

Supporting Information (2)

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

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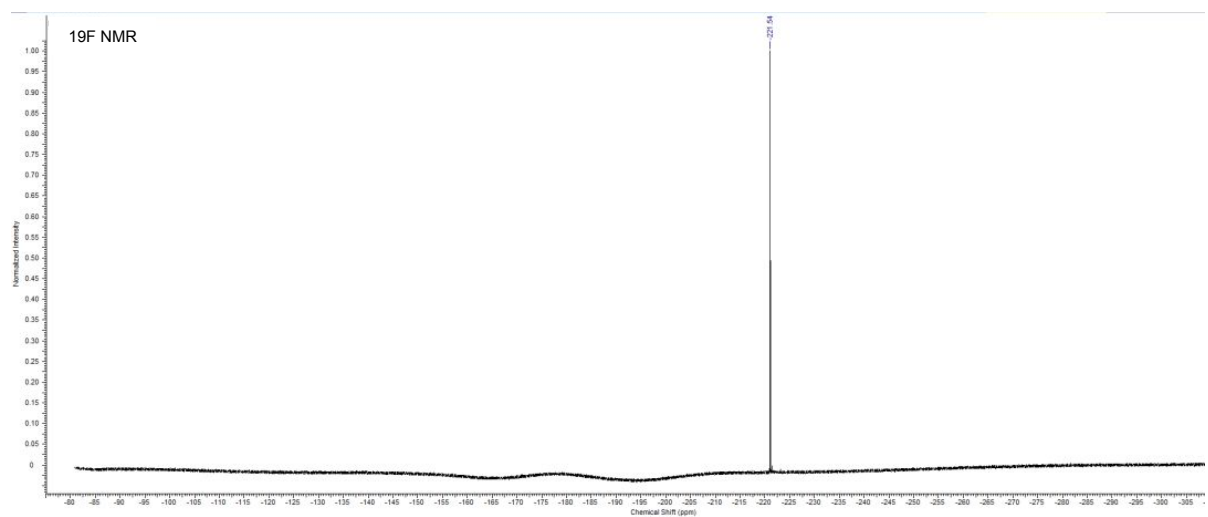
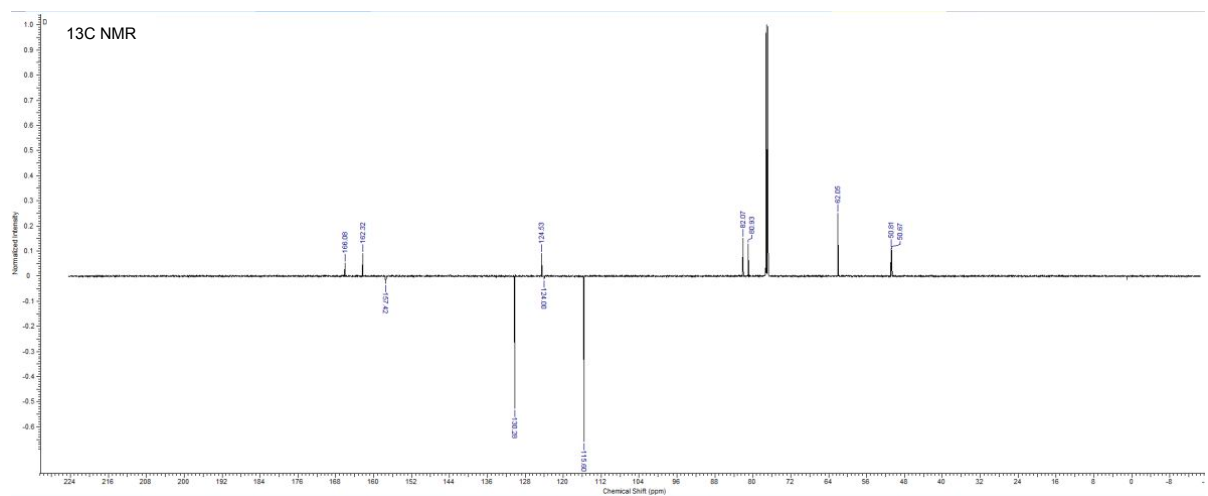
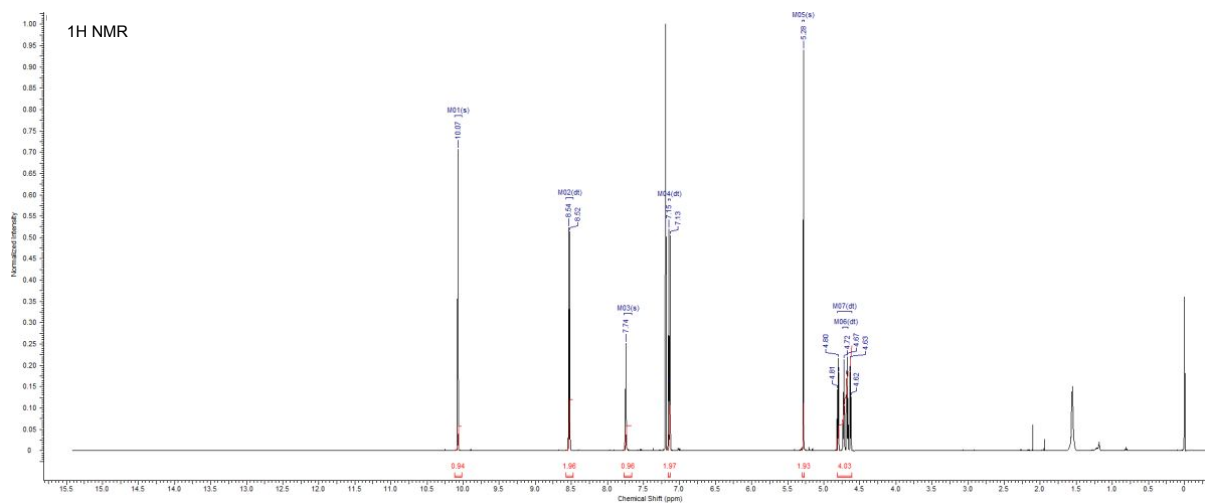
[†]Equally contributing authors

*Corresponding authors

NMR Spectra for compounds 1-45 (Tz library)

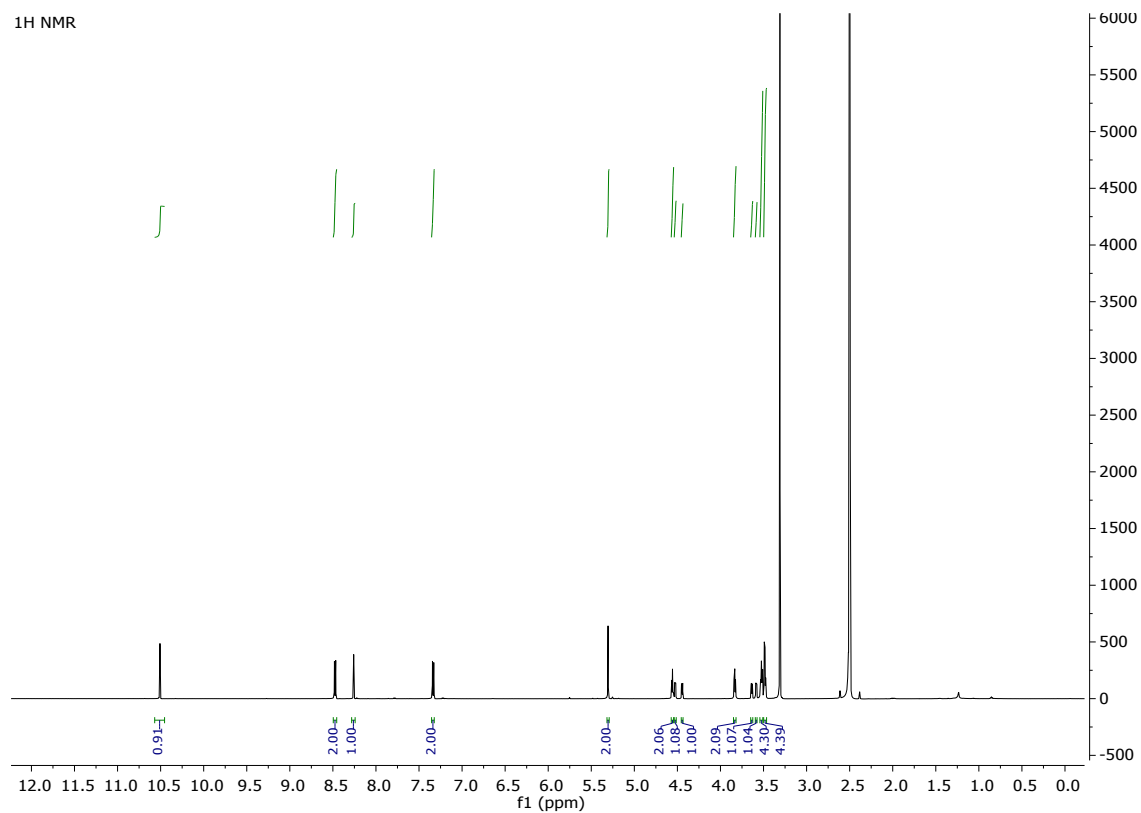
[except for tetrazines obtained as copper(II) complexes and purchased compounds]

3-(4-((1-(2-Fluoroethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1,2,4,5-tetrazine (1)

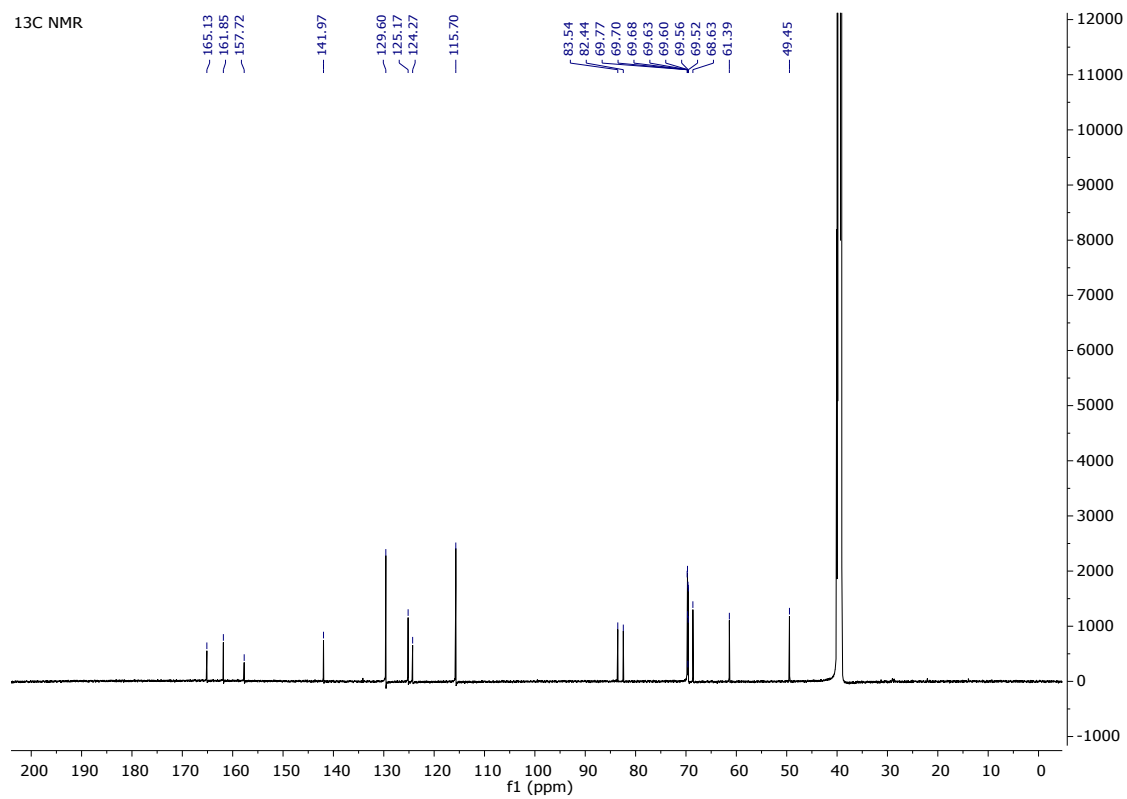


3-(4-((1-(2-(2-(2-(2-Fluoroethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1,2,4,5-tetrazine (2)

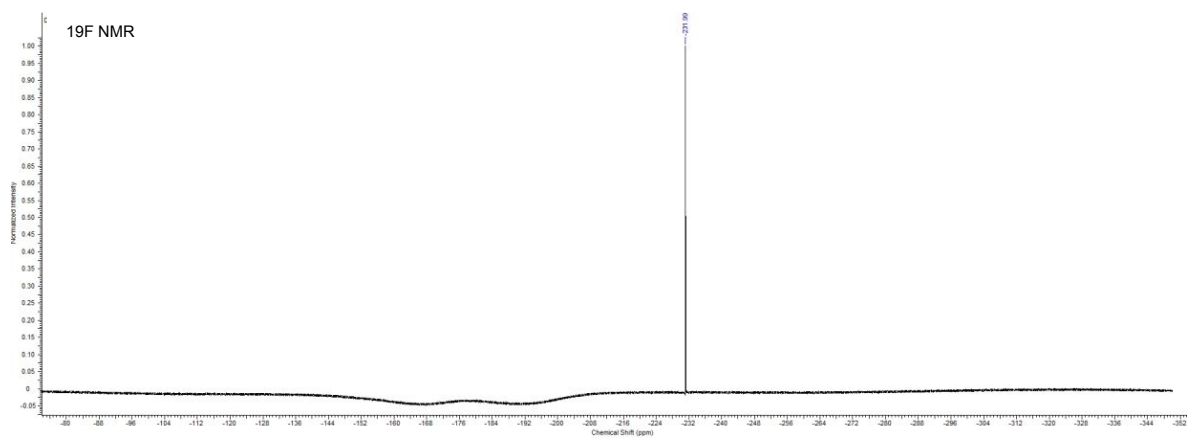
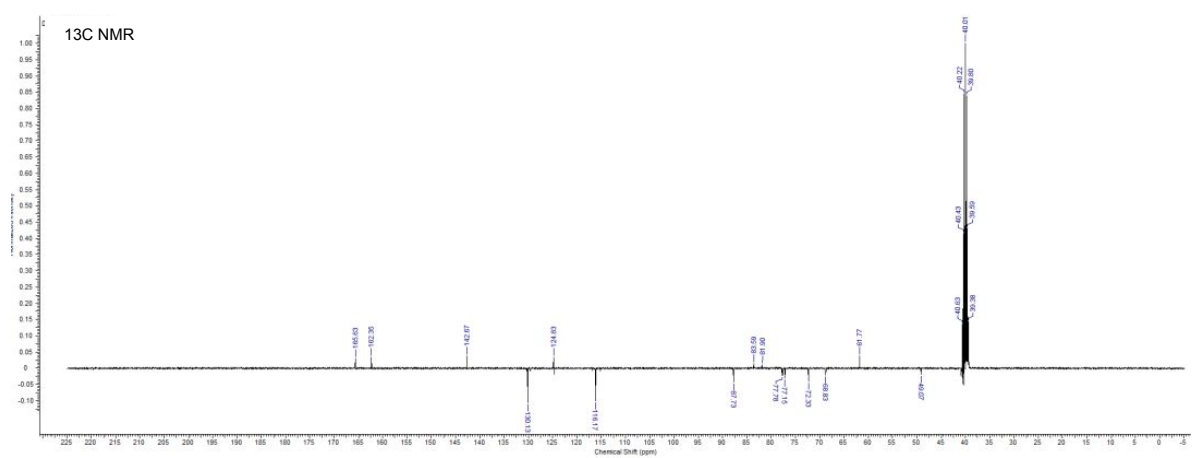
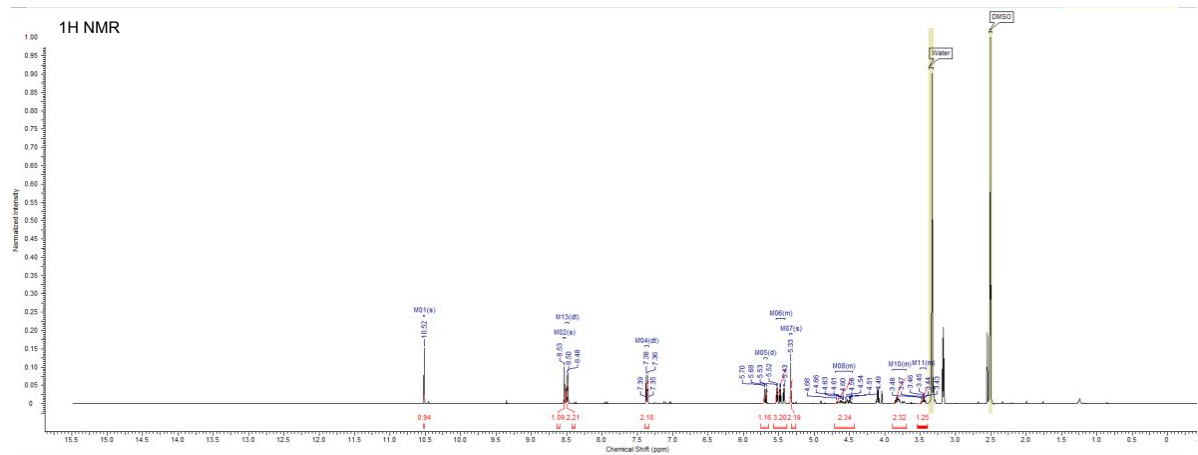
¹H NMR



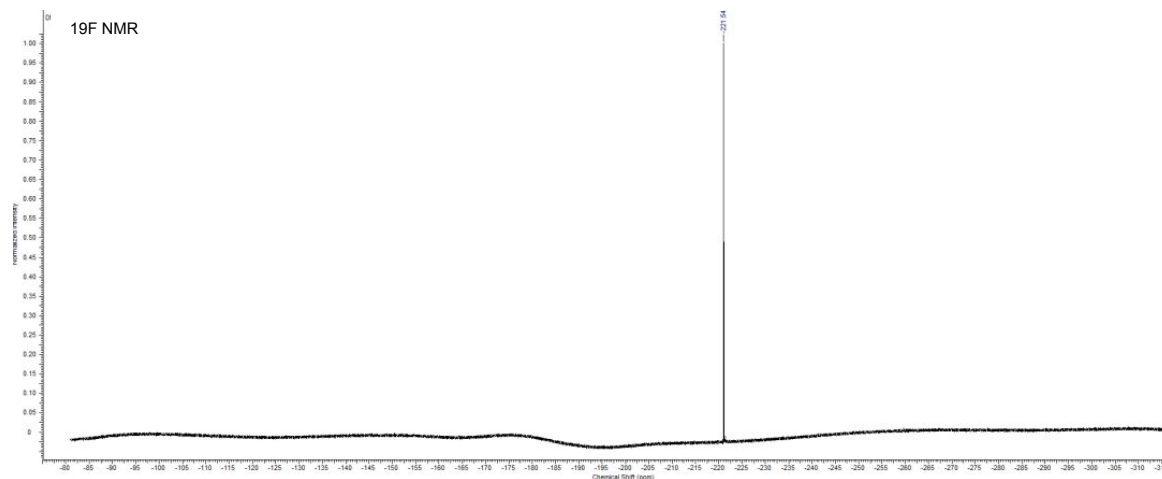
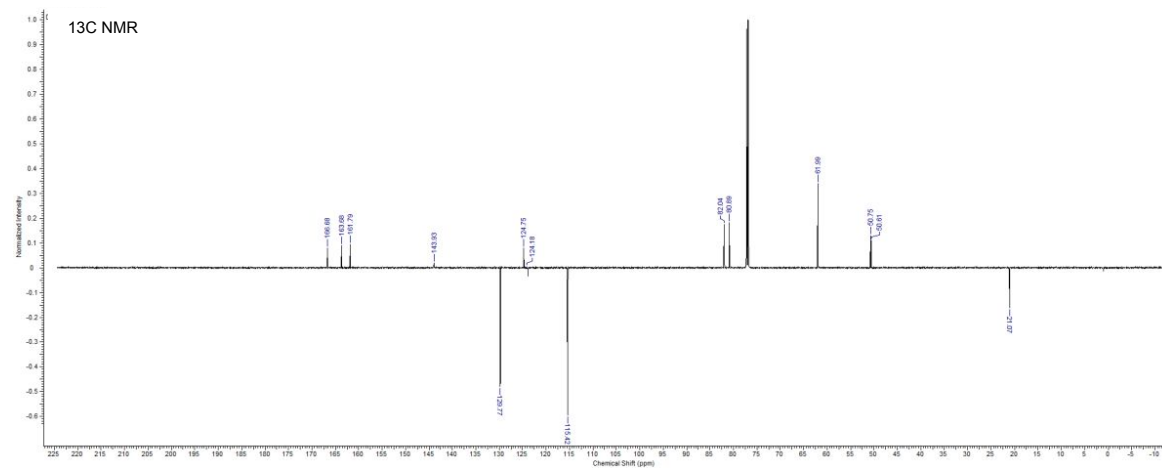
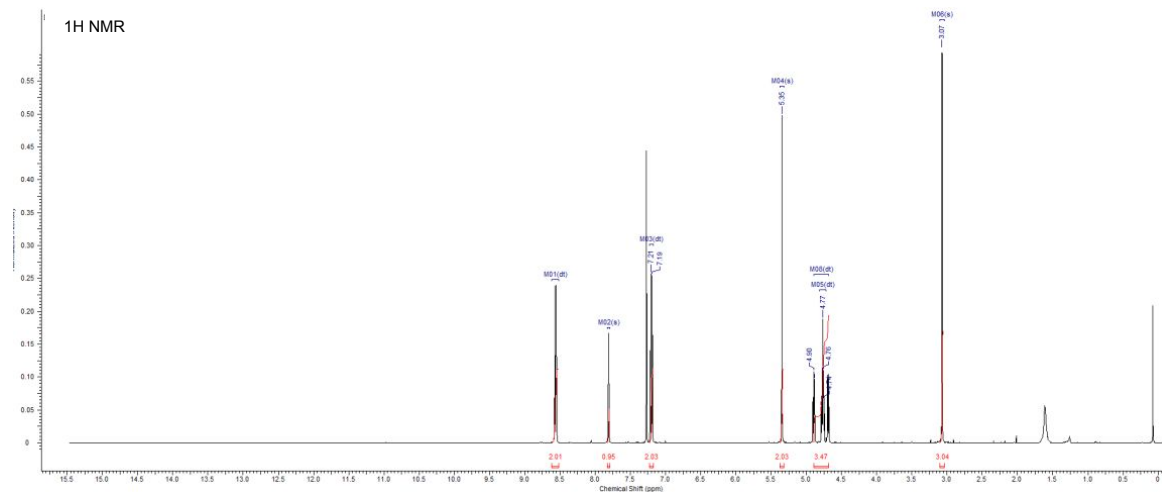
¹³C NMR



(2R,3R,4S,5S,6S)-2-(4-((1,2,4,5-Tetrazin-3-yl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)-6-(fluoromethyl)tetrahydro-2H-pyran-3,4,5-triol (3)

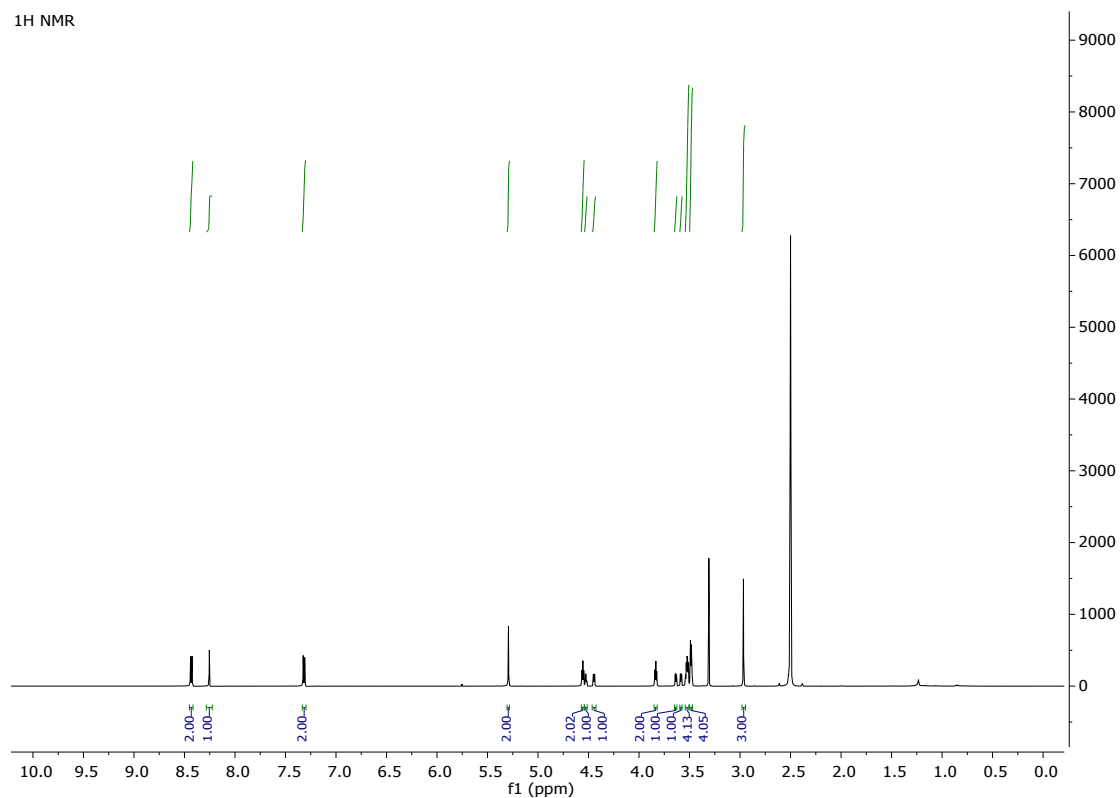


3-(4-((1-(2-Fluoroethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-6-methyl-1,2,4,5-tetrazine (5)

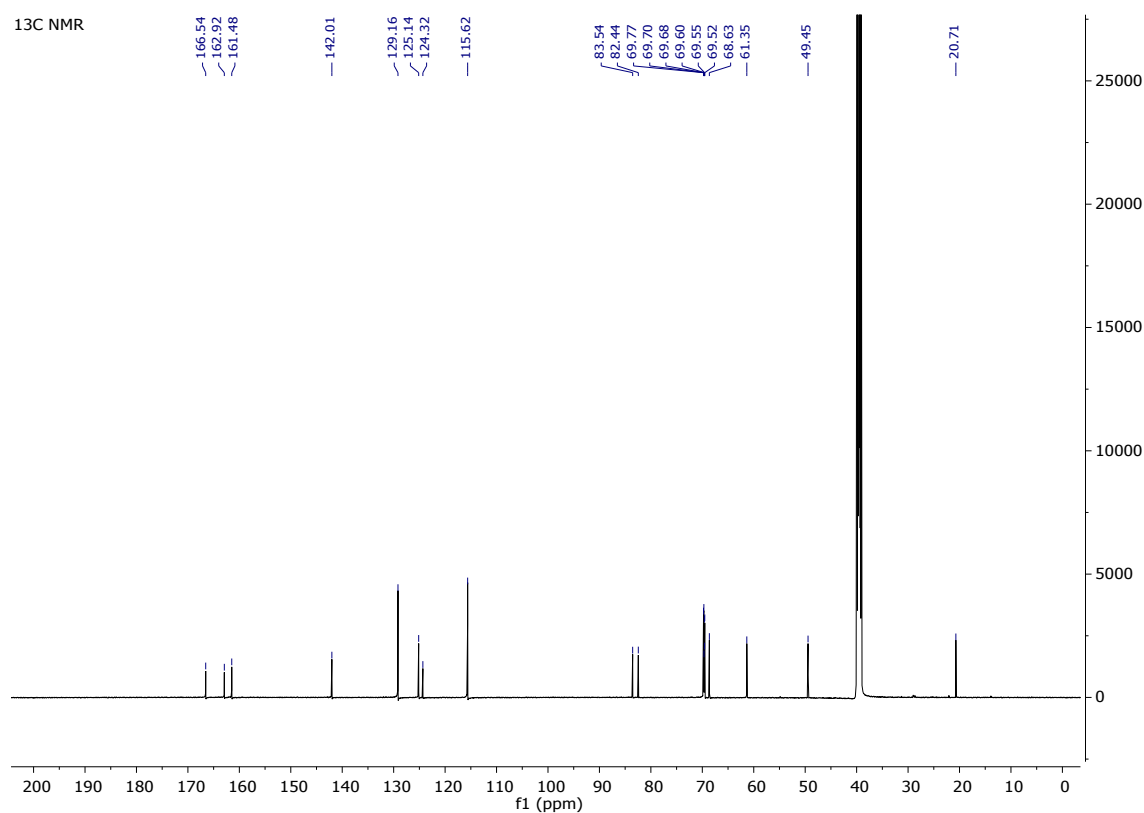


3-(4-((1-(2-(2-(2-(2-Fluoroethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-6-methyl-1,2,4,5-tetrazine (6)

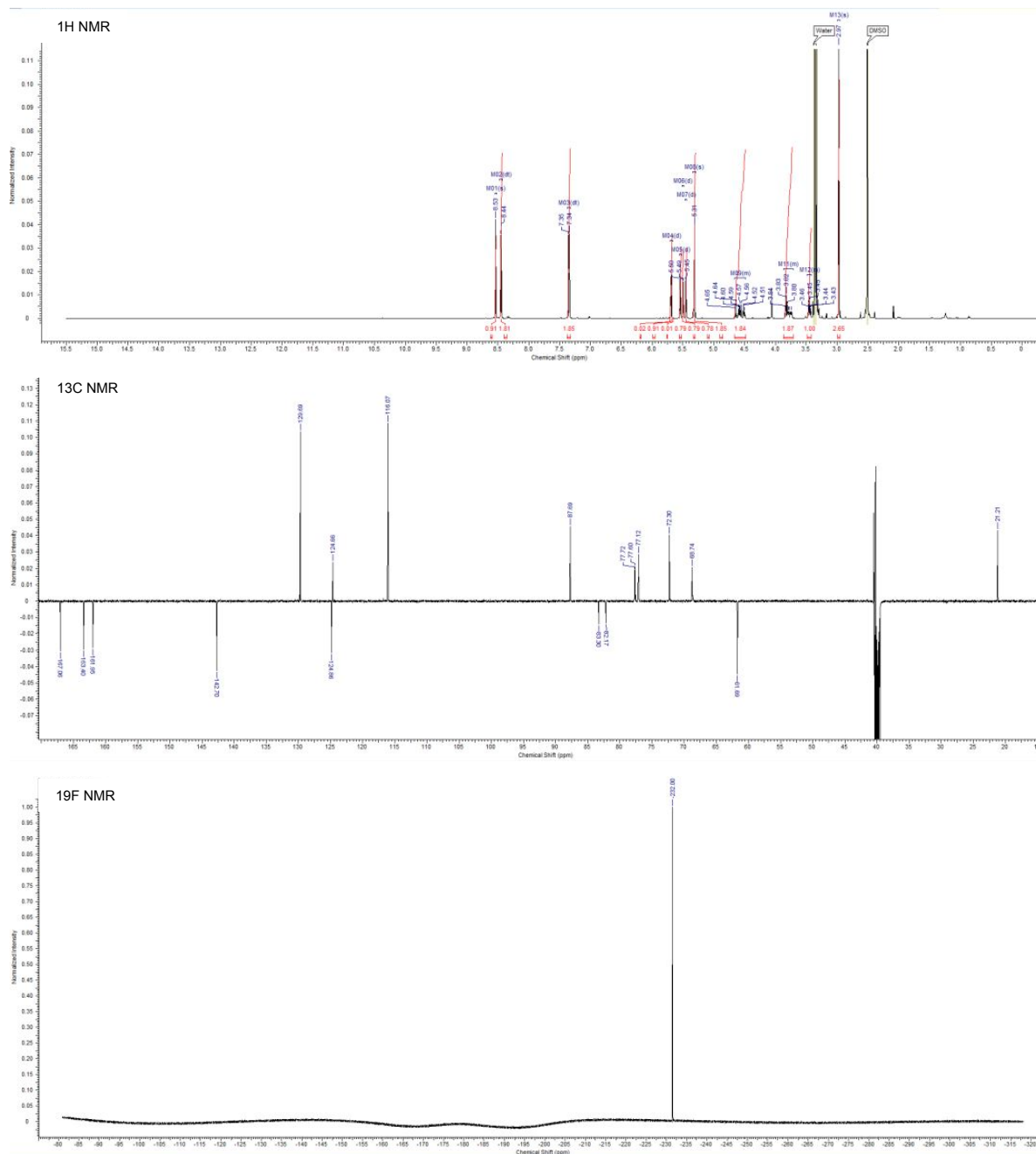
¹H NMR



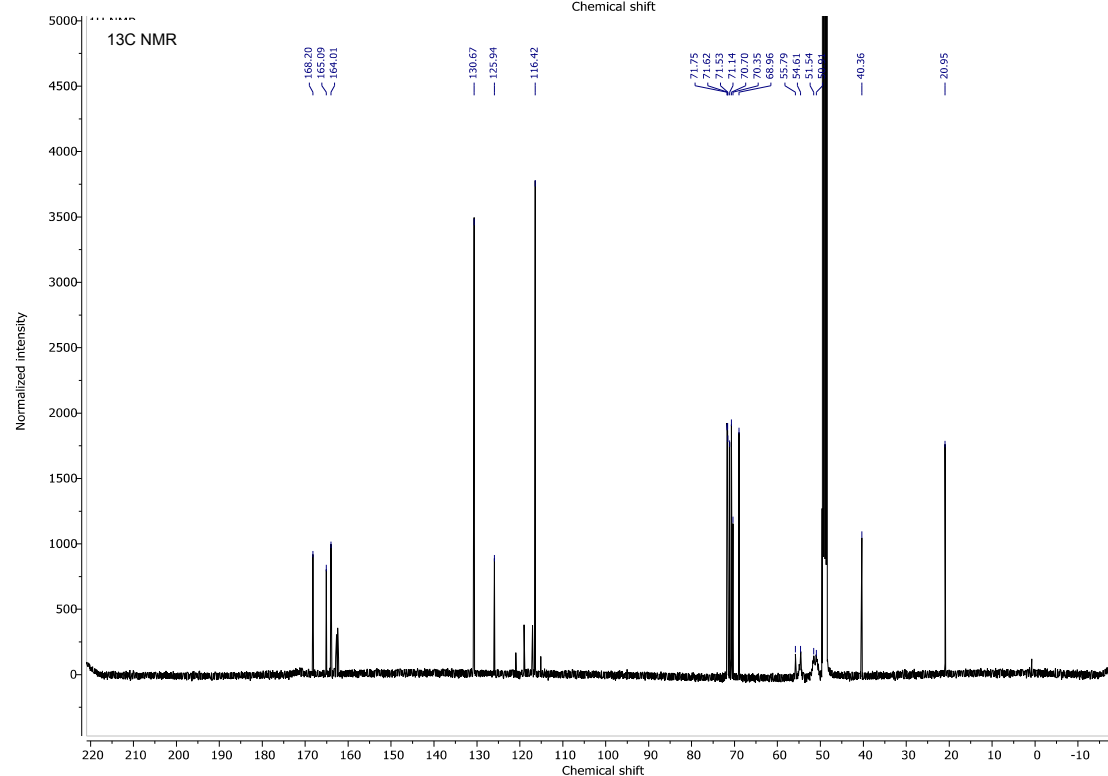
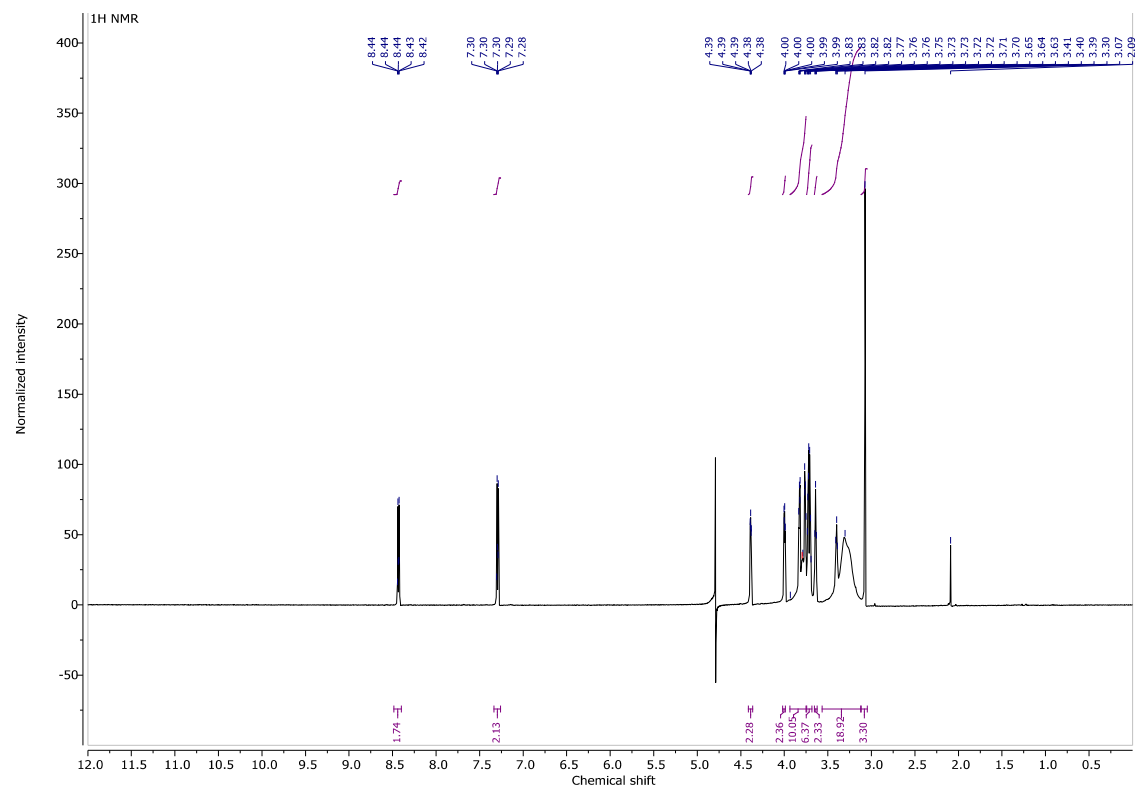
¹³C NMR



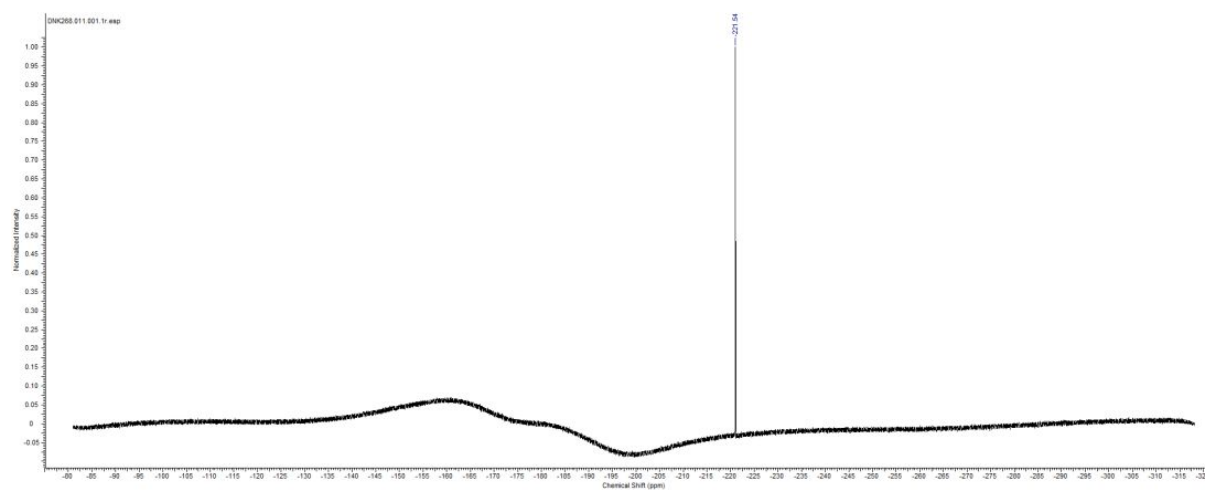
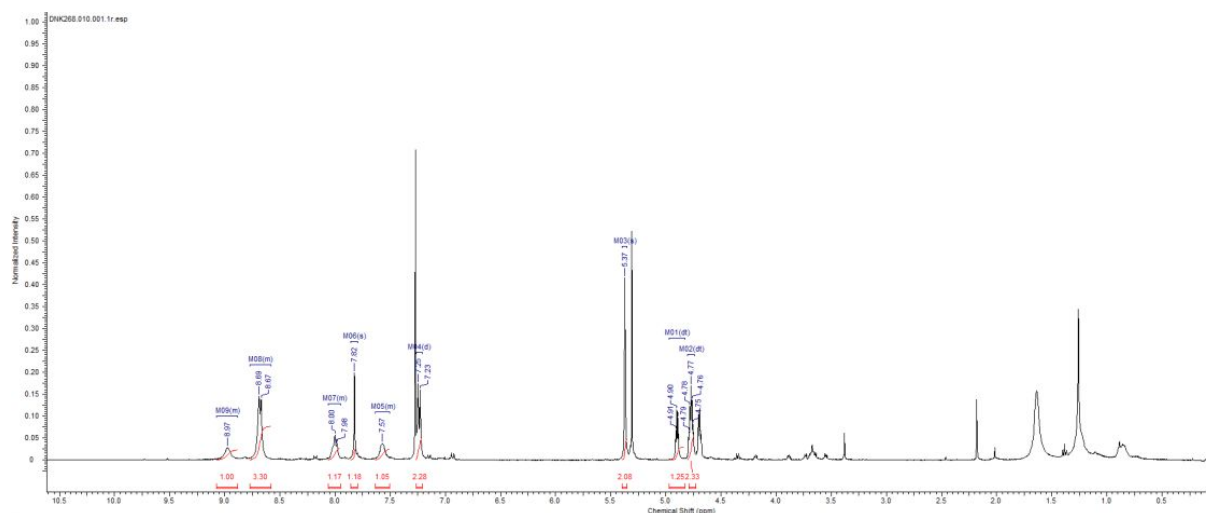
(2S,3S,4S,5R,6R)-2-(Fluoromethyl)-6-(4-((4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triol (7)



2,2',2''-(10-(14-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenoxy)-2-oxo-6,9,12-trioxa-3-azatetradecyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (10)

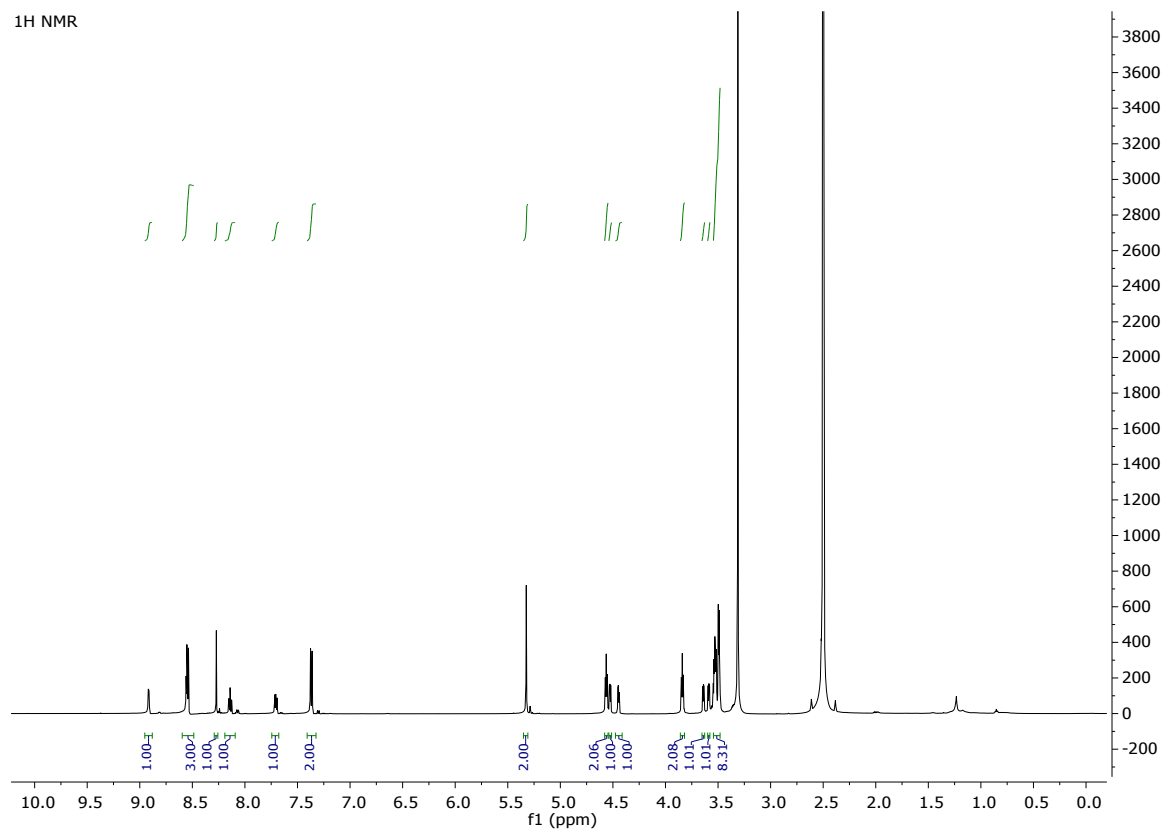


3-(4-((1-(2-Fluoroethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-6-(pyridin-2-yl)-1,2,4,5-tetrazine (12)

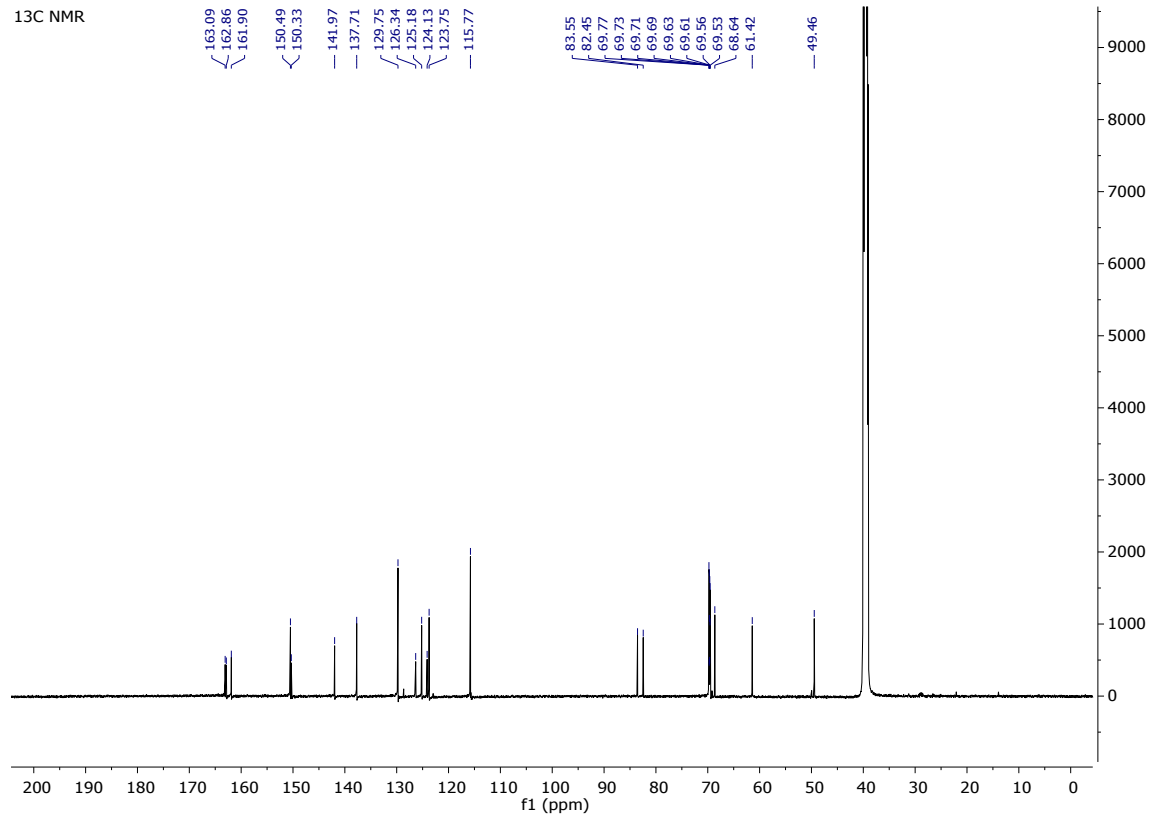


3-(4-((1-(2-(2-(2-(2-Fluoroethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-6-(pyridin-2-yl)-1,2,4,5-tetrazine (13)

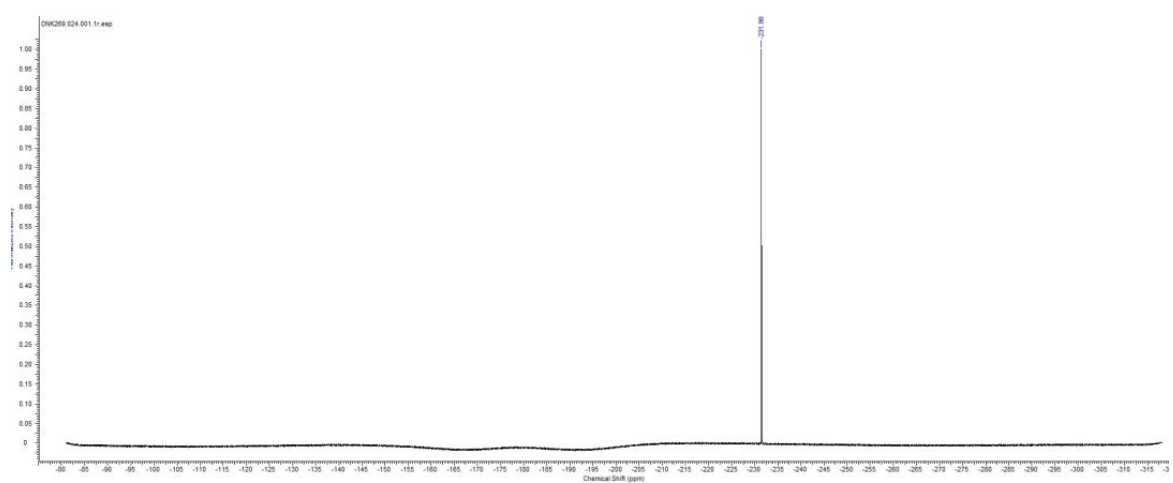
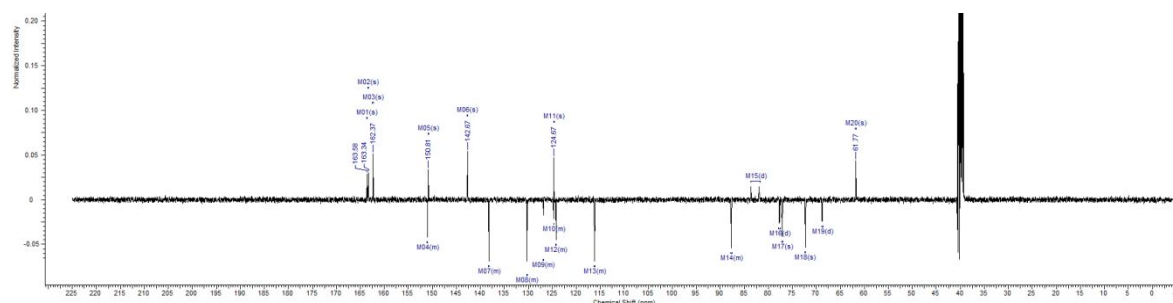
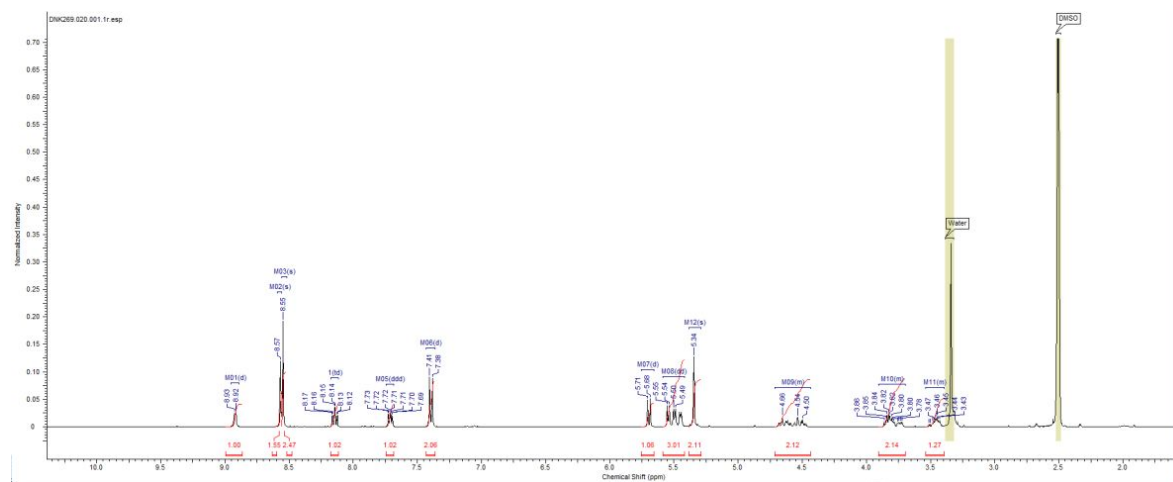
¹H NMR



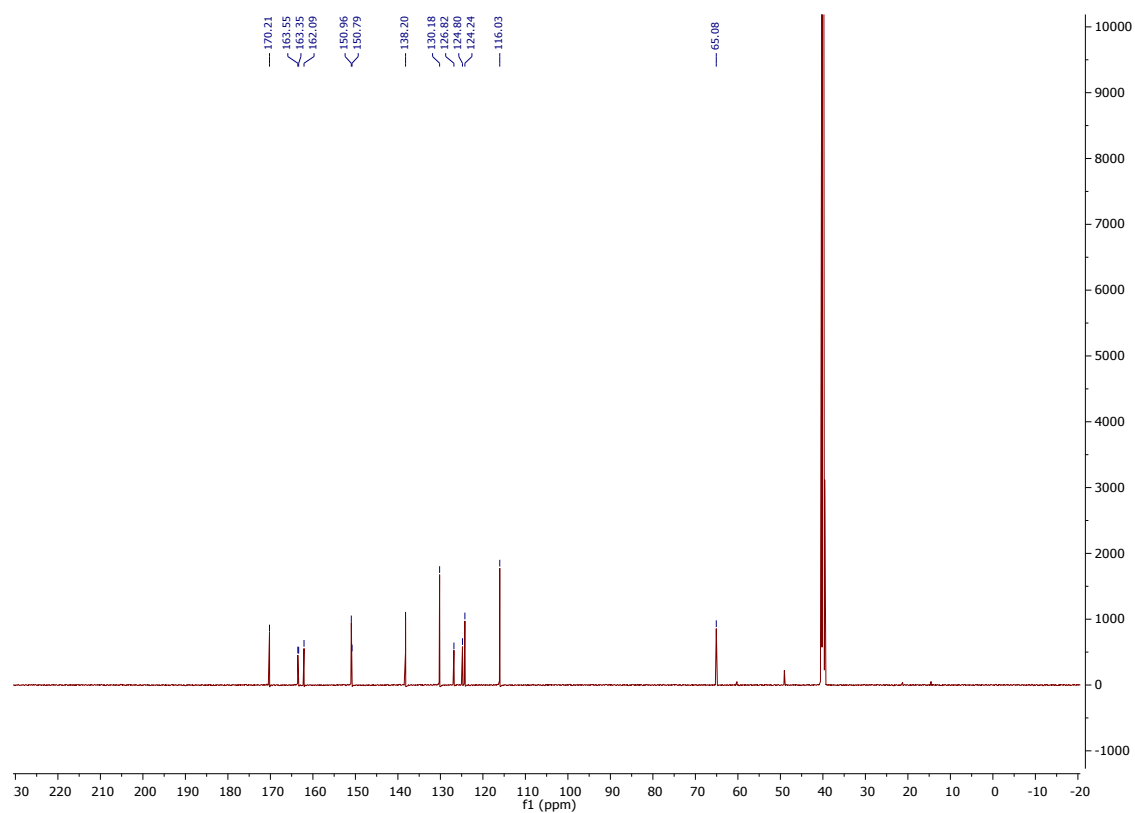
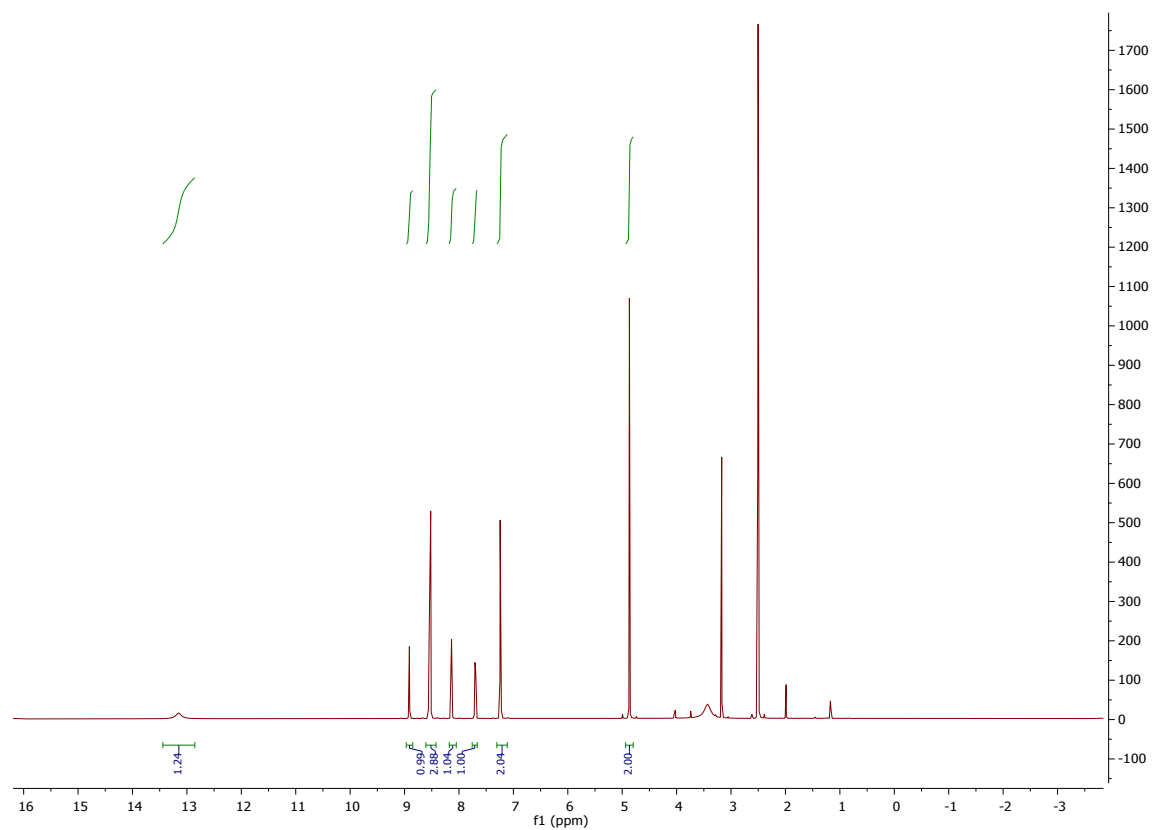
¹³C NMR



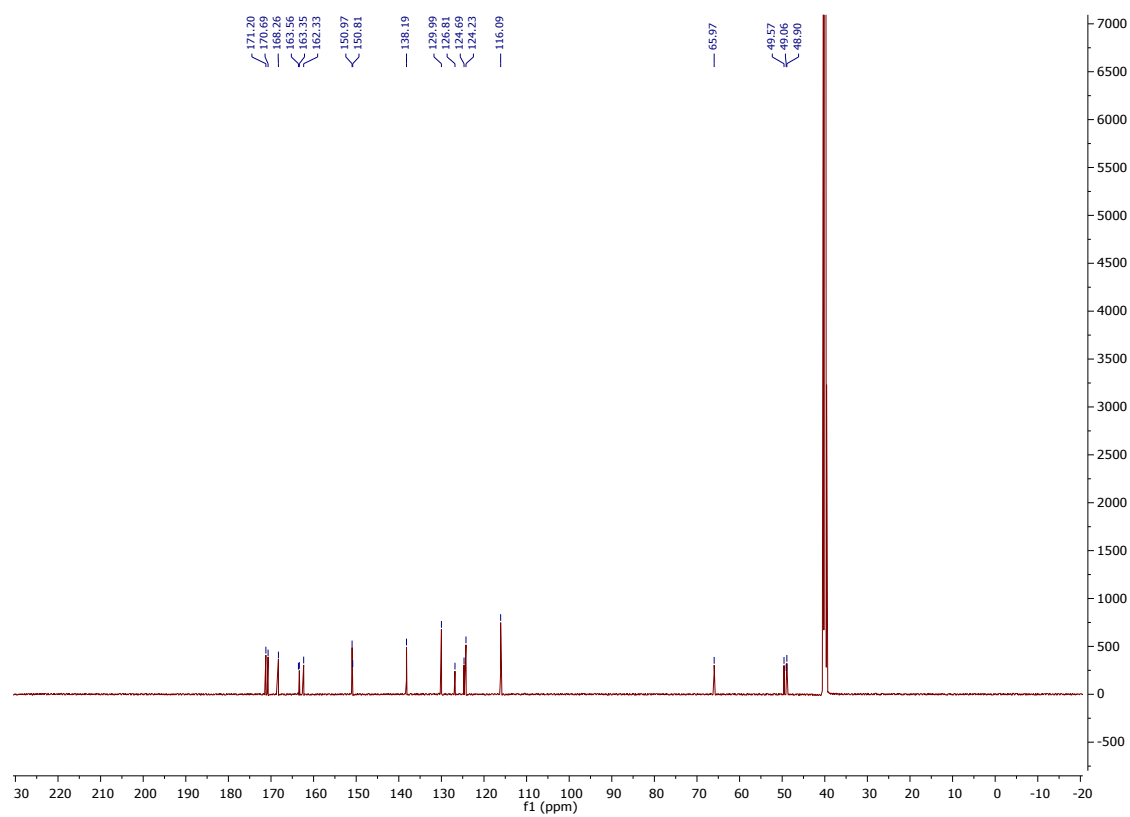
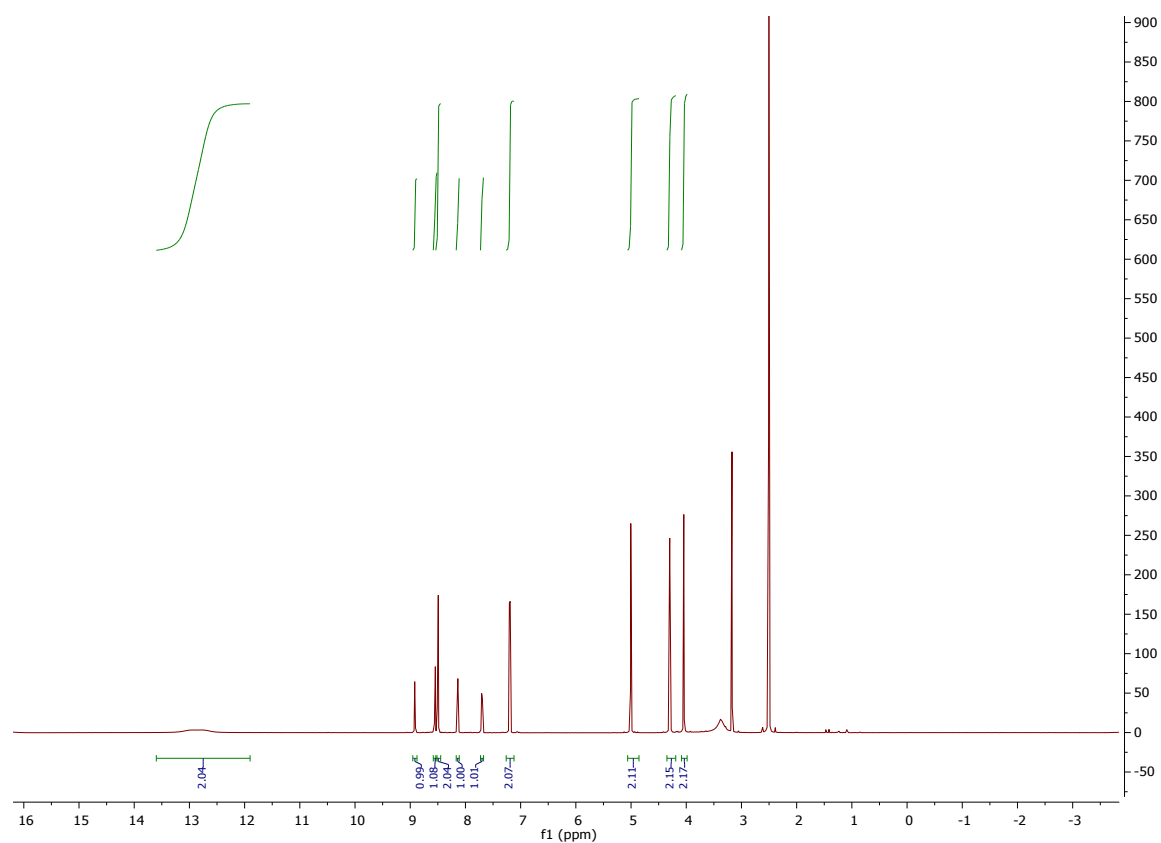
(2*S*,3*S*,4*S*,5*R*,6*R*)-2-(Fluoromethyl)-6-(4-((4-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)tetrahydro-2*H*-pyran-3,4,5-triol (**14**)



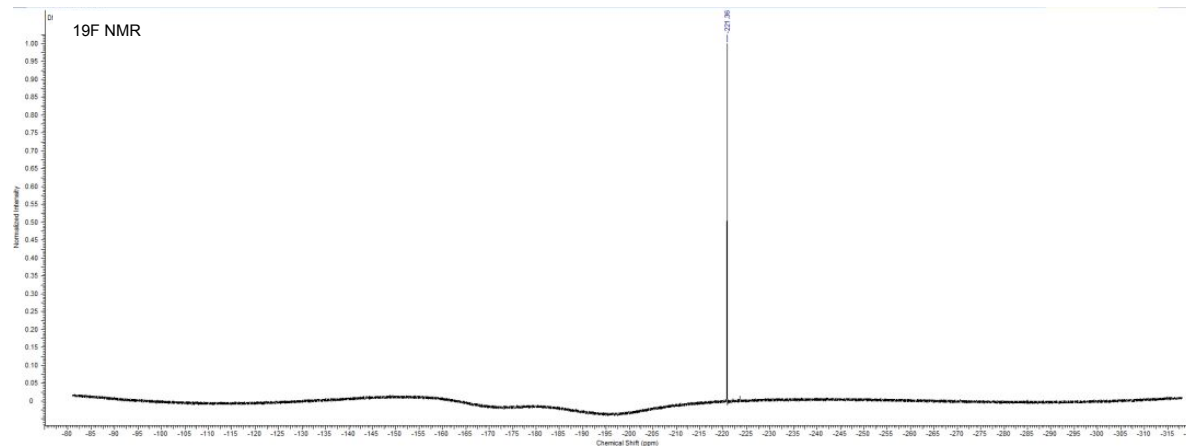
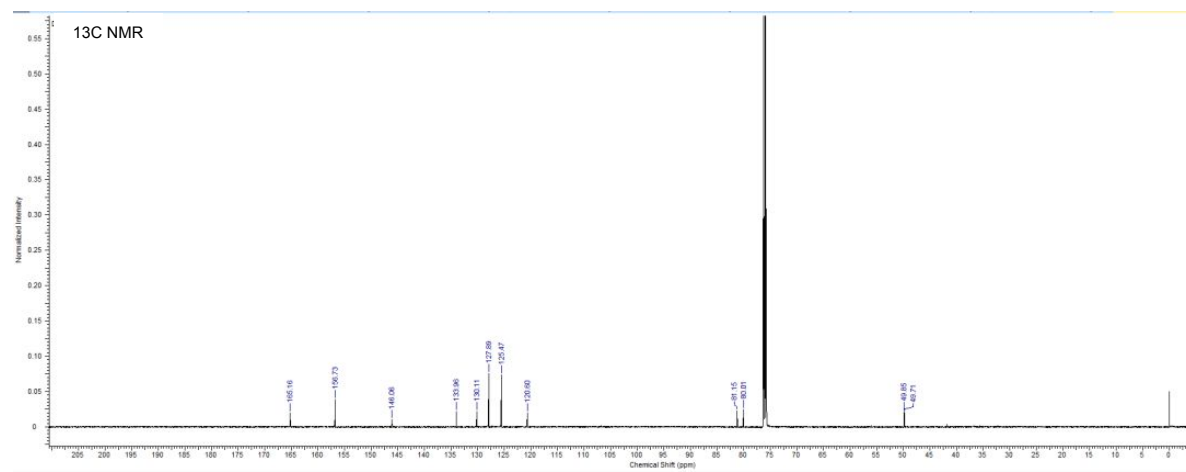
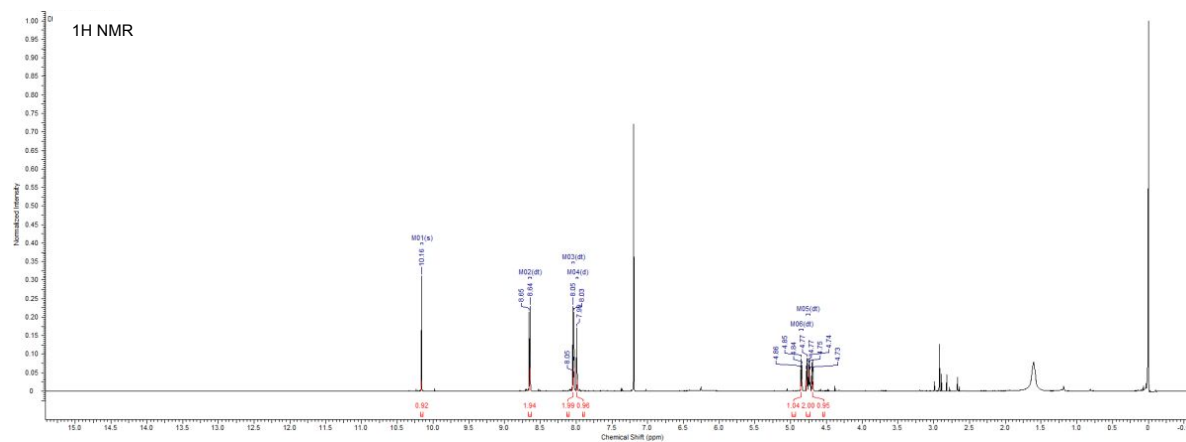
2-(4-(6-(Pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)phenoxy)acetic acid (15)



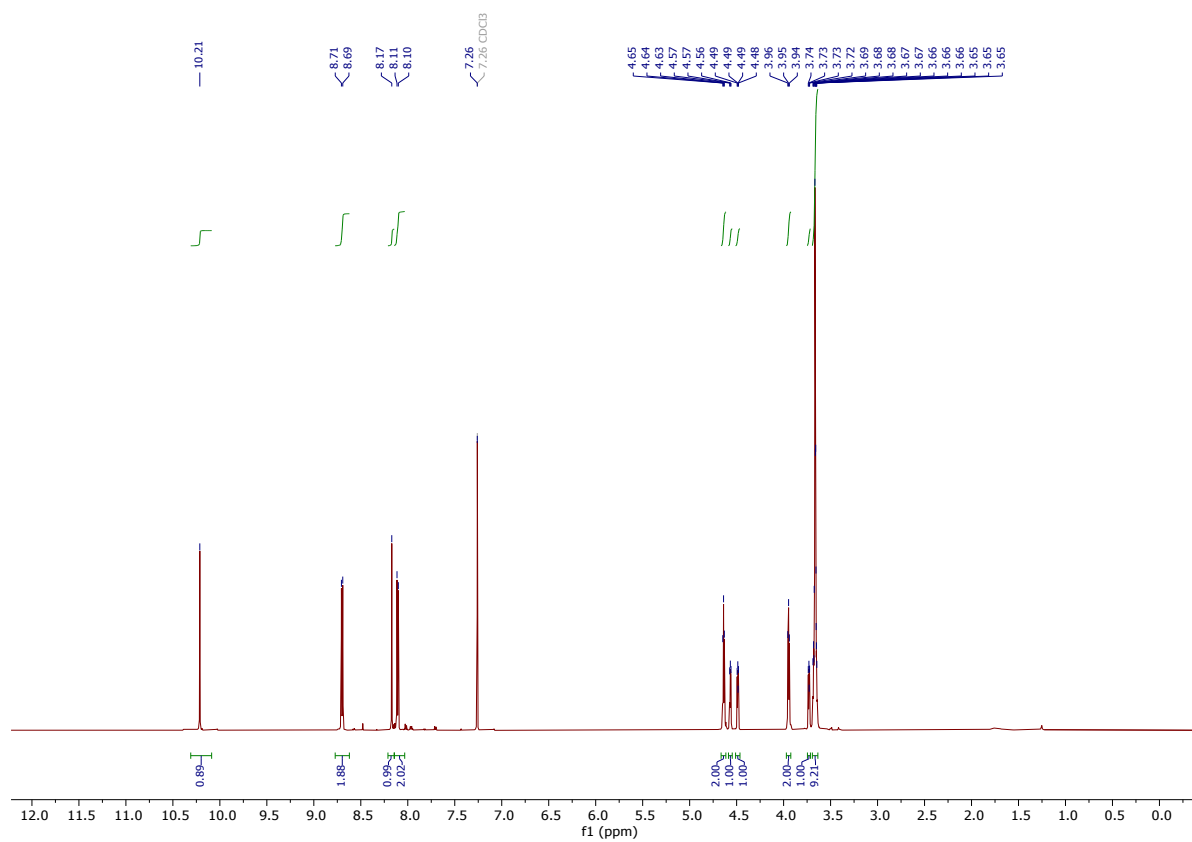
2,2'-((2-(4-(6-(Pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)phenoxy)acetyl)azanediyl)diacetic acid (**16**)

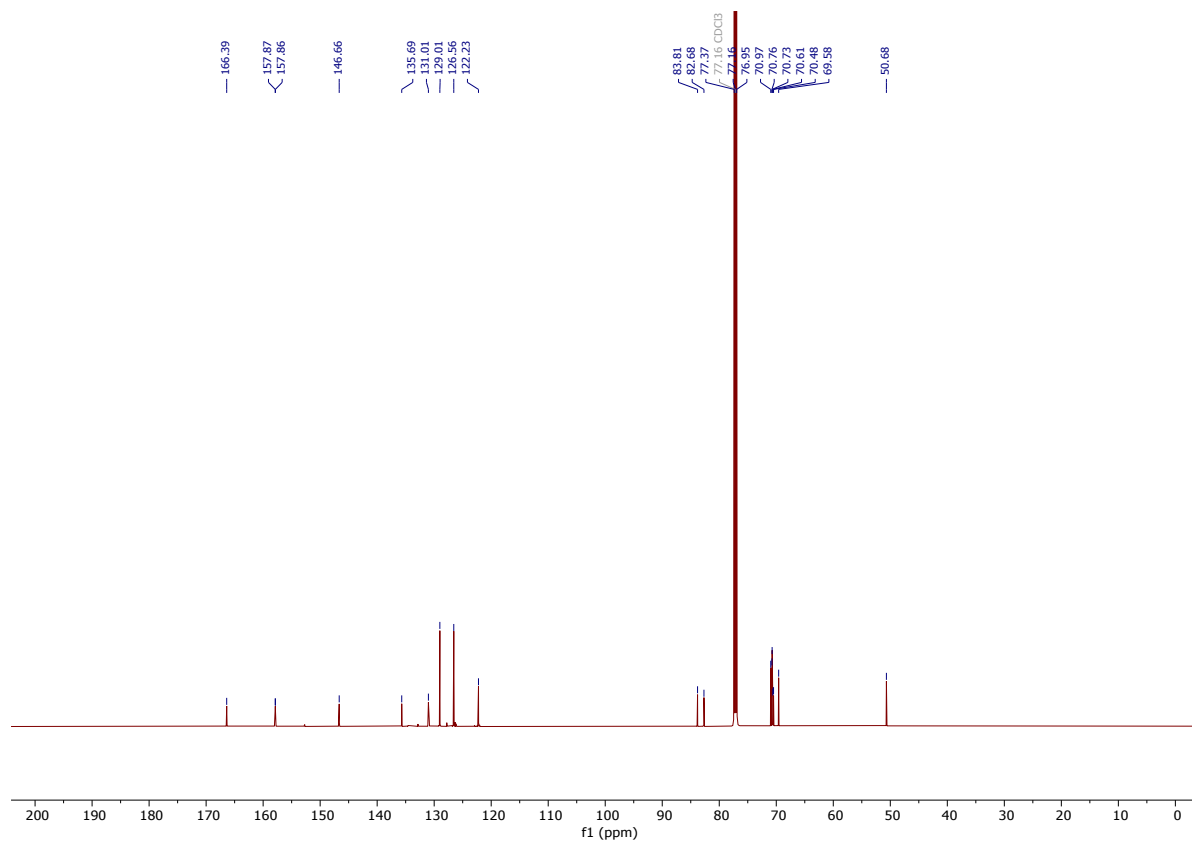


3-(4-(1-(2-Fluoroethyl)-1H-1,2,3-triazol-4-yl)phenyl)-1,2,4,5-tetrazine (17)

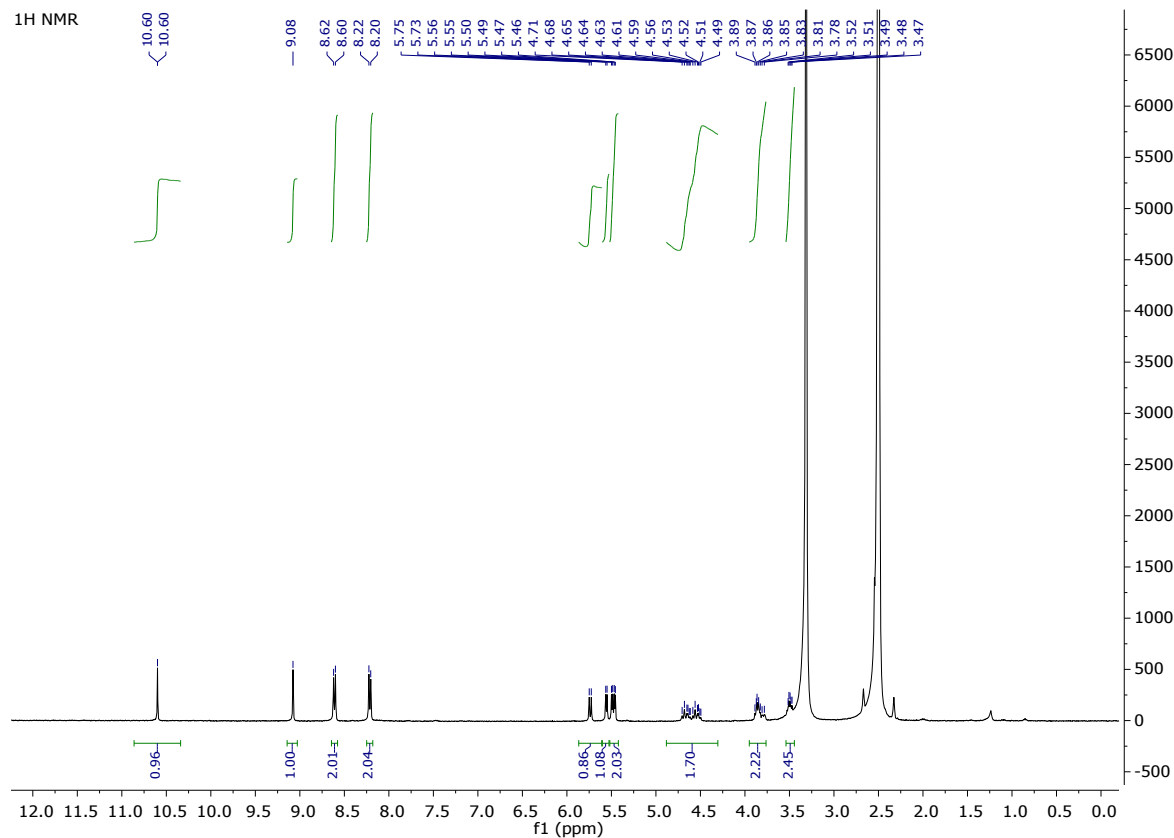


3-(4-(1-(2-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)phenyl)-1,2,4,5-tetrazine
(18)

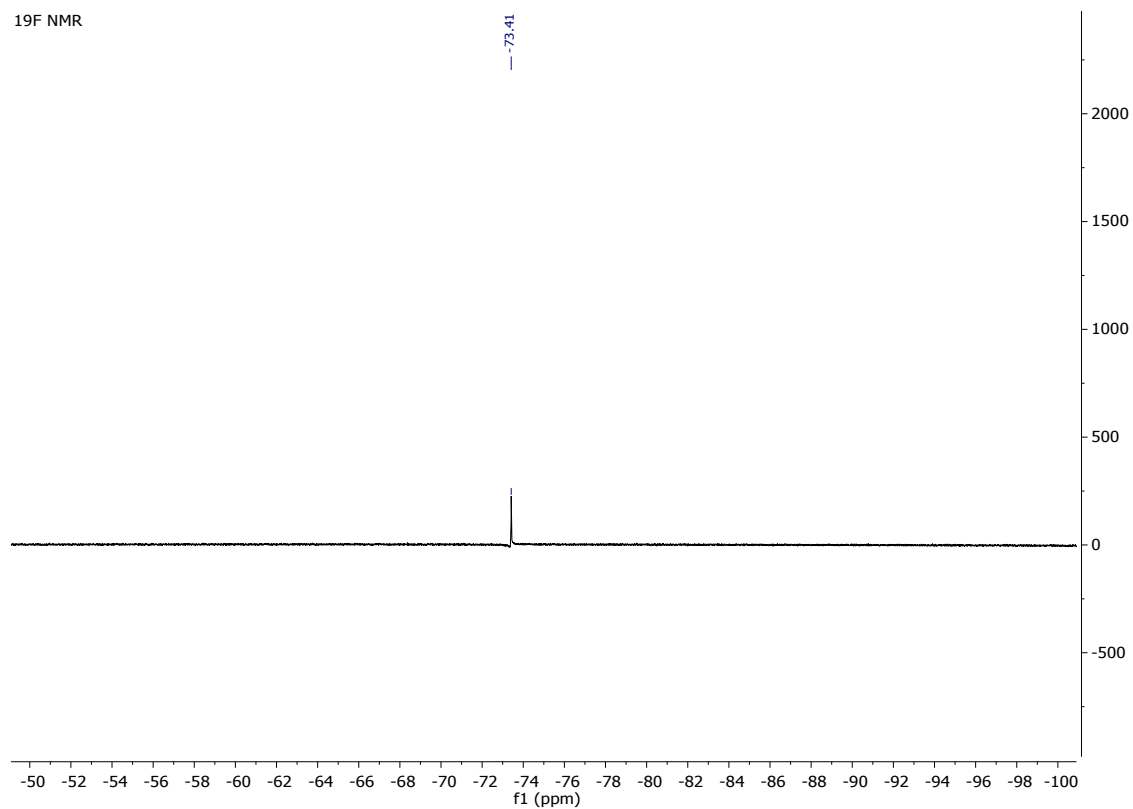




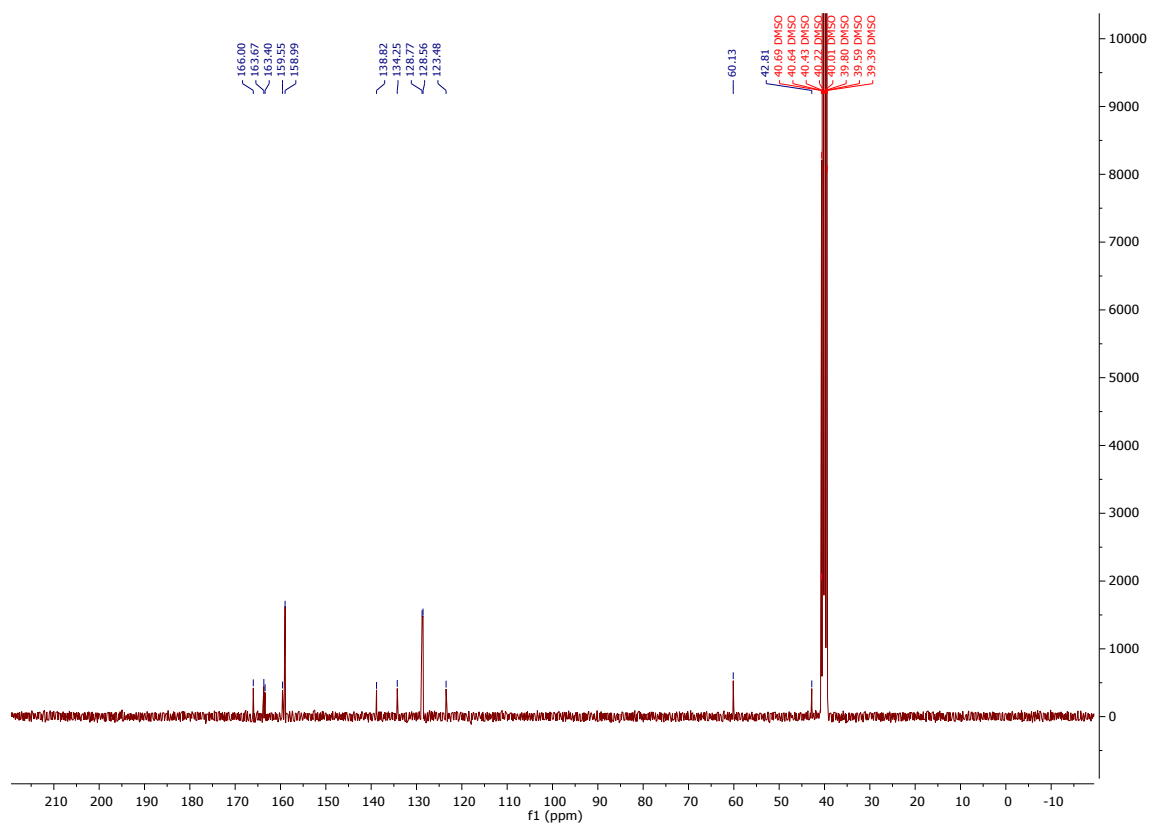
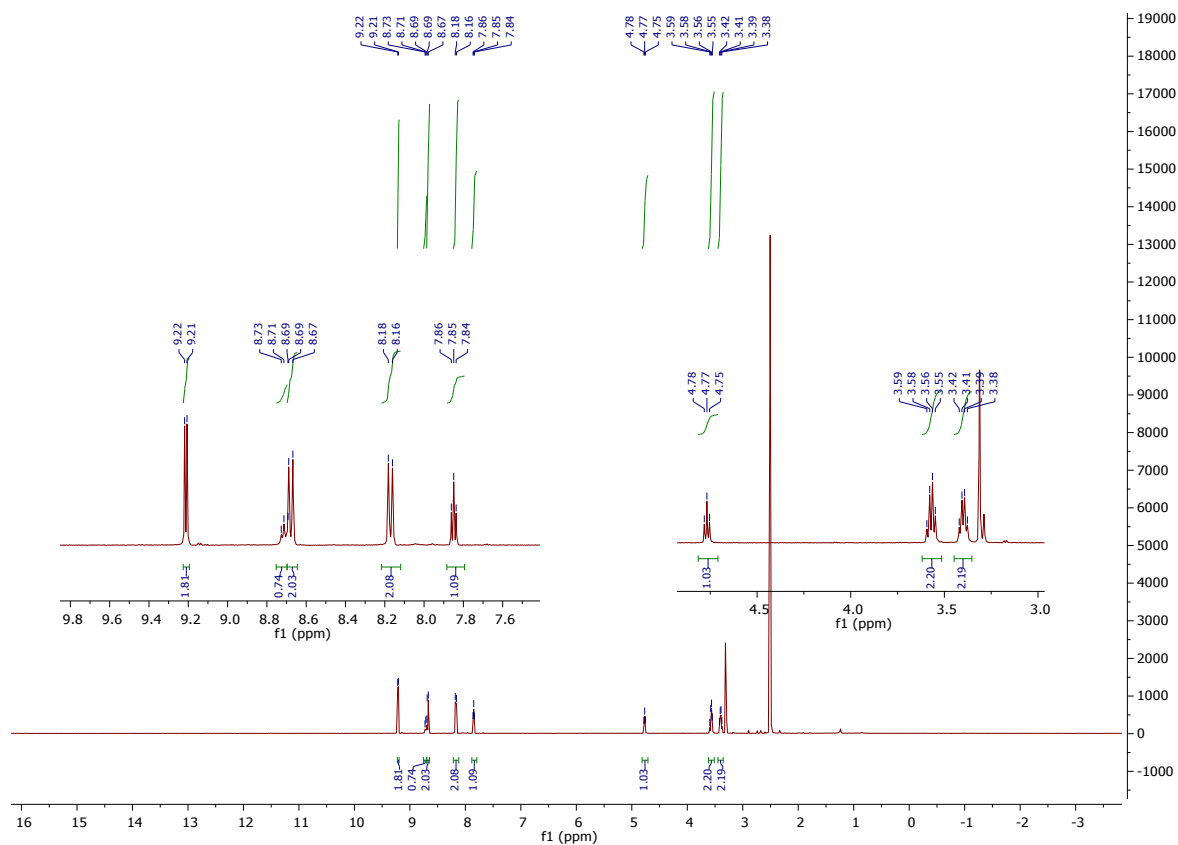
(2R,3R,4S,5S,6S)-2-(4-(4-(1,2,4,5-Tetrazin-3-yl)phenyl)-1H-1,2,3-triazol-1-yl)-6-(fluoromethyl)tetrahydro-2H-pyran-3,4,5-triol (**19**)



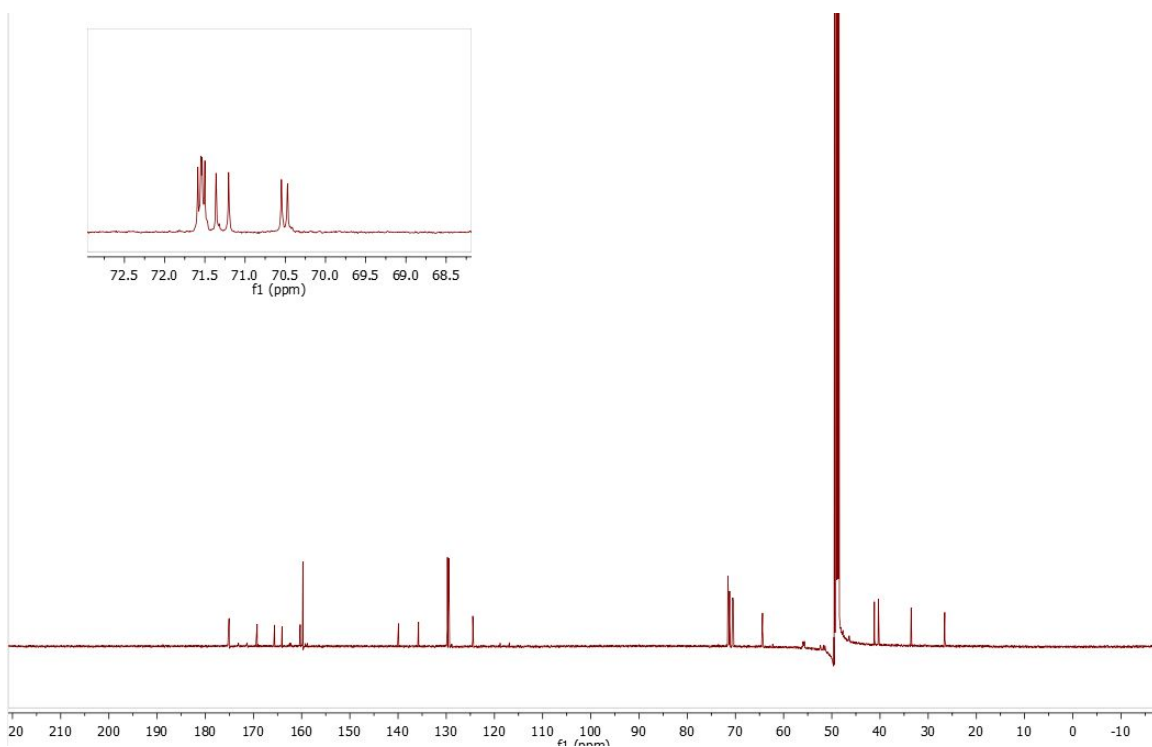
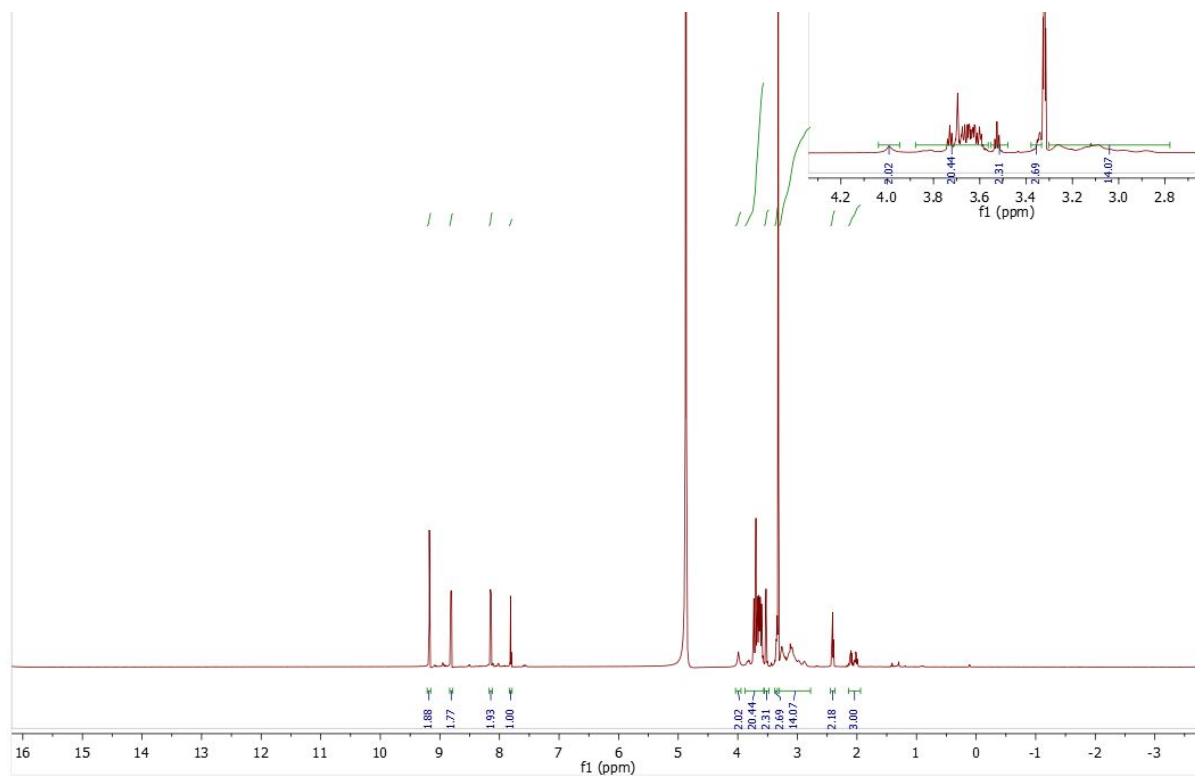
19F NMR



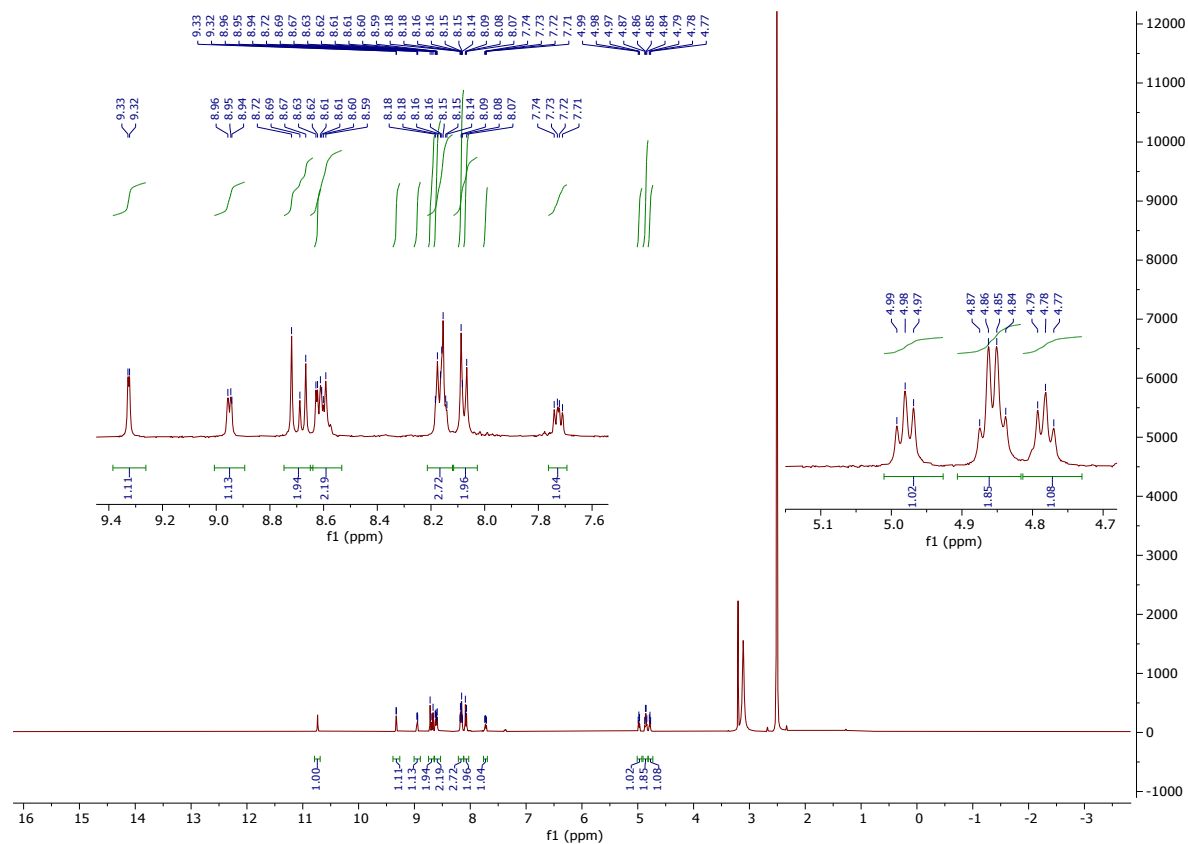
N-(2-Hydroxyethyl)-4-(6-(pyrimidin-2-yl)-1,2,4,5-tetrazin-3-yl)benzamide (**23**)



2,2'-(7-(21-Carboxy-1,18-dioxo-1-(4-(6-(pyrimidin-2-yl)-1,2,4,5-tetrazin-3-yl) phenyl)-5,8,11,14-tetraoxa-2,17-diazahenicosan-21-yl)-1,4,7-triazonane-1,4-diyl) diacetic acid (**24**)

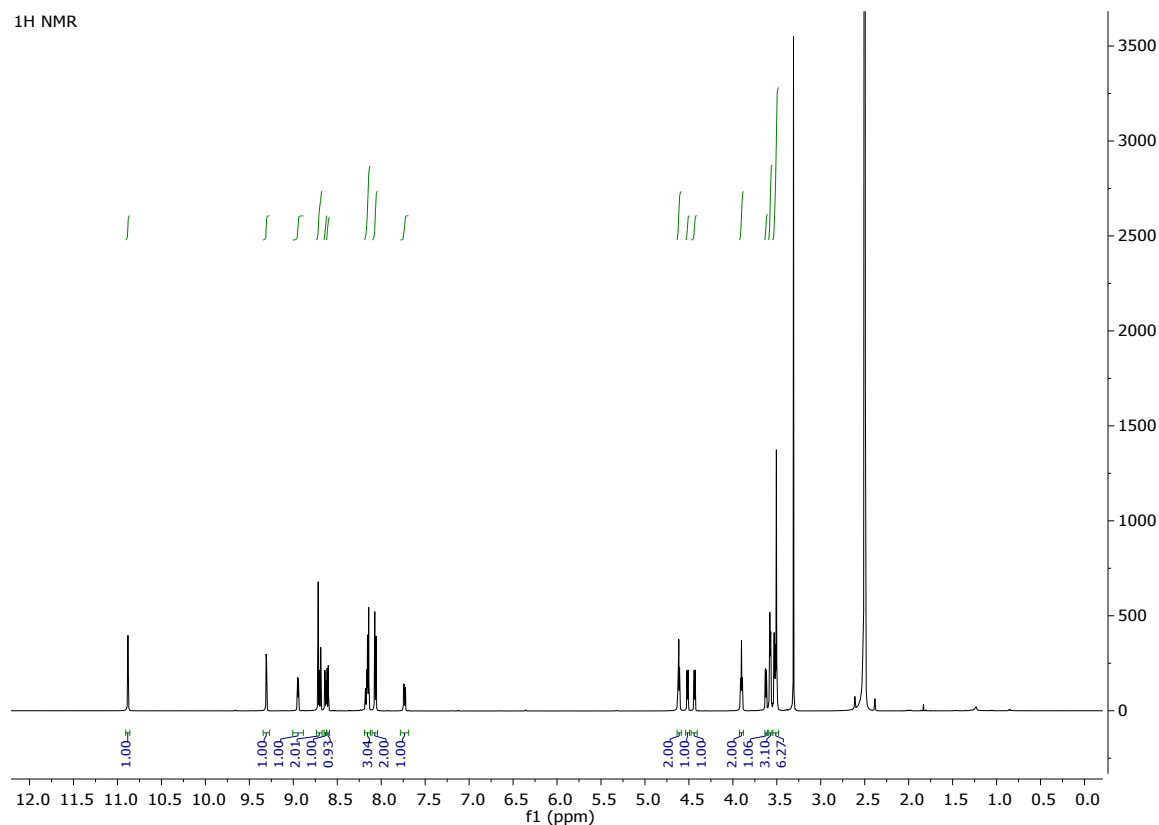


4-(1-(2-Fluoroethyl)-1H-1,2,3-triazol-4-yl)-N-(6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)benzamide (25)

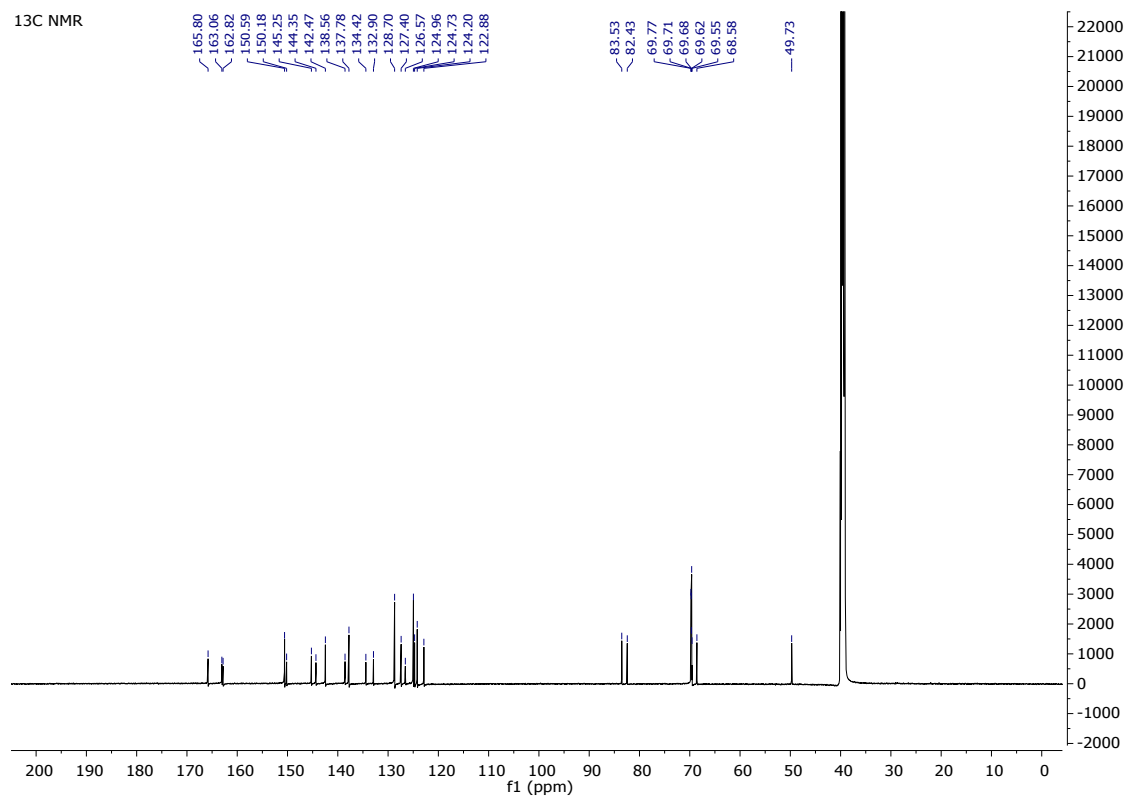


4-(1-(2-(2-(2-(2-Fluoroethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)-N-(6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)benzamide (26)

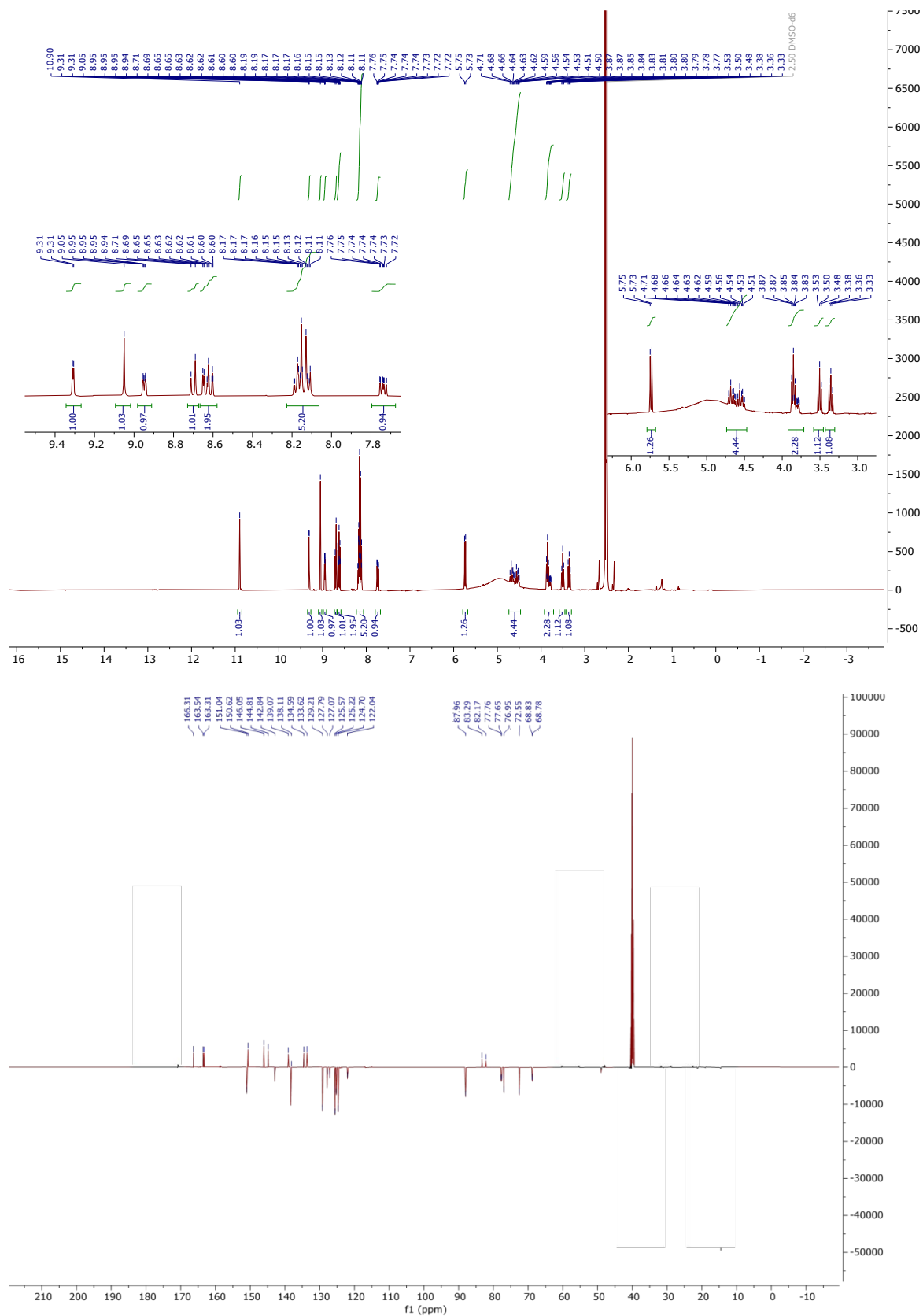
¹H NMR

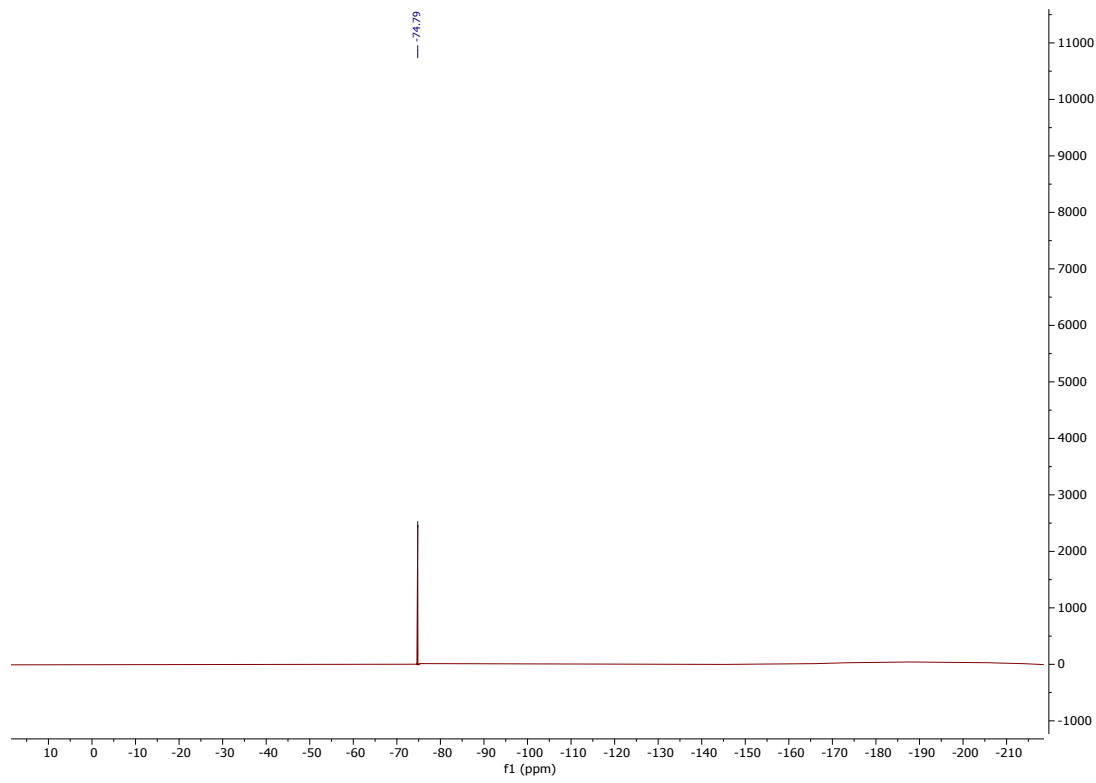


¹³C NMR

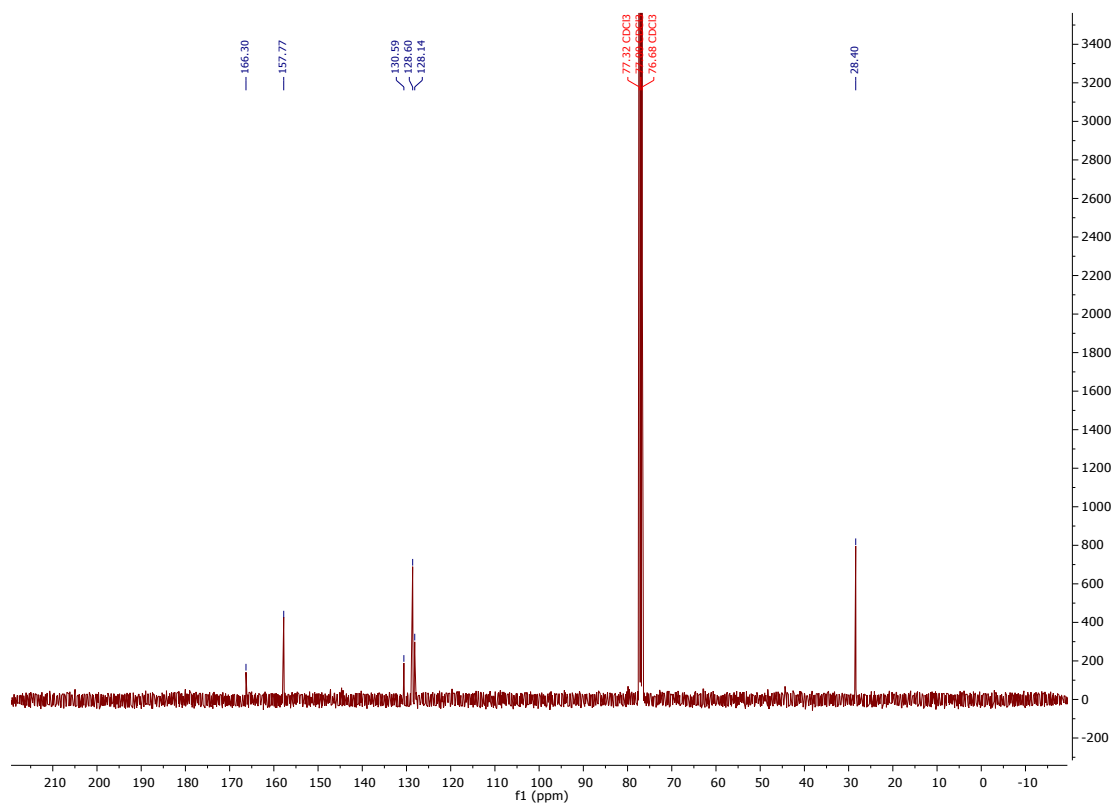
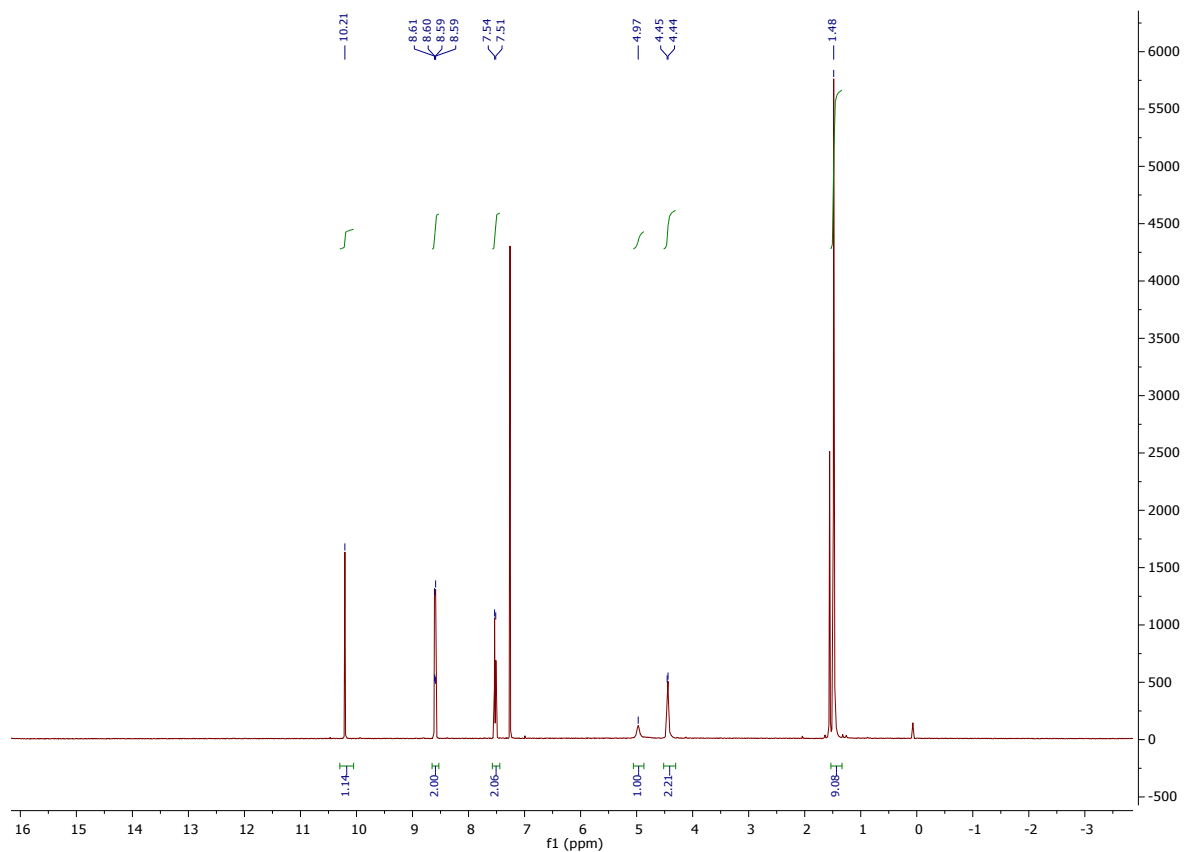


4-(1-((2R,3R,4S,5S,6S)-6-(fluoromethyl)-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)-1H-1,2,3-triazol-4-yl)-N-(6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)benzamide (27)

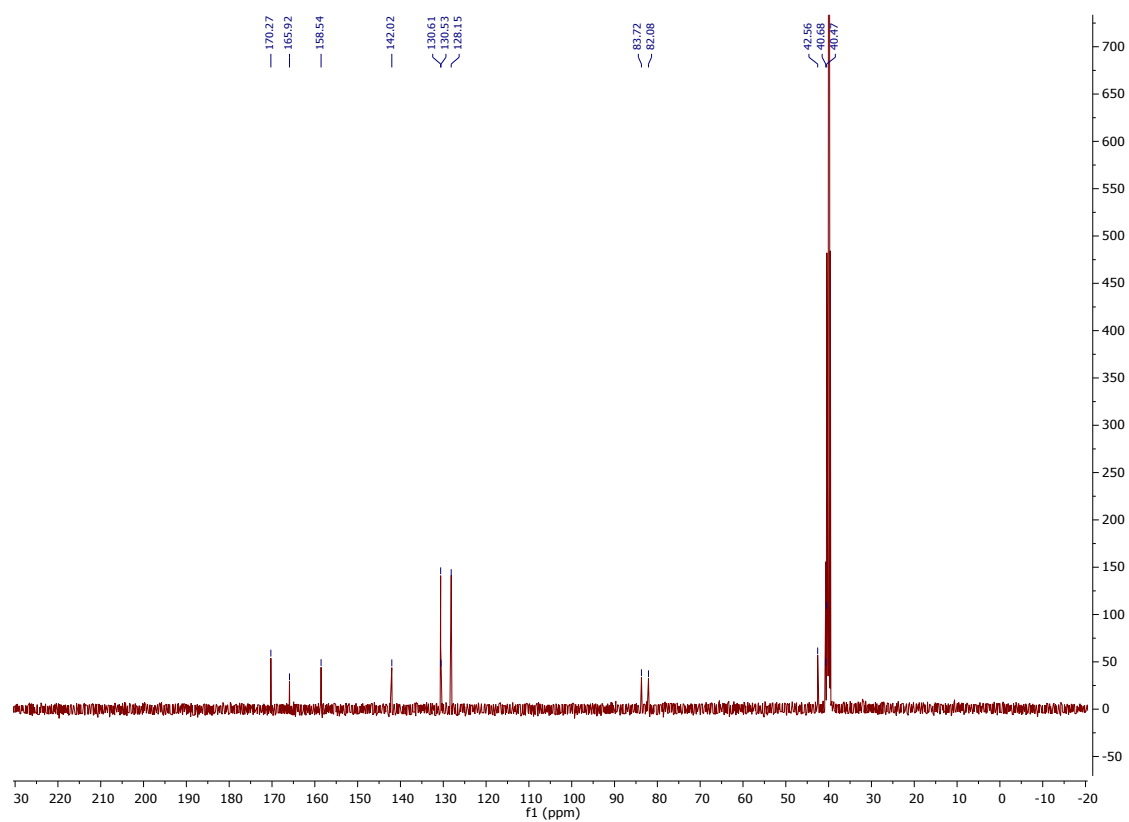
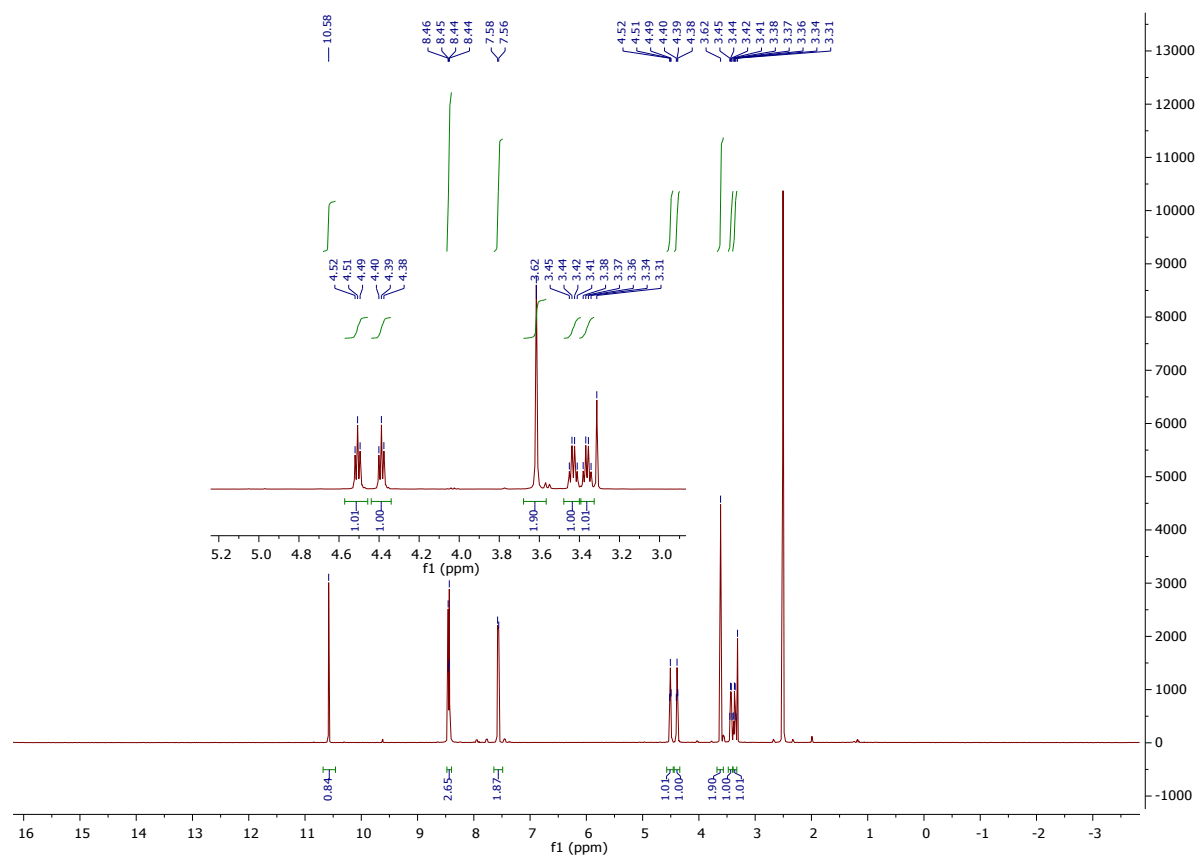




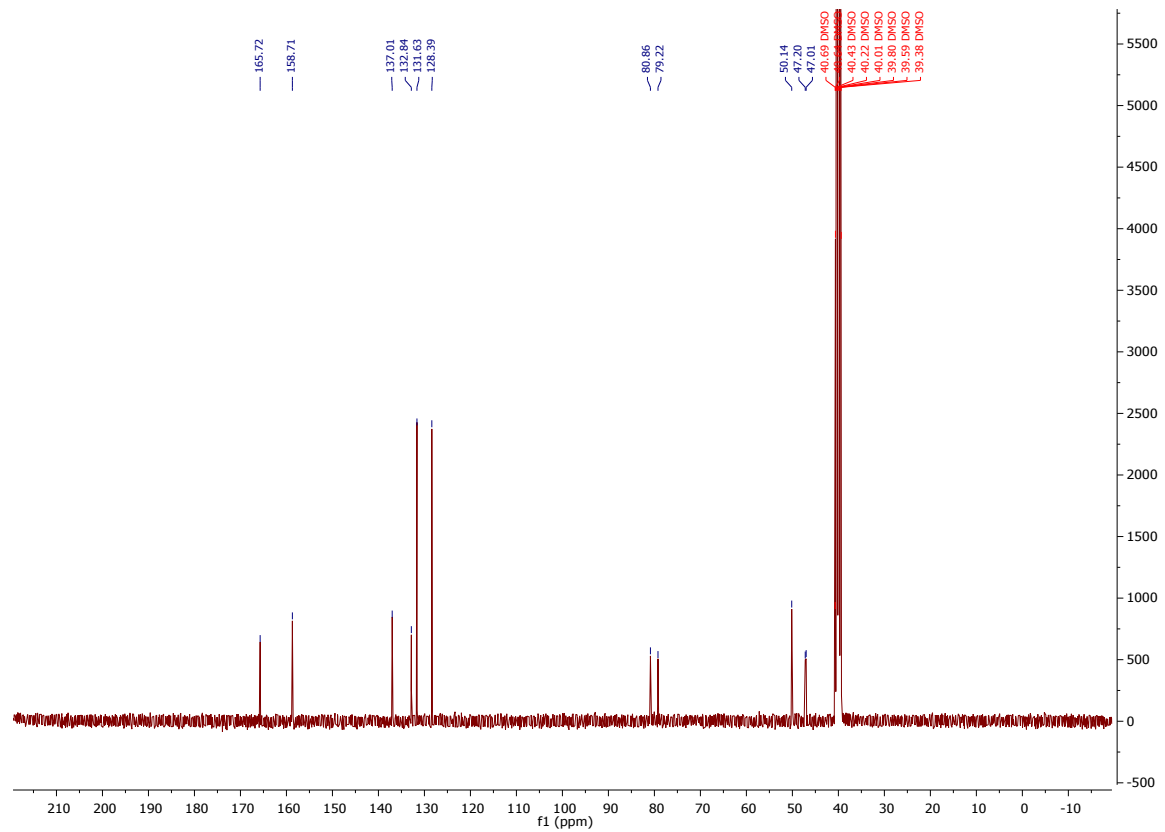
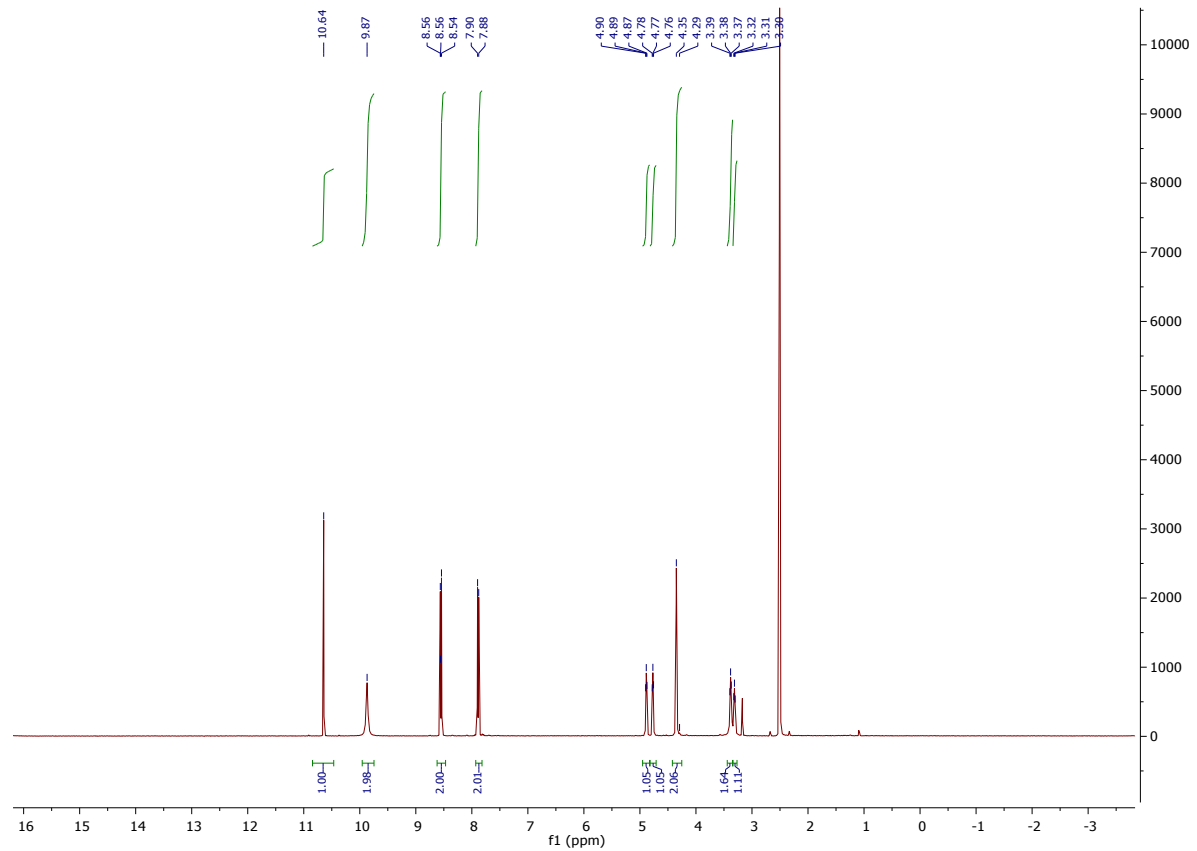
Tert-butyl 4-(1,2,4,5-tetrazin-3-yl)benzylcarbamate (28)



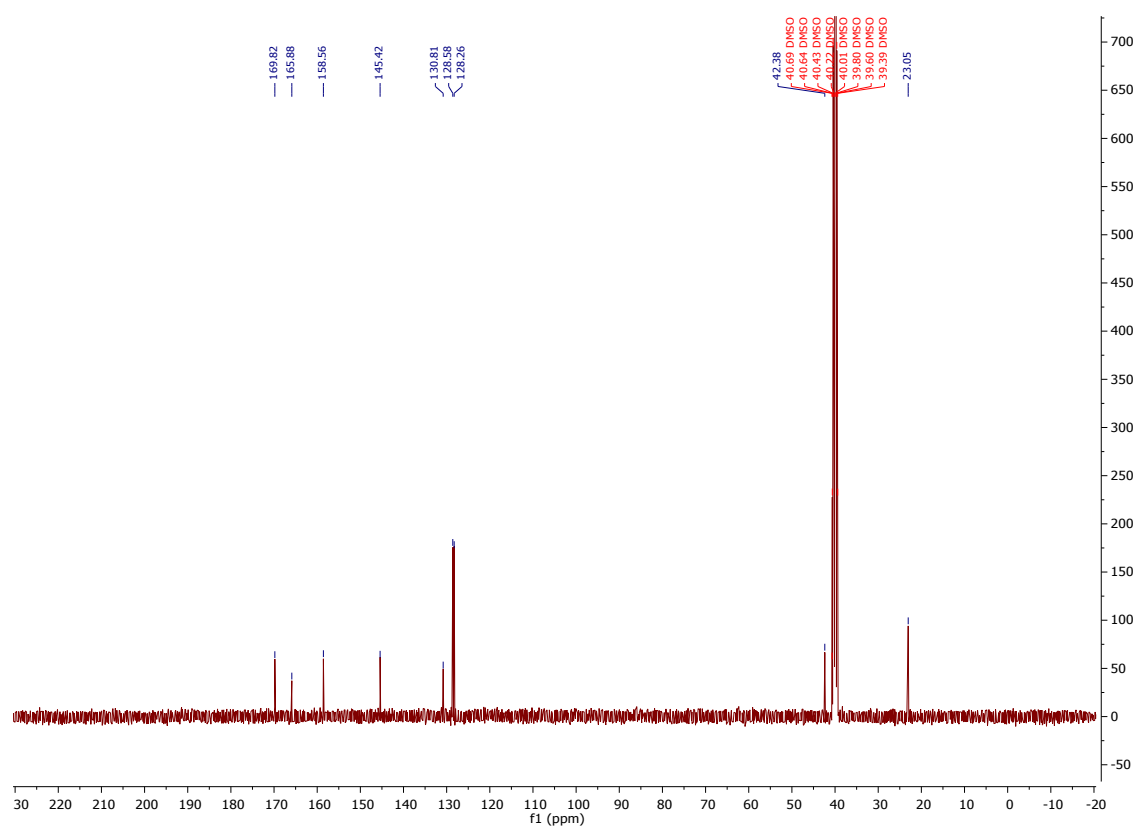
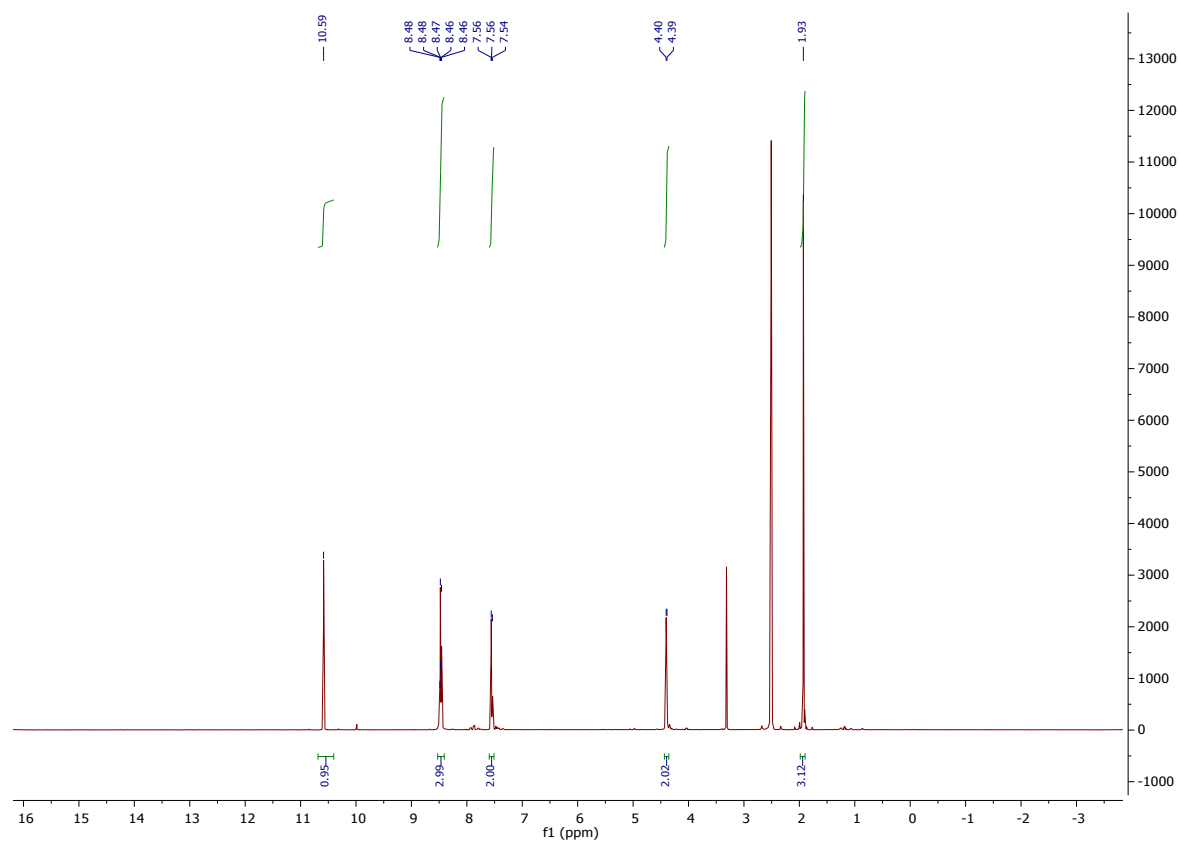
2-(4-(1,2,4,5-Tetrazin-3-yl)phenyl)-N-(2-fluoroethyl)acetamide (29)



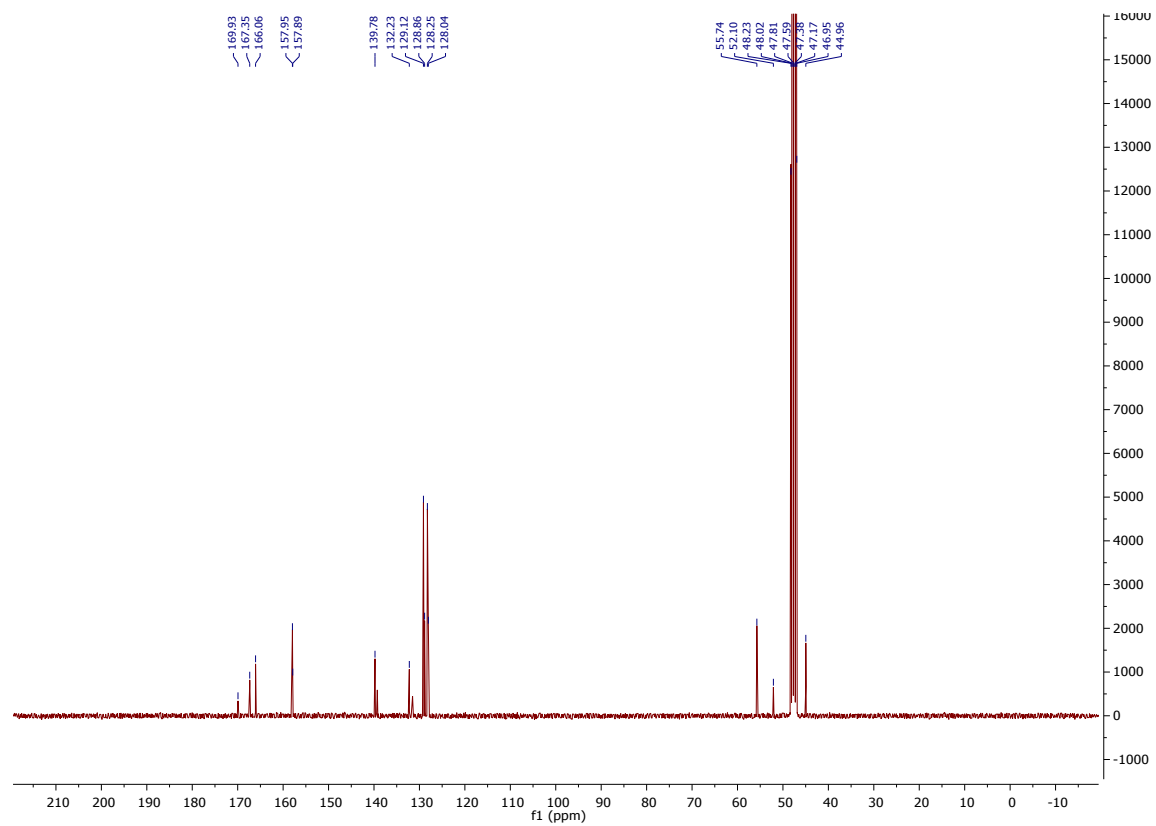
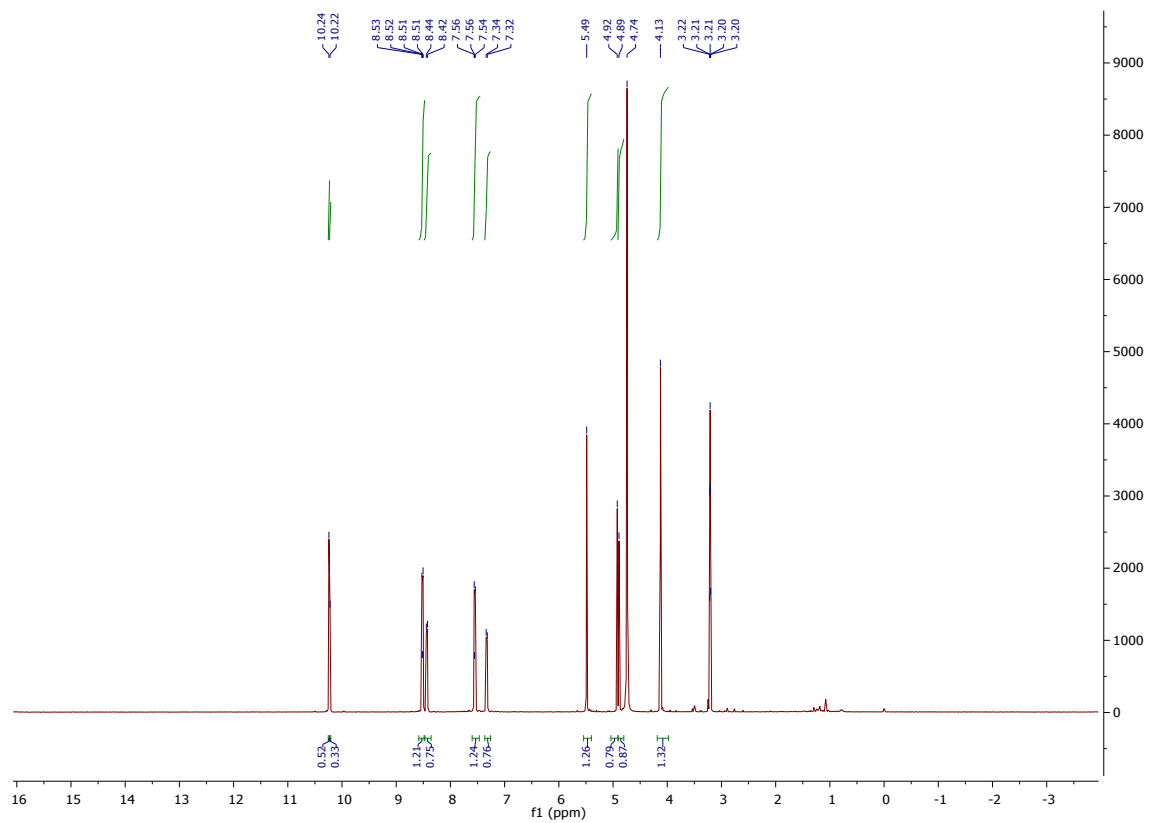
N-(4-(1,2,4,5-Tetrazin-3-yl)benzyl)-2-fluoroethanamine hydrochloride (30)



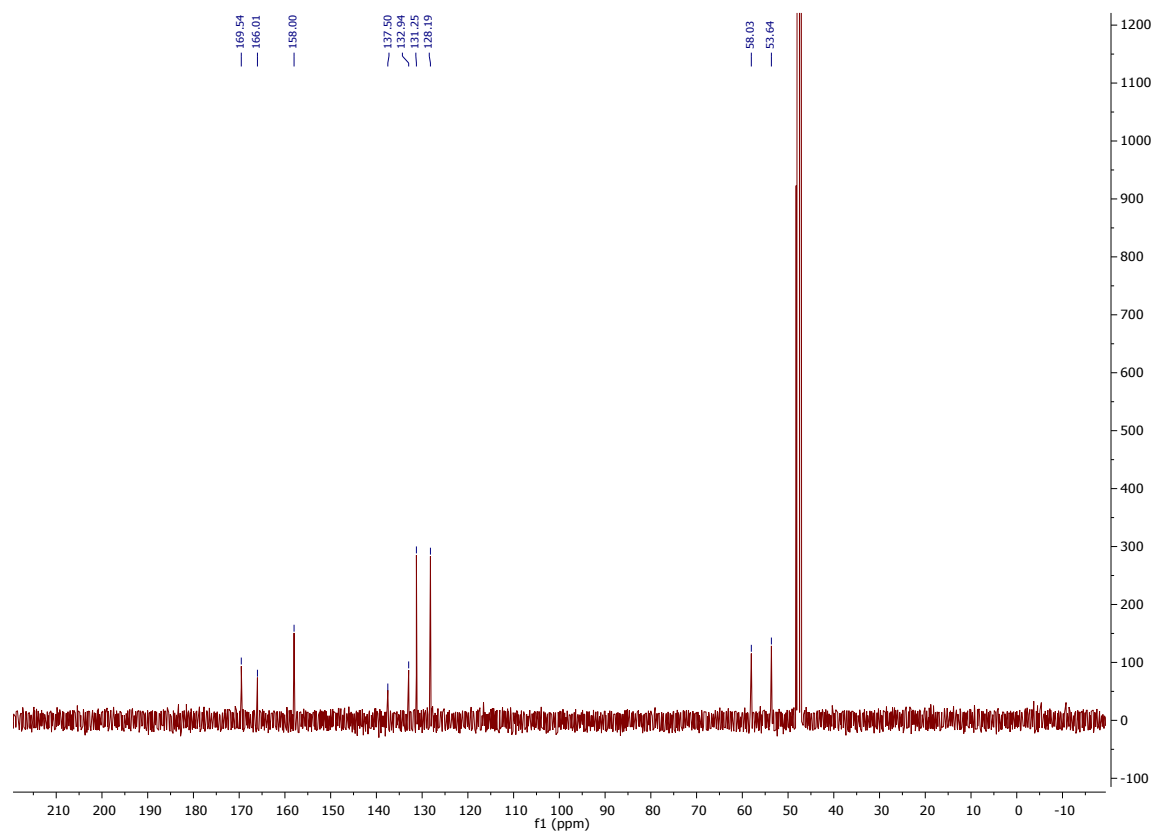
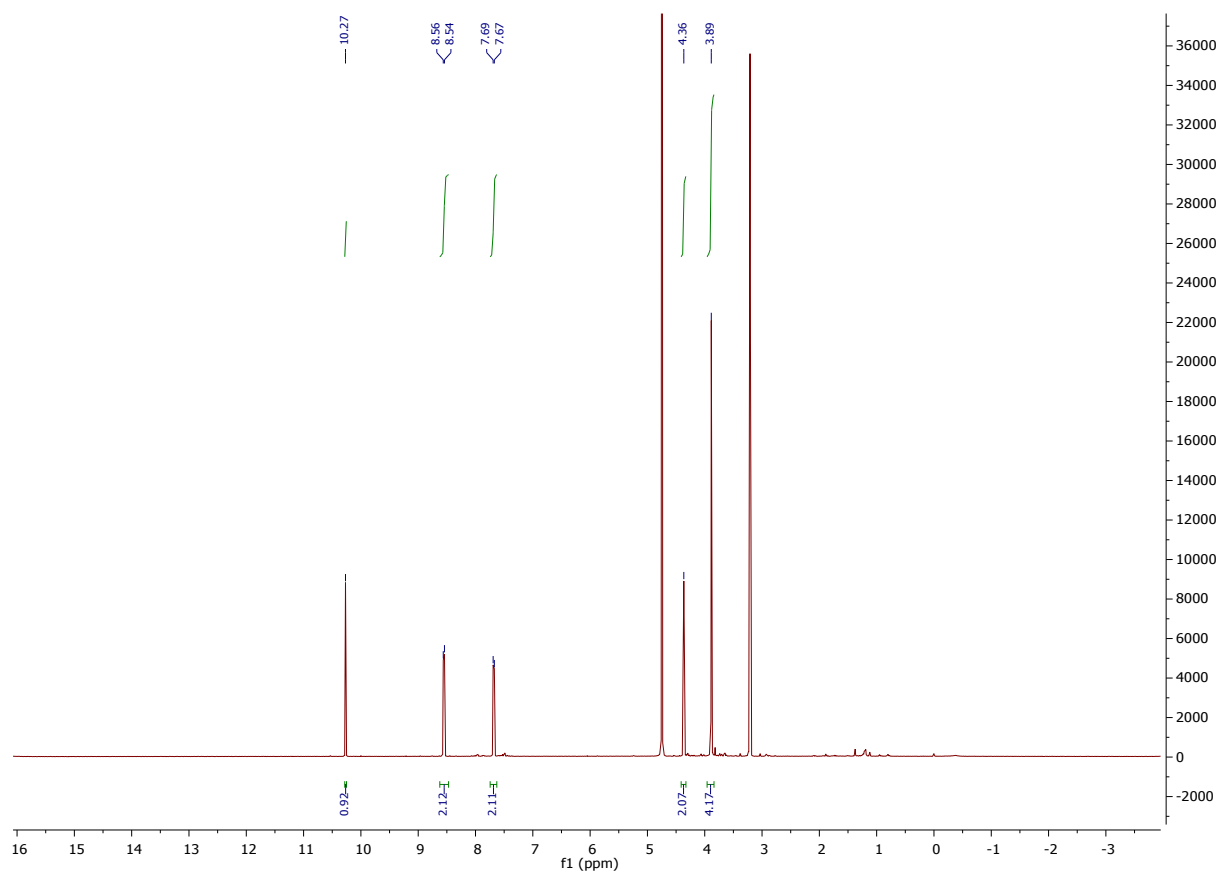
N-(4-(1,2,4,5-tetrazin-3-yl)benzyl)acetamide (**31**)



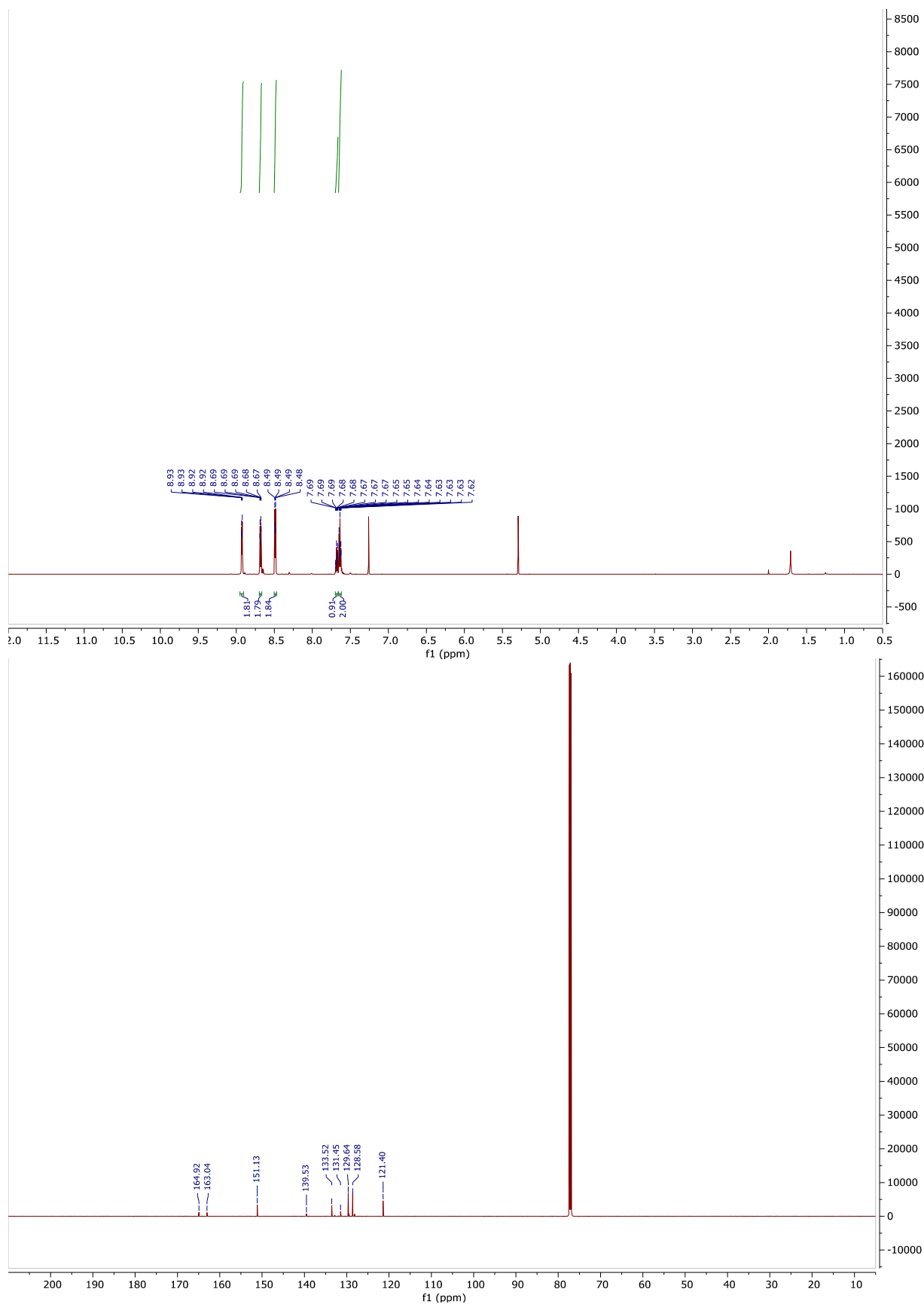
2-((4-(1,2,4,5-Tetrazin-3-yl)benzyl)amino)acetic acid (32)



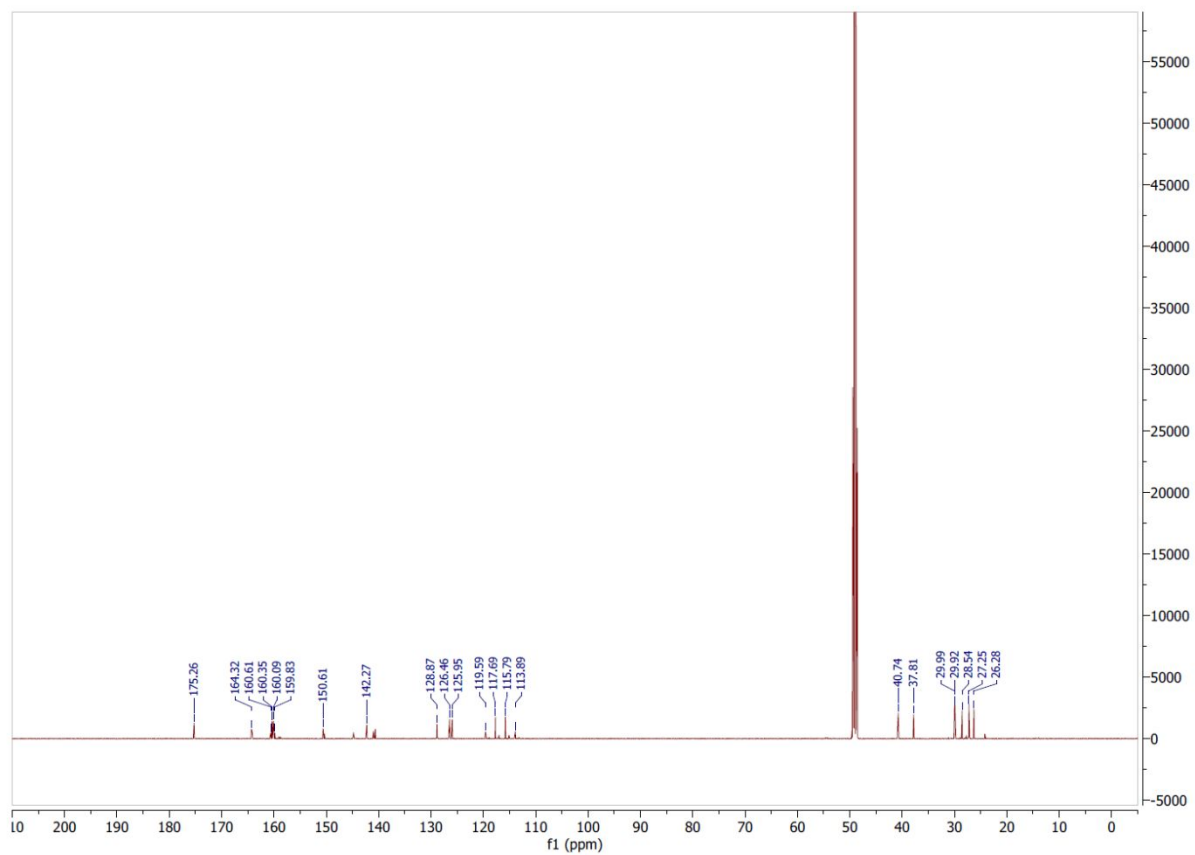
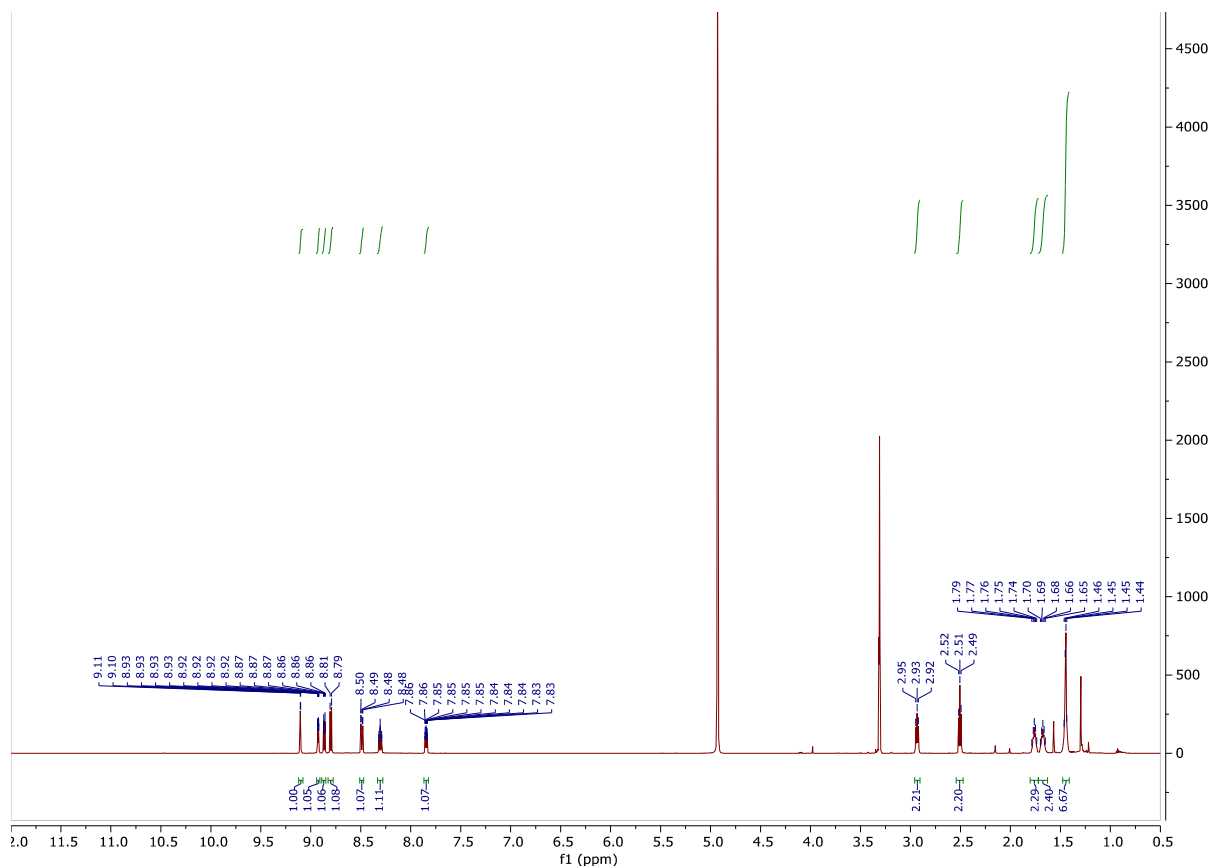
2,2'-((4-(1,2,4,5-Tetrazin-3-yl)benzyl)azanediyldiacetic acid (33)



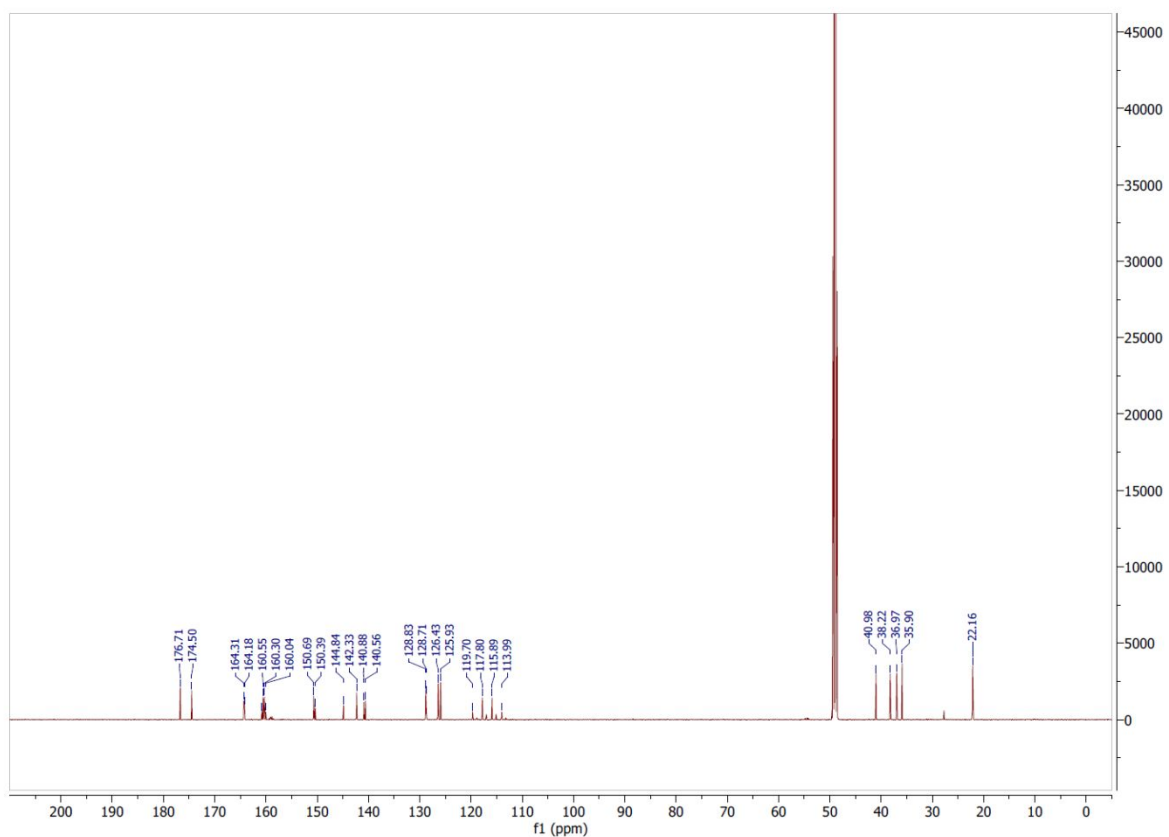
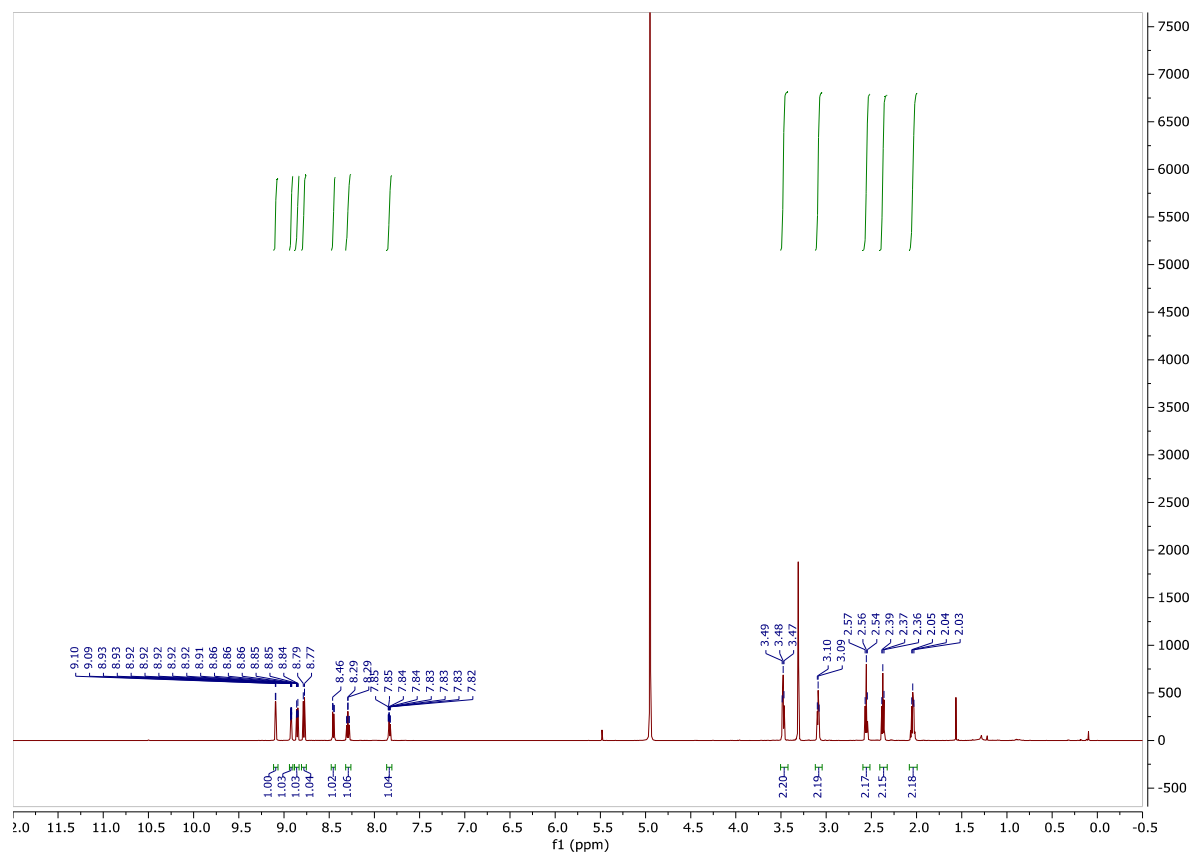
3-Phenyl-6-(pyridin-4-yl)-1,2,4,5-tetrazine (36)



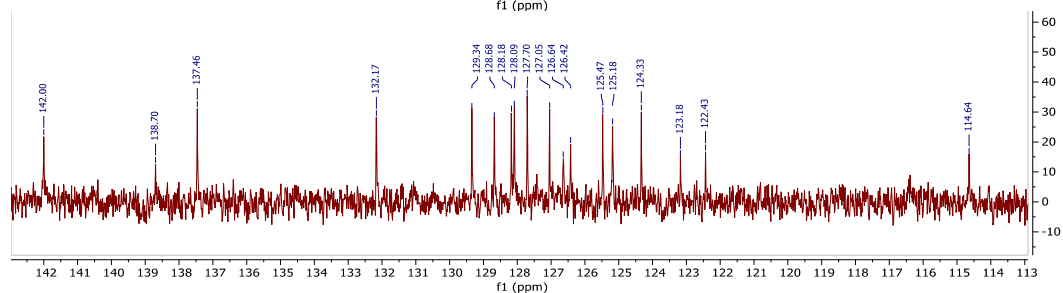
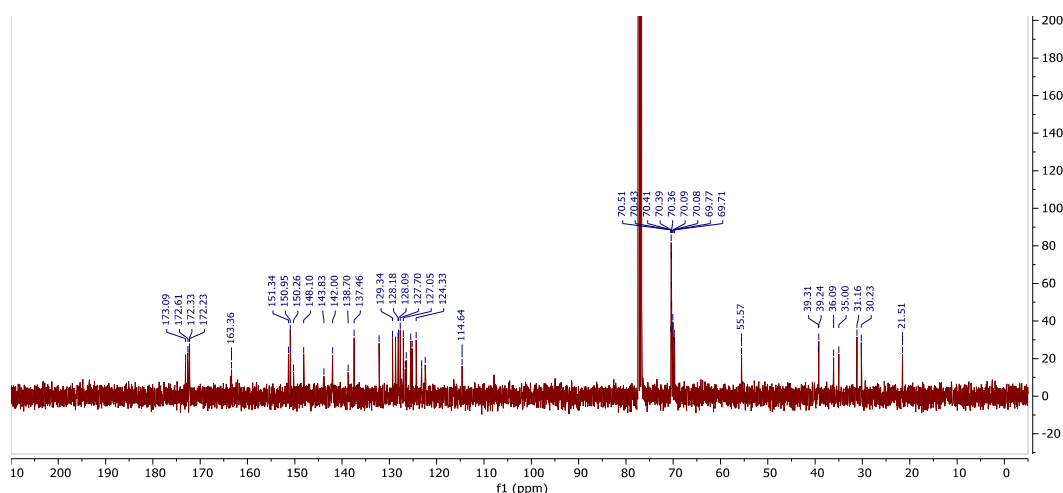
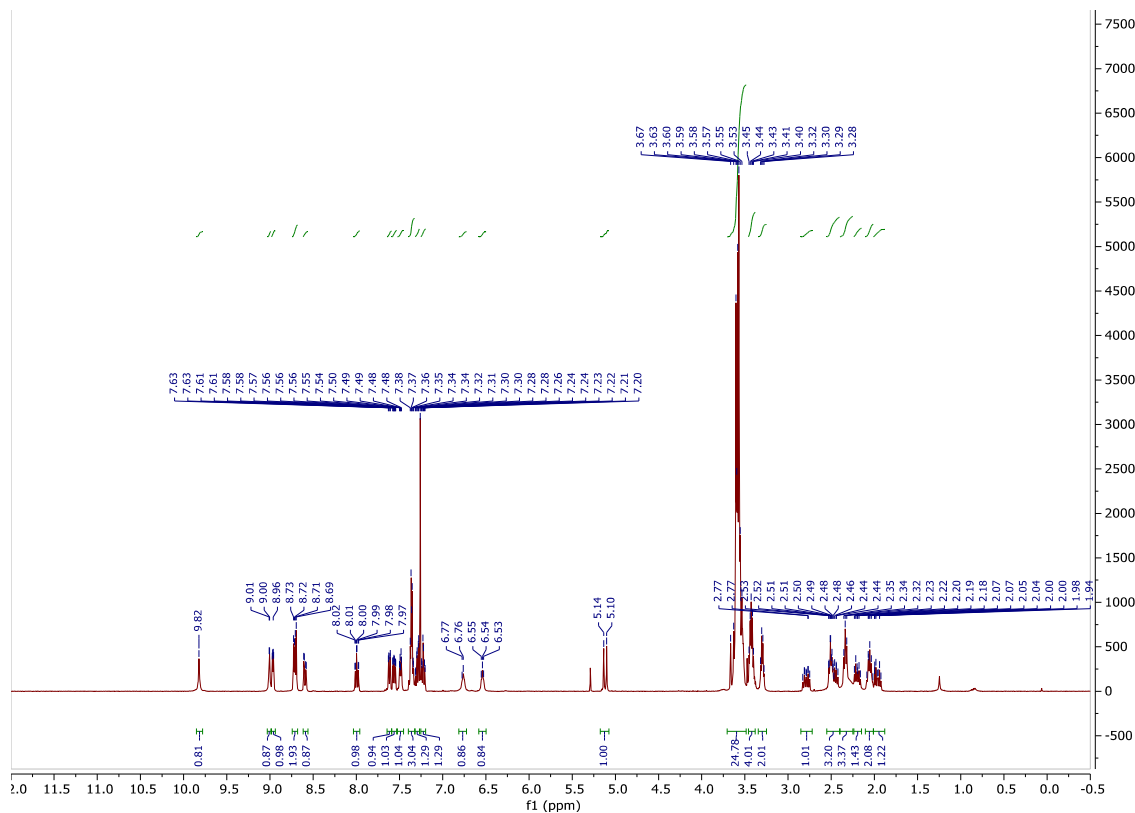
8-Amino-N-(6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)octanamide (37)



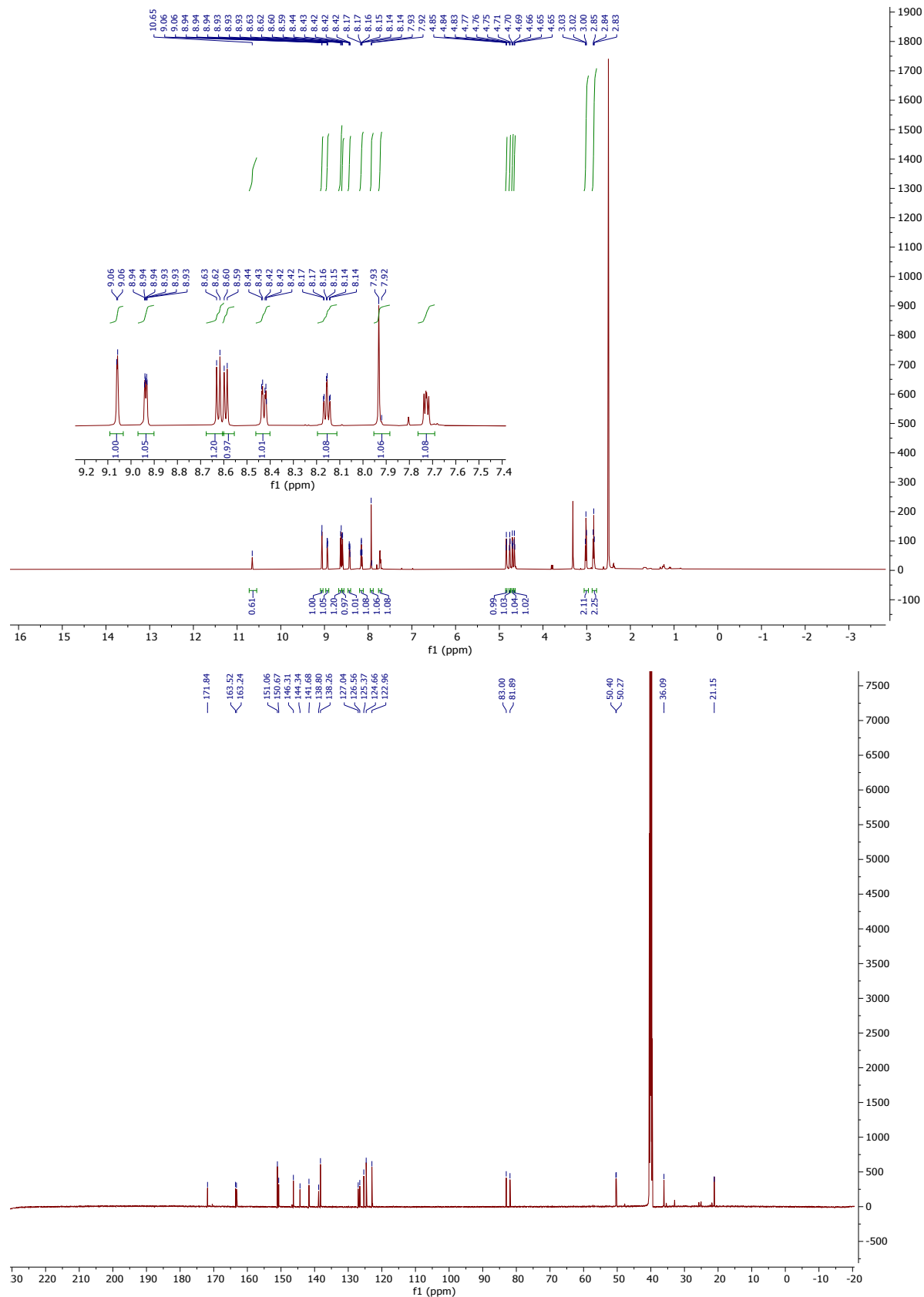
*N*¹-(2-Aminoethyl)-*N*⁵-(6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)glutaramide (**38**)



*N*¹-(25-(11,12-didehydrodibenzo[*b,f*]azocin-5(6*H*)-yl)-22,25-dioxo-3,6,9,12,15,18-hexaoxa-21-azapentacosyl)-*N*⁵-(6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)glutaramide (**42**)

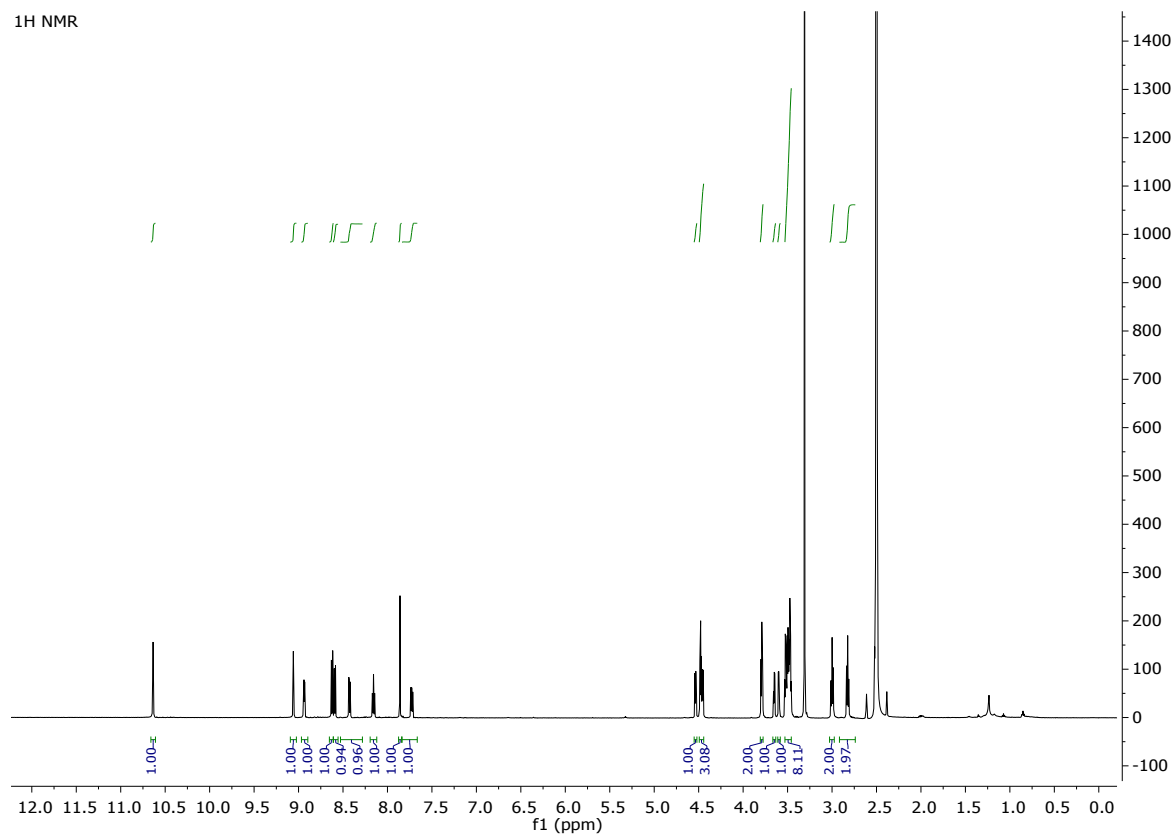


3-(1-(2-fluoroethyl)-1H-1,2,3-triazol-4-yl)-N-(6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)propanamide (**43**)

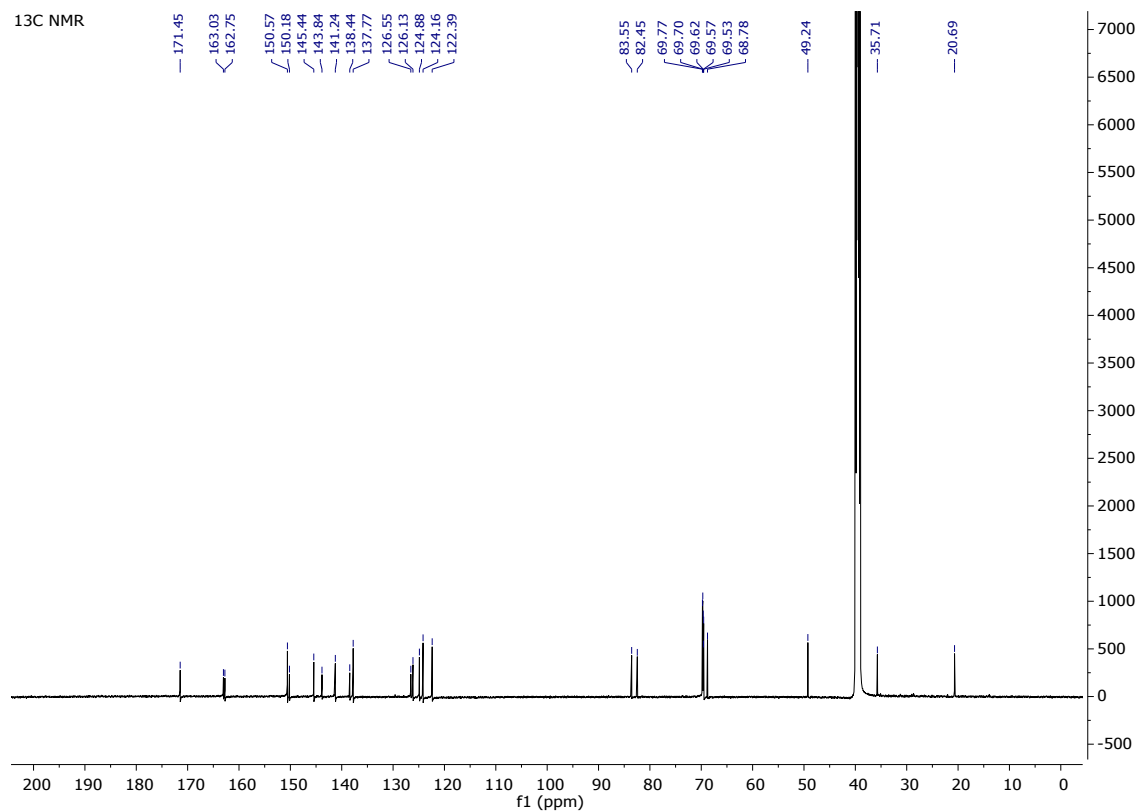


3-(1-(2-(2-(2-(2-Fluoroethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)-N-(6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)propanamide (44)

¹H NMR



¹³C NMR



3-(1-((2R,3R,4S,5S,6S)-6-(Fluoromethyl)-3,4,5-Trihydroxytetrahydro-2H-pyran-2-yl)-1H-1,2,3-triazol-4-yl)-N-(6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)propenamide (45)

