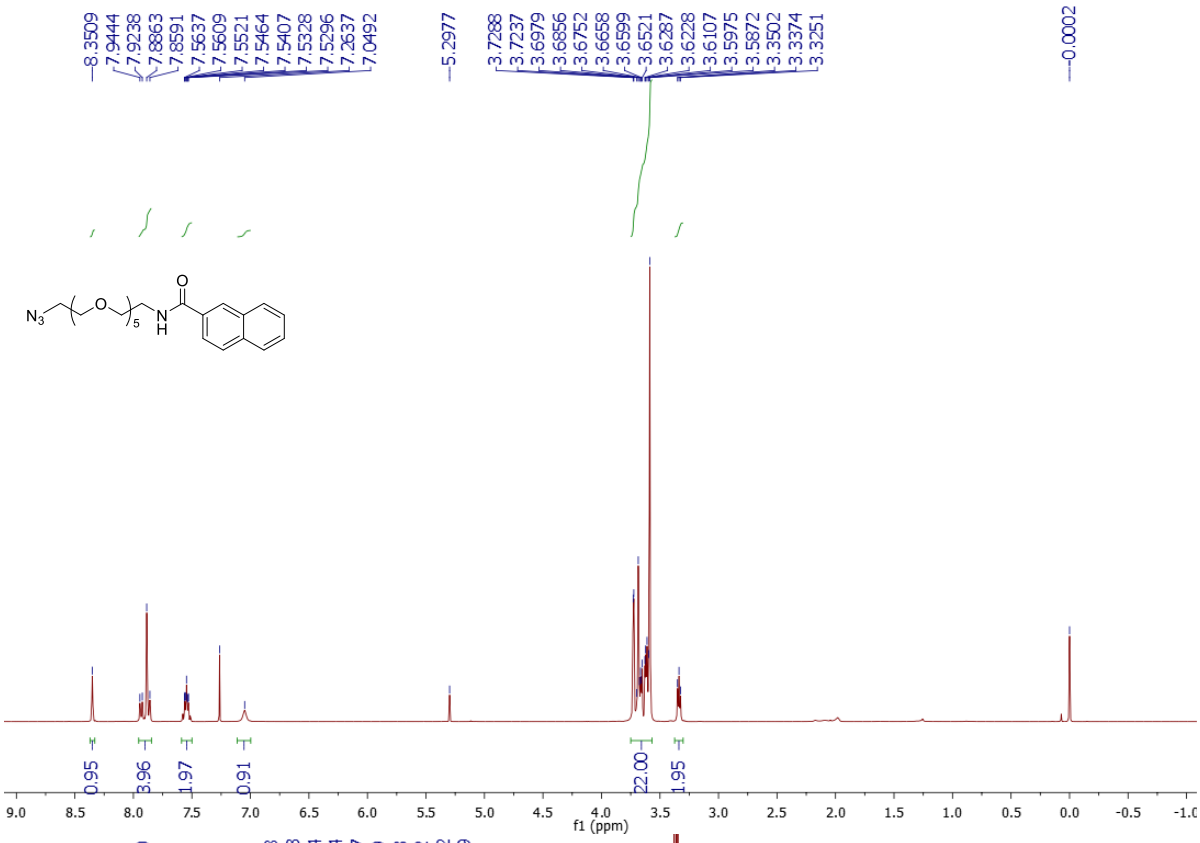
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7.5464
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7.5296
7.2637
7.0492
5.2977
3.7288
3.7237
3.6979
3.6856
3.6752
3.6658
3.6599
3.6521
3.6287
3.6228
3.6107
3.5975
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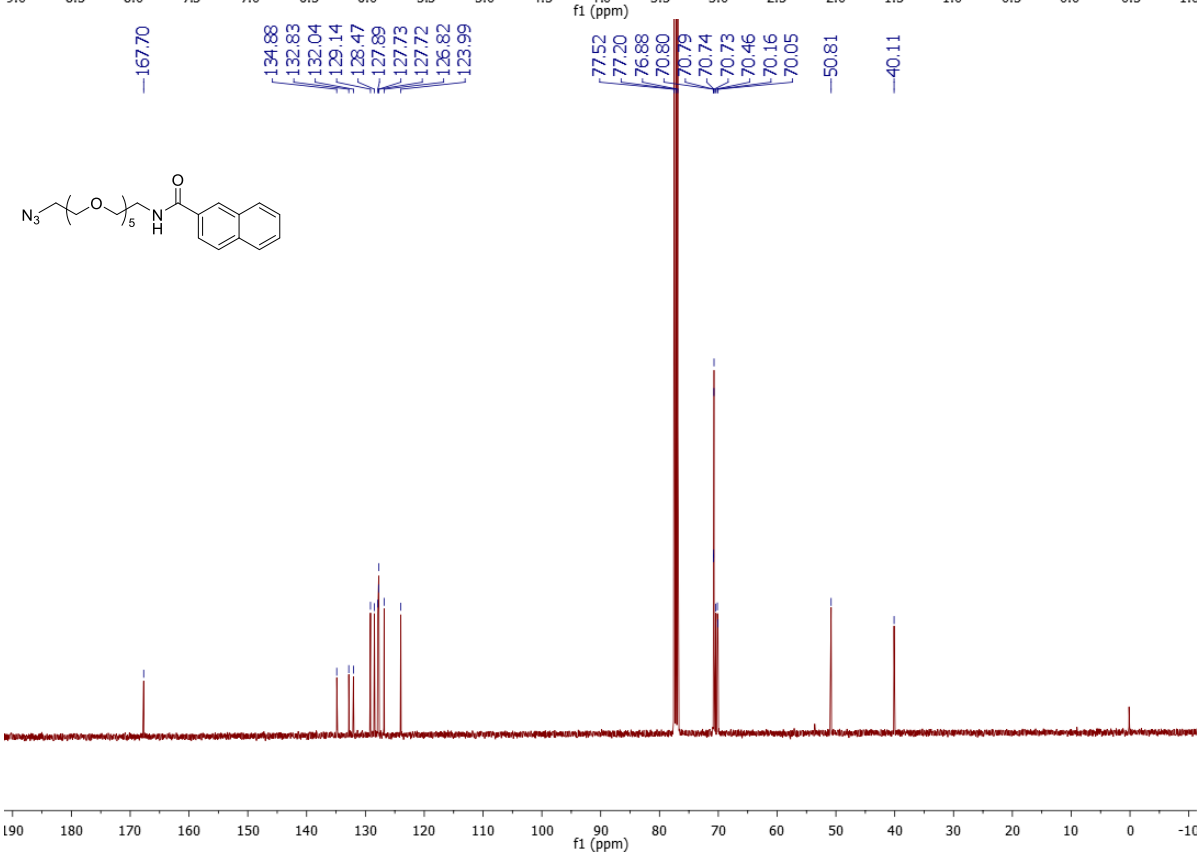
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127.73
127.72
126.82
123.99
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70.73
70.46
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70.05
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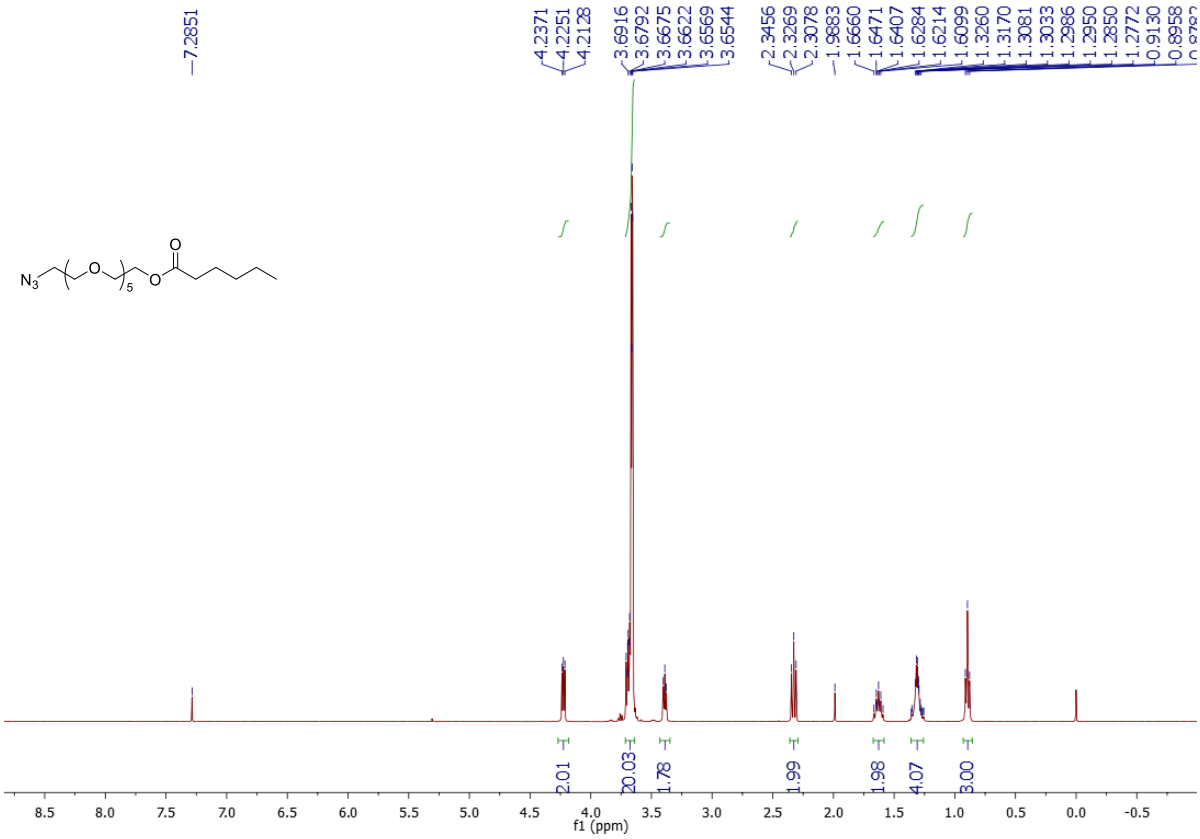
40.11
f1 (ppm)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2.3078
1.9883
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1.6471
1.6407
1.6284
1.6214
1.6099
1.3260
1.3170
1.3081
1.3033
1.2986
1.2950
1.2850
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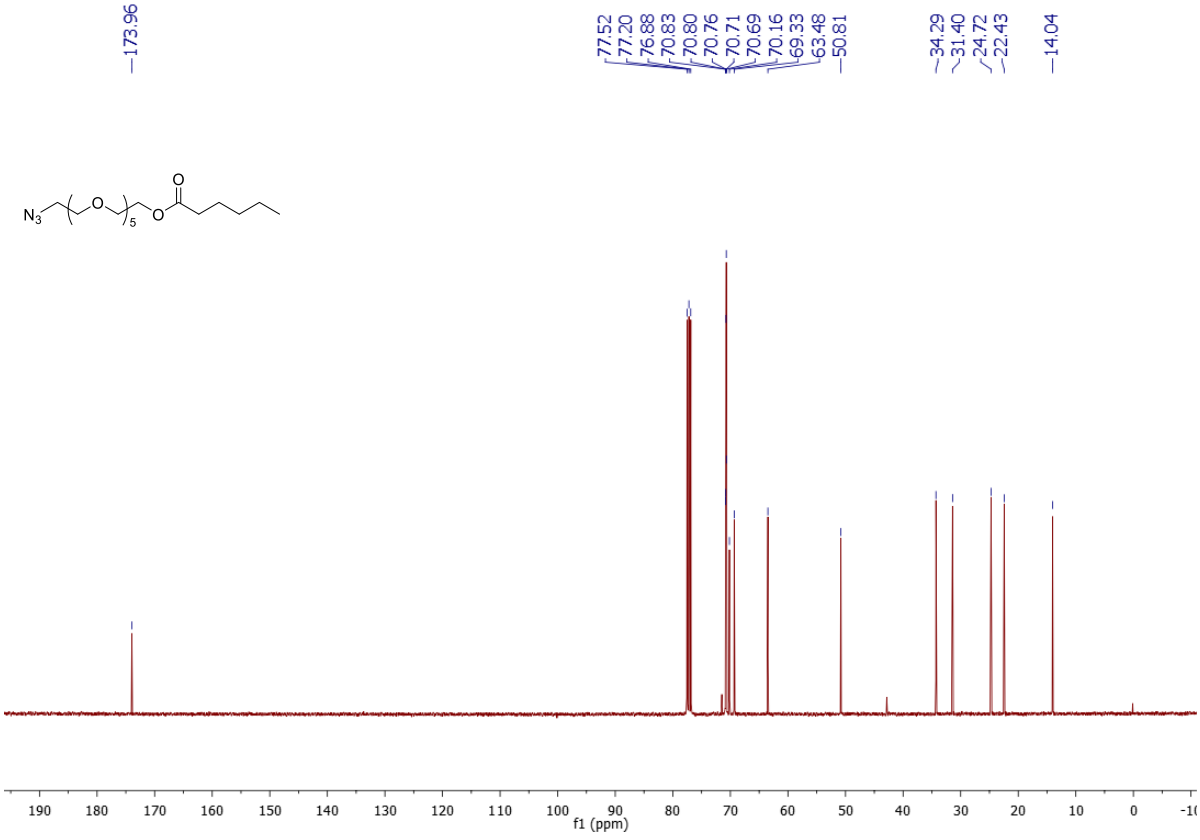
20.03
1.78
1.99
1.98
4.07
3.00
f1 (ppm)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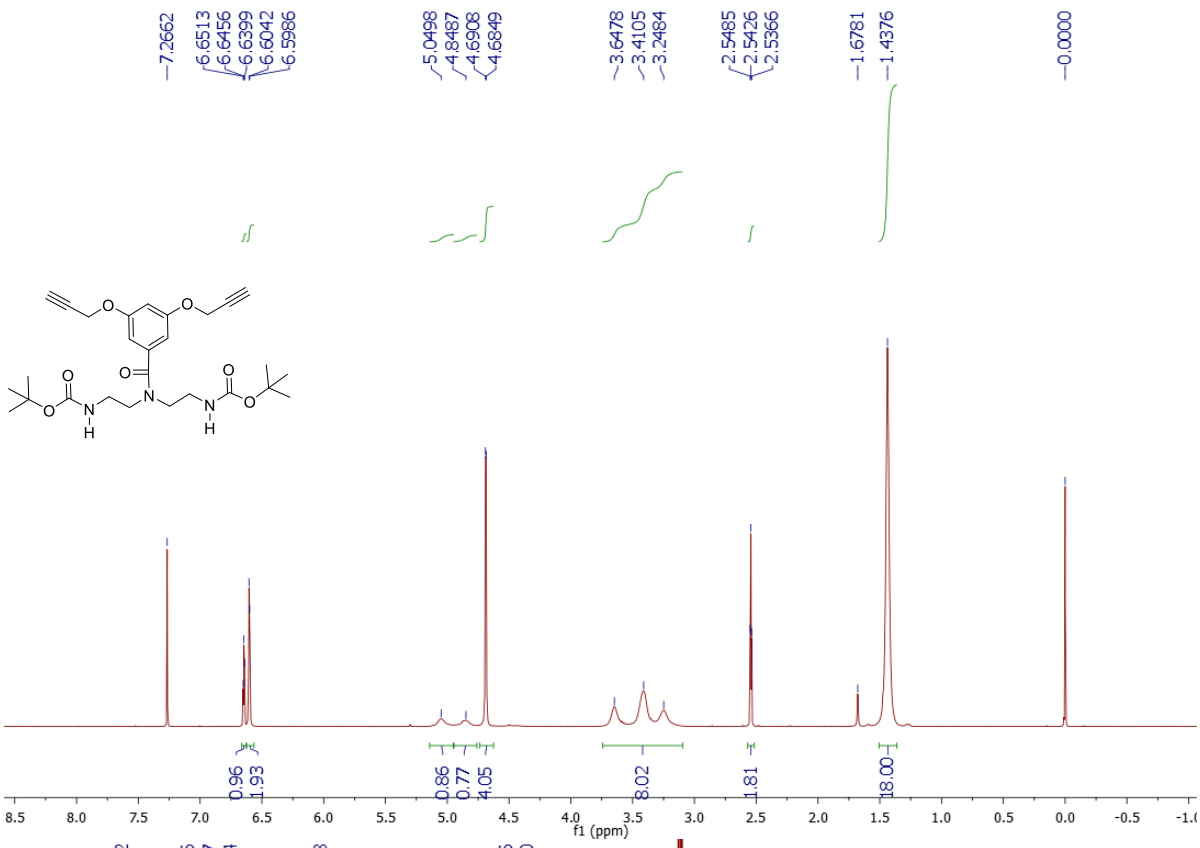
31.40
24.72
22.43
14.04
f1 (ppm)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3.6478
3.4105
3.2484
2.5485
2.5426
2.5366
1.6781
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1.93
0.86
0.77
4.05
8.02
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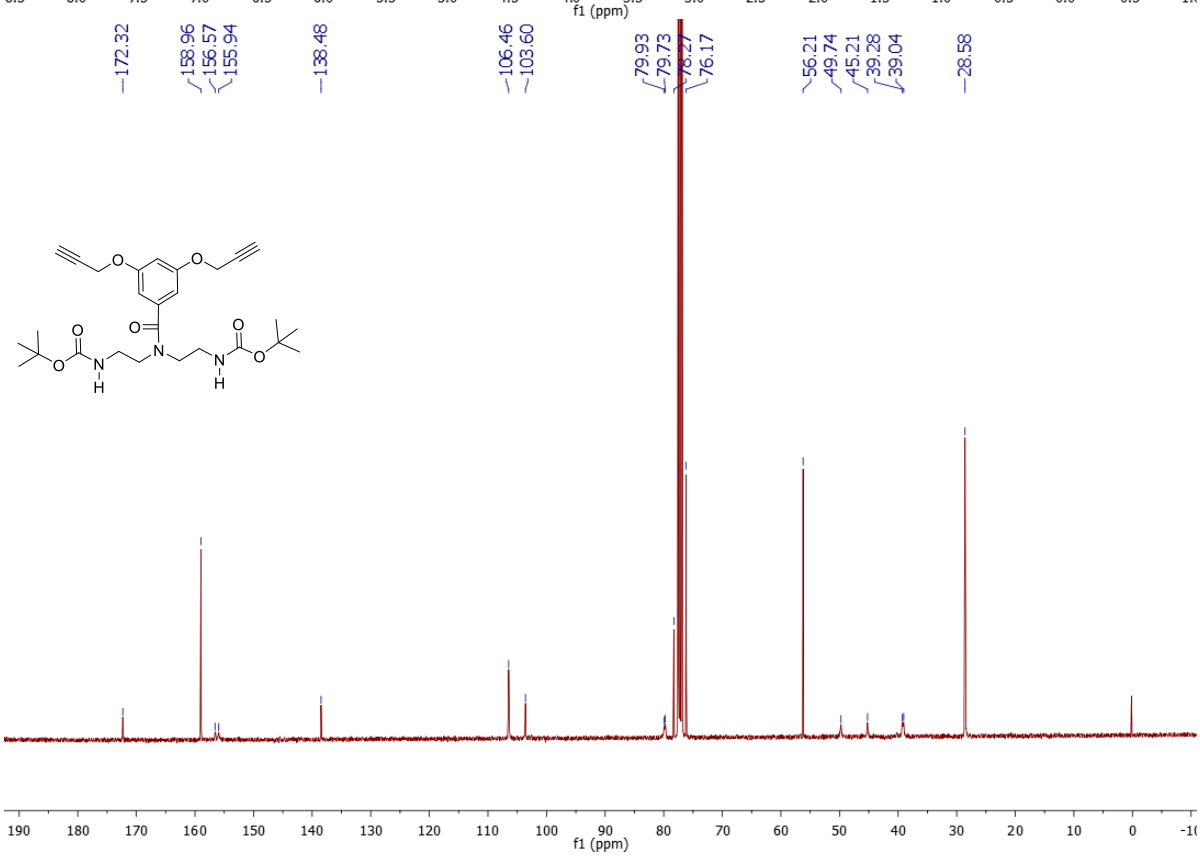
18.00
f1 (ppm)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79.73
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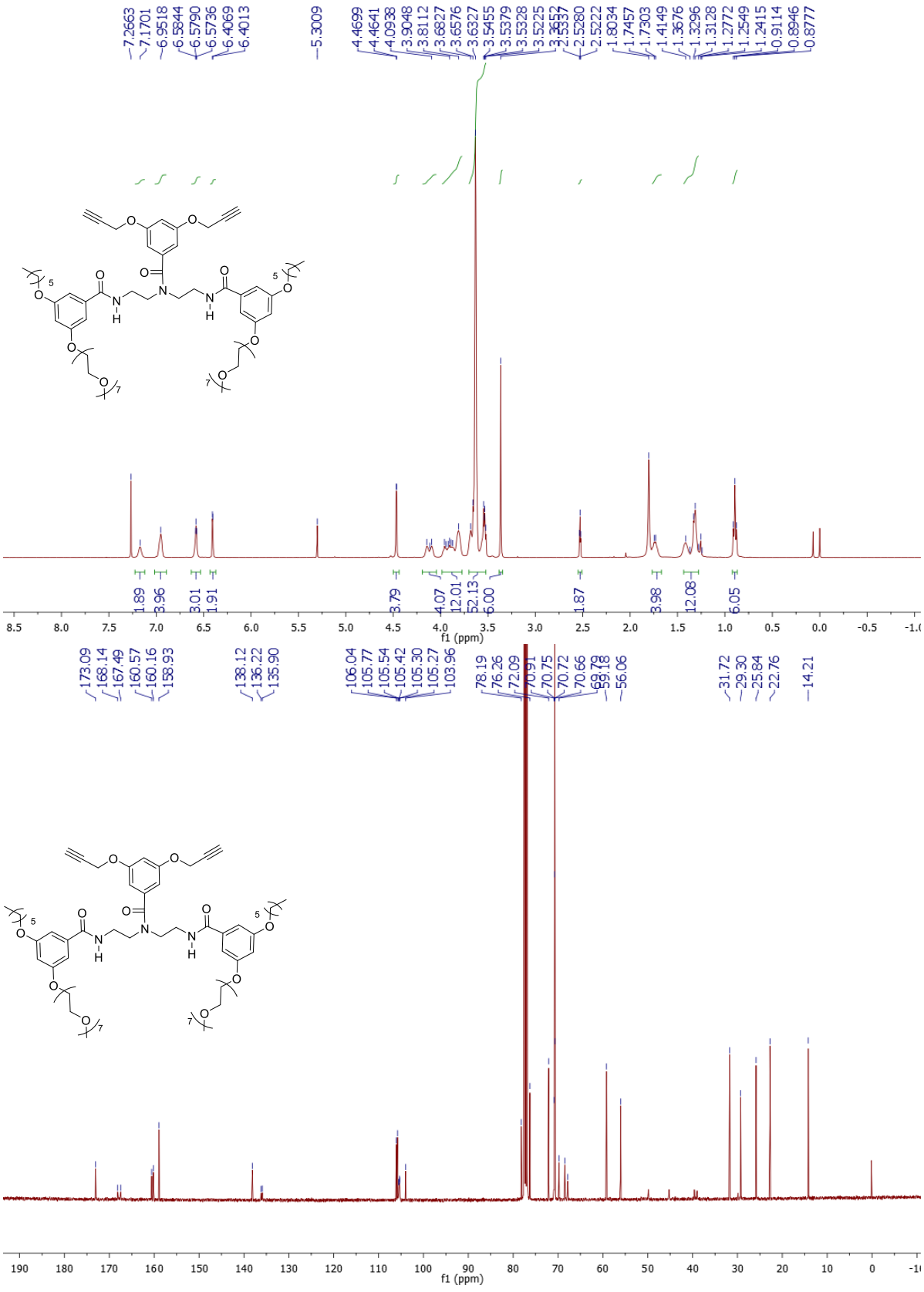
45.21
39.28
39.04
28.58
f1 (ppm)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3.5379
3.5328
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2.5222
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1.7303
1.4149
1.3676
1.3296
1.3128
1.2772
1.2549
1.2415
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0.8946
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1.89
3.96
3.01
1.91
3.79
4.07
12.01
52.13
6.00
1.87
3.98
12.08
6.05
f1 (ppm)
173.09
168.14
167.49
160.57
160.16
158.93
138.12
136.22
135.90
106.04
105.77
105.54
105.42
105.30
105.27
103.96
78.19
76.26
72.09
70.91
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56.06
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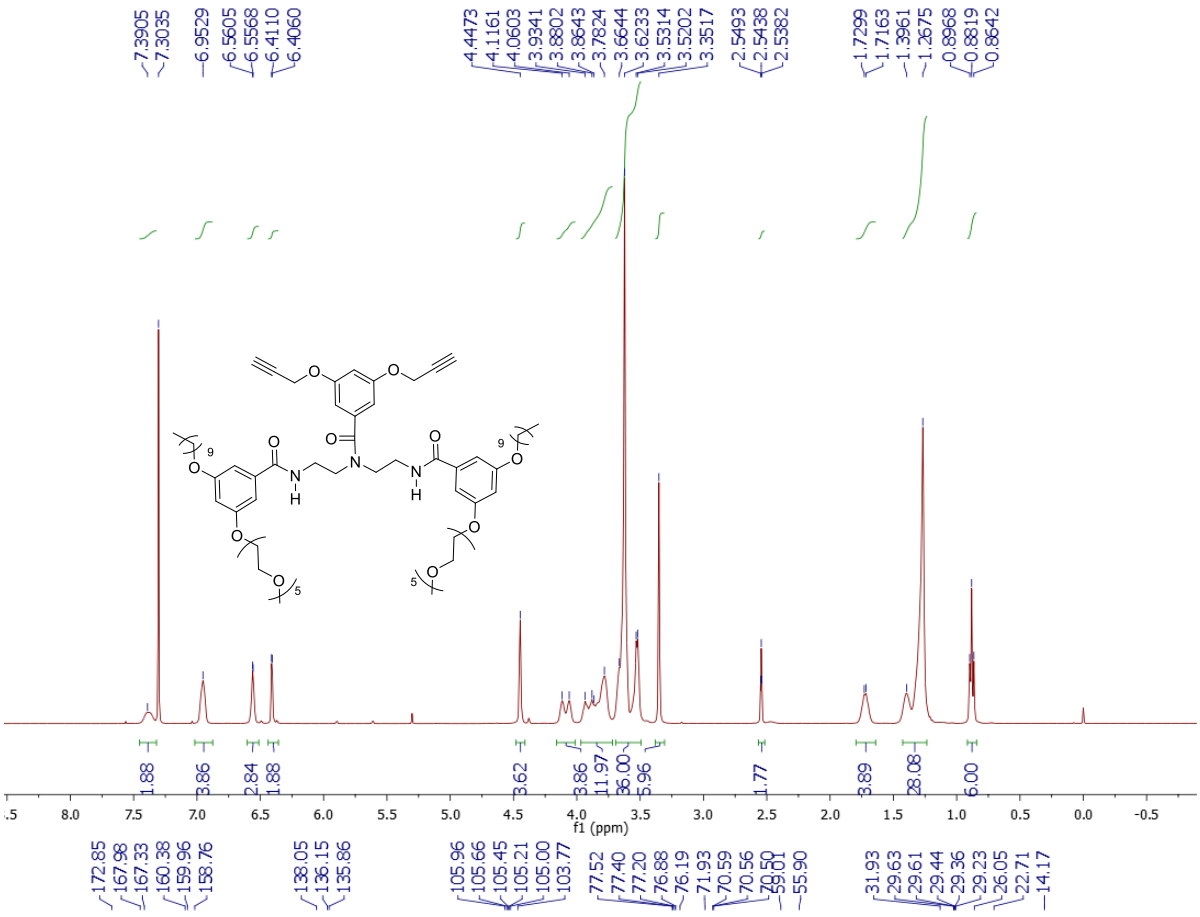
22.76
14.21
f1 (ppm)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3.8802
3.8643
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3.5314
3.5202
3.3517
2.5493
2.5438
2.5382
1.7299
1.7163
1.3961
1.2675
0.8968
0.8819
0.8642
1.88
3.86
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3.86
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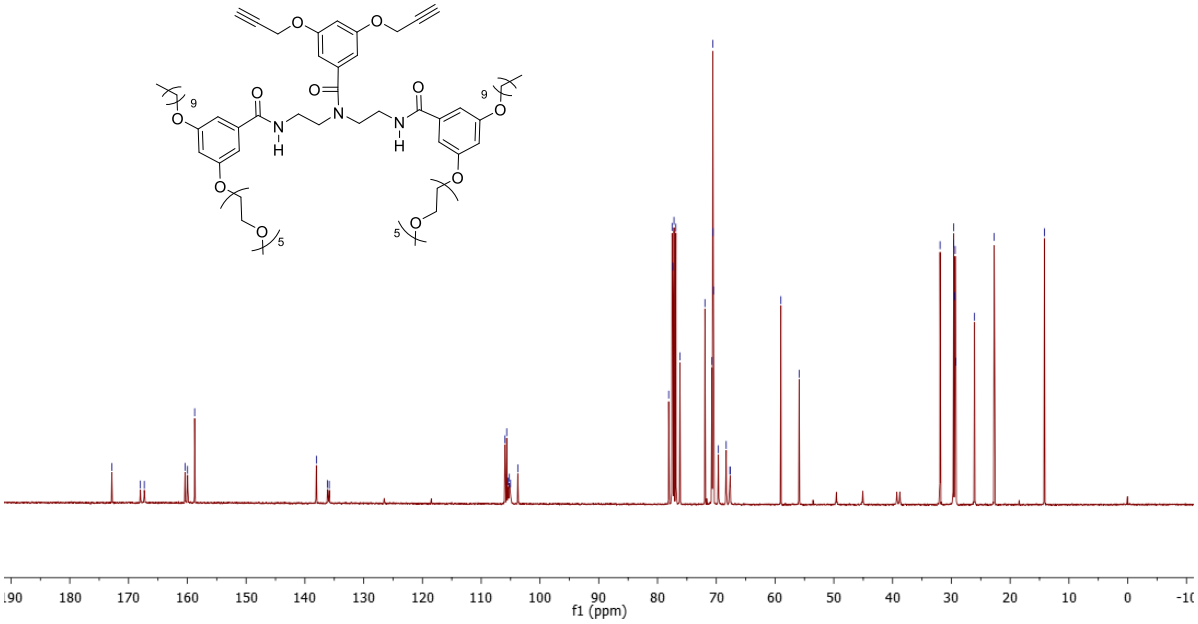
6.00
f1 (ppm)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105.66
105.45
105.21
105.00
103.77
77.52
77.40
77.20
76.88
76.19
71.93
70.59
70.56
59.01
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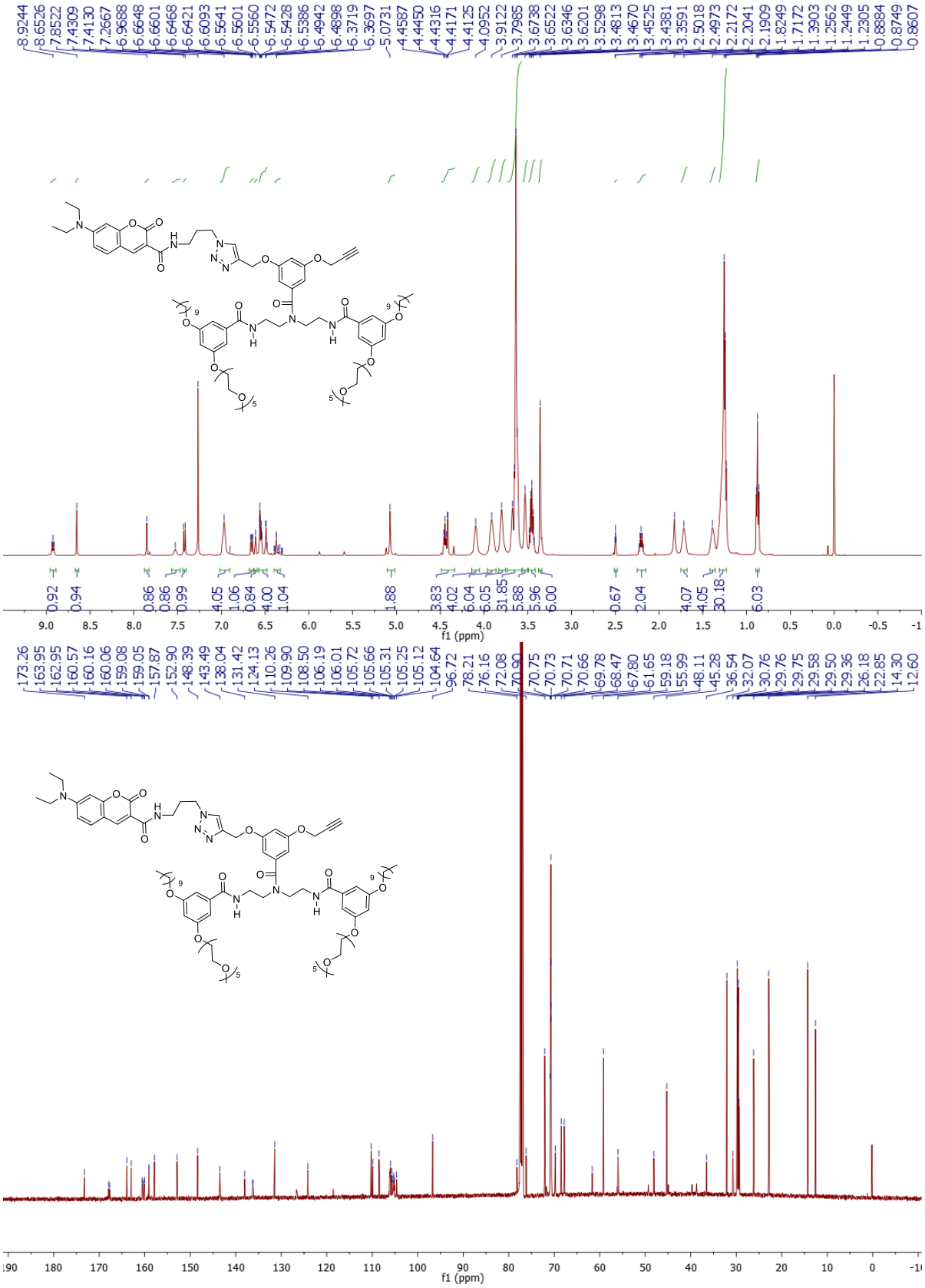
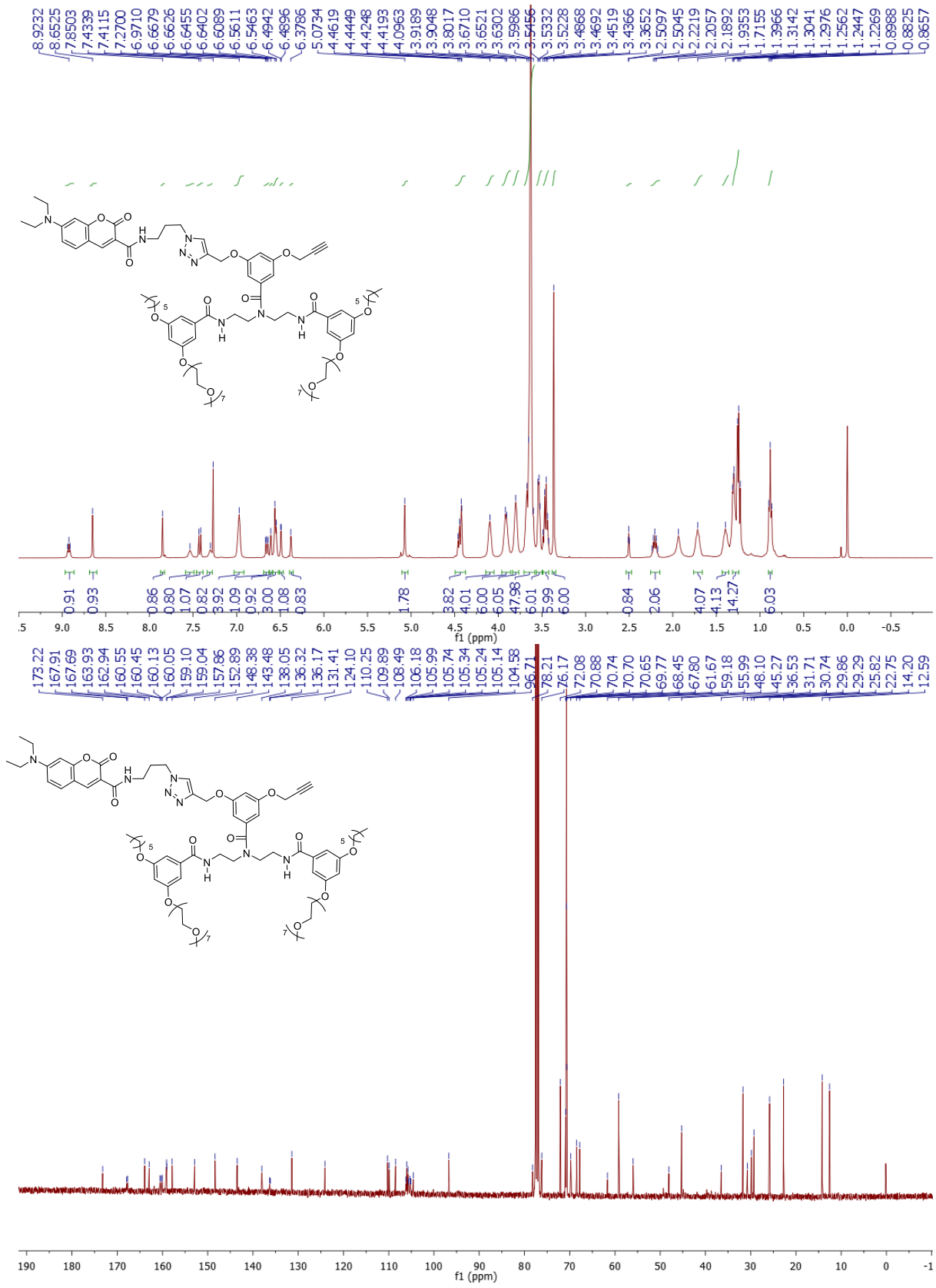
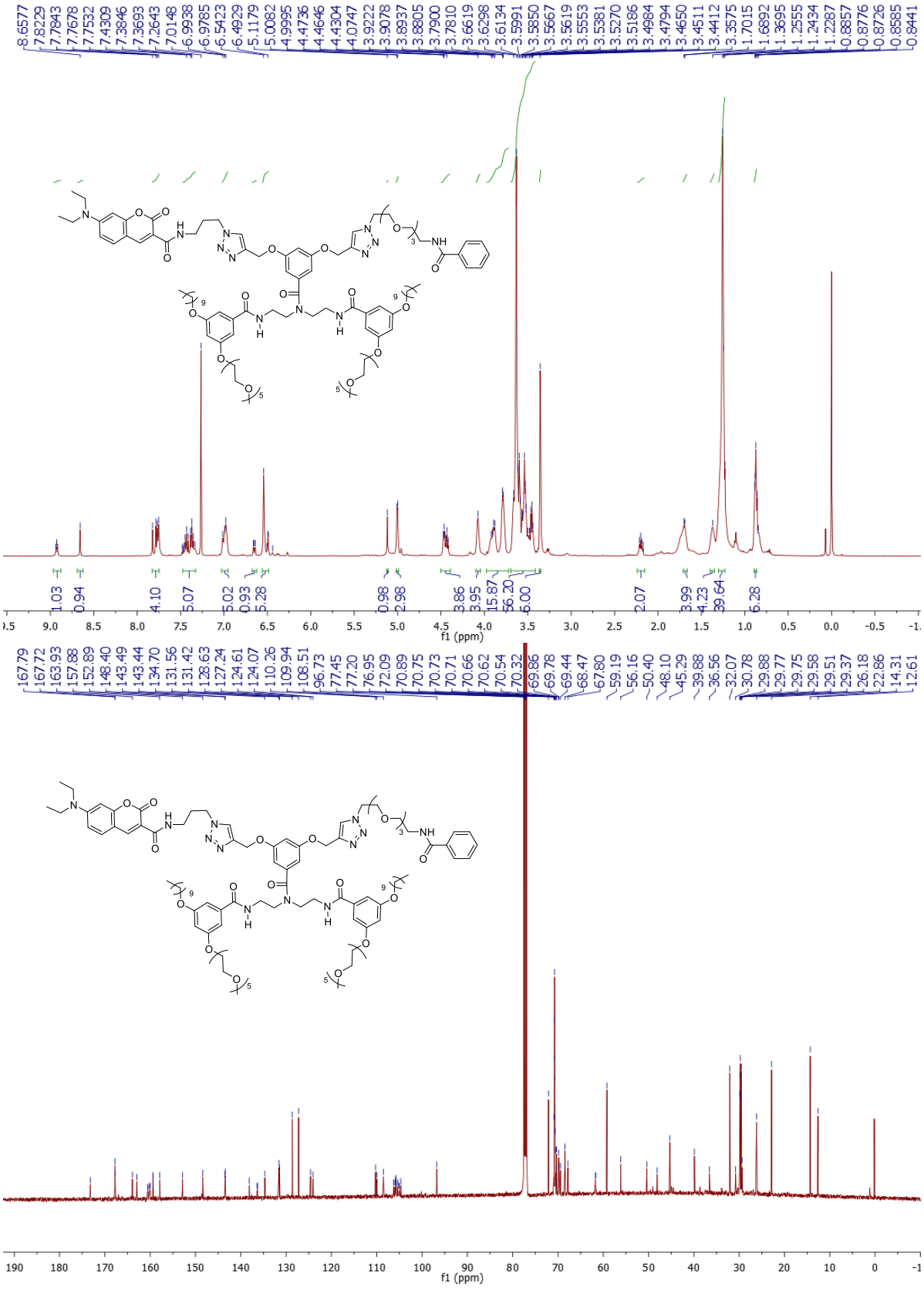
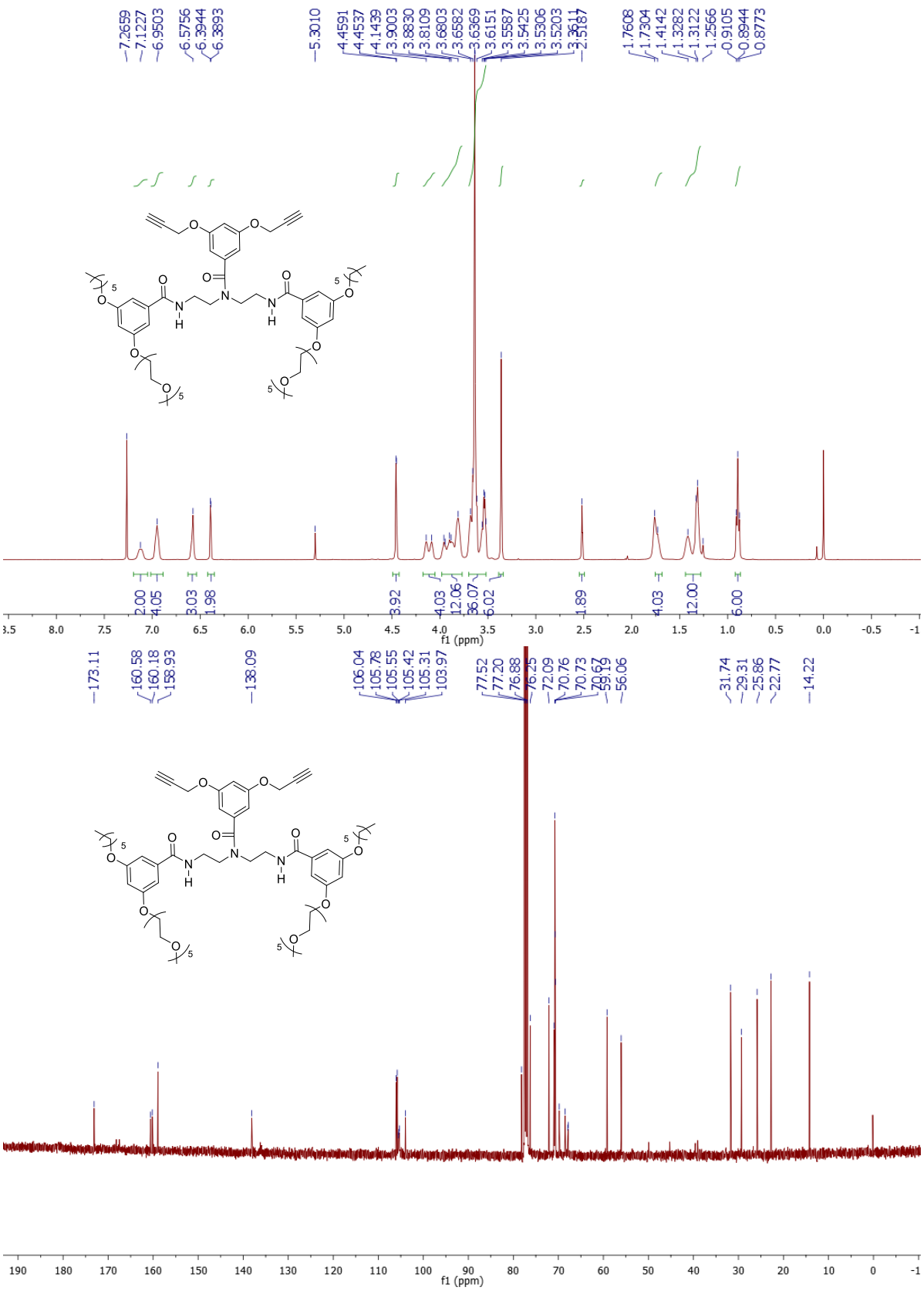
29.63
29.61
29.44
29.36
29.23
26.05
22.71
14.17
f1 (ppm)






f1 (ppm)
f1 (ppm)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3.03
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12.06
36.07
6.02
1.89
4.03
12.00
6.00
f1 (ppm)
173.11
160.58
160.18
158.93
138.09
106.04
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77.52
77.20
76.88
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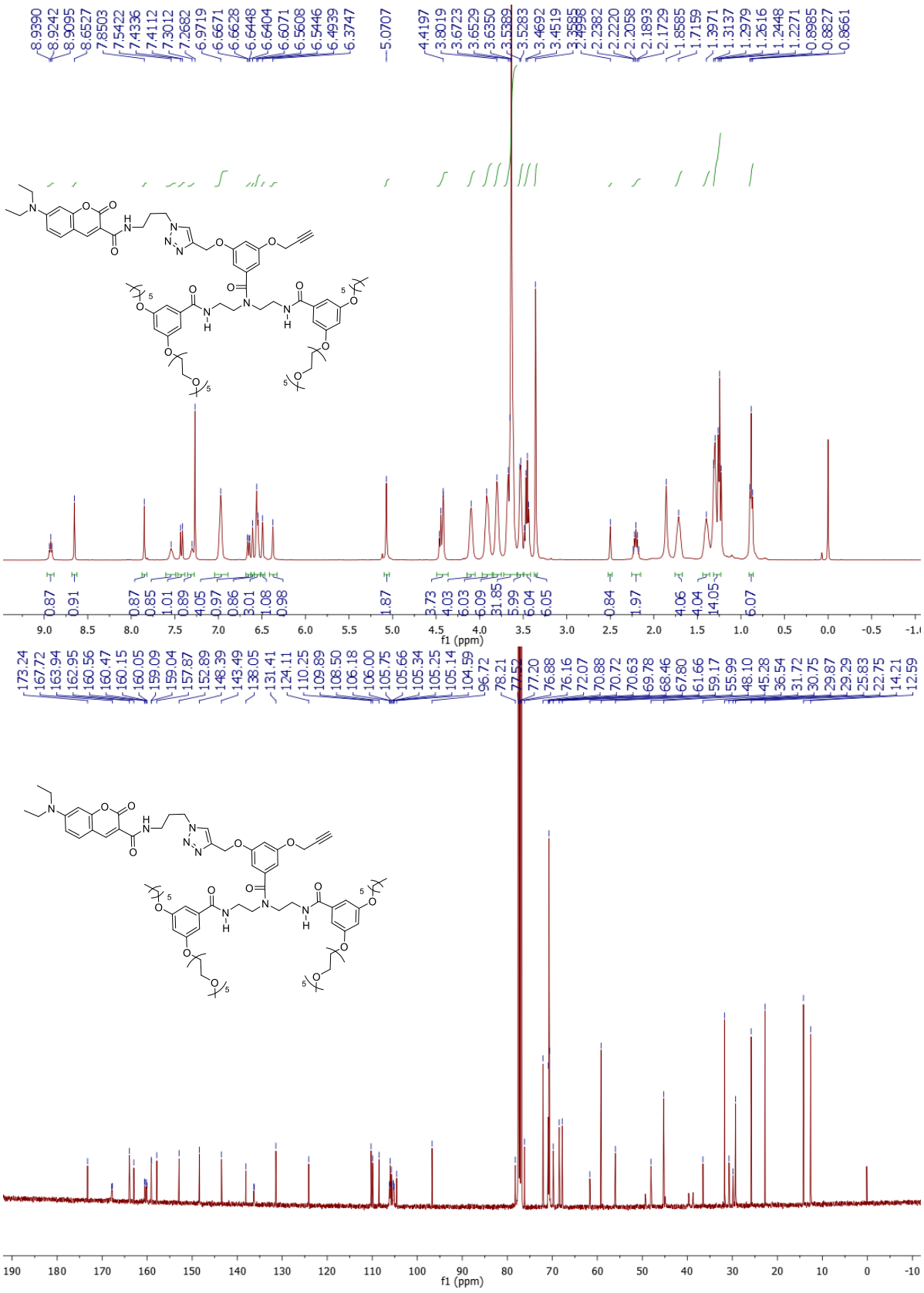
f1 (ppm)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3.01
1.08
0.98
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4.03
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6.09
31.85
5.99
6.04
6.05
0.84
1.97
4.06
4.04
14.05
6.07
f1 (ppm)
173.24
167.72
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162.95
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152.89
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105.25
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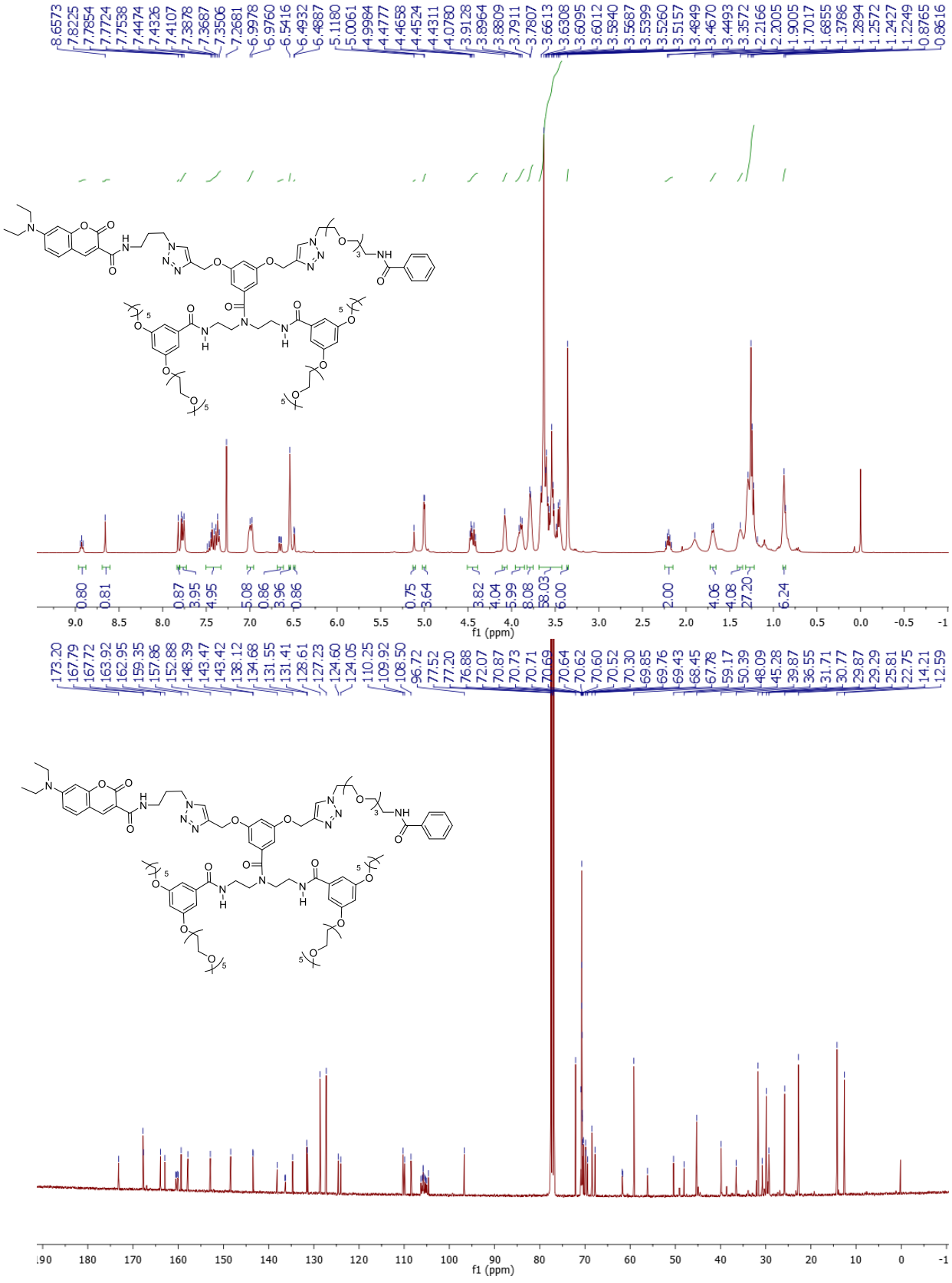
12.59
f1 (ppm)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6.24
f1 (ppm)
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167.72
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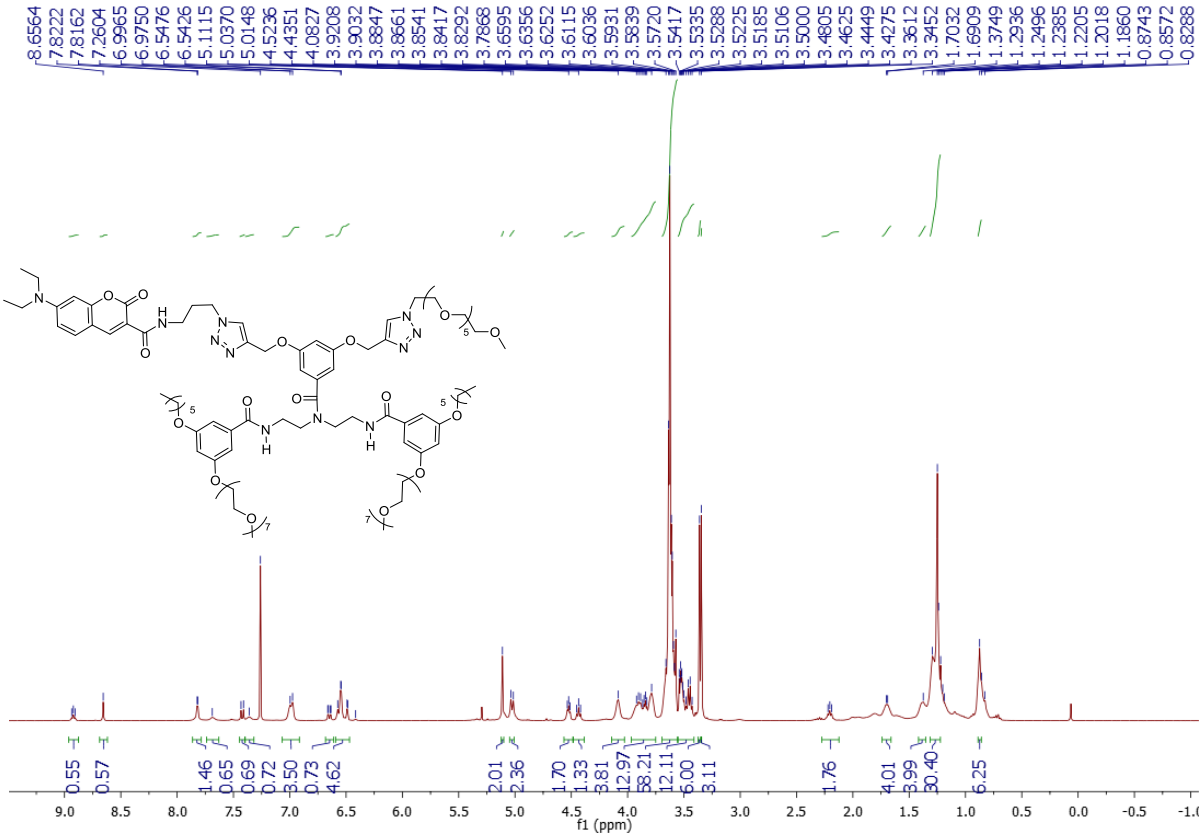

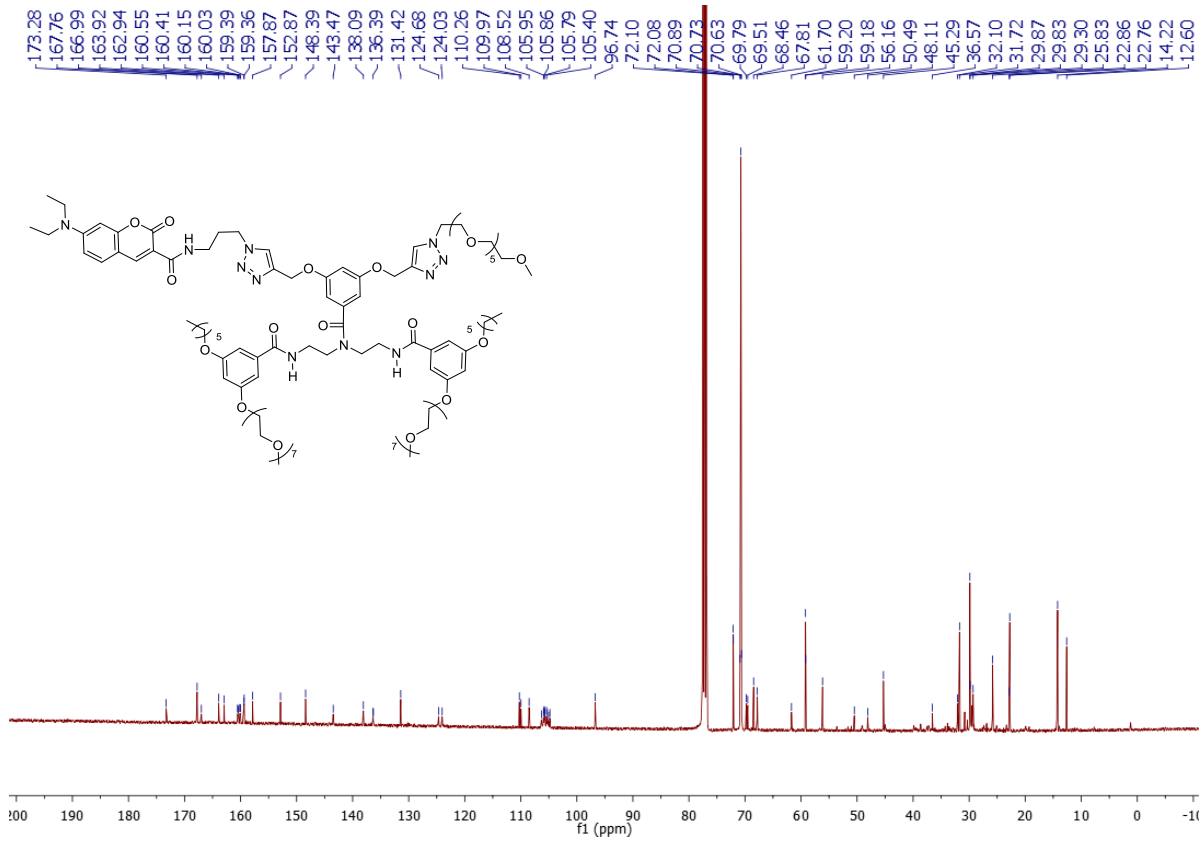

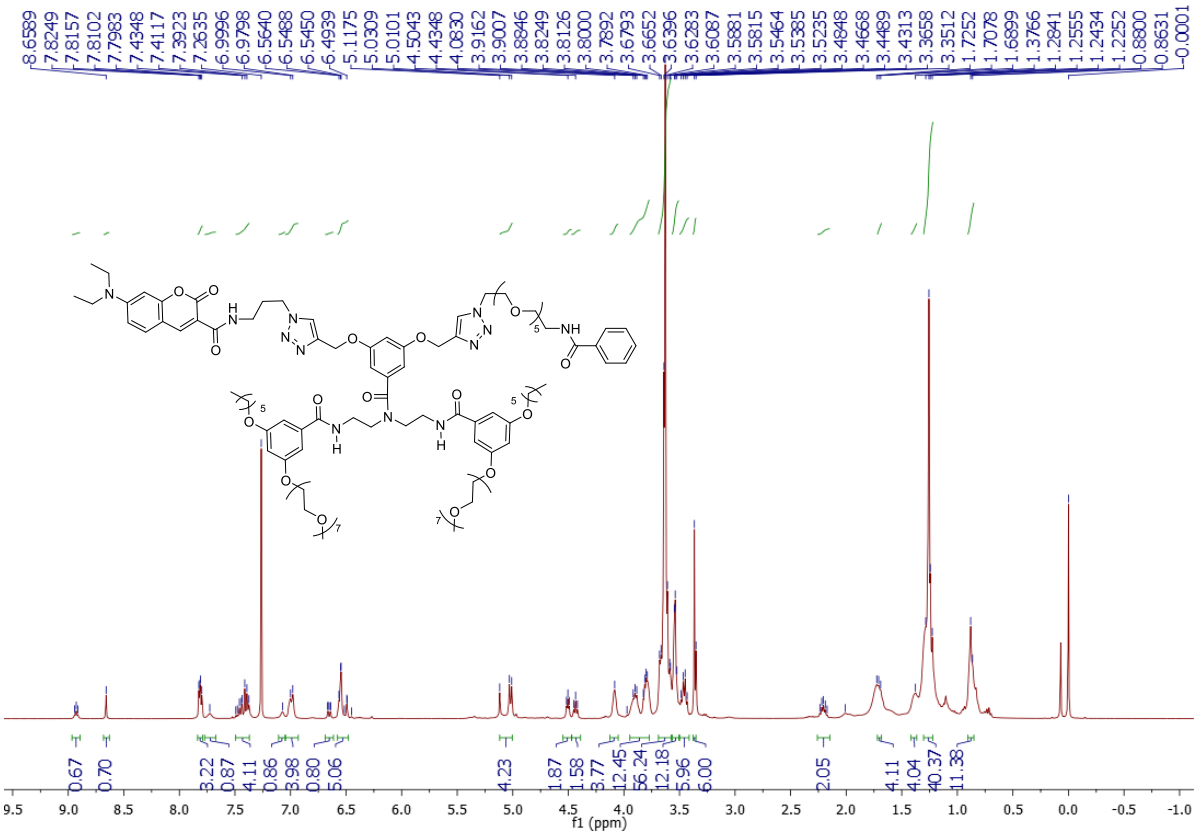

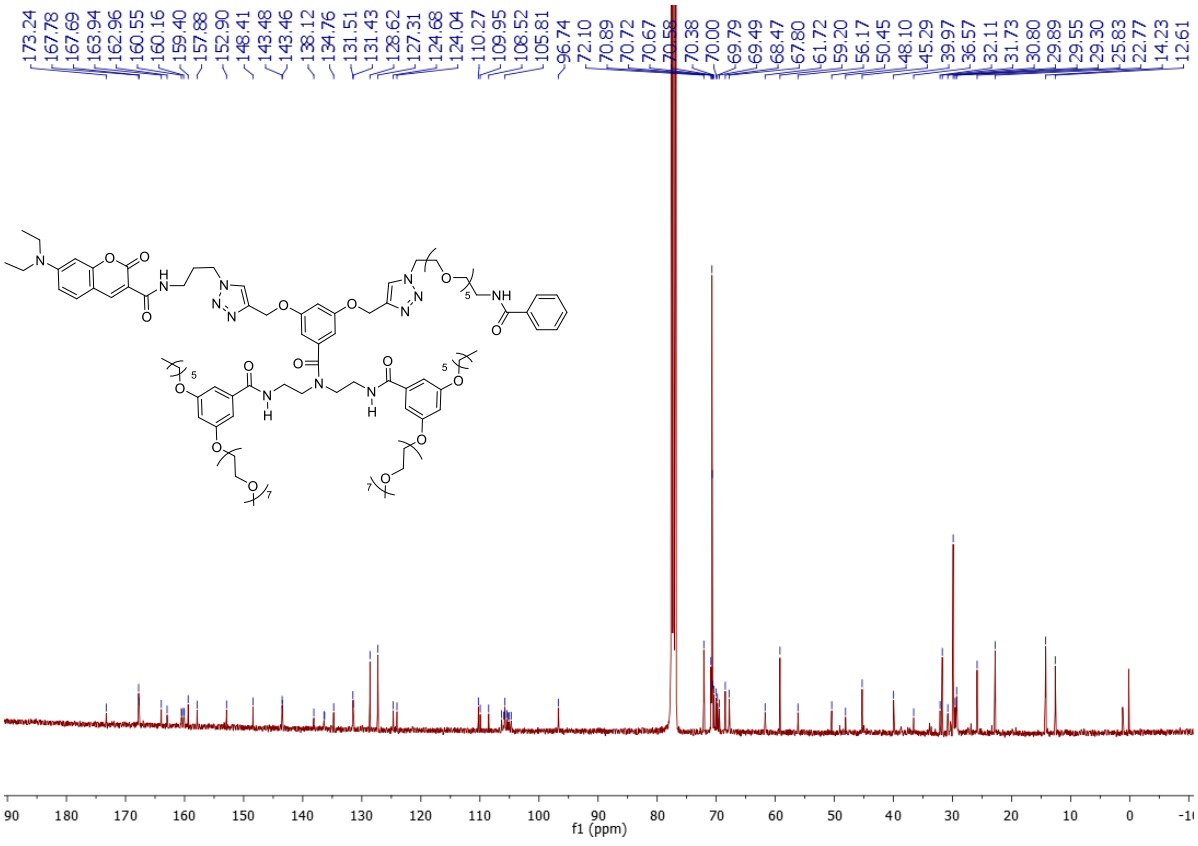

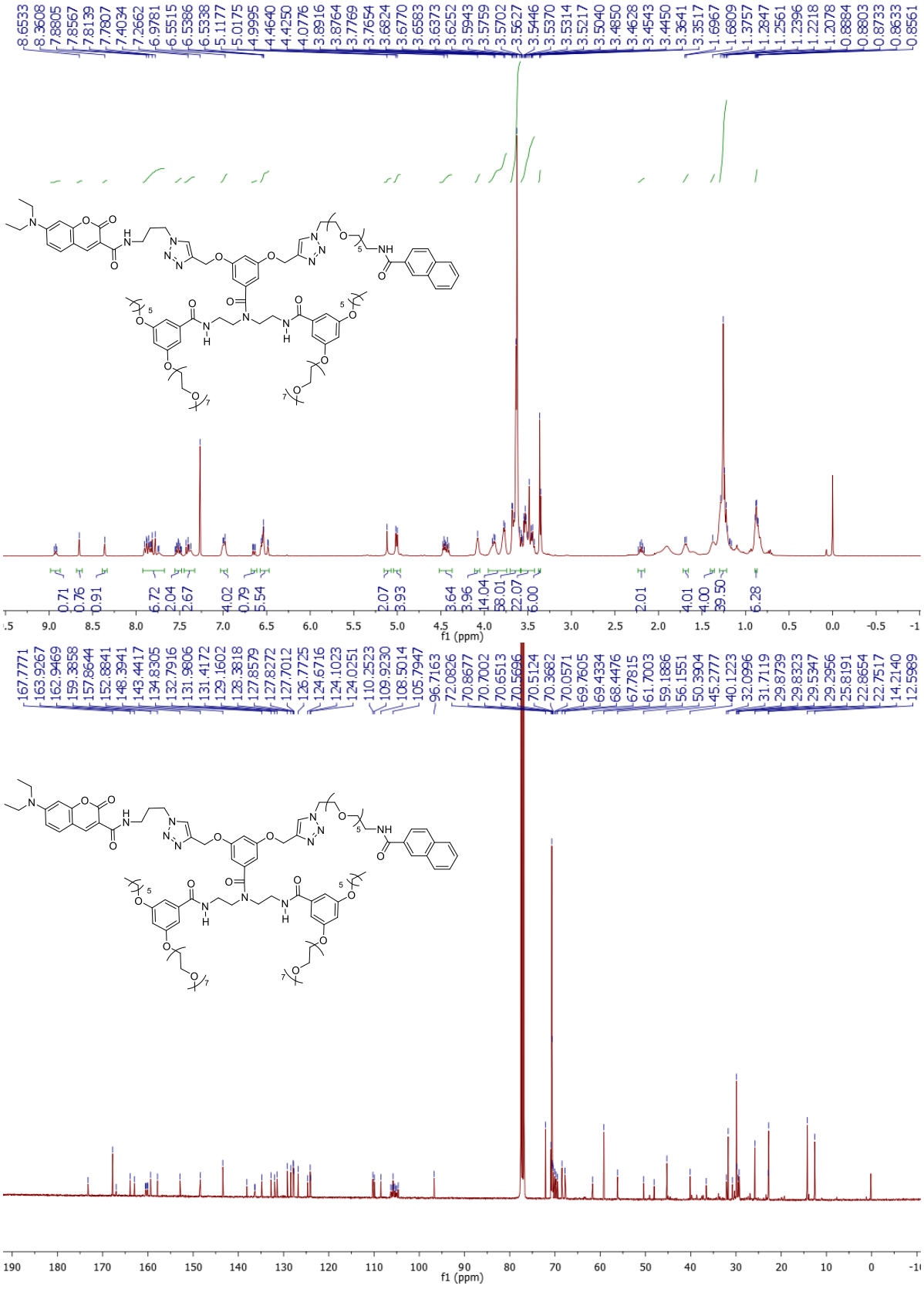

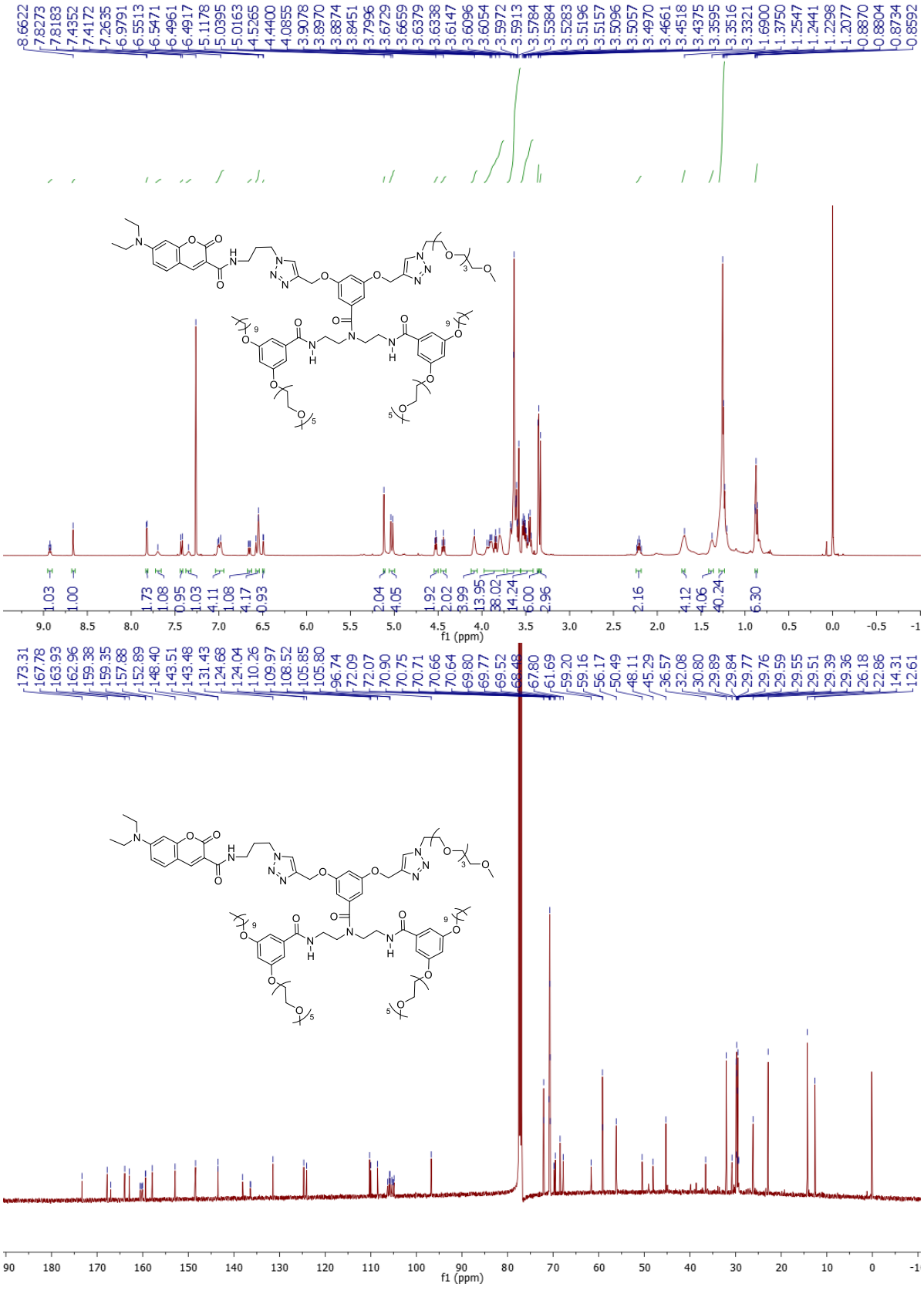
f1 (ppm)
f1 (ppm)