Supporting Information

Development of ^{18}F-Labeled Bispyridyl Tetrazines for In Vivo Pretargeted PET Imaging

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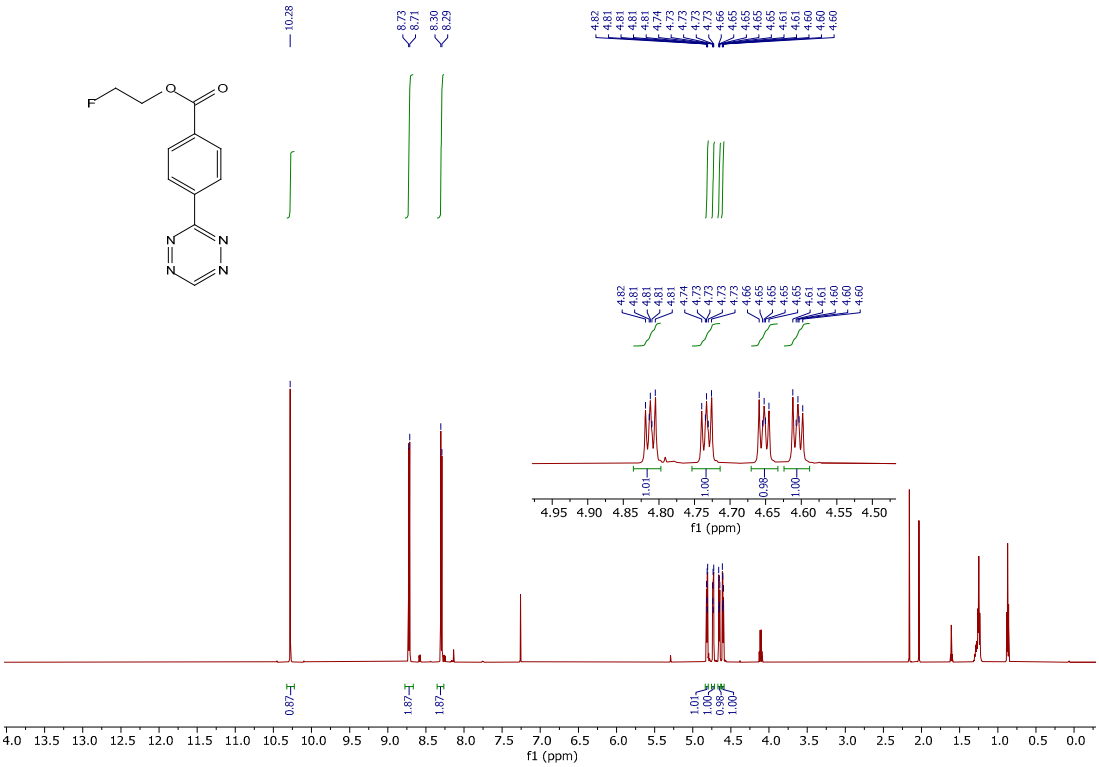
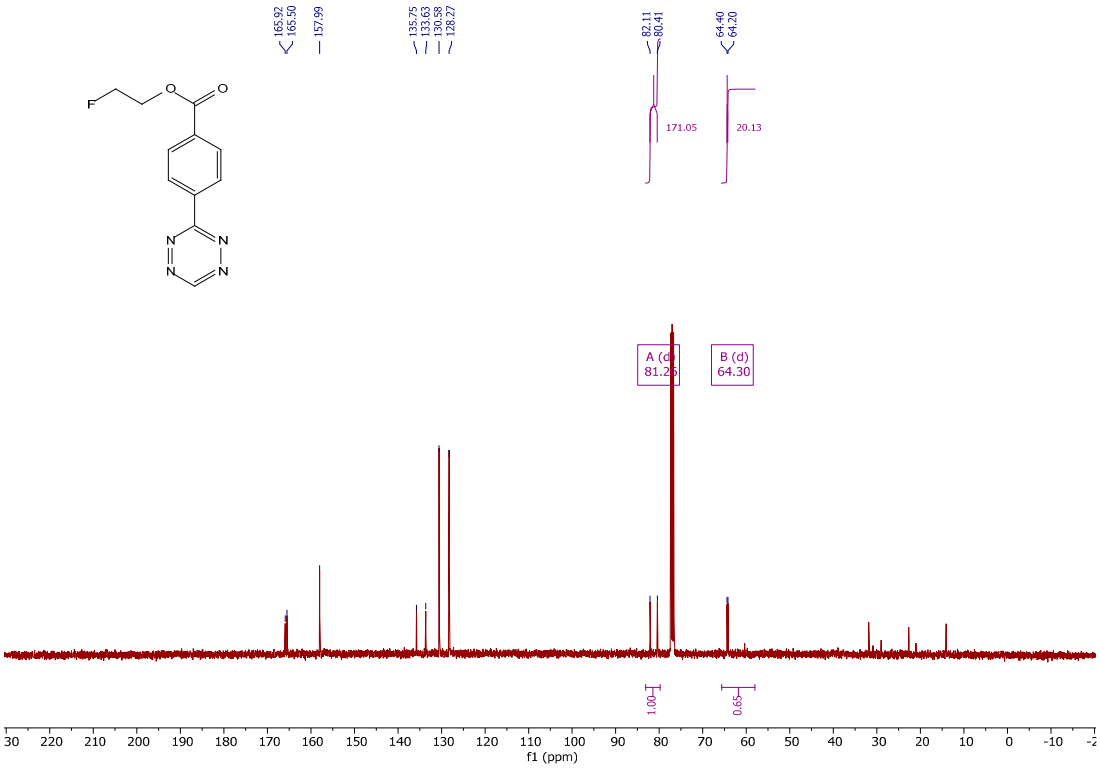
† These authors contributed equally to the work.

*2-Fluoroethyl 4-(1,2,4,5-tetrazin-3-yl)benzoate (**3**)*





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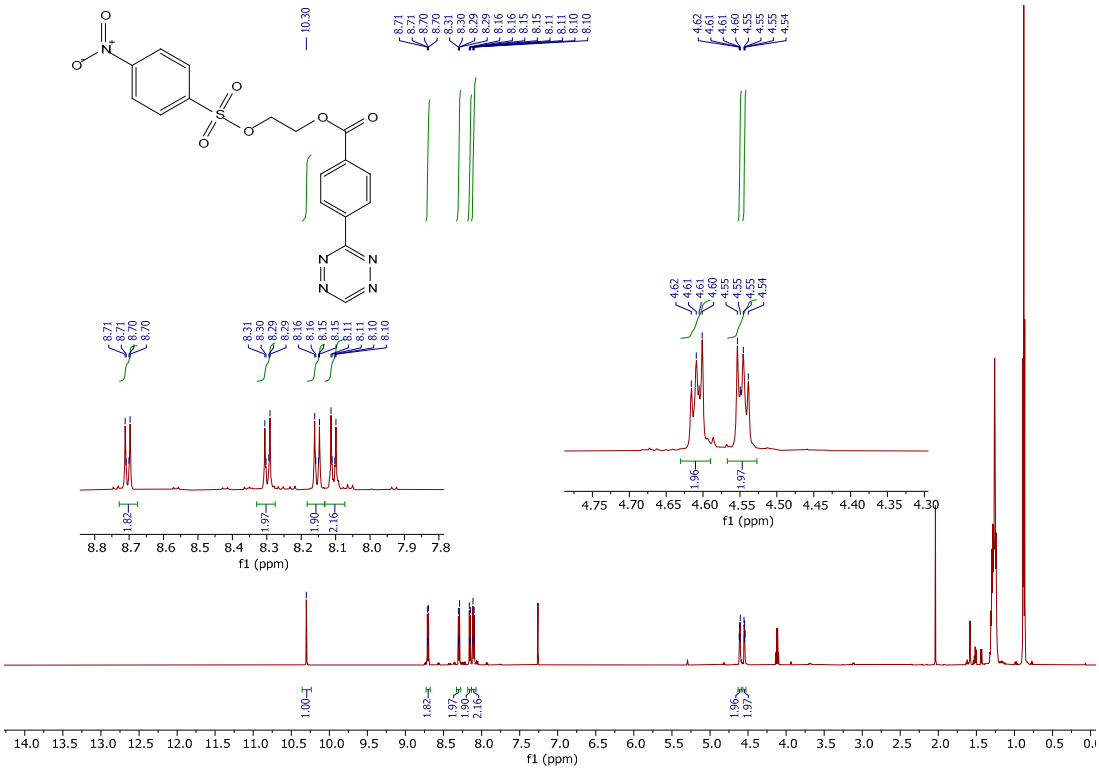

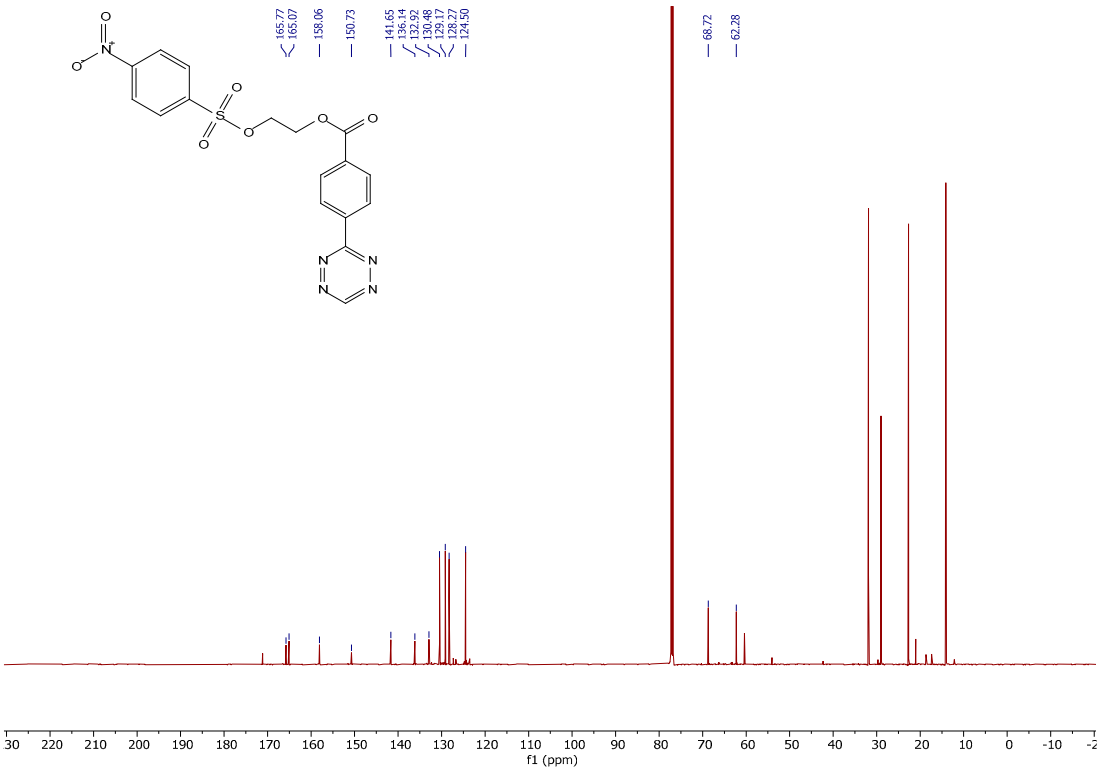

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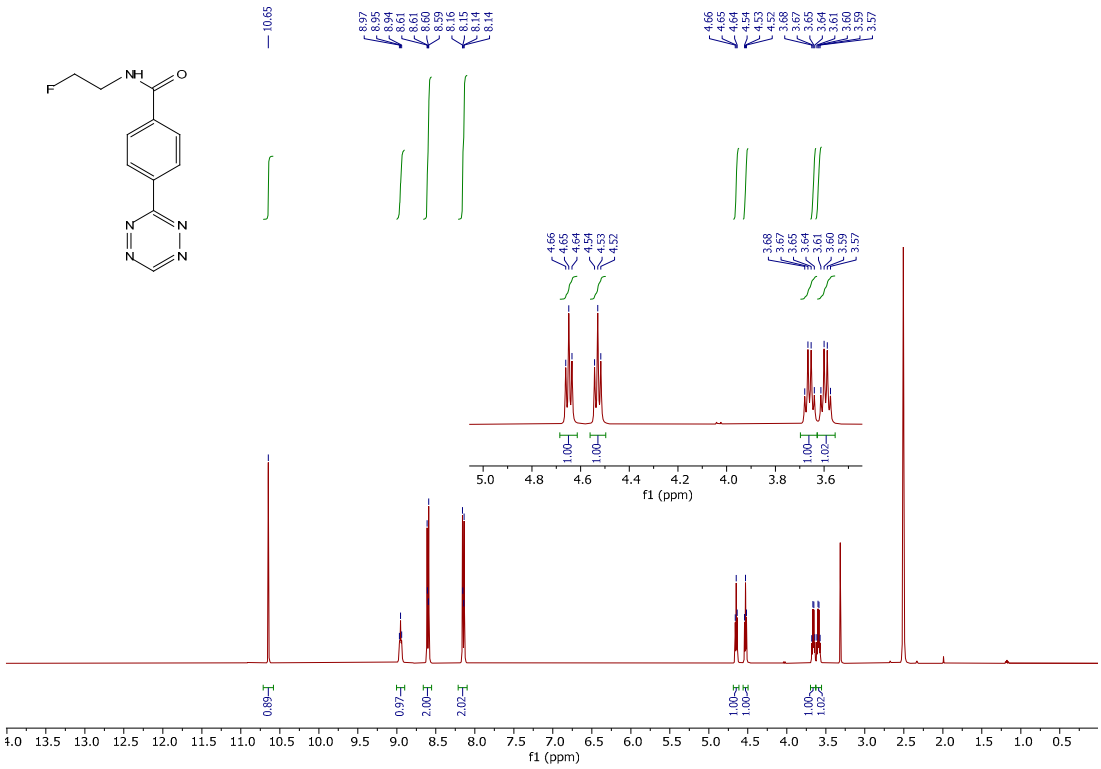

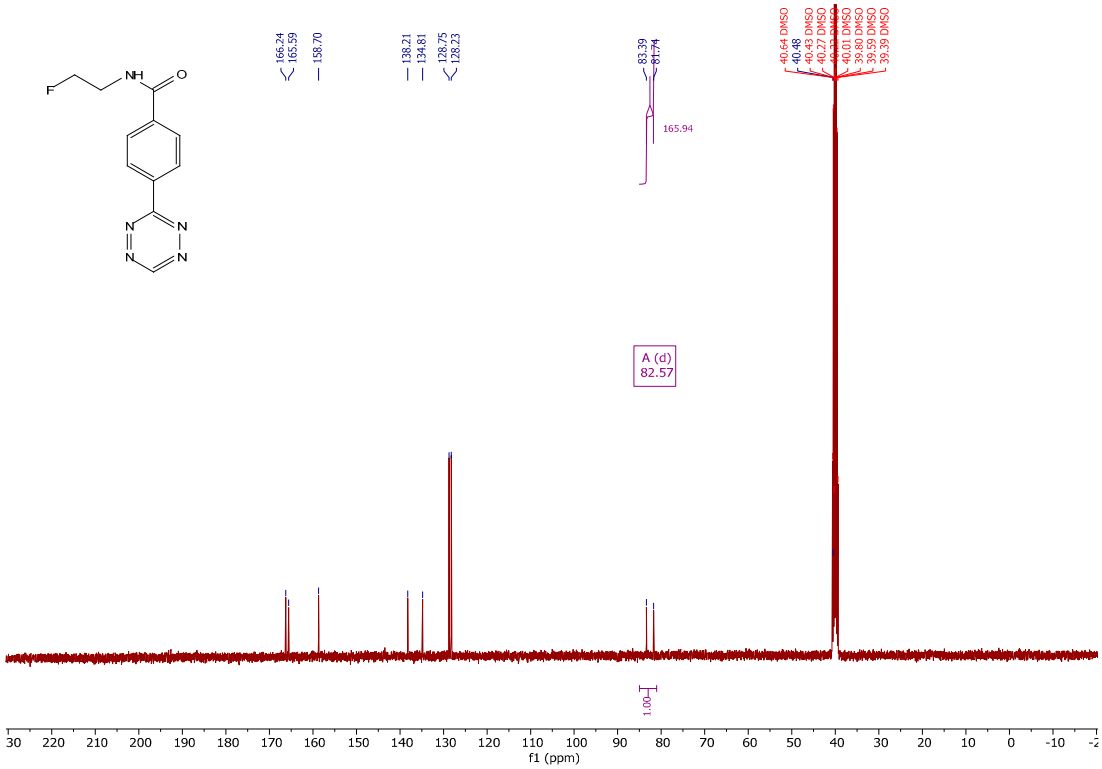

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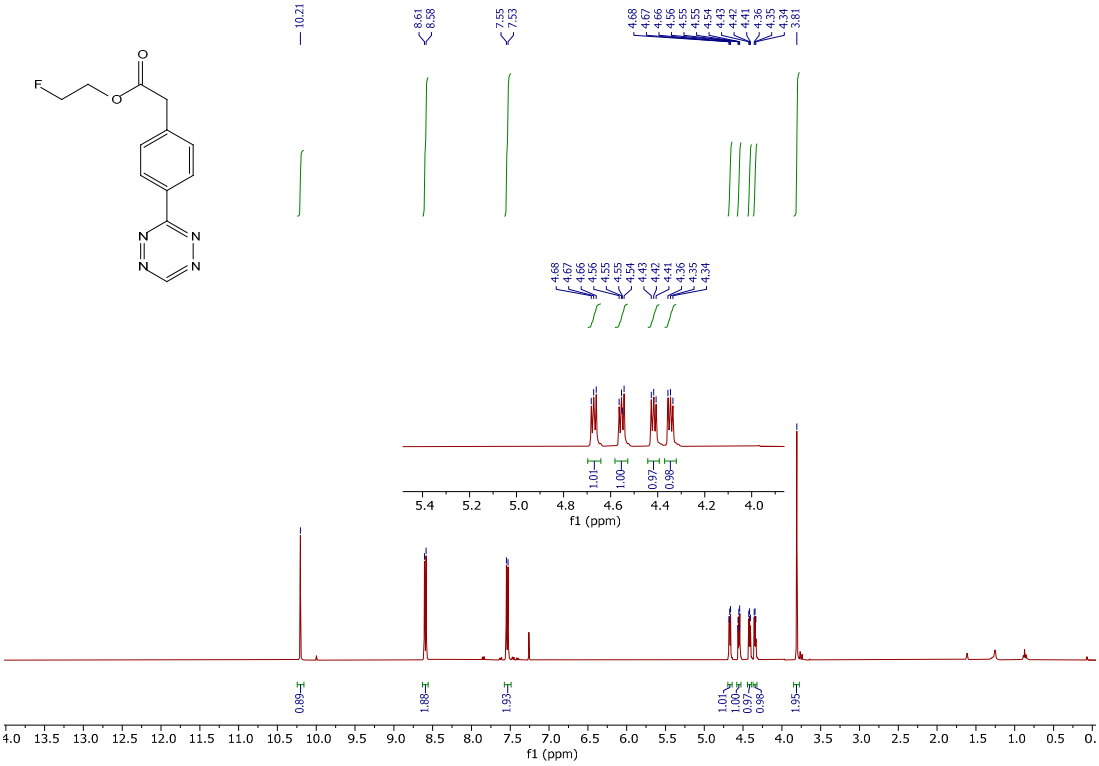

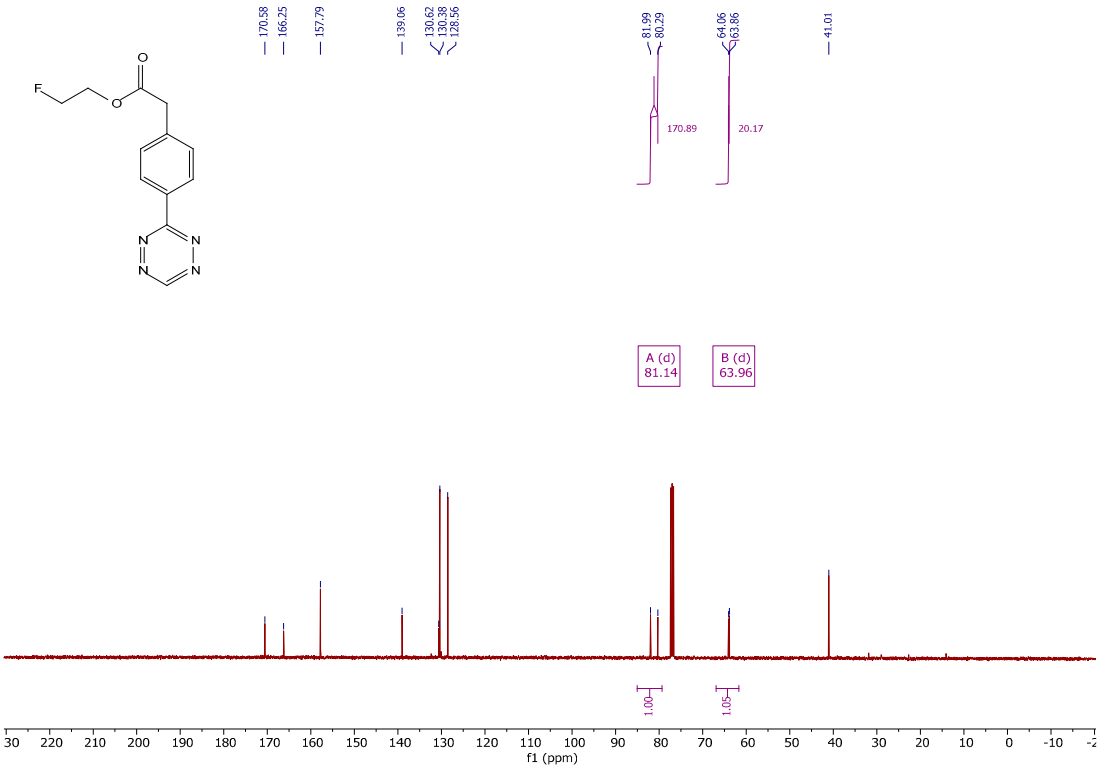

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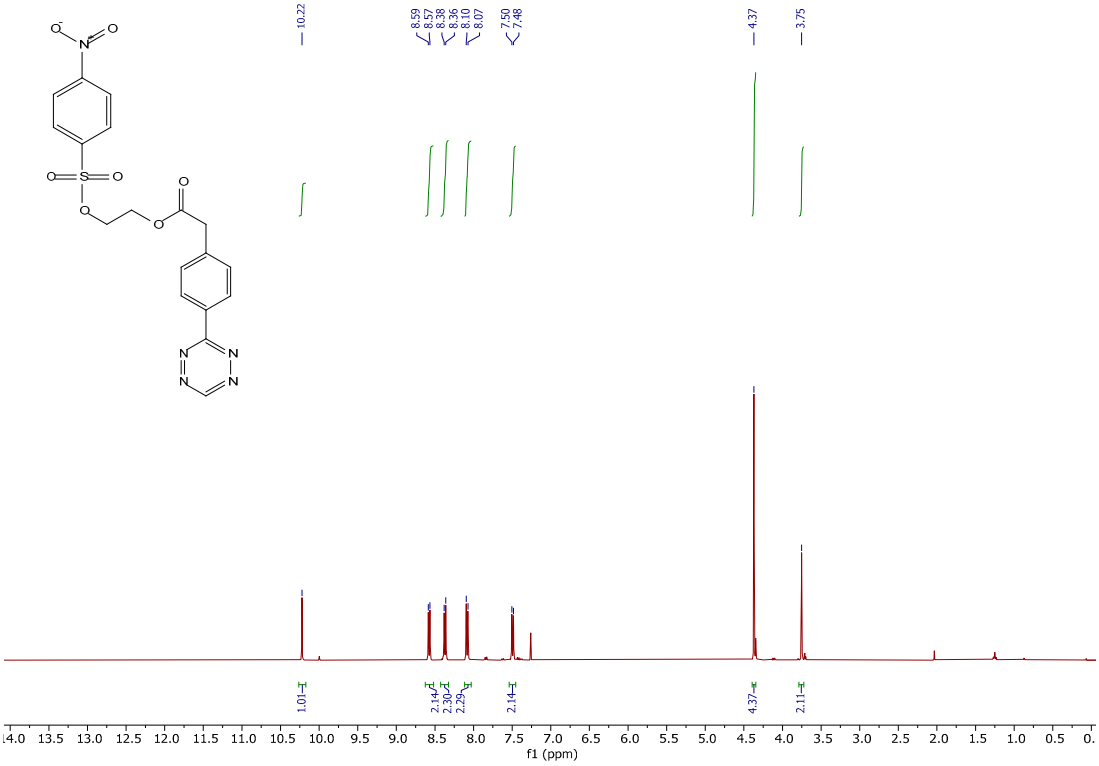

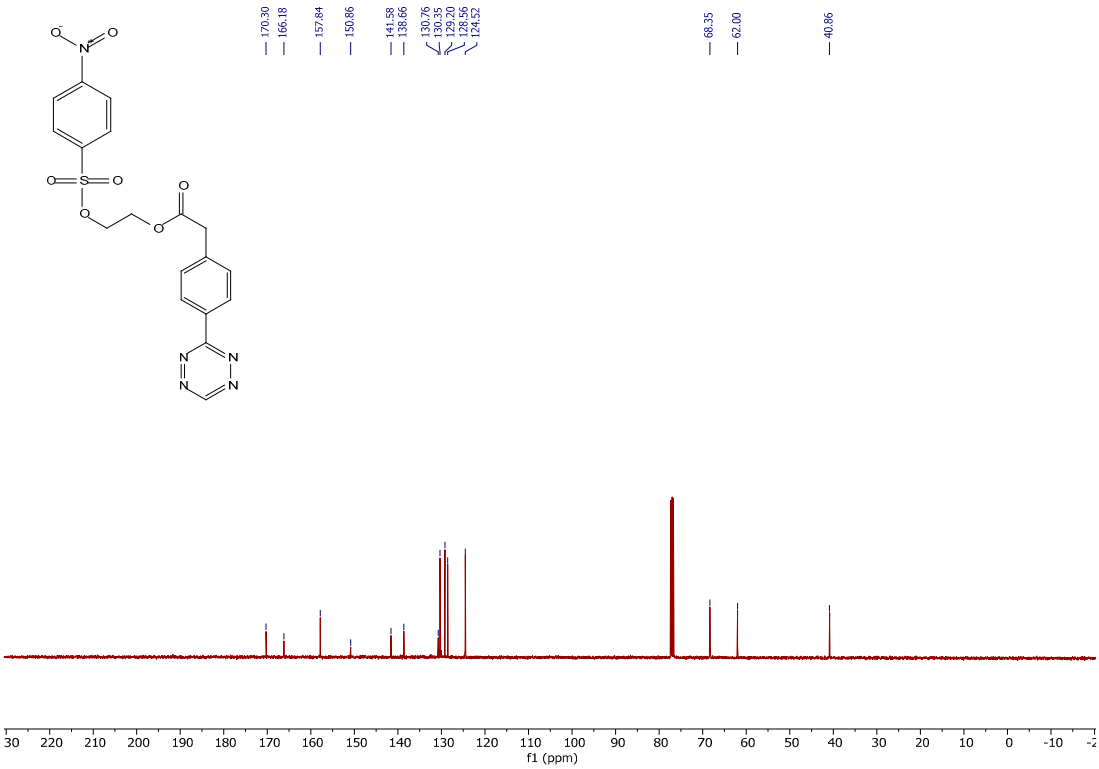

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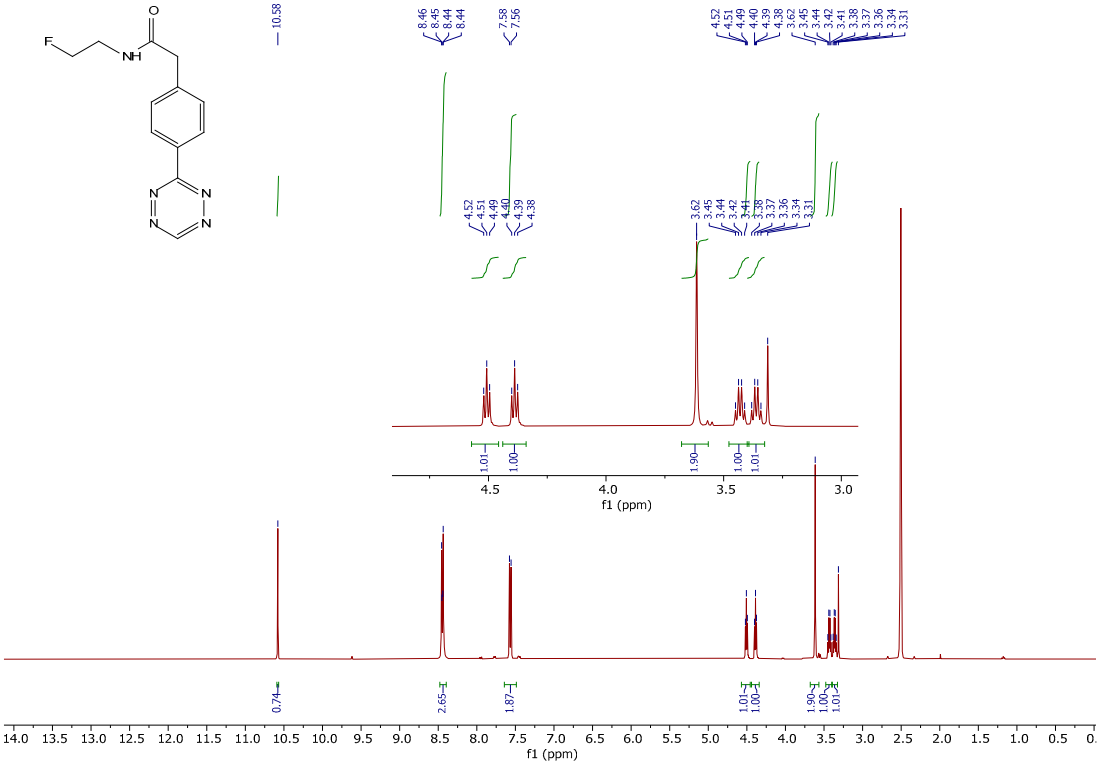

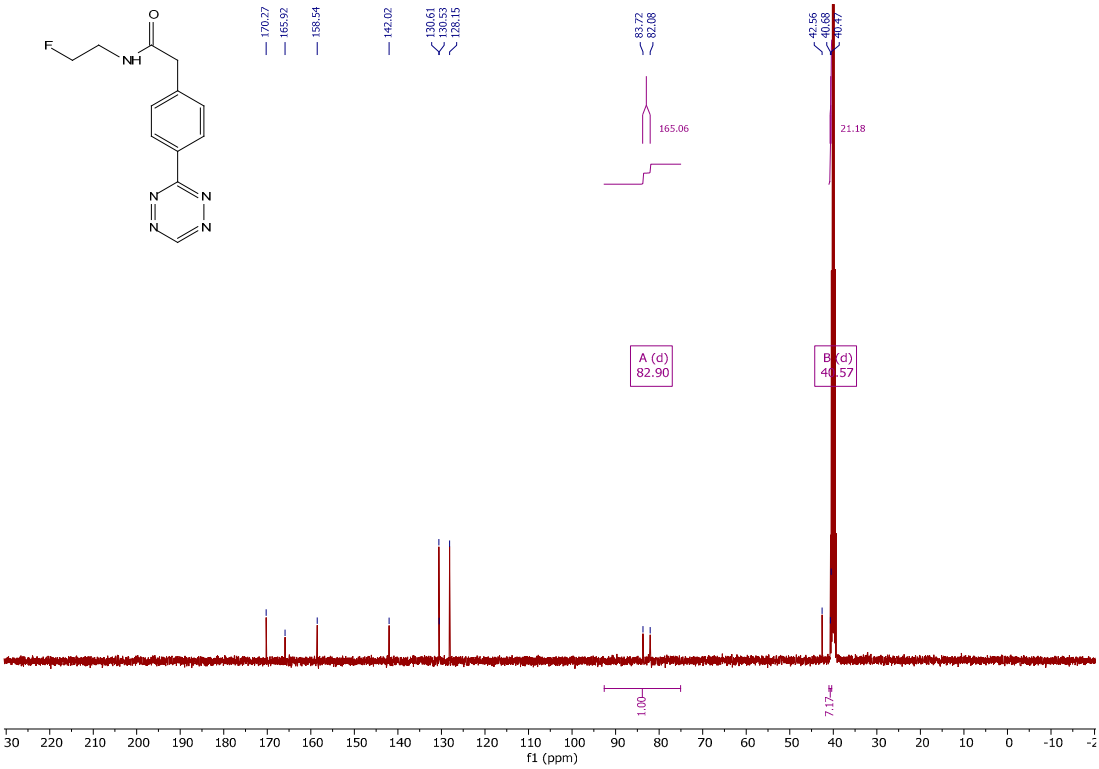

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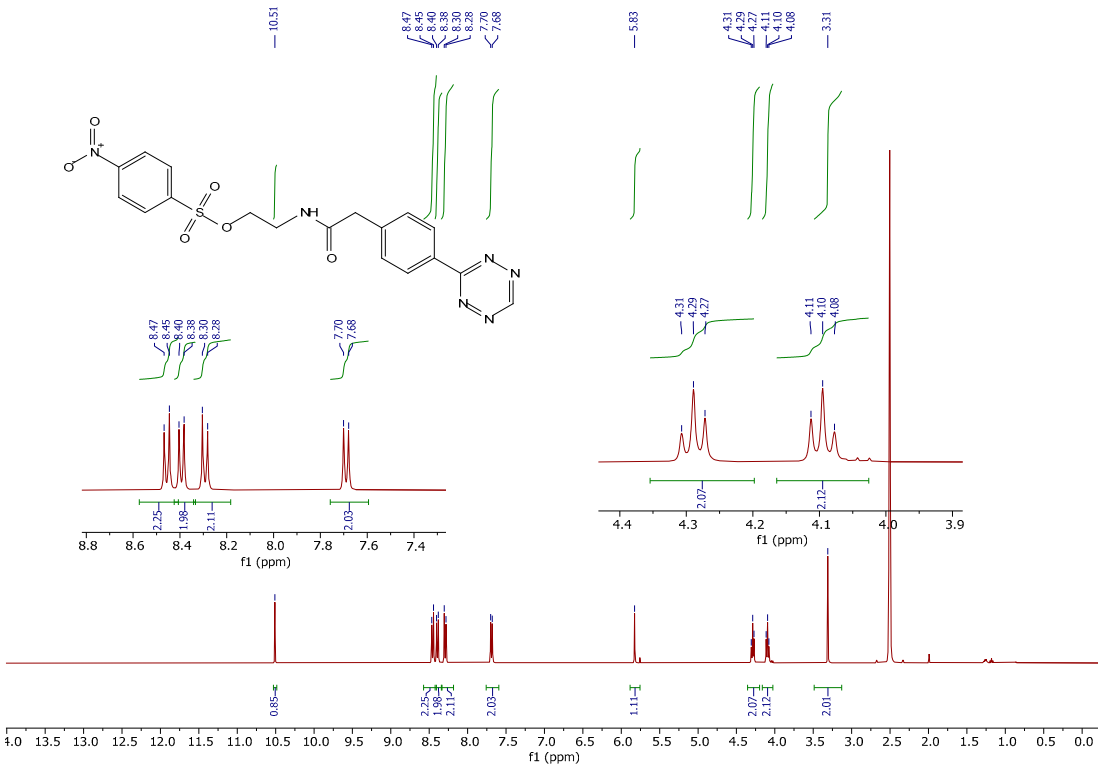
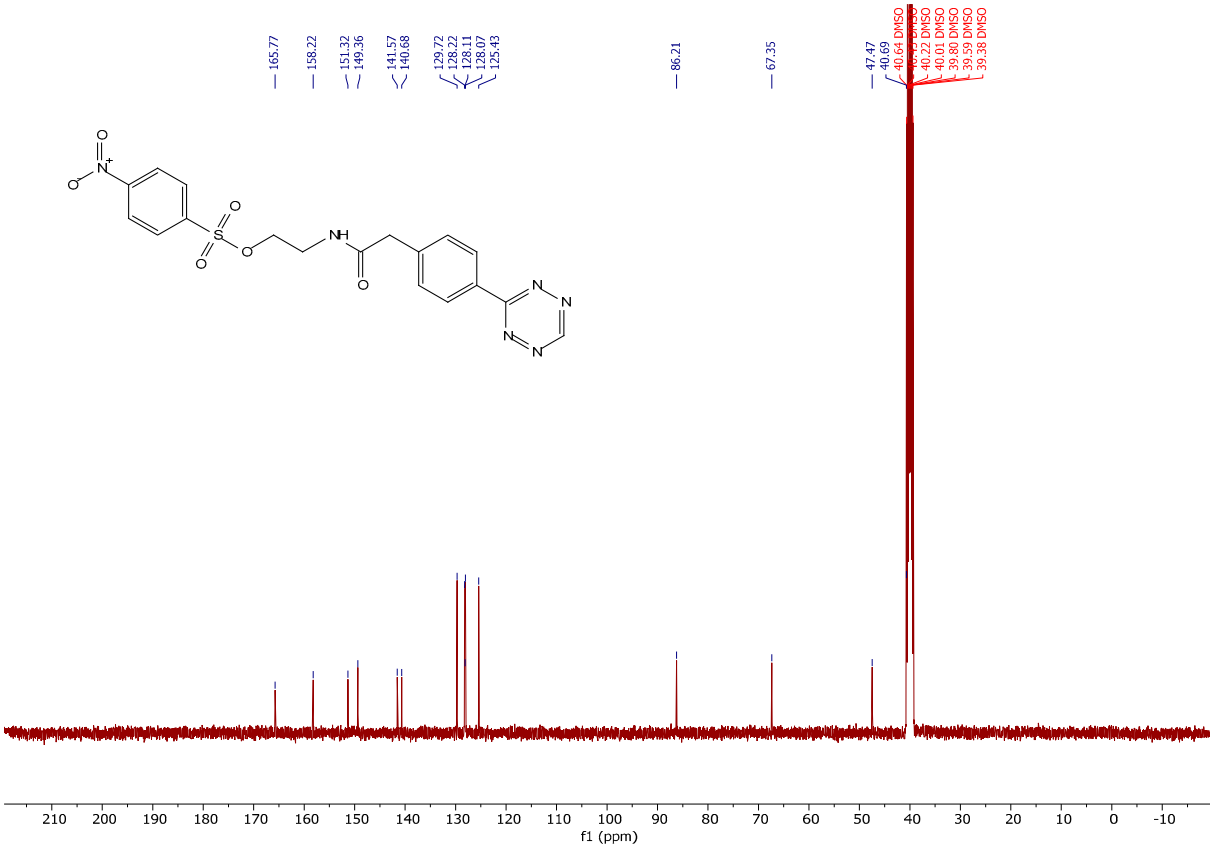
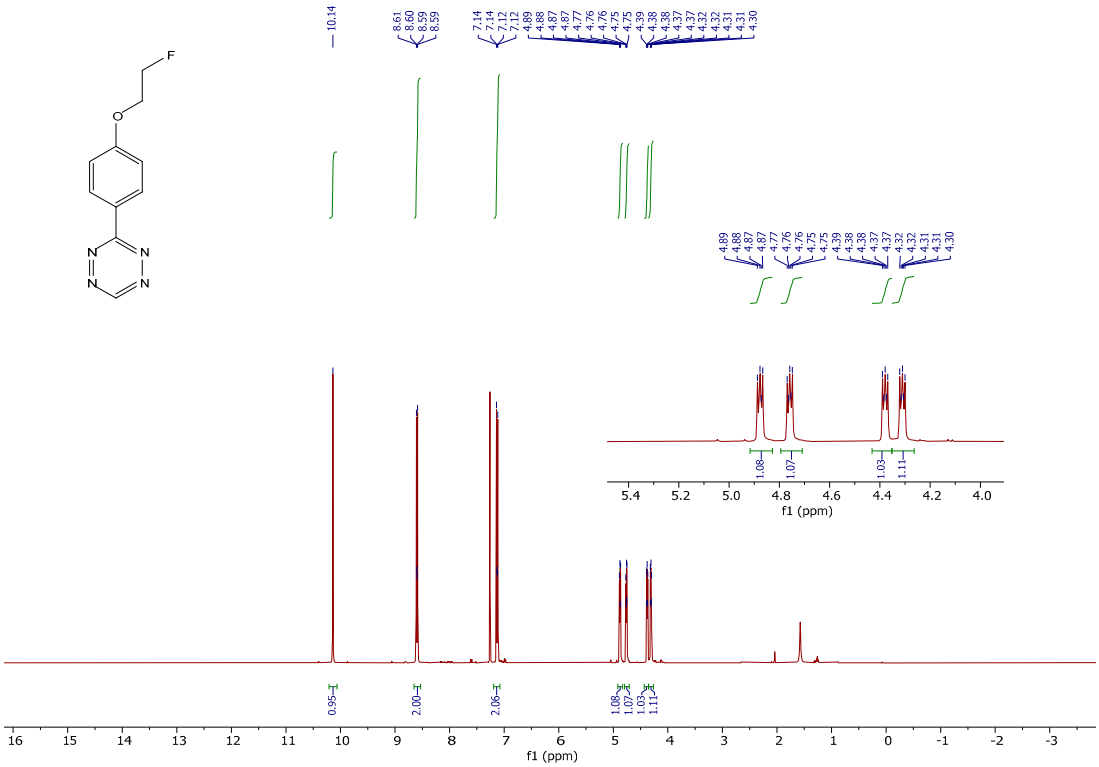
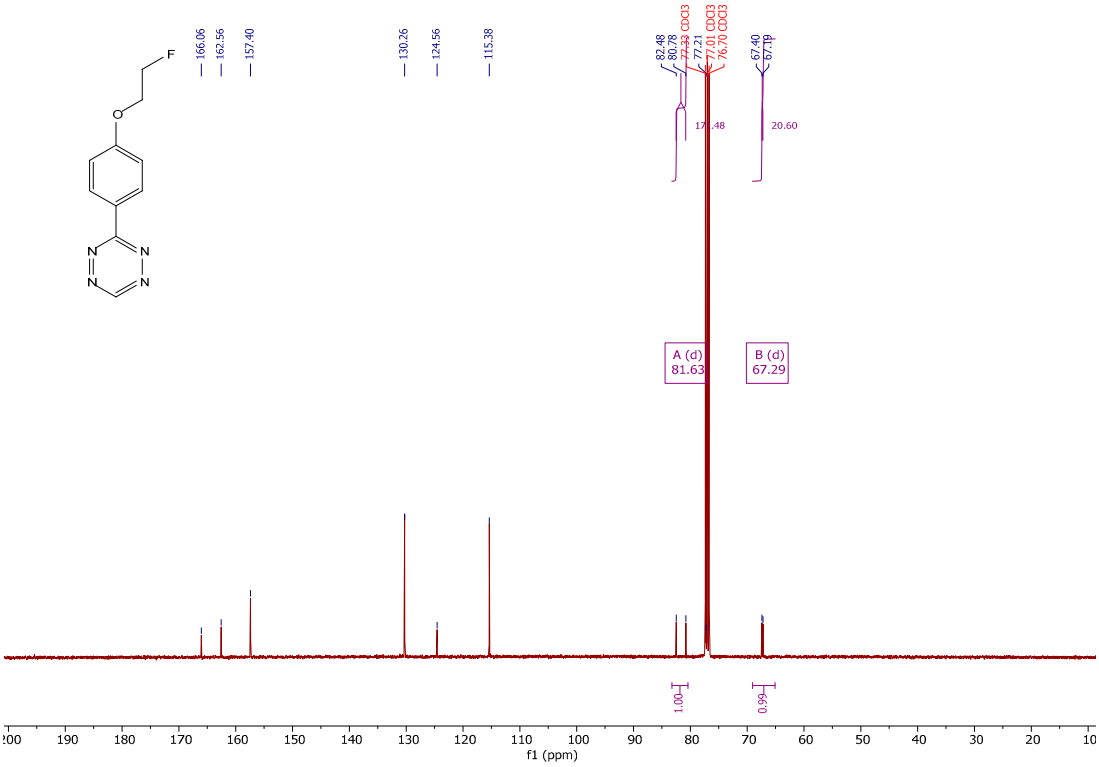




3-(4-(2-Fluoroethoxy)phenyl)-1,2,4,5-tetrazine (7)





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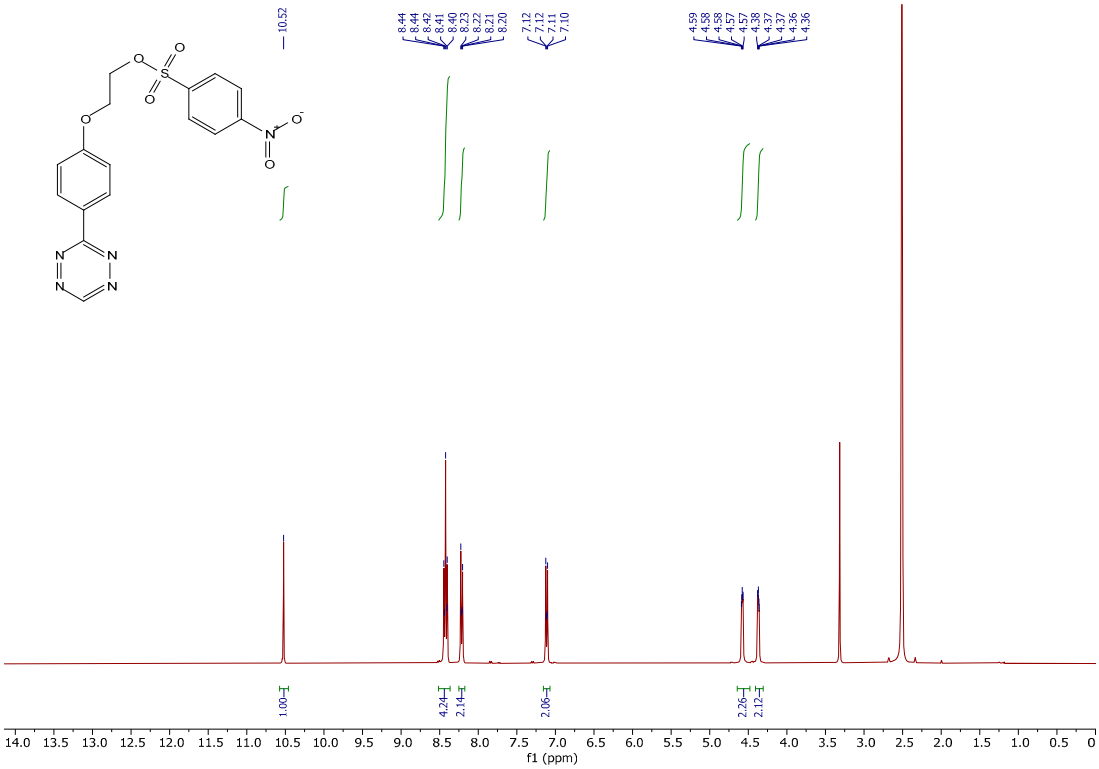
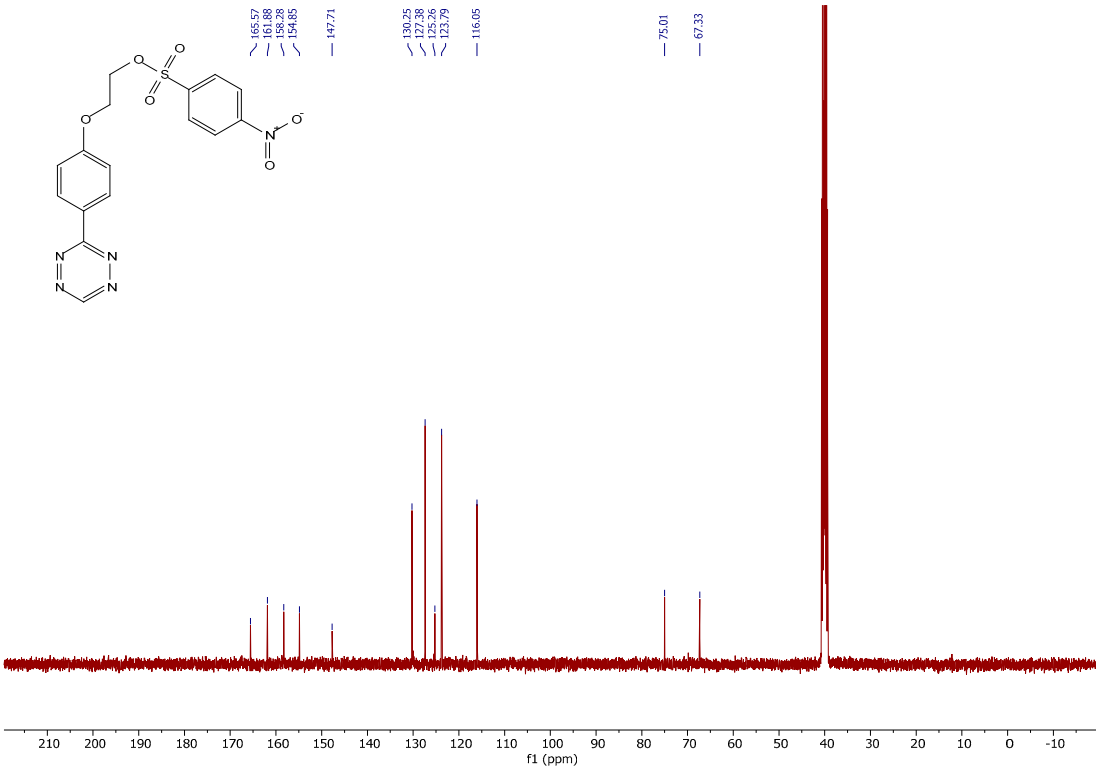
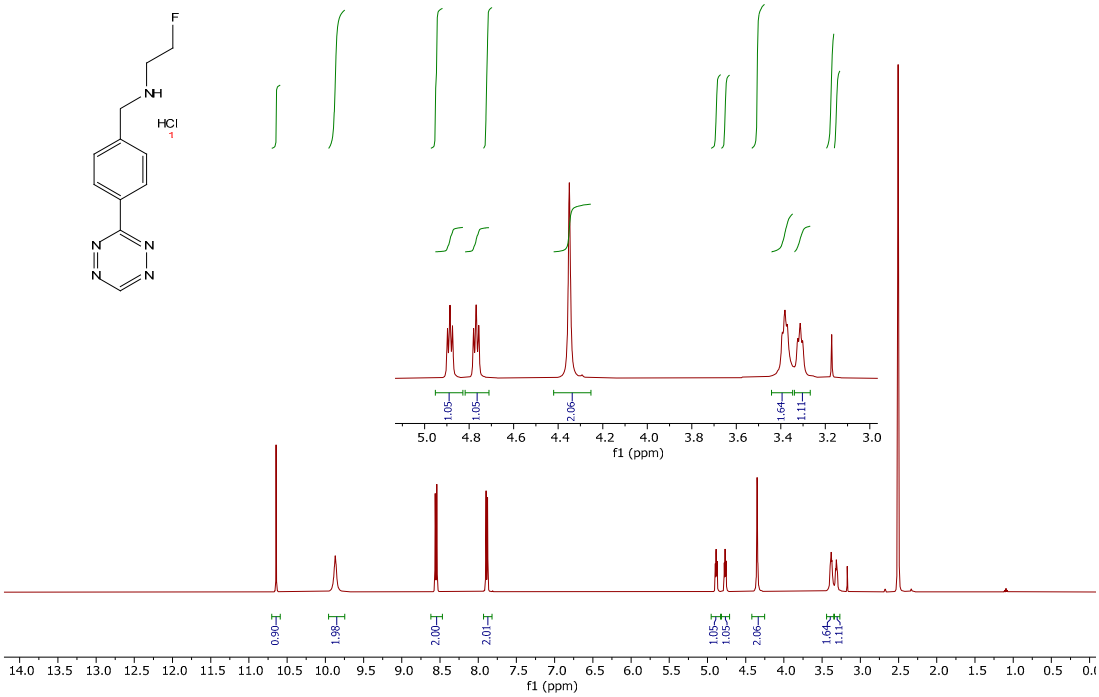
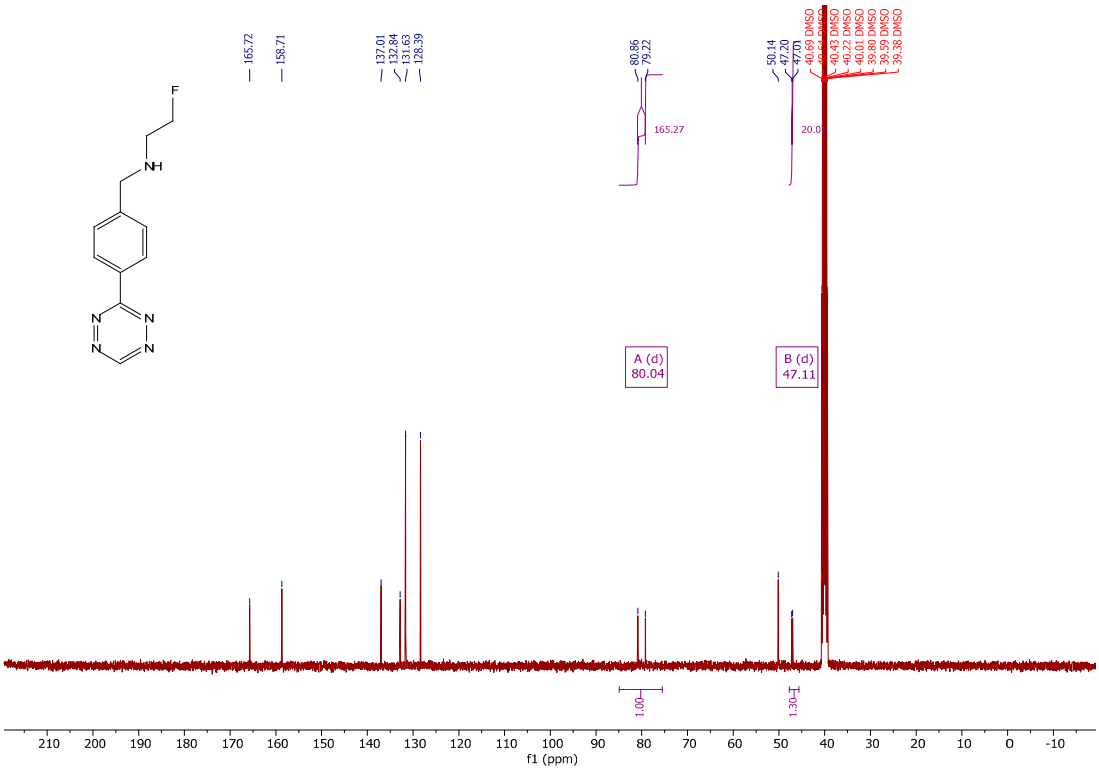




*N-(4-(1,2,4,5-tetrazin-3-yl)benzyl)-2-fluoroethan-1-amine (**9**)*





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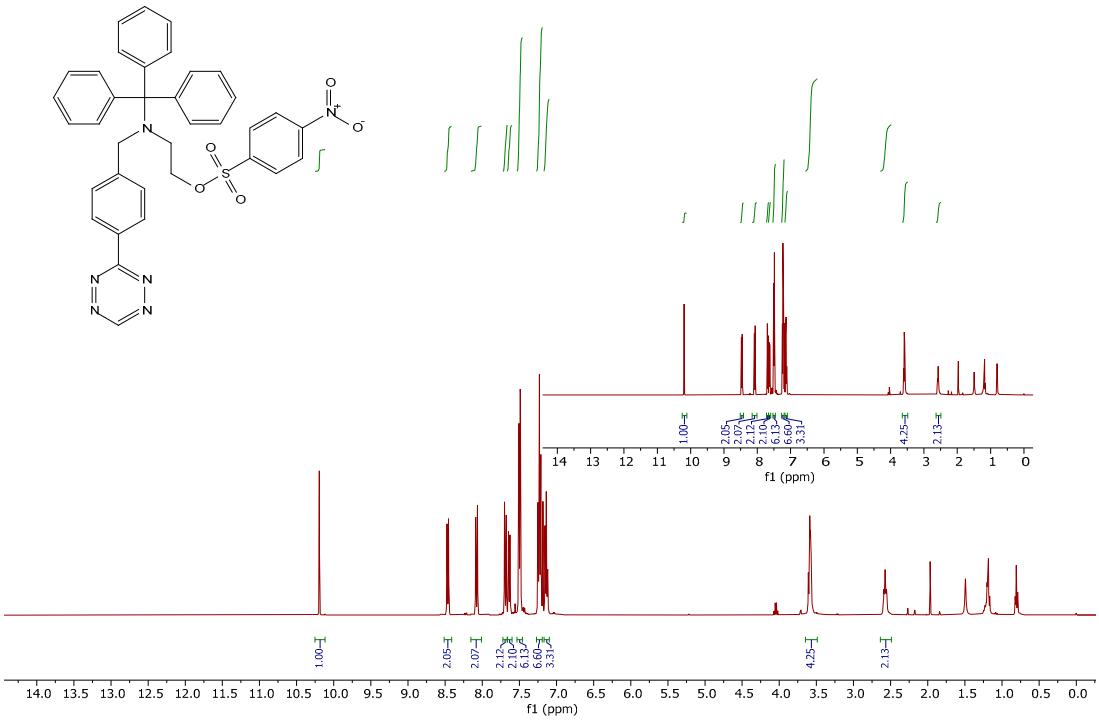
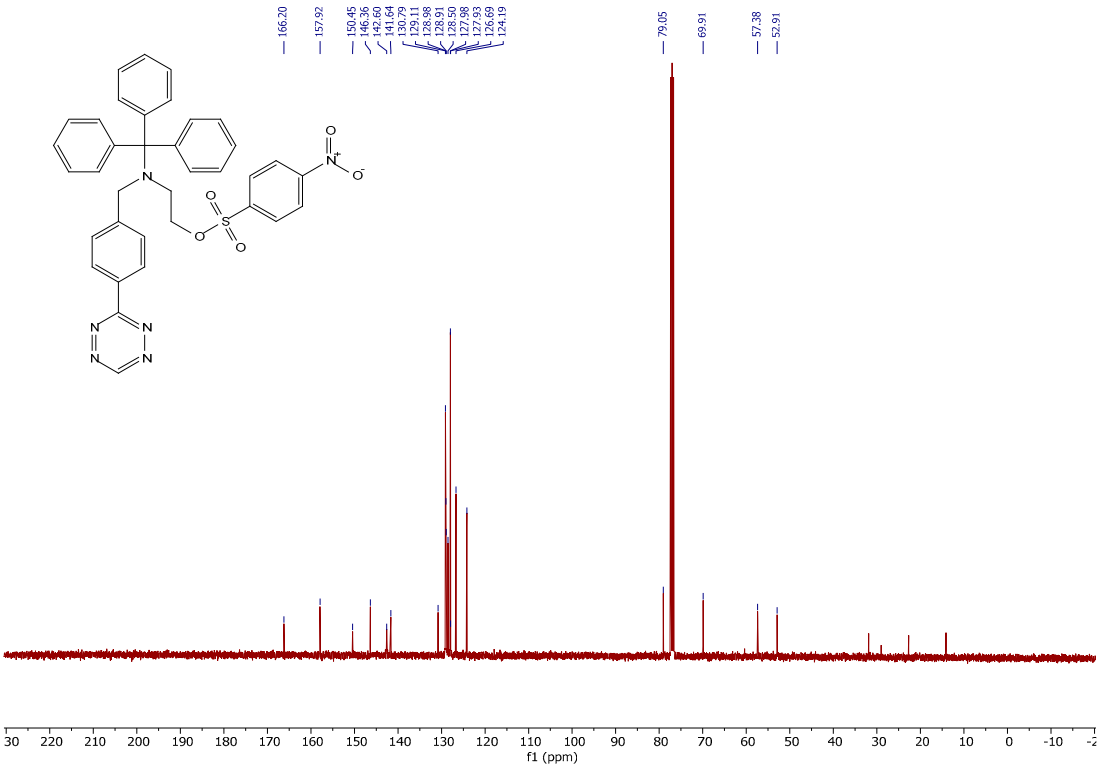
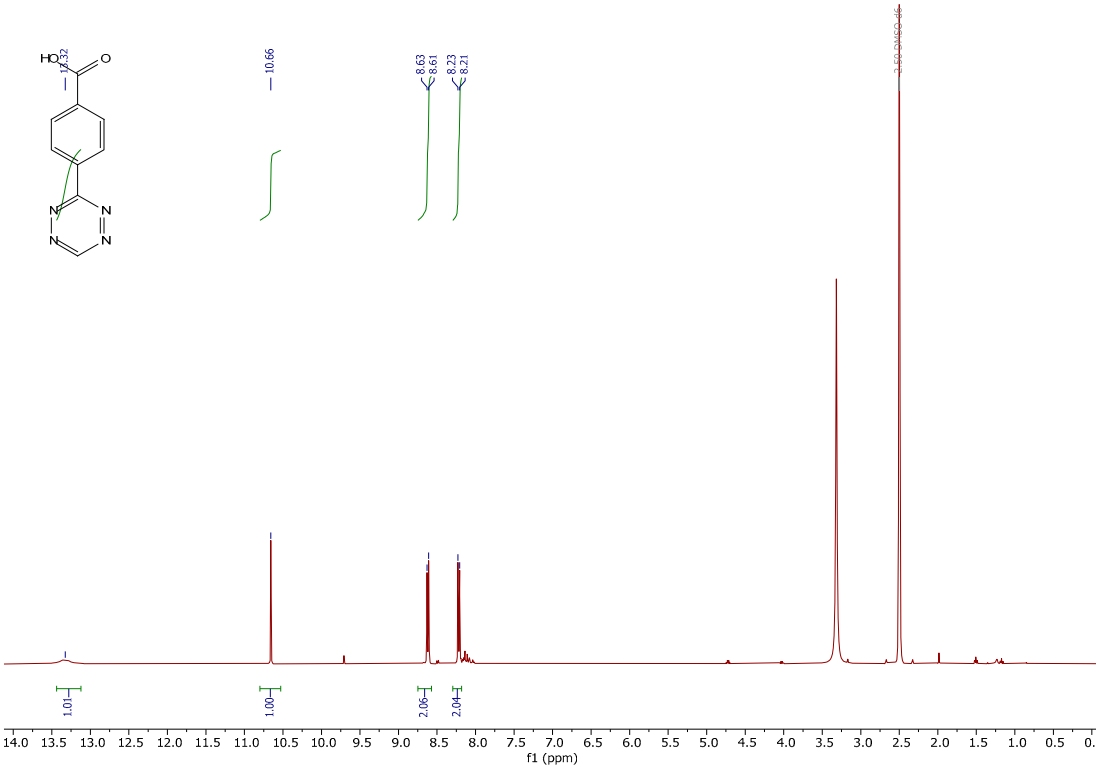
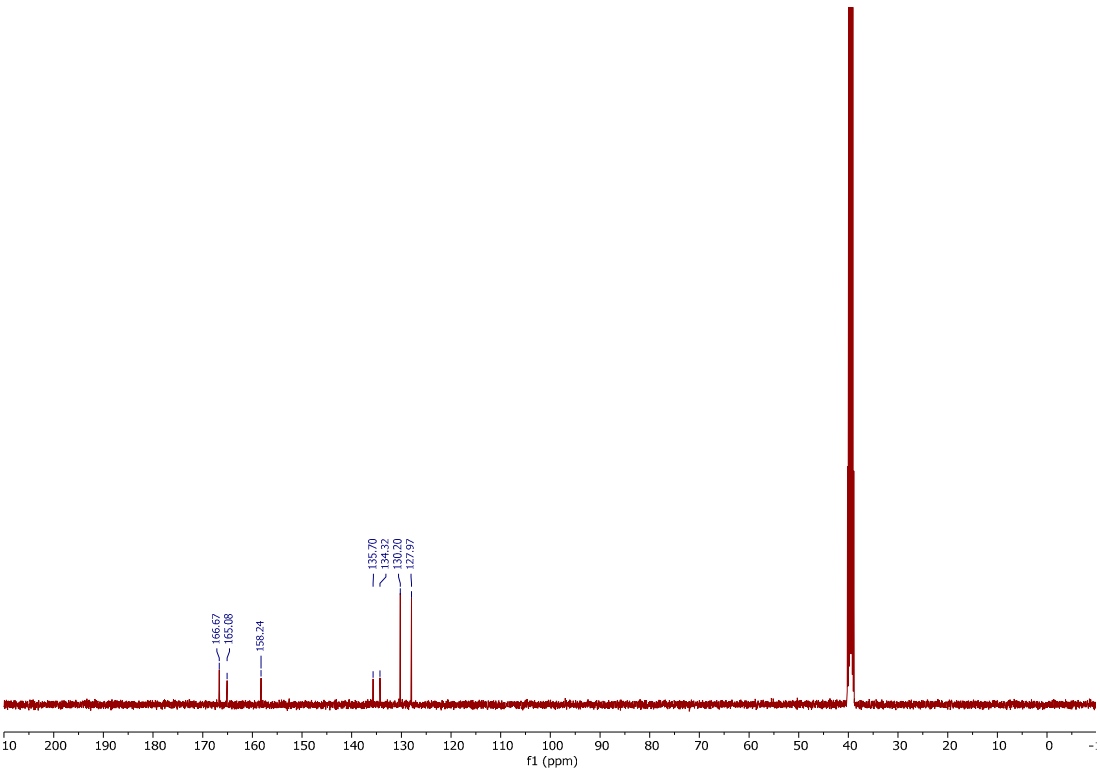




*4-(1,2,4,5-tetrazin-3-yl)benzoic acid (**10**)*





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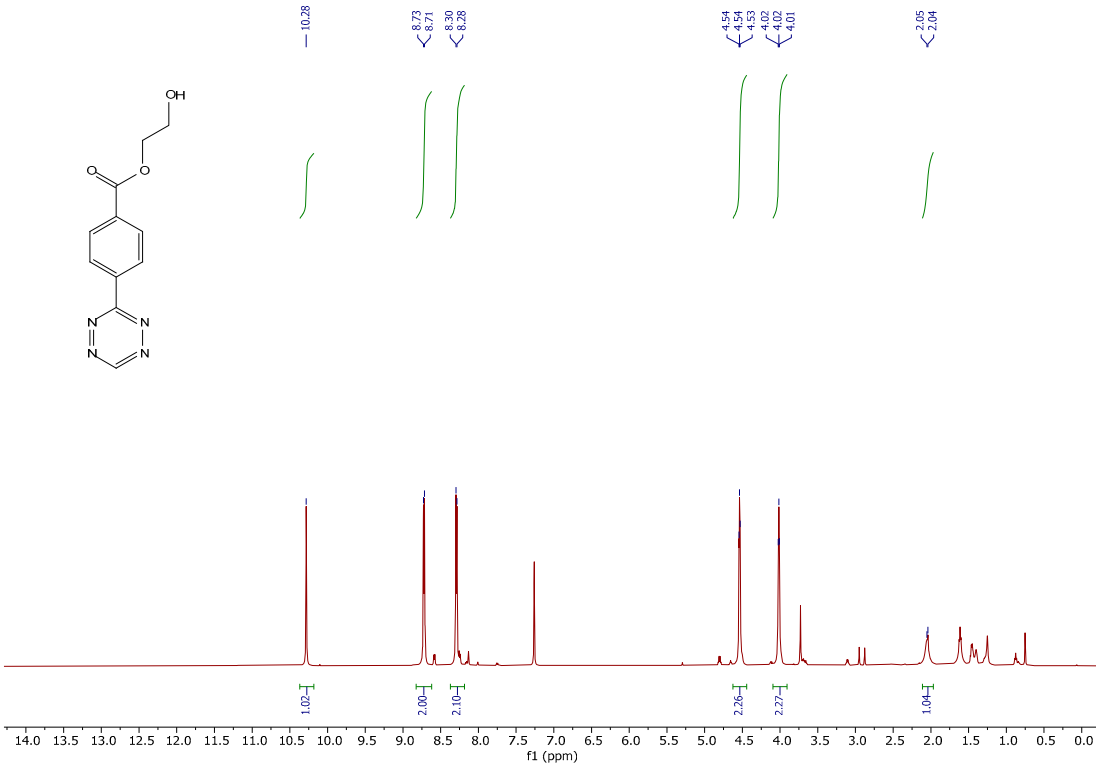
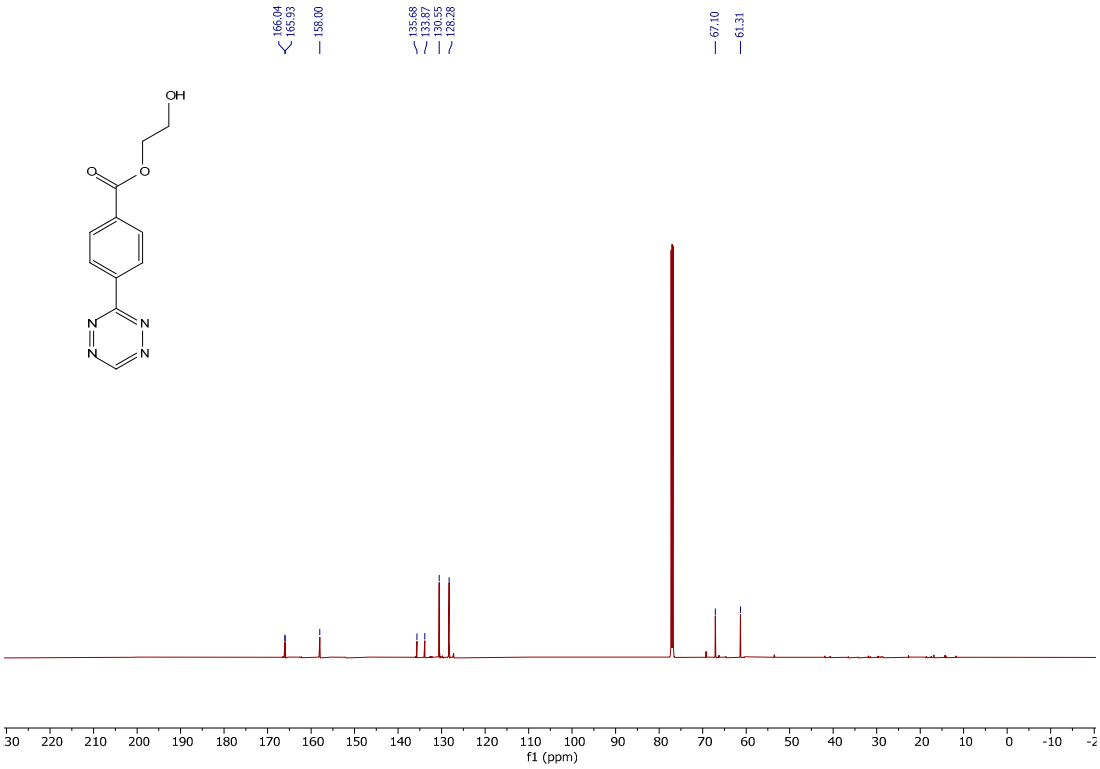
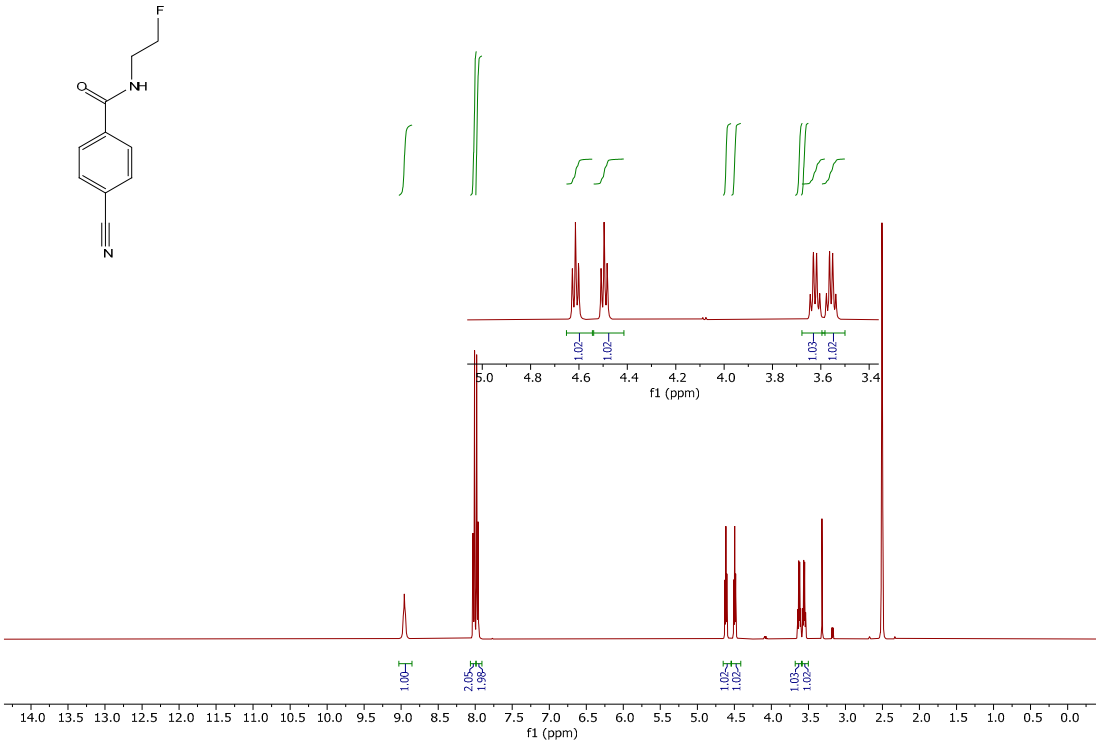
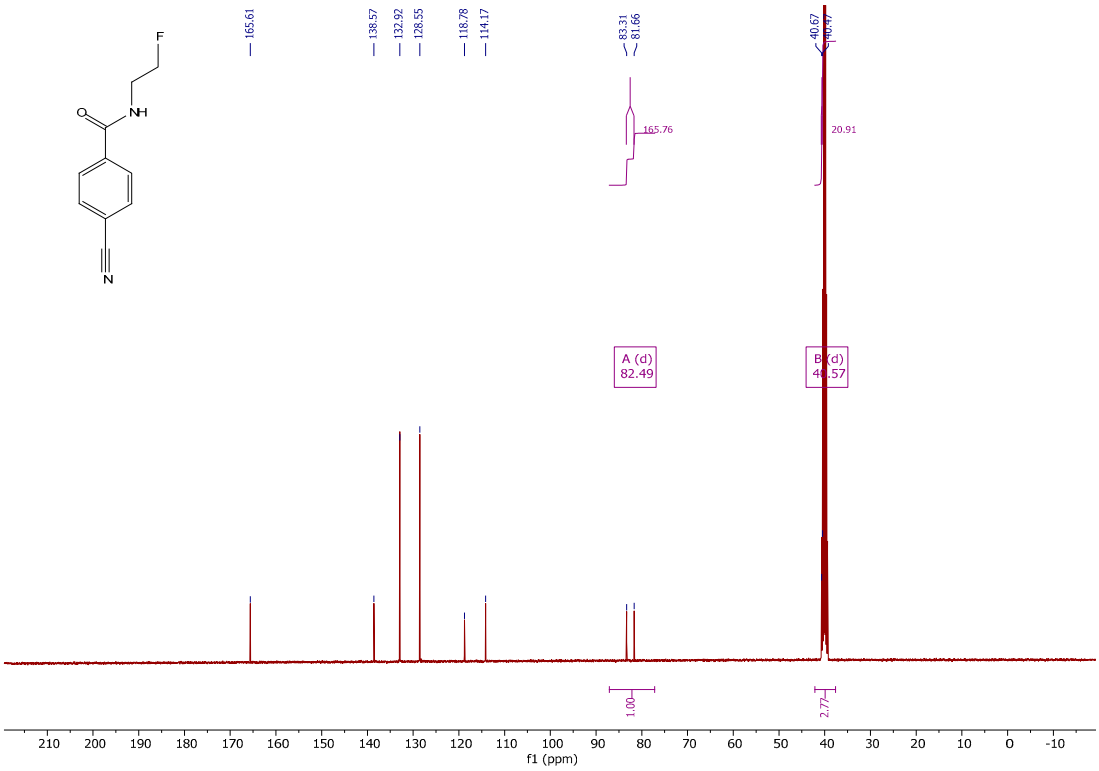




*4-Cyano-N-(2-fluoroethyl)benzamide (**12**)*





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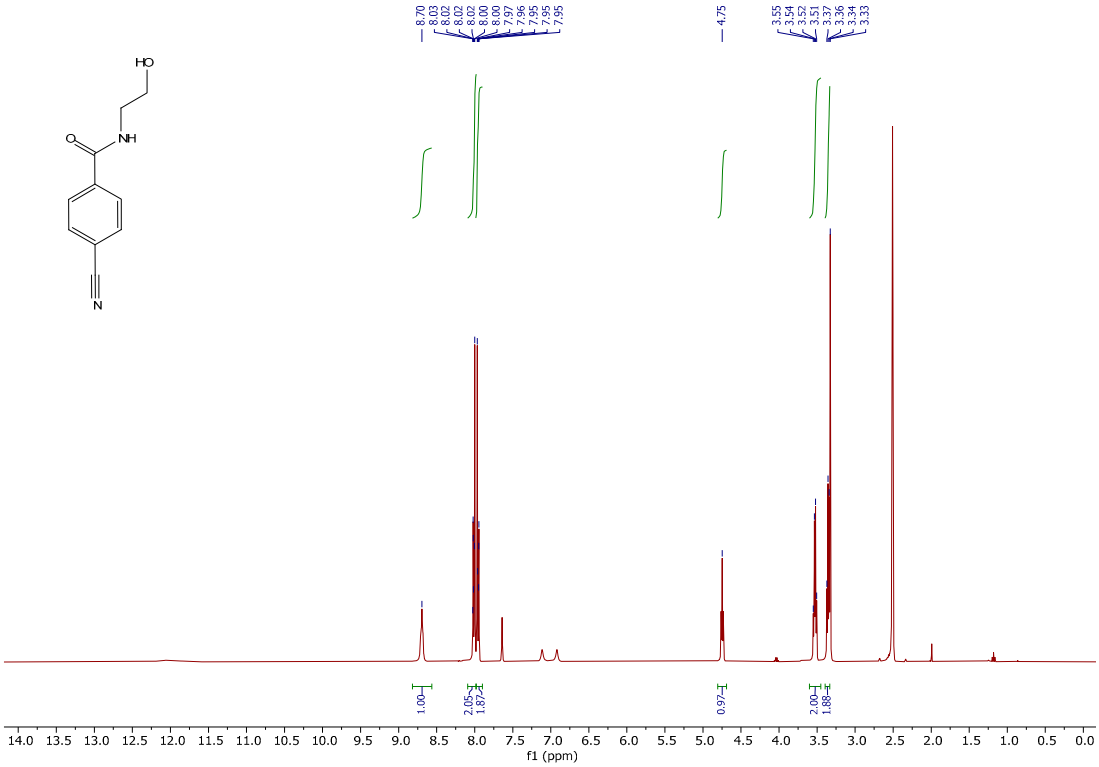
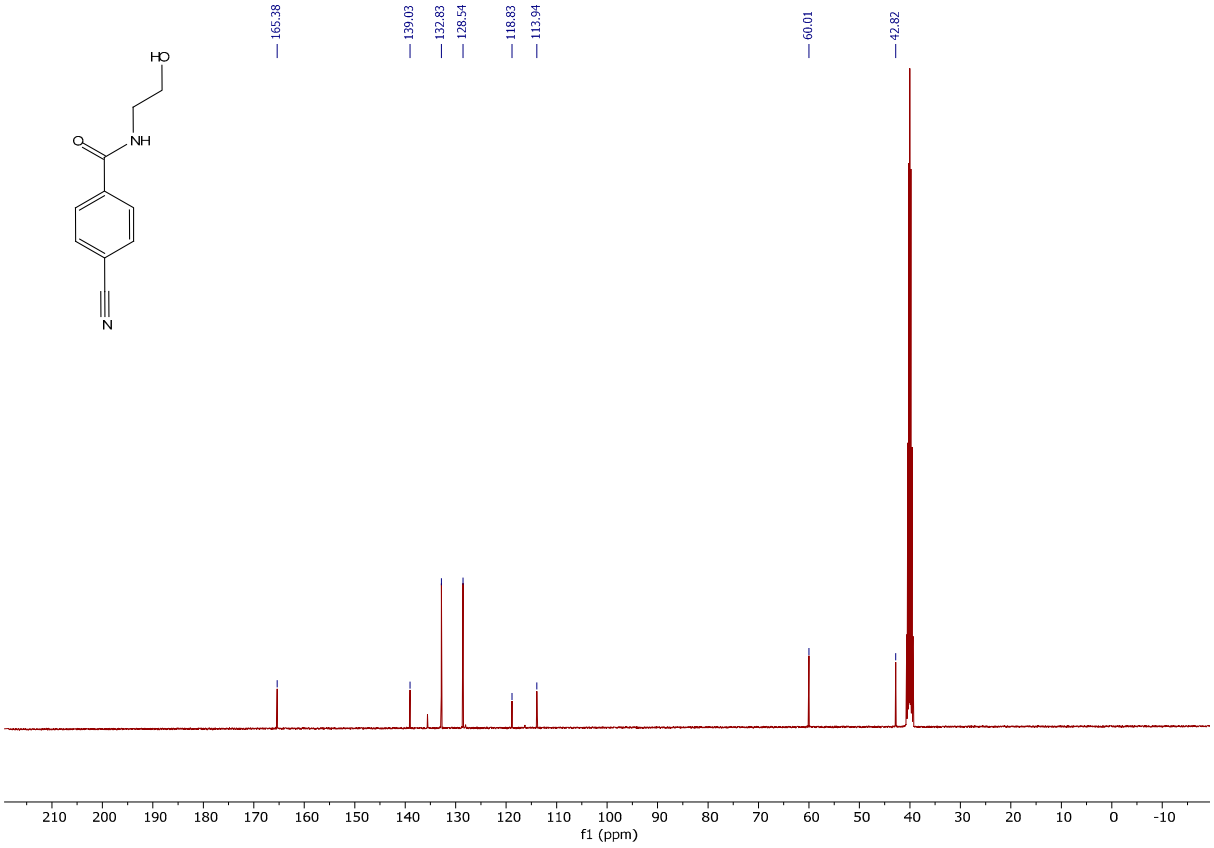
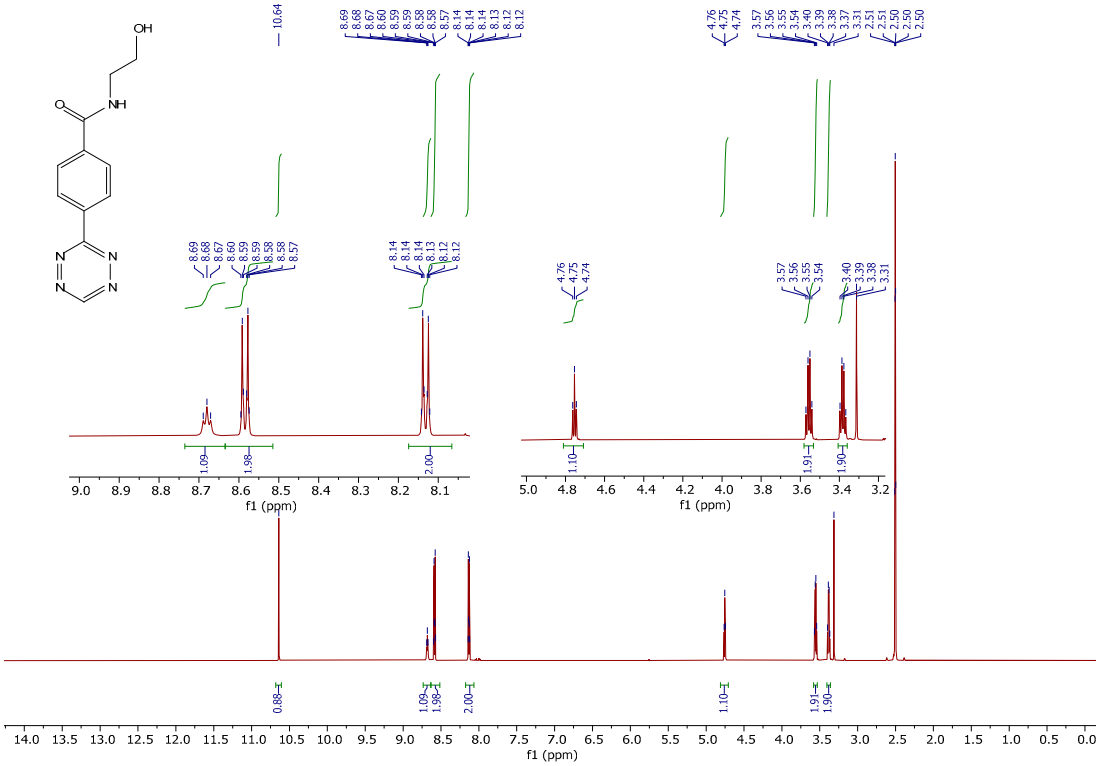
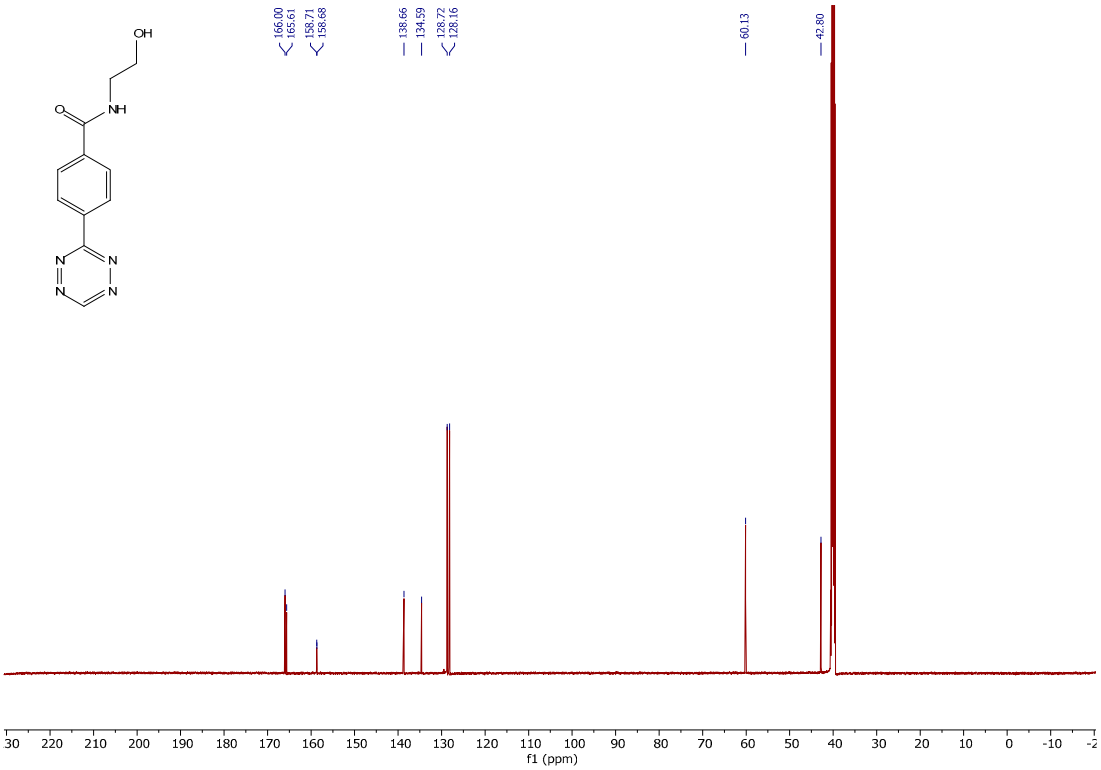


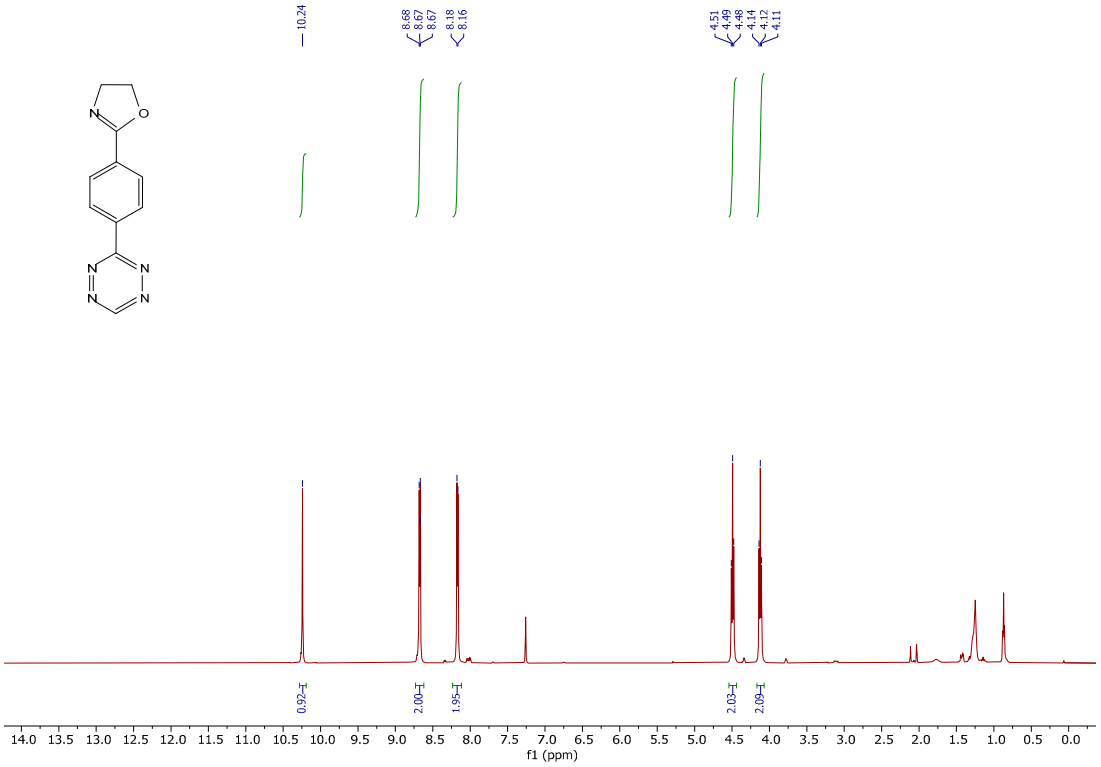


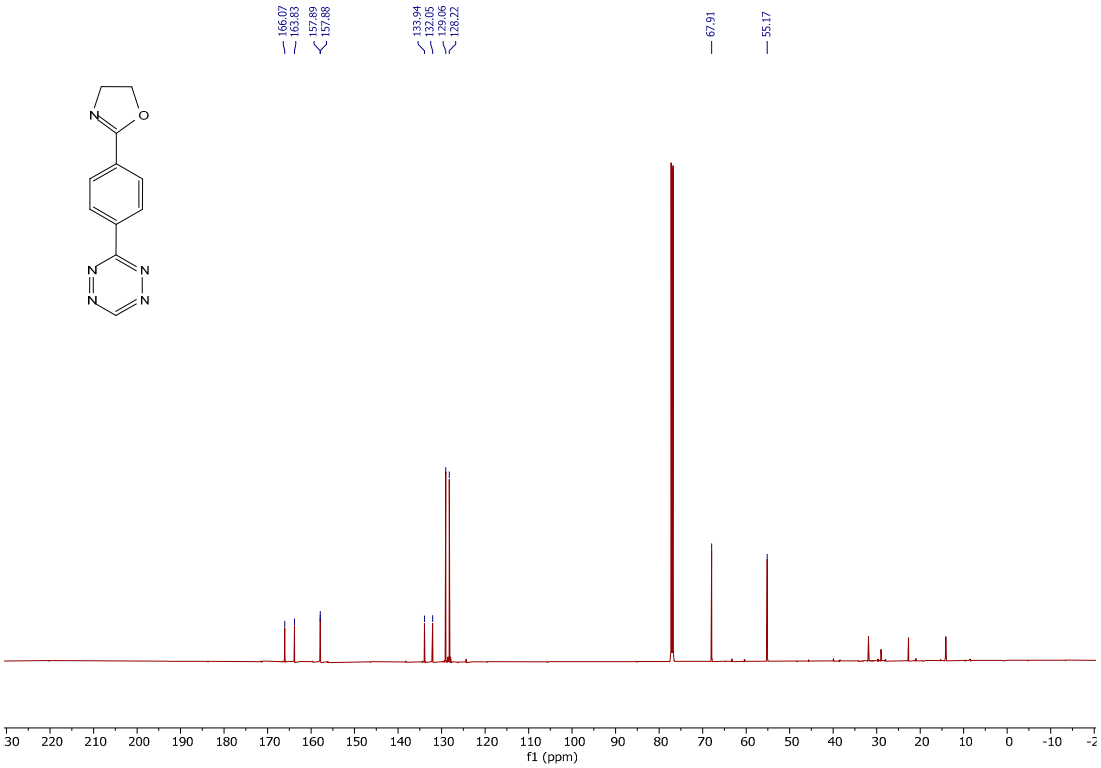
*N-(2-hydroxyethyl)-4-(1,2,4,5-tetrazin-3-yl)benzamide (**14**)*



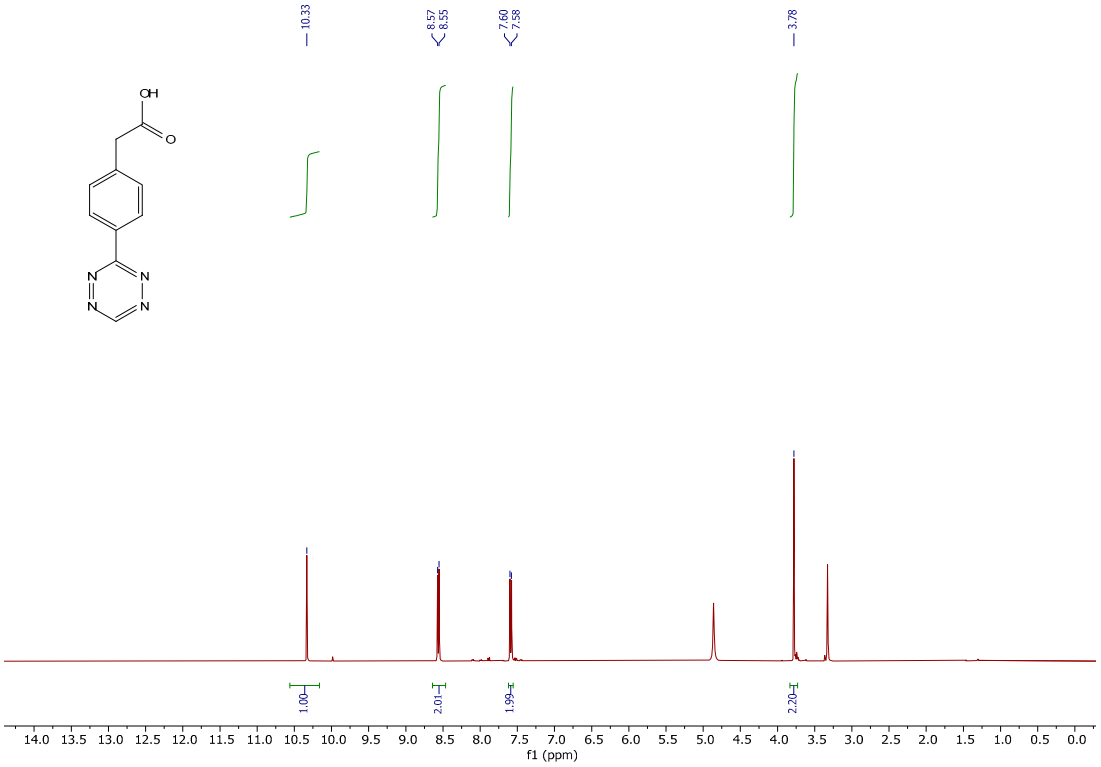


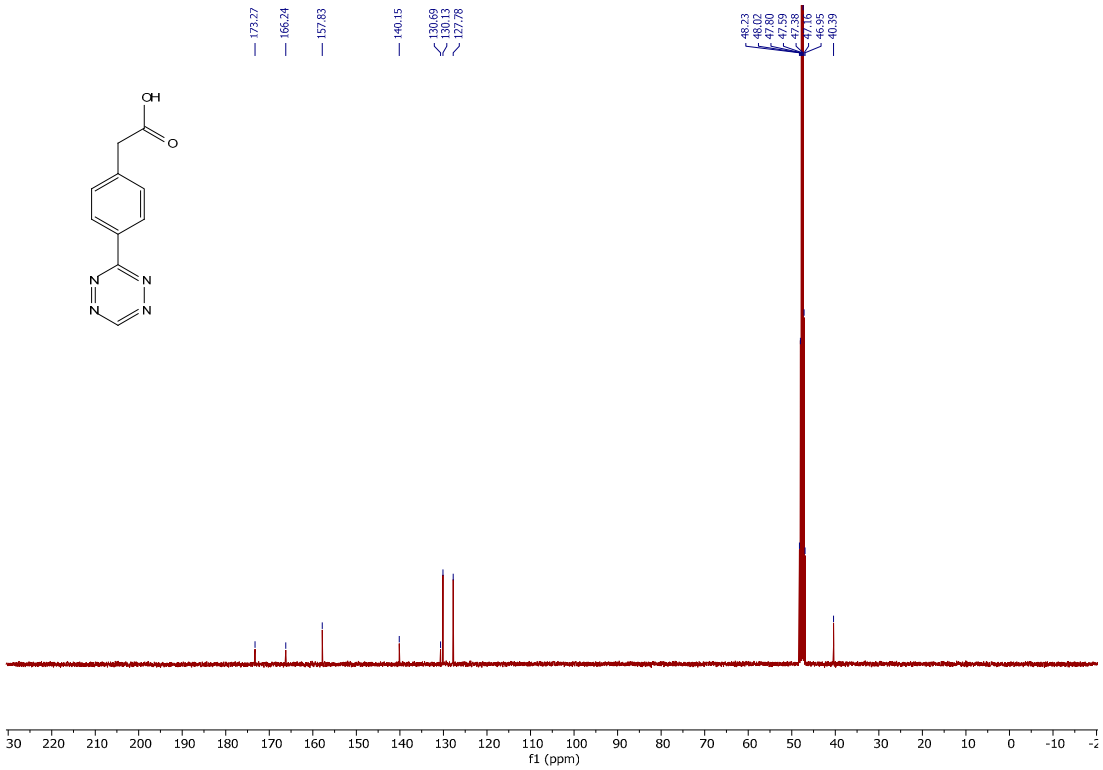
2-(4-(1,2,4,5-Tetrazin-3-yl)phenyl)-4,5-dihydrooxazole





*2-(4-(1,2,4,5-Tetrazin-3-yl)phenyl)acetic acid (**15**)*





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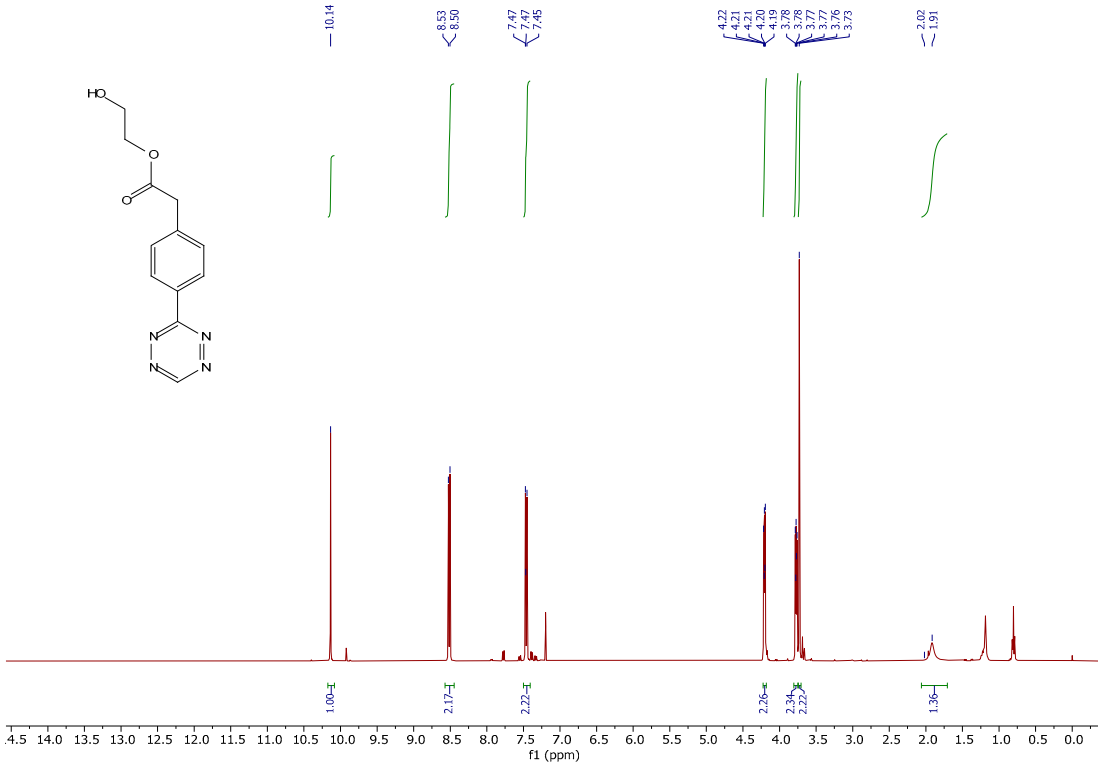
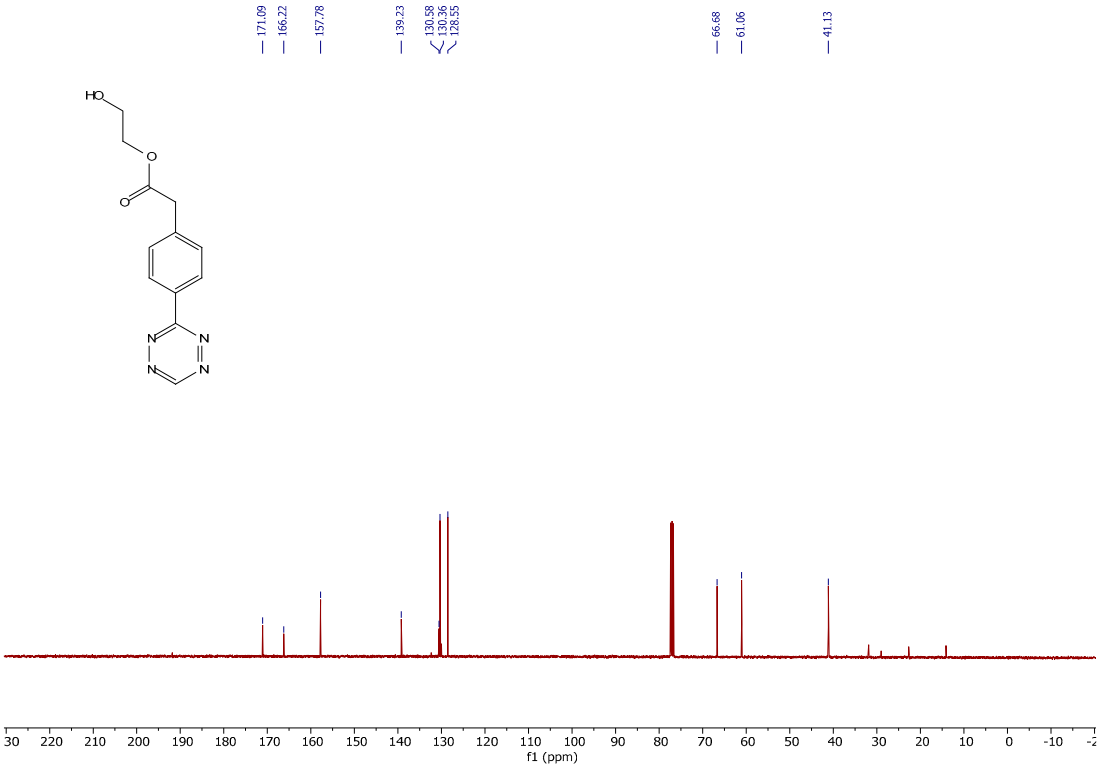
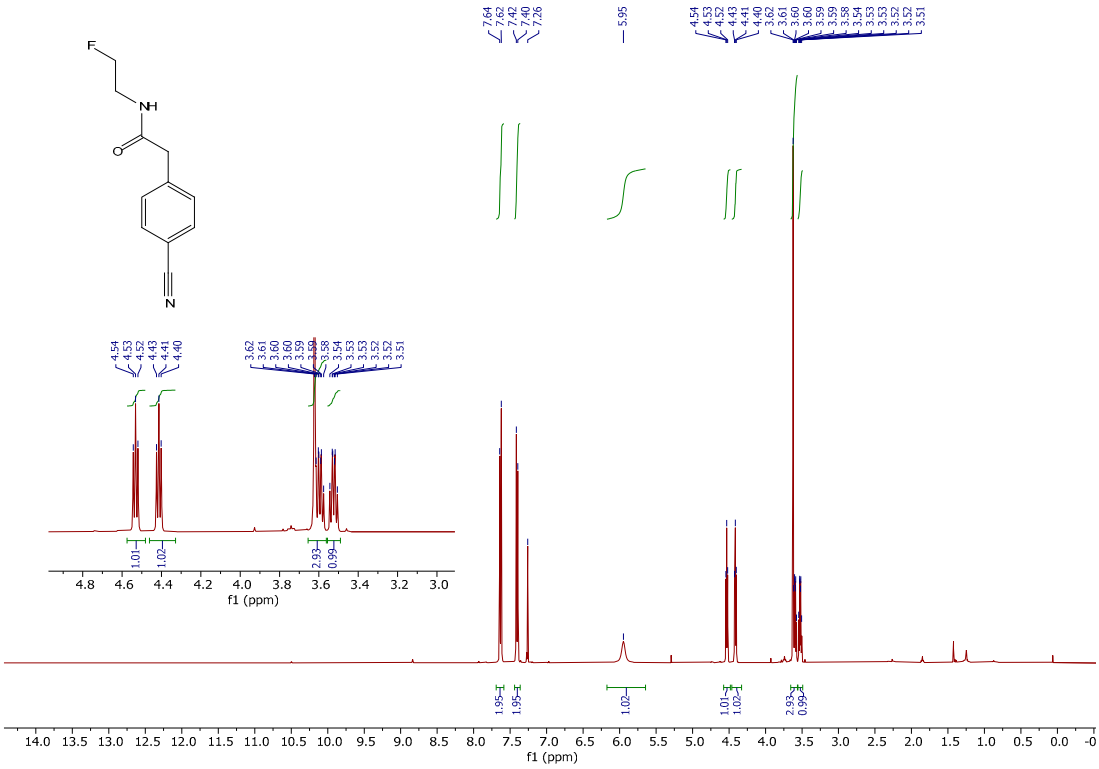
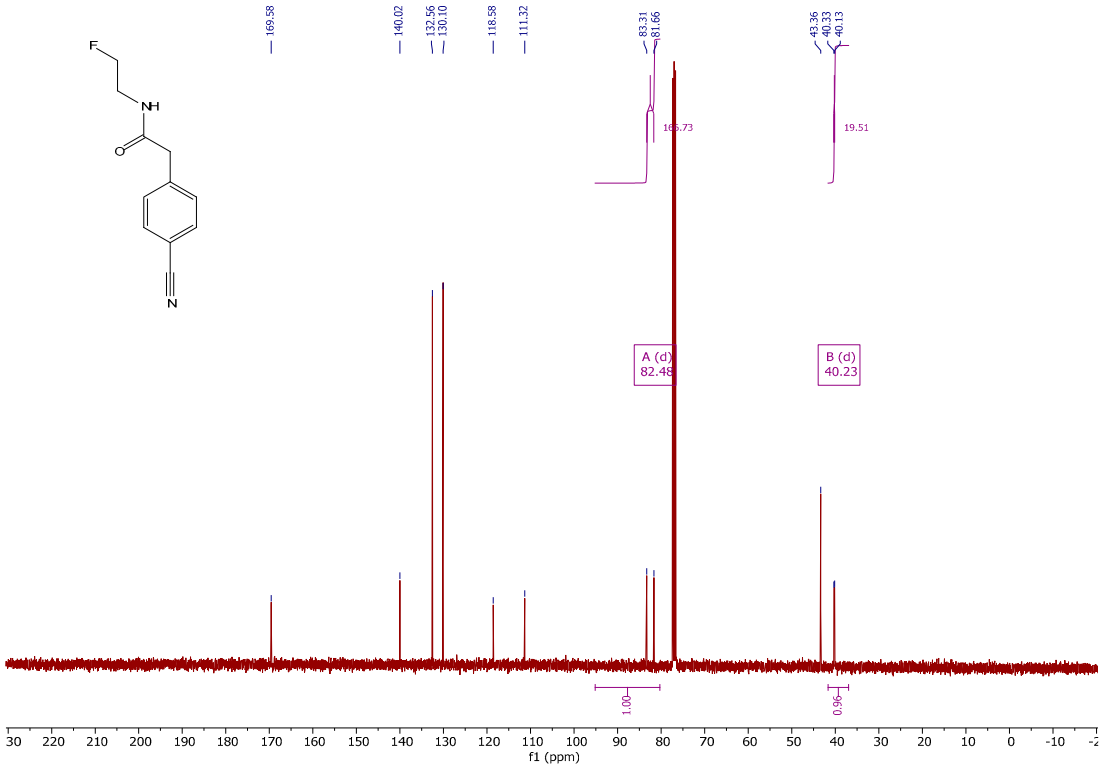


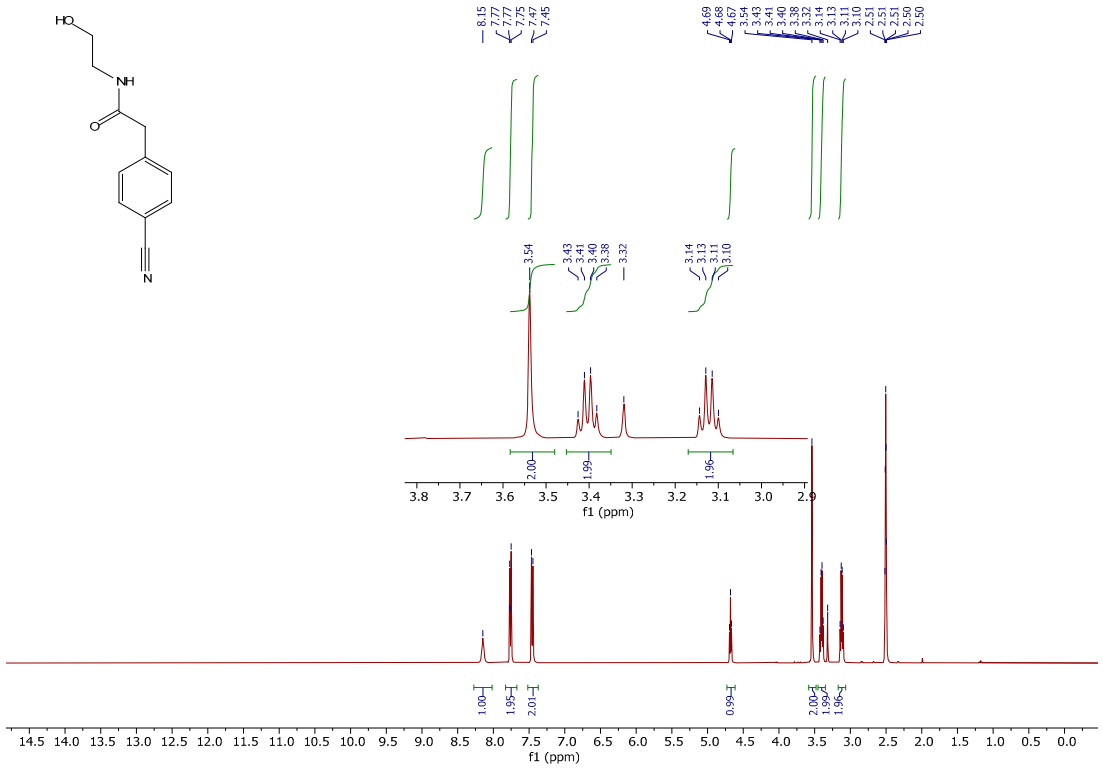


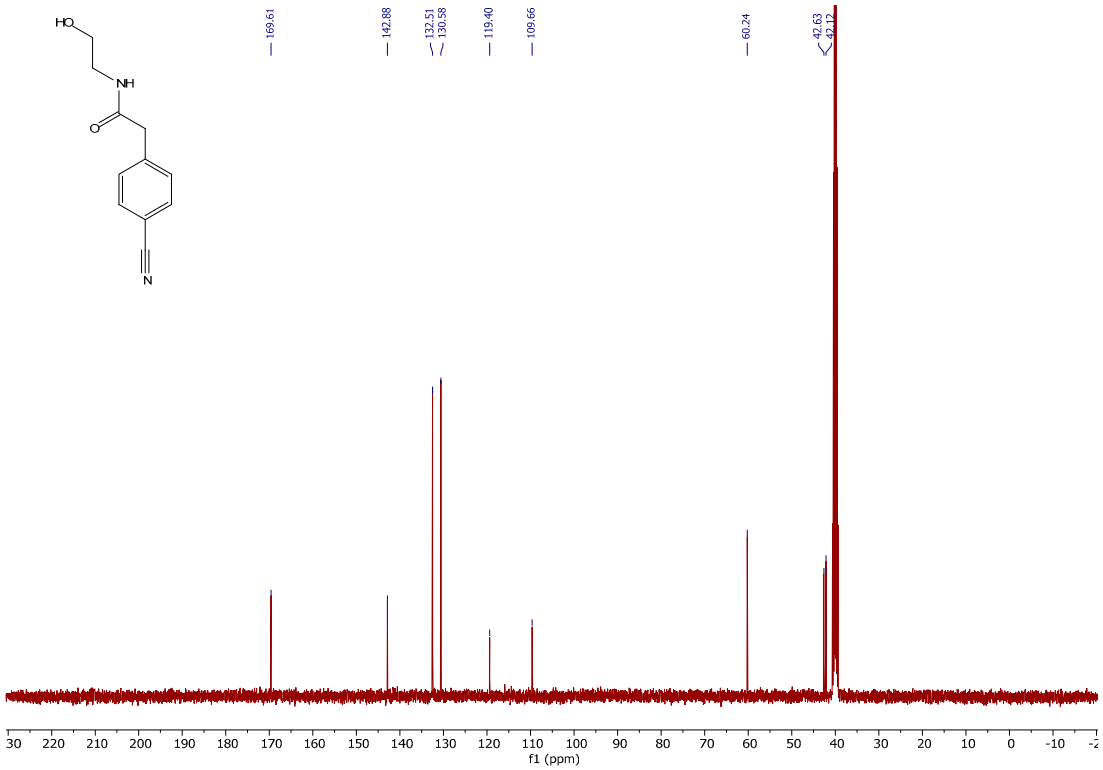
*2-(4-Cyanophenyl)-N-(2-fluoroethyl)acetamide (**17**)*



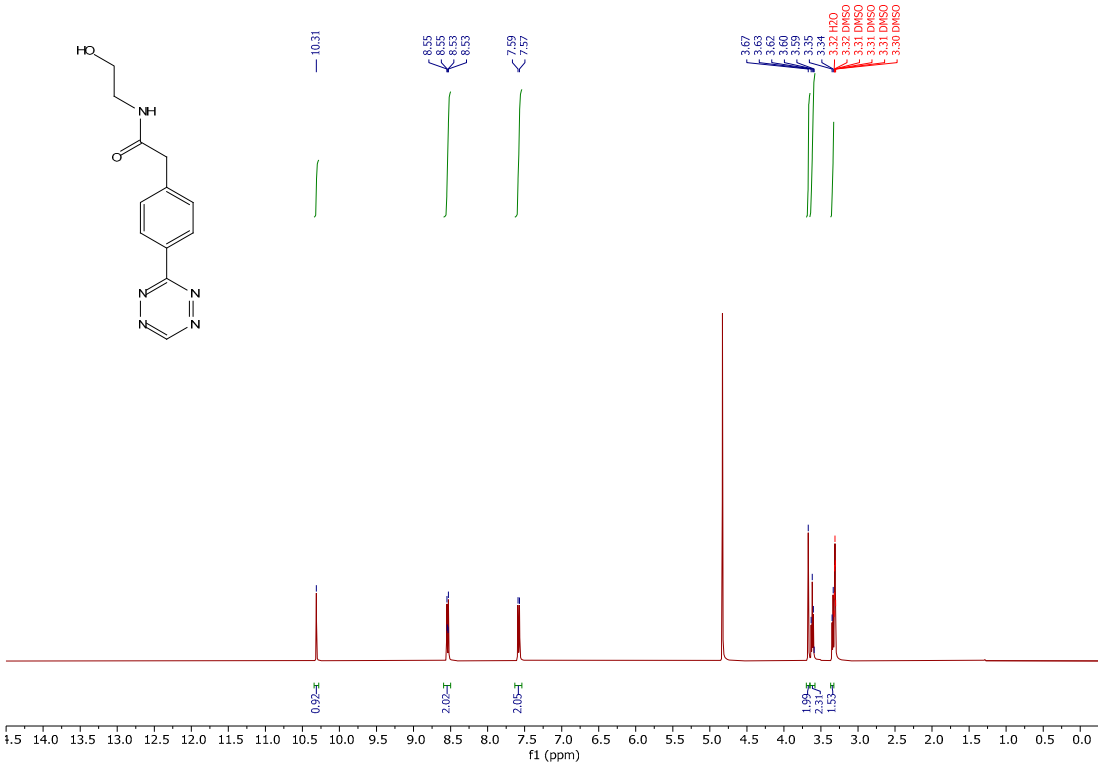


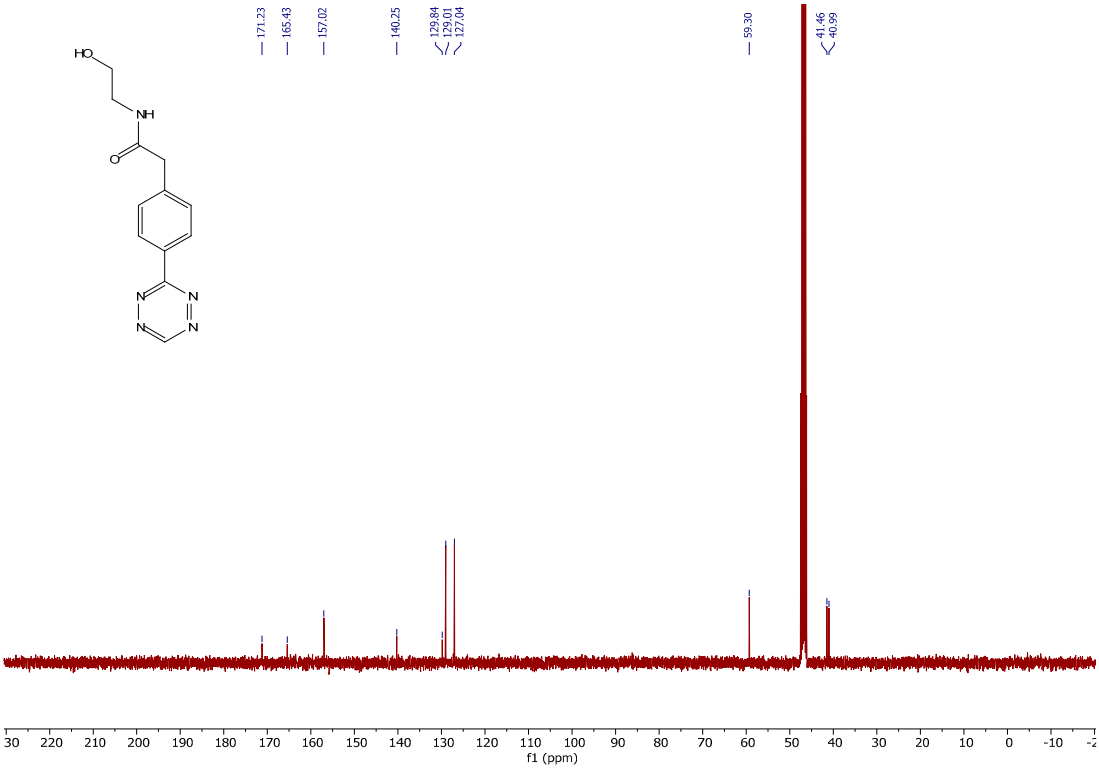
*2-(4-Cyanophenyl)-N-(2-hydroxyethyl)acetamide (**18**)*



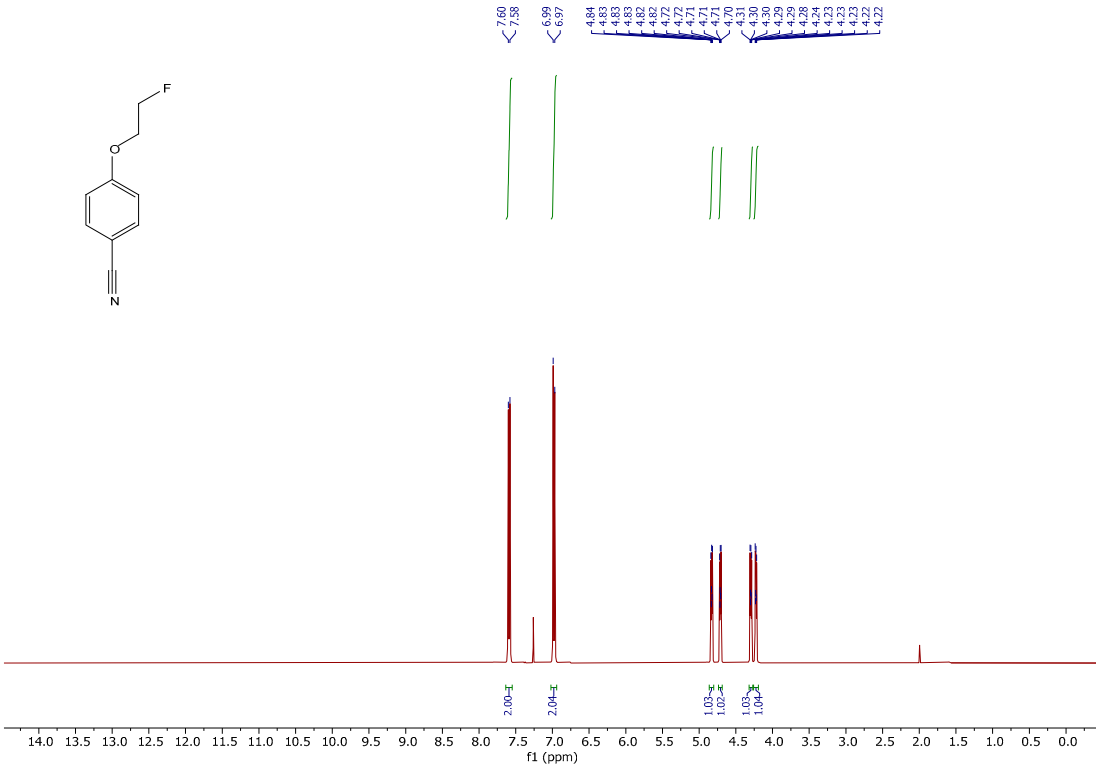


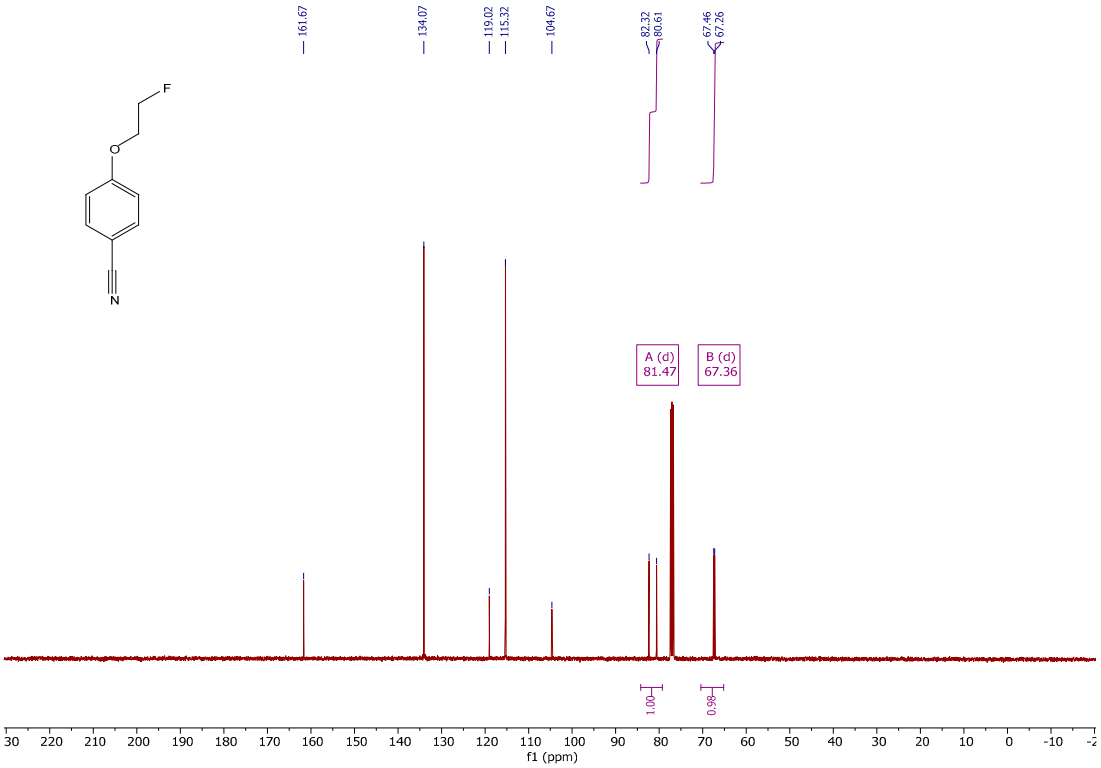
*2-(4-(1,2,4,5-Tetrazin-3-yl)phenyl)-N-(2-hydroxyethyl)acetamide (**19**)*



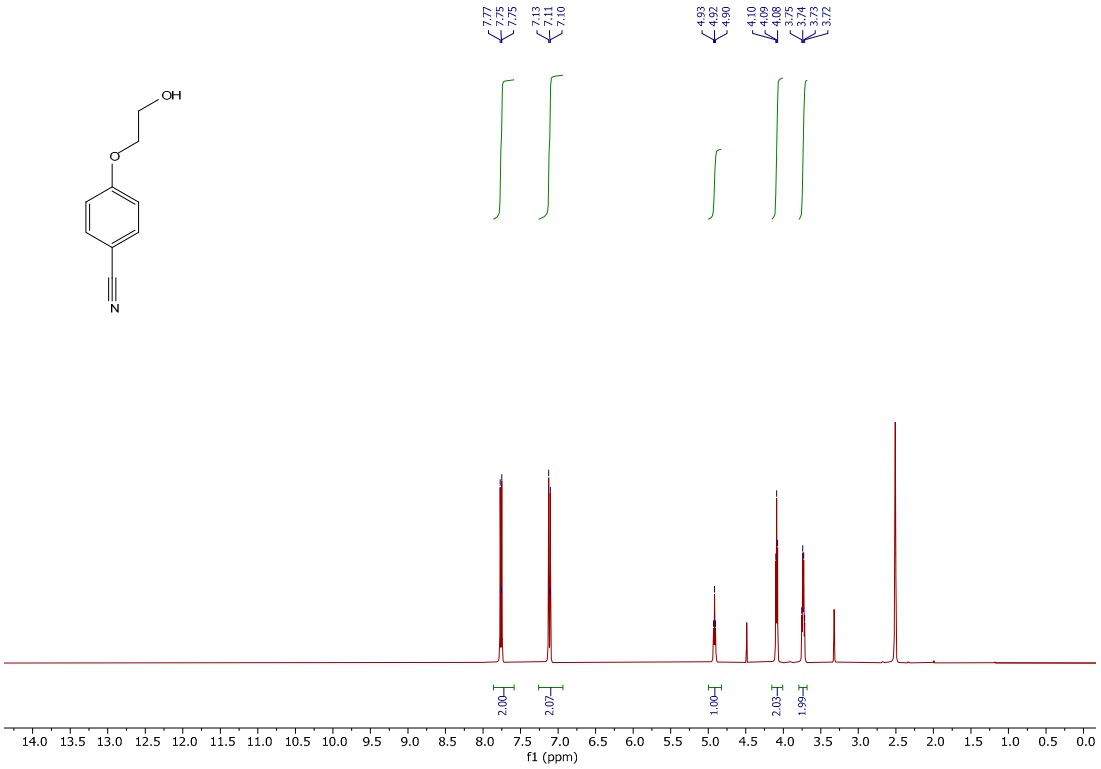


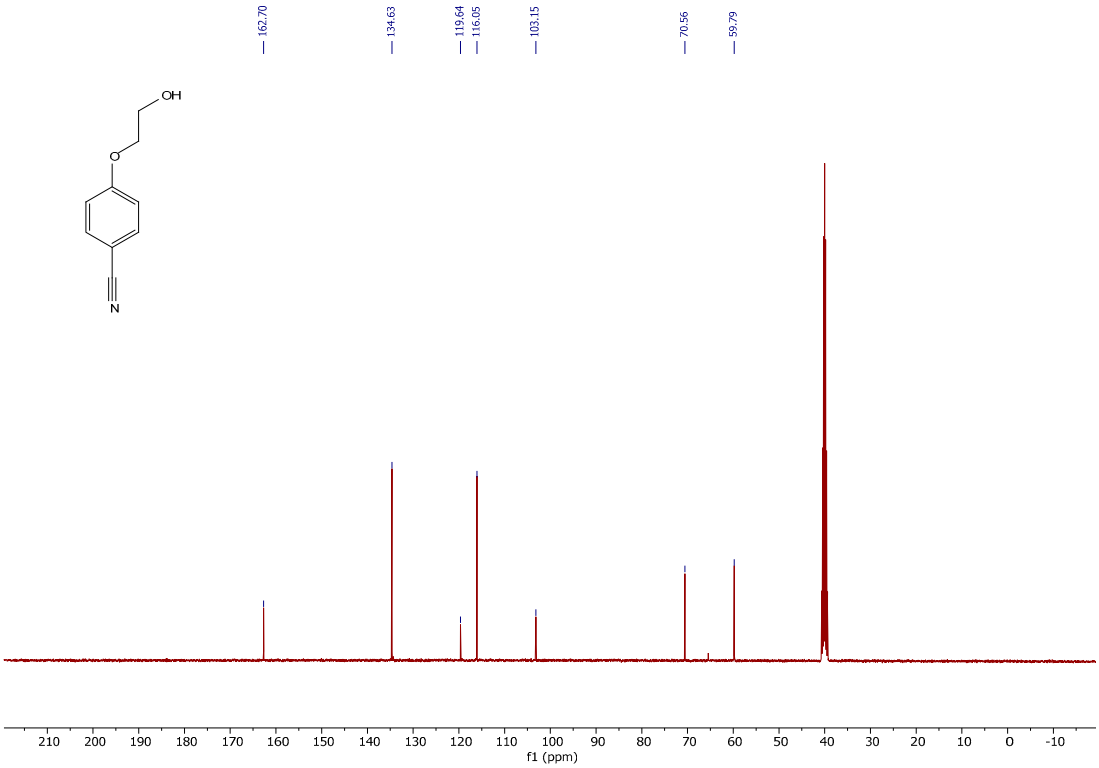
*4-(2-Fluoroethoxy)benzonitrile (**20**)*



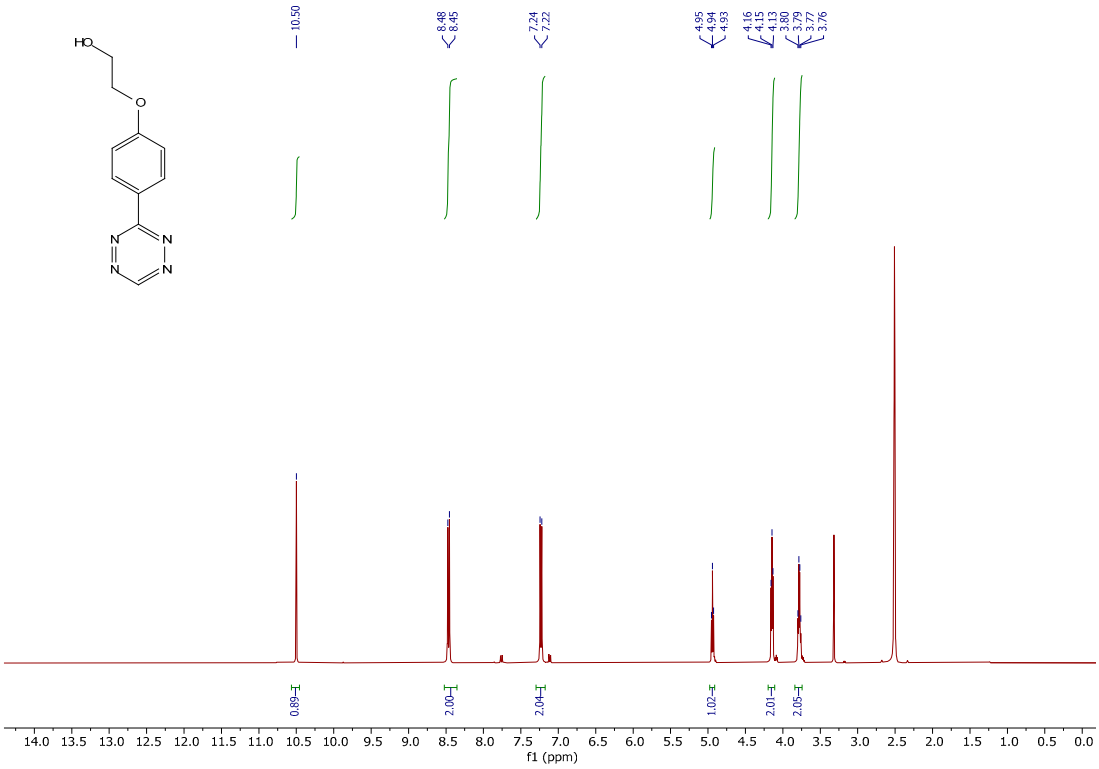


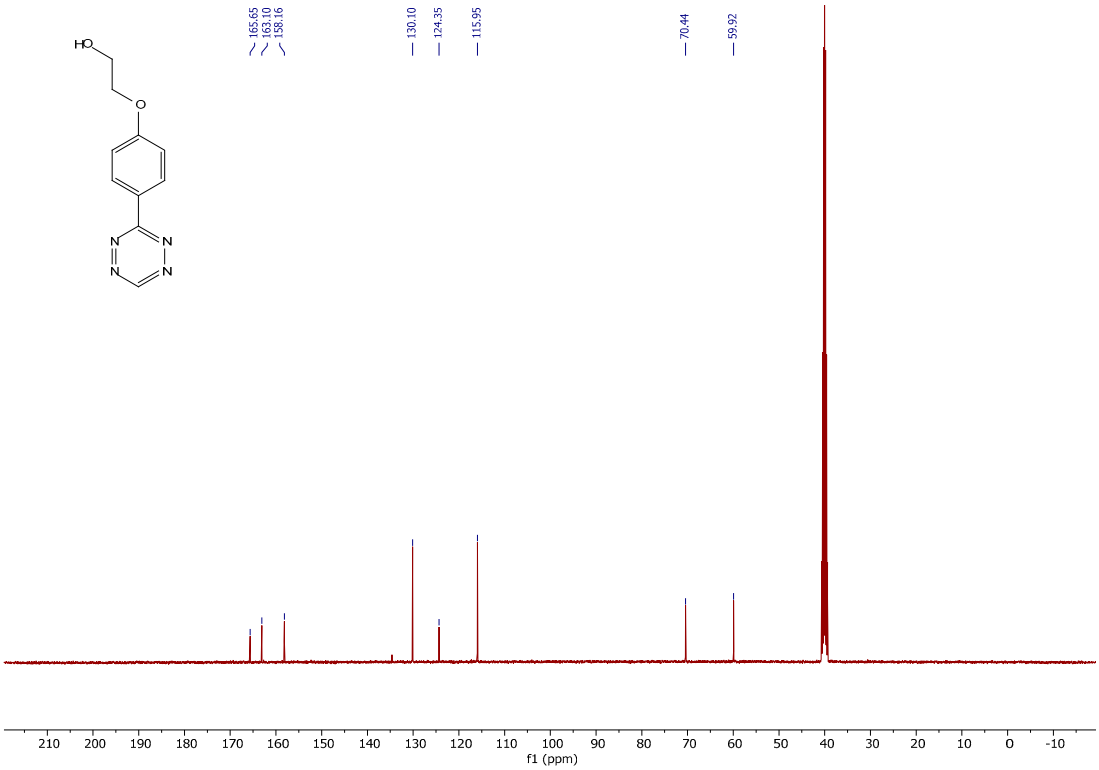
*4-(2-Hydroxyethoxy)benzonitrile (**21**)*



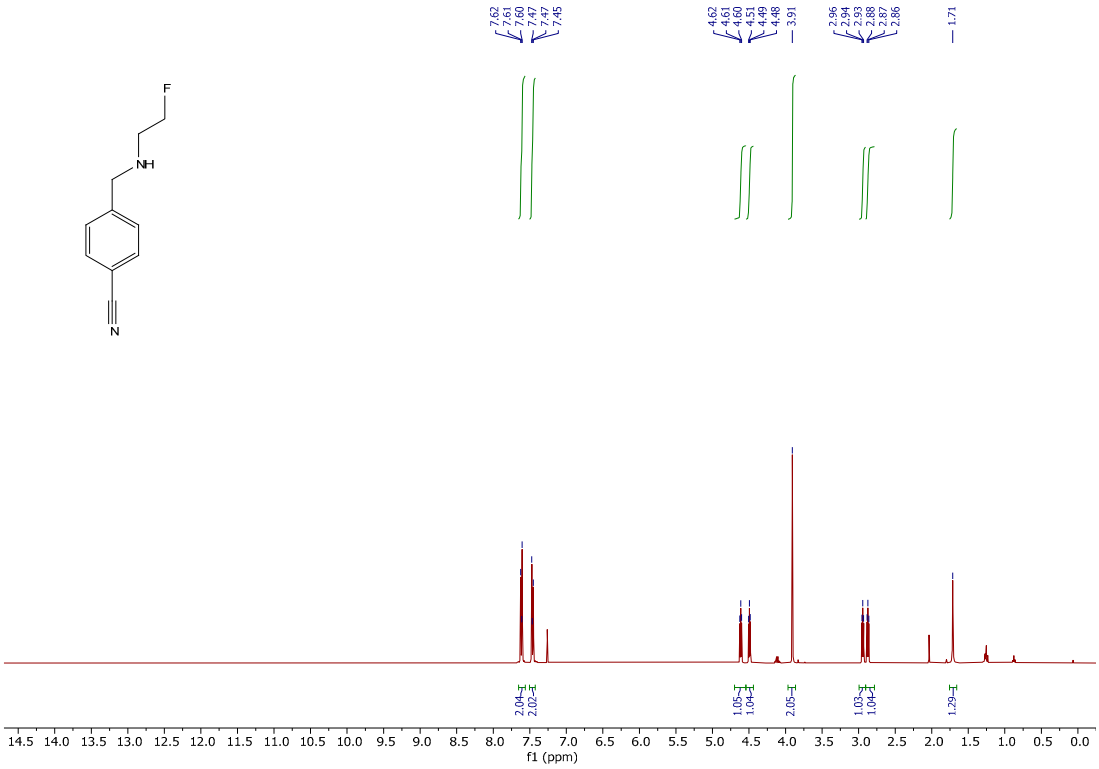


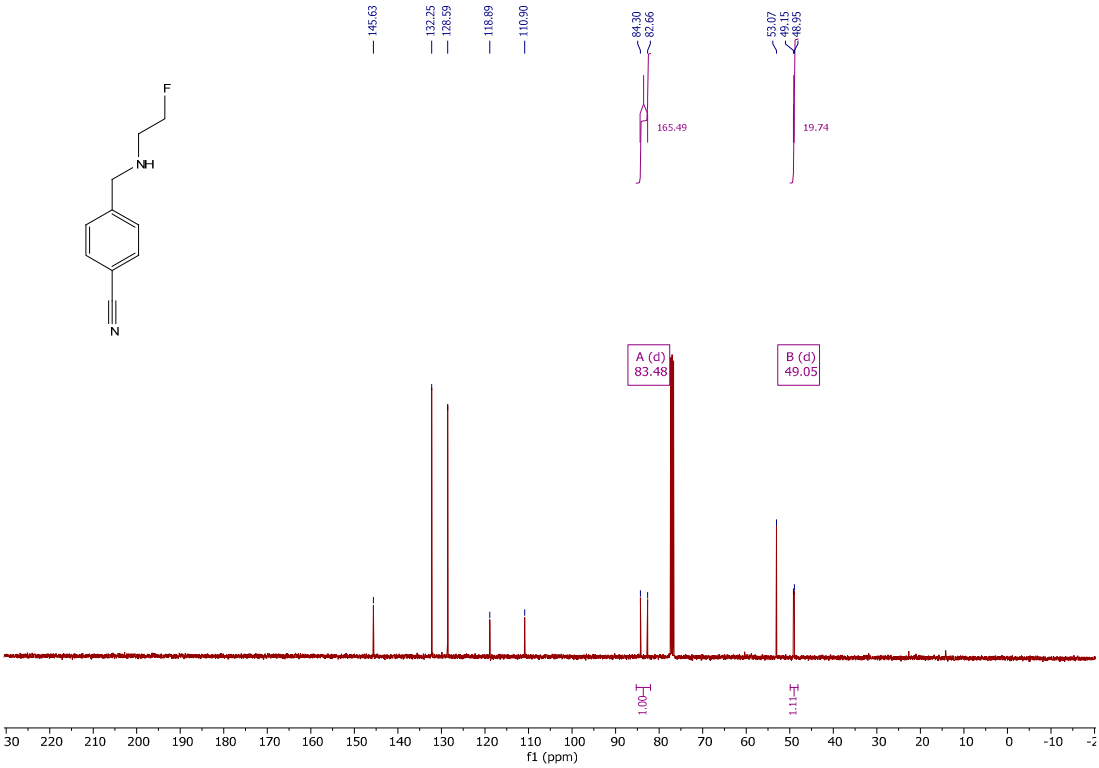
*2-(4-(1,2,4,5-Tetrazin-3-yl)phenoxy)ethan-1-ol (**22**)*



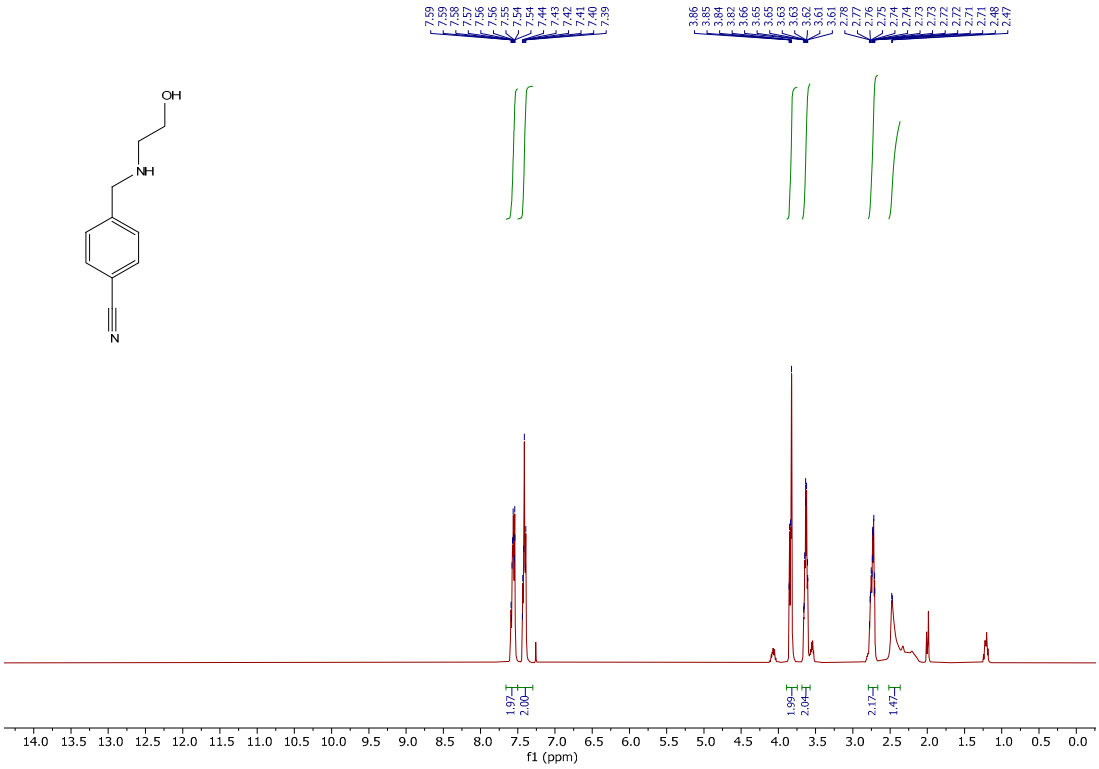


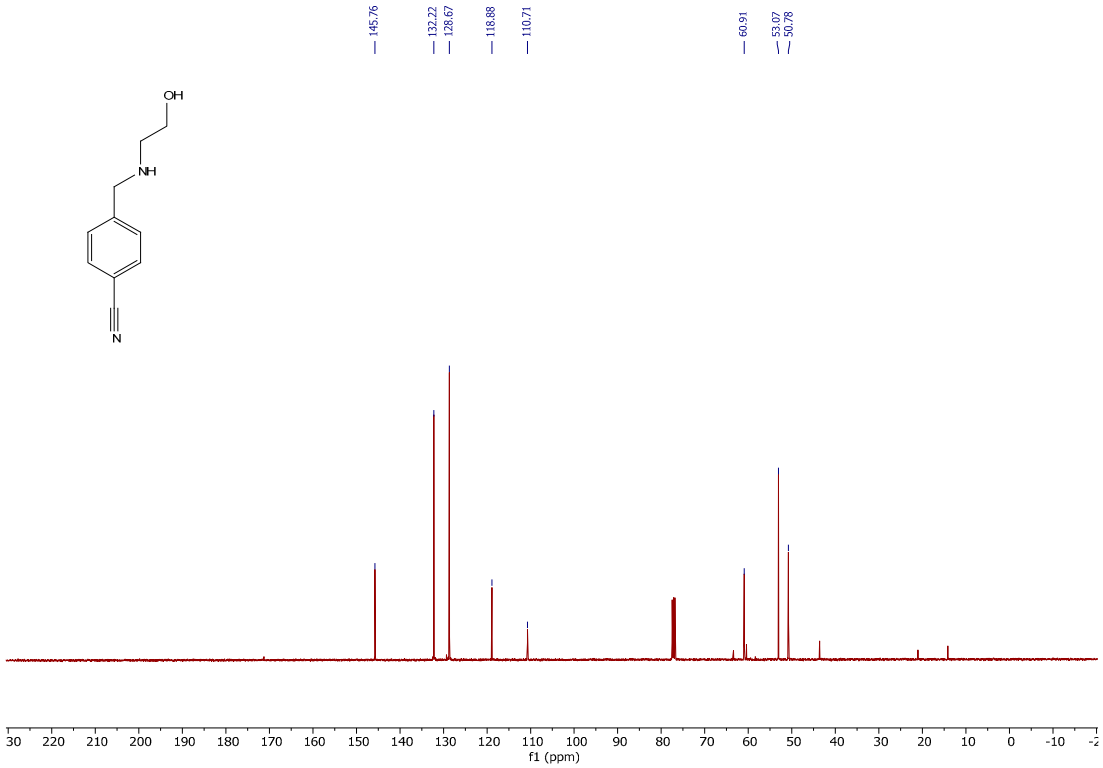
*4-(((2-Fluoroethyl)amino)methyl)benzonitrile (**23**)*



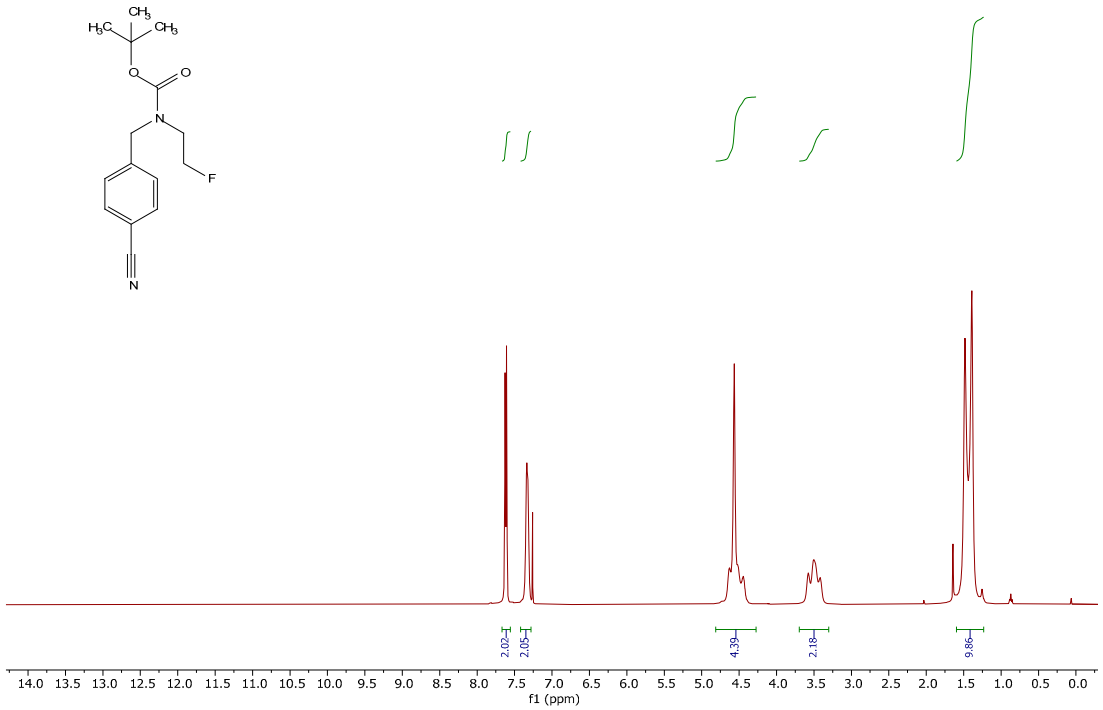


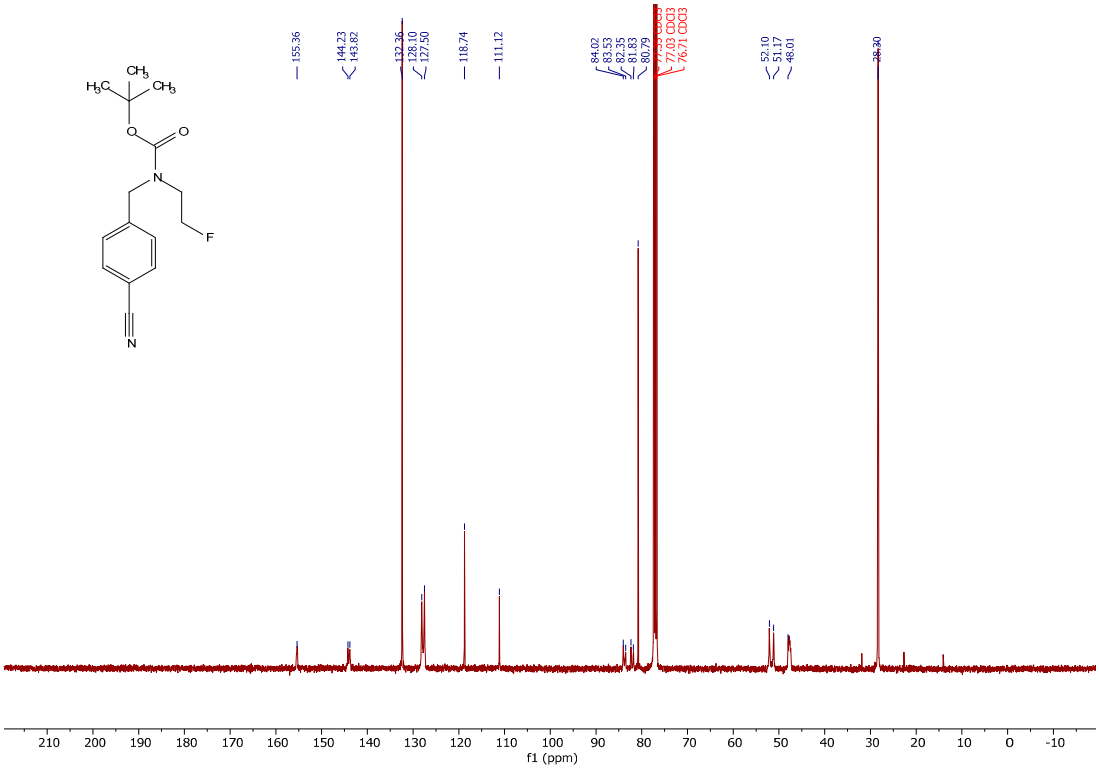
*4-(((2-Hydroxyethyl)amino)methyl)benzonitrile (**24**)*



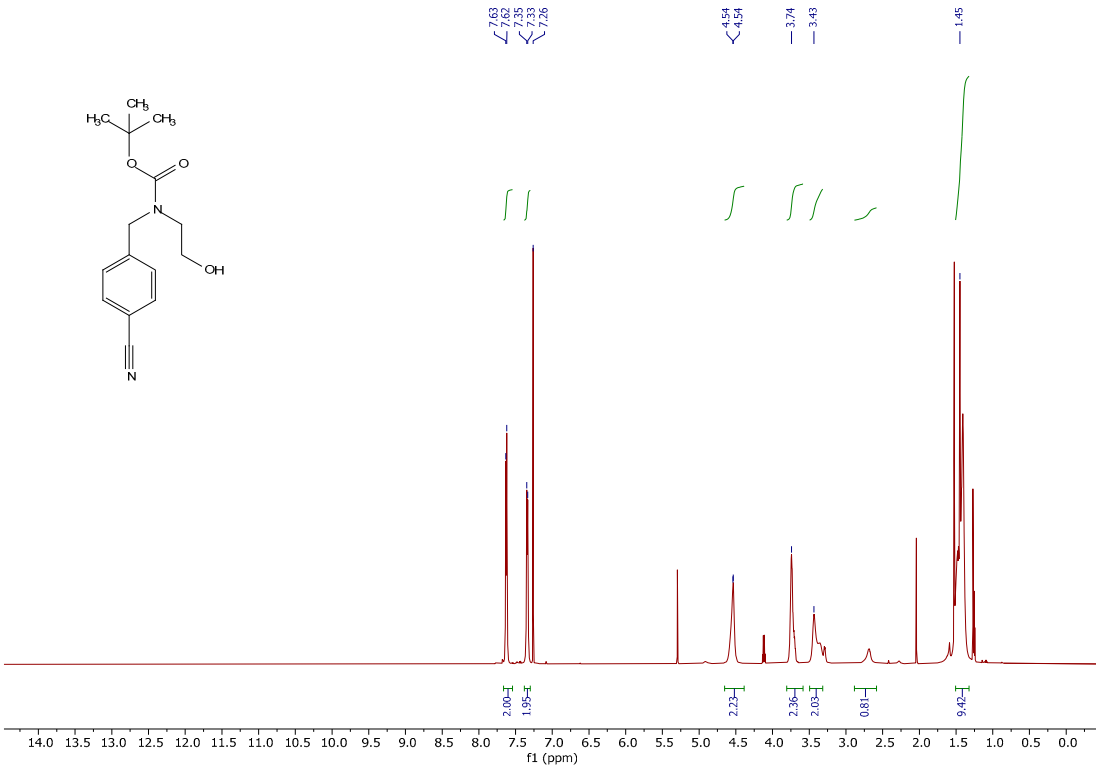


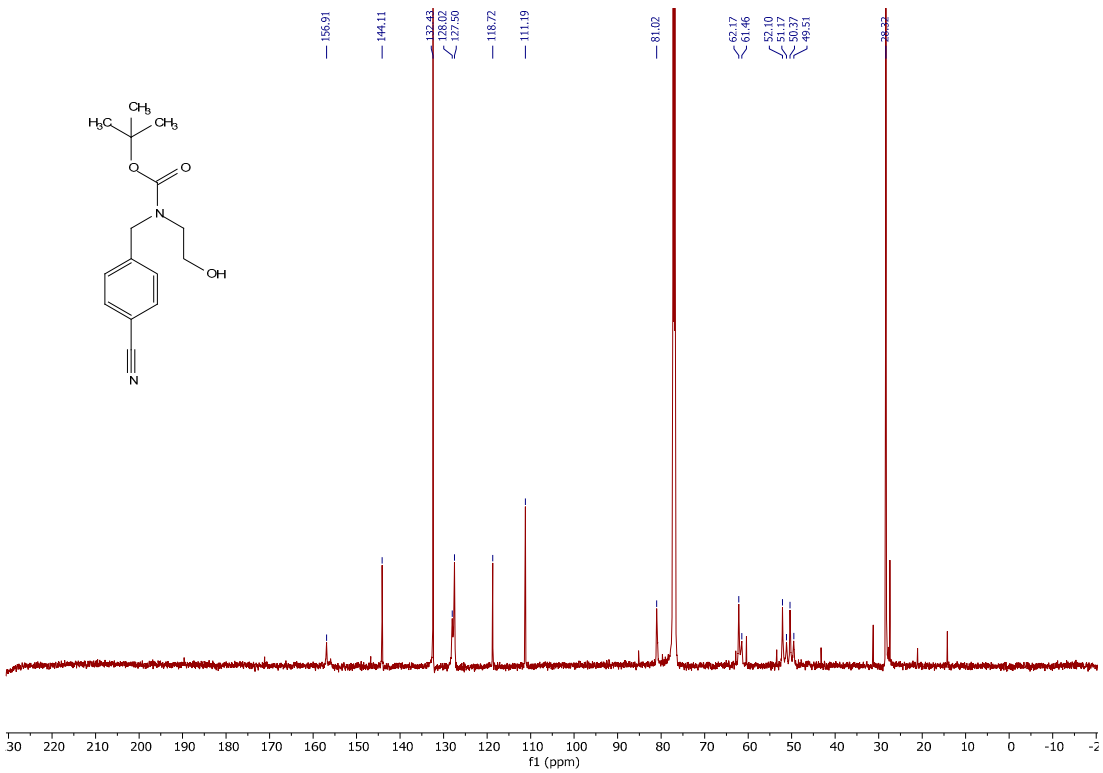
*Tert-butyl 4-cyanobenzyl(2-fluoroethyl)carbamate (**25**)*



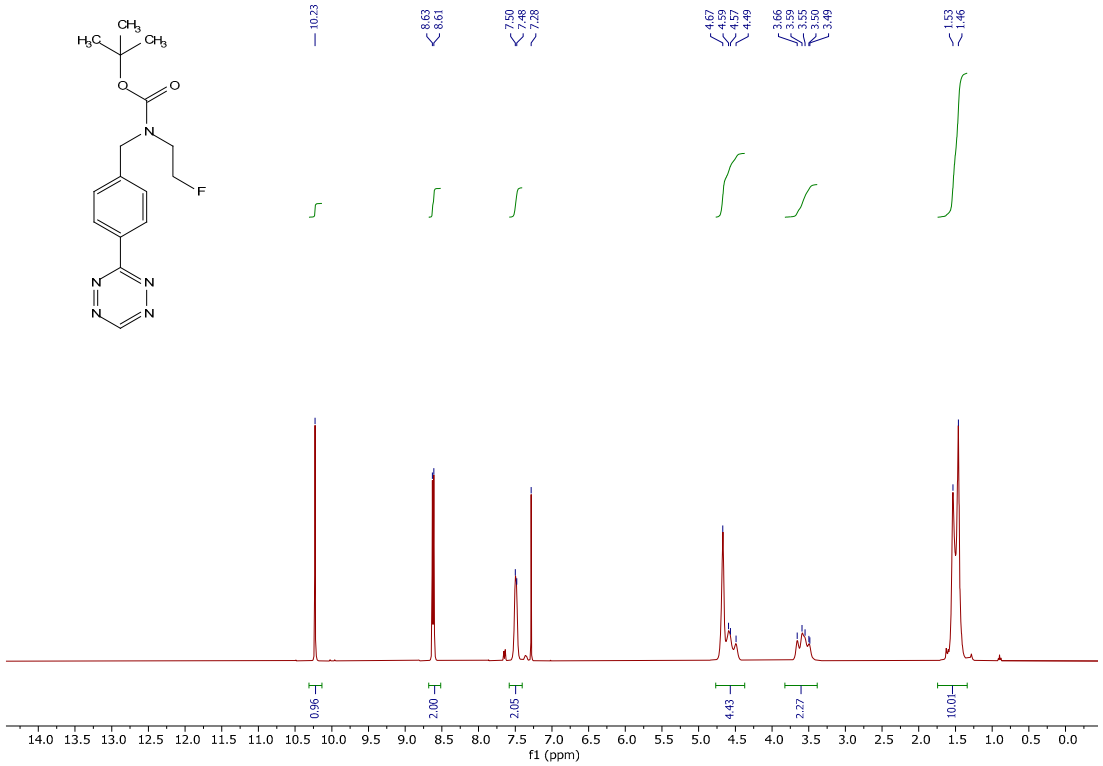


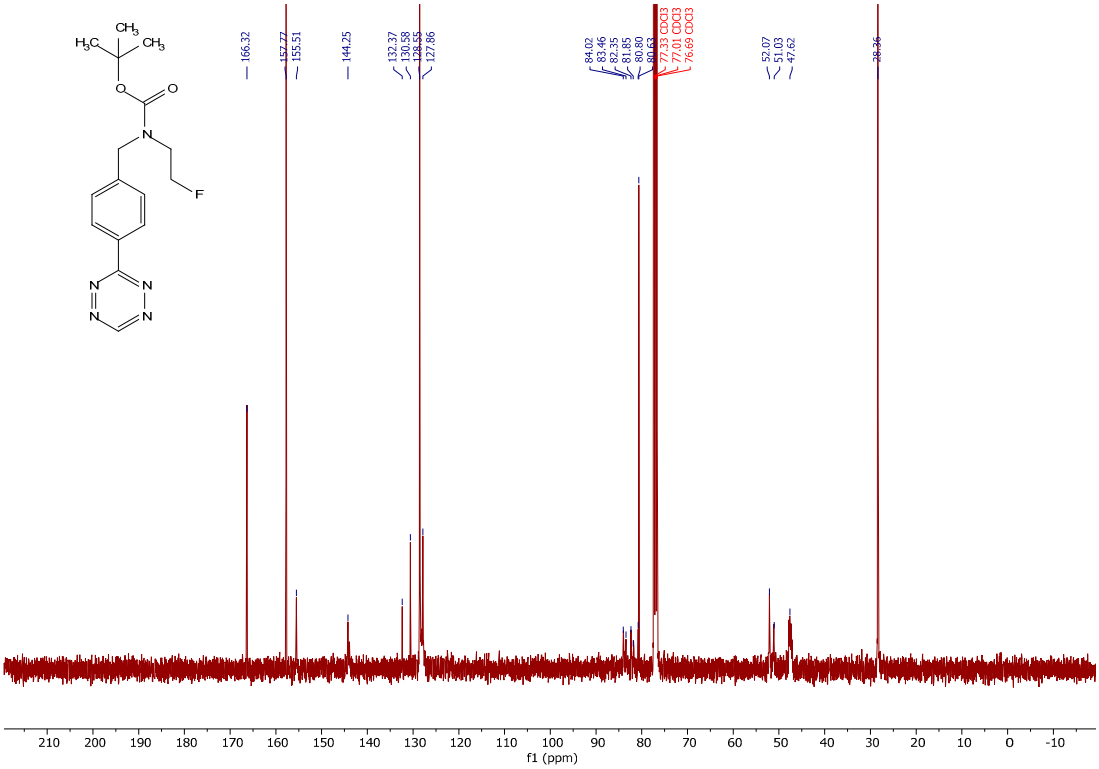
*Tert-butyl (4-cyanobenzyl)(2-hydroxyethyl)carbamate (**26**)*



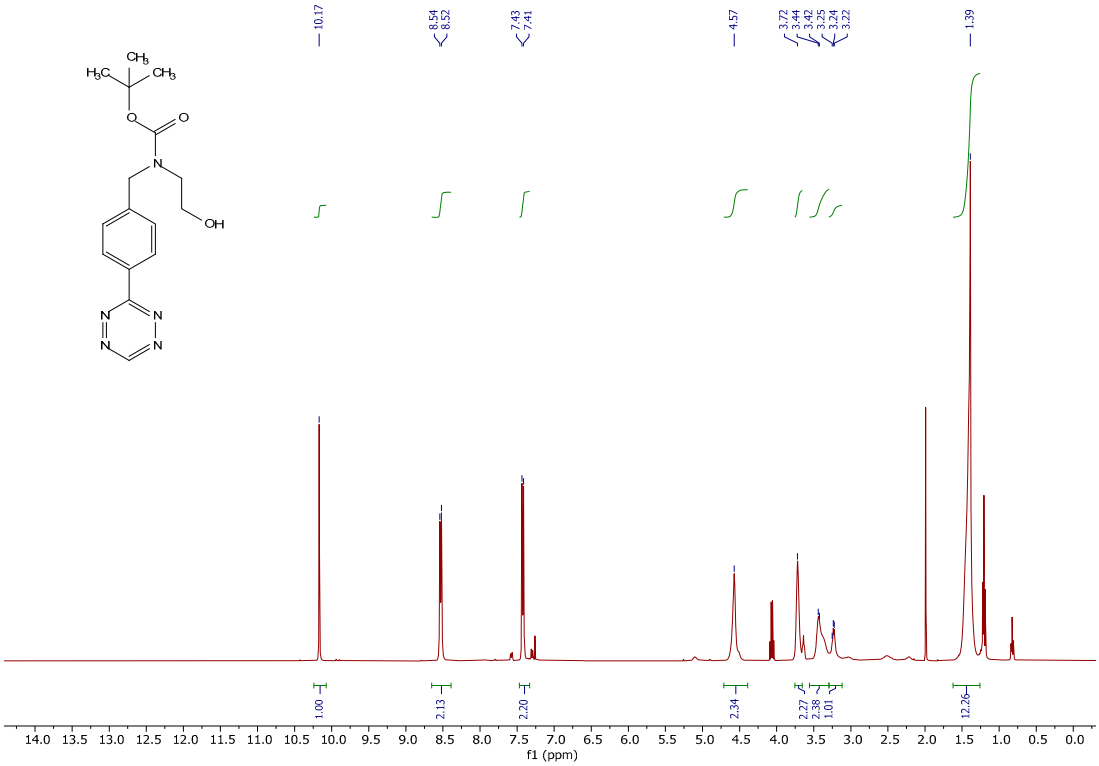


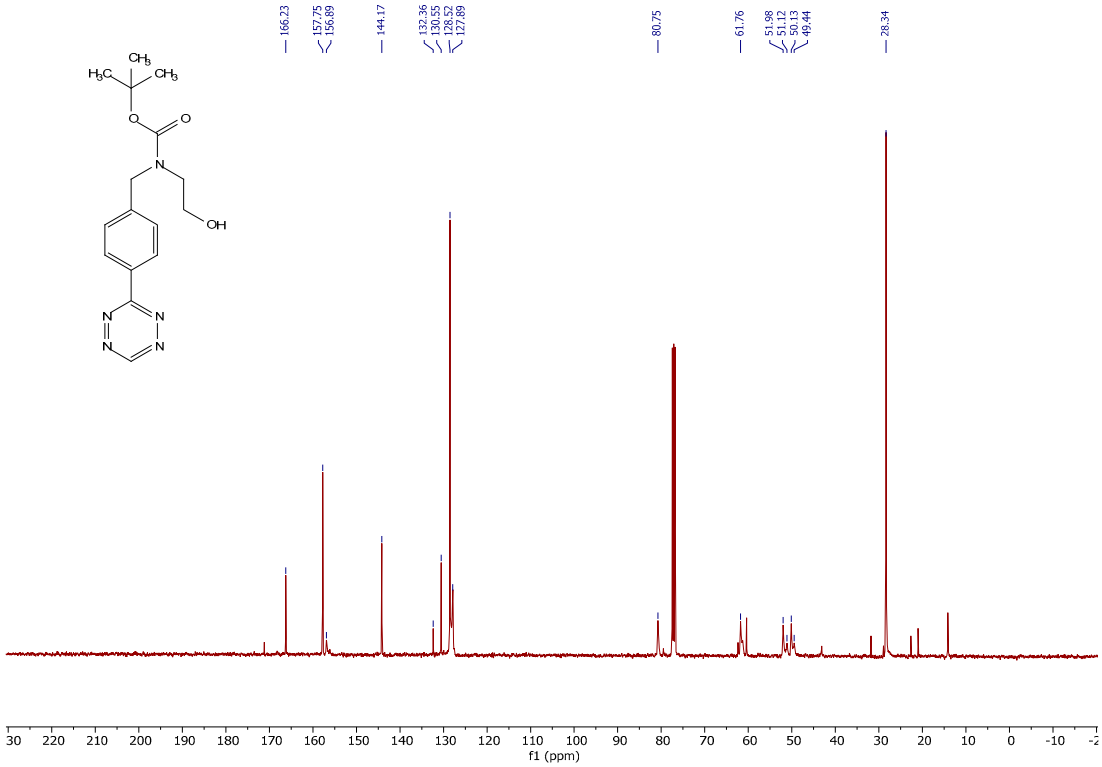
*Tert-butyl 4-(1,2,4,5-tetrazin-3-yl)benzyl(2-fluoroethyl)carbamate (**27**)*



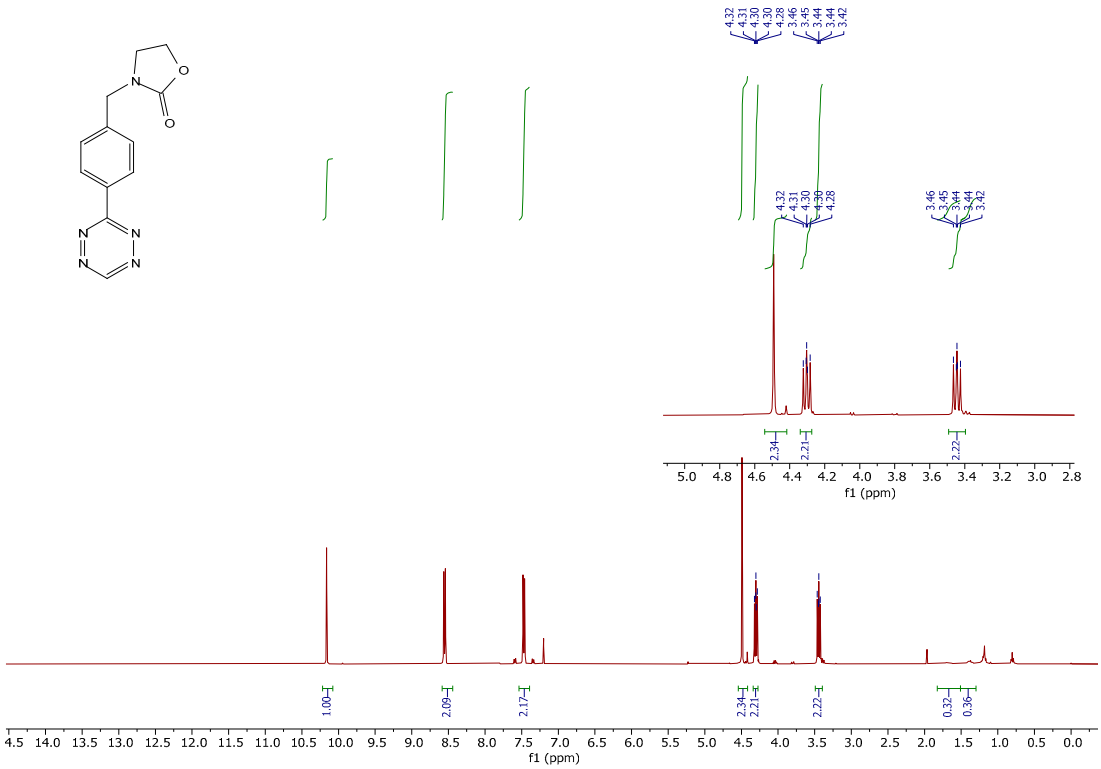


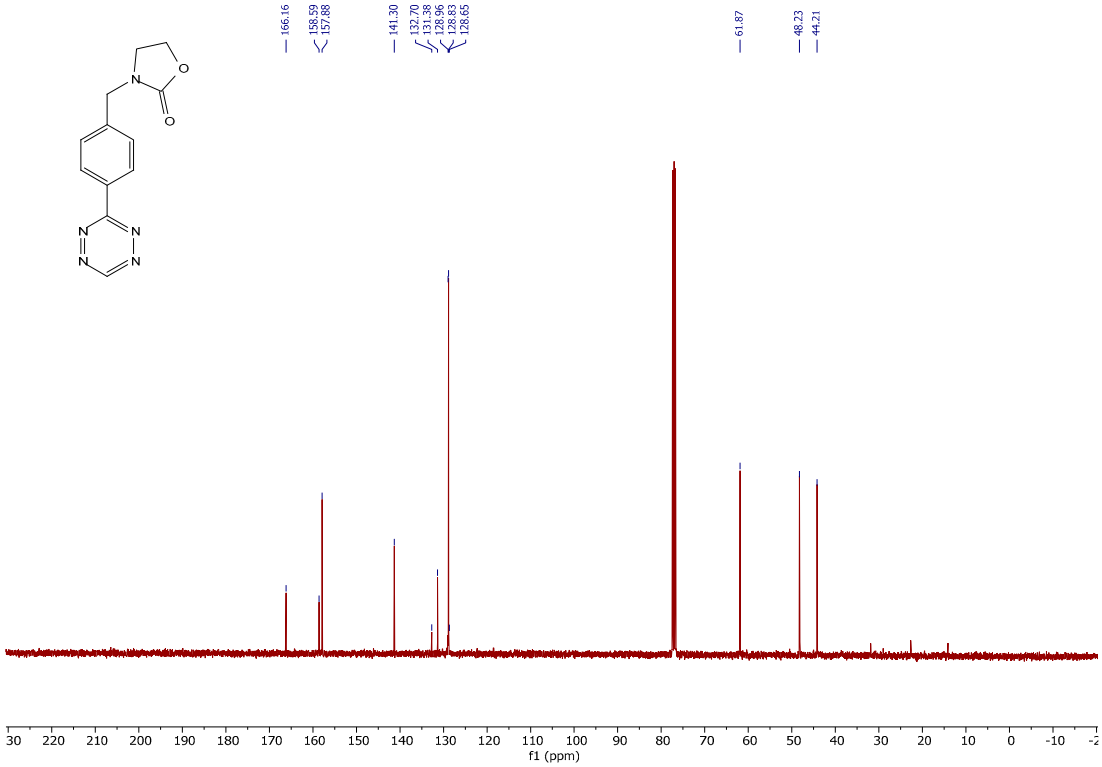
*Tert-butyl (4-(1,2,4,5-tetrazin-3-yl)benzyl)(2-hydroxyethyl)carbamate (**28**)*



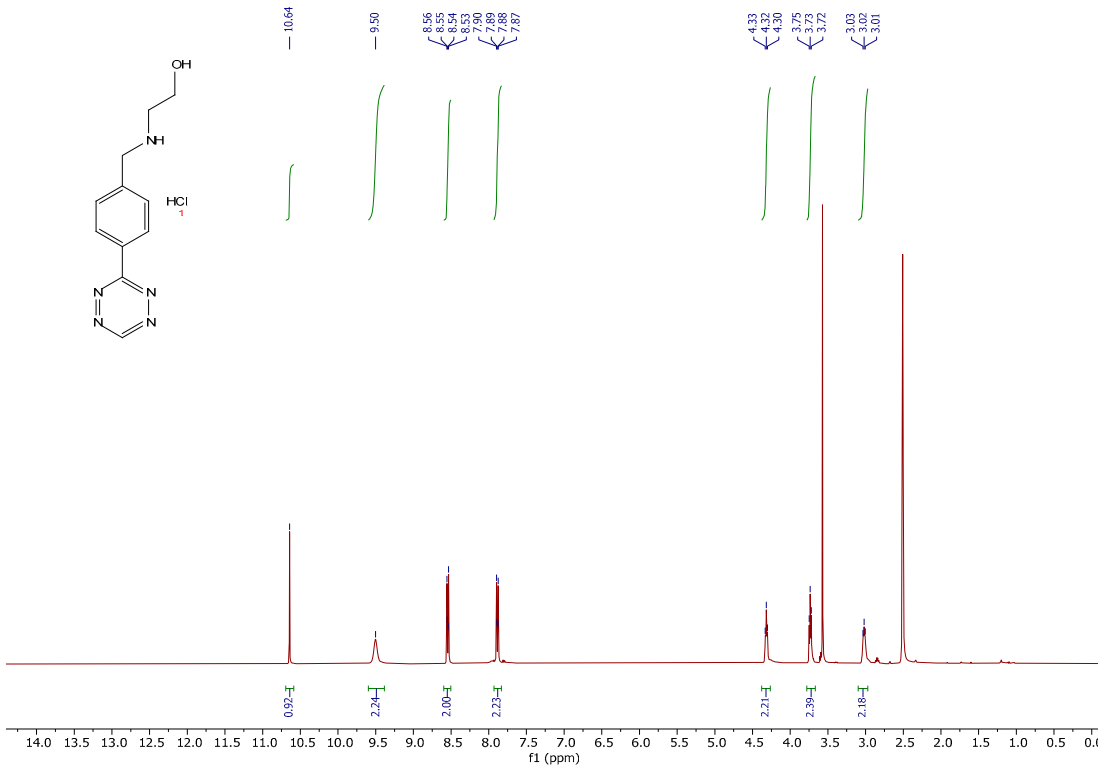


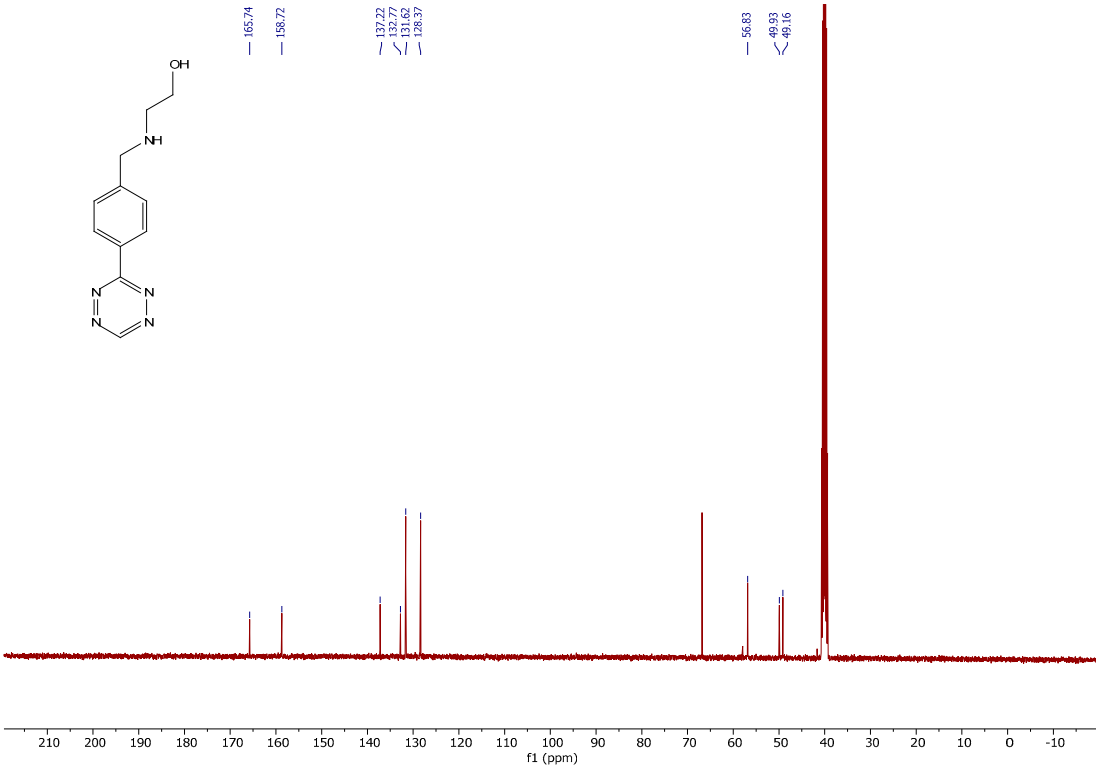
3-(4-(1,2,4,5-Tetrazin-3-yl)benzyl)oxazolidin-2-one



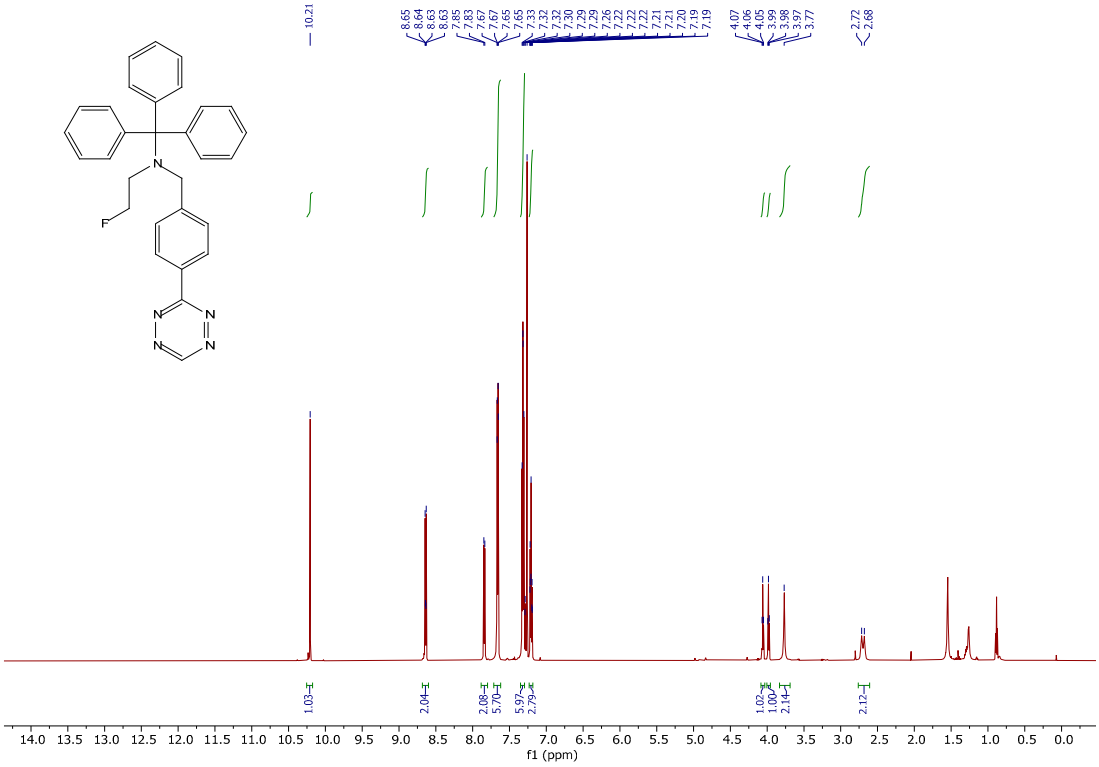


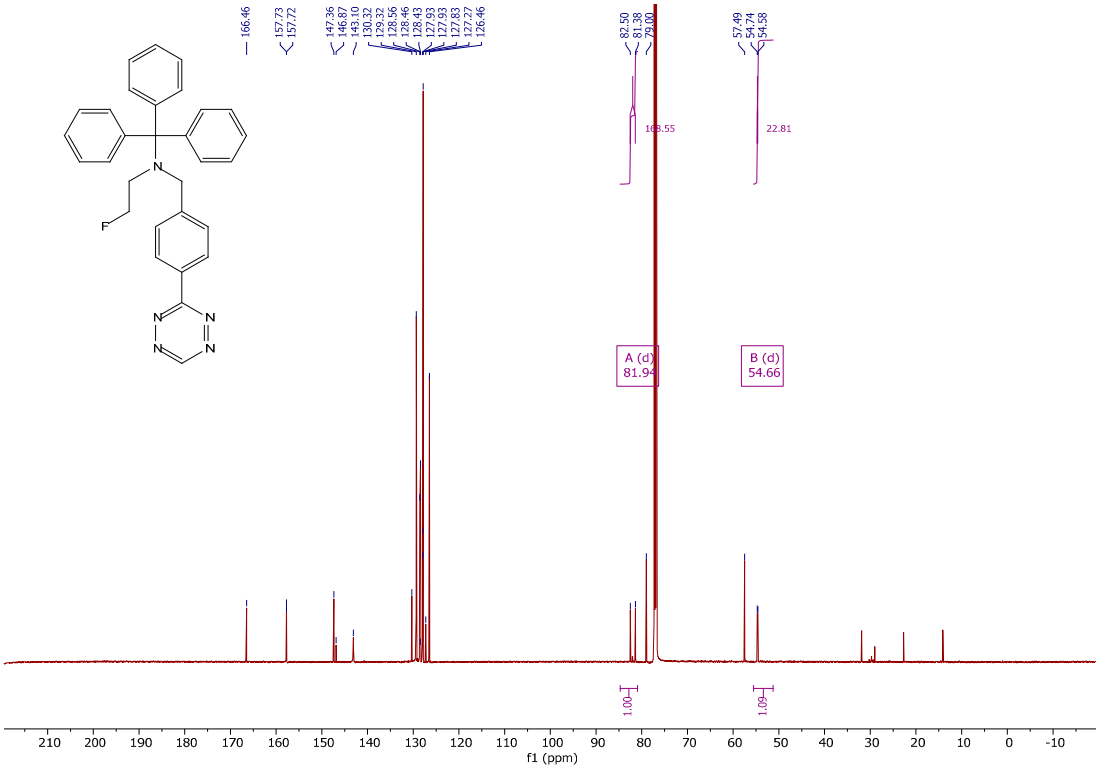
*2-((4-(1,2,4,5-Tetrazin-3-yl)benzyl)amino)ethan-1-ol (**29**)*



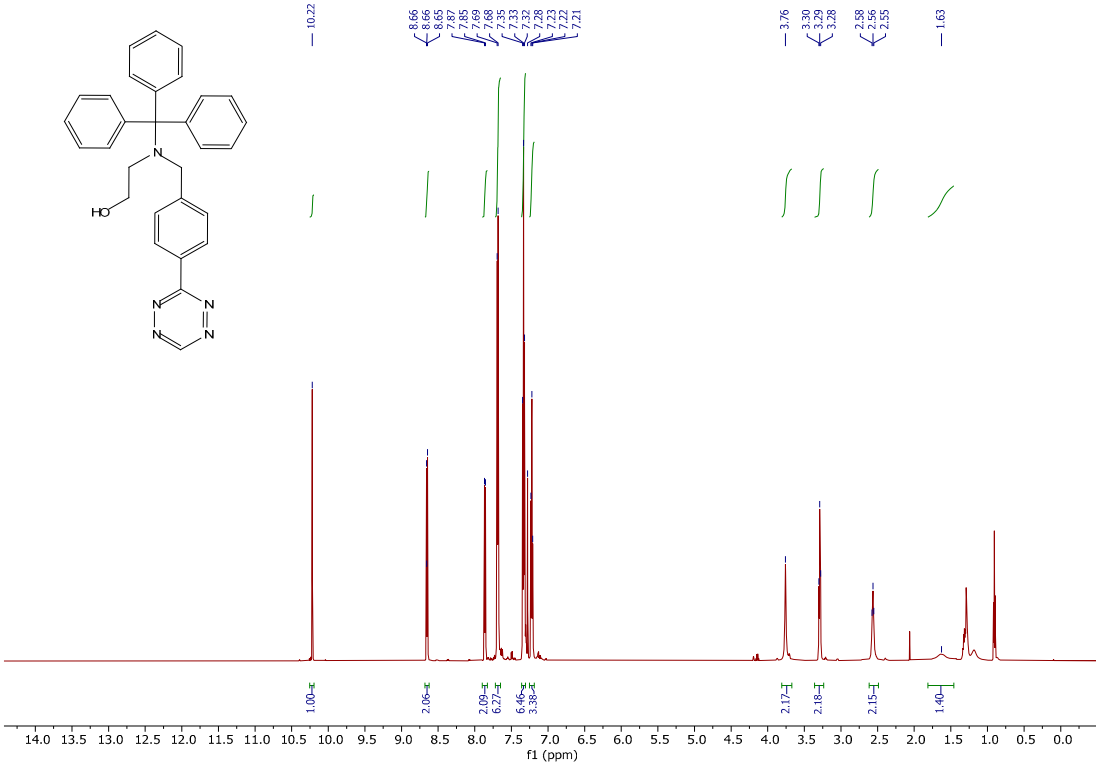


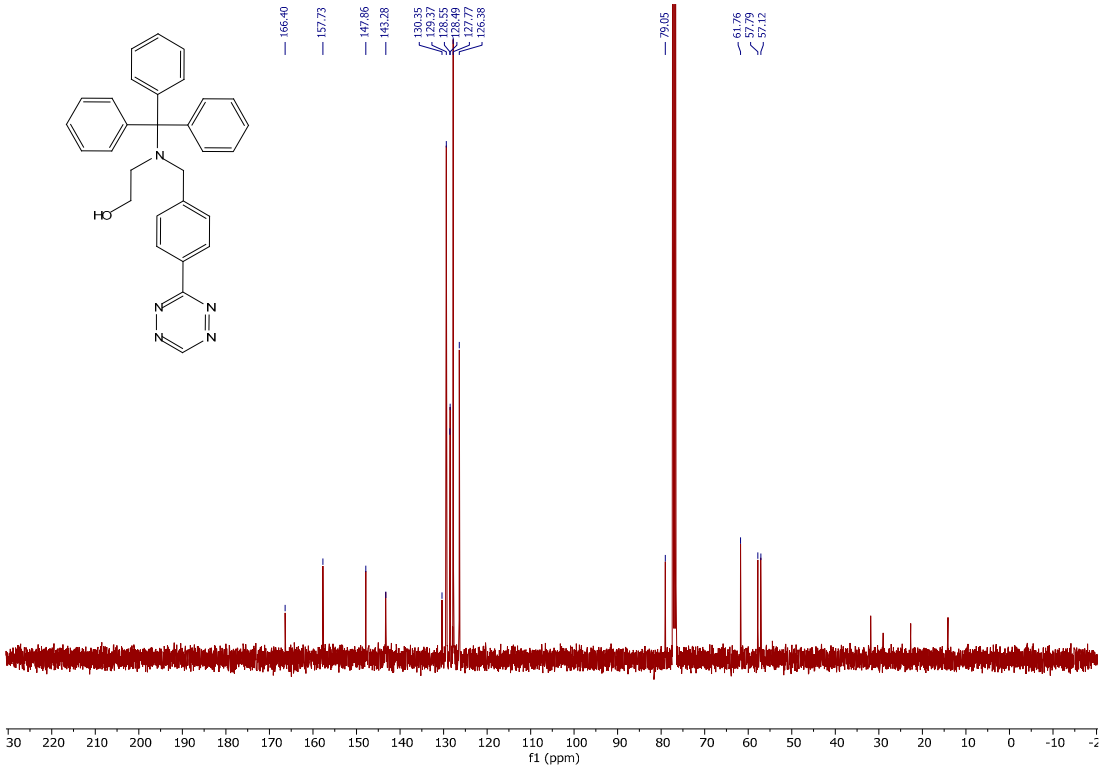
*N-(4-(1,2,4,5-tetrazin-3-yl)benzyl)-2-fluoro-N-tritylethan-1-amine (**30**)*



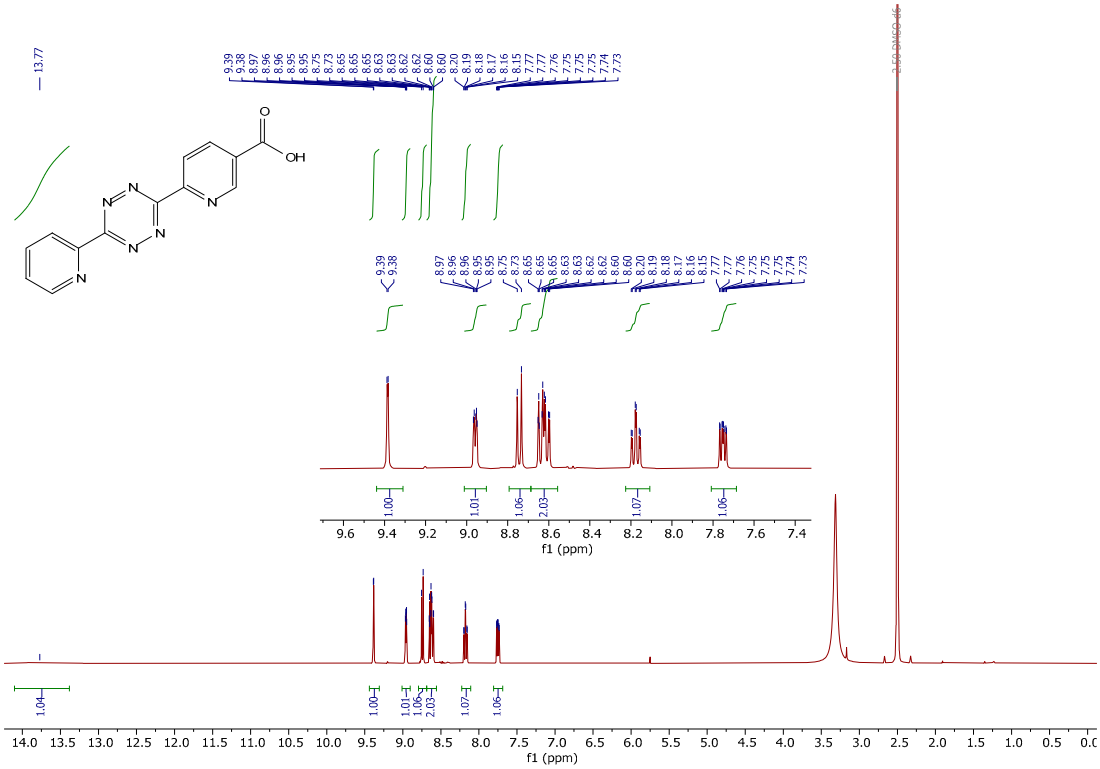


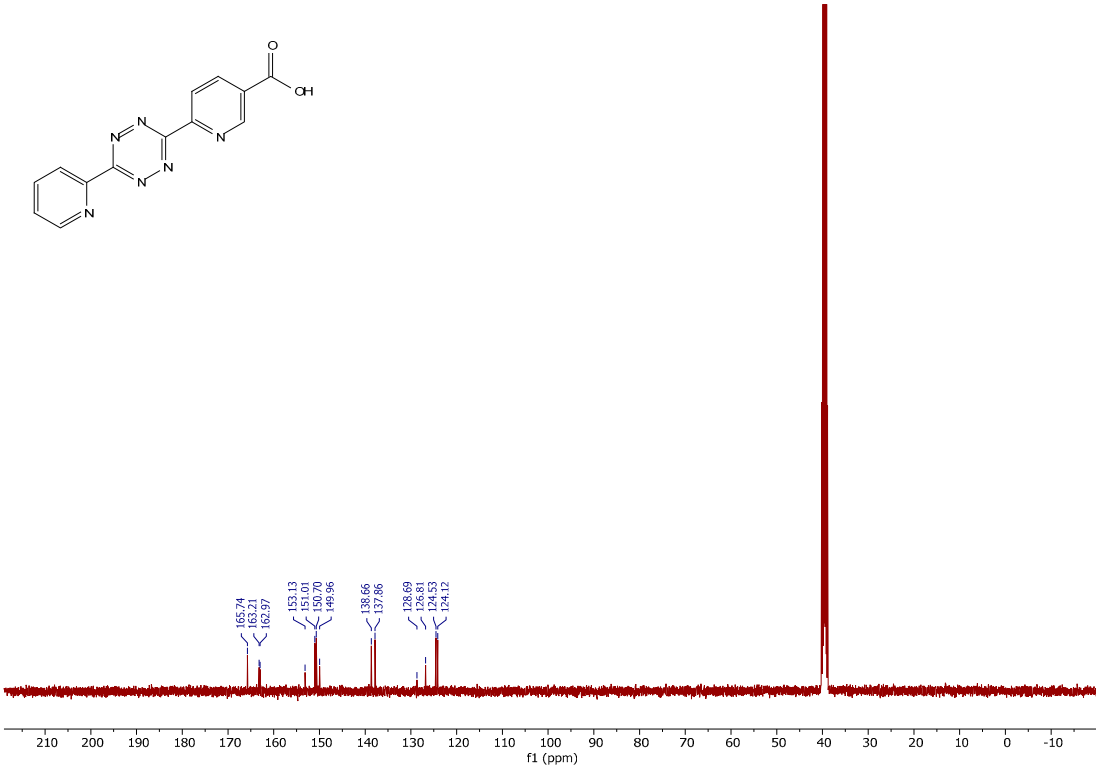
*2-((4-(1,2,4,5-Tetrazin-3-yl)benzyl)(trityl)amino)ethan-1-ol (**31**)*



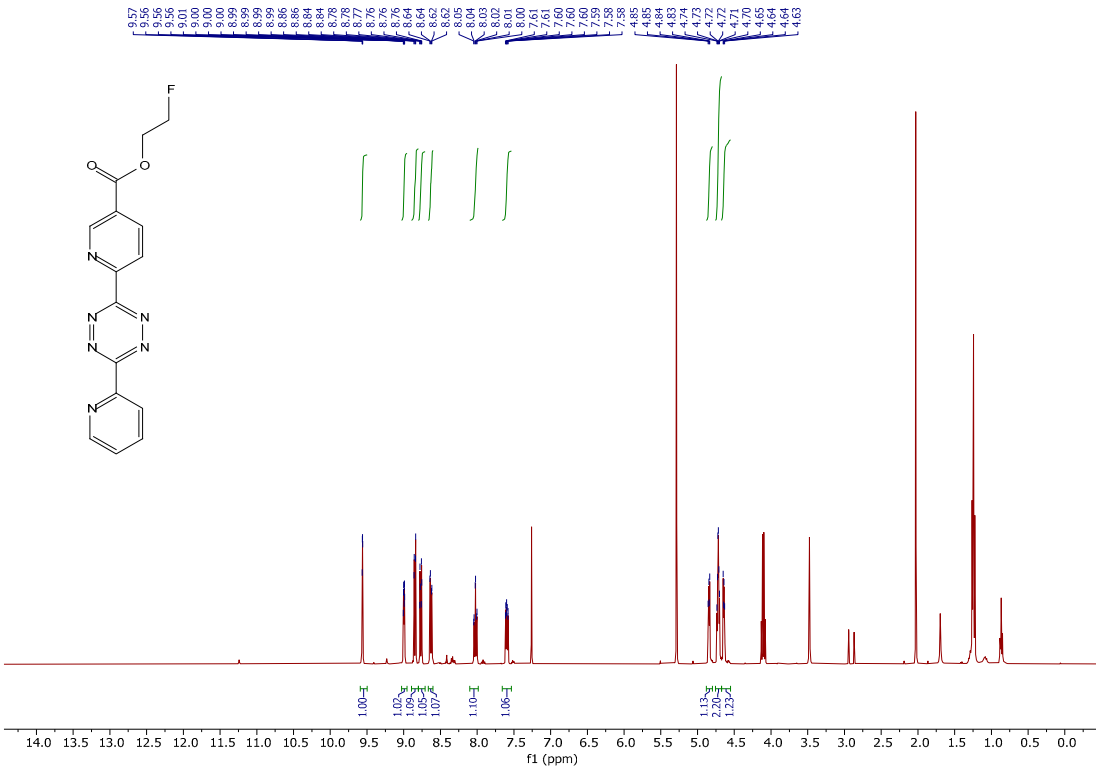


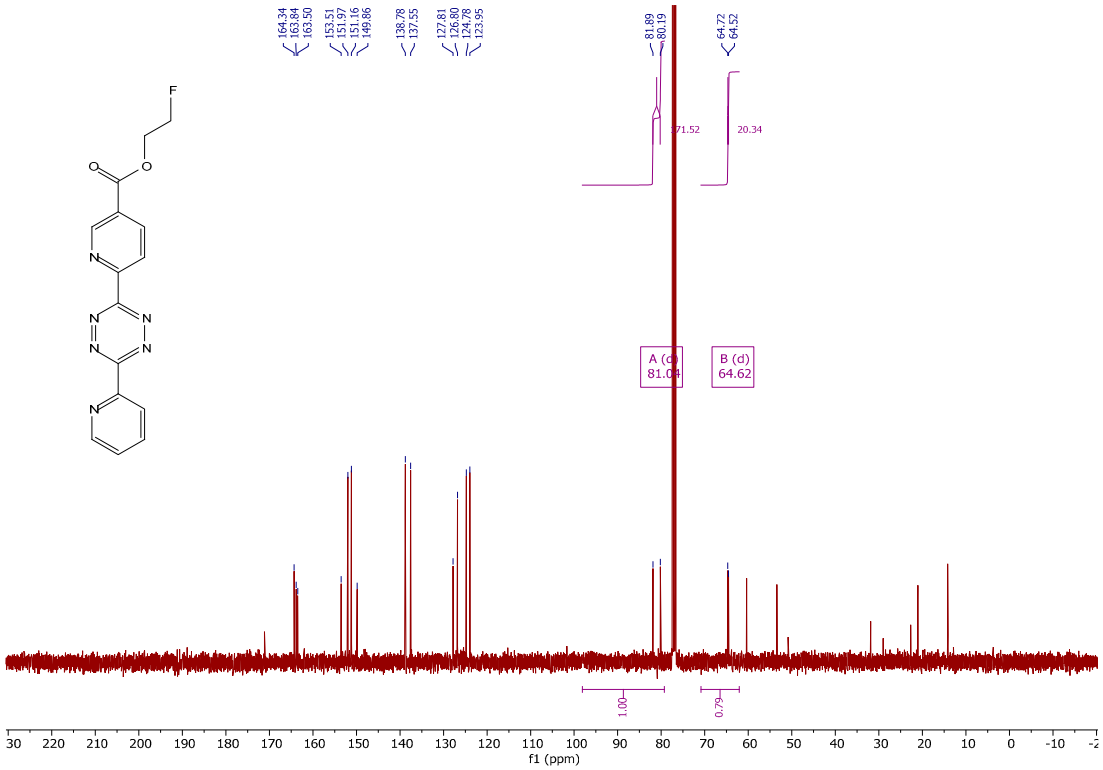
*6-(4-(Pyridin-2-yl)phenyl)nicotinic acid (**32**)*



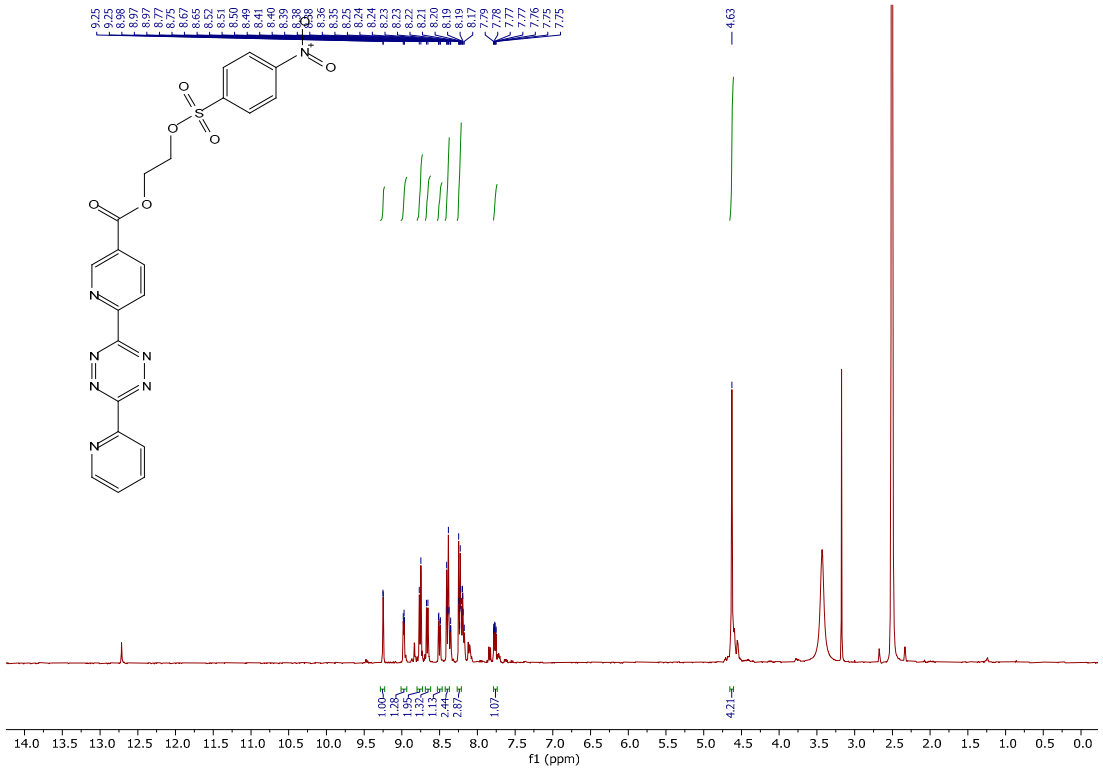


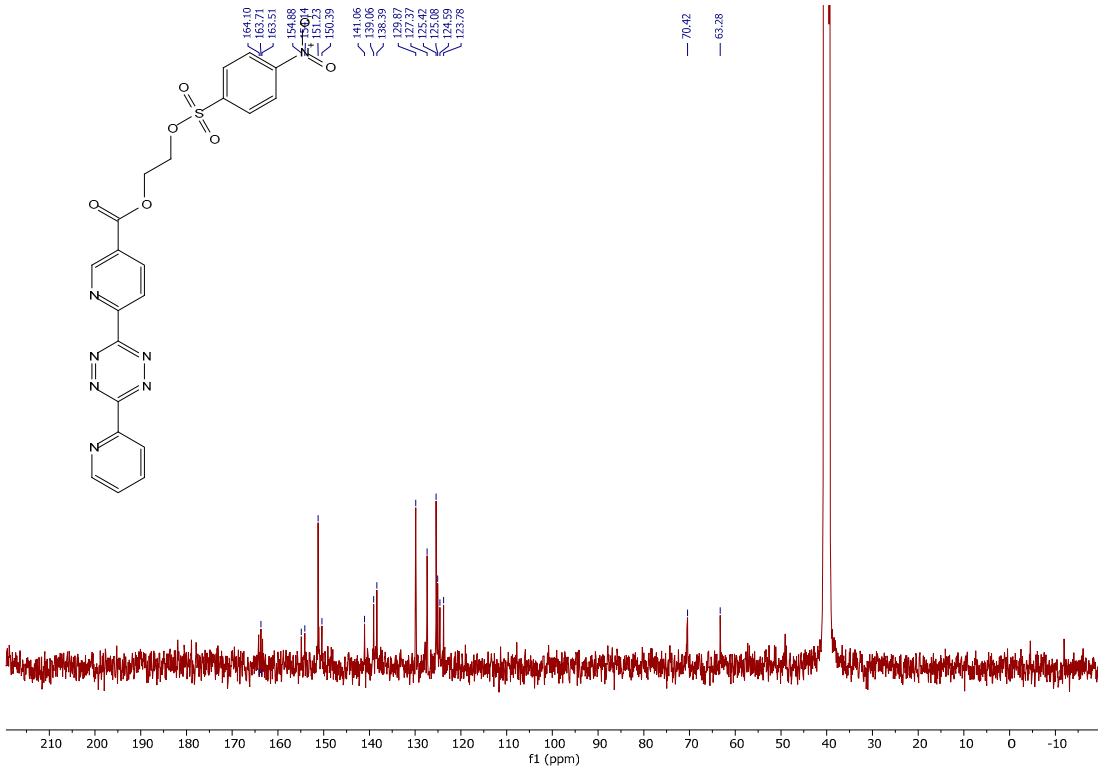
*2-Fluoroethyl 6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)nicotinate (**33**)*



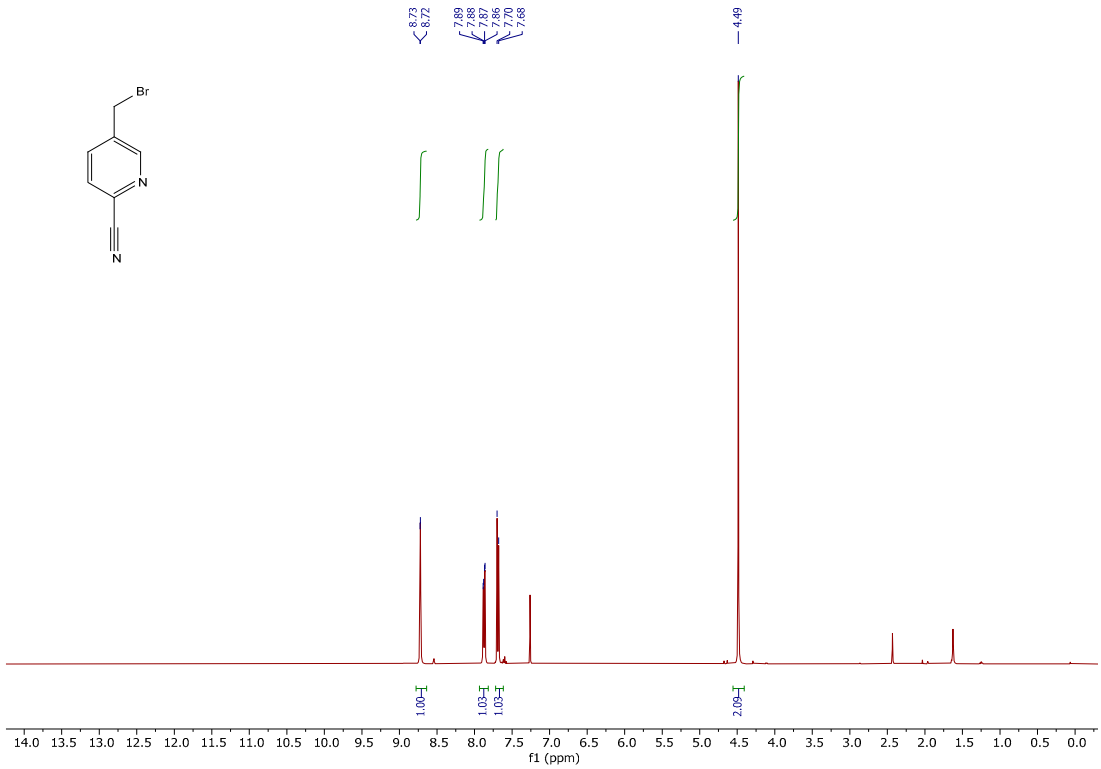


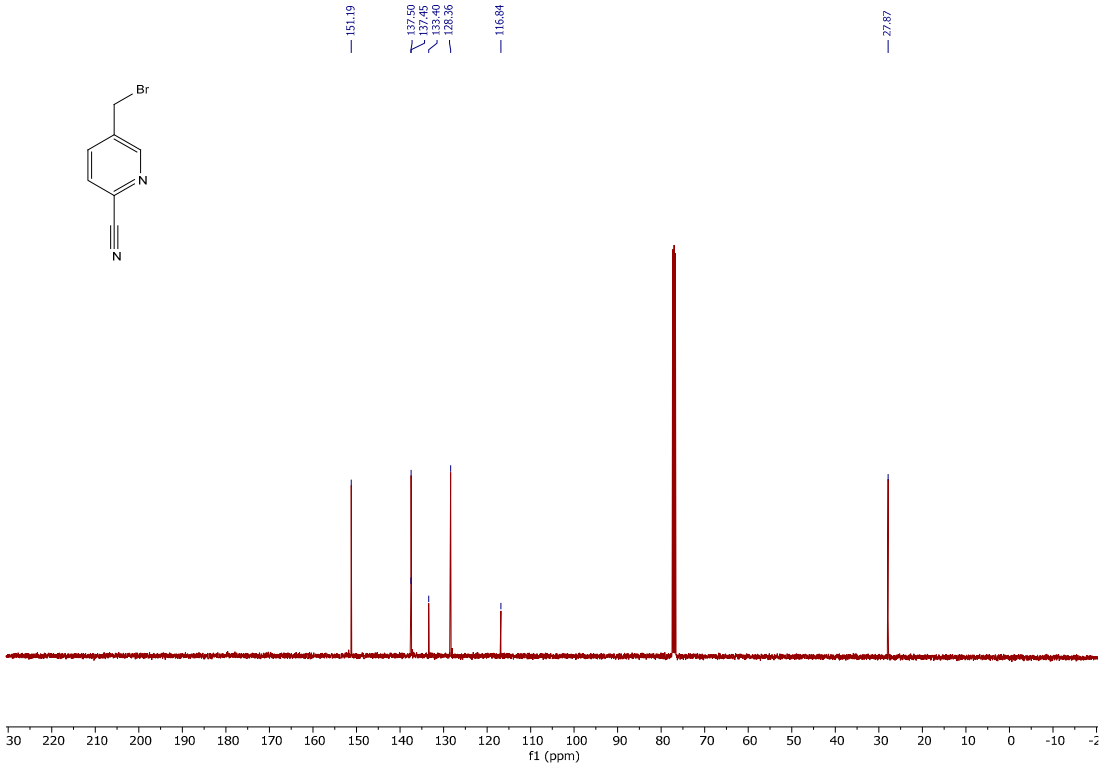
2-(((4-Nitrophenyl)sulfonyl)oxy)ethyl 6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)nicotinate ***(33a)***



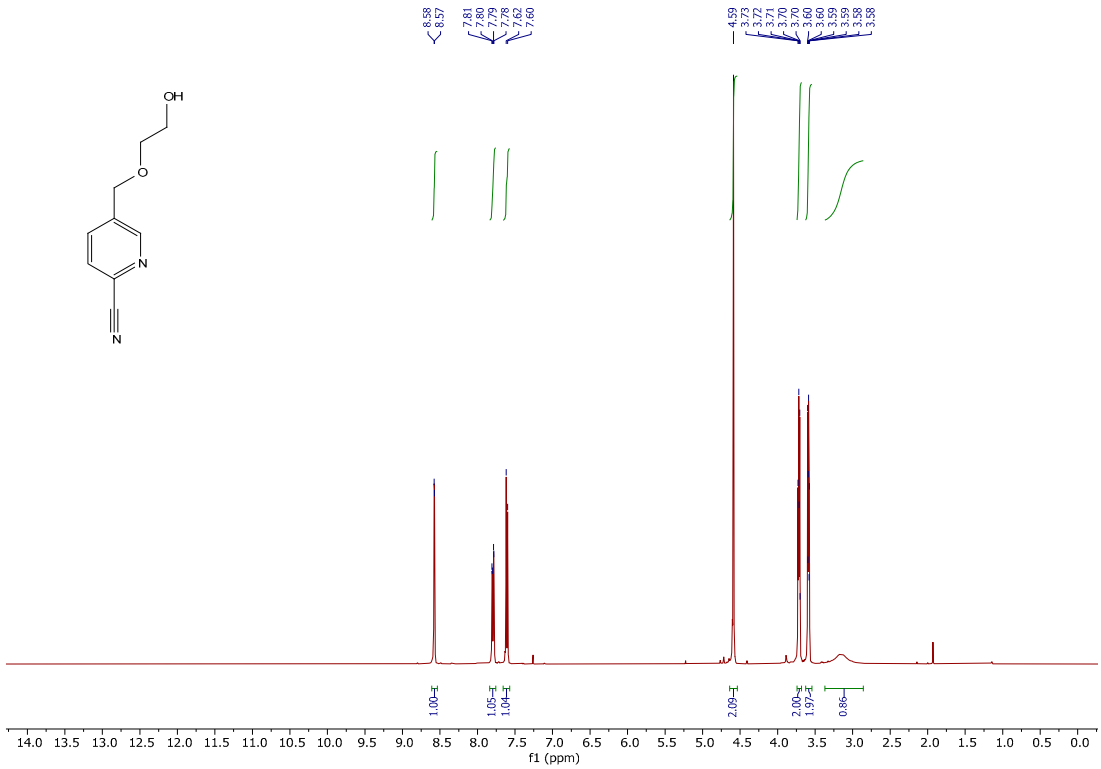


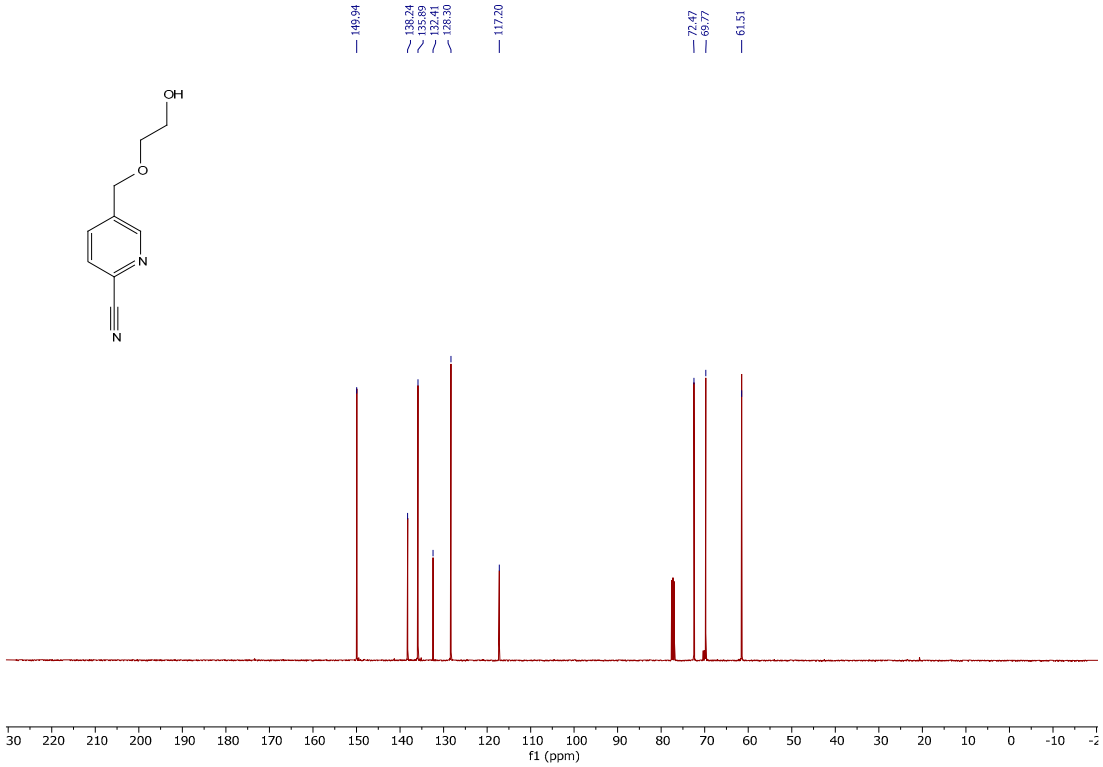
*5-(Bromomethyl)picolinonitrile (**35**)*



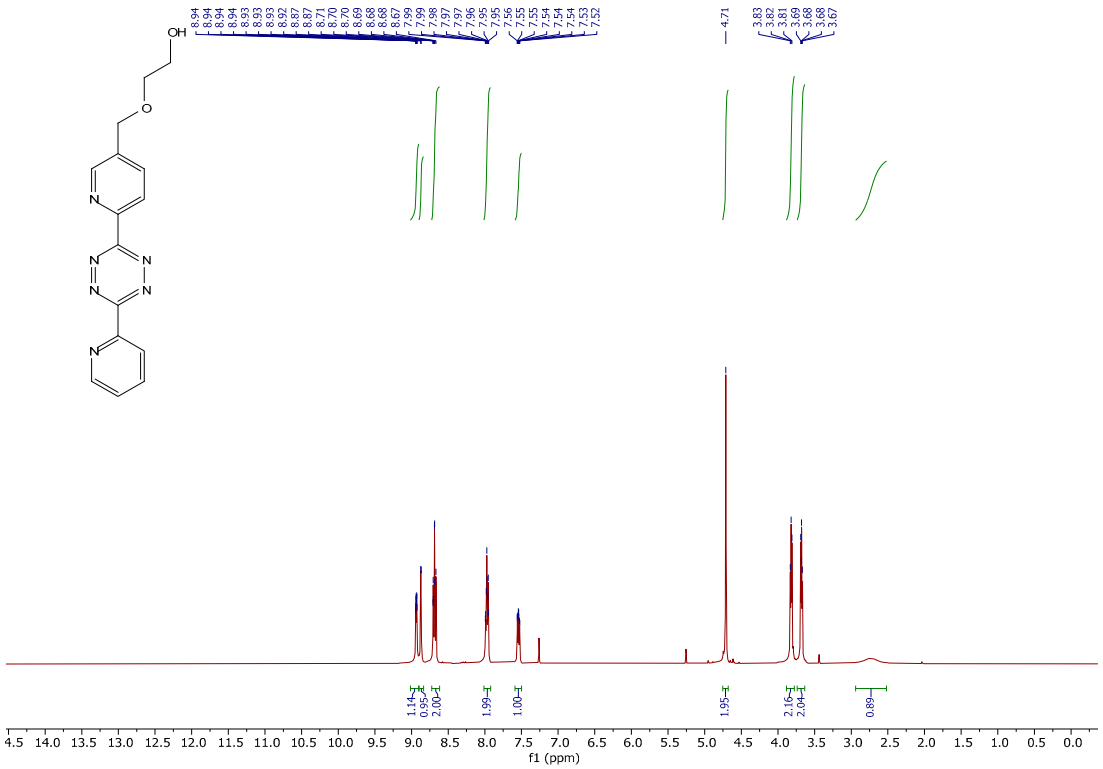


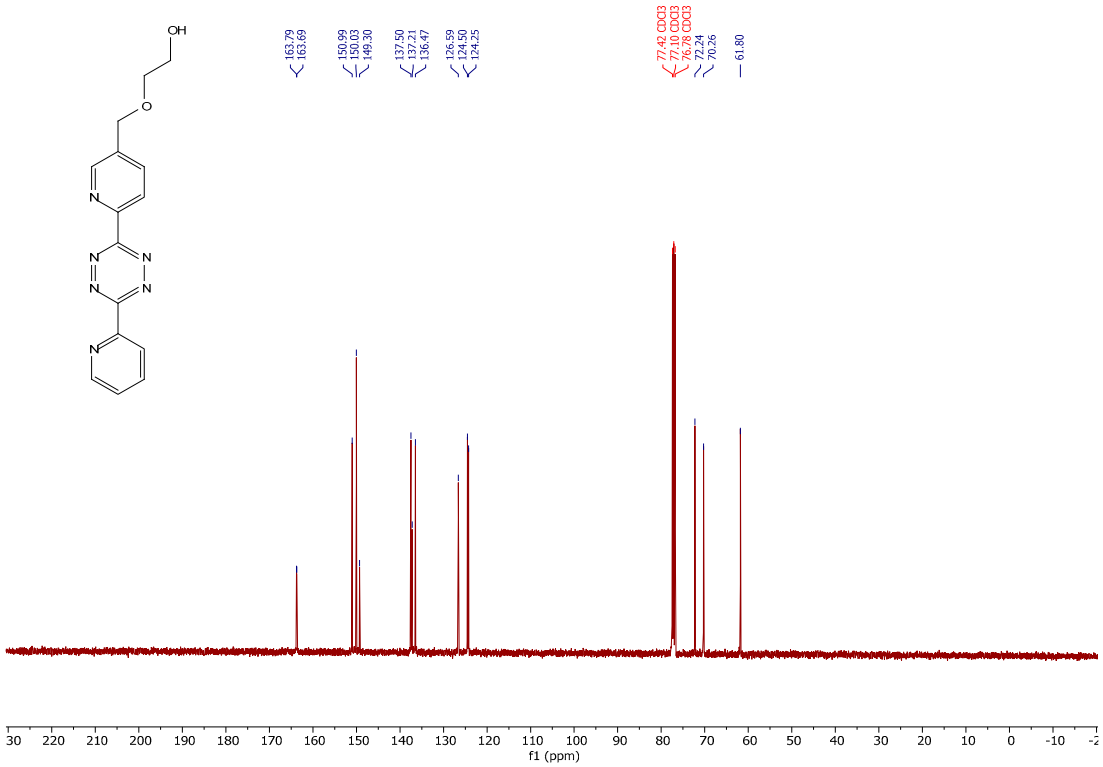
*5-((2-Hydroxyethoxy)methyl)picolinonitrile (**36**)*



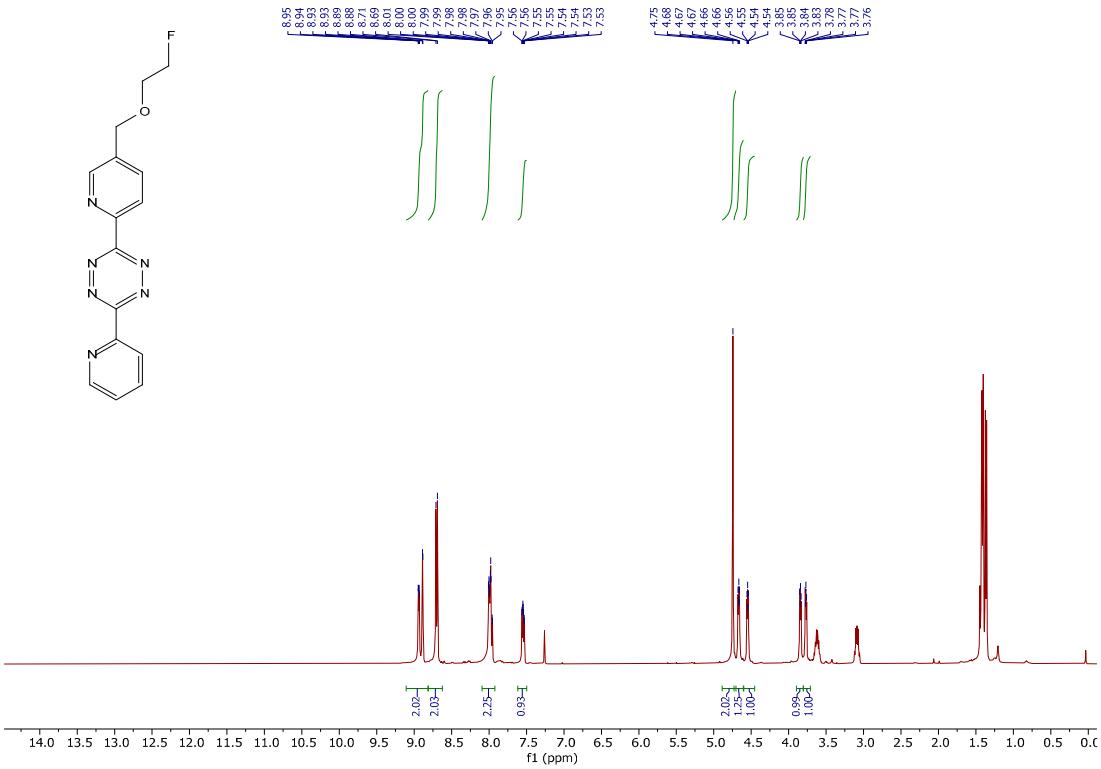


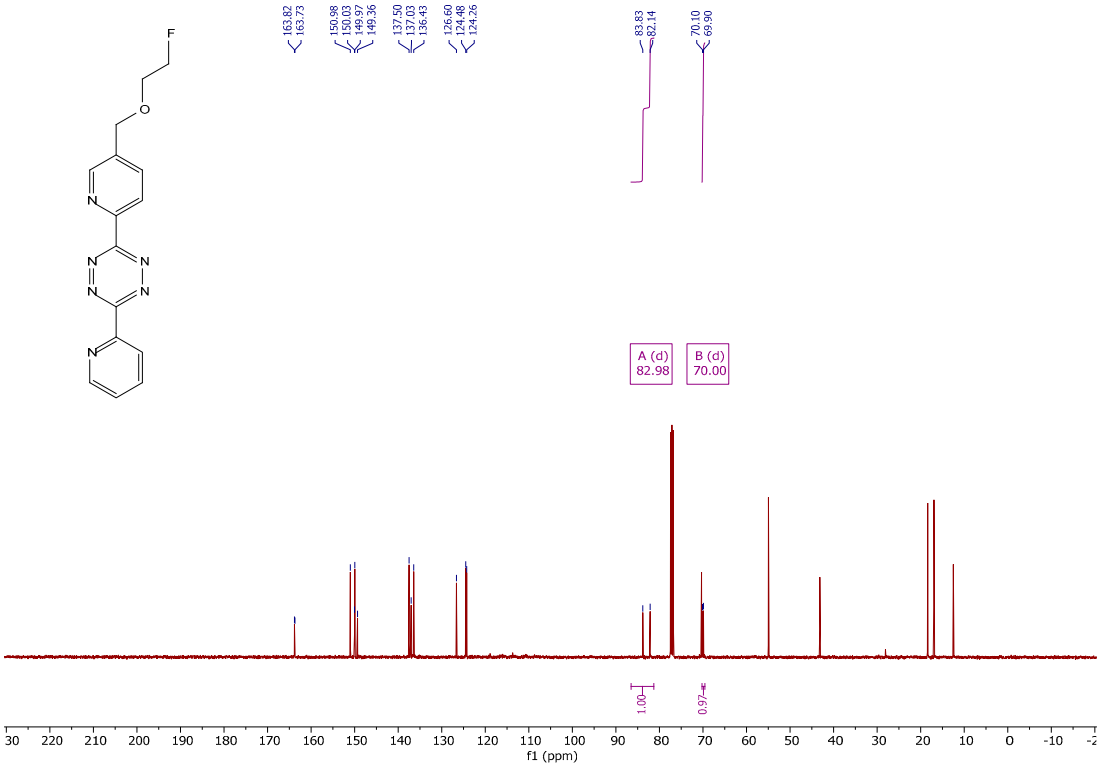
*2-((6-(6-(Pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)methoxy)ethan-1-ol (**37**)*



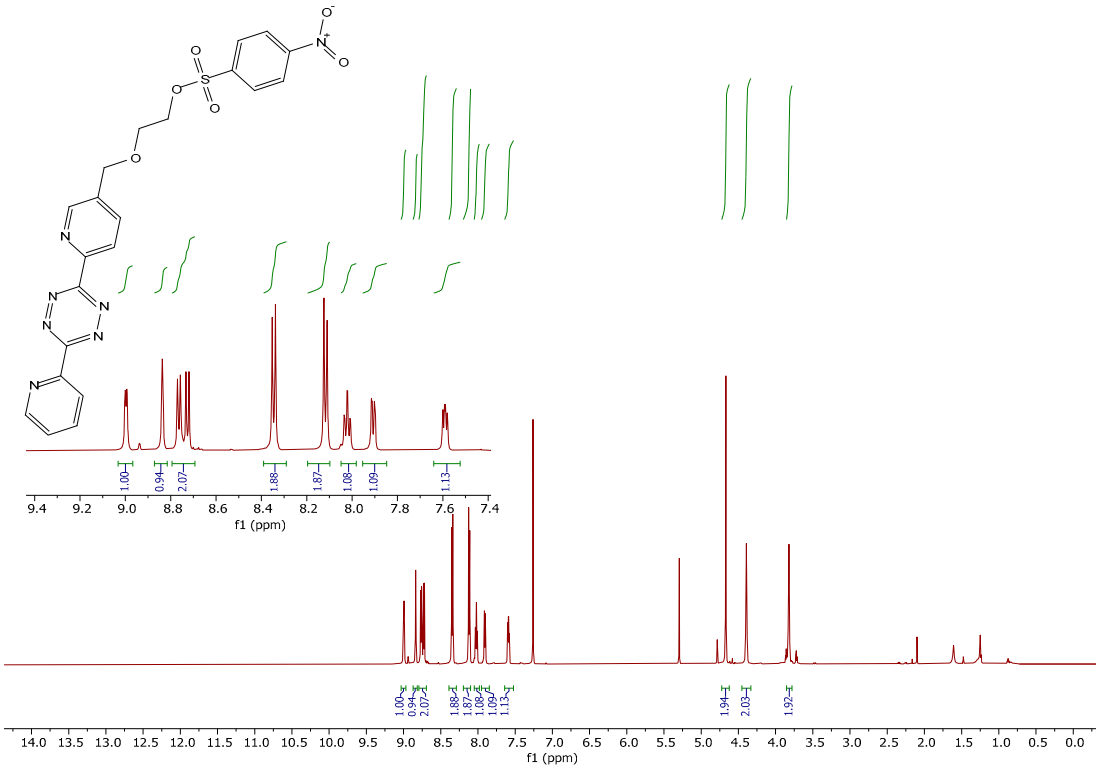


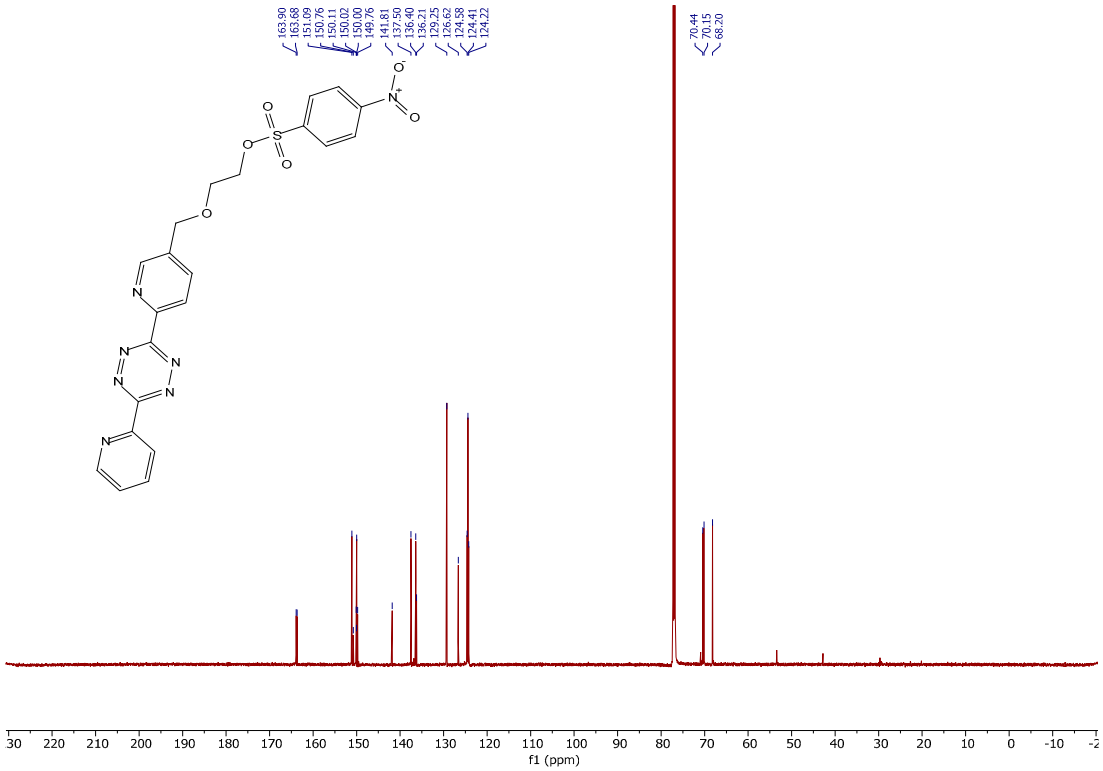
*3-(5-((2-fluoroethoxy)methyl)pyridin-2-yl)-6-(pyridin-2-yl)-1,2,4,5-tetrazine (**38**)*



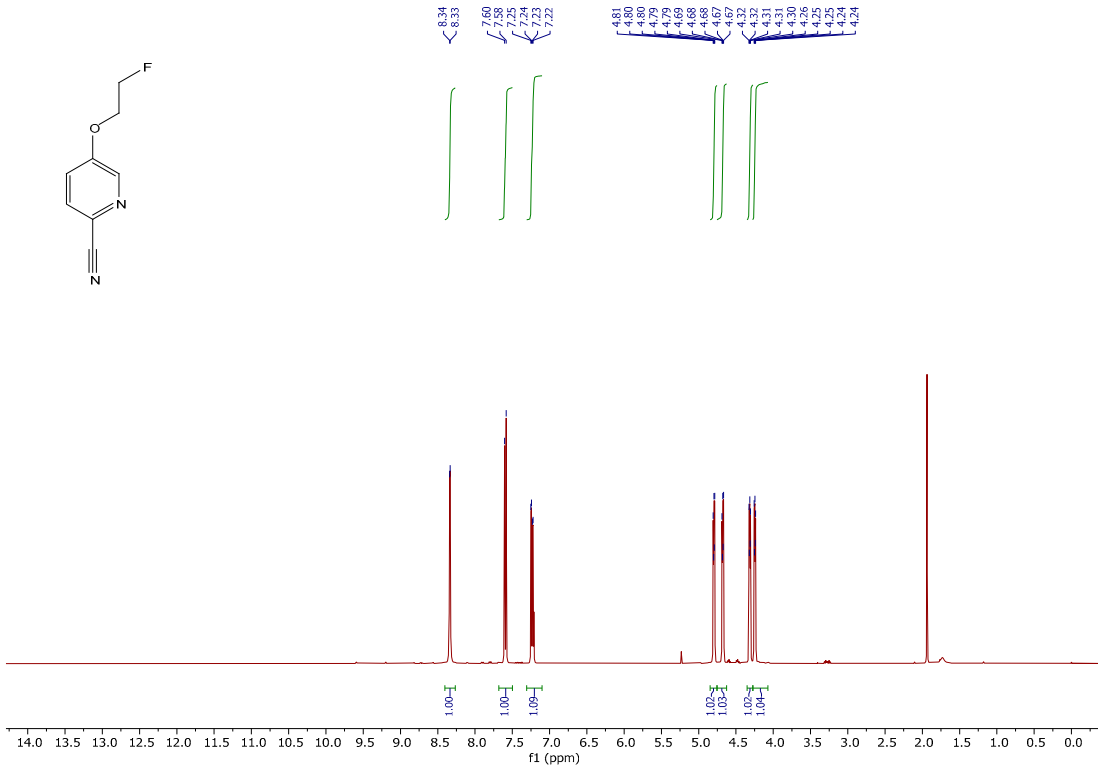


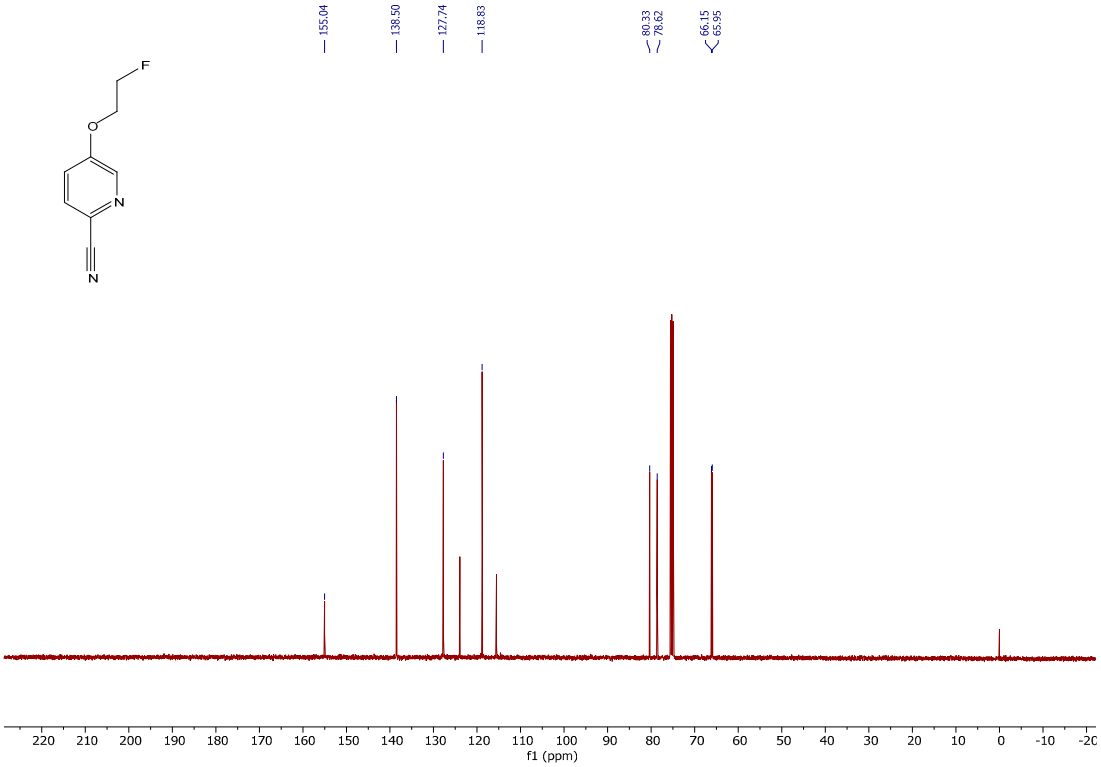
2-((6-(4-(pyridin-2-yl)phenyl)pyridin-3-yl)methoxy)ethyl 4-methylbenzene sulfonate ***(38a)***



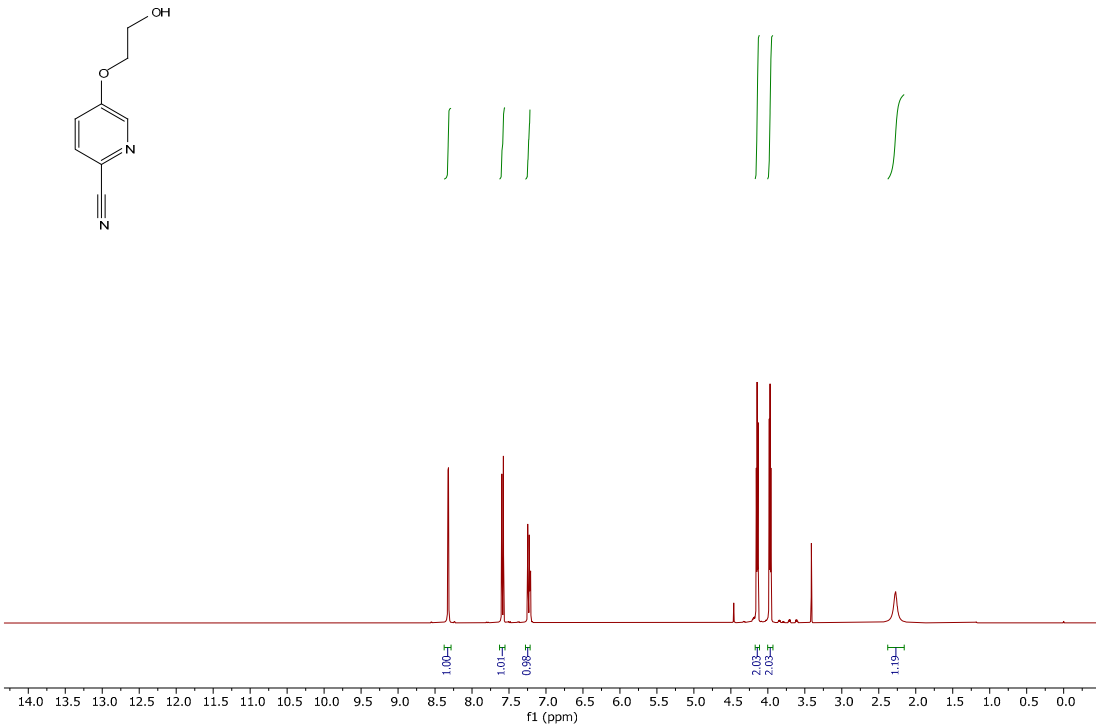


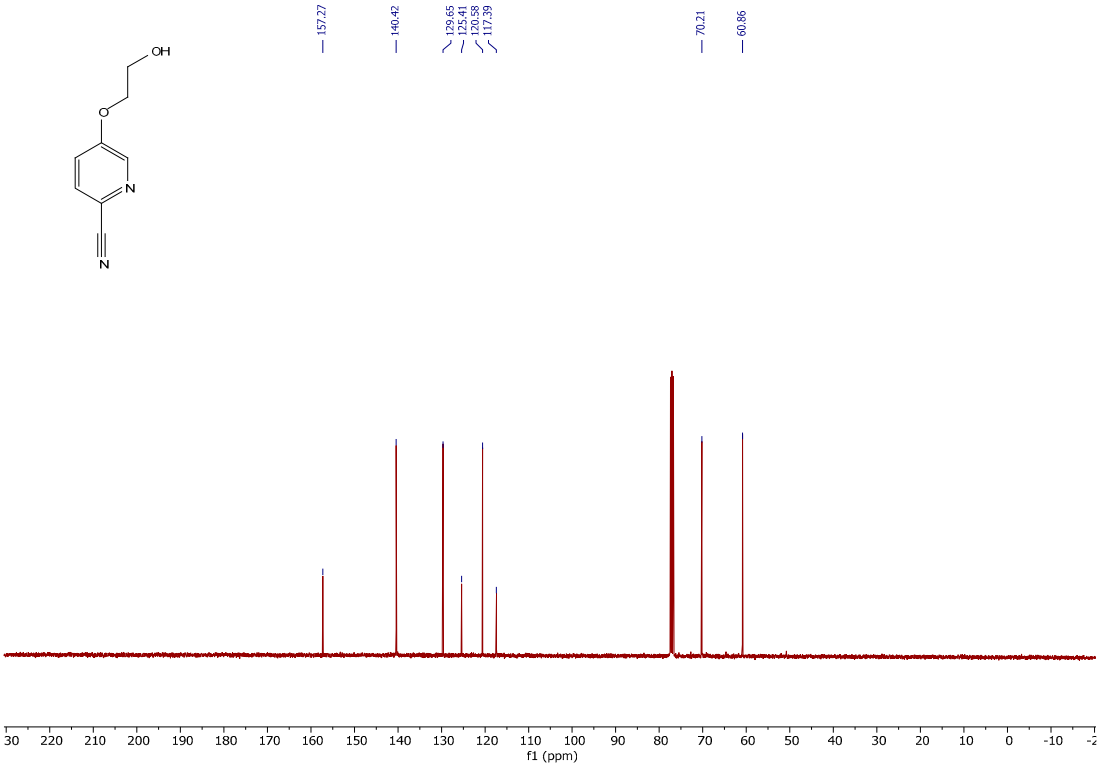
*5-(2-Fluoroethoxy)picolinonitrile (**39**)*



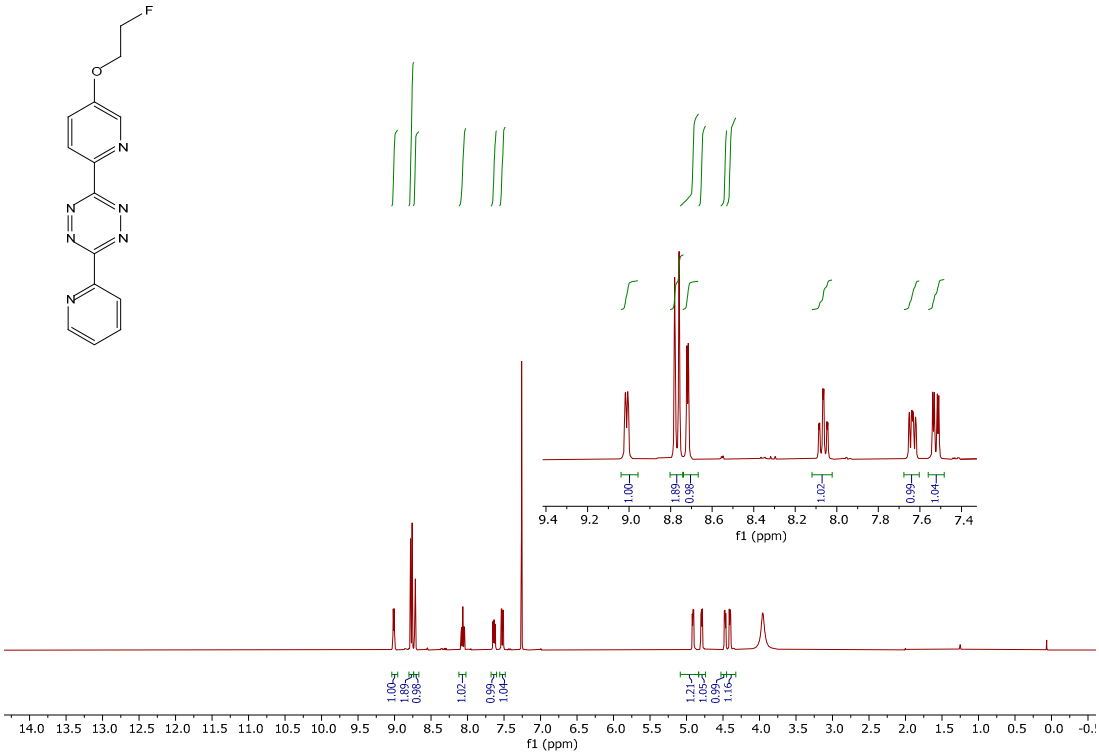


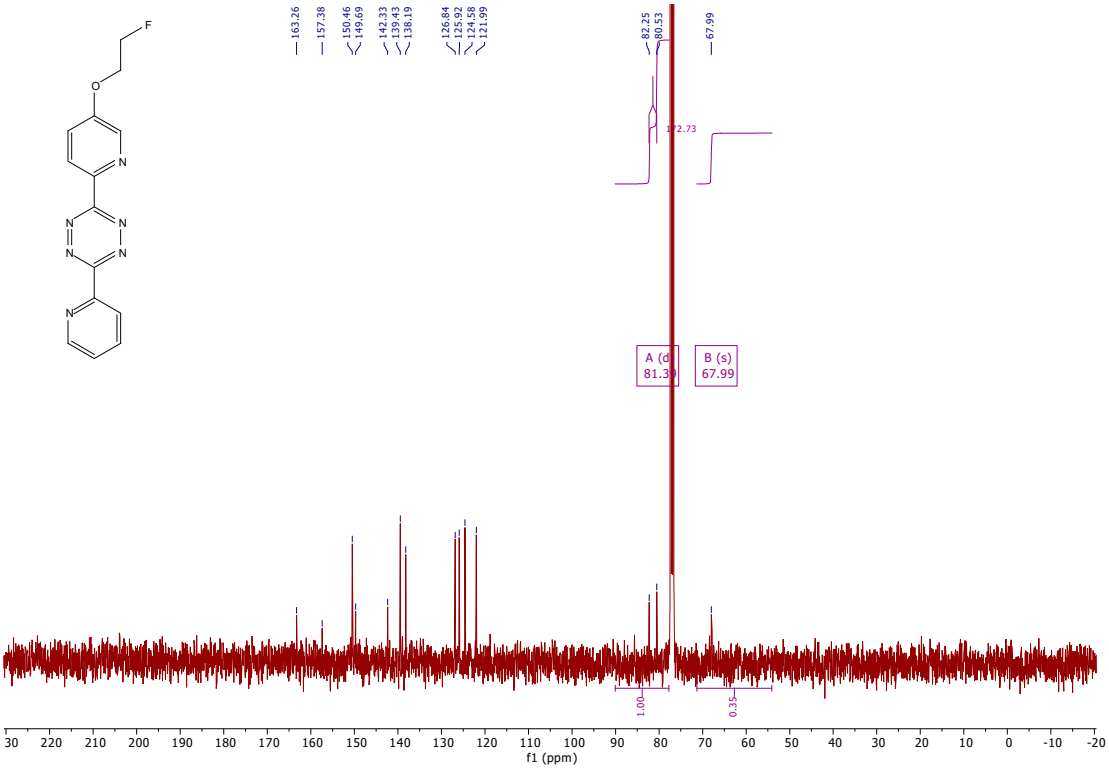
*5-(2-Hydroxyethoxy)picolinonitrile (**40**)*

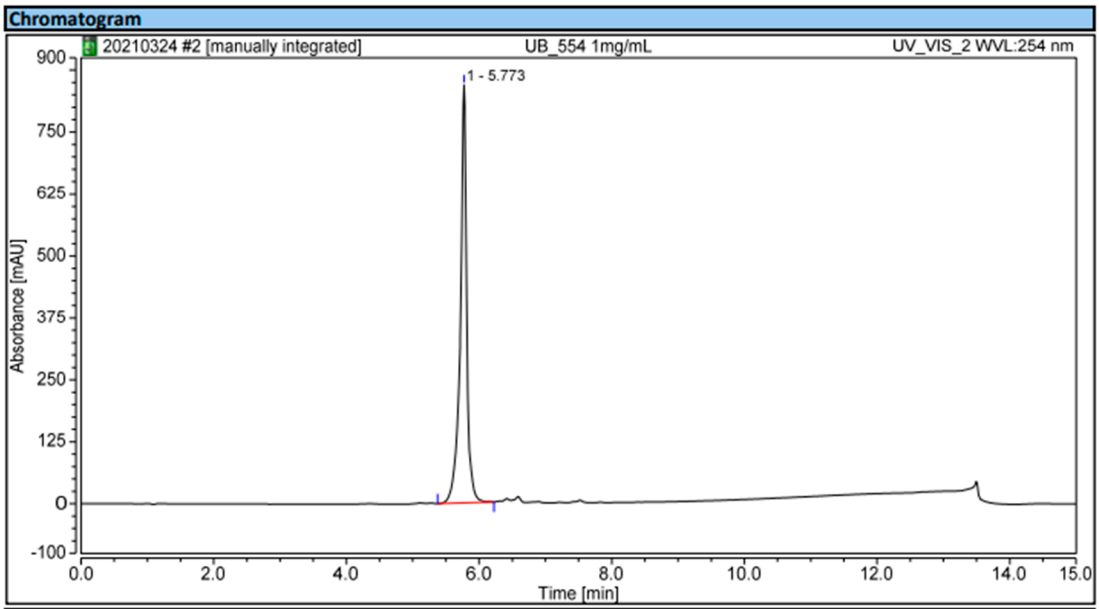




*3-(5-(2-Fluoroethoxy)pyridin-2-yl)-6-(pyridin-2-yl)-1,2,4,5-tetrazine (**41**)*



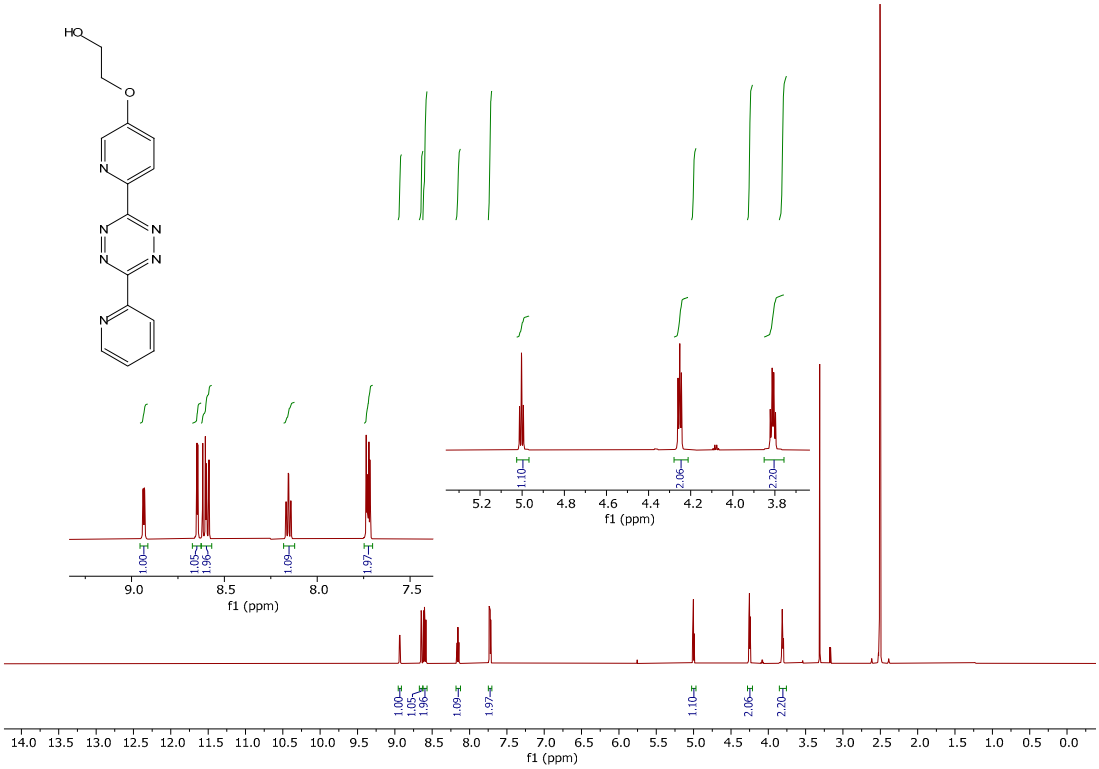


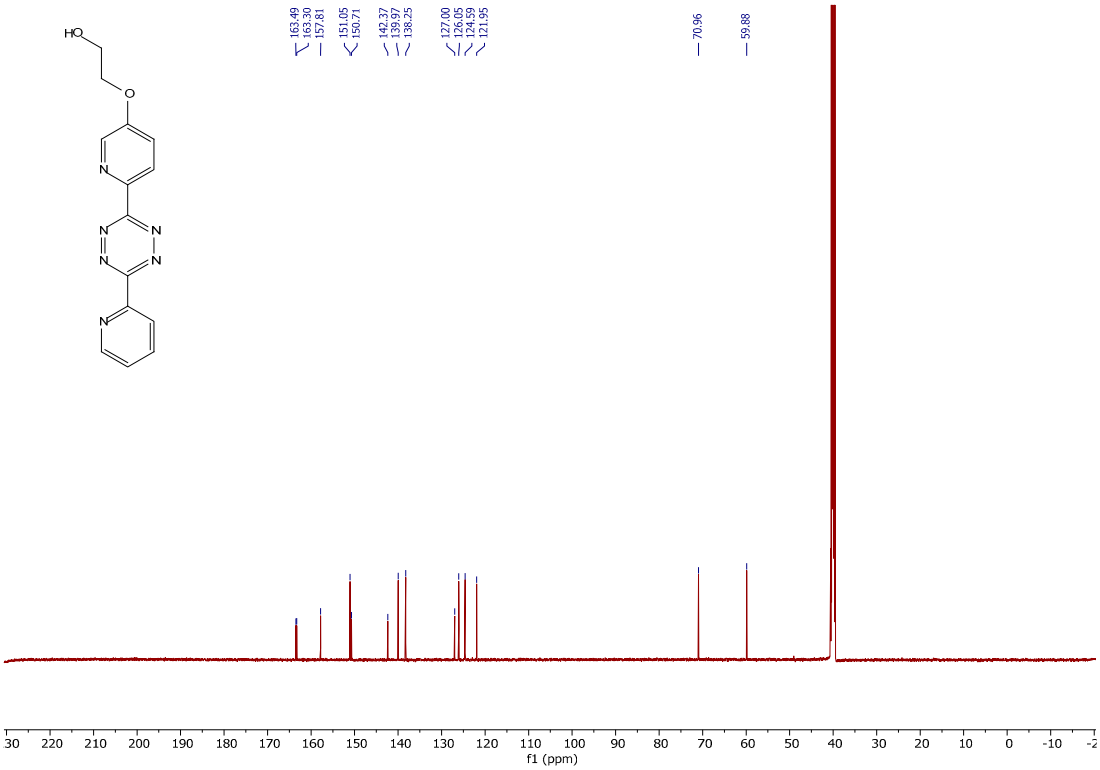


Integration Results

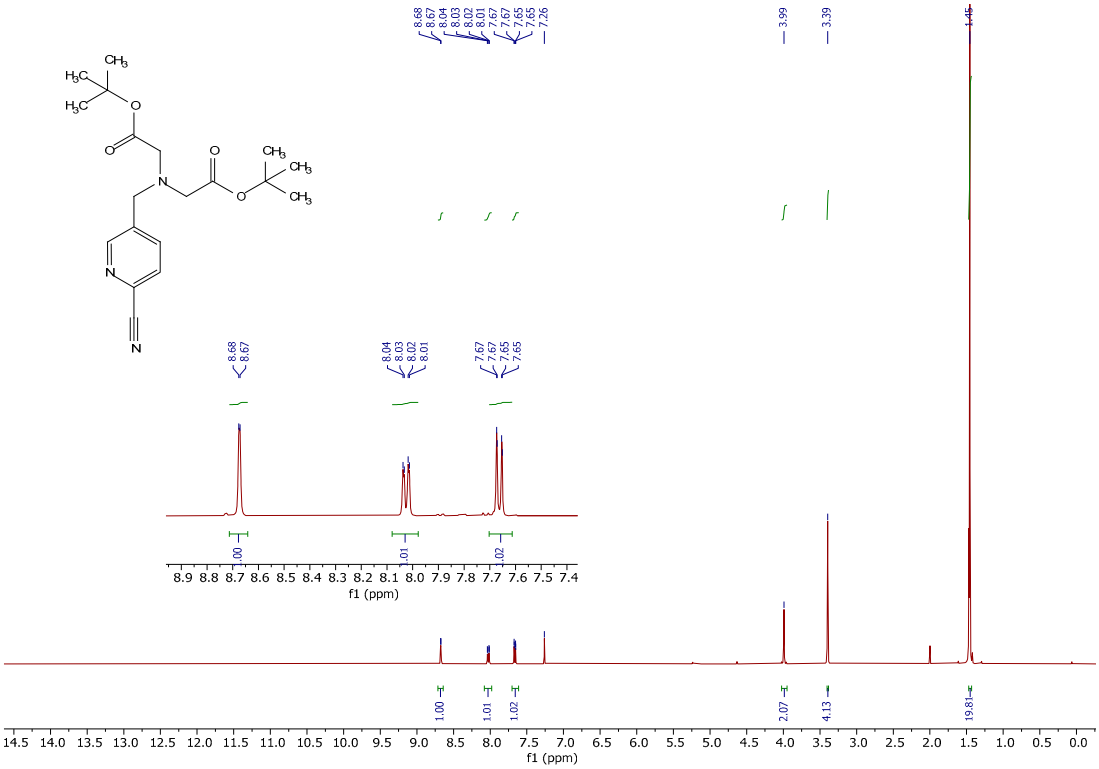
No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1		5.773	94.418	843.489	100.00	100.00
Total:			**94.418**	**843.489**	**100.00**	**100.00**

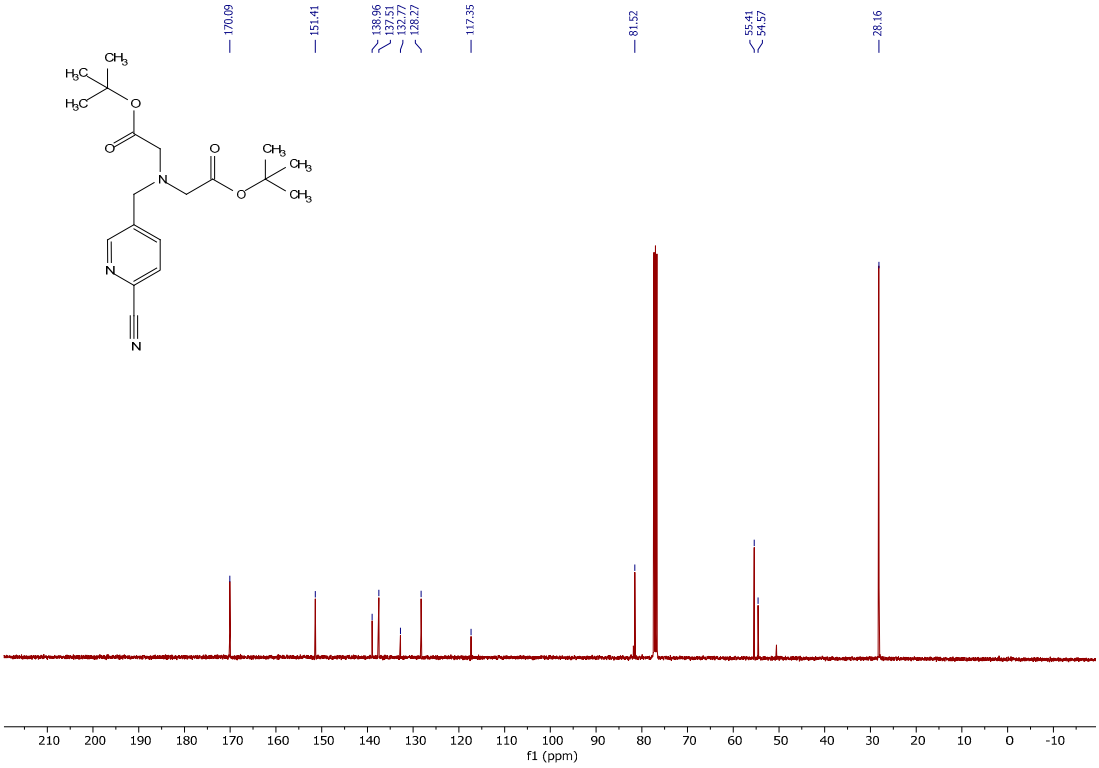
*2-((6-(6-(Pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)oxy)ethan-1-ol (**42**)*



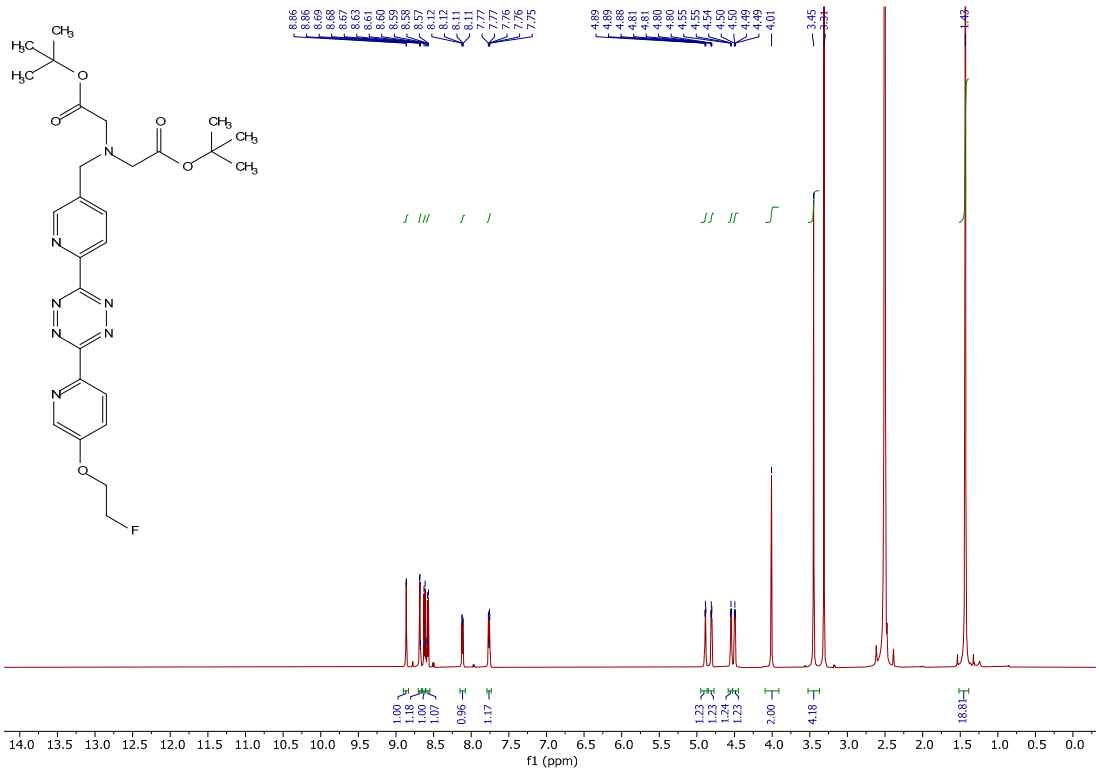


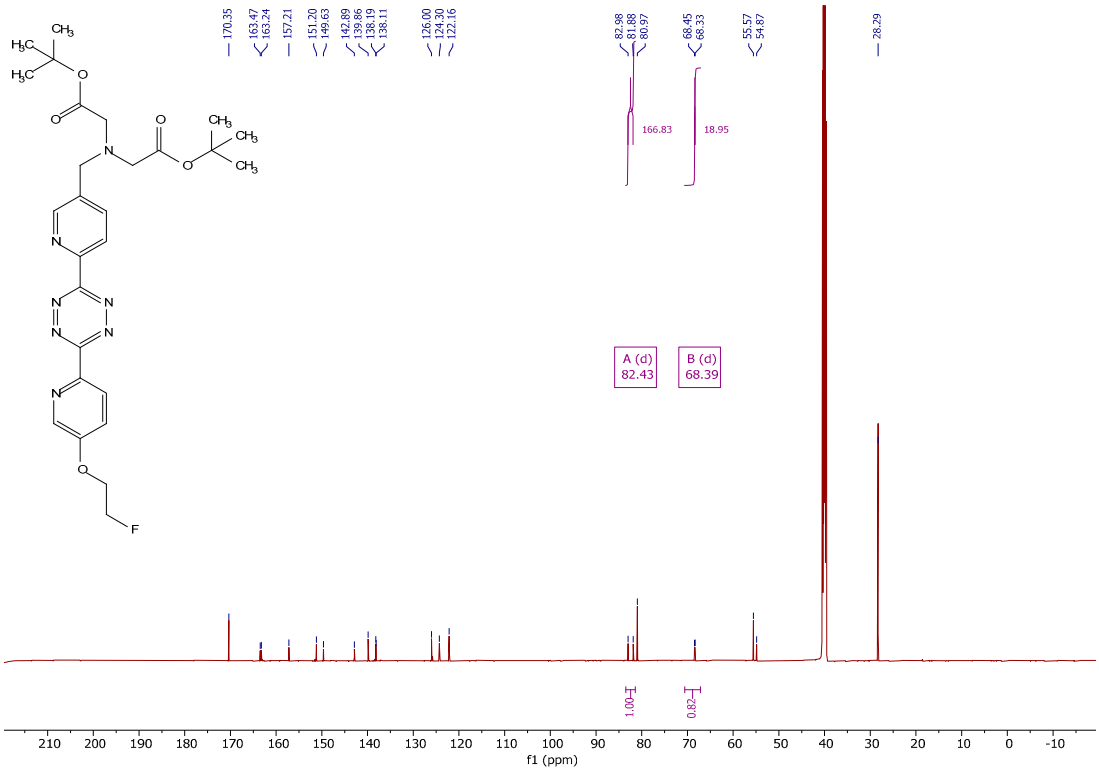
*Di-tert-butyl 2,2'-(((6-cyanopyridin-3-yl)methyl)azanediyl)diacetate (**43**)*

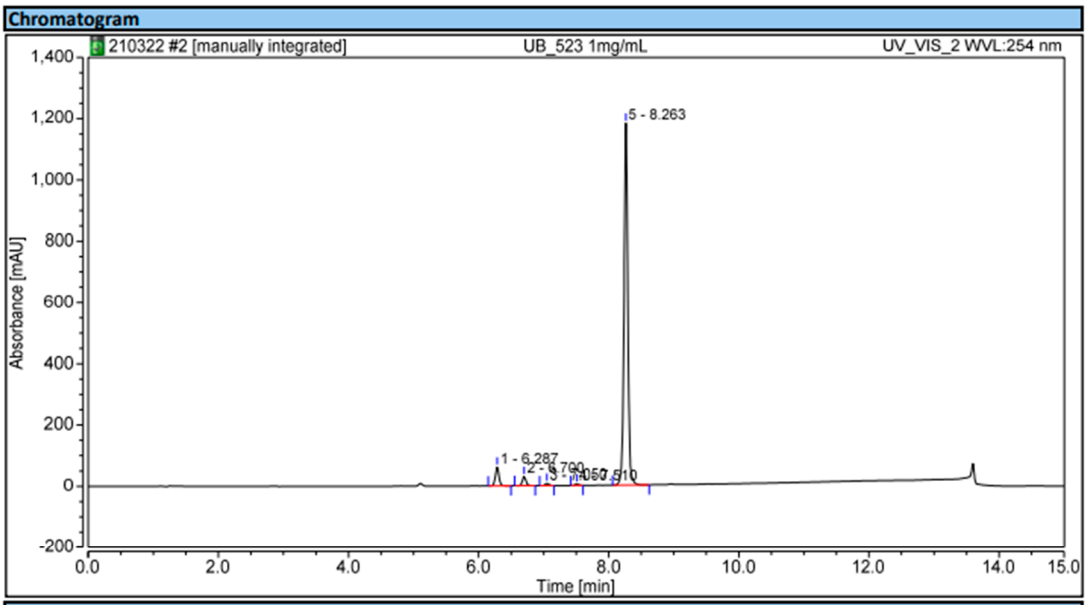

8.68
8.67
8.04
8.03
8.02
8.01
7.67
7.67
7.65
7.65
7.26
3.99
3.39
1.45
1.00
1.01
1.02
2.07
4.13
19.81
f1 (ppm)


170.09
151.41
138.96
137.51
132.77
128.27
117.35
81.52
55.41
54.57
28.16
f1 (ppm)

*Di-tert-butyl 2,2'-(((6-(6-(5-(2-fluoroethoxy)pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)methyl)- azanediyl)diacetate (**44**)*



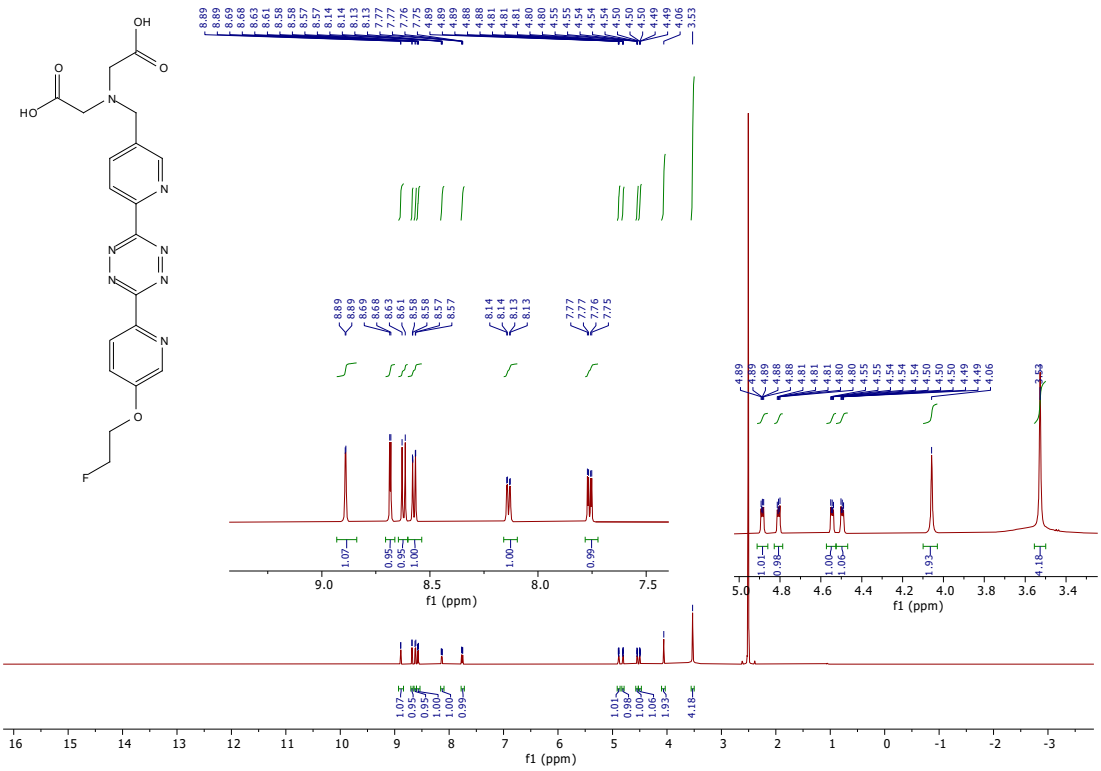


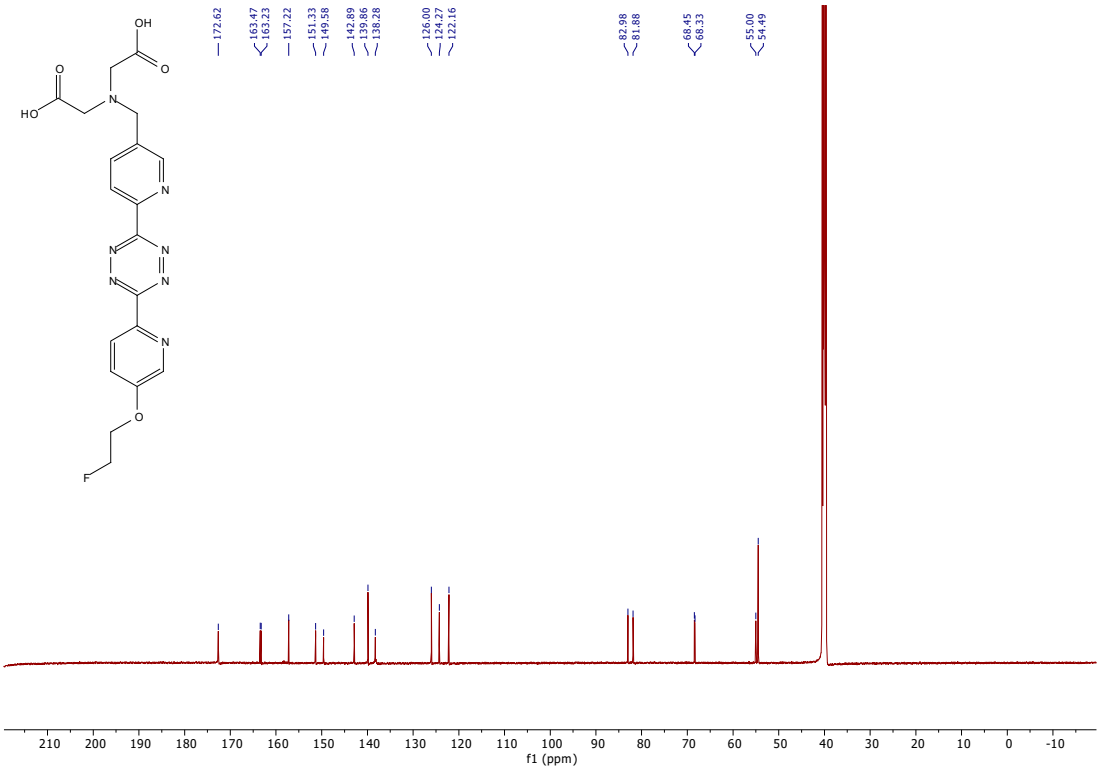


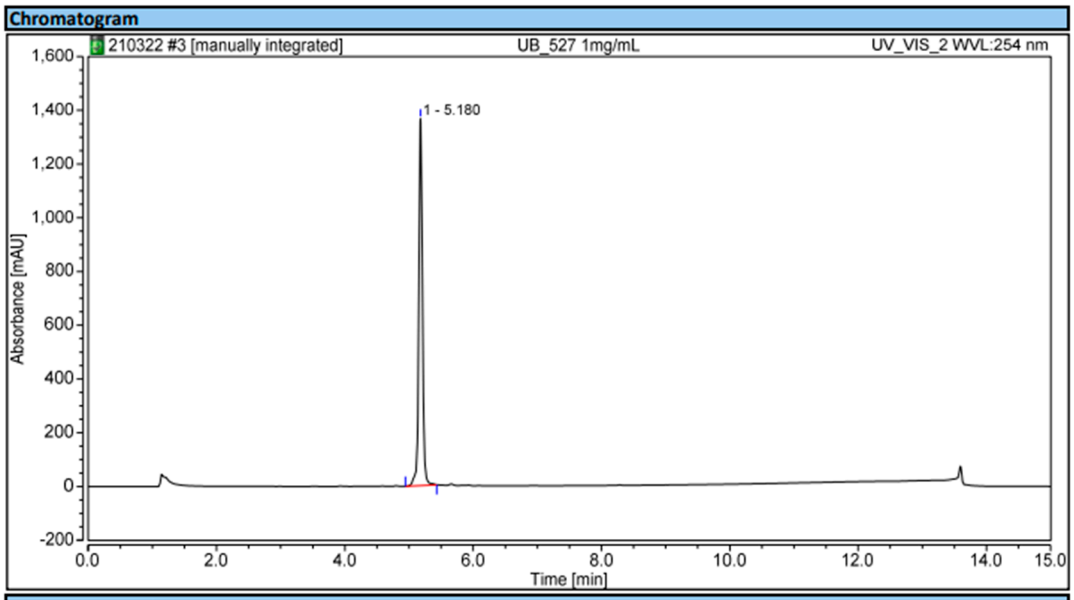
Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1		6.287	3.887	61.435	4.48	4.78
2		6.700	1.780	29.761	2.05	2.32
3		7.050	0.470	7.431	0.54	0.58
4		7.510	0.298	4.813	0.34	0.37
5		8.263	80.362	1181.589	92.59	91.95
Total:			**86.796**	**1285.028**	**100.00**	**100.00**

*2,2'-(((6-(6-(5-(2-fluoroethoxy)pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)methyl) azanediyl)diacetic acid (**45**)*



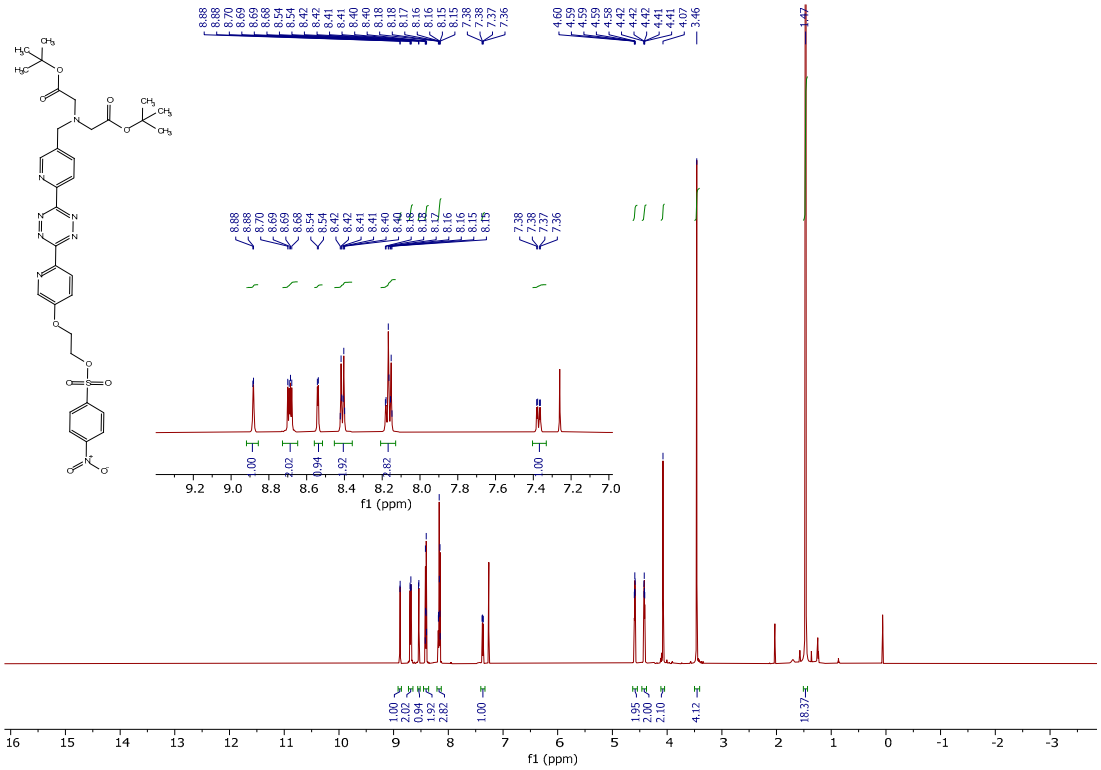


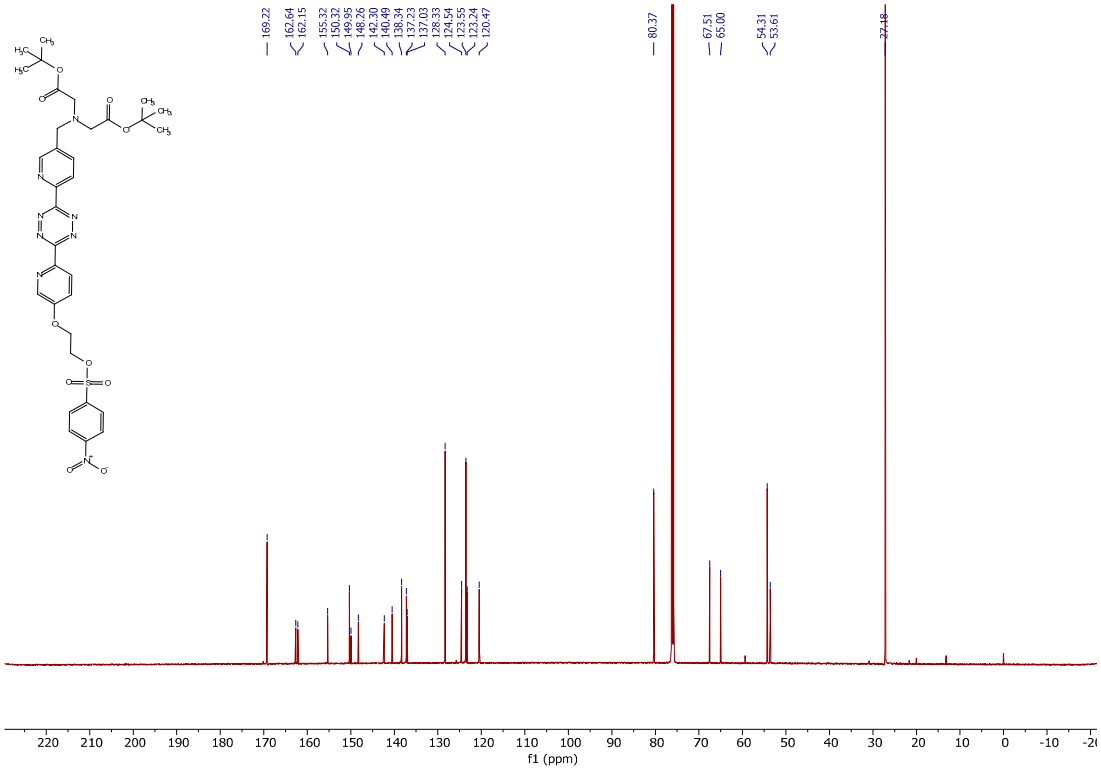


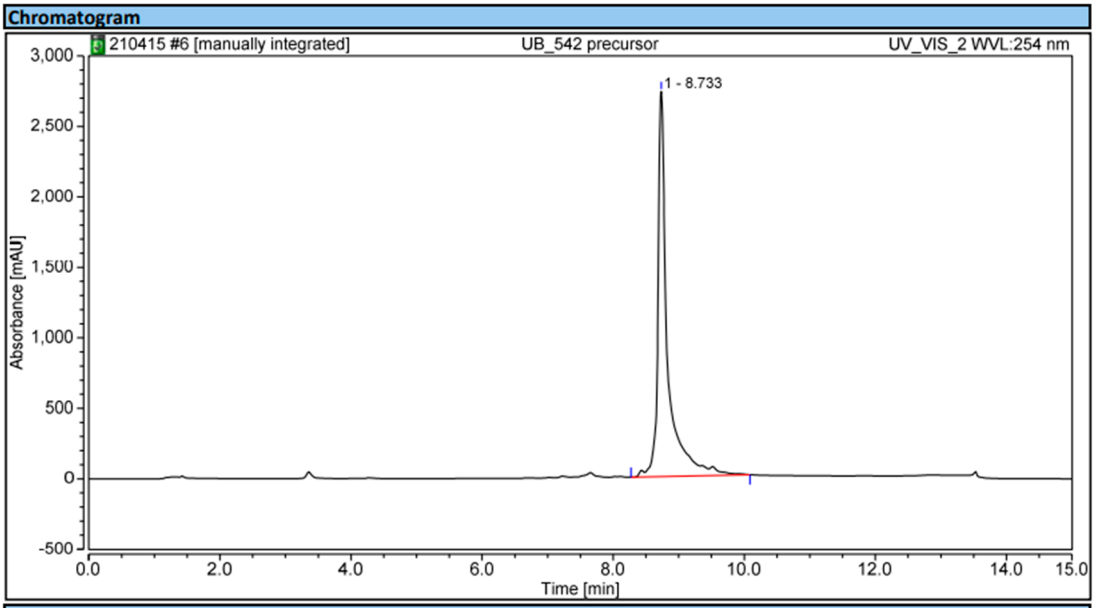
Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1		5.180	89.203	1364.904	100.00	100.00
Total:			**89.203**	**1364.904**	**100.00**	**100.00**

*Di-tert-butyl 2,2'-(((6-(6-(5-(2-(((4-nitrophenyl)sulfonyl)oxy)ethoxy)pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)methyl)azanediyl)diacetate (**45a**)*



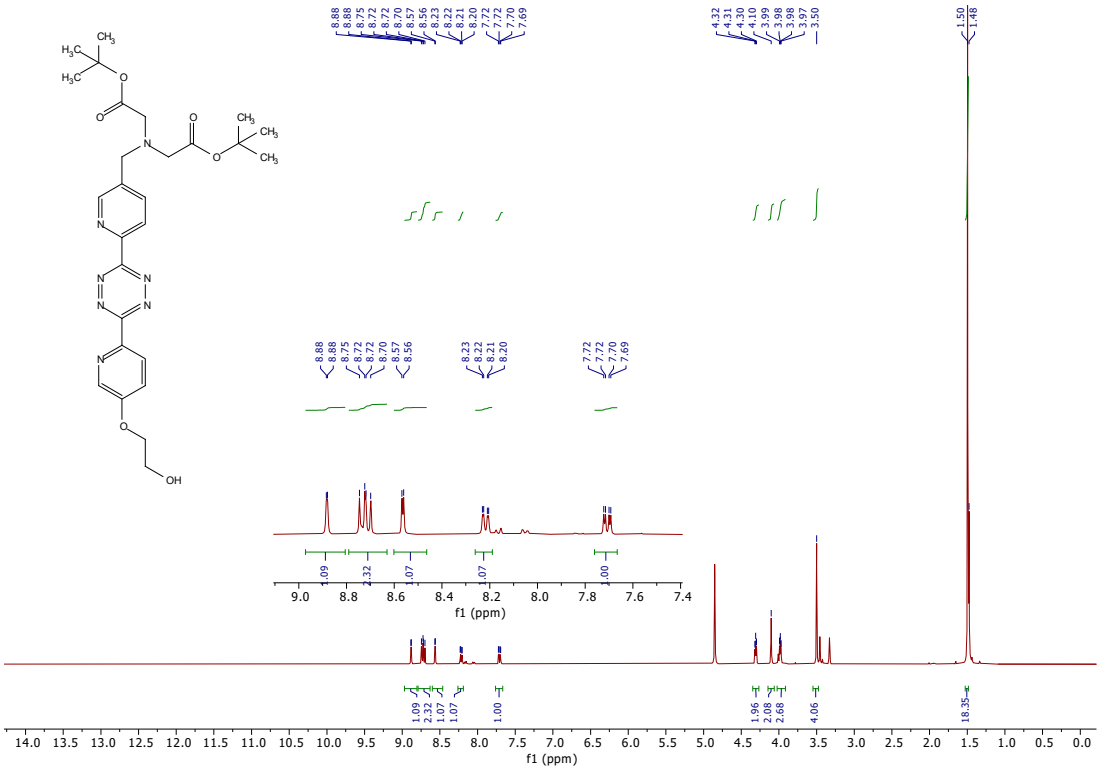


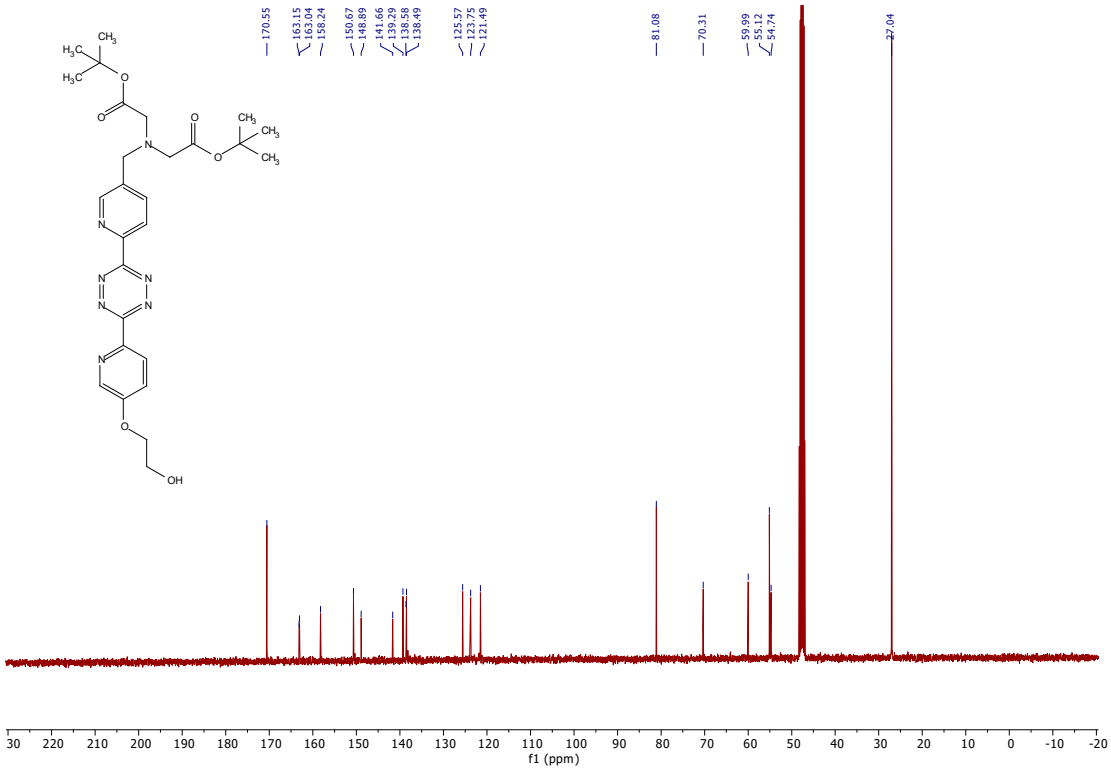


Integration Results

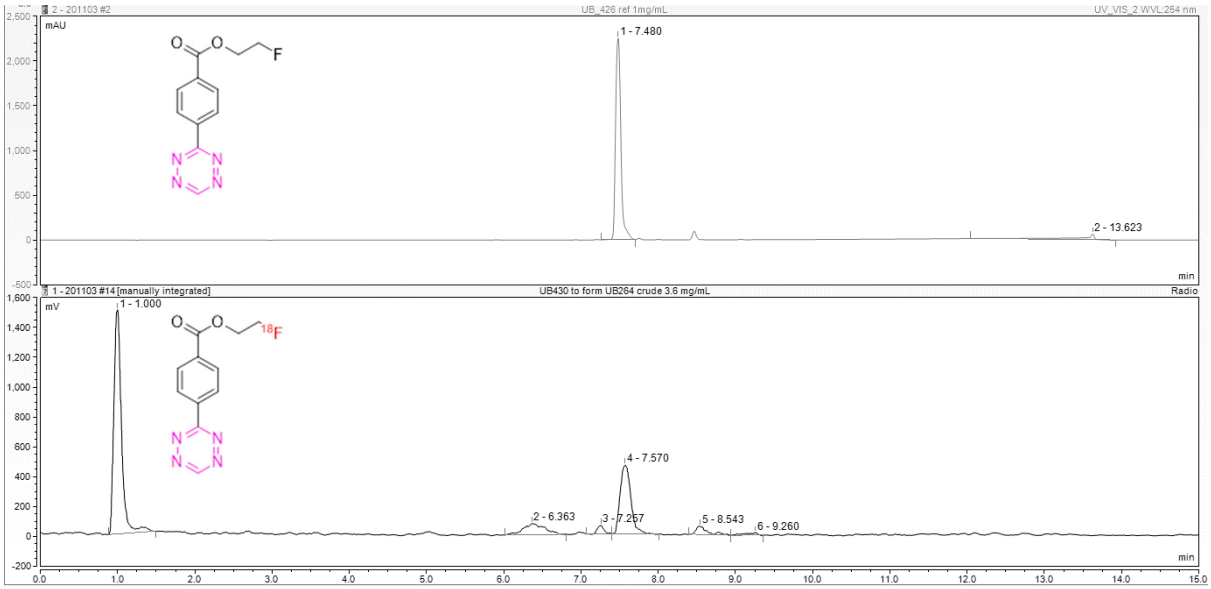
No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1		8.733	468.202	2731.135	100.00	100.00
Total:			**468.202**	**2731.135**	**100.00**	**100.00**

*Di-tert-butyl 2,2'-(((6-(6-(5-(2-hydroxyethoxy)pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)methyl)azanediyl)diacetate (**46**)*

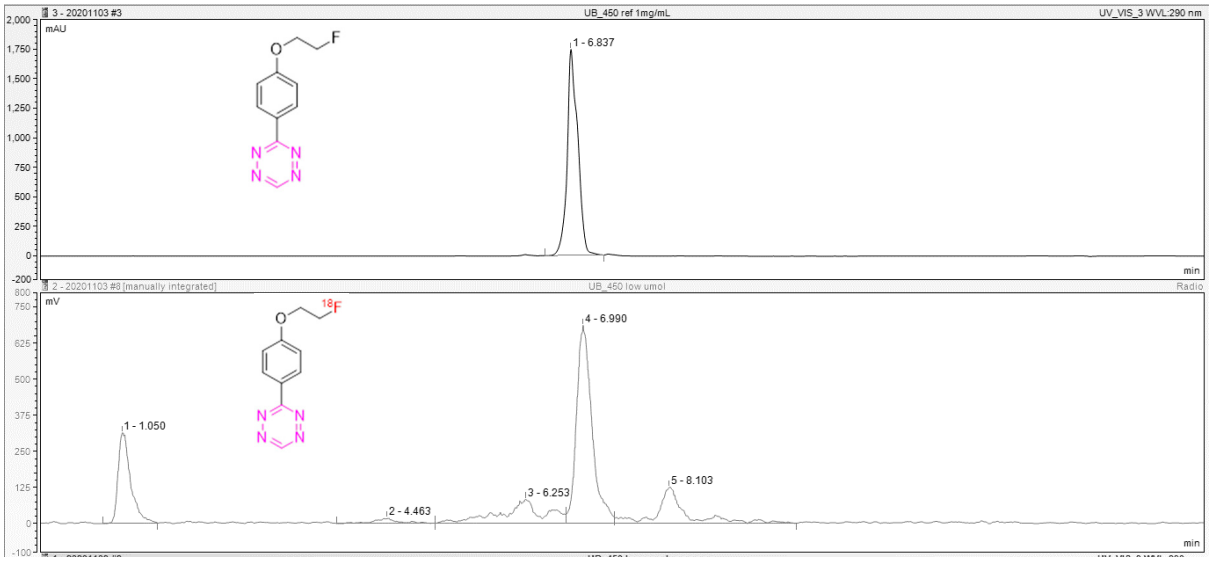




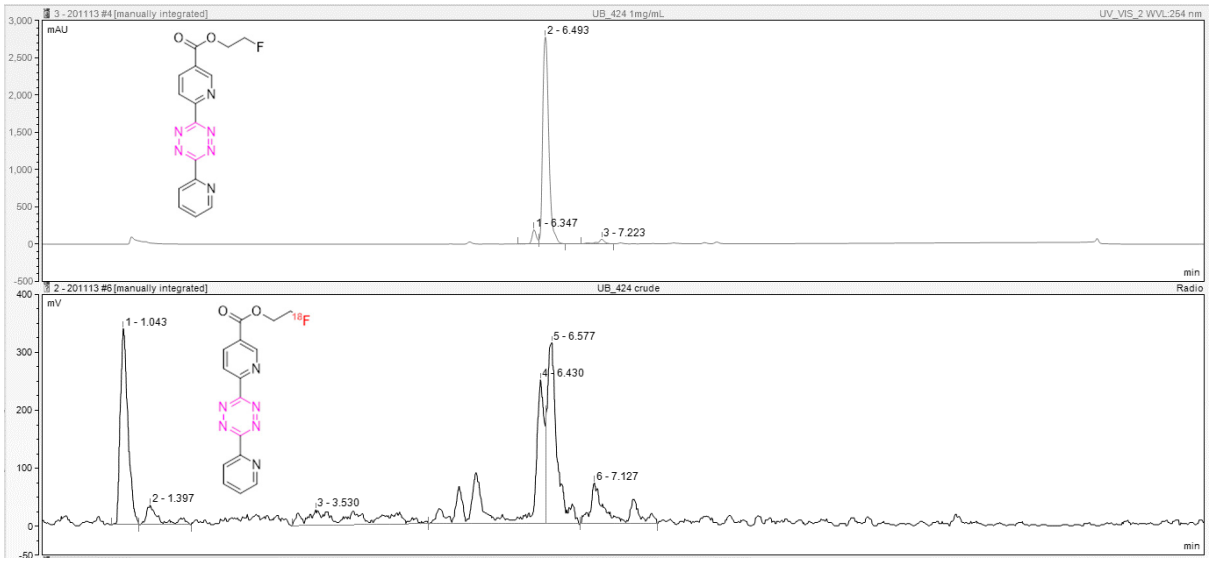
Labeling of compound [^{18}F]**1**



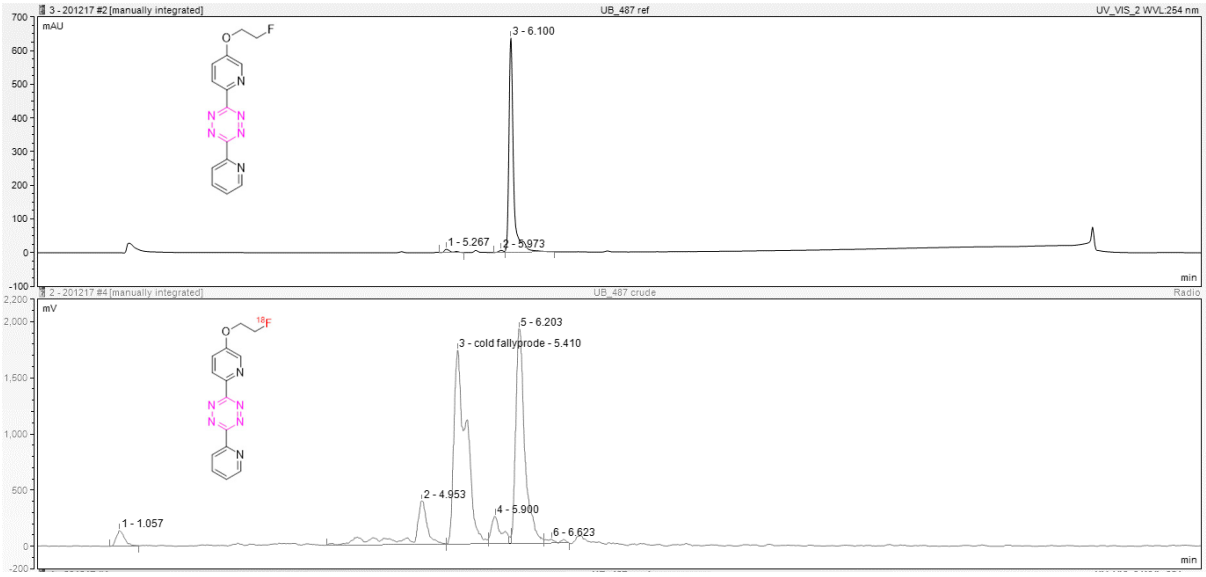
Labeling of compound [^{18}F]**8**



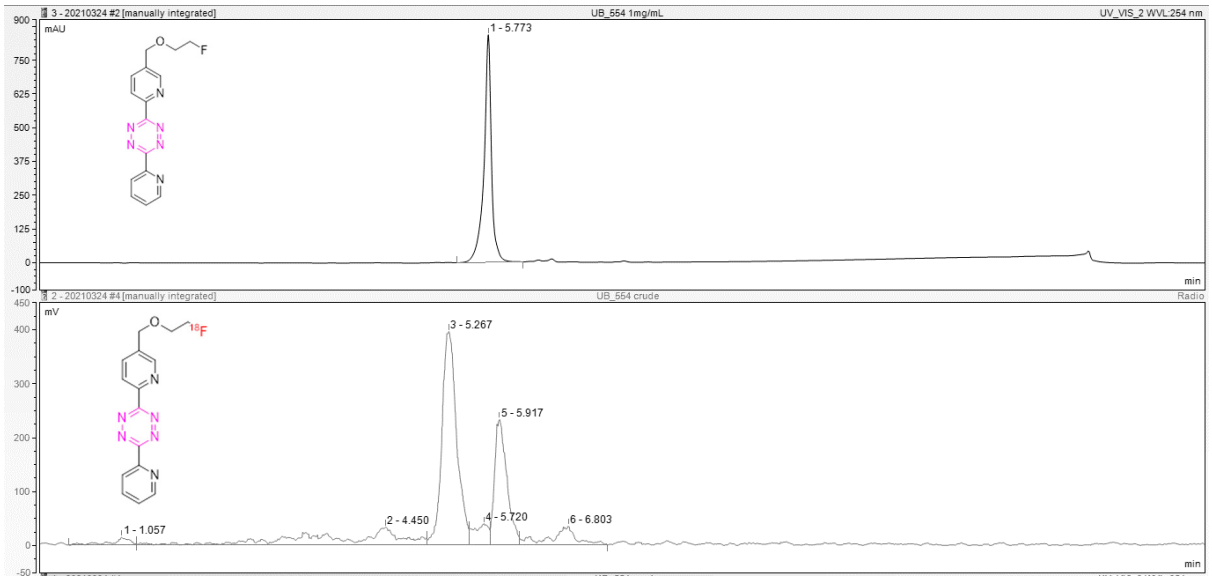
Labeling of compound [^{18}F]**33**



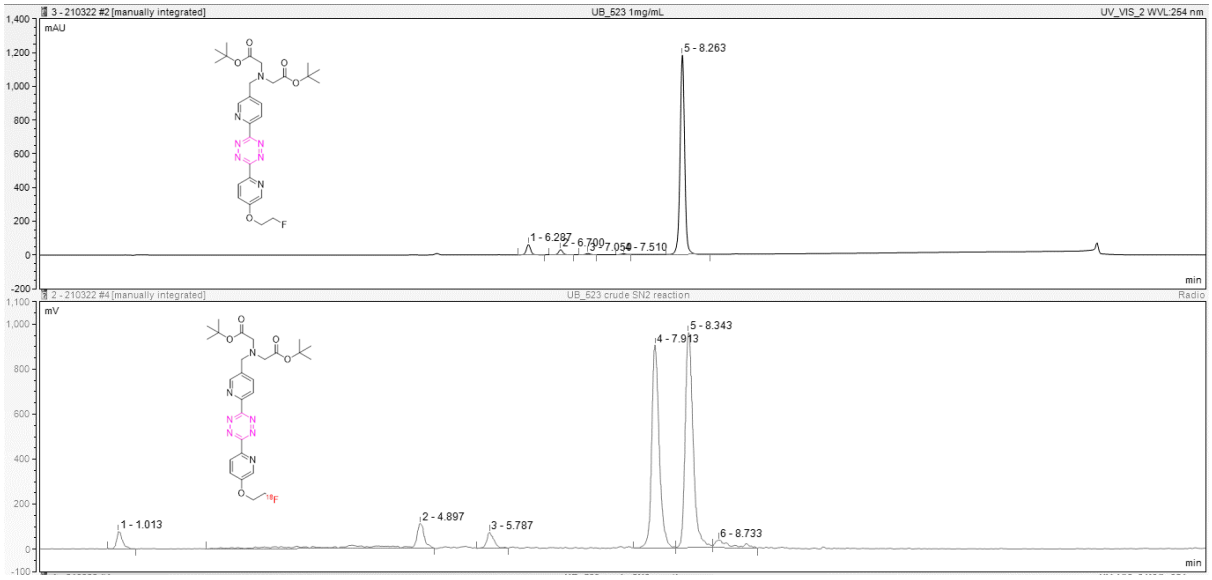
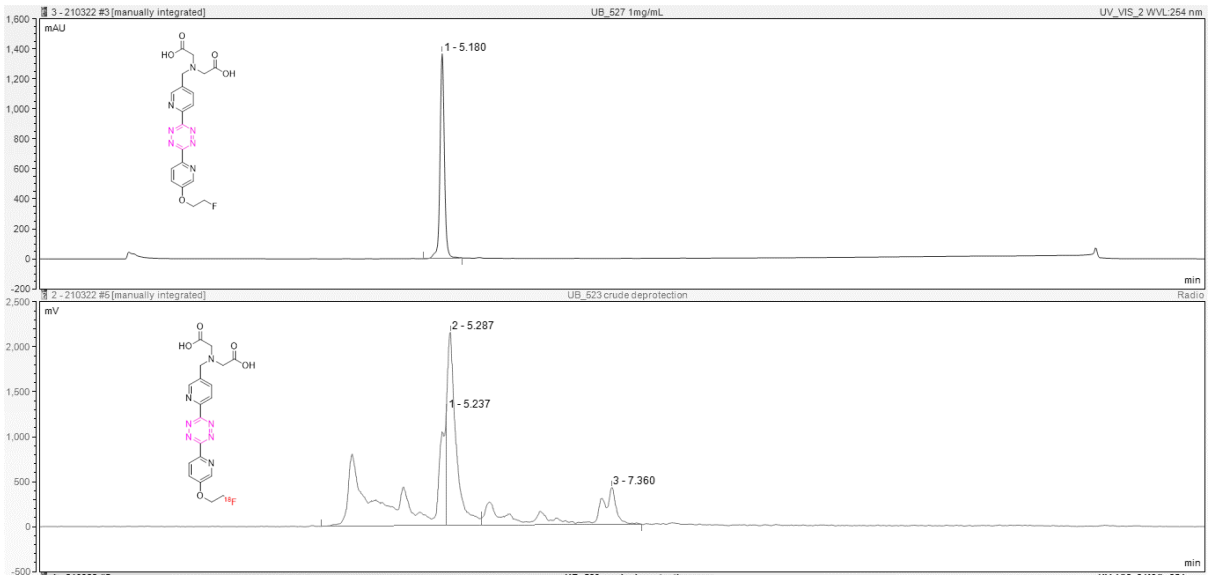
Labeling of compound [^{18}F]**38**



Labeling of compound [^{18}F]**41**



Labeling of compound [^{18}F]**44**



Deprotection of compound [^{18}F]**44**