SUPPORTING INFORMATION

Convergent synthesis and properties of photoactivable NADPH mimics targeting nitric oxide synthases

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Absorption spectra of NT compounds 1-7

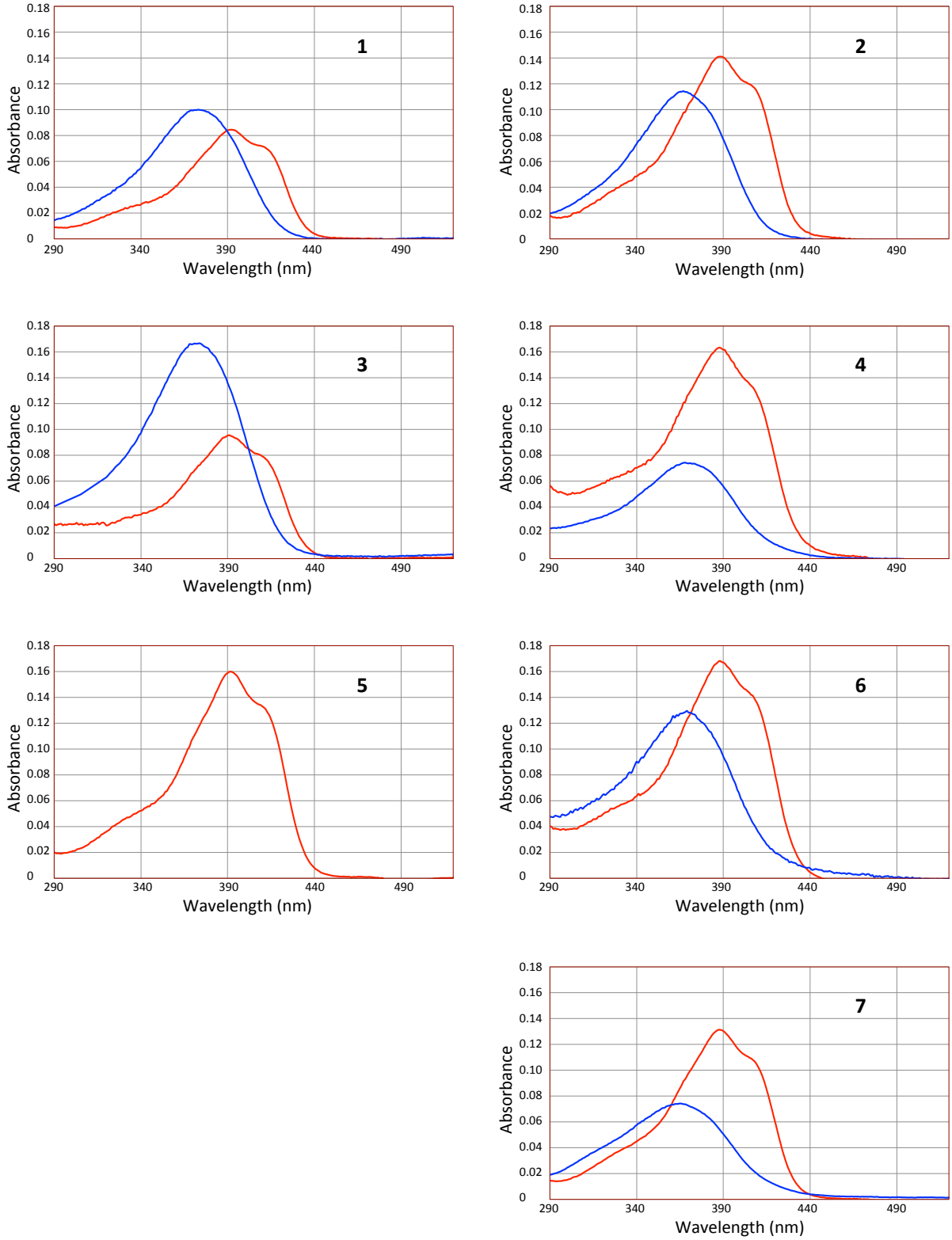
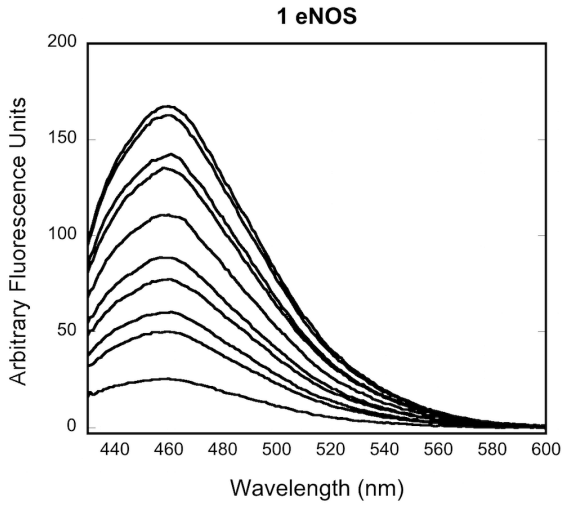
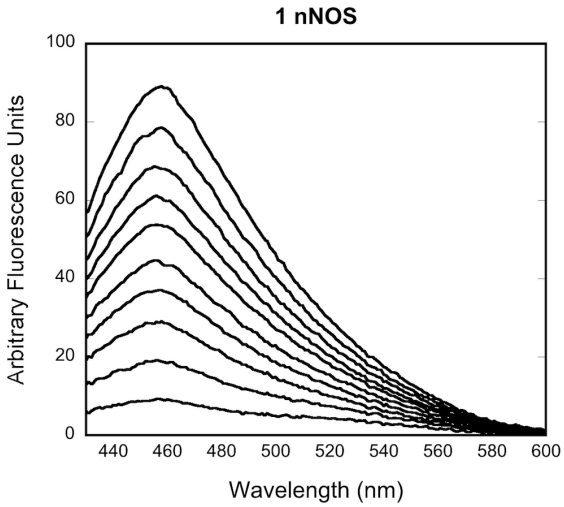
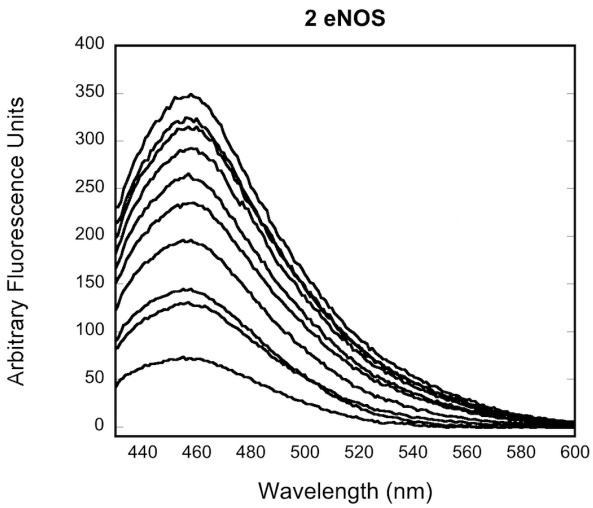


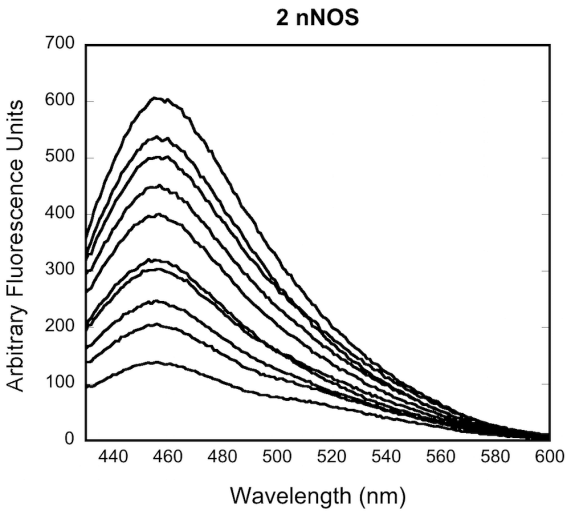
Figure S1: Absorption spectra of NT compounds **1-7** in Tris-HCl buffer (blue) or in DMSO (red). Left column: NT compounds with $R_1 = CH_3$ (**1**, **3**, **5**); right column: NT compounds with $R_1 = H$ (**2**, **4**, **6**, **7**). NT concentration was 2 μM.

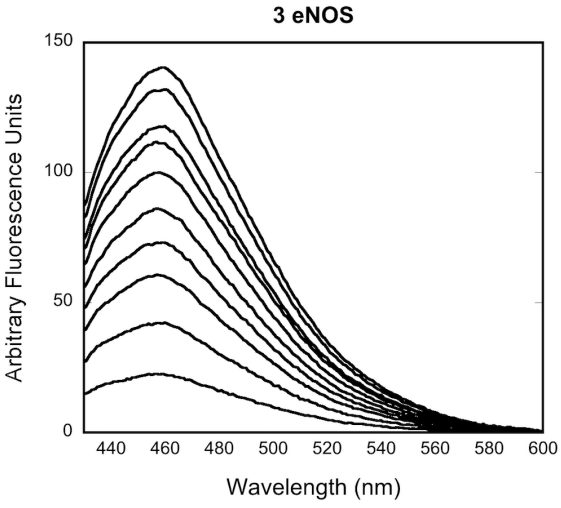
Fluorescence titration spectra of NT compounds 1-7 with NOS

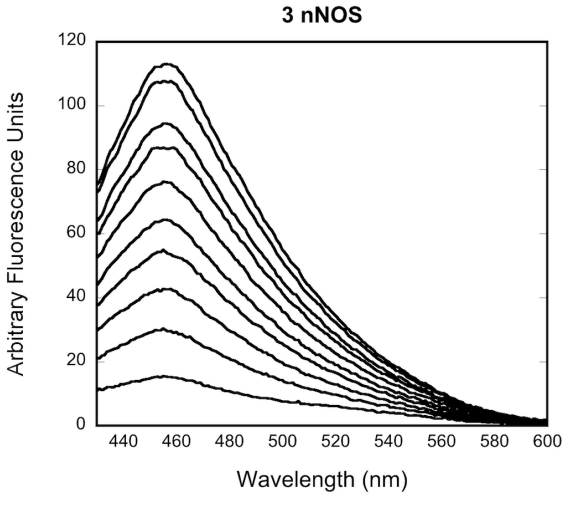












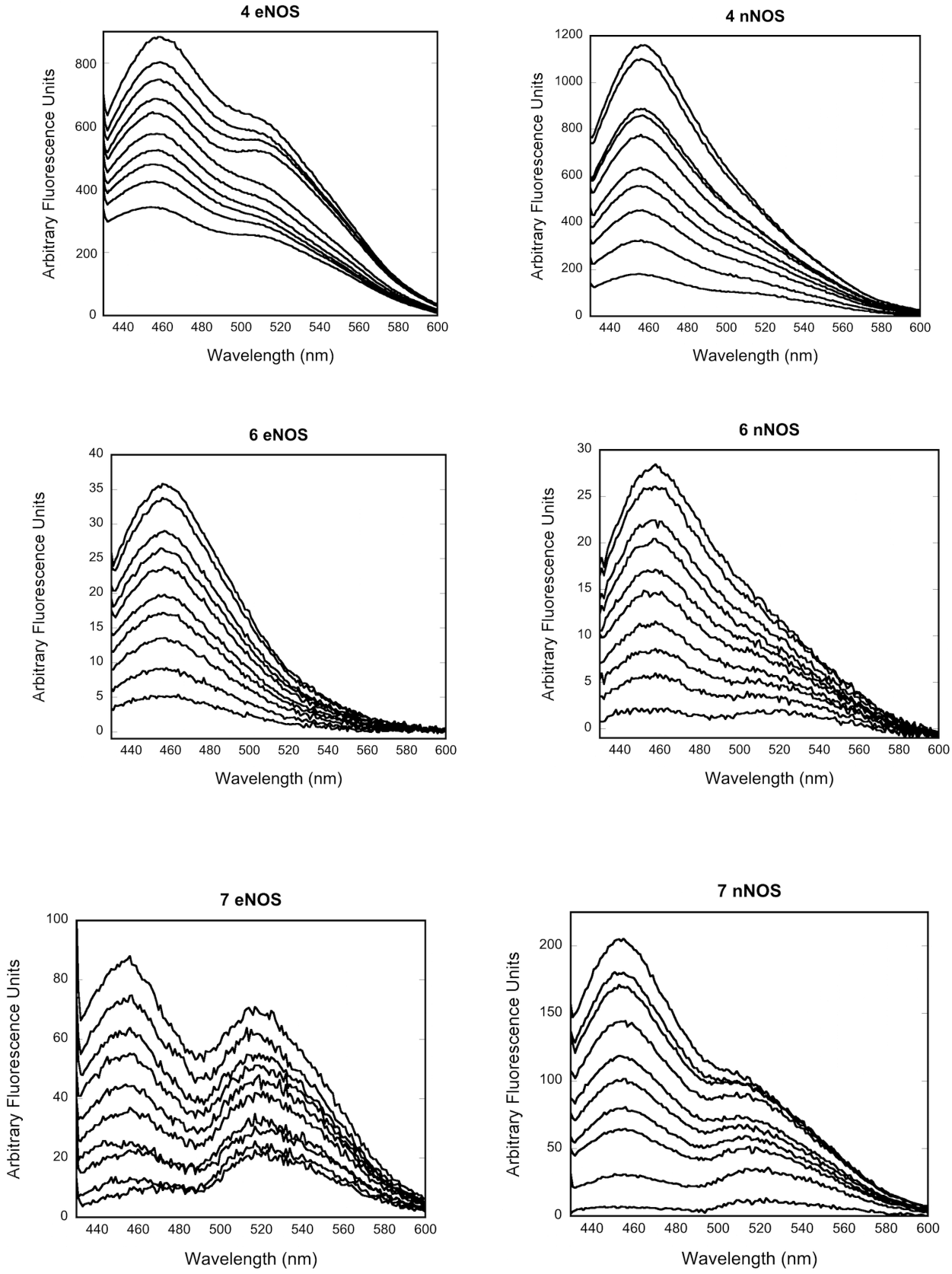


Figure S2: Fluorescence titration spectra of NT compounds **1-7** with NOS minus the spectra of free NOS and free NT. Data shown are representative from at least n = 3 titrations. NT concentrations are: 3, 6, 9, 12, 15, 20, 25, 30, 40, and 50 μM (spectra from bottom to top, respectively). NOS protein concentration was 5 μM. The excitation wavelength for all compounds was 420 nm and emission spectra were recorded from 430-700 nm.

Immuno-staining of eNOS on fixed cells.
The procedure for eNOS immuno-staining was previously described.[1] Briefly, PBS-0.5% Saponin was used as a permeabilization solution for 10 min at room temperature and the blocking solution was PBS-3% BSA. Purified mouse anti-eNOS/NOS Type III primary antibody (BD-Biosciences, France; ref. no. 610297) was used at a final concentration of 10 µg/mL in PBS-0.5% Saponin /3% BSA. The secondary antibody, AlexaFluor® 594 goat anti-mouse IgG (H+L) was purchased from Invitrogen (France; ref. no. A-11005) and used at a final concentration of 4 µg/mL in PBS-0.5% Saponin / 3% BSA. The fixation buffer BD CytofixTM was purchased from BD-Biosciences. Confocal images were obtained using a SP2 confocal microscope (Leica MicroSystems).

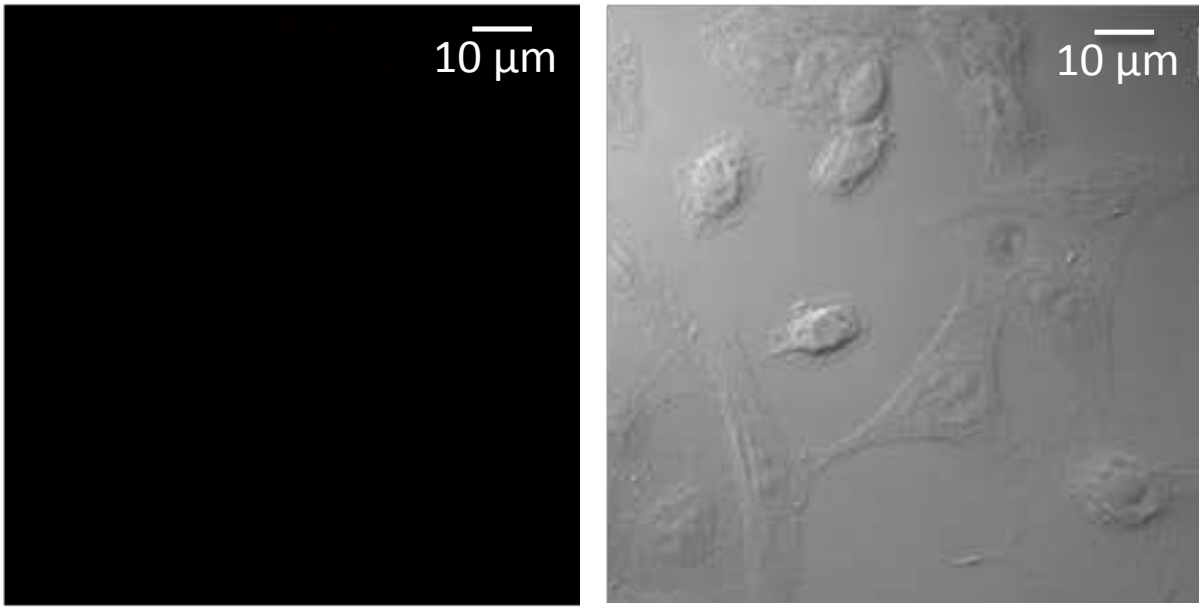


Figure S3: Immuno-staining of eNOS on fixed Hela cells. HeLa cells were treated with primary and secondary antibodies for immune-staining as indicated above. Primary and secondary antibodies were purified mouse anti-eNOS/NOS Type III and AlexaFluor 594 goat anti-mouse IgG (H+L), respectively. Left, confocal image using the imaging channel of AlexaFluor 594 (excitation, 543 nm; emission slit 590-700 nm); right, corresponding DIC (differential interference contrast) transmission image. No detectable emission signal was observed in the AlexaFluor channel, in sharp contrast to the result obtained with HUVECs using the same procedure (Fig. 5 in reference 1), confirming previous independent studies showing that Hela cells do not express eNOS.[2-5]

References:

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3. J. E. Fish, C. C. Matouk, A. Rachlis, S. Lin, S. C. Tai, C. D'Abreo and P. A. Marsden, *J. Biol. Chem.* 2005, **280**, 24824-24838.
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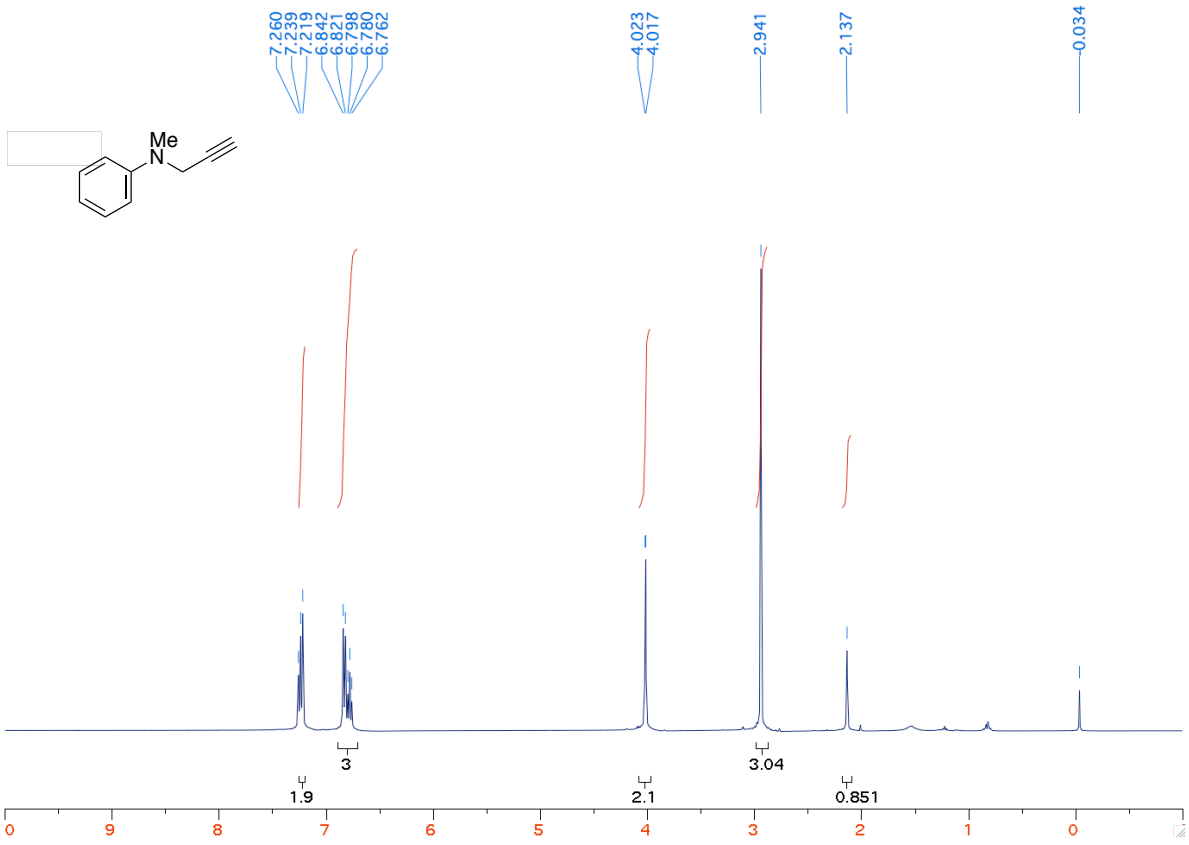
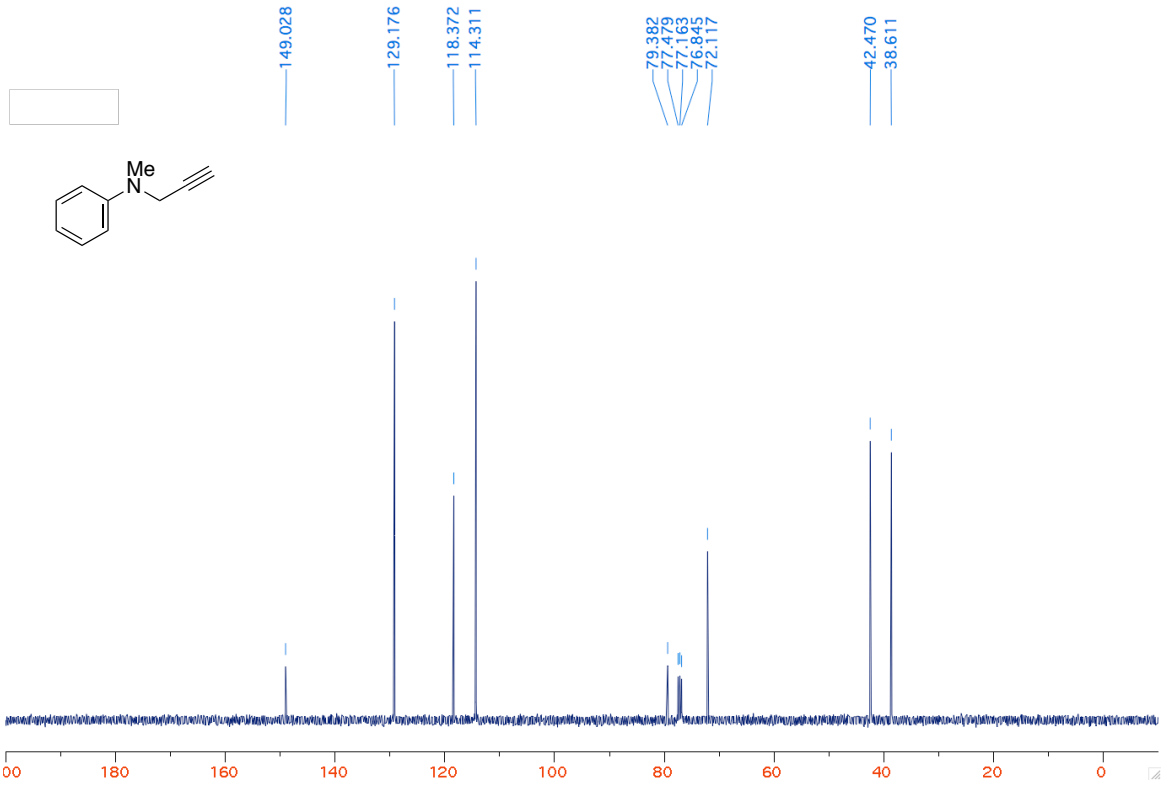
5. S. A. Ritchie, C. F. Kohlhaas, A. R. Boy, K. C. Yalla, K. Walsh, J. M. C. Connell and I. P. Salt, *Biochem. J.* 2010, **426**, 85-90.

^{1}H and ^{13}C NMR spectra of new compounds

^{1}H-NMR spectrum of **8** ($CDCl_3$, 400 MHz)



^{13}C-NMR spectrum of **8** ($CDCl_3$, 100 MHz)



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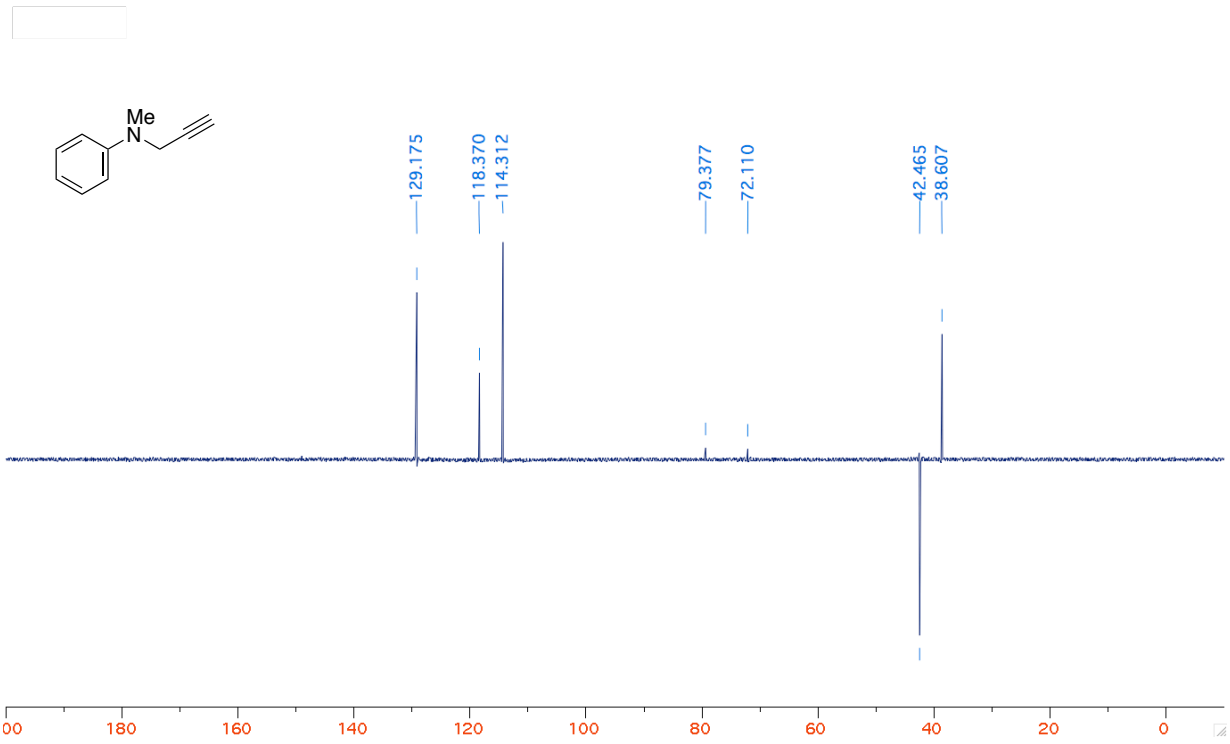

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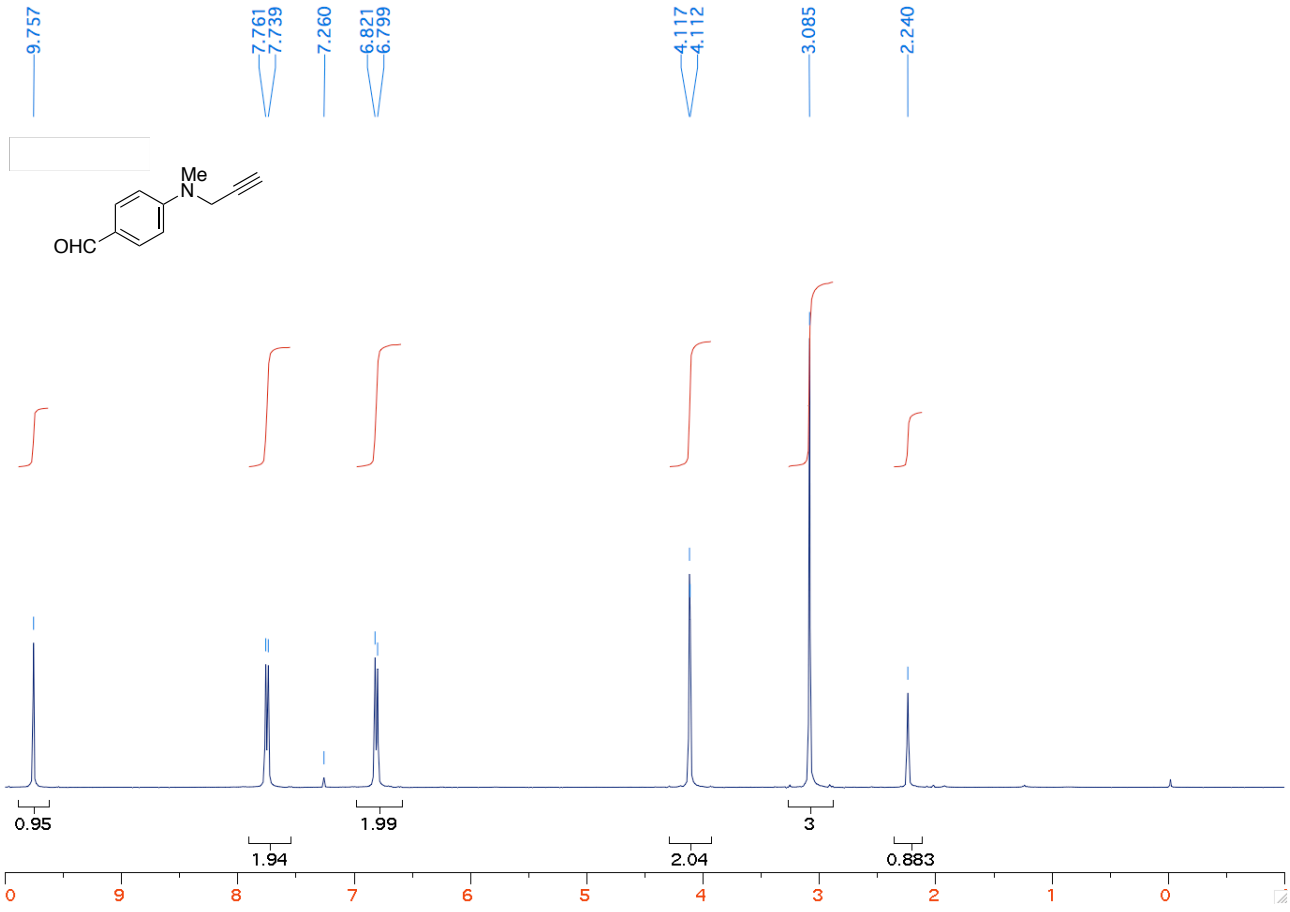

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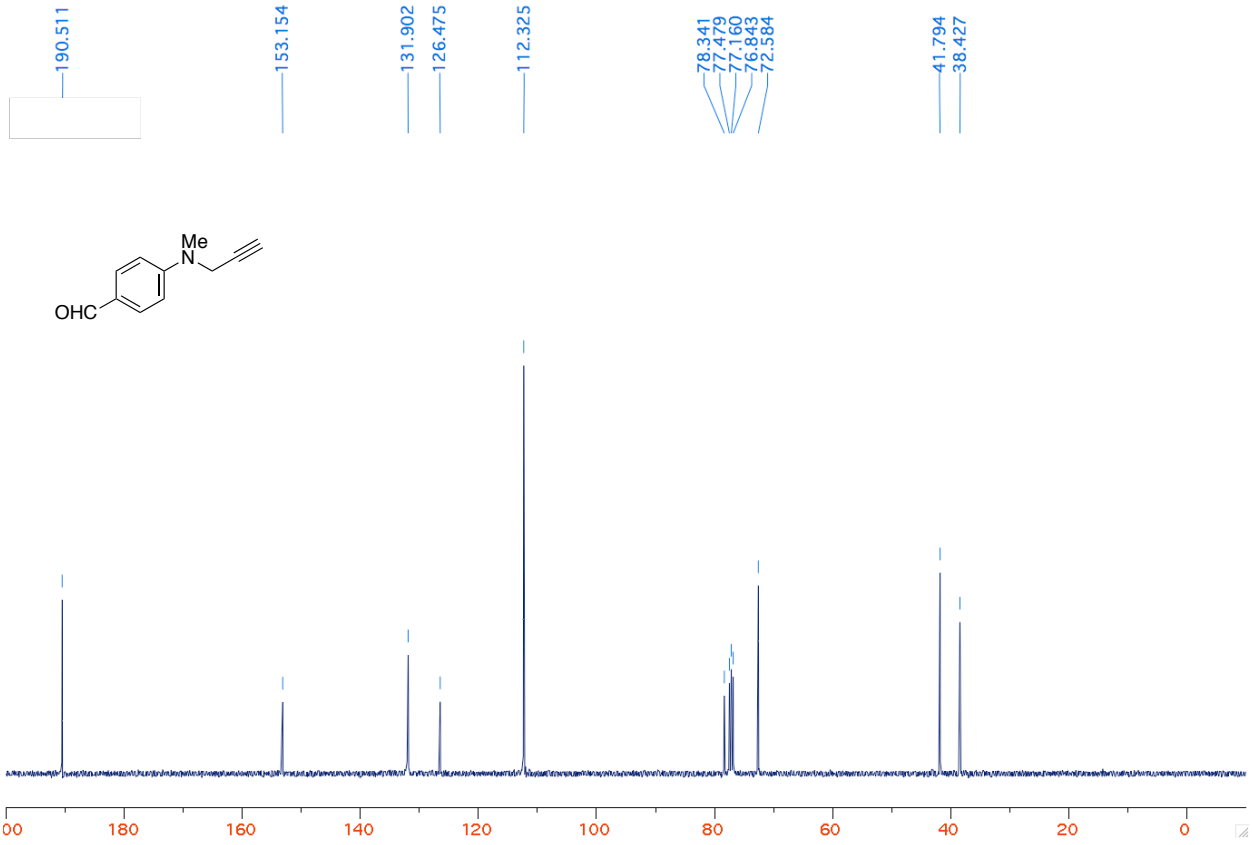

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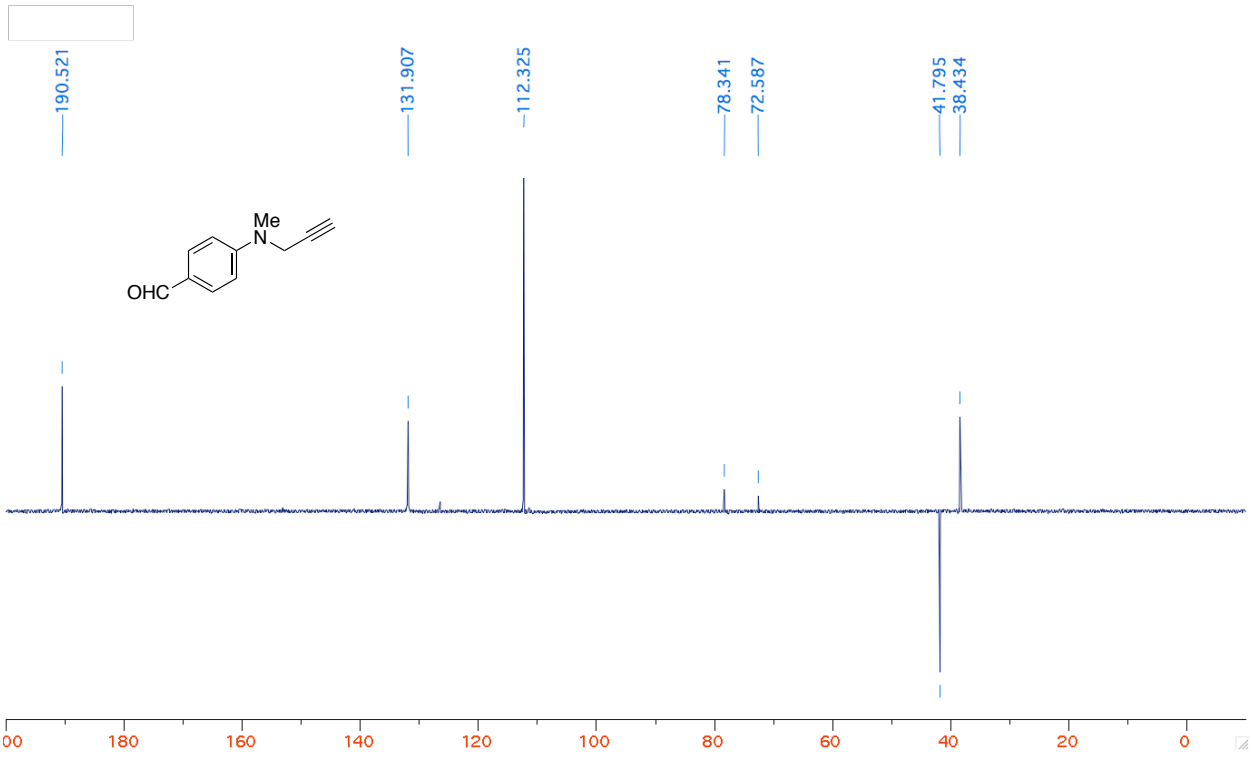

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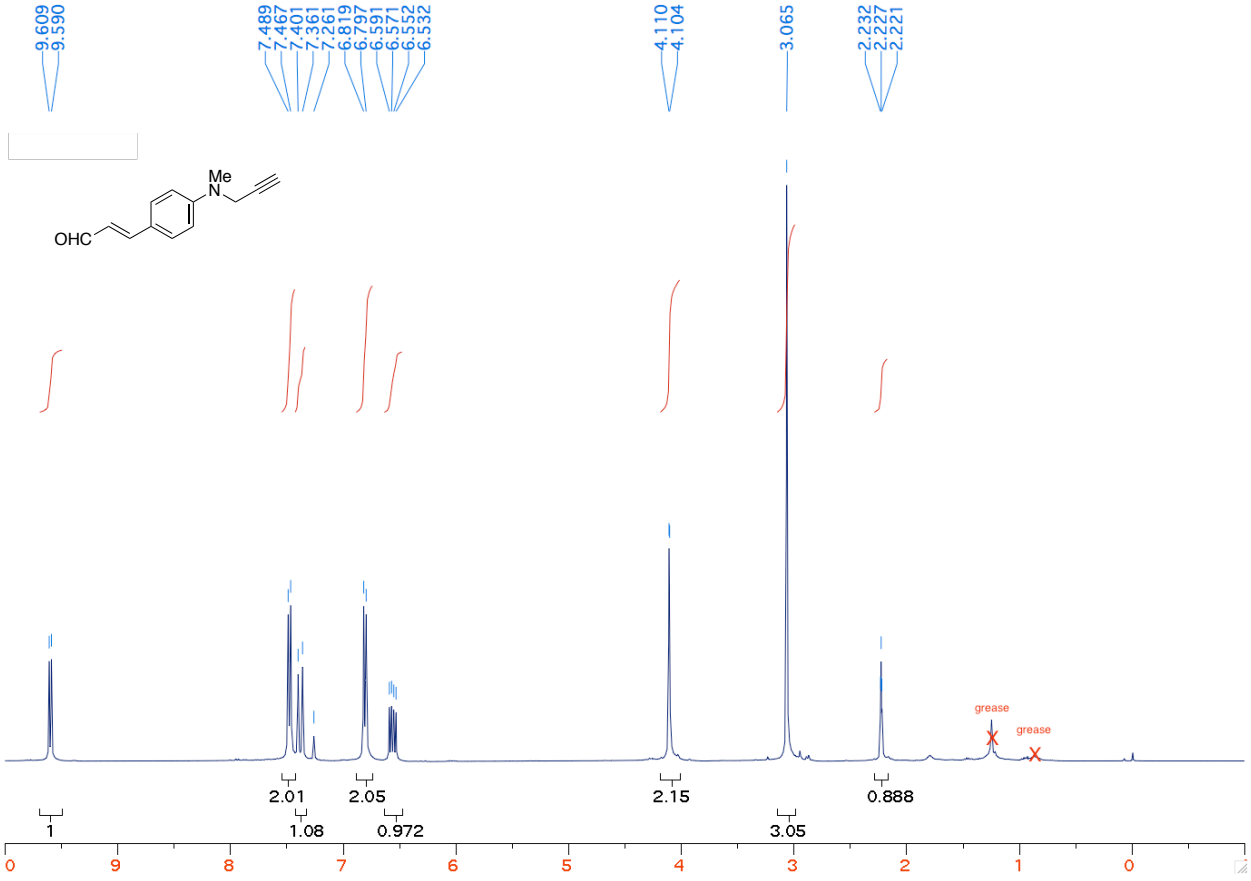

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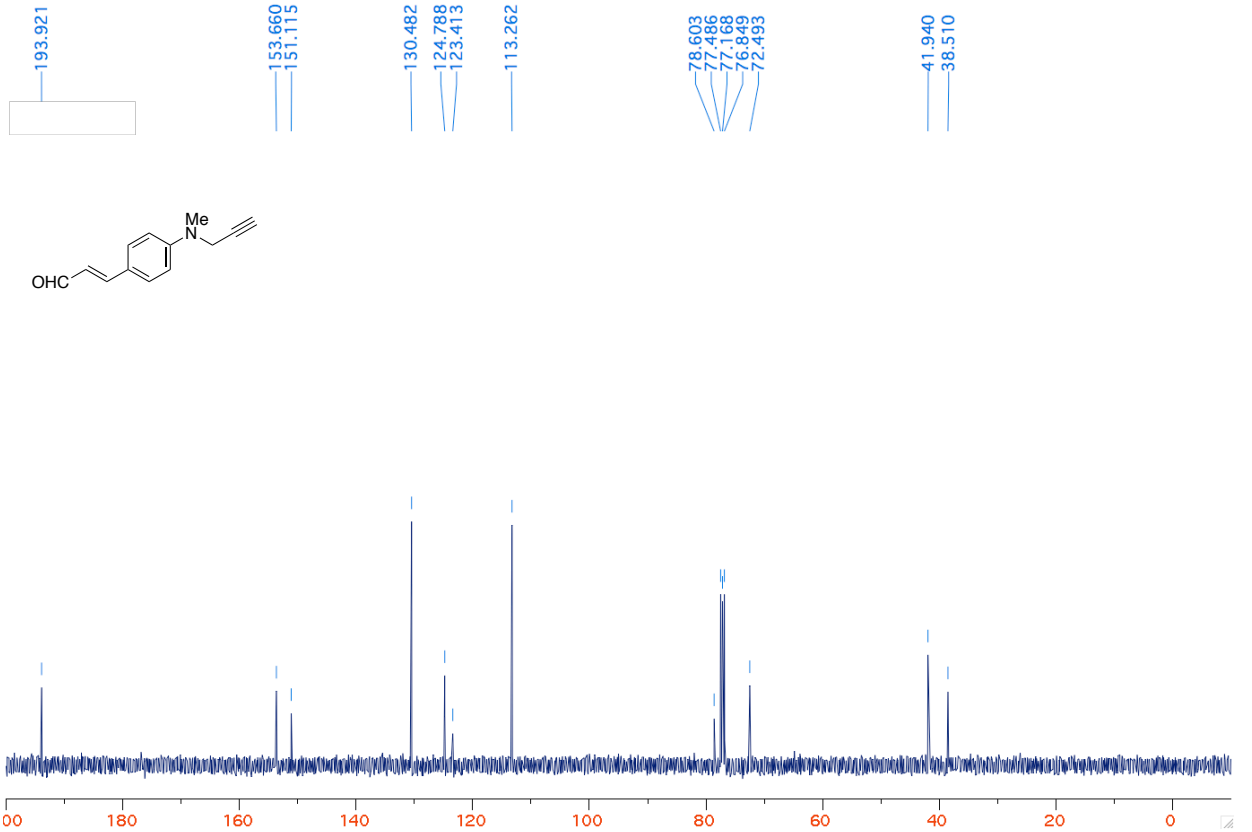

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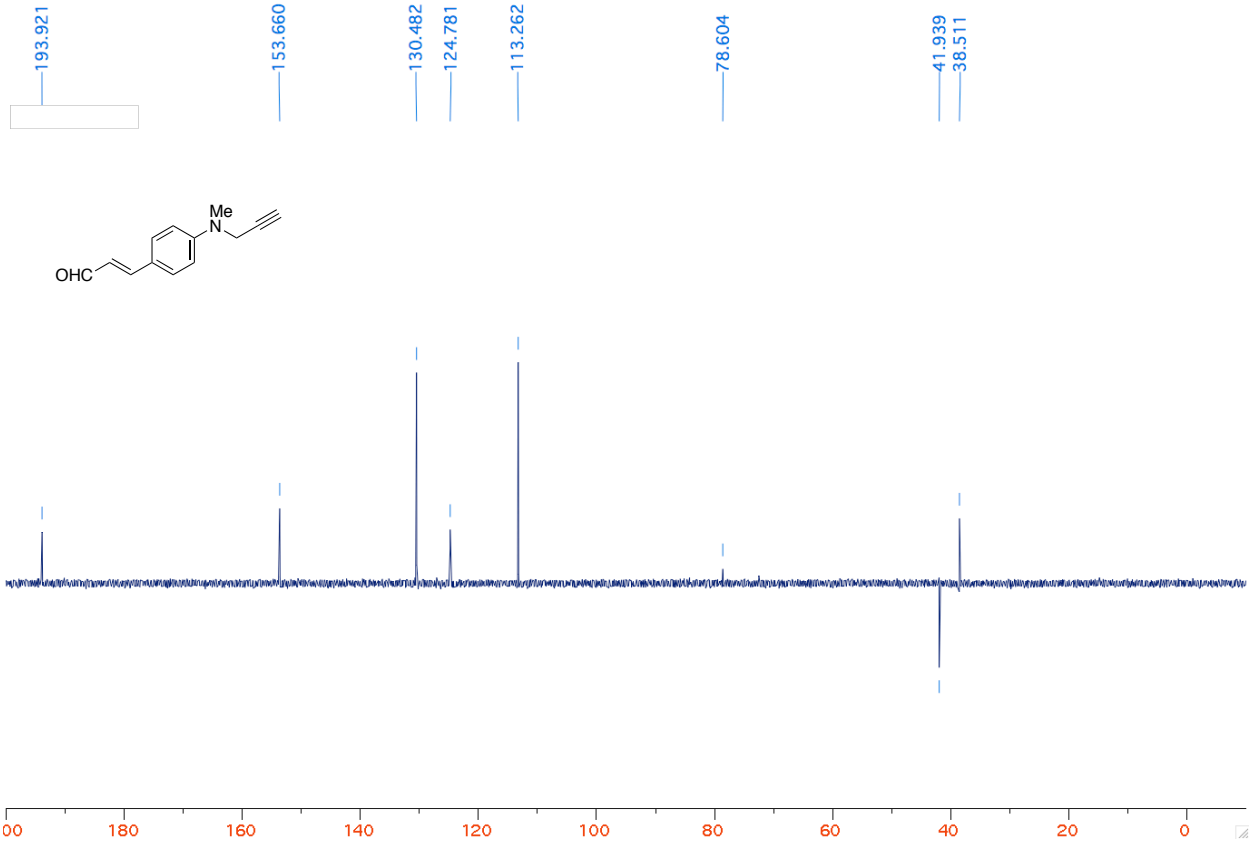

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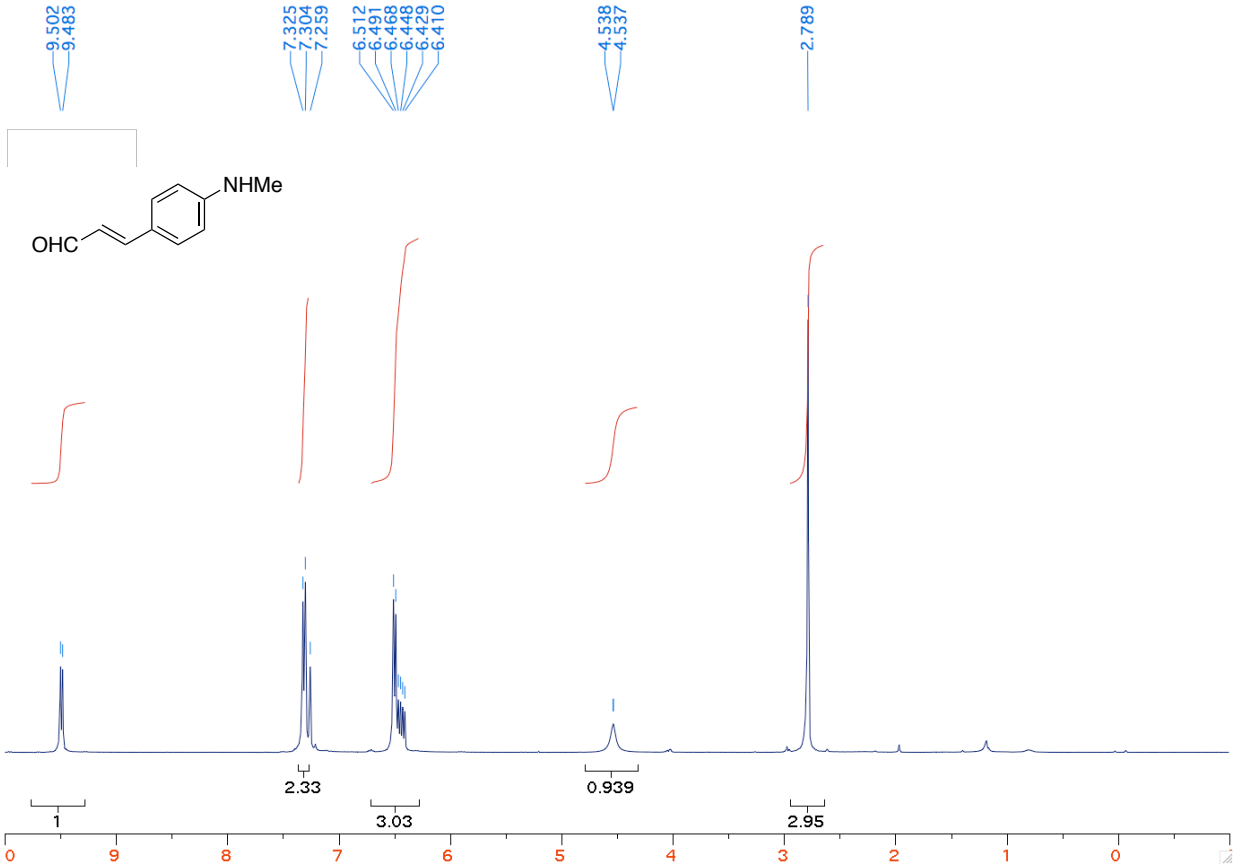

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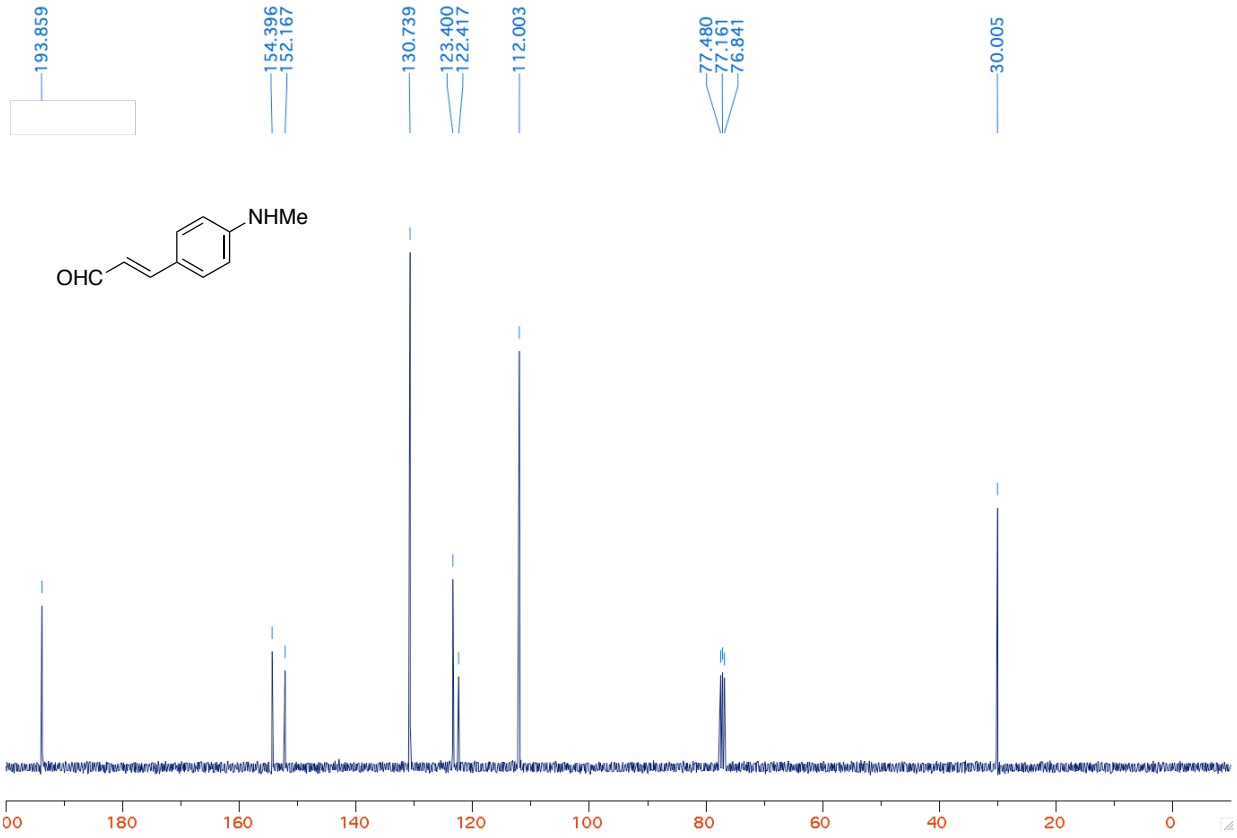

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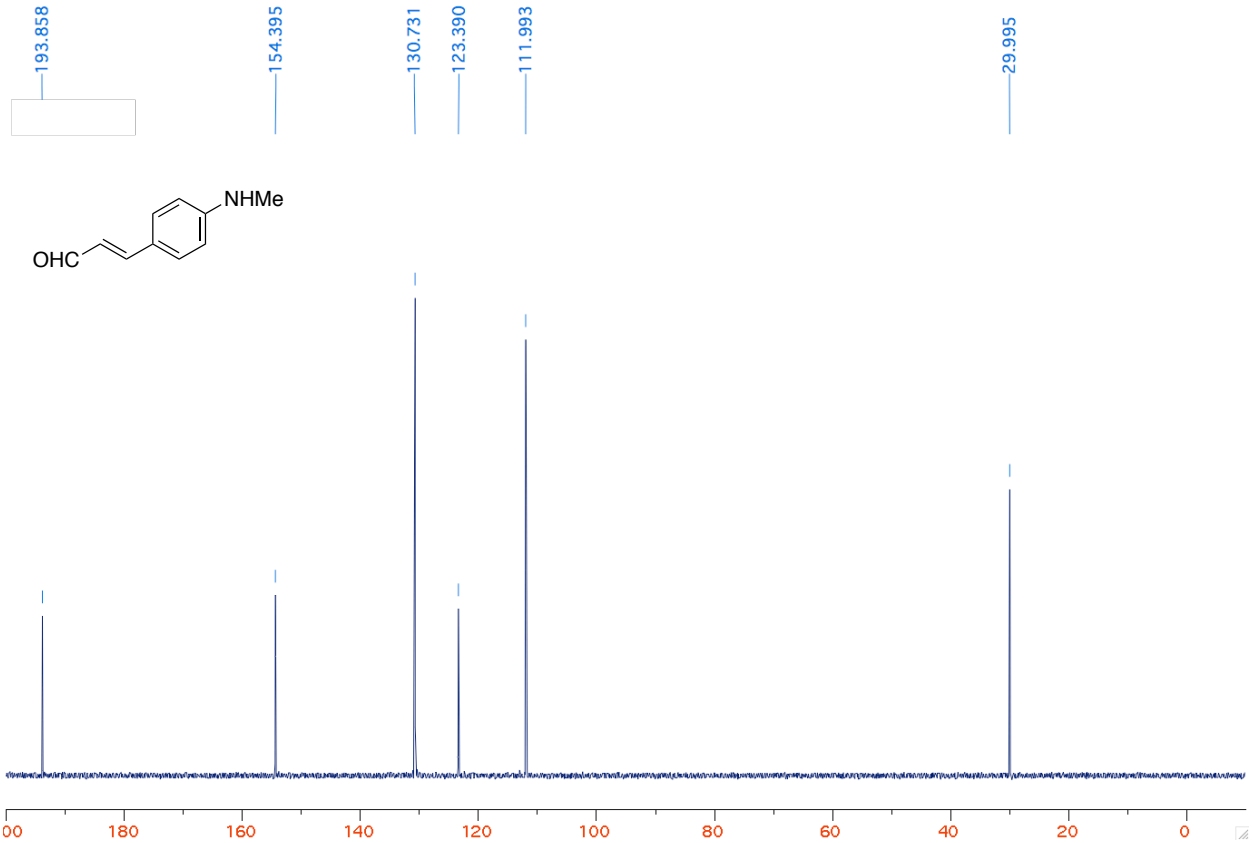

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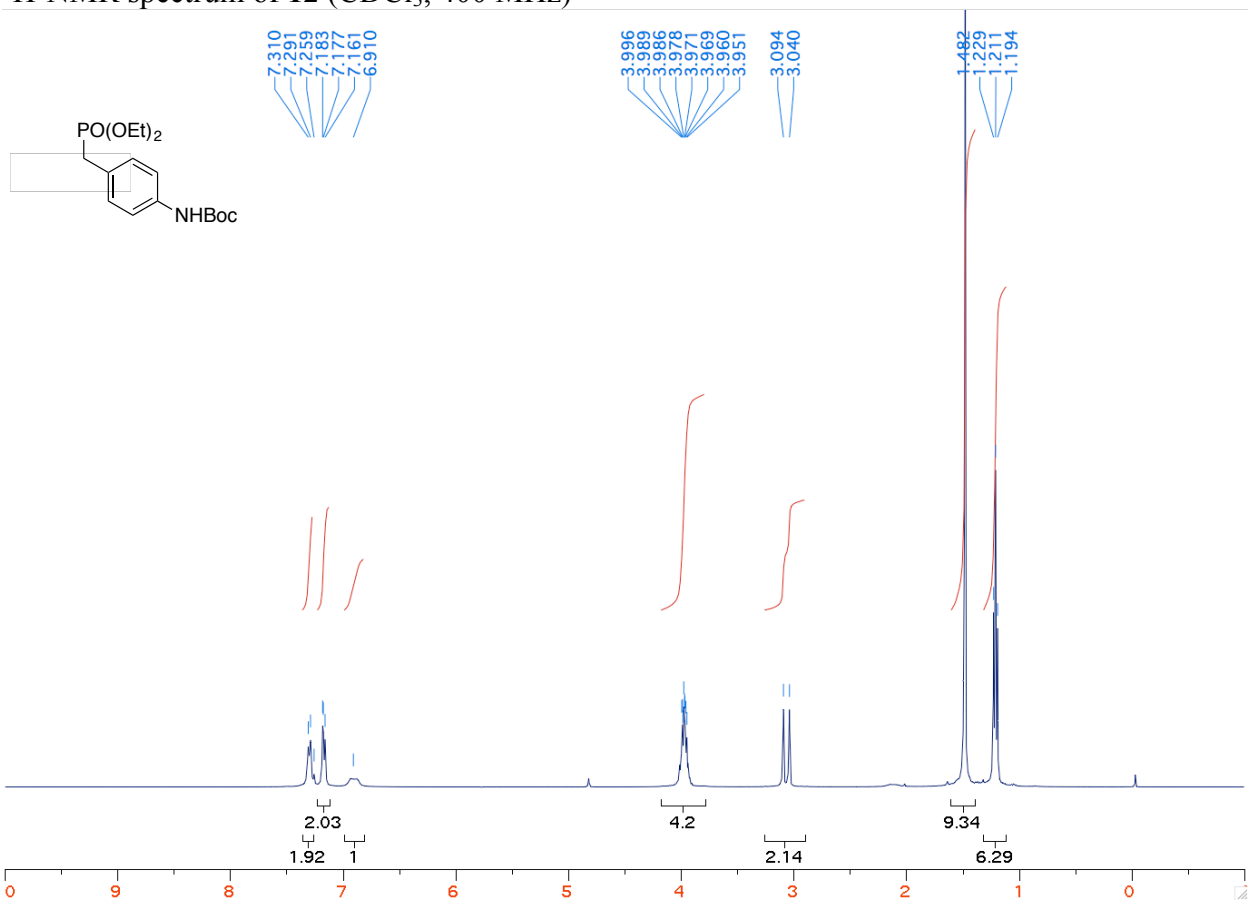

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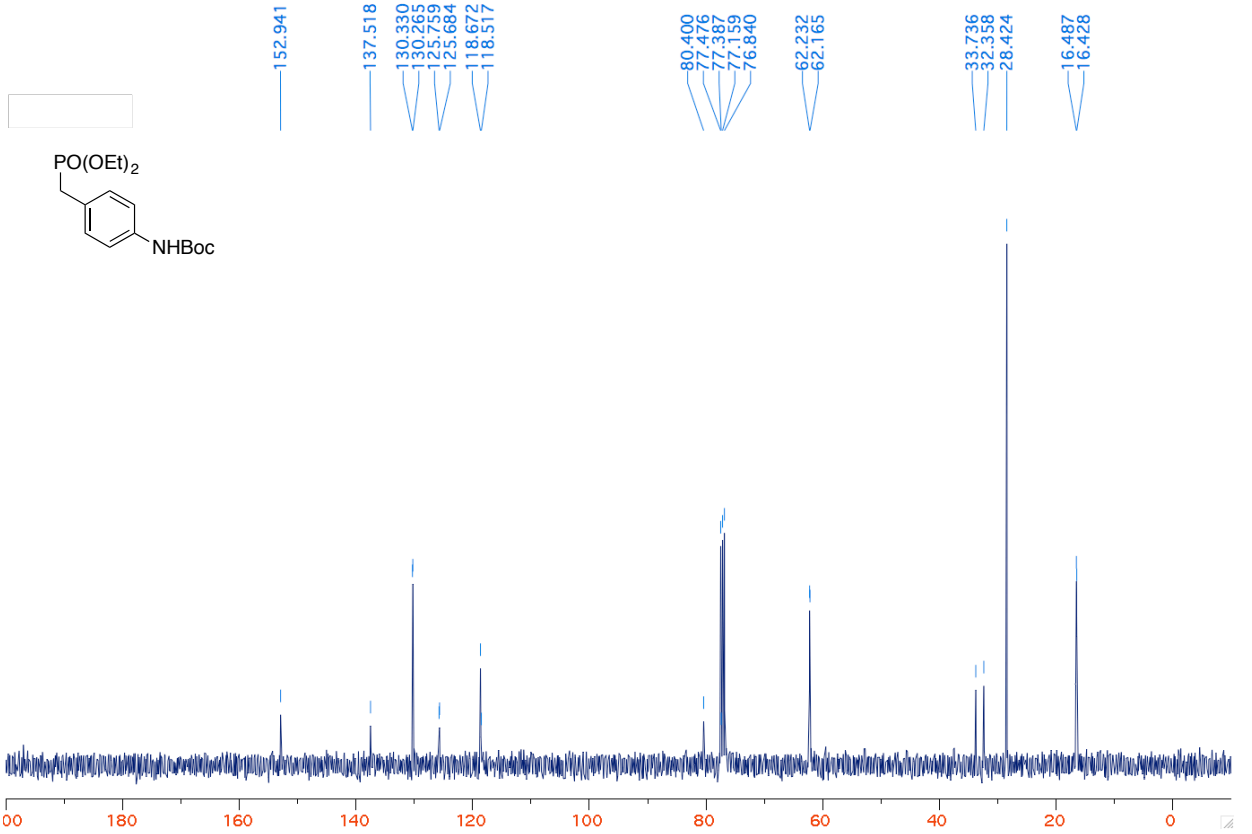

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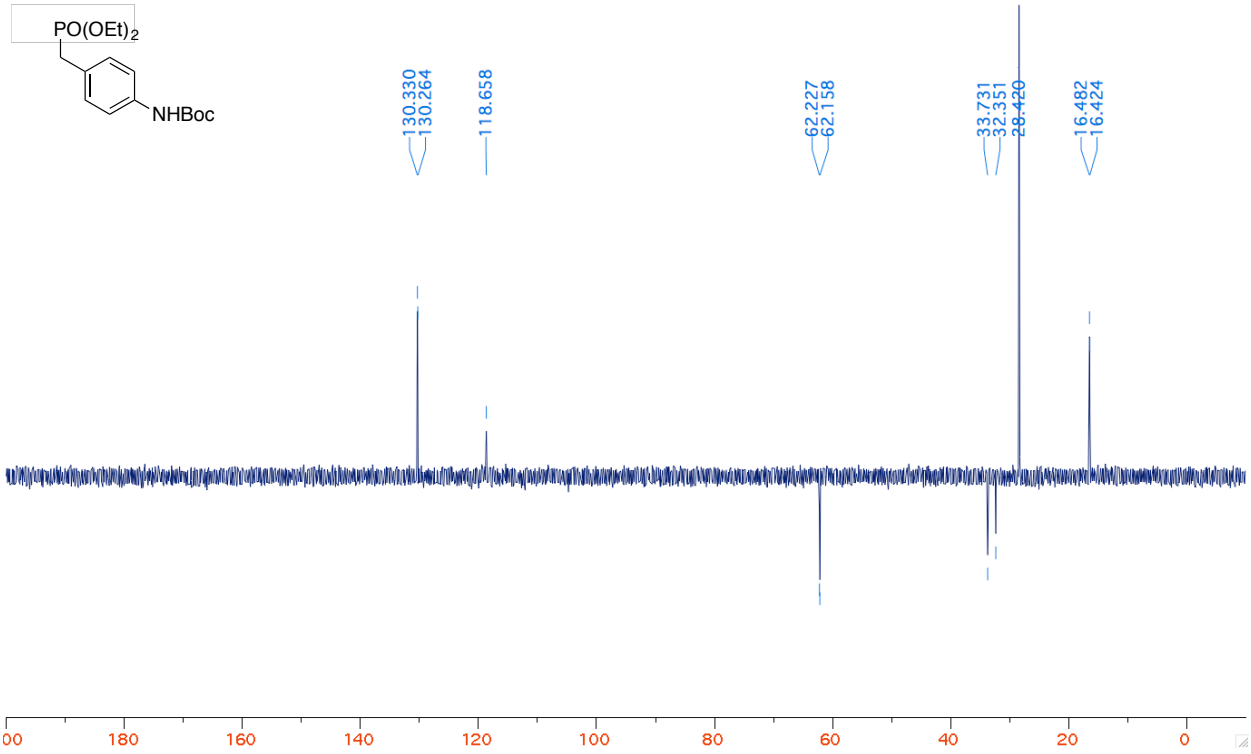

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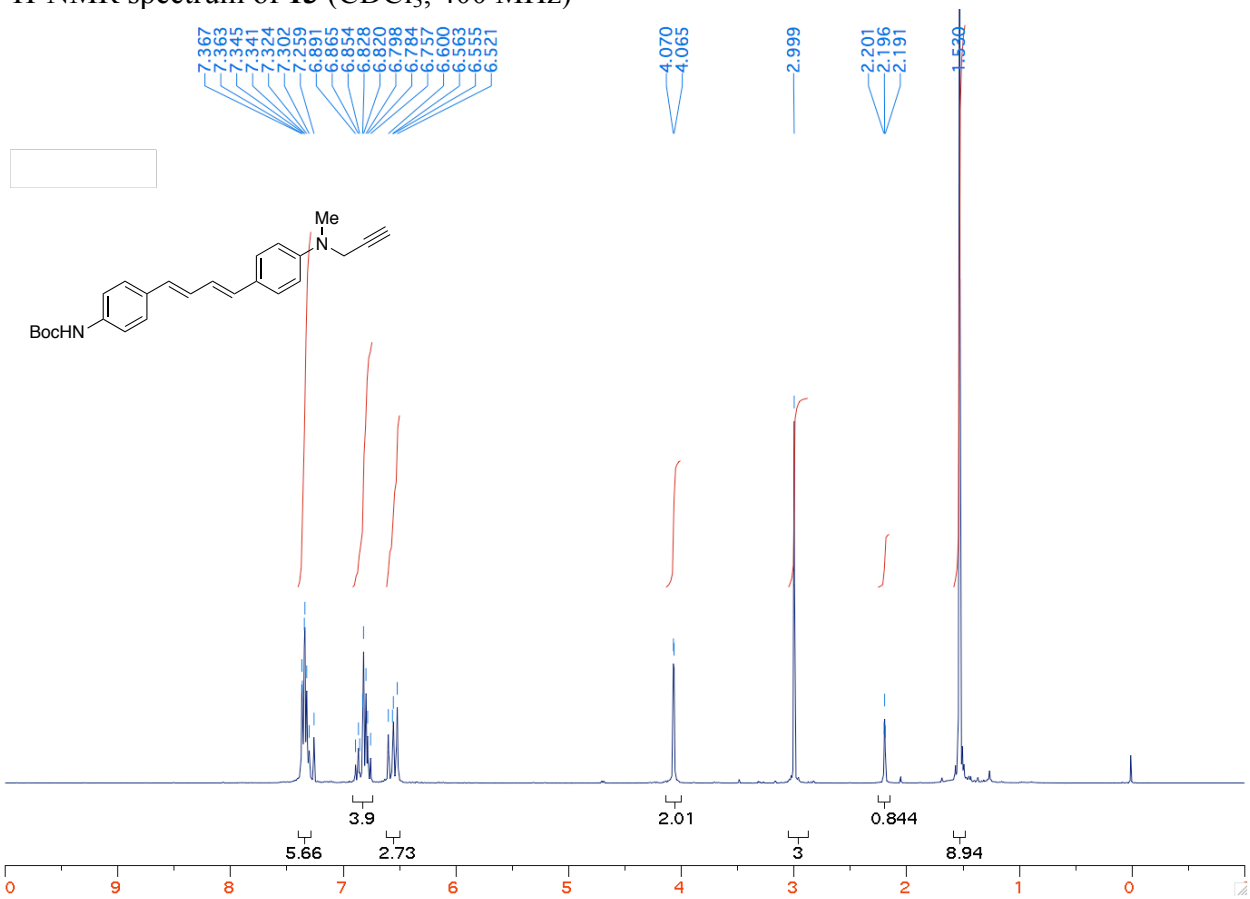

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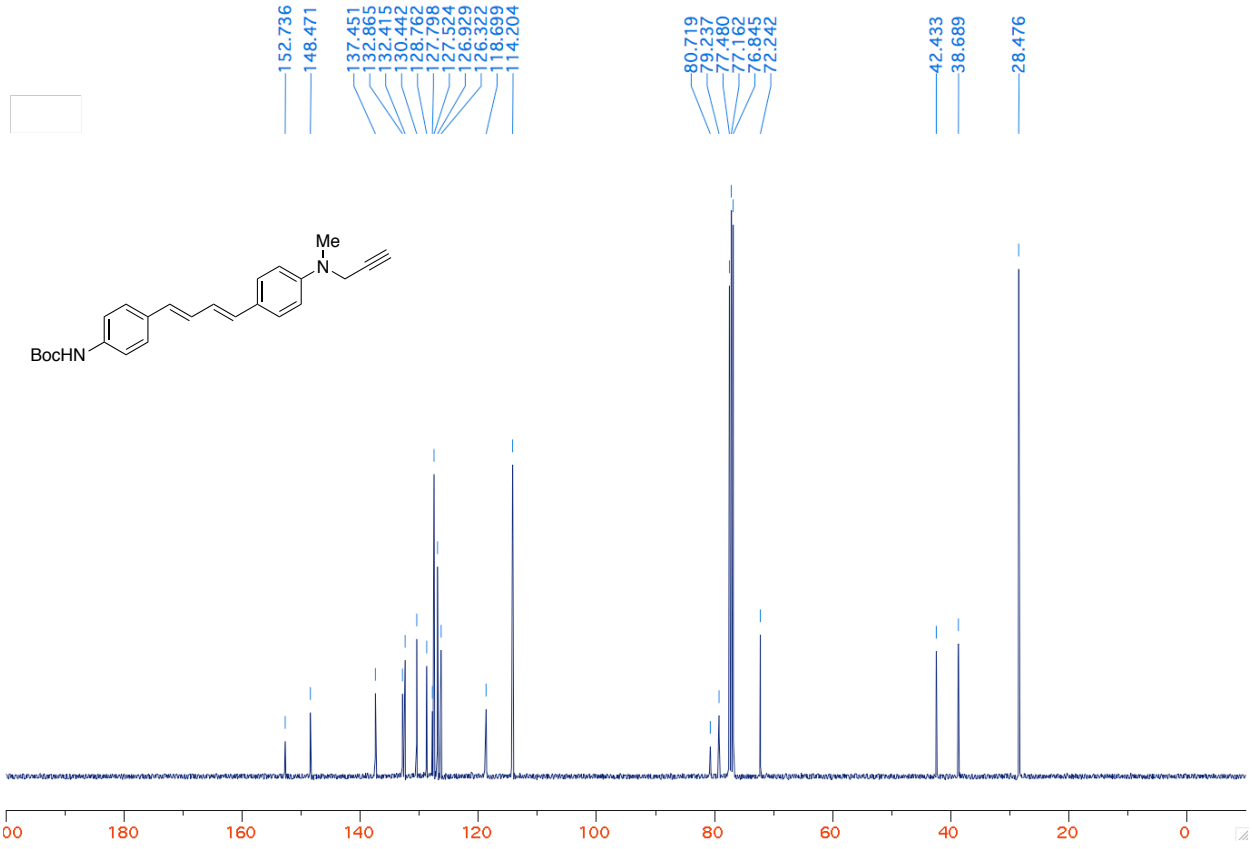

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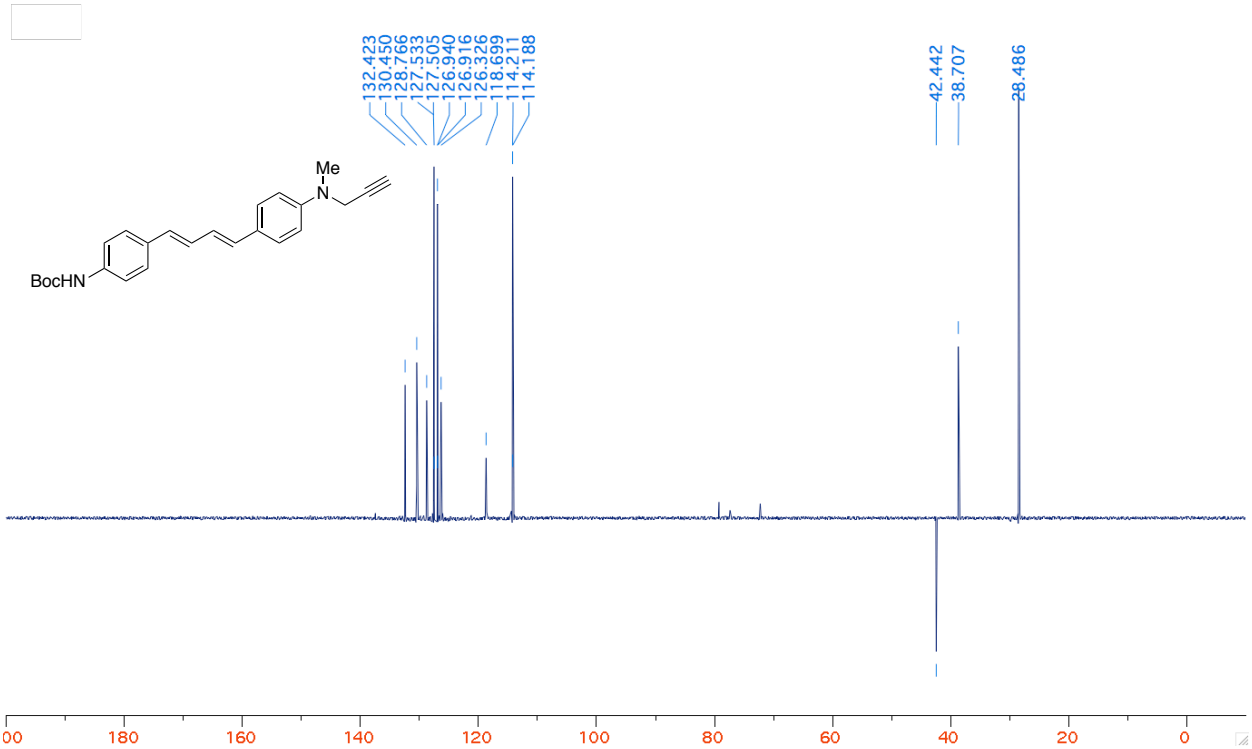

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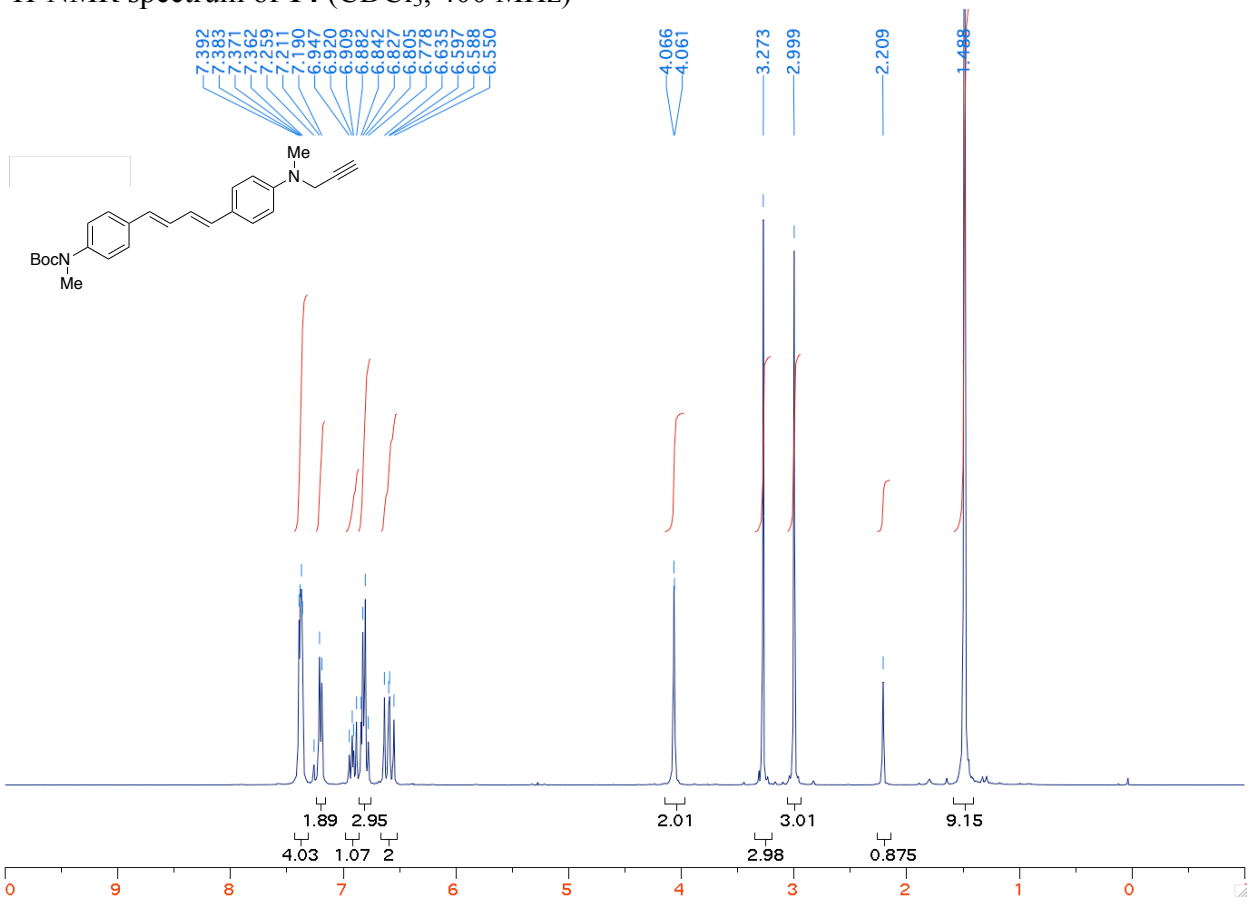

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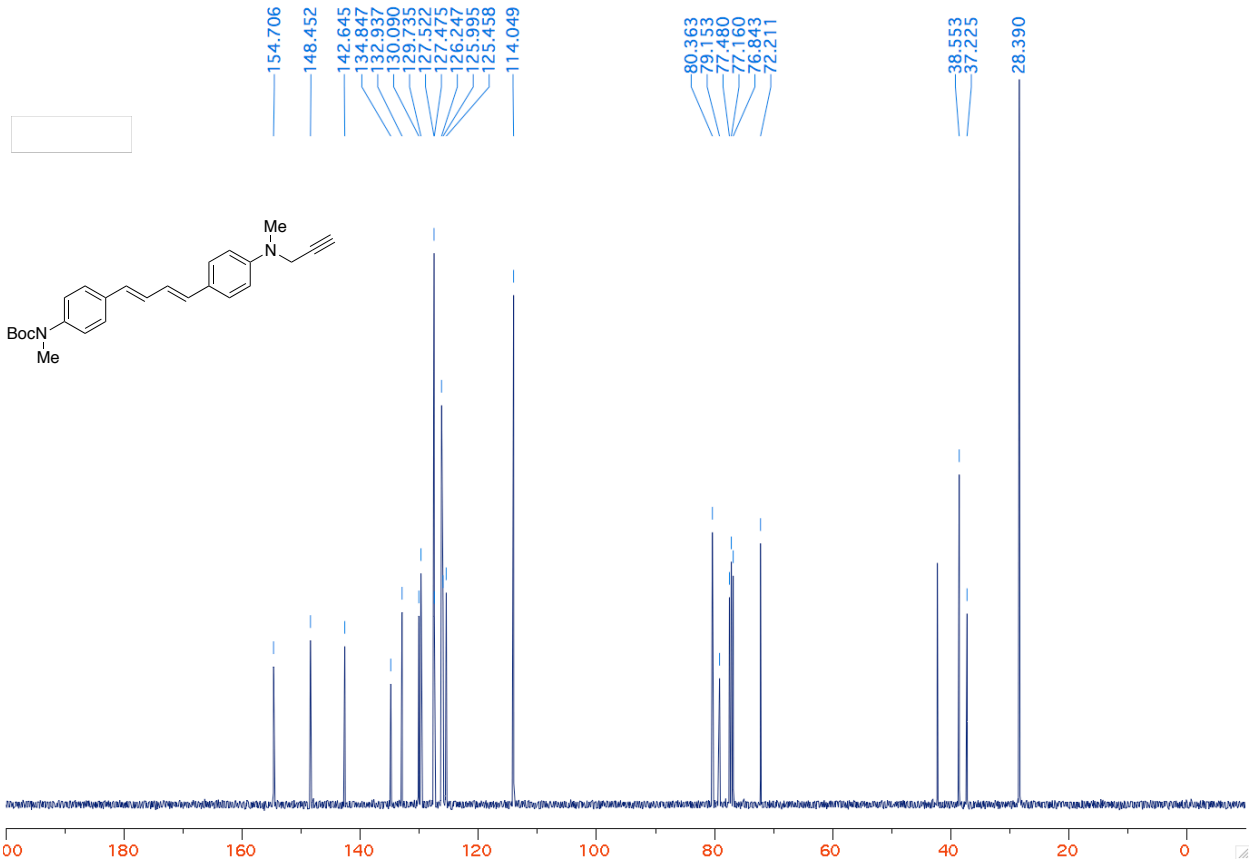

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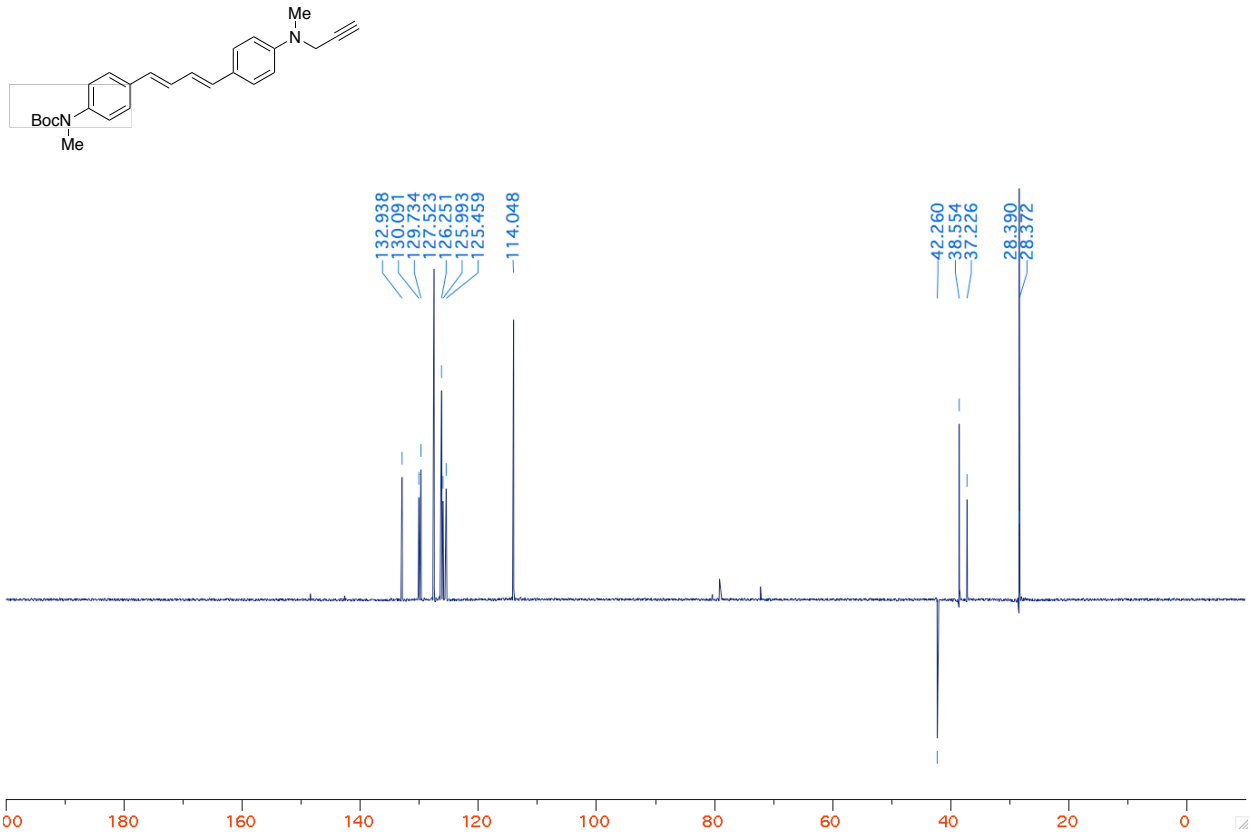

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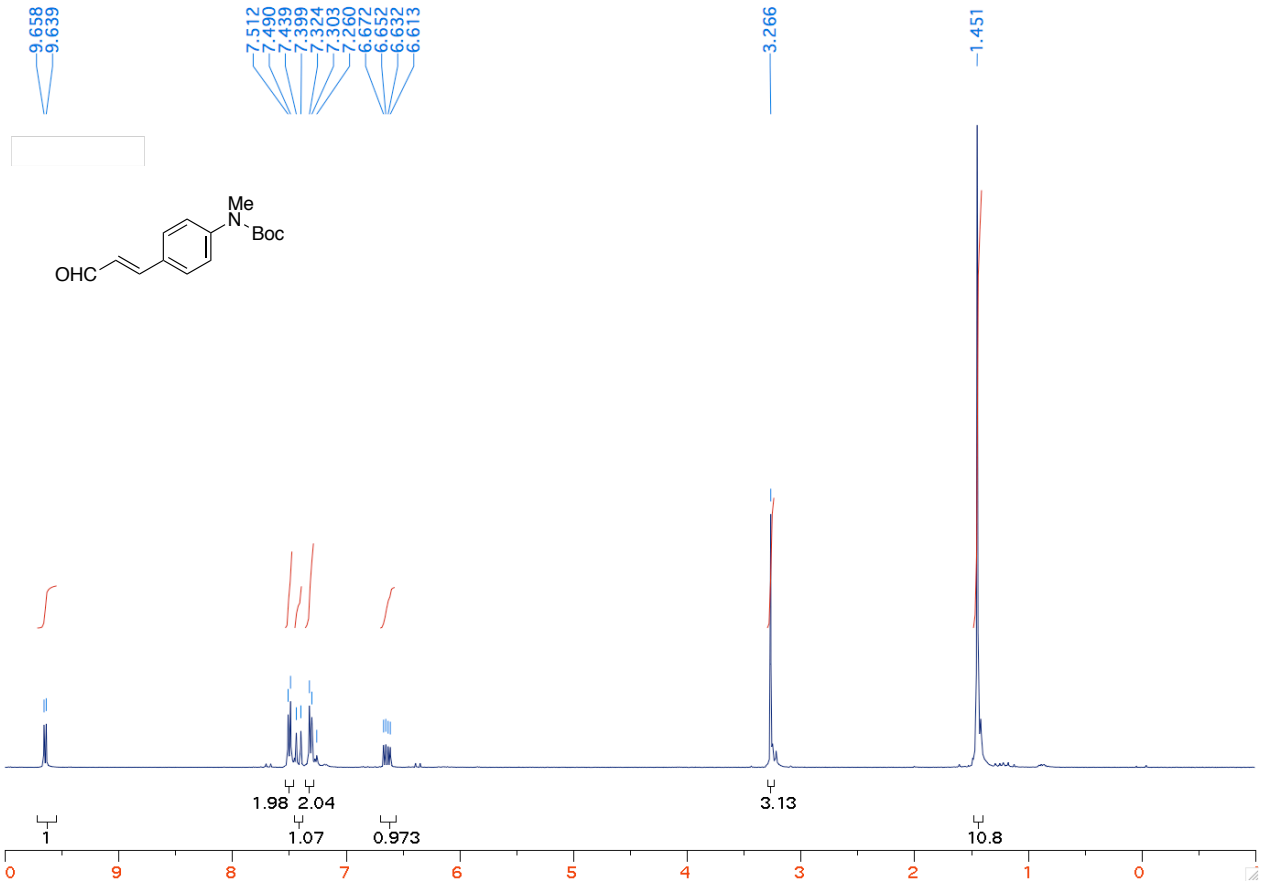

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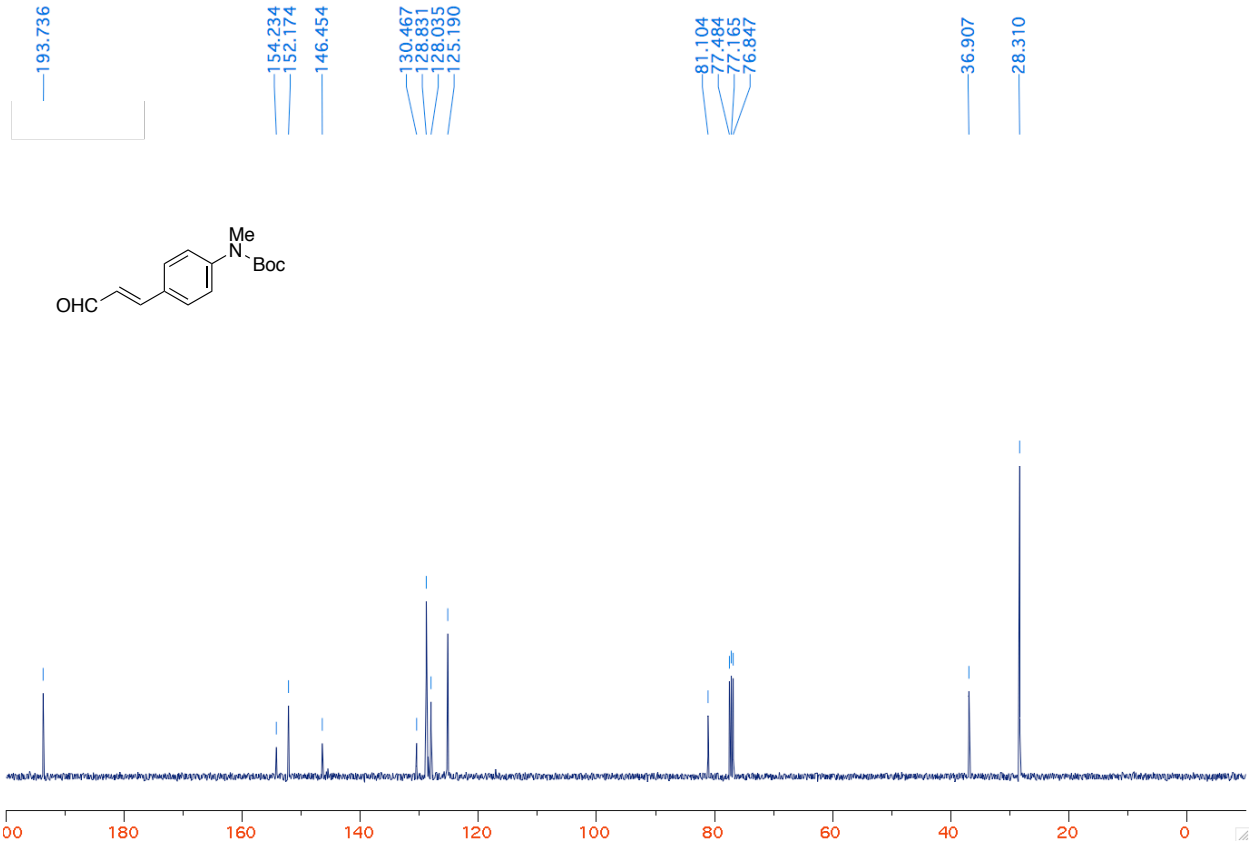

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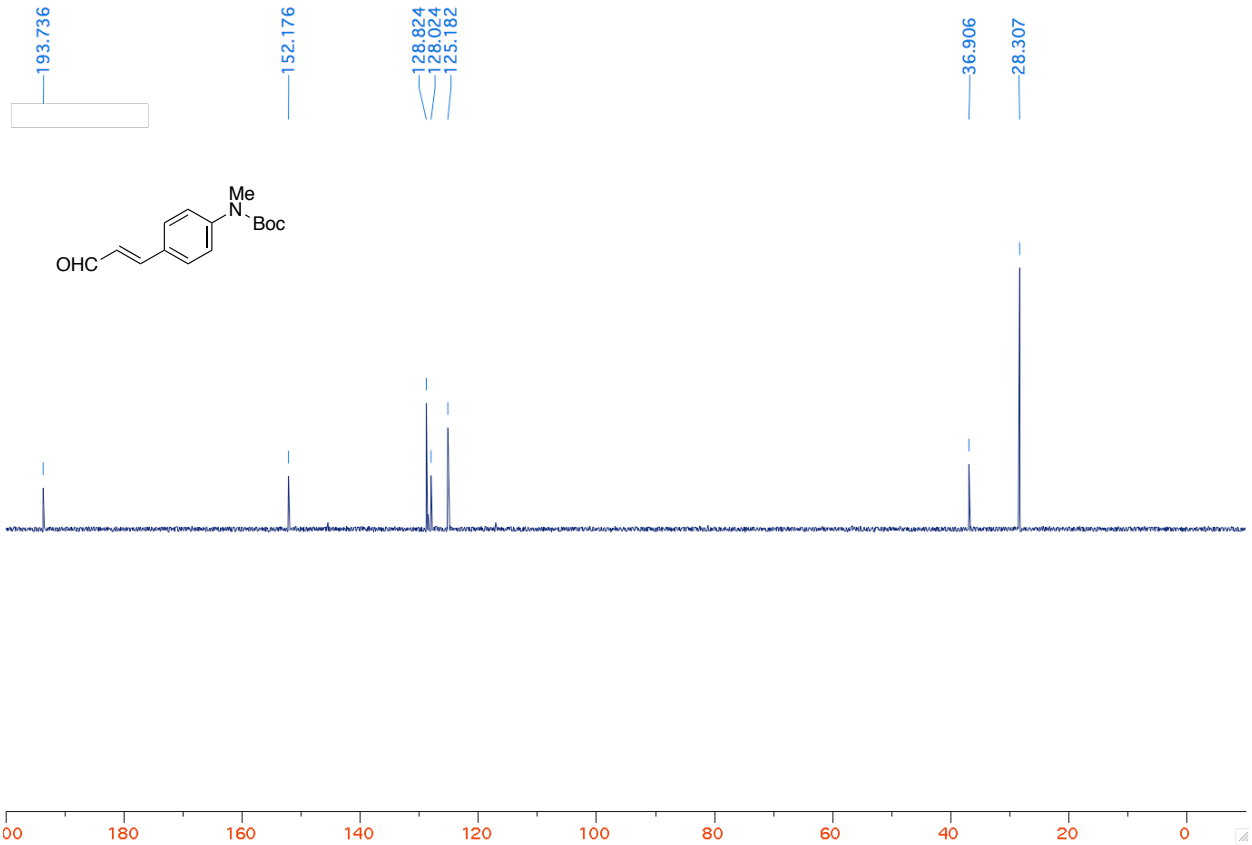

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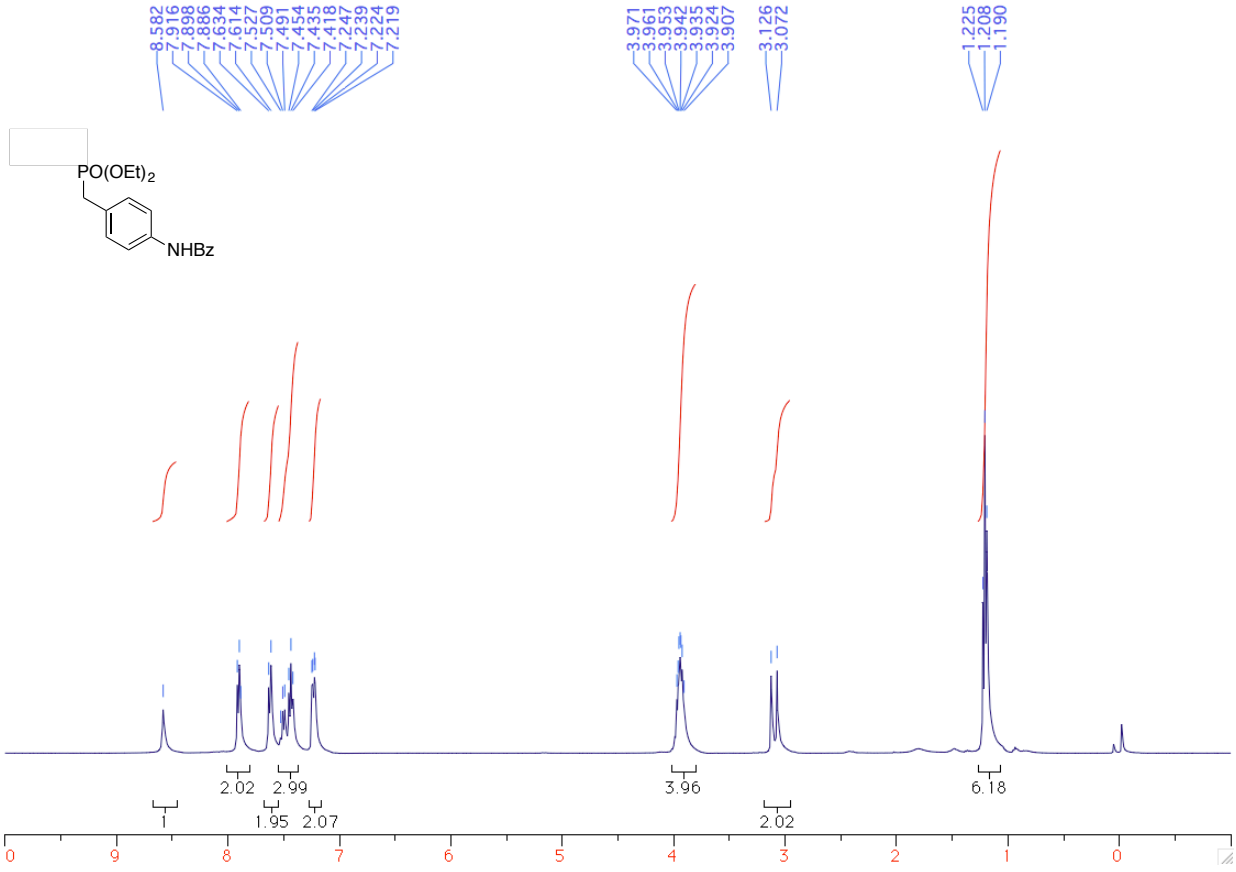
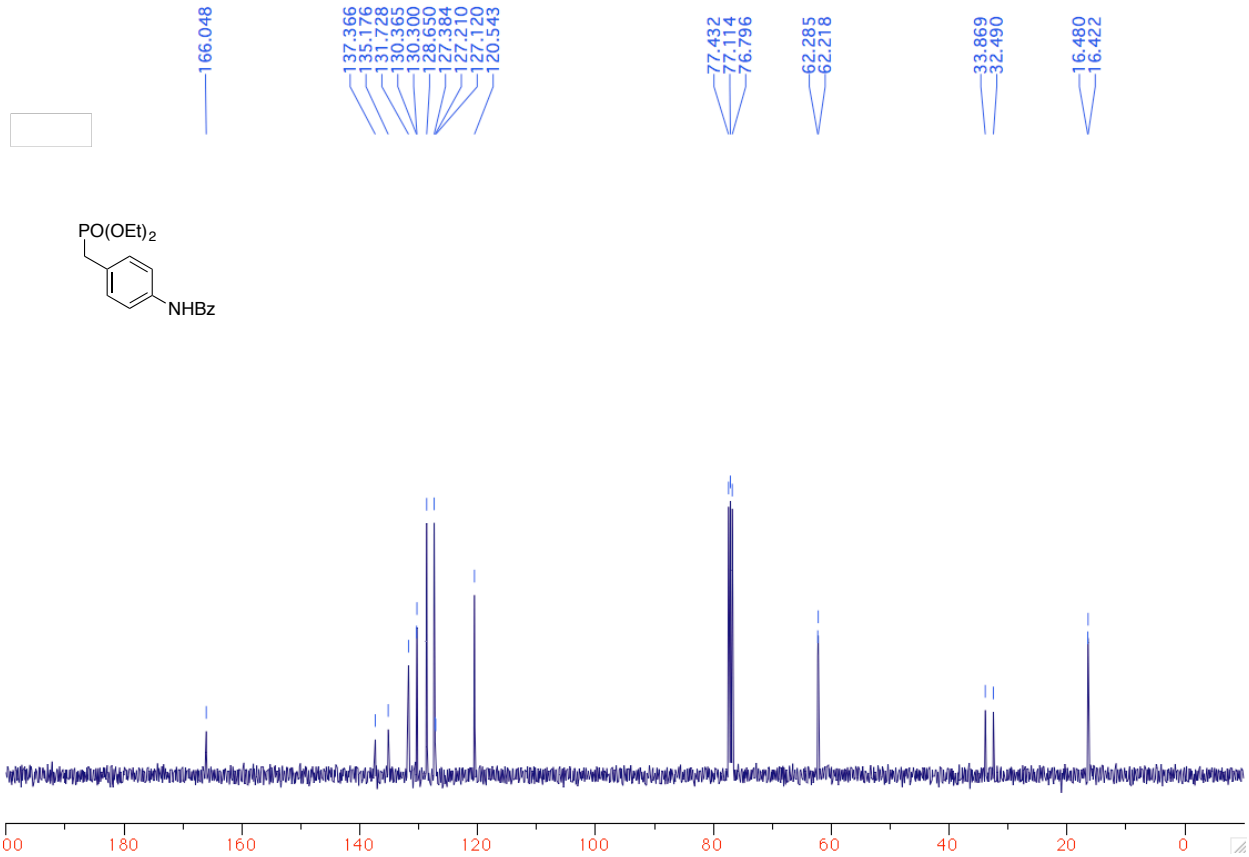
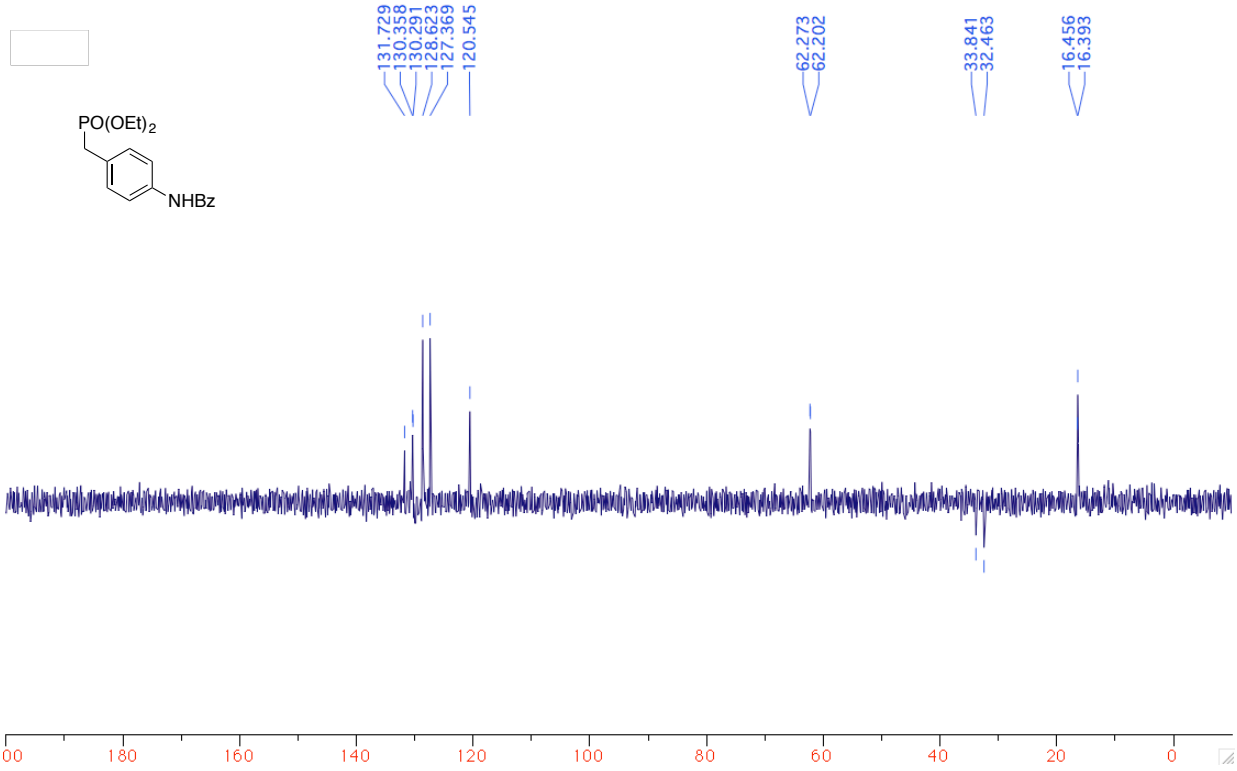


^{13}C-NMR spectrum of **16** ($CDCl_3$, 100 MHz)



DEPT 135 spectrum of **16** ($CDCl_3$, 100 MHz)



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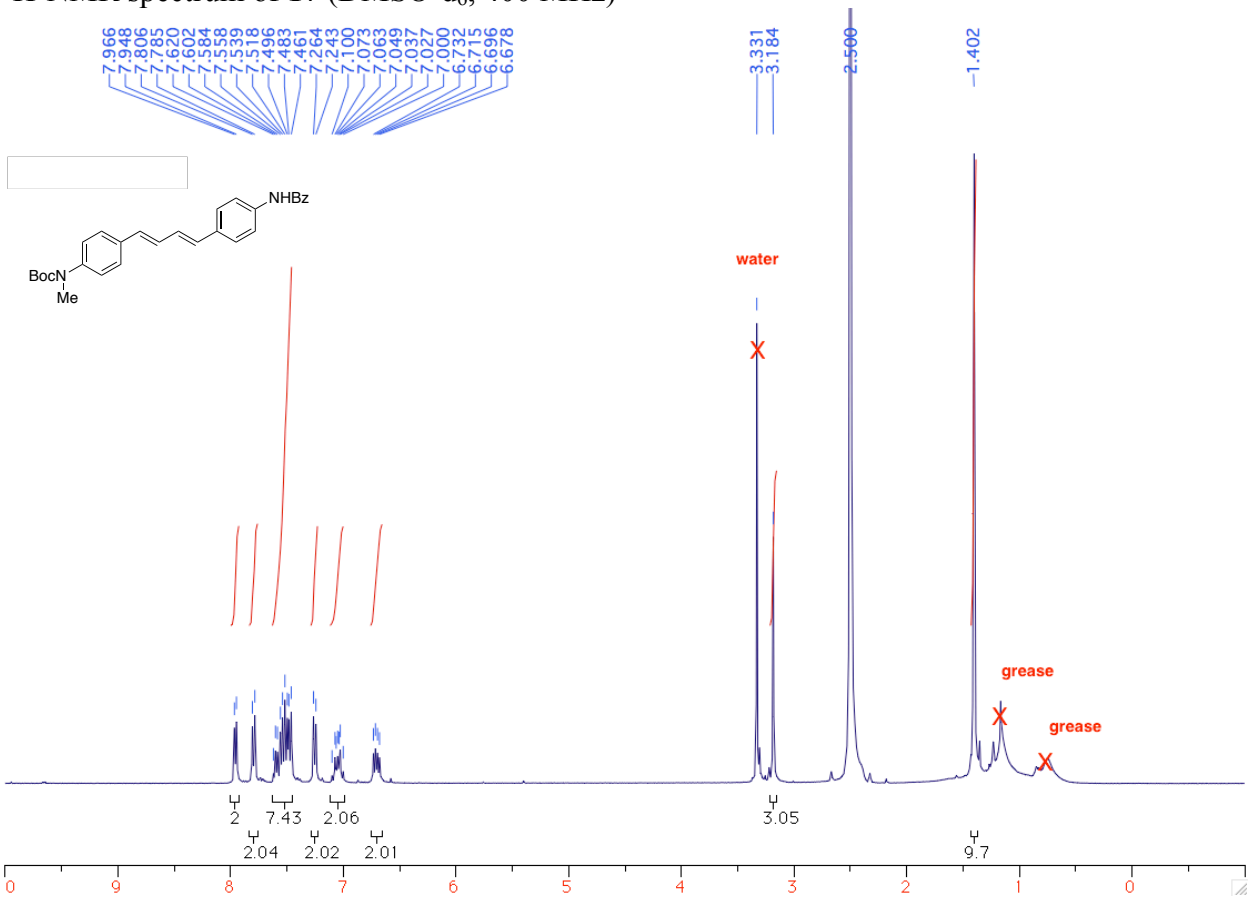
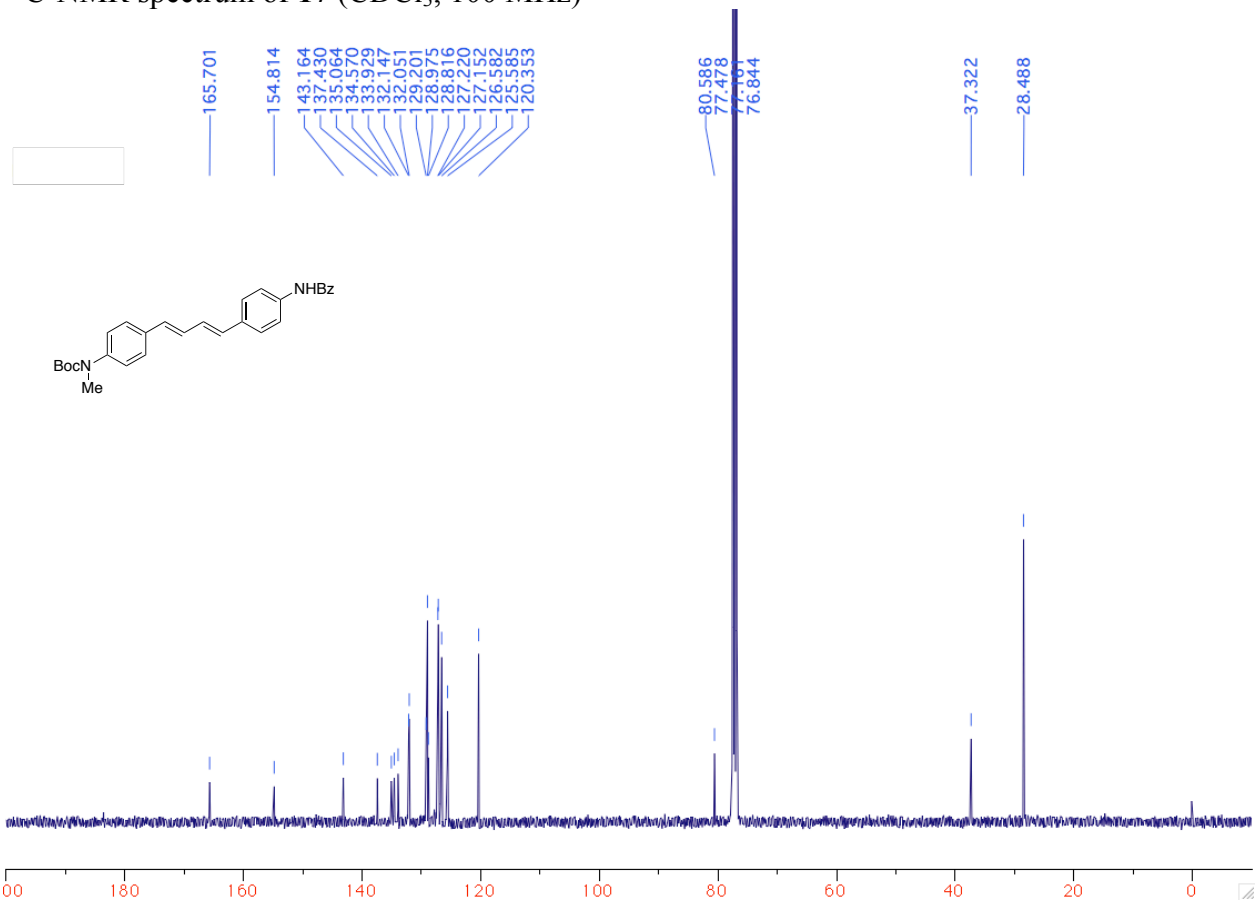
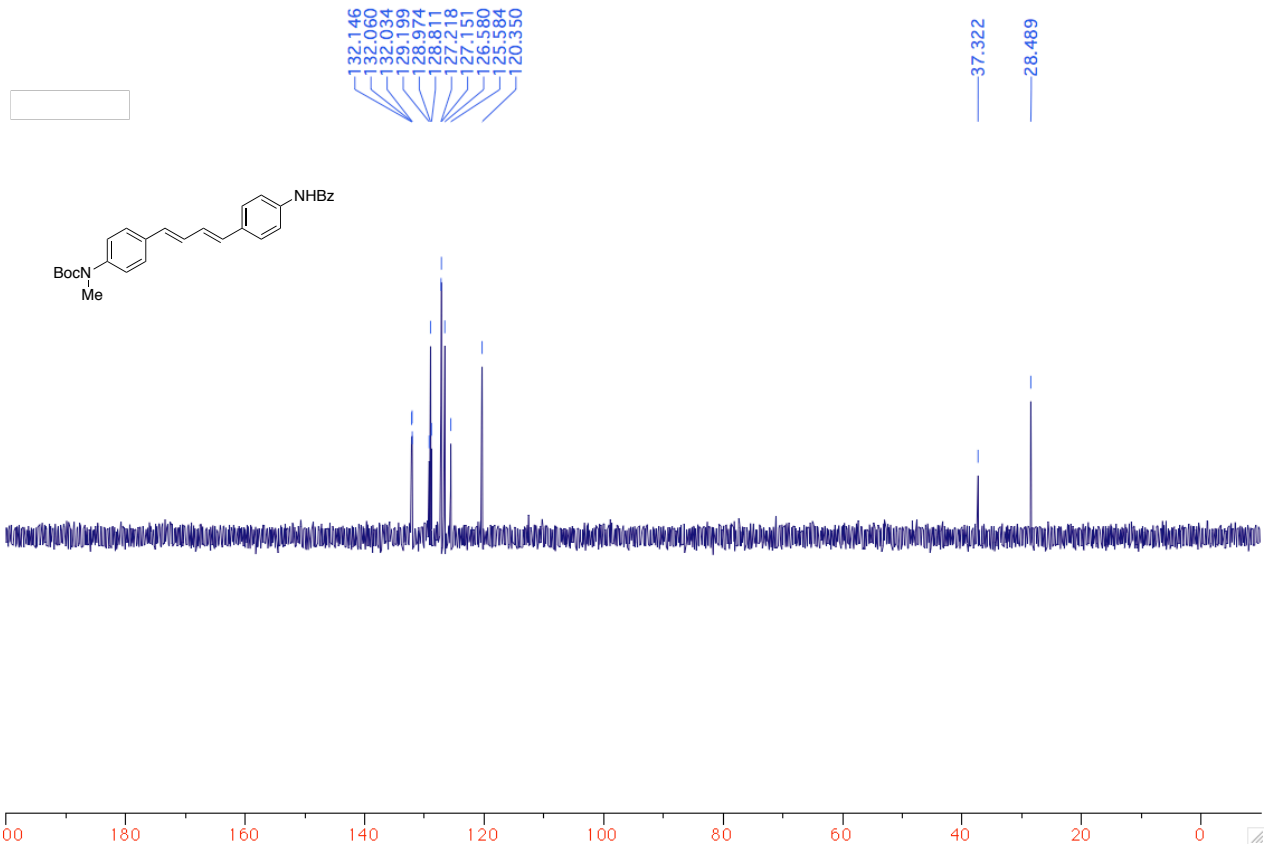


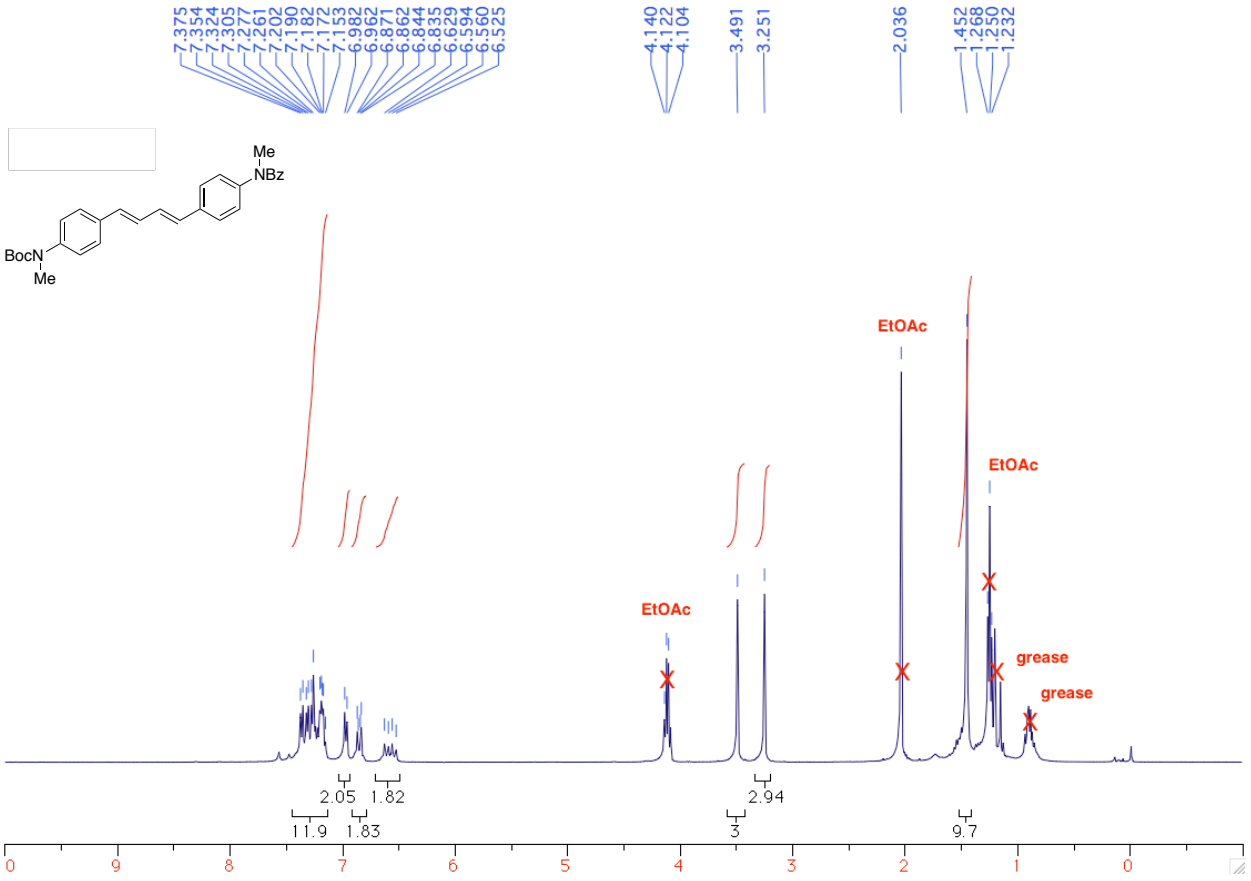
^{13}C-NMR spectrum of **17** ($CDCl_3$, 100 MHz)



DEPT 135 spectrum of **17** ($CDCl_3$, 100 MHz)



^{1}H-NMR spectrum of **18** ($CDCl_3$, 400 MHz)



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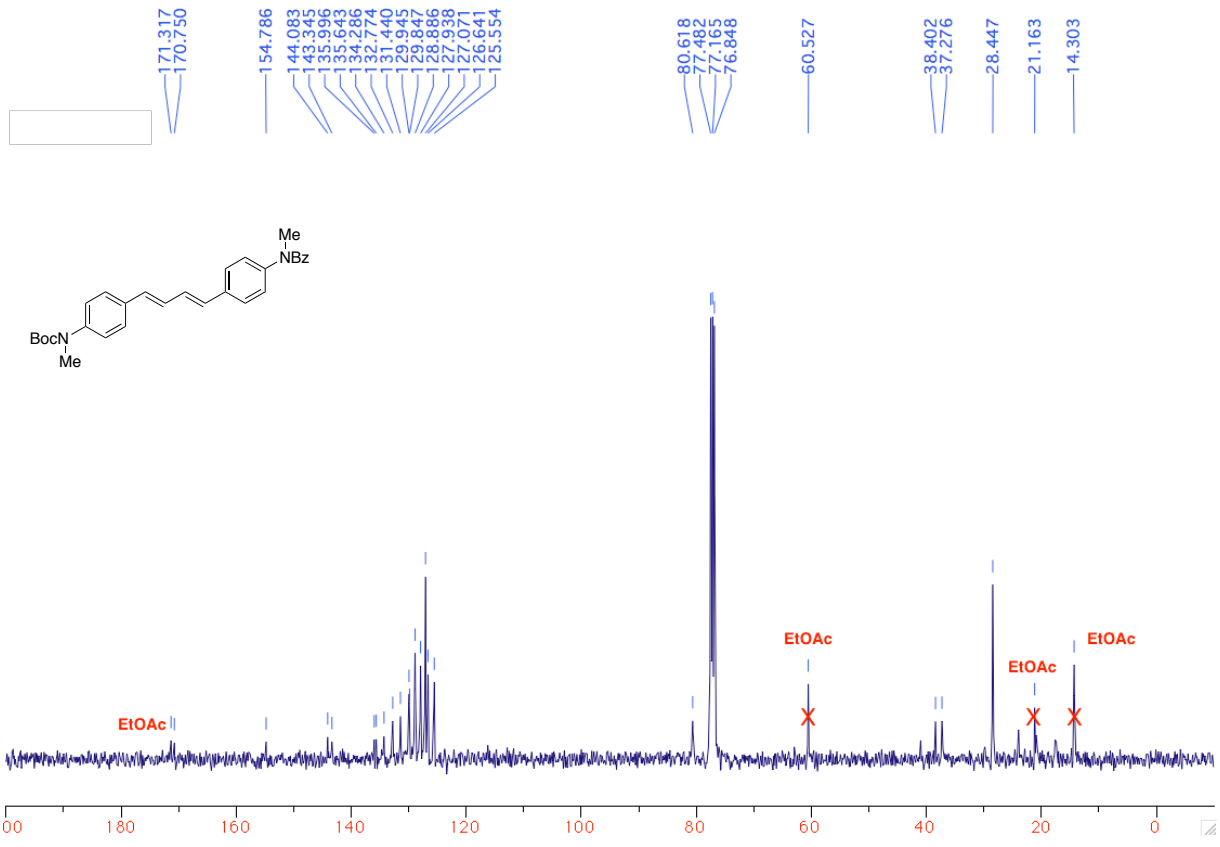
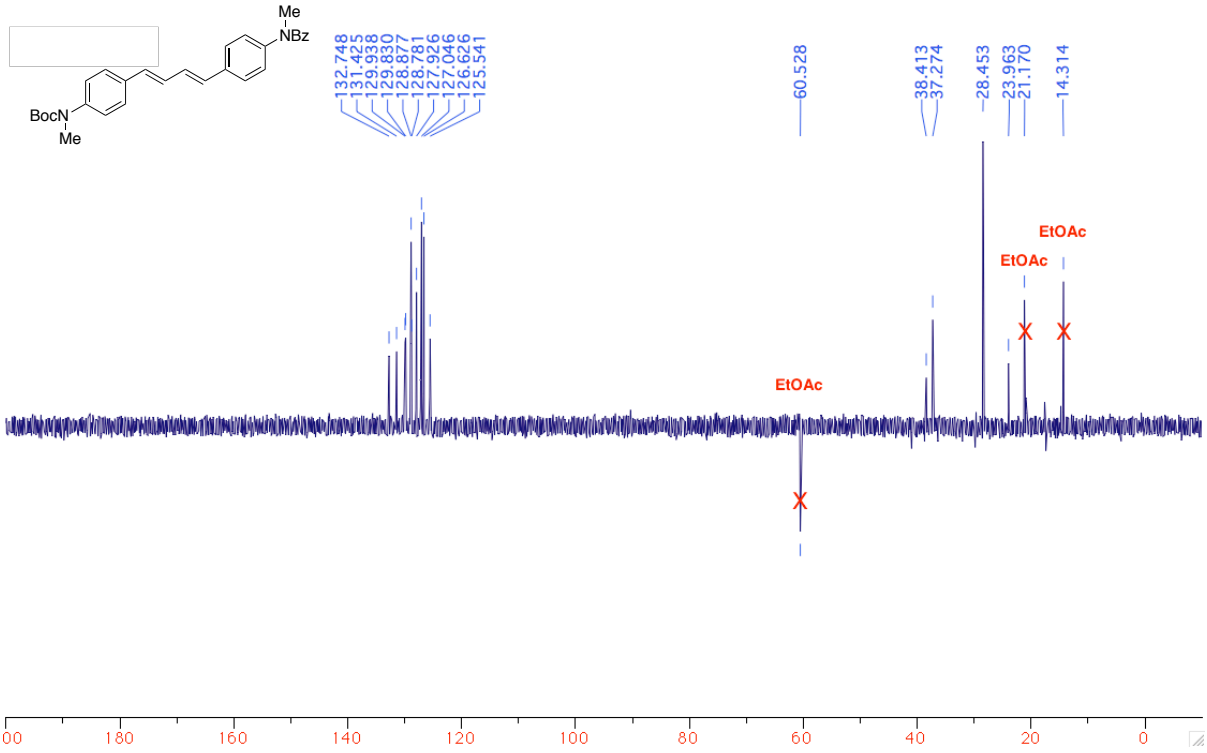
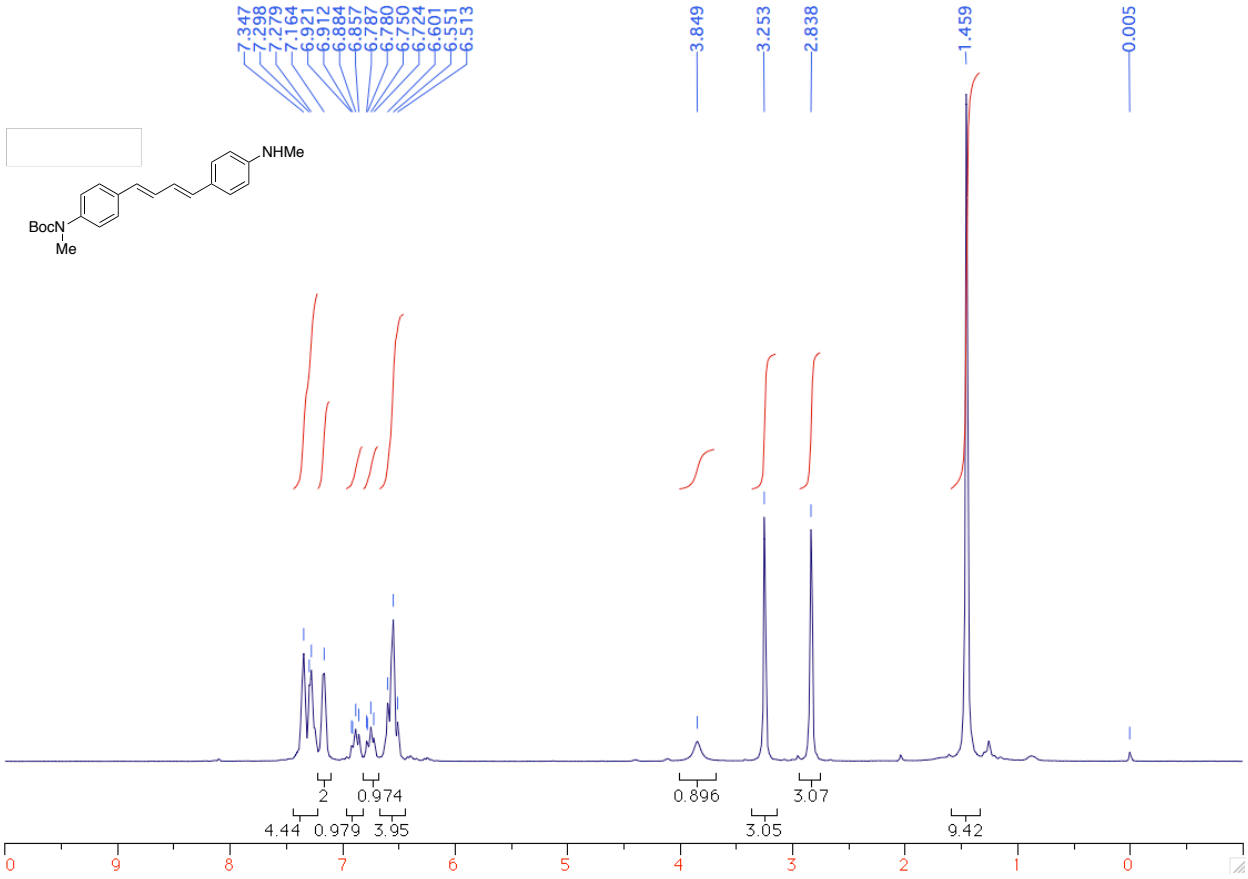


DEPT 135 spectrum of **18** ($CDCl_3$, 100 MHz)



^{1}H-NMR spectrum of **19** ($CDCl_3$, 400 MHz)



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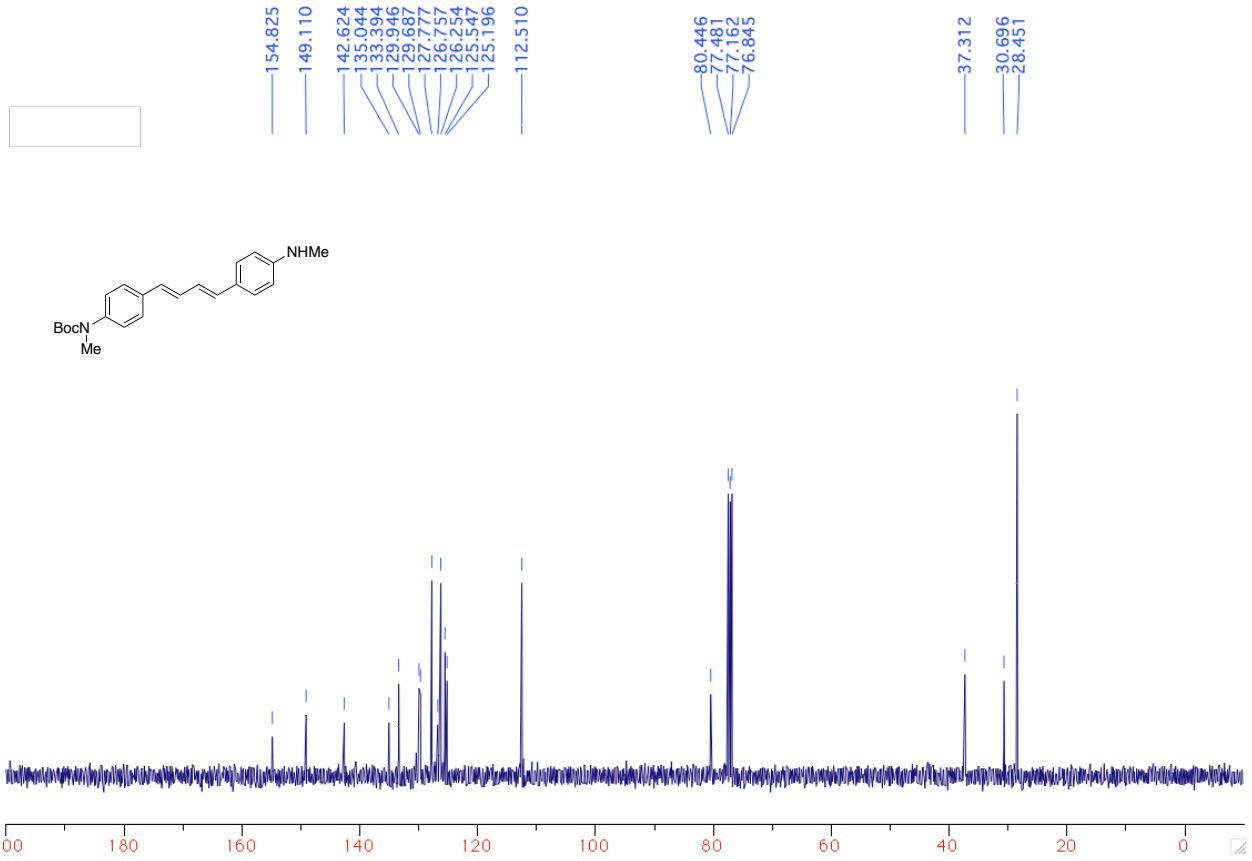

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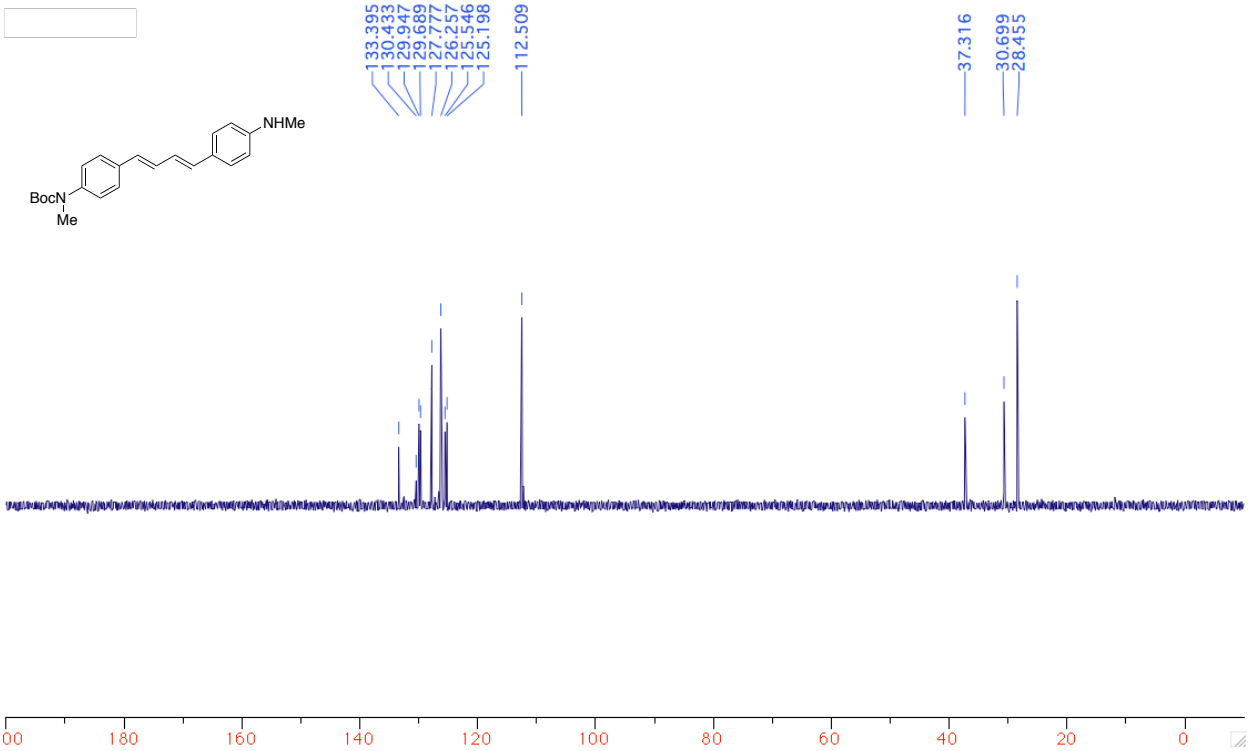

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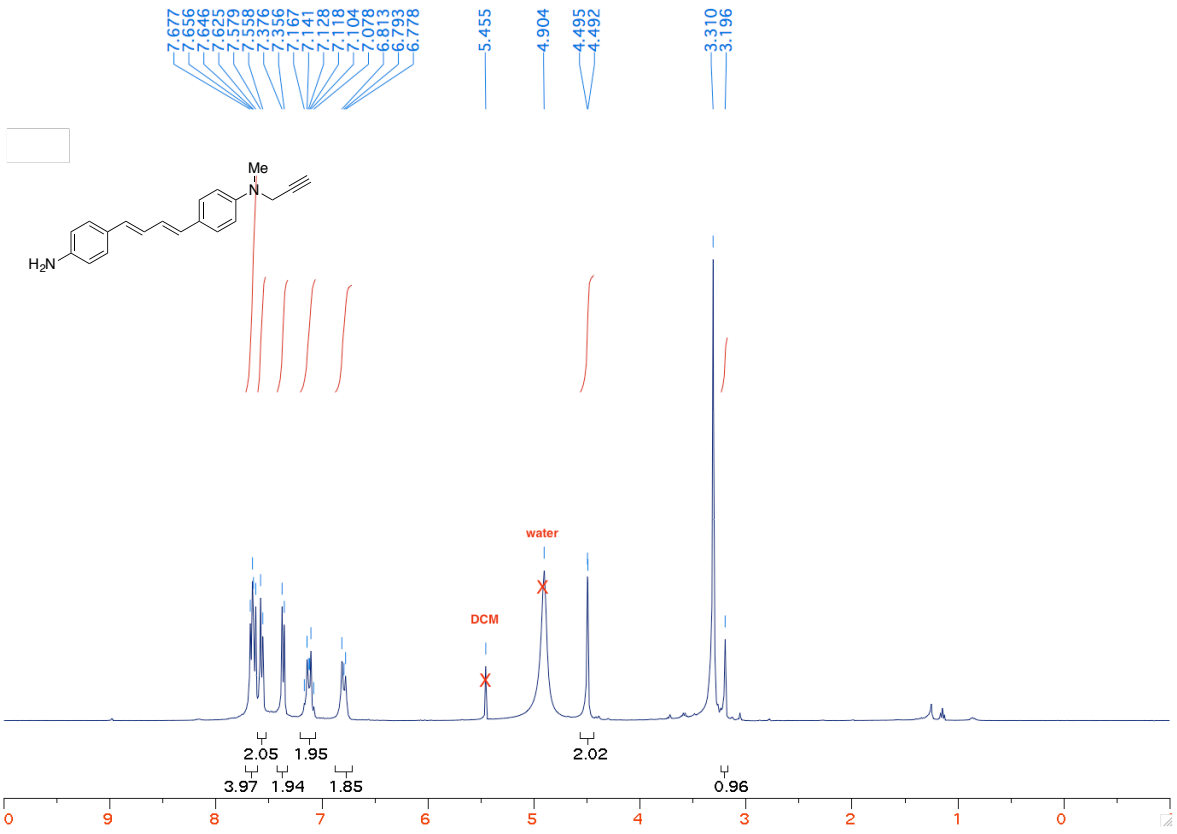

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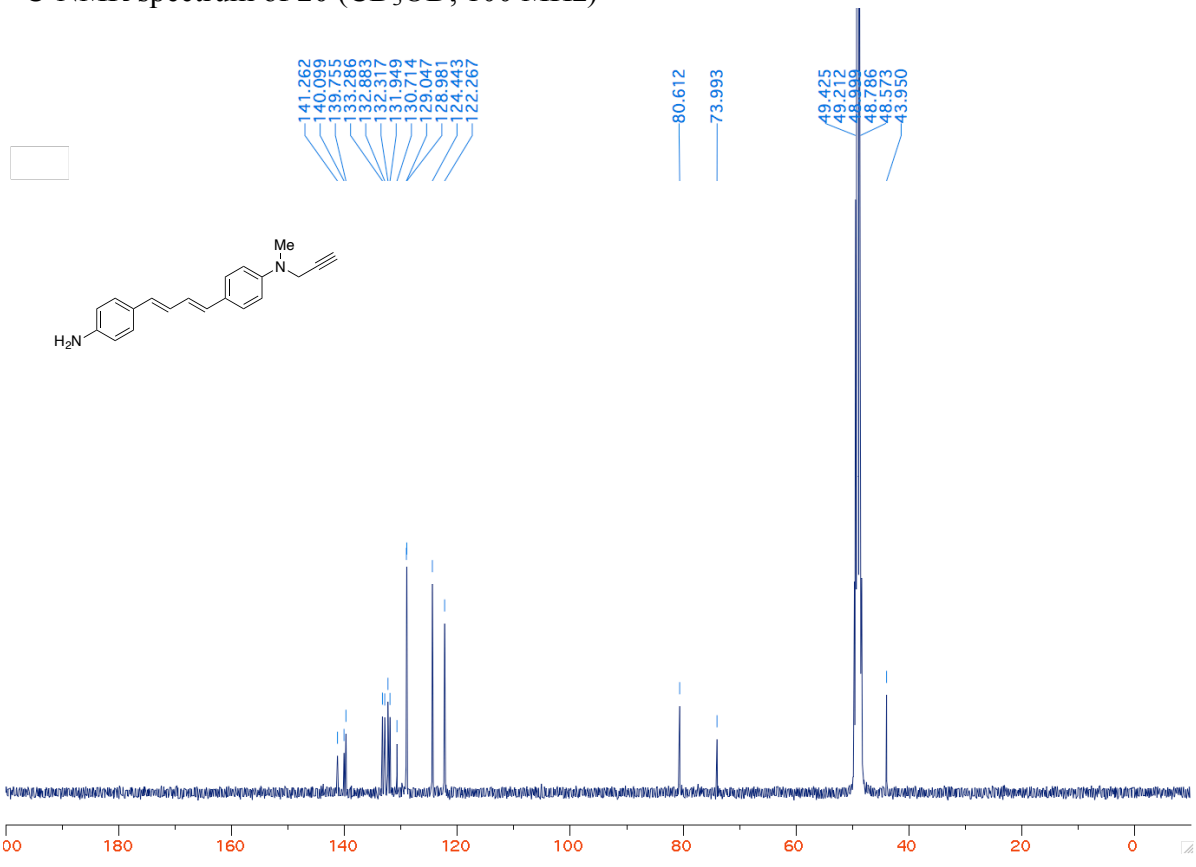
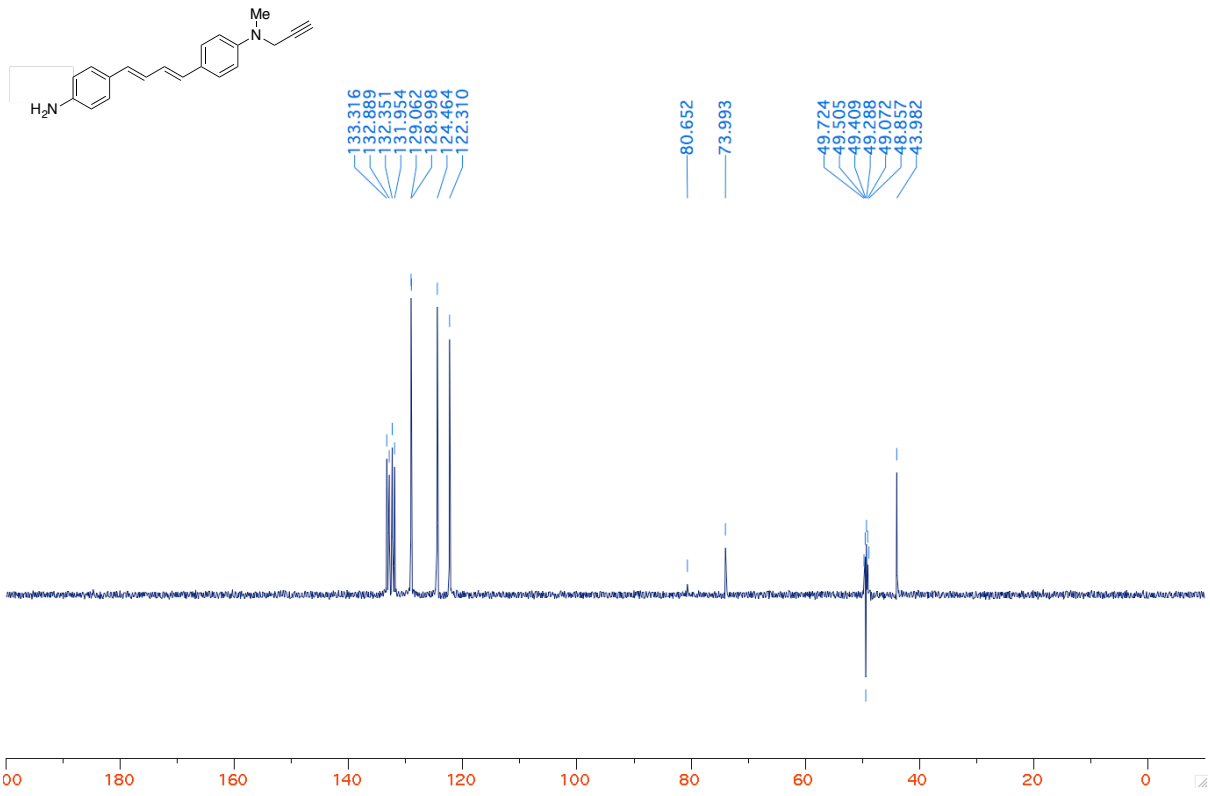
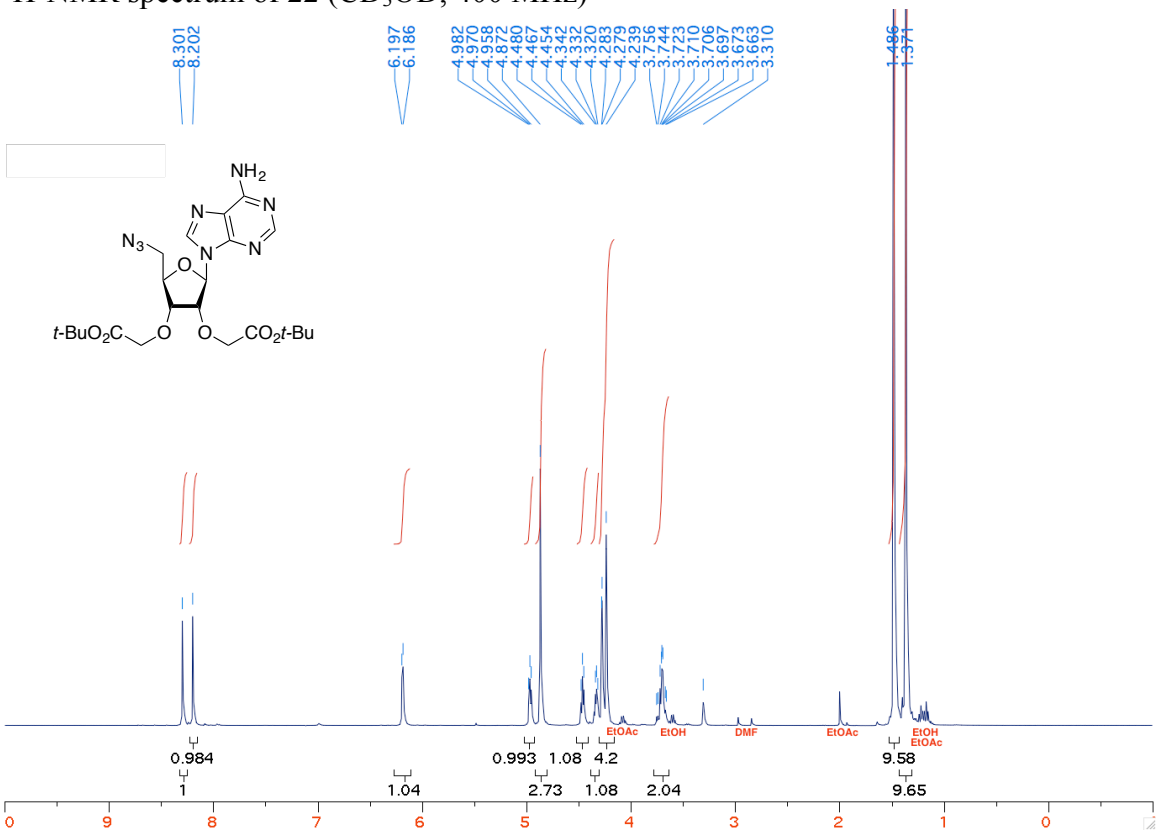


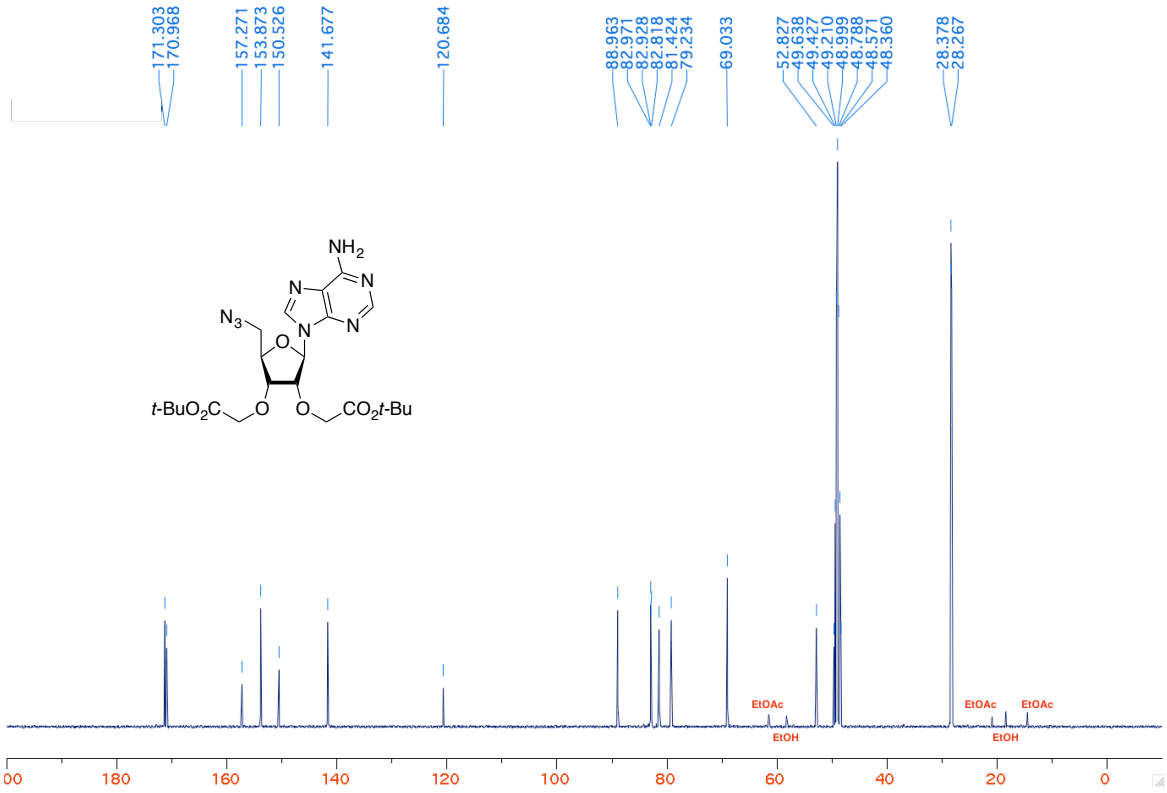
DEPT 135 spectrum of **20** (CD_3OD, 100 MHz)



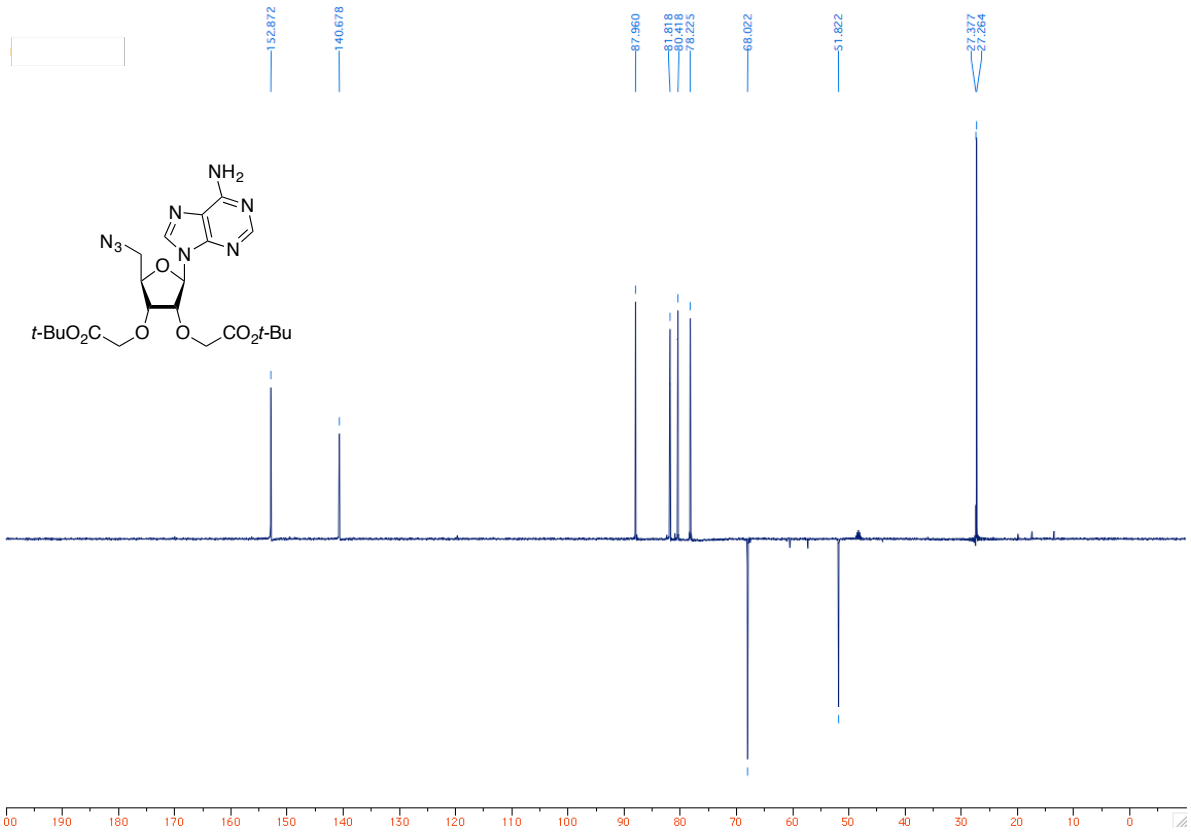
^{1}H-NMR spectrum of **22** (CD_3OD, 400 MHz)



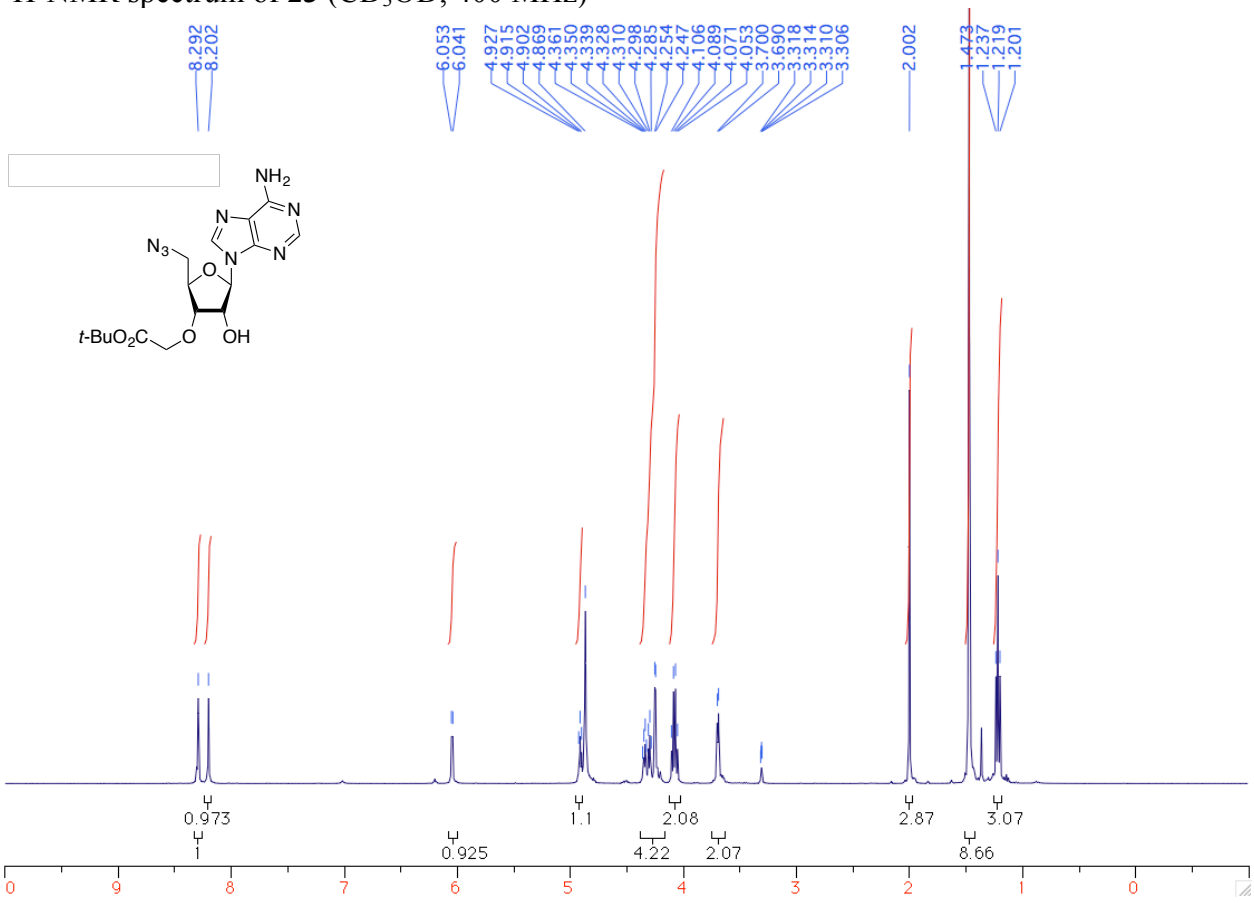
^{13}C-NMR spectrum of **22** (CD_3OD, 100 MHz)



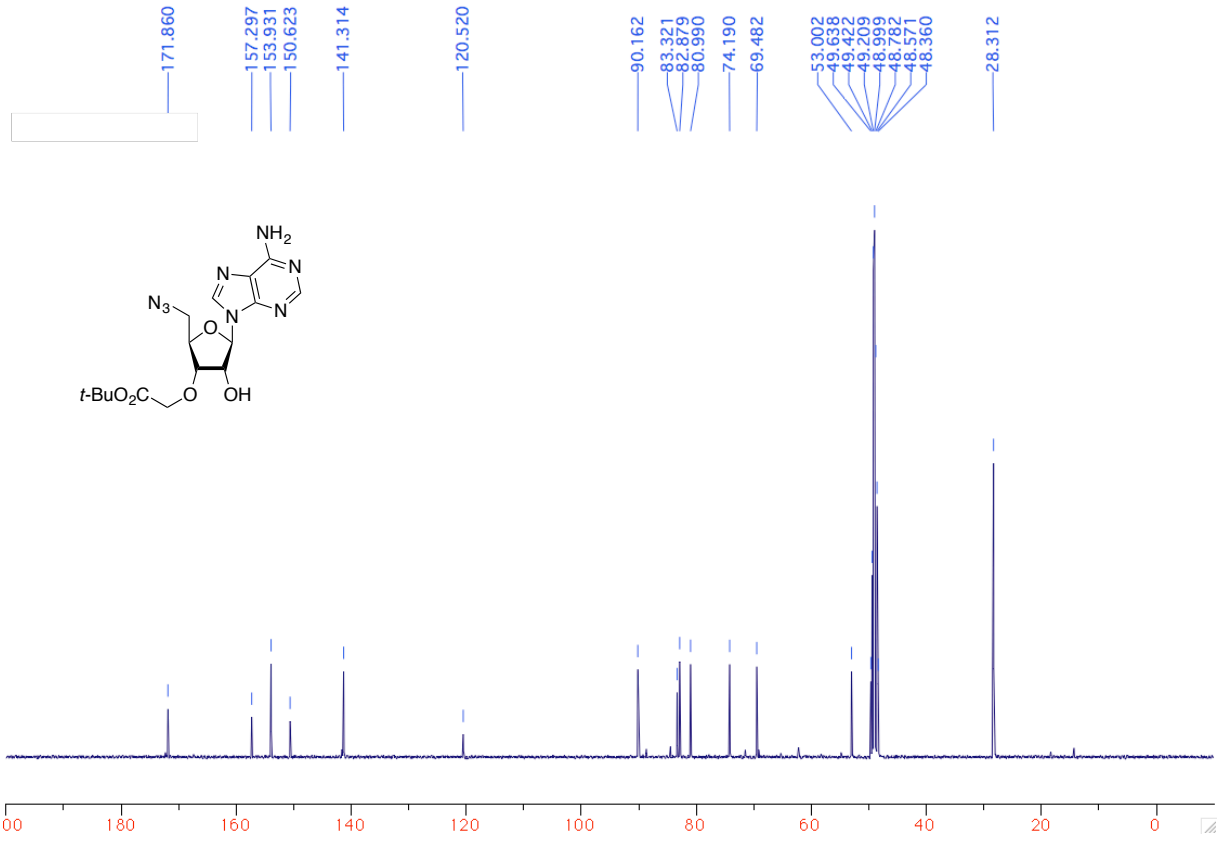
DEPT 135 spectrum of **22** (CD_3OD, 100 MHz)



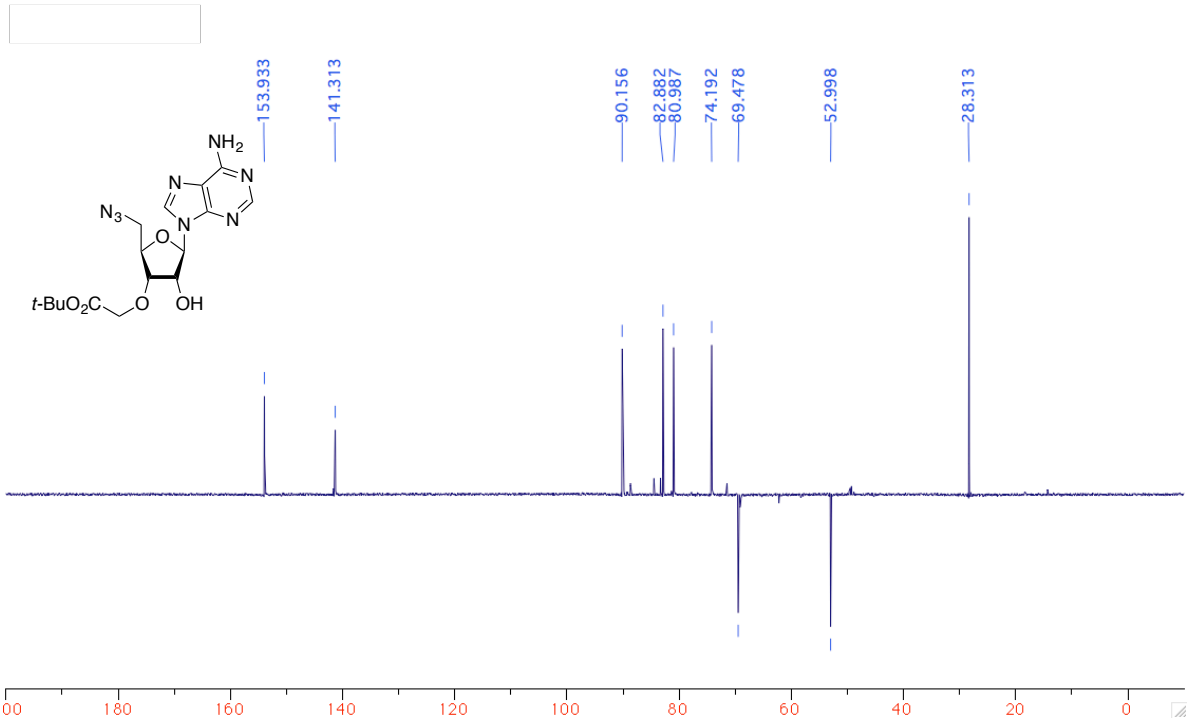
^{1}H-NMR spectrum of **23** (CD_3OD, 400 MHz)



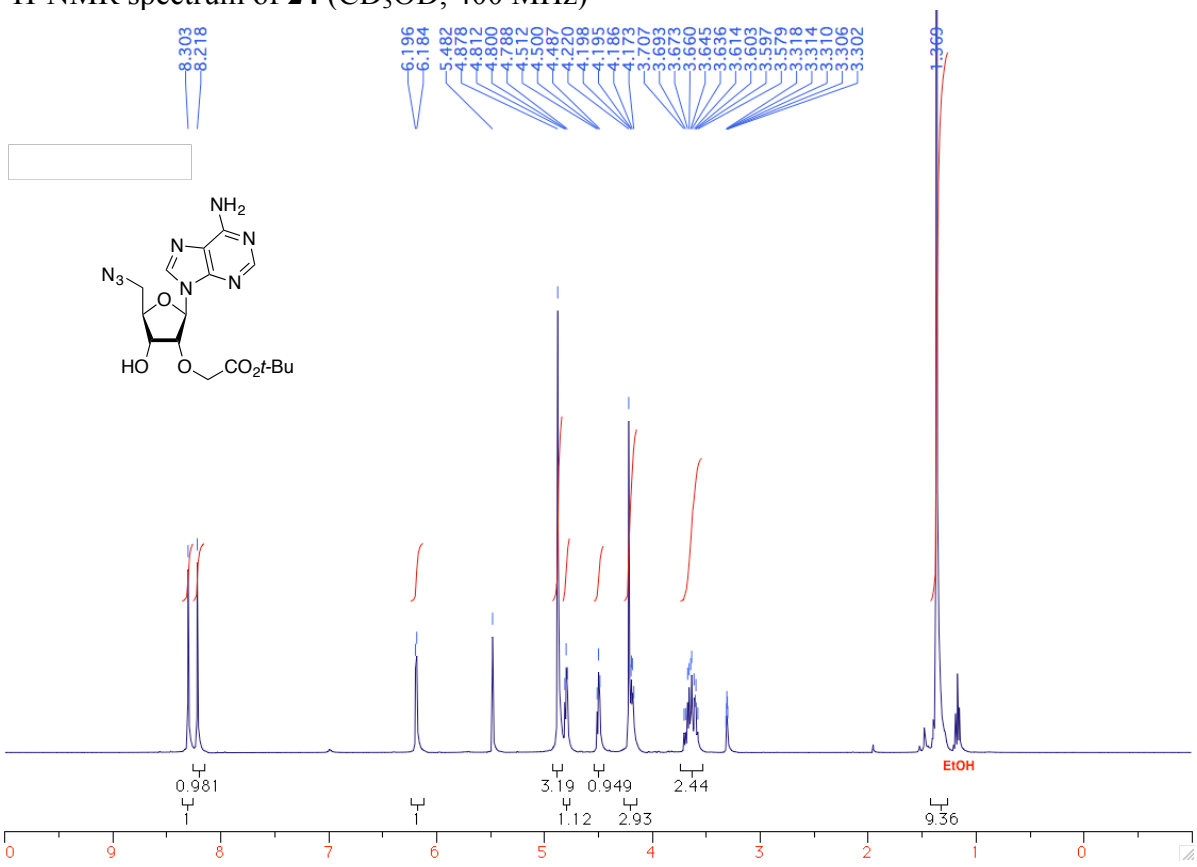
^{13}C-NMR spectrum of **23** (CD_3OD, 100 MHz)



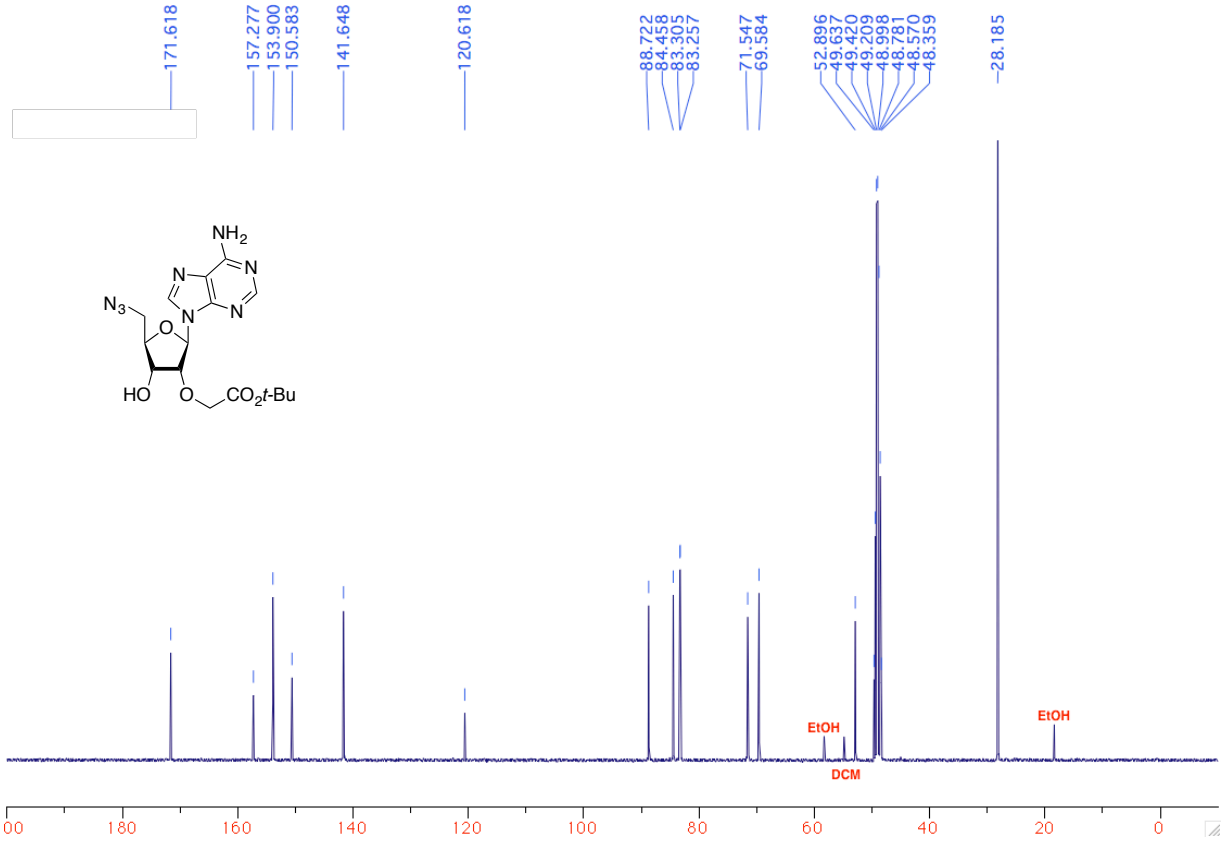
DEPT 135 spectrum of **23** (CD_3OD, 100 MHz)



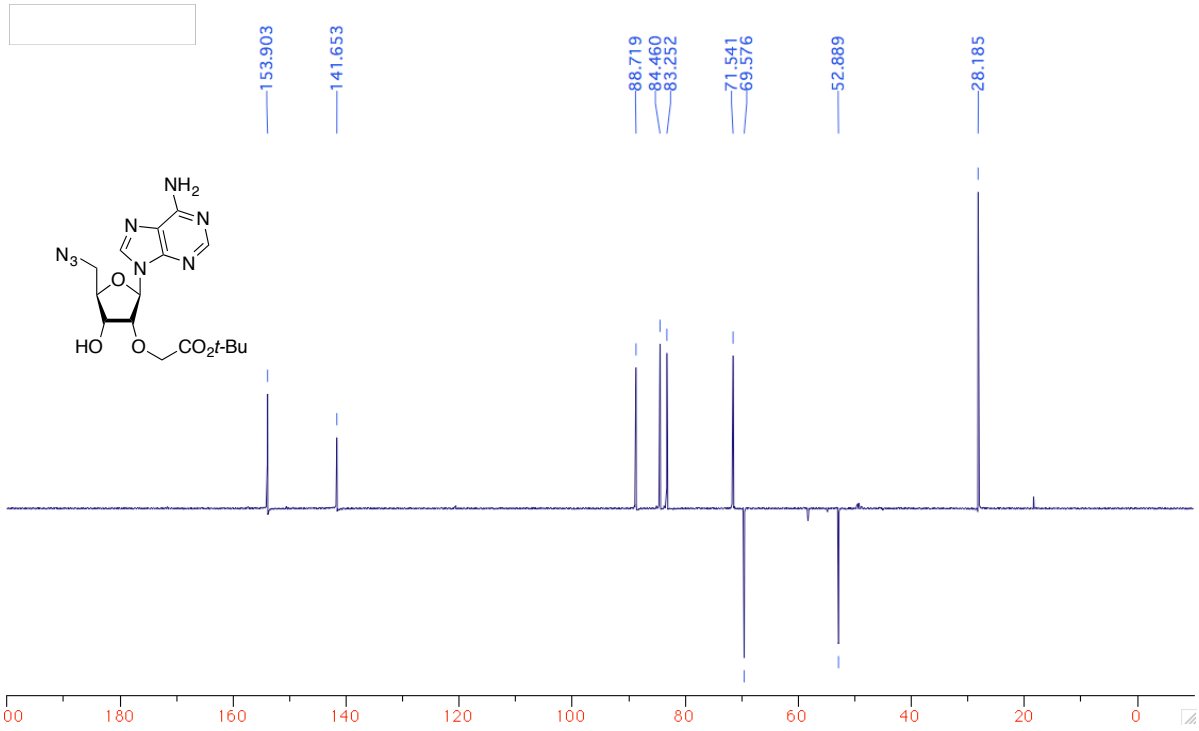
^{1}H-NMR spectrum of **24** (CD_3OD, 400 MHz)



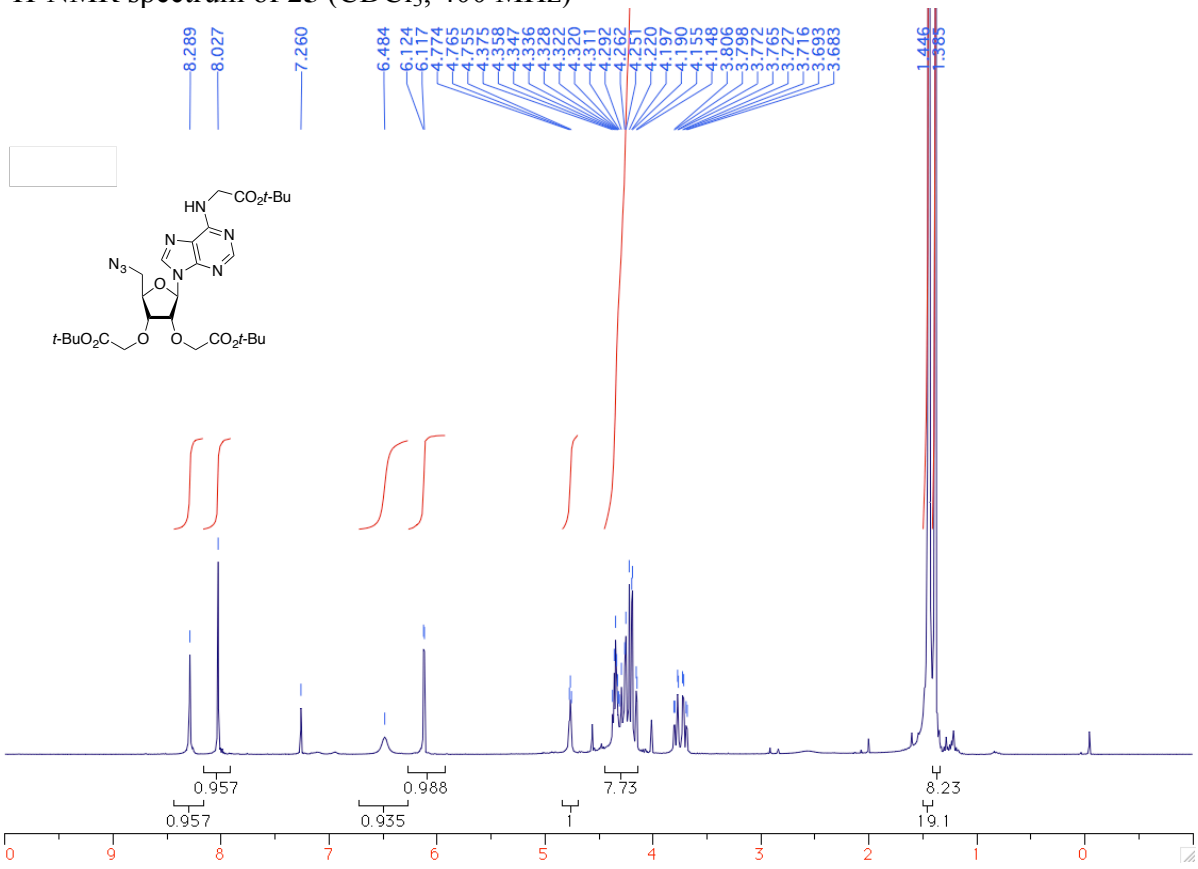
^{13}C-NMR spectrum of **24** (CD_3OD, 100 MHz)



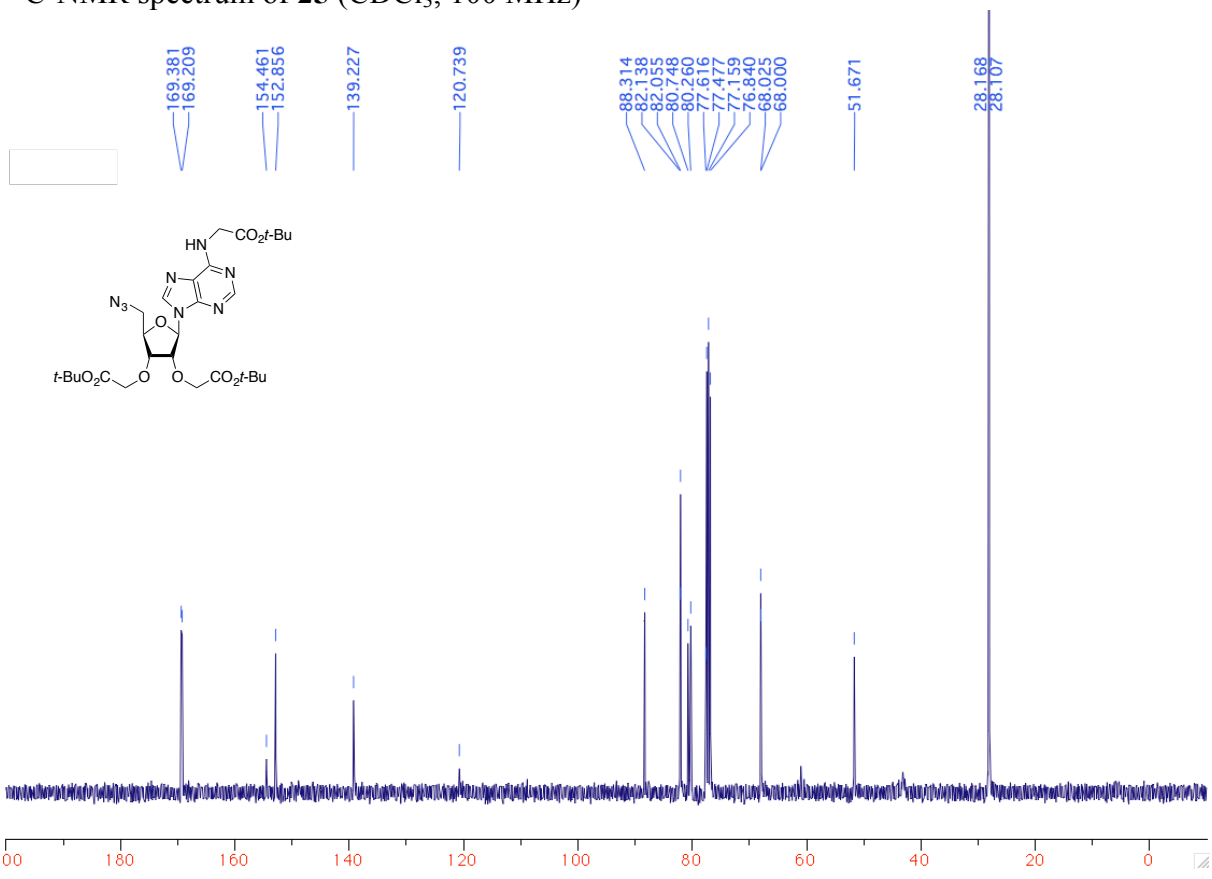
DEPT 135 spectrum of **24** (CD_3OD, 100 MHz)



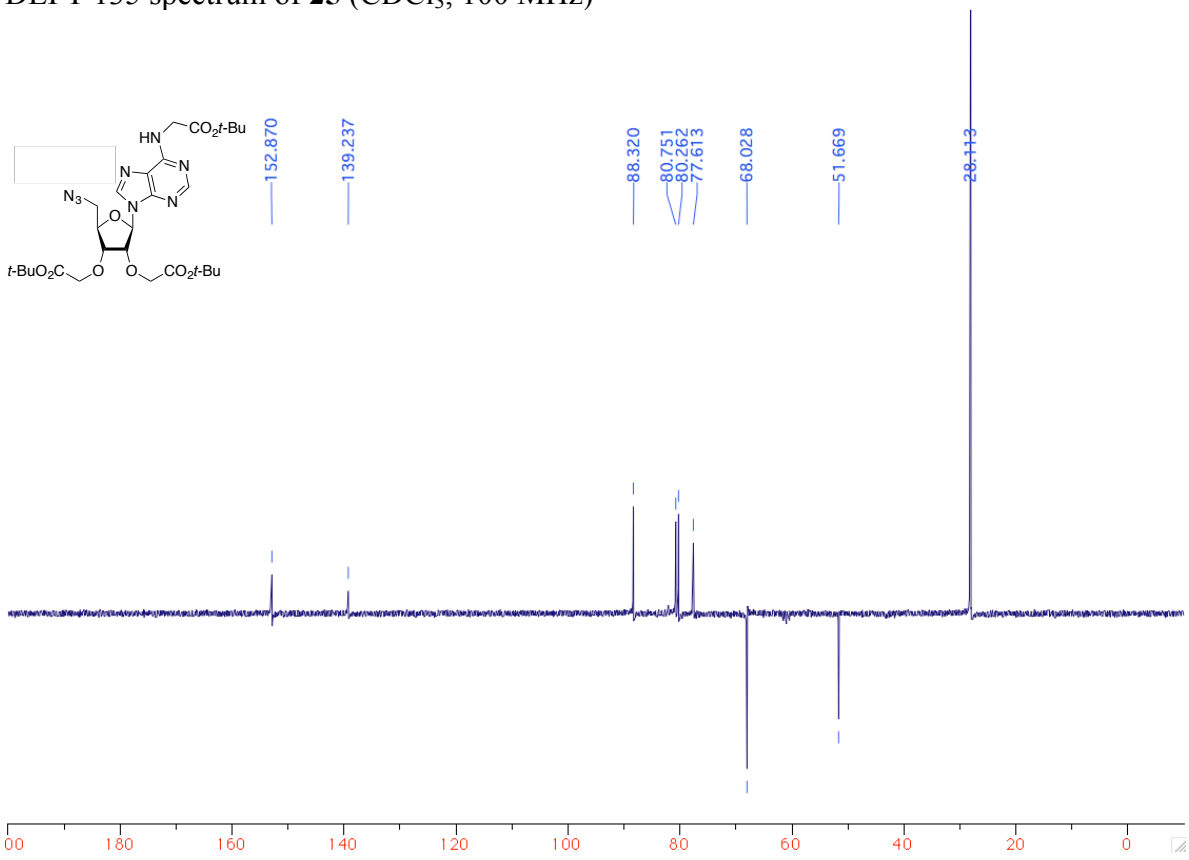
^{1}H-NMR spectrum of **25** ($CDCl_3$, 400 MHz)



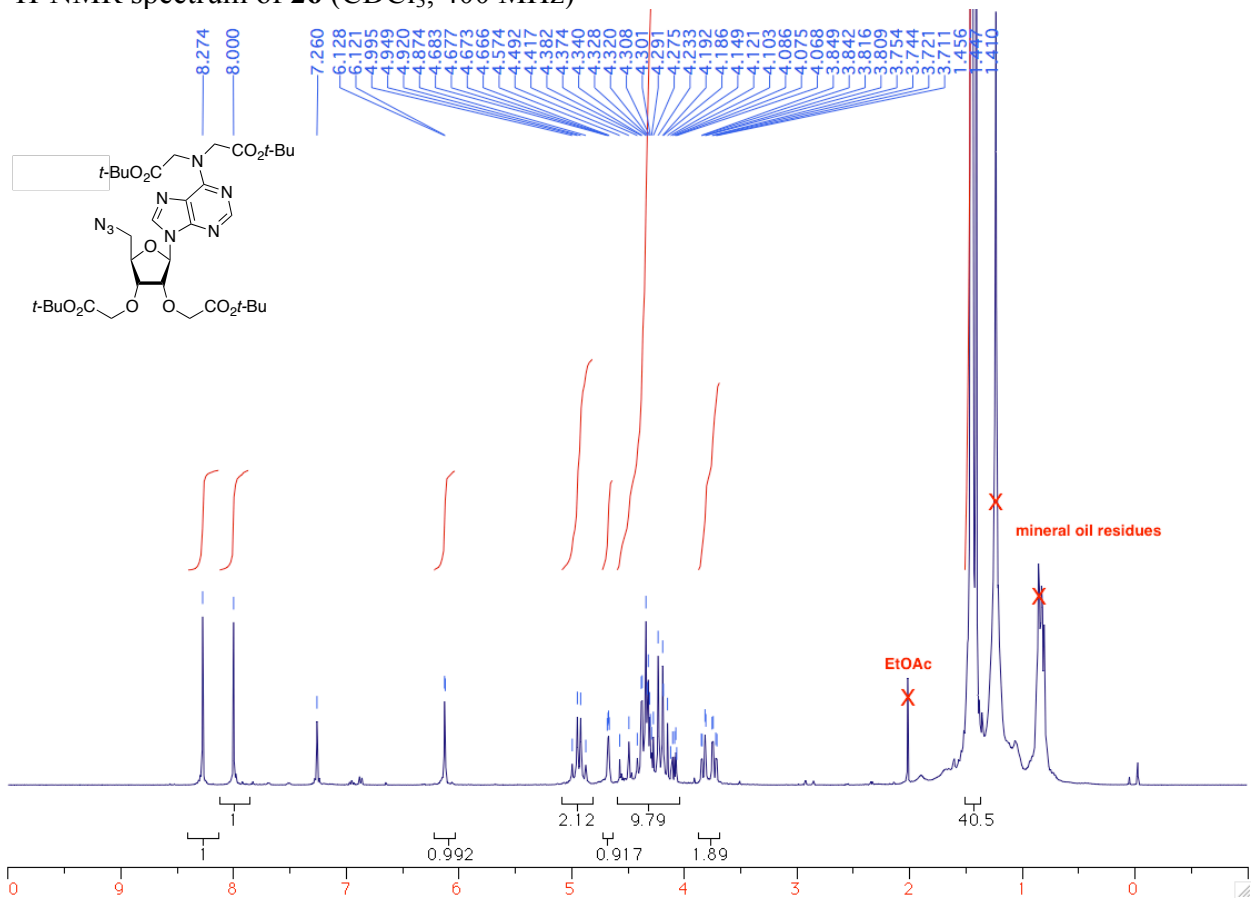
^{13}C-NMR spectrum of **25** ($CDCl_3$, 100 MHz)



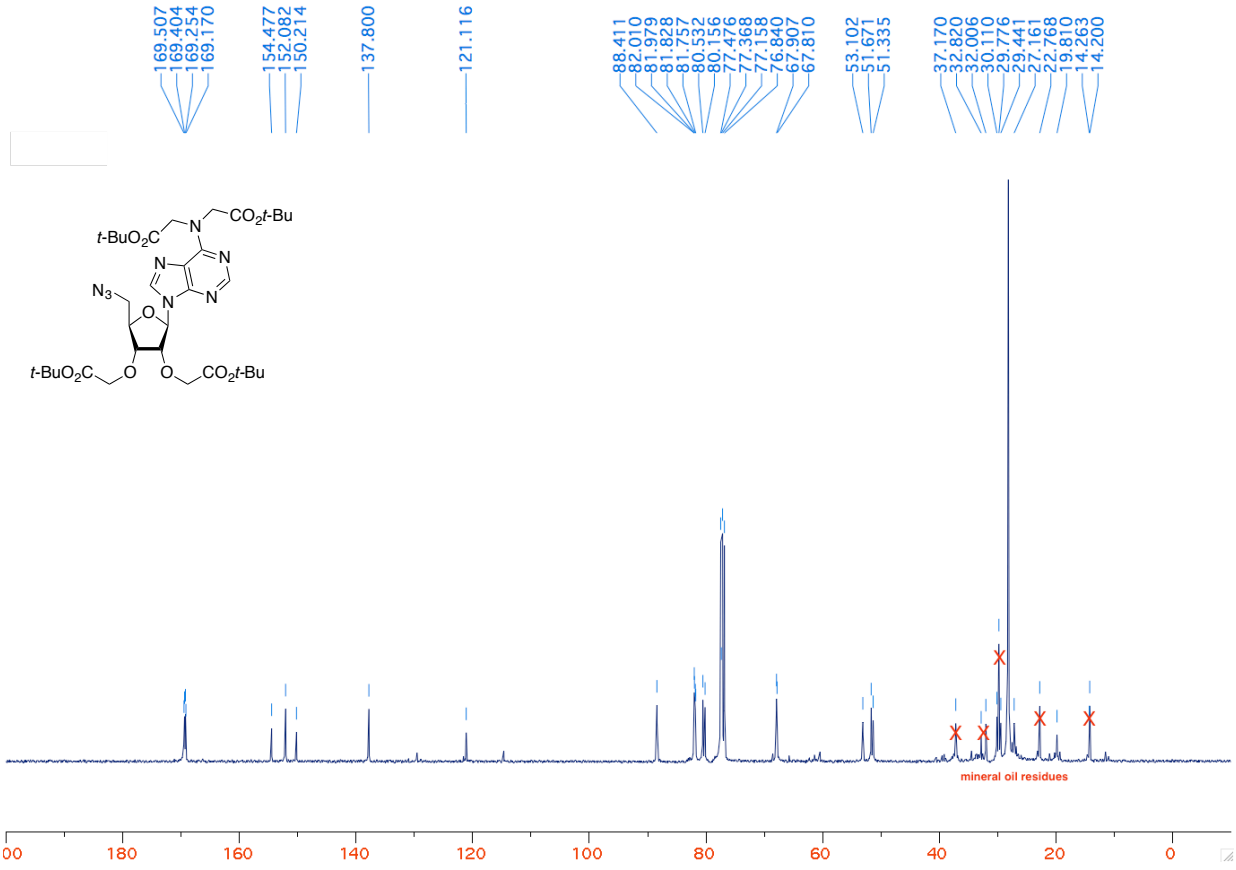
DEPT 135 spectrum of **25** ($CDCl_3$, 100 MHz)



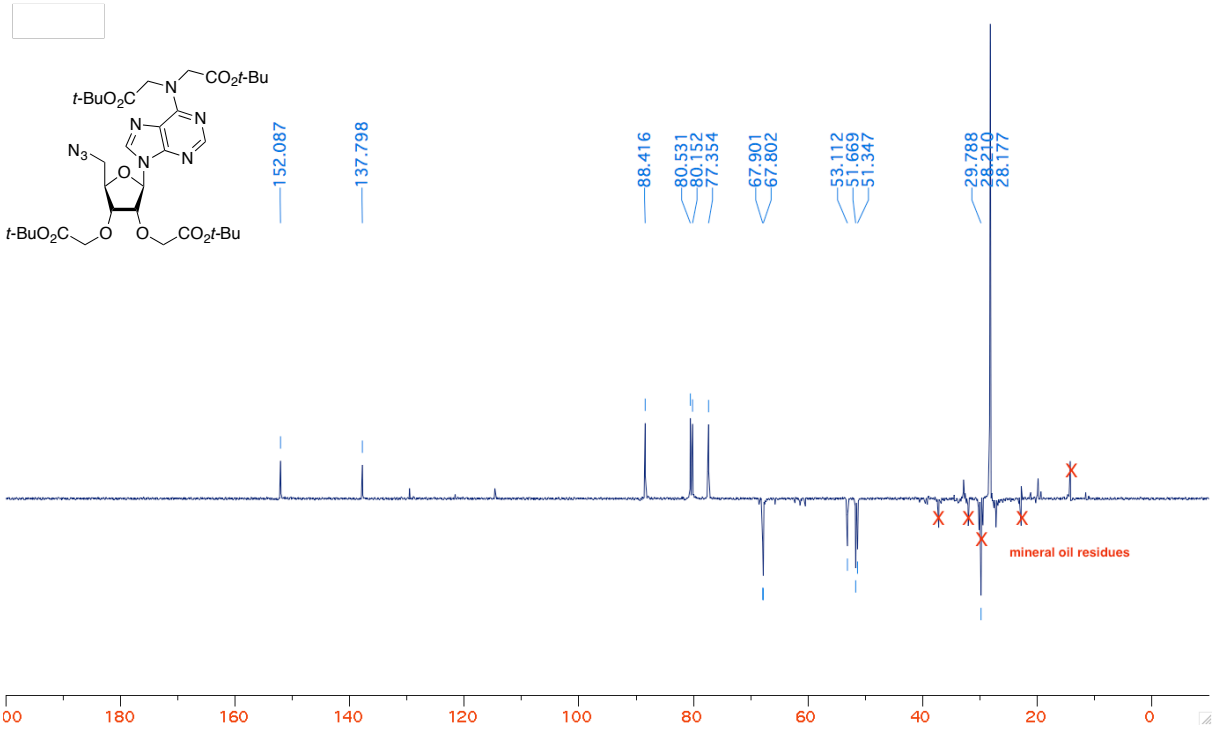
^{1}H-NMR spectrum of **26** ($CDCl_3$, 400 MHz)



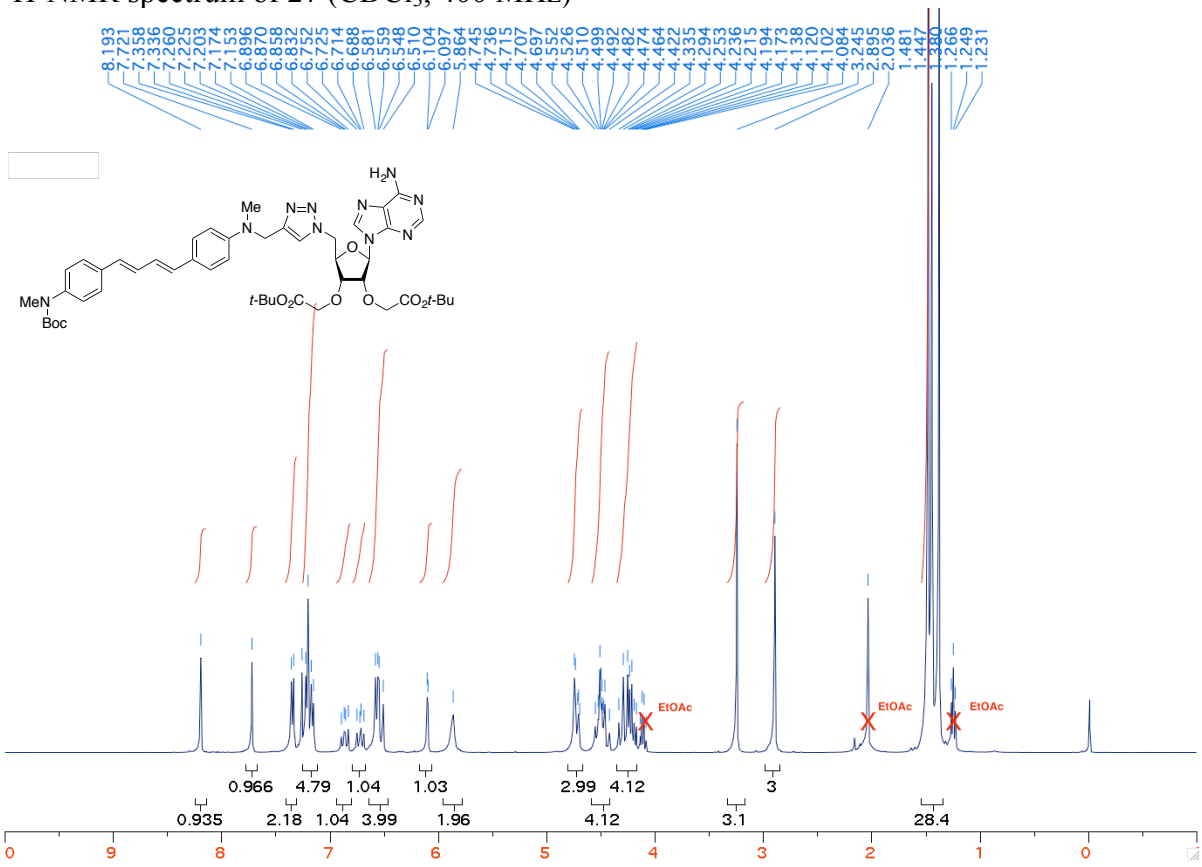
^{13}C-NMR spectrum of **26** ($CDCl_3$, 100 MHz)



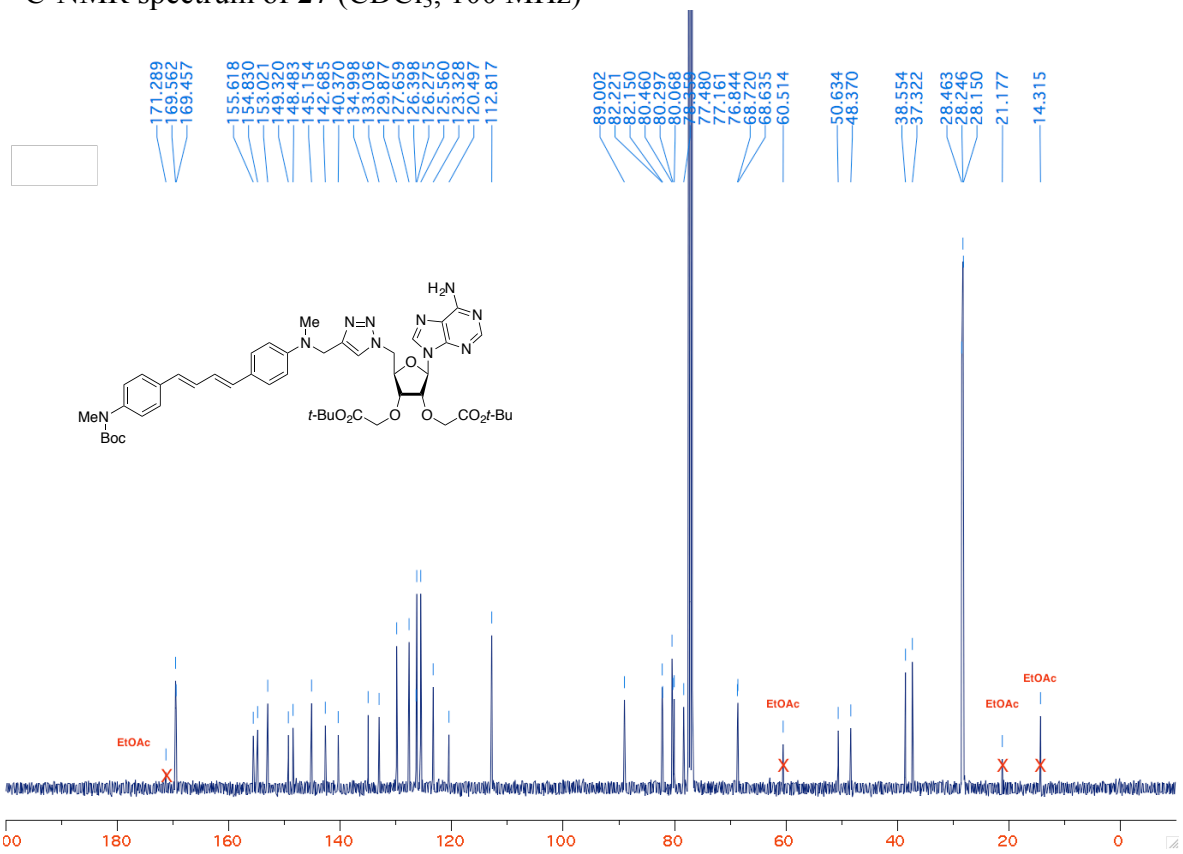
DEPT 135 spectrum of **26** ($CDCl_3$, 100 MHz)



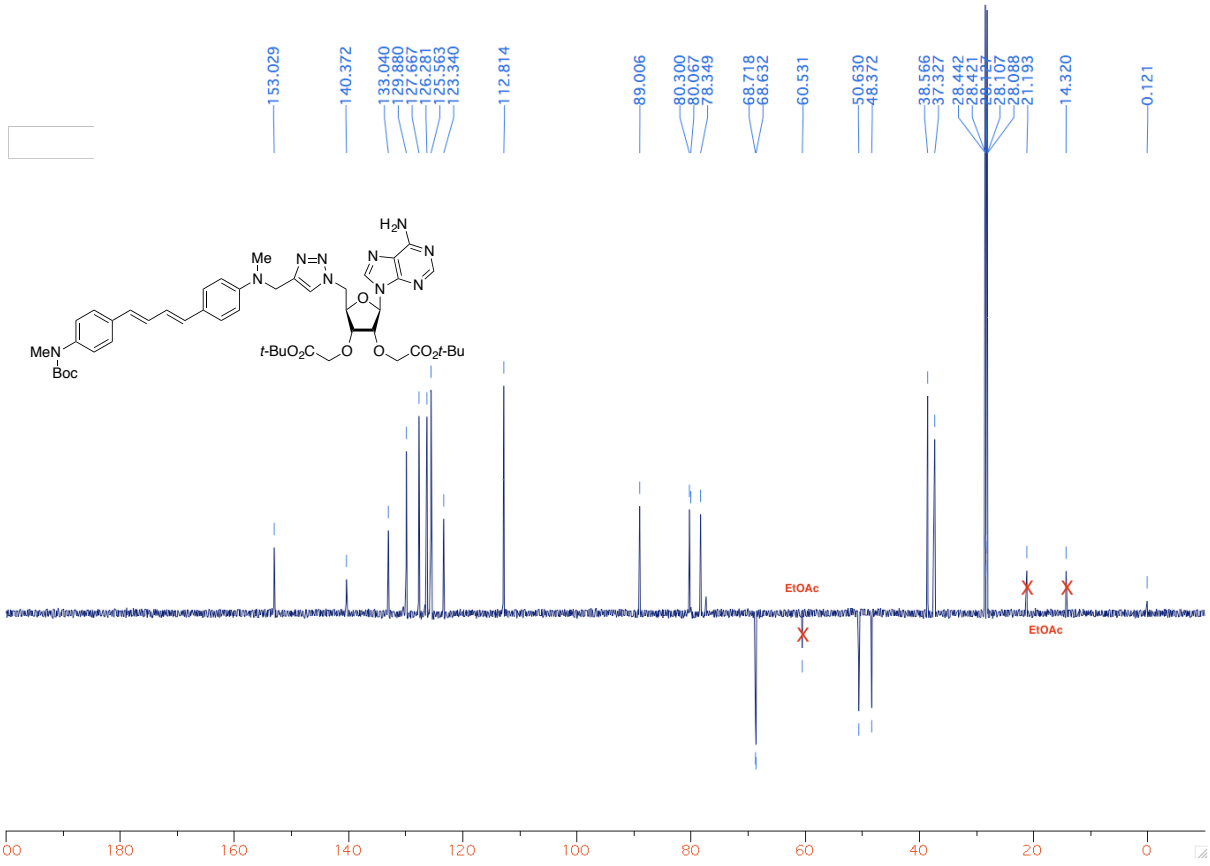
^{1}H-NMR spectrum of **27** ($CDCl_3$, 400 MHz)



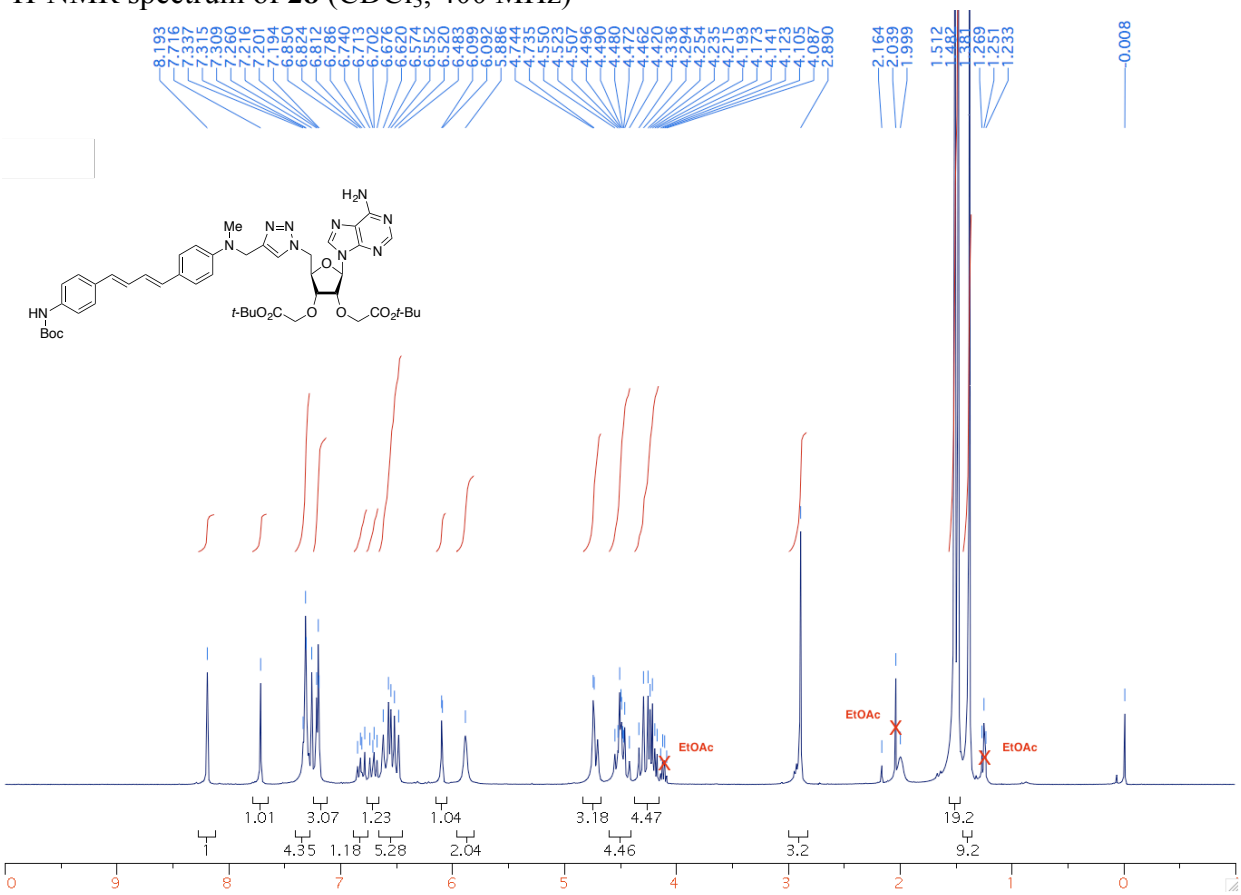
^{13}C-NMR spectrum of **27** ($CDCl_3$, 100 MHz)



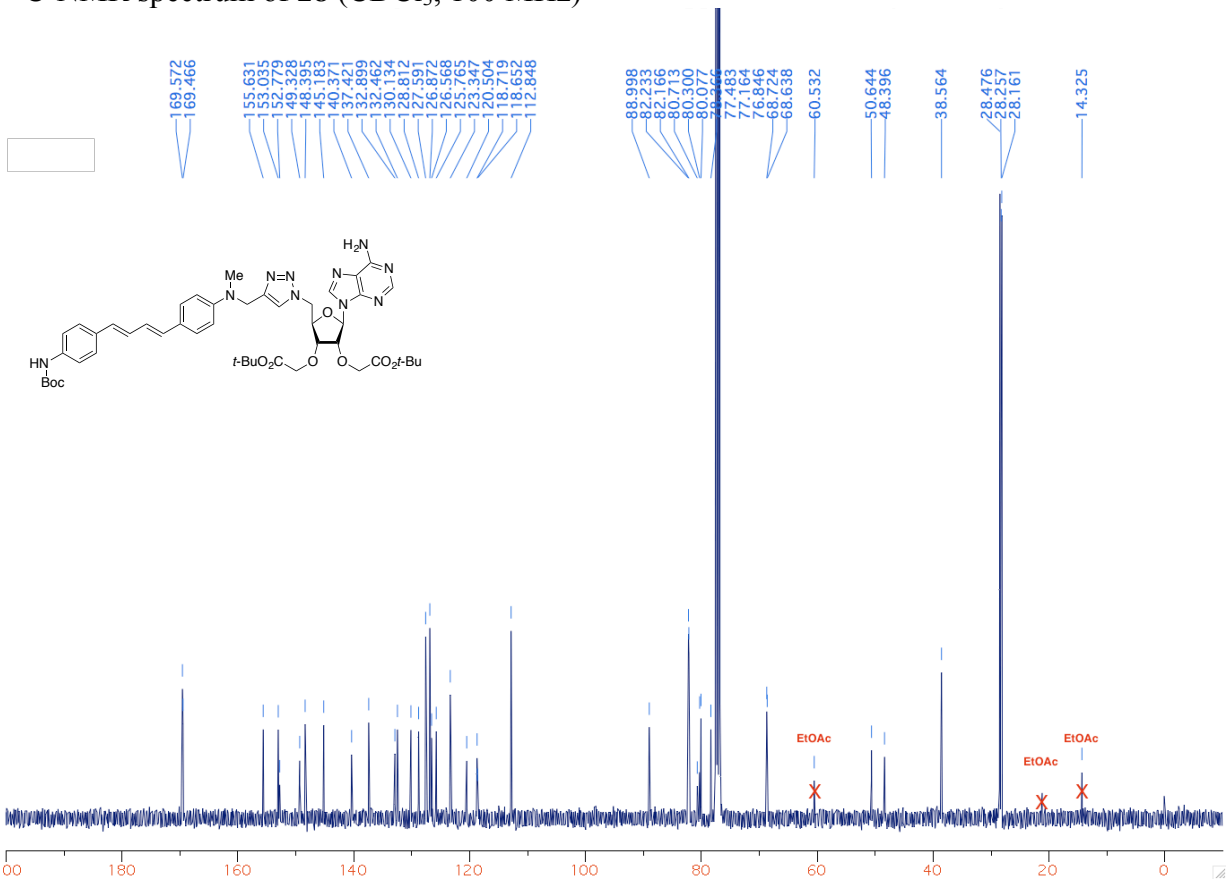
DEPT 135 spectrum of **27** ($CDCl_3$, 100 MHz)



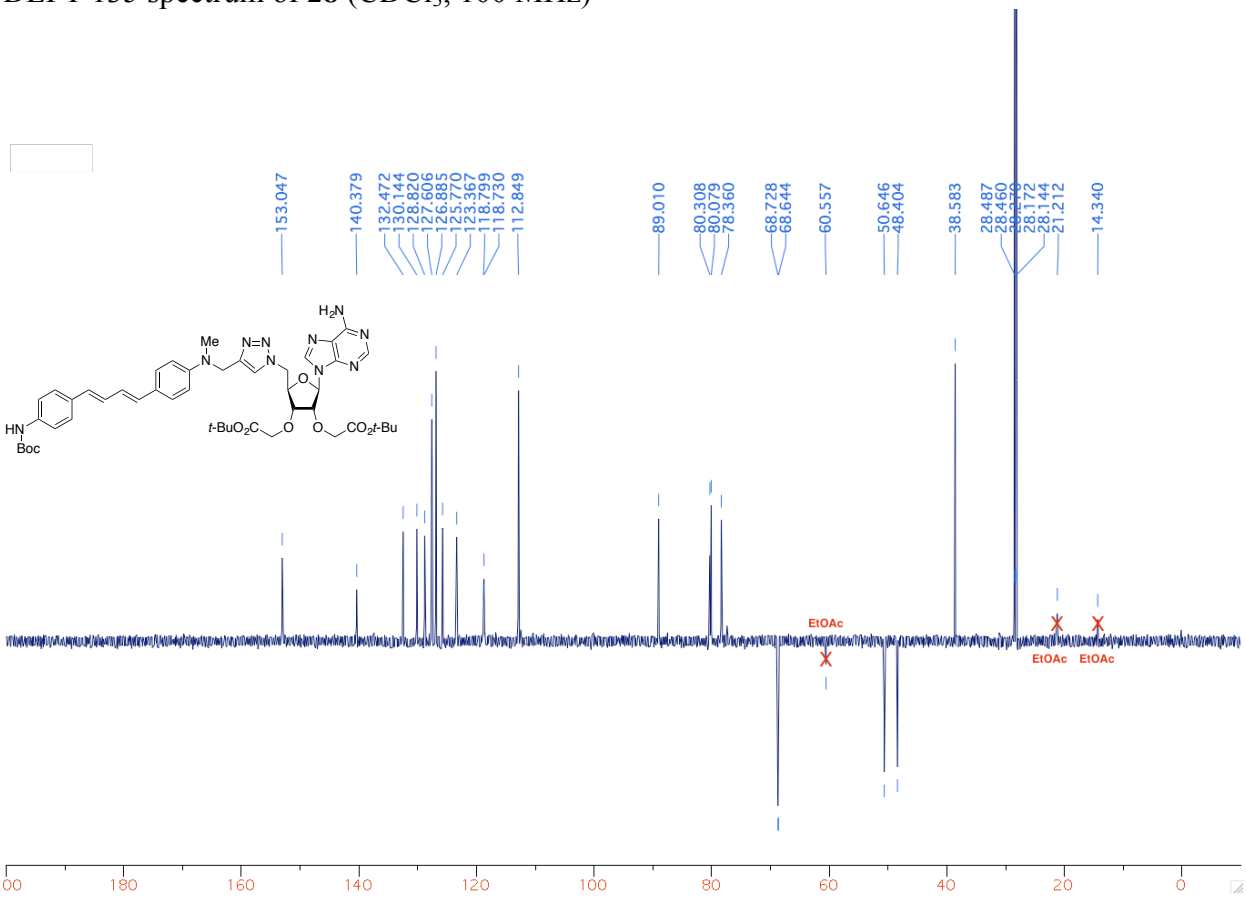
^{1}H-NMR spectrum of **28** ($CDCl_3$, 400 MHz)



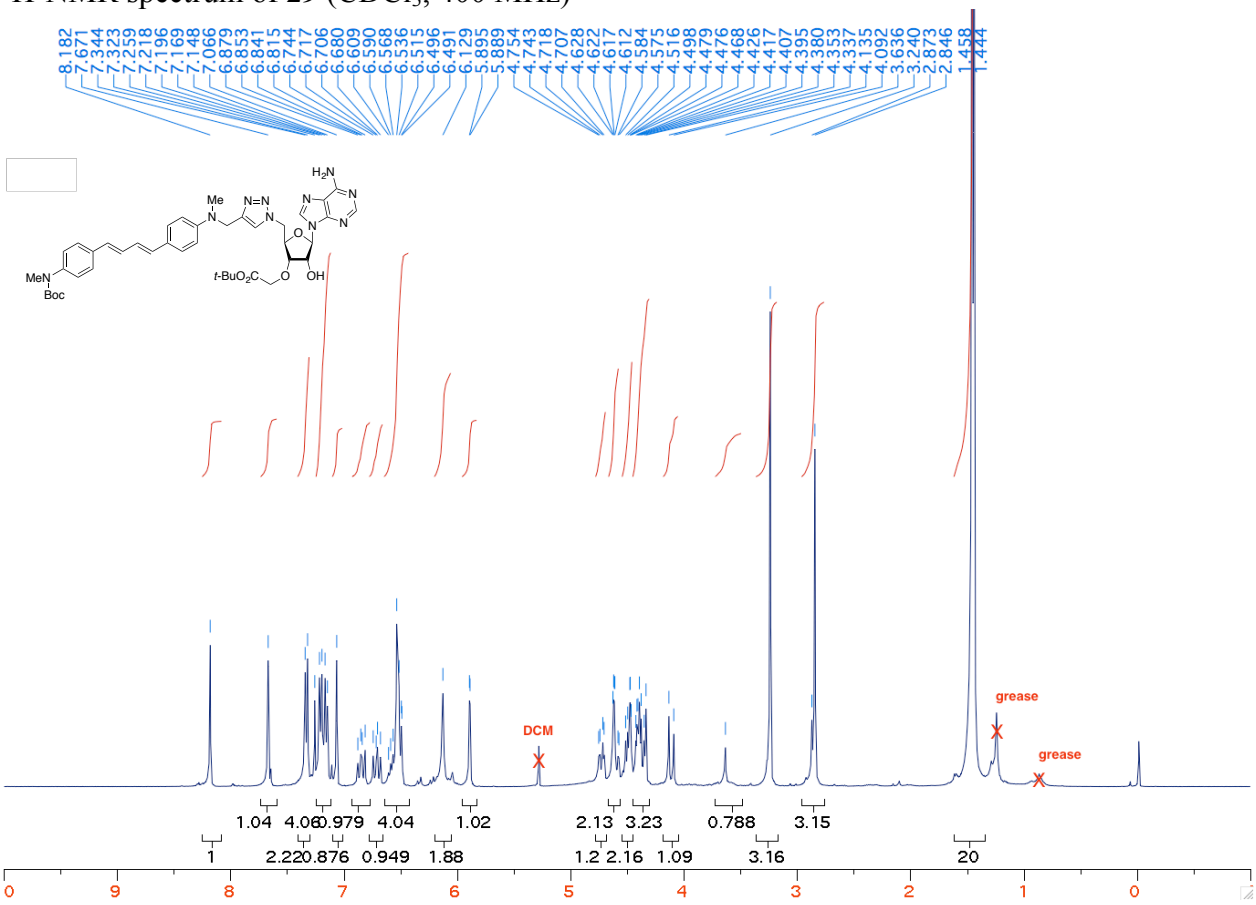
^{13}C-NMR spectrum of **28** ($CDCl_3$, 100 MHz)



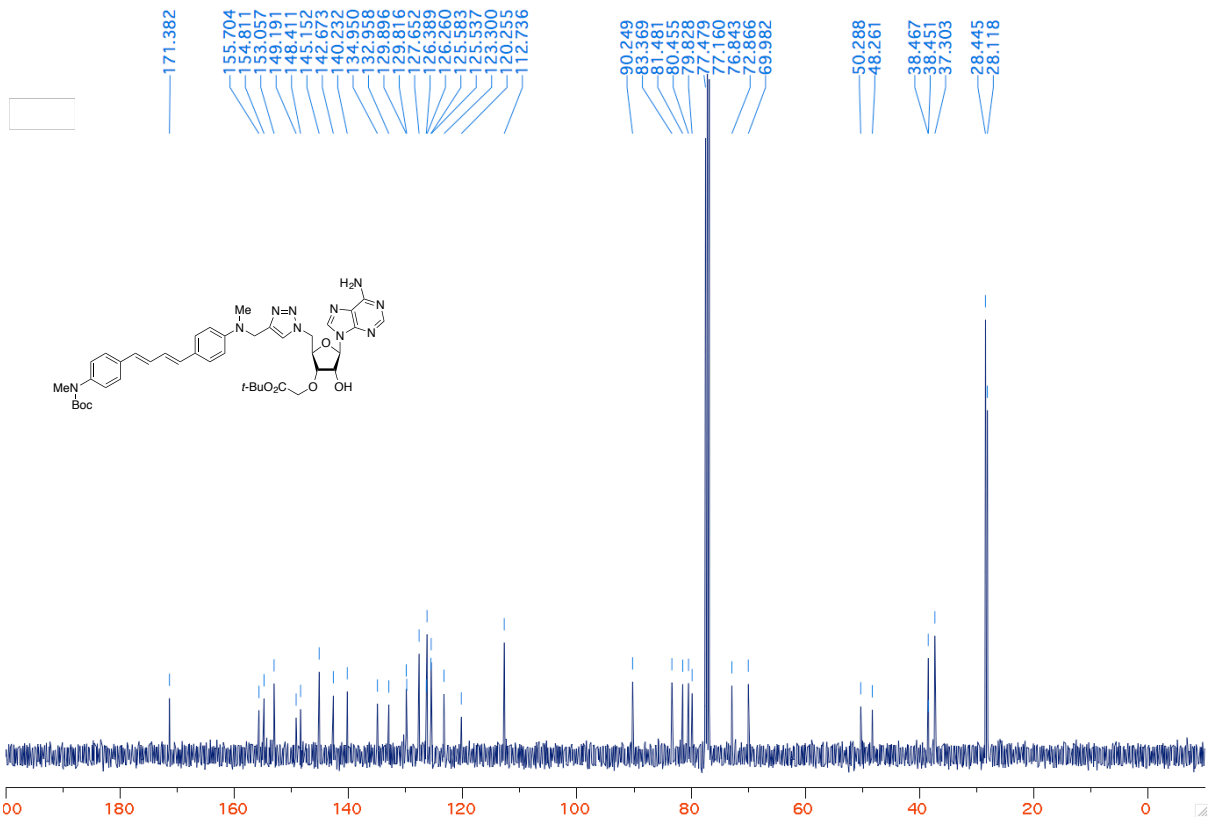
DEPT 135 spectrum of **28** ($CDCl_3$, 100 MHz)



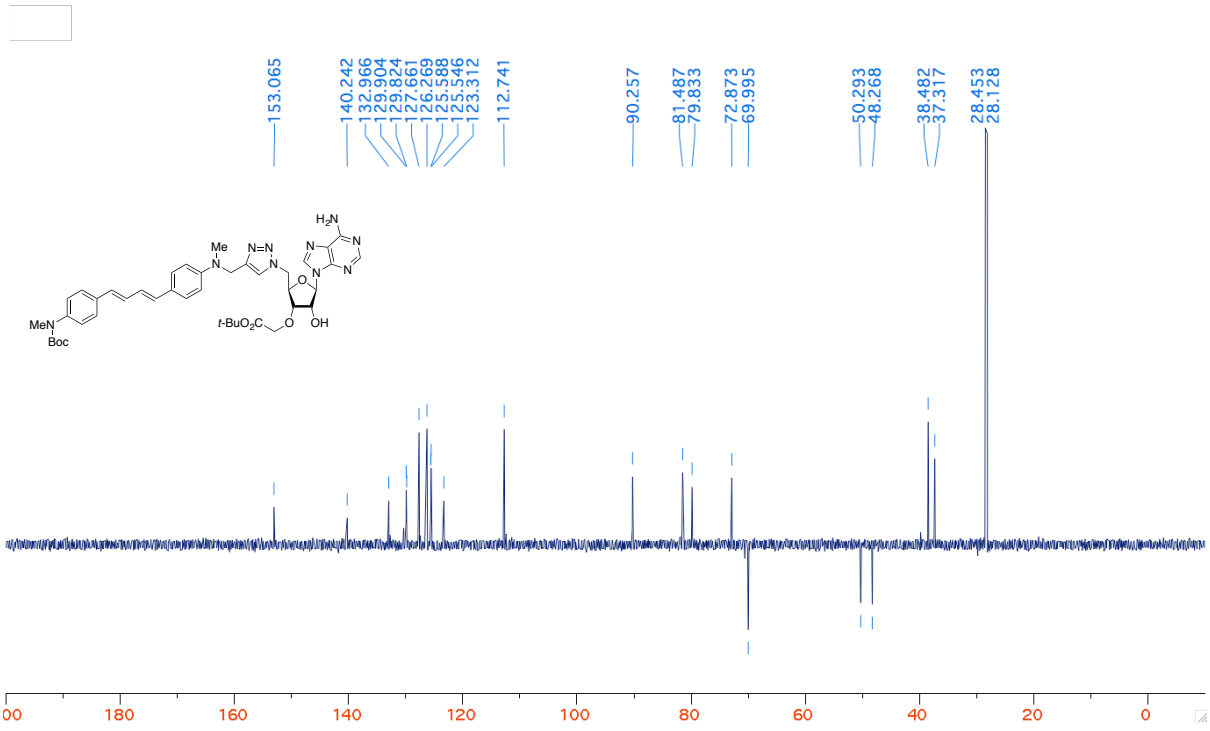
^{1}H-NMR spectrum of **29** ($CDCl_3$, 400 MHz)



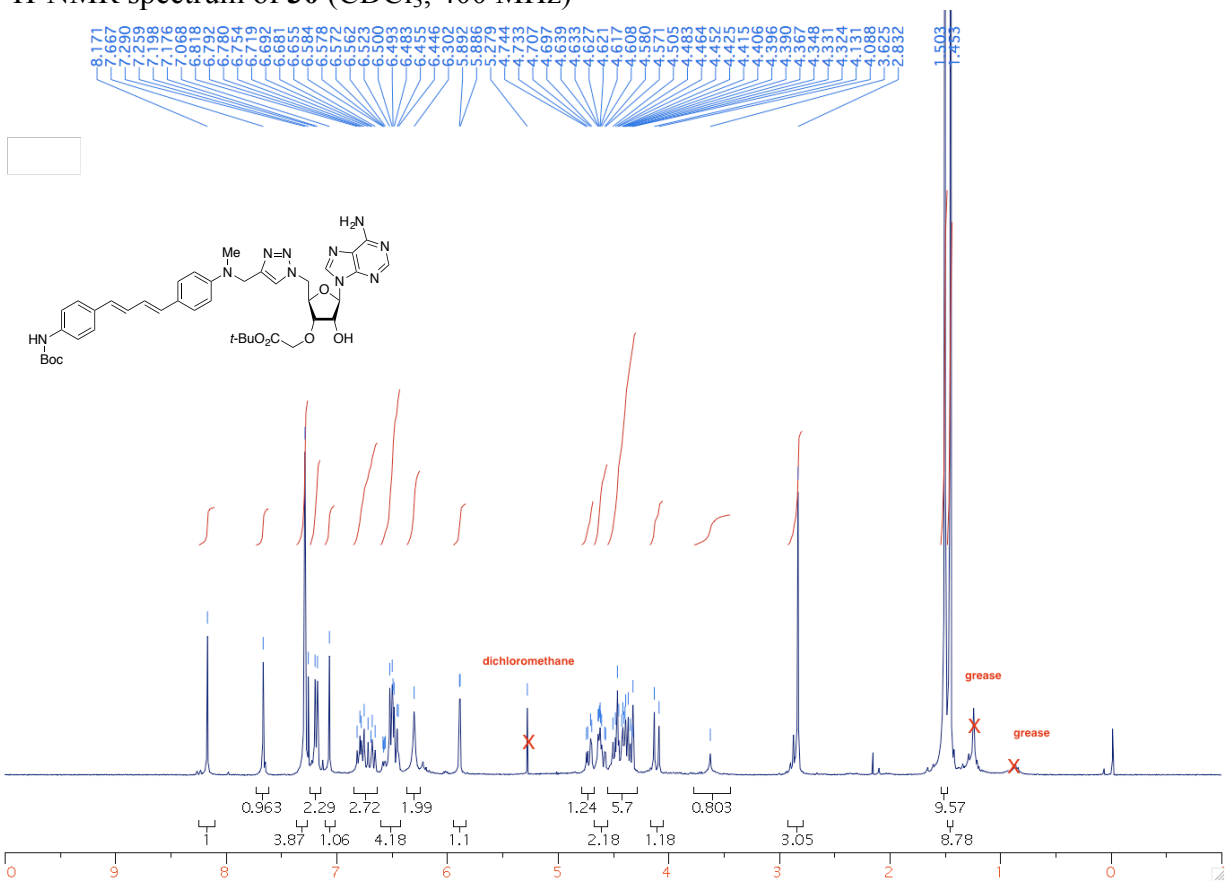
^{13}C-NMR spectrum of **29** ($CDCl_3$, 100 MHz)



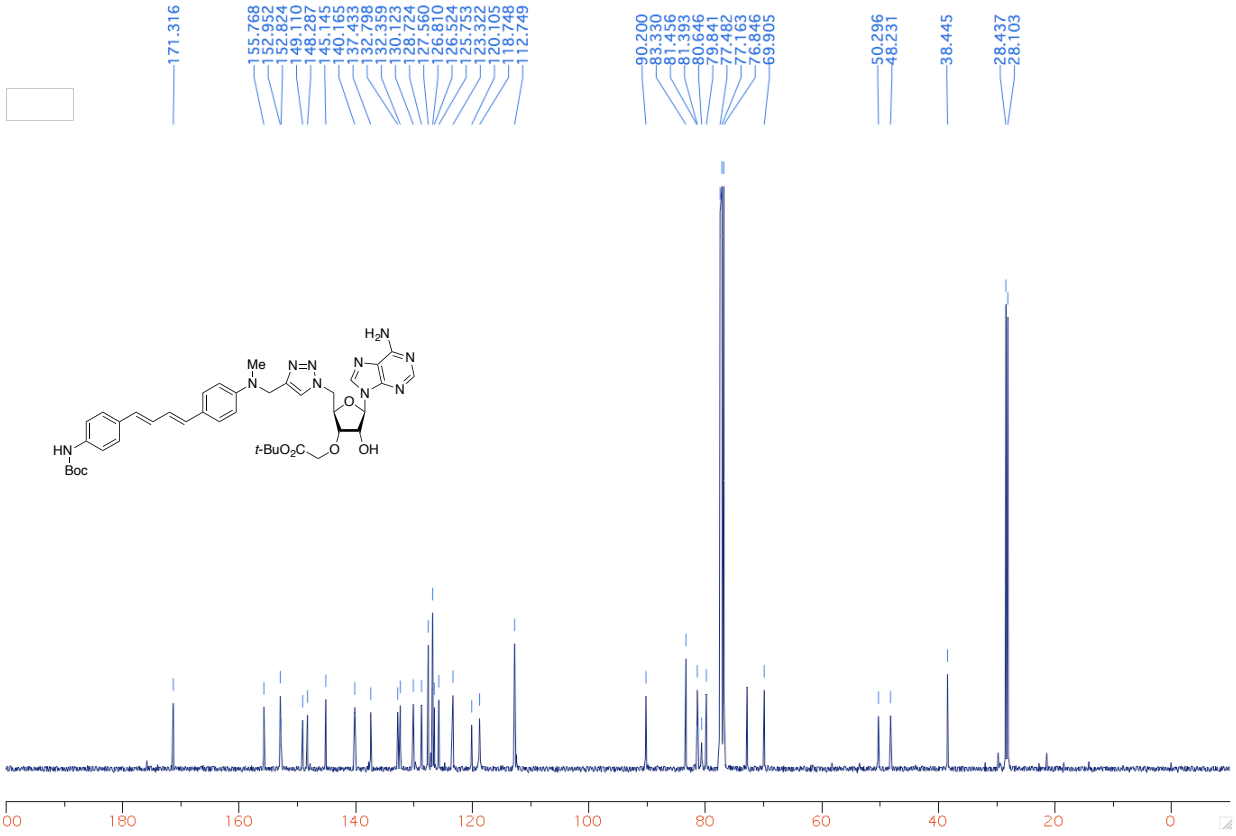
DEPT 135 spectrum of **29** ($CDCl_3$, 100 MHz)



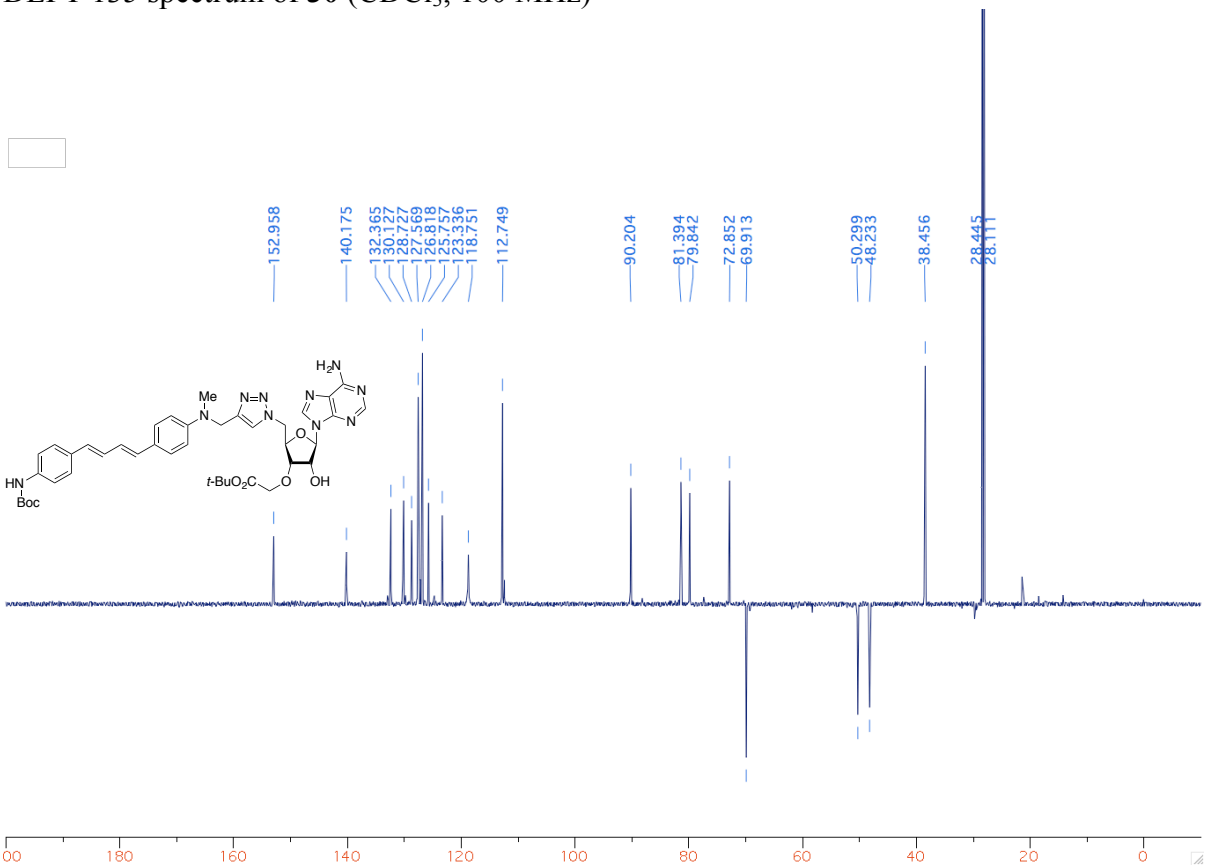
^{1}H-NMR spectrum of **30** ($CDCl_3$, 400 MHz)



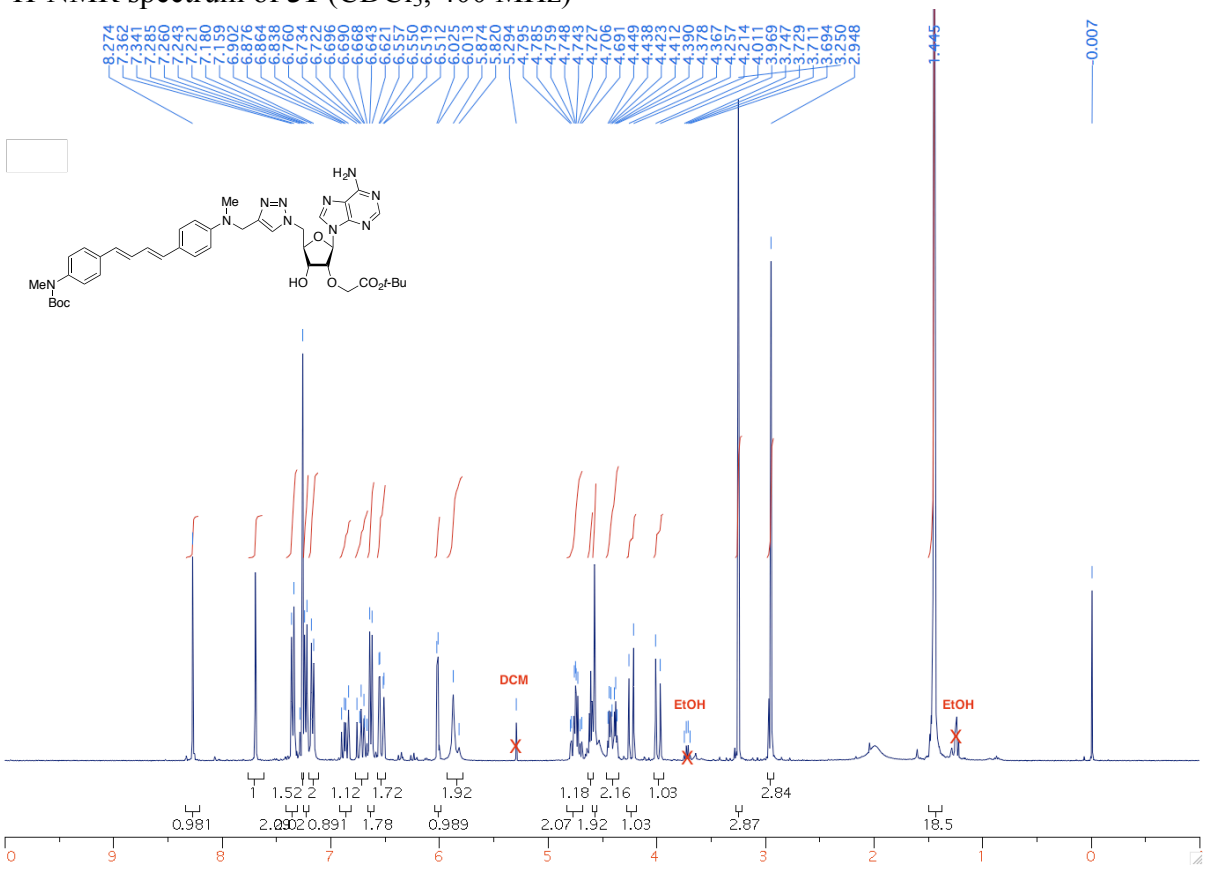
^{13}C-NMR spectrum of **30** ($CDCl_3$, 100 MHz)



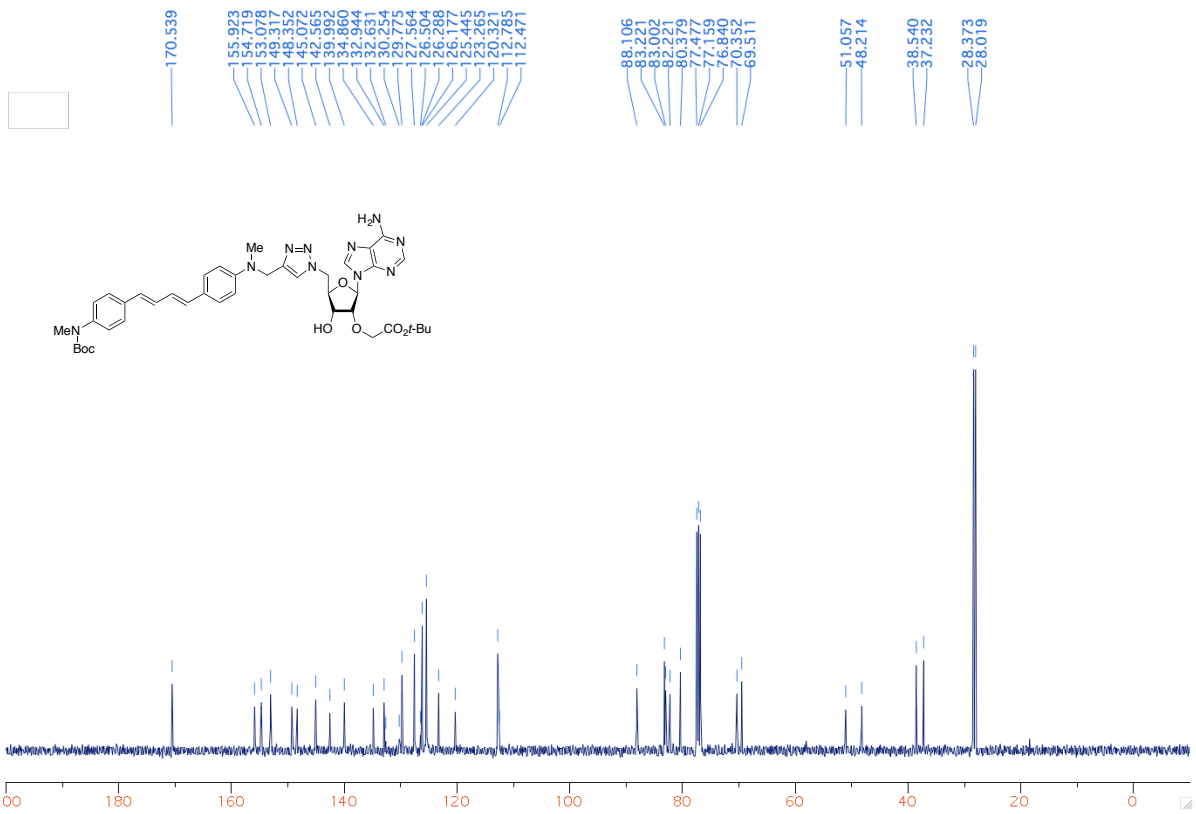
DEPT 135 spectrum of **30** ($CDCl_3$, 100 MHz)



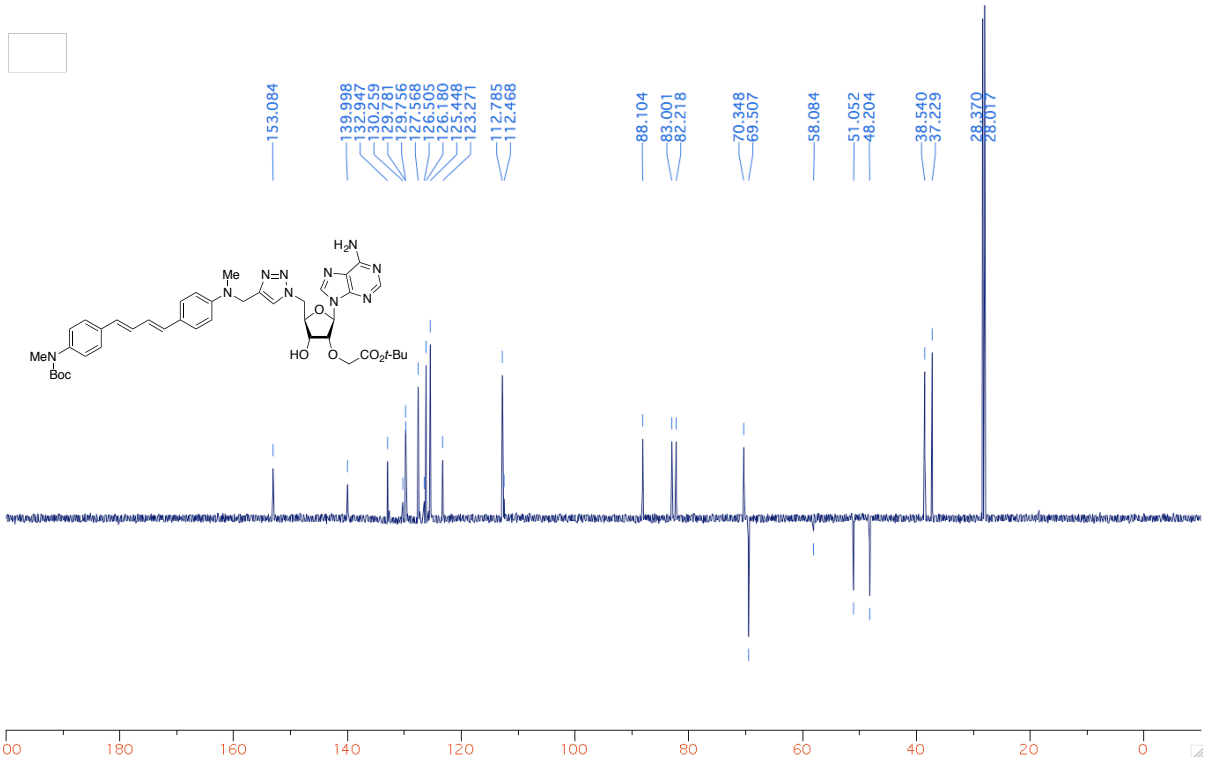
^{1}H-NMR spectrum of **31** ($CDCl_3$, 400 MHz)



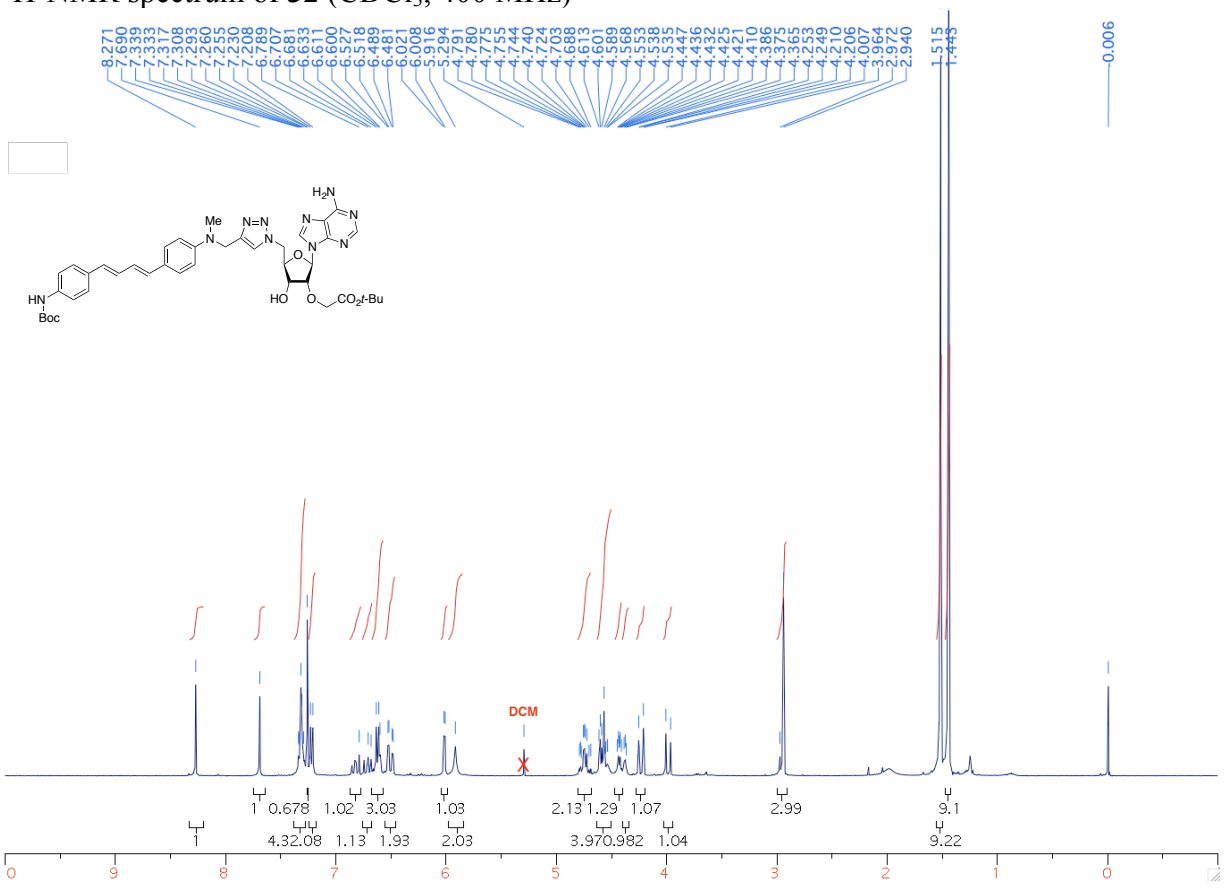
^{13}C-NMR spectrum of **31** ($CDCl_3$, 100 MHz)



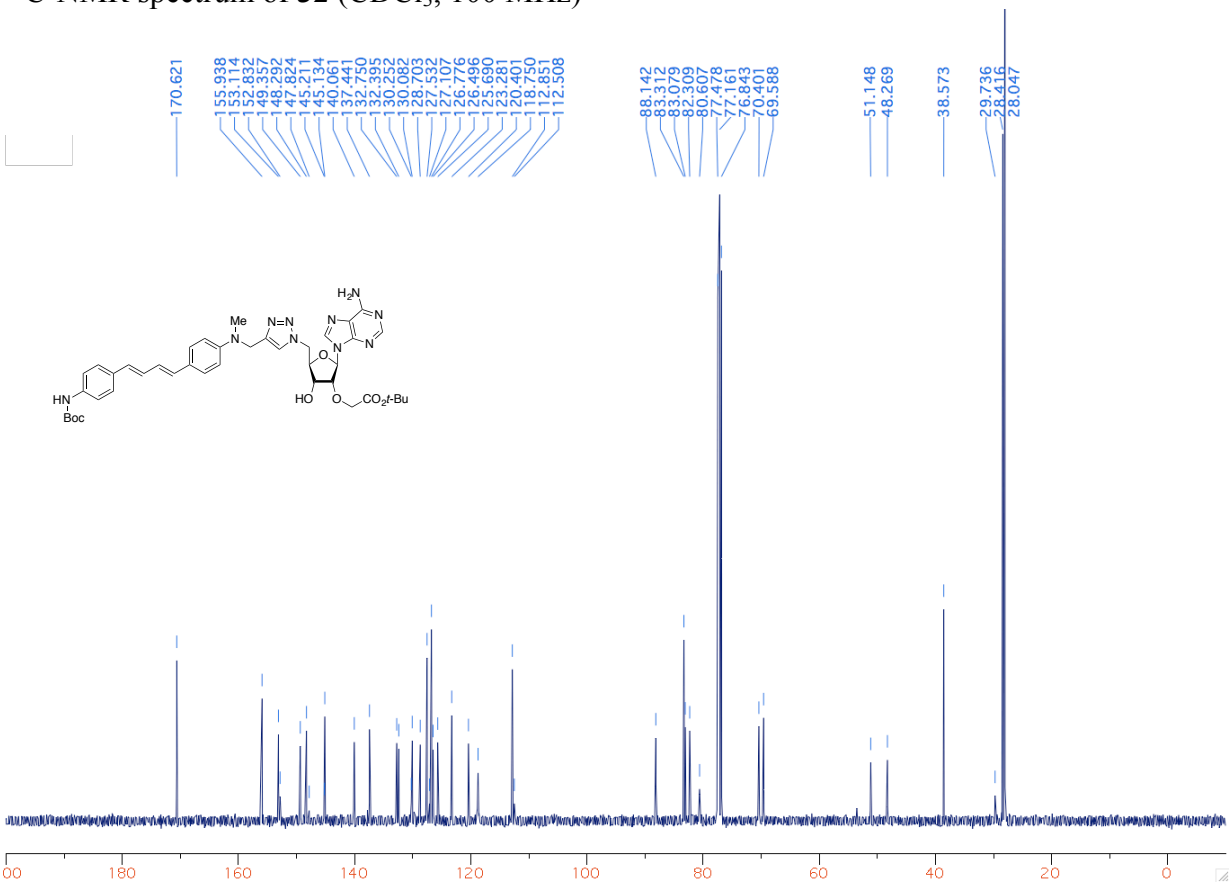
DEPT 135 spectrum of **31** ($CDCl_3$, 100 MHz)



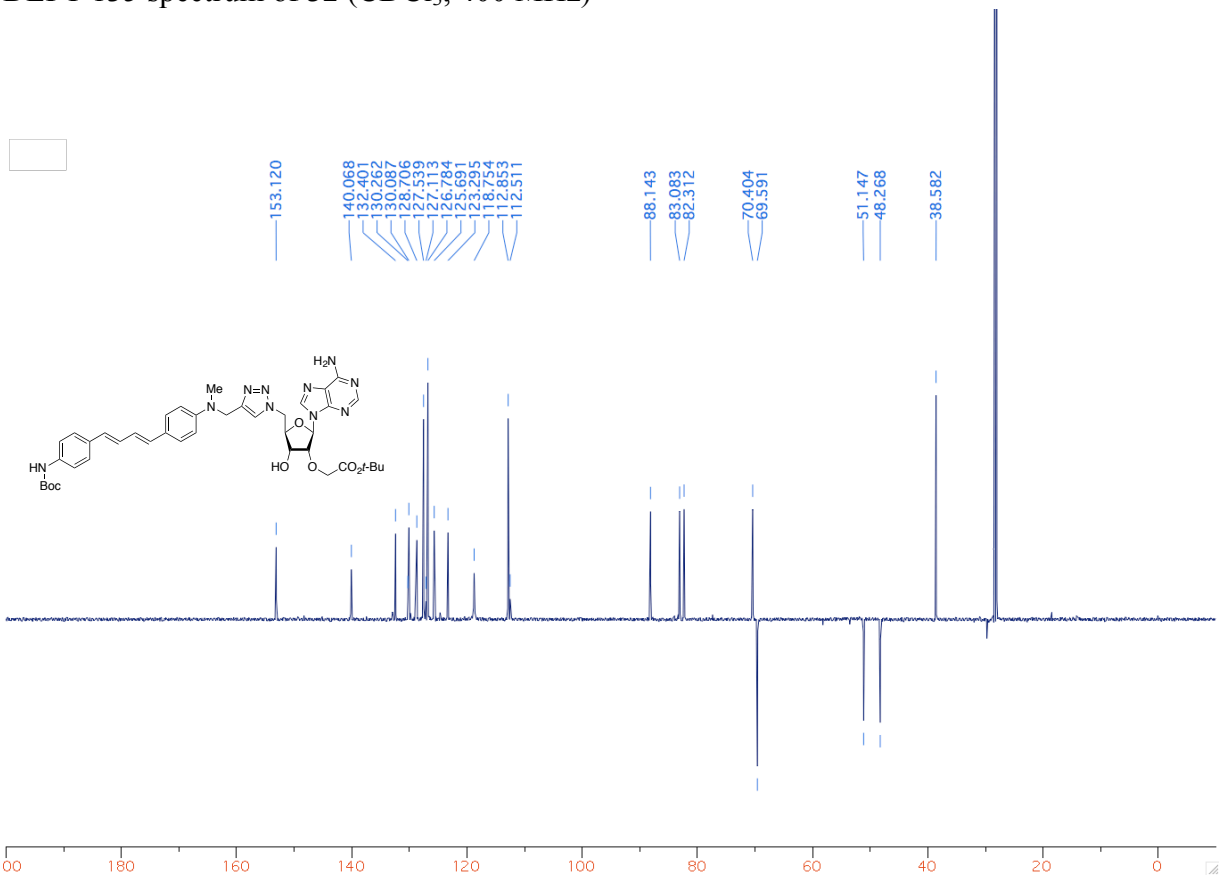
^{1}H-NMR spectrum of **32** ($CDCl_3$, 400 MHz)



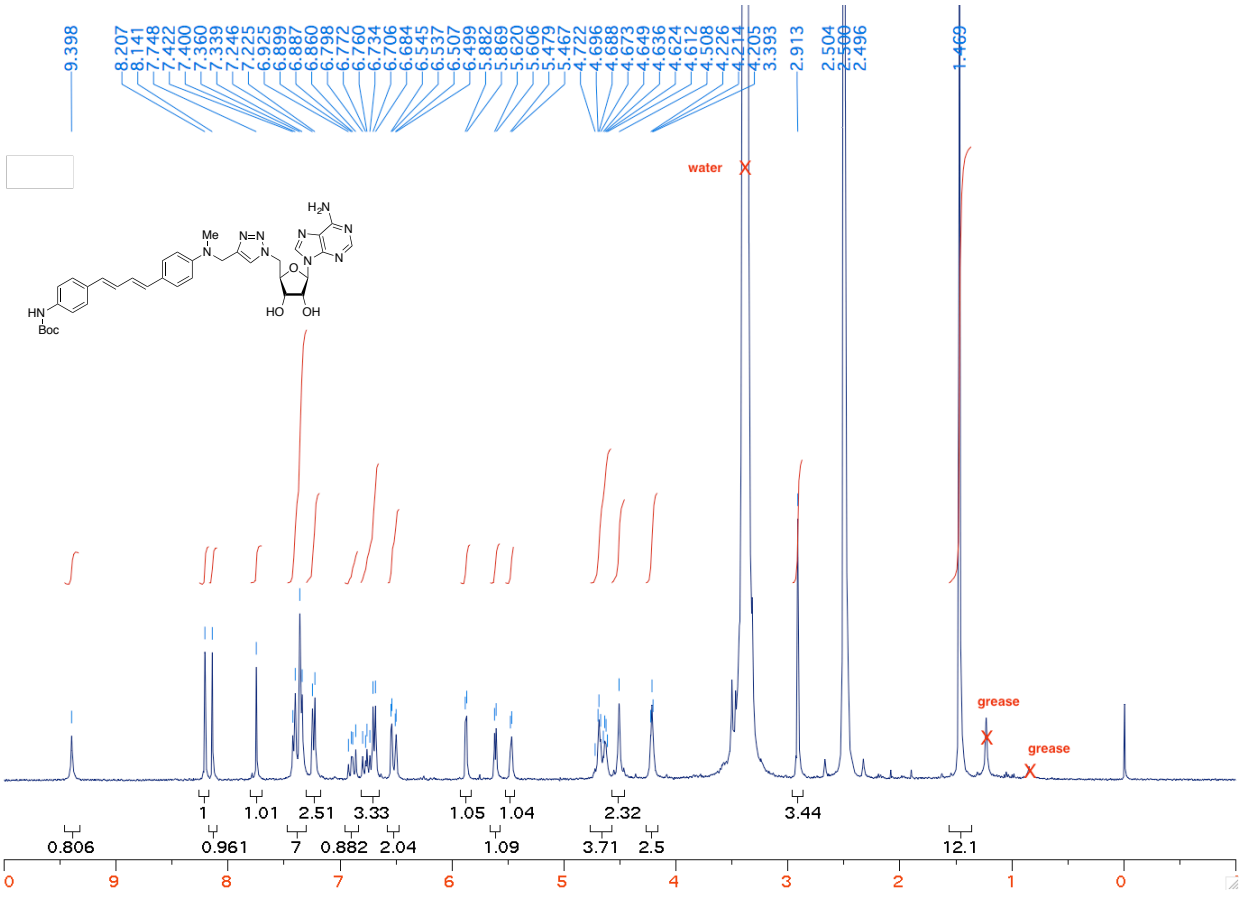
^{13}C-NMR spectrum of **32** ($CDCl_3$, 100 MHz)



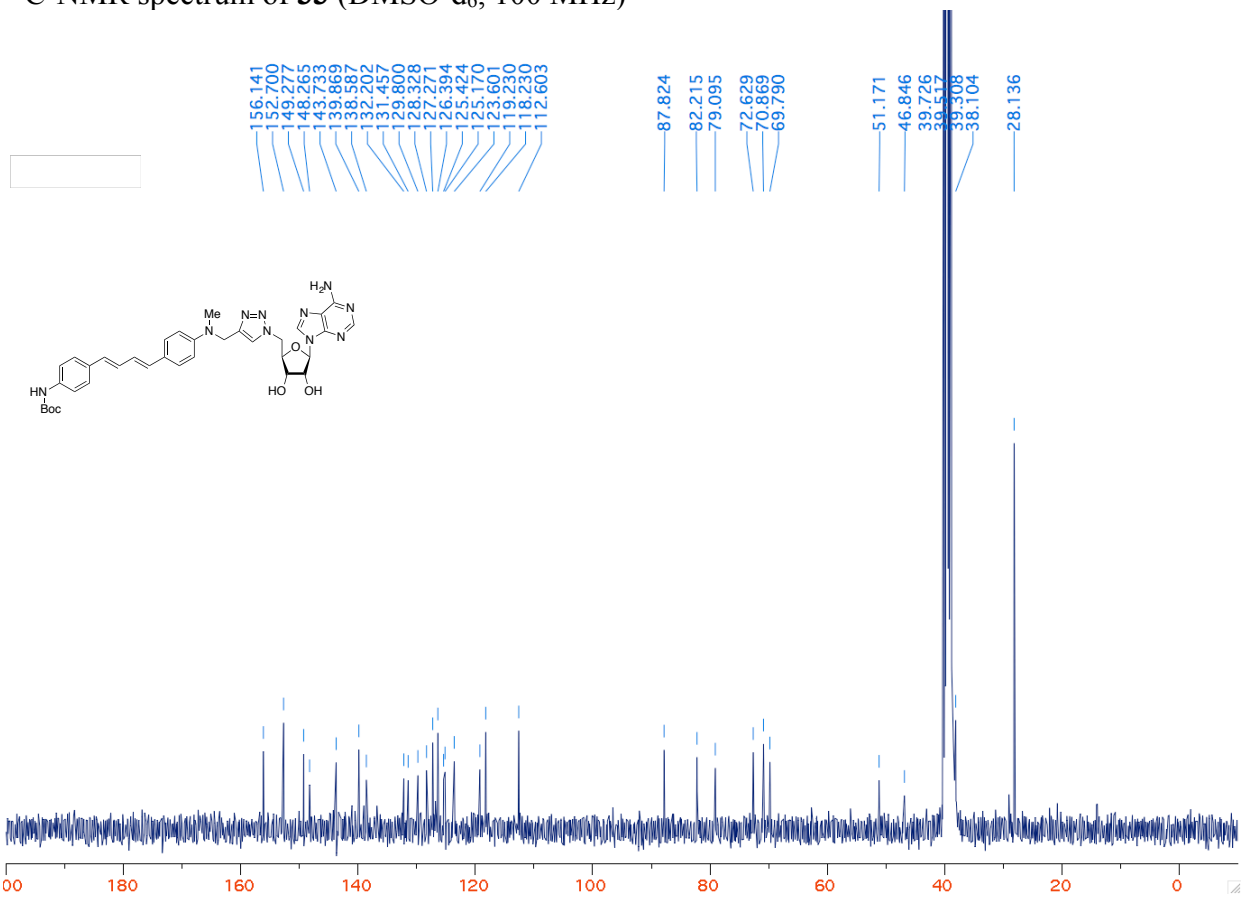
DEPT 135 spectrum of **32** ($CDCl_3$, 400 MHz)



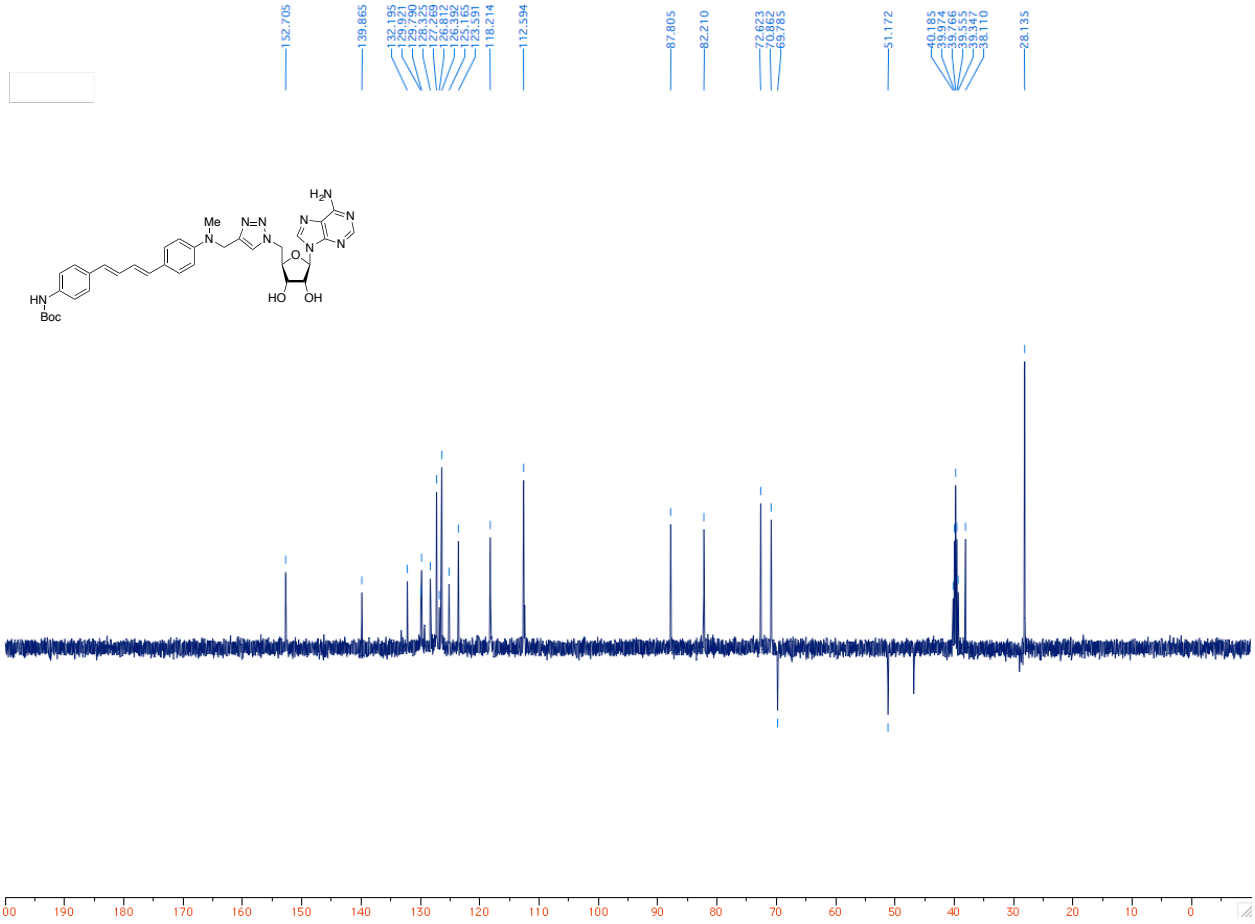
^{1}H-NMR spectrum of **33** (DMSO-d_6, 400 MHz)



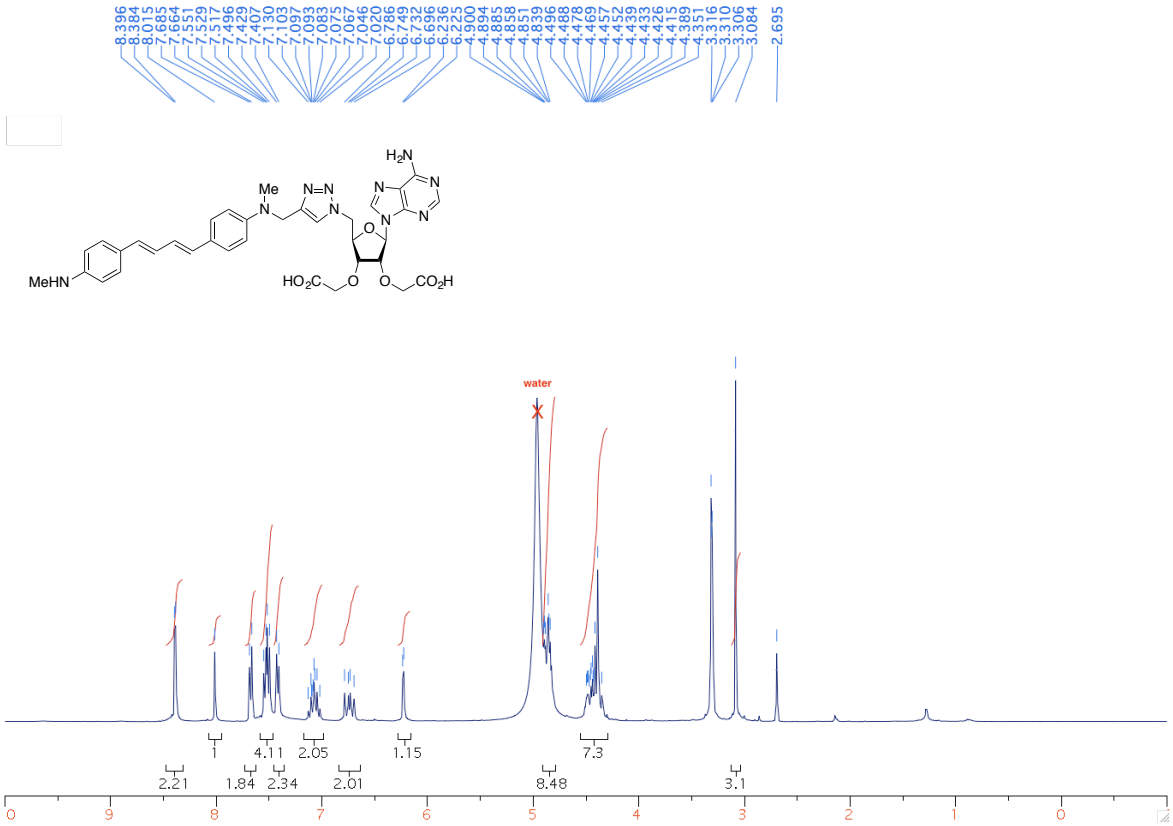
^{13}C-NMR spectrum of **33** (DMSO-d_6, 100 MHz)



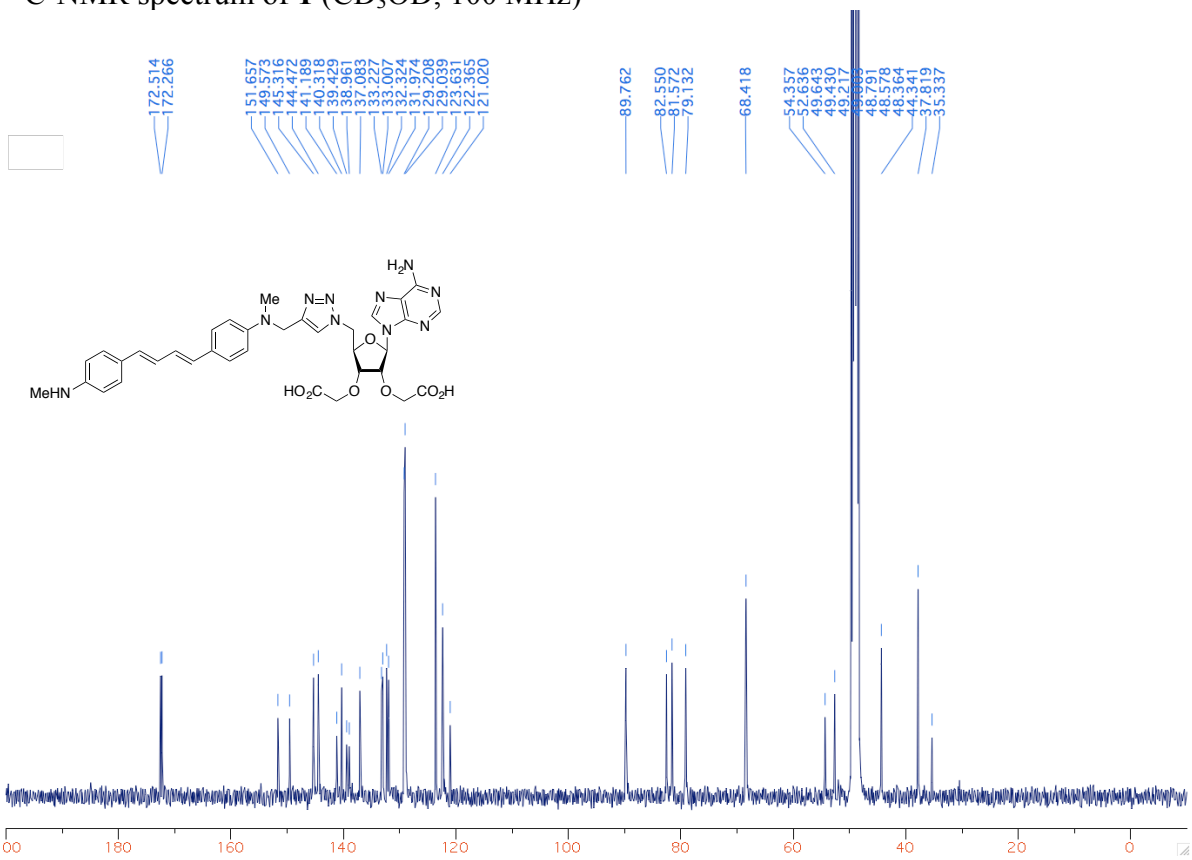
DEPT 135 spectrum of **33** (DMSO-d_6, 100 MHz)



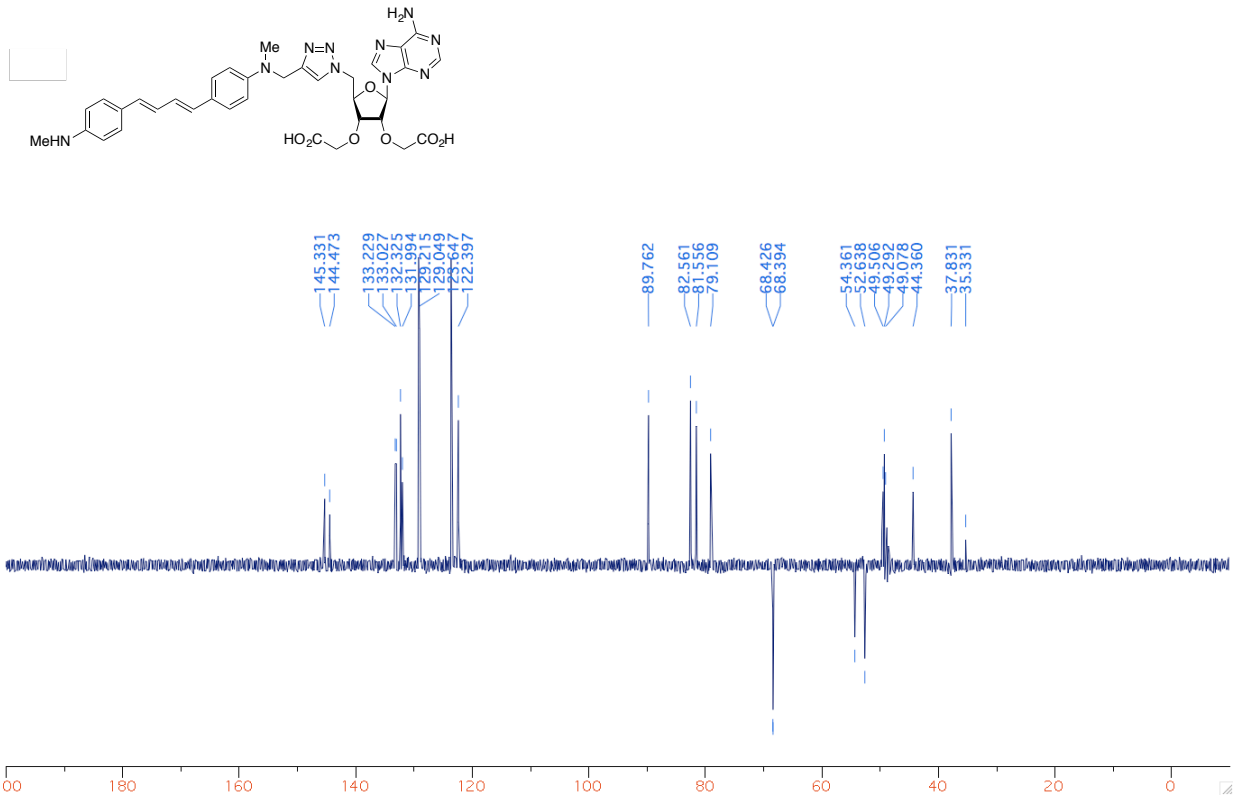
^{1}H-NMR spectrum of **1** (CD_3OD, 400 MHz)



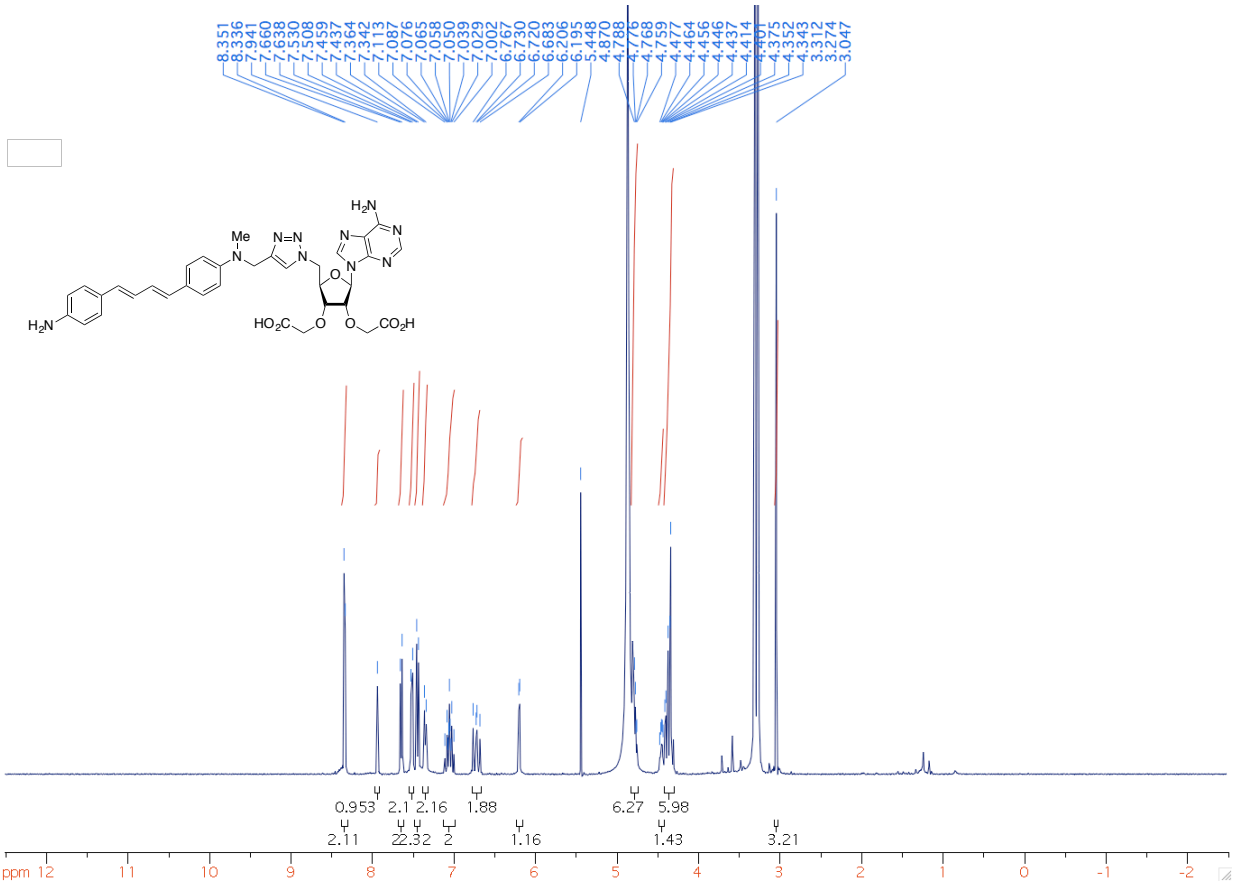
^{13}C-NMR spectrum of **1** (CD_3OD, 100 MHz)



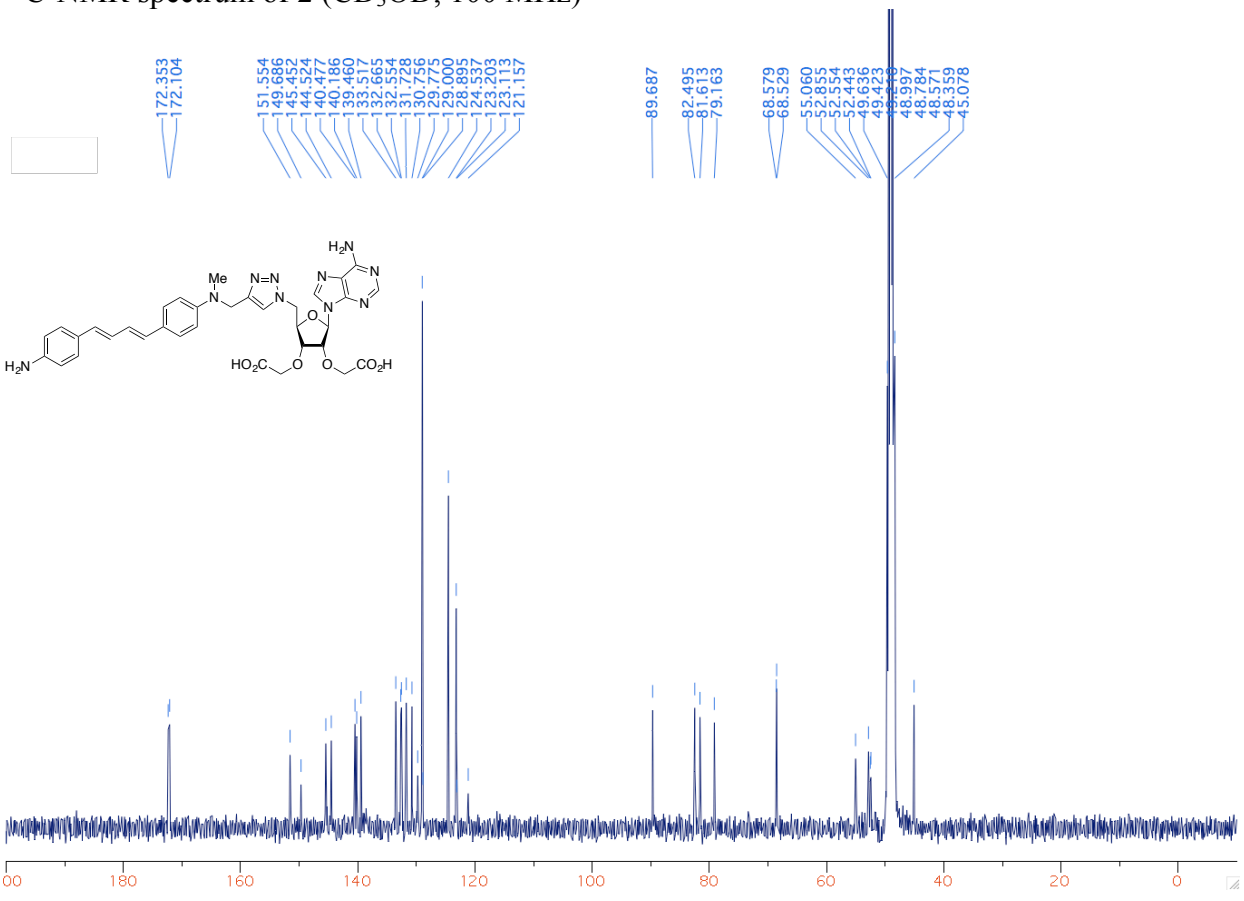
DEPT 135 spectrum of **1** (CD_3OD, 400 MHz)



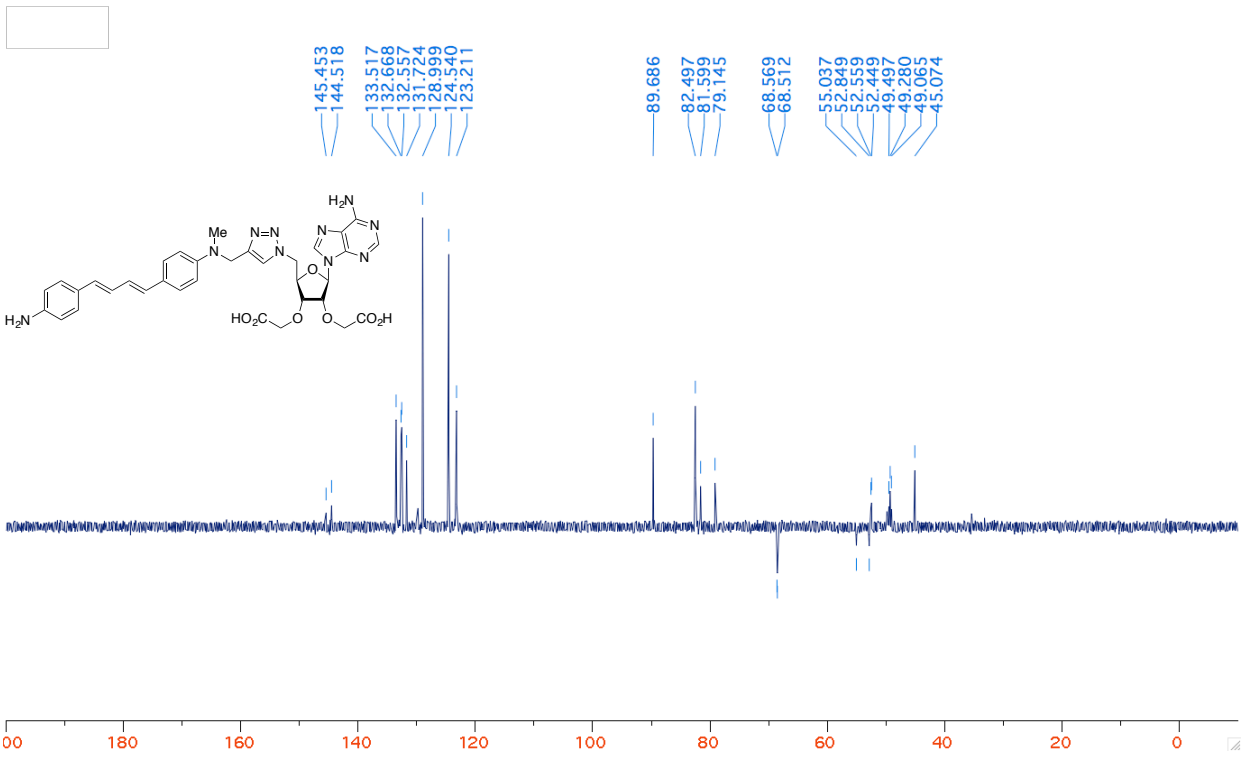
^{1}H-NMR spectrum of **2** (CD_3OD, 400 MHz)



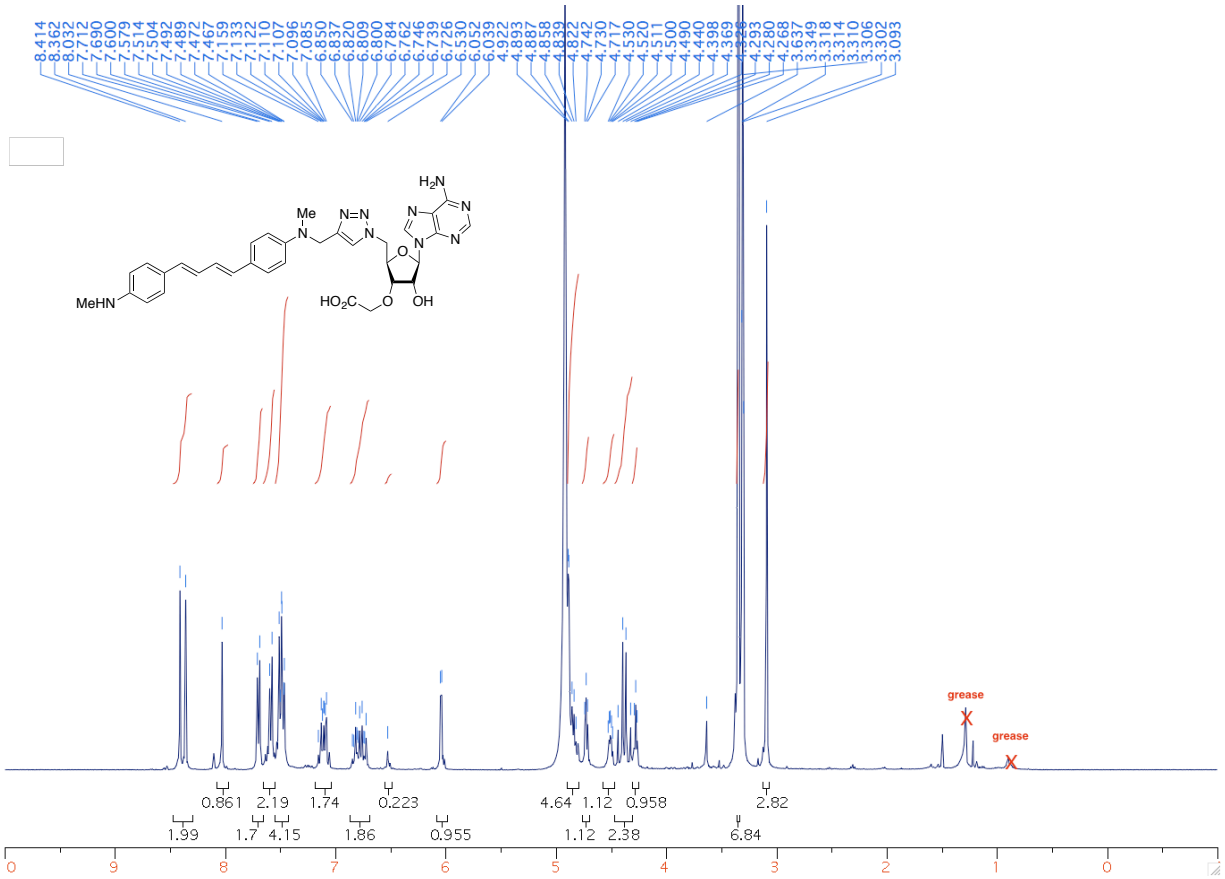
^{13}C-NMR spectrum of **2** (CD_3OD, 100 MHz)



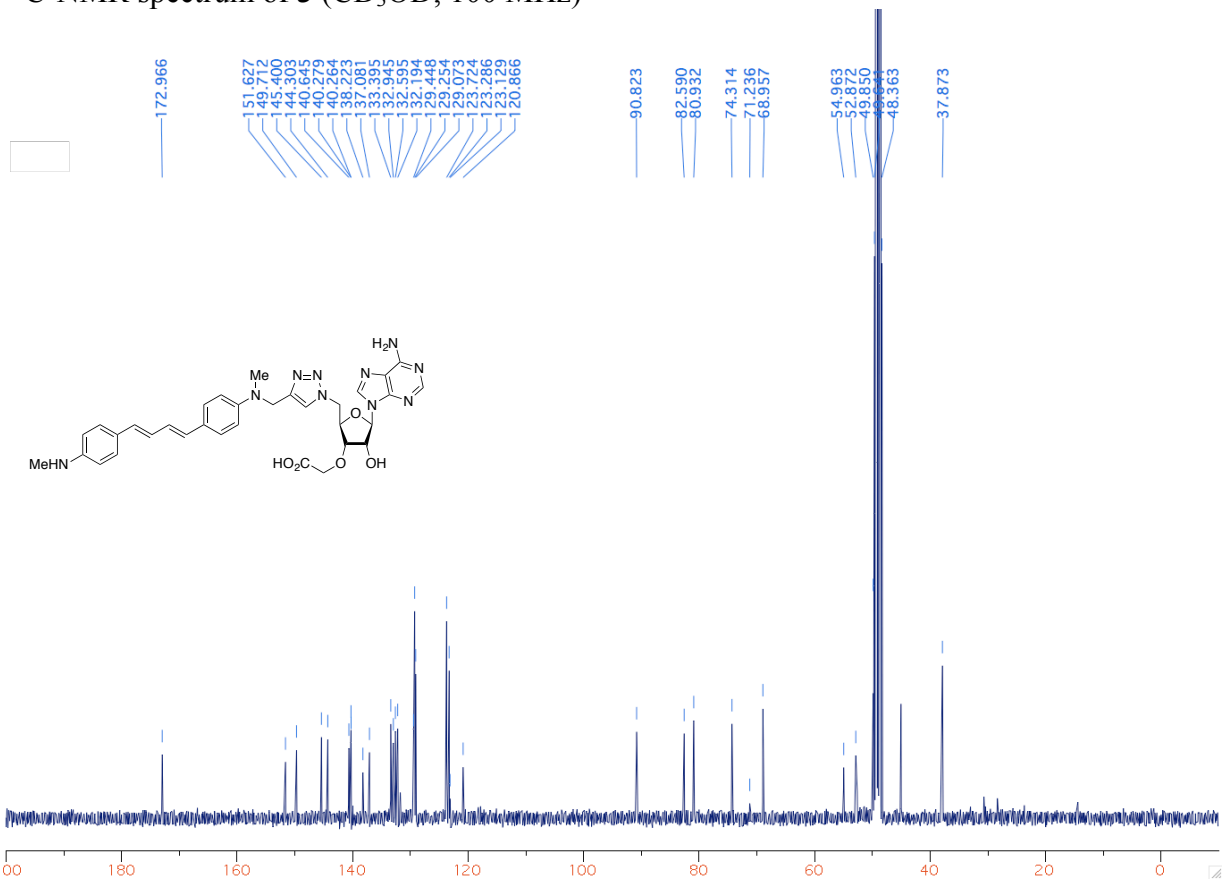
DEPT 135 spectrum of **2** (CD_3OD, 100 MHz)



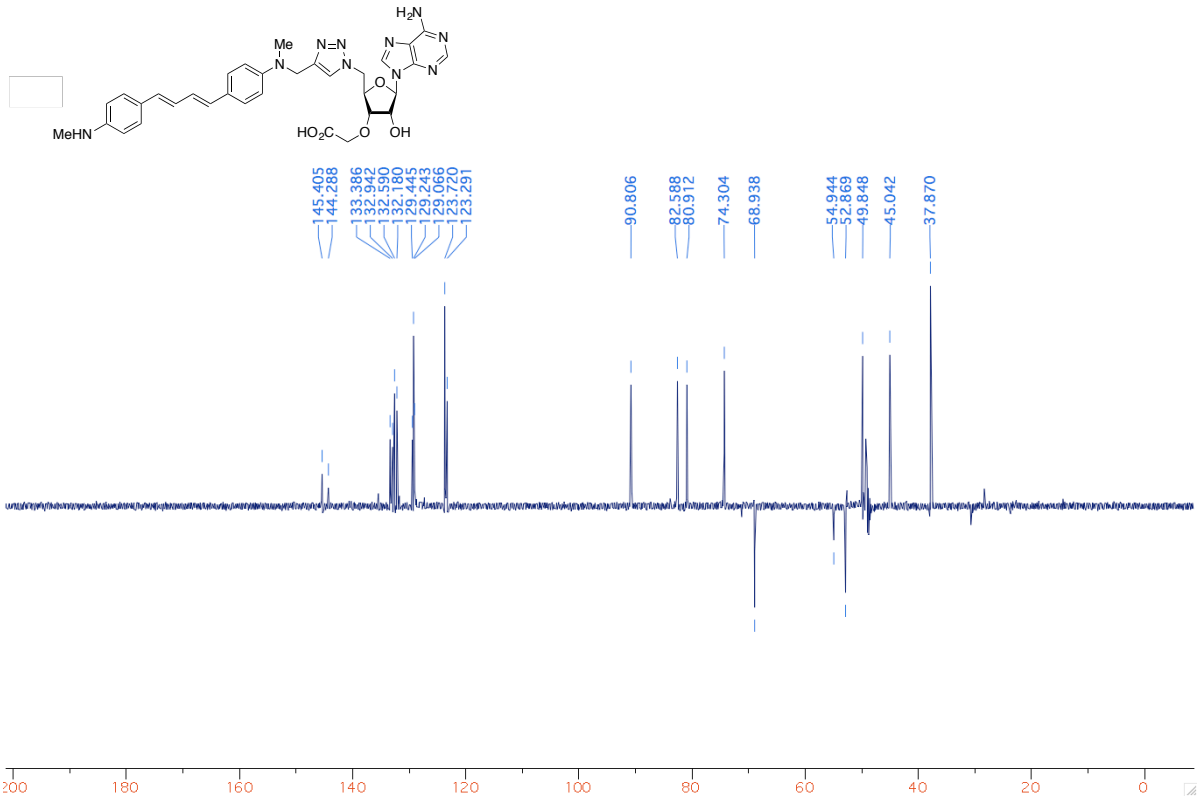
^{1}H-NMR spectrum of **3** (CD_3OD, 400 MHz)



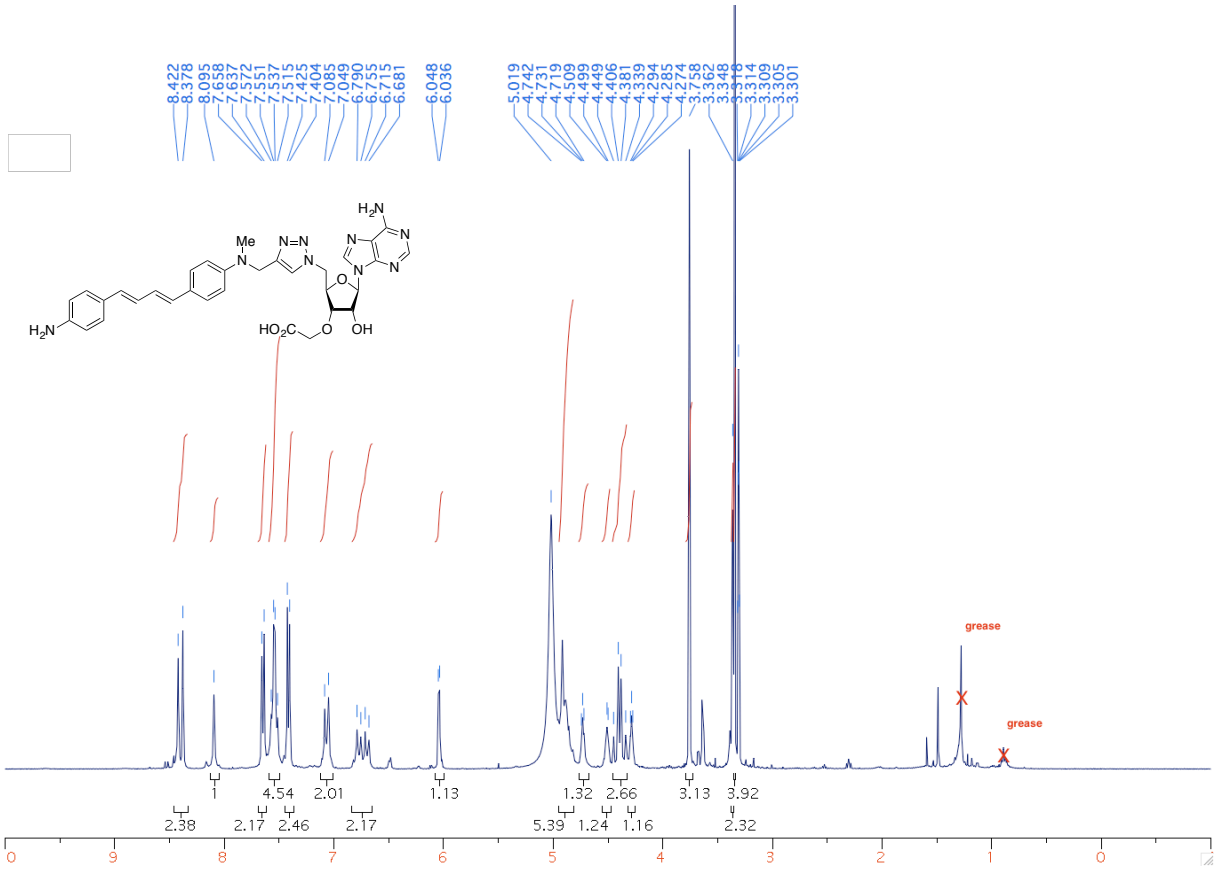
^{13}C-NMR spectrum of **3** (CD_3OD, 100 MHz)



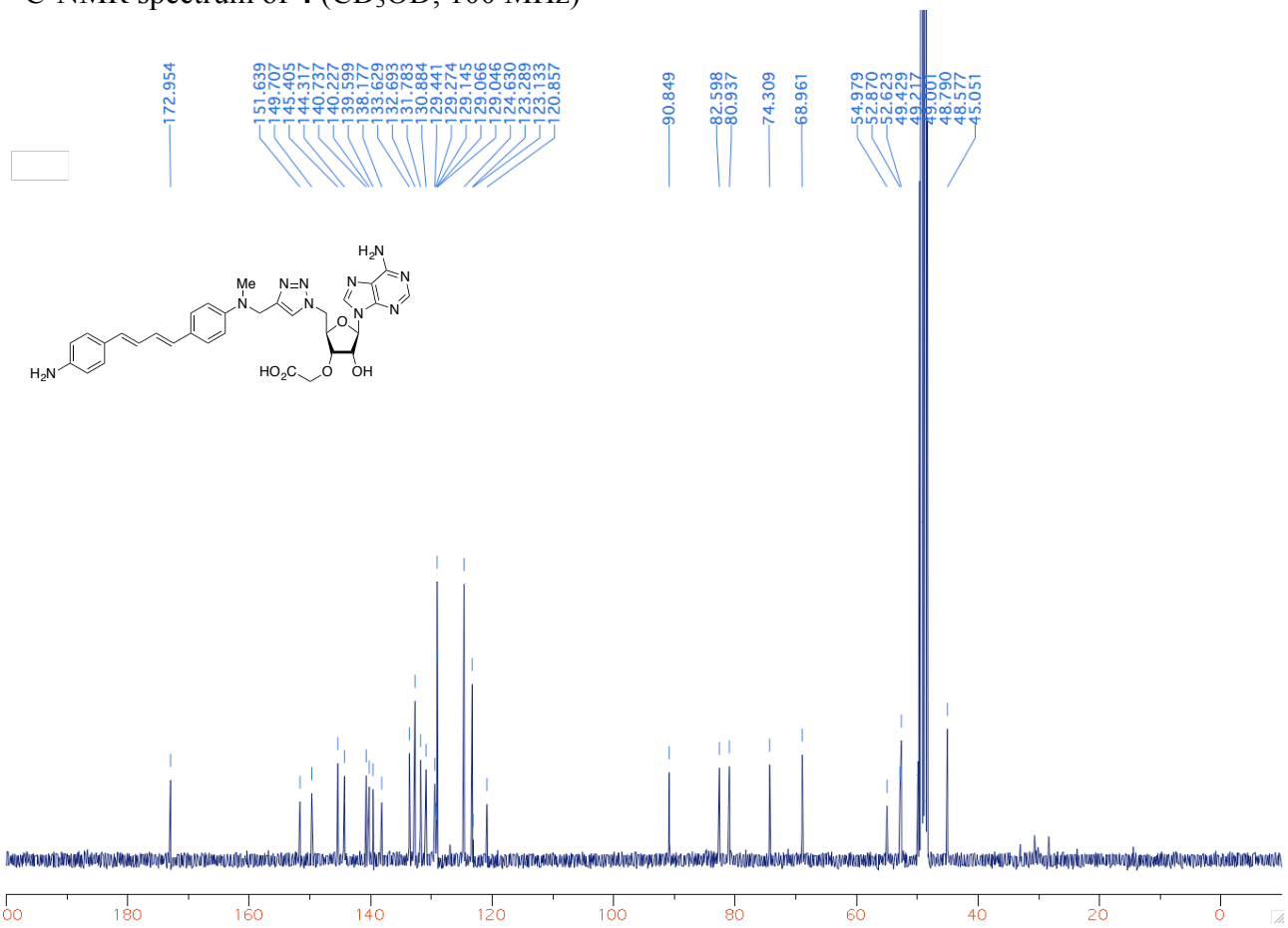
DEPT 135 spectrum of **3** (CD_3OD, 100 MHz)



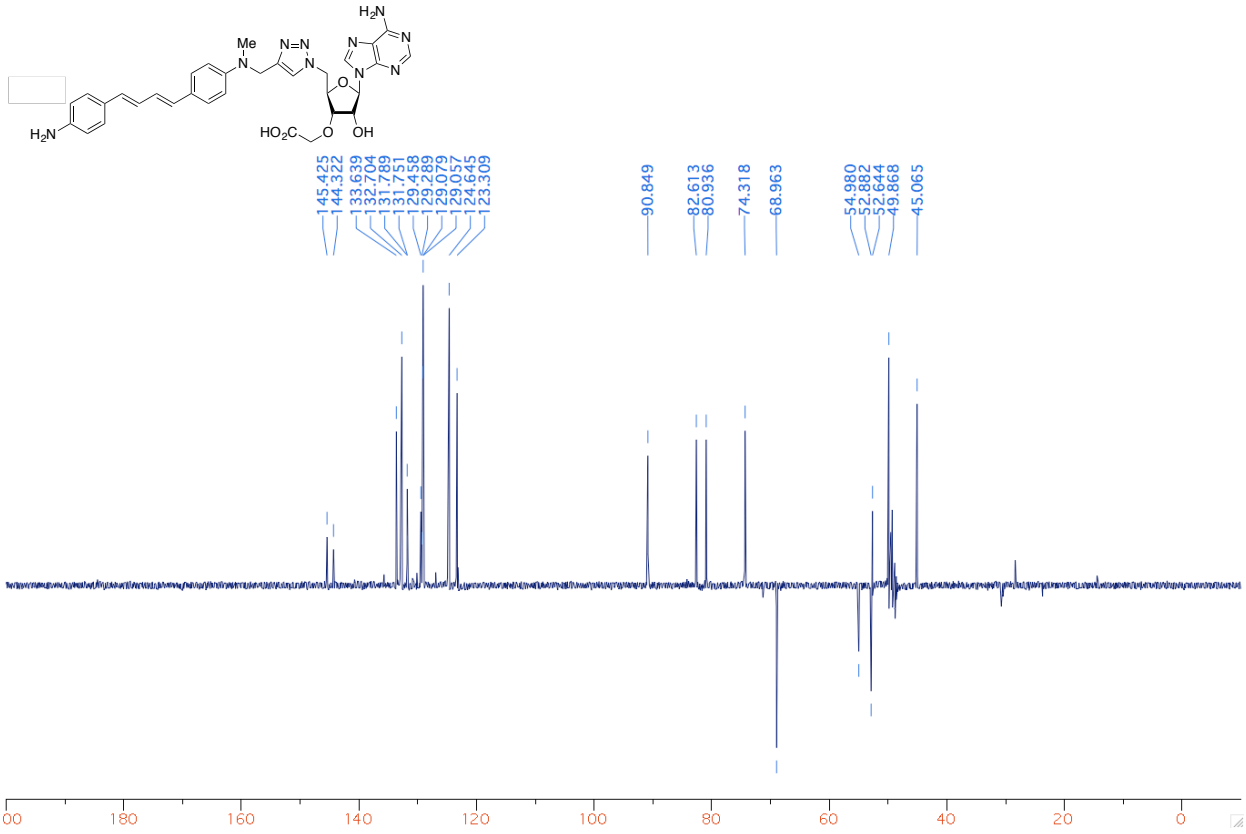
^{1}H-NMR spectrum of **4** (CD_3OD, 400 MHz)



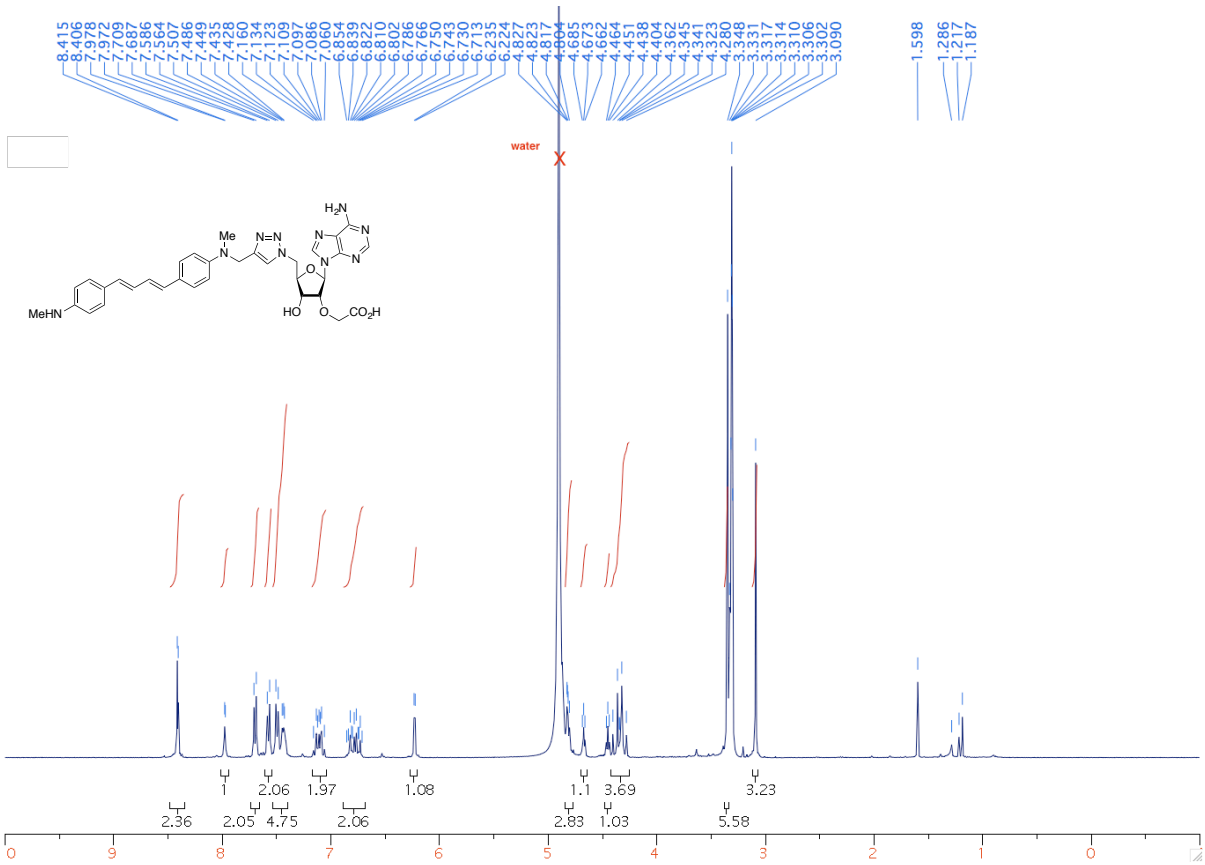
^{13}C-NMR spectrum of **4** (CD_3OD, 100 MHz)



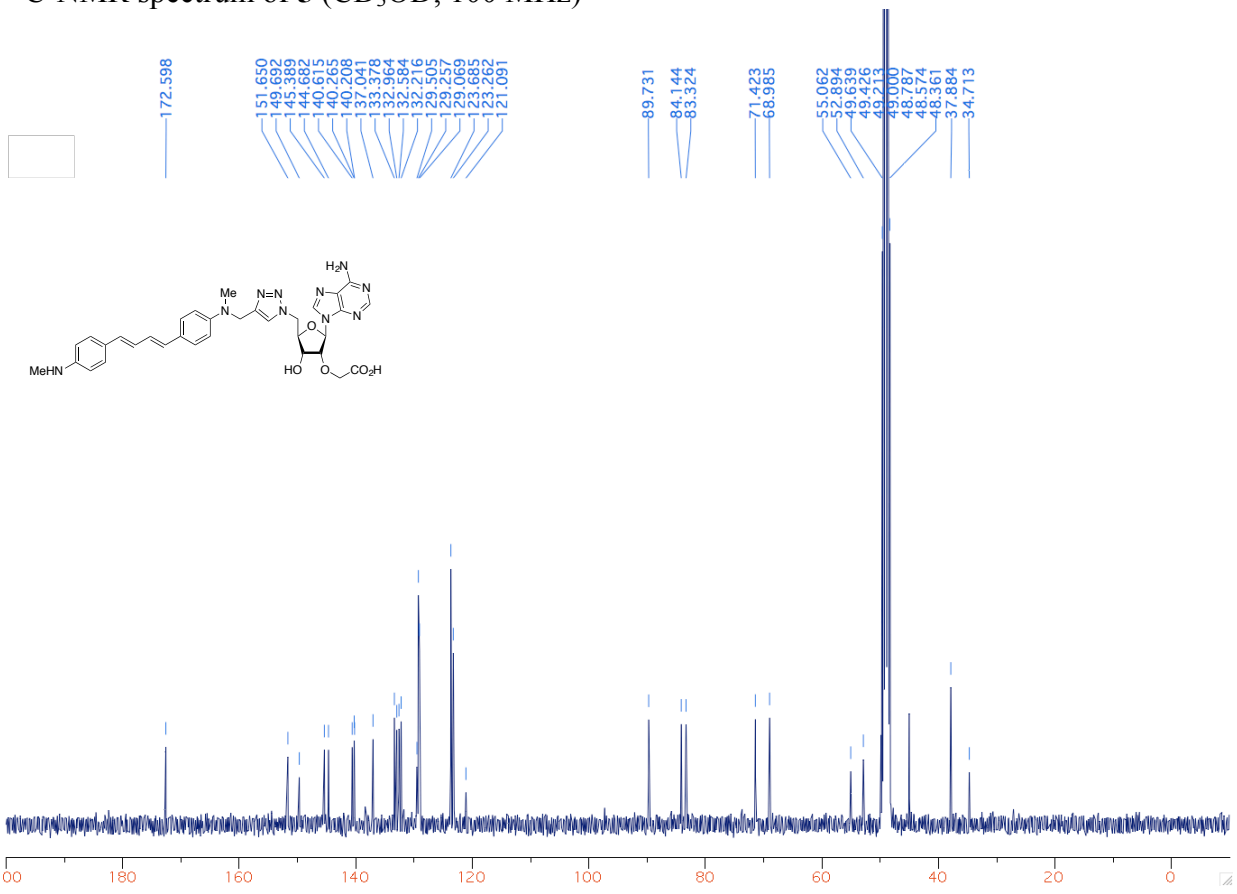
DEPT 135 spectrum of **4** (CD_3OD, 100 MHz)



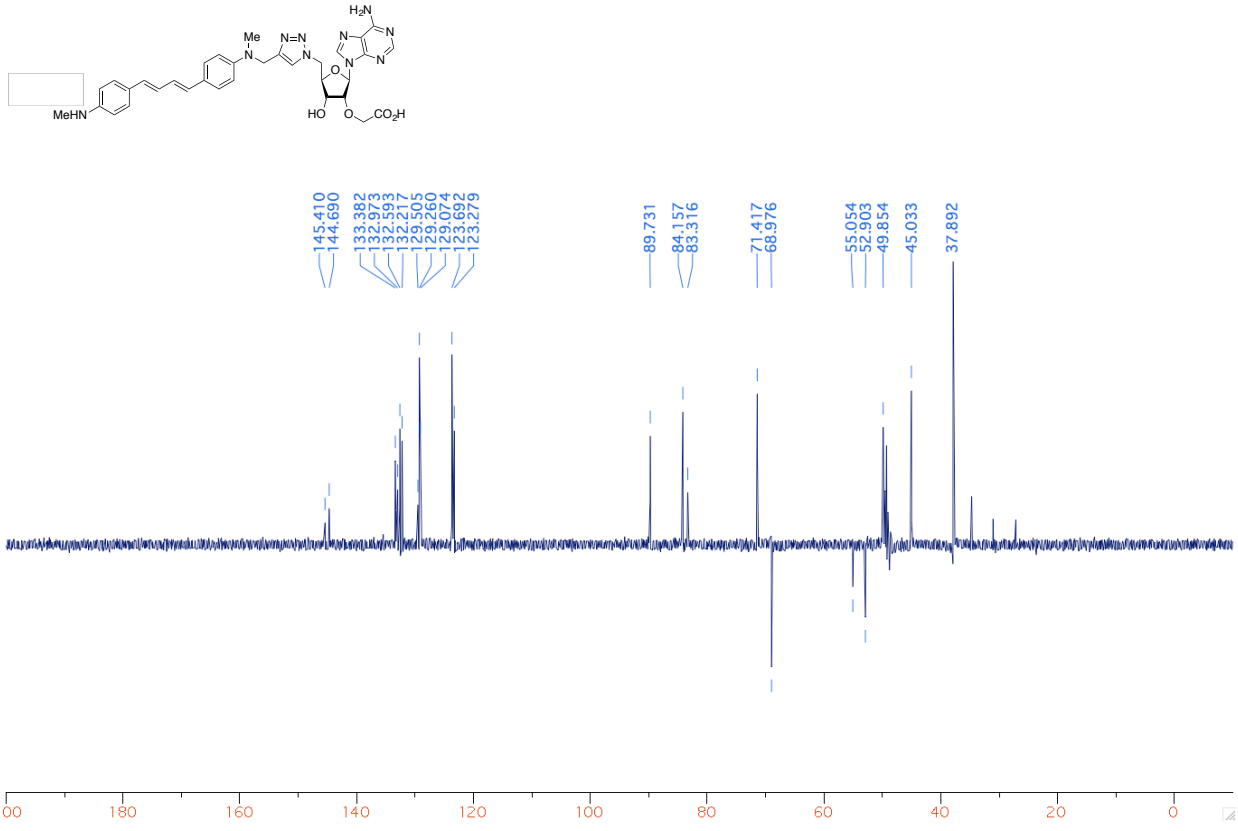
^{1}H-NMR spectrum of **5** (CD_3OD, 400 MHz)



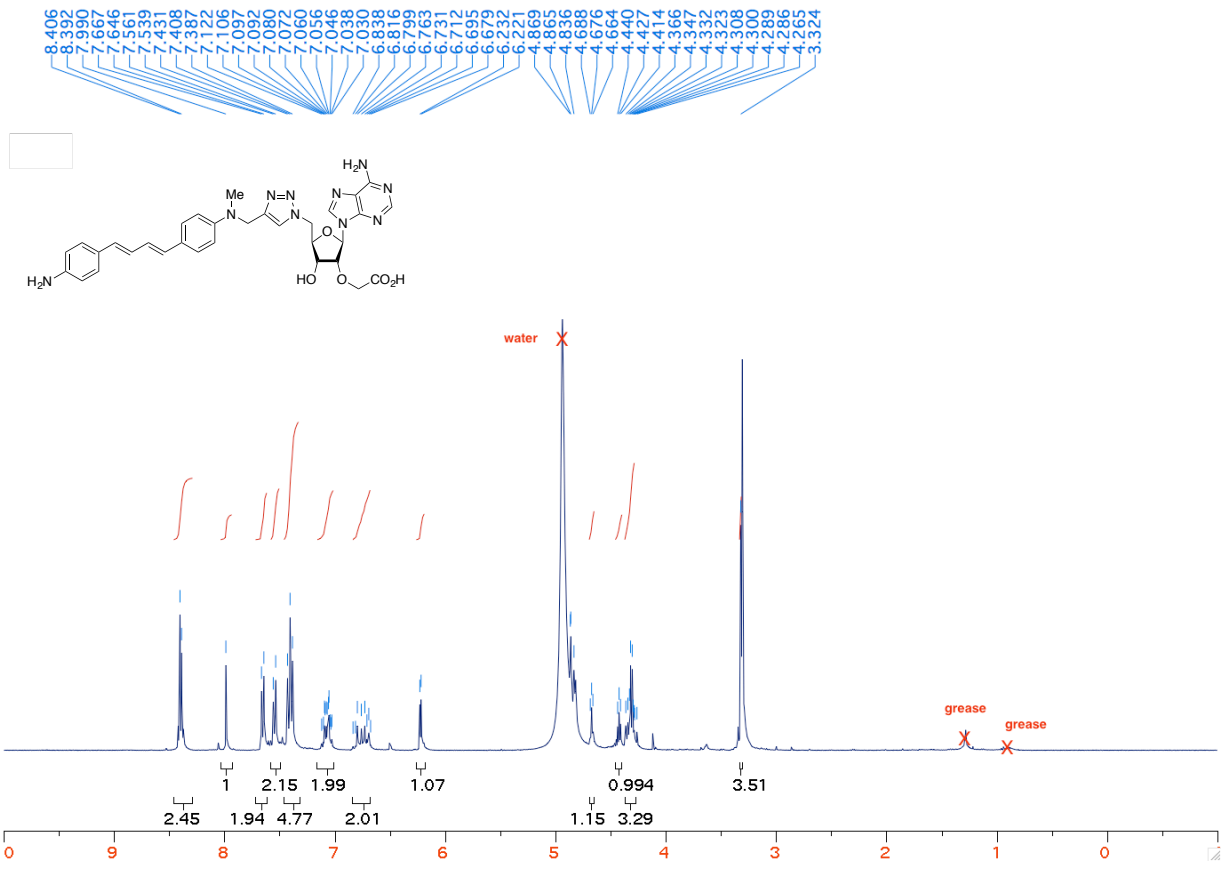
^{13}C-NMR spectrum of **5** (CD_3OD, 100 MHz)



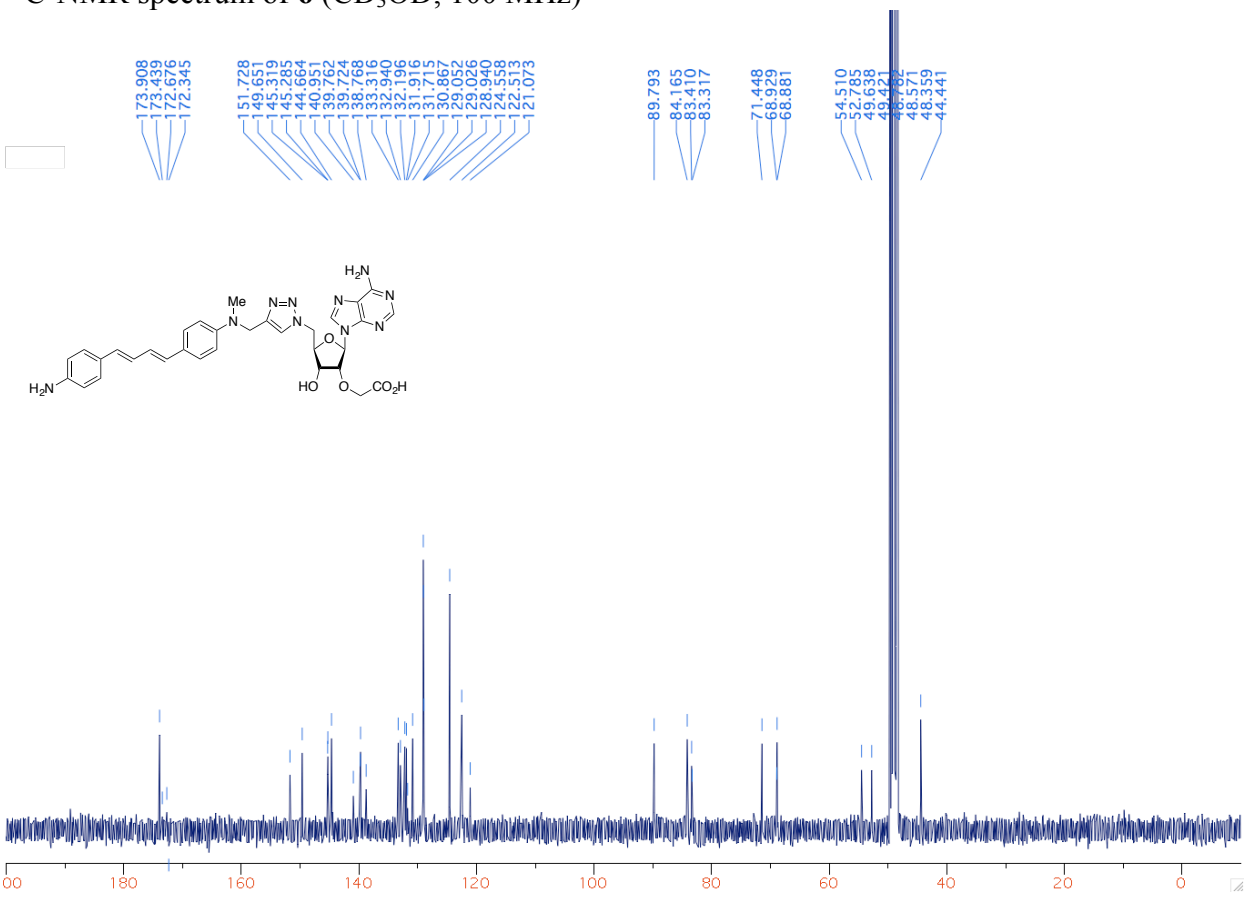
DEPT 135 spectrum of **5** (CD_3OD, 100 MHz)



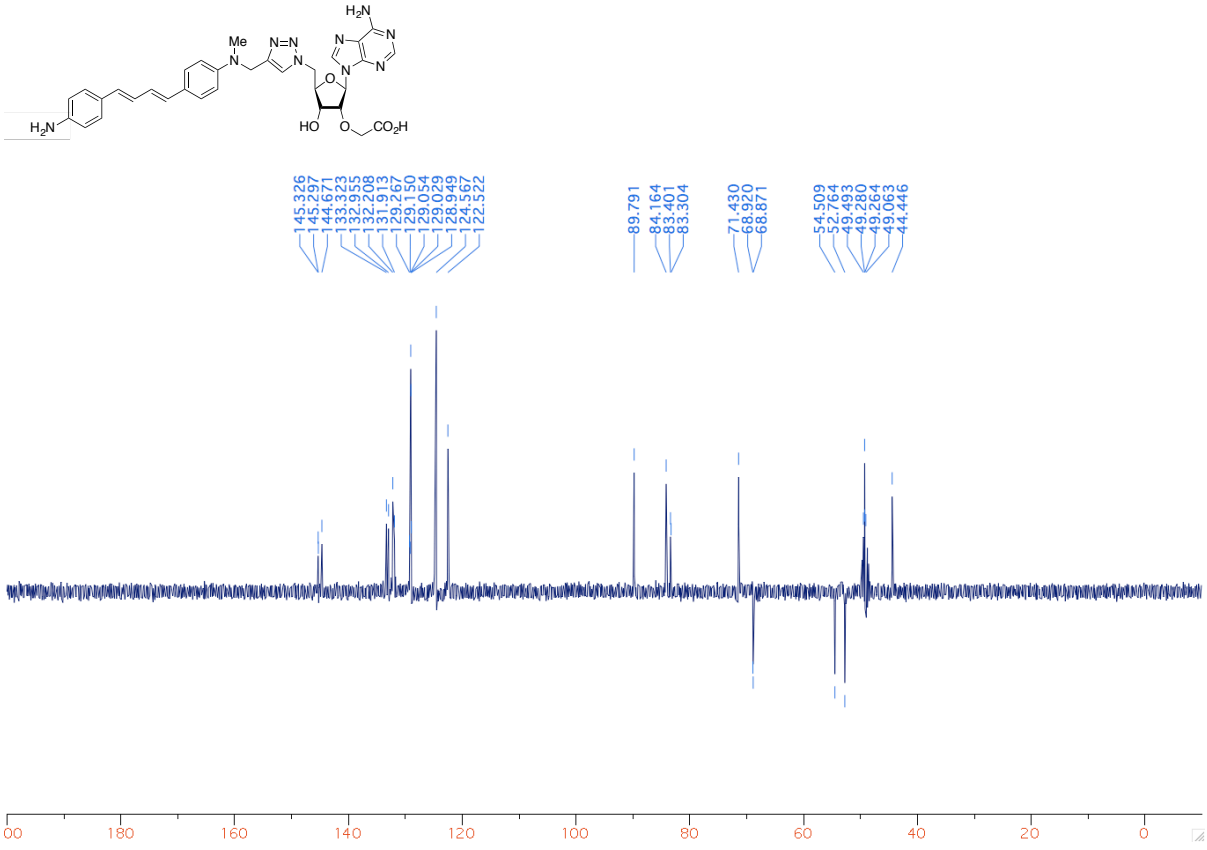
^{1}H-NMR spectrum of **6** (CD_3OD, 400 MHz)



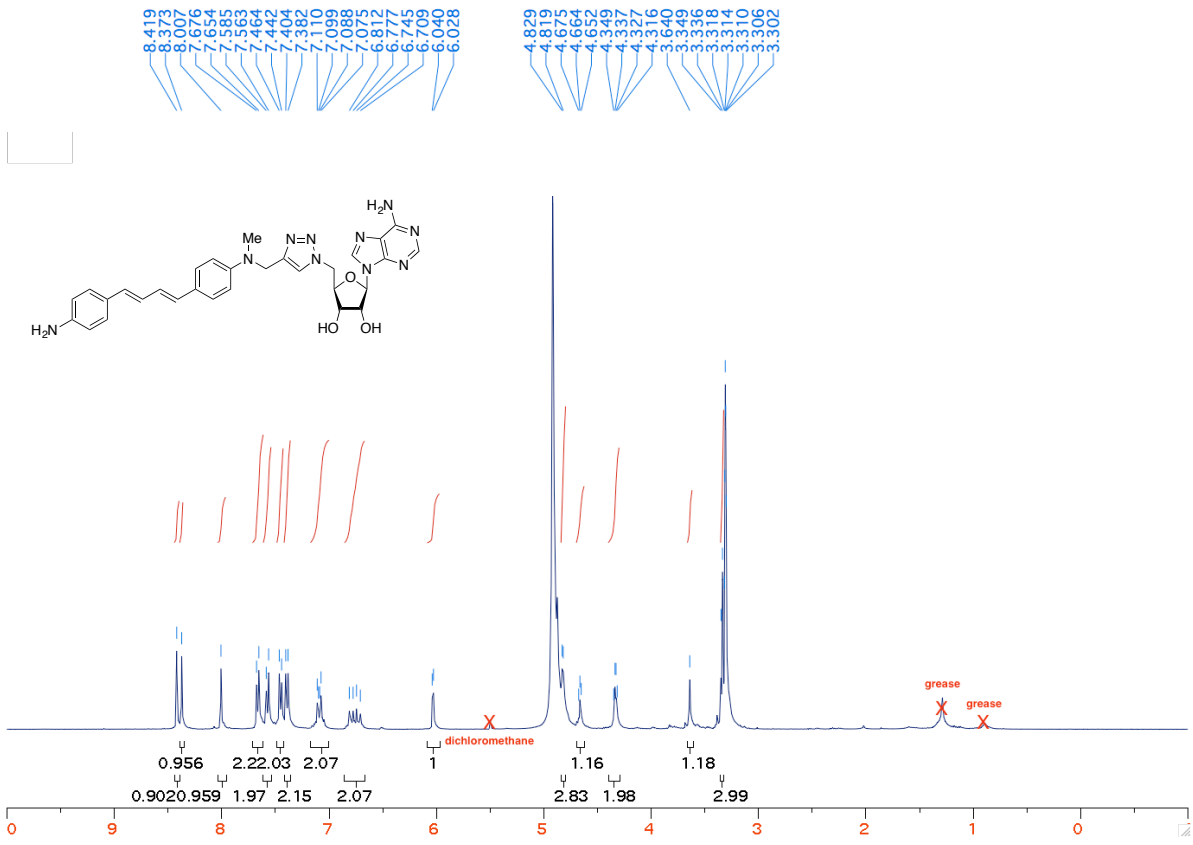
^{13}C-NMR spectrum of **6** (CD_3OD, 100 MHz)



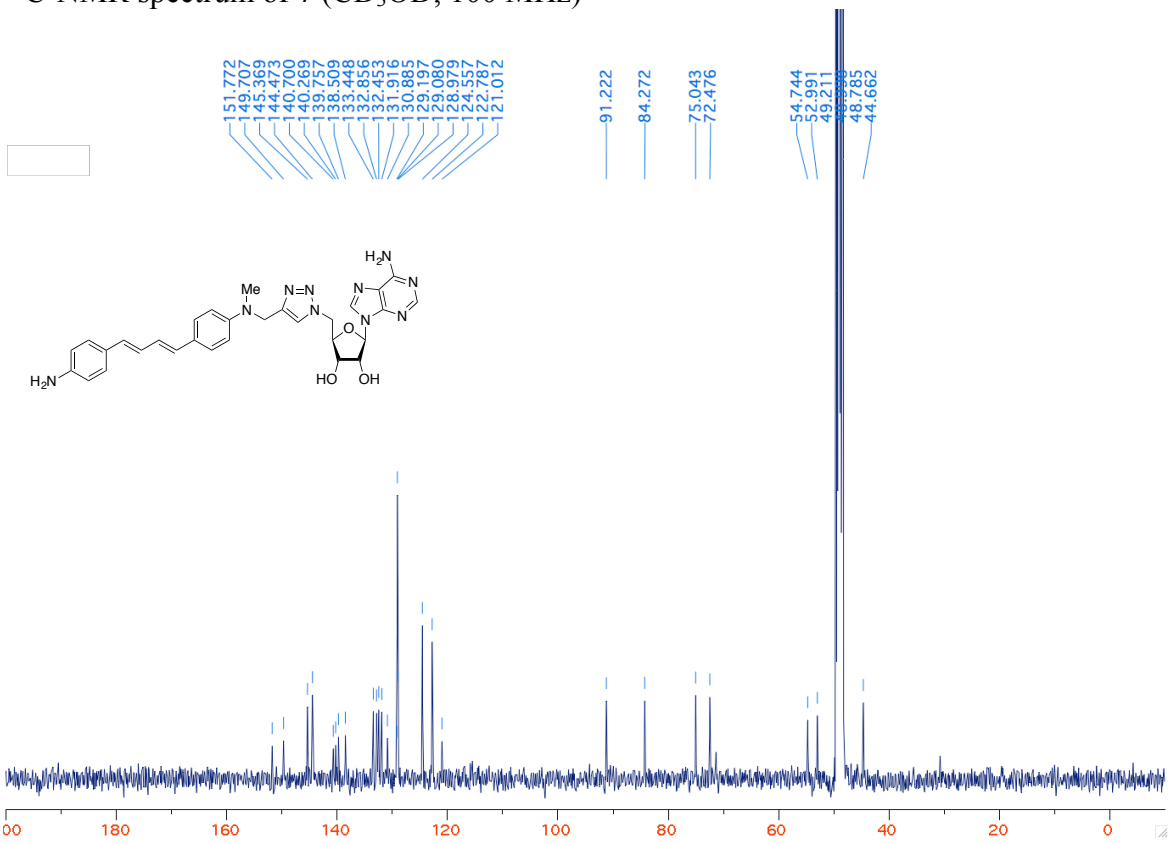
DEPT 135 spectrum of **6** (CD_3OD, 100 MHz)



^{1}H-NMR spectrum of **7** (CD_3OD, 400 MHz)



^{13}C-NMR spectrum of **7** (CD_3OD, 100 MHz)



DEPT 135 spectrum of **7** (CD_3OD, 100 MHz)