Imaging Drug Uptake by Bioorthogonal Stimulated Raman Scattering Microscopy

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Supplementary Figures, Supplementary Materials and Synthetic Procedures

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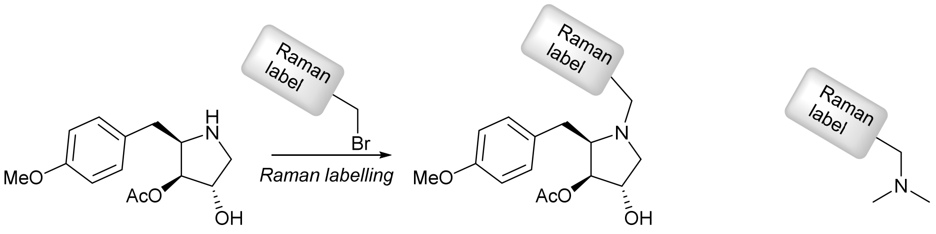
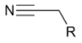
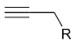
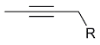
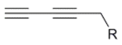
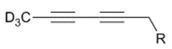
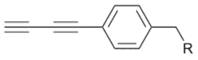
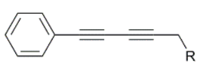
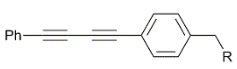


ANS **2b-i** **3b-i**

Figure S1 *N*-pyrrolidine labelled anisomycin derivatives (**2b-i**) used in the experimental determination of vibrational shifts and Raman scattering intensities. (**2h** = **PhDY-ANS**; **2i** = **BADY-ANS**) Dimethylamine counterparts (**3b-i**) used to as truncated models to enable rapid DFT calculations of vibrational shifts and Raman scattering activities (I_{Ram}).

Table S1 Anisomycin derivatives and their dimethylamine counterparts used in DFT studies.

Raman Label	**R**	**Compound**	**Raman shift** (cm^{-1}) [a]
	*N*-labelled anisomycin	**2b**	2234
	Me$_2$N	**3b**	-
	*N*-labelled anisomycin	**2c**	2136
	Me$_2$N	**3c**	-
	*N*-labelled anisomycin	**2d**	2249
	Me$_2$N	**3d**	-
	*N*-labelled anisomycin	**2e**	2239
	Me$_2$N	**3e**	-
	*N*-labelled anisomycin	**2f**	2260[b]
	Me$_2$N	**3f**	-
	*N*-labelled anisomycin	**2g**	N.D.[c]
	Me$_2$N	**3g**	-
	*N*-labelled anisomycin	**2h** **(PhDY-ANS)**	2236[d]
	Me$_2$N	**3h**	-
	*N*-labelled anisomycin	**2i** **(BADY-ANS)**	2219
	Me$_2$N	**3i**	-

[a] Values presented for the solid material in the bioorthogonal region 1800 – 2800 cm^{-1}. [b] The bioorthogonal Raman shift due to the CD$_3$ group is of much lower intensity and was not used in this study. [c] Compound **2g** was found to be unstable to laser irradiation. [d] Intracellular shift 2219 cm^{-1} determined by SRS imaging.

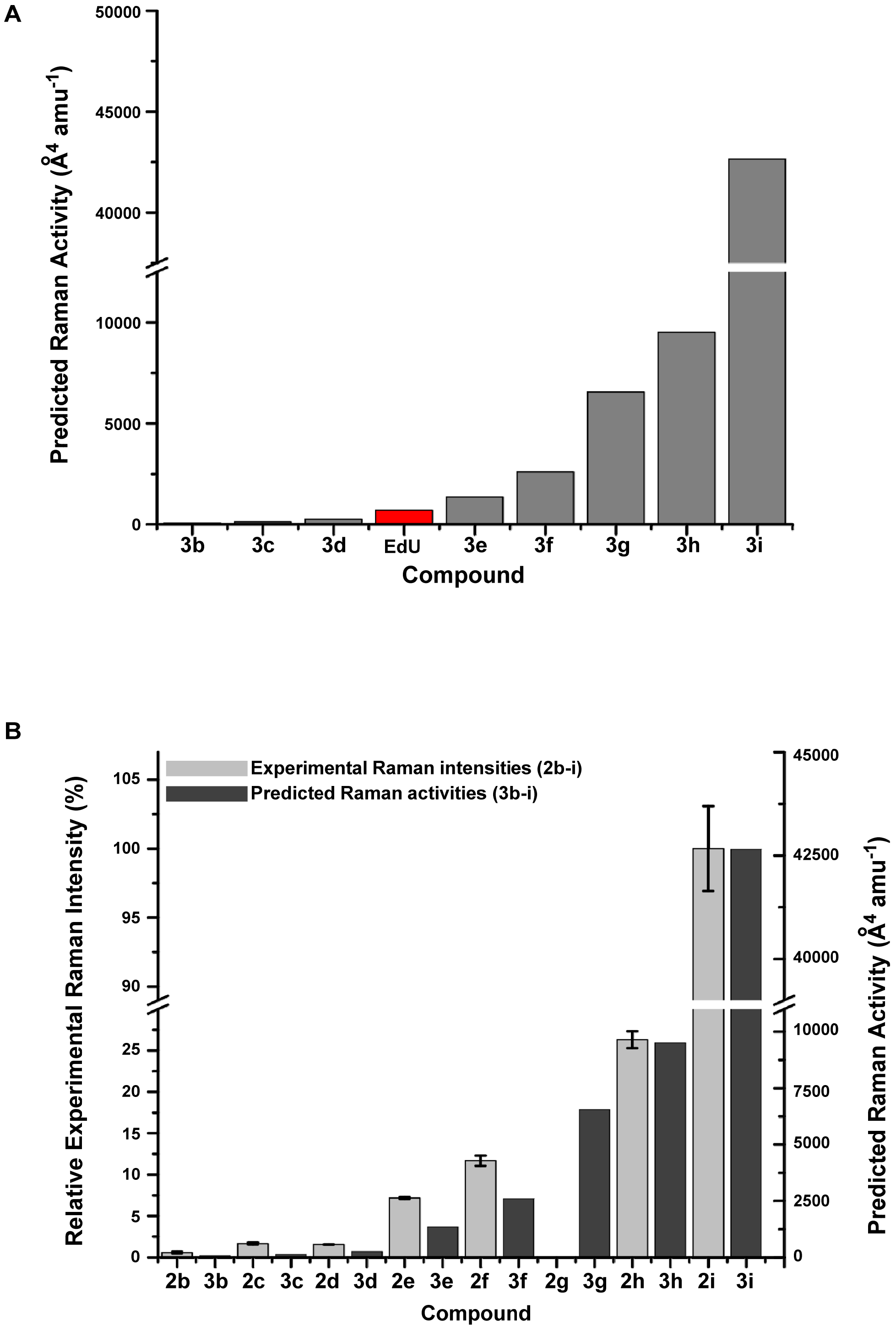


Figure S2 Raman activities anisomycin analogues and their dimethylamine counterparts. **A** Predicted Raman activity of dimethylamine adducts **3b-i**, and EdU **1**. DFT predicted Raman scattering activity (I_{ram}) of the peak maximum between 2100 – 2250 cm^{-1} [gas phase intensities calculated at B3LYP/6-31G(d,p)]. EdU is highlighted in red for clarity. **B** Comparison of the DFT-predicted Raman activities and experimental spontaneous Raman scattering intensities for peaks in the region 2100 – 2250 cm^{-1}. DFT gas phase activities (I_{Ram}) for dimethylamine adducts **3b-i** calculated at the B3LYP/6-31G(d,p) level. Relative spontaneous Raman scattering intensities for the solid anisomycin analogues **2b-i** normalised to the peak area$_{(819\ \text{cm-1})}$ for each analogue and expressed as a percentage of the BADY-ANS peak; mean of ten replicates with error bars ± S.D. (**2h** = **PhDY-ANS**; **2i** = **BADY-ANS**)

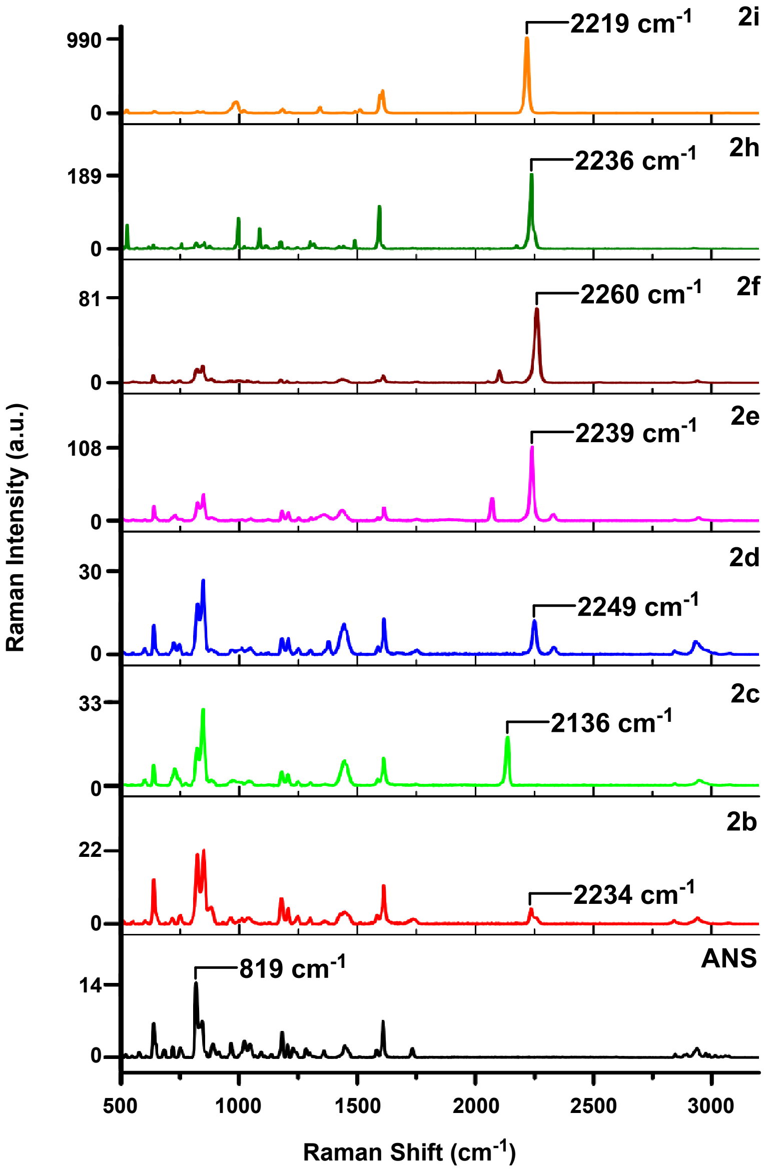


Figure S3 Spontaneous Raman spectroscopy of anisomycin **ANS** and analogues **2b-f**, **2h** and **2i** as solid material. Spectra normalised to the anisomycin peak at 819 cm^{-1} present in each sample, and offset for clarity. (**2h** = **PhDY-ANS**; **2i** = **BADY-ANS**)

Note: compound **2g** is unstable upon laser irradiation, and therefore no Raman spectrum is presented.

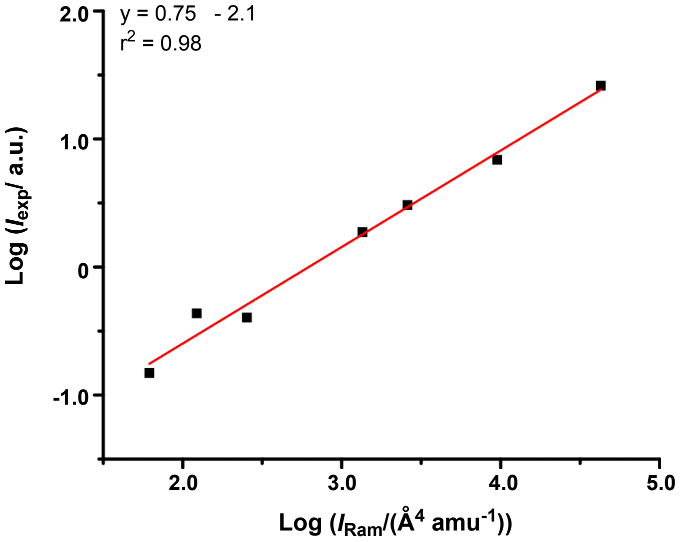


Figure S4 Correlation of the predicted Raman scattering activities calculated by Gaussian (I_{ram}), with experimental Raman intensities measured by spontaneous Raman scattering (I_{exp}). Due to the large dynamic ranges, these are plotted as their logarithms.

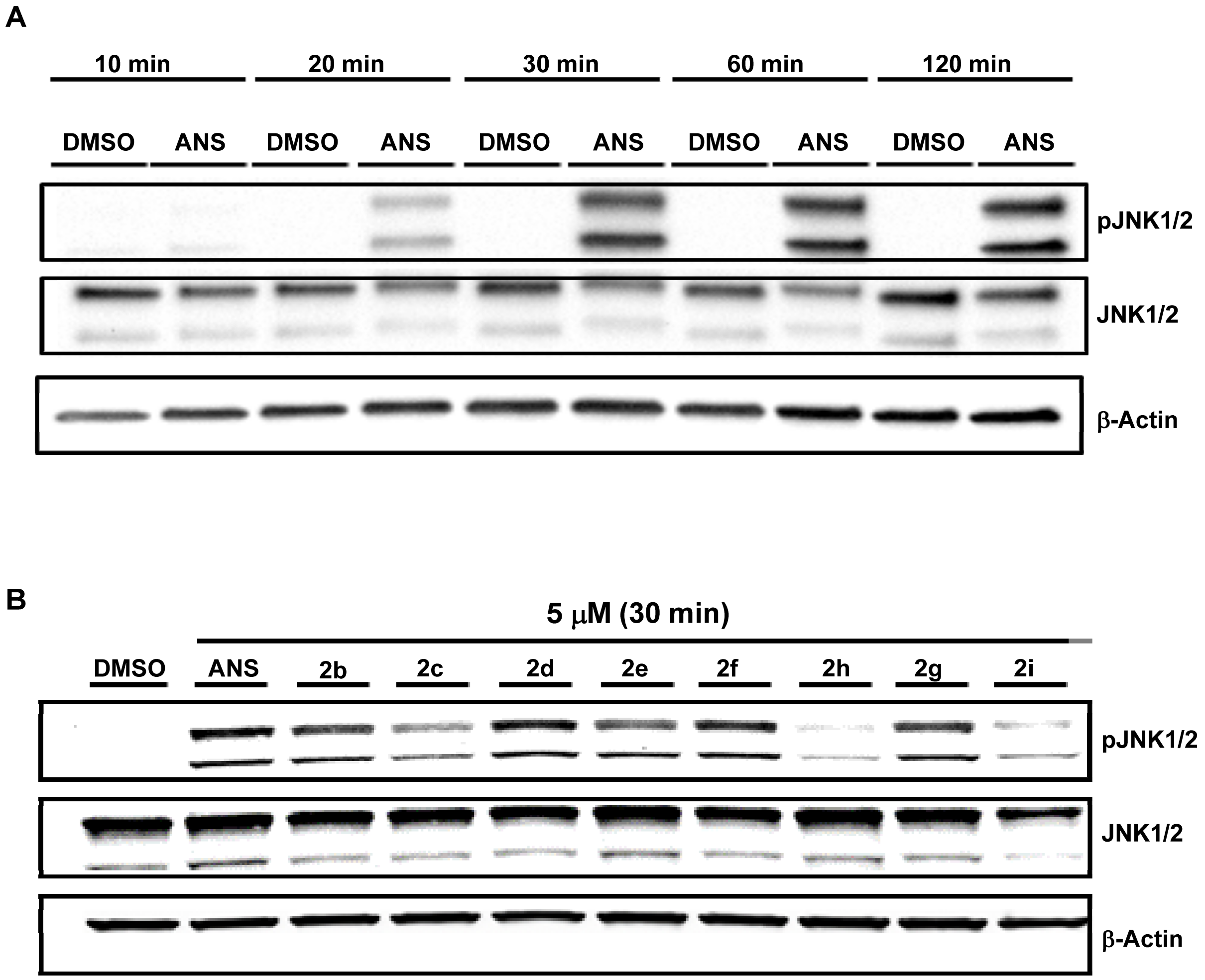


Figure S5 Effect of anisomycin **ANS** and analogues **2b-i** on the phosphorylation of JNK1/2 isoforms in SKBR3 cells. (**A**) Cells were exposed to either DMSO or anisomycin ANS (10 μM) at selected timepoints; (**B**) Cells were exposed to DMSO (lane 1), and 5 μM concentrations of anisomycin **ANS** (lane 2) and analogues **2b-i** (lanes 3-10) for 30 min. Western blot analysis was carried out with antibodies to phosphorylated JNK (pJNK1/2) and JNK1/2. β-actin was used as a loading control. (**2h** = **PhDY-ANS**; **2i** = **BADY-ANS**)

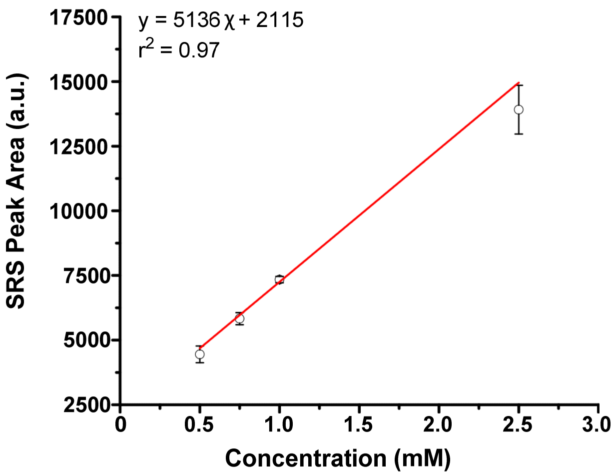


Figure S6 Correlation between SRS intensity measured at 2219 cm^{-1} (**BADY-ANS** on-resonance) and **BADY-ANS** concentration as DMSO stock solutions. Average pixel intensities were calculated using ImageJ software. Images were acquired at 512 × 512 pixels with a 20 µs pixel dwell time. Data represented as mean of 6 replicates with error bars ± SD.

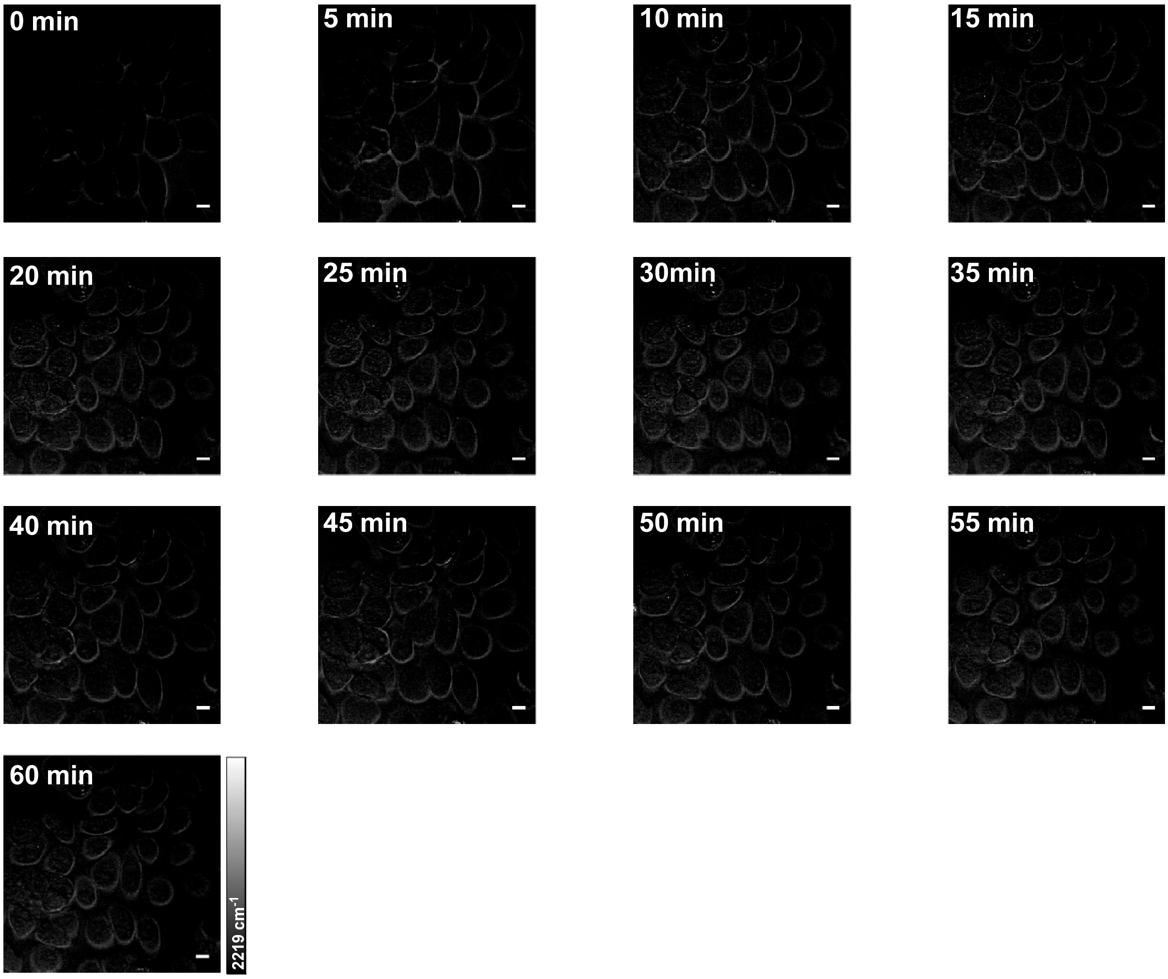


Figure S7 Time-lapse imaging of anisomycin **ANS** uptake into live SKBR3 cells. SKBR3 cells were treated with **ANS** (10 µM at t = 0 min) and imaged at 2219 cm^{-1} (C≡C, **BADY-ANS**) every minute for 60 mins. Images were acquired at 1024 × 1024 pixels, 20 µs pixel dwell time. Scale bars: 10 µm.

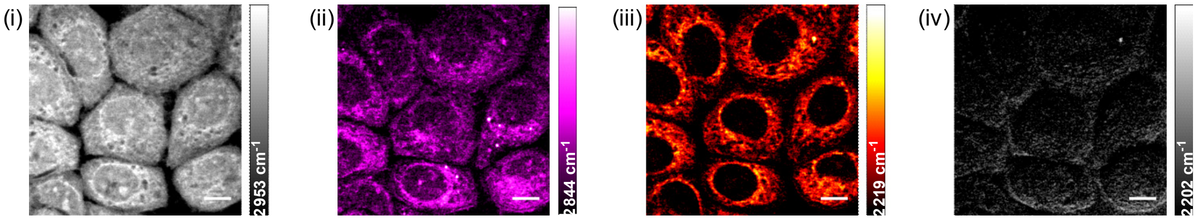


Figure S8. Fast-acquisition SRS images of fixed SKBR3 cells treated with **BADY-ANS**. SKBR3 cells treated with **BADY-ANS** (100 µM, 20 mins) and images acquired at (i) 2953 cm^{-1} (CH_3, proteins); (ii) 2844 cm^{-1} (CH_2, lipids); (iii) 2219 cm^{-1} (C≡C, **BADY-ANS**); and (iv) 2202 cm^{-1} (cell silent region). Images acquired at 512 × 512 pixels, 2 µs pixel dwell time, false colour for images applied to different detection wavenumbers. Scale bars: 10 µm.

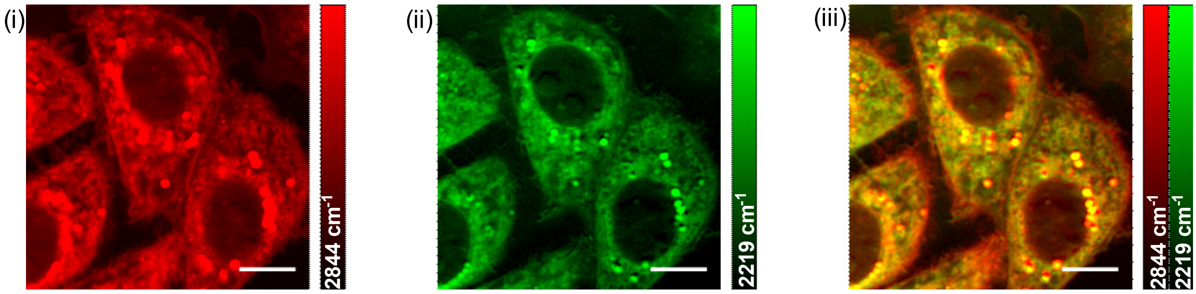


Figure S9 Multi-colour SRS imaging of fixed SKBR3 cells treated with **BADY-ANS**. SKBR3 cells were treated with **BADY-ANS** (10 µM, 30 min) and SRS images acquired at (i) 2844 cm^{-1} (CH_2, lipids) and (ii) 2219 cm^{-1} (C≡C, **BADY-ANS**) and (iii) overlay of (i) and (ii) showing that **BADY-ANS** is initially concentrated in lipid droplets in some of the cells. Images acquired at 1024 × 1024 pixels, 20 µs pixel dwell time, false colour for images applied to different detection wavenumbers. Scale bars: 10 µm.

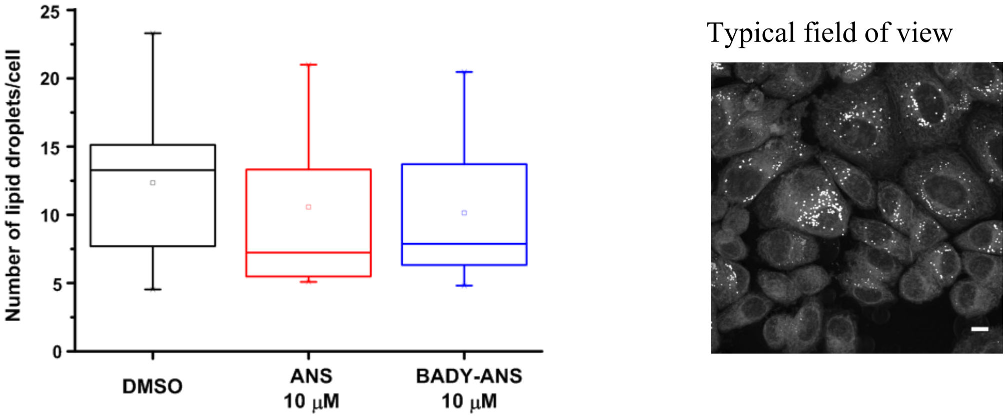


Figure S10 Quantification of the number of lipid droplets present in SKBR3 cells following treatment with (i) DMSO; (ii) **ANS** (10 µM, 30 min) and (iii) **BADY-ANS** (10 µM, 30 min). SRS images were acquired at 2844 cm^{-1} (CH_2, lipids) across a typical field of view (~40 cells, 20× objective lens, n = 9 repeats), and the number of lipid droplets >1 µm were counted using ImageJ. Inset: maximum intensity Z-projection for a typical field of view following acquisition of a Z-stack of SRS images at 2844 cm^{-1}. Scale bar: 10 µm. See Materials and Methods for further details. The average number of lipid droplets under each treatment is reported across n = 9 repeats.

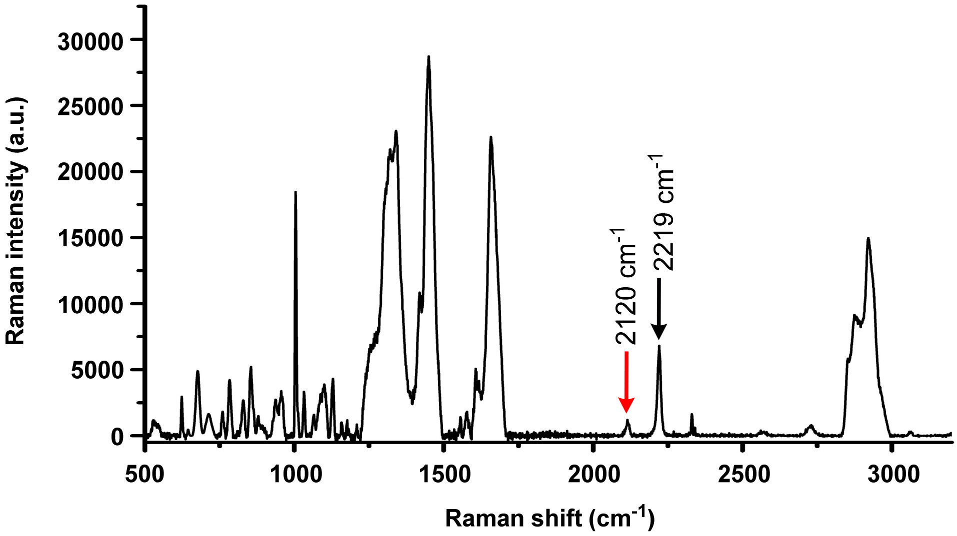


Figure S11 Dual colour alkyne-label imaging by spontaneous Raman spectrosocpy. Spontaneous Raman spectrum of SKBR3 cells treated with **EdU** (100 µM, 18 h) and **BADY-ANS** (100 µM, 20 min). Peak at 2120 cm^{-1} indicative of **EdU** (red) and peak at 2219 cm^{-1} indicative of **BADY-ANS** (black). Raman spectrum acquired at λ_{ex} = 785 nm for 60 s using a 50× objective.

Movie BADY-ANS

Live SKBR3 cells were treated with **BADY-ANS** (10 µM) at t=0 min and SRS images were acquired at 2219 cm^{-1} (C≡C, **BADY-ANS**) every minute for 60 minutes. The individual frames were compiled on ImageJ as an image stack to generate the movie. Scale bars: 50 µm.

Movie ANS

Live SKBR3 cells were treated with **ANS** (10 µM) at t=0 min and SRS images were acquired at 2219 cm^{-1} (C≡C, **BADY-ANS**) every minute for 60 minutes. The individual frames were compiled on ImageJ as an image stack to generate the movie. Scale bars: 50 µm.

General Methods

All non-aqueous reactions were carried out under an atmosphere of nitrogen or argon using oven-dried glassware that was cooled in a desiccator prior to use. Unless otherwise noted, starting materials and reagents were obtained from commercial suppliers and were used without further purification. Toluene, THF, CH_2Cl_2, and Et_2O were dried and purified by passage through activated alumina columns using a Glass Contour Solvent Purification System. Triethylamine was distilled from calcium hydride and stored over activated 4 Å molecular sieves under an argon atmosphere. Anhydrous DMF was purchased from Acros Organics. Saturated aqueous solutions of inorganic salts are represented as (volume, sat aq.). Nuclear magnetic resonance (**NMR**) spectra were recorded at ambient temperature (298 K, unless otherwise stated) on a Bruker AVA400, AVA500 or AVA600 spectrometer running at 400, 500, or 600 MHz (^{1}H spectra) or 101, 126, 151 Hz (^{13}C spectra), respectively. Chemical shifts (δ values) are reported in parts-per-million (ppm) relative to tetramethylsilane (^{1}H and ^{13}C spectra; $\delta_{TMS} = 0$) and are calibrated to the residual solvent peak. ^{1}H NMR data are reported as follows: chemical shift, relative intensity, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, m = multiplet, br = broad), coupling constants (J value, Hz), and interpretation. ^{13}C NMR data are reported as follows: chemical shift, relative intensity and assignment (Q = quaternary, CH = methane, CH_2 = methylene, CH_3 = methyl). Infra-red spectra (**IR**) were recorded neat on Shimadzu IRAffinity-1. The value of peaks at maximum absorbance (ν_{max}) are quoted in wavenumbers (cm^{-1}). Mass spectra were obtained by electrospray (ESI) on a Bruker microTOF II mass spectrometer, or by electron ionisation (EI) on a Kratos MS50TC mass spectrometer. Mass-to-charge ratios (***m/z***) of all parent (molecular) ions ($[M]^{+/-}$) and their intensities are reported, followed by (major) fragment or adduct ions and their intensities. Melting points (**mp**) were determined on a Gallenkamp Electrothermal Melting Point apparatus and are uncorrected the temperature range. R_f values (**R_f**) were recorded using Merck Silicagel 60 F254 aluminium backed plates. Flash chromatography was carried out using Merck Kieselgel 60 (Merck 9385) under positive pressure. Eluent compositions are quoted as v/v ratios. R_t values were recorded by analytical reverse phase HPLC analysis using a Waters 600E (100 µL) gradient pump using a 717plus autosampler and a Waters 996 PDA equipped with a Phenomenex Luna C18(2), 5 µm, 250 x 4.6 mm column at a flow rate of 1 mL min^{-1}. Preparative reverse phase HPLC was performed using a Waters 600 (225 µL) system using a Waters 486 tuneable absorbance detector recording at 254 nm equipped with a Phenomonex Luna C18(2), 5 µm, 250 x 21.2 mm column at a flow rate of 21.2 mL min^{-1}.

Preparative RP HPLC Method A

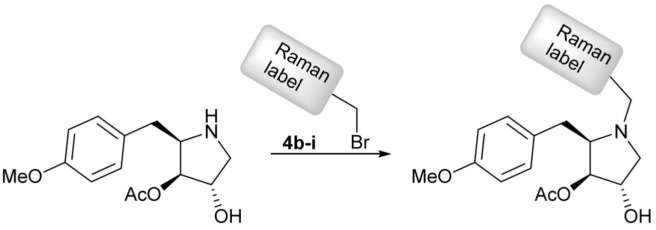
Flow Rate: 21.2 mL min^{-1}	λ: 275 nm	
Time (min)	% H_2O + 0.1% TFA	% MeCN + 0.1% TFA
0.0	80	20
20	60	40
50	60	40
55	5	95
65	5	95

Analytical RP HPLC Method B

Flow Rate: 1.0 mL min^{-1}	λ: 275 nm	
Time (min)	% H_2O + 0.1% TFA	% MeCN + 0.1% TFA
0	80	20
20	60	40
25	5	95
35	5	95
40	80	20
50	80	20

Analytical RP HPLC Method C

Flow Rate: 1.0 mL min^{-1}	λ: 275 nm	
Time (min)	% H_2O + 0.1% TFA	% MeCN + 0.1% TFA
0.0	95	5
30	5	95
35	5	95
40	95	5
50	95	5



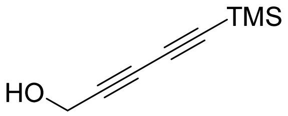
ANS

2b-i

Scheme S1 Synthesis of anisomycin analogues. Reagents and Conditions: **ANS** (60 μmol), **4b-i** (60 μmol), K_2CO_3, DMF, 9 h, 71-95%.[37] Labels **4e** and **4g** were reacted as their alkyne protected-counterparts **4e-TMS** and **4g-TMS** which were deprotected *in situ*.

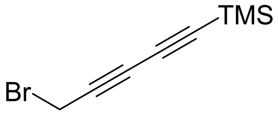
Synthesis of labels 4e-i

5-Trimethylsilyl-2,4-pentadiyn-1-ol, S1



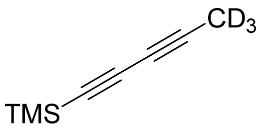
A solution of 1,4-bis(trimethylsilyl)butadiyne (486 mg, 2.50 mmol) and LiBr (271 mg, 3.13 mmol) in anhydrous Et_2O (25 mL) at -10 °C, was stirred for 10 min in darkness. MeLi (1.95 mL, 3.13 mmol; 1.6 M in Et_2O) was added dropwise and the mixture was stirred at -10 °C for 15 min, warmed to rt and stirred for a further 2 h in darkness. The mixture was cooled to 0 °C, and a suspension of paraformaldehyde (225 mg, 7.50 mmol) in dry Et_2O (30 mL) was added slowly, the mixture was warmed to rt and stirred for 18 h in darkness. The mixture was washed with NH_4Cl (50 mL; sat. aq.), $NaHCO_3$ (50 mL; sat. aq.), and brine (50 mL). The combined aqueous extracts were extracted with Et_2O (3 × 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The crude material was purified using flash column chromatography (Hexane:EtOAc, 5:1) to afford the product as a pale golden oil (199 mg, 52%). **R_f** (Hexane:EtOAc, 5:1) = 0.19; **IR** (neat, cm^{-1}) 3323 (OH), 2224 (C≡C), 2108 (C≡C); **^{1}H NMR** (CD_3OD, 500 MHz) δ 4.26 (2H, s, C*H*$_2$); 0.20 (9H, s, 3C*H*$_3$); **^{13}C NMR** (CD_3OD, 126 MHz) 87.2 (1C, Q), 85.5 (1C, Q), 76.5 (1C, Q), 68.7 (1C, Q), 49.6 (1C, CH_2), -1.9 (3C, $3CH_3$); ***m/z*** (EI) 152.1 ($[M]^+$, 22%), 137.0 (100), 109.0 (17), 77.0 (10), 75.0 (31), 63 (3); **HRMS** (EI) calcd. for $C_8H_{12}OSi$ $[M]^+$ 152.0652, found 152.0647. ^{1}H and ^{13}C NMR spectroscopic data were in good agreement with the literature.[1]

5-Bromo-1-trimethylsilyl-1,3-pentadiyne, 4e-TMS



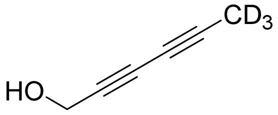
A solution of 5-trimethylsilyl-2,4-pentadiyn-1-ol **S1** (59.8 mg, 400 μmol) in anhydrous Et_2O (1.0 mL) at 0 °C, was treated successively with pyridine (2.5 μL, 32 μmol) and PBr_3 (15.2 μL, 160 μmol). The reaction mixture was warmed to rt and stirred for 18 h in darkness. The mixture was diluted with Et_2O (30 mL) and washed with Na_2CO_3 (15 mL; sat. aq.). The aqueous layer was separated and extracted with Et_2O (3 × 30 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified using flash column chromatography (Hexane) to afford the product as a pale yellow oil (68 mg, 79%). **R_f** (Hexane) = 0.74; **IR** (neat, cm^{-1}) 2358 (C≡C), 2112 (C≡C); **^{1}H NMR** (CD_3OD, 500 MHz) δ 4.15 (2H, s, C*H*$_2$); 0.22 (9H, s, 3C*H*$_3$); **^{13}C NMR** (CD_3OD, 126 MHz) δ 87.8 (1C, Q), 86.6 (1C, Q), 72.6 (1C, Q), 70.1 (1C, Q), 13.0 (1C, CH_2), -2.0 (3C, $3CH_3$); ***m/z*** (EI) 216.0 ($[^{81}BrM]^+$, 42%), 214.0 ($[^{79}BrM]^+$, 40), 201.0 (100), 199.0 (97), 172.9 (34), 170.9 (26), 135.1 (44), 64.1 (78); **HRMS** (EI) calcd. for $C_8H_{11}BrSi$ $[^{79}BrM]^+$ 213.9808, found 213.9807. ^{1}H and ^{13}C NMR spectroscopic data are in good agreement with the literature.[2]

[5-$^2H_3$]-1-Trimethylsilyl-1,3-pentadiyne, S2



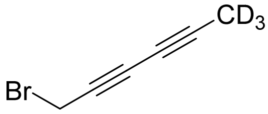
A solution of 1,4-bis(trimethylsilyl)butadiyne (972 mg, 5.00 mmol) in anhydrous THF (10 mL) was treated with MeLi•LiBr complex (3.78 mL, 5.67 mmol, 1.5 N in Et_2O), and the resultant mixture stirred at rt for 5 h in darkness. The reaction mixture was cooled to -78 °C, and CD_3I (335 μL, 5.38 mmol) was added dropwise. The mixture was stirred at -78 °C for 1 h, and slowly warmed to rt over 12 h. The reaction mixture was cooled to 0 °C, and slowly quenched with NH_4Cl (20 mL; sat. aq.). The aqueous mixture was then extracted with Et_2O (3 × 15 mL), and the combined organic extracts were washed with water (3 × 15 mL) and brine (15 mL). The resultant organic mixture was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified using flash column chromatography (Hexane) to give the product as a pale yellow oil (400 mg, 57%). **R*f***(Hexane) = 0.82; **IR** (neat, cm^{-1}) 2266 (C≡C), 2230 (C≡C), 2110 (CD); **^{1}H NMR** (CD_3OD, 500 MHz) δ 0.18 (9H, s, 3C*H*$_3$); **^{13}C NMR** (CD_3OD, 126 MHz) δ 88.4 (1C, Q), 80.8 (1C, Q), 75.1 (1C, Q), 64.0 (1C, Q), 1.6 (1C, sept, *J* 20.9 Hz, *C*D$_3$), -1.7 (3C, 3 × CH_3); ***m/z*** (EI) 139.0 ($[M]^+$, 39%), 124.0 (100); **HRMS** (EI) calcd. for $C_8H_9D_3Si$ $[M]^+$ 139.0891, found 139.0889.

[6-$^2H_3$]-2,4-Hexadiyn-1-ol, S3



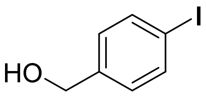
A solution of [5-$^2H_3$]-1-trimethylsilyl-1,3-pentadiyne **S2** (139 mg, 1.00 mmol) in anhydrous THF (10 mL) was cooled to -10 °C, and treated with MeLi•LiBr complex (830 µL, 1.25 mmol, 1.5 N in Et_2O). The mixture was stirred at -10 °C for 15 min, warmed to rt and stirred for an additional 2 h. After complete desilylation, the mixture was cooled to -10 °C, and a suspension of paraformaldehyde (60.9 mg, 2.03 mmol) in anhydrous THF (3.0 mL) was added. The mixture was warmed to rt and stirred for 18 h. The reaction was quenched by the slow addition of NH_4Cl (20 mL; sat. aq.), and the organic layer was washed with $NaHCO_3$ (20 mL; sat. aq.) and brine (20 mL). The combined aqueous layers were extracted with Et_2O (3 × 30 mL), and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified by flash column chromatography (Hexane:EtOAc, 10:1 → Hexane:EtOAc, 4:1) to afford the product as pale yellow needles (95 mg, 98%). **R*f*** (Hexane:EtOAc, 4:1) = 0.39; **IR** (neat, cm^{-1}) 3329 (OH), 2259 (C≡C); **^{1}H NMR** (CD_3OD, 500 MHz) δ 4.21 (2H, s, C*H*$_2$); **^{13}C NMR** (CD_3OD, 126 MHz) δ 75.8 (1C, Q), 73.1 (1C, Q), 69.1 (1C, Q), 63.2 (1C, Q), 49.6 (1C, CH_2), 1.5 (1C, sept., *J* 20.7 Hz, CD_3); ***m/z*** (EI) 97.0 ($[M]^+$, 100%), 80.0 (60), 77.9 (73), 69.0 (72), 68.0 (46), 62.9 (60), 52.9 (39); **HRMS** (EI) calcd. for $C_6H_3D_3O$ $[M]^+$ 97.0602, found 97.0598.

[6-$^2H_3$]-1-Bromo-2,4-hexadiyne, 4f



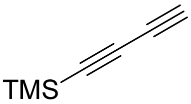
A solution of [6-$^2H_3$]-2,4-hexadiyn-1-ol **S3** (27.2 mg, 280 µmol) in anhydrous Et_2O (1.0 mL) at 0 °C, was treated successively with pyridine (1.9 µL, 24 µmol) and PBr_3 (10.5 µL, 110 µmol). The reaction mixture was warmed to rt and stirred for 18 h in darkness. The mixture was diluted with Et_2O (20 mL) and washed with Na_2CO_3 (10 mL; sat. aq.). The aqueous layer was separated and extracted with Et_2O (3 × 25 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified by flash column chromatography (Hexane) to afford the product as a colourless oil (36 mg, 80%). **R*f***(Hexane) = 0.85; **IR** (neat, cm^{-1}) 2257 (C≡C); **^{1}H NMR** (CD_3OD, 600 MHz) δ 4.10 (2H, s, C*H*$_2$Br); **^{13}C NMR** (CD_3OD, 151 MHz) δ 78.0 (1C, Q), 70.8 (1C, Q), 69.4 (1C, Q), 62.9 (1C, Q), 13.6 (1C, CH_2), 1.7 (1C, sept. *J* 20.1 Hz, CD_3); ***m/z*** (EI) 161.0 ($[^{81}BrM]^+$, 27%), 159.0 ($[^{79}BrM]^+$, 28), 80.1 (100), 78.1 (58), 63.0 (62) **HRMS** (EI) calcd. for $C_6H_2D_3Br$ $[^{79}BrM]^+$ 158.9757, found 158.9762.

4-Iodobenzyl alcohol, S4



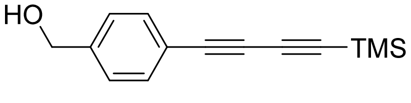
A solution of 4-iodobenzoic acid (1.44 g, 5.81 mmol) in anhydrous THF (12 mL) was treated with BH_3•THF (12.0 mL, 12.0 mmol, 1 N in THF), and the reaction stirred at rt for 4 h. The reaction was quenched by the careful addition of HCl (30 mL, 2 N aq.) and the resulting aqueous mixture extracted with CH_2Cl_2 (3 × 45 mL). The combined organic extracts were washed with $NaHCO_3$ (2 × 25 mL; sat. aq.) and brine (2 × 25 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified by flash column chromatography (Hexane:EtOAc, 10:1) to afford the product as a white solid (1.35 g, 99%). **R*f*** (CH_2Cl_2) = 0.42; **mp** 66 – 68 °C, lit. 68 – 70 °C; **IR** (neat, cm^{-1}) 3294 (OH), 1584 (C=C), 789 (C-I); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.72 (2H, d, *J* 8.3 Hz, 2 × Ar*H*), 7.14 (2H, d, *J* 8.3 Hz, 2 × Ar*H*), 4.68 (2H, s, C*H*$_2$), 1.72 (1H, s, O*H*); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 140.5 (1C, Q), 137.6 (2C, CH), 128.8 (2C, CH), 93.0 (1C, Q), 64.7 (1C, CH_2); ***m/z*** (EI) 234.0 ([M]$^+$, 100%), 107.1 (28) **HRMS** (EI) calcd. for C_7H_7OI [M]$^+$ 233.9536, found 233.9536. ^{1}H and ^{13}C NMR spectroscopic data are in good agreement with the literature.[3]

1-Trimethylsilyl-1,3-butadiyne, S5



A solution of 1,4-bis(trimethylsilyl)butadiyne (972 mg, 5.00 mmol) in anhydrous Et_2O (10 mL) at 0 °C was treated with MeLi•LiBr complex (5.0 mL, 7.50 mmol, 1.5 N in Et_2O). The reaction mixture was warmed to rt, and stirred for 5 h. The reaction was quenched by the addition of NH_4Cl (10 mL, sat. aq.), and the resulting mixture extracted with Et_2O (3 × 20 mL). The combined organic extracts were washed with water (20 mL) and brine (20 mL), dried ($MgSO_4$), filtered and concentrated at ambient conditions overnight. The product was found to be volatile and unstable when concentrating to dryness; approximate concentration was determined by ^{1}H NMR (0.16 N in Et_2O). **R*f*** (Hexane) = 0.98; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 2.13 (1H, s, *H*C≡CC≡C), 0.23 (9H, s, 3 × C*H*$_3$); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 88.0 (1C, Q), 84.7 (1C, Q), 68.3 (1C, CH), 66.6 (1C, Q), -0.6 (3C, $3CH_3$). ^{1}H and ^{13}C NMR spectroscopic data are in good agreement with the literature.[4]

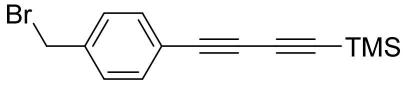
4-(4-Trimethylsilyl-1,3-butadiyn-1-yl)benzyl alcohol, S6



A mixture of 4-iodobenzyl alcohol **S4** (748 mg, 3.20 mmol), Et_3N (890 μL, 6.40 mmol), $PdCl_2(PPh_3)_2$ (45.5 mg, 65.0 μmol, 2 mol%) and CuI (34.2 mg, 180 μmol, 5.6 mol%) were dissolved in anhydrous Et_2O (4 mL). The reaction mixture was stirred for 10 minutes, after which 1-trimethylsilyl-1,3-butadiyne **S5** (20 mL, 3.2 mmol, 0.16 N in Et_2O) was added, and the mixture stirred at rt for 18 h. The resulting mixture was concentrated *in vacuo* and re-dissolved in CH_2Cl_2 (50 mL). The organic mixture was washed with HCl (2 × 40 mL, 1 N aq.) and water (40 mL). The combined aqueous washings were extracted CH_2Cl_2 (3 × 100 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified by flash column chromatography (CH_2Cl_2) to afford the product as a light brown solid (272 mg, 37%). **R*f***(CH_2Cl_2) = 0.36; **mp** 88 - 90 °C; **IR** (neat, cm^{-1}) 3256 (OH), 2203 (C≡C), 2102 (C≡C), 1508 (C=C); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.51 (2H, d, *J* 8.4 Hz, 2 × Ar*H*), 7.35 (2H, d, *J* 8.4 Hz, 2 × Ar*H*), 4.73 (2H, d, *J* 5.9 Hz, C*H*$_2$OH), 1.72 (1H, t, *J* 5.9 Hz, CH_2O*H*), 0.26 (9H, s, 3 × C*H*$_3$); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 142.2 (1C, Q), 132.9 (2C, CH), 126.8 (2C, CH), 120.6 (1C, Q), 90.8

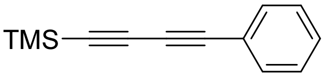
(1C, Q), 87.8 (1C, Q), 76.6 (1C, Q), 74.2 (1C, Q), 64.8 (1C, CH_2), -0.4 (3C, CH_3); ***m/z*** (EI) 228.1 ($[M]^+$, 39%), 213.1 (100); **HRMS** (EI) calcd. for $C_{14}H_{16}OSi$ $[M]^+$ 228.0965, found 228.0978. ^{1}H NMR spectroscopic data is in good agreement with the literature.[5]

4-(4-Trimethylsilyl-1,3-butadiyn-1-yl)benzyl bromide, 4g-TMS



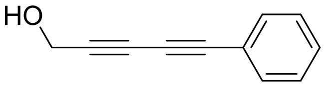
A solution of 4-(4-trimethylsilyl-1,3-butadiyn-1-yl)benzyl alcohol **S6** (91 mg, 400 μmol) in anhydrous Et_2O (1 mL) at 0 °C, was treated with pyridine (2.5 μL, 32 μmol) then PBr_3 (15.2 μL, 160 μmol). The reaction mixture was warmed to rt and stirred for 18 h in darkness. The reaction mixture was diluted with Et_2O (30 mL) and washed with Na_2CO_3 (15 mL; sat. aq.). The aqueous layer was separated and extracted with Et_2O (3 × 30 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo.* The crude material was purified by flash column chromatography (Hexane) to afford the product as a pale yellow solid (90 mg, 77%). **R*f*** (Hexane) = 0.48; **mp** 65 – 67 °C; **IR** (neat, cm^{-1}) 2203 (C≡C), 2099 (C≡C); **^{1}H NMR** (CD_3OD, 500 MHz) δ 7.50 (2H, d, *J* 8.4 Hz, 2 × Ar*H*), 7.44 (2H, d, *J* 8.4 Hz, 2 × Ar*H*), 4.59 (2H, s, C*H*$_2$Br), 0.24 (9H, s, 3 × C*H*$_3$); **^{13}C NMR** (CD_3OD, 126 MHz) δ 140.0 (1C, Q), 132.6 (2C, CH), 129.1 (2C, CH), 121.0 (1C, Q), 90.0 (1C, Q), 87.2 (1C, Q), 75.6 (1C, Q), 74.0 (1C, Q), 31.7 (1C, CH_2), -1.9 (3C, CH_3); ***m/z*** (EI) 292.0 ($[^{81}BrM]^+$, 14%), 290.0 ($[^{79}BrM]^+$, 14), 211.1 (100), 196.0 (27), 183.0 (24); **HRMS** (EI) calcd. for $C_{14}H_{15}BrSi$ $[^{79}BrM]^+$ 290.0121, found 290.0132.

4-Phenyl-1-trimethylsilyl-1,3-butadiyne, S7



A mixture of iodobenzene (180 μL, 1.6 mmol), Et_3N (450 μL, 3.2 mmol), $PdCl_2(PPh_3)_2$ (11.5 mg, 16.4 μmol, 1 mol%) and CuI (8.8 mg, 46.2 μmol, 3 mol%) were dissolved in anhydrous Et_2O (2 mL). The mixture was stirred for 10 min, after which 1-trimethylsilyl-1,3-butadiyne **S5** (10 mL, 1.6 mmol, 0.16 N in Et_2O) was added, and the mixture stirred at rt for 18 h. The mixture was concentrated *in vacuo* and re-dissolved in CH_2Cl_2 (25 mL). The organic mixture was washed with HCl (2 × 20 mL, 1 N aq.) and water (20 mL). The combined aqueous washings were extracted CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified by flash column chromatography (CH_2Cl_2) to afford the product as a pale yellow oil (260 mg, 82%). **R*f***(Hexane) = 0.64; **IR** (neat, cm^{-1}) 2207 (C≡C), 2102 (C≡C); **^{1}H NMR** (CD_3OD, 500 MHz) δ 7.53 - 7.49 (2H, m, 2 × Ar*H*), 7.46 – 7.41 (1H, m, Ar*H*), 7.38 (2H, t, *J* 7.5 Hz, 2 × Ar*H*), 0.24 (9H, s, 3 × C*H*$_3$); **^{13}C NMR** (CD_3OD, 126 MHz) δ 132.2 (2C, CH), 129.3 (1C, CH), 128.3 (2C, CH), 121.1 (1C, Q), 89.5 (1C, Q), 87.4 (1C, Q), 76.0 (1C, Q), 73.3 (1C, Q), -1.8 (3C, CH_3); ***m/z*** (EI) 198.1 ($[M]^+$, 32%), 183.2 (100); **HRMS** (EI) *m/z* calcd. for $C_{13}H_{14}Si$ $[M]^+$ 198.0859, found 198.0862. ^{1}H and ^{13}C NMR spectroscopic data are in good agreement with the literature.[6]

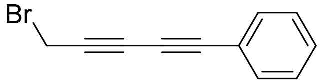
5-Phenyl-2,4-pentadiyn-1-ol, S8



A solution of 4-phenyl-1-trimethylsilyl-1,3-butadiyne **S7** (198.3 mg, 1.00 mmol) in anhydrous THF (10 mL) was cooled to -10 °C, and treated with MeLi•LiBr complex (1.3 mL, 2.00 mmol, 1.5 N in Et_2O).

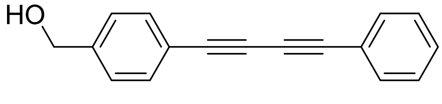
The mixture was stirred at -10 °C for 15 min, warmed to rt and stirred for an additional 2 h. After complete desilylation, the mixture was cooled to -10 °C, and a suspension of paraformaldehyde (90.7 mg, 3.02 mmol) in anhydrous THF (3.0 mL) was added. The mixture was warmed to rt and stirred for 18 h. The reaction was quenched by the slow addition of NH_4Cl (20 mL; sat. aq.), and the organic layer was washed with $NaHCO_3$ (20 mL; sat. aq.) and brine (20 mL). The combined aqueous layers were extracted with Et_2O (3 × 30 mL), and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified by flash column chromatography (Hexane:EtOAc, 10:1) to afford the product as a pale yellow oil (117.2 mg, 75%). **R_f** (Hexane:EtOAc, 5:2) = 0.54; **IR** (neat, cm^{-1}) 3293 (OH), 2241 (C≡C); **^{1}H NMR** (CD_3OD, 500 MHz) δ 7.53 – 7.48 (2H, m, 2 × Ar*H*), 7.45 – 7.35 (3H, m, 3 × Ar*H*), 4.34 (2H, s, C*H*$_2$OH); **^{13}C NMR** (CD_3OD, 126 MHz) δ 132.1 (2C, CH), 129.1 (1C, CH), 128.3 (2C, CH), 121.4 (1C, Q), 81.2 (1C, Q), 77.1 (1C, Q), 72.7 (1C, Q), 68.3 (1C, Q), 49.8 (1C, CH_2); ***m/z*** (EI) 156.0 ($[M]^+$, 100%), 139.0 (22), 128.1 (65), 102.0 (64); **HRMS** (EI) *m/z* calcd. for $C_{11}H_8O$ $[M]^+$ 156.0570, found 156.0565. ^{1}H and ^{13}C NMR spectroscopic data are in good agreement with the literature.[7]

5-Bromo-1-phenyl-1,3-pentadiyne, 4h



A solution of 5-phenyl-2,4-pentadiyn-1-ol **S8** (117.2 mg, 750 µmol) in anhydrous Et_2O (3 mL) at 0 °C, was treated with pyridine (4.8 µL, 60 µmol) then PBr_3 (28.5 µL, 300 µmol). The reaction mixture was warmed to rt and stirred for 18 h in darkness. The mixture was diluted with Et_2O (30 mL) and washed with Na_2CO_3 (15 mL; sat. aq.). The aqueous layer was separated and extracted with Et_2O (3 × 30 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo.* The crude material was purified by flash column chromatography (Hexane) to afford the product as a yellow oil (97 mg, 59 %). **R_f** (Hexane) = 0.47; **IR** (neat, cm^{-1}) 2243 (C≡C), 2222 (C≡C); **^{1}H NMR** (CD_3OD, 500 MHz) δ 7.55 – 7.51 (2H, m, 2 × Ar*H*), 7.47 – 7.42 (1H, m, Ar*H*), 7.41 – 7.37 (2H, m, 2 × Ar*H*), 4.24 (2H, s, C*H*$_2$Br); **^{13}C NMR** (CD_3OD, 126 MHz) δ 132.2 (2C, CH), 129.4 (1C, CH), 128.3 (2C, CH), 121.0 (1C, Q), 78.9 (1C, Q), 77.3 (1C, Q), 72.3 (1C, Q), 69.8 (1C, Q), 13.4 (1C, CH_2); ***m/z*** (EI) 219.9 ($[^{81}BrM]^+$, 11%), 217.9 ($[^{79}BrM]^+$, 11), 139.0 (100); **HRMS** (EI) calcd. for $C_{11}H_7Br$ $[^{79}BrM]^+$ 217.9726, found 217.9711.

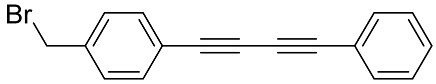
4-(4-Phenyl-1,3-butadiyn-1-yl)benzyl alcohol, S9



A solution of 4-phenyl-1-trimethylsilyl-1,3-butadiyne **S7** (100.1 mg, 500 µmol) in THF:MeOH (2 mL, 1:1 v/v) was treated with K_2CO_3 (138.2 mg, 1.00 mmol) and the mixture stirred at rt for 2 h. The reaction was quenched by the addition of NH_4Cl (2.0 mL, sat. aq.) and Et_2O (4.0 mL). The organic phase was separated, washed with brine (2 × 5 mL), dried ($MgSO_4$) and filtered. To the deprotected alkyne was added Et_3N (4.2 mL, 30.0 mmol), 4-iodobenzyl alcohol **S4** (117.1 mg, 500 µmol), $PdCl_2(PPh_3)_2$ (3.5 mg, 5.0 µmol, 1 mol%) and CuI (2.7 mg, 14 µmol, 2.8 mol%), and the mixture stirred at rt for 18 h. The reaction was quenched by the addition of NH_4Cl (10 mL, sat. aq.) and the resulting mixture extracted with Et_2O (3 × 20 mL). The combined organic extracts were washed with brine (15 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified by flash column chromatography (Hexane:EtOAc, 5:2) to give the product as a white solid (97.6 mg, 84%). **R_f** (Hexane:EtOAc, 5:2) = 0.35; **mp** 126 – 128 °C;

IR (neat, cm^{-1}) 3246 (OH), 2216 (C≡C), 2145 (C≡C); **^{1}H NMR** (CD$_3$OD, 500 MHz) δ 7.58 – 7.51 (4H, m, 4 × Ar*H*), 7.48 – 7.35 (5H, m, 5 × Ar*H*), 4.65 (2H, s, C*H*$_2$OH); **^{13}C NMR** (CD$_3$OD, 126 MHz) δ 143.3 (1C, Q), 132.1 (2C, CH), 132.0 (2C, CH), 129.1 (1C, CH), 128.3 (2C, CH), 126.6 (2C, CH), 121.6 (1C, Q), 120.2 (1C, Q), 80.9 (1C, Q), 80.7 (1C, Q), 73.0 (1C, Q), 72.8 (1C, Q), 63.2 (1C, CH$_2$); ***m/z*** (EI) 232.1 ([M]$^+$, 100%), 202.1 (44); **HRMS** (EI) calcd. for C$_{17}$H$_{12}$O [M]$^+$ 232.0883, found 232.0889. ^{1}H and ^{13}C NMR spectroscopic data are in good agreement with the literature.[8]

4-(4-Phenyl-1,3-butadiyn-1-yl)benzyl bromide, 4i



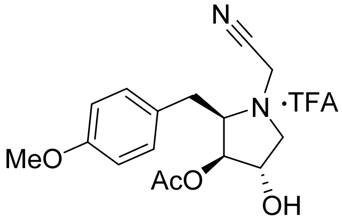
A solution of 4-(4-phenyl-1,3-butadiyn-1-yl)benzyl alcohol **S9** (116.1 mg, 500 μmol) in anhydrous Et$_2$O (3 mL) at 0 °C, was treated with pyridine (4.8 μL, 60 μmol) then PBr$_3$ (28.5 μL, 300 μmol). The reaction mixture was warmed to rt and stirred for 18 h in darkness. The reaction mixture was diluted with Et$_2$O (30 mL) and washed with Na$_2$CO$_3$ (15 mL; sat. aq.). The aqueous layer was separated and extracted with Et$_2$O (3 × 30 mL). The combined organic extracts were dried (MgSO$_4$), filtered and concentrated *in vacuo.* The crude material was purified by flash column chromatography (Hexane) to afford the product as white solid (91 mg, 62 %). **R$_f$** (Hexane) = 0.25; **IR** (neat, cm^{-1}) 2216 (C≡C), 2145 (C≡C); **^{1}H NMR** (CD$_3$OD, 500 MHz) δ 7.57 – 7.51 (4H, m, 4 × Ar*H*), 7.49 – 7.38 (5H, m, 5 × Ar*H*), 4.60 (2H, s, C*H*$_2$Br); **^{13}C NMR** (CD$_3$OD, 126 MHz) δ 139.8 (1C, Q), 132.4 (2C, CH), 132.1 (2C, CH), 129.2 (1C, CH), 129.1 (2C, CH), 128.3 (2C, CH), 121.4 (1C, Q), 121.4 (1C, Q), 81.2 (1C, Q), 80.4 (1C, Q), 73.7 (1C, Q), 72.9 (1C, Q), 31.7 (1C, CH$_2$); ***m/z*** (EI) 295.9 ([^{81}BrM]$^+$, 12%), 293.9 ([^{79}BrM]$^+$, 12), 215.0 (100), 213.0 (44), 107.4 (10); **HRMS** (EI) calcd. for C$_{17}$H$_{11}$Br [^{79}BrM]$^+$ 294.0039, found 294.0034.

Characterisation of anisomycin analogues 2b-i

General procedure for the synthesis of Raman-labelled anisomycin derivatives

To a solution of **ANS** (60.0 μmol) in DMF (1.5 mL) was added potassium carbonate (60.0 μmol) and **4b-i** (60.0 μmol), the solution was stirred at room temperature for 9 h. The solution was then concentrated *in vacuo*, and the residue was purified by RP HPLC, and freeze dried to afford the product.

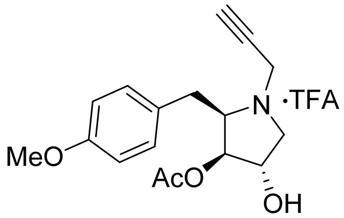
N-Cyanomethyl anisomycin, 2b



Purification (Method A); colourless oil (20.9 mg, 87%). **R$_t$** (Method B) = 19.4 min; **IR** (neat, cm^{-1}) 3410 (OH), 2363 (C≡N), 1748 (C=O), 1672 (C=O, TFA), 1613 (C=C), 1585 (C=C), 1514 (C=C); **^{1}H NMR** (CDCl$_3$, 500 MHz) δ 7.17 (2H, d, *J* 8.6 Hz, 2 × Ar*H*), 6.89 (2H, d, *J* 8.6 Hz, 2 × Ar*H*), 4.82 (1H, d, *J* 3.7 Hz, C$_3$*H*), 4.31 – 4.28 (1H, m C$_4$*H*), 3.89 – 3.70 (2H, dd, *J* 41.5, 17.5 Hz, NC*H*$_2$C≡N), 3.82 (3H, s, O*Me*), 3.79 (1H, dd, *J* 11.2, 6.1 Hz, C$_5$*H*$_a$H$_b$), 3.65 (1H, dd, *J* 12.4, 7.4 Hz, C$_2$*H*), 3.01 – 2.92 (2H, m, C*H*$_2$Ar), 2.86 (1H, dd, *J* 11.2, 6.1 Hz, C$_5$H$_a$*H*$_b$), 2.19 (3H, s, O*Ac*); **^{13}C NMR** (CDCl$_3$, 126 MHz) δ 170.9 (1C, Q), 158.8 (1C, Q), 129.8 (2C, CH), 127.9 (1C, Q), 114.5 (2C, CH), 112.8 (1C, Q), 80.1 (1C, CH), 73.5 (1C, CH), 65.8 (1C, CH), 59.1 (1C, CH$_2$), 55.3

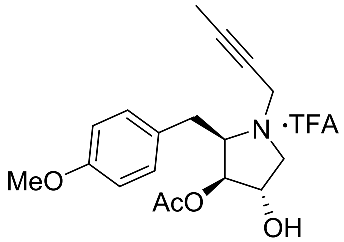
(1C, CH_3), 40.6 (1C, CH_2), 31.4 (1C, CH_2), 20.7 (1C, CH_3); ***m/z*** (ESI) 305.2 ($[M + H]^+$, 100%), 263.1 (11), 179.0 (41), 117.0 (22); **HRMS** (ESI) calcd. for $C_{16}H_{20}O_4N_2Na$ $[M + Na]^+$ 327.1315, found 327.1315.

N-Propargyl anisomycin, 2c



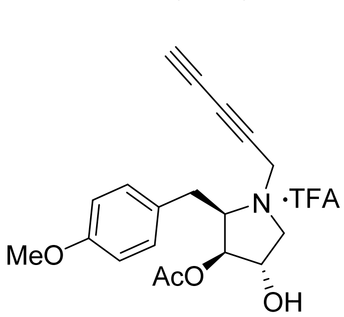
Purification (Method A); colourless oil (21.6 mg, 90%). **R$_t$** (Method B) = 16.6 min; **IR** (neat, cm^{-1}) 3277 (OH), 2131 (C≡C), 1749 (C=O), 1672 (C=O, TFA), 1613 (C=C), 1585 (C=C), 1514 (C=C); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.14 (1H, d, *J* 8.6 Hz, 2 × ArH), 6.88 (1H, d, *J* 8.6 Hz, 2 × ArH), 4.82 (1H, d, *J* 4.0 Hz, C_3H), 4.40 – 4.36 (1H, m, C_4H), 4.22 – 4.15 (1H, m, $C_5\mathit{H}_aH_b$), 4.03 – 4.00 (1H, m, C_2H), 3.95 (2H, ddd, *J* 38.2, 17.2, 2.1 Hz, N$C\mathit{H}_2$C≡CH), 3.82 (3H, s, O*Me*), 3.21 – 3.15 (1H, m, $C_5H_a\mathit{H}_b$), 3.15 – 3.07 (1H, m, C*H*$_2$Ar), 2.62 (1H, t, *J* 2.1 Hz, NCH_2C≡C*H*), 2.20 (3H, s, O*Ac*); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 170.4 (1C, Q), 158.9 (1C, Q), 129.8 (2C, CH), 127.2 (1C, Q), 114.5 (2C, CH), 78.74 (1C, Q), 78.68 (1C, CH), 77.2 (1C, CH),* 72.3 (1C, CH), 63.3 (1C, CH), 57.8 (1C, CH_2), 55.3 (1C, CH_3), 41.2 (1C, CH_2), 29.6 (1C, CH_2), 20.6 (1C, CH_3); ***m/z*** (ESI) 326.1 ($[M + Na]^+$, 100%), 304.2 ($[M + H]^+$, 8), 284.1 (18), 262.1 (15), 244.1 (6); **HRMS** (ESI) calcd. for $C_{17}H_{22}O_4N$ $[M + H]^+$ 304.1549, found 304.1547. *Obscured by solvent peak – observed in HSQC.

N-2-Butyn-1-yl anisomycin, 2d



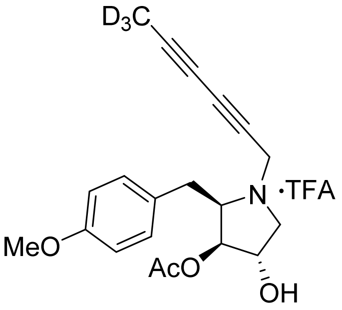
Purification (Method A); colourless oil (17.6 mg, 71%). **R$_t$** (Method B) = 23.1 min; **IR** (neat, cm^{-1}) 3271 (OH), 2247 (C≡C), 1751 (C=O), 1670 (C=O, TFA), 1612 (C=C), 1585 (C=C), 1514 (C=C); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.11 (2H, d, *J* 8.2 Hz, 2 × Ar*H*), 6.87 (2H, d, *J* 8.2 Hz, 2 × Ar*H*), 4.87 (1H, s, $C_3\mathit{H}$), 4.34 (1H, s, $C_4\mathit{H}$), 4.18 (1H, br. s, $C_5\mathit{H}_aH_b$), 4.08 (1H, br. s, C_2H), 3.91 (2H, dd, *J* 31.0, 17.0 Hz, N$C\mathit{H}_2$C≡CMe), 3.81 (3H, s, O*Me*), 3.24 (1H, d, *J* 11.6 Hz, $C_5H_a\mathit{H}_b$), 3.17 – 3.06 (2H, m, C*H*$_2$Ar), 2.17 (3H, s, O*Ac*), 1.94 (3H, s, NCH_2C≡C*Me*); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 170.2 (1C, Q), 158.9 (1C, Q), 129.8 (2C, CH), 127.1 (1C, Q), 114.5 (2C, CH), 87.8 (1C, Q), 78.0 (1C, CH), 71.7 (1C, CH), 66.7 (1C, Q), 65.7 (1C, CH), 58.0 (1C, CH_2), 55.3 (1C, CH_3), 42.5 (1C, CH_2), 29.4 (1C, CH_2), 20.6 (1C, CH_3), 3.6 (1C, CH_3); ***m/z*** (ESI) 318.2 ($[M + H]^+$, 100%); **HRMS** (ESI) calcd. for $C_{18}H_{24}O_4N$ $[M + H]^+$ 318.1700, found 318.1705.

N-2,4-Pentadiyn-1-yl anisomycin, 2e



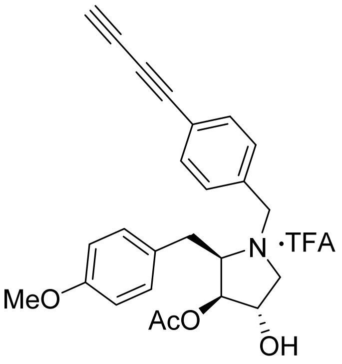
Purification (Method A); pale yellow oil (18.8 mg, 74%). **R$_t$** (Method B) = 24.2 min; **IR** (neat, cm^{-1}) 3271 (OH), 2236 (C≡C), 1751 (C=O), 1670 (C=O, TFA), 1613 (C=C), 1585 (C=C), 1514 (C=C); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.15 (2H, d, *J* 8.6 Hz, 2 × Ar*H*), 6.89 (2H, d, *J* 8.6 Hz, 2 × Ar*H*), 4.90 – 4.85 (1H, m, $C_3\mathit{H}$), 4.39 – 4.35 (1H, m, $C_4\mathit{H}$), 4.12 (1H, dd, *J* 12.1, 5.7 Hz, $C_5\mathit{H}_aH_b$), 4.00 – 3.94 (1H, m, $C_2\mathit{H}$), 3.97 (2H, dd, *J* 59.7, 18.0 Hz, N$C\mathit{H}_2$C≡C-), 3.83 (3H, s, O*Me*), 3.17 – 3.06 (3H, m, $C_5H_a\mathit{H_b}$ + $C\mathit{H_2}Ar$), 2.29 (1H, s, -C≡C*H*), 2.20 (3H, s, O*Ac*); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 170.2 (1C, Q), 158.9 (1C, Q), 129.8 (2C, CH), 127.1 (1C, Q), 114.6 (2C, CH), 78.3 (1C, CH), 74.1 (1C, Q), 72.0 (1C, CH), 69.6 (1C, Q), 66.5 (1C, Q), 66.3 (1C, CH), 64.9 (1C, Q), 58.6 (1C, CH_2), 55.3 (1C, CH_3), 42.6 (1C, CH_2), 29.9 (1C, CH_2), 20.6 (1C, CH_3); ***m/z*** (ESI) 328.2 ($[M + H]^+$, 100%), 179.0 (7); **HRMS** (EI) calcd. for $C_{19}H_{22}O_4N$ $[M + H]^+$ 328.1543, found 328.1544.

N-[6-$^2H_3$]-2,4-Hexadiyn-1-yl anisomycin, 2f



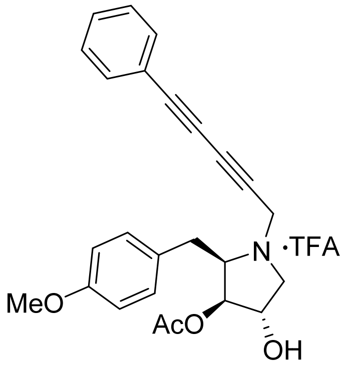
Purification (Method A); colourless oil (20.4 mg, 77%). **R_t** (Method C) = 21.1 min; **IR** (neat, cm^{-1}) 3289 (OH), 2264 (C≡C), 1751 (C=O), 1670 (C=O, TFA), 1613 (C=C), 1585 (C=C), 1514 (C=C); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.15 (2H, d, *J* 8.6 Hz, 2 × Ar*H*), 6.89 (2H, d, *J* 8.6 Hz, 2 × Ar*H*), 4.90 (1H, d, *J* 3.7 Hz, $C_3\mathit{H}$), 4.41 – 4.38 (1H, m, $C_4\mathit{H}$), 4.23 (1H, dd, *J* 12.5, 5.6 Hz, $C_5\mathit{H_a}H_b$), 4.12 – 4.07 (1H, m, $C_2\mathit{H}$) 4.04 (2H, dd, *J* 51.5, 17.8 Hz, $NC\mathit{H_2}$C≡C-), 3.83 (3H, s, O*Me*), 3.24 (1H, d, *J* 12.5 Hz, $C_5H_a\mathit{H_b}$), 3.19 – 3.09 (2H, m, $C\mathit{H_2}Ar$), 2.20 (3H, s, O*Ac*); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 170.2 (1C, Q), 159.0 (1C, Q), 129.8 (2C, CH), 126.8 (1C, Q), 114.6 (2C, CH), 79.1 (1C, Q), 78.1 (1C, CH), 75.8 (1C, Q), 71.9 (1C, CH), 66.0 (1C, CH), 63.0 (1C, Q), 62.0 (1C, Q), 58.2 (1C, CH_2), 55.3 (1C, CH_3), 42.6 (1C, CH_2), 29.5 (1C, CH_2), 20.5 (1C, CH_3) 3.73 (1C, m, $C\mathit{D_3}$); ***m/z*** (ESI) 345.2 ($[M + H]^+$, 100%), 179.0 (6); **HRMS** (ESI) calcd. for $C_{20}H_{21}D_3O_4N$ $[M + H]^+$ 345.1888, found 345.1888.

N-4-(1,3-Butadiyn-1-yl)benzyl anisomycin, 2g



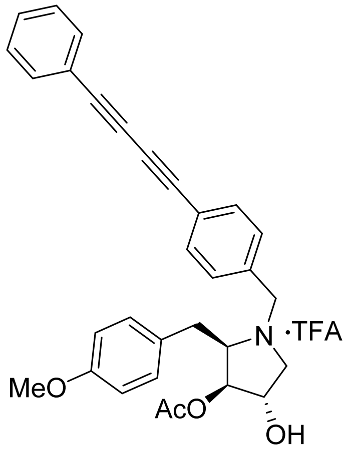
Purification (Method A); light brown oil (28.5 mg, 95%). **R_t** (Method B) = 28.2 min; **IR** (neat, cm^{-1}) 3273 (OH), 1751 (C=O), 1670 (C=O, TFA), 1613 (C=C), 1585 (C=C), 1514 (C=C); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.58 (2H, d, *J* 8.2 Hz, 2 × Ar*H*), 7.49 (2H, d, *J* 8.2 Hz, 2 × Ar*H*), 6.99 (2H, d, *J* 8.6 Hz, 2 × Ar*H*), 6.84 (2H, d, *J* 8.6 Hz, 2 × Ar*H*), 4.93 (1H, d, *J* 3.2 Hz, $C_3\mathit{H}$), 4.32 – 4.23 (3H, m, $C_4\mathit{H}$ + $NC\mathit{H_2}Ar$), 4.03 – 3.89 (2H, m, $C_2\mathit{H}$ + $C_5\mathit{H_a}H_b$), 3.79 (3H, s, O*Me*), 3.15 – 3.08 (2H, m, $C_5H_a\mathit{H_b}$ + $C\mathit{H_X}H_YAr$), 2.91 – 2.84 (1H, m, $CH_X\mathit{H_Y}Ar$), 2.55 (1H, s, -C≡C*H*), 2.16 (3H, s, O*Ac*); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 169.9 (1C, Q), 158.9 (1C, Q), 133.5 (2C, CH), 130.9 (2C, CH), 129.7 (2C, CH), 127.2 (1C, Q), 123.0 (1C, Q), 117.9 (1C, Q), 114.5 (2C, CH), 77.8 (1C, Q), 77.2 (1C, CH),* 75.3 (1C, Q), 74.0 (1C, Q), 72.3 (1C, CH), 72.3 (1C, CH), 67.8 (1C, CH), 59.9 (1C, CH_2), 58.8 (1C, CH_2), 55.3 (1C, CH_3), 30.4 (1C, CH_2), 20.6 (1C, CH_3); ***m/z*** (ESI) 404.2 ($[M + H]^+$, 100%), 179.0 (17); **HRMS** (ESI) calcd. for $C_{25}H_{26}O_4N$ $[M + H]^+$ 404.1856, found 404.1869. * Obscured by solvent peak – observed in HSQC.

N-5-Phenyl-2,4-pentadiyn-1-yl anisomycin, 2h (PhDY-ANS)



Purification (isocratic 60:40 H_2O: MeCN 0.1% TFA), light brown oil (21.0 mg, 73%). **R_t** (Method C) = 31.3 min; **IR** (neat, cm^{-1}) 3284 (OH), 2253 (C≡C), 1751 (C=O), 1672 (C=O, TFA), 1612 (C=C), 1585 (C=C), 1514 (C=C); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.58 – 7.55 (2H, m, 2 × Ar*H*), 7.47 – 7.43 (1H, m, Ar*H*), 7.41 – 7.34 (2H, m, 2 × Ar*H*), 7.18 (2H, d, *J* 8.6 Hz, 2 × Ar*H*), 6.90 (2H, d, *J* 8.6 Hz, 2 × Ar*H*), 4.90 (1H, d, *J* 3.8 Hz, C_3H), 4.41 (1H, br s, C_4H), 4.23 – 4.18 (1H, m, $C_5H_aH_b$), 4.15 – 4.02 (3H, m, C_2H + $NCH_2C{\equiv}C$-), 3.83 (3H, s, O*Me*), 3.25 – 3.22 (1H, m, $C_5H_aH_b$), 3.19 – 3.10 (2H, m, CH_2Ar), 2.21 (3H, s, O*Ac*); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 170.3 (1C, Q), 159.0 (1C, Q), 132.9 (2C, CH), 130.1 (1C, CH), 129.8 (2C, CH), 128.6 (2C, CH), 127.1 (1C, Q), 120.5 (1C, Q), 114.6 (2C, CH), 79.5 (1C, Q), 78.6 (1C, CH),* 74.9 (1C, Q), 72.3 (1C, CH), 72.3 (1C, Q), 65.7 (1C, CH), 58.1 (1C, CH_2), 55.3 (1C, CH_3), 42.5 (1C, CH_2), 29.7 (1C, CH_2), 20.6 (1C, CH_3); ***m/z*** (ESI) 404.2 ($[M + H]^+$, 100%), 324.9 (4), 246.9 (5), 179.0 (9); **HRMS** (ESI) calcd. for $C_{25}H_{26}O_4N$ $[M + H]^+$ 404.1856, found 404.1861. *Q peak expected at 77.2 ppm obscured by solvent peak.

N-4-(4-Phenyl-1,3-butadiyn-1-yl)benzyl anisomycin, 2i (BADY-ANS)



Purification (isocratic 50:50 H_2O:MeCN 0.1% TFA), pale yellow oil (28.0 mg, 81%). **R_t** (Method C) = 29.9 min; **IR** (neat, cm^{-1}) 3284 (OH), 2218 (C≡C), 1751 (C=O), 1670 (C=O, TFA), 1613 (C=C), 1585 (C=C), 1514 (C=C); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.61 – 7.54 (4H, m, 4 × Ar*H*), 7.50 (2H, d, *J* 7.9 Hz, 2 × Ar*H*), 7.43 – 7.34 (3H, m, 3 × Ar*H*), 6.99 (2H, d, *J* 8.6 Hz, 2 × Ar*H*), 6.84 (2H, d, *J* 8.6 Hz, 2 × Ar*H*), 4.95 (1H, s, C_3H), 4.32 – 4.25 (3H, m, C_4H + NCH_2Ar), 4.05 – 3.87 (2H, m, C_2H + $C_5H_aH_b$), 3.79 (3H, s, O*Me*), 3.17 – 3.06 (2H, m, CH_XH_YAr + $C_5H_aH_b$), 2.91 – 2.81 (1H, m, CH_XH_YAr), 2.15 (3H, s, O*Ac*); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 169.9 (1C, Q), 158.9 (1C, Q), 133.2 (2C, CH), 132.6 (2C, CH), 131.0 (2C, CH), 129.7 (2C, CH), 129.5 (1C, CH), 128.5 (2C, CH), 127.3 (1C, Q), 123.8 (1C, Q), 121.5 (2C, Q), 114.5 (2C, CH), 82.6 (1C, Q), 80.2 (1C, Q), 77.2 (1C, CH),* 75.0 (1C, Q), 73.6 (1C, Q), 72.3 (1C, CH), 69.2 (1C, CH), 60.2 (1C, CH_2), 58.9 (1C, CH_2), 55.3 (1C, CH_3), 30.5 (1C, CH_2), 20.6 (1C, CH_3); ***m/z*** (ESI) 480.2 ($[M + H]^+$, 100%), 324.9 (4), 246.9 (6); **HRMS** (ESI) calcd. for $C_{31}H_{30}O_4N$ $[M + H]^+$ 480.2169, found 480.2179. *Obscured by solvent peak – observed in HSQC.

References

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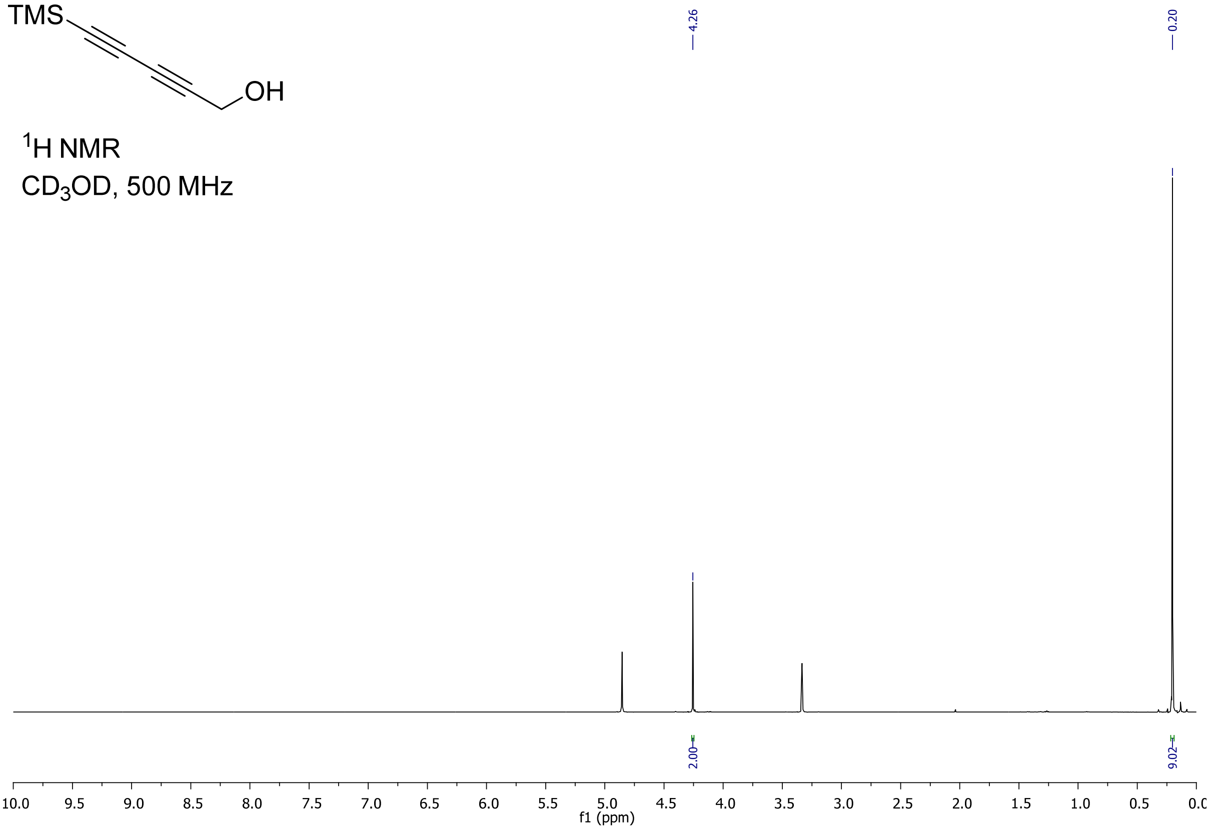
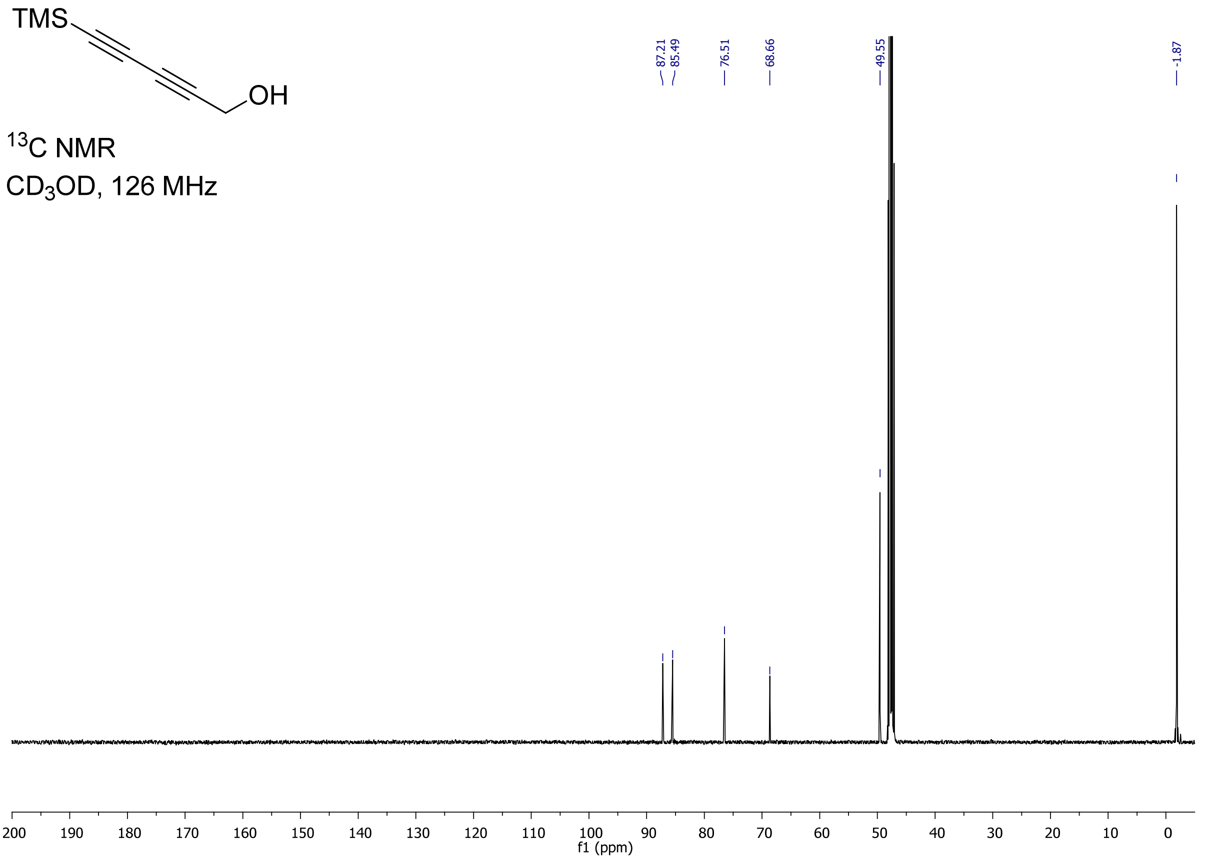
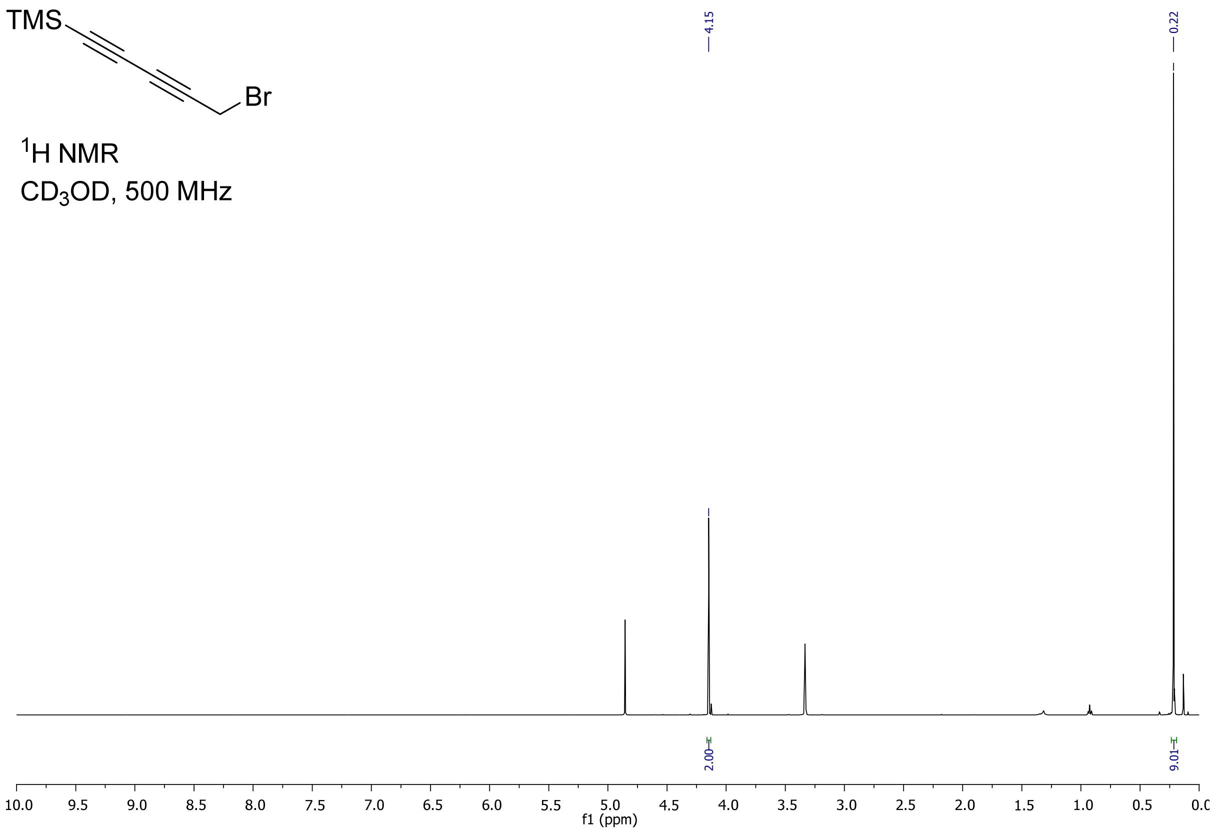
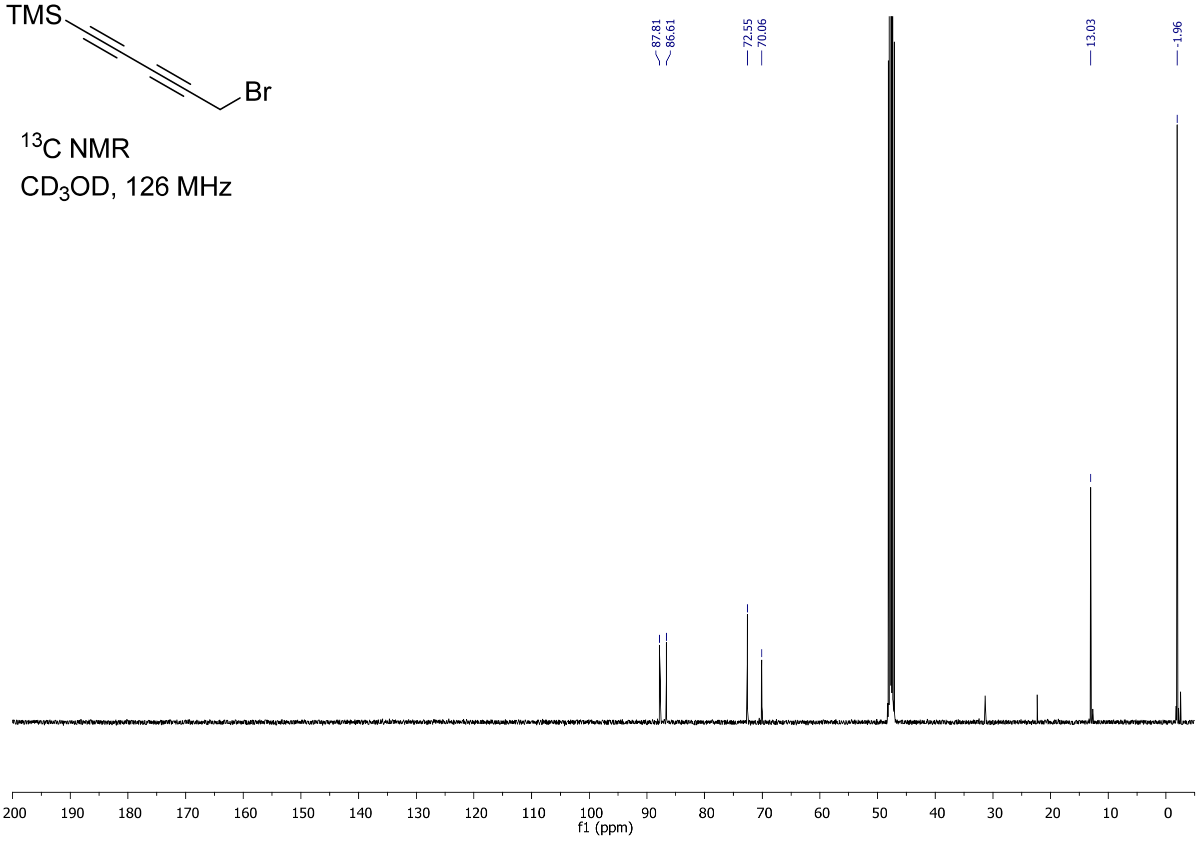

TMS
OH
^{1}H NMR
CD_3OD, 500 MHz
4.26
0.20
2.00
9.02
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


TMS
OH
^{13}C NMR
CD_3OD, 126 MHz
87.21
85.49
76.51
68.66
49.55
-1.87
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



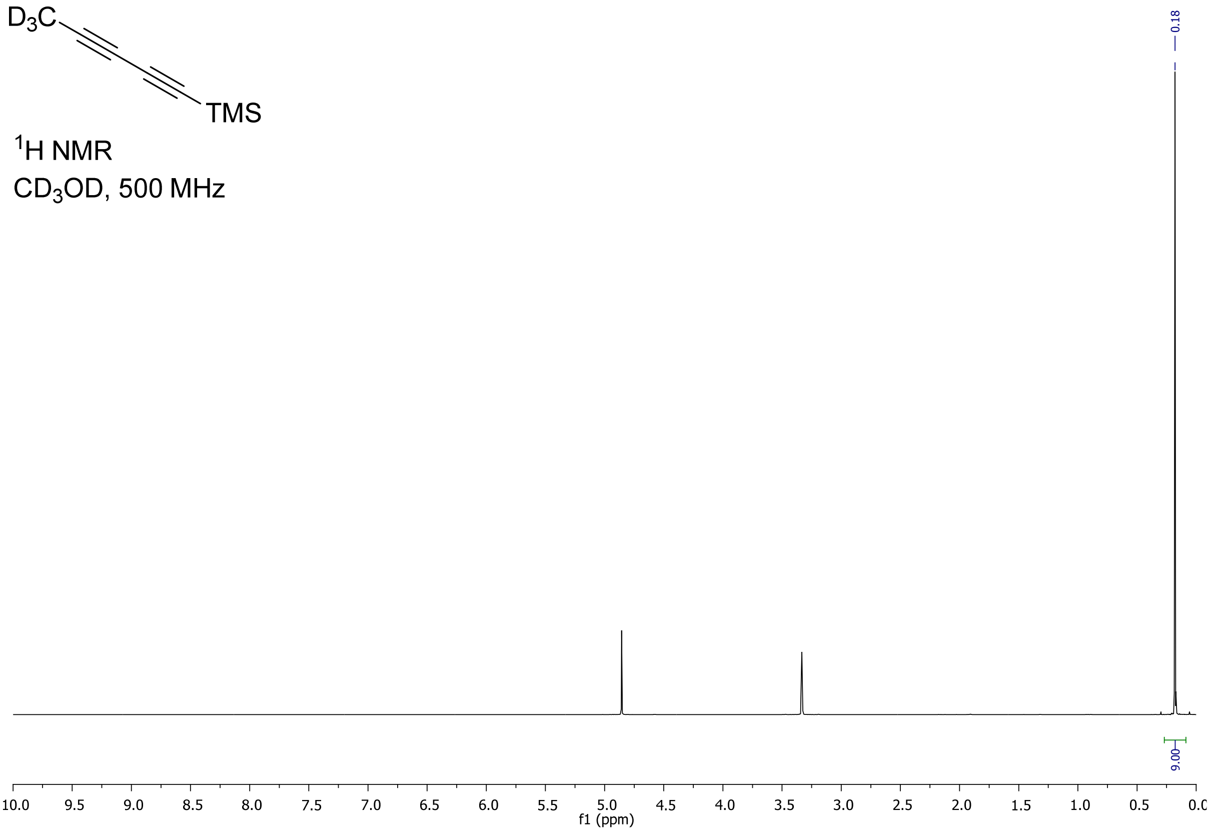
^{1}H NMR

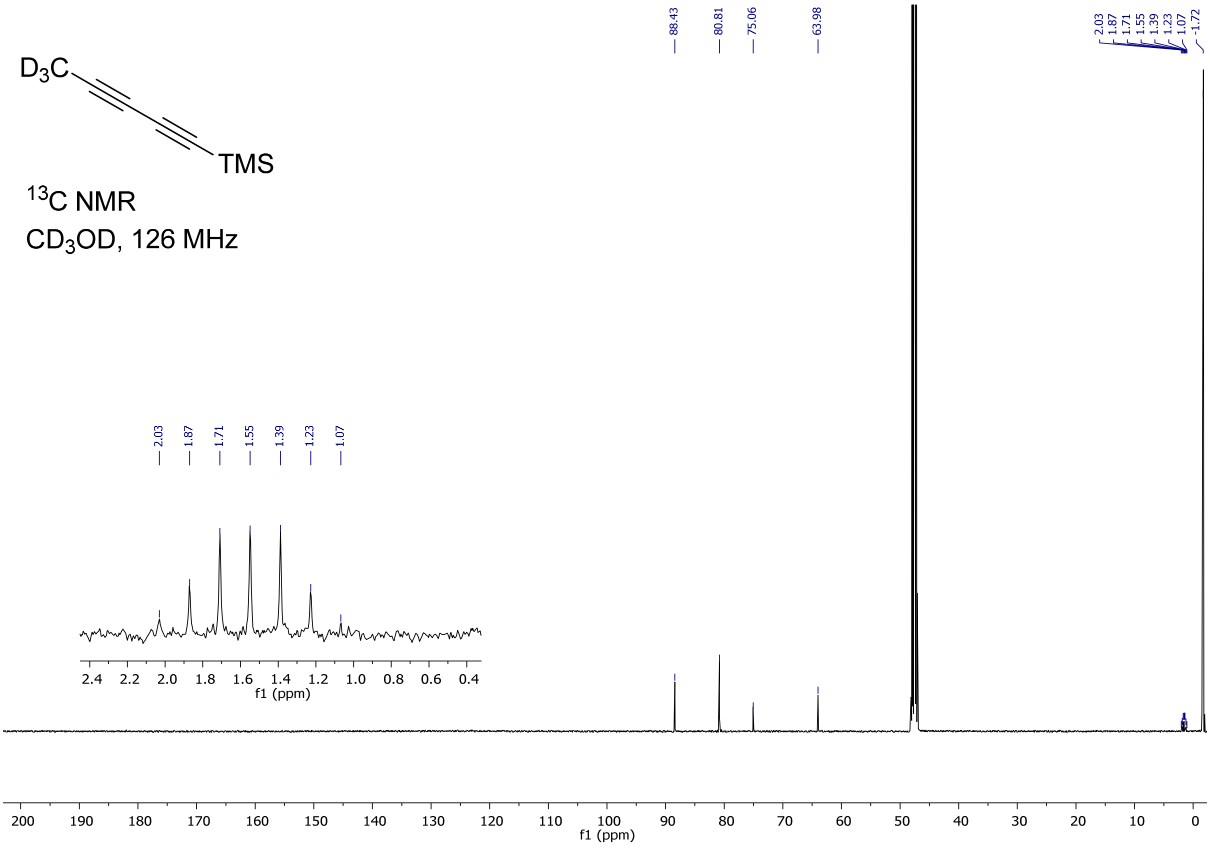
CD_3OD, 500 MHz

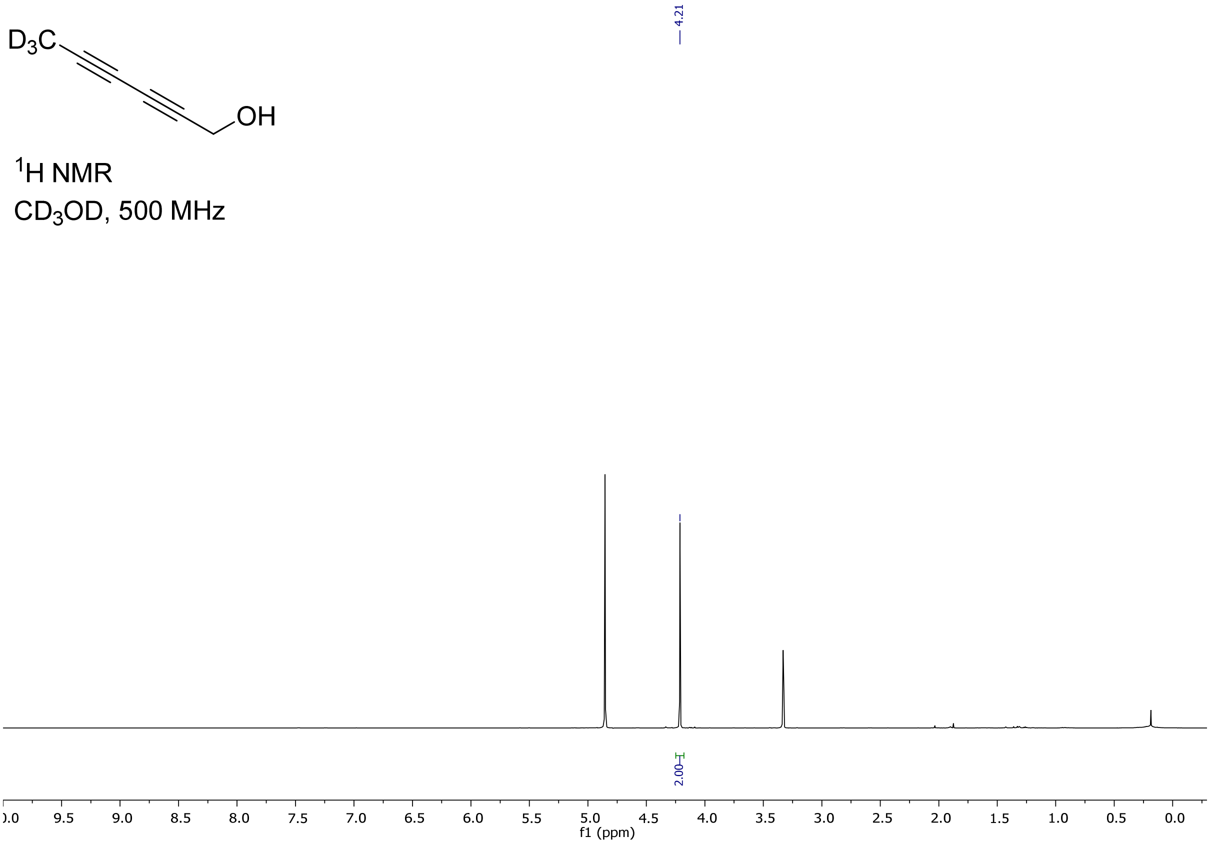


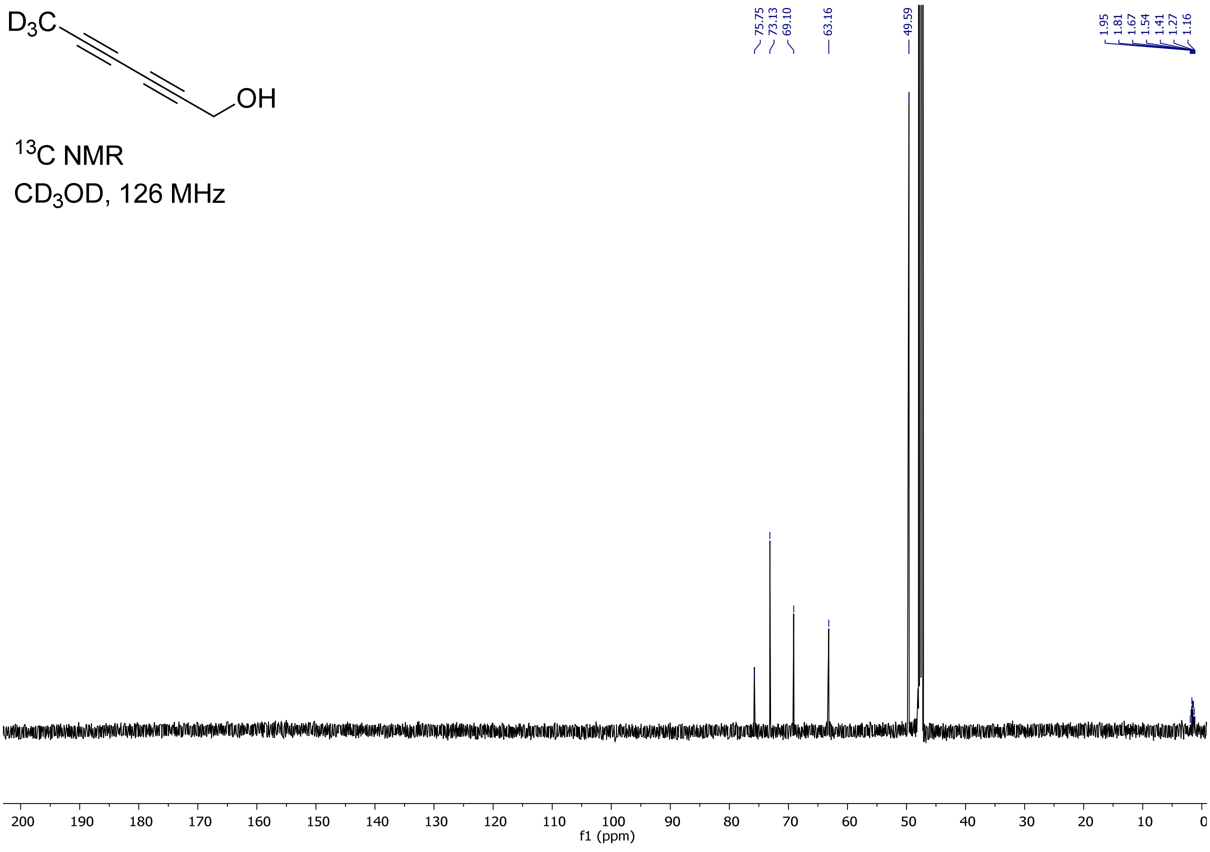
^{13}C NMR

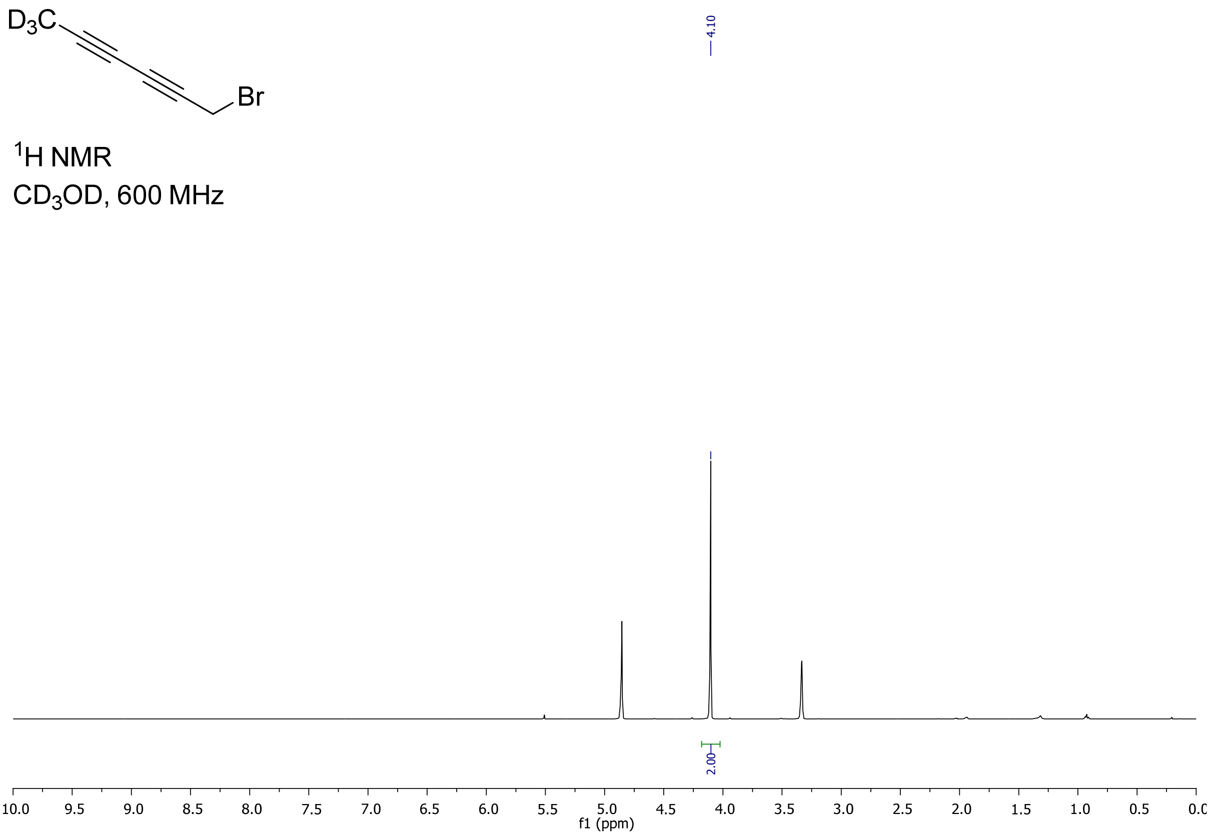
CD_3OD, 126 MHz

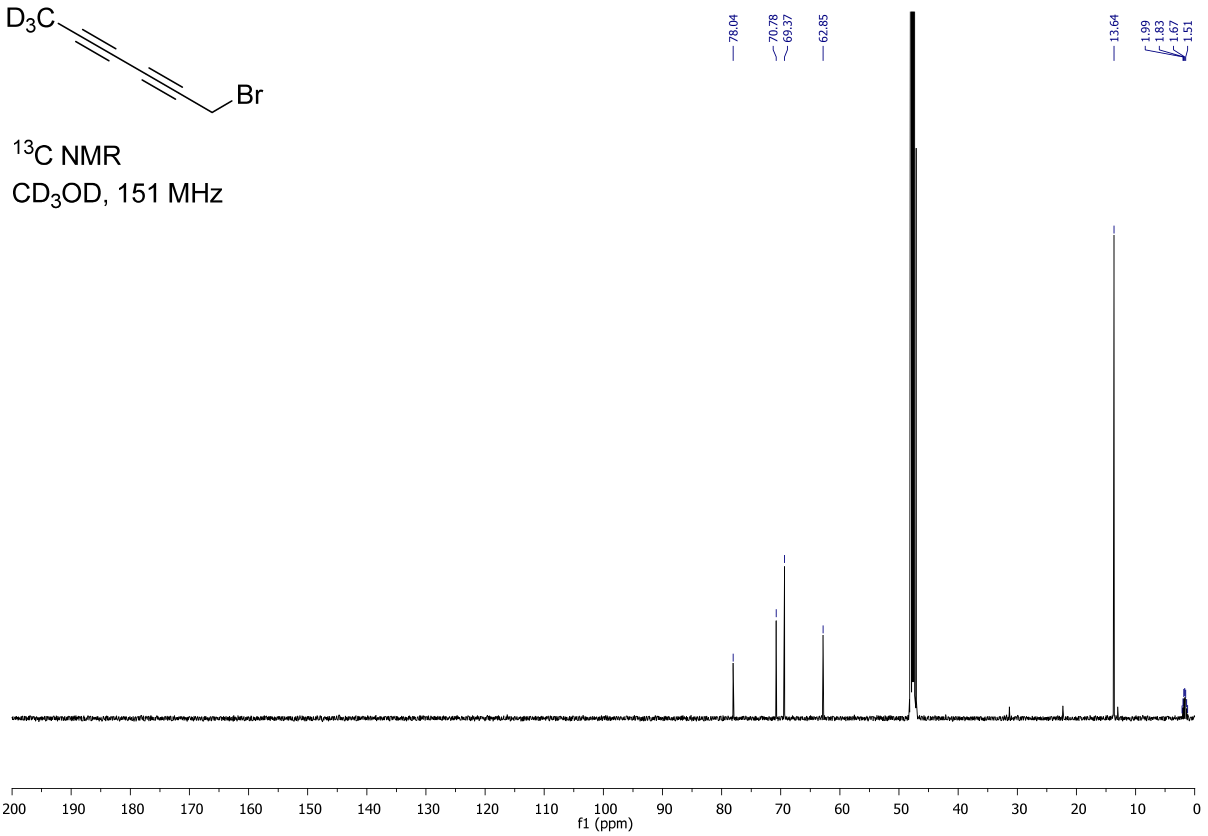

D$_3$C
TMS
^{1}H NMR
CD$_3$OD, 500 MHz
0.18
9.00
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

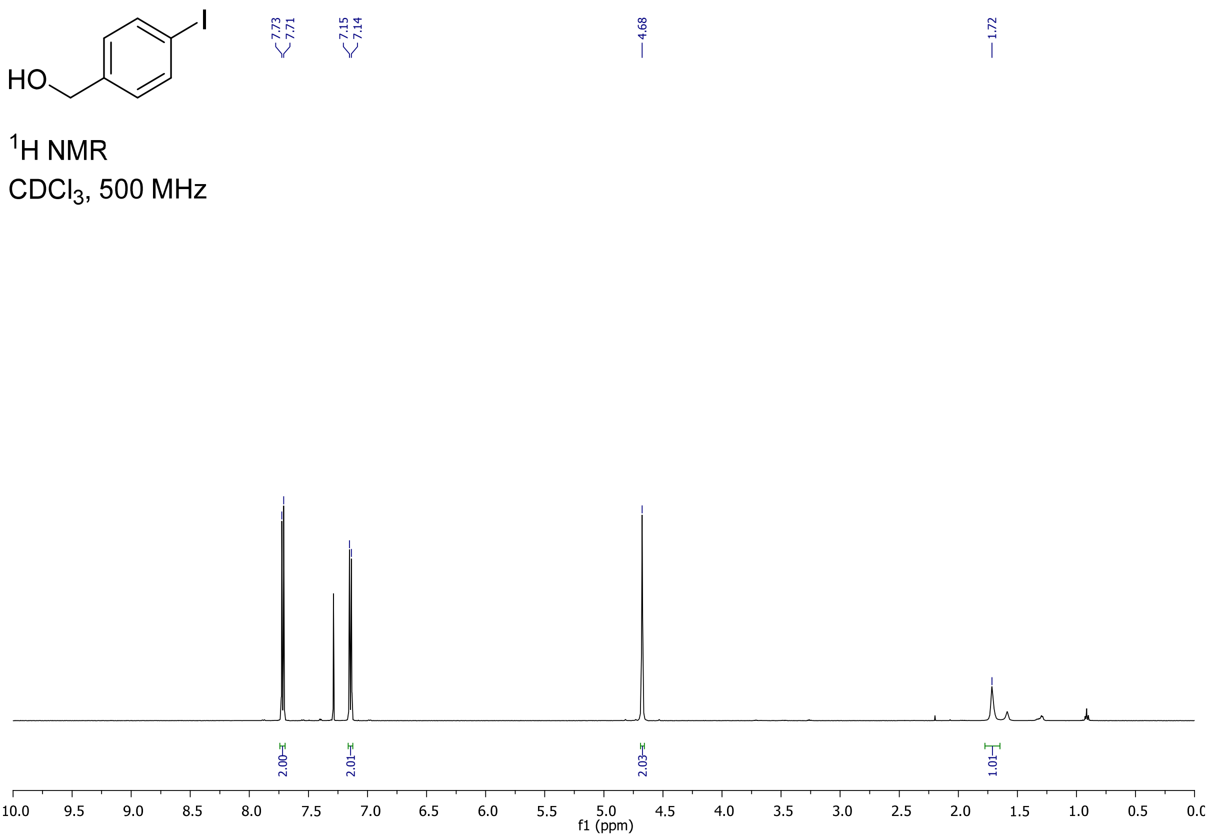

88.43
80.81
75.06
63.98
2.03
1.87
1.71
1.55
1.39
1.23
1.07
-1.72
D$_3$C
TMS
^{13}C NMR
CD$_3$OD, 126 MHz
2.03
1.87
1.71
1.55
1.39
1.23
1.07
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
f1 (ppm)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


D3C
OH
^{1}H NMR
CD_3OD, 500 MHz
4.21
2.00
).0
9.5
9.0
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
0.5
0.0
f1 (ppm)

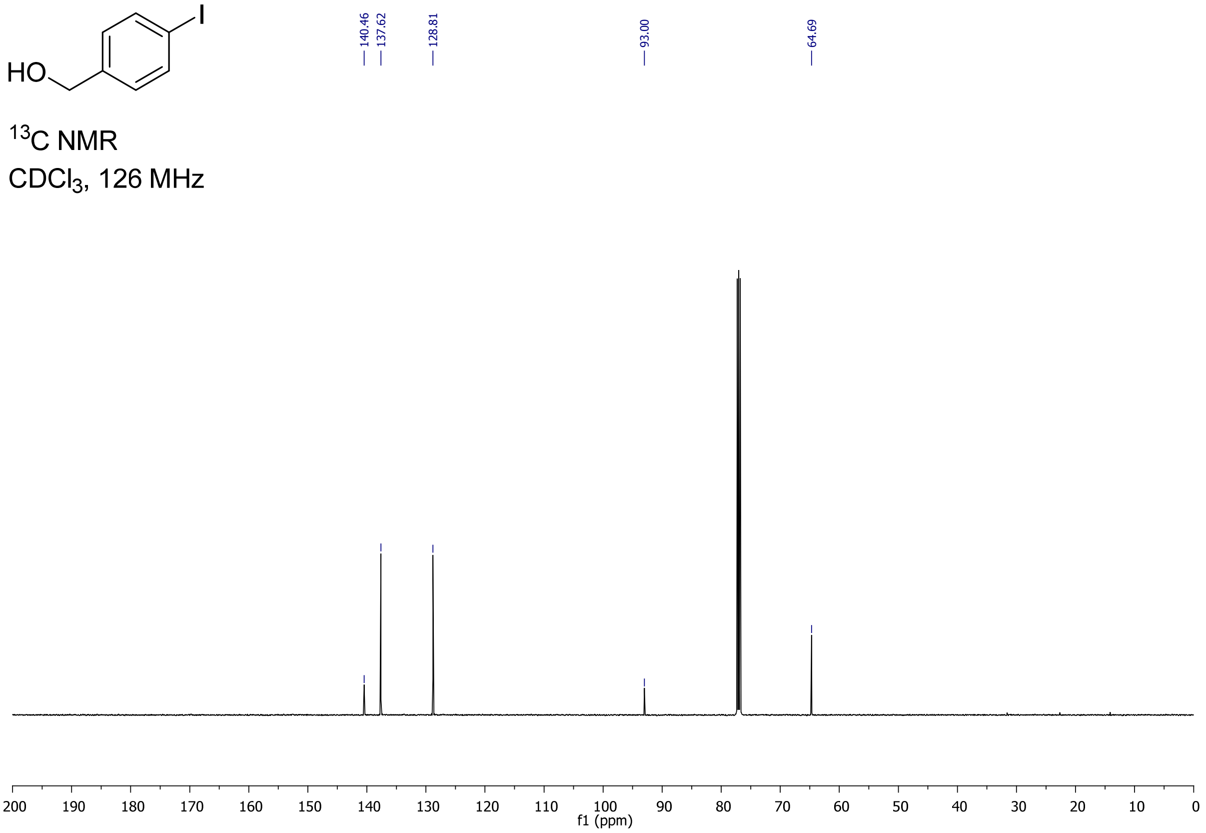

D3C
OH
^{13}C NMR
CD_3OD, 126 MHz
75.75
73.13
69.10
63.16
49.59
1.95
1.81
1.67
1.54
1.41
1.27
1.16
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


D3C
Br
1H NMR
CD3OD, 600 MHz
4.10
2.00
10.0
9.5
9.0
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
0.5
0.0
f1 (ppm)

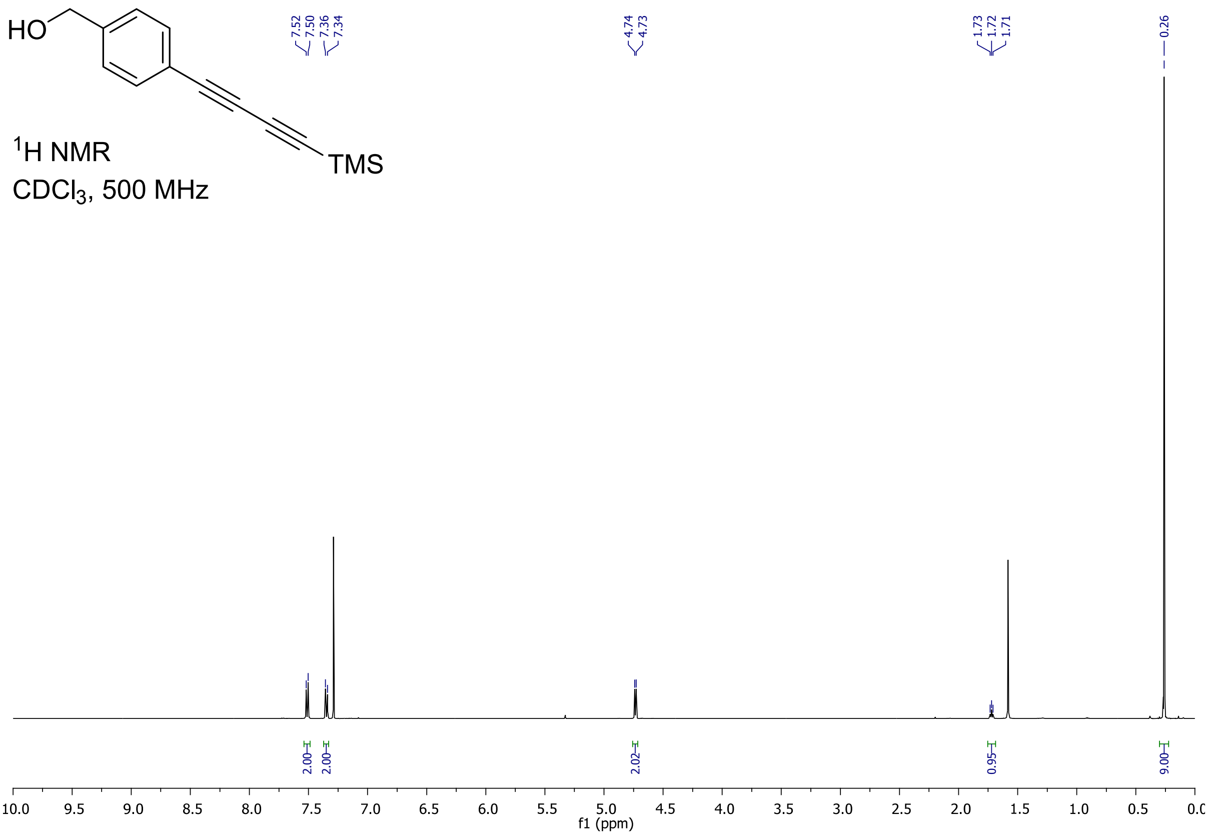

D3C
Br
13C NMR
CD3OD, 151 MHz
78.04
70.78
69.37
62.85
13.64
1.99
1.83
1.67
1.51
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

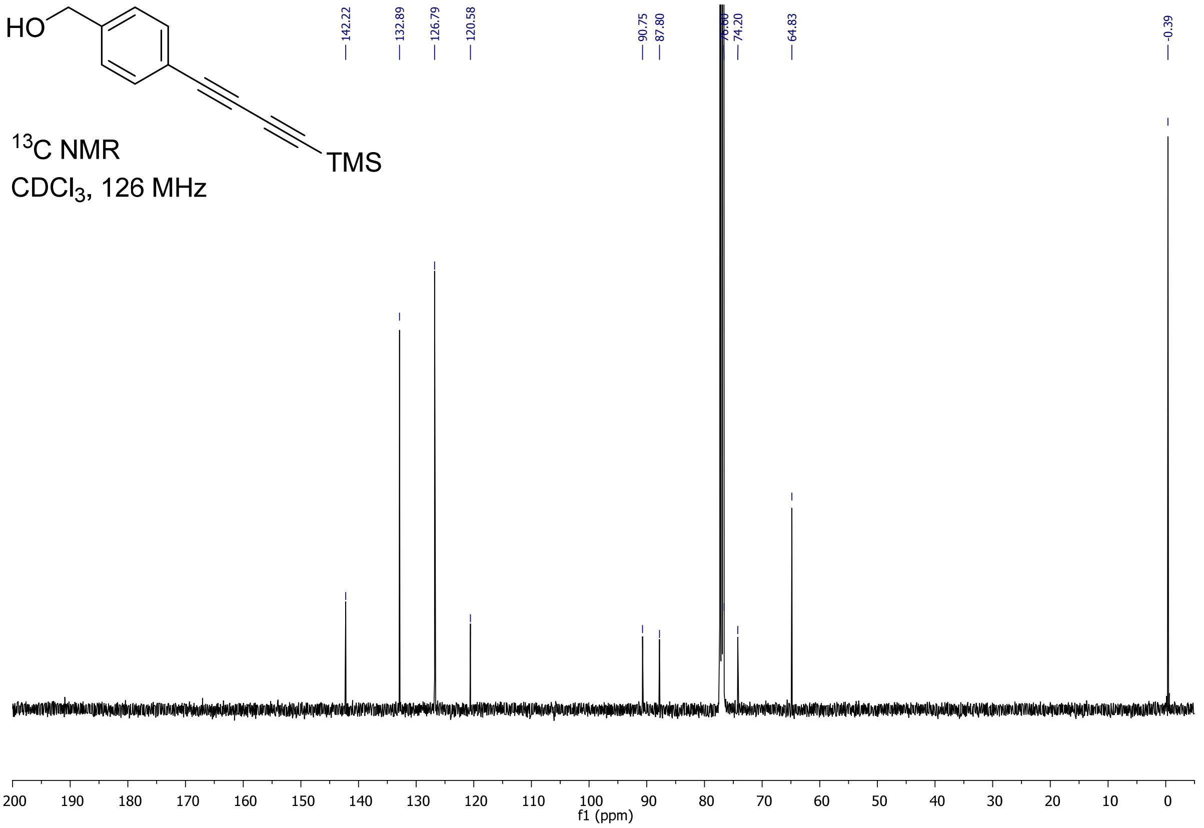


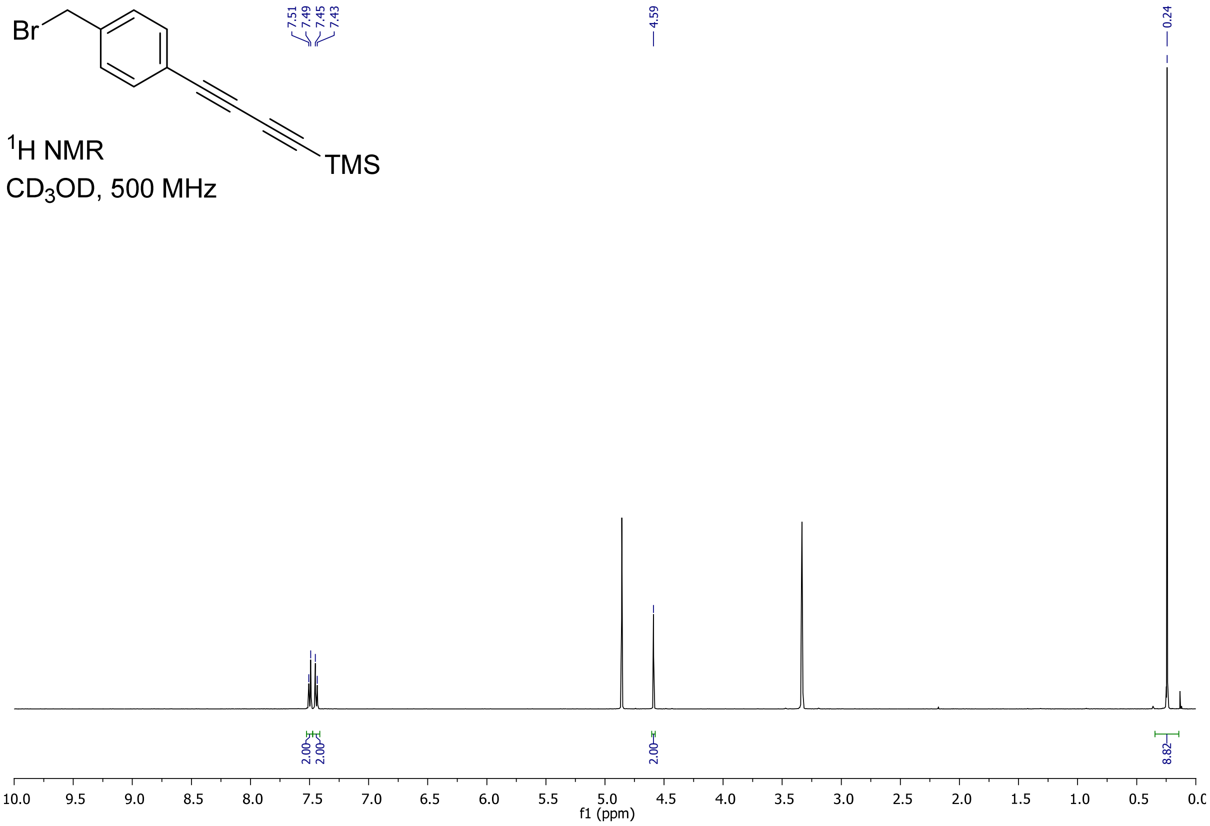
^{1}H NMR
CDCl$_3$, 500 MHz

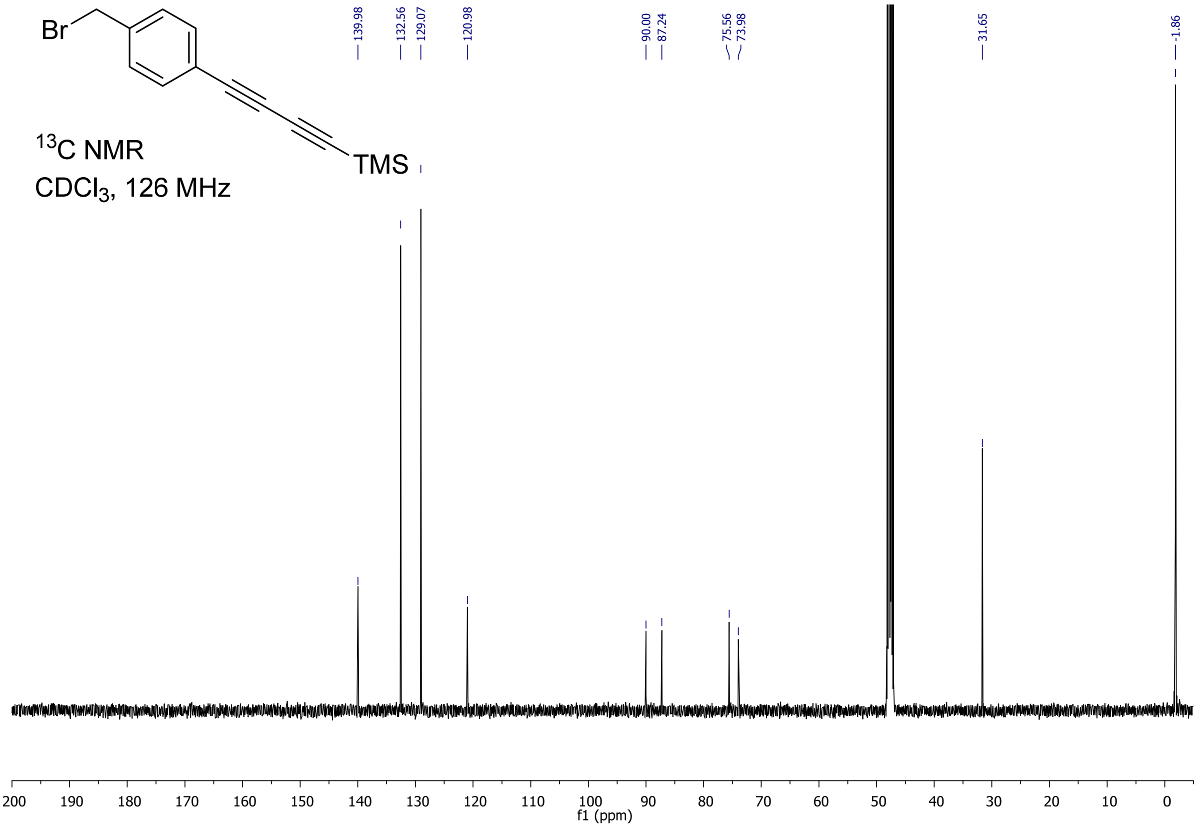


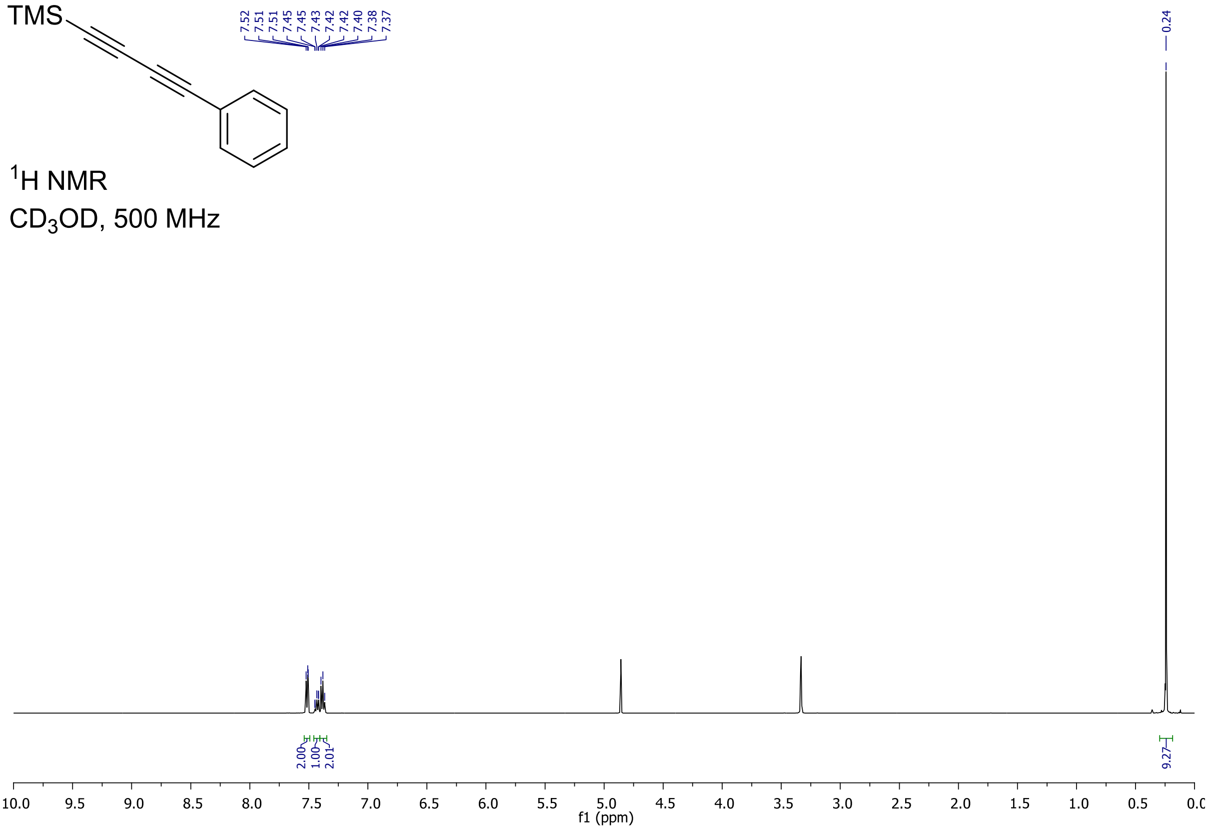
^{13}C NMR
CDCl$_3$, 126 MHz

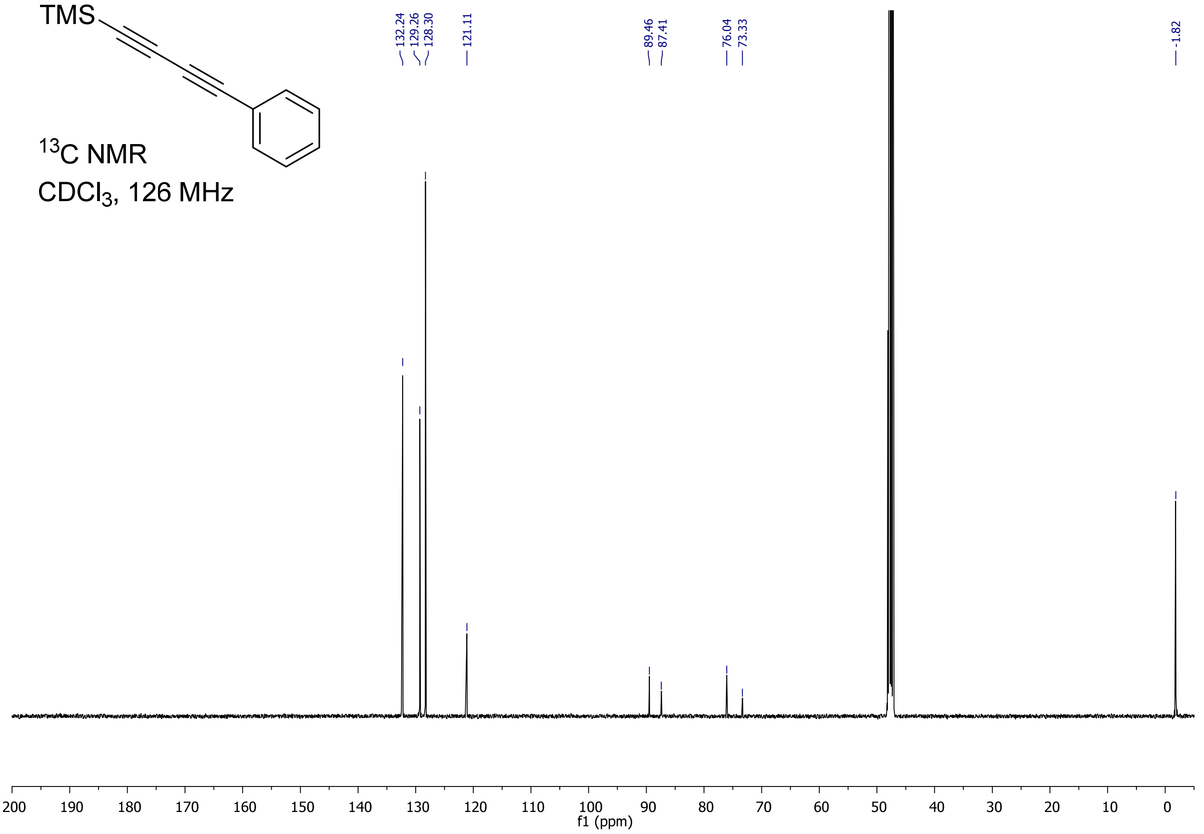

HO
TMS
^{1}H NMR
CDCl$_3$, 500 MHz
7.52
7.50
7.36
7.34
4.74
4.73
1.73
1.72
1.71
0.26
2.00
2.00
2.02
0.95
9.00
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

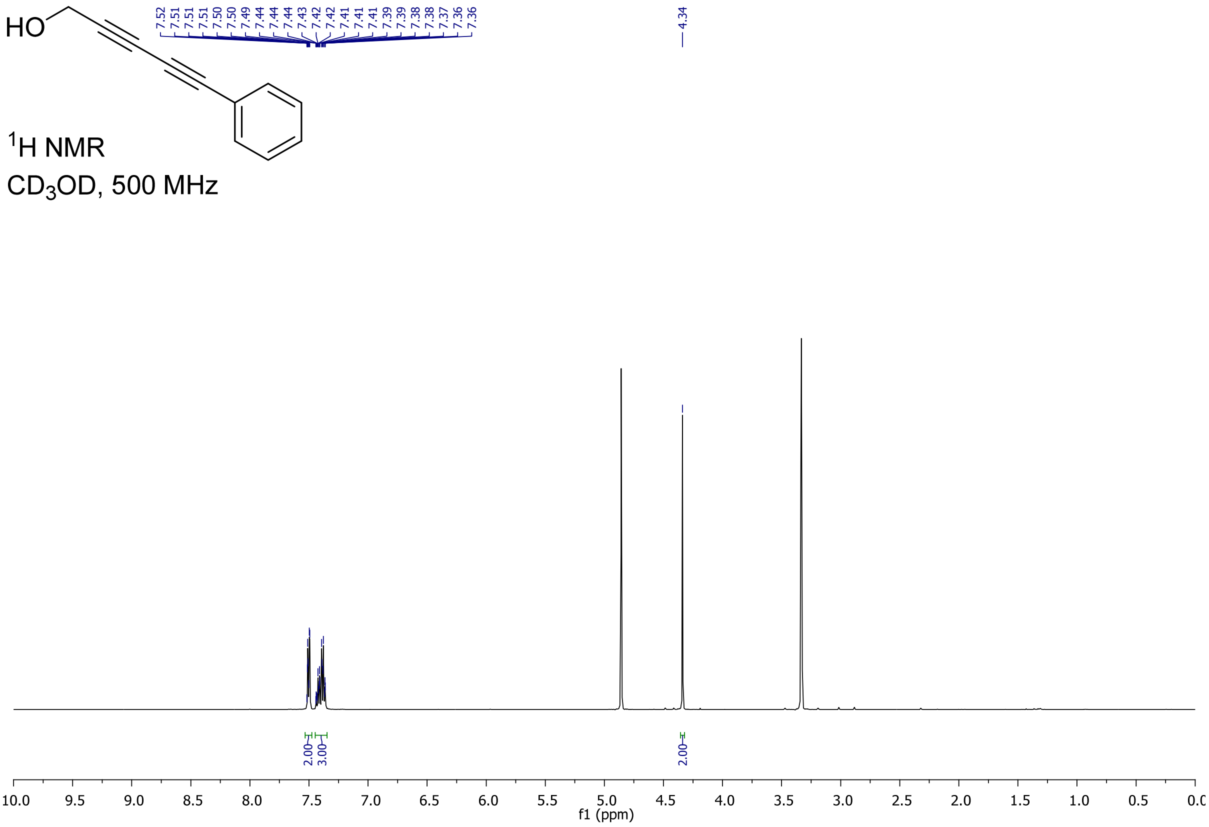

HO
TMS
^{13}C NMR
CDCl$_3$, 126 MHz
142.22
132.89
126.79
120.58
90.75
87.80
76.60
74.20
64.83
-0.39
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

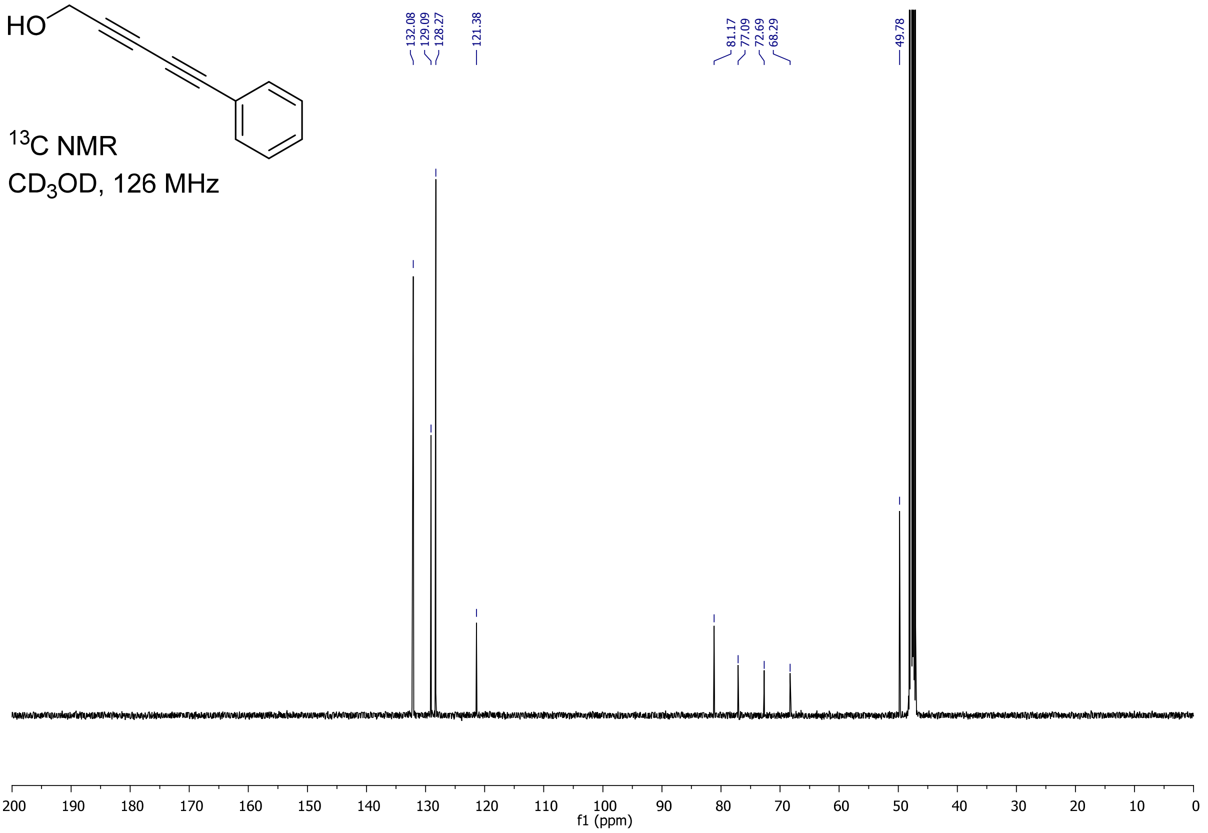

Br
TMS
^{1}H NMR
CD_3OD, 500 MHz
7.51
7.49
7.45
7.43
4.59
0.24
2.00
2.00
2.00
8.82
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

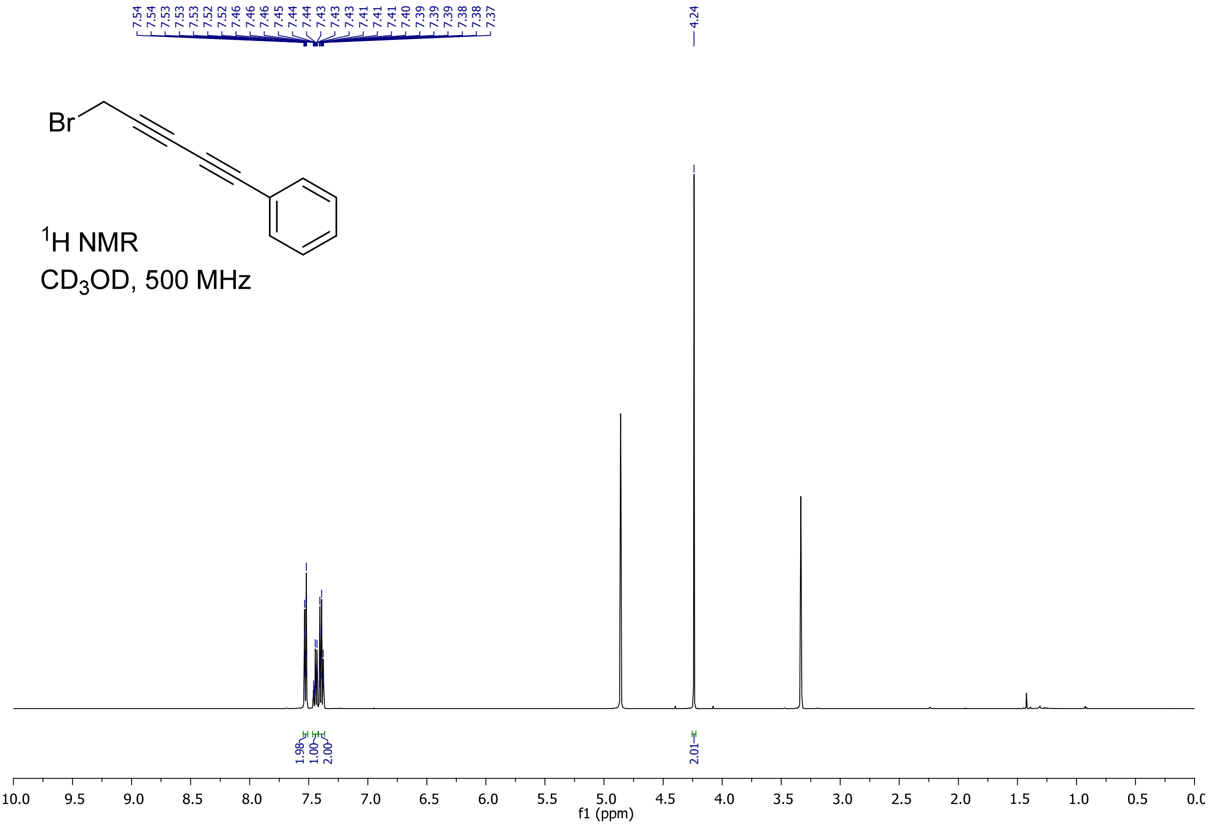

Br
TMS
^{13}C NMR
$CDCl_3$, 126 MHz
139.98
132.56
129.07
120.98
90.00
87.24
75.56
73.98
31.65
-1.86
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

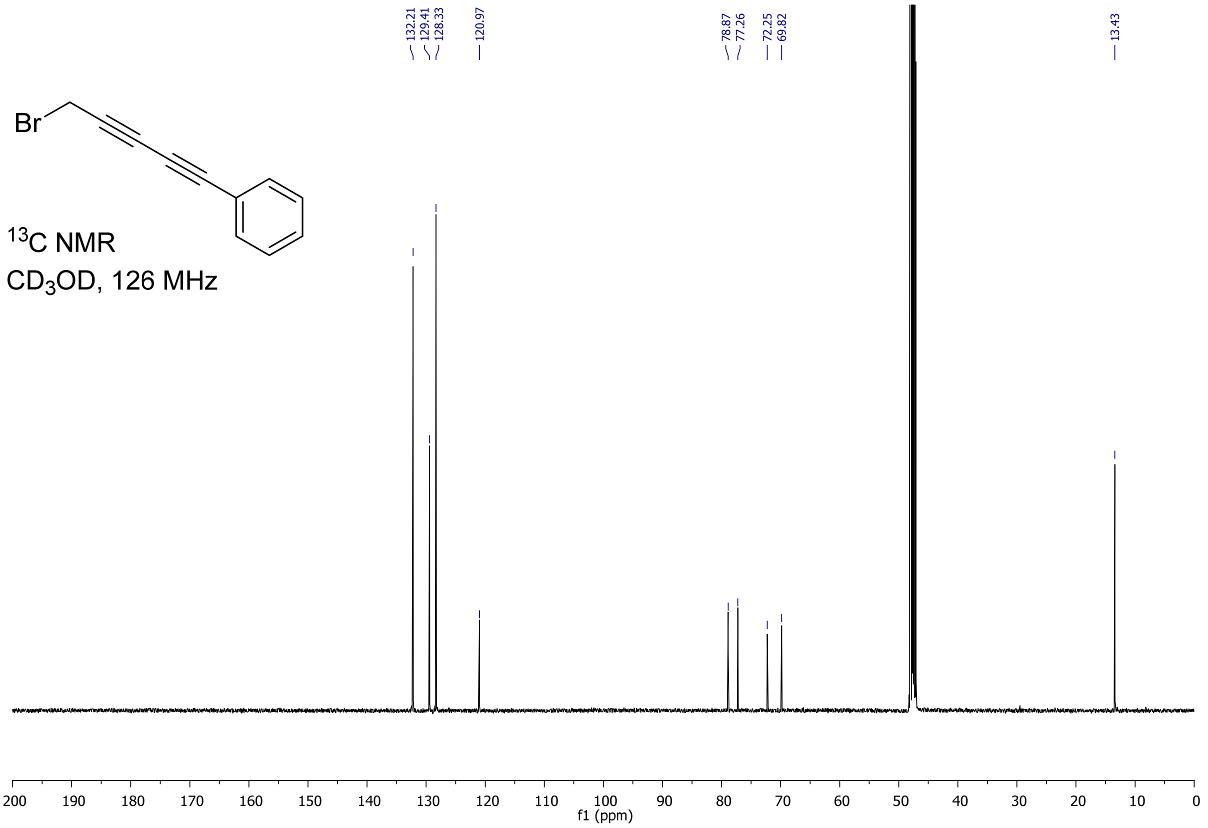

TMS
^{1}H NMR
CD$_3$OD, 500 MHz
7.52
7.51
7.51
7.45
7.43
7.43
7.42
7.42
7.40
7.38
7.37
0.24
2.00
1.00
2.01
9.27
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

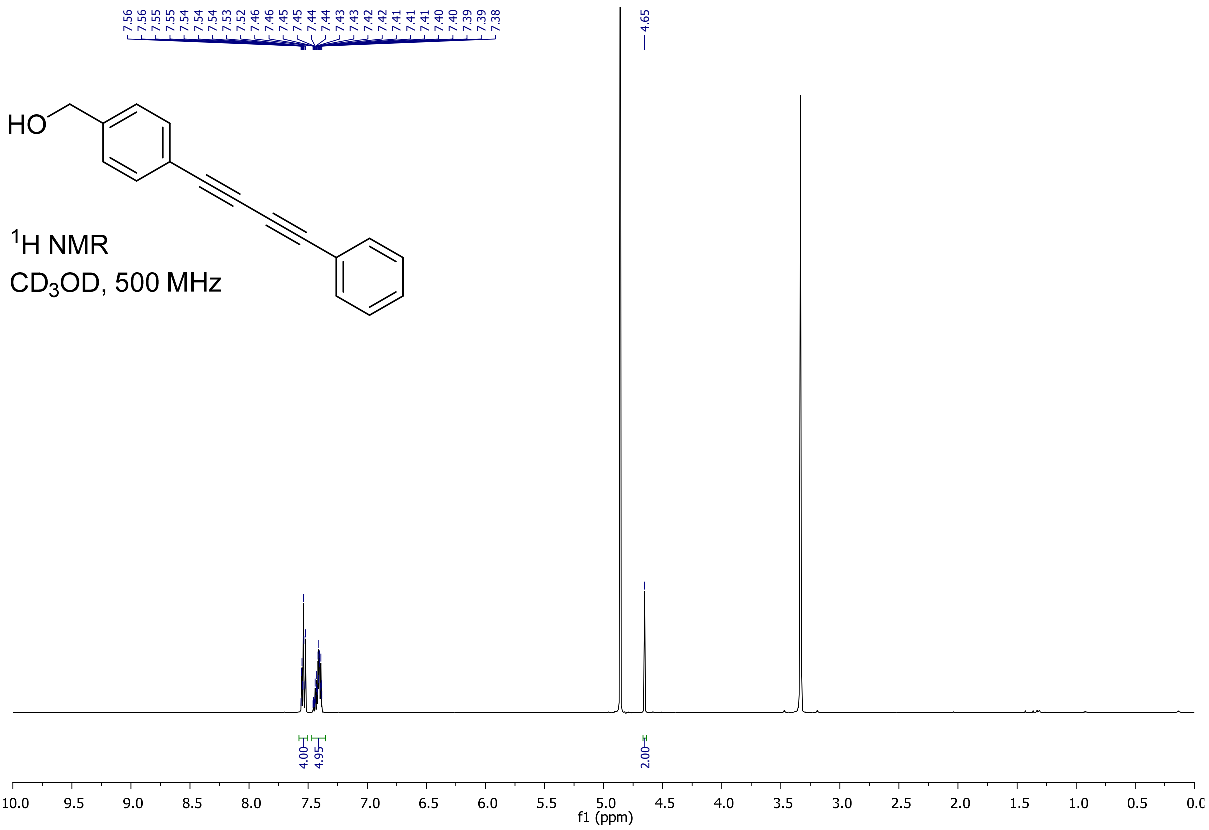

TMS
^{13}C NMR
CDCl$_3$, 126 MHz
132.24
129.26
128.30
121.11
89.46
87.41
76.04
73.33
-1.82
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

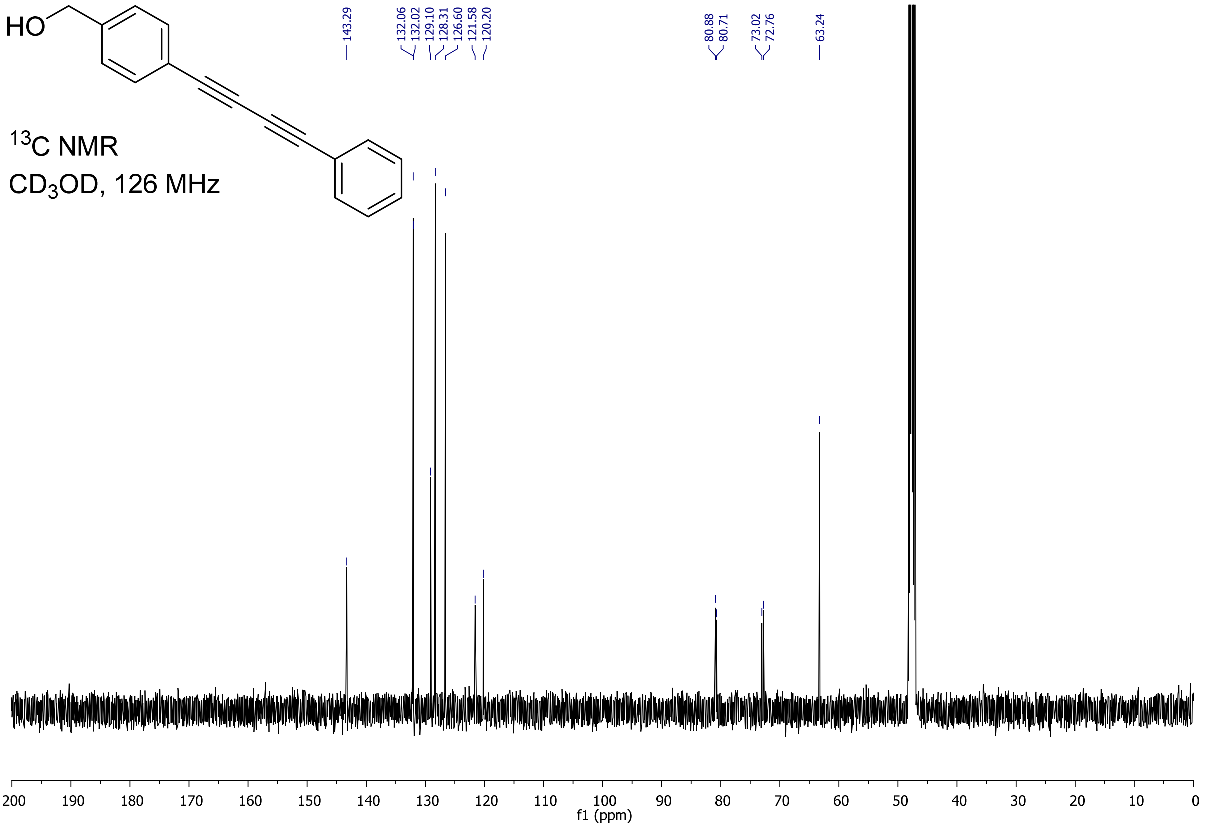

HO
^{1}H NMR
CD$_3$OD, 500 MHz
7.52
7.51
7.51
7.51
7.50
7.49
7.44
7.44
7.44
7.43
7.42
7.42
7.41
7.41
7.41
7.39
7.39
7.38
7.38
7.37
7.36
7.36
4.34
2.00
3.00
2.00
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

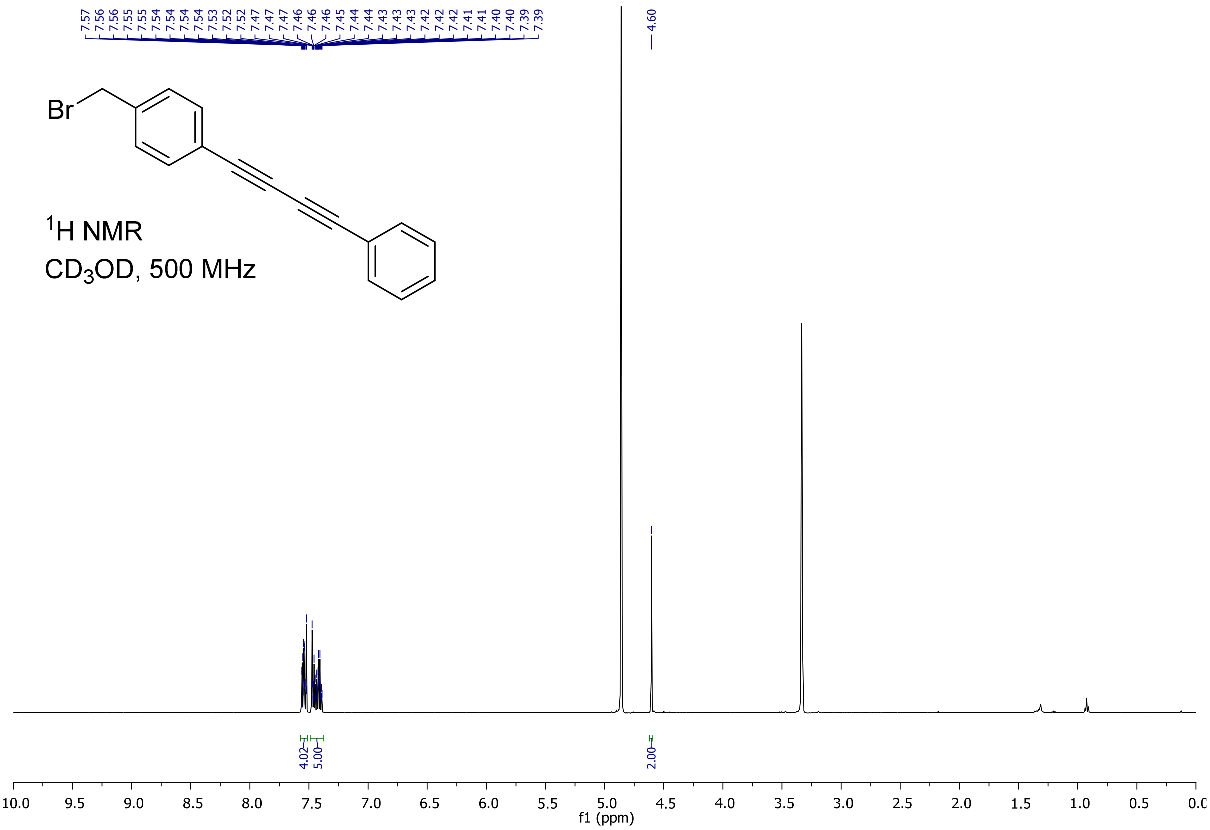

HO
^{13}C NMR
CD$_3$OD, 126 MHz
132.08
129.09
128.27
121.38
81.17
77.09
72.69
68.29
49.78
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

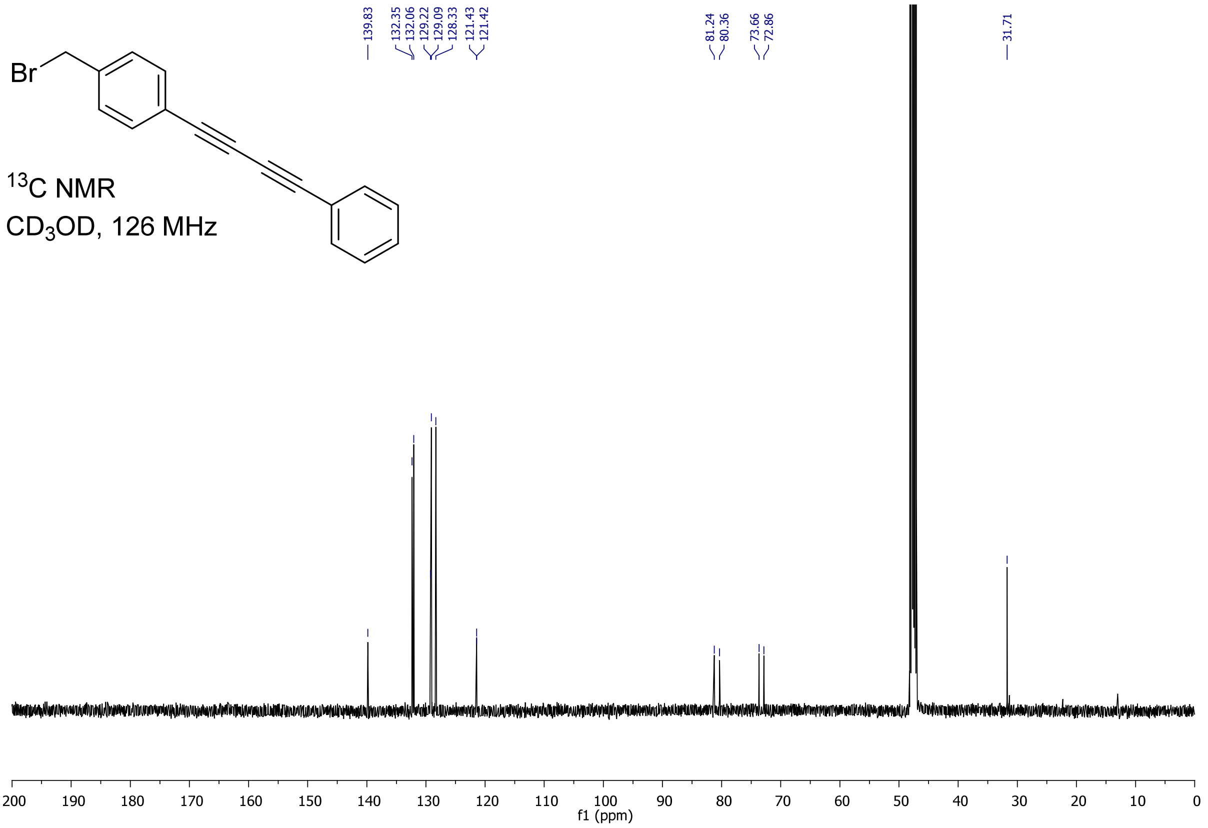

7.54
7.54
7.53
7.53
7.53
7.52
7.52
7.46
7.46
7.46
7.45
7.44
7.44
7.43
7.43
7.43
7.41
7.41
7.41
7.40
7.39
7.39
7.39
7.38
7.38
7.37
4.24
Br
1H NMR
CD3OD, 500 MHz
1.98
1.00
2.00
2.01
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

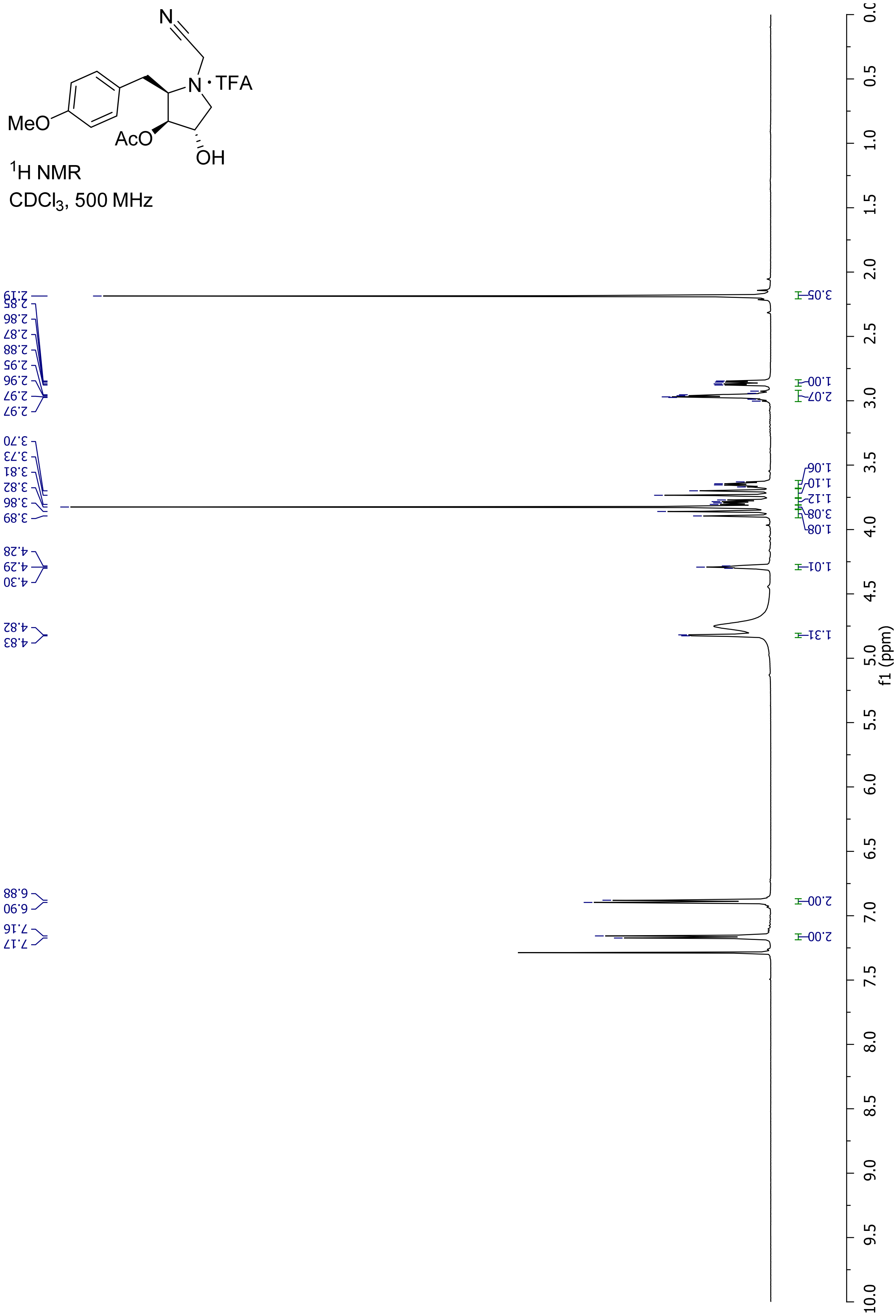

132.21
129.41
128.33
120.97
78.87
77.26
72.25
69.82
13.43
Br
13C NMR
CD3OD, 126 MHz
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.56
7.56
7.55
7.55
7.54
7.54
7.54
7.53
7.52
7.46
7.46
7.45
7.45
7.44
7.44
7.43
7.43
7.42
7.42
7.41
7.41
7.41
7.40
7.40
7.39
7.39
7.38
4.65
HO
1H NMR
CD3OD, 500 MHz
4.00
4.95
2.00
10.0
9.5
9.0
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
0.5
0.0
f1 (ppm)


HO
13C NMR
CD3OD, 126 MHz
143.29
132.06
132.02
129.10
128.31
126.60
121.58
120.20
80.88
80.71
73.02
72.76
63.24
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

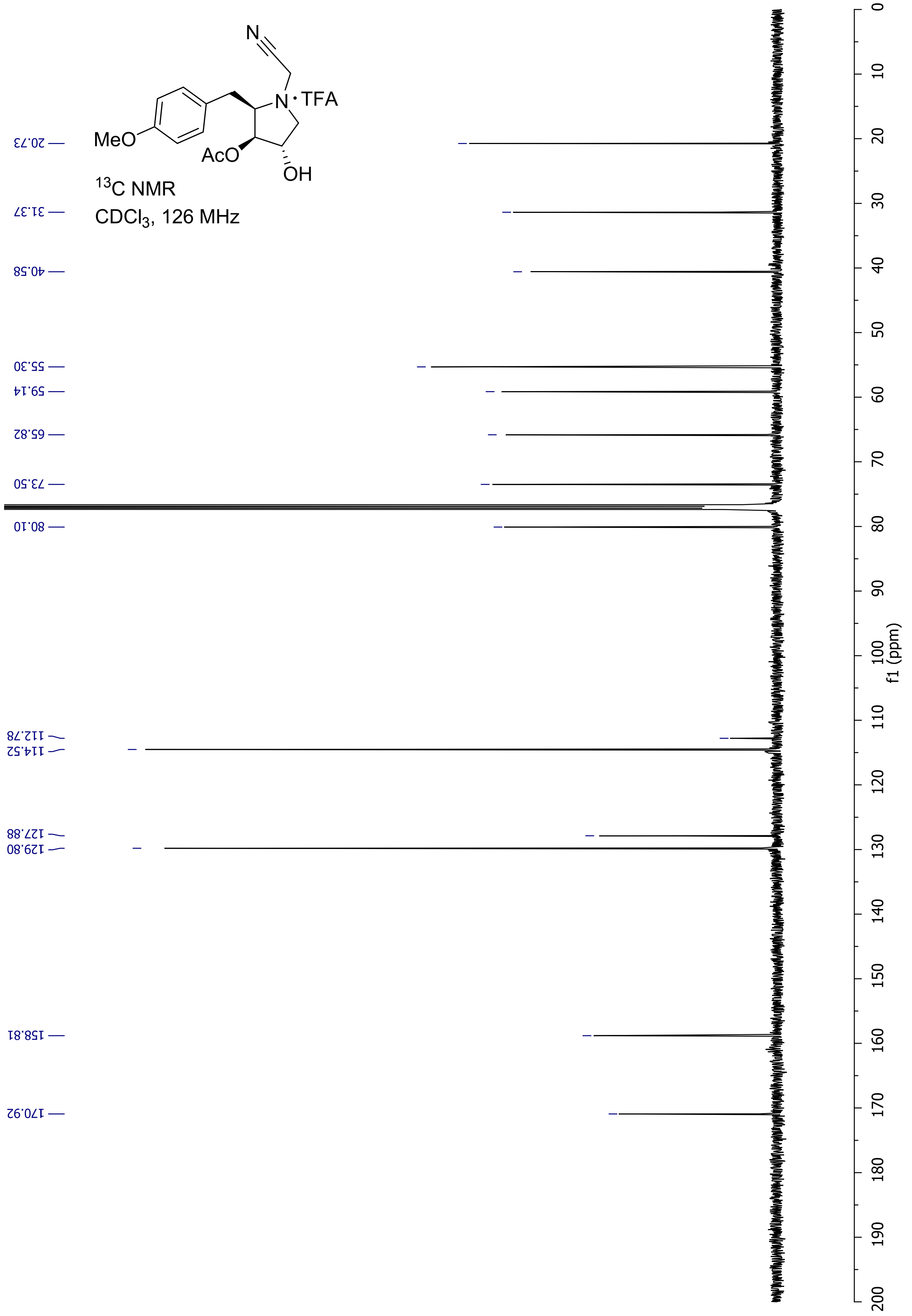

7.57 7.56 7.56 7.55 7.54 7.54 7.54 7.54 7.53 7.52 7.52 7.47 7.47 7.47 7.46 7.46 7.46 7.45 7.44 7.44 7.43 7.43 7.42 7.42 7.42 7.41 7.41 7.40 7.40 7.39 7.39
4.60
Br
^{1}H NMR
CD$_3$OD, 500 MHz
4.02
5.00
2.00
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


139.83
132.35
132.06
129.22
129.09
128.33
121.43
121.42
81.24
80.36
73.66
72.86
31.71
Br
^{13}C NMR
CD$_3$OD, 126 MHz
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



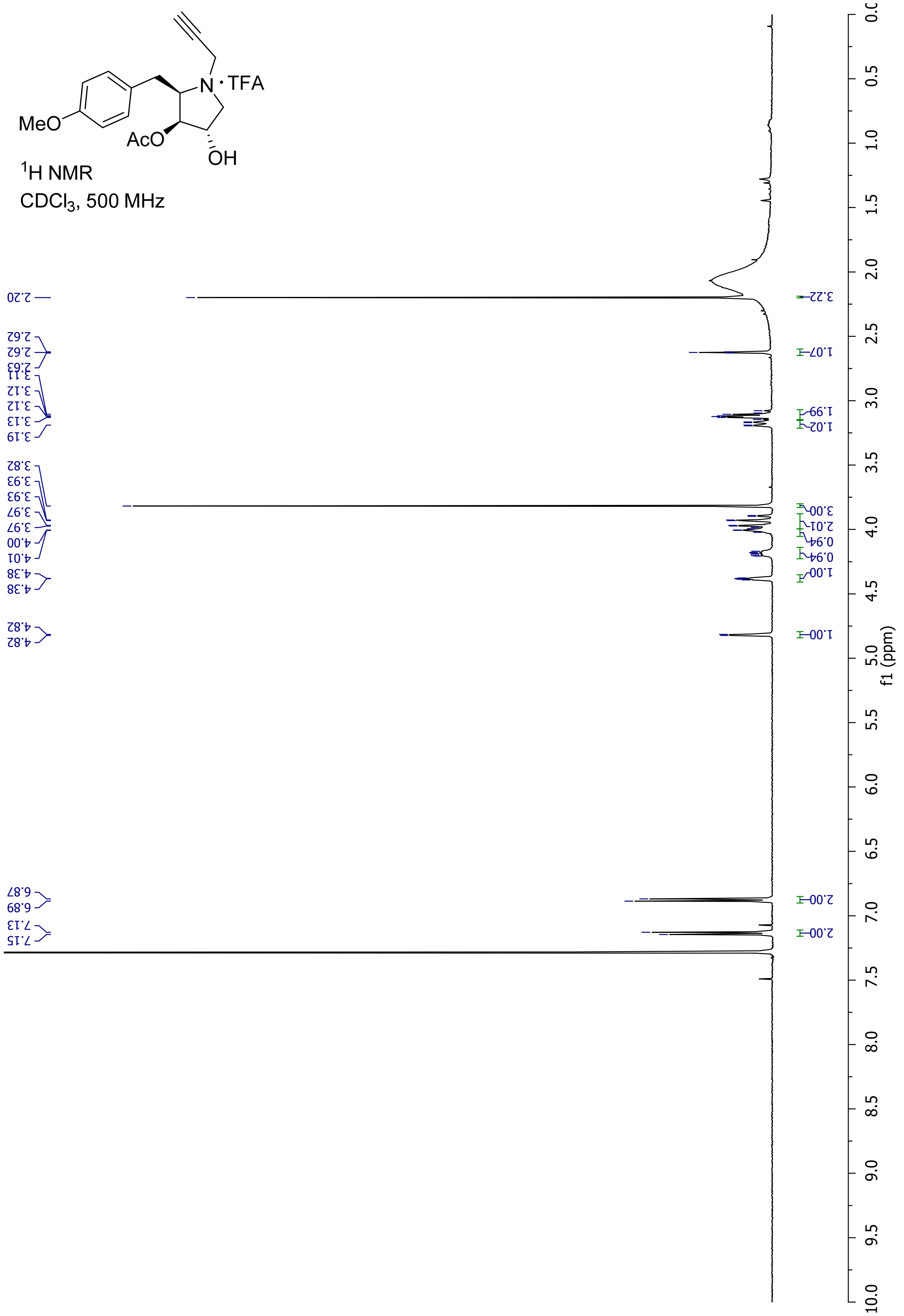
^{1}H NMR

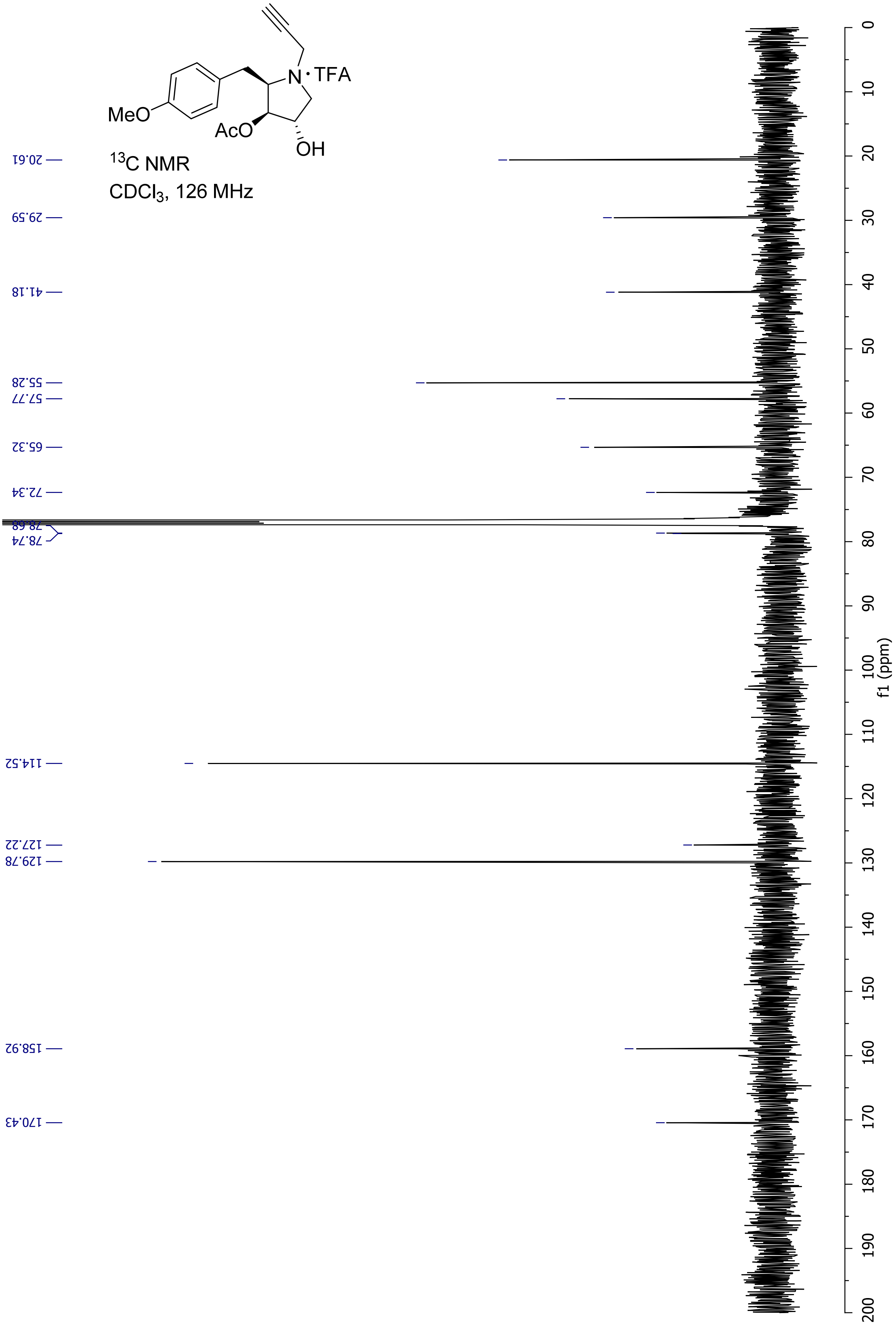
$CDCl_3$, 500 MHz

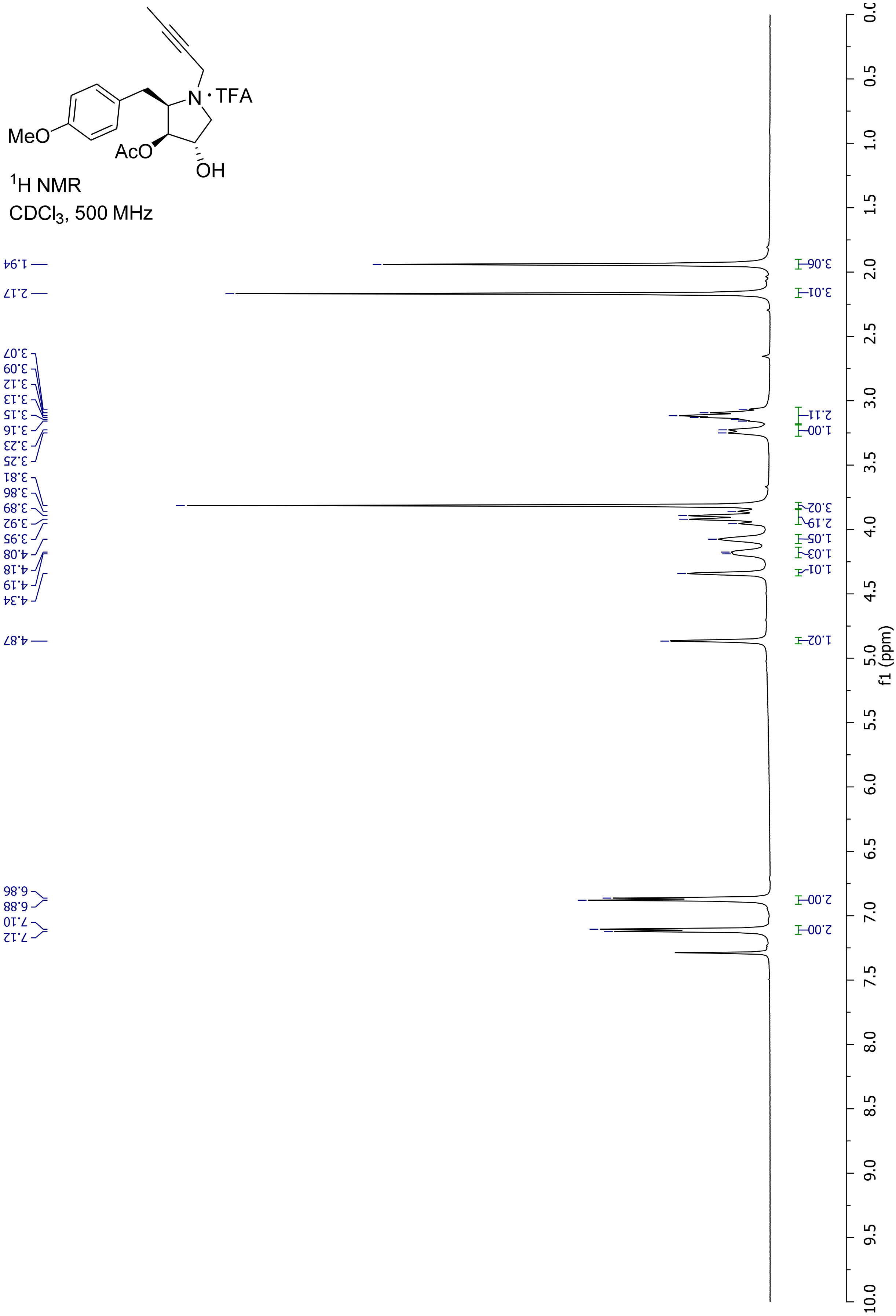


^{13}C NMR

$CDCl_3$, 126 MHz

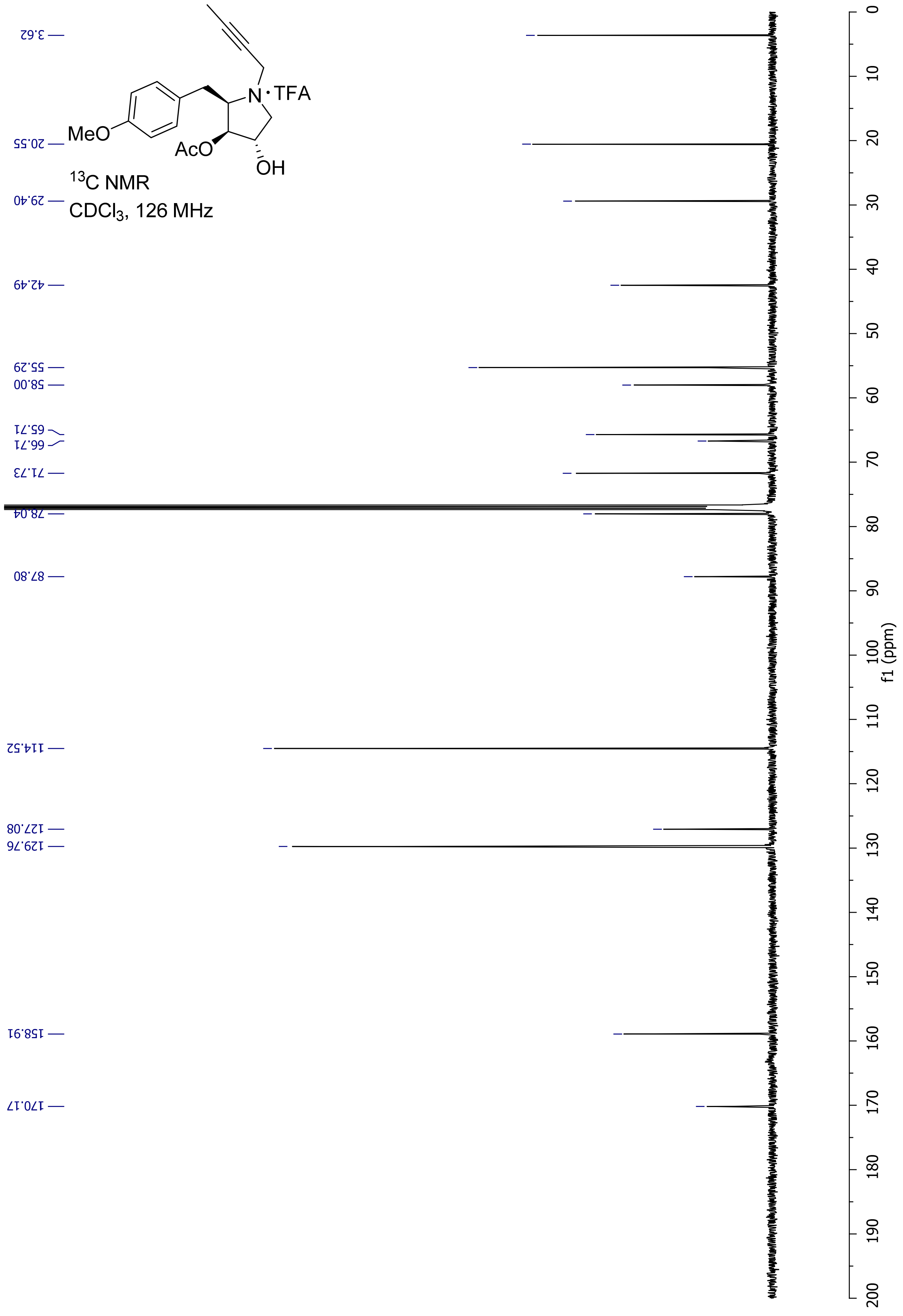

MeO
AcO
OH
N
TFA
^{1}H NMR
$CDCl_3$, 500 MHz
2.20
2.62
2.62
2.63
3.11
3.12
3.12
3.13
3.19
3.82
3.93
3.93
3.97
3.97
4.00
4.01
4.38
4.38
4.82
4.82
6.87
6.89
7.13
7.15
3.22
1.07
1.99
1.02
3.00
2.01
0.94
0.94
1.00
1.00
2.00
2.00
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
f1 (ppm)

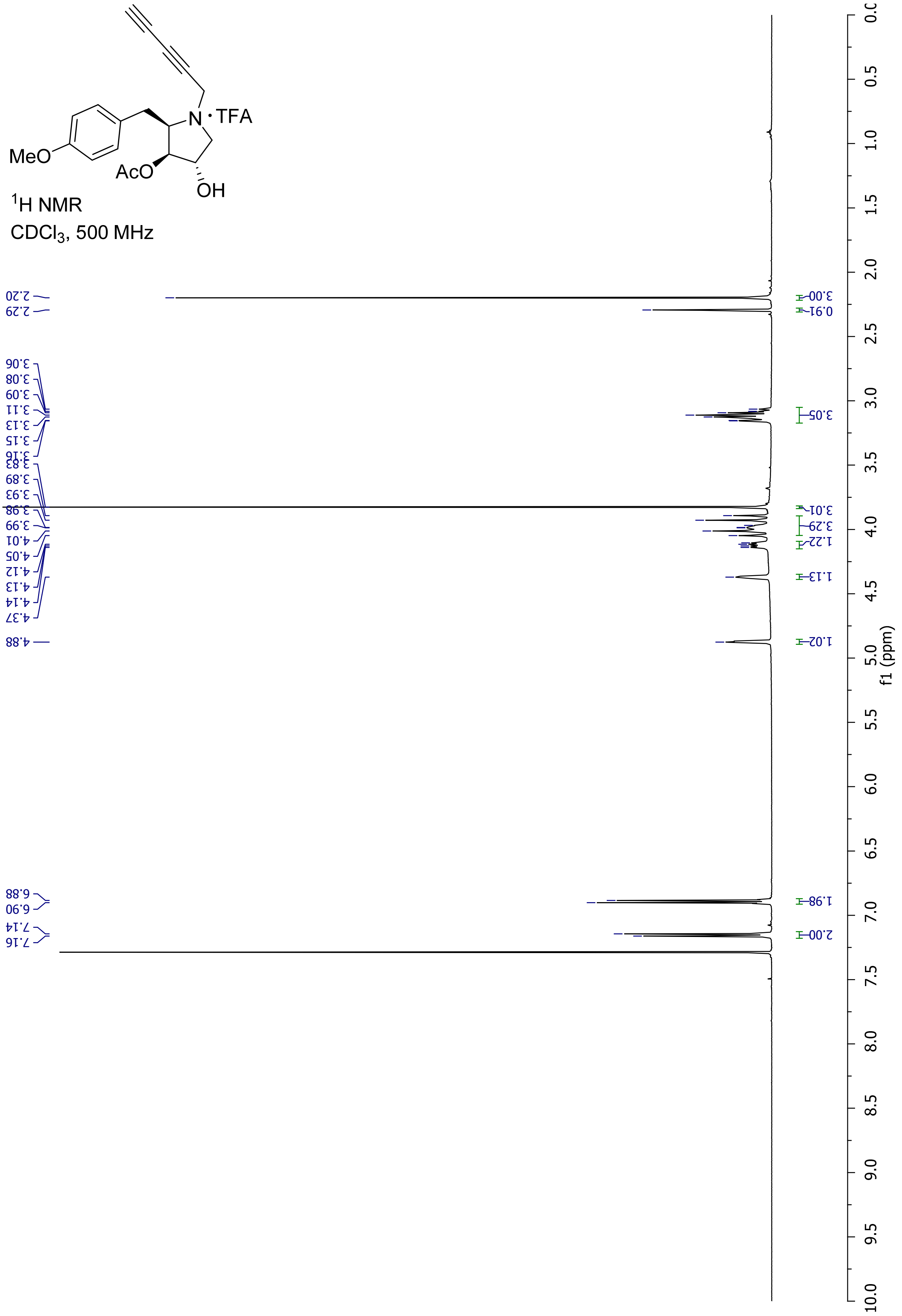
MeO
AcO
OH
N·TFA
¹³C NMR
CDCl₃, 126 MHz
20.61
29.59
41.18
55.28
57.77
65.32
72.34
78.68
78.74
114.52
127.22
129.78
158.92
170.43
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
f1 (ppm)

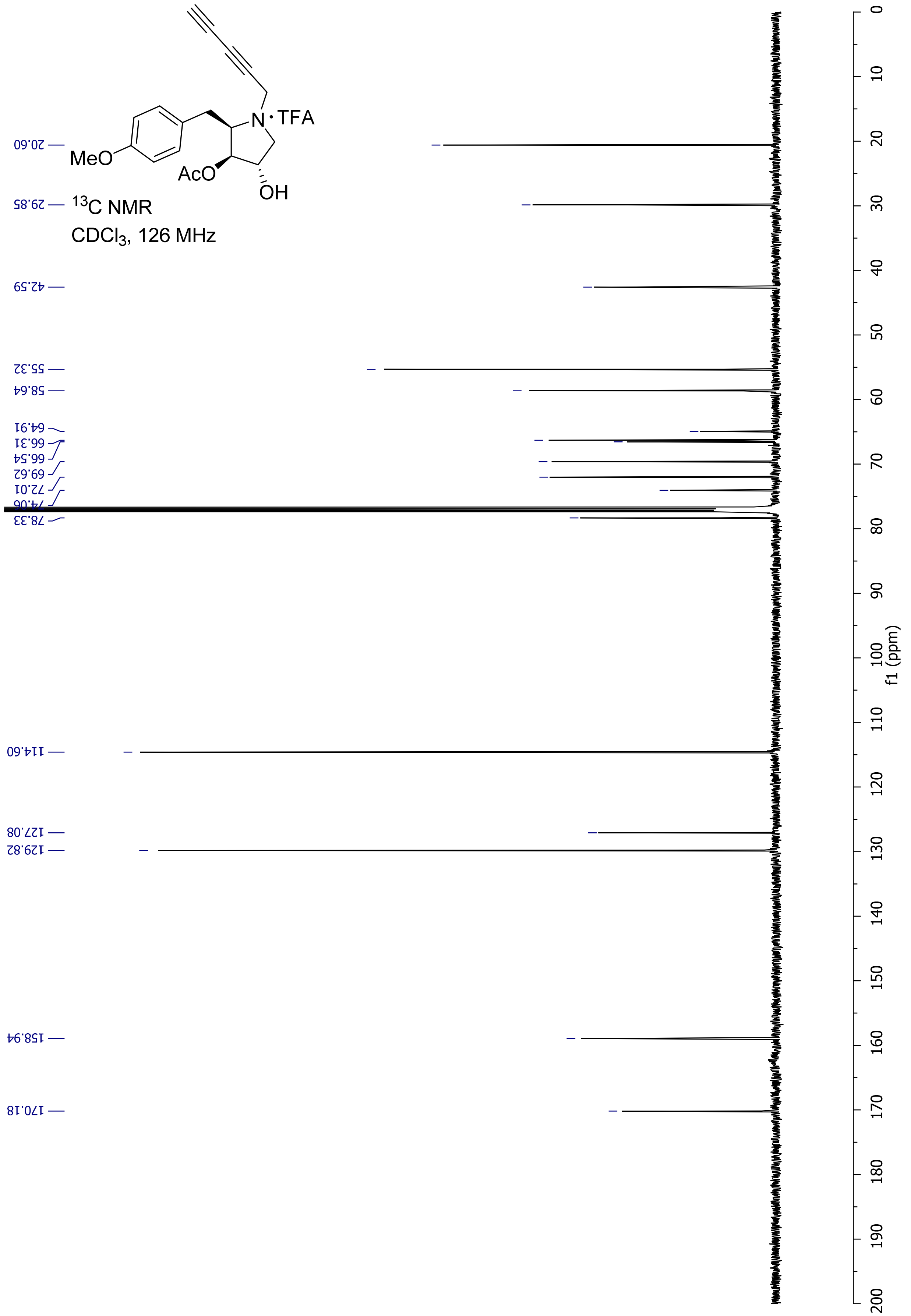


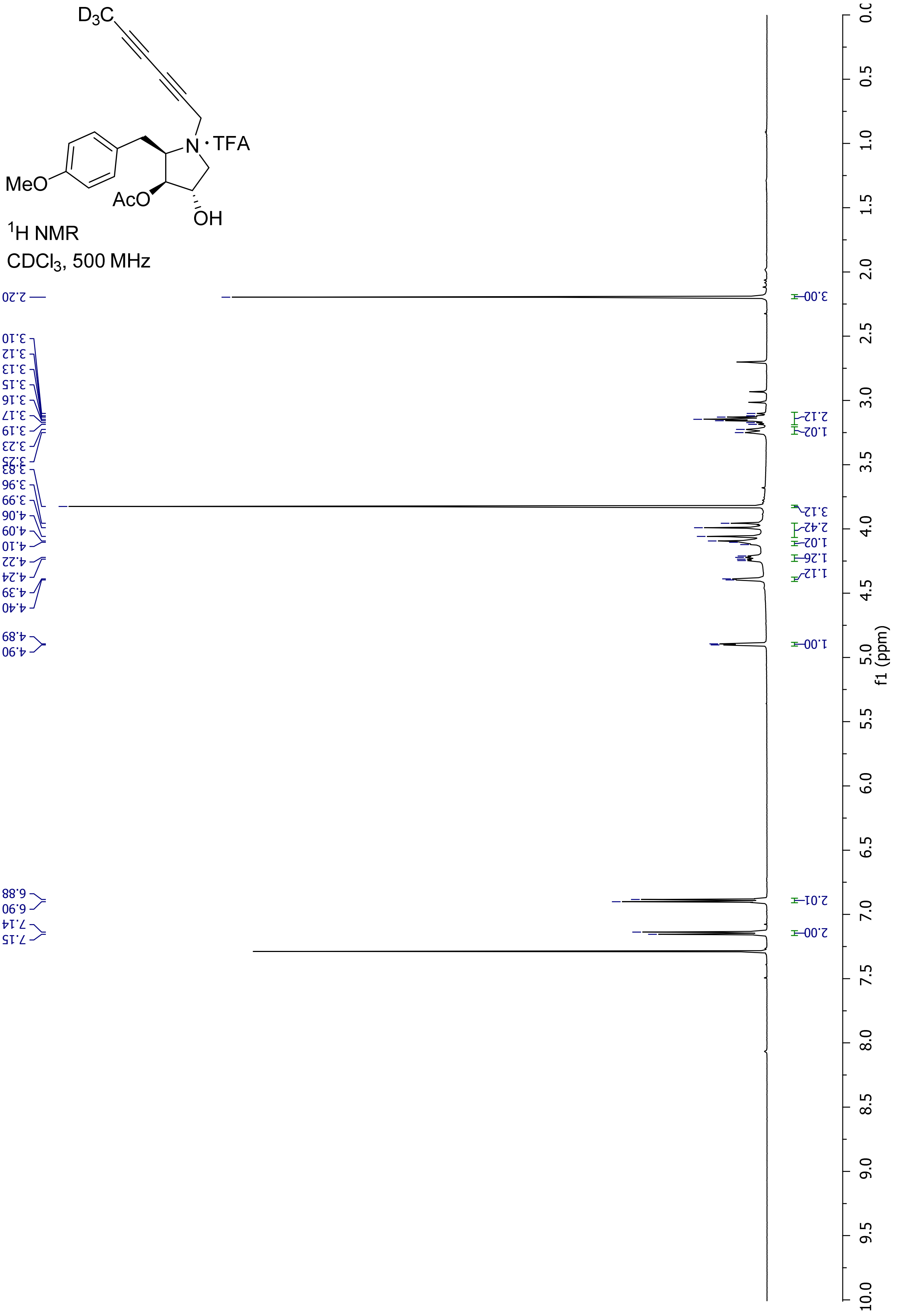
^{1}H NMR

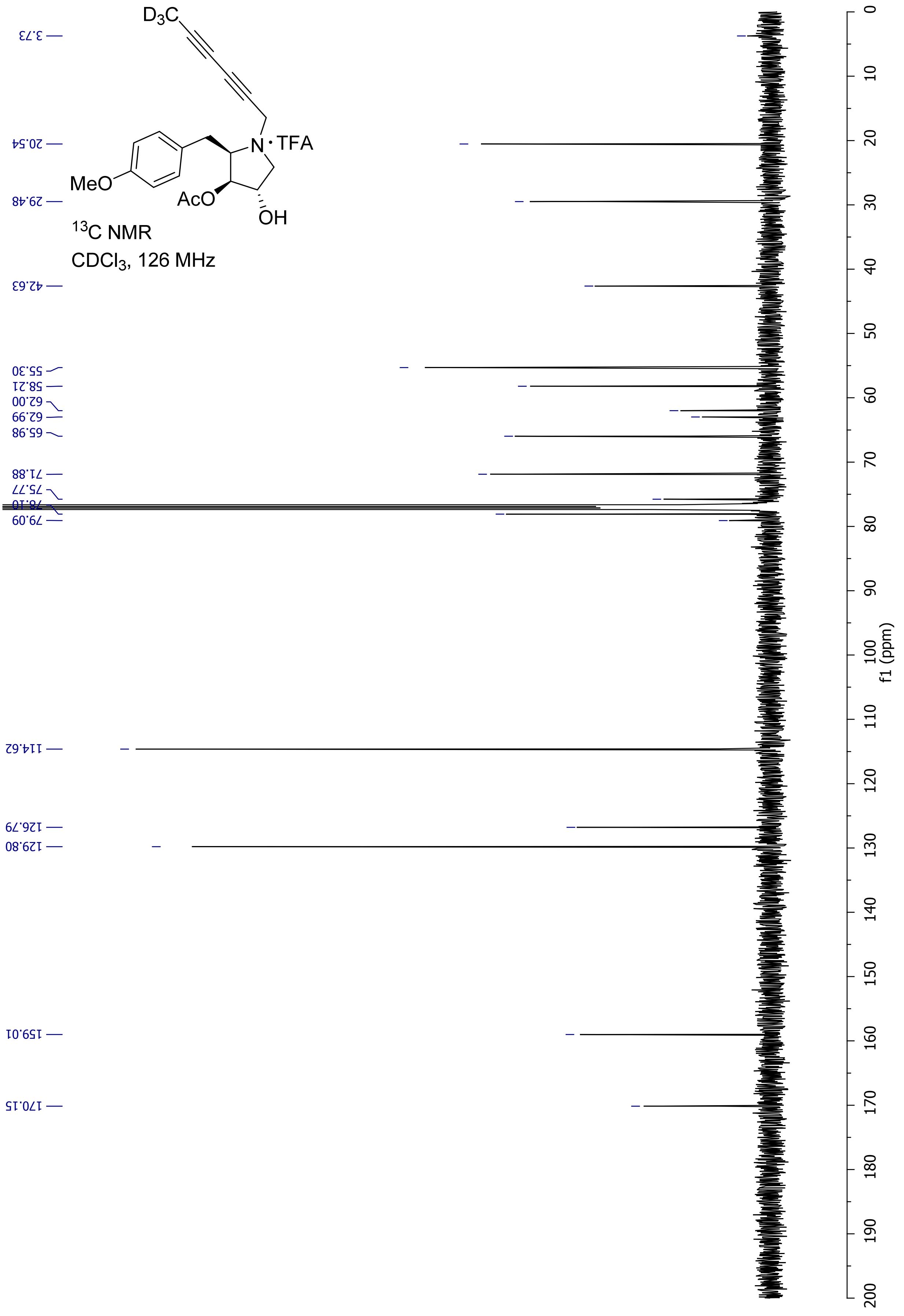
CDCl$_3$, 500 MHz


3.62
20.55
29.40
42.49
55.29
58.00
65.71
66.71
71.73
78.04
87.80
114.52
127.08
129.76
158.91
170.17
MeO
AcO
OH
N·TFA
13C NMR
CDCl3, 126 MHz
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
f1 (ppm)

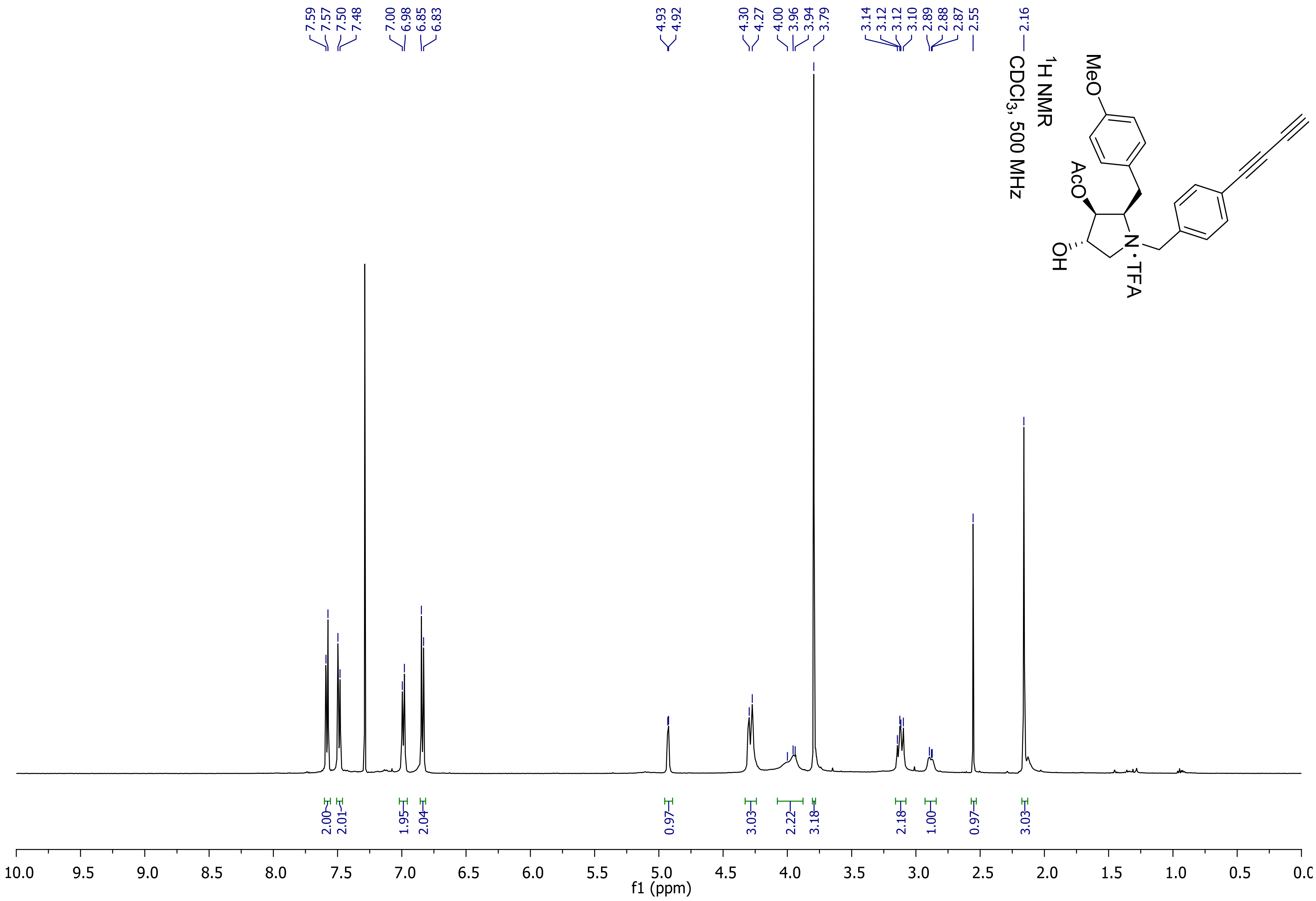

MeO
AcO
OH
N · TFA
¹H NMR
CDCl₃, 500 MHz
2.20
2.29
3.06
3.08
3.09
3.11
3.13
3.15
3.16
3.83
3.89
3.93
3.98
3.99
4.01
4.05
4.12
4.13
4.14
4.37
4.88
6.88
6.90
7.14
7.16
3.00
0.91
3.05
3.01
3.29
1.22
1.13
1.02
1.98
2.00
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
f1 (ppm)

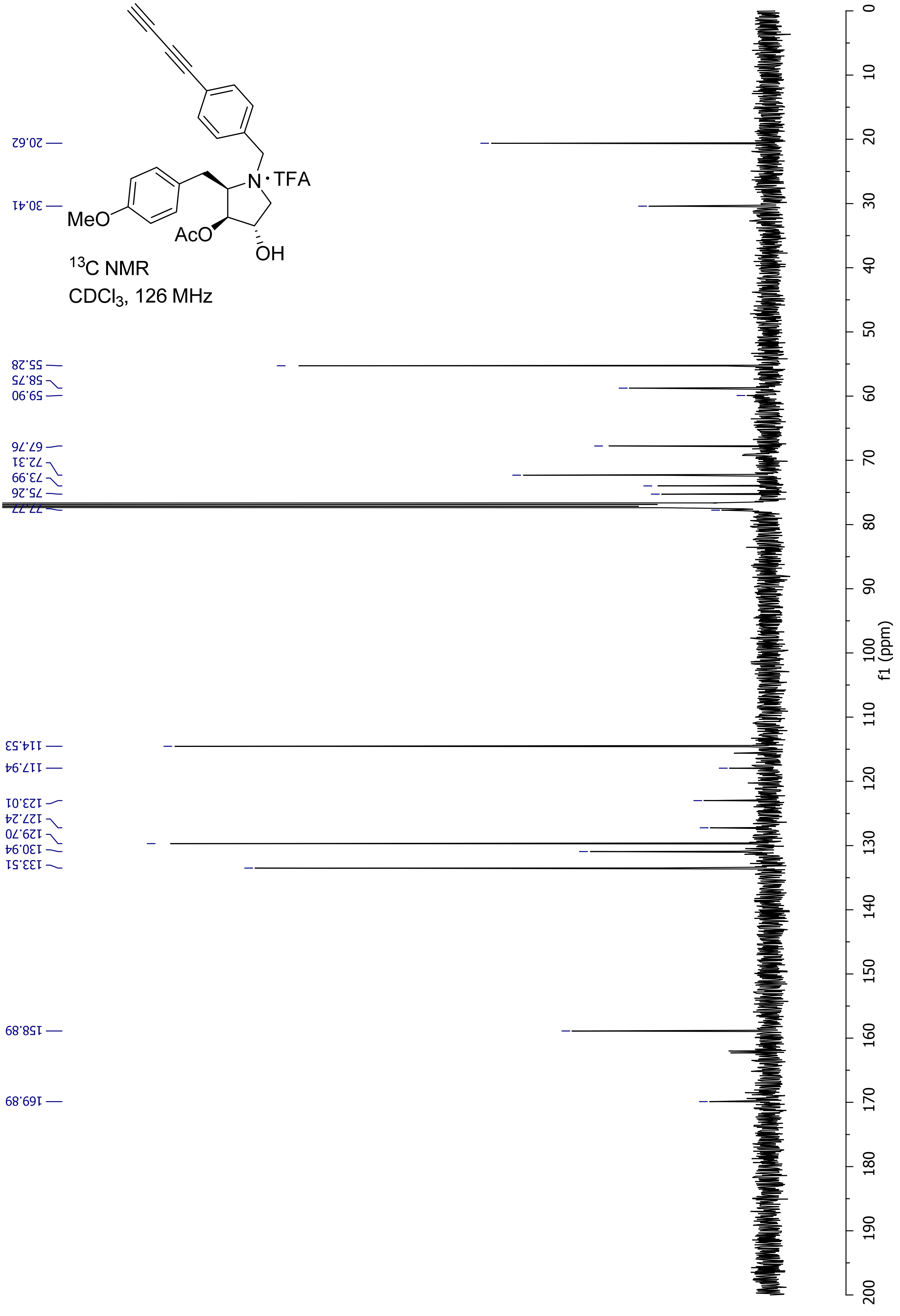

N · TFA
MeO
AcO
OH
13C NMR
CDCl3, 126 MHz
20.60
29.85
42.59
55.32
58.64
64.91
66.31
66.54
69.62
72.01
74.06
78.33
114.60
127.08
129.82
158.94
170.18
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
f1 (ppm)


D3C
N · TFA
MeO
AcO
OH
1H NMR
CDCl3, 500 MHz
2.20
3.10
3.12
3.13
3.15
3.16
3.17
3.19
3.23
3.25
3.83
3.96
3.99
4.06
4.09
4.10
4.22
4.24
4.39
4.40
4.89
4.90
6.88
6.90
7.14
7.15
3.00
2.12
1.02
3.12
2.42
1.02
1.26
1.12
1.00
2.01
2.00
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
f1 (ppm)

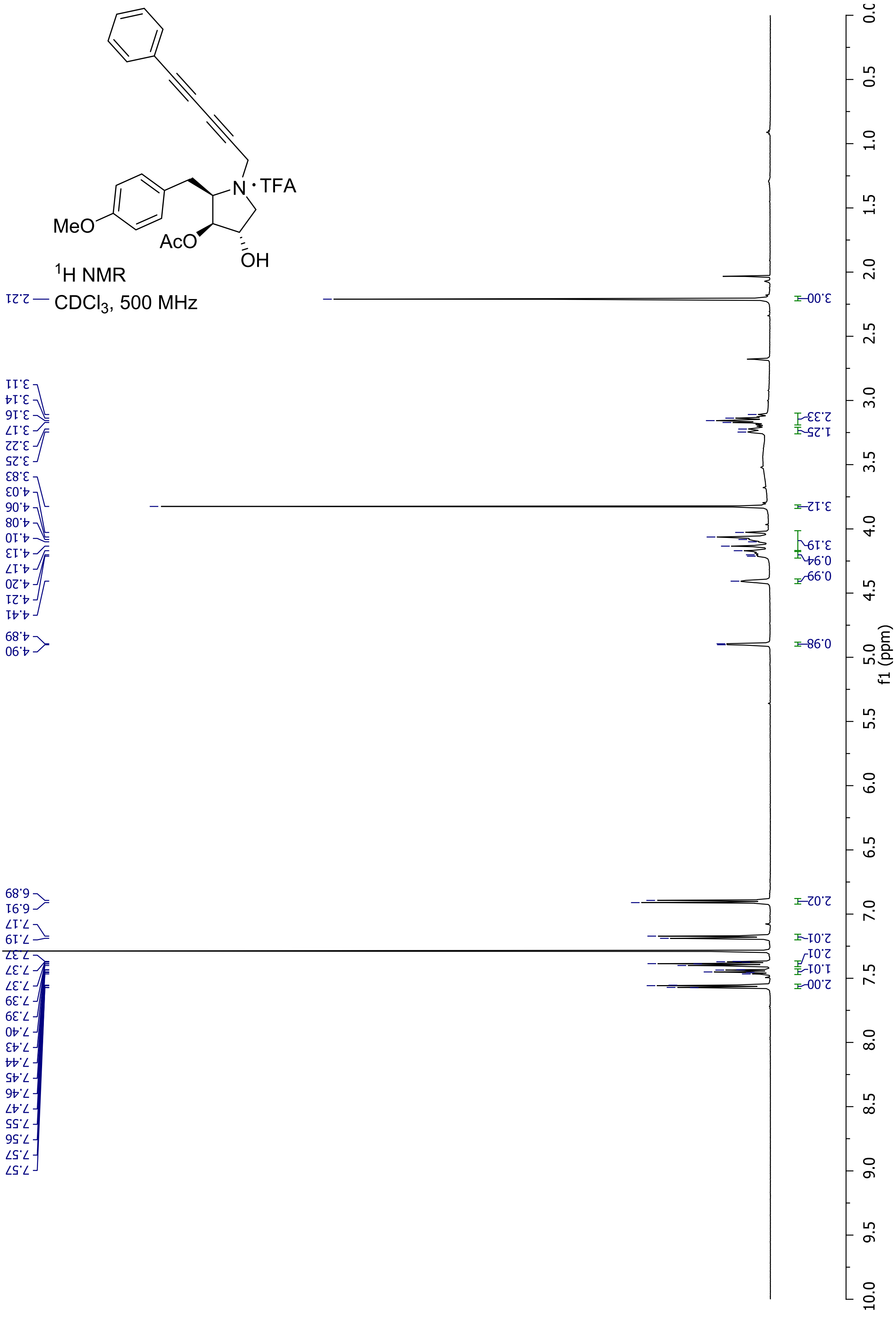


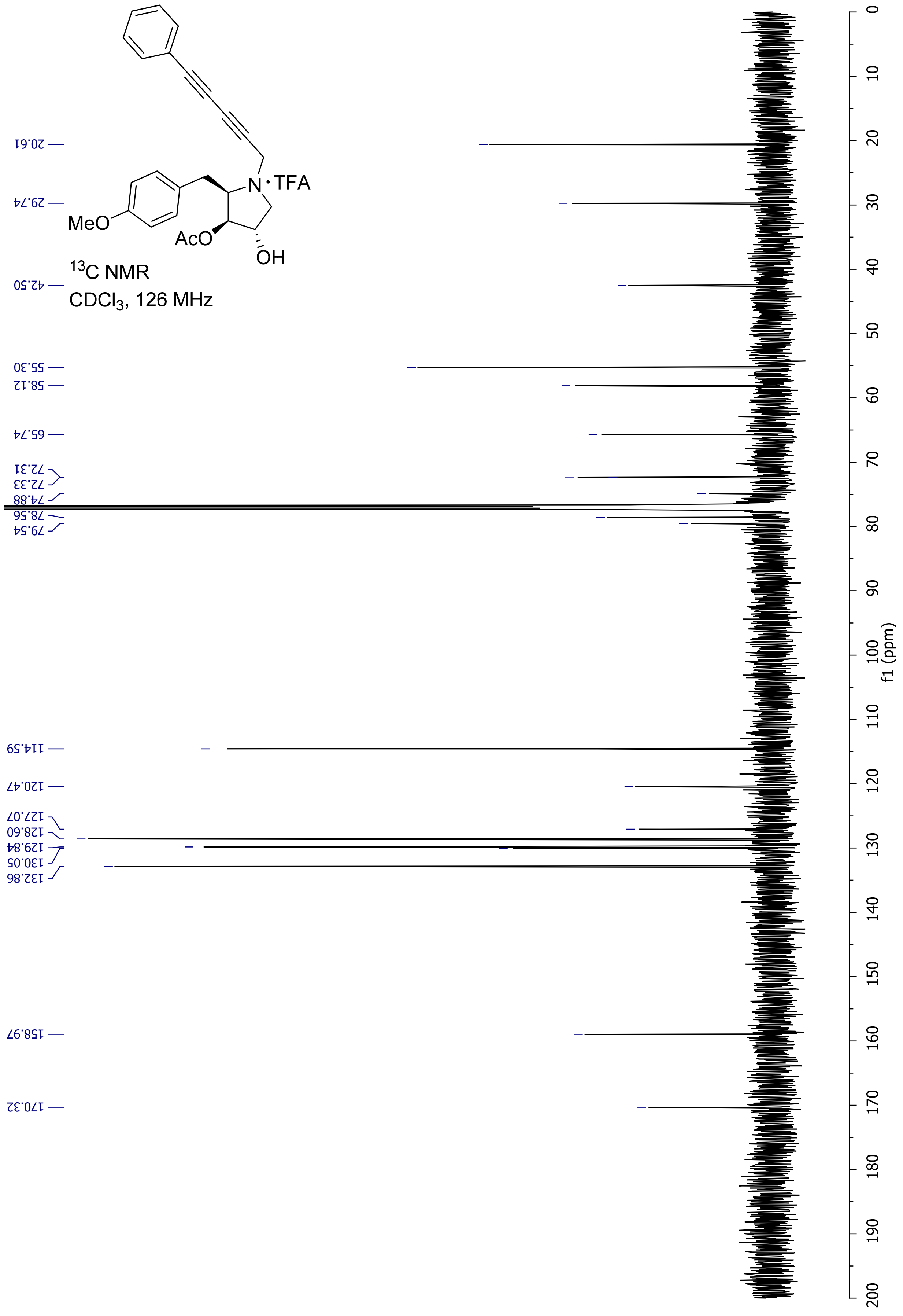
D_3C
N · TFA
MeO
AcO
OH
^{13}C NMR
$CDCl_3$, 126 MHz
3.73
20.54
29.48
42.63
55.30
58.21
62.00
62.99
65.98
71.88
75.77
78.10
79.09
114.62
126.79
129.80
159.01
170.15
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
f1 (ppm)

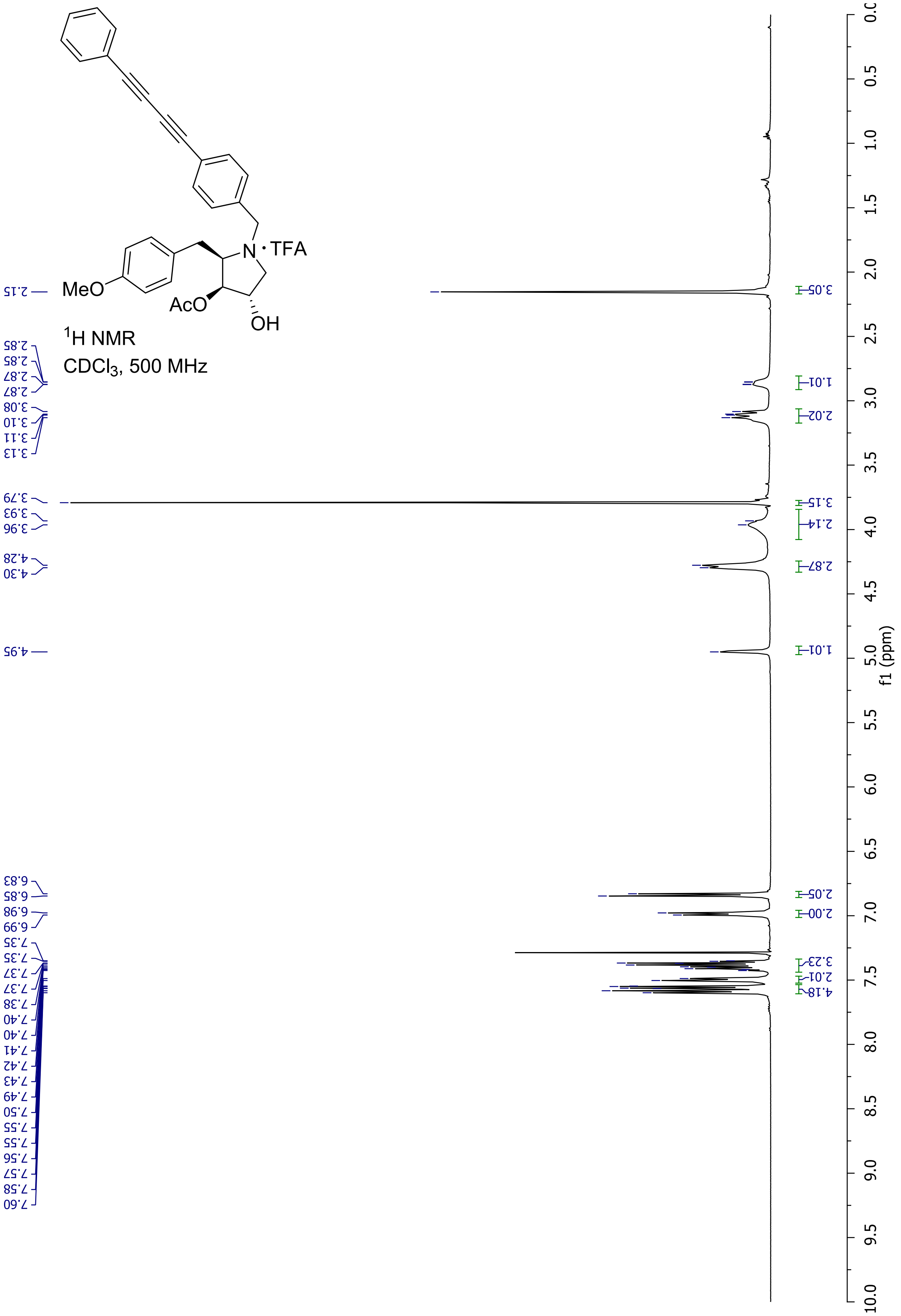

MeO
AcO
OH
N · TFA
^{1}H NMR
CDCl$_3$, 500 MHz
7.59
7.57
7.50
7.48
7.00
6.98
6.85
6.83
4.93
4.92
4.30
4.27
4.00
3.96
3.94
3.79
3.14
3.12
3.12
3.10
2.89
2.88
2.87
2.55
2.16
2.00
2.01
1.95
2.04
0.97
3.03
2.22
3.18
2.18
1.00
0.97
3.03
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

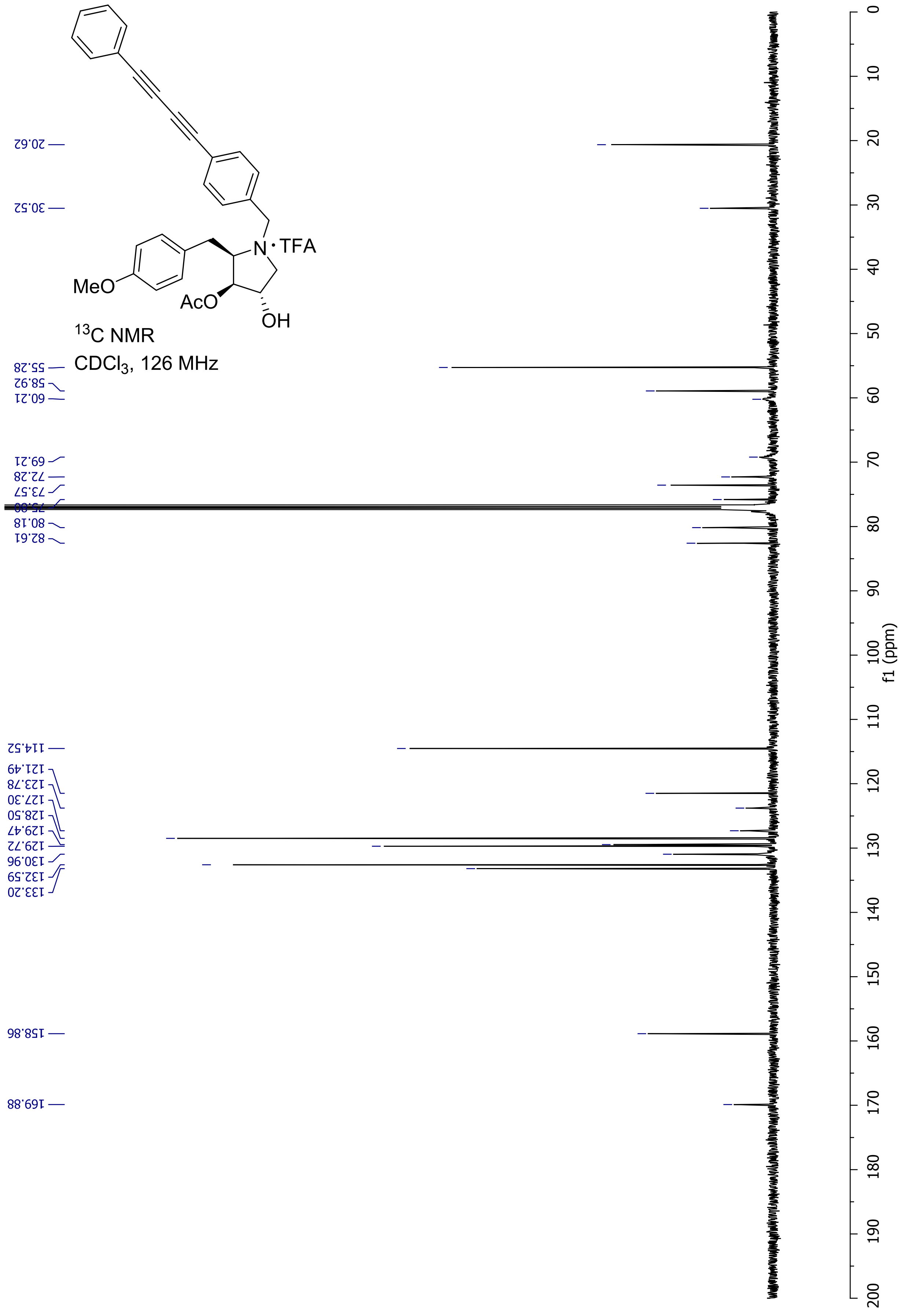


MeO
AcO
OH
N · TFA
^{13}C NMR
CDCl$_3$, 126 MHz
20.62
30.41
55.28
58.75
59.90
67.76
72.31
73.99
75.26
77.27
114.53
117.94
123.01
127.24
129.70
130.94
133.51
158.89
169.89
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
f1 (ppm)


MeO
AcO
N
TFA
OH
^{1}H NMR
CDCl$_3$, 500 MHz
2.21
3.11
3.14
3.16
3.17
3.22
3.25
3.83
4.03
4.06
4.08
4.10
4.13
4.17
4.20
4.21
4.41
4.89
4.90
6.89
6.91
7.17
7.19
7.32
7.37
7.37
7.39
7.39
7.40
7.43
7.44
7.45
7.46
7.47
7.55
7.56
7.57
7.57
3.00
2.33
1.25
3.12
3.19
0.94
0.99
0.98
2.02
2.01
2.01
1.01
2.00
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
f1 (ppm)

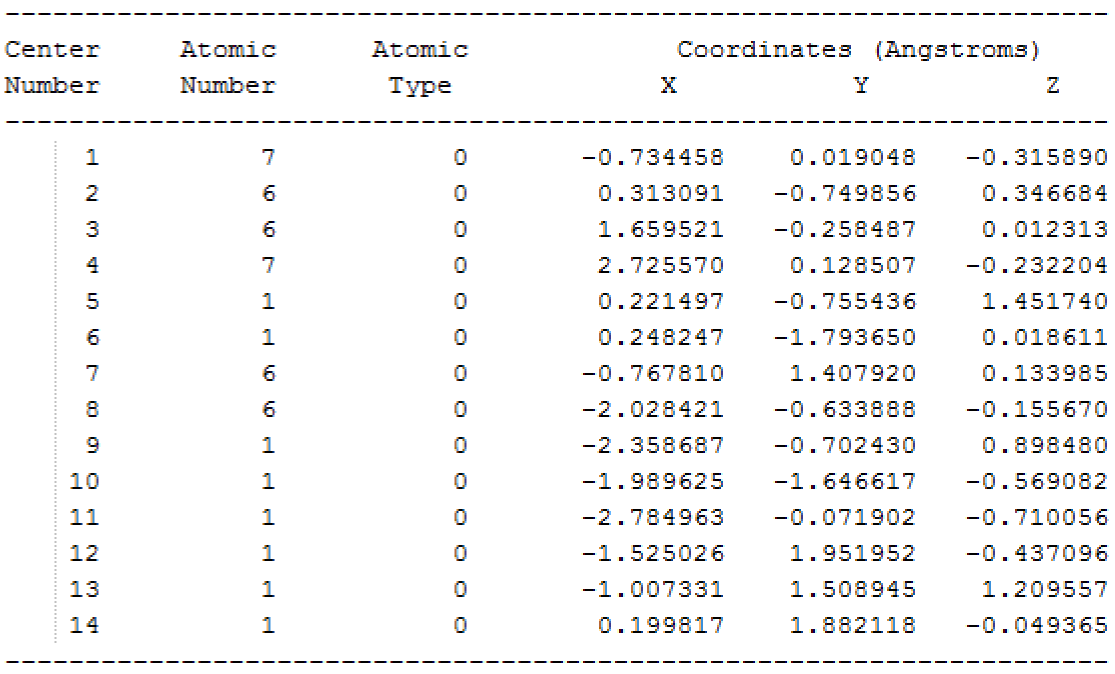

13C NMR
CDCl3, 126 MHz
N · TFA
MeO
AcO
OH
20.61
29.74
42.50
55.30
58.12
65.74
72.31
72.33
74.88
78.56
79.54
114.59
120.47
127.07
128.60
129.84
130.05
132.86
158.97
170.32
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
f1 (ppm)


MeO
AcO
OH
N
· TFA
1H NMR
CDCl3, 500 MHz
2.15
2.85
2.85
2.87
2.87
3.08
3.10
3.11
3.13
3.79
3.93
3.96
4.28
4.30
4.95
6.83
6.85
6.98
6.99
7.35
7.35
7.37
7.37
7.38
7.40
7.40
7.41
7.42
7.43
7.49
7.50
7.55
7.55
7.56
7.57
7.58
7.60
3.05
1.01
2.02
3.15
2.14
2.87
1.01
2.05
2.00
3.23
2.01
4.18
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
f1 (ppm)


20.62
30.52
55.28
58.92
60.21
69.21
72.28
73.57
75.80
80.18
82.61
114.52
121.49
123.78
127.30
128.50
129.47
129.72
130.96
132.59
133.20
158.86
169.88
N
TFA
MeO
AcO
OH
^{13}C NMR
CDCl$_3$, 126 MHz
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
f1 (ppm)

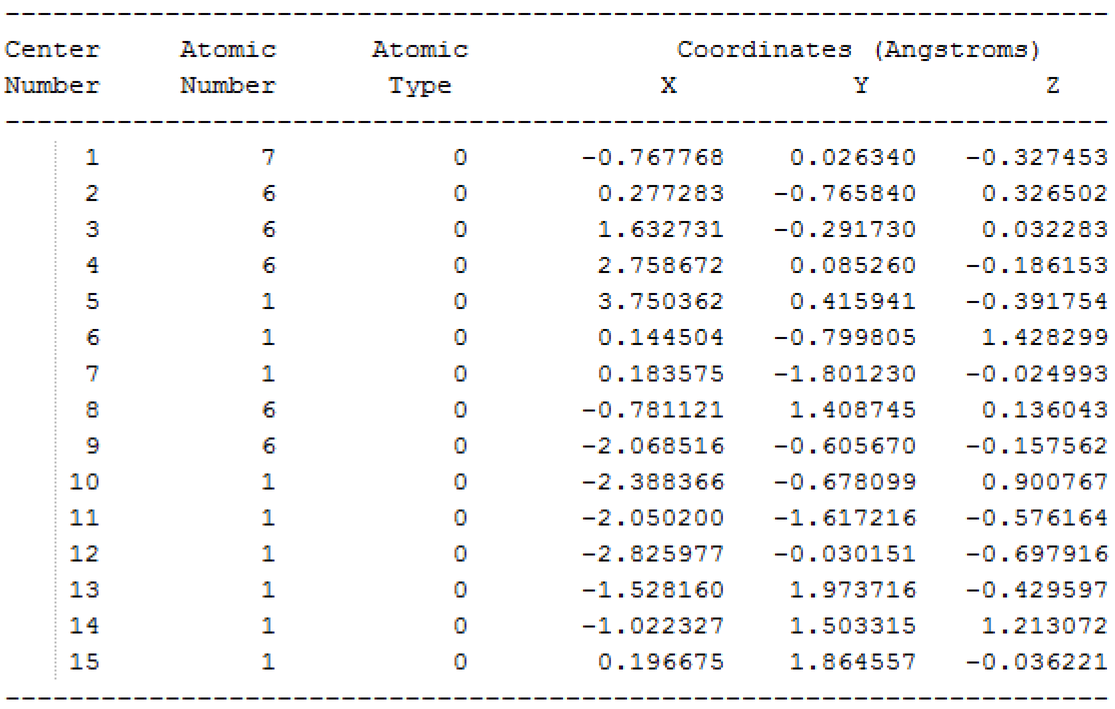
Optimised geometries for compounds **3b – i** and EdU **1** reported in standard orientation.

Compound **3b**



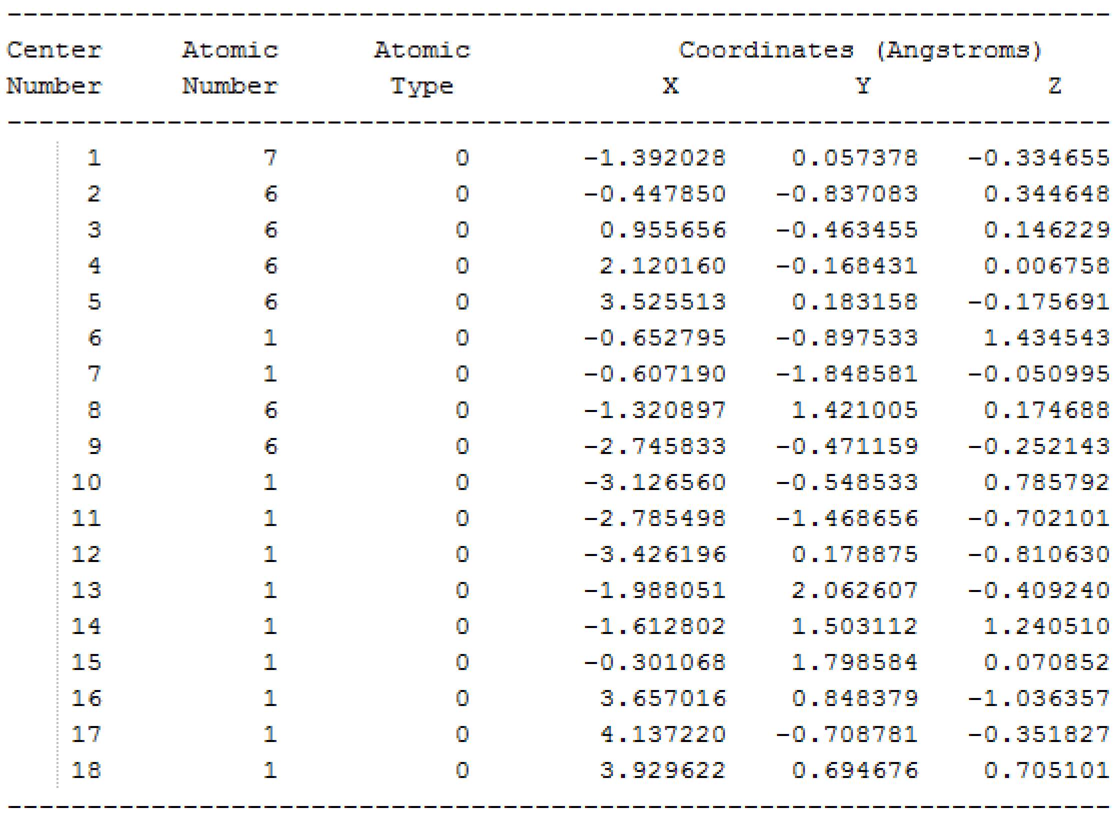
```
 ---------------------------------------------------------------------
 Center     Atomic      Atomic             Coordinates (Angstroms)
 Number     Number       Type             X           Y           Z
 ---------------------------------------------------------------------
      1          7           0       -0.734458    0.019048   -0.315890
      2          6           0        0.313091   -0.749856    0.346684
      3          6           0        1.659521   -0.258487    0.012313
      4          7           0        2.725570    0.128507   -0.232204
      5          1           0        0.221497   -0.755436    1.451740
      6          1           0        0.248247   -1.793650    0.018611
      7          6           0       -0.767810    1.407920    0.133985
      8          6           0       -2.028421   -0.633888   -0.155670
      9          1           0       -2.358687   -0.702430    0.898480
     10          1           0       -1.989625   -1.646617   -0.569082
     11          1           0       -2.784963   -0.071902   -0.710056
     12          1           0       -1.525026    1.951952   -0.437096
     13          1           0       -1.007331    1.508945    1.209557
     14          1           0        0.199817    1.882118   -0.049365
 ---------------------------------------------------------------------
```

Compound **3c**



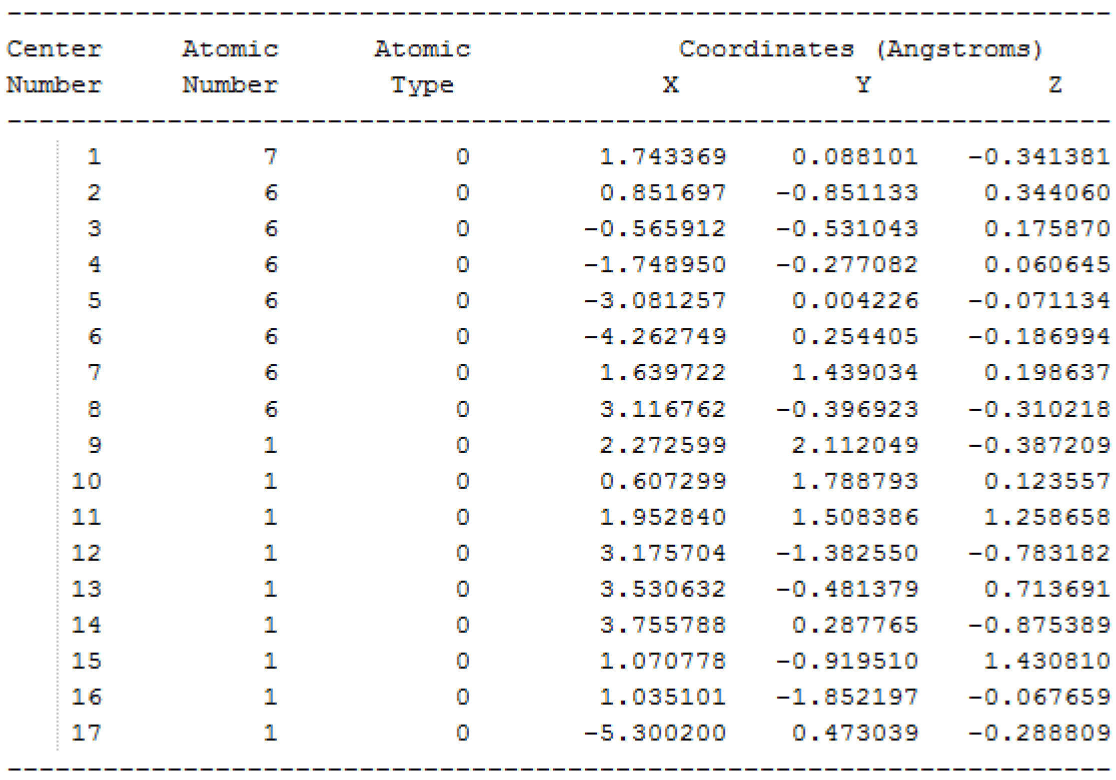
```
 ---------------------------------------------------------------------
 Center     Atomic      Atomic             Coordinates (Angstroms)
 Number     Number       Type             X           Y           Z
 ---------------------------------------------------------------------
      1          7           0       -0.767768    0.026340   -0.327453
      2          6           0        0.277283   -0.765840    0.326502
      3          6           0        1.632731   -0.291730    0.032283
      4          6           0        2.758672    0.085260   -0.186153
      5          1           0        3.750362    0.415941   -0.391754
      6          1           0        0.144504   -0.799805    1.428299
      7          1           0        0.183575   -1.801230   -0.024993
      8          6           0       -0.781121    1.408745    0.136043
      9          6           0       -2.068516   -0.605670   -0.157562
     10          1           0       -2.388366   -0.678099    0.900767
     11          1           0       -2.050200   -1.617216   -0.576164
     12          1           0       -2.825977   -0.030151   -0.697916
     13          1           0       -1.528160    1.973716   -0.429597
     14          1           0       -1.022327    1.503315    1.213072
     15          1           0        0.196675    1.864557   -0.036221
 ---------------------------------------------------------------------
```

Compound **3d**



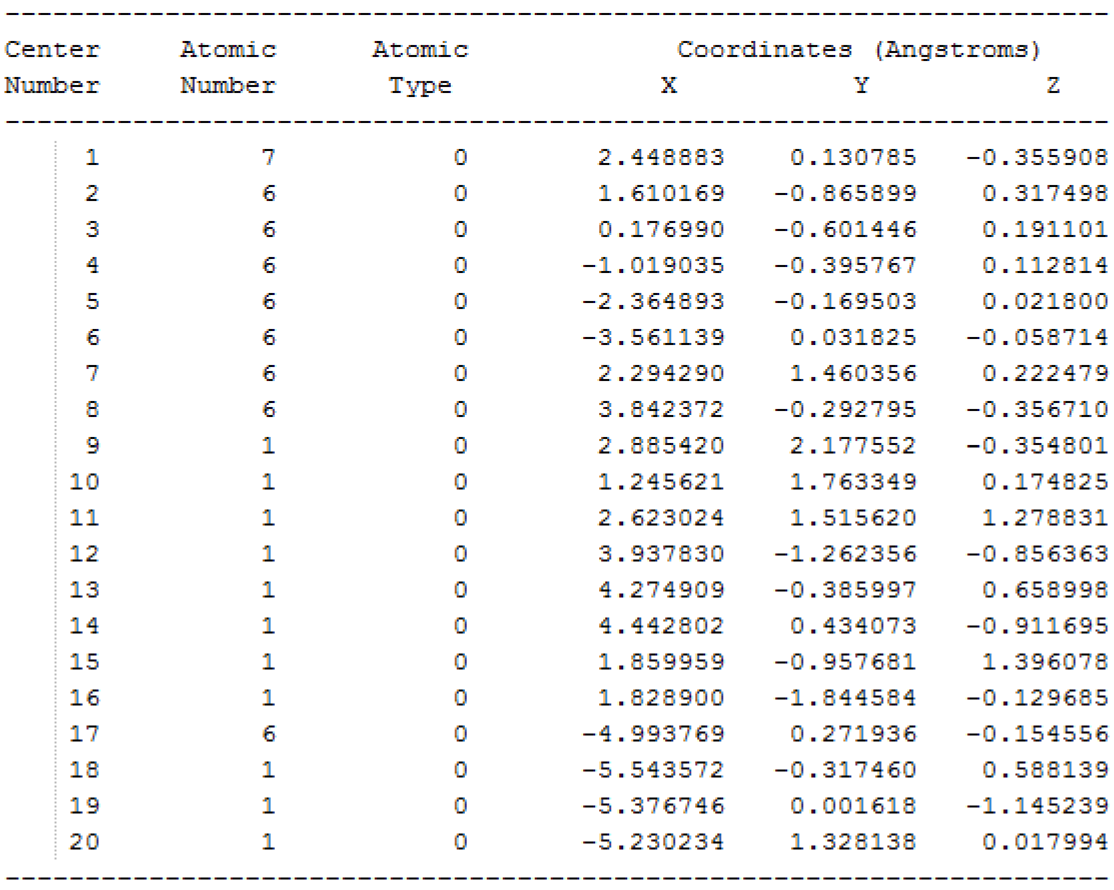
```
 ---------------------------------------------------------------------
 Center     Atomic      Atomic             Coordinates (Angstroms)
 Number     Number       Type             X           Y           Z
 ---------------------------------------------------------------------
      1          7           0       -1.392028    0.057378   -0.334655
      2          6           0       -0.447850   -0.837083    0.344648
      3          6           0        0.955656   -0.463455    0.146229
      4          6           0        2.120160   -0.168431    0.006758
      5          6           0        3.525513    0.183158   -0.175691
      6          1           0       -0.652795   -0.897533    1.434543
      7          1           0       -0.607190   -1.848581   -0.050995
      8          6           0       -1.320897    1.421005    0.174688
      9          6           0       -2.745833   -0.471159   -0.252143
     10          1           0       -3.126560   -0.548533    0.785792
     11          1           0       -2.785498   -1.468656   -0.702101
     12          1           0       -3.426196    0.178875   -0.810630
     13          1           0       -1.988051    2.062607   -0.409240
     14          1           0       -1.612802    1.503112    1.240510
     15          1           0       -0.301068    1.798584    0.070852
     16          1           0        3.657016    0.848379   -1.036357
     17          1           0        4.137220   -0.708781   -0.351827
     18          1           0        3.929622    0.694676    0.705101
 ---------------------------------------------------------------------
```

Compound **3e**



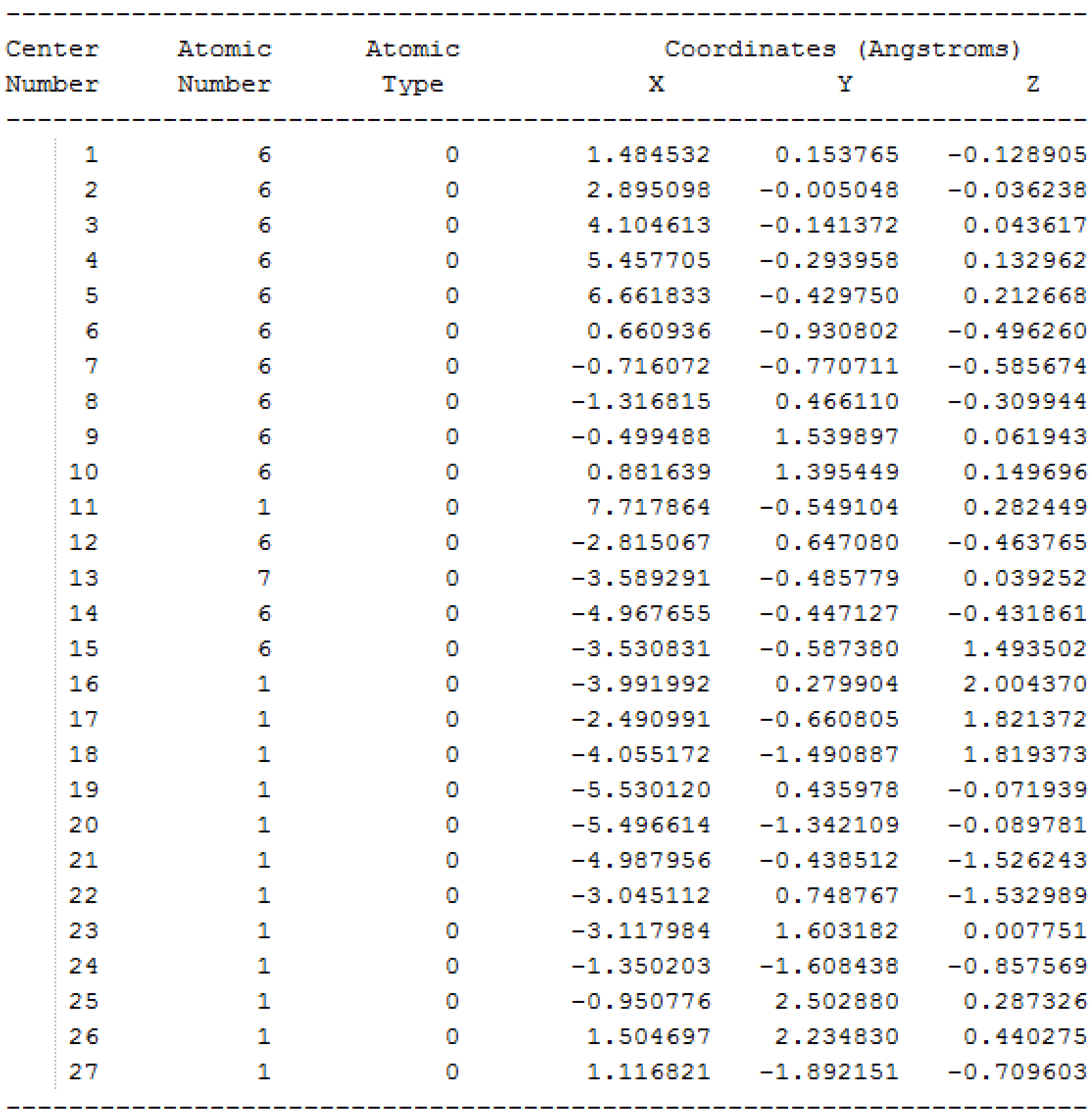
```
 ---------------------------------------------------------------------
 Center     Atomic      Atomic             Coordinates (Angstroms)
 Number     Number       Type             X           Y           Z
 ---------------------------------------------------------------------
      1          7           0        1.743369    0.088101   -0.341381
      2          6           0        0.851697   -0.851133    0.344060
      3          6           0       -0.565912   -0.531043    0.175870
      4          6           0       -1.748950   -0.277082    0.060645
      5          6           0       -3.081257    0.004226   -0.071134
      6          6           0       -4.262749    0.254405   -0.186994
      7          6           0        1.639722    1.439034    0.198637
      8          6           0        3.116762   -0.396923   -0.310218
      9          1           0        2.272599    2.112049   -0.387209
     10          1           0        0.607299    1.788793    0.123557
     11          1           0        1.952840    1.508386    1.258658
     12          1           0        3.175704   -1.382550   -0.783182
     13          1           0        3.530632   -0.481379    0.713691
     14          1           0        3.755788    0.287765   -0.875389
     15          1           0        1.070778   -0.919510    1.430810
     16          1           0        1.035101   -1.852197   -0.067659
     17          1           0       -5.300200    0.473039   -0.288809
 ---------------------------------------------------------------------
```

Compound **3f** (note the CD_3 group in compound **3f** was modelled as a CH_3 group).



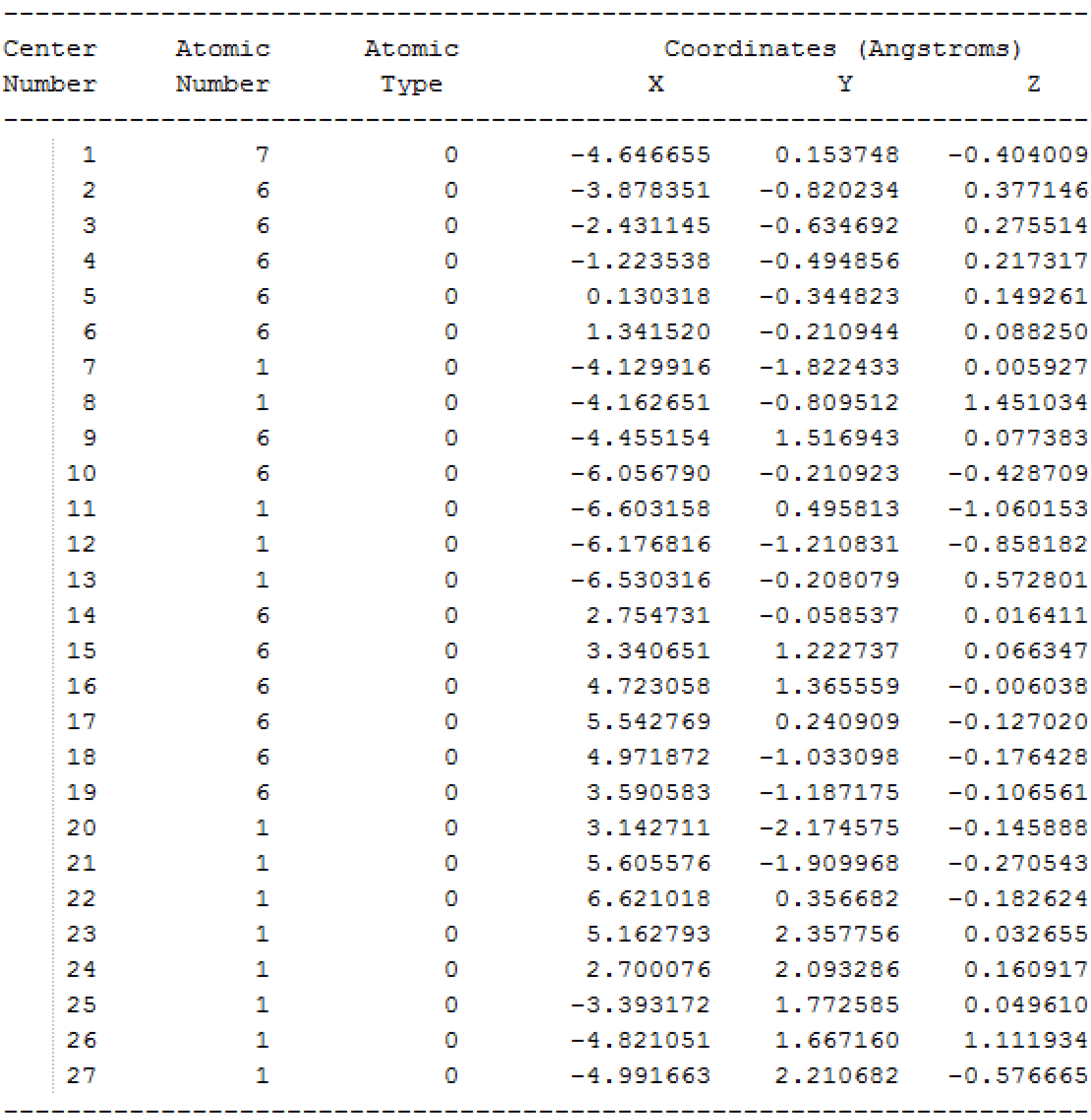
```
 ---------------------------------------------------------------------
 Center     Atomic      Atomic             Coordinates (Angstroms)
 Number     Number       Type             X           Y           Z
 ---------------------------------------------------------------------
      1          7           0        2.448883    0.130785   -0.355908
      2          6           0        1.610169   -0.865899    0.317498
      3          6           0        0.176990   -0.601446    0.191101
      4          6           0       -1.019035   -0.395767    0.112814
      5          6           0       -2.364893   -0.169503    0.021800
      6          6           0       -3.561139    0.031825   -0.058714
      7          6           0        2.294290    1.460356    0.222479
      8          6           0        3.842372   -0.292795   -0.356710
      9          1           0        2.885420    2.177552   -0.354801
     10          1           0        1.245621    1.763349    0.174825
     11          1           0        2.623024    1.515620    1.278831
     12          1           0        3.937830   -1.262356   -0.856363
     13          1           0        4.274909   -0.385997    0.658998
     14          1           0        4.442802    0.434073   -0.911695
     15          1           0        1.859959   -0.957681    1.396078
     16          1           0        1.828900   -1.844584   -0.129685
     17          6           0       -4.993769    0.271936   -0.154556
     18          1           0       -5.543572   -0.317460    0.588139
     19          1           0       -5.376746    0.001618   -1.145239
     20          1           0       -5.230234    1.328138    0.017994
 ---------------------------------------------------------------------
```

Compound **3g**



```
 ---------------------------------------------------------------------
 Center     Atomic      Atomic             Coordinates (Angstroms)
 Number     Number       Type             X           Y           Z
 ---------------------------------------------------------------------
      1          6           0        1.484532    0.153765   -0.128905
      2          6           0        2.895098   -0.005048   -0.036238
      3          6           0        4.104613   -0.141372    0.043617
      4          6           0        5.457705   -0.293958    0.132962
      5          6           0        6.661833   -0.429750    0.212668
      6          6           0        0.660936   -0.930802   -0.496260
      7          6           0       -0.716072   -0.770711   -0.585674
      8          6           0       -1.316815    0.466110   -0.309944
      9          6           0       -0.499488    1.539897    0.061943
     10          6           0        0.881639    1.395449    0.149696
     11          1           0        7.717864   -0.549104    0.282449
     12          6           0       -2.815067    0.647080   -0.463765
     13          7           0       -3.589291   -0.485779    0.039252
     14          6           0       -4.967655   -0.447127   -0.431861
     15          6           0       -3.530831   -0.587380    1.493502
     16          1           0       -3.991992    0.279904    2.004370
     17          1           0       -2.490991   -0.660805    1.821372
     18          1           0       -4.055172   -1.490887    1.819373
     19          1           0       -5.530120    0.435978   -0.071939
     20          1           0       -5.496614   -1.342109   -0.089781
     21          1           0       -4.987956   -0.438512   -1.526243
     22          1           0       -3.045112    0.748767   -1.532989
     23          1           0       -3.117984    1.603182    0.007751
     24          1           0       -1.350203   -1.608438   -0.857569
     25          1           0       -0.950776    2.502880    0.287326
     26          1           0        1.504697    2.234830    0.440275
     27          1           0        1.116821   -1.892151   -0.709603
 ---------------------------------------------------------------------
```

Compound **3h**

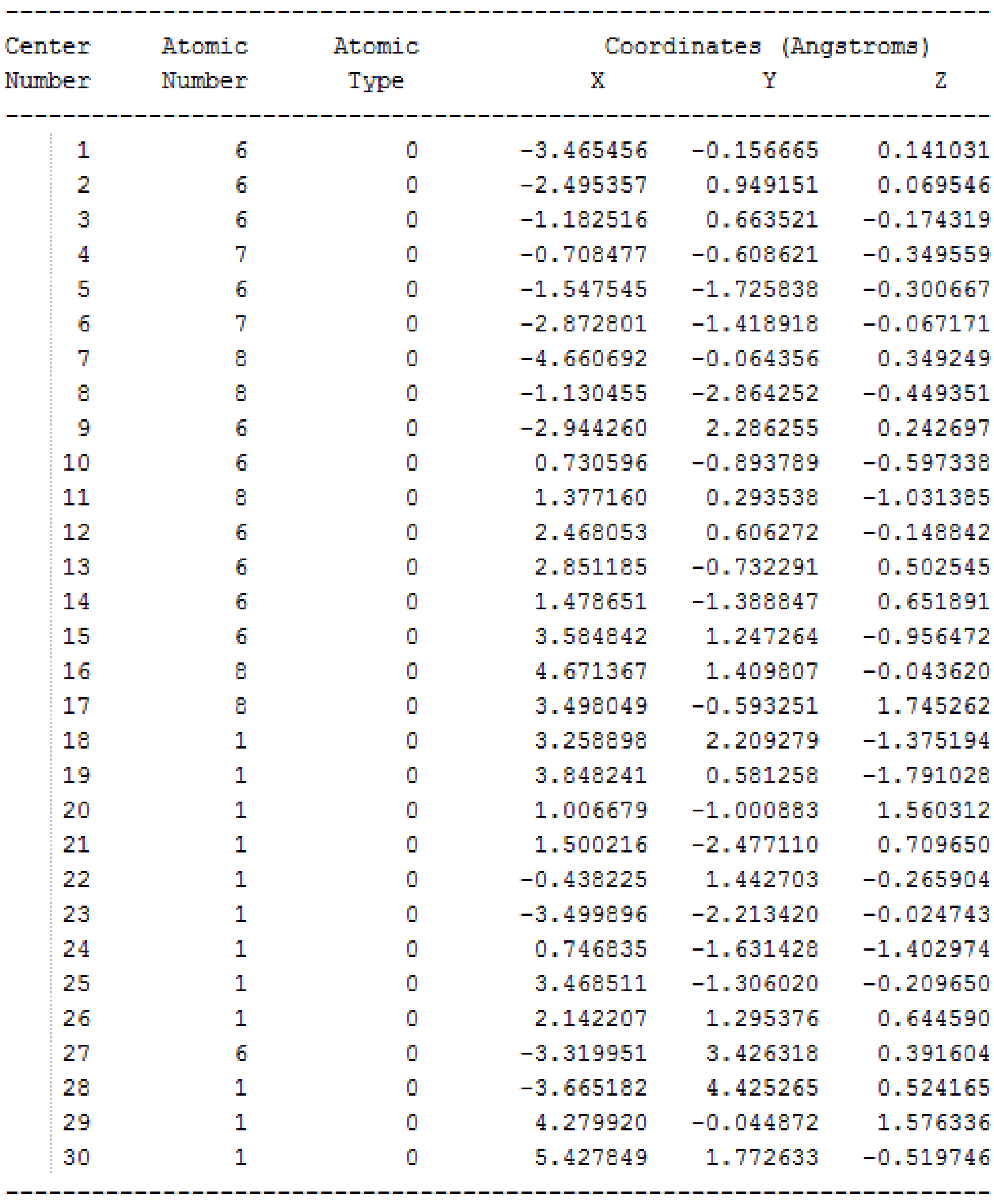


```
 ---------------------------------------------------------------------
 Center     Atomic      Atomic             Coordinates (Angstroms)
 Number     Number       Type             X           Y           Z
 ---------------------------------------------------------------------
      1          7           0       -4.646655    0.153748   -0.404009
      2          6           0       -3.878351   -0.820234    0.377146
      3          6           0       -2.431145   -0.634692    0.275514
      4          6           0       -1.223538   -0.494856    0.217317
      5          6           0        0.130318   -0.344823    0.149261
      6          6           0        1.341520   -0.210944    0.088250
      7          1           0       -4.129916   -1.822433    0.005927
      8          1           0       -4.162651   -0.809512    1.451034
      9          6           0       -4.455154    1.516943    0.077383
     10          6           0       -6.056790   -0.210923   -0.428709
     11          1           0       -6.603158    0.495813   -1.060153
     12          1           0       -6.176816   -1.210831   -0.858182
     13          1           0       -6.530316   -0.208079    0.572801
     14          6           0        2.754731   -0.058537    0.016411
     15          6           0        3.340651    1.222737    0.066347
     16          6           0        4.723058    1.365559   -0.006038
     17          6           0        5.542769    0.240909   -0.127020
     18          6           0        4.971872   -1.033098   -0.176428
     19          6           0        3.590583   -1.187175   -0.106561
     20          1           0        3.142711   -2.174575   -0.145888
     21          1           0        5.605576   -1.909968   -0.270543
     22          1           0        6.621018    0.356682   -0.182624
     23          1           0        5.162793    2.357756    0.032655
     24          1           0        2.700076    2.093286    0.160917
     25          1           0       -3.393172    1.772585    0.049610
     26          1           0       -4.821051    1.667160    1.111934
     27          1           0       -4.991663    2.210682   -0.576665
 ---------------------------------------------------------------------
```

Compound **3i**

```
 ---------------------------------------------------------------------
 Center     Atomic      Atomic             Coordinates (Angstroms)
 Number     Number       Type             X           Y           Z
 ---------------------------------------------------------------------
      1          6           0       -1.315577    0.256791   -0.253756
      2          6           0        0.102430    0.194002   -0.184730
      3          6           0        1.321562    0.136091   -0.129048
      4          6           0        2.678051    0.070319   -0.068597
      5          6           0        3.897006    0.010016   -0.015175
      6          6           0       -2.084959   -0.922580   -0.347723
      7          6           0       -3.470461   -0.857154   -0.419874
      8          6           0       -4.135809    0.377372   -0.397025
      9          6           0       -3.373221    1.546765   -0.294047
     10          6           0       -1.984414    1.496473   -0.226808
     11          6           0        5.315475   -0.063999    0.045713
     12          6           0        6.083557    1.090020    0.305092
     13          6           0        7.471530    1.010723    0.363626
     14          6           0        8.117246   -0.212249    0.165926
     15          6           0        7.365588   -1.361306   -0.091903
     16          6           0        5.977147   -1.293657   -0.152558
     17          6           0       -5.644785    0.442378   -0.535417
     18          7           0       -6.341919   -0.583998    0.237408
     19          6           0       -7.730790   -0.721703   -0.180849
     20          6           0       -6.243209   -0.349965    1.673983
     21          1           0       -6.743122    0.585148    1.992799
     22          1           0       -5.193237   -0.292310    1.971200
     23          1           0       -6.704863   -1.182158    2.214256
     24          1           0       -8.333520    0.191533   -0.011332
     25          1           0       -8.200973   -1.539754    0.373797
     26          1           0       -7.777030   -0.964492   -1.247186
     27          1           0       -5.905045    0.286370   -1.591170
     28          1           0       -5.991810    1.464019   -0.281928
     29          1           0       -4.060407   -1.766112   -0.481429
     30          1           0       -3.874374    2.511101   -0.265512
     31          1           0       -1.404625    2.410082   -0.146821
     32          1           0       -1.579384   -1.882607   -0.365200
     33          1           0        5.389318   -2.183329   -0.352625
     34          1           0        5.577856    2.037610    0.458229
     35          1           0        8.052149    1.906263    0.564220
     36          1           0        9.200600   -0.269551    0.212369
     37          1           0        7.863699   -2.313849   -0.246507
 ---------------------------------------------------------------------
```

EdU **1**



```
 ---------------------------------------------------------------------
 Center     Atomic      Atomic             Coordinates (Angstroms)
 Number     Number       Type             X           Y           Z
 ---------------------------------------------------------------------
      1          6           0       -3.465456   -0.156665    0.141031
      2          6           0       -2.495357    0.949151    0.069546
      3          6           0       -1.182516    0.663521   -0.174319
      4          7           0       -0.708477   -0.608621   -0.349559
      5          6           0       -1.547545   -1.725838   -0.300667
      6          7           0       -2.872801   -1.418918   -0.067171
      7          8           0       -4.660692   -0.064356    0.349249
      8          8           0       -1.130455   -2.864252   -0.449351
      9          6           0       -2.944260    2.286255    0.242697
     10          6           0        0.730596   -0.893789   -0.597338
     11          8           0        1.377160    0.293538   -1.031385
     12          6           0        2.468053    0.606272   -0.148842
     13          6           0        2.851185   -0.732291    0.502545
     14          6           0        1.478651   -1.388847    0.651891
     15          6           0        3.584842    1.247264   -0.956472
     16          8           0        4.671367    1.409807   -0.043620
     17          8           0        3.498049   -0.593251    1.745262
     18          1           0        3.258898    2.209279   -1.375194
     19          1           0        3.848241    0.581258   -1.791028
     20          1           0        1.006679   -1.000883    1.560312
     21          1           0        1.500216   -2.477110    0.709650
     22          1           0       -0.438225    1.442703   -0.265904
     23          1           0       -3.499896   -2.213420   -0.024743
     24          1           0        0.746835   -1.631428   -1.402974
     25          1           0        3.468511   -1.306020   -0.209650
     26          1           0        2.142207    1.295376    0.644590
     27          6           0       -3.319951    3.426318    0.391604
     28          1           0       -3.665182    4.425265    0.524165
     29          1           0        4.279920   -0.044872    1.576336
     30          1           0        5.427849    1.772633   -0.519746
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