

Supporting Information for

Synthesis of a Simplified Triazole Analogue of Pateamine A

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General Experimental Methods

Unless otherwise stated, the following conditions apply. All reactions were performed under an argon or nitrogen (dry and oxygen free) atmosphere, in oven- or heat gun-dried glassware, using dry solvents and standard syringe techniques. THF, DCM and toluene were either taken from solvent purification system (using Innovative Technology's PureSolv-EN system), or freshly distilled from CaH₂ (for DCM or toluene) or sodium metal and benzoquinone (for THF). DMSO, acetonitrile, diisopropylamine, triethylamine and pyridine were distilled from CaH₂. Anhydrous dimethylformamide (DMF) was purchased from Aldrich Chemical Company and used without further purification. All other reagents were of commercial quality and distilled prior to use if necessary. Distilled water was used for all aqueous solutions (*i.e.* the reaction solvent or aqueous work-up). Unless stated otherwise, organic solvents were removed by rotary evaporation with water bath temperature between 40 and 50 °C. The reaction progress was monitored using polyester-backed TLC plates pre-coated with silica UV₂₅₄ (Macherey-Nagel) and visualised by UV irradiation (254 nm), in combination with vanillin, phosphomolybdic acid (PMA) or potassium permanganate (KMnO₄) dip.¹⁴⁵ Purification of products *via* silica gel flash chromatography were conducted using a column packed with silica gel 60 (220-240 mesh) eluted with the solvent systems indicated. ¹H NMR spectra were recorded on either a Varian Unity Inova 300 Spectrometer at 300 MHz or a Varian Unity Inova 500 Spectrometer at 500 MHz. Data are listed as follows: chemical shift in ppm using residual CHCl₃ as an internal reference (at δ 7.26 ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet or overlap of non-equivalent resonances, br = broad), integration. ¹³C NMR spectra were recorded on a Varian Unity Inova 500 spectrometer at 125 MHz with proton decoupled. Data are listed with chemical shift in ppm using CDCl₃ as an internal reference (at δ 77.16 ppm). Infrared spectra were obtained on a Bruker Tensor 27 FTIR spectrometer (ATR). High-resolution mass spectrometry was recorded on a 6530 Accurate Mass Q-TOF LC/MS instrument (Agilent Technologies).

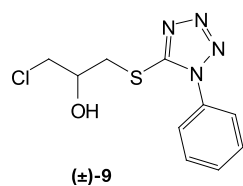
Mammalian cell culture and MTT assay

Cells were cultured as previously described,¹ using RPMI-1640 medium (Life Technologies) supplemented with 10% fetal bovine serum (Hyclone Laboratories, Logan, UT) and Pen-Strep (Life Technologies). For the ovarian 1A9 cell line the medium was supplemented with 0.25 U/mL insulin (Sigma Chemical Company, St. Louis, MO). The cell growth effects of the analogues were assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenoyltetrazolium bromide) cell proliferation assay as previously described.² In brief, cells were seeded into 96-well plates at a concentration of 1 x 10⁴ cells per well, and treated with compound (2-fold serial dilution) for either 48 h (HL-60 cells) or 96 h (1A9 cells). MTT tetrazolium dye (20 µL, 5 mg/mL) was then added to each well, followed by incubation for 2 h at 37 °C. The purple formazan crystals were solubilized in 10% sodium dodecyl sulfate, 45% dimethylformamide, pH 5.5, and the absorbance read at 570 nm in a multiplate reader (EnVision, PerkinElmer, Waltham, MA).

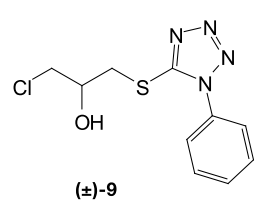
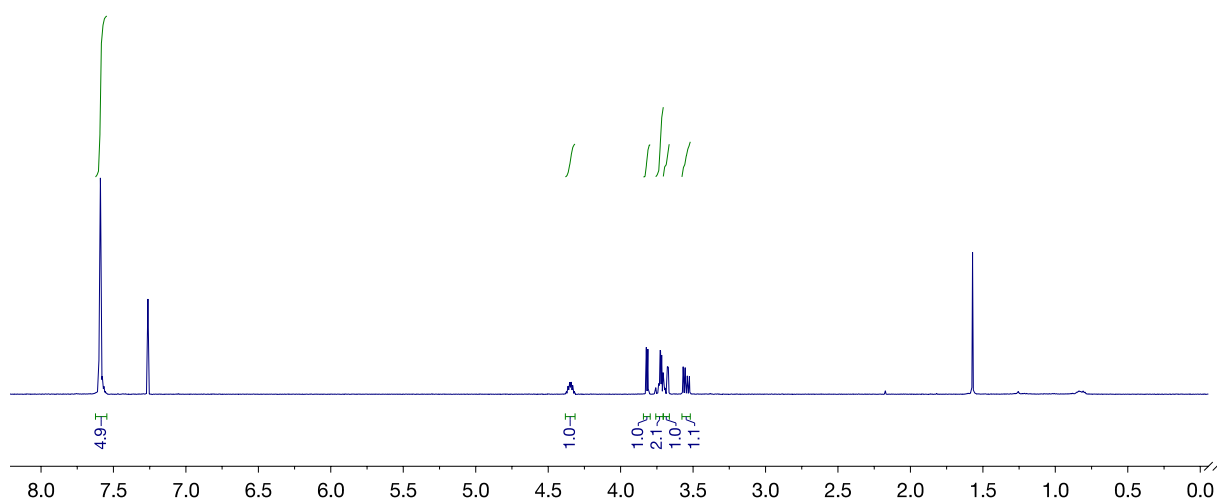
¹ Field, J. J.; Singh, A. J.; Kanakkanthara, A.; Halafihi, T.; Northcote, P. T.; Miller, J. H. *J Med Chem.* 2009, 52, 7328.

² Hood, K. A.; West, L. M.; Northcote, P. T.; Berridge, M. V.; Miller, J. H. *Apoptosis* 2001, 6, 207

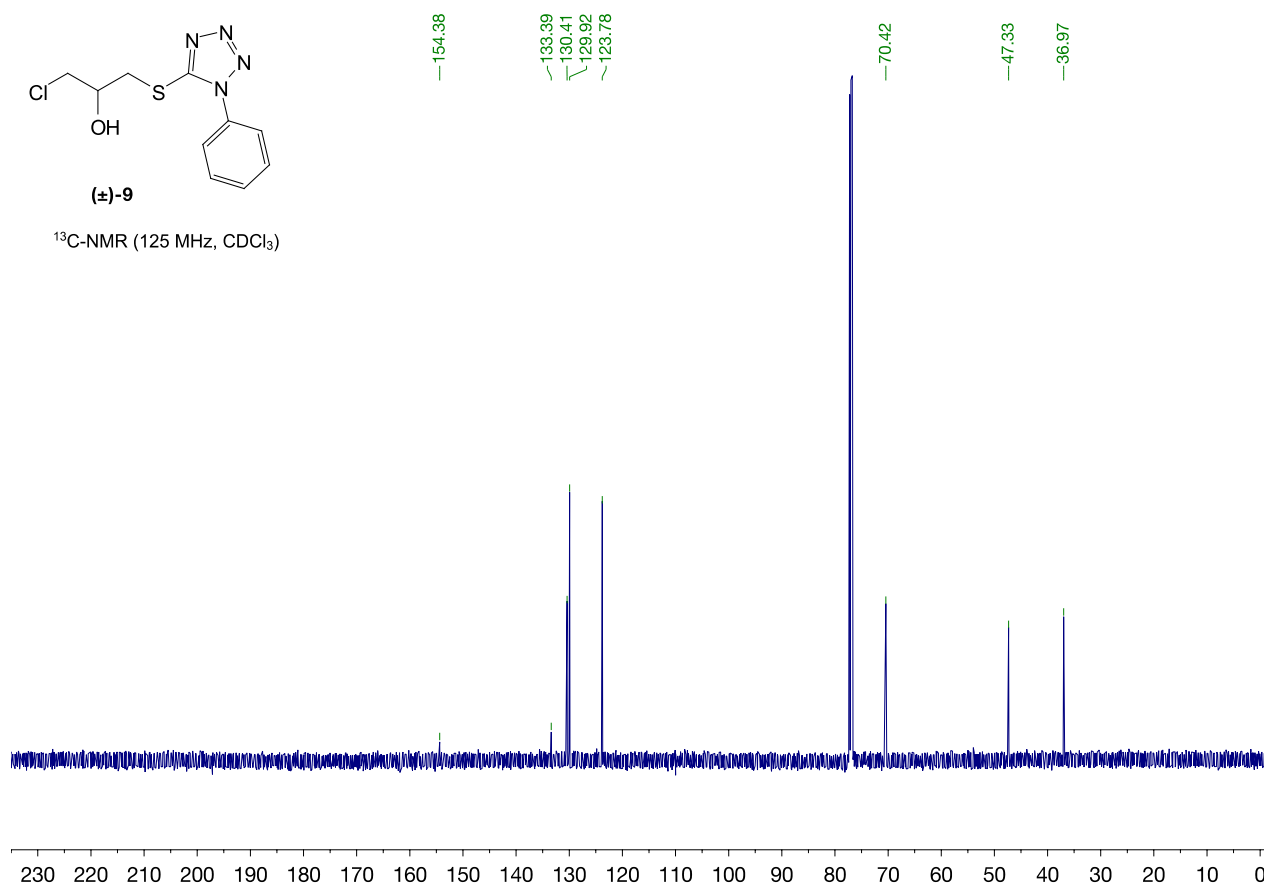
¹H-NMR and ¹³C-NMR spectra of novel compounds

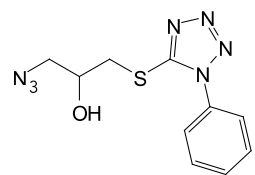


¹H-NMR (500 MHz, CDCl₃)



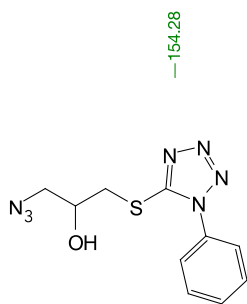
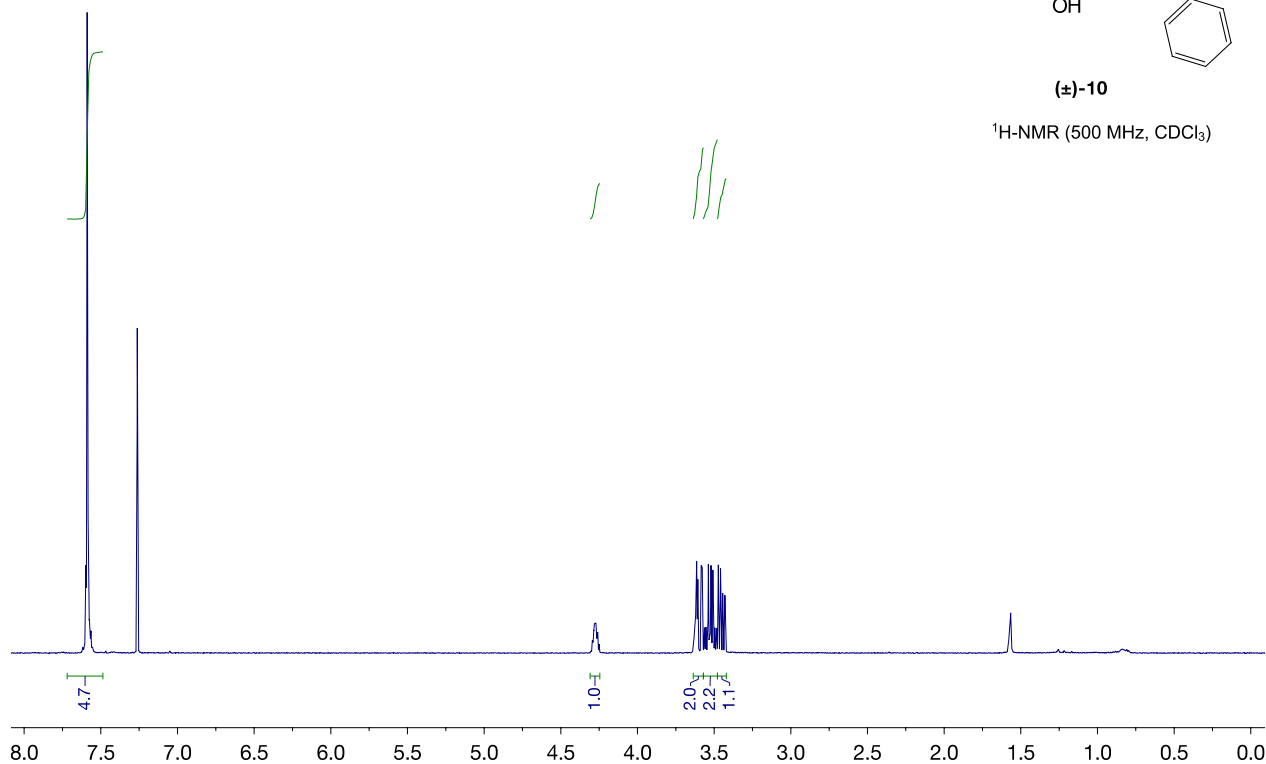
¹³C-NMR (125 MHz, CDCl₃)





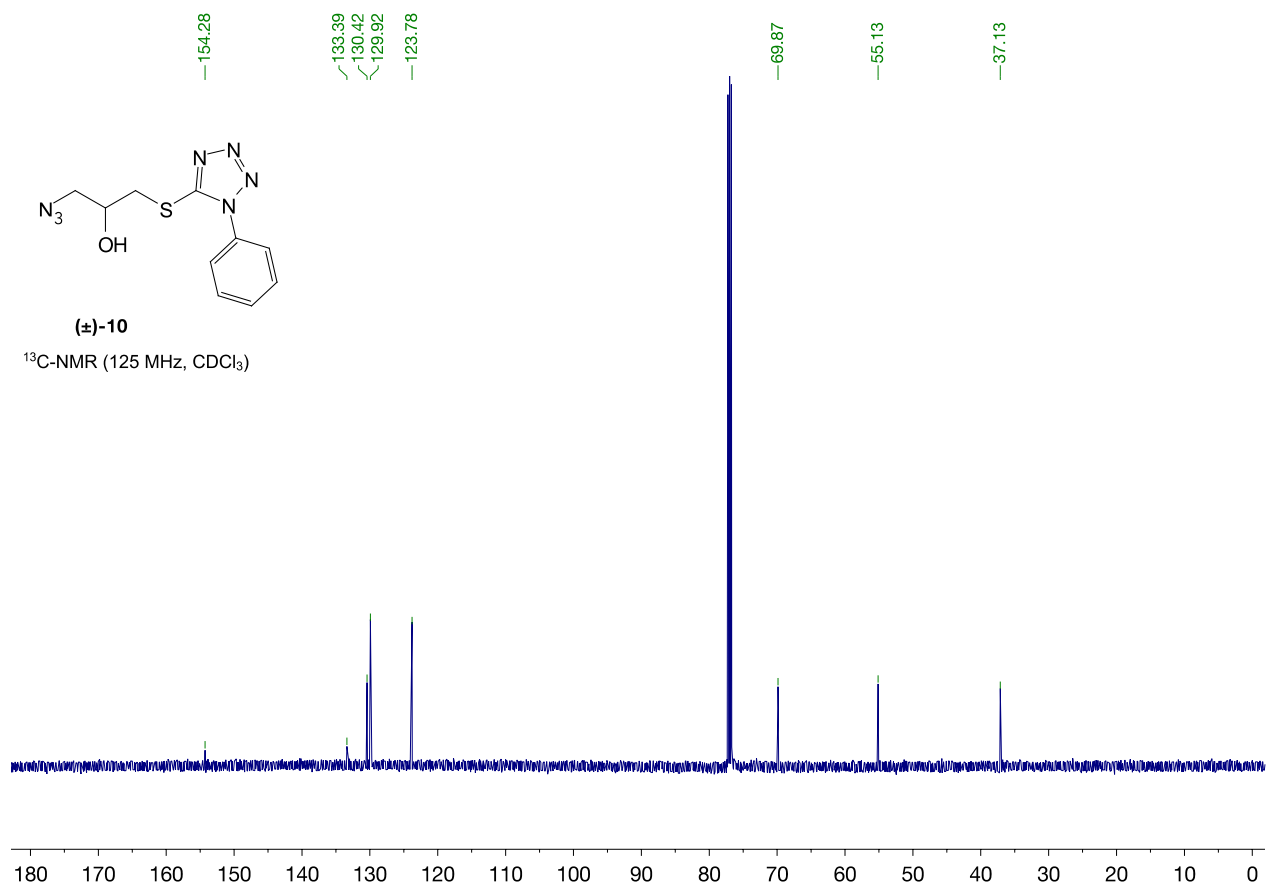
(±)-10

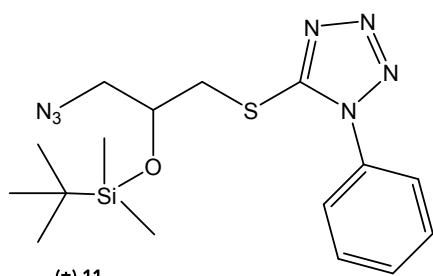
¹H-NMR (500 MHz, CDCl₃)



(±)-10

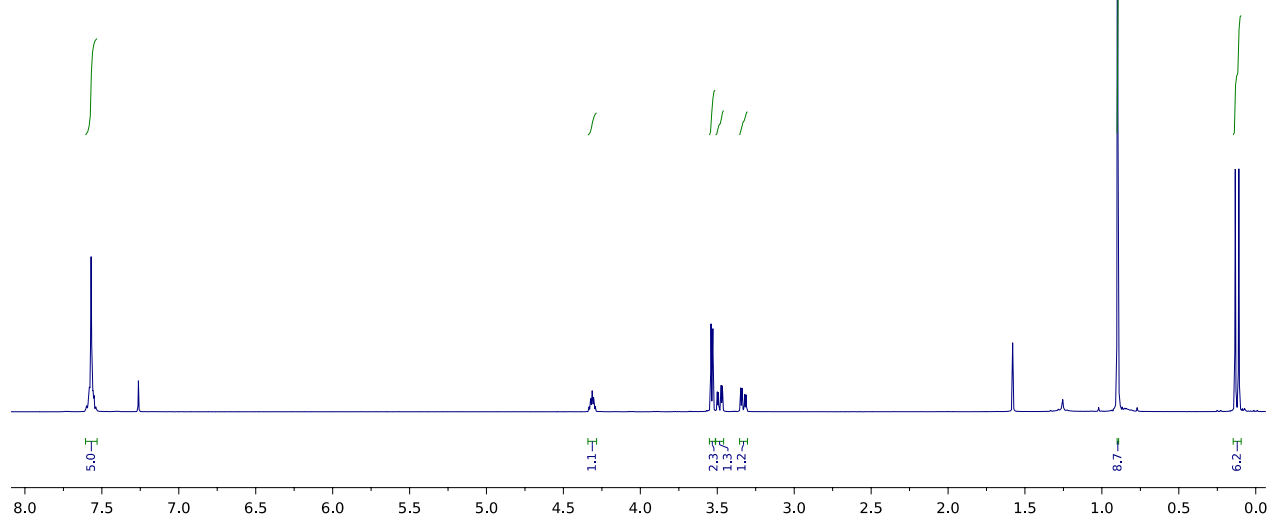
¹³C-NMR (125 MHz, CDCl₃)





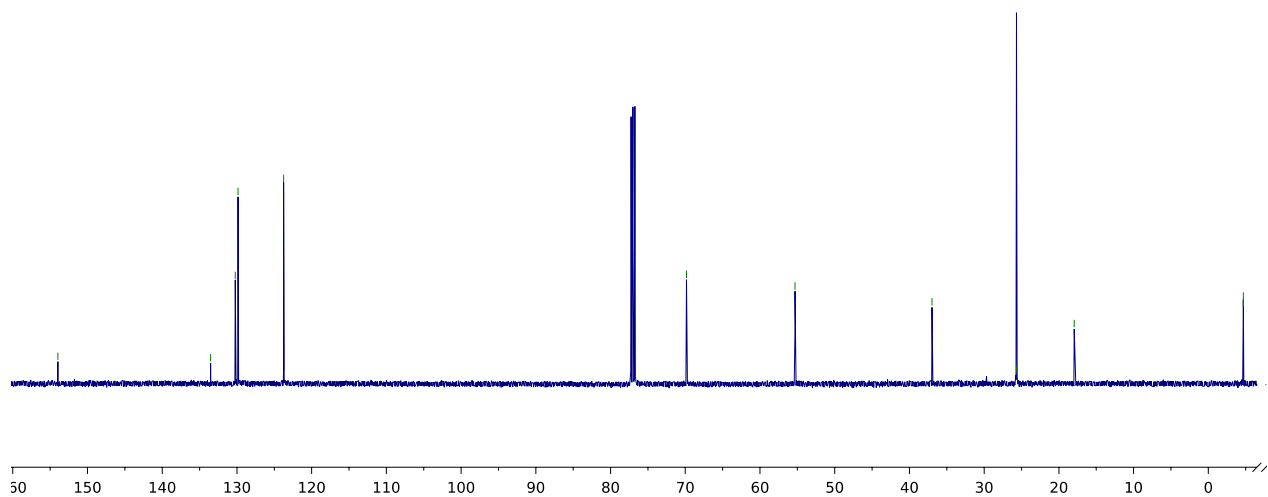
(±)-11

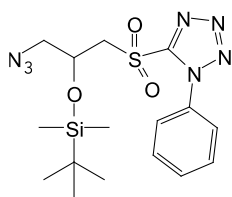
$^1\text{H-NMR}$ (500 MHz, CDCl_3)



(±)-11

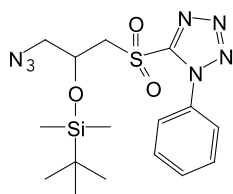
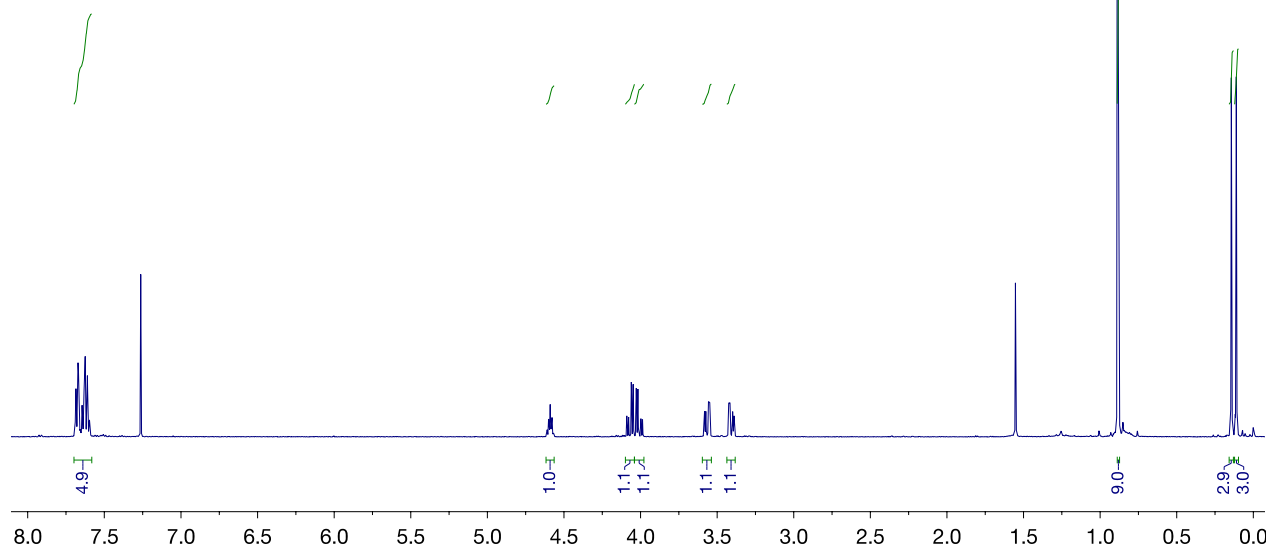
$^{13}\text{C-NMR}$ (125 MHz, CDCl_3)





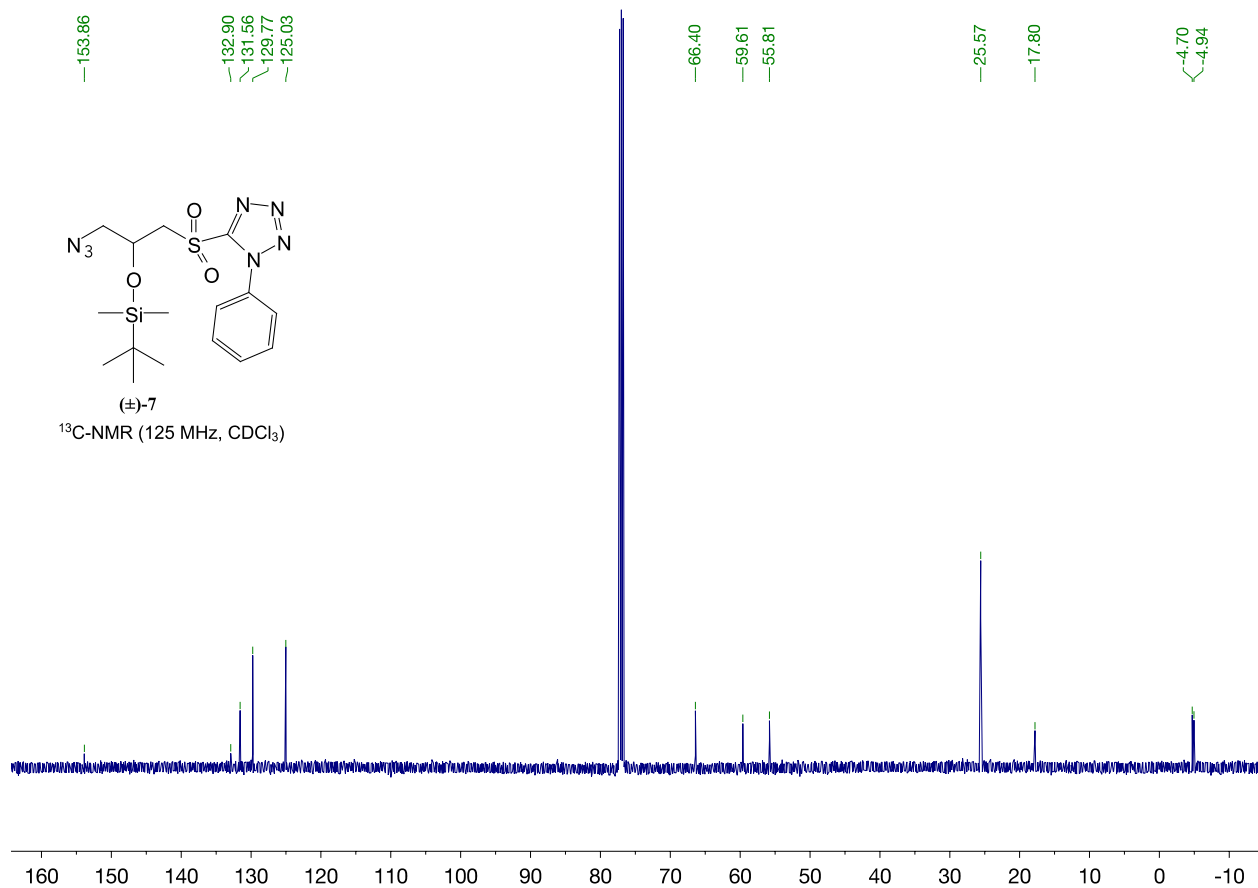
(±)-7

¹H-NMR (500 MHz, CDCl₃)



(±)-7

¹³C-NMR (125 MHz, CDCl₃)



¹H-NMR (500 MHz, CDCl₃)

CCOC(=O)/C=C/C=C/C=C/C(OTBS)C[N+]=[N-]
(±)-15

CC1=CC=CC=C1N2N=NS(=O)(=O)C2(C(OTBS)C[N+]=[N-])
(±)-7

Chemical shift markers (ppm): 166.95, 143.98, 139.18, 137.26, 130.61, 130.59, 121.55, 77.27, 77.02, 76.77, 72.63, 60.35, 56.96, 25.75, 18.13, 14.30, -4.57, -4.97.

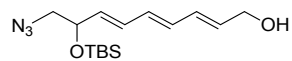
CCOC(=O)/C=C/C=C/C=C/C=C/C(OTBS)C[N+]=[N-]
(±)-15

CC1=NC(=N2C(=N1)N(C2)S(=O)(=O)C(OTBS)C[N+]=[N-])C3=CC=CC=C3
(±)-7

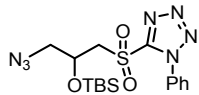
1H NMR spectrum (CDCl₃) of compound **7**. The spectrum shows several peaks, including aromatic signals (7H each), a methyl singlet (3H), and a TMS reference peak (0 ppm). Integration values are shown above the peaks: 7.00, 7.00, 7.00, 7.00, 3.00, 3.00, 3.00. Asterisks (*) indicate peaks that are not integrated.

16 crude from reaction mixture

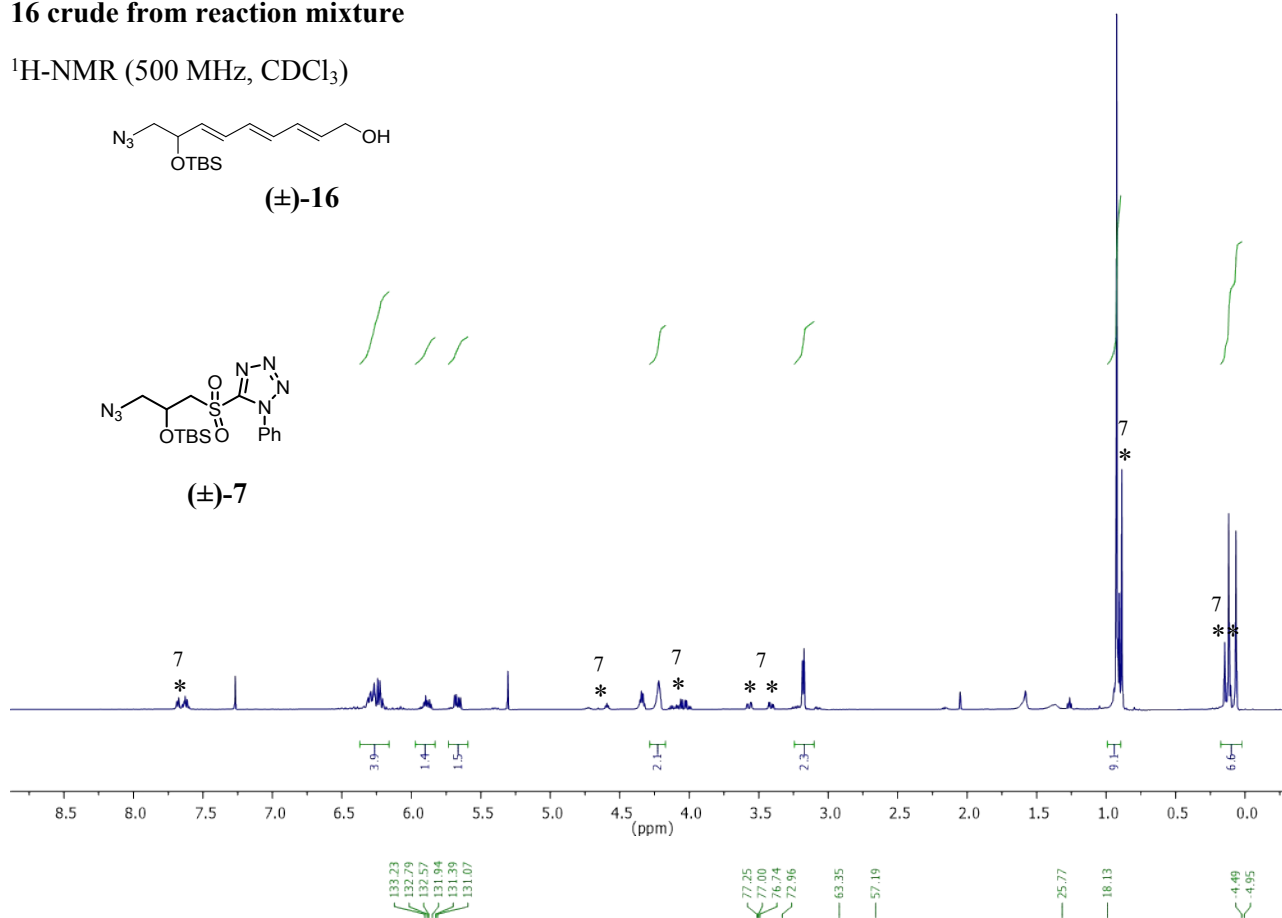
$^1\text{H-NMR}$ (500 MHz, CDCl_3)



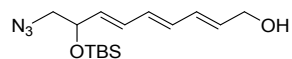
(±)-16



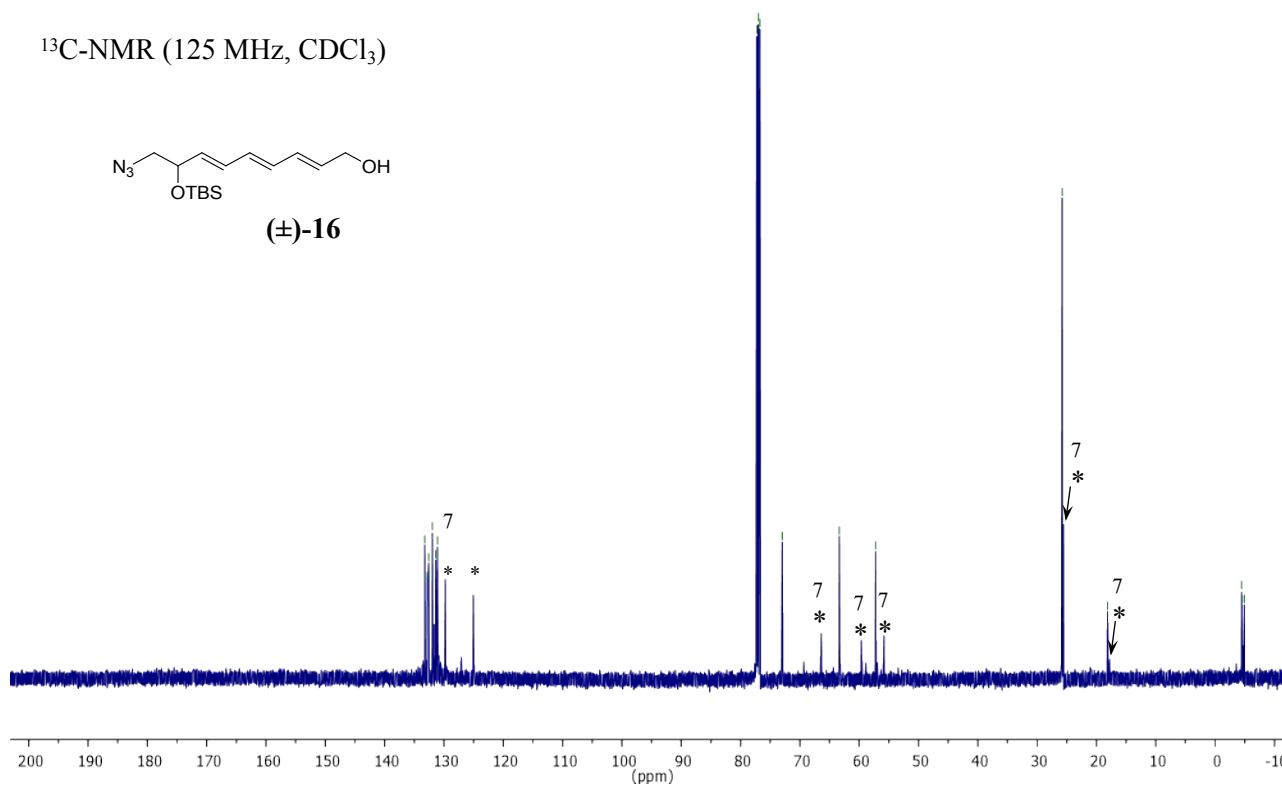
(±)-7



$^{13}\text{C-NMR}$ (125 MHz, CDCl_3)



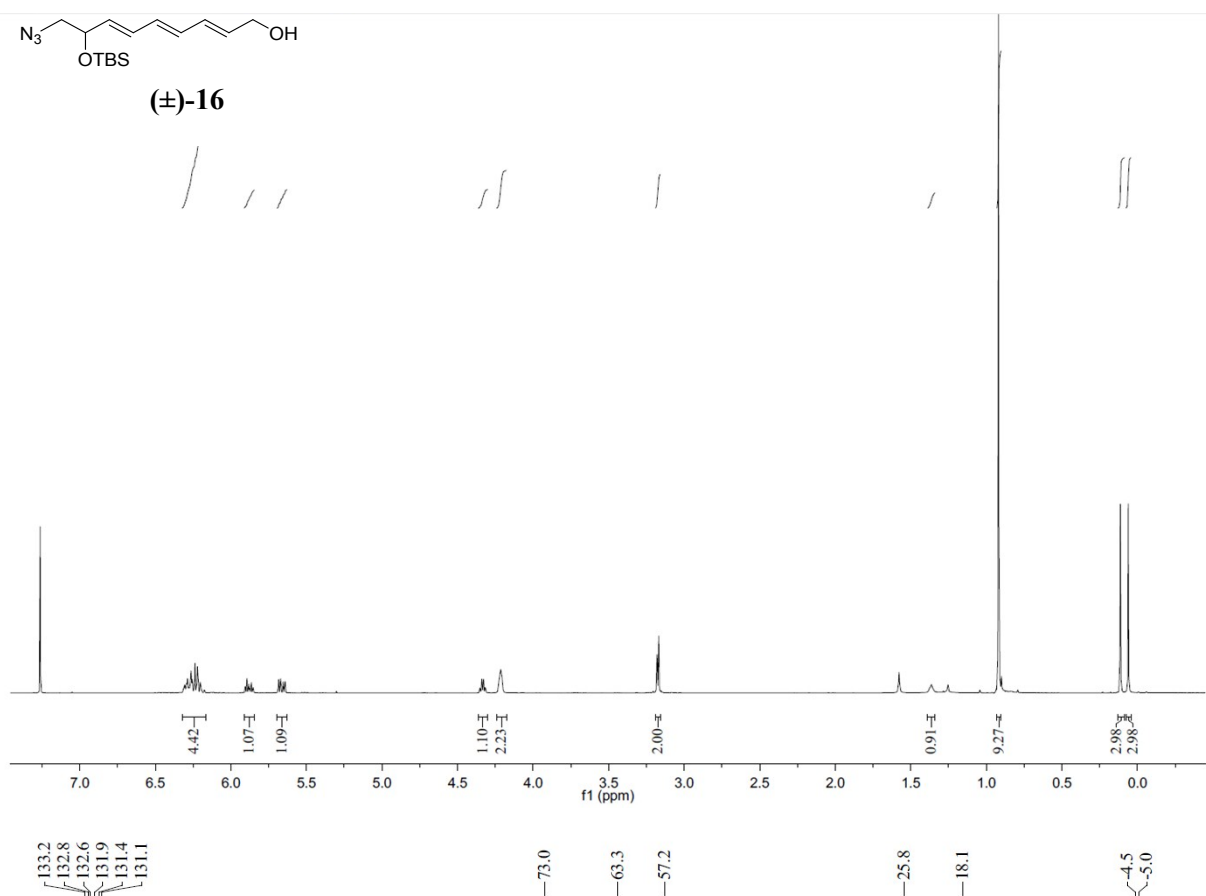
(±)-16



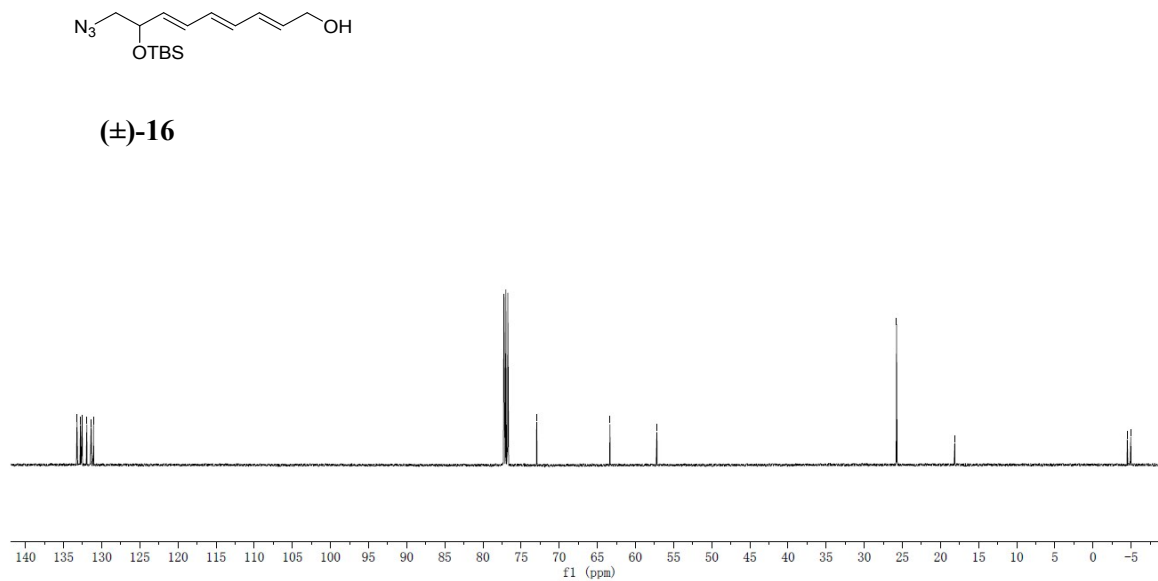
16 following purification.

Extensive purification of **16** removes traces of **7**, but retains a minor impurity. The loss of product required to achieve purification at this stage is not warranted in terms overall yield for the reaction sequence.

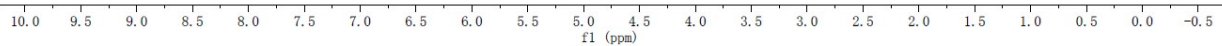
^1H -NMR (500 MHz, CDCl_3)



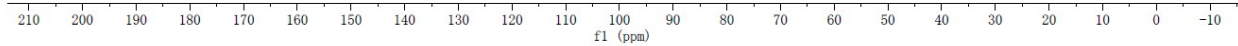
^{13}C -NMR (125 MHz, CDCl_3)

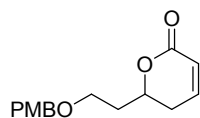


1 1 1 1 1



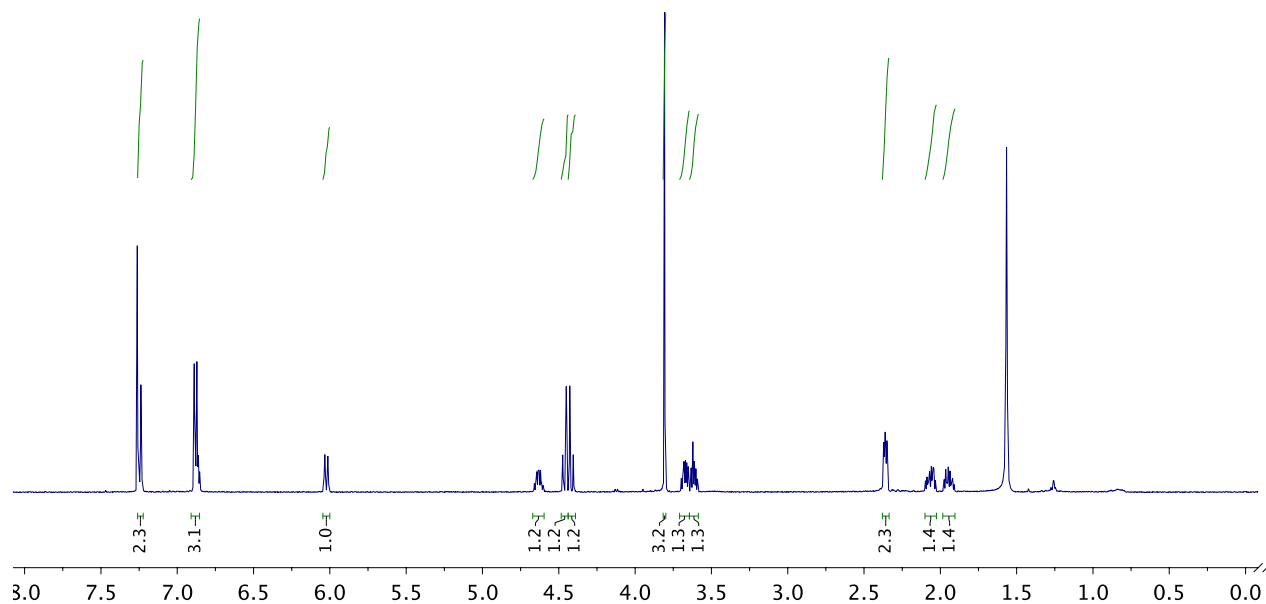
—193.5
—151.4
141.1
139.1
131.8
130.5
130.3



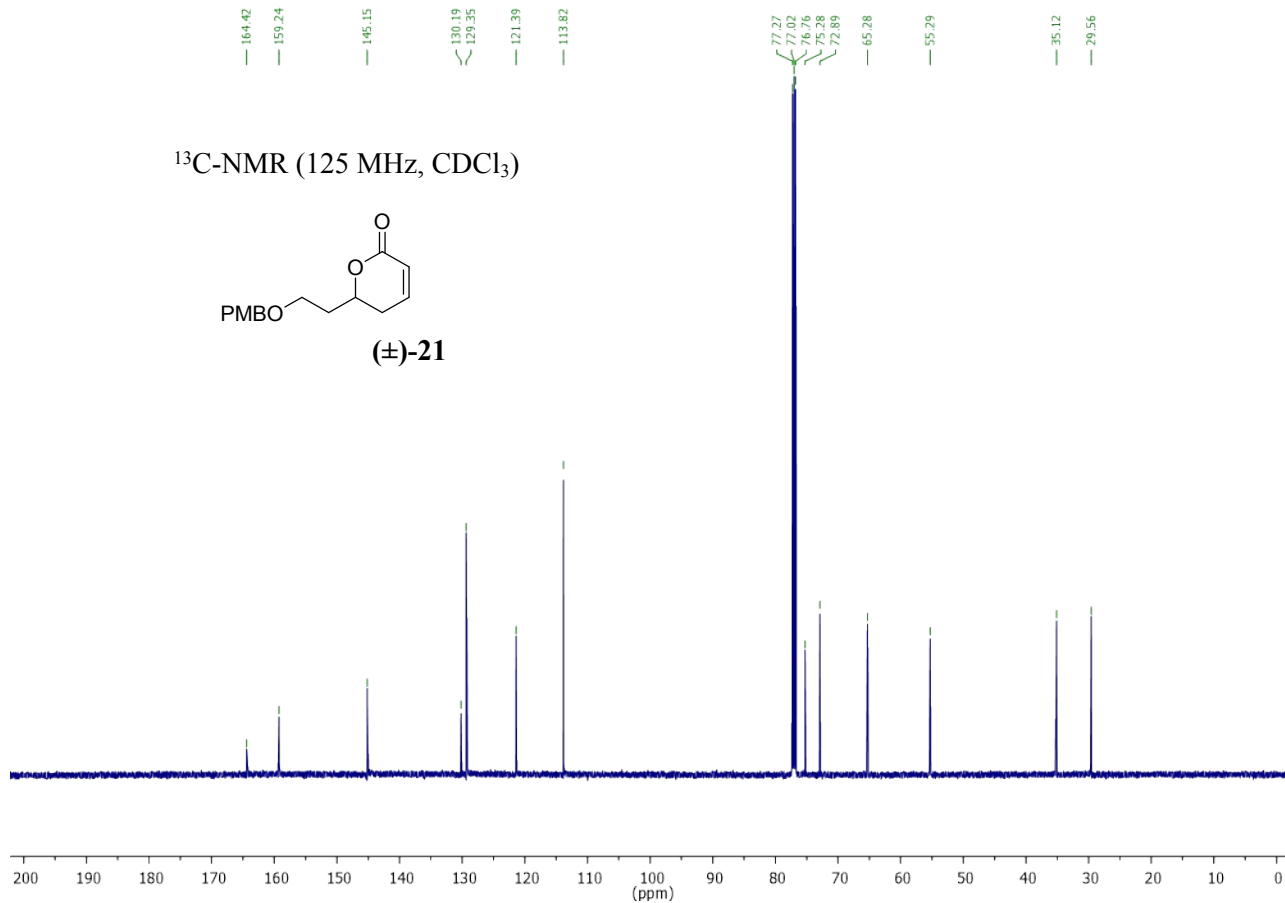
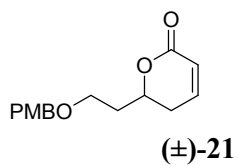


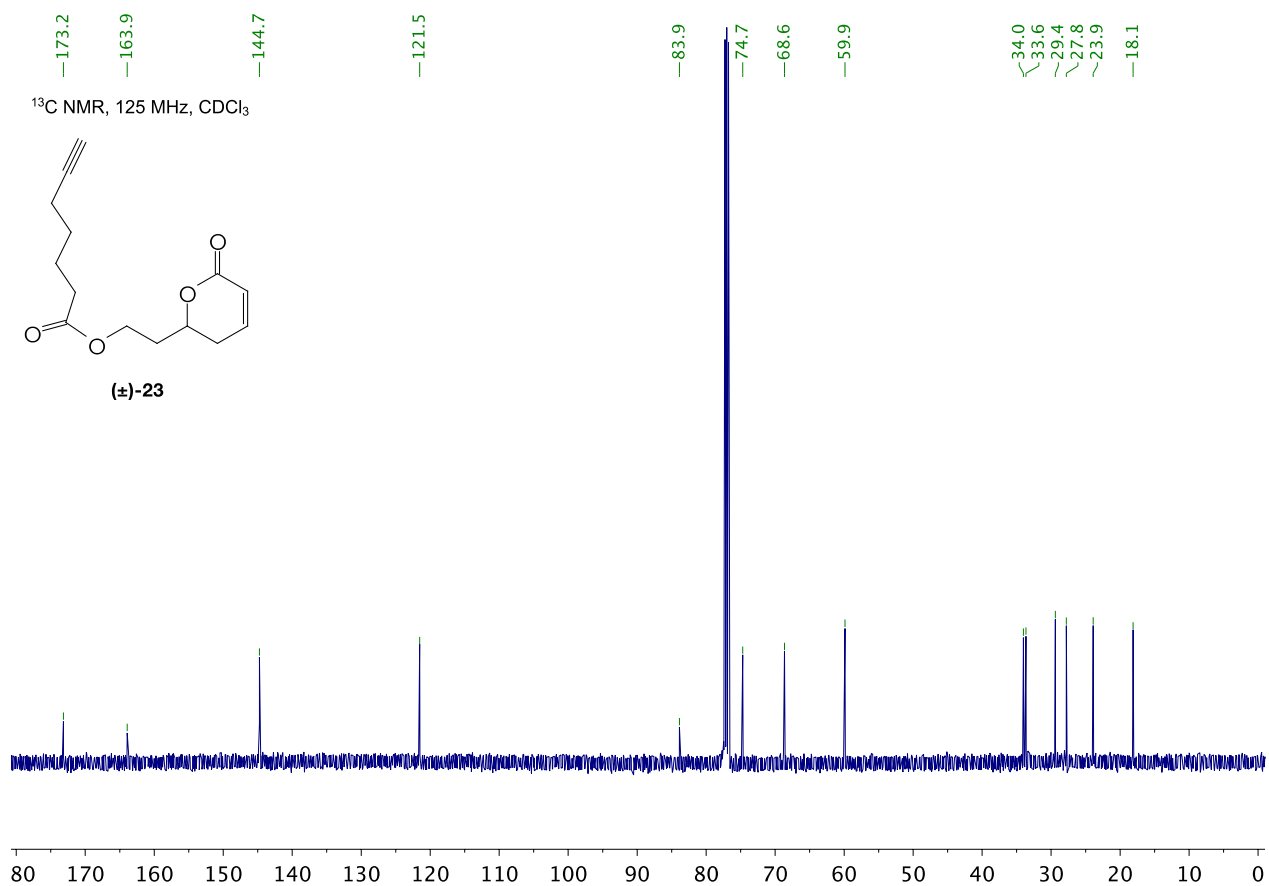
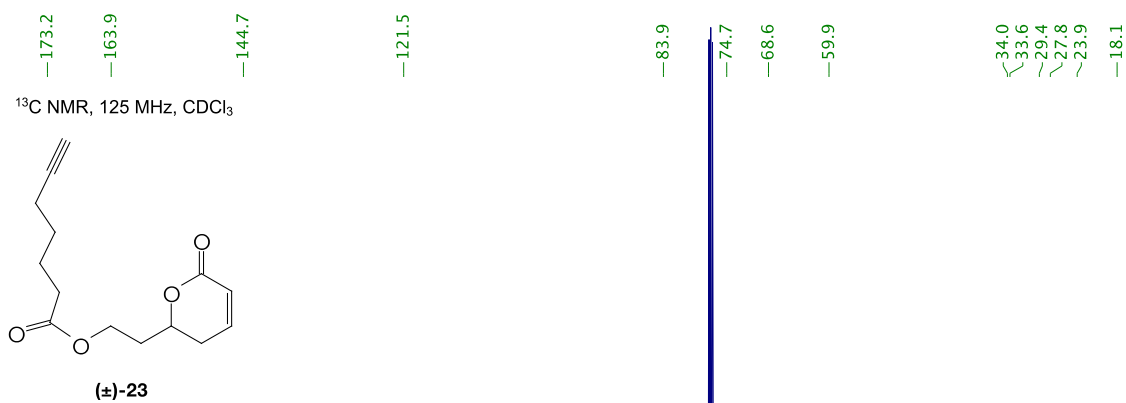
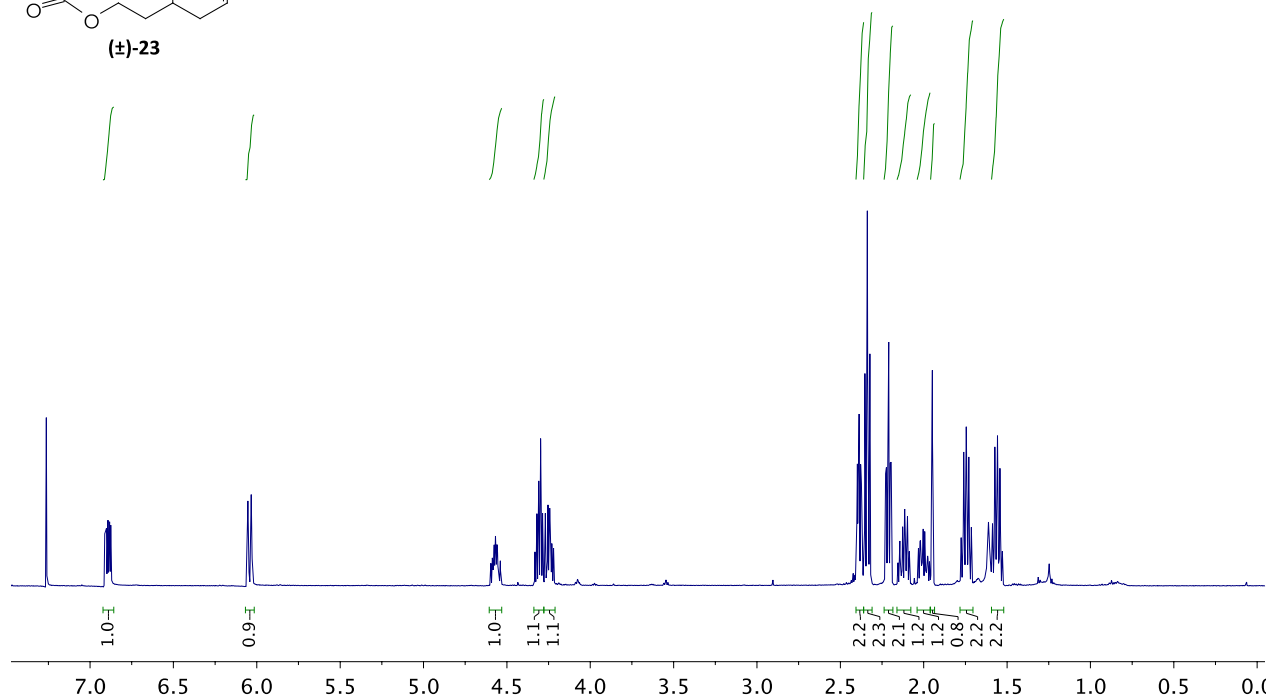
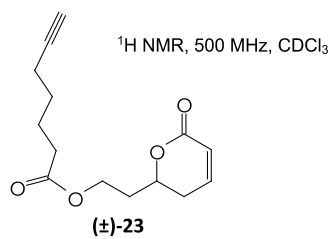
(±)-21

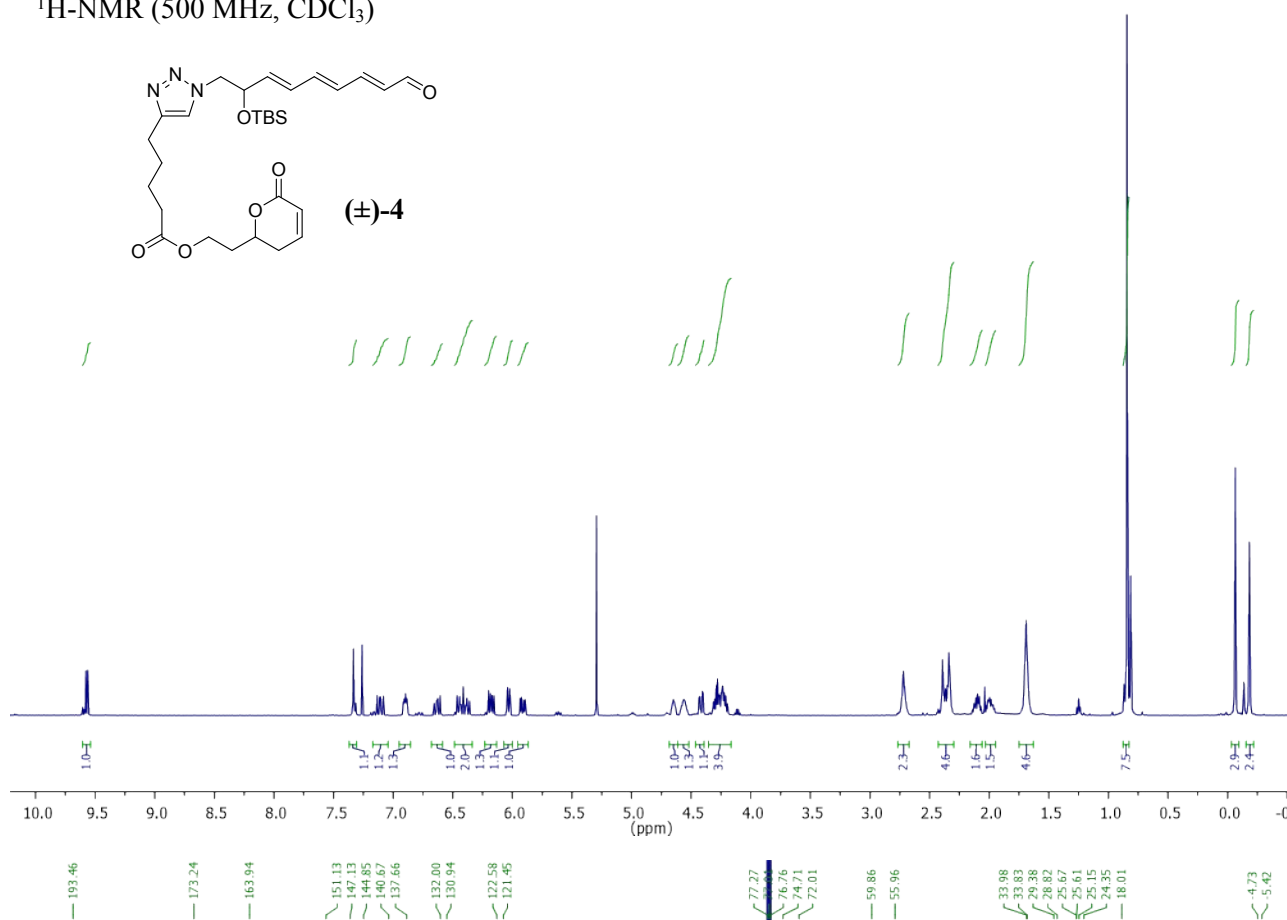
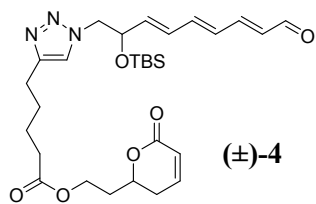
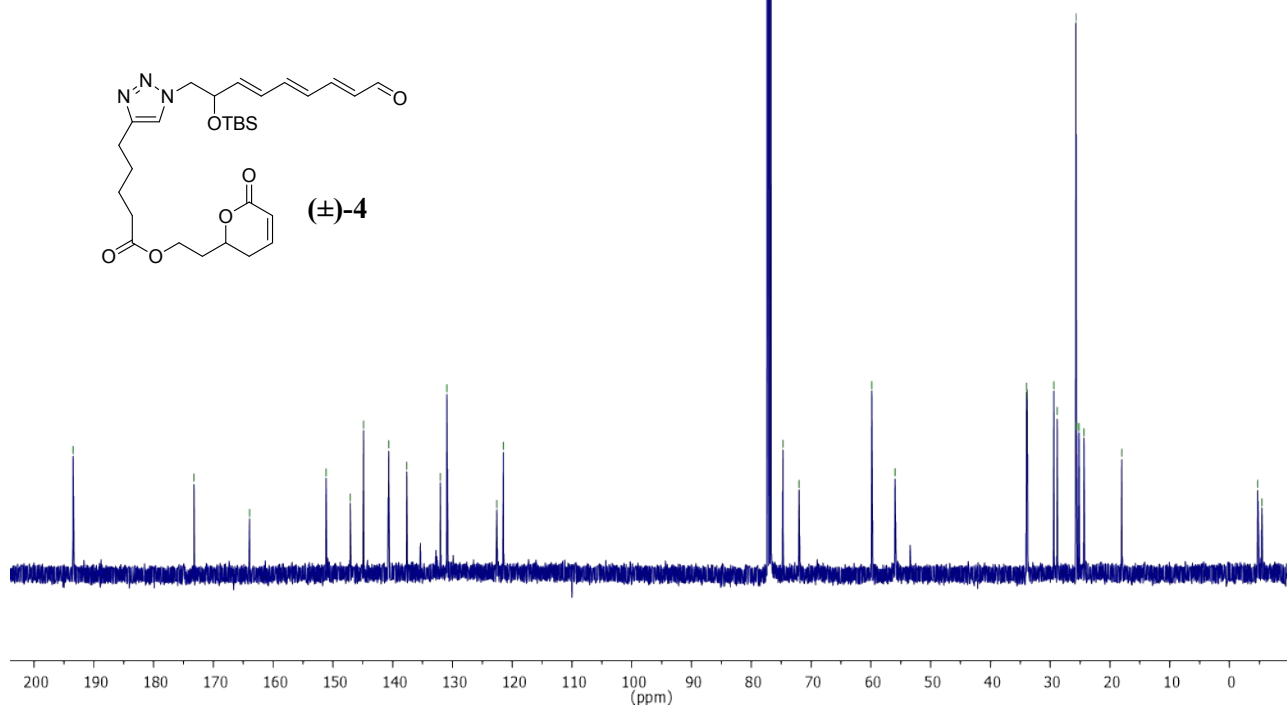
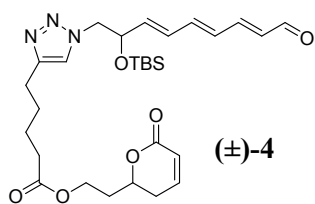
¹H NMR, 500 MHz, CDCl₃



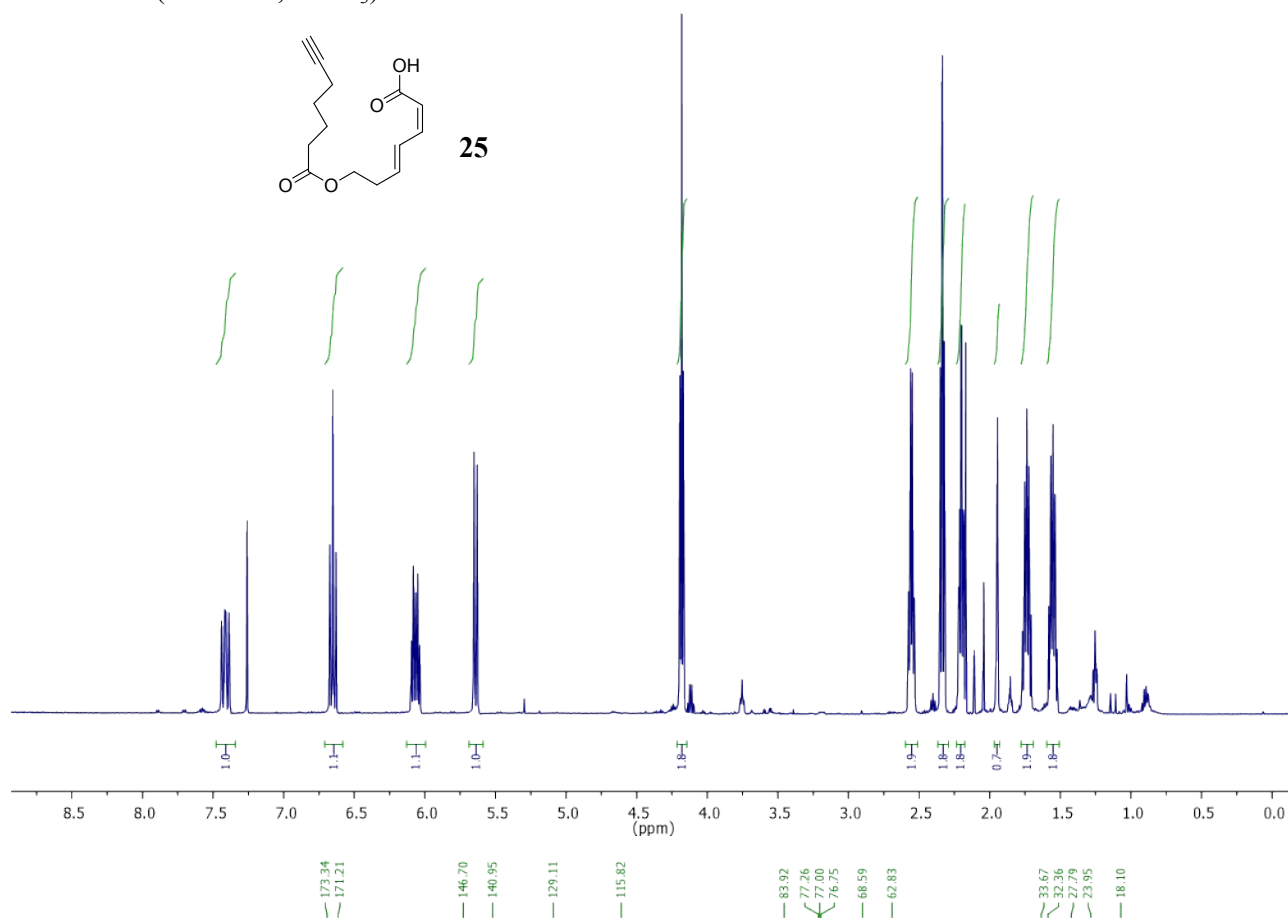
¹³C-NMR (125 MHz, CDCl₃)



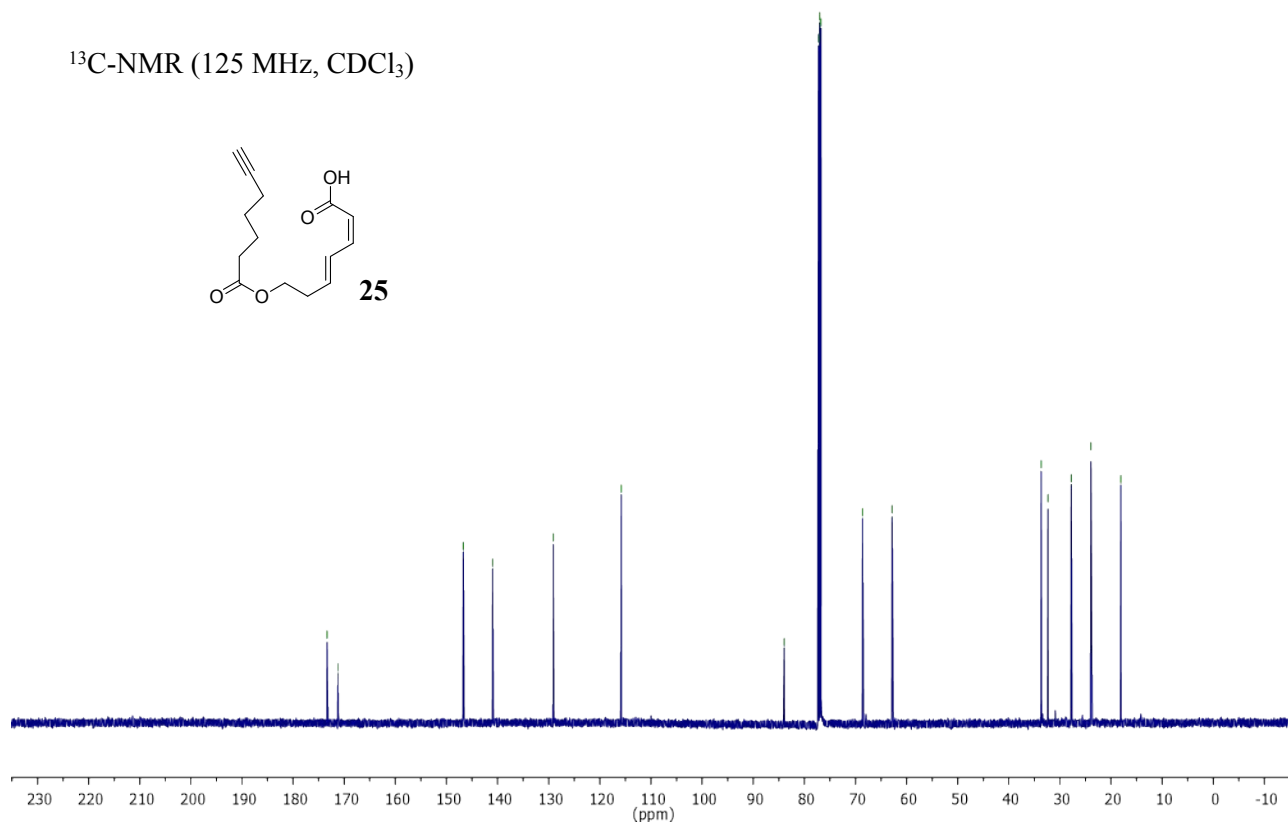


¹H-NMR (500 MHz, CDCl₃) ^{13}C -NMR (125 MHz, CDCl_3)

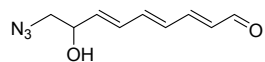
^1H -NMR (500 MHz, CDCl_3)



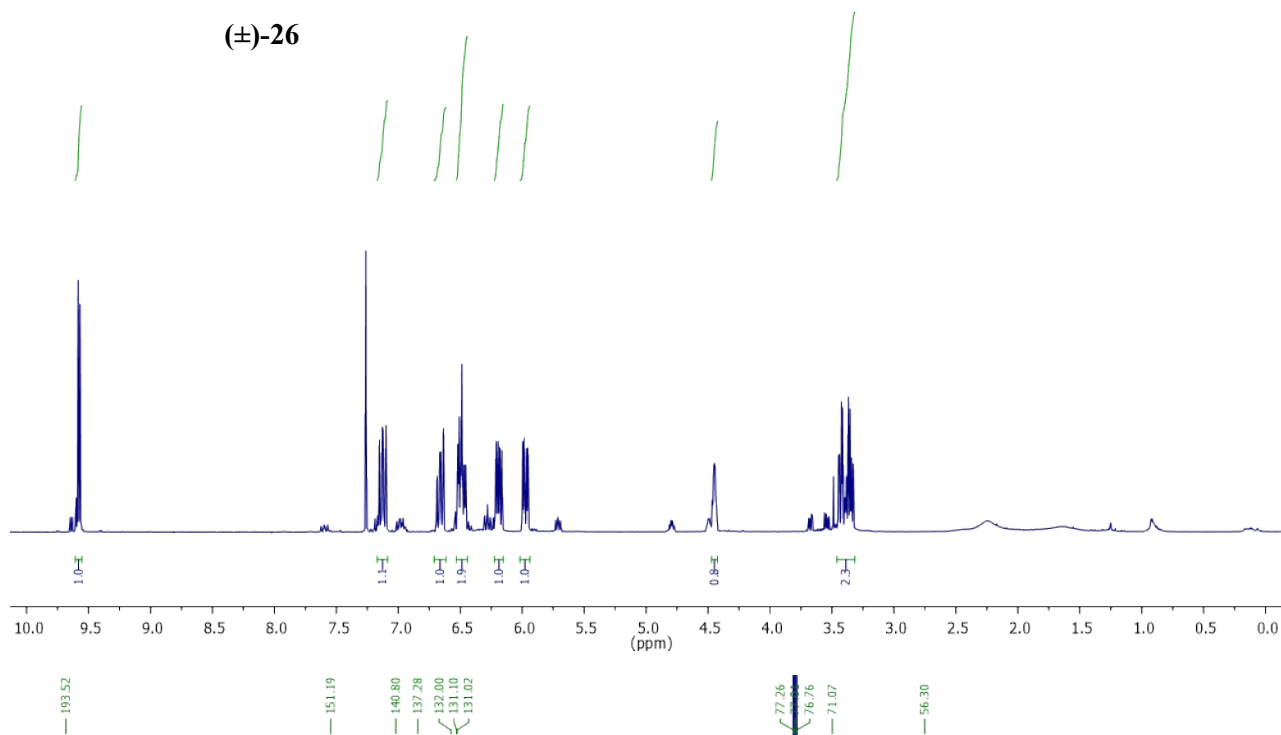
^{13}C -NMR (125 MHz, CDCl_3)



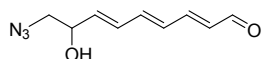
^1H -NMR (500 MHz, CDCl_3)



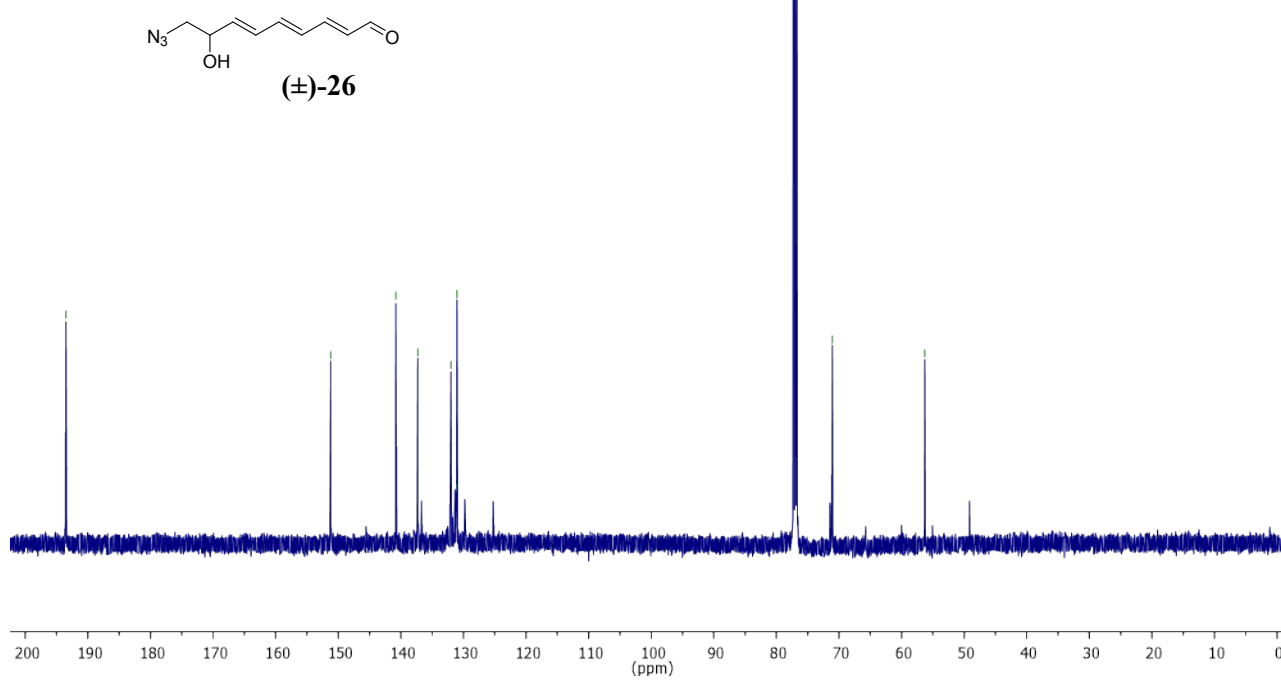
(\pm)-26



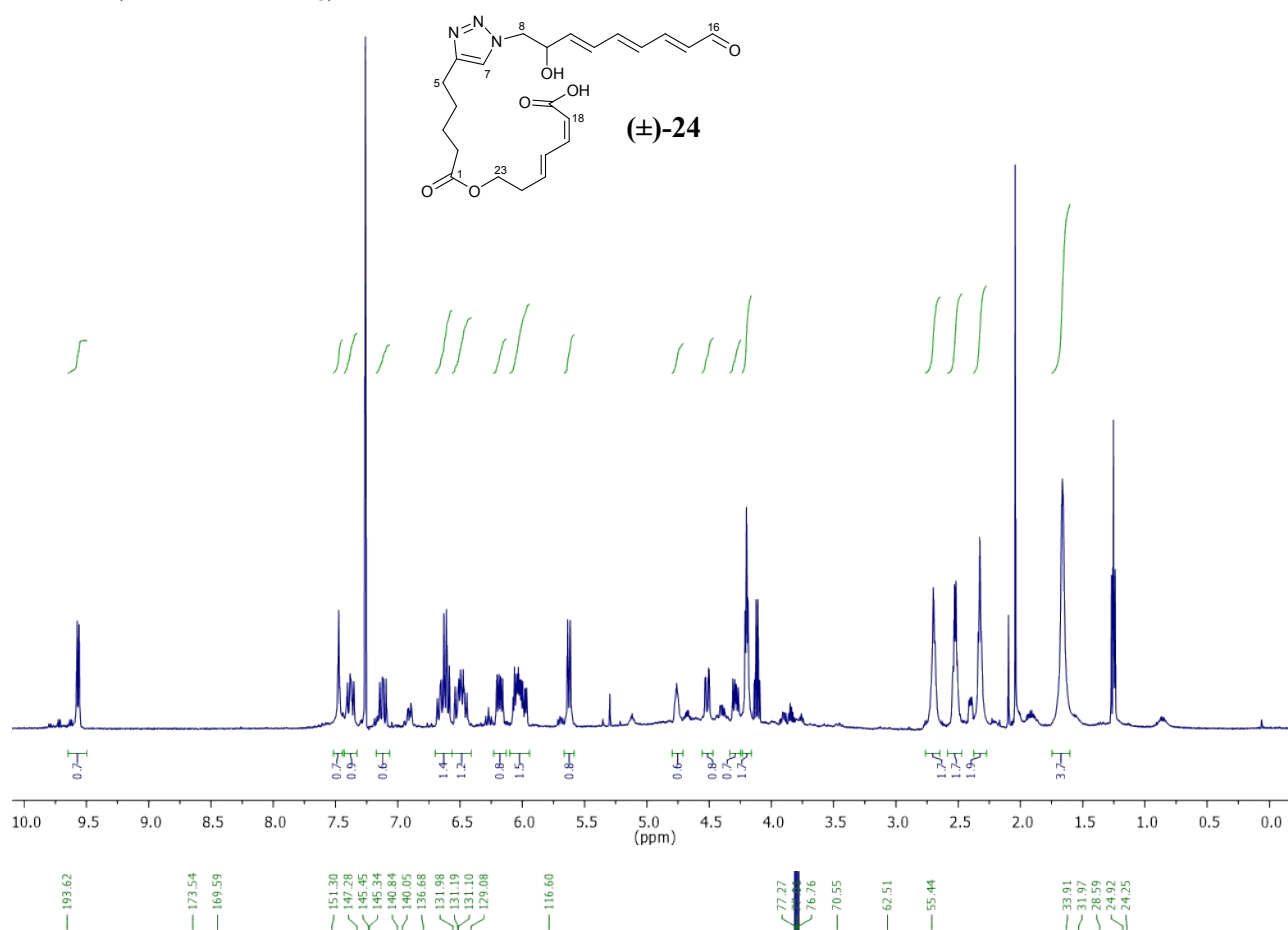
^{13}C -NMR (125 MHz, CDCl_3)



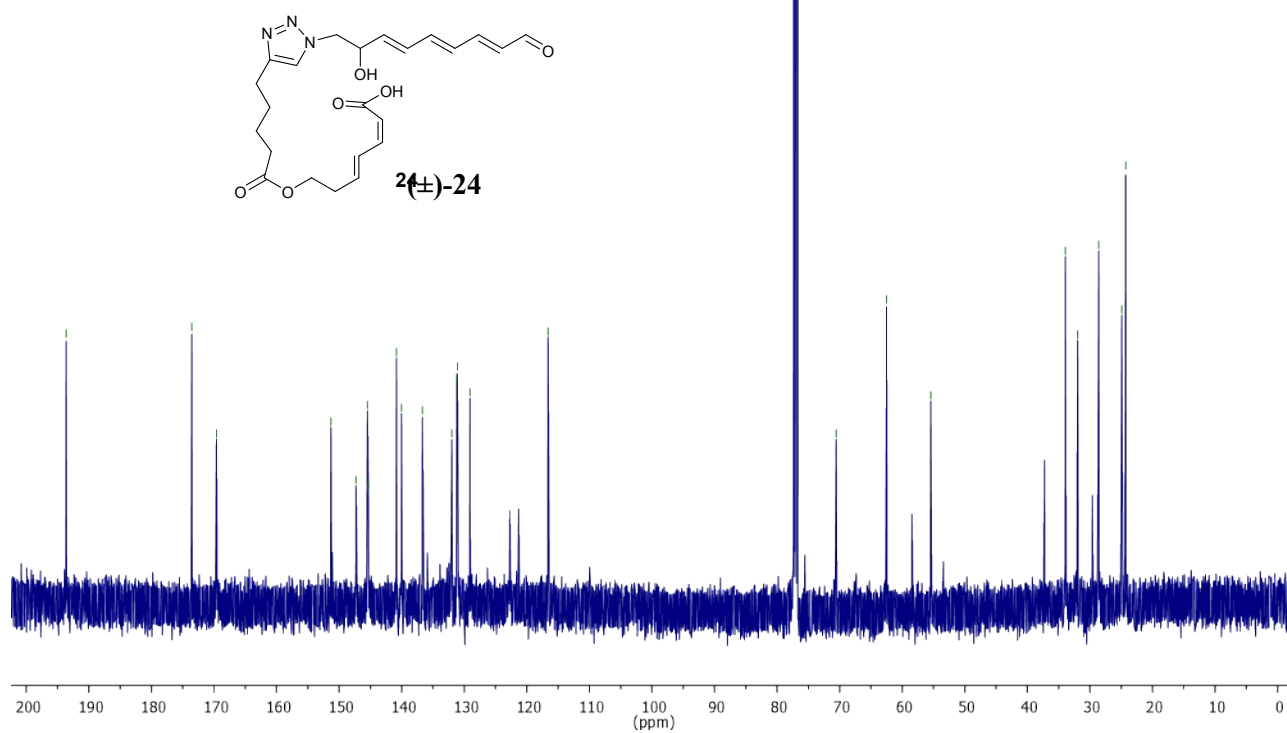
(\pm)-26



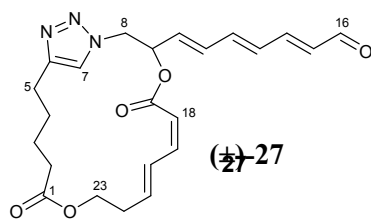
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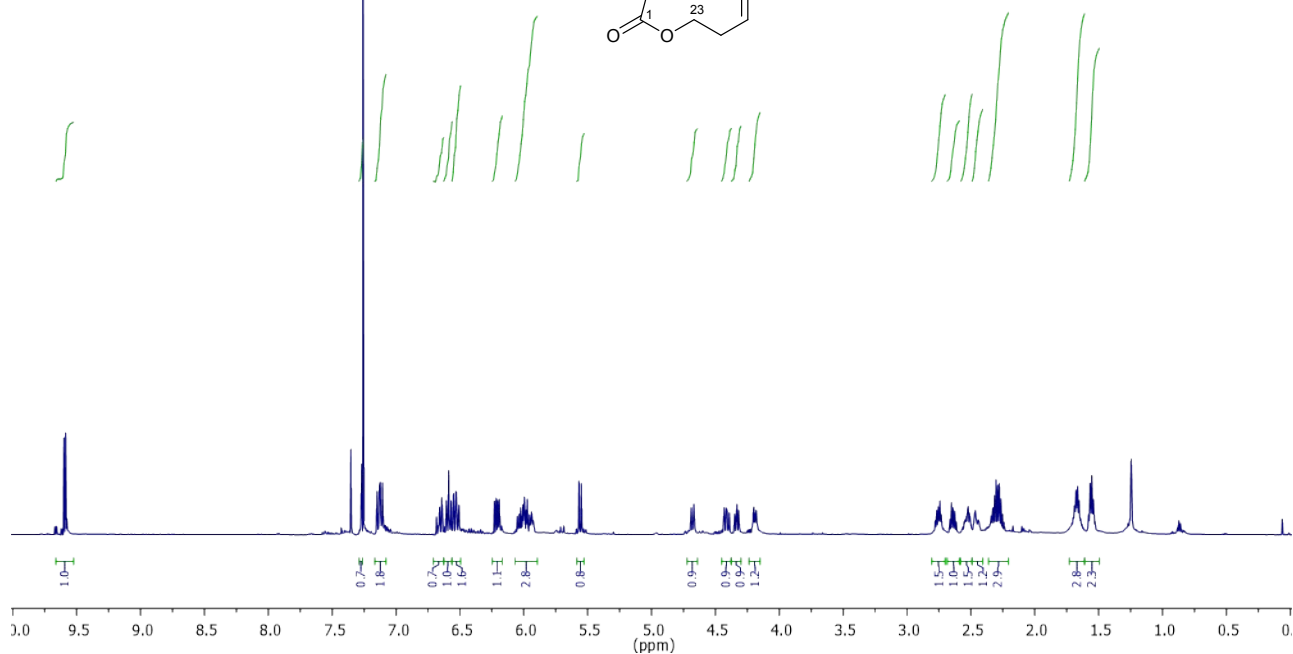
^{13}C -NMR (125 MHz, CDCl_3)



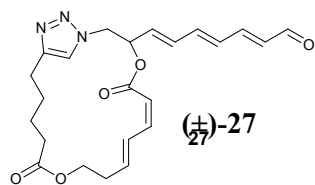
¹H-NMR (600 MHz, CDCl₃)



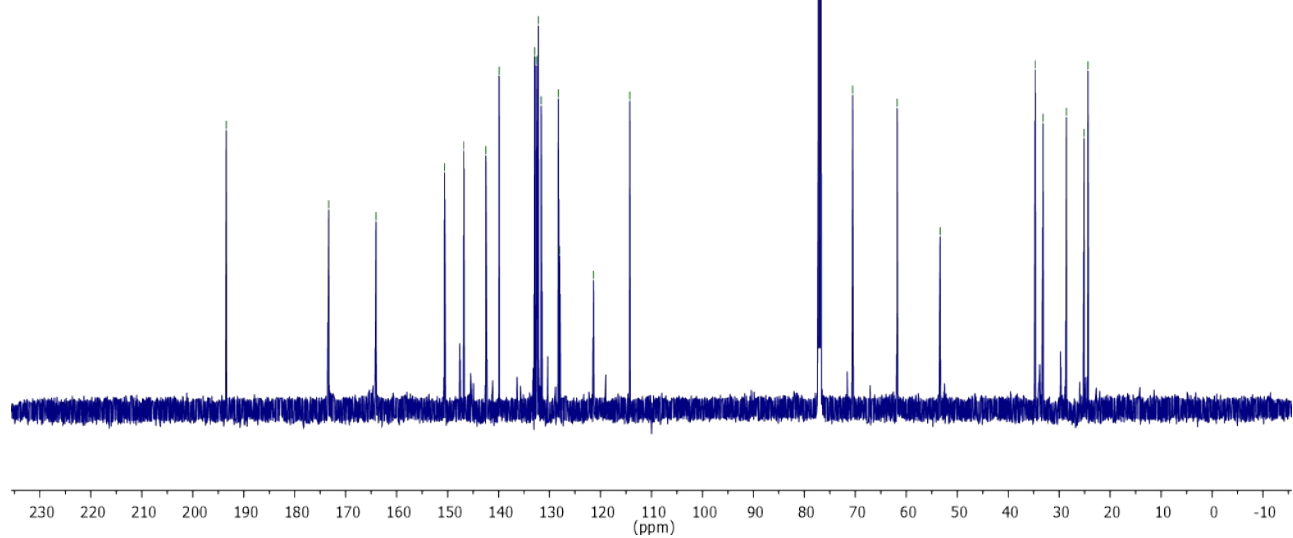
(±)-27



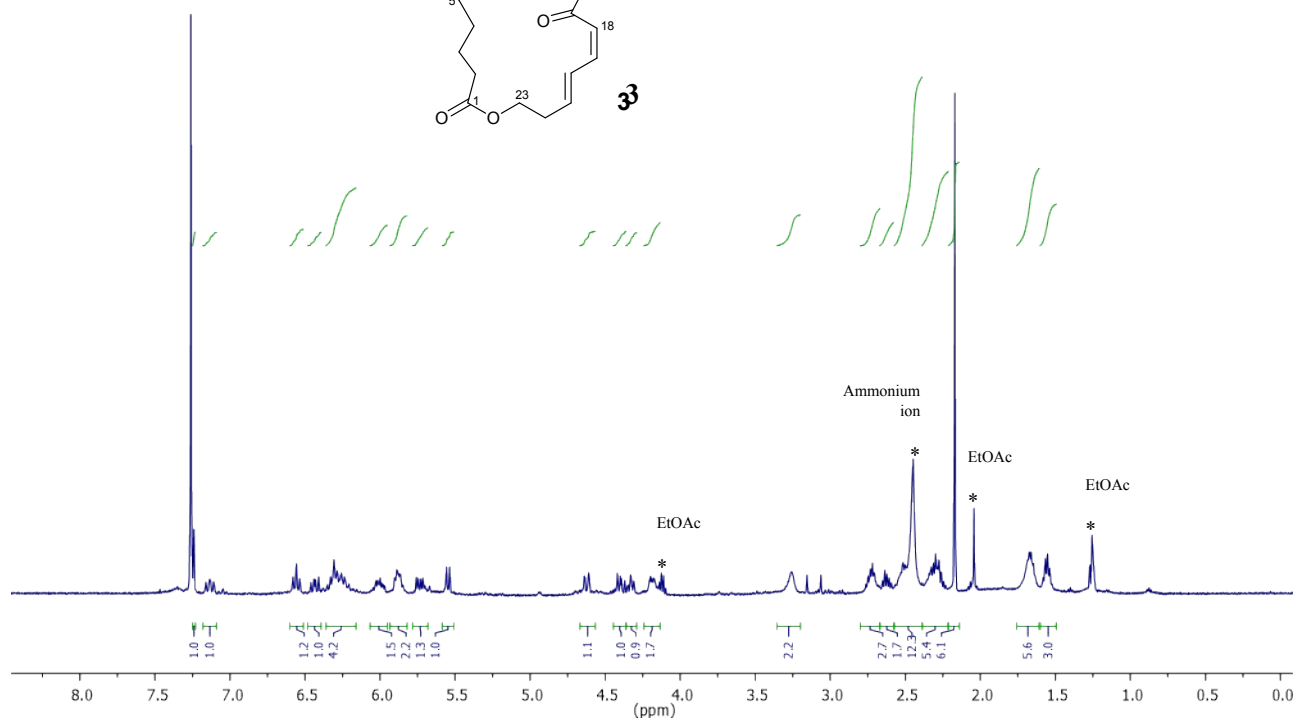
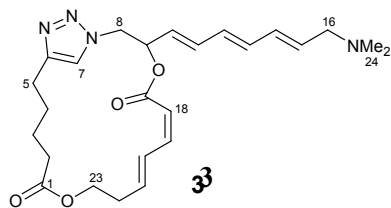
¹³C-NMR (151 MHz, CDCl₃)



(±)-27



$^1\text{H-NMR}$ (500 MHz, CDCl_3)



2D COSY (500 MHz, CDCl_3)

