

Supporting Information (3)

Lipophilicity and click reactivity determine the performance of bioorthogonal tetrazine tools in pretargeted *in vivo* chemistry

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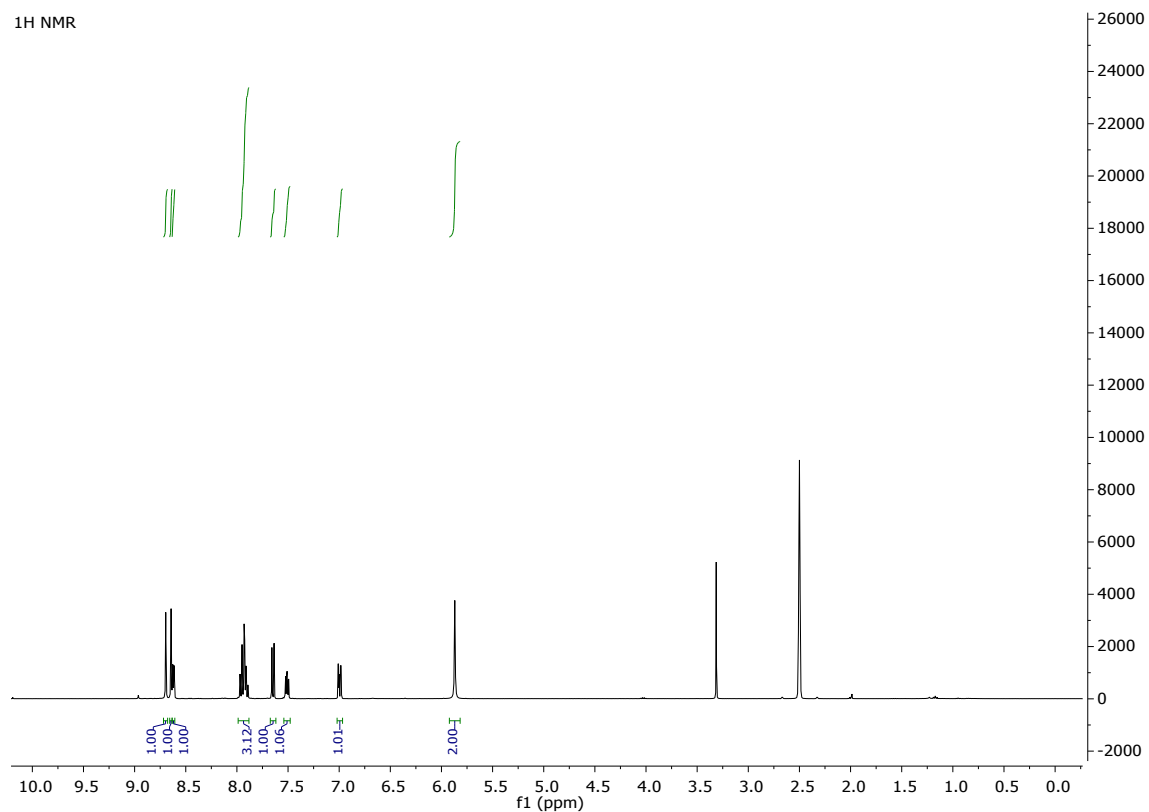
[†]Equally contributing authors

*Corresponding authors

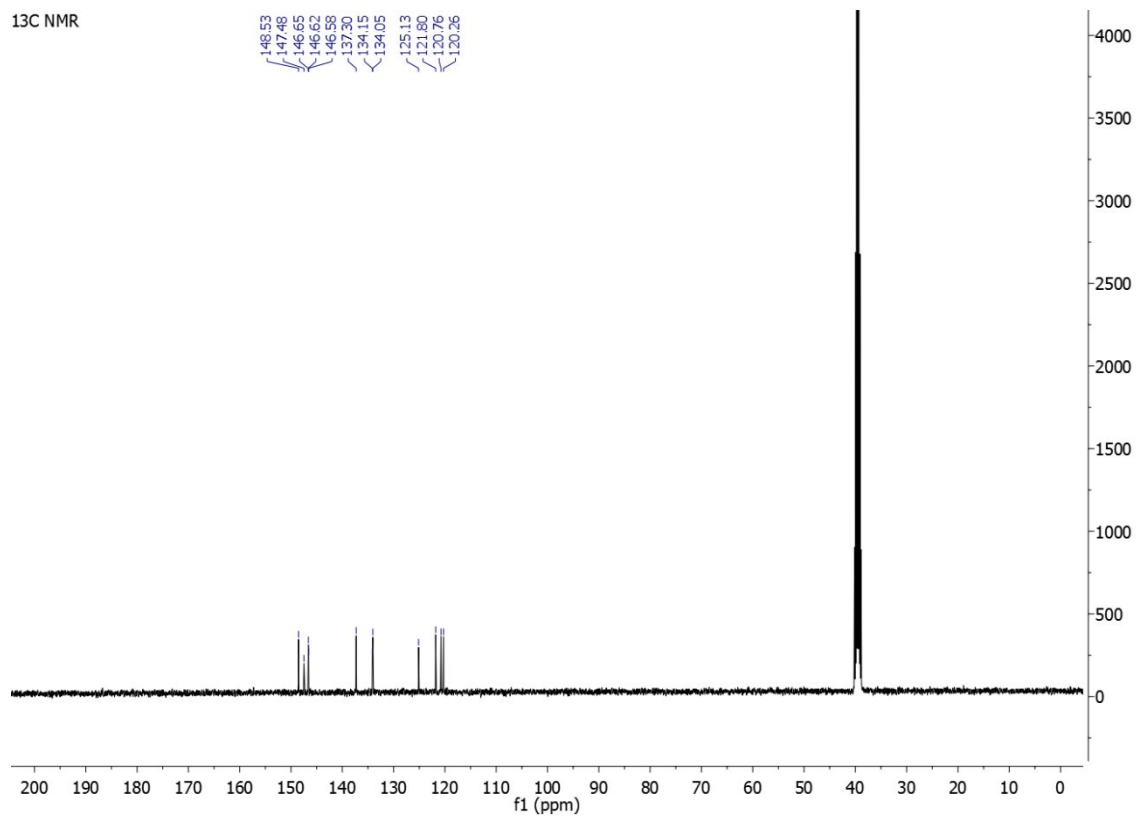
NMR Spectra for Tz I-VI, precursor and supplementary compounds

6-(6-(Pyridin-2-yl)-1,4-dihydro-1,2,4,5-tetrazin-3-yl)pyridin-3-amine (S1)

¹H NMR

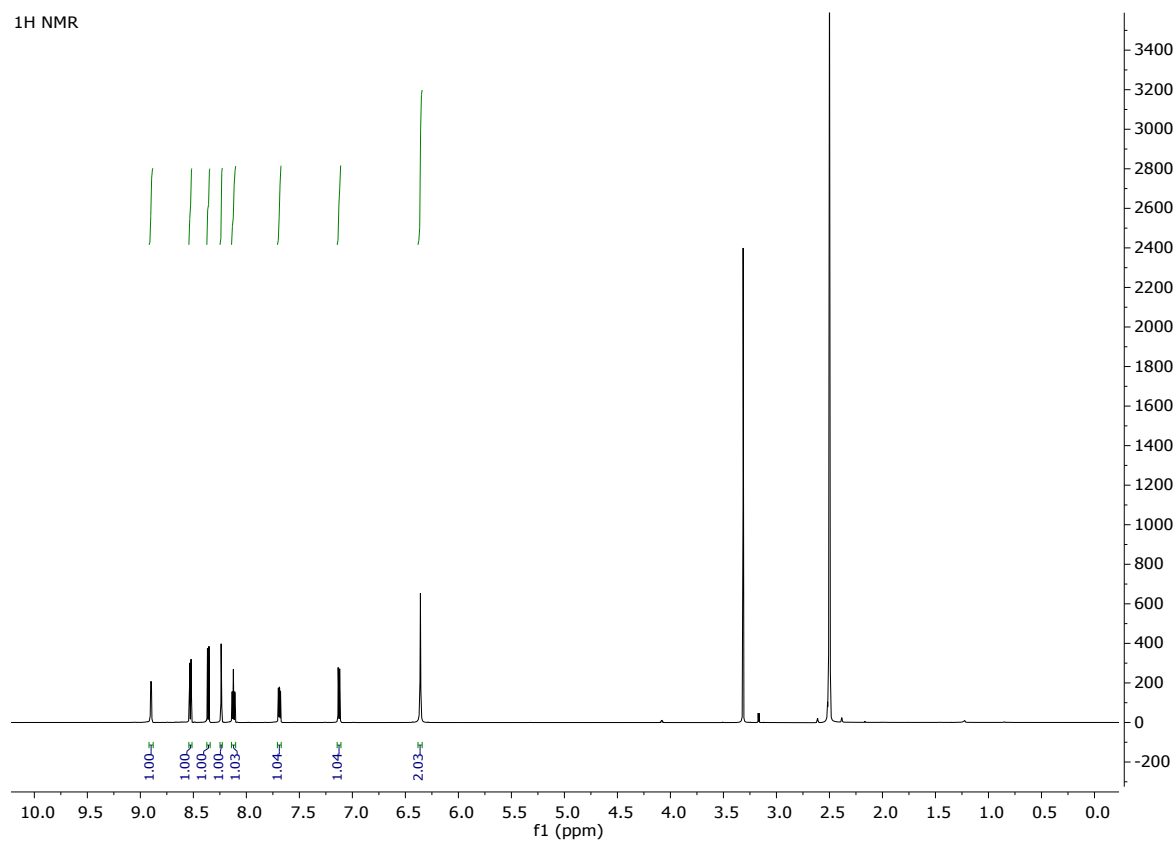


¹³C NMR

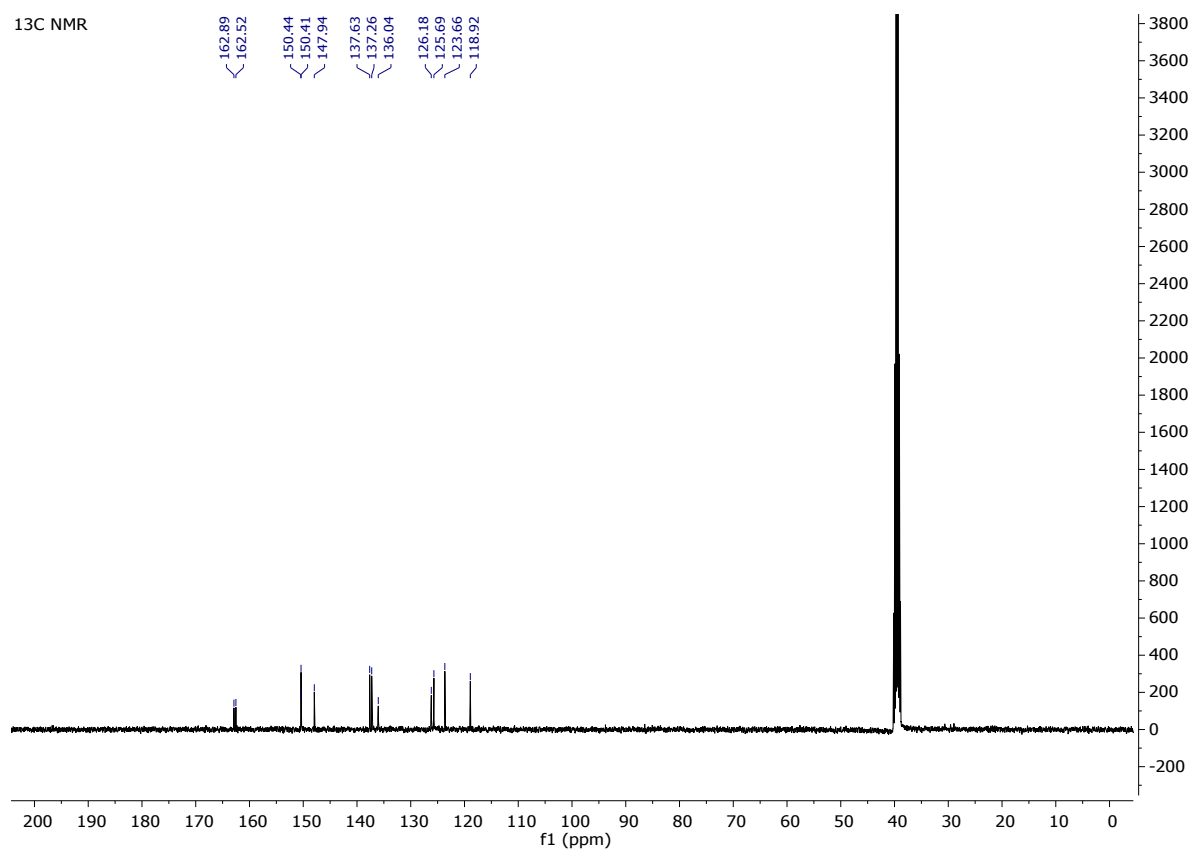


6-(6-(Pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-amine (S2)

¹H NMR

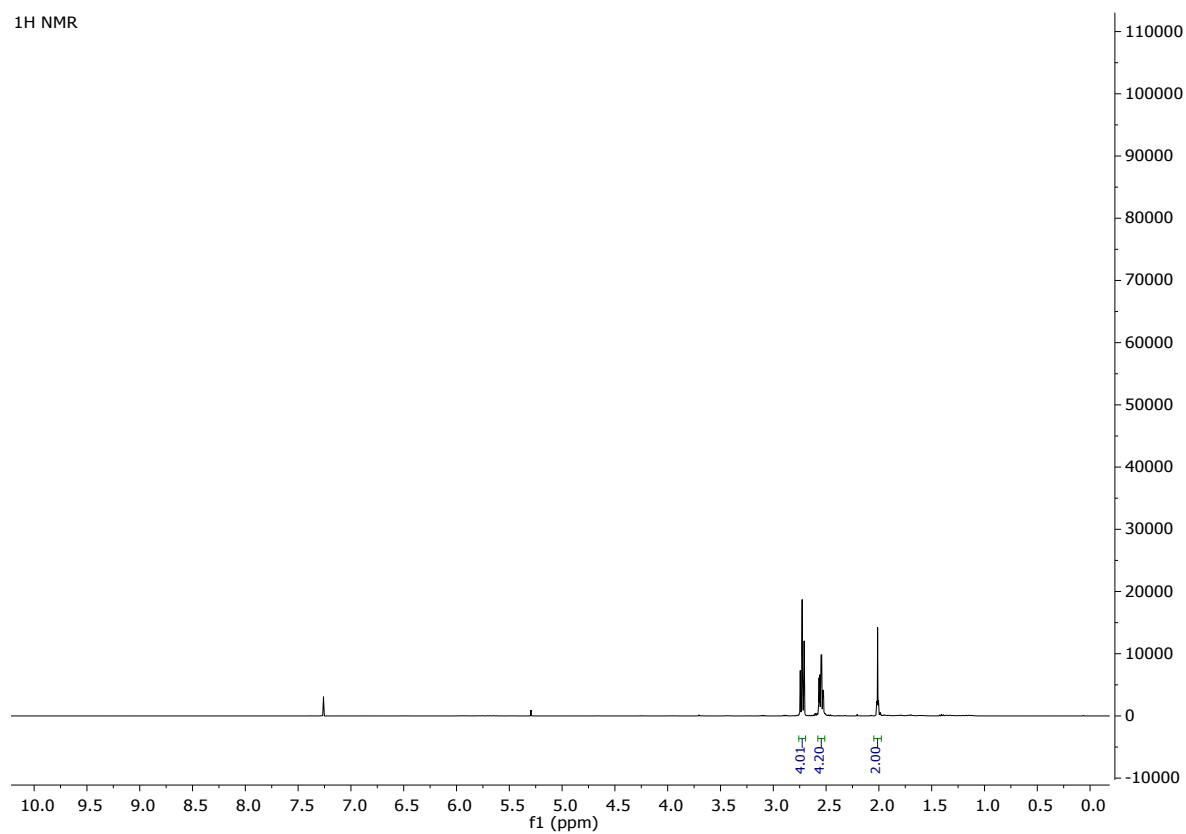


¹³C NMR

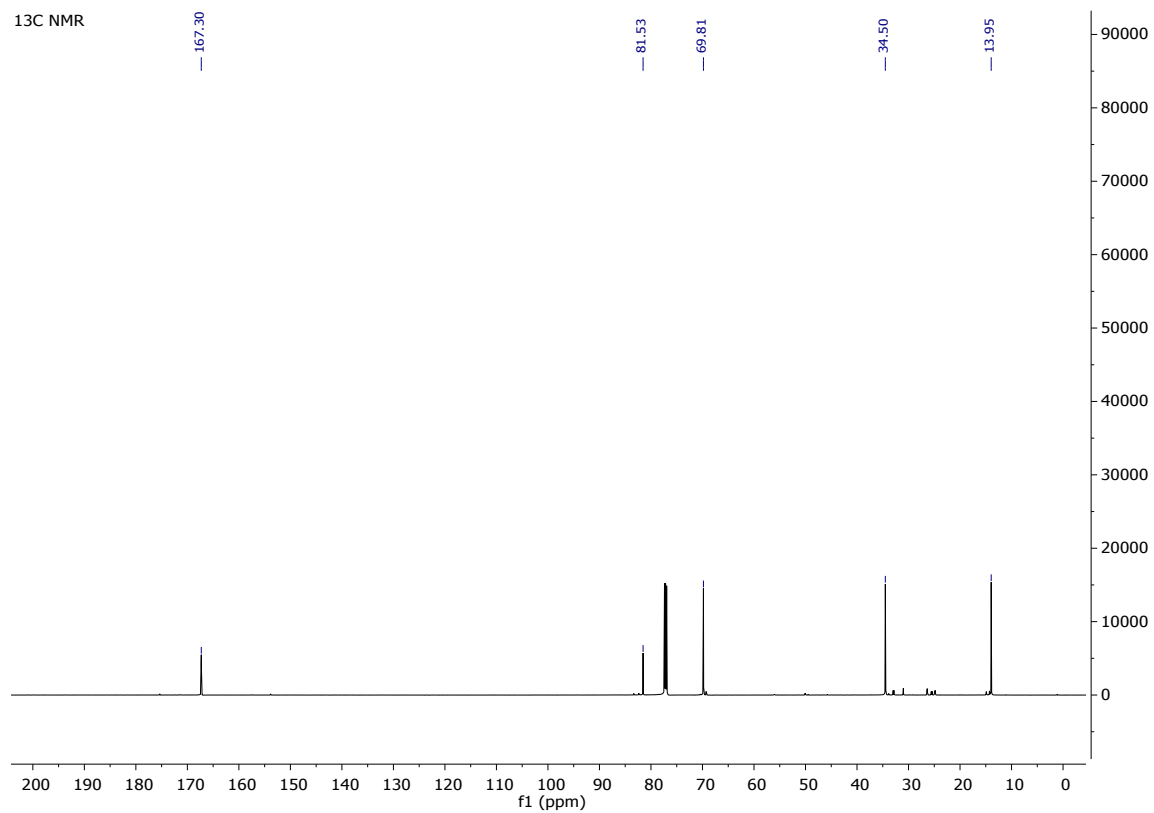


4-Pentynoic anhydride (S3)

¹H NMR

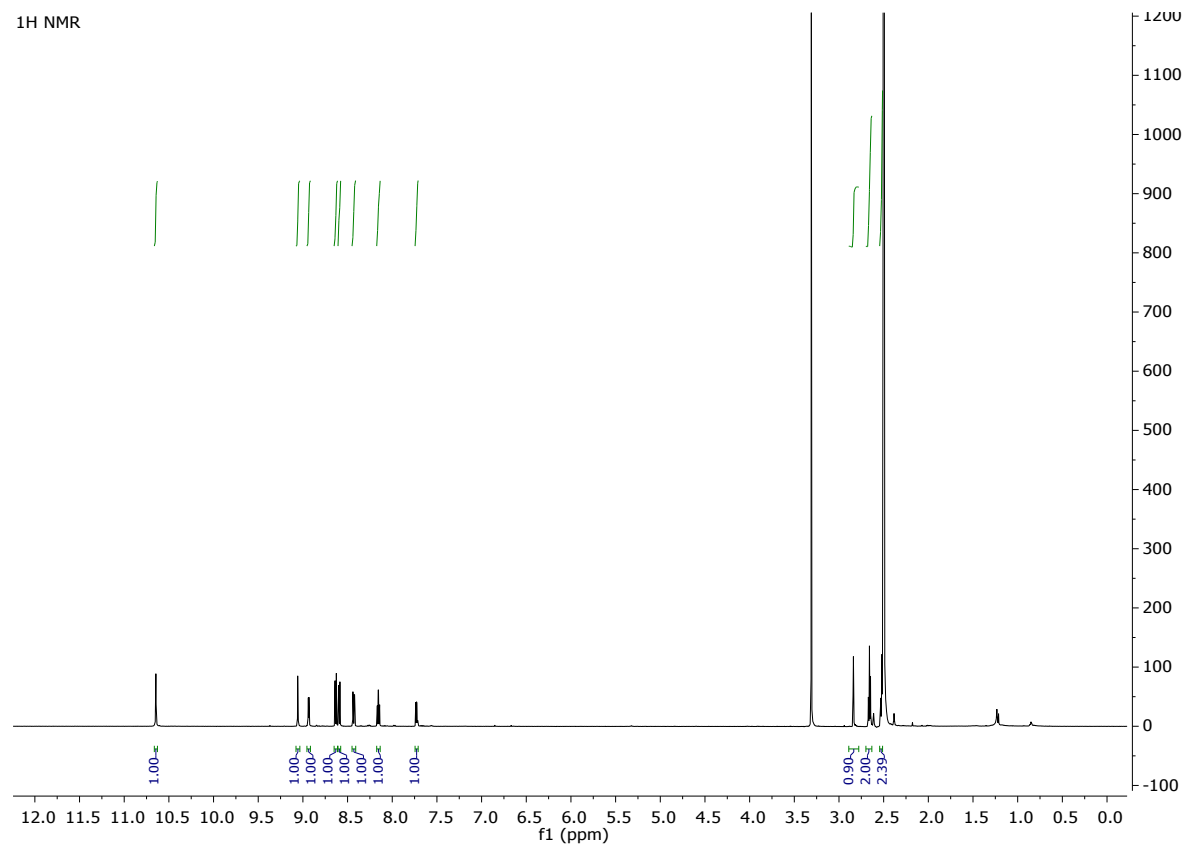


¹³C NMR

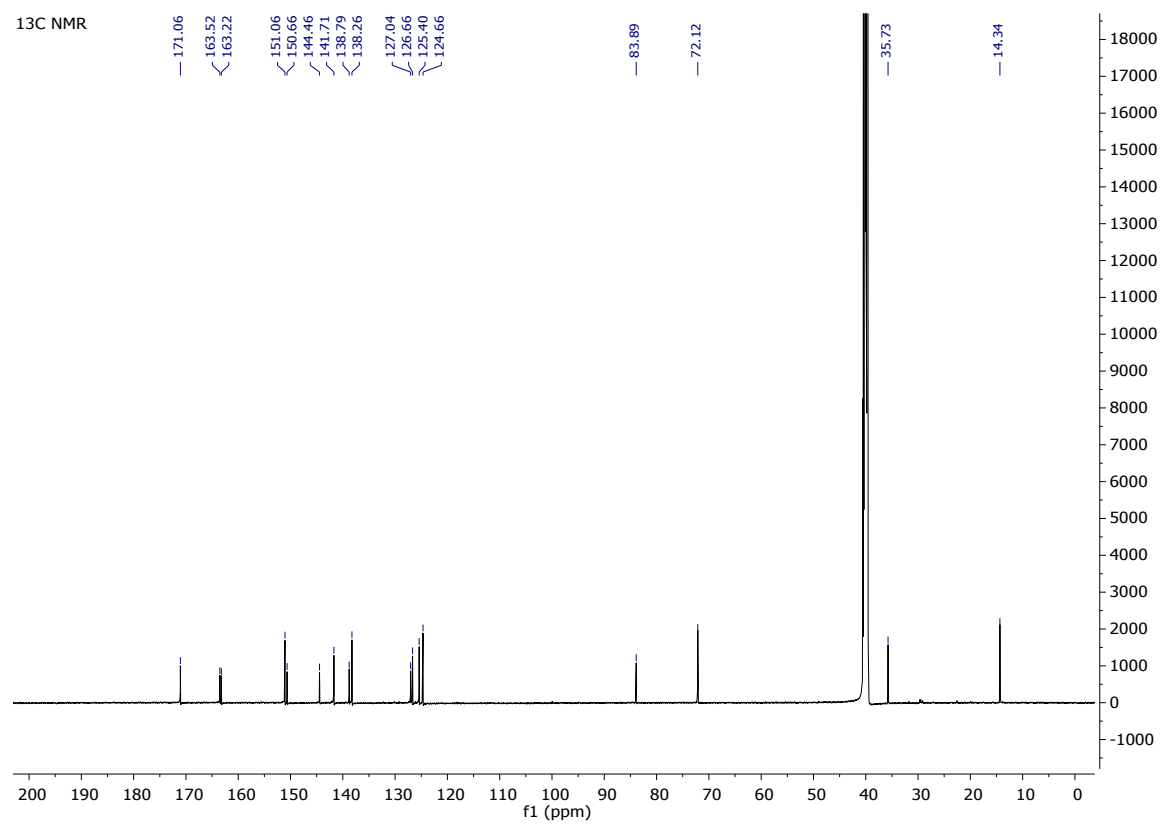


N-(6-(6-(Pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)pent-4-ynamide (**1**)

¹H NMR

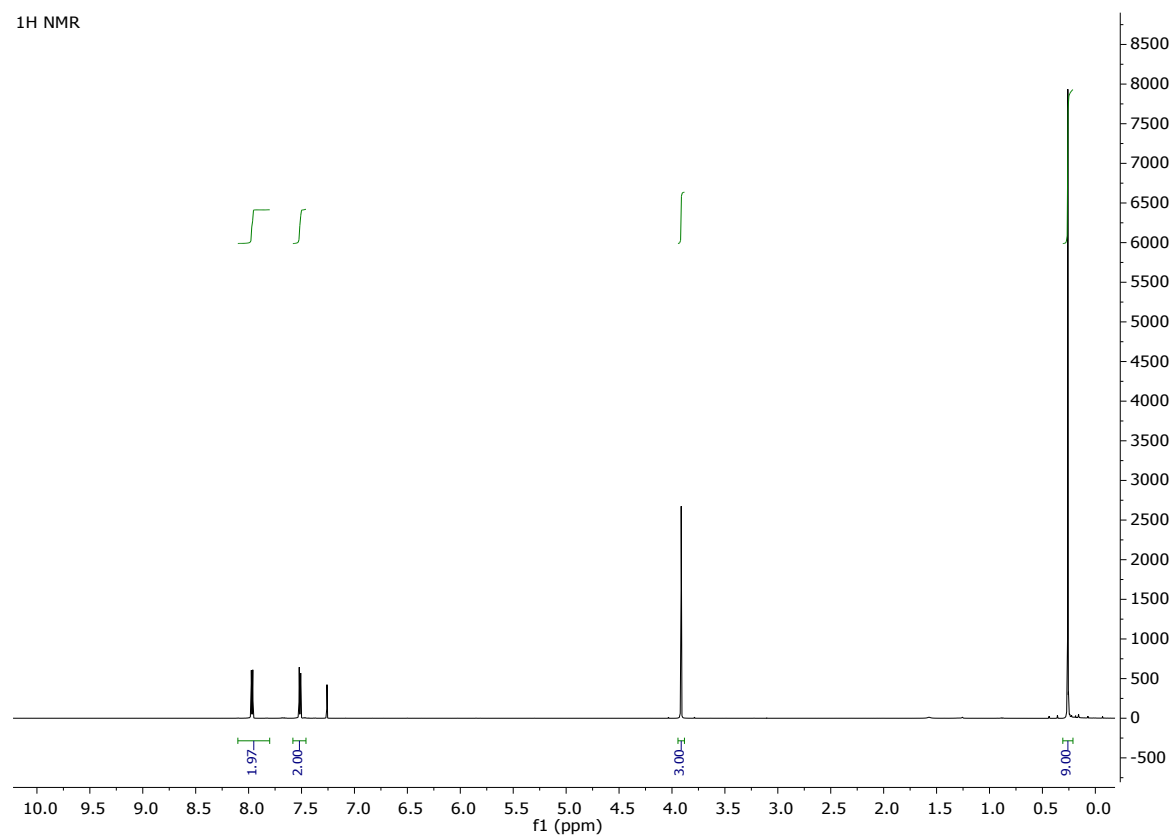


¹³C NMR

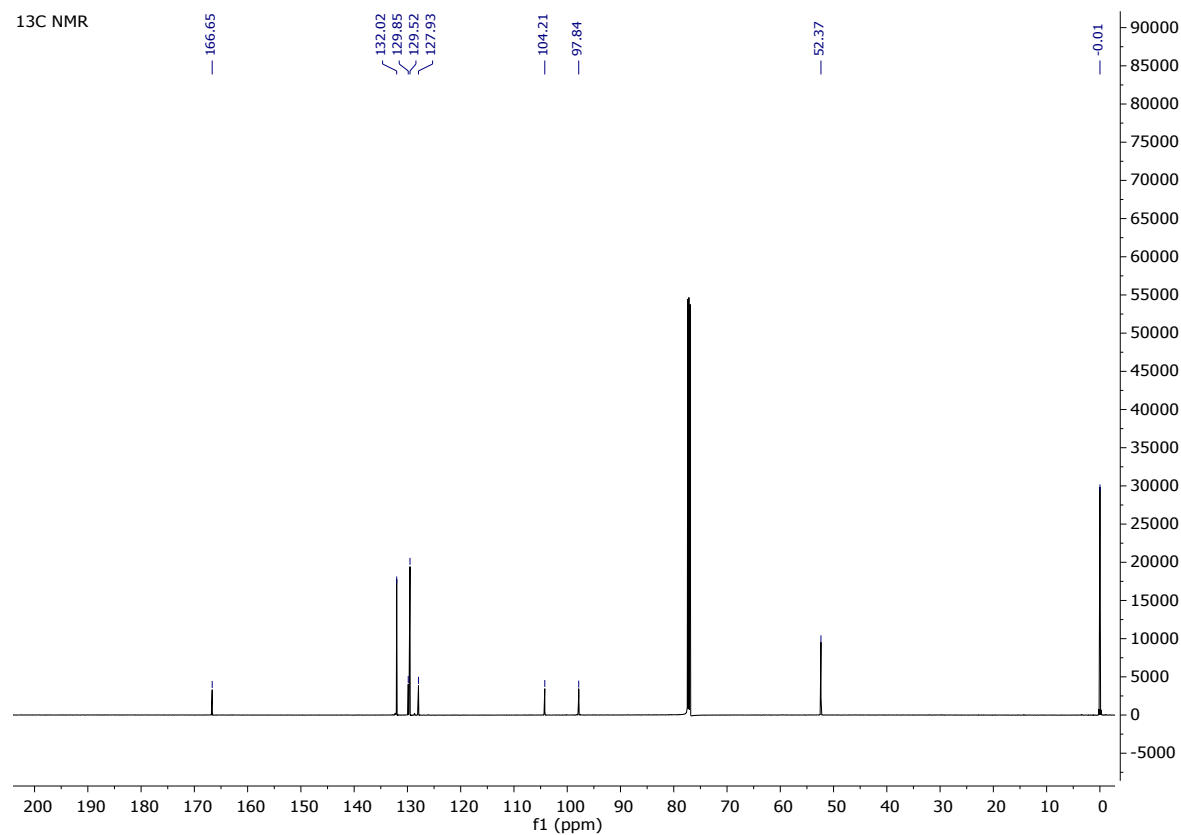


Methyl 4-((trimethylsilyl)ethynyl)benzoate (starting material for carboxylic acid S4)

¹H NMR

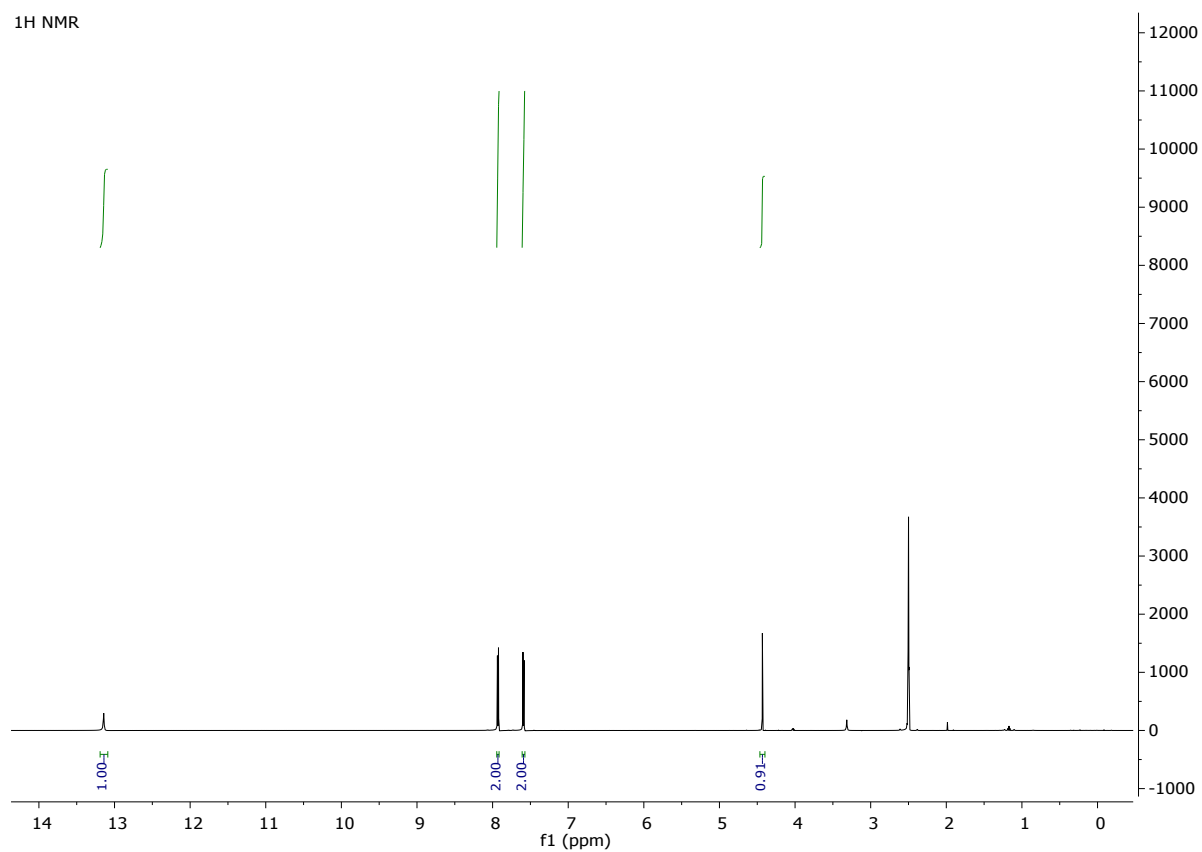


¹³C NMR

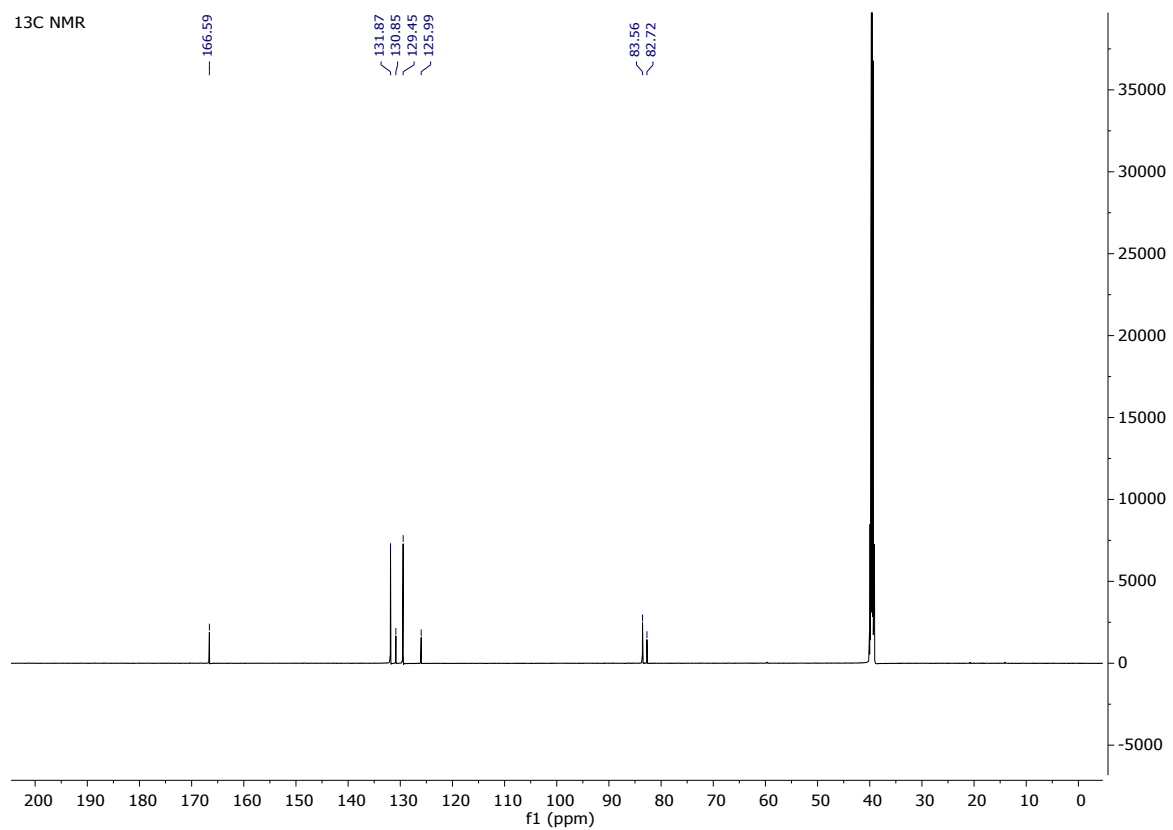


4-Ethynylbenzoic acid (S4)

¹H NMR

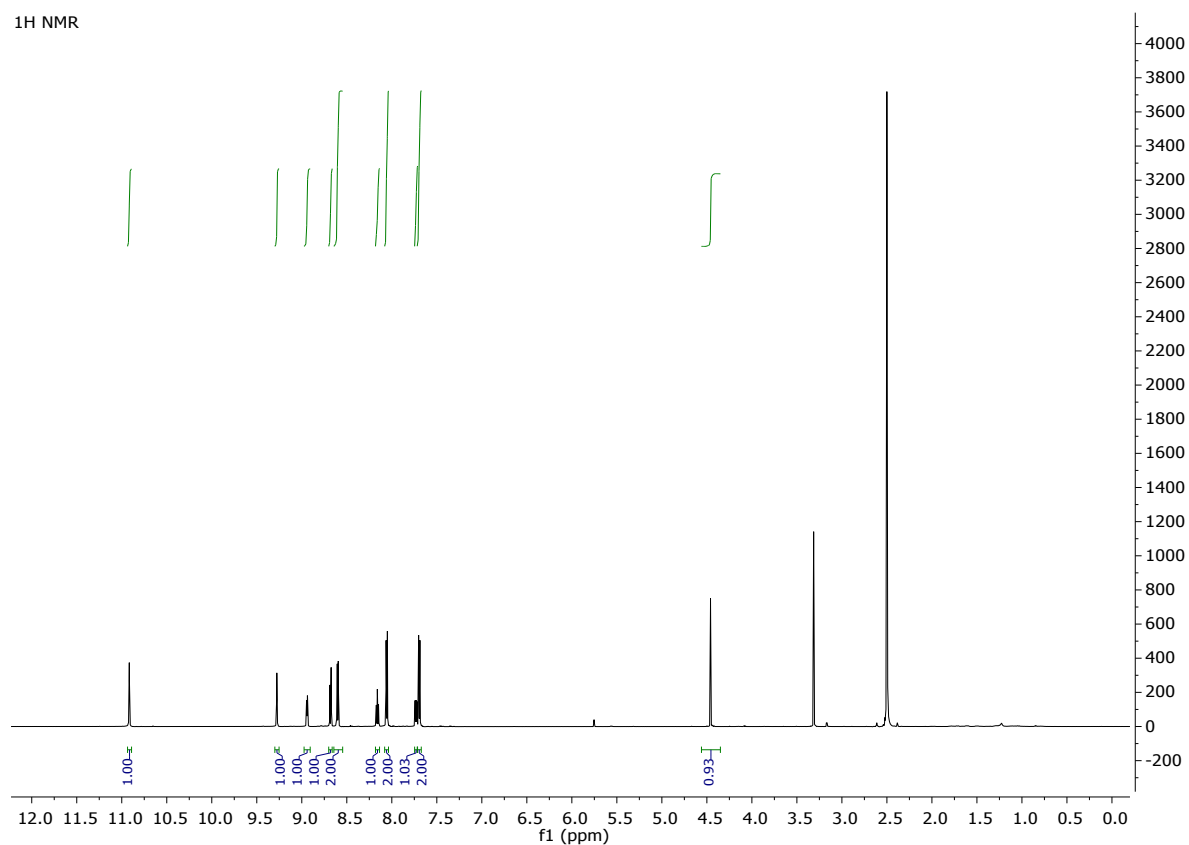


¹³C NMR

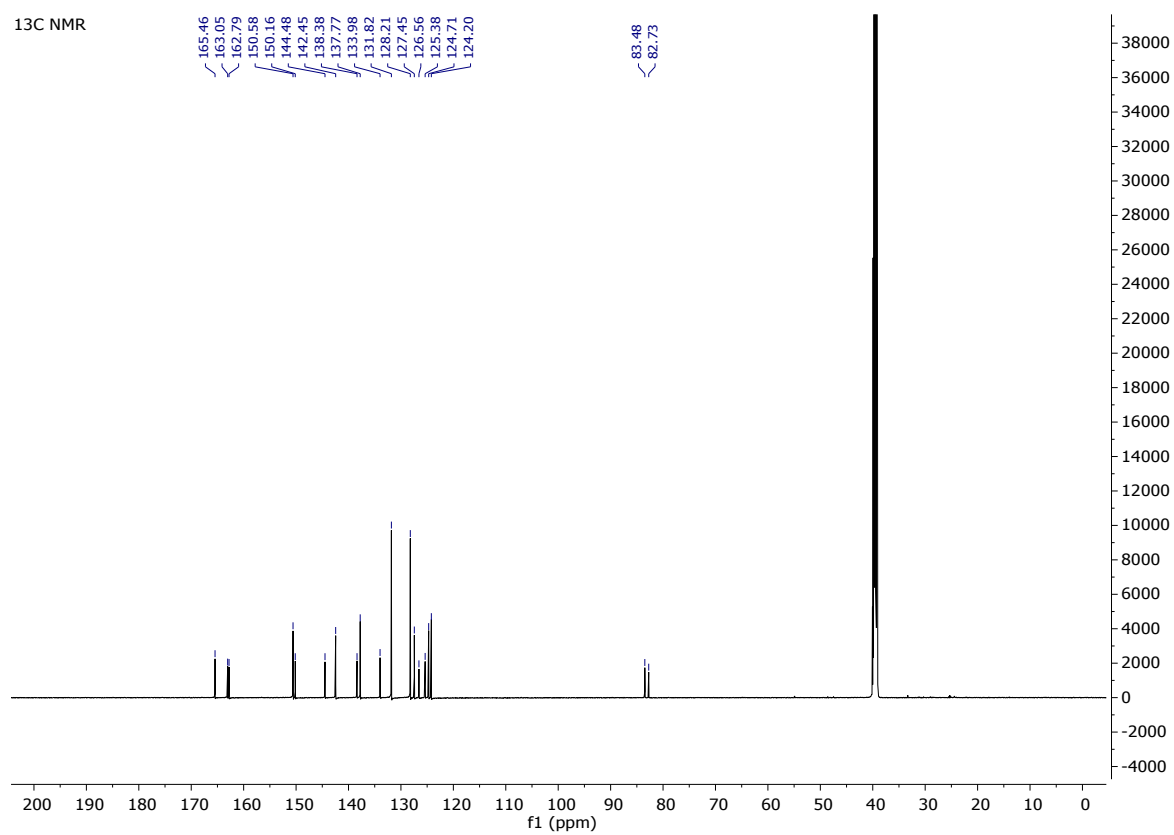


4-Ethynyl-N-(6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)benzamide (II)

¹H NMR

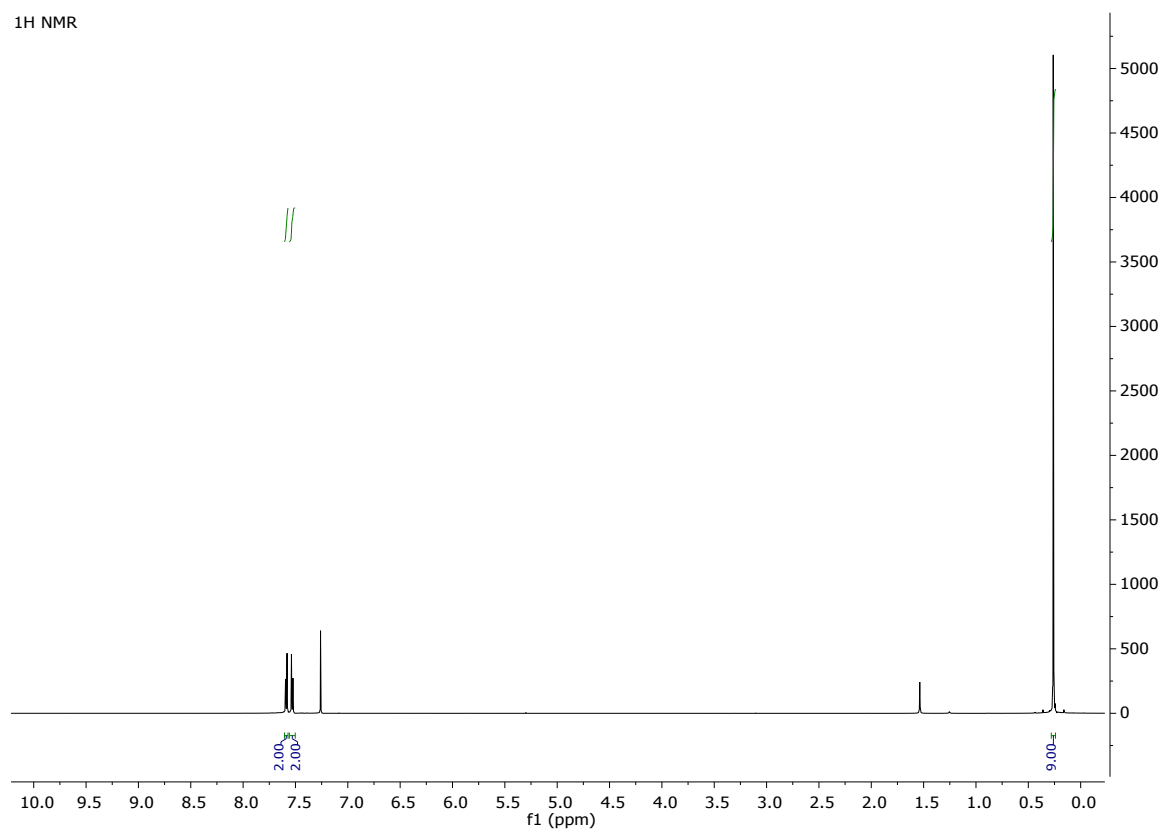


¹³C NMR

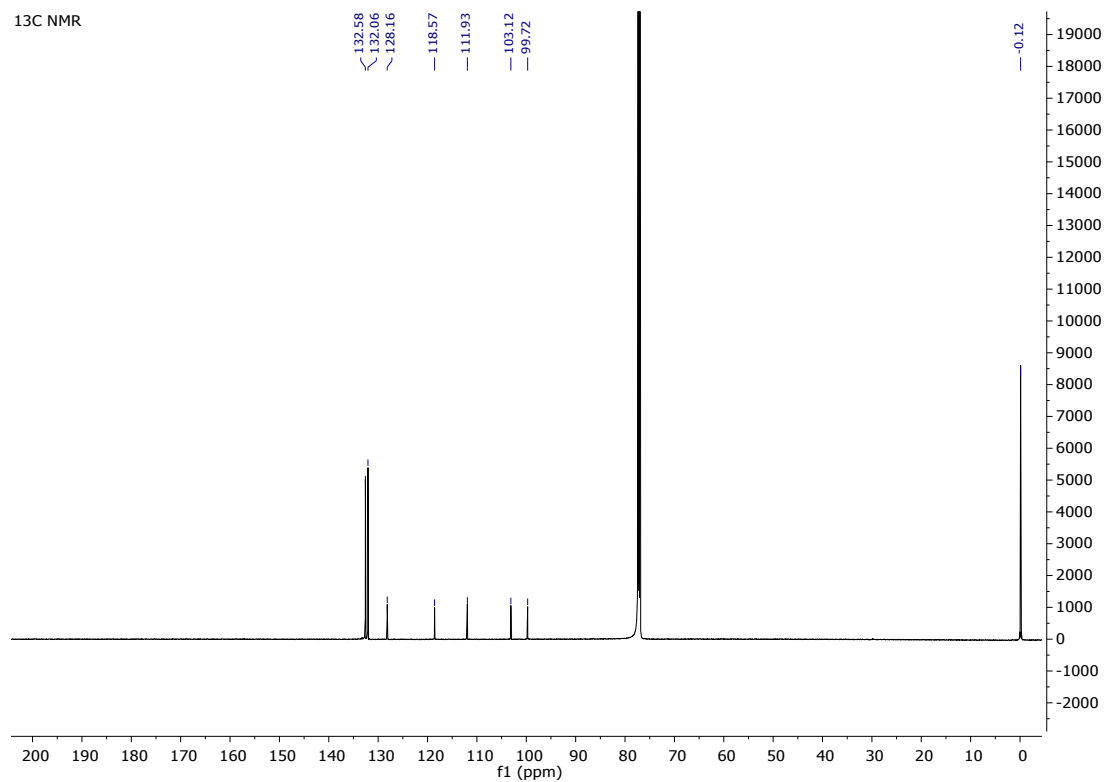


4-((Trimethylsilyl)ethynyl)benzonitrile (**S5**)

¹H NMR

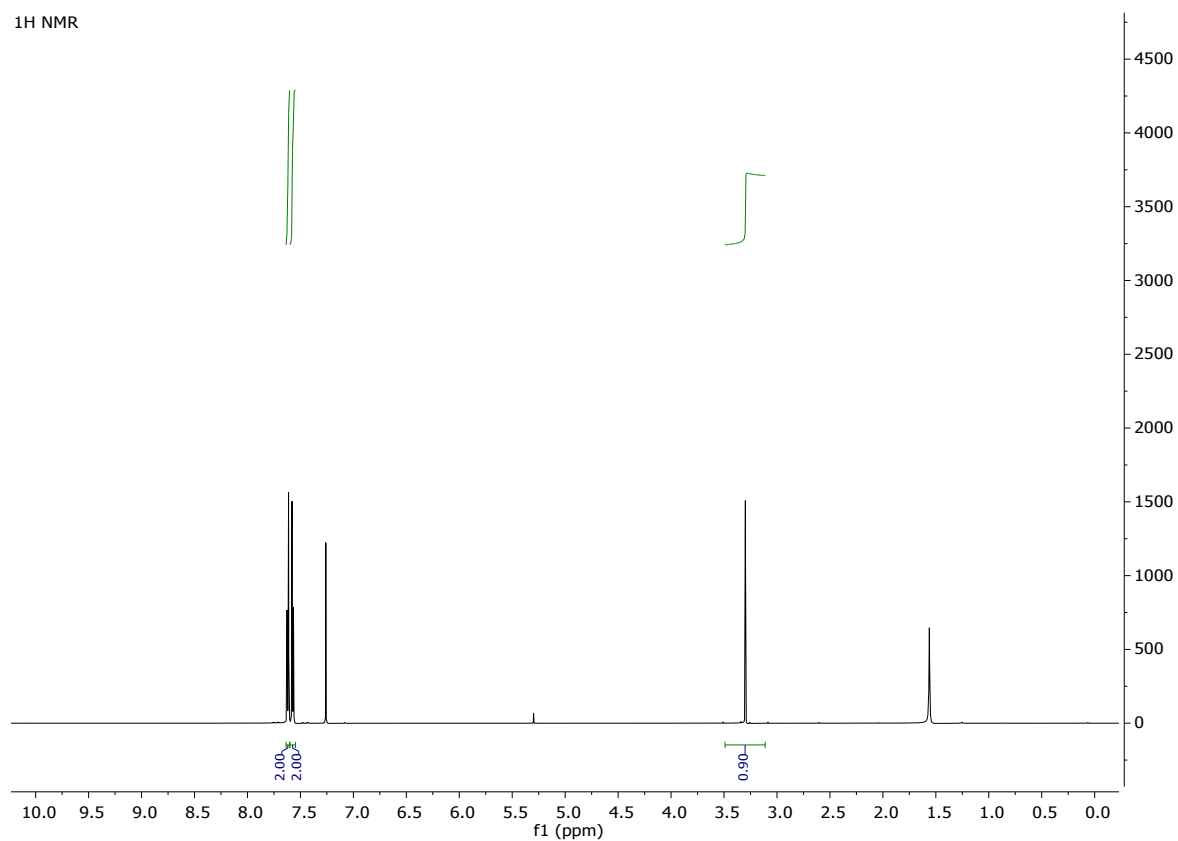


¹³C NMR

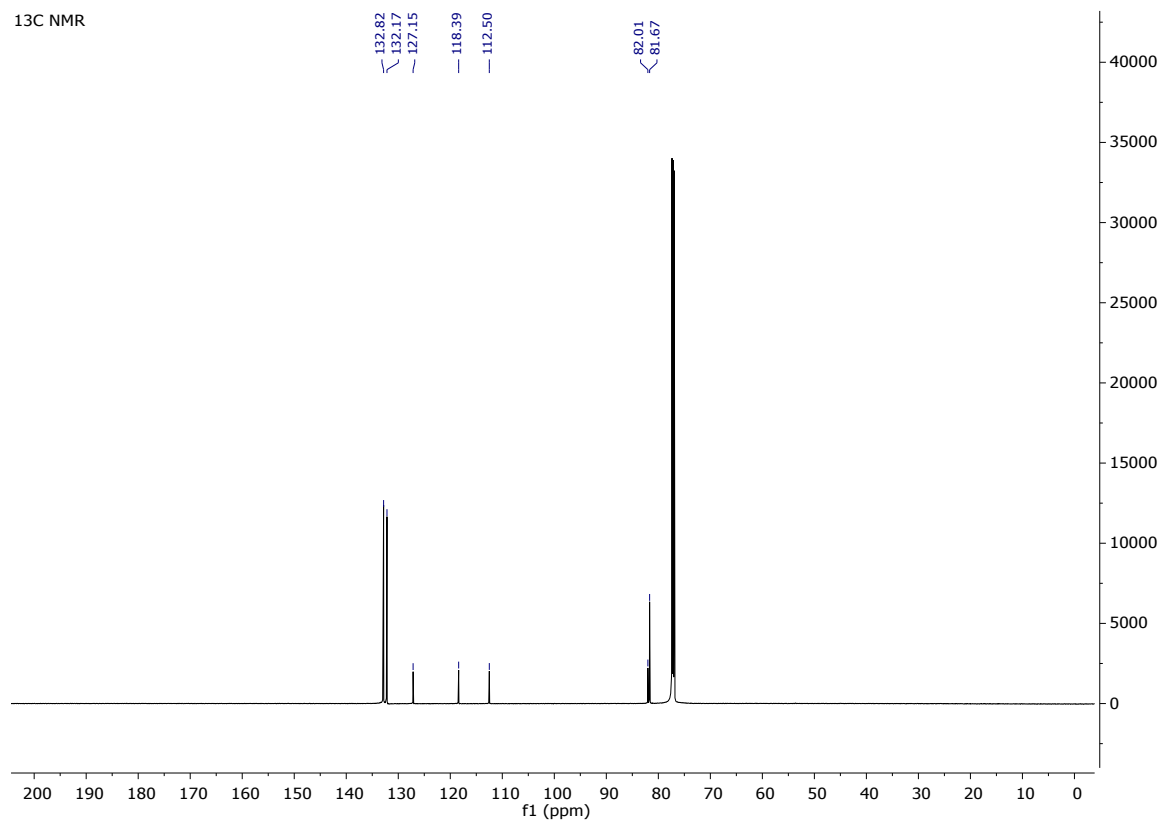


4-Ethynylbenzonitrile (S6)

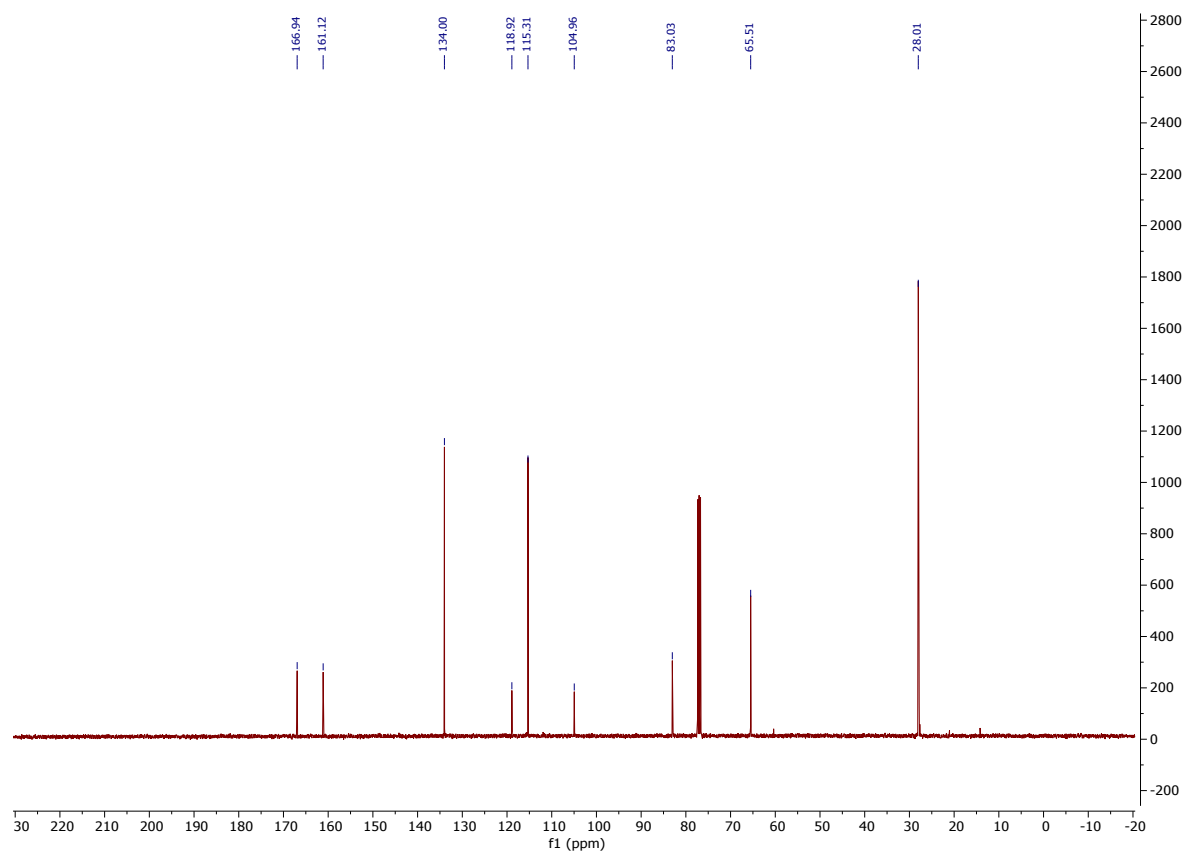
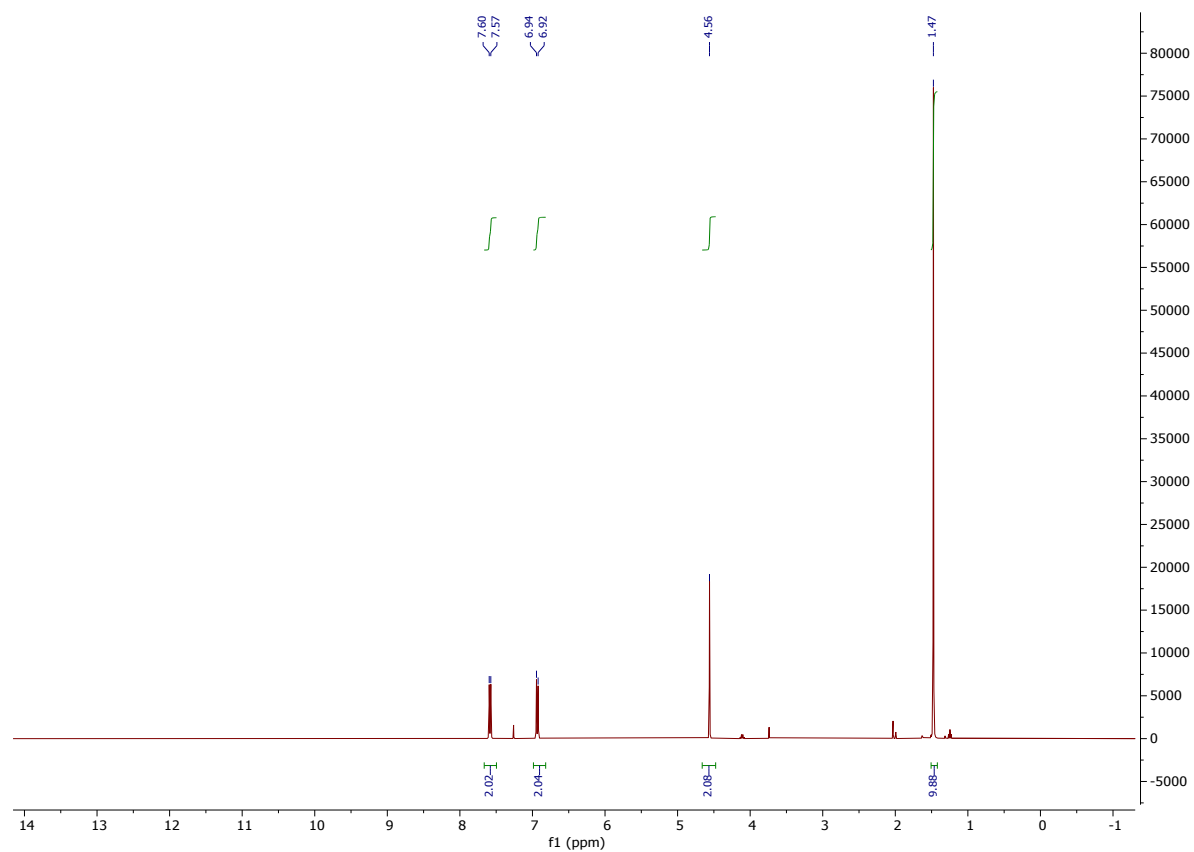
¹H NMR



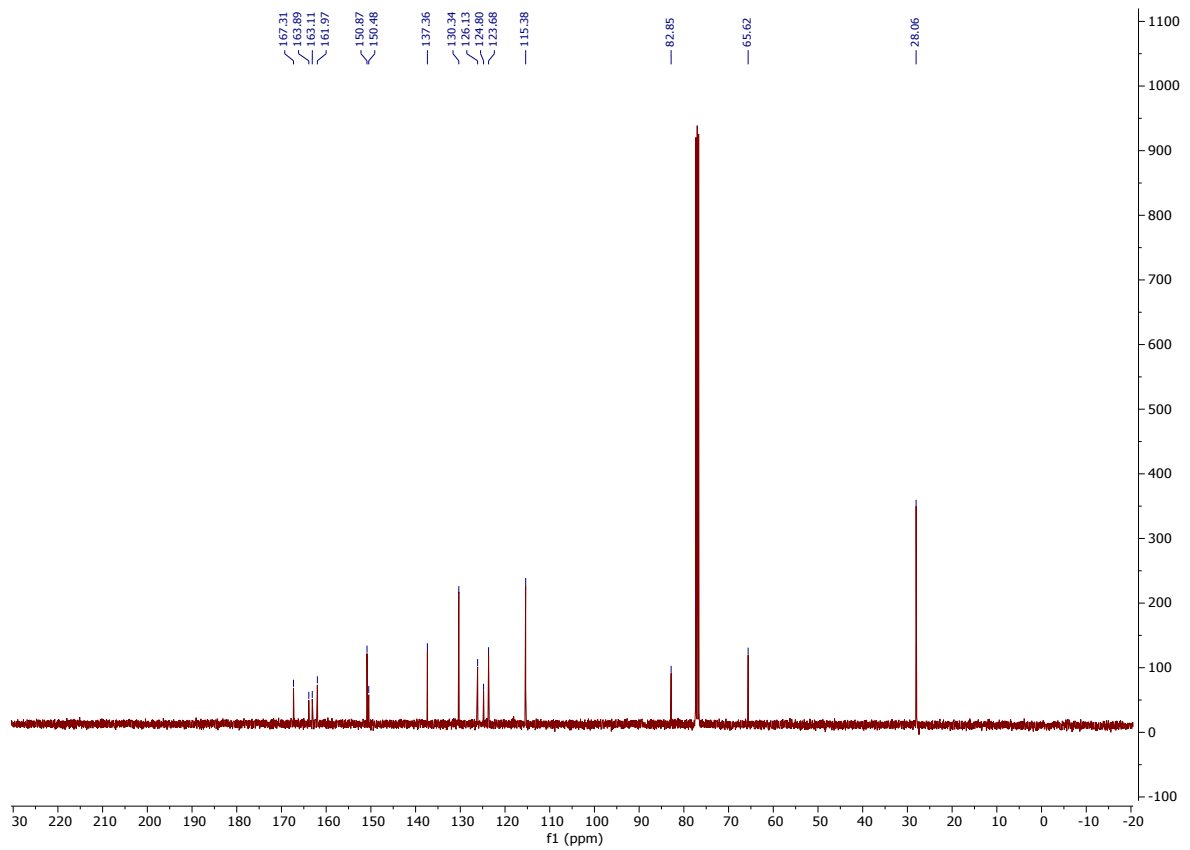
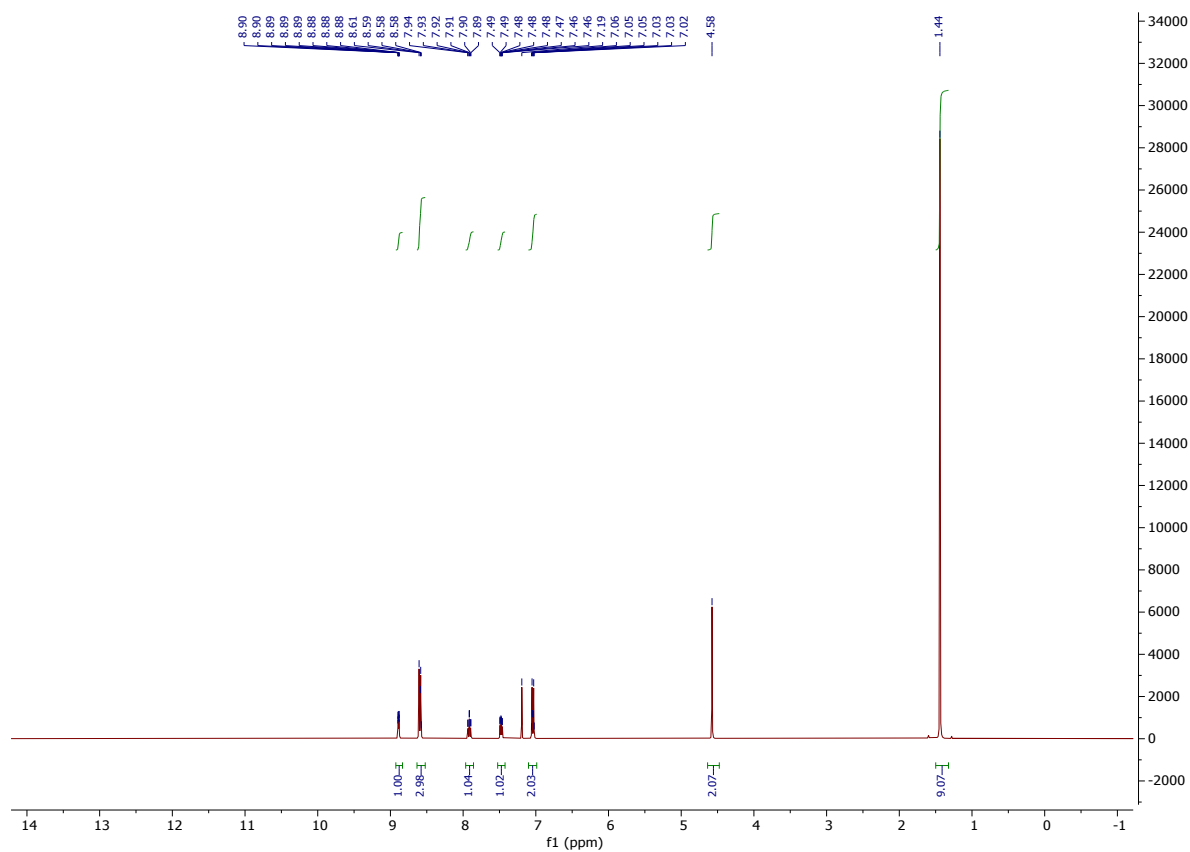
¹³C NMR



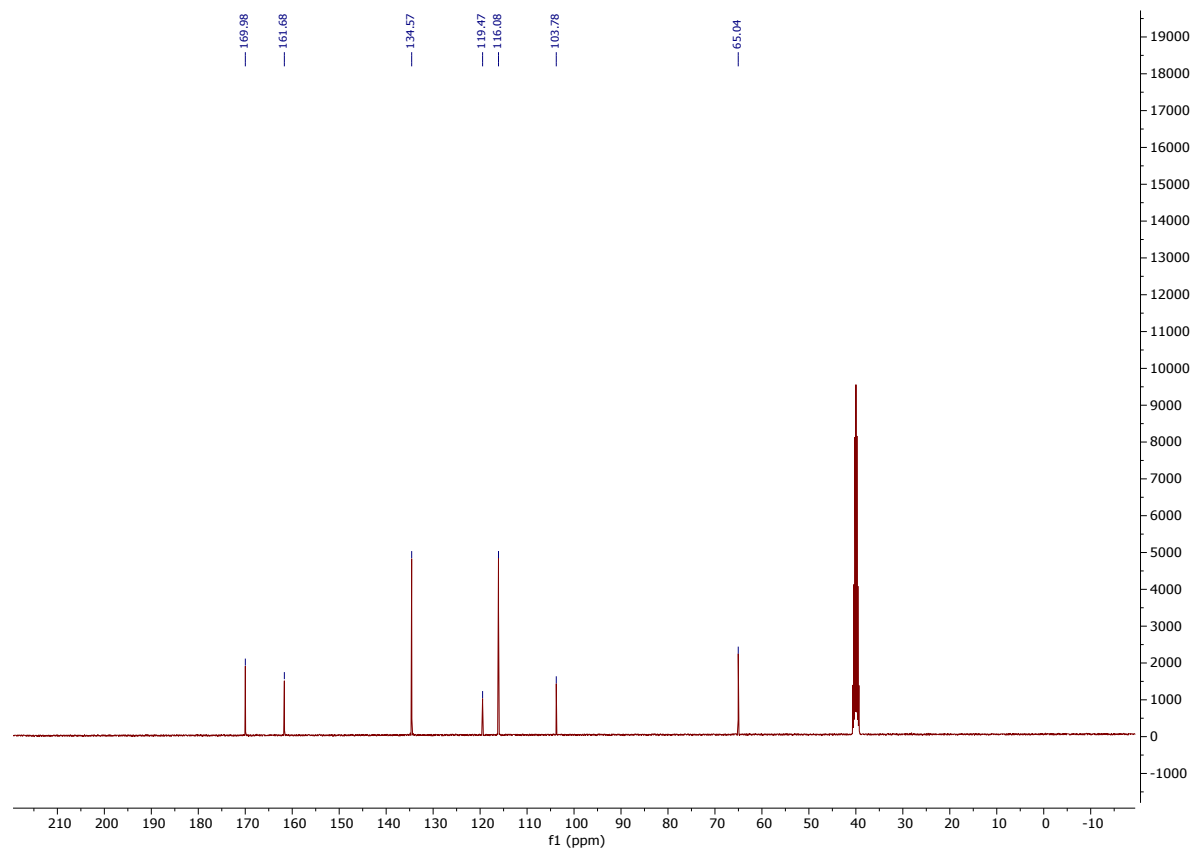
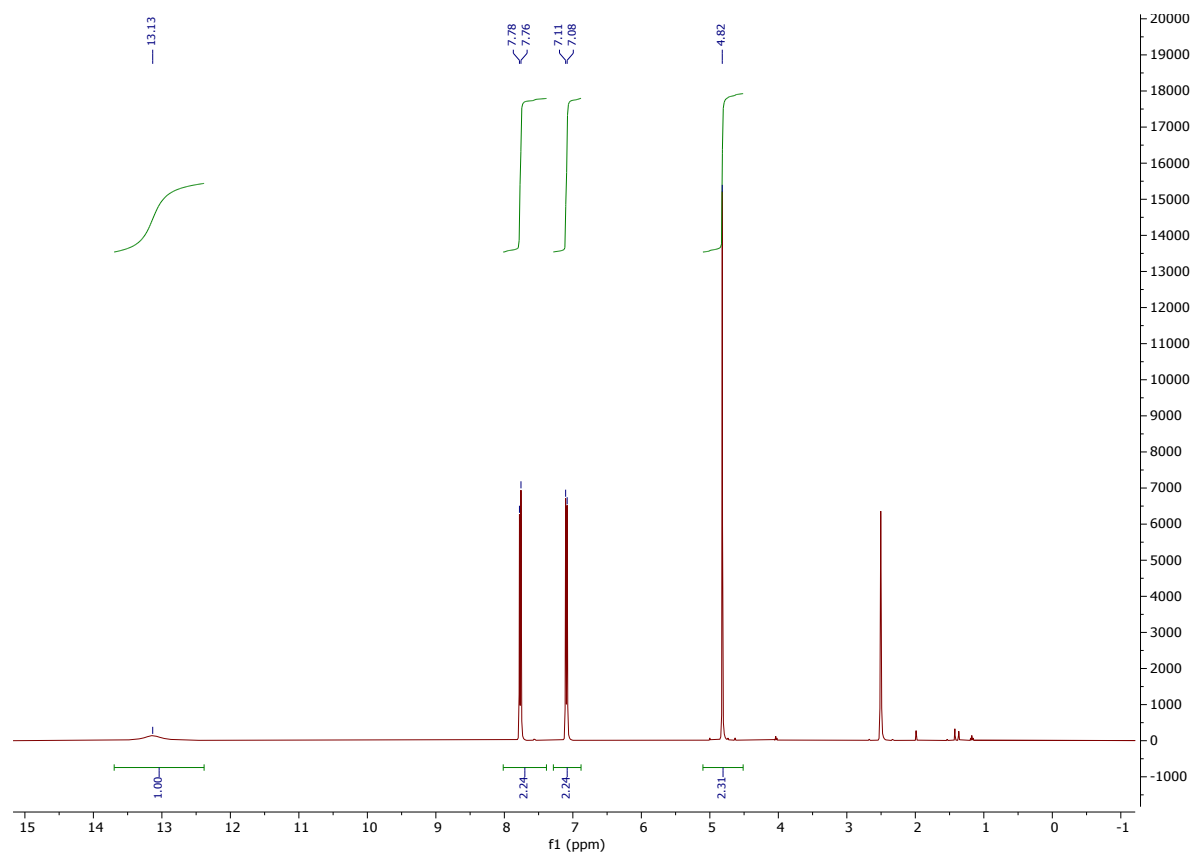
Tert-butyl 2-(4-cyanophenoxy)acetate (S11)



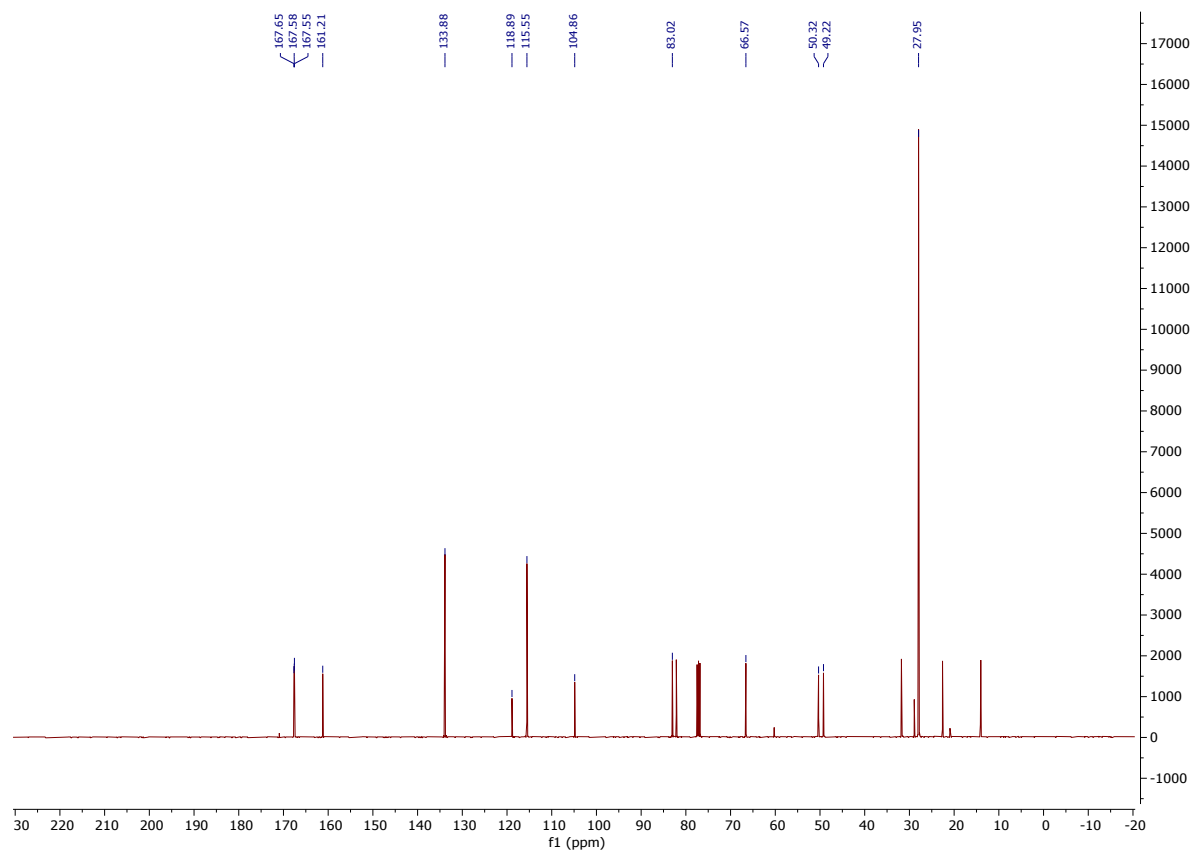
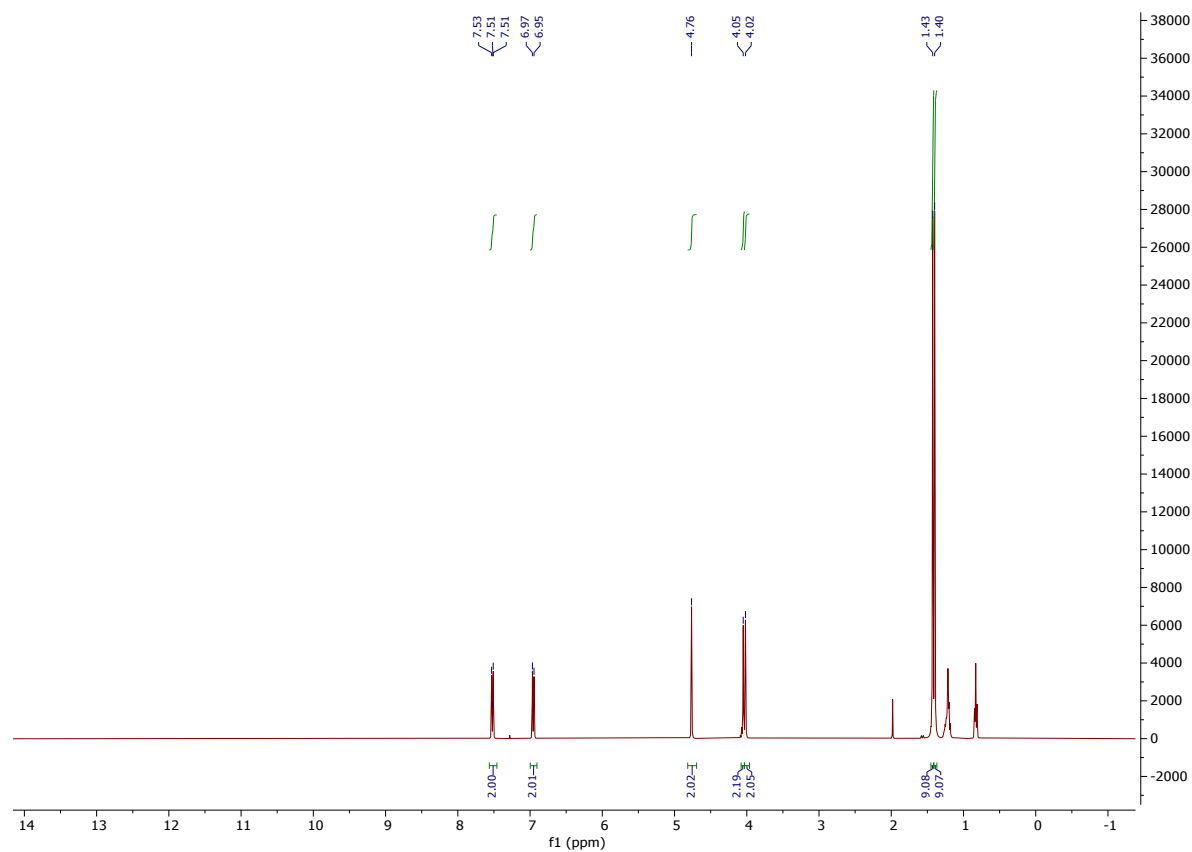
Tert-butyl 2-(4-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)phenoxy)acetate (S12)



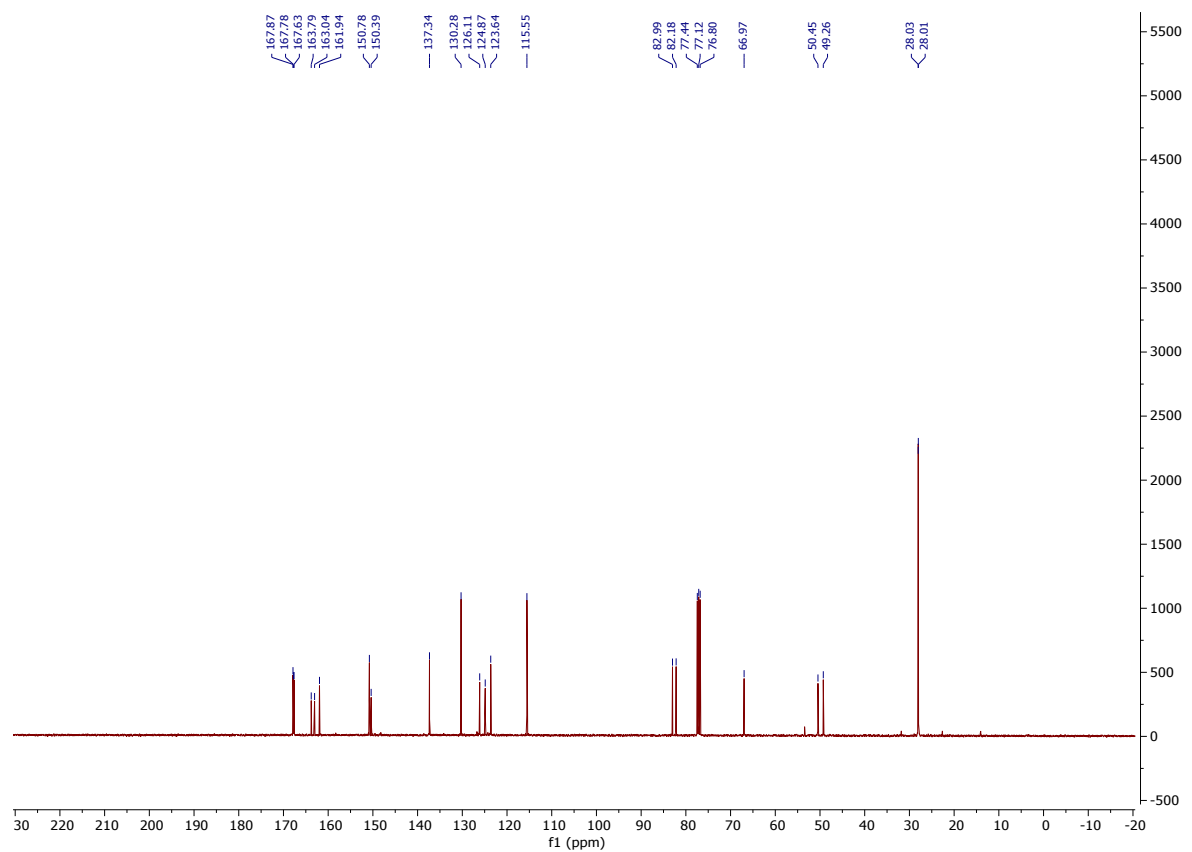
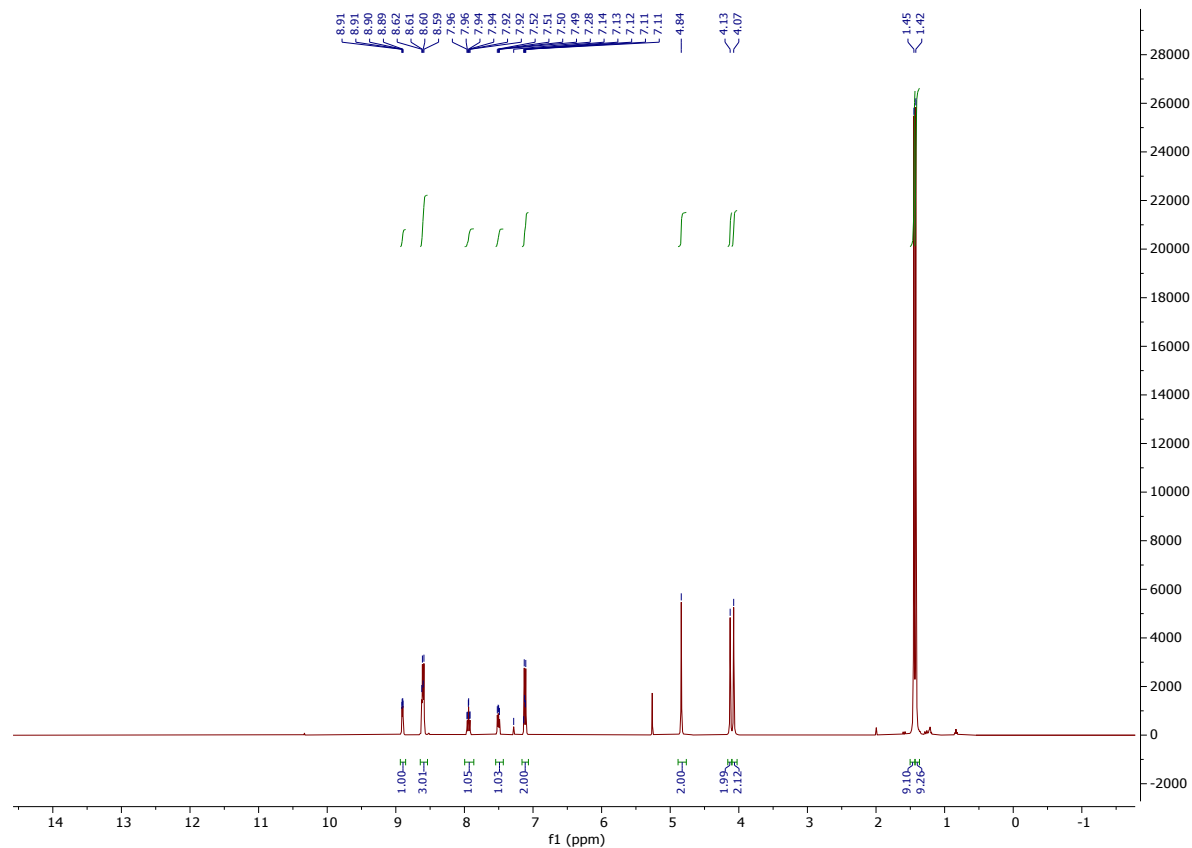
2-(4-Cyanophenoxy)acetic acid (S13)



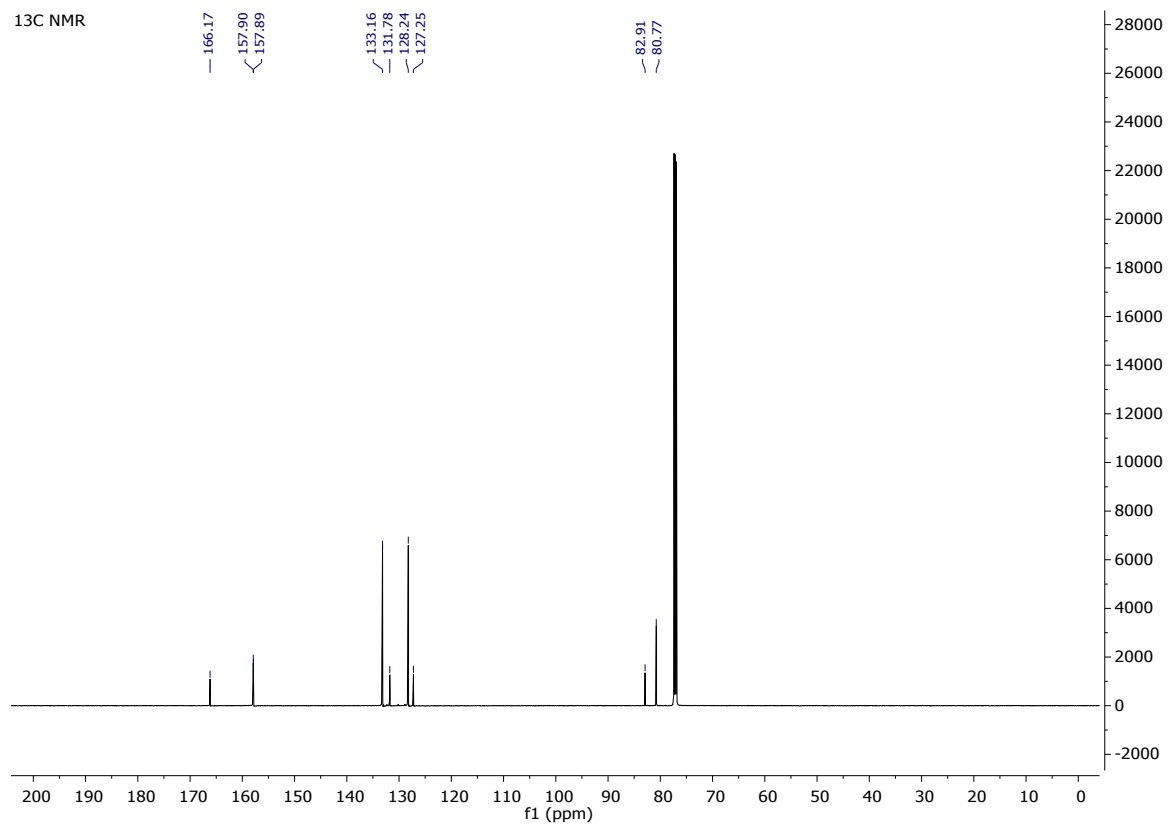
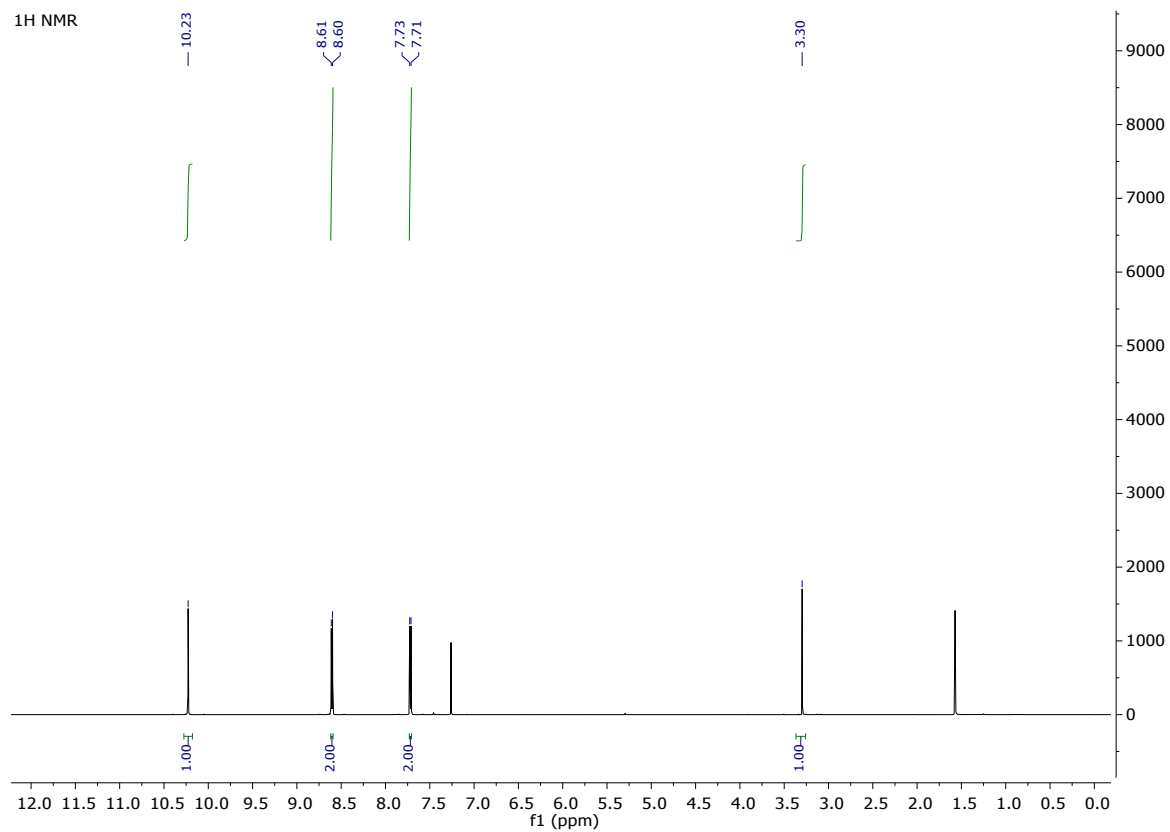
Di-tert-butyl 2,2'-((2-(4-cyanophenoxy)acetyl)azanediyl)diacetate (S14)



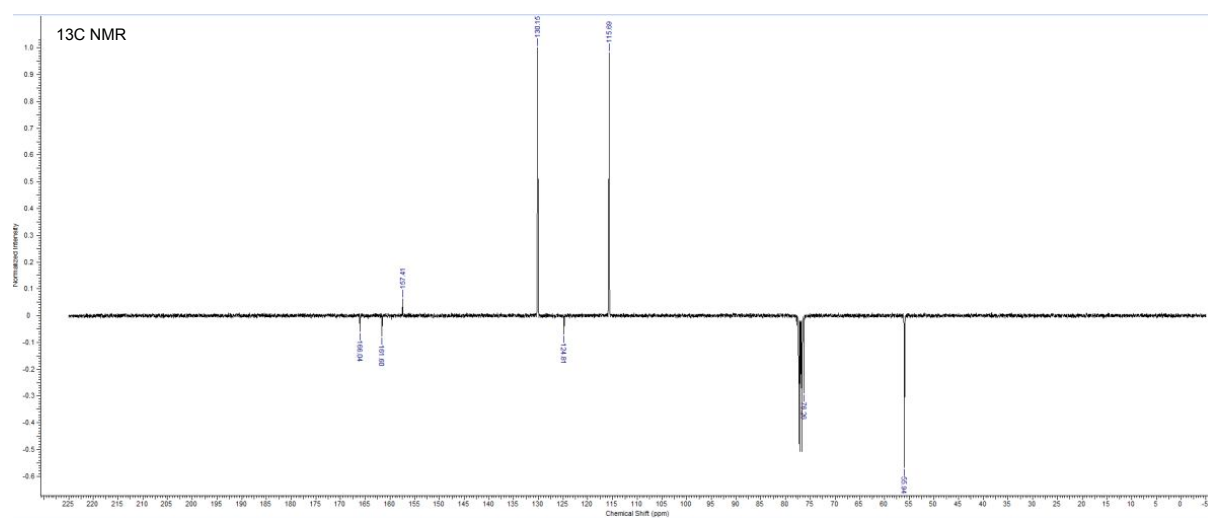
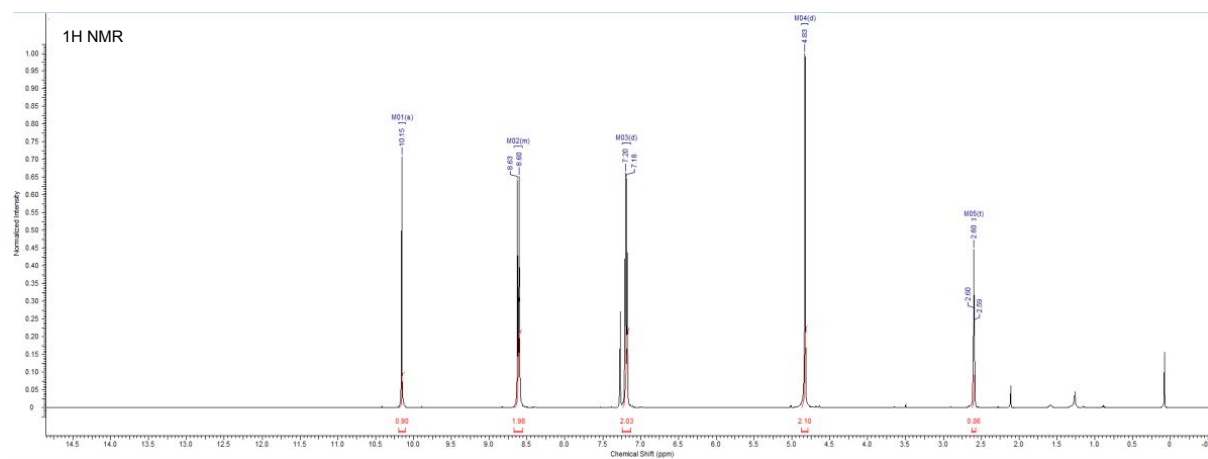
Di-tert-butyl 2,2'-((2-(4-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)phenoxy)acetyl)azanediyldiacetate (S15)



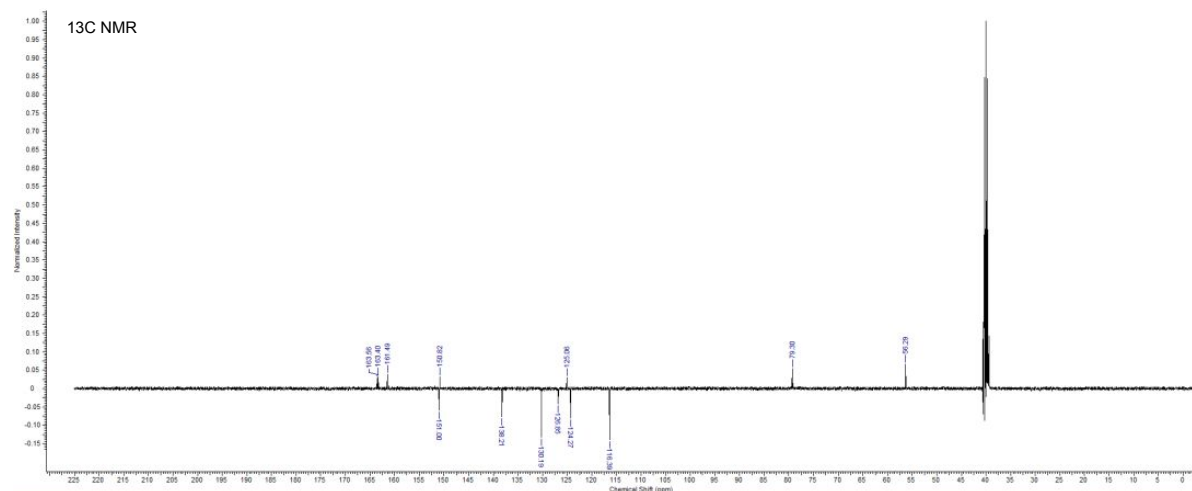
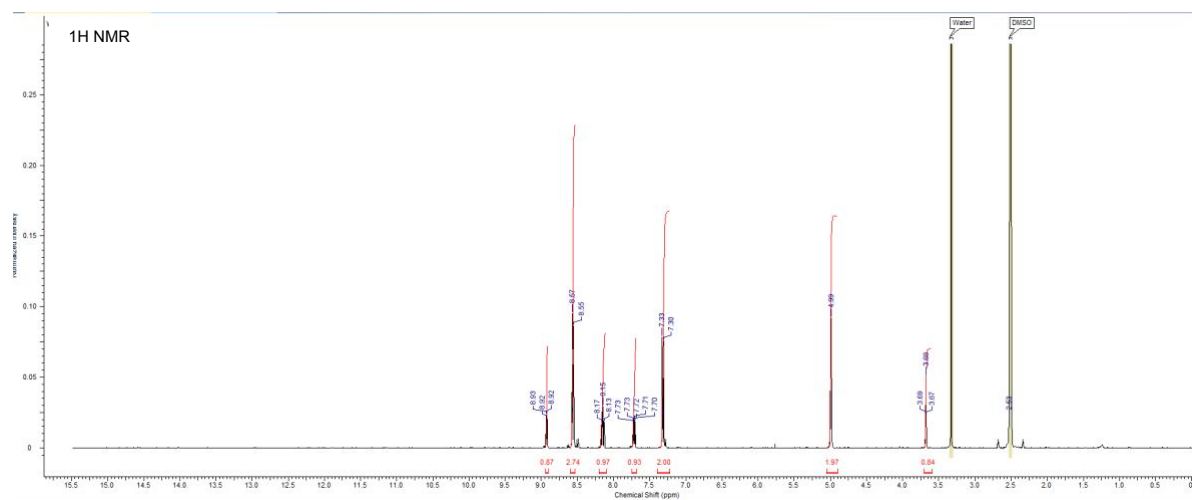
3-(4-Ethynylphenyl)-1,2,4,5-tetrazine (III)



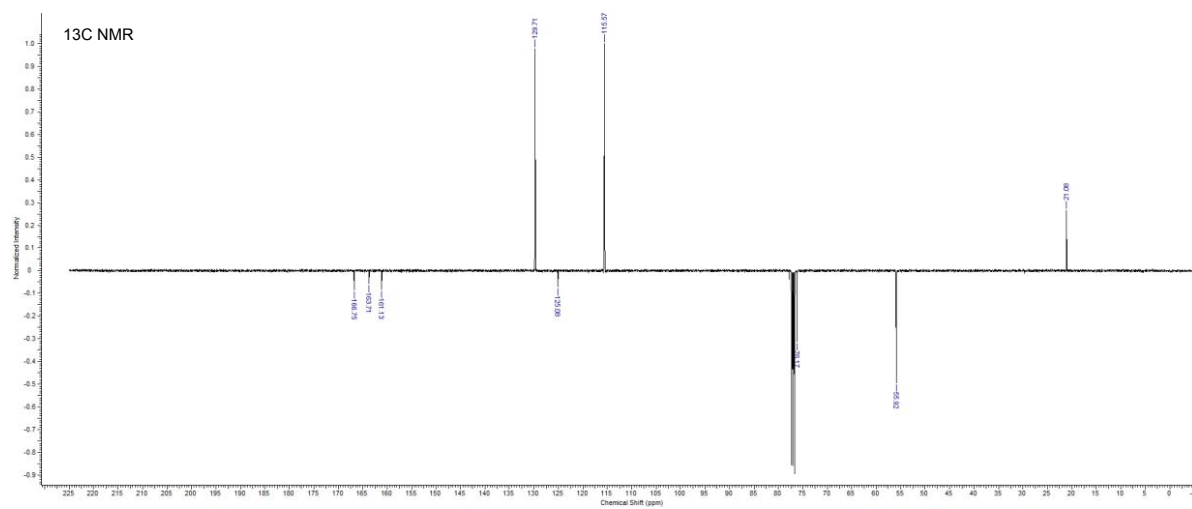
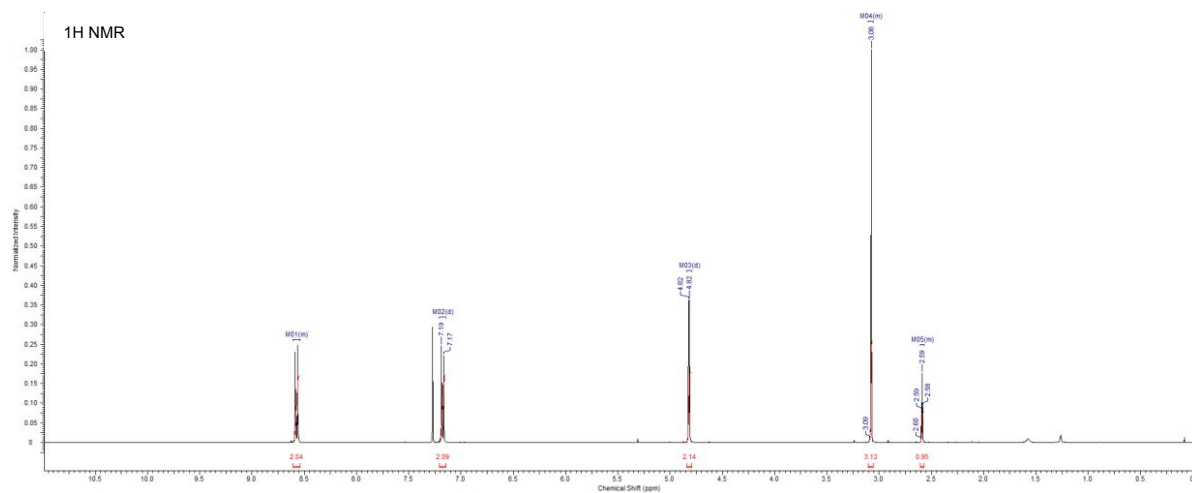
3-(4-(Prop-2-yn-1-yloxy)phenyl)-1,2,4,5-tetrazine (IV)



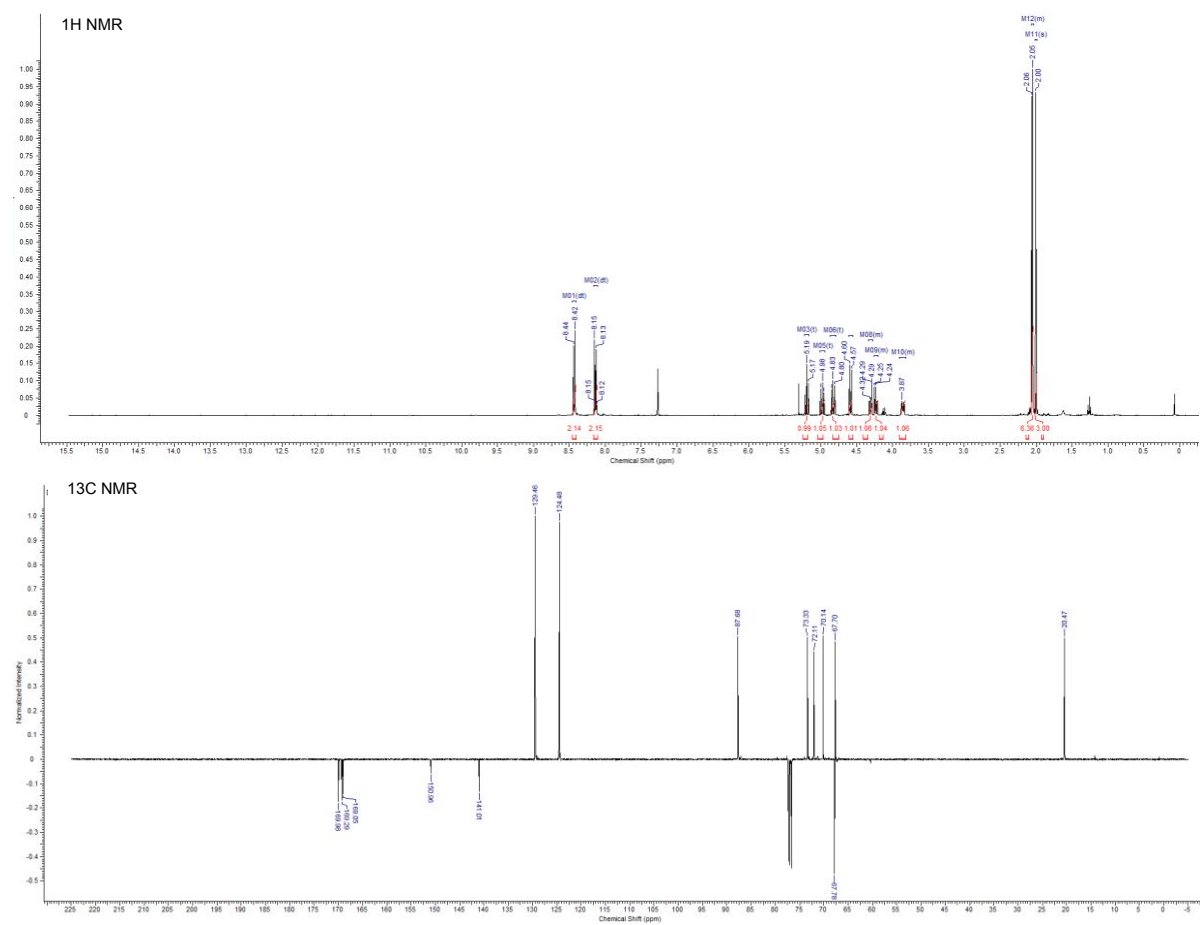
3-(4-(Prop-2-yn-1-yloxy)phenyl)-6-(pyridin-2-yl)-1,2,4,5-tetrazine (V)



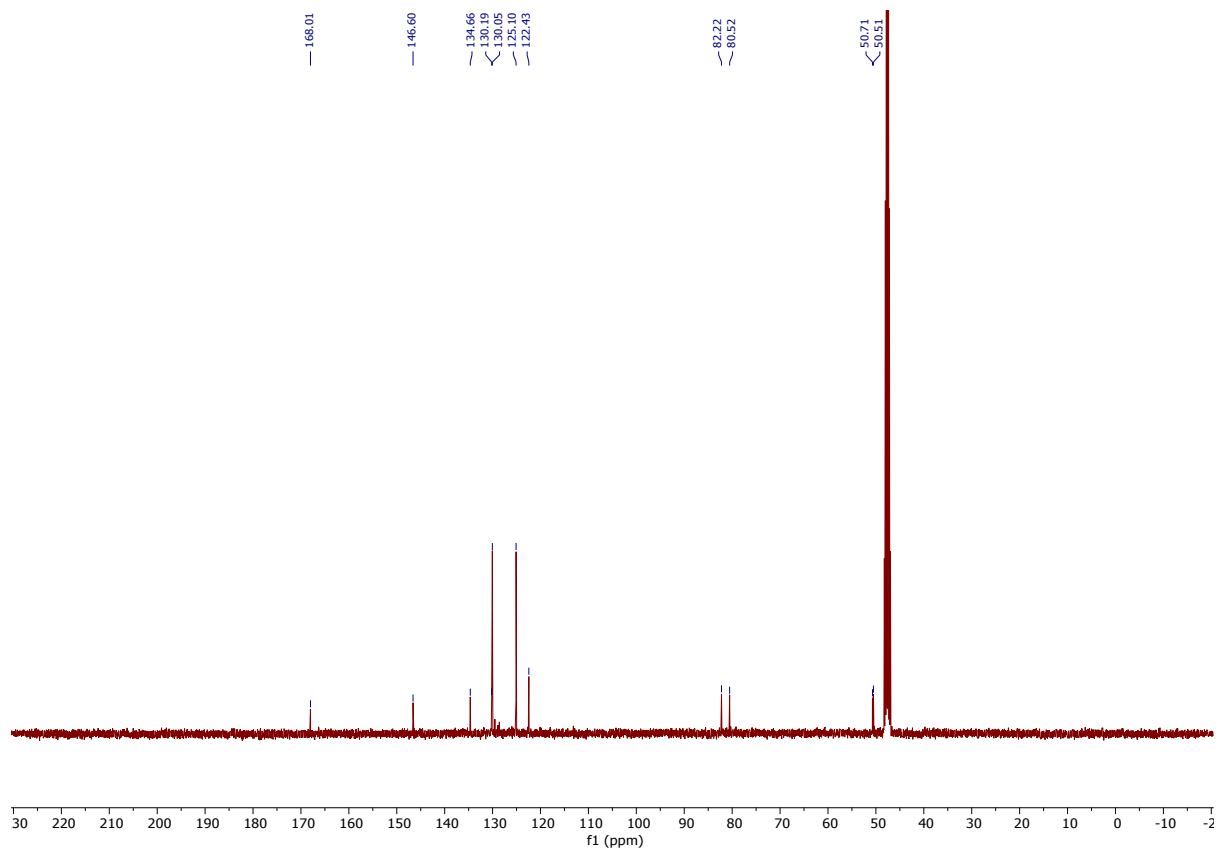
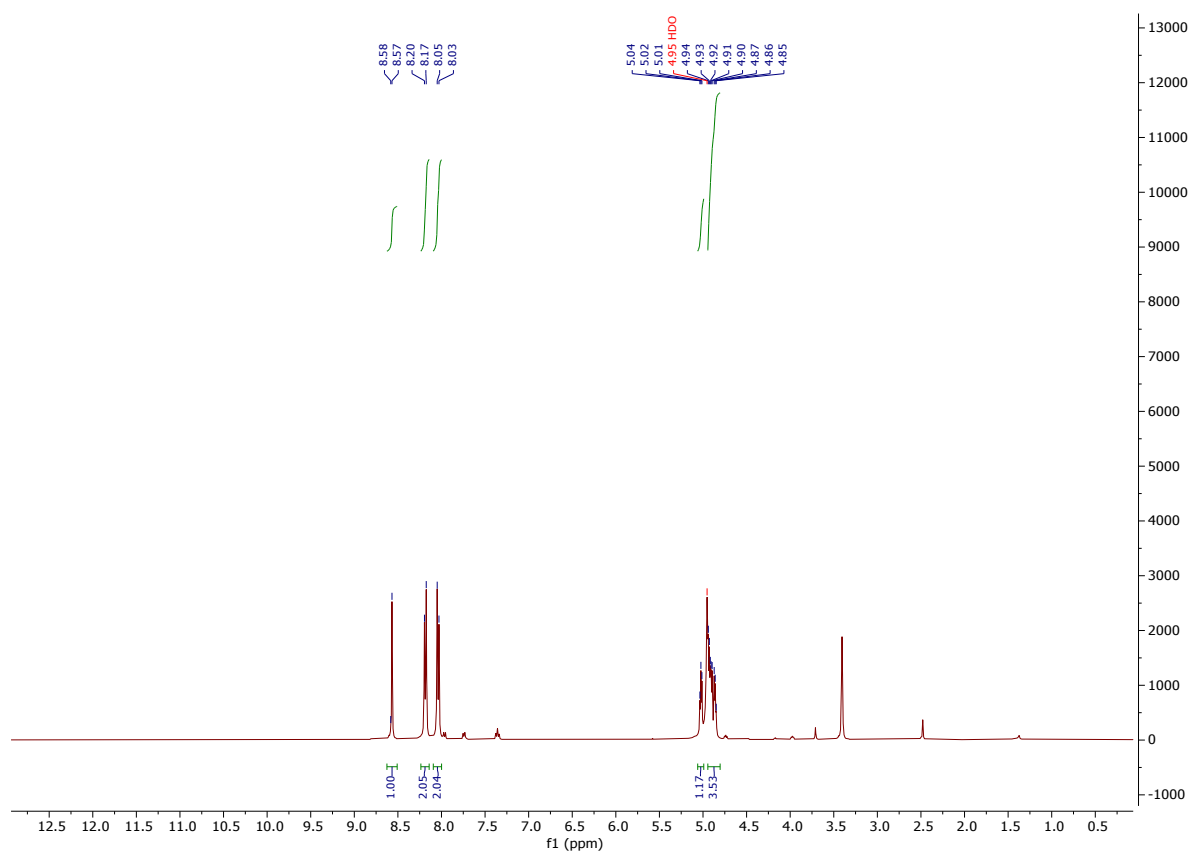
3-methyl-6-(4-(prop-2-yn-1-yloxy)phenyl)-1,2,4,5-tetrazine (VI)



2,3,4-Tri-O-acetyl-6-O-(4-nitrobenzoyl)- β -D-glucosyl azide (**pAz3**)

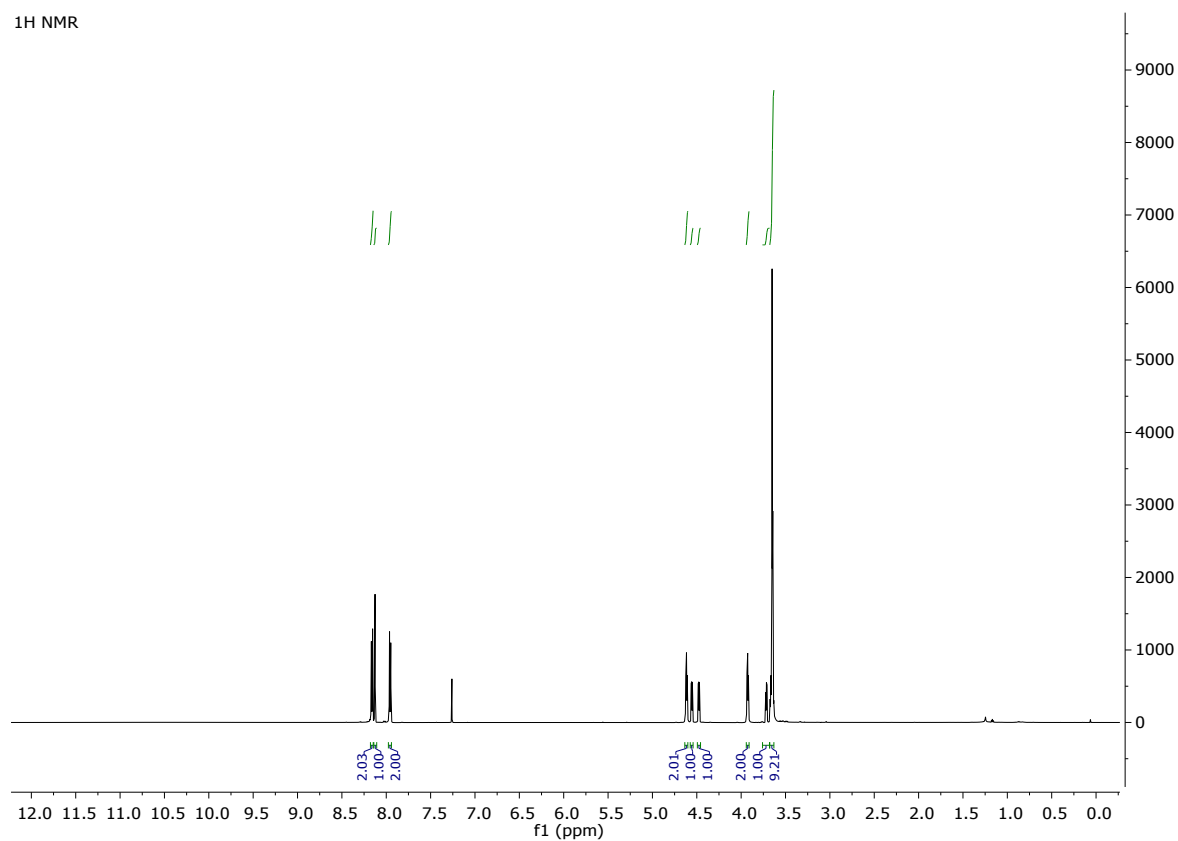


4-(1-(2-Fluoroethyl)-1H-1,2,3-triazol-4-yl)benzoic acid (S18)

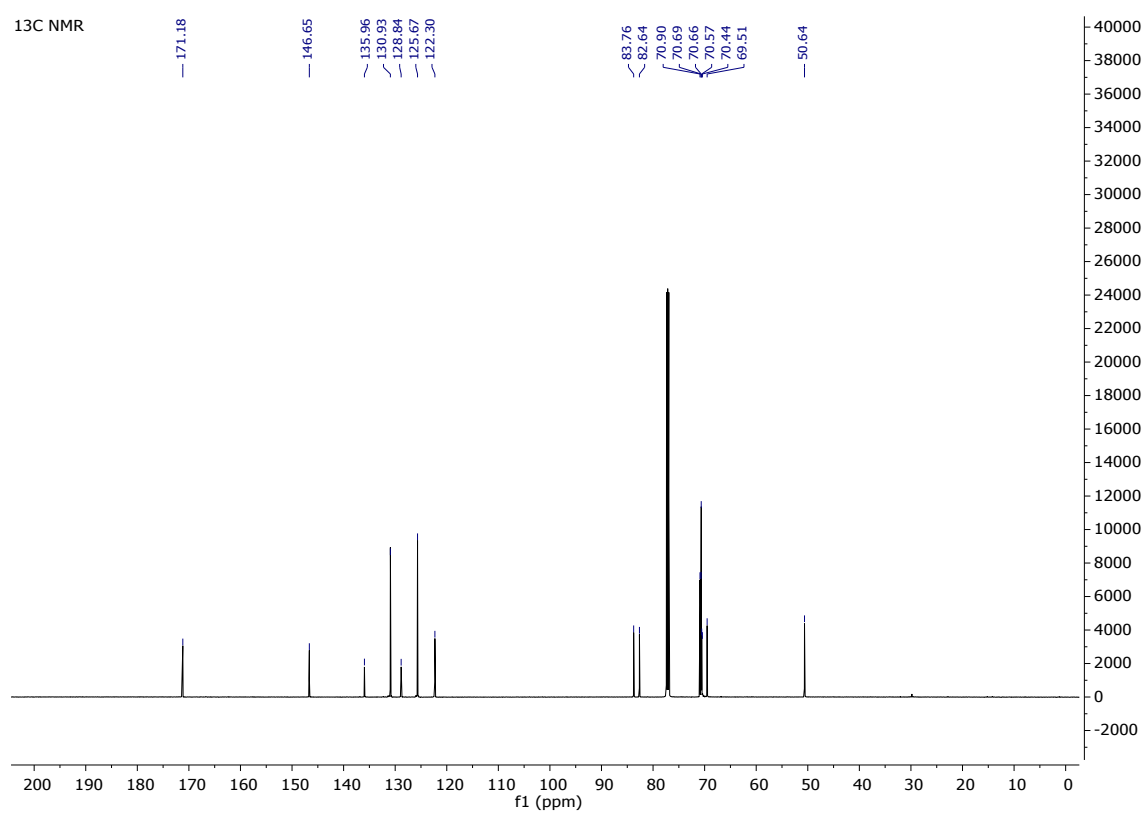


4-(1-(2-(2-(2-(2-Fluoroethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)benzoic acid (S19)

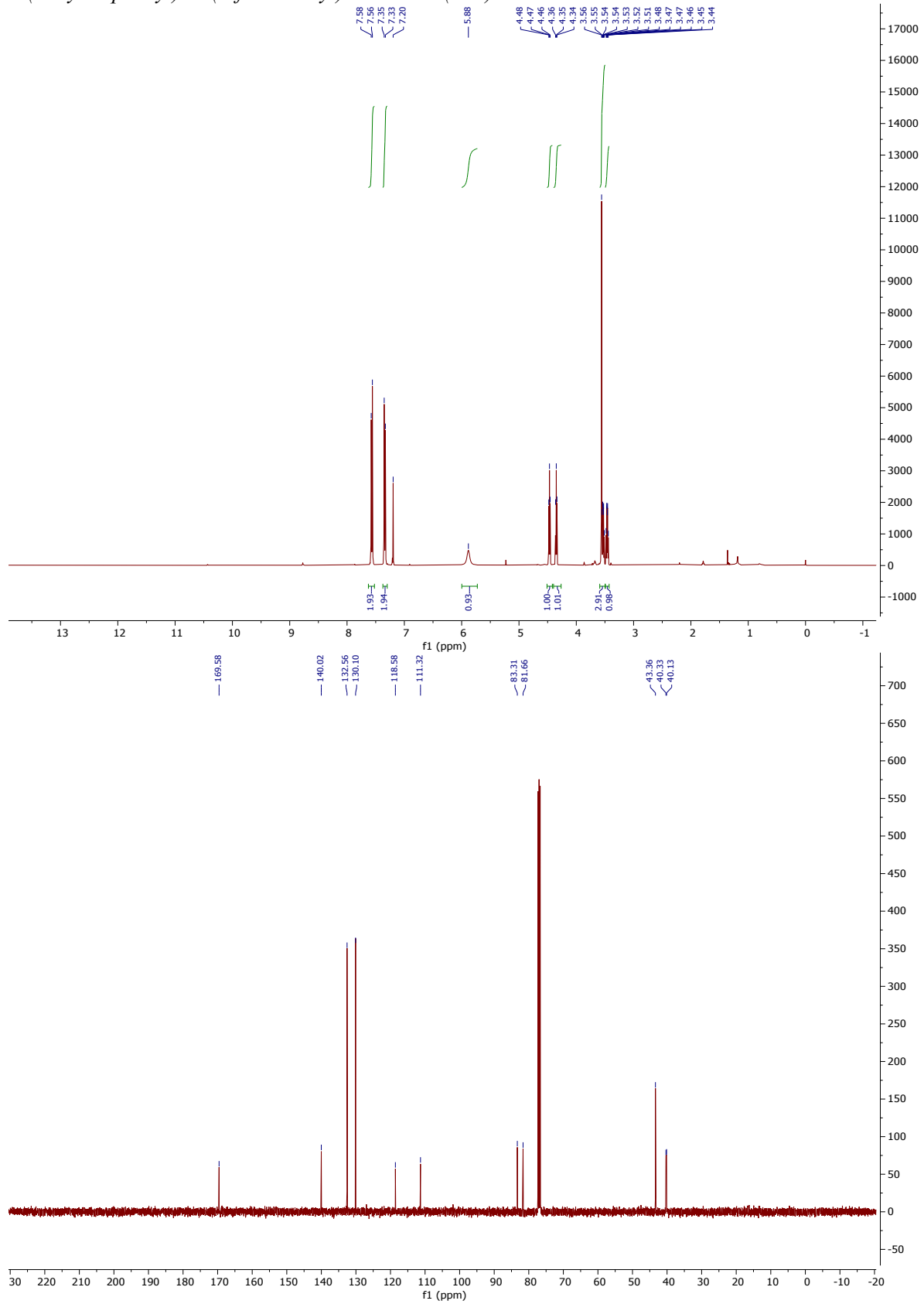
¹H NMR



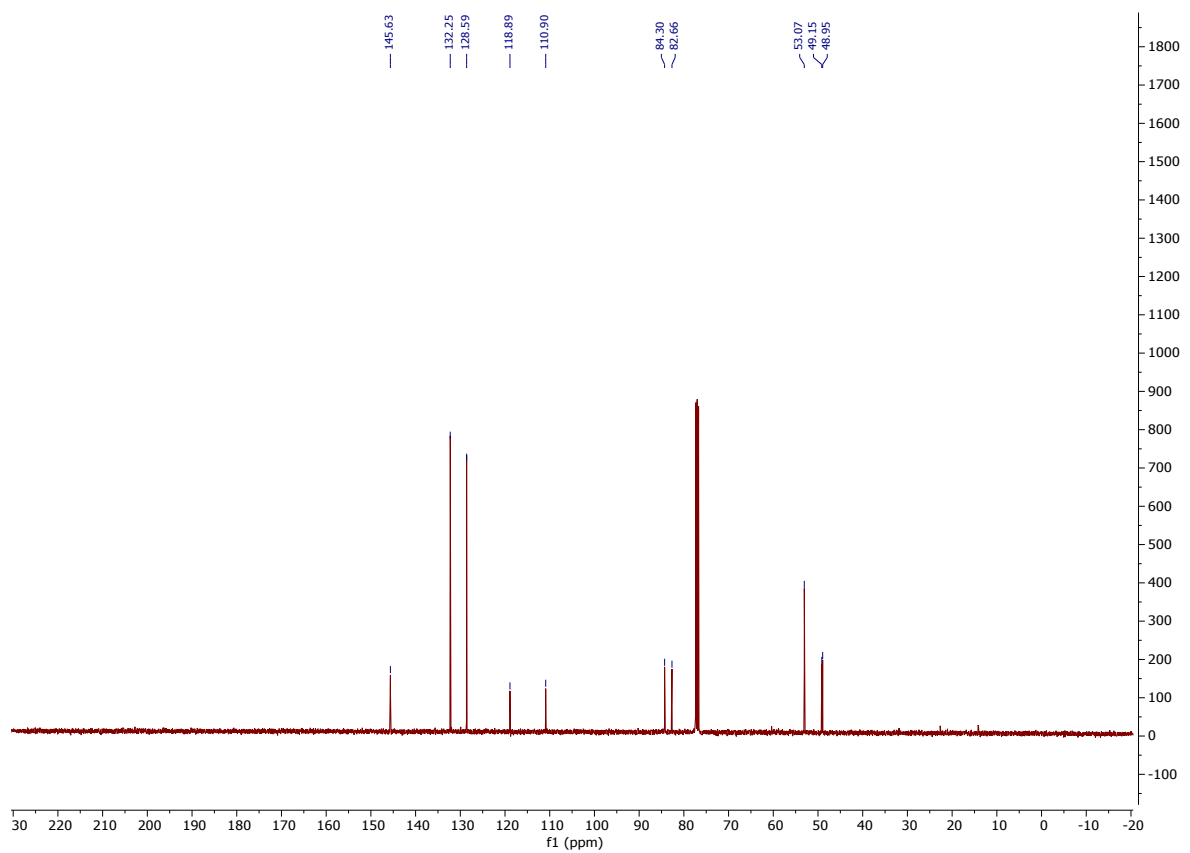
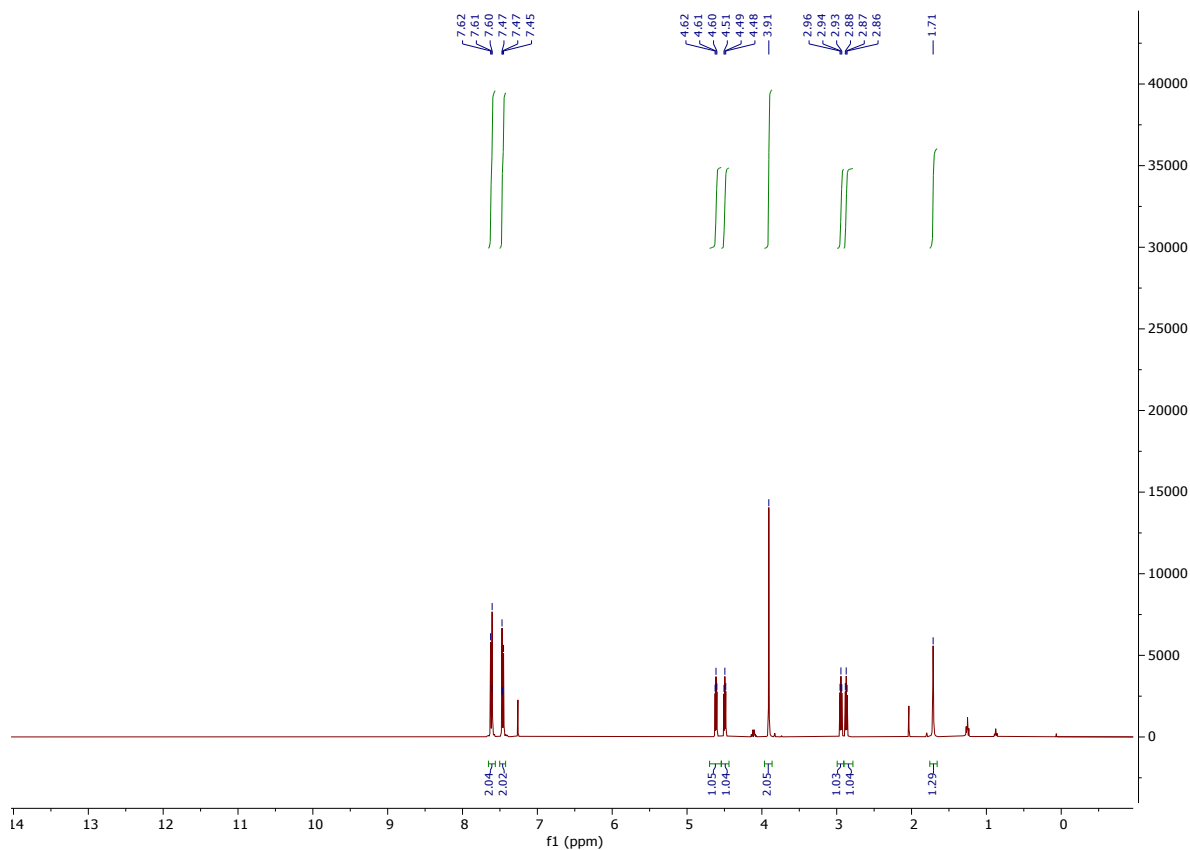
¹³C NMR



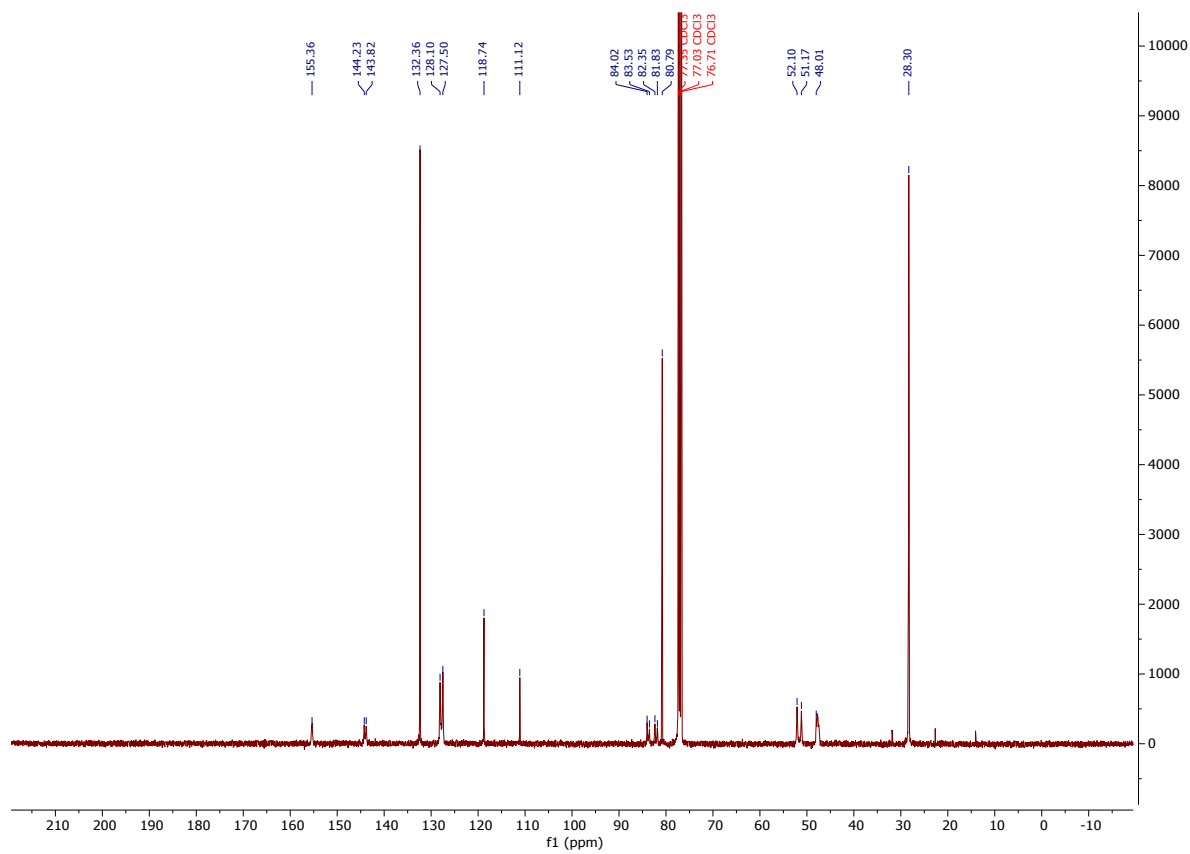
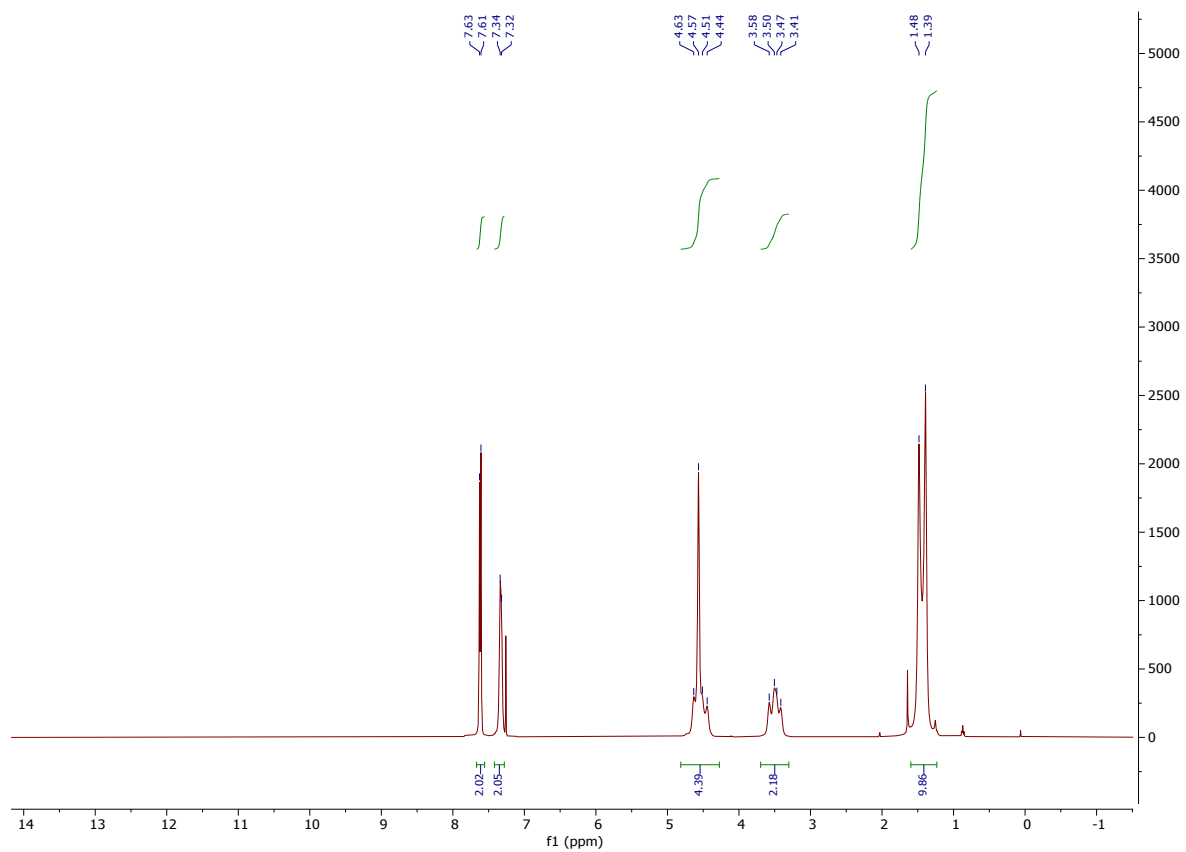
2-(4-Cyanophenyl)-N-(2-fluoroethyl)acetamide (S23)



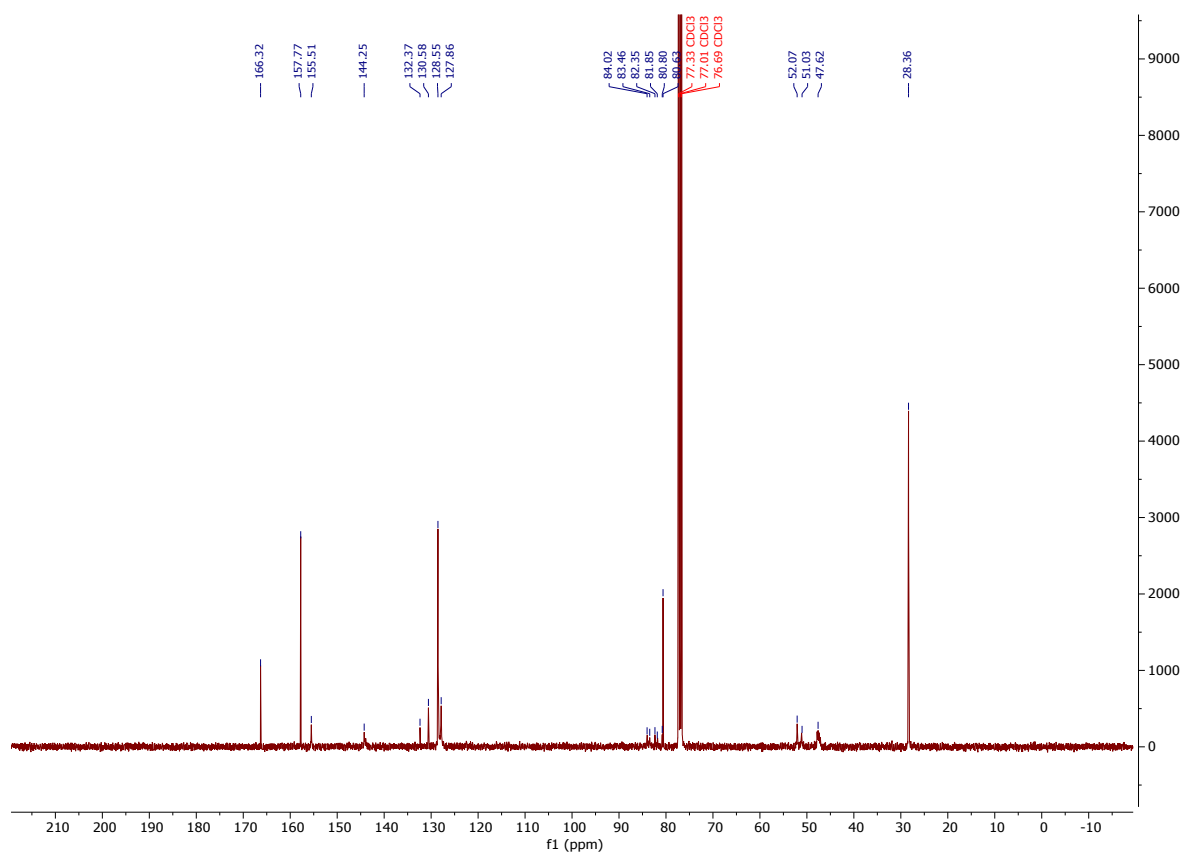
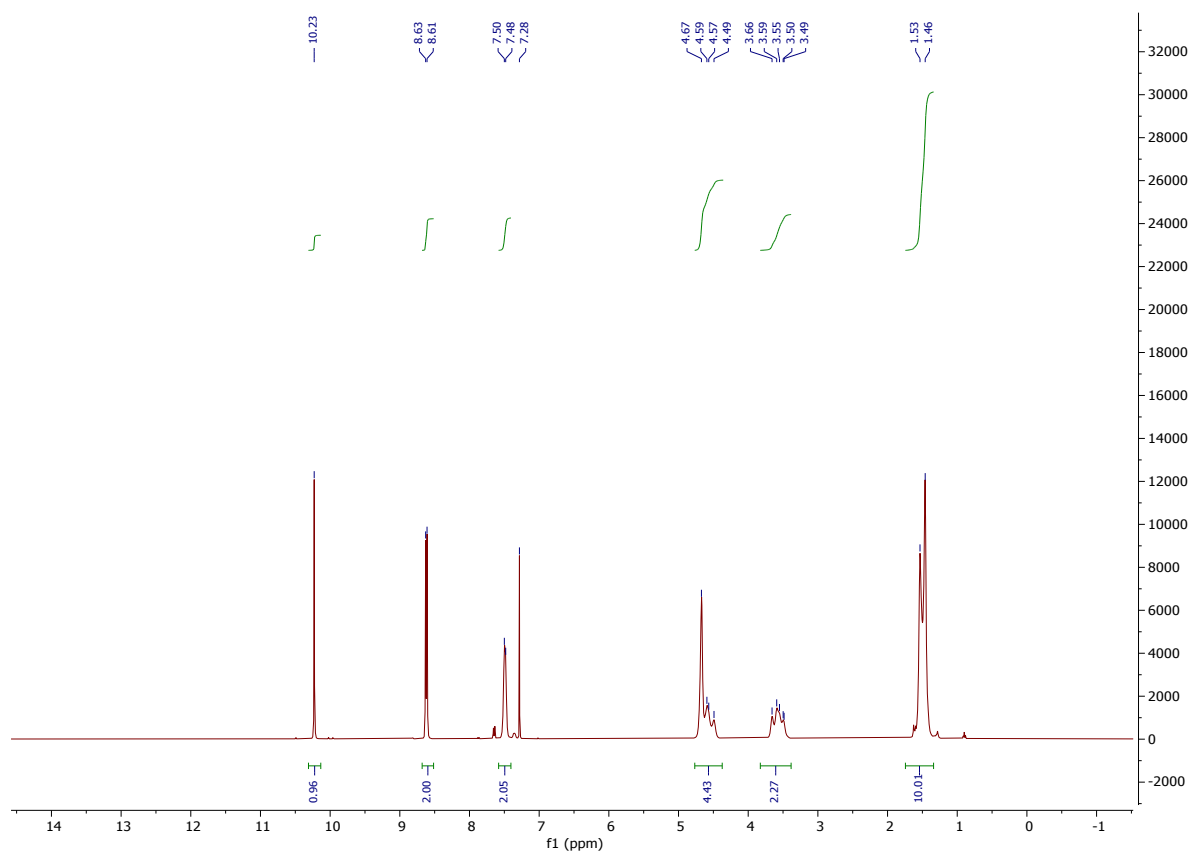
4-(((2-Fluoroethyl)amino)methyl)benzonitrile (S25)



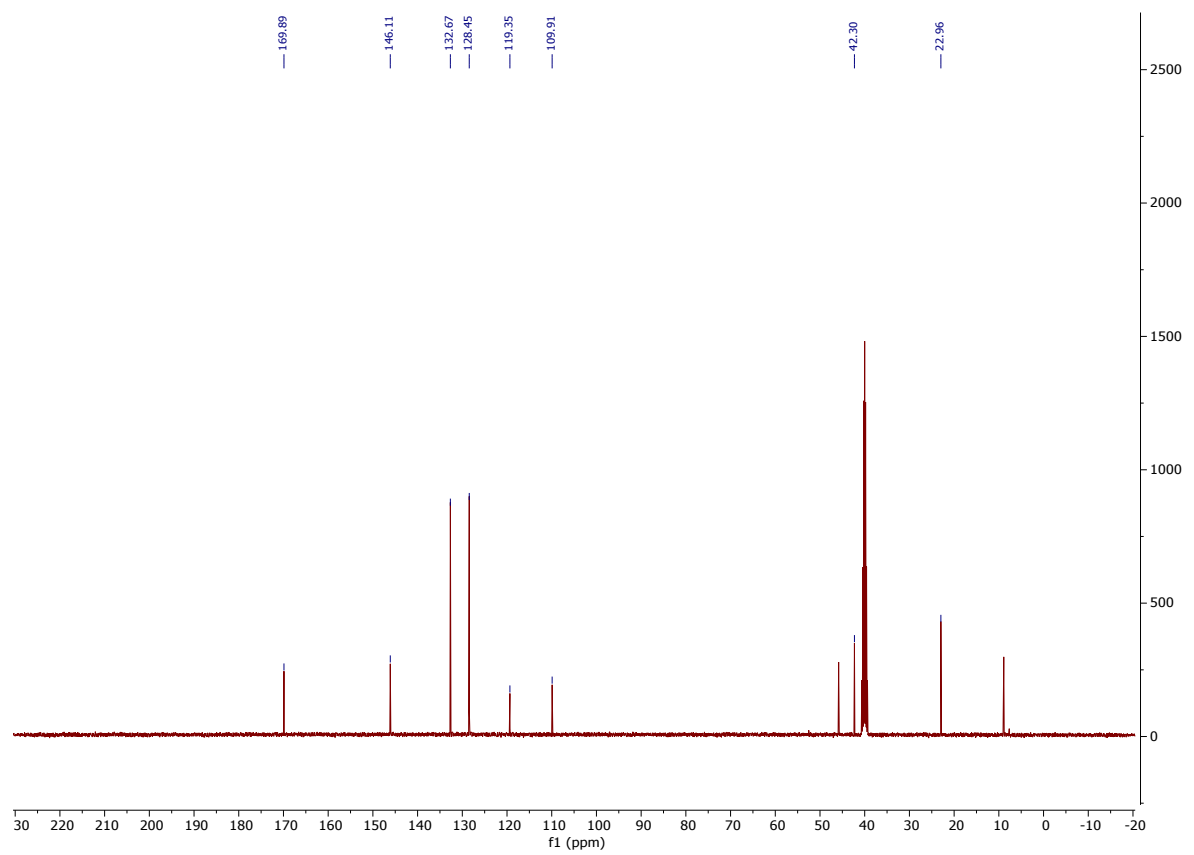
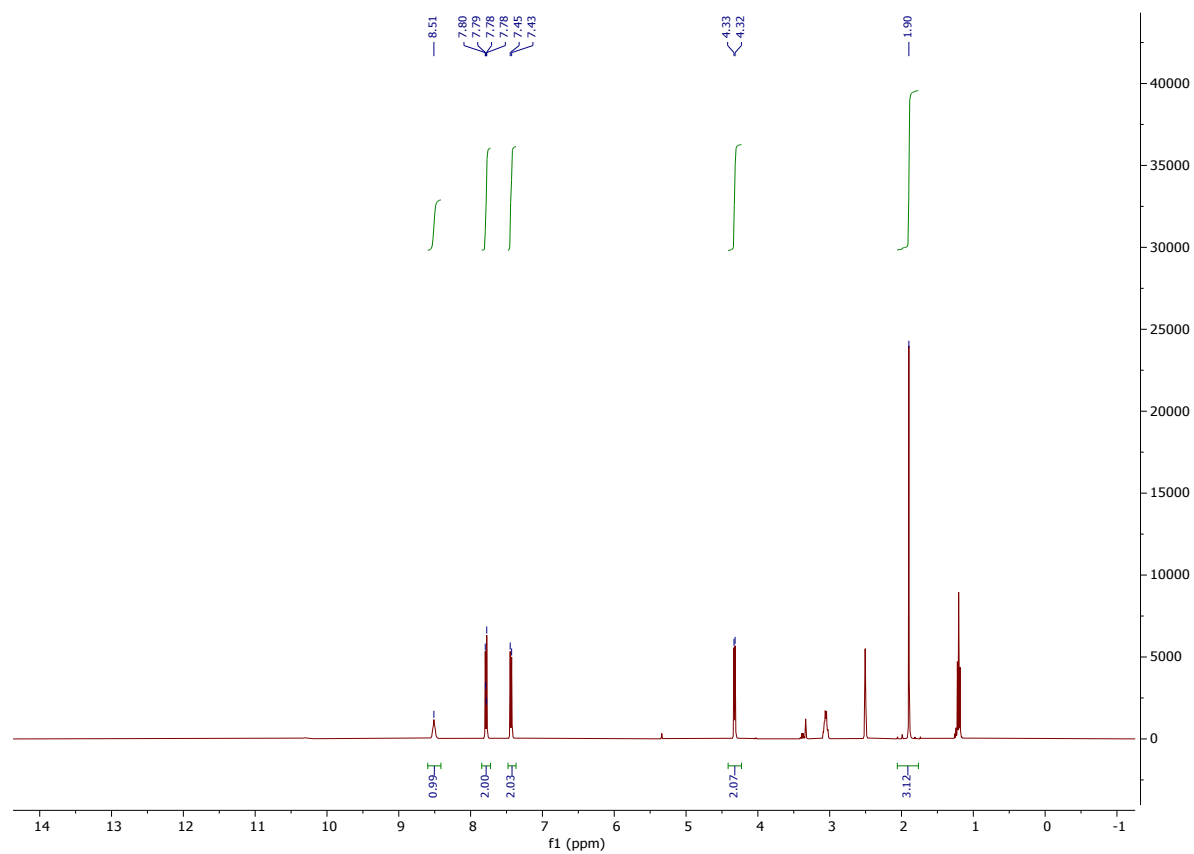
Tert-butyl 4-cyanobenzyl(2-fluoroethyl)carbamate (S26)



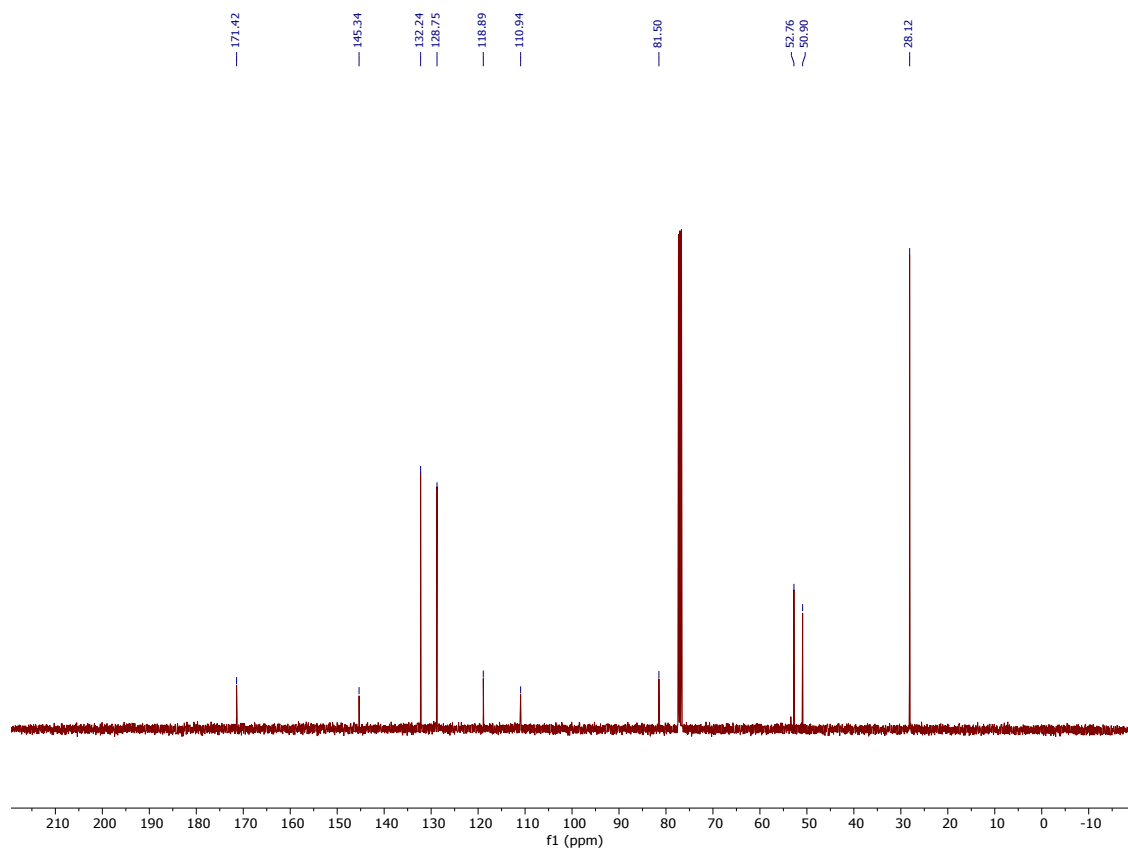
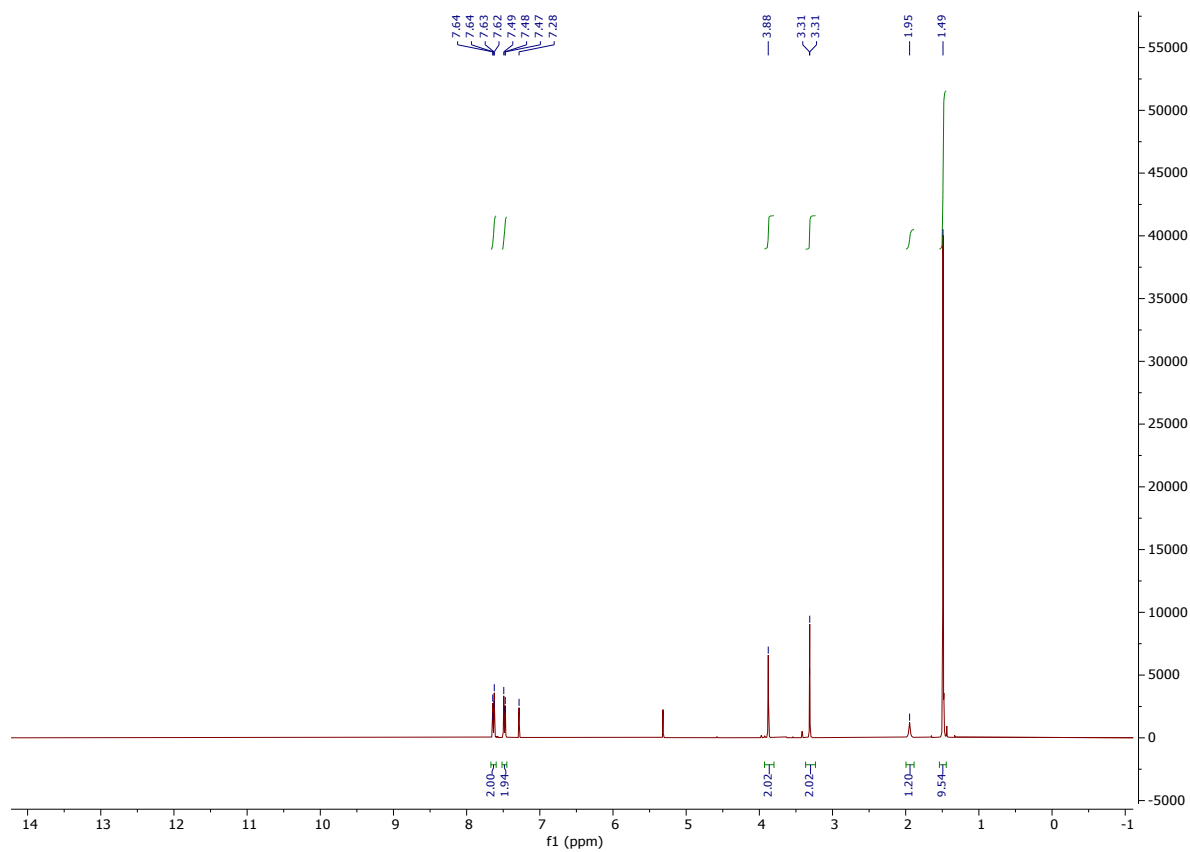
Tert-butyl 4-(1,2,4,5-tetrazin-3-yl)benzyl(2-fluoroethyl)carbamate (S27)



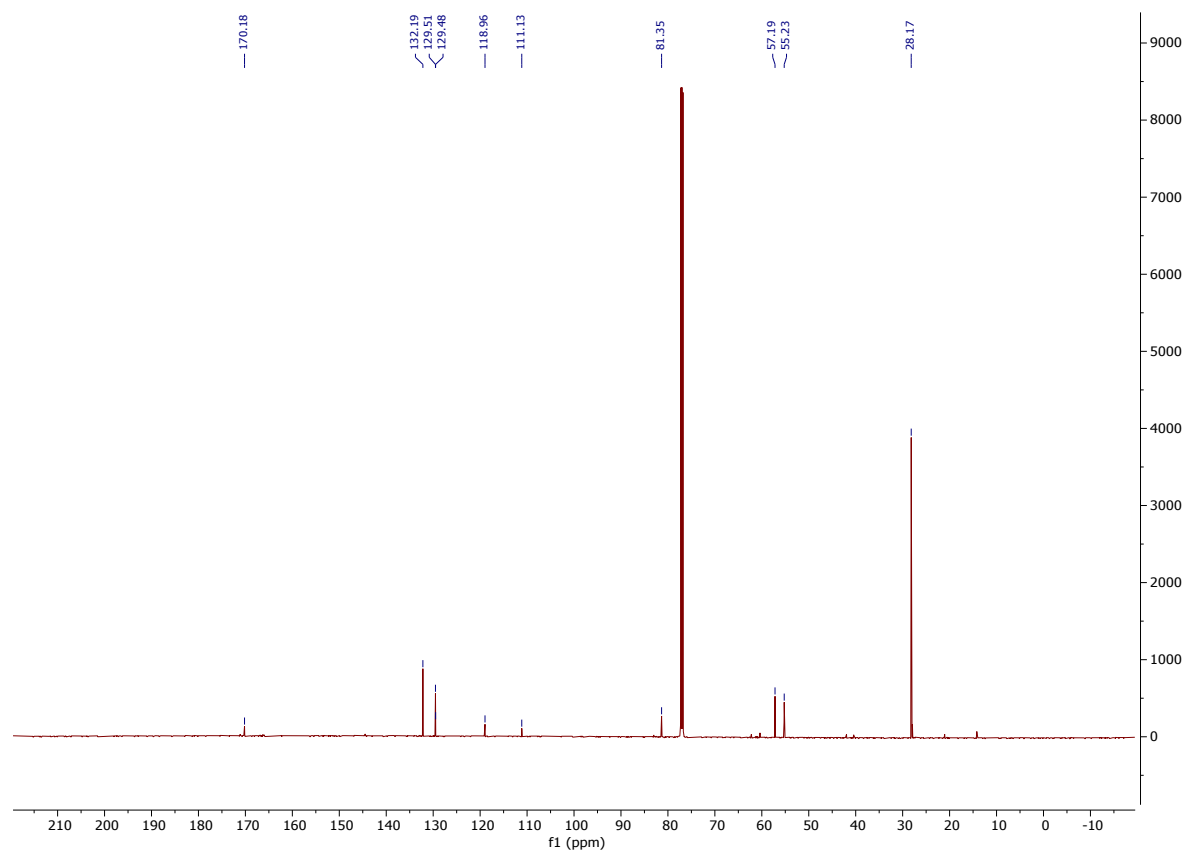
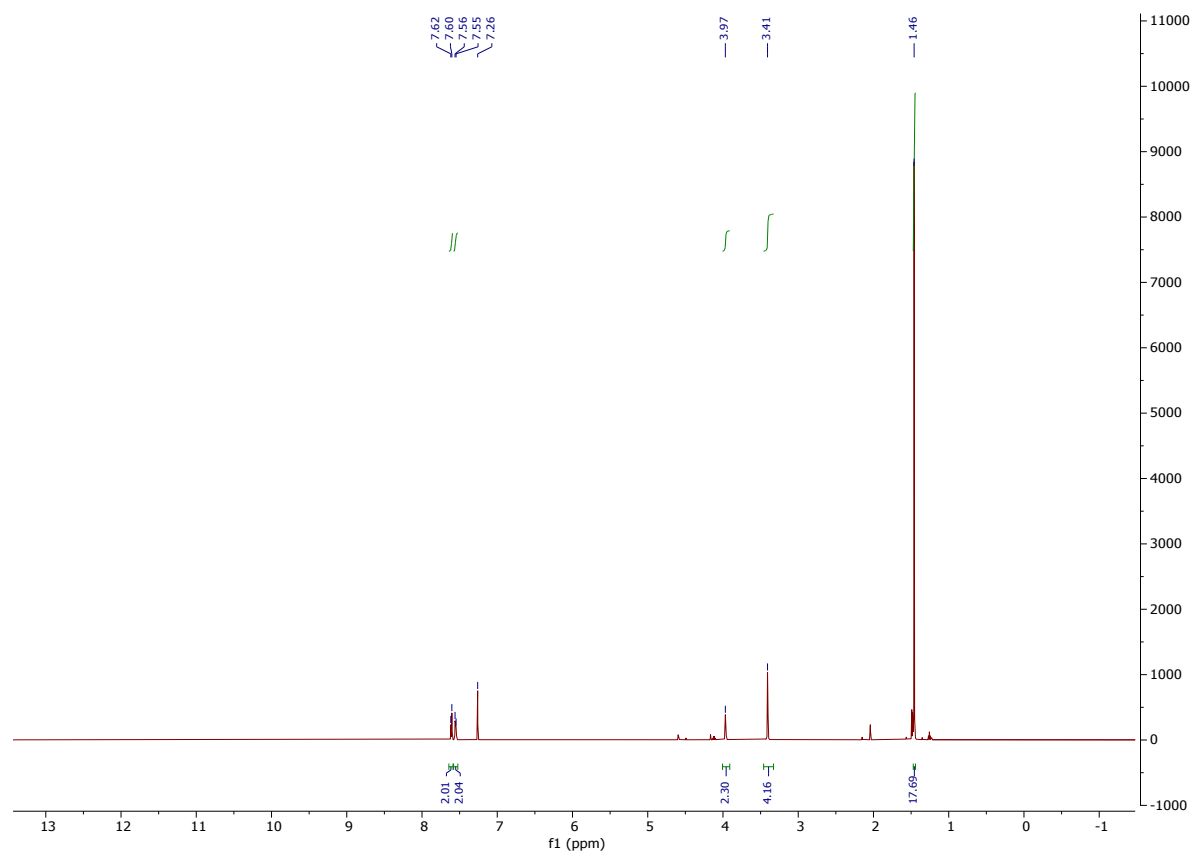
N-(4-Cyanobenzyl)acetamide (**S29**)



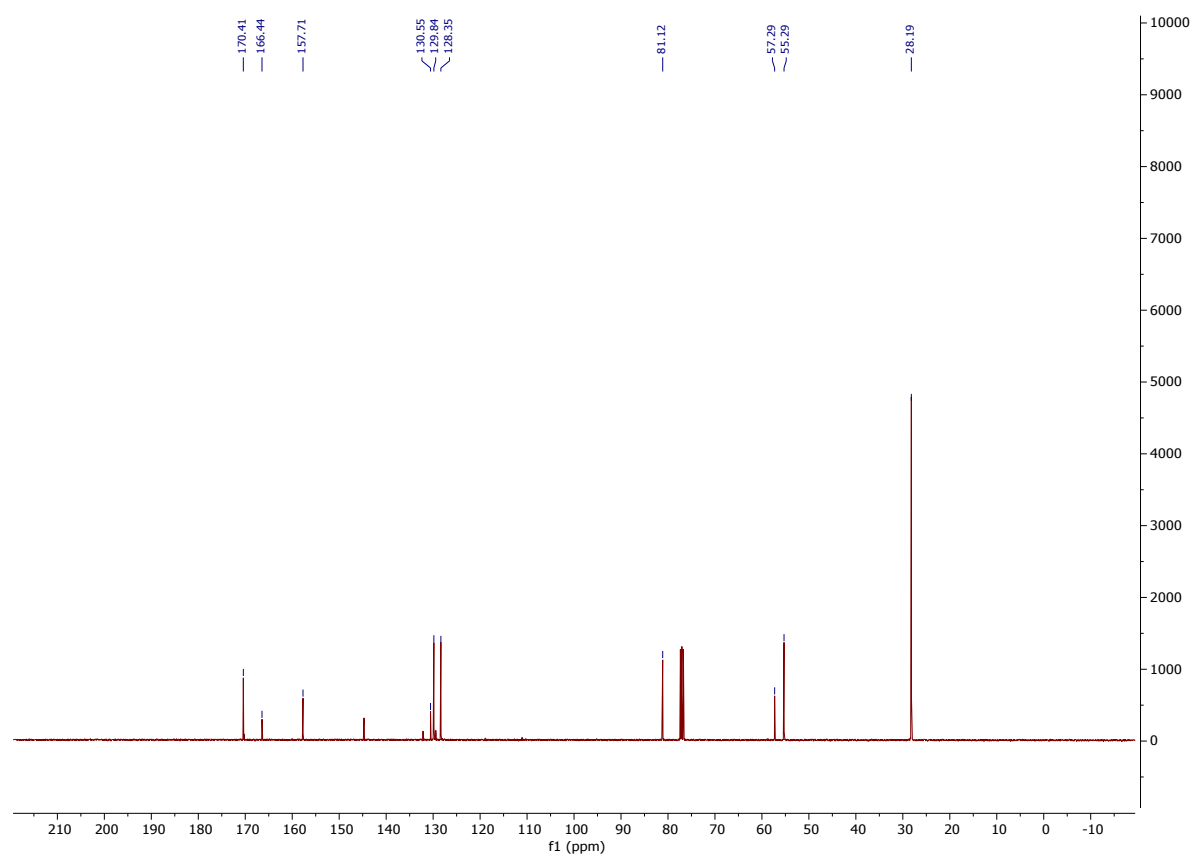
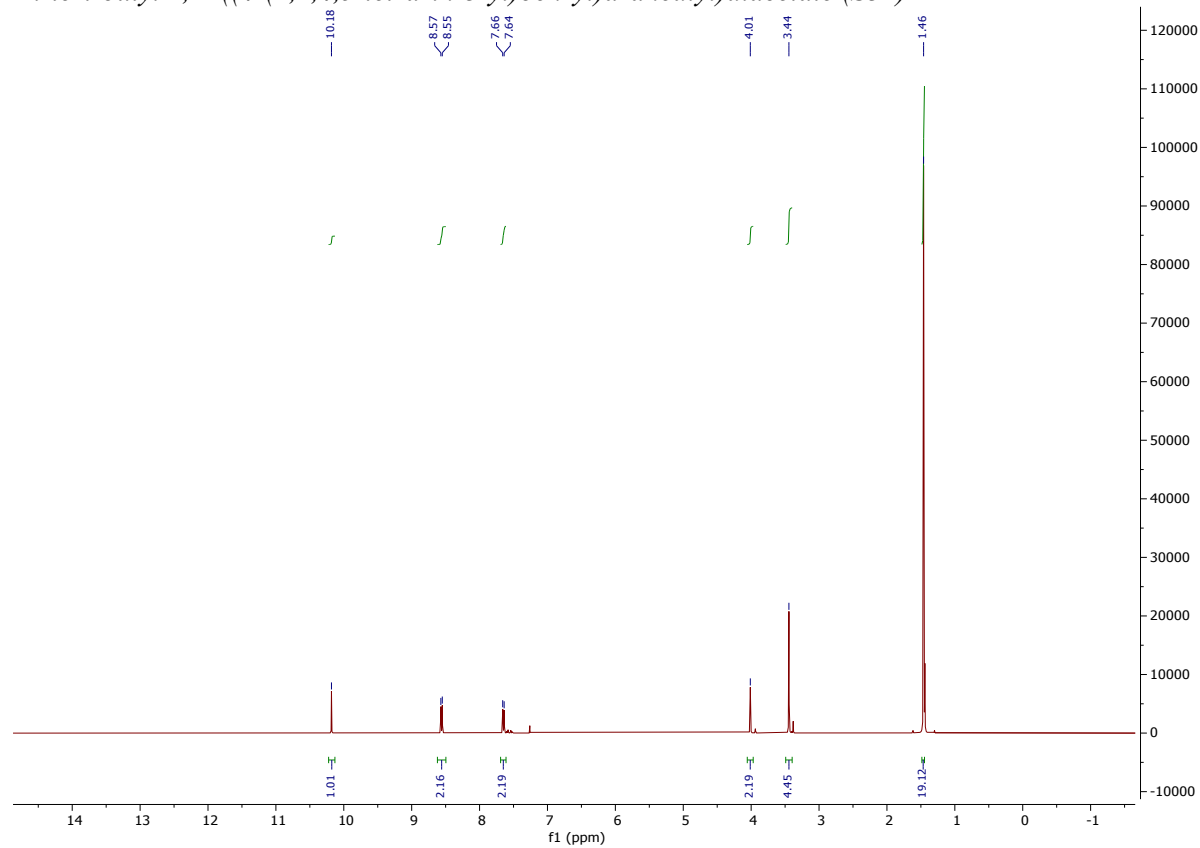
Tert-butyl 2-((4-cyanobenzyl)amino)acetate (S30)



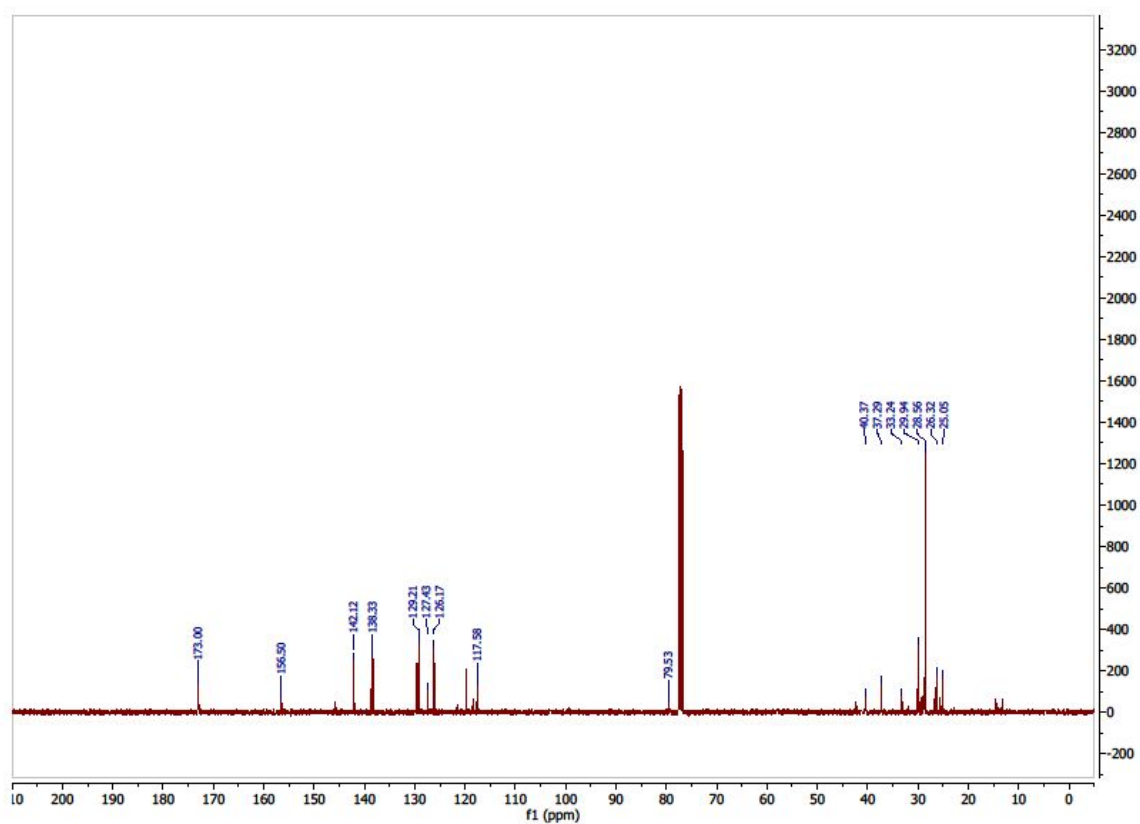
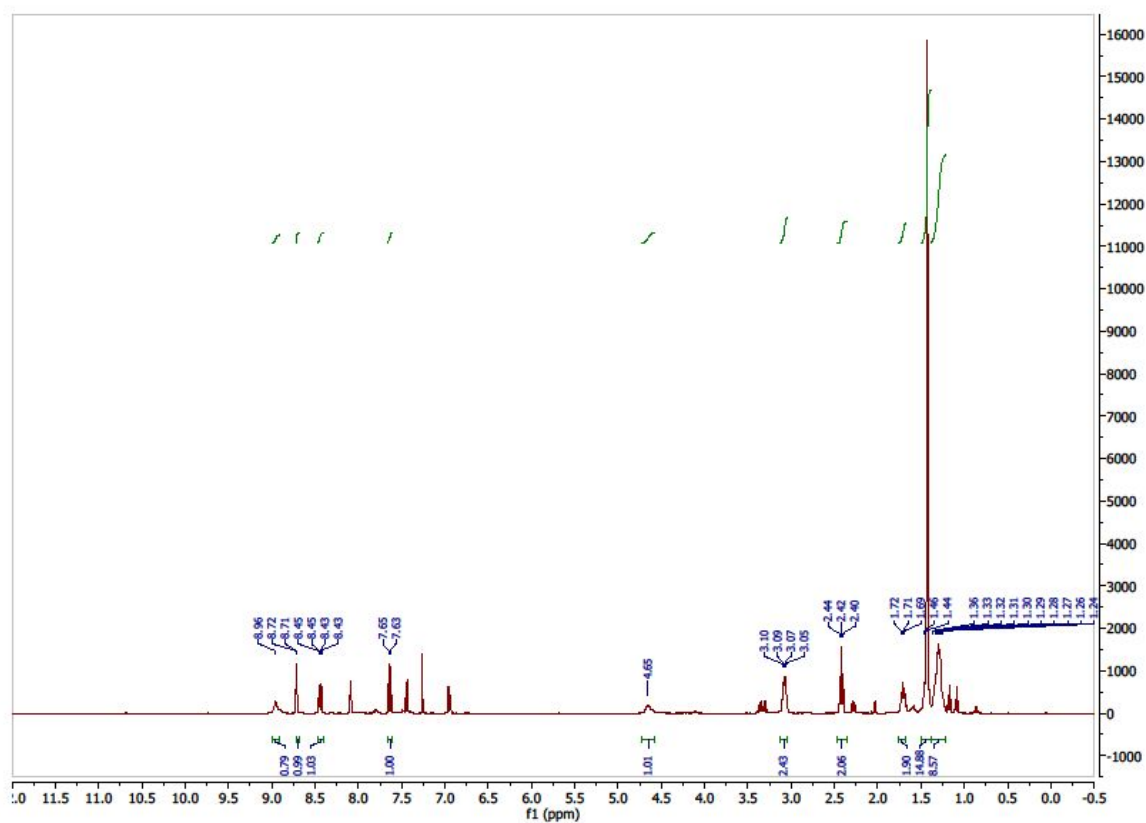
Di-tert-butyl 2,2'-((4-cyanobenzyl)azanediyl)diacetate (S31)



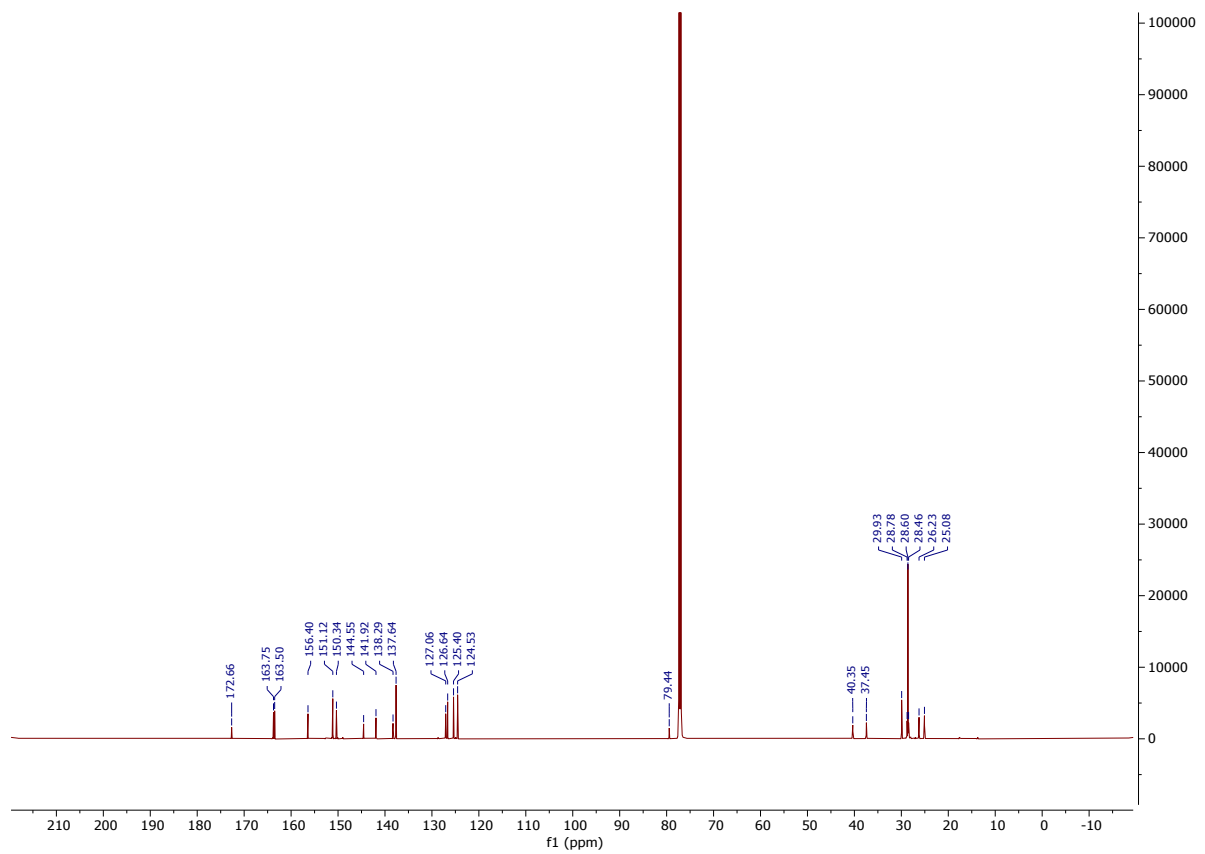
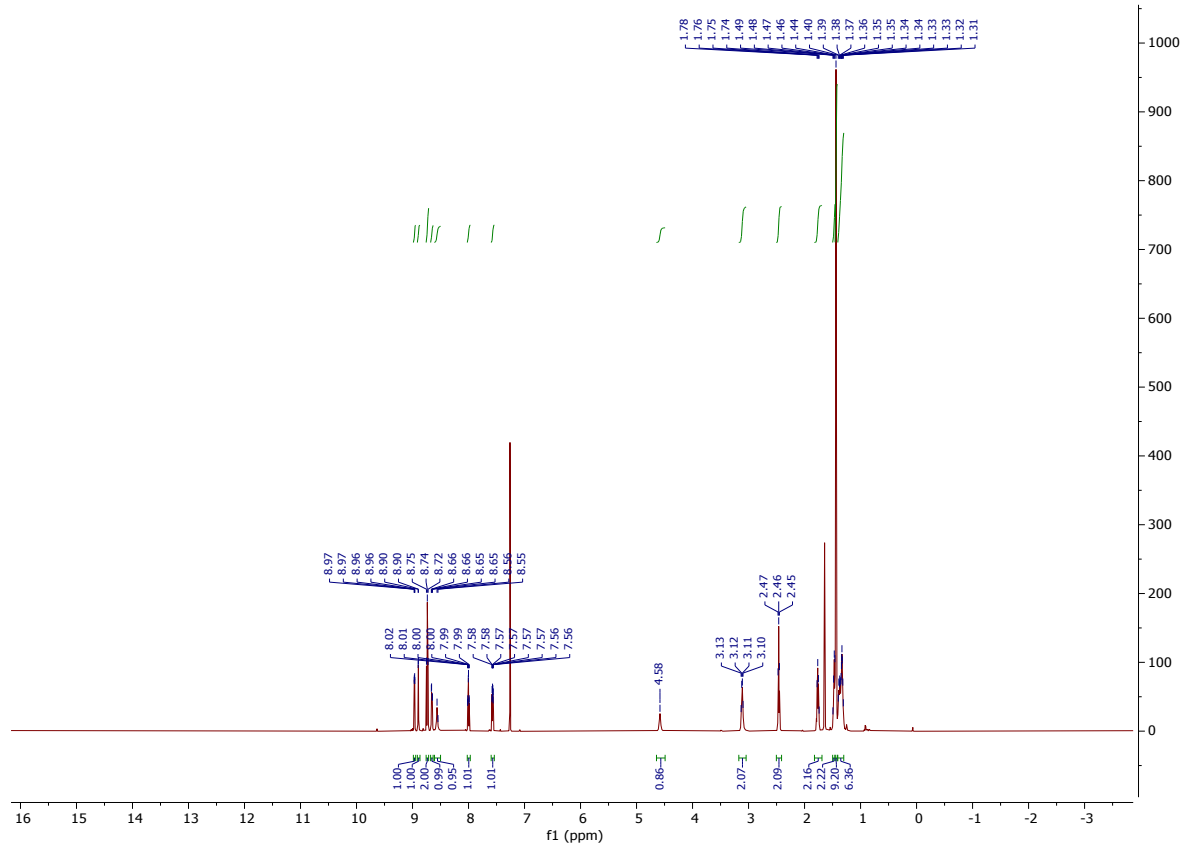
Di-tert-butyl 2,2'-((4-(1,2,4,5-tetrazin-3-yl)benzyl)azanediyl)diacetate (S32)



Tert-butyl (8-((6-cyanopyridin-3-yl)amino)-8-oxooctyl)carbamate (S34)

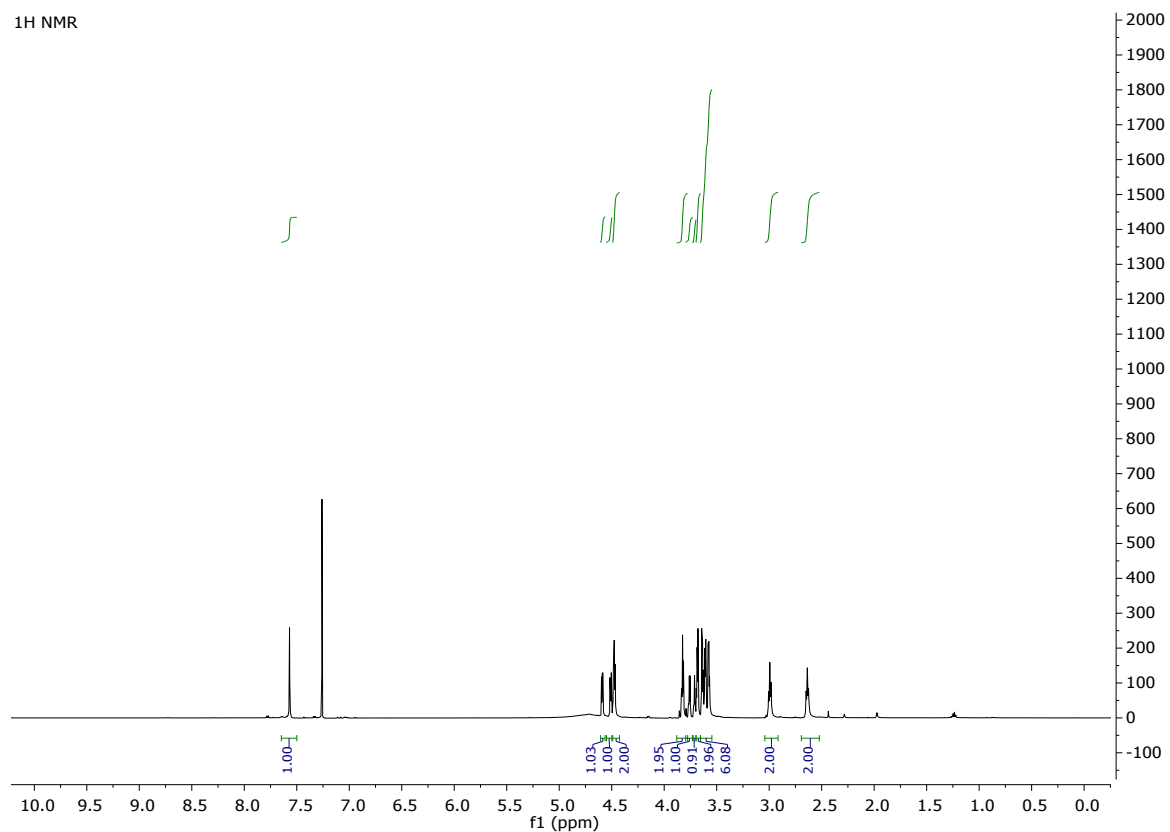


Tert-Butyl (8-oxo-8-((6-(6-(pyridin-2-yl)-tetrazin-3-yl)pyridin-3-yl)amino)octyl)carbamate (S36)

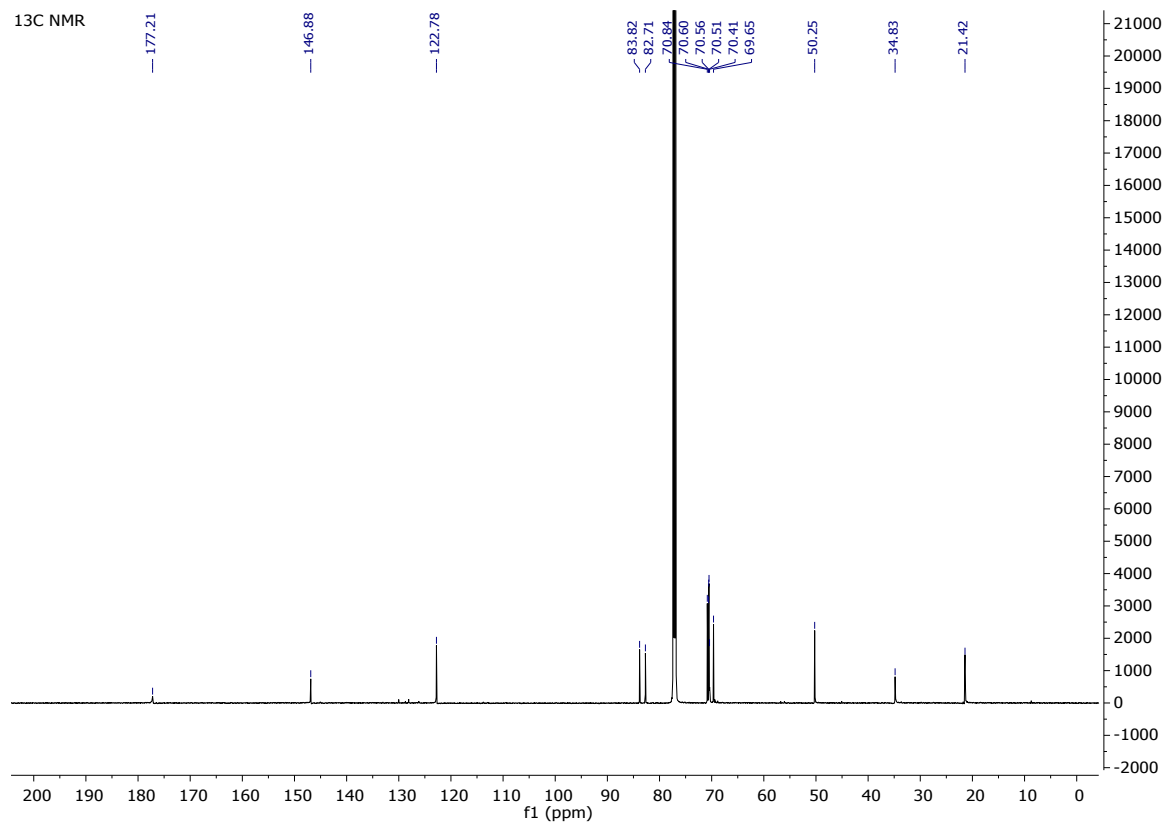


3-(1-(2-(2-(2-(2-Fluoroethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)propanoic acid (S40)

¹H NMR



¹³C NMR



19F NMR

