

## Supporting information

### A binding mode hypothesis of tiagabine confirms liothyronine effect on $\gamma$ -aminobutyric acid transporter 1 (GAT1)

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model	DOPE[2]	DFire[3]	QMEAN[4]	ZScore	PROCHECK[1]			
					core	allow.	gener.	disall.
7	-75432.88	-894.54	0.457	-3.704	94.4	4.7	0.4	0.4
22	-75120.70	-892.59	0.455	-3.723	93.6	6.0	0.4	0.0
34	-75127.98	-891.54	0.454	-3.737	93.8	5.3	0.7	0.2
38	-75029.60	-889.12	0.423	-4.110	93.1	5.8	0.7	0.4
55	-75581.91	-896.77	0.461	-3.652	92.7	6.2	0.9	0.2
57	-75156.31	-891.34	0.431	-4.011	93.1	5.8	1.1	0.0
77	-75214.13	-891.46	0.465	-3.609	93.3	5.6	0.7	0.4
81	-75454.95	-892.85	0.466	-3.592	92.9	5.8	1.1	0.2
82	-75010.28	-888.80	0.477	-3.464	91.3	6.2	1.3	1.1
96	-75220.47	-892.17	0.456	-3.707	93.6	5.6	0.7	0.2
template		-999.51	0.585	-2.180	94.9	5.1	0.0	0.0

**Table S1.** DOPE and SWISSModel[5] results for ten top-ranked hGAT1 homology models. PROCHECK results reporting % of the residues in core, allowed, generally allowed and disallowed regions. Template structure 3F3A was assessed for comparison. Model 55 was further investigated being best in DOPE and DFire scores, model 82 according to QMEAN and ZScore, model 7 having best PROCHECK results. Visual inspection of polar residues in putative membrane areas, as well as side chain orientations of reportedly important amino acids, finally led to the selection of model 55.

Name	ph4 fit score	ph4 rank	docking score	docking rank	chosen
tiagabine	46.96	2	-12.06	1	x
bepotastine	47.08	1	-9.91	2	x
liothyronine	46.54	4	-9.39	4	x
levothyroxine	46.58	3	-8.37	6	
dextrothyroxine	46.15	7	-8.76	5	
ramipril	46.31	6	-9.57	3	x
perindopril	46.43	5	-6.67	7	
lymecycline	46.10	8	(no pose)	-	

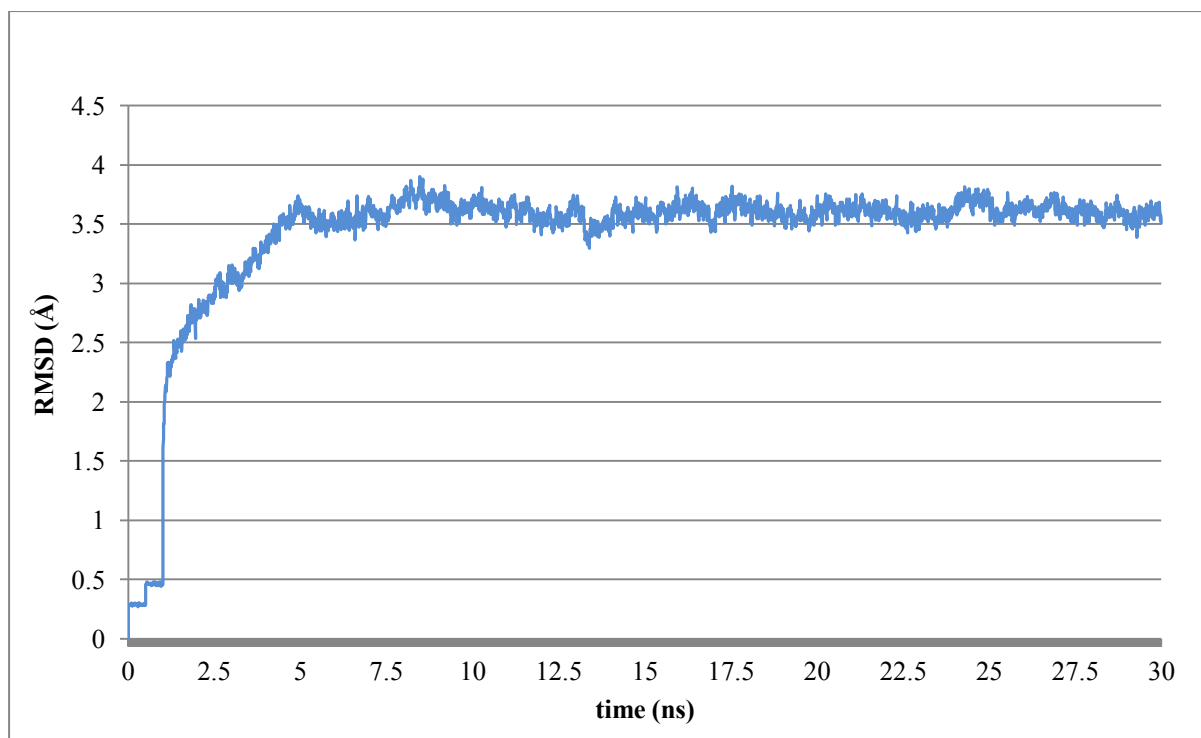
**Table S2.** Score ranking of DrugBank hit compounds and primary selection for testing. Upon detection of liothyronine's GAT modulating potential, the other two related derivatives (L- and D-thyroxine) and reverse liothyronine (3,3',5'-triiodothyronine), not present in the database, were additionally purchased for rationalizing thyroid hormone SAR in GAT-1.

	<b>% uptake</b>	<b>std.dev.</b>	<b>MACCS</b>	<b>FP2</b>	<b>FP4</b>
<b>12</b>	105.0	8.2	0.464	0.220	0.520
<b>13</b>	93.2	9.6	0.485	0.228	0.650
<b>14</b>	99.0	4.9	0.585	0.238	0.550
<b>15</b>	103.5	11.0	0.500	0.176	0.565
<b>16</b>	95.1	12.8	0.615	0.220	0.579
<b>17</b>	102.5	7.8	0.635	0.262	0.579
<b>18</b>	79.3	7.9	0.515	0.212	0.524
<b>19</b>	91.0	21.4	0.415	0.170	0.500
<b>20</b>	93.9	9.6	0.525	0.175	0.545
<b>21</b>	94.7	7.9	0.590	0.388	0.619
<b>22</b>	103.7	9.8	0.480	0.216	0.600
<b>23</b>	107.7	10.0	0.352	0.114	0.364
<b>24</b>	104.9	5.4	0.294	0.280	0.458
<b>25</b>	103.8	5.0	0.533	0.269	0.600
<b>26</b>	107.7	9.6	0.492	0.263	0.500
<b>27a</b>	31.5	5.4	0.254	0.147	0.409
<b>27b</b>	54.2	4.1	0.254	0.147	0.409
<b>27c</b>	126.6	5.1	0.254	0.147	0.409
<b>27d</b>	88.6	16.8	0.254	0.147	0.409

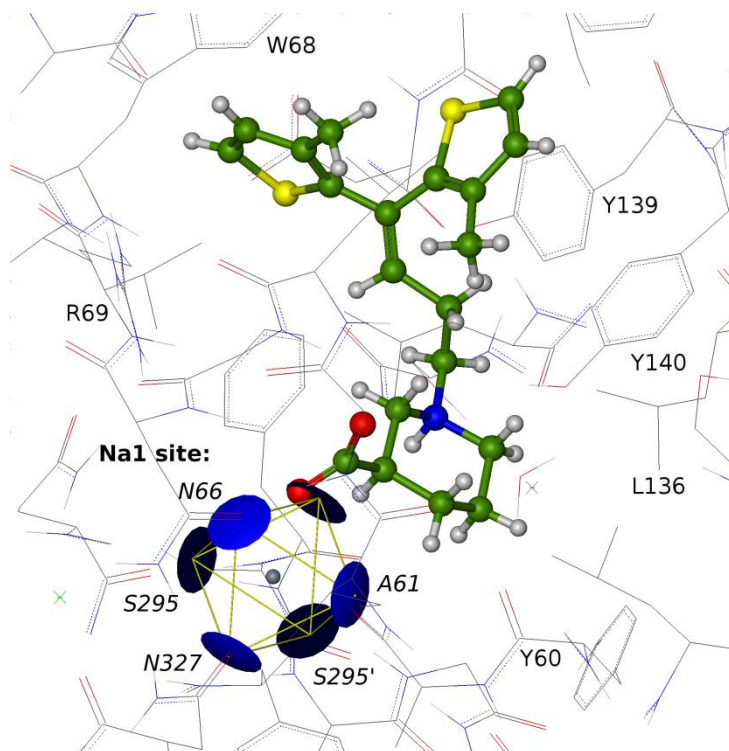
**Table S3.** Activity values (% remaining uptake in presence of 100  $\mu$ M of **5**) and Tanimoto coefficients, derived from substructure-based (MACCS and FP4) and path-based (FP2) fingerprints, of the tested screening compounds. Average MACCS Tanimoto coefficients to Tiagabine, complete screening databases: DrugBank: 0.346; Enamine Advanced & HTS collections: 0.396.

compound	vendor	catalog number	purity (%)	purity data	id. data
12	Enamine	Z728862468	100	LC/MS	NMR
13	Enamine	Z1459949930	99.0	LC/MS	NMR
14	Enamine	Z1459991978	100	LC/MS	NMR
15	Enamine	Z1459967721	95.6	LC/MS	NMR
16	Enamine	Z1459983749	100	LC/MS	NMR
17	Enamine	Z1465347397	100	LC/MS	NMR
18	Enamine	Z1459927845	100	LC/MS	NMR
19	Enamine	Z1523065505	100	LC/MS	NMR
20	Enamine	Z1459962146	100	LC/MS	NMR
21	Enamine	Z1459930349	100	LC/MS	NMR
22	Enamine	Z53113106	100	LC/MS	NMR
23	Enamine	Z317025968	98.3	LC/MS	NMR
24	Enamine	Z1541759893	100	LC/MS	NMR
27	AvaChem	2571	99.9	CoA	CoA
26	AvaChem	2593	100	CoA	CoA
27a	Sigma-Aldrich	T2877	98.0	CoA	CoA
27b	Fluka	89430	99.1	HPLC	CoA
27c	Fluka	89425	98.2	HPLC	CoA
27d	Sigma-Aldrich	T0281	97.9	CoA	CoA

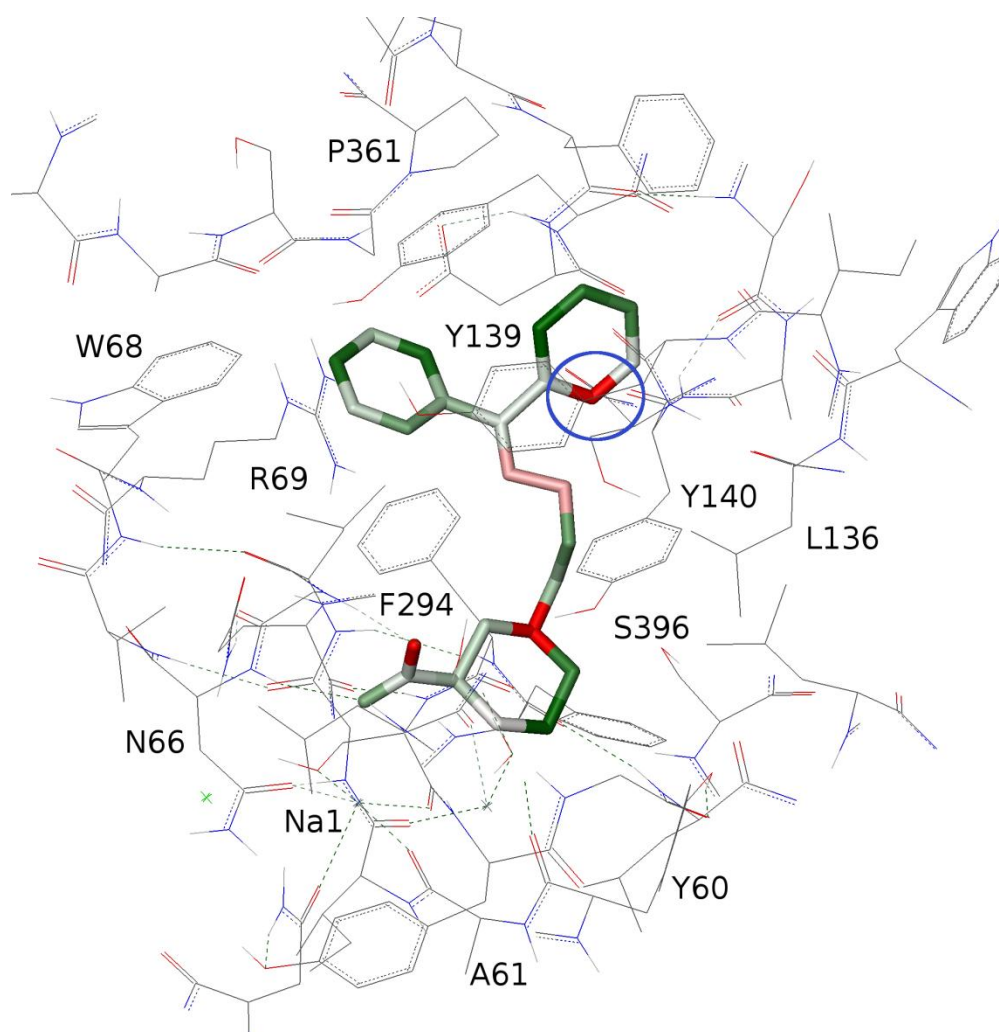
**Table S4.** Specifications of screening compounds and method of verification, as provided by vendors. Purity stated by coupled liquid chromatography/mass spectrometry (LC/MS), high performance liquid chromatography (HPLC) or certificate of analysis (CoA). Identity stated by nuclear magnetic resonance (NMR) spectra or CoA.



**Figure S1.** Protein backbone RMSD during 30 ns of MD simulation of the complex with tiagabine. Release phase visible in two 500 ps steps of the first nanosecond.

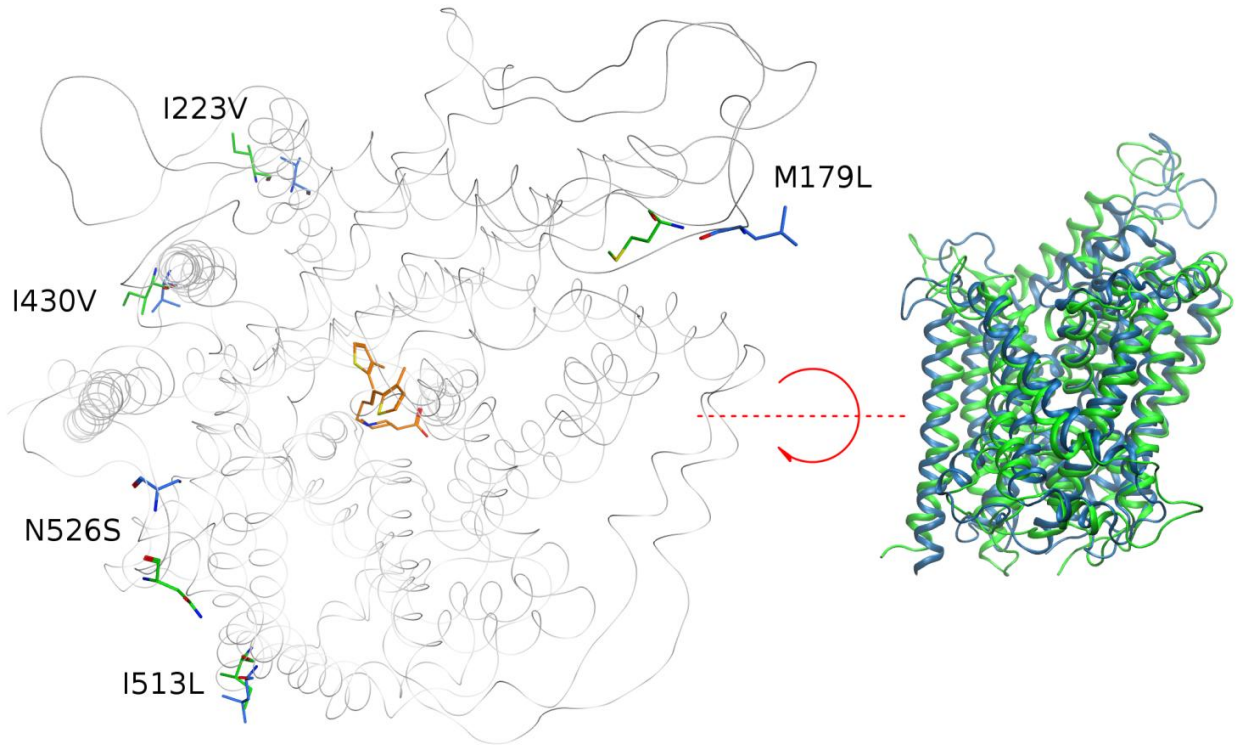


**Figure S2.** Octahedral geometry of the sodium site. Coordination points taken by the ligand carboxylic acid, backbone carbonyl groups of A61 and S295, as well as side chains of N66, S295 and N327 are highlighted in blue.



**Figure S3.** Compound **11** assessed with HYDE.[6] Example for an unfavorably located pyridine nitrogen atom in context with one of the hydrophobic cavities is highlighted in red / blue circle.





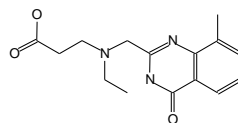
**Figure S4.** Non-conserved residues hGAT-1 (green) – rGAT-1 (blue). View from extracellular side.

## REFERENCES

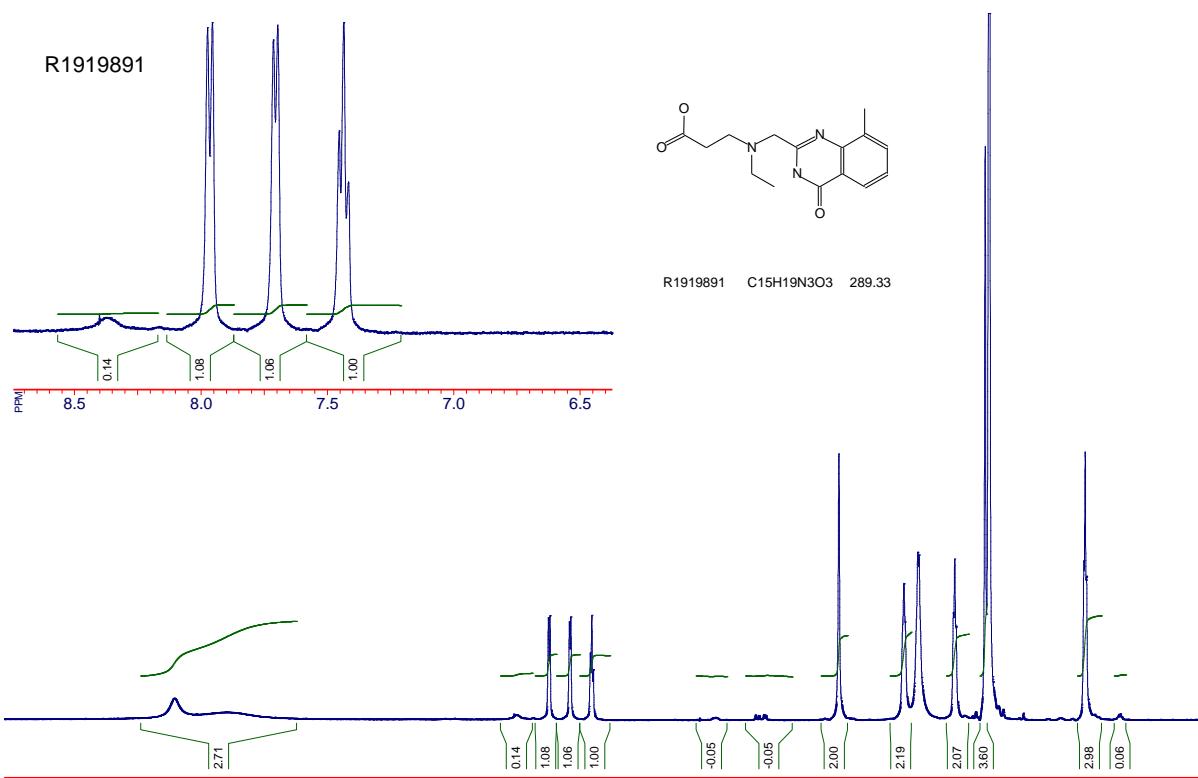
1. Laskowski, R.A., et al., *PROCHECK: a program to check the stereochemical quality of protein structures*. J. Appl. Cryst., 1993. **26**(2): p. 283-291.
2. Shen, M.Y. and A. Sali, *Statistical potential for assessment and prediction of protein structures*. Protein Sci, 2006. **15**(11): p. 2507-24.
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5. Arnold, K., et al., *The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling*. Bioinformatics, 2006. **22**(2): p. 195-201.
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Z728862468

R1919891



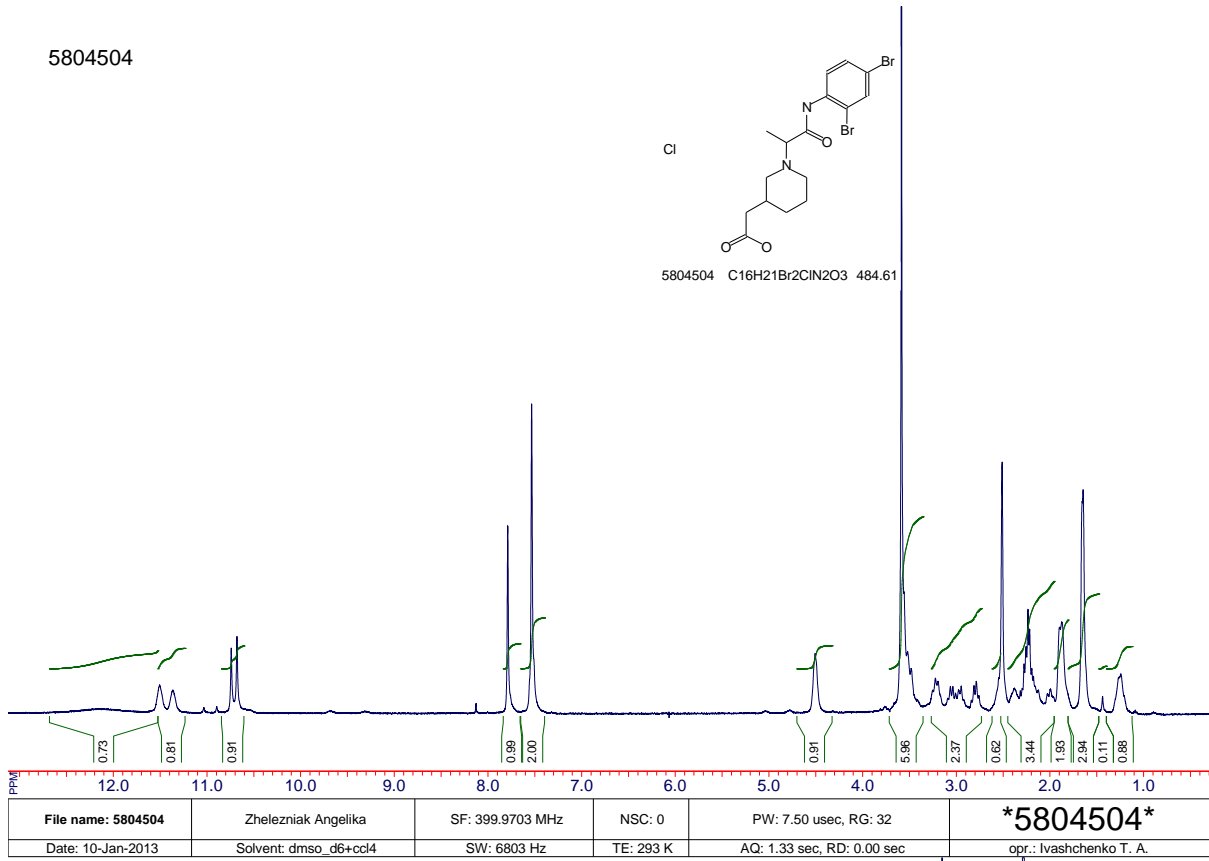
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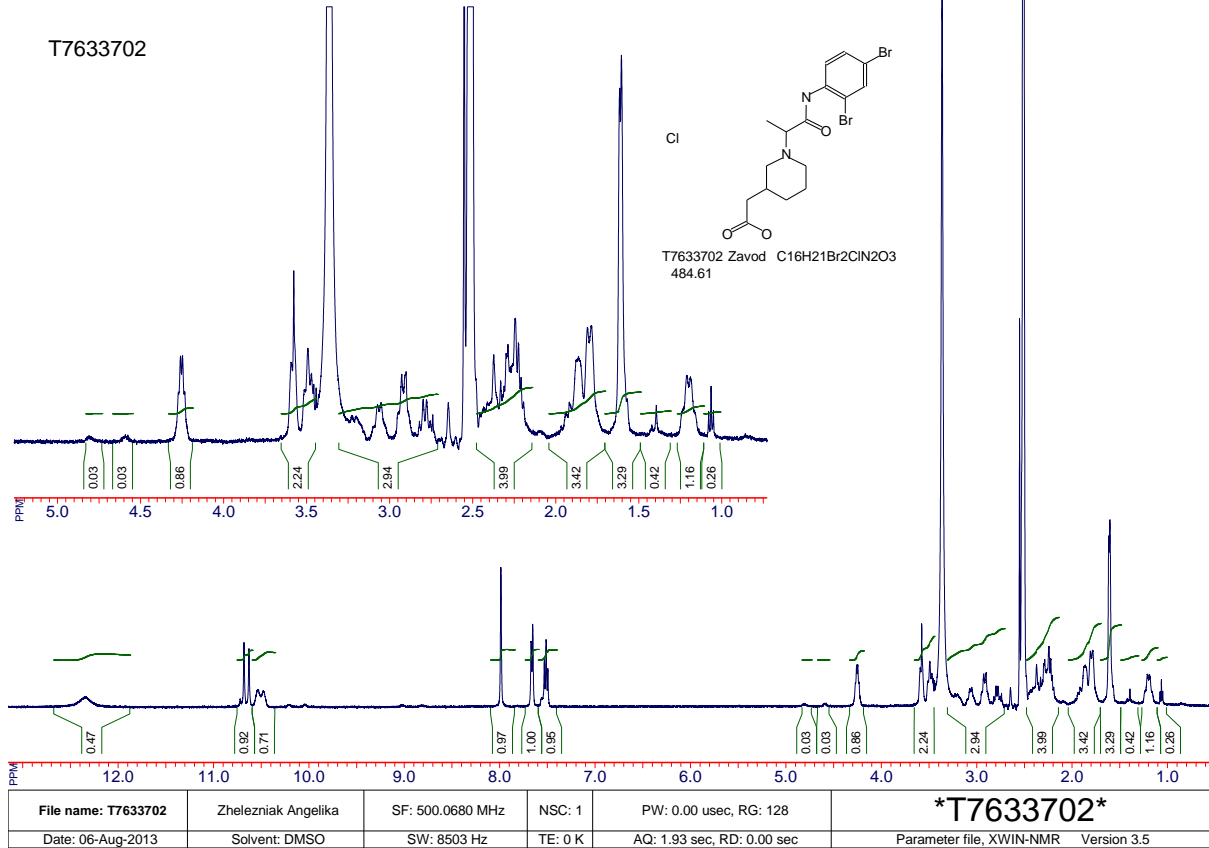
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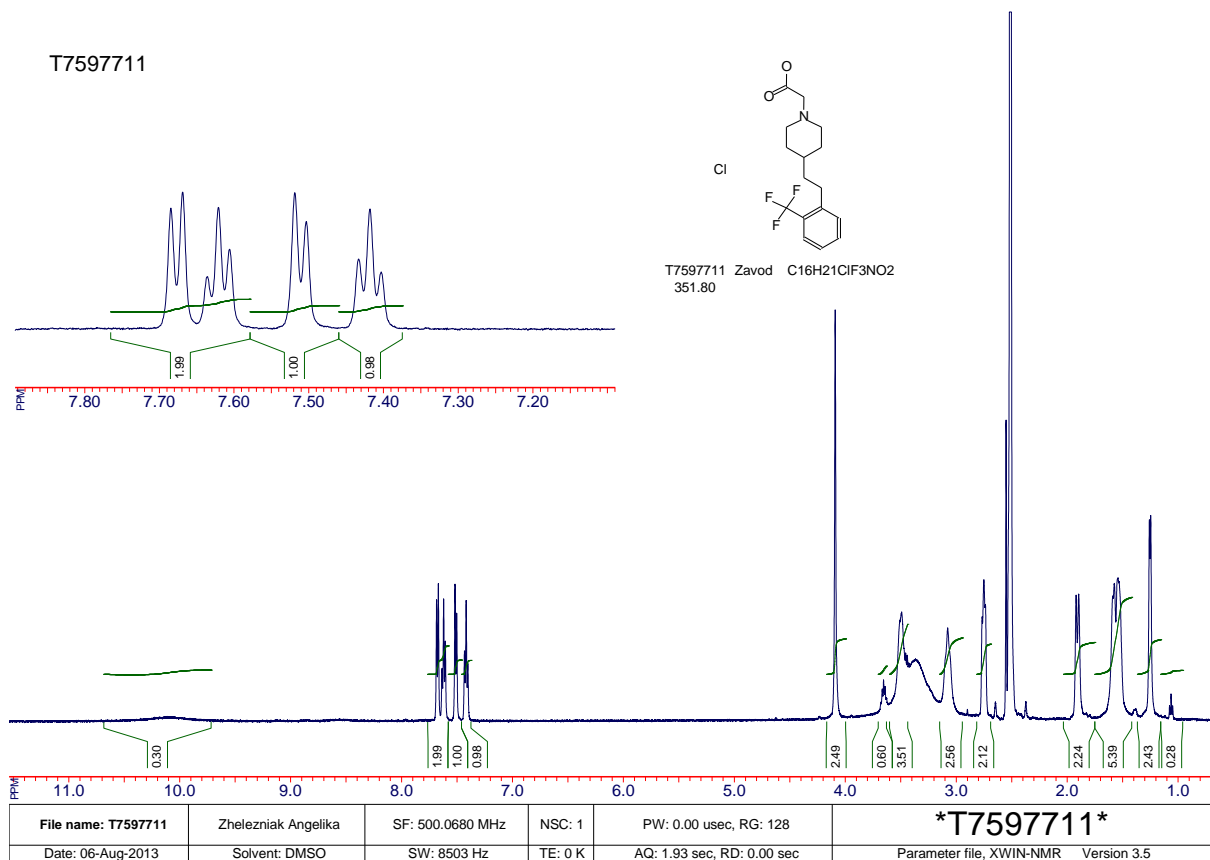
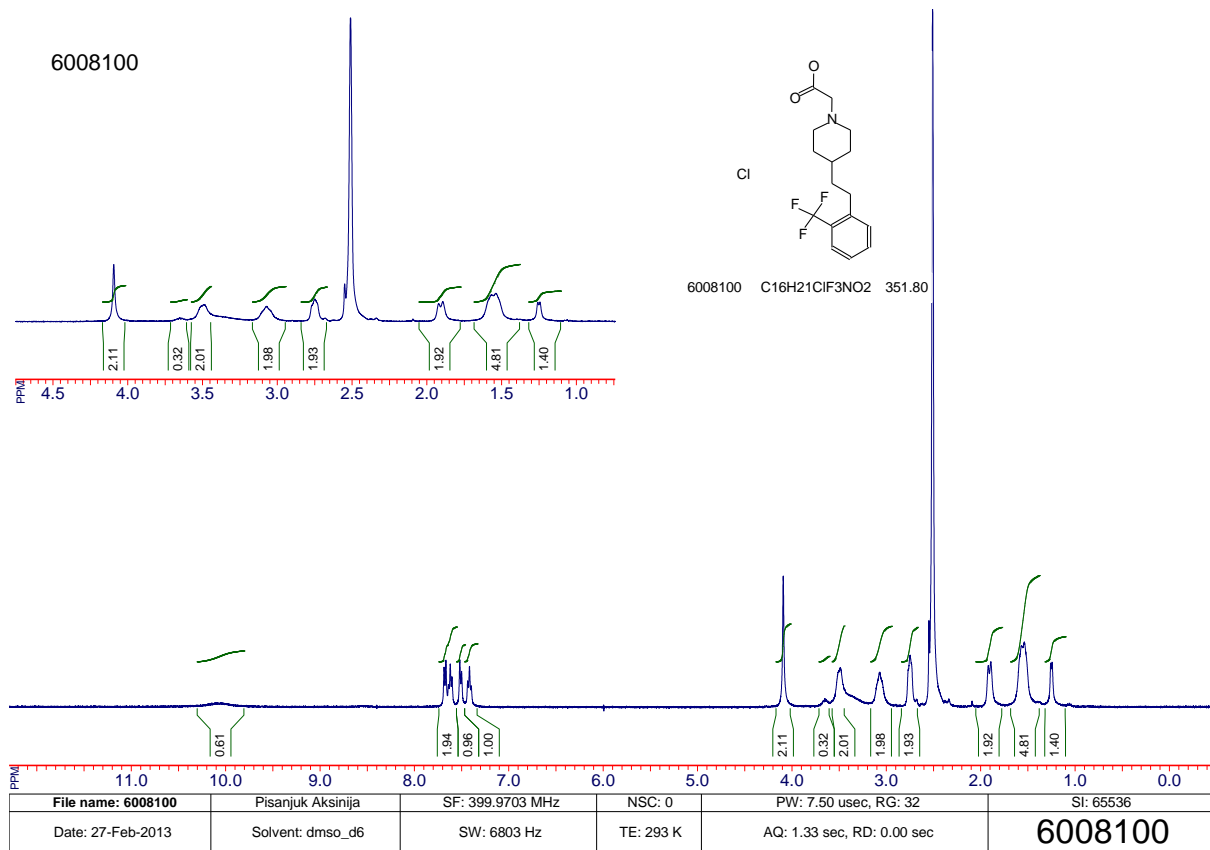
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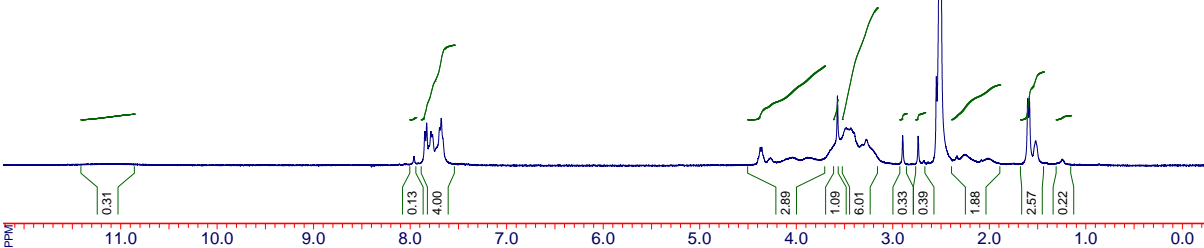
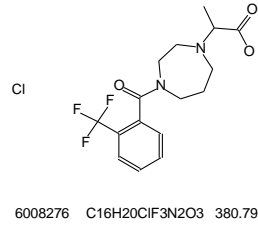
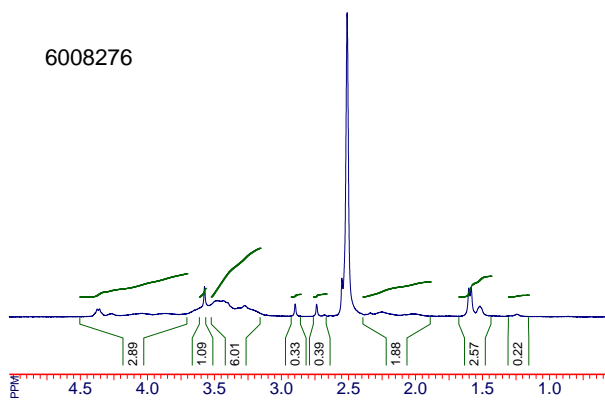


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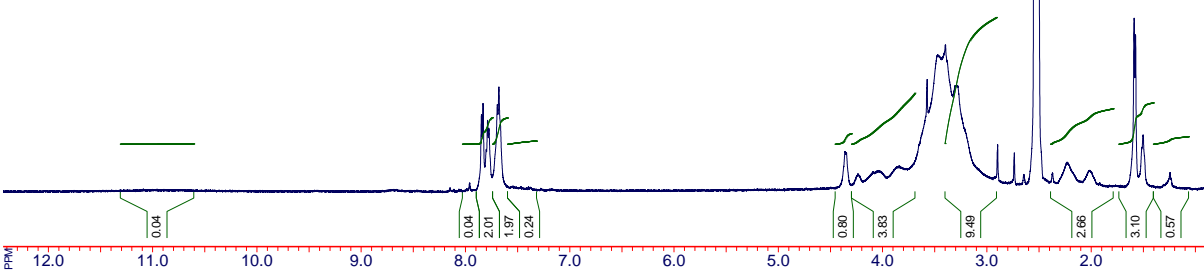
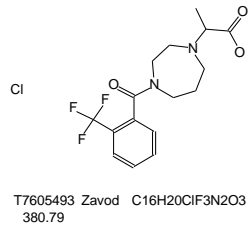
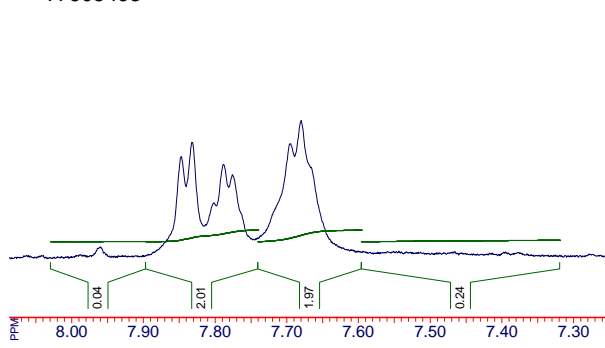
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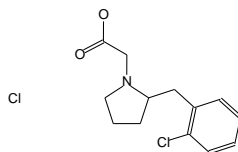
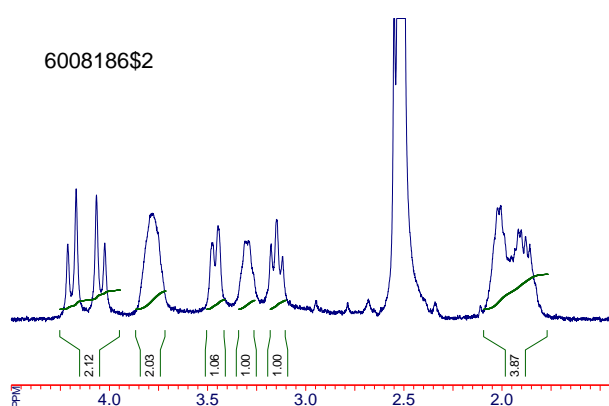
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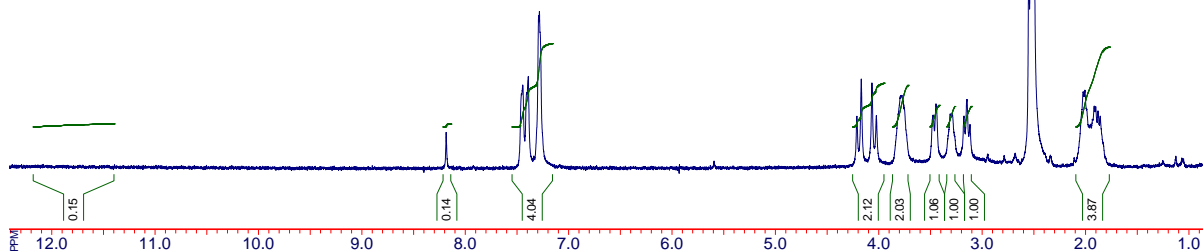
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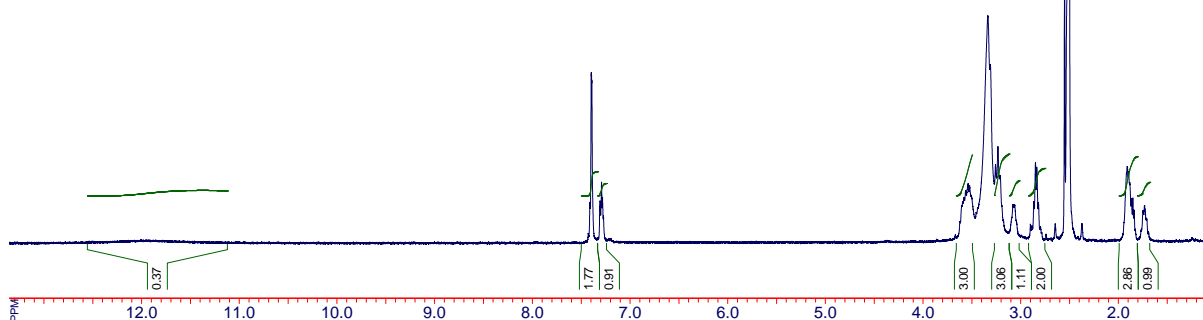
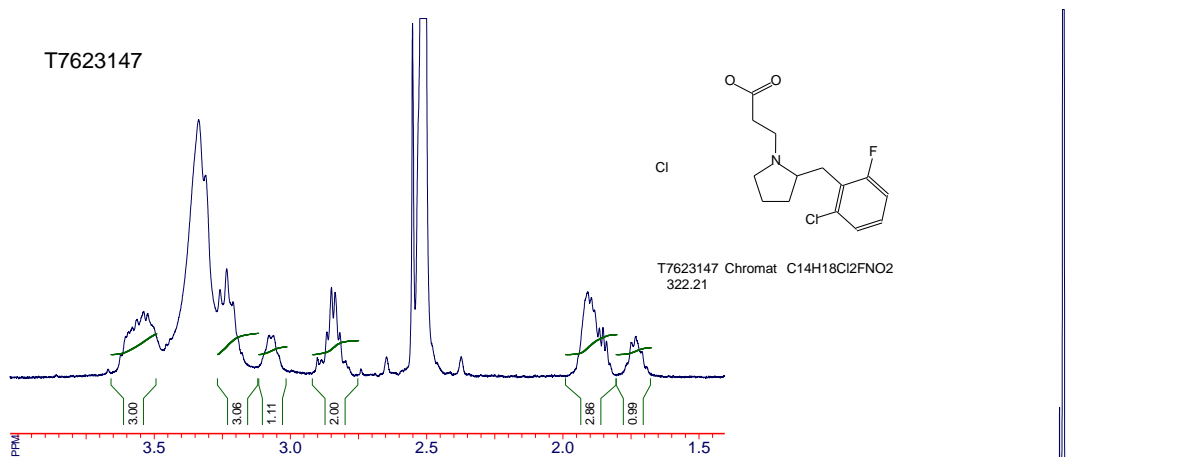
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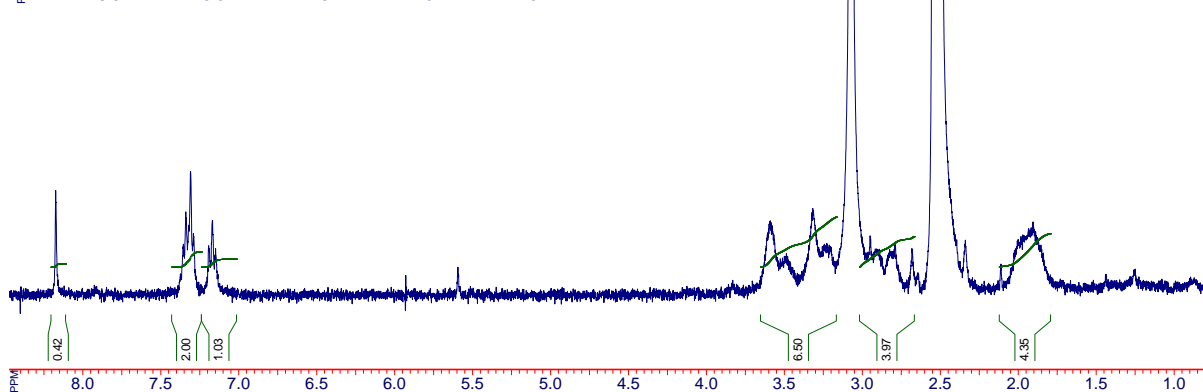
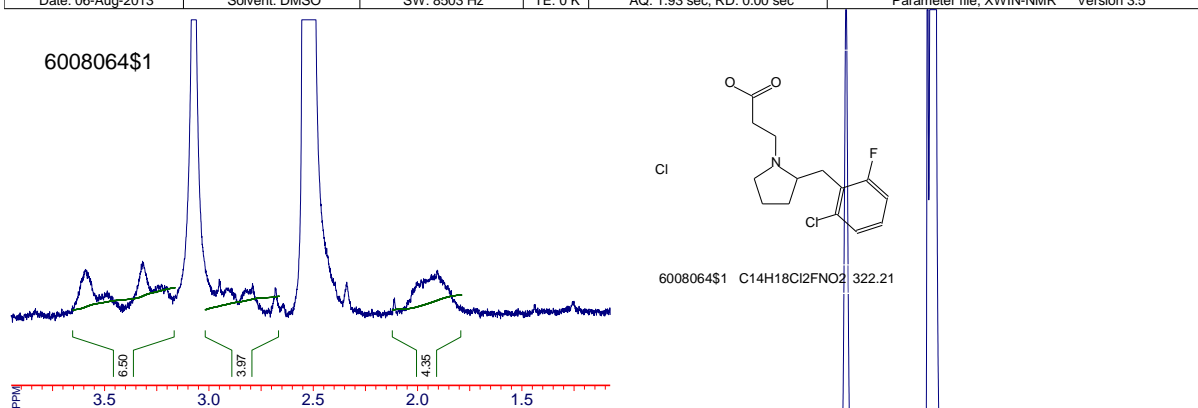
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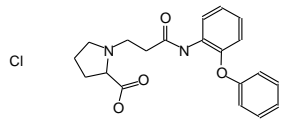
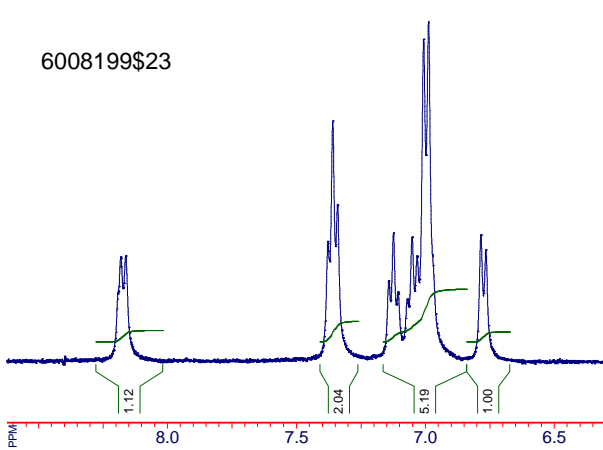


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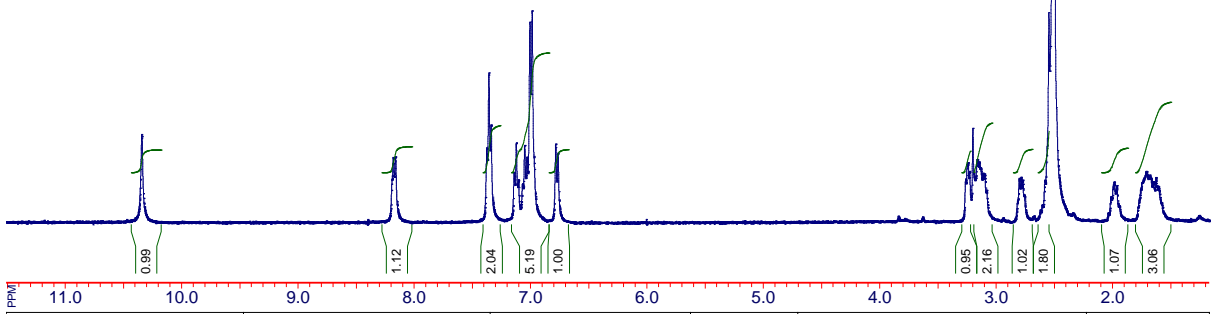


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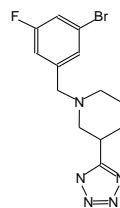
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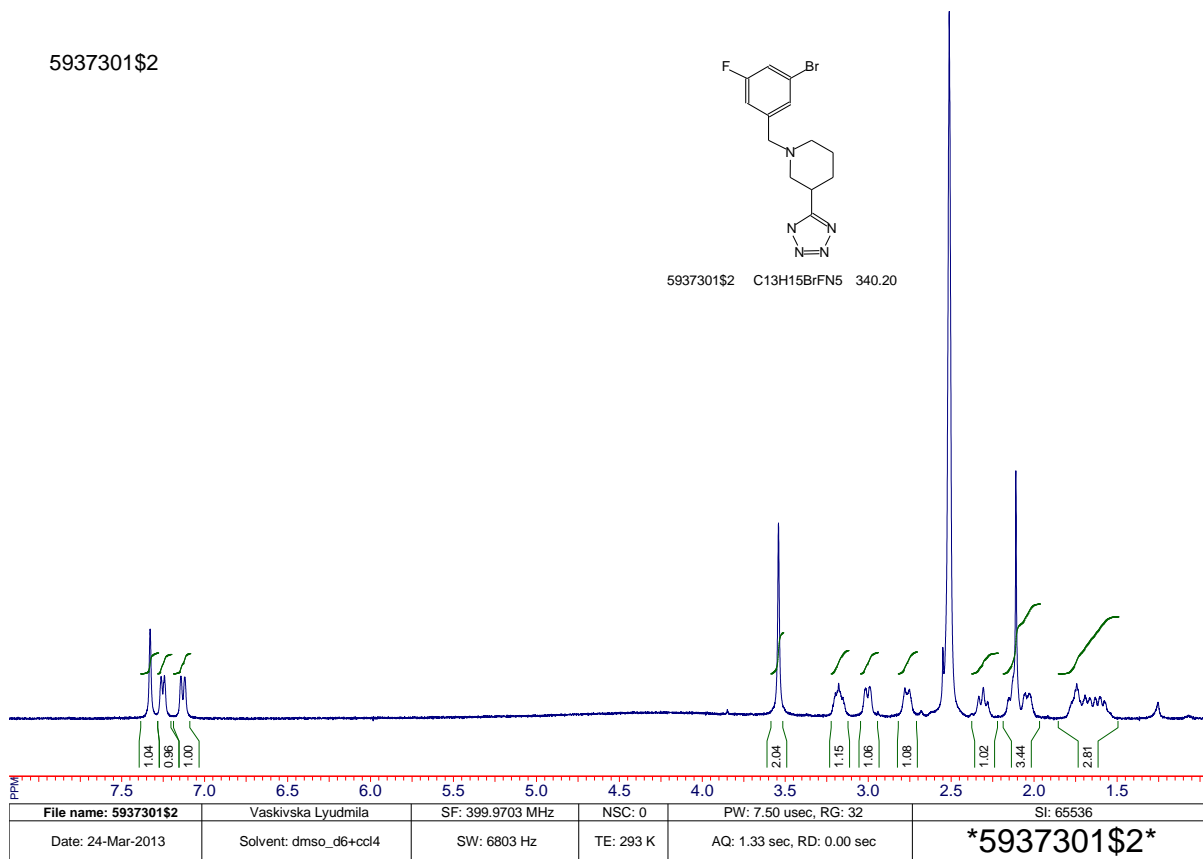
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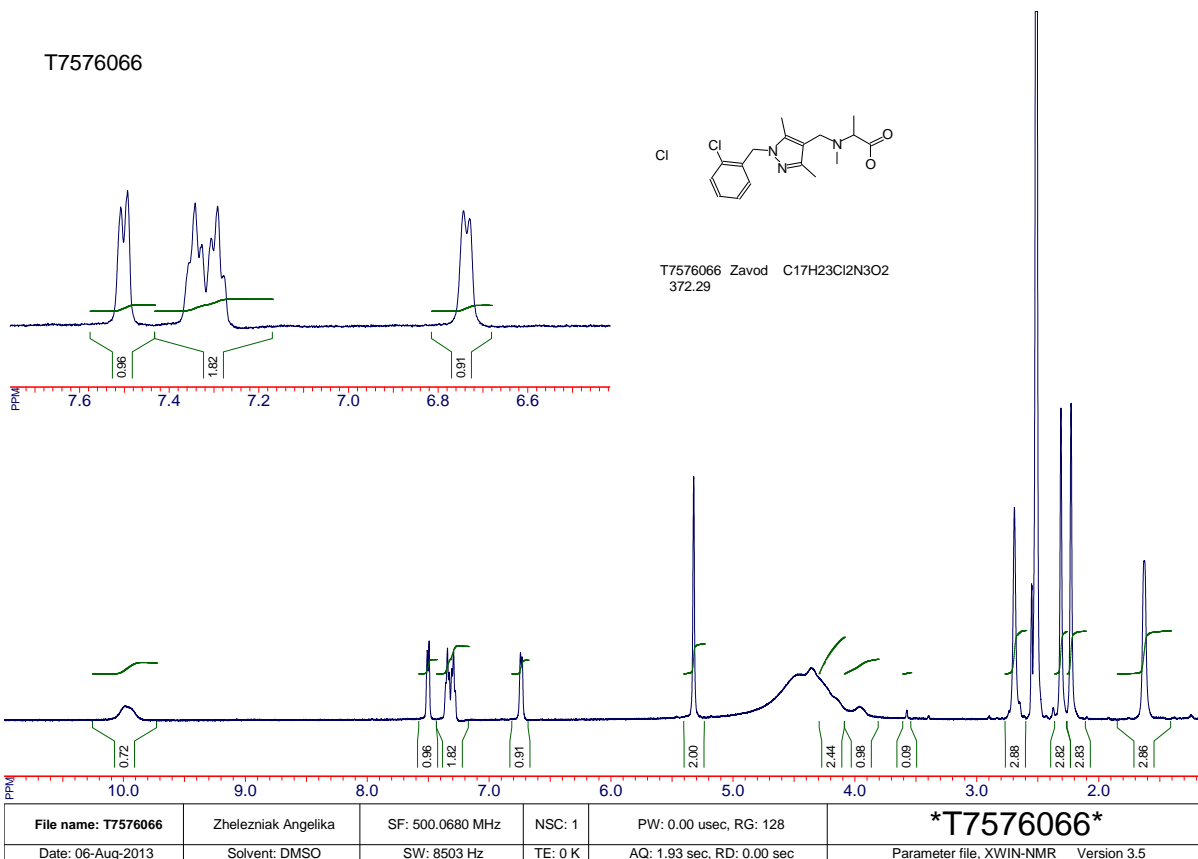


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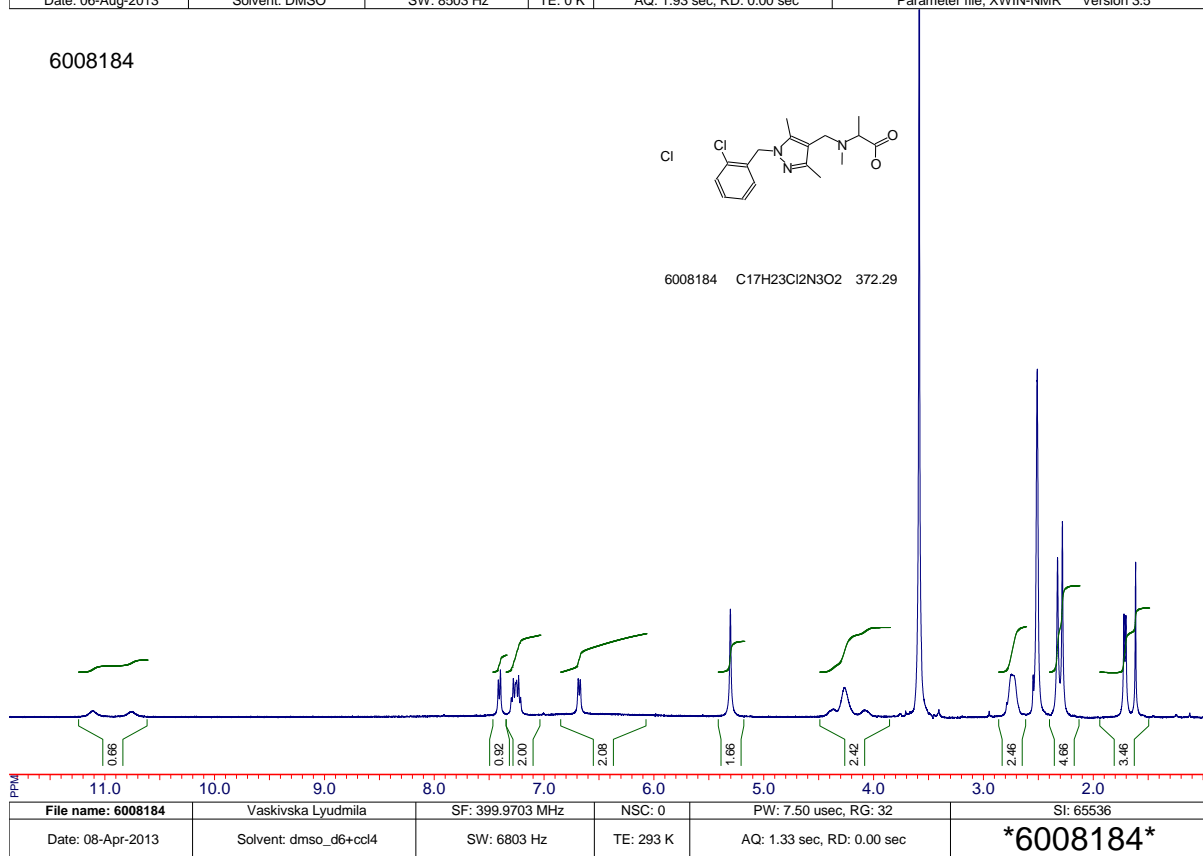


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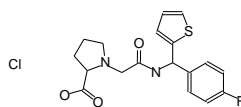


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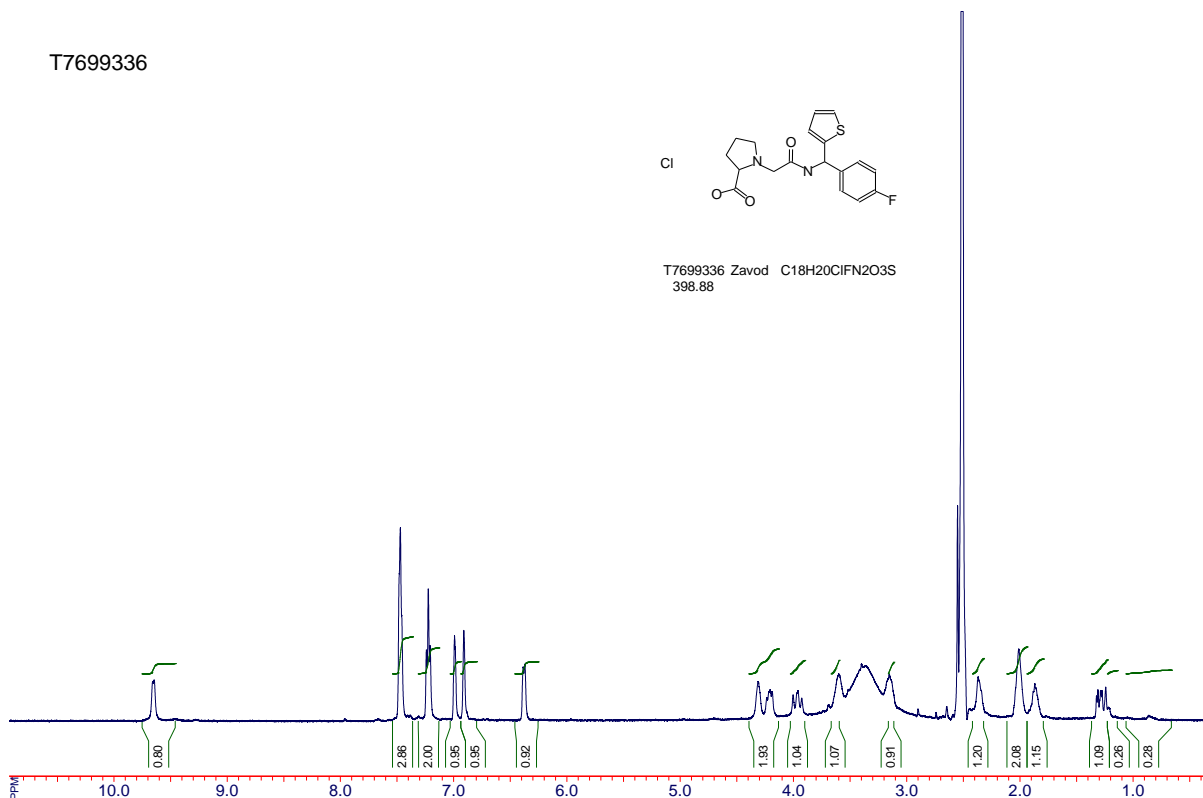


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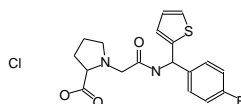


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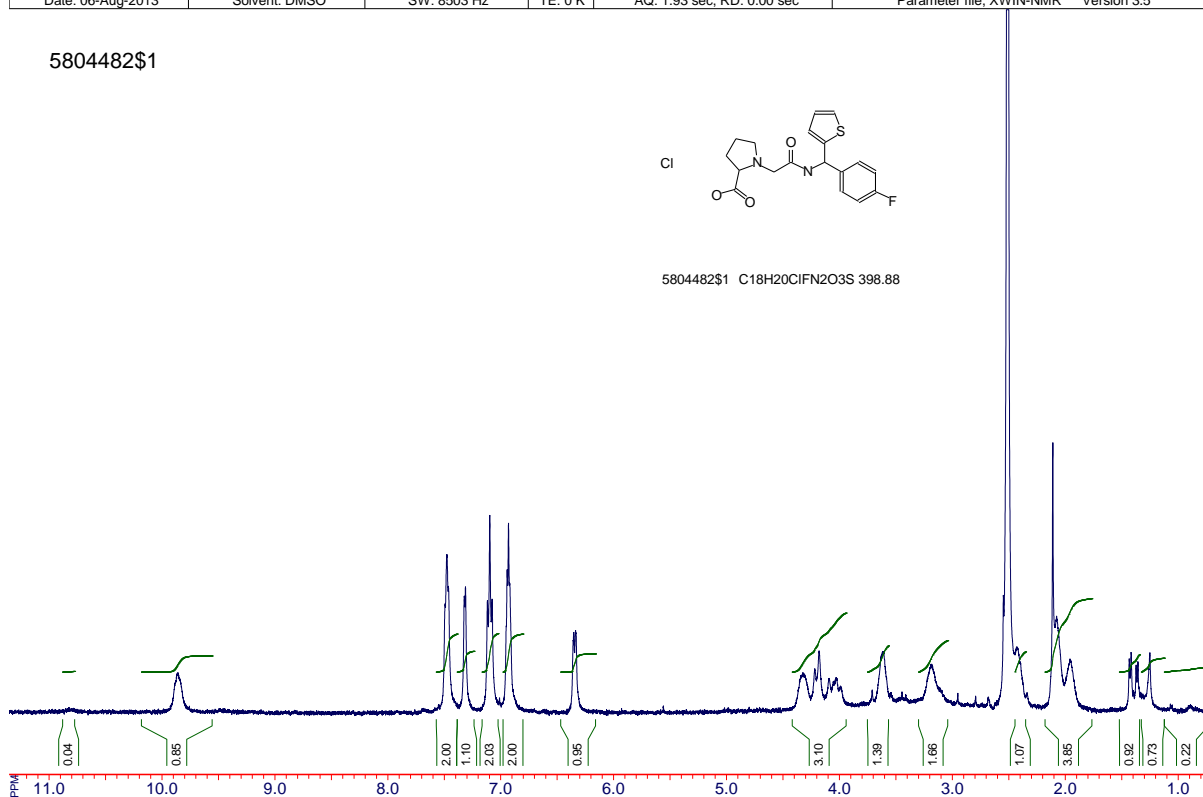


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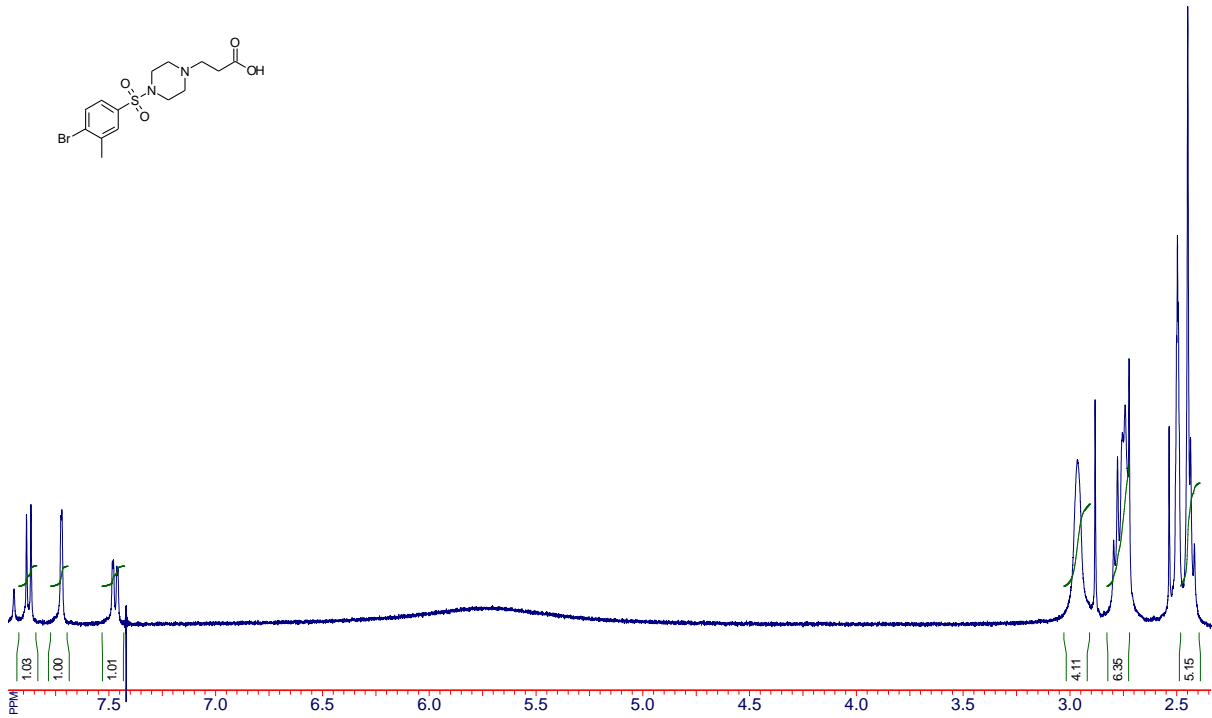
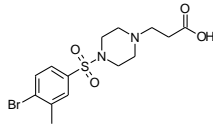
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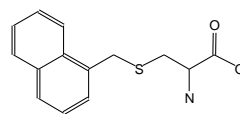
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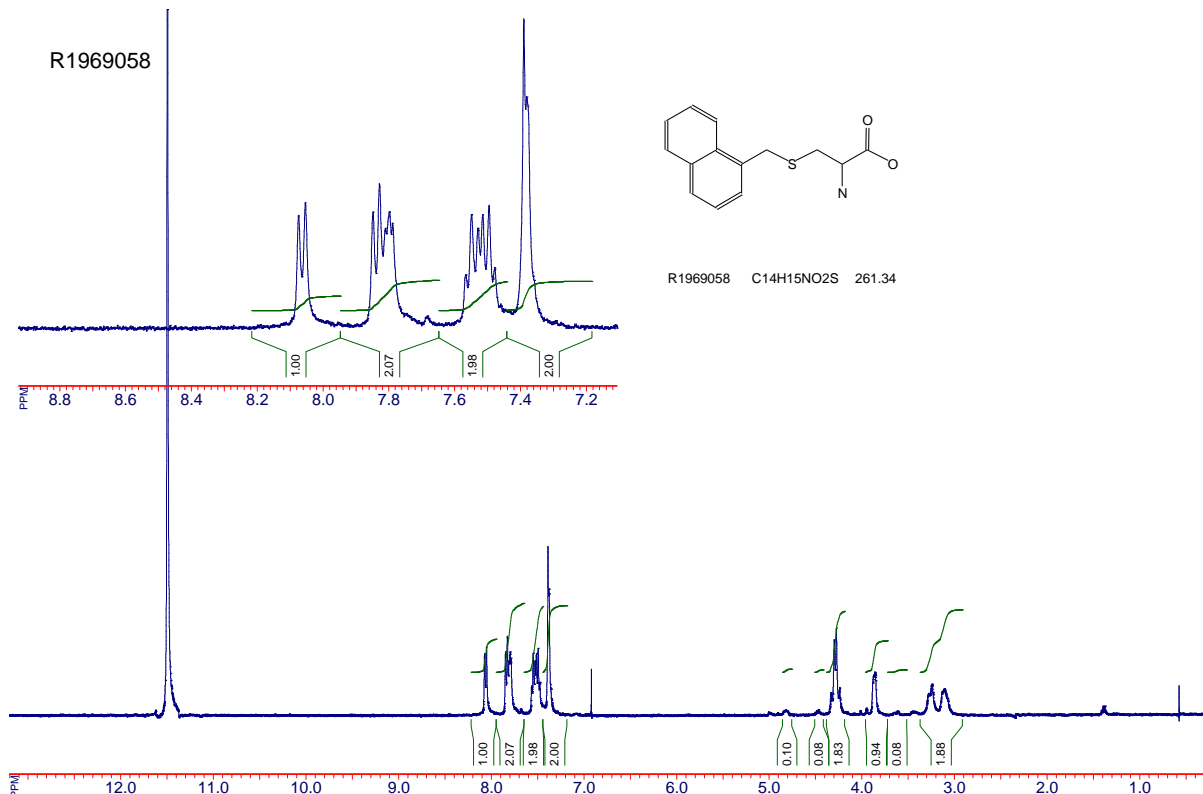
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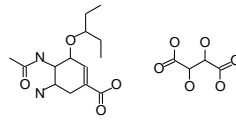
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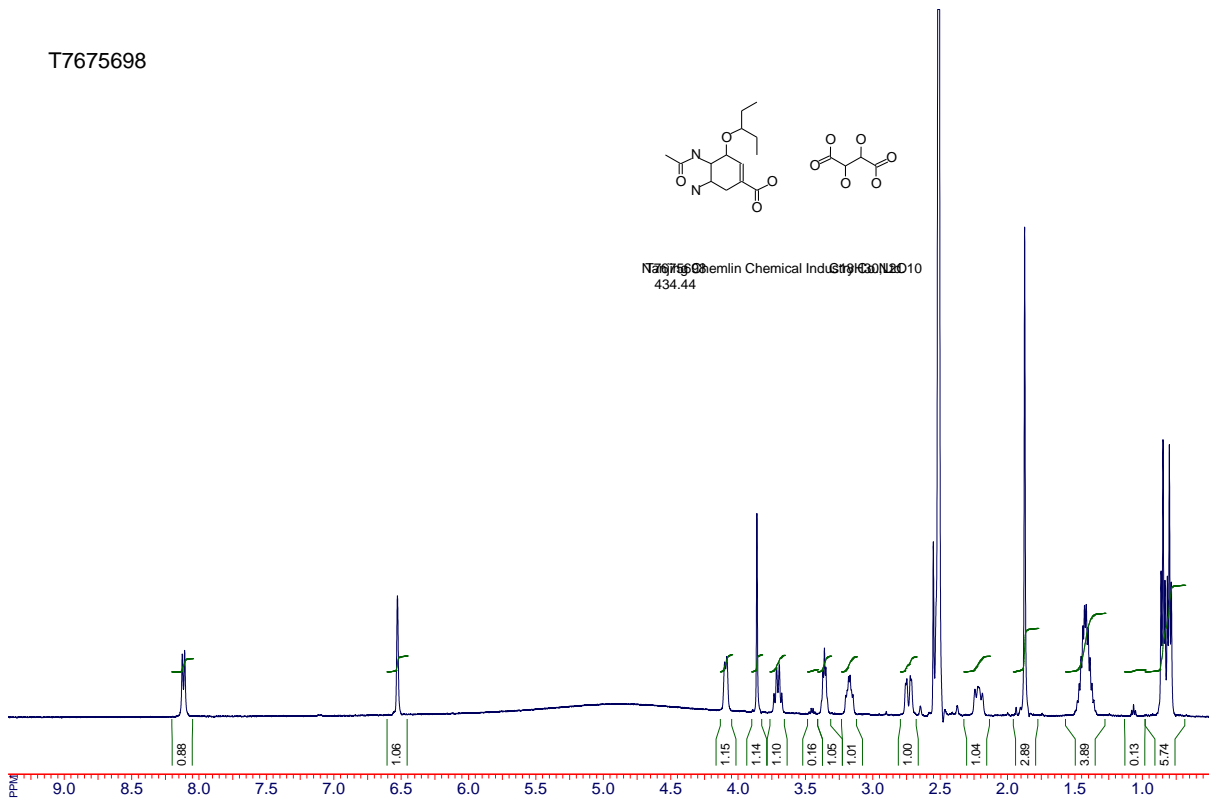
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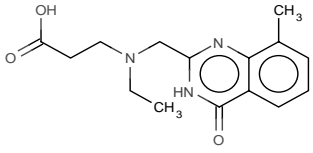
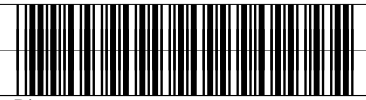
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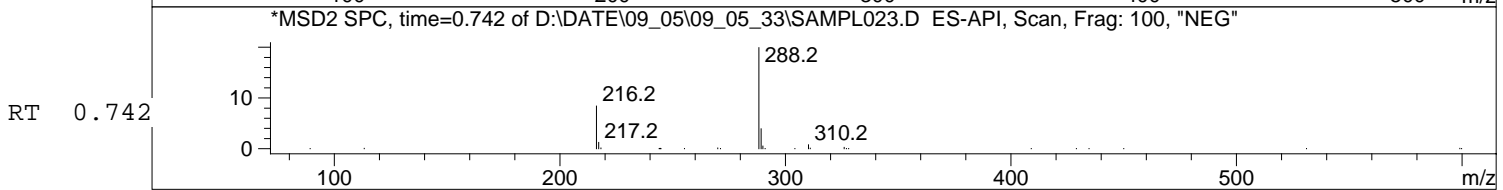
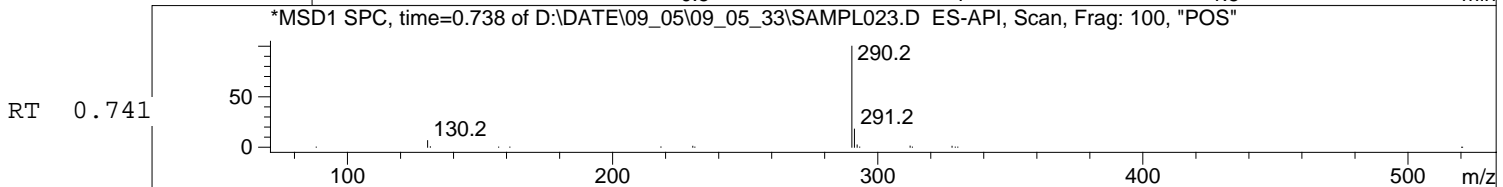
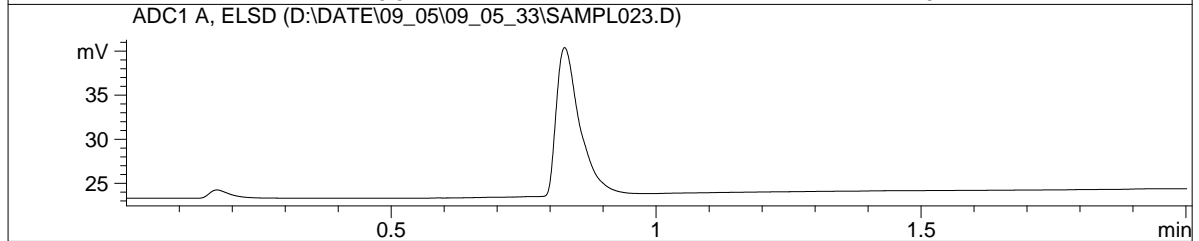
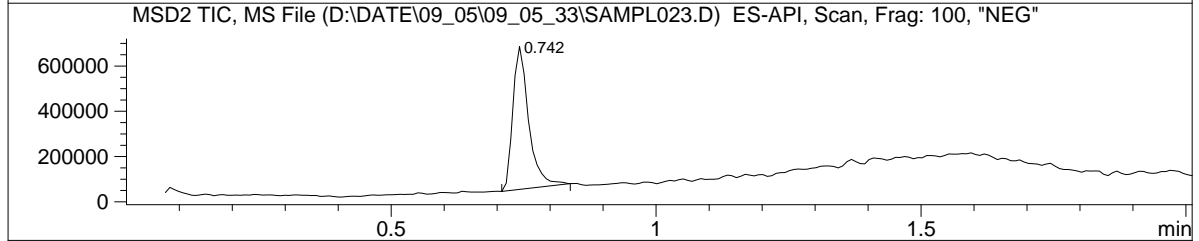
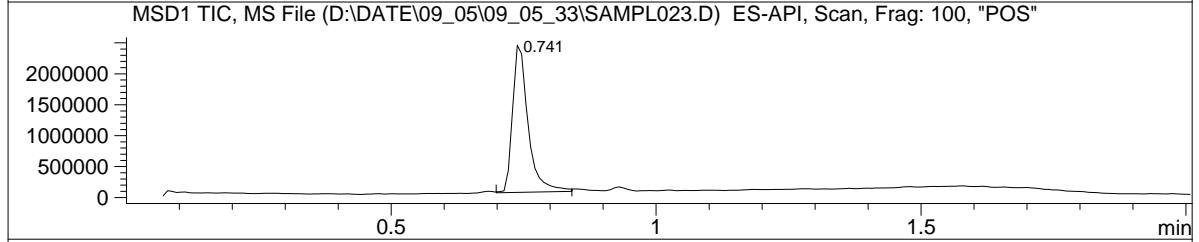
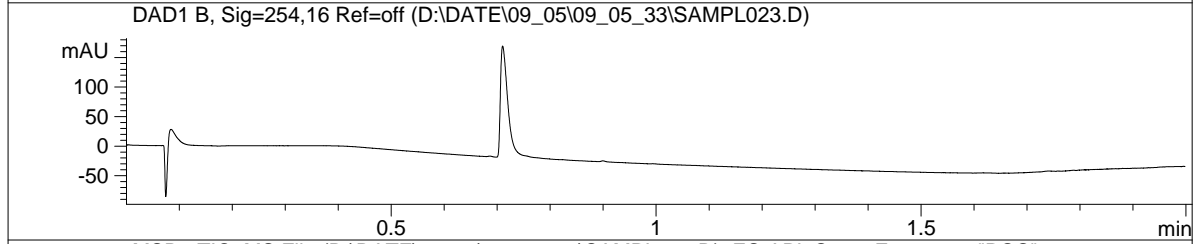
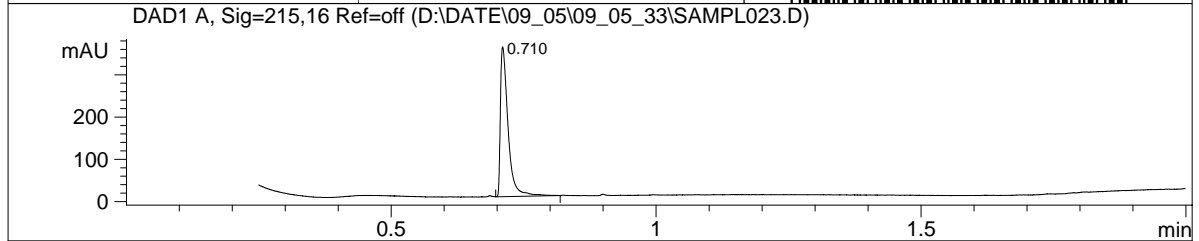
MaxPeak: 100.00%  
Ret\_Time: 0.710 min

# R1919891



**Mol Wt** 289.33  
**Exact Mass** 289.16

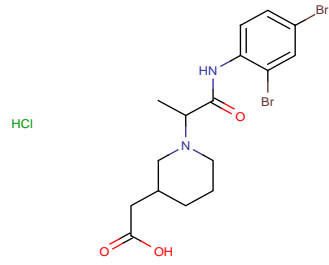
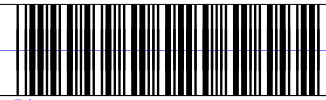
#	Time	Area%
1	0.710	100.00





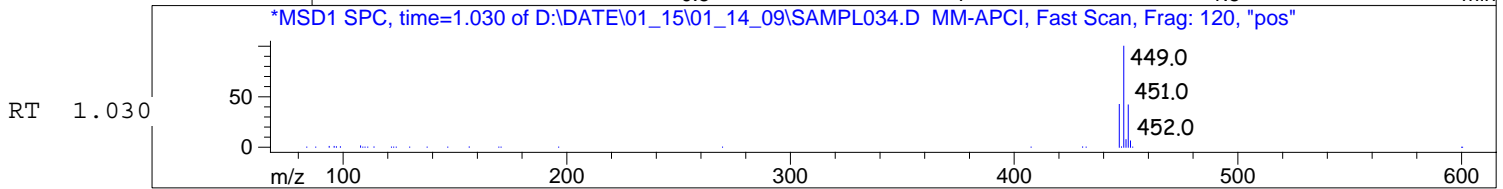
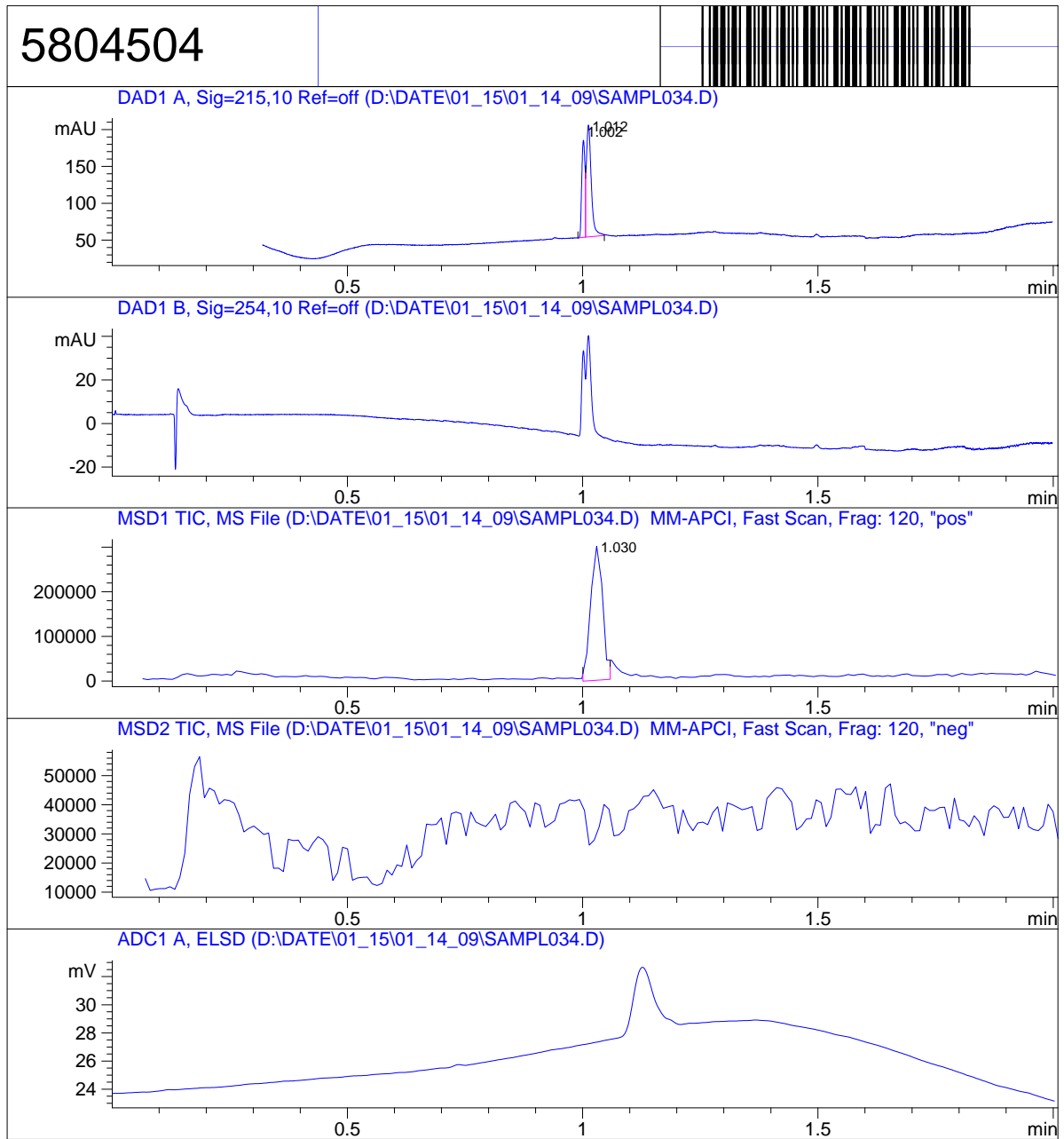
MaxPeak: 62.64%  
Ret\_Time: 1.012 min

# 5804504



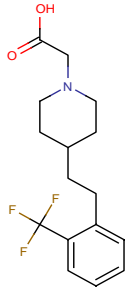
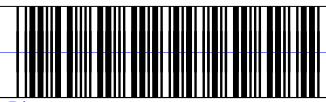
**Mol Wt** 484.61  
**Exact Mass** 448.01

#	Time	Area%
1	1.002	37.36
2	1.012	62.64



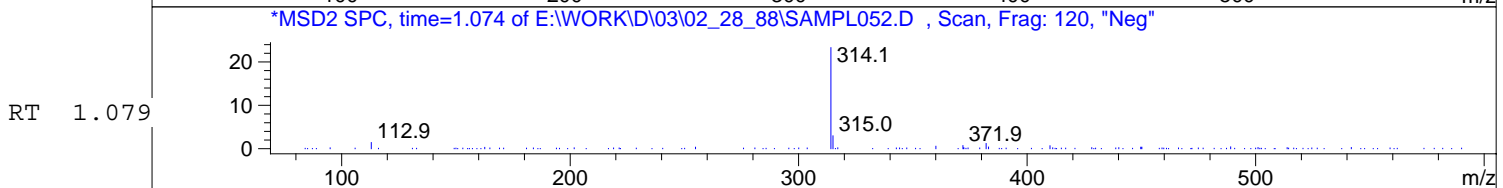
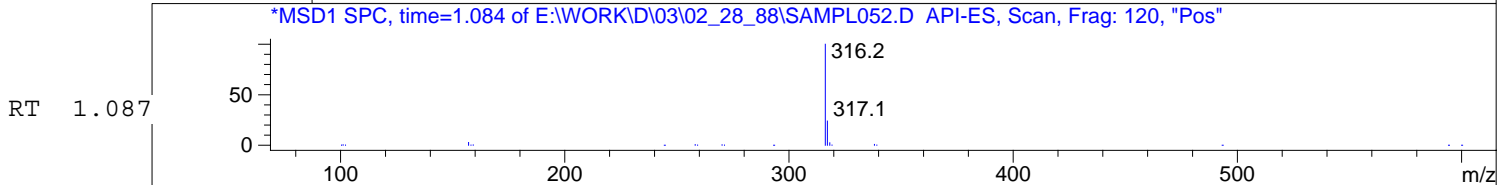
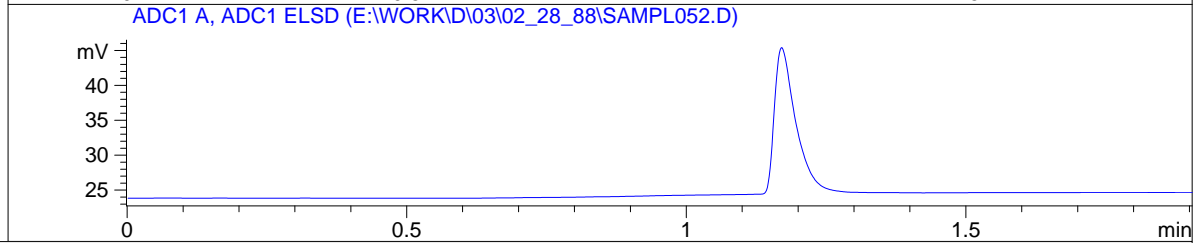
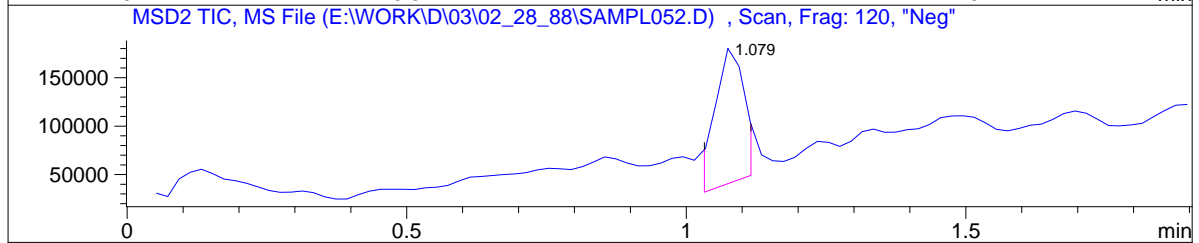
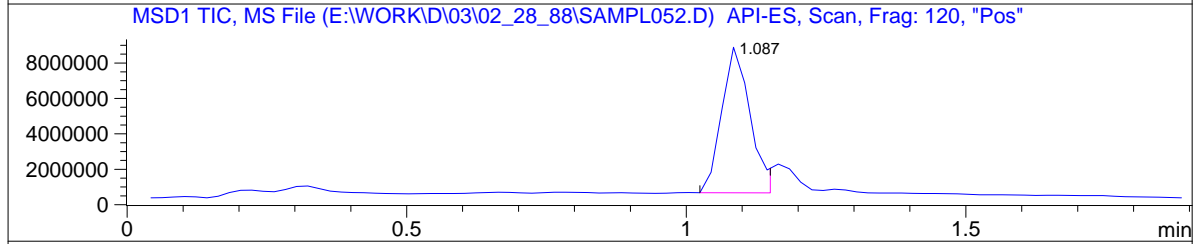
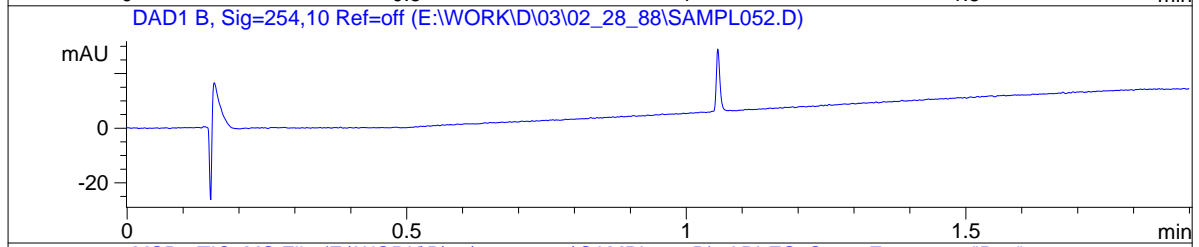
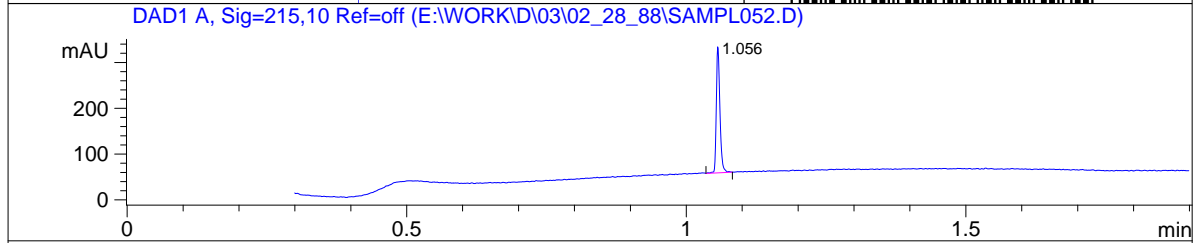
MaxPeak: 100.00%  
Ret\_Time: 1.056 min

# 6008100



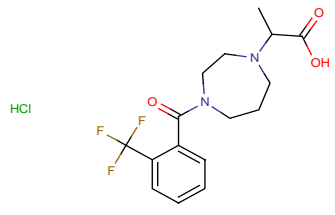
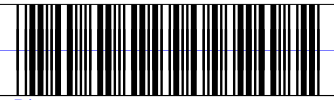
**Mol Wt** 351.79  
**Exact Mass** 315.18

#	Time	Area%
1	1.056	100.00



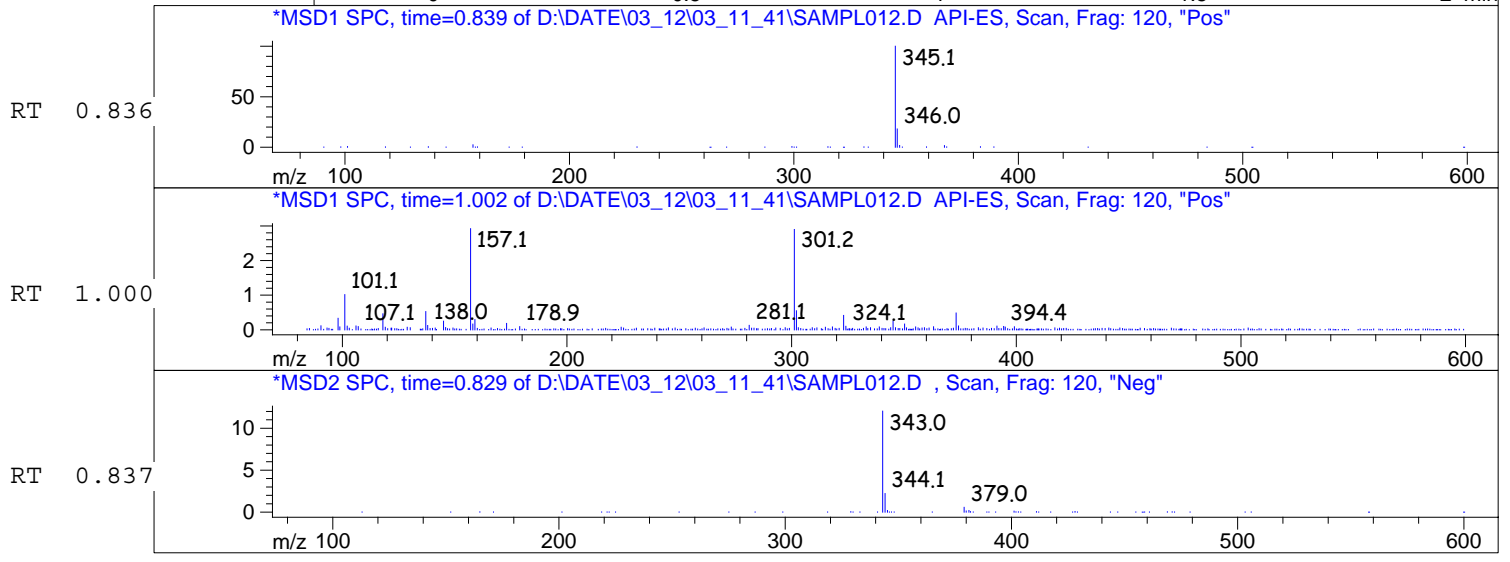
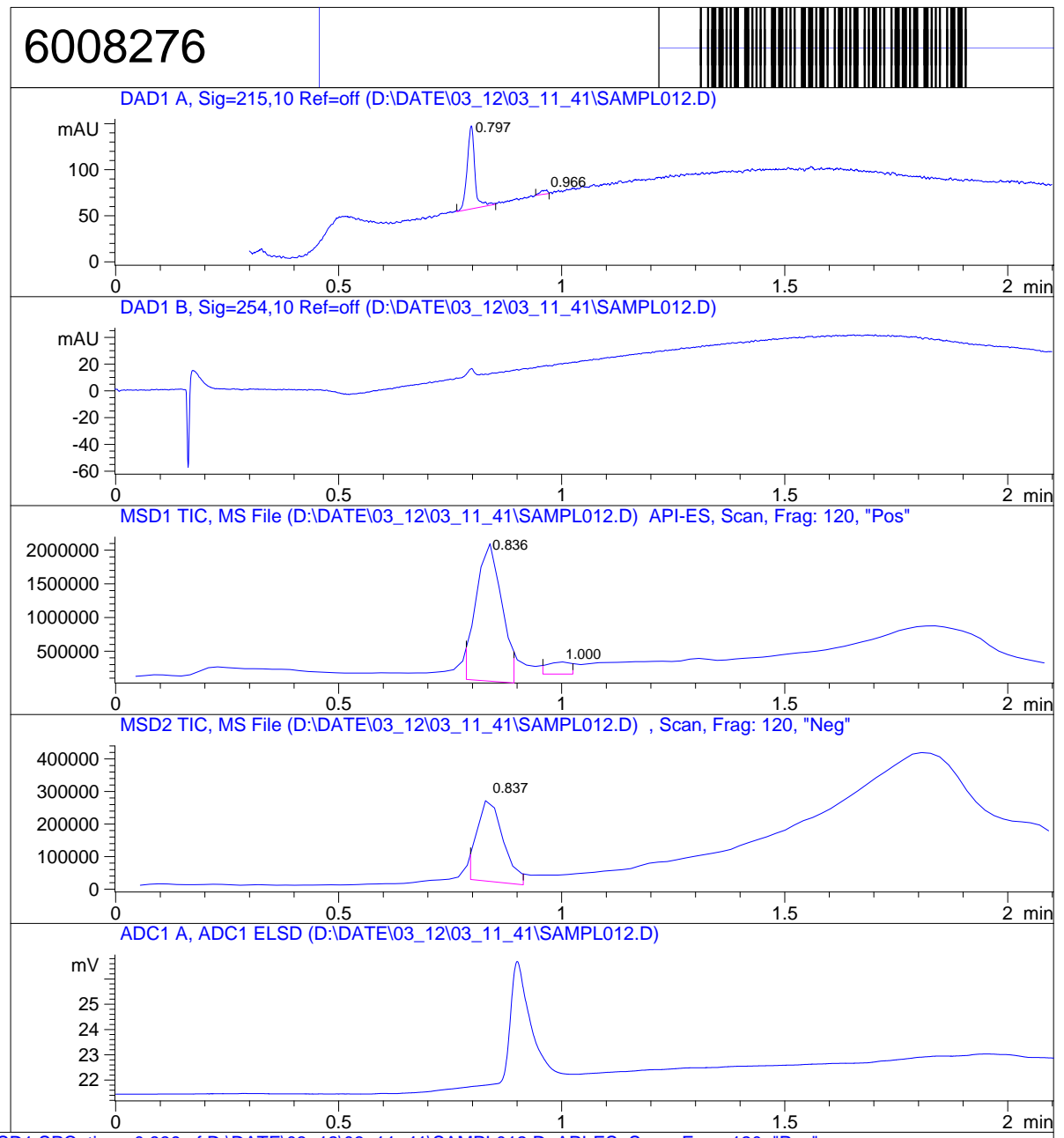
MaxPeak: 95.58%  
Ret\_Time: 0.797 min

6008276



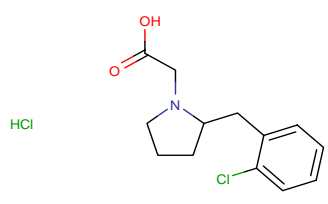
Mol Wt **380.79**  
Exact Mass **344.16**

#	Time	Area%
1	0.797	95.58
2	0.966	4.42



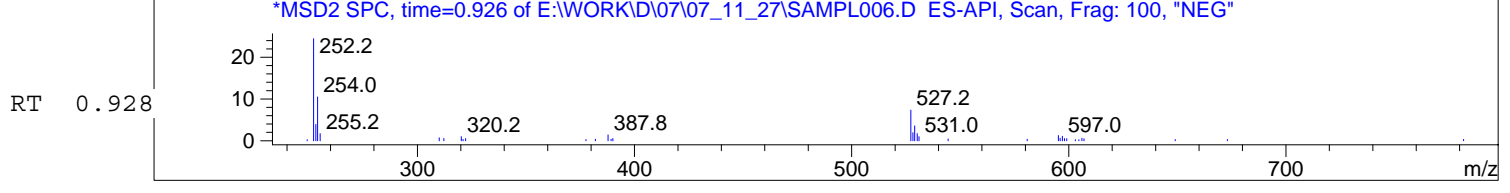
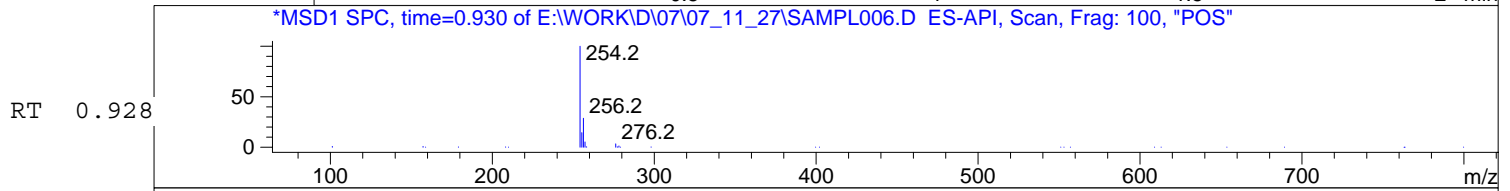
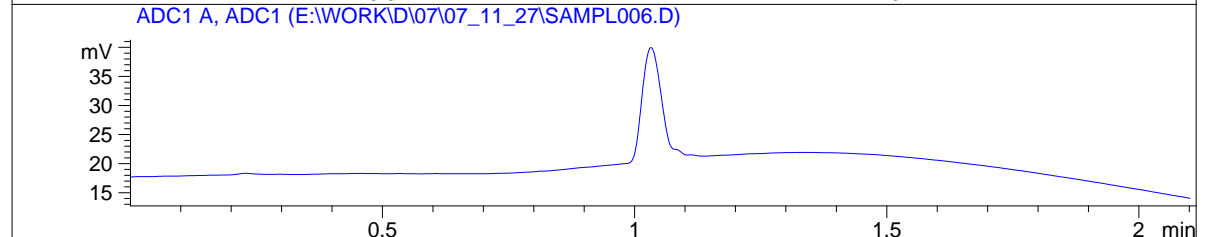
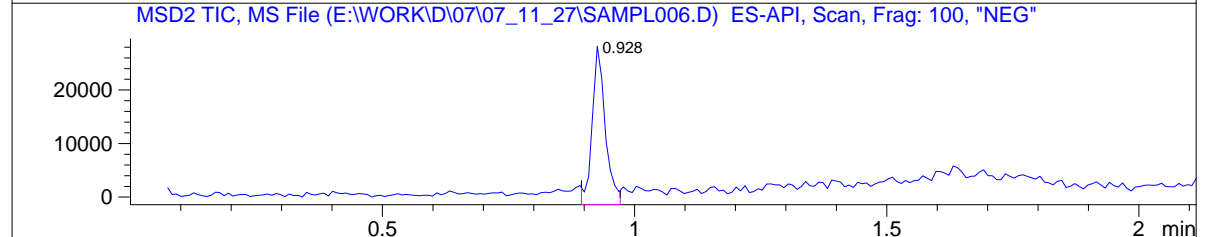
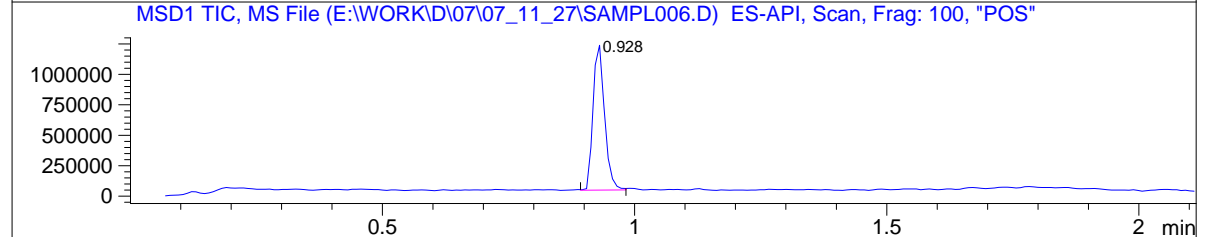
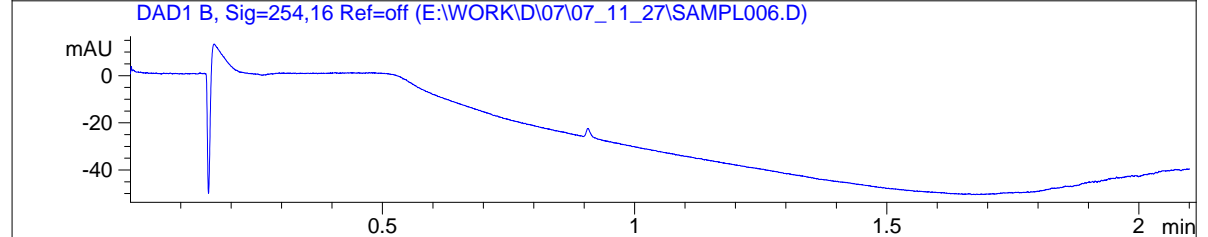
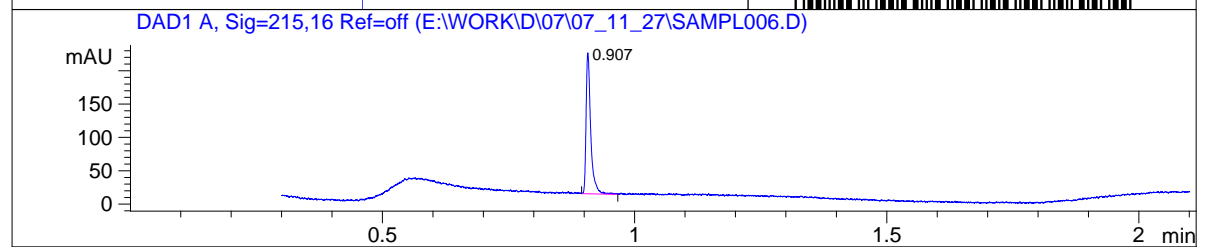
MaxPeak: 100.00%  
Ret\_Time: 0.907 min

# T7621214



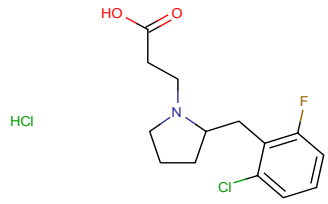
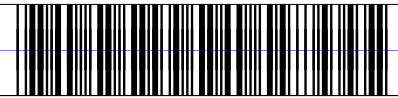
**Mol Wt** 290.19  
**Exact Mass** 253.11

#	Time	Area%
1	0.907	100.00



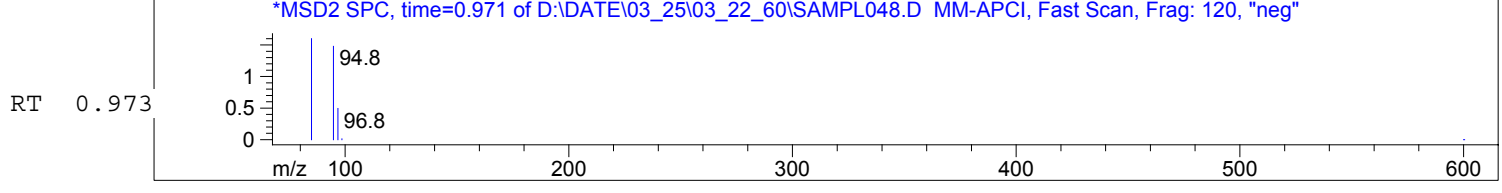
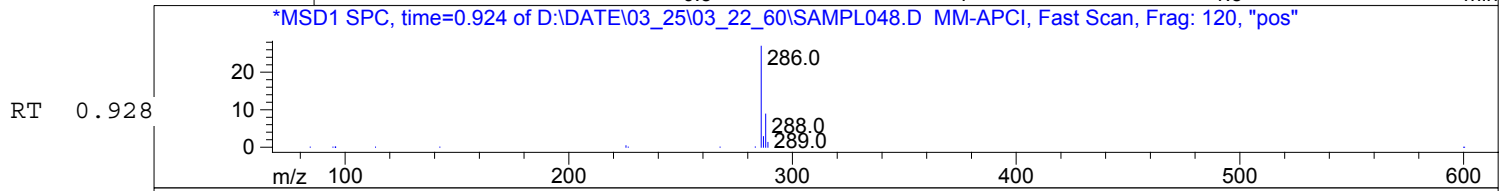
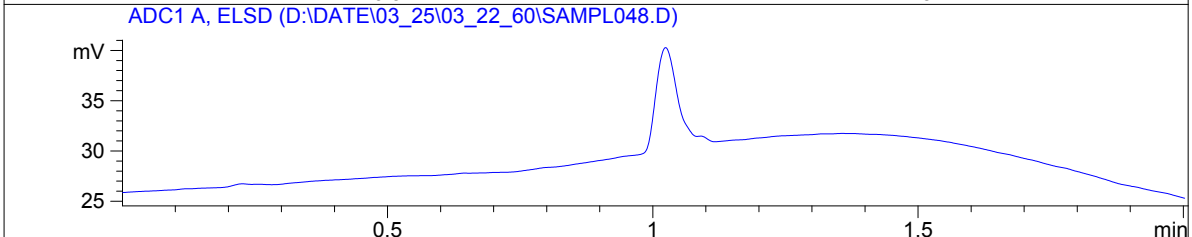
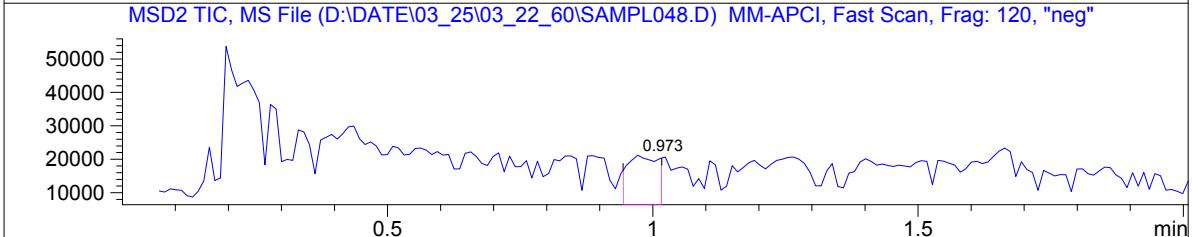
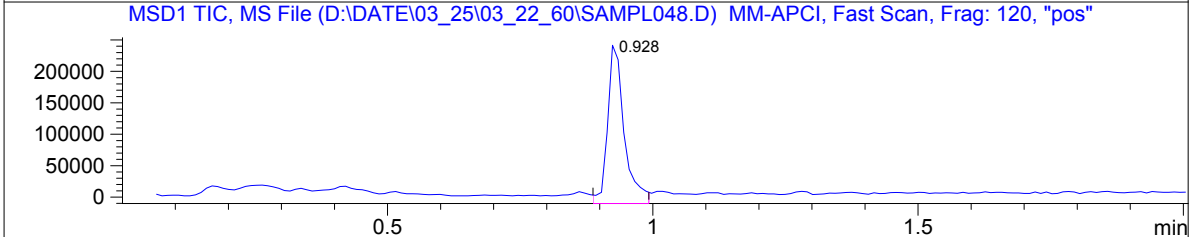
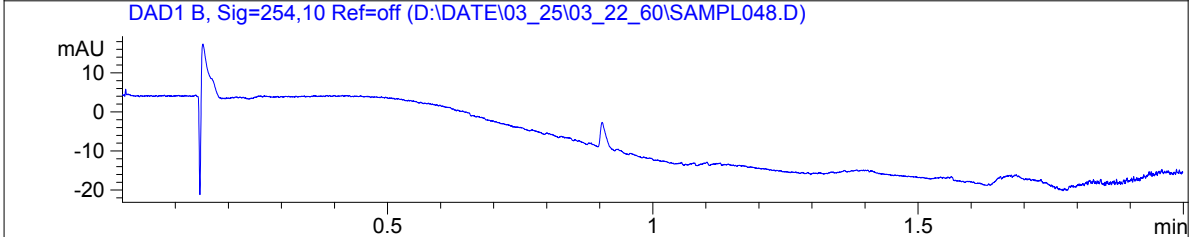
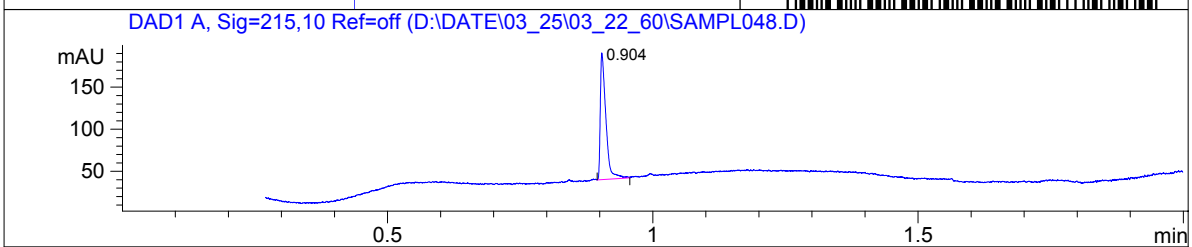
MaxPeak: 100.00%  
Ret\_Time: 0.904 min

# 6008064\$1



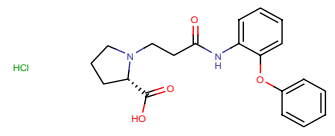
**Mol Wt** 322.2  
**Exact Mass** 285.12

#	Time	Area%
1	0.904	100.00



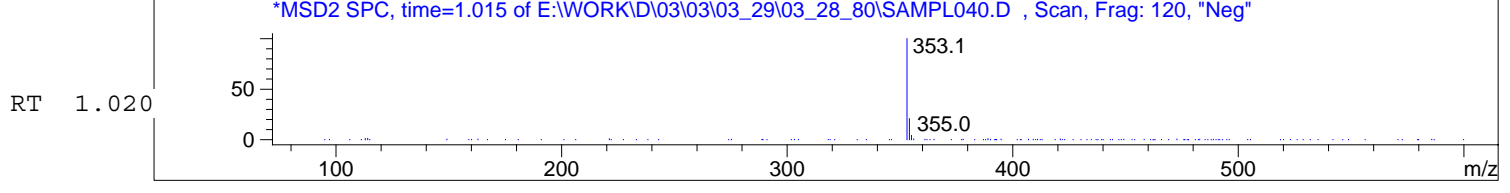
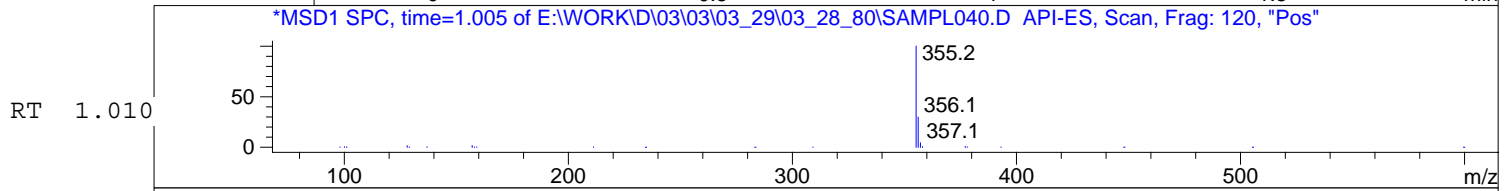
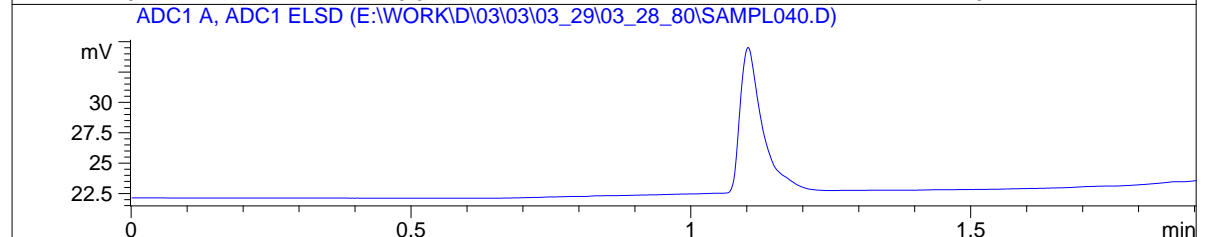
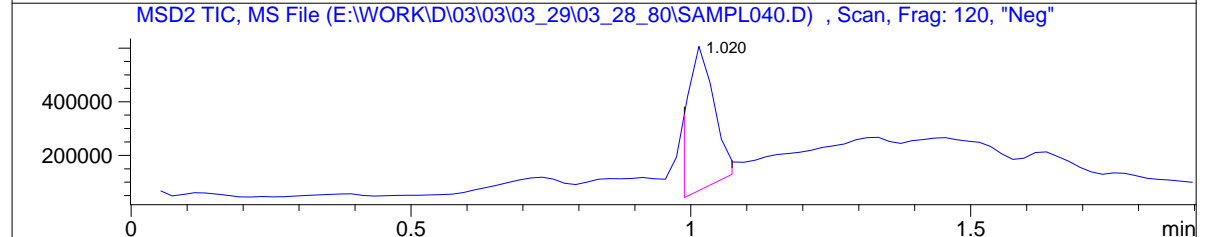
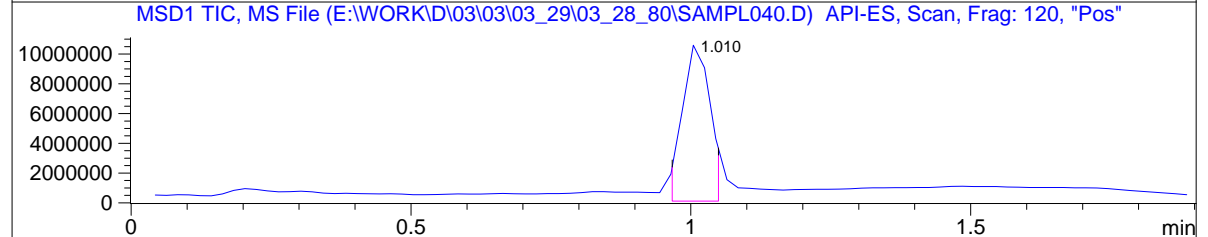
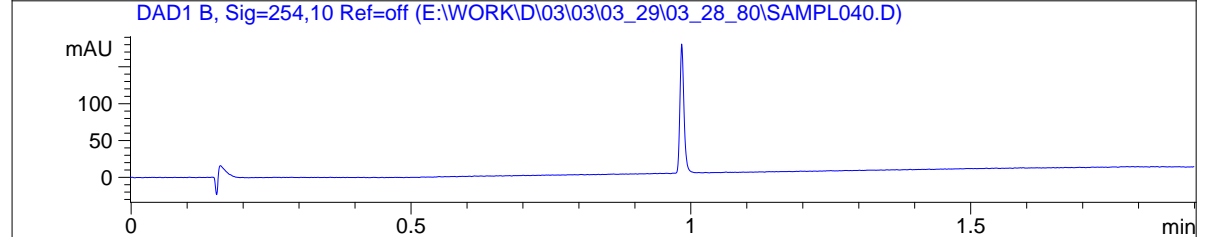
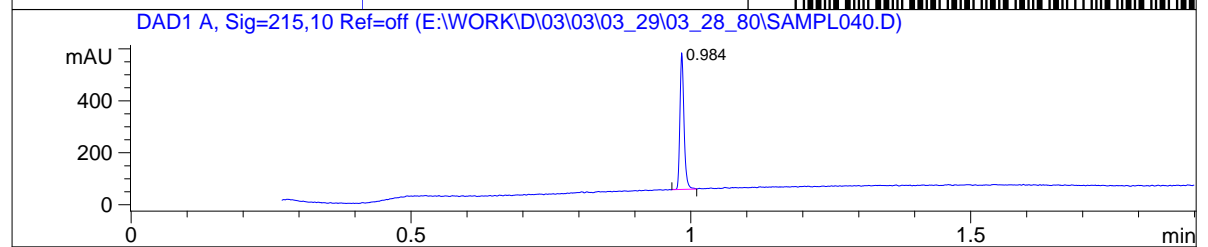
MaxPeak: 100.00%  
Ret\_Time: 0.984 min

6008199\$22



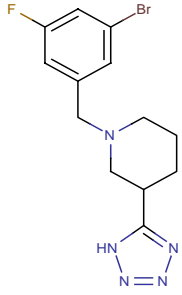
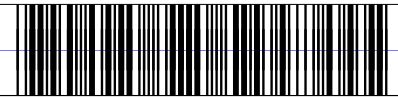
Mol Wt 390.86  
Exact Mass 354.18

#	Time	Area%
1	0.984	100.00



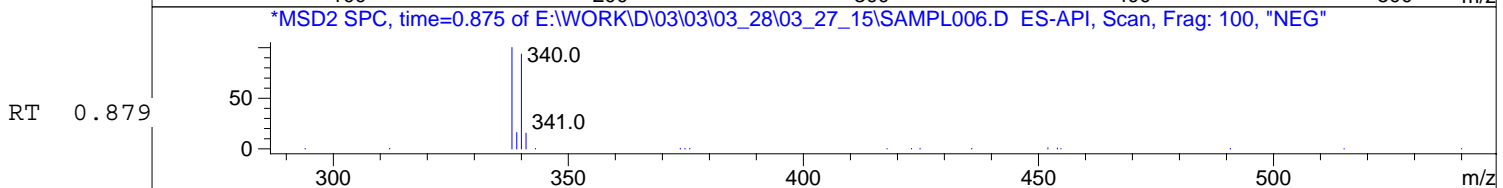
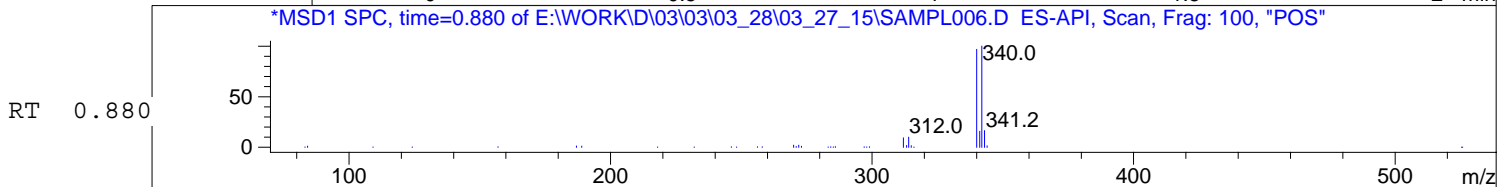
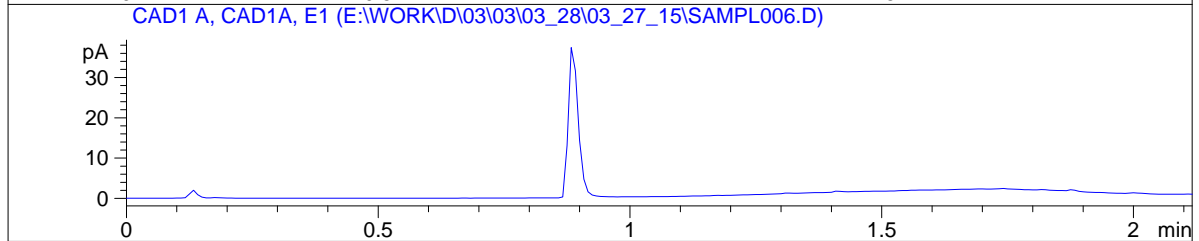
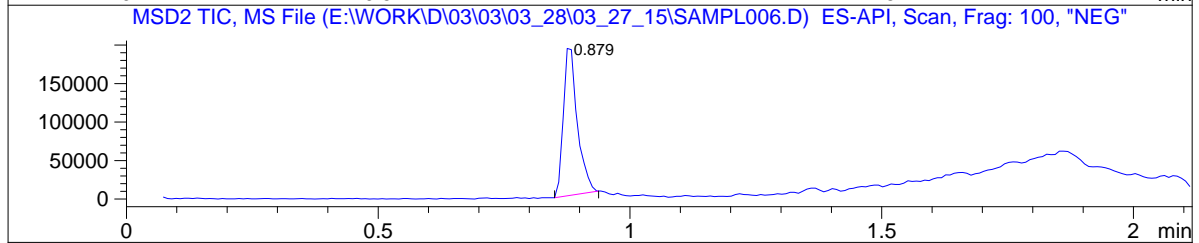
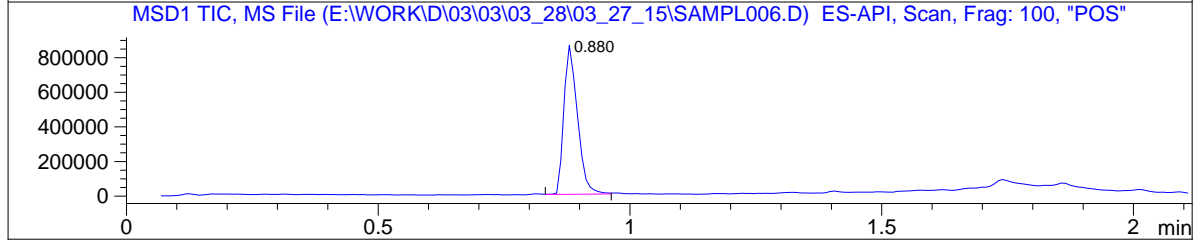
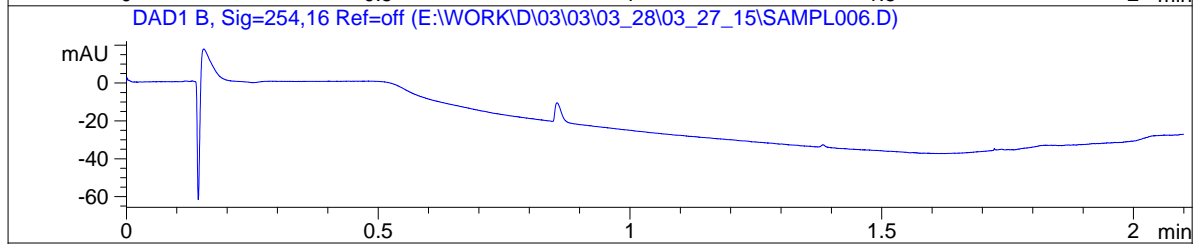
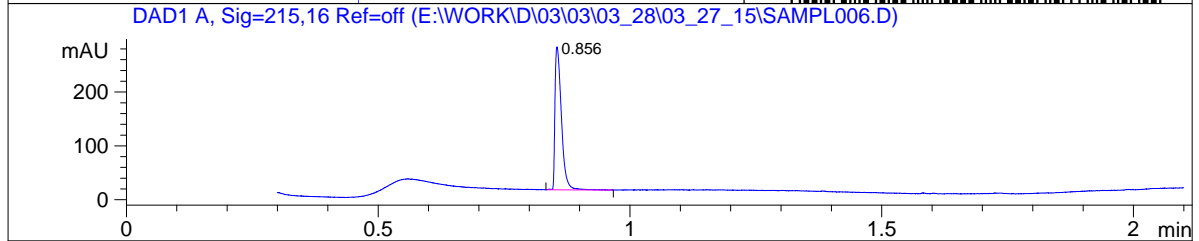
MaxPeak: 100.00%  
Ret\_Time: 0.856 min

5937301\$2



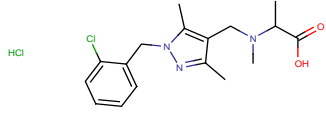
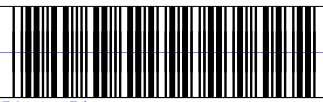
Mol Wt 340.19  
Exact Mass 339.07

#	Time	Area%
1	0.856	100.00



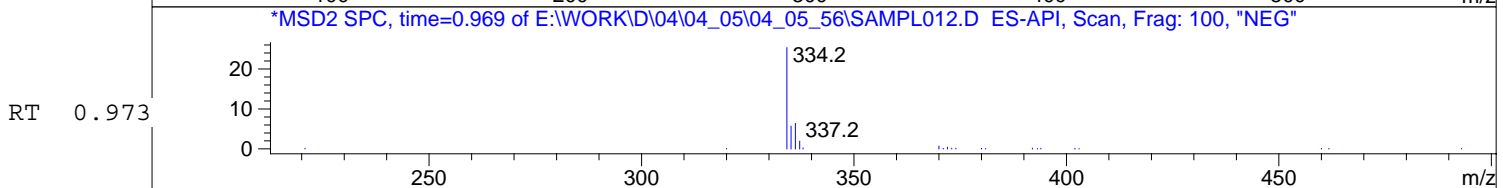
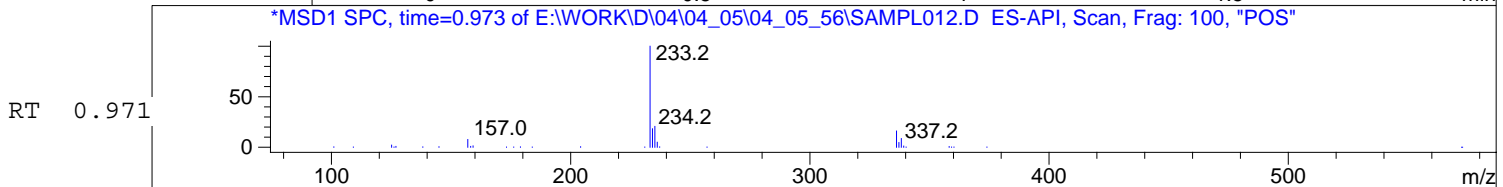
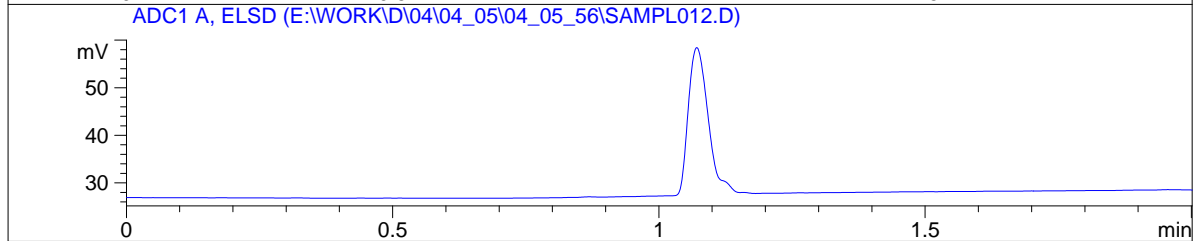
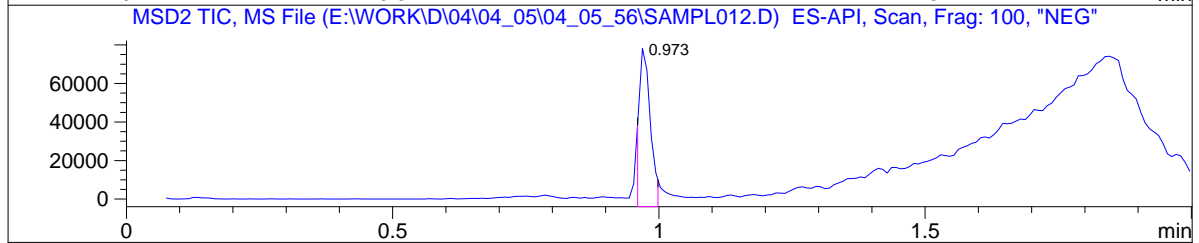
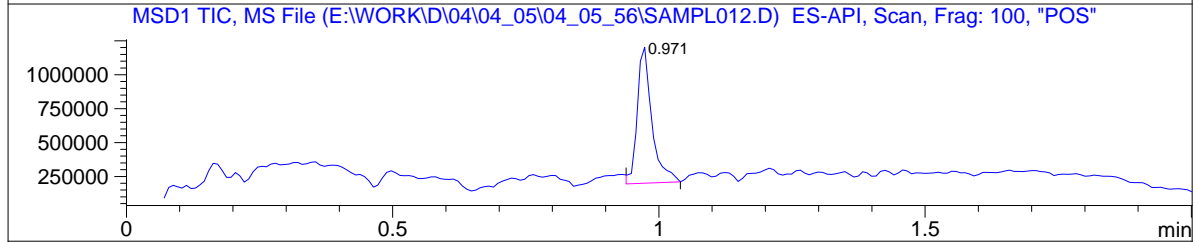
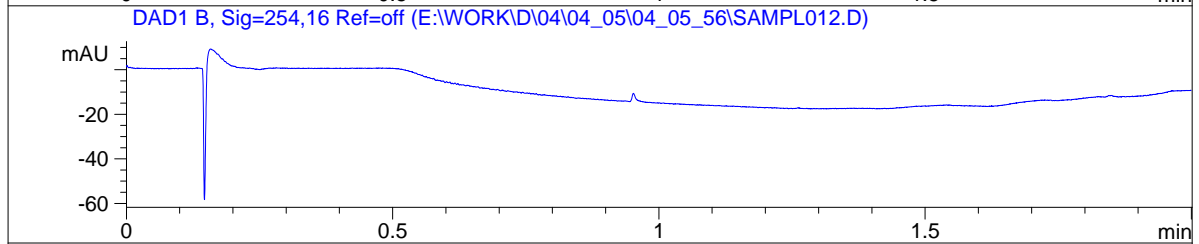
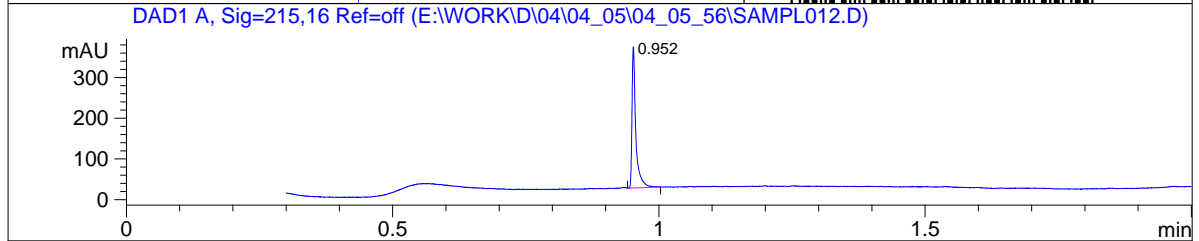
MaxPeak: 100.00%  
Ret\_Time: 0.952 min

# 6008184



**Mol Wt** 372.29  
**Exact Mass** 335.17

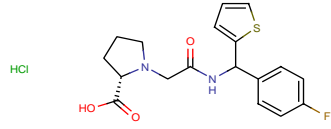
#	Time	Area%
1	0.952	100.00





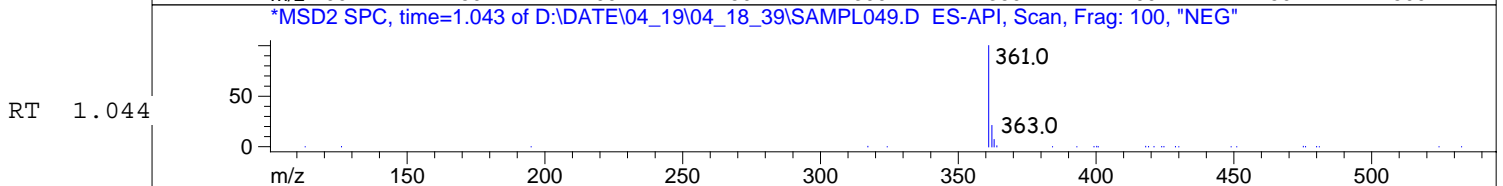
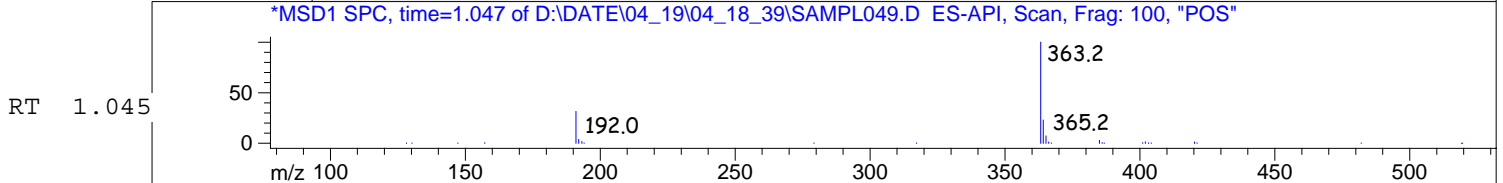
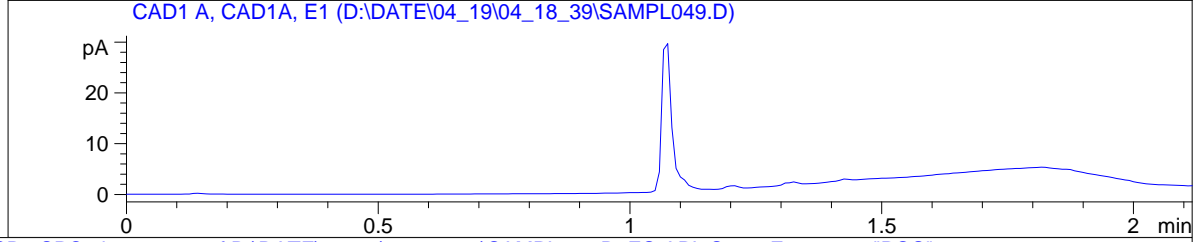
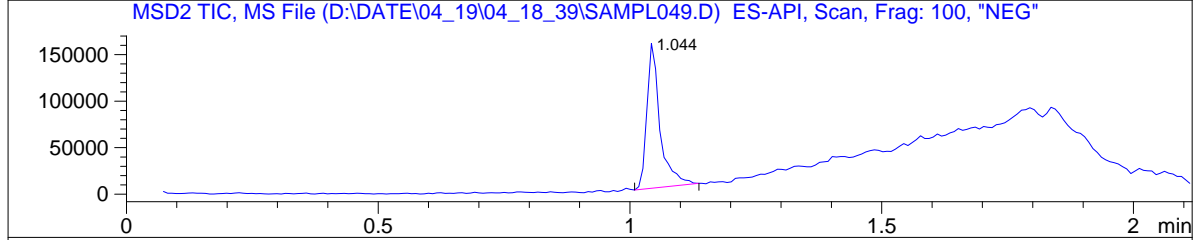
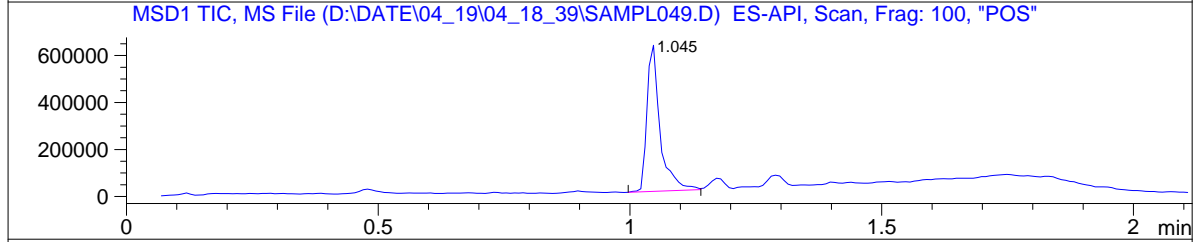
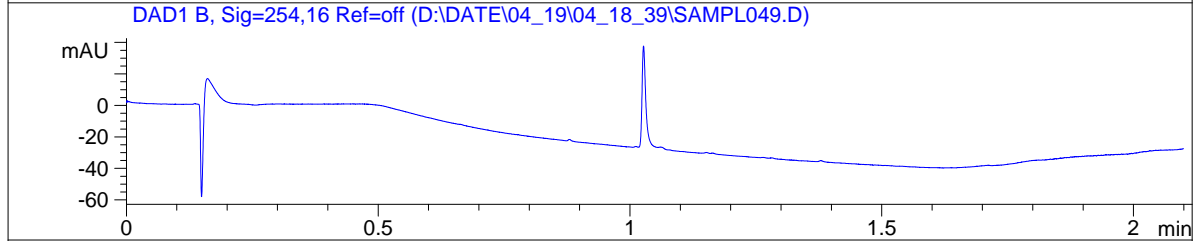
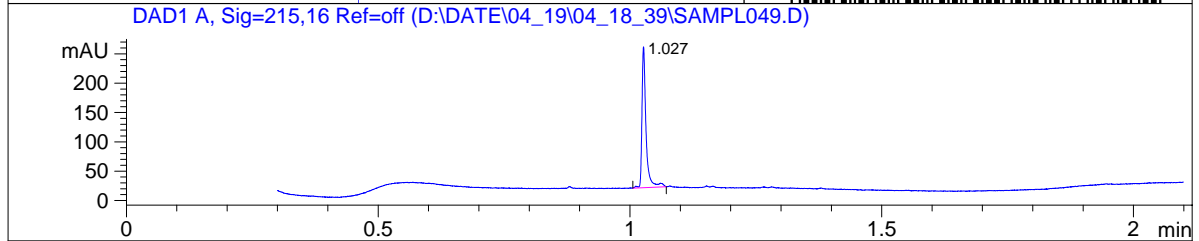
MaxPeak: 100.00%  
Ret\_Time: 1.027 min

# 5804482\$1



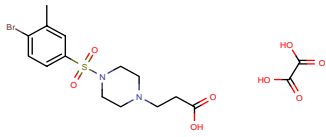
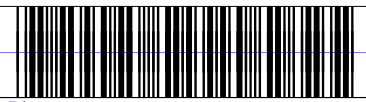
**Mol Wt** 398.88  
**Exact Mass** 362.13

#	Time	Area%
1	1.027	100.00



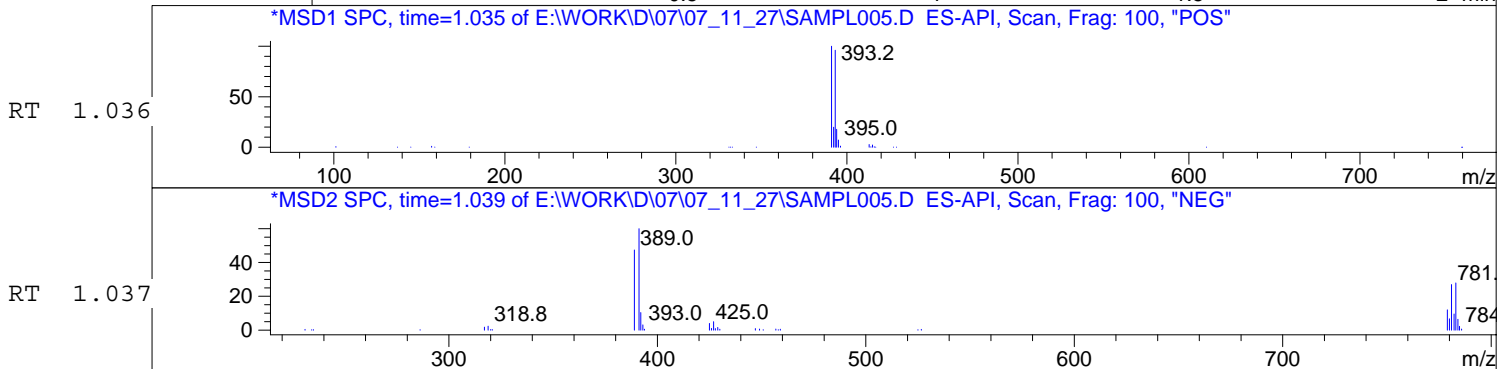
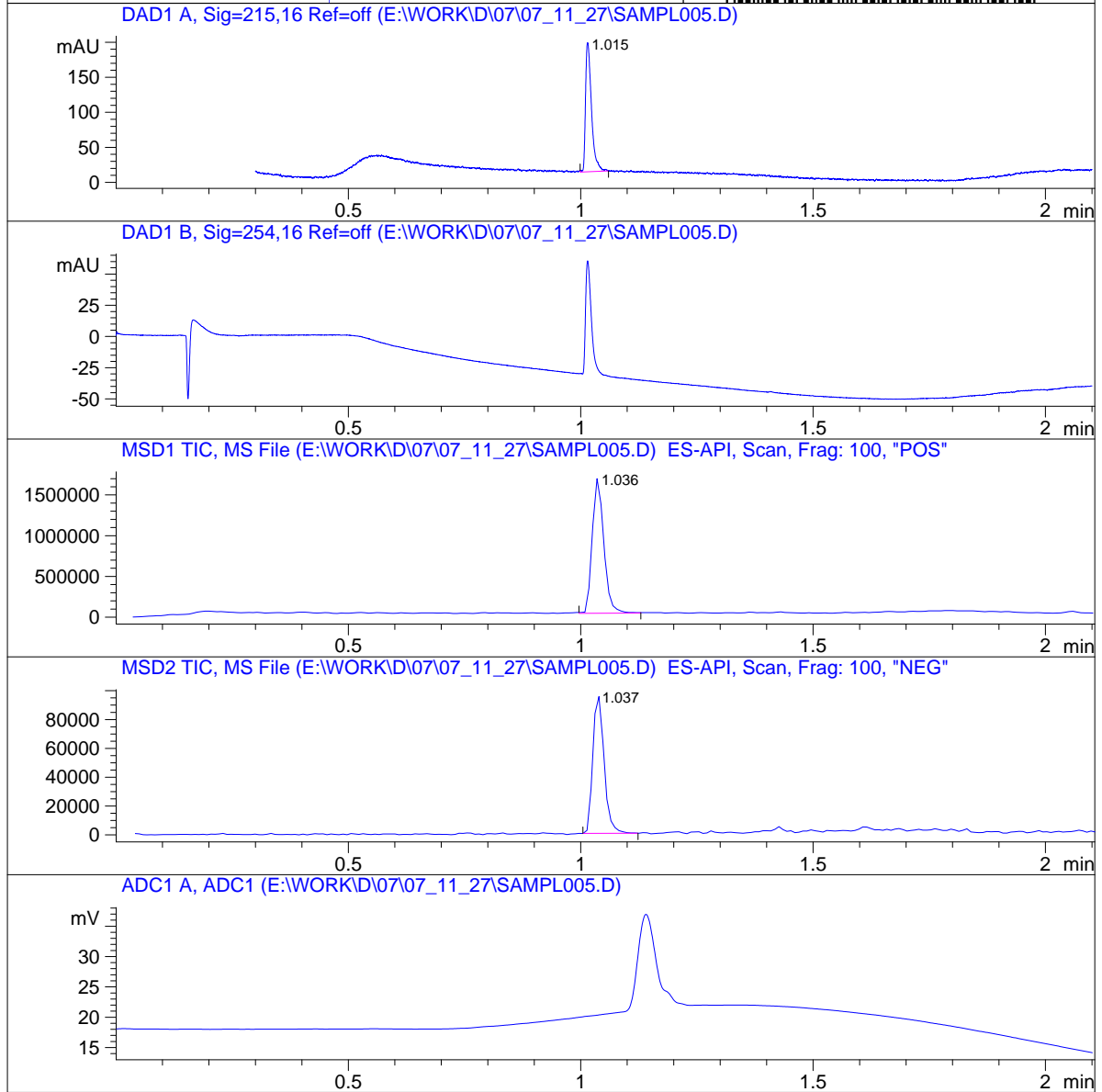
MaxPeak: 100.00%  
Ret\_Time: 1.015 min

# T5308507



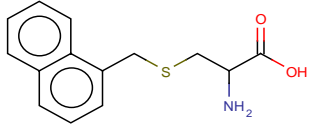
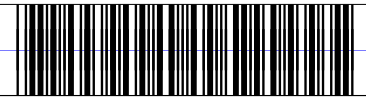
**Mol Wt** 481.32  
**Exact Mass** 392.04

#	Time	Area%
1	1.015	100.00



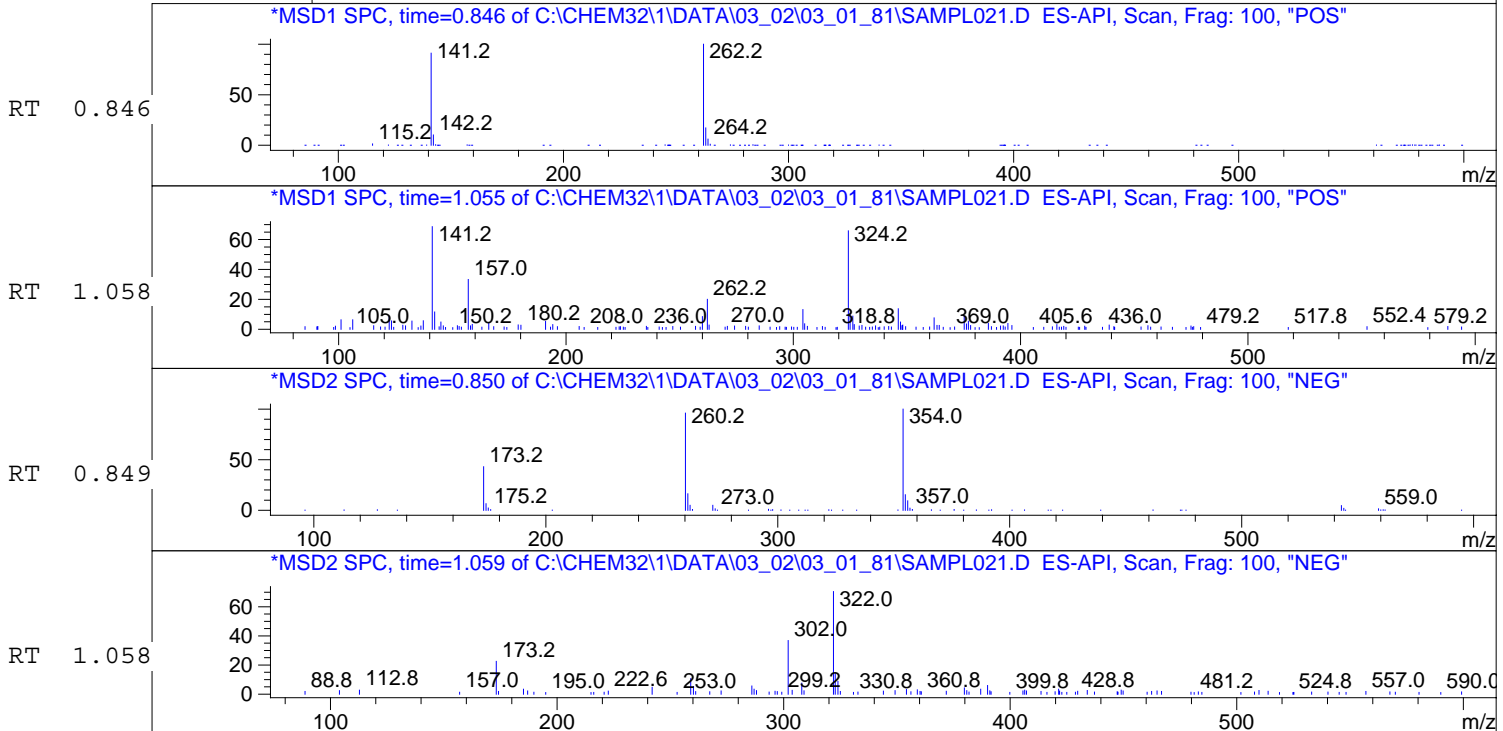
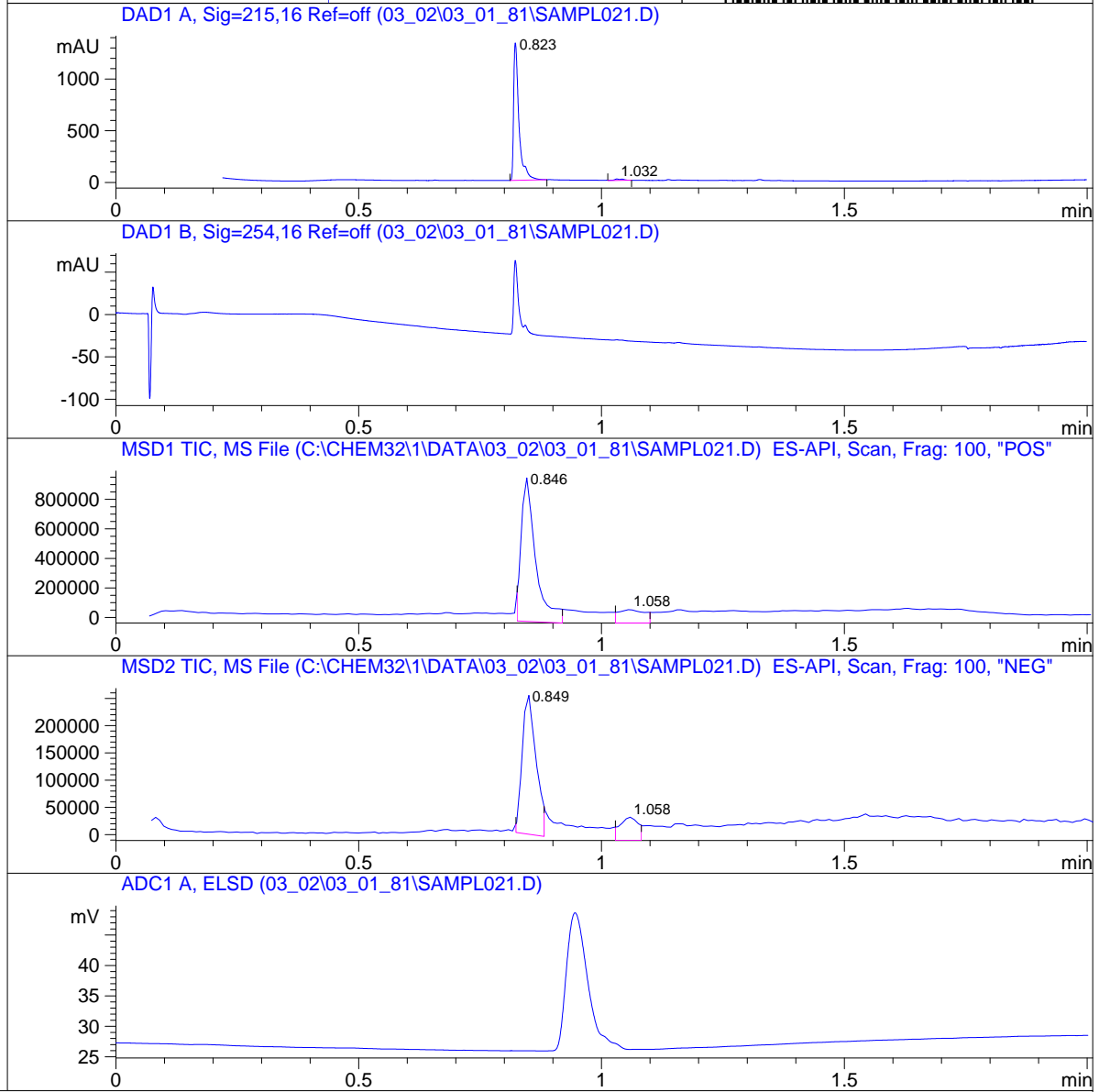
MaxPeak: 98.32%  
Ret\_Time: 0.823 min

# R1969058



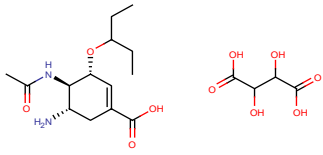
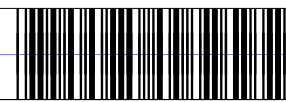
Mol Wt **261.339**  
Exact Mass **261.1**

#	Time	Area%
1	0.823	98.32
2	1.032	1.68



MaxPeak: 100.00%  
Ret\_Time: 0.748 min

R23970



**Mol Wt** 434.44  
**Exact Mass** 284.2

#	Time	Area%
1	0.748	100.00

