Electronic Supporting Information (ESI)

Polar Dibenzocyclooctynes for Selective Labeling of Extracellular Glycoconjugates of Living Cells

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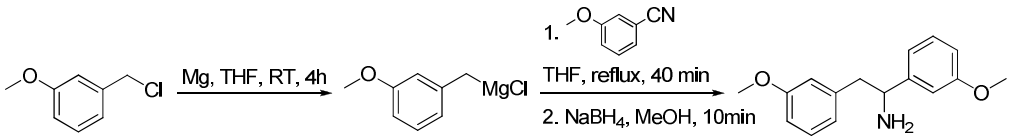
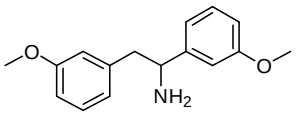
Materials and Methods

All solvents were of reagent grade and used as received. Dichloromethane was distilled over calcium hydride. All reagents were purchased from Sigma-Aldrich® unless stated otherwise. Aluminum chloride ultra dry 99.99% (metal basis) was purchased from Alfa Aesar®. Room temperature refers to ambient temperature (20-22 °C). Reactions were monitored by Thin Layer Chromatography (TLC) using aluminum backed silica gel 60 (F254) plates, visualized using UV254nm and potassium permanganate, ninhydrin and cerium molibdate dips as appropriate. Column chromatography was carried out using silica gel G60 (SiliCycle, 60-200μm, 60 Å) as the stationary phase. Sulfated compounds were purified using C-18 silica gel (Waters Bondapak C-18, 37-55 μm, 125 Å) as the stationary phase. Dowex Na^+ resin was prepared as follows: the Dowex 50 WX8 200 mesh H^+ resin from Sigma-Aldrich® was washed with two volumes (relative to the resin) of H_2O, five volumes of 1N NaOH, and four volumes of H_2O. IR spectra were recorded on a FT-IR Thermo-Nicolet 6700 spectrophotometer. The NMR spectra were recorded on a Varian Mercury 300 MHz and Varian Inova 500 MHz spectrometers. Chemical shifts are reported in δ units, parts per million (ppm) downfield from TMS. Coupling constants (*J*) are measured in Hertz (Hz) and are unadjusted; therefore, due to limits in resolution, in some cases there are small differences (<1 Hz) in the measured *J* value of the same coupling constant determined from different signals. Splitting patterns are designed as follows: s – singlet, d – doublet, t – triplet, dd – doublet of doublets, dt – doublet of triplets, td – triplet of doublets, ddd – doublet of doublet of doublets, tt – triplet of triplets, sp – septet, m – multiplet, br – broad. Various 2D techniques and DEPT experiments were used to establish the structures and to assign the signals. High-resolution mass spectra were obtained by using MALDI-ToF (Applied Biosystems SciEx 5800 instrument) with 2,5-dihydroxybenzoic acid as a matrix. Reverse-Phase HPLC was performed on an Agilent 1200 series system equipped with an automated injector, UV-detector, fraction-collector and Agilent Eclipse XDB-C18 column (5 μm, 4.6 × 150 mm). The rate measurements of cycloaddition of various dibenzocyclooctynes derivatives with benzyl azide were conducted by using Cary 100 UV-Vis spectrophotometer at 25 ± 0.1°C. Photoreactions were performed on a Rayonet RMR 600 photoreactor using 4×350 nm lamps.

Experimental Procedures



1,2-Bis(3-methoxyphenyl)ethylamine 9.

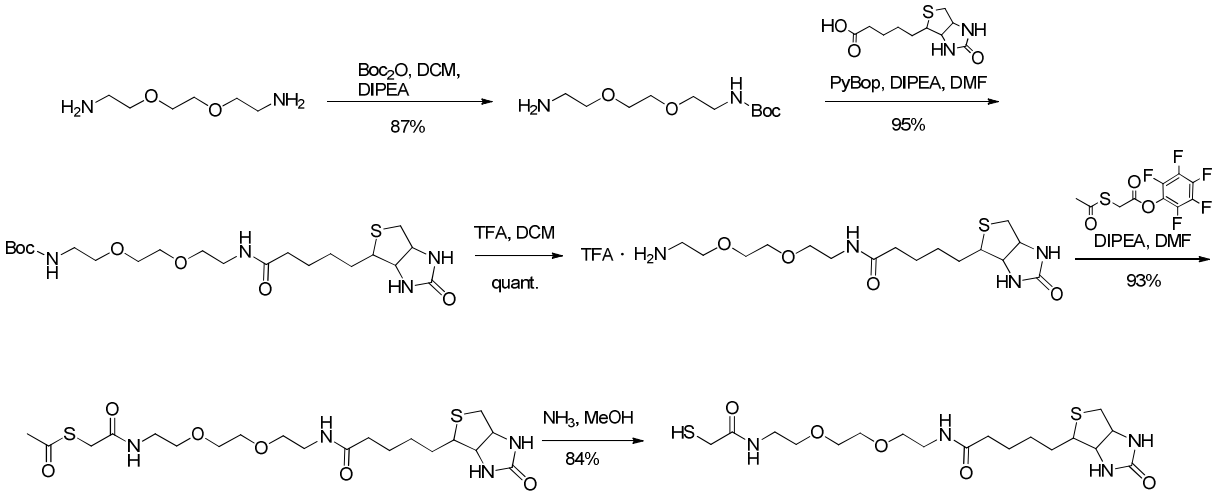


Method A: From commercially available 3-methoxybenzylmagnesium chloride. 3-Methoxybenzonitrile 1 (50 µL, 0.4 mmol) was added to a solution of 3-methoxybenzylmagnesium chloride in THF (0.25 M, 4.8 mL, 1.2 mmol). The reaction mixture was then heated at 100 °C for 10 min under microwave irradiation. After cooling down to room temperature , the mixture was added to a solution of sodium borohydride (31 mg, 0.8 mmol) in methanol (2 mL). The reaction mixture was then stirred at room temperature for 5 min. An aqueous solution of NaOH (0.25 M, 5 mL) was then added and the organic phase was extracted with ethyl acetate (3 × 5 mL), dried over $MgSO_4$ and concentrated under vacuum. The residue was purified by flash chromatography on silica gel (7 g) using 5% MeOH in CH_2Cl_2, affording pure amine **9** as colorless oil (101 mg, 98%).

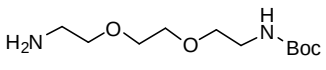
Method B: With formation of 3-methoxybenzylmagnesium chloride. A solution of 3-methoxybenzyl chloride (3 mL, 20 mmol) in dry THF (30 mL) was added drop wise to a mixture of magnesium turnings (2.4 g, 100 mmol) and 1,2-dibromoethane (ca. 100 µL) in dry THF (5 mL) over a period of 3.5 h. (Slow addition of 3-methoxybenzyl chloride and dilute condition suppress the formation of homocoupling byproduct. For the same reason the reaction temperature should not exceed the ambient temperature at any time except for the few min following the activation of magnesium with 1,2-dibromoethane.) After stirring for an additional 30 min, the resulting solution of 3-methoxybenzylmagnesium chloride was transferred to a 100 mL flask containing 3-methoxybenzonitrile (1 g, 10 mmol). The reaction mixture was then refluxed for 40 min (After TLC monitoring showed the complete consumption of the nitrile). The resulting mixture was then added slowly to a solution of sodium borohydride (0.8 g, 20 mmol) in dry MeOH (50 mL). The reaction mixture was stirred for 10 min and concentrated under vacuum. The residue was suspended in water (30 mL) and the organic phase was extracted with CH_2Cl_2 (3 × 50 mL), dried over $MgSO_4$, concentrated under reduced pressure and the

resulting residue was purified by flash chromatography on silica gel using a gradient of 3 to 10% MeOH in CH_2Cl_2 as eluent, affording pure amine **9** as colorless oil (2.4g, 95% based on the nitrile): **^{1}H NMR** (300 MHz, $CDCl_3$) δ 1.59 (brs, 2H, NH_2), 2.66 (dd, J = 13.3, 8.9 Hz, 1H, C*H*HCHNH_2), 2.86 (dd, J = 13.3, 4.8 Hz, 1H, CH*H*CHNH_2), 3.63 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 4.04 (dd, J = 8.9, 4.8 Hz, 1H, C*H*NH_2), 6.58-6.70 (m, 4H, CH_{Ar}), 6.79-6.85 (m, 2H, CH_{Ar}), 7.04-7.16 (m, 2H, CH_{Ar}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 46.40 (CH_2), 55.06 (OCH_3), 55.15 (OCH_3), 57.37 (CH_{Ar}), 111.88 (CH_{Ar}), 111.92 (CH_{Ar}), 112.53 (CH_{Ar}), 118.76 (CH_{Ar}), 114.85 (CH_{Ar}), 121.66 (CH_{Ar}), 129.35 (2×CH_{Ar}), 140.56 (C_{Ar}), 147.28 (C_{Ar}), 159.59 (C-OMe), 159.69 (C-OMe); **HRMS** (MALDI) 258.1219 ($C_{16}H_{20}NO_2$ (MH^+) requires 258.1494).

*Synthesis of the biotinylated thiol **17**:*

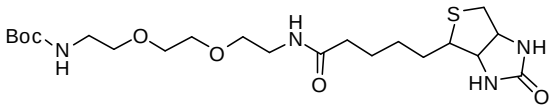


tert-Butyl 2-(2-(2-aminoethoxy)ethoxy)ethylcarbamate 20.



A solution of di-*tert*-butyl dicarbonate (1.0 g, 4.6 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise to a stirred solution of 1,2-bis(2-aminoethoxy)ethane (2.0 g, 13.7 mmol) and *N*-ethyldiisopropylamine (0.8 mL, 4.6 mmol) in dry CH_2Cl_2 (15 mL) at 0 ˚C. The reaction mixture was allowed to warm to room temperature and stirred for 18 h. Volatiles were removed under reduced pressure and the residue was purified by flash column chromatography on silica gel using a gradient of 5 to 15% methanol in CH_2Cl_2:NH_4OH (1000:5, v:v) to give pure **20** as a colorless oil (1.0 g, 87%): **^{1}H NMR** (300 MHz, $CDCl_3$) δ 1.43 (s, 9H, 3×CH_3), 1.57 (s, 2H, NH), 2.87 (t, J = 5.2 Hz, 2H, C$H_2$$NH_2$), 3.31 (dd, J = 10.2, 5.1 Hz, 2H, CH_2O), 3.49-3.56 (m, 4H, 2×CH_2O), 3.61 (s, 4H, 2×CH_2O), 5.14 (brs, 1H, NH); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 28.40 (3×CH_3), 40.33 (CH_2NH_2), 41.72 (CH_2NH), 70.19 (3×CH_2O), 73.41 (CH_2O), 79.16 (C(CH_3)$_3$), 155.98 (C=O); **HRMS** (MALDI) 249.0294 ($C_{11}H_{25}N_2O_4$ (MH^+) requires 249.1809).

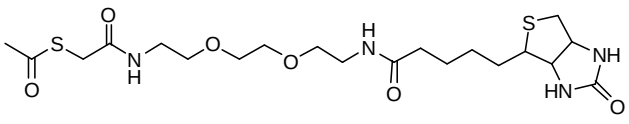
***N*-Boc-*N*'-biotinyl-3,6-dioxaoctane-1,8-diamine 21.**



A solution of tert-butyl 2-(2-(2-aminoethoxy)ethoxy)ethylcarbamate **20** (500 mg, 2 mmol) in dry DMF (5 mL) was added to a stirred solution of Biotin (580 mg, 2.4 mmol), (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBop) (1.25 g, 2.4 mmol) and *N*-ethyldiisopropylamine (0.7 mL, 4.0 mmol) in dry DMF (10 mL). The reaction mixture was stirred at room temperature for 18 h. Volatiles were removed under reduced pressure and the residue was purified by flash column chromatography on silica gel using a gradient of 5 to 10% methanol in CH_2Cl_2 to give pure **21** as an amorphous solid (900 mg, 95%): **IR** (KBr) ν 3300 (s, br, NH), 3117 (w), 2929 (s), 2866 (s), 1712 (s, C=O), 1646 (s), 1552 (m), 1461 (w), 1366 (w), 1278 (w), 1249 (w), 1169 (w), 1119 (m), 859 (w) cm^{-1}; **^{1}H NMR** (300 MHz, CD_3OD) δ 1.44 (s, 11H, CH_2, 3×CH_3), 1.55-1.80 (m, 4H, 2×CH_2), 2.23 (t, J = 7.3 Hz, 2H, CH_2CO), 2.71 (d, J = 12.7 Hz, 1H, SCHH), 2.93 (dd, J = 12.7, 4.9 Hz, 1H, SCHH), 3.19-3.26 (m, 3H, CHS, CH_2NH), 3.33-3.40 (m, 2H, CH_2NH), 3.52-3.58 (m, 4H, 2×CH_2O), 3.62 (s, 4H, 2×CH_2O), 4.32 (dd, J = 7.8, 4.4 Hz, 1H, CHNH), 4.50 (dd, J = 7.7, 4.8 Hz, 1H, CHNH); **^{13}C NMR** (75.5 MHz, CD_3OD) δ 26.80 (CH_2), 28.78 (3×CH_3), 29.45 (CH_2), 29.72 (CH_2), 36.70 ($C$$H_2$CO), 40.25 ($CH_2NH$), 41.03 ($CH_2NH$), 41.19 ($SCH_2$), 56.96 (SCH), 61.56 (CHNH), 63.30

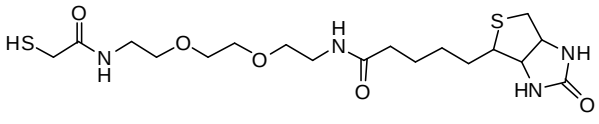
(CHNH), 70.59 (CH_2O), 71.03 (CH_2O), 71.23 (2×CH_2O), 80.03 ($C(CH_3)_3$), 158.33 (C=O), 166.00 (C=O), 176.03 (C=O); **HRMS** (MALDI) 497.1021 ($C_{21}H_{38}N_4NaO_6S$ ($M+Na^+$) requires 497.2404).

***N*-Acetyl-ethanethiolate-*N*'-biotinyl-3,6-dioxaoctane-1,8-diamine 22.**



Trifluoroacetic acid (2 mL) was added to a stirred suspension of biotin derivative **21** (500 mg, 1.05 mmol) in dry CH_2Cl_2 (8 mL). The resulting mixture was stirred at room temperature for 2 h, then concentrated under reduced pressure, coevaporated with toluene (3×30 mL) and the residue was dried overnight in vacuum to give a TFA salt of unprotected **21**. A solution of the resulting TFA salt (500 mg, 1.05 mmol) in DMF (5 mL) was added to a solution of *S*-acetylthioglycolic acid pentafluorophenyl ester (470 mg, 1.57 mmol) and *N*-ethyldiisopropylamine (0.7 mL, 4.0 mmol) in dry DMF (5 mL). The resulting mixture was then stirred at room temperature for 18 h. The volatiles were evaporated under reduced pressure and the residue was purified by flash column chromatography on silica gel using a gradient of 5 to 10% methanol in CH_2Cl_2 to give pure **22** as a white solid (480 mg, 93%): **IR** (KBr) *v* 3277 (s, br, NH), 2931 (m), 2856 (m), 1710 (s), 1695 (s), 1649 (s), 1550 (m), 1462 (w), 1426 (w), 1303 (w), 1241 (w), 1134 (s), 978 (w) cm^{-1}; **^{1}H NMR** (300 MHz, d_6-DMSO) δ 1.23-1.81 (m, 6H, 3×CH_2), 2.06 (t, J = 7.3 Hz, 2H, CH_2CO), 2.35 (s, 3H, CH_3), 2.57 (d, J = 12.4 Hz, 1H, SC*H*H), 2.81 (dd, J = 12.4, 5.0 Hz, 1H, SCH*H*), 3.06-3.22 (m, 5H, CHS, 2×CH_2NH), 3.34-3.41 (m, 4H, 2×CH_2O), 3.50 (s, 4H, 2×CH_2O), 3.59 (s, 2H, CH_2S), 4.10-4.14 (m, 1H, C*H*NH), 4.28-4.32 (m, 1H, C*H*NH), 6.37-6.43 (m, 2H, 2×NH), 7.84 (t, J = 5.2 Hz, 1H, NH), 8.18 (t, J = 4.5 Hz, 1H, NH); **^{13}C NMR** (75.5 MHz, d_6-DMSO) δ 25.33 (CH_2), 28.10 (CH_2), 28.27 (CH_2), 30.16 (CH_3), 32.61 (CH_2), 35.16 (CH_2CO), 38.51 (CH_2NH), 39.09 (CH_2NH), 39.94 (SCH_2), 55.50 (SCH), 59.28 (CHNH), 61.12 (CHNH), 68.92 (CH_2O), 69.23 (CH_2O), 69.57 (CH_2O), 69.61 (CH_2O), 162.85 (C=O), 167.00 (C=O), 172.24 (C=O), 194.53 (C=O); **HRMS** (MALDI) 513.0952 ($C_{20}H_{34}N_4NaO_6S_2$ ($M+Na^+$) requires 513.1812).

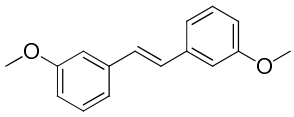
***N*-2-Sulfanylacetamide-*N*'-biotinyl-3,6-dioxaoctane-1,8-diamine 17.**



Biotin derivative **22** (400 mg, 0.82 mmol) was dissolved in a solution of ammonia in methanol (7N, 10 mL) and the resulting mixture was stirred at room temperature for 2 h. The volatiles were evaporated under reduced pressure and the residue was purified by flash column chromatography on silica gel using a gradient of 5 to 15% methanol in CH_2Cl_2 to give pure **17** as a white solid (310 mg, 84%): **^{1}H NMR** (300 MHz, CD_3OD) δ 1.37-1.82 (m, 6H, 3×CH_2), 2.27 (t, J = 7.3 Hz, 2H, CH_2CO), 2.75 (d, J = 12.7 Hz, 1H, SC*H*H), 2.97 (dd, J = 12.7, 4.9 Hz, 1H, SCH*H*), 3.22-3.32 (m, 3H, CH_2SH, CHS), 3.40-3.43 (m, 4H, 2×CH_2NH), 3.55-3.60 (m, 4H, 2×CH_2O), 3.67 (s, 4H, 2×CH_2O), 4.36 (dd, J = 7.9, 4.4 Hz, 1H, C*H*NH), 4.55 (dd, J = 7.8, 4.3 Hz, 1H, C*H*NH); **^{13}C NMR** (75.5 MHz, CD_3OD) δ 26.83 (CH_2), 28.22 (CH_2), 29.48 (CH_2), 29.74 (CH_2), 36.74 (*C*H_2CO), 40.26 (CH_2NH), 40.68 (CH_2NH), 41.06 (SCH_2), 56.98 (SCH), 61.59 (CHNH), 63.33 (CHNH), 70.38 (CH_2O), 70.59 (CH_2O), 71.26 (CH_2O), 71.32 (CH_2O), 166.03 (C=O), 173.33 (C=O), 176.09 (C=O); **HRMS** (MALDI) 471.0500 ($C_{18}H_{32}N_4NaO_5S_2$ (MNa^+) requires 471.1706).

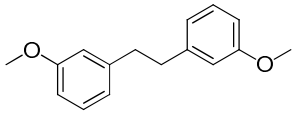
For the purpose of kinetic data, the following compounds were synthesized:

(*E*)-3,3'-Dimethoxystilbene 23.



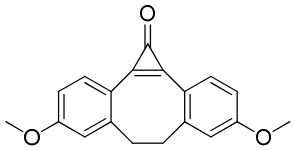
A solution of 3-vinylanisole (0.7 mL, 5 mmol) and Grubbs' catalyst 2nd generation (105 mg, 0.125 mmol) in CH_2Cl_2 (10 mL) was refluxed overnight. After being cooled to room temperature, the reaction mixture was concentrated under vacuum and the residue was purified by flash chromatography on silica gel (16 g) using a mixture of hexanes and ethyl acetate (6:1) affording pure alkene **23** (589 mg, 98%) as a white solid: **^{1}H NMR** (300 MHz, $CDCl_3$) δ 3.86 (s, 6H, 2×OCH_3), 6.84 (dd, J = 7.8, 2.2 Hz, 2H, 2×H_4), 7.06 (brs, 2H, 2×H_2), 7.08 (s, 2H, CH=CH), 7.12 (d, J = 7.8 Hz, 2H, 2×H_6), 7.29 (t, J = 7.8 Hz, 2H, 2×H_5); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 55.40 (2×CH_3), 111.91 (2×CH), 113.52 (2×CH), 119.42 (2×CH), 129.05 (CH=CH), 129.78 (2×CH), 138.83 (2×C-1), 160.04 (2×C-3); **HRMS** (MALDI) 240.1113 ($C_{16}H_{16}O_2$ (M^+) requires 240.1150).

1-Methoxy-3-[2-(3-methoxyphenyl)ethyl]benzene 24.



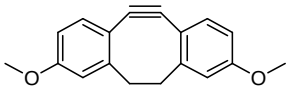
A suspension of dimethoxystilbene **23** (589 mg, 2.45 mmol) and 10% Pd/C (118 mg, 20% w/w) in methanol (40 mL) was stirred at room temperature for 5 h under H_2 (1 atm). The reaction mixture was then filtered over celite and the filtrate was concentrated under vacuum. The residue was then purified by flash chromatography on silica gel (16 g) using a mixture of hexanes and ethyl acetate (6:1) affording pure alkane **24** (587 mg, 99%) as a colorless oil: **^{1}H NMR** (300 MHz, $CDCl_3$) δ 2.99 (s, 4H, 2×CH_2), 3.85 (s, 6H, 2×OCH_3), 6.81-6.85 (m, 4H, 2×H_4, 2×H_2), 6.88 (d, *J* = 7.6 Hz, 2H, 2×H_6), 7.29 (dd, *J* = 8.7, 7.6 Hz, 2H, 2×H_5); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 37.93 (2×CH_2), 55.19 (2×CH_3), 111.38 (2×CH), 114.28 (2×CH), 120.94 (2×CH), 129.39 (2×CH), 143.45 (2×C-1), 159.72 (2×C-3); **HRMS** (MALDI) 265.1241 ($C_{16}H_{18}O_2$ ($M+Na^+$) requires 265.1204).

4,9-Dimethoxy-6,7-dihydro-1H-dibenzo[a,e]cyclopropa[c]cycloocten-1-one 25.



A solution of aluminum chloride (808 mg, 6.06 mmol) and tetrachlorocyclopropene (0.36 mL, 2.90 mmol) in CH_2Cl_2 (15 mL) was stirred at room temperature for 20 min. The reaction mixture was then cooled to –20 °C and a solution of alkane **24** (587 mg, 2.42 mmol) in CH_2Cl_2 (5 mL) was added. The reaction mixture was stirred at –20 °C for 1 h, was then allowed to warm to room temperature over a period of 2 h and was stirred for an extra hour at room temperature. Water (20 mL) was then added and the reaction mixture was stirred for an extra 30 min. The organic layer was then extracted with CH_2Cl_2 (3×20 mL), dried over $MgSO_4$ and concentrated under vacuum. The residue was then purified by flash chromatography on silica gel (16 g) using a mixture of a mixture of 2% MeOH in CH_2Cl_2 affording pure cyclopropenone **25** (453 mg, 64%) as a white solid: **IR** (KBr) *ν* 3008 (w), 2948 (w), 2837 (w), 1846 (s, C=O cyclopropenone), 1600 (s), 1560 (s), 1426 (m), 1342 (w), 1319 (w), 1274 (w), 1251 (m), 1138 (m), 1050 (w), 1023 (m), 872 (w), 812 (m) cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 2.58 (d, *J* = 10.5 Hz, 2H, CH_2), 3.32 (d, *J* = 10.5 Hz, 2H, CH_2), 3.85 (s, 6H, 2×OCH_3), 6.83-6.89 (m, 4H, 2×H_4 ,2×H_2), 7.90 (d, *J* = 9.2 Hz, 2H, 2×H_5); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 37.23 (2×CH_2), 55.59 (2×CH_3), 111.91 (2×CH), 115.82 (2×CH), 116.51 (2×C-6), 135.79 (2×CH), 142.36 (2×C-1), 147.86 (C=C), 153.75 (C=O), 162.47 (2×C-OMe); **HRMS** (MALDI) 292.1001 ($C_{19}H_{16}O_3$ (M^+) requires 292.1099).

2,9-Dimethoxy-5,6-didehydro-11,12-dihydrodibenzo[a,e]cyclooctyne 6.



A solution of cyclopropenone **25** (30 mg, 0.1 mmol) in MeOH (2 mL) was irradiated at 350 nm for 6 h. The solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography on silica gel using a gradient of 10 to 20% acetone in hexanes to give **6** as white solid (24 mg, 91%)**:** ^{1}**H NMR** (300 MHz, $CDCl_3$) δ 2.44 (d, *J* = 11.3 Hz, 2H, CH_2), 3.19 (d, *J* = 11.3 Hz, 2H, CH_2), 3.80 (s, 6H, 2×OCH_3), 6.76 (dd, *J* = 8.4, 2.6 Hz, 2H, 2×H_4), 6.87 (d, *J* = 2.6 Hz, 2H, 2×H_2), 7.20 (d, *J* = 8.4 Hz, 2H, 2×H_5); ^{13}**C NMR** (75.5 MHz, $CDCl_3$) δ 36.76 (2×CH_2), 55.44 (2×CH_3), 110.50 (C≡C), 111.37 (2×CH), 116.26 (2×CH), 116.34 (2×C-6), 126.78 (2×CH), 154.97 (2×C-1), 159.17 (2×C-OMe); **HRMS** (MALDI) 264.1142 ($C_{18}H_{16}O_2$ (M^+) requires 264.1150).

Kinetic Measurements

The rate measurements of cycloaddition of various dibenzocyclooctynes derivatives with benzyl azide were conducted by using Cary 100 UV-Vis spectrophotometer at 25 ± 0.1°C. A calculated amount of 0.25 M solutions of benzyl azide required to achieve the desired concentration ($2.5 \times 10^{-3} - 1.25 \times 10^{-2}$ M) was added to a thermally equilibrated solution of dibenzocyclooctynes (6×10^{-5} M) in MeOH. Reactions were monitored by following the decay of the characteristic absorbance of dibenzocyclooctynes ca. 312 nm. Consumption of starting material followed a first order equation and the pseudo first order rate constants were obtained by least-squares fitting of the data to a single exponential equation (**5**, **6, 7**).

Table S1. Pseudo first order rate constants for the cycloaddition of dibenzocyclooctyne **6** with various concentrations of benzyl azide in MeOH.

Concentration of Benzyl Azide (x 10^{-3}M)	k (s^{-1})
2.5	(1.51 ± 0.02) x 10^{-4}
3.5	(2.77 ± 0.10) x 10^{-4}
5.0	(3.64 ± 0.10) x 10^{-4}
8.0	(6.96 ± 0.57) x 10^{-4}
1.25	(8.83 ± 0.29) x 10^{-4}

Linear plots for determining second order rate constants.

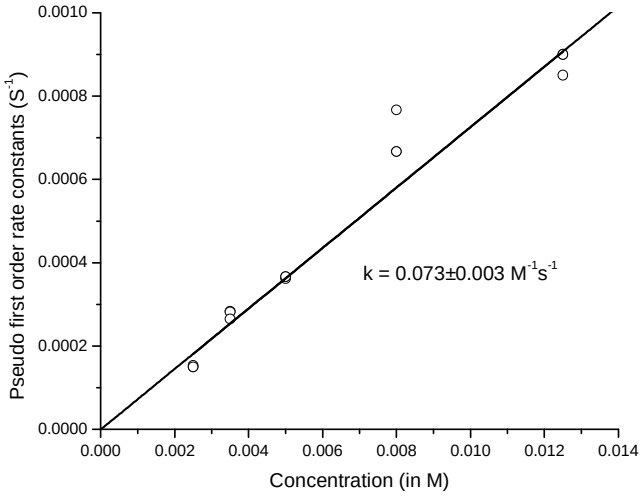


Table S2. Pseudo first order rate constants for the cycloaddition of dibenzocyclooctyne **5** with various concentrations of benzyl azide in MeOH.

Concentration of Benzyl Azide (x 10^{-3}M)	k (s^{-1})
2.5	(3.11 ± 0.02) x 10^{-4}
3.5	(4.07 ± 0.37) x 10^{-4}
5.0	(7.02 ± 0.07) x 10^{-4}
7.5	(8.27 ± 0.11) x 10^{-4}
1.25	(16.80 ± 0.91) x 10^{-4}

Linear plots for determining second order rate constants.

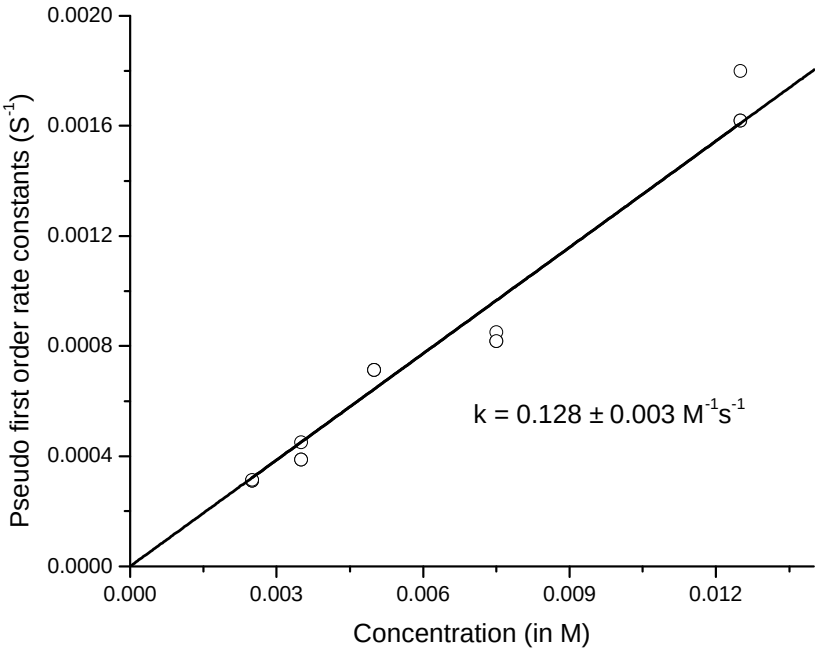
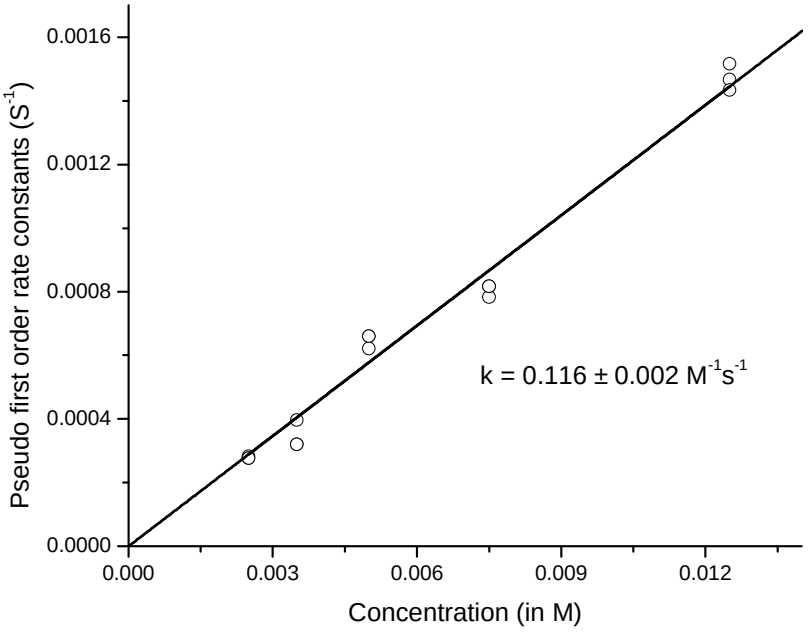


Table S3. Pseudo first order rate constants for the cycloaddition of dibenzocyclooctyne **7** with various concentrations of benzyl azide in MeOH.

Concentration of Benzyl Azide (x 10^{-3}M)	k (s^{-1})
2.5	(2.78 ± 0.05) x 10^{-4}
3.5	(3.45 ± 0.44) x 10^{-4}
5.0	(6.43 ± 0.23) x 10^{-4}
7.5	(8.06 ± 0.20) x 10^{-4}
1.25	(14.70 ± 0.50) x 10^{-4}

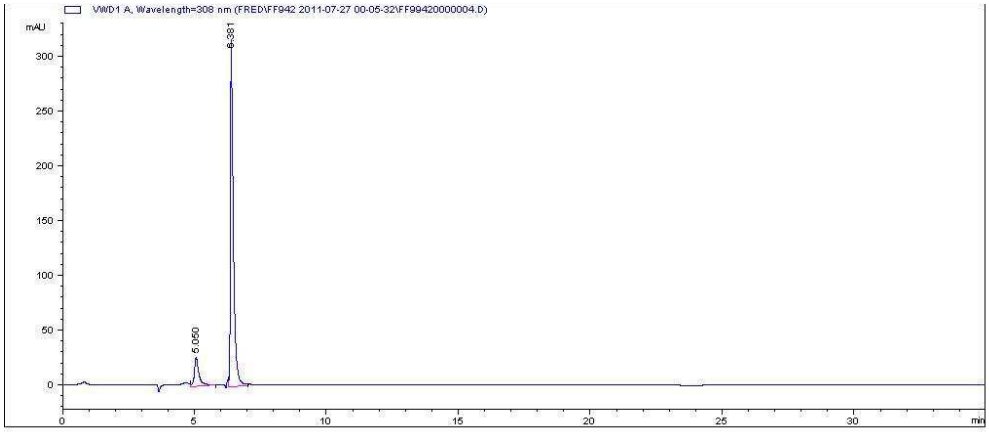
Linear plots for determining second order rate constants.

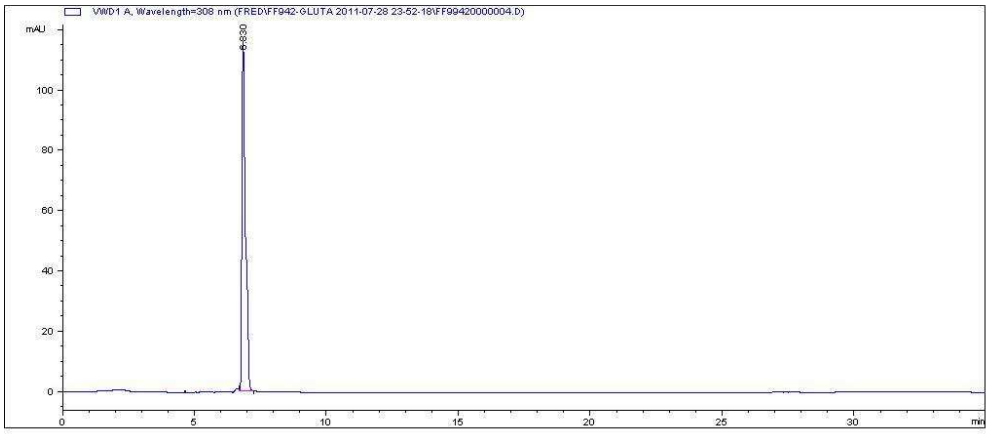


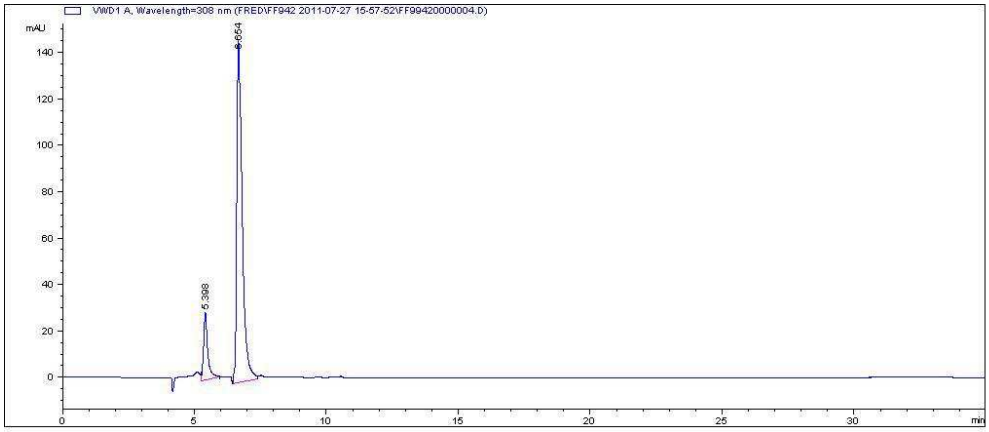
HPLC Stability Study

The following gradient program was used for all analyses: linear gradient 0-20% CH_3CN in water for the first 20 min, followed by a gradient of 20-100% CH_3CN in water for the remaining 10 min; flow: 1.0 mL/min. Visualization at 308 nm.

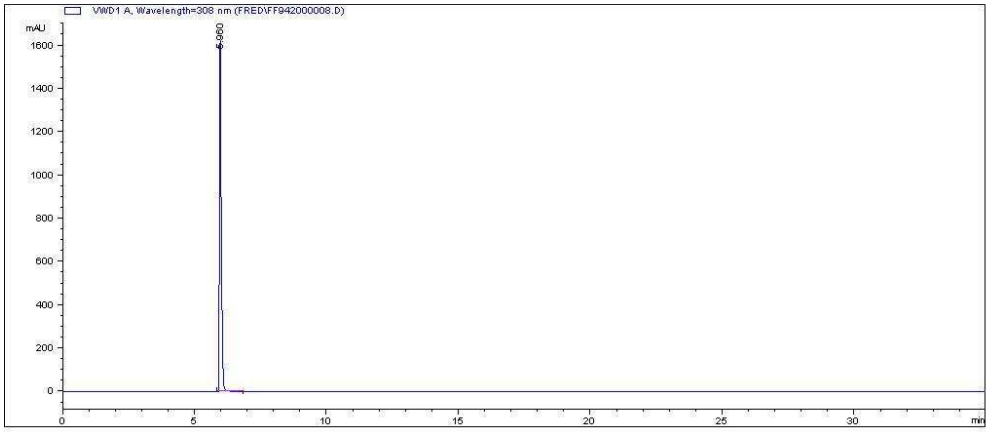
7 in PBS buffer at t=0, t=12h, and t=24h: $t_R \approx 6.3$ min.

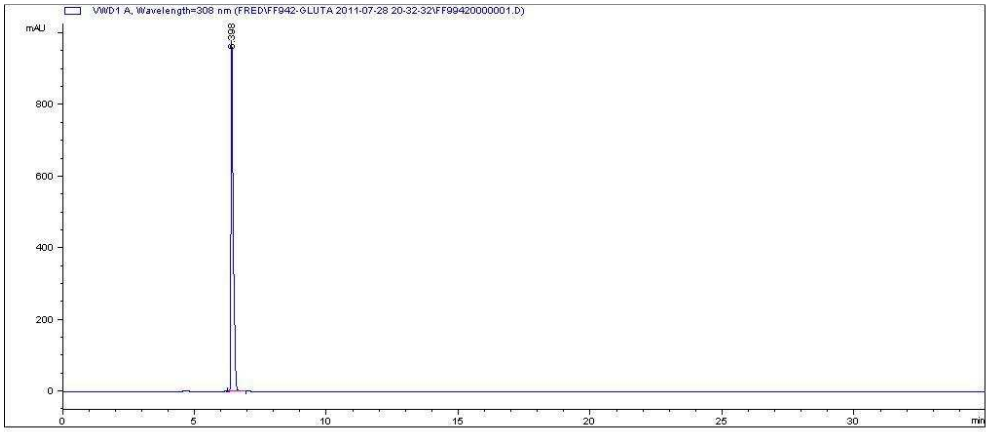


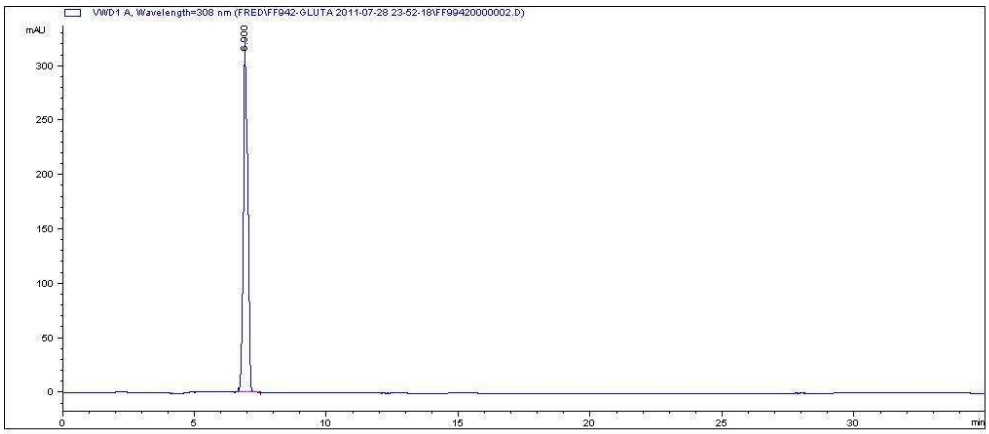




7 in the presence of 10mM of Glutathione at t=0, t=3h, and t=6h: $t_R \approx 6.3$ min.







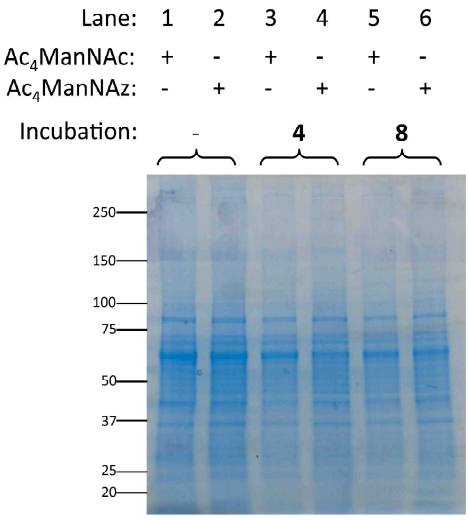
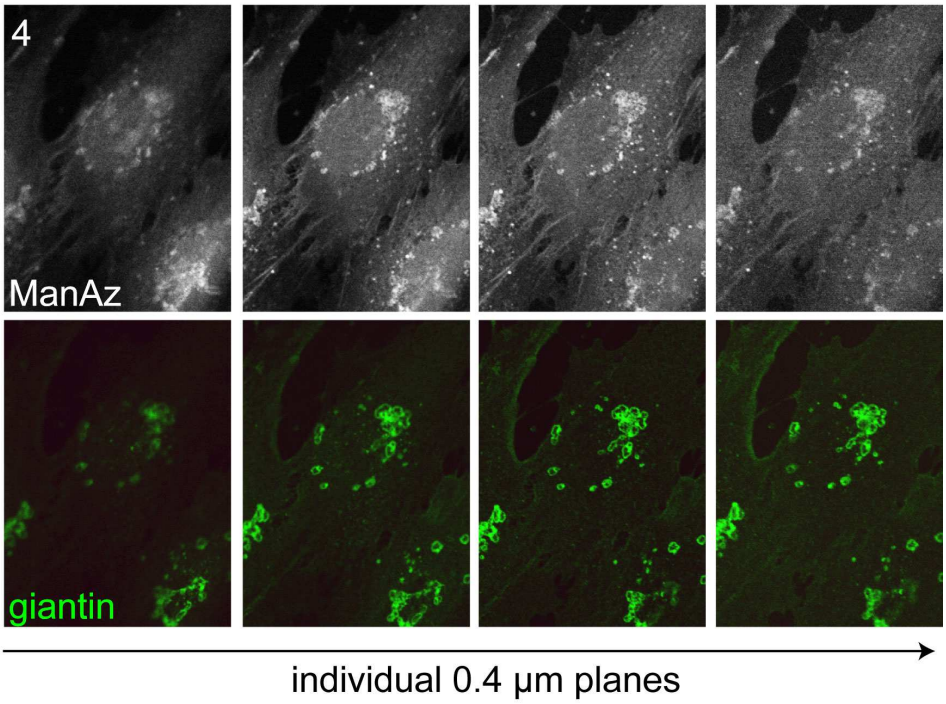


Figure S1. Coomassie staining of cell lysates indicating comparable levels of protein loading in Figure 2c.



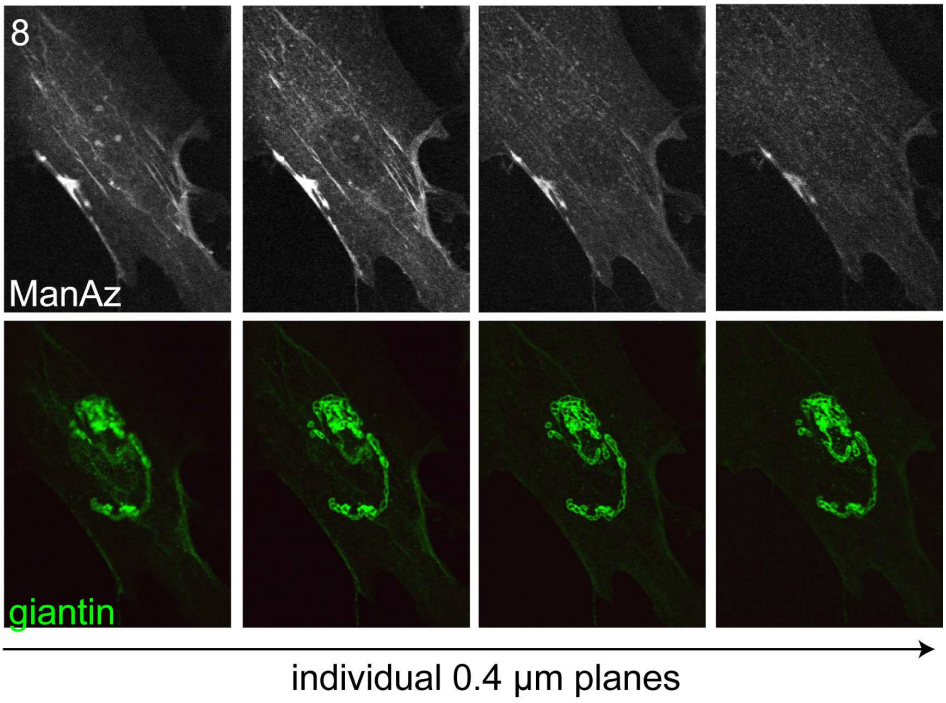
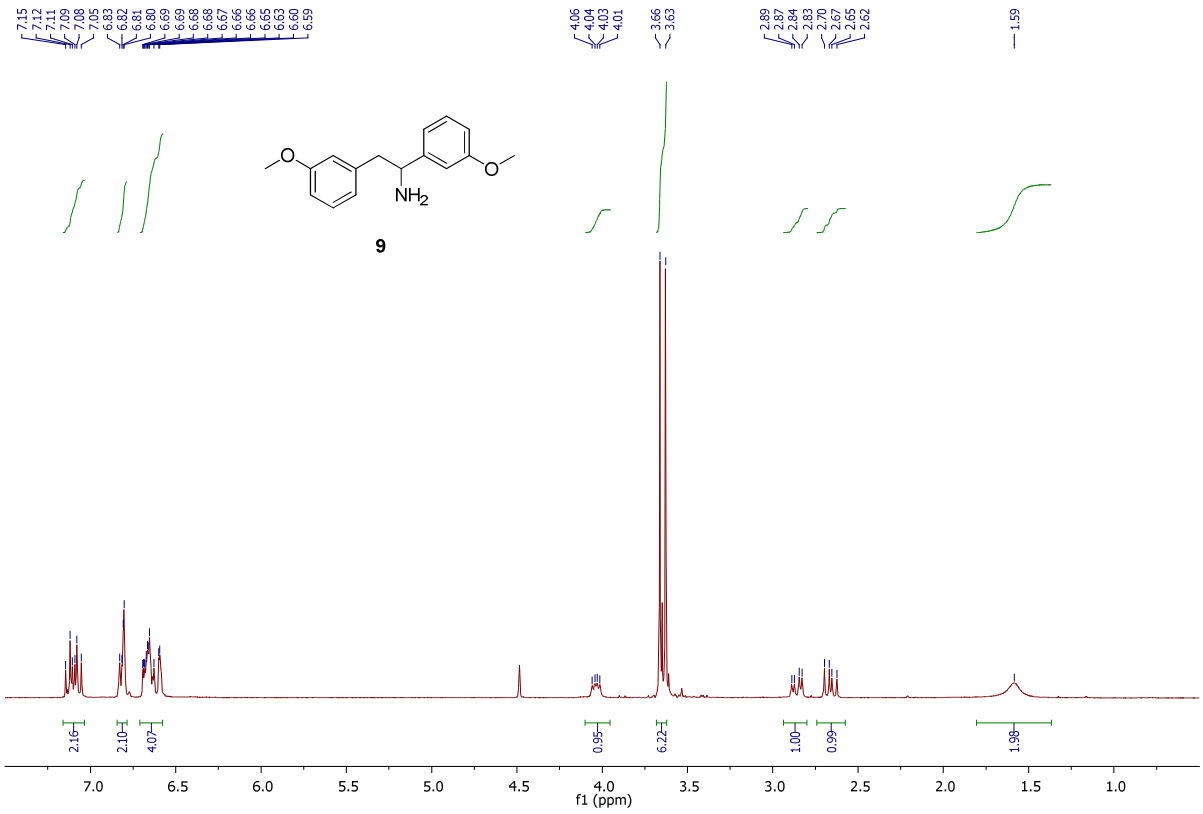


Figure S2. A montage of individual 0.4 µm confocal stacks taken from the original images in Figure 3A. Note the complete lack of co-localization between giantin and ManNAz staining in these stacks when compound **8** is used.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6.66
6.65
6.63
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4.06
4.04
4.03
4.01
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2.65
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O
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2.10
4.07
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6.22
1.00
0.99
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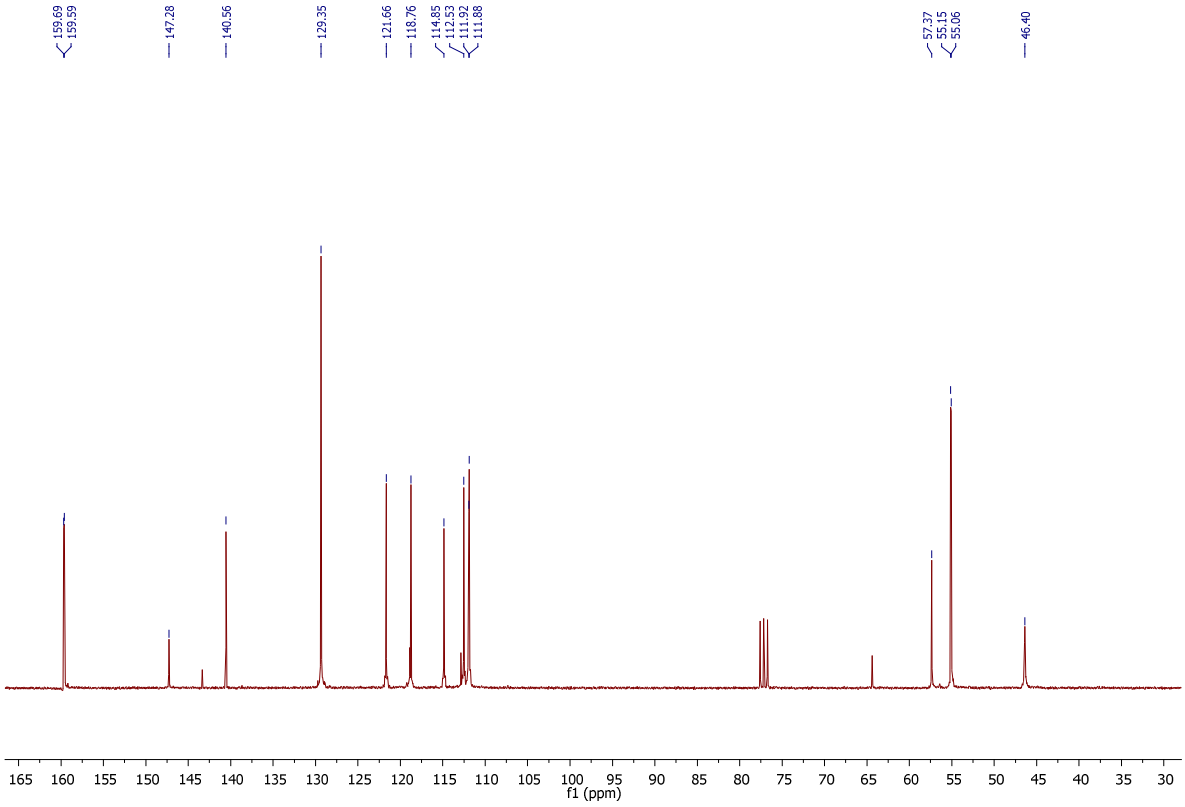
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55.06
46.40
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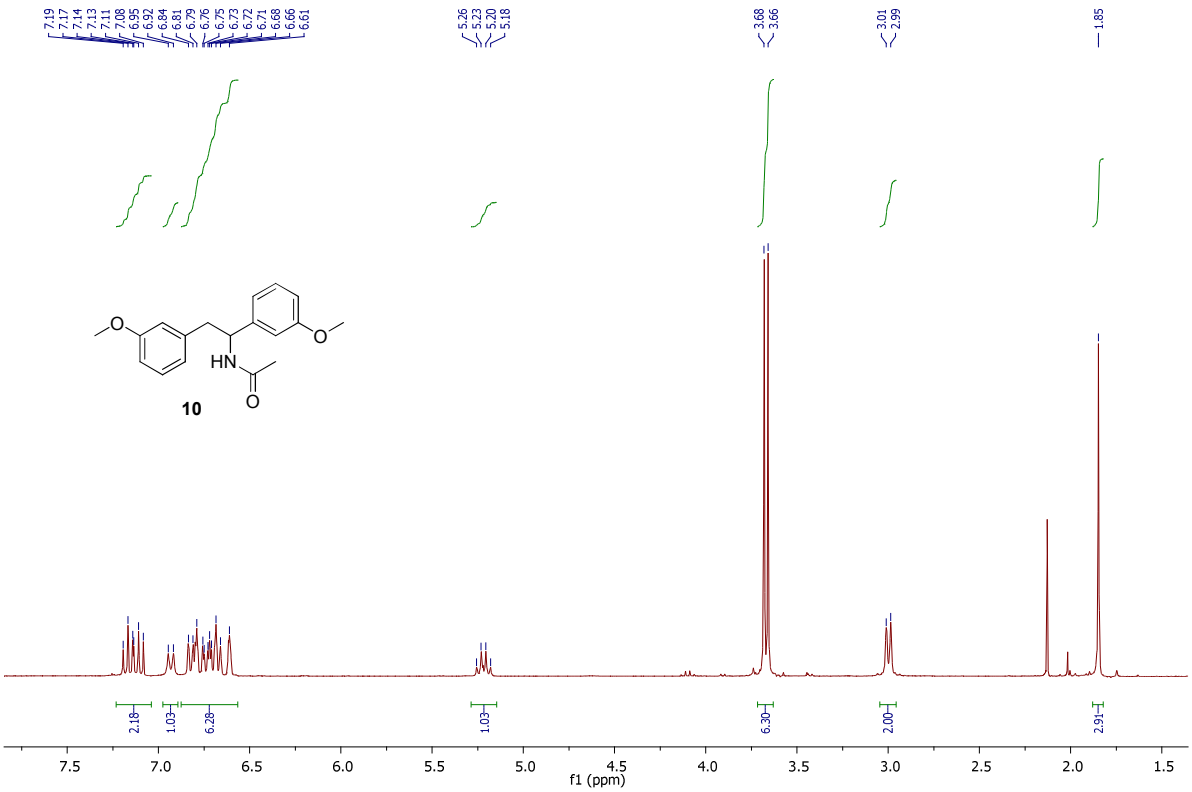
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6.72
6.71
6.68
6.66
6.61
5.26
5.23
5.20
5.18
3.68
3.66
3.01
2.99
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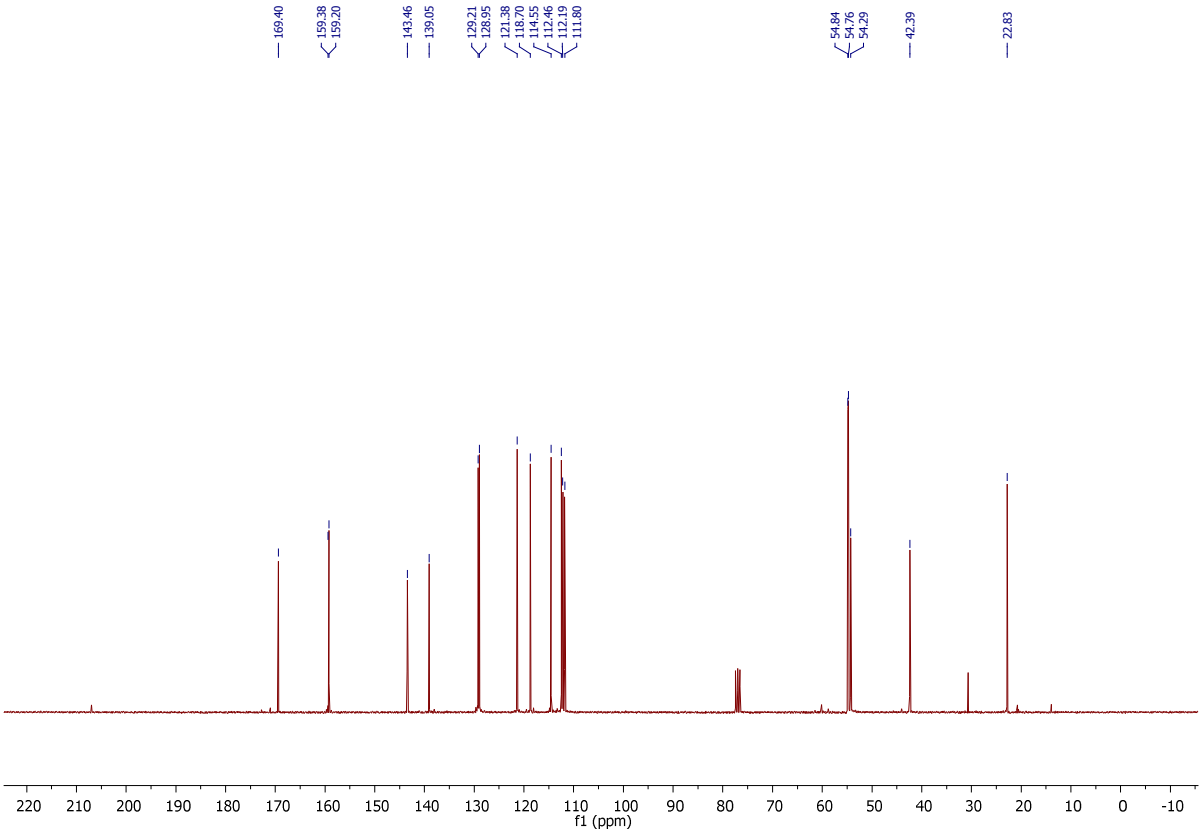
f1 (ppm)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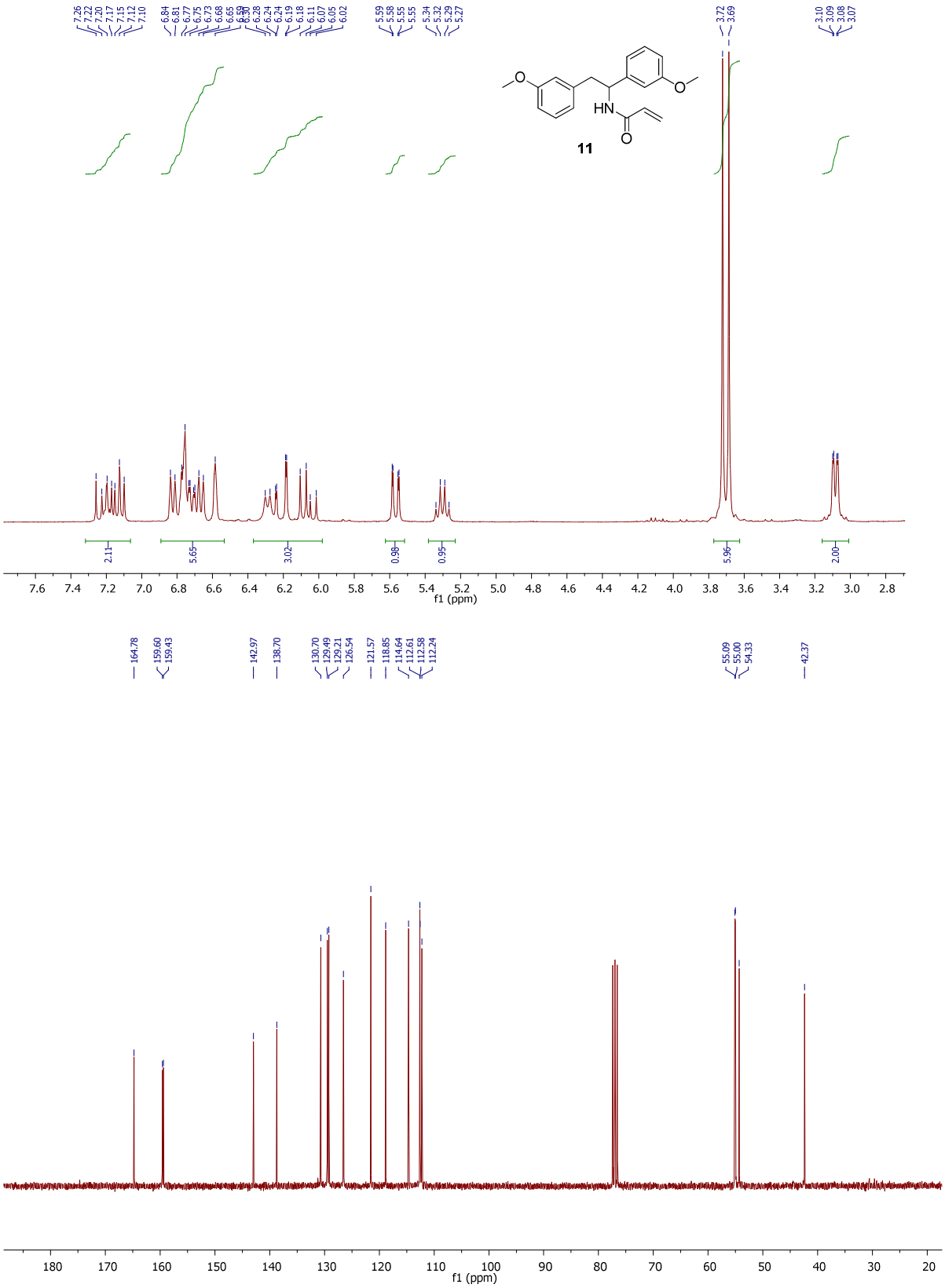
f1 (ppm)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f1 (ppm)
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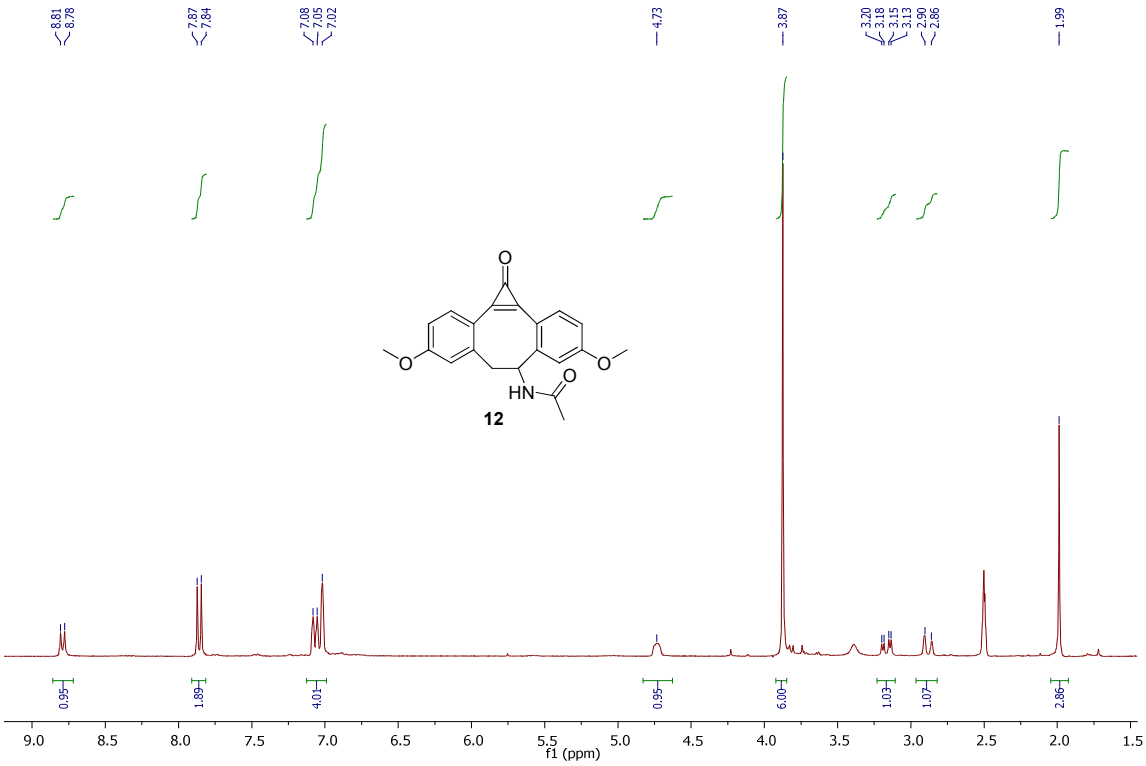
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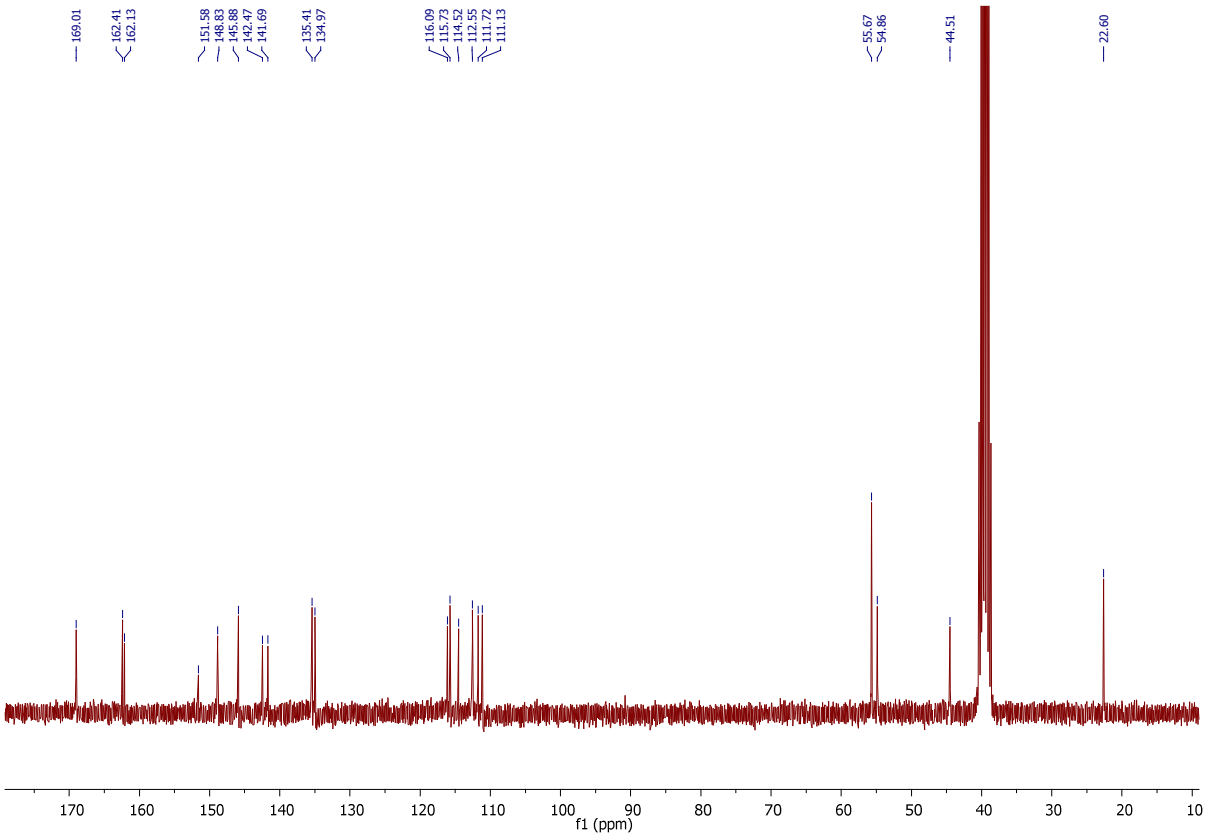
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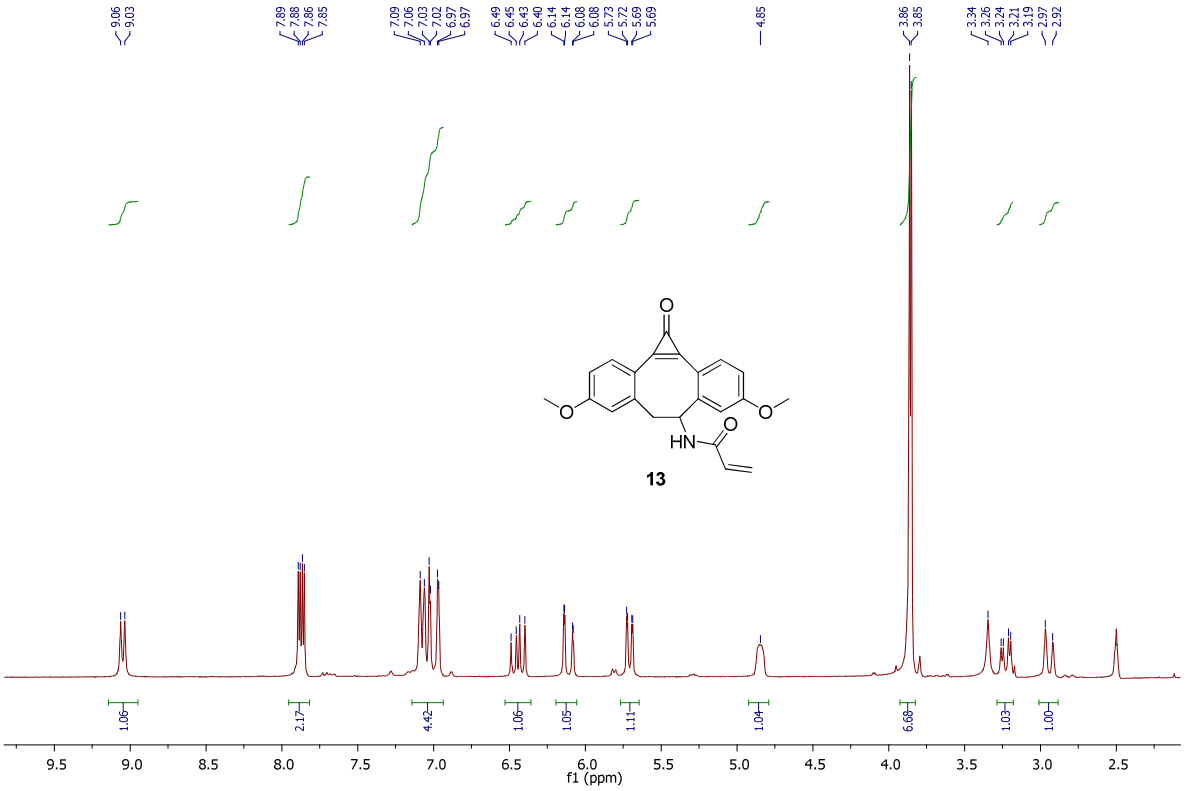
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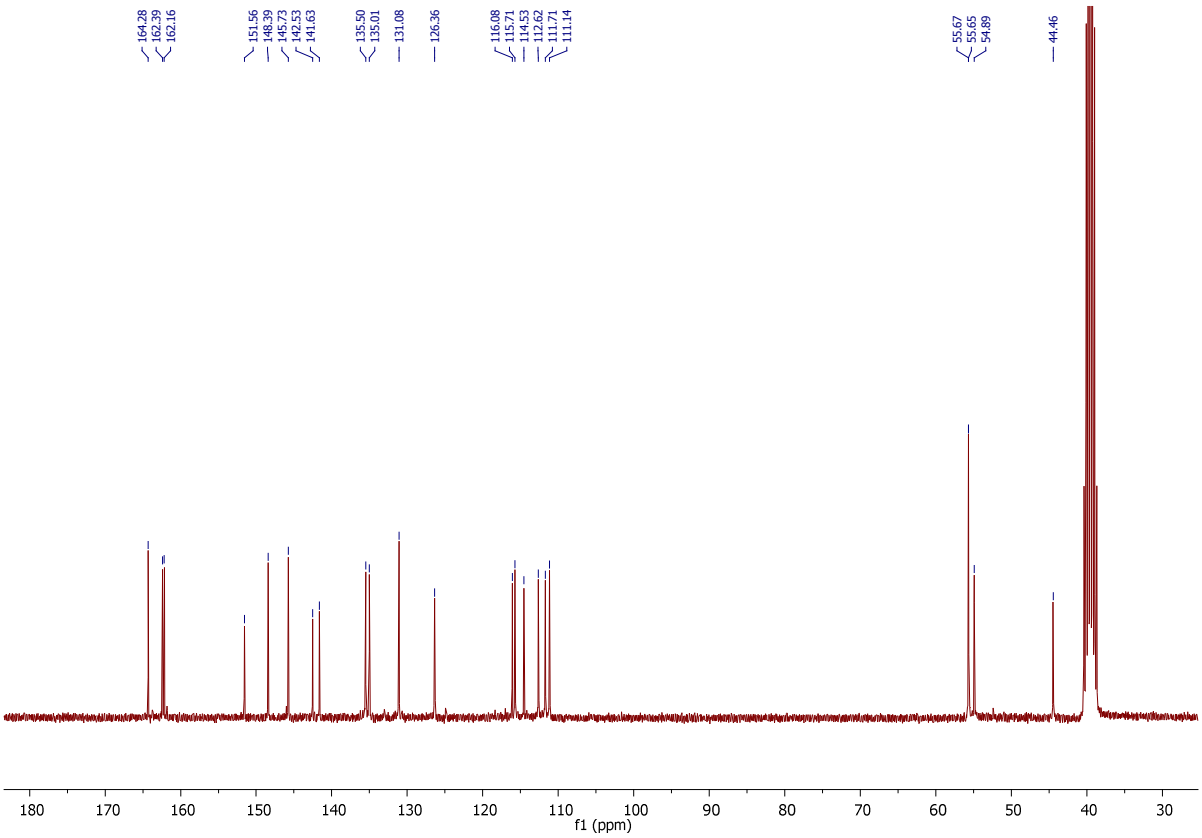
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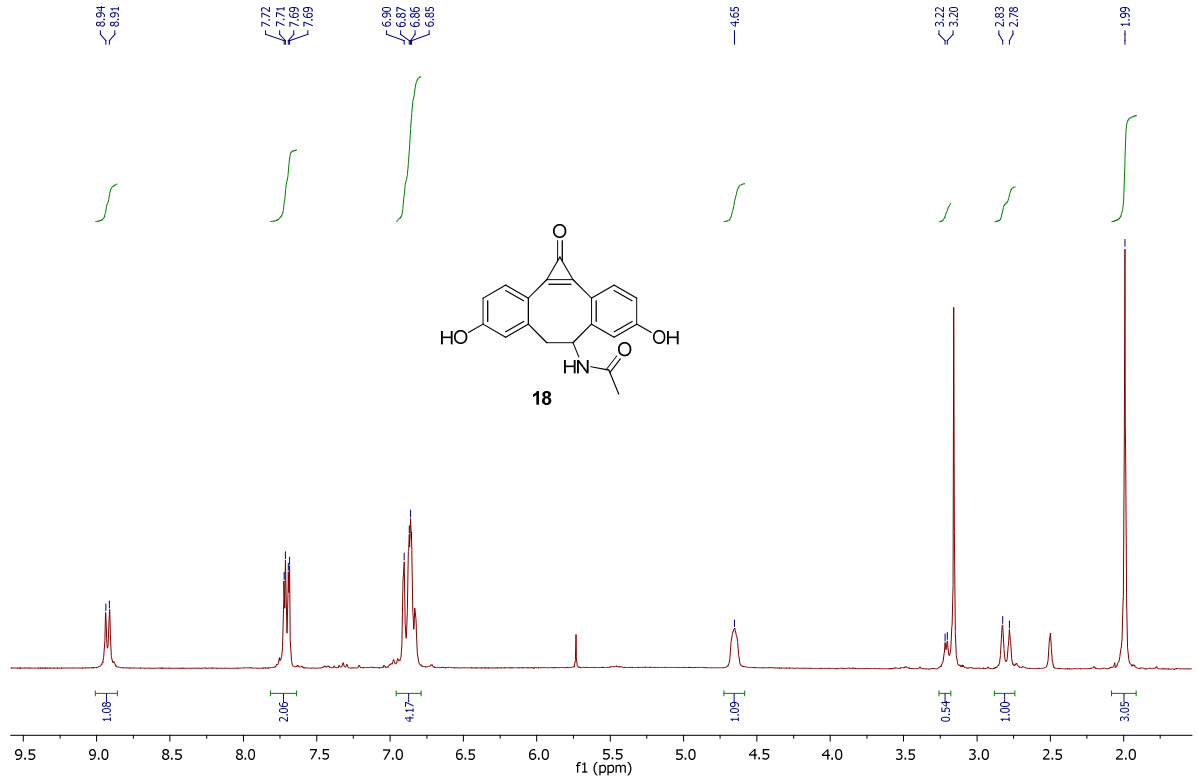
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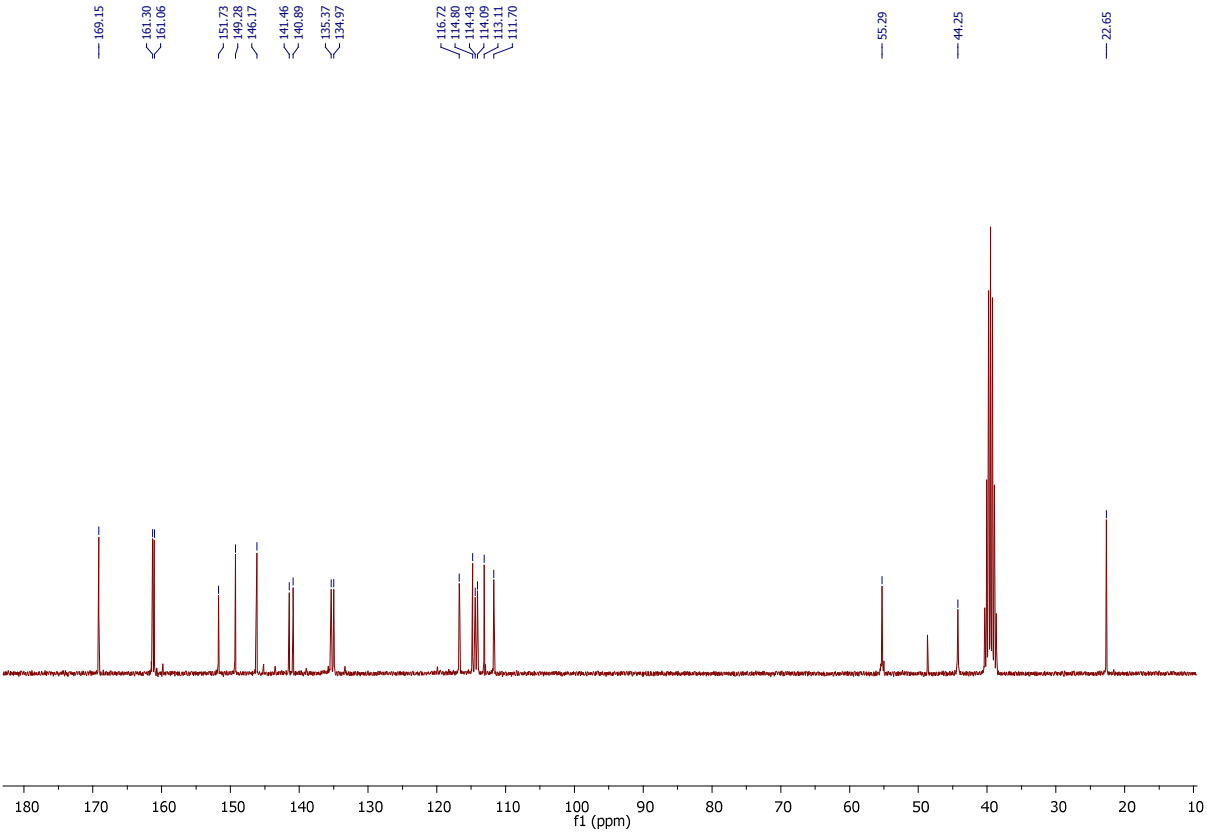
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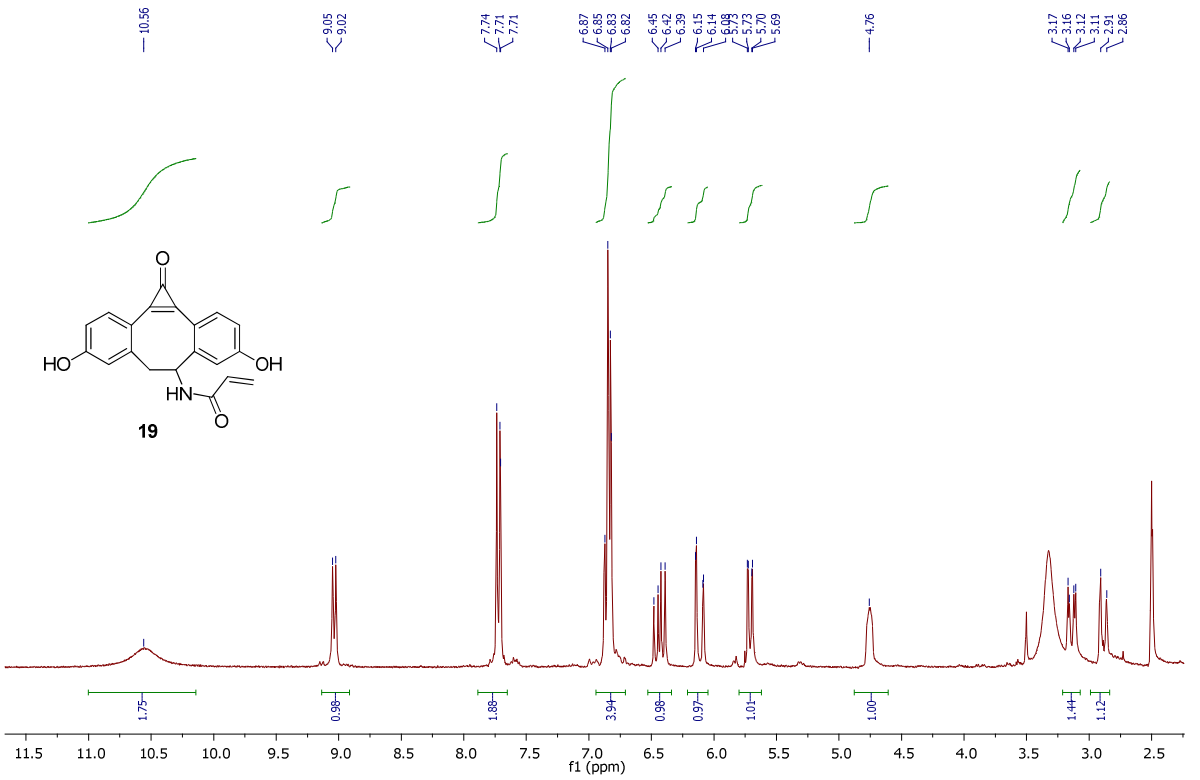
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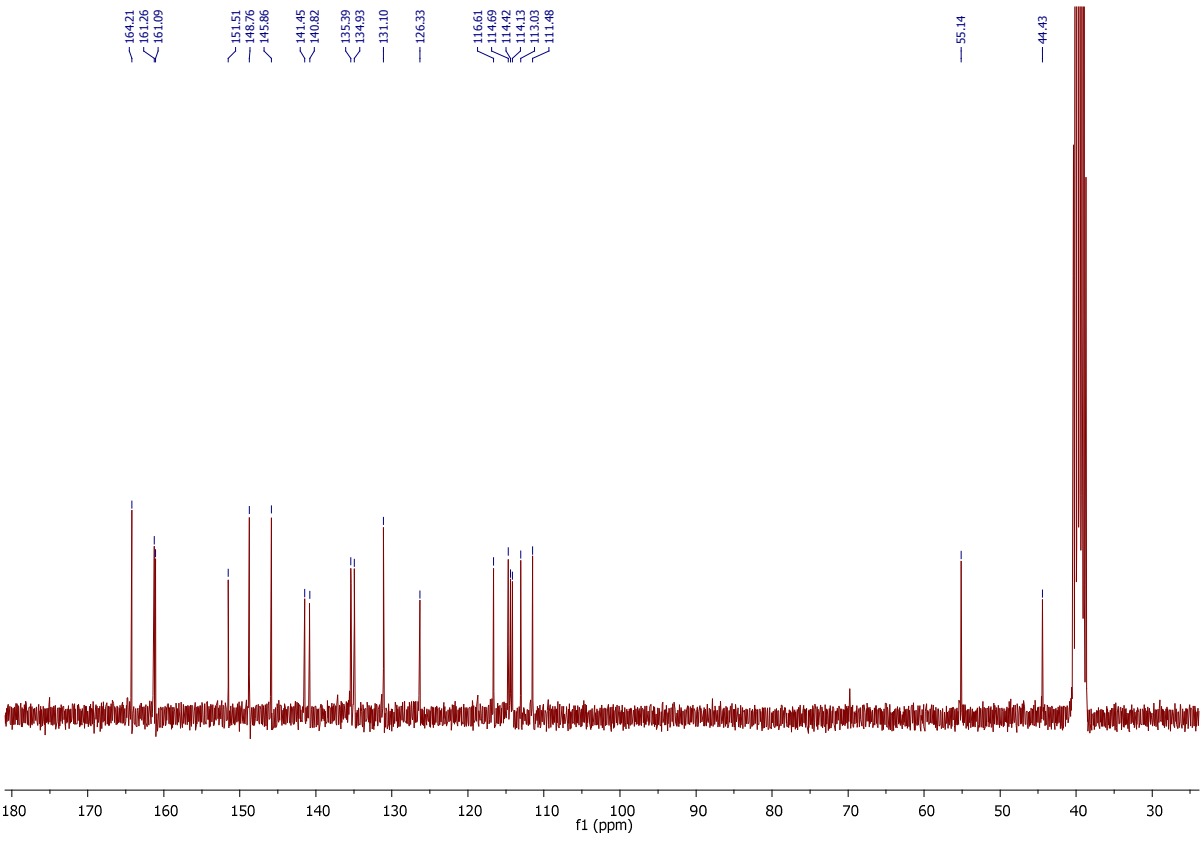
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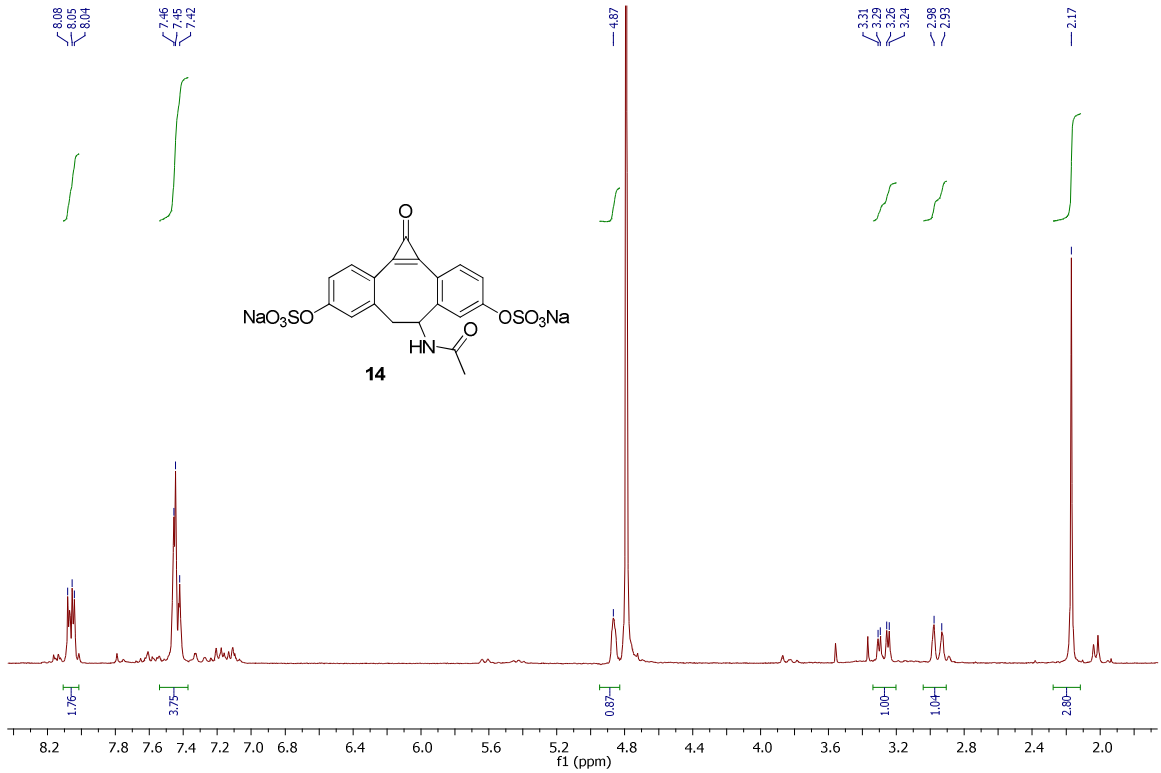
f1 (ppm)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OSO3Na
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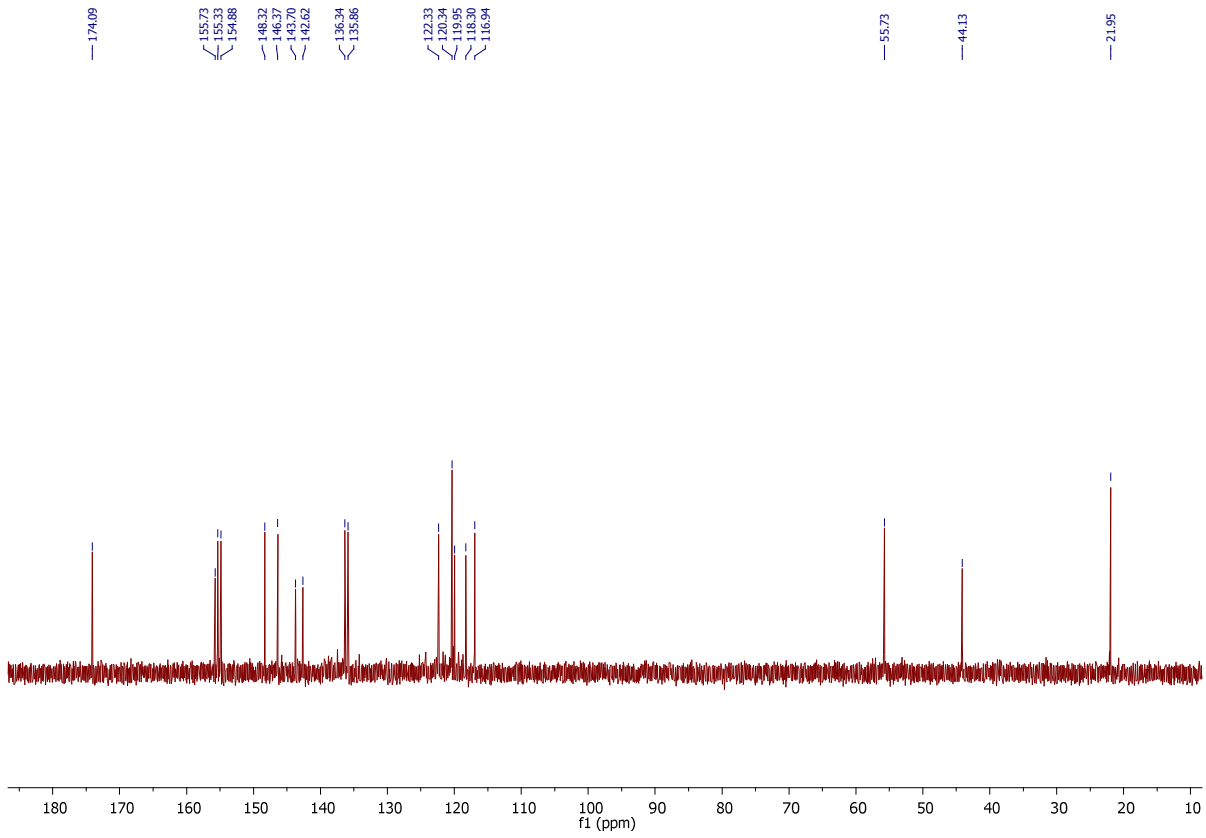
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100
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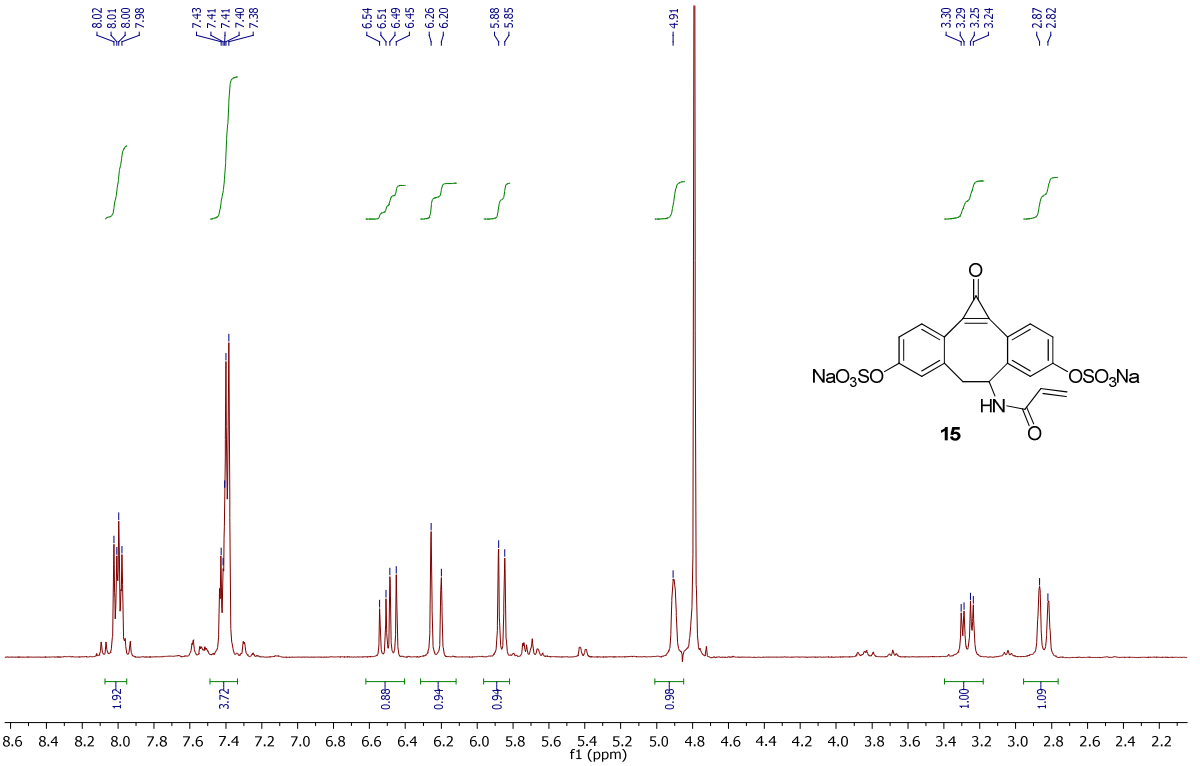
40
30
20
10
f1 (ppm)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8.01
8.00
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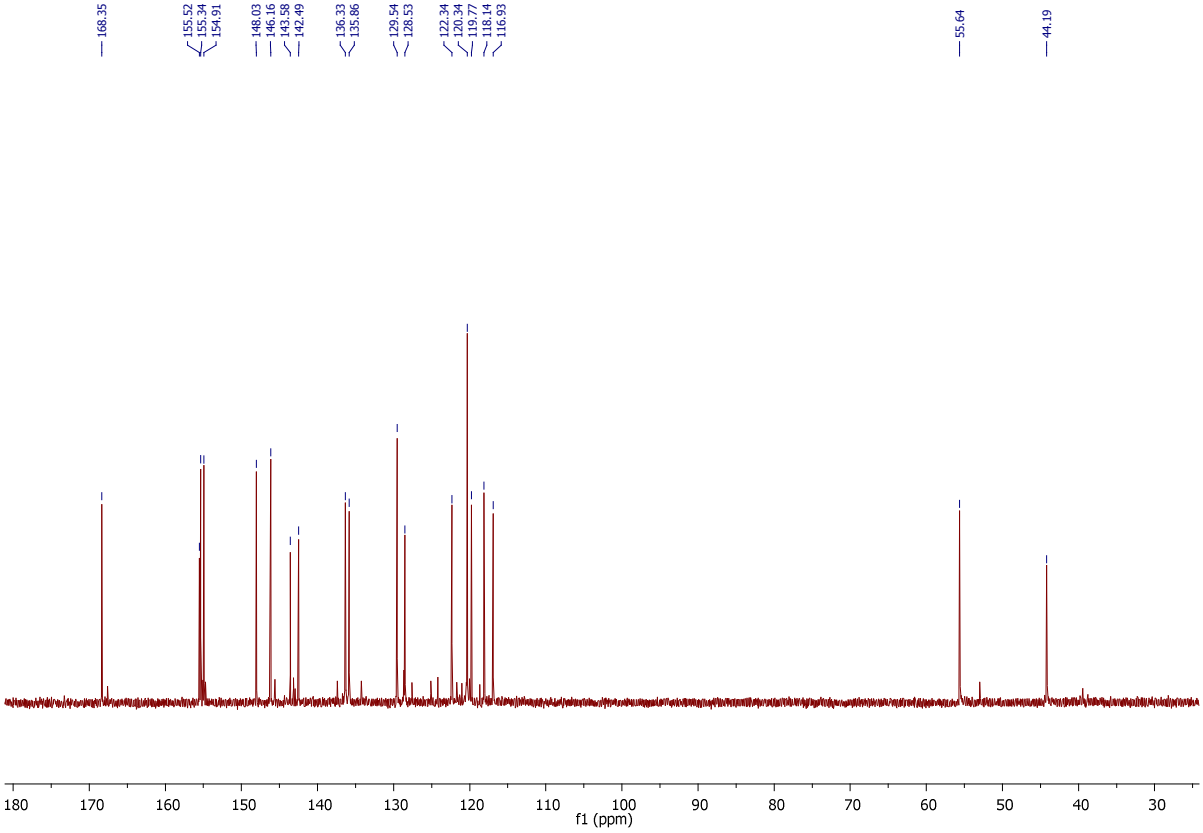
7.41
7.41
7.40
7.38
6.54
6.51
6.49
6.45
6.26
6.20
5.88
5.85
4.91
3.30
3.29
3.25
3.24
2.87
2.82
O
NaO3SO
OSO3Na
HN
O
15
1.92
3.72
0.88
0.94
0.94
0.98
1.00
1.09
f1 (ppm)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155.52
155.34
154.91
148.03
146.16
143.58
142.49
136.33
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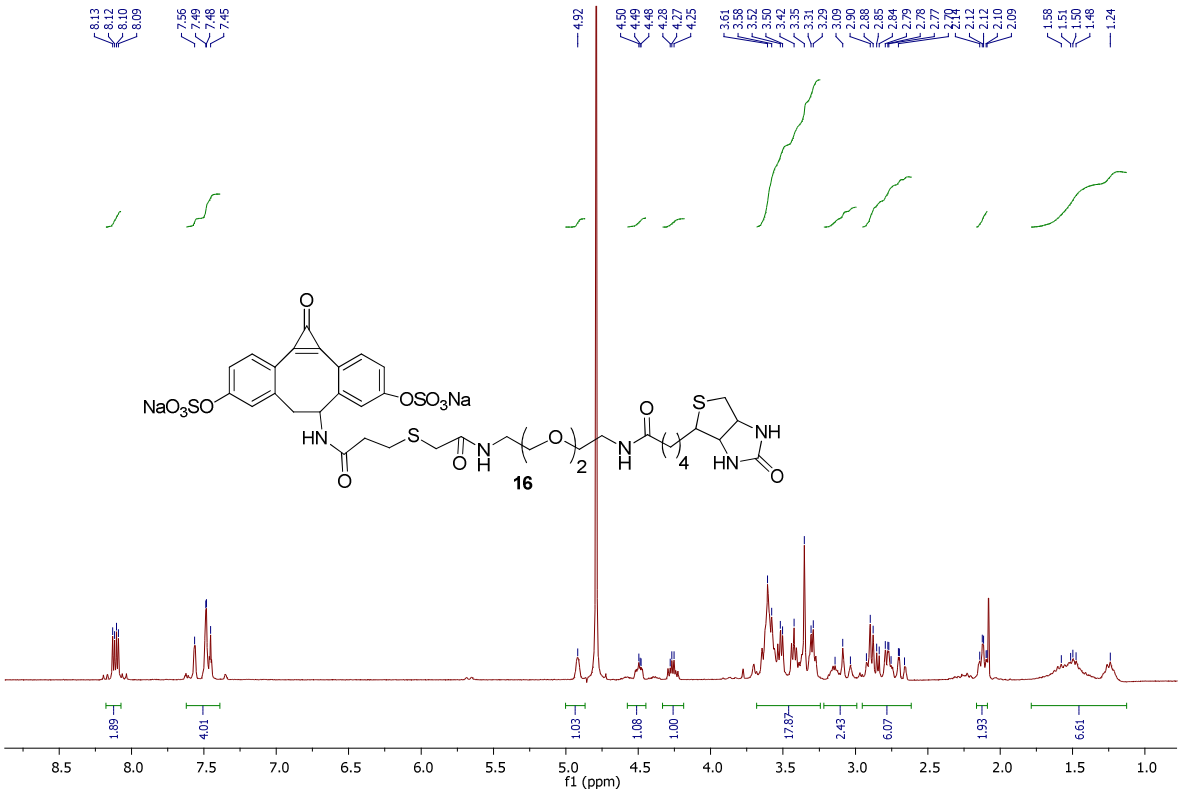
128.53
122.34
120.34
119.77
118.14
116.93
55.64
44.19
f1 (ppm)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7.45
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4.50
4.49
4.48
4.28
4.27
4.25
3.61
3.58
3.52
3.50
3.42
3.35
3.31
3.29
3.09
2.90
2.88
2.85
2.84
2.79
2.78
2.77
2.70
2.14
2.12
2.12
2.10
2.09
1.58
1.51
1.50
1.48
1.24
NaO3SO
OSO3Na
HN
S
O
N
H
O
2
4
NH
HN
16
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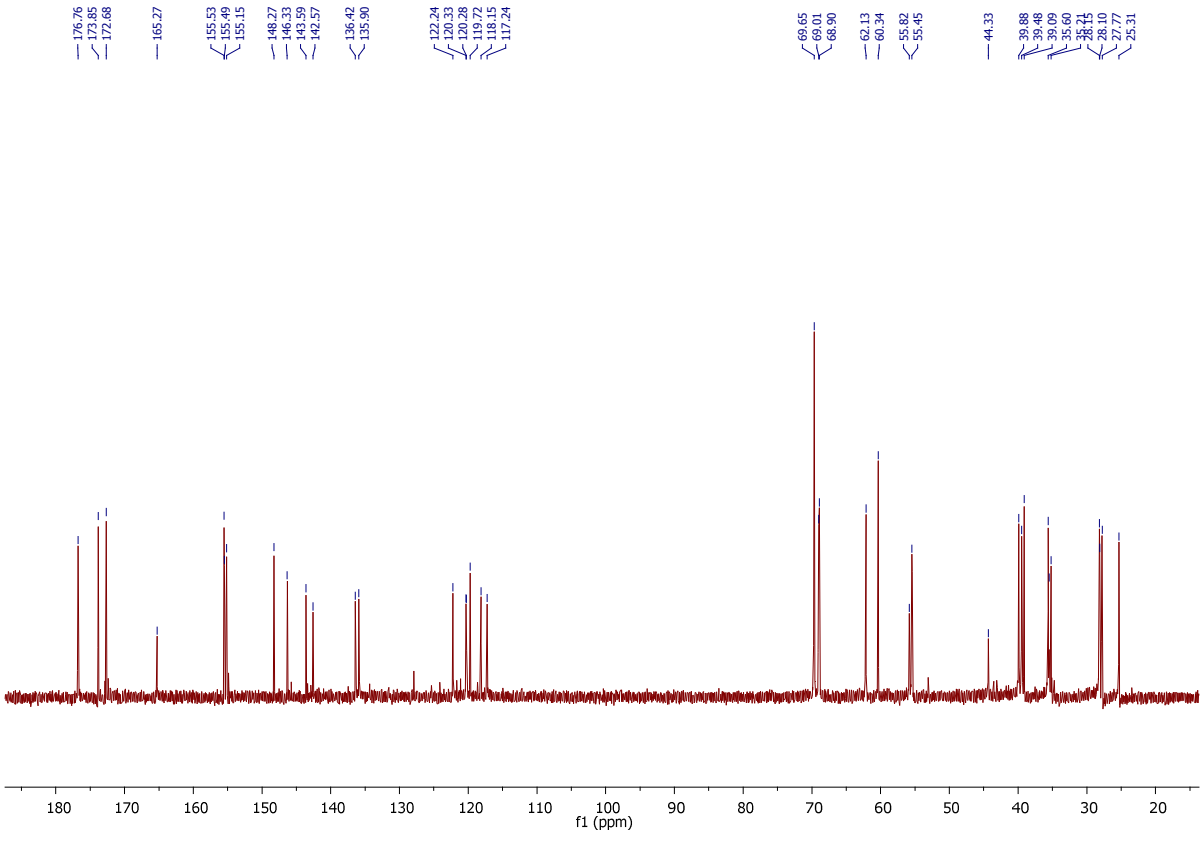
1.03
1.08
1.00
17.87
2.43
6.07
1.93
6.61
f1 (ppm)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122.24
120.33
120.28
119.72
118.15
117.24
69.65
69.01
68.90
62.13
60.34
55.82
55.45
44.33
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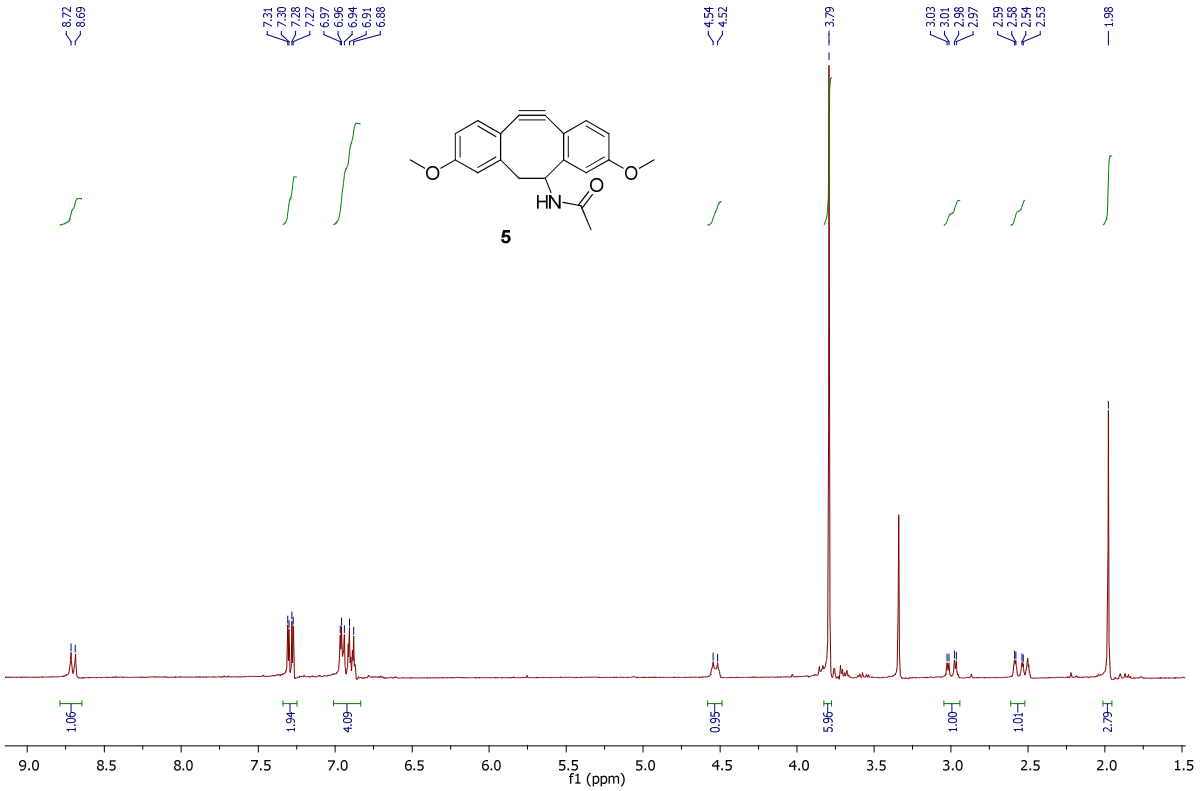
39.48
39.09
35.60
28.71
28.15
28.10
27.77
25.31
f1 (ppm)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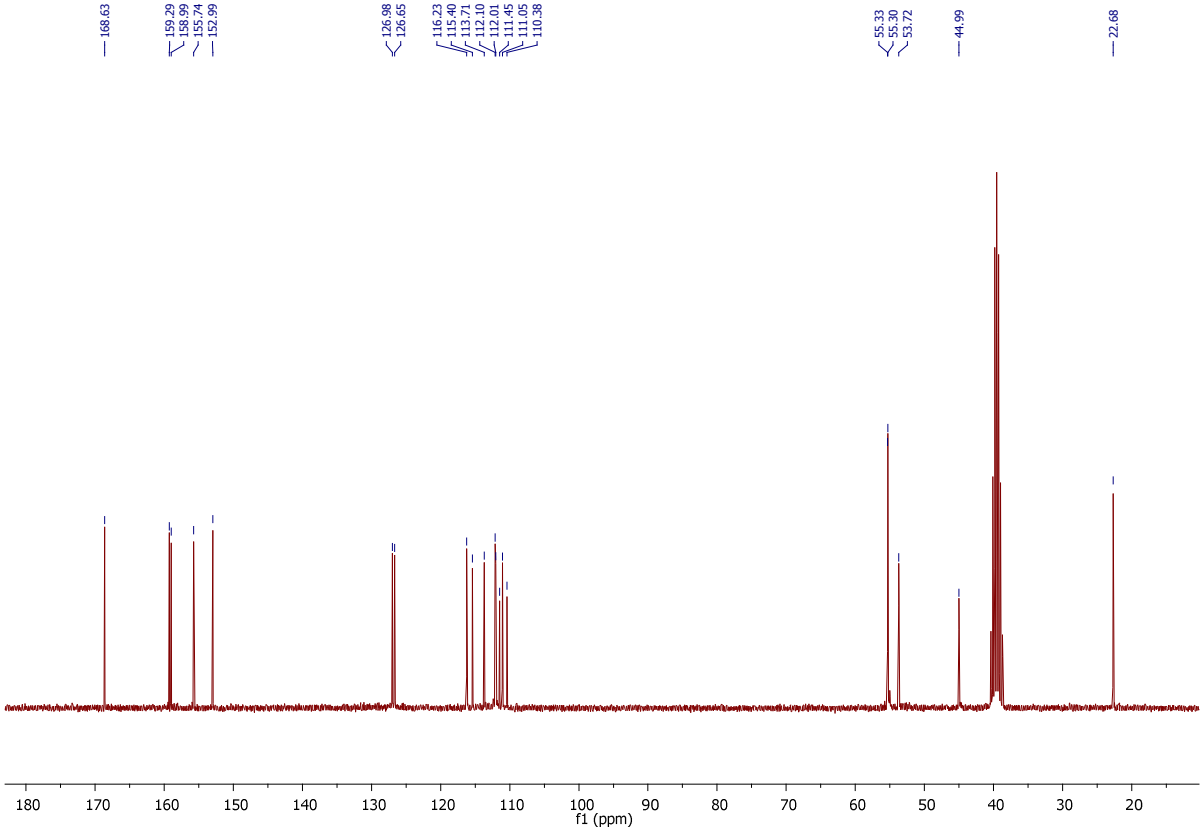
7.31
7.30
7.28
7.27
6.97
6.96
6.94
6.91
6.88
4.54
4.52
3.79
3.03
3.01
2.98
2.97
2.59
2.58
2.54
2.53
1.98
O
O
HN
O
5
1.06
1.94
4.09
0.95
5.96
1.00
1.01
2.79
f1 (ppm)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152.99
126.98
126.65
116.23
115.40
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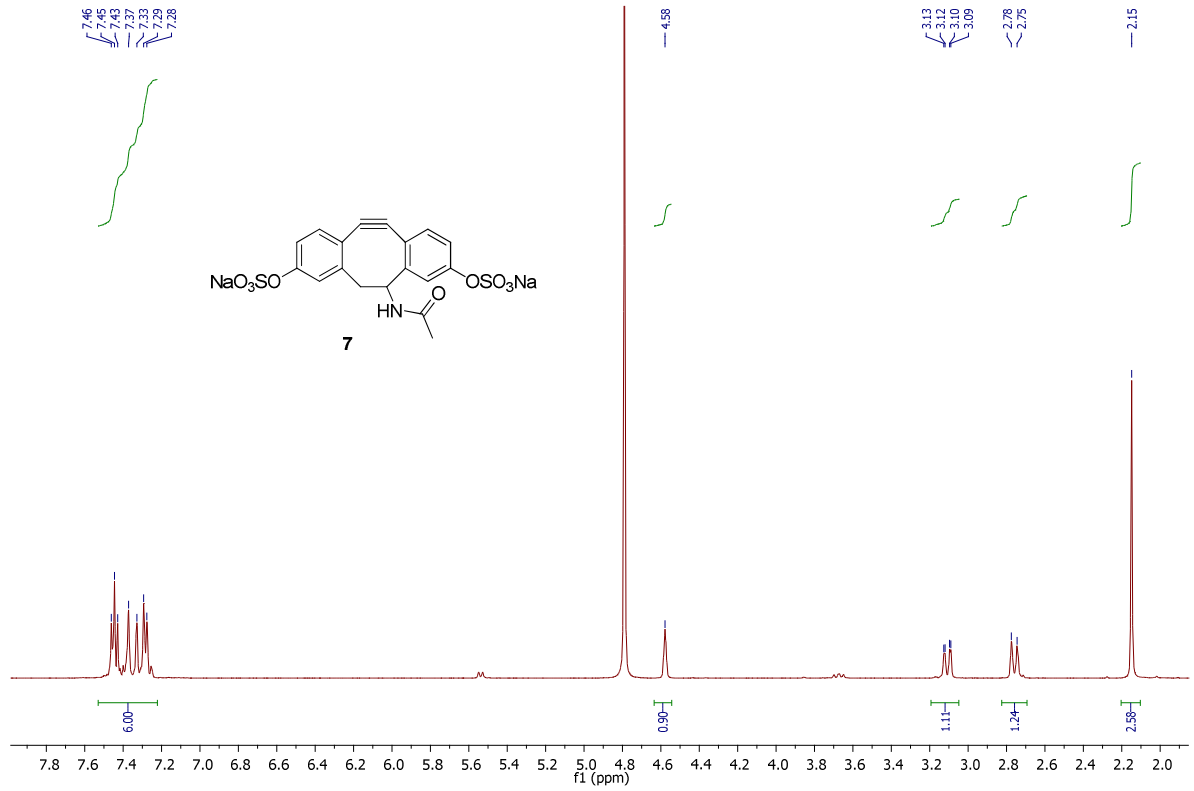
112.10
112.01
111.45
111.05
110.38
55.33
55.30
53.72
44.99
22.68
f1 (ppm)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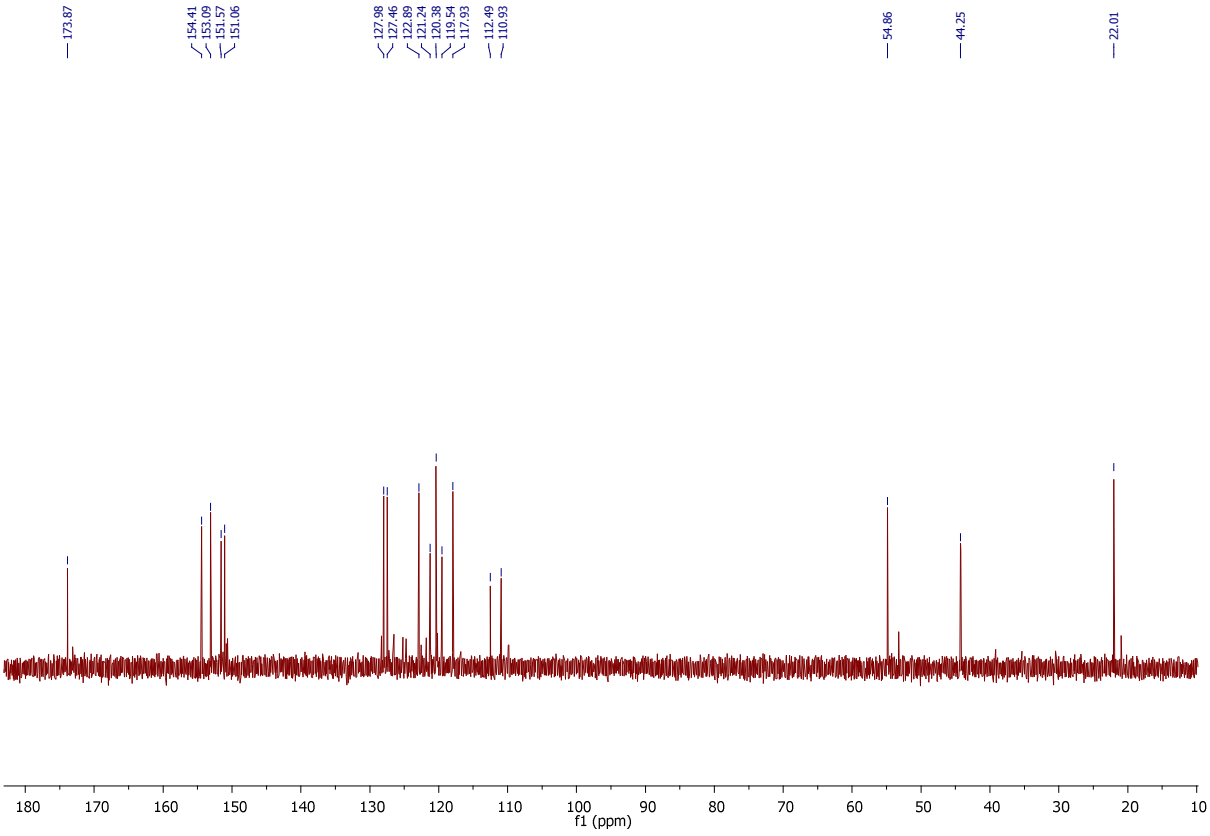
7.45
7.43
7.37
7.33
7.29
7.28
4.58
3.13
3.12
3.10
3.09
2.78
2.75
2.15
NaO3SO
OSO3Na
HN
O
7
6.00
0.90
1.11
1.24
2.58
f1 (ppm)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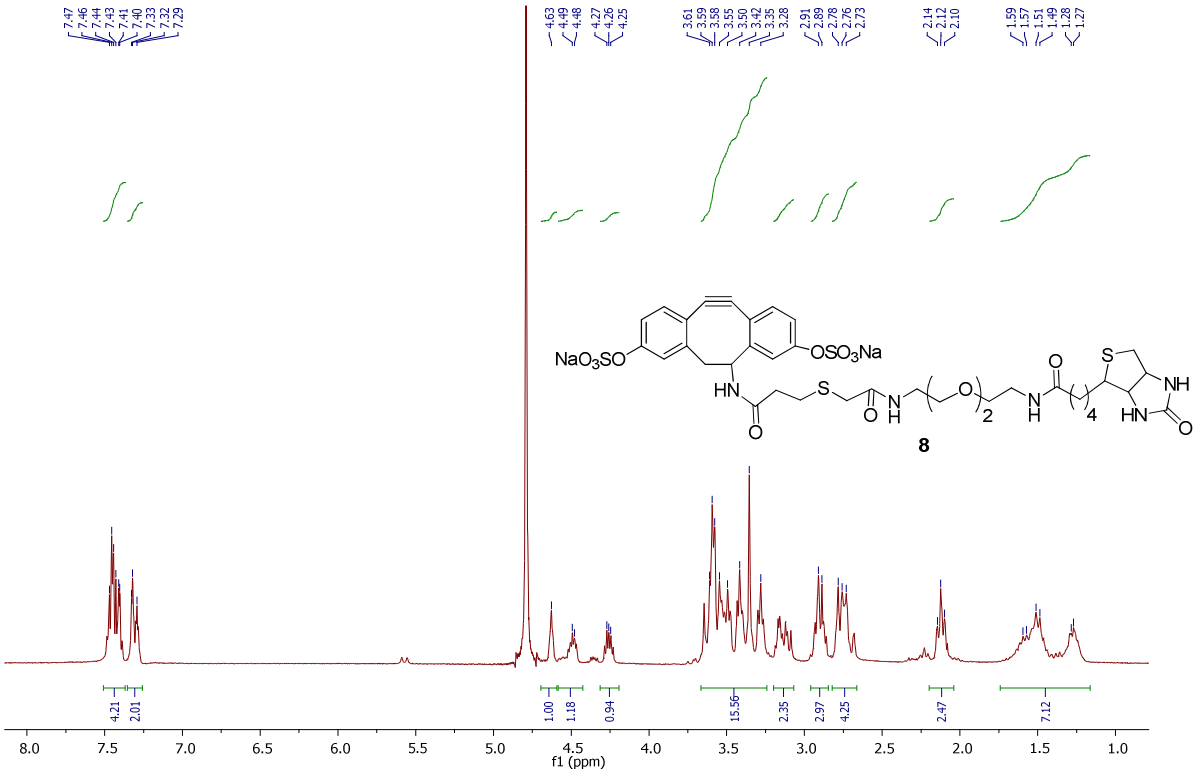
154.41
153.09
151.57
151.06
127.98
127.46
122.89
121.24
120.38
119.54
117.93
112.49
110.93
54.86
44.25
22.01
f1 (ppm)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7.43
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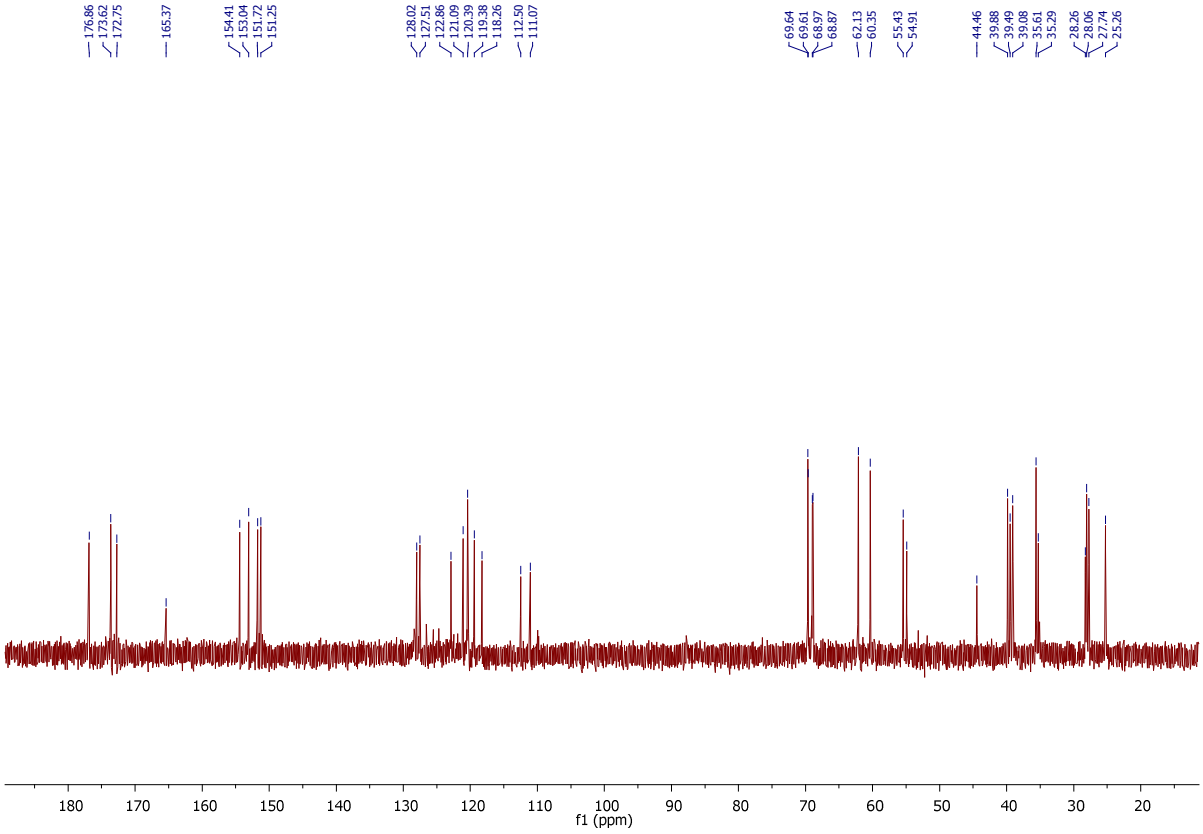
7.40
7.33
7.32
7.29
4.63
4.49
4.48
4.27
4.26
4.25
3.61
3.59
3.58
3.55
3.50
3.42
3.35
3.28
2.91
2.89
2.78
2.76
2.73
2.14
2.12
2.10
1.59
1.57
1.51
1.49
1.28
1.27
NaO3SO
OSO3Na
HN
S
O
N
H
O
2
N
H
O
4
S
NH
HN
O
8
4.21
2.01
1.00
1.18
0.94
15.56
2.35
2.97
4.25
2.47
7.12
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)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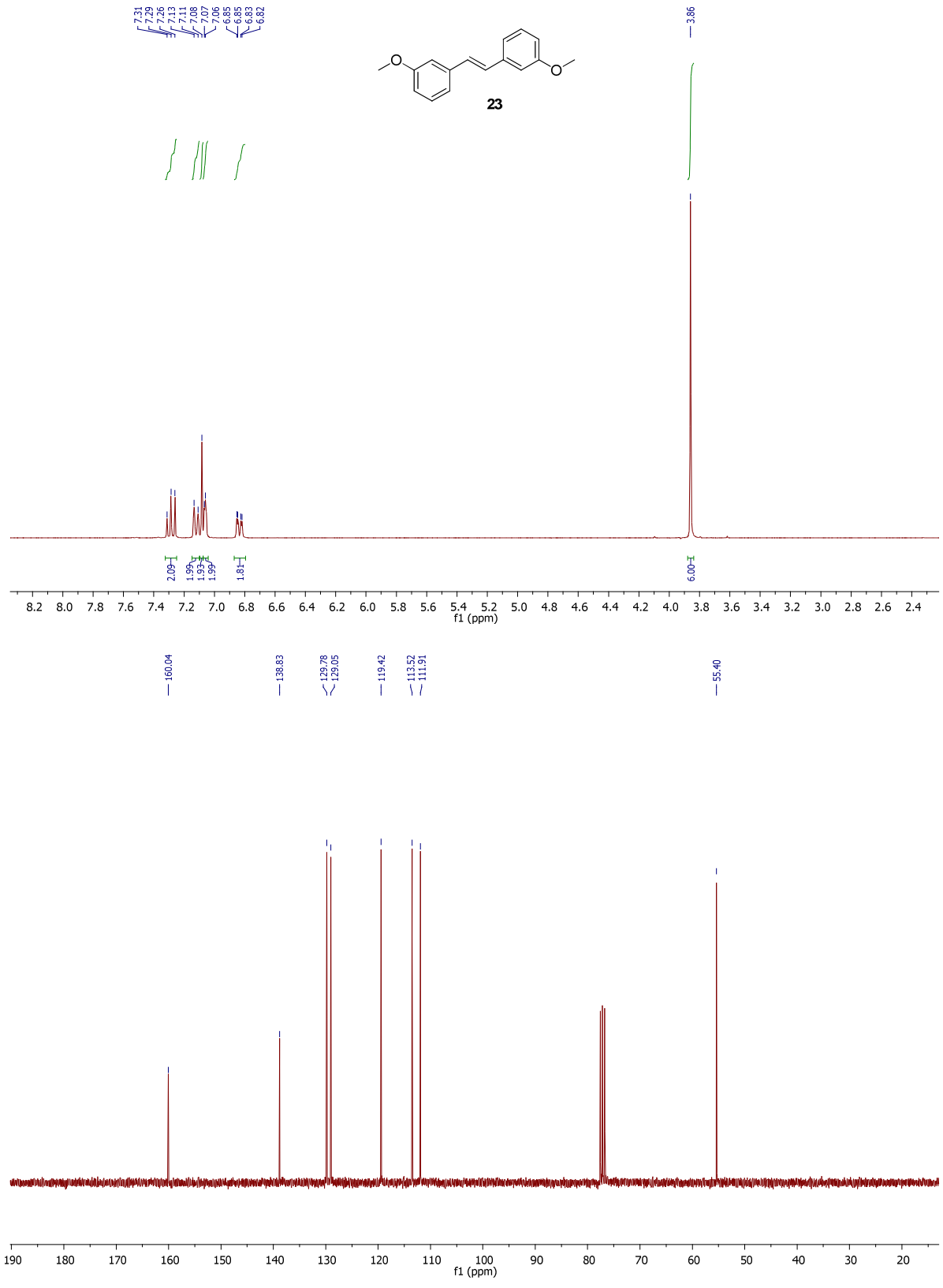
173.62
172.75
165.37
154.41
153.04
151.72
151.25
128.02
127.51
122.86
121.09
120.39
119.38
118.26
112.50
111.07
69.64
69.61
68.97
68.87
62.13
60.35
55.43
54.91
44.46
39.88
39.49
39.08
35.61
35.29
28.26
28.06
27.74
25.26
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)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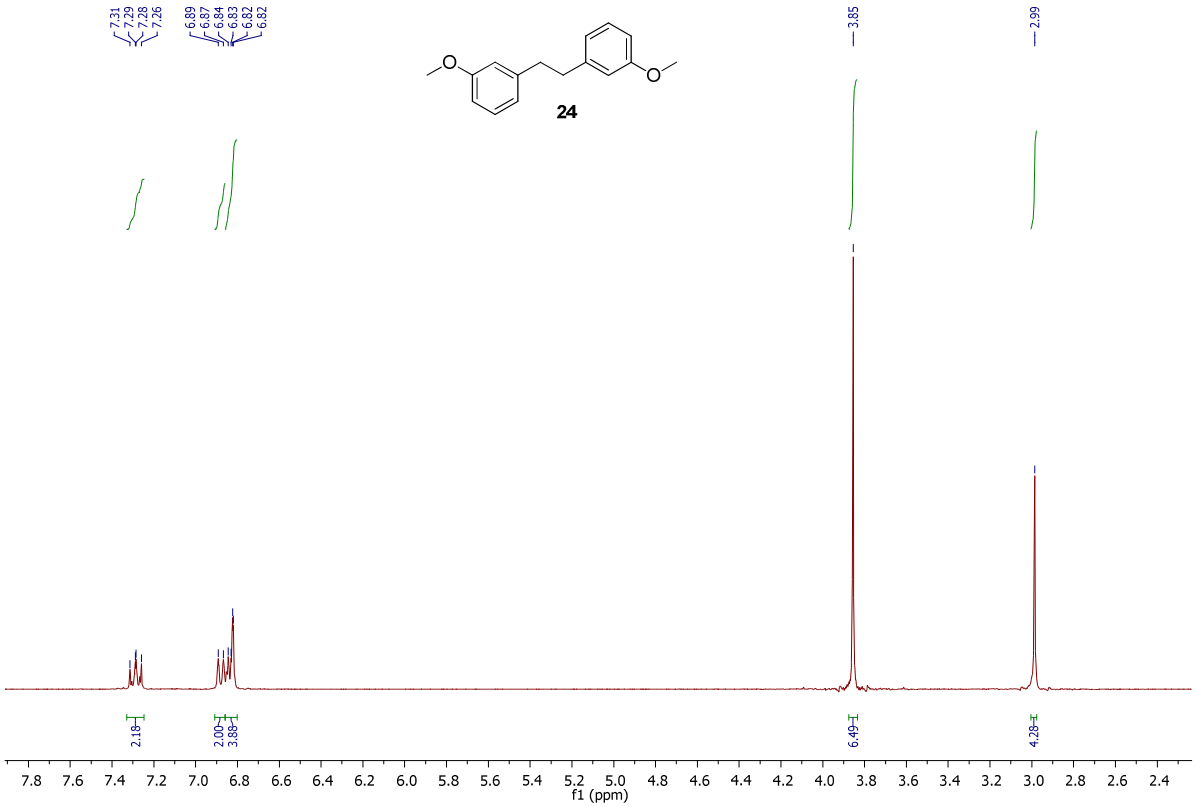
7.29
7.26
7.13
7.11
7.08
7.07
7.06
6.85
6.85
6.83
6.82
3.86
23
2.09
1.99
1.93
1.99
1.84
6.00
f1 (ppm)
160.04
138.83
129.78
129.05
119.42
113.52
111.91
55.40
f1 (ppm)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7.29
7.28
7.26
6.89
6.87
6.84
6.83
6.82
6.82
3.85
2.99
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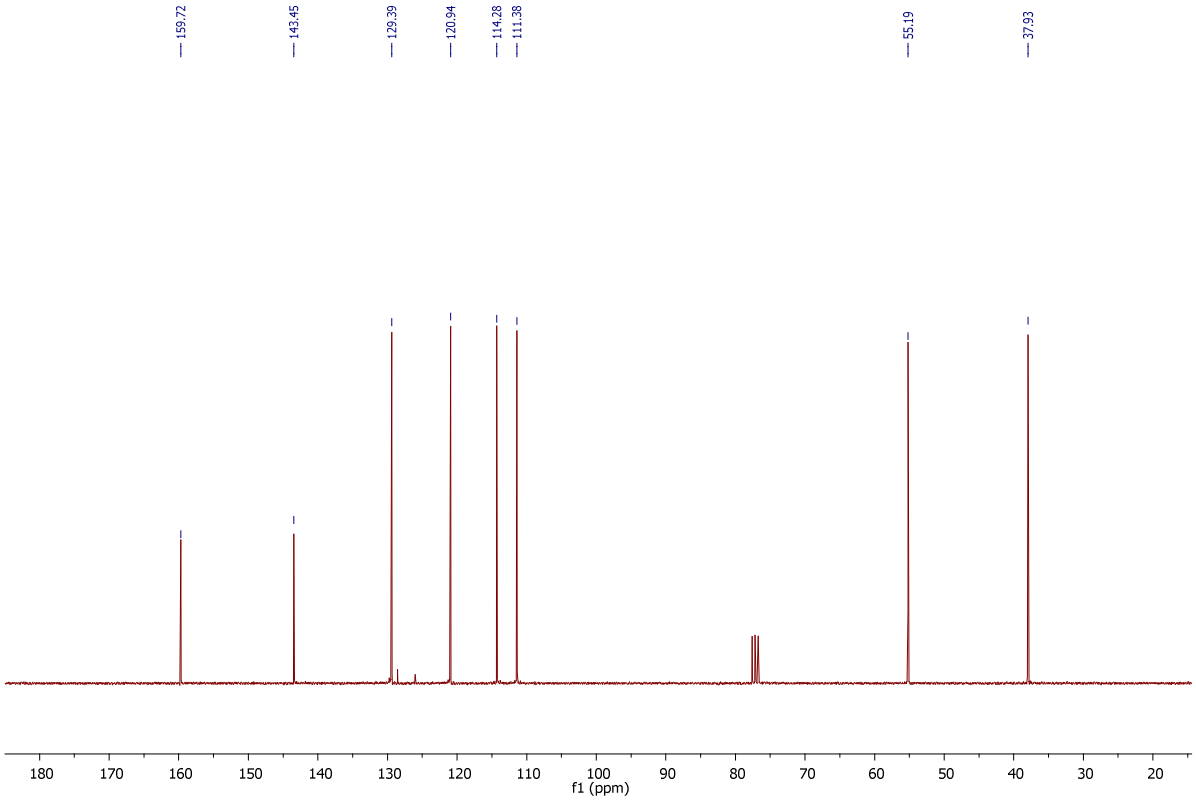
2.00
3.88
6.49
4.28
f1 (ppm)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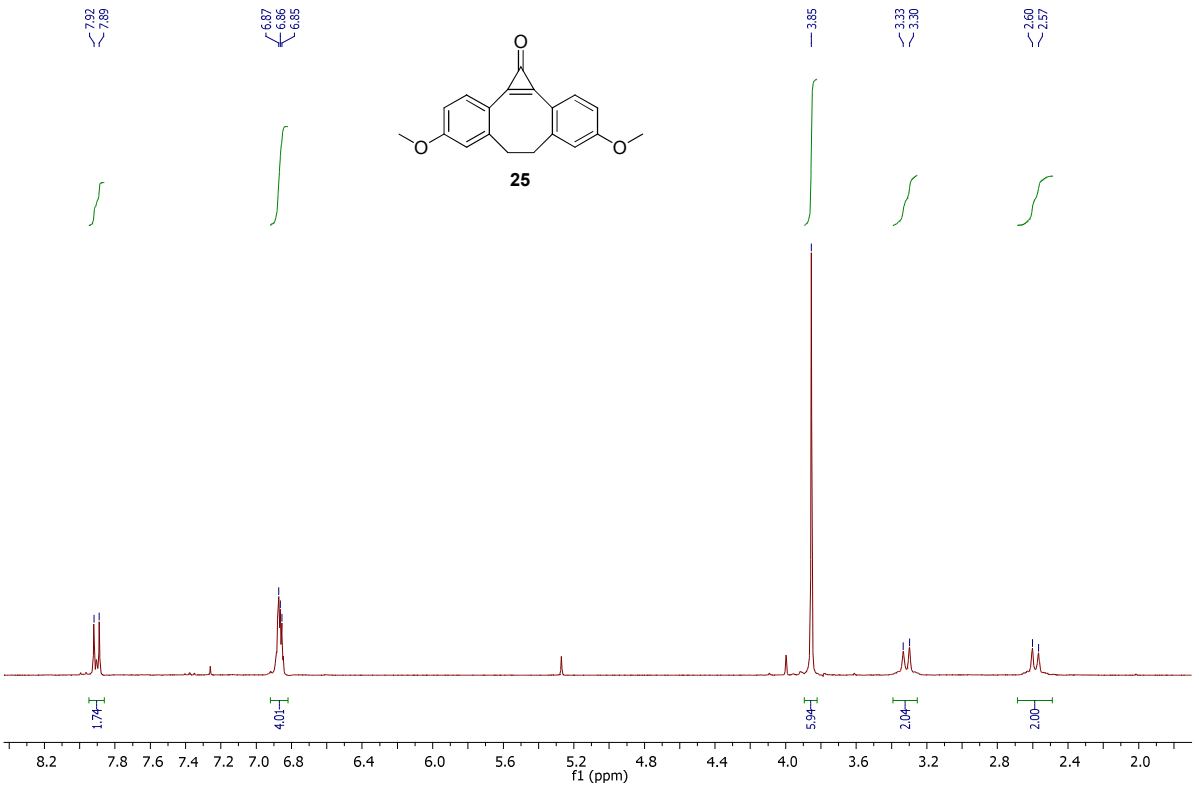
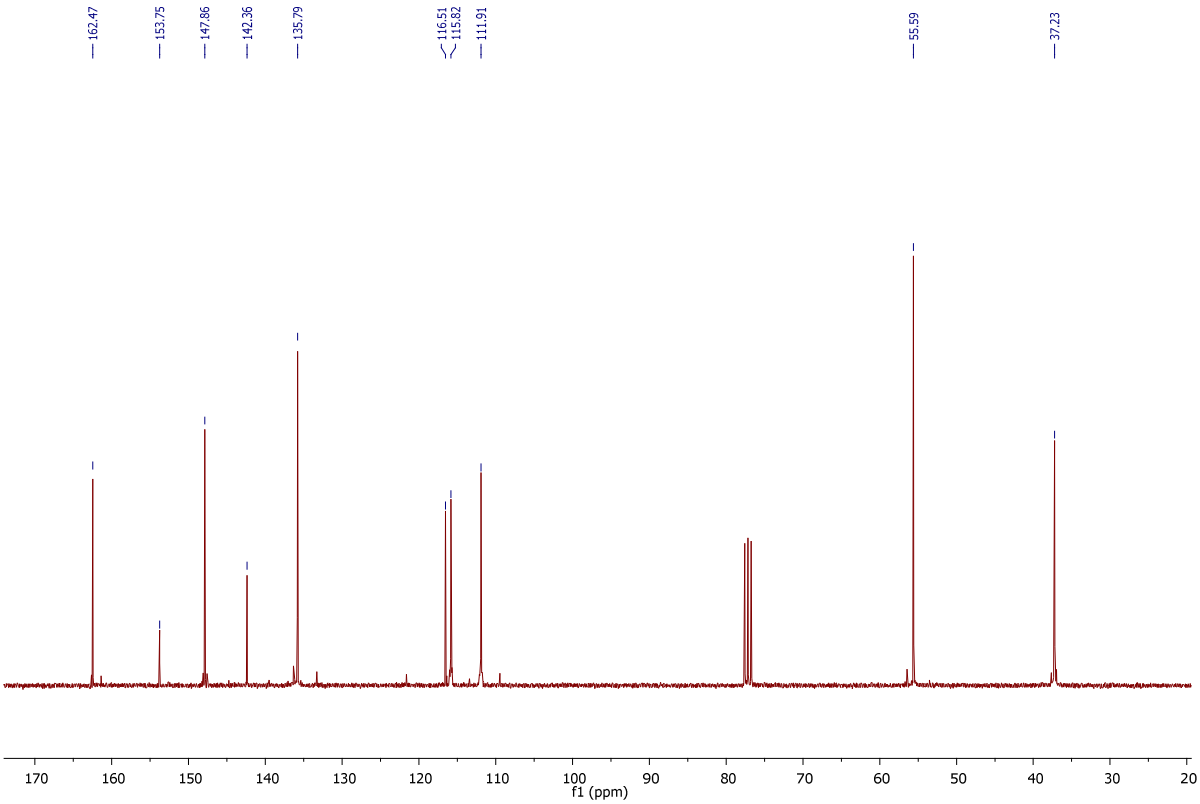
143.45
129.39
120.94
114.28
111.38
55.19
37.93
f1 (ppm)


7.92
7.89
6.87
6.86
6.85
3.85
3.33
3.30
2.60
2.57
O
O
O
25
1.74
4.01
5.94
2.04
2.00
8.2
7.8
7.6
7.4
7.2
7.0
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
f1 (ppm)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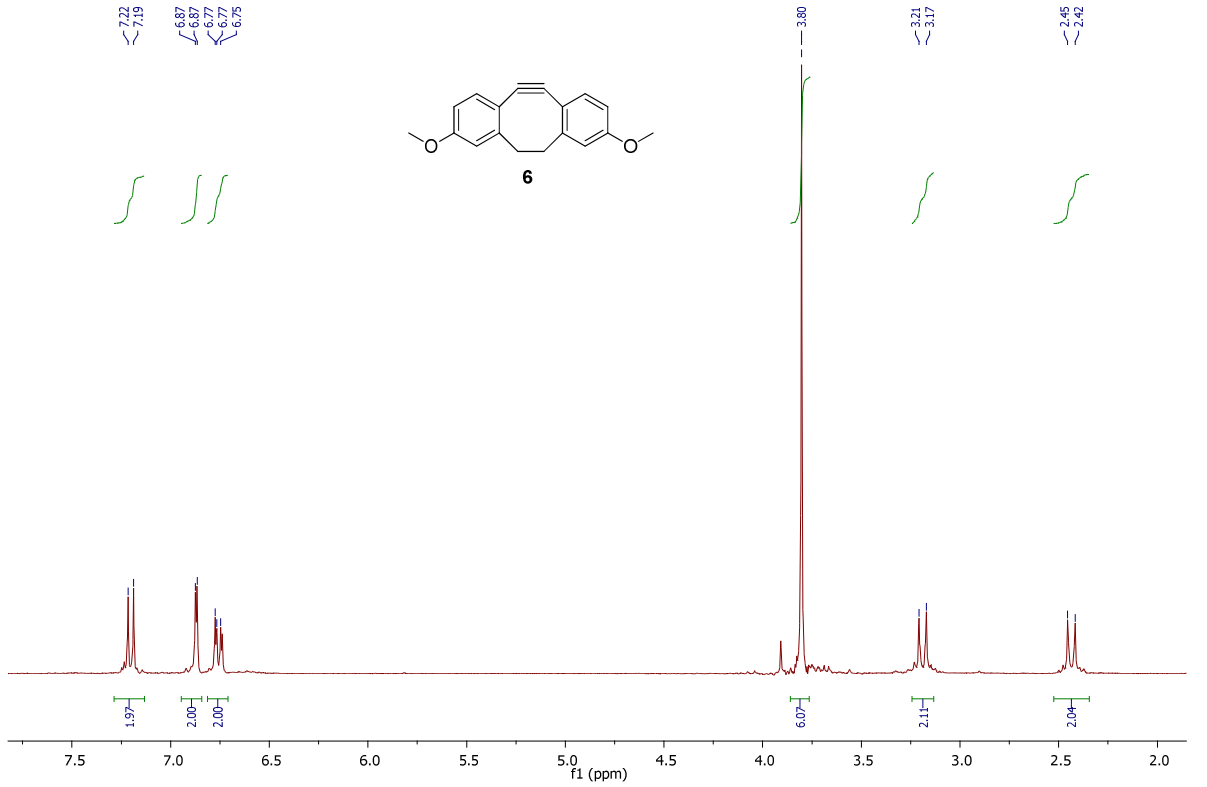
147.86
142.36
135.79
116.51
115.82
111.91
55.59
37.23
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)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7.19
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6.87
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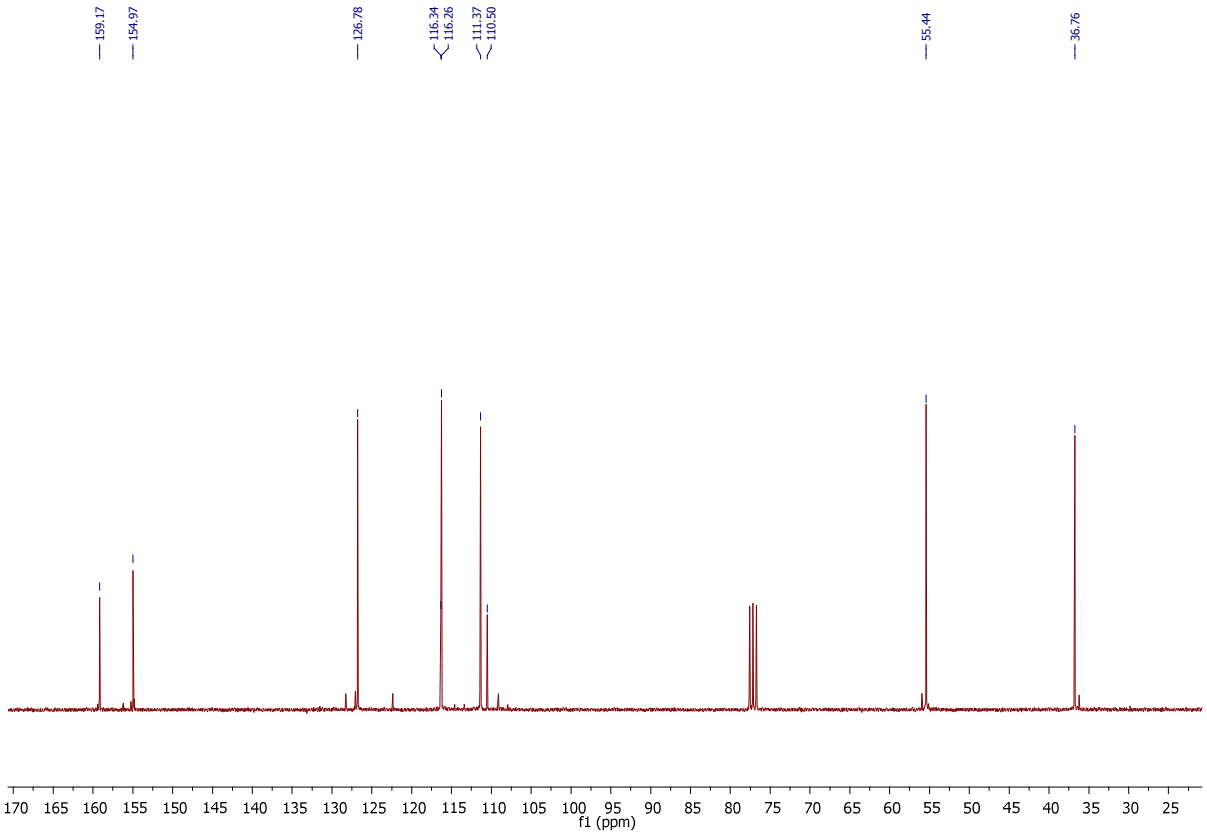
6.77
6.75
3.80
3.21
3.17
2.45
2.42
6
1.97
2.00
2.00
6.07
2.11
2.04
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)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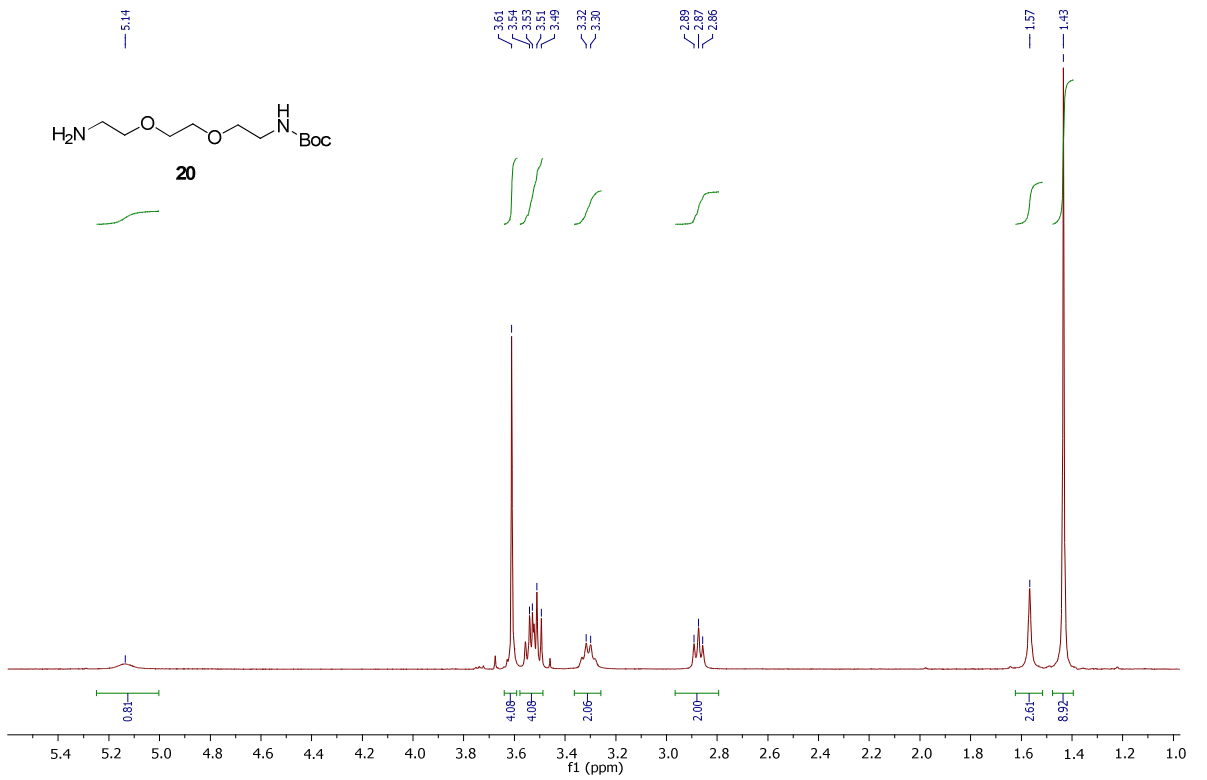
126.78
116.34
116.26
111.37
110.50
55.44
36.76
170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25
f1 (ppm)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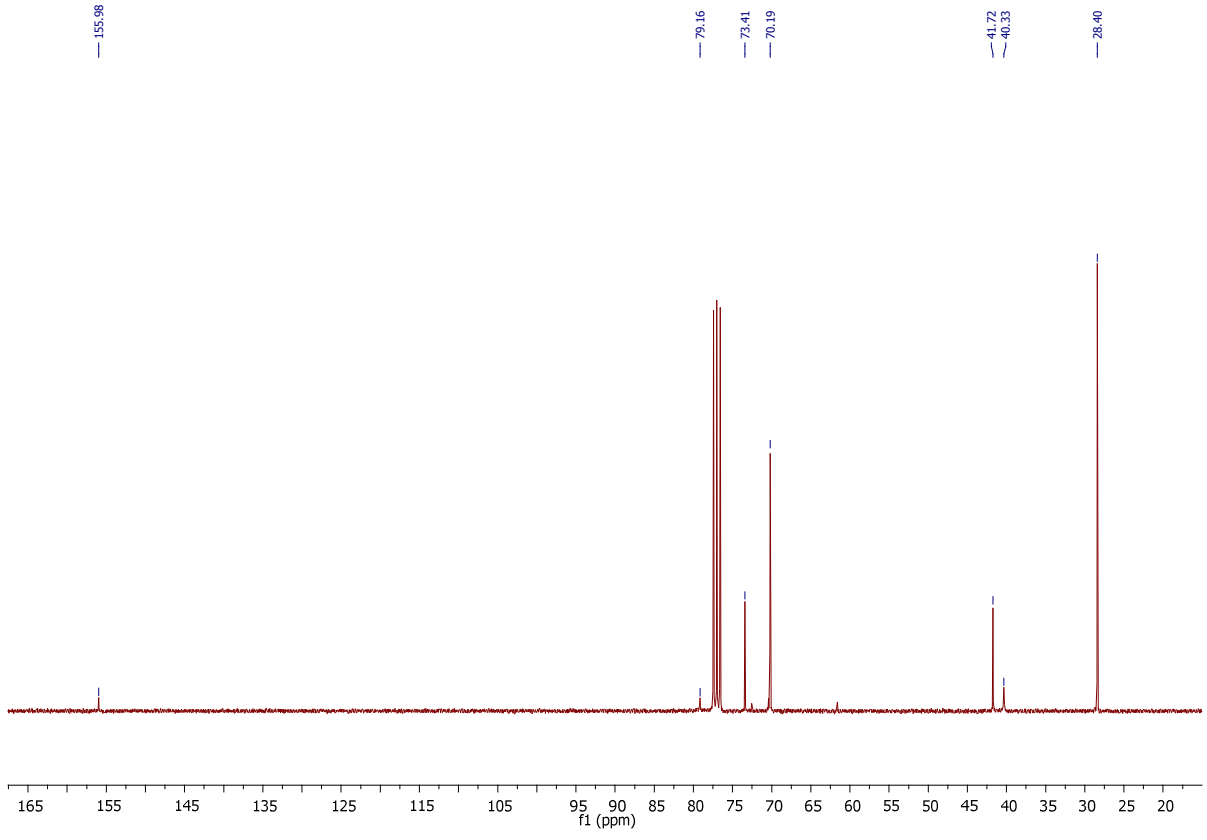
3.61
3.54
3.53
3.51
3.49
3.32
3.30
2.89
2.87
2.86
1.57
1.43
H2N
O
O
H
N
Boc
20
0.81
4.08
4.08
2.06
2.00
2.61
8.92
5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0
f1 (ppm)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79.16
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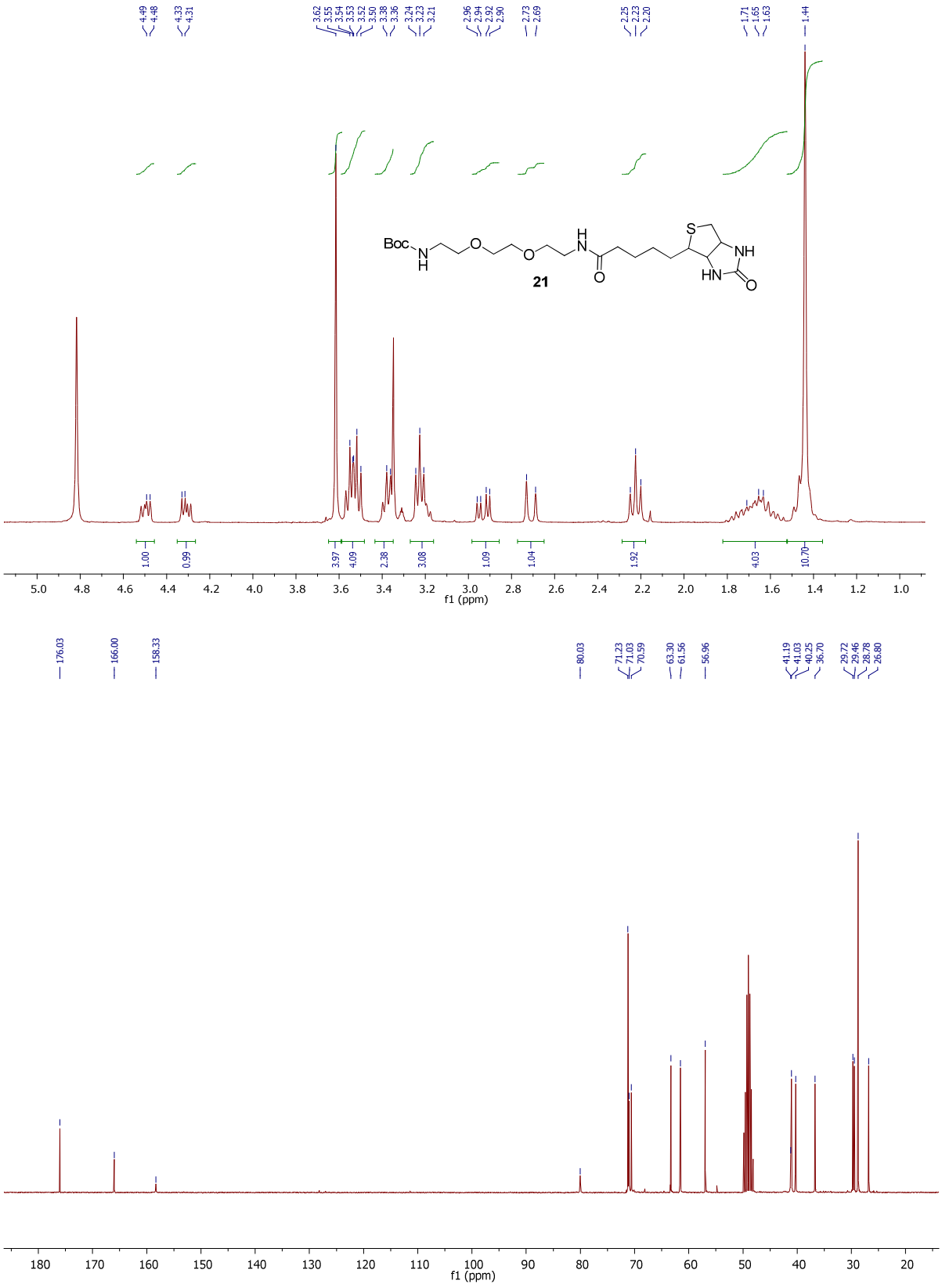
70.19
41.72
40.33
28.40
165 155 145 135 125 115 105 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20
f1 (ppm)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4.48
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4.31
3.62
3.55
3.54
3.53
3.52
3.50
3.38
3.36
3.24
3.23
3.21
2.96
2.94
2.92
2.90
2.73
2.69
2.25
2.23
2.20
1.71
1.65
1.63
1.44
Boc
N
H
O
O
H
N
O
S
NH
HN
O
21
1.00
0.99
3.97
4.09
2.38
3.08
1.09
1.04
1.92
4.03
10.70
f1 (ppm)
176.03
166.00
158.33
80.03
71.23
71.03
70.59
63.30
61.56
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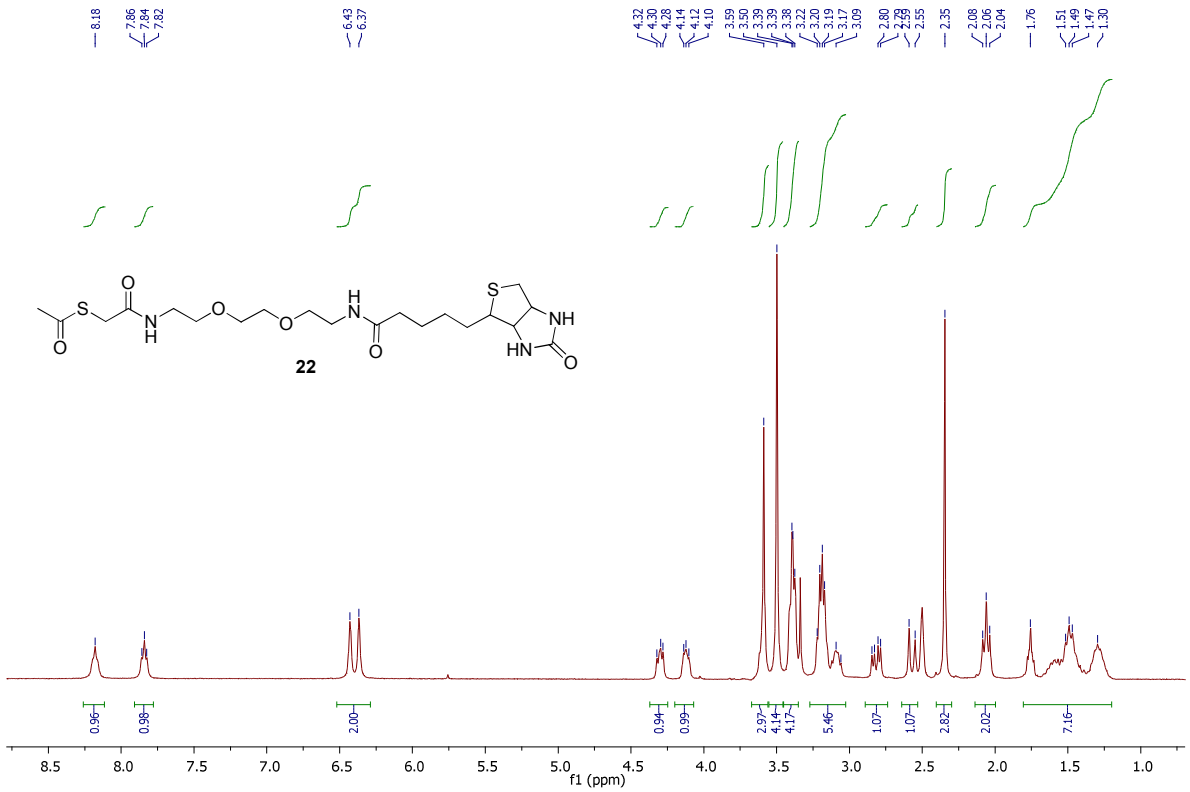
41.19
41.03
40.25
36.70
29.72
29.46
28.78
26.80
f1 (ppm)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7.84
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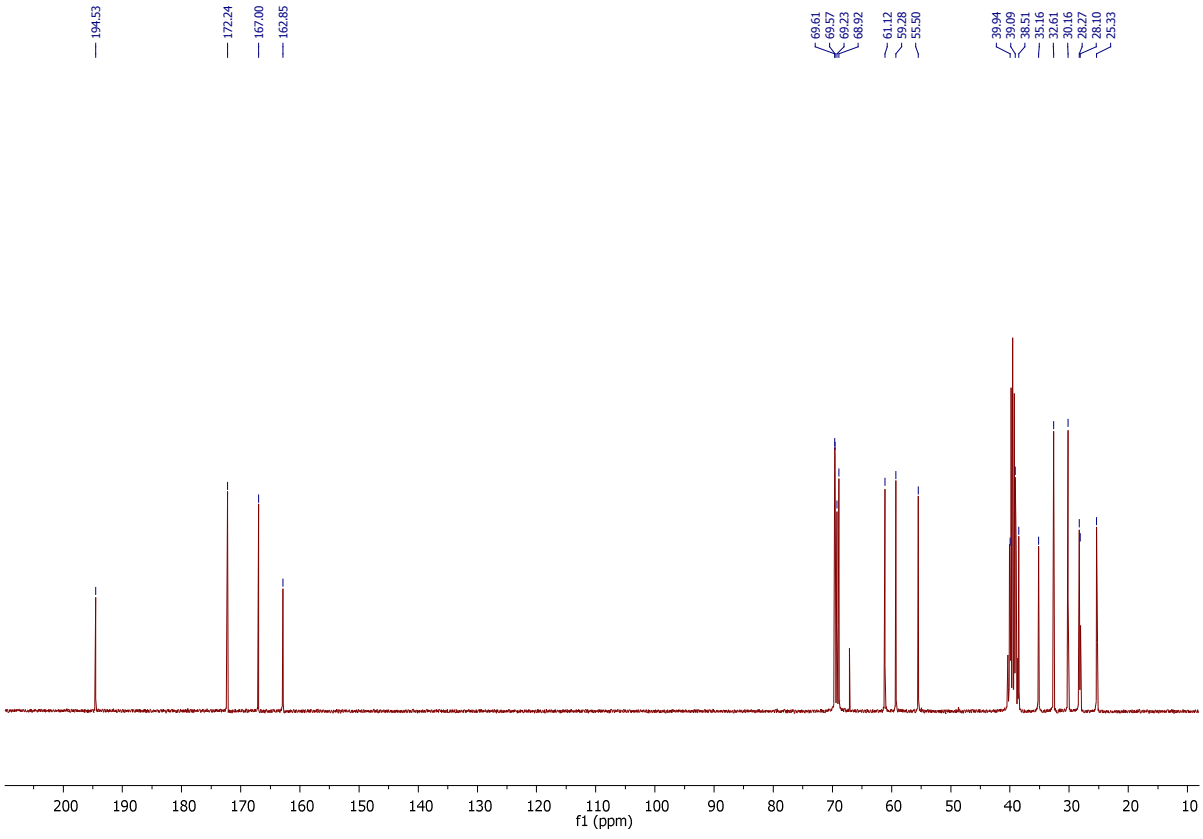
6.43
6.37
4.32
4.30
4.28
4.14
4.12
4.10
3.59
3.50
3.39
3.39
3.38
3.22
3.20
3.19
3.17
3.09
2.80
2.78
2.55
2.35
2.08
2.06
2.04
1.76
1.51
1.49
1.47
1.30
0.96
0.98
2.00
0.94
0.99
2.97
4.14
4.17
5.46
1.07
1.07
2.82
2.02
7.16
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

22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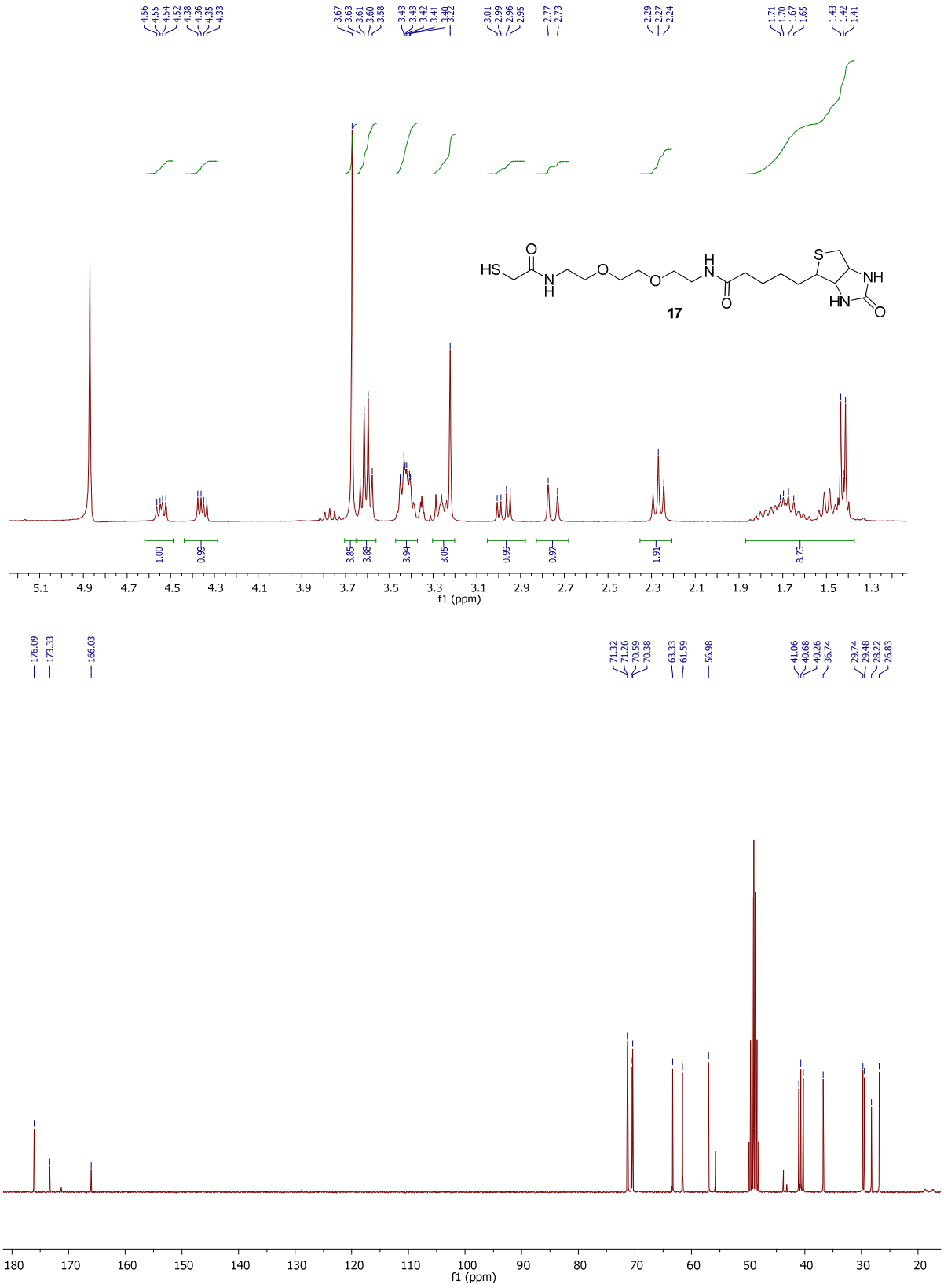
167.00
162.85
69.61
69.57
69.23
68.92
61.12
59.28
55.50
39.94
39.09
38.51
35.16
32.61
30.16
28.27
28.10
25.33
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)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4.54
4.52
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4.35
4.33
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3.63
3.61
3.60
3.58
3.43
3.43
3.42
3.41
3.40
3.42
3.01
2.99
2.96
2.95
2.77
2.73
2.29
2.27
2.24
1.71
1.70
1.67
1.65
1.43
1.42
1.41
HS
O
N
H
O
O
H
N
O
S
NH
HN
O
17
1.00
0.99
3.85
3.88
3.94
3.05
0.99
0.97
1.91
8.73
5.1
4.9
4.7
4.5
4.3
4.1
3.9
3.7
3.5
3.3
3.1
2.9
2.7
2.5
2.3
2.1
1.9
1.7
1.5
1.3
f1 (ppm)
176.09
173.33
166.03
71.32
71.26
70.59
70.38
63.33
61.59
56.98
41.06
40.68
40.26
36.74
29.74
29.48
28.22
26.83
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)