

Supporting information

C-terminal 1-aminoethyltetrazole containing oligopeptides as novel alanine racemase inhibitors

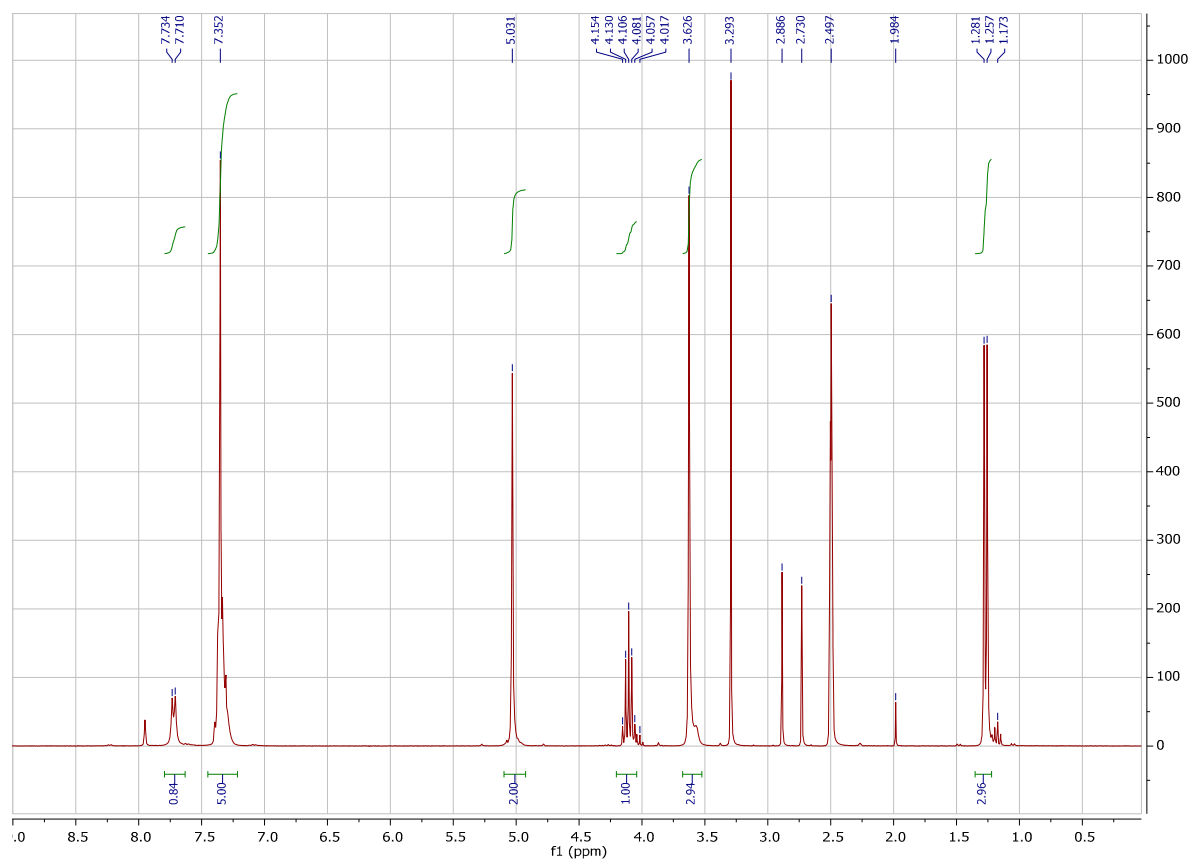
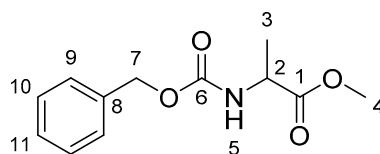
Laszlo A. Kondacs ¹, Sylvain Orenge ², The late Rosaleen J. Anderson ¹, Emma C. L. Marrs ³, John D. Perry ³, Mark Gray ^{1*}

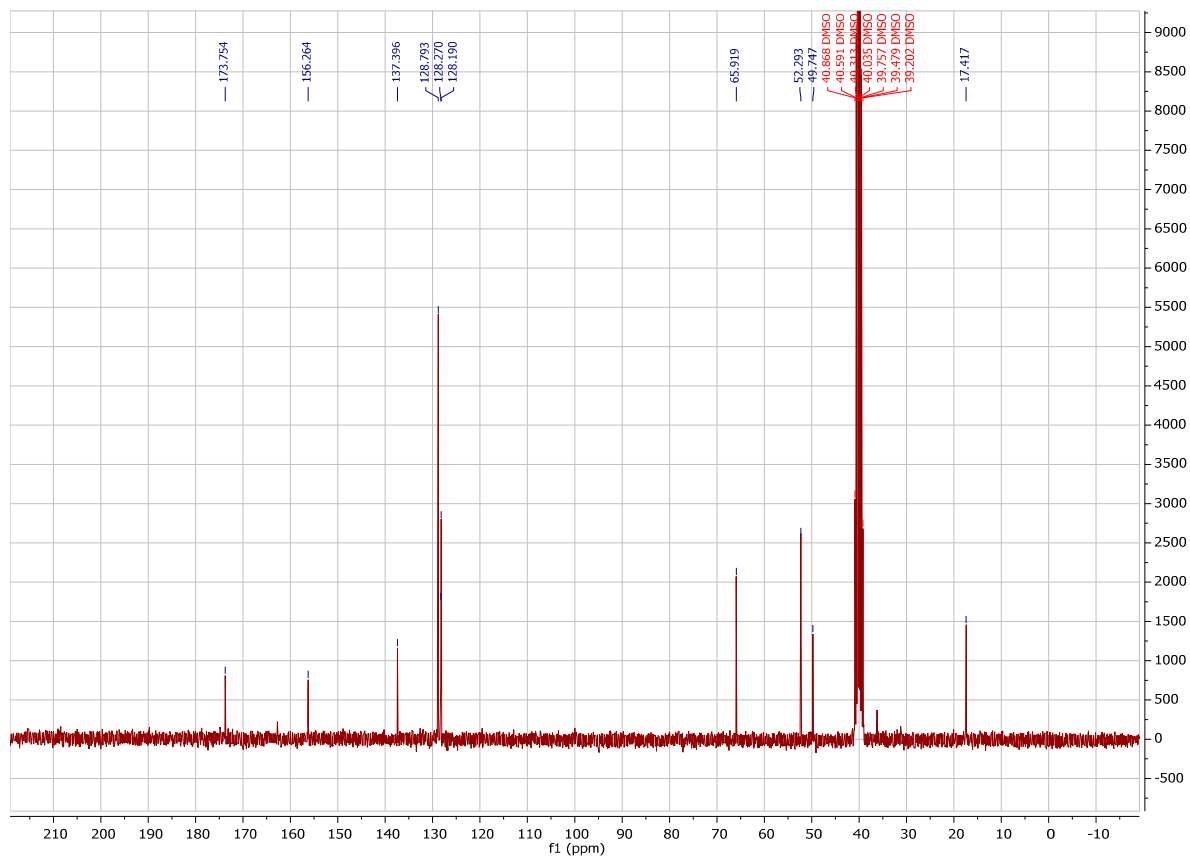
¹ Sunderland Pharmacy School, University of Sunderland, Sunderland SR1 3SD, U.K.

² Research & Development Microbiology, bioMérieux, 38390 La Balme-les-Grottes, France

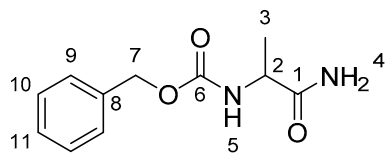
³ Department of Microbiology, Freeman Hospital, Newcastle upon Tyne NE7 7DN, U.K.

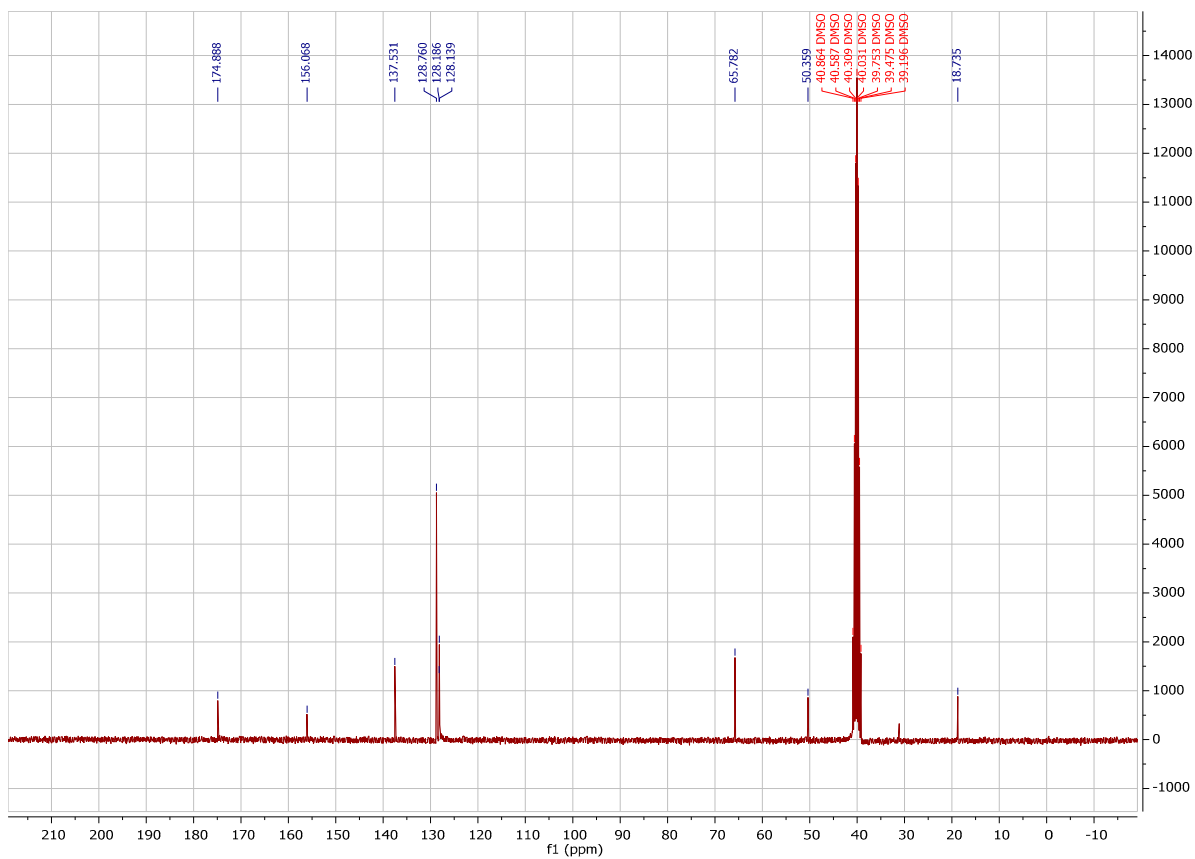
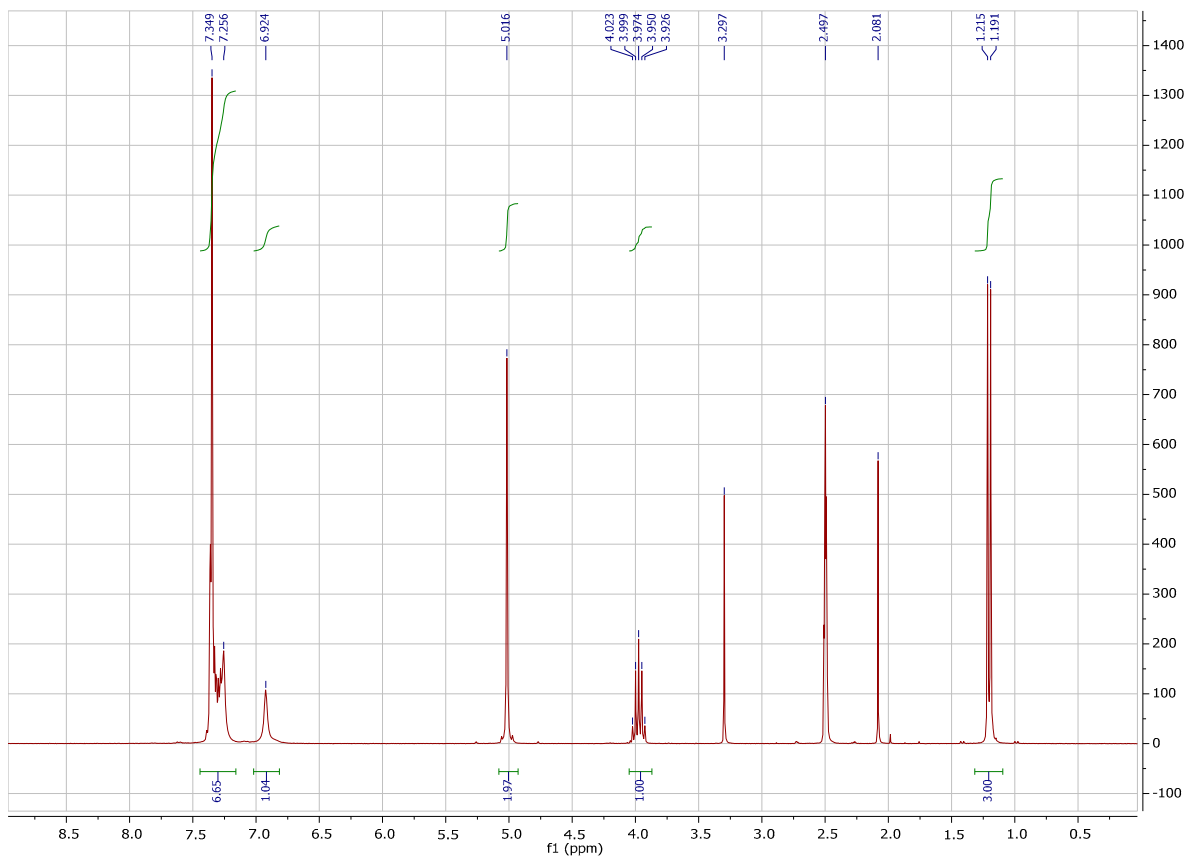
Methyl-(2S or R)-2-[(benzyloxy)carbonyl]amino}propanoate **6a**:



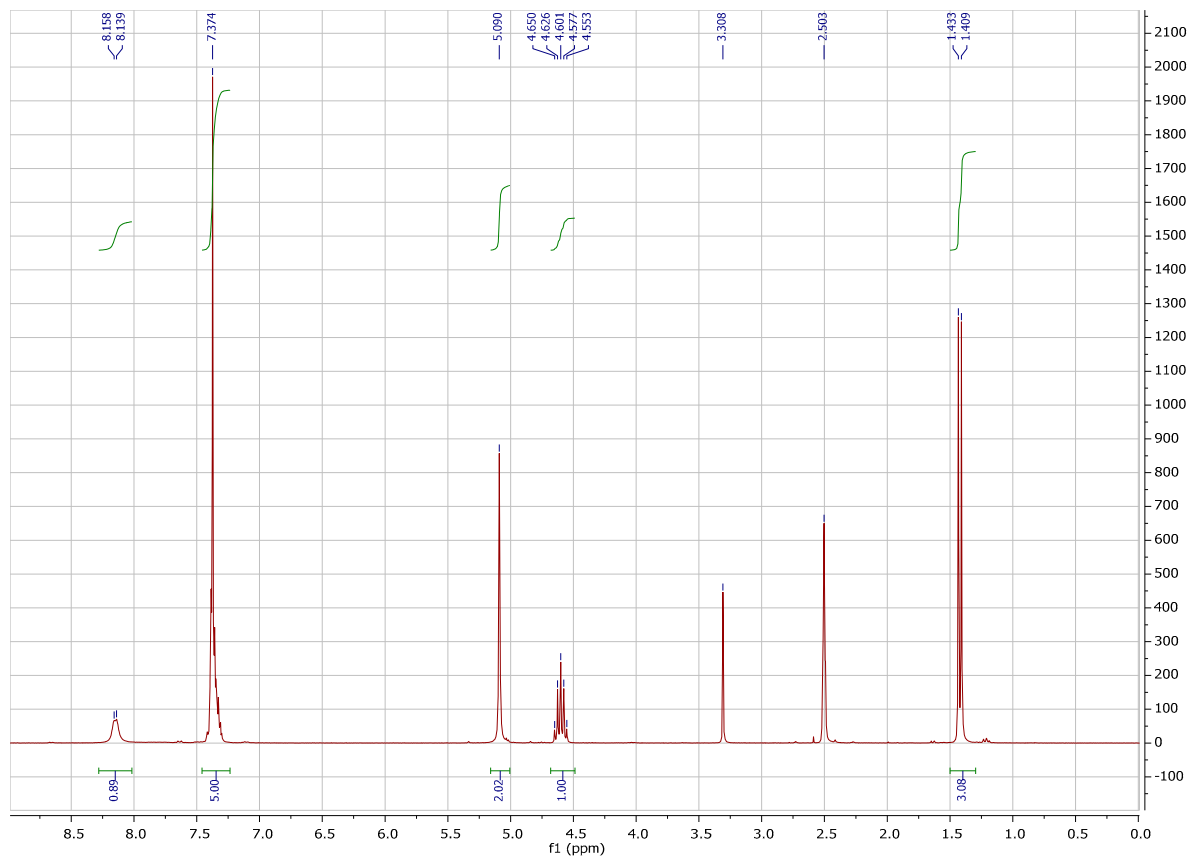
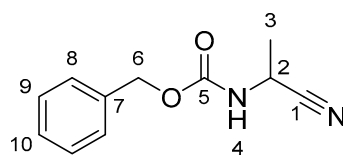


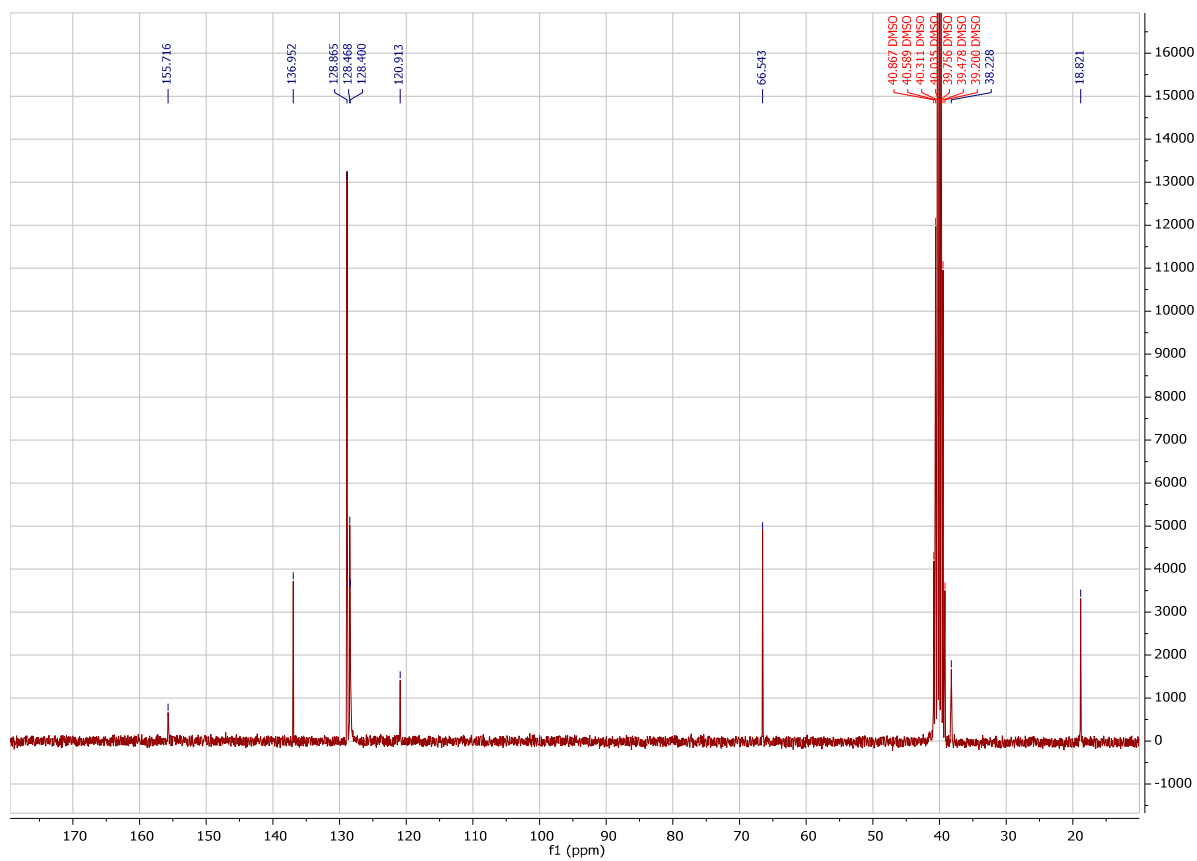
Benzyl-N-[(1S or R)-1-carbamoyl]ethyl]carbamate 7a:



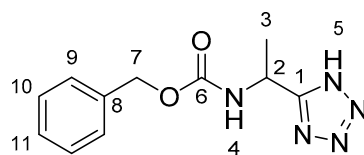


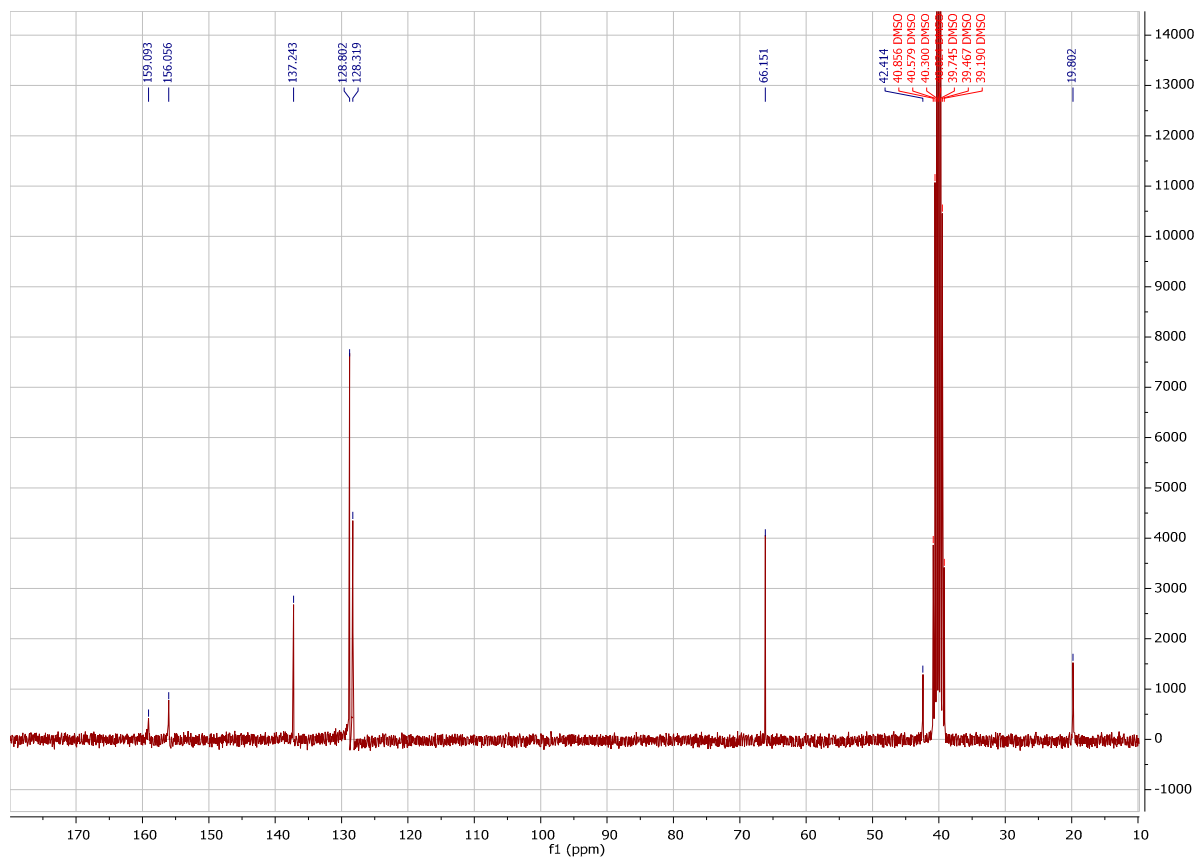
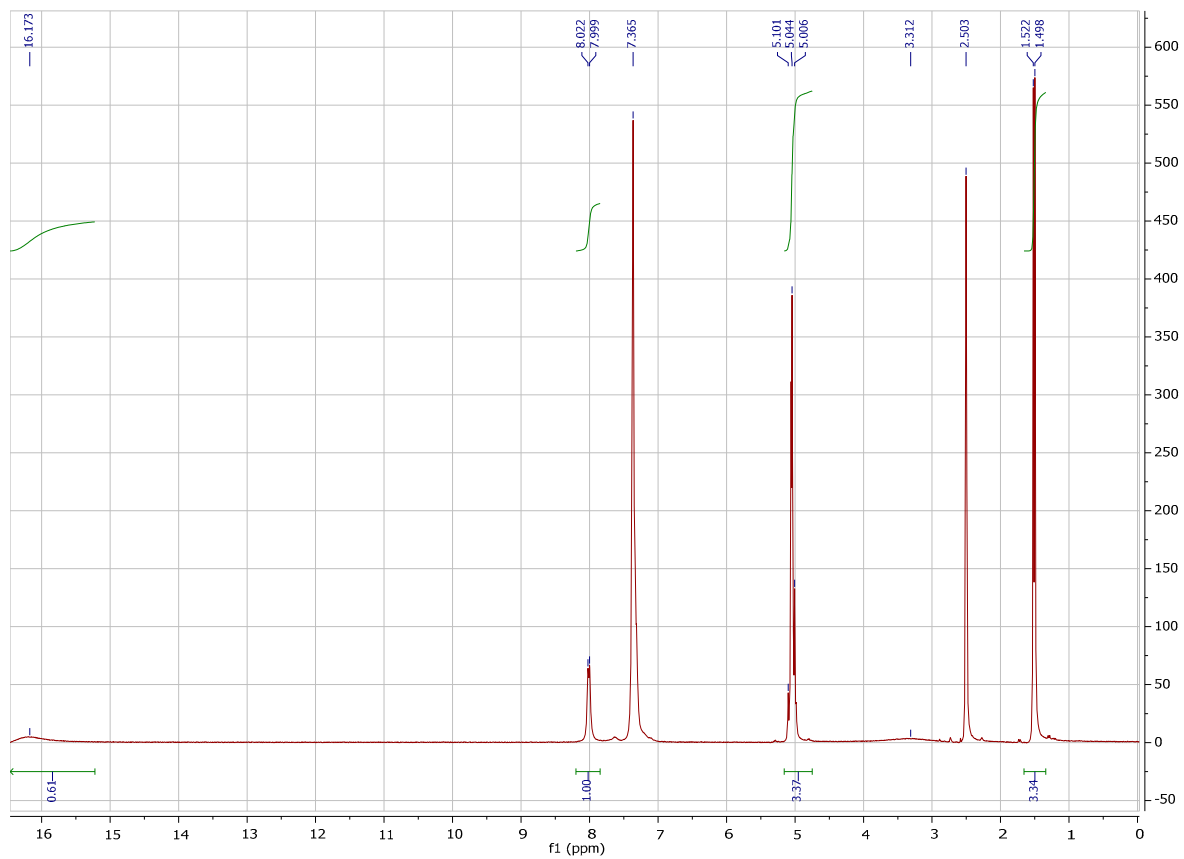
Benzyl-N-[(1S or R)-1-cyanoethyl]carbamate **8a**:



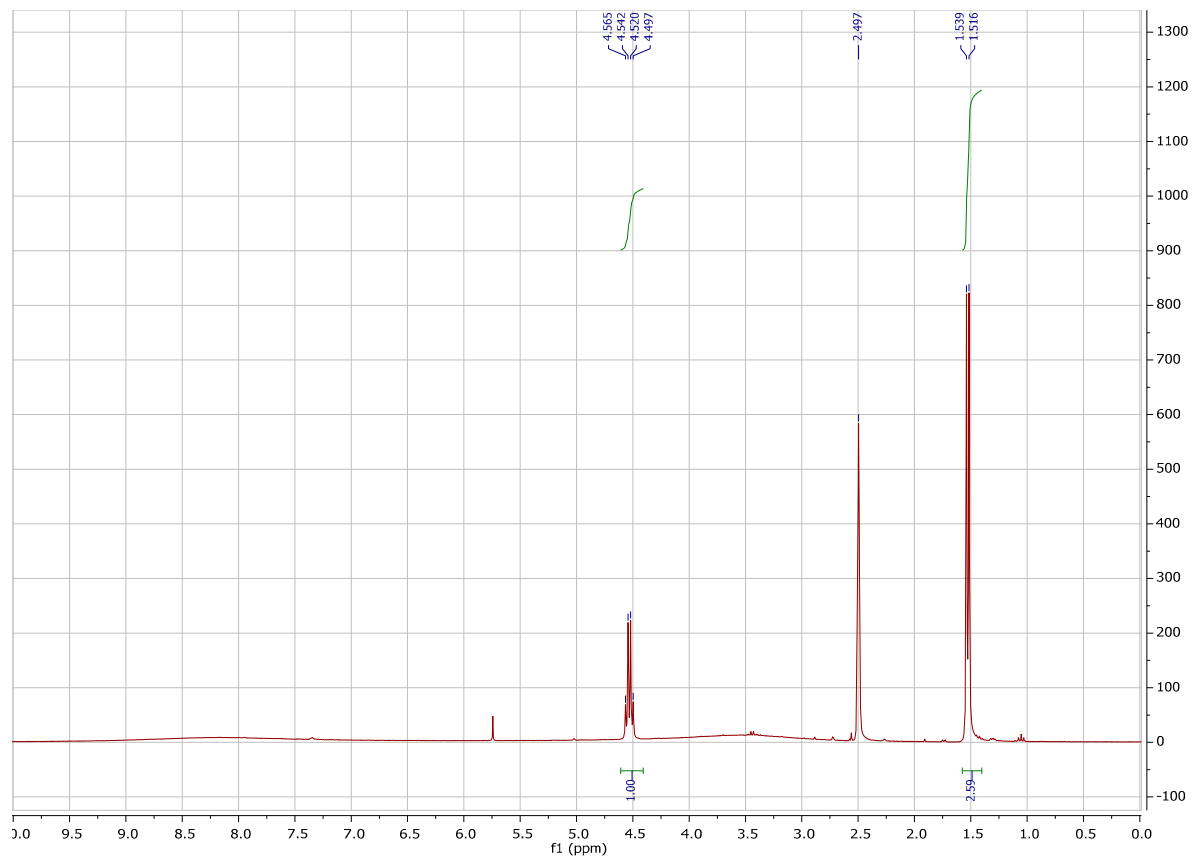
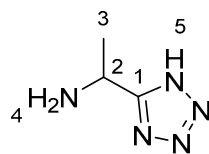


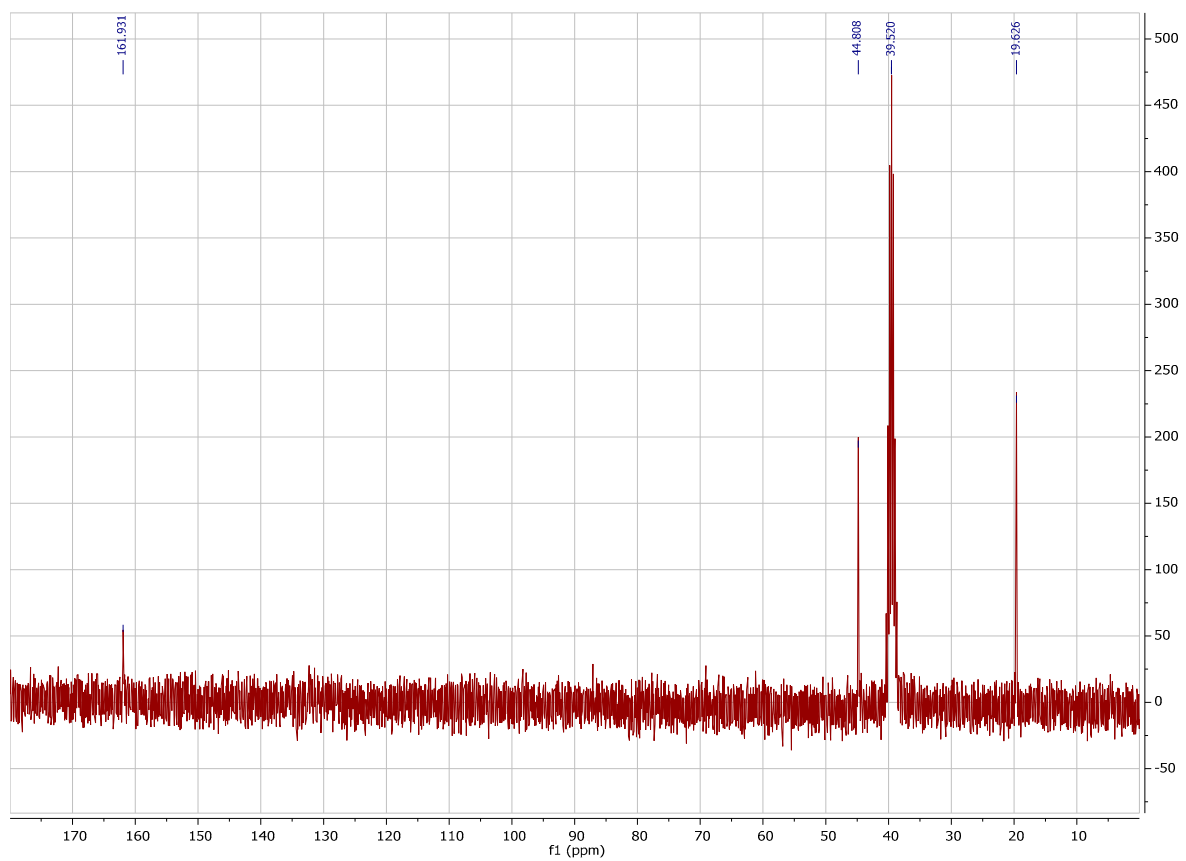
Benzyl-*N*-[(1*S* or *R*)-1-(1*H*-1,2,3,4-tetrazol-5-yl)ethyl]carbamate **9a**:



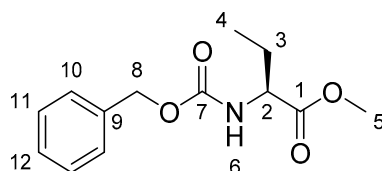


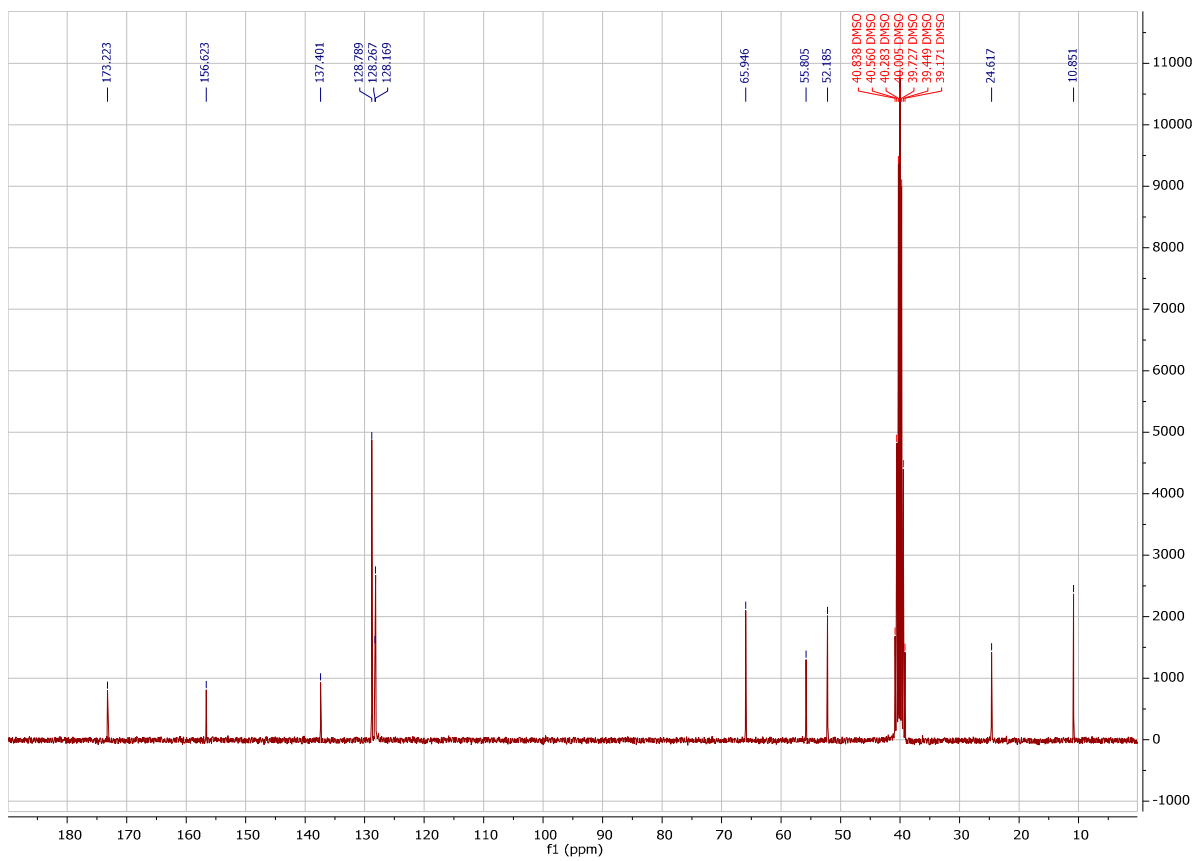
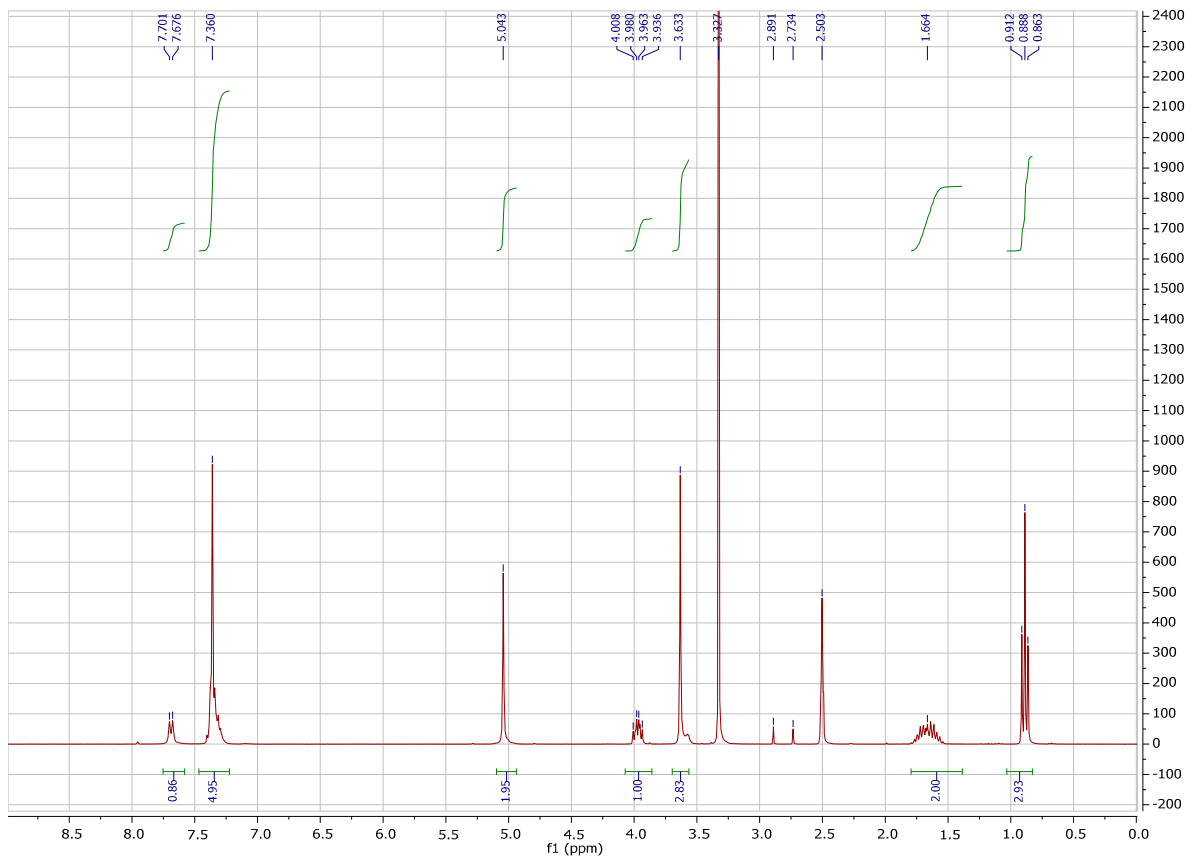
(1S or R)-1-(1H-1,2,3,4-tetrazol-5-yl)ethan-1-amine **4a**:



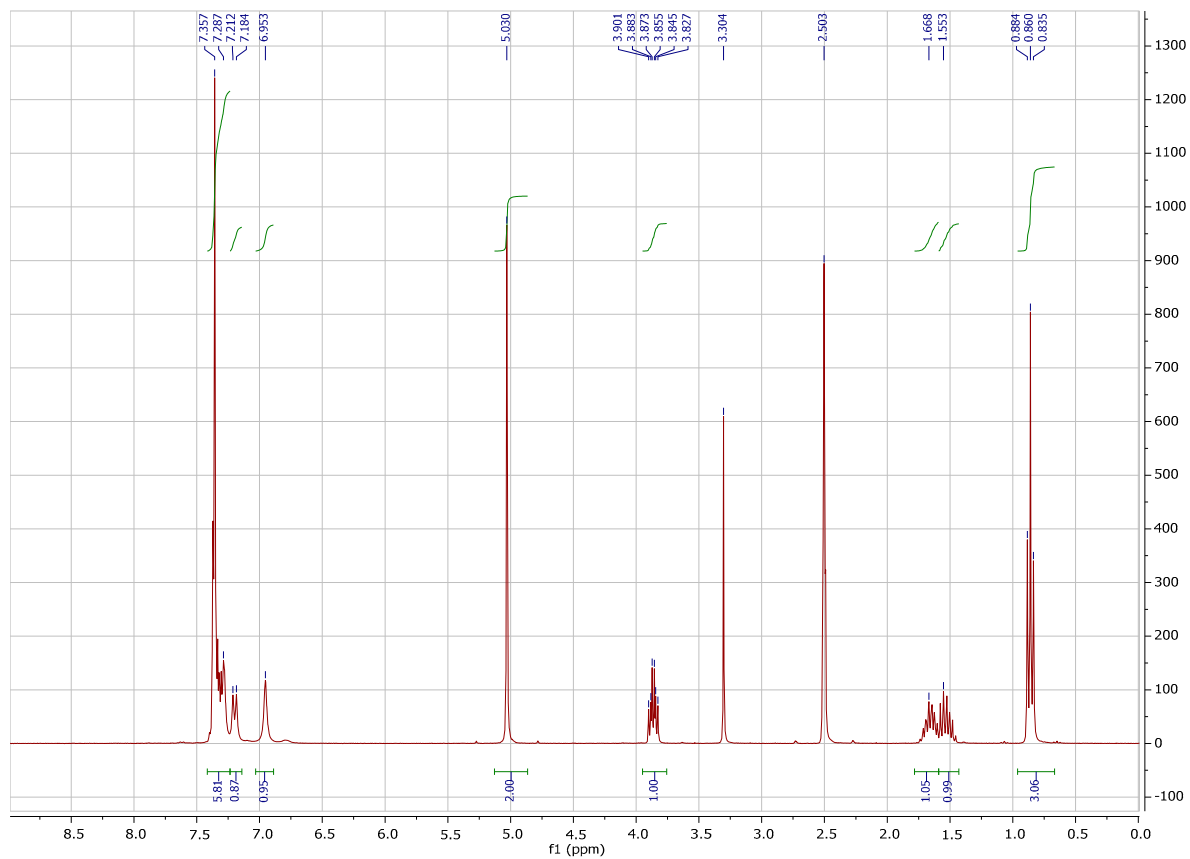
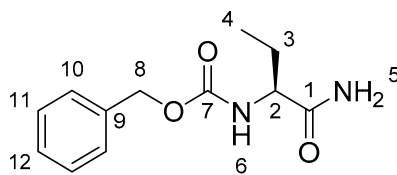


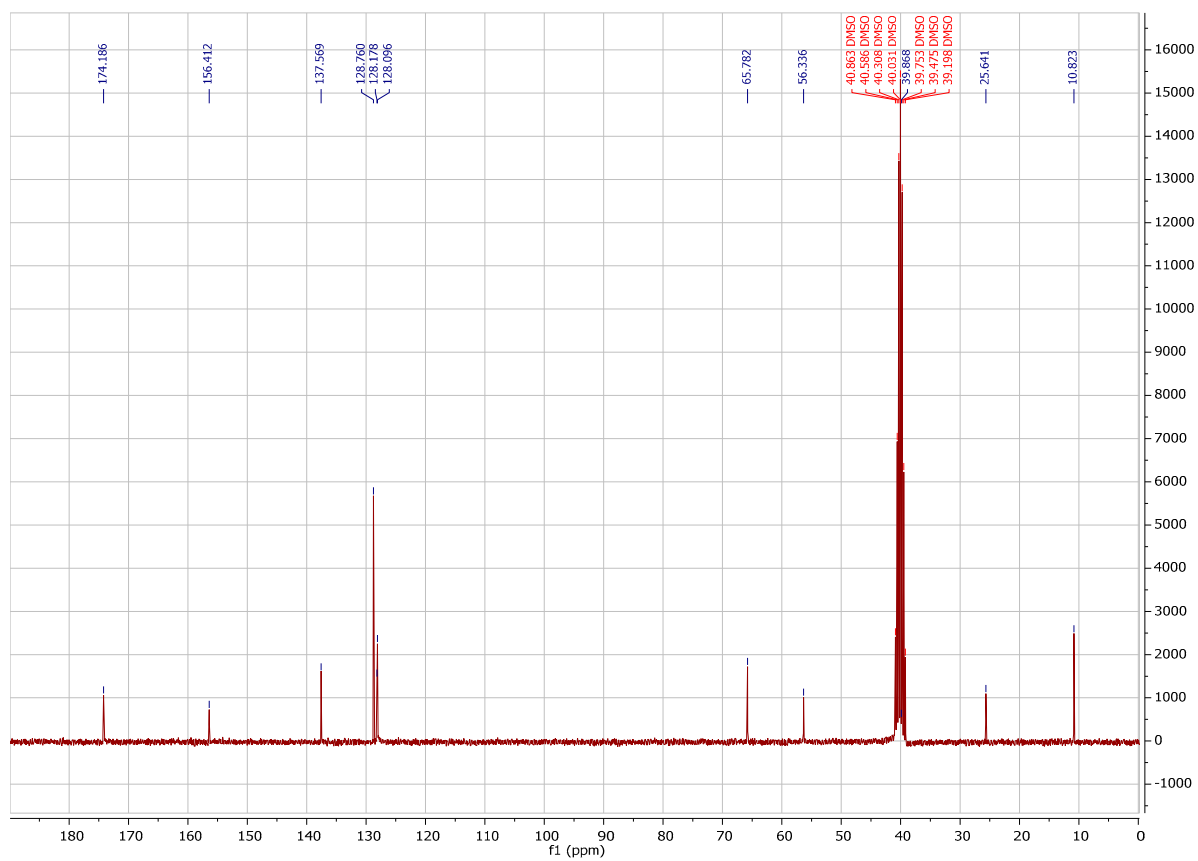
Methyl-(2S)-2-[(benzyloxy)carbonyl]butanoate **6b-L**:



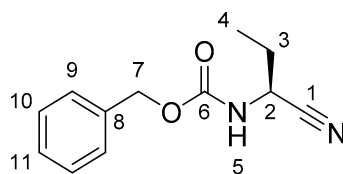


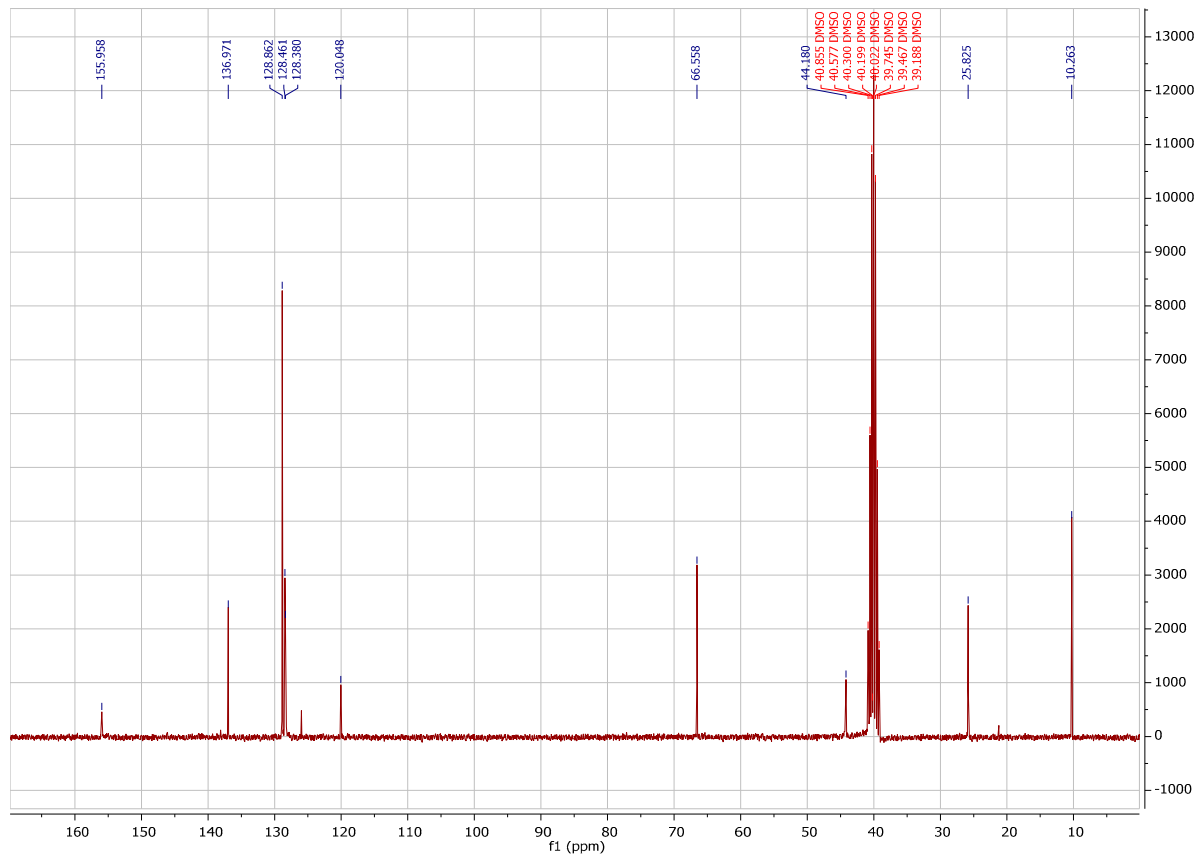
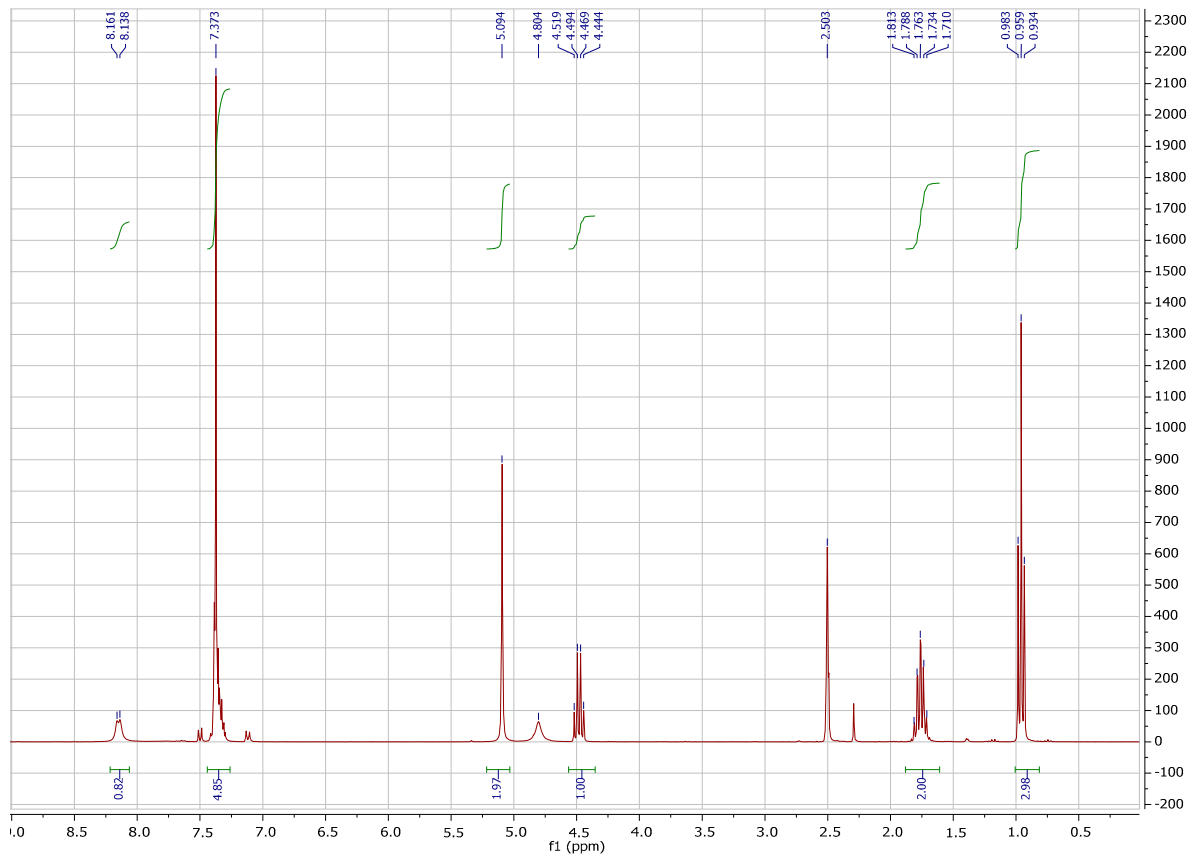
Benzyl-N-[(1S)-1-carbamoylpropyl]carbamate **7b-L**:



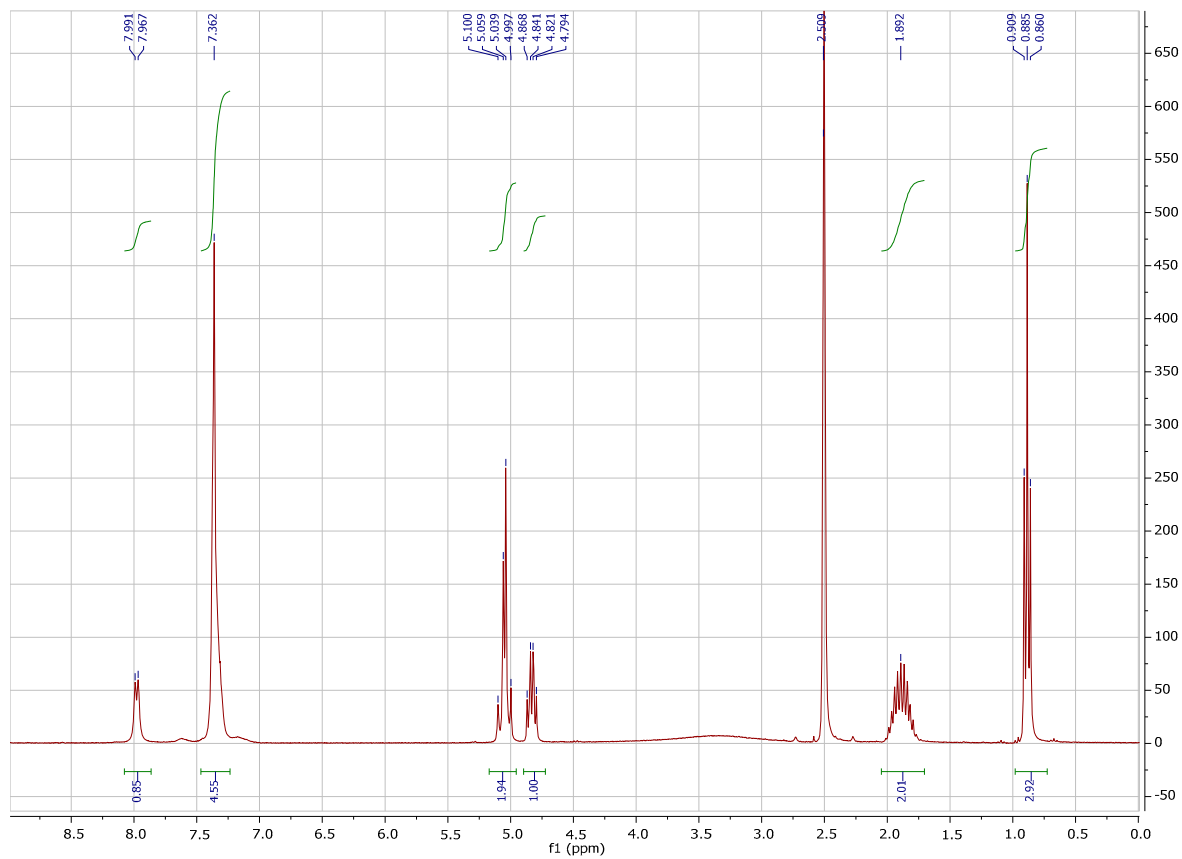
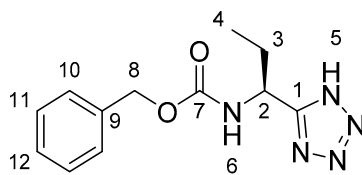


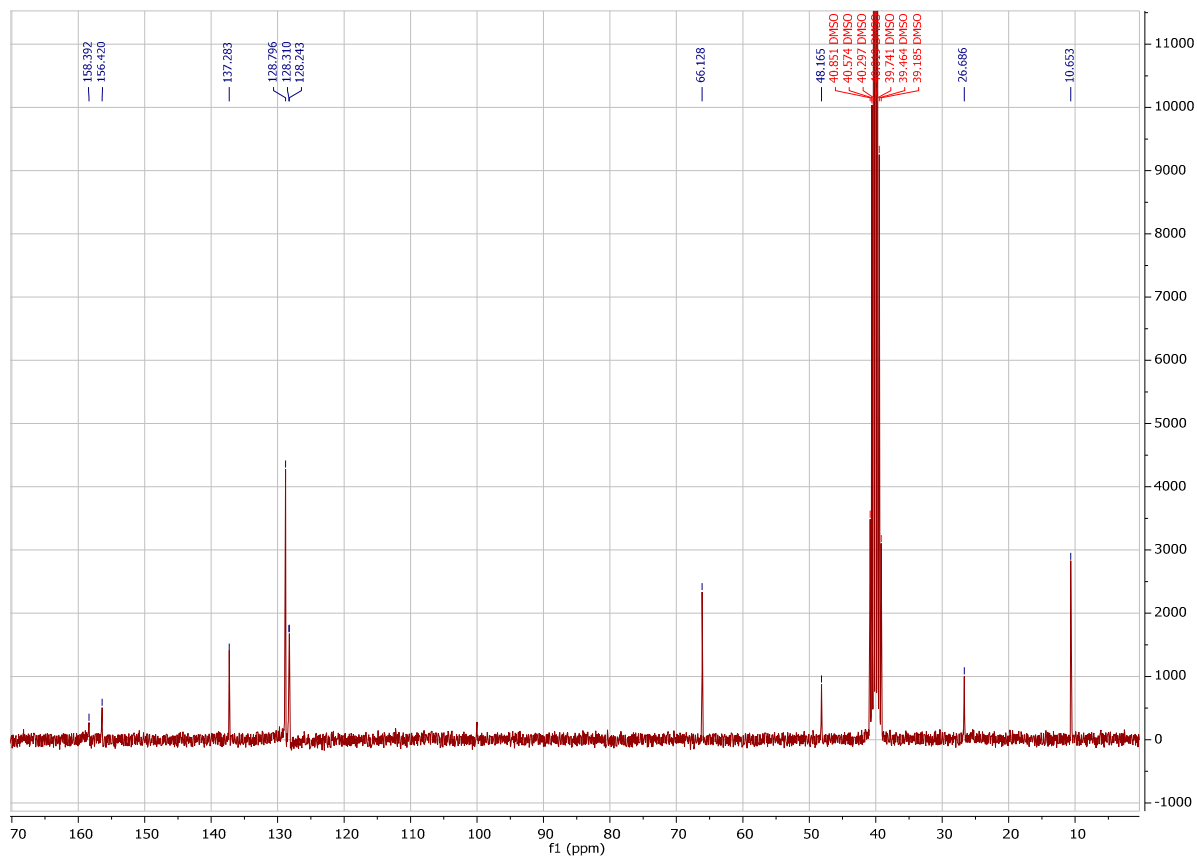
Benzyl-N-[(1S)-1-cyanopropyl]carbamate **8b-L**:



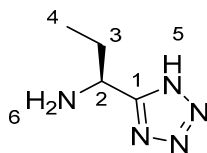


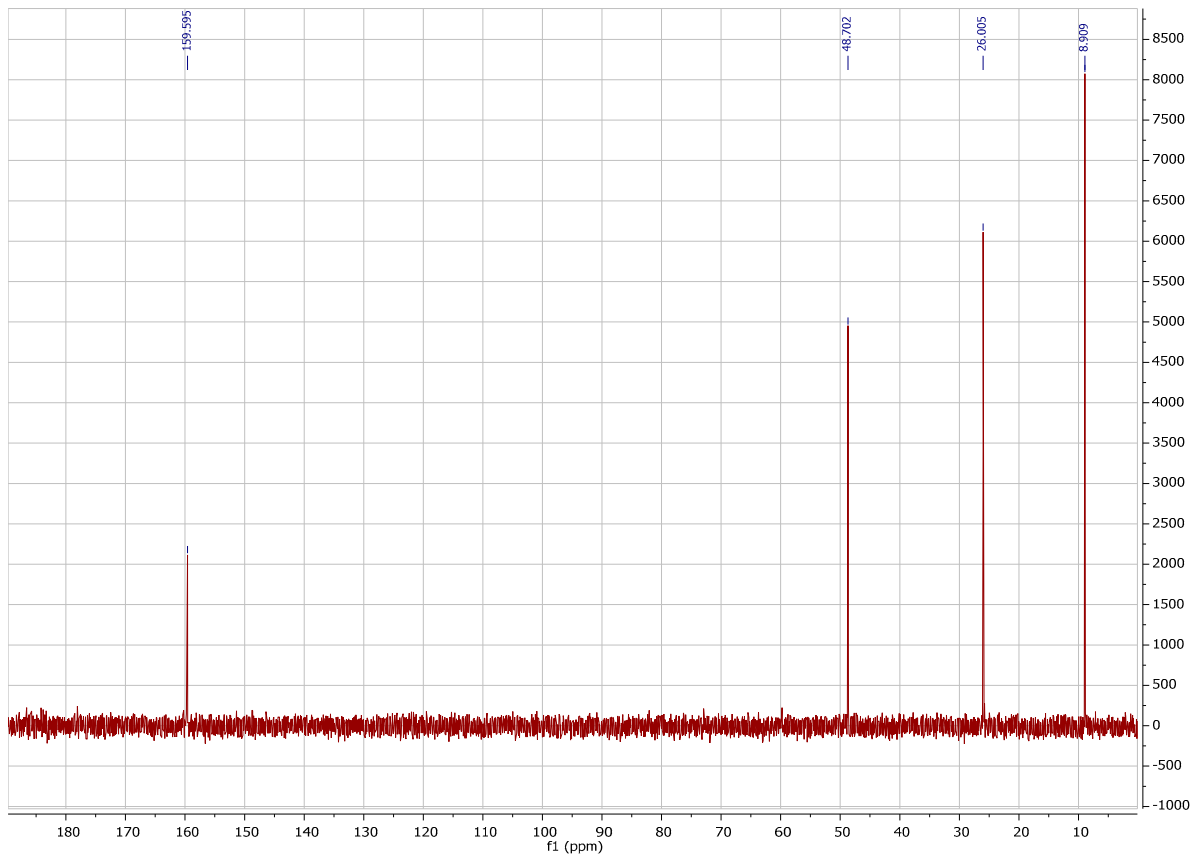
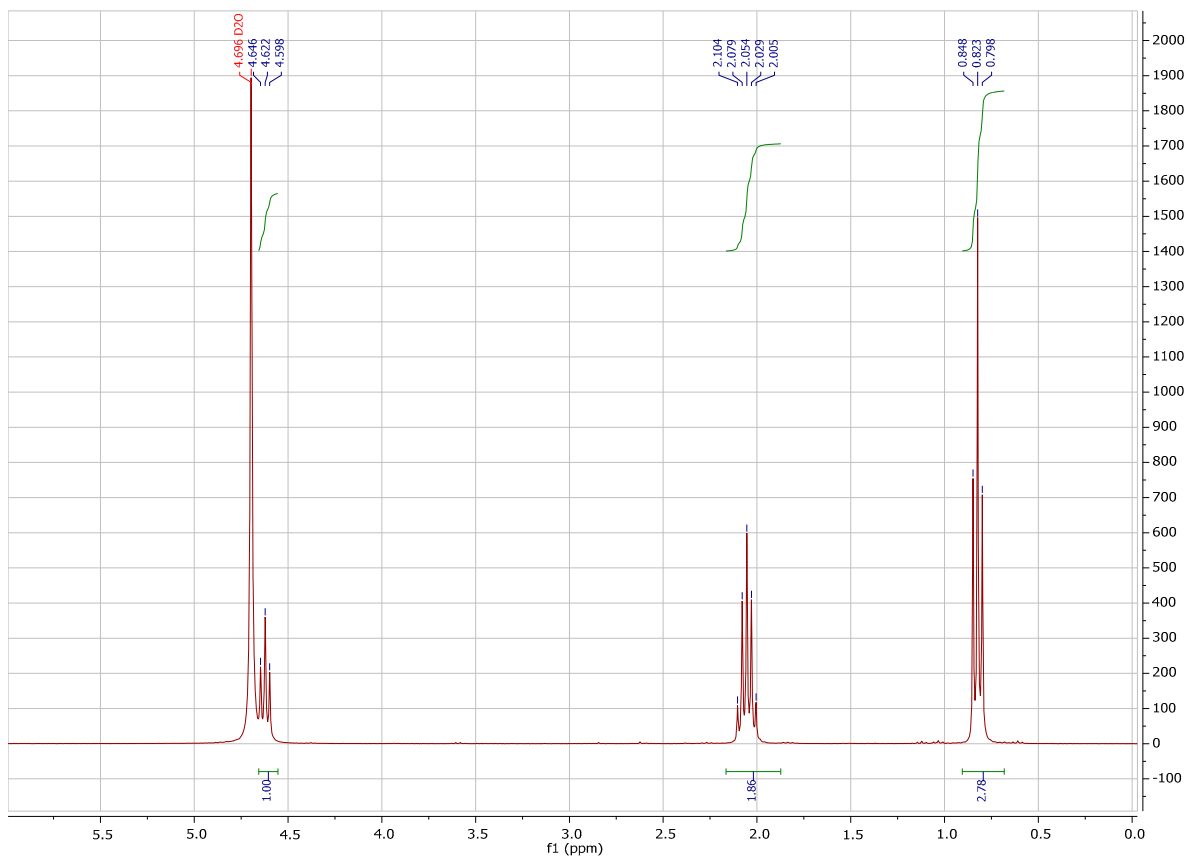
Benzyl-N-[(1S)-1-(1H-1,2,3,4-tetrazol-5-yl)propyl]carbamate **9b-L**:



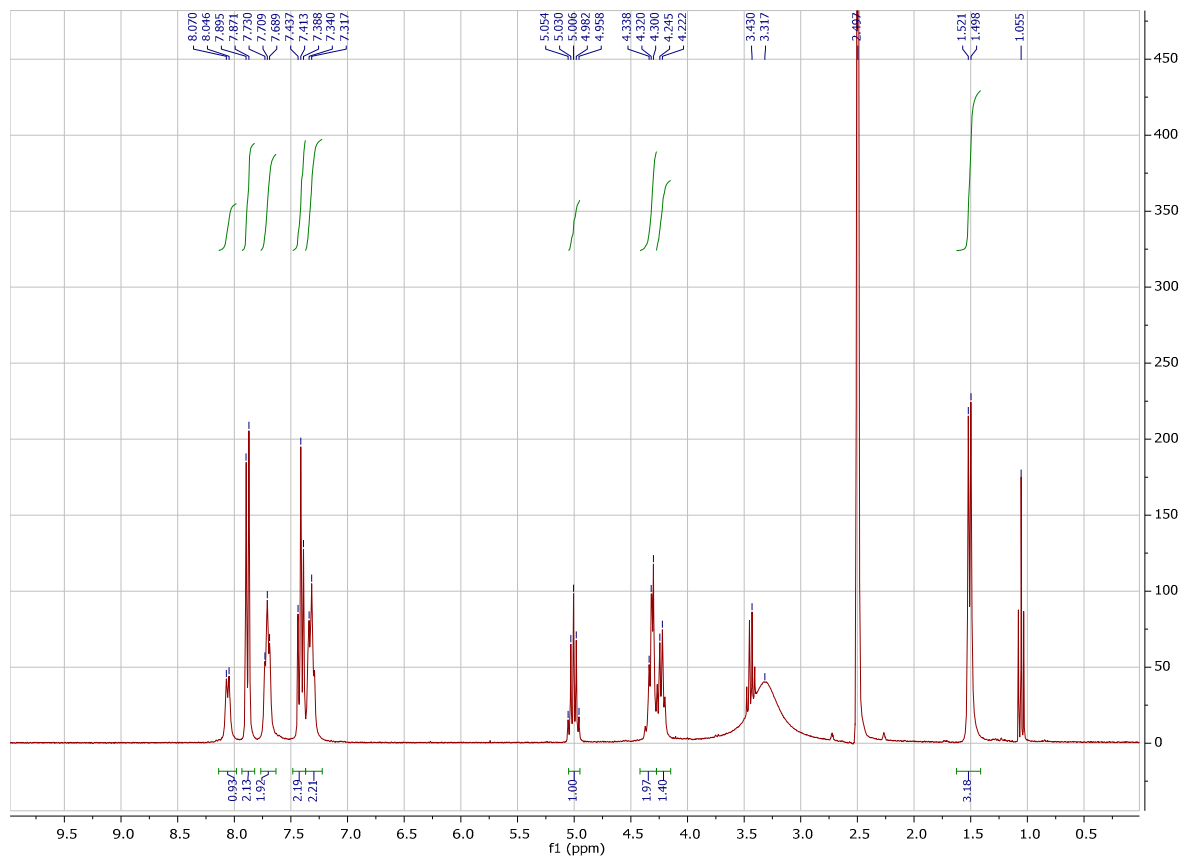
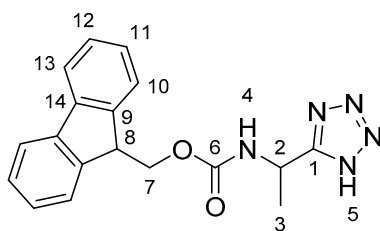


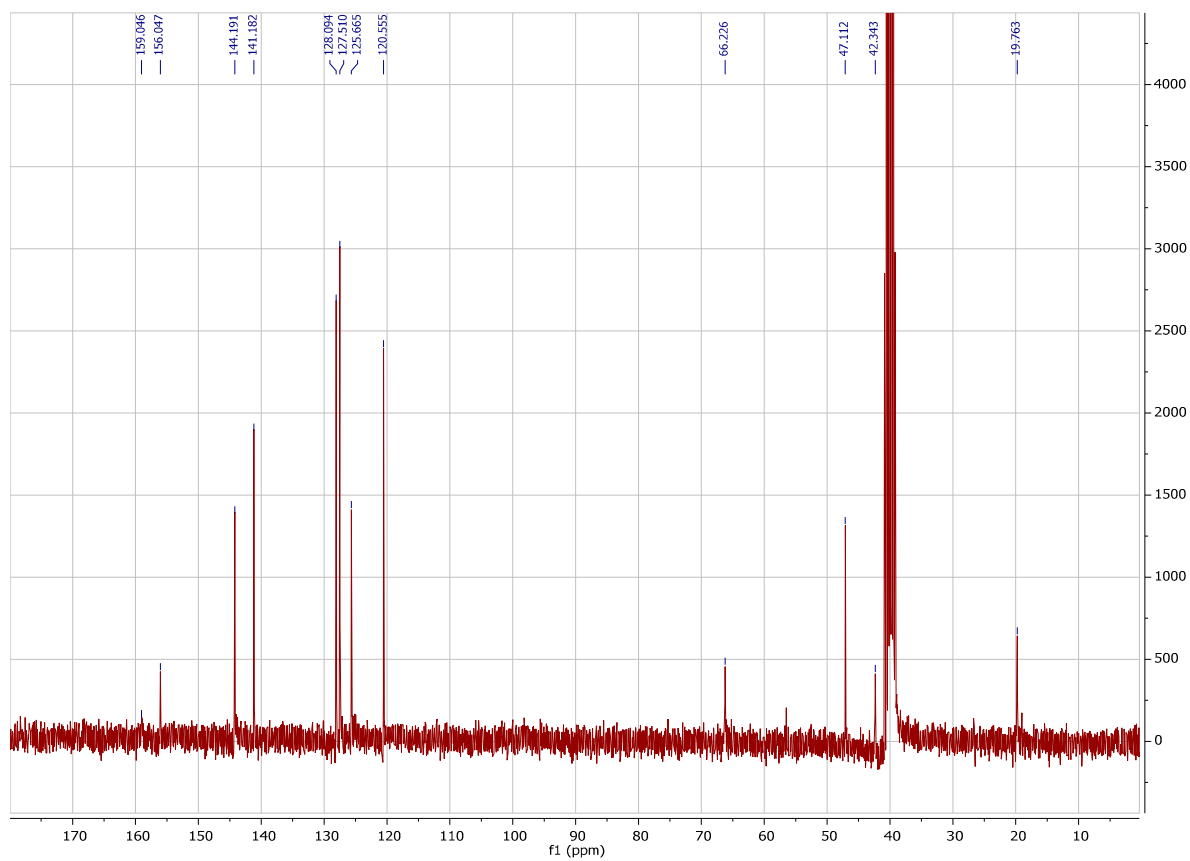
(1S)-1-(1H-1,2,3,4-tetrazol-5-yl)propane-1-amine **4b-L**:



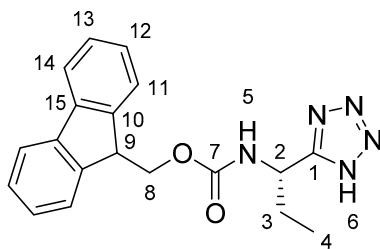


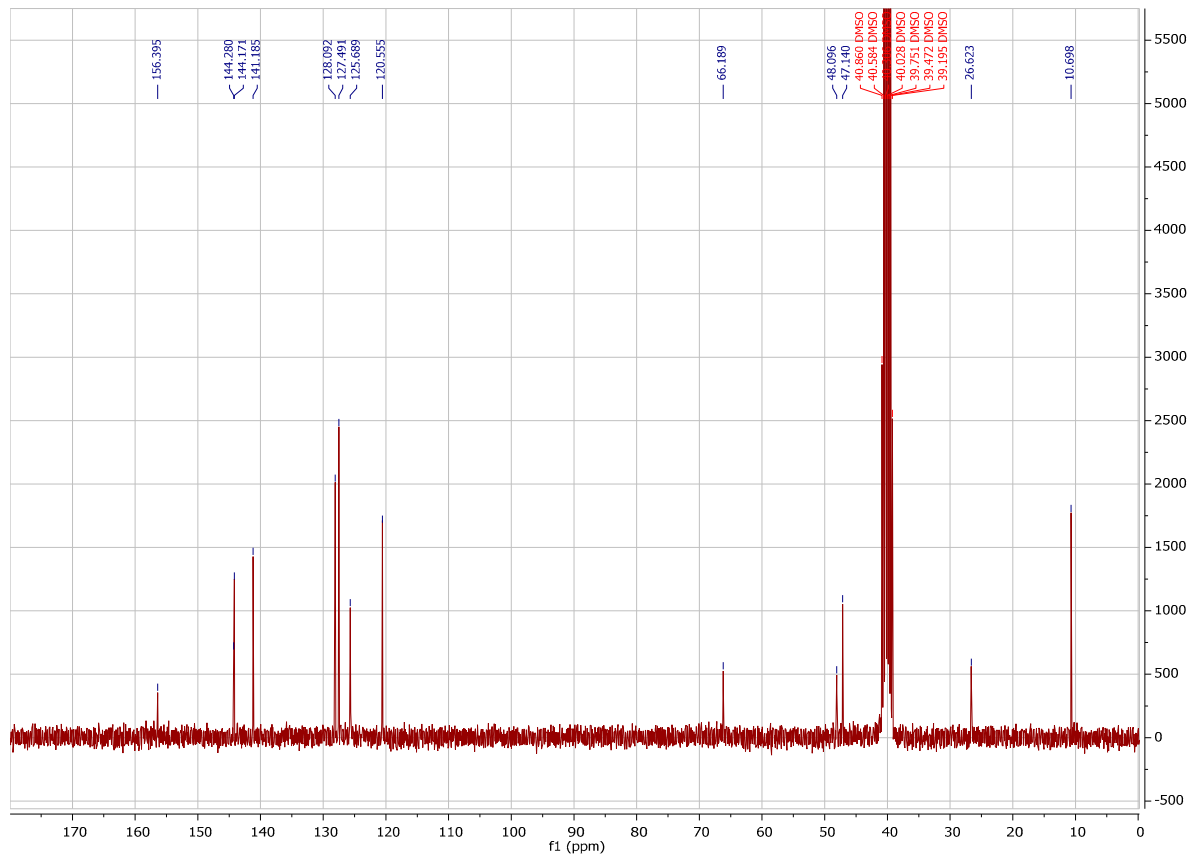
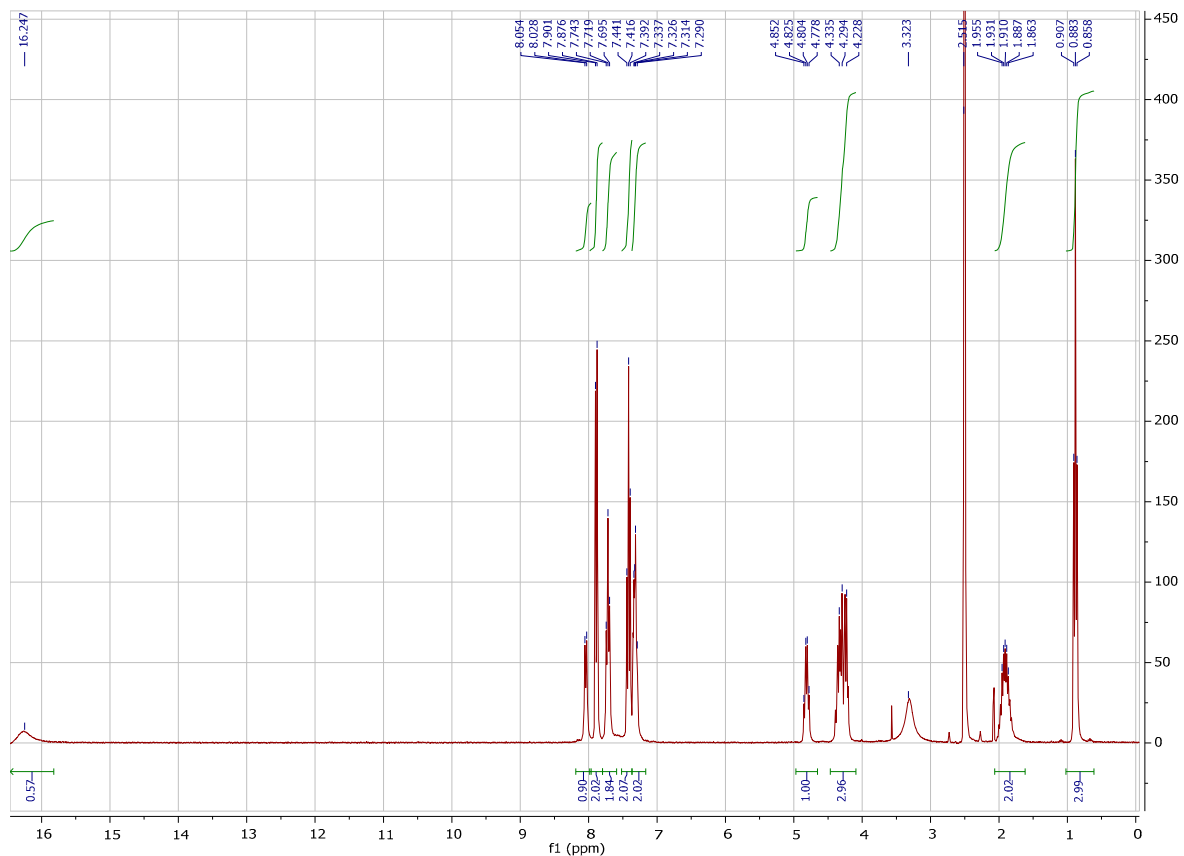
9H-Fluoren-9-ylmethyl-N-[(1S or R)-1-(1H-1,2,3,4-tetrazol-5-yl)ethyl]carbamate **10a**:



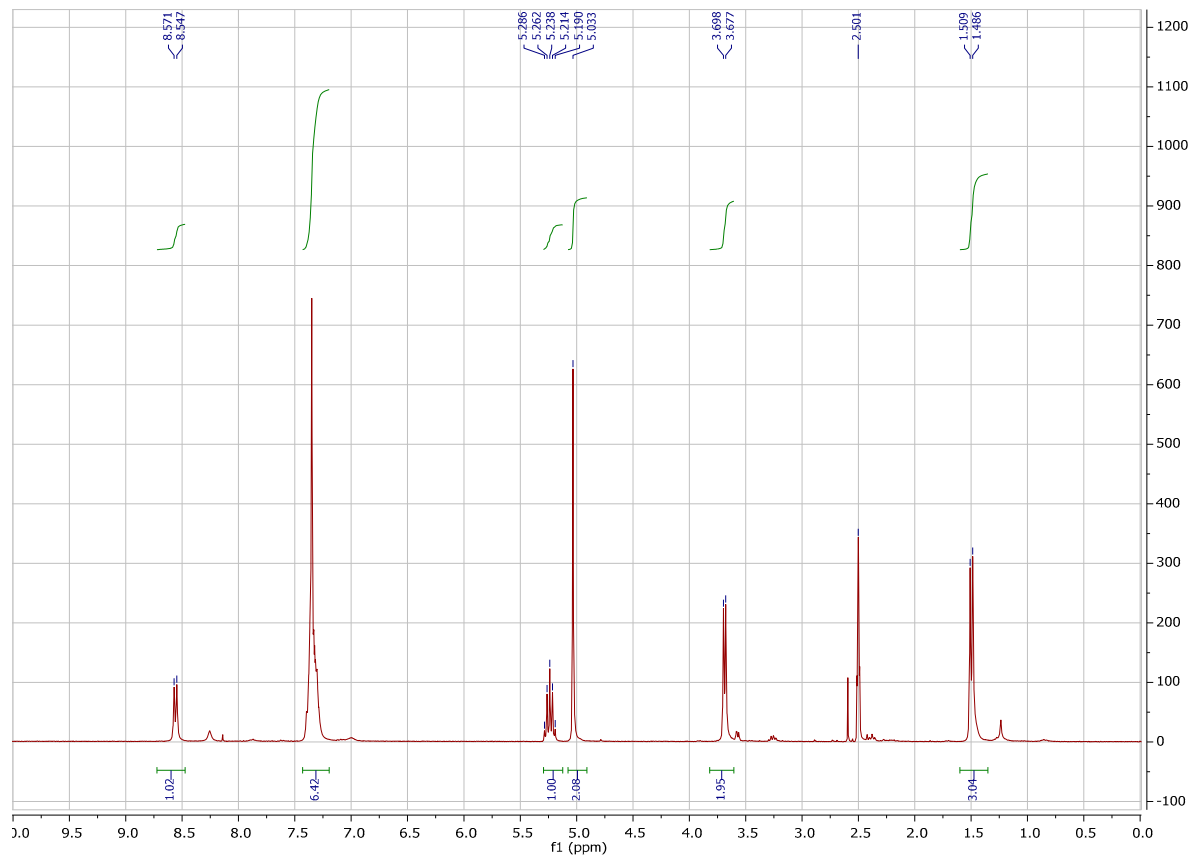
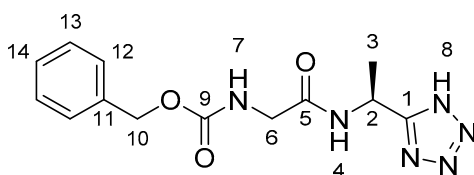


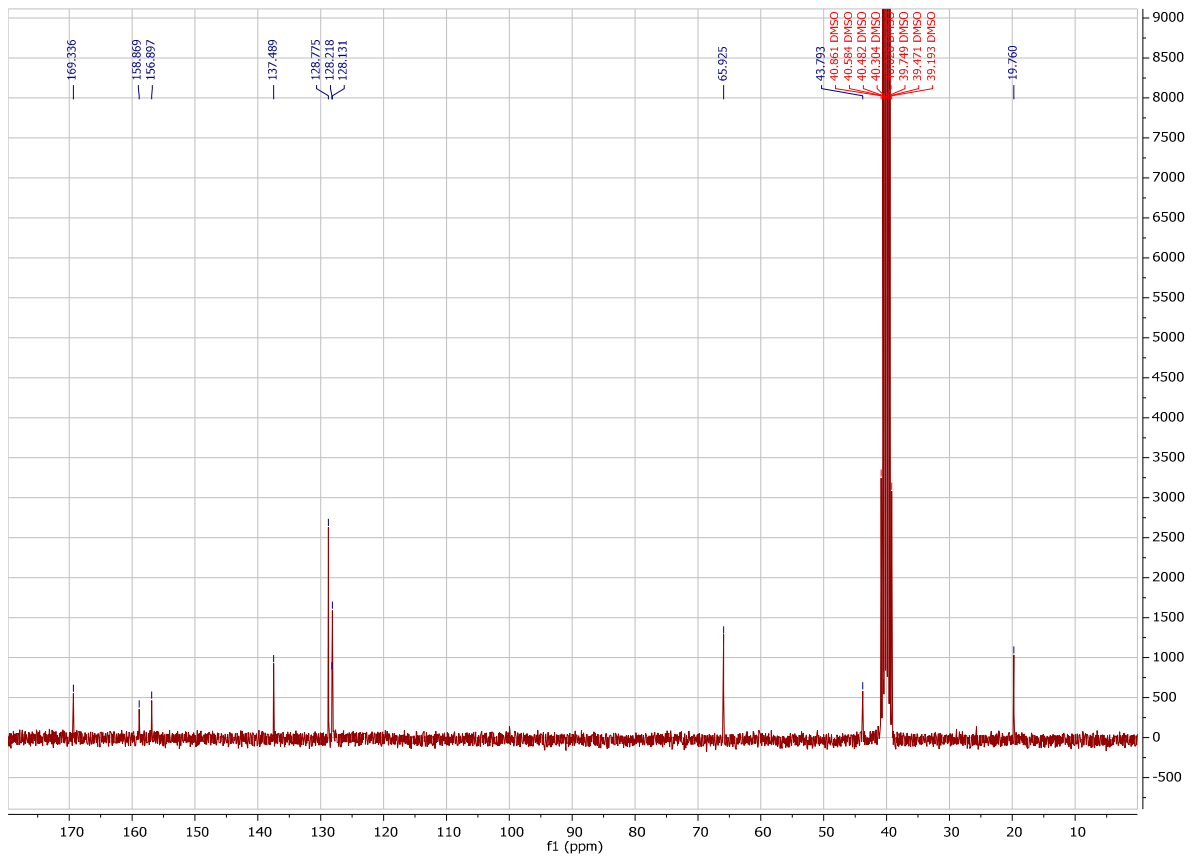
9H-Fluoren-9-ylmethyl-N-[(1S)-1-(1H-1,2,3,4-tetrazol-5-yl)propyl]carbamate **10b-L**



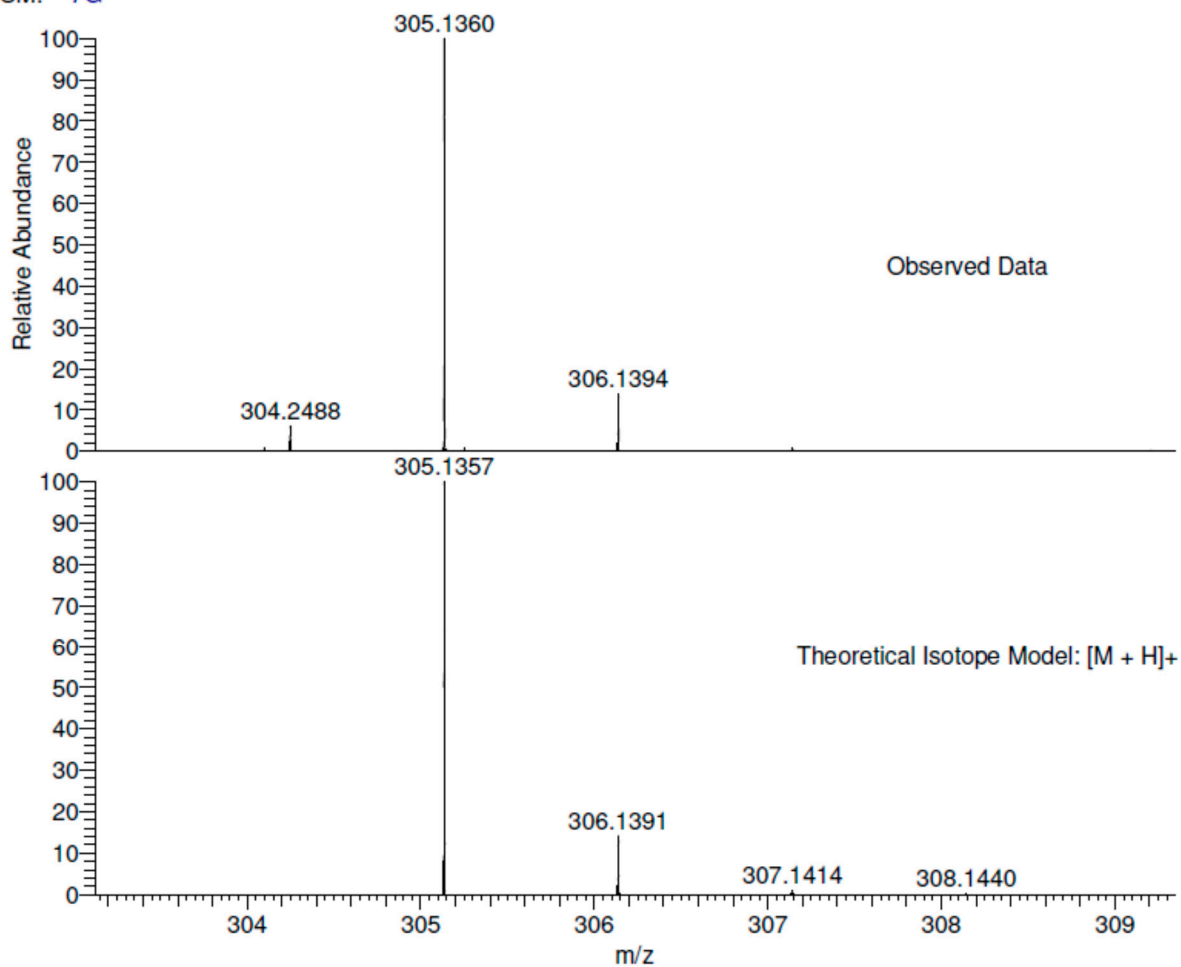


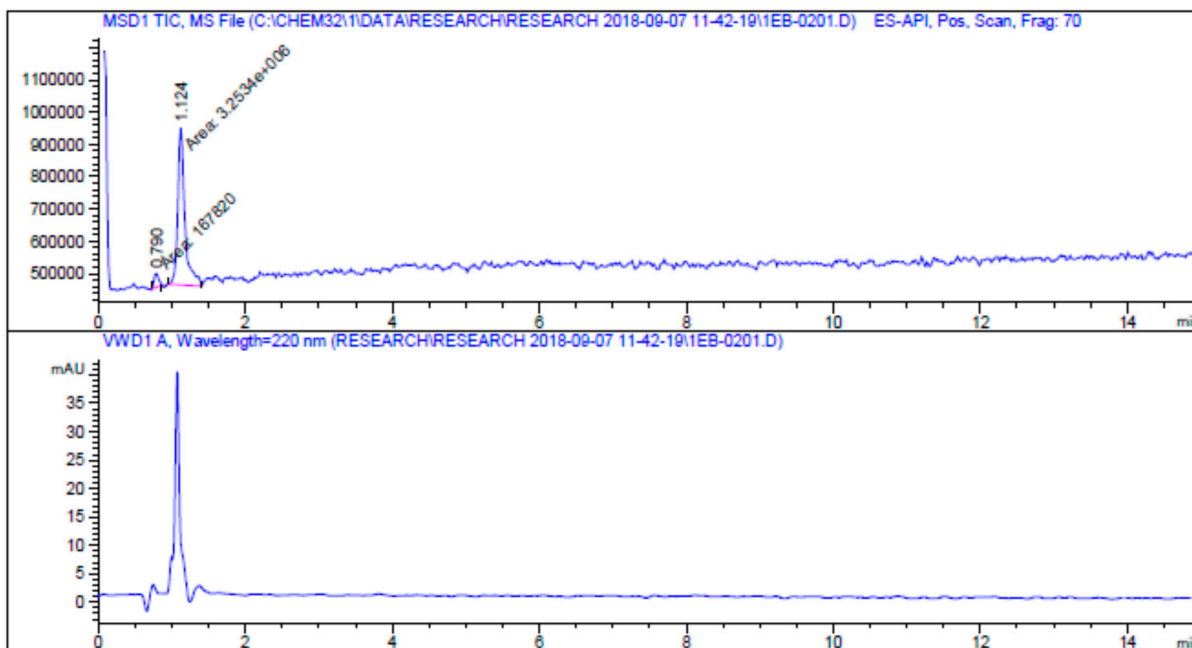
Benzyl-N-(((1S)-1-(1H-1,2,3,4-tetrazol-5-yl)ethyl)carbamoyl)methyl)carbamate **13g-L**:





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 Area Percent Report
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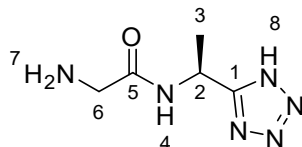
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 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

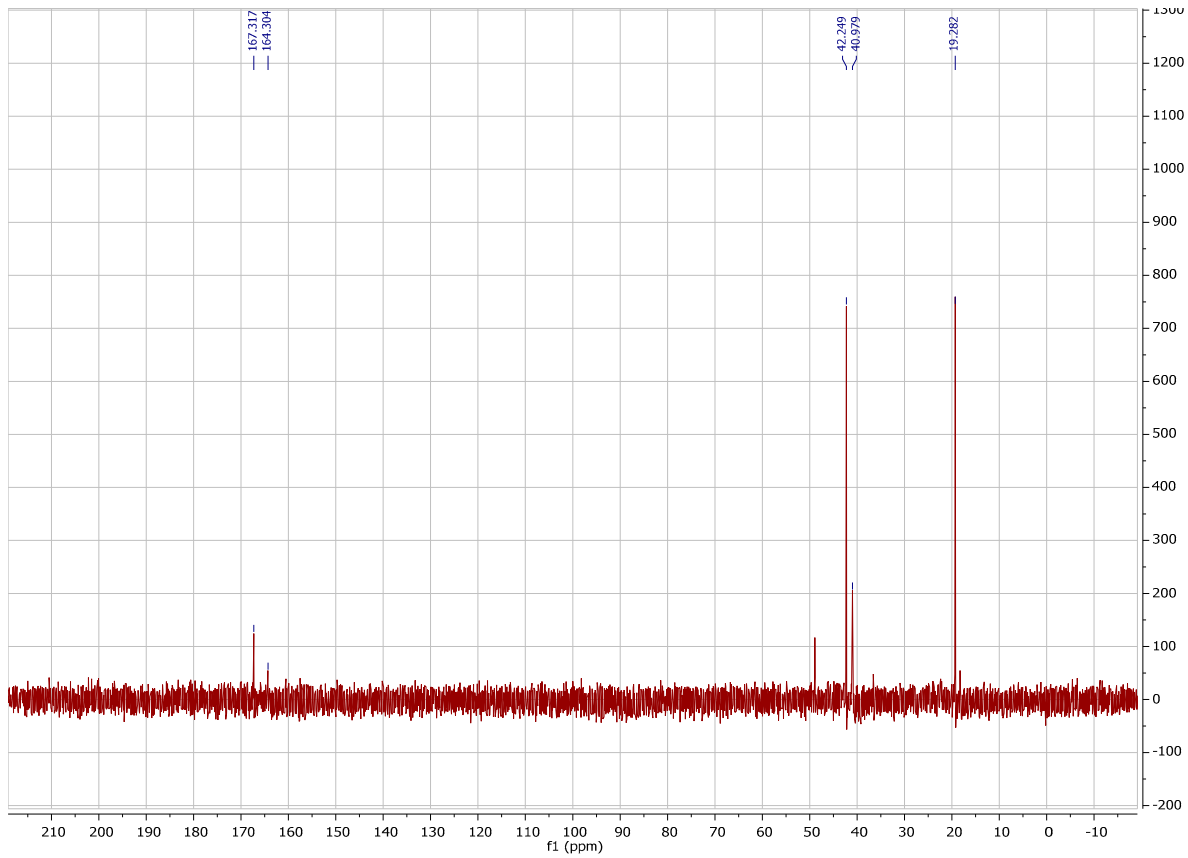
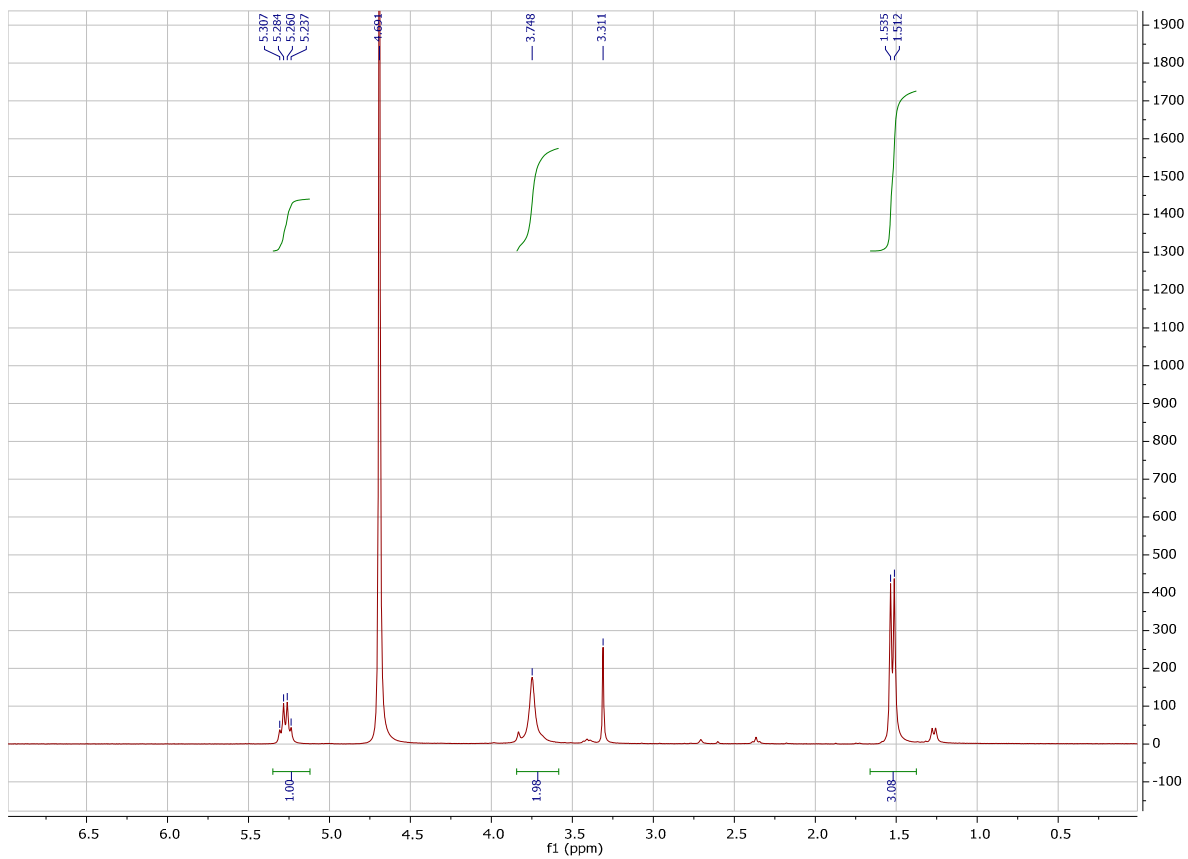
Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	0.790	MM	0.0623	1.67820e5	4.49148e4	4.9053
2	1.124	MM	0.1104	3.25340e6	4.91366e5	95.0947

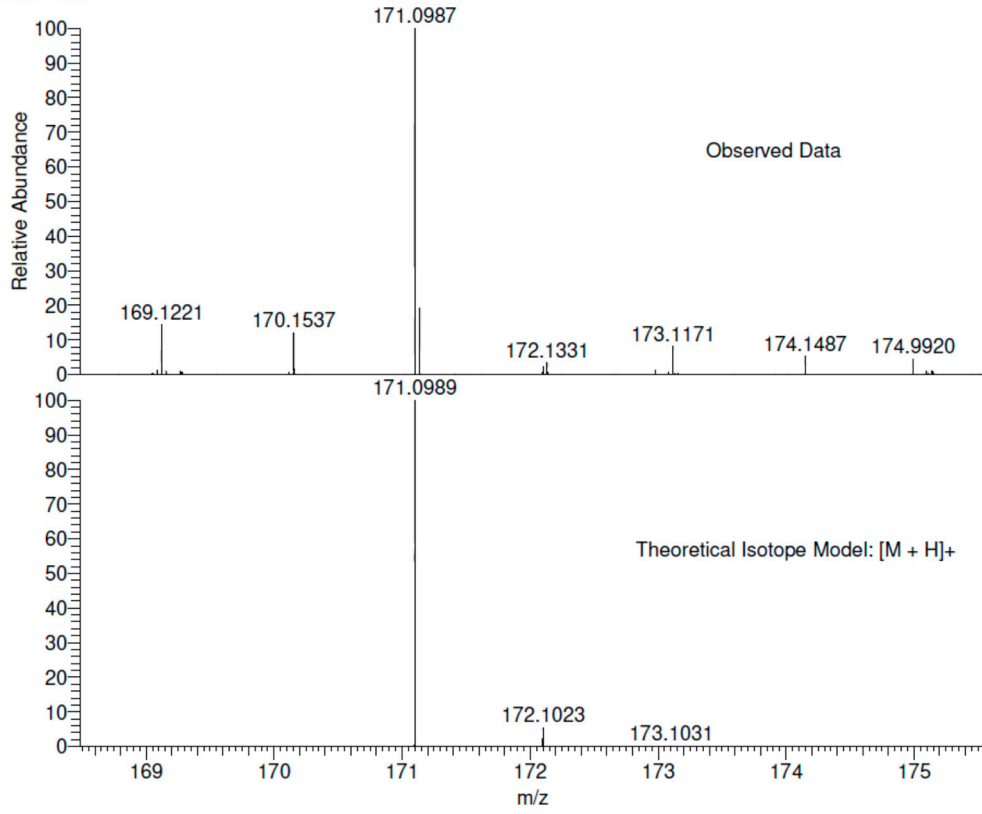
Totals : 3.42122e6 5.36281e5

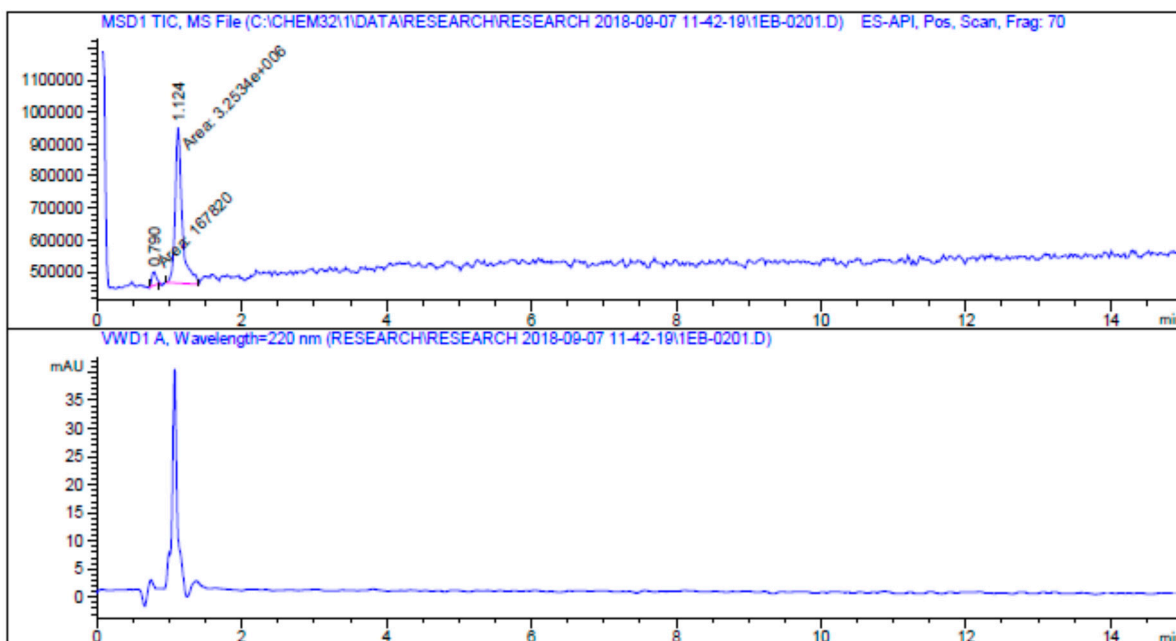
2-Amino-N-[(1S)-1-(1H-1,2,3,4-tetrazol-5-yl)ethyl]acetamide **14g-L**:





SM: 7G





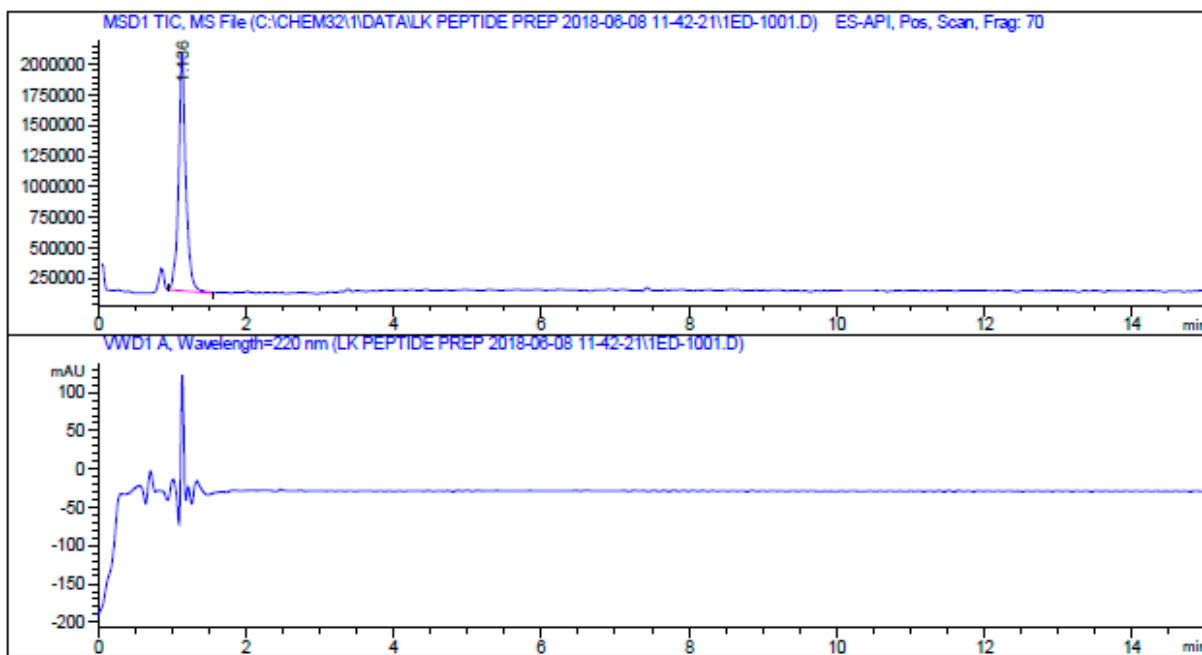
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 Area Percent Report
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Sorted By : Signal
 Multiplier: : 1.0000
 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	0.790	MM	0.0623	1.67820e5	4.49148e4	4.9053
2	1.124	MM	0.1104	3.25340e6	4.91366e5	95.0947

Totals : 3.42122e6 5.36281e5



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 Area Percent Report
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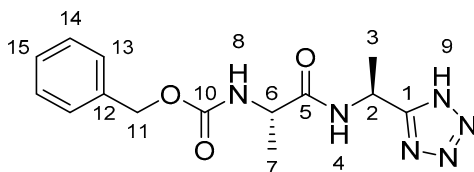
Sorted By : Signal
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 Use Multiplier & Dilution Factor with ISTDs

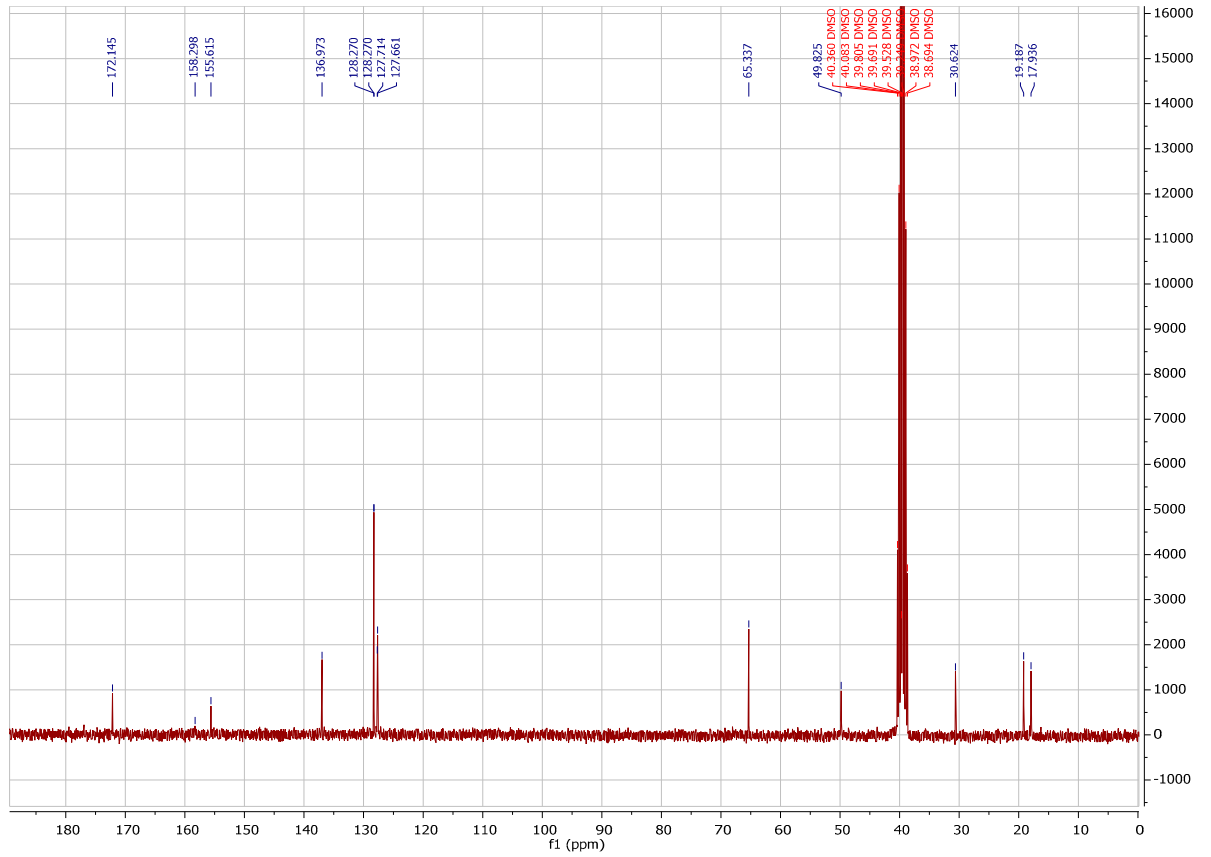
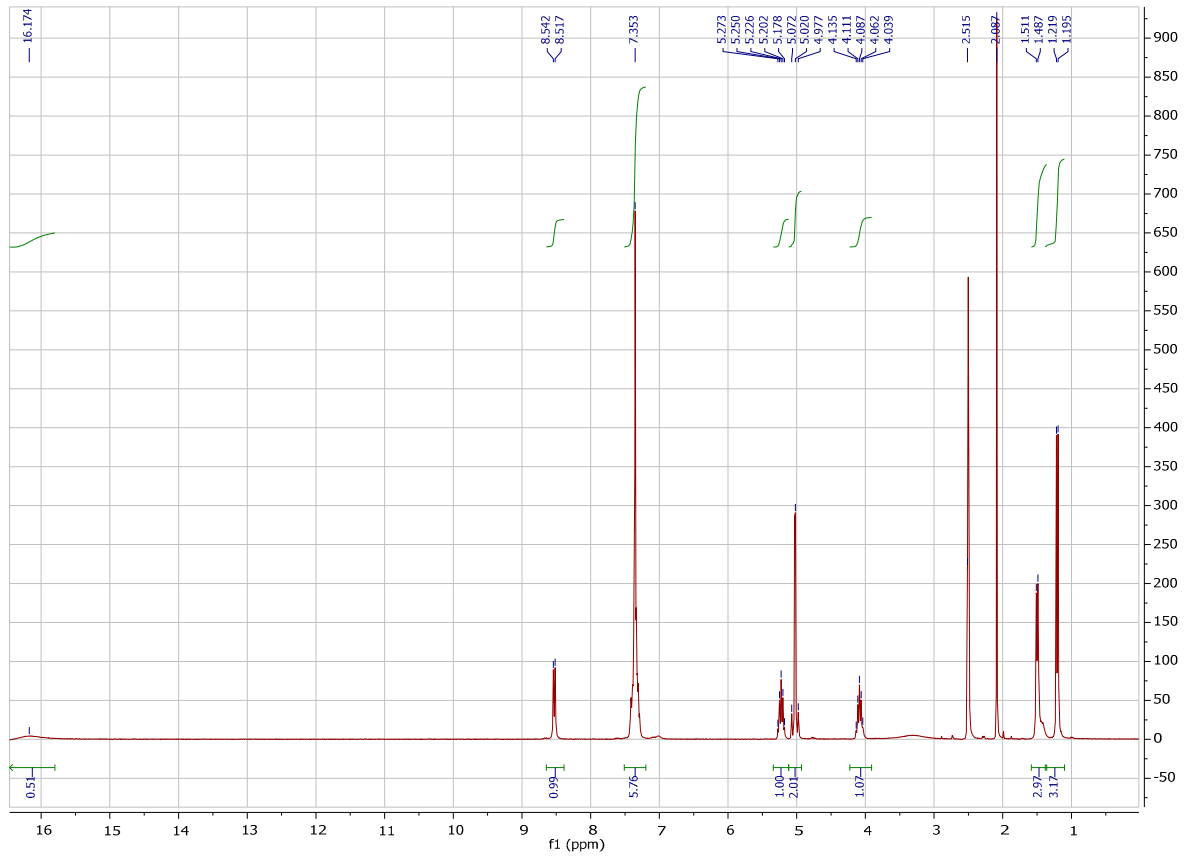
Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	1.136	BB	0.1009	1.34913e7	1.99147e6	100.0000

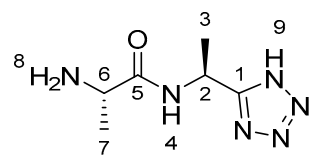
Totals : 1.34913e7 1.99147e6

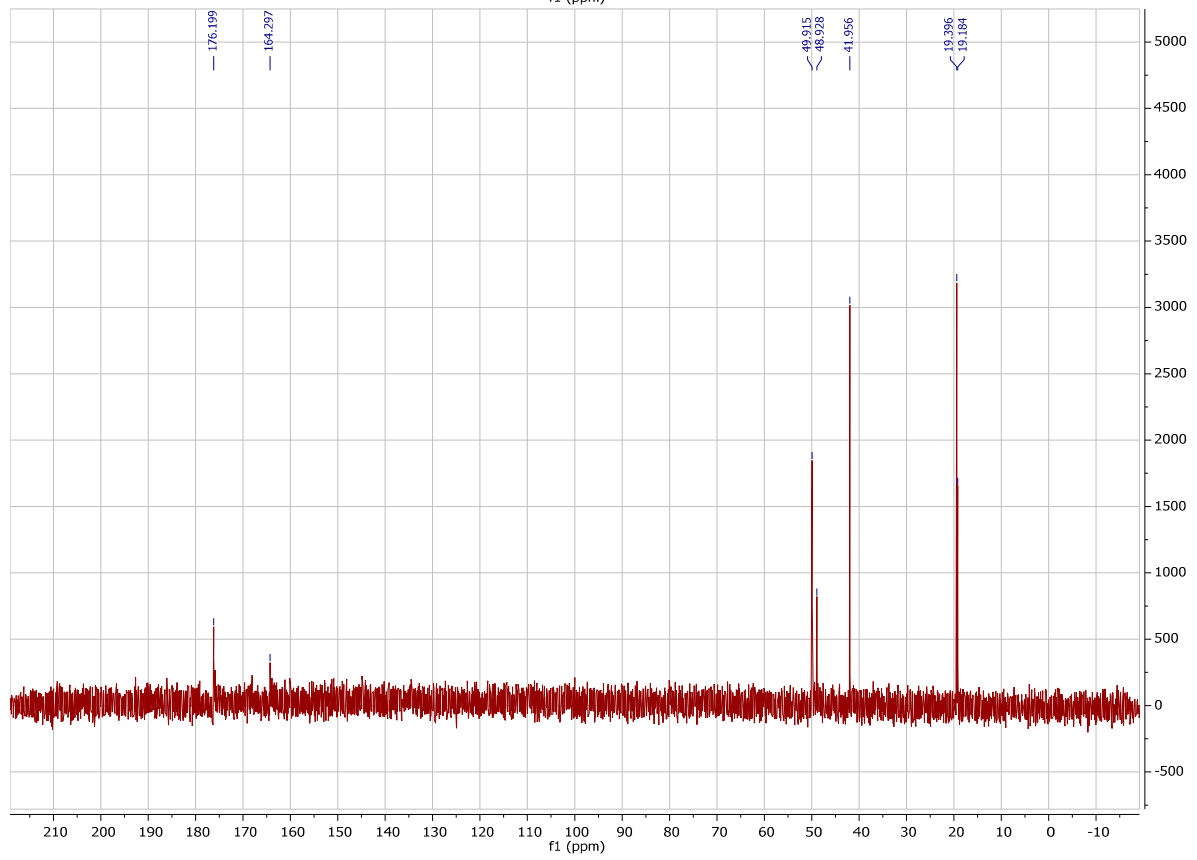
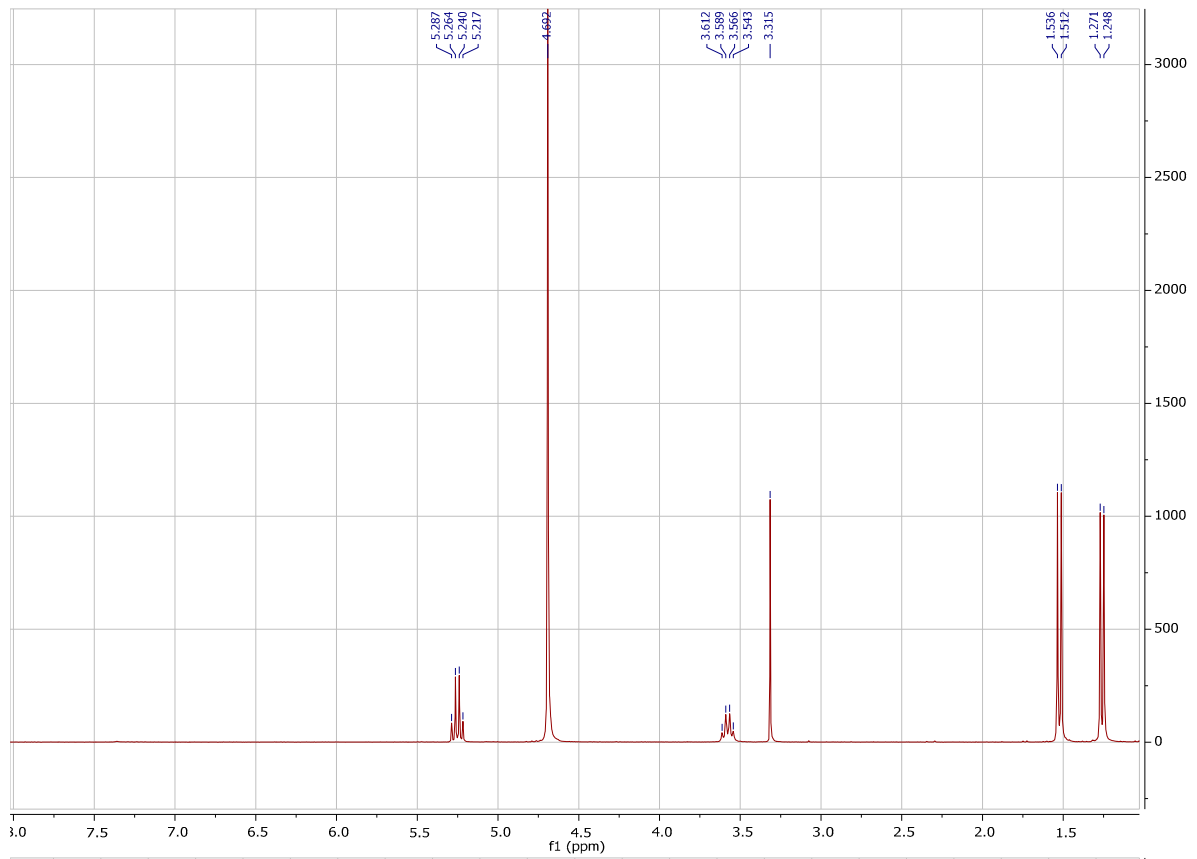
Benzyl-N-[(1S)-1-[(1S)-1-(1H-1,2,3,4-tetrazol-5-yl)ethyl]carbamoyl]ethyl]carbamate **13a-LL**:



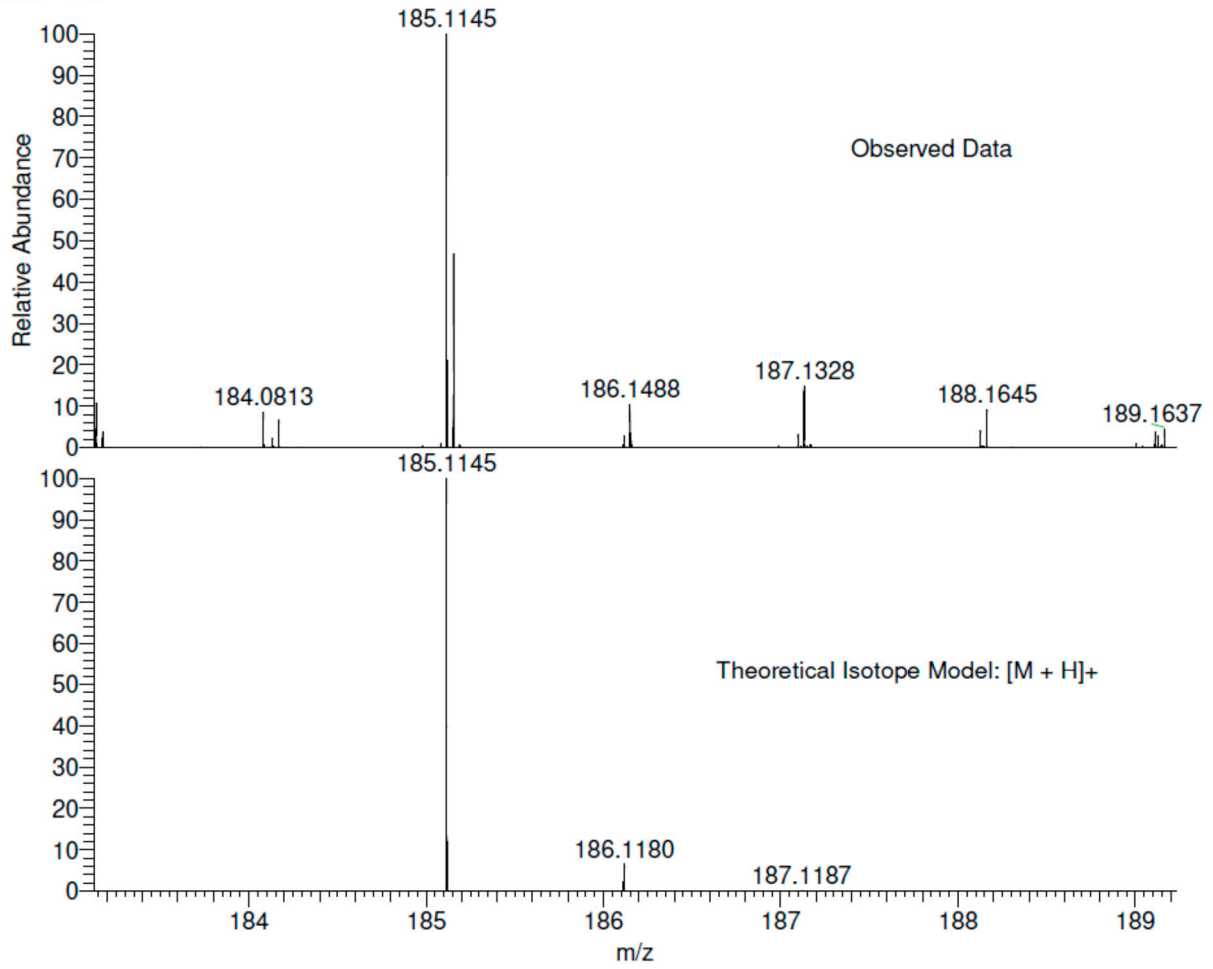


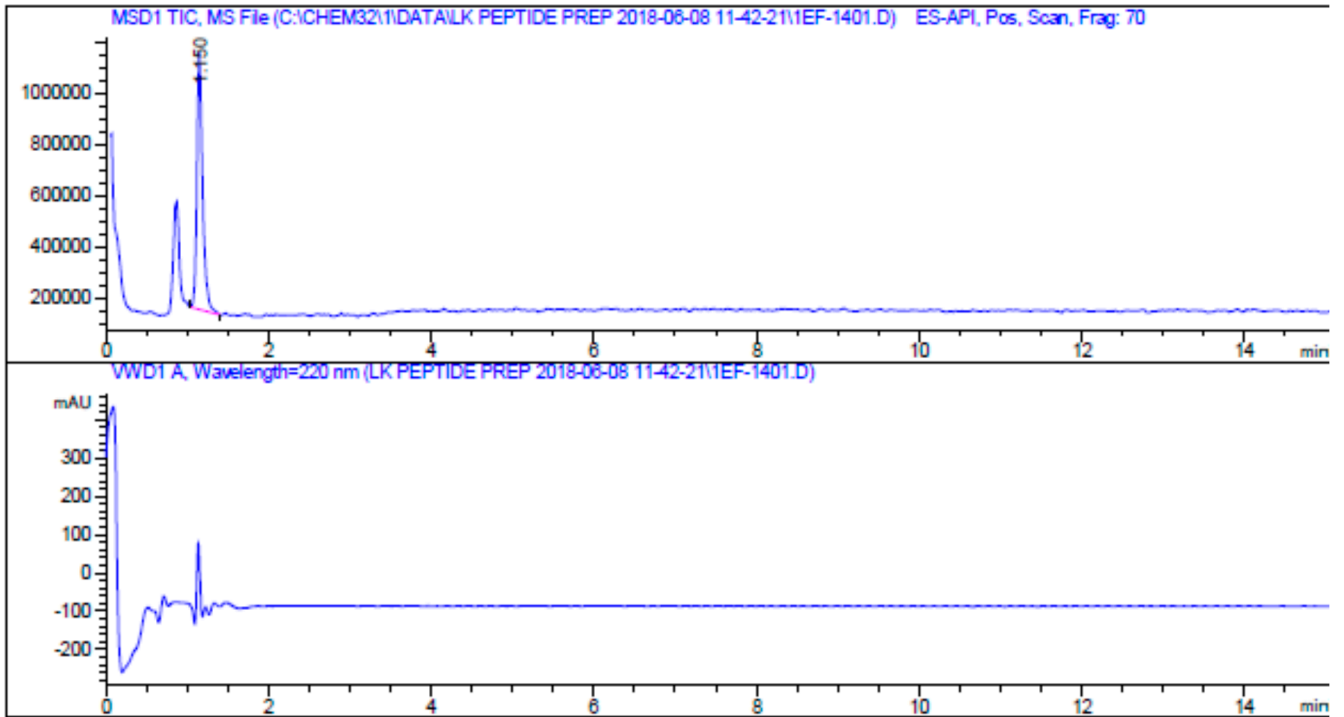
(2*S*)-2-amino-*N*-[(1*S*)-1-(1*H*-1,2,3,4-tetrazol-5-yl)ethyl]propanamide **14a-LL**:





SM: 7G





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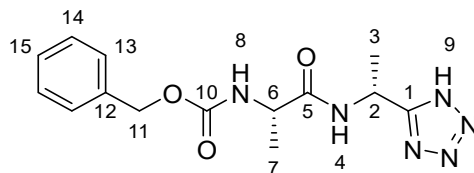
Sorted By : Signal
 Multiplier: : 1.0000
 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

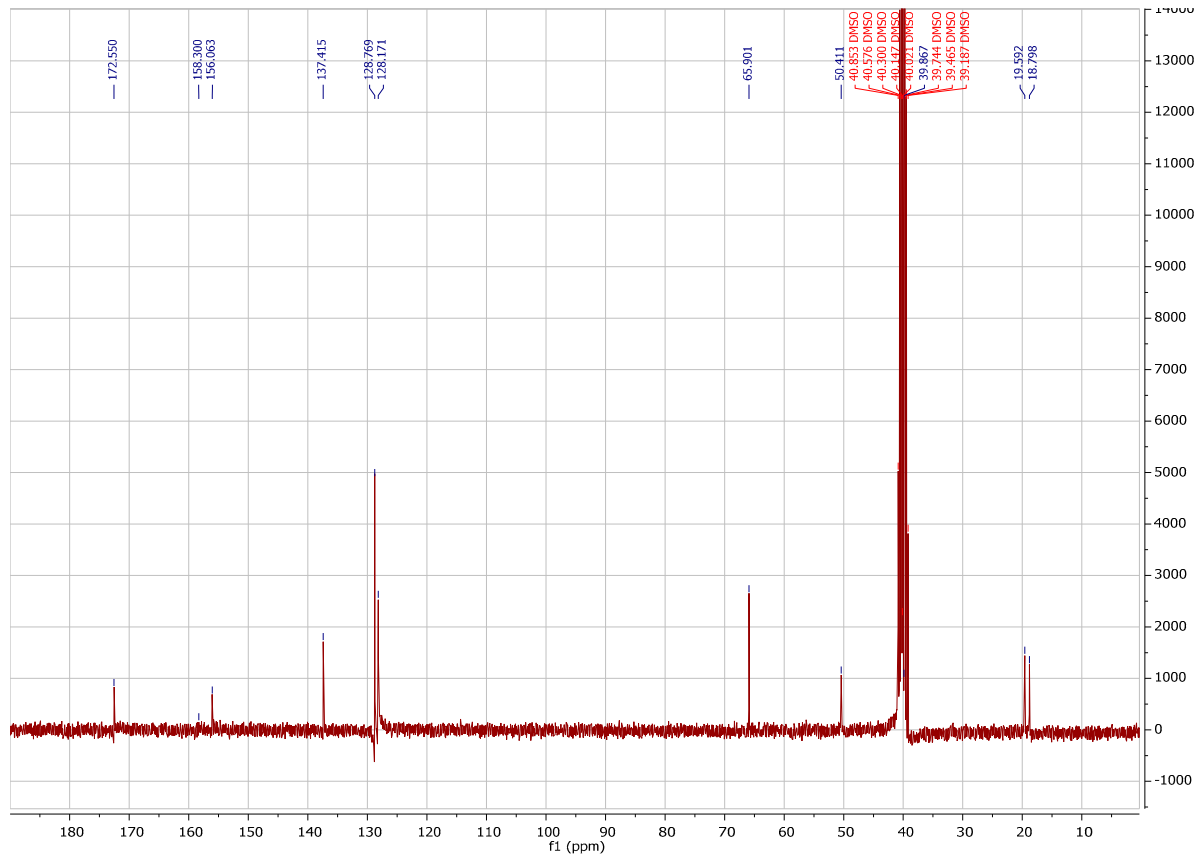
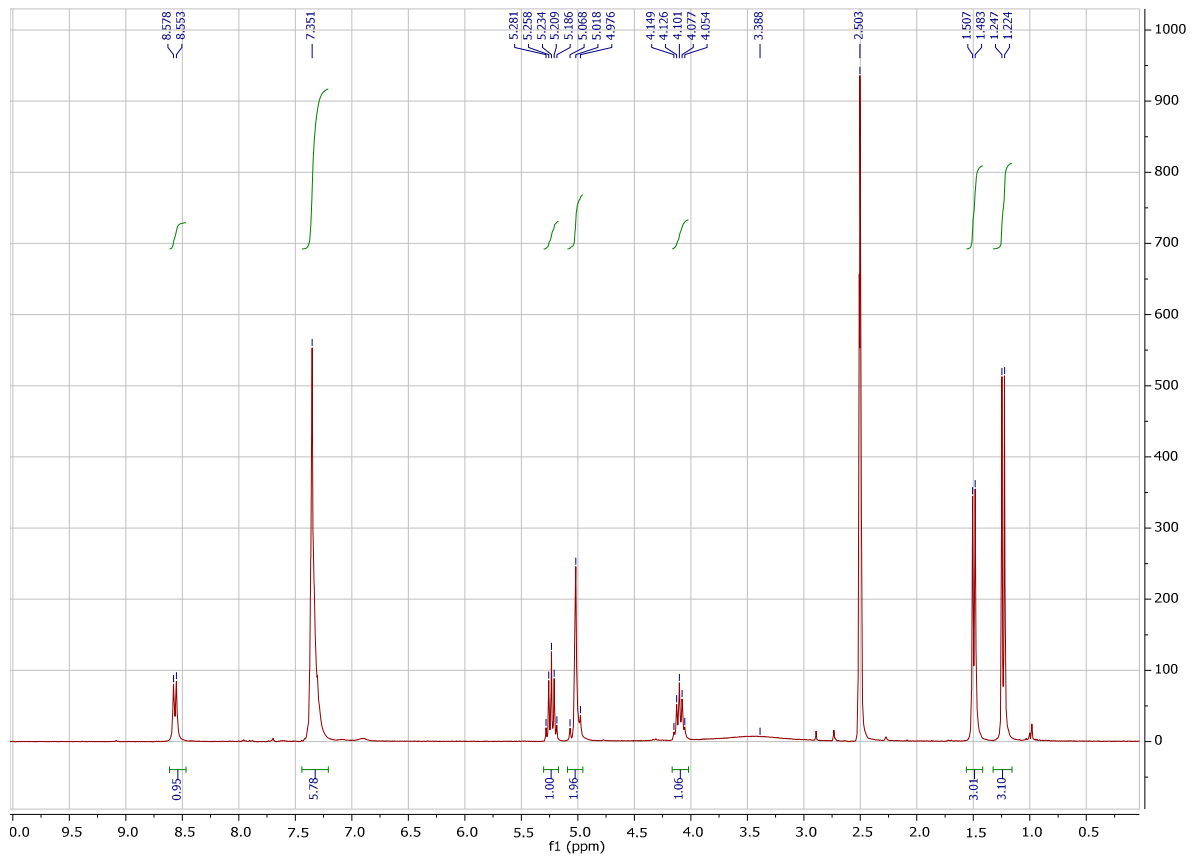
Signal 1: MSD1 TIC, MS File

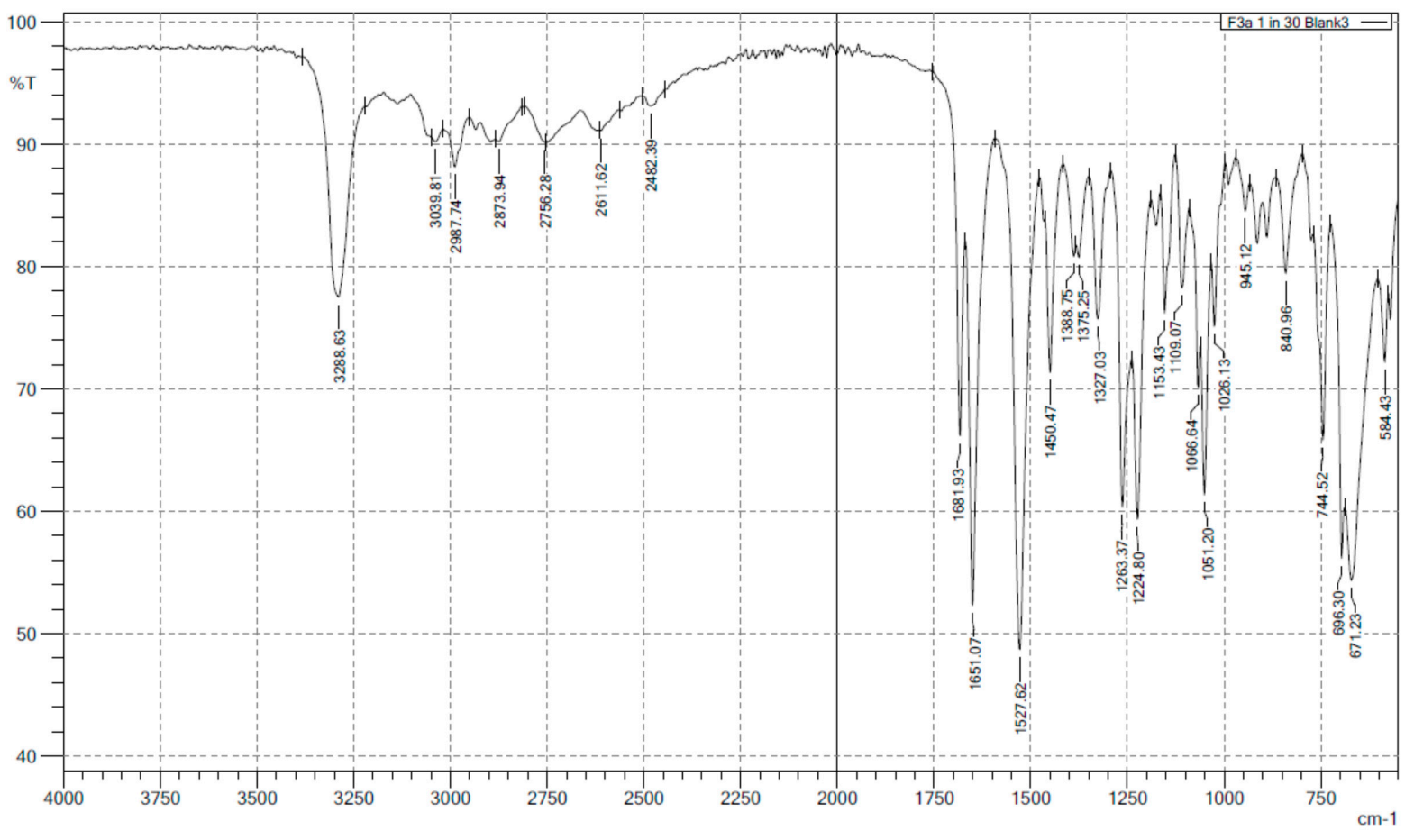
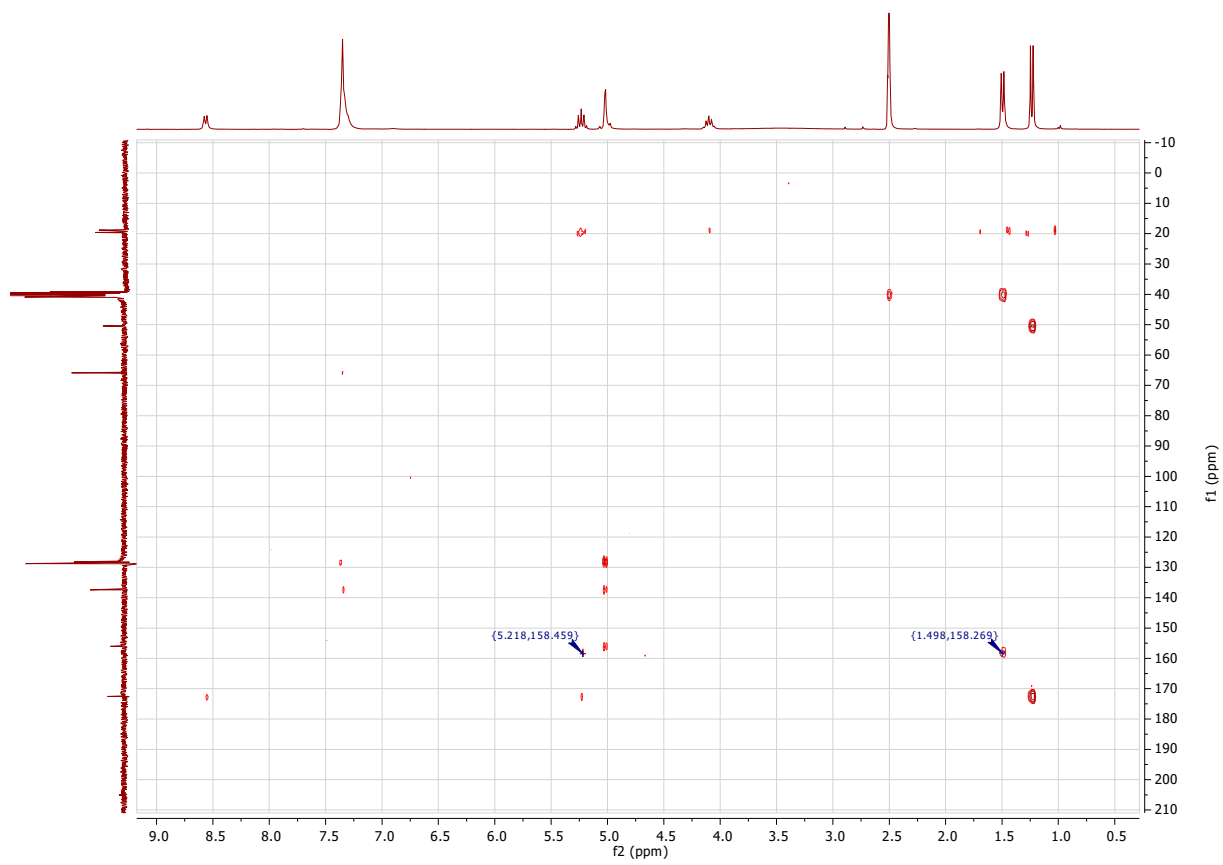
Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	1.150	BB	0.0730	4.93549e6	1.01672e6	100.0000

Totals : 4.93549e6 1.01672e6

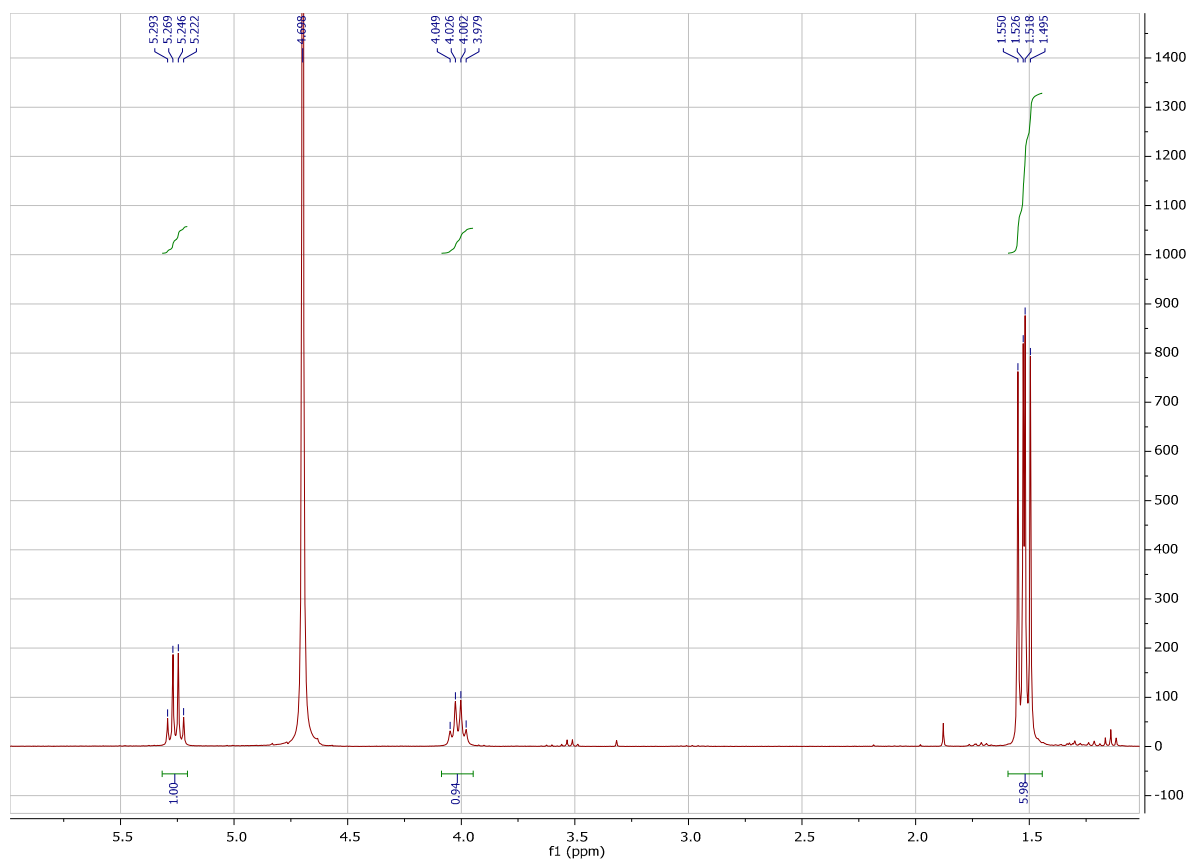
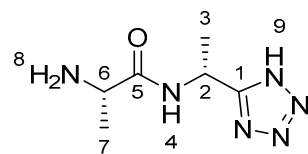
Benzyl-*N*-[(1*S*)-1-[(1*R*)-1-(1*H*-1,2,3,4-tetrazol-5-yl)ethyl]carbamoyl]ethyl]carbamate **13a-LD**:

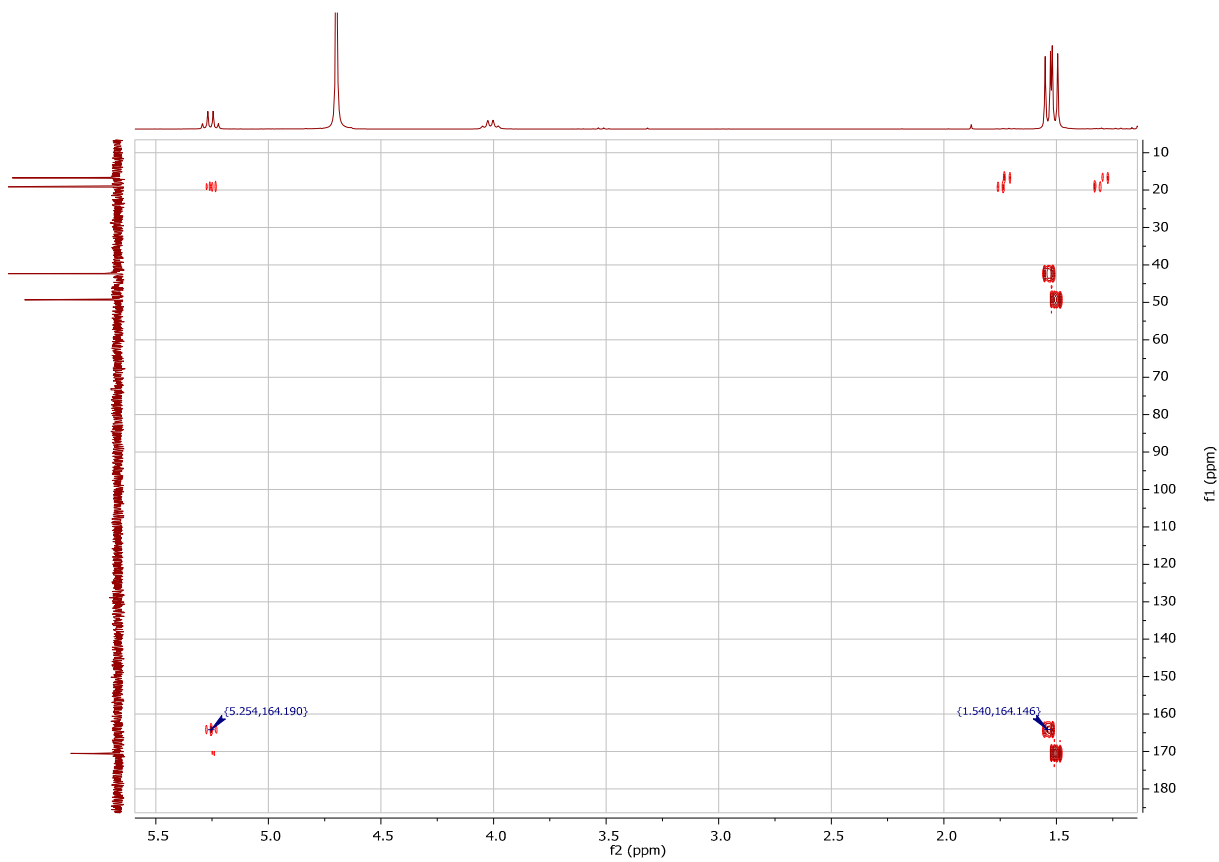
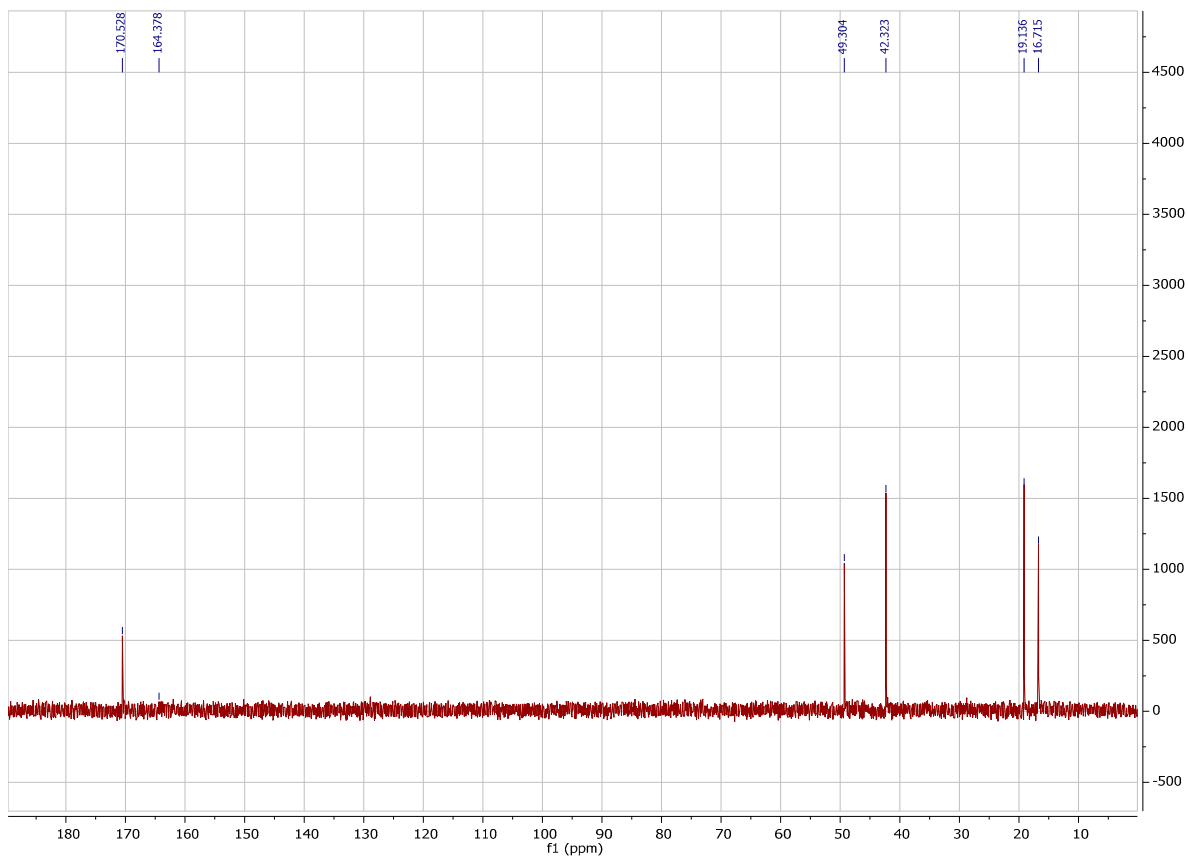


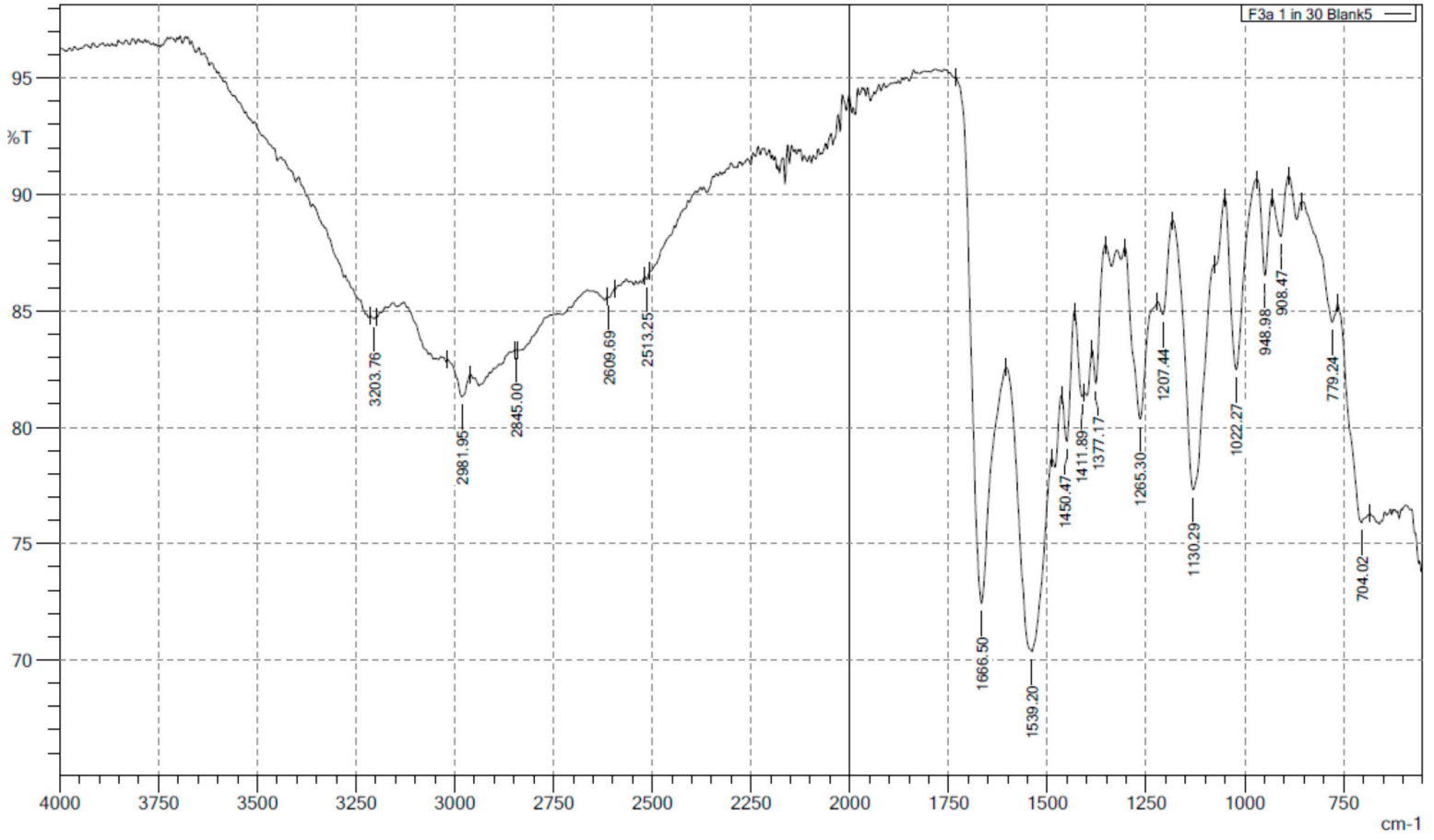




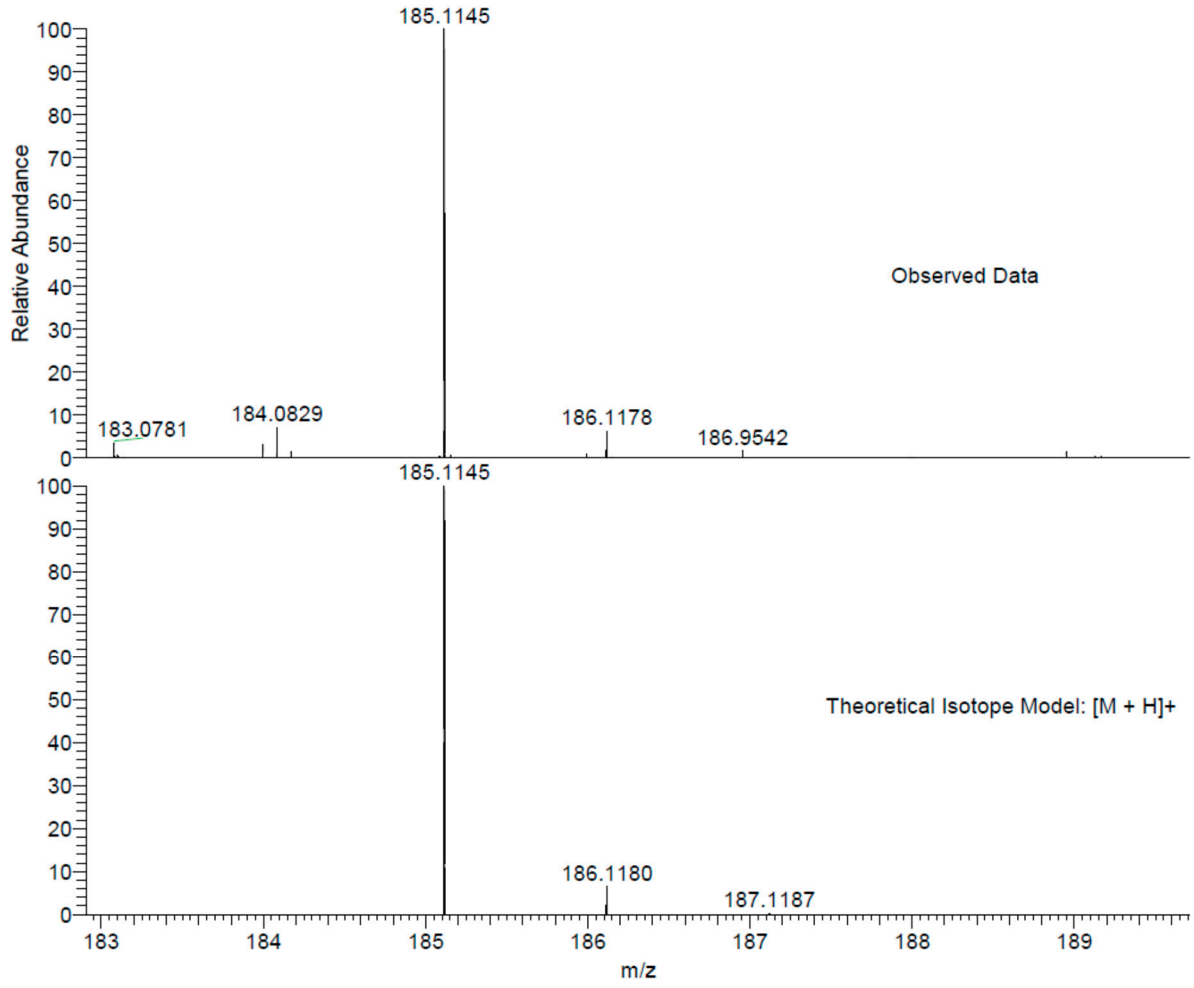
(2S)-2-amino-N-[(1R)-1-(1H-1,2,3,4-tetrazol-5-yl)ethyl]propanamide **14a-LD**:

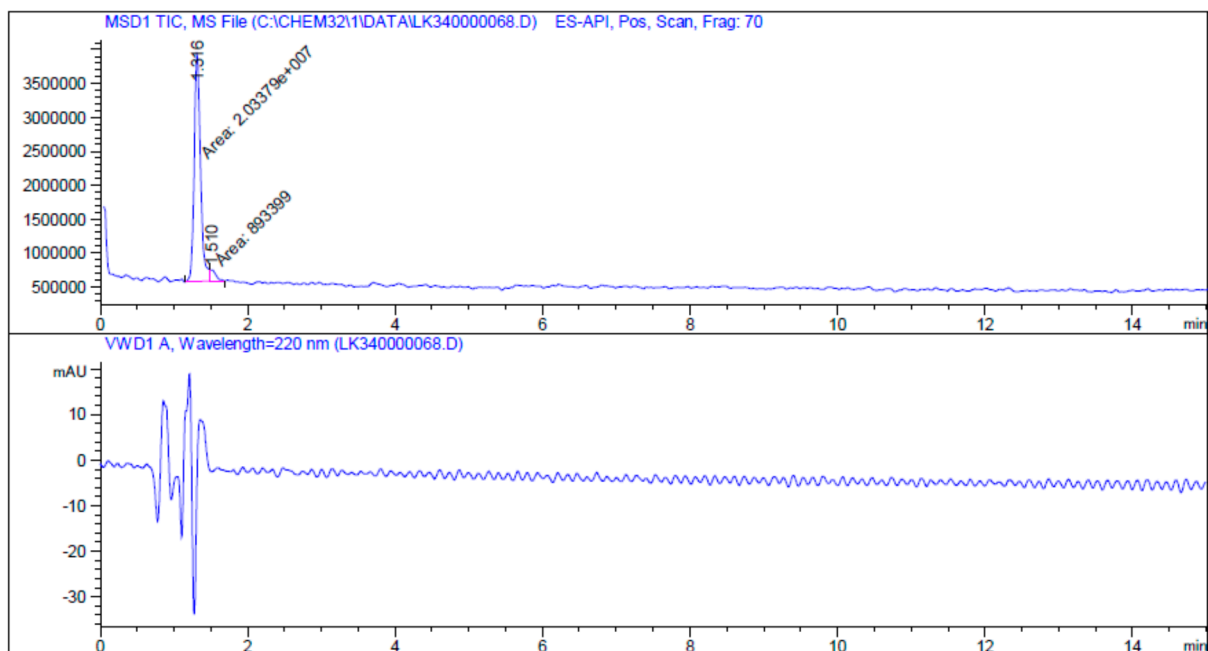






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 Area Percent Report
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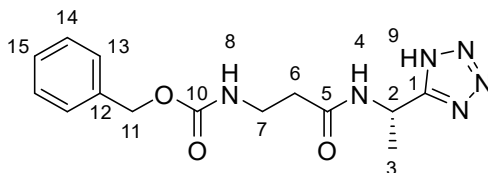
Sorted By : Signal
 Multiplier: : 1.0000
 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

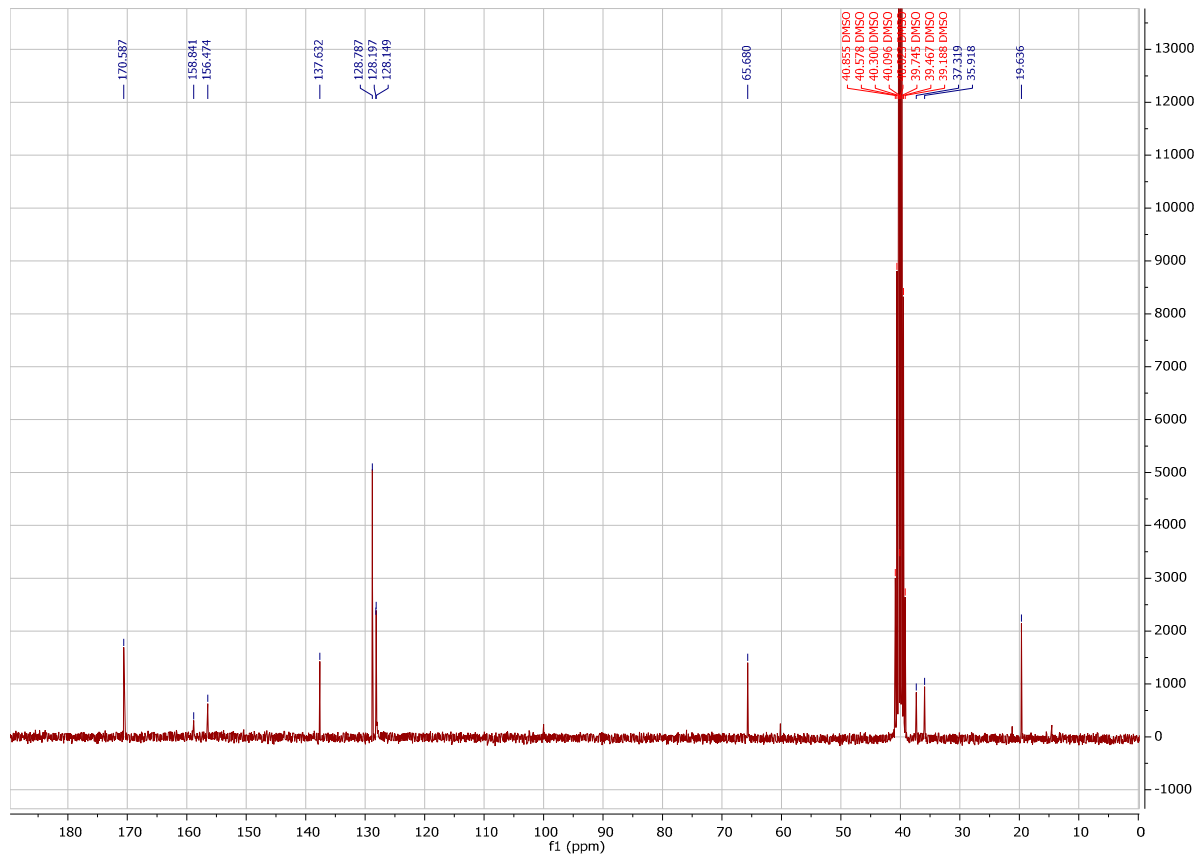
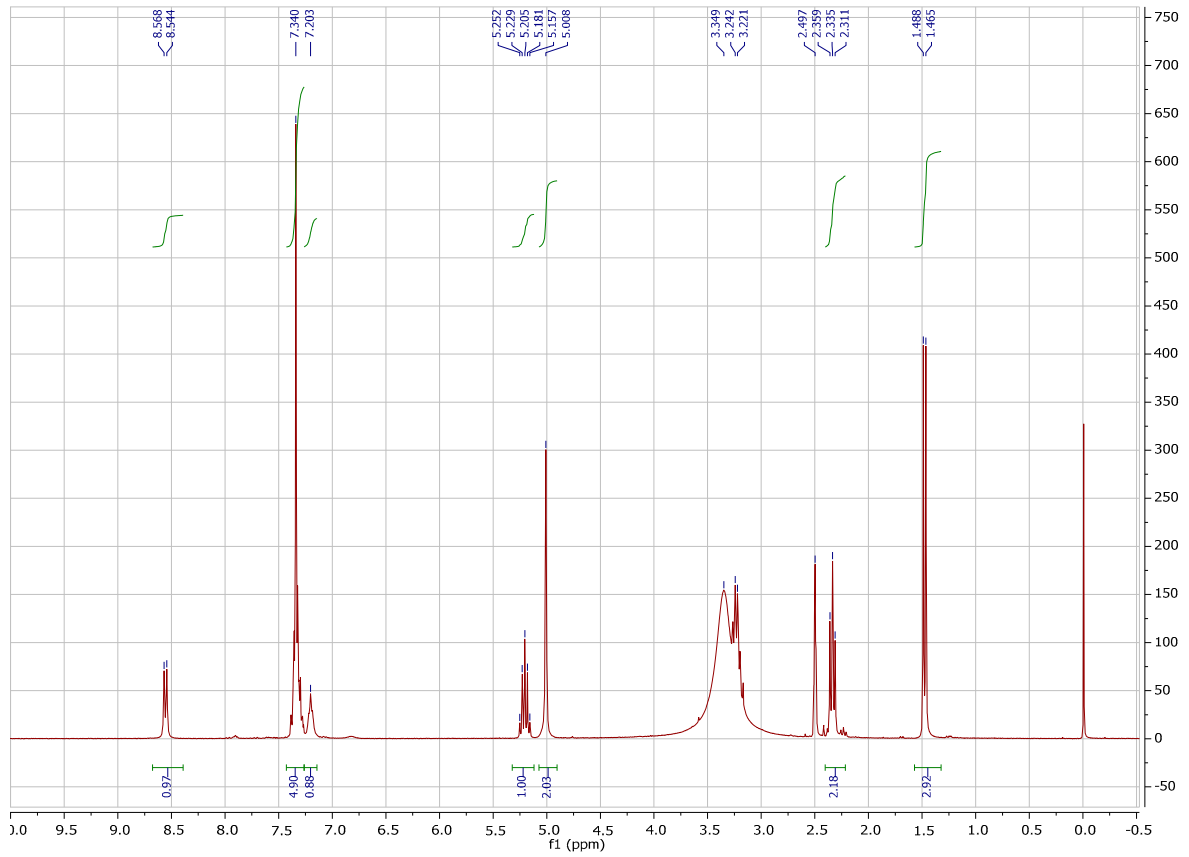
Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	1.316	MF	0.0976	2.03379e7	3.47199e6	95.7921
2	1.510	FM	0.0882	8.93399e5	1.68763e5	4.2079

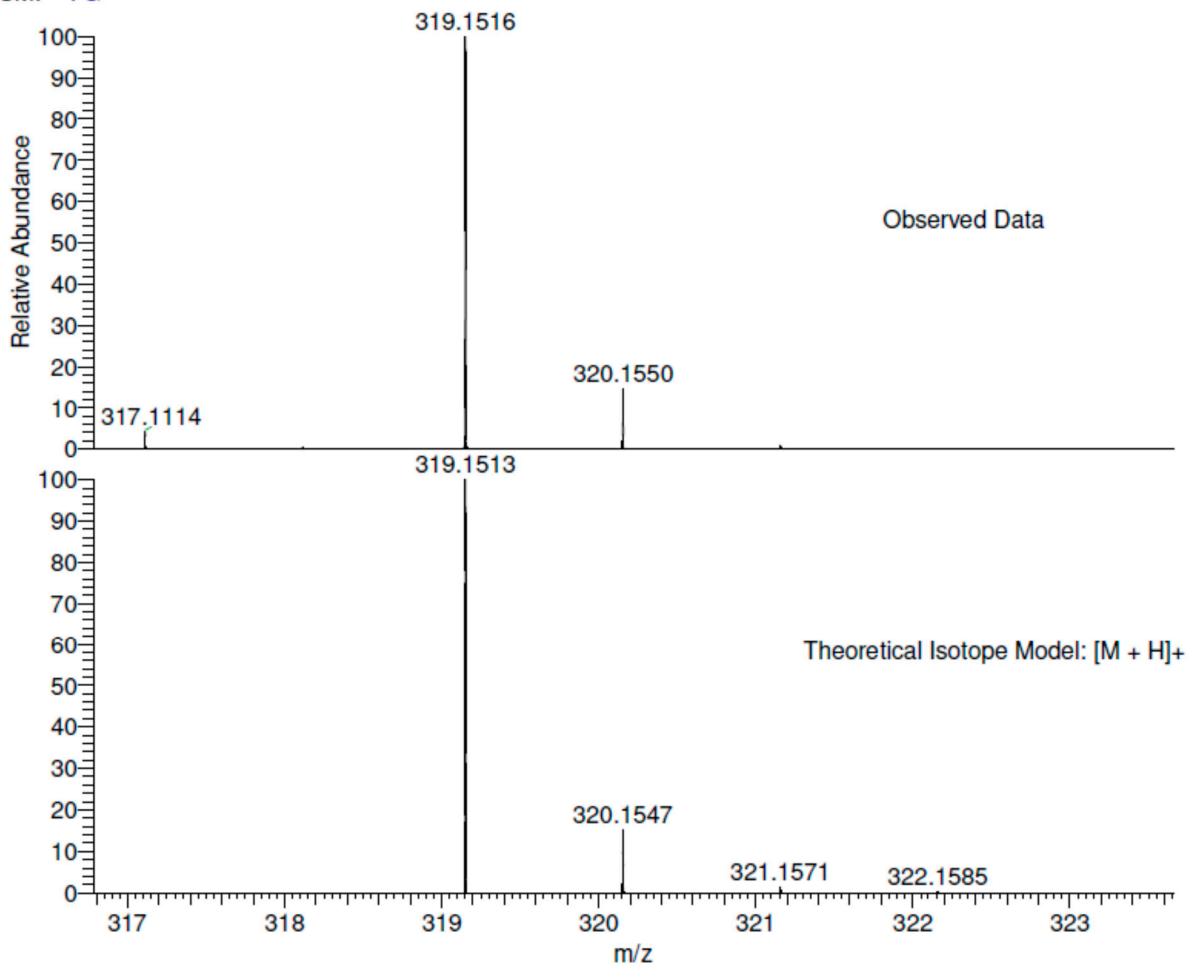
Totals : 2.12313e7 3.64076e6

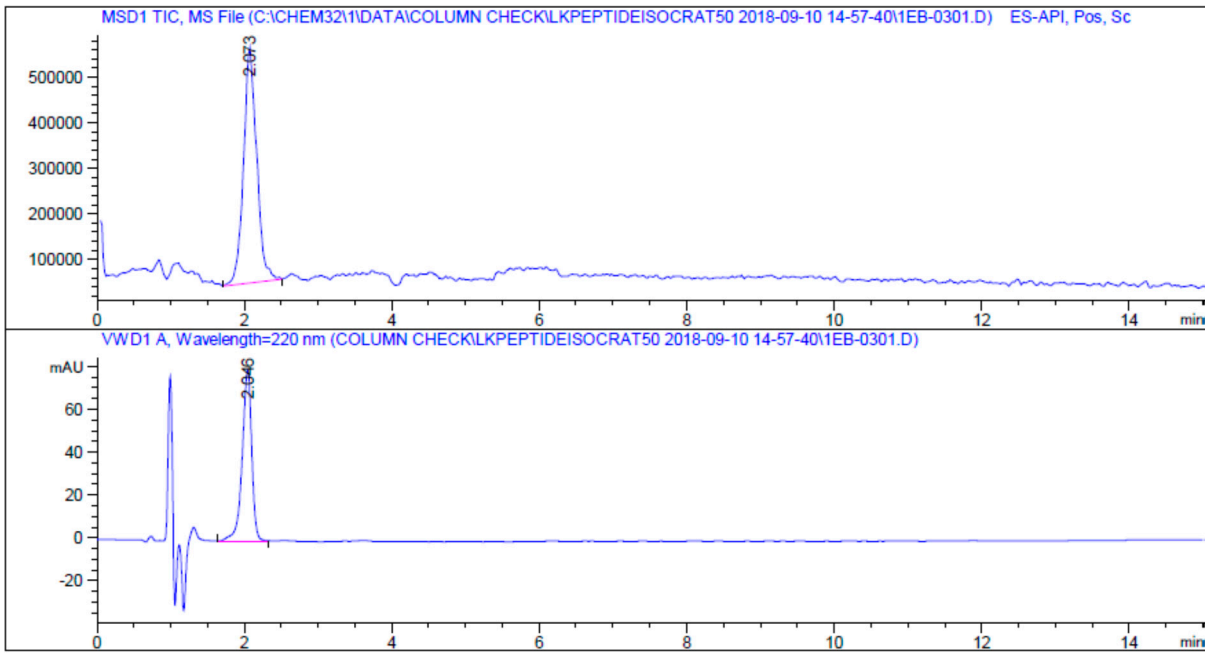
(S)-Benzyl-(3-((1-(1H-tetrazol-5-yl)ethyl)amino)-3-oxopropyl)carbamate **13d-L**:





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 Area Percent Report
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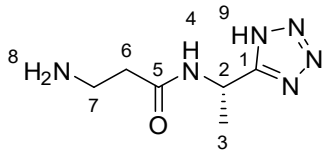
Sorted By : Signal
 Multiplier: : 1.0000
 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

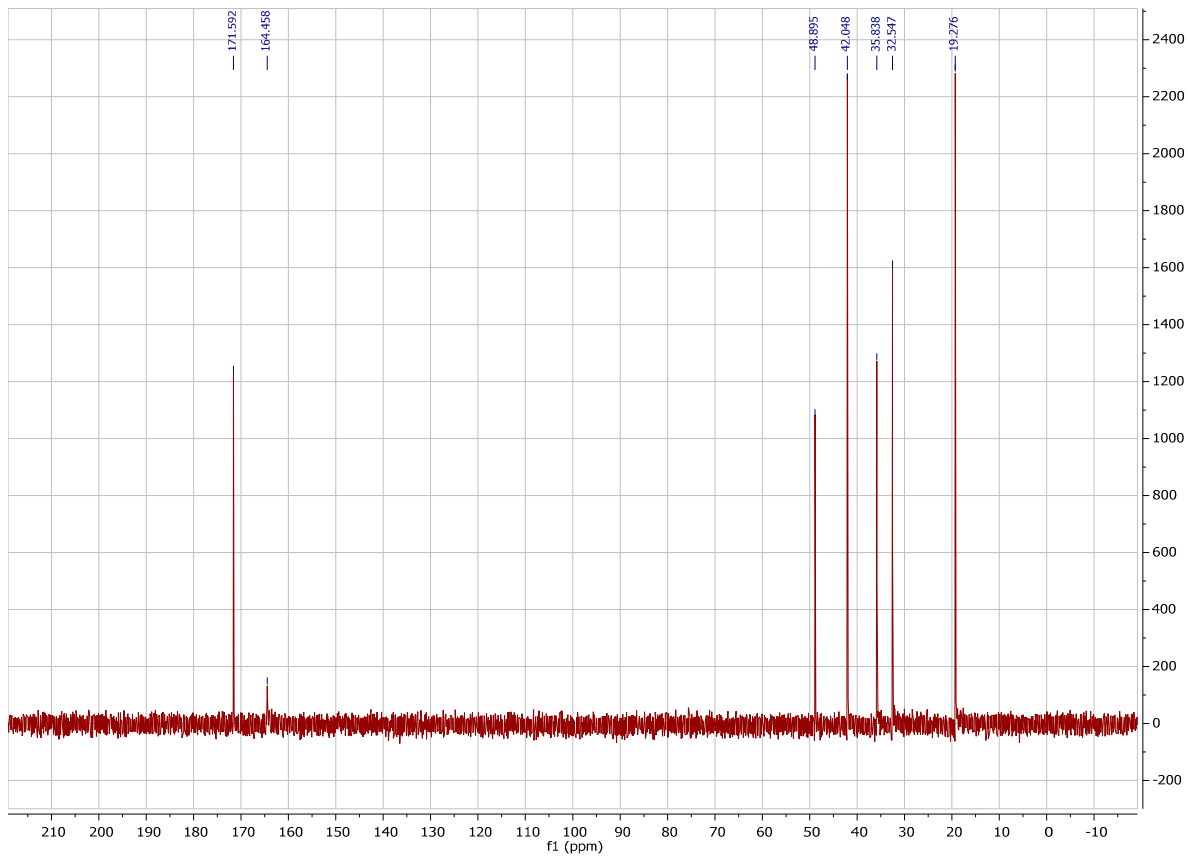
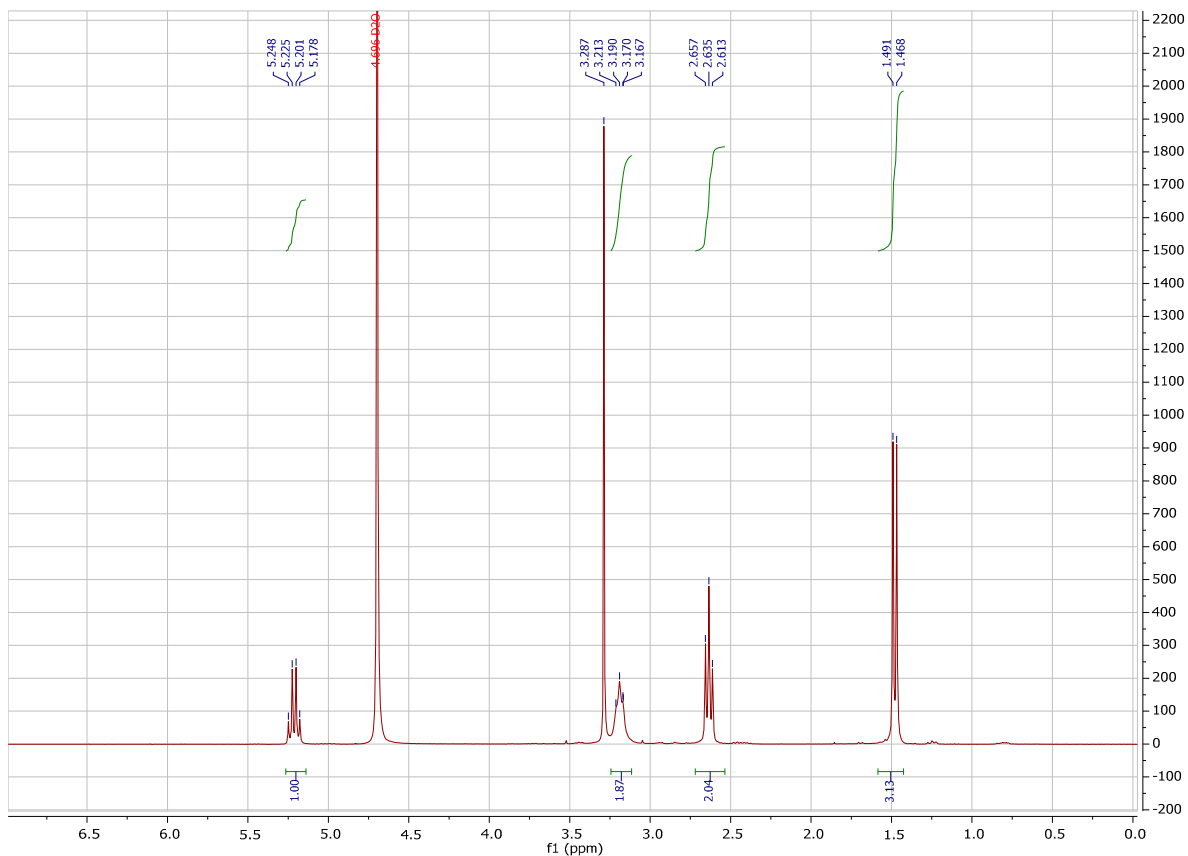
Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	2.073	BB	0.1717	6.38522e6	5.16189e5	100.0000

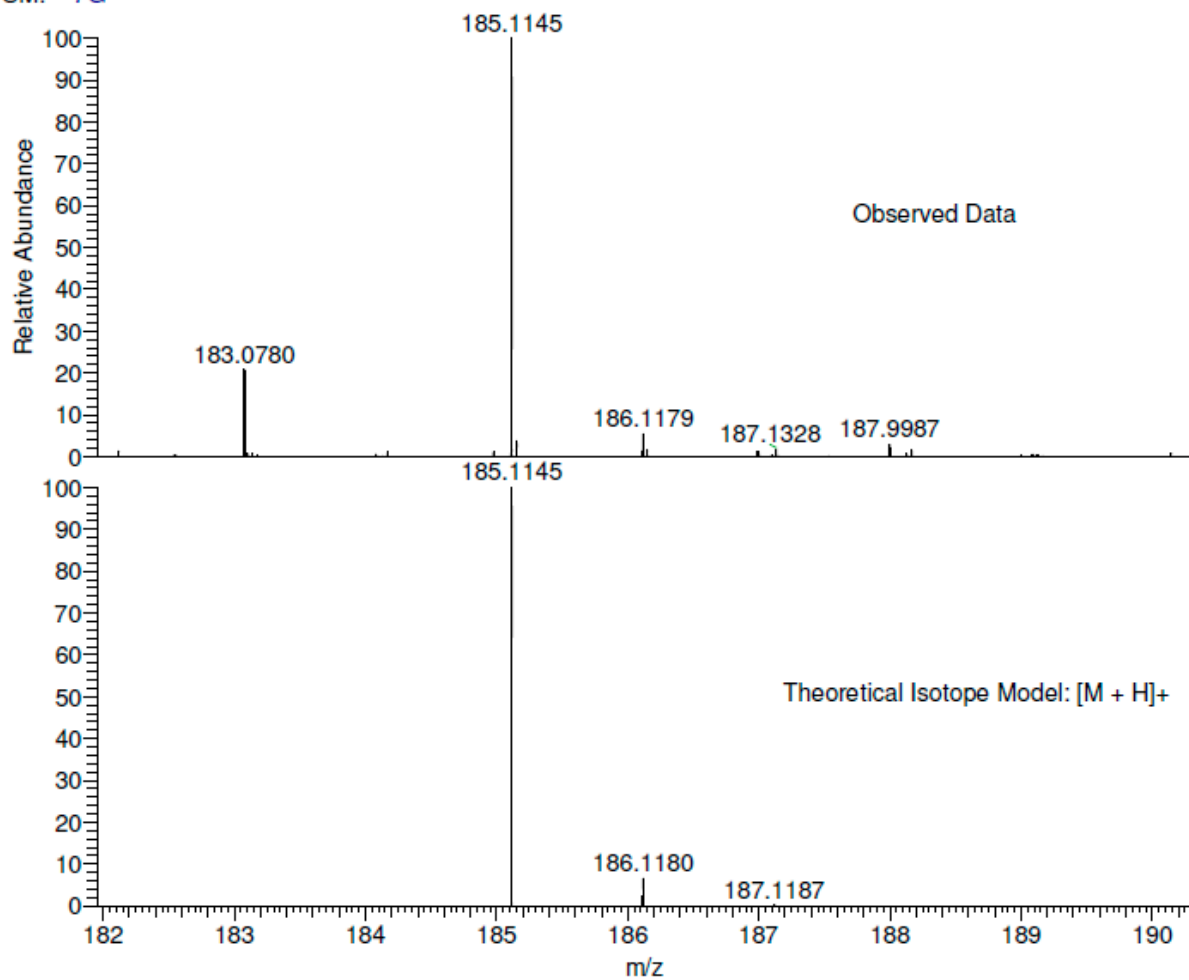
Totals : 6.38522e6 5.16189e5

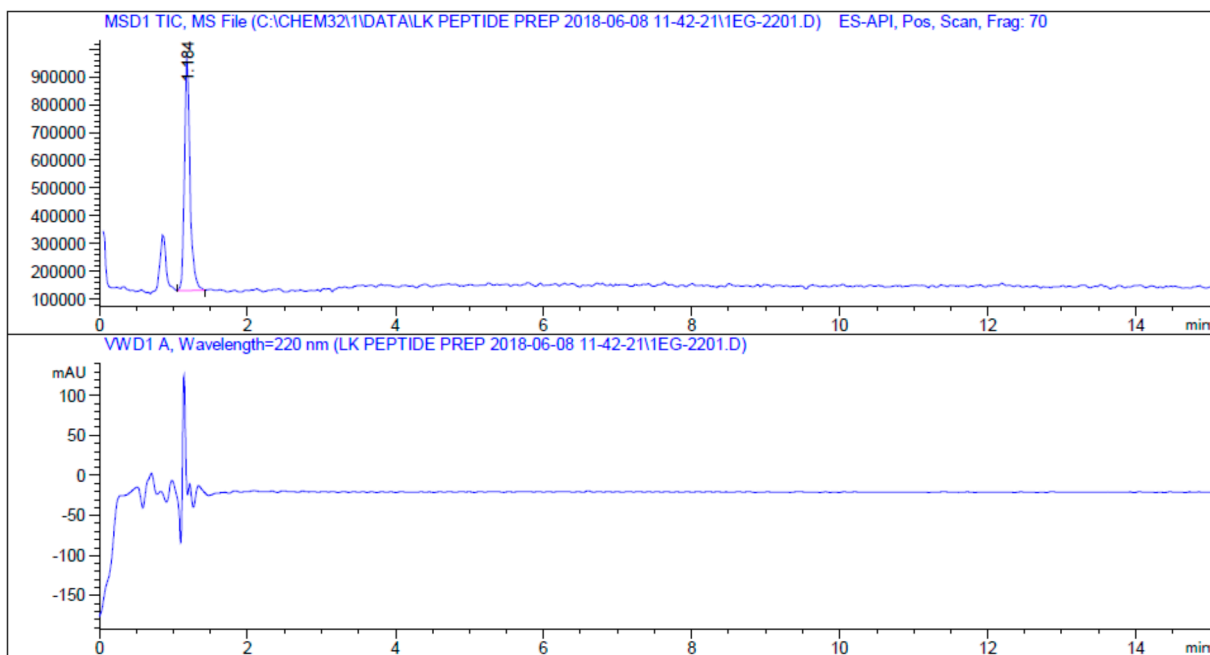
3-Amino-N-[(1S)-1-(1H-1,2,3,4-tetrazol-5-yl)ethyl]propanamide **14d-L**:





SM: 7G





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 Area Percent Report
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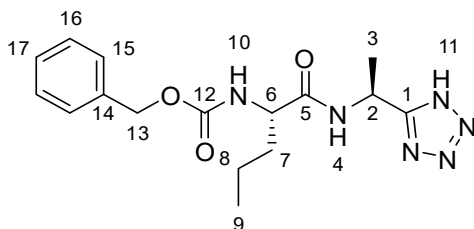
Sorted By : Signal
 Multiplier: : 1.0000
 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

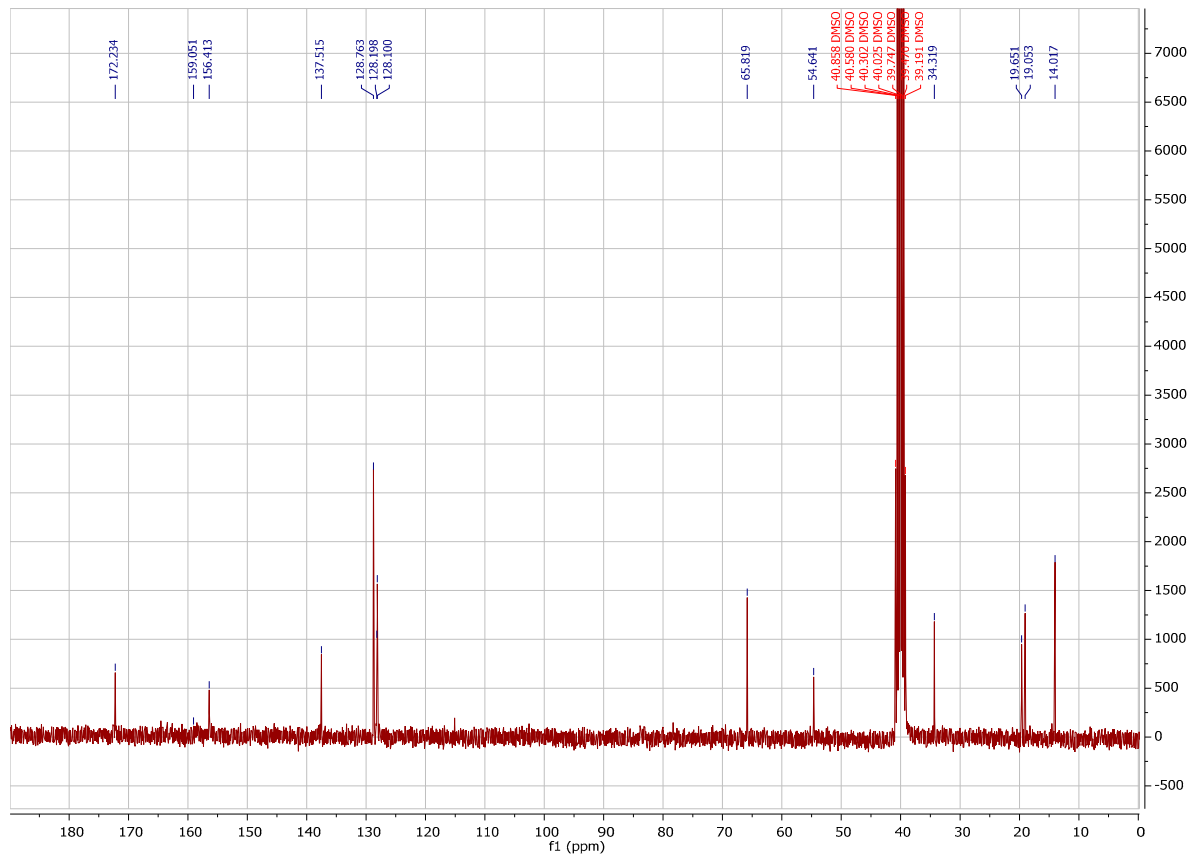
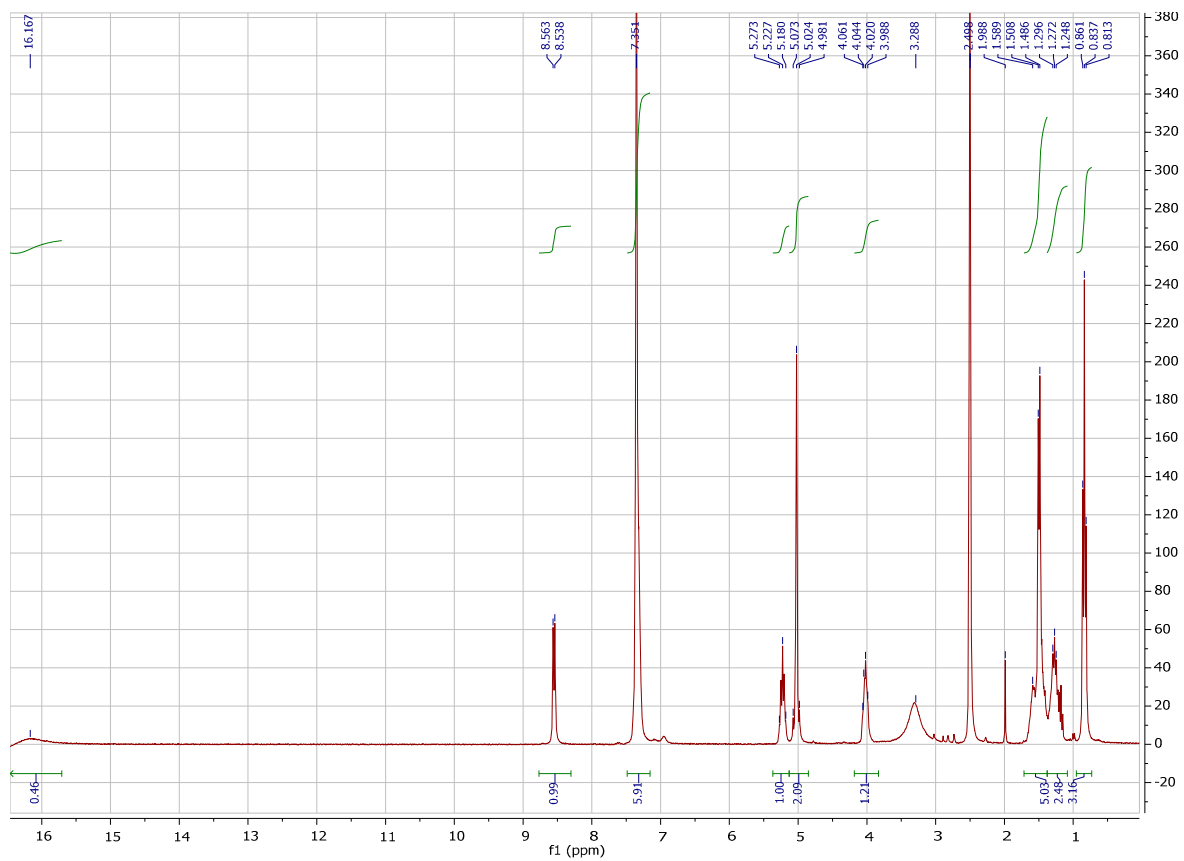
Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	1.184	BB	0.0759	4.37722e6	8.57835e5	100.0000

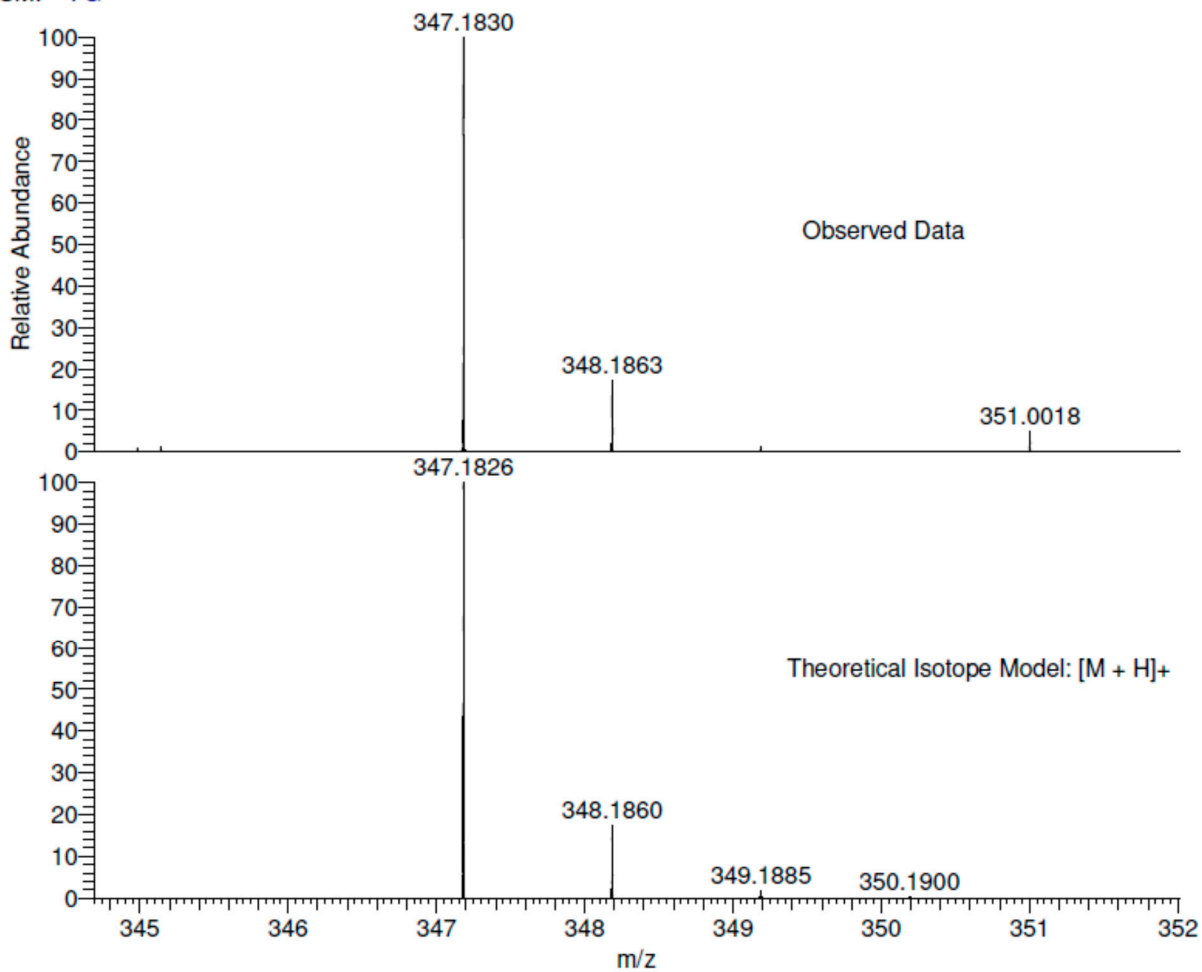
Totals : 4.37722e6 8.57835e5

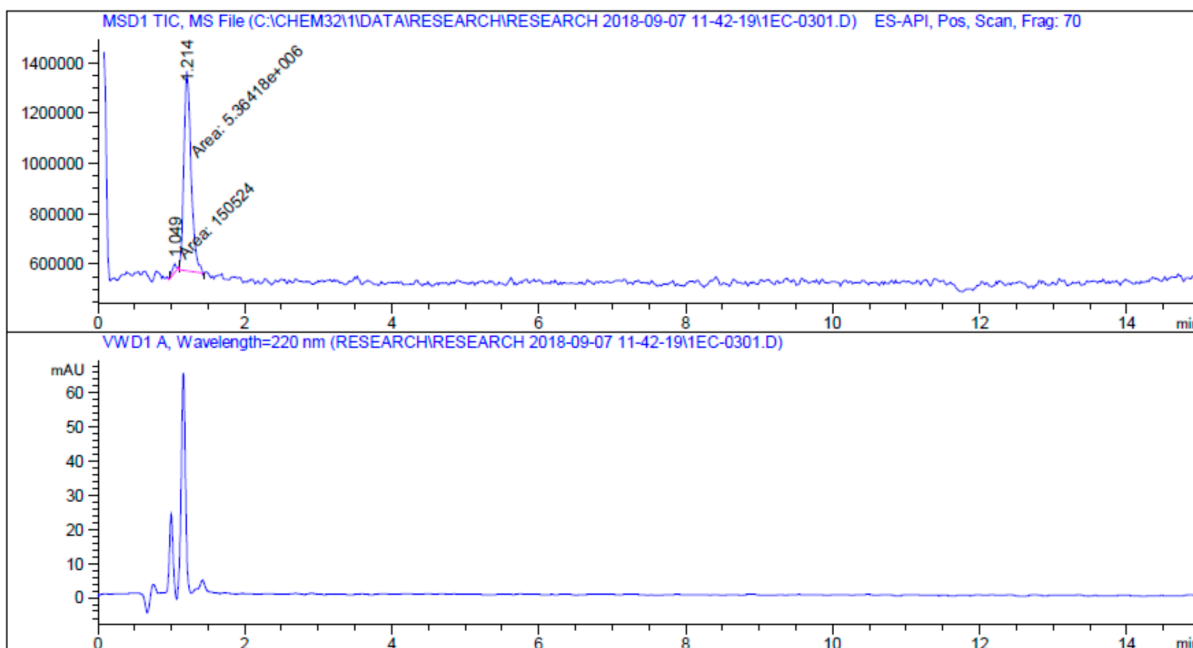
Benzyl-*N*-[(1*S*)-1-[(1*S*)-1-(1*H*-1,2,3,4-tetrazol-5-yl)ethyl]carbamoyl]butyl]carbamate **13e-LL**:





SM: 7G





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 Area Percent Report
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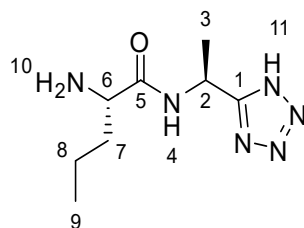
Sorted By : Signal
 Multiplier: : 1.0000
 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

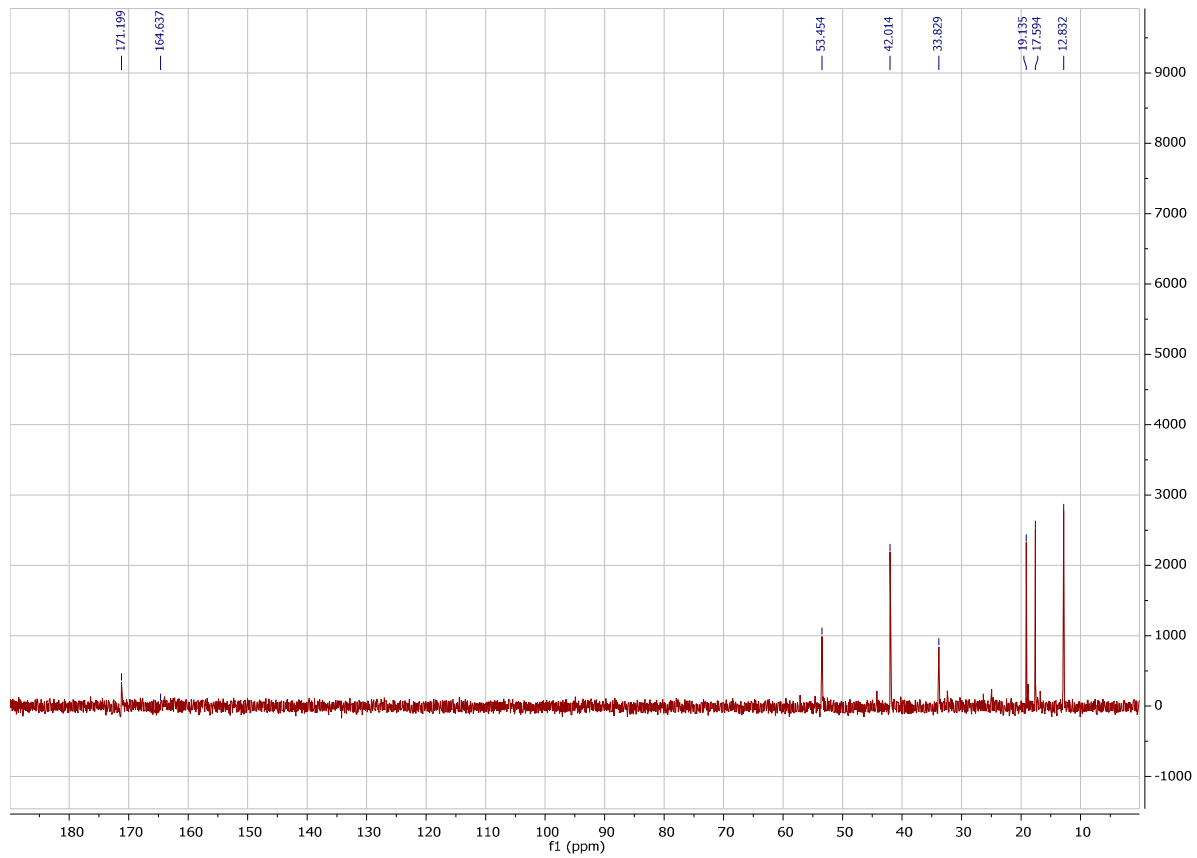
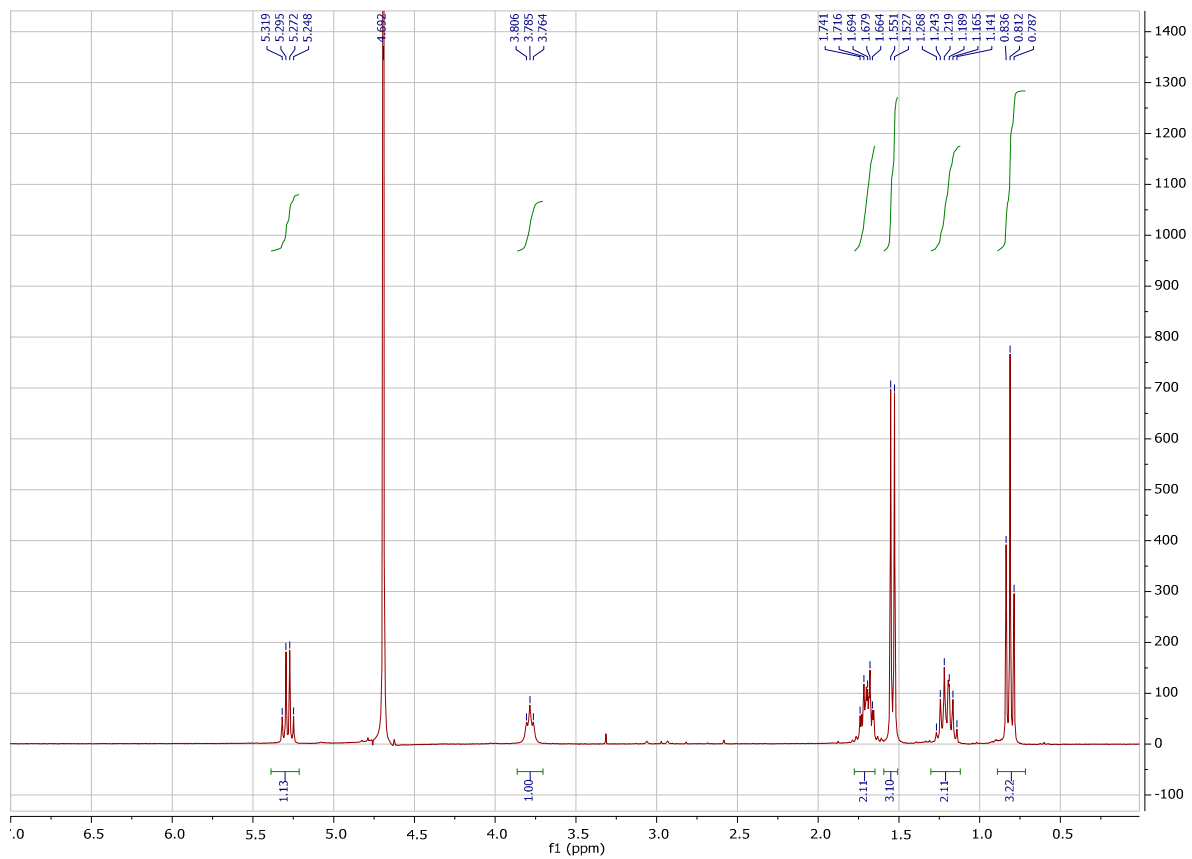
Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	1.049	MM	0.0621	1.50524e5	4.04039e4	2.7295
2	1.214	MM	0.1118	5.36418e6	7.99972e5	97.2705

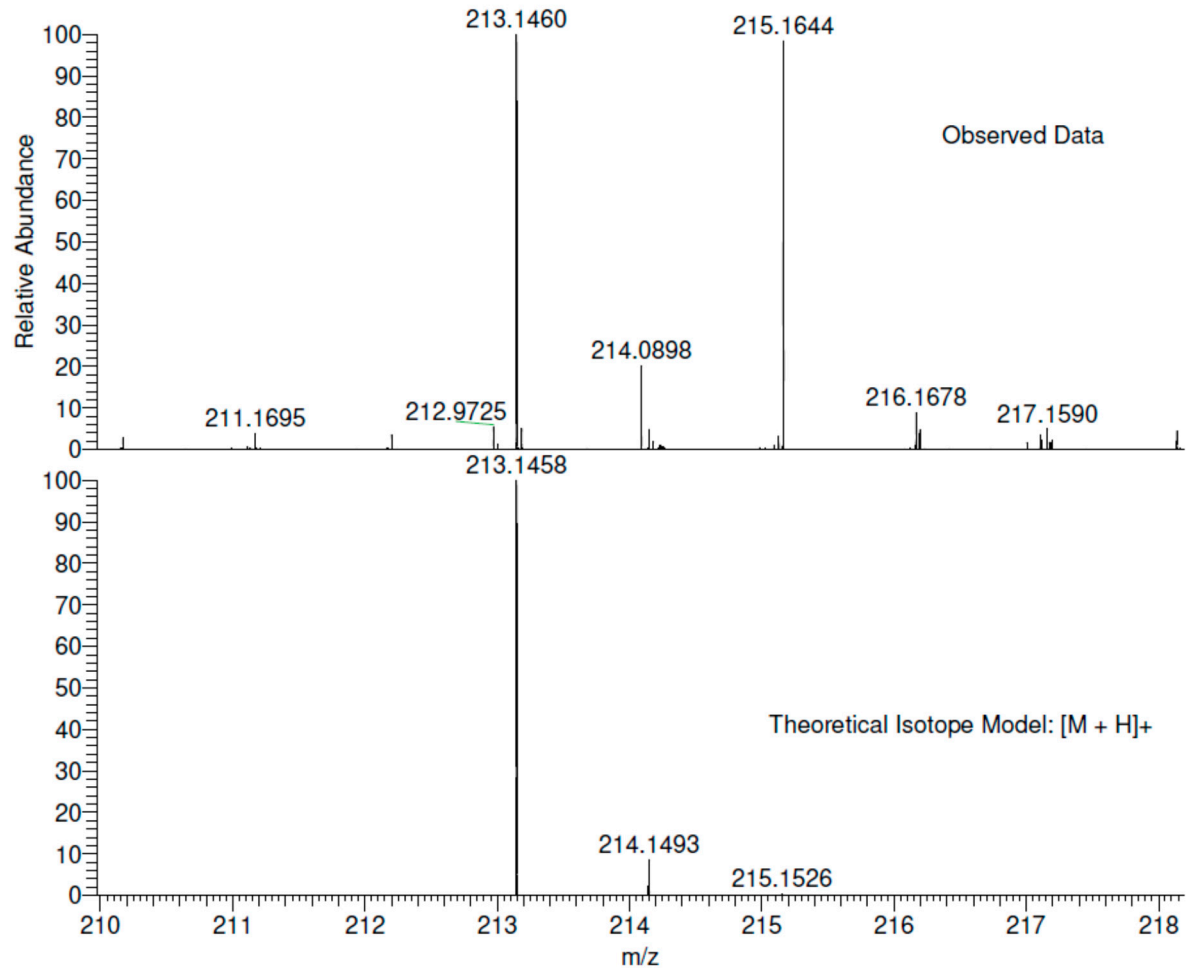
Totals : 5.51471e6 8.40376e5

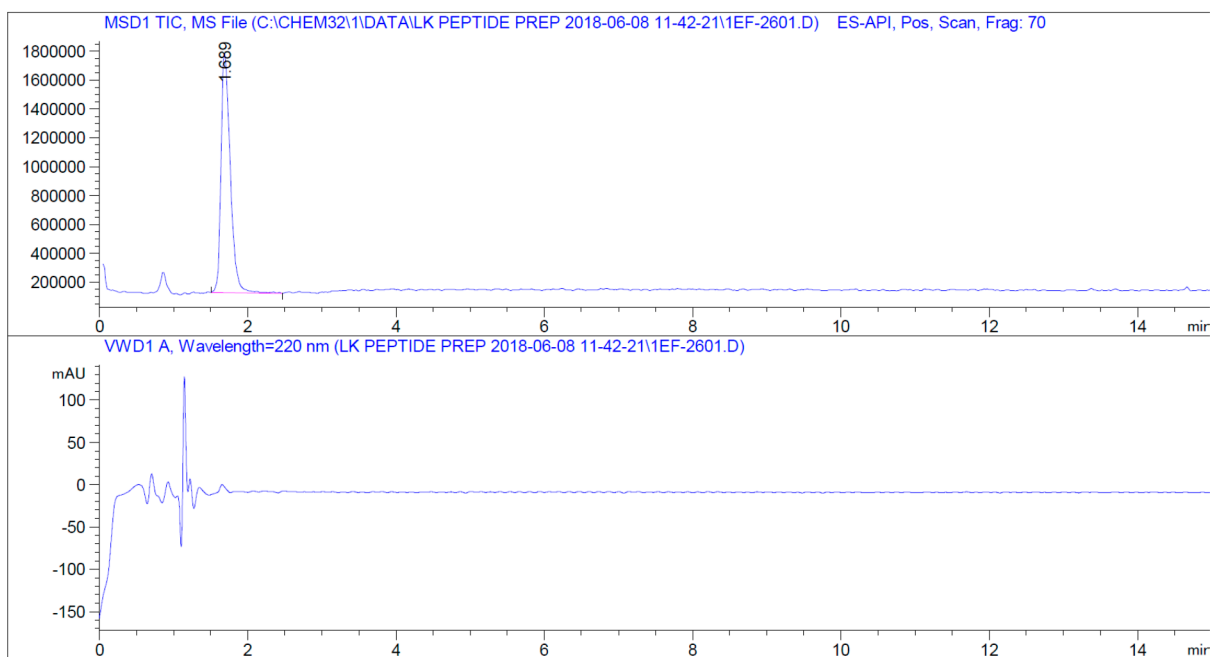
(S)-N-((S)-1-(1H-tetrazol-5-yl)ethyl)-2-aminopentanamide **14e-LL**:





SM: 7G





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 Area Percent Report
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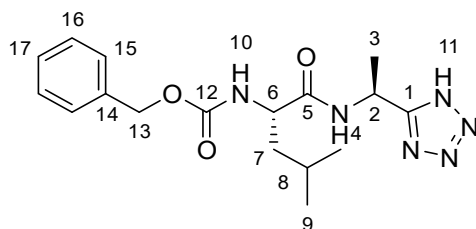
Sorted By : Signal
 Multiplier: : 1.0000
 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

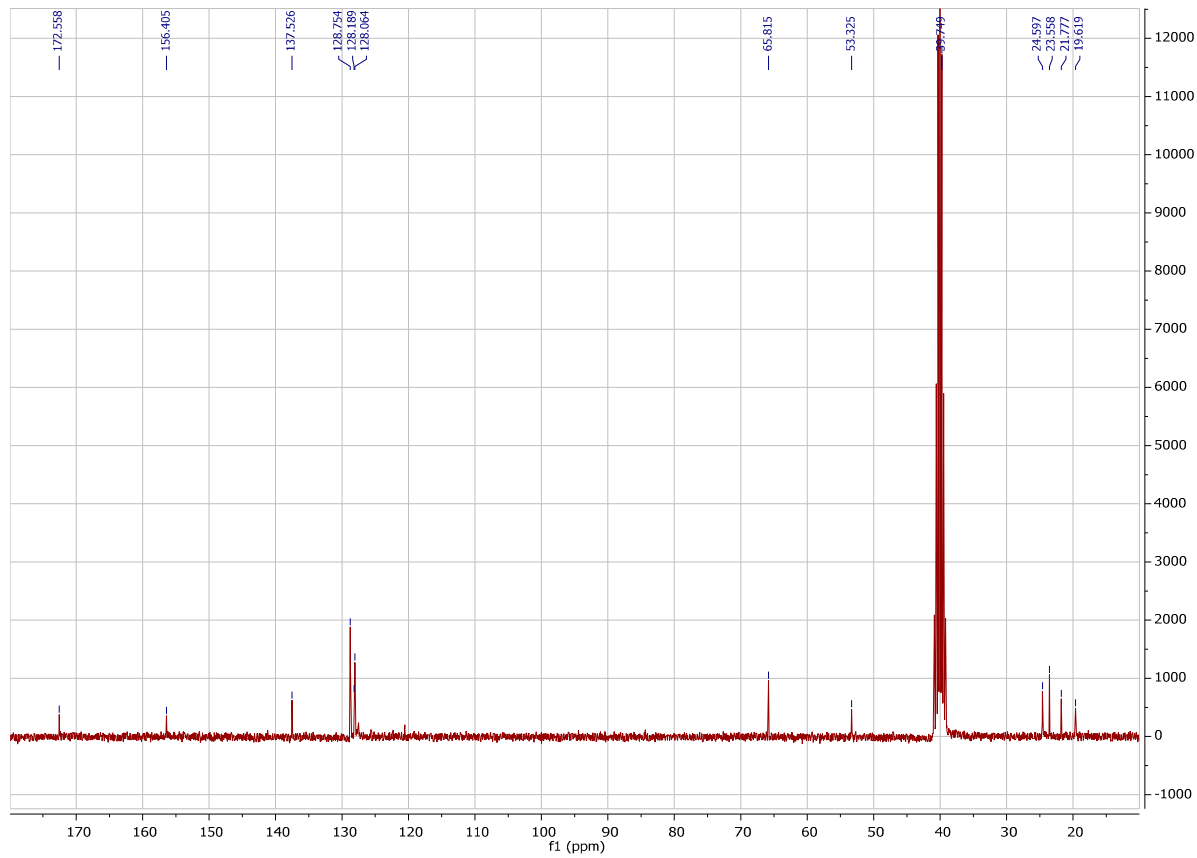
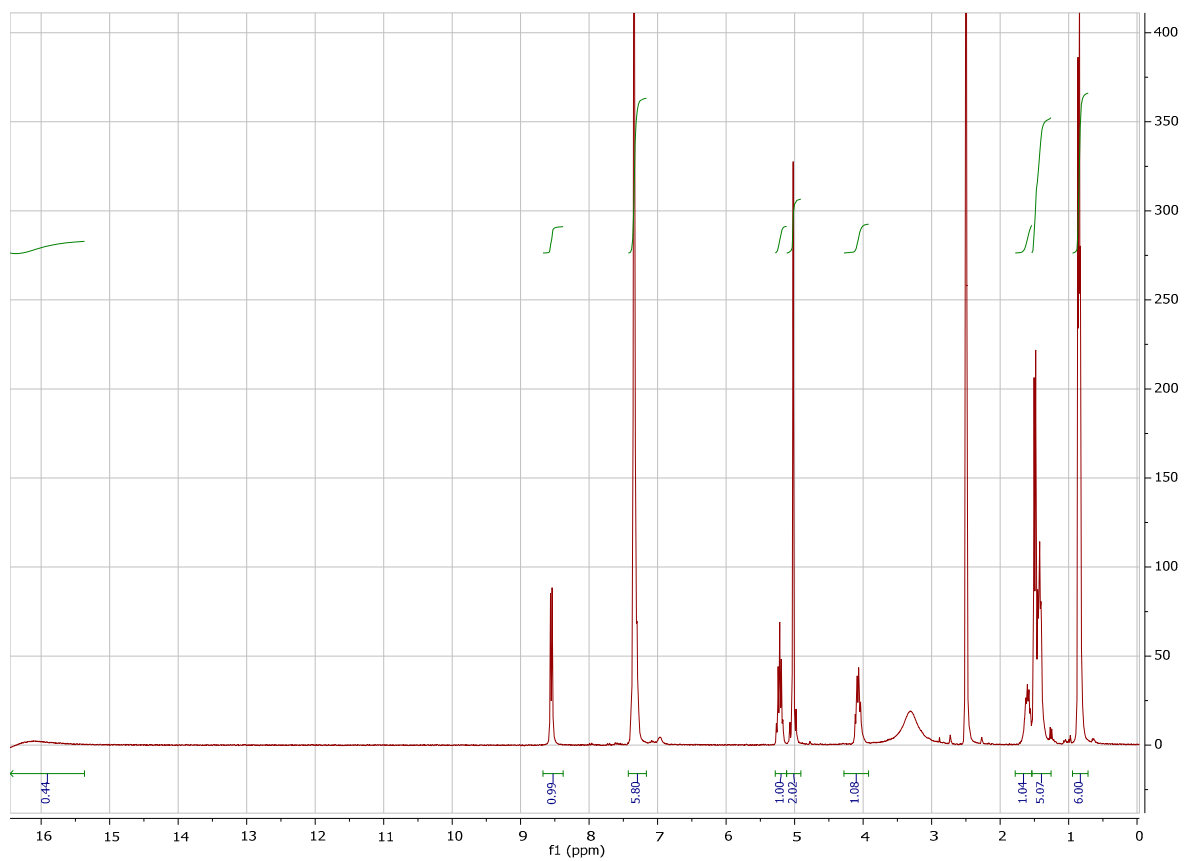
Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	1.689	BB	0.1337	1.43625e7	1.66034e6	100.0000

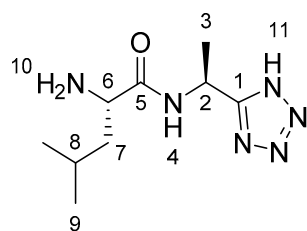
Totals : 1.43625e7 1.66034e6

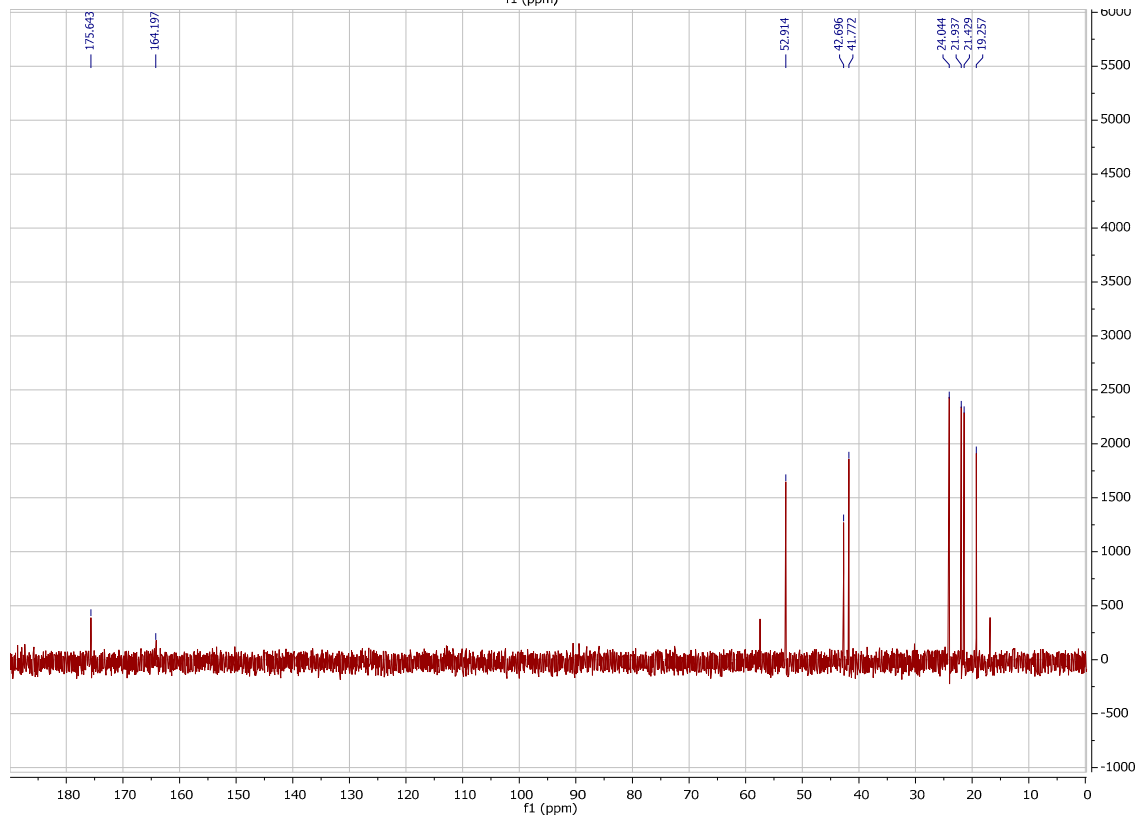
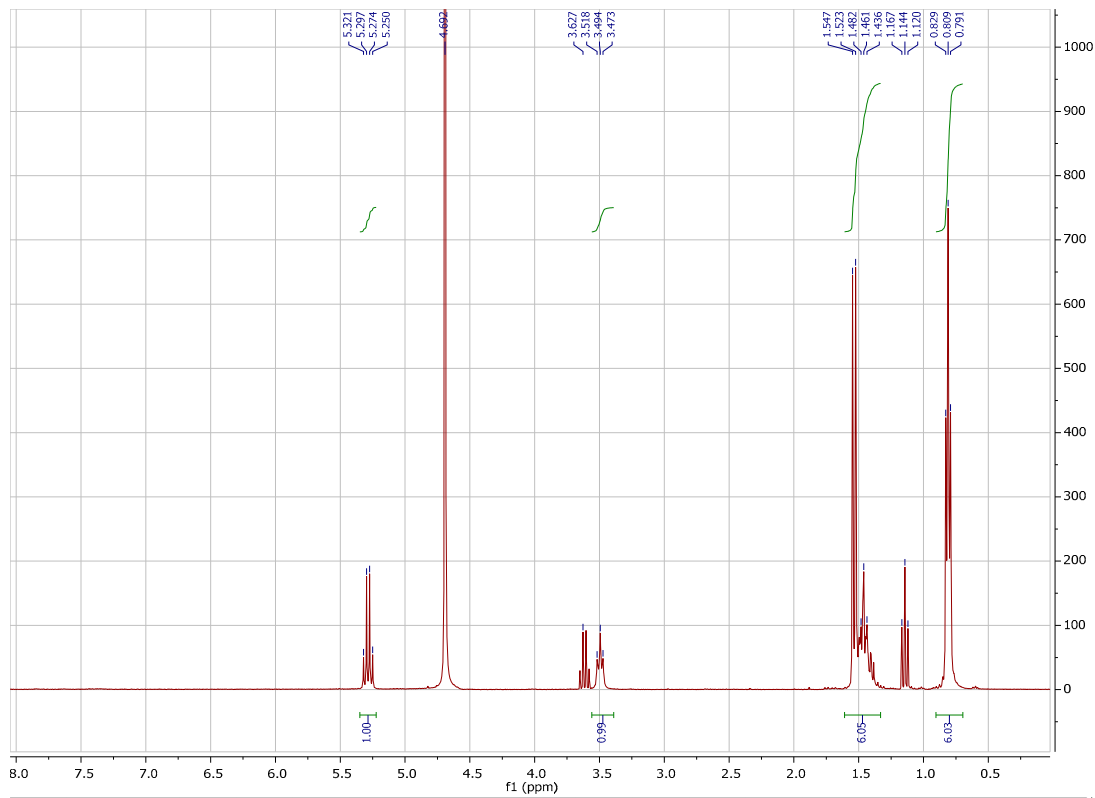
Benzyl-*N*-[(1*S*)-3-methyl-1-[(1*S*)-1-(1*H*-1,2,3,4-tetrazol-5-yl)ethyl]carbamoyl]butyl]carbamate **13h-LL**:

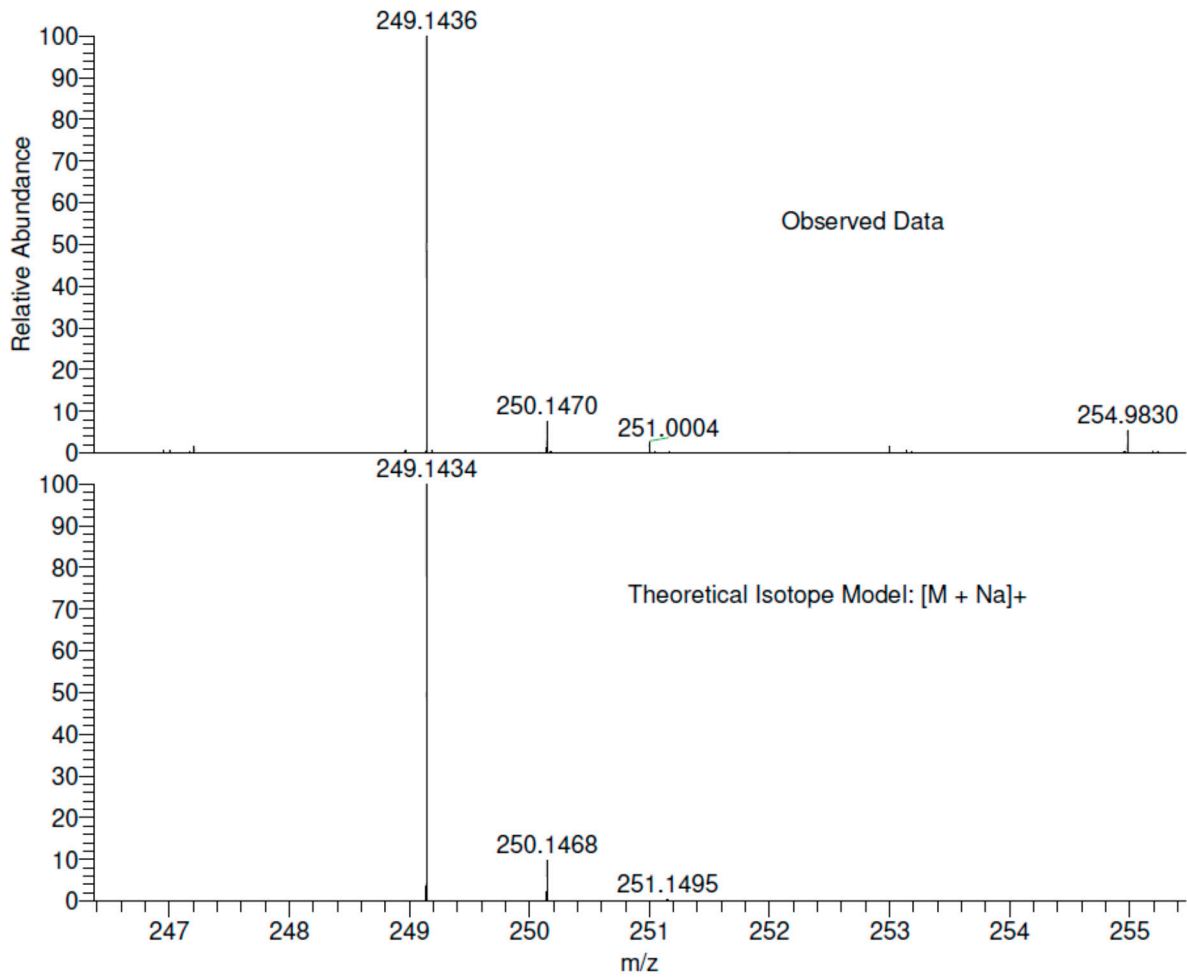


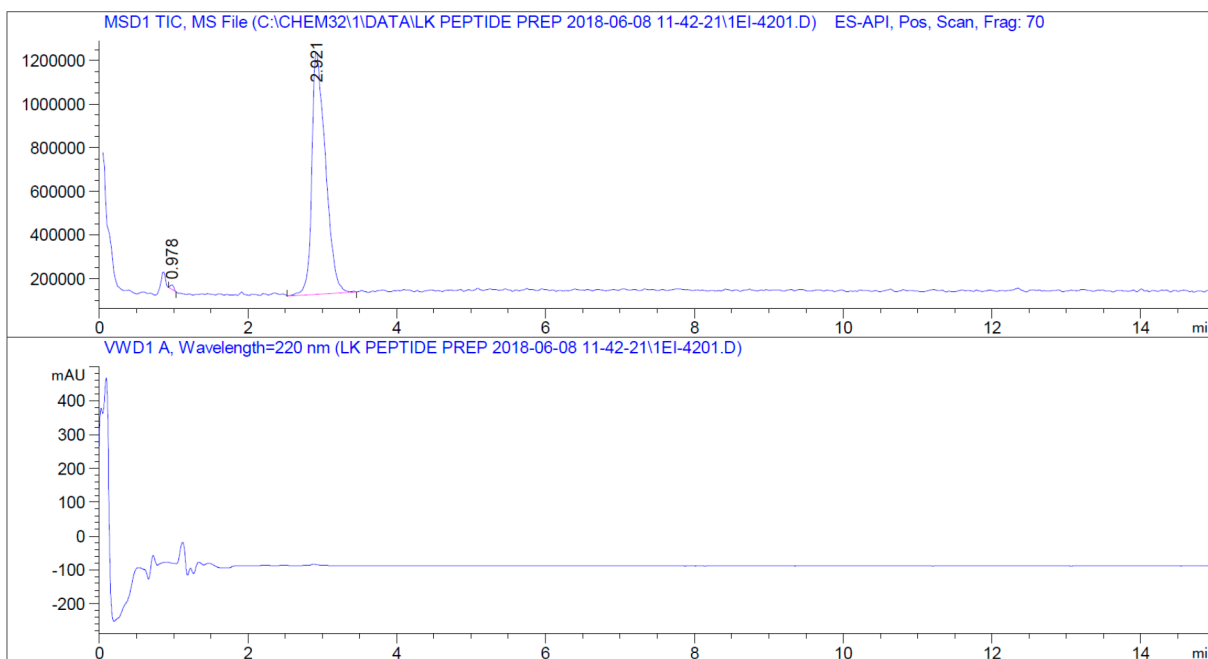


(2*S*)-2-Amino-4-methyl-*N*-[(1*S*)-1-(1*H*-1,2,3,4-tetrazol-5-yl)ethyl]pentanamide **14h-LL**:









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 Area Percent Report
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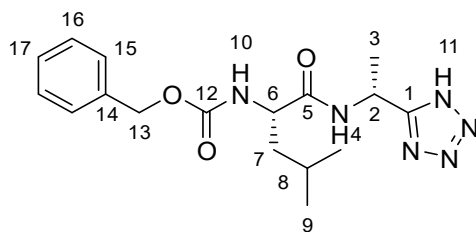
Sorted By : Signal
 Multiplier: : 1.0000
 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

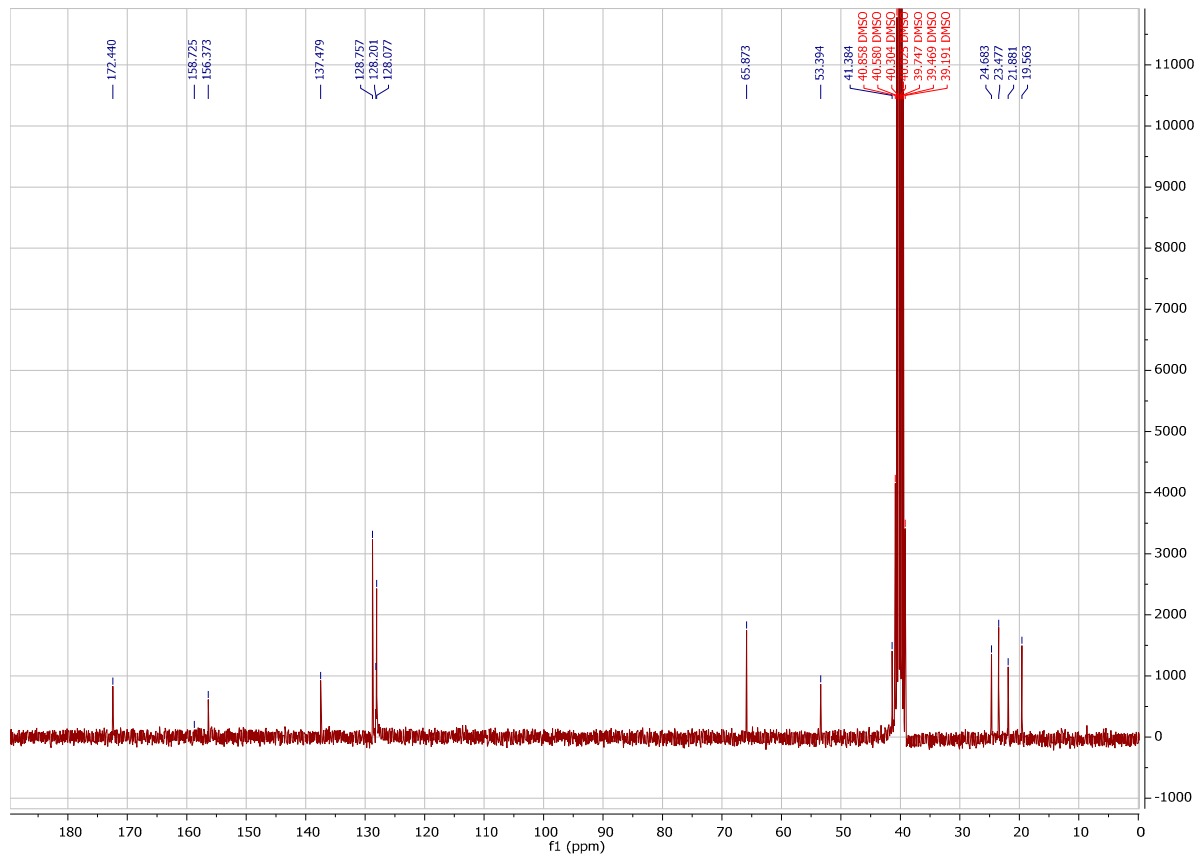
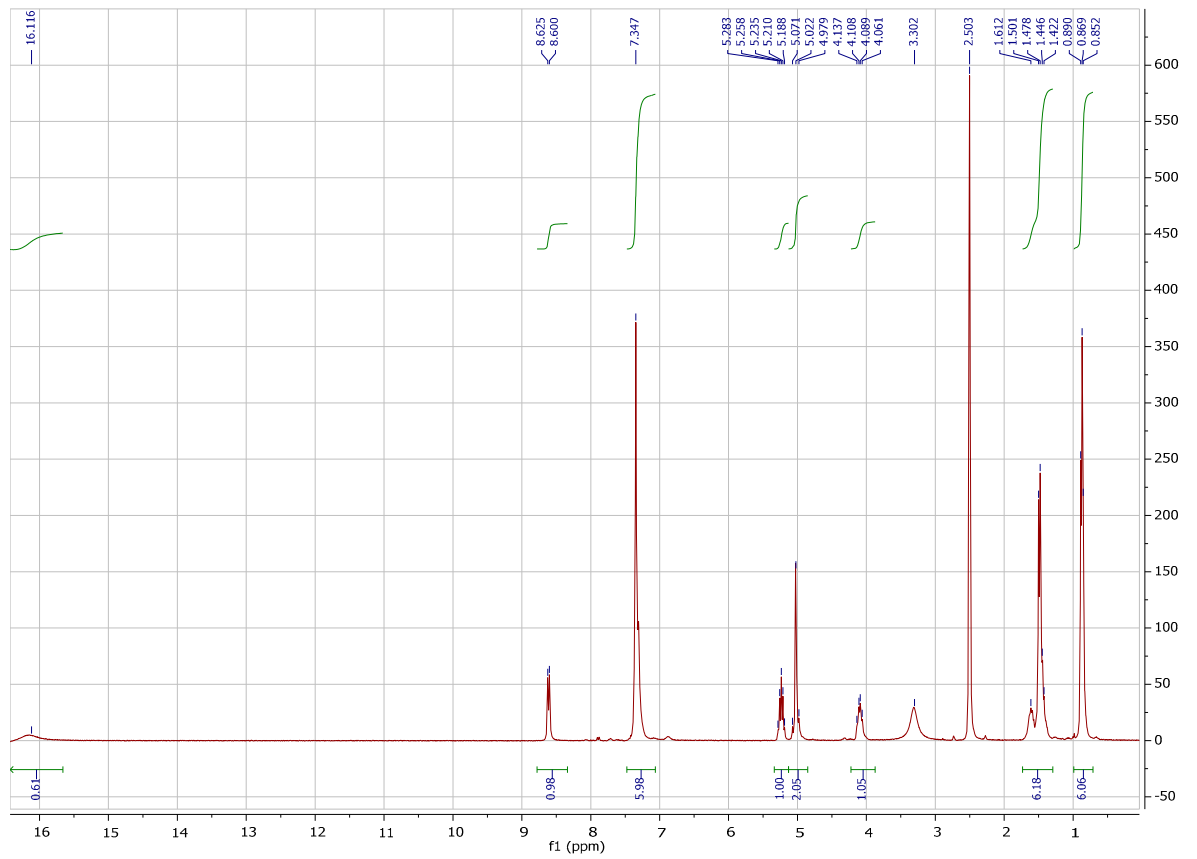
Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	0.978	BB	0.0522	6.68819e4	2.18223e4	0.4687
2	2.921	BB	0.1758	1.42027e7	1.11638e6	99.5313

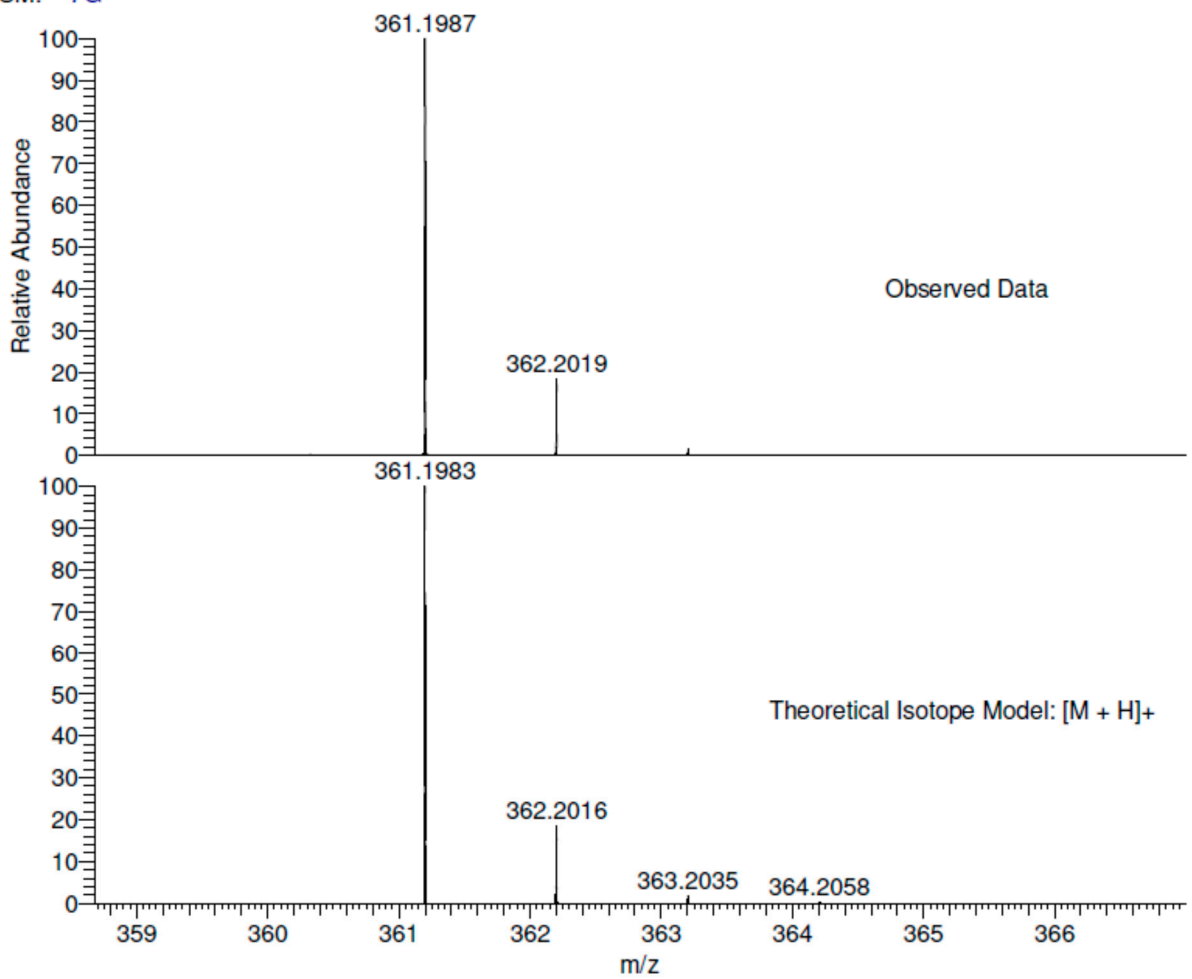
Totals : 1.42696e7 1.13821e6

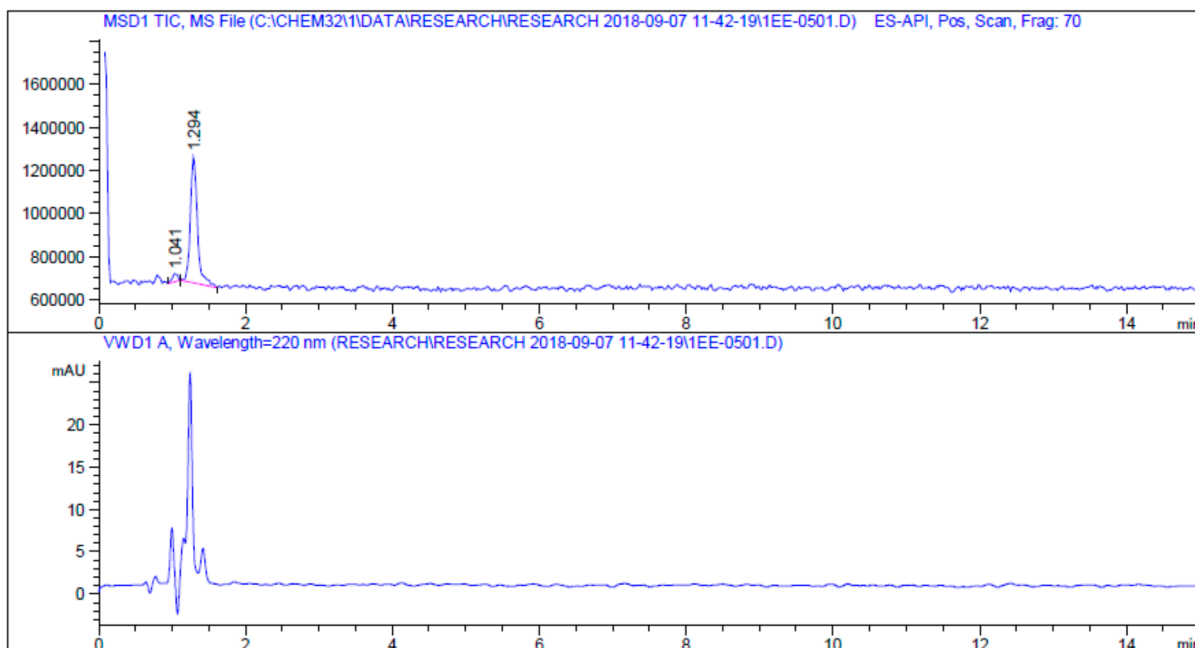
Benzyl-N-[(1S)-3-methyl-1-[(1R)-1-(1H-1,2,3,4-tetrazol-5-yl)ethyl]carbamoyl]butyl]carbamate **13h-LD**:





SM: 7G





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 Area Percent Report
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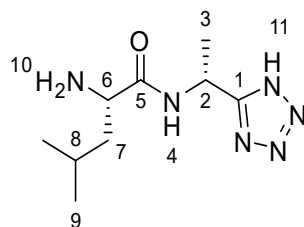
Sorted By : Signal
 Multiplier: : 1.0000
 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

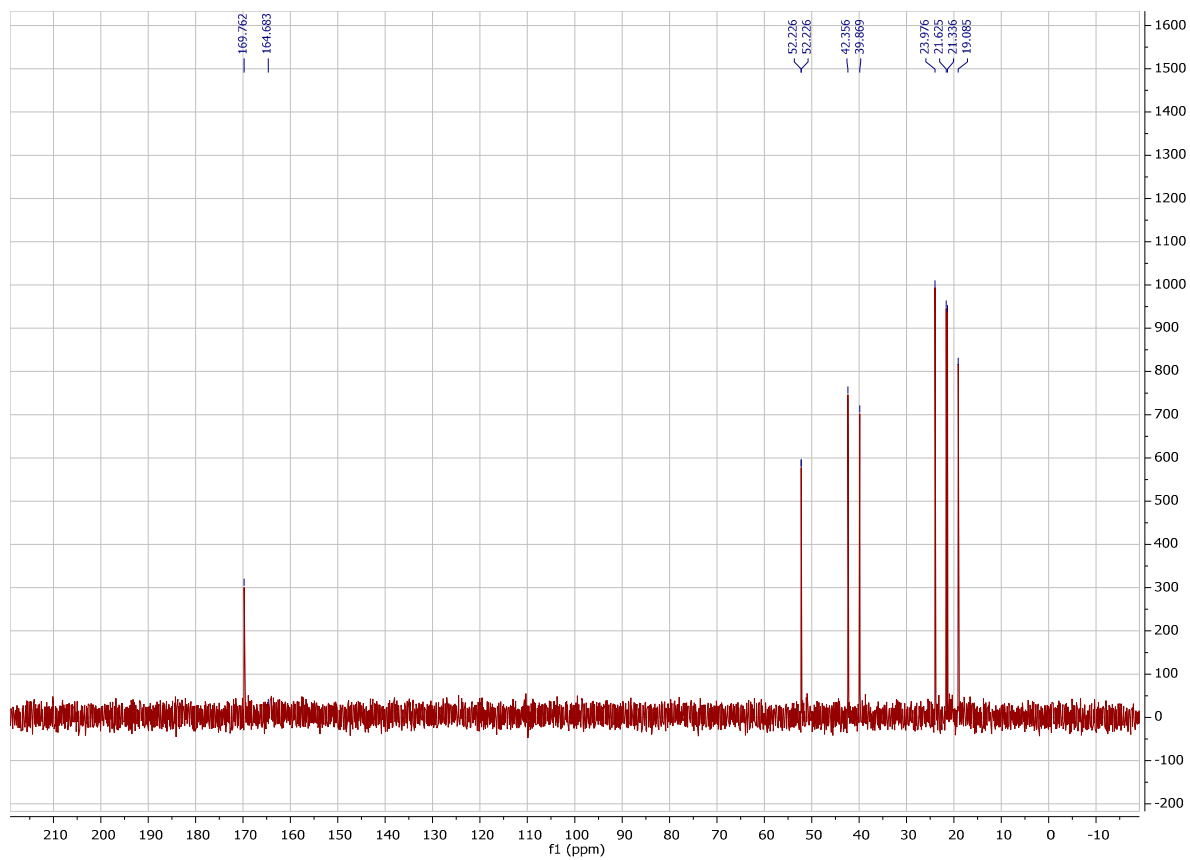
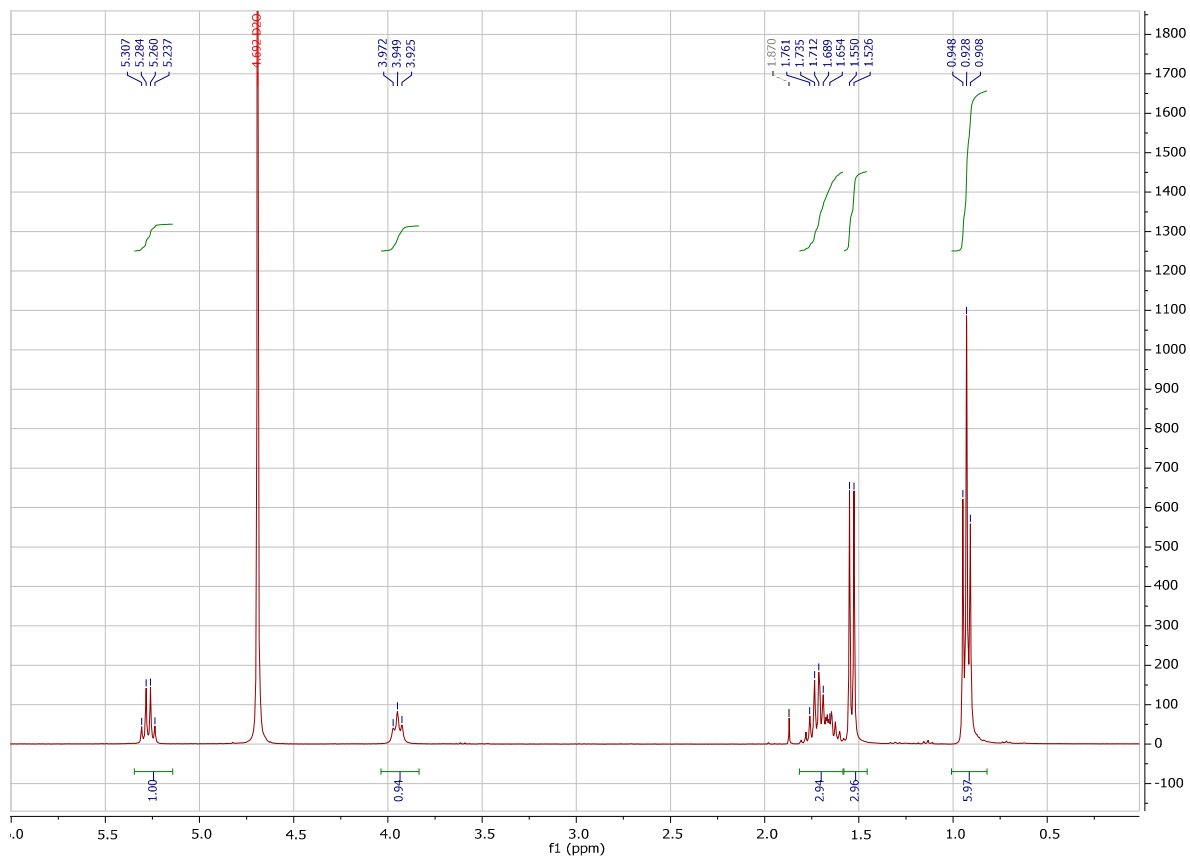
Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	1.041	BB	0.0982	1.84721e5	3.75385e4	4.5083
2	1.294	BB	0.0992	3.91267e6	5.89957e5	95.4917

Totals : 4.09739e6 6.27495e5

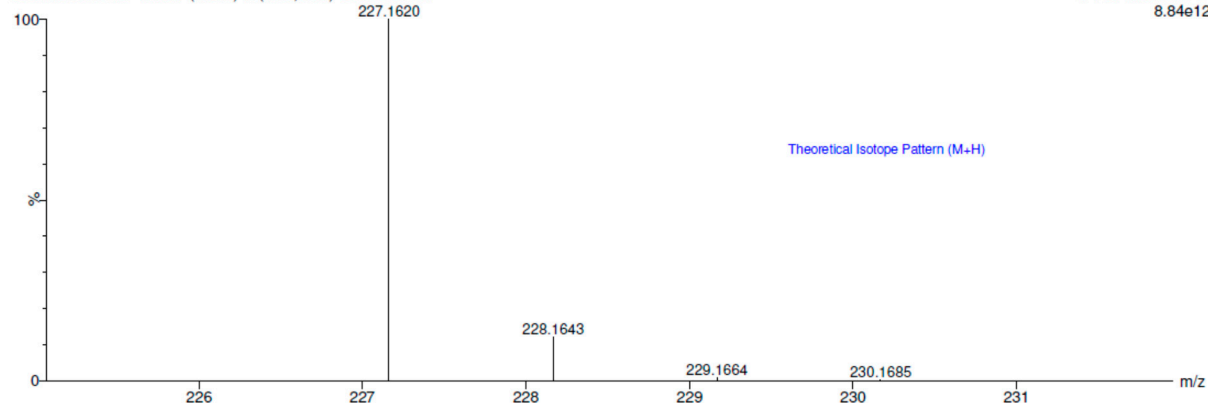
(2S)-2-Amino-4-methyl-N-[(1R)-1-(1H-1,2,3,4-tetrazol-5-yl)ethyl]pentanamide **14h-LD**:





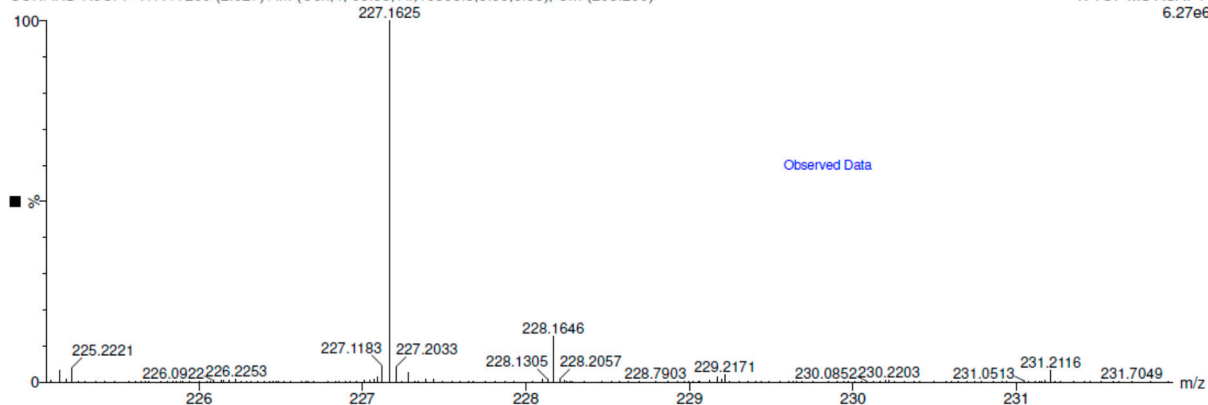
SUNAND-NJCFP-WR-A (0.037) Is (1.00,0.01) C9H18N6OH

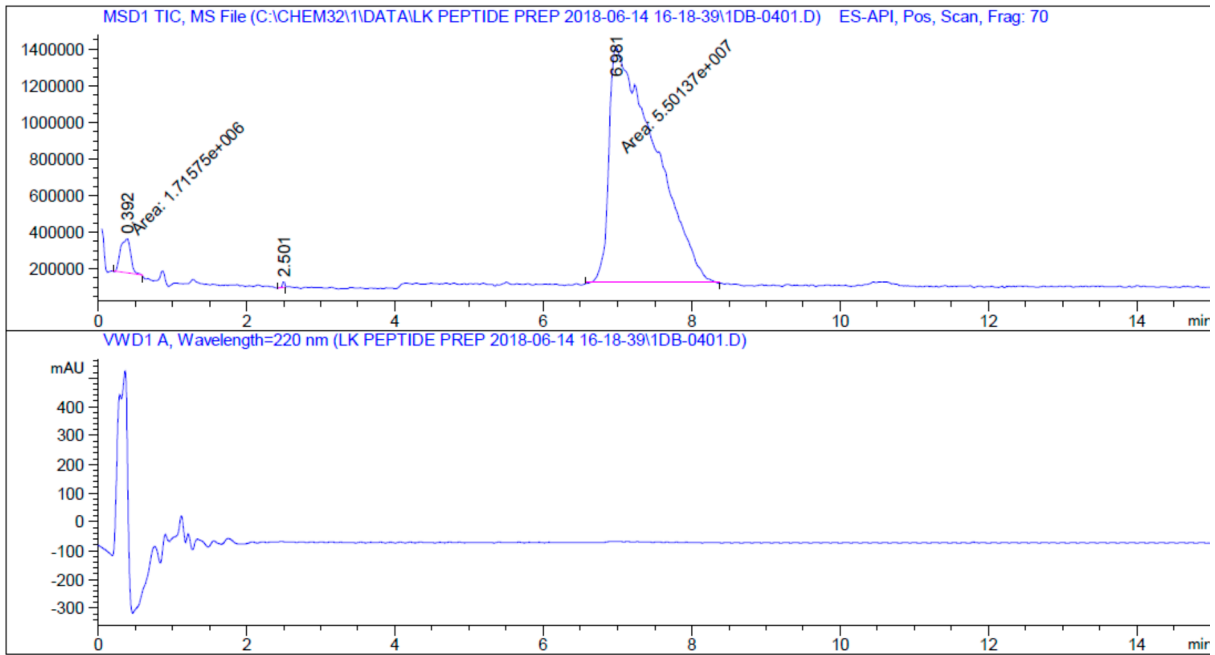
1: TOF MS ASAP+
8.84e12



SUNAND-NJCFP-WR-A 283 (2.627) AM (Cen.4, 80.00, Ar, 10000.0,0.00,0.00); Cm (280:288)

1: TOF MS ASAP+
6.27e6





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 Area Percent Report
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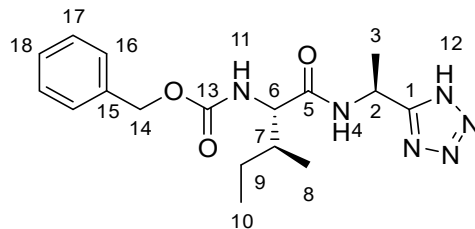
Sorted By : Signal
 Multiplier: : 1.0000
 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

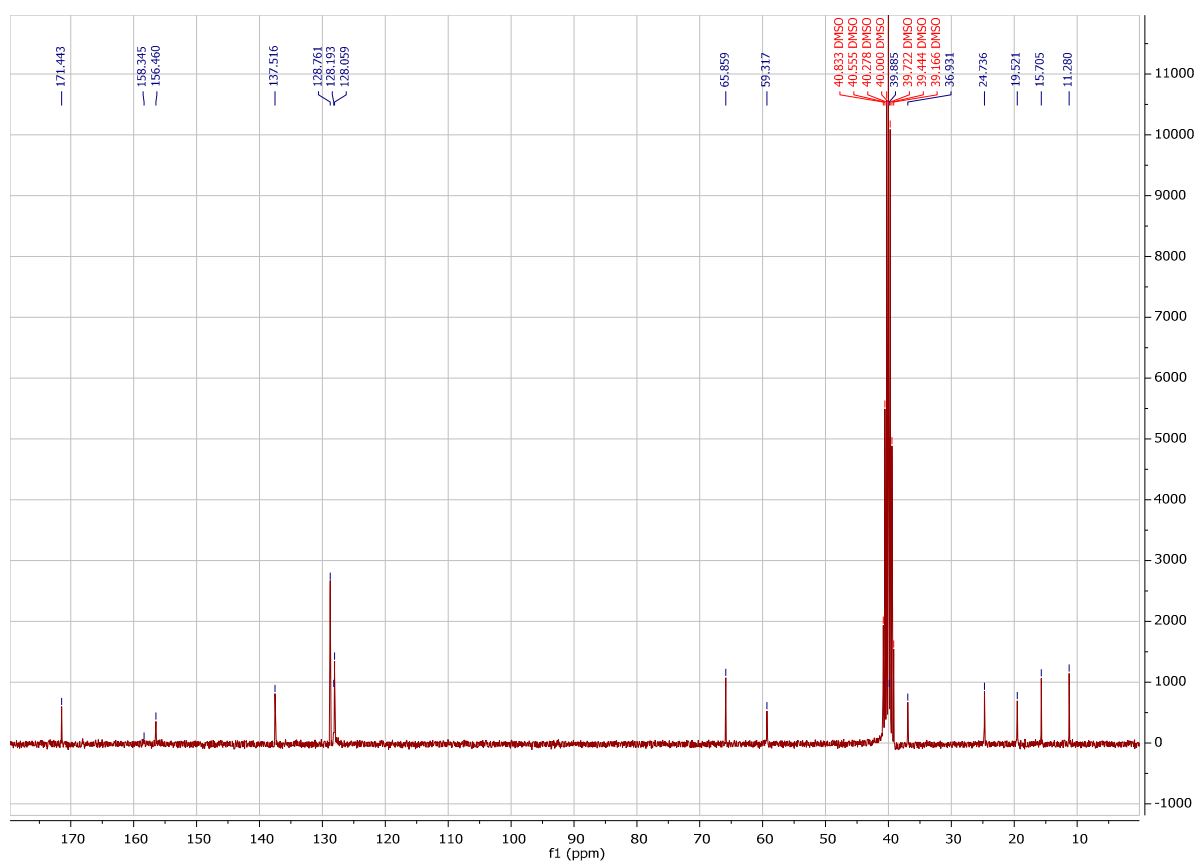
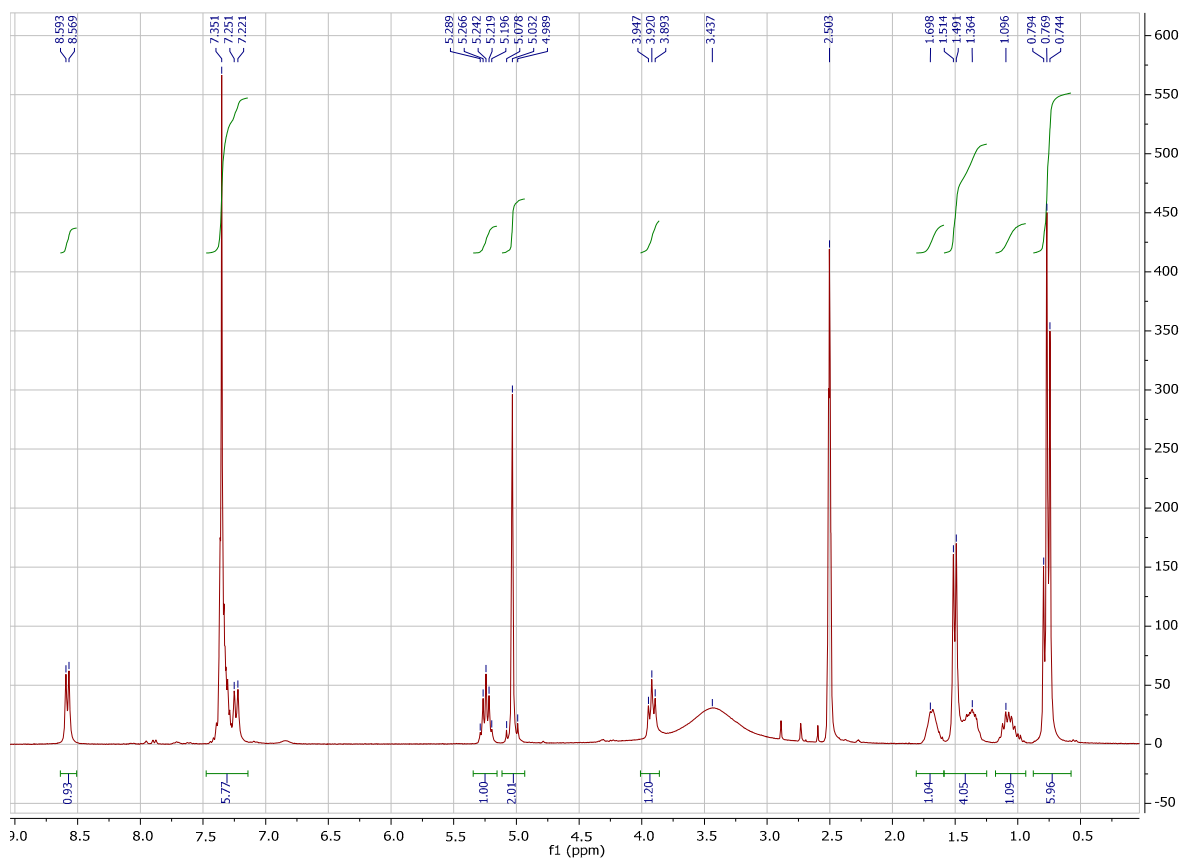
Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	0.392	MM	0.1524	1.71575e6	1.87597e5	3.0210
2	2.501	BB	0.0337	6.38562e4	3.15880e4	0.1124
3	6.981	MM	0.7142	5.50137e7	1.28389e6	96.8665

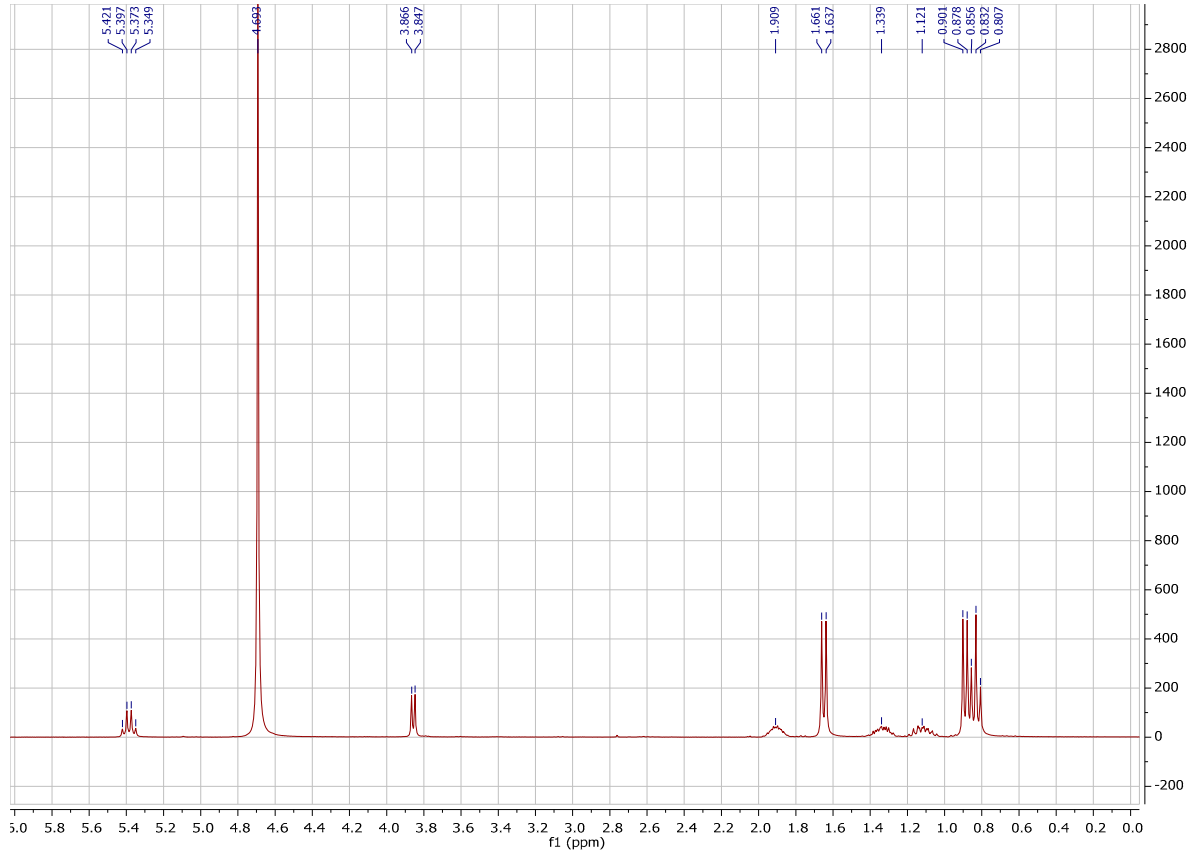
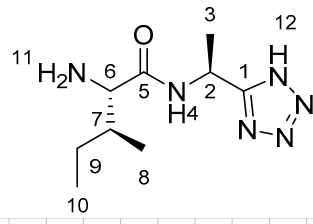
Totals : 5.67933e7 1.50308e6

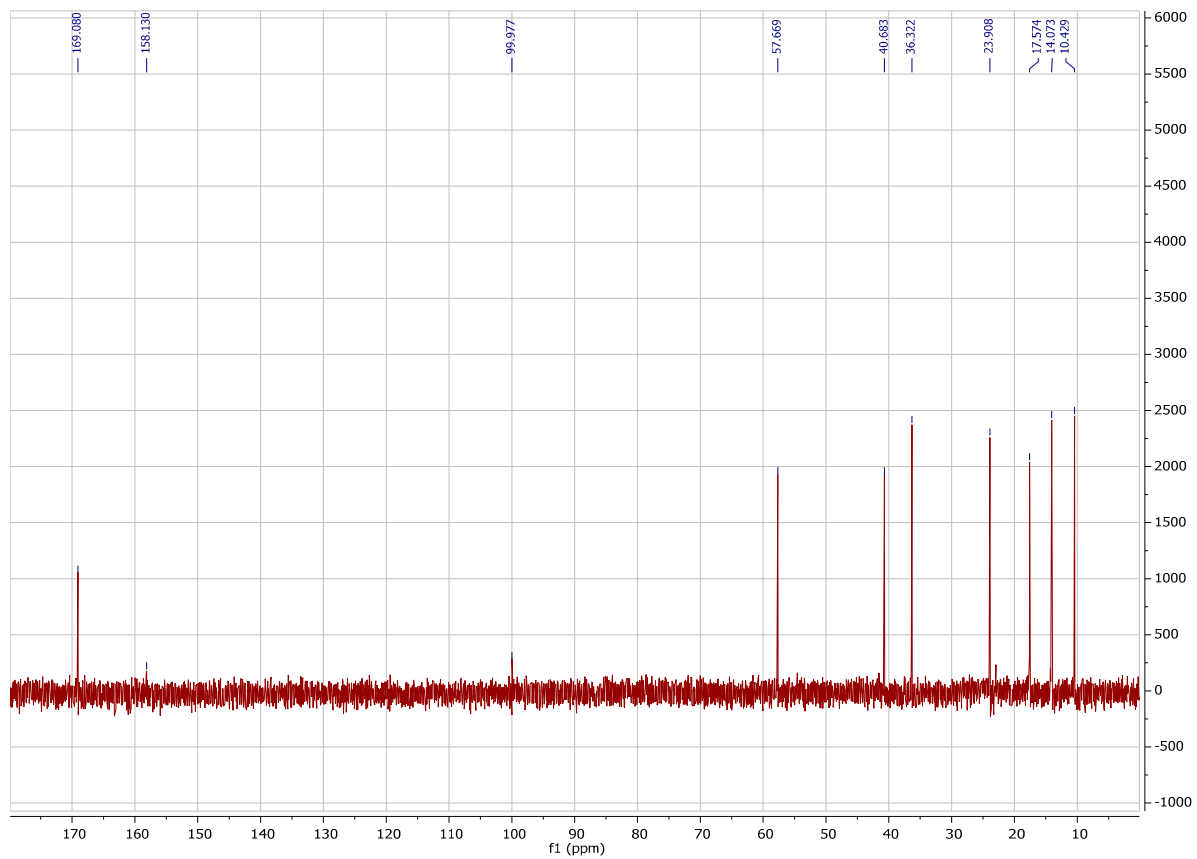
Benzyl-*N*-[(1*S*,2*S*)-2-methyl-1-[(1*S*)-1-(1*H*-1,2,3,4-tetrazol-5-yl)ethyl]carbamoyl]butyl]carbamate **13i-LL**:



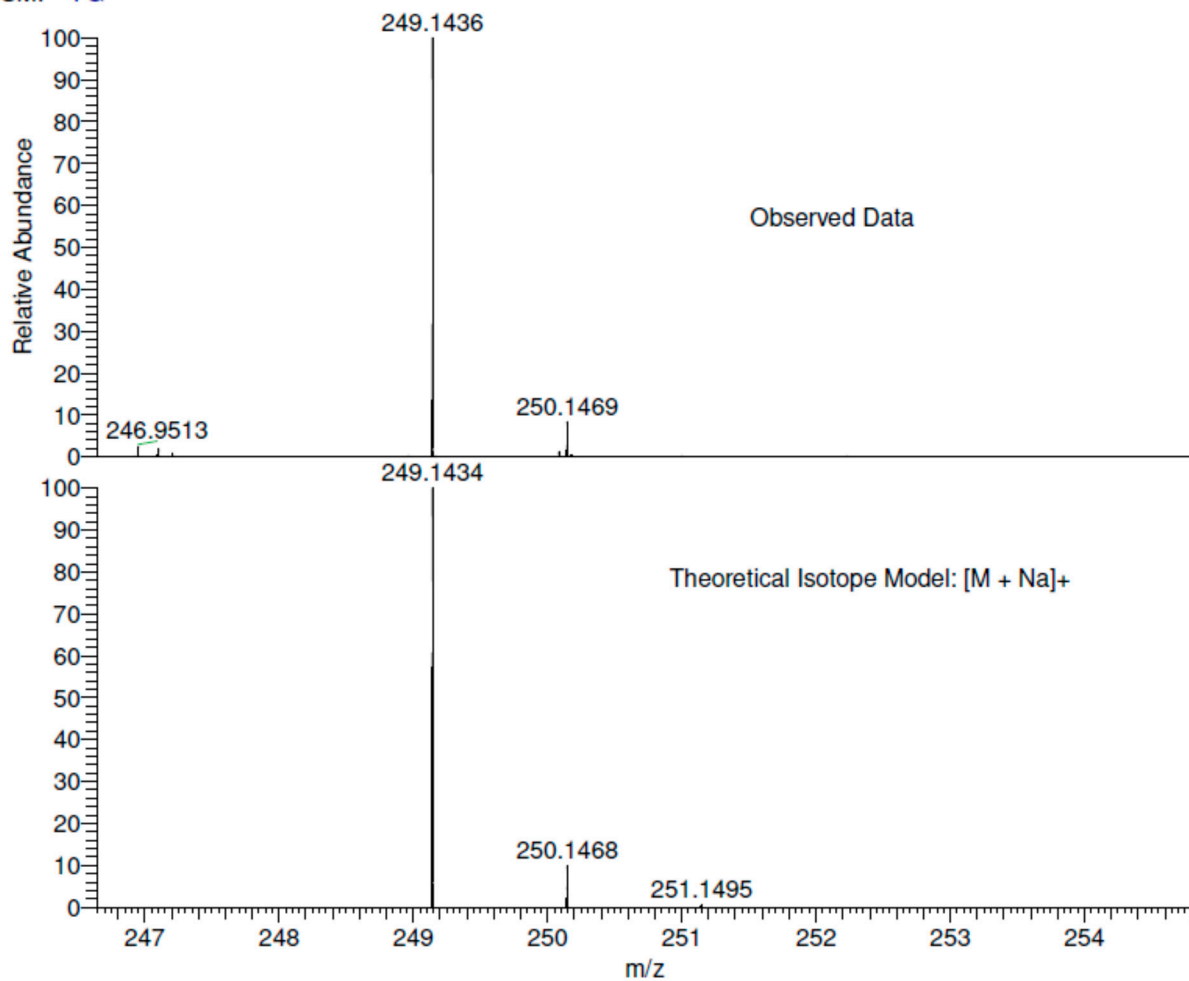


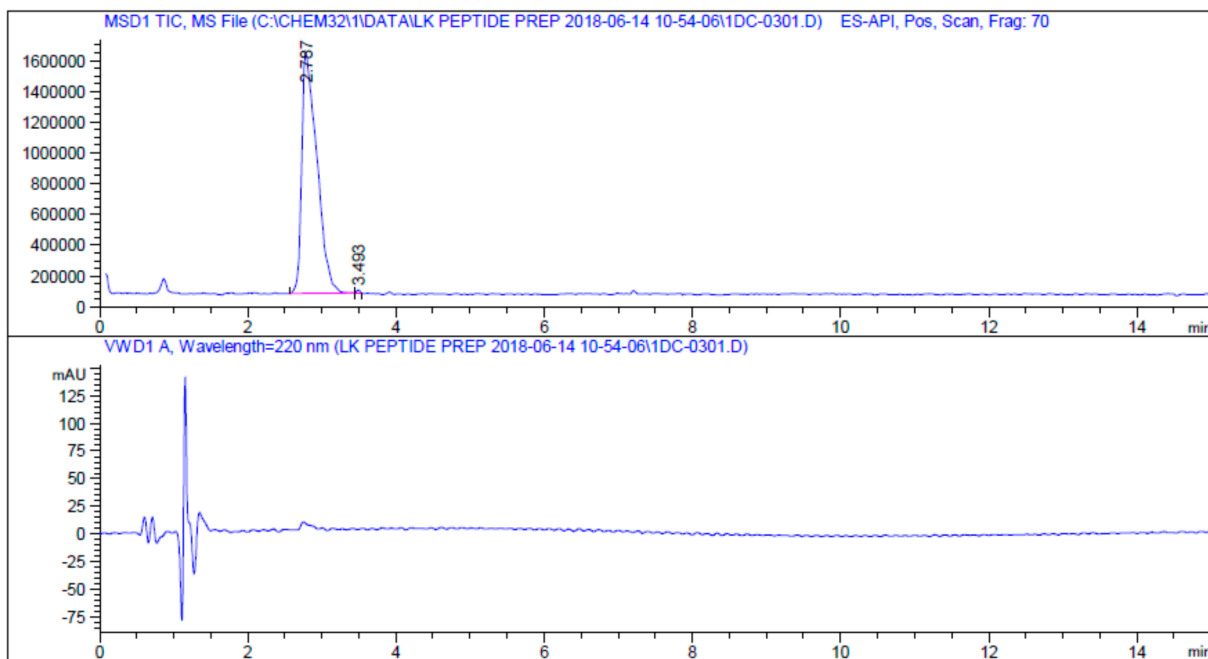
(2S,3S)-2-amino-3-methyl-N-[(1S)-1-(1H-1,2,3,4-tetrazol-5-yl)ethyl]pentanamide **14i-LL**





SM: 7G





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 Area Percent Report
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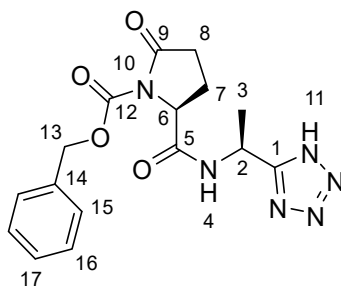
Sorted By : Signal
 Multiplier: : 1.0000
 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

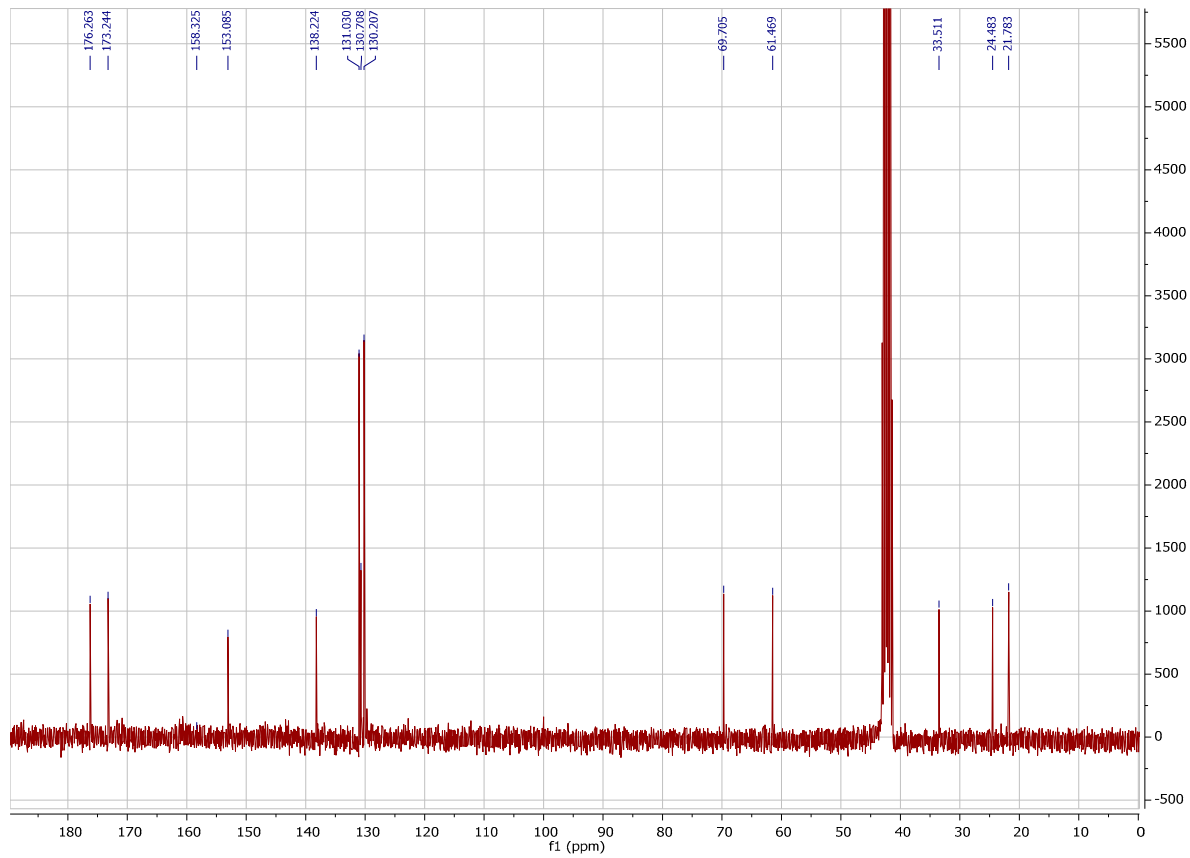
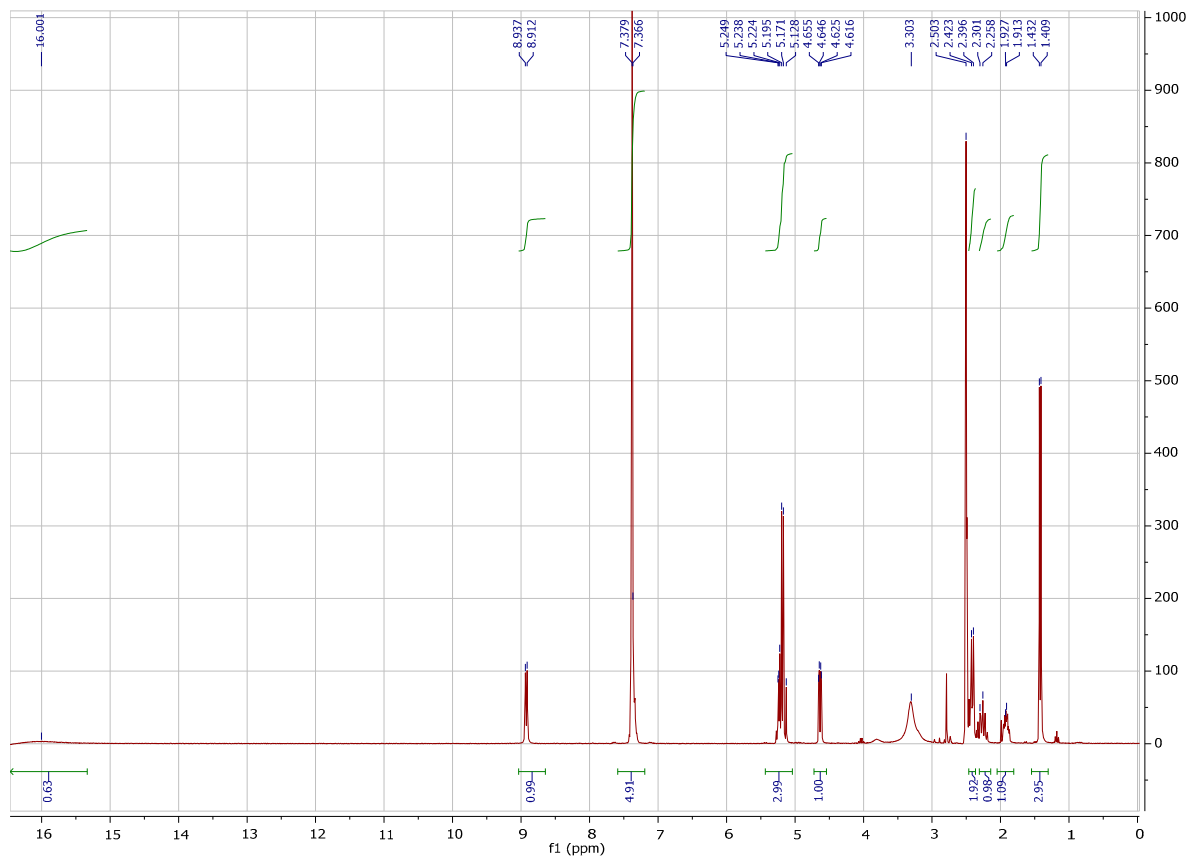
Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	2.787	BB	0.1981	2.22201e7	1.56789e6	99.6797
2	3.493	BB	0.0522	7.13974e4	2.32827e4	0.3203

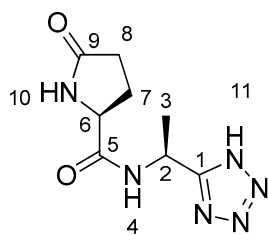
Totals : 2.22915e7 1.59117e6

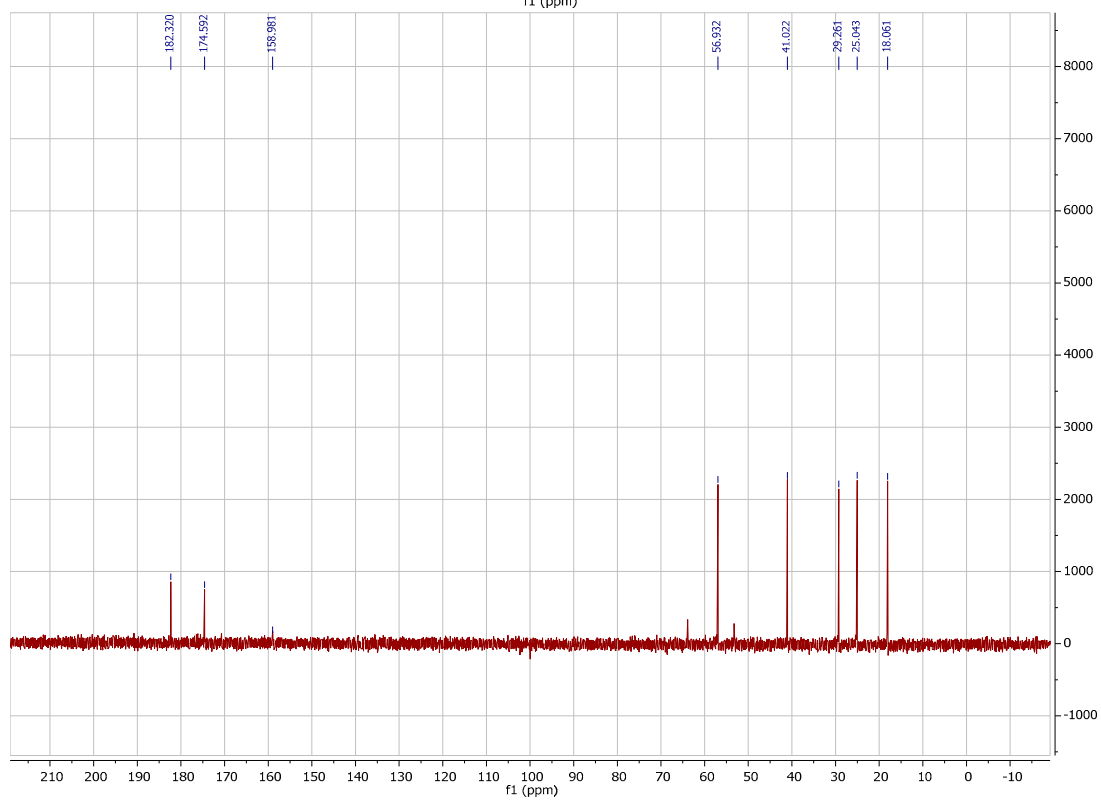
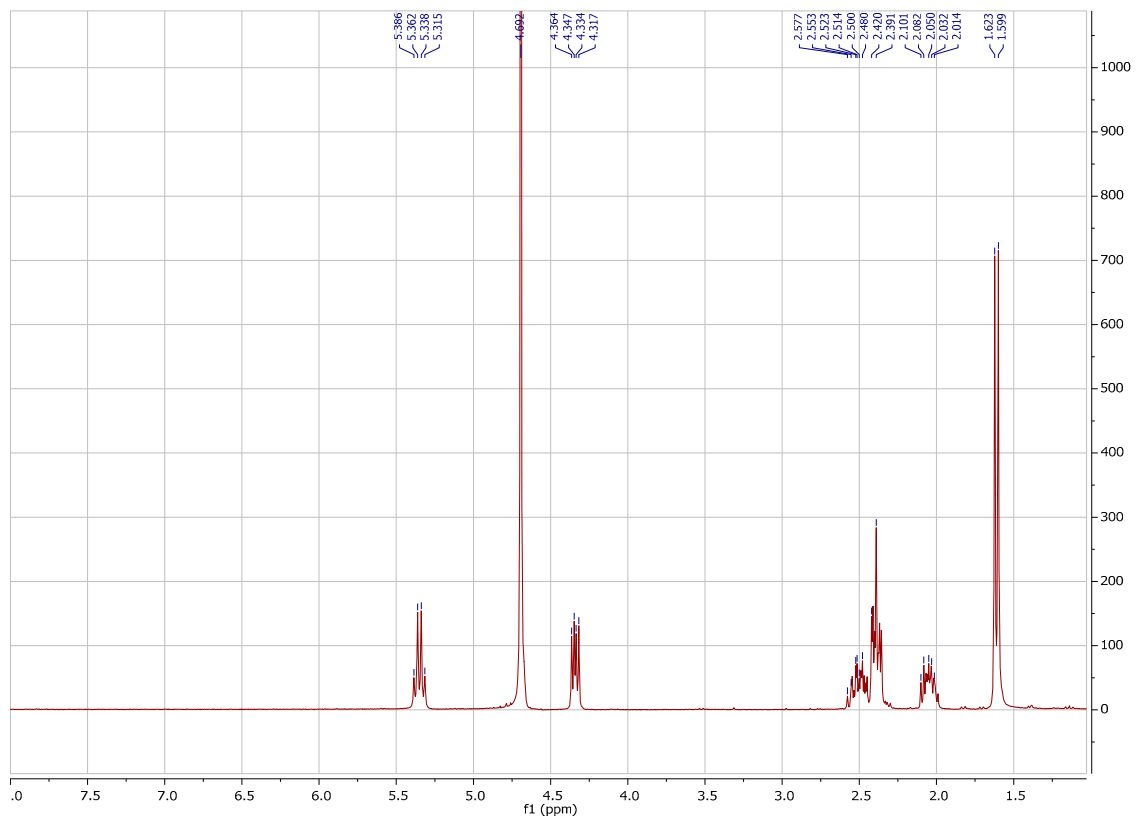
Benzyl-(5S)-2-oxo-5-[[1(1S)-1-(1H-1,2,3,4-tetrazol-5-yl)ethyl]carbamoyl]pyrrolidine-1-carboxylate 13f-LL:

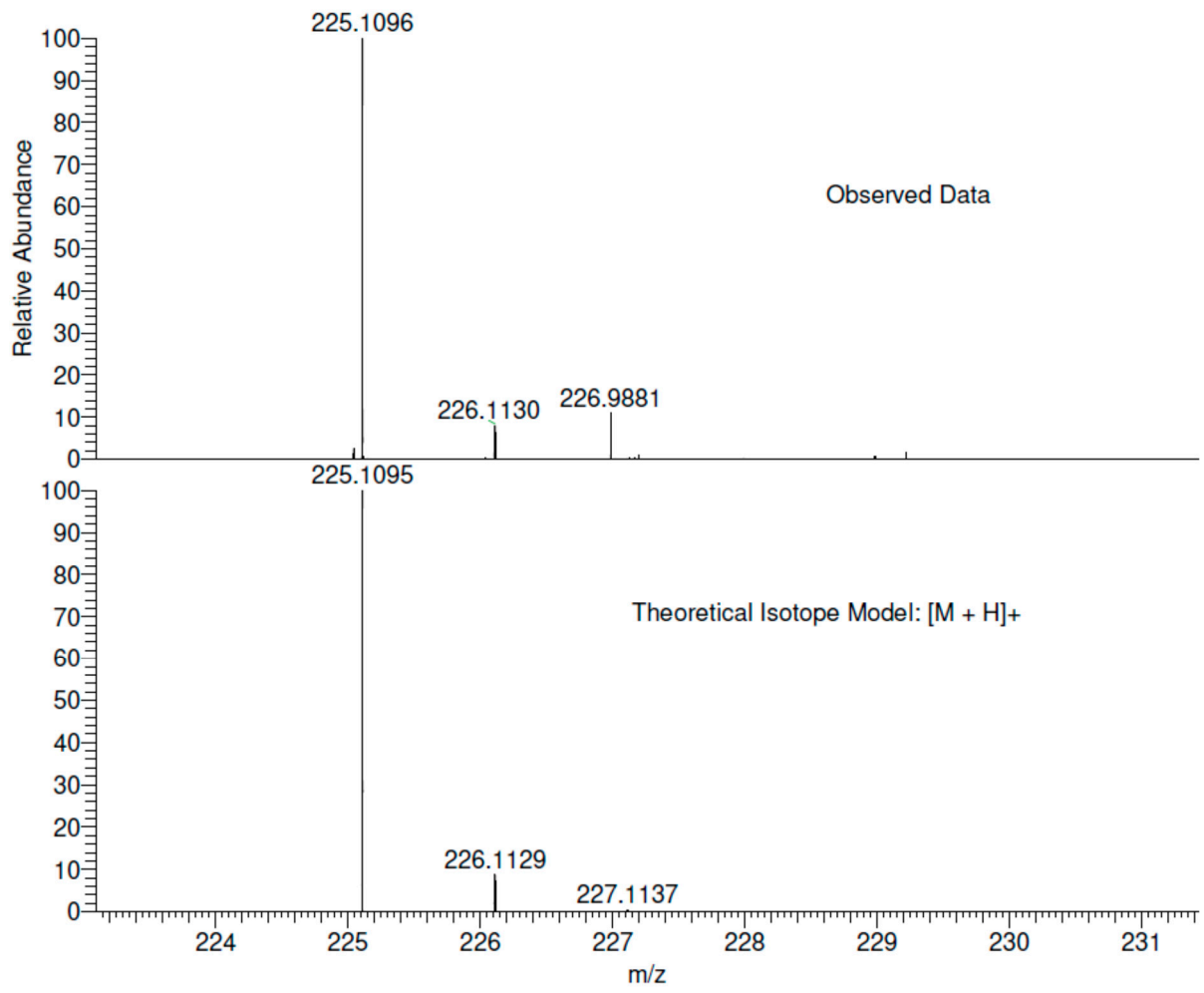


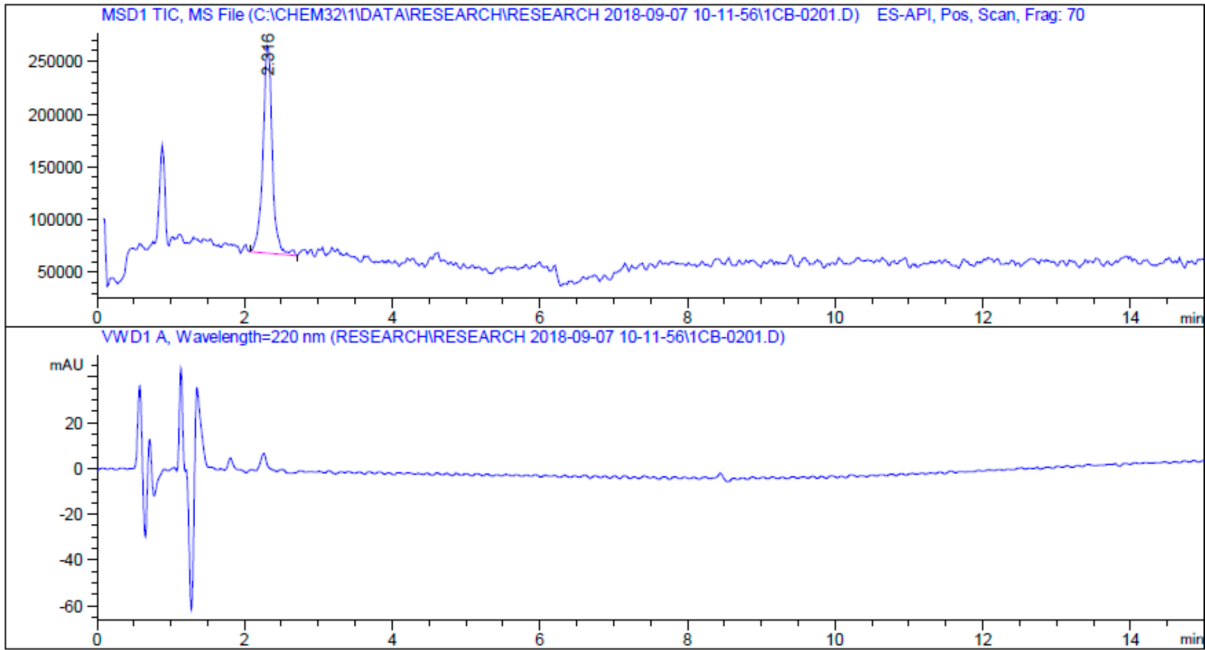


(S)-N-((S)-1-(1H-tetrazol-5-yl)ethyl)-5-oxopyrrolidine-2-carboxamide **14f-LL**:









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 Area Percent Report
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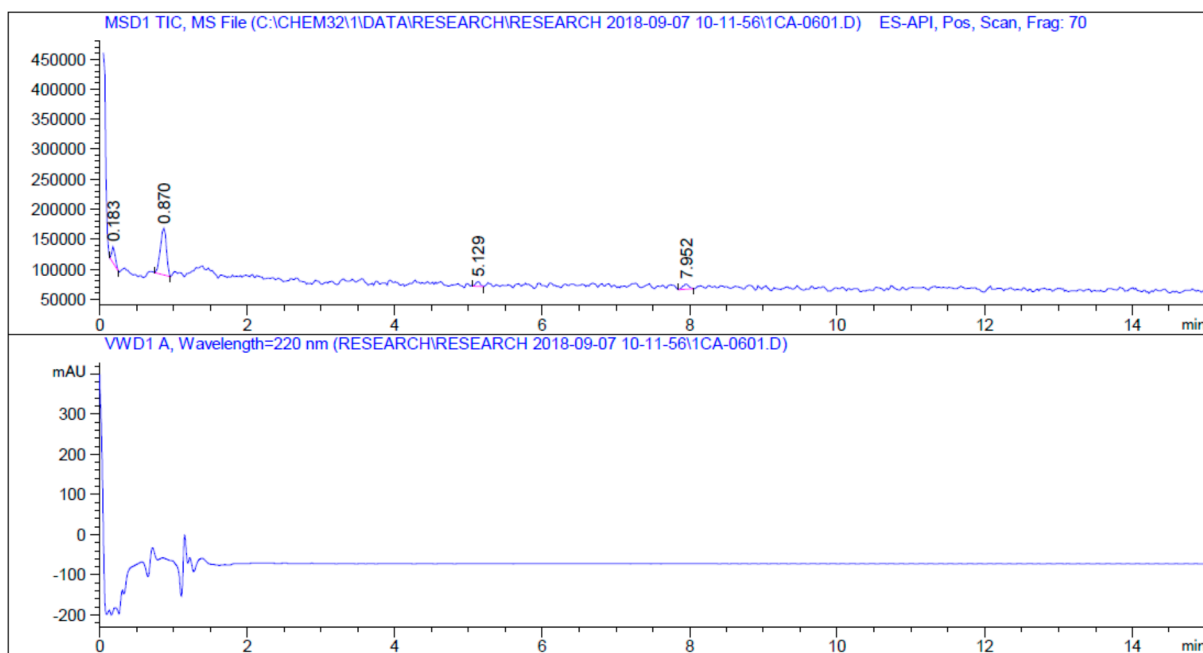
Sorted By : Signal
 Multiplier: : 1.0000
 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

Signal 1: MSD1 TIC, MS File

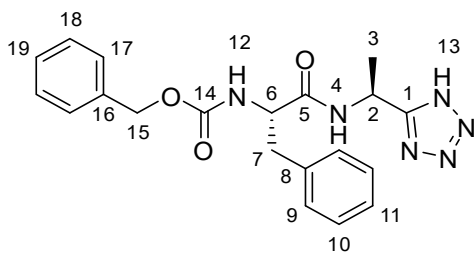
Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	2.316	BB	0.1342	1.72300e6	1.98295e5	100.0000

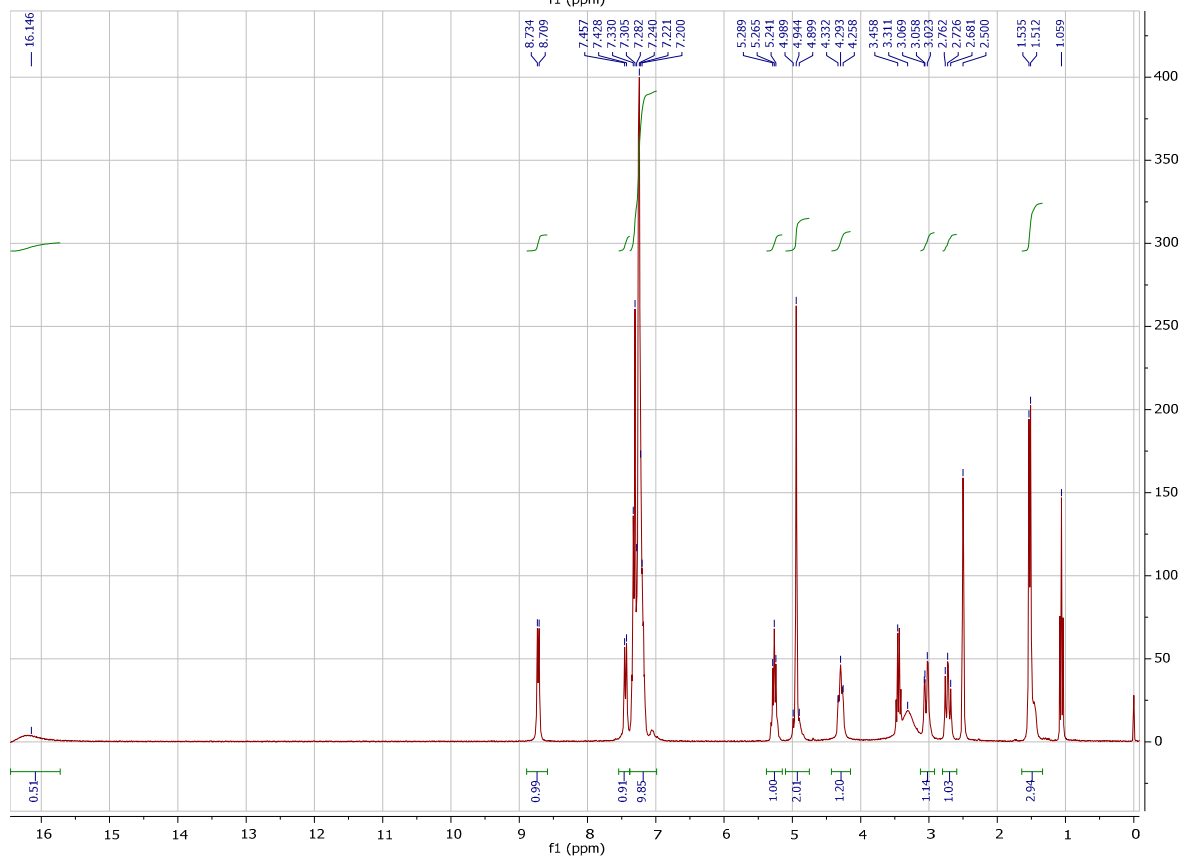
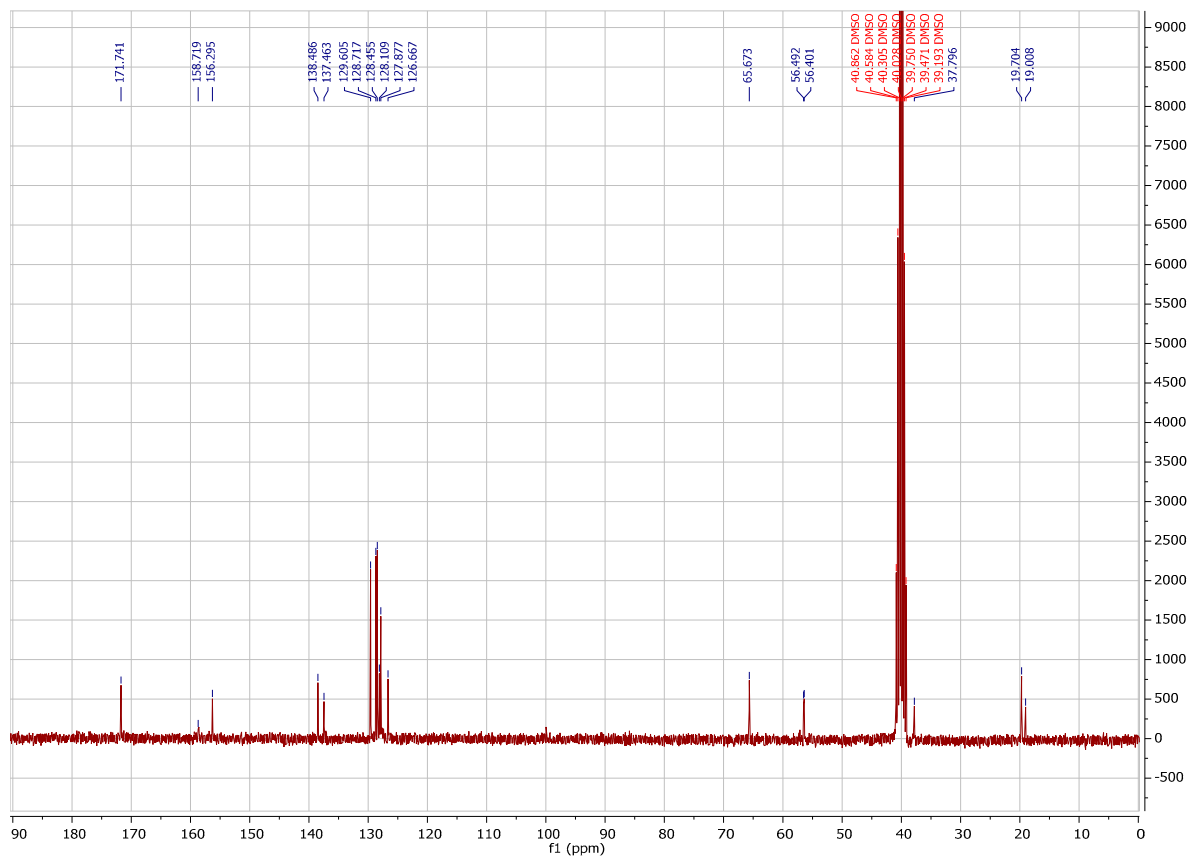
Totals : 1.72300e6 1.98295e5

Blank for 16f-LL:

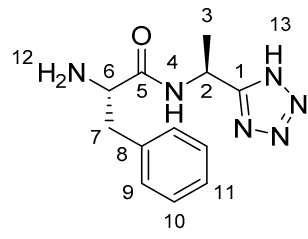


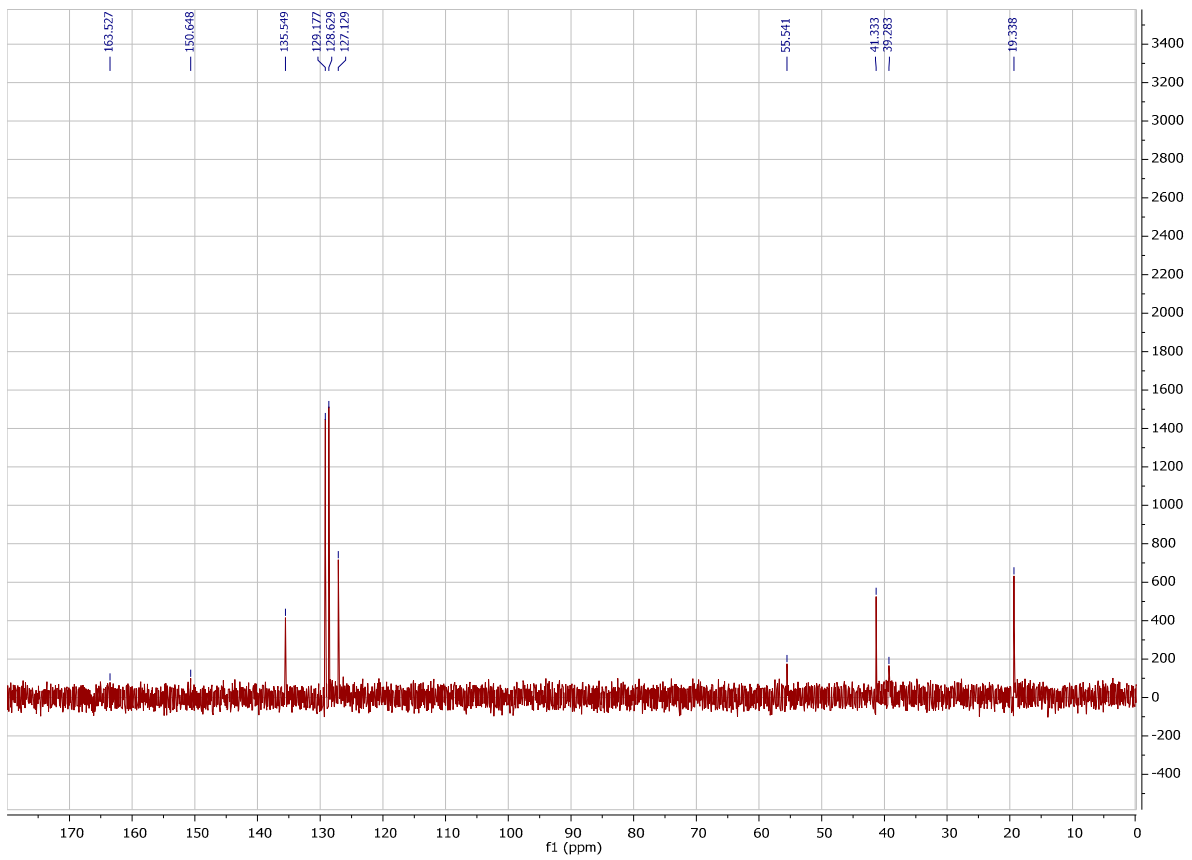
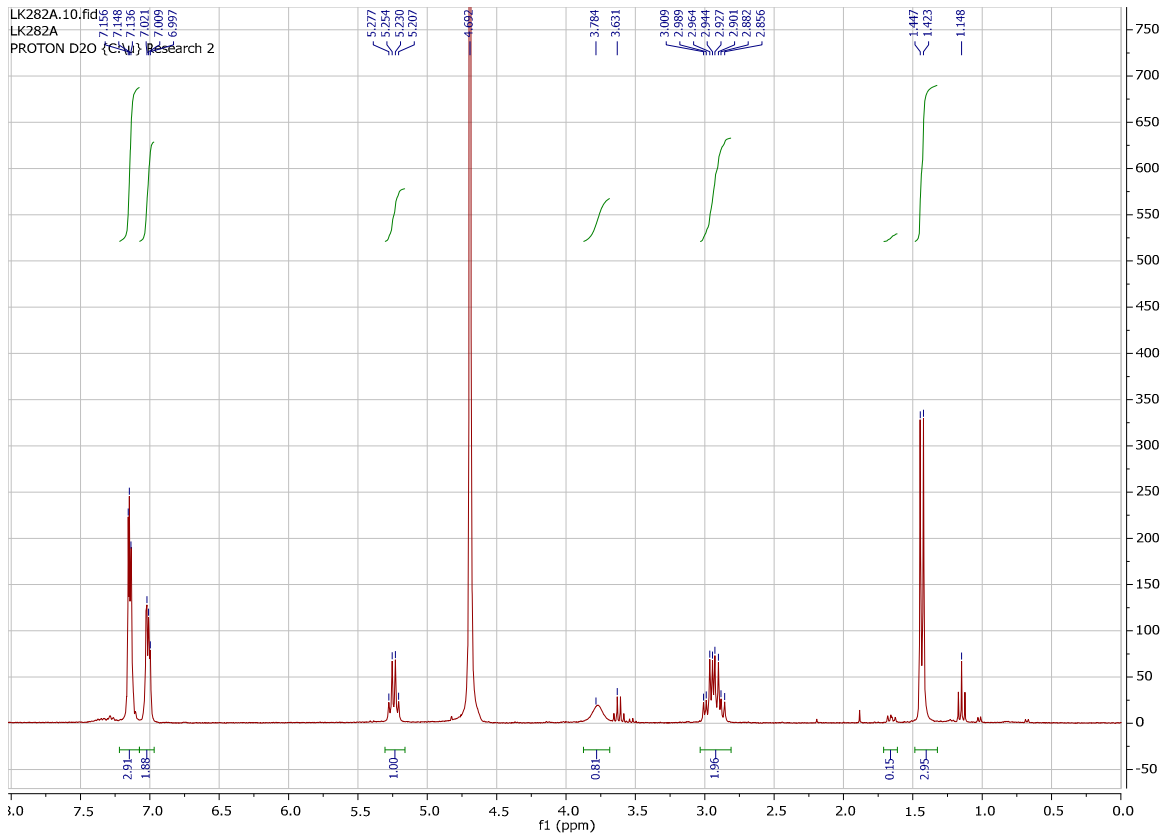
Benzyl-*N*-[(1*S*)-2-phenyl-1-[(1*S*)-1-(1*H*-1,2,3,4-tetrazol-5-yl)ethyl]carbamoyl]ethyl]carbamate
13j-LL:



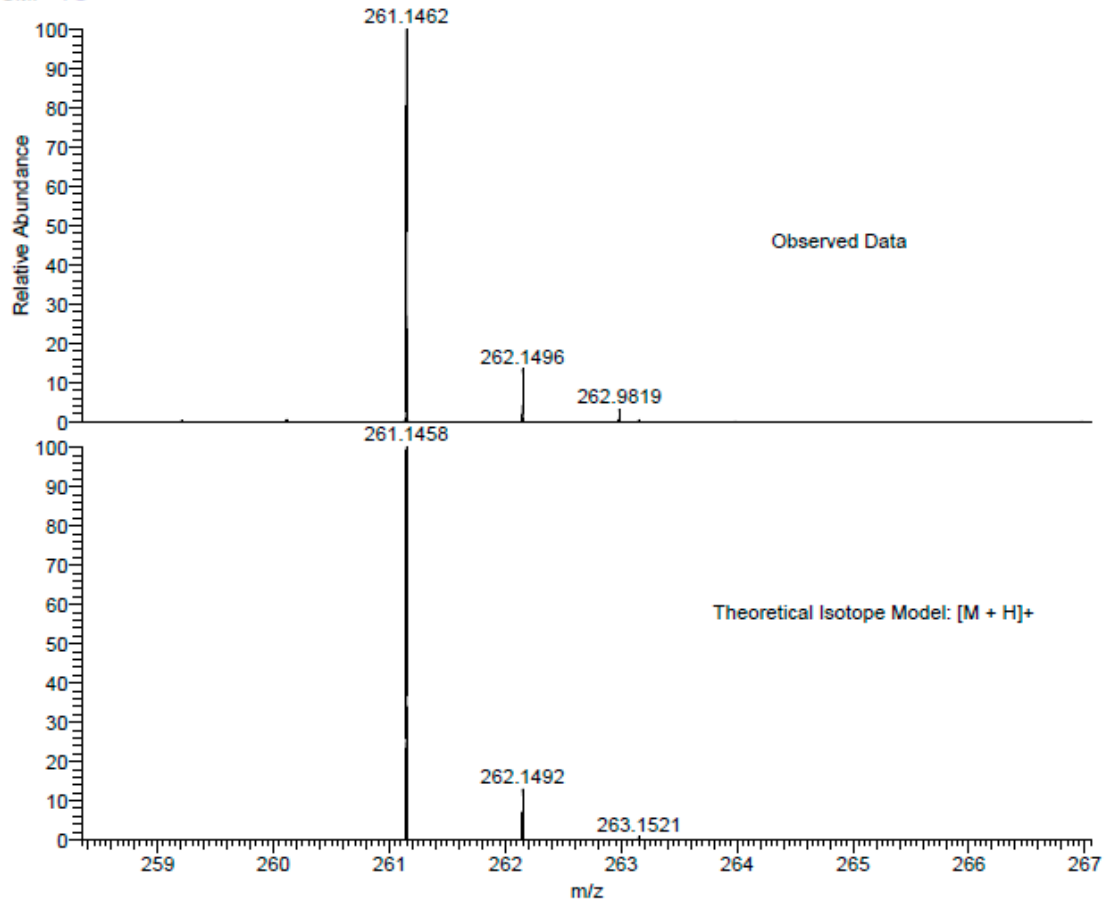


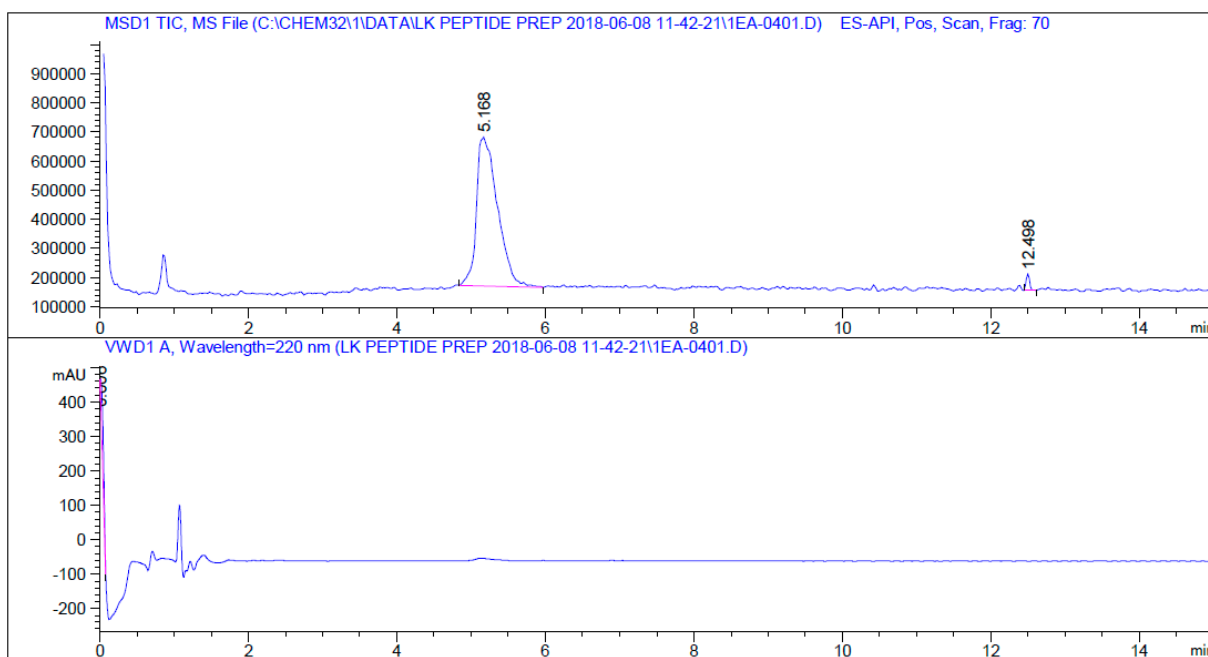
(2S)-2-Amino-3-phenyl-N-[(1S)-1-(1H-1,2,3,4-tetrazol-5-yl)ethyl]propanamide **14j-LL**:





SM: 7G





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 Area Percent Report
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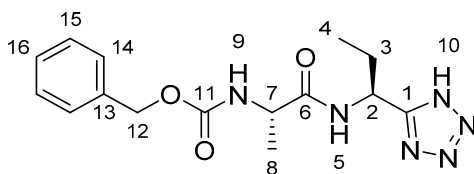
Sorted By : Signal
 Multiplier: : 1.0000
 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

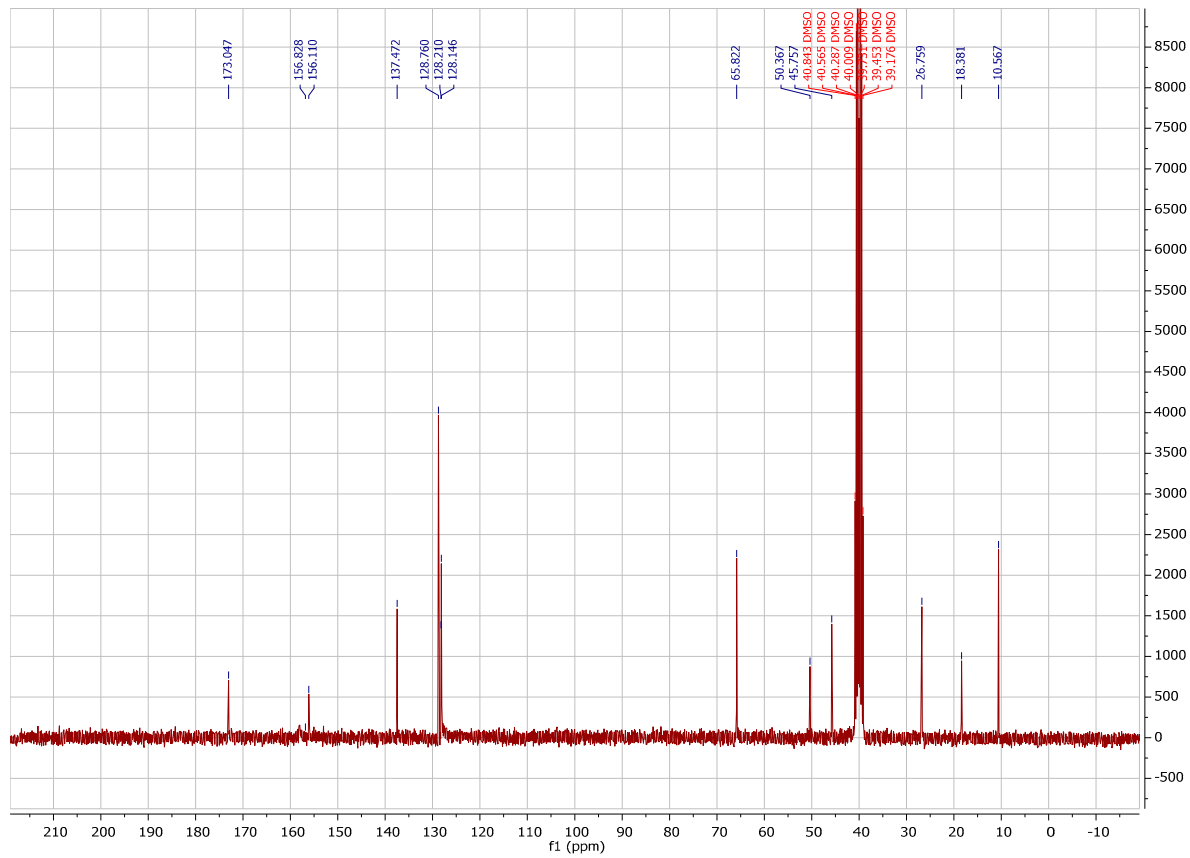
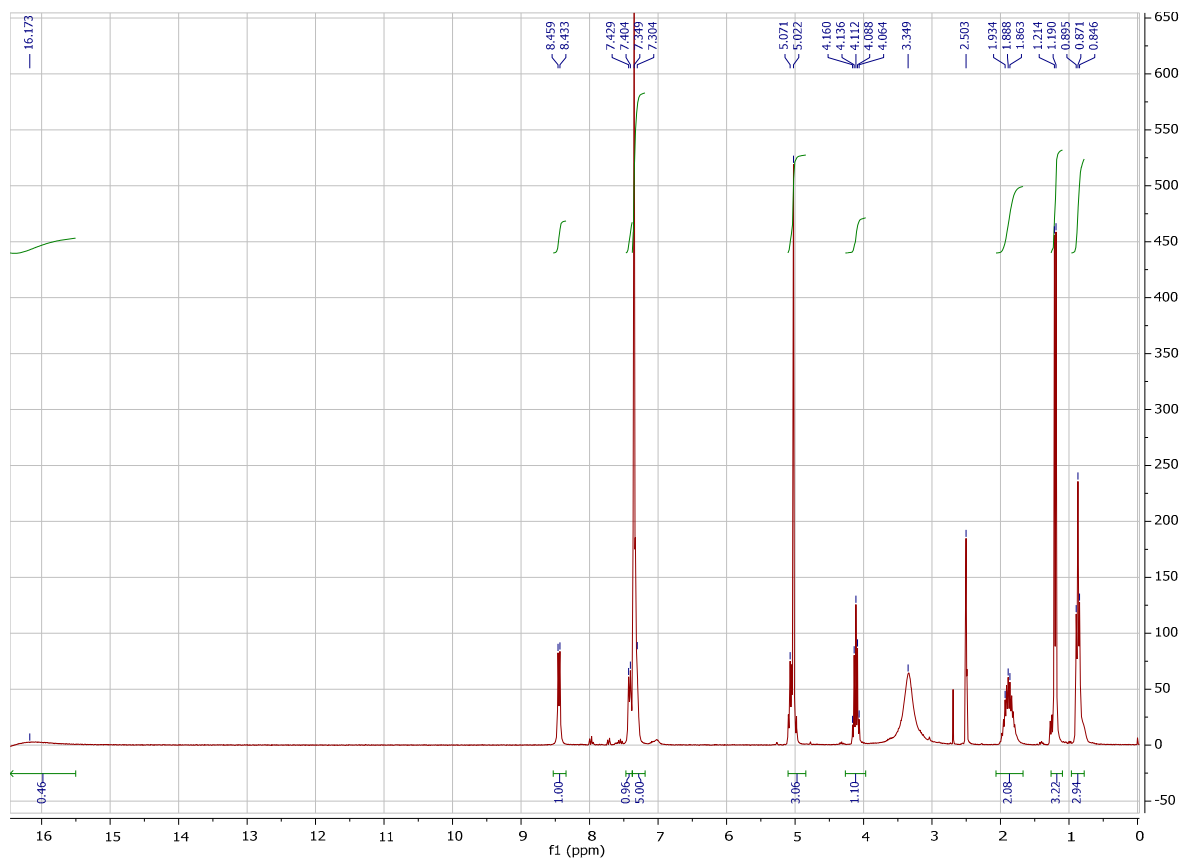
Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	5.168	BB	0.2626	1.00788e7	5.11773e5	98.1589
2	12.498	BB	0.0551	1.89042e5	5.70412e4	1.8411

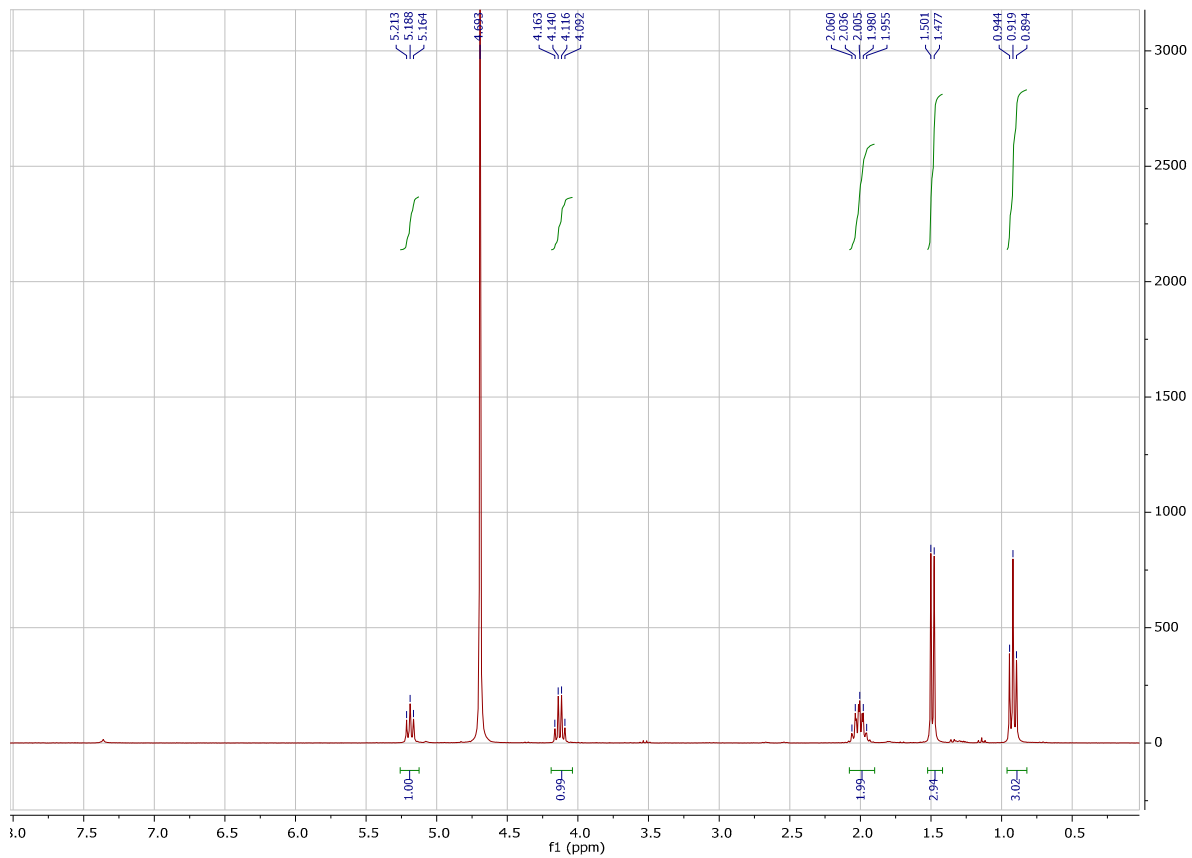
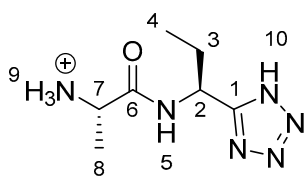
Totals : 1.02678e7 5.68814e5

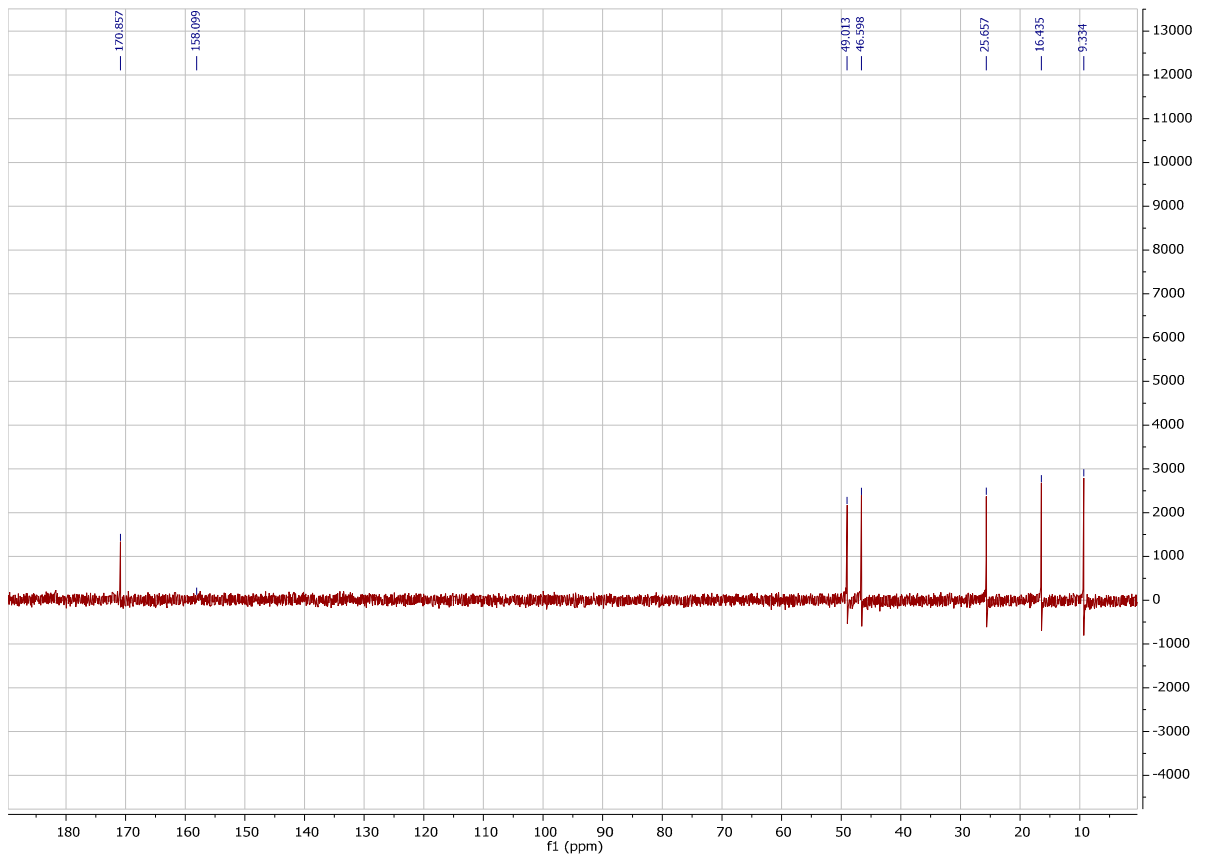
Benzyl-*N*-[(1*S*)-1-[(1*S*)-1-(1*H*-1,2,3,4-tetrazol-5-yl)propyl]carbamoyl]ethyl]carbamate **13b-LL**:



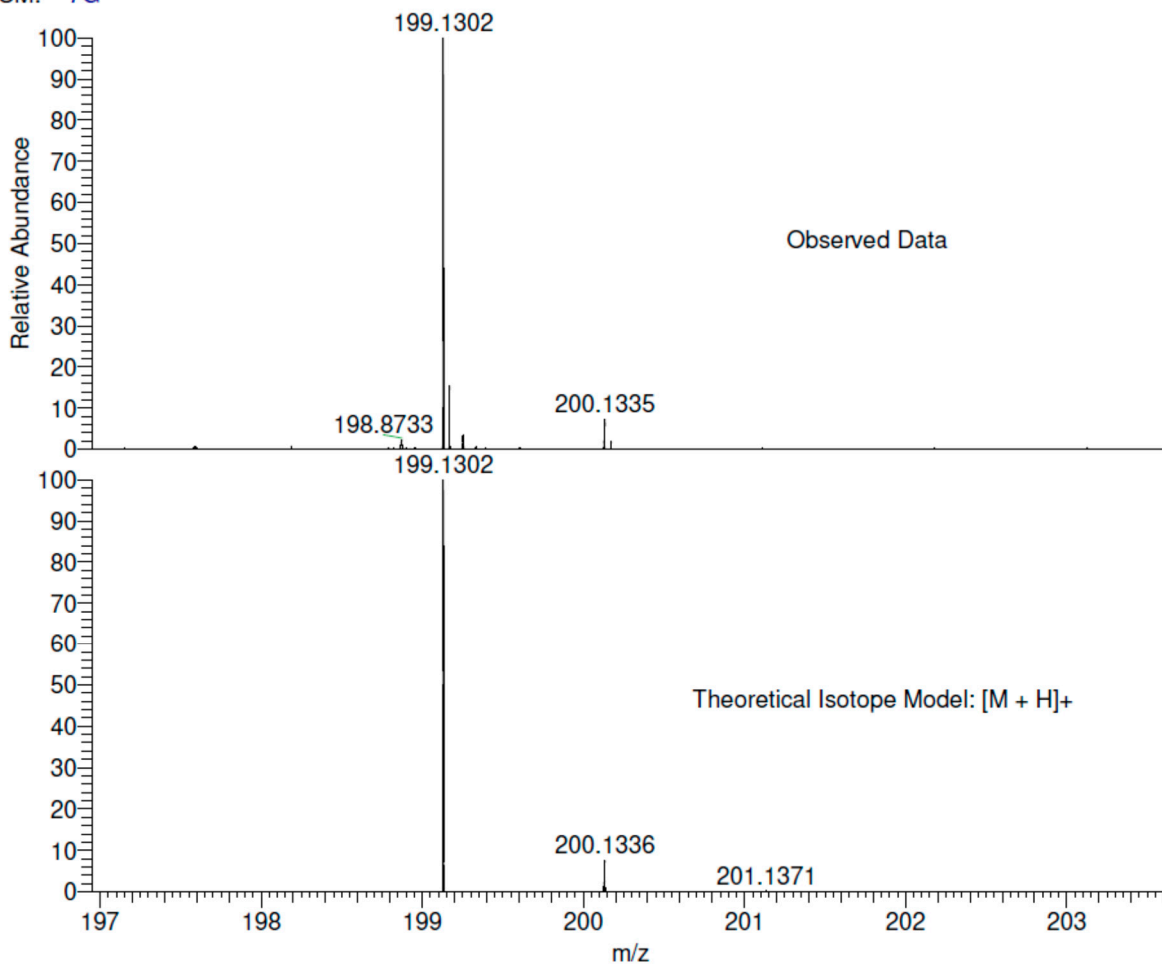


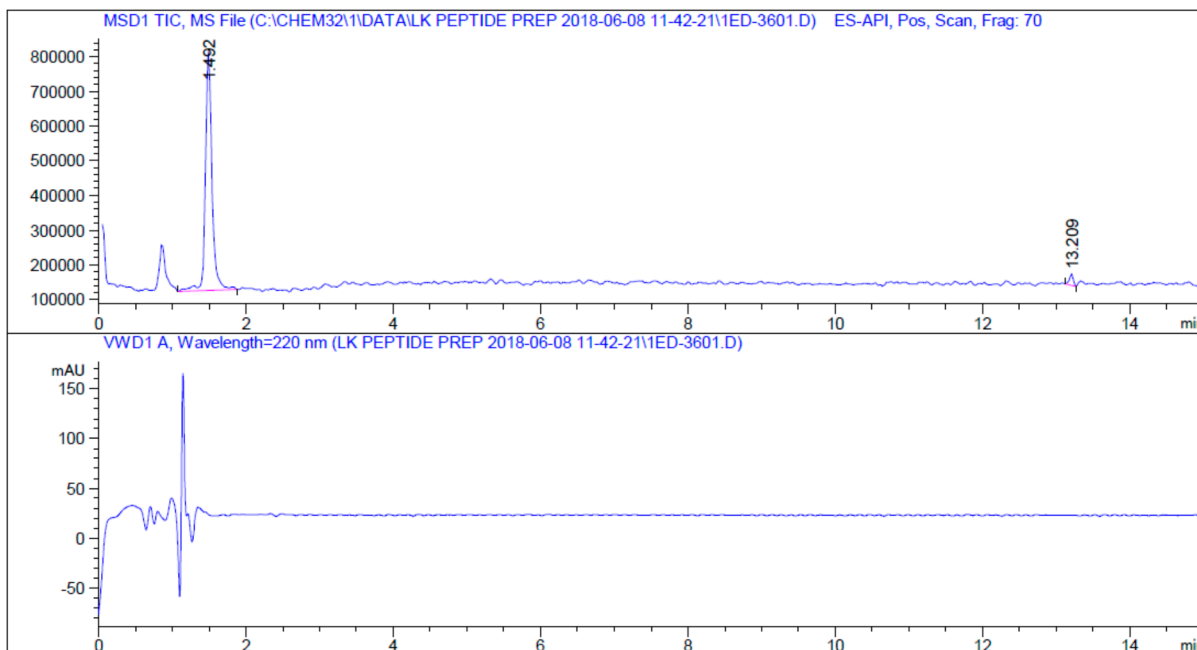
(2S)-2-Amino-N-[(1S)-1-(1H-1,2,3,4-tetrazol-5-yl)propyl]propanamide **14b-LL**:





SM: 7G





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 Area Percent Report
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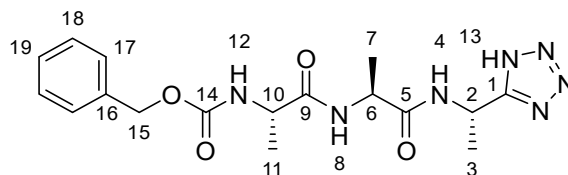
Sorted By : Signal
 Multiplier: : 1.0000
 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

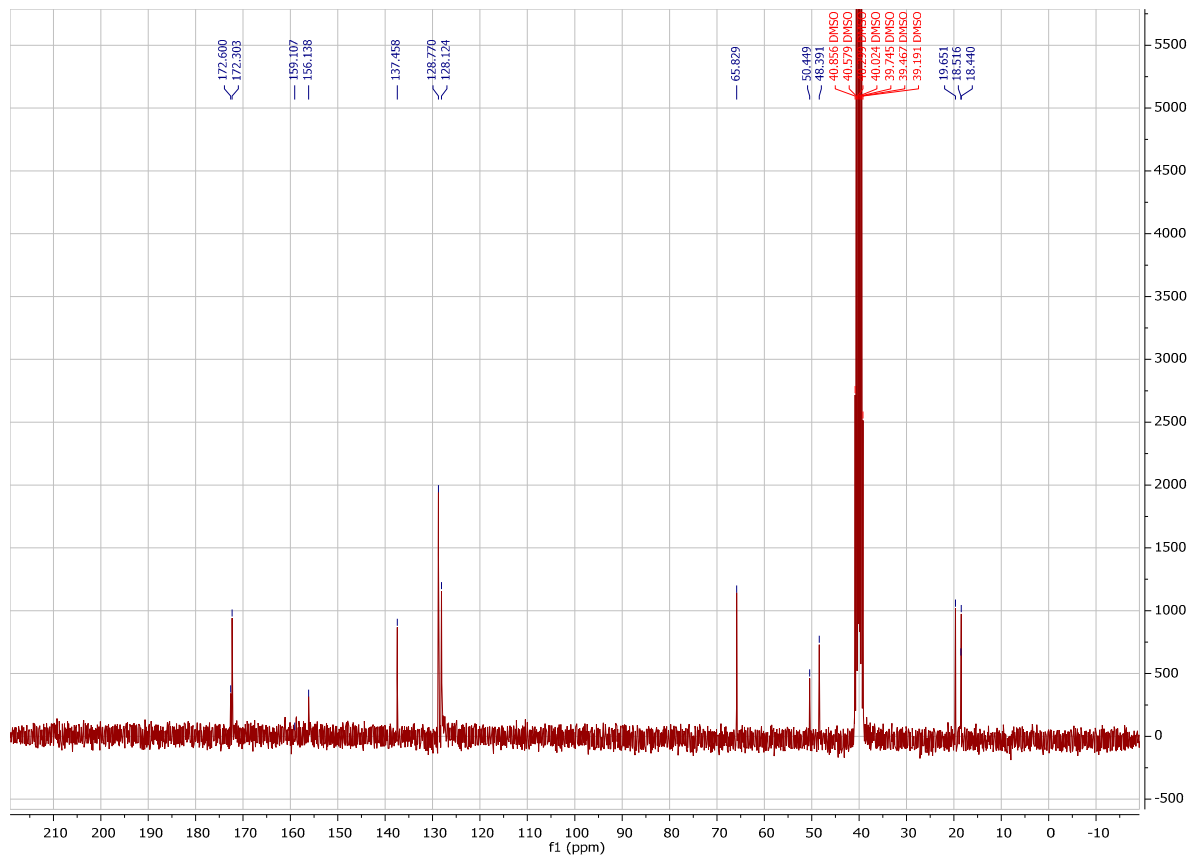
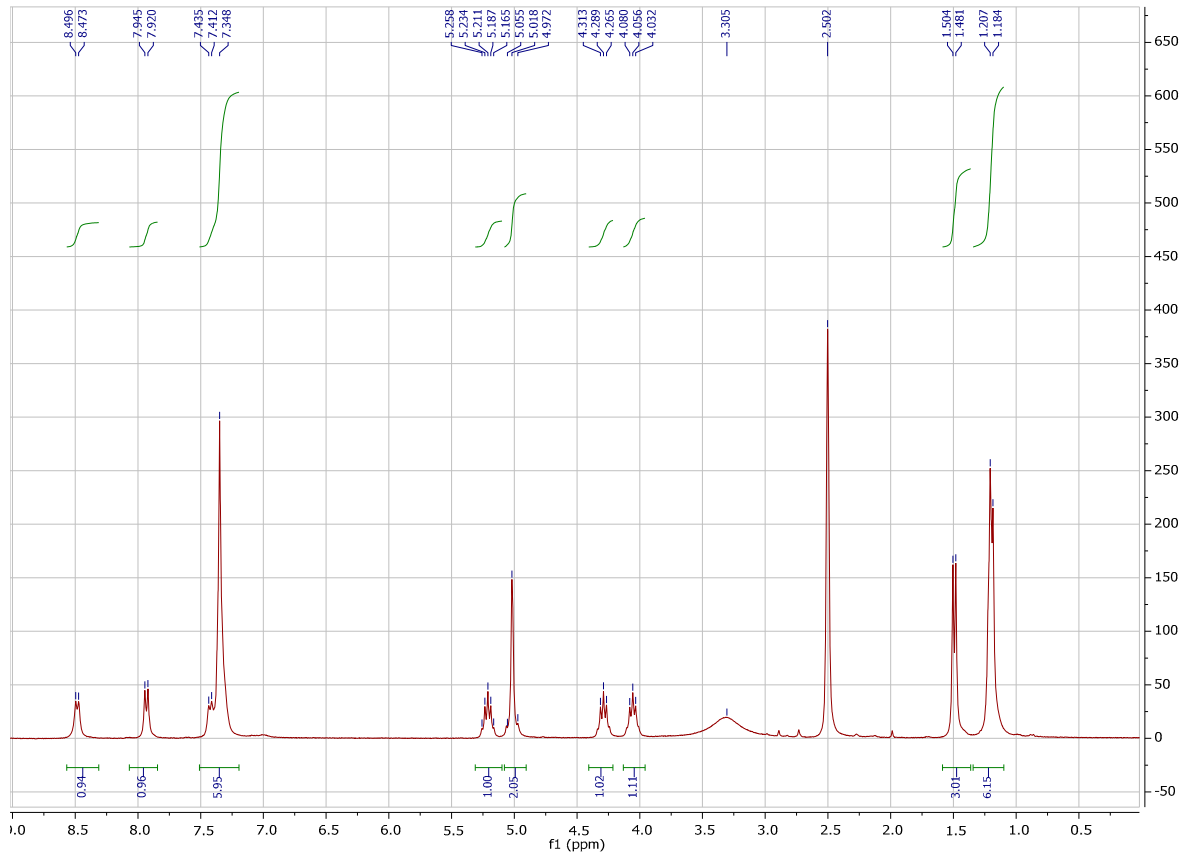
Signal 1: MS1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	1.492	BB	0.0888	4.28483e6	6.90281e5	97.1740
2	13.209	BB	0.0588	1.24610e5	3.43131e4	2.8260

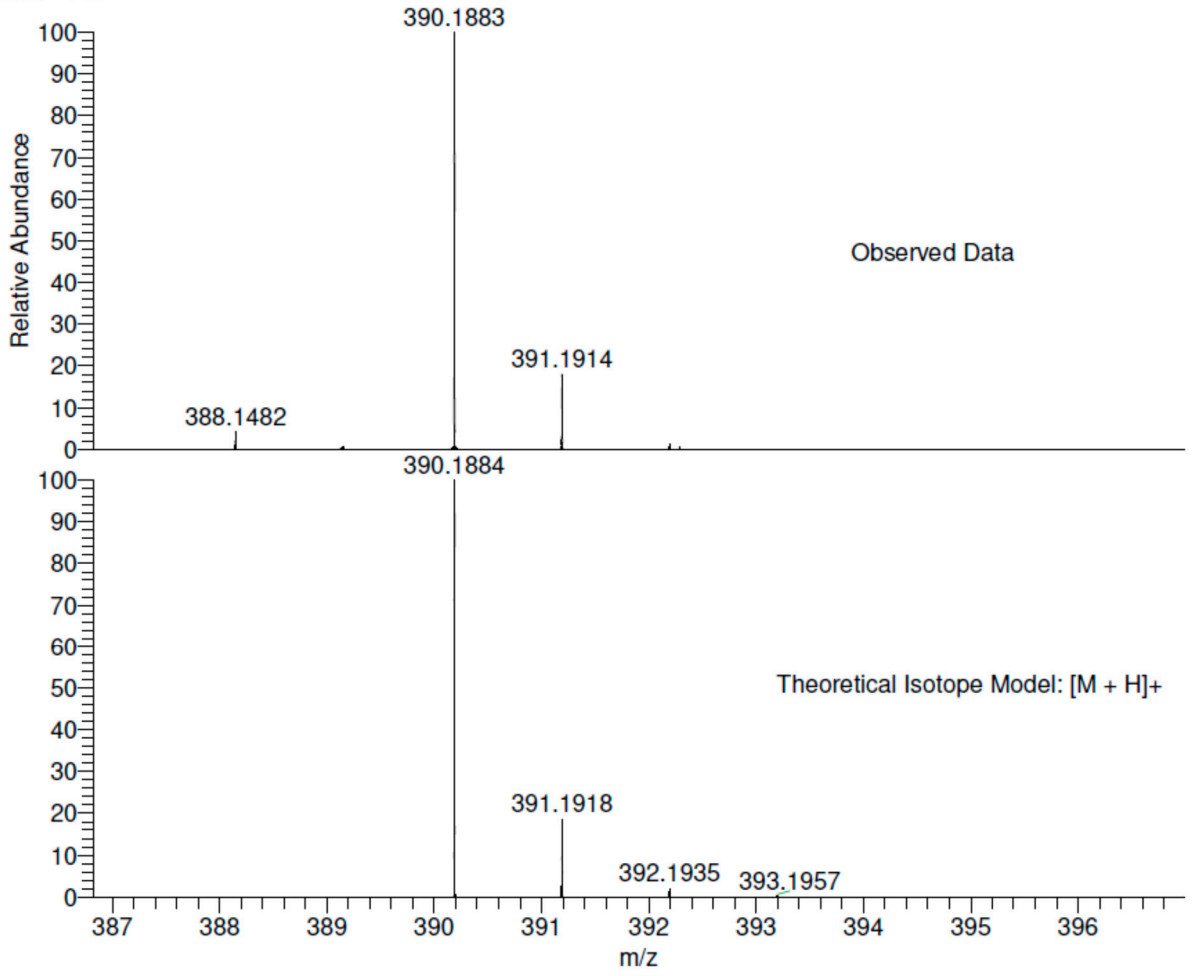
Totals : 4.40944e6 7.24594e5

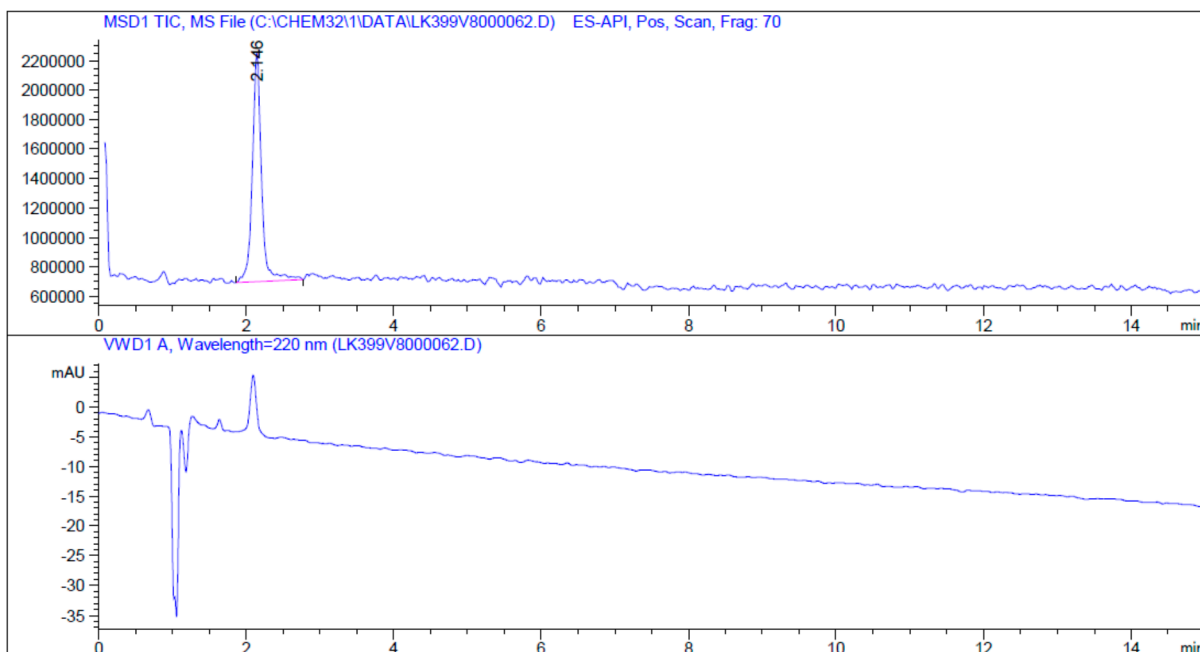
N-[(1*S*)-1-[[*(1S)*-1-[[*(1S)*-1-(1*H*-1,2,3,4-tetrazol-5-yl)ethyl]carbamoyl]ethyl]carbamoyl]ethyl]carbamate **13c-LLL**:





SM: 7G





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 Area Percent Report
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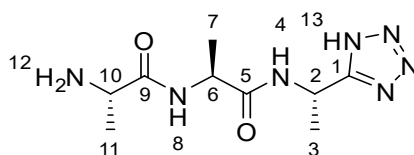
Sorted By : Signal
 Multiplier: : 1.0000
 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

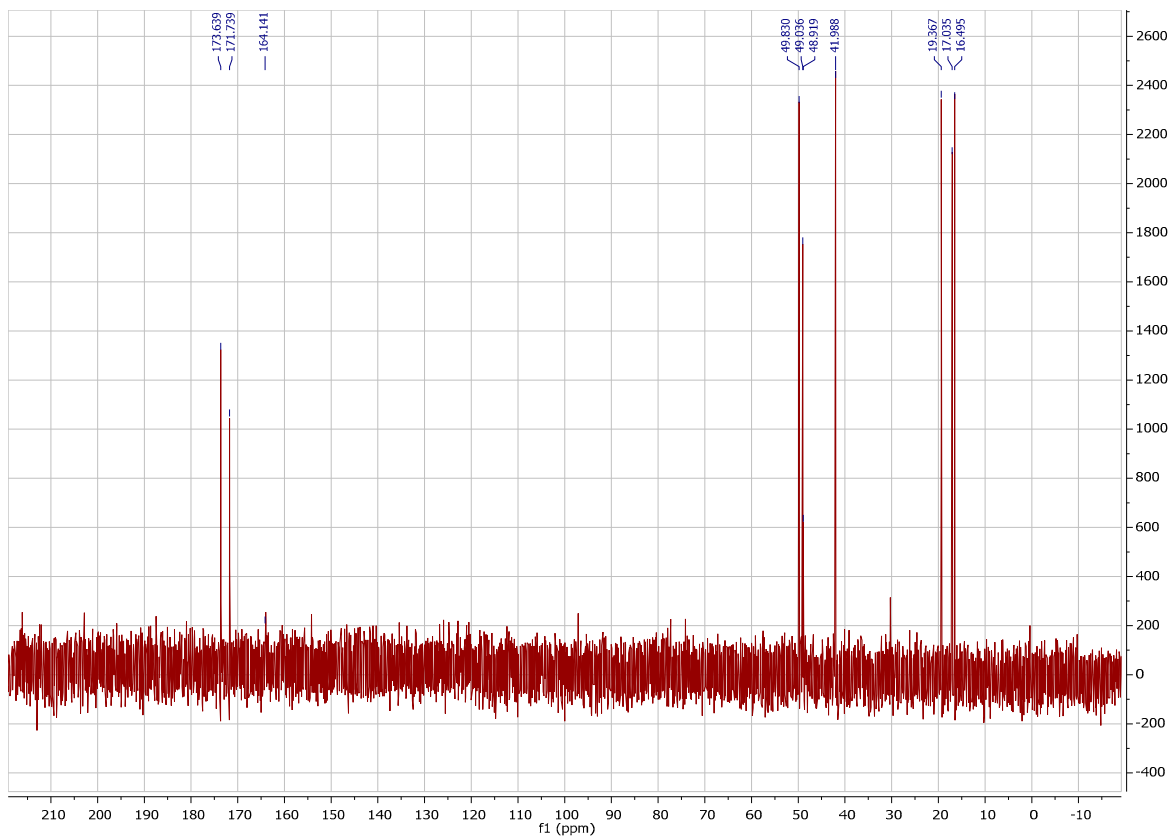
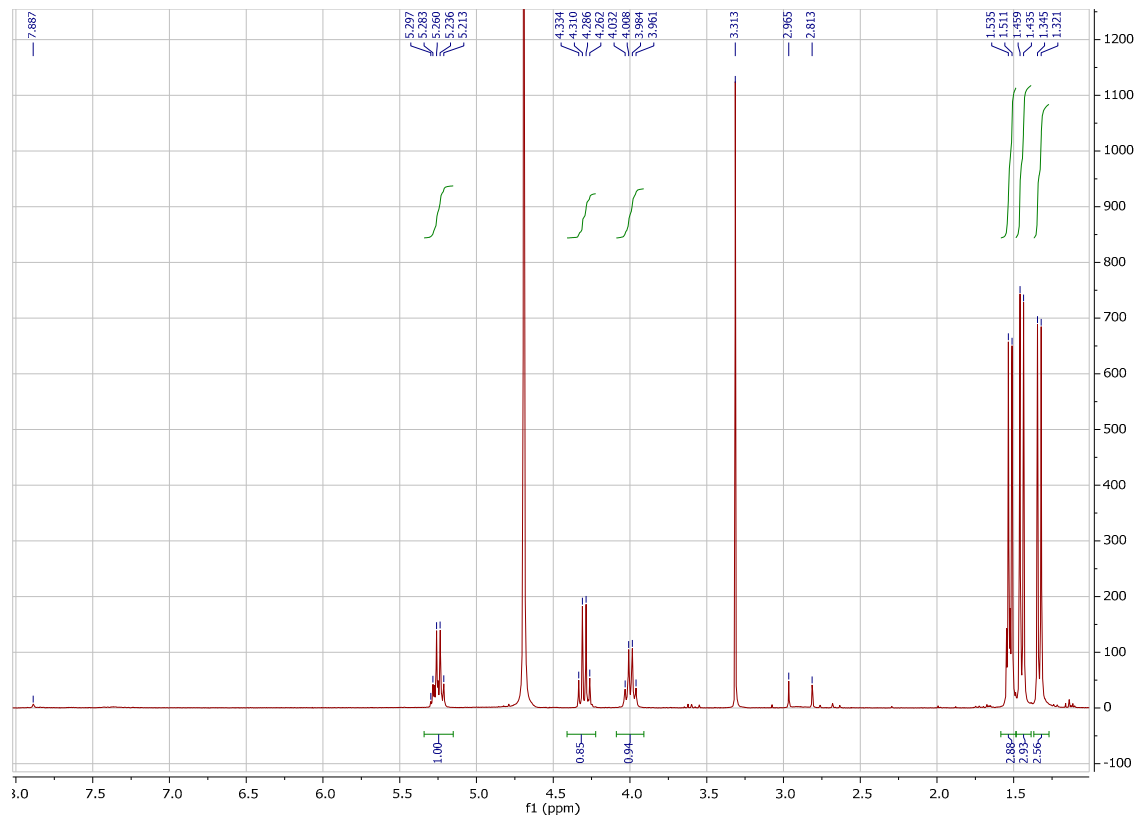
Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	2.146	BB	0.1280	1.34620e7	1.55971e6	100.0000

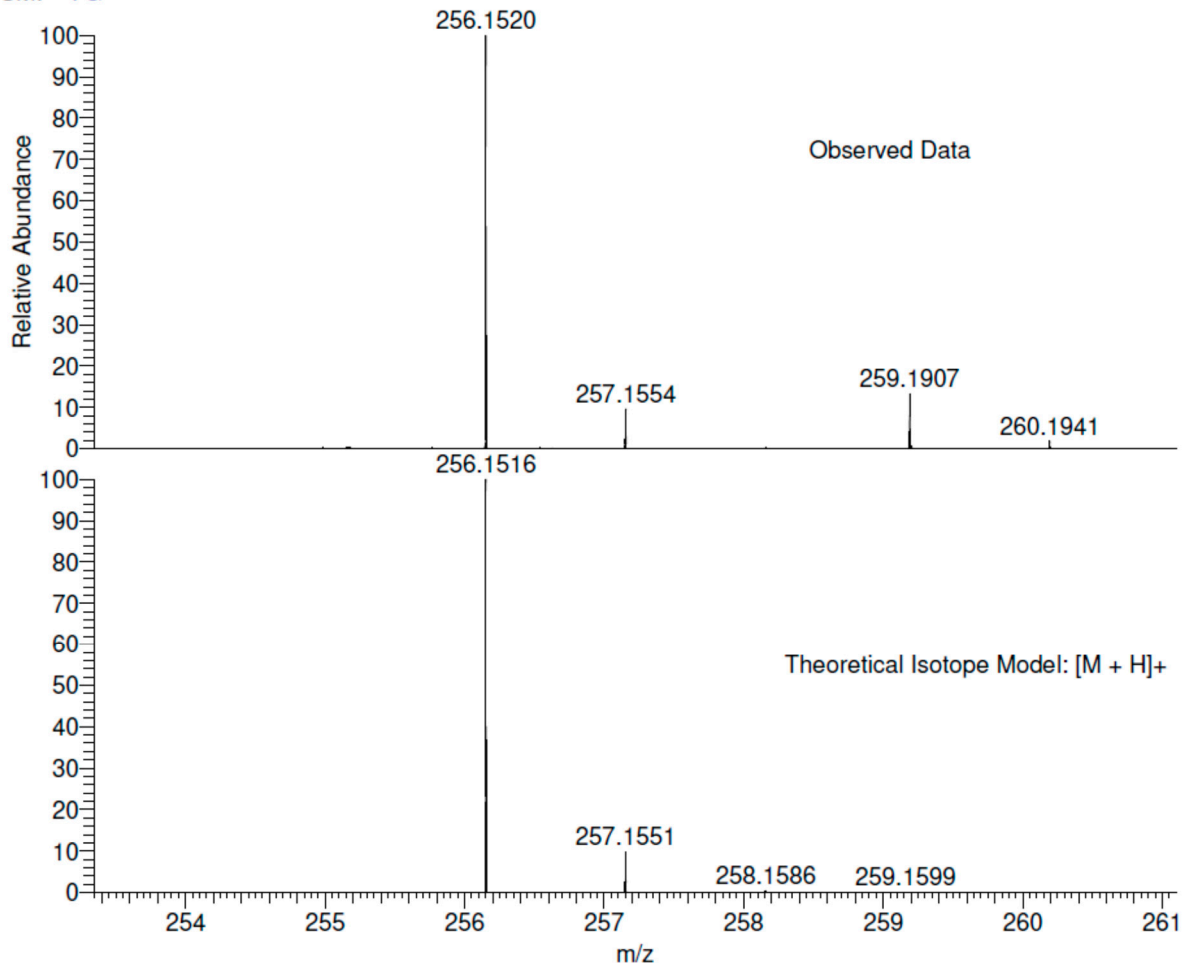
Totals : 1.34620e7 1.55971e6

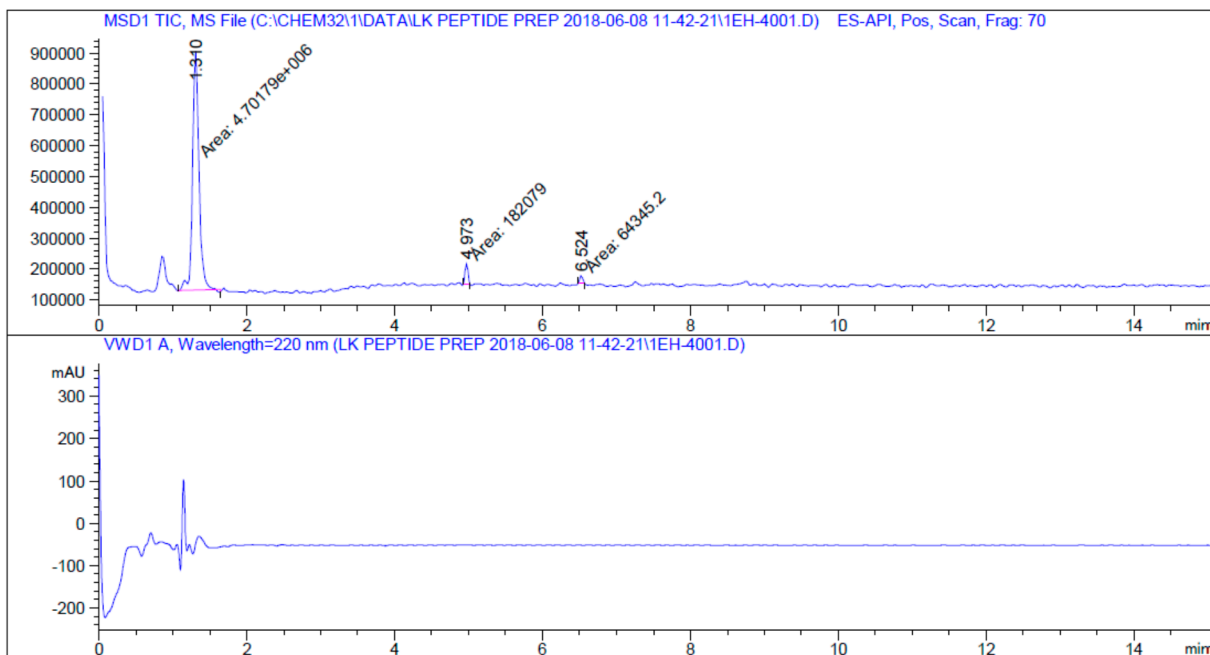
(S)-N-((S)-1-(1H-tetrazol-5-yl)ethyl)-2-((S)-2-aminopropanamido)propanamide **14c-LLL**:





SM: 7G





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 Area Percent Report
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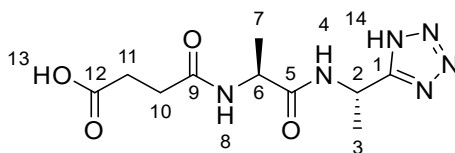
Sorted By : Signal
 Multiplier: : 1.0000
 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

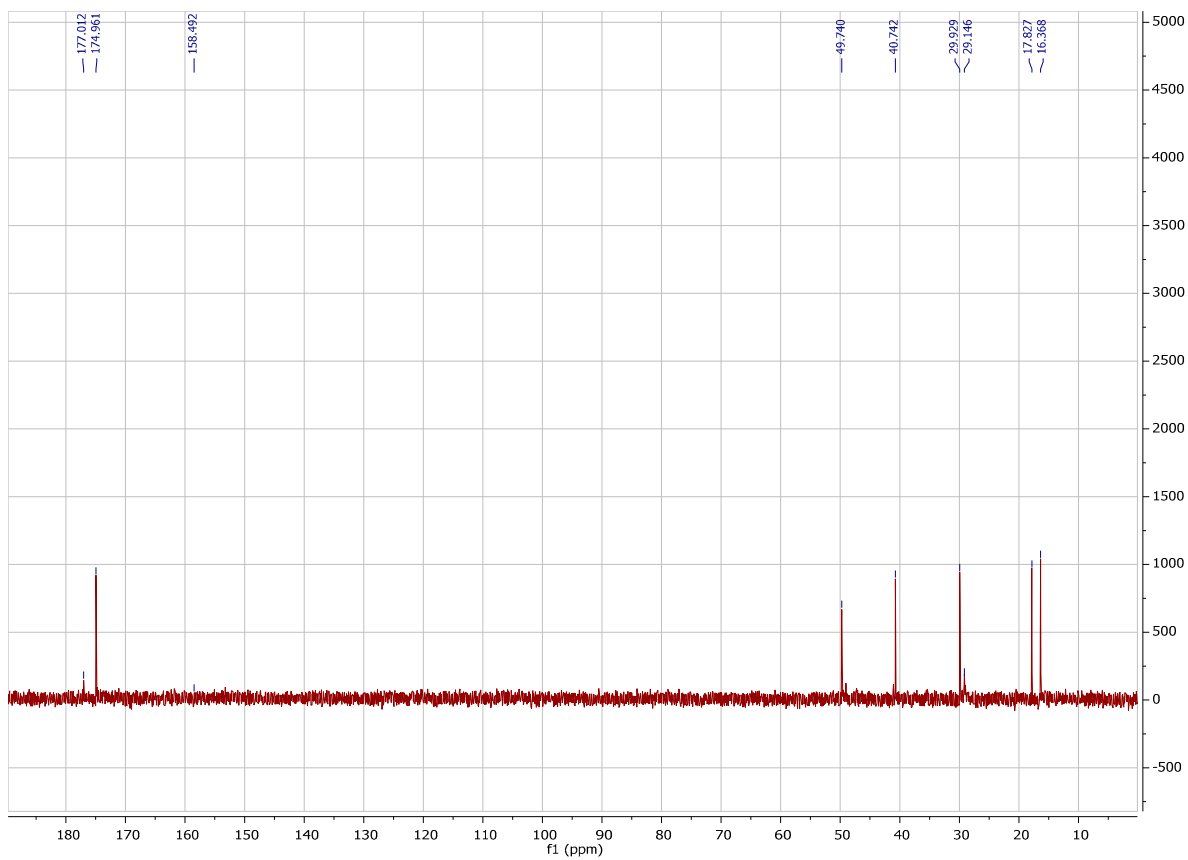
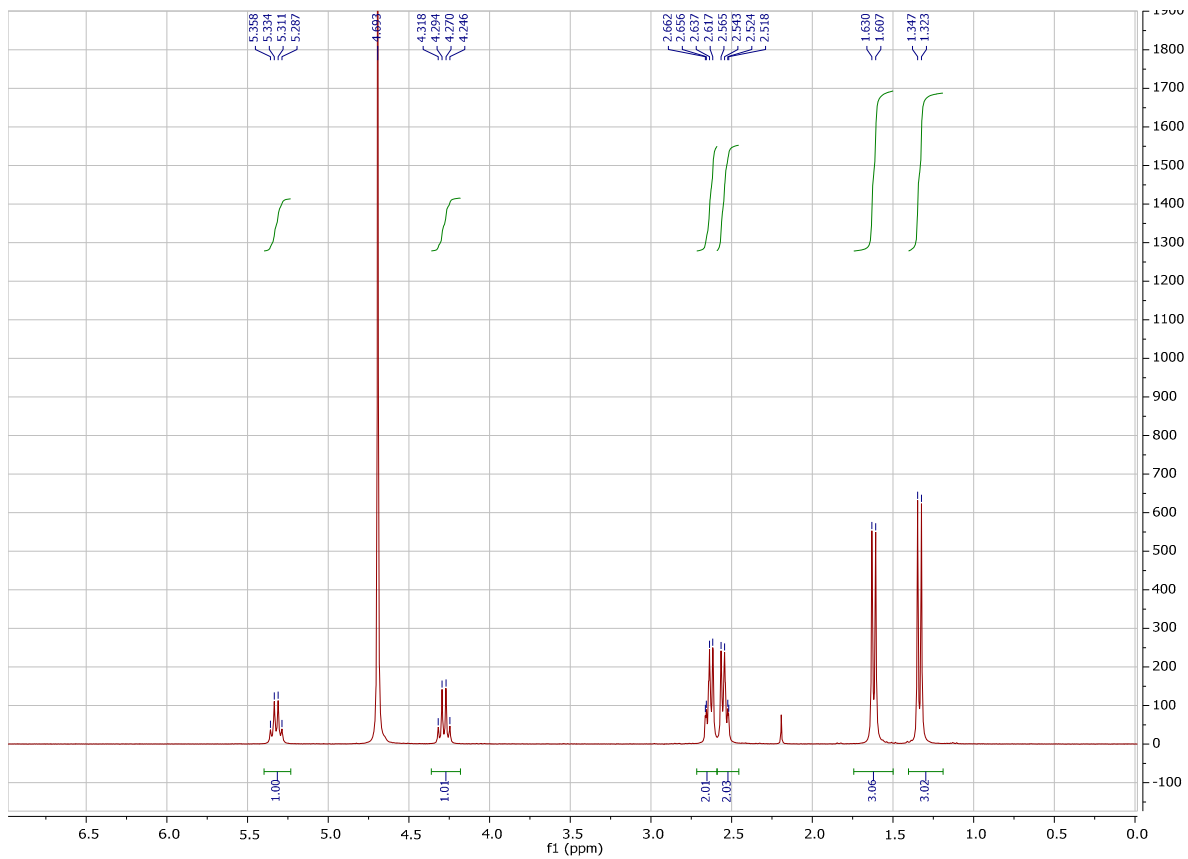
Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	1.310	MM	0.1002	4.70179e6	7.82248e5	95.0199
2	4.973	MM	0.0457	1.82079e5	6.63530e4	3.6797
3	6.524	MM	0.0427	6.43452e4	2.51431e4	1.3004

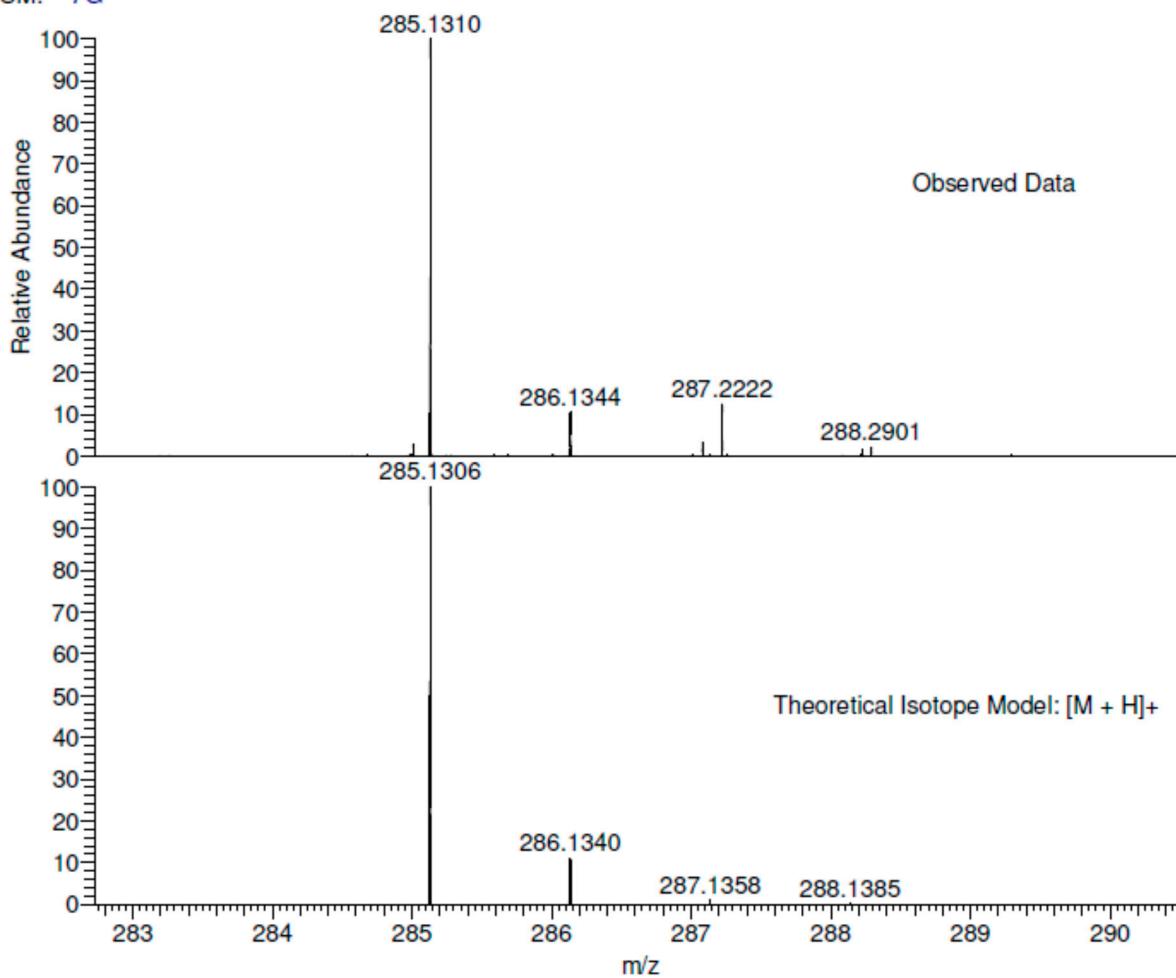
Totals : 4.94821e6 8.73744e5

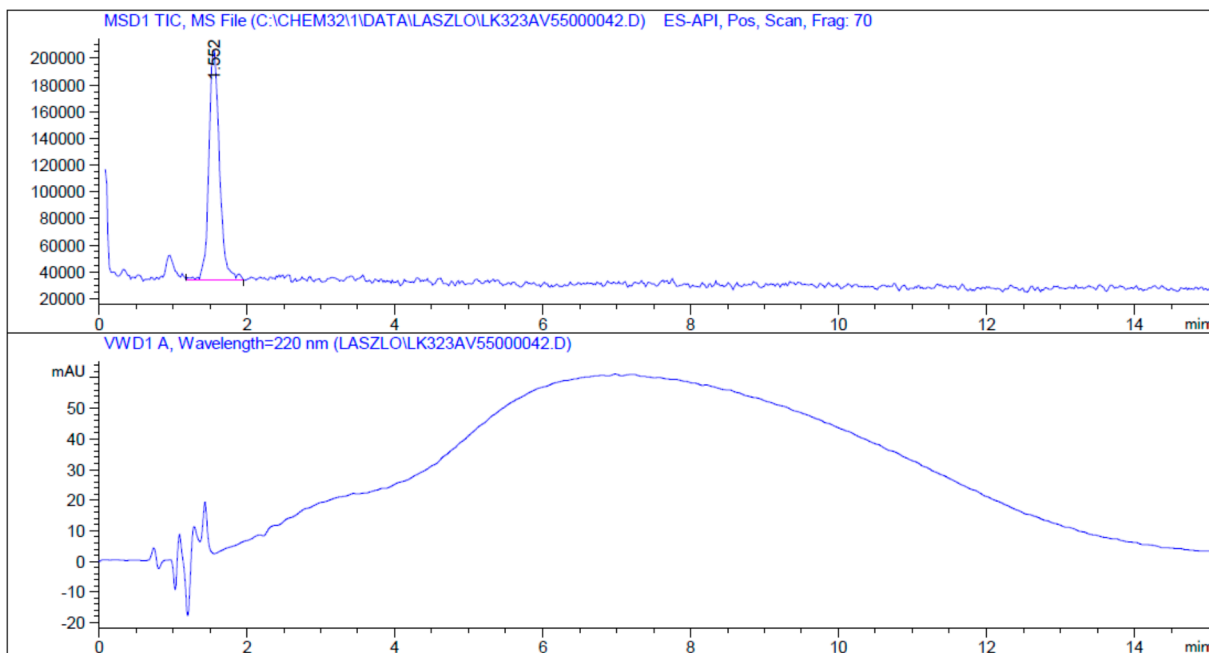
3-[[[(1S)-1-[[[(1S)-1-(1H-1,2,3,4-tetrazol-5-yl)ethyl]carbamoyl]ethyl]carbamoyl]propanoic acid
16-LL:





SM: 7G





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 Area Percent Report
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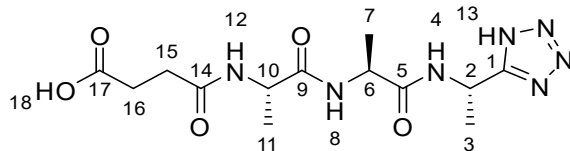
Sorted By : Signal
 Multiplier: : 1.0000
 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

Signal 1: MSD1 TIC, MS File

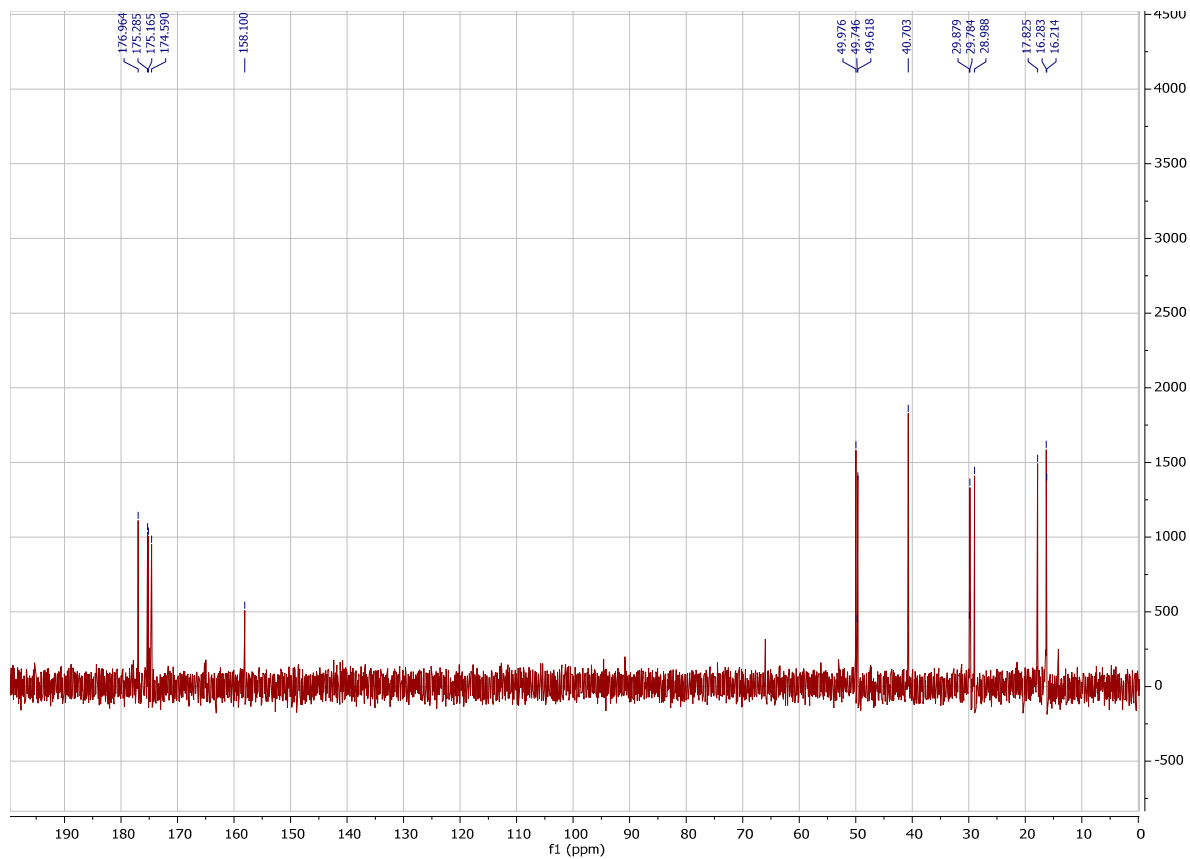
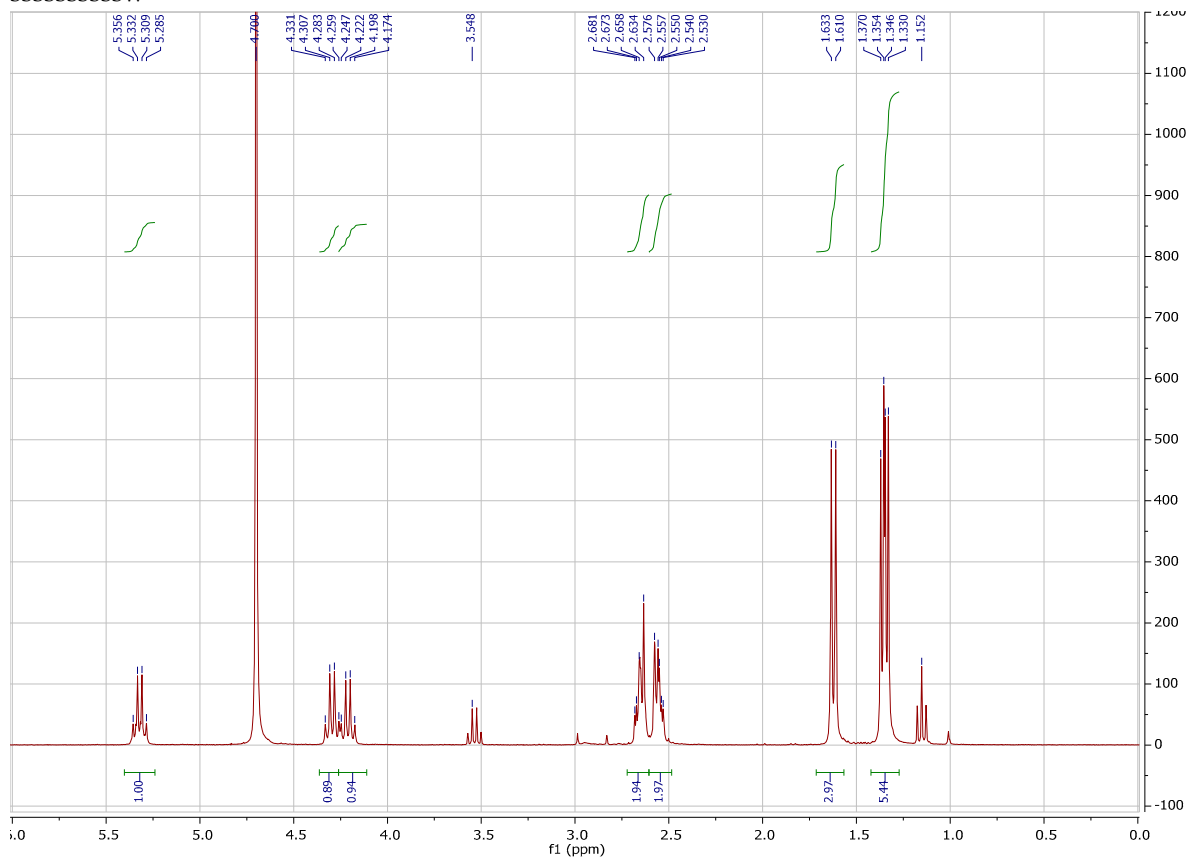
Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	1.552	BB	0.1551	1.73583e6	1.73335e5	100.0000

Totals : 1.73583e6 1.73335e5

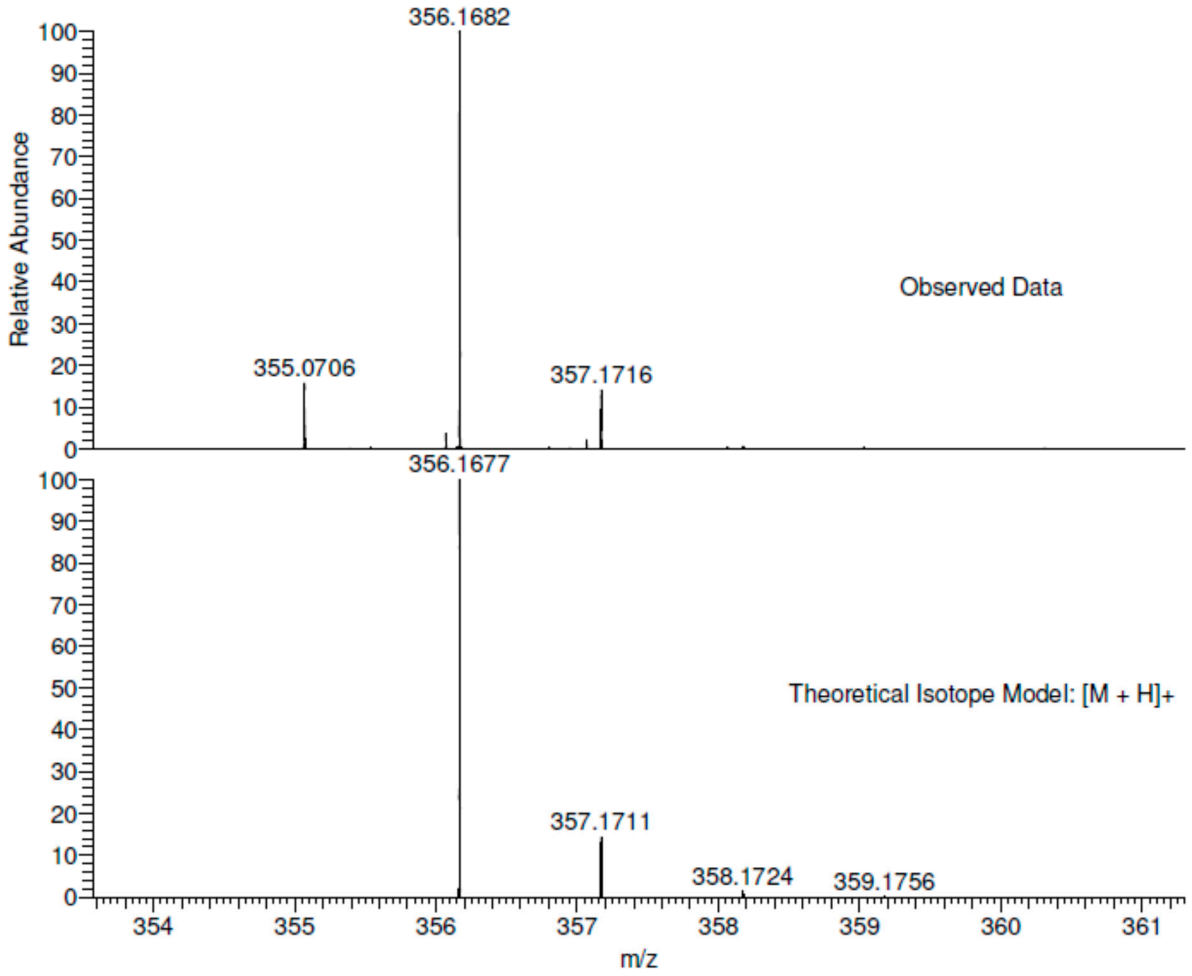
3-[[[(1S)-1-[[[(1S)-1-[[[(1S)-1-(1H-1,2,3,4-tetrazol-5-yl)ethyl]carbamoyl]ethyl]carbamoyl]ethyl]carbamoyl]propanoic acid **17-LLL**:

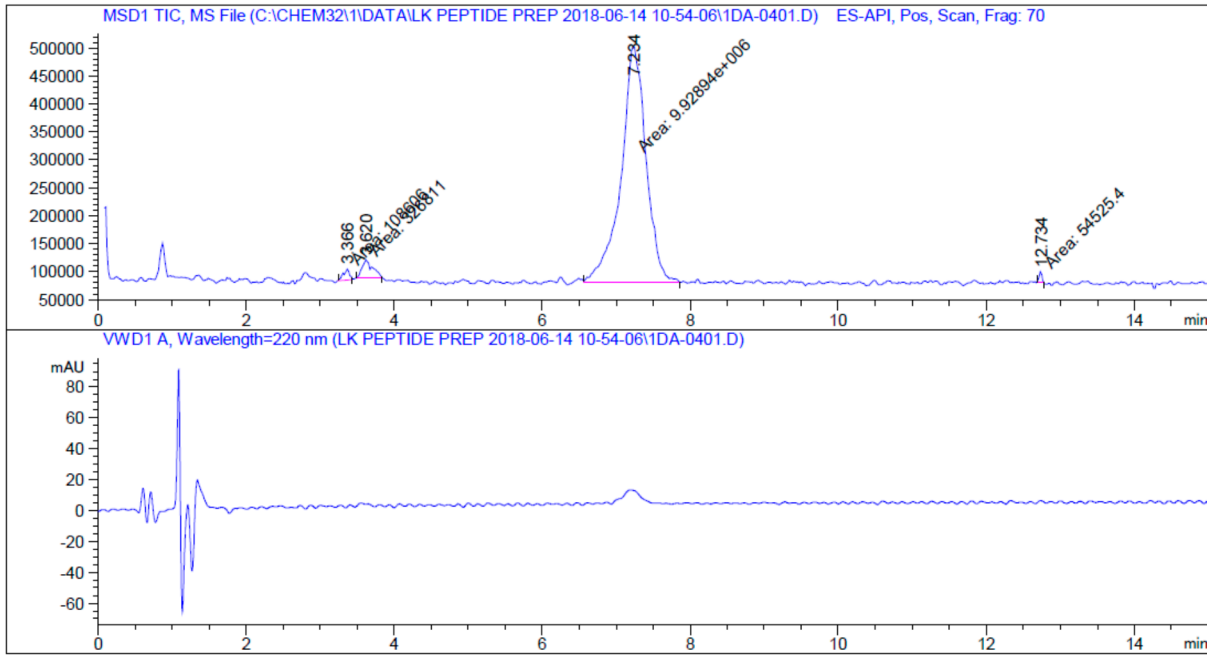


SSSSSSSSSSW



SM: 7G





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 Area Percent Report
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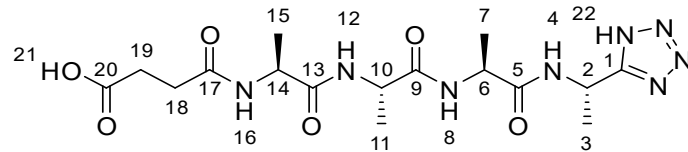
Sorted By : Signal
 Multiplier: : 1.0000
 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

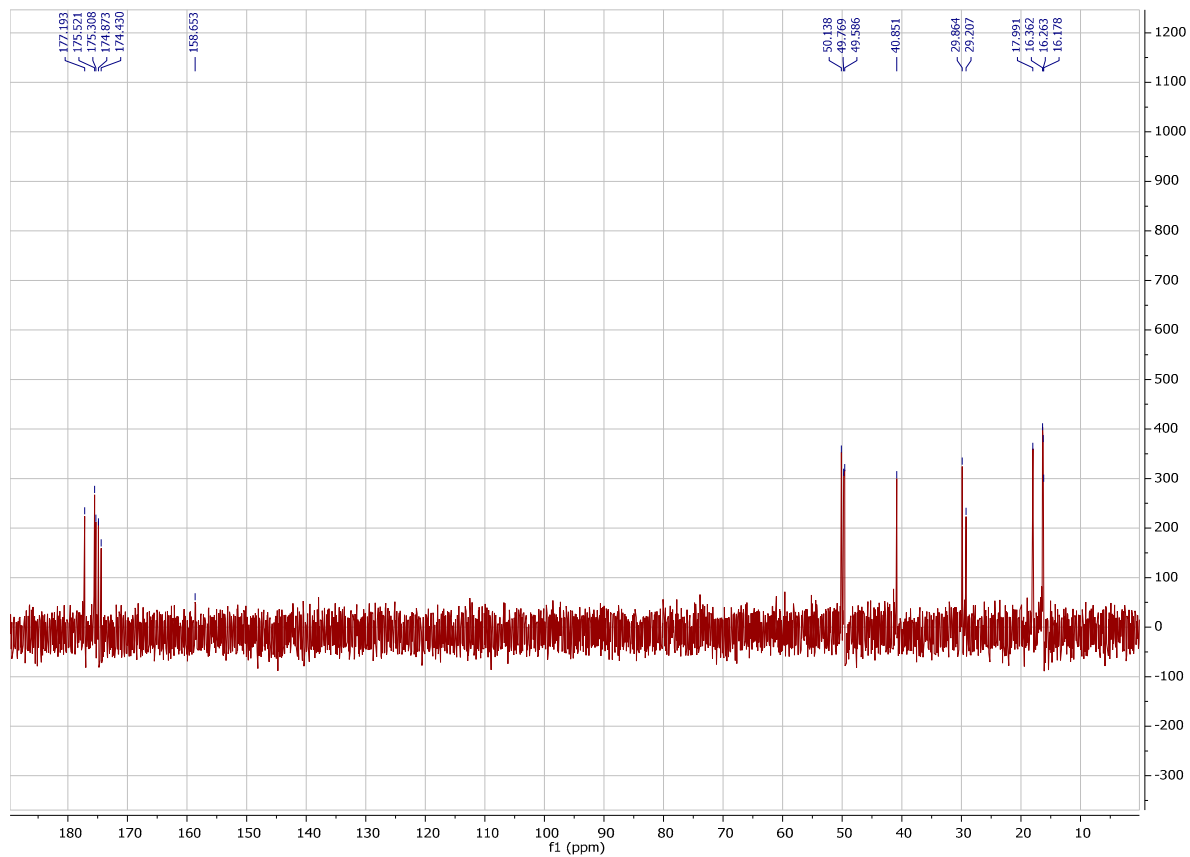
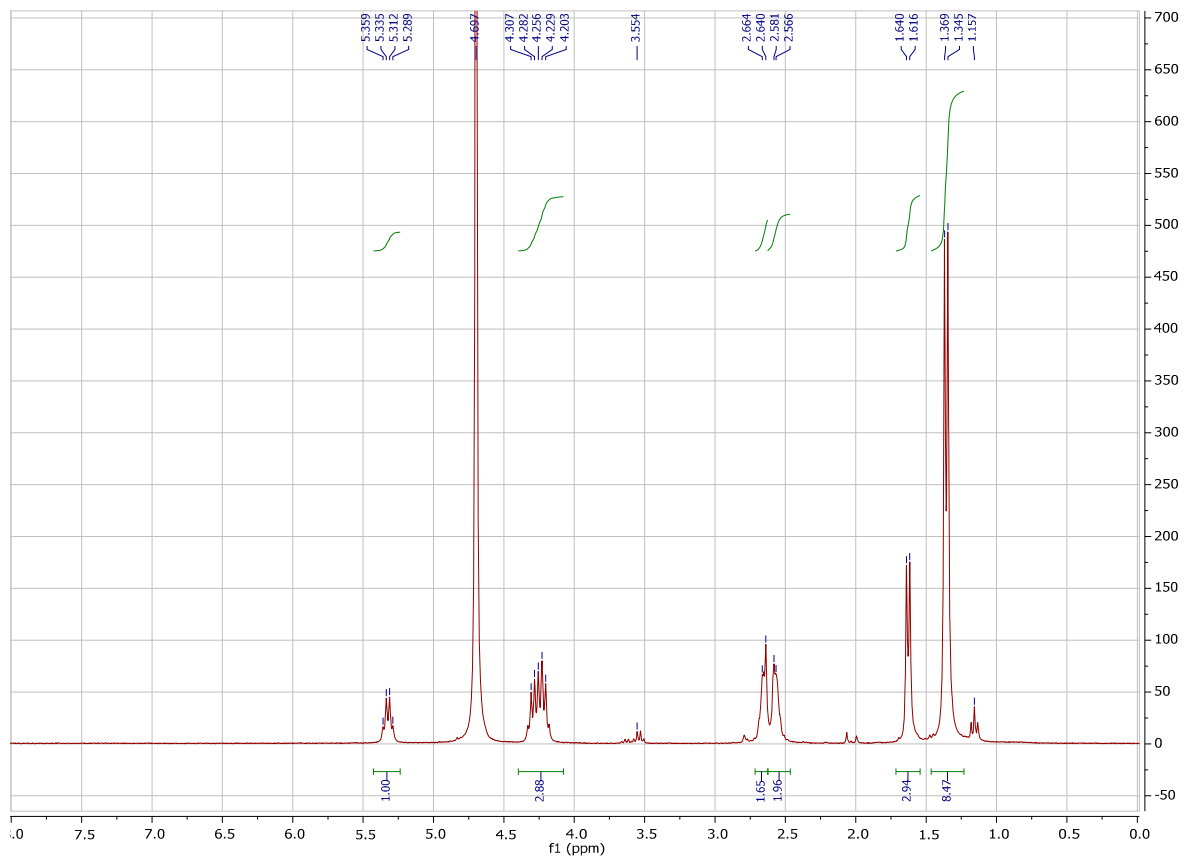
Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	3.366	MM	0.0884	1.08606e5	2.04785e4	1.0424
2	3.620	MM	0.1675	3.26811e5	3.25102e4	3.1367
3	7.234	MM	0.3902	9.92894e6	4.24108e5	95.2976
4	12.734	MM	0.0456	5.45254e4	1.99175e4	0.5233

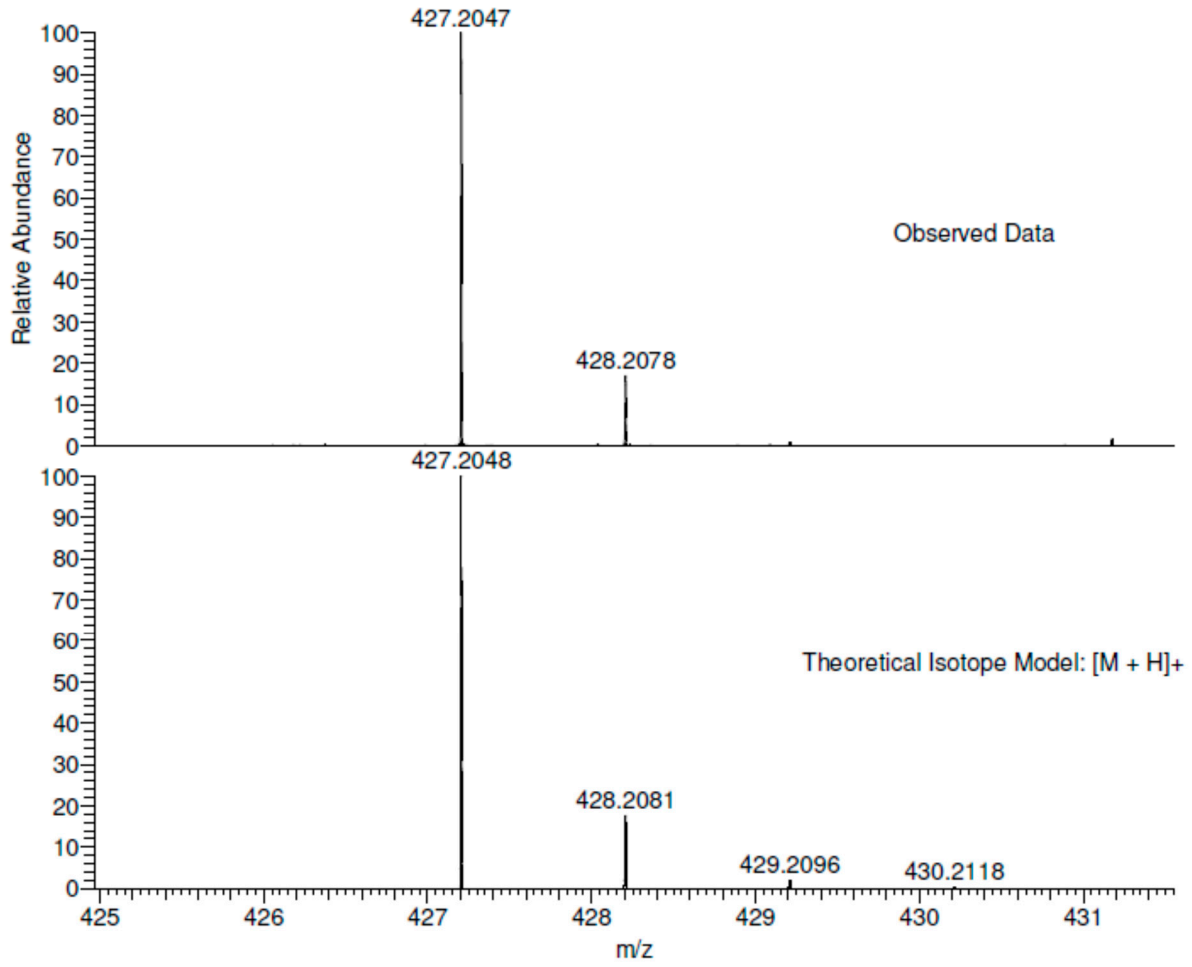
Totals : 1.04189e7 4.97014e5

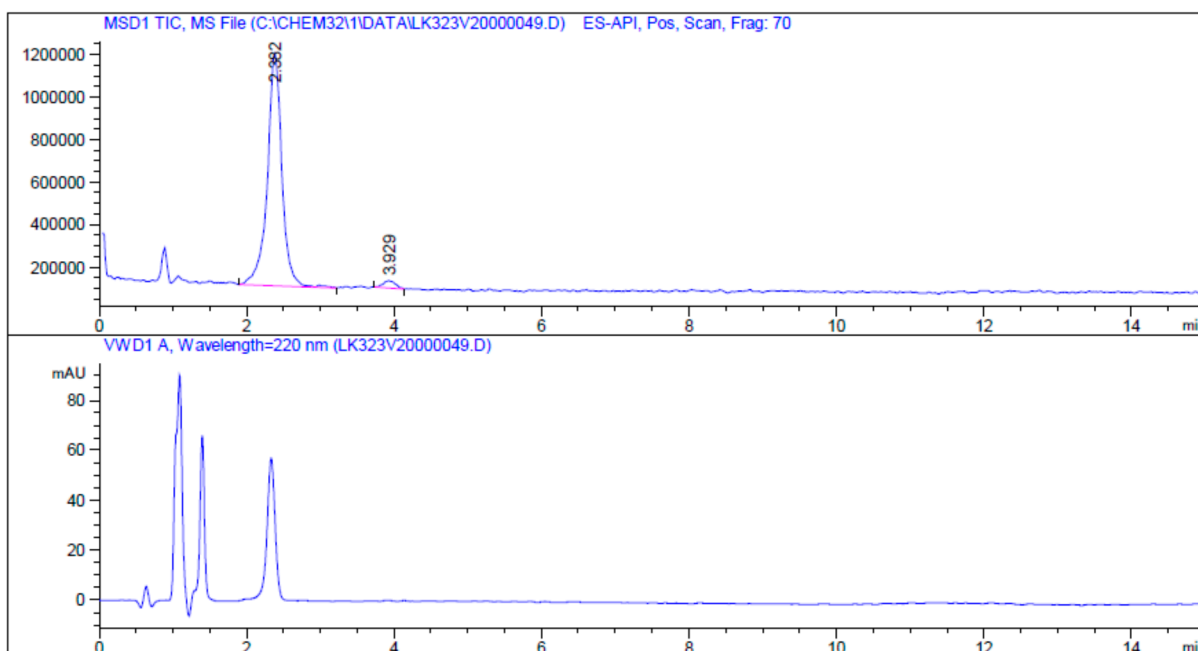
3-[[[(1S)-1-[[[(1S)-1-[[[(1S)-1-((1H-1,2,3,4-tetrazol-5-yl)ethyl]carbamoyl]ethyl]carbamoyl]ethyl]carbamoyl]propanoic acid **18-LLLL**:





SM: 7G





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 Area Percent Report
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Sorted By : Signal
 Multiplier: : 1.0000
 Dilution: : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	2.382	BB	0.2094	1.50151e7	1.09338e6	97.3823
2	3.929	BB	0.1539	4.03623e5	3.56642e4	2.6177

Totals : 1.54188e7 1.12904e6

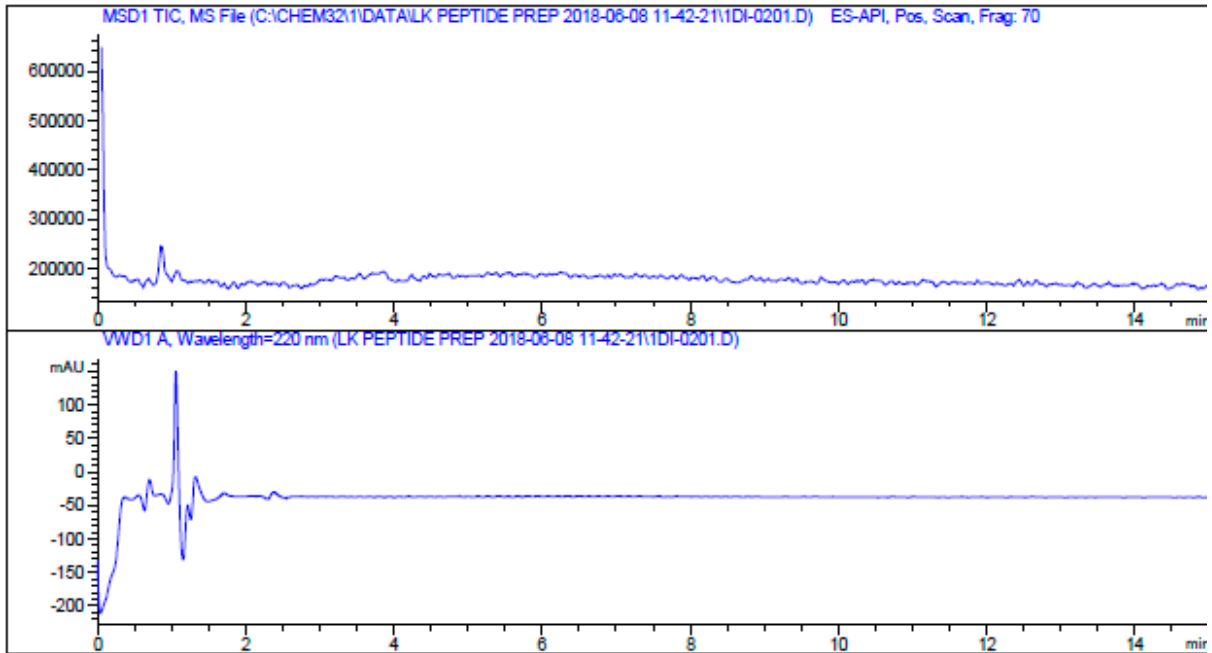
Microbiology

All MICs were determined using an agar dilution method.¹⁶ This necessitated the use of a defined antagonist-free medium (peptone-free), prepared as previously described with the inclusion of 2% saponin-lysed horse blood, 25 µg/ml NAD and 25 µg/ml haemin.⁴⁶ Test compounds were dissolved in sterile deionised water and incorporated into the antagonist-free medium at a final concentration range of 1 – 128 mg/l. All bacteria were prepared from fresh (18 h) subcultures on blood agar. Each isolate was suspended in sterile deionised water to a density equivalent to 0.5 McFarland units using a densitometer (approx. 1.5×10^8 CFU/ml) and then diluted 1 in 15. A 1 µl aliquot of each diluted suspension was then delivered onto plates with a multipoint inoculator to give a final inoculum of approximately 10 000 or 150 000 CFU/spot as recommended.¹⁶ All plates (including antimicrobial-free

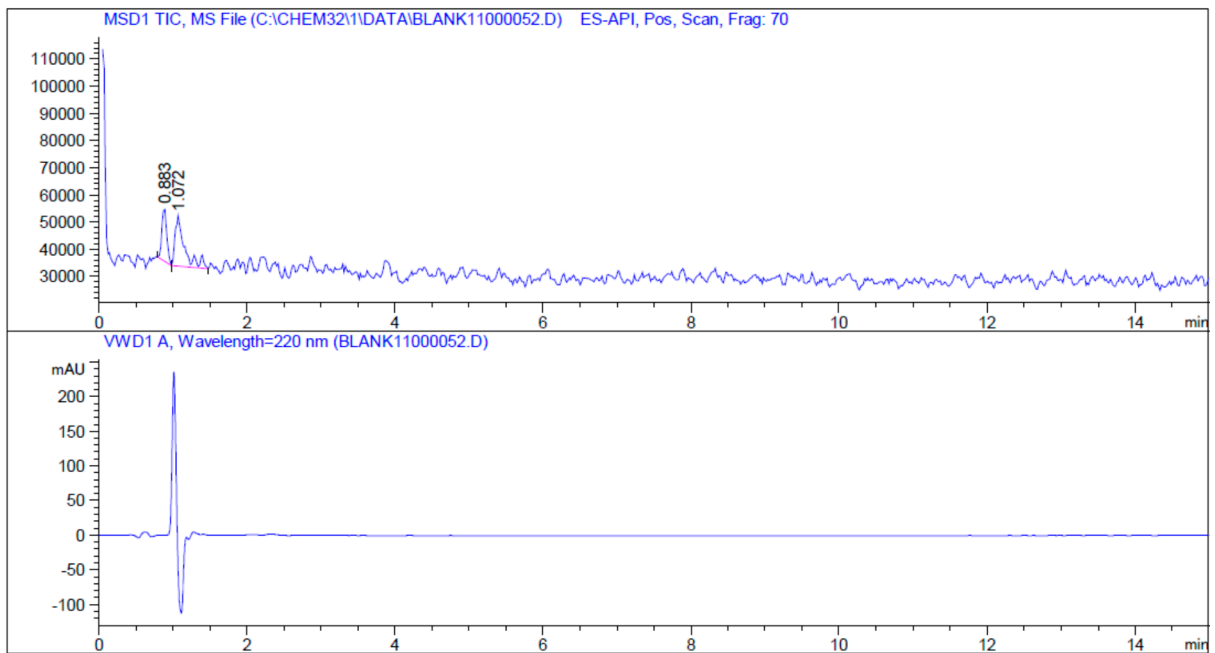
controls) were incubated for 22 h at 37°C. The minimum inhibitory concentration (MIC) was recorded as the lowest concentration of test compound that resulted in complete inhibition of bacterial growth.

HPLC blanks

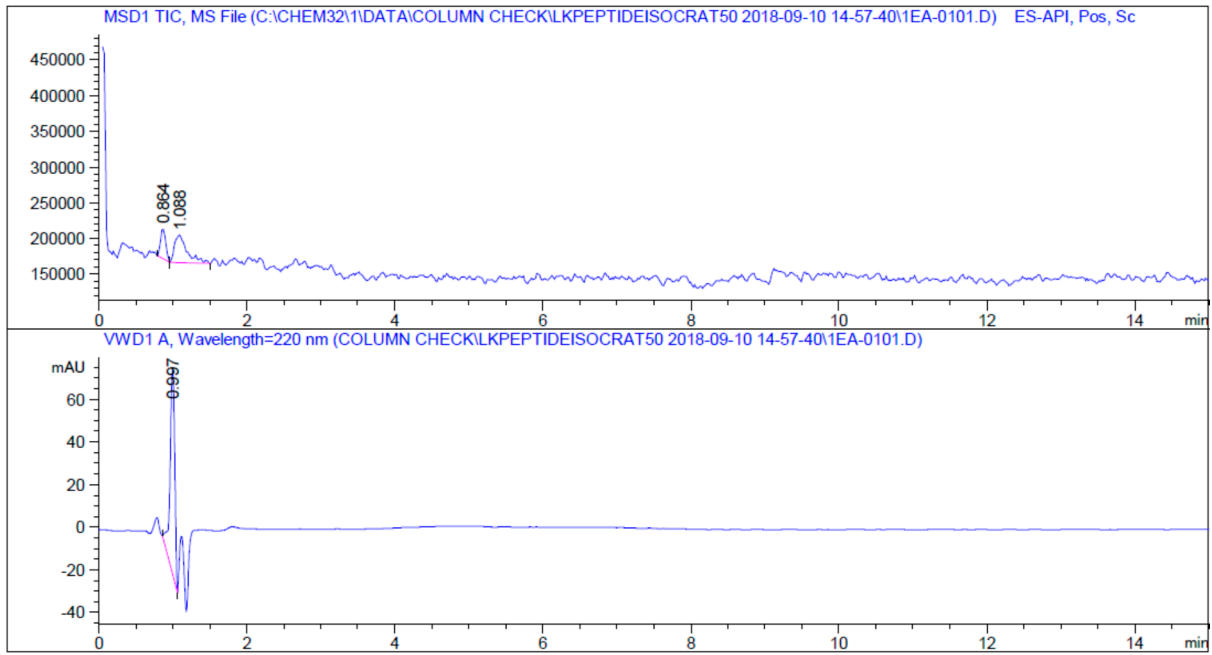
HPLC method A



HPLC method B



HPLC method C



HPLC method D

