

Supporting Information

Tumor-targeted delivery of 6-Diazo-5-oxo-L-norleucine (DON) using substituted acetylated lysine prodrugs

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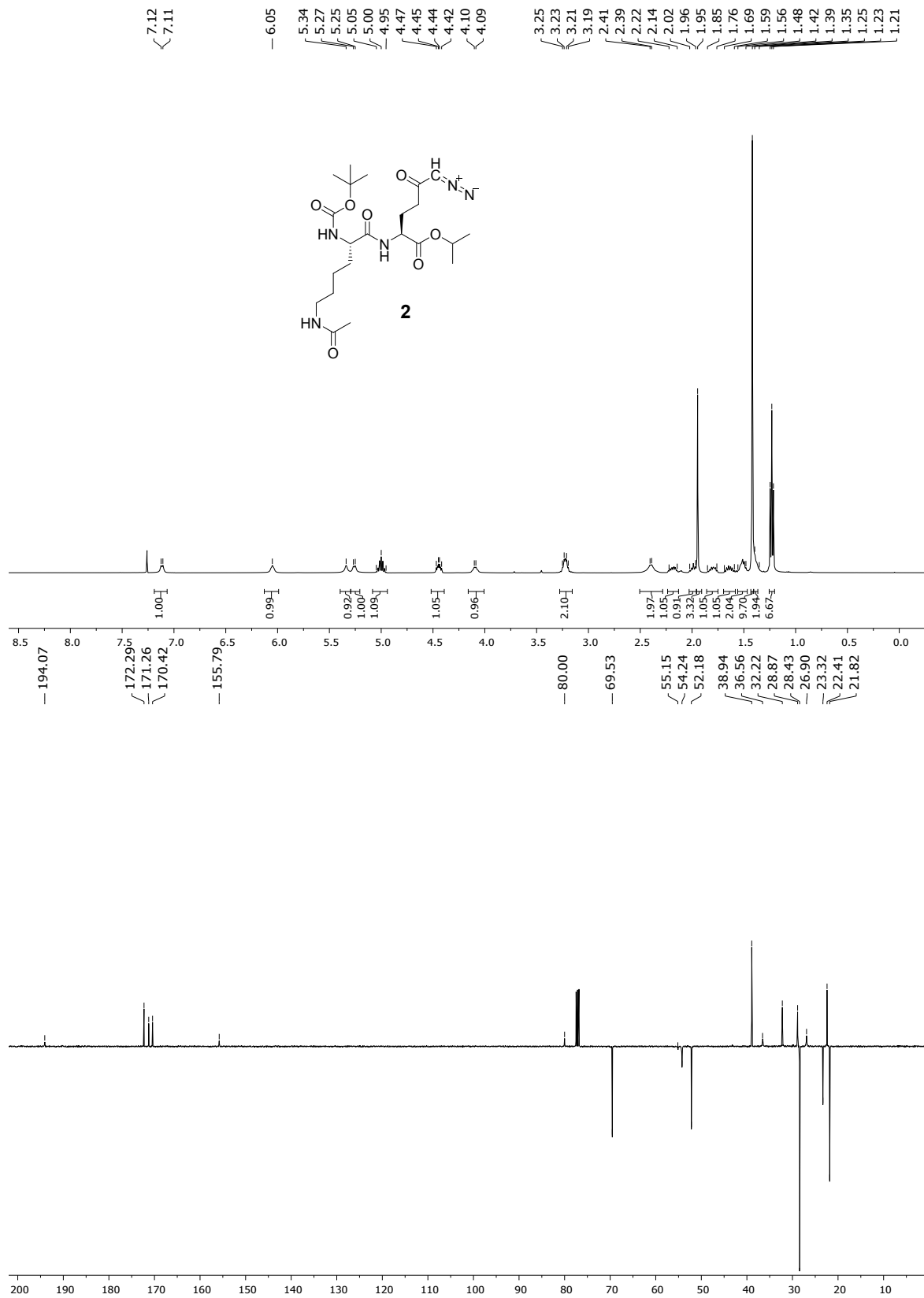
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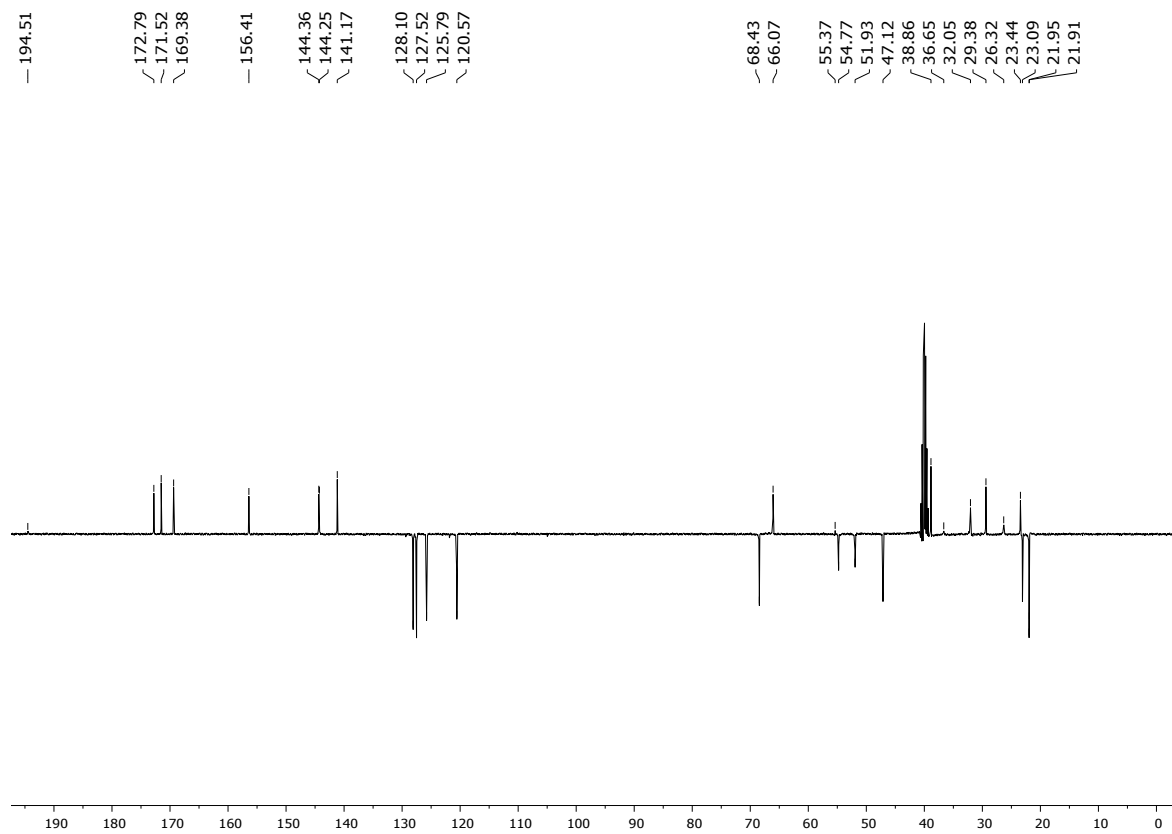
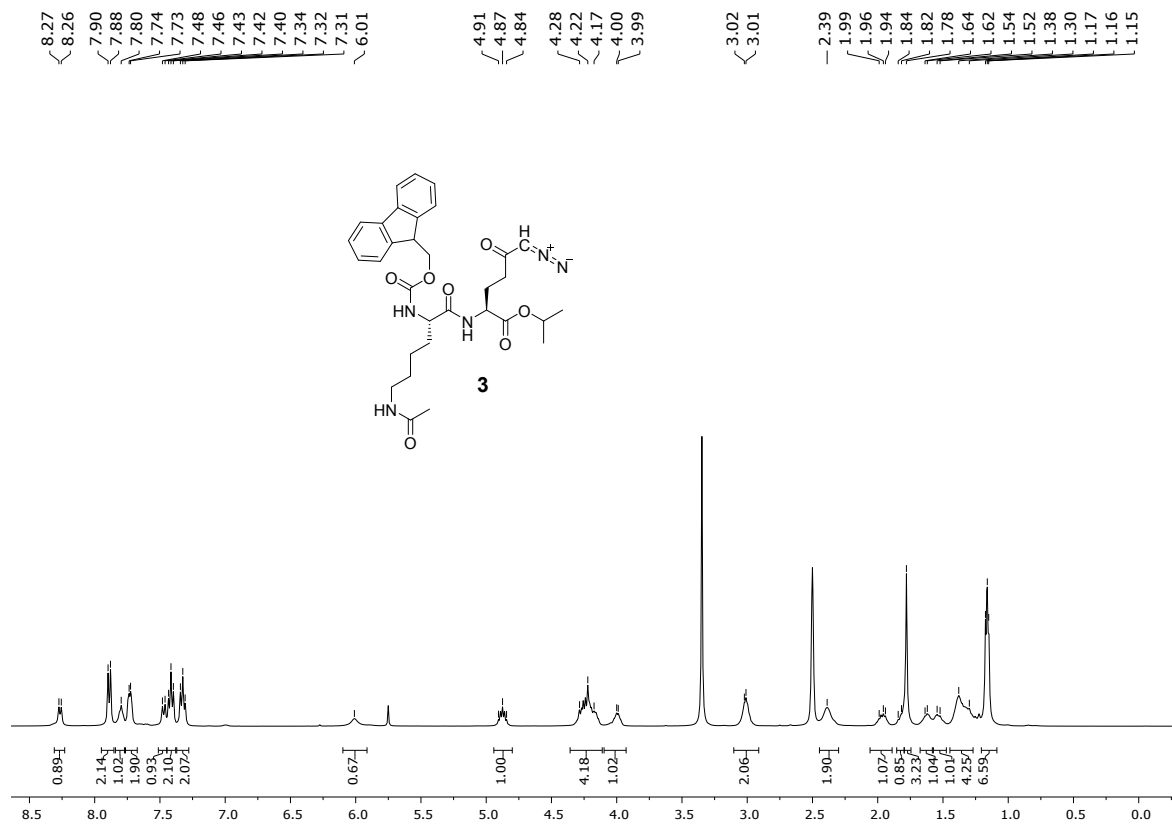
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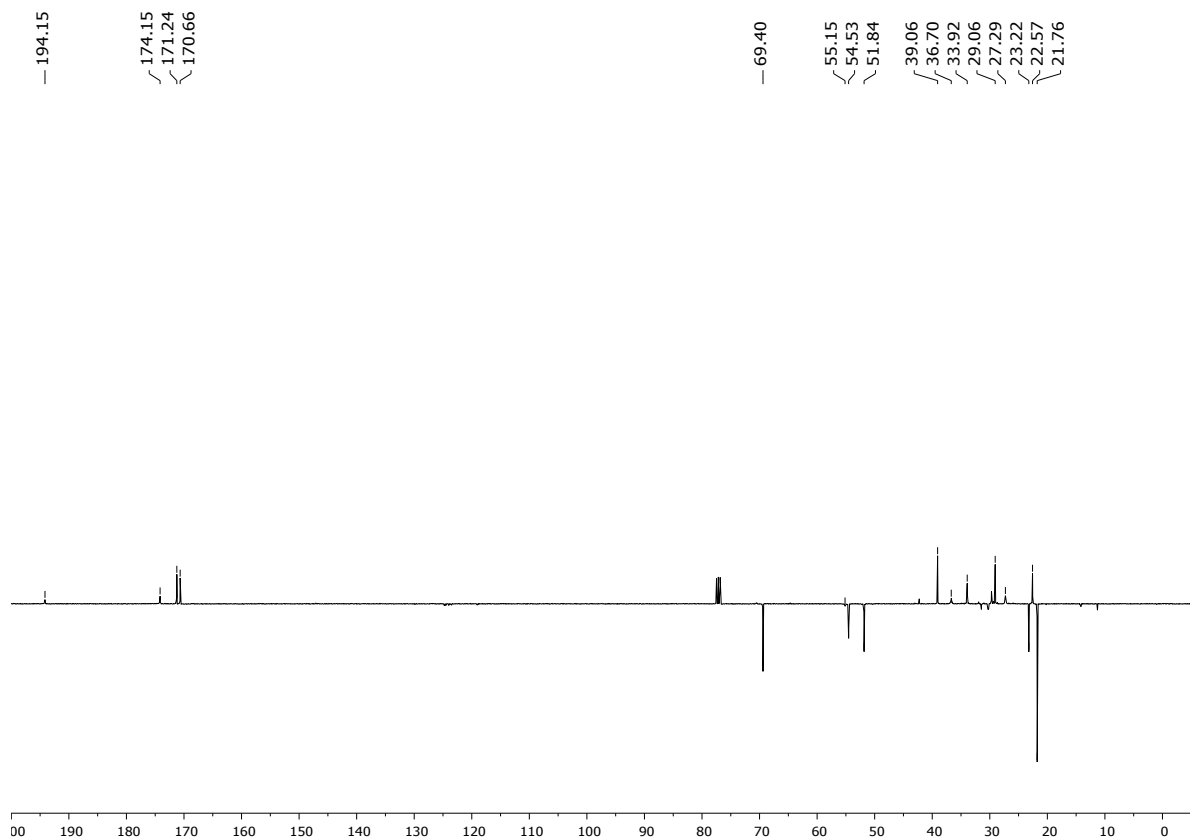
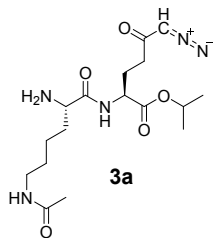
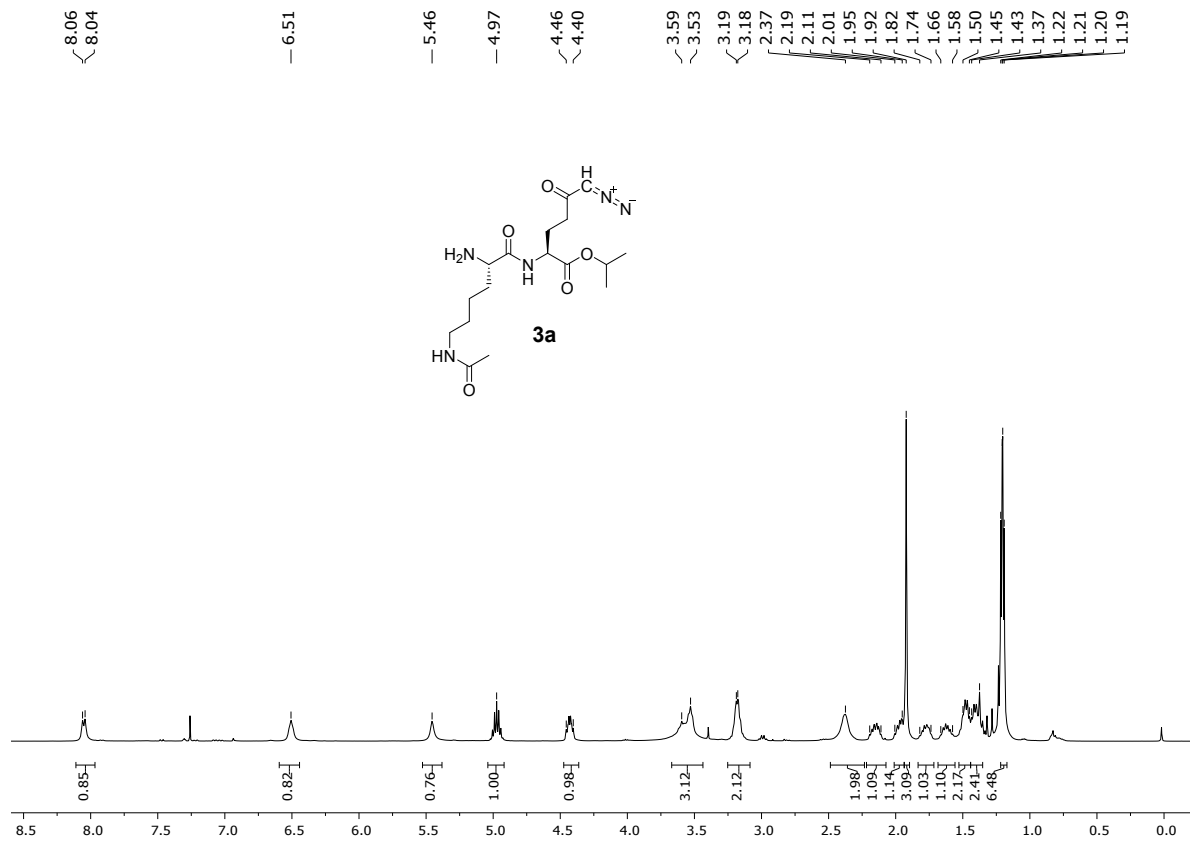
The ^1H NMR spectra were measured at 400.1 MHz and ^{13}C NMR spectra at 100.8 MHz.

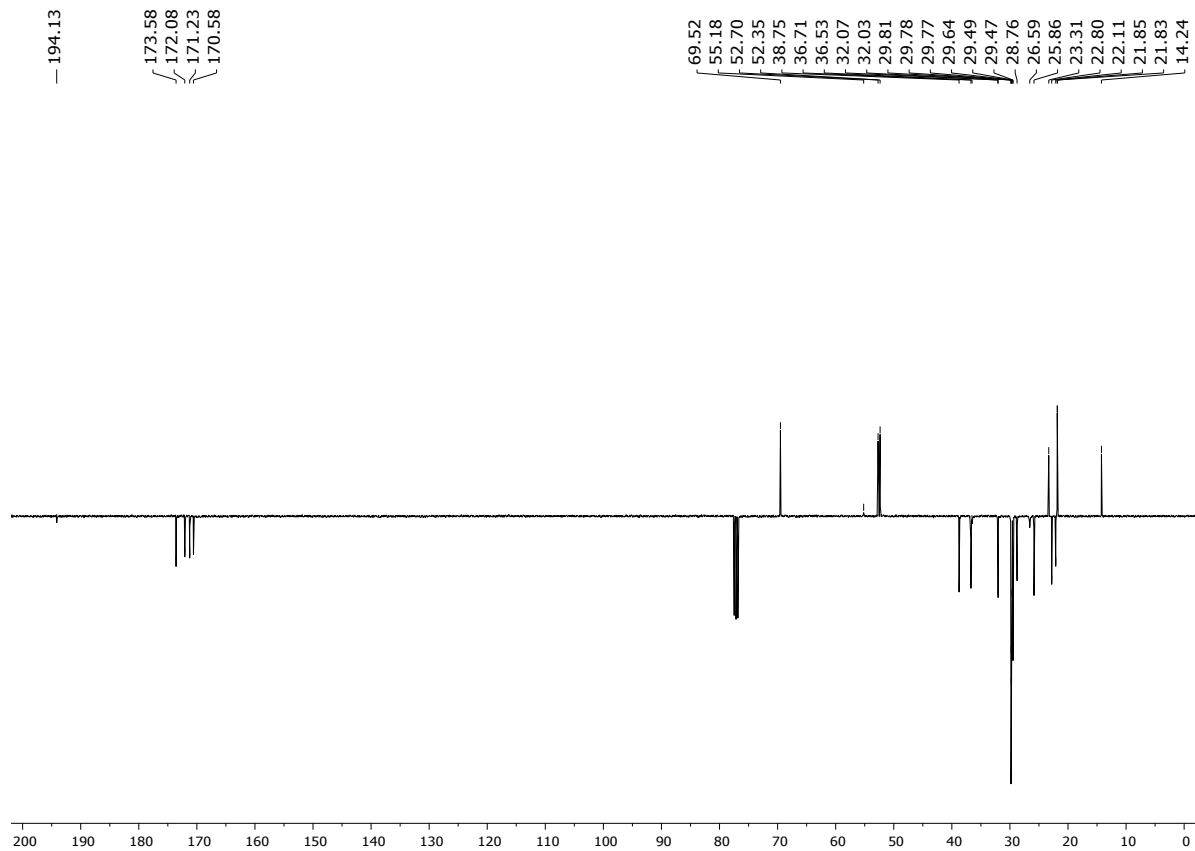
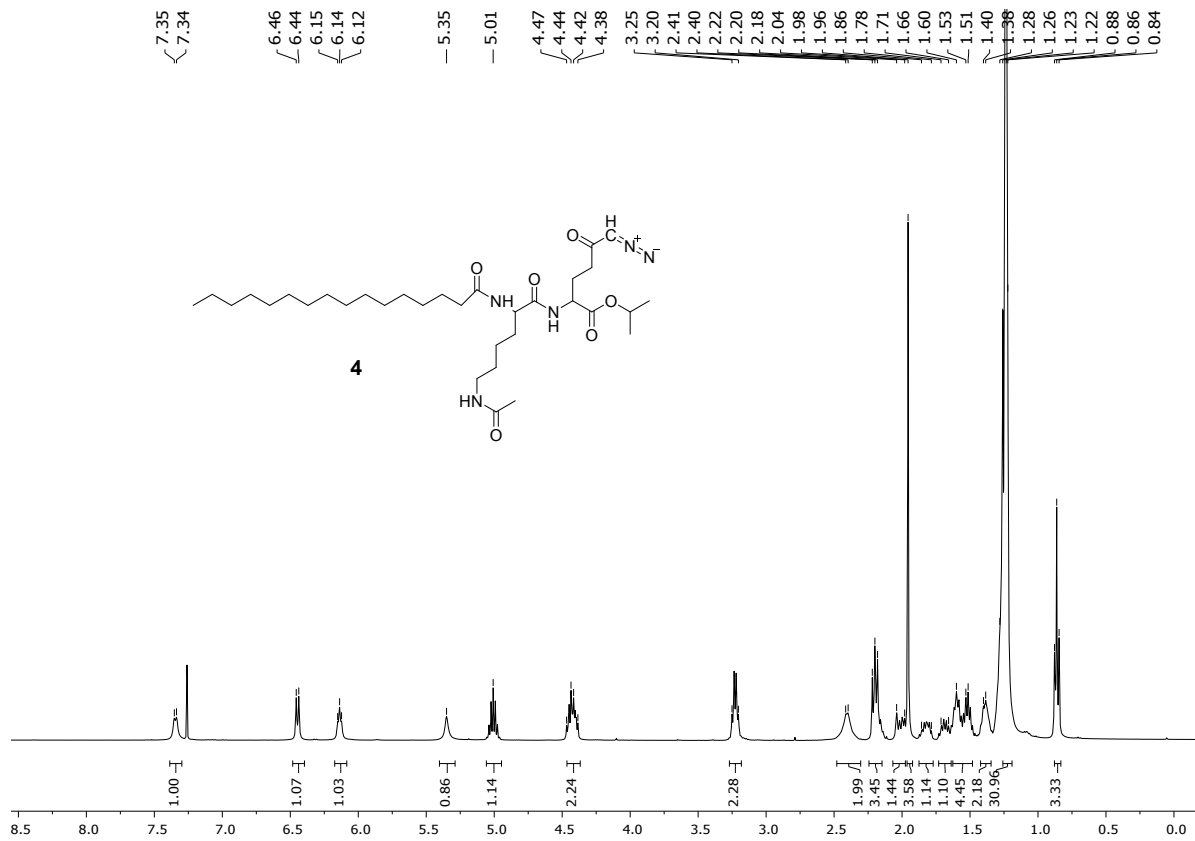
Conditions for HPLC analysis:

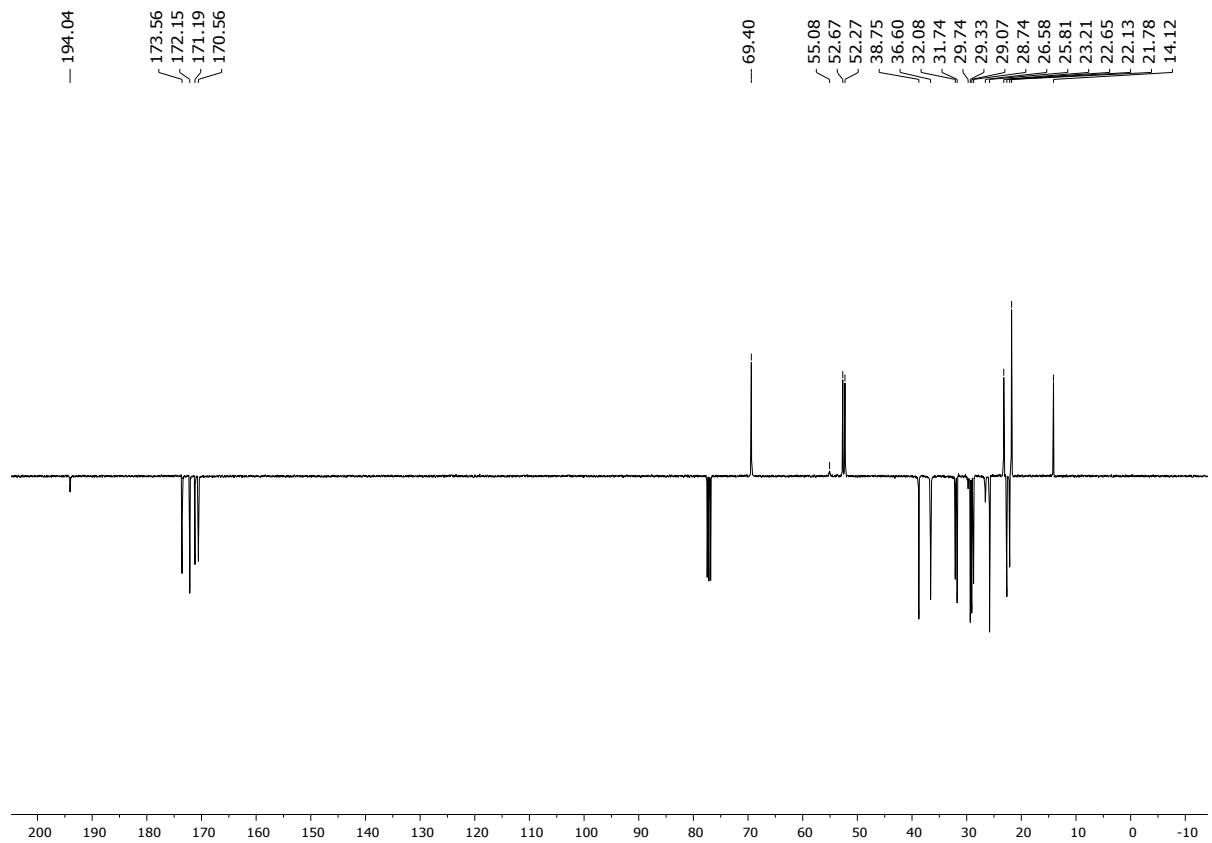
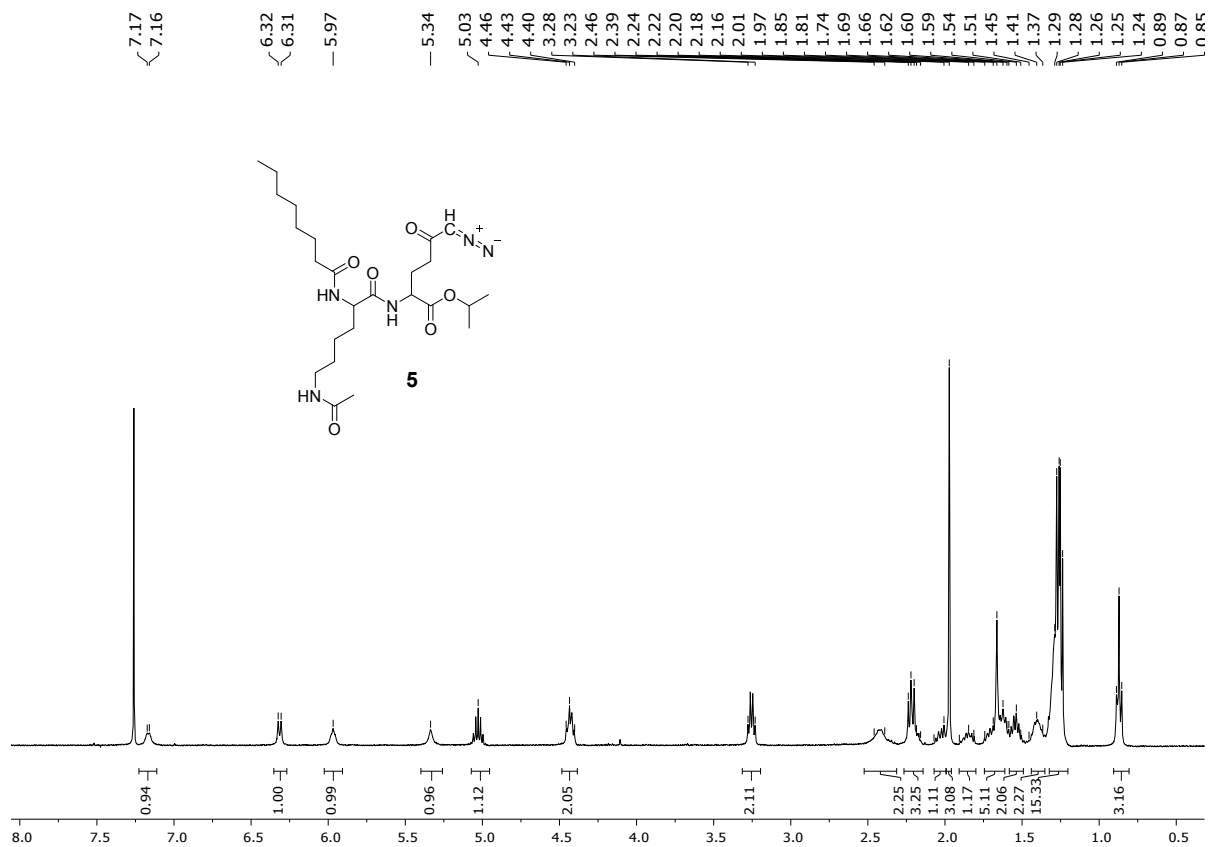
We utilized an HPLC (Jasco Inc.) equipped with a Reprosil 100 C18, 5 μm , 250 mm \times 4 mm column. The analysis was performed using a gradient of 2% $\text{CH}_3\text{CN}/98\%$ H_2O with 0.1% formic acid \rightarrow 100% CH_3CN , with UV detection, $\lambda = 210$ nm.

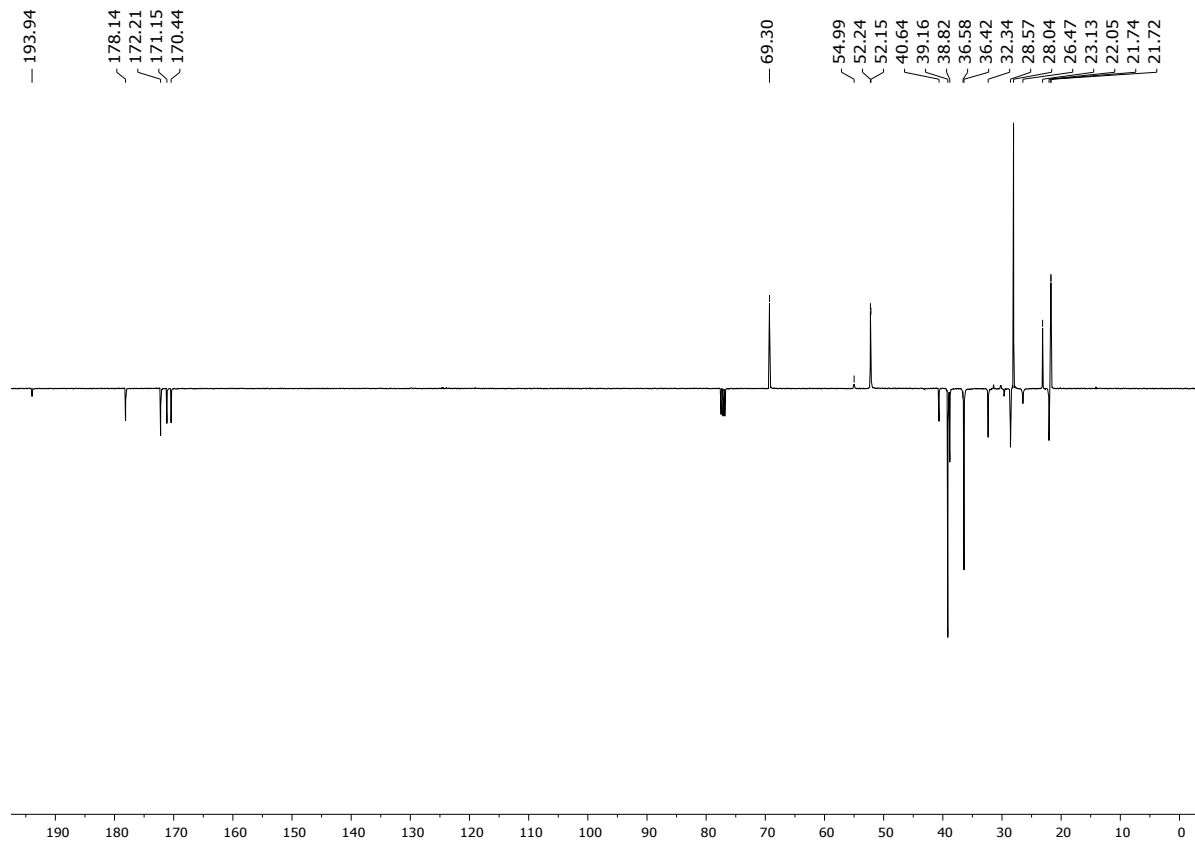
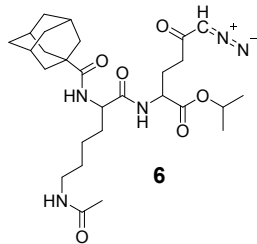
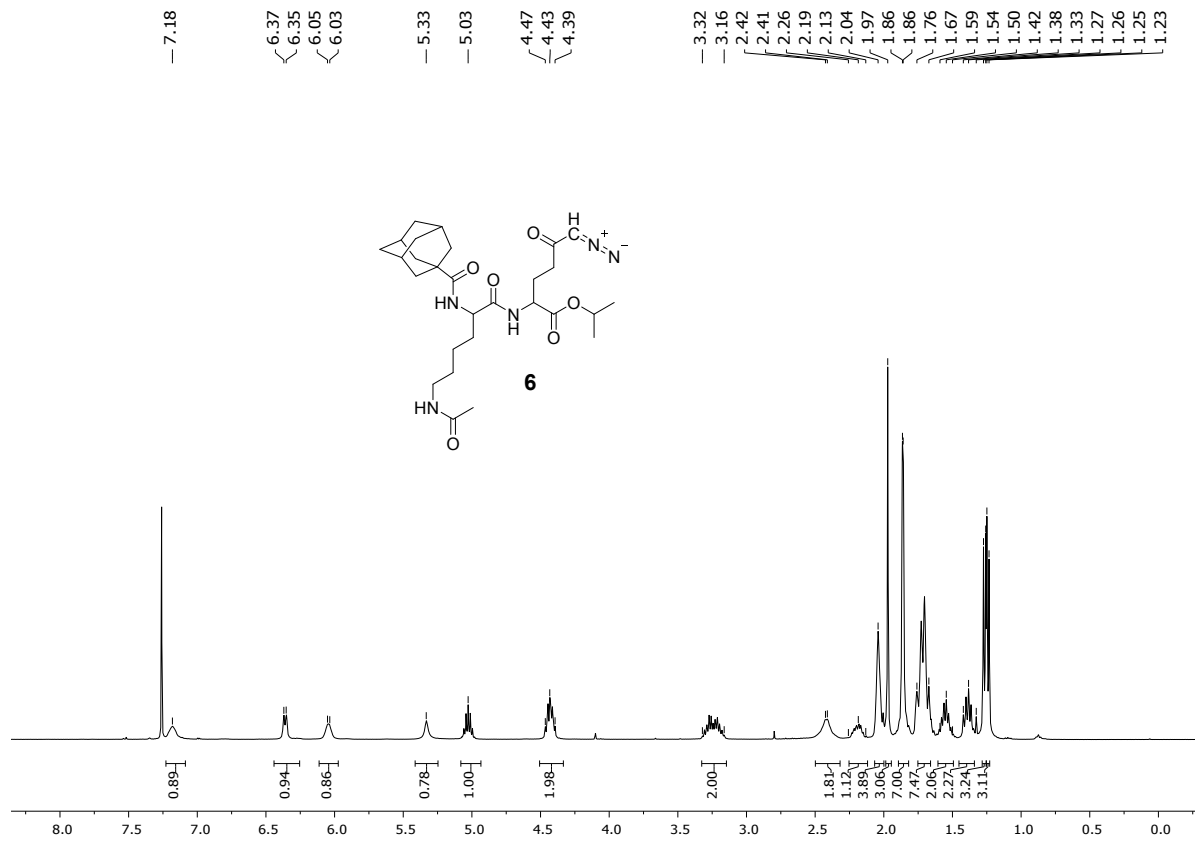


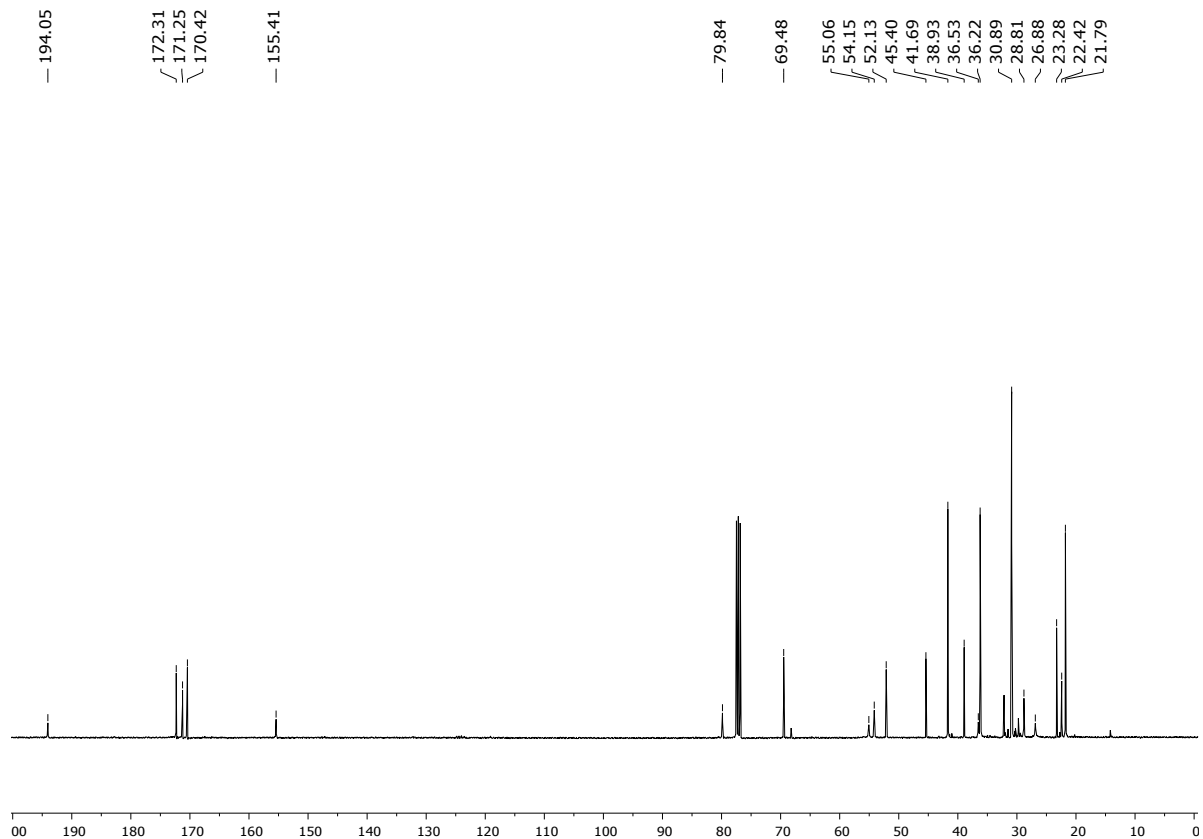
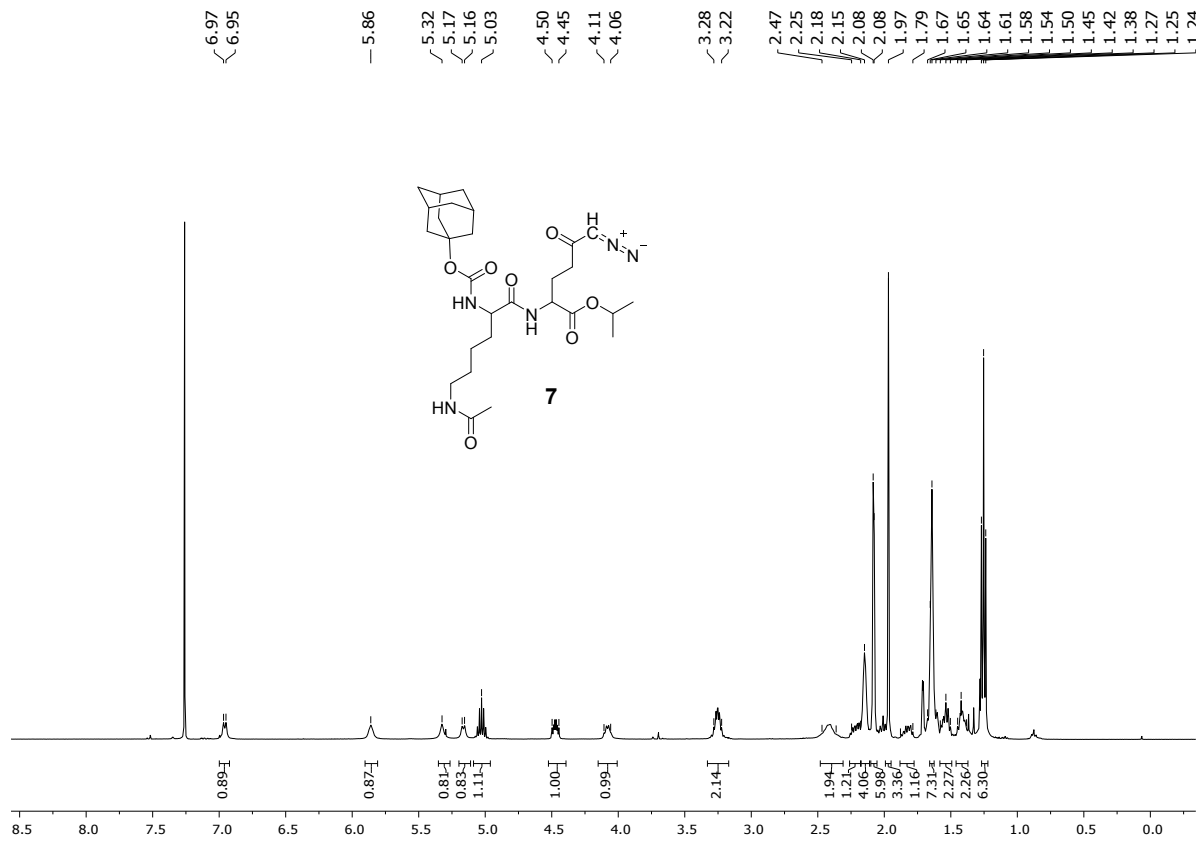


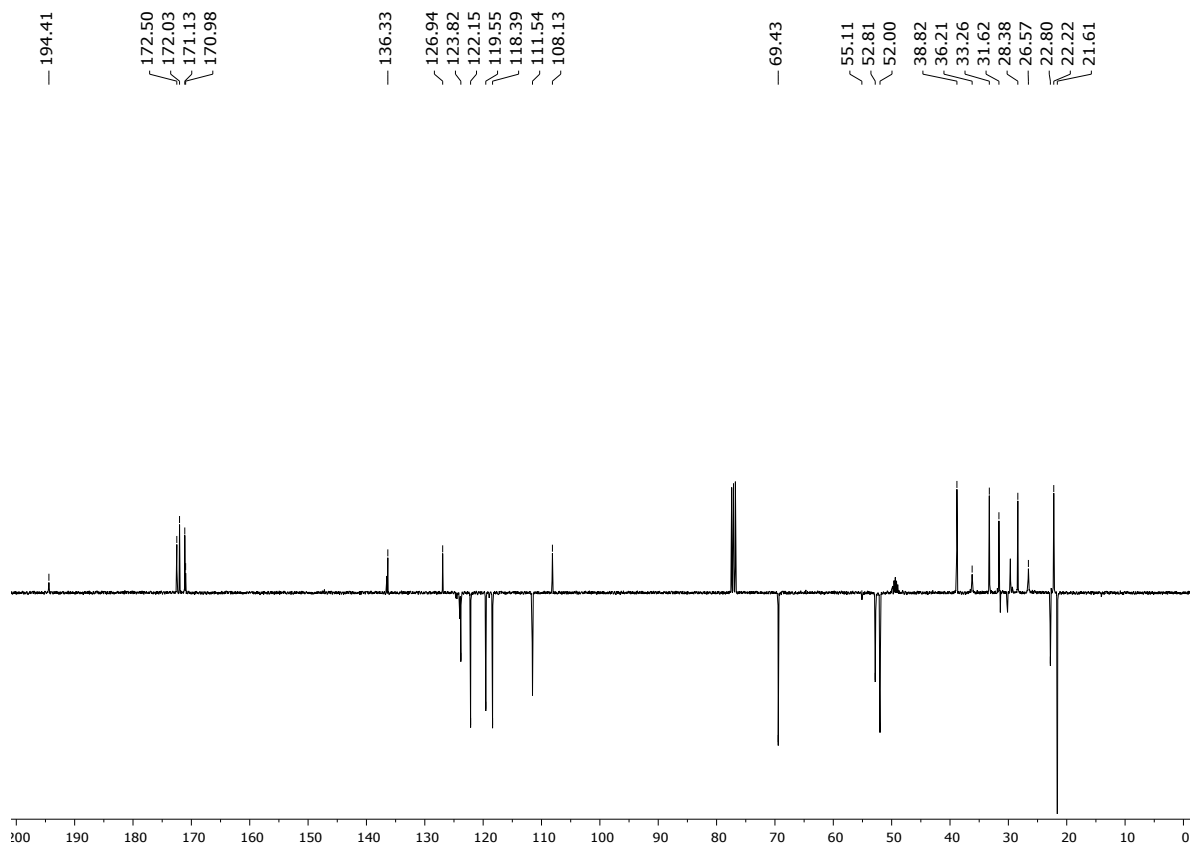
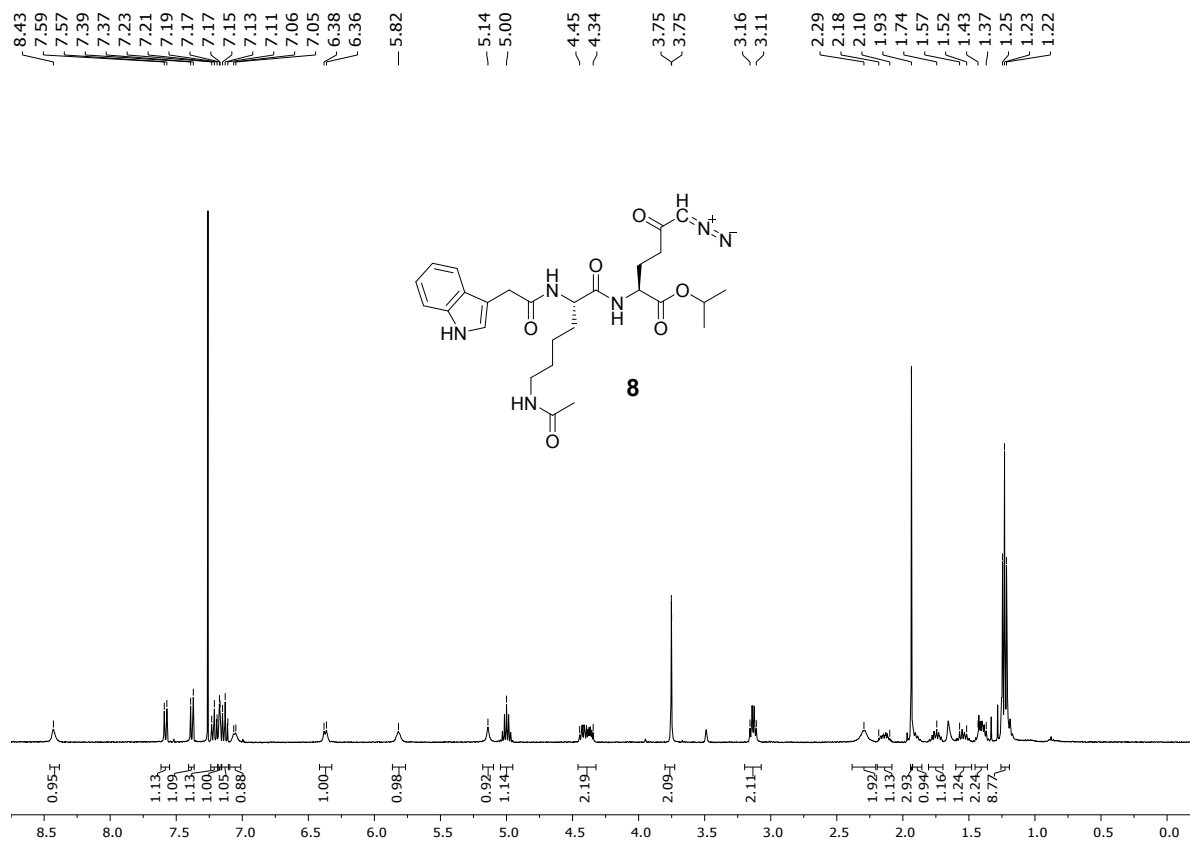


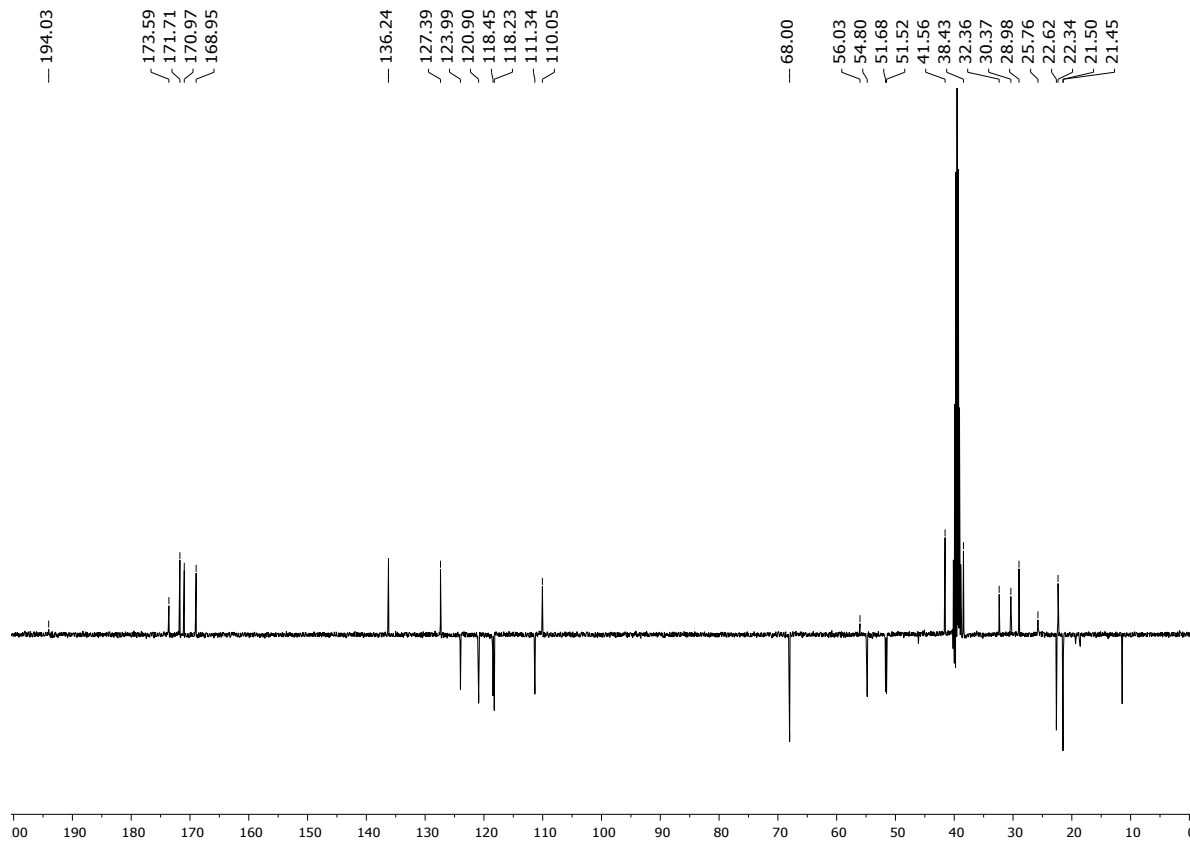
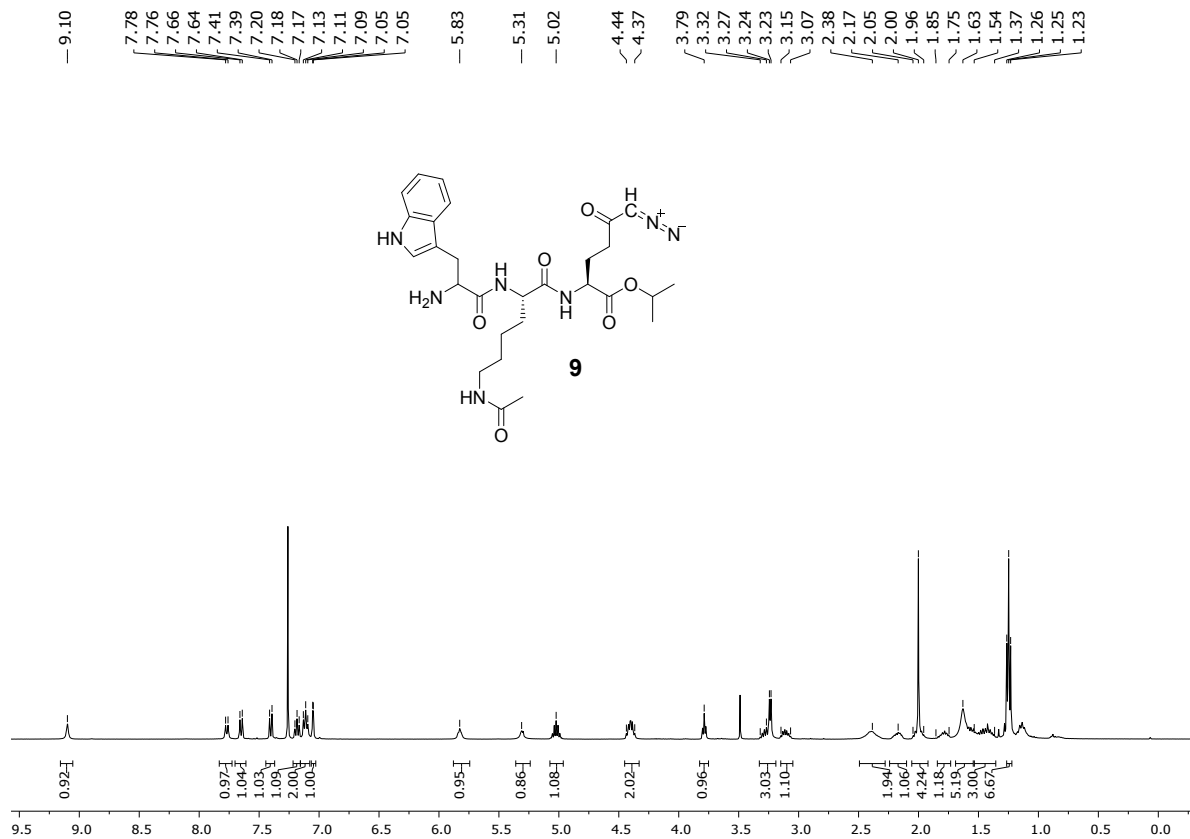


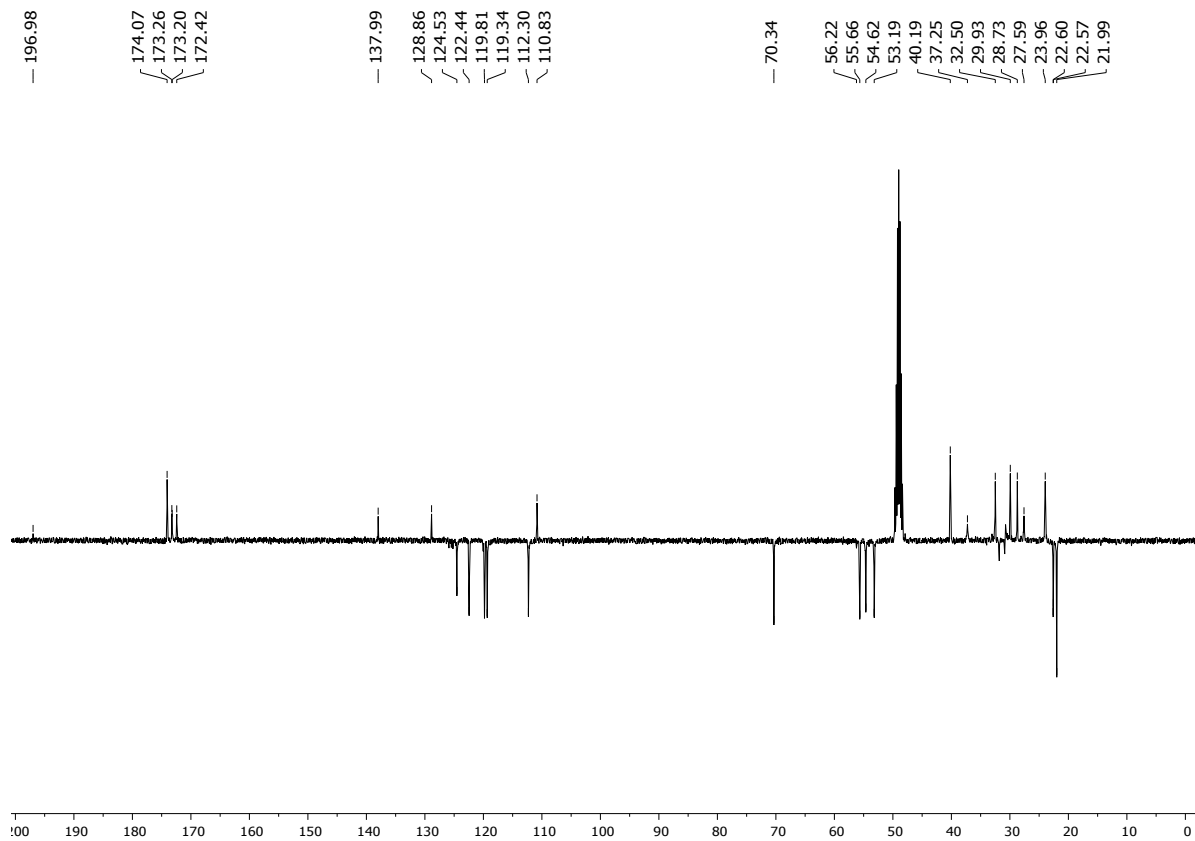
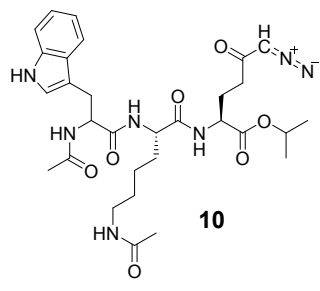
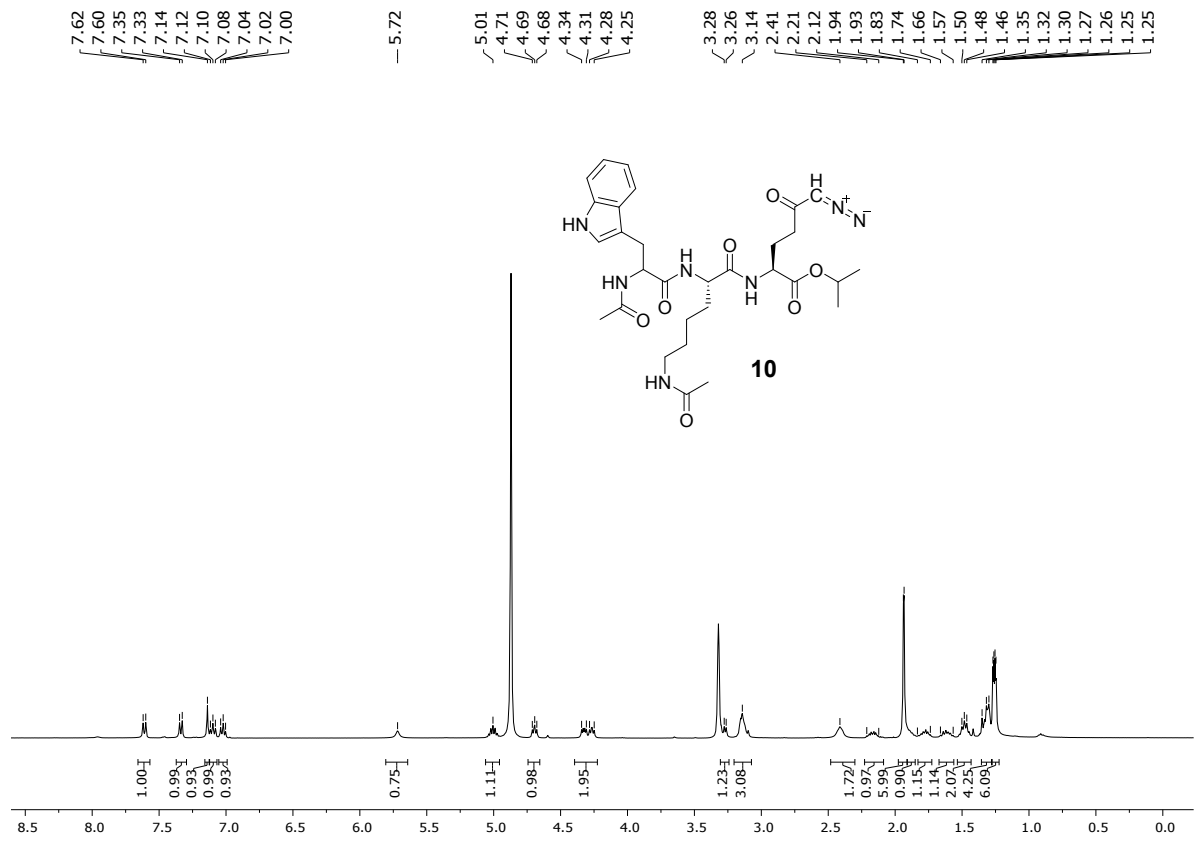


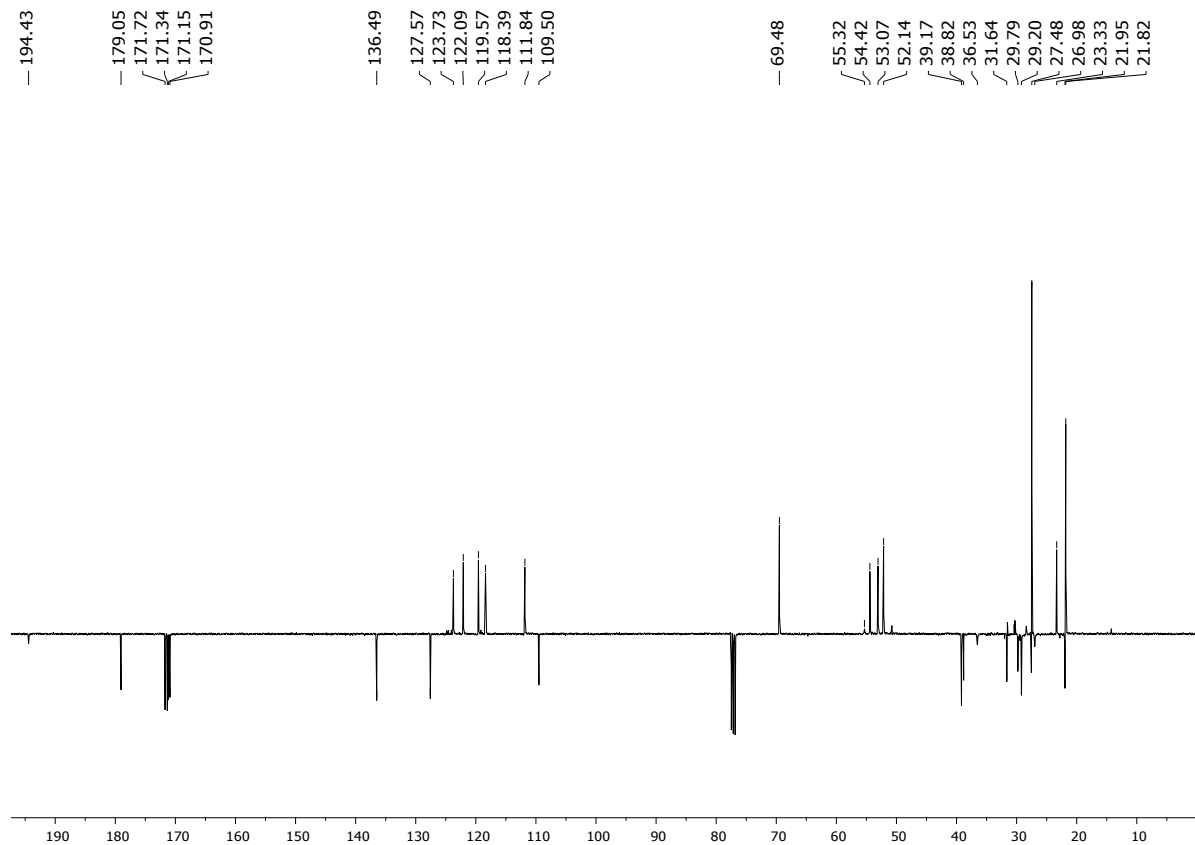
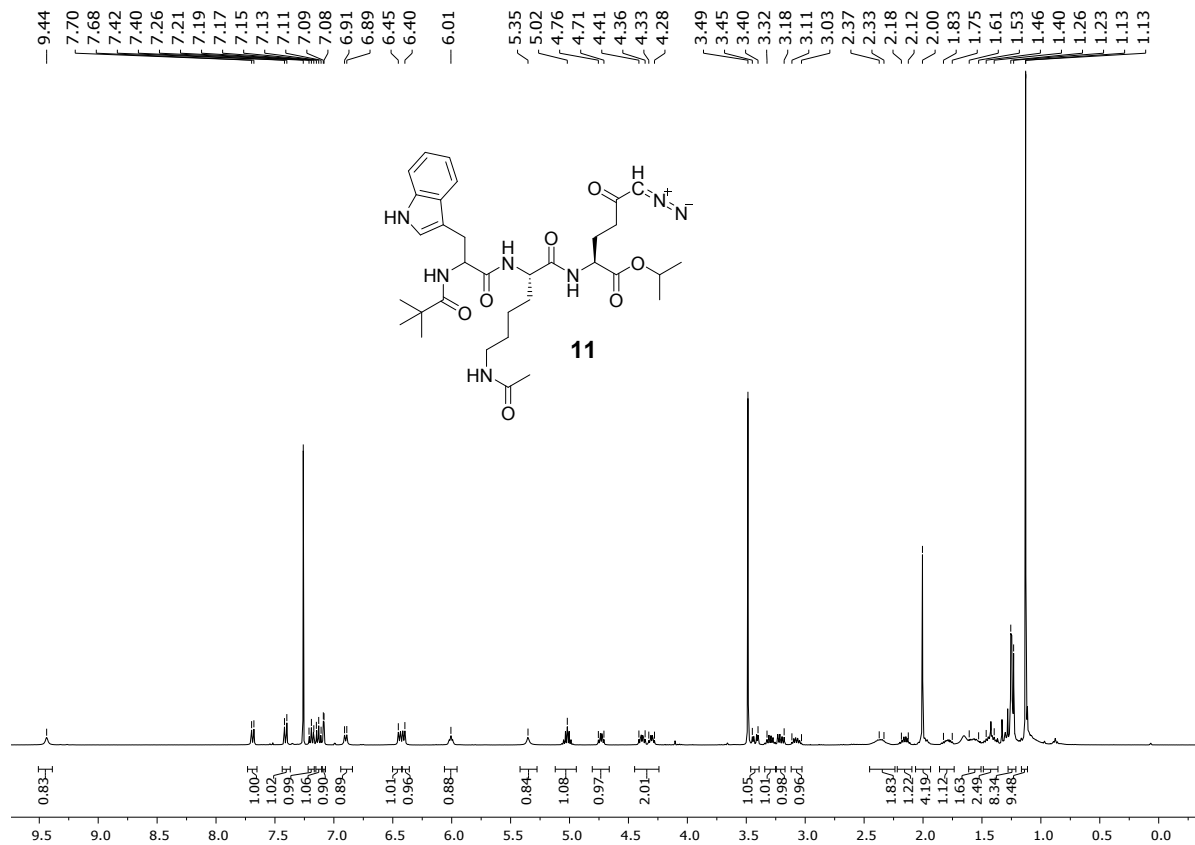


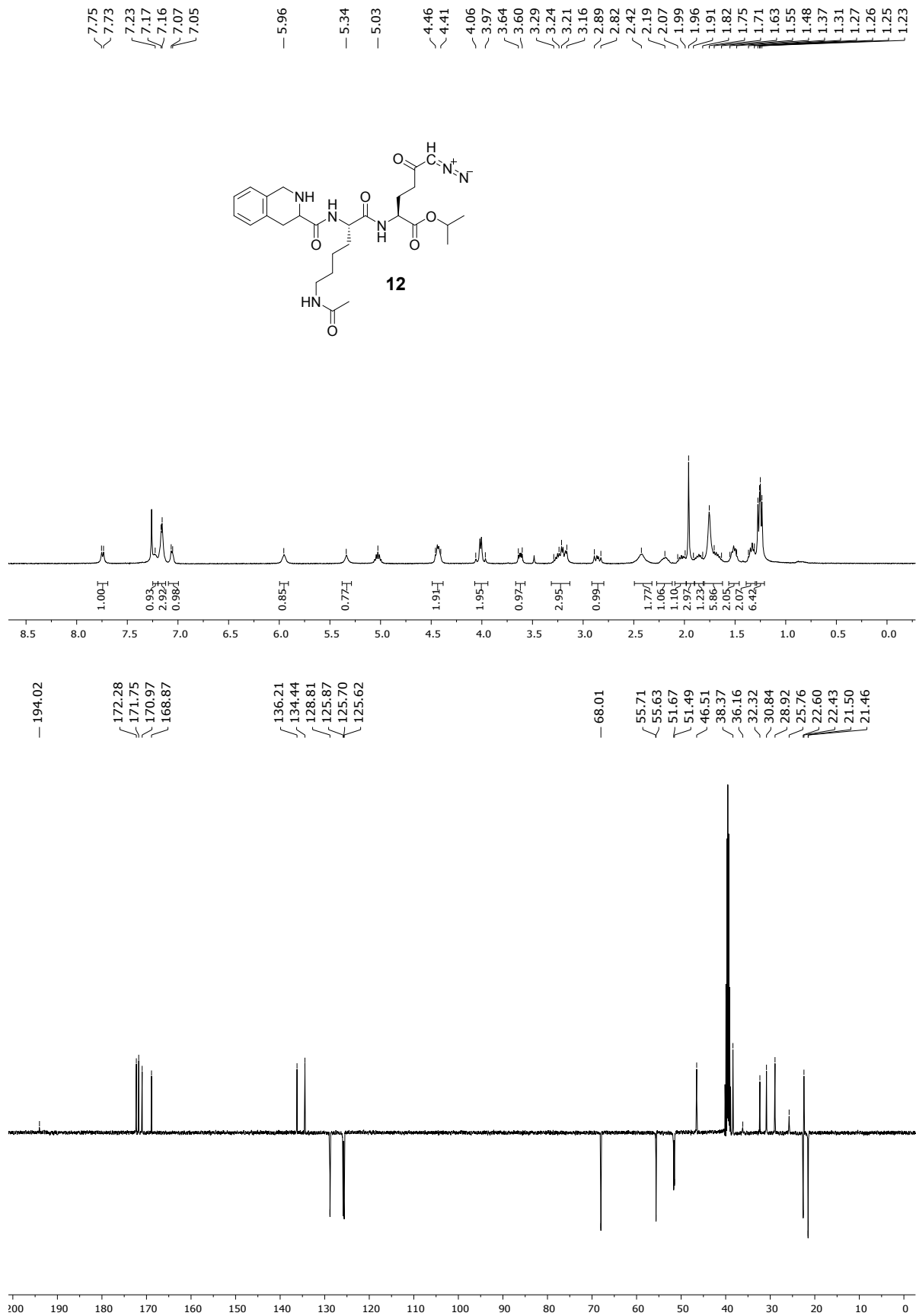


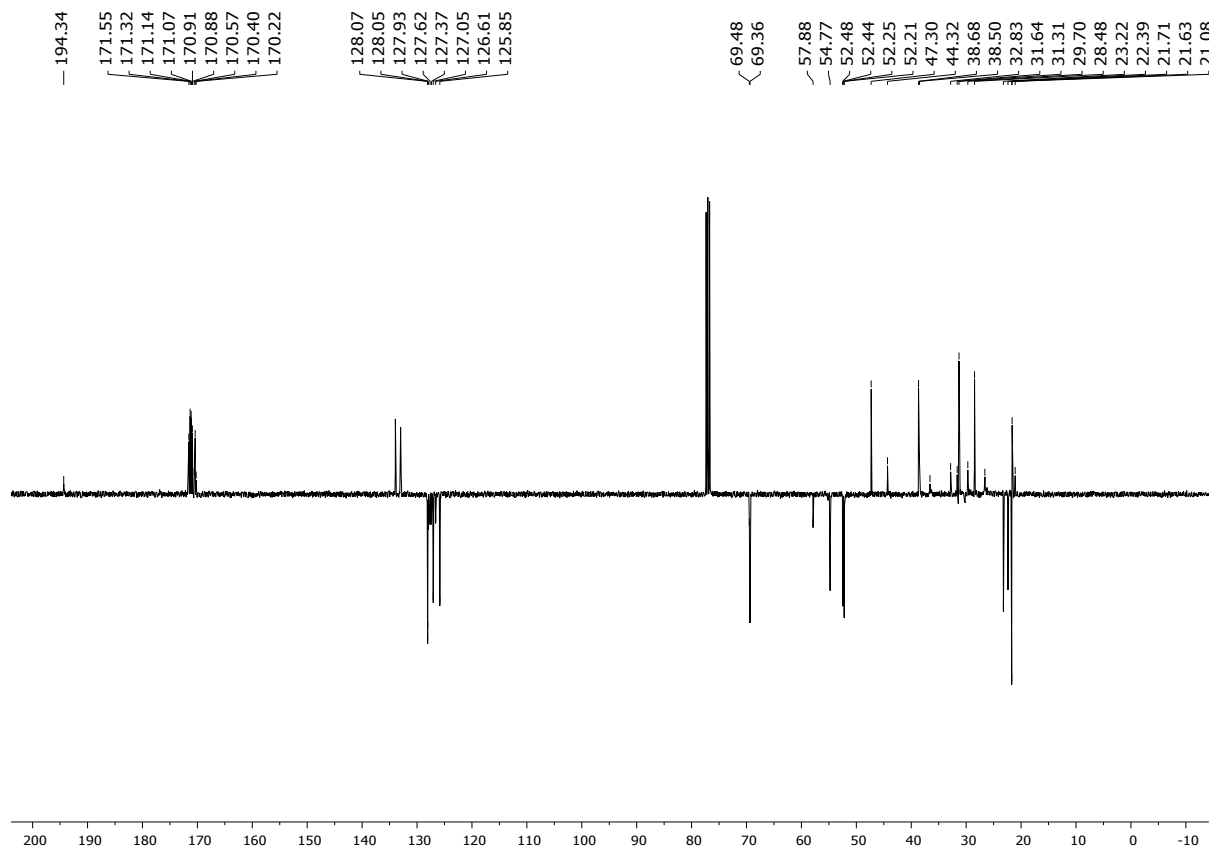
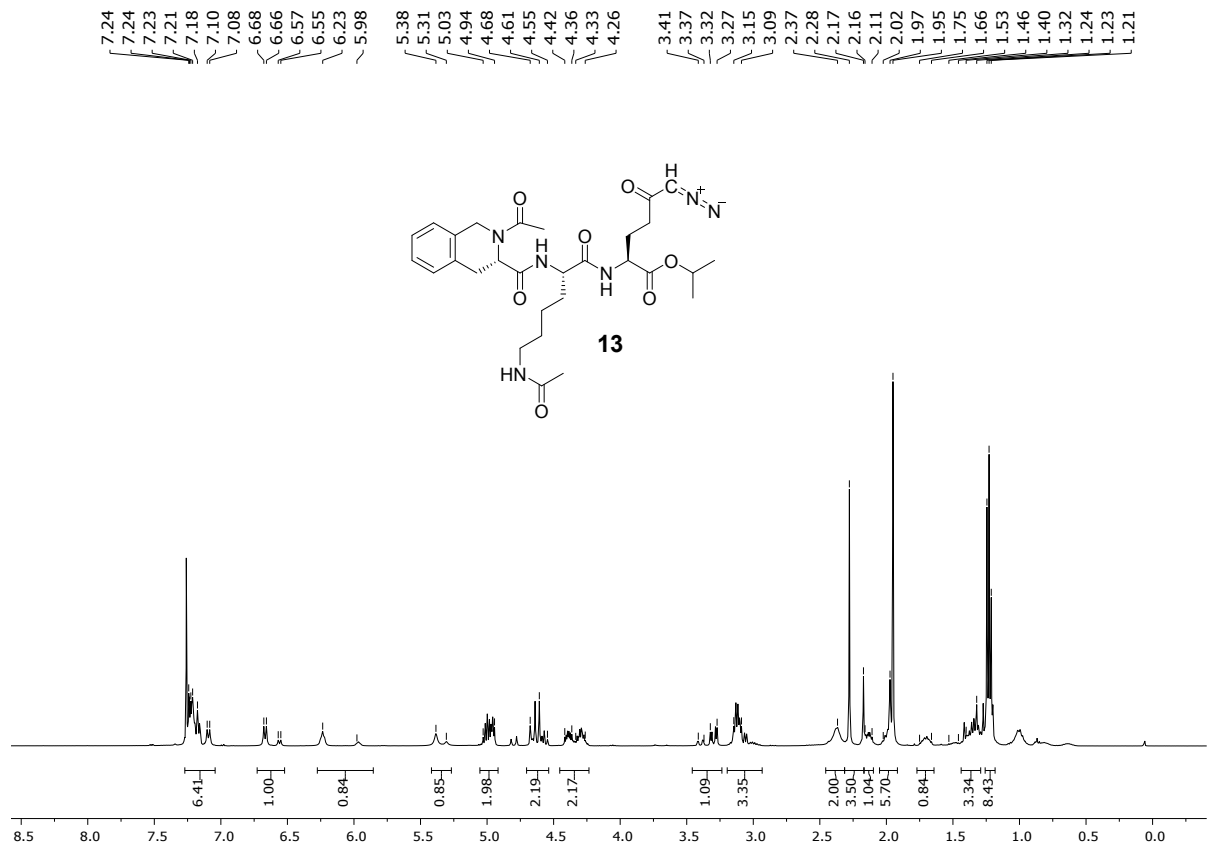


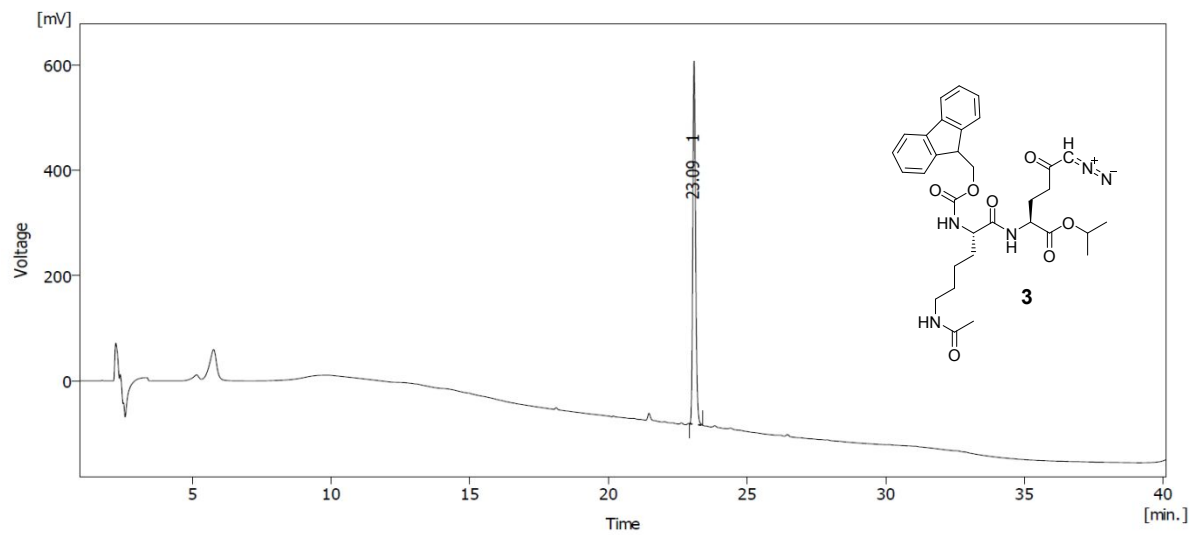
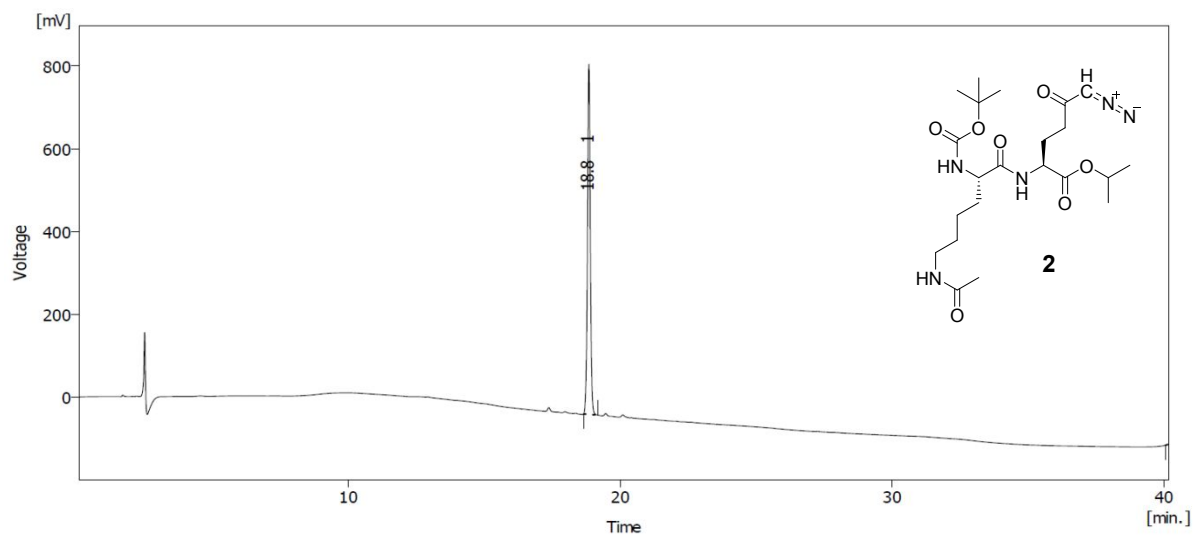


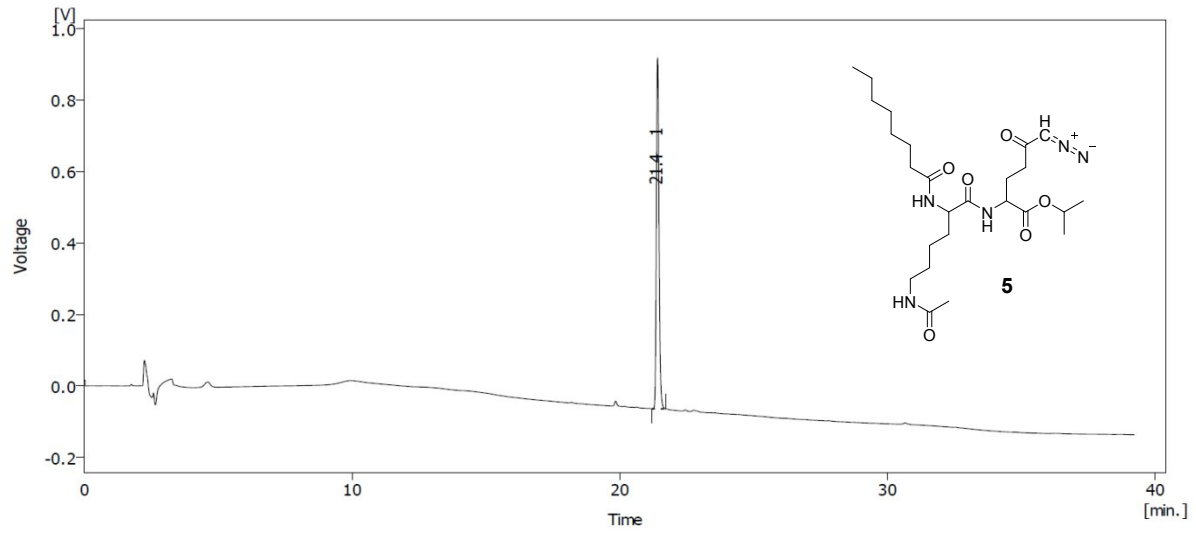
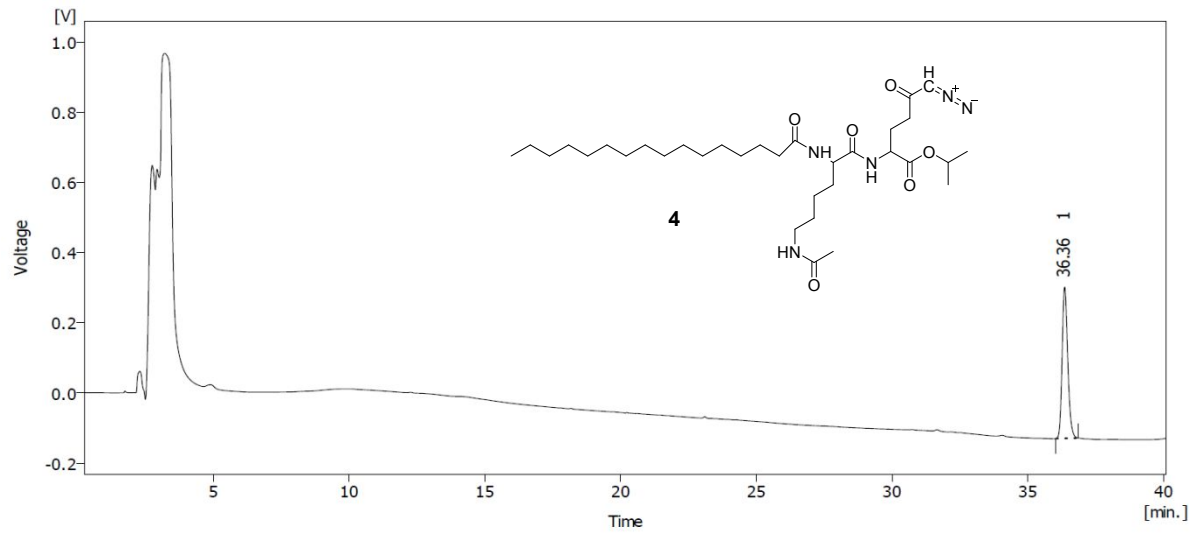


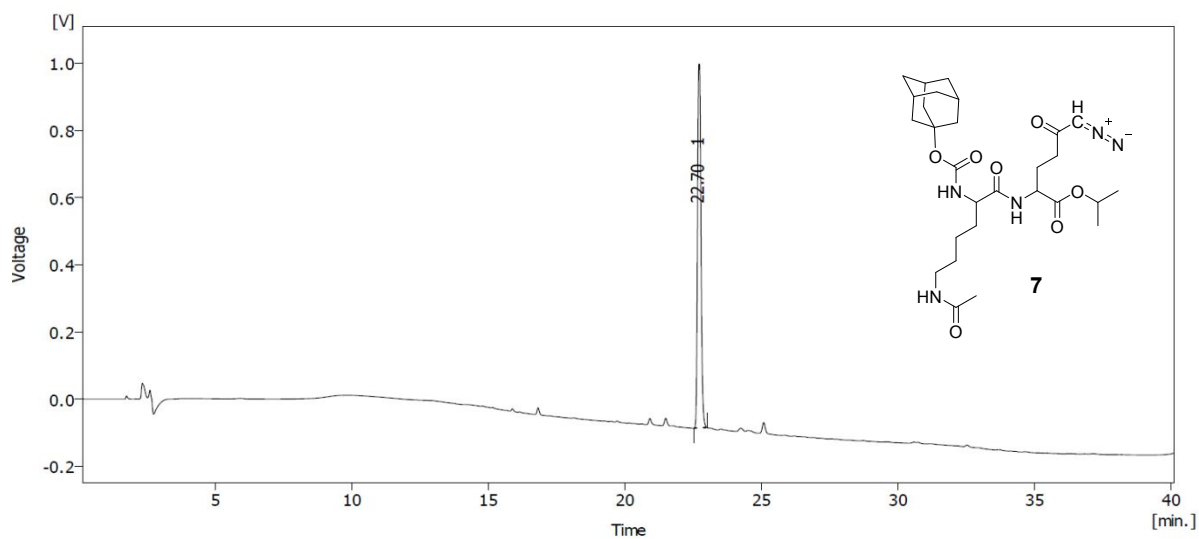
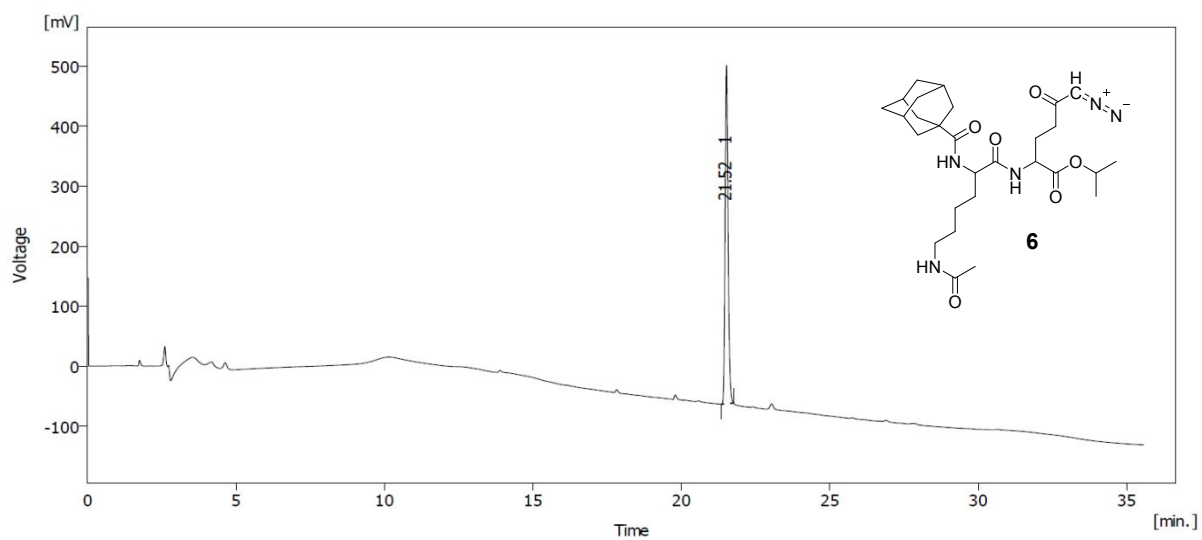


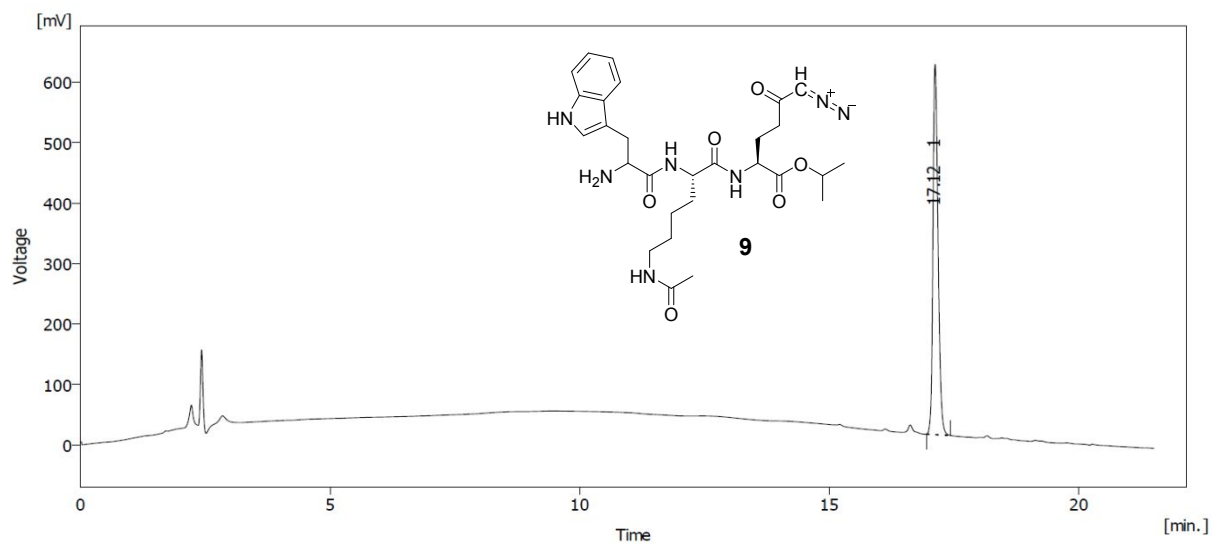
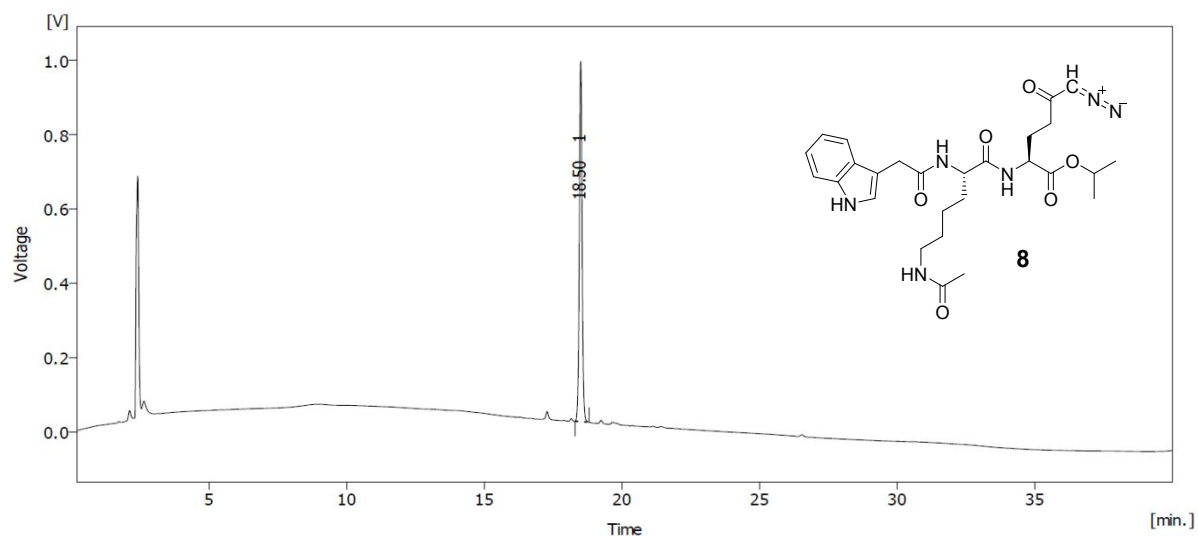


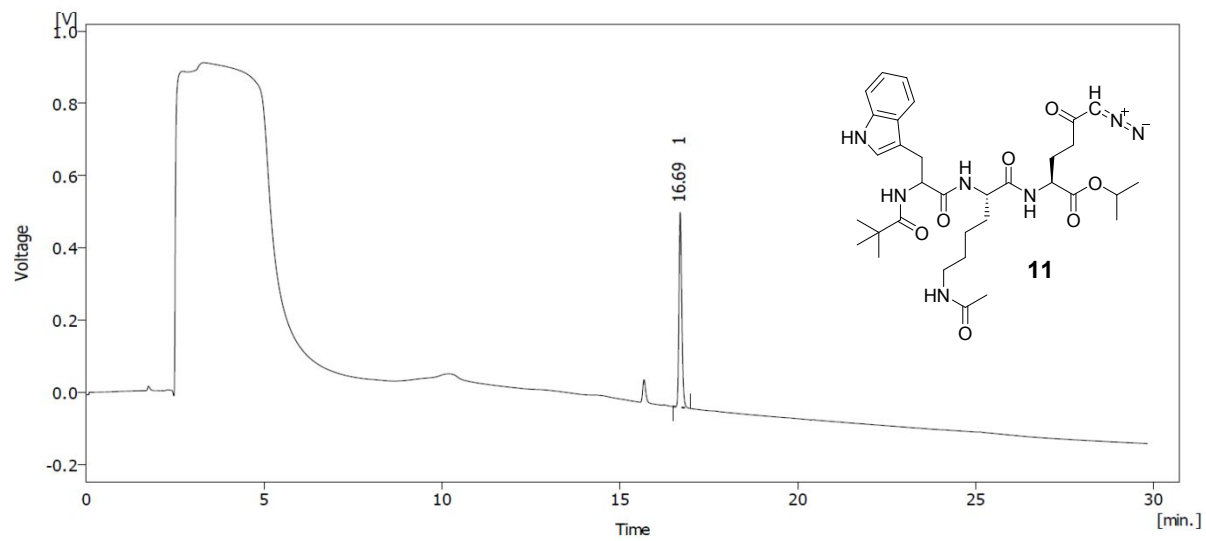
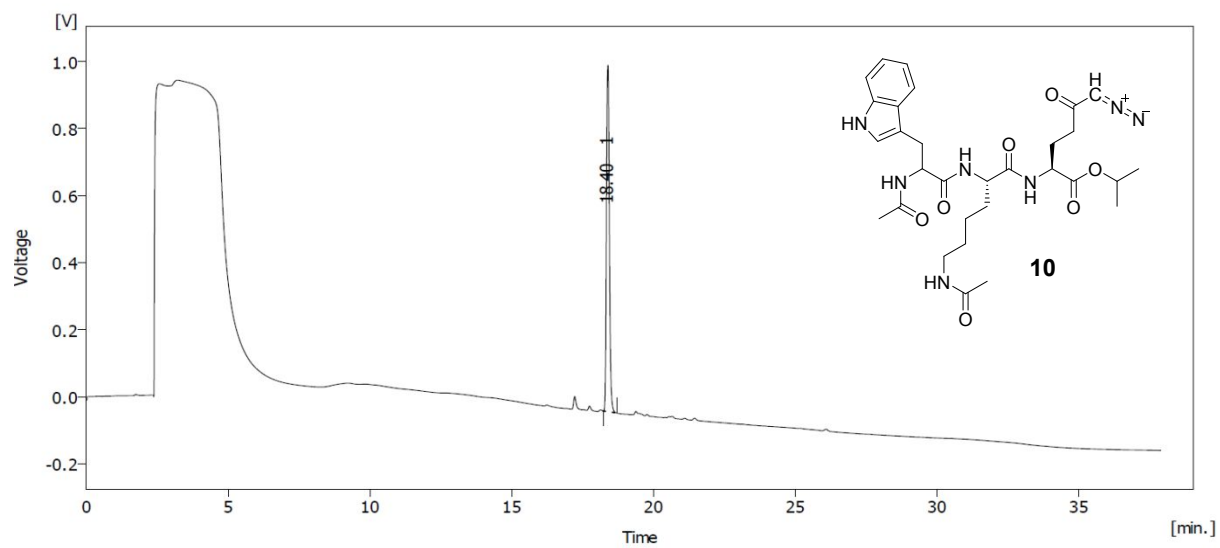












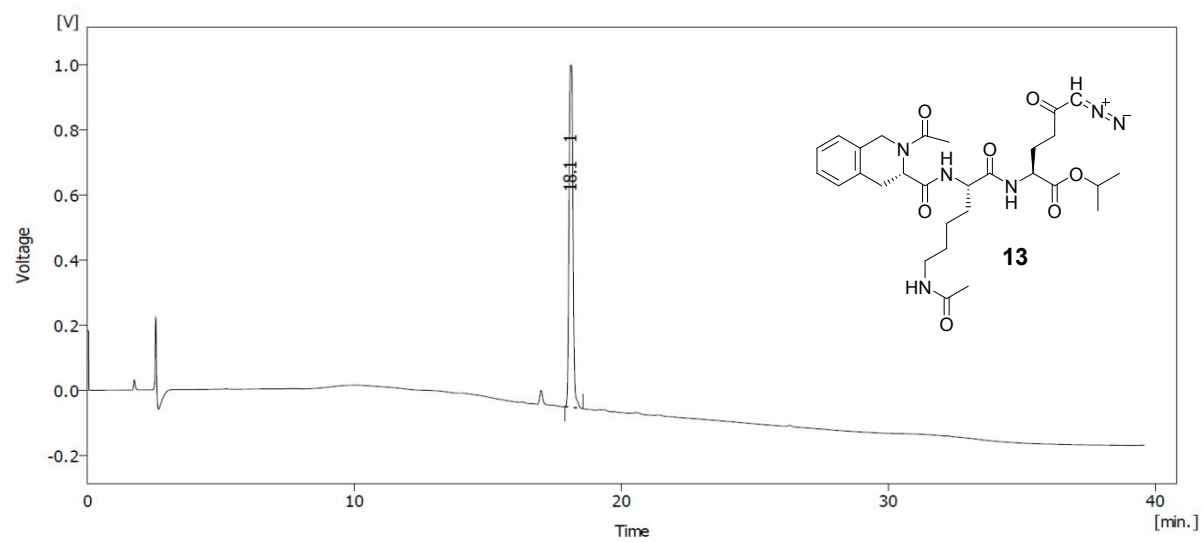
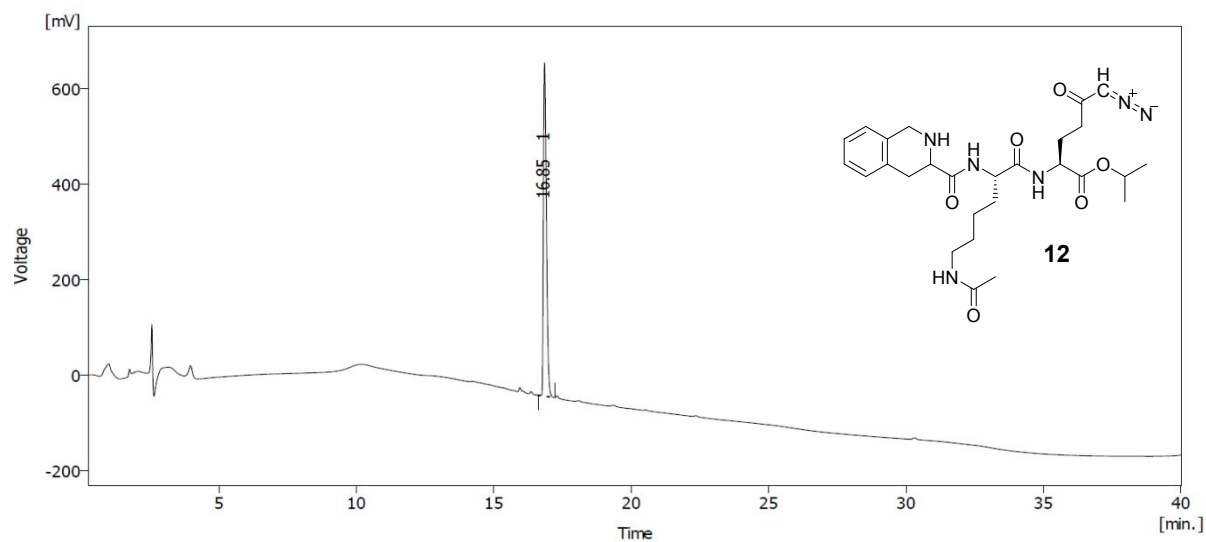
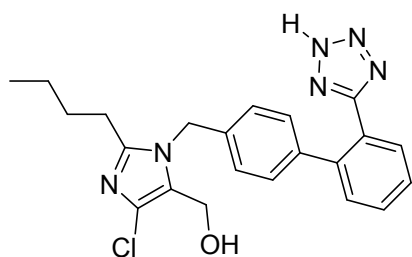


Table S1. Mass transitions and conditions for 2-13 and internal standard (IS) used for LC-MS/MS analysis

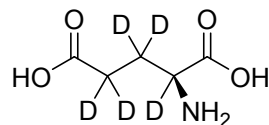
Ionization Mode	ESI, MRM(+)			
Compound	Q1	Q3	CE	S-Lens
2	506.317	165.100, 378.316	26, 16	83
3	578.185	154.043, 518.358	38, 26	145
4	594.237	153.959	40	135
5	482.158	153.981, 464.386	35, 18	112
6	518.142	153.976, 500.401	40, 22	121
7	534.174	135.066, 516.425	37, 20	124
8	513.003	158.980, 495.283	34, 18	146
9	542.225	153.964, 524.378	39, 17	139
10	583.996	158.930	39	169
11	561.001	158.958	36	166
12	515.220	126.008, 132.006, 497.357	27, 38, 18	143
13	556.941	131.993, 539.304	51, 20	118
Losartan (IS)	422.938	184.580, 209.275	37, 46	120

A



Chemical Formula: $C_{22}H_{23}ClN_6O$
Molecular Weight: 422.92

B



Chemical Formula: $C_5H_4D_5NO_4$
Molecular Weight: 152.16

Figure S1. (A) Structure of internal standard, losartan, used in LC-MS/MS analysis for **2-13. (B).**

Structure of internal standard, L-glutamic acid-2,3,3,4,4-*d*₅, used in LC/MS/MS for DON (**1**)

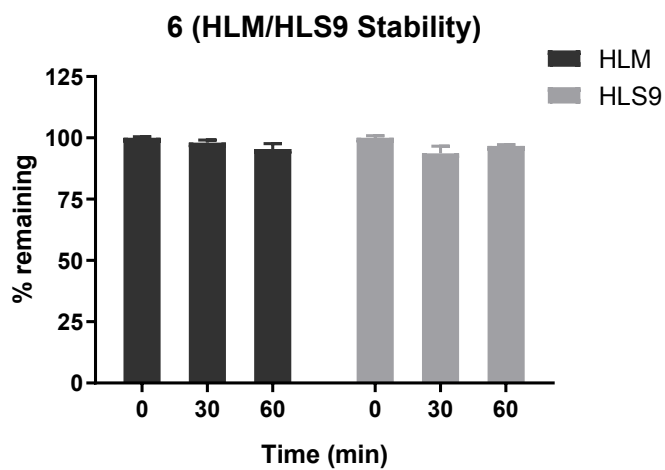


Figure S2. Stability of 6 in human liver fractions. 6 was incubated at 1 μ M concentration in both human liver microsomes (0.2 mg/mL) and S9 fractions (0.5 mg/mL). 6 was found to be stable in both assays.

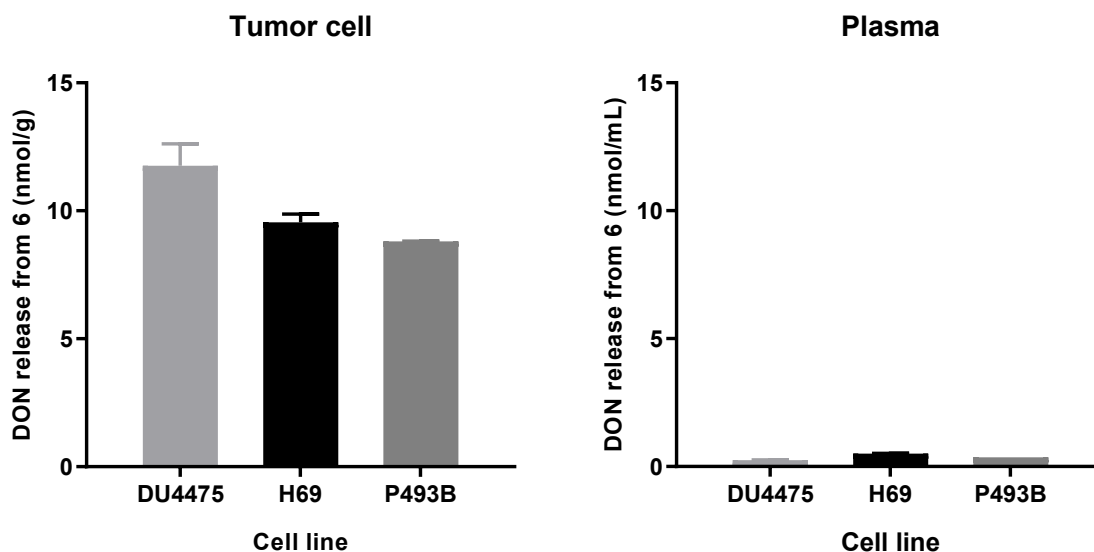


Figure S3. Human tumor cell to plasma partitioning of 6 in DU4475, H69 and P493B cell lines. (A) DON levels were similar in tumor cells from all three cell lines. (B) Plasma levels of DON were relatively lower in DU4475 cells giving the best cell-to-plasma ratio of 47 followed by a ratio of 19 and 24 for H69 and P493B cells, respectively.

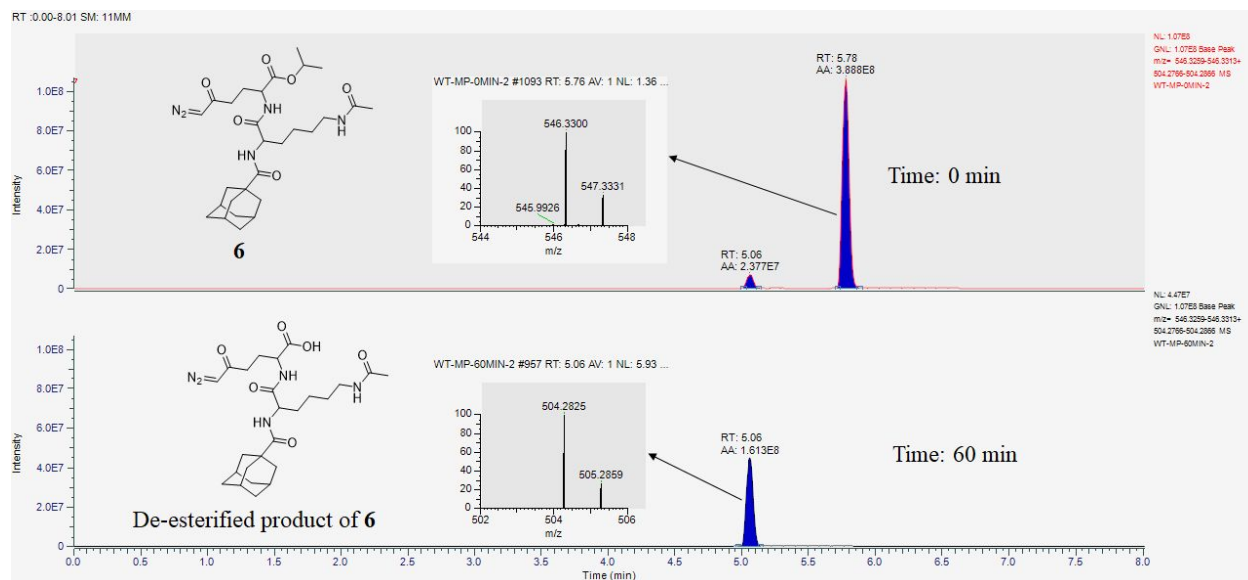


Figure S4. Extracted chromatogram and corresponding spectra obtained from HRMS analysis of in vitro wild type mouse plasma stability study samples showing the intact **6** at 0 min and complete de-esterification of the isopropyl ester of compound **6** following 60 min incubation at 37 °C.