

Multifunctional multivalency: a focused library of polymeric cholera toxin antagonists

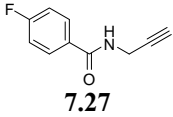
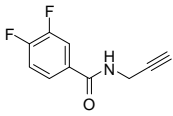
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ELISA Assay for Inhibition of Heat-labile Toxin (LT)

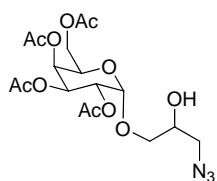
To PVC microtiter plates (Gibco BRL Inc.) solution of coating compound **9** (1 µg/mL, 100 µL/well) in PBS buffer was added and incubated at room temperature overnight, then washed (6×) with PBST (0.05 % Tween 20 in phosphate buffer saline, PBS). An inhibitor at decreasing concentrations (starting at 20 mg/mL, dilution factor 3.16; 50 µL/well) was mixed with LT (Sigma, 2 ng/mL) and the mixtures were applied to the plates. After incubation at room temperature for 2 h the plates were washed (6×) with PBST. Rabbit anti-LT polyclonal antibody (Abcam Inc., dilution 1:10000, 100 µL/well) was added, incubated at room temperature for 1 h then the plates were washed (6×) with PBST. Goat anti-rabbit IgG conjugated to horseradish peroxidase (Kirkegaard and Rerry Laboratories, dilution 1:5000, 100 µL/well) was added, incubated at room temperature for 30 min then the plates were washed (6×) with PBST. 3,3',5,5'-Tetramethylbenzidine solution (TMB purchased from KPL, 100 µL/well) was added. After 15 min incubation the reaction was stopped by addition of H₃PO₄ (100 µL/well) and absorbance was measured at 450 nm.

Table 1S. Inhibitory activities of heterobifunctional polymers against LT.

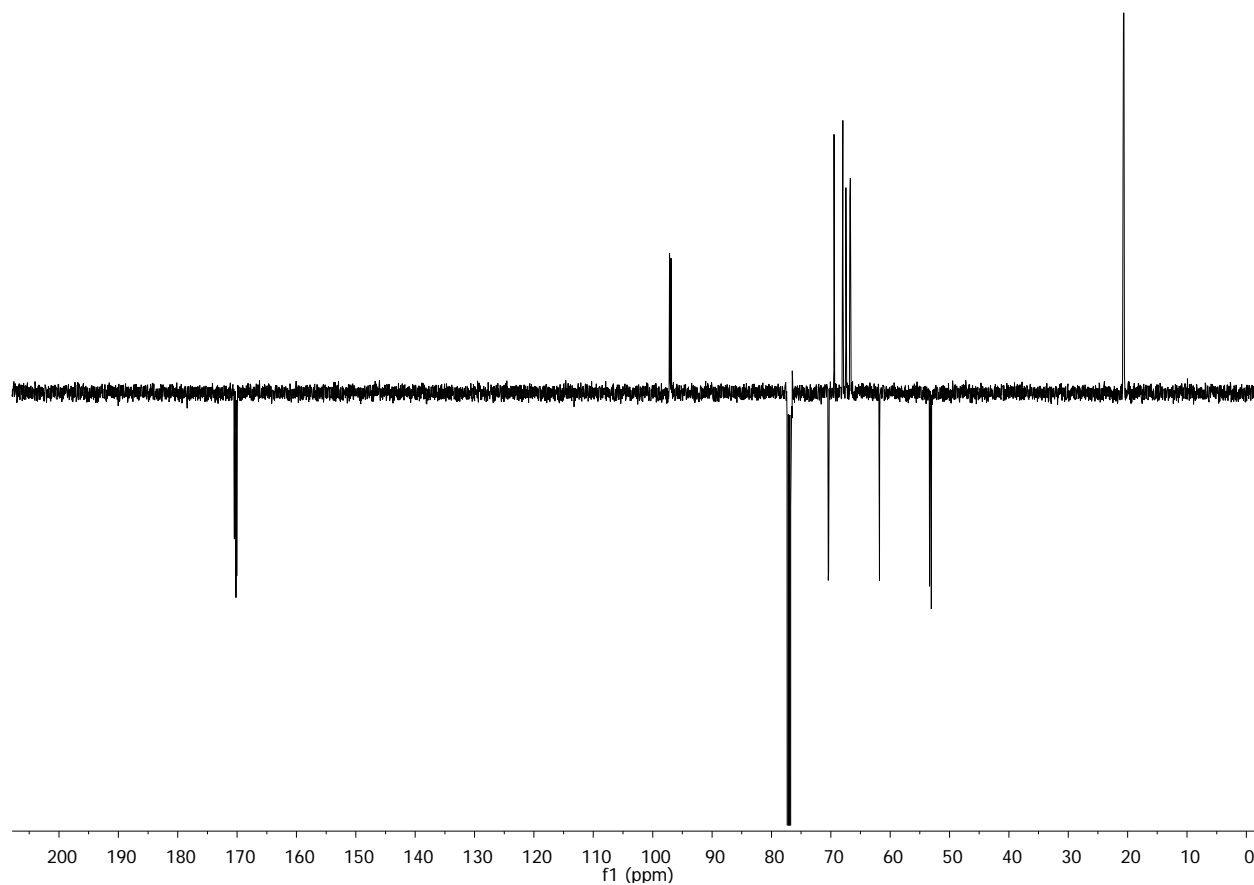
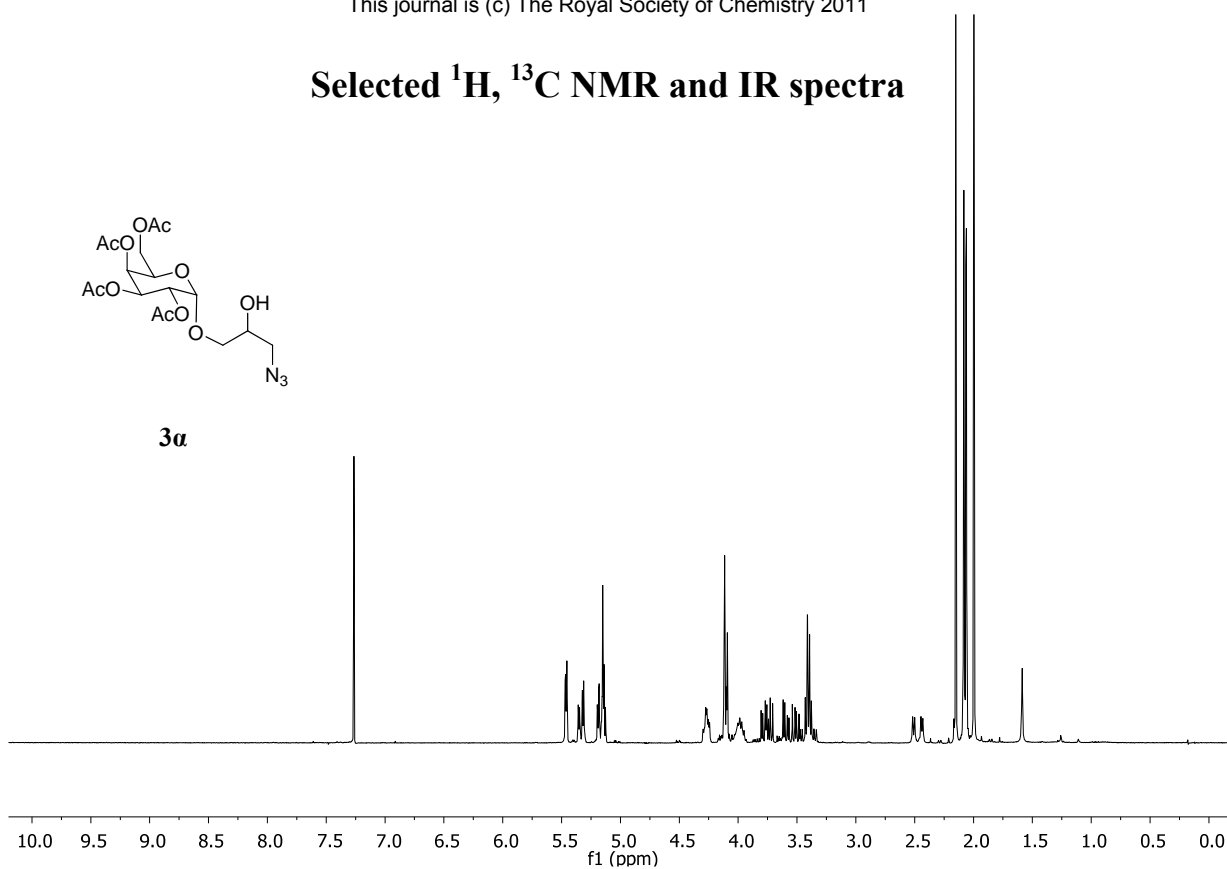
Alkyne derivative 7.n	α-Gal polymer	IC ₅₀ , µM ^a	β-Gal polymer	IC ₅₀ , µM ^a
 7.27	5α27 (3%)	0.00099 (h ^b =0.56)	5β27 (3%)	0.0011 (h ^b =0.8)
	5α27 (5%)	0.00007 (h=0.54)	5β27 (5%)	0.0004 (h=0.95)
	5α27 (9%)	0.00015 (h=1.04)	5β27 (9%)	0.00023 (h=1.3)
	6α27 (6.6%)	0.0006 (h=0.76)	6β27 (6.6%)	0.0017 (h=1.0)
 7.28	5α28 (5%)	0.0029 (h=1.3)	5β28 (5%)	0.0034 (h=1.1)
	5α28 (9%)	0.00007 (h=1.3)	5β28 (9%)	0.00013 (h=1.4)
	6α28 (6.6%)	0.00095 (h=0.8)	6β28 (6.6%)	0.001 (h=0.74)
	6α28 (15%)	0.00019 (h=0.7)	6β28 (15%)	0.00084 (h=0.7)

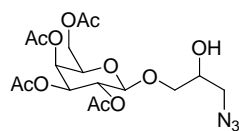
^a activities per pendant ligand. ^b Hill coefficient

Selected ^1H , ^{13}C NMR and IR spectra

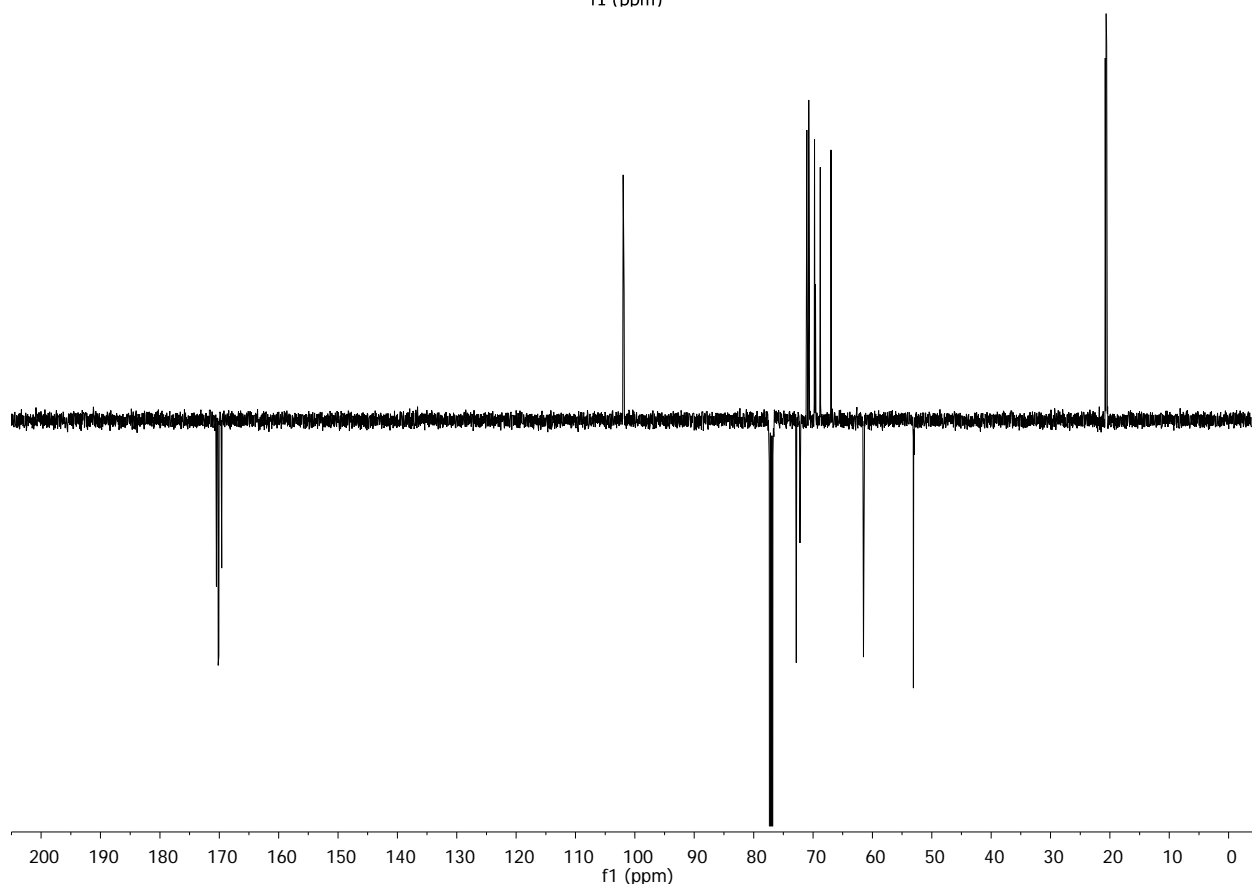
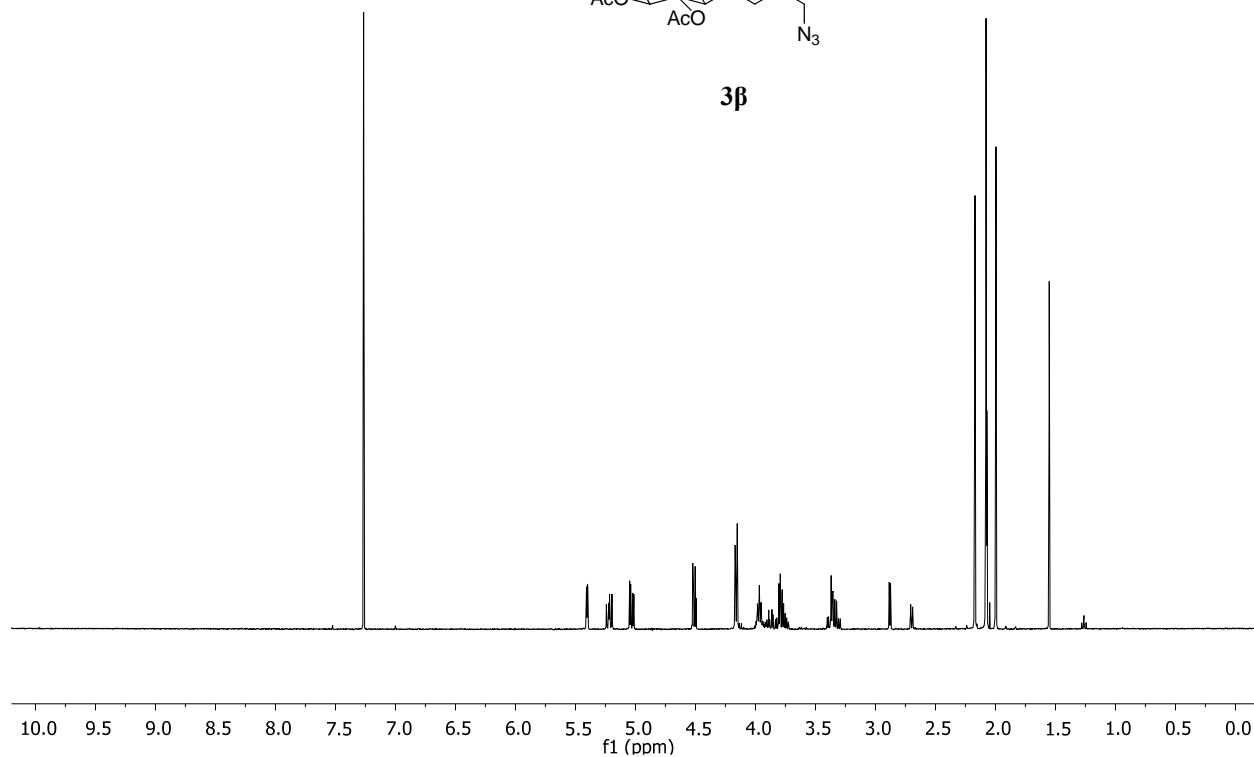


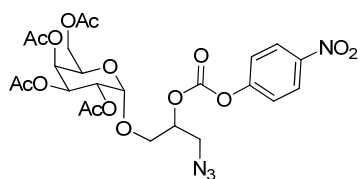
3a



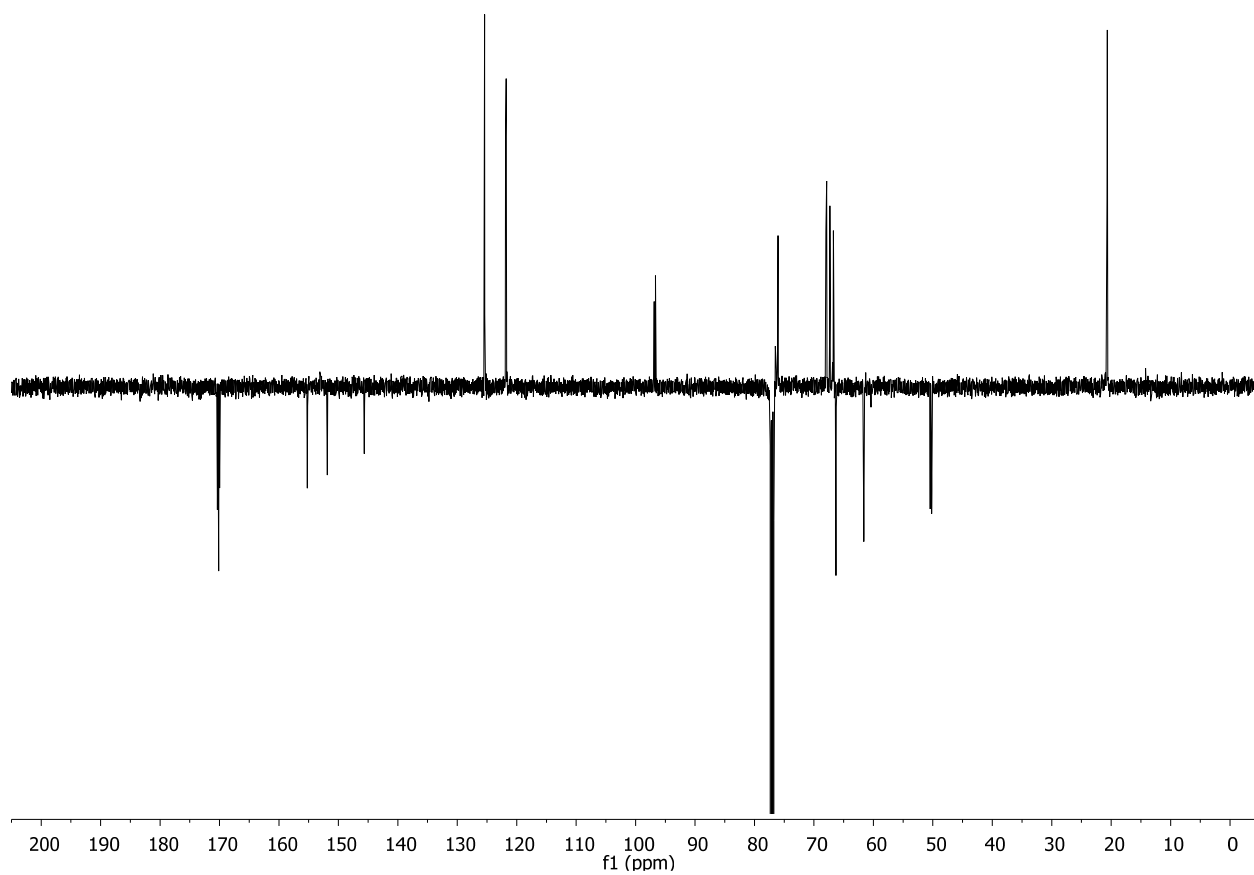
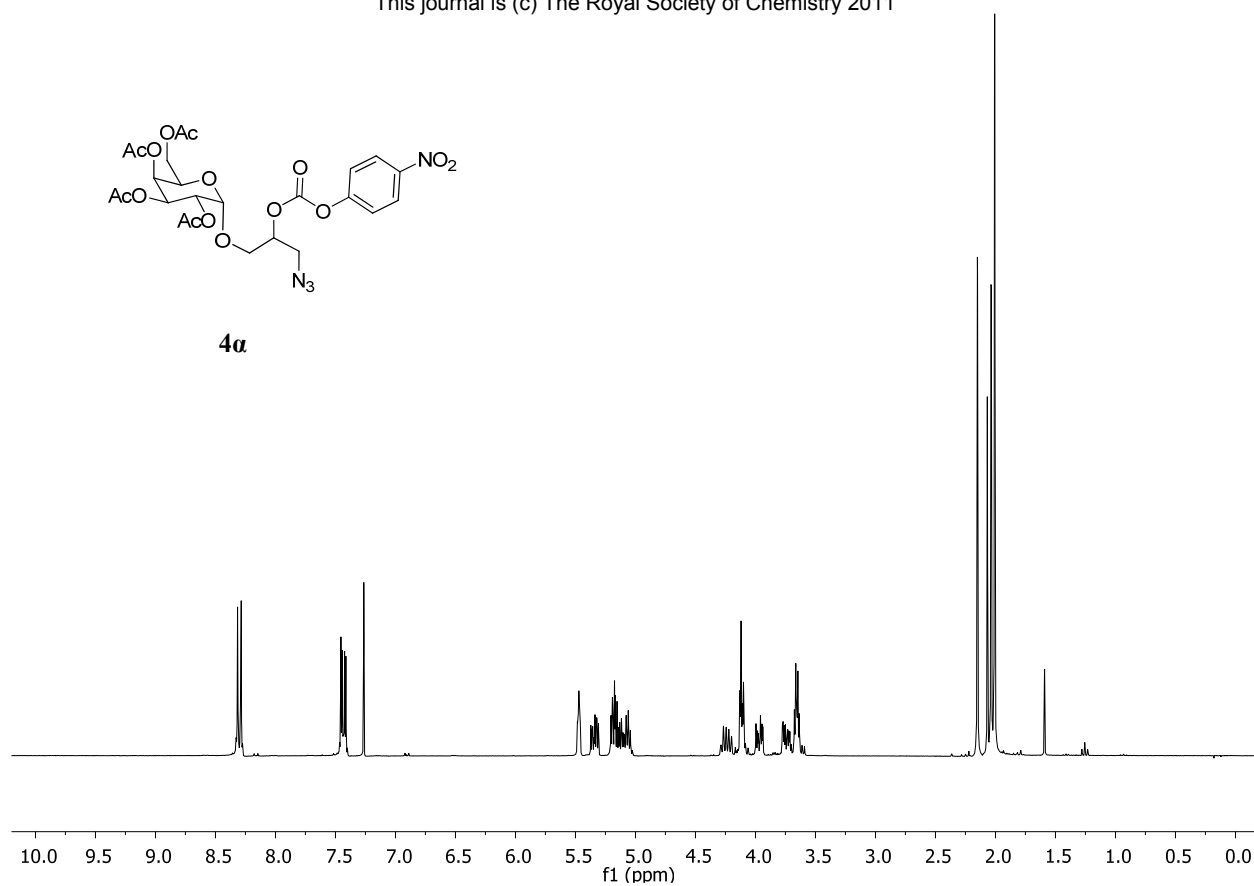


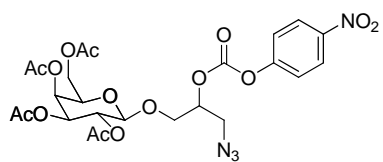
3β



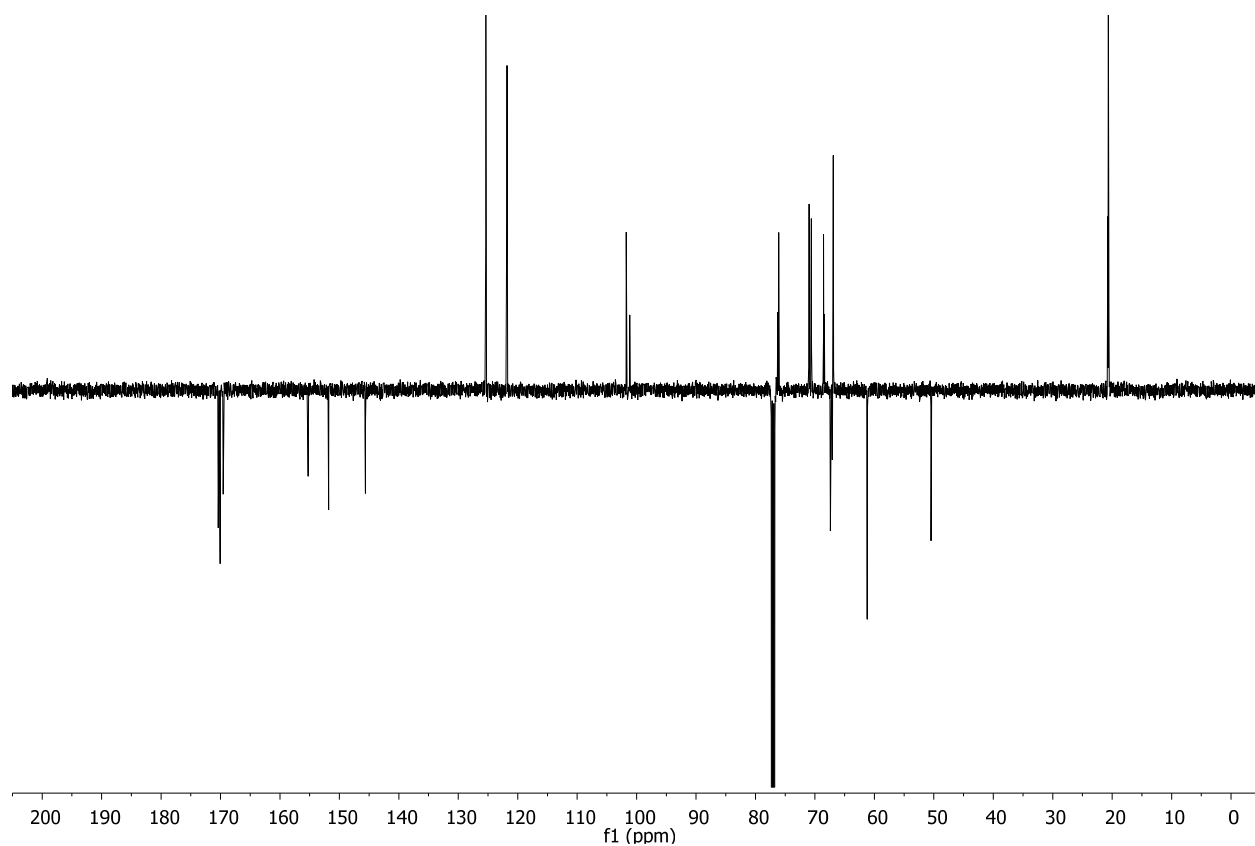
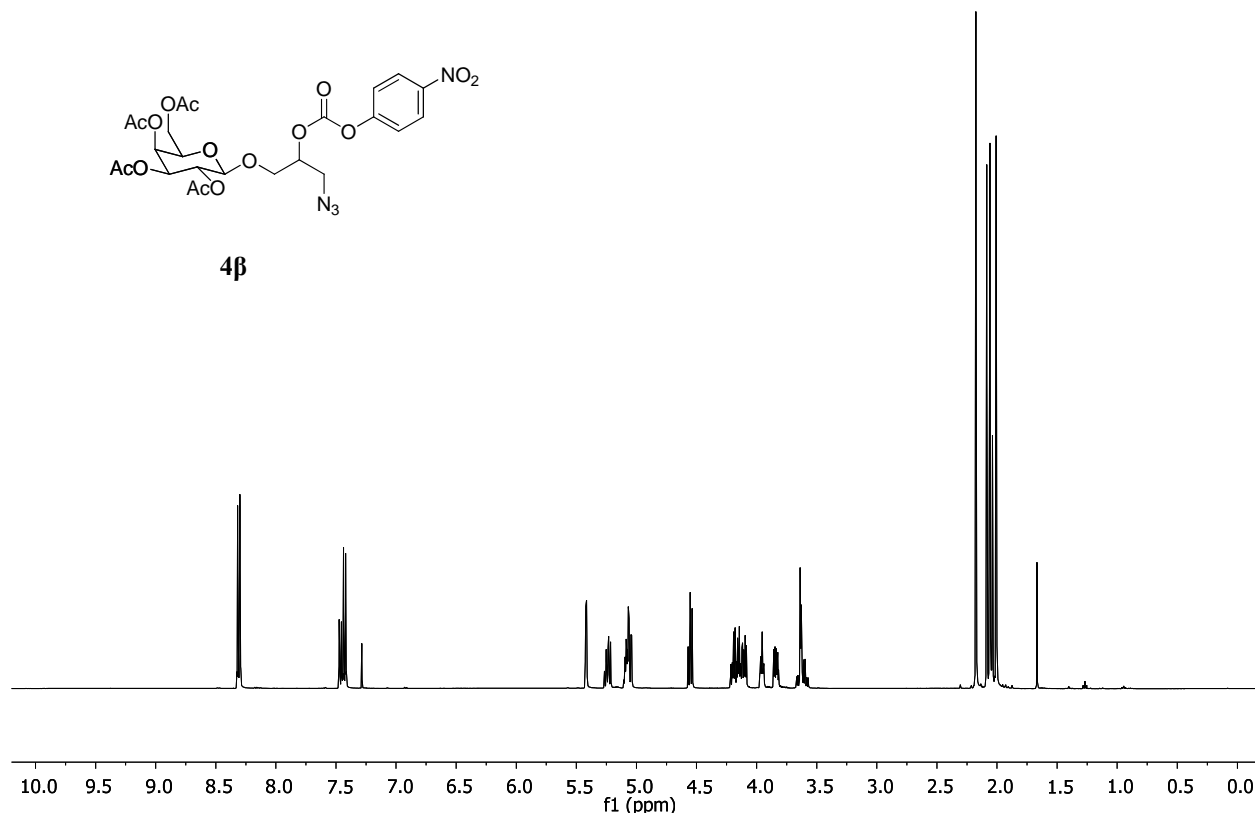


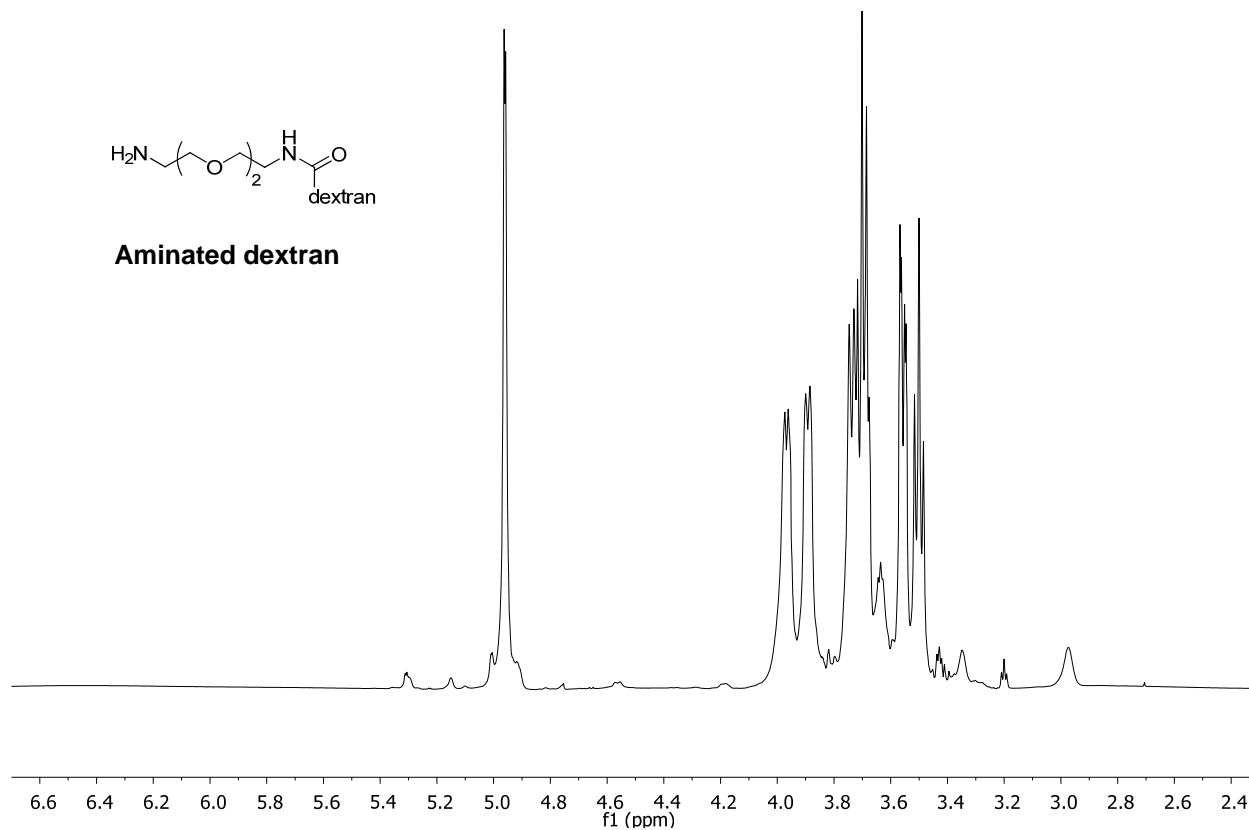
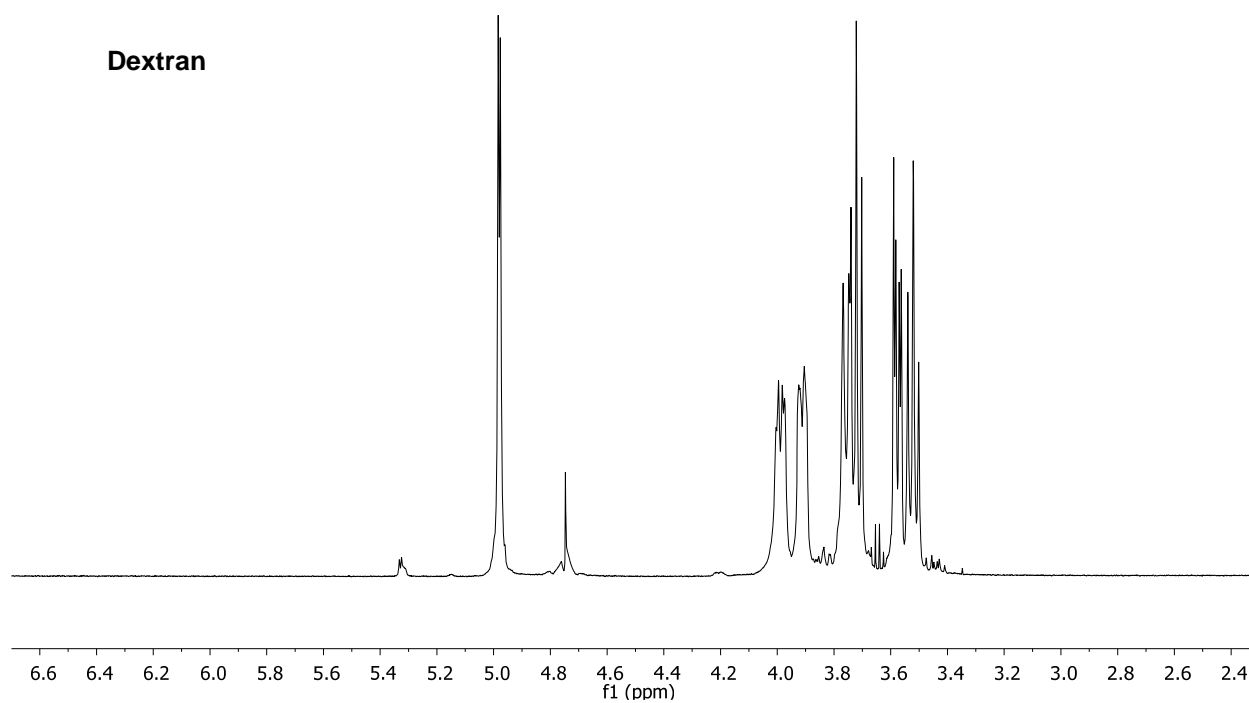
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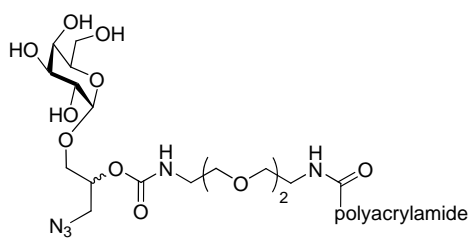




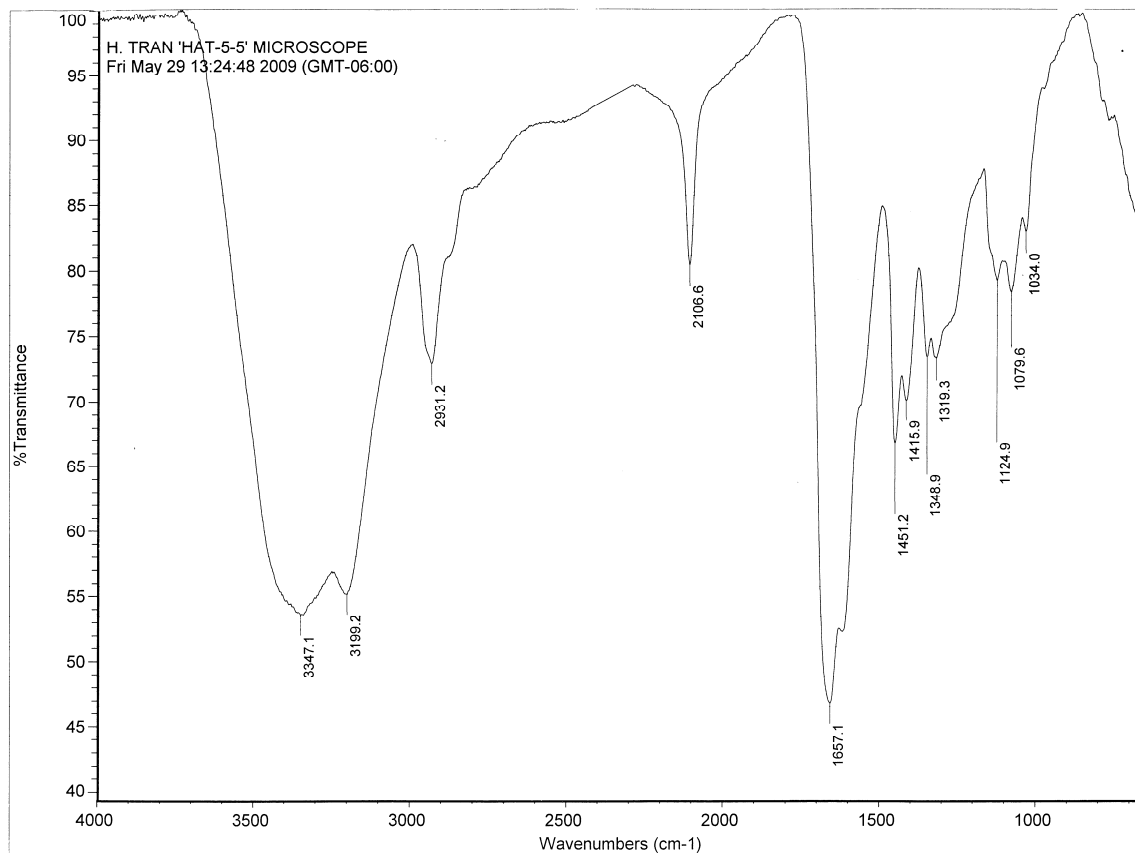
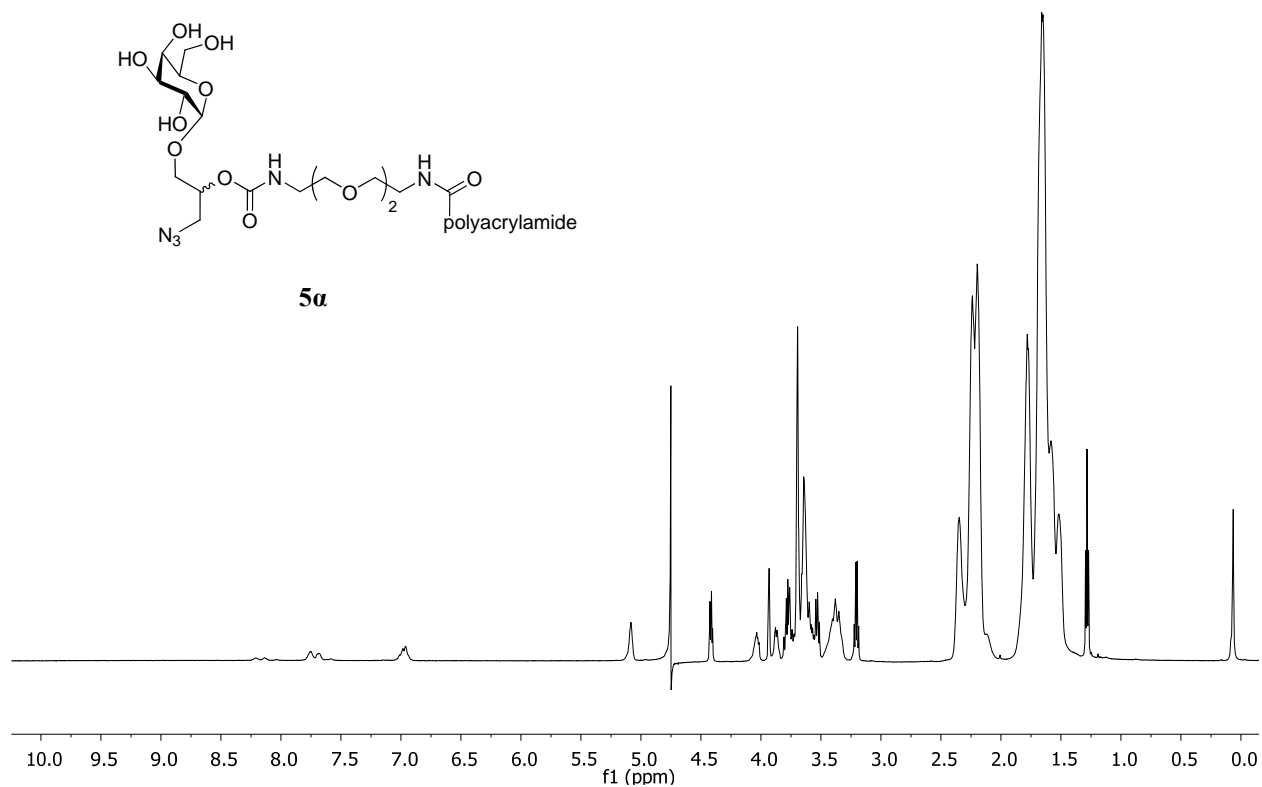
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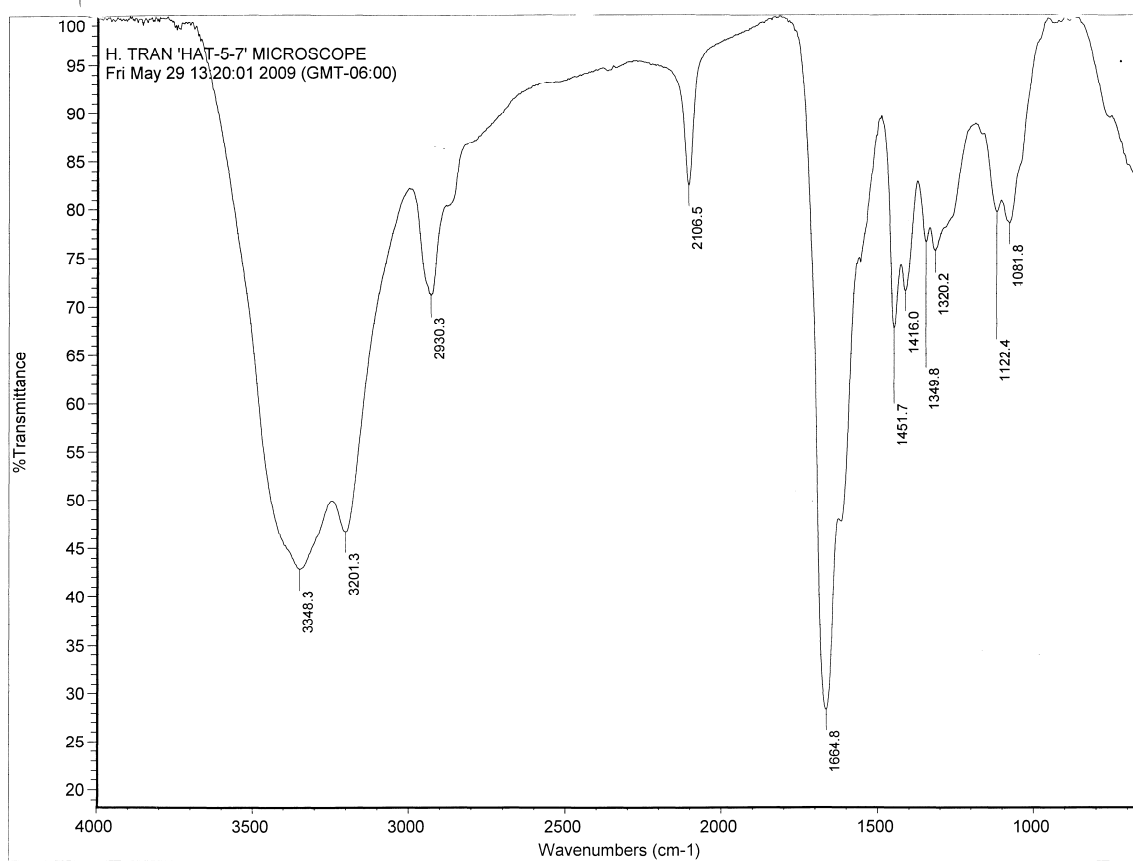
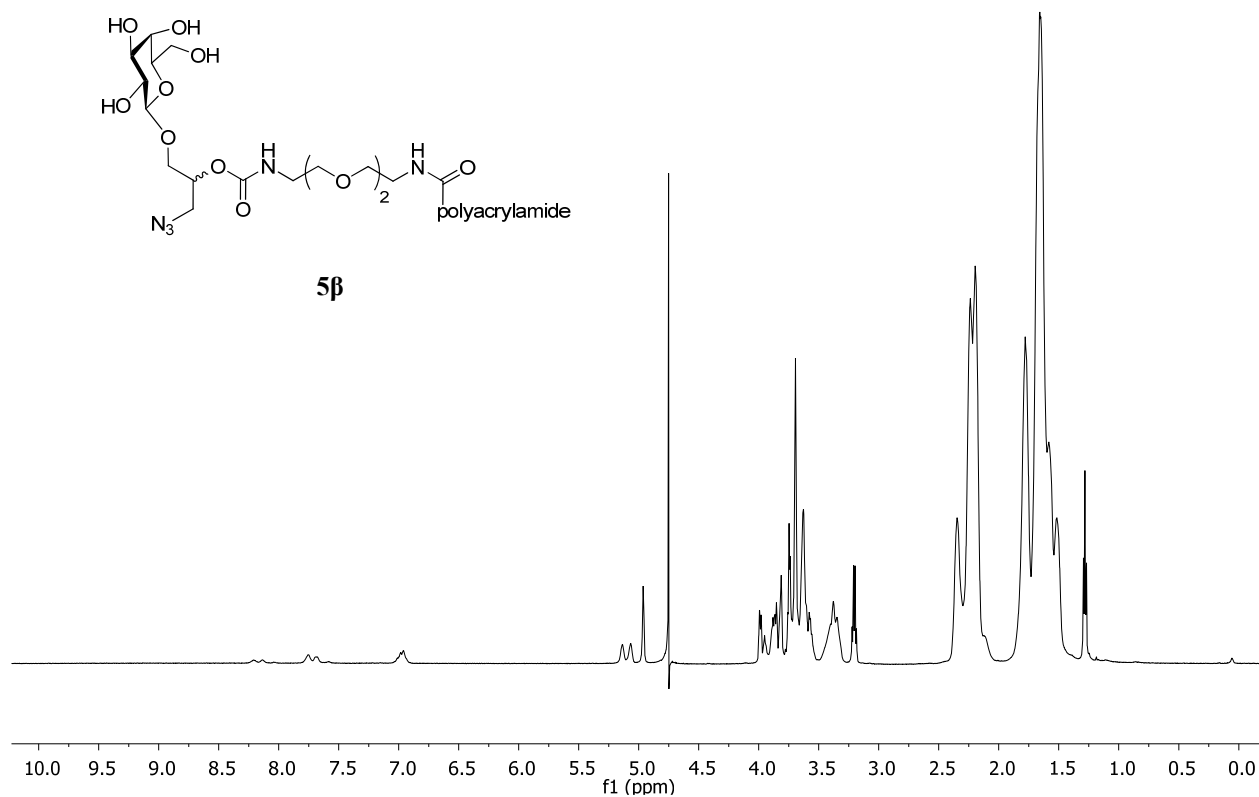


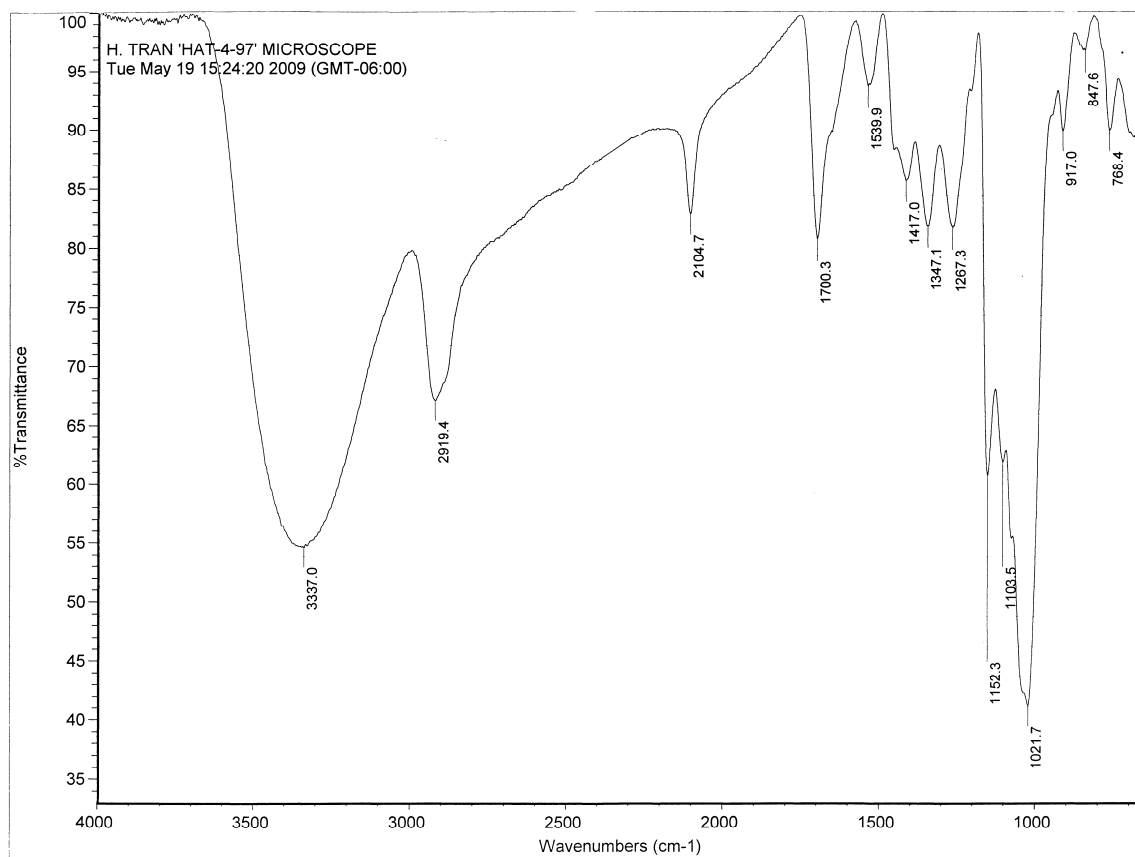
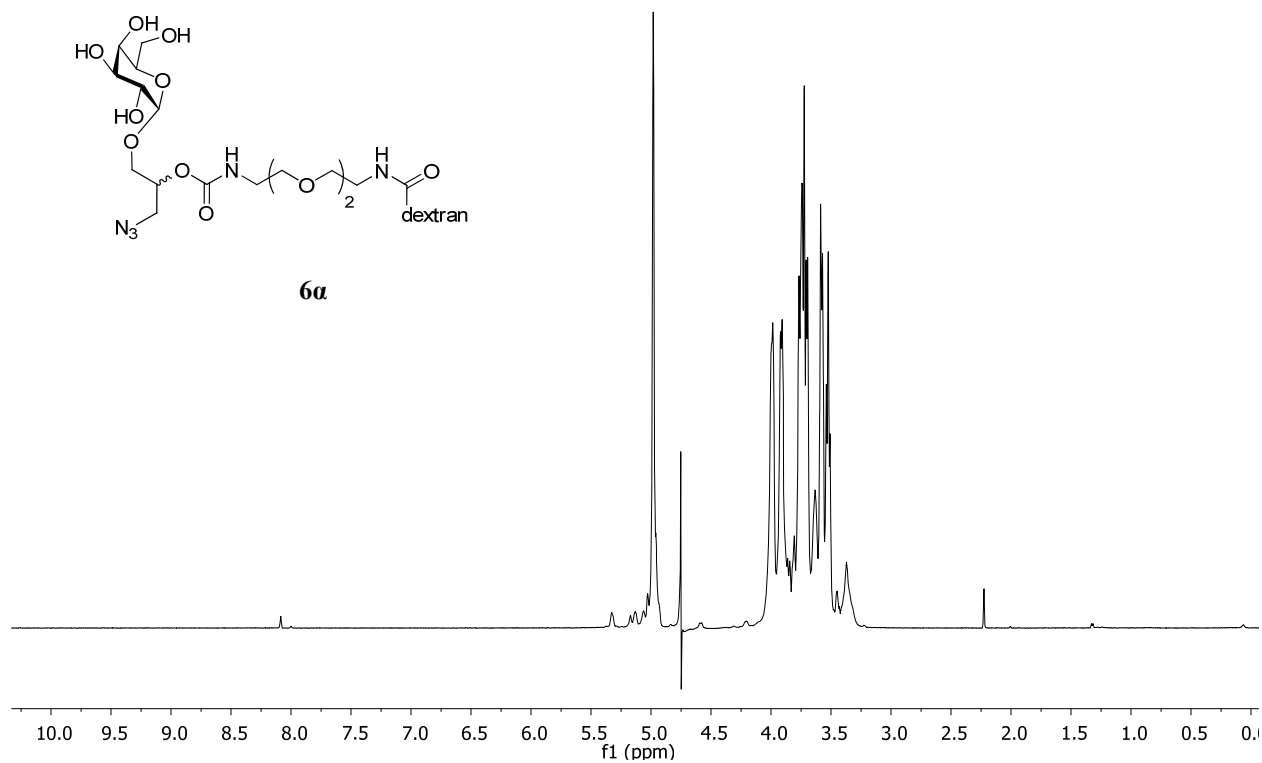


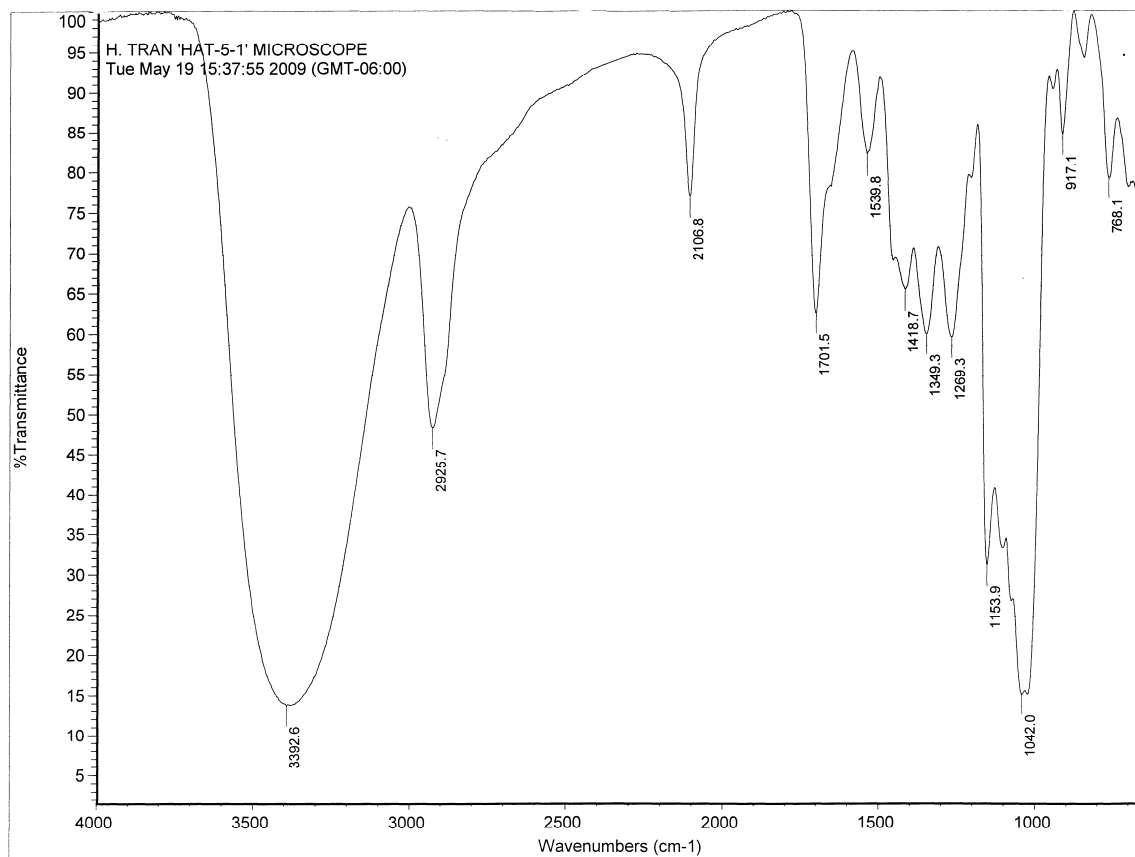
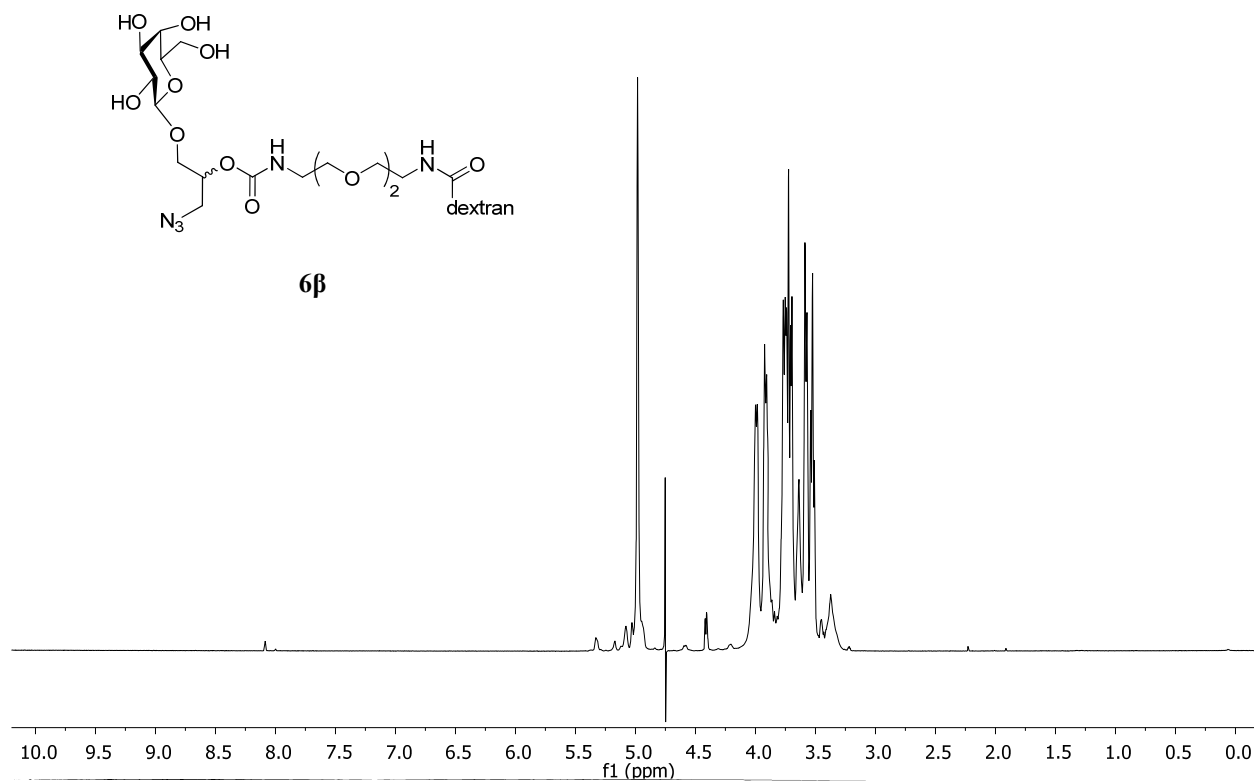


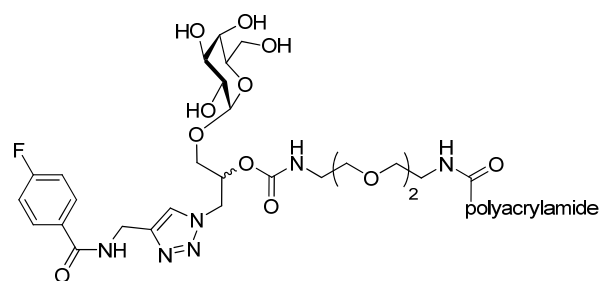
5a



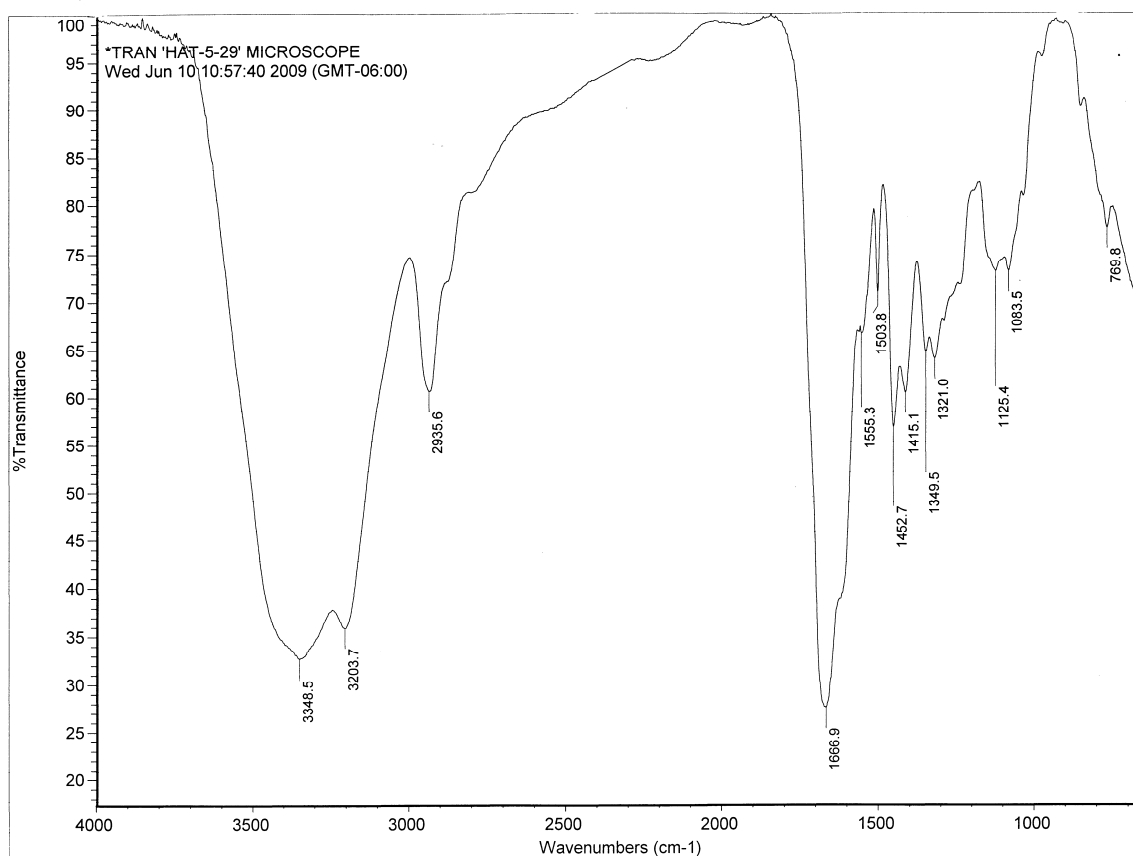
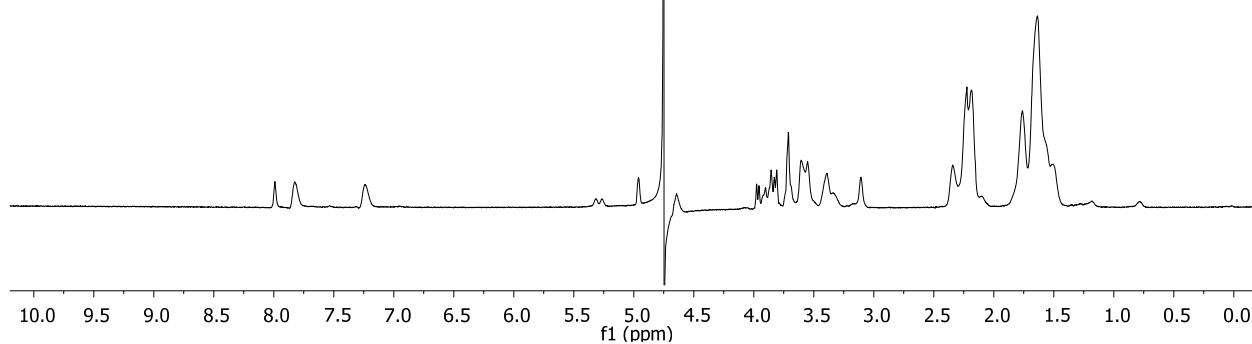


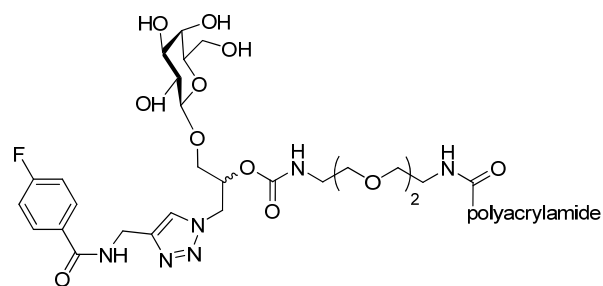




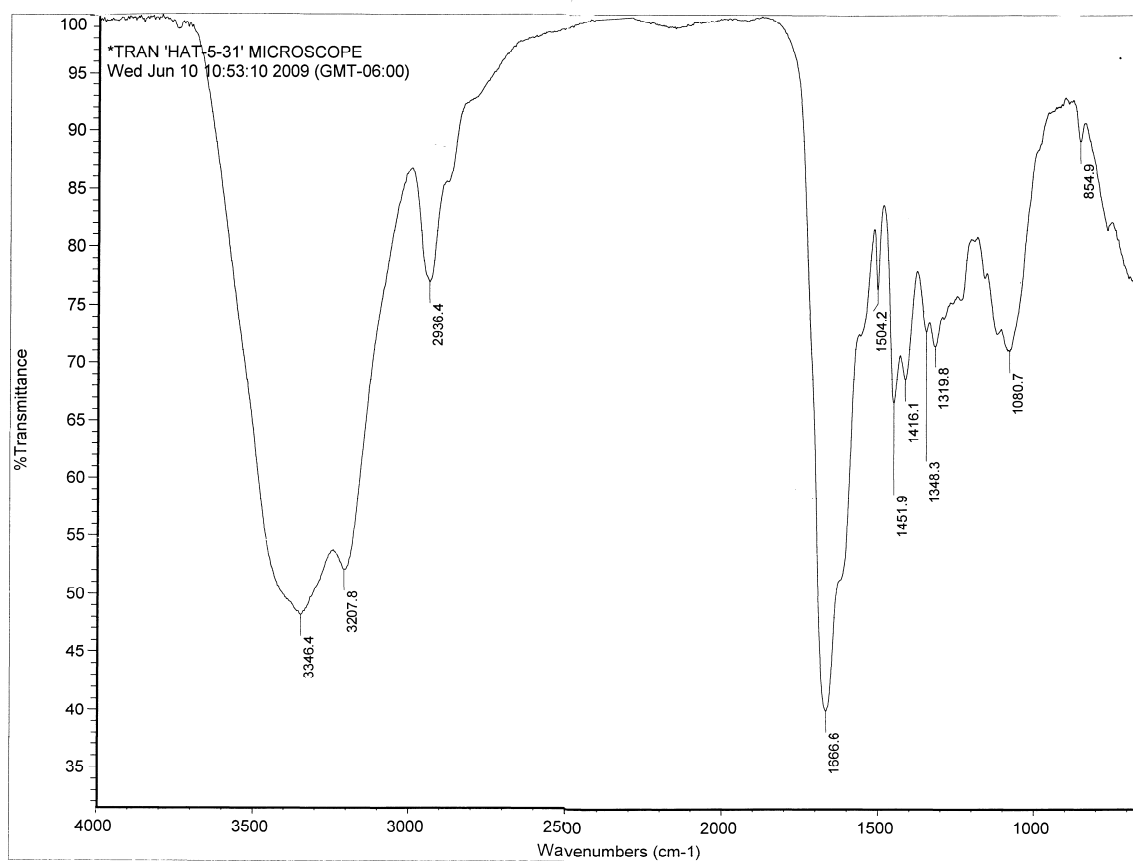
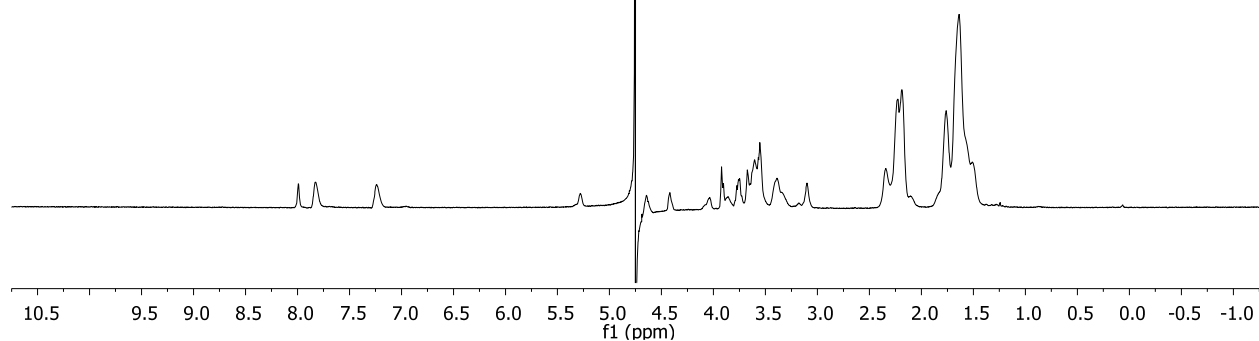


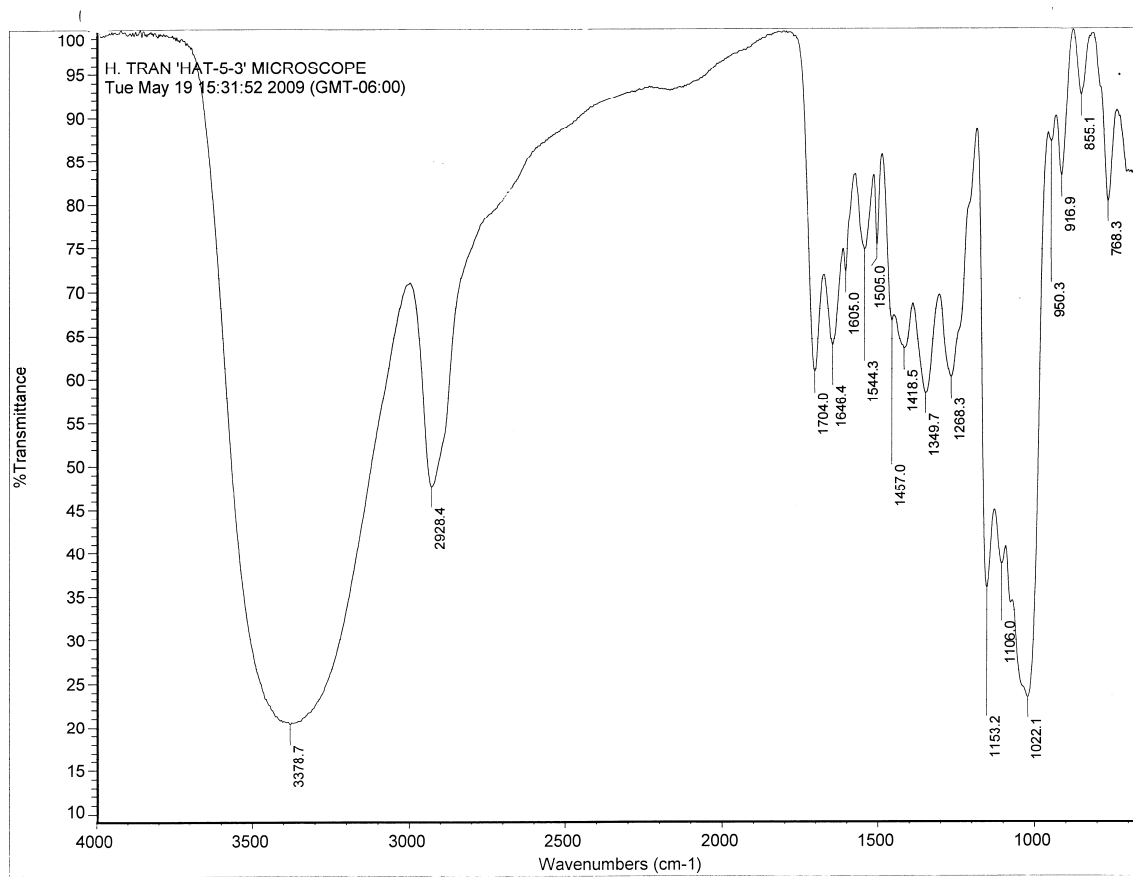
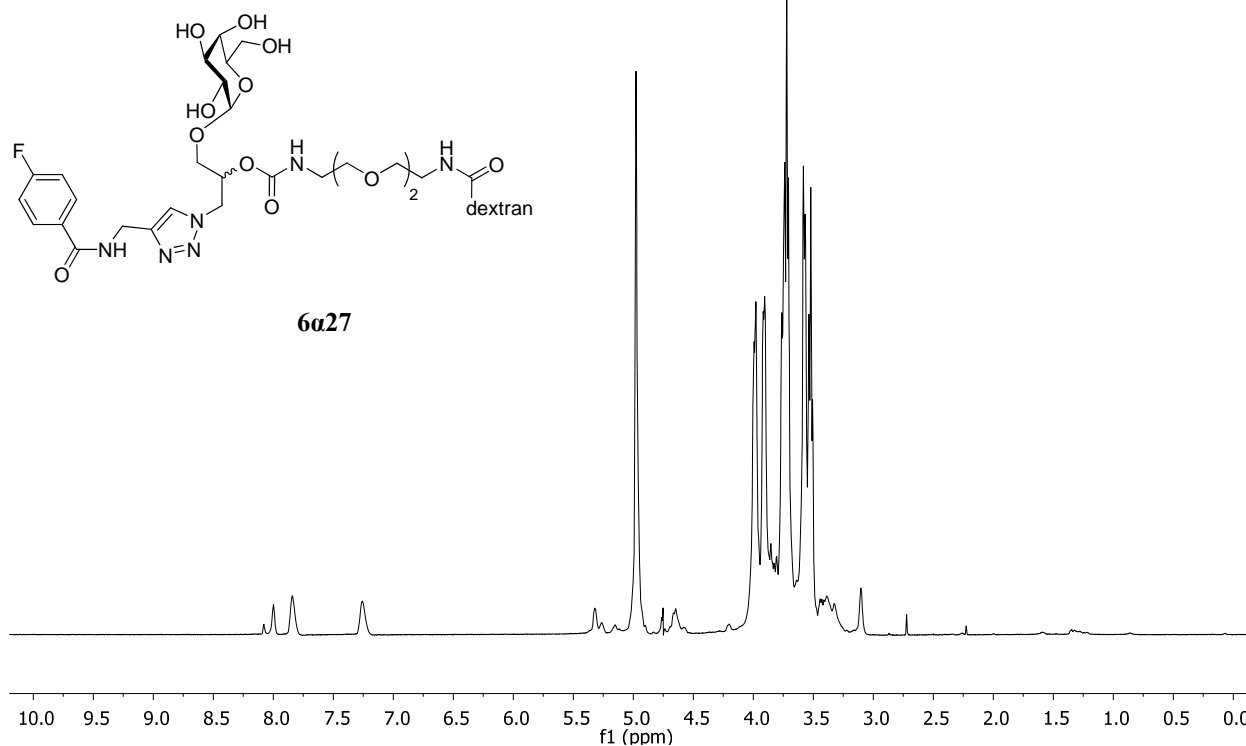
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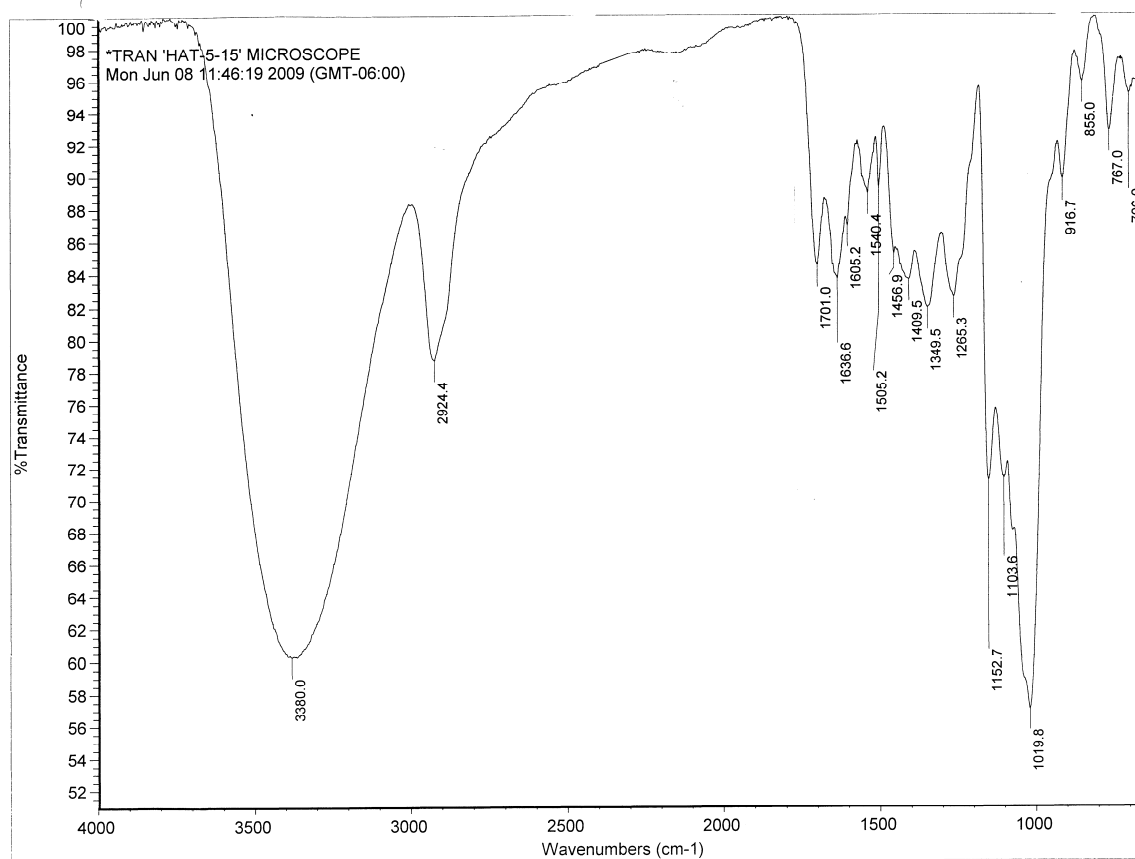
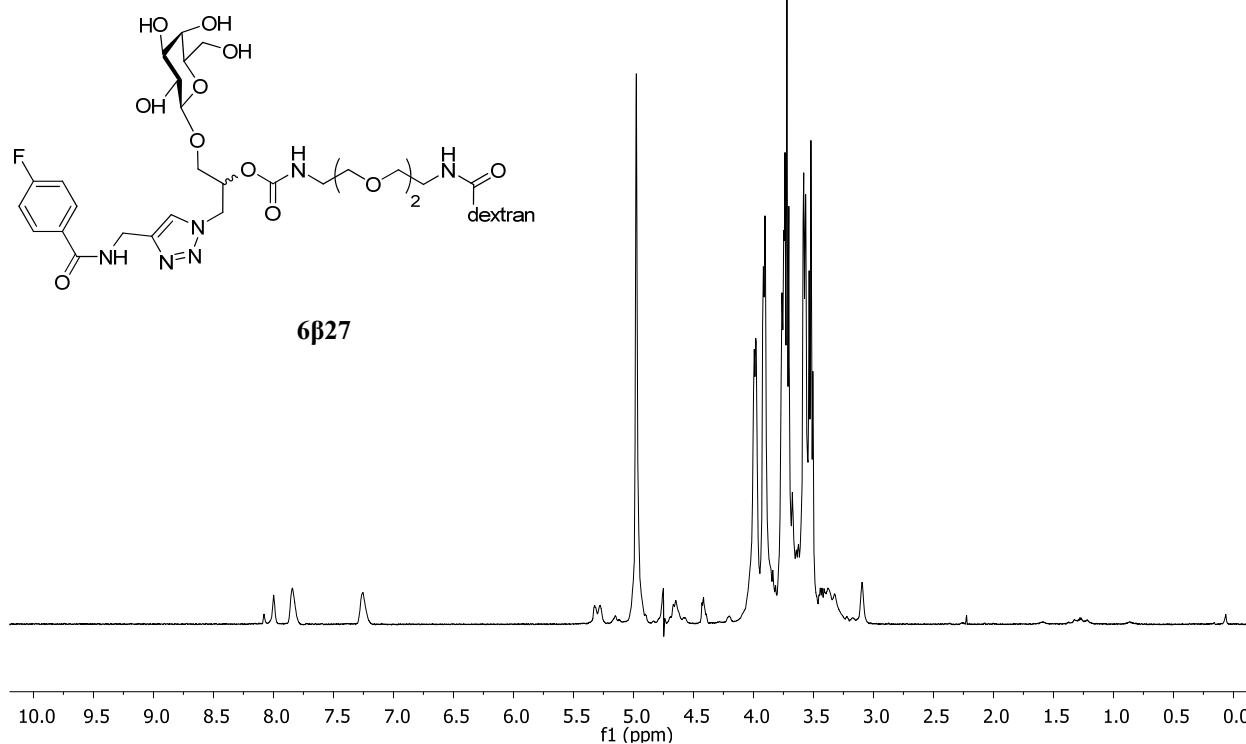


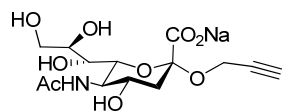


5β27

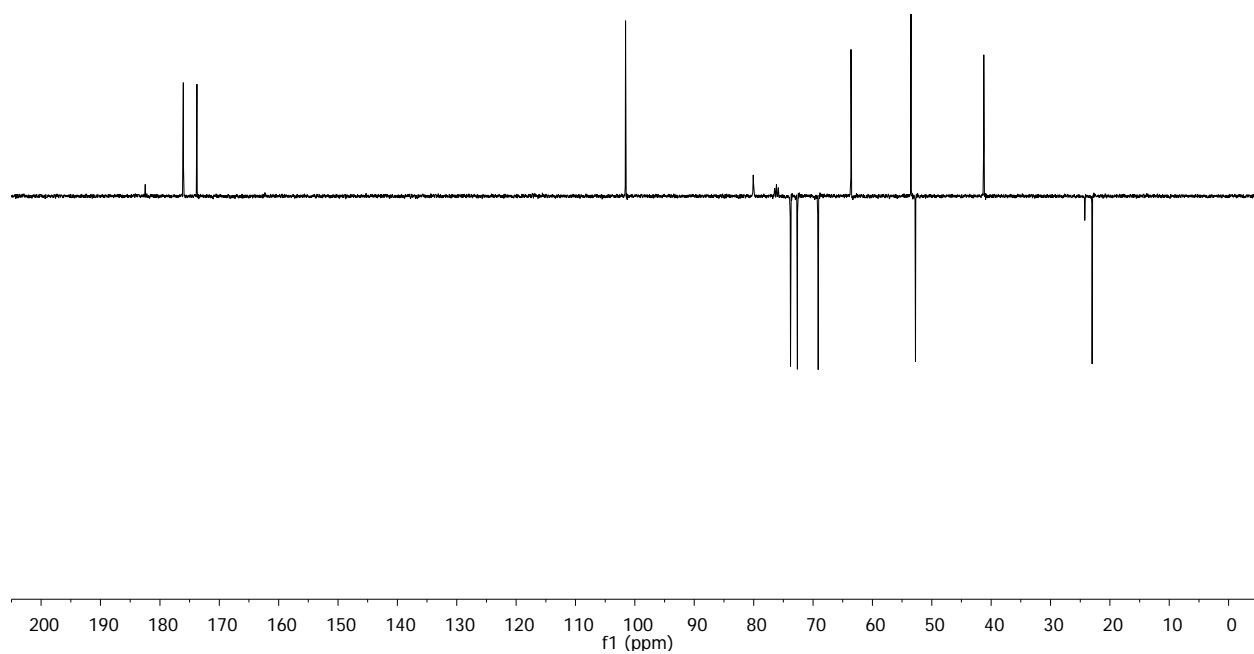
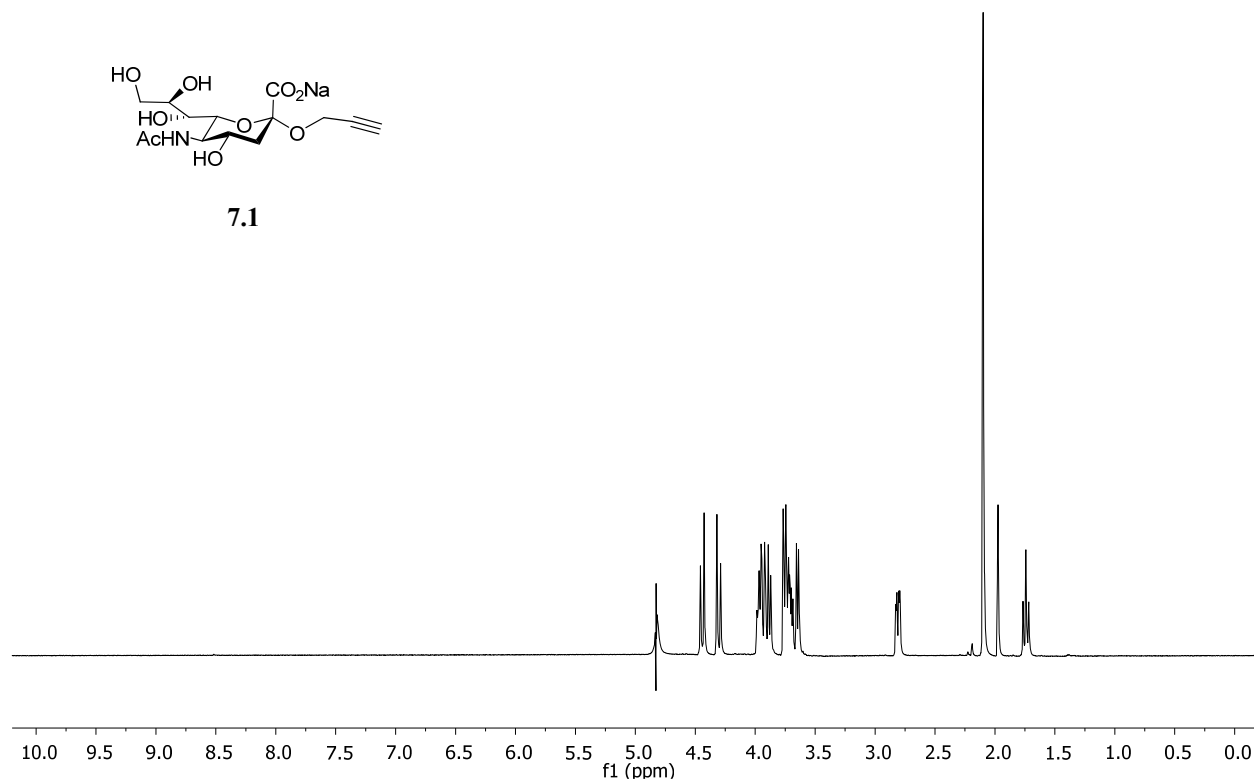


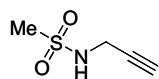




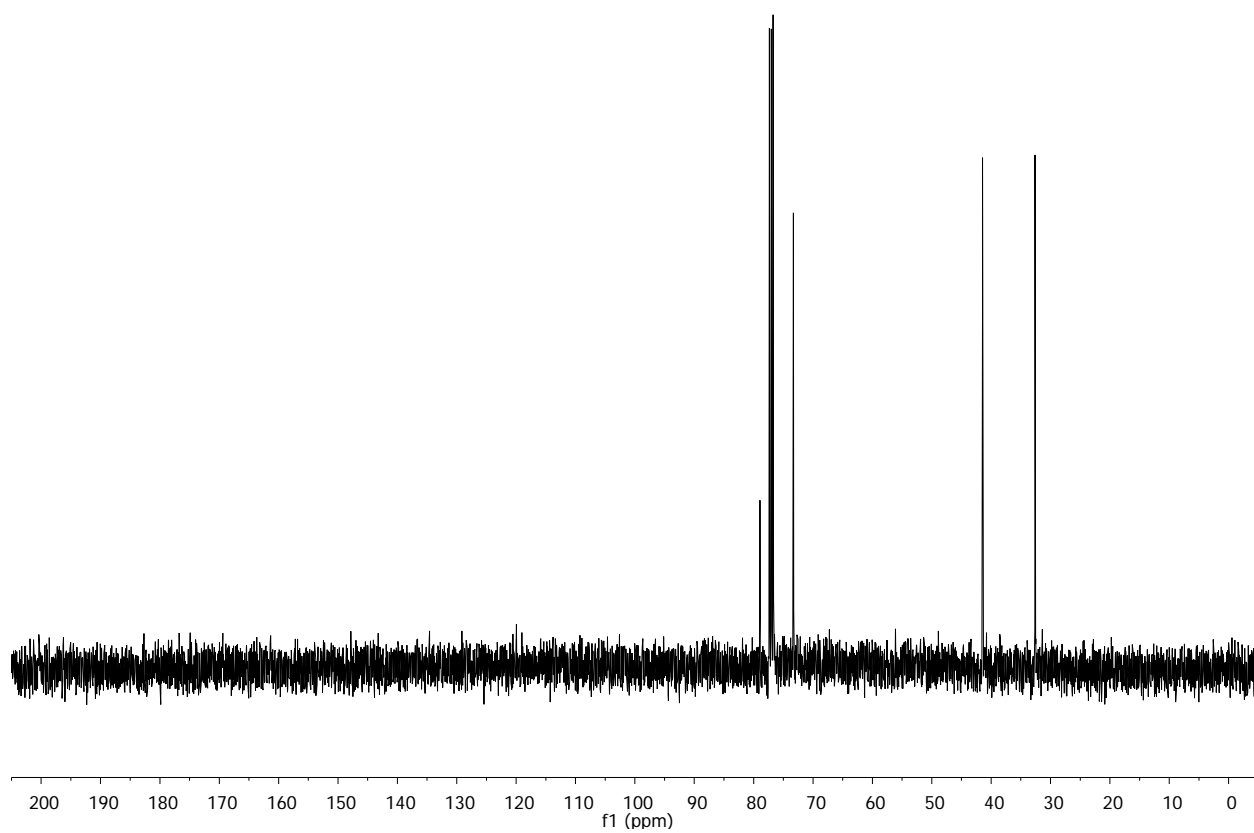
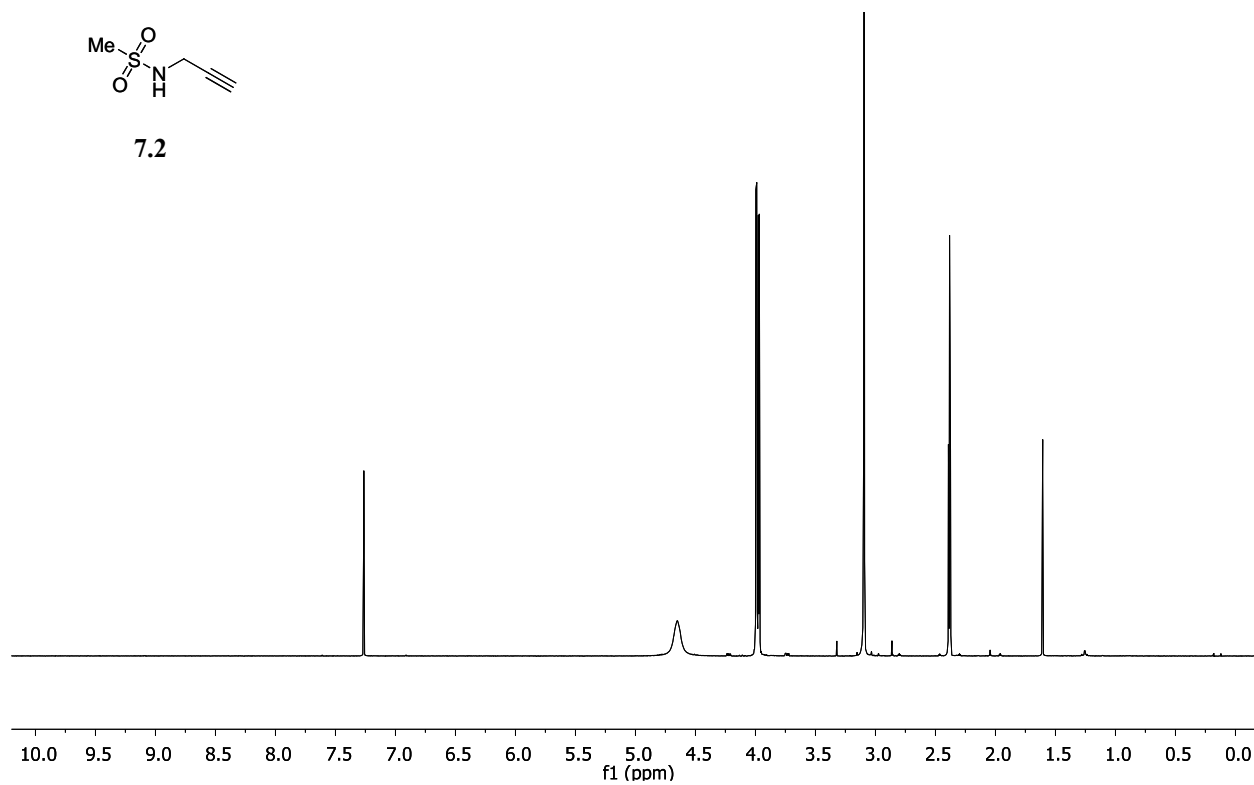


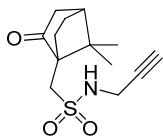
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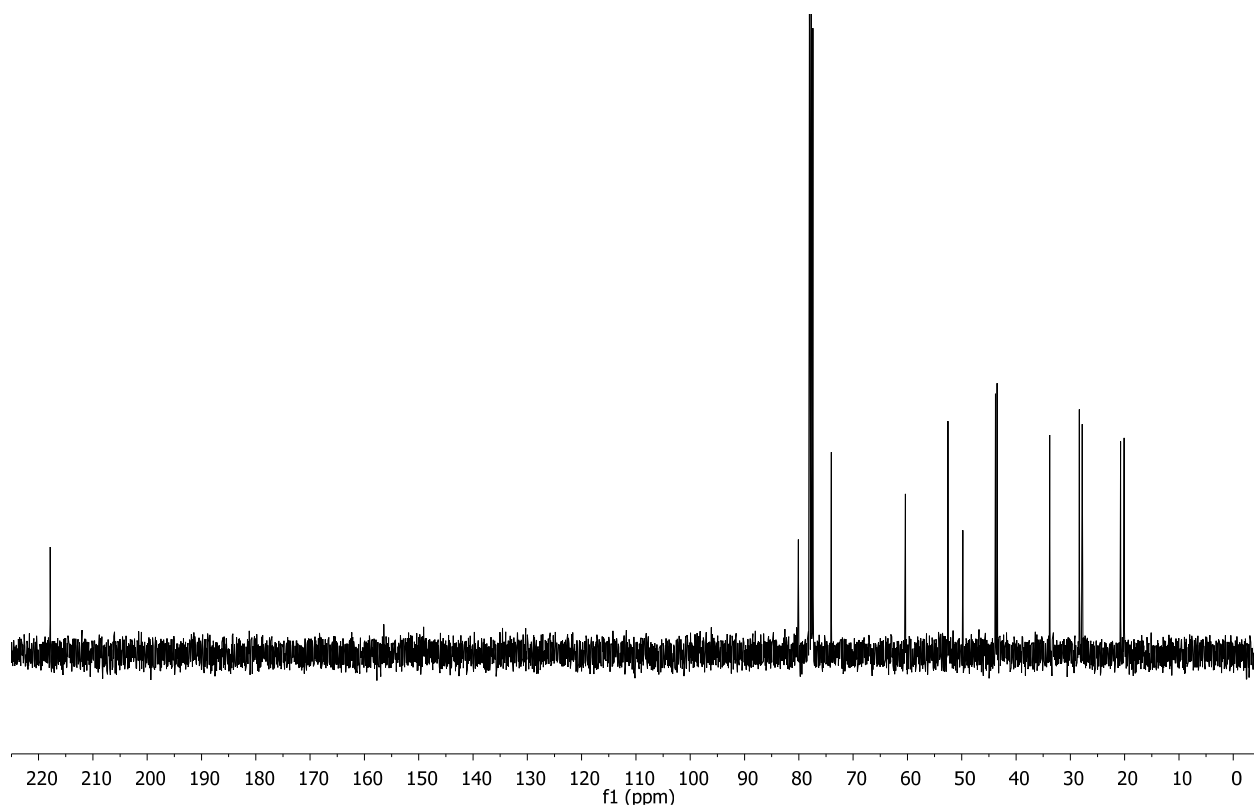
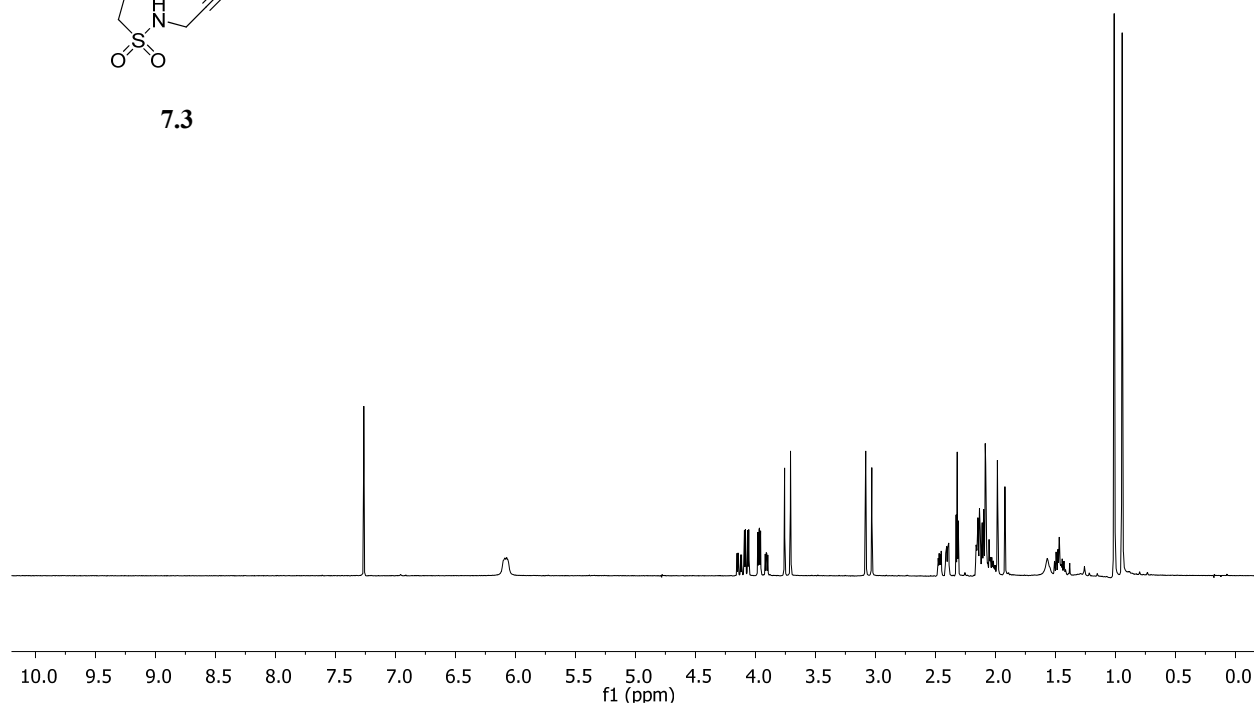


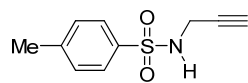
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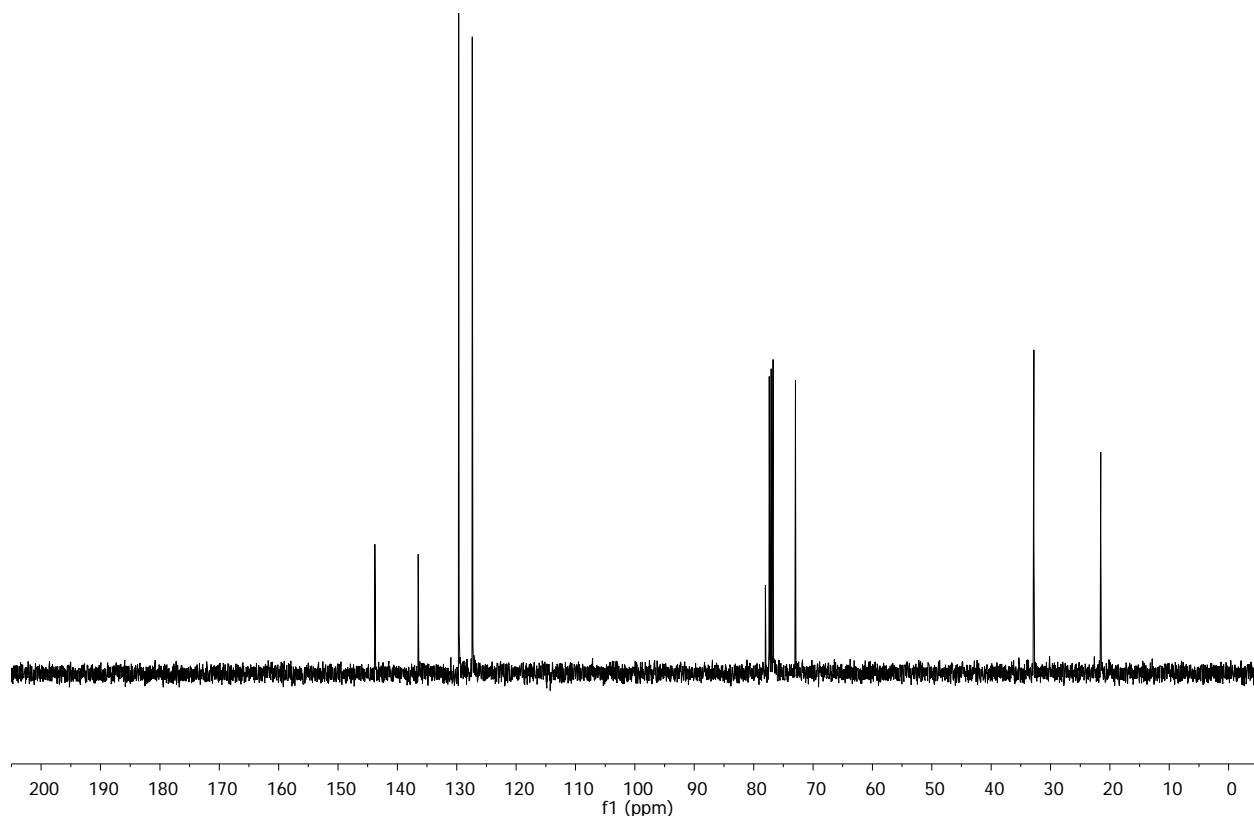
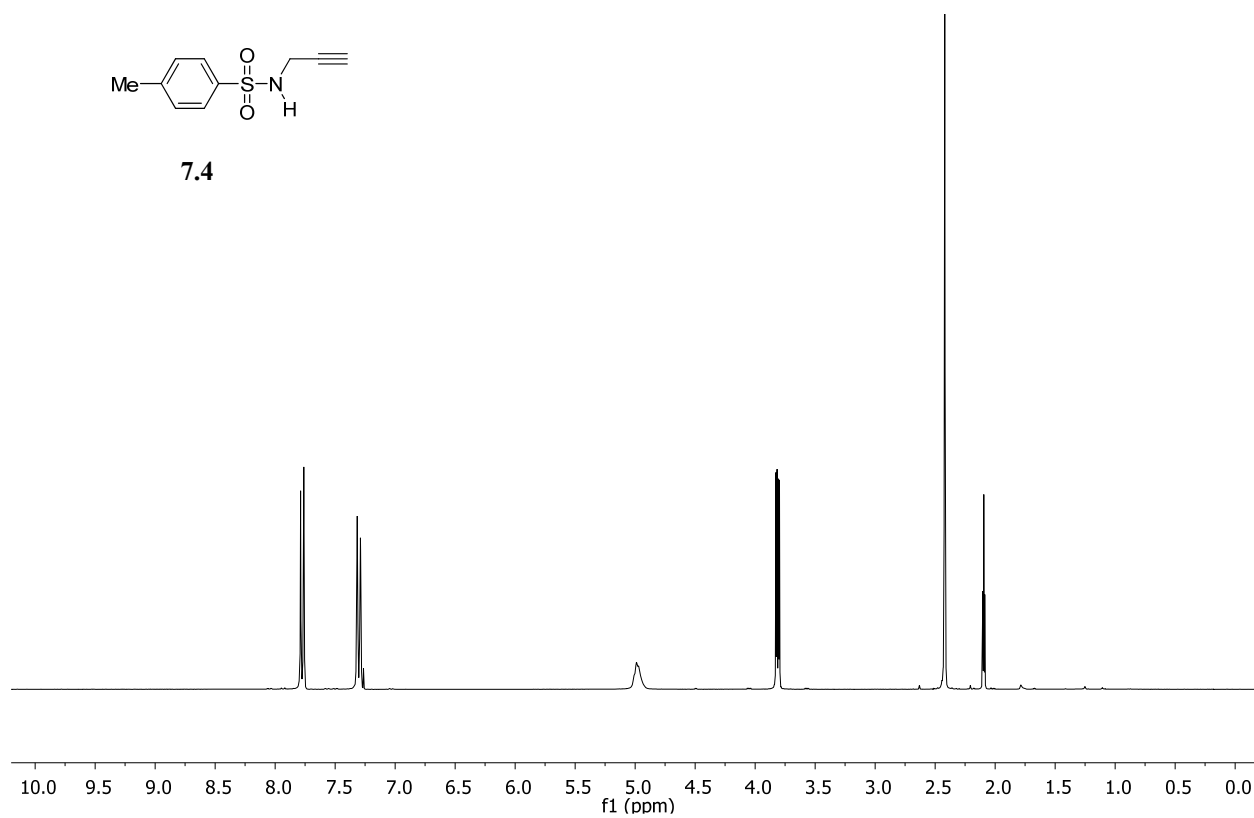


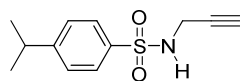
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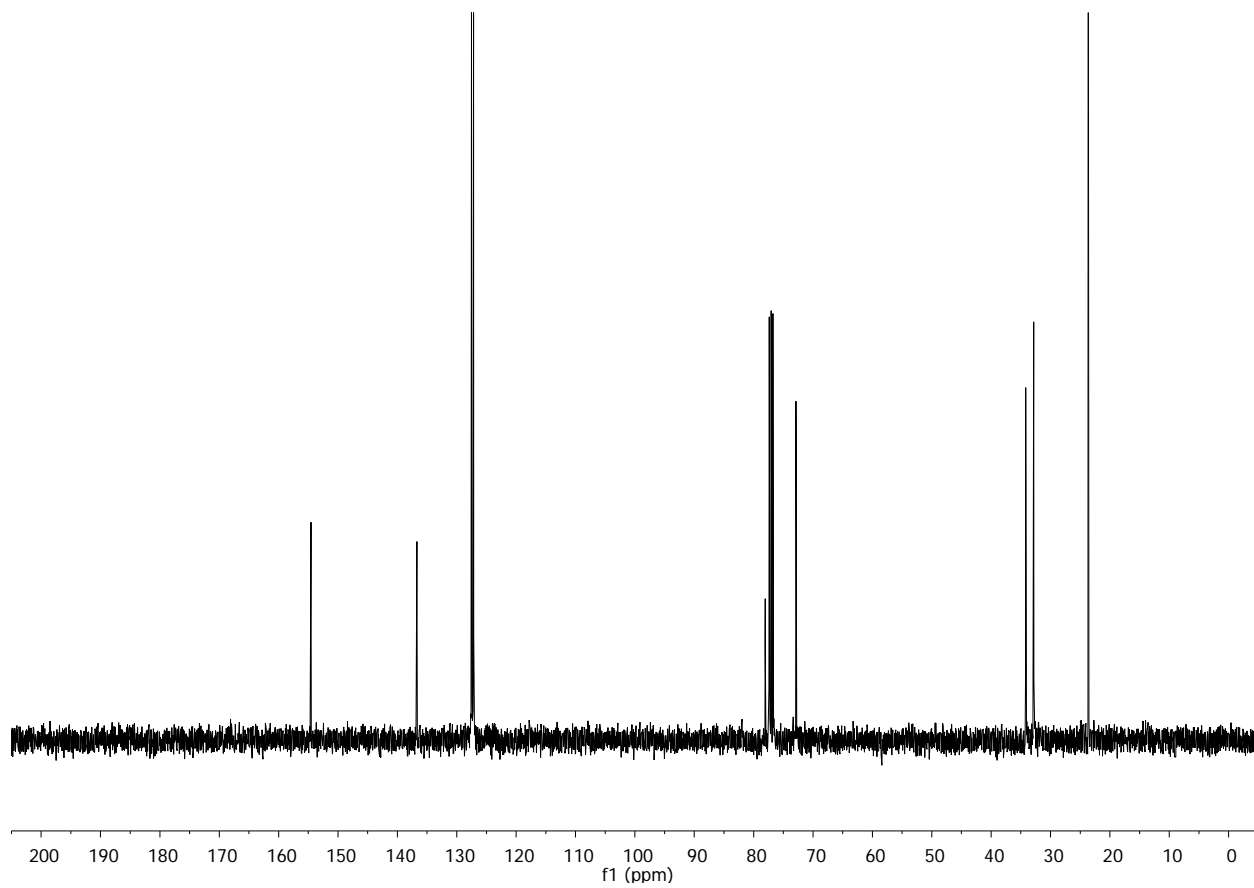
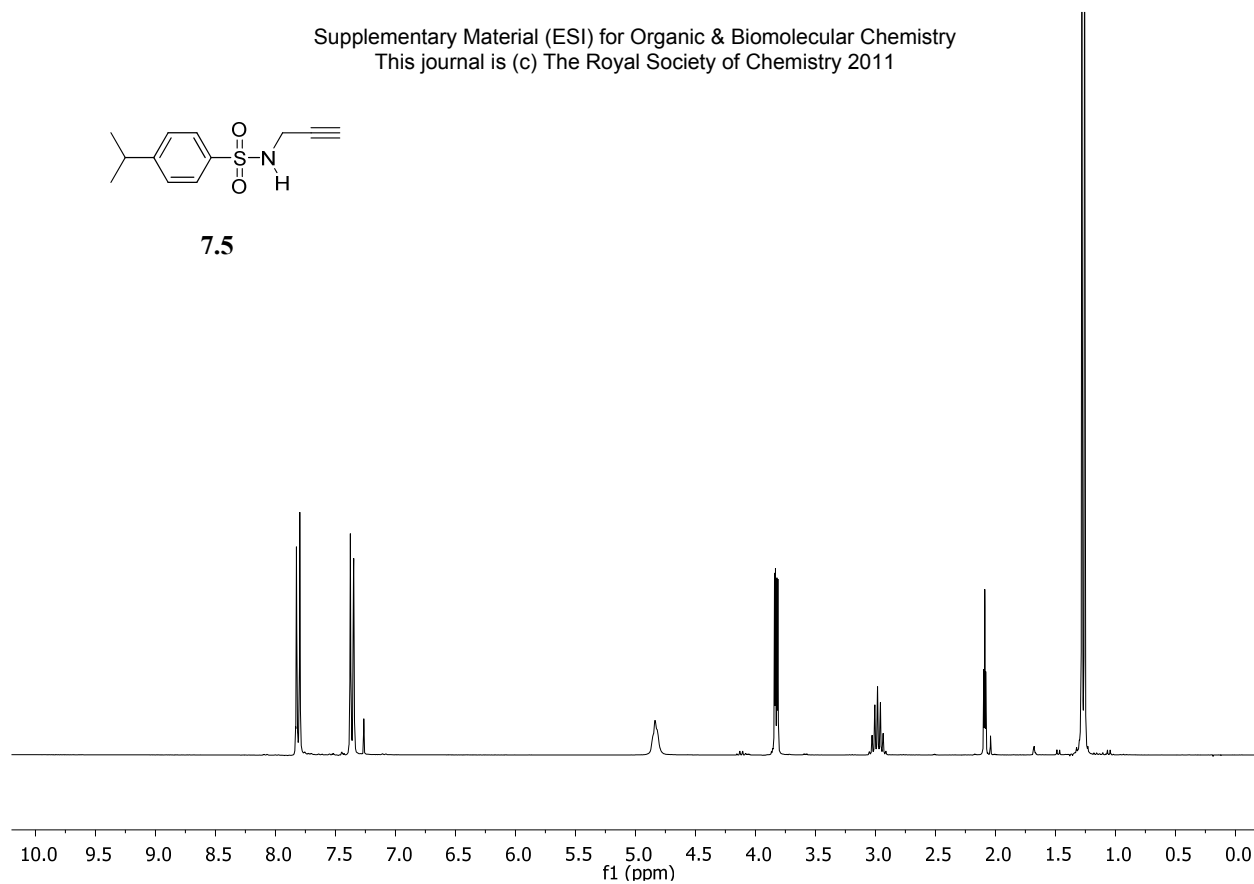


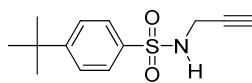
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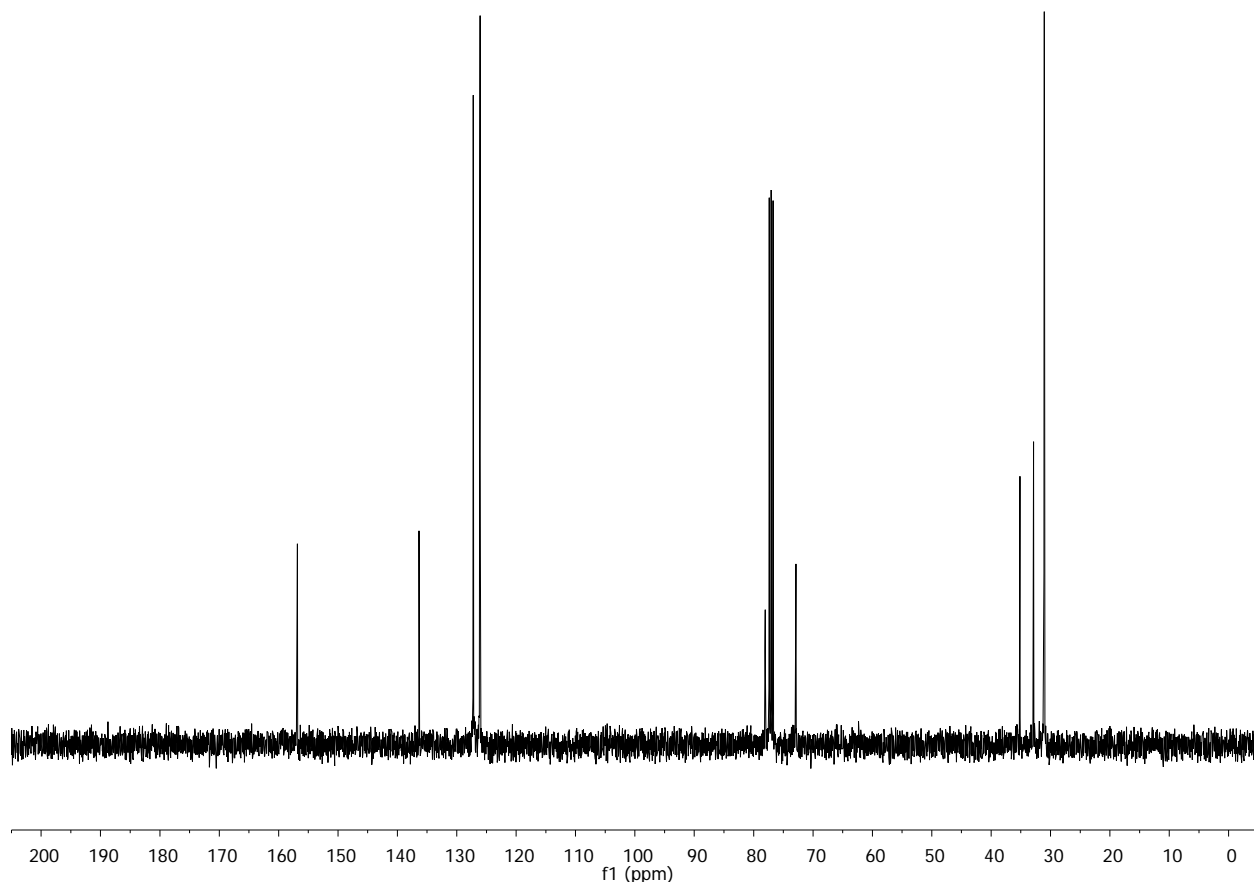
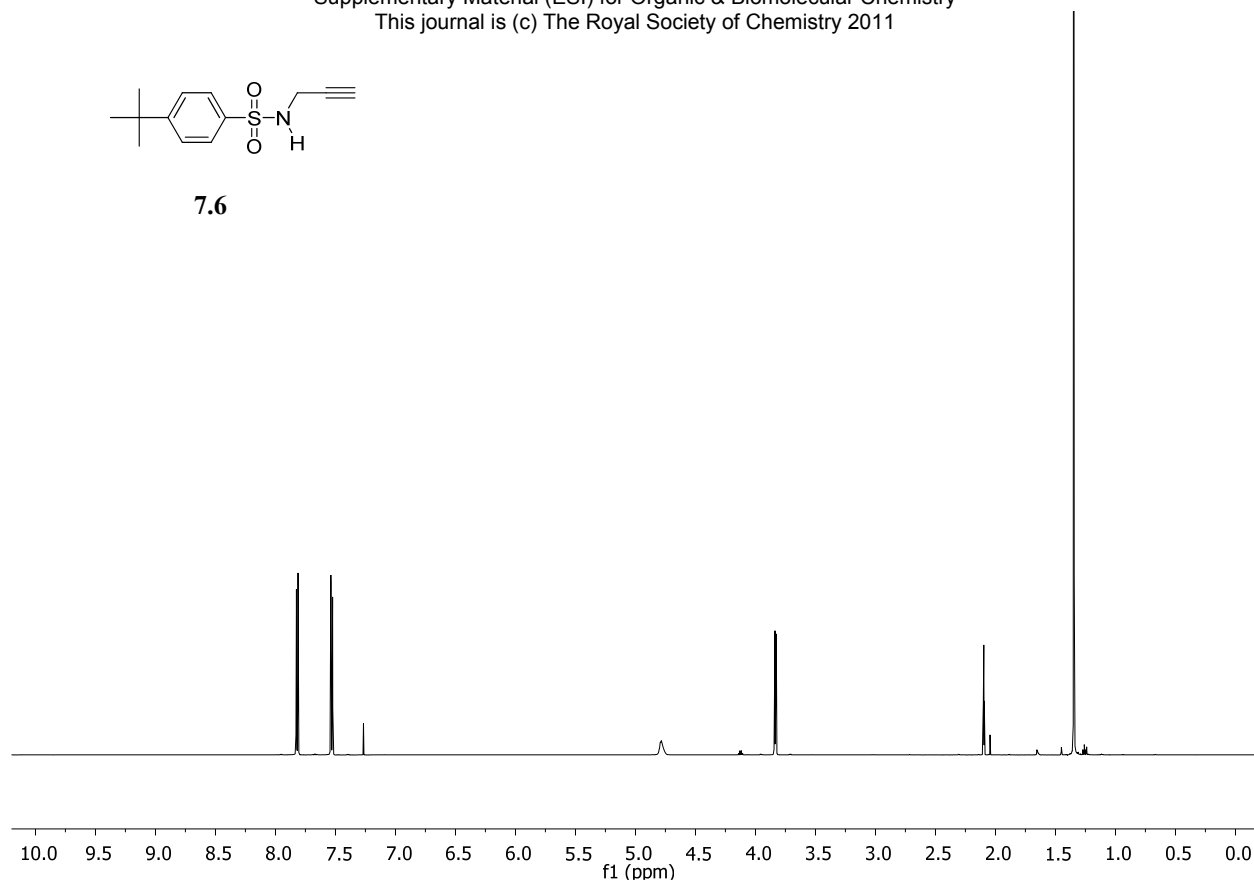


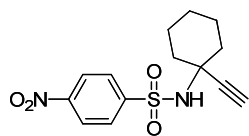
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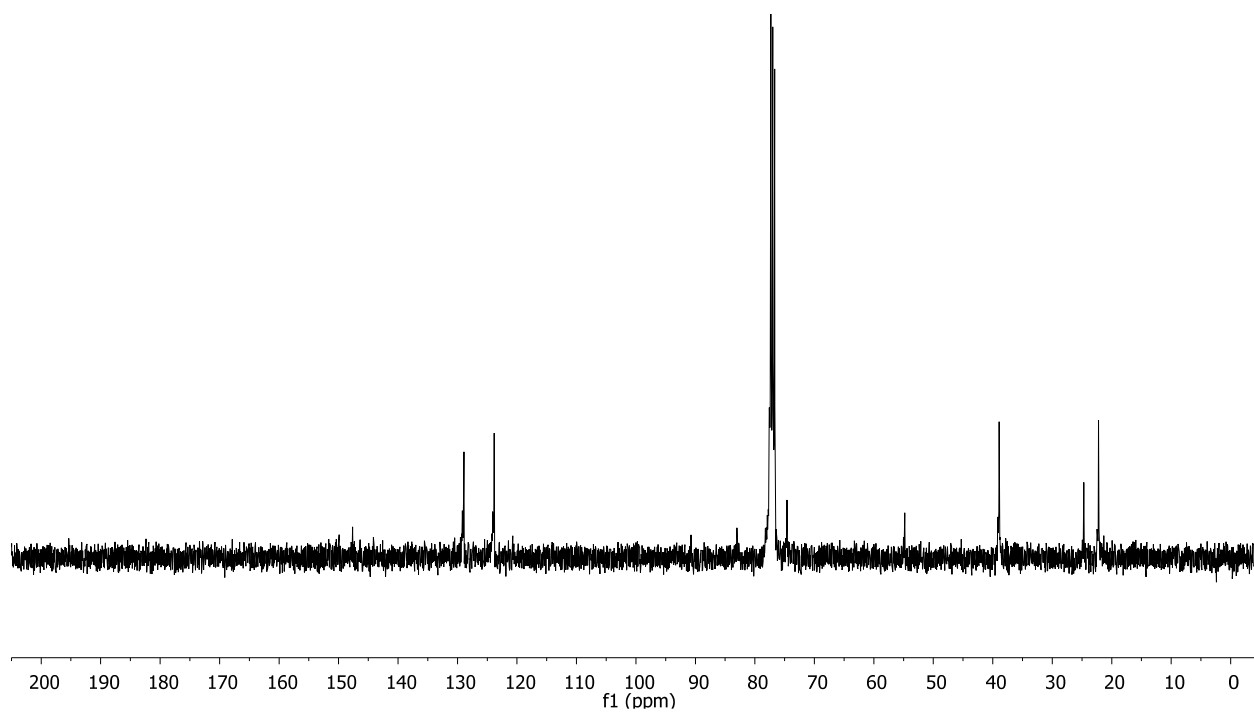
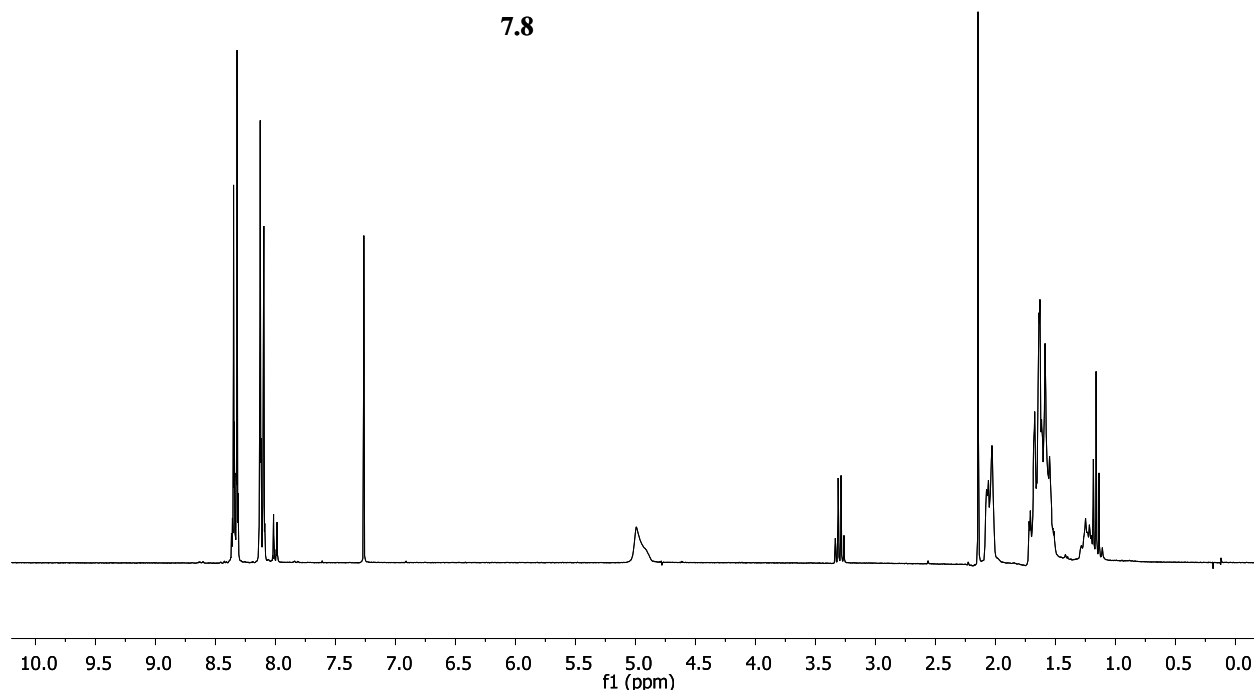


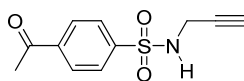
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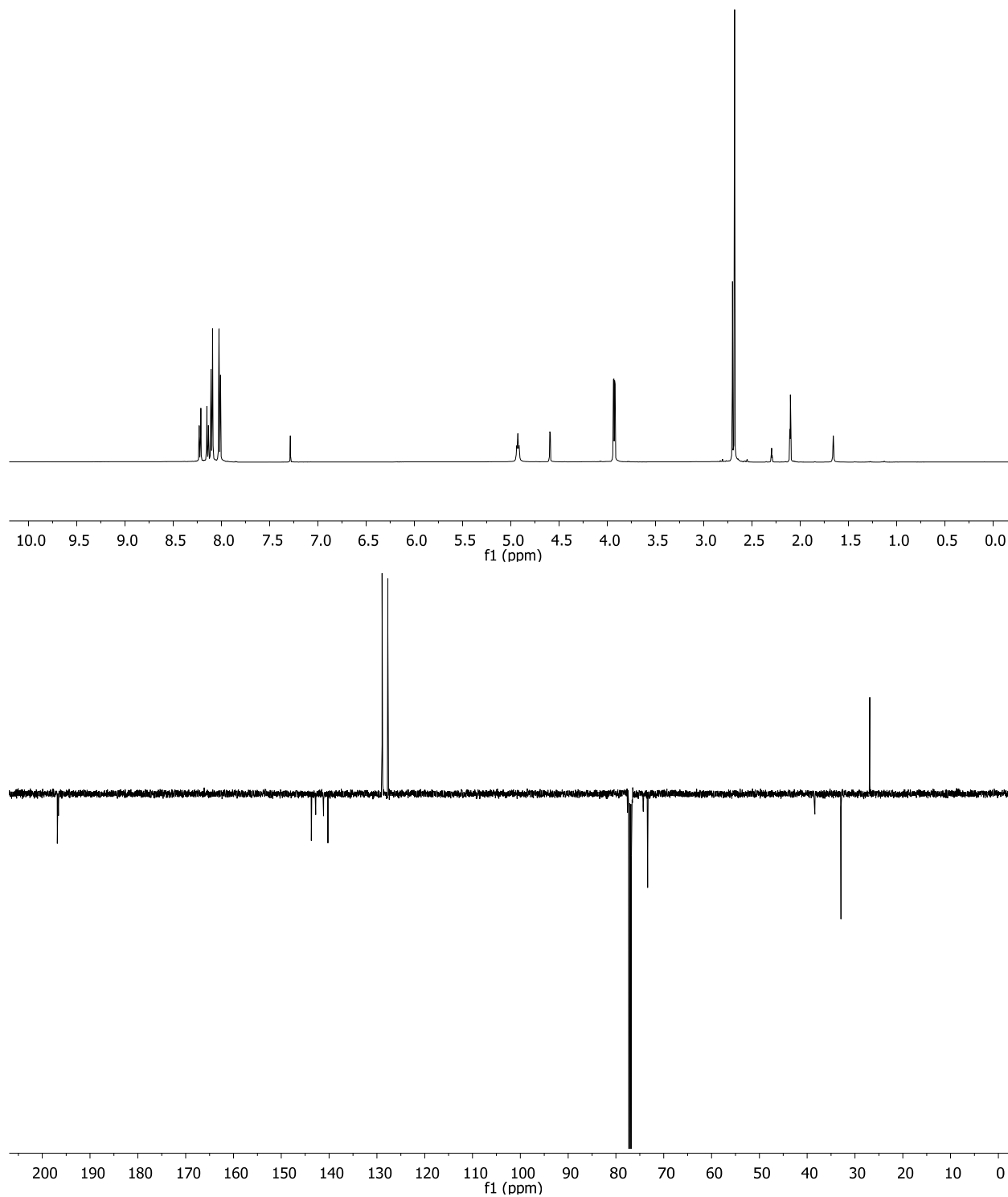


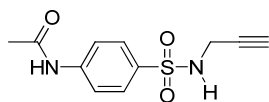
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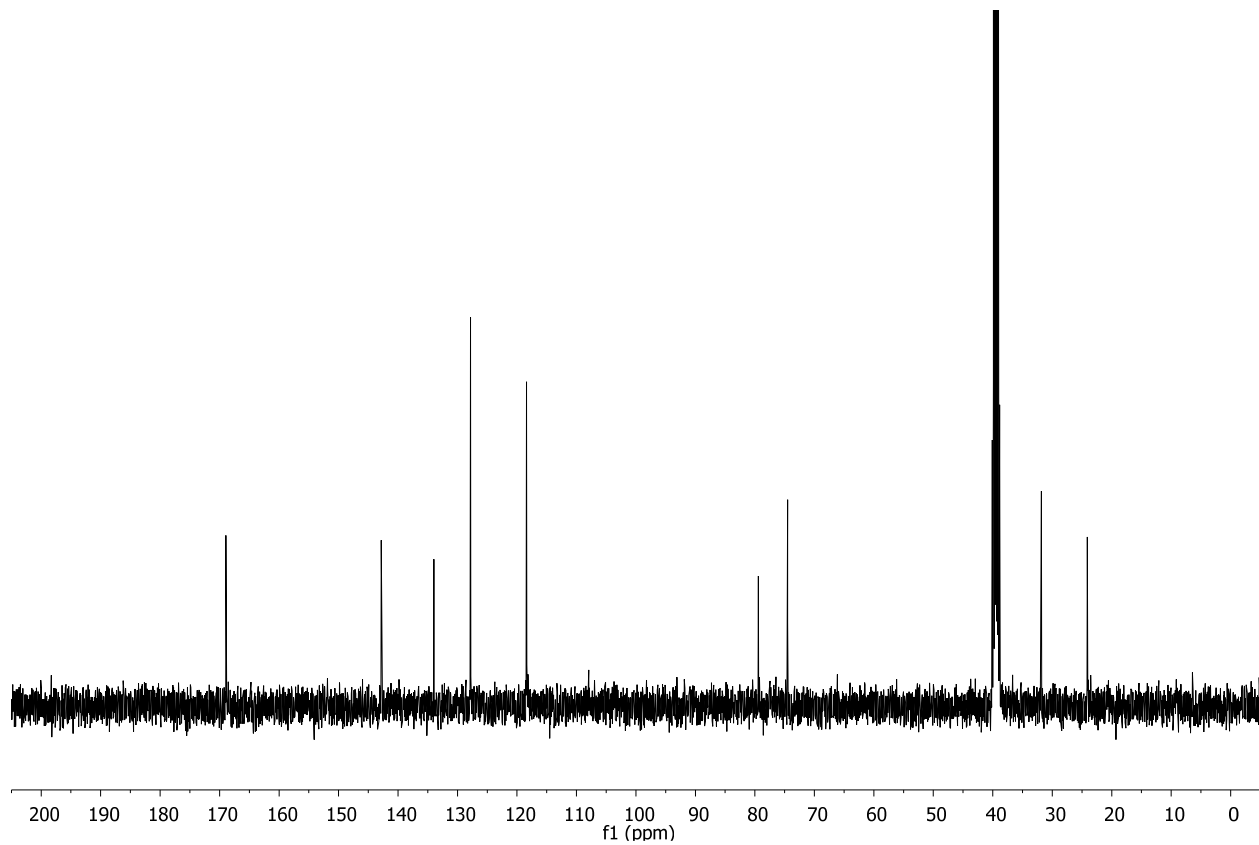
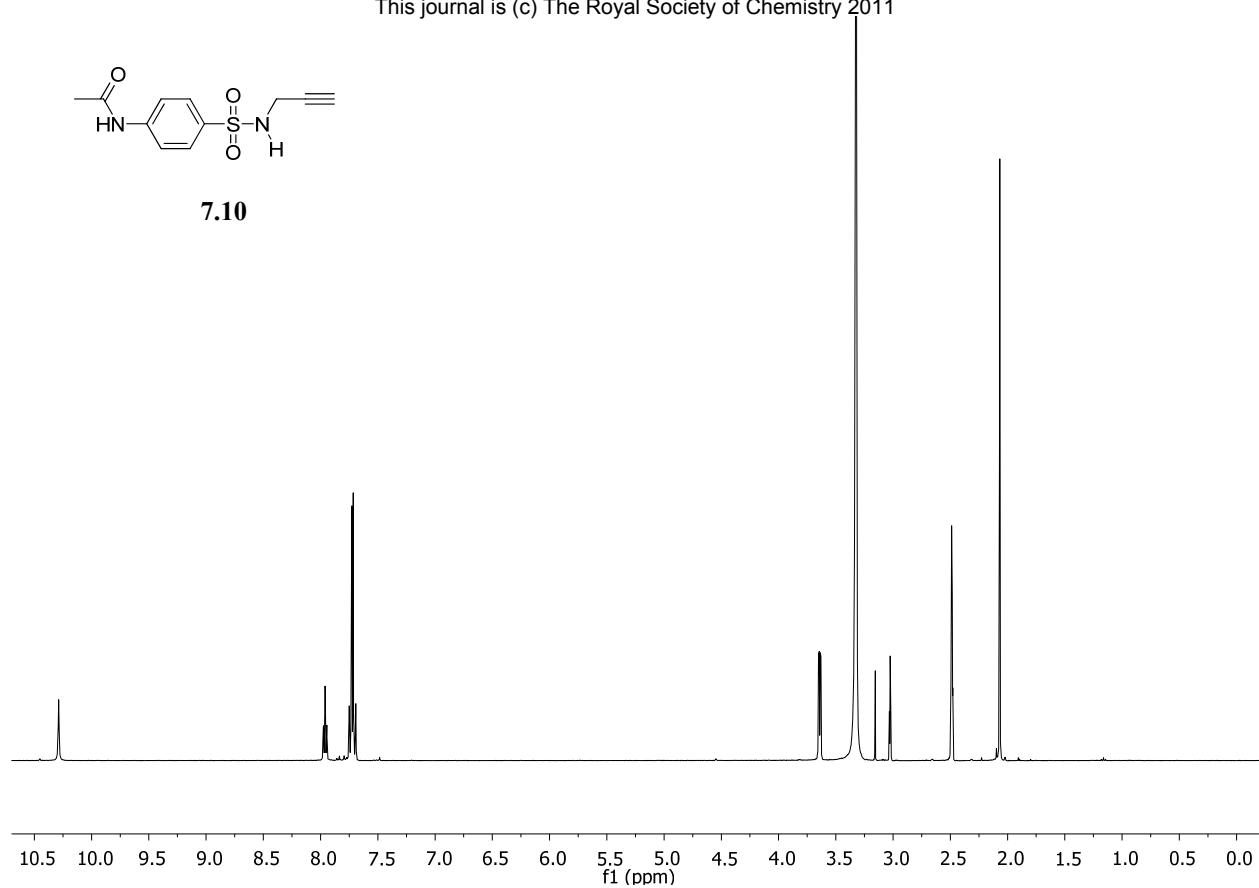


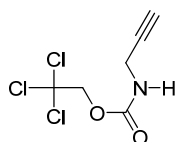
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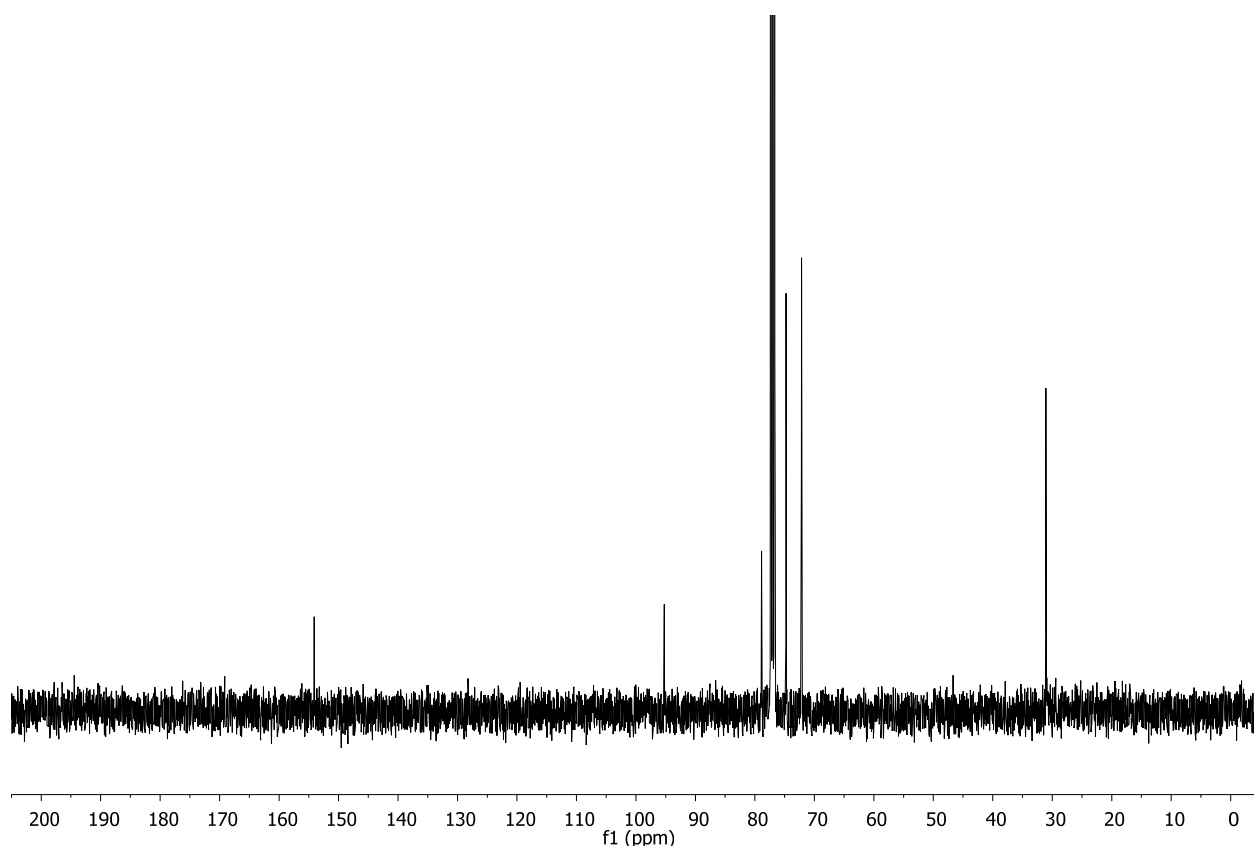
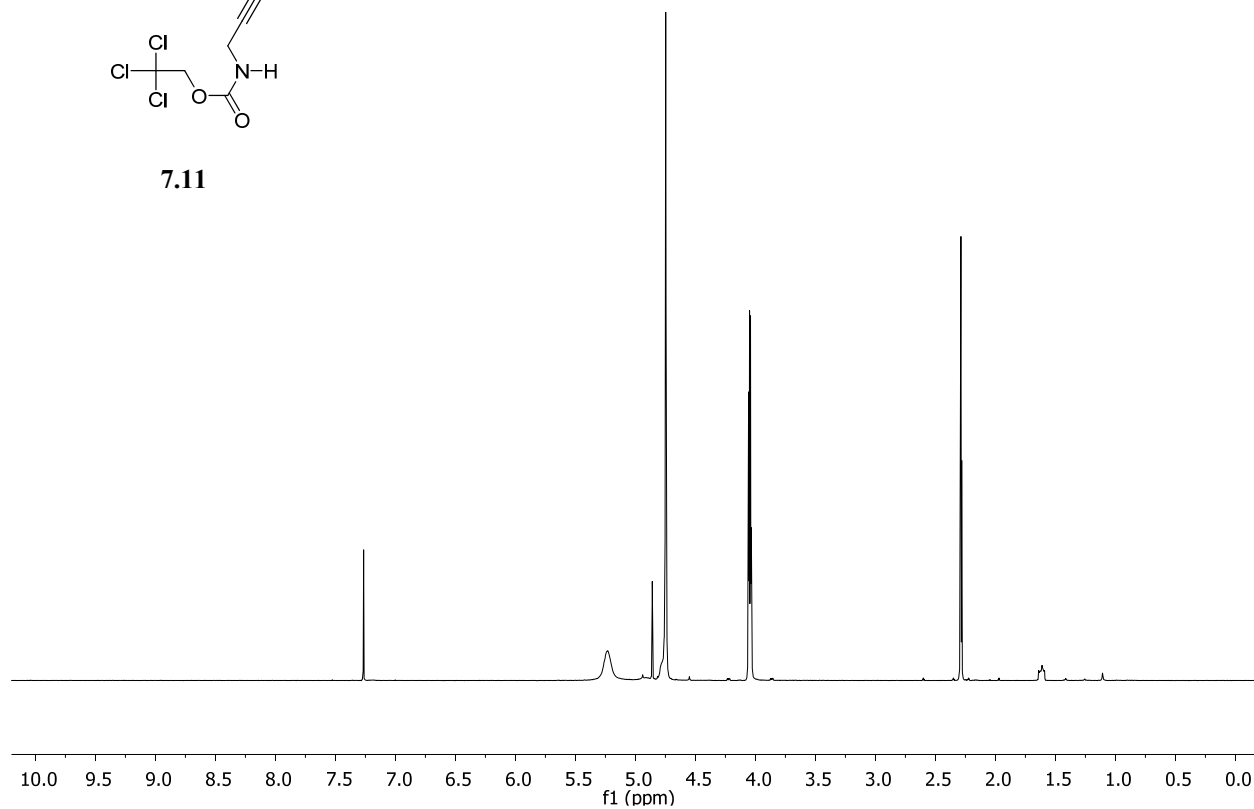


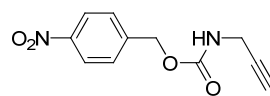
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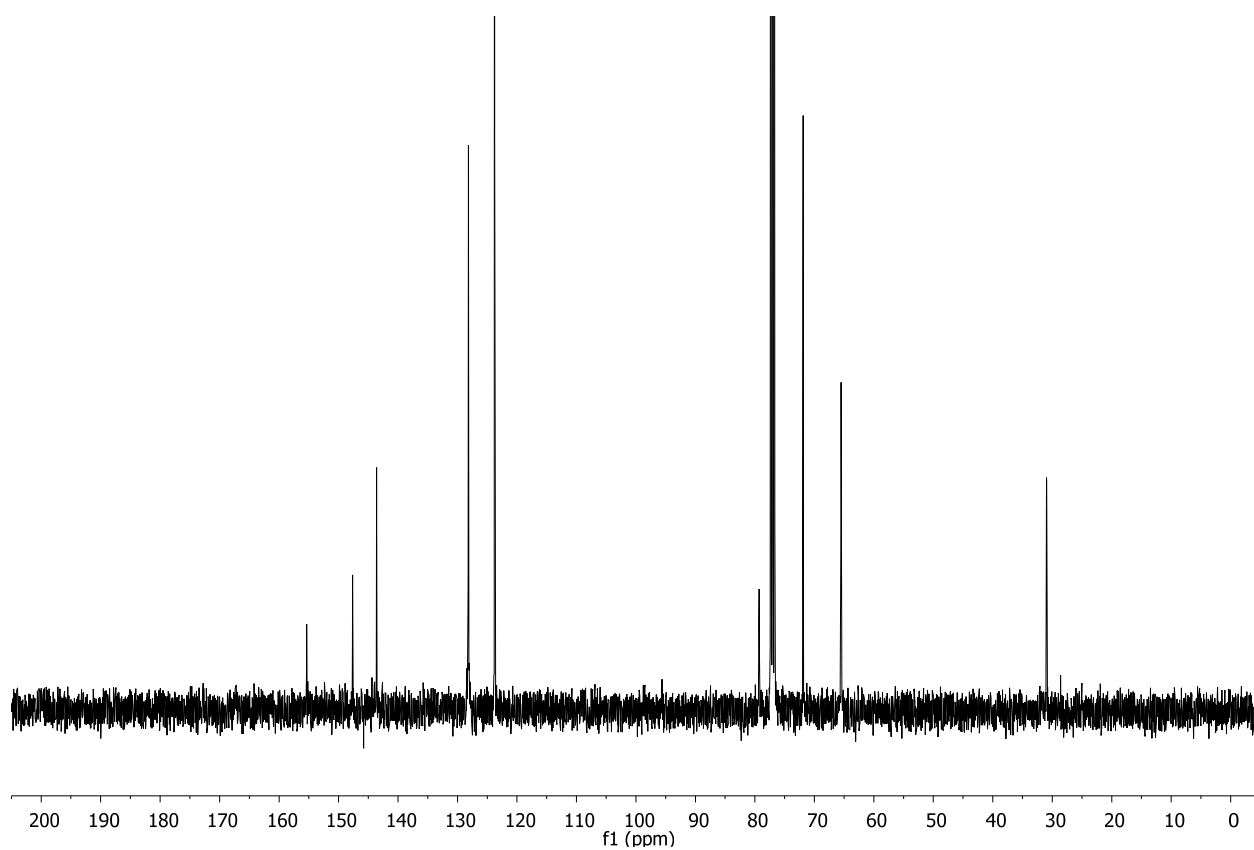
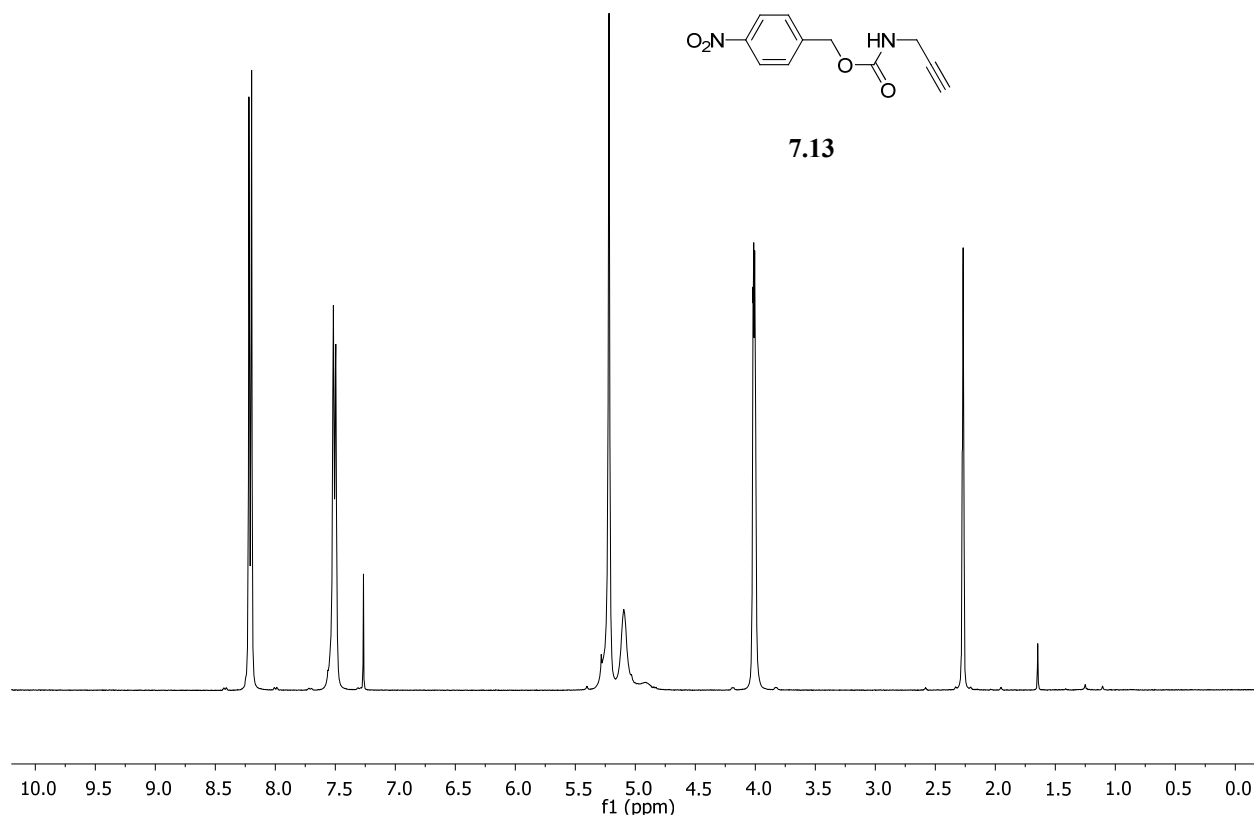


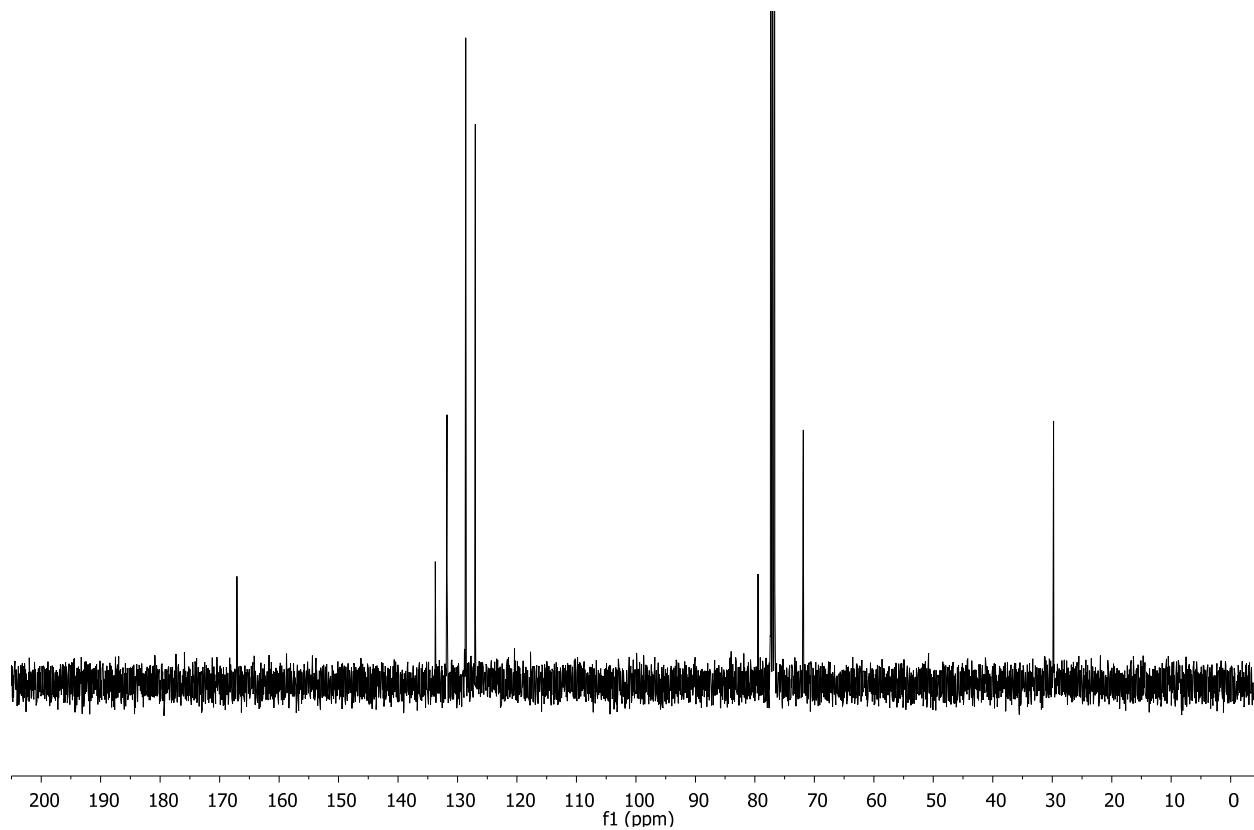
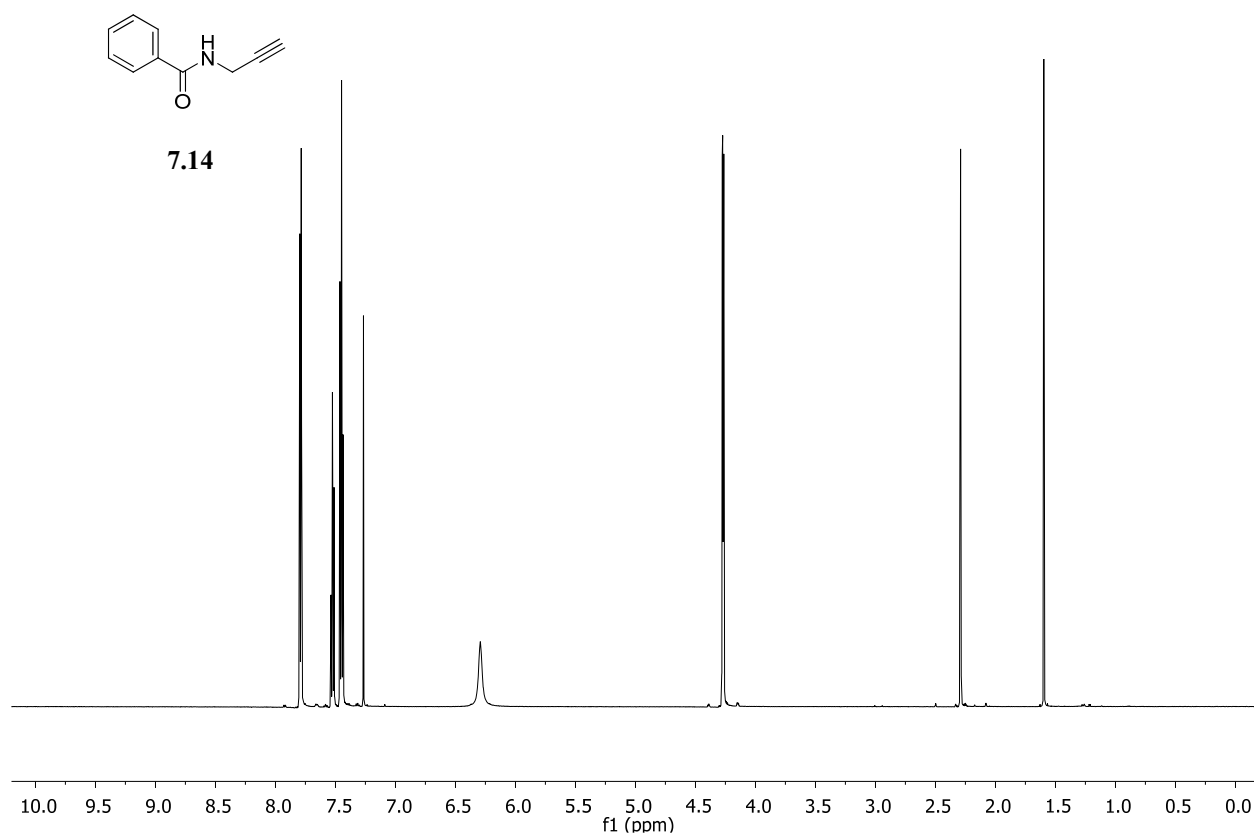
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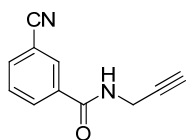




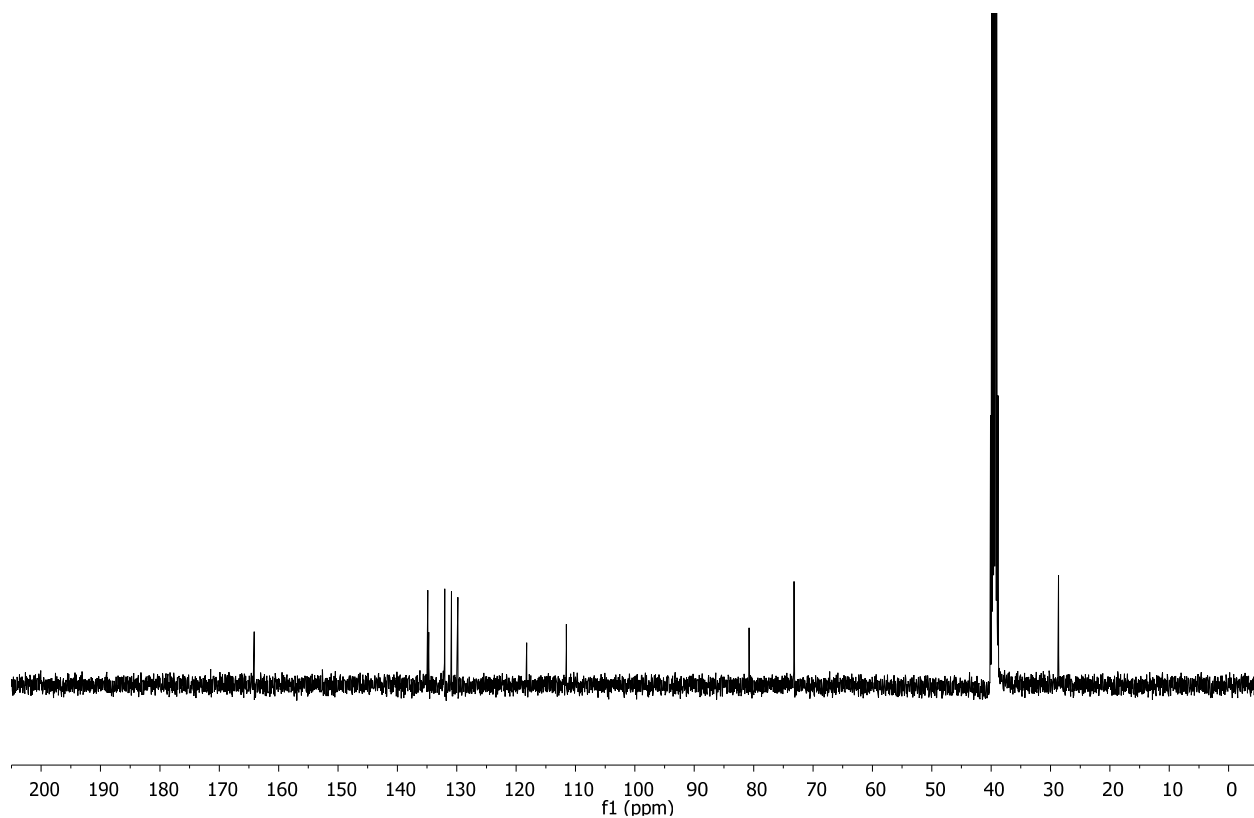
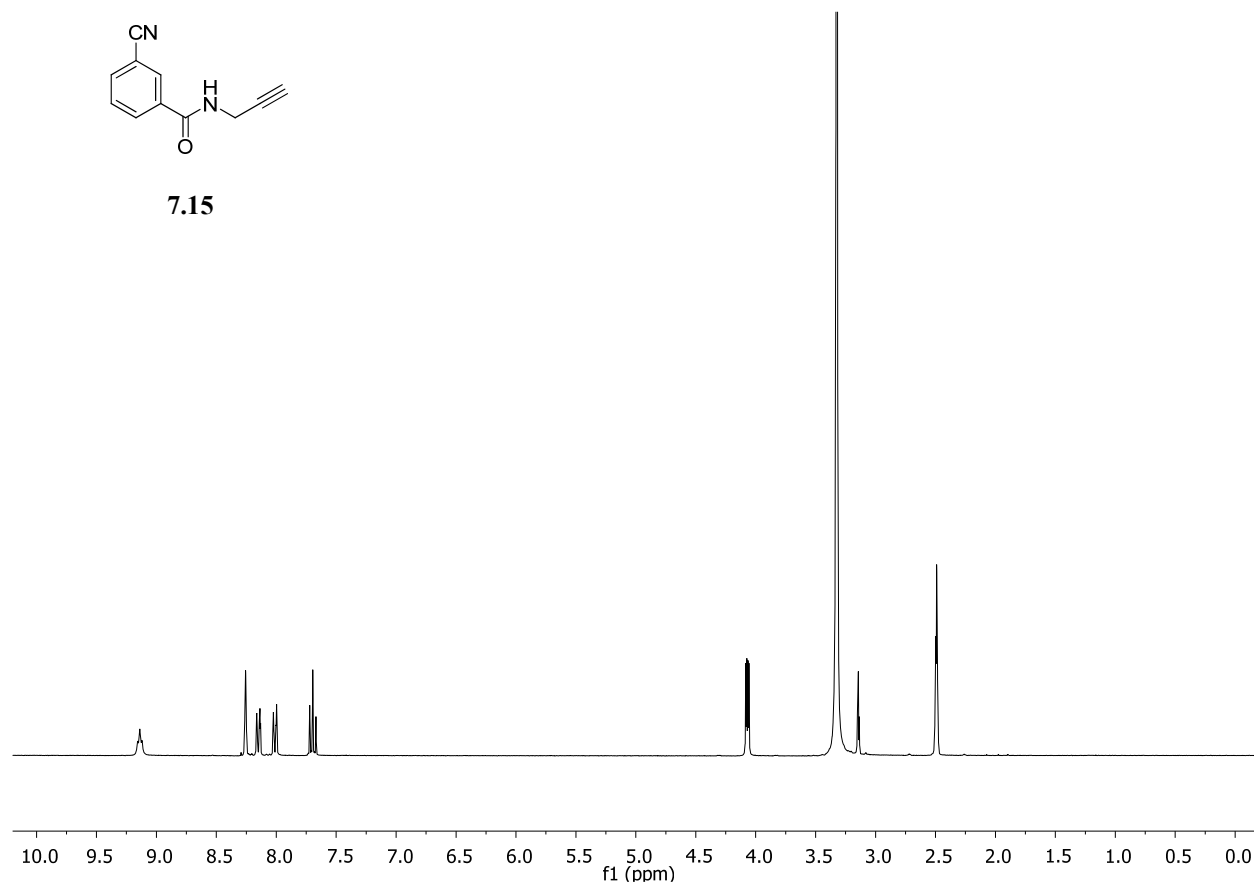
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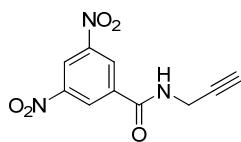




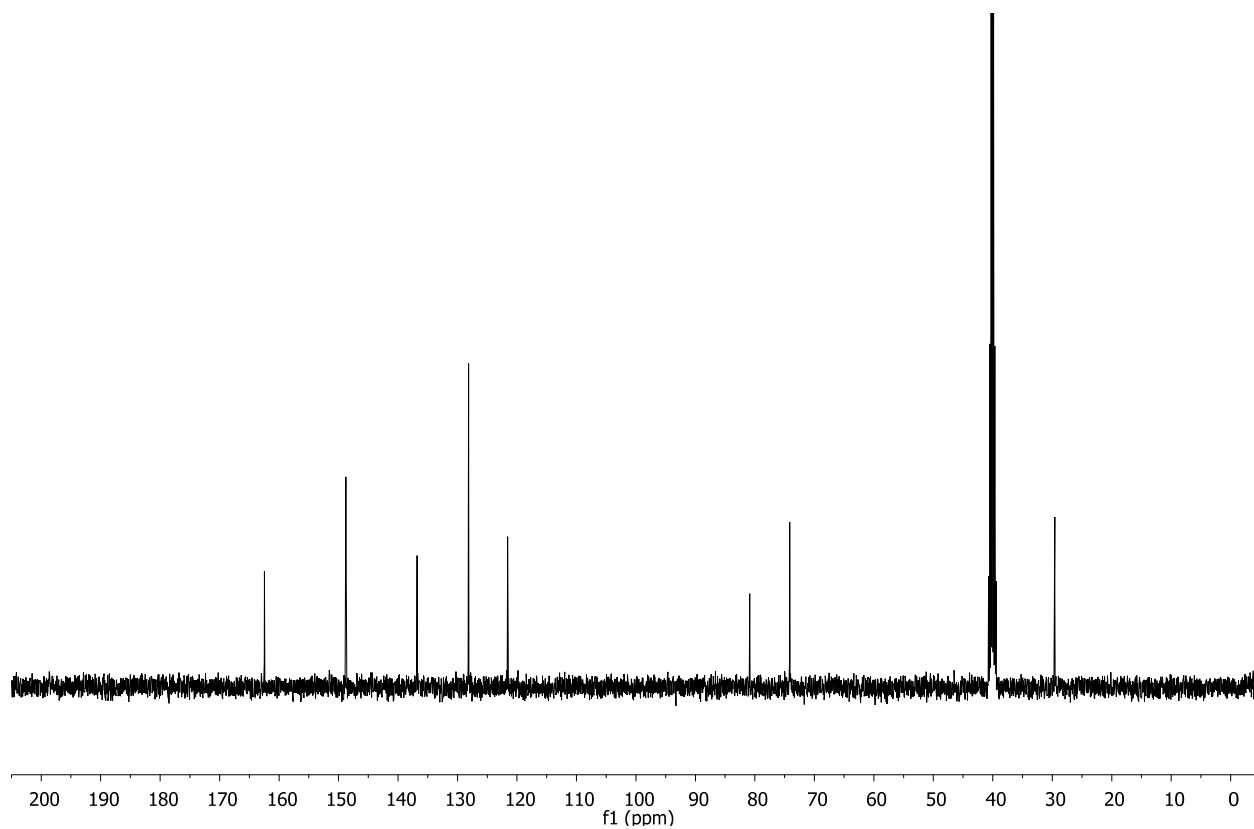
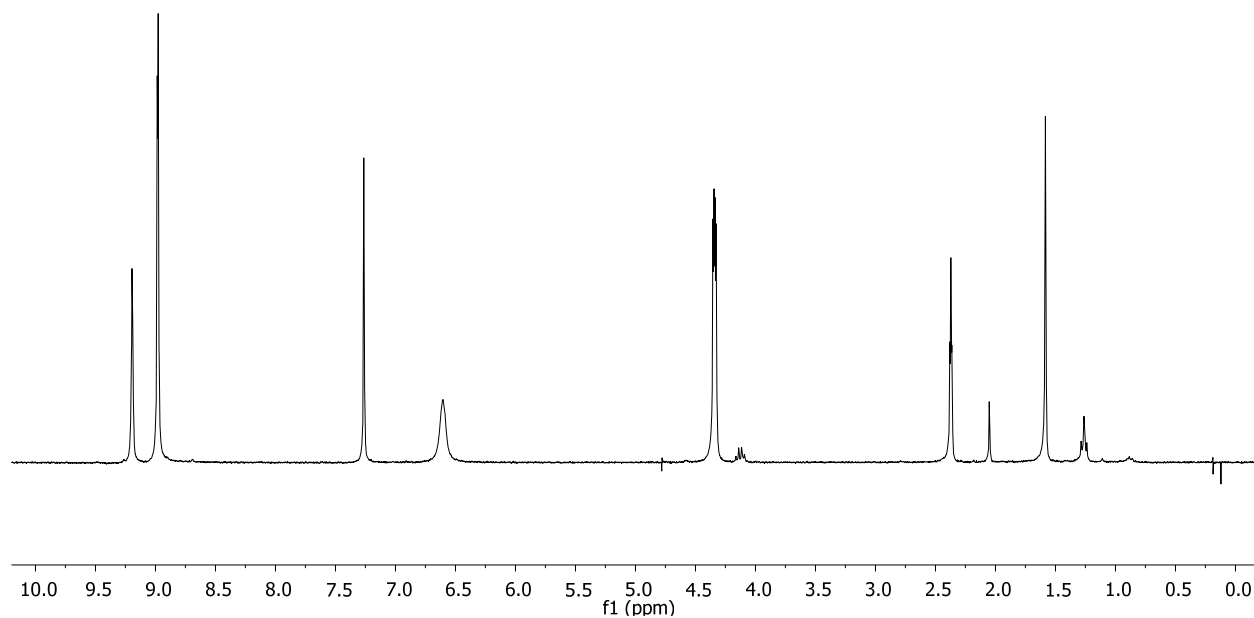


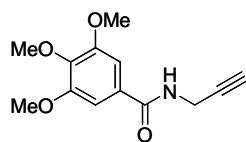
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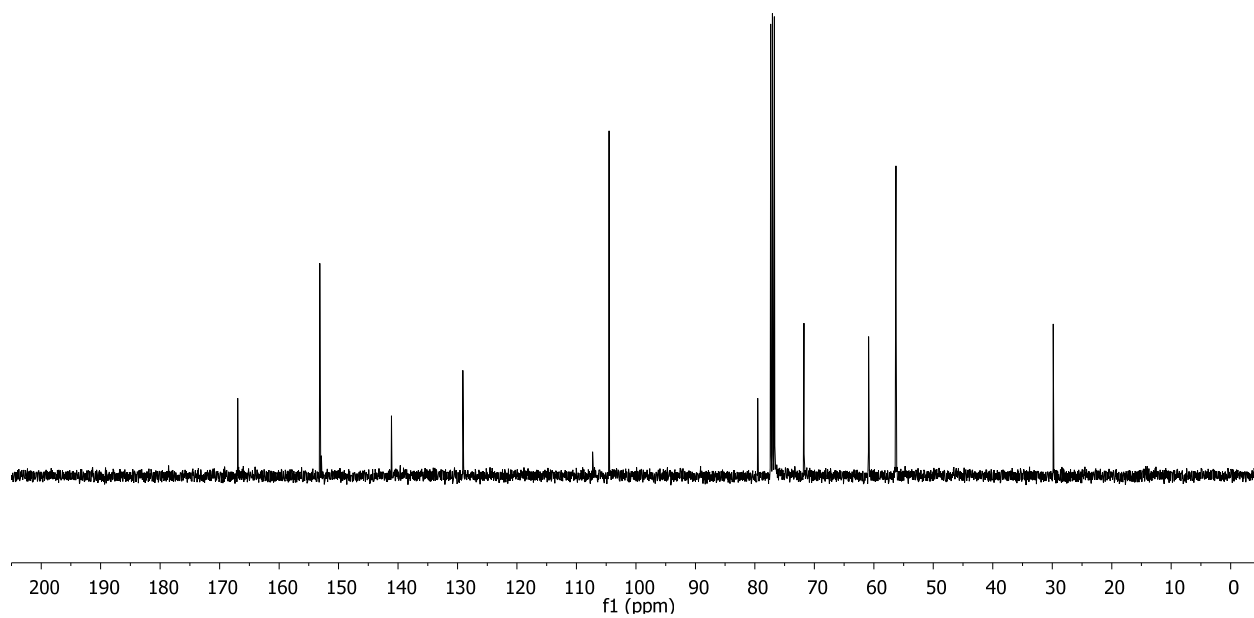
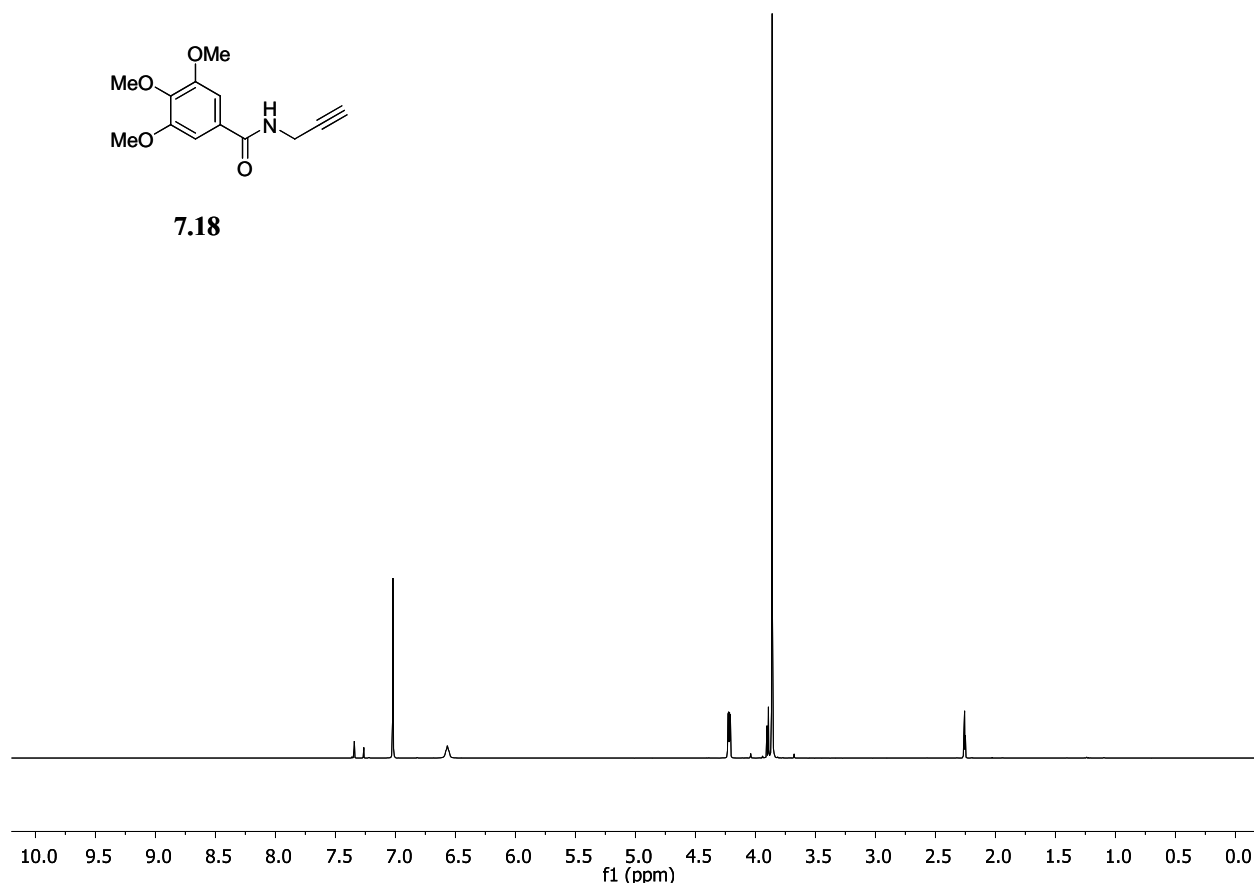


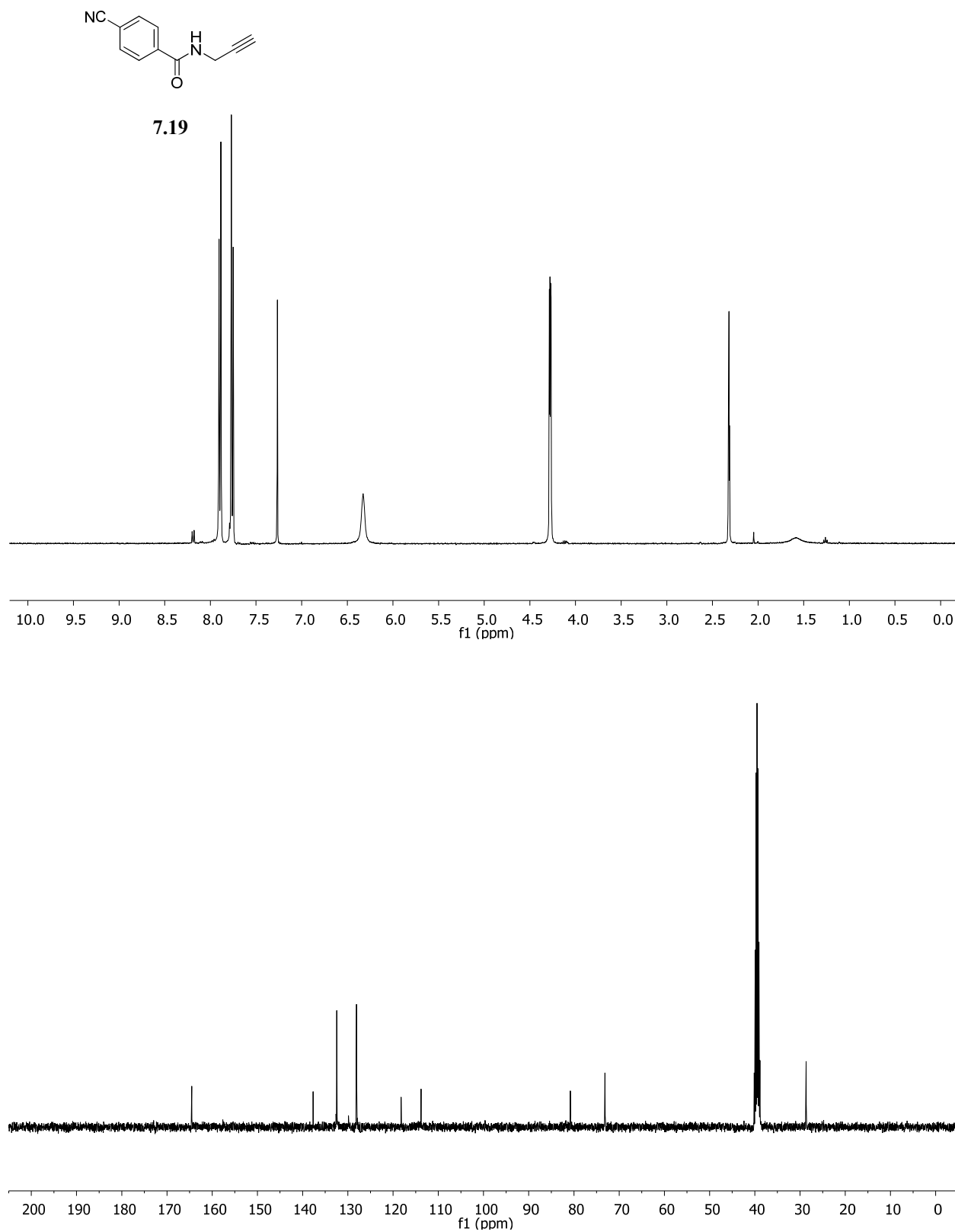
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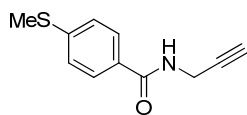




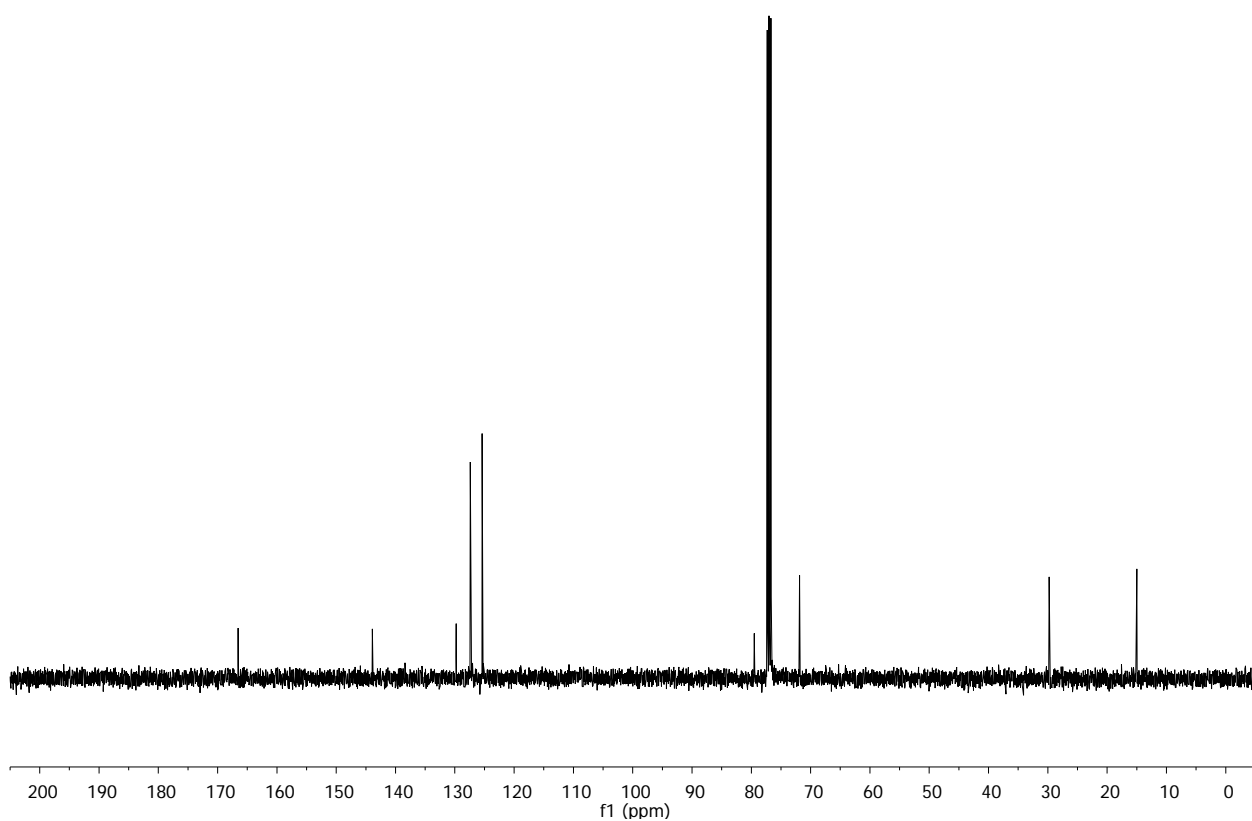
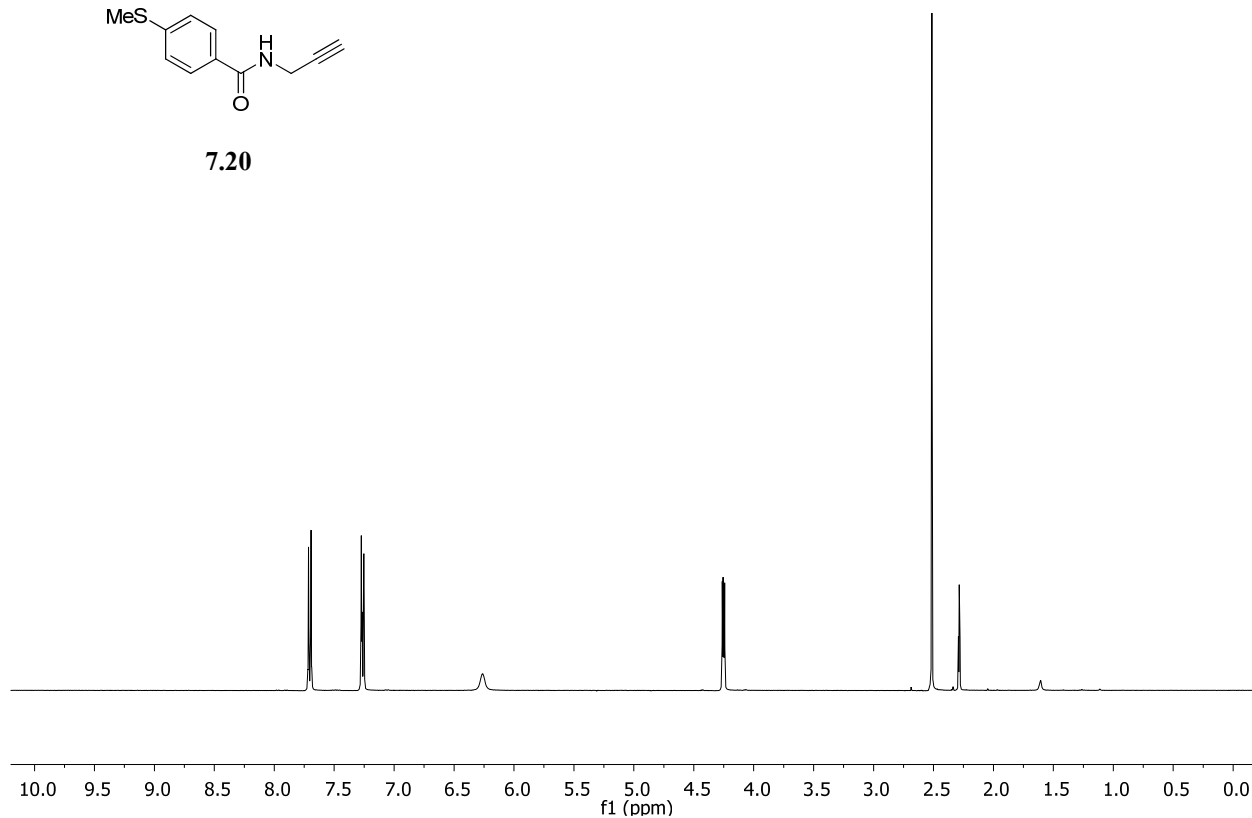
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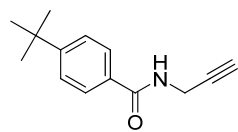




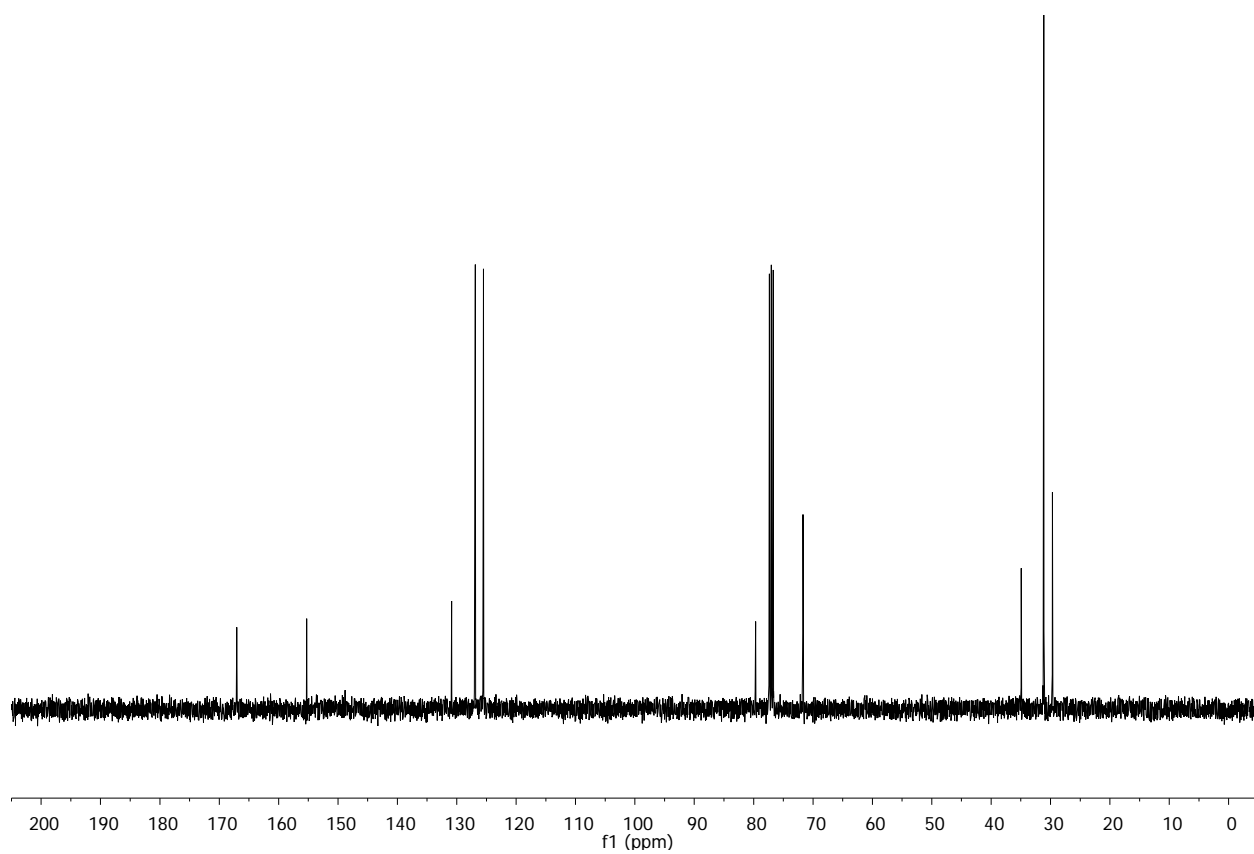
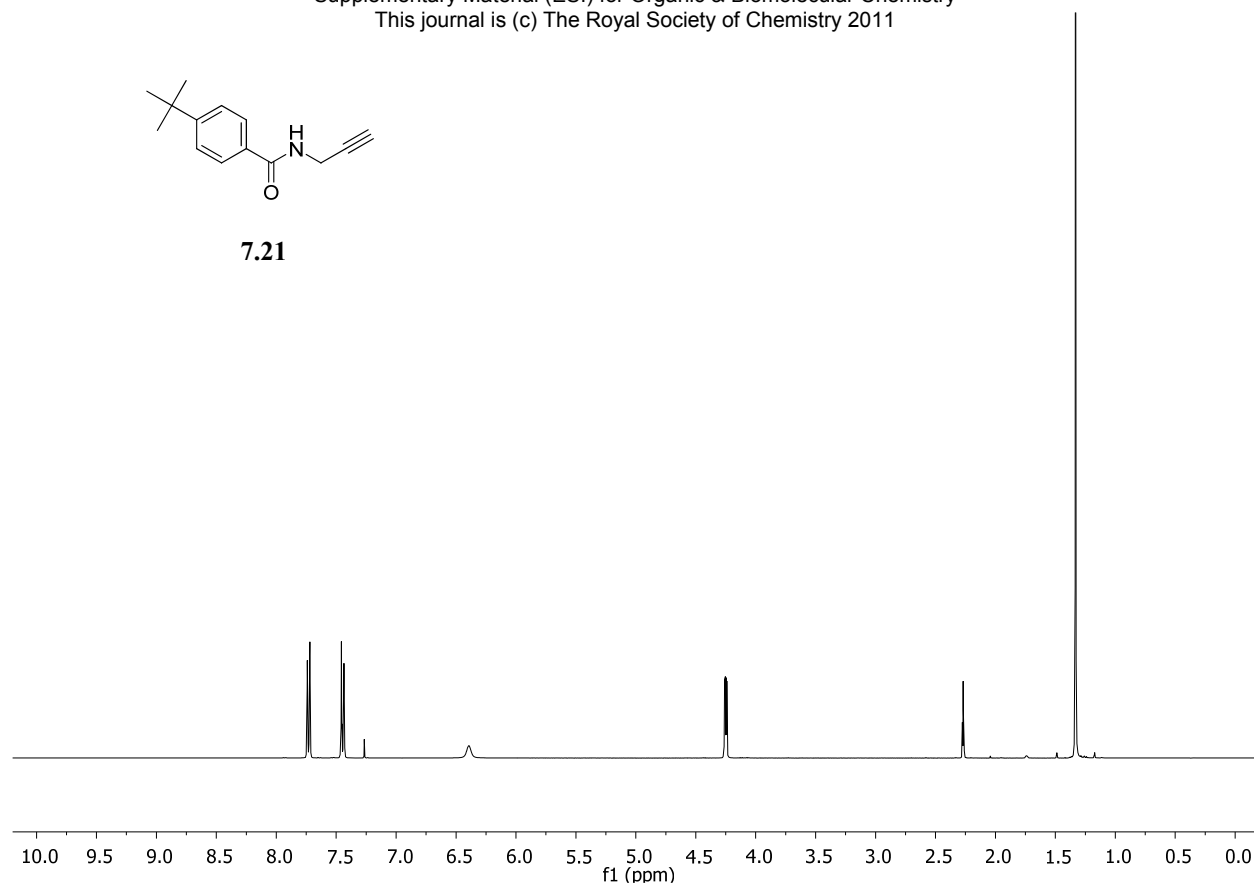


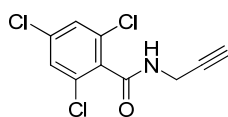
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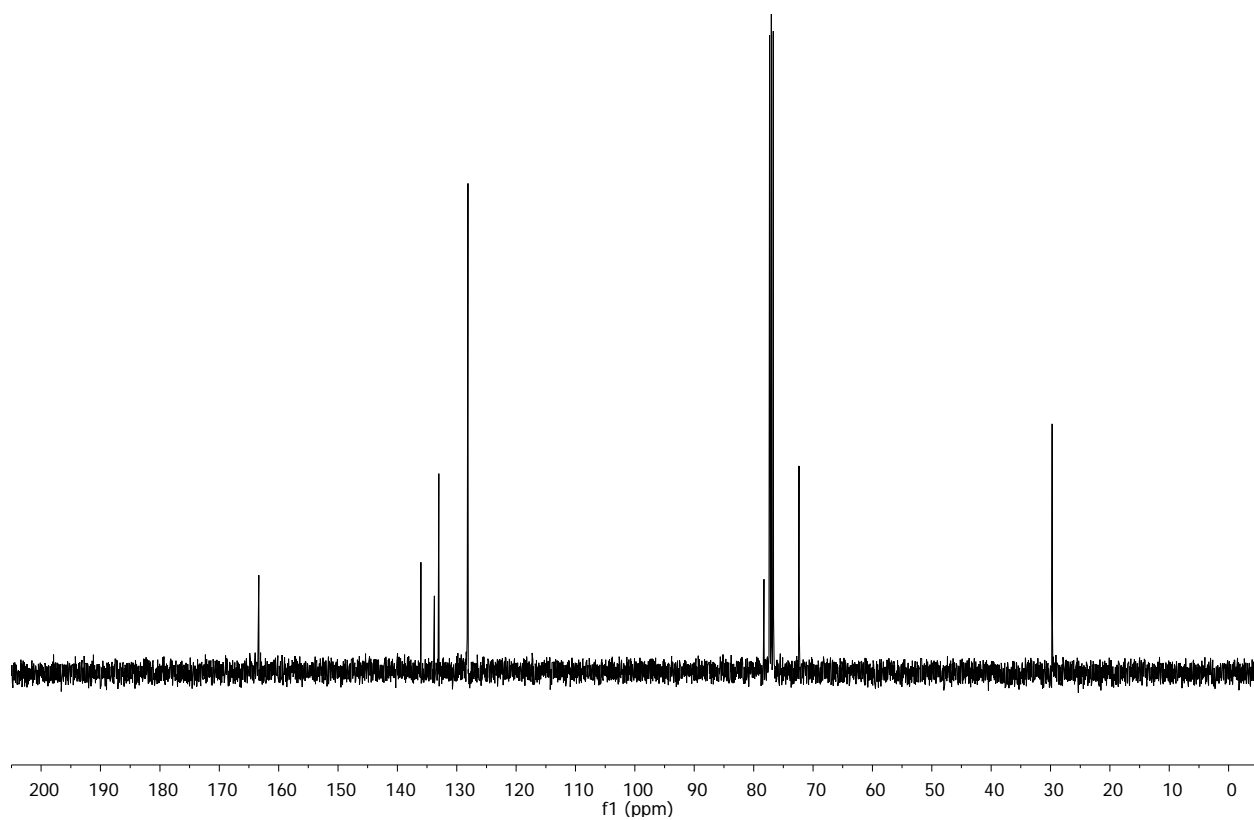
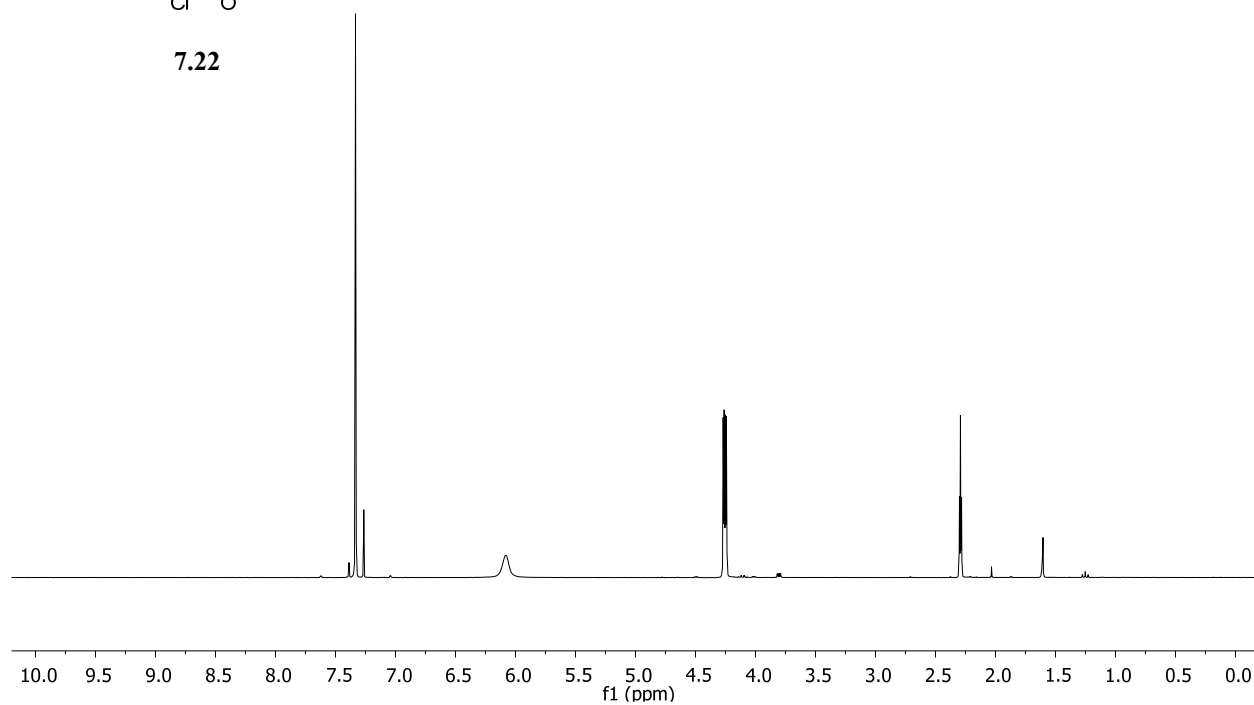


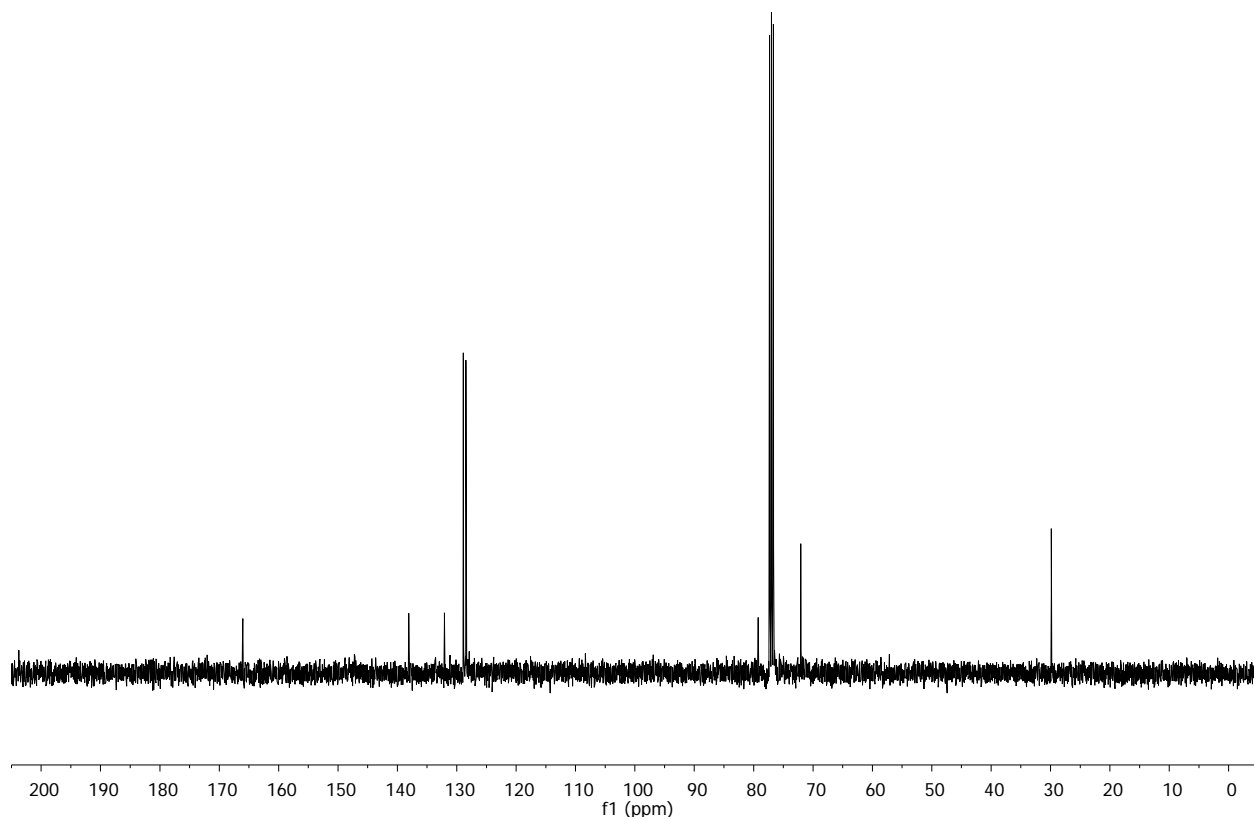
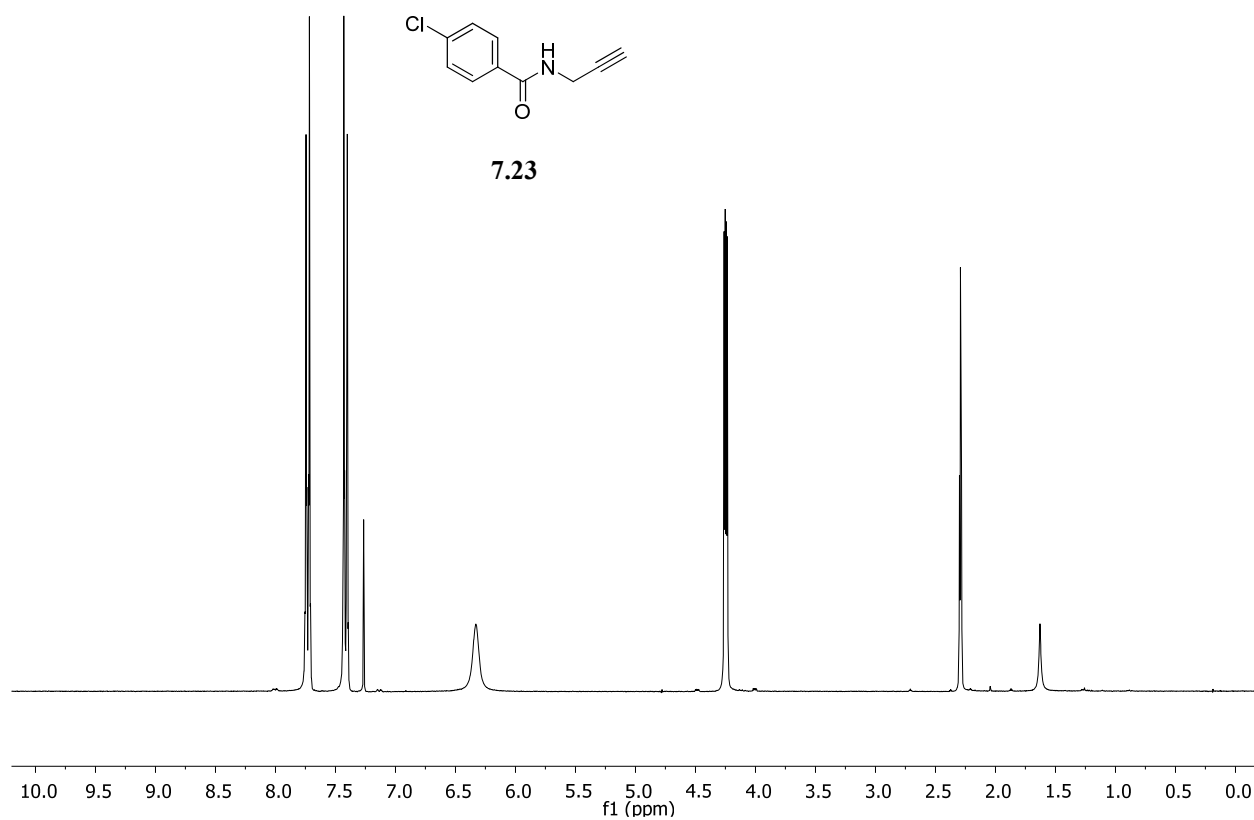
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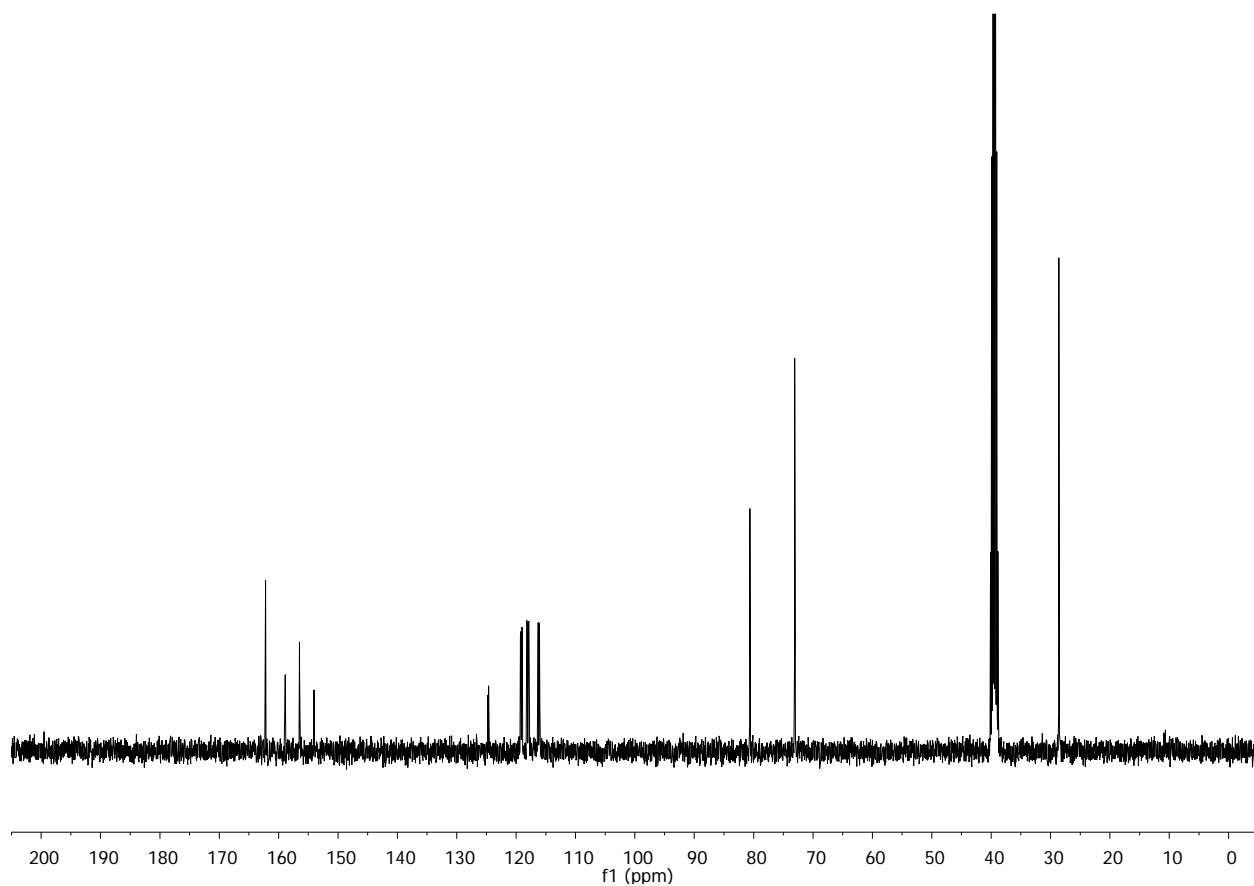
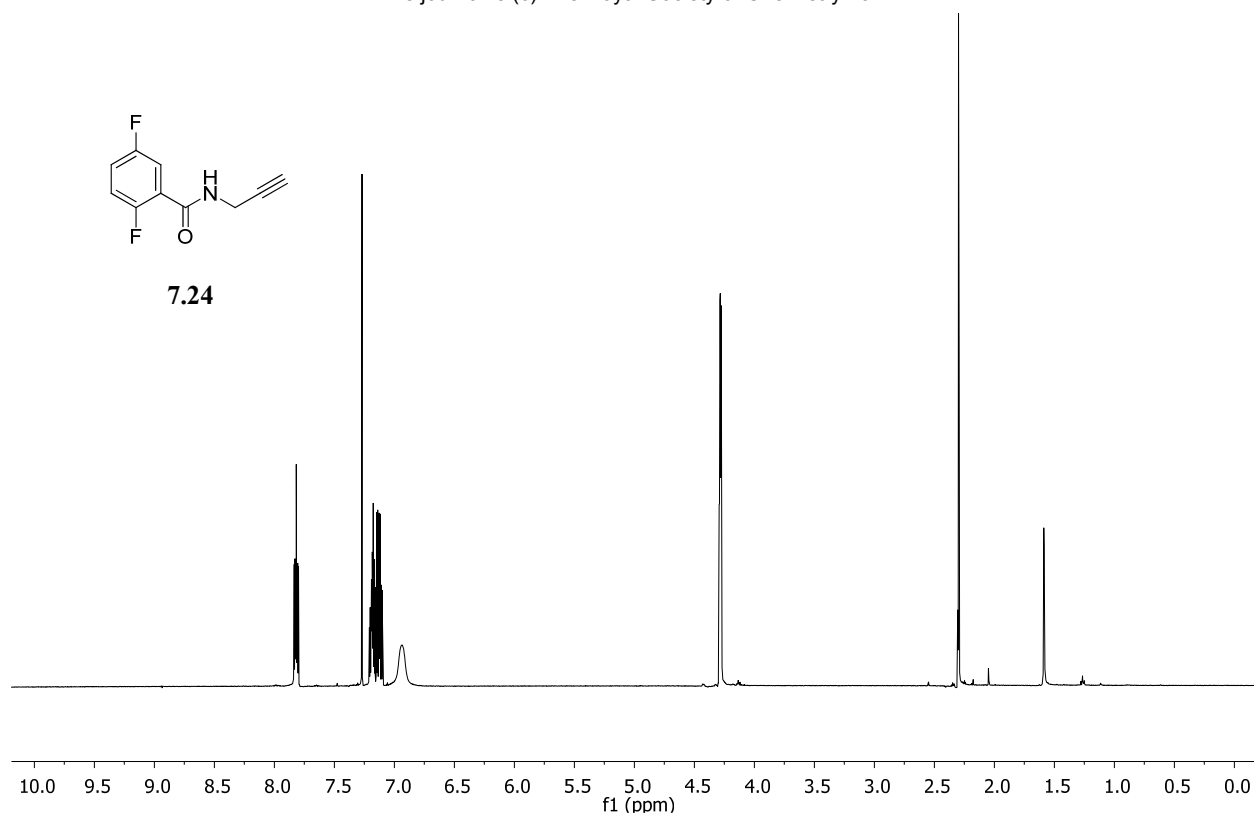


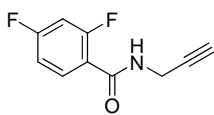


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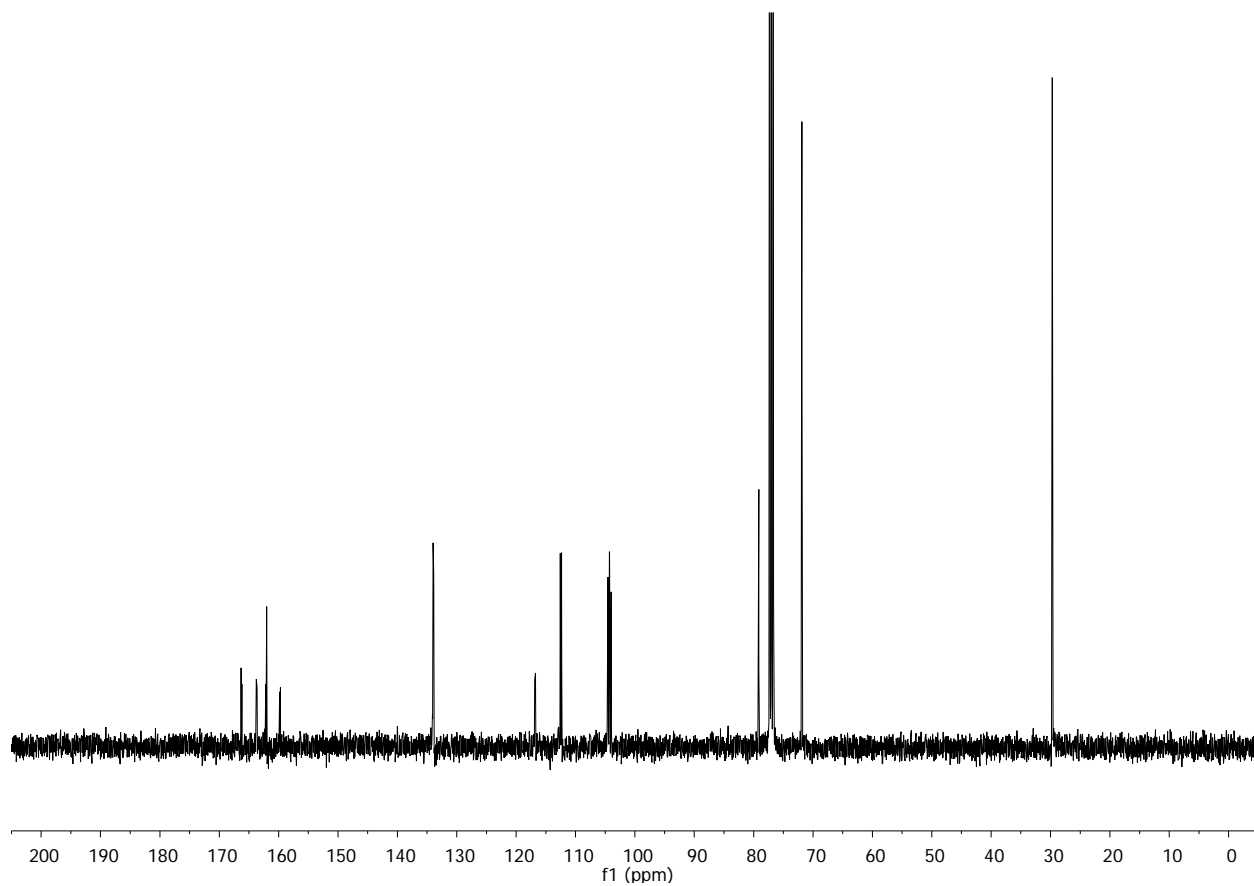
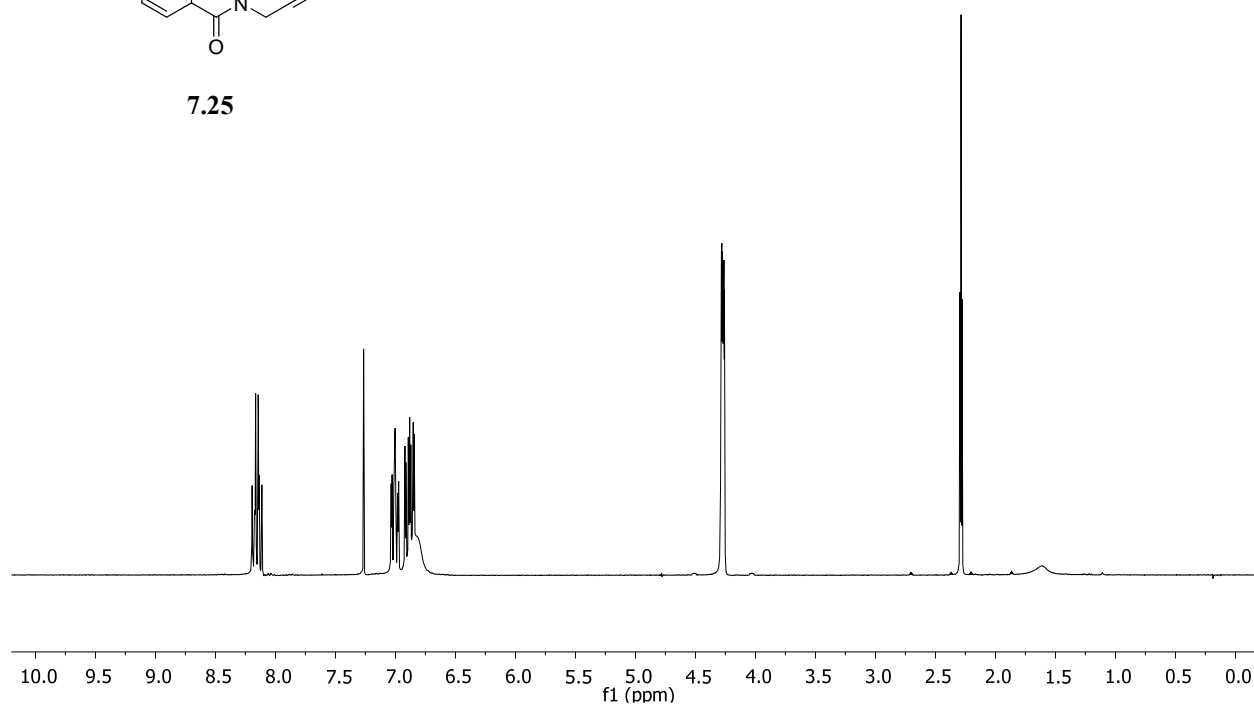


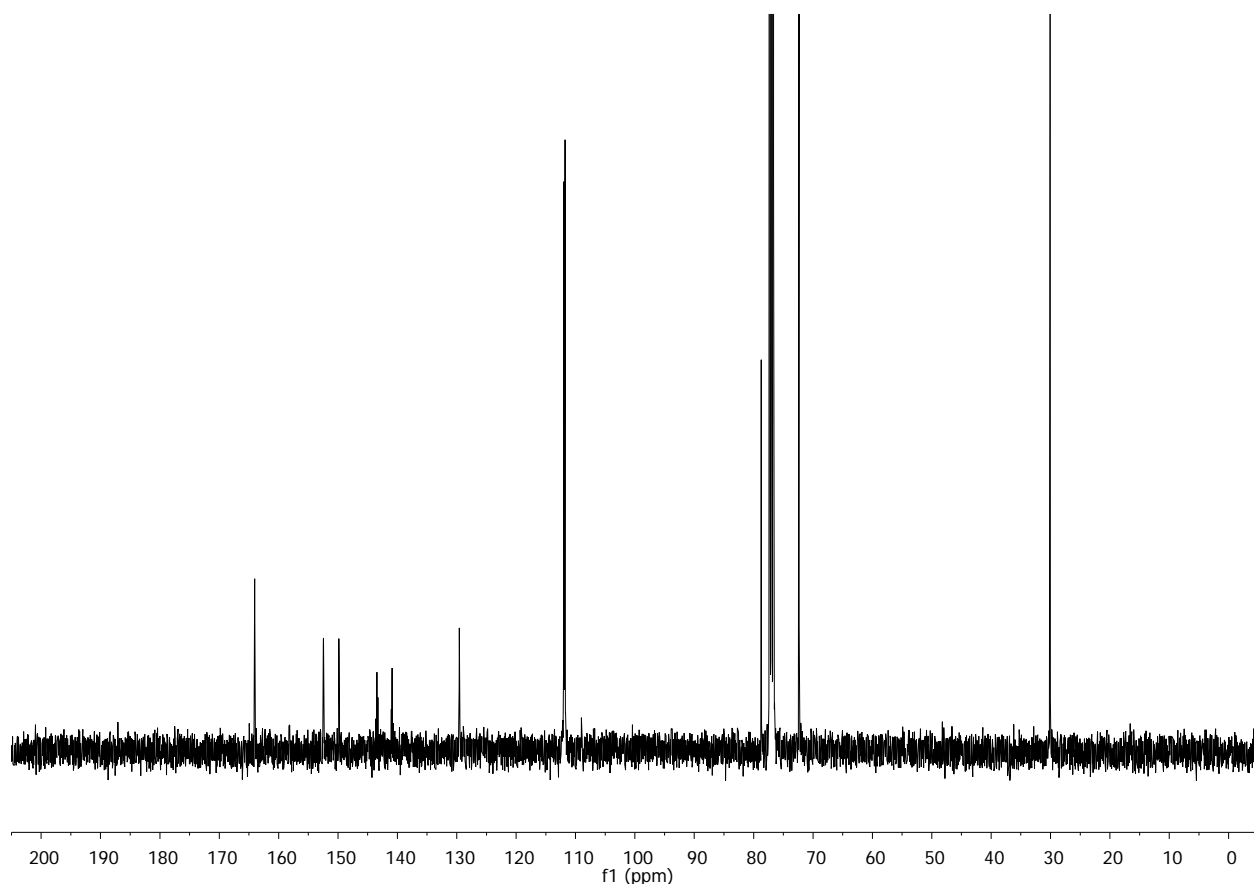
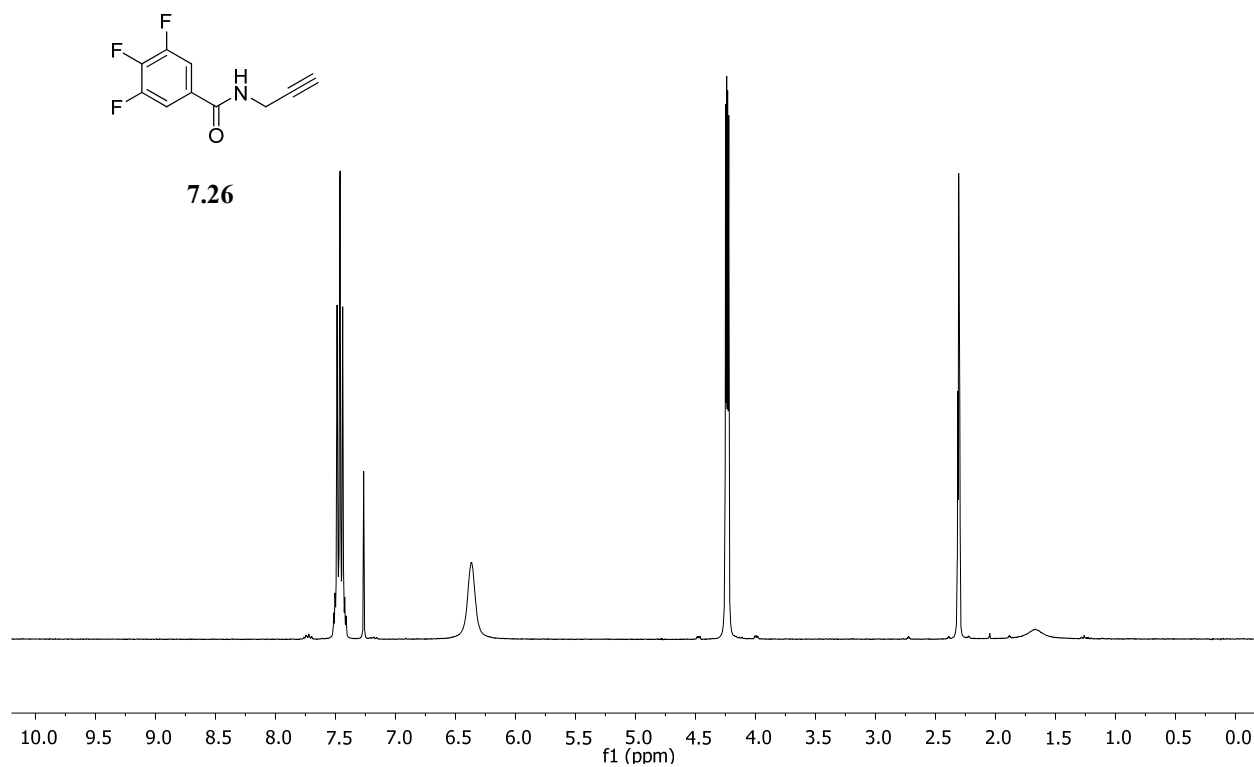


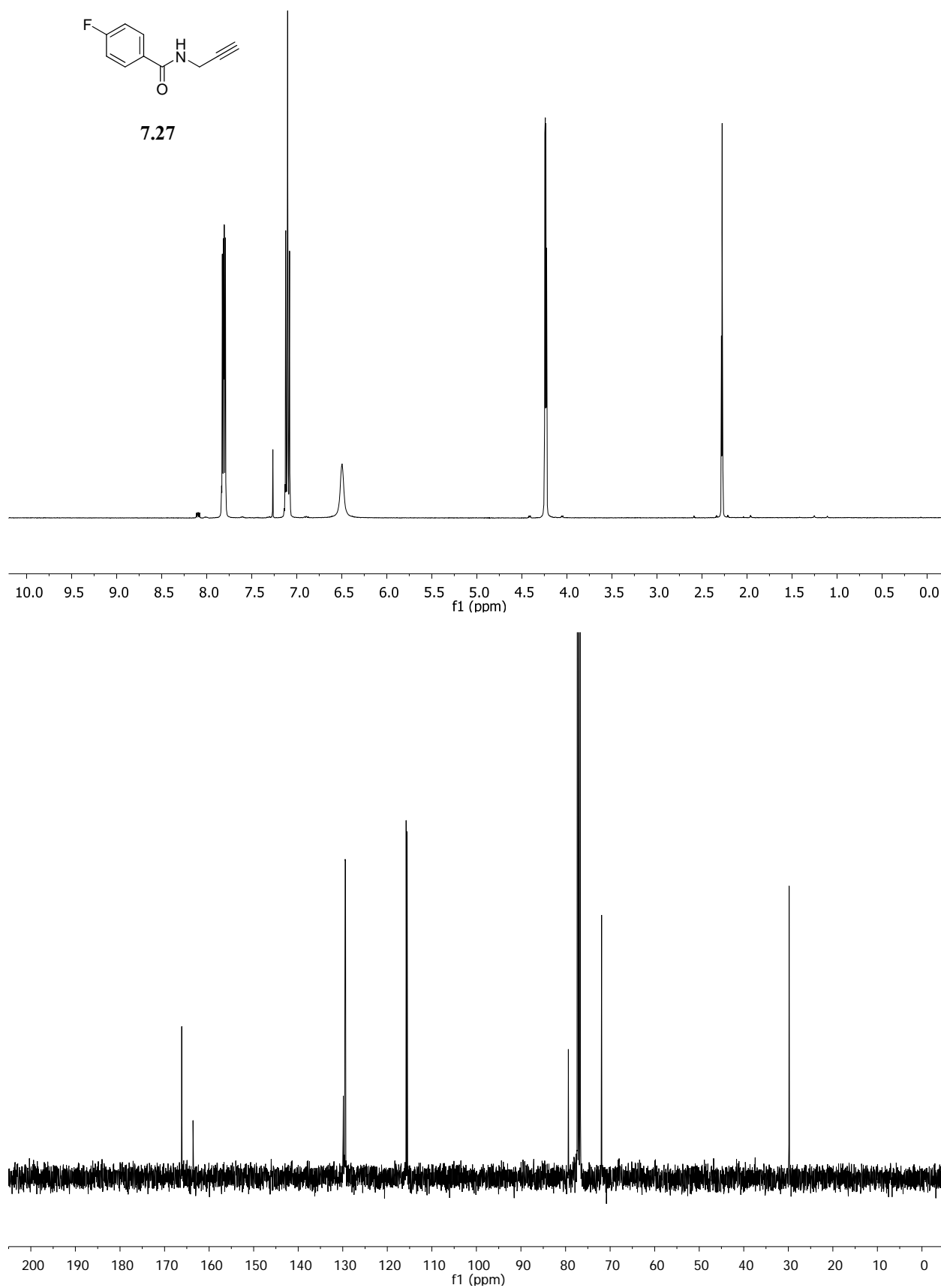


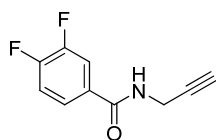


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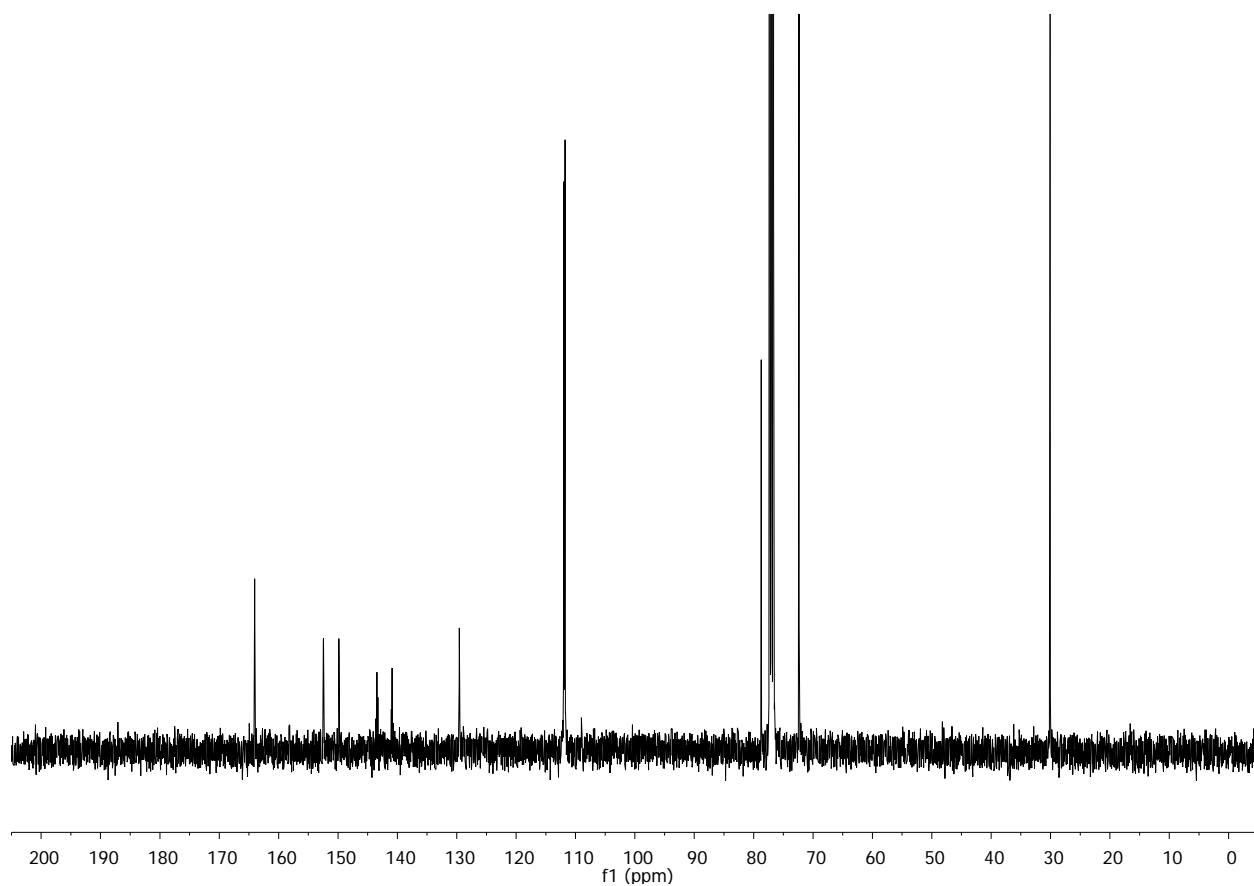
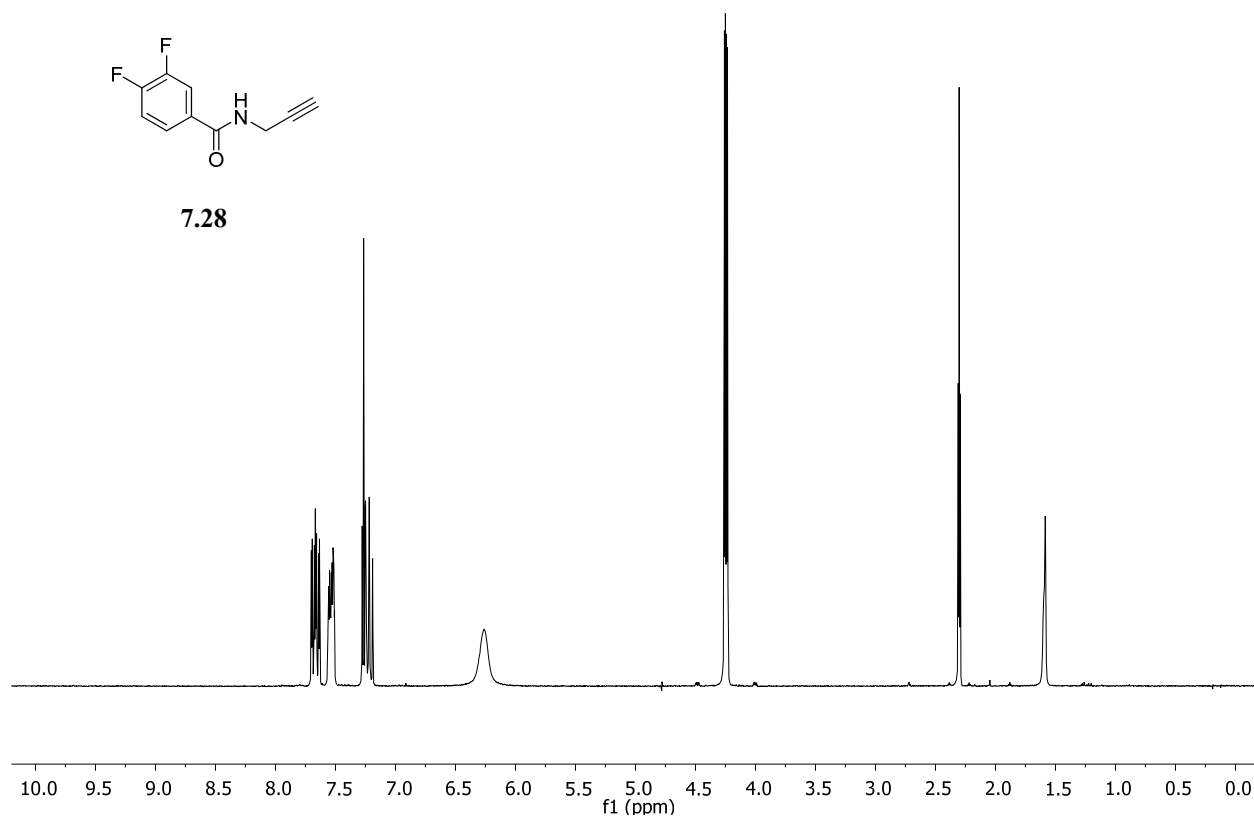


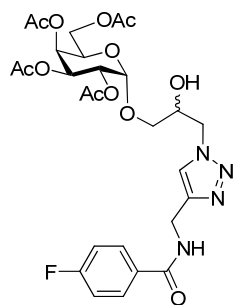




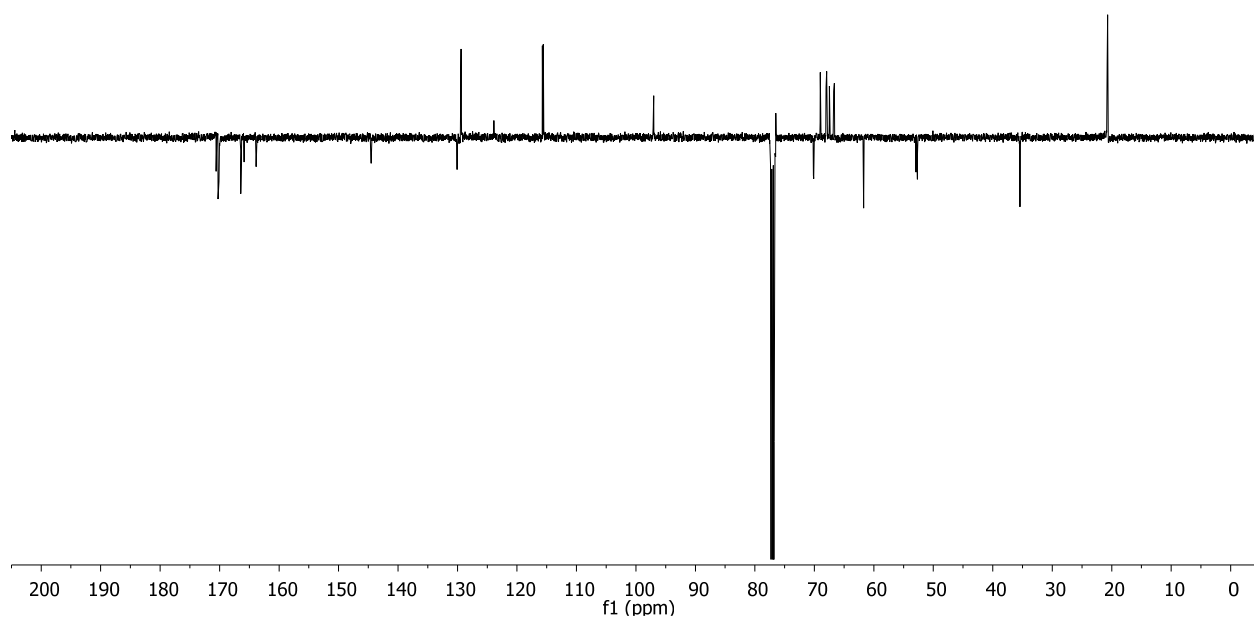
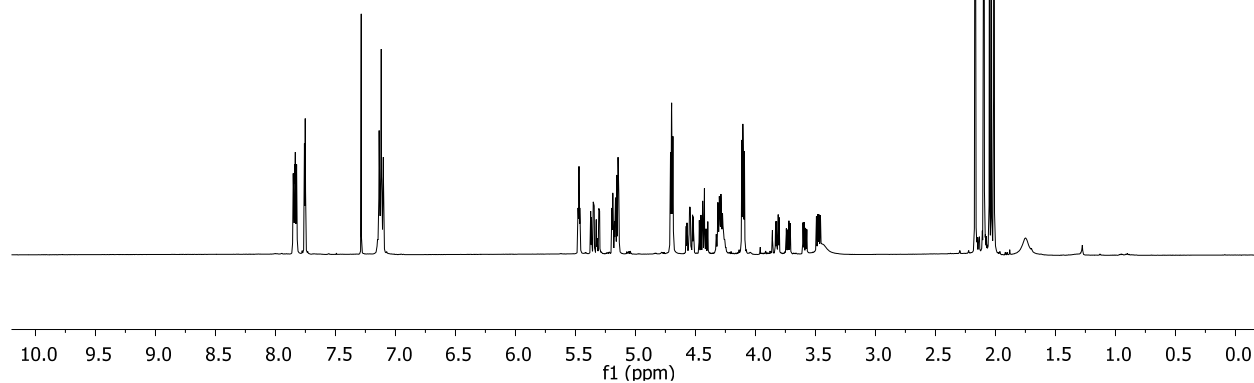


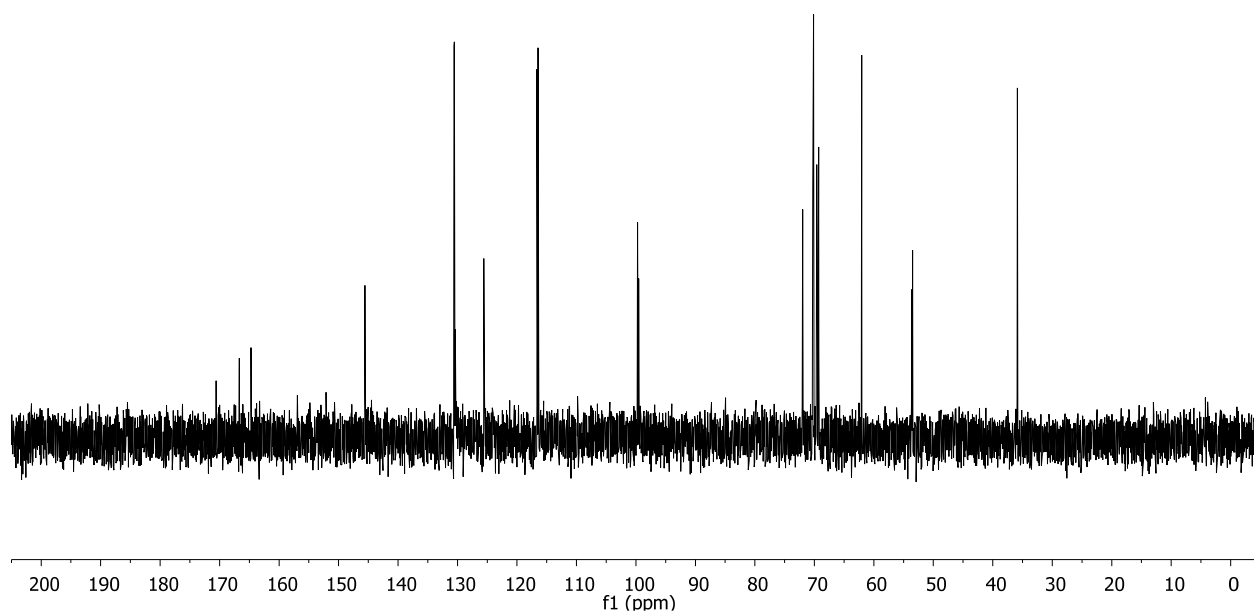
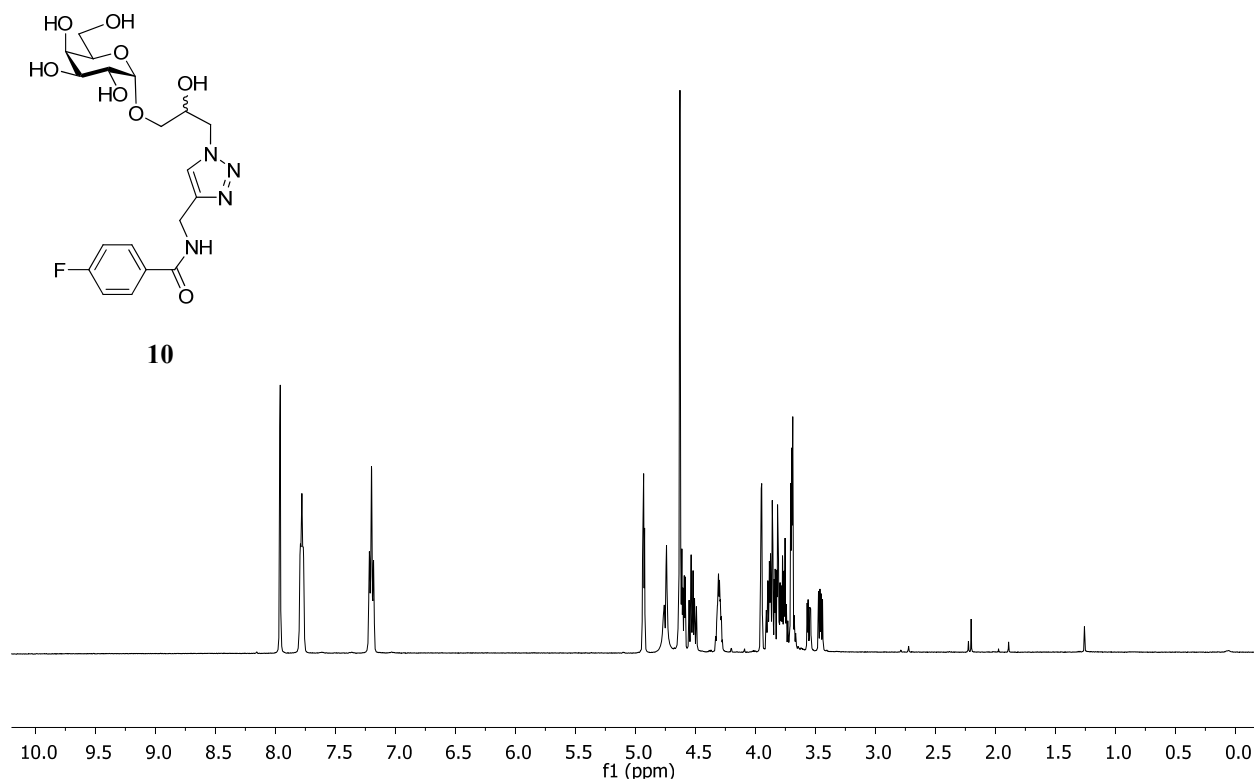
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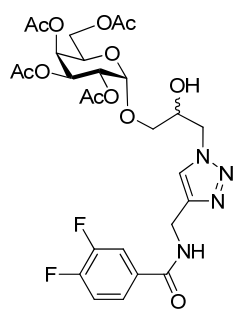




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