

**The War on hTG2: Warhead Optimization in Small Molecule Human Tissue
Transglutaminase Inhibitors**

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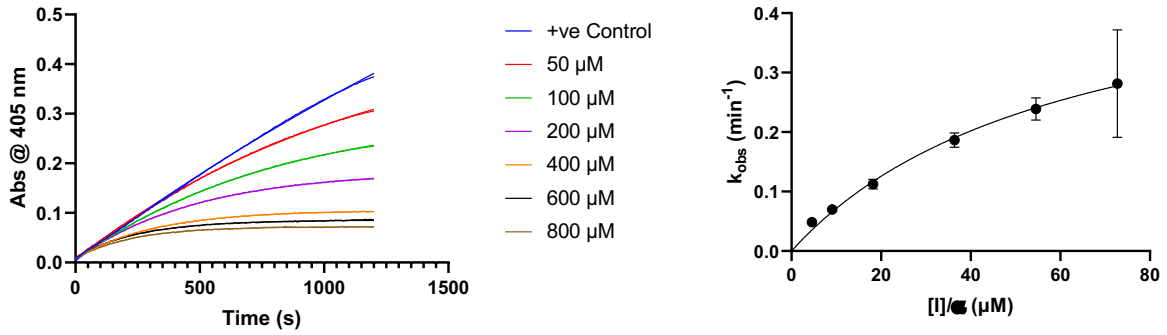
Supplementary Information

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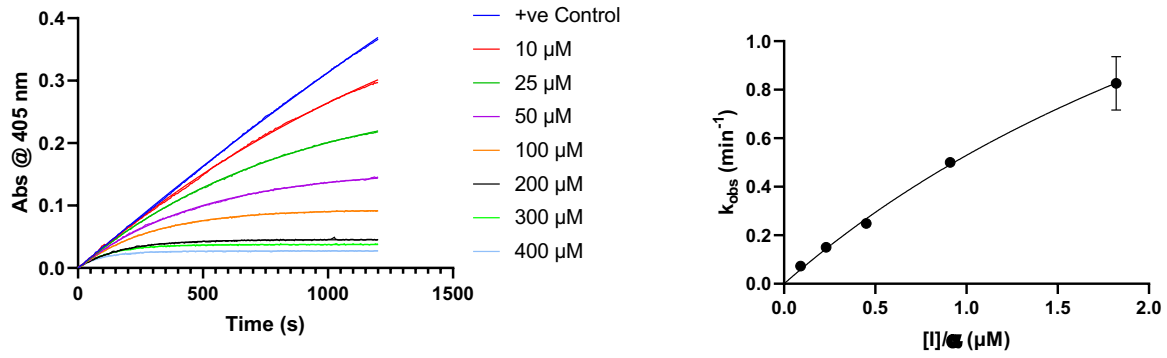
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hTG2 Inhibition Kinetics Data:

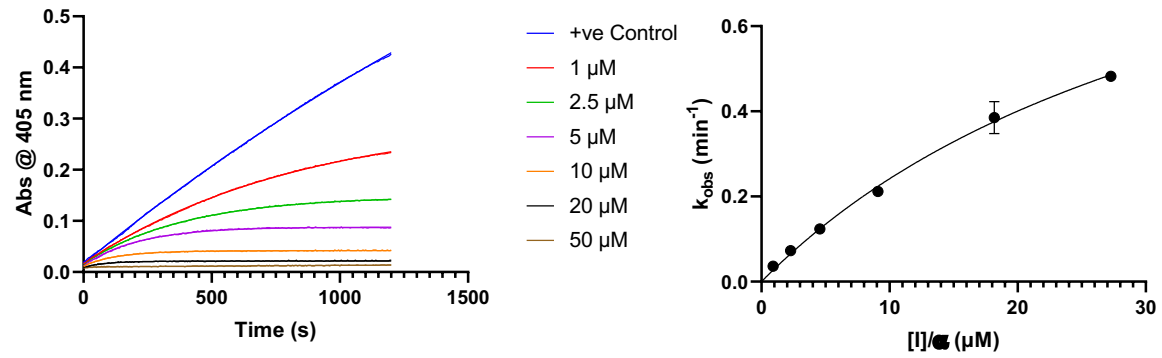
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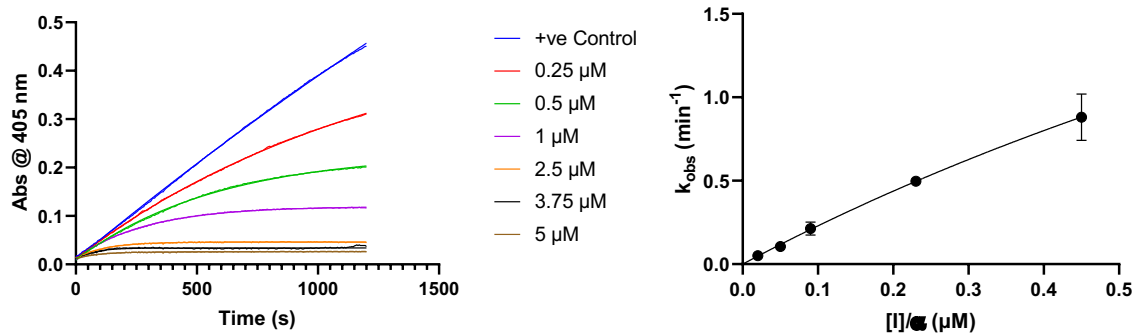
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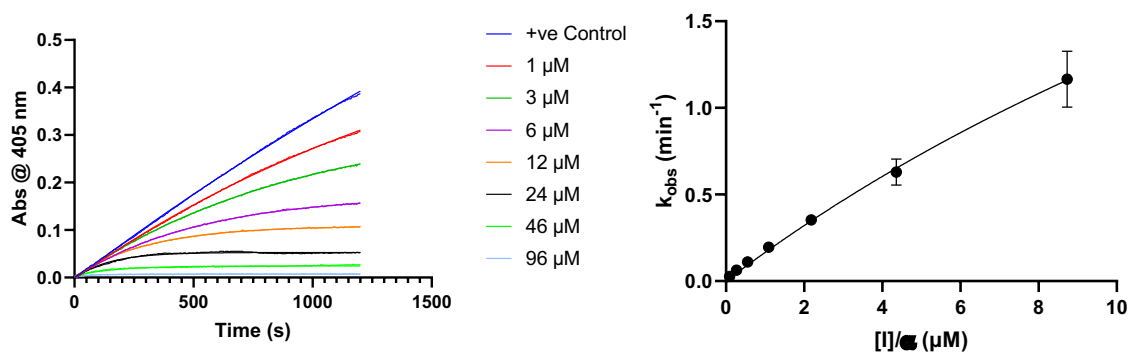
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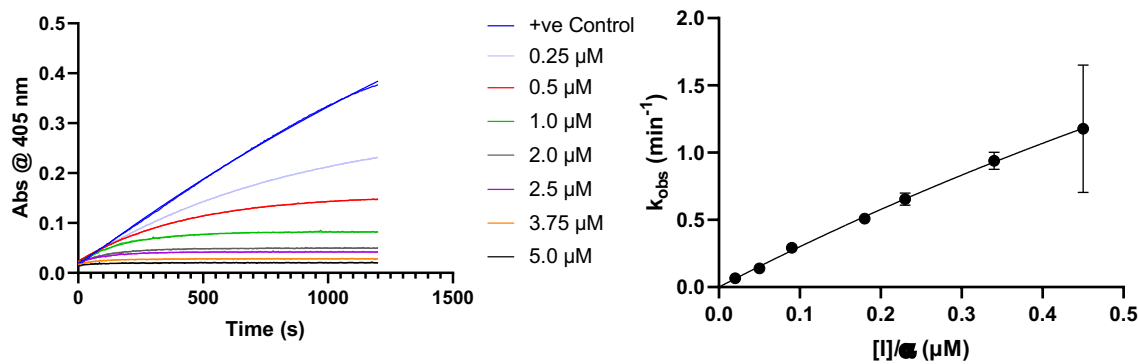
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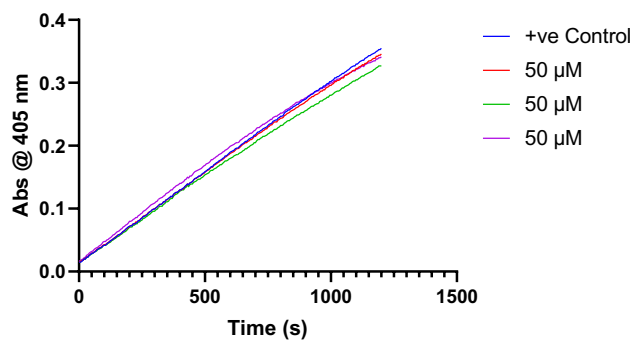
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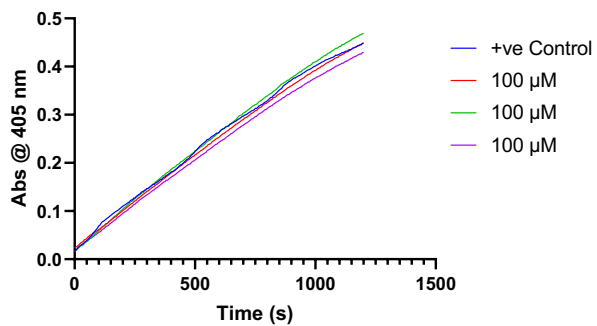
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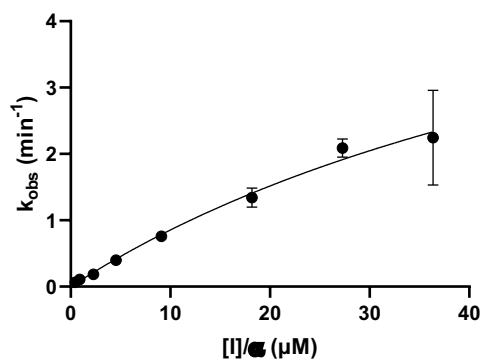
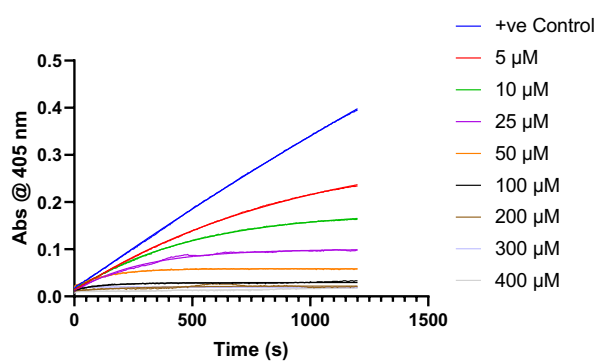
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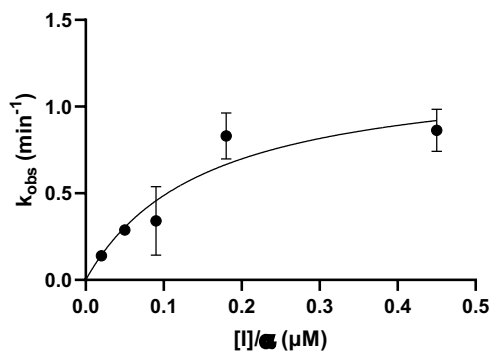
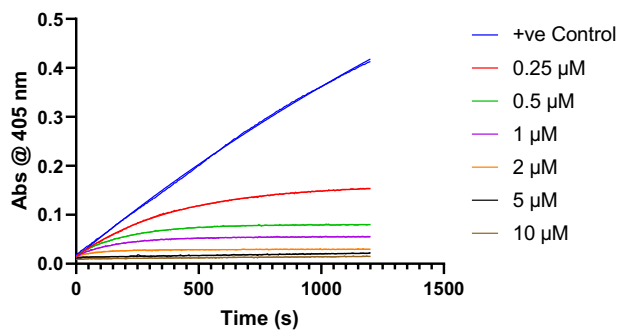
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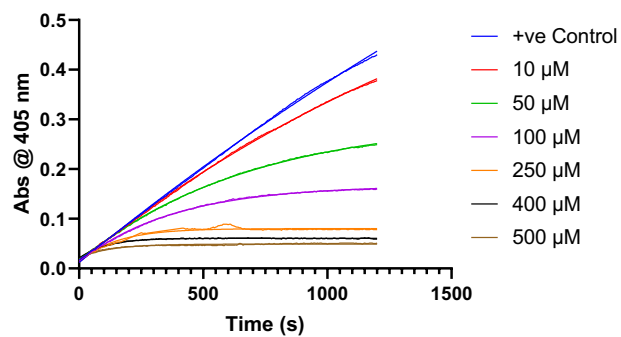
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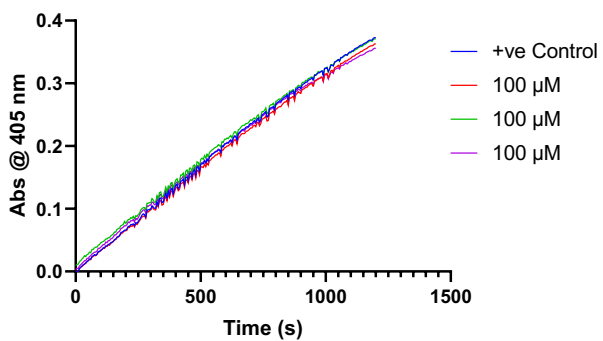
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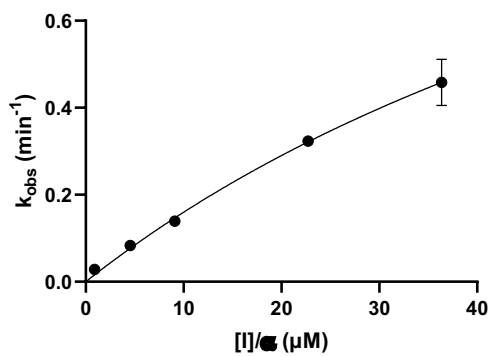
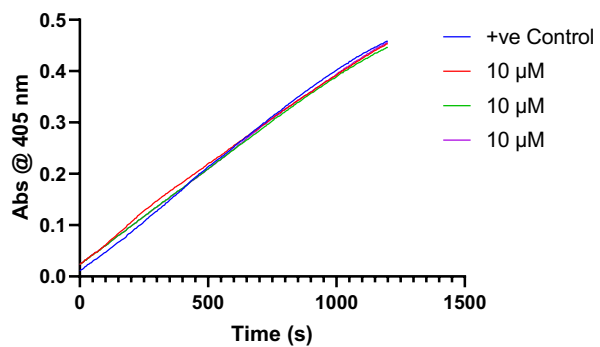
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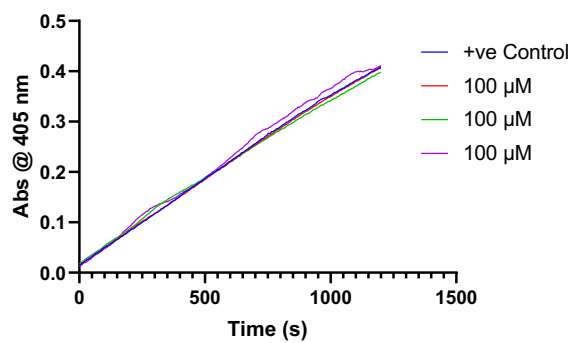
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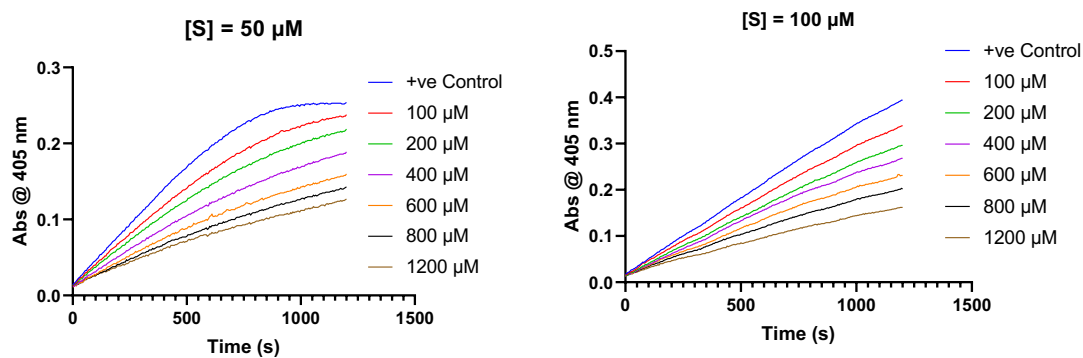
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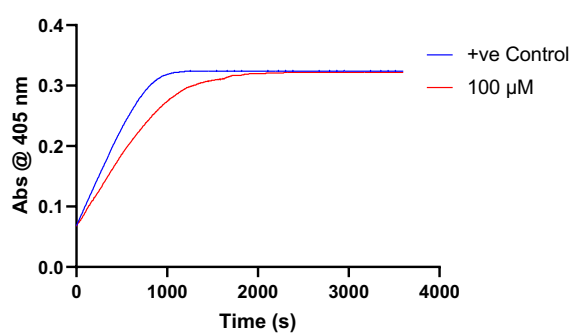
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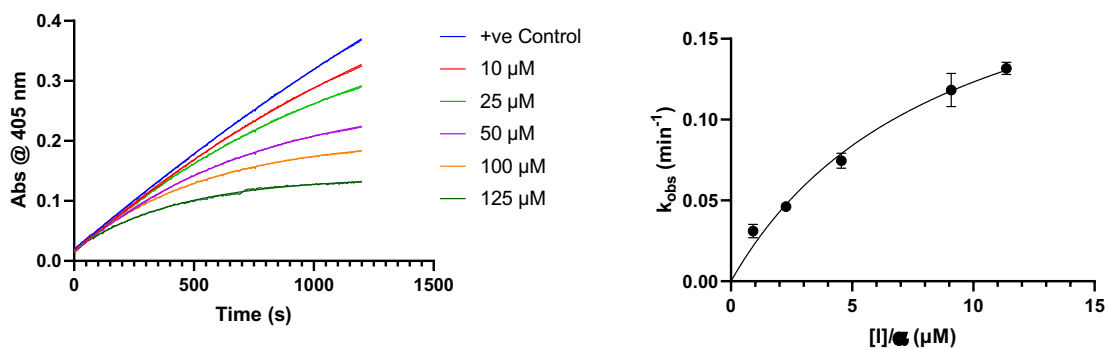
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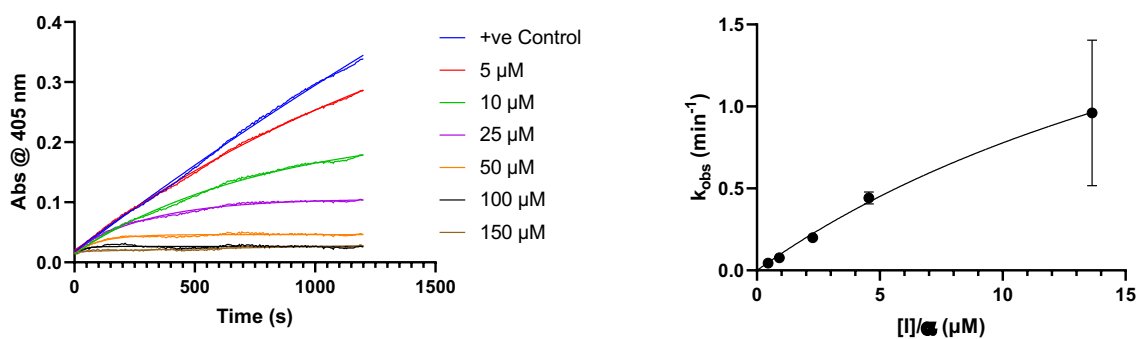
Reversibility Check ([S] = 50 μM)



7p



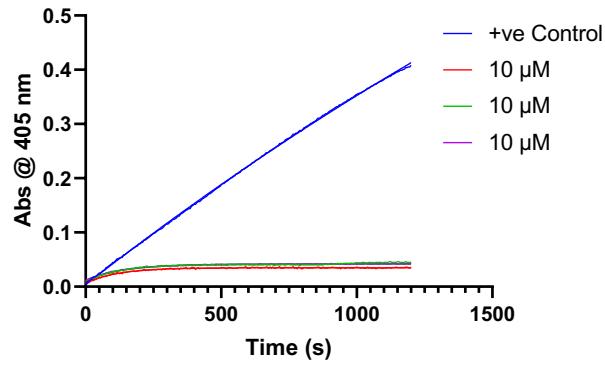
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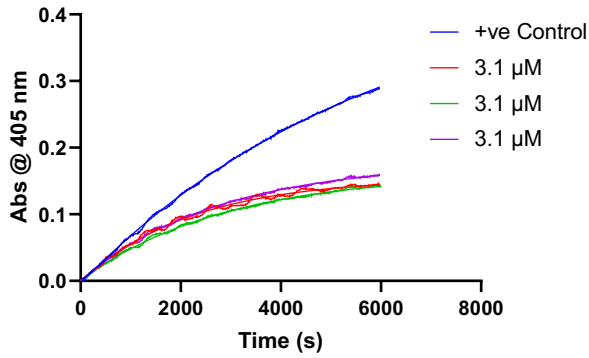
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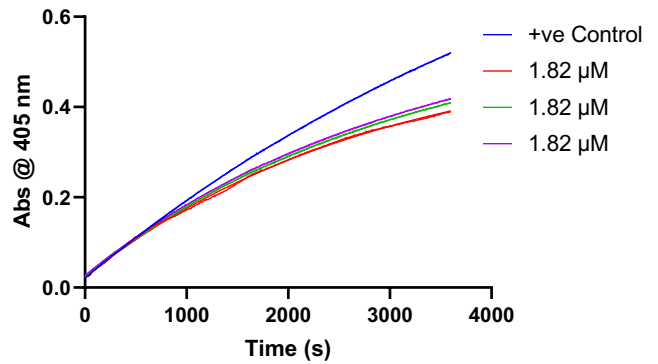
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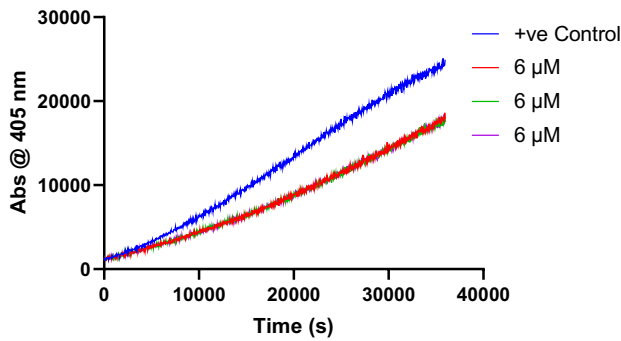
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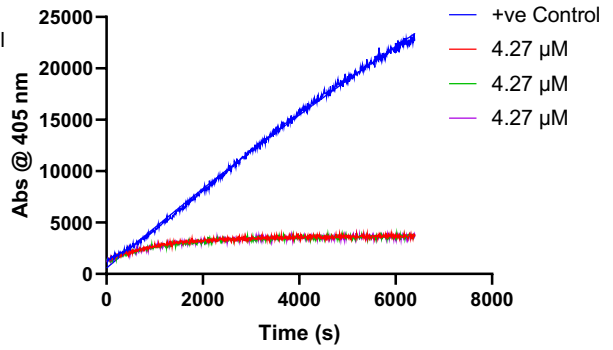
TG6



TG3a

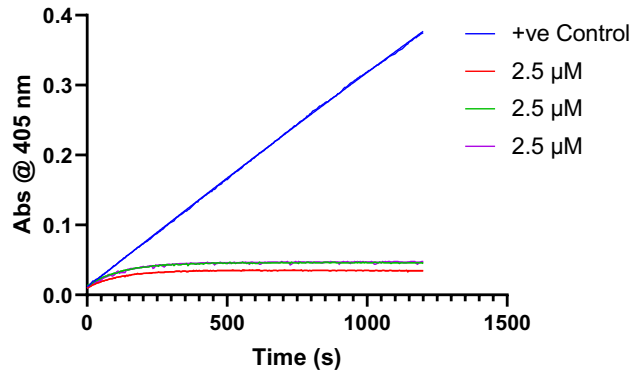


hFXIIIa

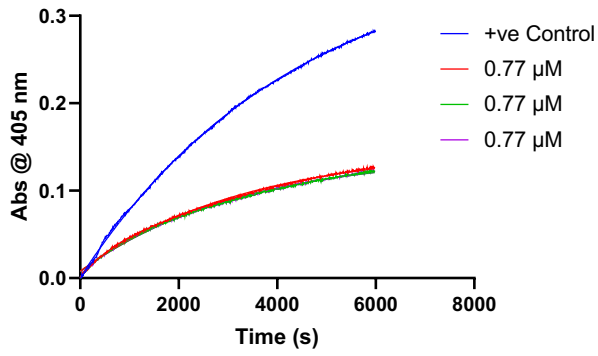


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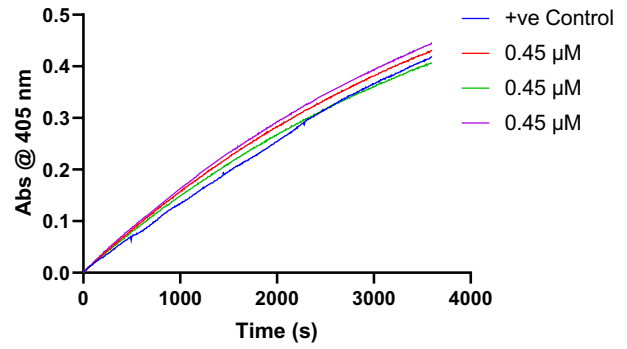
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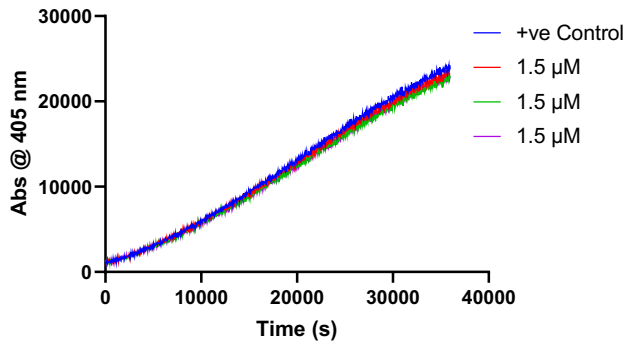
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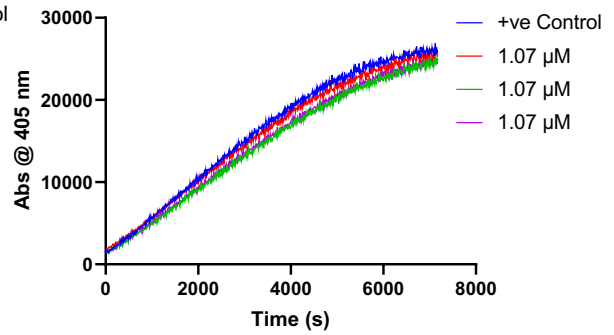
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TG3a

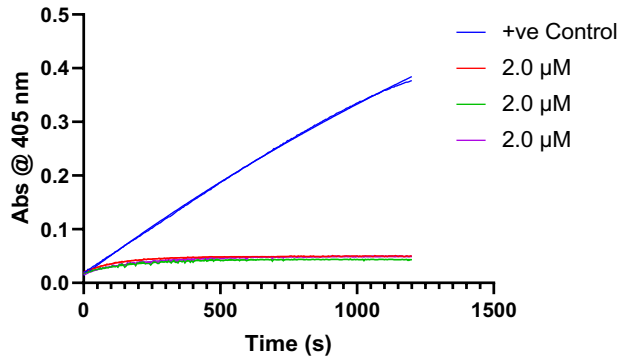


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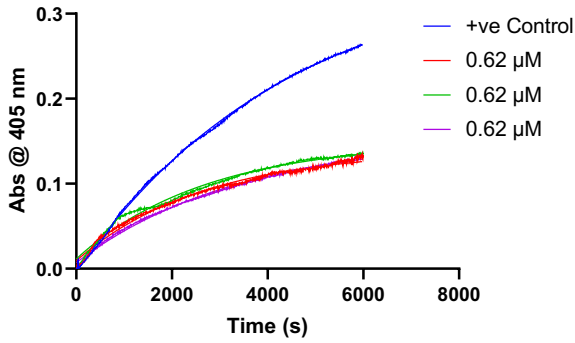


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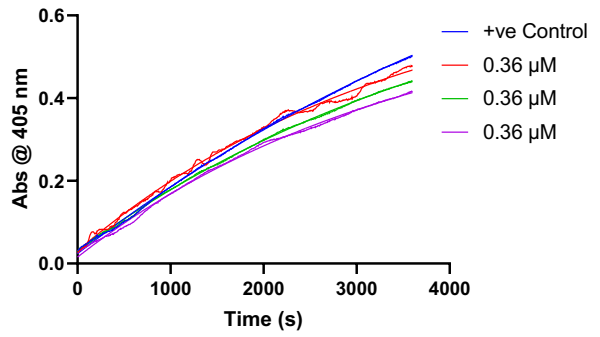
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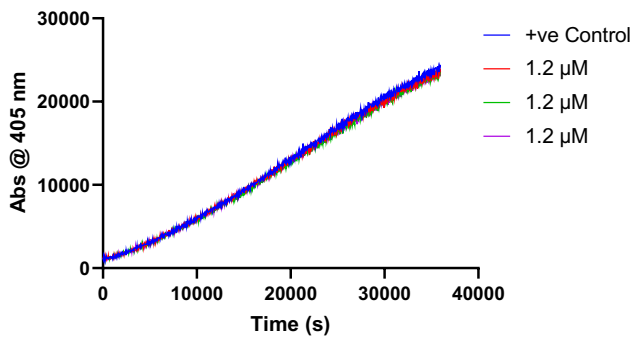
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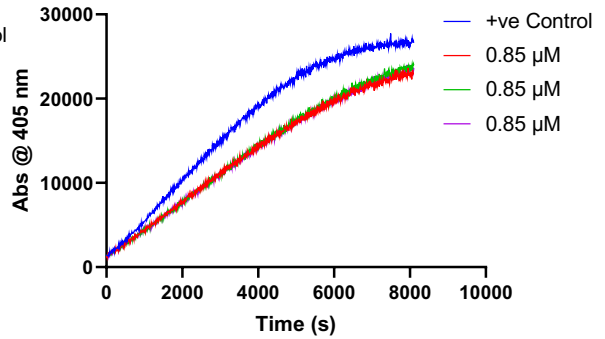
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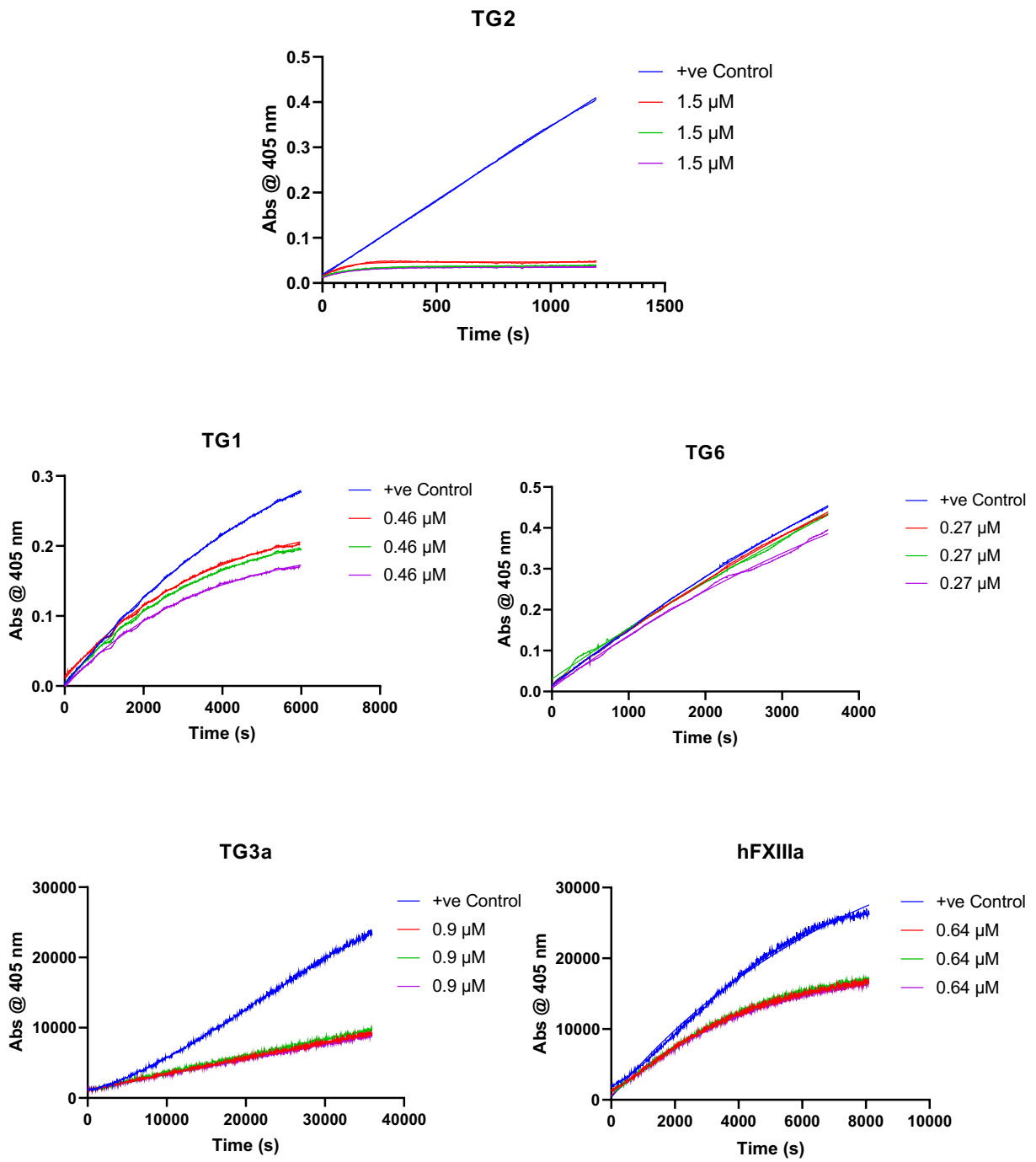


TG3a



hFXIIIa





Intrinsic Reactivity Data:

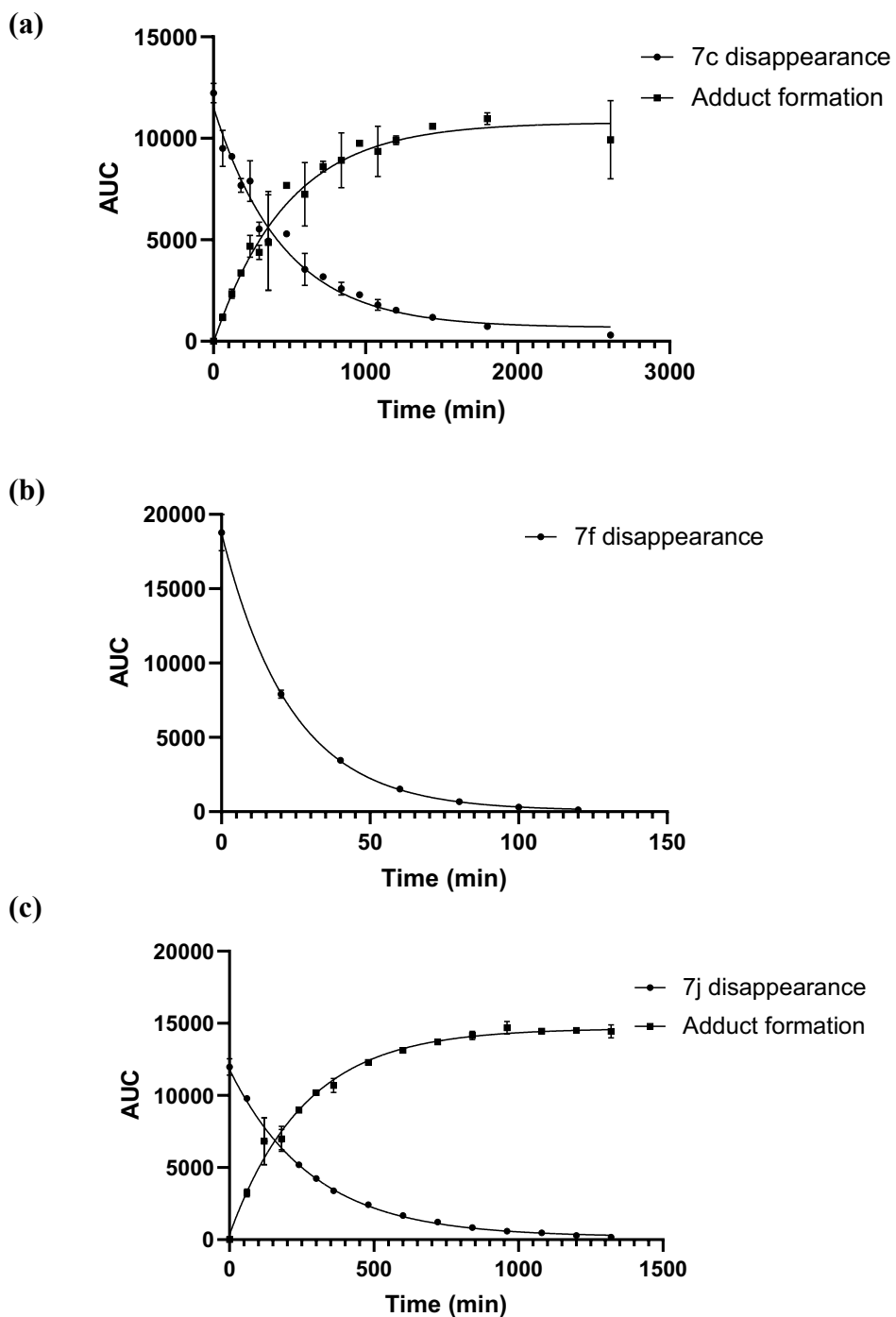


Figure S1: Plots representing the disappearance of inhibitor and/or appearance of thiol-inhibitor adduct. The area under the curve (AUC) were plotted over time and fitted to a mono-exponential equation to give pseudo-first order reaction rates (k_{obs}), (a) 7c + GSH, (b) 7f + GSH, (c) 7j + GSH.

LC-MS Data of Reaction Between 7f and GSH:

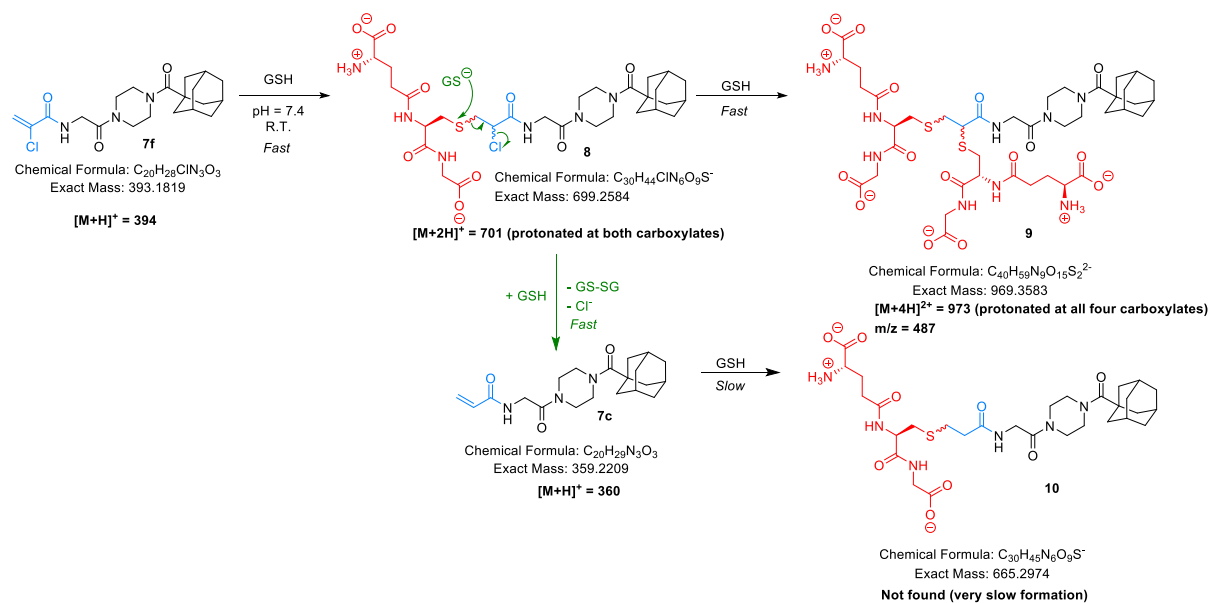
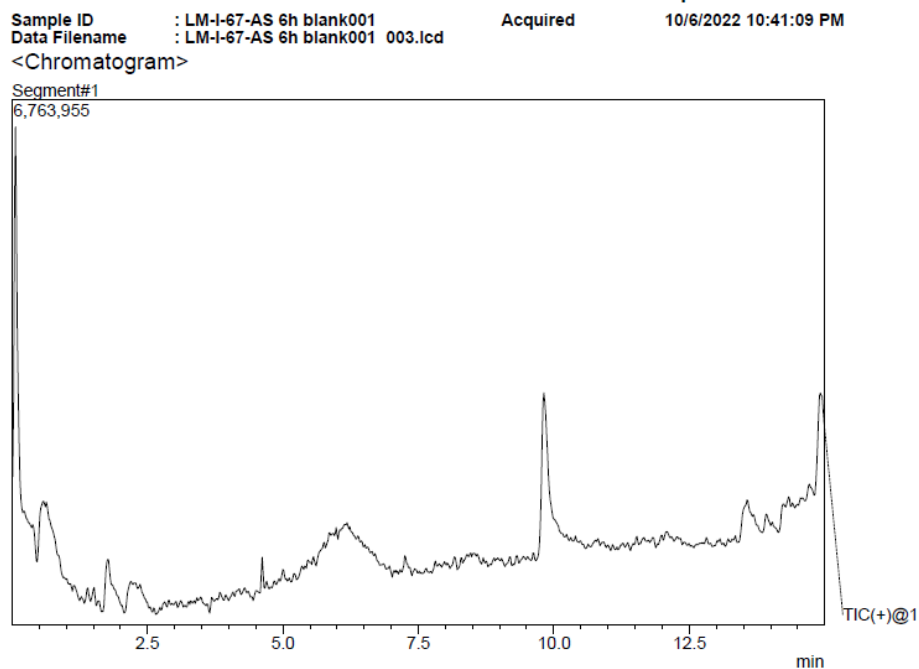


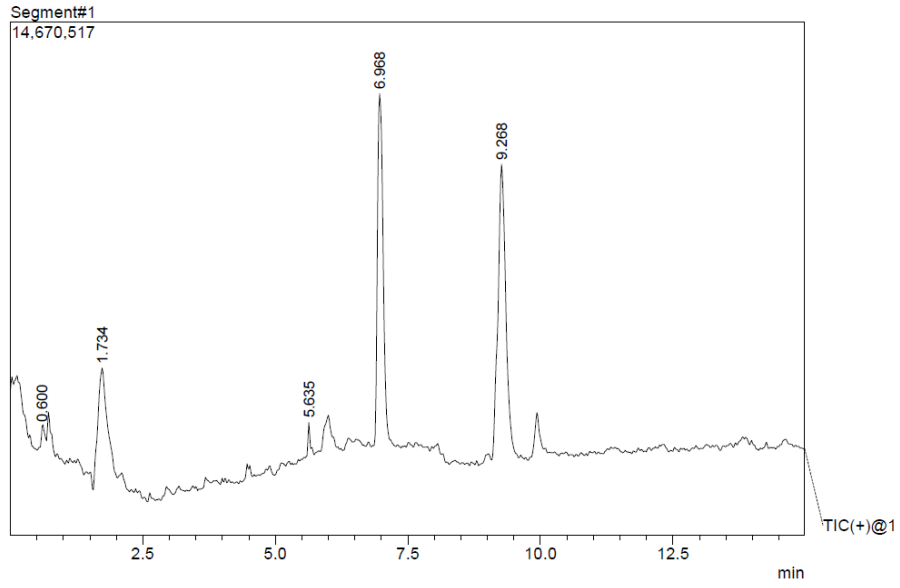
Figure S2: Products of reaction of 7f with GSH with m/z values found by LC-MS.

Chromatograms:

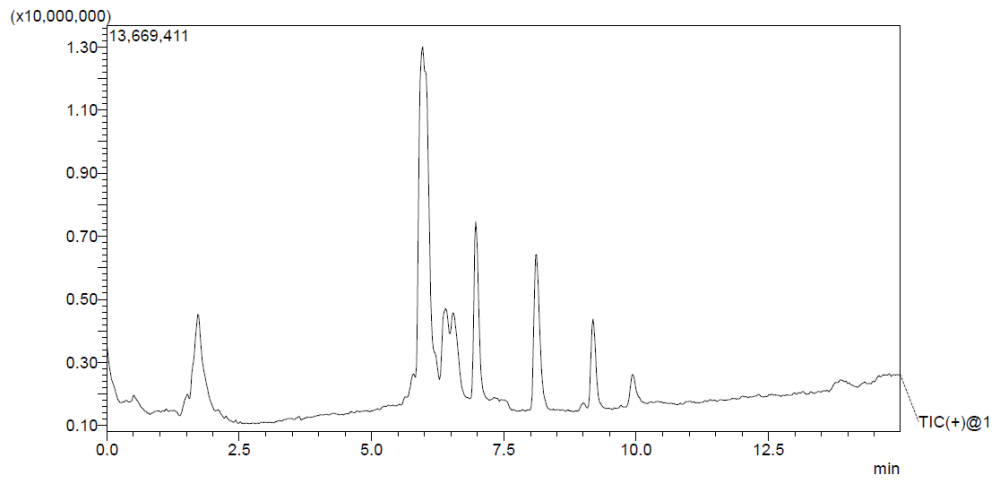
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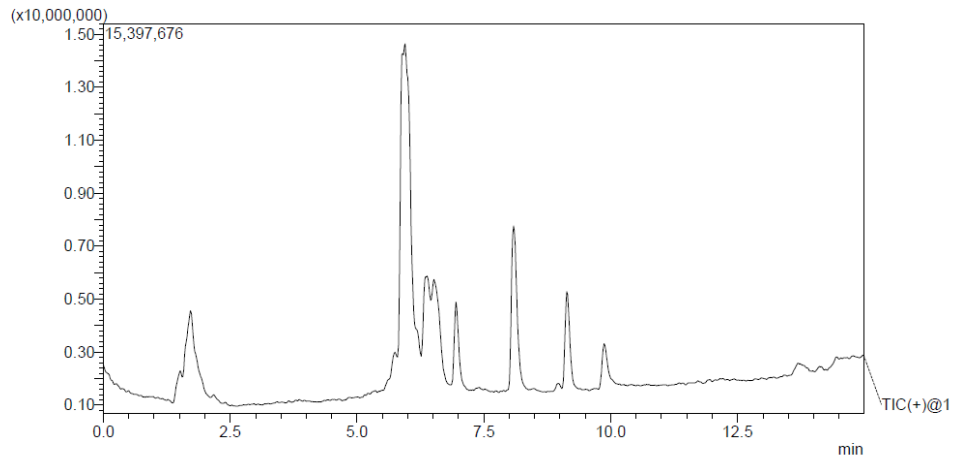
(T = 0)



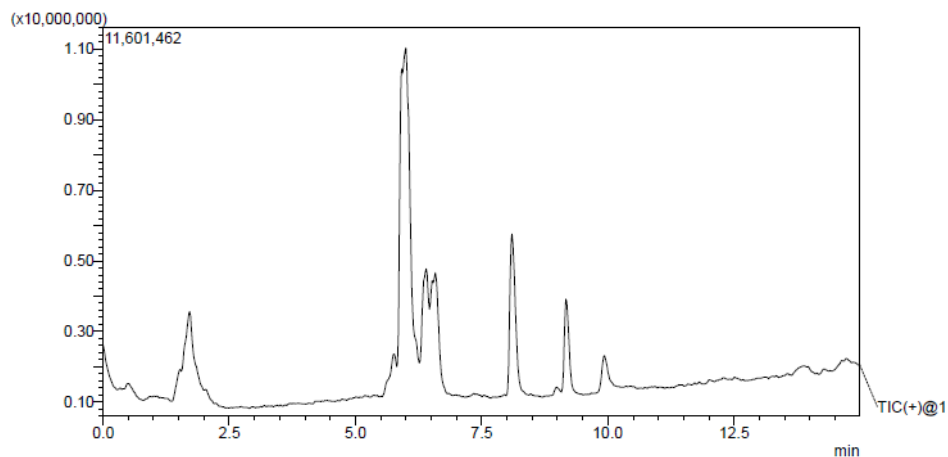
(T = 3h)



(T = 6h)



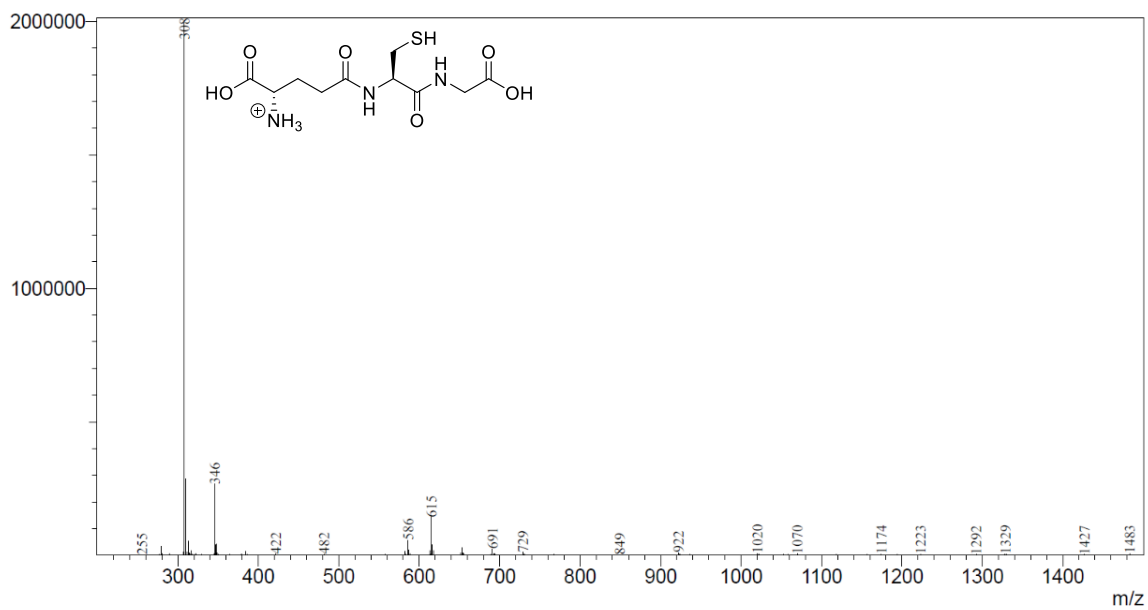
(T = 24h)



Mass Spectra of identifiable compounds (Compound, Retention Time):

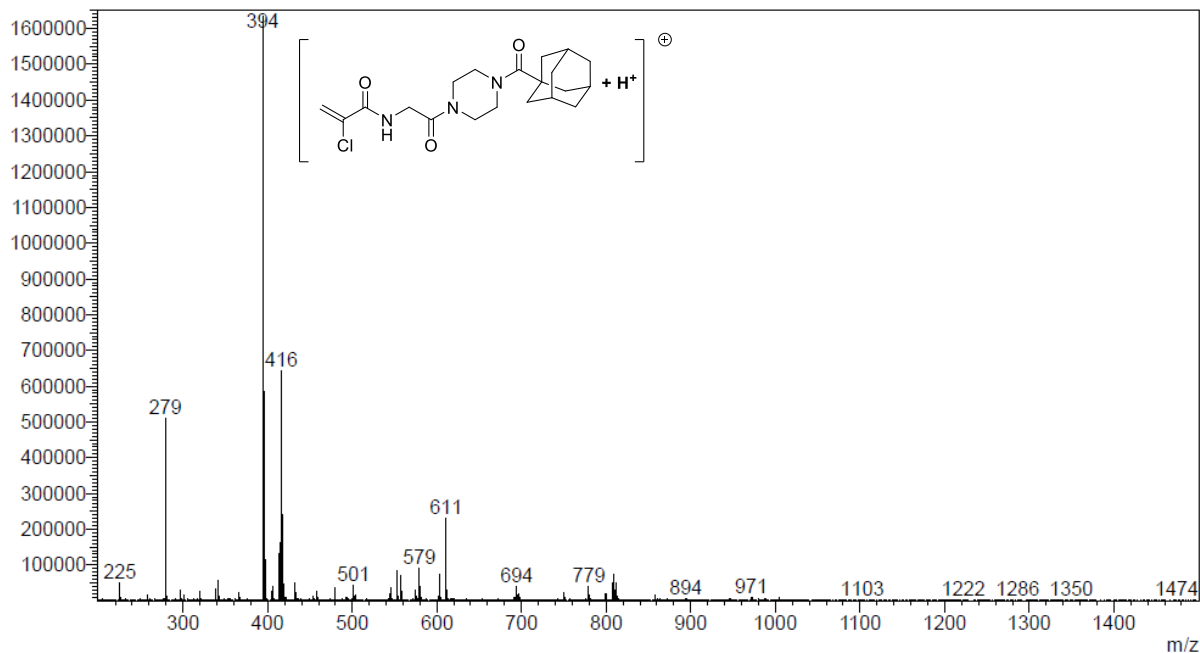
(GSH, 1.717 min)

Line#:1 R.Time:1.717(Scan#:104)
MassPeaks:1368
RawMode:Single 1.717(104) BasePeak:308(1994432)
BG Mode:None Segment 1 - Event 1



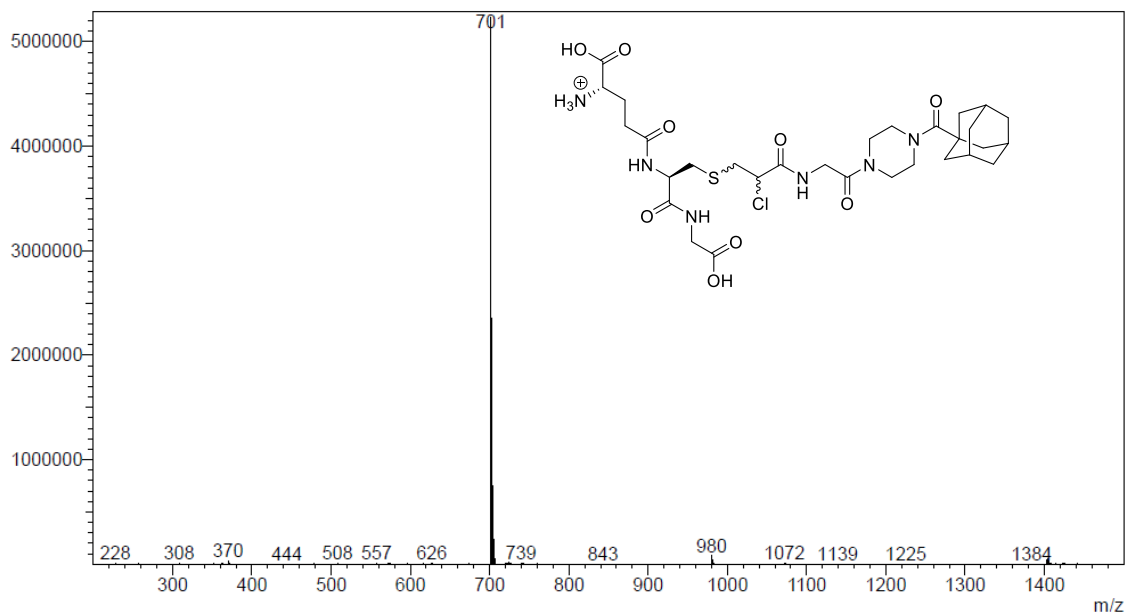
(7f, 9.333 min)

R.Time:9.333(Scan#:561)
MassPeaks:1340 BasePeak:394(1636407)
Spectrum Mode:Single 9.333(561)
BG Mode:None Polarity:Positive Segment 1 - Event 1



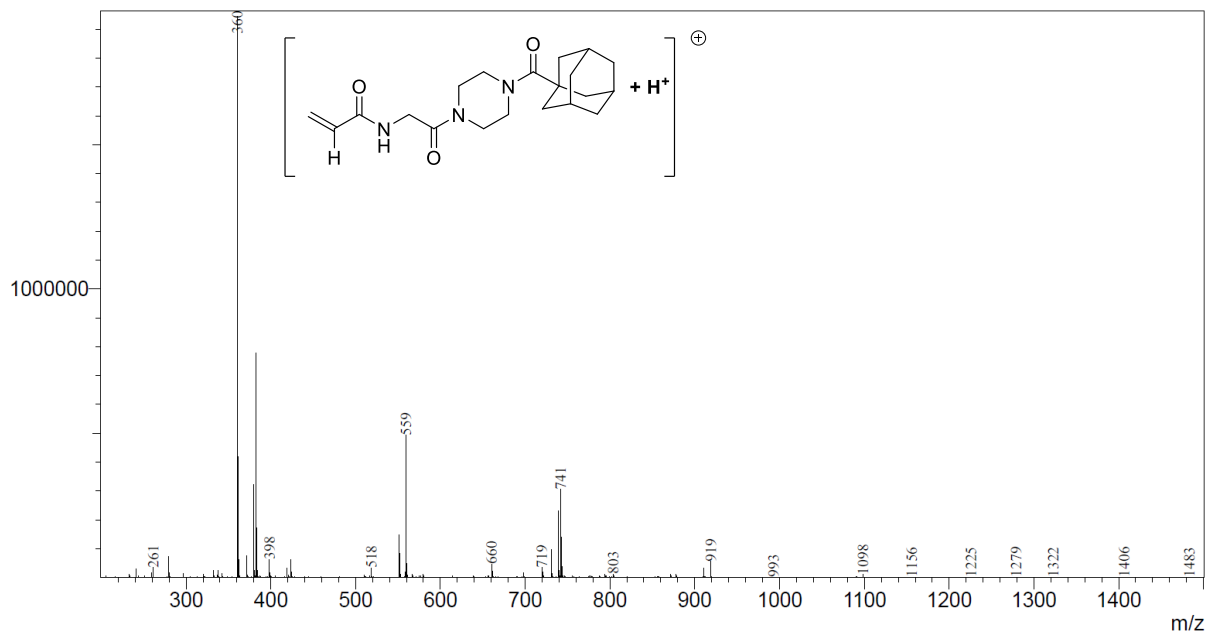
(8, 7.350 min)

R.Time:----(Scan#:----)
MassPeaks:437 BasePeak:701(5239228)
Spectrum Mode:Averaged 6.950-6.983(418-420)
BG Mode:Calc Polarity:Positive Segment 1 - Event 1



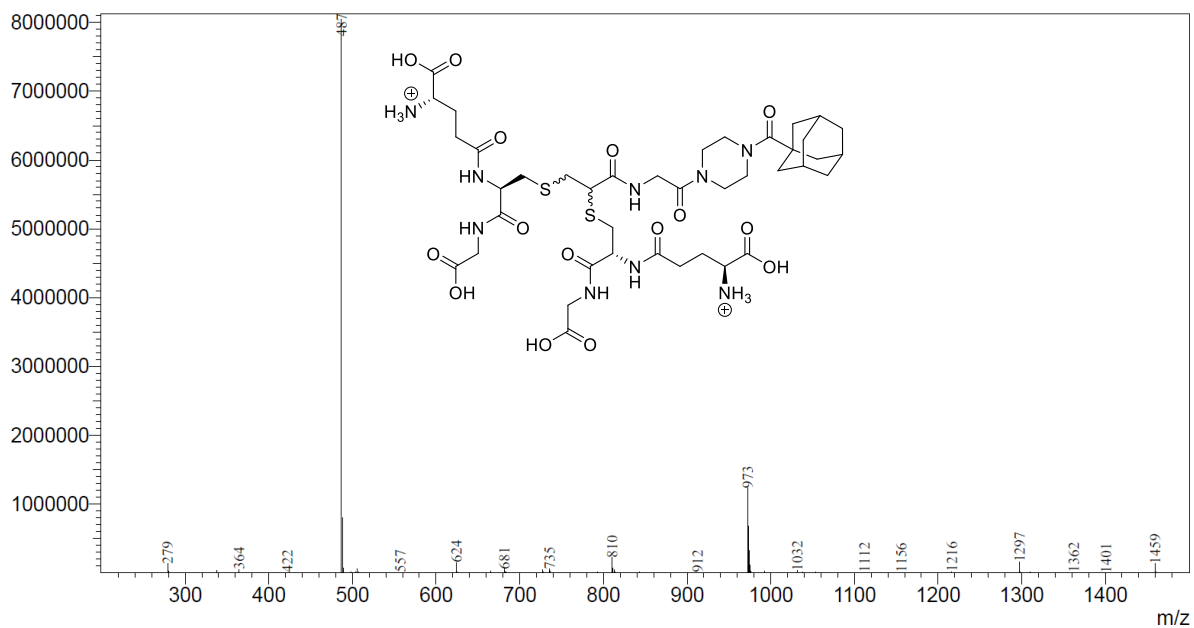
(7c, 8.083 min)

Line#:1 R.Time:8.083(Scan#:486)
MassPeaks:1344
RawMode:Single 8.083(486) BasePeak:360(1944438)
BG Mode:None Segment 1 - Event 1



(9, 5.933 min)

Line#:1 R.Time:5.933(Scan#:357)
MassPeaks:1365
RawMode:Single 5.933(357) BasePeak:487(8044516)
BG Mode:None Segment 1 - Event 1

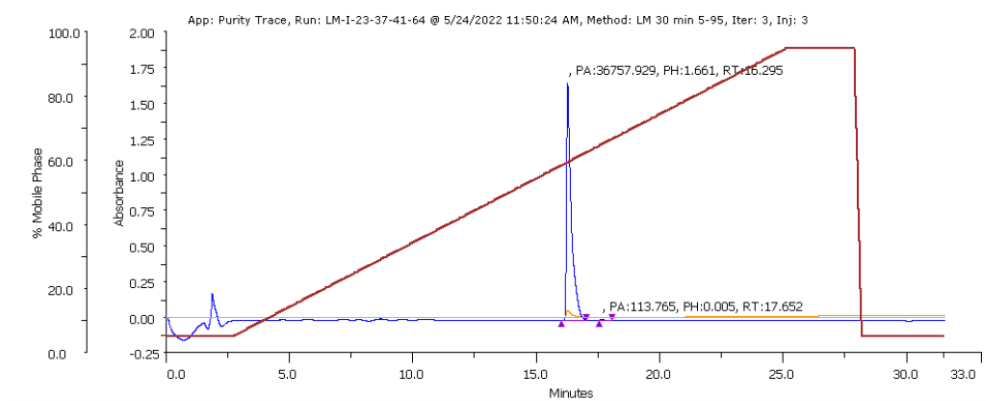


HPLC Purity Analysis of Final Inhibitors:

| Inhibitor | Retention Time (min) | Purity (%) |
|-----------|----------------------|------------|
| 7a | 16.295 | 99.691 |
| 7b* | 15.017 | 100 |
| 7c* | 12.520 | 96.285 |
| 7d | 18.468 | 99.670 |
| 7e | 18.838 | 93.586 |
| 7f | 17.996 | 99.741 |
| 7g | 17.374 | 99.824 |
| 7h | 16.823 | 100 |
| 7i | 16.075 | 100 |
| 7j* | 14.270 | 98.501 |
| 7k | 17.477 | 90.763 |
| 7l | 15.803 | 99.702 |
| 7m | 27.028 | 100 |
| 7n | 18.817 | 97.148 |
| 7o | 16.550 | 98.985 |
| 7p | 20.905 | 100 |
| 7q | 14.142 | 91.349 |

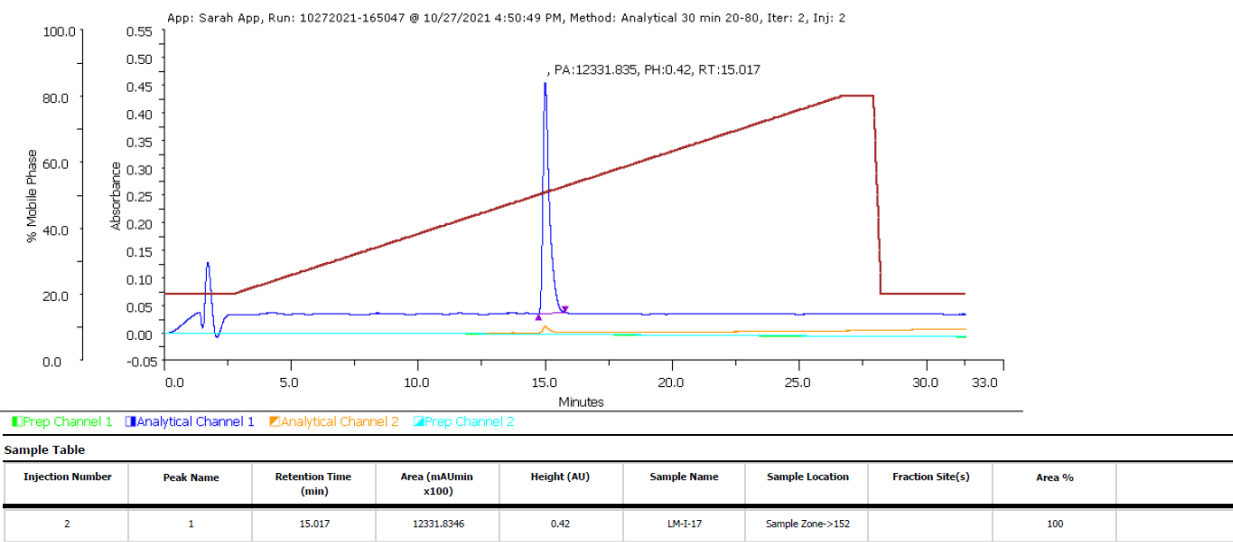
Table S1: The purity of the final inhibitors determined by Gilson-Mandel GXP271 high performance liquid chromatography (HPLC) with UV detection at 214 and 254 nm (Phenomenex Luna, 150 mm × 4.6 mm, 30 min, 1.5 mL/min flow rate, 5-95% 0.1% TFA in MeCN/0.1% TFA in H₂O unless otherwise indicated). *20-80% 0.1% TFA in MeCN/0.1% TFA in H₂O

7a

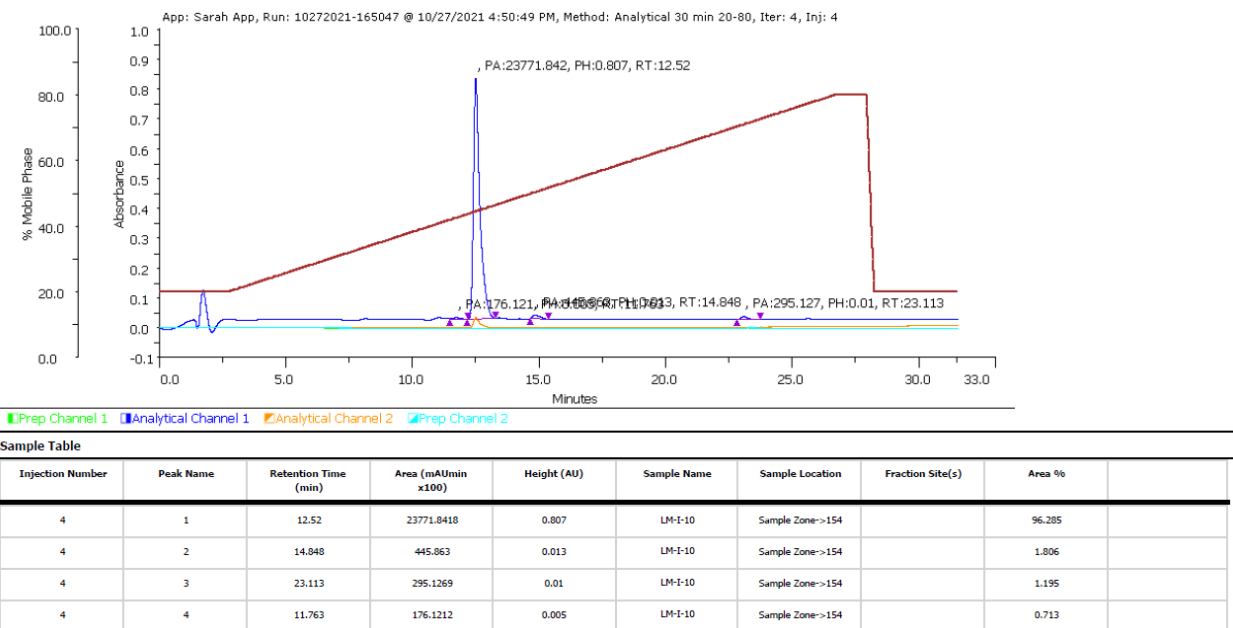


| Injection Number | Peak Name | Retention Time (min) | Area (mAUmin x100) | Height (AU) | Sample Name | Sample Location | Fraction Site(s) | Area % |
|------------------|-----------|----------------------|--------------------|-------------|-------------|------------------|------------------|--------|
| 3 | 1 | 16.295 | 36757.9292 | 1.661 | LM-1-23 | Sample Zone->161 | | 99.691 |
| 3 | 2 | 17.652 | 113.7647 | 0.005 | LM-1-23 | Sample Zone->161 | | 0.309 |

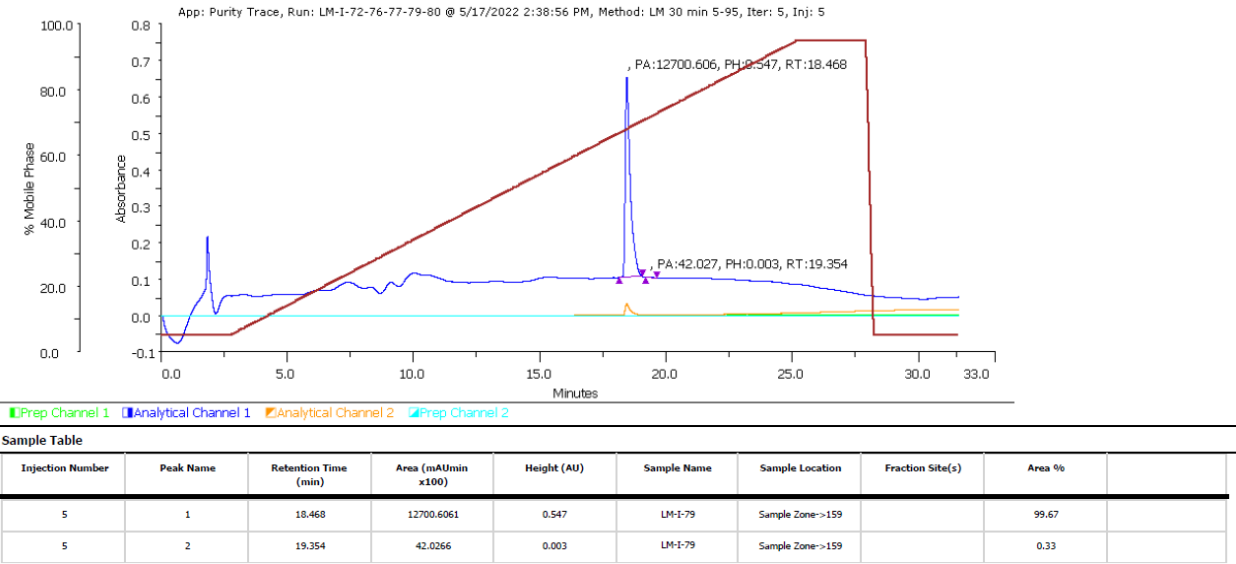
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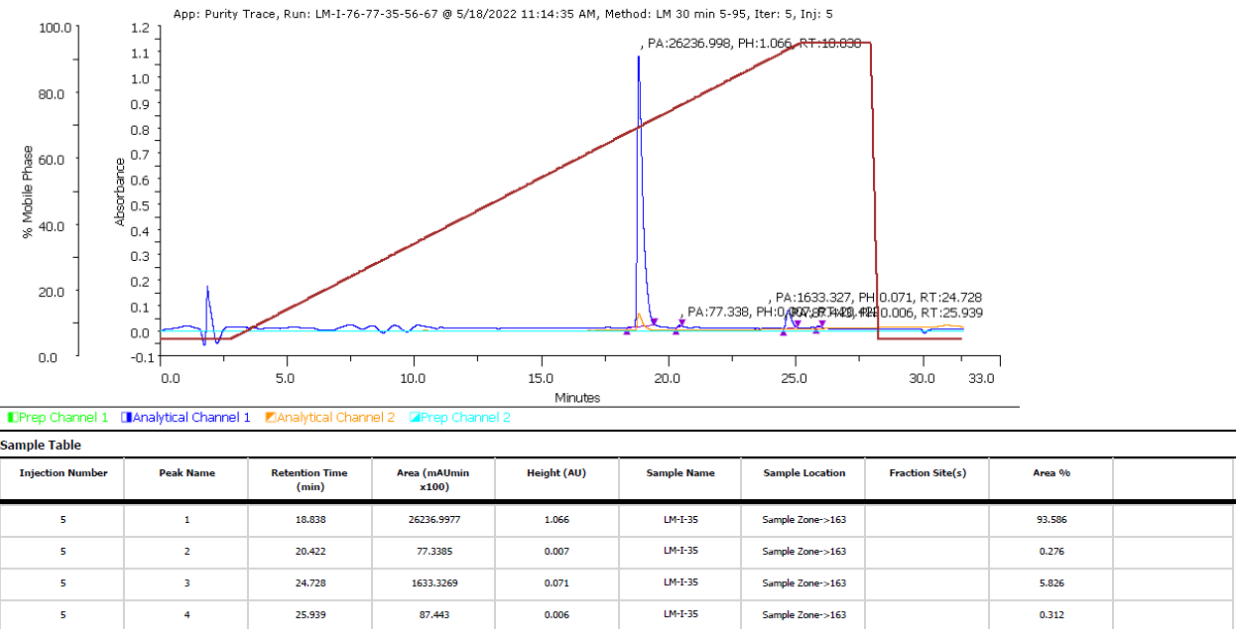
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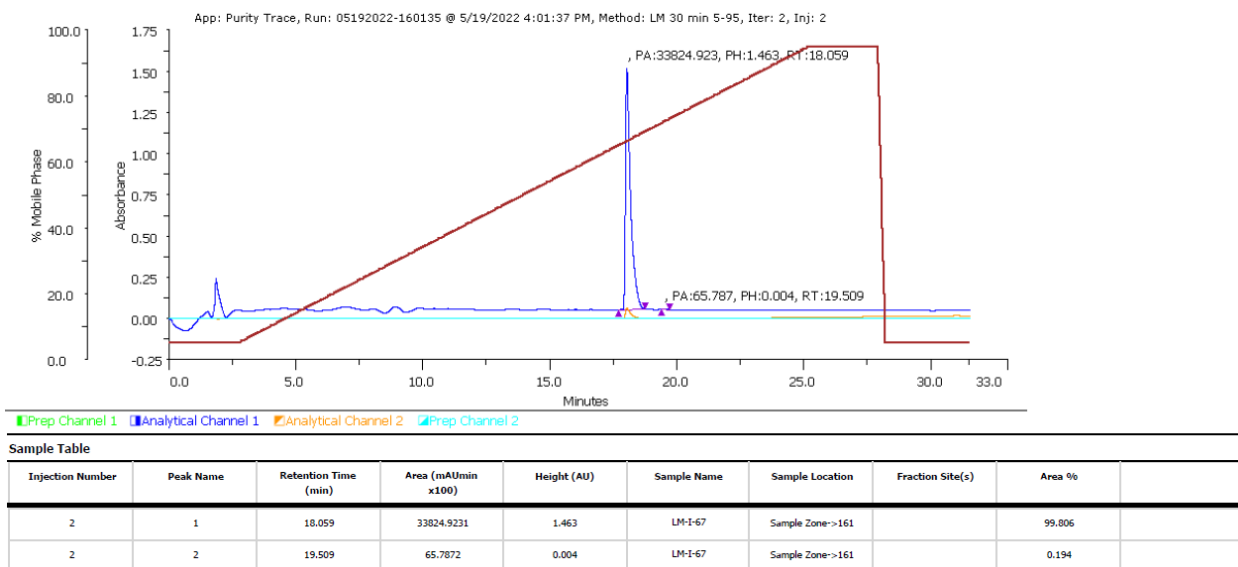
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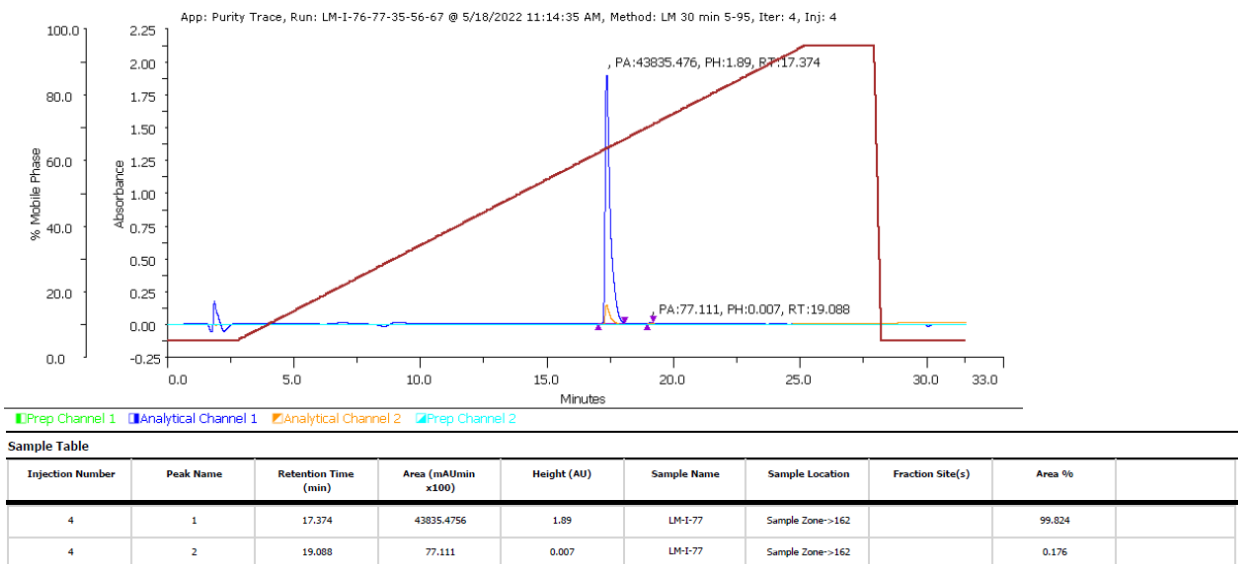
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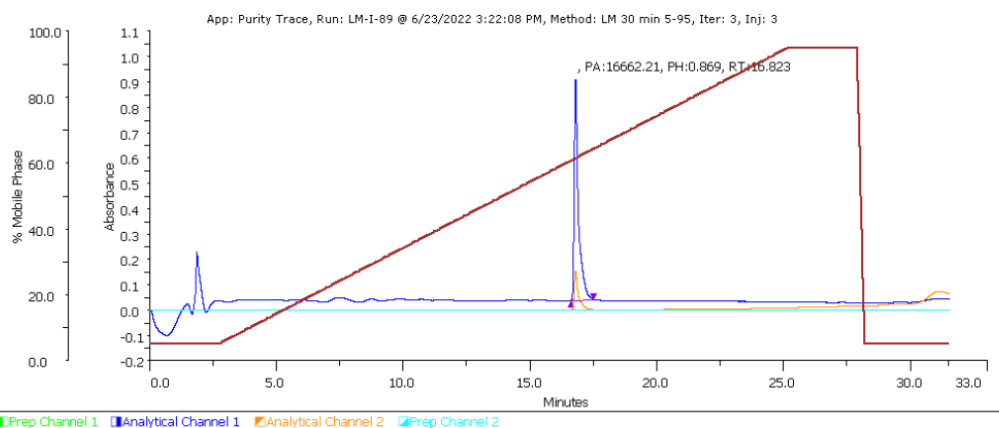
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7g



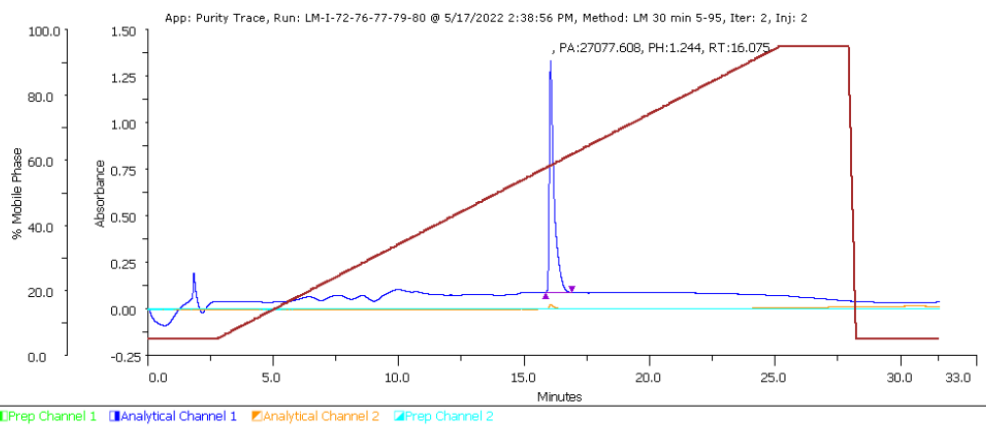
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Sample Table

| Injection Number | Peak Name | Retention Time (min) | Area (mAUmin x100) | Height (AU) | Sample Name | Sample Location | Fraction Site(s) | Area % |
|------------------|-----------|----------------------|--------------------|-------------|-------------|------------------|------------------|--------|
| 3 | 1 | 16.823 | 16662.2096 | 0.869 | LM-1-89 | Sample Zone->161 | | 100 |

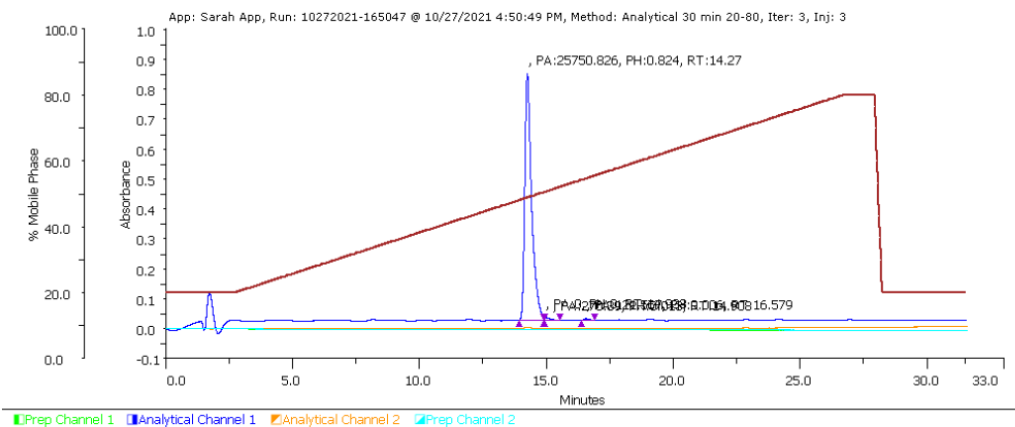
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Sample Table

| Injection Number | Peak Name | Retention Time (min) | Area (mAUmin x100) | Height (AU) | Sample Name | Sample Location | Fraction Site(s) | Area % |
|------------------|-----------|----------------------|--------------------|-------------|-------------|------------------|------------------|--------|
| 2 | 1 | 16.075 | 27077.6078 | 1.244 | LM-1-72 | Sample Zone->161 | | 100 |

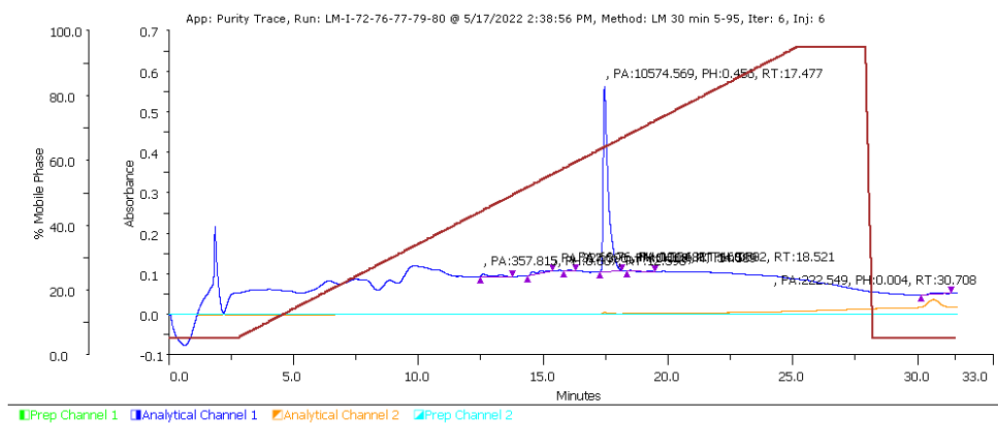
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Sample Table

| Injection Number | Peak Name | Retention Time (min) | Area (nAUmin x100) | Height (AU) | Sample Name | Sample Location | Fraction Site(s) | Area % |
|------------------|-----------|----------------------|--------------------|-------------|-------------|------------------|------------------|--------|
| 3 | 1 | 14.27 | 25750.8256 | 0.824 | LM-I-13 | Sample Zone->153 | | 98.501 |
| 3 | 2 | 16.579 | 121.5673 | 0.006 | LM-I-13 | Sample Zone->153 | | 0.465 |
| 3 | 3 | 14.933 | 0 | 0 | LM-I-13 | Sample Zone->153 | | 0 |
| 3 | 4 | 14.908 | 270.3904 | 0.013 | LM-I-13 | Sample Zone->153 | | 1.034 |

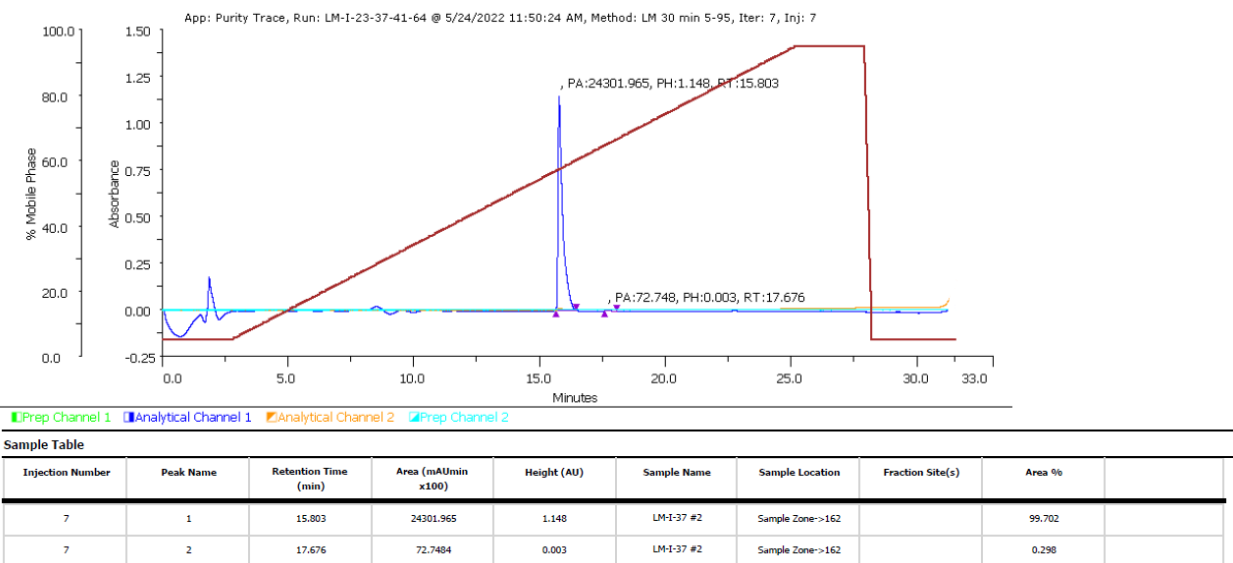
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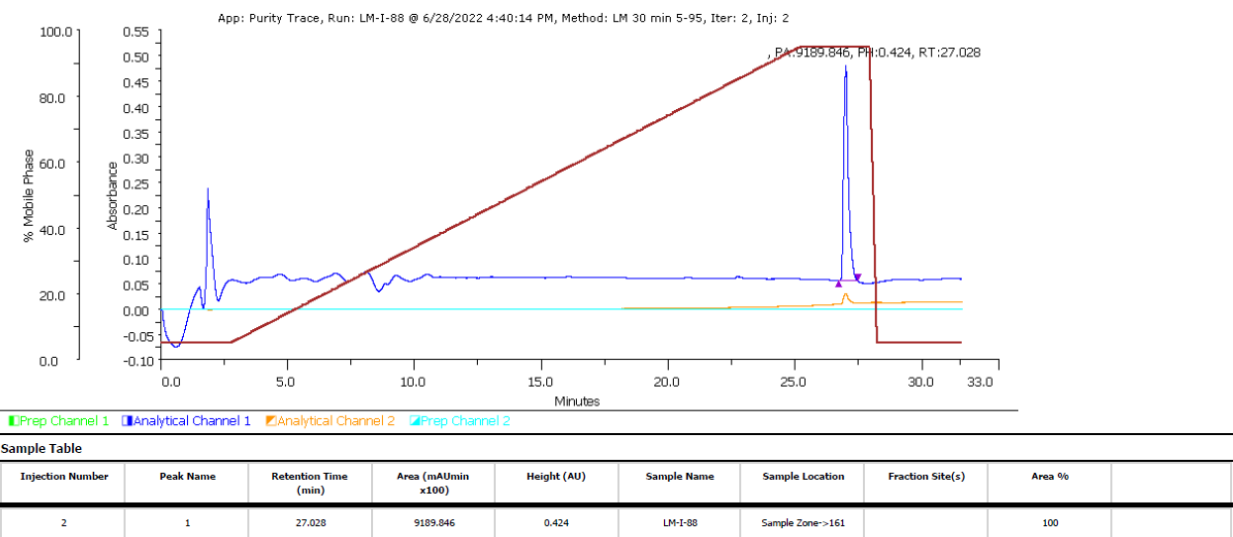
Sample Table

| Injection Number | Peak Name | Retention Time (min) | Area (nAUmin x100) | Height (AU) | Sample Name | Sample Location | Fraction Site(s) | Area % |
|------------------|-----------|----------------------|--------------------|-------------|-------------|------------------|------------------|--------|
| 6 | 1 | 17.477 | 10574.5667 | 0.456 | LM-I-80 | Sample Zone->158 | | 90.763 |
| 6 | 2 | 12.598 | 357.8152 | 0.007 | LM-I-80 | Sample Zone->158 | | 3.071 |
| 6 | 3 | 14.589 | 327.396 | 0.008 | LM-I-80 | Sample Zone->158 | | 2.81 |
| 6 | 4 | 16.058 | 66.7605 | 0.004 | LM-I-80 | Sample Zone->158 | | 0.573 |
| 6 | 5 | 18.521 | 101.6815 | 0.002 | LM-I-80 | Sample Zone->158 | | 0.873 |
| 6 | 6 | 30.708 | 222.5489 | 0.004 | LM-I-80 | Sample Zone->158 | | 1.91 |

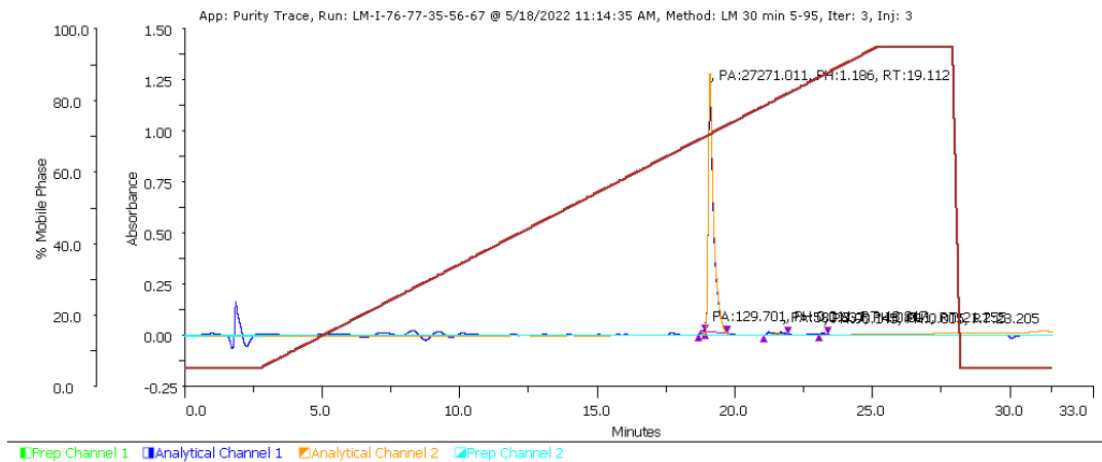
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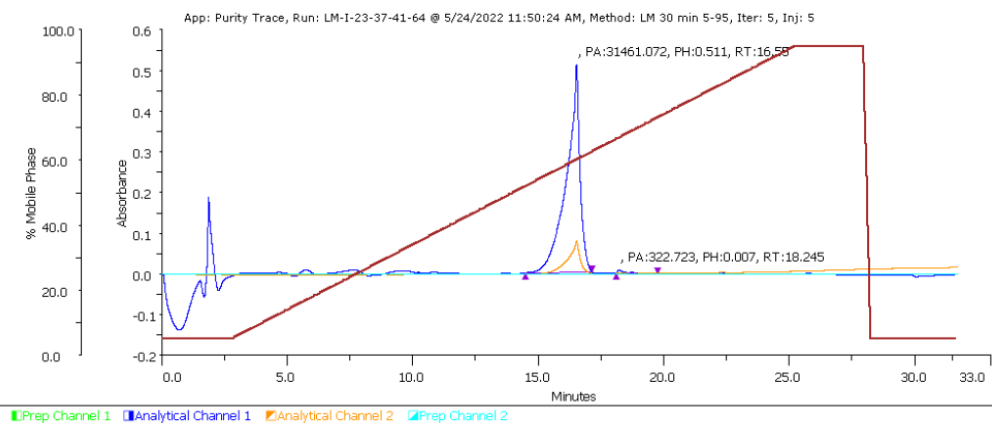
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Sample Table

| Injection Number | Peak Name | Retention Time (min) | Area (mAUmin x100) | Height (AU) | Sample Name | Sample Location | Fraction Site(s) | Area % |
|------------------|-----------|----------------------|--------------------|-------------|-------------|------------------|------------------|--------|
| 3 | 1 | 18.817 | 129.7009 | 0.011 | LM-I-76 | Sample Zone->161 | | 0.462 |
| 3 | 2 | 19.112 | 27271.0106 | 1.186 | LM-I-76 | Sample Zone->161 | | 97.148 |
| 3 | 3 | 21.255 | 580.8367 | 0.011 | LM-I-76 | Sample Zone->161 | | 2.069 |
| 3 | 4 | 23.205 | 90.1447 | 0.005 | LM-I-76 | Sample Zone->161 | | 0.321 |

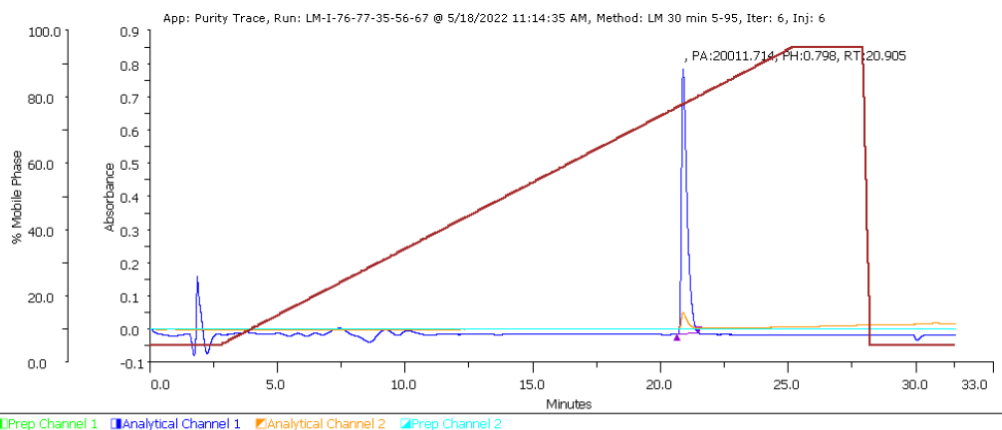
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Sample Table

| Injection Number | Peak Name | Retention Time (min) | Area (mAUmin x100) | Height (AU) | Sample Name | Sample Location | Fraction Site(s) | Area % |
|------------------|-----------|----------------------|--------------------|-------------|-------------|------------------|------------------|--------|
| 5 | 1 | 16.55 | 31461.072 | 0.511 | LM-I-41 | Sample Zone->163 | | 98.985 |
| 5 | 2 | 18.245 | 322.7227 | 0.007 | LM-I-41 | Sample Zone->163 | | 1.015 |

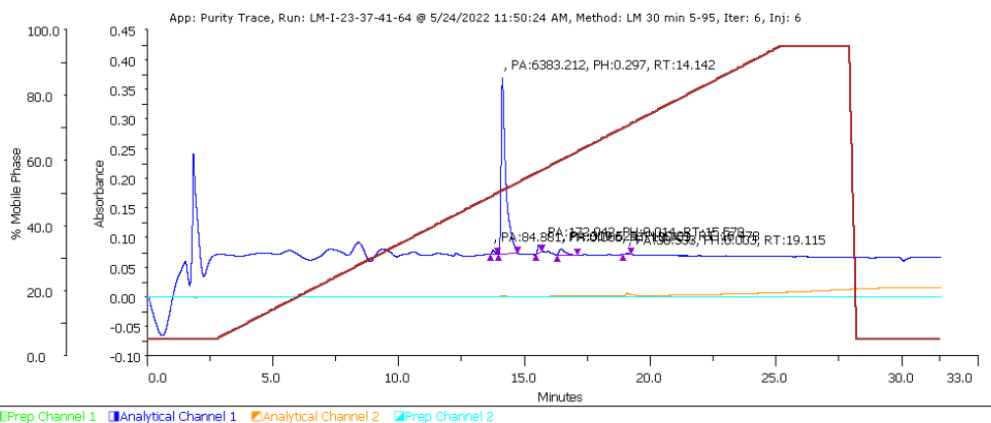
7p



Sample Table

| Injection Number | Peak Name | Retention Time (min) | Area (mAUmin x100) | Height (AU) | Sample Name | Sample Location | Fraction Site(s) | Area % |
|------------------|-----------|----------------------|--------------------|-------------|-------------|------------------|------------------|--------|
| 6 | 1 | 20.905 | 20011.7141 | 0.798 | LM-1-56 | Sample Zone->159 | | 100 |

7q



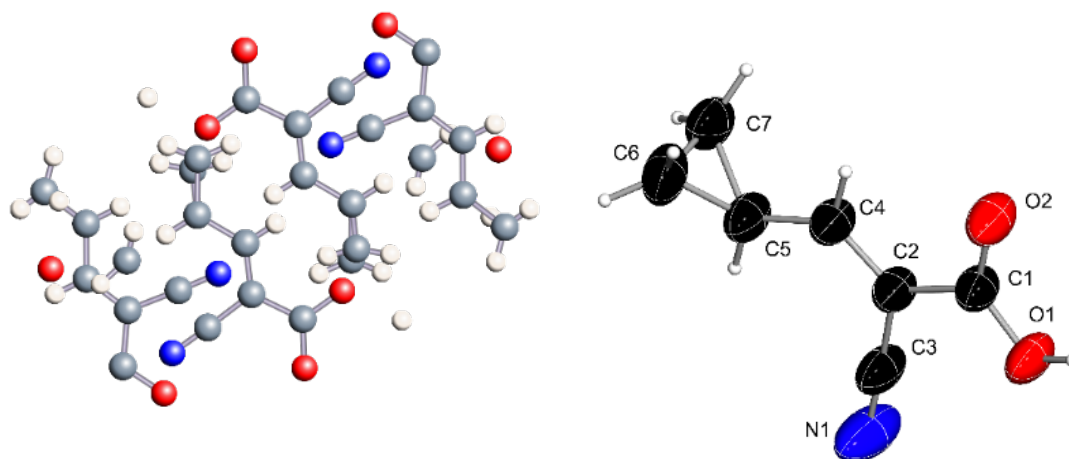
Sample Table

| Injection Number | Peak Name | Retention Time (min) | Area (mAUmin x100) | Height (AU) | Sample Name | Sample Location | Fraction Site(s) | Area % |
|------------------|-----------|----------------------|--------------------|-------------|-------------|------------------|------------------|--------|
| 6 | 1 | 14.142 | 6383.212 | 0.297 | LM-1-64 | Sample Zone->159 | | 91.349 |
| 6 | 2 | 15.578 | 172.0418 | 0.014 | LM-1-64 | Sample Zone->159 | | 2.462 |
| 6 | 3 | 16.478 | 309.5251 | 0.01 | LM-1-64 | Sample Zone->159 | | 4.43 |
| 6 | 4 | 13.763 | 84.381 | 0.006 | LM-1-64 | Sample Zone->159 | | 1.208 |
| 6 | 5 | 19.115 | 38.5334 | 0.003 | LM-1-64 | Sample Zone->159 | | 0.551 |

Synthesis of N-2-[4-[[1-Adamantanecarbonyl]-1-piperazinyl]-2-oxoethyl]-2-methylpropenamide (7r):

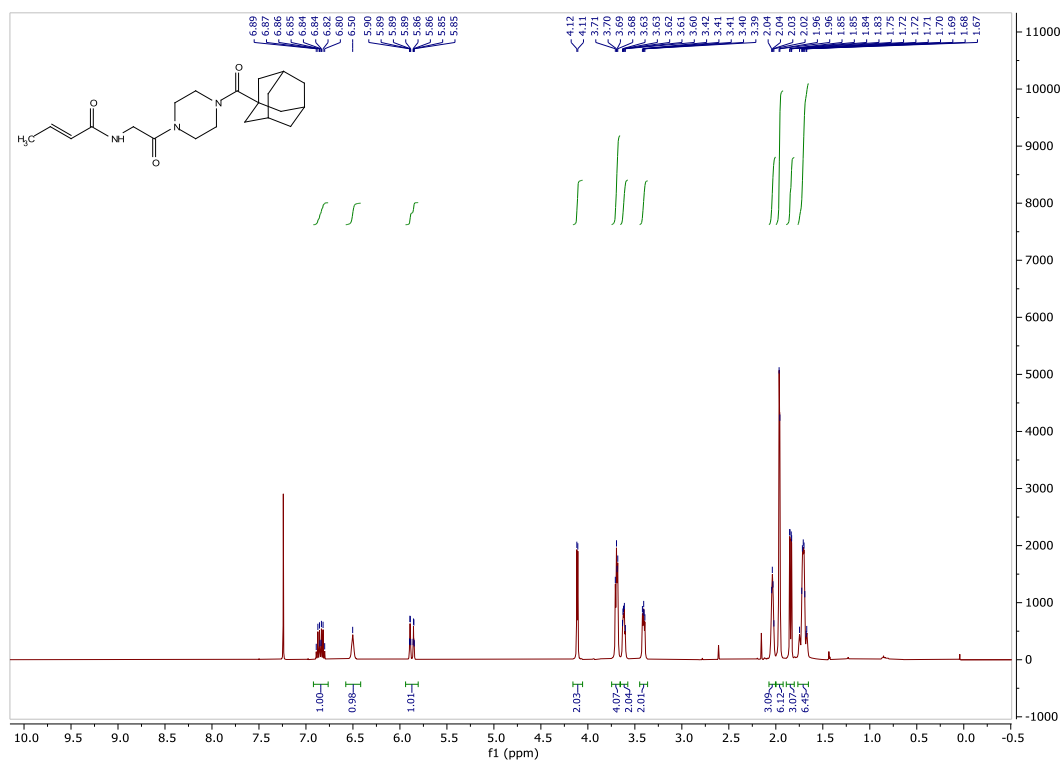
Methacrylic acid (0.031 mL, 0.361 mmol, 1.1 eq), HBTU (0.2 g, 0.524 mmol, 1.6 eq), Hünig's base (0.17 mL, 0.98 mmol, 3.0 eq), and **6** (0.1 g, 0.327 mmol, 1.0 eq) were stirred in anhydrous DCM (5 mL) at R.T. for 16 h, at which point the reaction was confirmed complete by TLC analysis. The reaction was concentrated *in vacuo* and the residue was redissolved in EtOAc (50 mL). The organic phase was washed with 5% AcOH (3 × 15 mL), brine (25 mL), NaHCO₃ (25 mL), and brine again (25 mL). The organic phase was dried over MgSO₄ and concentrated *in vacuo* to give a light orange solid, which was purified by silica gel column chromatography (5% MeOH/EtOAc, dry load using celite, R_f = 0.41) and then washing with pentane to give a white solid (0.028 g, 23%). ¹H NMR (300 MHz, CDCl₃) δ 5.78-5.77 (m, 1H), 5.37-5.36 (m, 1H), 4.07 (s, 2H), 3.71-3.61 (m, 4H), 3.60-3.57 (m, 2H), 3.44-3.40 (m, 2H), 2.02-2.00 (m, 3H), 1.96-1.93 (m, 9H), 1.74-1.64 (m, 6H). ¹³C NMR (75 MHz, CDCl₃) δ 176.2, 168.2, 167.0, 139.2, 120.6, 45.3, 44.9, 44.7, 42.3, 41.9, 41.5, 39.2, 36.6, 28.5, 18.6. HRMS (ESI) calc'd for C₂₁H₃₁N₃O₃Na [MNa]⁺: 396.2263, found: 396.2243.

Crystal structure of 2-cyano-3-cyclopropylacrylic acid (I3):

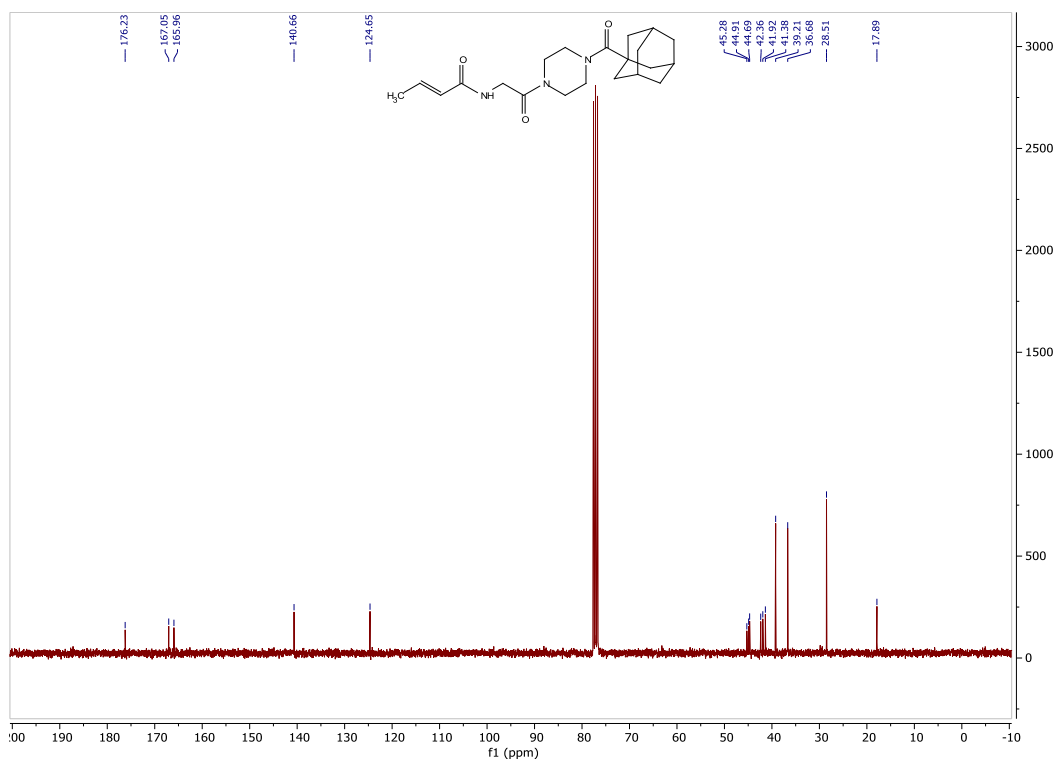


NMR Spectra of Final Inhibitors and New Synthetic Intermediates:

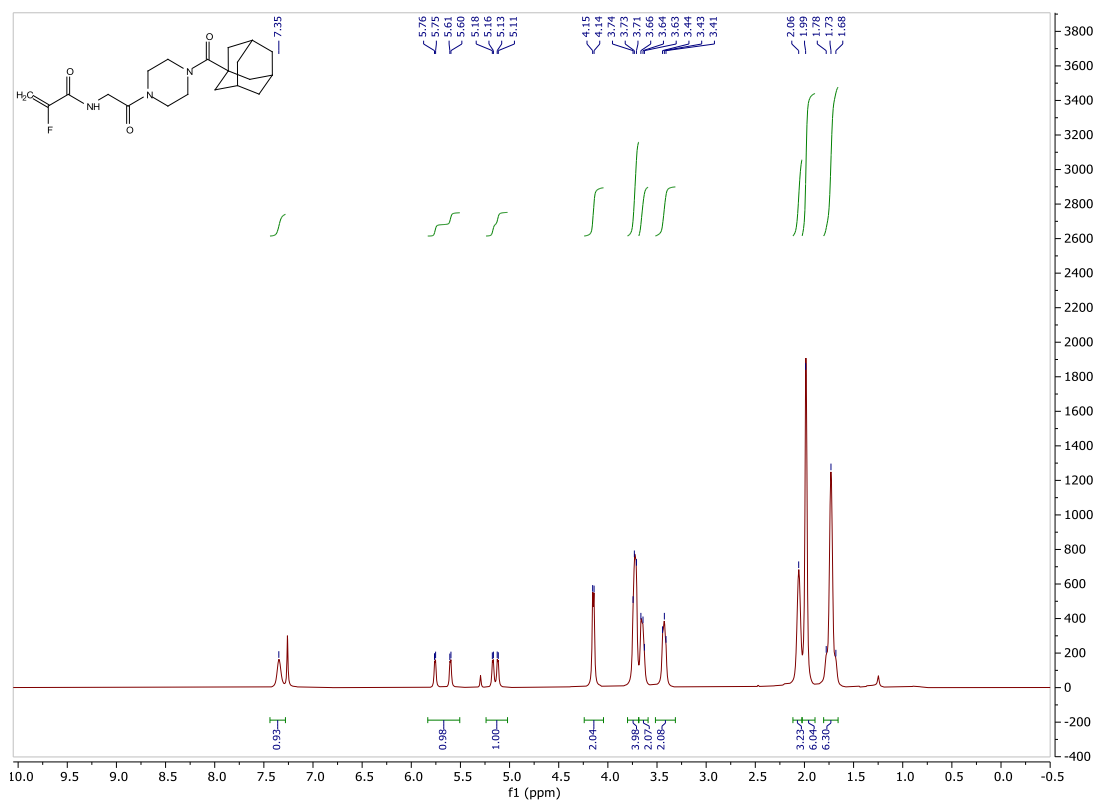
^1H NMR (300 MHz, CDCl_3) Spectra of Compound 7a



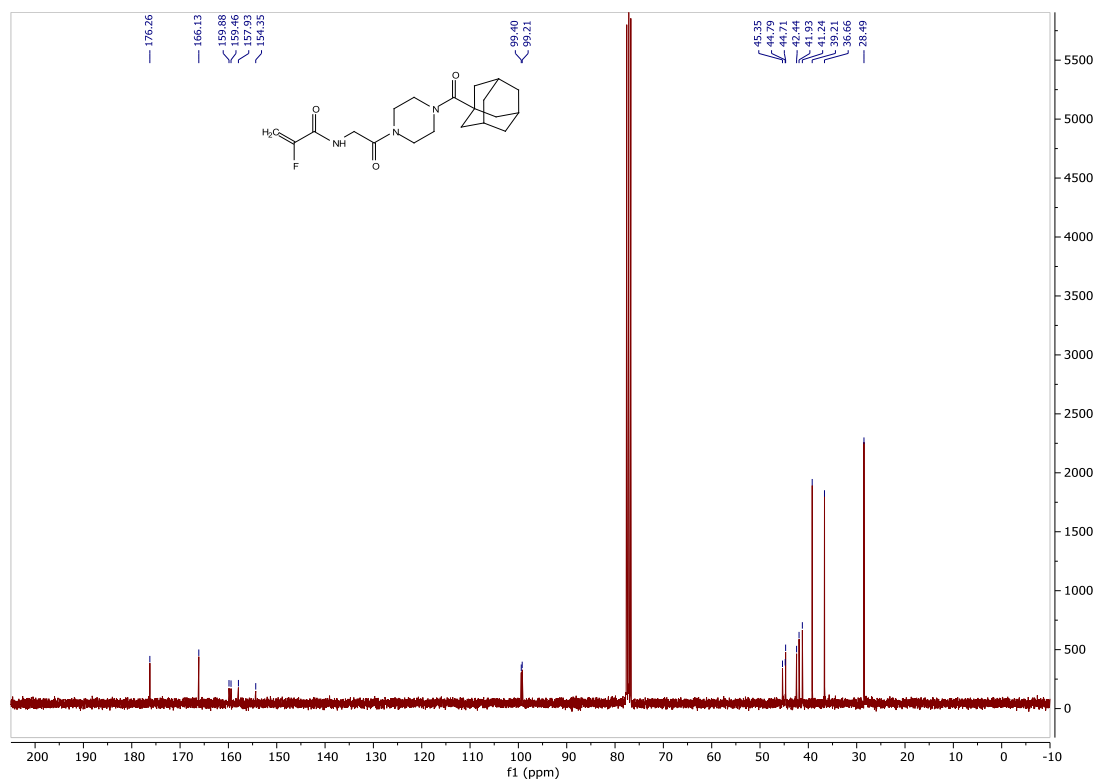
^{13}C NMR (75 MHz, CDCl_3) Spectra of Compound 7a



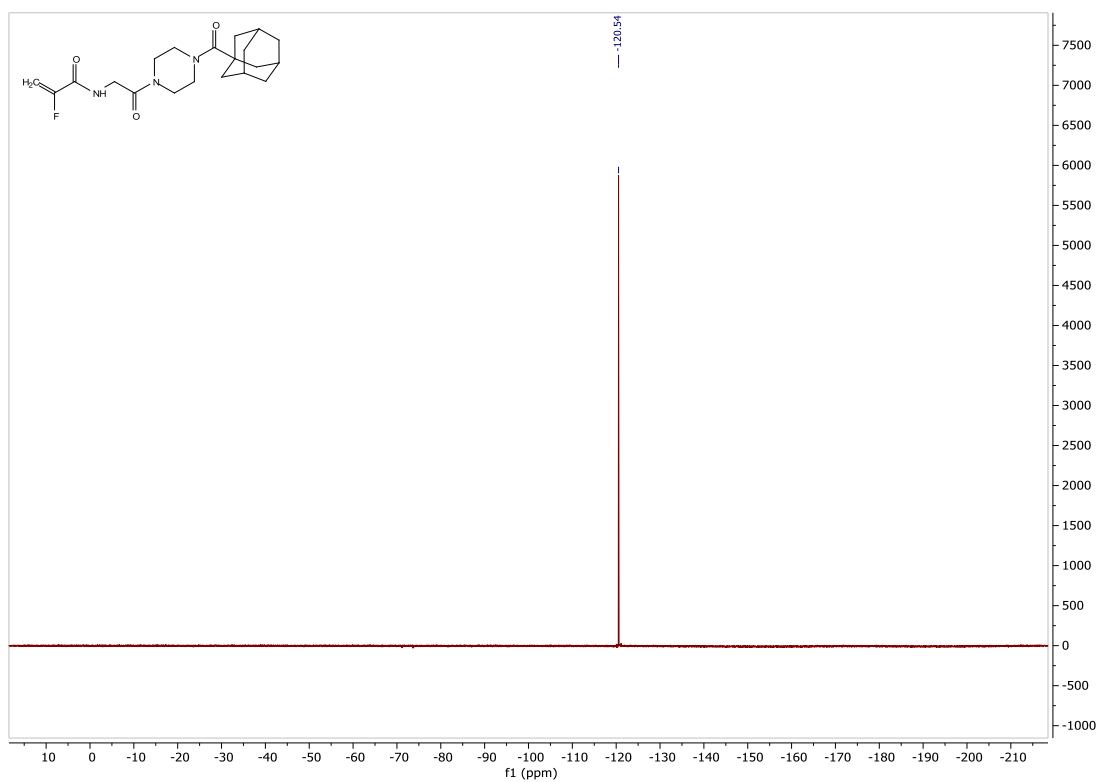
¹H NMR (300 MHz, CDCl₃) Spectra of Compound **7b**



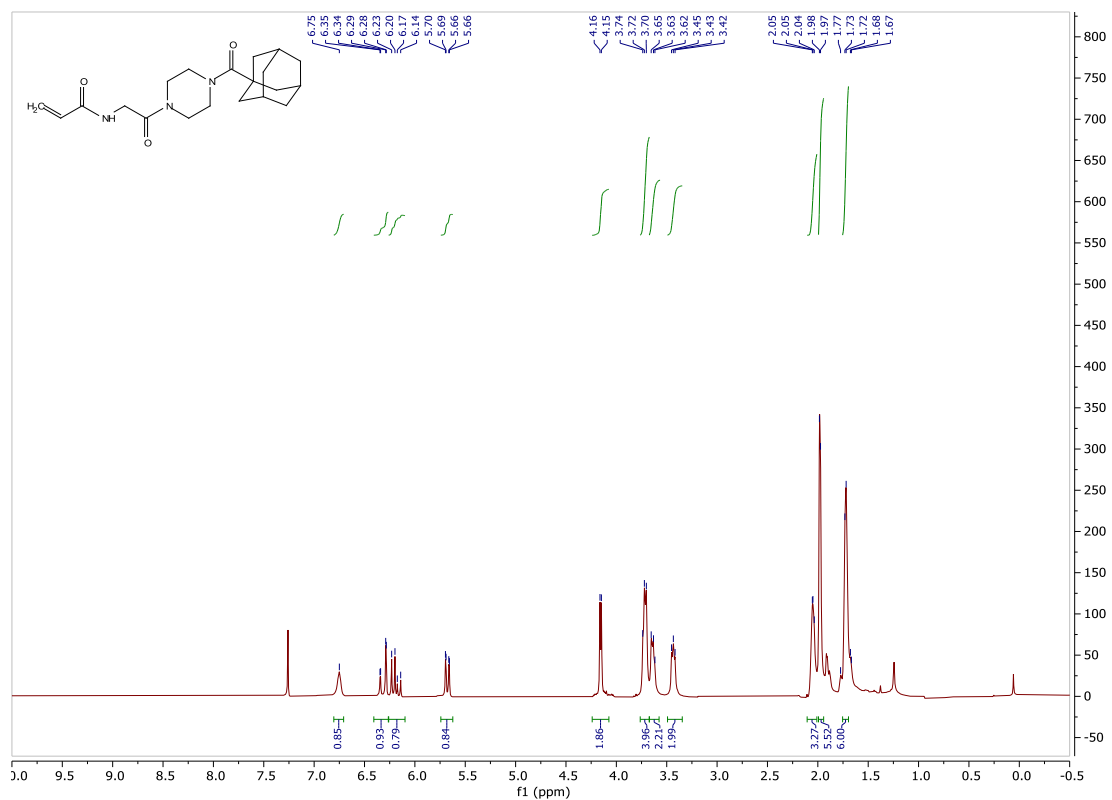
¹³C NMR (75 MHz, CDCl₃) Spectra of Compound **7b**



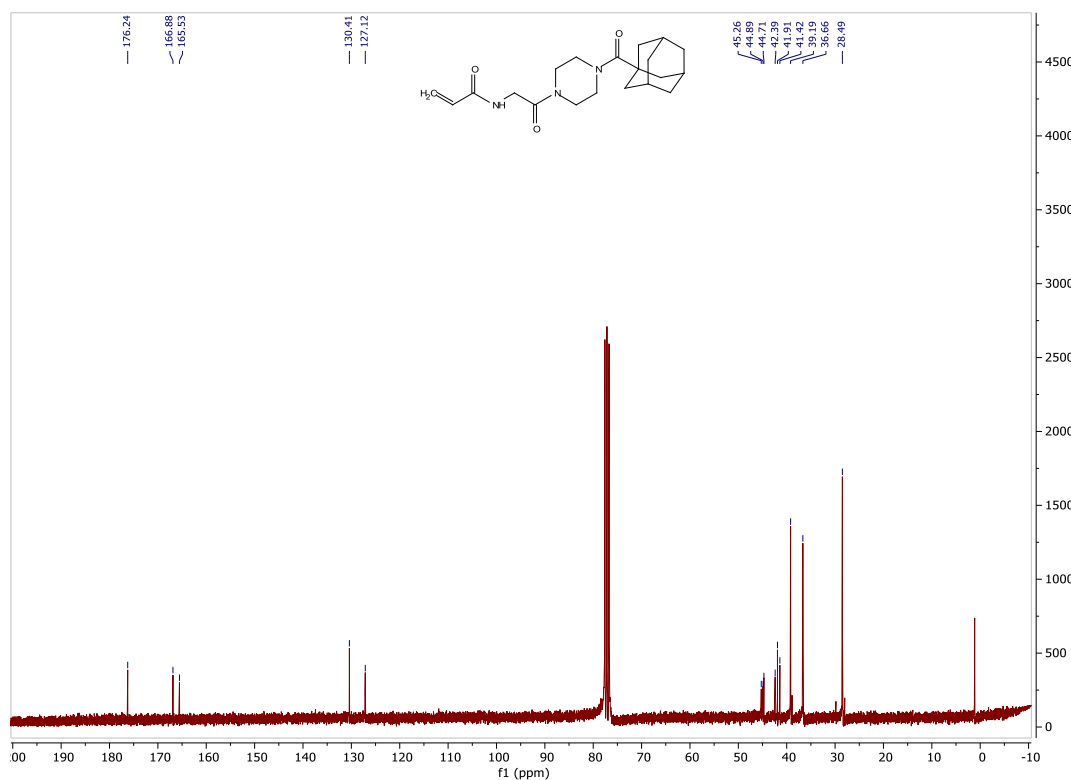
^{19}F NMR (283 MHz, CDCl_3) Spectra of Compound **7b**



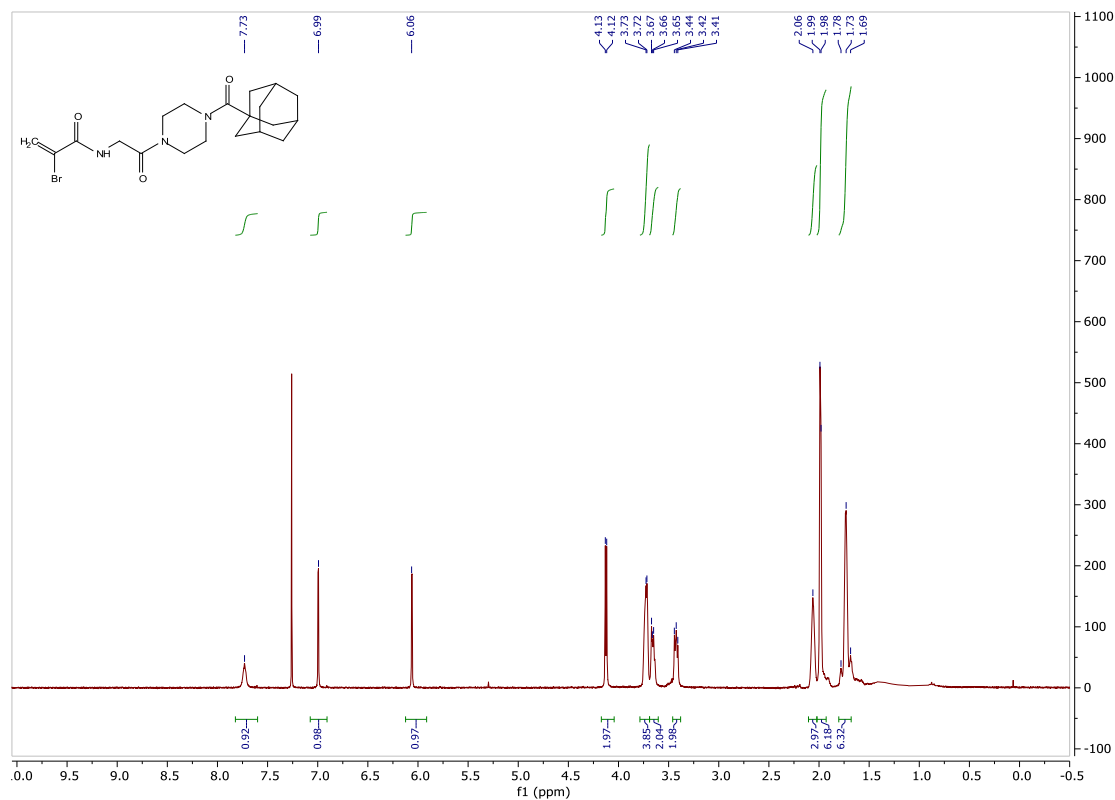
^1H NMR (300 MHz, CDCl_3) Spectra of Compound **7c**



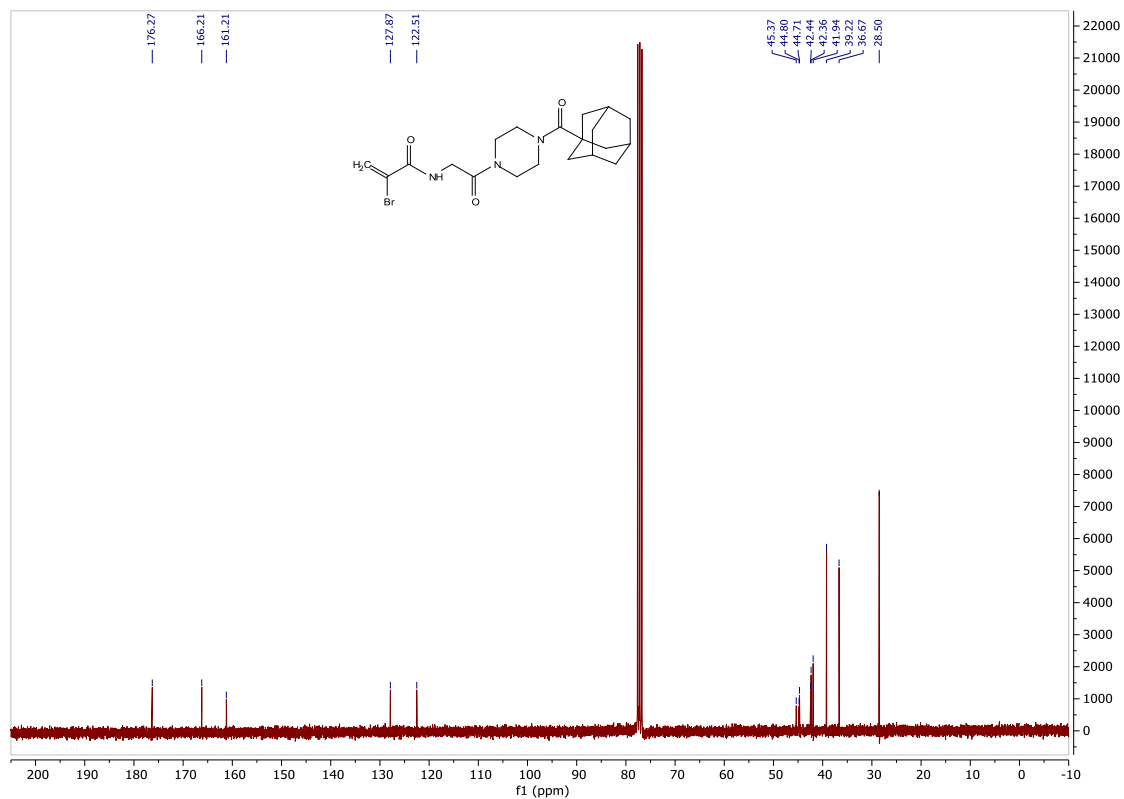
^{13}C NMR (75 MHz, CDCl_3) Spectra of Compound **7c**



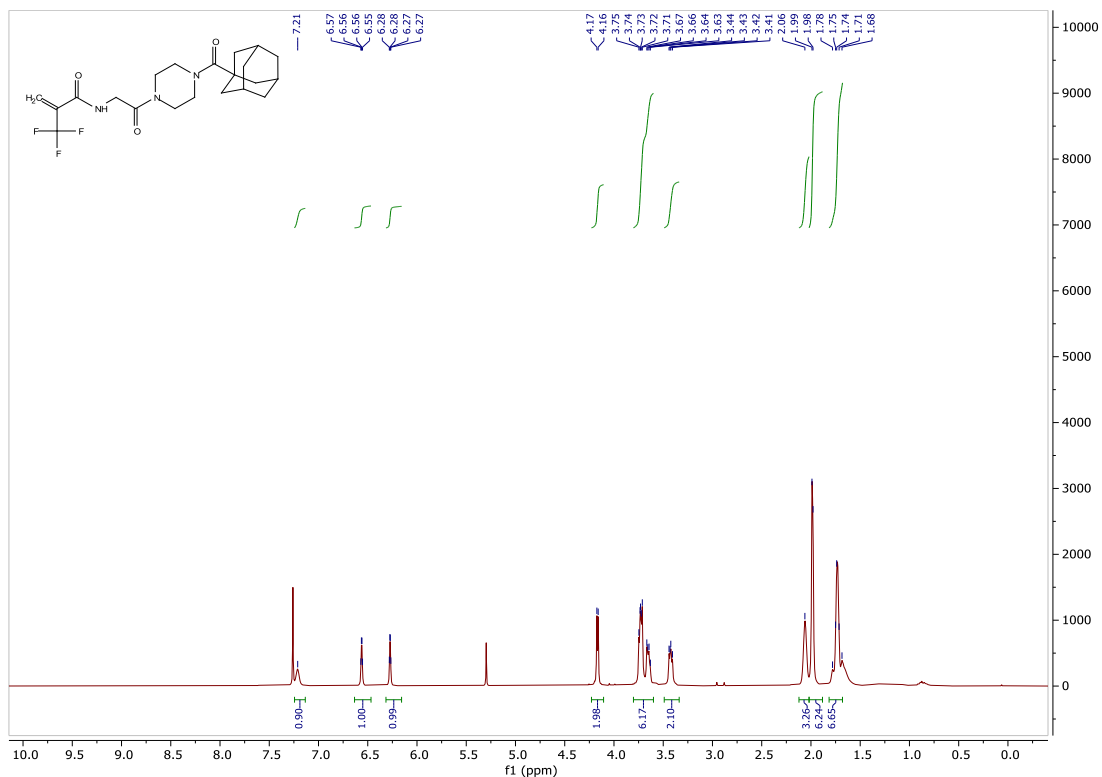
^1H NMR (300 MHz, CDCl_3) Spectra of Compound **7d**



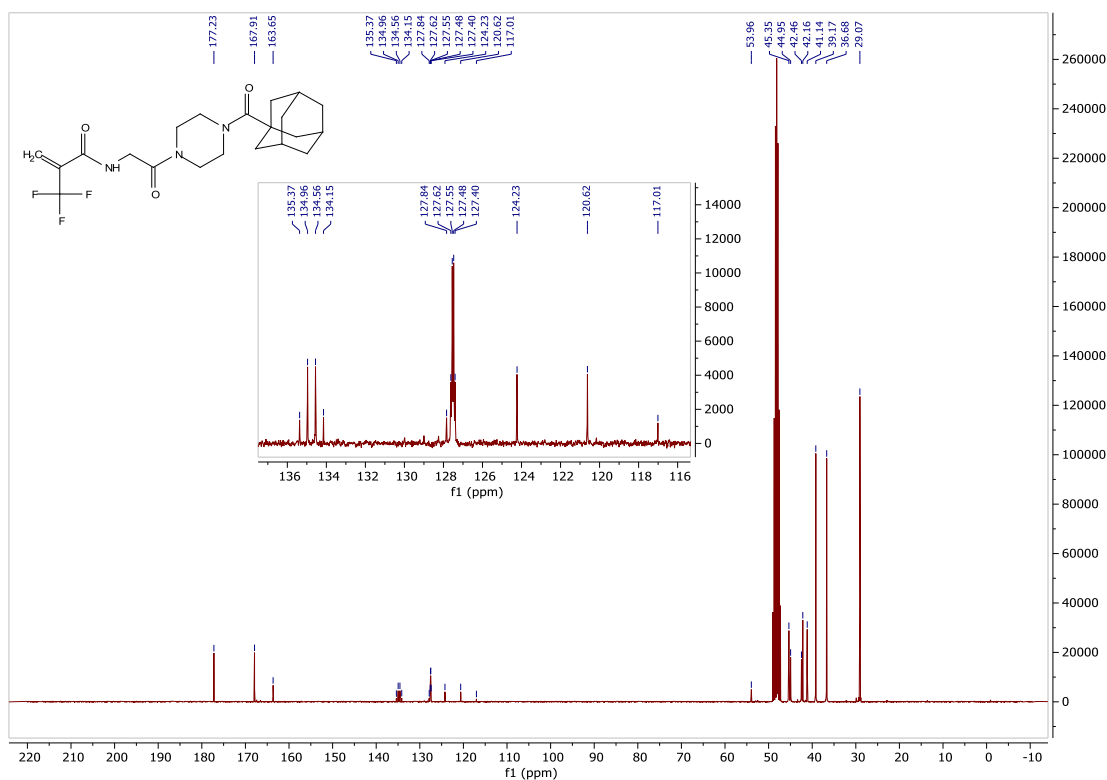
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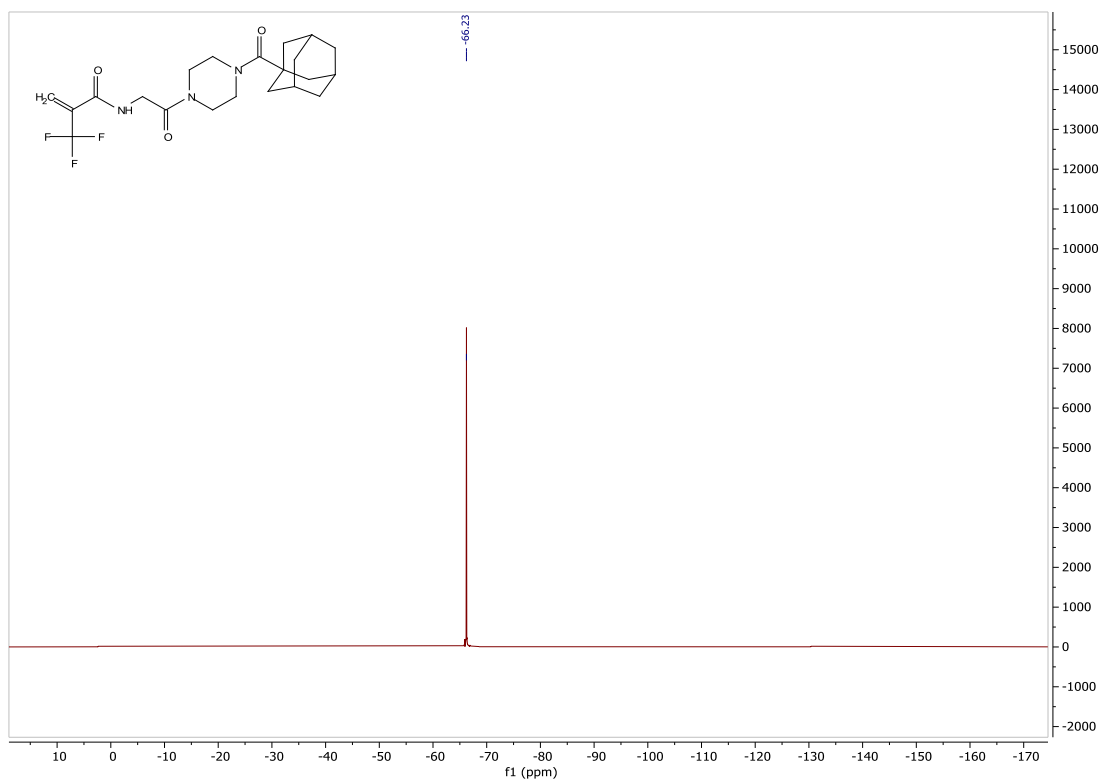
¹H NMR (300 MHz, CDCl₃) Spectra of Compound 7e



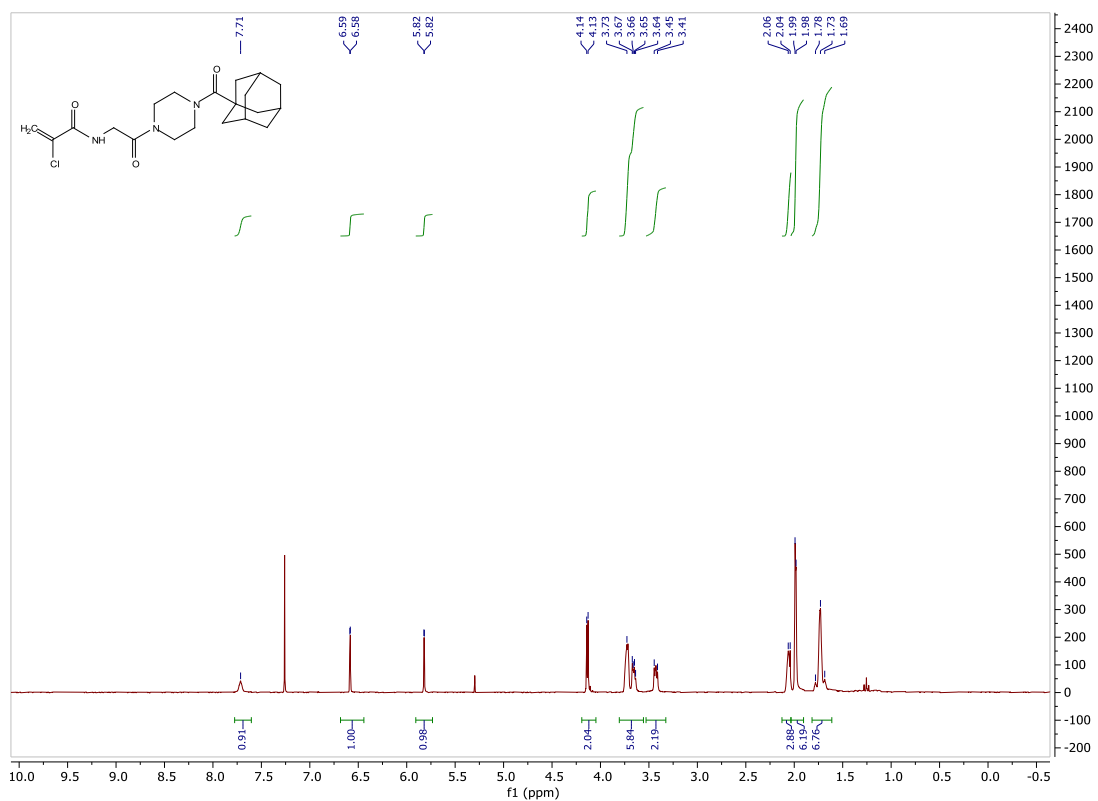
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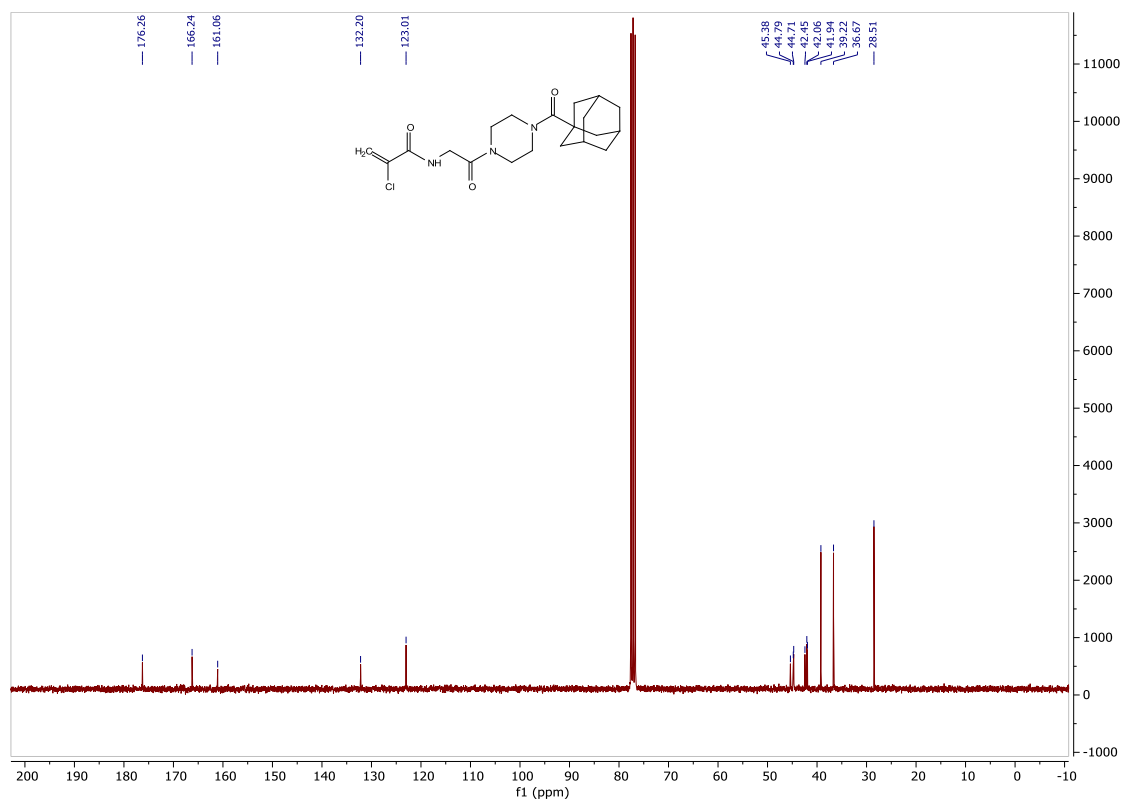
¹⁹F NMR (283 MHz, CDCl₃) Spectra of Compound 7e



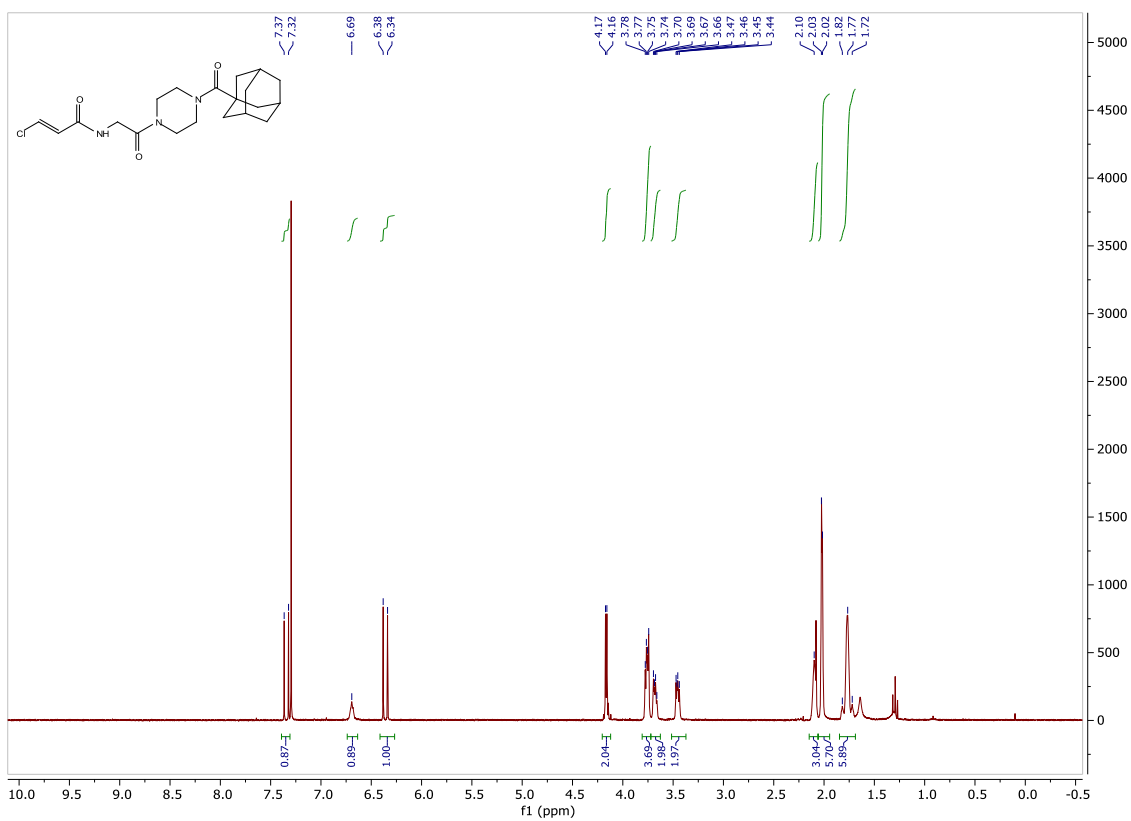
^1H NMR (300 MHz, CDCl_3) Spectra of Compound **7f**



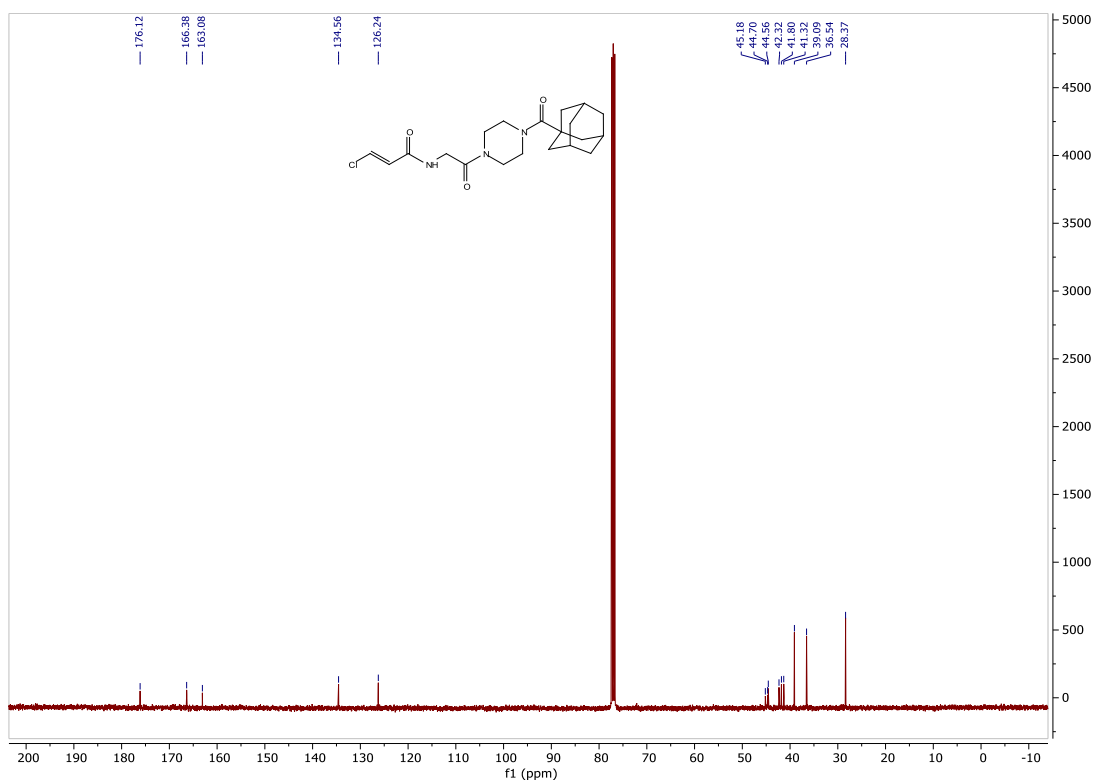
^{13}C NMR (75 MHz, CDCl_3) Spectra of Compound **7f**



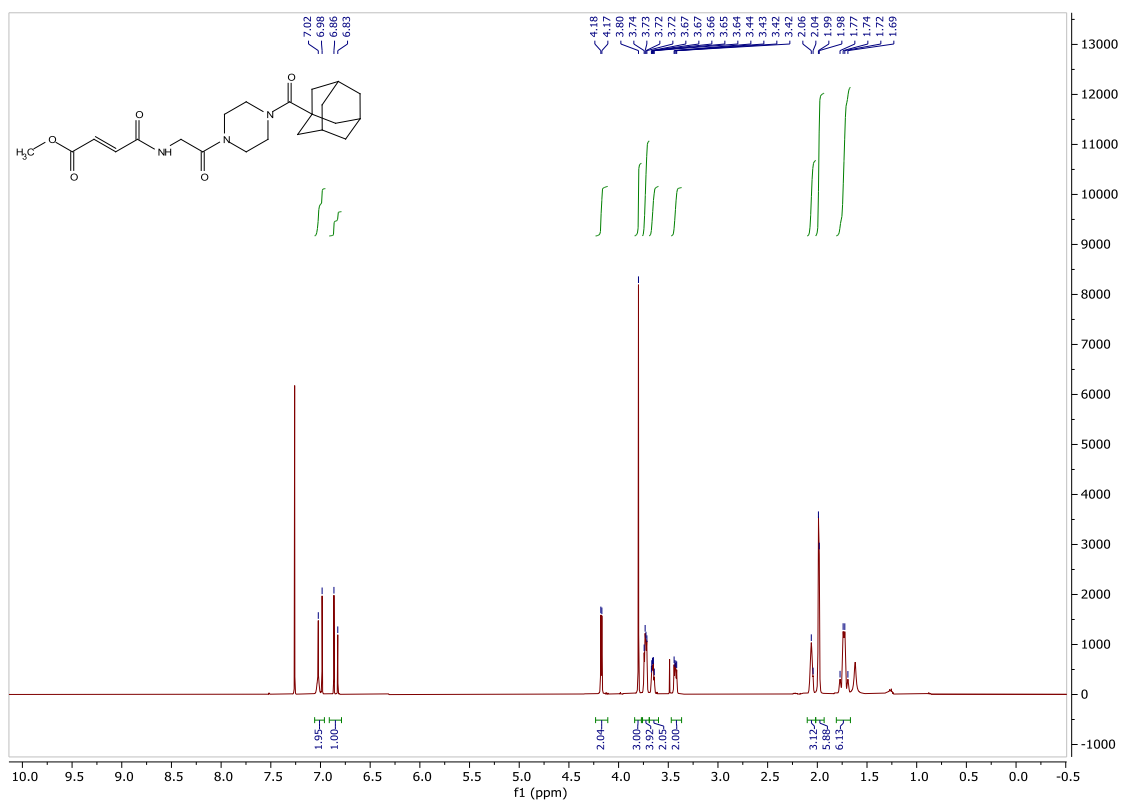
^1H NMR (300 MHz, CDCl_3) Spectra of Compound **7g**



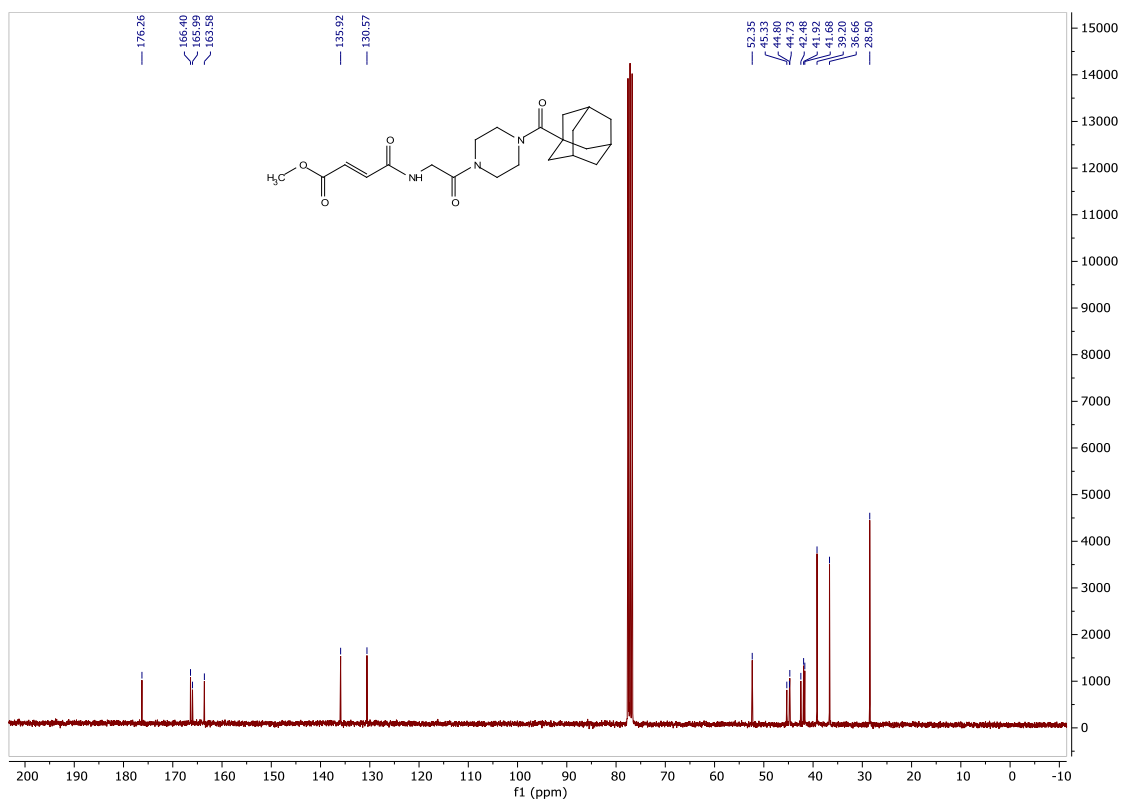
^{13}C NMR (75 MHz, CDCl_3) Spectra of Compound **7g**



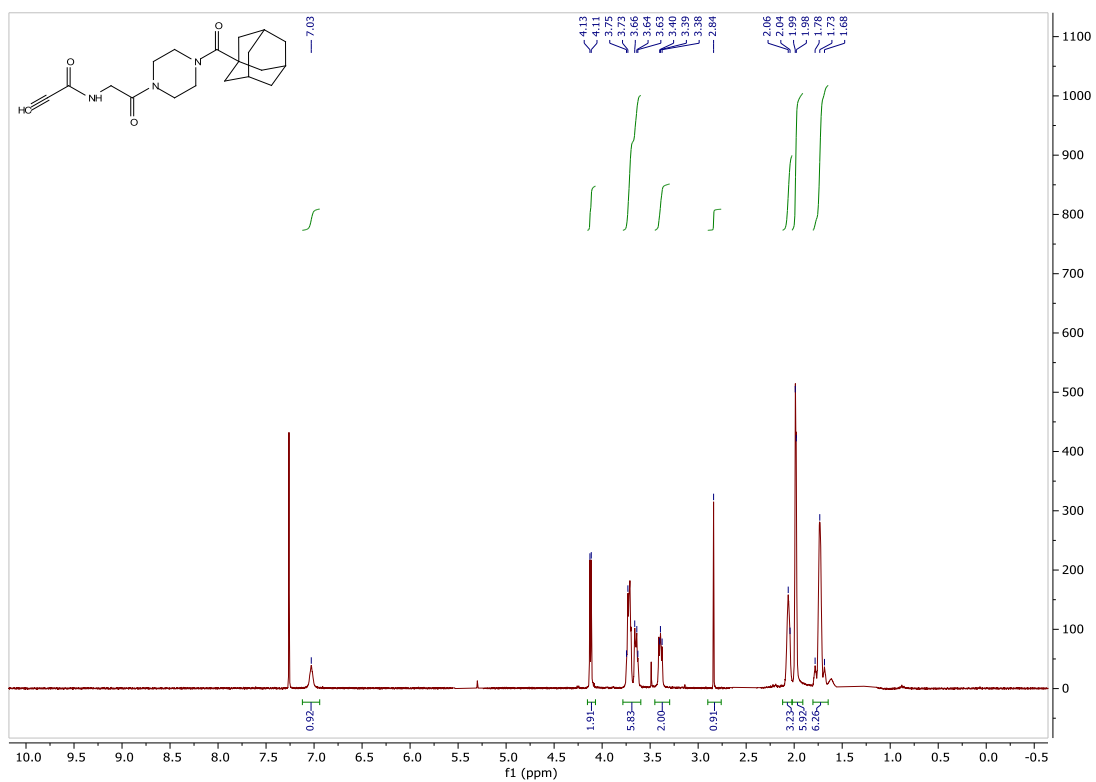
¹H NMR (300 MHz, CDCl₃) Spectra of Compound 7h



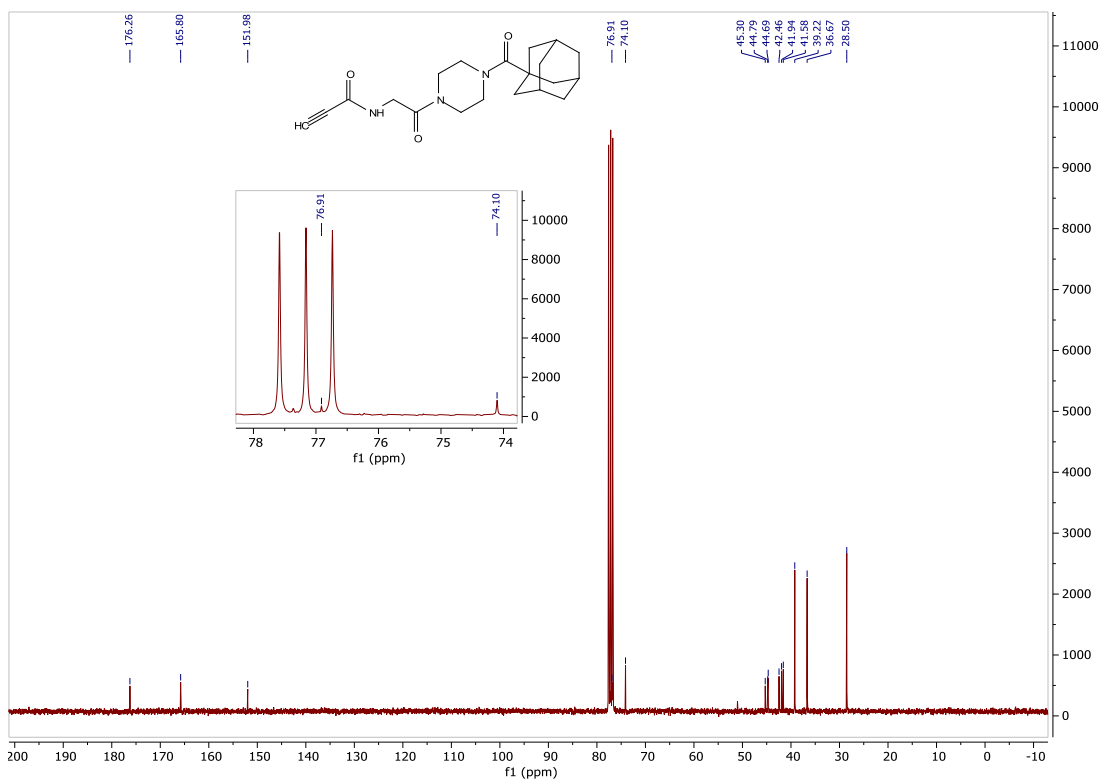
¹³C NMR (75 MHz, CDCl₃) Spectra of Compound 7h



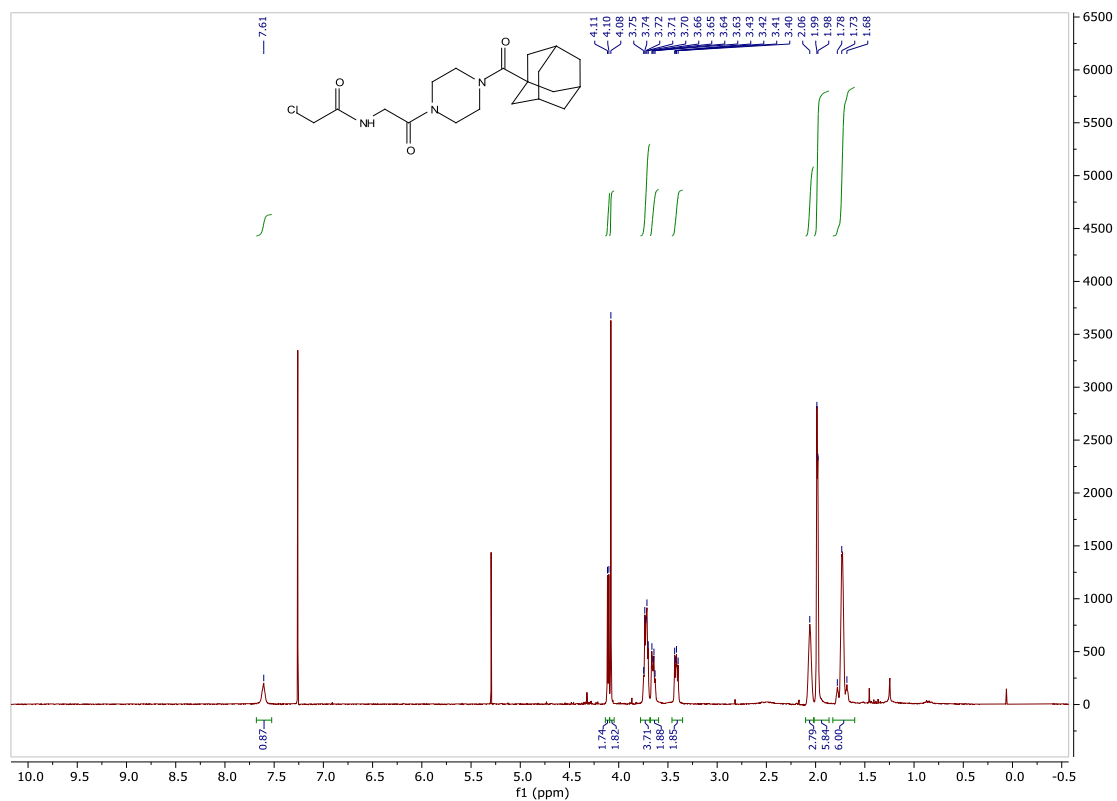
¹H NMR (300 MHz, CDCl₃) Spectra of Compound **7i**



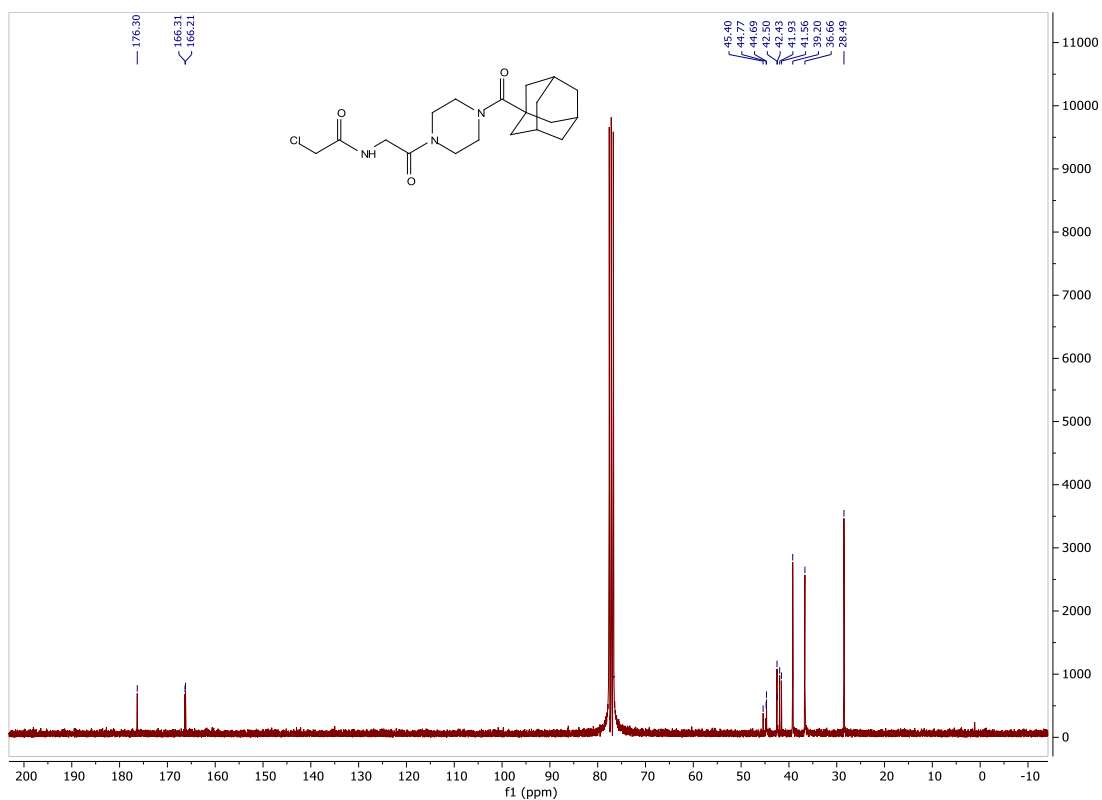
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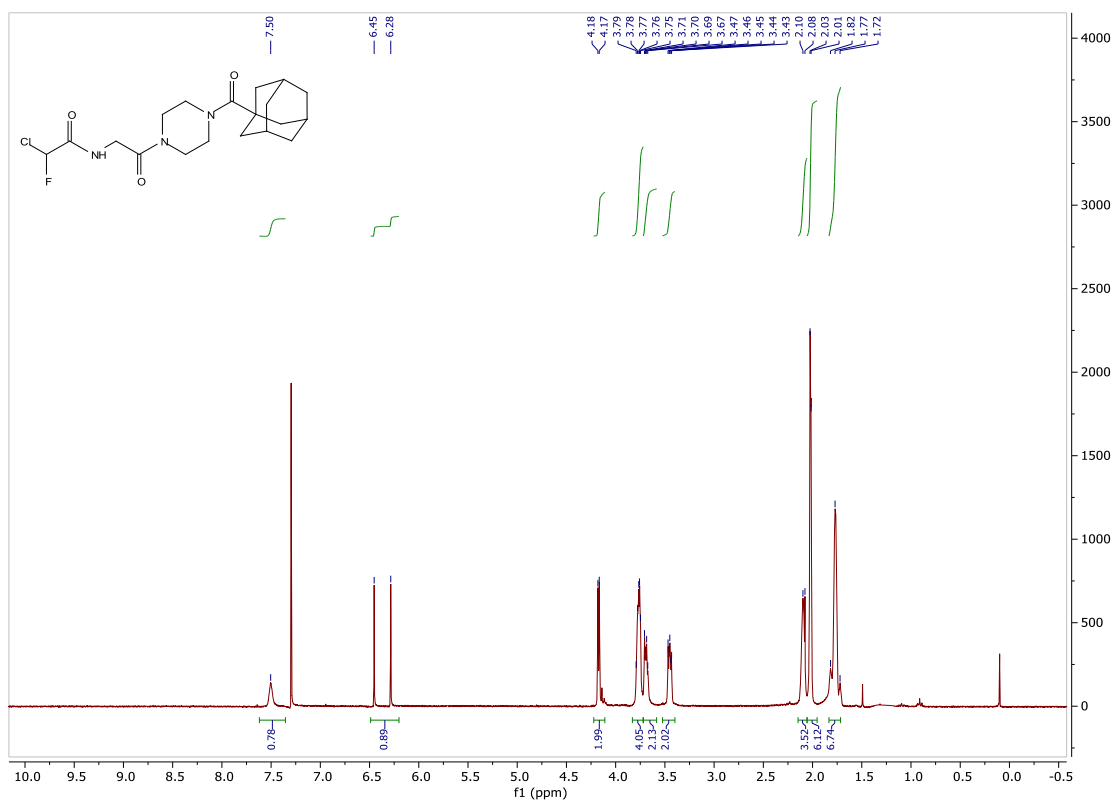
¹H NMR (300 MHz, CDCl₃) Spectra of Compound 7j



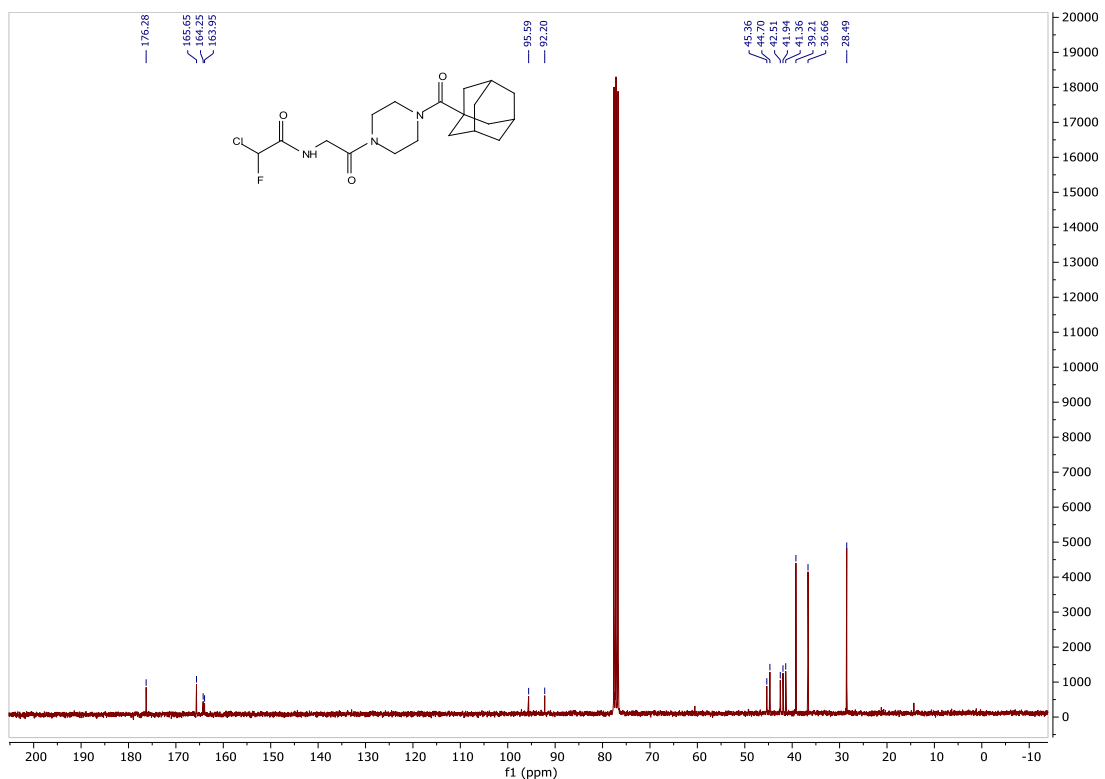
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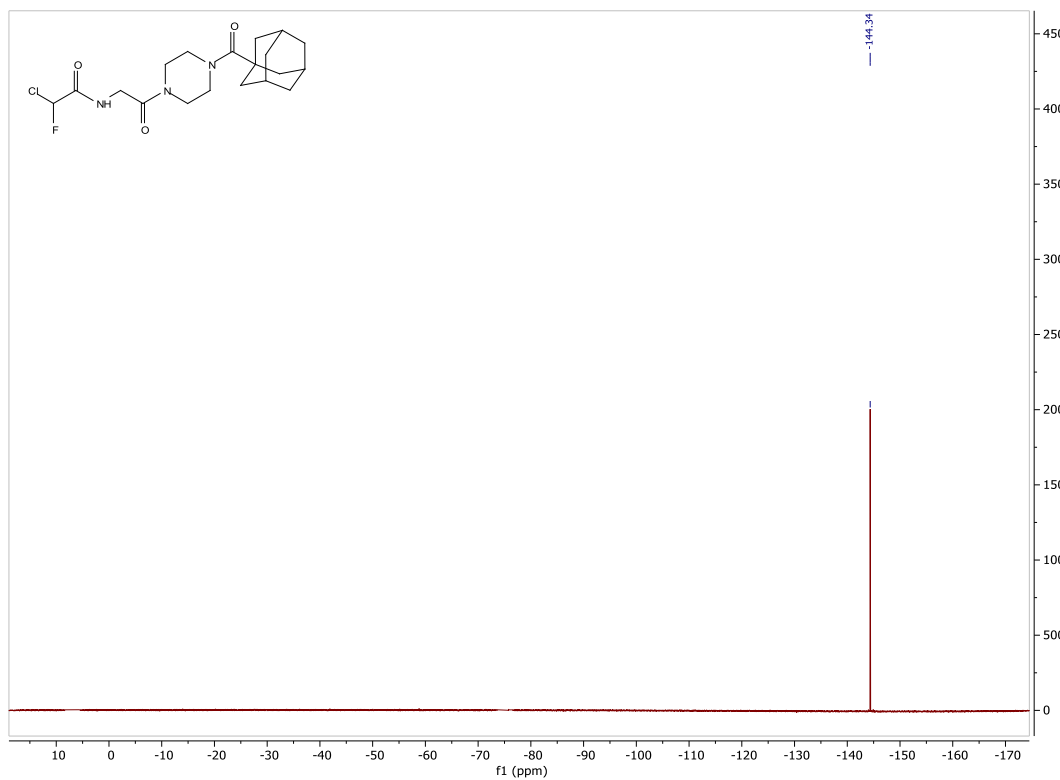
¹H NMR (300 MHz, CDCl₃) Spectra of Compound 7k



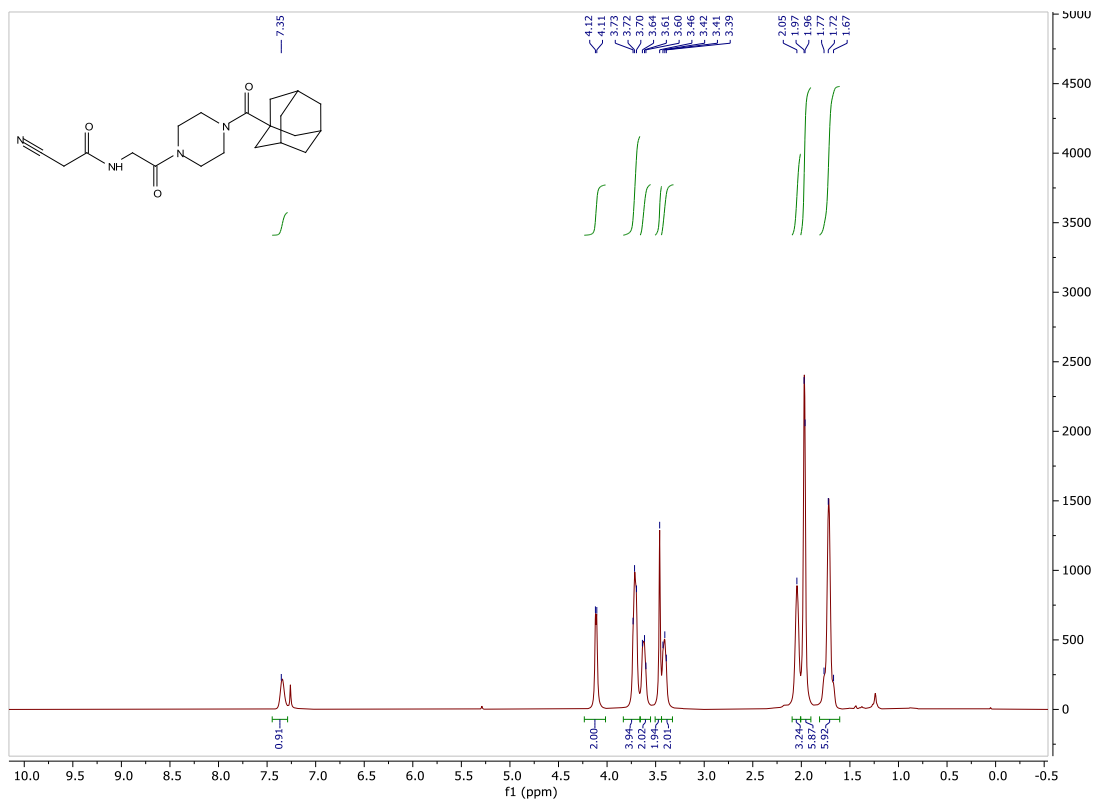
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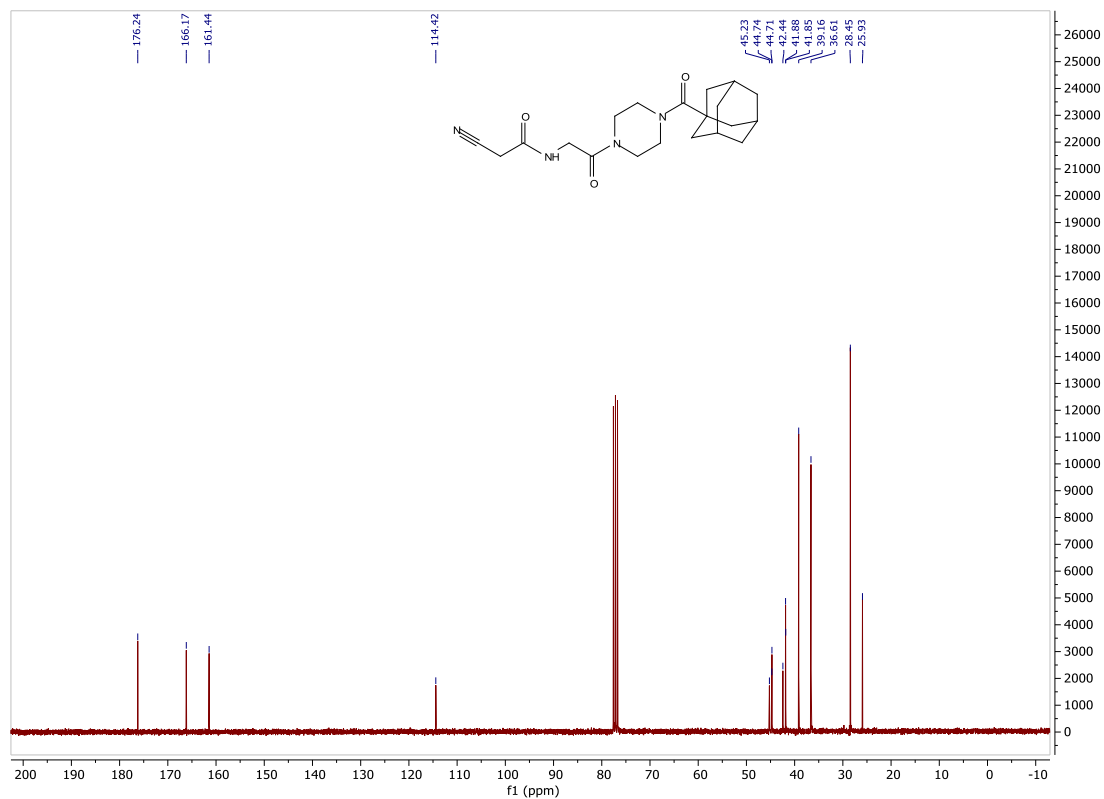
^{19}F NMR (283 MHz, CDCl_3) Spectra of Compound **7k**



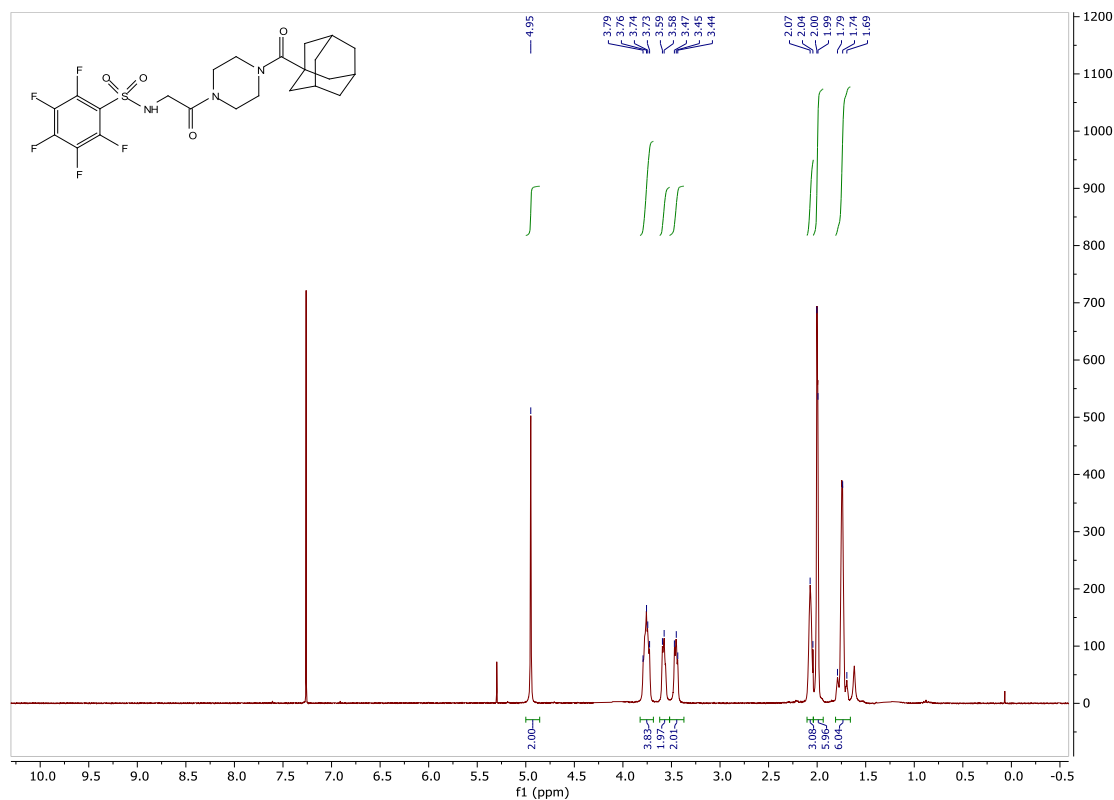
^1H NMR (300 MHz, CDCl_3) Spectra of Compound **7l**



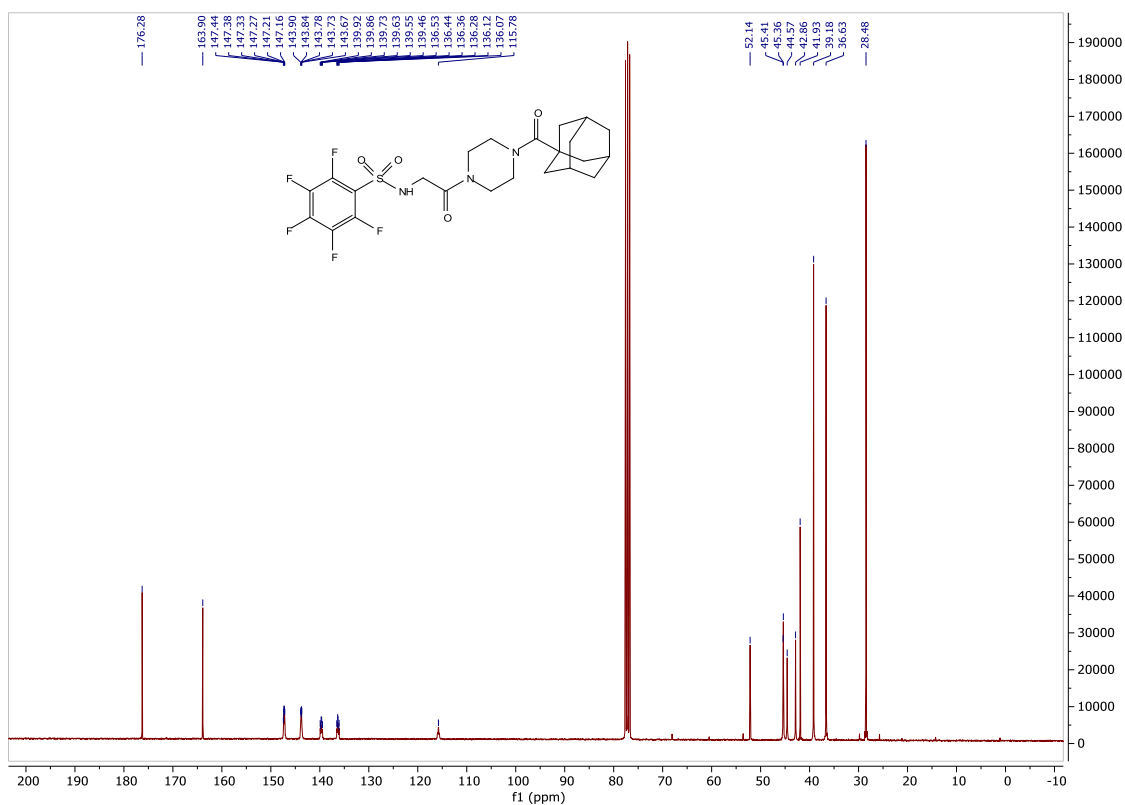
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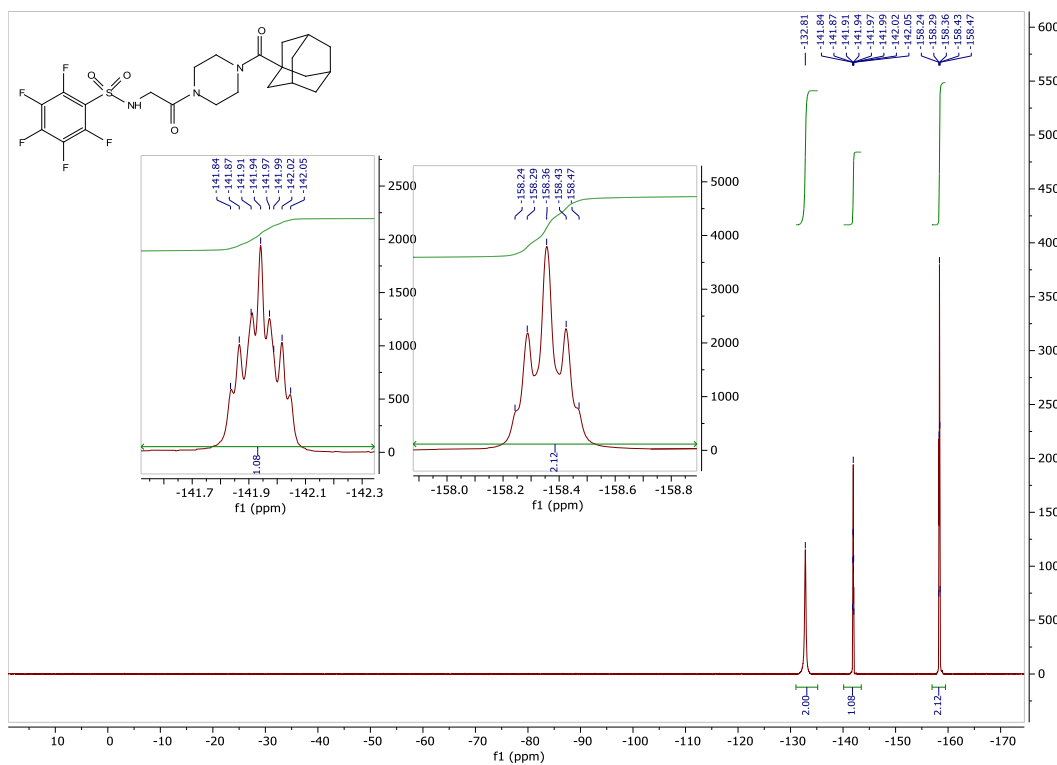
^1H NMR (300 MHz, CDCl_3) Spectra of Compound **7m**



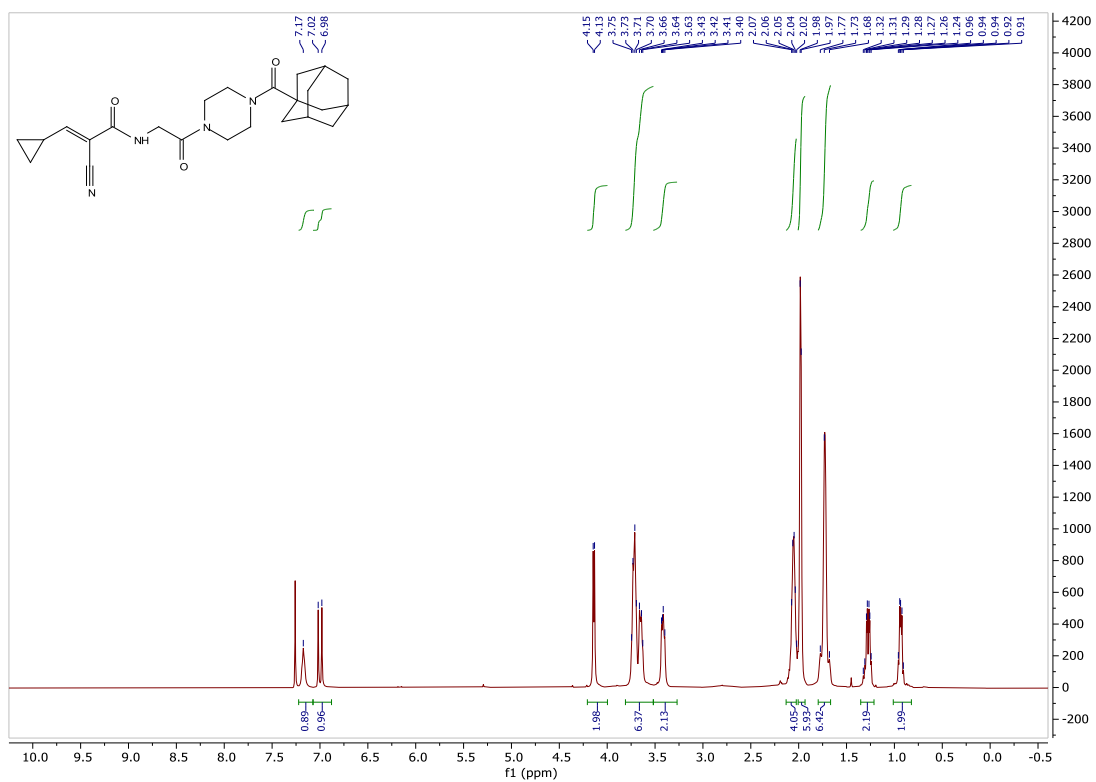
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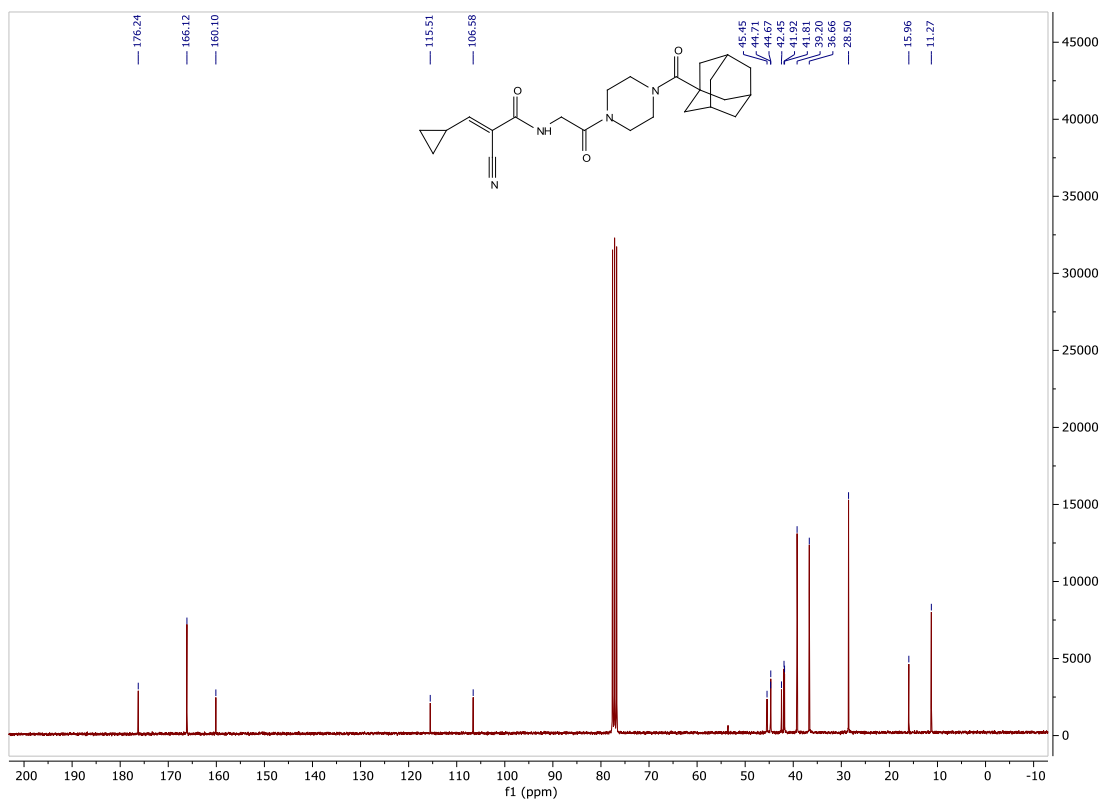
¹⁹F NMR (283 MHz, CDCl₃) Spectra of Compound 7m



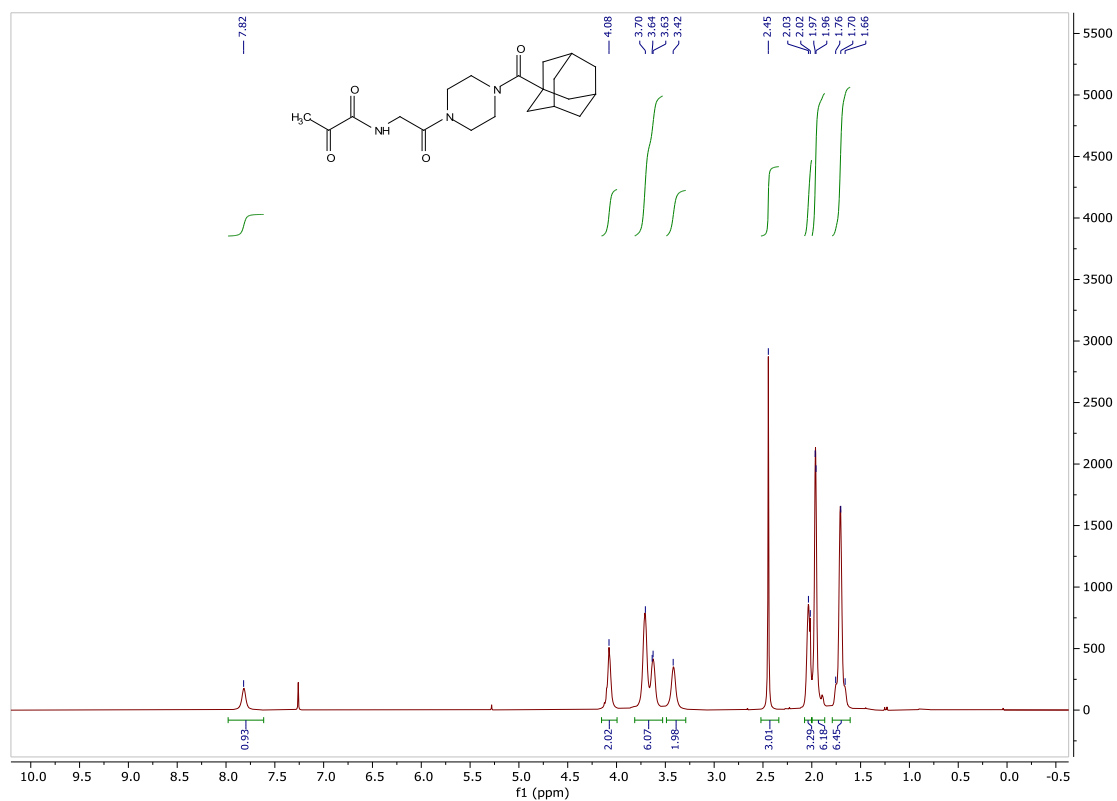
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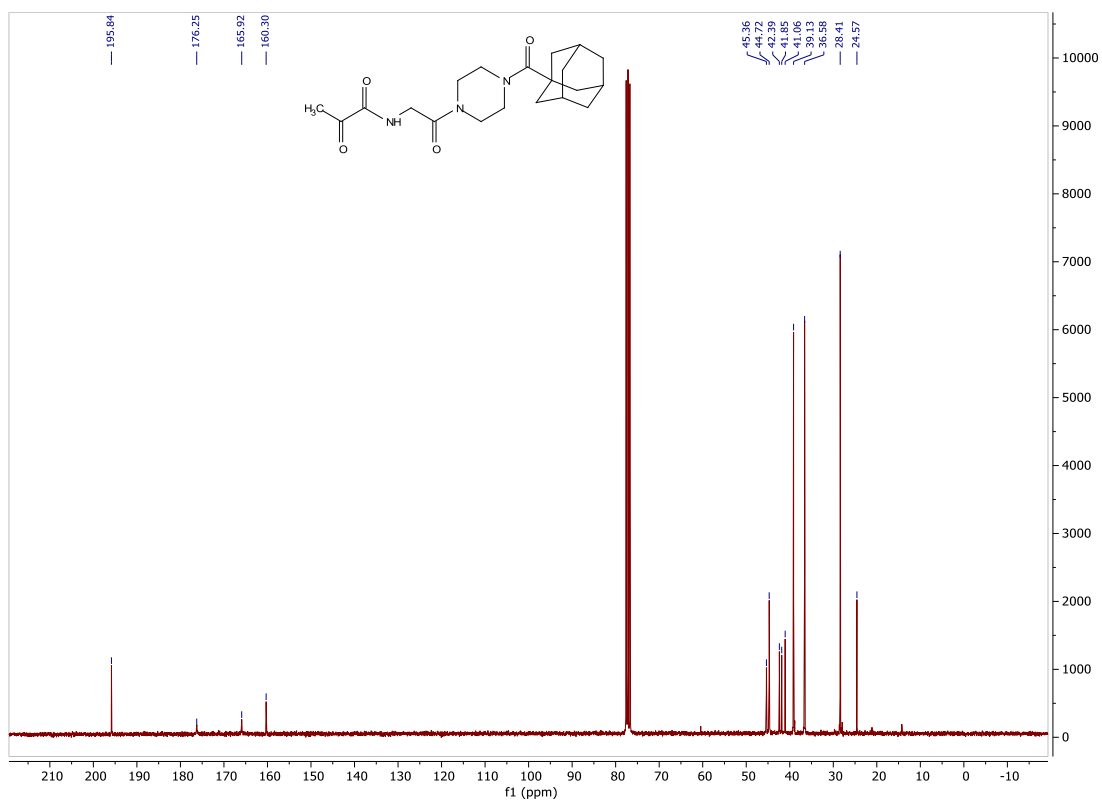
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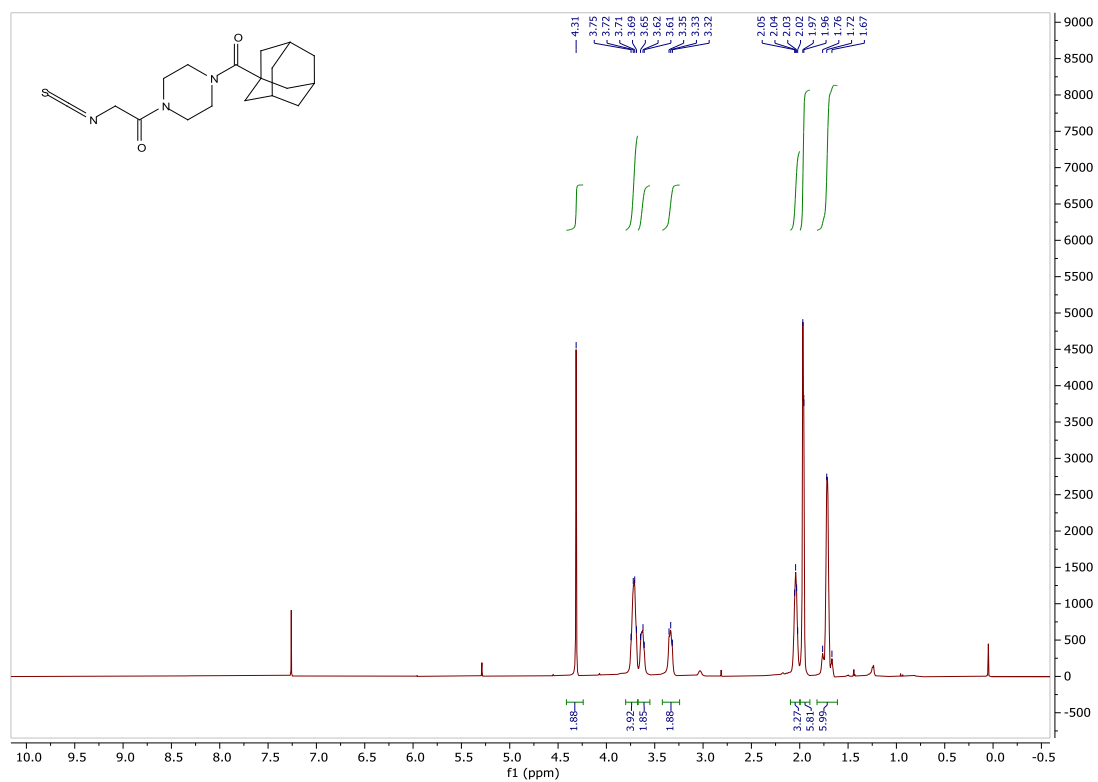
¹H NMR (300 MHz, CDCl₃) Spectra of Compound **7o**



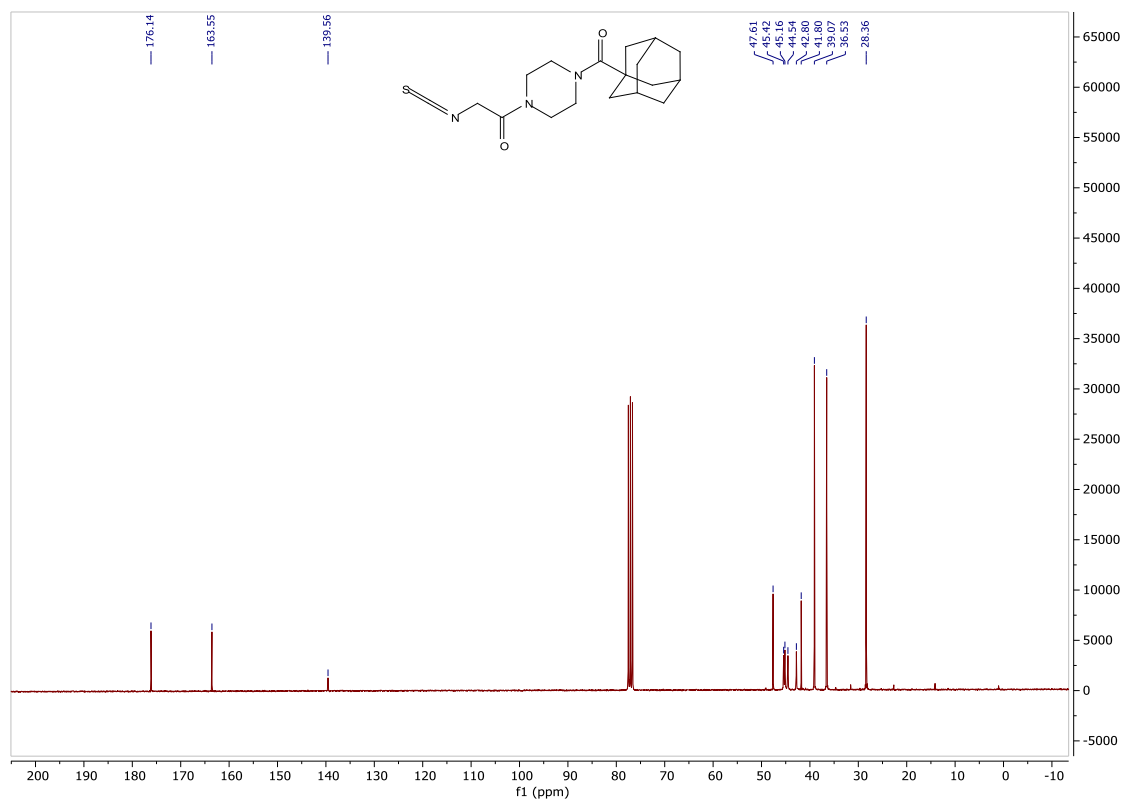
¹³C NMR (75 MHz, CDCl₃) Spectra of Compound **7o**



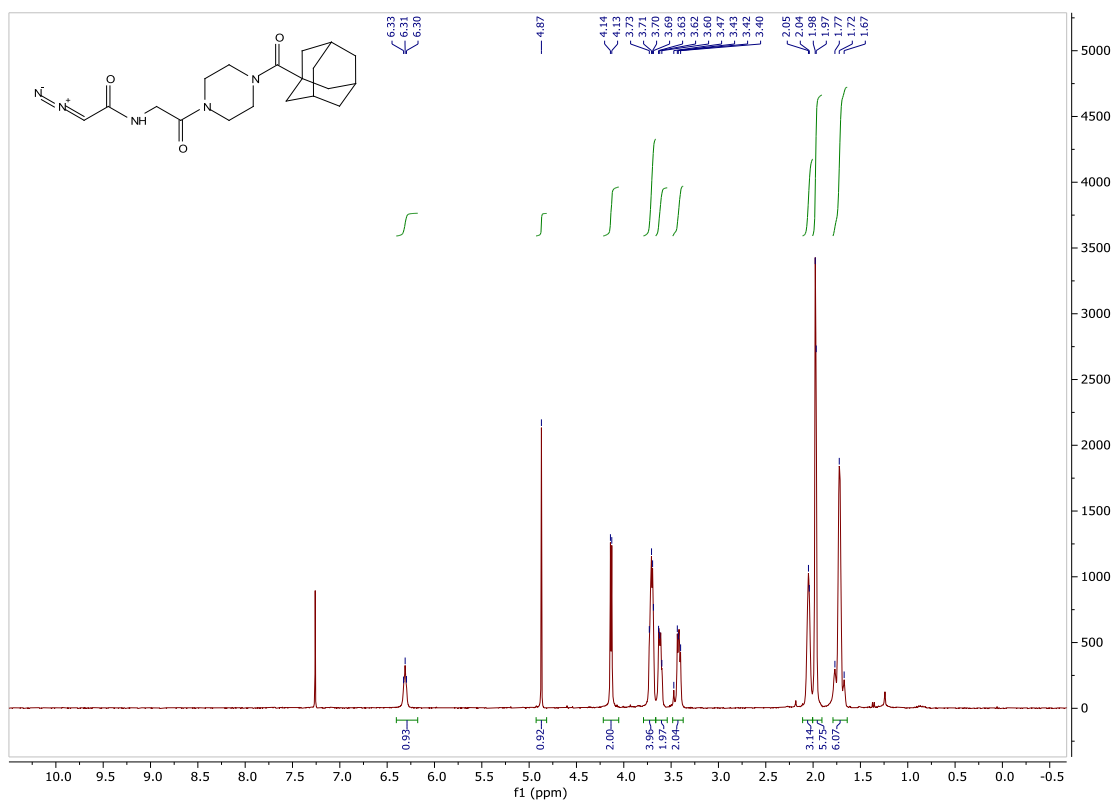
^1H NMR (300 MHz, CDCl_3) Spectra of Compound **7p**



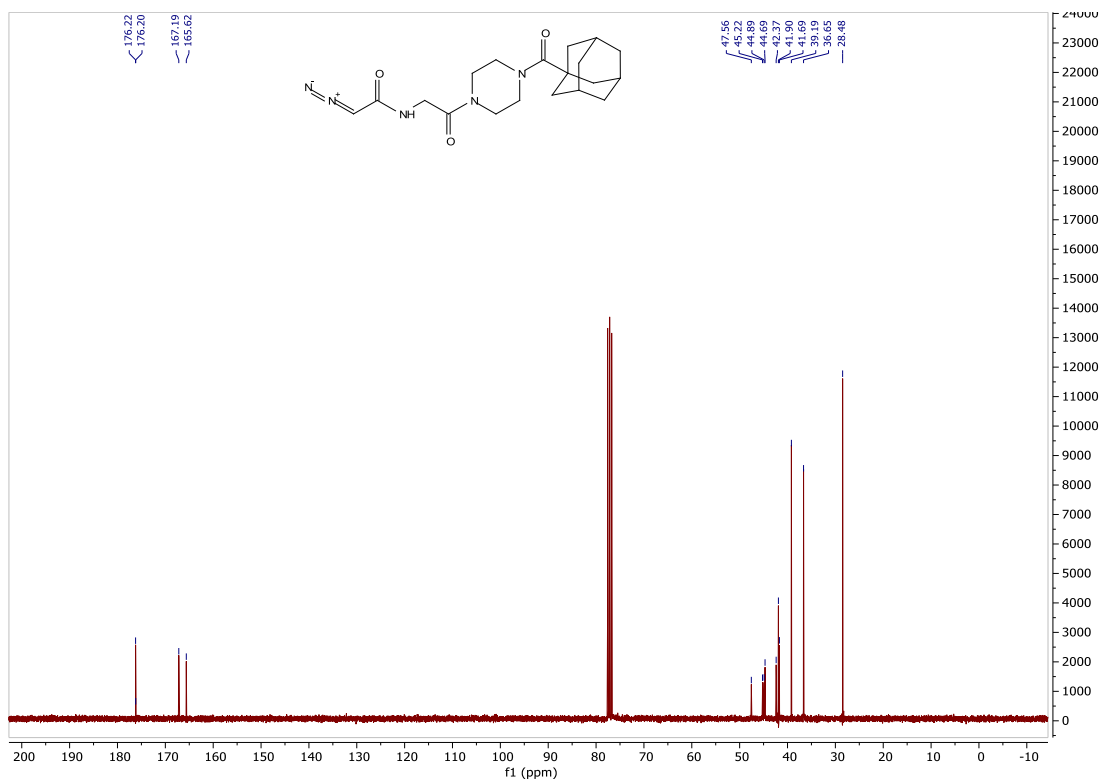
^{13}C NMR (75 MHz, CDCl_3) Spectra of Compound **7p**



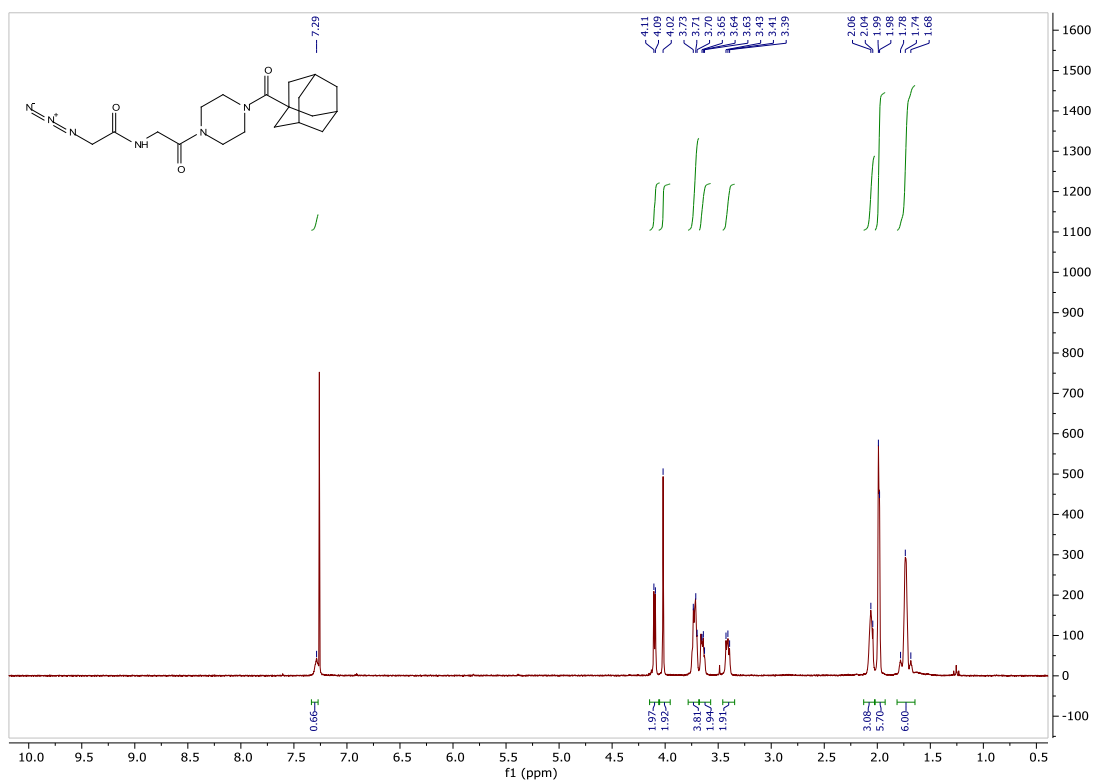
¹H NMR (300 MHz, CDCl₃) Spectra of Compound 7q



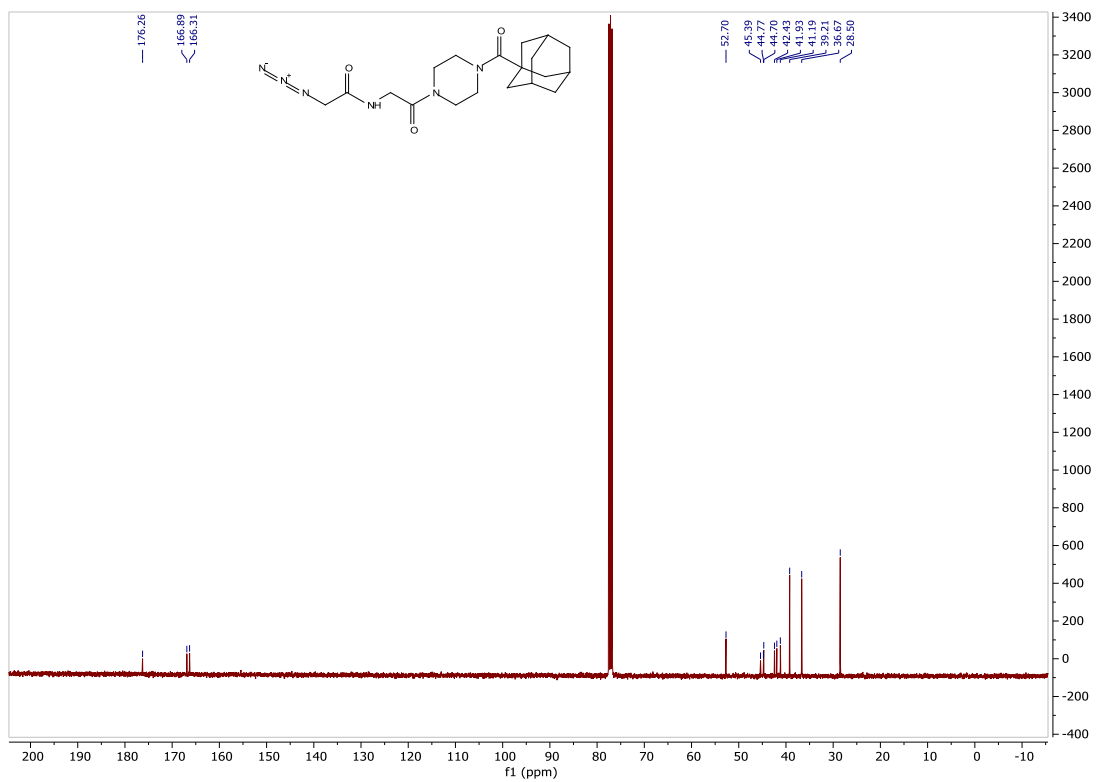
¹³C NMR (75 MHz, CDCl₃) Spectra of Compound 7q



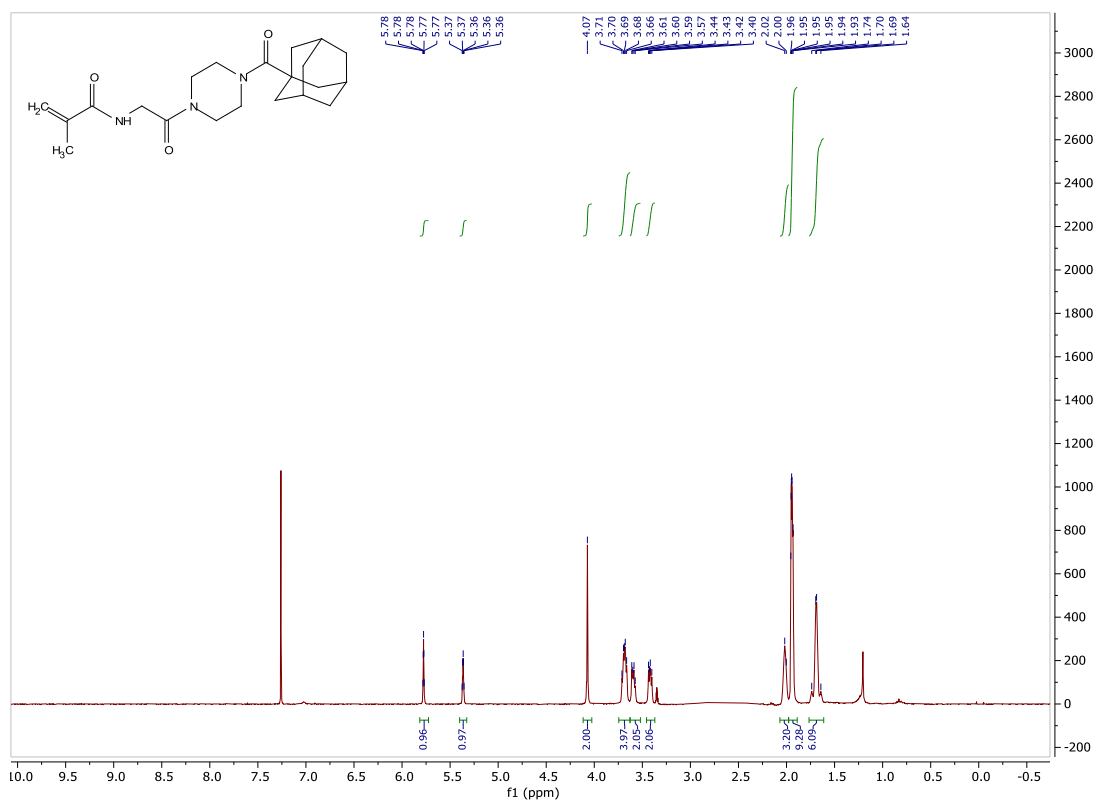
¹H NMR (300 MHz, CDCl₃) Spectra of Compound **I5**



¹³C NMR (75 MHz, CDCl₃) Spectra of Compound **I5**



^1H NMR (300 MHz, CDCl_3) Spectra of Compound **7r**



^{13}C NMR (75 MHz, CDCl_3) Spectra of Compound **7r**

