Supporting Information

Self-Masked Aldehyde Inhibitors: A Novel Strategy for Inhibiting Cysteine Proteases

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Table S1. Kinetic parameters obtained from rapid dilution assays

Compound	k_{obs} ($\times 10^{-4}$ s^{-1})	Residence time τ (s)[a]	Estimated k_4 ($\times 10^{-4}$ s^{-1})[b]
1	3.18 ± 0.24	3141	2.89
2	30.2 ± 1.9	331	27.5
6	67.4 ± 4.8	148	61.3
7	54.3 ± 3.5	184	49.4
8	62.4 ± 2.5	160	56.8
9	32.1 ± 1.3	312	29.2
10	53.1 ± 5.7	188	48.3
11	3.71 ± 0.21	2695	3.37
12	6.33 ± 0.42	1580	5.75

[a]Residence time $\tau = 1/k_{obs}$; [b]k_4 values were estimated from k_{obs} based on eq. 3

Table S2. The details of peaks in ^{1}H-^{13}C HSQC NMR

peak	^{1}H (ppm)	^{13}C (ppm)	Width 1	Width 2	Volume
		12 *only*			
A	5.34	90.84	192.6	31.4	40225129.1
B	5.13	90.80	192.59	26.24	52937692.9
		12 *+ cruzain*			
A'	6.13	76.25	137.58	49.49	5030193.12
B'	5.91	79.69	165.06	63.22	6245466.52
A (residual)	5.33	90.81	192.6	40.23	1725522.38

Table S3. Anti-CoV-2 activity of **18** on SARS-CoV-2 infected A549/ACE2 cells[a]

Compound **18** (μM)	A549/ACE2 cells + CoV-2 infection + inhibitor	A549/ACE2 cells + CoV-2 infection + inhibitor	A549/ACE2 cells + CoV-2 infection no inhibitor	A549/ACE2 Cells no CoV-2 infection + inhibitor
20	Viable	Viable	CPE	Viable
10	Viable	Viable	CPE	Viable
5	CPE	Viable	CPE	Viable
2.5	CPE	CPE	CPE	Viable
1.25	CPE	CPE	CPE	Viable
0.625	CPE	CPE	CPE	Viable
0.313	CPE	CPE	CPE	Viable
0.156	CPE	CPE	CPE	Viable

[a]Viable cells or CPE: microscopic examination of treated cells indicated a majority of either fully viable cells, or a majority of cells which exhibited SARS-CoV-2-induced cytopathic effect (CPE).

Table S4. Statistic summary of co-crystal structure of $3CL^{pro}$ complexed with **18**.

	$3CL^{pro}$-**18**
Data collection	
Space group	I 1 2 1
Cell dimensions	
a, b, c (Å)	54.20, 80.74, 85.76
α, β, γ (°)	90.00, 97.12, 90.00
Resolution (Å)	48.25-1.70 (1.76-1.70)
R_{merge}	0.084 (1.632)
$<I/\sigma I>$	1.18 (at 1.70Å)
$CC_{1/2}$	0.996 (0.475)
Completeness (%)	99.8 (99.3)
Redundancy	6.4 (5.6)
Refinement	
Resolution (Å)	48.25-1.70
No. Reflections	40267 (4001)
R_{work}/R_{free}	0.186/0.206
RMSD in bond lengths (Å)	0.008
RMSD in bond angles (°)	1.17
No. atoms	
Protein	2395
Ligand	40
Water	173
B factors	
Protein	41.6
Ligand	43.4
Water	44.9

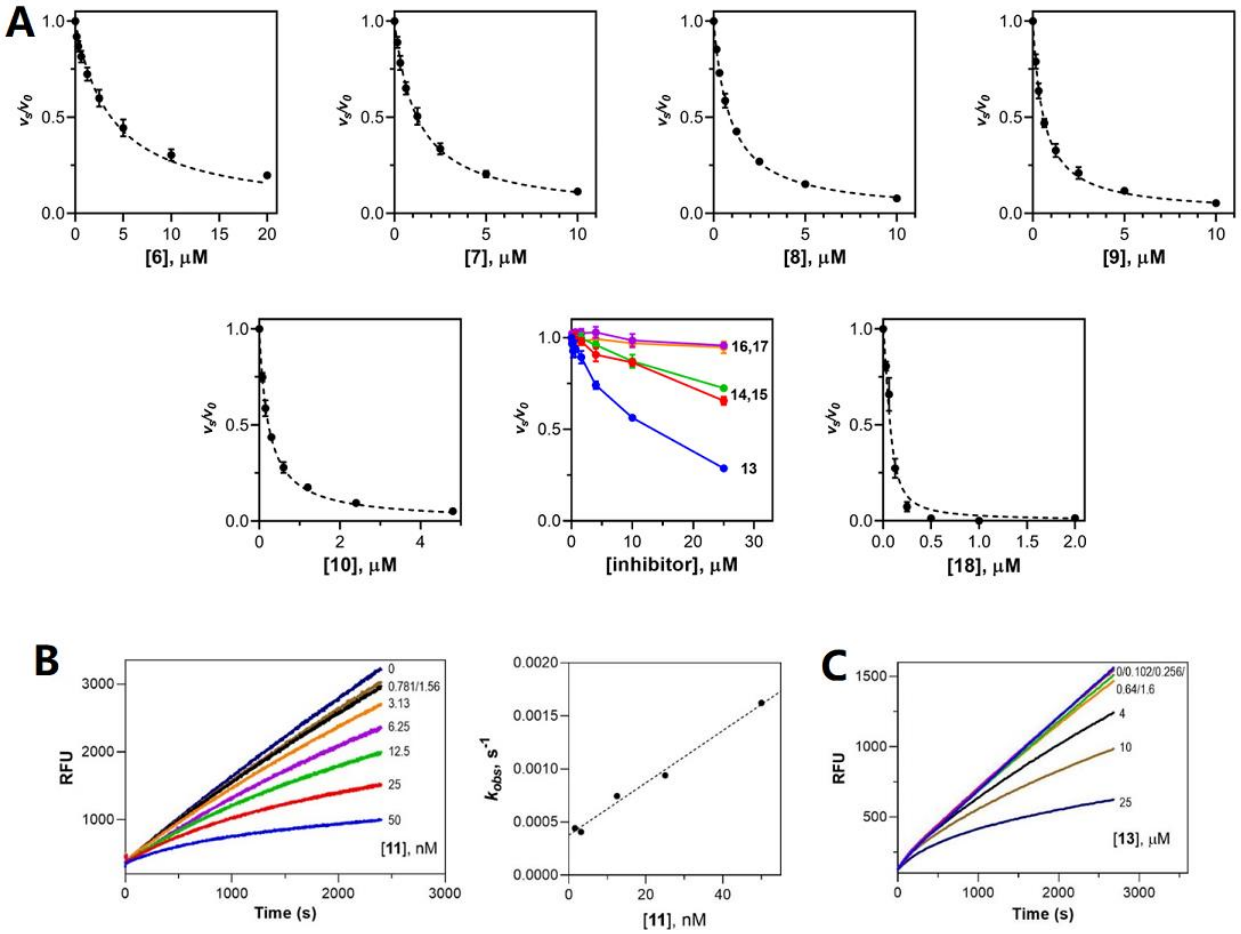

Figure S1. Kinetic data of cruzain inhibition by **6** – **11** and **13** – **17**, and $3CL^{pro}$ inhibition by **18**. (A) Concentration-inhibition curves for SMAIs and their prodrugs. (B) Time-courses of cruzain inhibition by free aldehyde **11**. The k_{obs} values were obtained by fitting progress curves to eq. 1. Replot of k_{obs} vs. [**11**] with the line drawn through data points from fitting to eq. 3 which afforded $k_4 = (3.8 \pm 0.4) \times 10^{-4}$ s^{-1}. (C) Time-courses of cruzain inhibition by **13**.

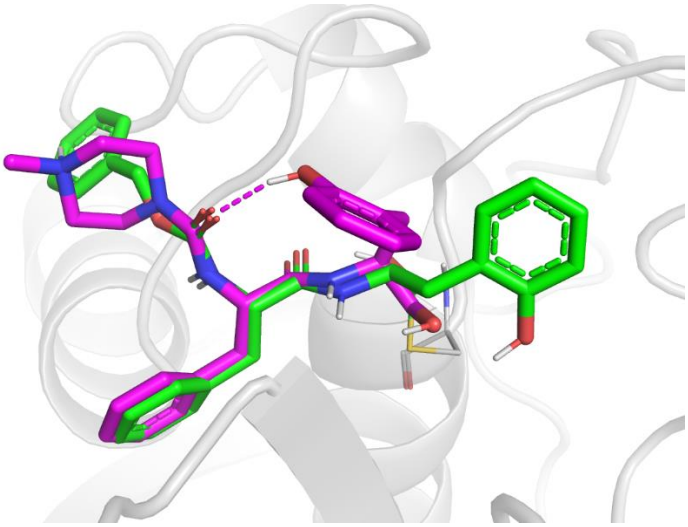

Figure S2. Compound **12** (magenta) was covalently docked to cruzain, and was superimposed with compound **2** (green). The intramolecular hydrogen bond between P_1 phenol and P_3 carbonyl group was drawn as magenta dashed lines.

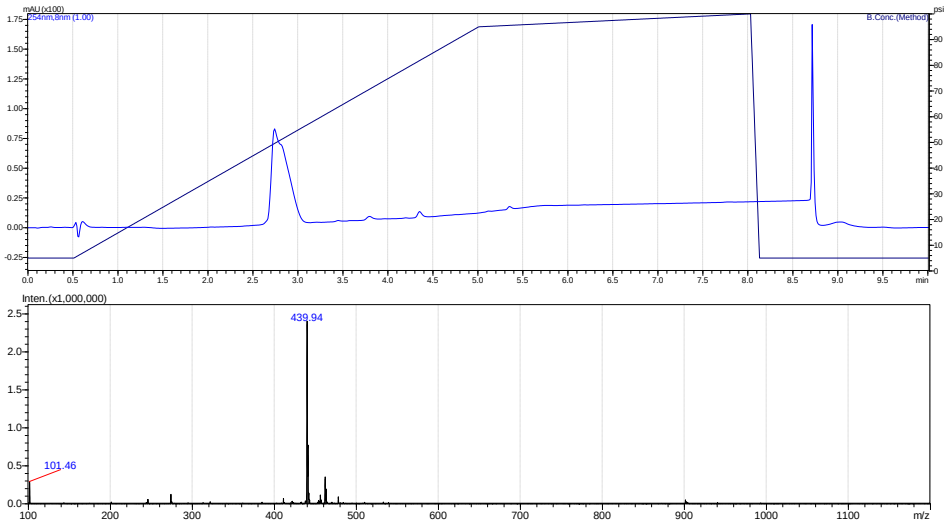

Figure S3. LC-MS of ^{13}C-labeled **12**. Calculated m/z for molecular ion is 440.24 for hemiacetal and 458.25 for hydrate.

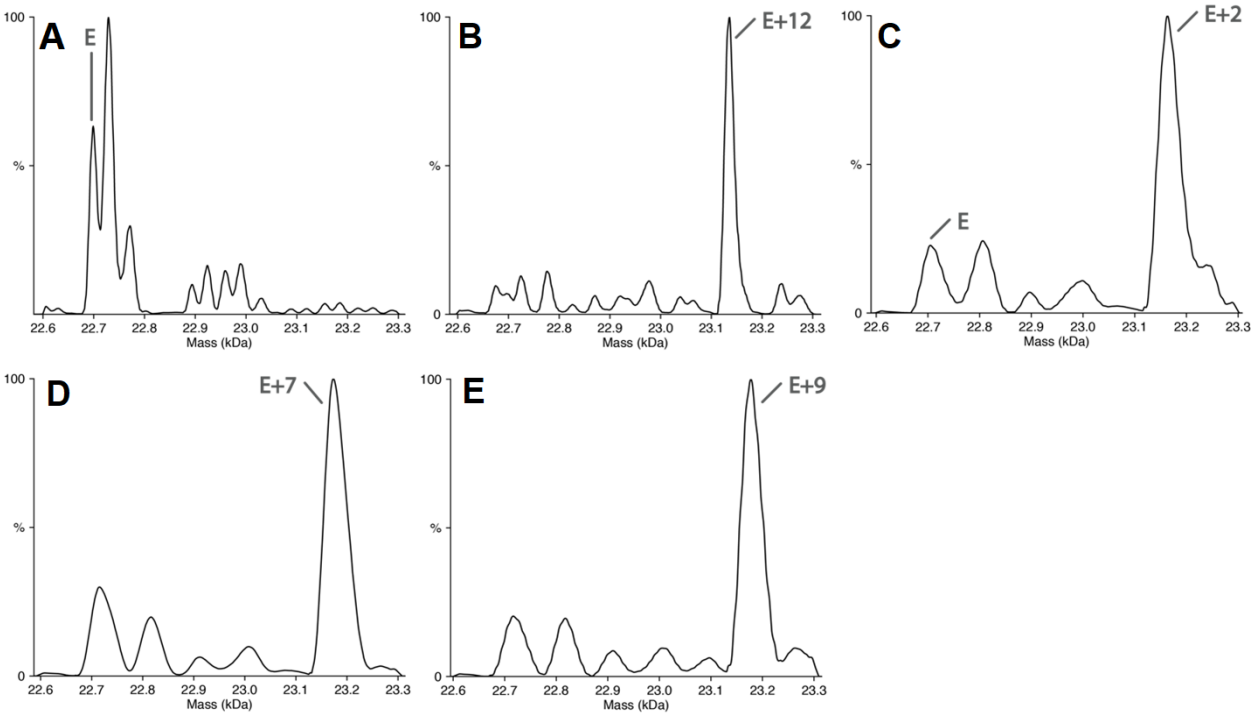

Figure S4. Native mass spectra of cruzain pre-incubated with selected SMAIs. The deconvoluted mass spectra are shown for 25 µM cruzain (A) alone or treated with 50 µM compounds (B) **12**, (C) **2**, (D) **7** and (E) **9**. The addition of compound **12** shows that compound is bound to apo-enzyme with a measured mass of 438.8 Da, consistent with the theoretical mass of **12** (438 Da).

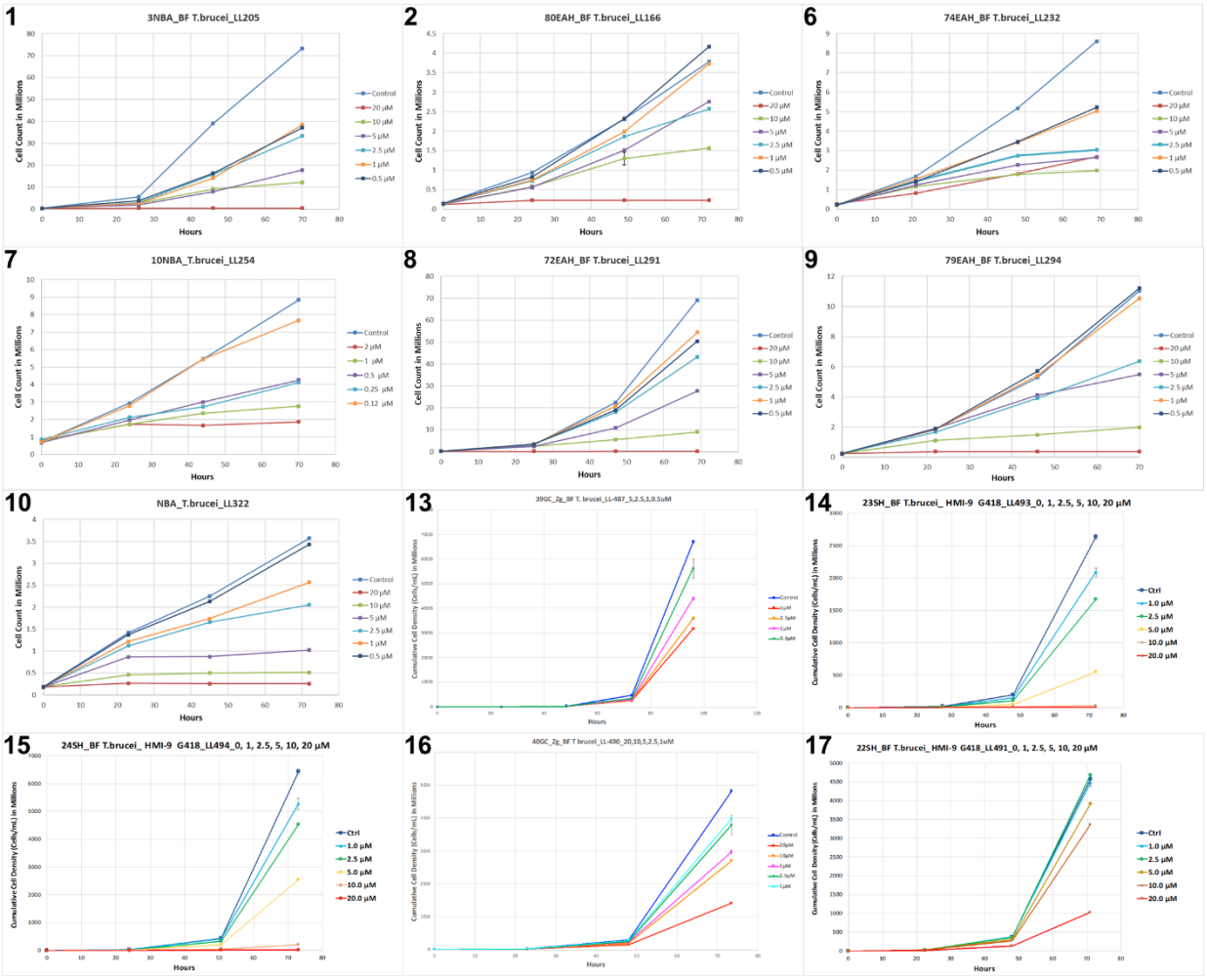

Figure S5. Growth curves for *T. b. brucei* BSFs treated with SMAIs and their prodrugs. Each diagram only represents one of the duplicate experiments.

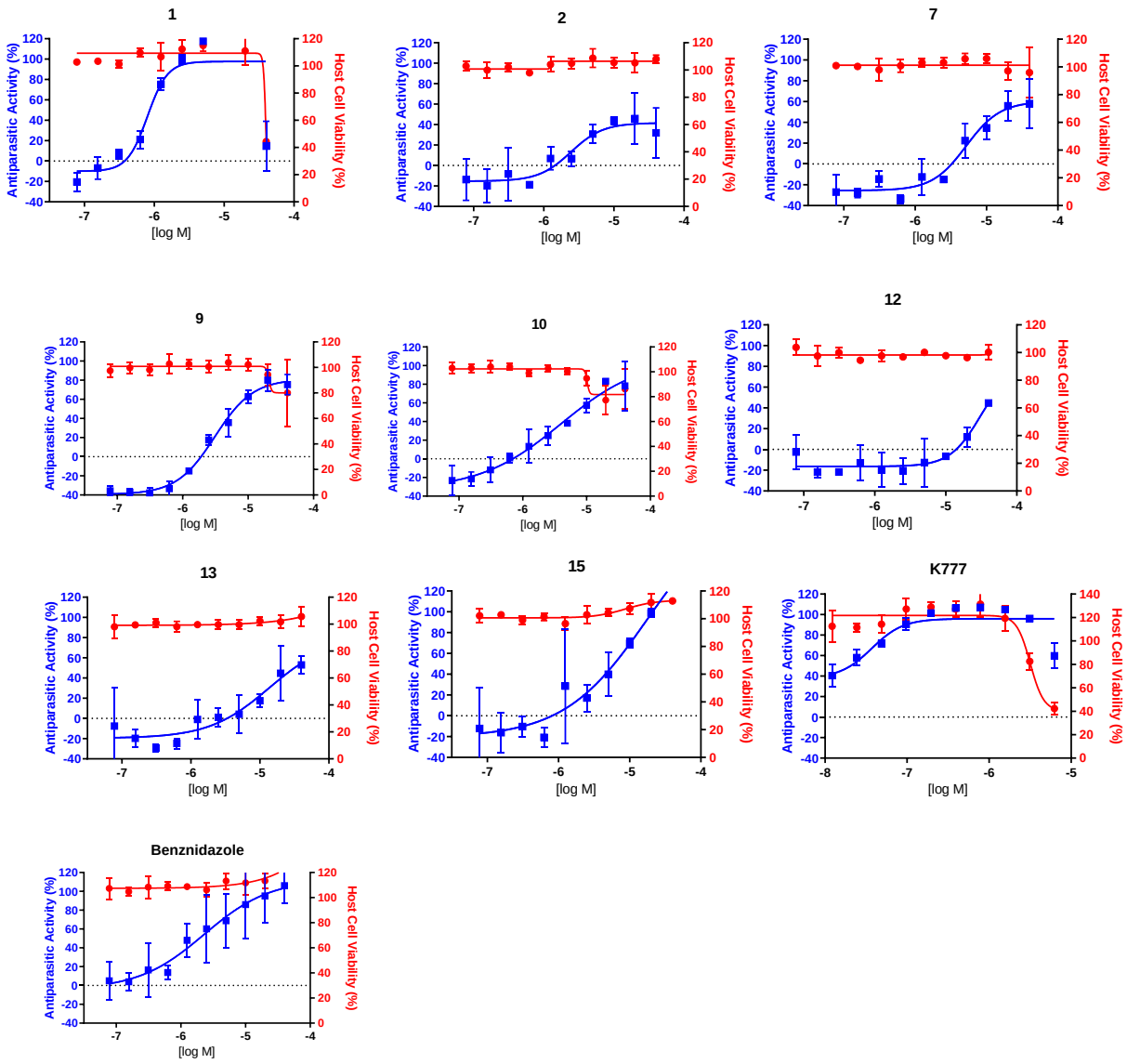

Figure S6. Cell viability of *T. cruzi*-infected murine cardiomyoblasts in presence of **1**, **2**, **7**, **9**, **10**, **12**, **13**, **15**, **K777**, and benznidazole.

NMR spectra

(Note: The presence of lactol anomers in many SMAIs led to complicated splitting/overlapping and low-quality NMR spectra; therefore, the NMR spectra of corresponding precursors (the second last products) were provided.)

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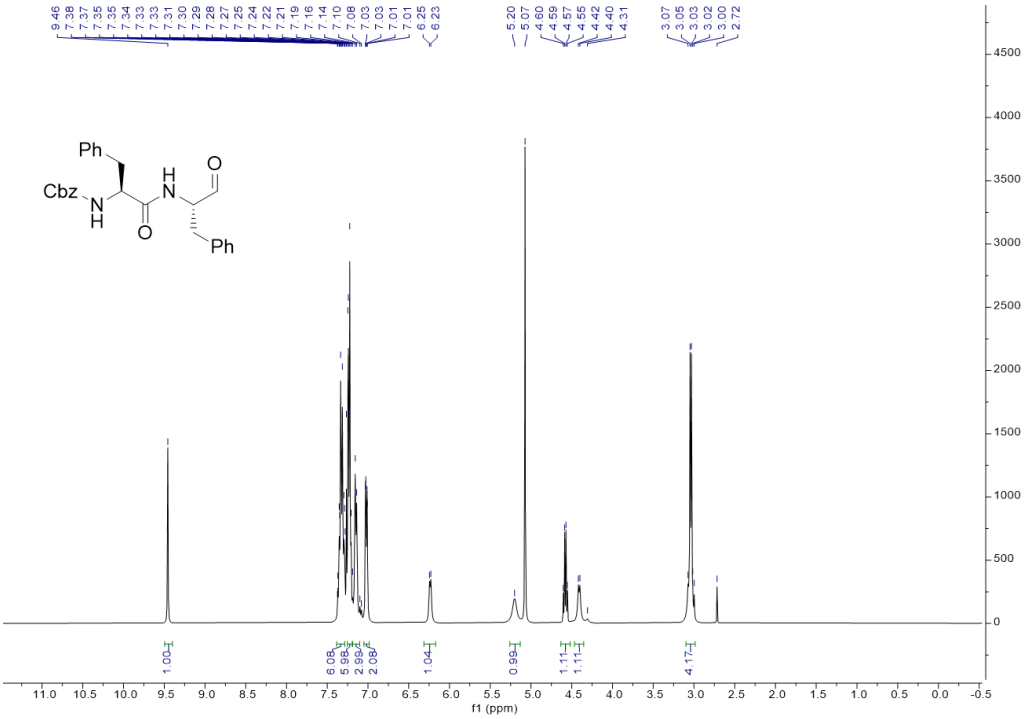

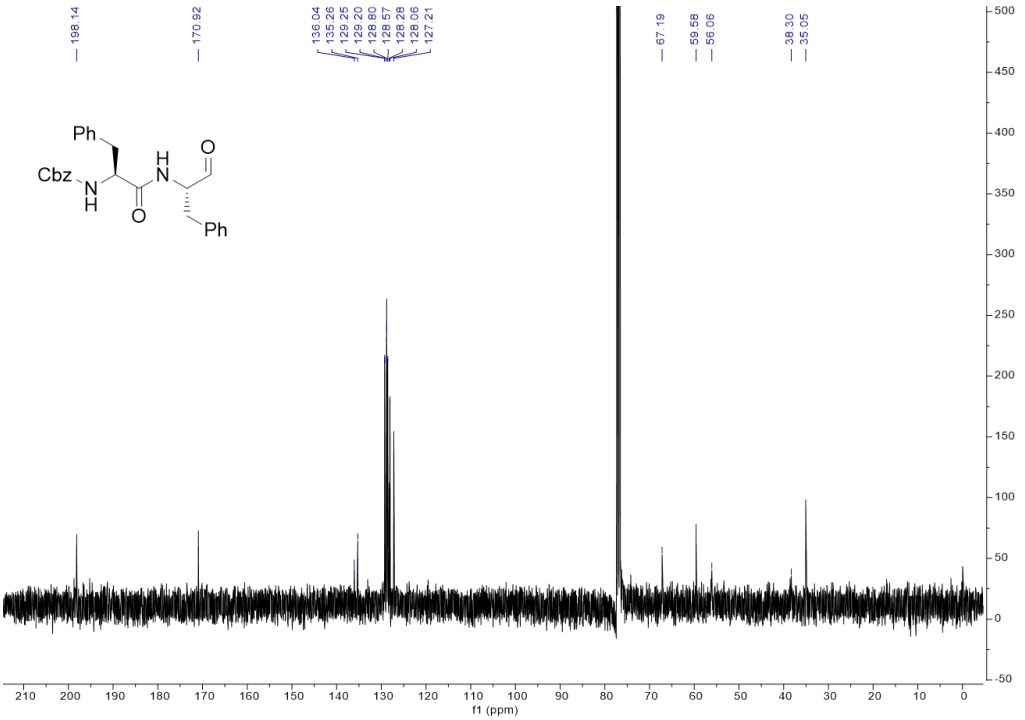

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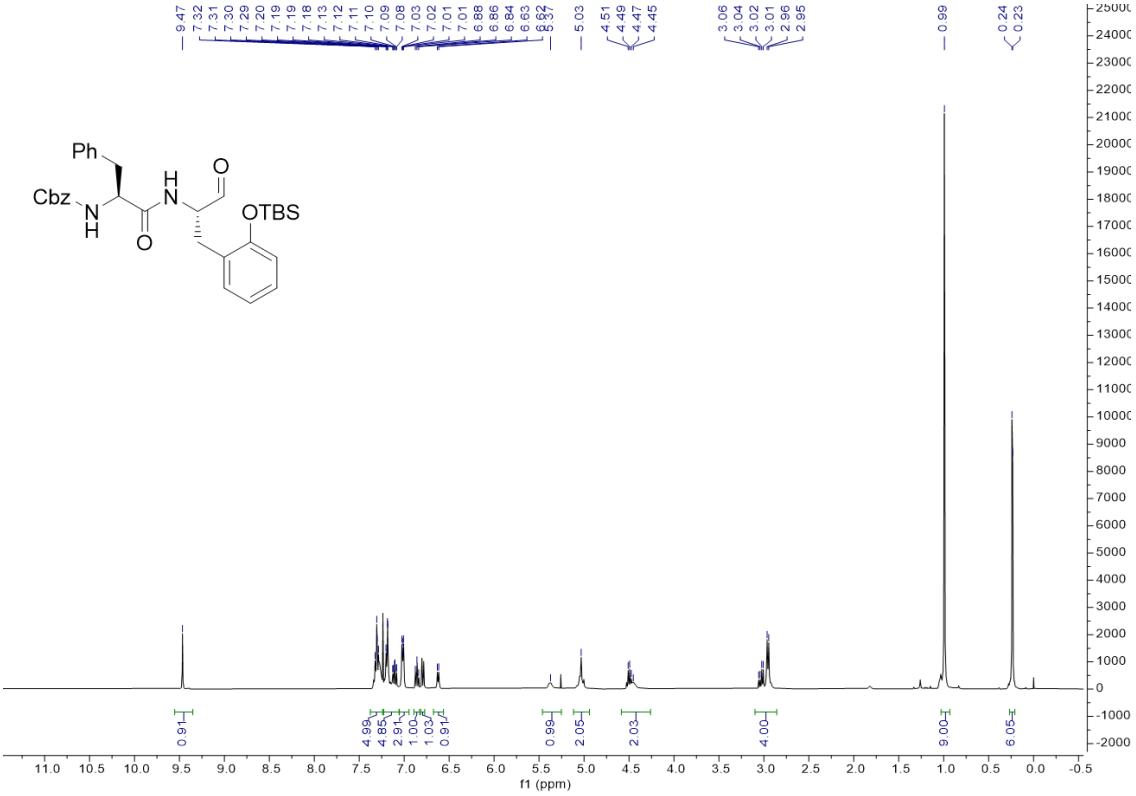
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7.11
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7.01
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6.62
5.03
4.51
4.49
4.47
4.45
3.06
3.04
3.02
3.01
2.96
2.95
0.99
0.24
0.23
Ph
Cbz
N
H
O
H
N
O
OTBS
0.91
4.99
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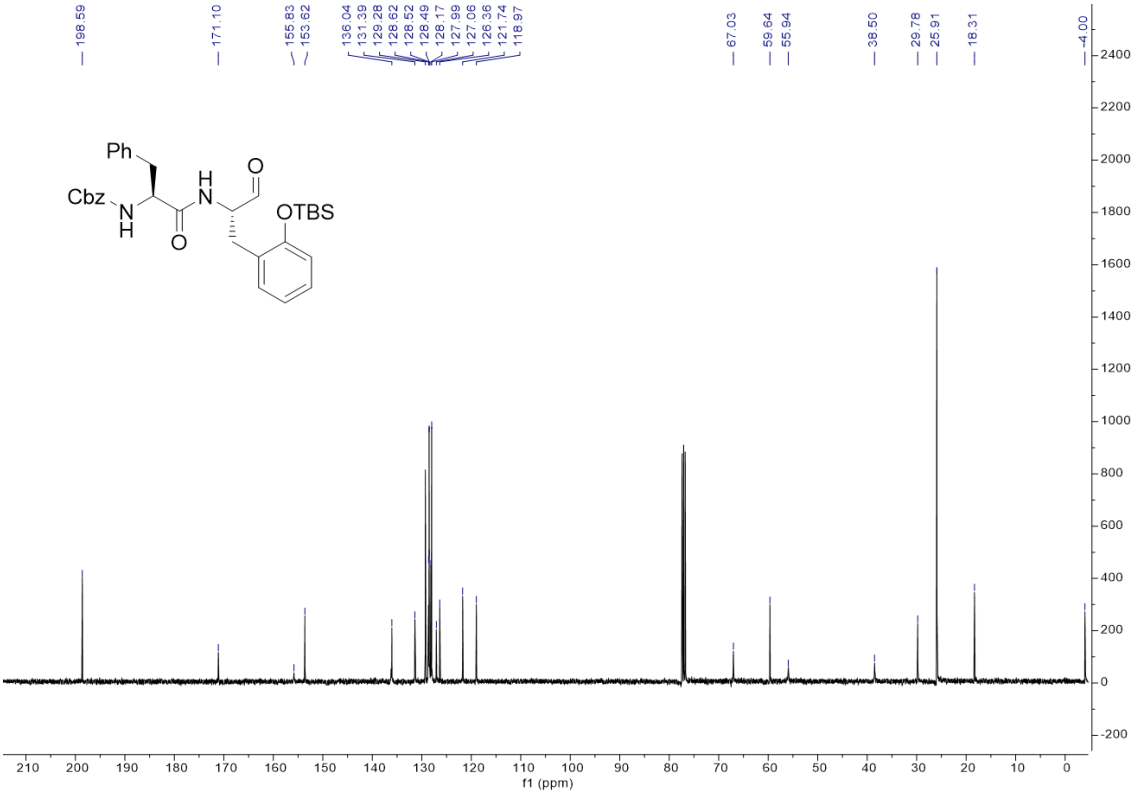
6.05
f1 (ppm)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59.64
55.94
38.50
29.78
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18.31
-4.00
Ph
Cbz
N
H
O
H
N
O
OTBS
f1 (ppm)

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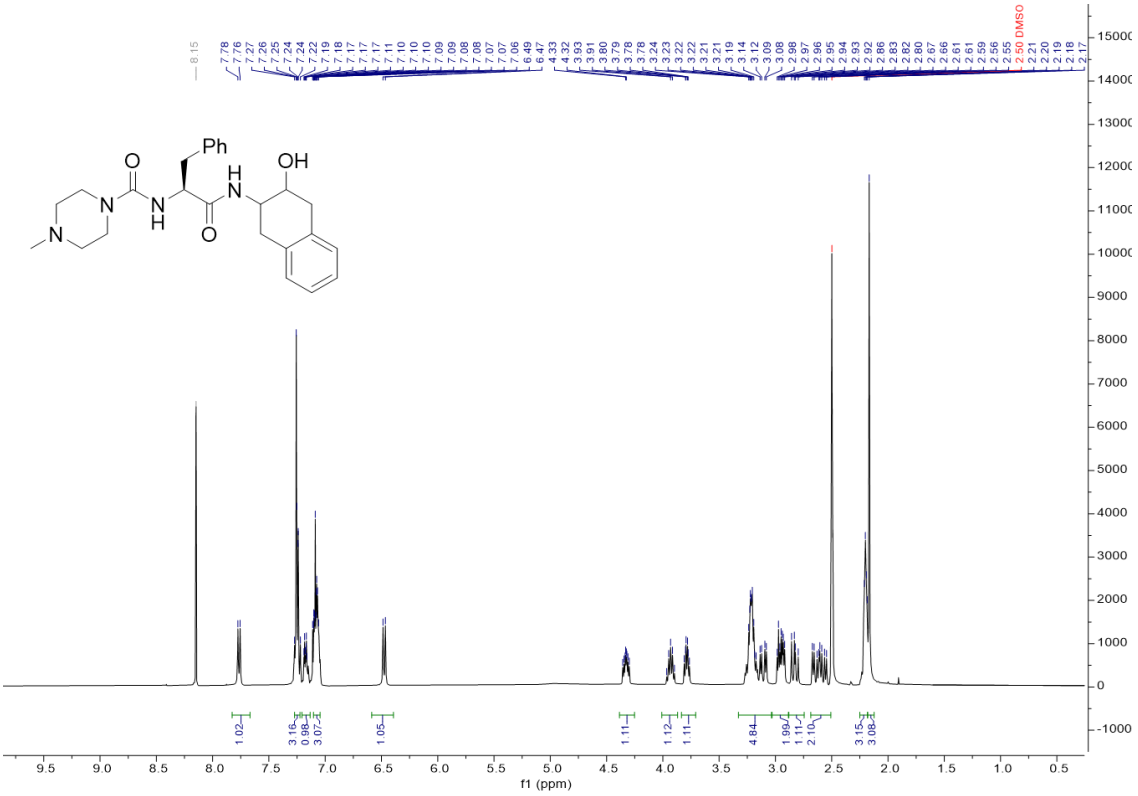

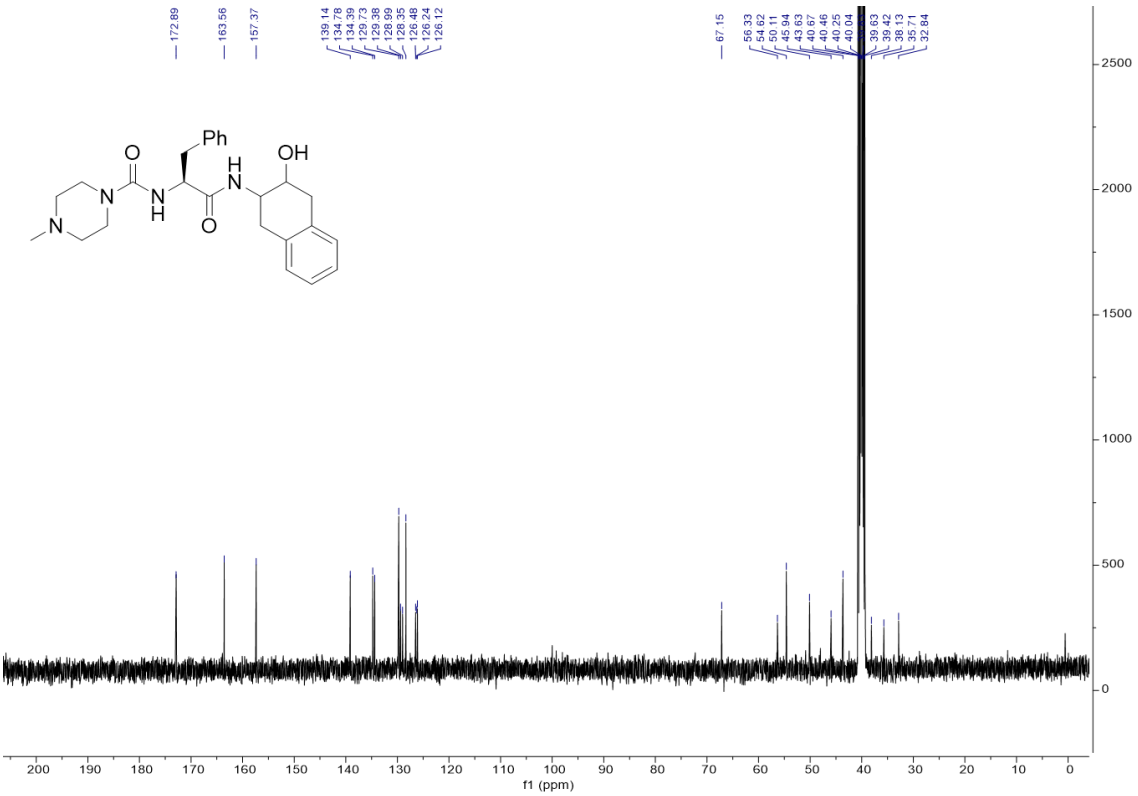

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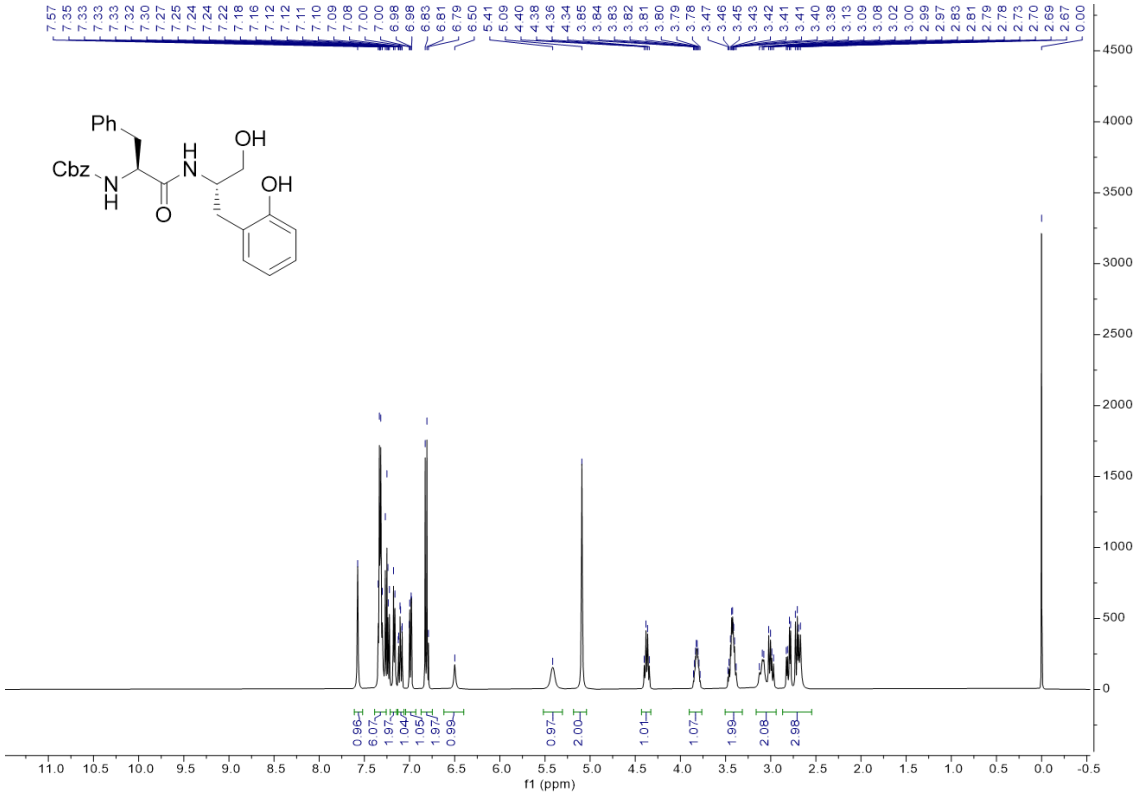

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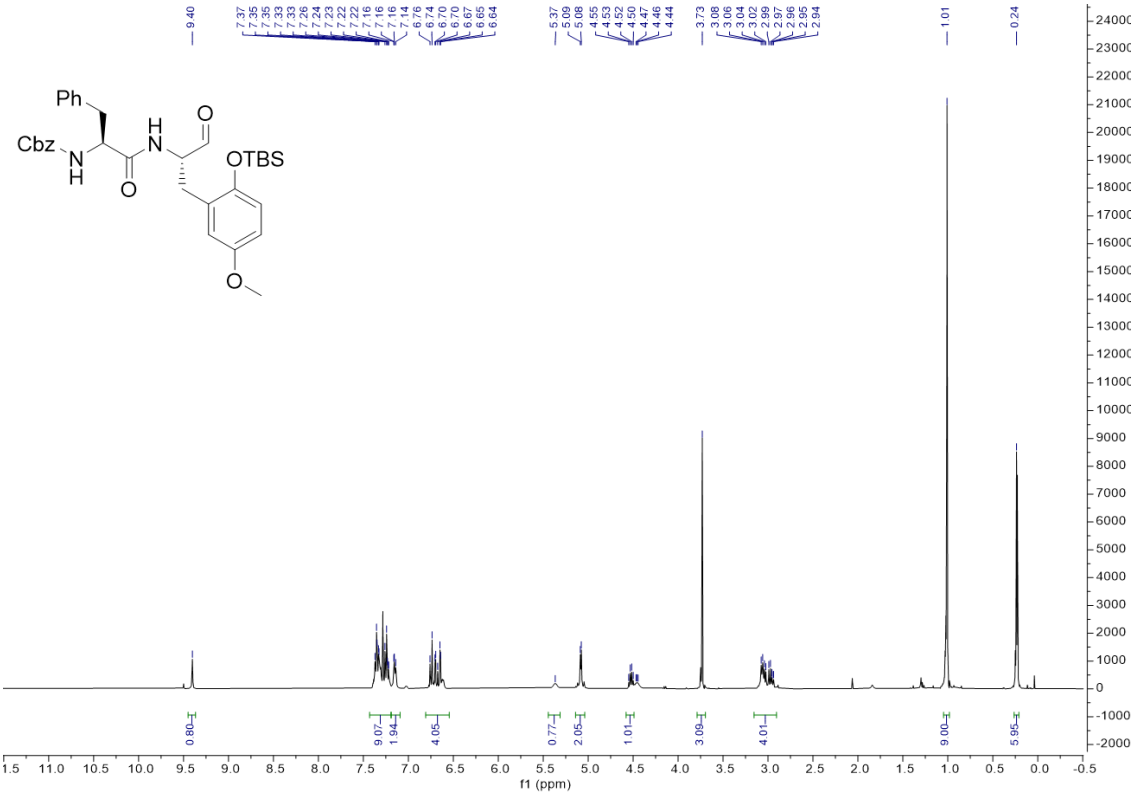

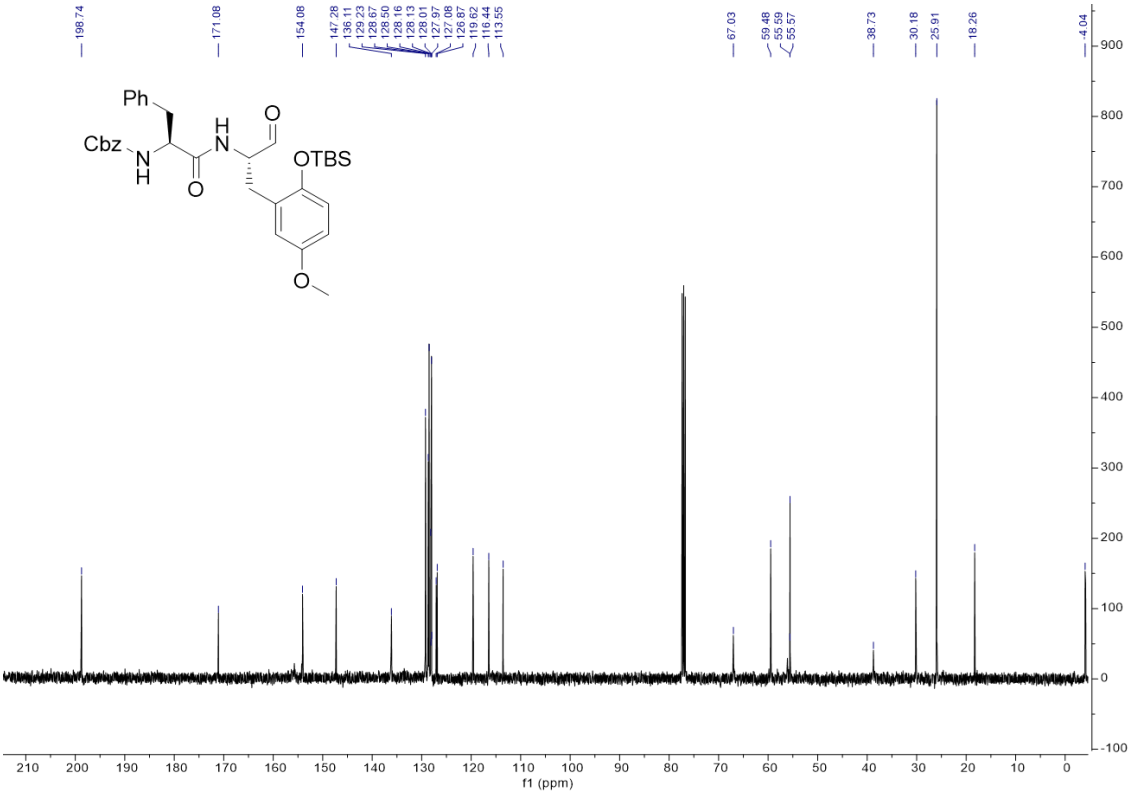

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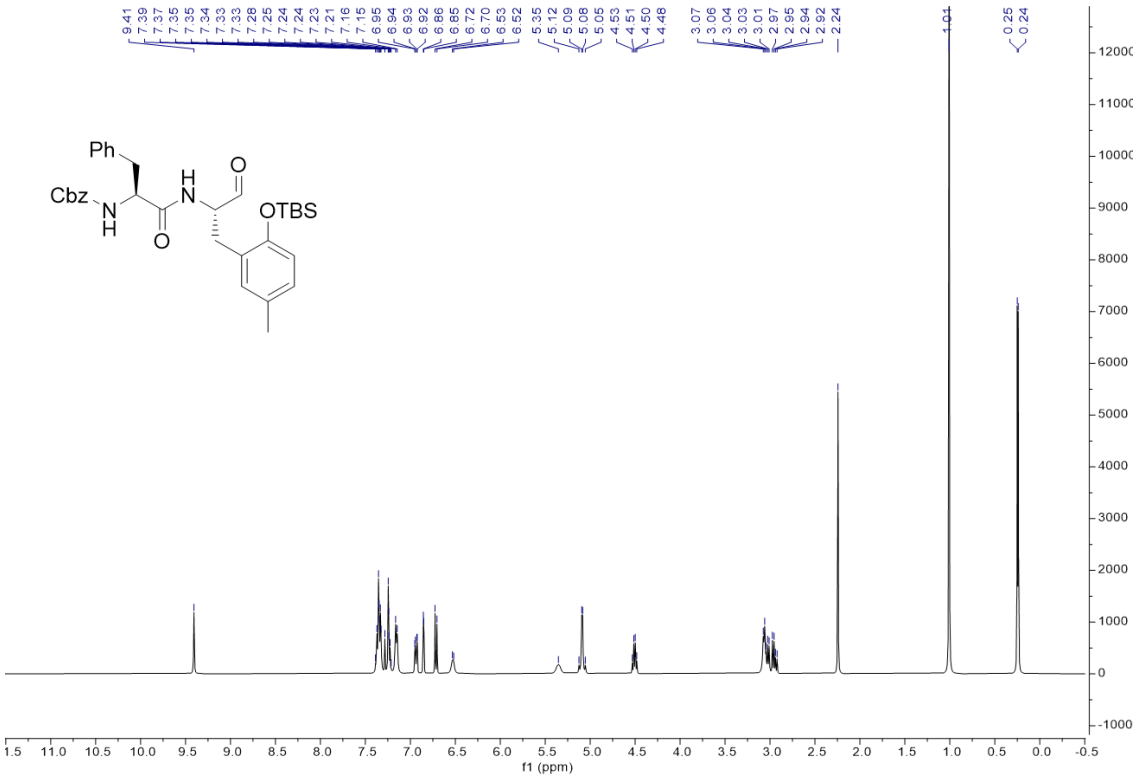
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3.04
3.03
3.01
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2.92
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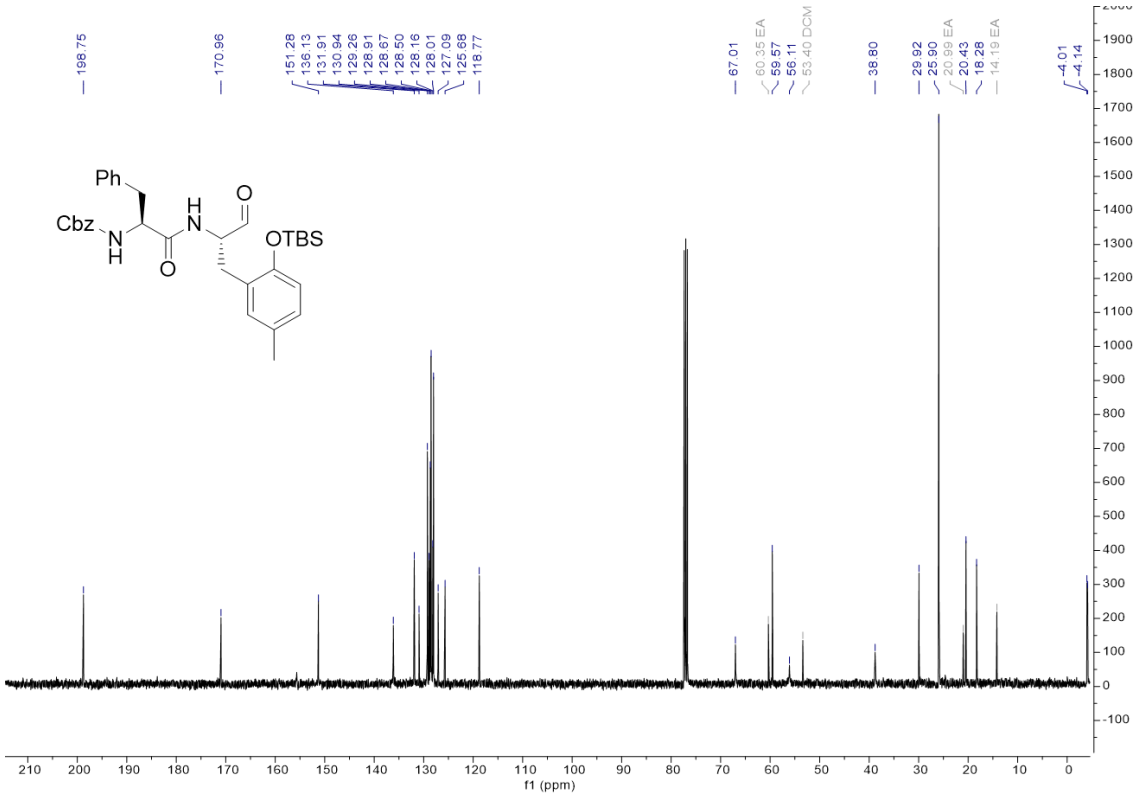
OTBS
f1 (ppm)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67.01
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59.57
56.11
53.40 DCM
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20.99 EA
20.43
18.28
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N
O
O
O
OTBS
f1 (ppm)

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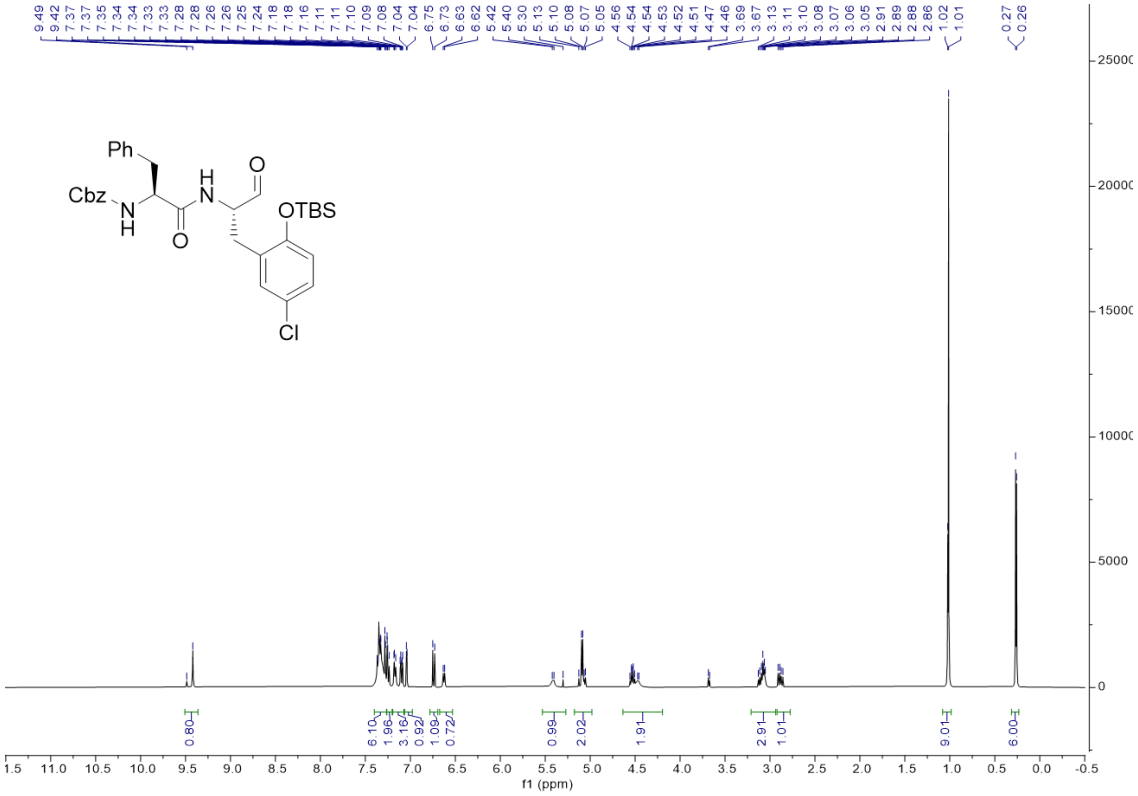

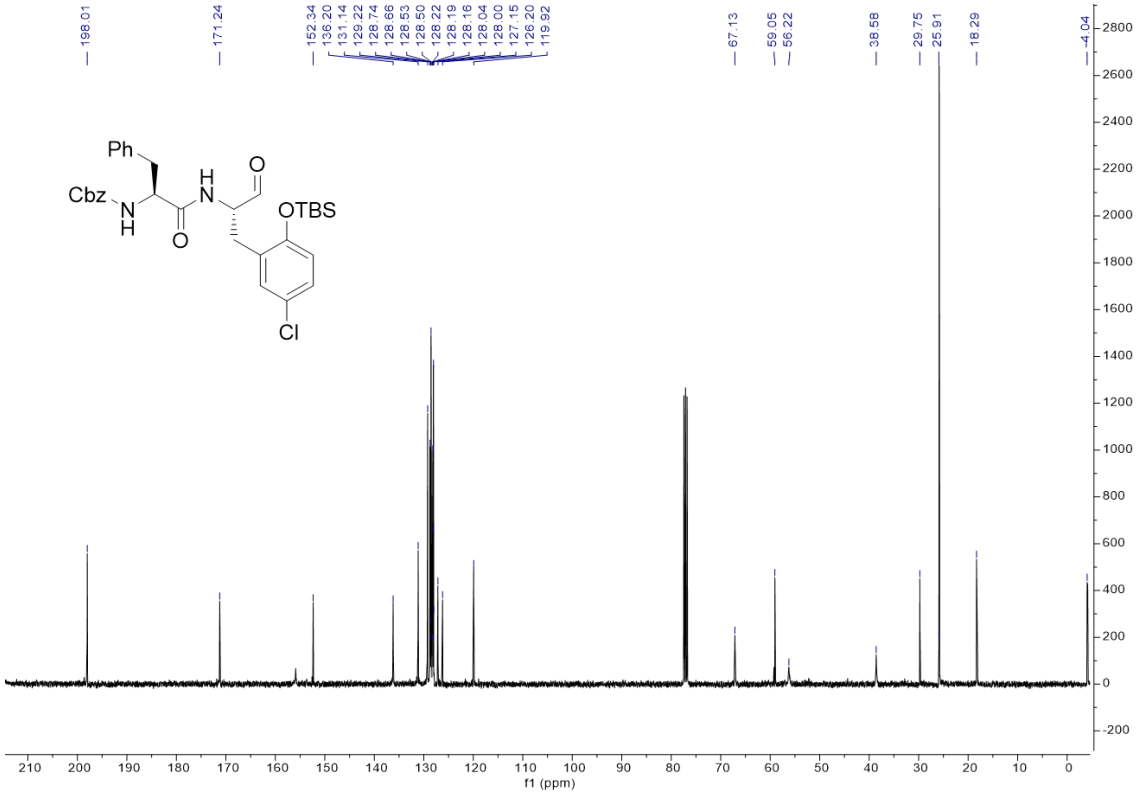

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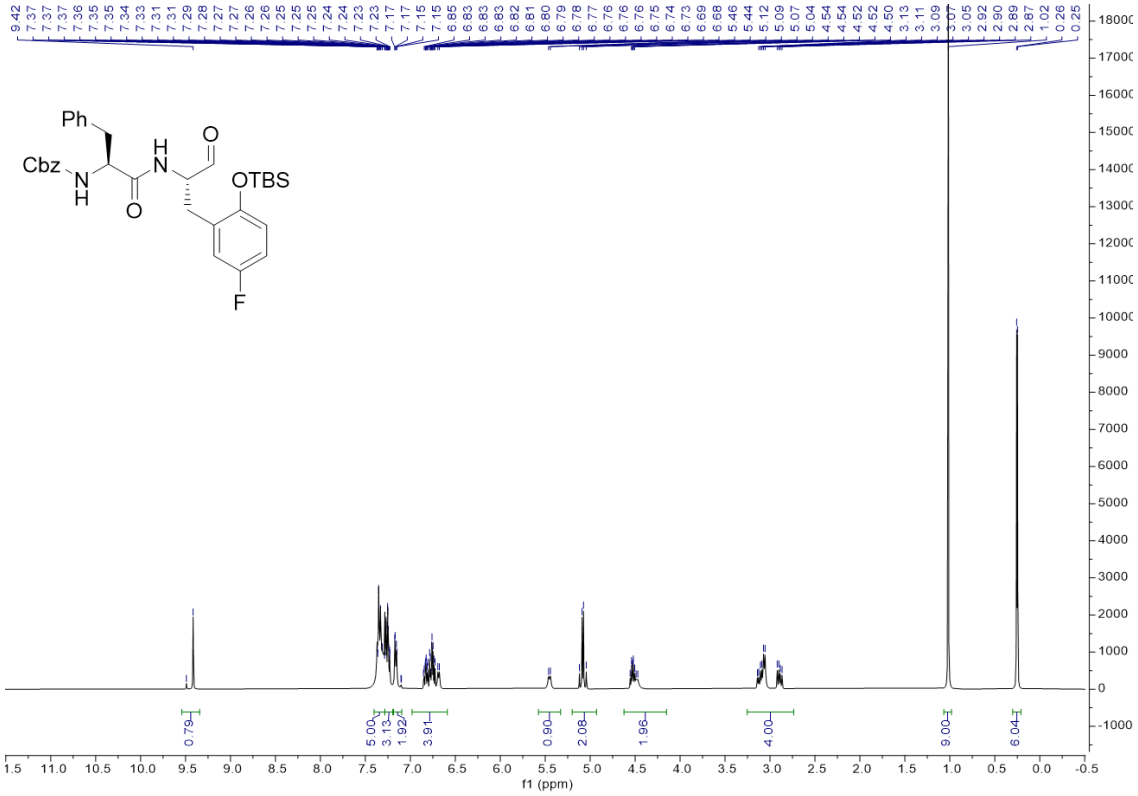

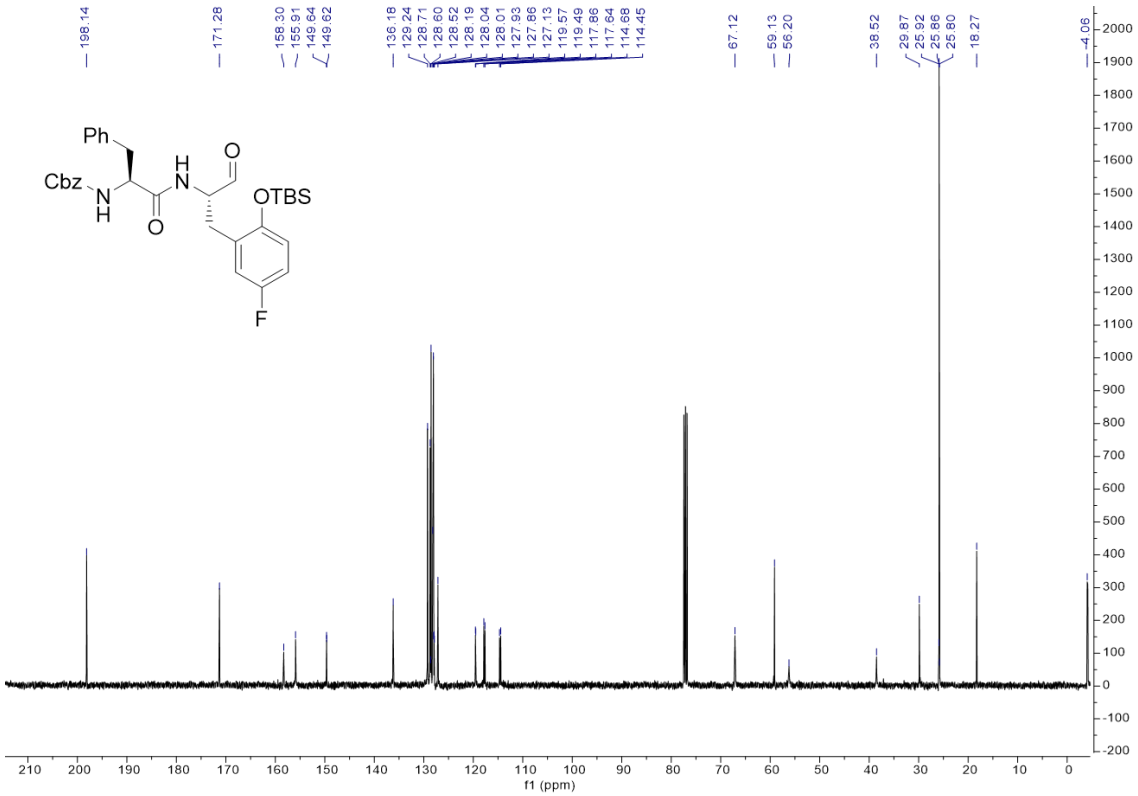

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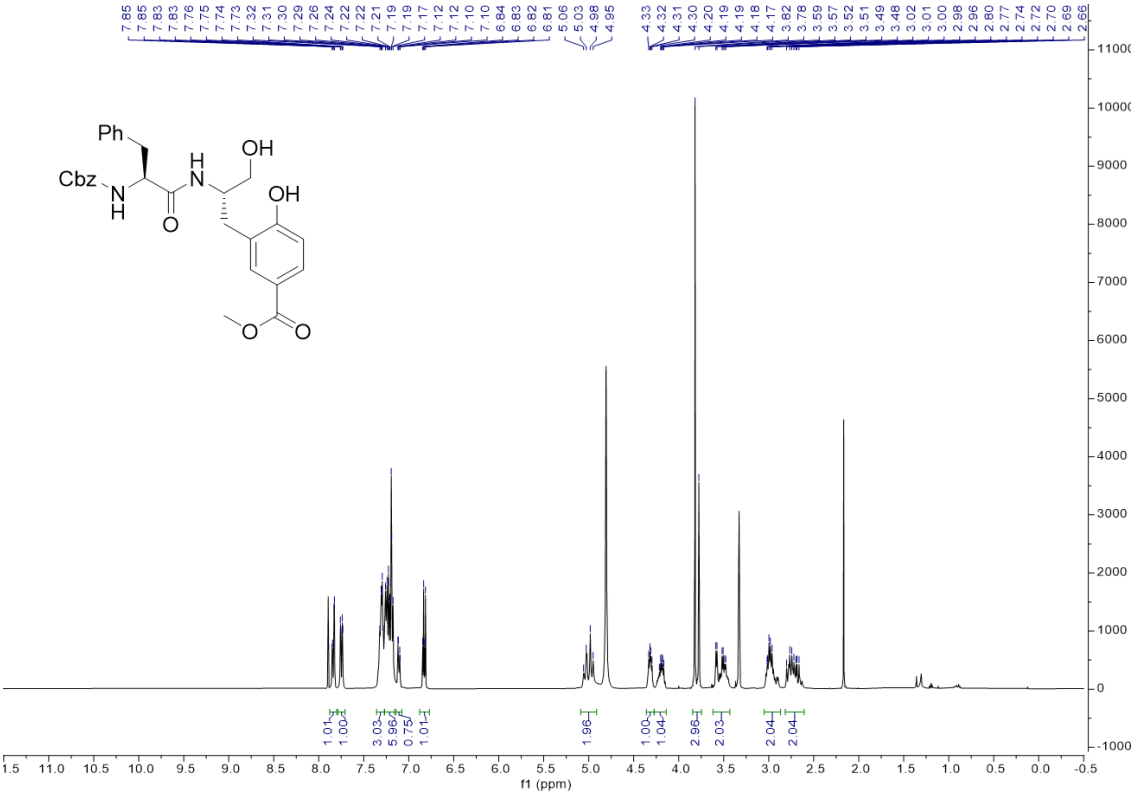

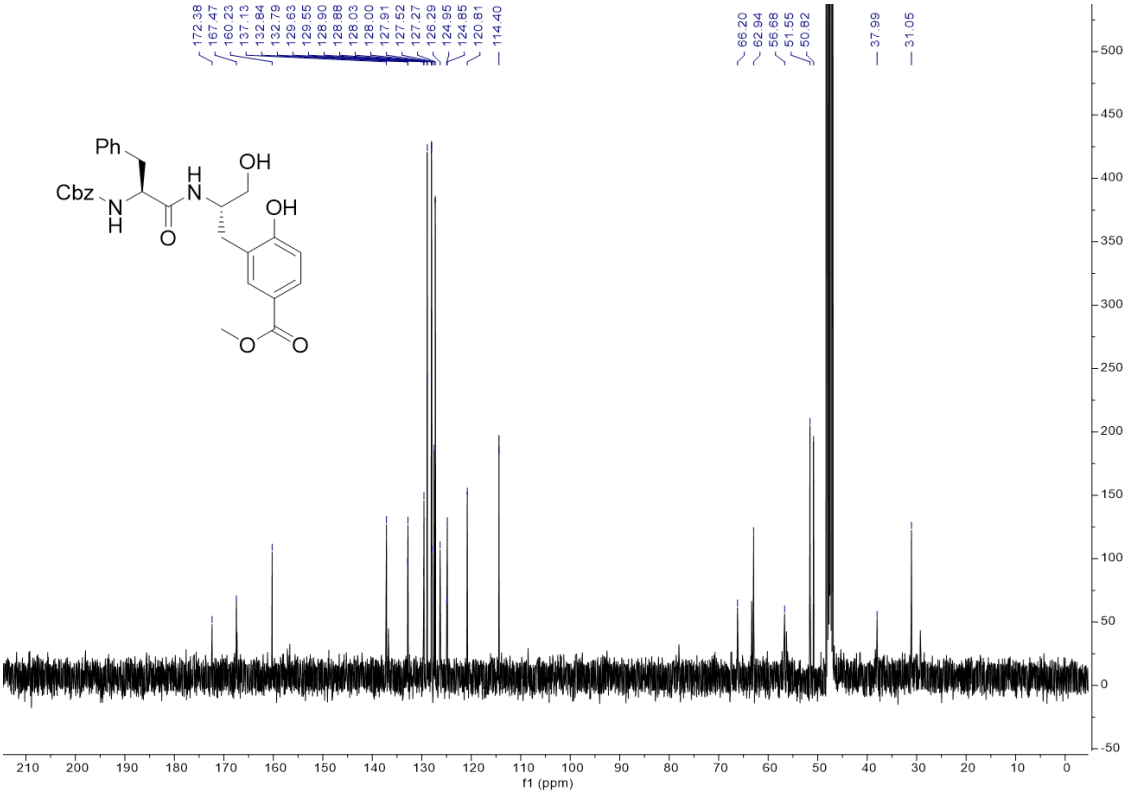

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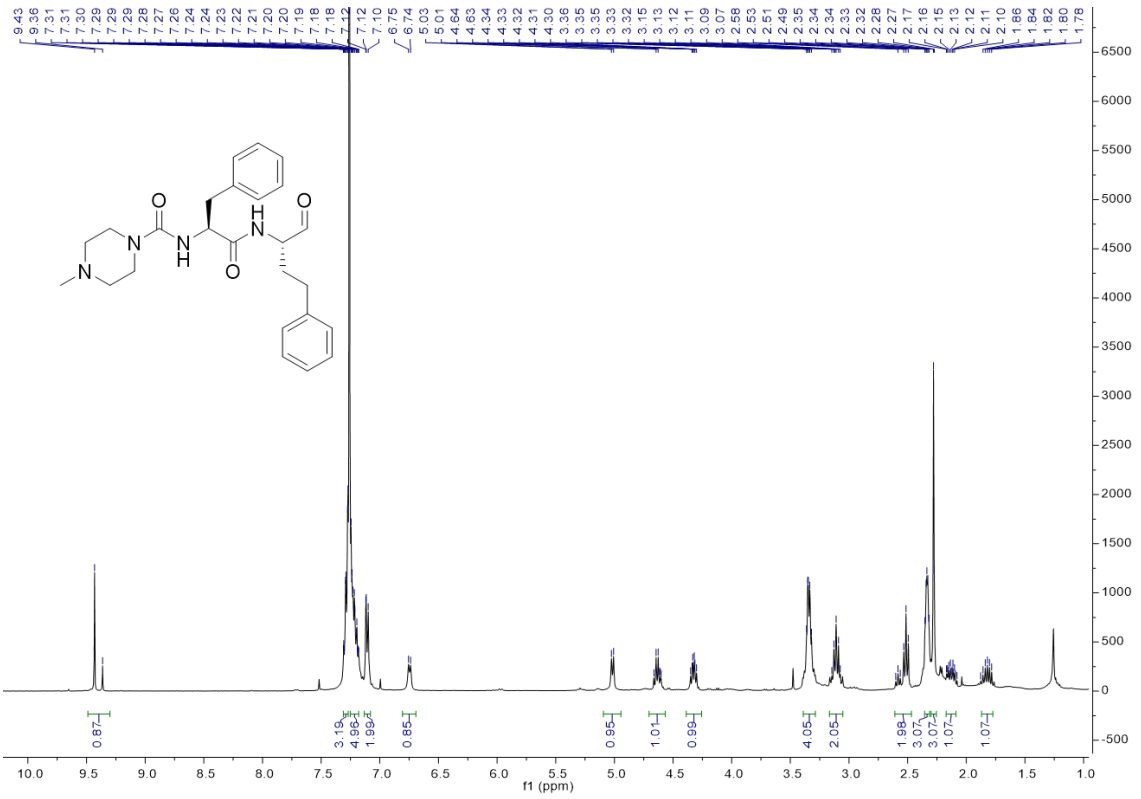
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O
O
H
N
N
N
N
H
O
0.87 3.19 4.96 1.99 0.85 0.95 1.01 0.99 4.05 2.05 1.98 3.07 3.07 1.07 1.07
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
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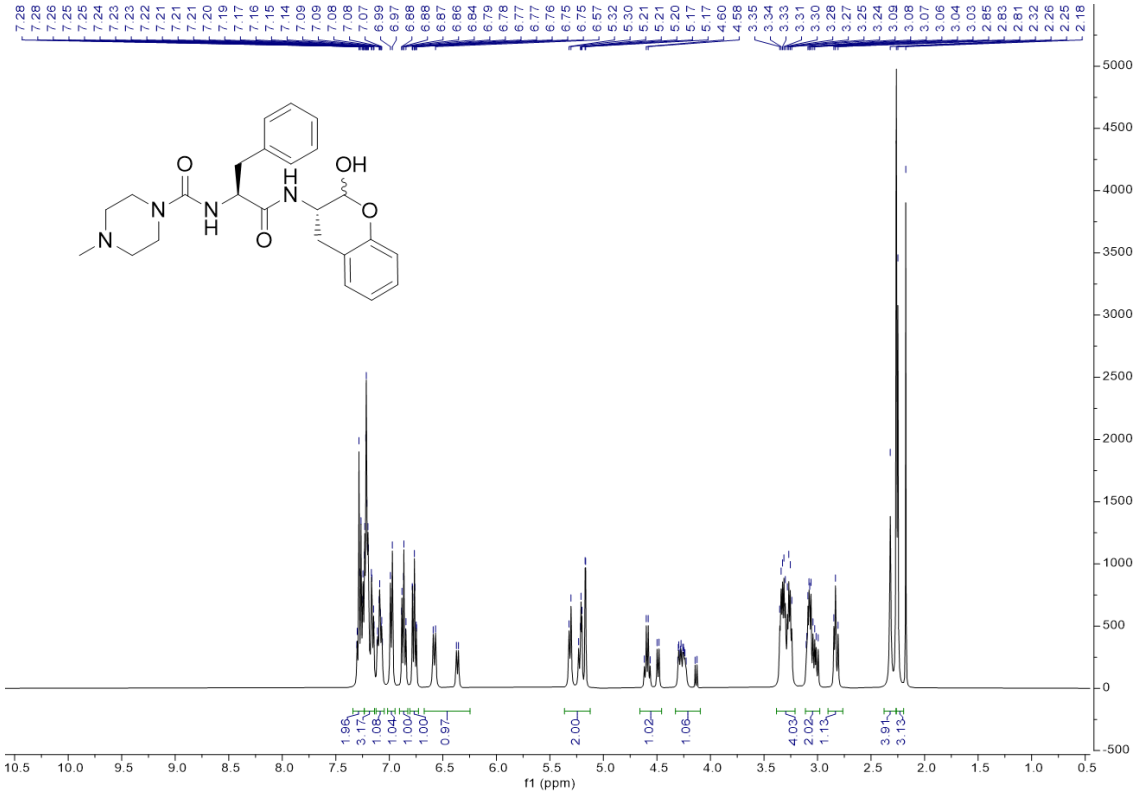

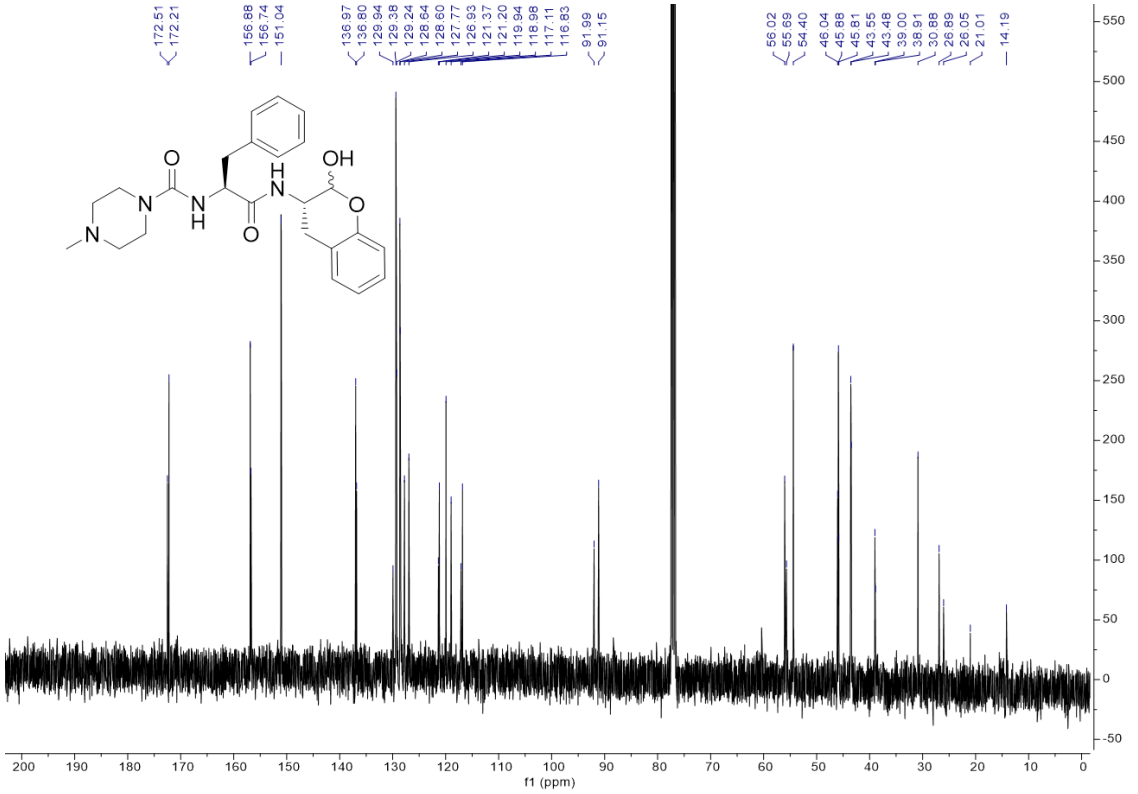

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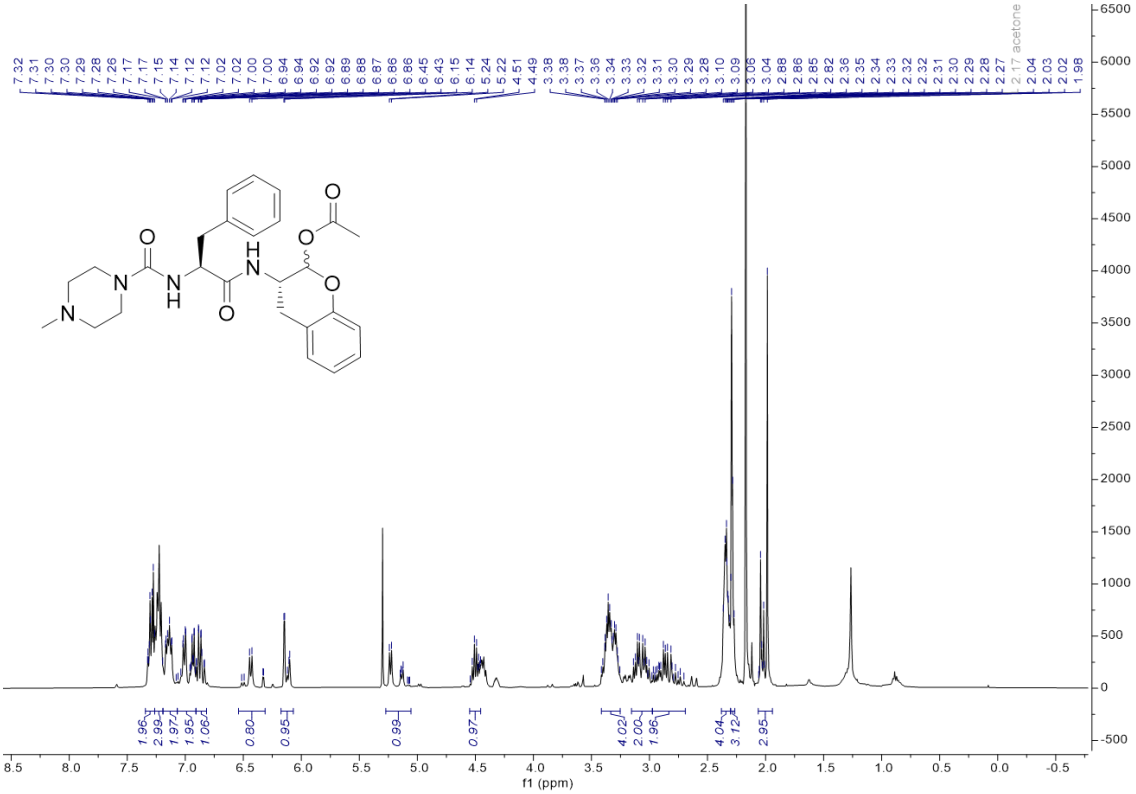
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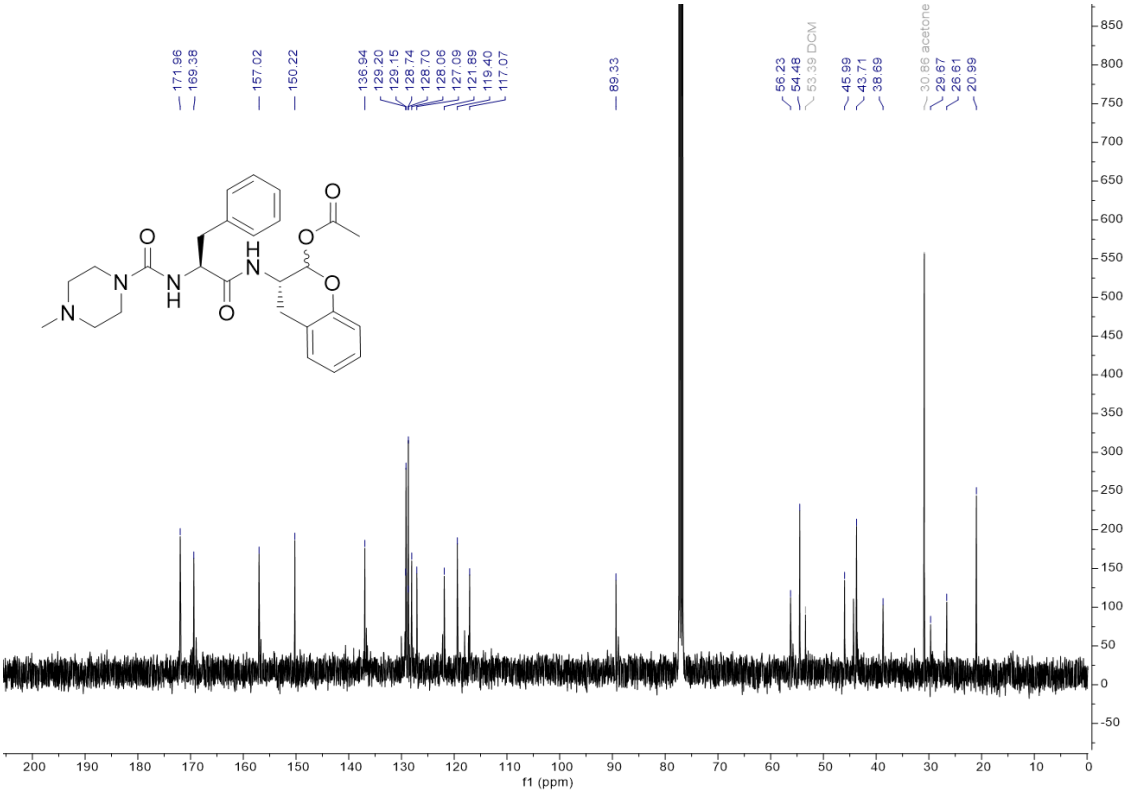
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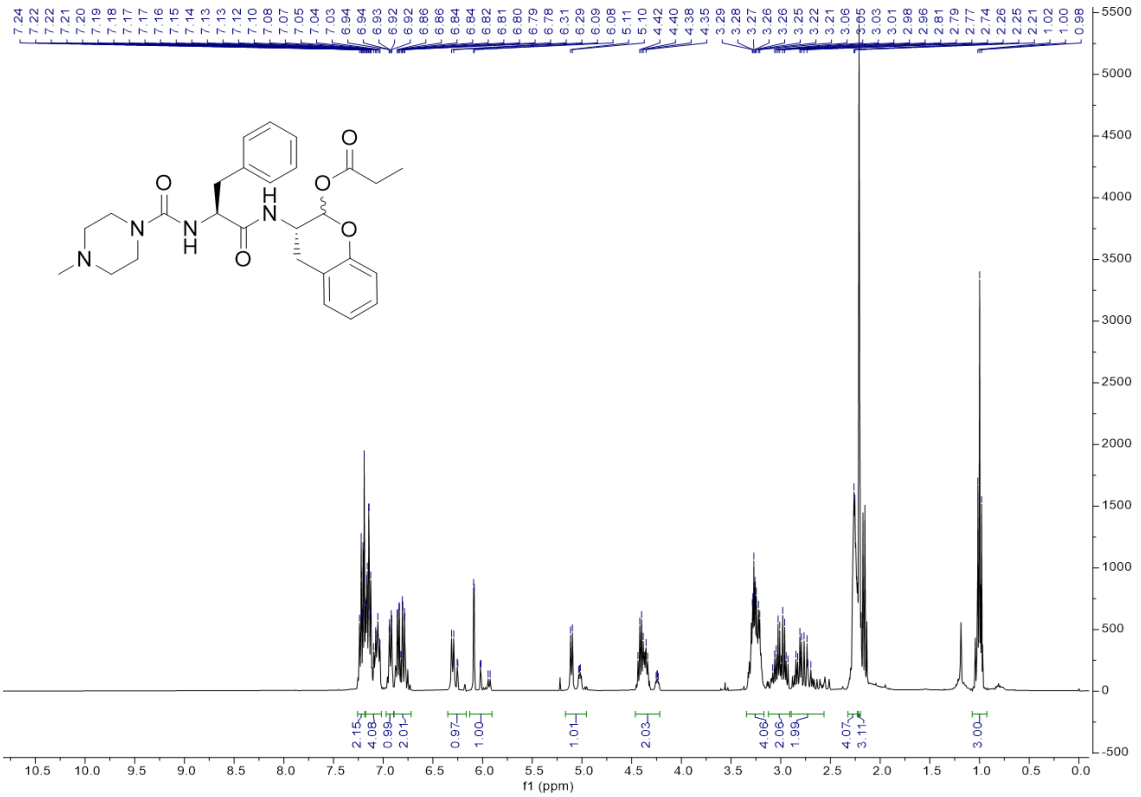

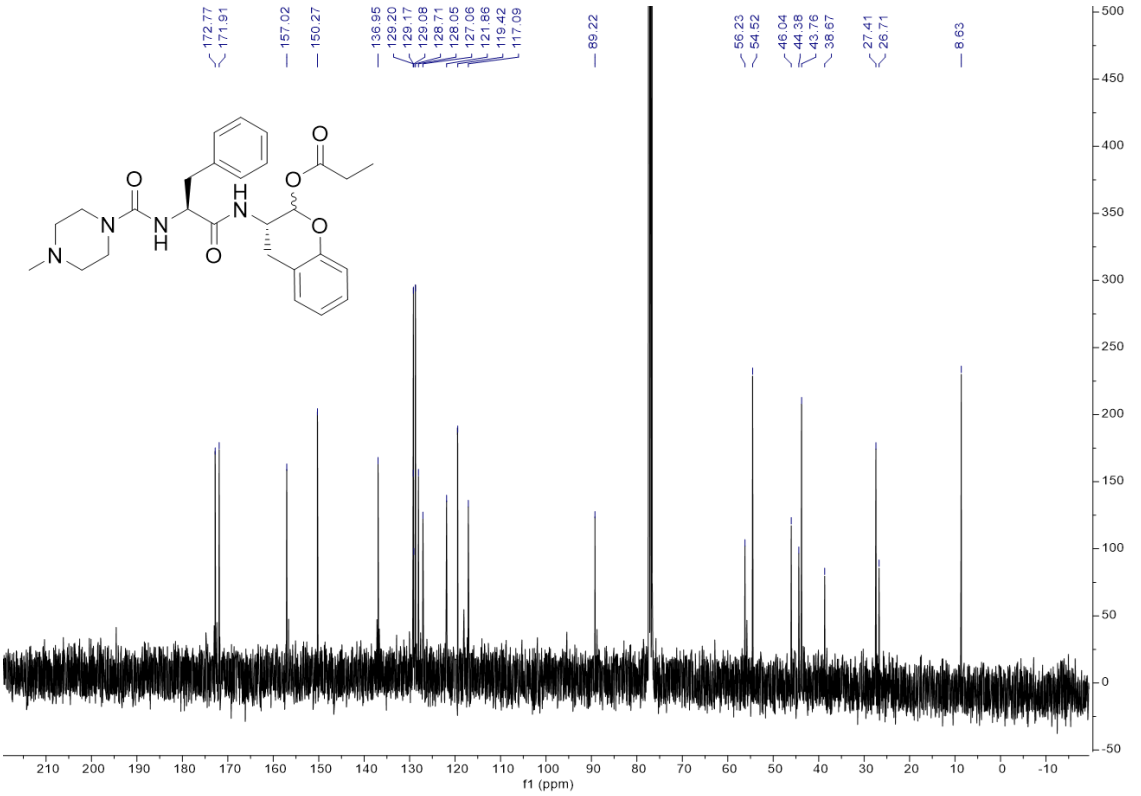

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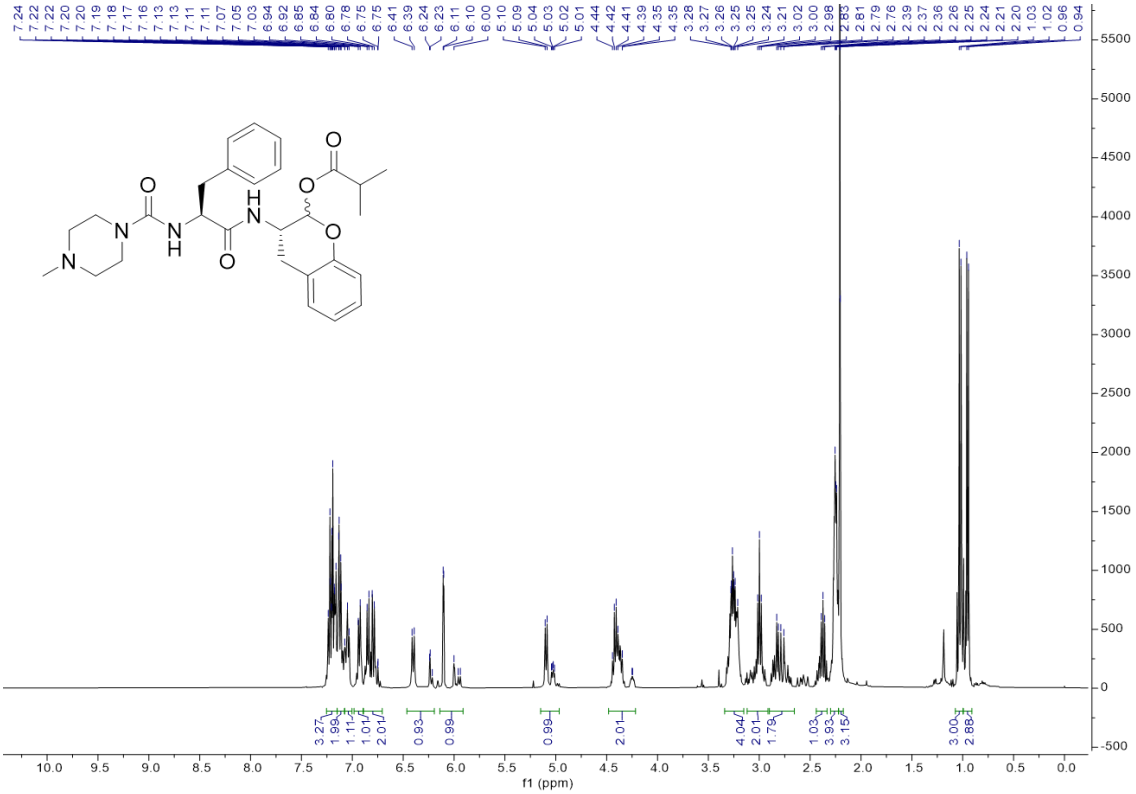
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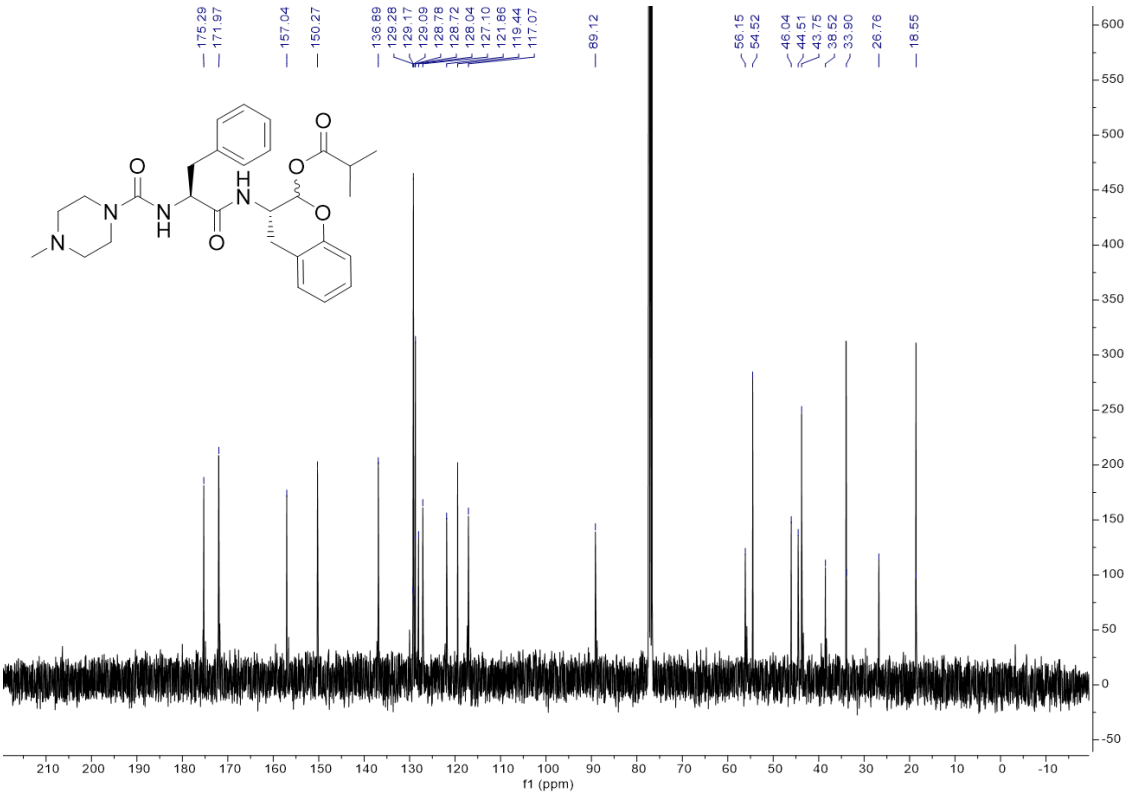
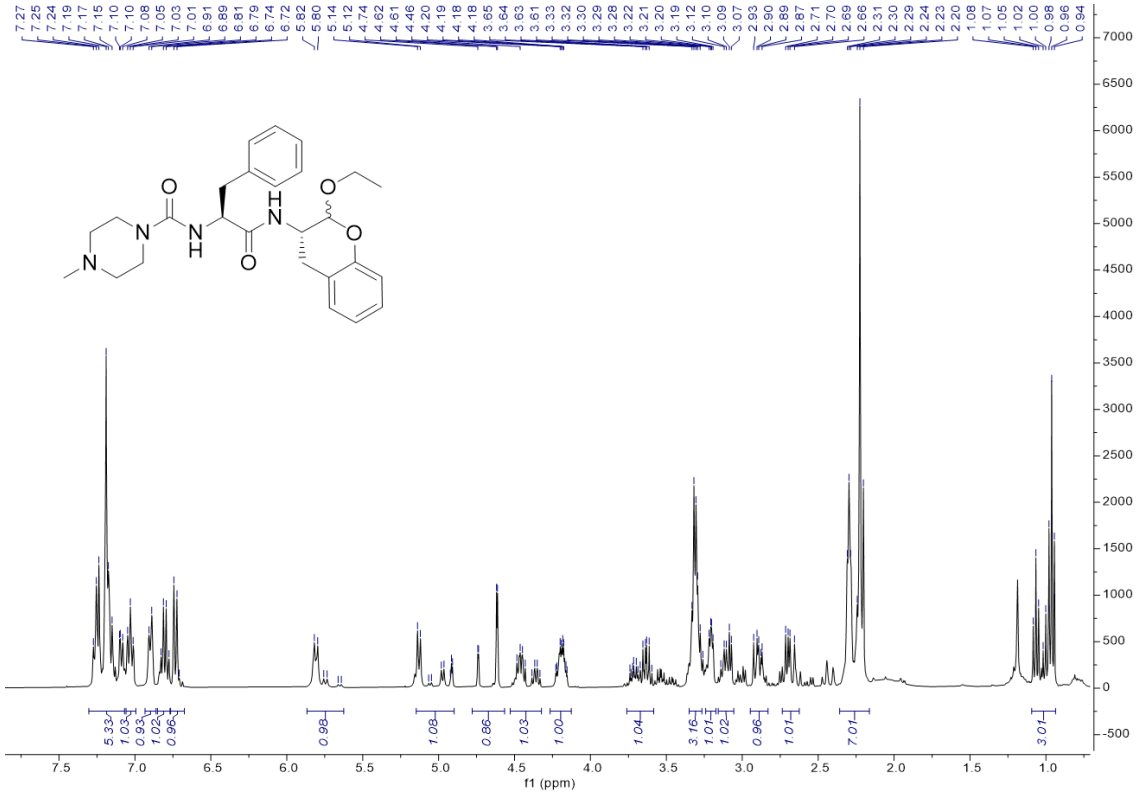
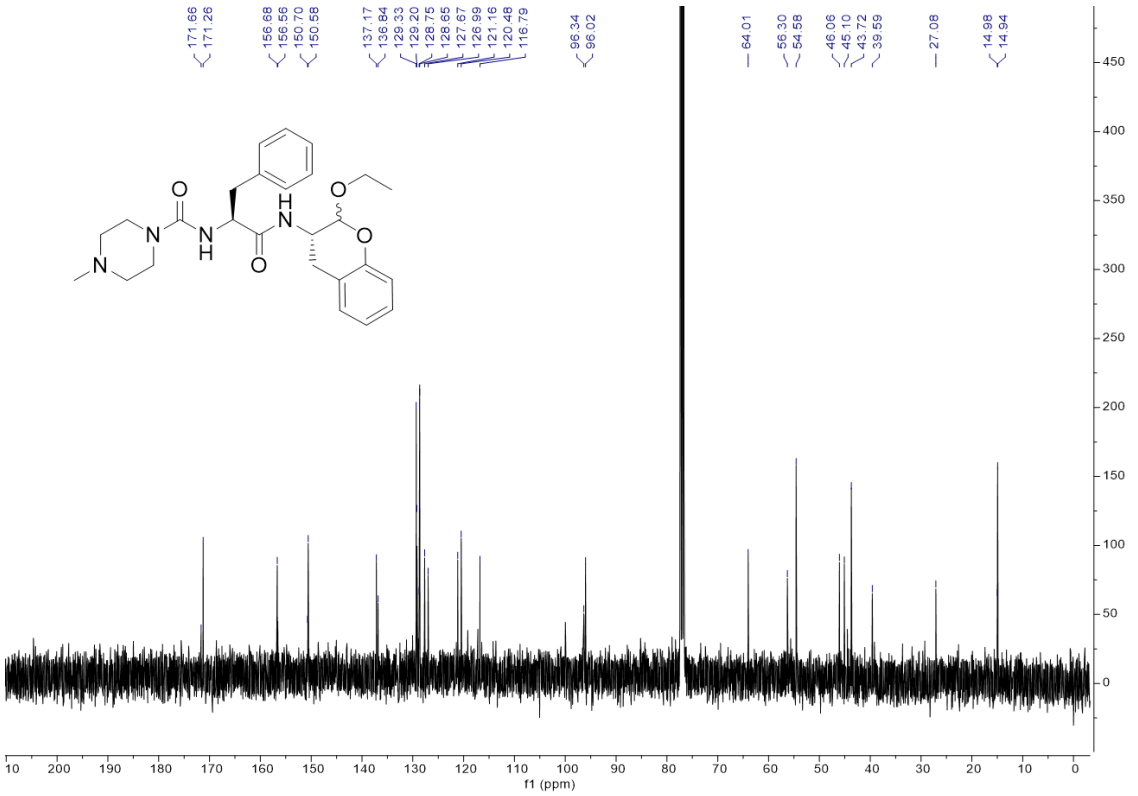
3.27 1.99 1.11 1.01 2.01 0.93 0.99 0.99 2.01 4.04 2.01 1.79 1.03 3.93 3.15 3.00 2.88
f1 (ppm)

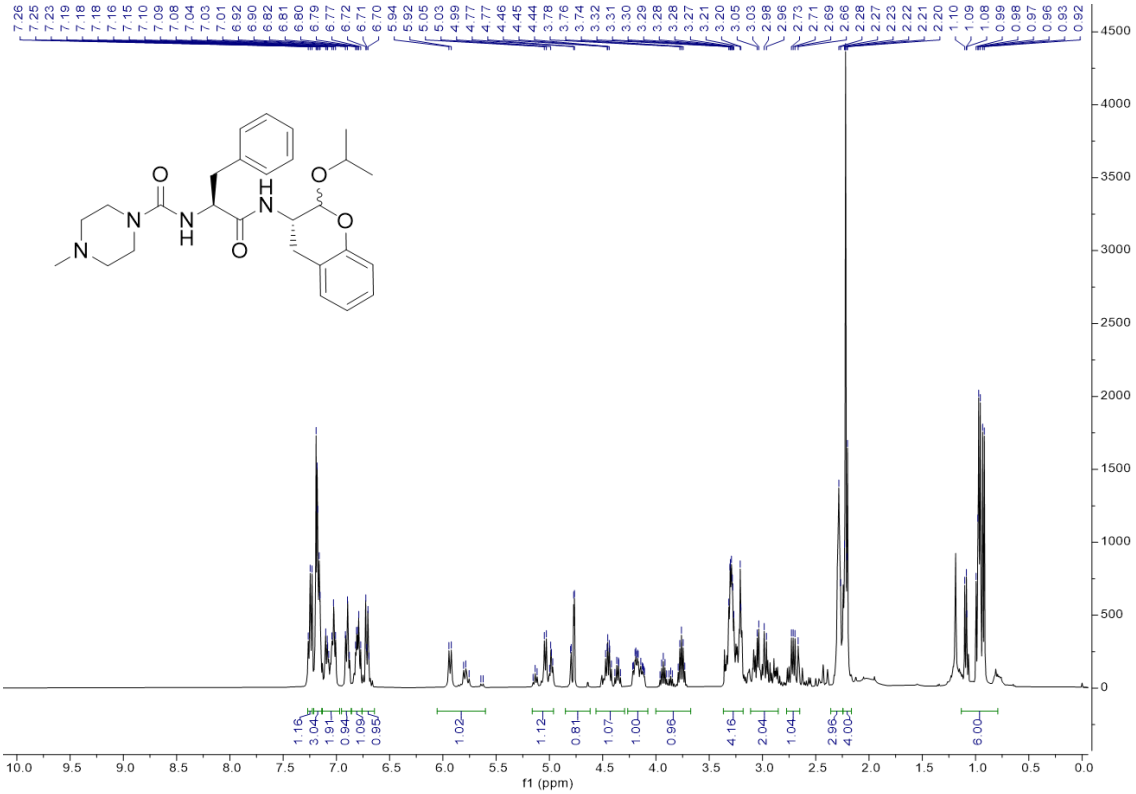

175.29 171.97 157.04 150.27 136.89 129.28 129.17 129.09 128.78 128.72 128.04 127.10 121.86 119.44 117.07 89.12 56.15 54.52 46.04 44.51 43.75 38.52 33.90 26.76 18.55
f1 (ppm)

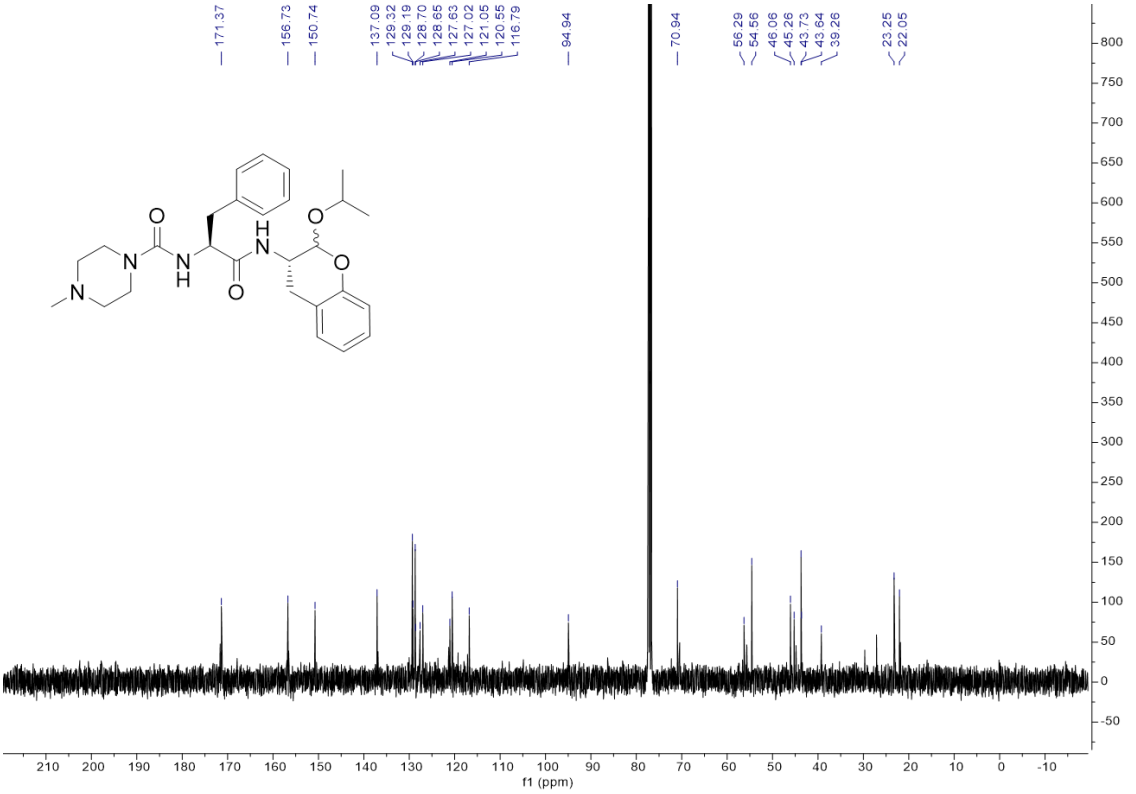
16:


7.27 7.25 7.24 7.19 7.17 7.15 7.10 7.10 7.08 7.05 7.03 7.01 6.91 6.89 6.81 6.79 6.74 6.72 5.82 5.80 5.14 5.12 4.74 4.62 4.61 4.46 4.20 4.19 4.18 4.18 3.65 3.64 3.63 3.61 3.33 3.32 3.30 3.29 3.28 3.22 3.21 3.20 3.19 3.12 3.10 3.09 3.07 2.93 2.90 2.89 2.87 2.71 2.70 2.69 2.66 2.31 2.30 2.29 2.24 2.23 2.20 1.08 1.07 1.05 1.02 1.00 0.98 0.96 0.94
5.33 1.03 0.93 1.02 0.96 0.98 1.08 0.86 1.03 1.00 1.04 3.16 1.01 1.02 0.96 1.01 7.01 3.01
f1 (ppm)

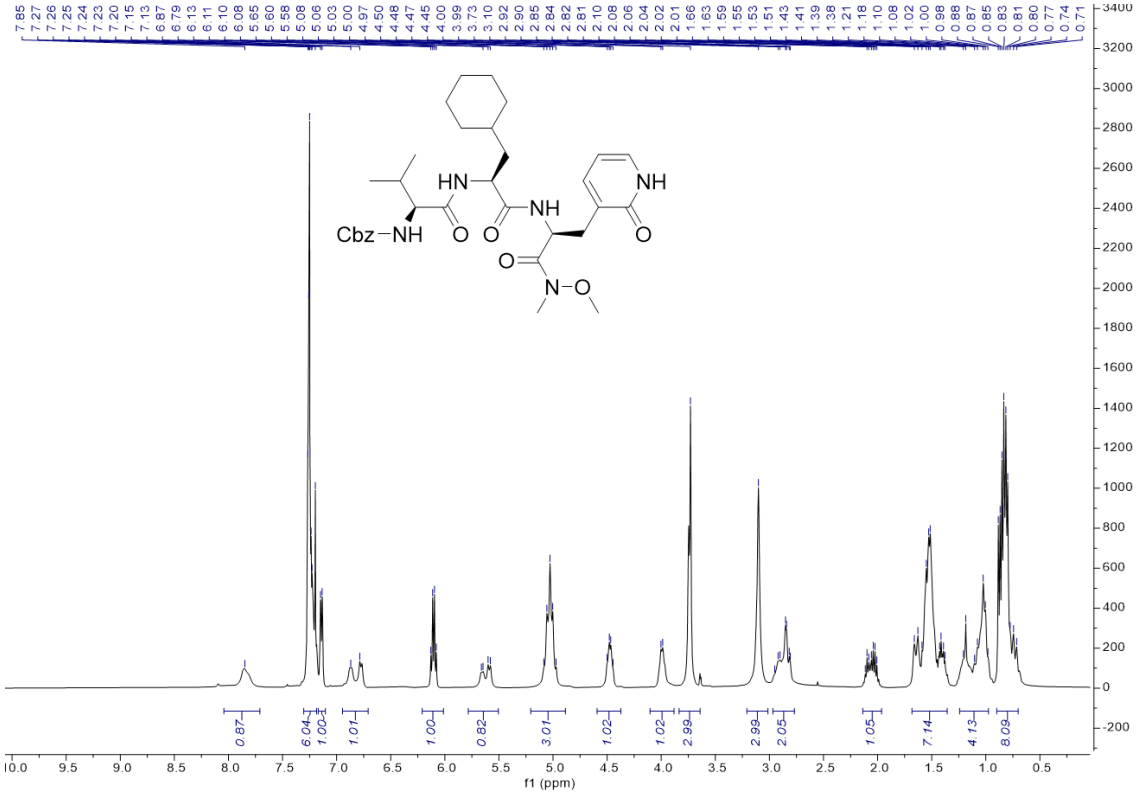

171.66 171.26 156.68 156.56 150.70 150.58 137.17 136.84 129.33 129.20 128.75 128.65 127.67 126.99 121.16 120.48 116.79 96.34 96.02 64.01 56.30 54.58 46.06 45.10 43.72 39.59 27.08 14.98 14.94
f1 (ppm)

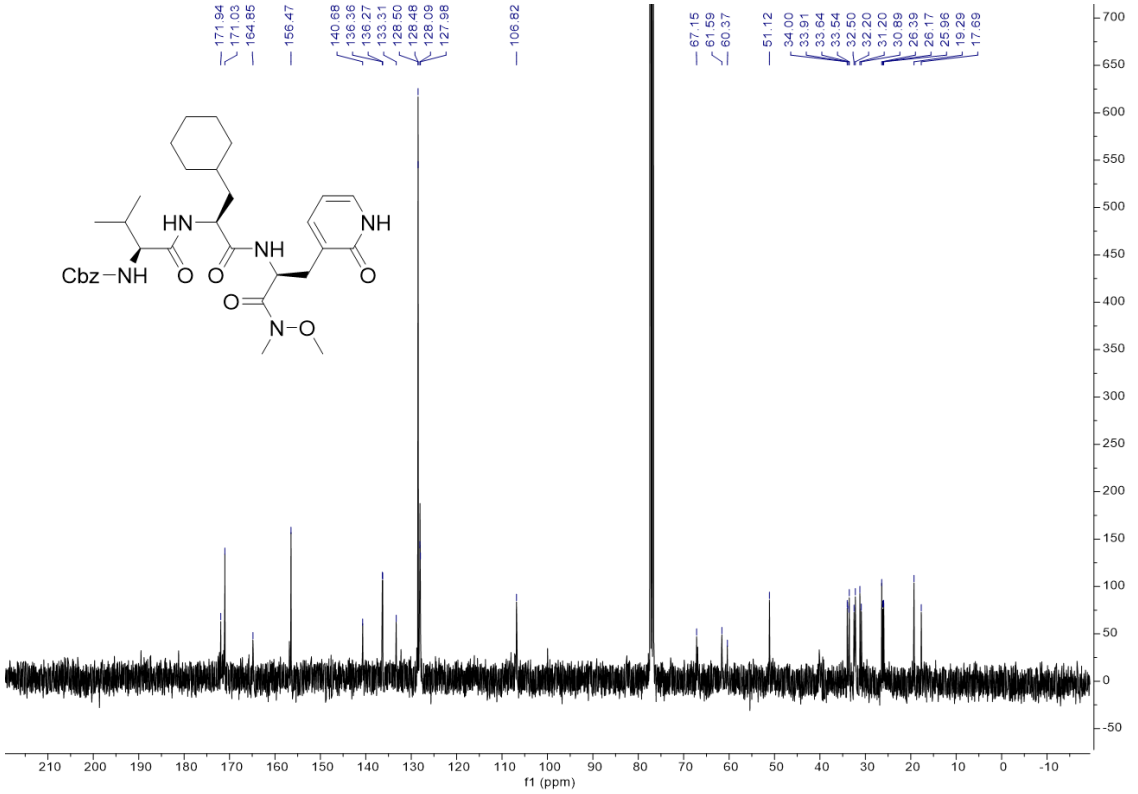
17:

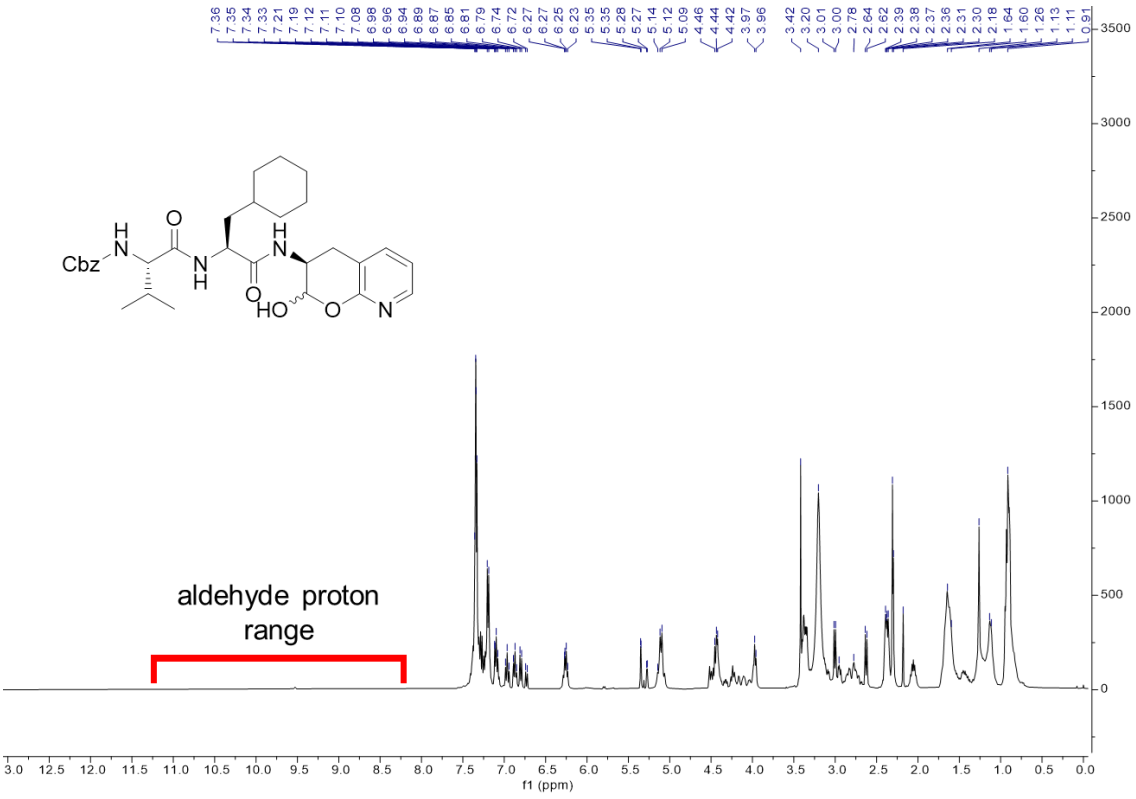




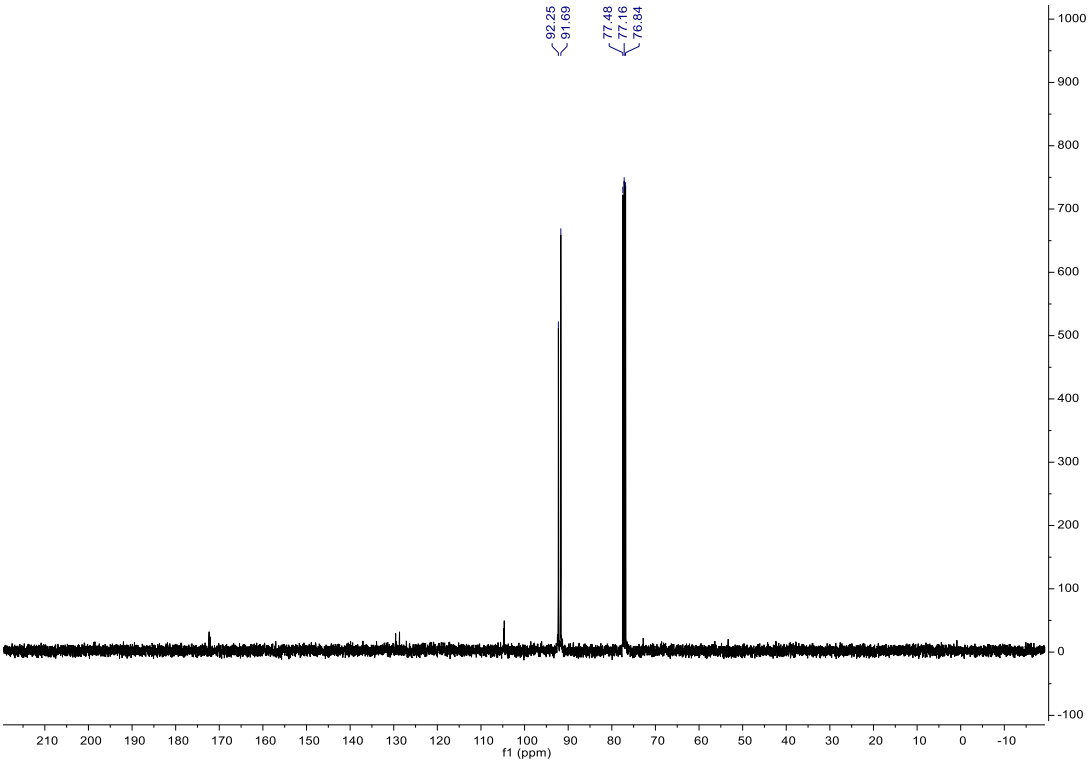
18:



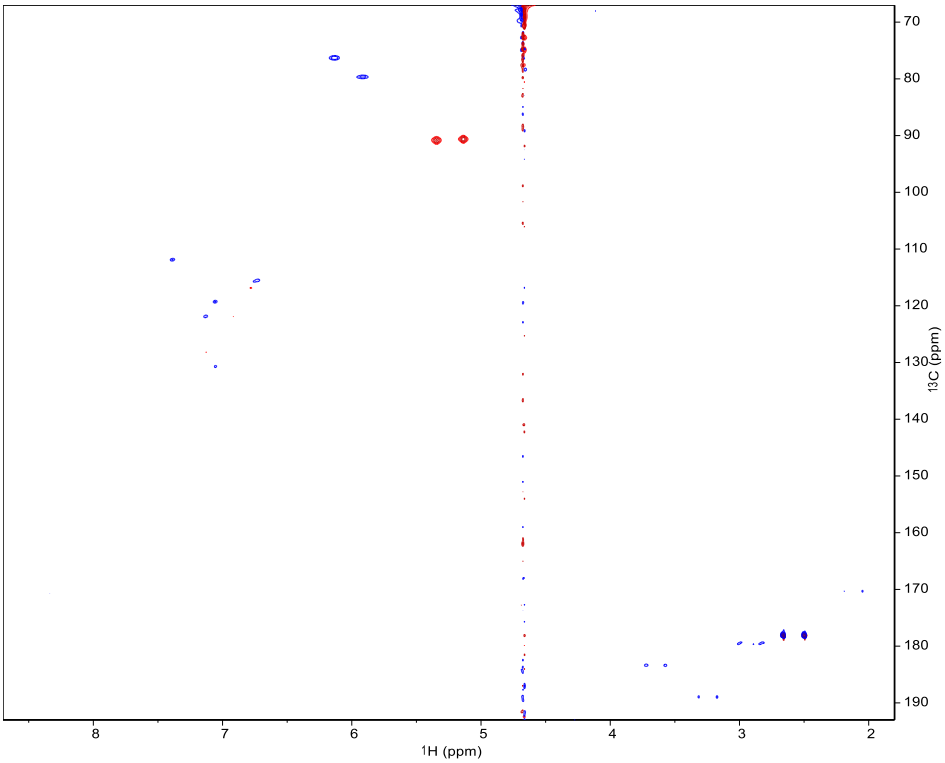



Cbz
HO
aldehyde proton range
f1 (ppm)

^{13}C-labeled **12** in chloroform-d_3:

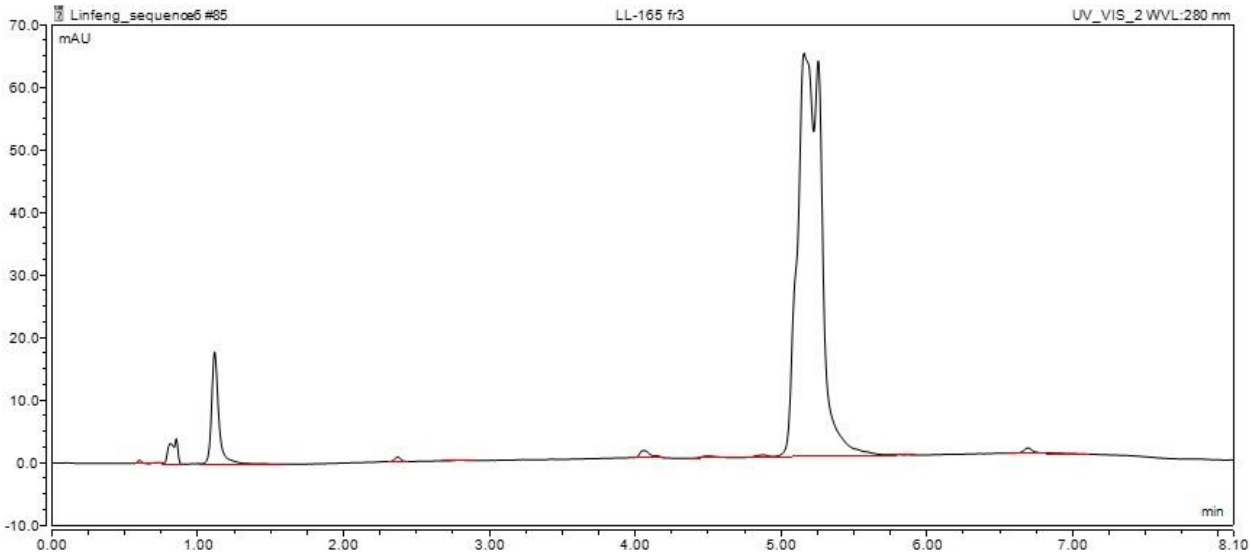


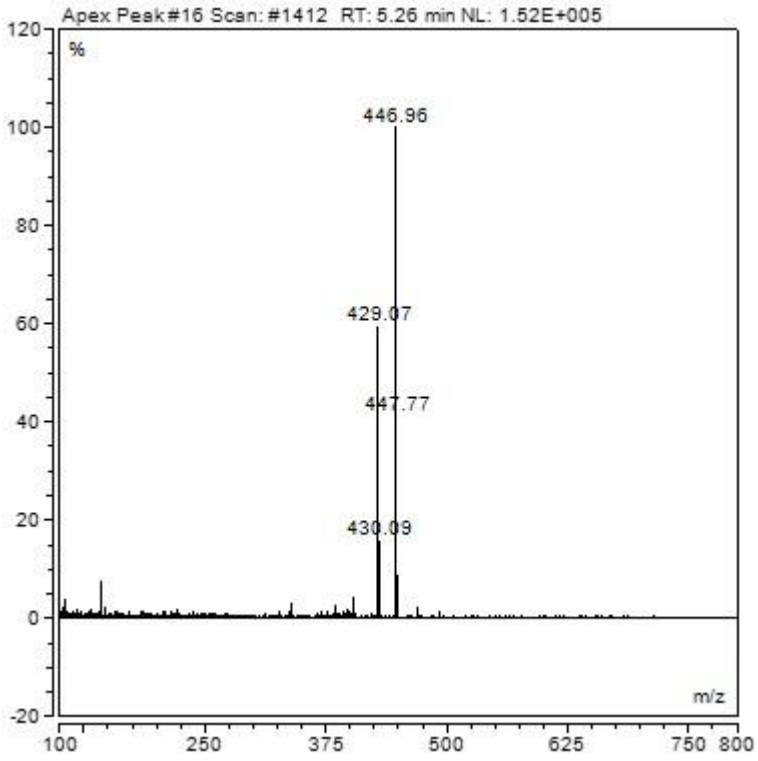
^{1}H-^{13}C HSQC NMR of ^{13}C-labeled **12** with and without cruzain in buffer (full view):



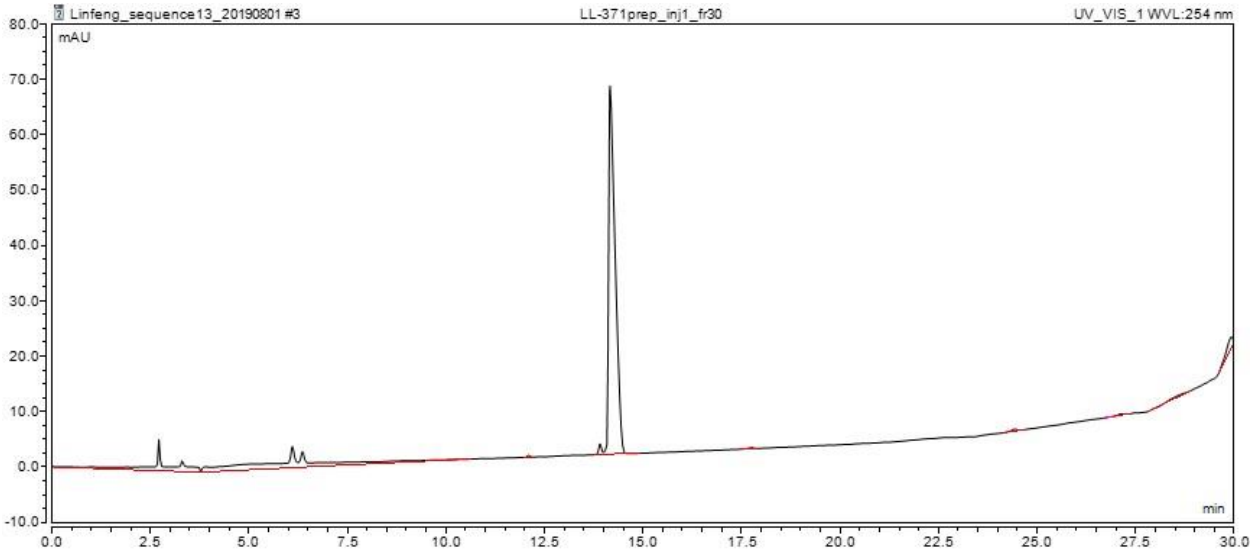
HPLC traces and MS data

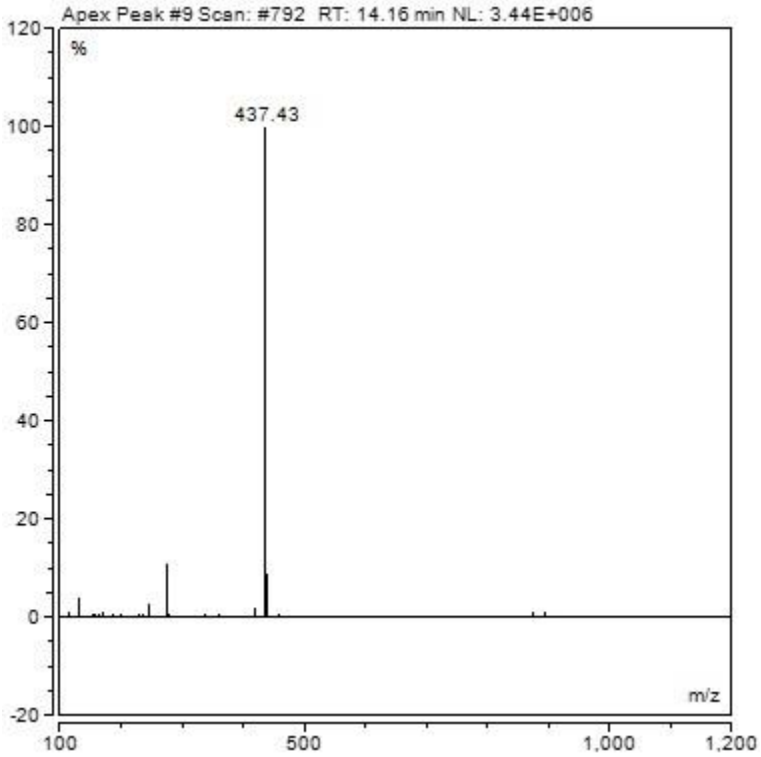
2: exact mass = 446.18



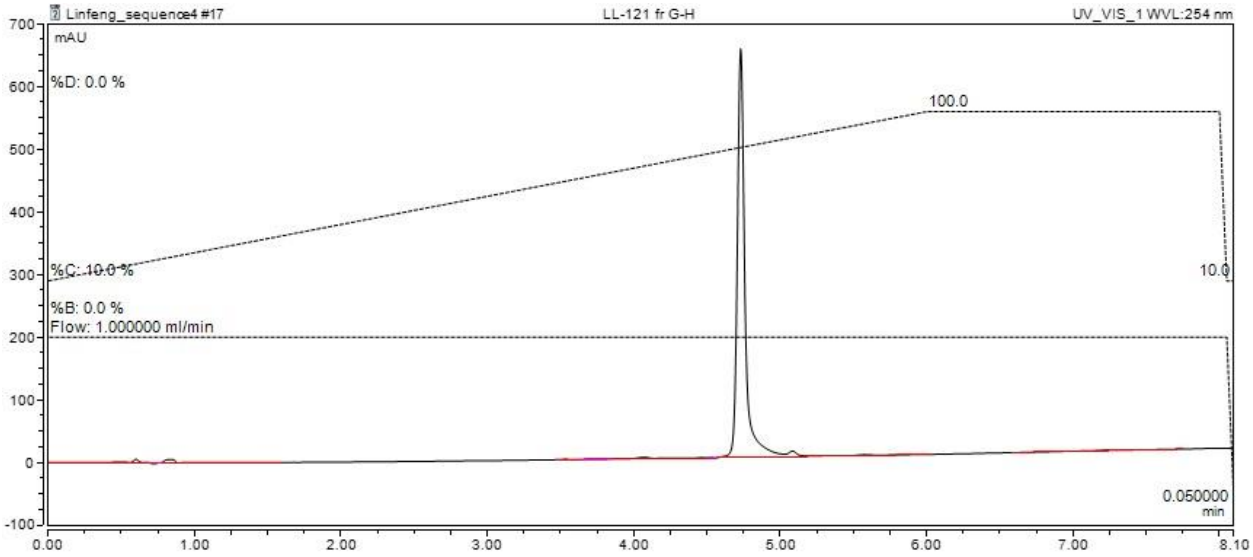


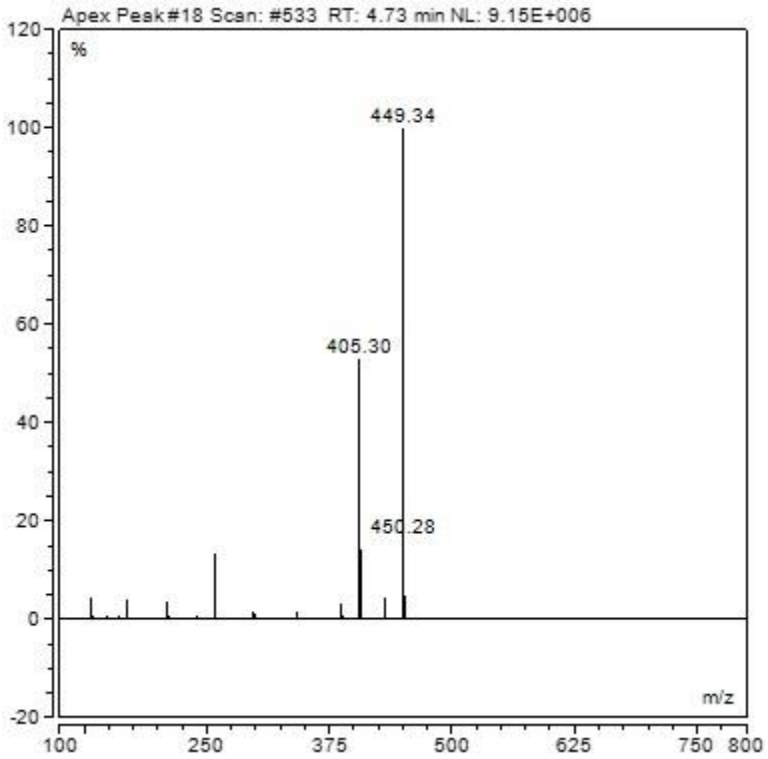
3: exact mass = 436.25



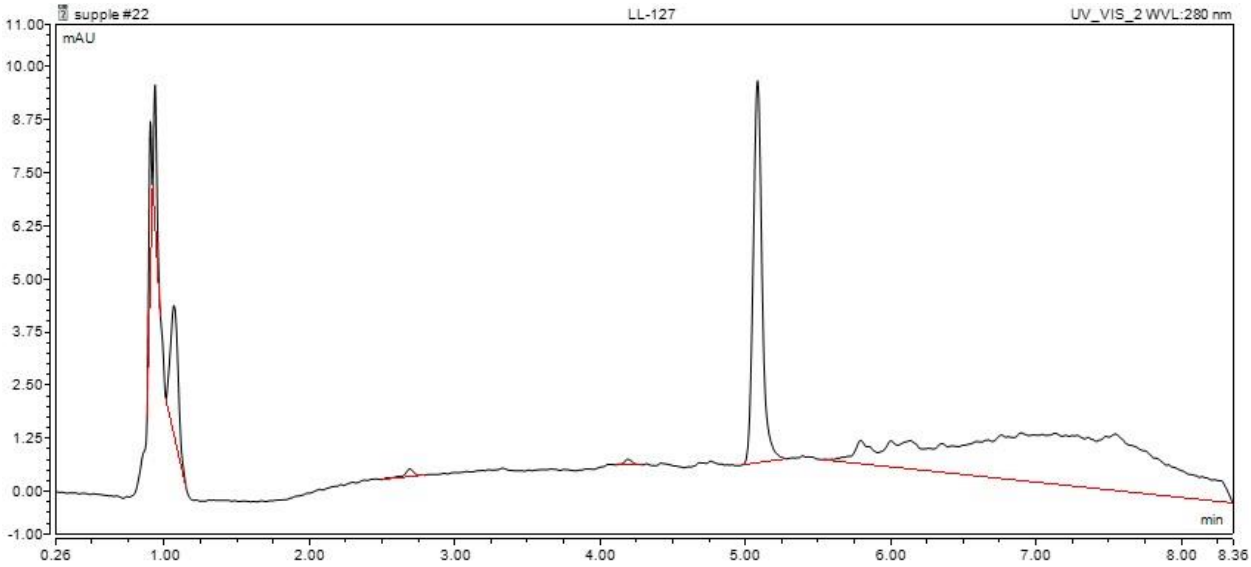


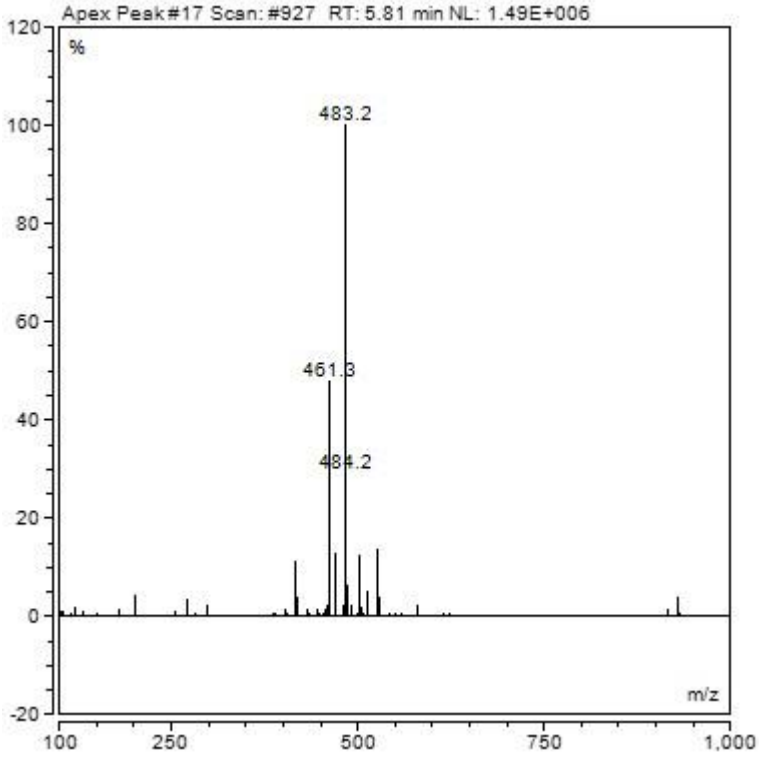
4: exact mass = 448.20



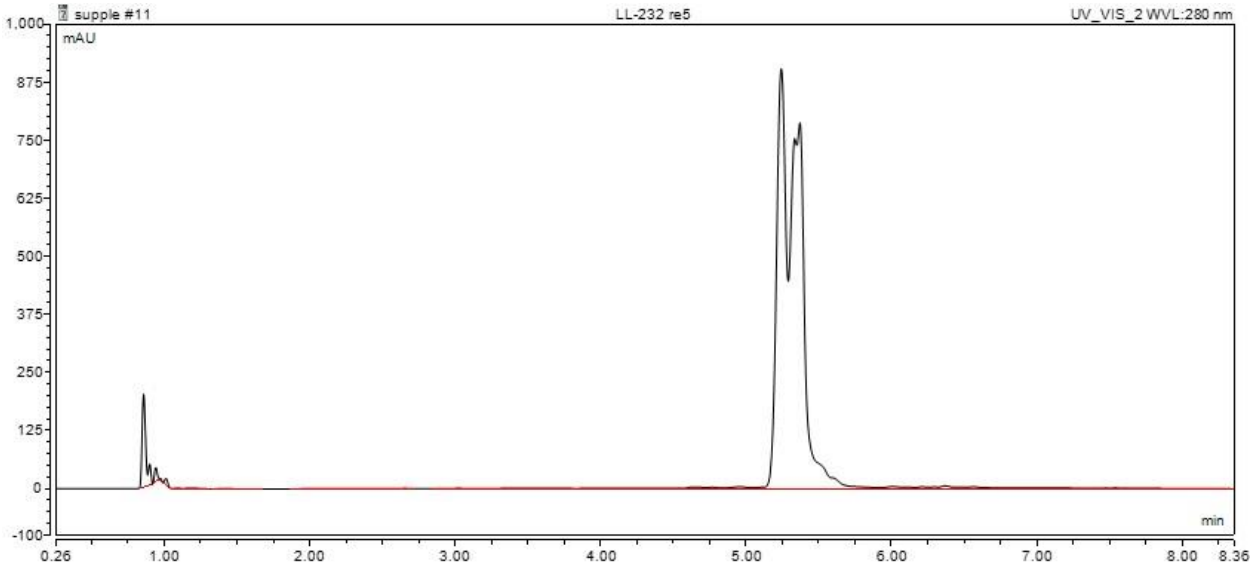


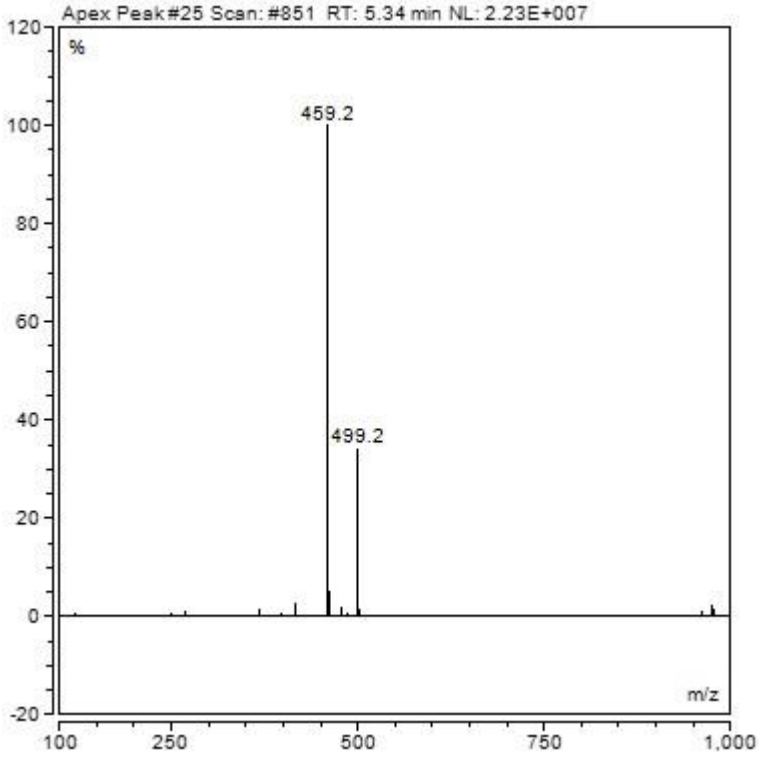
5: exact mass = 460.20



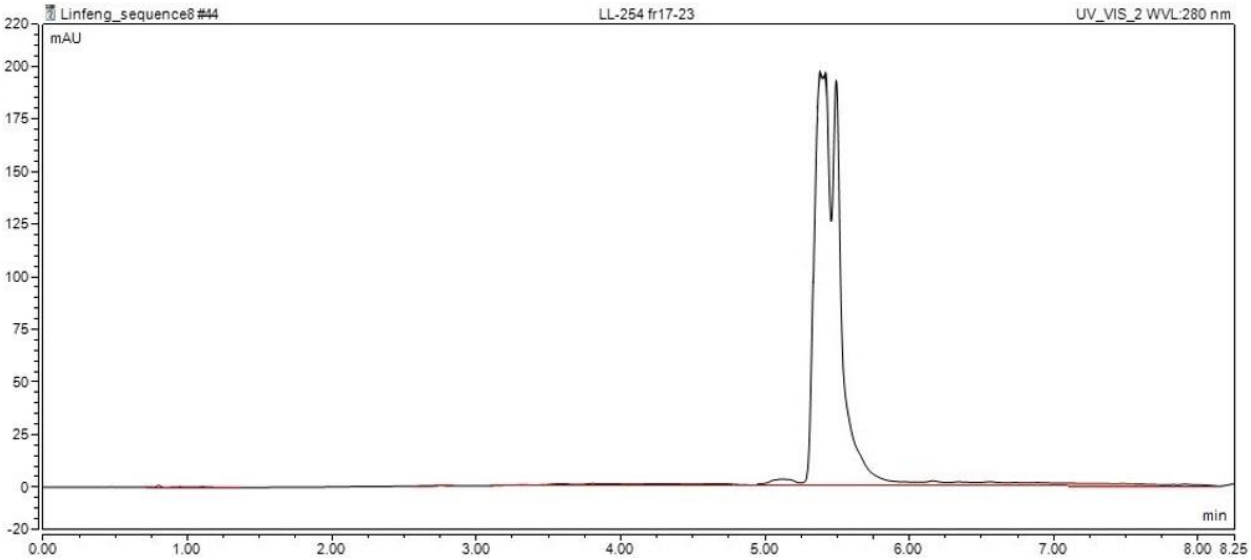


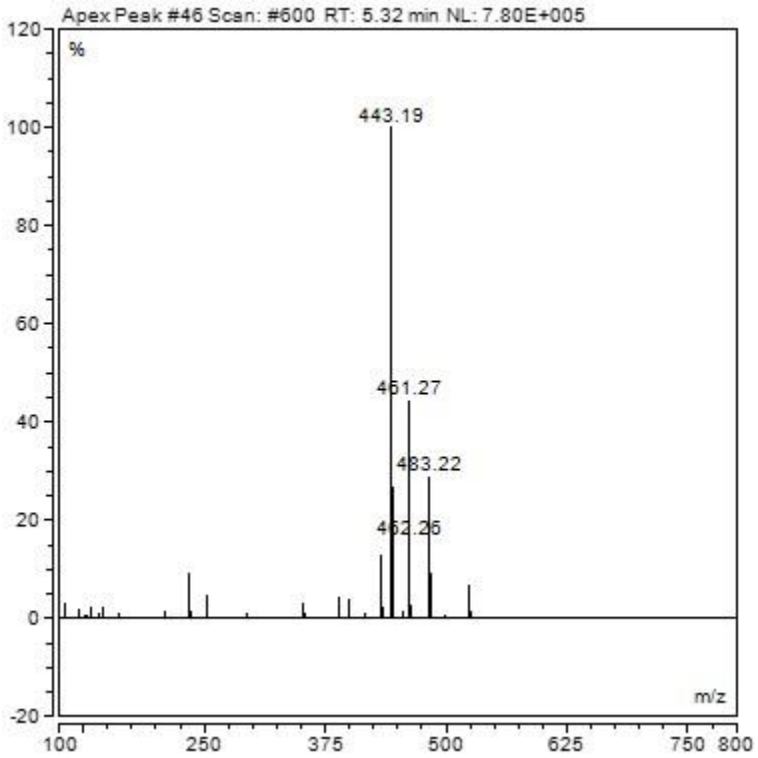
6: exact mass = 476.19



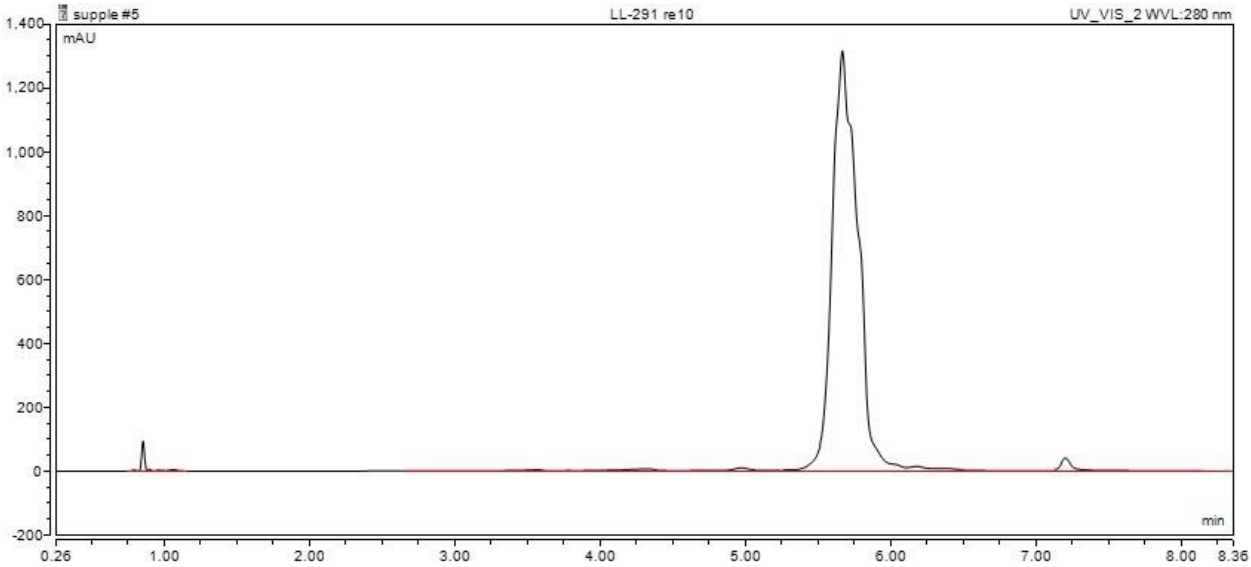


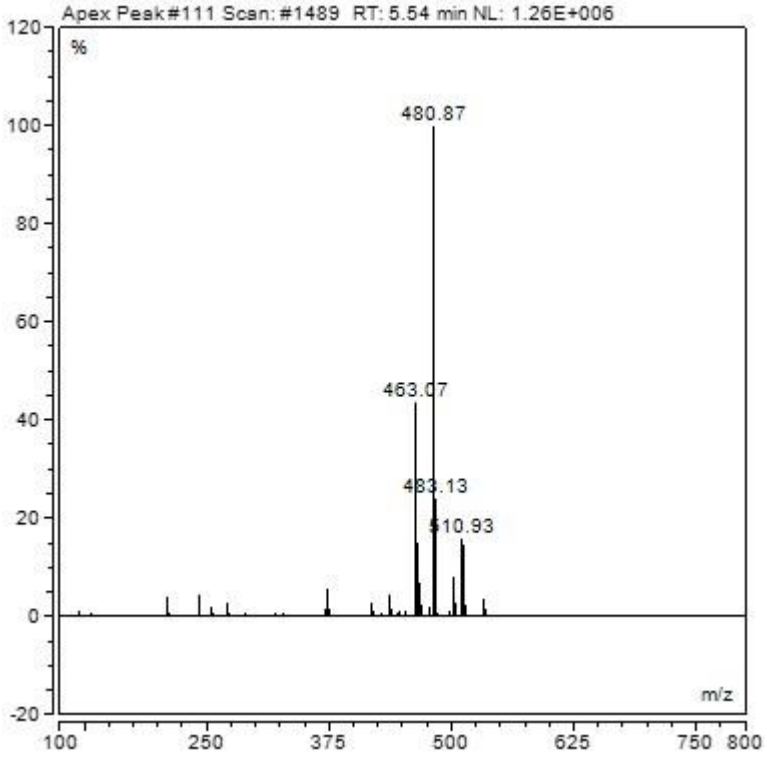
7: exact mass = 460.20



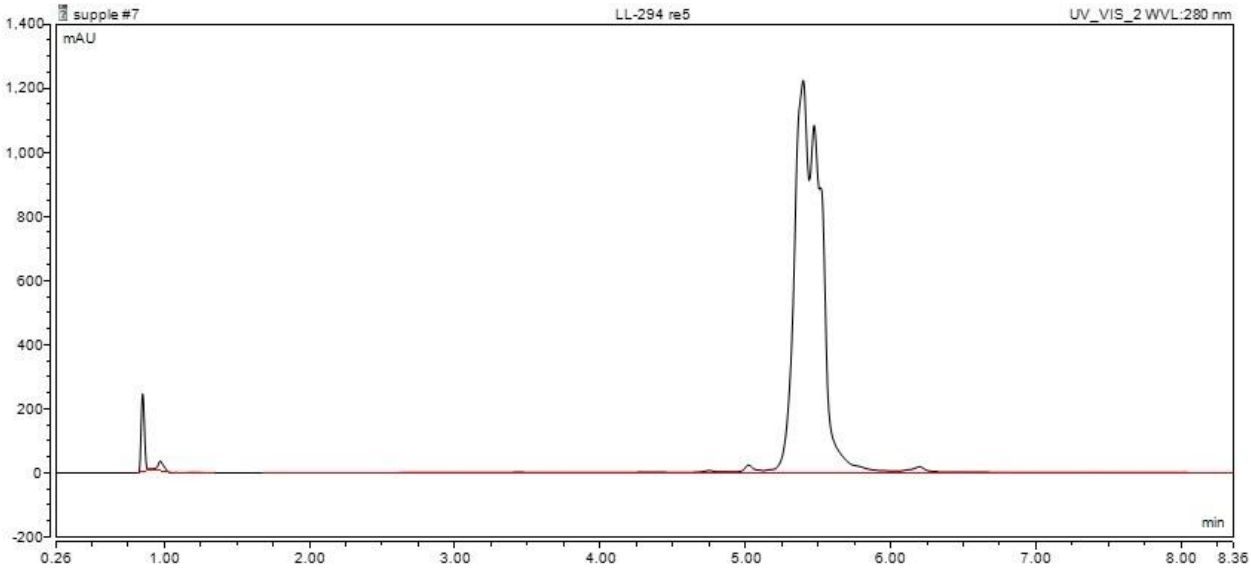


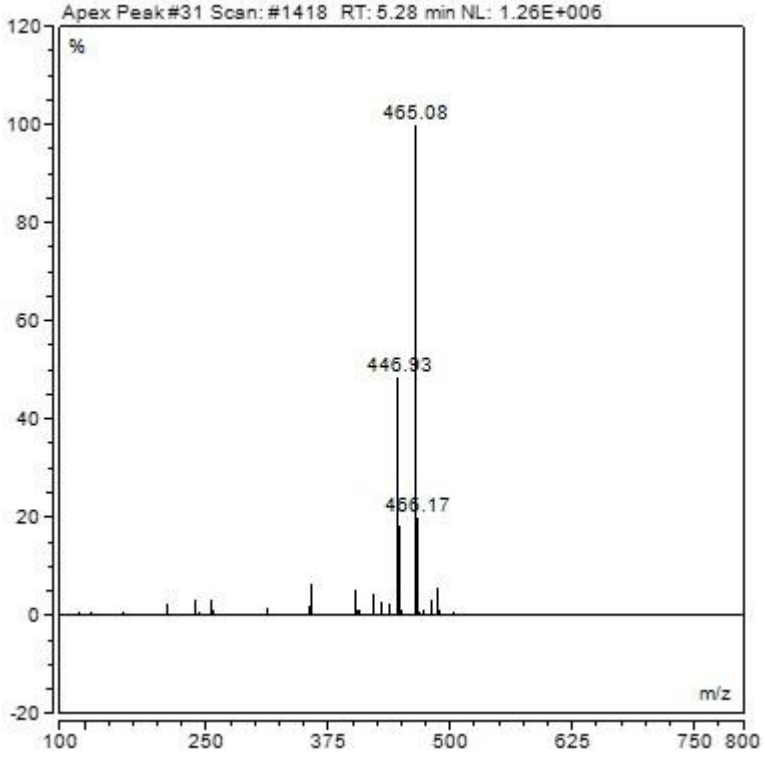
8: exact mass = 480.16



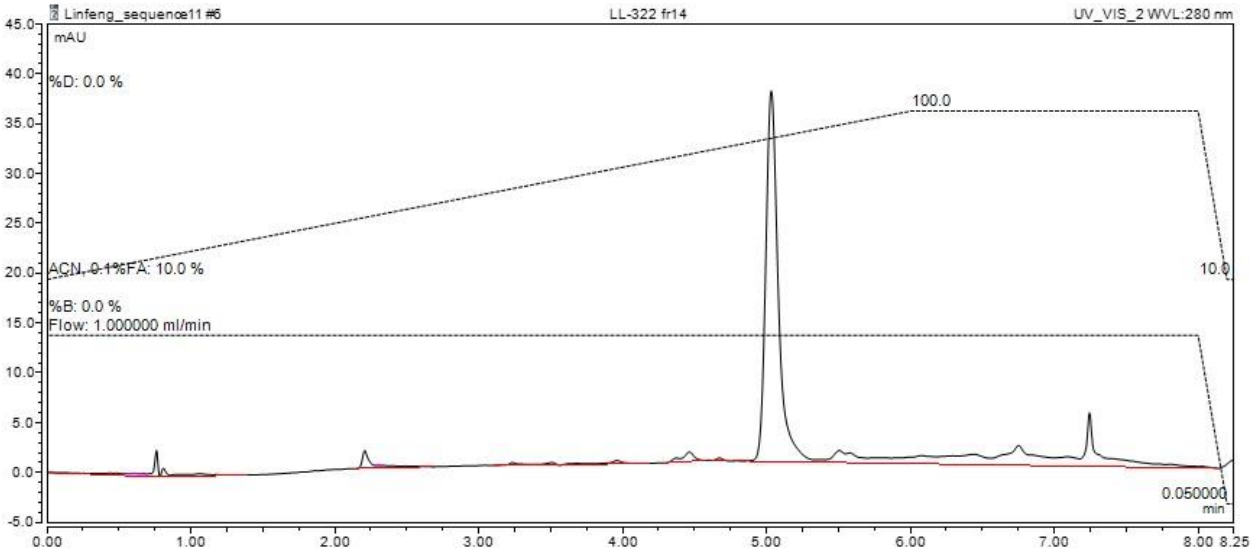


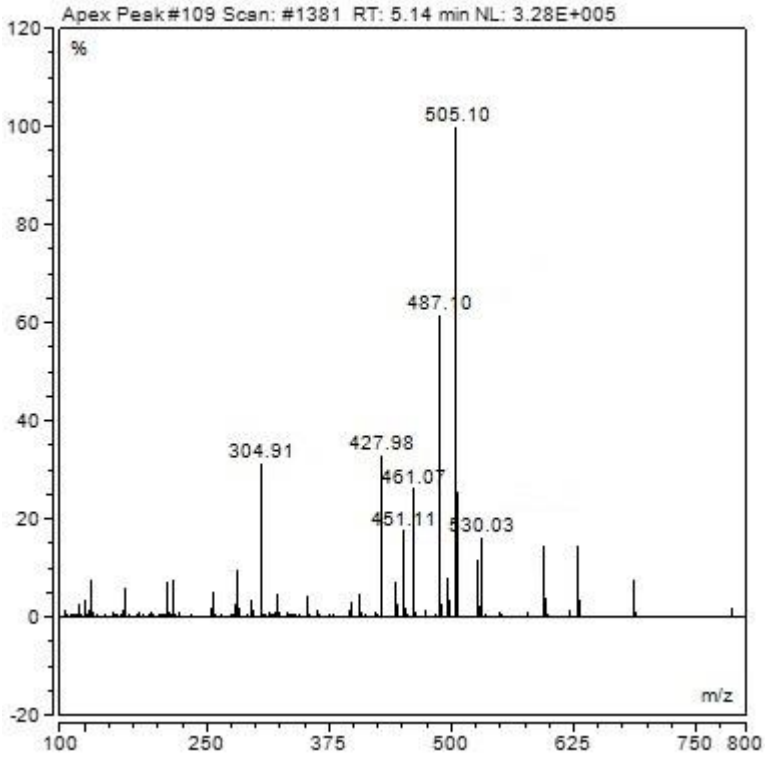
9: exact mass = 464.17



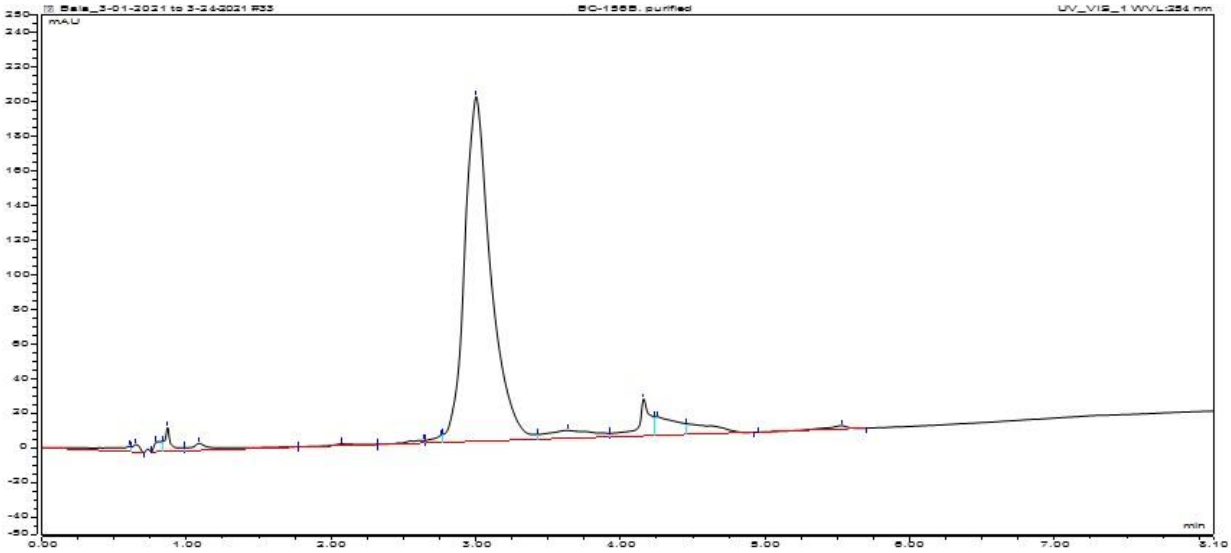


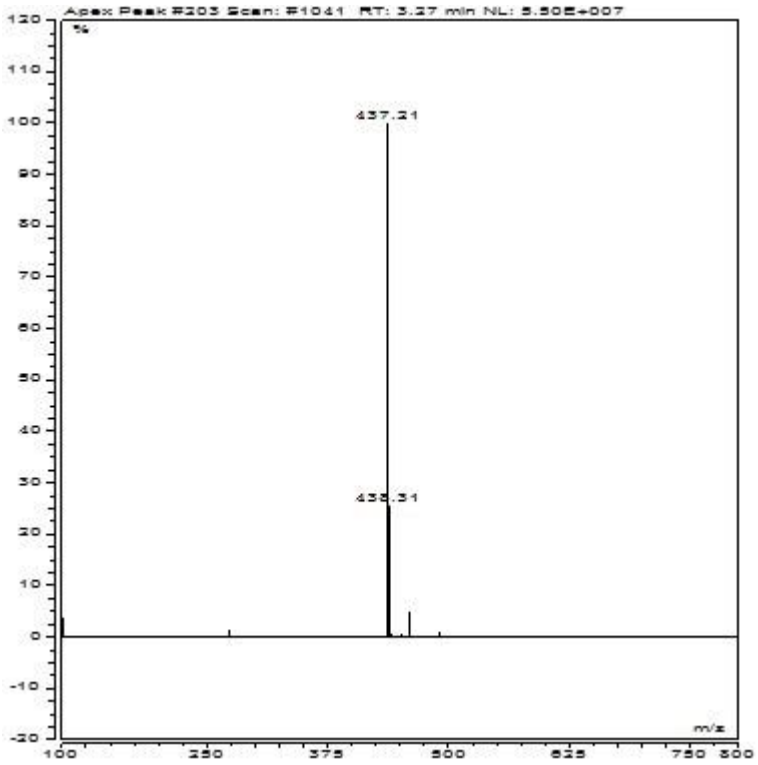
10: exact mass = 504.19



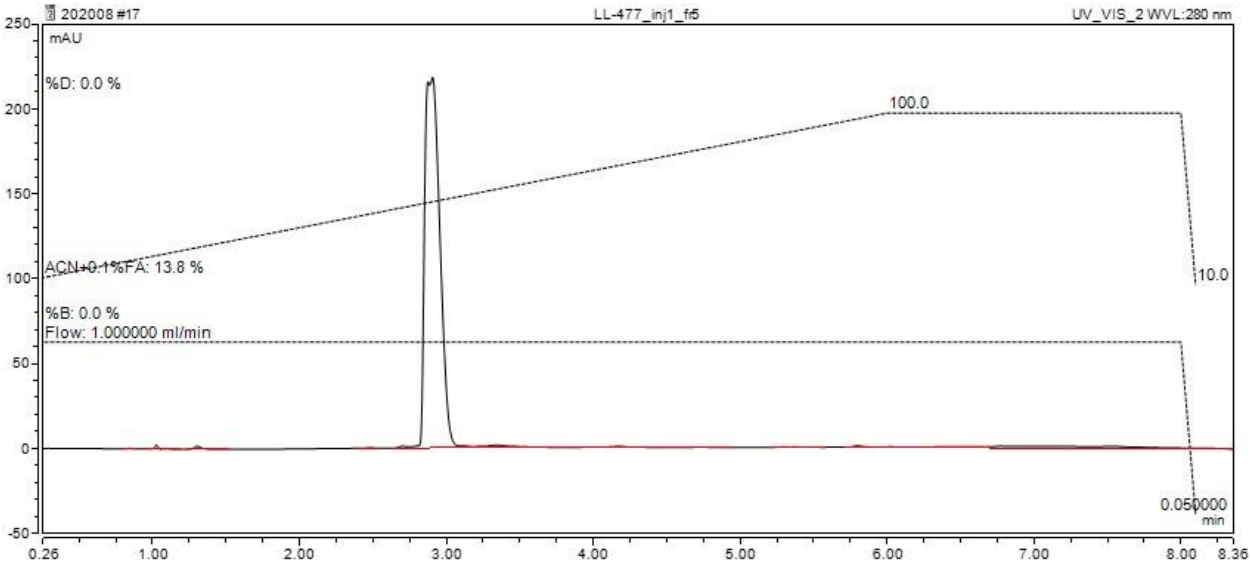


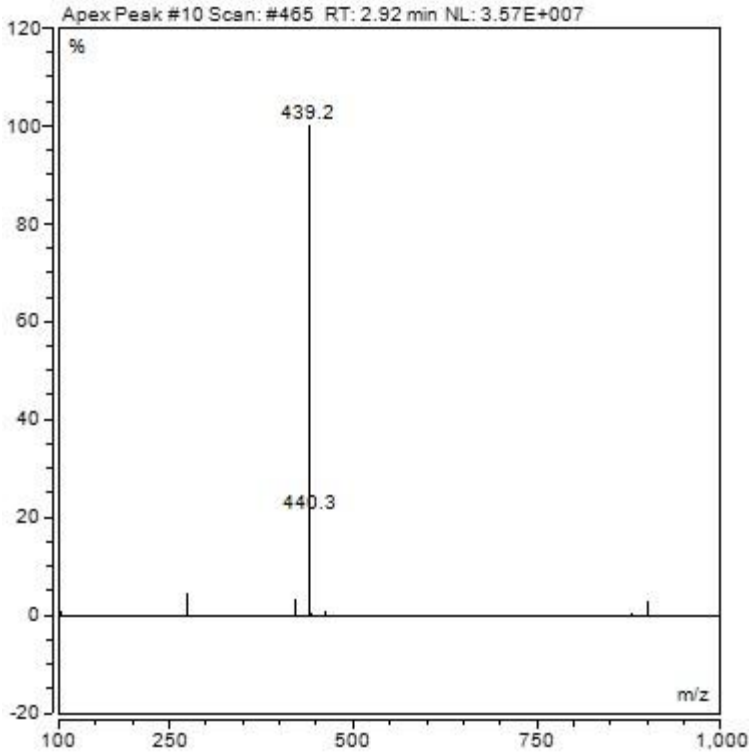
11: exact mass = 436.25



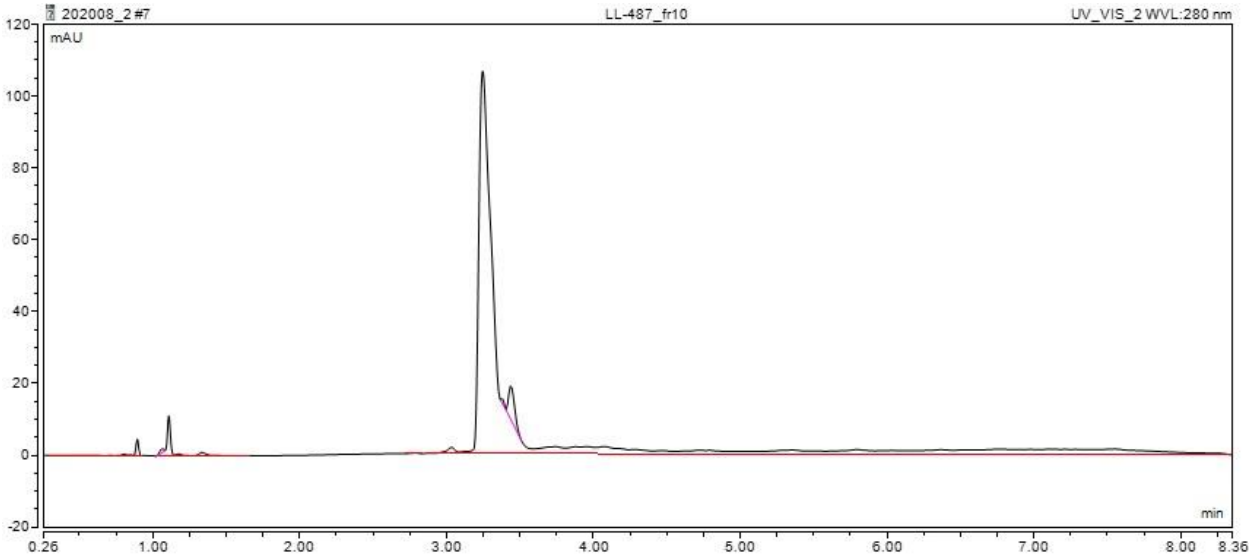


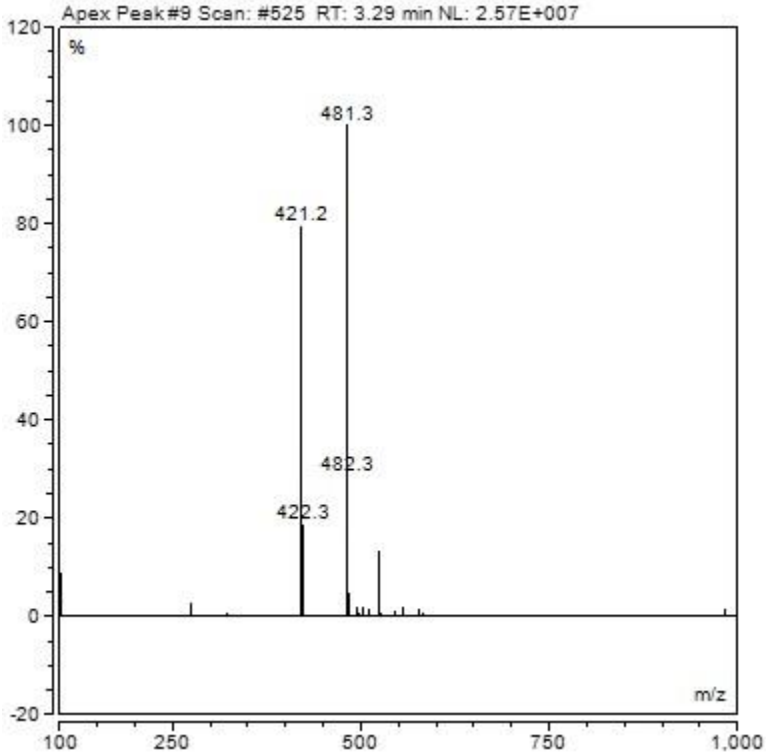
12: exact mass = 438.23



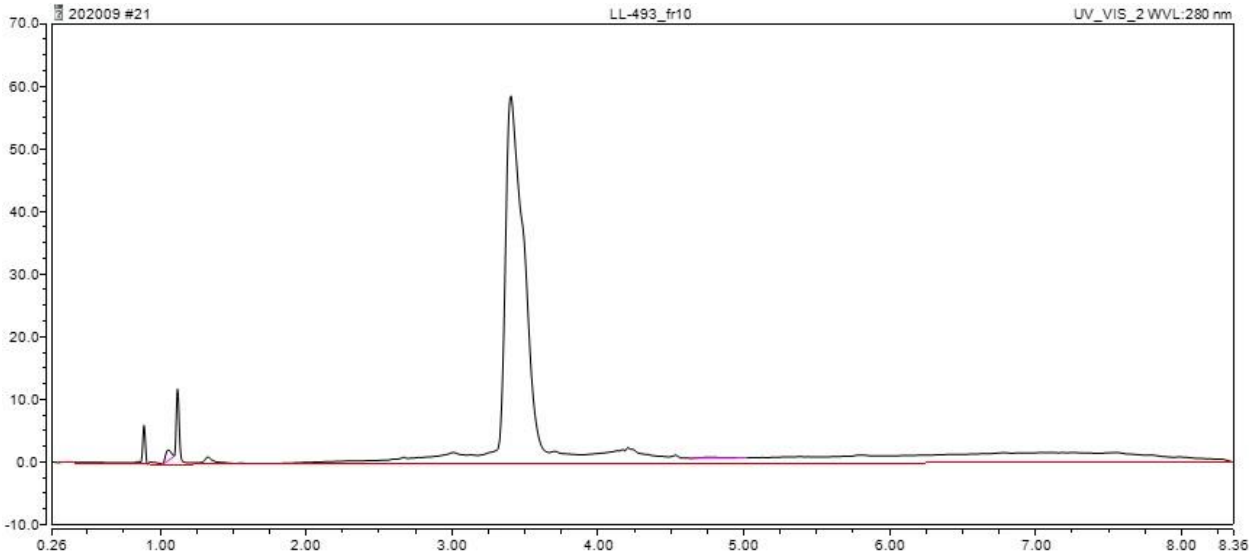


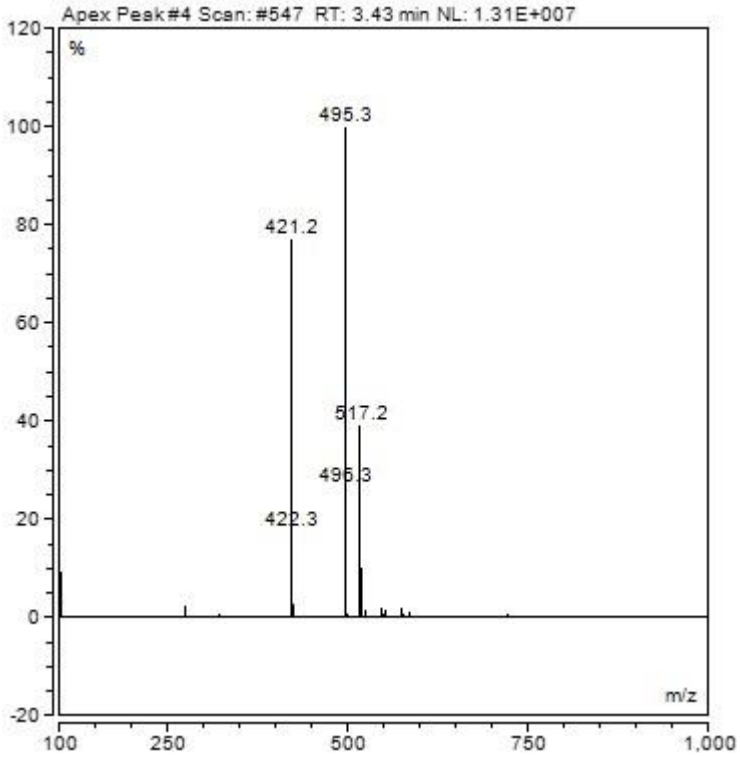
13: exact mass = 480.24



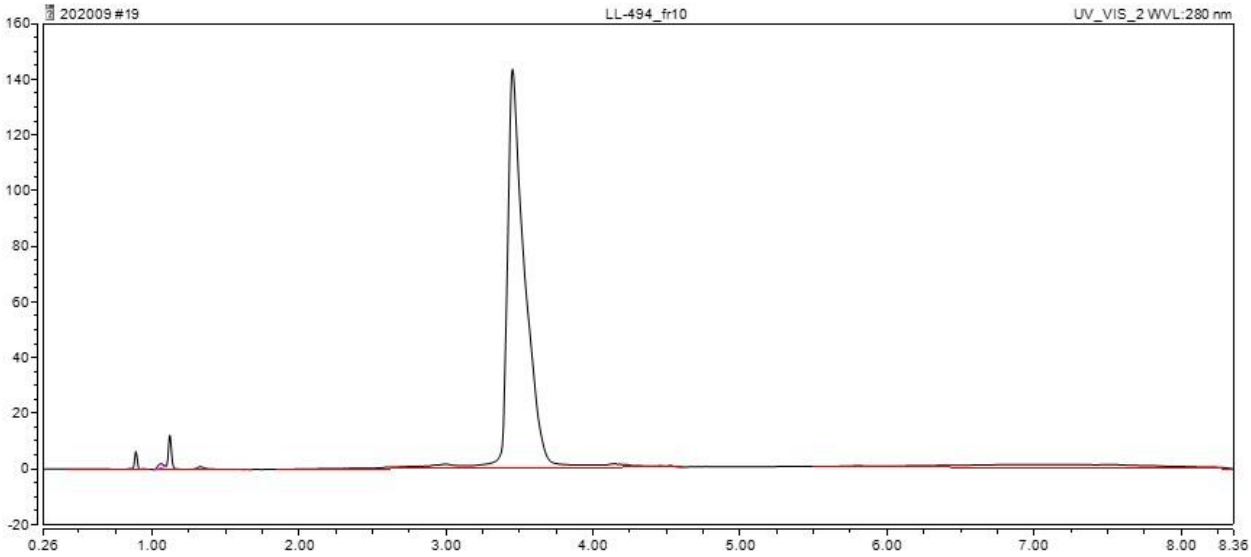


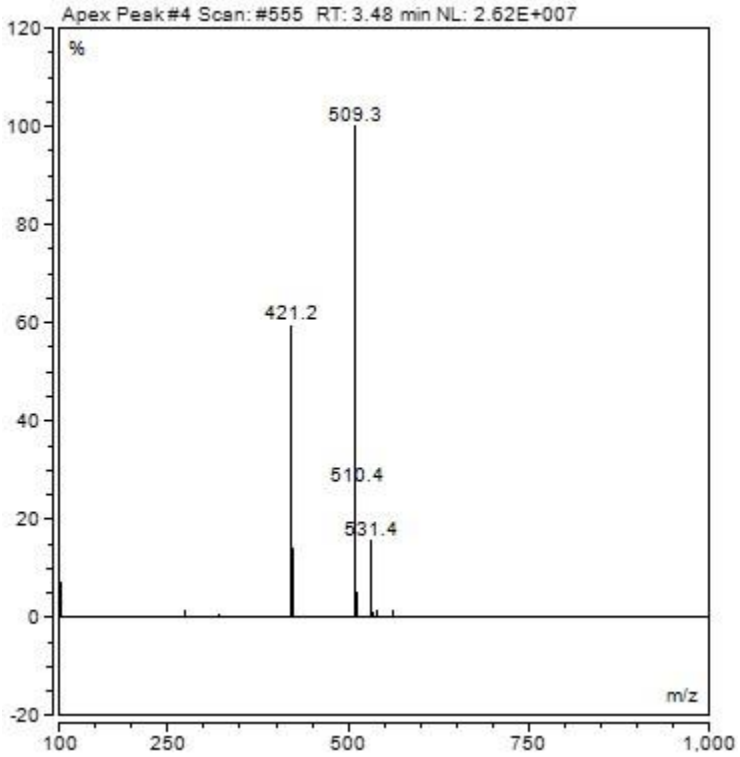
14: exact mass = 494.25



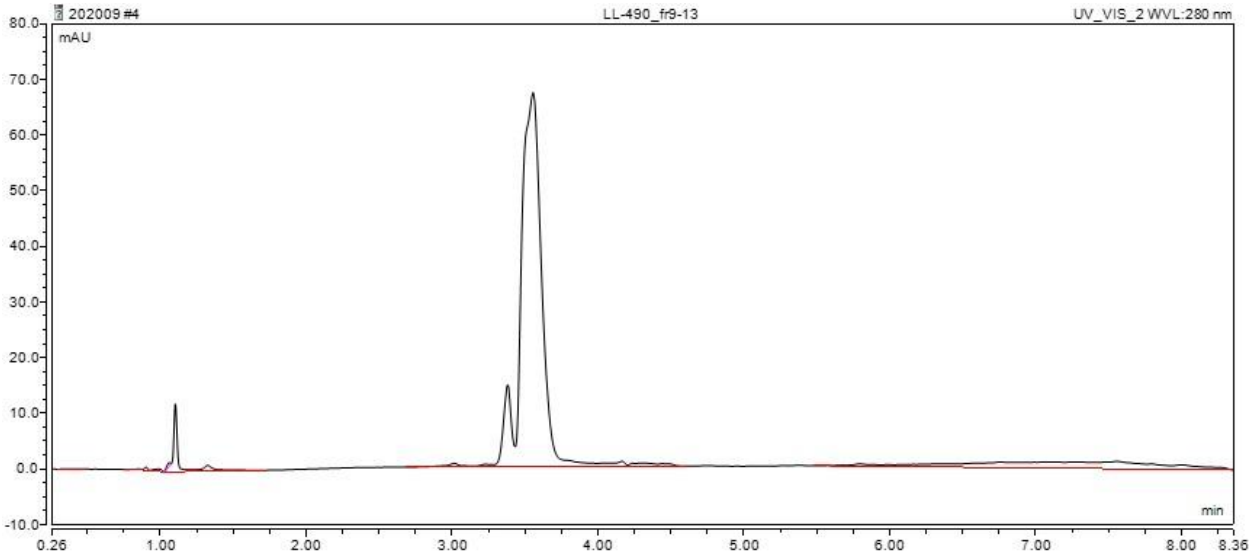


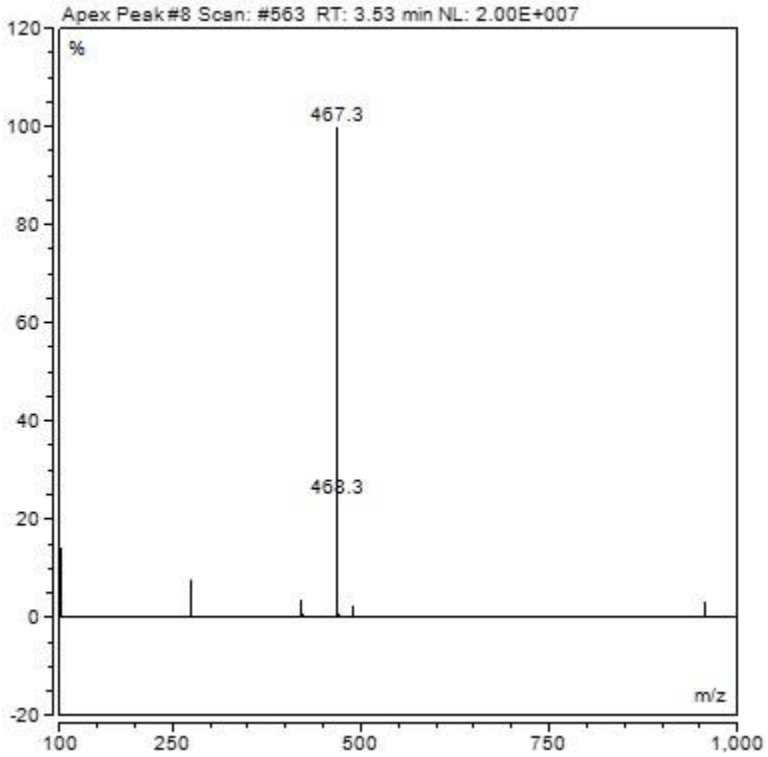
15: exact mass = 508.27



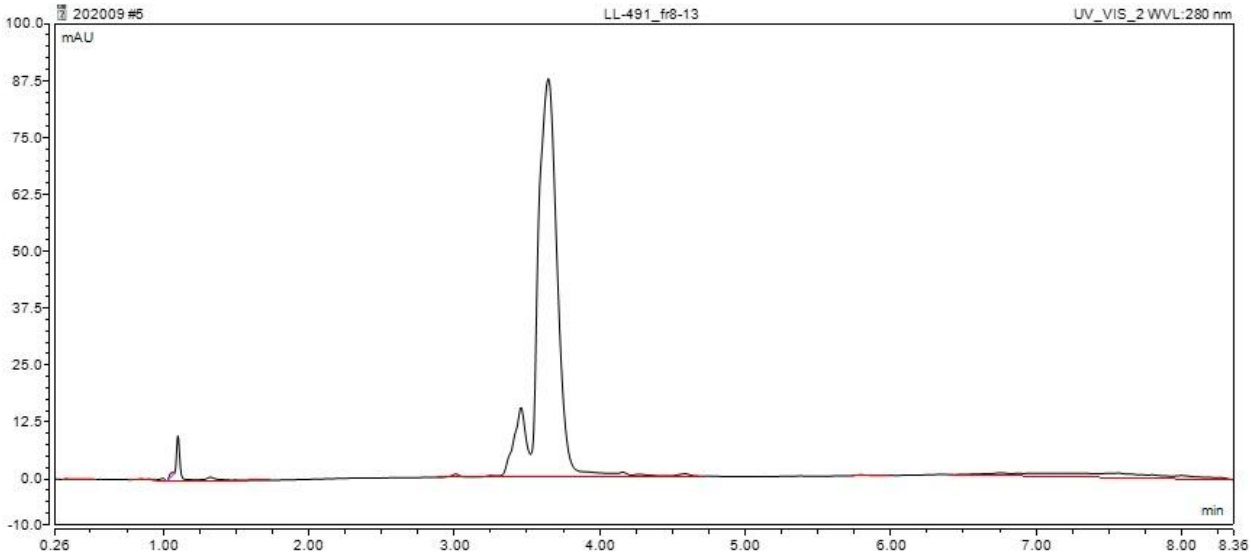


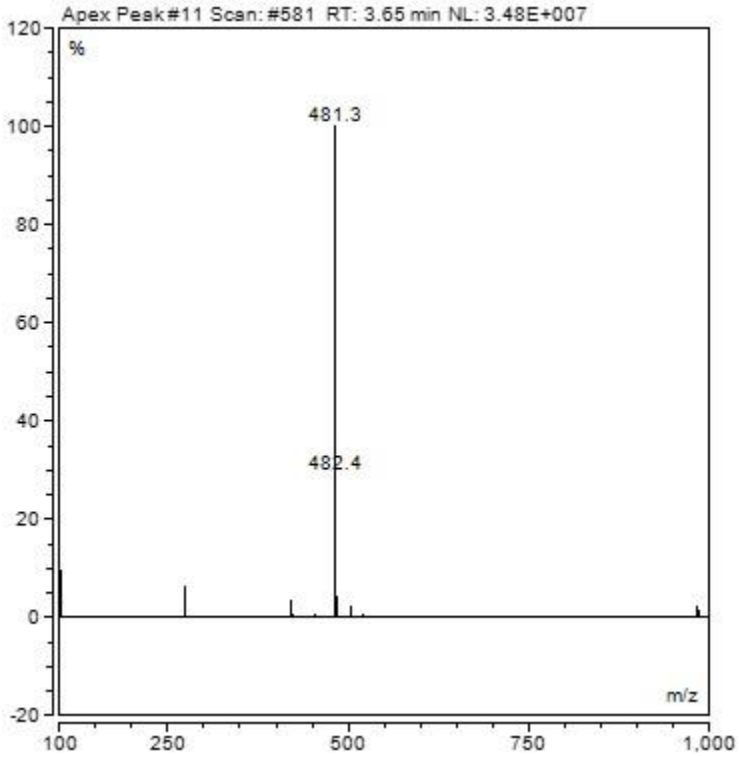
16: exact mass = 466.26





17: exact mass = 480.27





18: exact mass = 552.29