

Metal Free Sequential [3+2]-Dipolar Cycloadditions using Cyclooctynes and 1,3-Dipoles of Different Reactivity

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General Methods.

Room temperature refers to ambient room temperature (20-22 °C). Reactions were monitored by Thin Layer Chromatography (TLC) using aluminium backed silica gel 60 (F254) plates, visualised using UV254nm and potassium permanganate and ninhydrin dips as appropriate. Flash chromatography was carried out routinely using silica gel G60 (SiliCycle, 60-200µm 60 Å) as the stationary phase unless otherwise stated. The NMR spectra were recorded on a Varian Mercury (300 MHz) spectrometer. 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 either MALDI-ToF (Applied Biosystems 4700 Proteomics Analyzer) with 2,5-dihydroxybenzoic acid as a matrix or a Sciex API-1 Plus quadrupole mass spectrometer with an electron ionization source. Reverse Phase HPLC purification was performed on an Agilent 1200 series system equipped with an automated injector, UV-detector, fraction-collector and Agilent Zorbax Eclipse XD8-C18 column (5 µm, 9.4 × 250 mm). The eluents used for all purifications were: A 0.1% TFA in water; B 0.1% TFA in CH₃CN, the flow was set to 1.5 ml/min.

Materials.

All solvents were of reagent grade. All aldehydes, hydroxylamine hydrochloride, BAIB were purchased from Sigma-Aldrich[®]. Benzyl azide was purchased from Alfa Aesar[®].

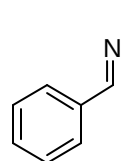
Experimental Procedures.

Due to the inseparable complex mixture of regioisomers and diastereoisomers of all click products, the ^1H -NMR and ^{13}C -NMR spectra were difficult to analyze in details. Therefore, only ^1H -NMR as well as HRMS were recorded and are therefore depicted.

General Procedure for the Formation of Aldoximes **14a-f**.

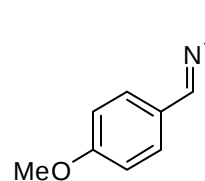
Hydroxylamine hydrochloride (77 mg, 1.1 mmol) was added to a solution of aldehyde **13a-f** (1.0 mmol, 1.0 equiv) and sodium hydroxide (44 mg, 1.1 mmol) in a mixture of water and ethanol (1:1, 5 mL). The reaction mixture was stirred at room temperature for 3 h. The organic phase was extracted with diethyl ether (3×5 mL), dried over MgSO_4 and concentrated under vacuum. The residue was then purified by flash column chromatography on silica gel using an appropriate mixture of hexane and ethyl acetate to give pure aldoxime **14a-f**.

Benzaldehyde oxime (**14a**).



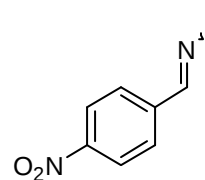
(112 mg, 92%): ^1H NMR (300 MHz, CDCl_3) δ 7.35-7.45 (m, 3H, aromH), 7.55-7.65 (m, 2H, aromH), 8.18 (s, 1H, $\text{CH}=\text{N}$), 8.68 (brs, 1H, OH); ^{13}C NMR (75.5 MHz, CDCl_3) δ 127.18 ($2 \times \text{CHm}$), 128.93 ($2 \times \text{CHo}$), 130.22 (CHp), 132.04 (C), 150.50 ($\text{C}=\text{N}$) in agreement with the literature data.ⁱ

4-Methoxybenzaldehyde oxime (**14b**).



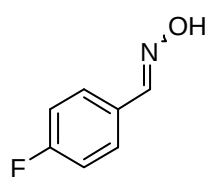
(135 mg, 89%): ^1H NMR (300 MHz, CDCl_3) δ 3.83 (s, 3H, OMe), 6.91 (d, $J = 8.8$ Hz, 2H, $2 \times \text{Hm}$), 7.52 (d, $J = 8.8$ Hz, 2H, $2 \times \text{Ho}$), 8.12 (s, 1H, $\text{CH}=\text{N}$); ^{13}C NMR (75.5 MHz, CDCl_3) δ 55.47 (CH_3), 114.39 ($2 \times \text{CHm}$), 124.69 (C-1), 128.66 ($2 \times \text{CHo}$), 150.03 ($\text{C}=\text{N}$), 161.21 (C-4) in agreement with the literature data.ⁱ

4-Nitrobenzaldehyde oxime (**14c**).

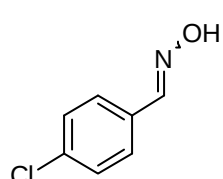


(143 mg, 86%): ^1H NMR (300 MHz, CD_3OD) δ 7.79 (d, $J = 8.8$ Hz, 2H, $2 \times \text{Ho}$), 8.16 (s, 1H, $\text{CH}=\text{N}$), 8.21 (d, $J = 8.8$ Hz, 2H, $2 \times \text{Hm}$); ^{13}C NMR (75.5 MHz, CD_3OD) δ 124.81 ($2 \times \text{CHm}$), 128.37 ($2 \times \text{CHo}$), 132.57 (C-1), 140.94 (C-4), 147.99 ($\text{C}=\text{N}$) in agreement with the literature data.ⁱ

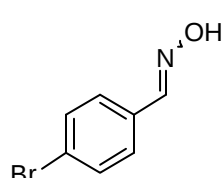
4-Fluorobenzaldehyde oxime (14d).

 (124 mg, 89%): $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.08 (t, $J = 8.7$ Hz, 2H, 2 $\times$ Hm), 7.57 (dd, $J = 8.7, 5.4$ Hz, 2H, 2 $\times$ Ho), 8.14 (s, 1H, CH=N), 8.70 (brs, 1H, OH); $^{13}\text{C NMR}$ (75.5 MHz, CDCl_3) δ 116.11 (d, $J = 22.1$ Hz, 2 $\times$ CHm), 129.03 (d, $J = 8.4$ Hz, 2 $\times$ CHo), 133.42 (d, $J = 8.6$ Hz, C-1), 149.43 (C=N), 163.93 (d, $J = 250.4$ Hz, C-4) in agreement with the literature data.ⁱ

4-Chlorobenzaldehyde oxime (14e).

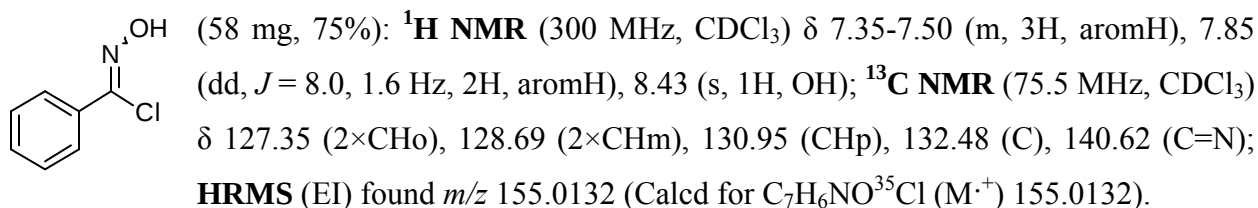
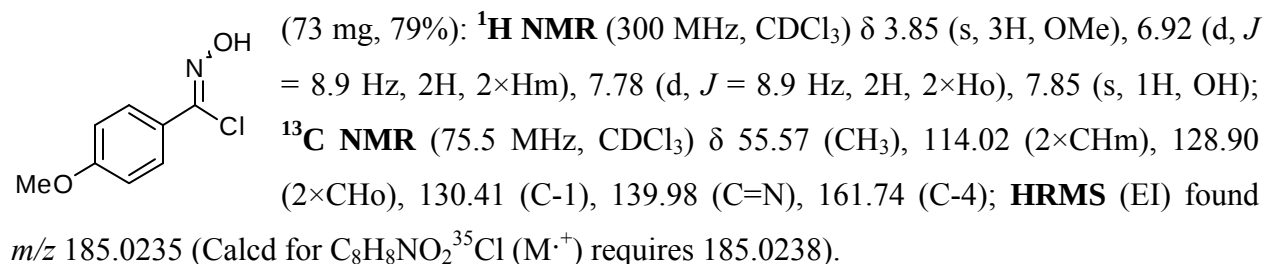
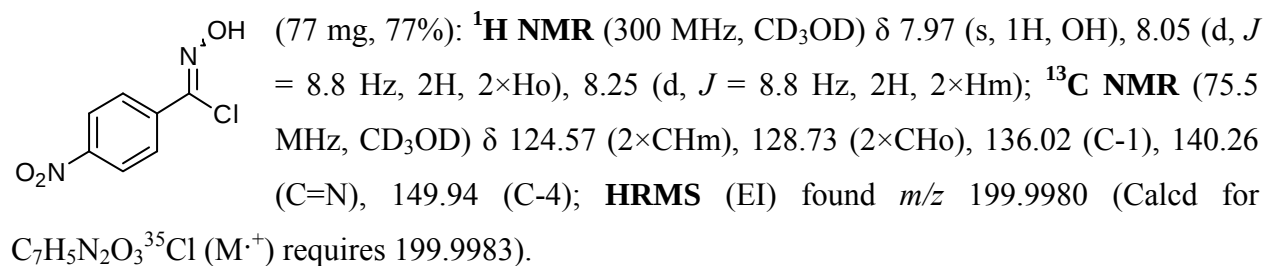
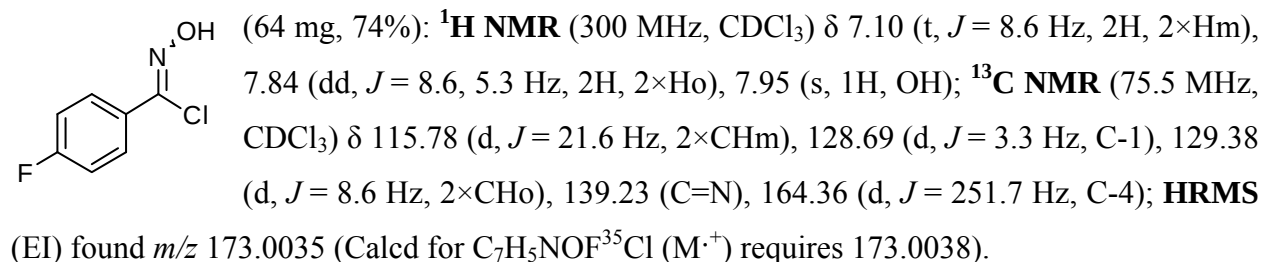
 (148 mg, 95%): $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.36 (d, $J = 8.6$ Hz, 2H, 2 $\times$ Ho), 7.51 (d, $J = 8.6$ Hz, 2H, 2 $\times$ Hm), 8.12 (s, 1H, CH=N); $^{13}\text{C NMR}$ (75.5 MHz, CDCl_3) δ 128.35 (2 $\times$ CHo), 129.22 (2 $\times$ CHm), 130.56 (C-1), 136.12 (C-4), 149.43 (C=N) in agreement with the literature data.ⁱ

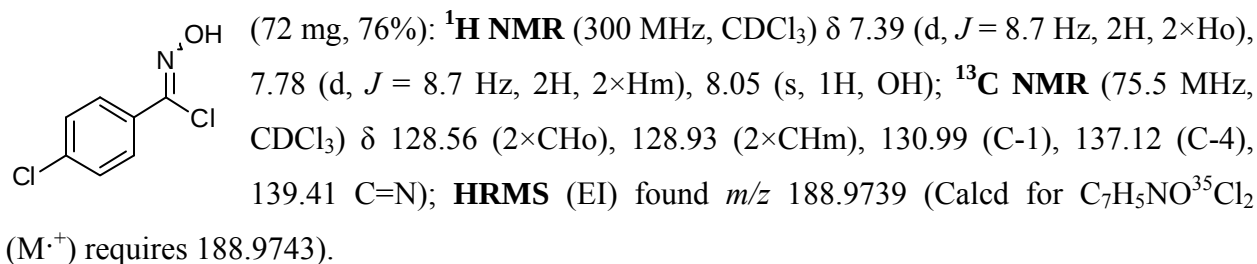
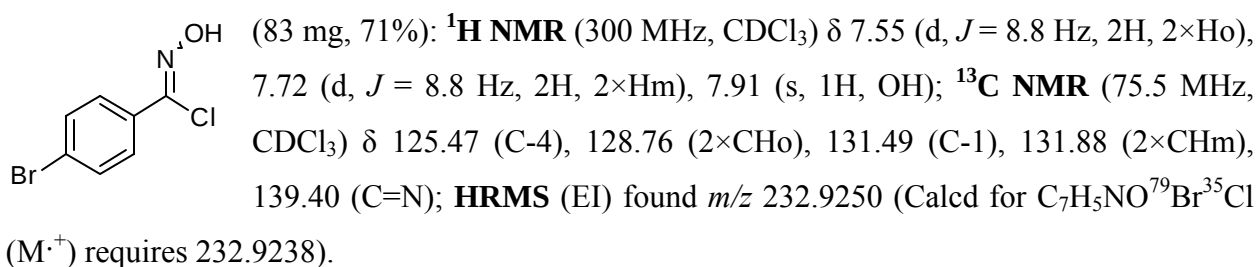
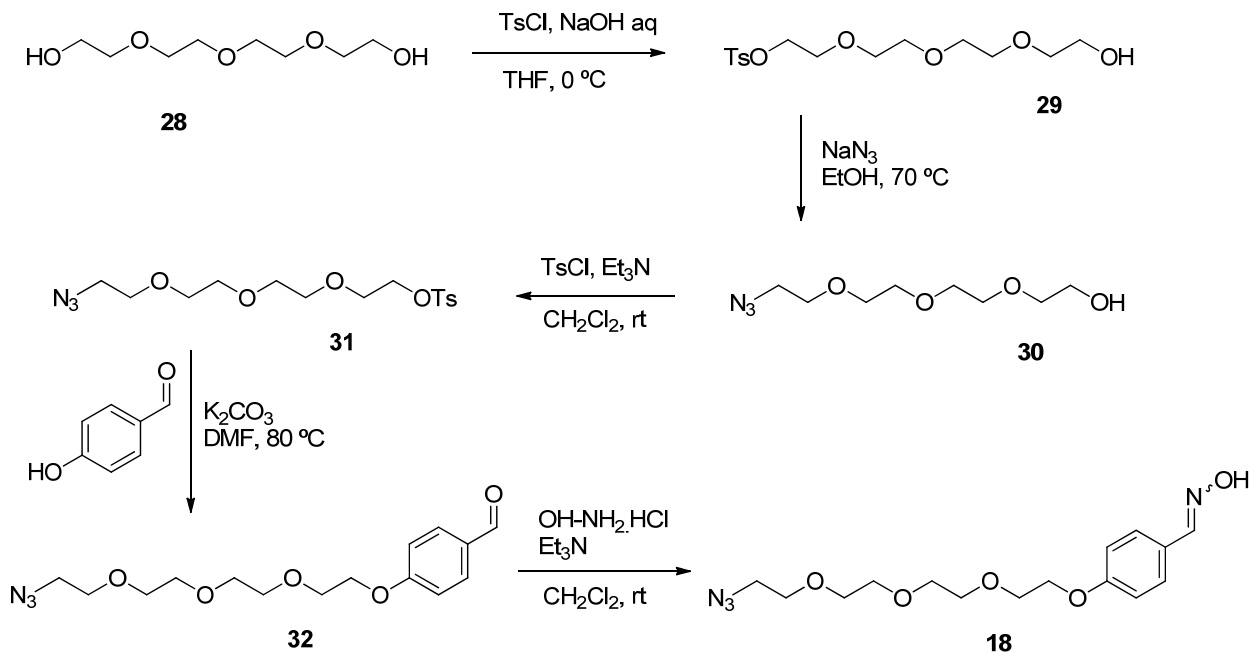
4-Bromobenzaldehyde oxime (14f).

 (182 mg, 91%): $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.44 (d, $J = 8.6$ Hz, 2H, 2 $\times$ Ho), 7.52 (d, $J = 8.6$ Hz, 2H, 2 $\times$ Hm), 8.11 (s, 1H, CH=N); $^{13}\text{C NMR}$ (75.5 MHz, CDCl_3) δ 124.46 (C-4), 128.57 (2 $\times$ CHm), 130.94 (C-1), 132.18 (2 $\times$ CHo), 149.54 (C=N) in agreement with the literature data.ⁱ

General Procedure for the Formation of Imidoyl Chlorides 5a-f

N-Chlorosuccinimide (67 mg, 0.5 mmol) was added portion-wise to a cold (0 °C) solution of aldoxime **14a-f** (0.5 mmol) in DMF (3 mL). The reaction mixture was then allowed to reach room temperature and stirred for 2 h. The reaction was then quenched by addition of water (3 mL). The organic phase was extracted with diethyl ether (3 $\times$ 5 mL), dried over MgSO_4 and concentrated under vacuum. The residue was then purified by flash column chromatography on silica gel using an appropriate mixture of hexane and ethyl acetate, to afford pure imidoyl chloride **5a-f** as a mixture of *Z/E* isomers (only the major isomer is described within the NMR data).

N-Hydroxybenzenecarboximidoyl chloride (5a).**N-Hydroxy-4-methoxybenzenecarboximidoyl chloride (5b).****N-Hydroxy-4-nitrobenzenecarboximidoyl chloride (5c).****N-Hydroxy-4-fluorobenzenecarboximidoyl chloride (5d).**

N*-Hydroxy-4-chlorobenzenecarboximidoyl chloride (5e).**N*-Hydroxy-4-bromobenzenecarboximidoyl chloride (5f).****Azido-oxime Linker Synthesis.**

2-{2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy}ethyl 4-methylbenzenesulfonate (29).

Sodium hydroxide (0.69 g, 17.13 mmol) was added to a solution of tetraethylene glycol (28) (21.95 g, 113 mmol) in THF (5 mL) at 0 °C, followed by a slow addition of a solution of *p*-toluenesulfonylchloride (2.08 g, 10.93 mmol) in THF (20 mL). The reaction mixture was then stirred for 2 h at 0 °C and poured into a mixture of ice and water. The organic layer was separated, and the aqueous layer was extracted with CH₂Cl₂ (3×100 mL). The combined organic layers were washed with water (2×50 mL), dried over MgSO₄ and evaporated in vacuo to yield 29 as a yellow oil (3.29 g, 86%): ¹H NMR (300 MHz, CDCl₃) δ 2.33 (s, 3H, CH₃), 2.89 (t, *J* = 6.0 Hz, 1H, OH), 3.40-3.70 (m, 14H, 7×CH₂), 4.00-4.10 (m, 2H, CH₂OTs), 7.24 (d, *J* = 8.0 Hz, 2H, 2×H_m), 7.68 (d, *J* = 8.0 Hz, 2H, 2×H_o); ¹³C NMR (75.5 MHz, CDCl₃) δ 21.41 (CH₃), 61.40 (CH₂OH), 68.44 (CH₂OTs), 69.17 (CH₂), 70.10 (CH₂), 70.22 (CH₂), 70.41 (CH₂), 70.46 (CH₂), 72.34 (CH₂), 127.73 (2×CH_o), 129.68 (2×CH_m), 132.76 (C), 144.68 (C) in agreement with the literature data.ⁱⁱ

2-{2-[2-(2-Azidoethoxy)ethoxy]ethoxy}ethanol (30).

Sodium azide (3.0 g, 46.15 mmol) was added to a solution of 29 (3.2 g, 9.18 mmol) in ethanol (50 mL) at room temperature. The reaction mixture was stirred overnight at 70 °C. The reaction was then quenched by addition of water (50 mL), and concentrated under vacuum to a third of its volume. The aqueous layer was extracted with ethyl acetate (3×50 mL). The combined organic layers were then dried over MgSO₄ and concentrated under vacuum. The residue was then purified by flash column chromatography on silica gel using a mixture of acetone and hexane (2:3) to yield 30 as an oil (1.63 g, 81%): ¹H NMR (300 MHz, CDCl₃) δ 2.91 (t, *J* = 6.0 Hz, 1H, OH), 3.30 (t, *J* = 5.0 Hz, 2H, CH₂N₃), 3.49-3.65 (m, 14H, 7×CH₂); ¹³C NMR (75.5 MHz, CDCl₃) δ 50.54 (CH₂N₃), 61.51 (CH₂OH), 69.91 (CH₂), 70.21 (CH₂), 70.46 (CH₂), 70.52 (CH₂), 70.56 (CH₂), 72.43 (CH₂); HRMS (MALDI- ToF) 258.0880 (C₈H₁₇N₃O₄K (M+K⁺) requires 258.0856).

2-{2-[2-(2-Azidoethoxy)ethoxy]ethoxy}ethyl 4-methylbenzenesulfonate (31).

p-Toluenesulfonylchloride (2.13 g, 11.15 mmol) was added to a solution of azide 30 (1.63 g, 7.43 mmol) and triethylamine (1.55 mL, 11.15 mmol) in CH₂Cl₂ (20 mL) at room temperature.

The reaction mixture was stirred at room temperature for 6 h. Water (20 mL) was then added to the reaction mixture, the organic layer was separated and the aqueous layer was extracted with CH₂Cl₂ (3×20 mL). The combined organic layers were dried over MgSO₄ and concentrated under vacuum. The residue was then purified by flash column chromatography on silica gel using a mixture of 2% methanol in CH₂Cl₂ to afford pure **31** as an oil (2.47 g, 89%): **¹H NMR** (300 MHz, CDCl₃) δ 2.44 (s, 3H, CH₃), 3.38 (t, *J* = 5.0 Hz, 2H, CH₂N₃), 3.55-3.75 (m, 12H, 6×CH₂), 4.10-4.20 (m, 2H, CH₂OTs), 7.34 (d, *J* = 8.4 Hz, 2H, 2×Hm), 7.80 (d, *J* = 8.4 Hz, 2H, 2×Ho); **¹³C NMR** (75.5 MHz, CDCl₃) δ 21.77 (CH₃), 50.82 (CH₂N₃), 68.82 (CH₂), 69.37 (CH₂OTs), 70.18 (2×CH₂), 70.74 (CH₂), 70.81 (CH₂), 70.90 (CH₂), 128.11 (2×CHo), 129.94 (2×CHm), 133.16 (C), 144.92 (C); **HRMS** (MALDI- ToF) 412.0954 (C₁₅H₂₃N₃O₆SK (M+K⁺) requires 412.0945).

4-(2-{2-[2-(2-Azidoethoxy)ethoxy]ethoxy}ethoxy)benzaldehyde (**32**).

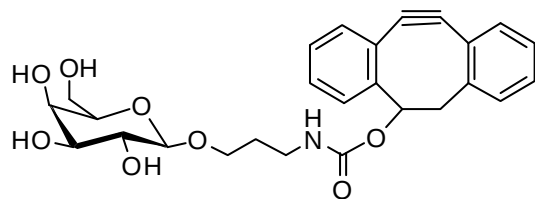
Potassium carbonate (1.36 g, 9.84 mmol) was added to a solution of 4-hydroxybenzaldehyde (1.20 g, 9.84 mmol) and azido-tosyl **31** (2.45 g, 6.56 mmol) in DMF (10 mL) at room temperature. The reaction mixture was then stirred at 80 °C overnight. Water (15 mL) was then added to the reaction mixture, the organic layer was separated and the aqueous layer was extracted with CH₂Cl₂ (3×10 mL). The combined organic layers were dried over MgSO₄ and concentrated under vacuum. The residue was then purified by flash column chromatography on silica gel using a mixture of 2% methanol in CH₂Cl₂ to afford pure **32** as an oil (1.27 g, 60%): **¹H NMR** (300 MHz, CDCl₃) δ 3.37 (t, *J* = 5.0 Hz, 2H, CH₂N₃), 3.60-3.75 (m, 10H, 5×CH₂), 3.88 (t, *J* = 5.0 Hz, 2H, CH₂CH₂OAr), 4.21 (t, *J* = 5.0 Hz, CH₂CH₂OAr), 7.01 (d, *J* = 8.7 Hz, 2H, 2×Ho), 7.82 (d, *J* = 8.7 Hz, 2H, 2×Hm), 9.87 (s, 1H, CH=O); **¹³C NMR** (75.5 MHz, CDCl₃) δ 50.80 (CH₂N₃), 67.89 (CH₂OAr), 69.59 (CH₂ CH₂OAr), 70.17 (CH₂), 70.80 (CH₂), 70.84 (2×CH₂), 71.03 (CH₂), 115.00 (2×CHo), 130.17 (C), 132.08 (2×CHm), 163.98 (C), 190.95 (C=O); **HRMS** (MALDI- ToF) 362.1096 (C₁₅H₂₁N₃O₅K (M+K⁺) requires 362.1118).

4-(2-{2-[2-(2-Azidoethoxy)ethoxy]ethoxy}ethoxy)benzaldehyde oxime (**18**).

N-Hydroxylamine hydrochloride (410 mg, 5.91 mmol) was added to a solution of aldehyde **32** (1.27 g, 3.94 mmol) and triethylamine (0.83 mL, 5.91 mmol) in CH₂Cl₂ (10 mL) at room temperature. The reaction mixture was then stirred at room temperature overnight. Water (10

mL) was then added to the reaction mixture, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried over MgSO_4 and concentrated under vacuum. The residue was then purified by flash column chromatography on silica gel using a mixture of 2% methanol in CH_2Cl_2 to afford pure **18** as an oil (1.17 g, 88%): ^1H NMR (300 MHz, CDCl_3) δ 3.36 (t, $J = 5.2$ Hz, 2H, CH_2N_3), 3.63-3.76 (m, 10H, $5 \times \text{CH}_2$), 3.86 (t, $J = 5.0$ Hz, 2H, $\text{CH}_2\text{CH}_2\text{OAr}$), 4.14 (t, $J = 5.0$ Hz, $\text{CH}_2\text{CH}_2\text{OAr}$), 6.89 (d, $J = 8.7$ Hz, 2H, $2 \times \text{H}_o$), 7.46 (d, $J = 8.7$ Hz, 2H, $2 \times \text{H}_m$), 8.06 (s, 1H, $\text{CH}=\text{N}$); ^{13}C NMR (75.5 MHz, CDCl_3) δ 50.79 (CH_2N_3), 67.60 (CH_2OAr), 69.75 ($\text{CH}_2\text{CH}_2\text{OAr}$), 70.15 (CH_2), 70.79 (CH_2), 70.80 (CH_2), 70.82 (CH_2), 70.97 (CH_2), 114.99 ($2 \times \text{CH}_o$), 124.96 (C), 128.55 ($2 \times \text{CH}_m$), 149.86 ($\text{C}=\text{NOH}$), 160.35 (C); HRMS (MALDI- ToF) 361.1449 ($\text{C}_{15}\text{H}_{22}\text{N}_4\text{O}_5\text{Na}$ ($\text{M}+\text{Na}^+$) requires 361.1488).

DIBO-Galactose **19**

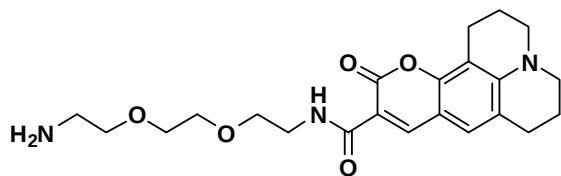


3-Azidopropyl β -D-galactopyranosideⁱⁱⁱ (93 mg, 0.35 mmol) was dissolved in a mixture of THF and H_2O (9:1, v/v, 5 mL). A 1 M solution of trimethylphosphine in THF (1.75 mL, 1.75 mmol)

was added to the solution and the resulting mixture was stirred for 3 h. The solution was concentrated *in vacuo* and coevaporated with toluene (3×10 mL). The resulting residue was treated with 11,12-didehydro-5,6-dihydrodibenzo[a,e]cycloocten-5-yl carbonic acid 4-nitrophenyl ester^{iv} (135 mg, 0.35 mmol) and DIPEA (61 μL , 0.35 mmol) in DMF (5mL). After stirring for 8 h, the solvent was evaporated under reduced pressure and the resulting residue was purified by silica gel column chromatography (10% MeOH/DCM) to give **19** as a colorless oil (123 mg, 73%): ^1H NMR (300 MHz, CD_3OD) δ 1.76-1.85 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2$), 2.80 (dd, $J = 14.9, 3.8$ Hz, 1H, CHHCHO), 3.18-3.32 (m, 3 H, CH_2NH , CHHCHO), 3.43-3.85 (m, 7H, CH_2_{gal} , $\text{CH}-3_{\text{gal}}$, $\text{CH}-5_{\text{gal}}$, $\text{CHHCH}_2\text{CH}_2\text{NH}$, $\text{CH}_2-6_{\text{gal}}$, $\text{CH}-4_{\text{gal}}$), 3.93-4.00 (m, 1H, $\text{CHHCH}_2\text{CH}_2\text{NH}$), 4.23 (dd, $J = 7.4, 2.7$ Hz, 1H, $\text{CH}-1_{\text{gal}}$), 5.43 (s, 1H, CH_2CHO), 7.29-7.41 (m, 7H, aromH), 7.57 (d, $J = 7.4$ Hz, 1H, aromH); ^{13}C NMR (75.5 MHz, CD_3OD) δ 30.84 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}$), 38.92 (CH_2NH), 47.17 (CH_2CHO), 62.53 ($\text{CH}_2-6_{\text{gal}}$), 68.10 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}$), 70.29 ($\text{CH}-4_{\text{gal}}$), 72.55 ($\text{CH}-2_{\text{gal}}$), 74.98 ($\text{CH}-3_{\text{gal}}$), 76.63 ($\text{CH}-5_{\text{gal}}$), 77.86 (CH_2CHO), 104.95 ($\text{CH}-1_{\text{gal}}$), 110.99 ($\text{C}\equiv\text{C}$), 113.84 ($\text{C}\equiv\text{C}$), 122.37 (C), 124.94 (CH, C), 126.87

(CH), 127.15 (CH), 128.22 (CH), 128.28 (CH), 129.25 (CH), 129.31 (CH), 131.05 (CH), 152.42 (C), 153.65 (C), 157.98 (C=O); **MS** (MALDI- ToF) 506.2457 ($C_{26}H_{29}NO_8Na$ ($M+Na^+$) requires 506.1785).

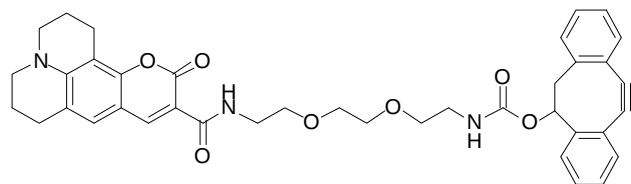
Amino PEG Coumarin derivative **33**



Coumarin 343 (100 mg, 0.35 mmol), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBop) (273 mg, 0.53 mmol) and *N,N*-diisopropylethylamine (DIPEA)

(182 μ L, 1.05 mmol) were dissolved in CH_2Cl_2 (5 mL). The resulting mixture was added dropwise to a stirred solution of 2,2'-(ethylenedioxy)bis(ethylamine) (511 μ L, 3.5 mmol) in CH_2Cl_2 (5 mL) over a period of 5 min. After stirring for 4 h, the solvent was evaporated under reduced pressure and the resulting residue was purified by column chromatography on silica gel (gradient MeOH/ CH_2Cl_2 / NH_4OH 8/91/1, v/v/v to MeOH/ CH_2Cl_2 / NH_4OH 10/89/1, v/v/v) yielding **33** as an orange glass-like solid (104 mg, 72%): **1H NMR** (300 MHz, CD_3OD) δ 1.84-1.98 (m, 4H, $2\times NCH_2CH_2$), 2.59-2.73 (m, 4H, $2\times N(CH_2)_2CH_2$), 2.79-2.94 (m, 2H, CH_2NH_2), 3.23-3.36 (m, 4H, $2\times NCH_2(CH_2)_2$), 3.60 (t, $J = 5.21$ Hz, 4H, $2\times NHCH_2CH_2$), 3.68-3.71 (m, 6H, $2\times CH_2O$, $NHCH_2$), 6.83 (s, 1H, aromH), 8.21 (s, 1H, vinylH); **^{13}C NMR** (75.5 MHz, CD_3OD) δ 20.87 ($N(CH_2)_2CH_2$), 20.99 (NCH_2CH_2), 21.98 (NCH_2CH_2), 28.28 ($N(CH_2)_2CH_2$), 40.51 ($NHCH_2$), 42.13 (CH_2NH_2), 50.66 (NCH_2), 51.18 (NCH_2), 70.63 (CH_2O), 71.38 (CH_2O), 71.55 ($2\times CH_2O$), 106.12 (C), 108.02 (C), 109.09 (C), 121.35 (C), 128.29 (CH), 148.64 (CH), 149.76 (C), 153.62 (C), 163.91 (C=O), 165.38 (C=O); **HRMS** (MALDI-ToF) 416.2057 ($C_{22}H_{30}N_3O_5$ ($M+H^+$) requires 416.2180).

Coumarin-DIBO derivative **22**

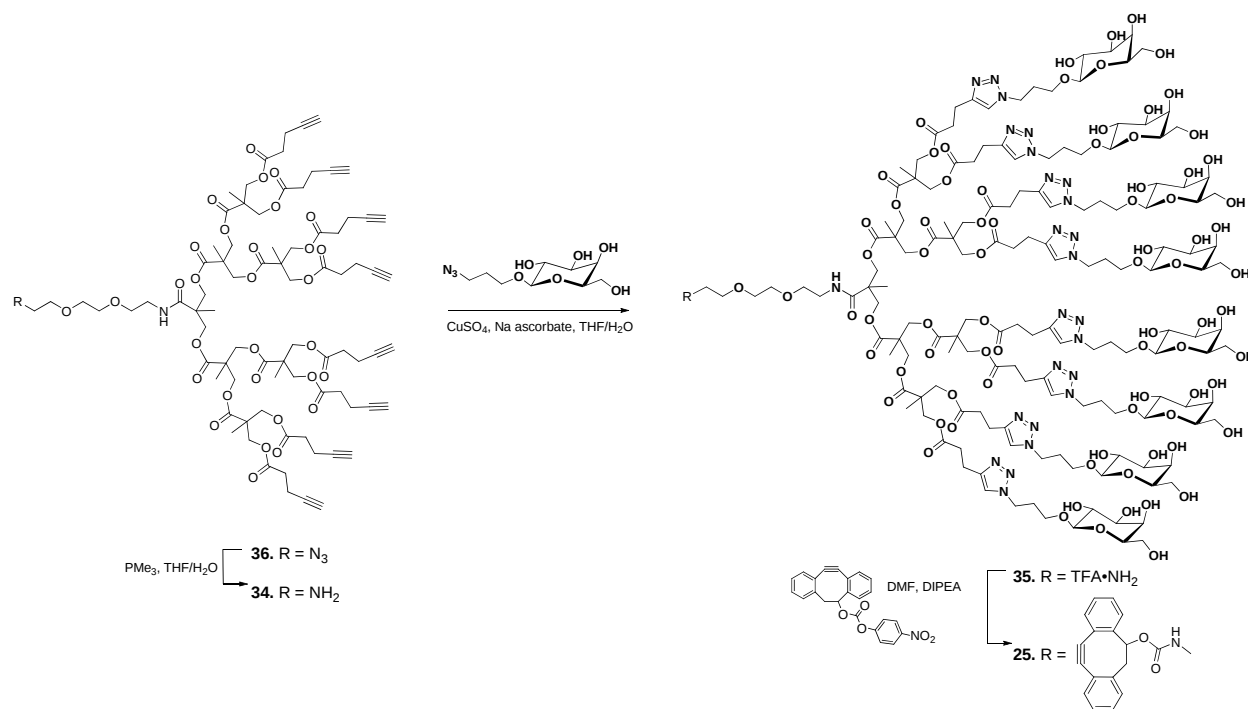


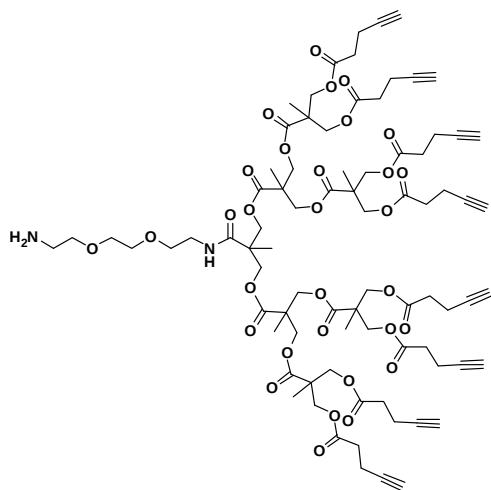
Coumarin derivative **33** (37.0 mg, 0.09 mmol), 11,12-didehydro-5,6-dihydrodibenzo[a,e]cycloocten-5-yl carbonic acid 4-nitrophenyl ester (41 mg, 0.11 mmol)

and DIPEA (63 μ L, 0.36 mmol) were dissolved in CH_2Cl_2 (3 mL). The reaction mixture was

stirred for 24h and concentrated *in vacuo*. The resulting residue was purified by column chromatography on silica gel (50% acetone in hexanes) to give **22** as a yellow glass-like solid (54 mg, 91%): **¹H NMR** (300 MHz, CDCl₃) δ 1.83-1.98 (m, 4H, 2×NCH₂CH₂), 2.69-2.85 (m, 5H, 2×N(CH₂)₂CH₂, CHHCHO), 3.11 (d, *J* = 15 Hz, 1H, CHHCHO), 3.22-3.30 (m, 4H, 2×NCH₂(CH₂)₂), 3.35-3.46 (m, 2H, NHCH₂), 3.54-3.79 (m, 10H, 4×CH₂O, NHCH₂), 5.44 (brs, 1H, CH₂CHO), 6.01 (t, *J* = 5.0 Hz, 1H, NH), 6.93 (s, 1H, aromH), 7.20-7.32 (m, 7H, aromH), 7.50 (d, *J* = 6.5 Hz, 1H, aromH), 8.58 (s, 1H, vinylH), 9.17 (brs, 1H, NH); **¹³C NMR** (75.5 MHz, CDCl₃) δ 19.99 (N(CH₂)₂CH₂, NCH₂CH₂), 21.00 (NCH₂CH₂), 27.32 (N(CH₂)₂CH₂), 39.30 (NHCH₂), 41.00 (NHCH₂), 46.06 (CH₂CHO), 49.66 (NCH₂), 50.09 (NCH₂), 69.75 (CH₂O), 70.12 (CH₂O), 70.32 (CH₂O), 70.40 (CH₂O), 76.51 (CH₂CHO), 105.51 (C), 108.10 (C), 108.78 (C), 109.90 (C≡C), 112.71 (C≡C), 119.53 (C), 121.04 (C), 123.69 (C), 123.88 (CH), 125.70 (CH), 125.93 (CH), 126.81 (2×CH), 126.89 (CH), 127.84 (CH), 127.91 (CH), 129.92 (CH), 147.97 (CH), 148.03 (C), 151.06 (C=O), 152.19 (C), 152.54 (C), 155.62 (C) 162.95 (C=O), 163.60 (C=O); **HRMS** (MALDI-ToF) 684.2219 (C₃₉H₃₉N₃O₇Na (M+Na⁺) requires 684.2680).

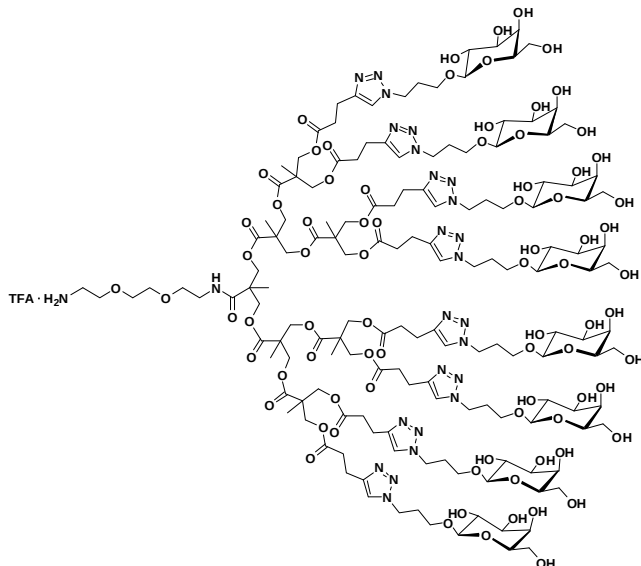
Synthesis of DIBO-glycodendron **25**



Dendron 34.

Azido-dendron **36^v** (100 mg, 0.061 mmol) was dissolved in a mixture of THF and H₂O (9:1, v/v, 5 mL). A 1 M solution of trimethylphosphine in THF (0.61 mL, 0.61 mmol) was added to the latter solution and the resulting mixture was stirred for 3 h. The solution was concentrated *in vacuo* and coevaporated with toluene (3×10 mL). The resulting residue was purified by silica gel column chromatography (10% MeOH in CH₂Cl₂) to give **34** as a colorless oil (76 mg, 78%): ¹H NMR (300 MHz, CDCl₃) δ 1.22-1.27 (m, 21H, 7×CH₃), 1.97 (t, *J* =

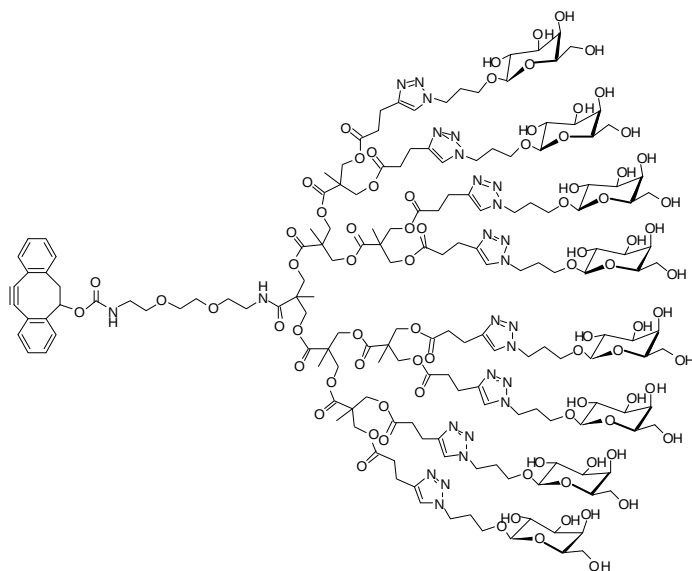
2.5 Hz, 8H, 8×C≡CH), 2.41-2.56 (m, 32H, 8×CH₂CH₂C≡CH), 2.61-3.00 (m, 4H, CH₂NH₂), 3.37-3.59 (m, 10H, CH₂NH, 4×CH₂O), 4.17 - 4.25 (m, 28H, 14×OCH₂), 6.90 (t, *J* = 5.2 Hz, 1H, NH); ¹³C NMR (75.5 MHz, CDCl₃) δ 14.19 (8×CH₂C≡CH), 17.43 (2×CH₃), 17.53 (CH₃), 17.72 (4×CH₃), 33.04 (8×CH₂CH₂C≡CH), 39.45 (NHCH₂), 41.21 (CH₂NH₂), 46.23 (CH₂C), 46.28 (4×CH₂C), 46.64 (2×CH₂C), 65.18 (8×OCH₂C), 65.30 (4×OCH₂C), 67.07 (2×OCH₂C), 69.27 (8×C≡CH, CH₂O), 69.59 (CH₂O), 69.94 (CH₂O), 70.07 (CH₂O), 82.22 (8×C≡CH), 171.07 (8×C=O), 171.42 (C=O), 171.46 (2×C=O), 171.84 (4×C=O); MS (MALDI-ToF) 1601.8 (C₈₁H₁₀₅N₂O₃₁ (M+H⁺) requires 1601.7).

Glycodendron 35.

Dendron **34** (40 mg, 0.025 mmol), 3-azidopropyl β-D-galactopyranosideⁱⁱⁱ (78.6mg, 0.300 mmol) and tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) (10.6mg, 0.020mmol) were dissolved in THF (2 mL). An aqueous solution of (+)-sodium L-ascorbate (0.1 M, 0.6 mL, 0.060 mmol) and CuSO₄ (0.1 M, 0.2 mL, 0.020 mmol) were added, and the resulting solution was stirred

for 18 h. Solvents were evaporated under reduced pressure and the residue was purified *via* RP-HPLC (0-5min 0% B, 5-30 min gradient of 0-100% B, t_r =20.8 min). Fractions of interest were combined and lyophilized to give pure **35** as TFA salt (65mg, 70%): ^1H NMR (500 MHz, D_2O) δ 1.12 (s, 12H, $4\times\text{CH}_3$), 1.23 (s, 6H, $2\times\text{CH}_3$), 1.30 (s, 3H, CH_3), 2.20-2.22 (m, 16H, $8\times\text{CH}_2\text{CH}_2\text{CH}_2\text{-triazole}$), 2.77-2.80 (m, 16H, $8\times\text{CH}_2\text{CH}_2\text{-triazole}$), 3.03 (brs, 16H, $8\times\text{CH}_2\text{CH}_2\text{-triazole}$), 3.20 (t, $J = 4.9$ Hz, 2H, CH_2NH_2), 3.41 (t, $J = 5.3$ Hz, 2H, CH_2NH), 3.50 (dd, $J = 9.8, 7.9$ Hz, 8H, $8\times\text{CH-2}_{\text{gal}}$), 3.57-3.77 (m, 48H, $4\times\text{CH}_2\text{O}$, $8\times\text{CH-3}_{\text{gal}}$, $8\times\text{CH-5}_{\text{gal}}$, $8\times\text{CH}_2\text{-6}_{\text{gal}}$, $8\times\text{CHHCH}_2\text{CH}_2\text{-triazole}$), 3.87-3.94 (m, 16H, $8\times\text{CH-4}_{\text{gal}}$, $8\times\text{CHHCH}_2\text{CH}_2\text{-triazole}$), 4.12-4.27 (m, 28H, $14\times\text{OCH}_2$), 4.34 (d, $J = 7.9$ Hz, 8H, $8\times\text{CH-1}_{\text{gal}}$), 4.57 (t, $J = 6.8$ Hz, 16H, $8\times\text{CH}_2\text{-triazole}$), 8.04 (s, 8H, $8\times\text{CH}_{\text{triazole}}$); ^{13}C NMR (75.5 MHz, D_2O) δ 16.98 ($7\times\text{CH}_3$), 19.61 ($8\times\text{CH}_2\text{CH}_2\text{-triazole}$), 29.53 ($8\times\text{CH}_2\text{CH}_2\text{CH}_2\text{-triazole}$), 32.57 ($8\times\text{CH}_2\text{CH}_2\text{-triazole}$), 39.13 (NHCH_2), 39.25 (CH_2NH), 46.38 (CH_2C), 46.47 ($4\times\text{CH}_2\text{C}$), 46.76 ($2\times\text{CH}_2\text{C}$), 48.40 ($8\times\text{CH}_2\text{-triazole}$), 61.12 ($8\times\text{CH}_2\text{-6 of Gal}$), 65.82 ($8\times\text{OCH}_2\text{C}$), 66.34 ($8\times\text{CH}_2\text{CH}_2\text{CH}_2\text{-triazole}$), 66.56 ($4\times\text{OCH}_2\text{C}$), 67.11 ($2\times\text{OCH}_2\text{C}$), 68.76 ($8\times\text{CH-4}_{\text{gal}}$, CH_2O), 68.98 (CH_2O), 69.66 ($2\times\text{CH}_2\text{O}$), 70.92 ($8\times\text{CH-2}_{\text{gal}}$), 72.91 ($8\times\text{CH-3}_{\text{gal}}$), 75.26 ($8\times\text{CH-5}_{\text{gal}}$), 102.96 ($8\times\text{CH-1}_{\text{gal}}$), 125.02 ($8\times\text{CH}_{\text{triazole}}$), 145.12 ($8\times\text{C}_{\text{triazole}}$), 162.86 (q, $J = 35.4$ Hz, CF_3), 173.13 ($2\times\text{C=O}$), 173.59 ($4\times\text{C=O}$), 173.65 ($8\times\text{C=O}$), 174.10 (C=O); MS (MALDI-ToF) 3706.5576 ($\text{C}_{153}\text{H}_{241}\text{N}_{26}\text{O}_{79}$ ($\text{M}+\text{H}^+$) requires 3706.5635).

DIBO-glycodendron **25**



Glycodendron **35** (65.0 mg, 0.017 mmol) and 11,12-didehydro-5,6-dihydrodibenzo[a,e]cycloocten-5-yl carbonic acid 4-nitrophenyl ester^{iv} (13.5 mg, 0.035 mmol) were dissolved in DMF (3 mL). DIPEA (9 μL , 0.051 mmol) was added to the solution and the resulting mixture was stirred for 24 h. The solvent was evaporated under reduced pressure to give a residue, which

was purified by RP-HPLC (0-2min 0% B, 2-5 min gradient of 0-20% B, 5-40 min gradient of 20-55% B, t_r =27.8 min). Fractions of interest were combined and lyophilized to give **25** as a white powder (43.0 mg, 64%): **¹H NMR** (600 MHz, D₂O) δ 1.05-1.18 (m, 21H, 7 $\times$ CH₃), 2.10-2.19 (m, 16H, 8 $\times$ CH₂CH₂CH₂-triazole), 2.55 (d, J = 14.6 Hz, 1H, CHHCHO), 2.70 (t, J = 6.9 Hz, 16H, 8 $\times$ CH₂CH₂-triazole), 2.95 (t, J = 6.8 Hz, 16H, 8 $\times$ CH₂CH₂-triazole), 3.09 (d, J = 14.0 Hz, 1H, CHHCHO), 3.26-3.33 (m, 4H, 2 $\times$ CH₂NH), 3.49 (dd, J = 9.6, 8.1 Hz, 8H, 8 $\times$ CH-2_{gal}), 3.55-3.74 (m, 48H, 4 $\times$ CH₂O, 8 $\times$ CH-3_{gal}, 8 $\times$ CH-5_{gal}, 8 $\times$ CH₂-6_{gal}, 8 $\times$ CHHCH₂CH₂-triazole), 3.83-3.91 (m, 16H, 8 $\times$ CH-4_{gal}, 8 $\times$ CHHCH₂CH₂-triazole), 4.01-4.22 (m, 28H, 14 $\times$ OCH₂), 4.30 (d, J = 7.9Hz, 8H, 8 $\times$ CH-1_{gal}), 4.50 (t, J = 6.8 Hz, 16H, 8 $\times$ CH₂-triazole), 5.16 (brs, 1H, CH₂CHO), 7.18-7.33 (m, 7H, aromH), 7.48 (d, J = 6.8 Hz, 1H, aromH), 7.91 (s, 8H, 8 $\times$ CH_{triazole}); **¹³C NMR** (125 MHz, CD₃OD:CDCl₃, 4/1, v/v) δ 17.77 (CH₃), 18.03 (2 $\times$ CH₃), 18.15 (4 $\times$ CH₃), 21.48 (8 $\times$ CH₂CH₂-triazole), 31.19 (8 $\times$ CH₂CH₂CH₂-triazole), 34.00 (8 $\times$ CH₂CH₂-triazole), 40.25 (CH₂NH), 41.44 (CH₂NH), 46.85 (CH₂CHO), 47.27 (CH₂C), 47.32 (4 $\times$ CH₂C), 47.63 (2 $\times$ CH₂C), 47.90 (8 $\times$ CH₂-triazole), 62.14 (8 $\times$ CH₂-6_{gal}), 66.11 (12 $\times$ OCH₂C), 66.60 (8 $\times$ CH₂CH₂CH₂-triazole), 67.87 (2 $\times$ OCH₂C), 69.81 (8 $\times$ CH-4_{gal}), 70.20 (CH₂O), 70.67 (CH₂O), 70.92 (2 $\times$ CH₂O), 72.16 (8 $\times$ CH-2_{gal}), 74.52 (8 $\times$ CH-3_{gal}), 76.11 (8 $\times$ CH-5_{gal}), 77.63 (CH₂CHO), 104.47 (8 $\times$ CH-1_{gal}), 110.54 (C $\equiv$ C), 113.33 (C $\equiv$ C), 121.92 (C), 123.89 (8 $\times$ CH_{triazole}), 124.50 (CH), 124.65 (C), 126.88 (CH), 127.15 (CH), 128.02 (CH), 128.08 (CH), 129.11 (2 $\times$ CH), 130.81 (CH), 151.80 (C), 152.98 (C), 157.42 (C=O), 146.86 (8 $\times$ C_{triazole}), 172.79 (2 $\times$ C=O), 173.17 (4 $\times$ C=O), 173.23 (8 $\times$ C=O), 173.54 (C=O); **MS** (MALDI-ToF) 3974.7 (C₁₇₀H₂₅₀N₂₆O₈₁Na (M+Na⁺) requires 3974.6).

UV-experiments

Table S1 Observed rate constants for the cycloaddition of dibenzocyclooctynol with various concentrations of benzyl azide (**7**) in acetonitrile.

Concentration of Benzyl Azide ($\times 10^{-3}\text{M}$)	k (s^{-1})
3	1.41×10^{-4}
8	4.32×10^{-4}
13	6.97×10^{-4}
20	1.06×10^{-3}
27	1.32×10^{-3}

Linea Plot:

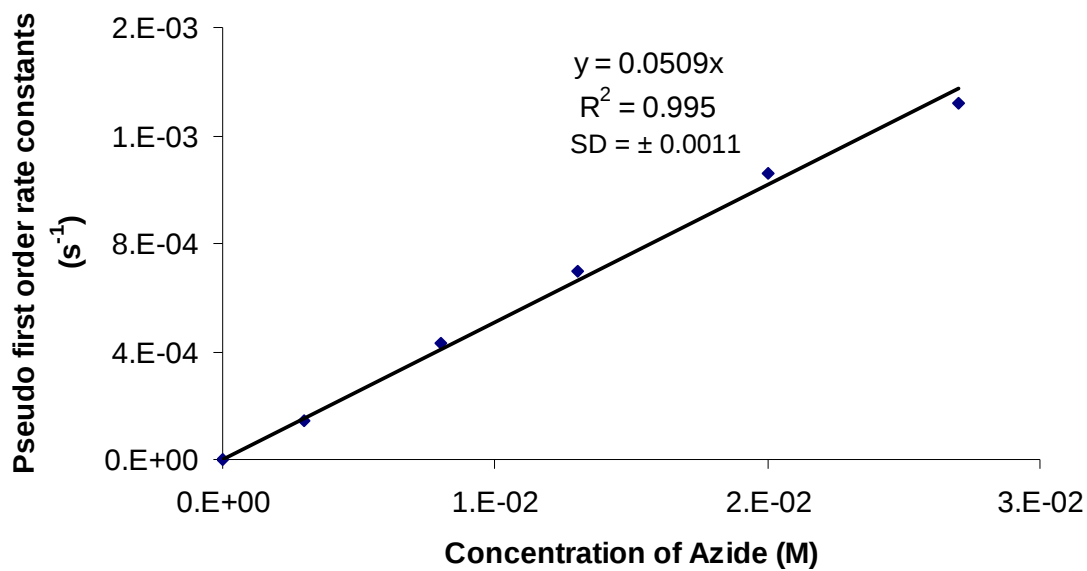


Table S2. Observed rate constants for the cycloaddition of dibenzocyclooctynol with various concentrations of benzyl (7) azide in methanol.

Concentration of Benzyl Azide ($\times 10^{-3}\text{M}$)	k (s^{-1})
3	1.87×10^{-4}
8	4.28×10^{-4}
13	7.67×10^{-4}
20	1.20×10^{-3}
27	1.60×10^{-3}

Linea Plot:

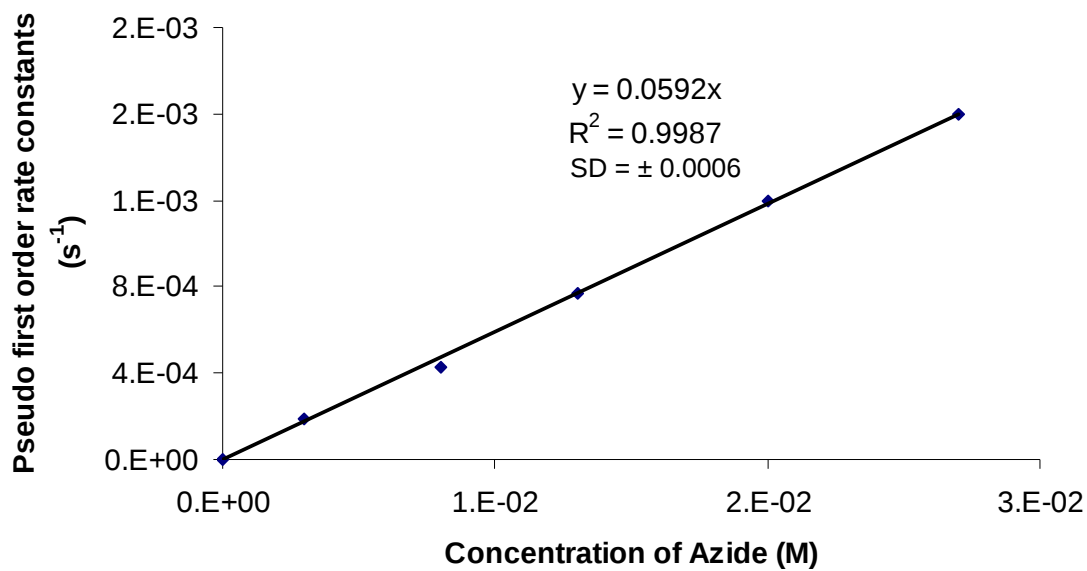


Table S3. Observed rate constants for the cycloaddition of dibenzocyclooctynol with various concentrations of nitrile oxide **5a** in acetonitrile.

Concentration of nitrile oxide 5a ($\times 10^{-3}$ M)	k (s^{-1})
1.5	3.80×10^{-3}
2.5	6.30×10^{-3}
3.0	7.70×10^{-3}
3.5	8.30×10^{-3}
4.5	1.10×10^{-2}

Linea Plot:

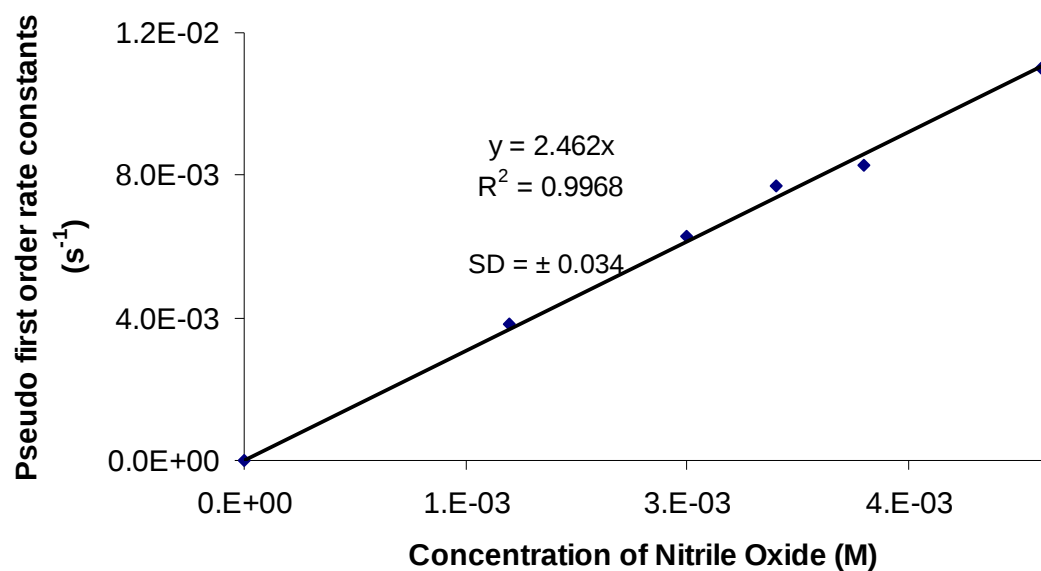


Table S4. Observed rate constants for the cycloaddition of dibenzocyclooctynol with various concentrations of nitrile oxide **5a** in methanol.

Concentration of nitrile oxide 5a ($\times 10^{-3}$ M))	k (s^{-1})
1.5	5.1×10^{-3}
2.5	8.7×10^{-3}
3.0	1.0×10^{-2}
3.5	1.2×10^{-2}
4.5	1.5×10^{-2}

Linea Plot:

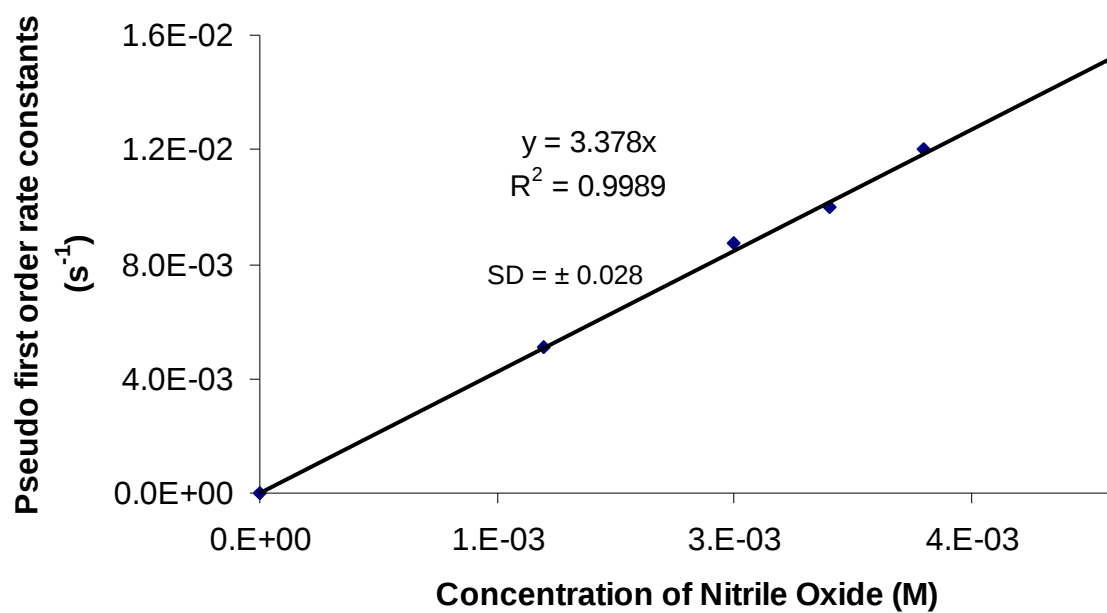


Table S5. Observed rate constants for the cycloaddition of dibenzocyclooctynol with various concentrations of nitrile oxide **5b** in methanol.

Concentration of nitrile oxide 5b ($\times 10^{-4}$ M)	k (s^{-1})
2.50	5.26×10^{-4}
3.00	6.33×10^{-4}
3.75	8.03×10^{-4}
4.25	9.00×10^{-4}
5.00	1.10×10^{-3}

Linea Plot:

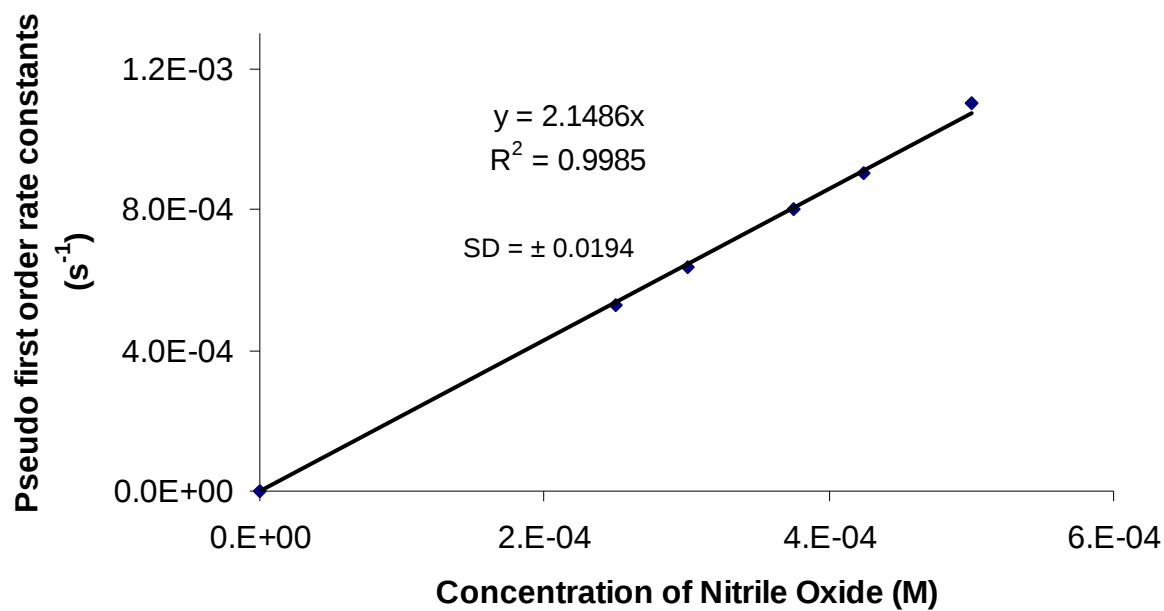


Table S6. Observed rate constants for the cycloaddition of dibenzocyclooctynol with various concentrations of nitrile oxide **5d** in methanol.

Concentration of nitrile oxide 5d ($\times 10^{-4}\text{M}$)	k (s^{-1})
6	2.40×10^{-3}
8	3.00×10^{-3}
10	4.00×10^{-3}
11	4.50×10^{-3}
13	5.20×10^{-3}

Linea Plot:

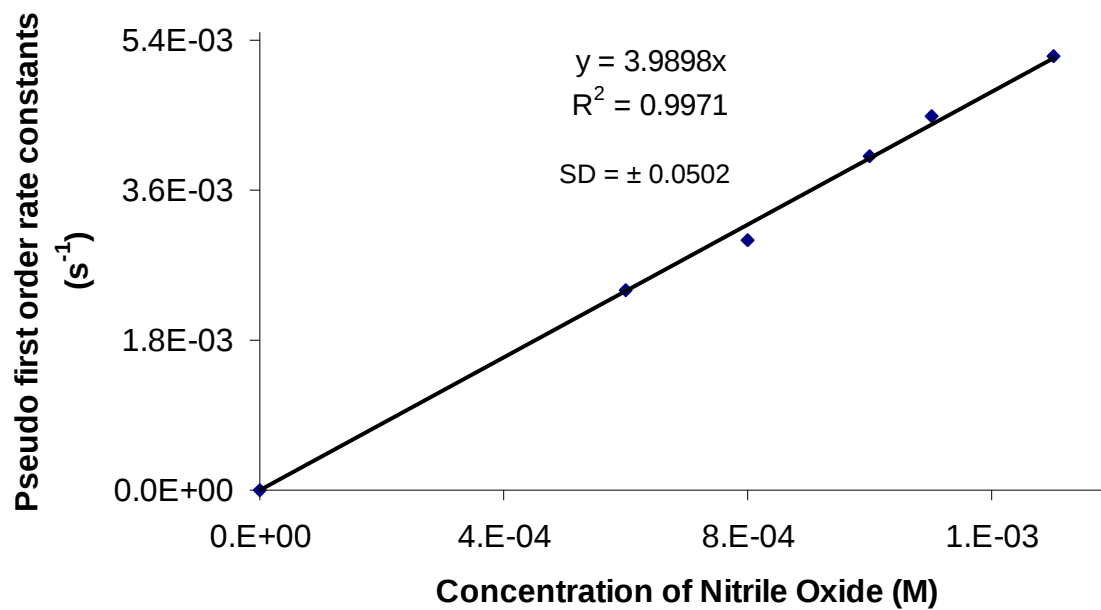


Table S7 Observed rate constants for the cycloaddition of dibenzocyclooctynol with various concentrations of nitrile oxide **5e** in methanol.

Concentration of nitrile oxide 5e ($\times 10^{-4}$ M)	k (s^{-1})
6	2.00×10^{-3}
8	2.70×10^{-3}
10	3.40×10^{-3}
12	4.20×10^{-3}
15	5.10×10^{-3}

Linea Plot:

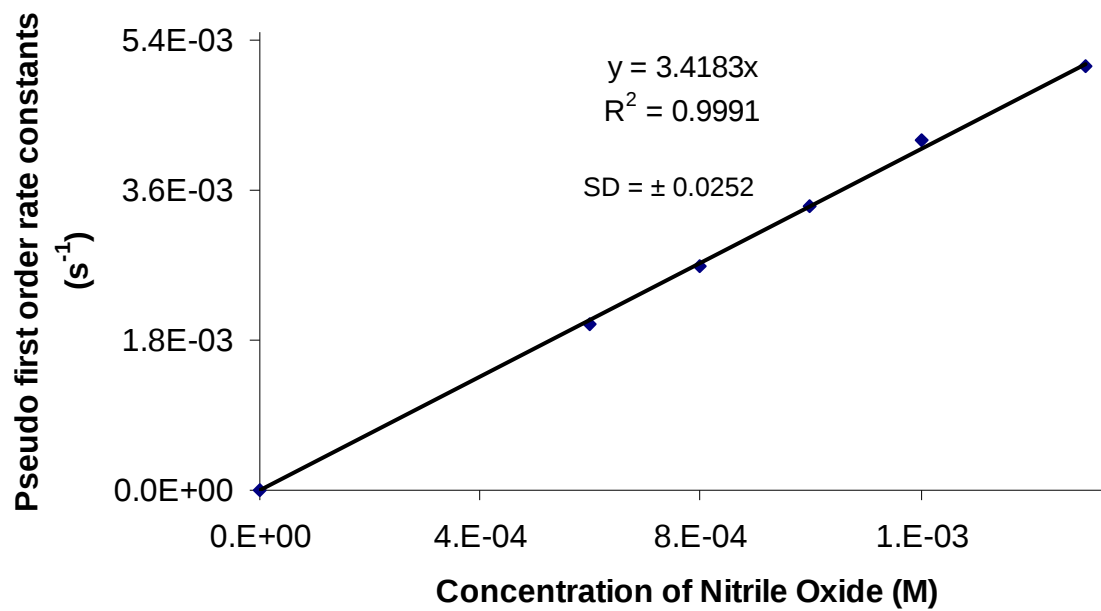


Table S8. Observed rate constants for the cycloaddition of dibenzocyclooctynol with various concentrations of nitrile oxide **5f** in methanol.

Concentration of nitrile oxide 5f ($\times 10^{-4}$ M)	k (s^{-1})
8	2.80×10^{-3}
14	4.50×10^{-3}
19	6.30×10^{-3}
24	7.60×10^{-3}
29	9.90×10^{-3}

Linea Plot:

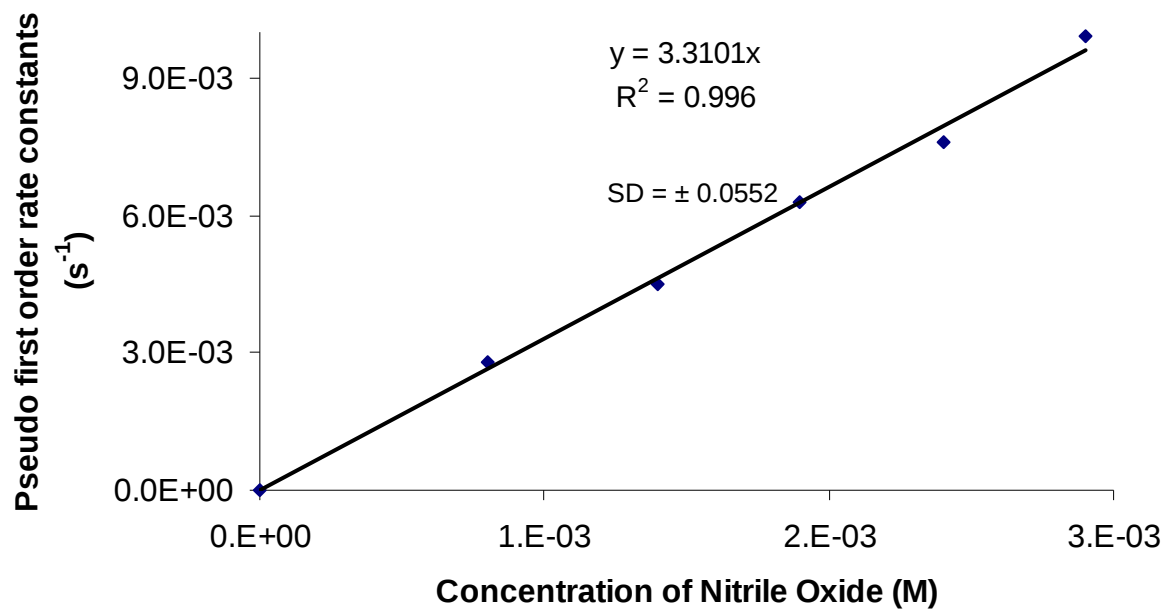


Table S9. Observed rate constants for the cycloaddition of dibenzocyclooctynol with various concentrations of nitrile oxide **13h** in methanol.

Concentration of nitrile oxide 13h ($\times 10^{-4}$ M)	k (s^{-1})
6	8.78×10^{-4}
9	1.26×10^{-3}
12	1.64×10^{-3}
15	2.10×10^{-3}
18	2.44×10^{-3}

Linea Plot:

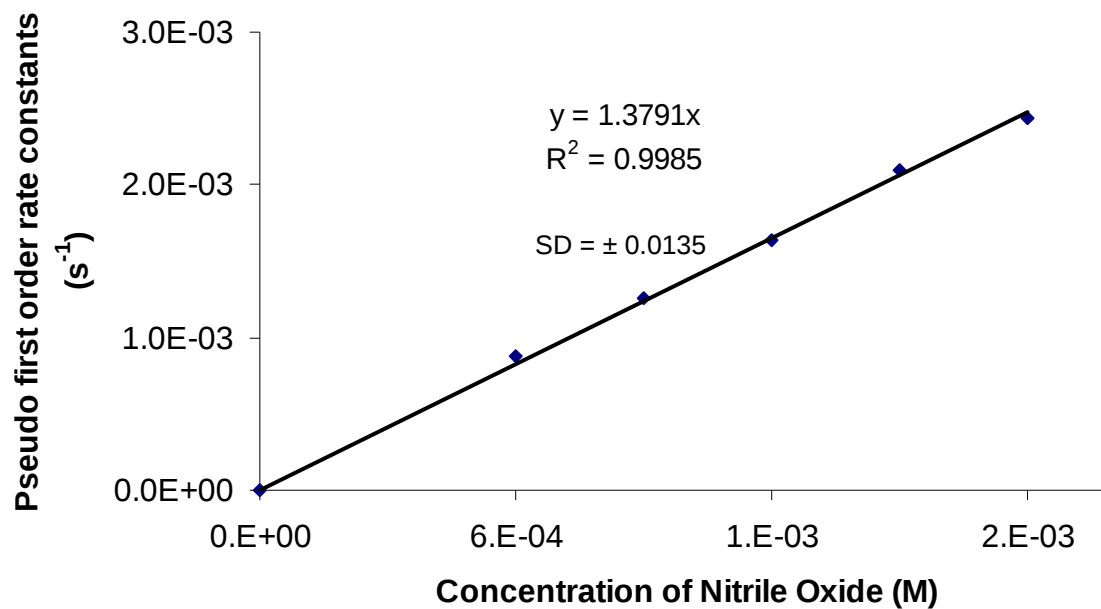


Table S10. Observed rate constants for the cycloaddition of dibenzocyclooctynol with various concentrations of nitrile oxide **13g** in methanol.

Concentration of nitrile oxide 13g ($\times 10^{-3}$ M)	k (s^{-1})
1.80	5.80×10^{-3}
2.55	8.20×10^{-3}
3.30	1.02×10^{-2}
4.05	1.29×10^{-2}
4.80	1.57×10^{-2}

Linea Plot:

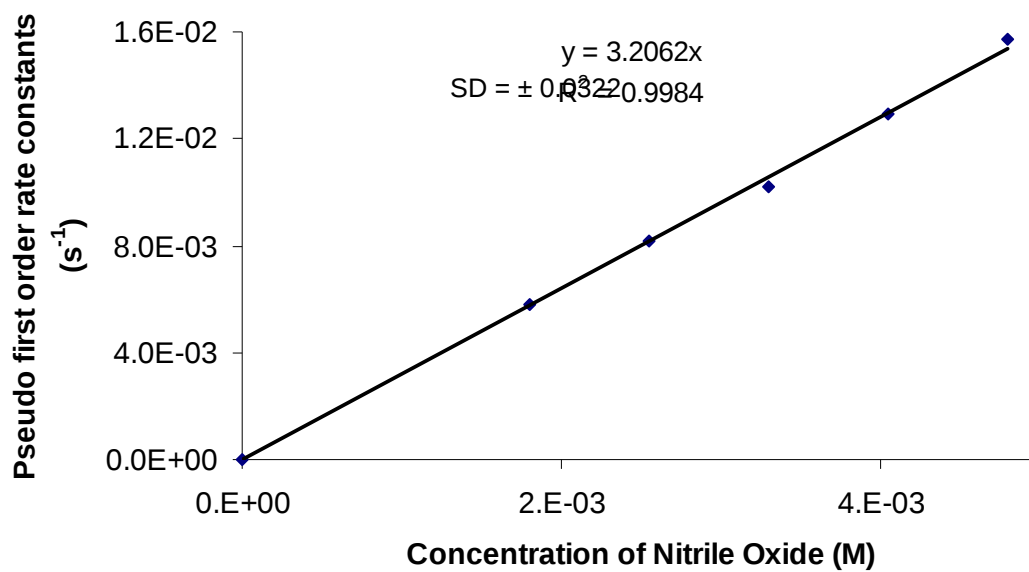


Table S11. Observed rate constants for the cycloaddition of dibenzocyclooctynol with various concentrations of nitrile oxide **5c** in methanol.

Concentration of nitrile oxide 5c ($\times 10^{-4}$ M)	k (s^{-1})
7.0	5.70×10^{-3}
10.0	8.60×10^{-3}
12.5	1.10×10^{-2}
15.0	1.23×10^{-2}
17.5	1.49×10^{-2}

Linea Plot:

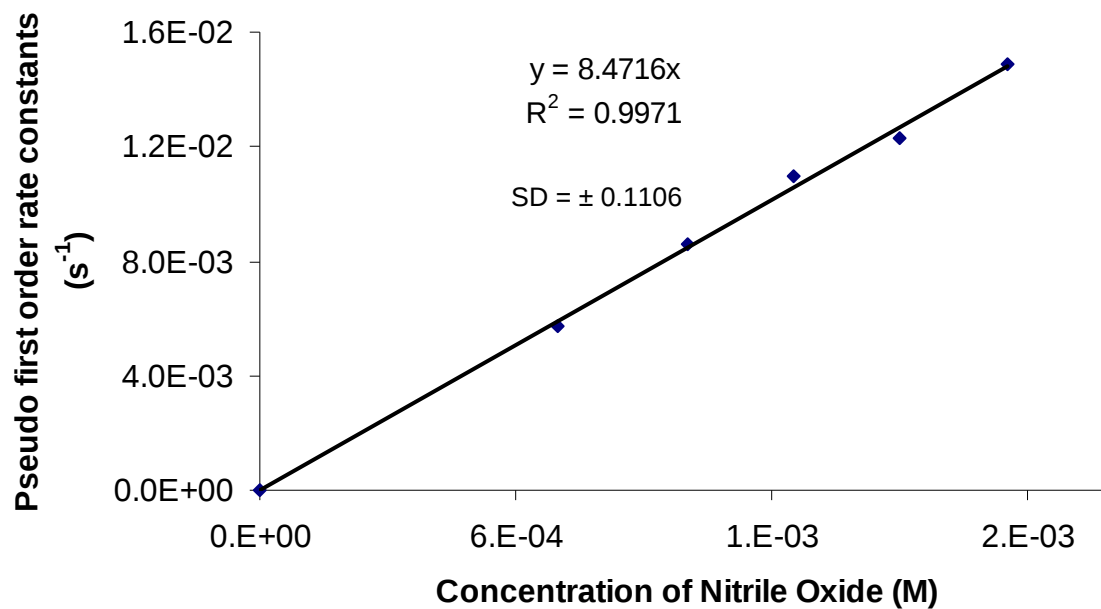


Table S12. Observed rate constants for the cycloaddition of dibenzocyclooctynol with various concentrations of nitrile oxide **13a** in methanol.

Concentration of nitrile oxide 13a ($\times 10^{-3}$ M)	k (s^{-1})
1.6	5.30×10^{-3}
2.0	6.90×10^{-3}
2.4	8.50×10^{-3}
2.8	9.50×10^{-3}
3.2	1.10×10^{-2}

Linea Plot:

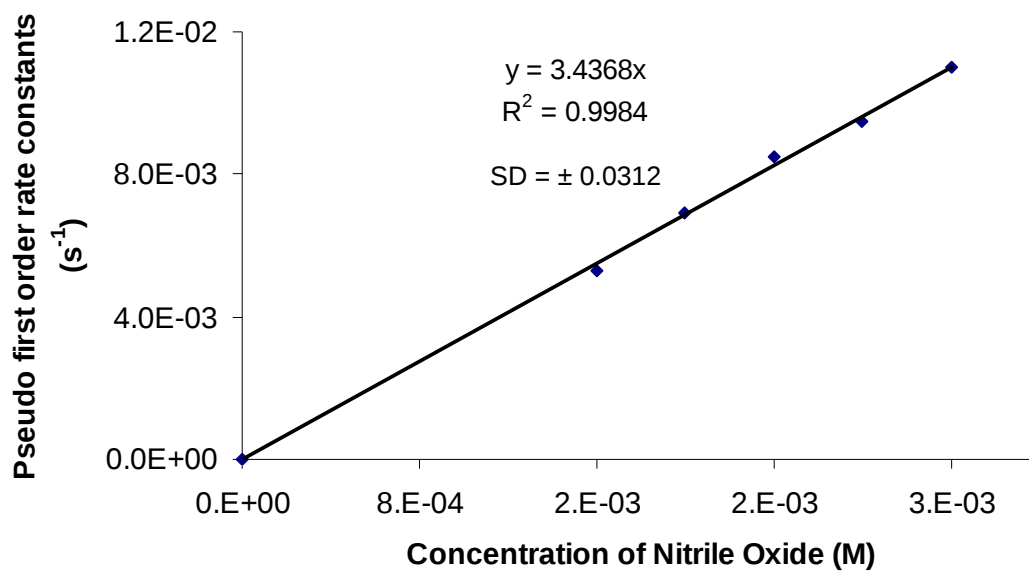


Table S13. Observed rate constants for the cycloaddition of dibenzocyclooctynol with various concentrations of Phenylethyl Nitron **9** in methanol.

Concentration of Phenylethyl Nitron 9 ($\times 10^{-3}\text{M}$)	k (s^{-1})
3.0	9.43×10^{-5}
4.5	1.25×10^{-4}
6.0	1.83×10^{-4}
9.0	2.70×10^{-4}
15.0	4.28×10^{-4}

Linea Plot:

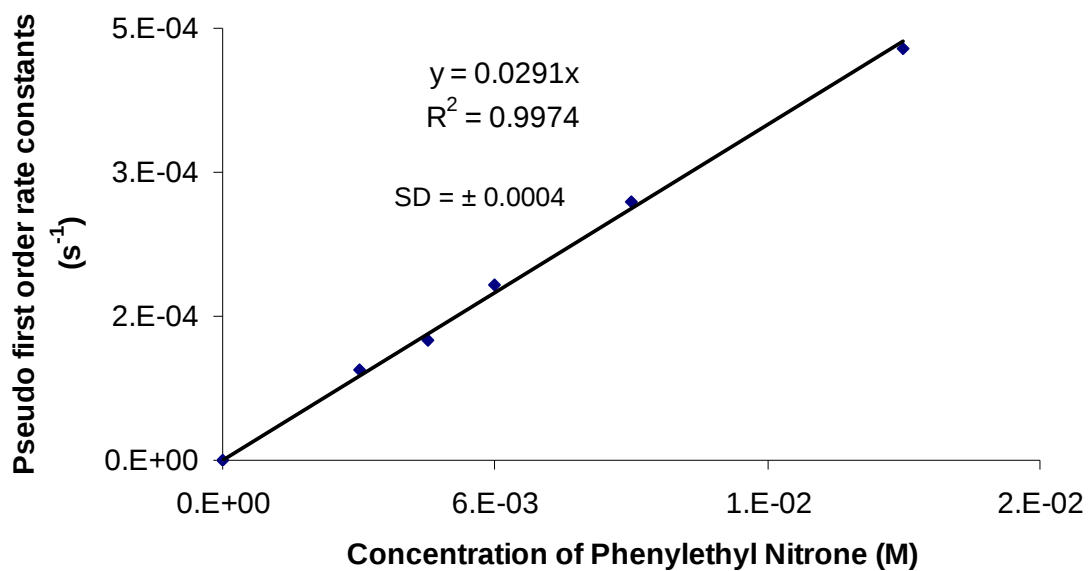


Table S14. Observed rate constants for the cycloaddition of dibenzocyclooctynol with various concentrations of diazocarbonyl derivative **11** in methanol.

Concentration of diazocarbonyl derivative 11 ($\times 10^{-3}\text{M}$)	k (s^{-1})
1.9	5.94×10^{-5}
2.2	6.71×10^{-5}
2.6	8.34×10^{-5}
3.0	9.44×10^{-5}
3.4	1.04×10^{-4}

Linea Plot:

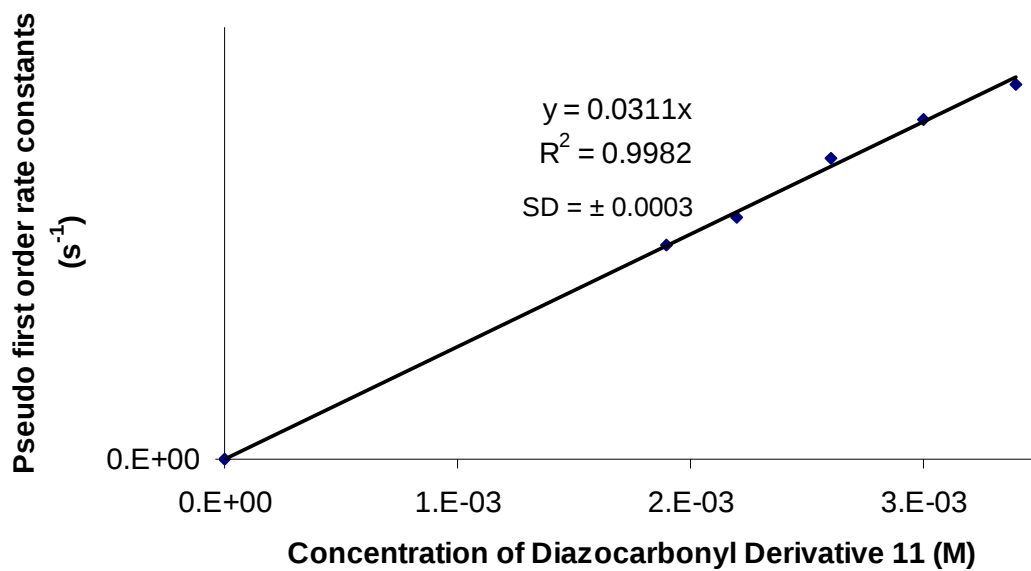
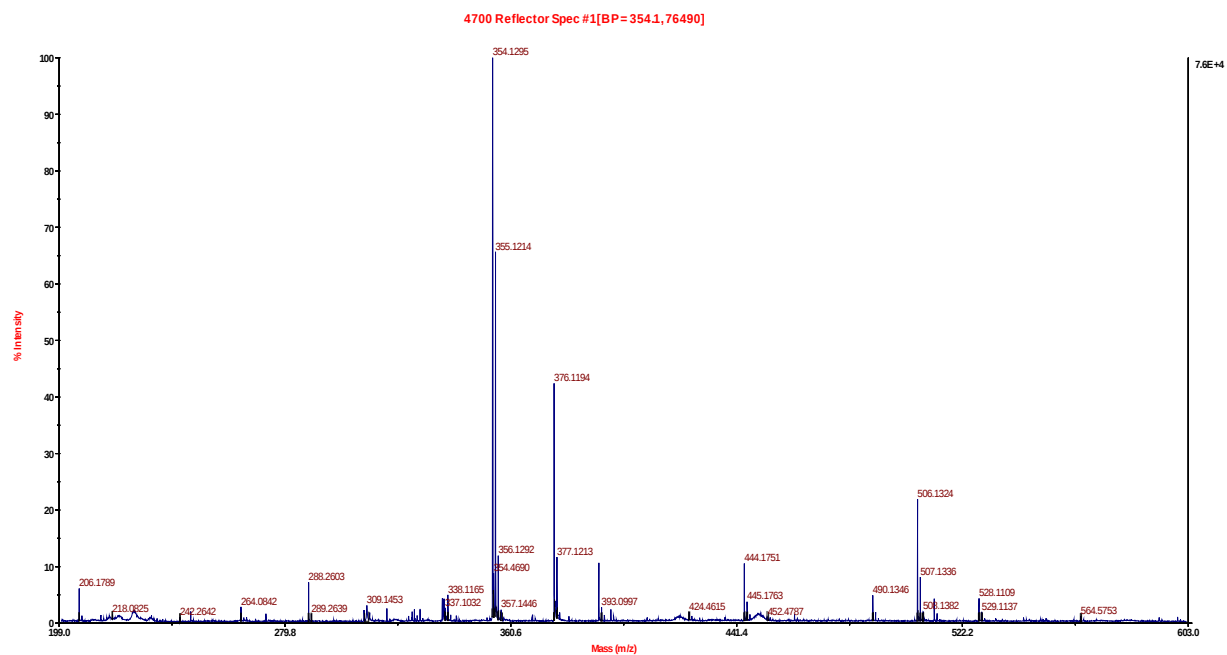
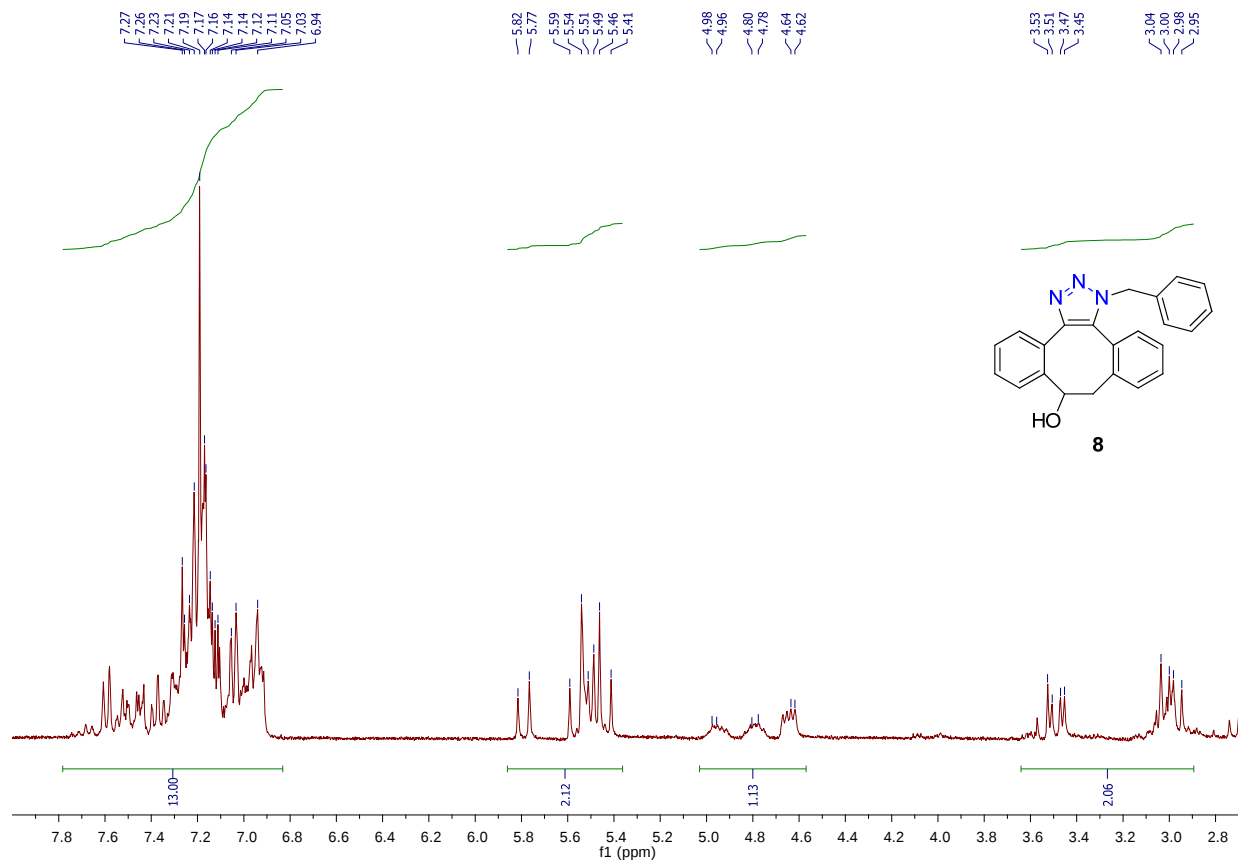
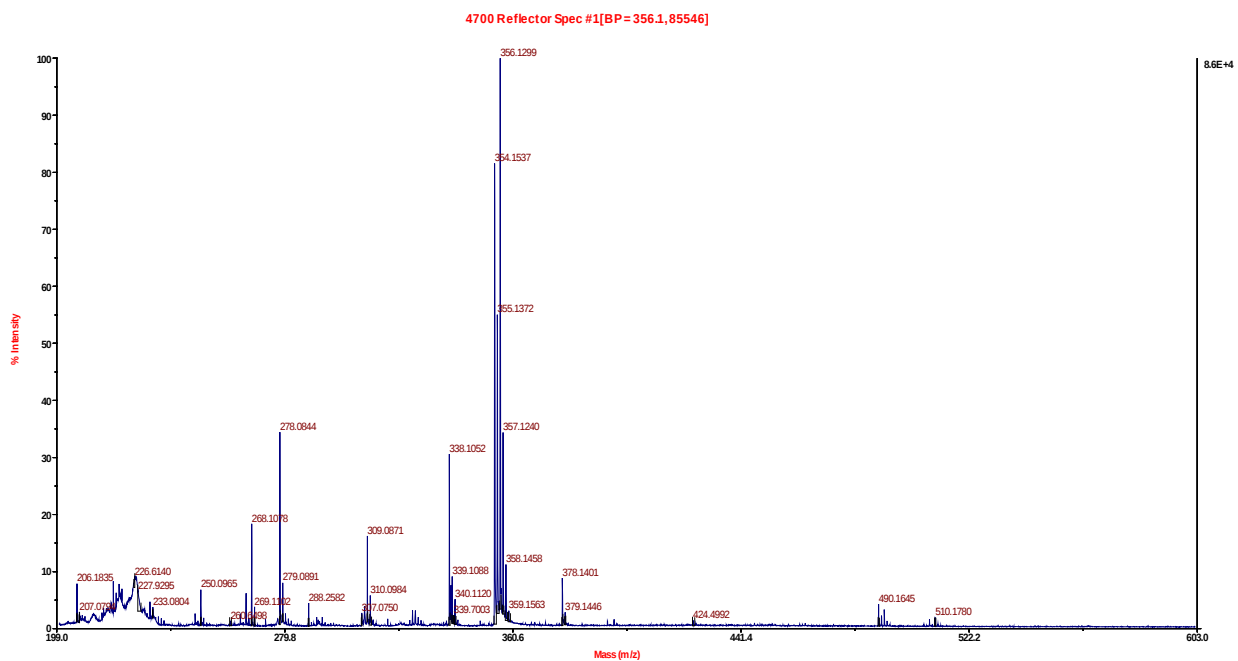
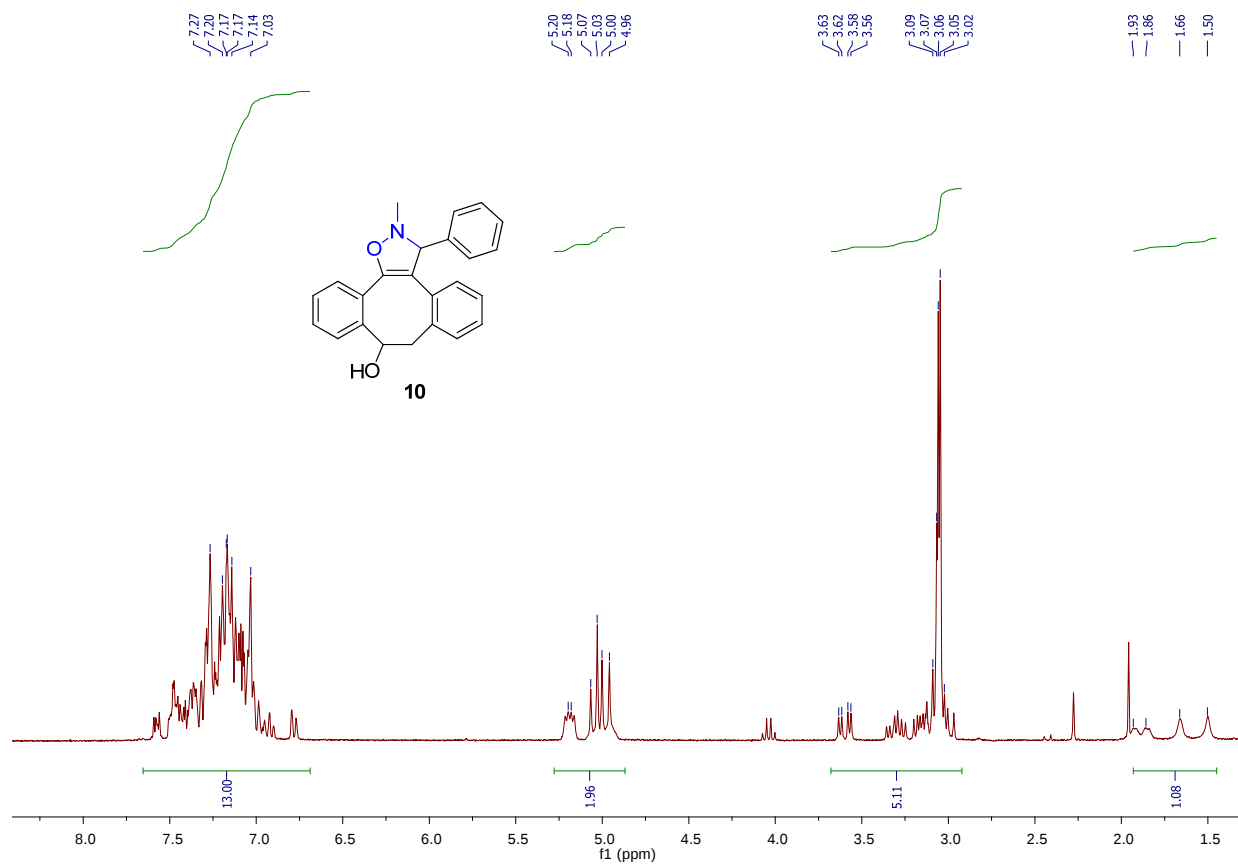
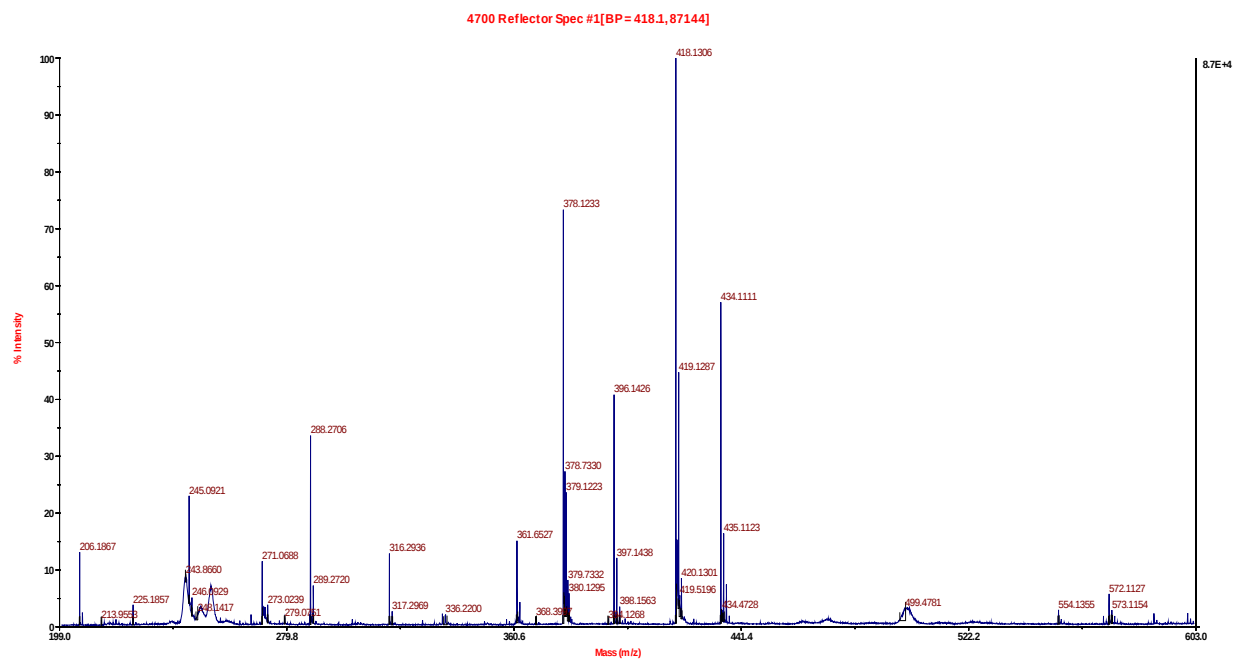
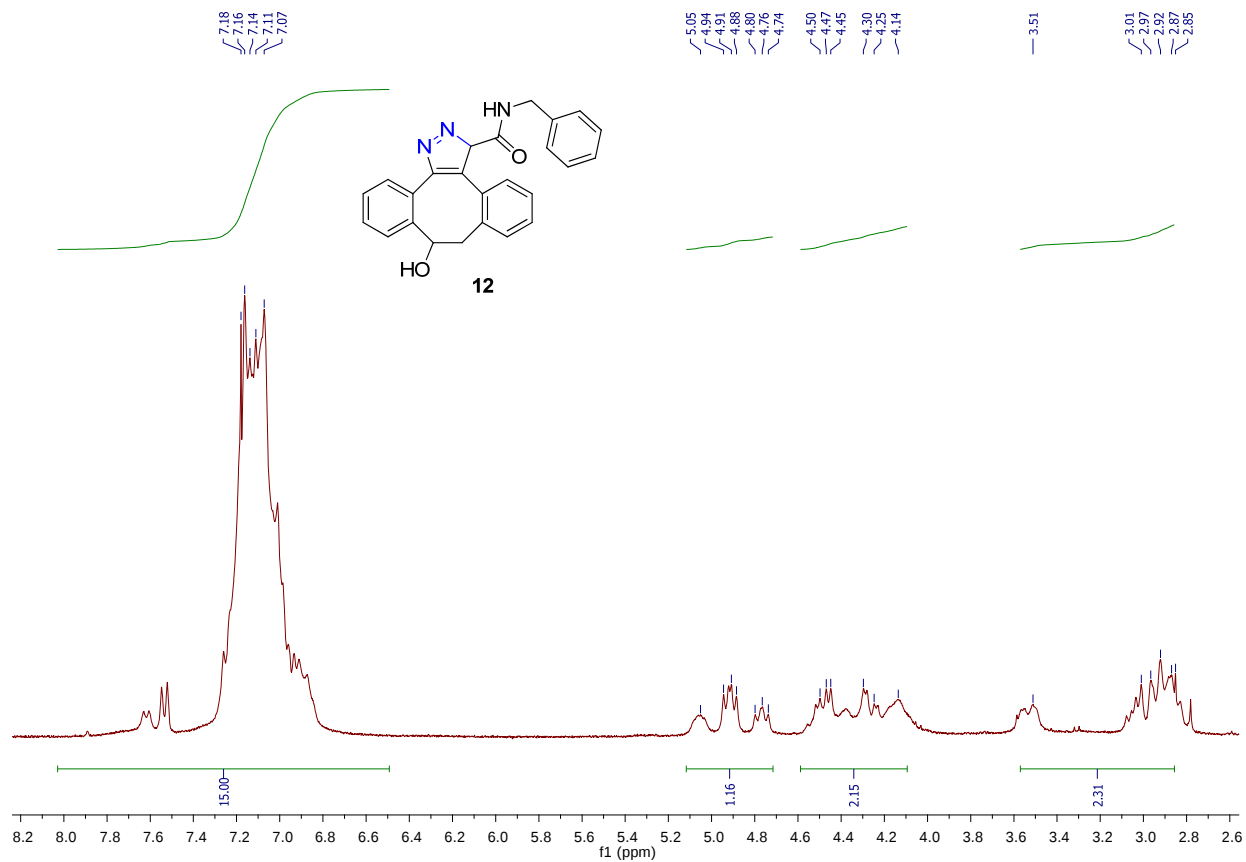


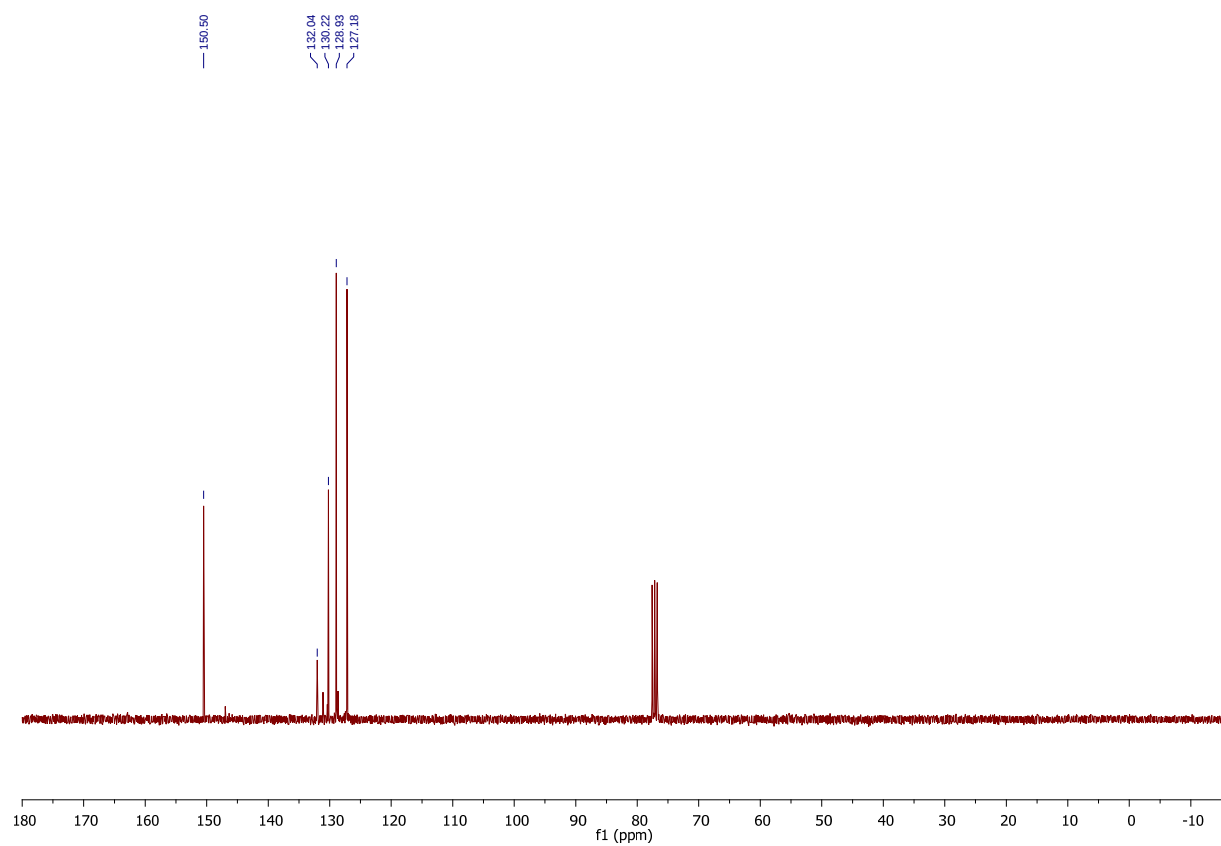
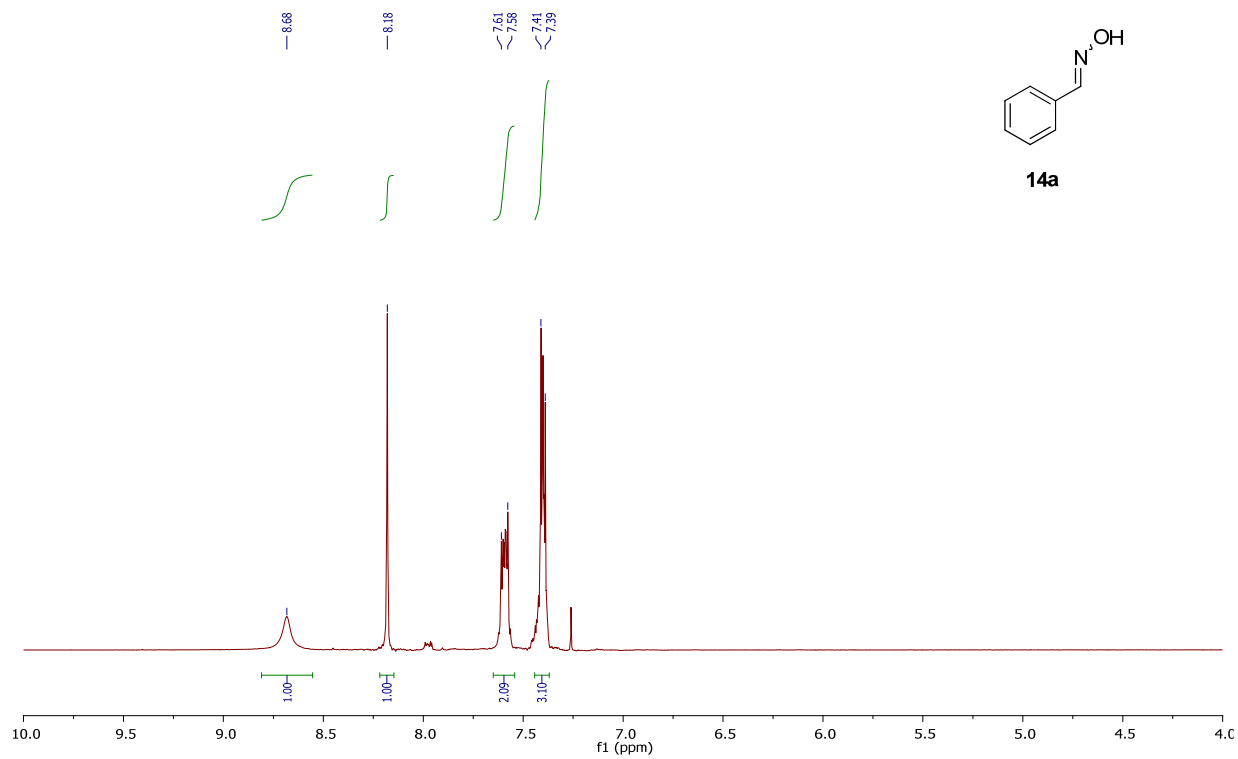
Table S15. Second order rate constants for the cycloaddition of dibenzocyclooctynol with phenyl Nitron **9** in Methanol.

Concentration of 2	Concentration of Phenyl Nitron (9)	k (M ⁻¹ s ⁻¹)
1 mM	1 mM	2.44 x 10 ⁻²
1 mM	1 mM	2.33 x 10 ⁻²
1 mM	1 mM	2.54 x 10 ⁻²

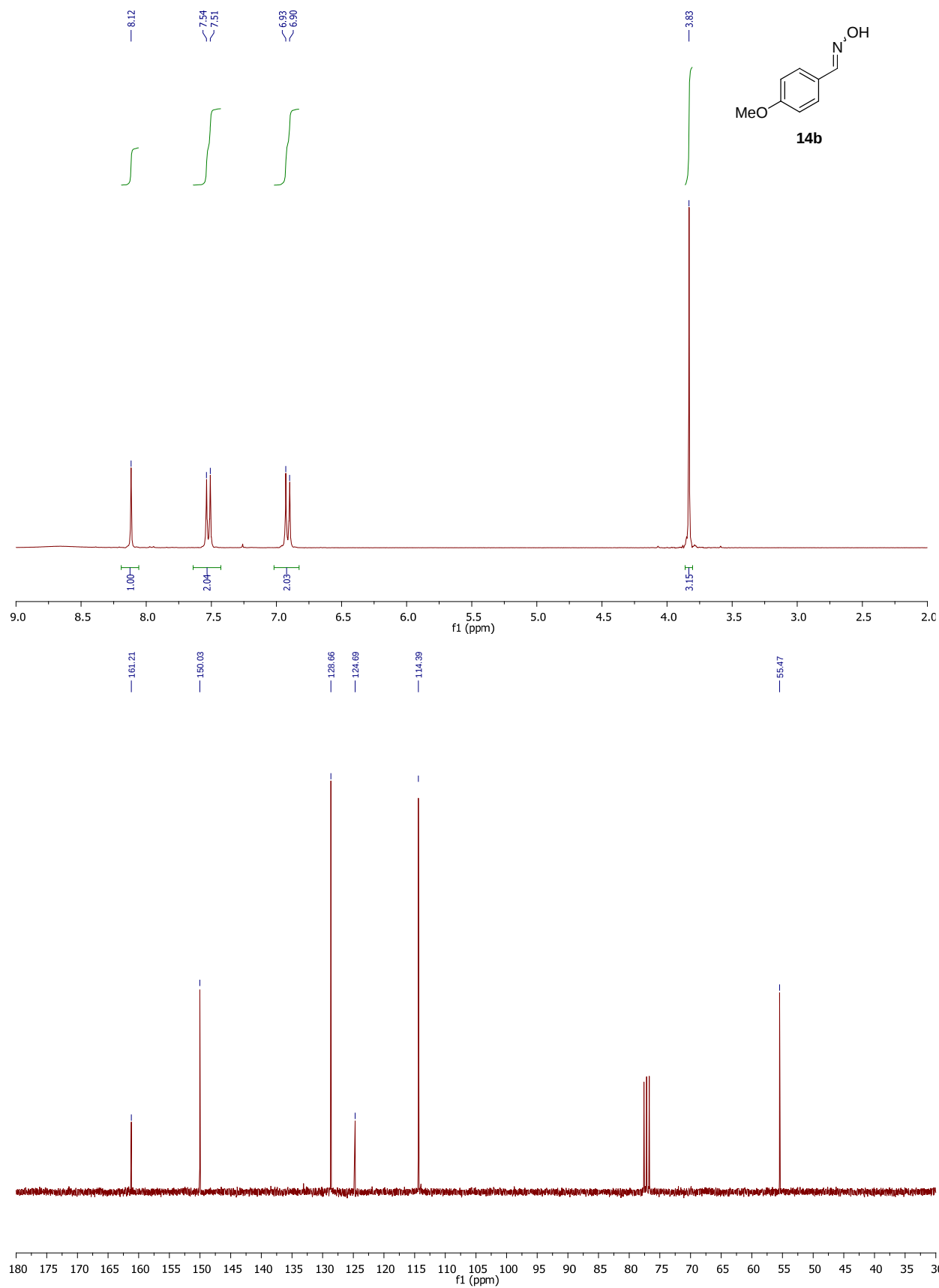


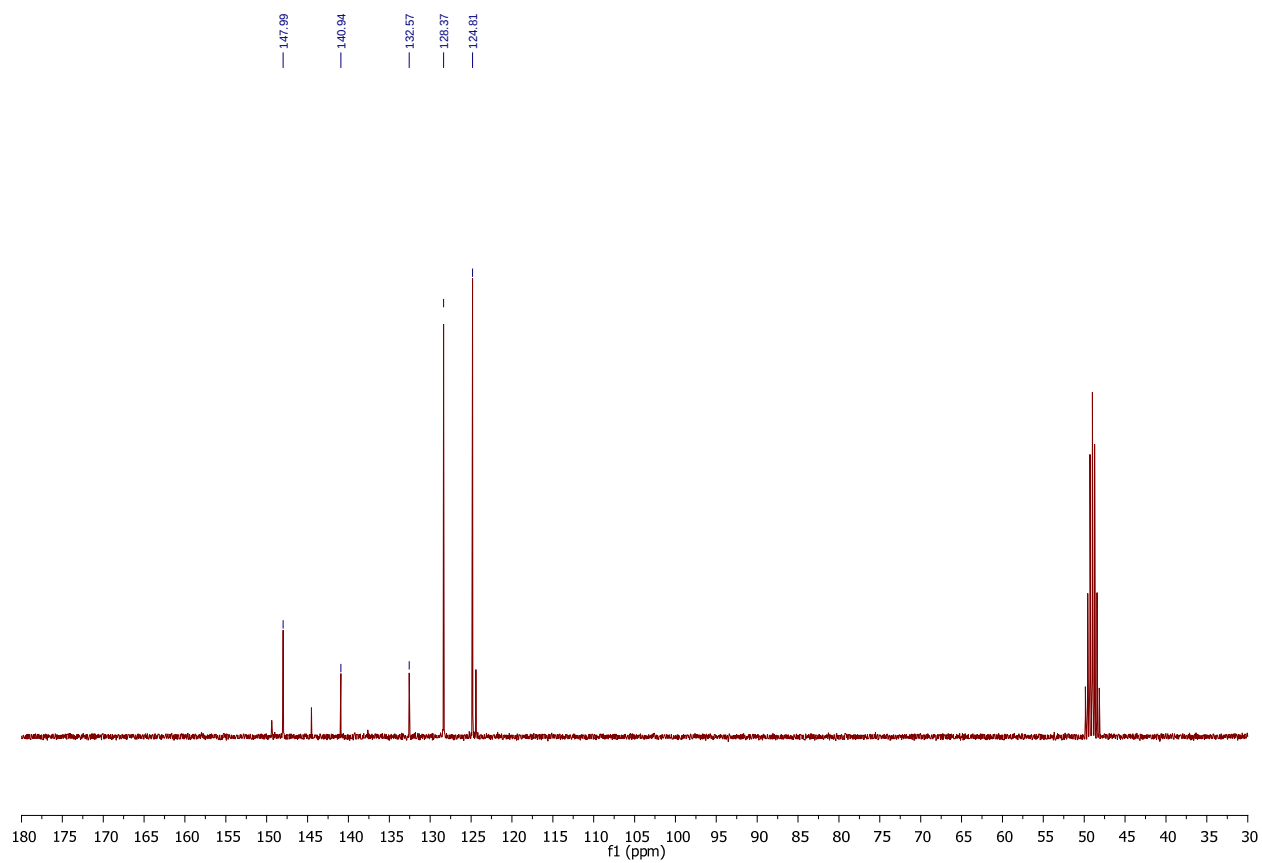
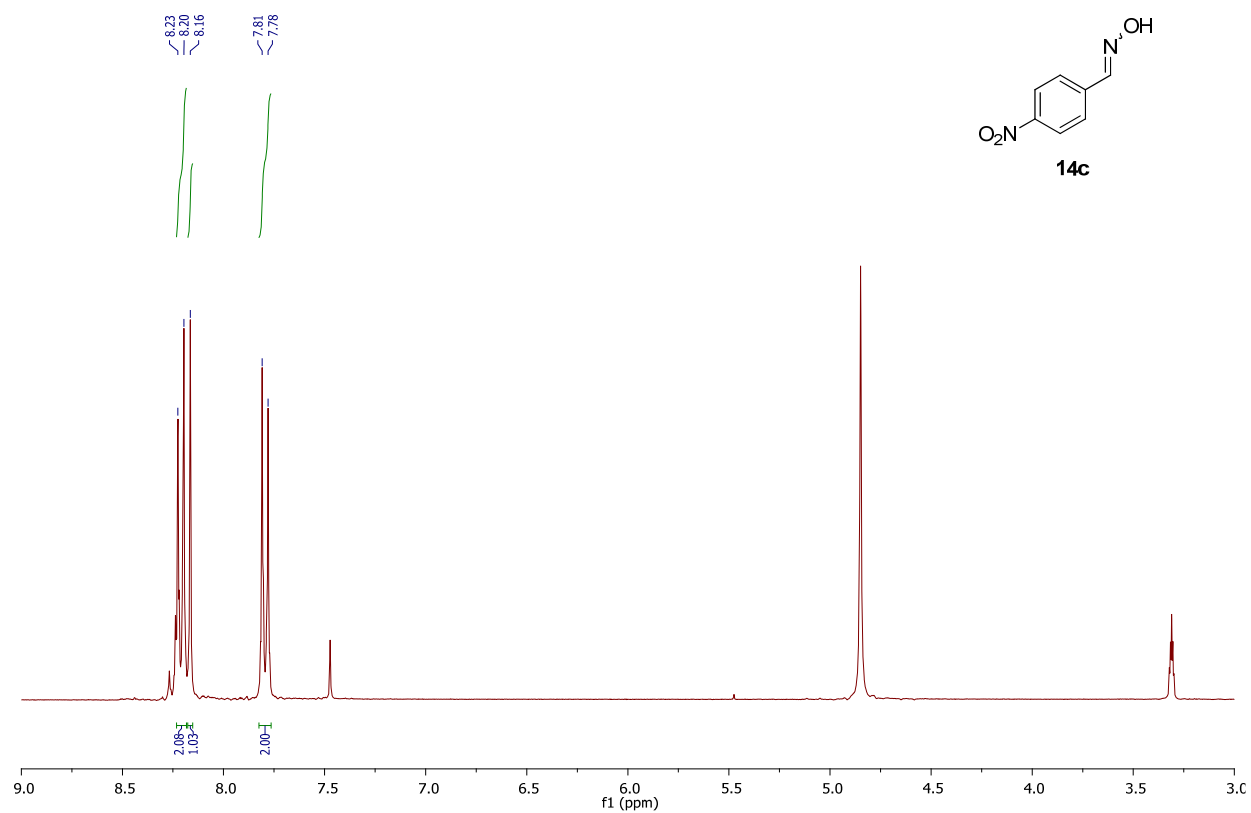


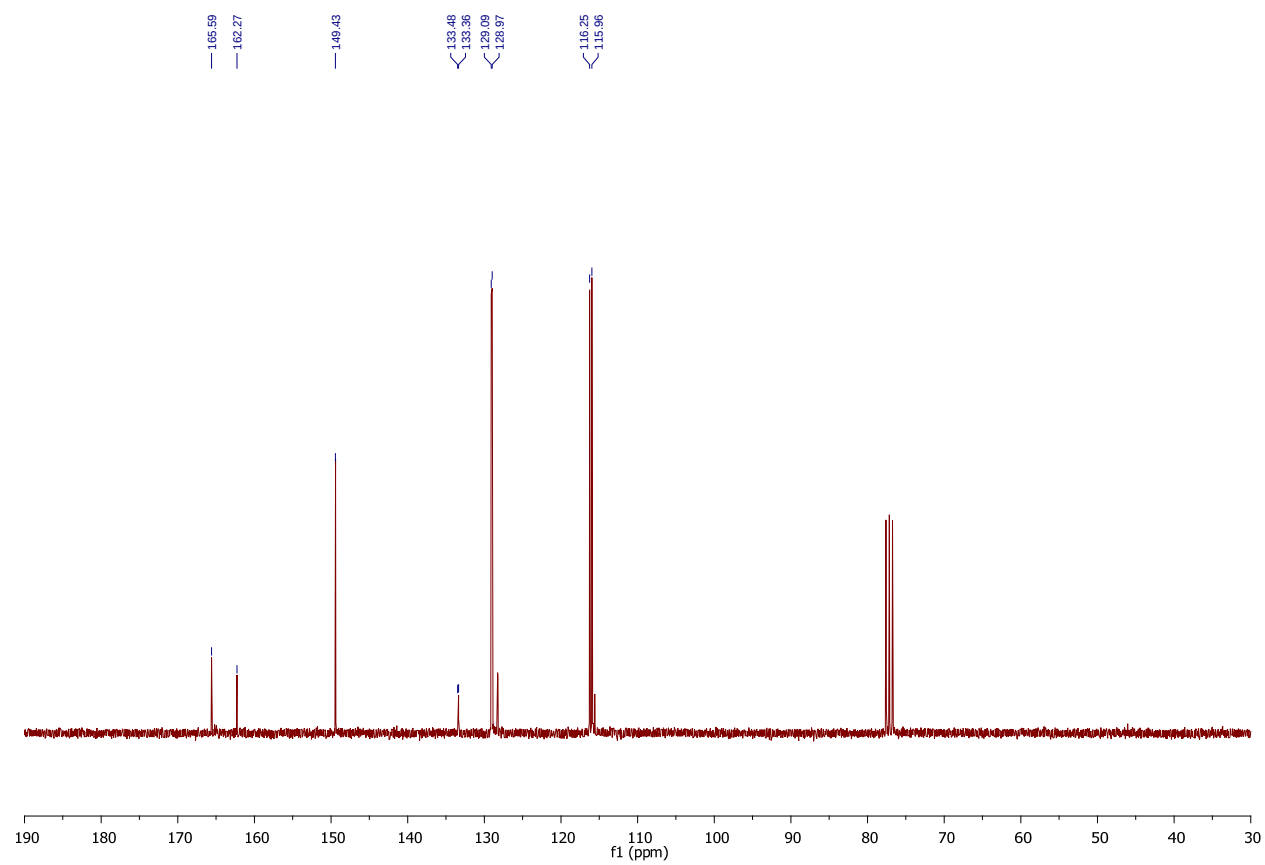
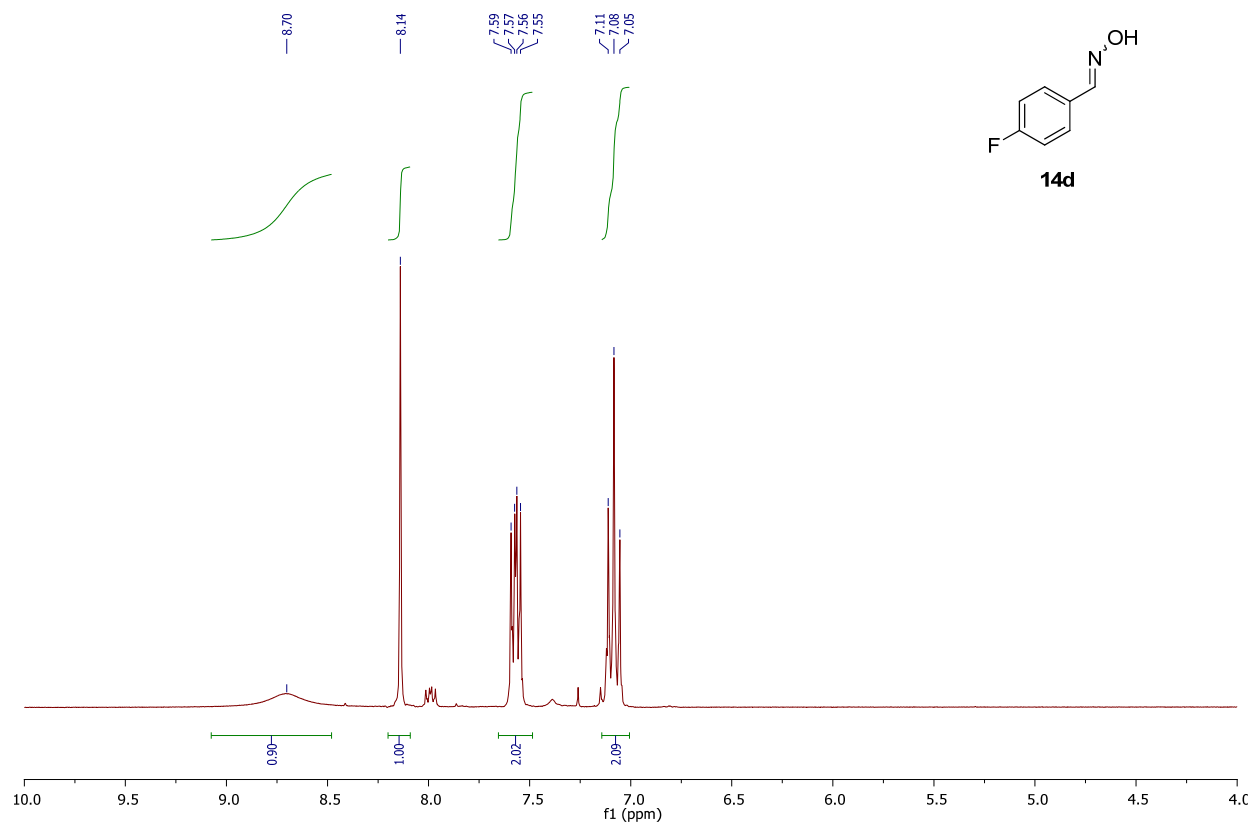


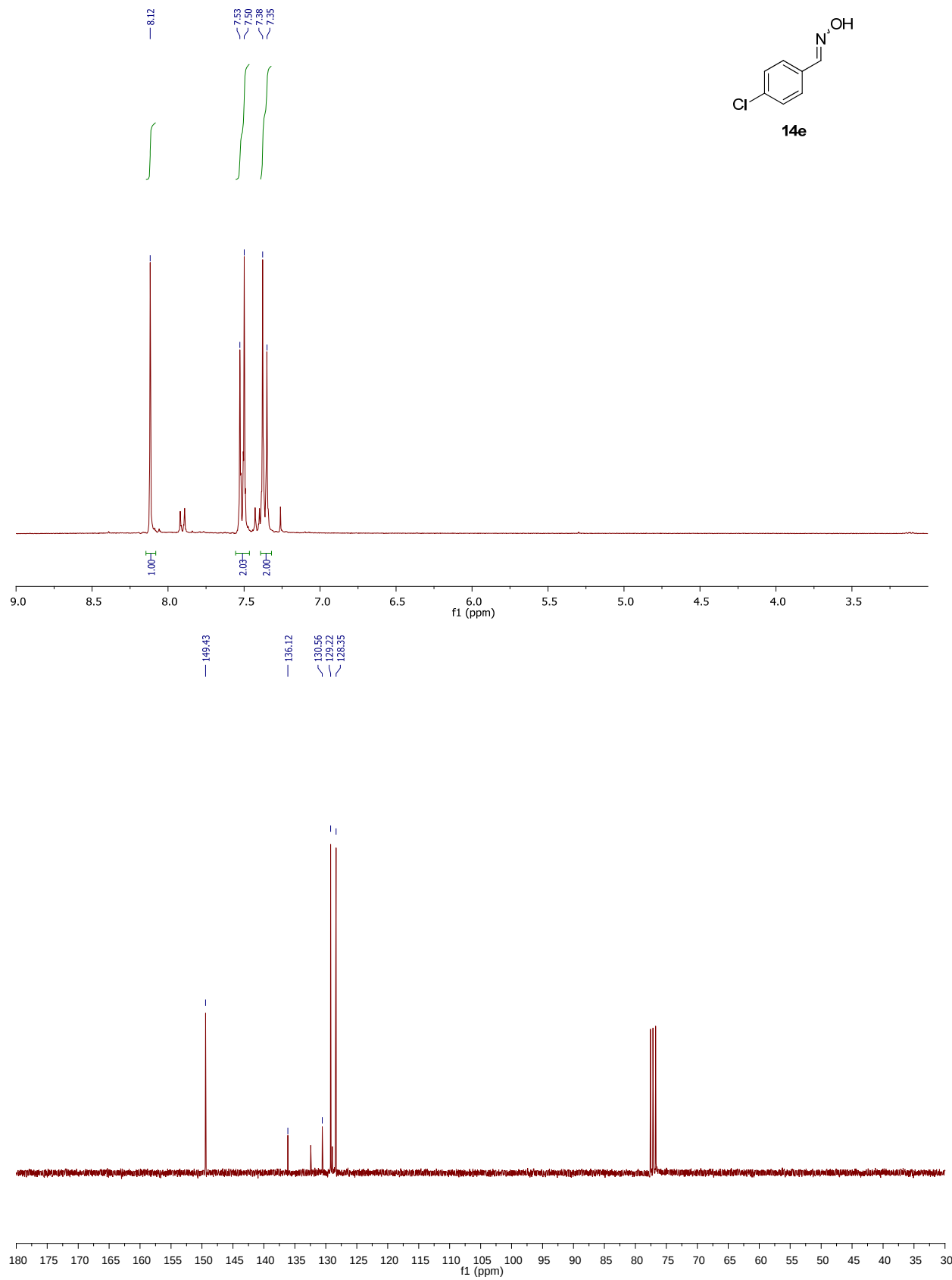


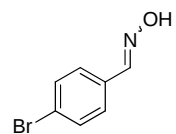
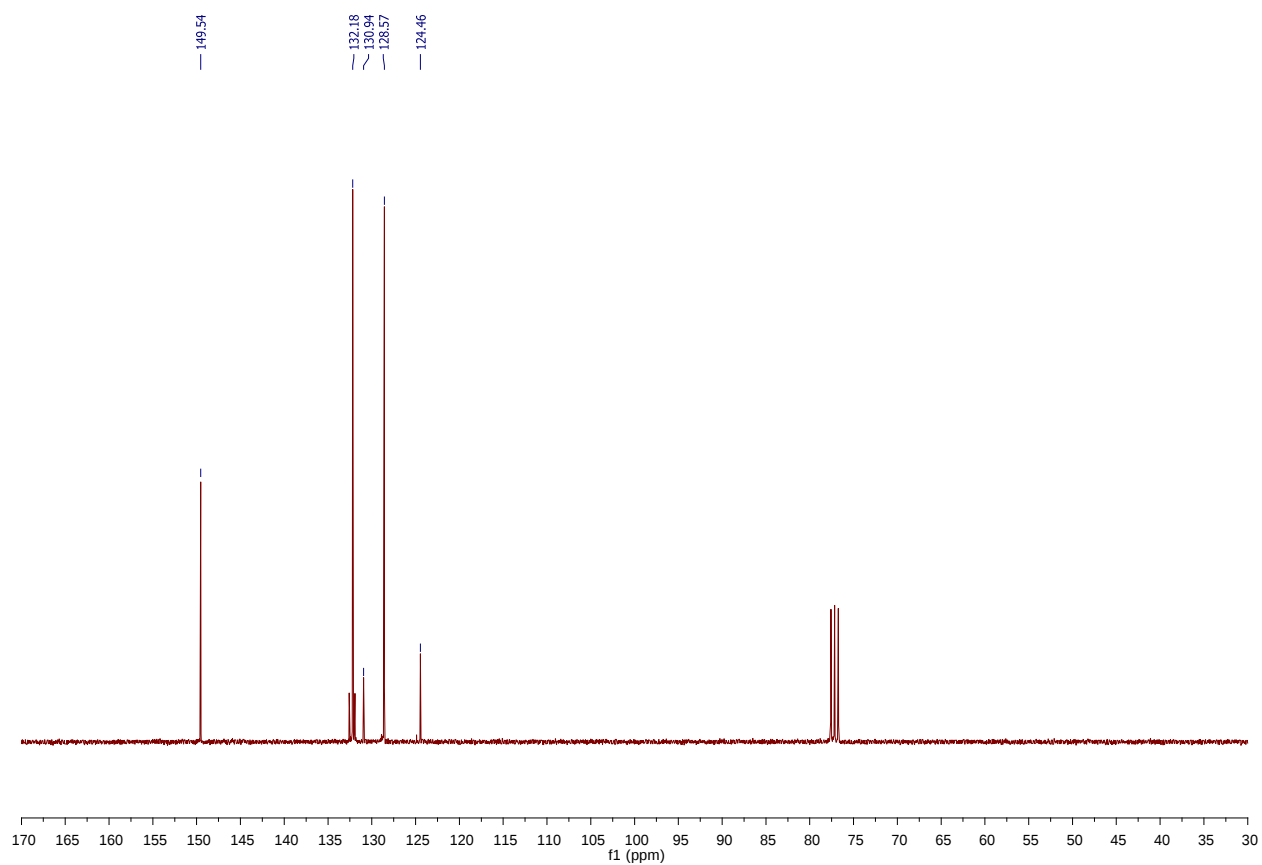
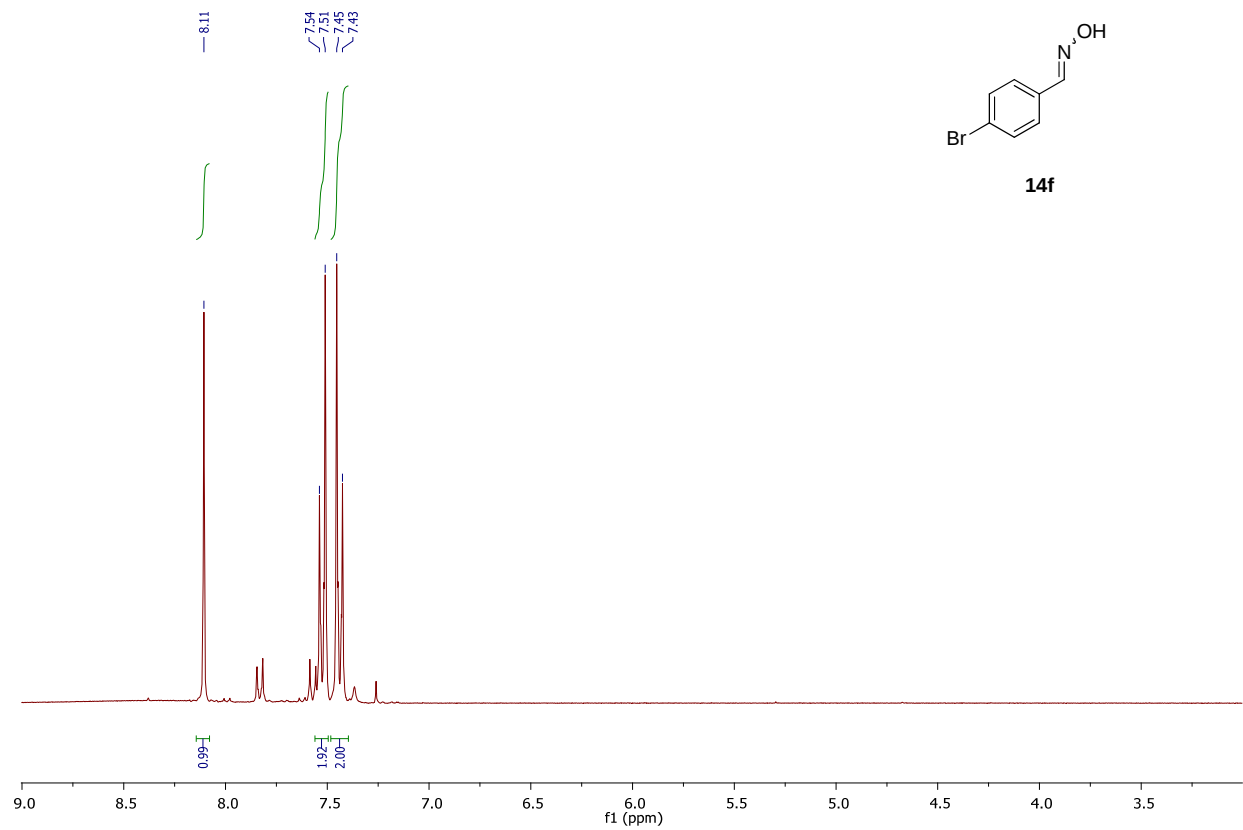
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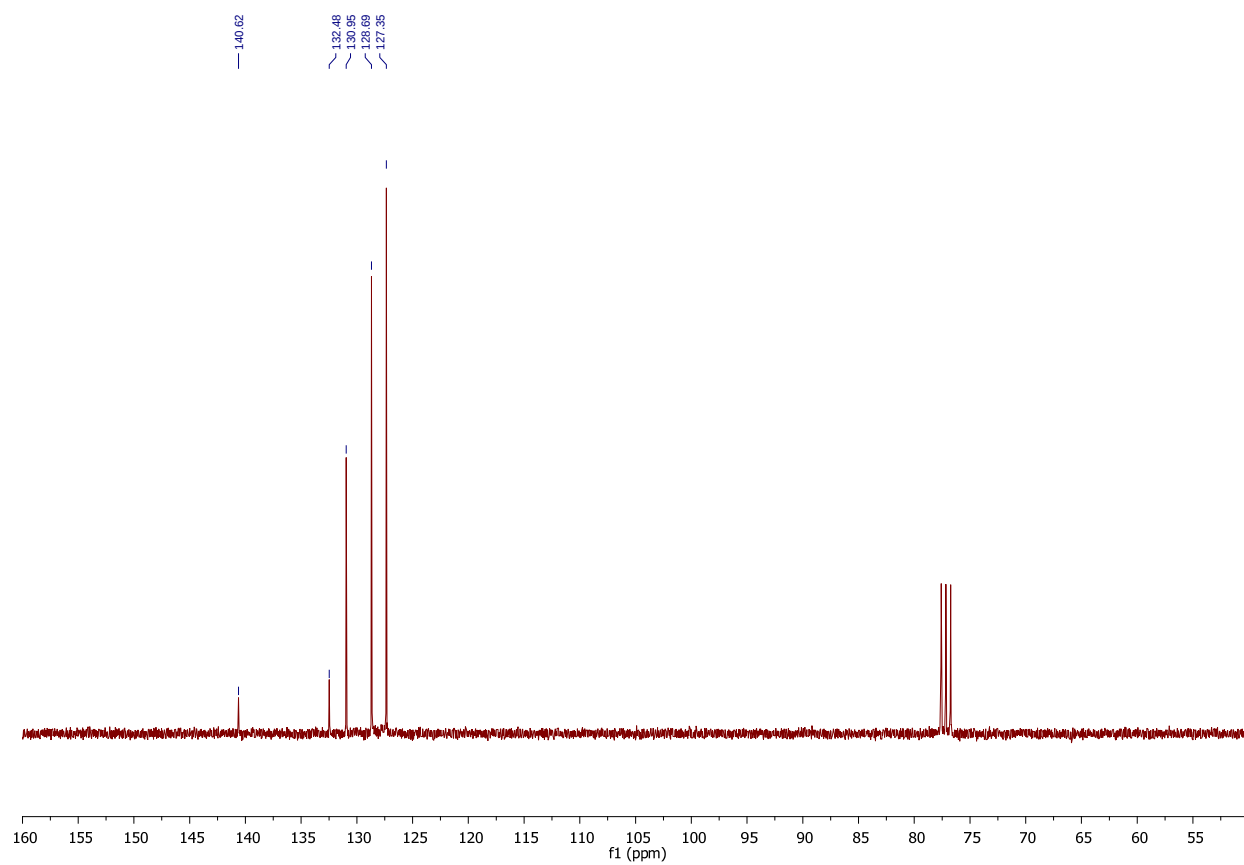
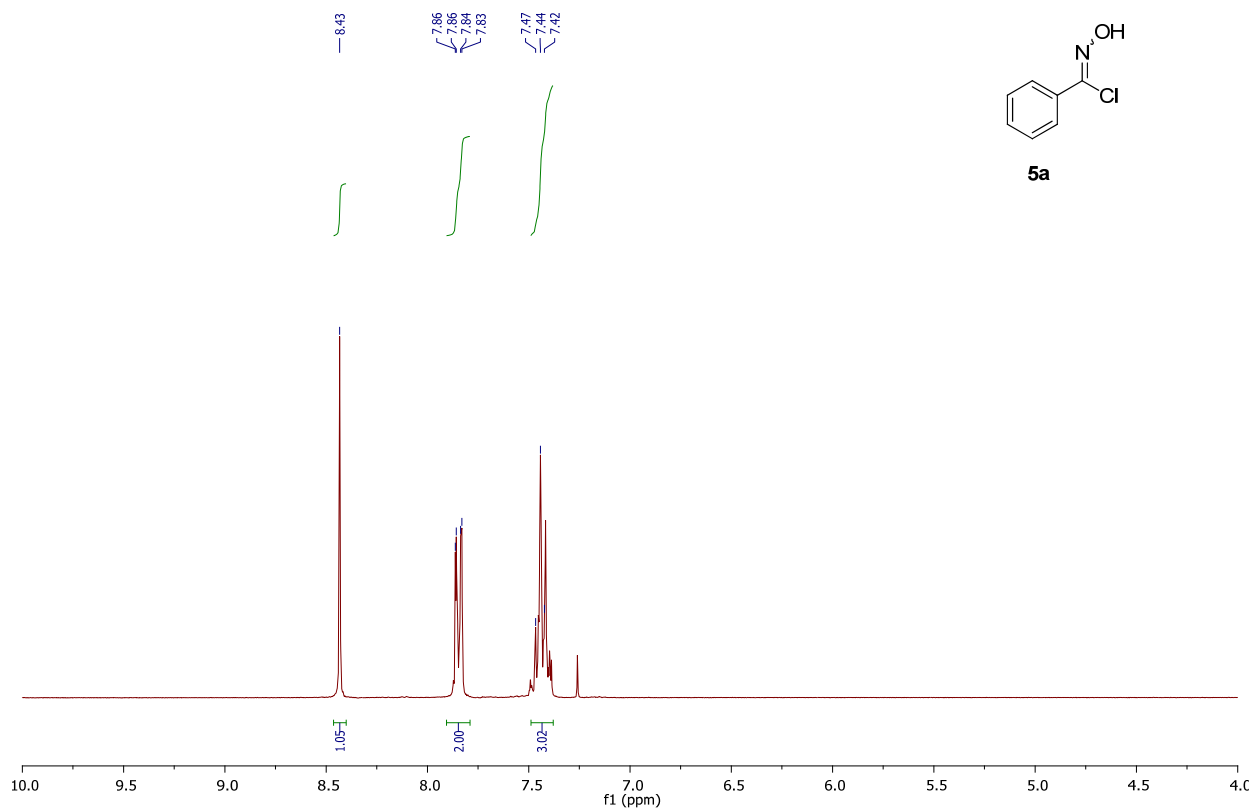
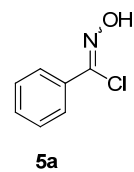




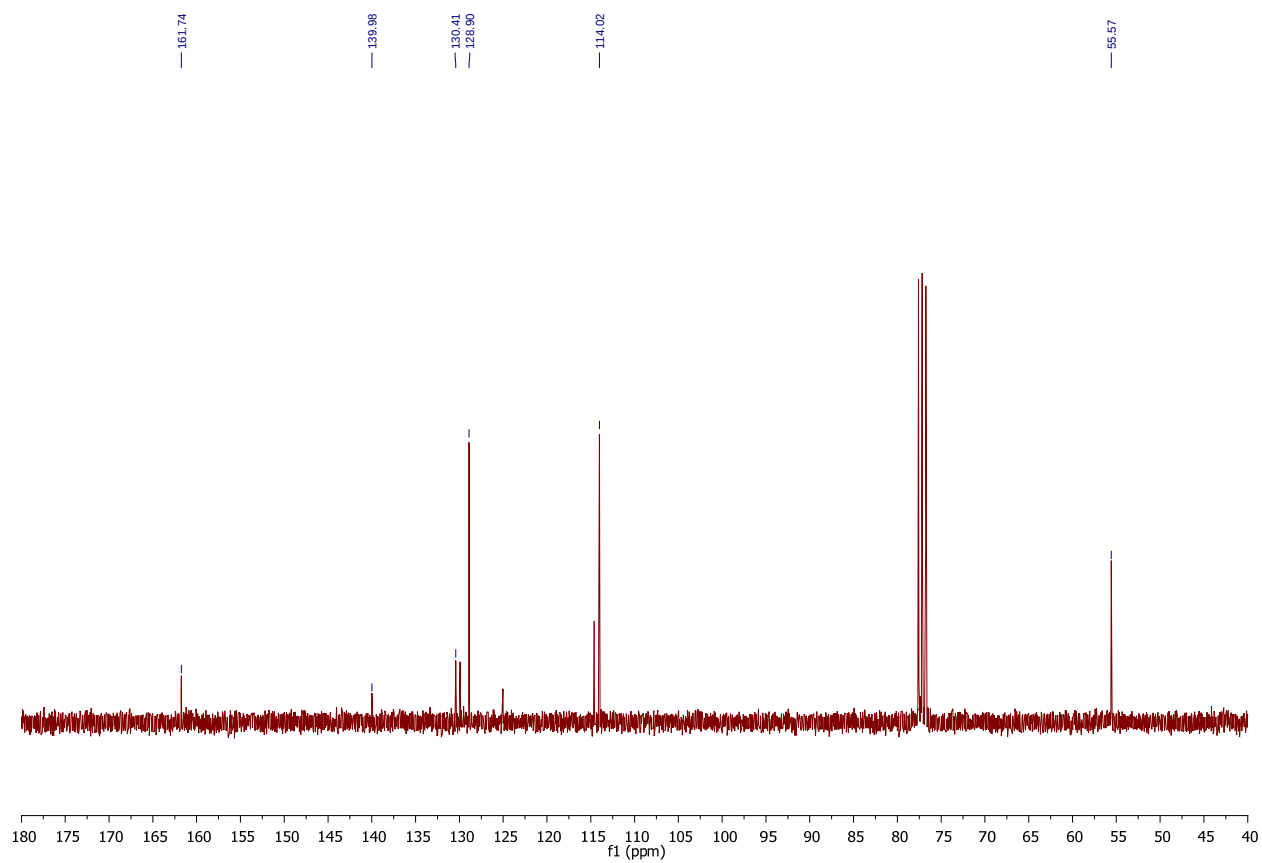
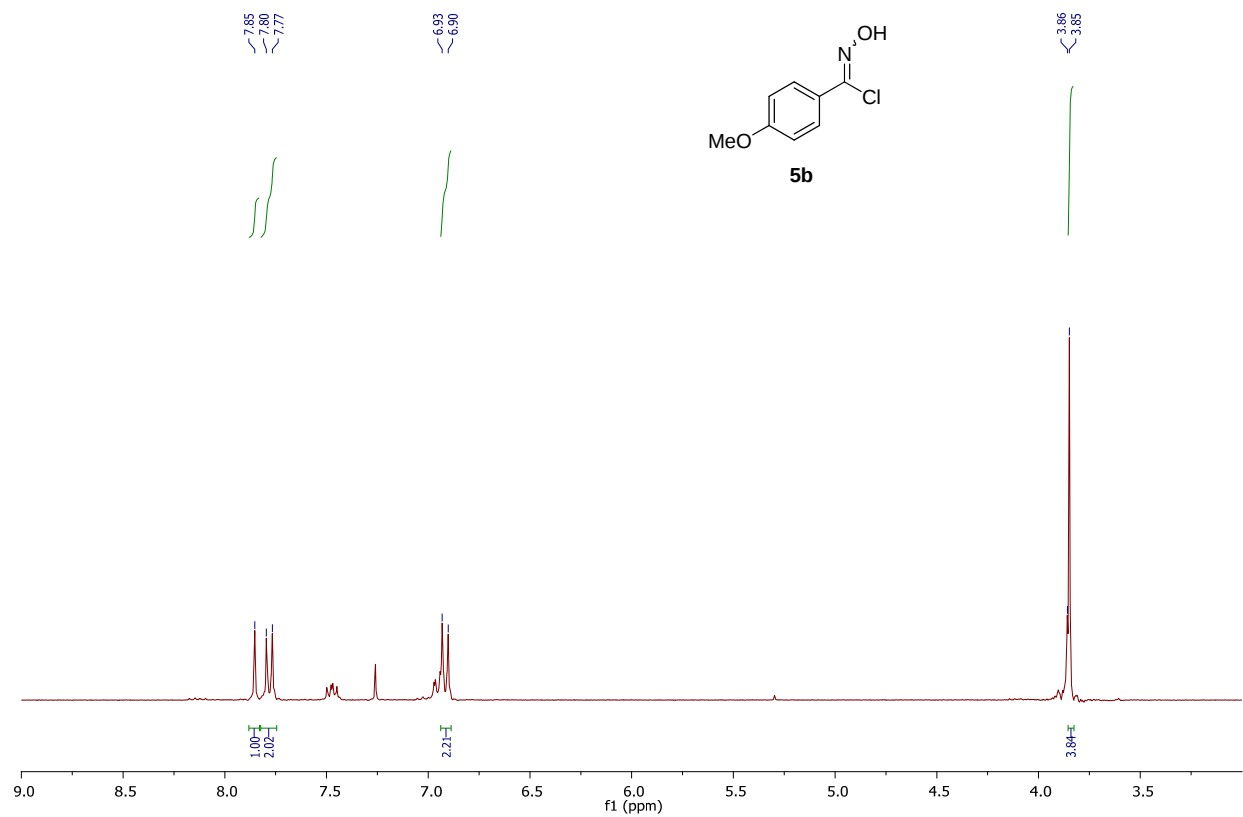
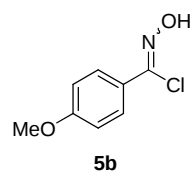




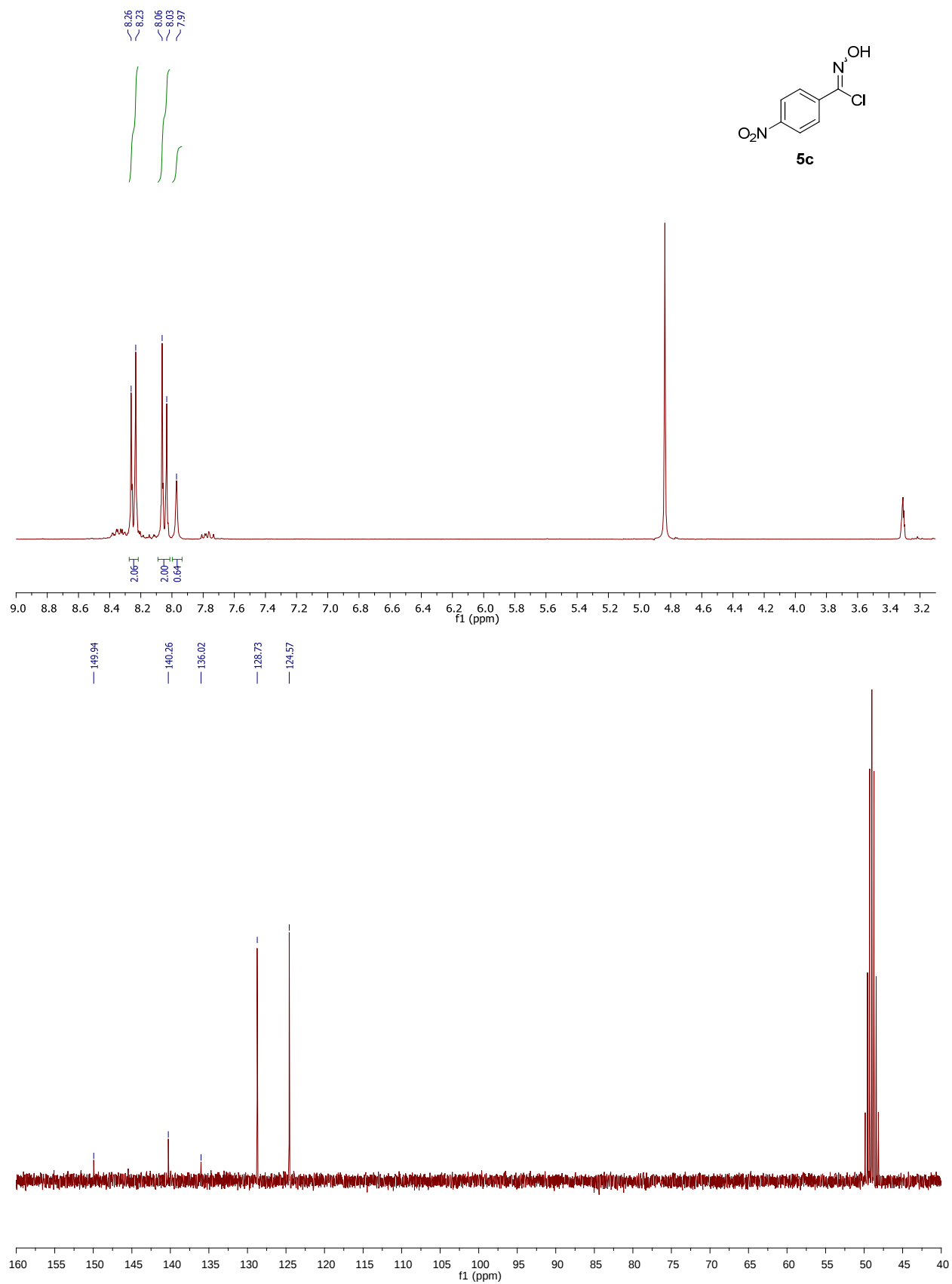
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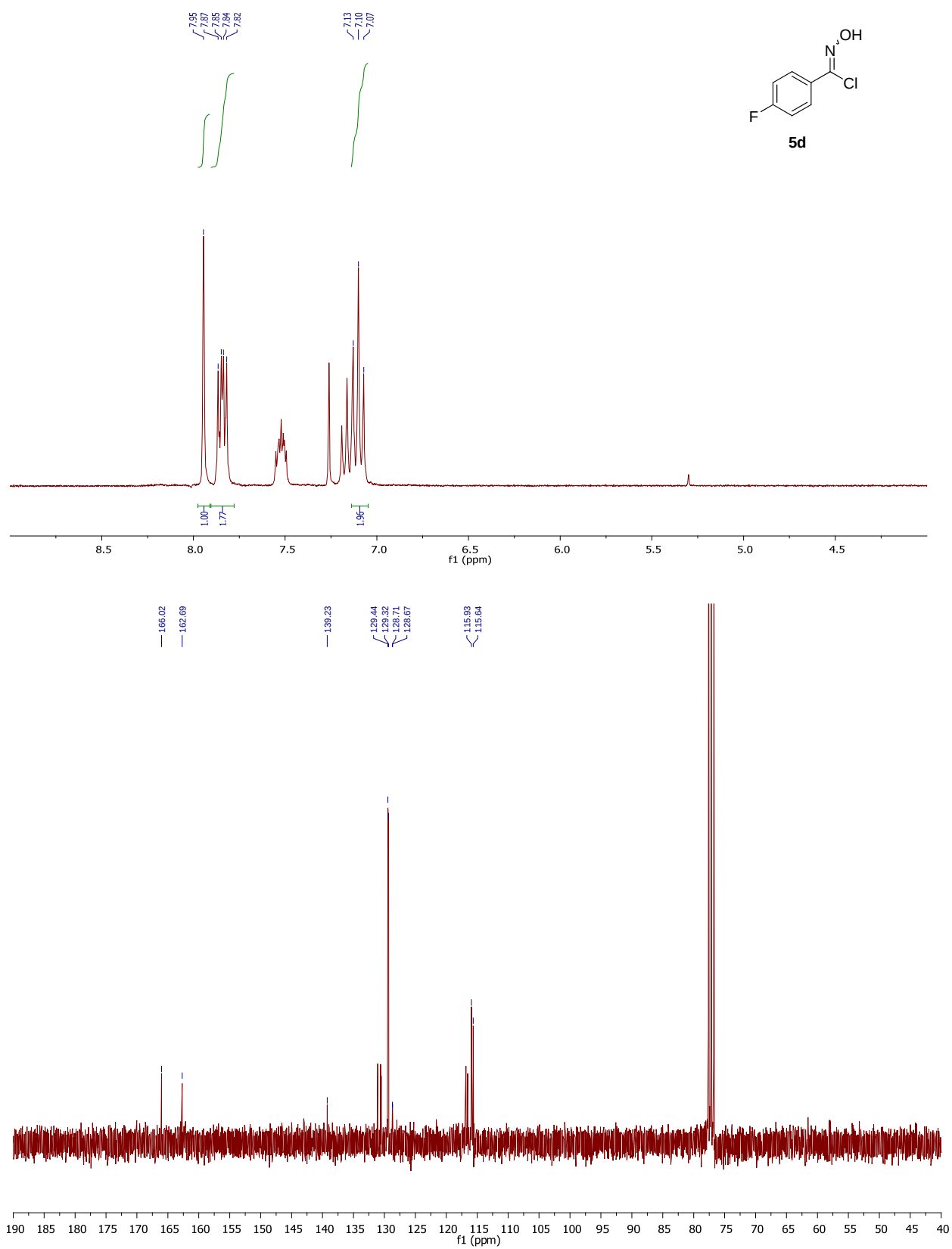


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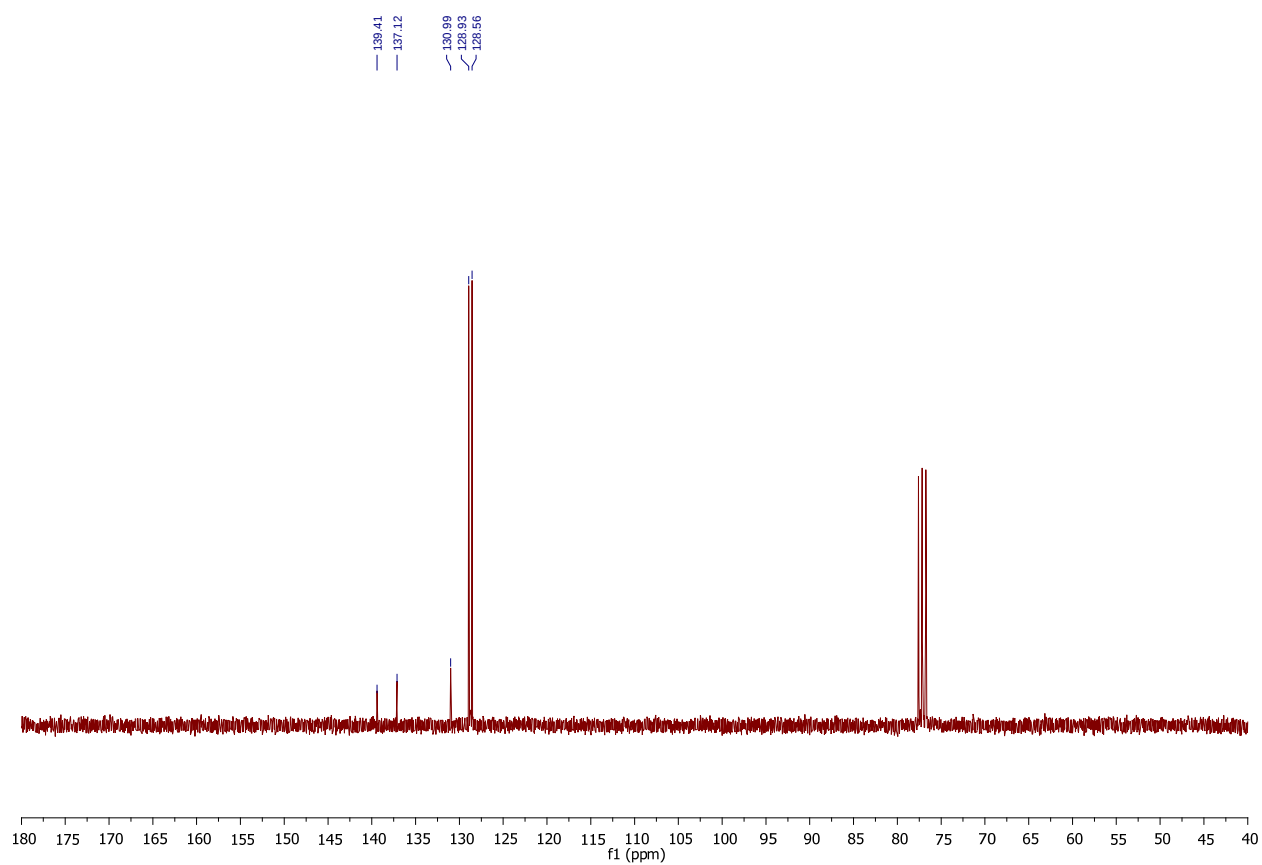
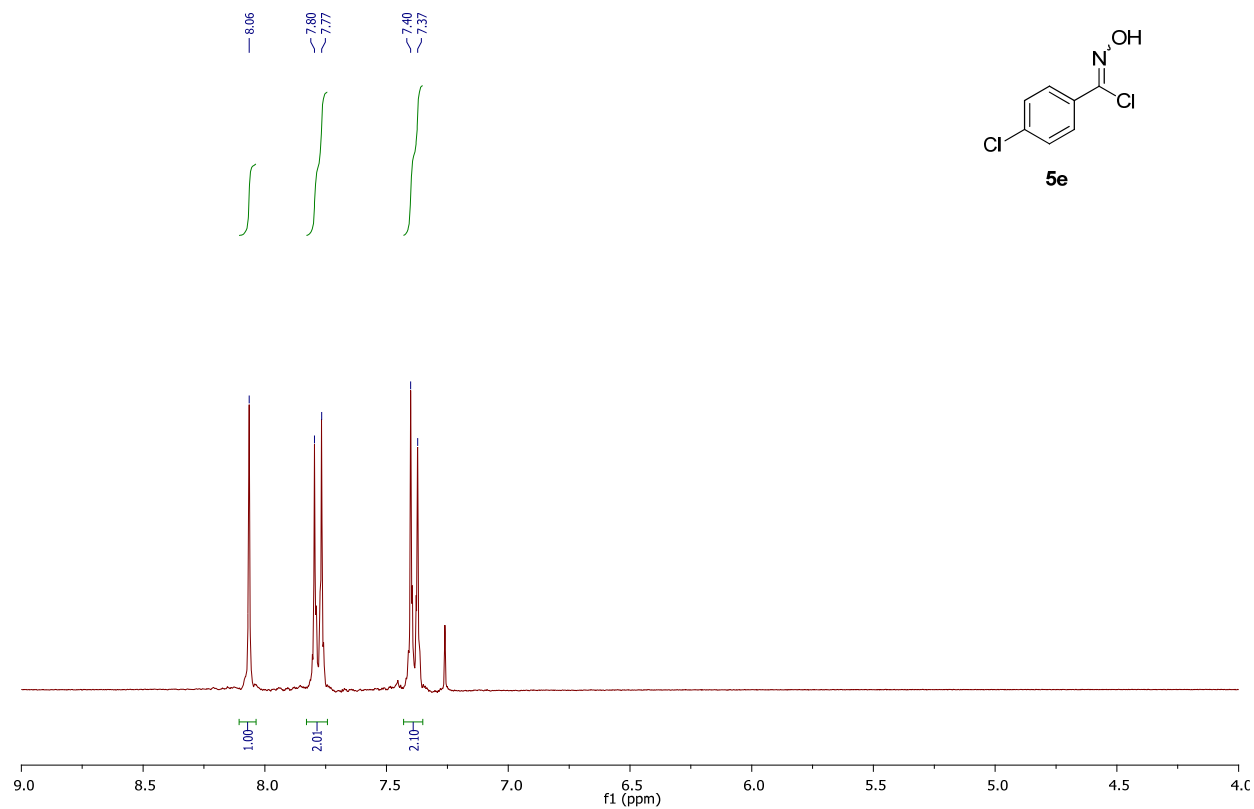
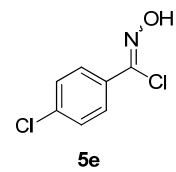


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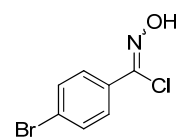




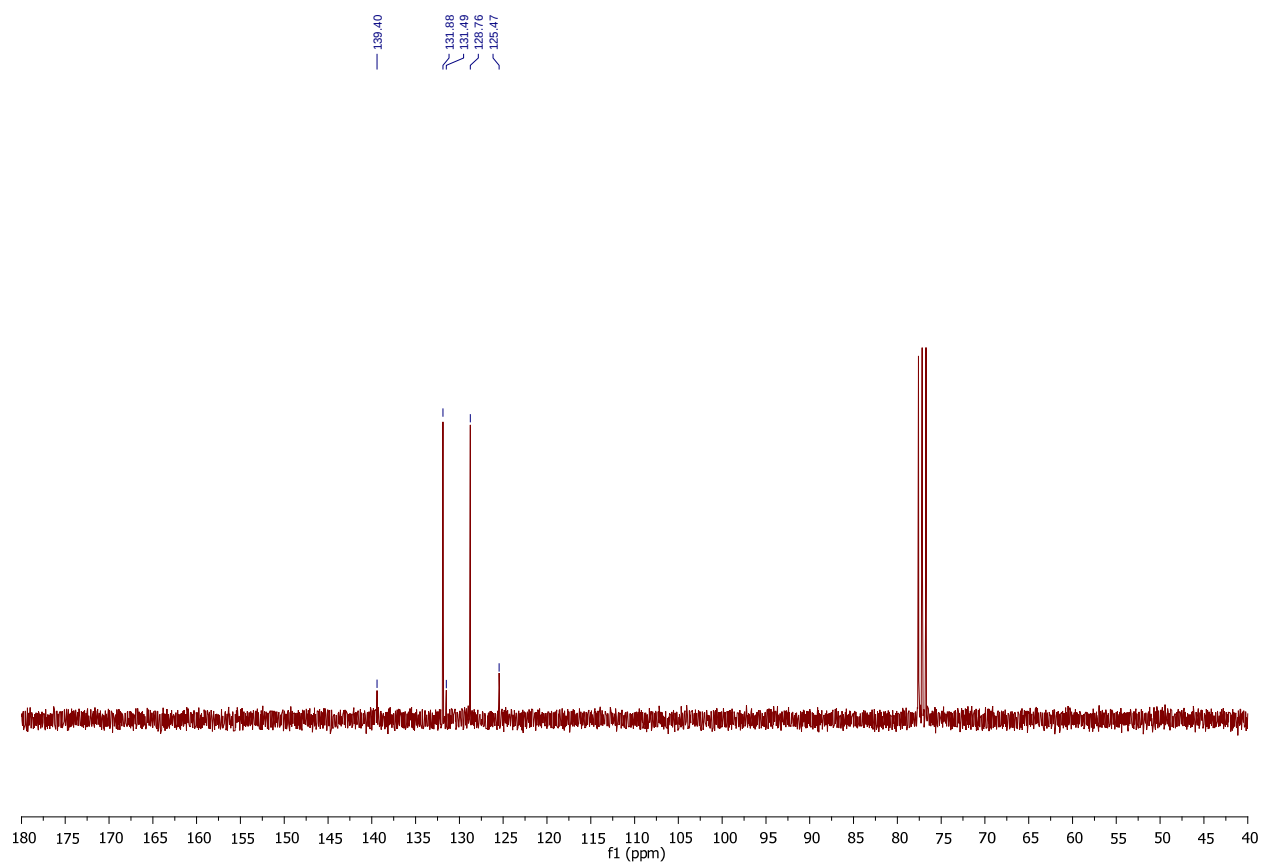
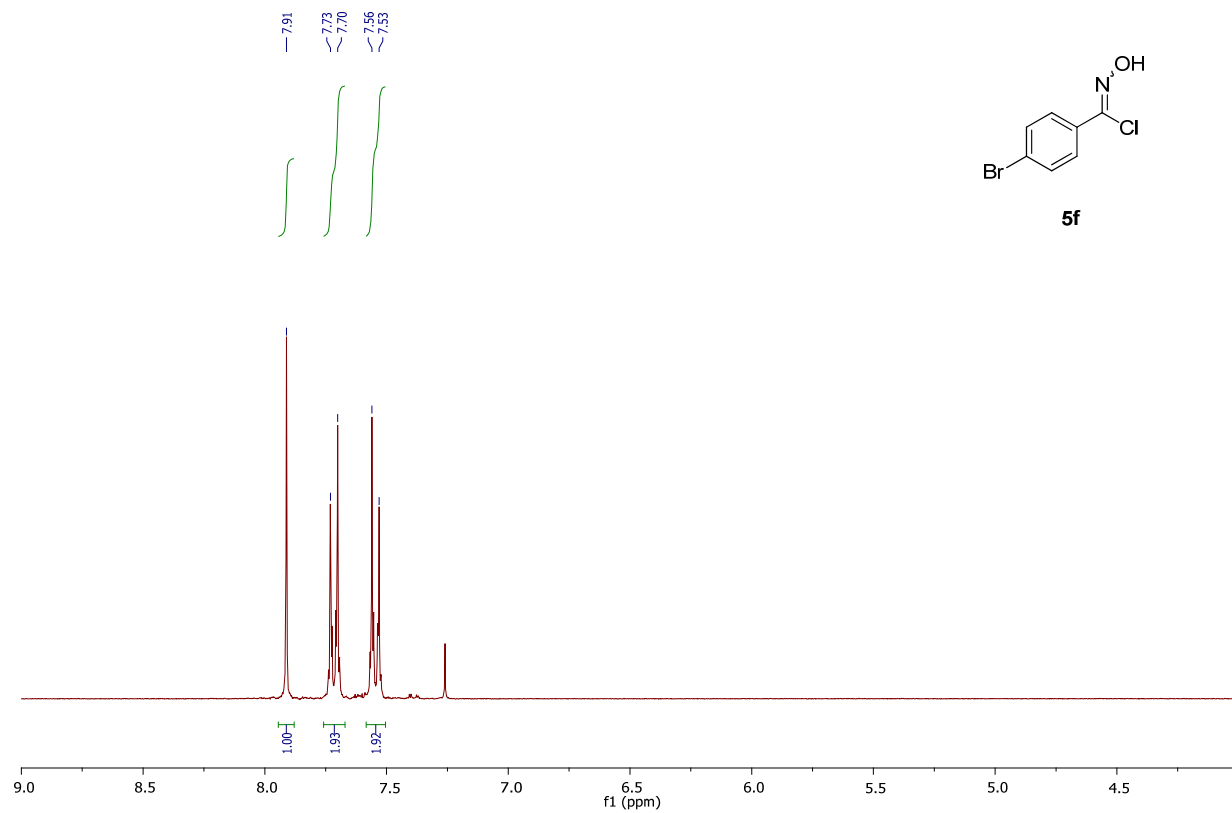
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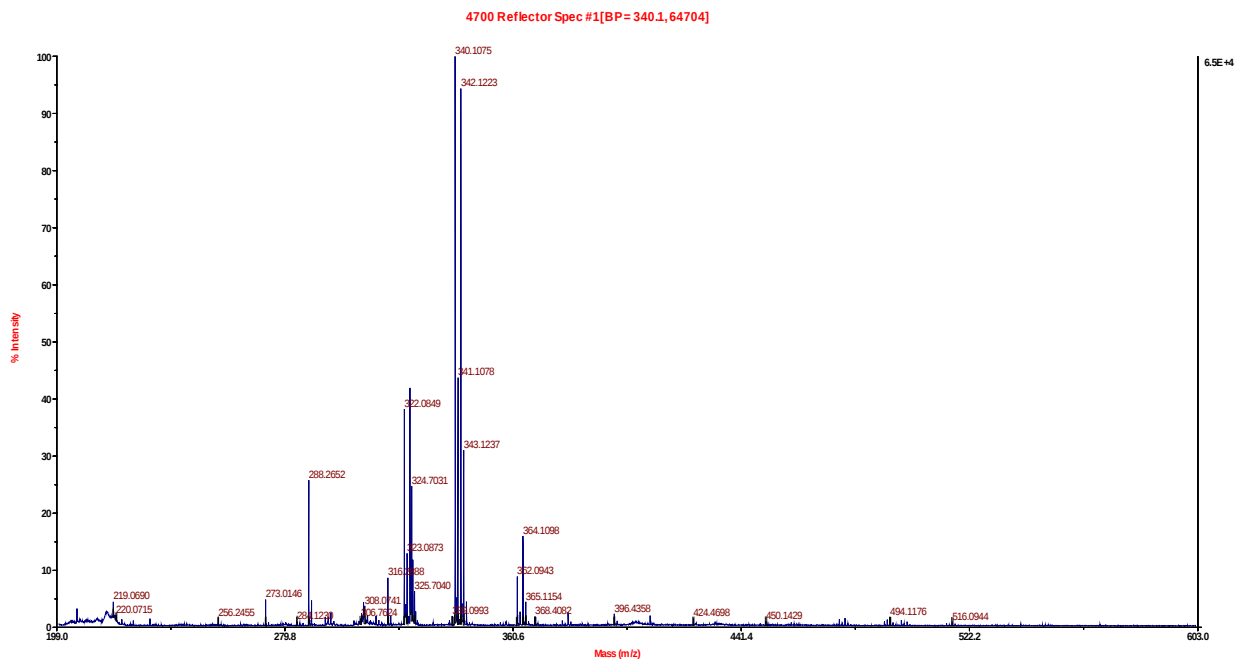
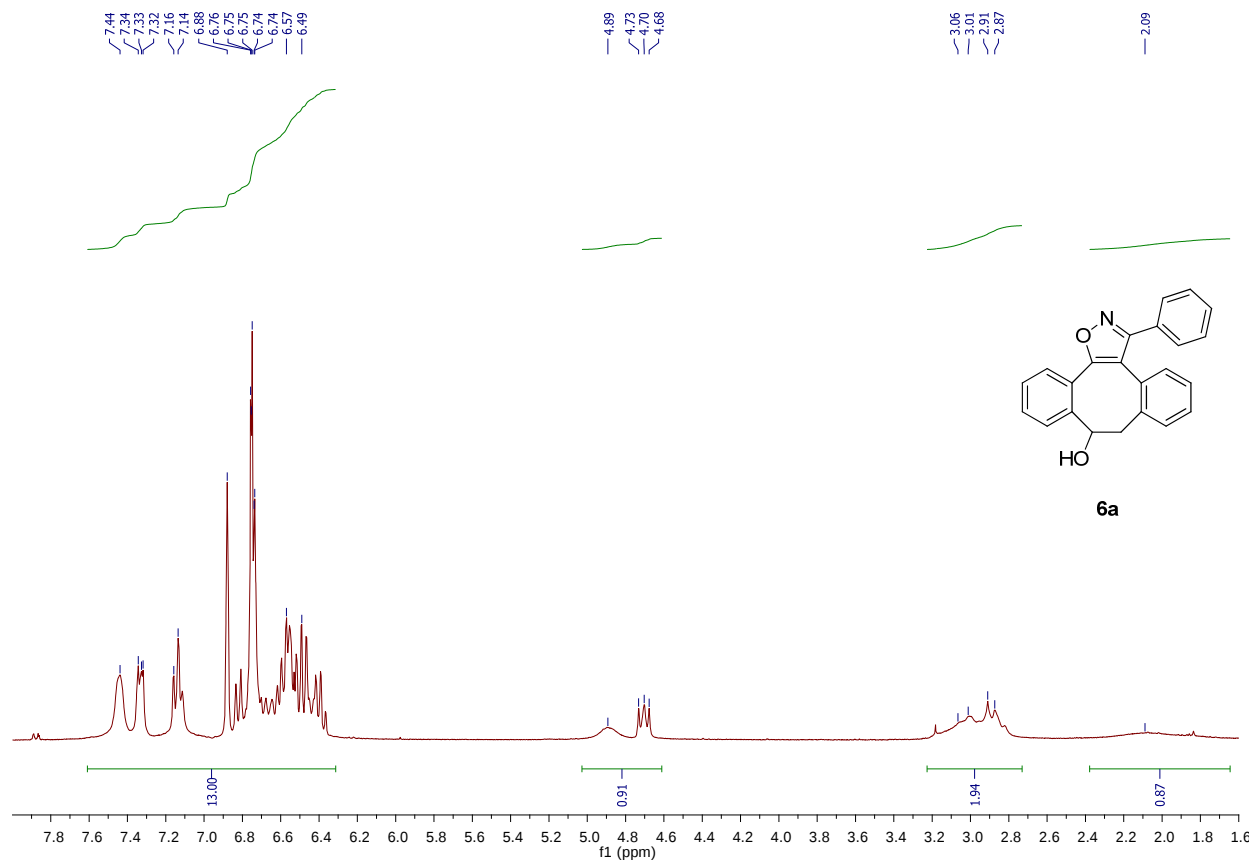
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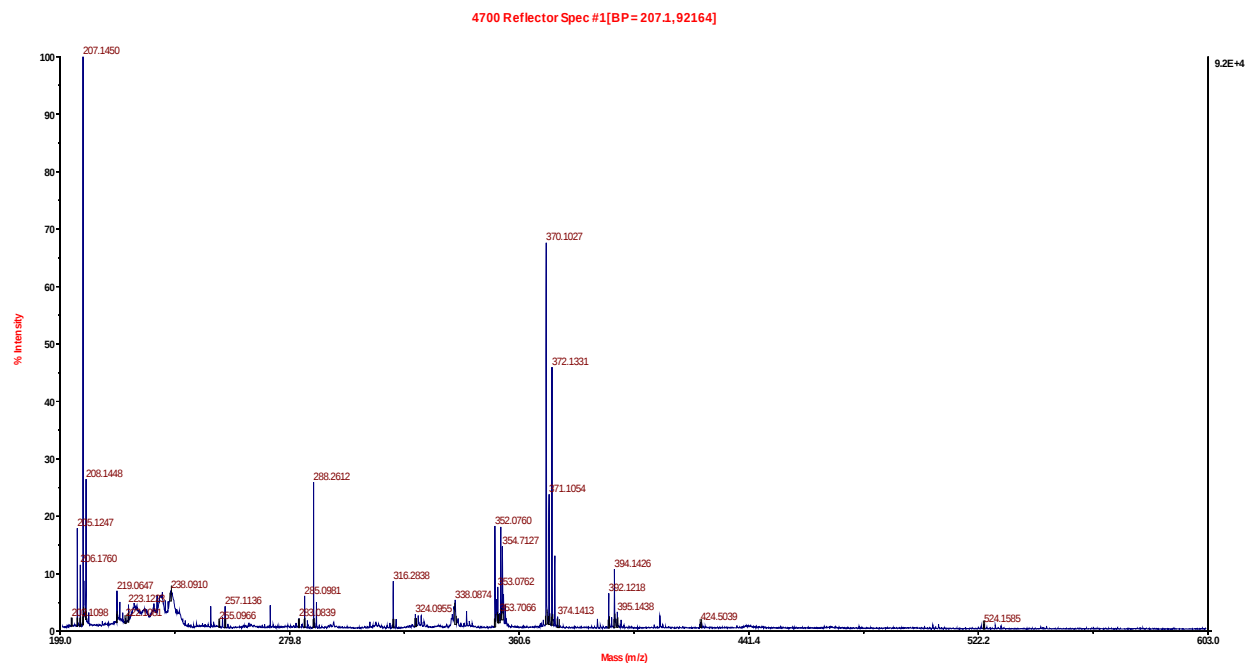
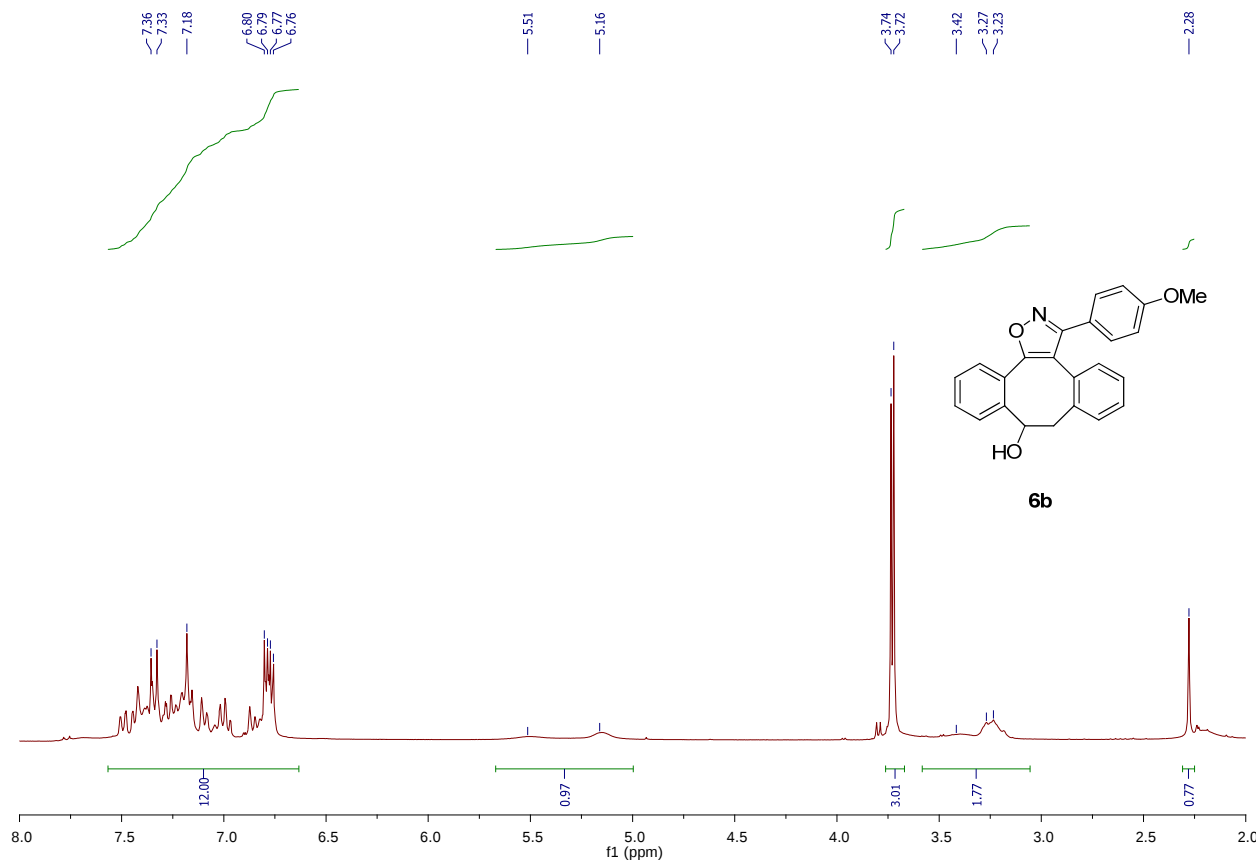
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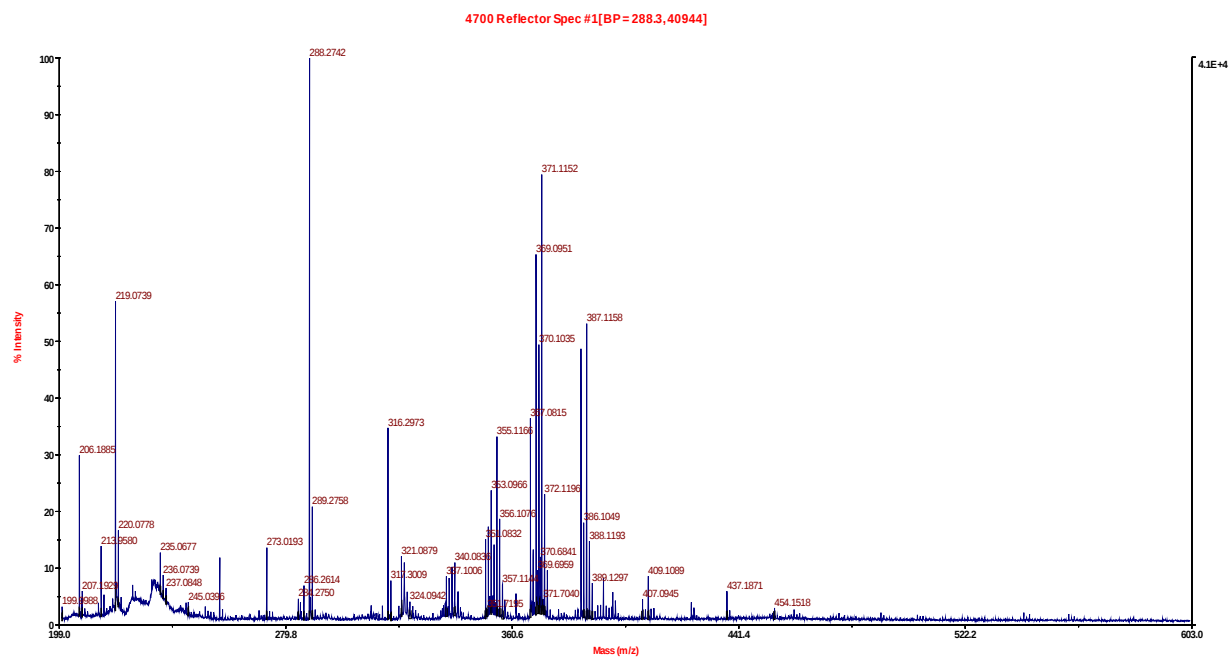
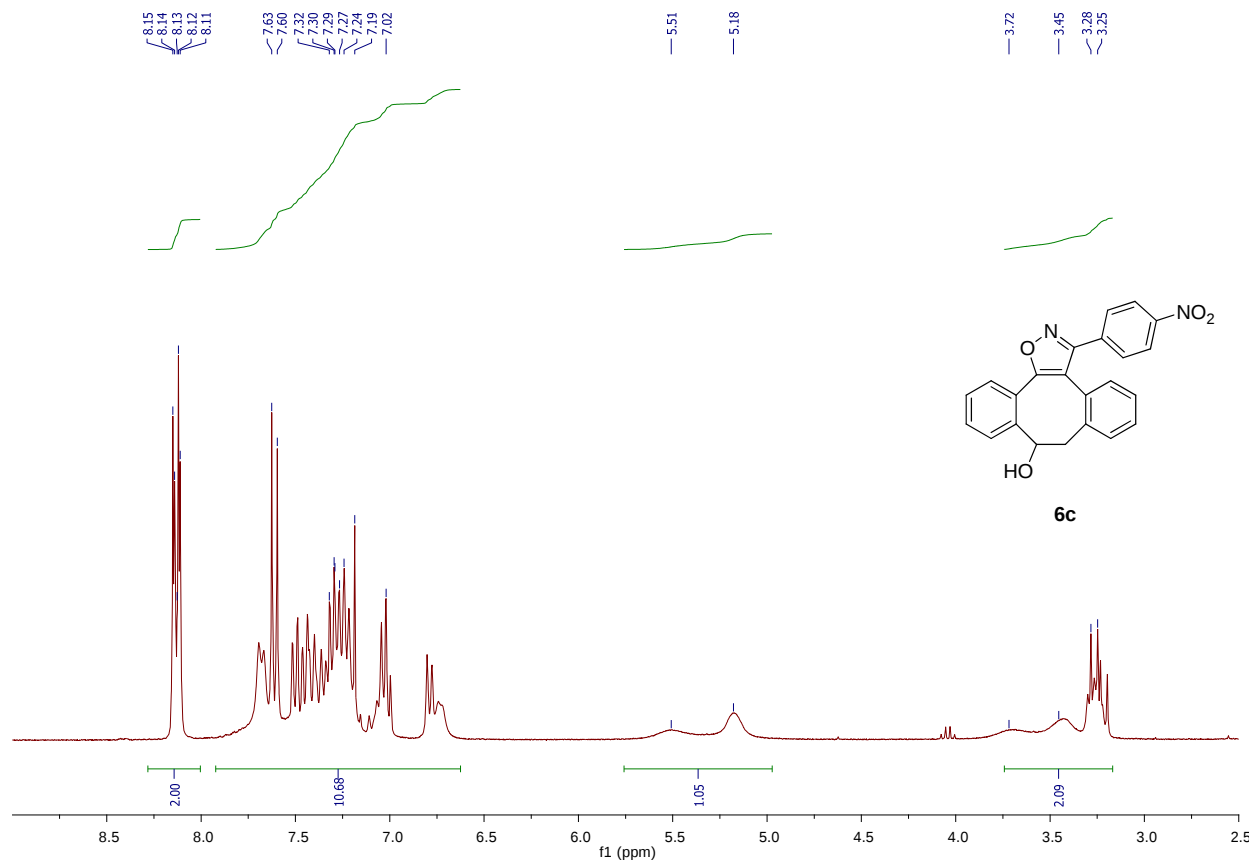
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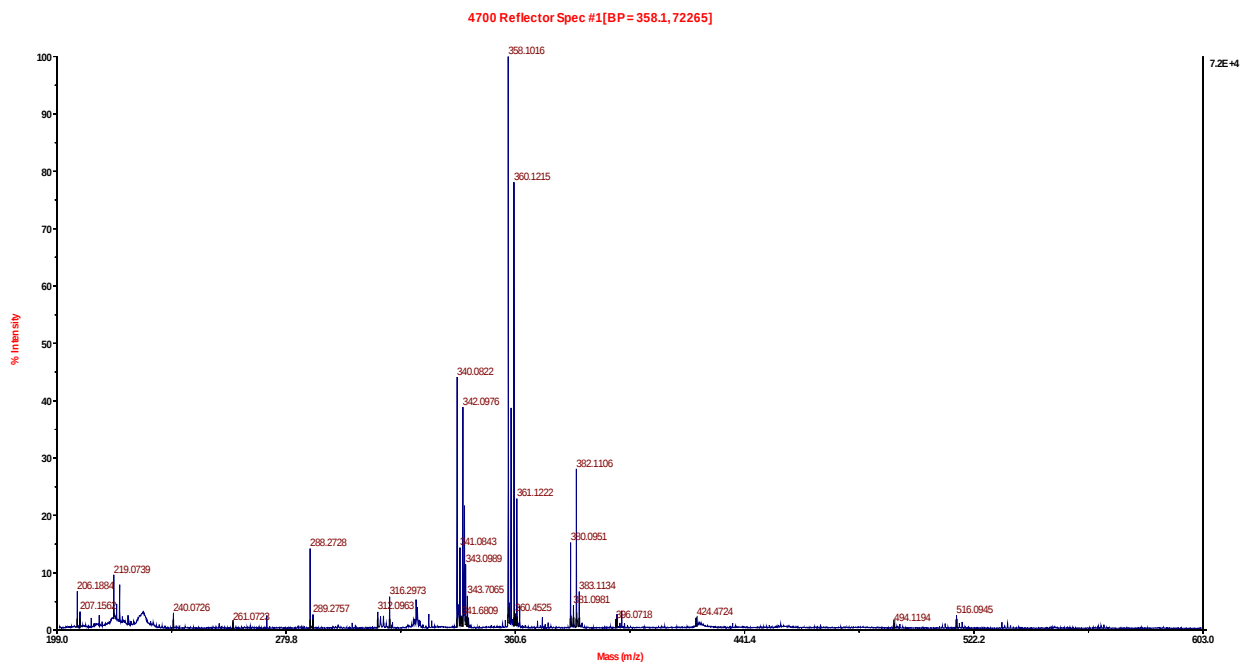
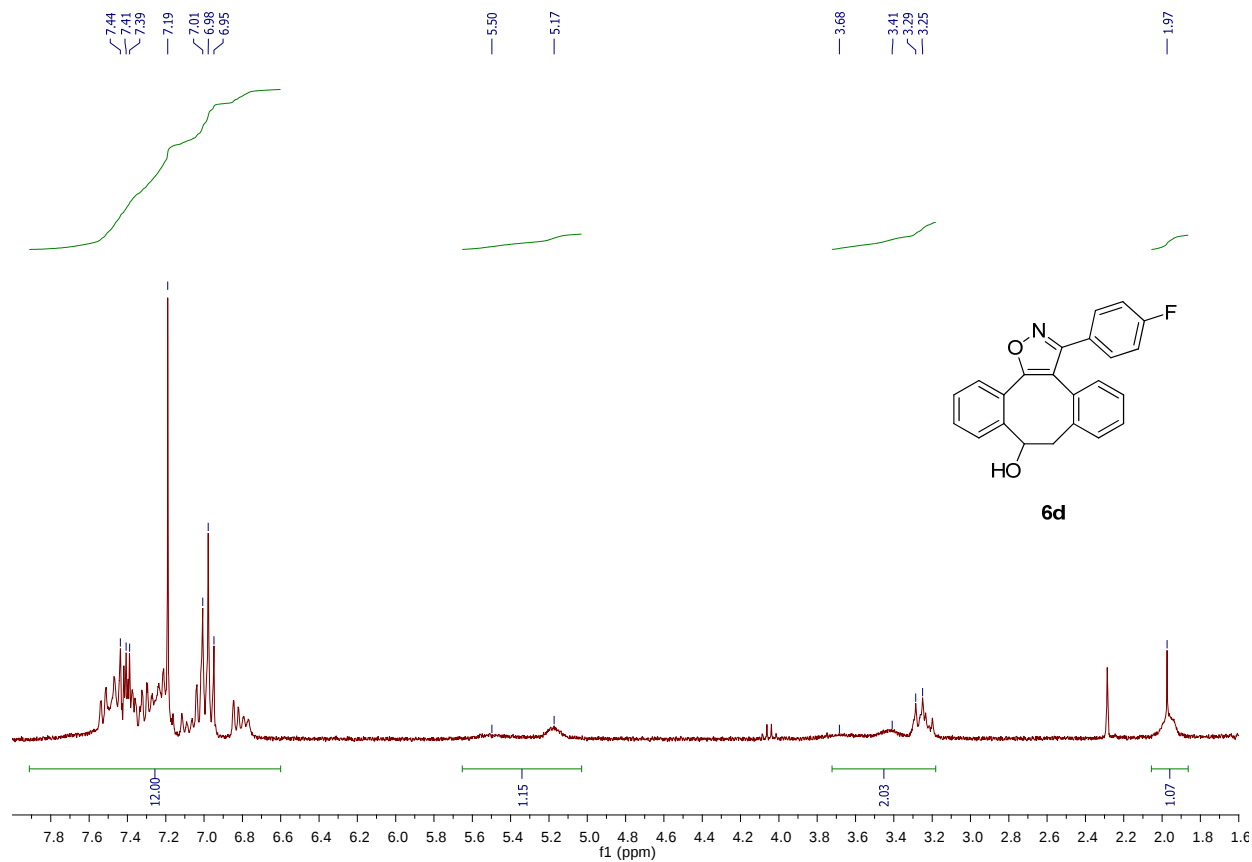
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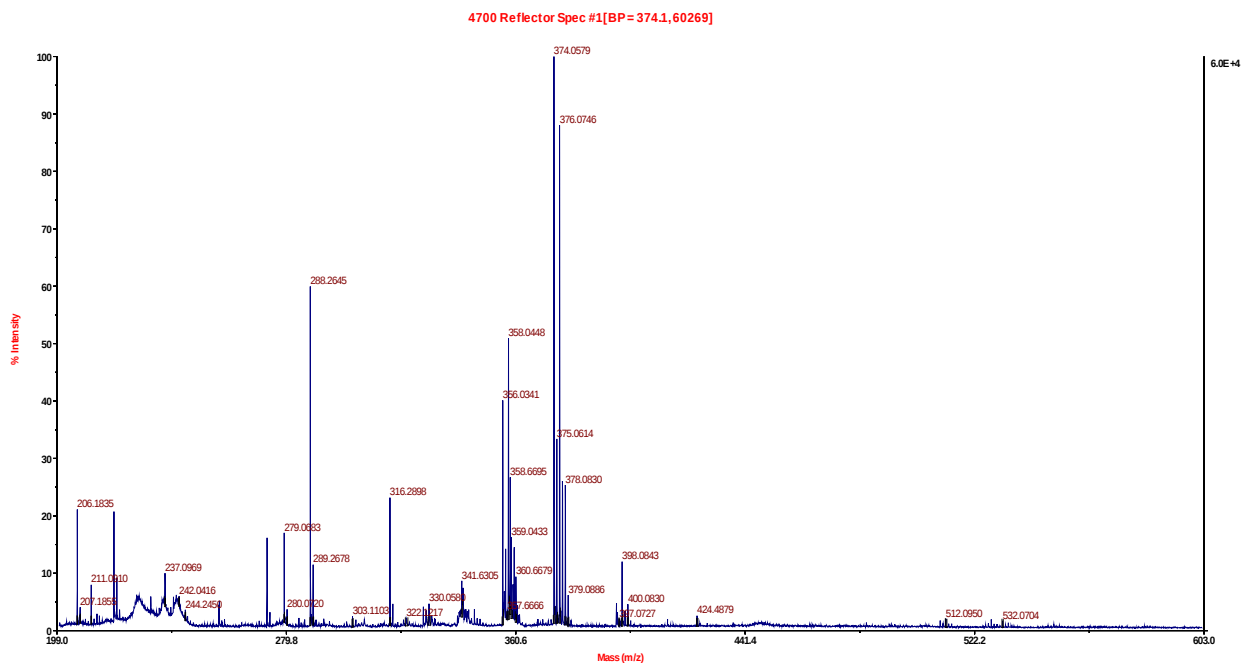
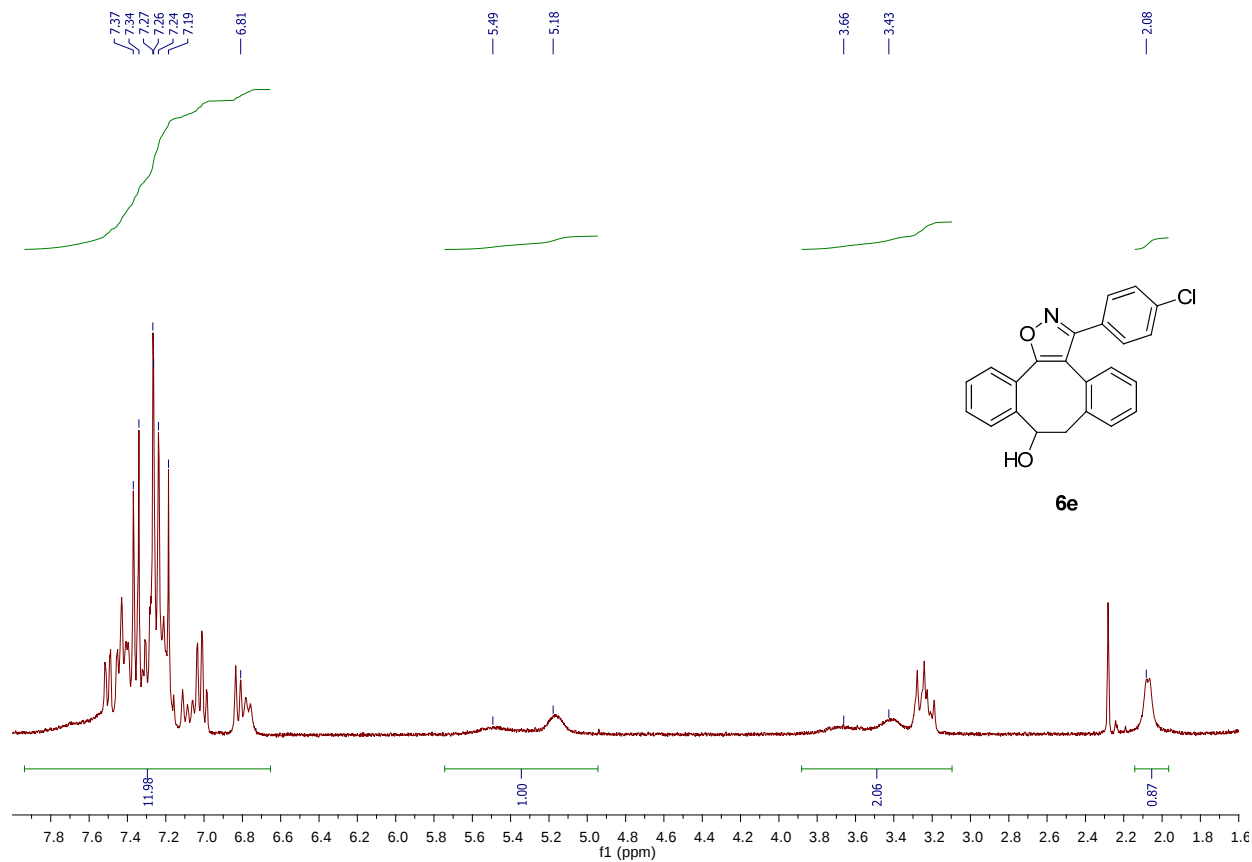


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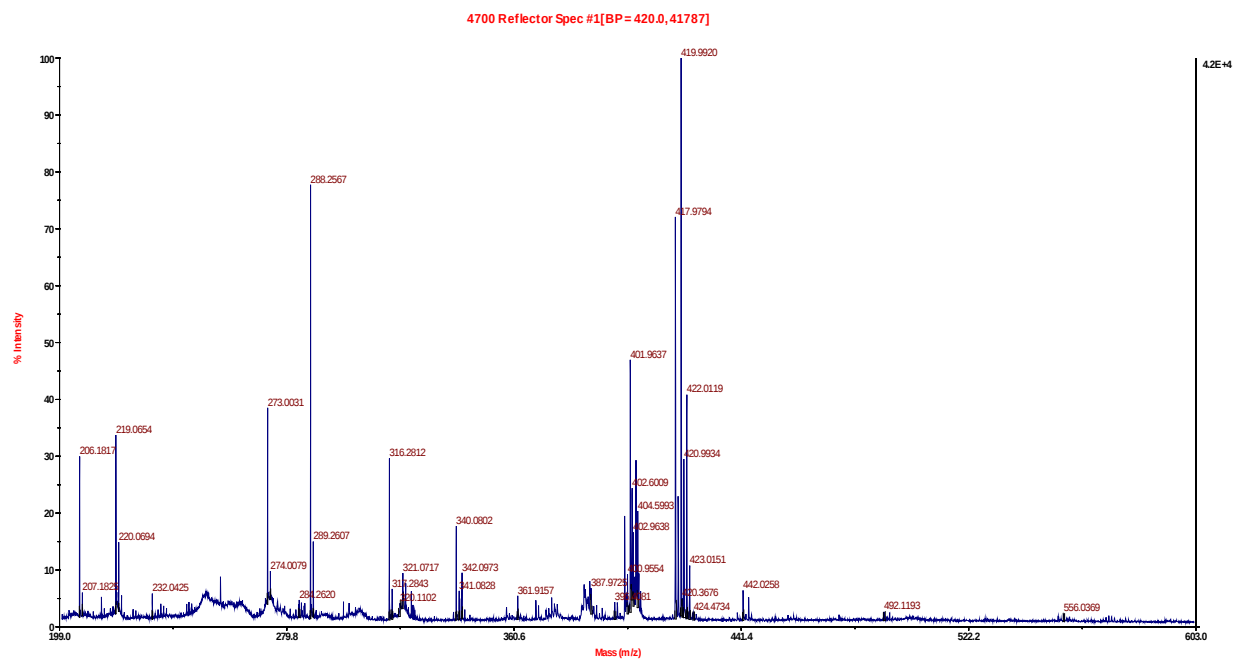
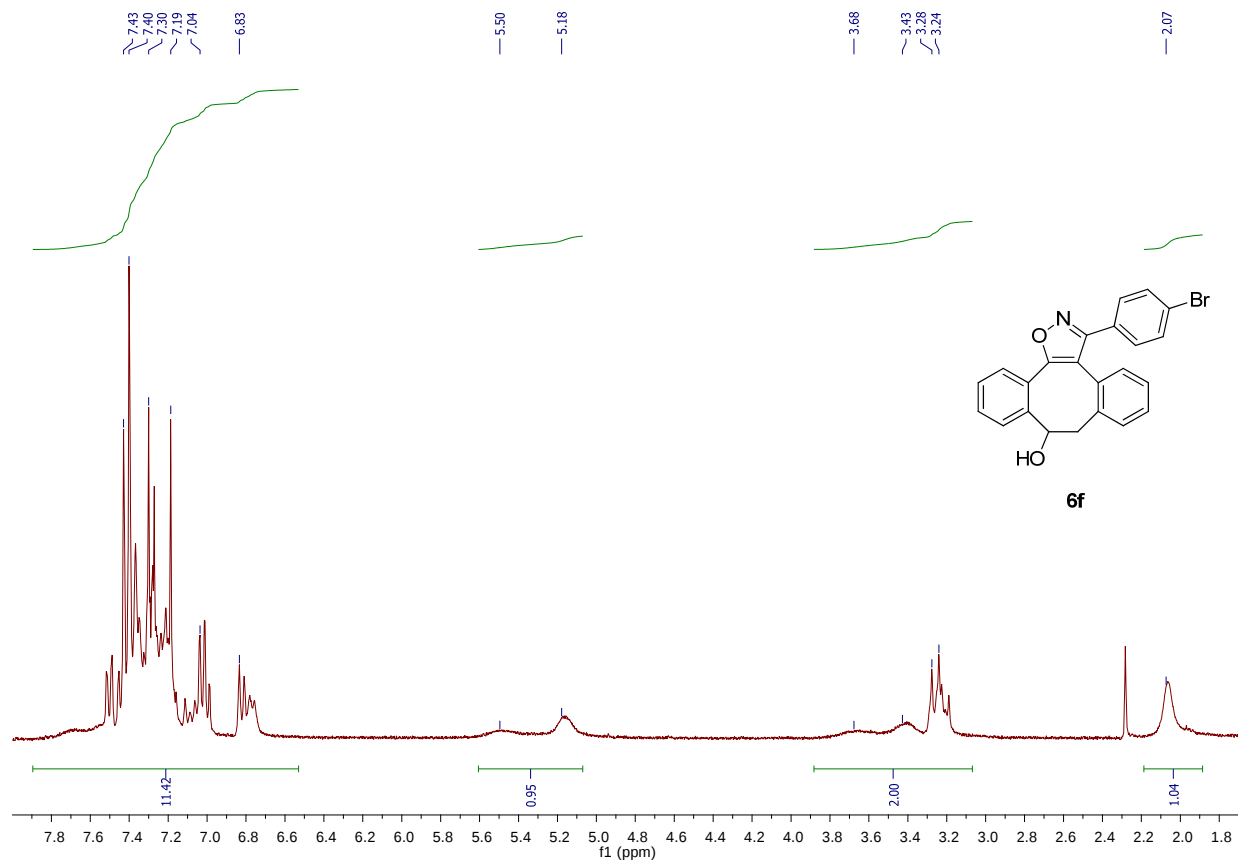


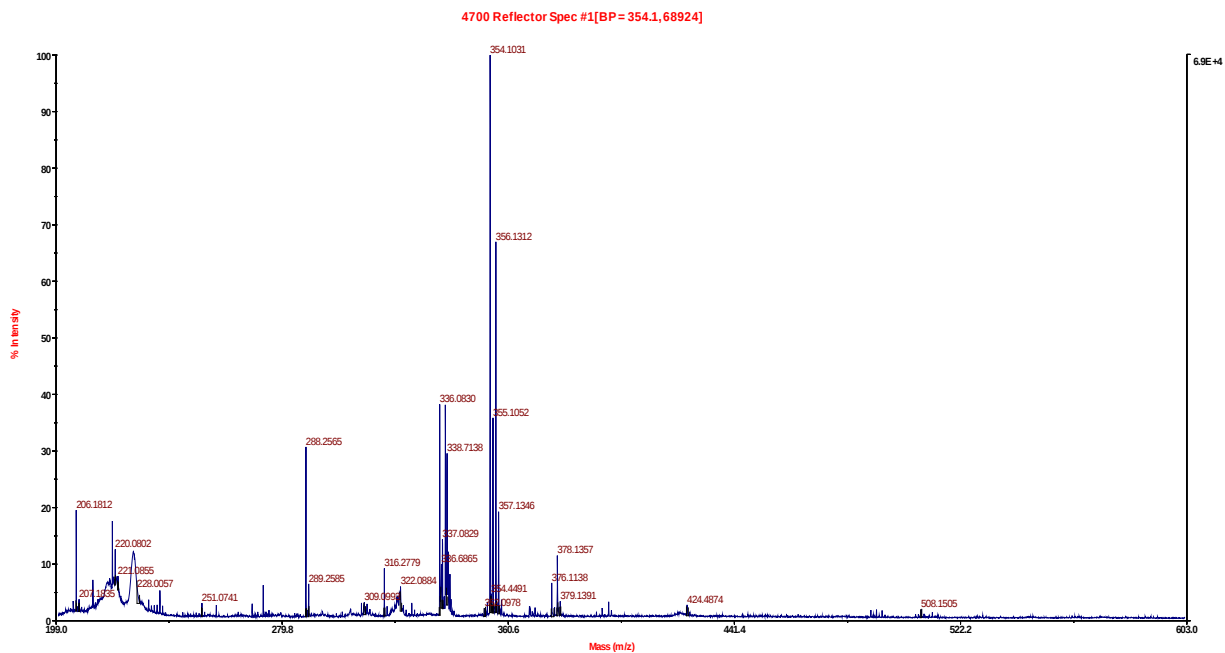
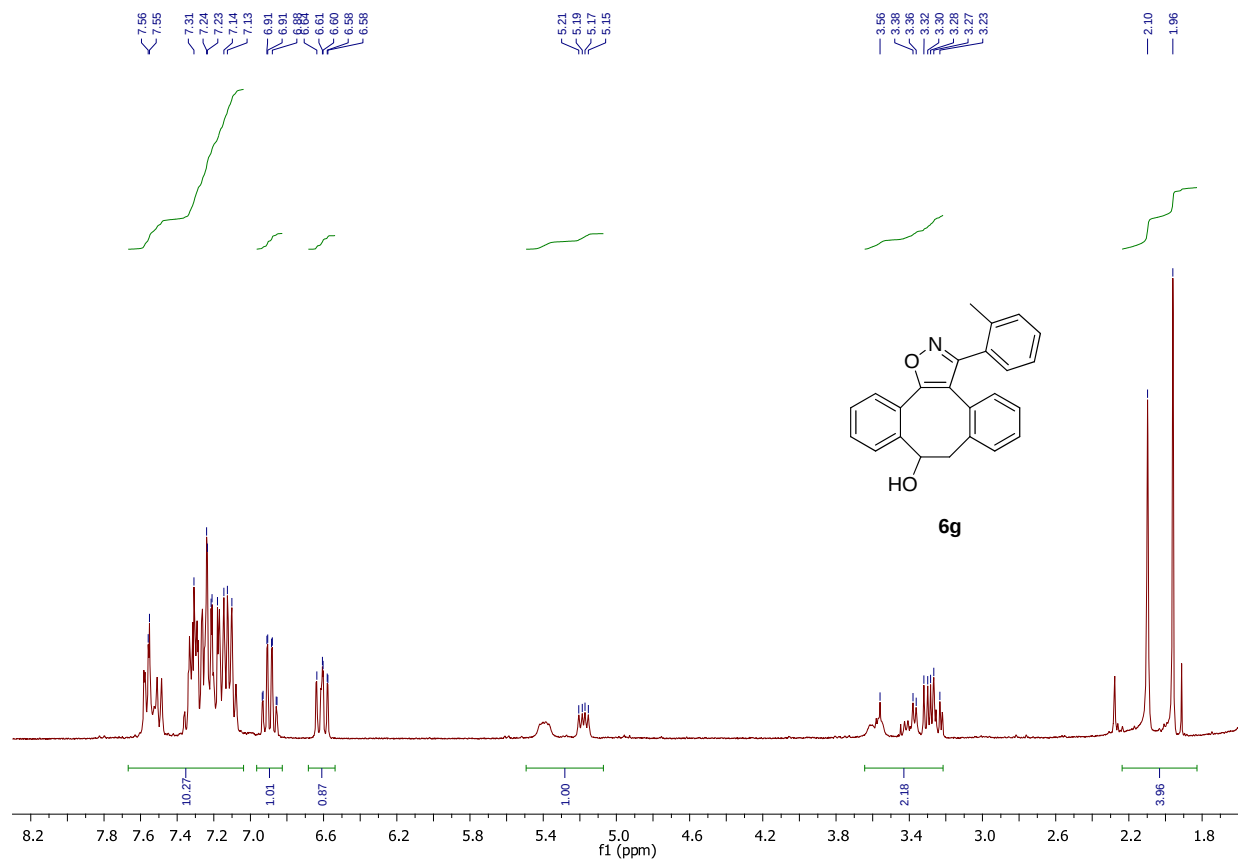
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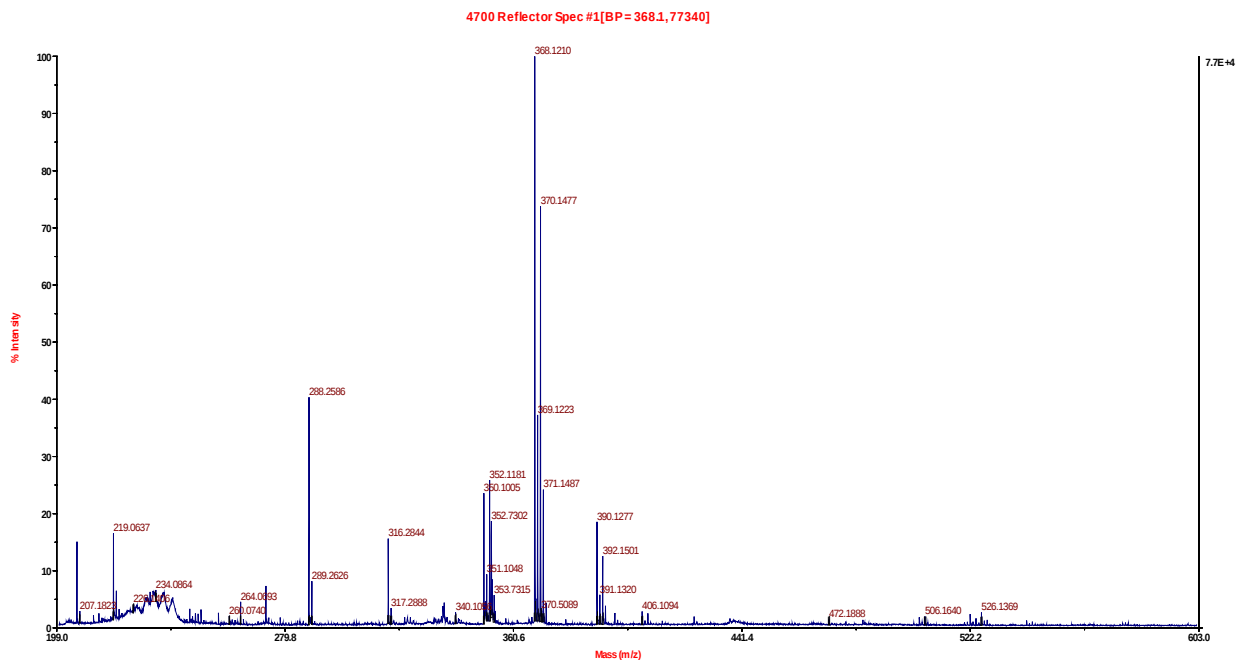
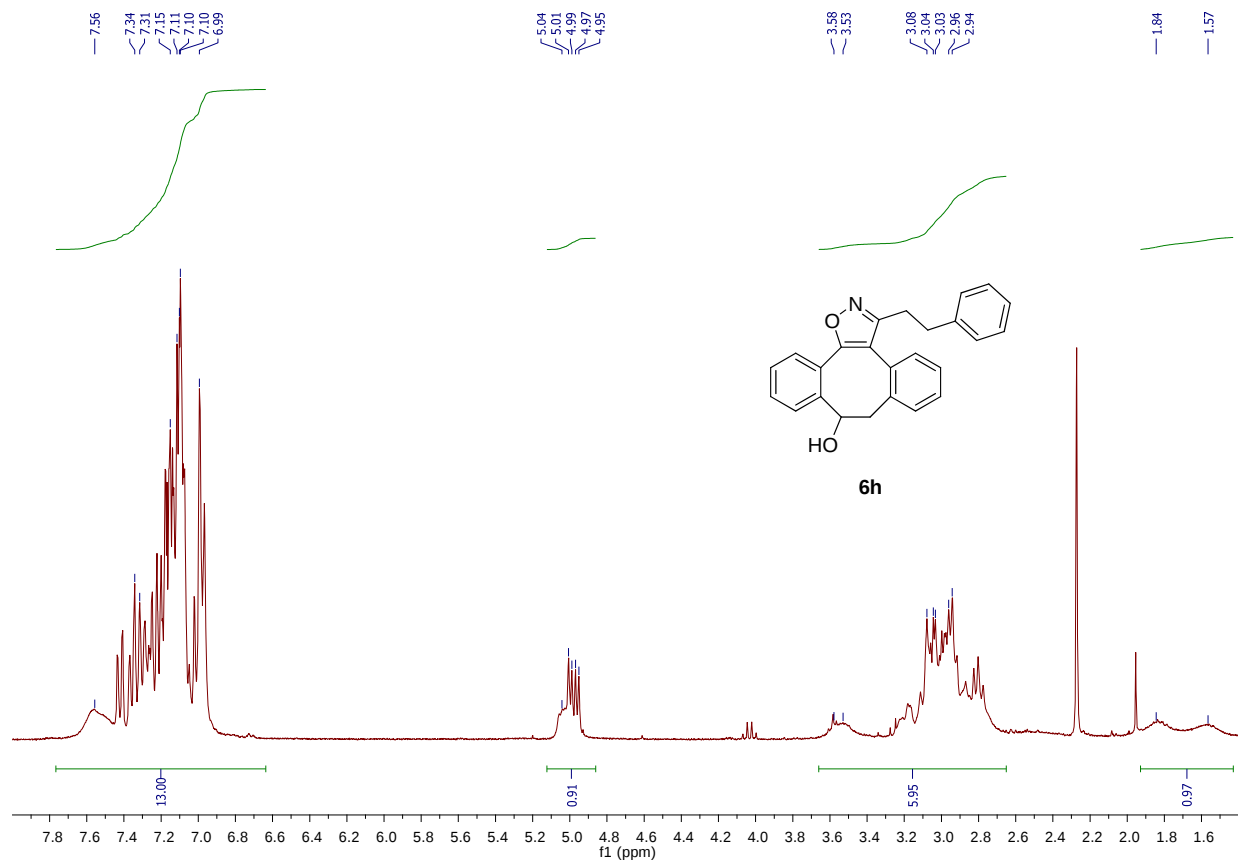


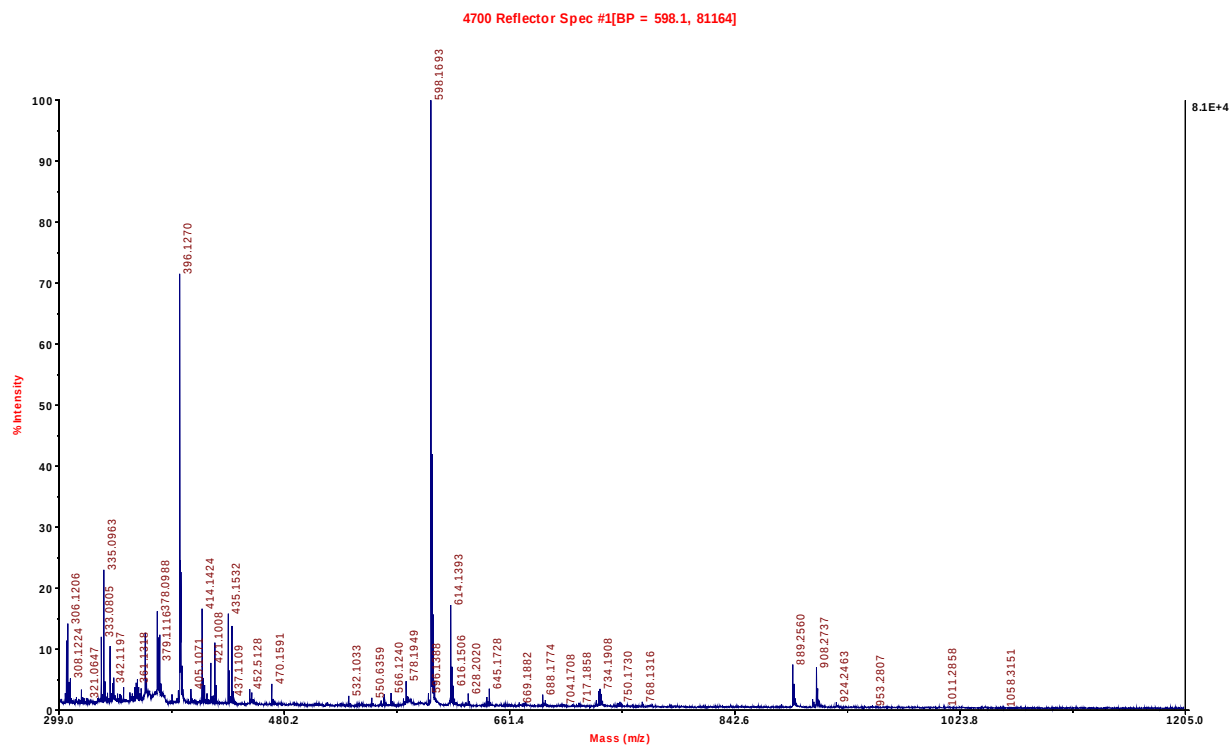
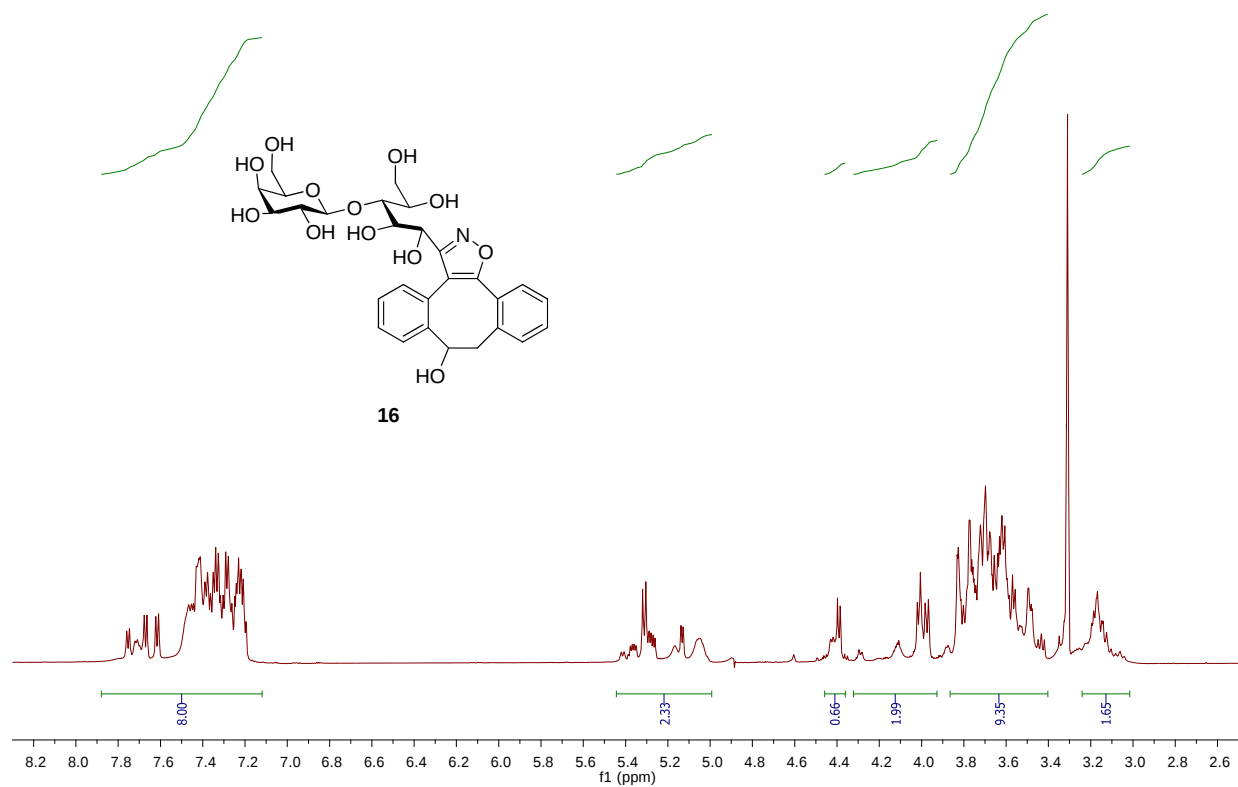


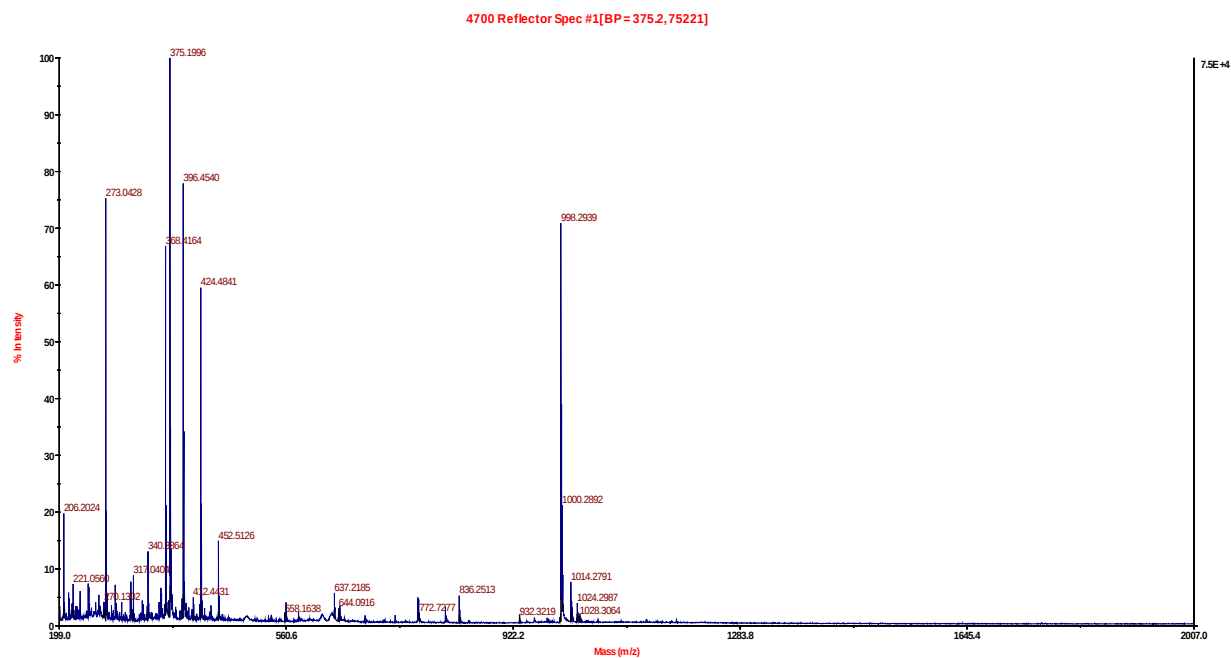
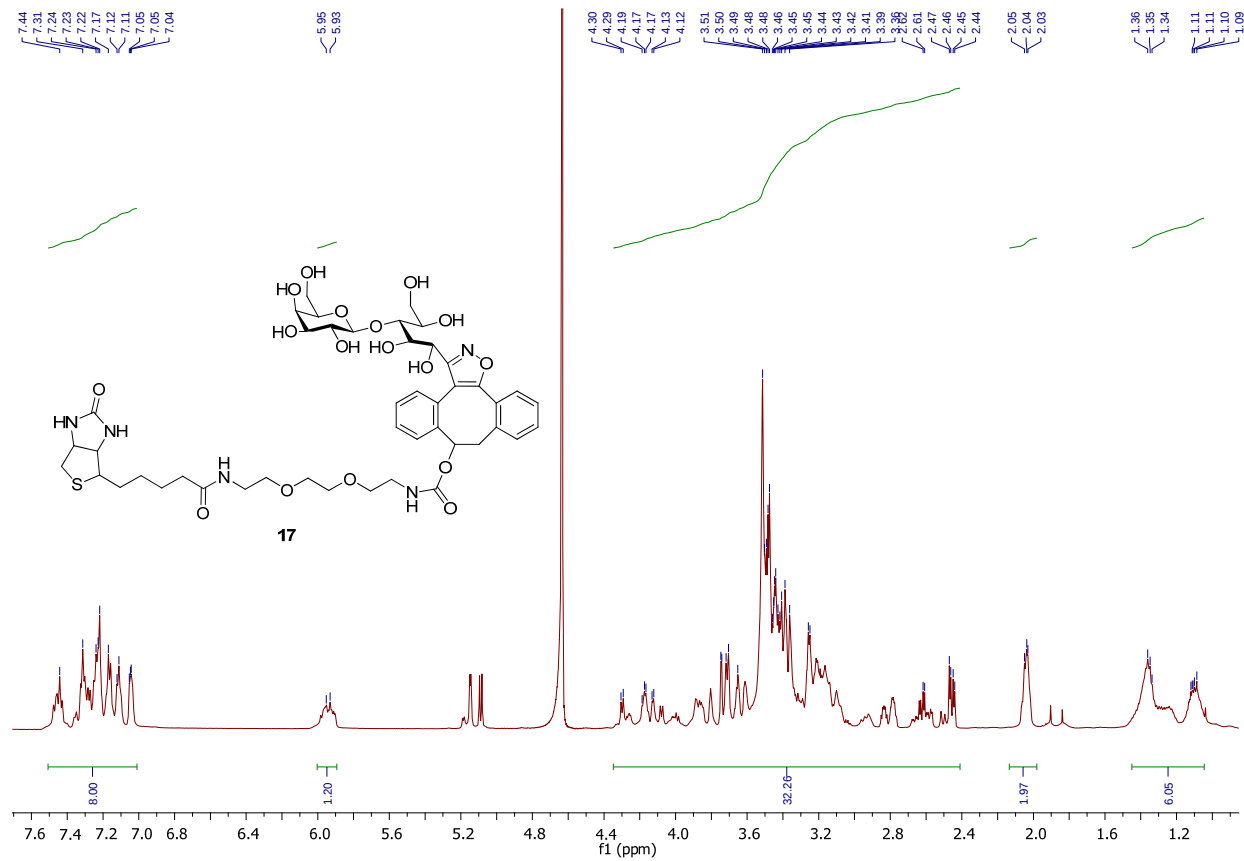
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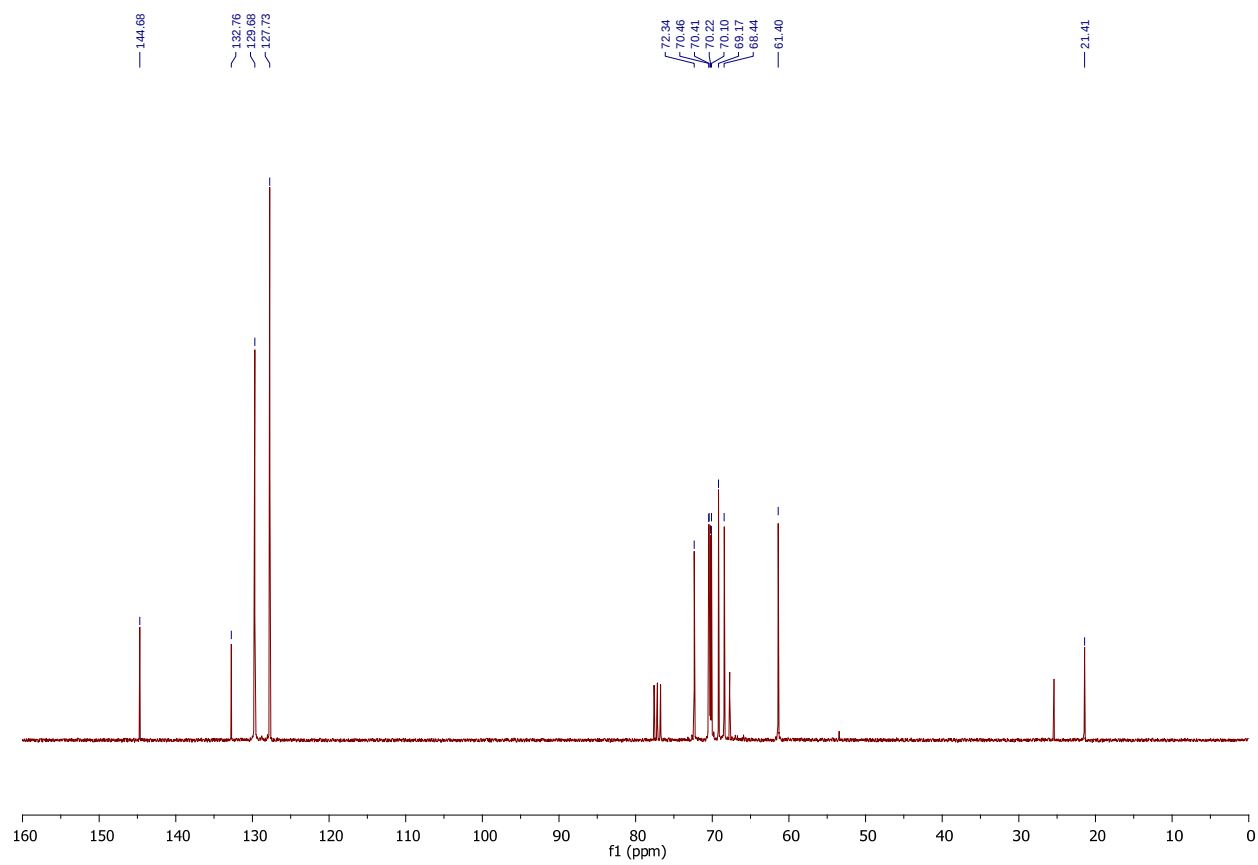
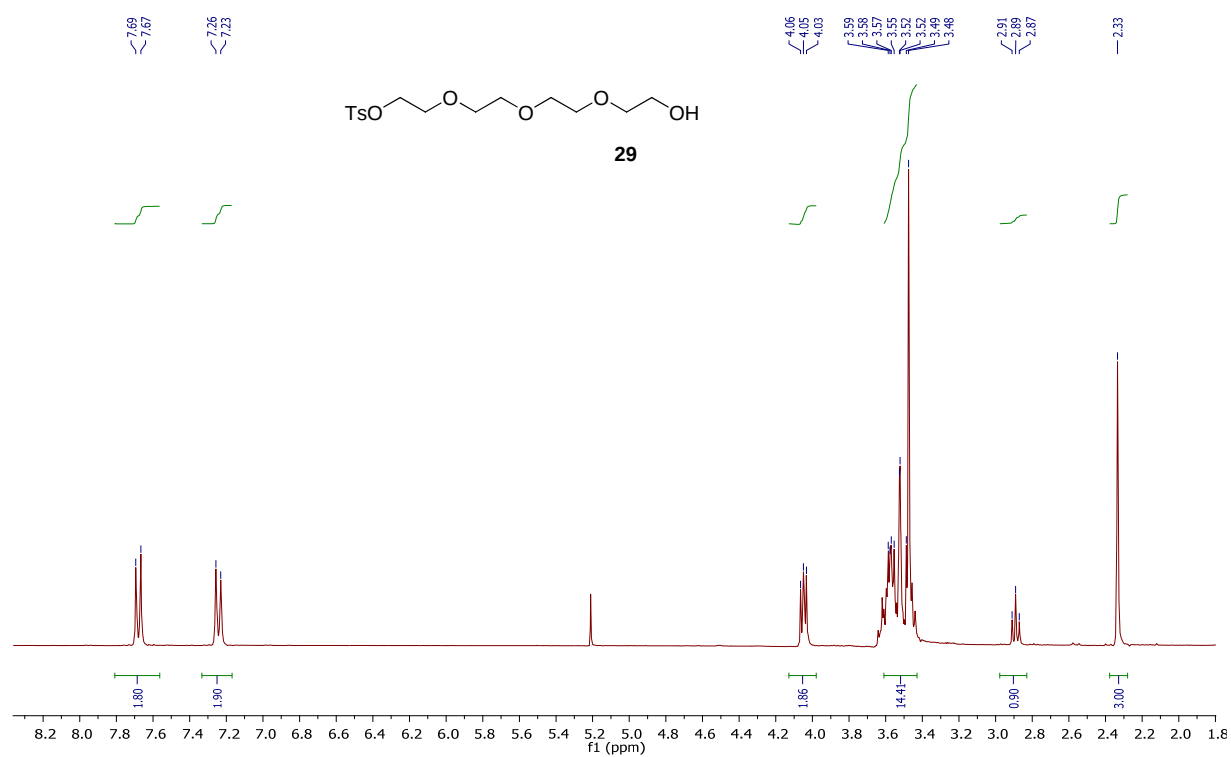


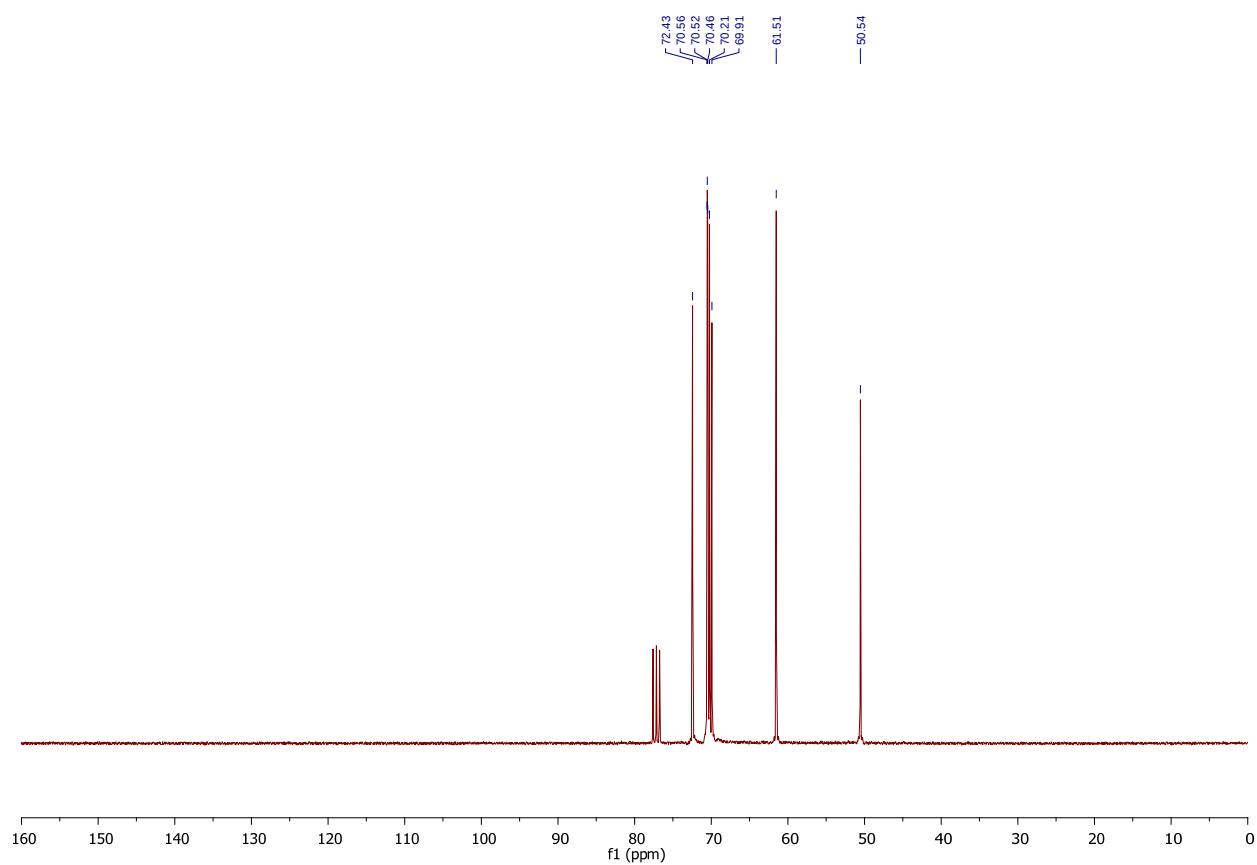
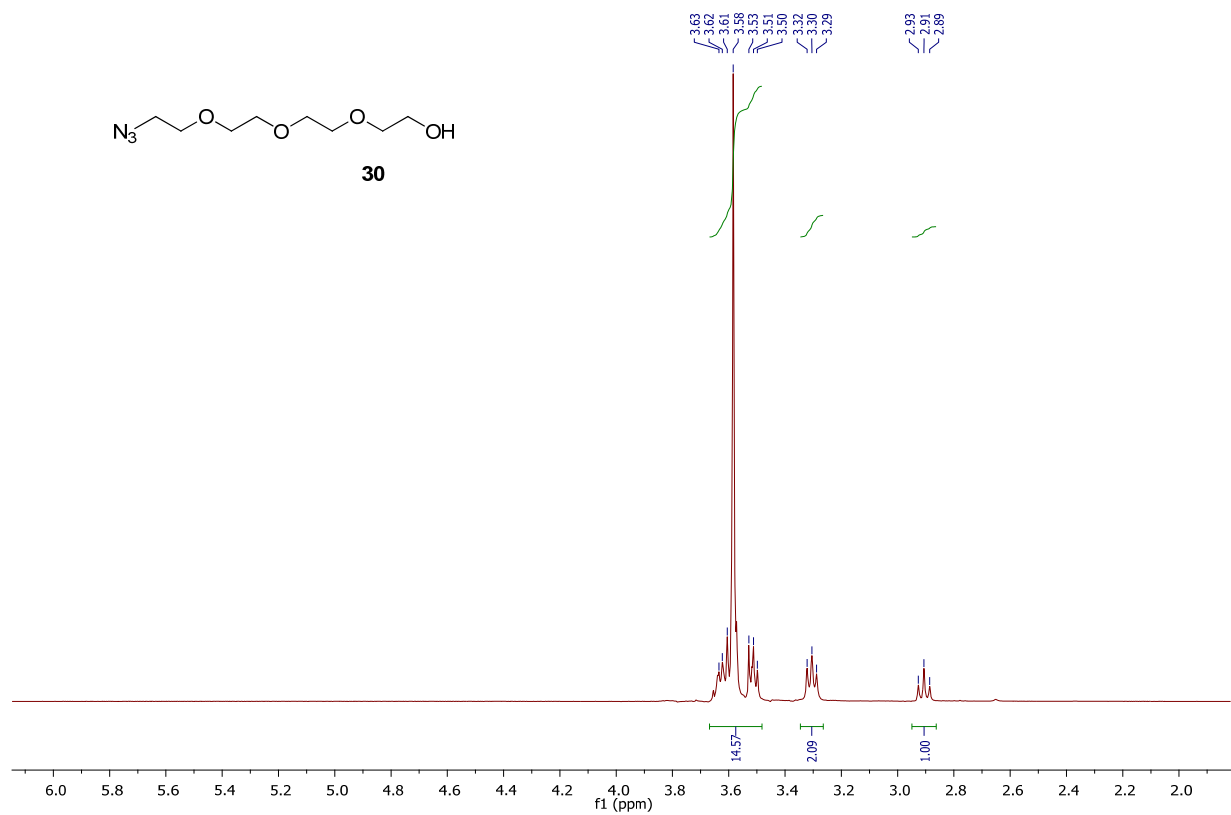
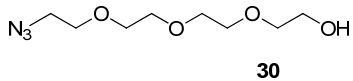


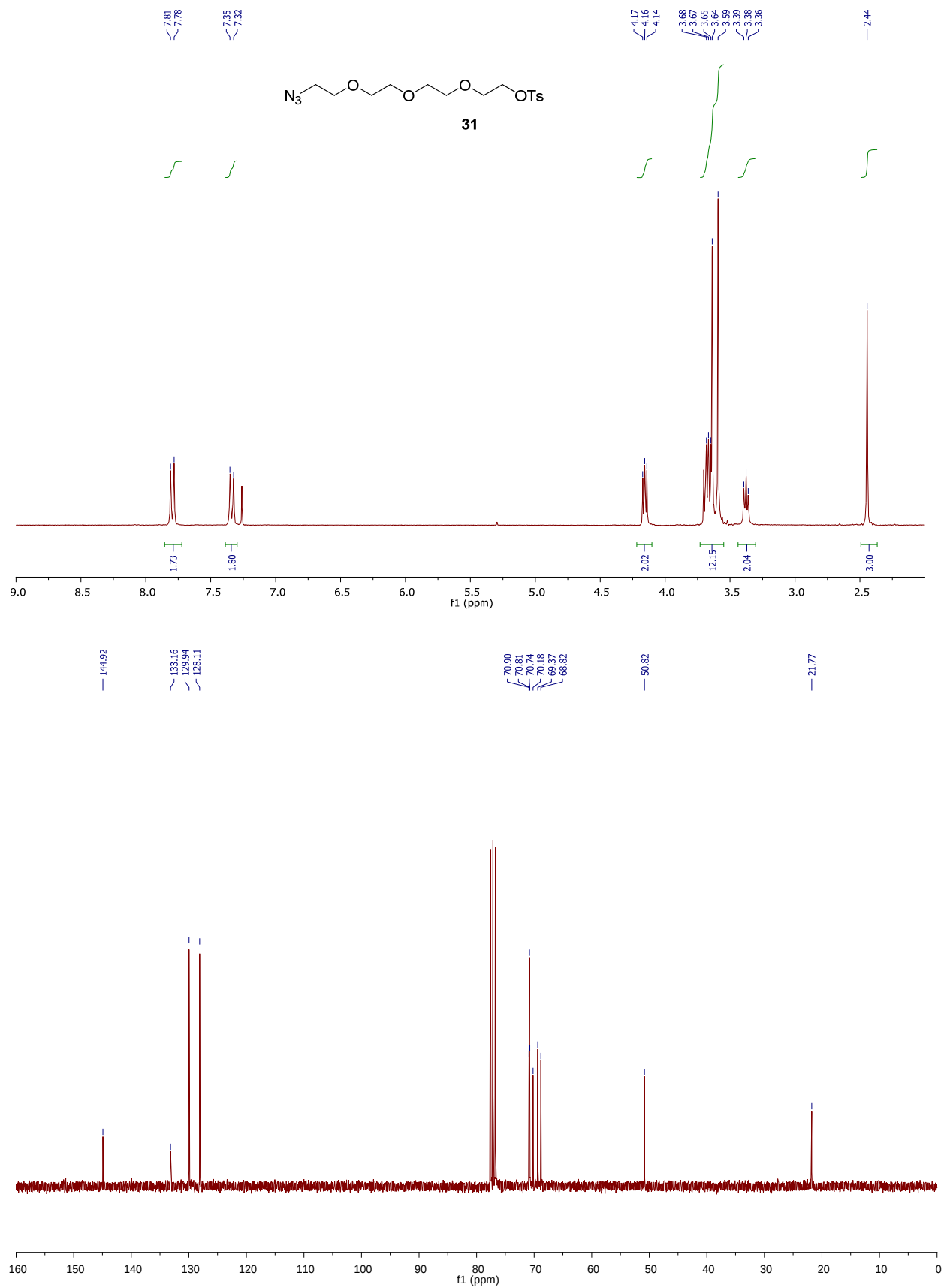


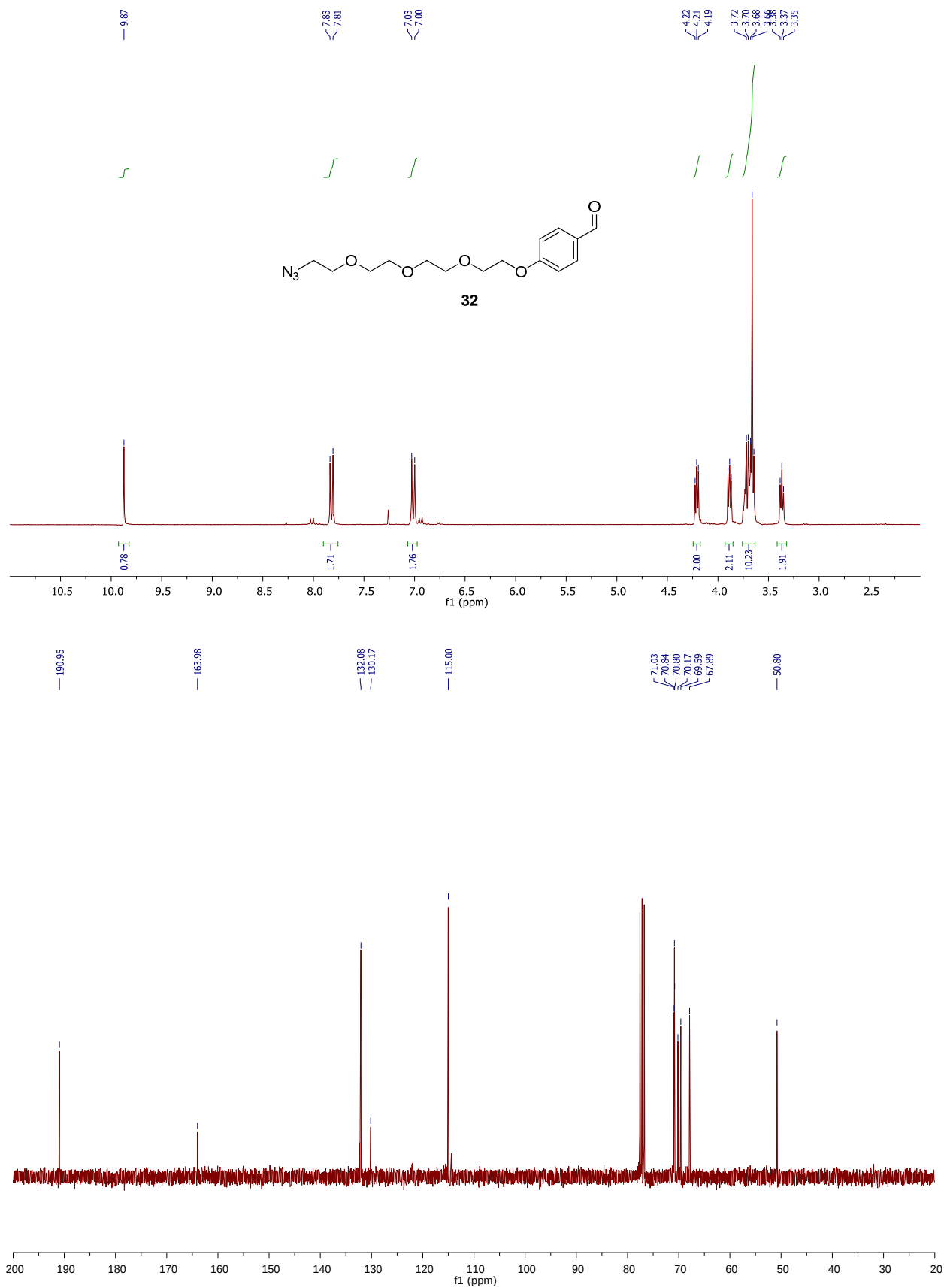


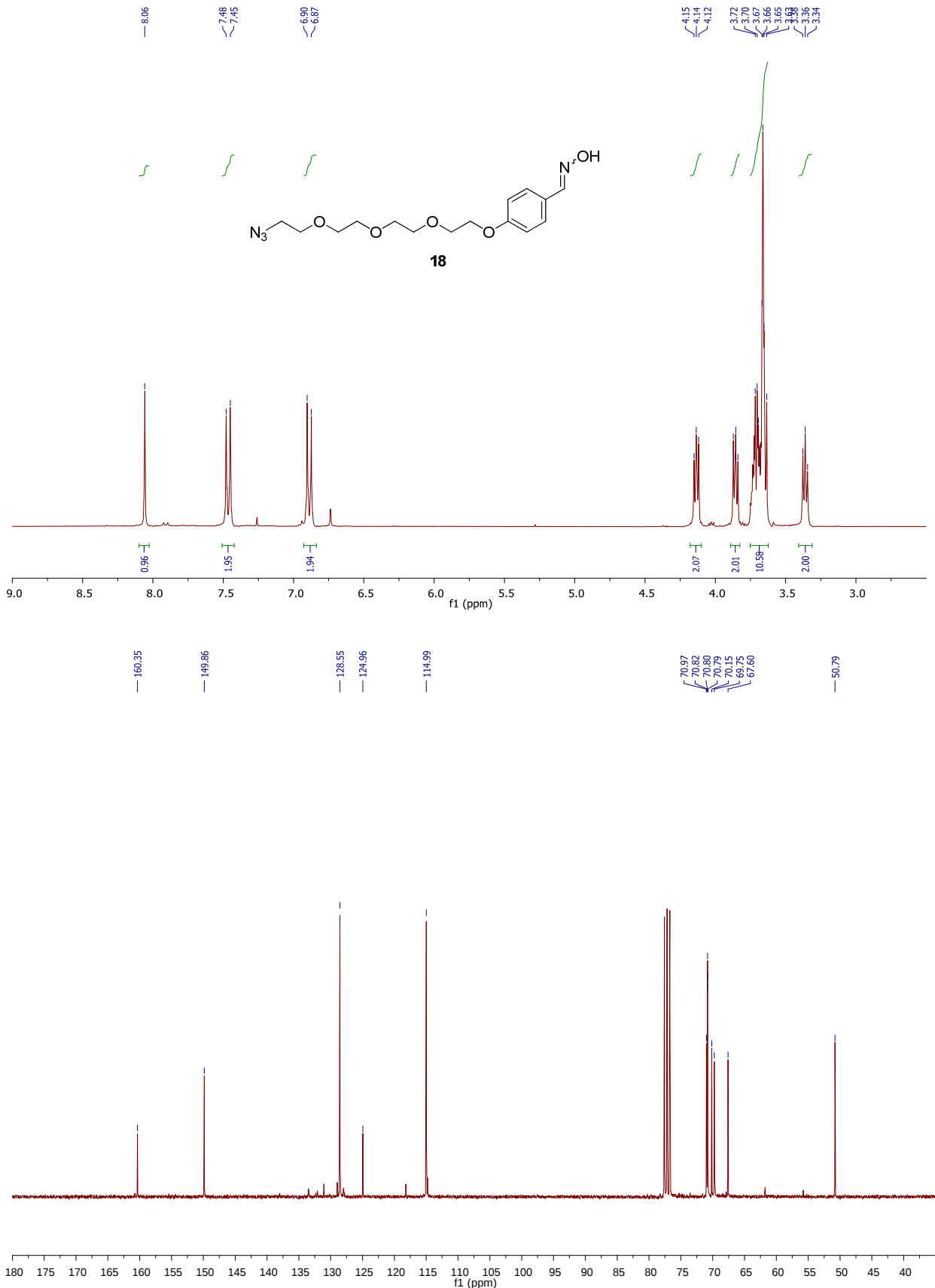


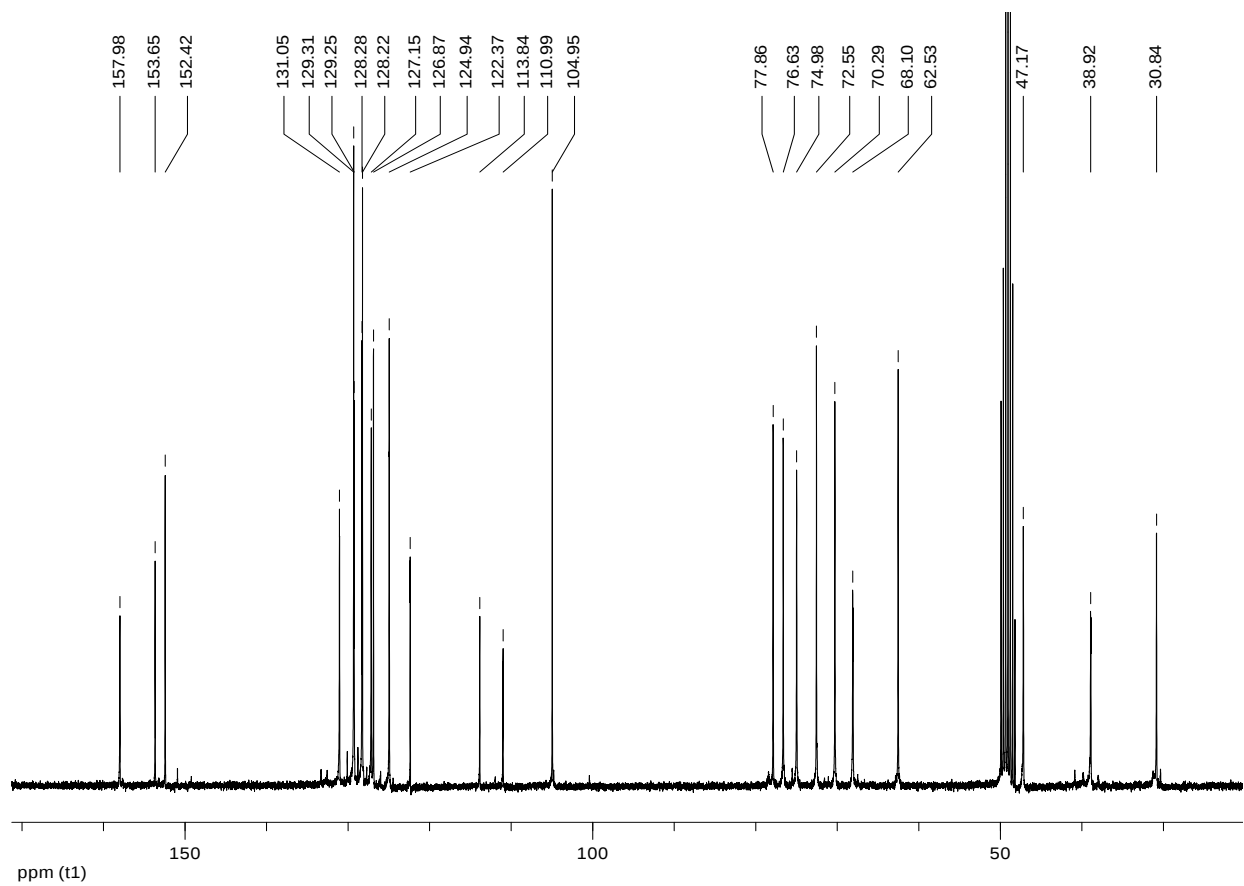
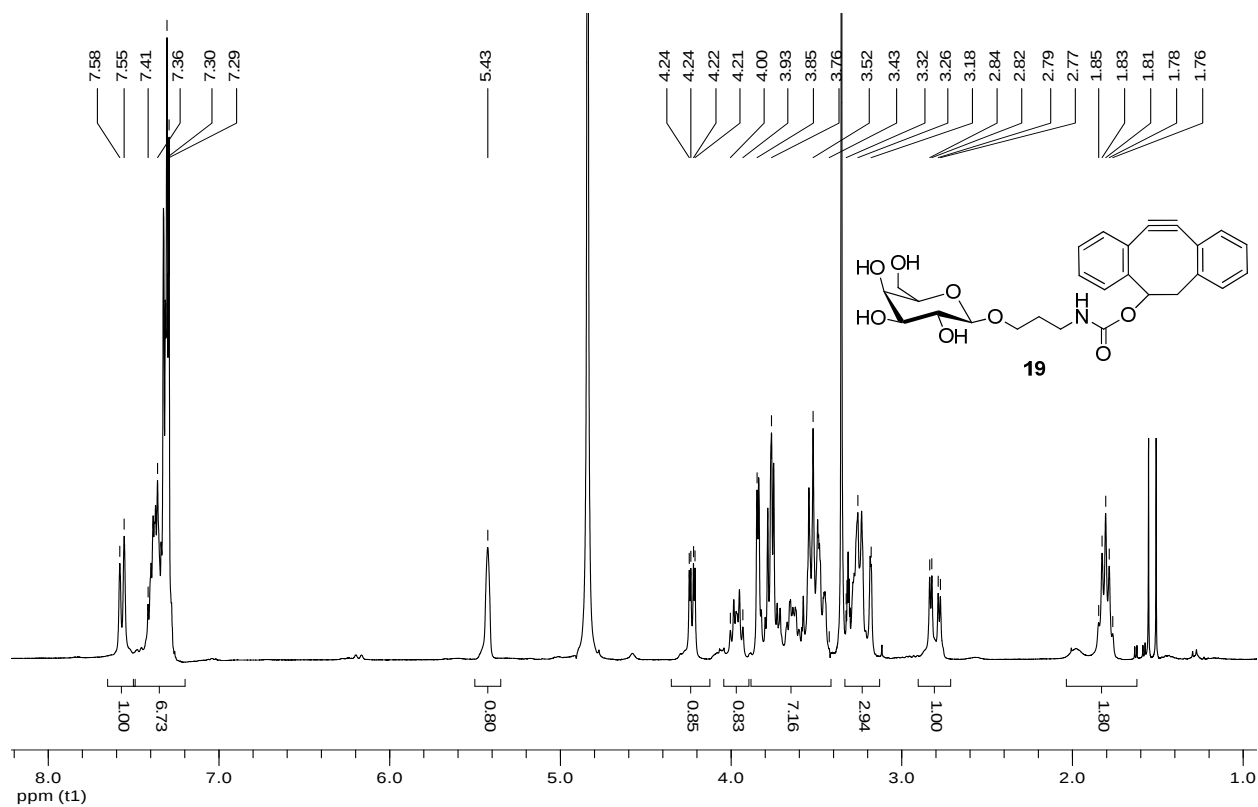


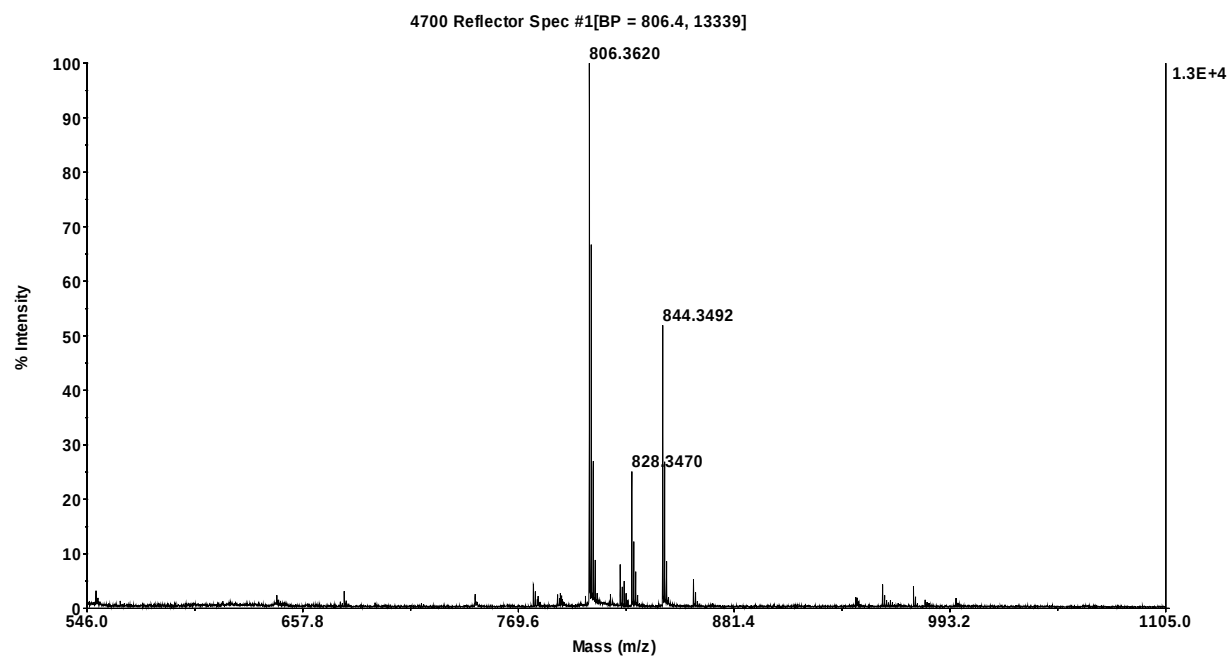
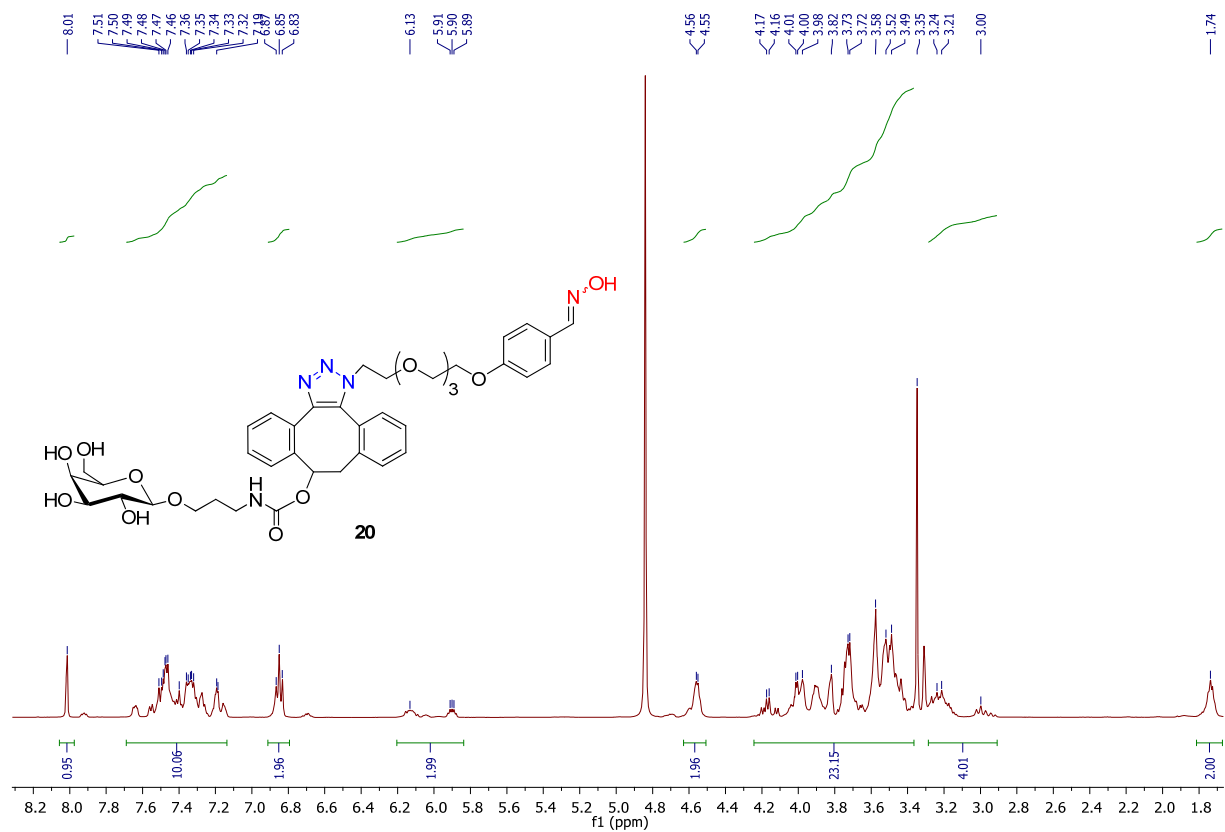


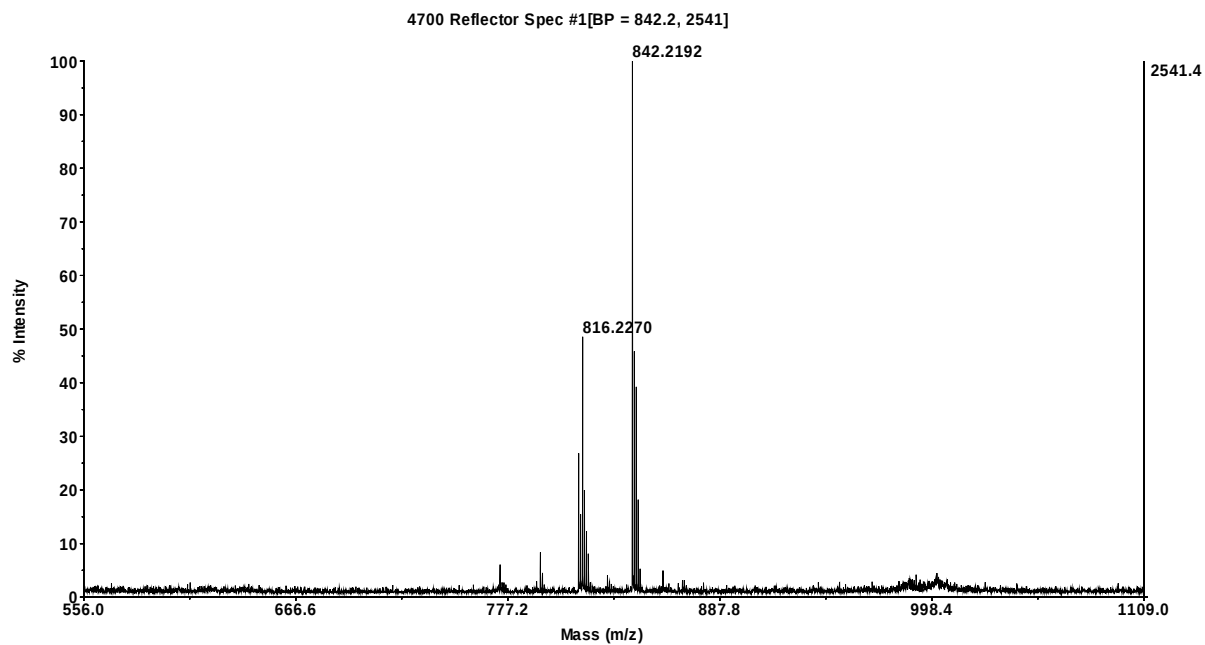
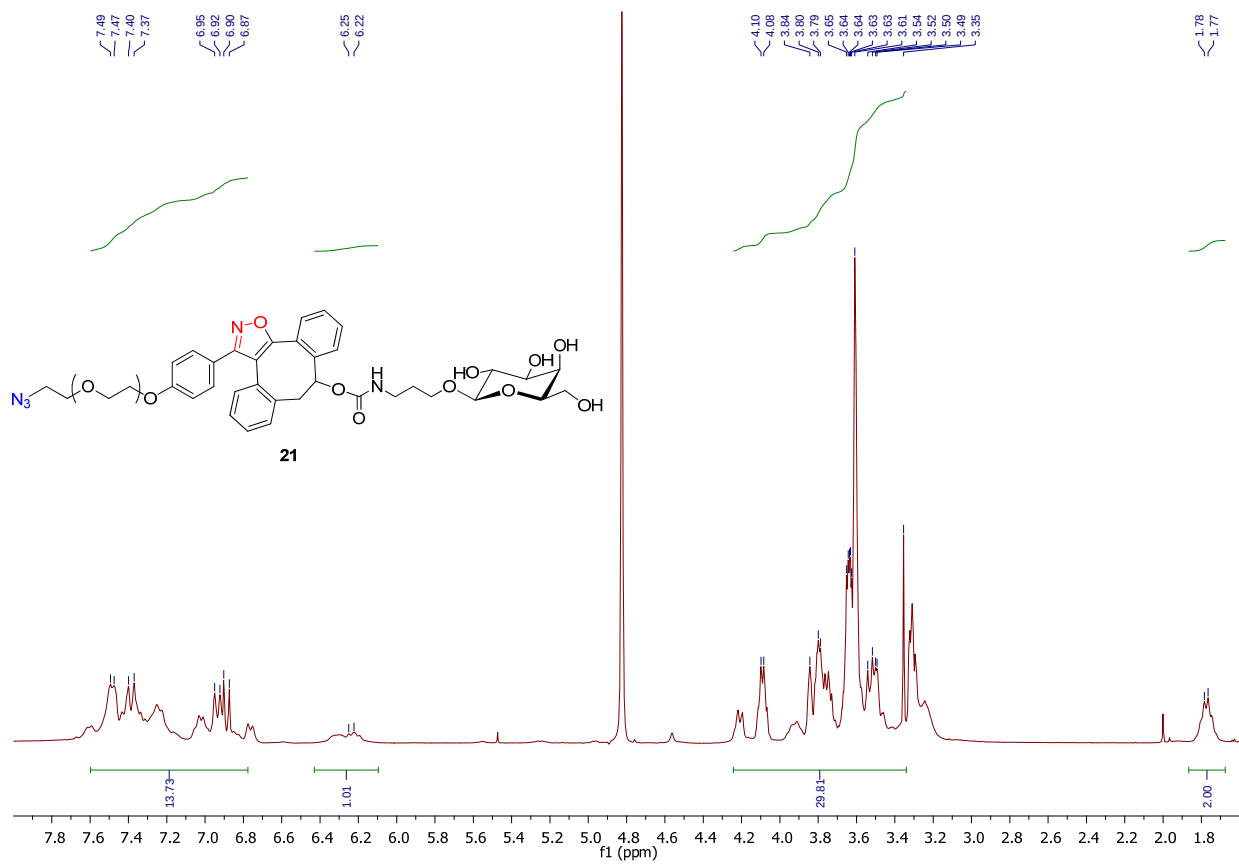


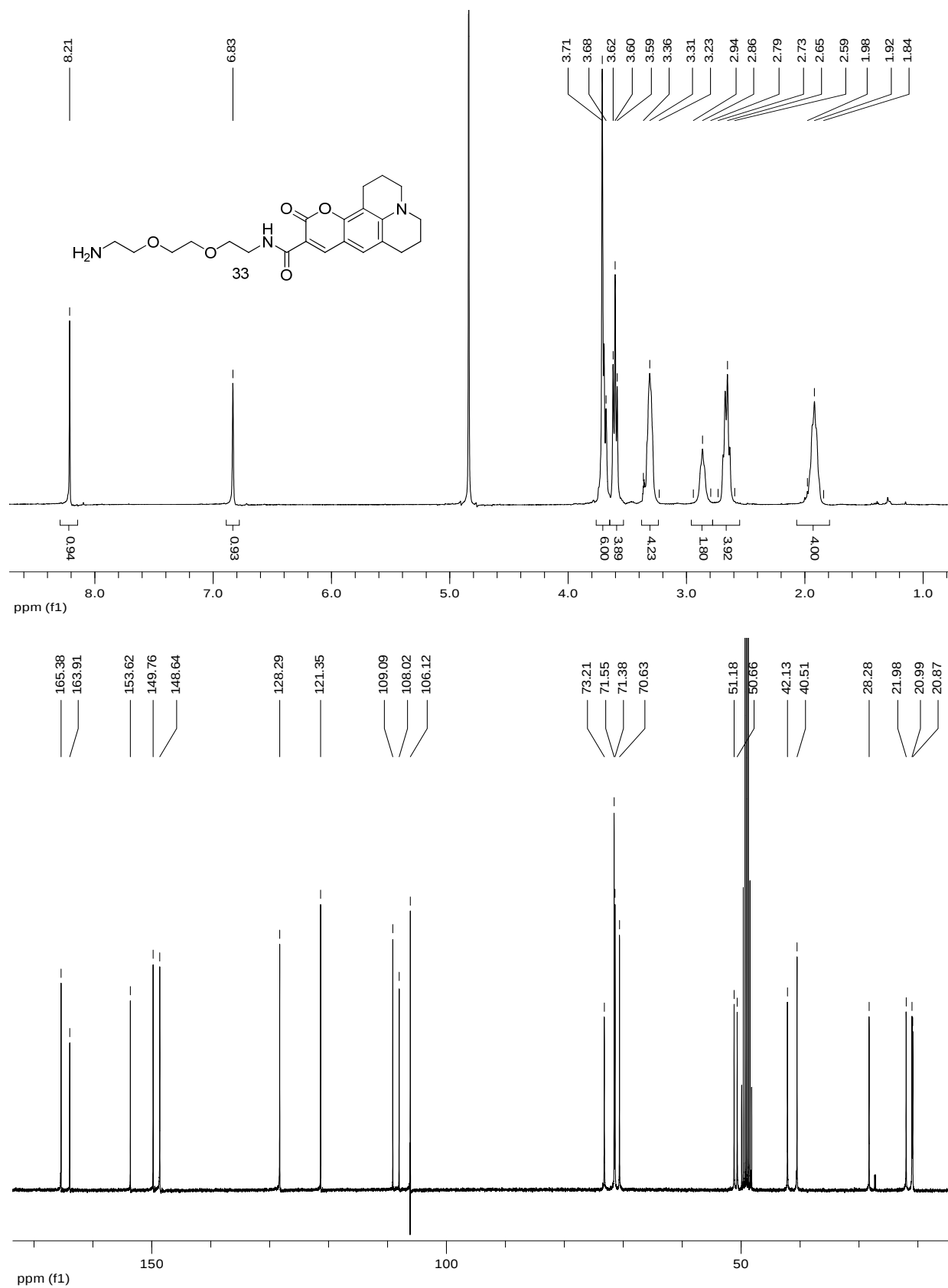


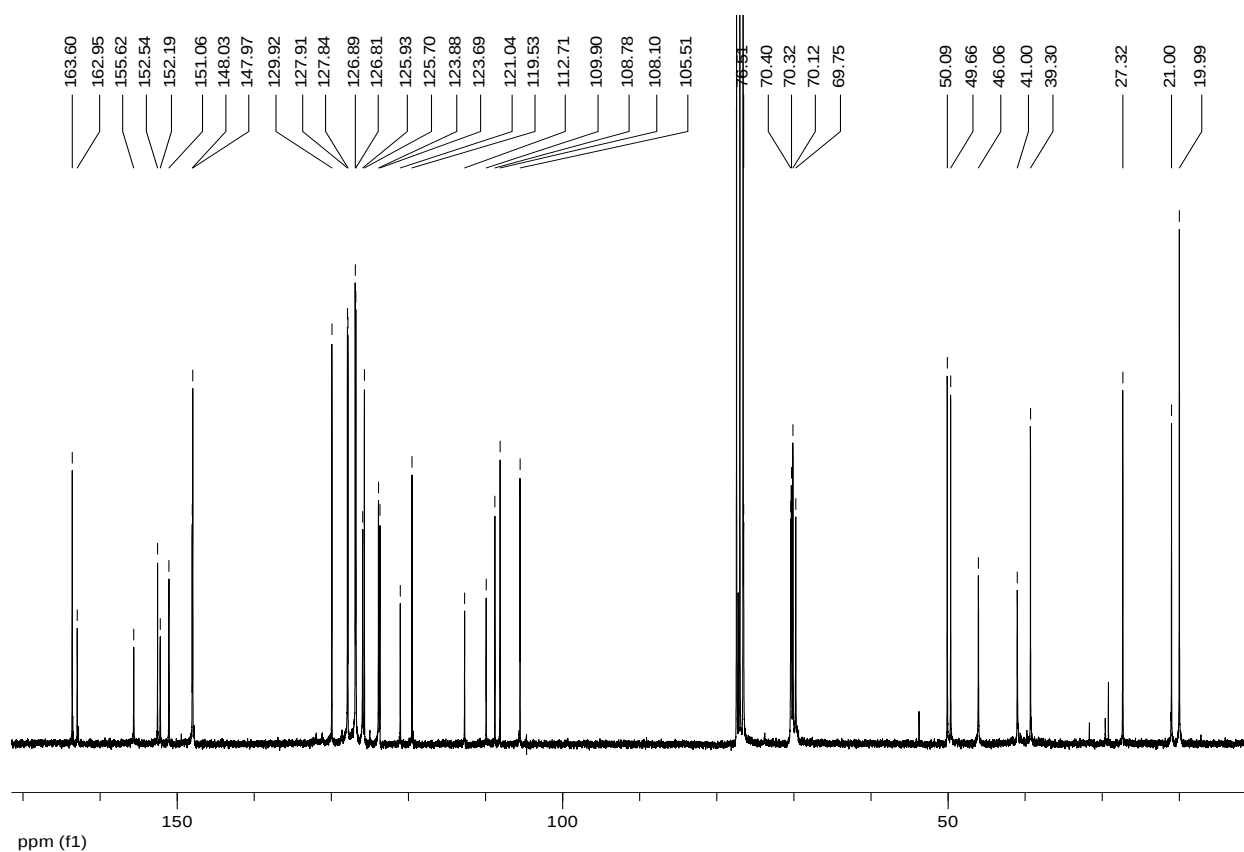
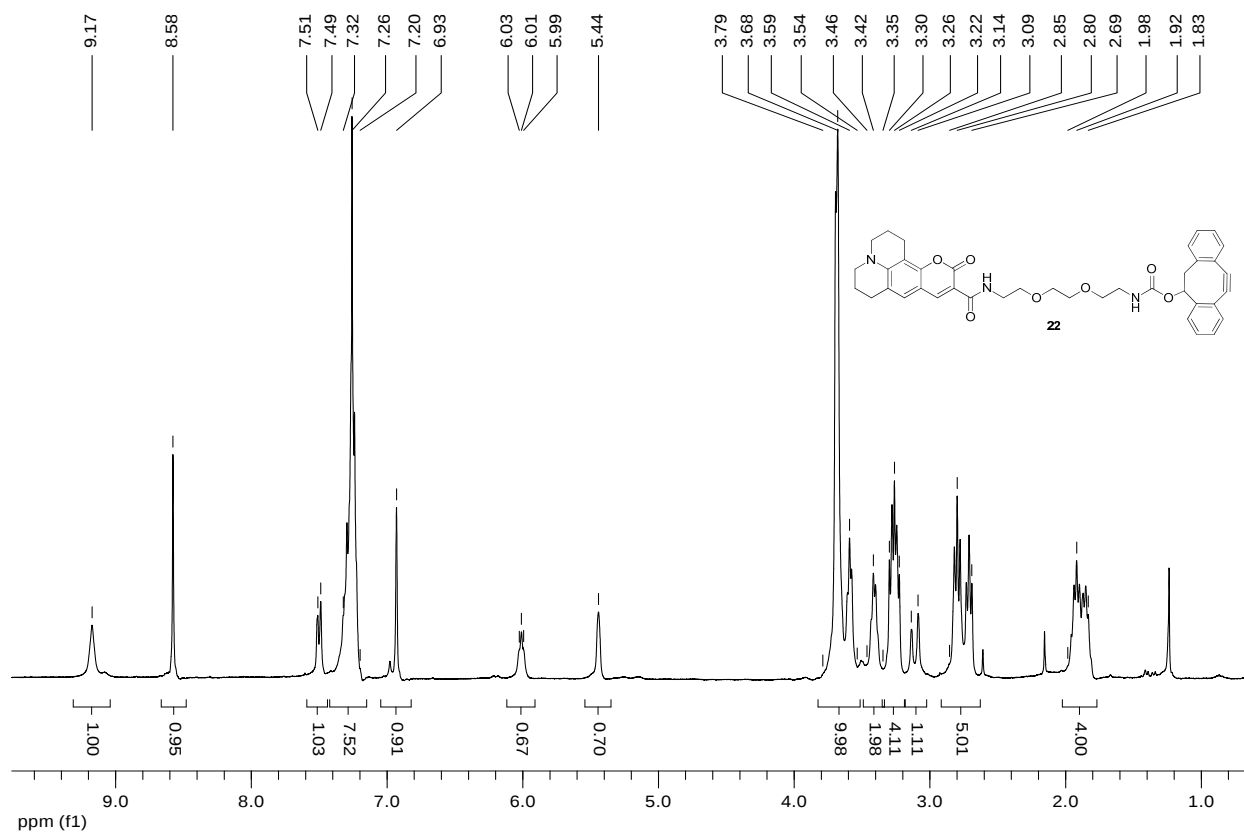


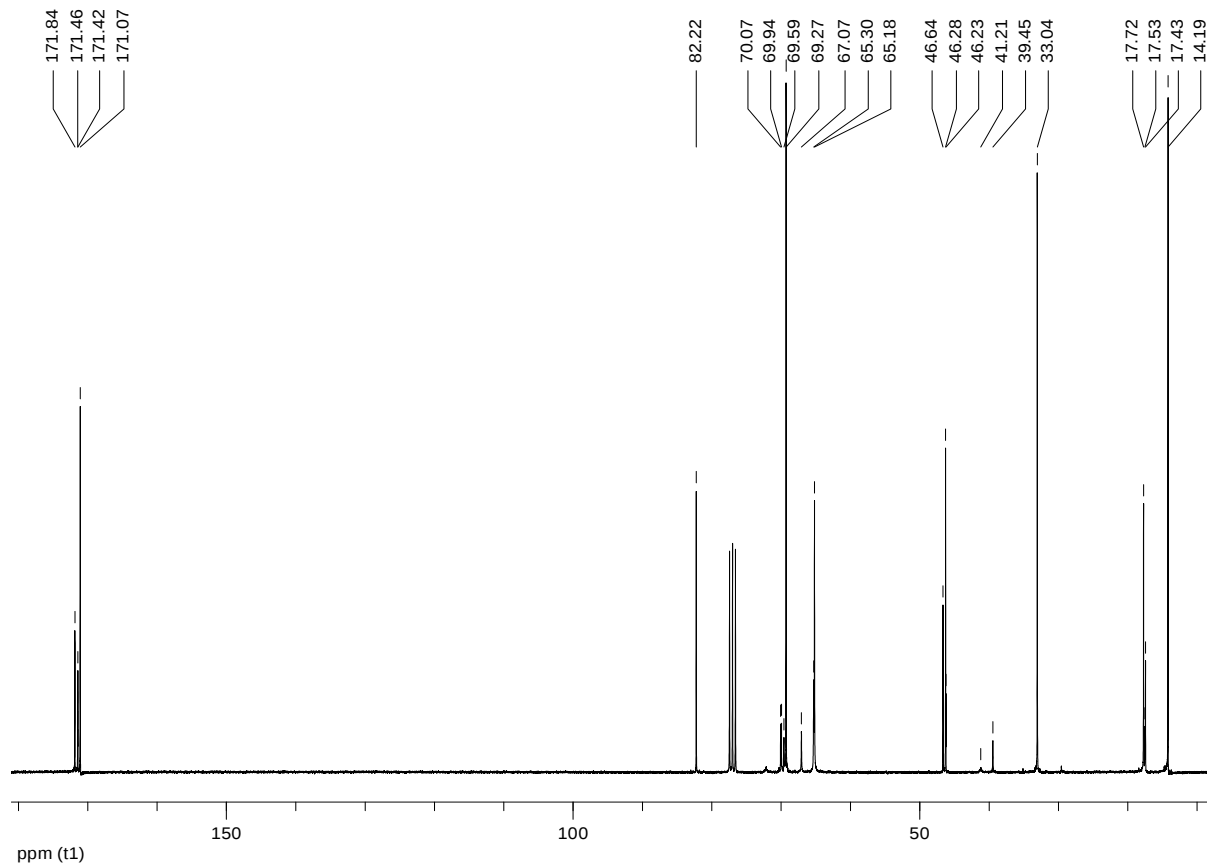
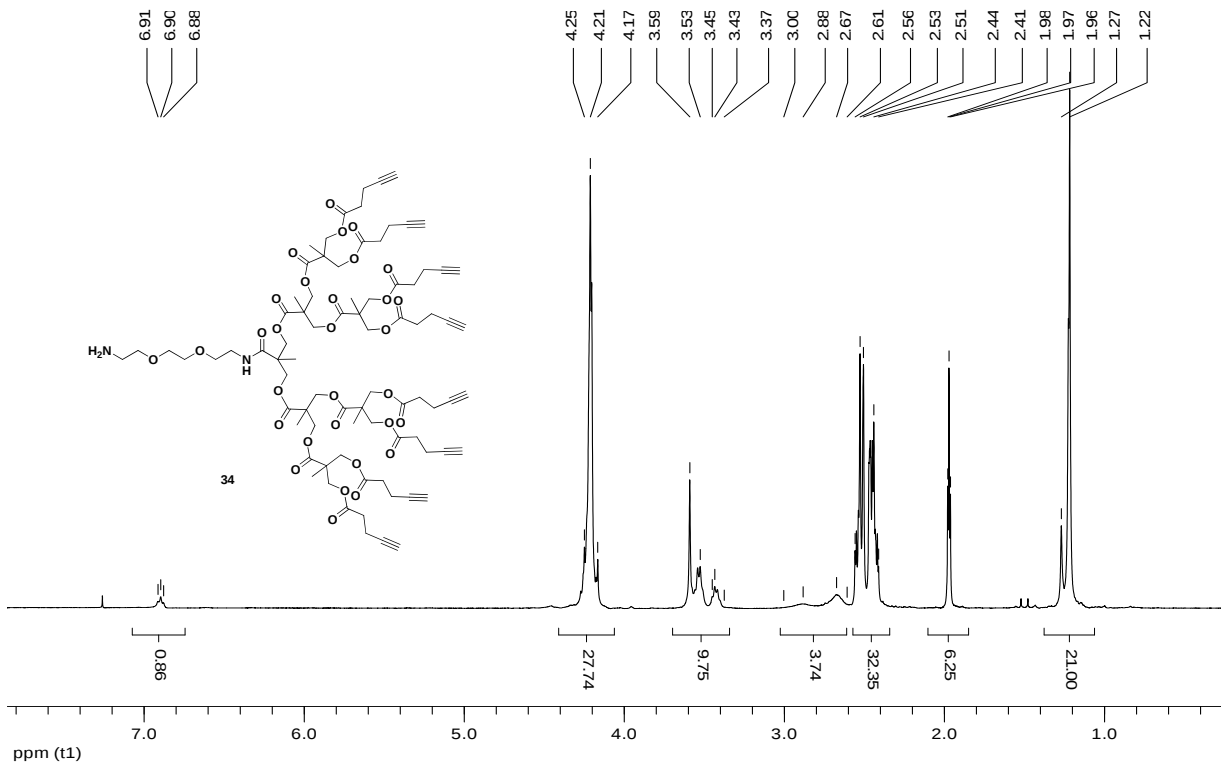


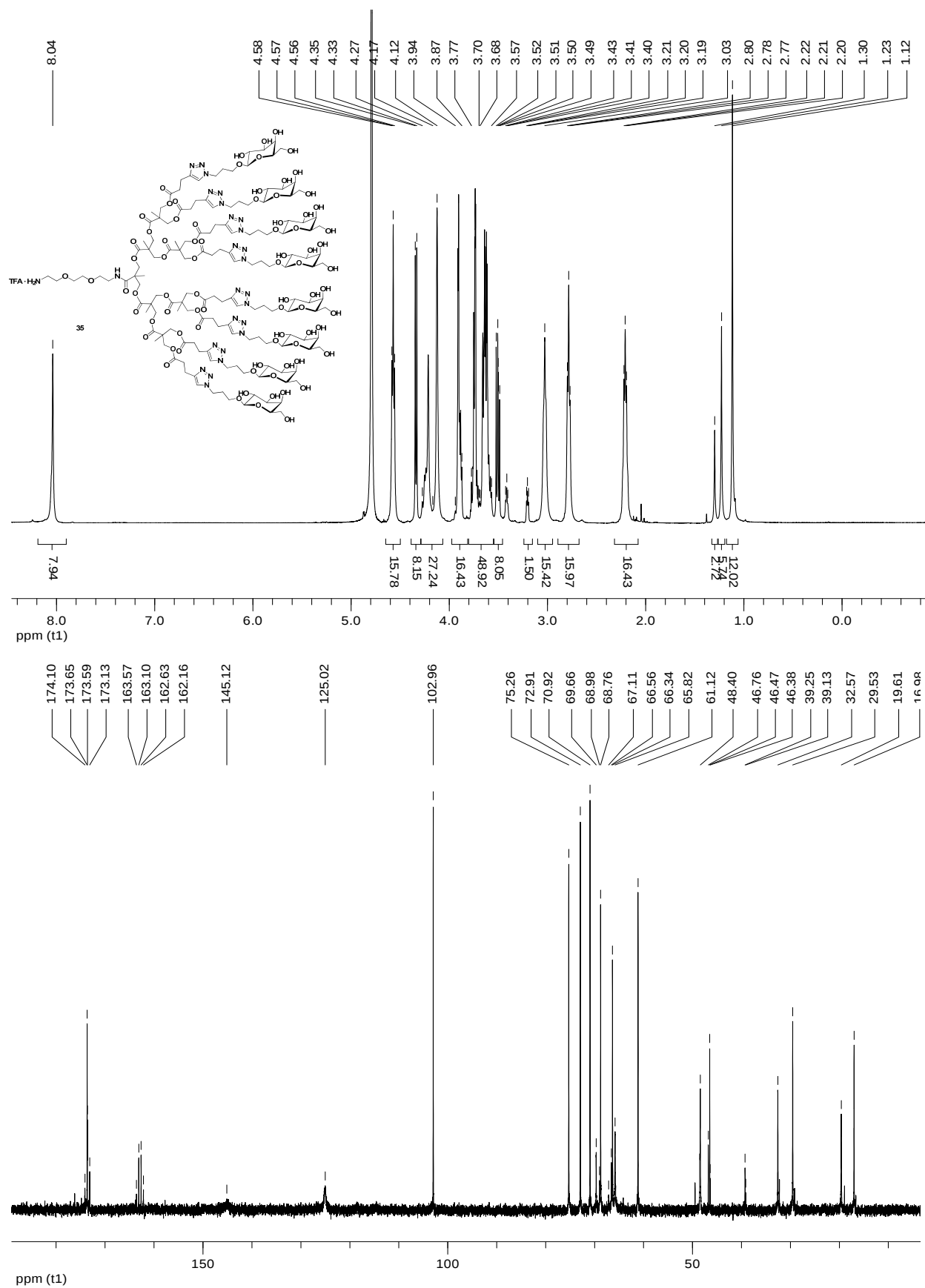


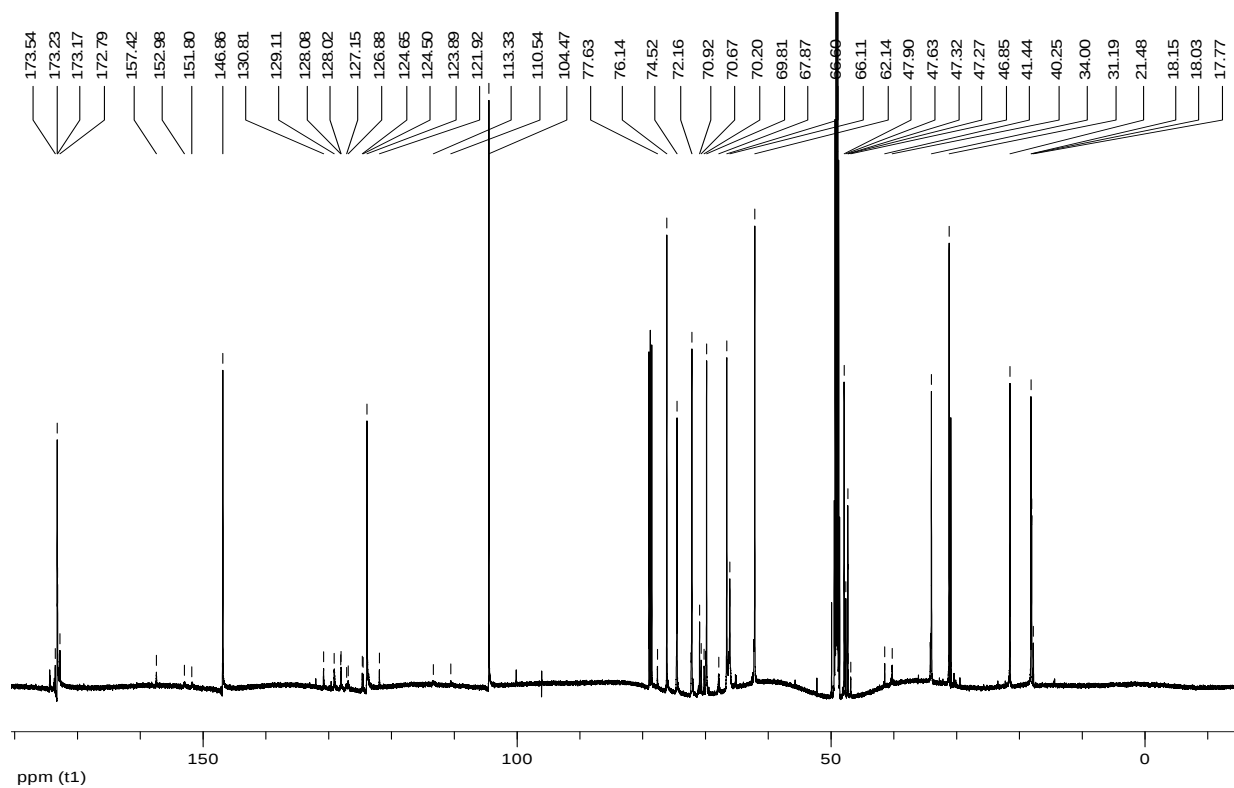
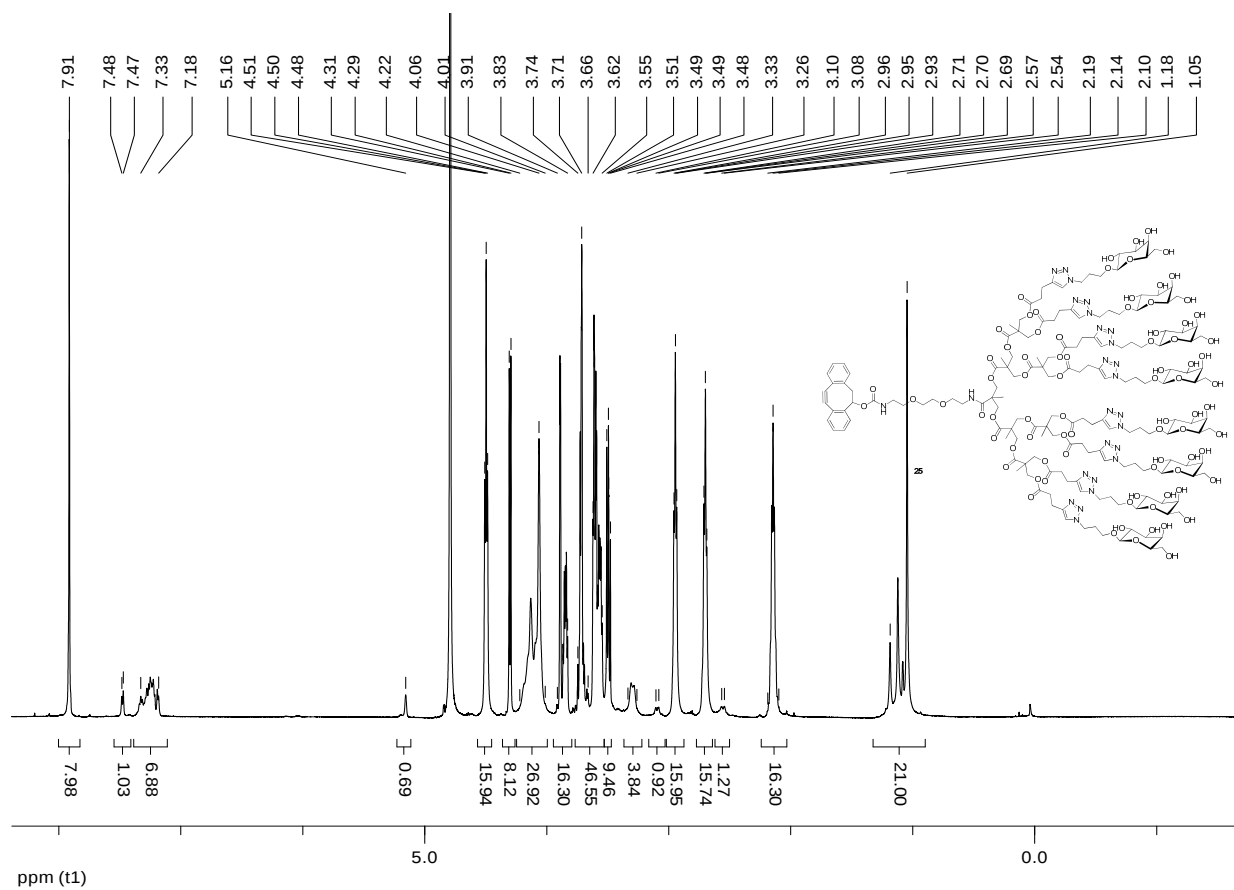


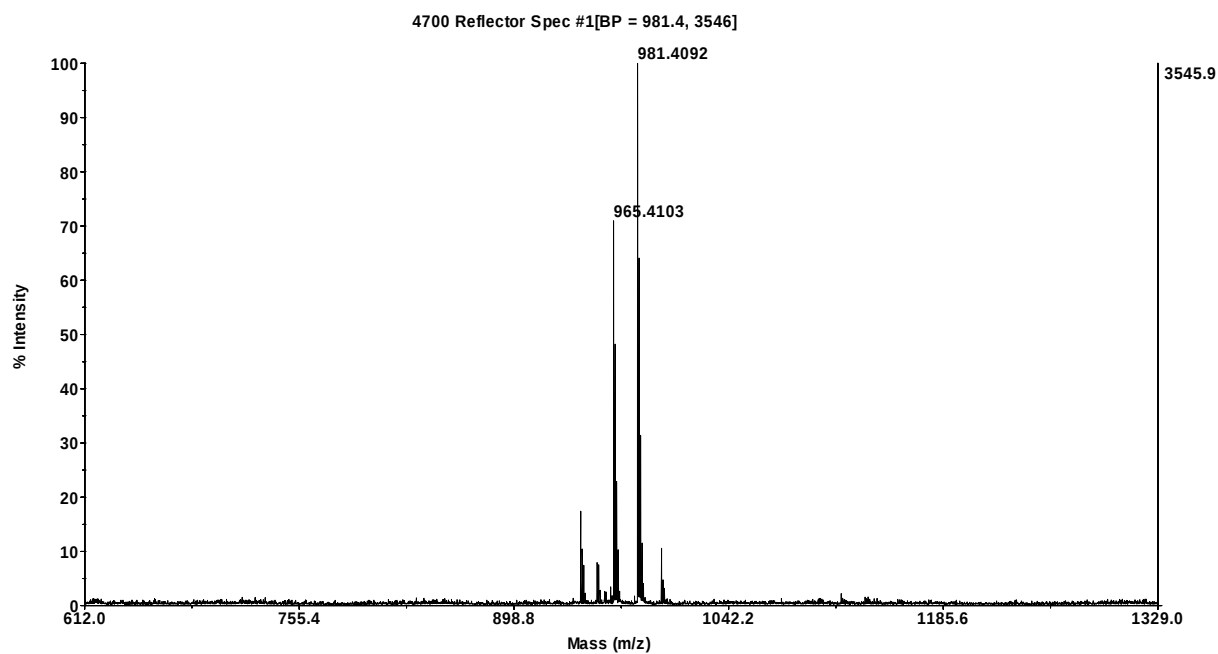


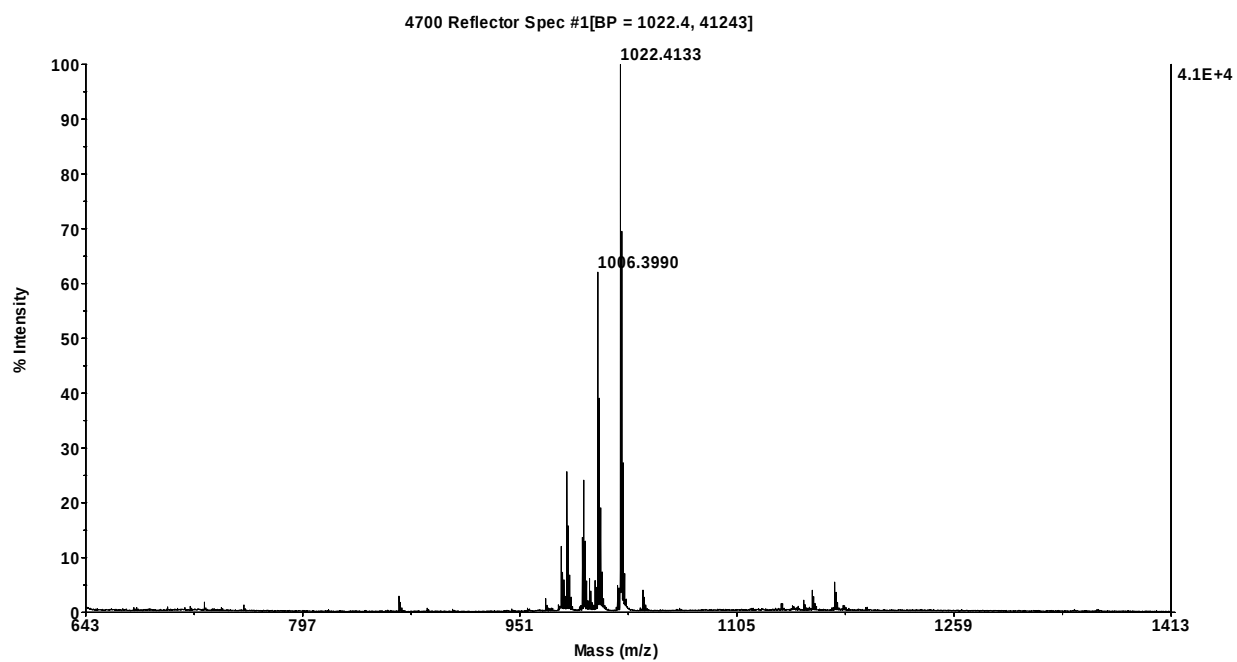
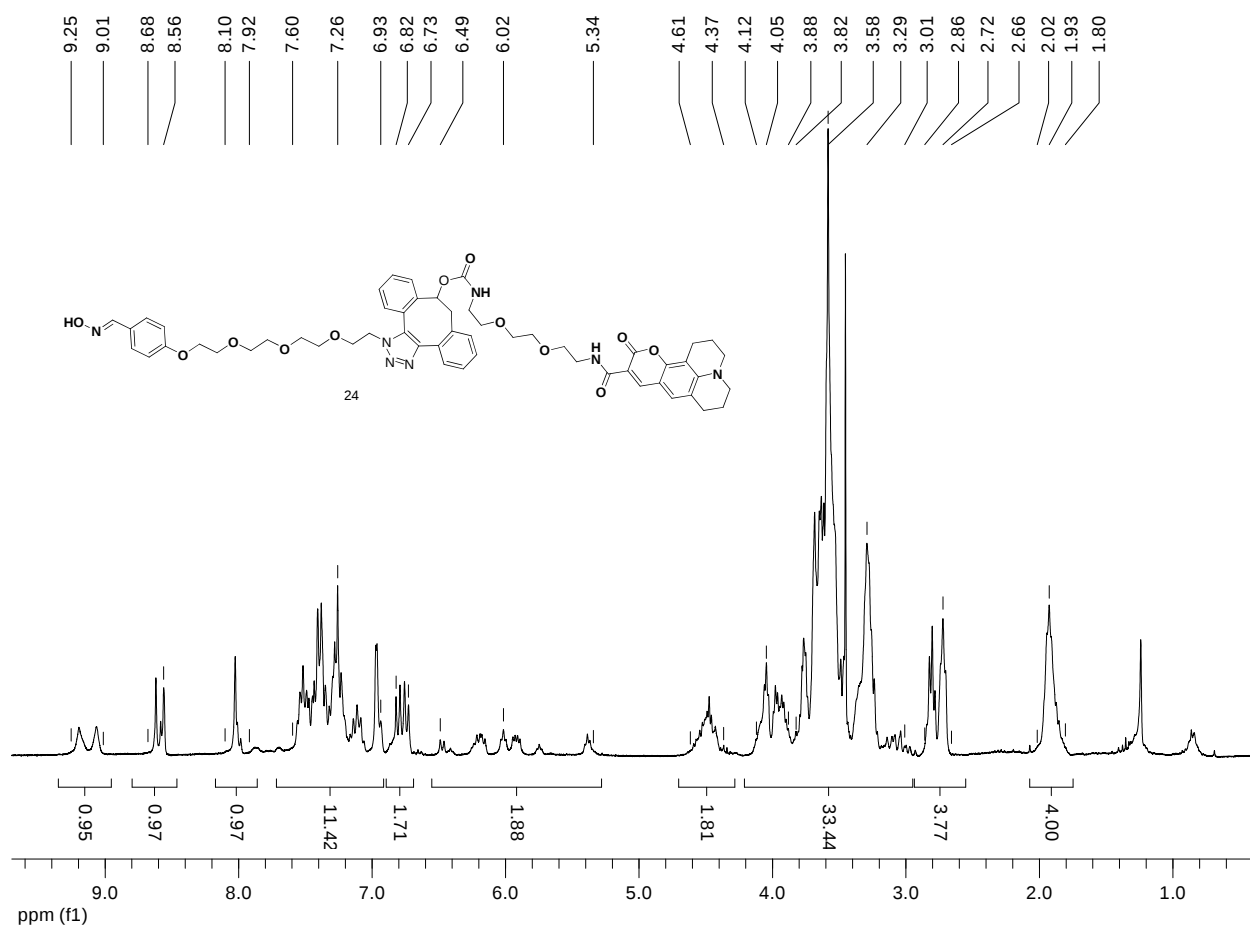


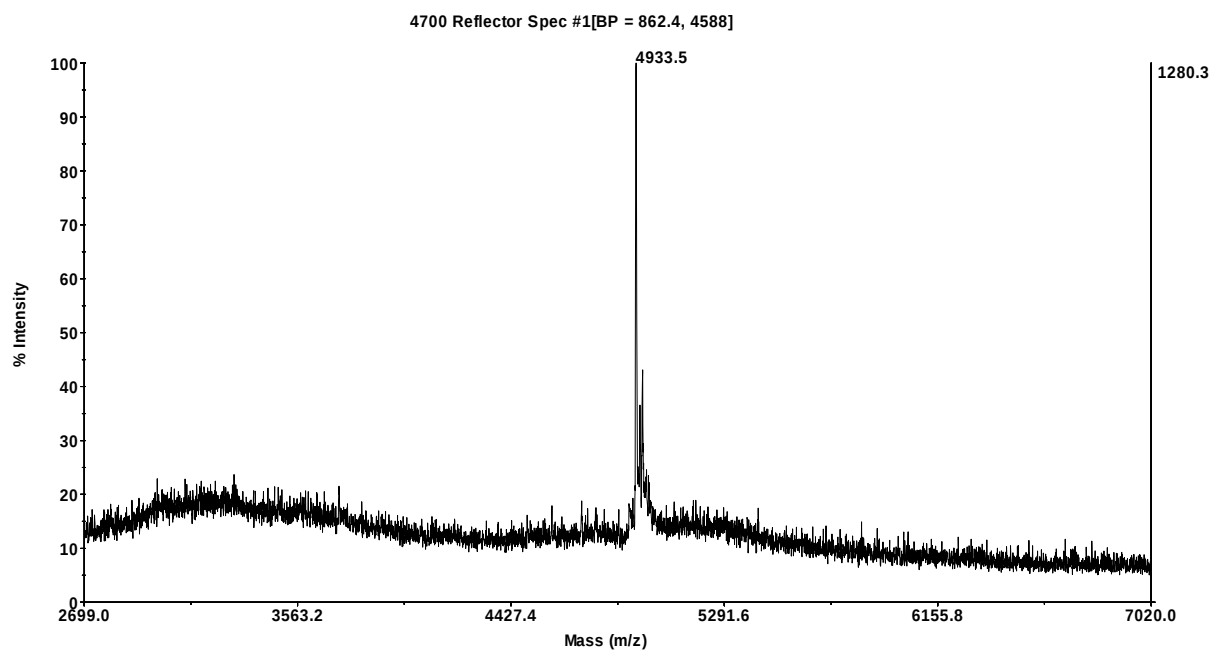
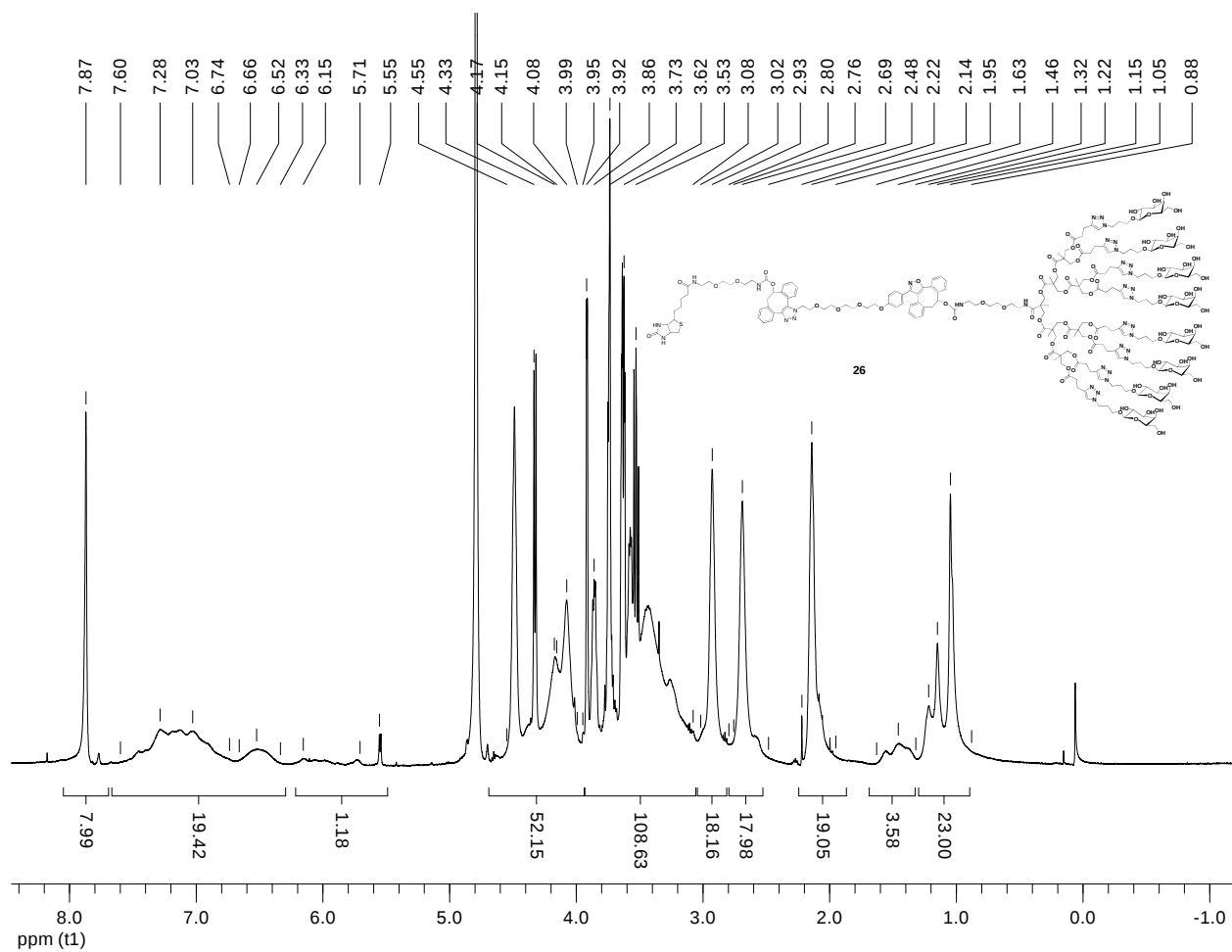


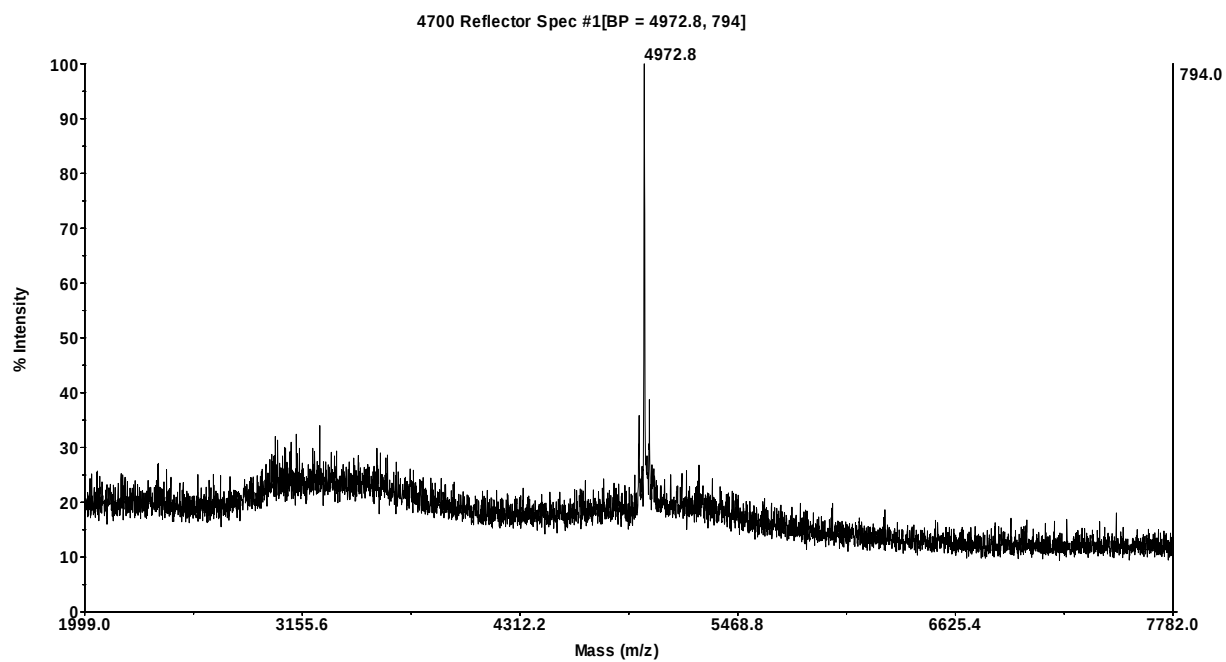












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