Discovery of thienoquinolone derivatives as selective and ATP non-competitive CDK5/p25 inhibitors by structure-based virtual screening

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Table T1. Ensemble docking of CDK5/p25 crystal bound ligands

Protein PDB id	Crystal bound Ligands	GlideScores
3O0G	Ligand_3O0G	-10.570
	Ligand_1UNH	-8.542
	Ligand_1UNG	-8.401
	Ligand_1UNL	-5.530
1UNL	Ligand_1UNL	-9.968
	Ligand_1UNG	-8.498
	Ligand_3O0G	-8.325
	Ligand_1UNH	-7.678
1UNG	Ligand_1UNG	-9.208
	Ligand_1UNH	-6.737
	Ligand_1UNL	-4.612
	Ligand_3O0G	-3.912
1UNH	Ligand_1UNH	-8.263
	Ligand_3O0G	-6.594
	Ligand_1UNL	-5.999
	Ligand_1UNG	-5.883

Table T2. Ensemble docking of CDK2 crystal bound ligands

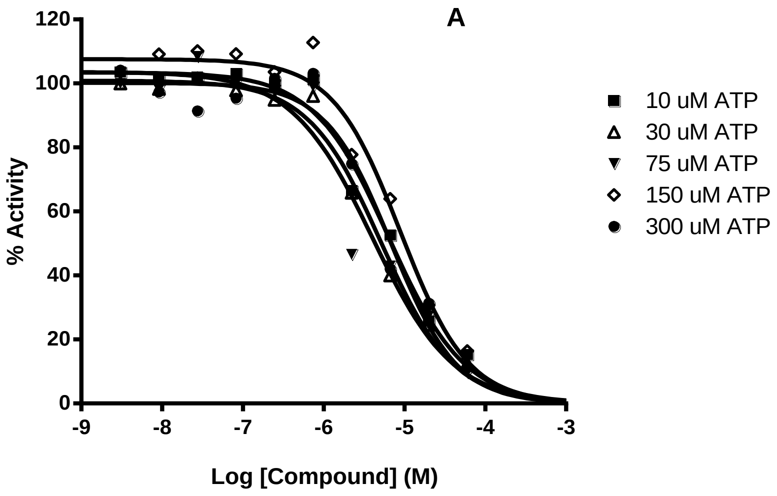
Protein Structures	Crystal bound Ligands	GlideScores
1AQ1	Ligand_1AQ1	-13.324
	Ligand_2VTP	-10.099
	Ligand_1OIT	-9.906
	Ligand_1R78	-8.256
	Ligand_2A4L	-8.117
	Ligand_2W17	-4.511
2W17	Ligand_2W17	-11.763
	Ligand_1OIT	-11.185
	Ligand_2A4L	-10.681
	Ligand_2VTP	-8.965
	Ligand_1R78	-6.146
	Ligand_1AQ1	-1.955
1OIT	Ligand_1OIT	-11.324
	Ligand_2W17	-10.102
	Ligand_1R78	-9.914
	Ligand_2VTP	-8.477
	Ligand_2A4L	-6.960
	Ligand_1AQ1	*
2A4L	Ligand_2W17	-10.450
	Ligand_1R78	-9.762
	Ligand_2A4L	-9.592
	Ligand_1OIT	-7.258
	Ligand_2VTP	-5.645
	Ligand_1AQ1	-3.240
2VTP	Ligand_1OIT	-11.279
	Ligand_2VTP	-10.470
	Ligand_2W17	-9.874
	Ligand_1R78	-9.102
	Ligand_2A4L	-7.590
	Ligand_1AQ1	-7.434
1R78	Ligand_2W17	-10.488
	Ligand_2A4L	-9.256
	Ligand_1OIT	-8.886
	Ligand_1R78	-8.724
	Ligand_2VTP	-8.418
	Ligand_1AQ1	-5.067

* Ligand did not dock in the selected grid

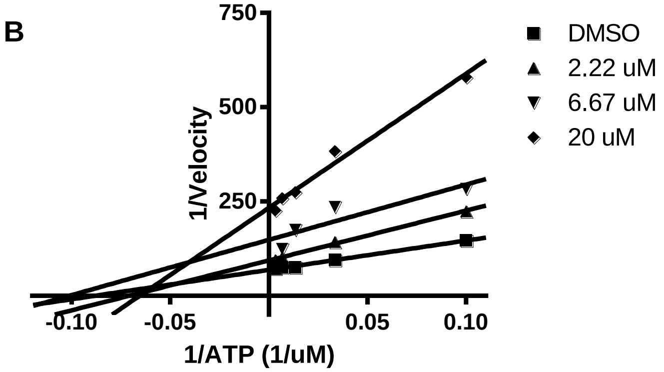
Table T3. Hypotheses generated from E-pharmacophores

E-pharmacophore	Hypotheses	Pharmacophoric features
1UNG	1UNG_hypo 1	A1A2D5H6R8R9
	1UNG_hypo 2	A1A2D5R8R9
	1UNG_hypo 3	A2D5R8R9
1UNH	1UNH_hypo 1	A3D4H8R10
	1UNH_hypo 2	A1A3D4H8R10
1UNL	1UNL_hypo 1	A2D5D6R11R12
	1UNL_hypo 2	A2D6H8R11R12
	1UNL_hypo 3	A2A4H8D6R11R12
	1UNL_hypo 4	A2D5D6H8R11R12
	1UNL_hypo 5	A2A4D5D6H8R11R12
3O0G	3O0G_hypo 1	A1A2D3D4R7R8R9
	3O0G_hypo 2	A1A2D3D4R7R8

Figure F1. IC50 curves (A) and Lineweaver-Burk Plot (B) of **25o**

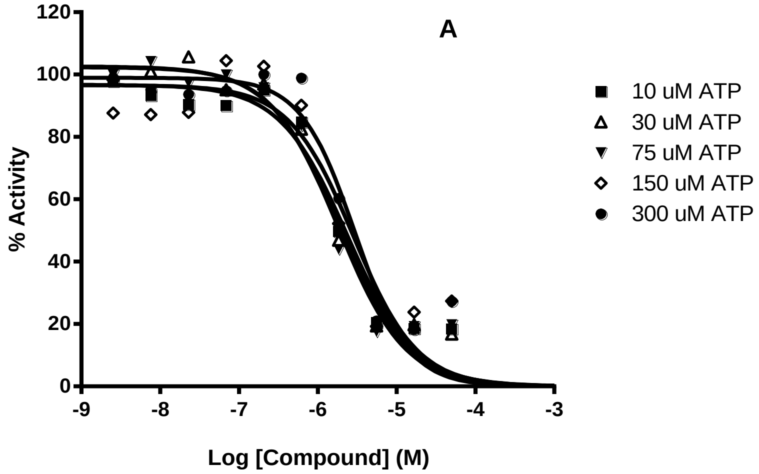


	10 uM ATP	30 uM ATP	75 uM ATP	150 uM ATP	300 uM ATP
HillSlope	-0.9278	-0.9563	-0.8668	-1.031	-1.059
EC50	6.433e-006	5.114e-006	4.003e-006	8.864e-006	6.805e-006

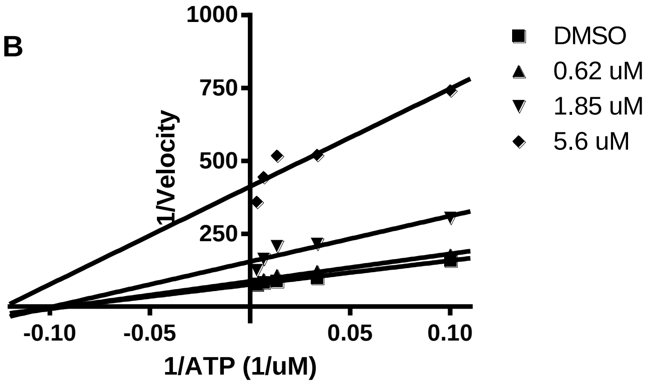


	DMSO	2.22 uM	6.67 uM	20 uM
Y-intercept when X=0.0	68.73 ± 1.464	93.73 ± 3.056	148.4 ± 26.90	233.1 ± 13.20
X-intercept when Y=0.0	-0.08840	-0.07094	-0.1013	-0.06550
1/slope	0.001286	0.0007569	0.0006826	0.0002810

Figure F2. IC50 curves (A) and Lineweaver-Burk Plot (B) of **25s**

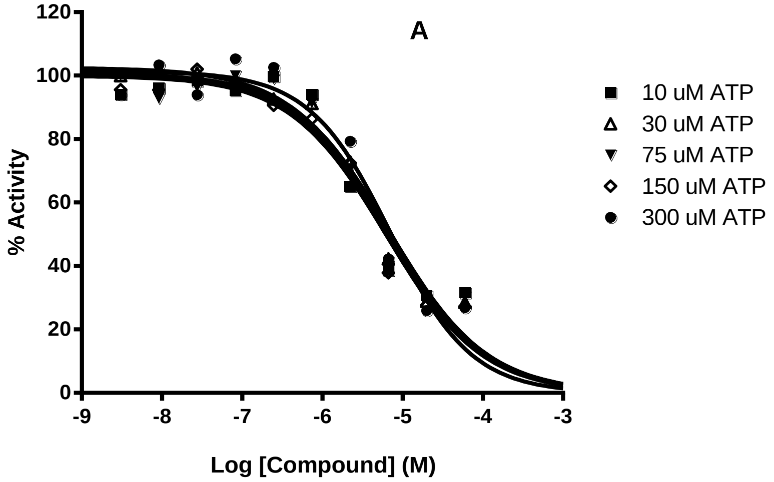


	10 uM ATP	30 uM ATP	75 uM ATP	150 uM ATP	300 uM ATP
HillSlope	-1.017	-0.9749	-1.021	-1.067	-1.264
EC50	2.365e-006	1.907e-006	1.807e-006	2.814e-006	2.944e-006

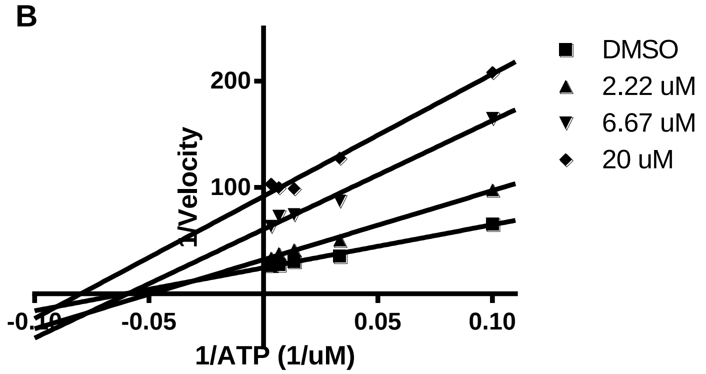


	DMSO	0.62 uM	1.85 uM	5.6 uM
Y-intercept when X=0.0	75.74 ± 1.735	86.77 ± 5.961	154.3 ± 16.54	412.0 ± 30.56
X-intercept when Y=0.0	-0.09230	-0.09192	-0.09850	-0.1227
1/slope	0.001219	0.001059	0.0006381	0.0002978

Figure F3. IC50 curves (A) and Lineweaver-Burk Plot (B) of **25w**

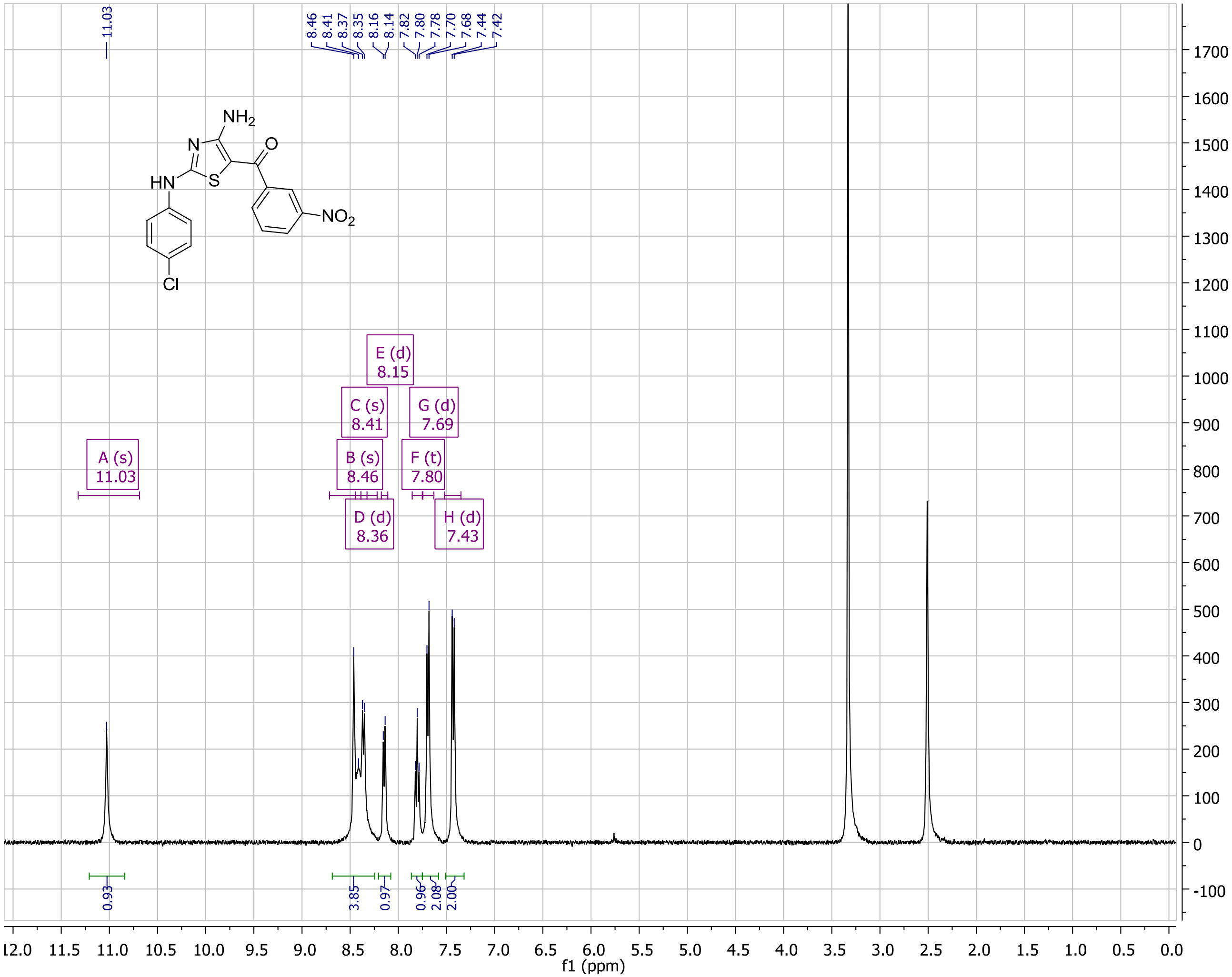


	10 uM ATP	30 uM ATP	75 uM ATP	150 uM ATP	300 uM ATP
HillSlope	-0.7101	-0.7108	-0.7318	-0.7229	-0.8565
EC50	6.863e-006	6.686e-006	6.997e-006	6.191e-006	7.009e-006

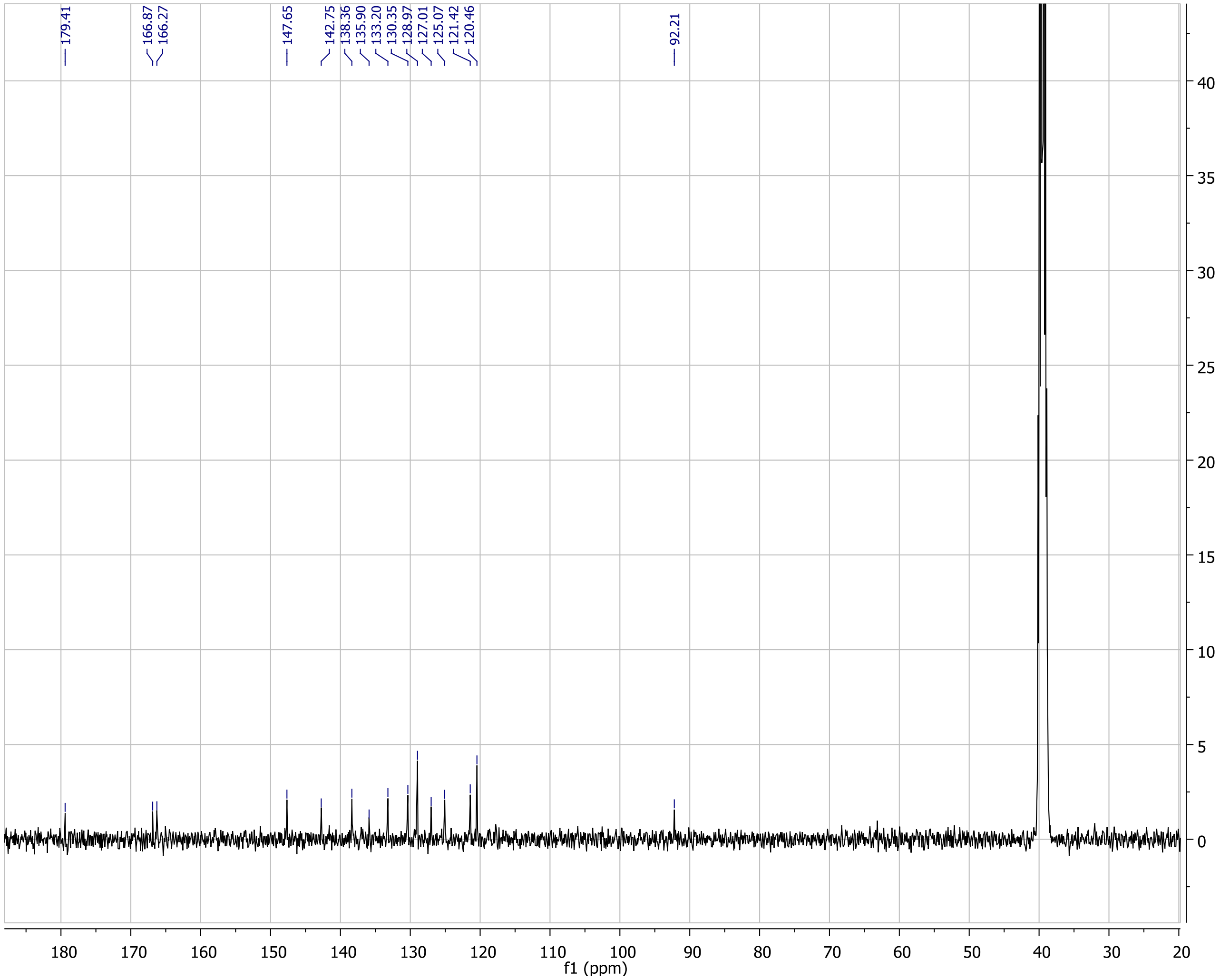


	DMSO	2.22 uM	6.67 uM	20 uM
Y-intercept when X=0.0	24.52 ± 0.9928	32.11 ± 1.289	60.66 ± 3.273	91.78 ± 3.963
X-intercept when Y=0.0	-0.06060	-0.04940	-0.05928	-0.07981
1/slope	0.002471	0.001539	0.0009772	0.0008696

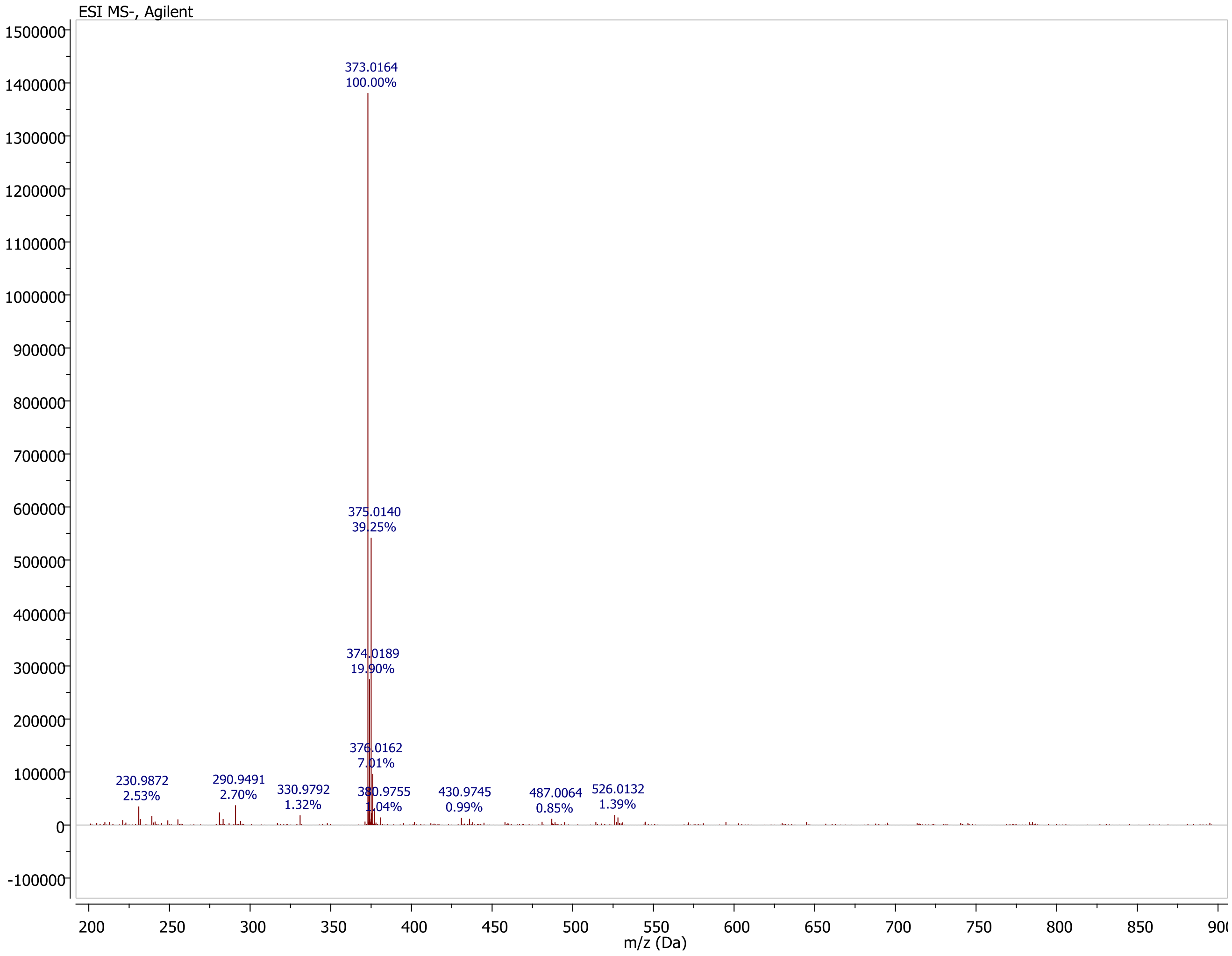
^{1}H & ^{13}C NMR, HRMS and HPLC data



^{1}H NMR spectrum of 4



^{13}**C NMR spectrum of 4**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1.04%
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2.53%
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2.70%
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1.32%
430.9745
0.99%
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0.85%
526.0132
1.39%
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250
300
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400
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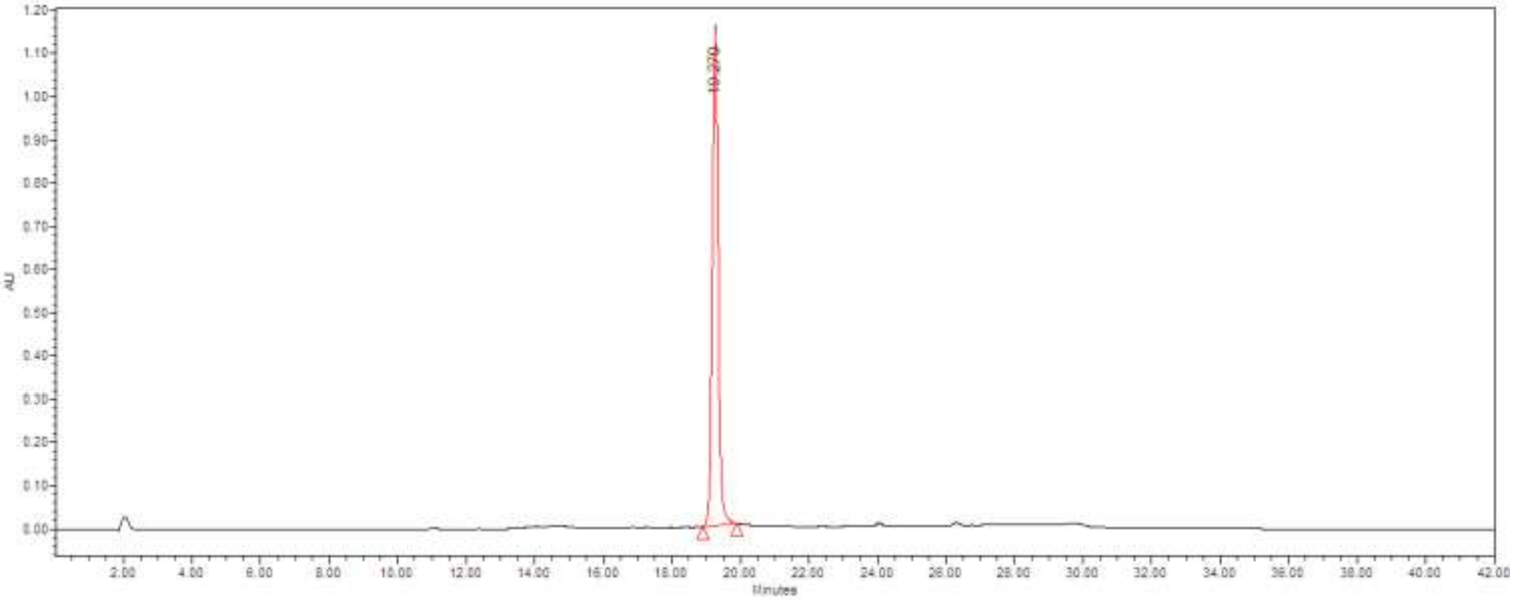
m/z (Da)

HRMS (TOF) of 4



Retention time (min)	**% Area**
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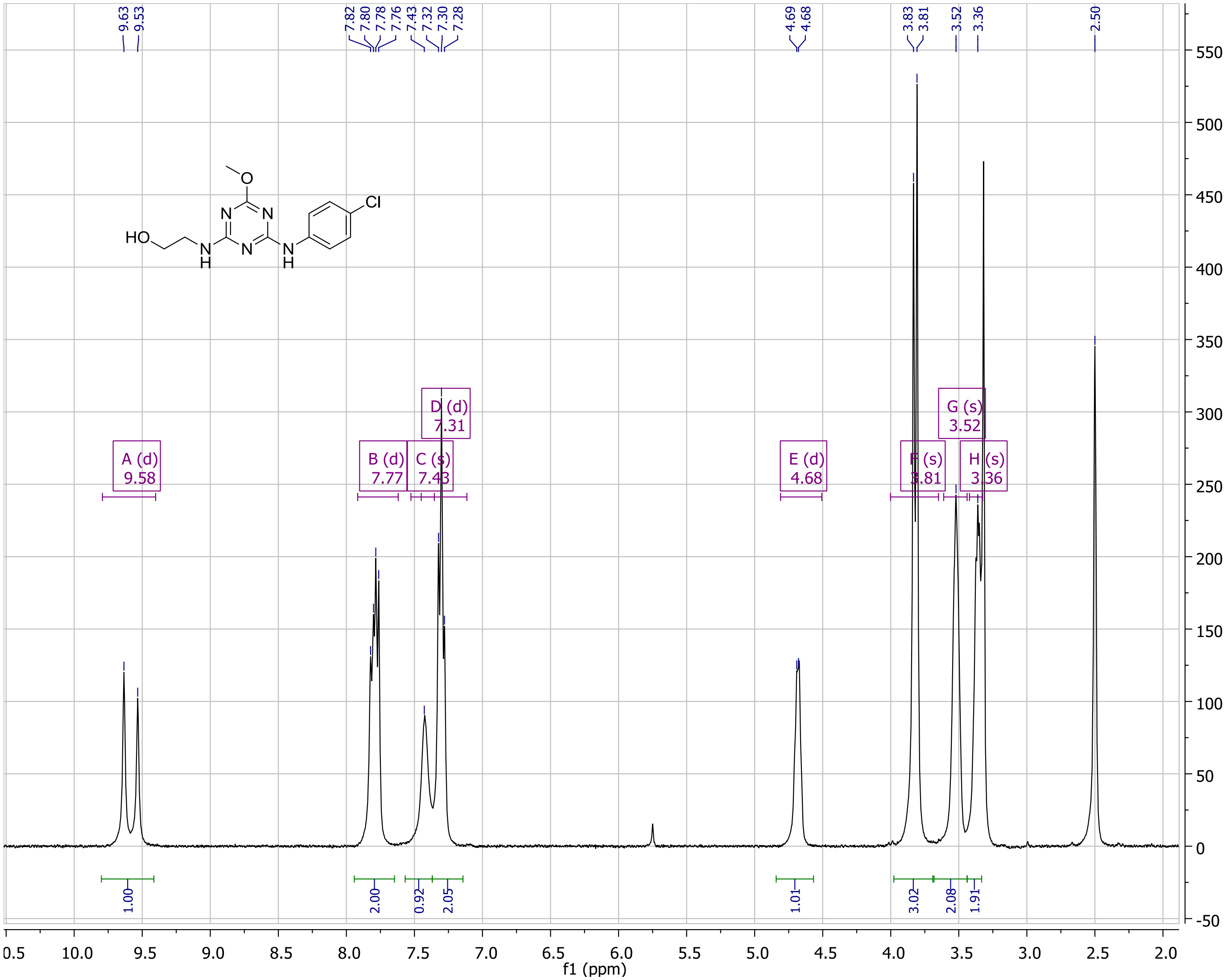
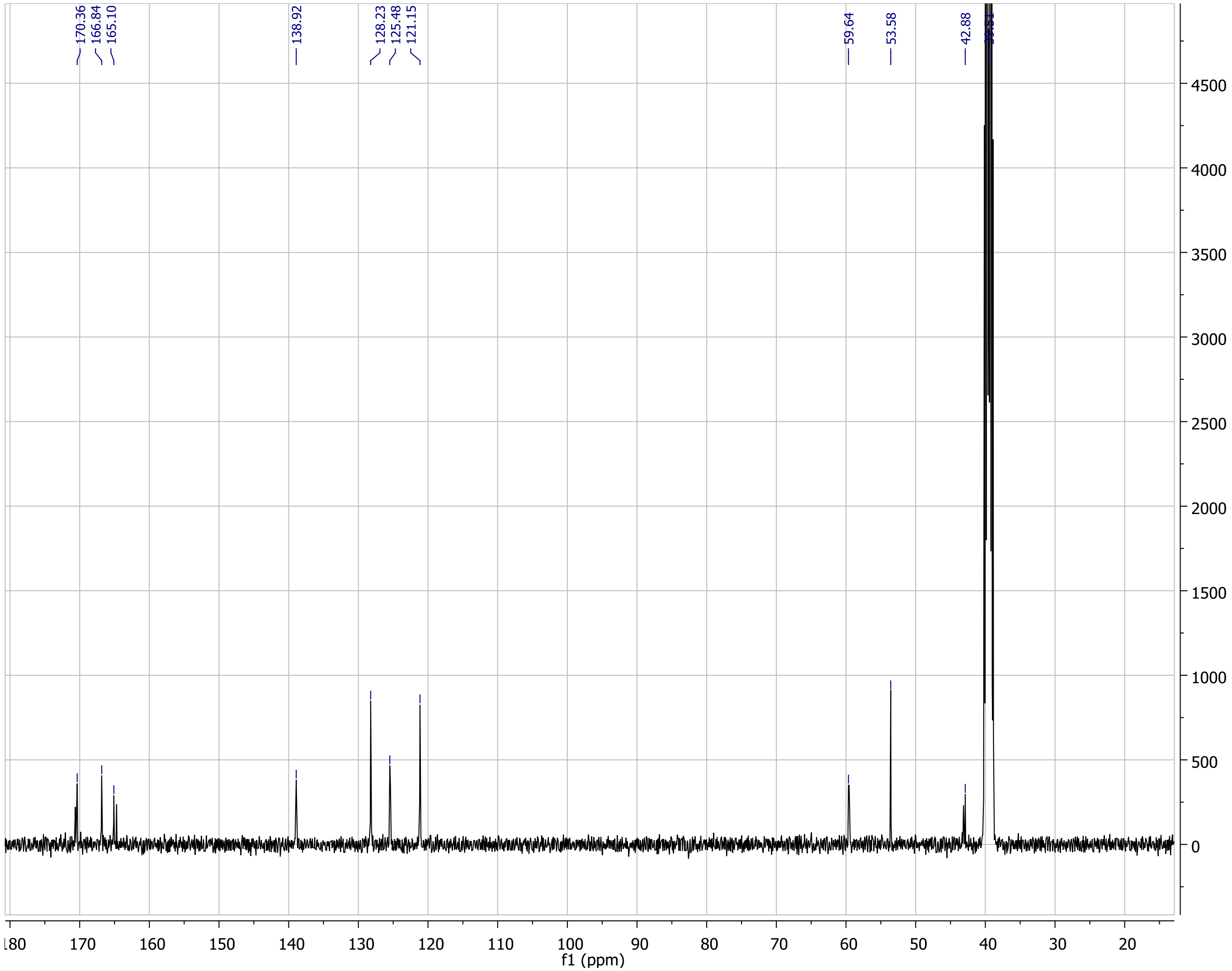
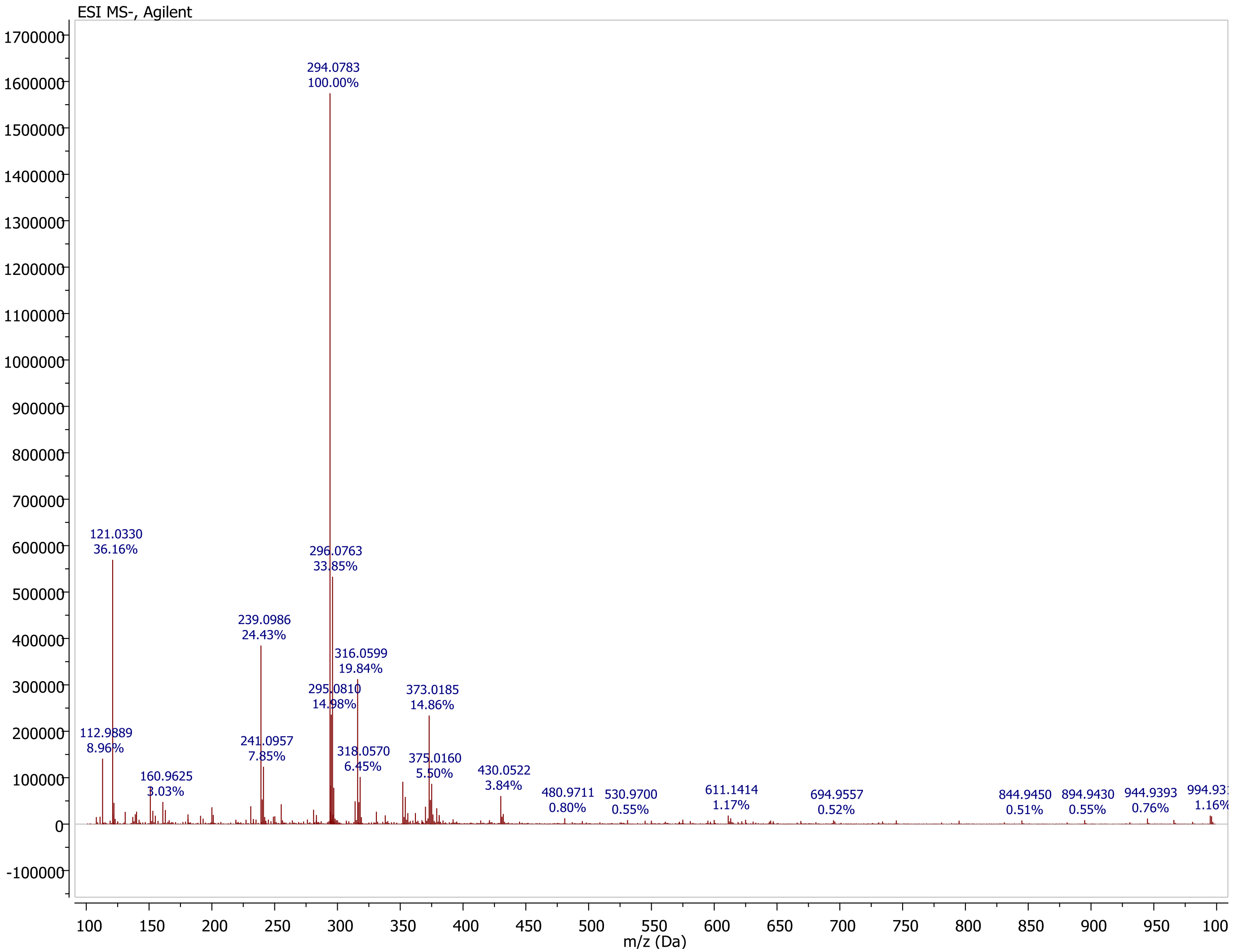
HPLC of **4**



^{1}H NMR spectrum of 5



^{13}C NMR spectrum of 5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100.00%
121.0330
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296.0763
33.85%
239.0986
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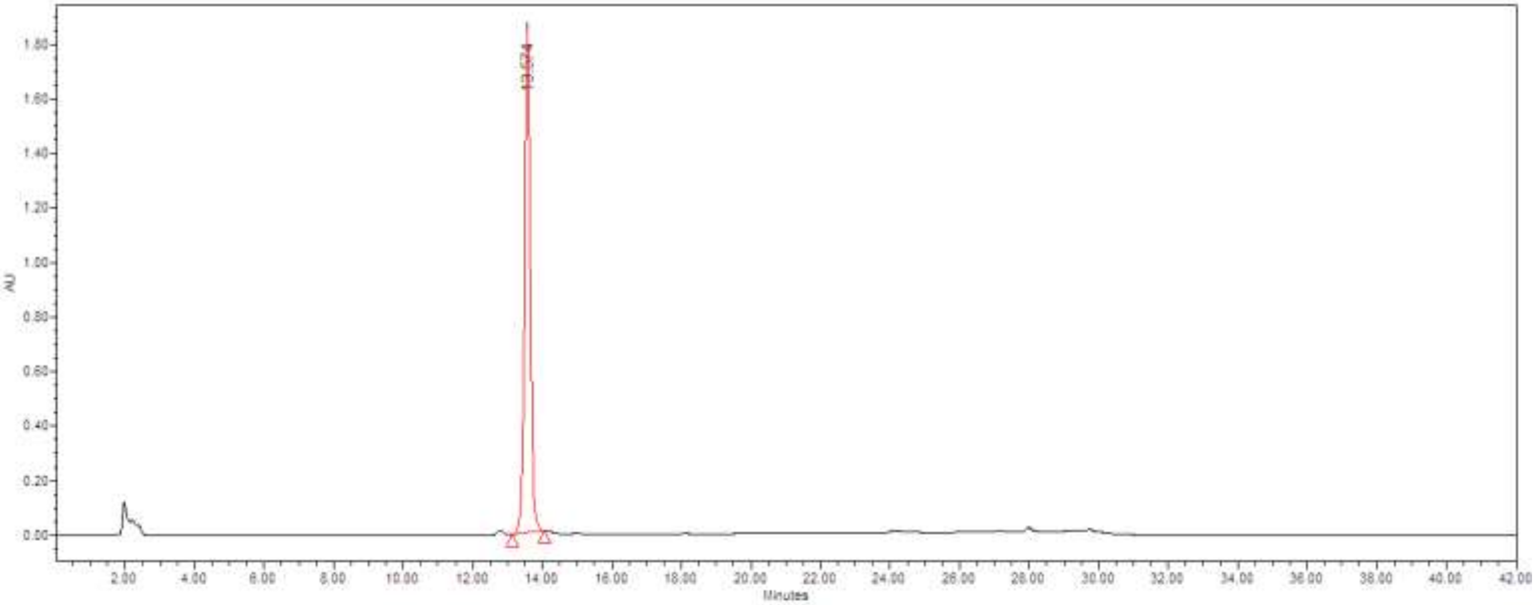
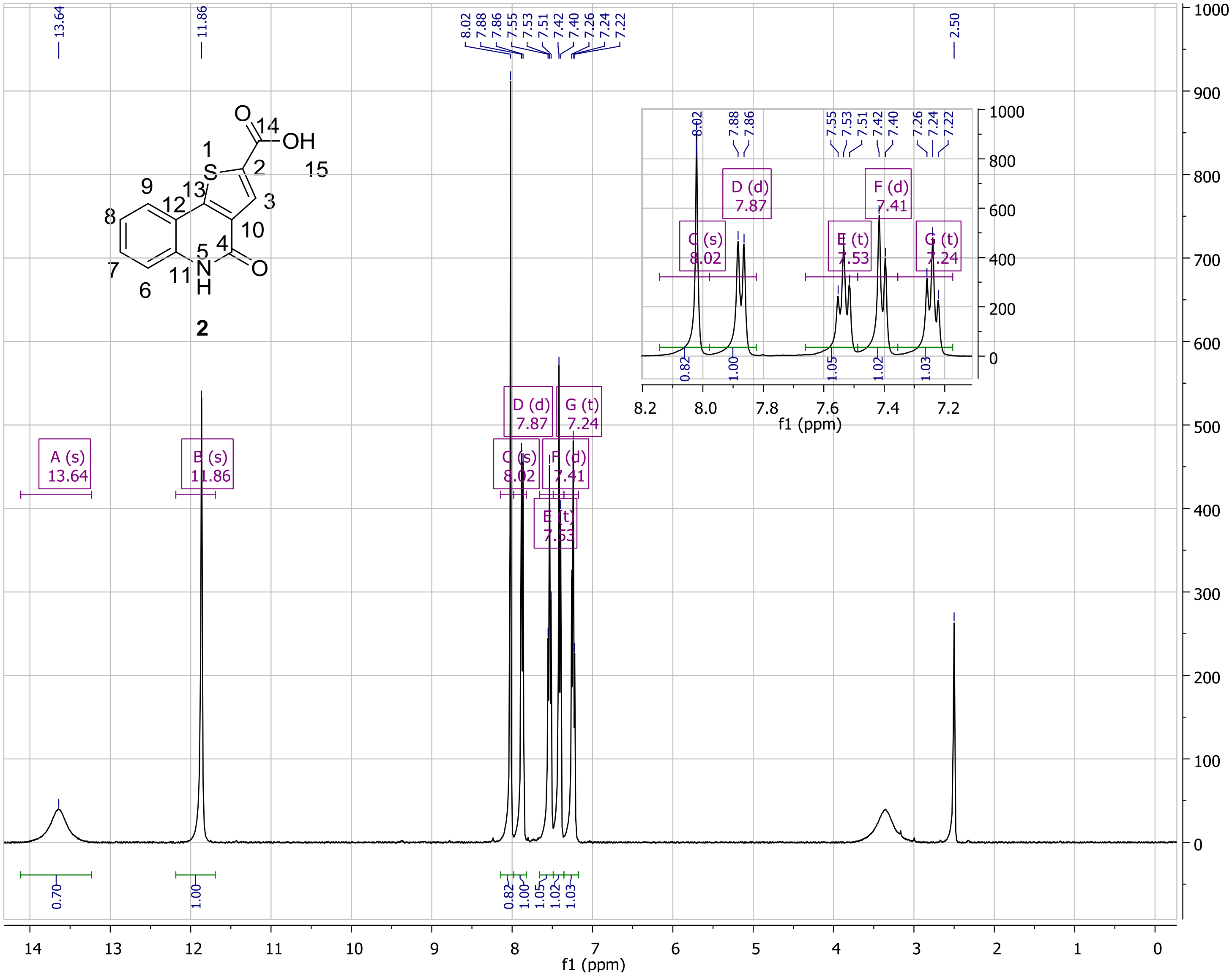
m/z (Da)

HRMS (TOF) of 5

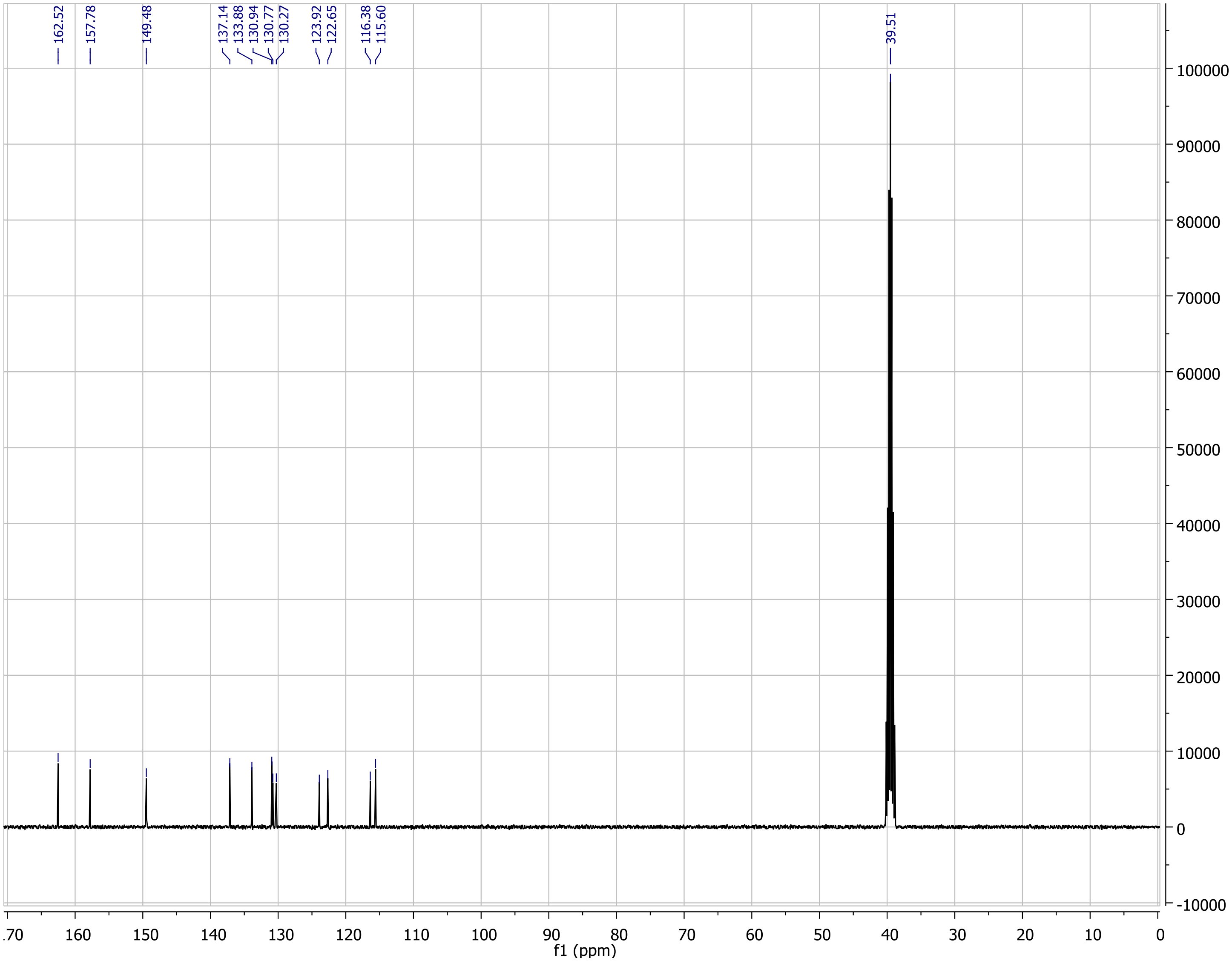


Retention time (min)	% Area
13.574	99.9

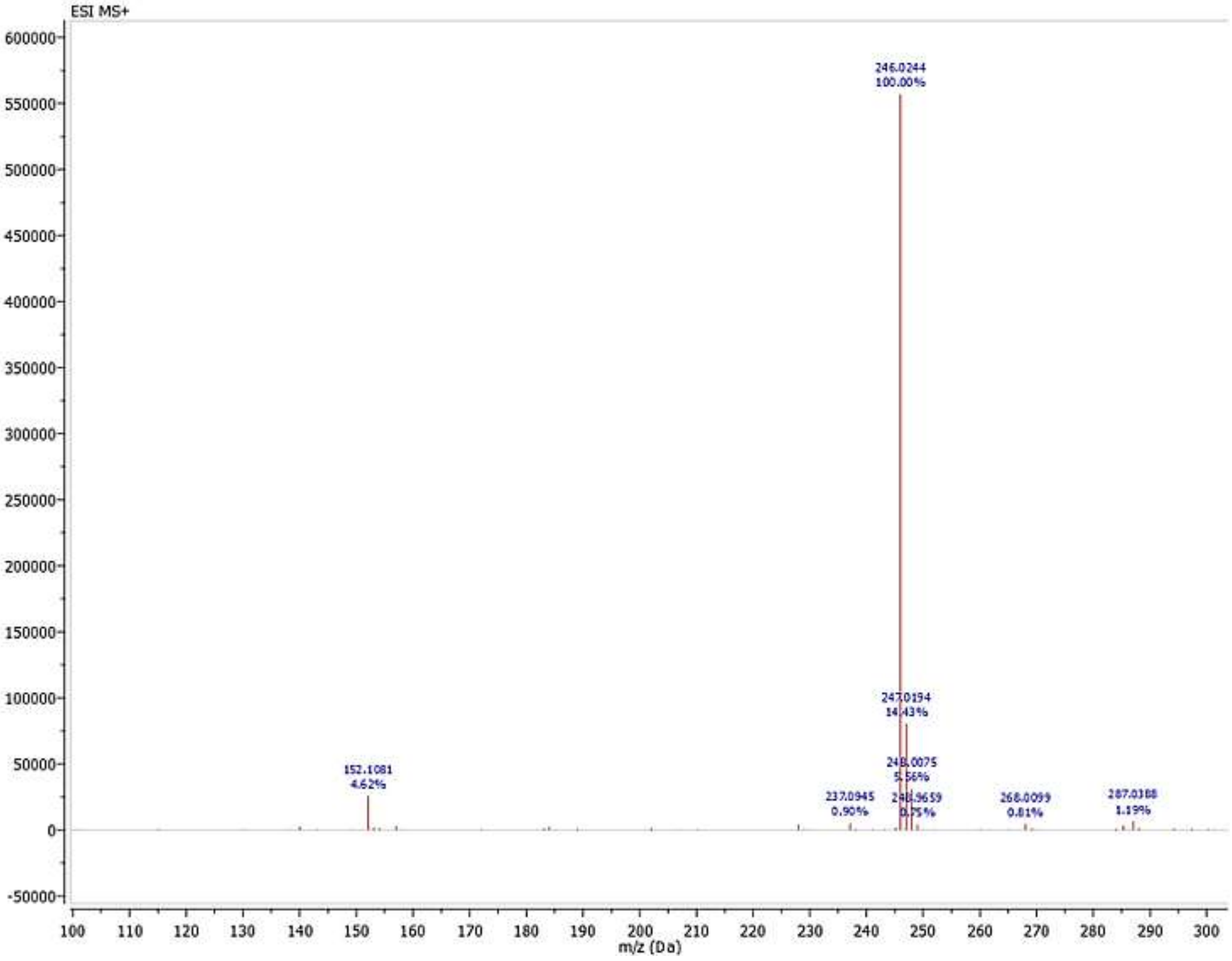
HPLC of **5**



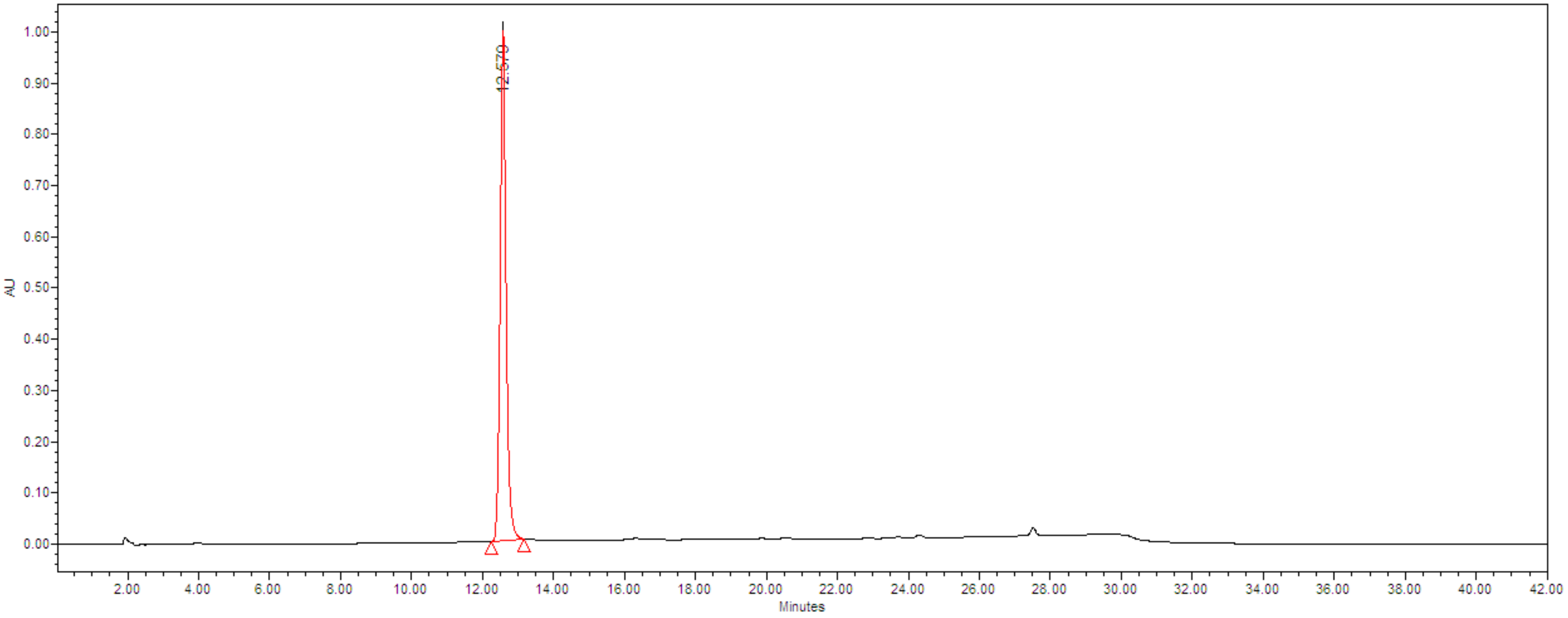
^{1}H NMR spectrum of 14



^{13}C NMR spectrum of 14



HRMS (TOF) of 14



HPLC scan of 14

Retention time (min)	% Area
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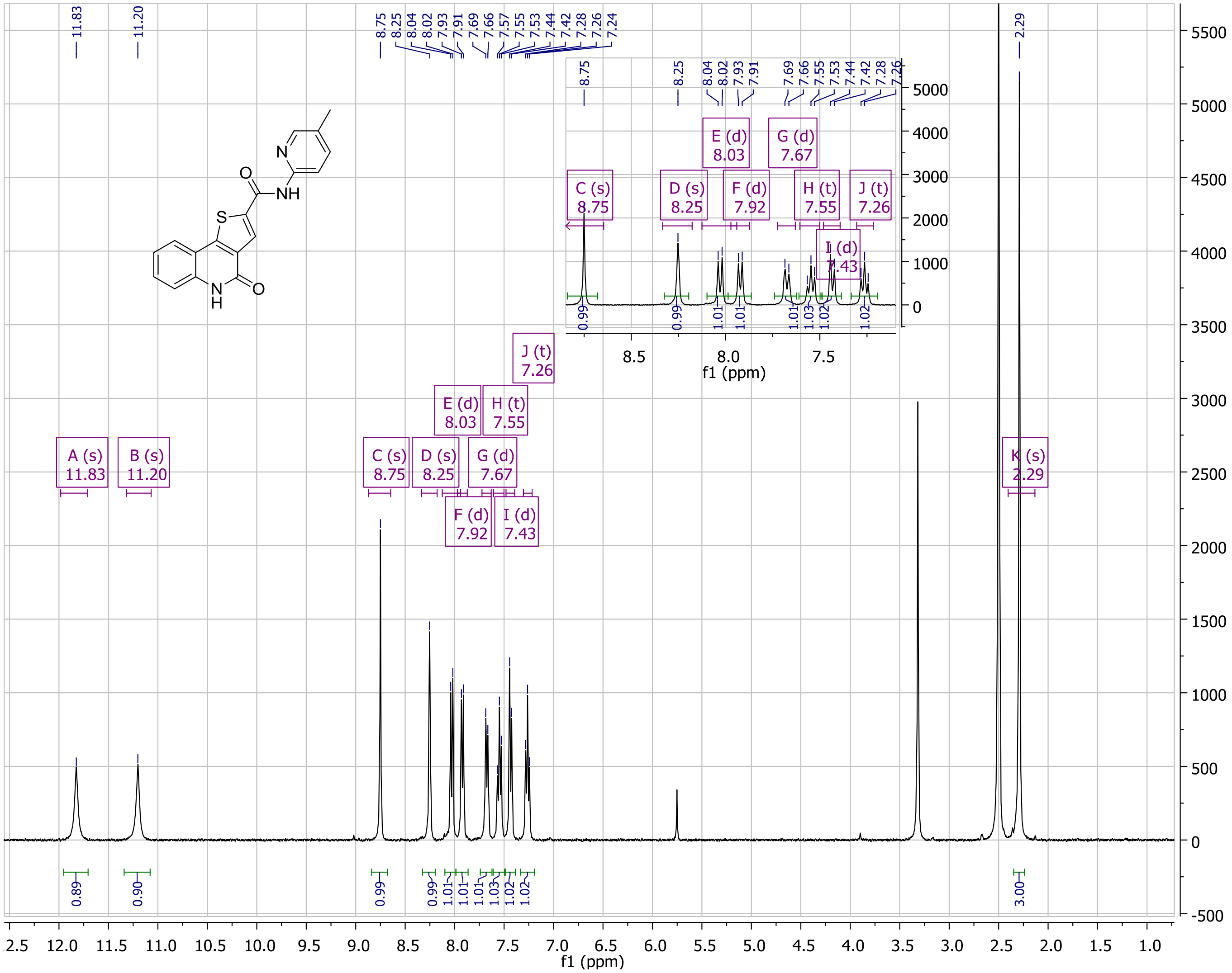
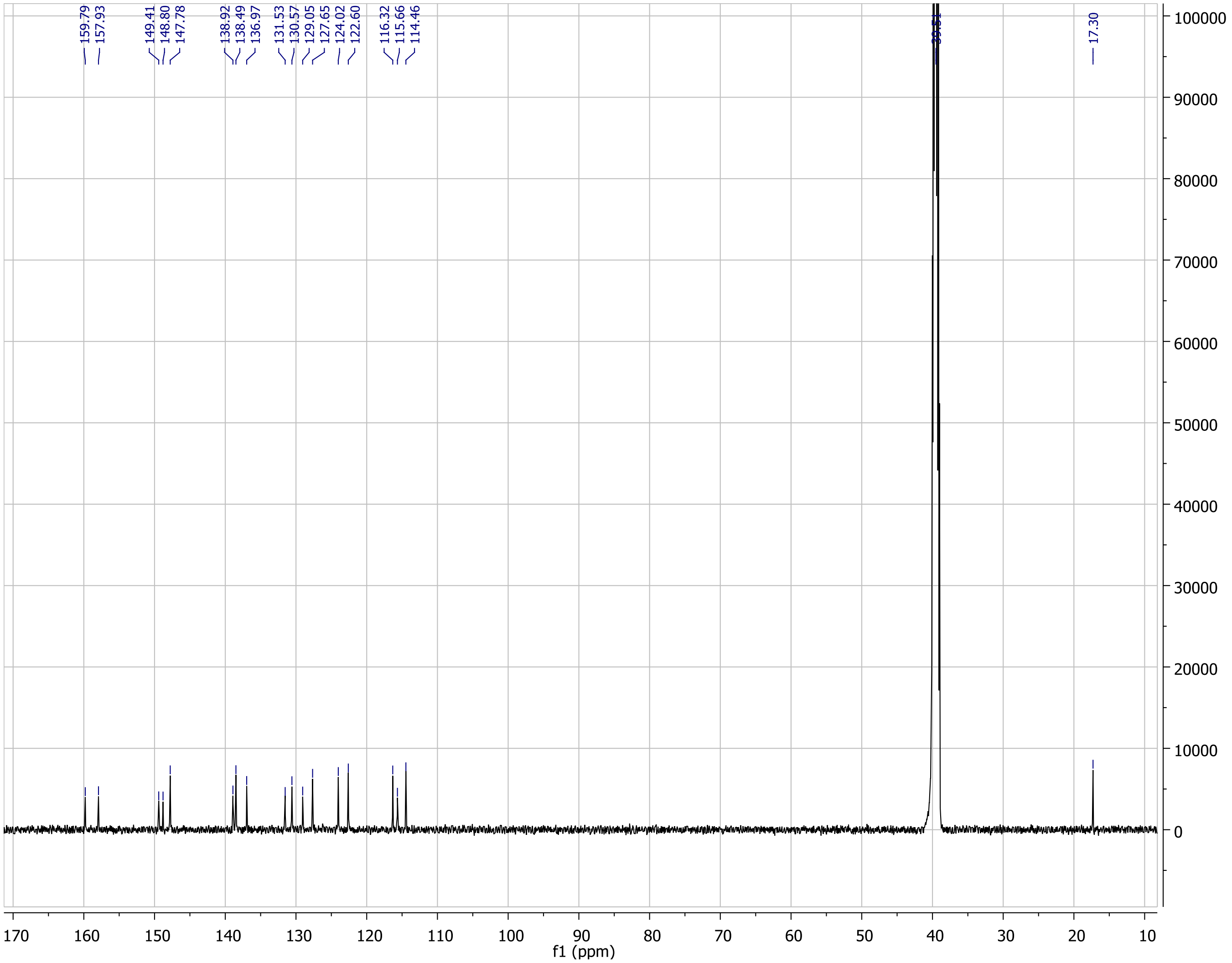


^{1}H NMR spectrum of 10



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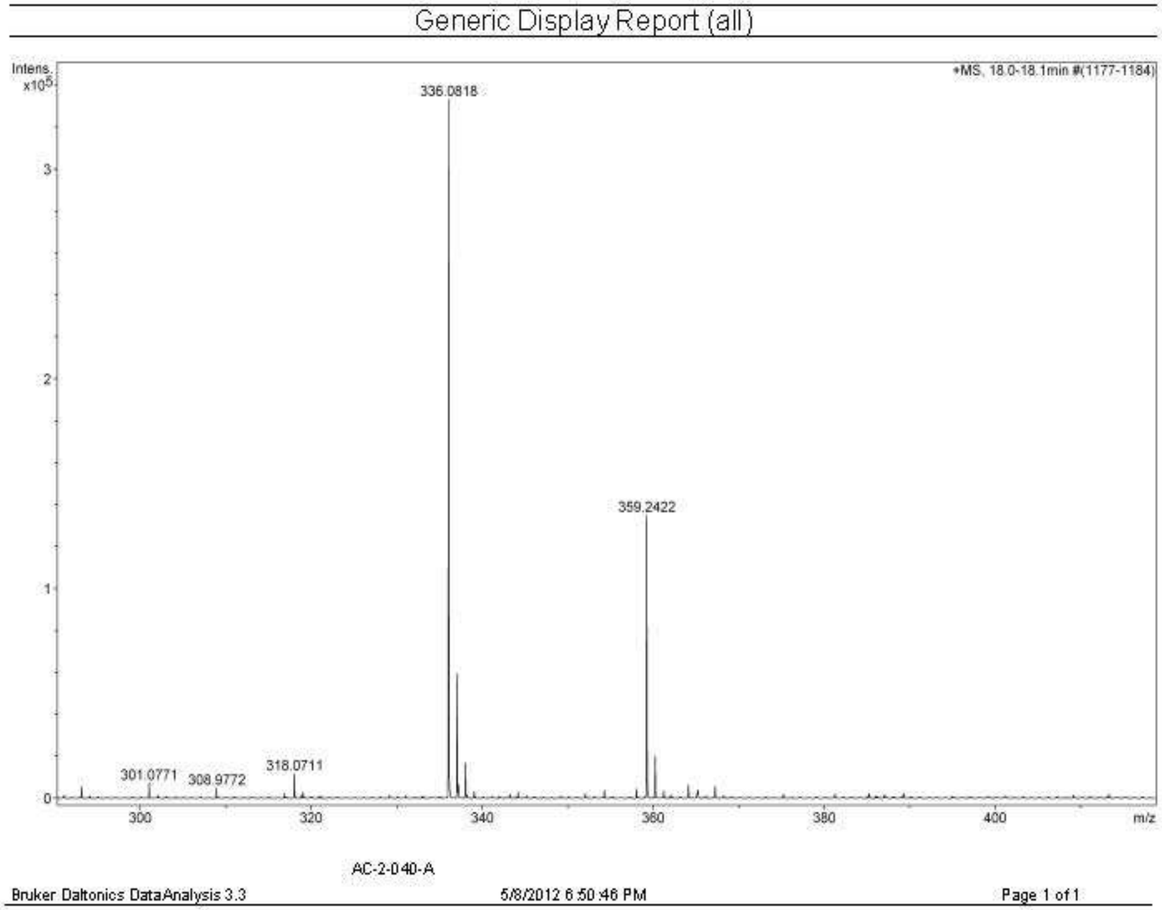

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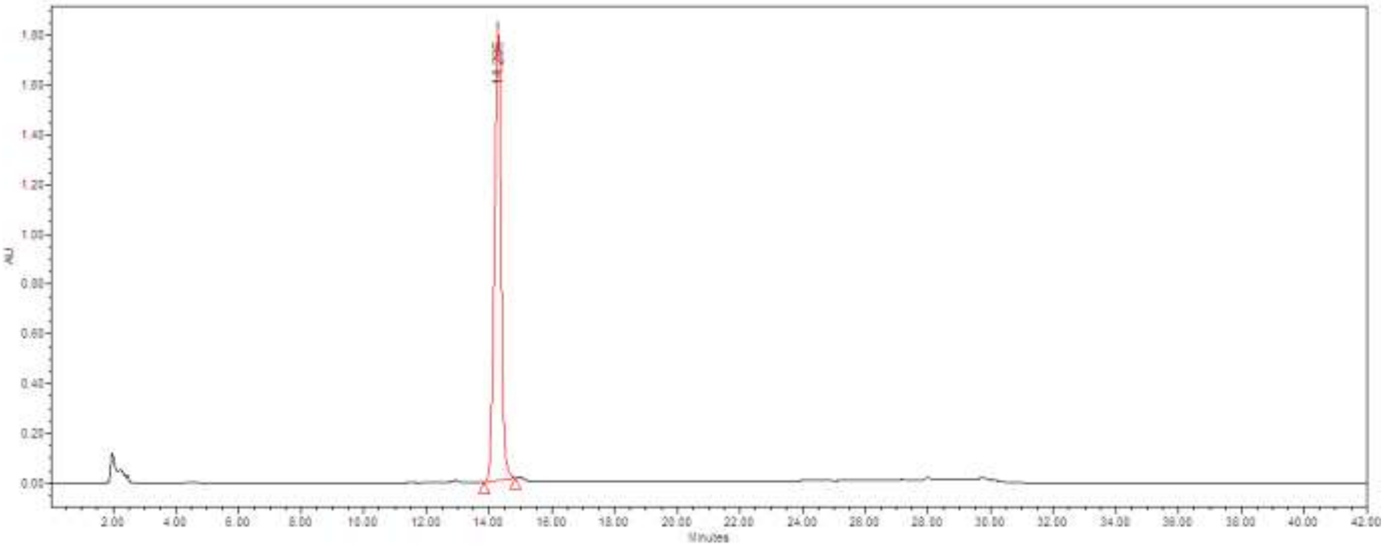
HRMS (TOF) of 10



HPLC scan of 10

Retention time (min)	% Area
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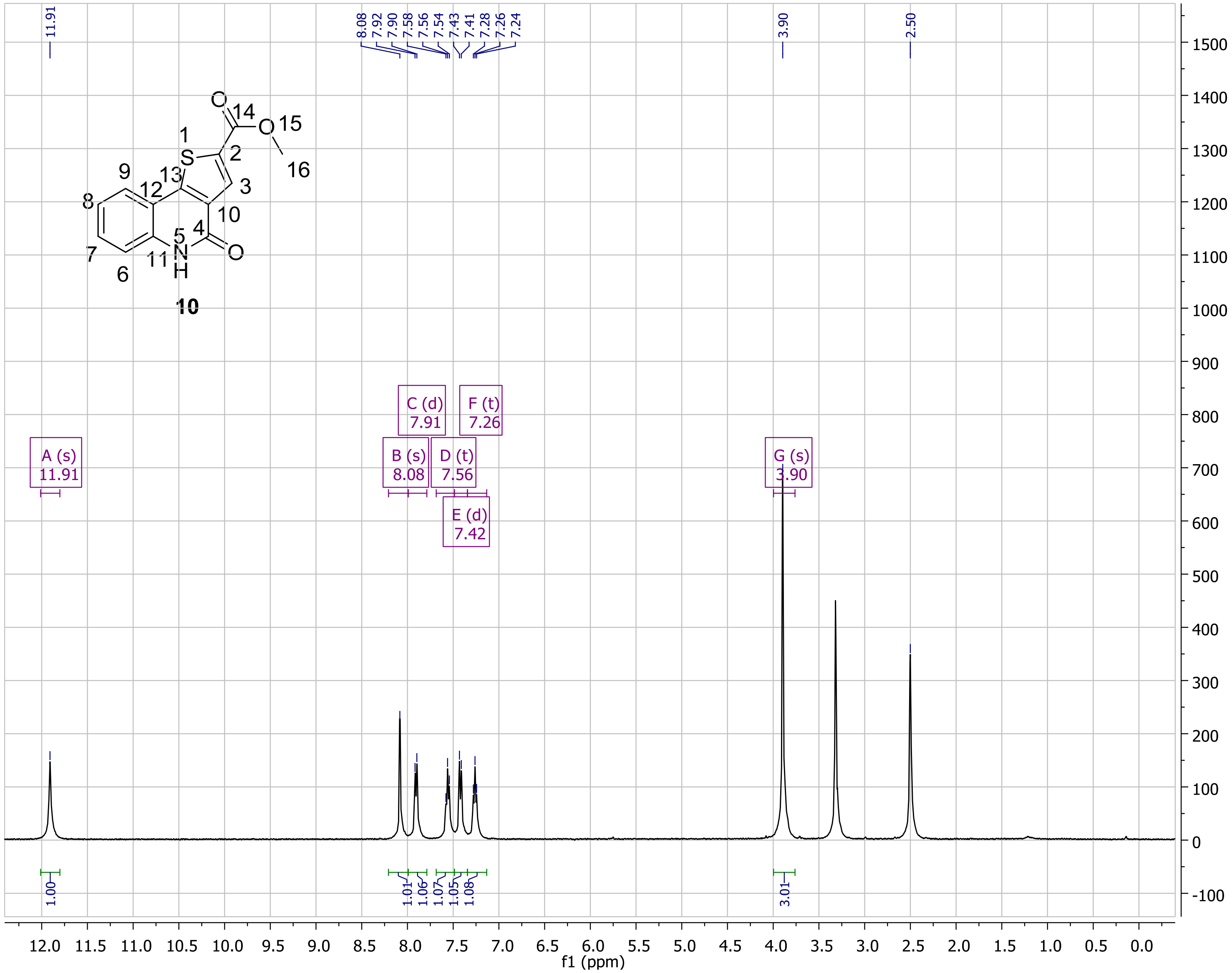
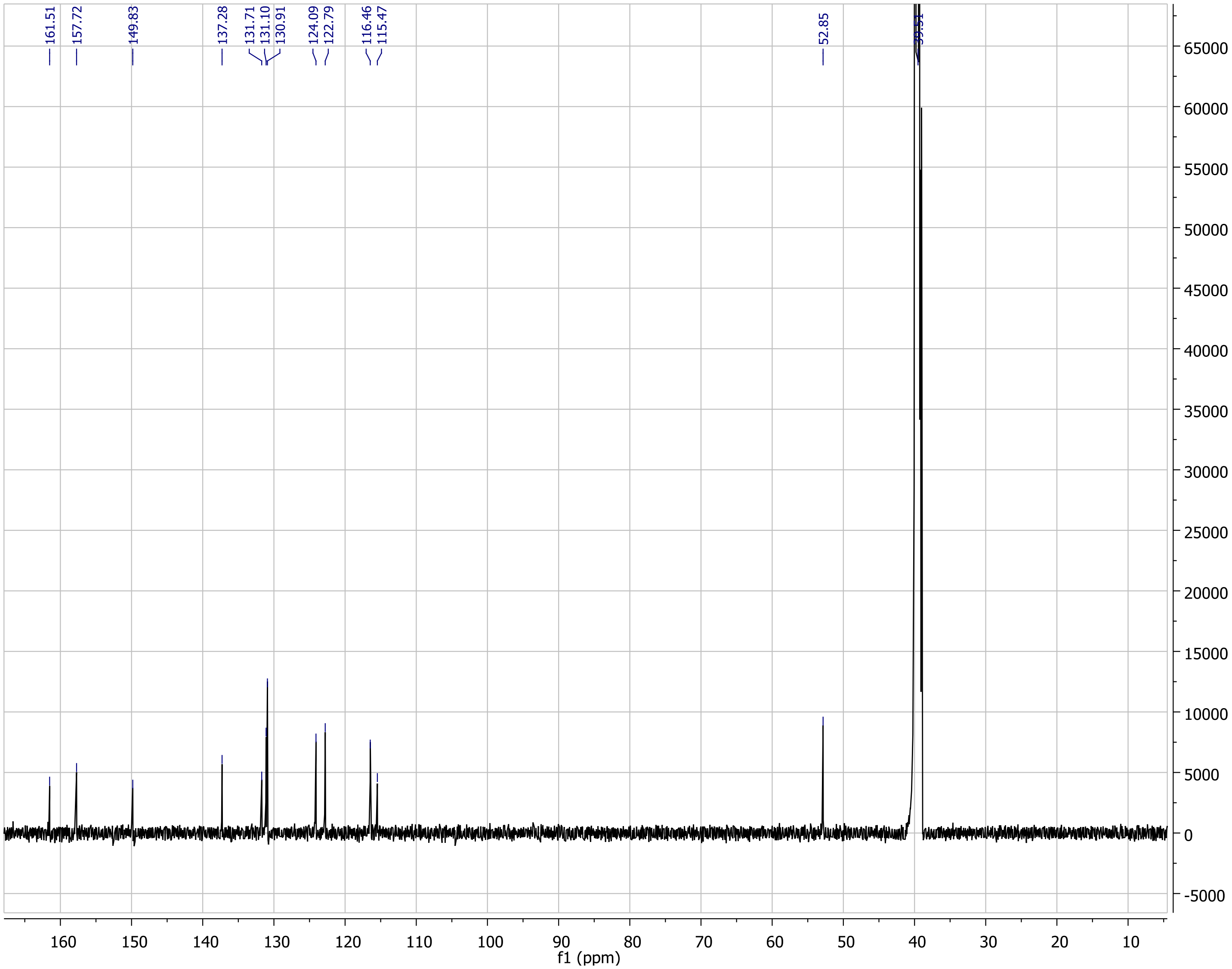
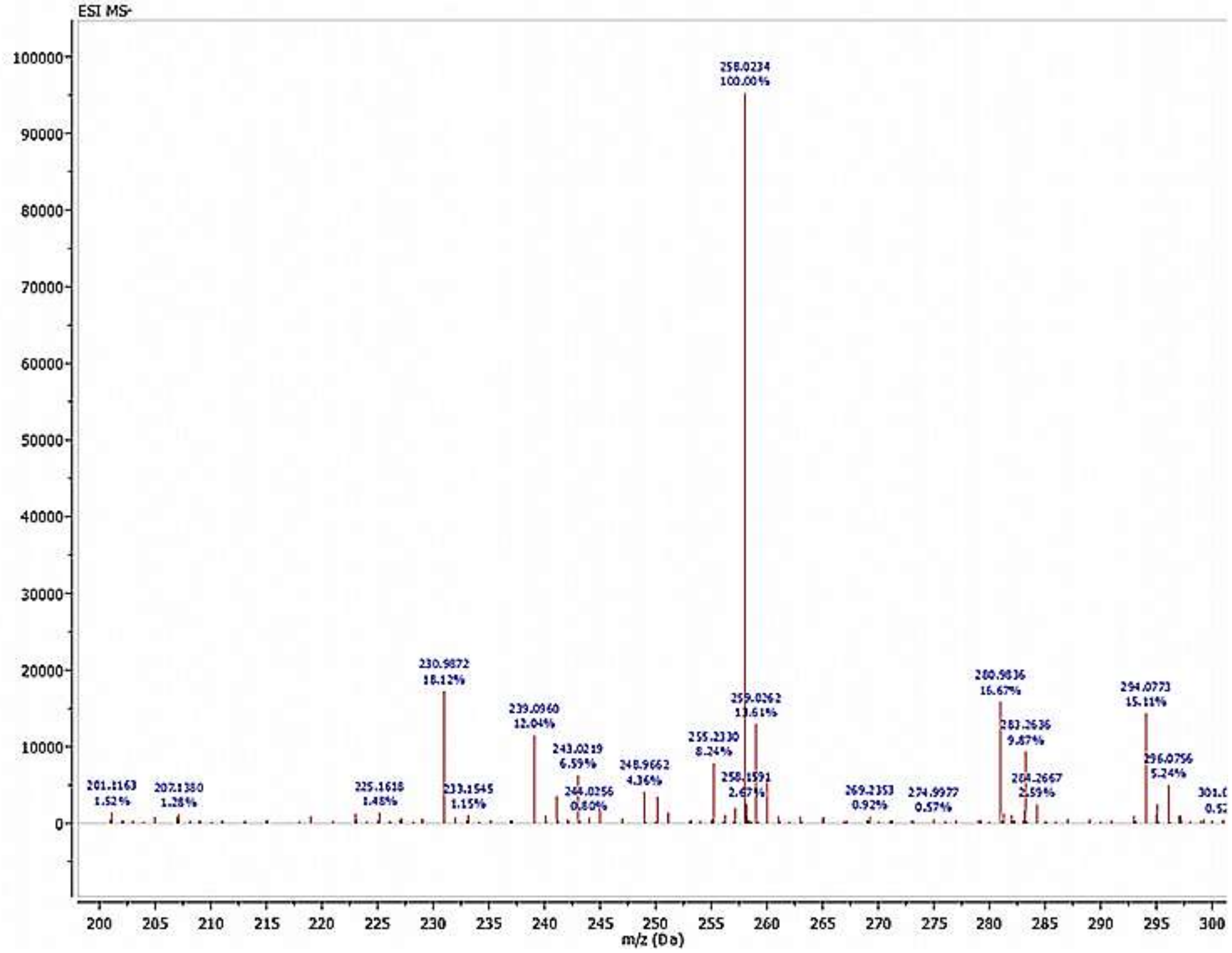


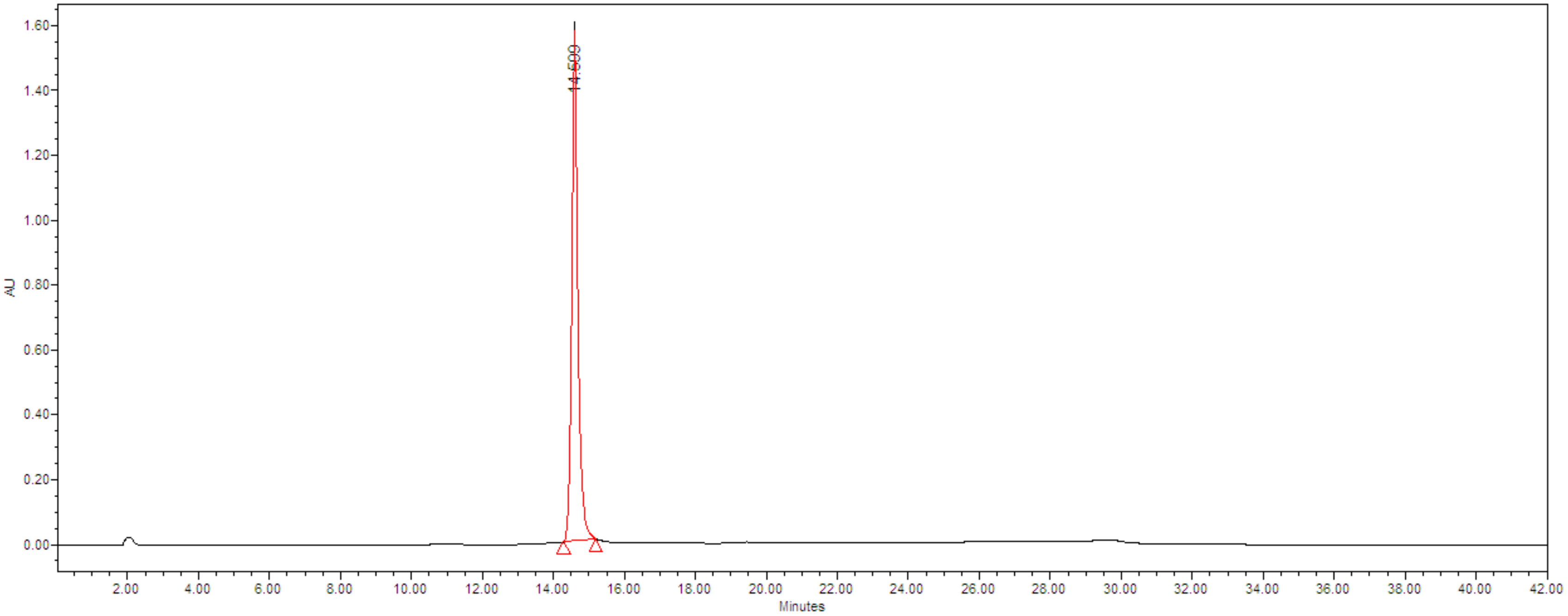
^{1}H NMR spectrum of 24



^{13}C NMR spectrum of 24



HRMS (TOF) of 24



HPLC scan of 24

Retention time (min)	**% Area**
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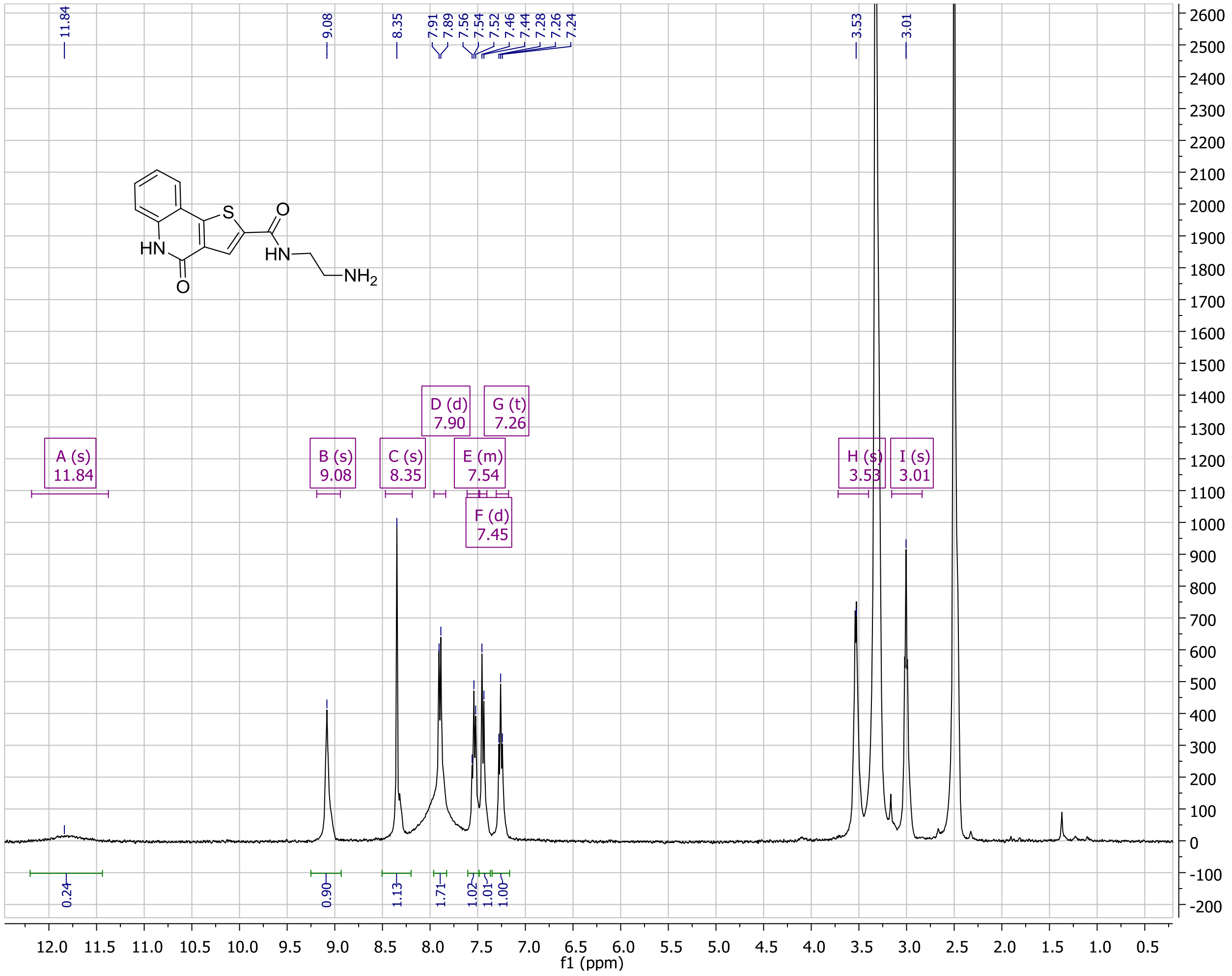
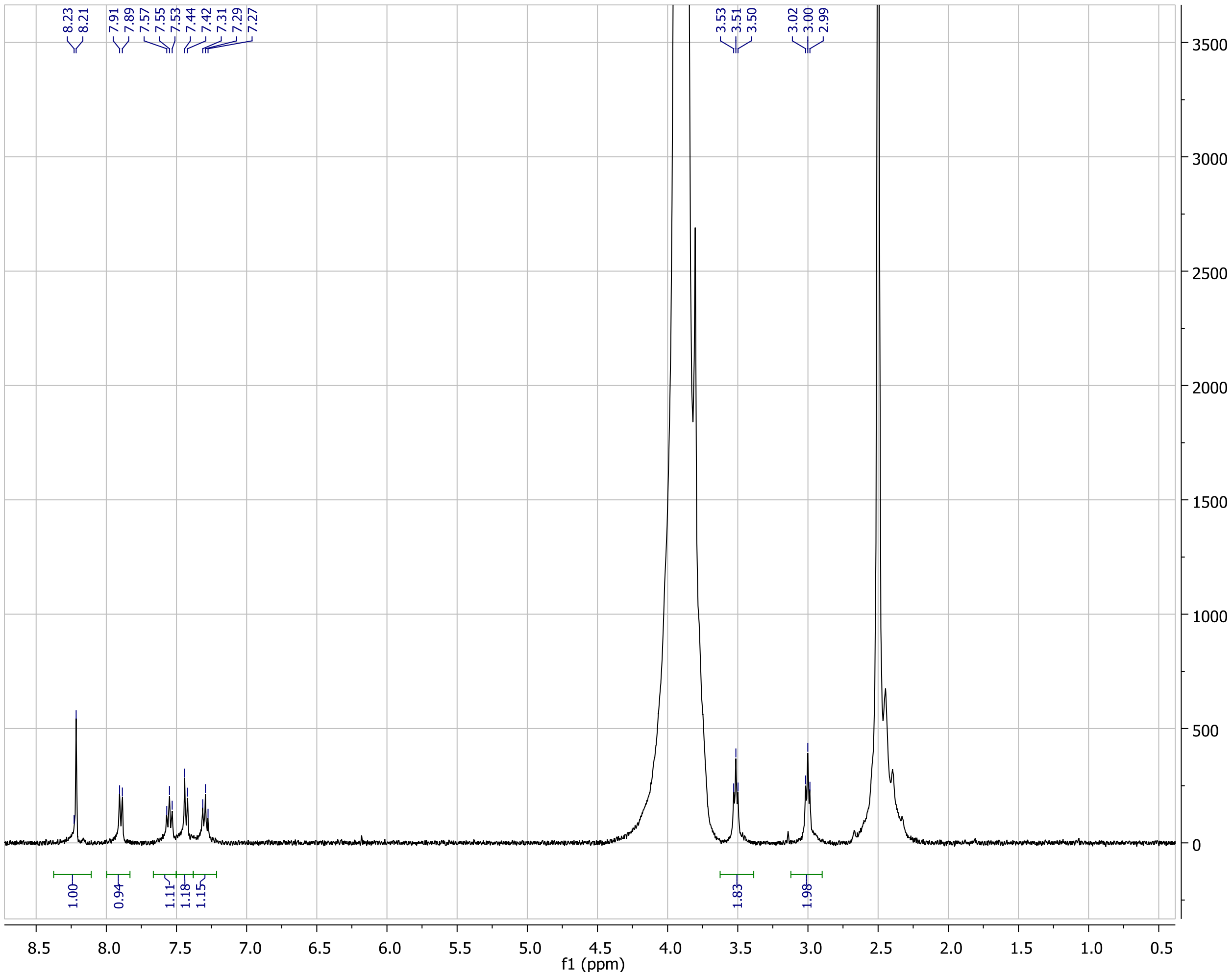
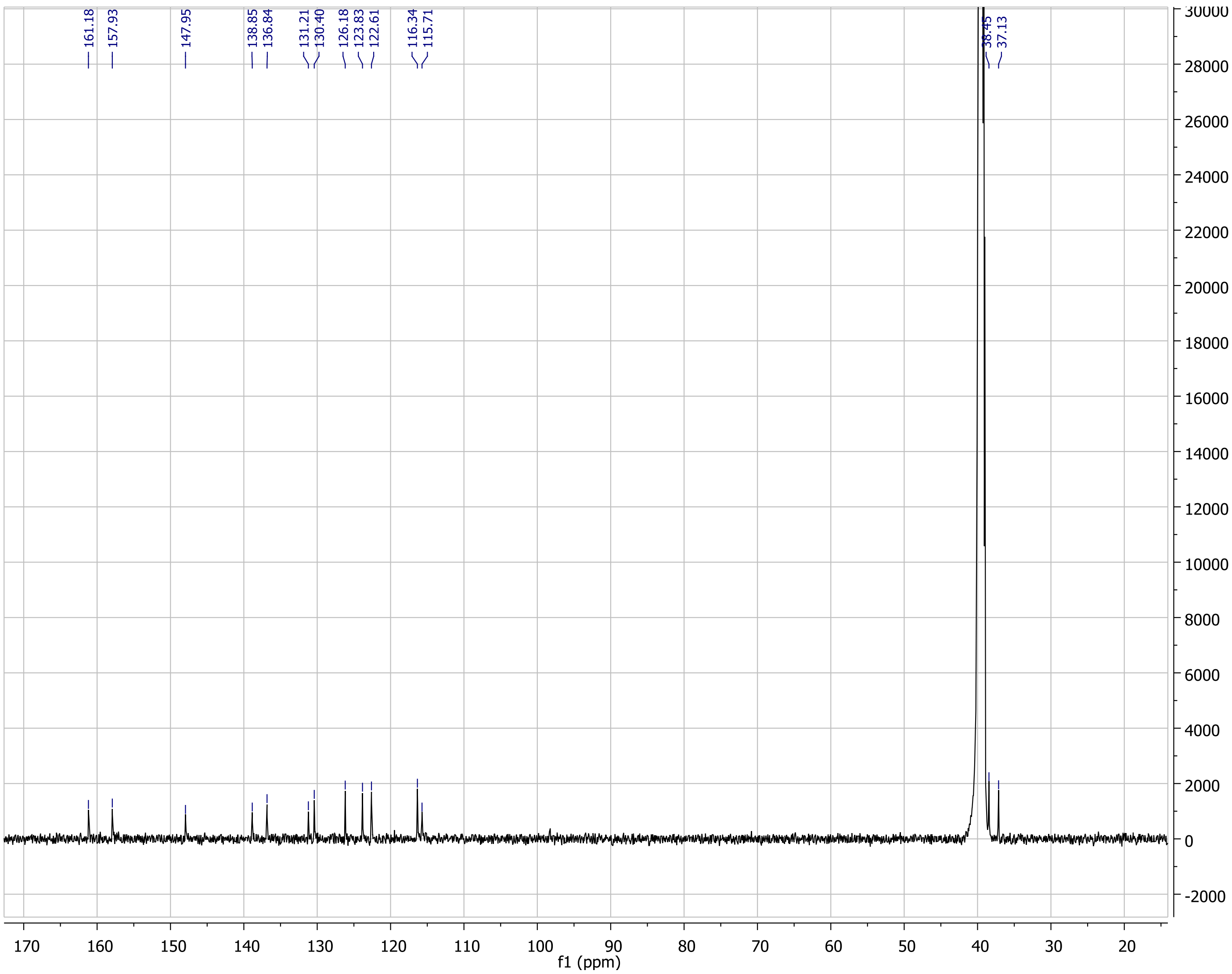


^{1}H NMR spectrum of 25i



^{1}H NMR (D_2O wash) spectrum of 25i



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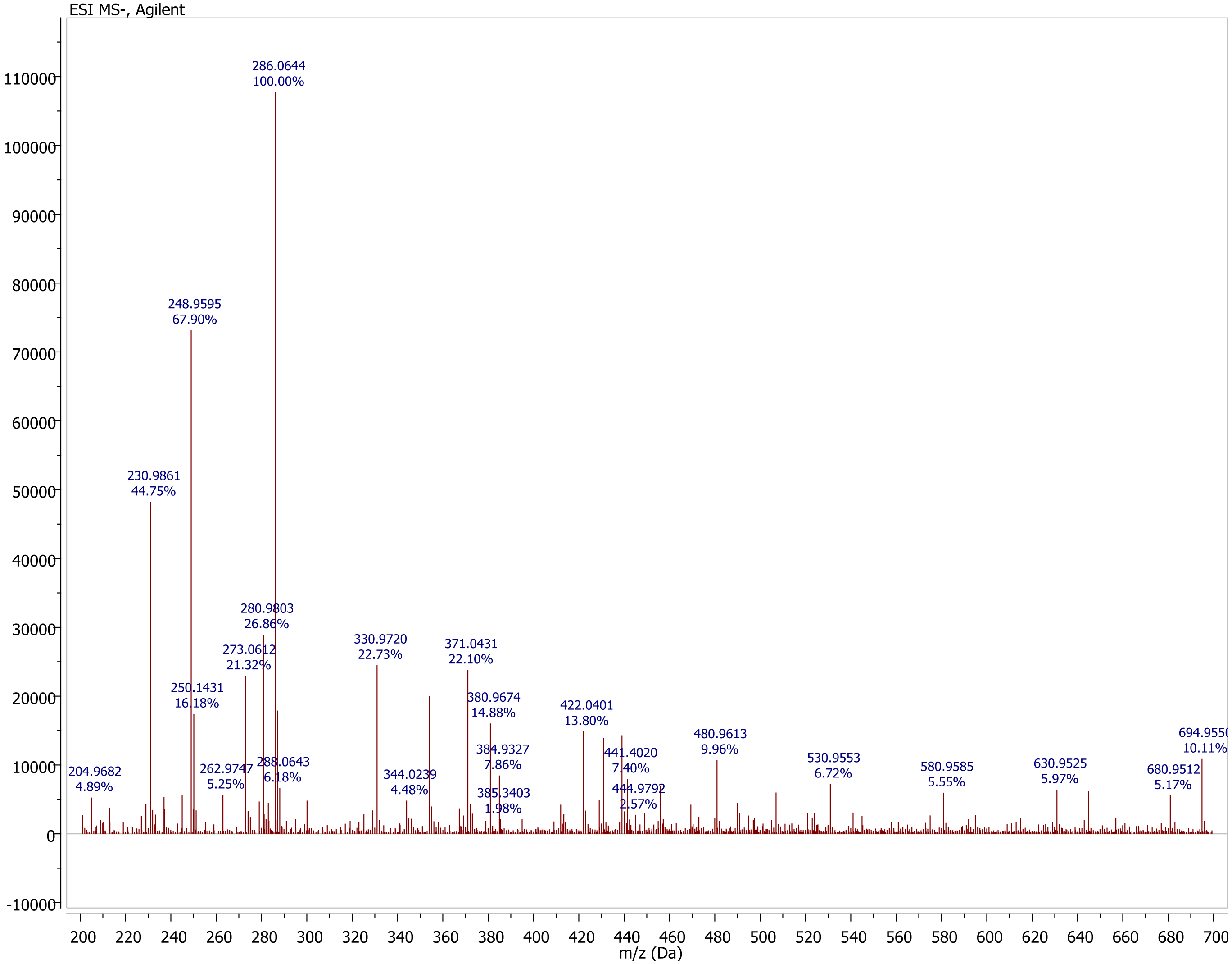

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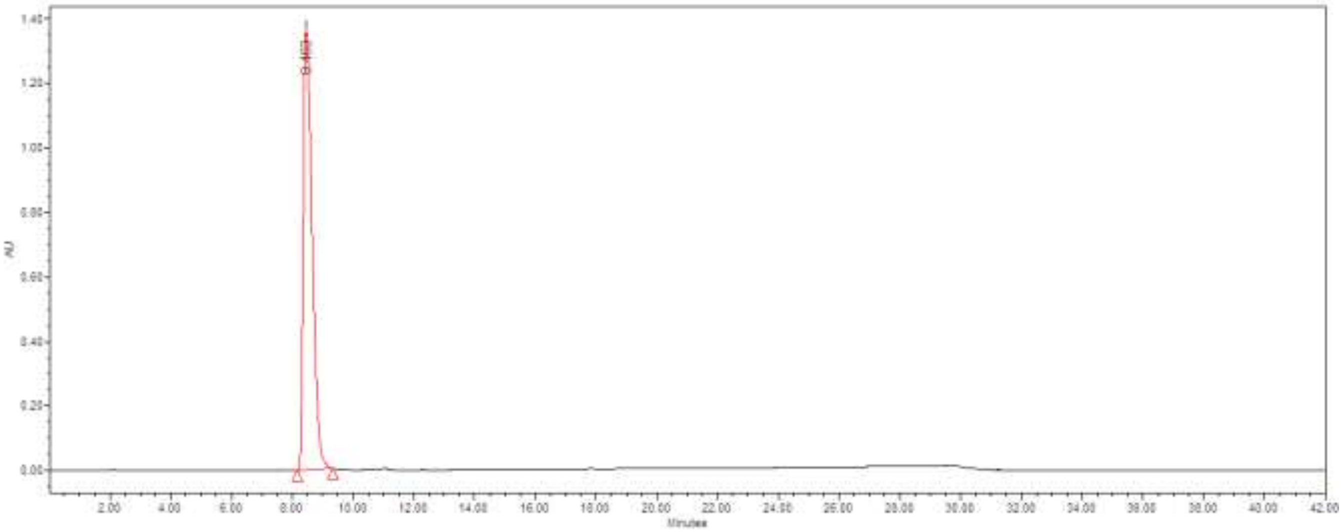

HPLC scan of 25i

Retention time (min)	**% Area**
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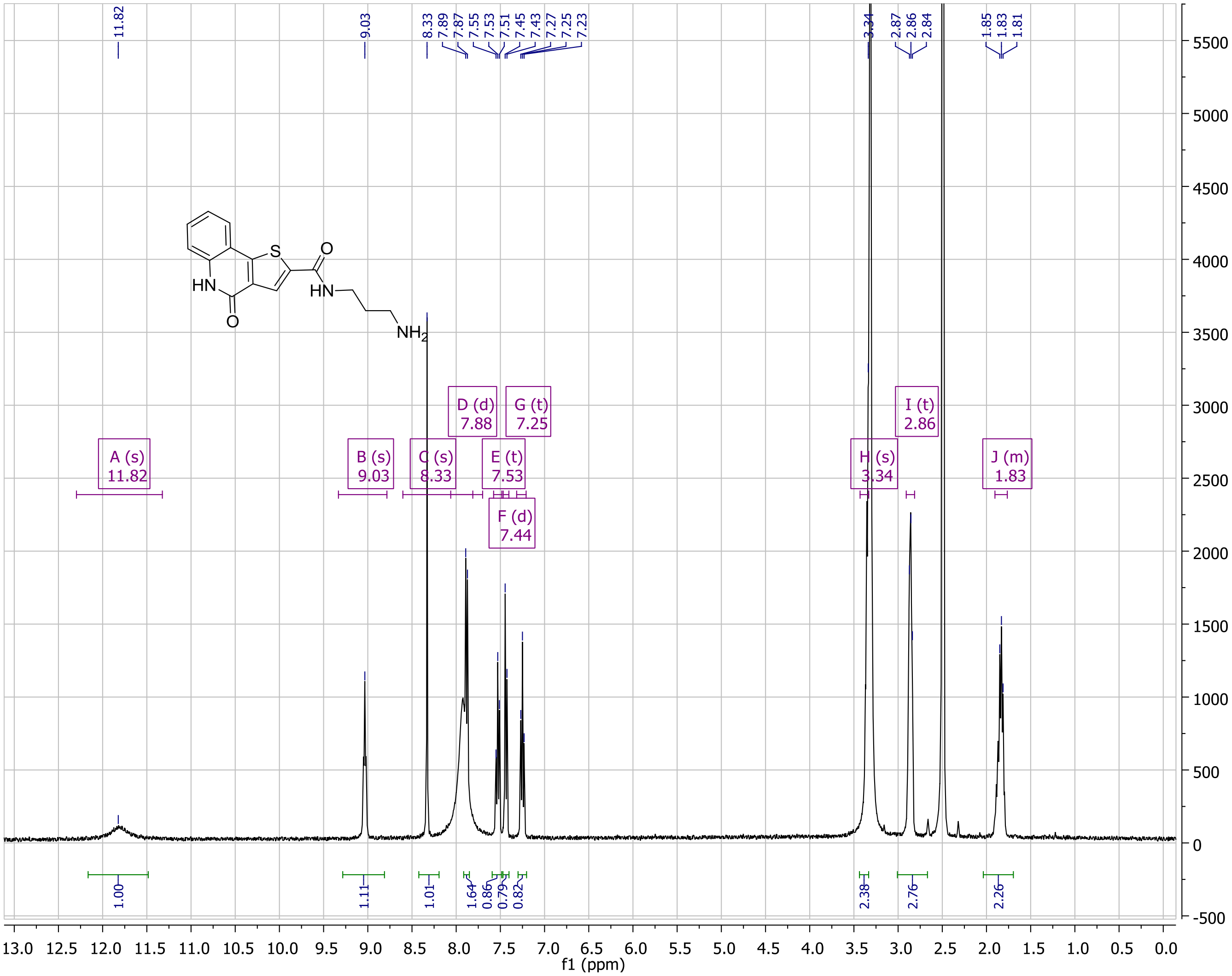
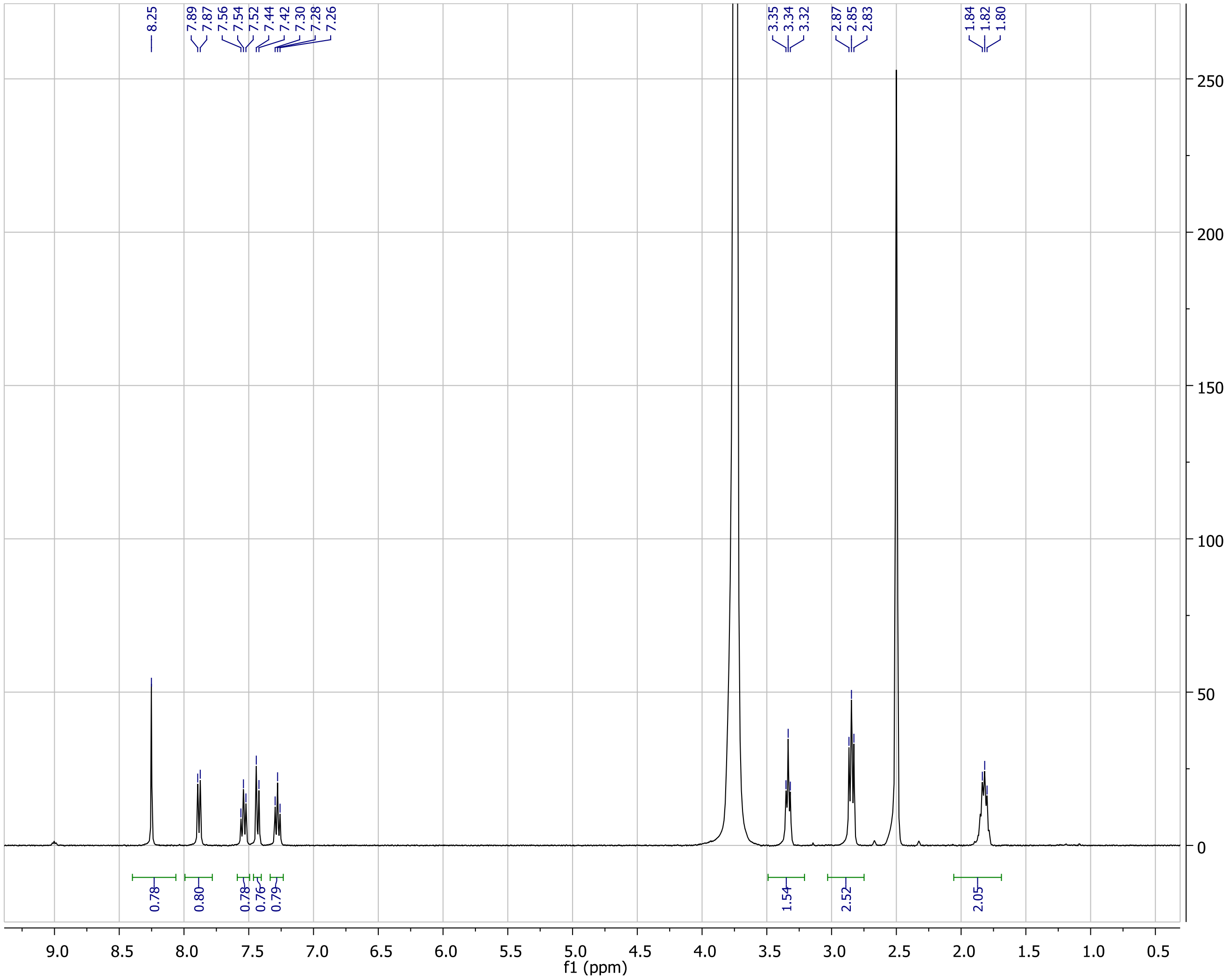


^{1}H NMR spectrum of 25k



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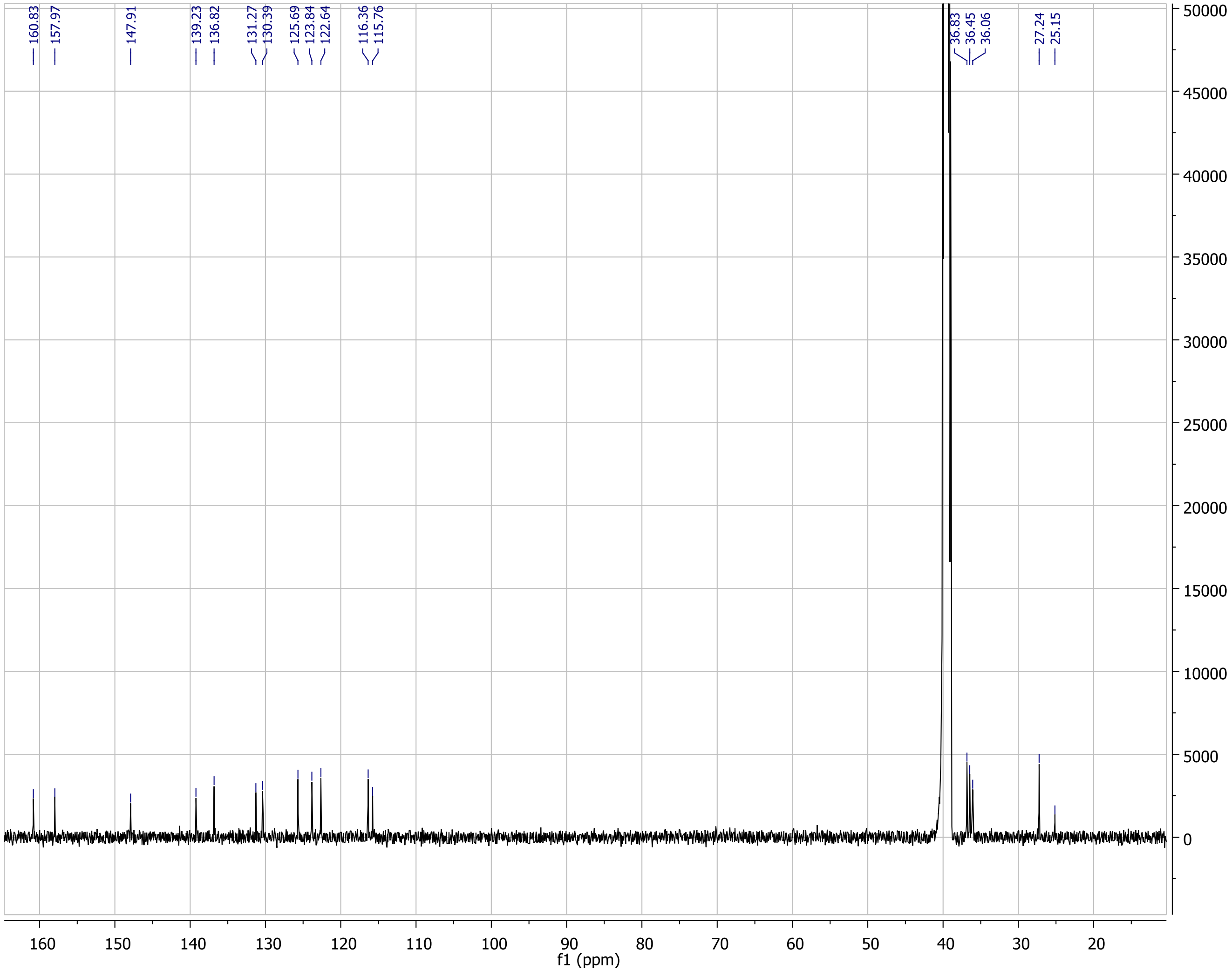

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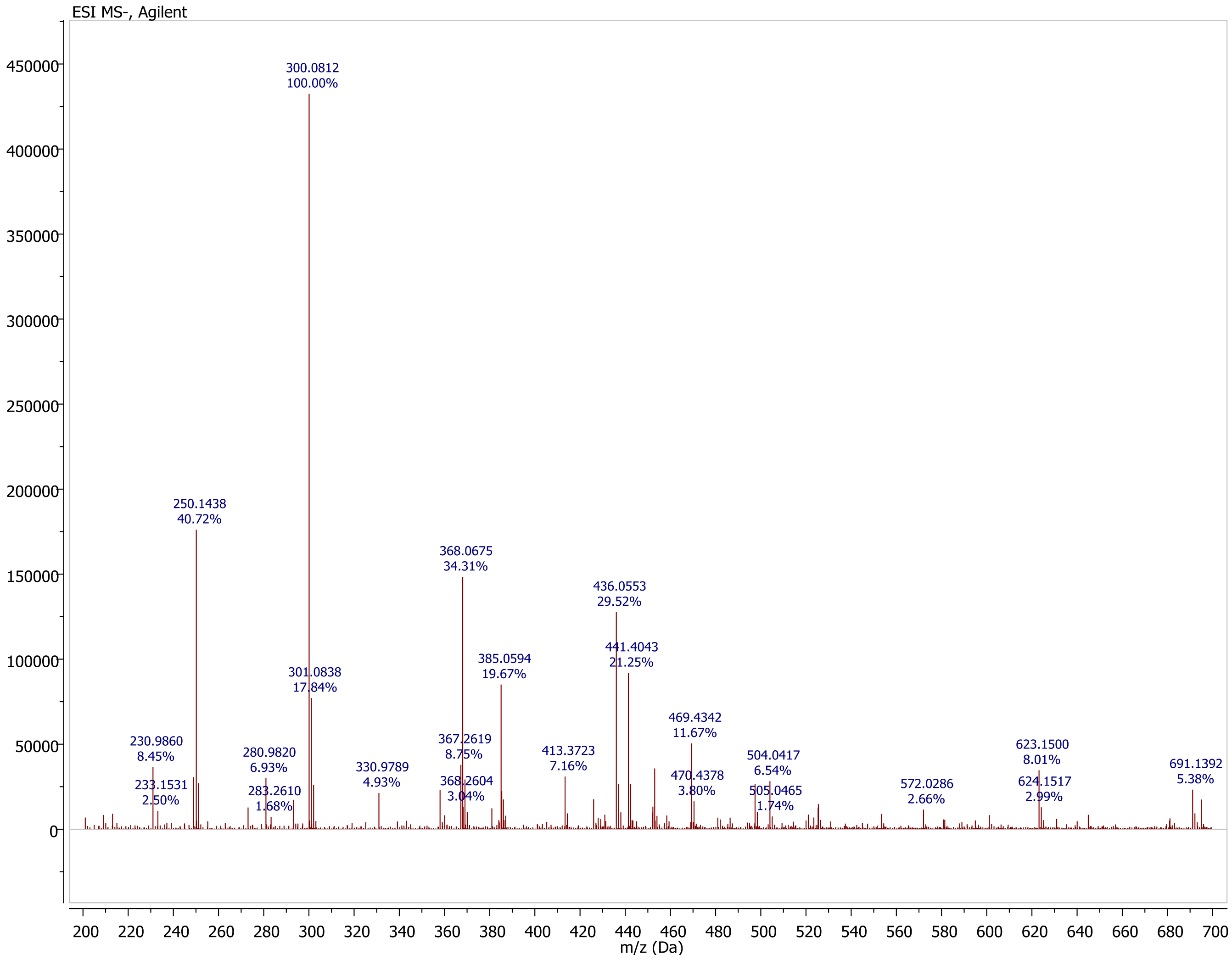

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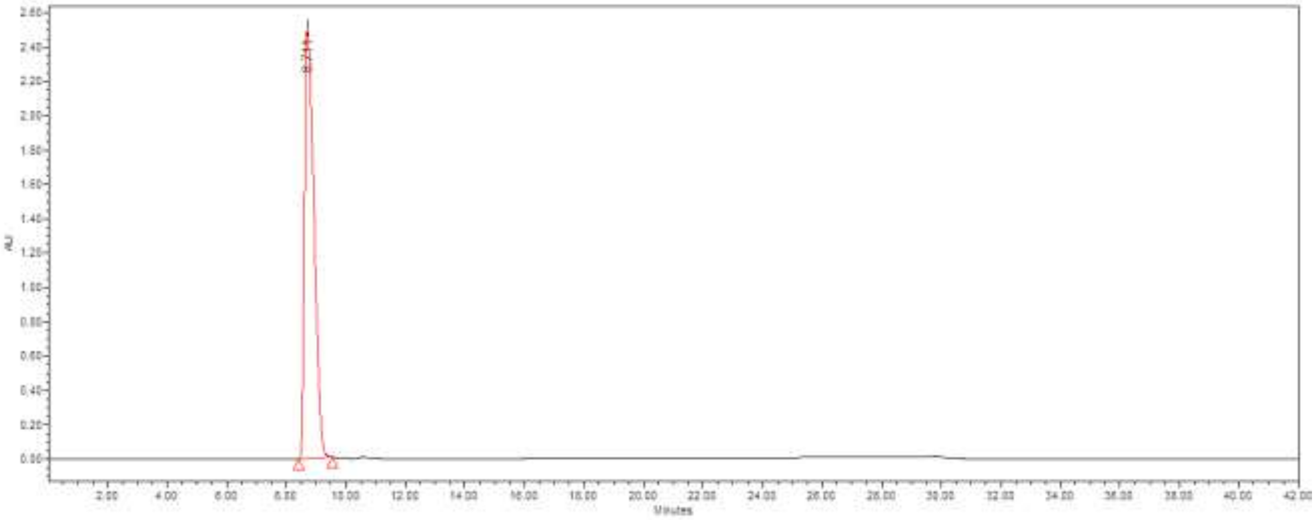

HPLC scan of 25k

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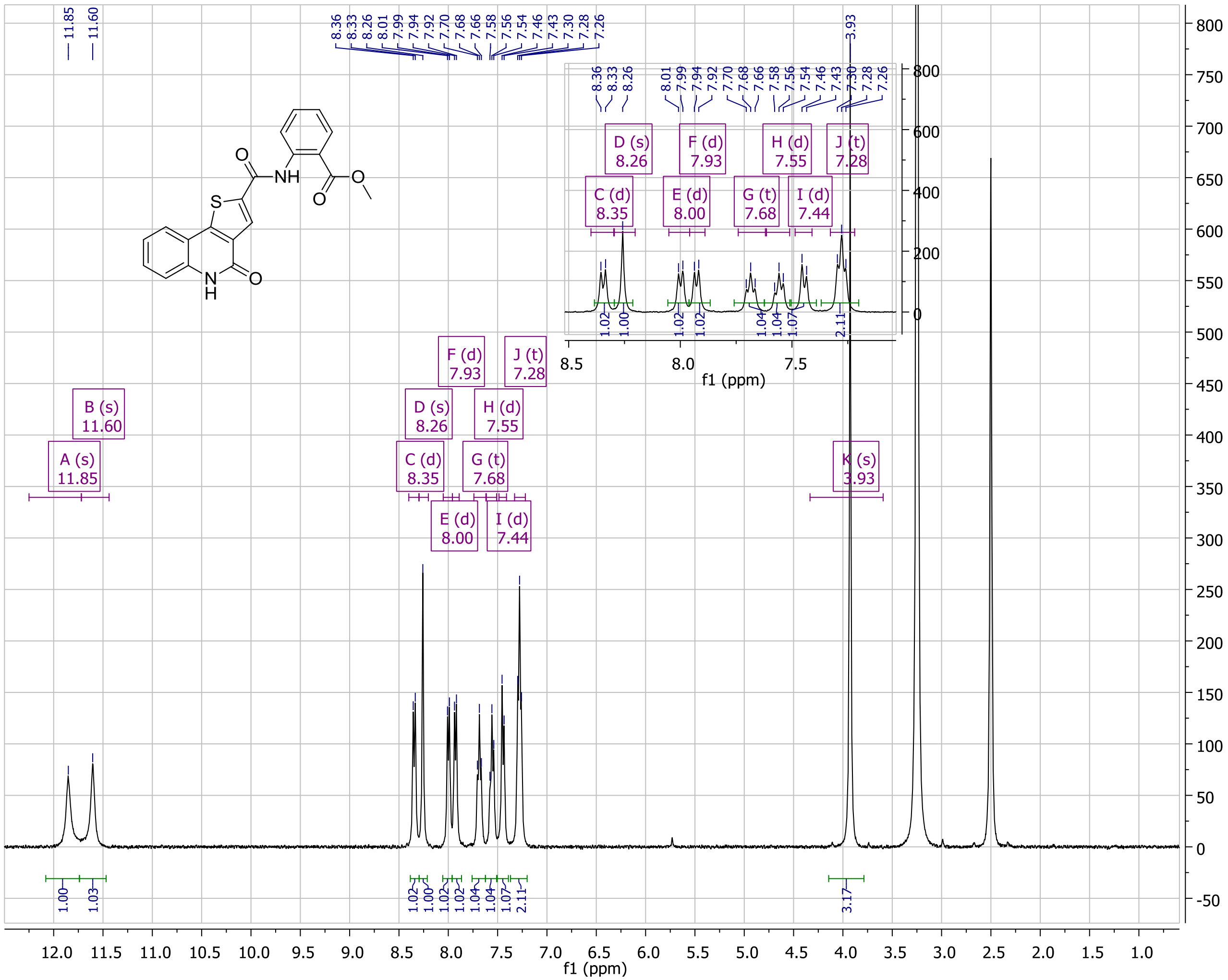
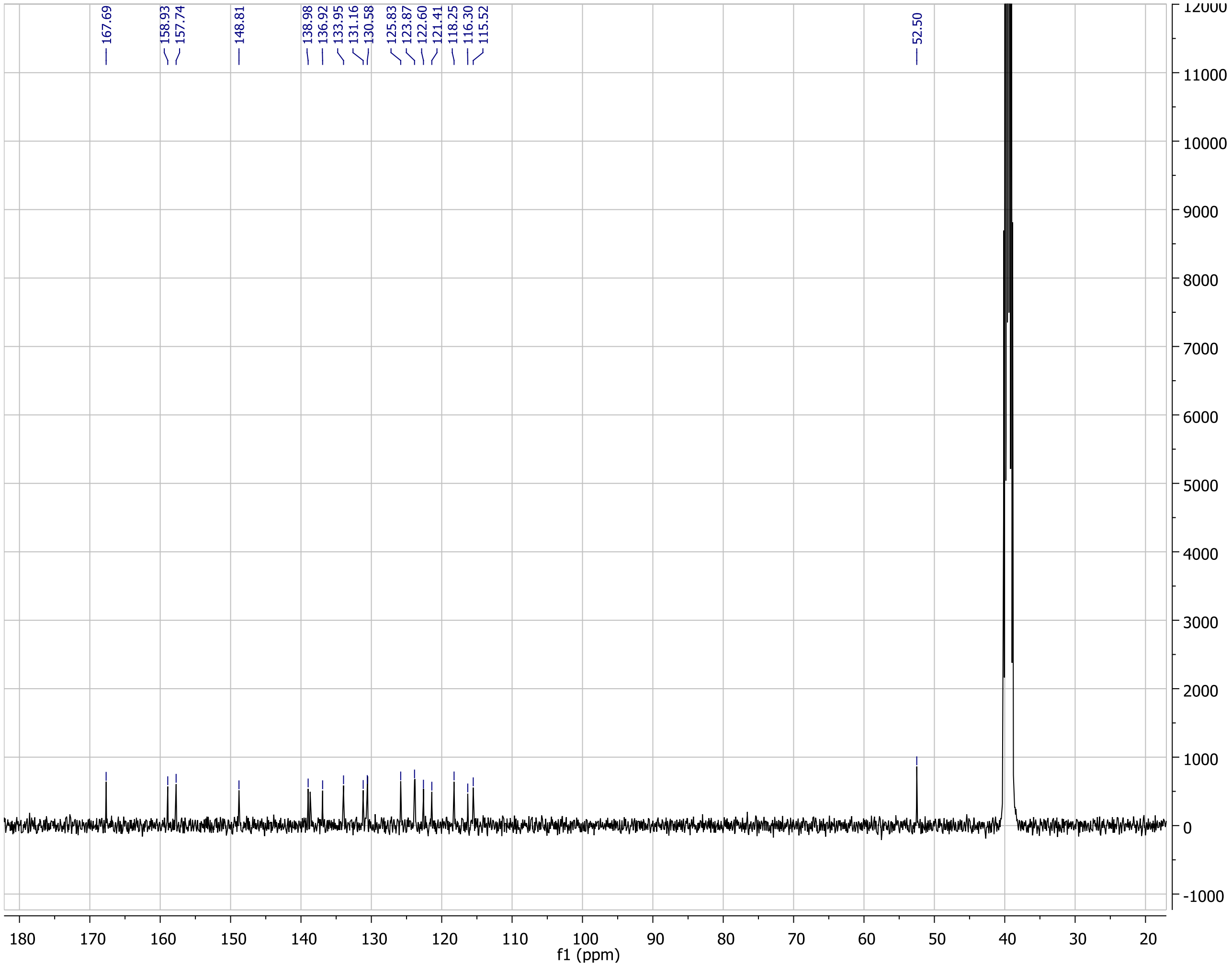
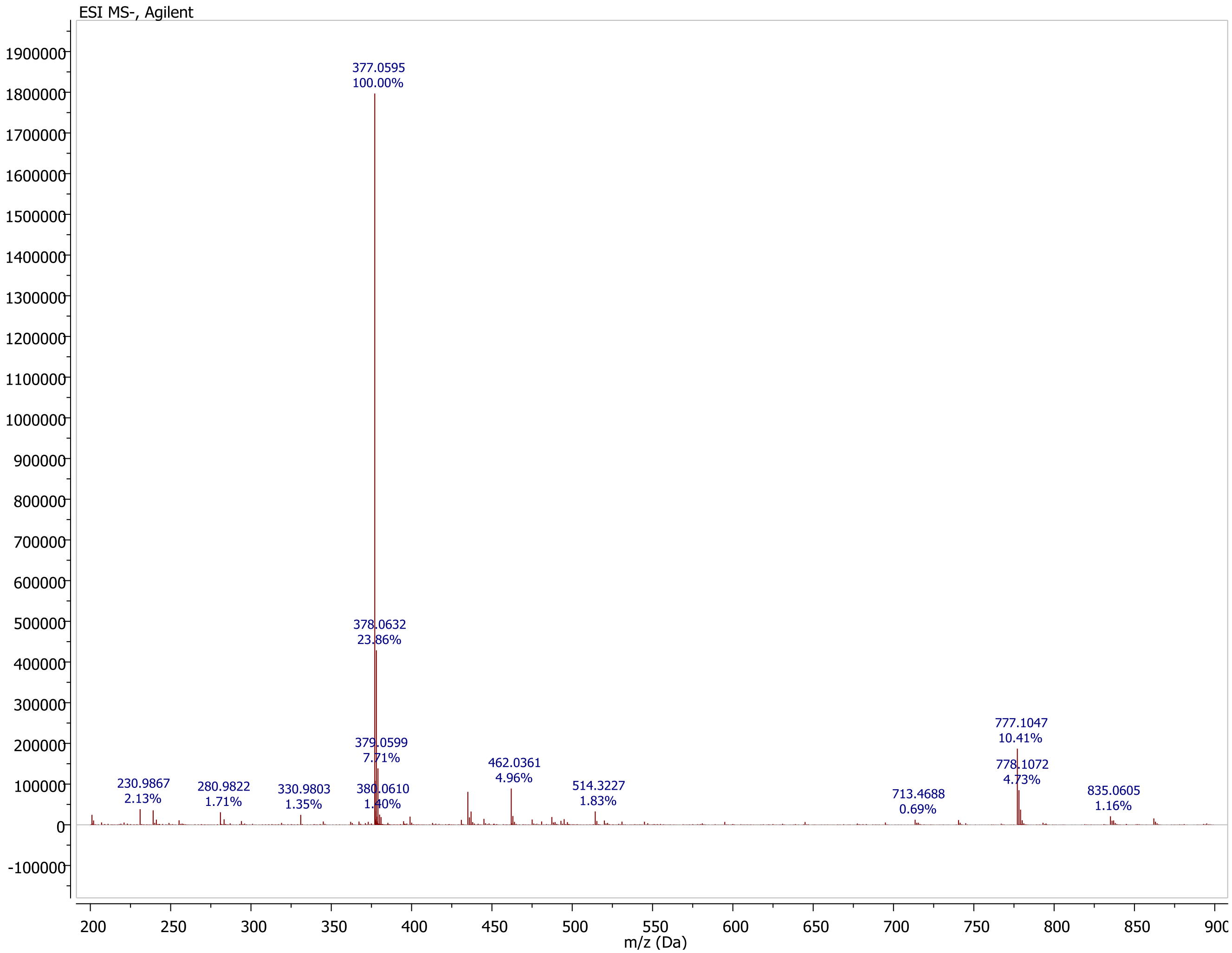


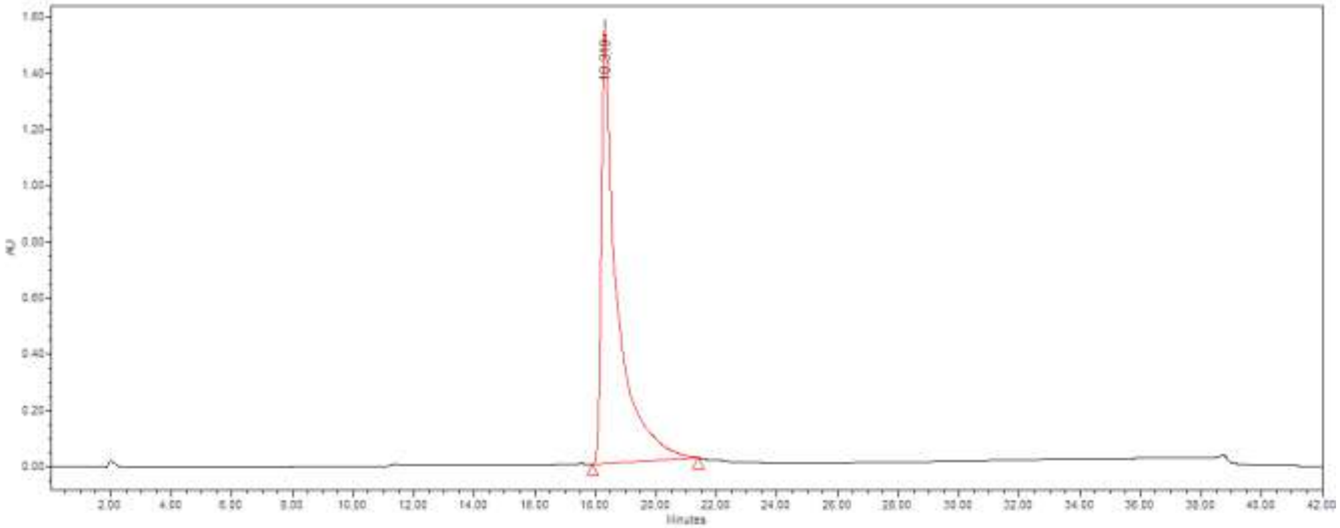
^{1}H NMR spectrum of 25r



^{13}C NMR spectrum of 25r



HRMS (TOF) of 25r



HPLC scan of 25r

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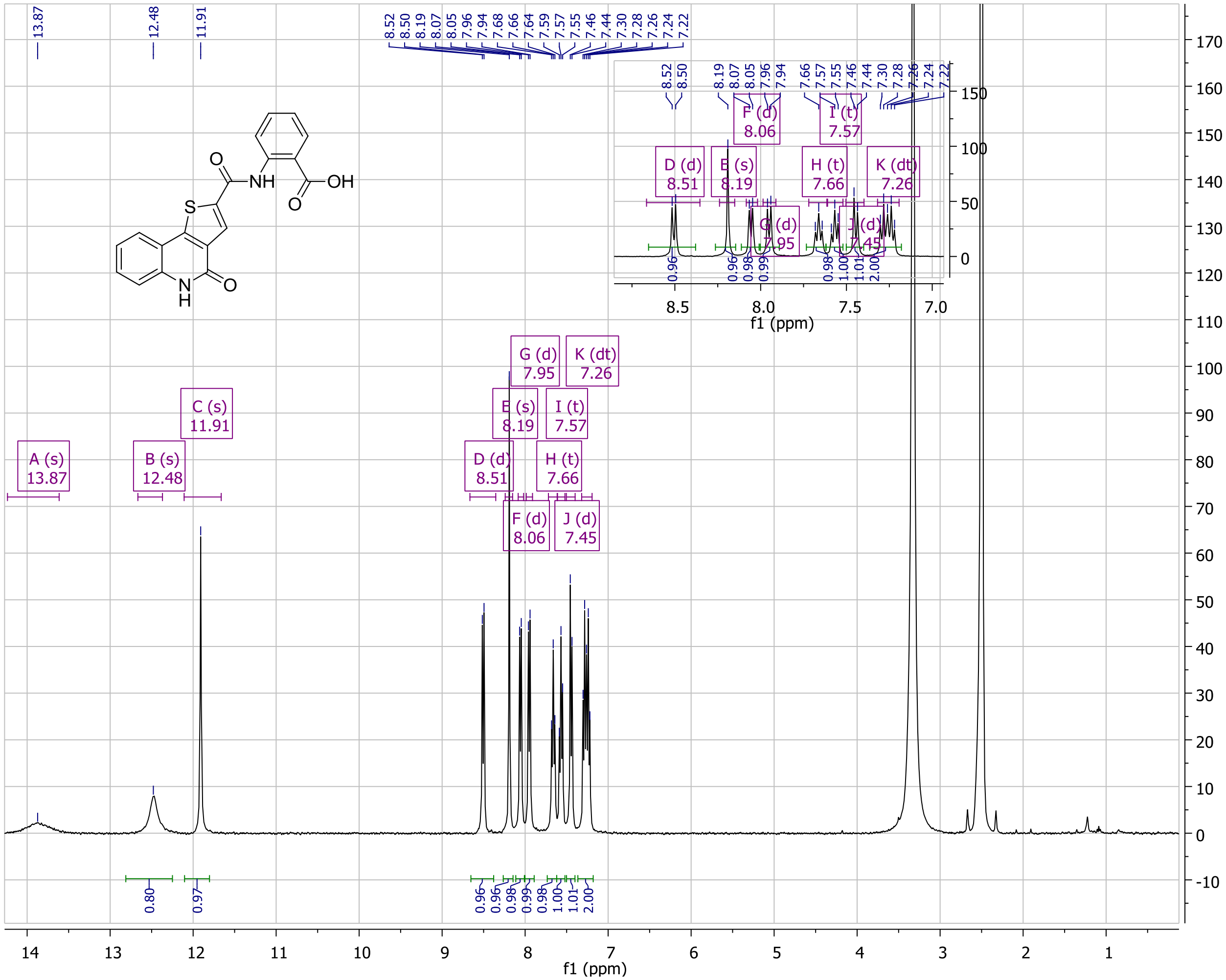

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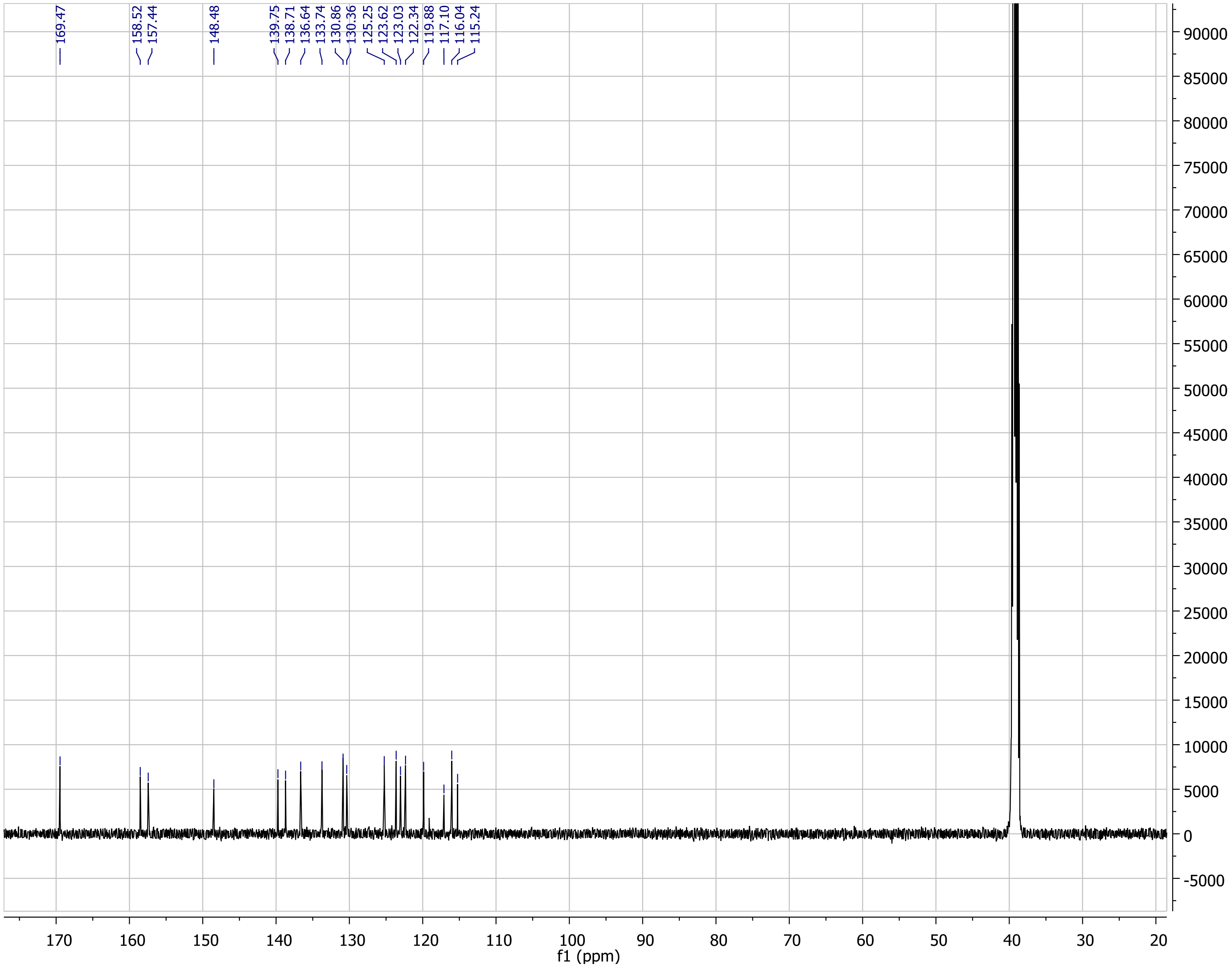

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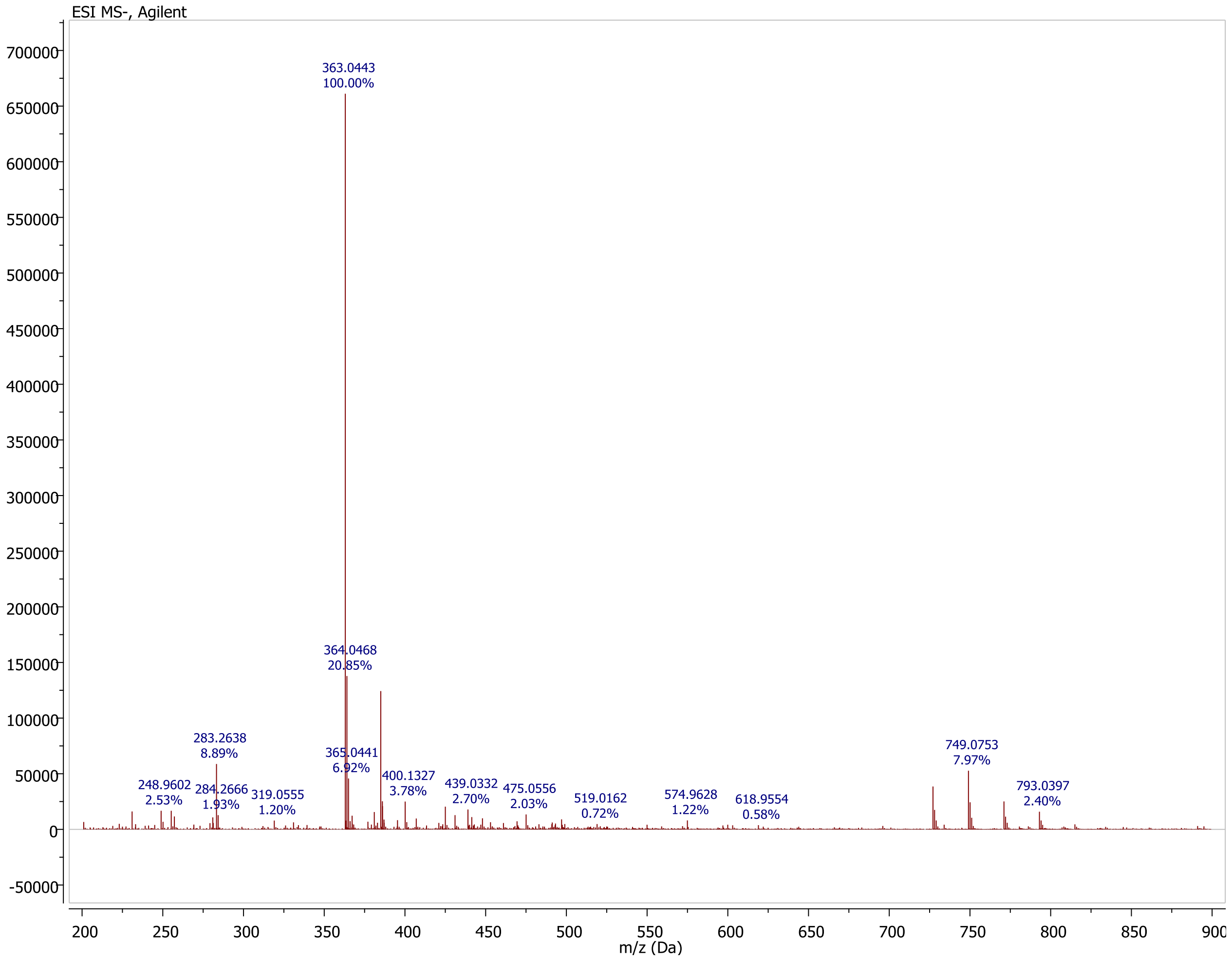
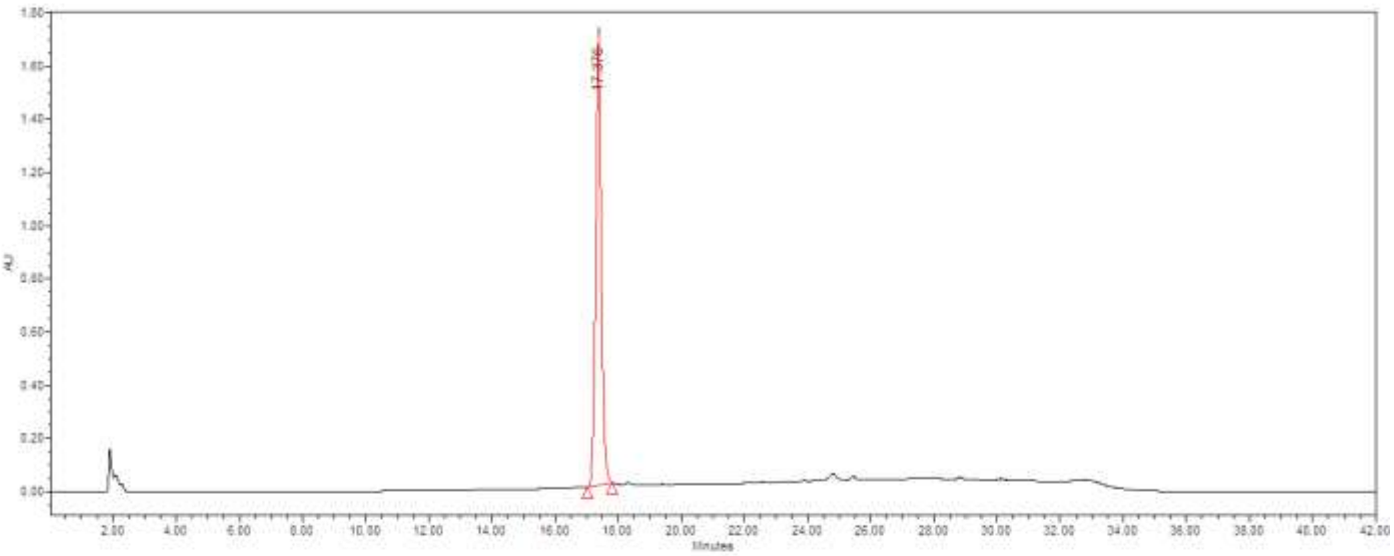
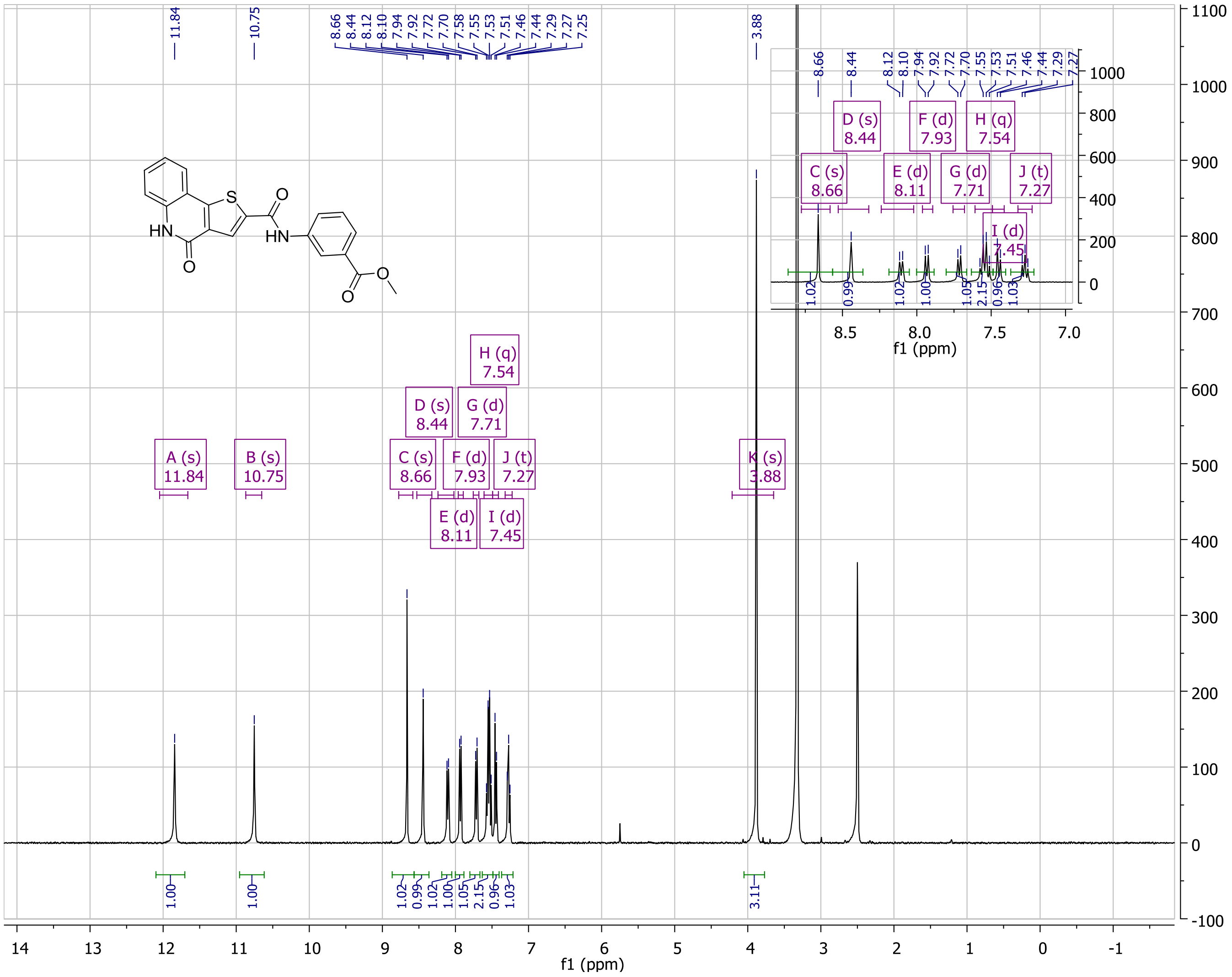


HRMS (TOF) of 25r

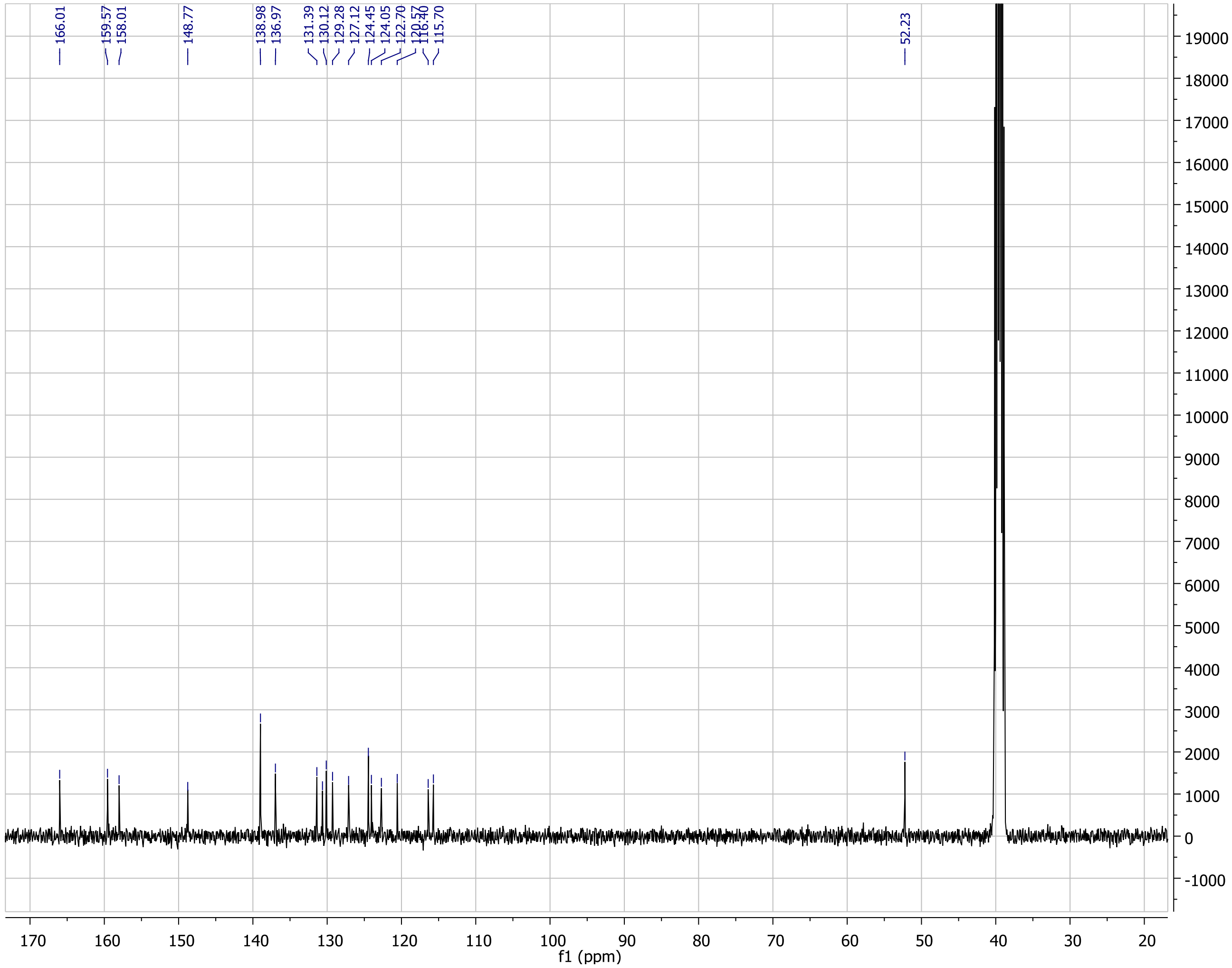


HPLC scan of 25s

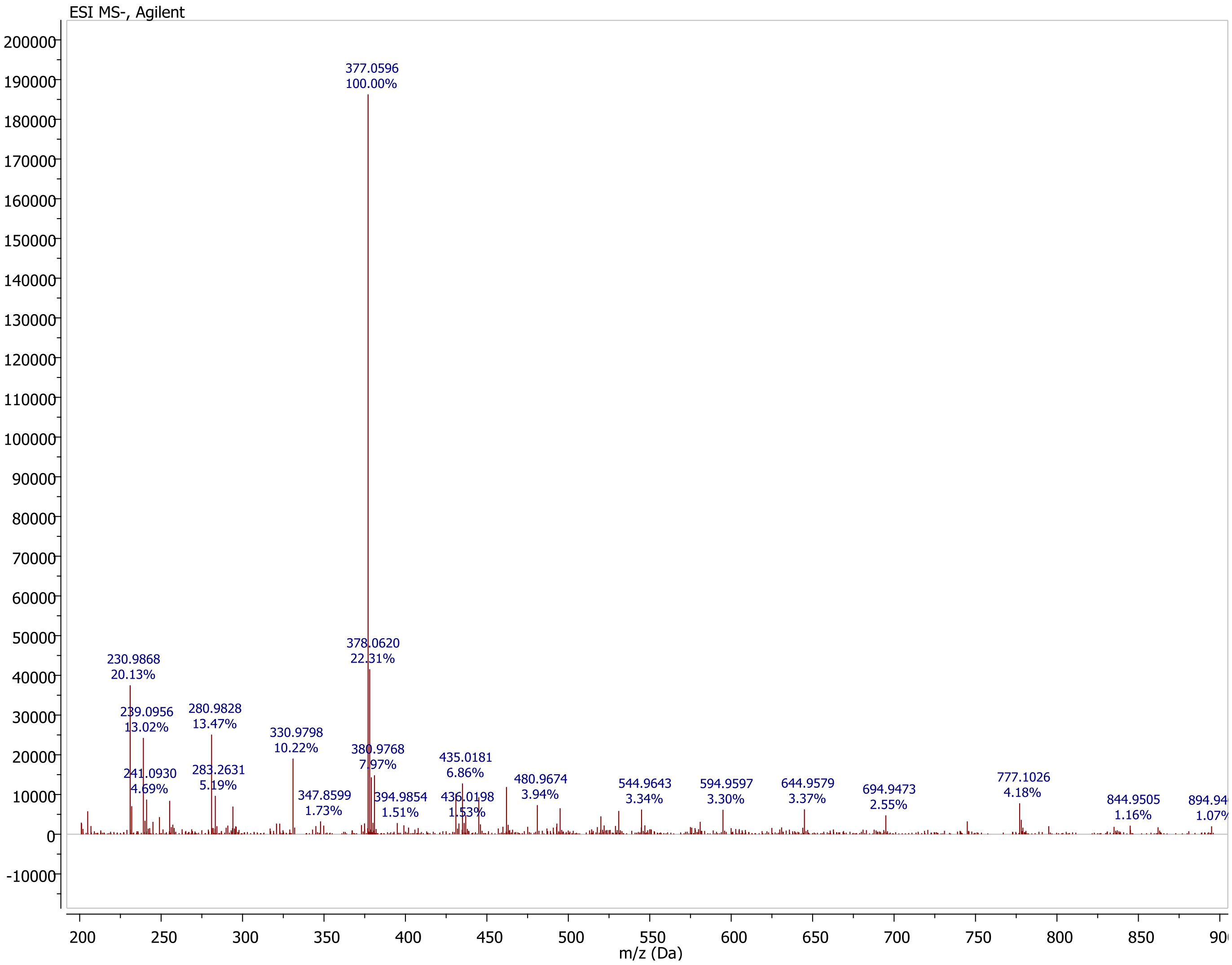
Retention time (min)	% Area
17.376	100



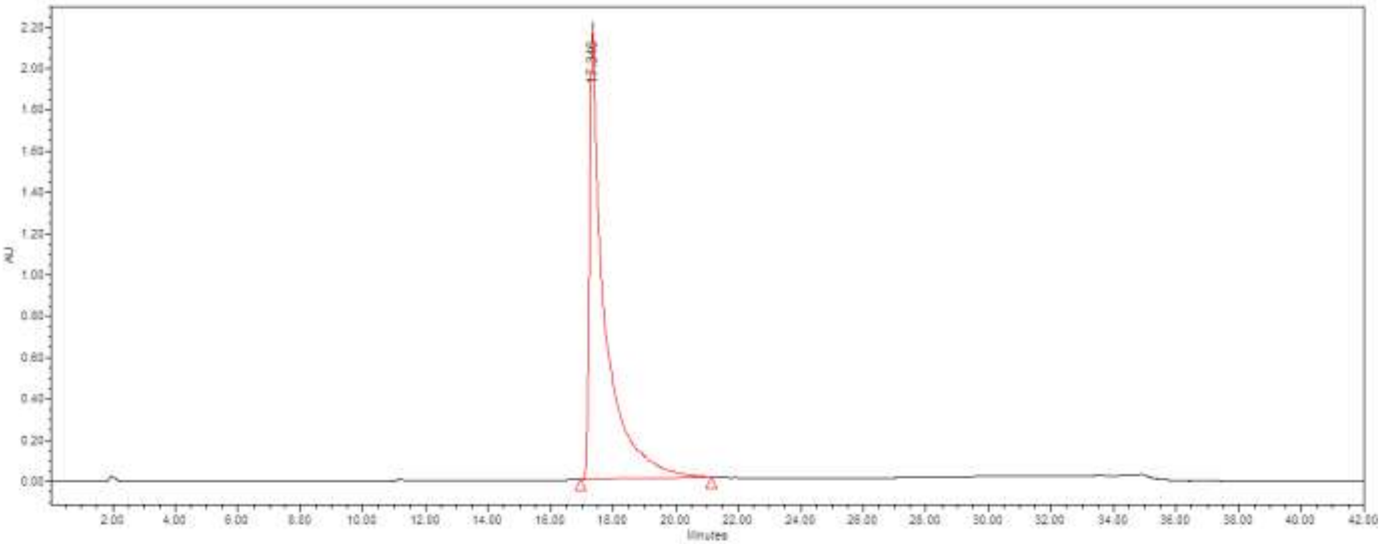
^{1}H NMR spectrum of 25t



^{13}C NMR spectrum of 25t

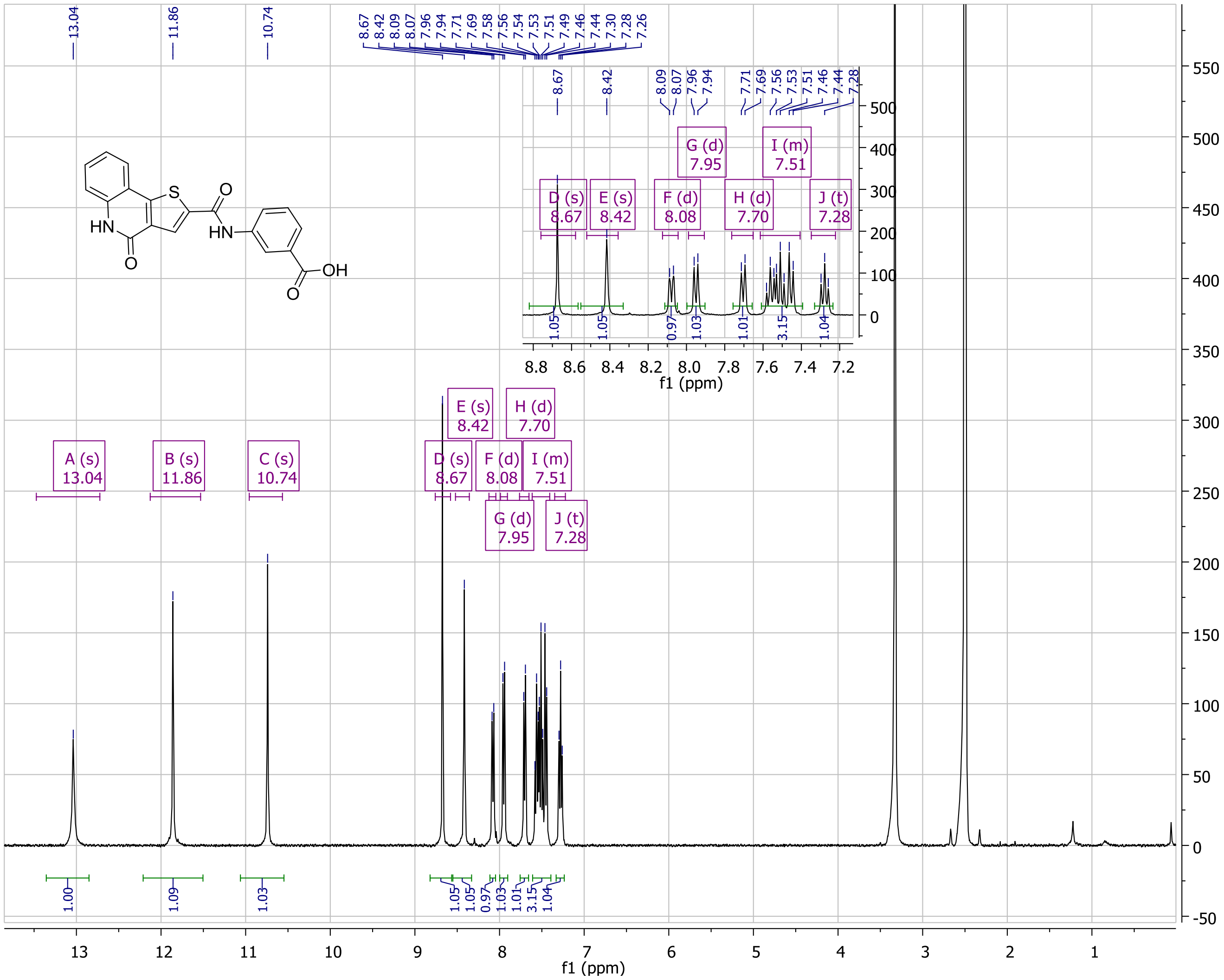


HRMS (TOF) of 25t

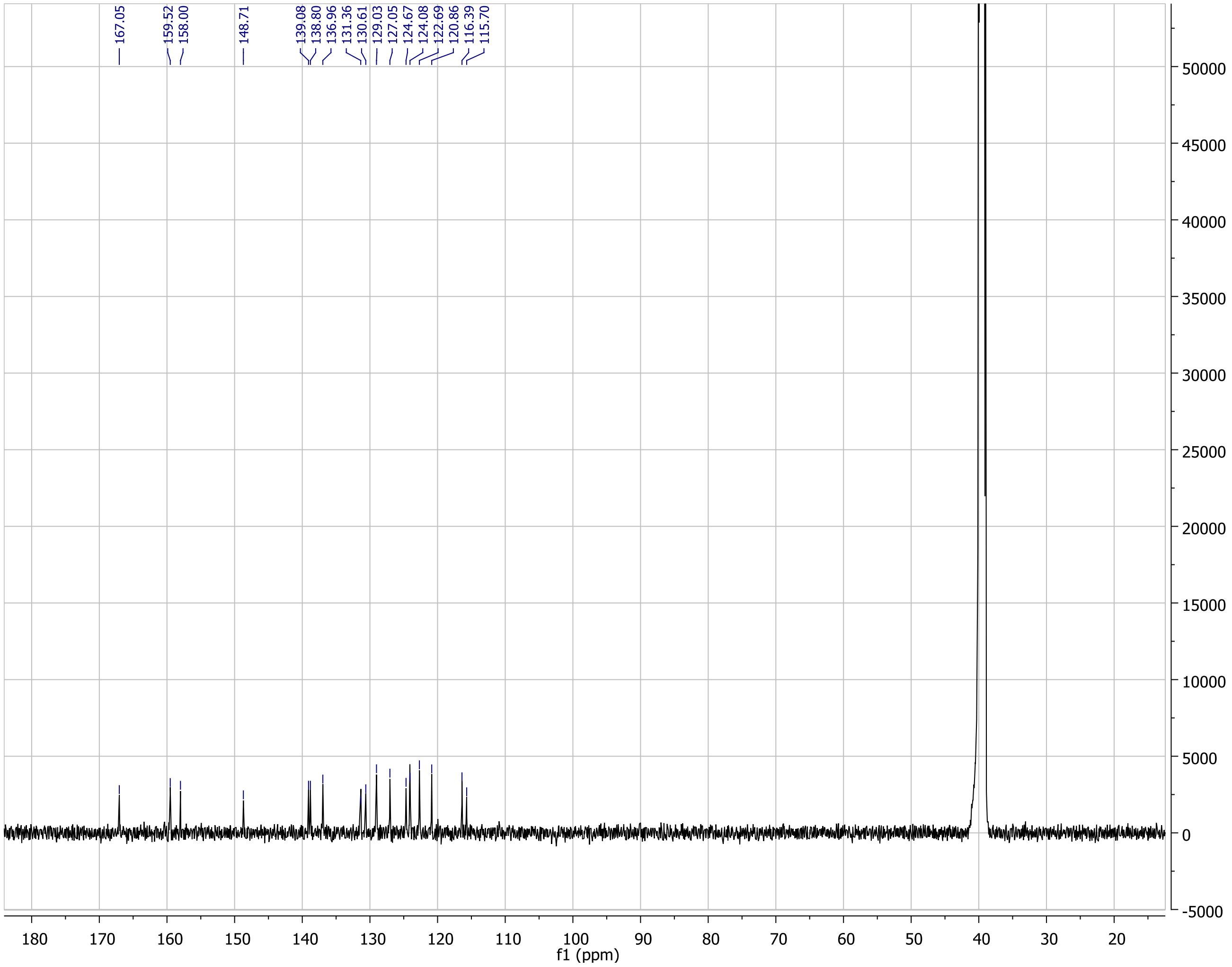


HPLC scan of 25t

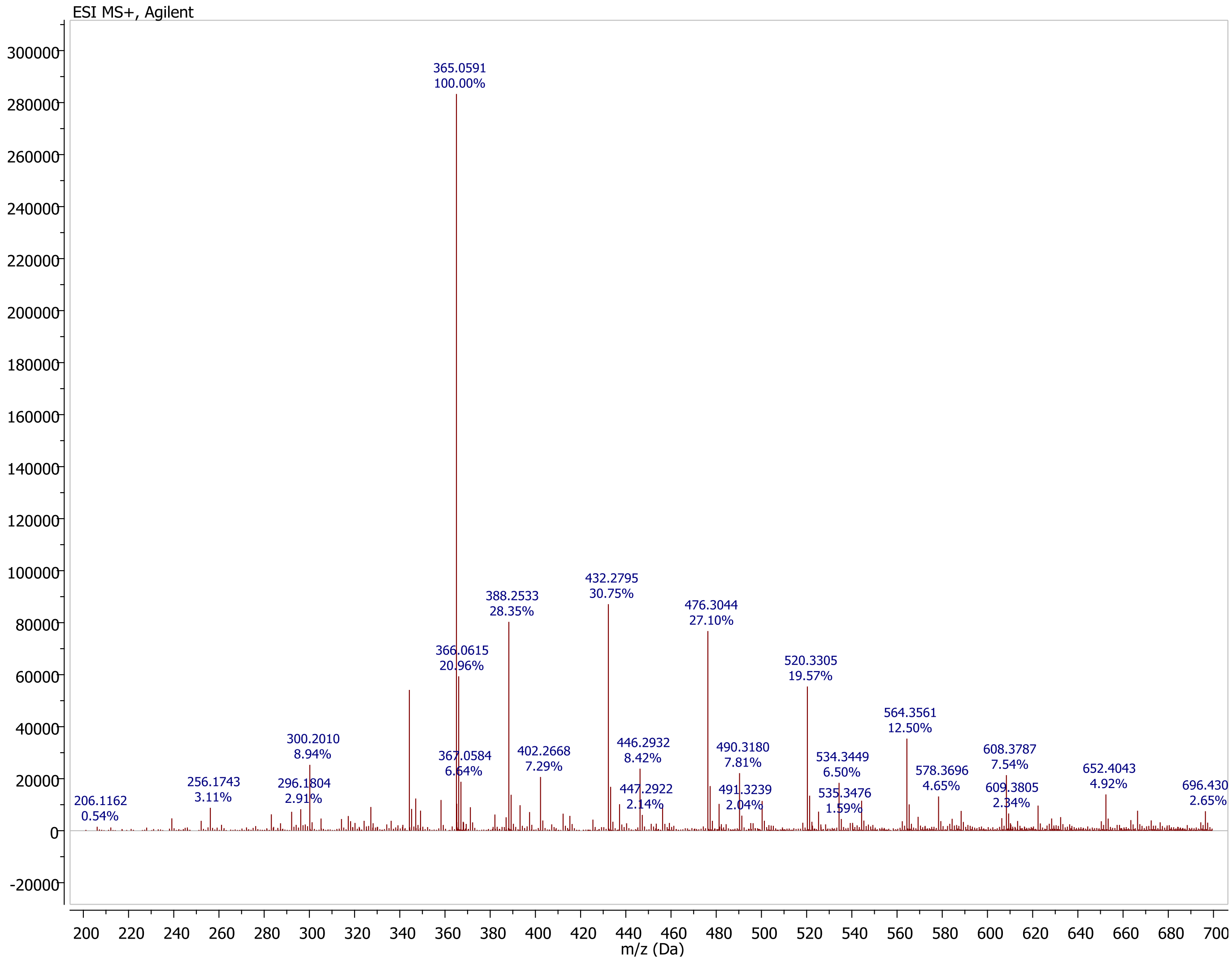
Retention time (min)	% Area
17.346	100



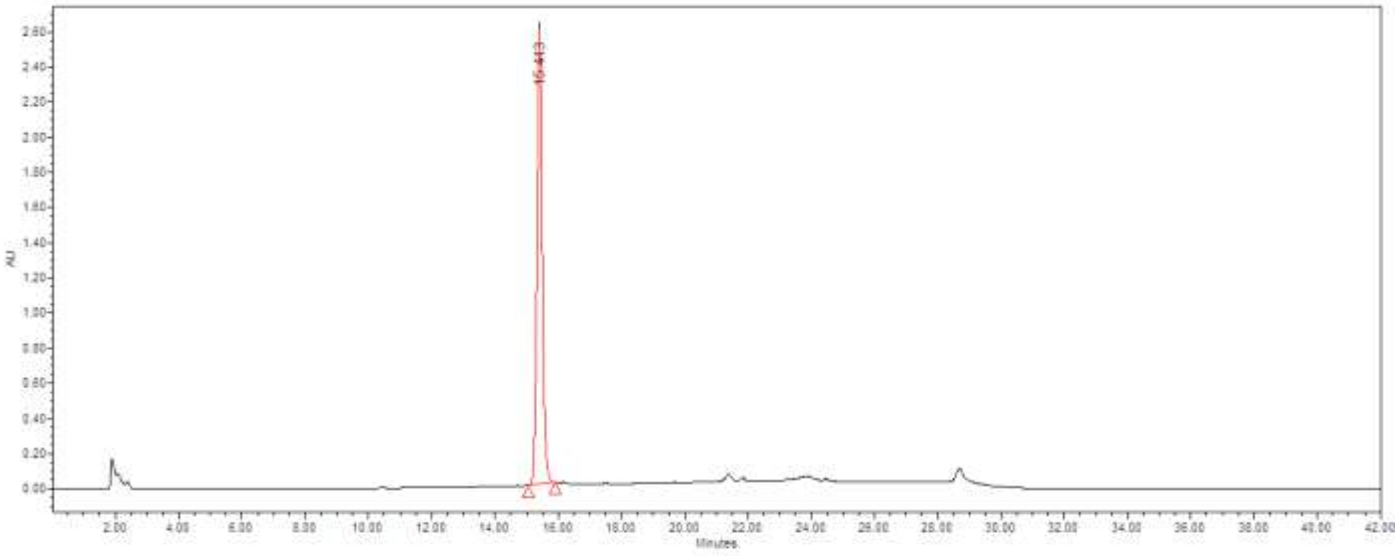
^{1}H NMR spectrum of 25u



^{13}C NMR spectrum of 25u

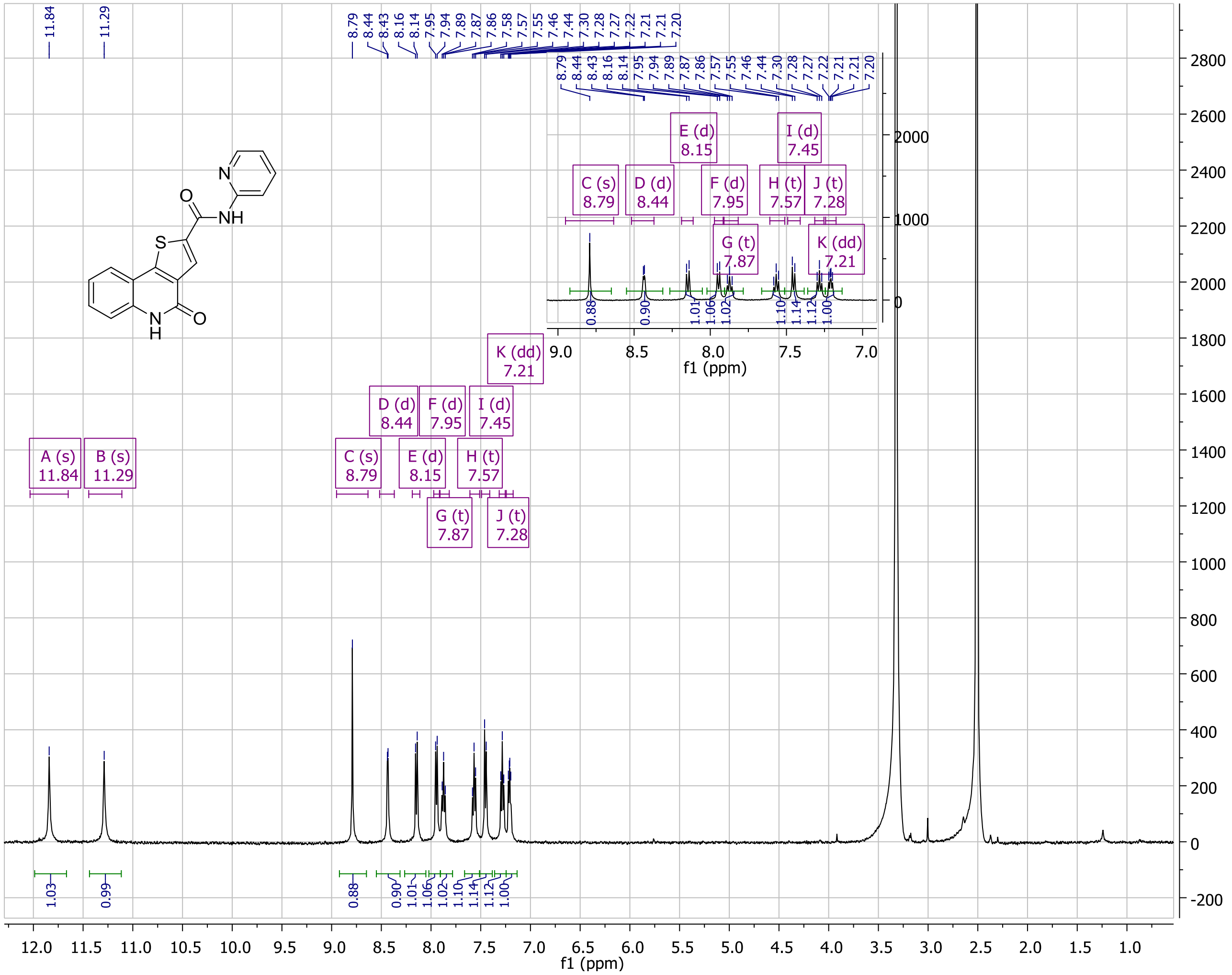


HRMS (TOF) of 25u

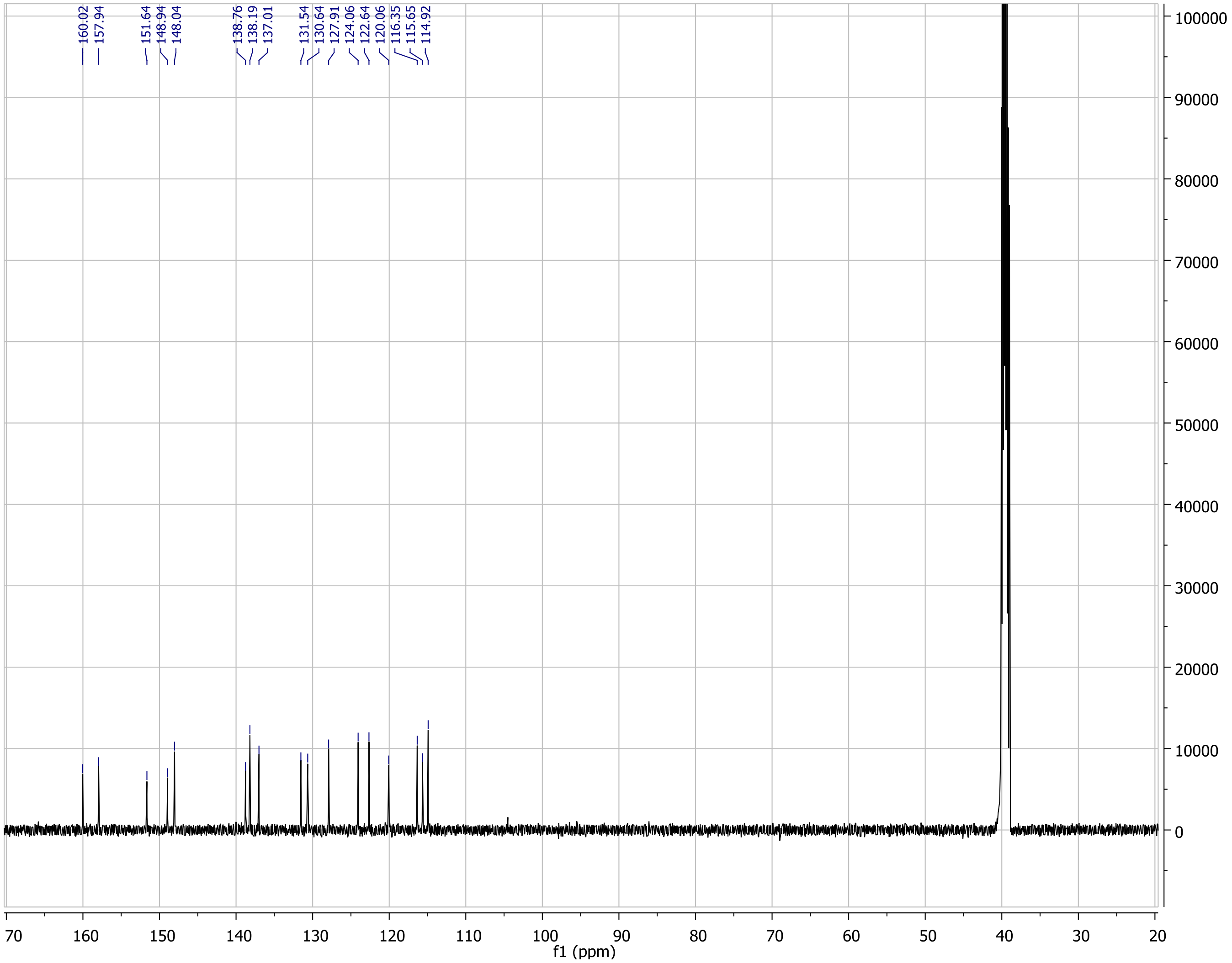


HPLC scan of 25u

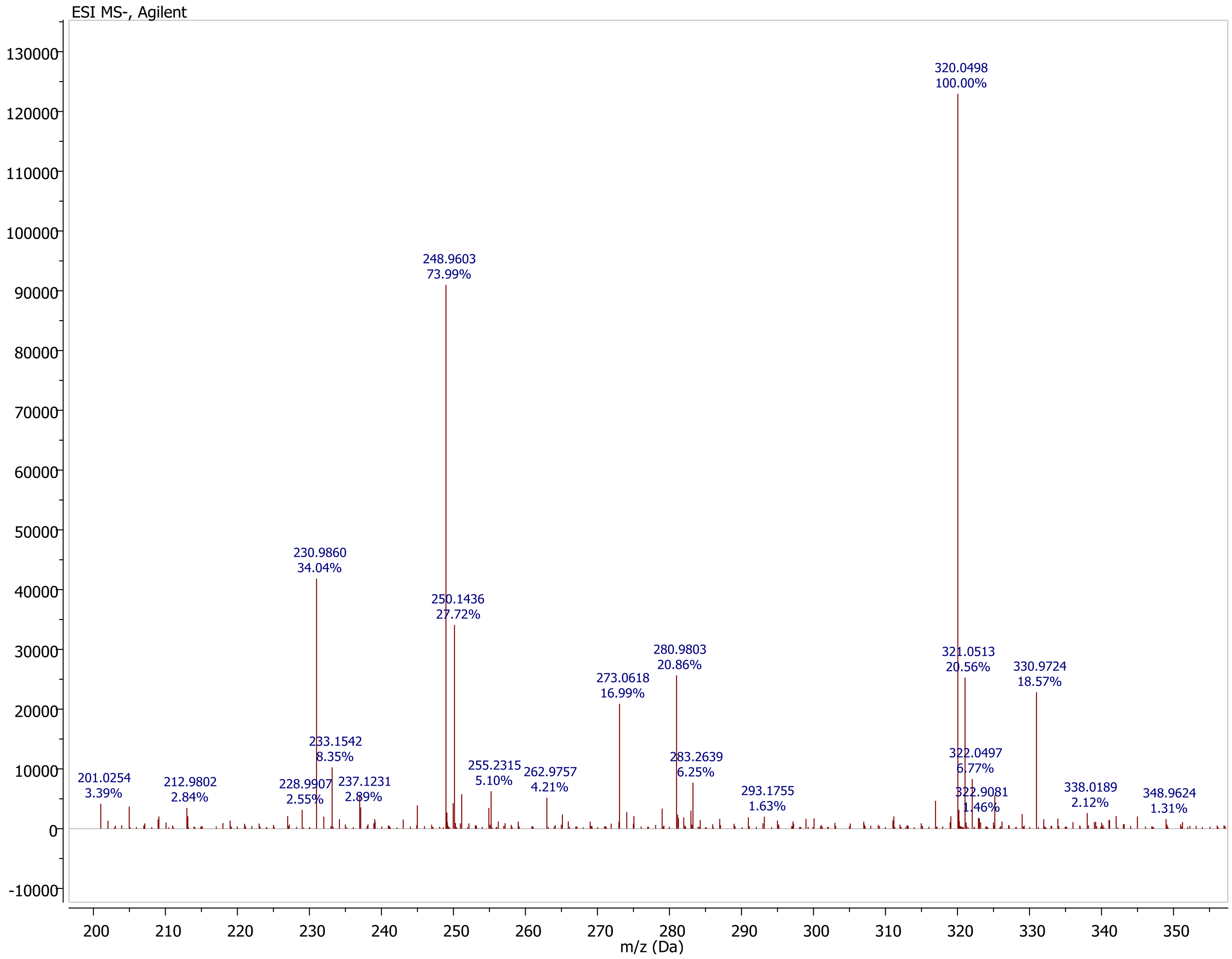
Retention time (min)	**% Area**
15.413	100



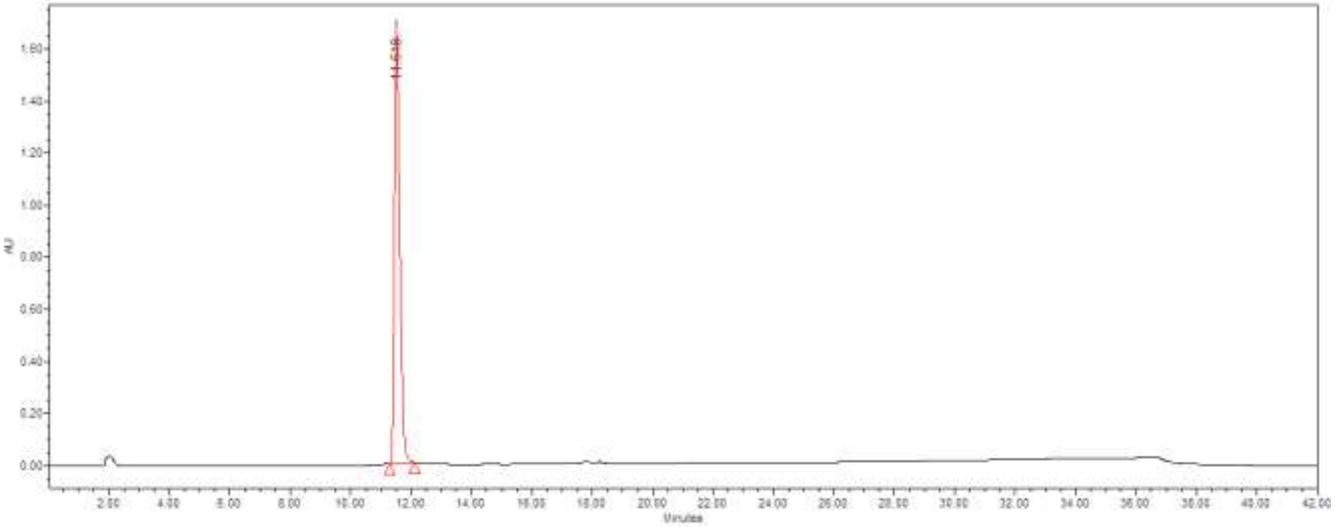
^{1}H NMR spectrum of 25v



^{13}**C NMR spectrum of 25v**

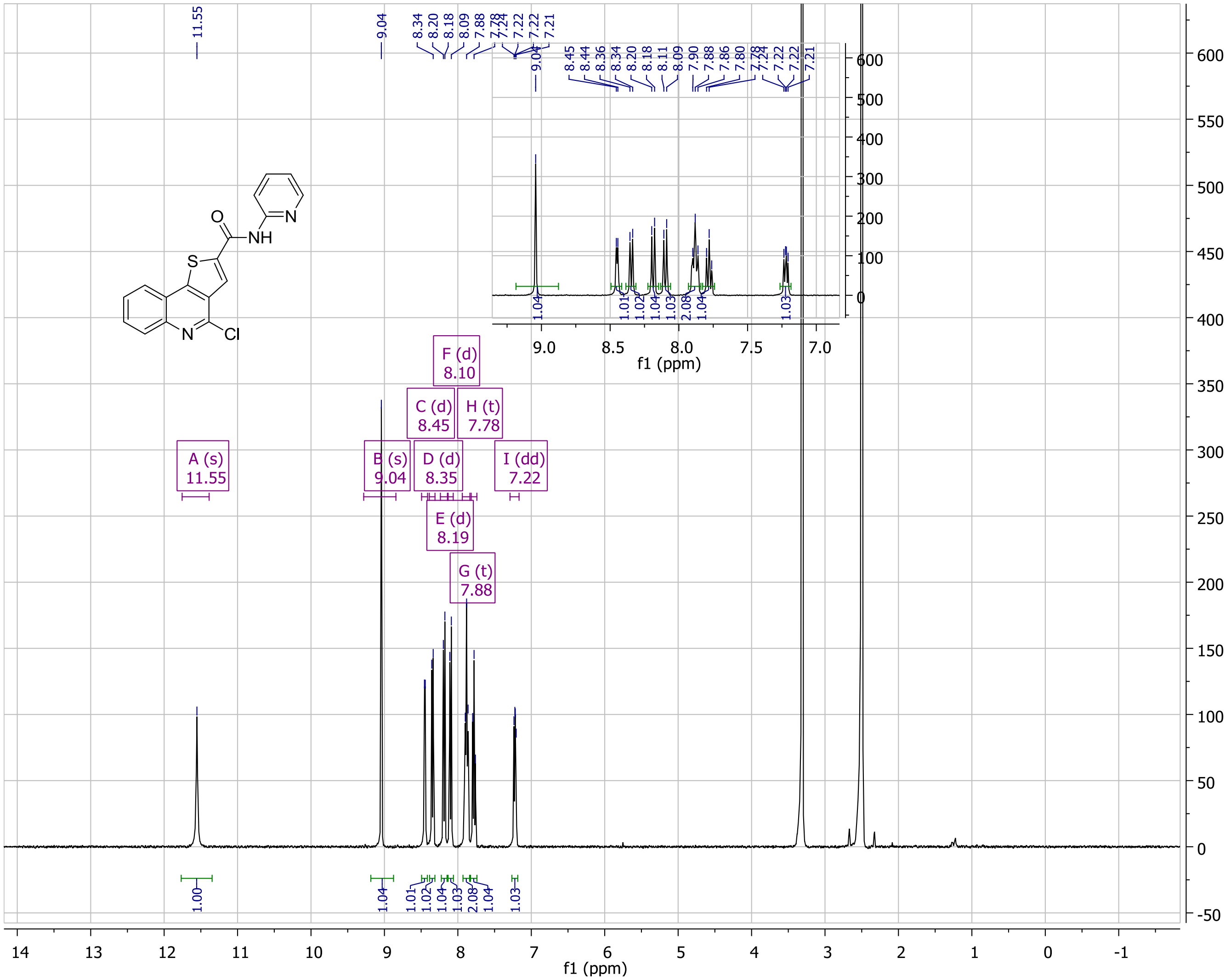


HRMS (TOF) of 25v

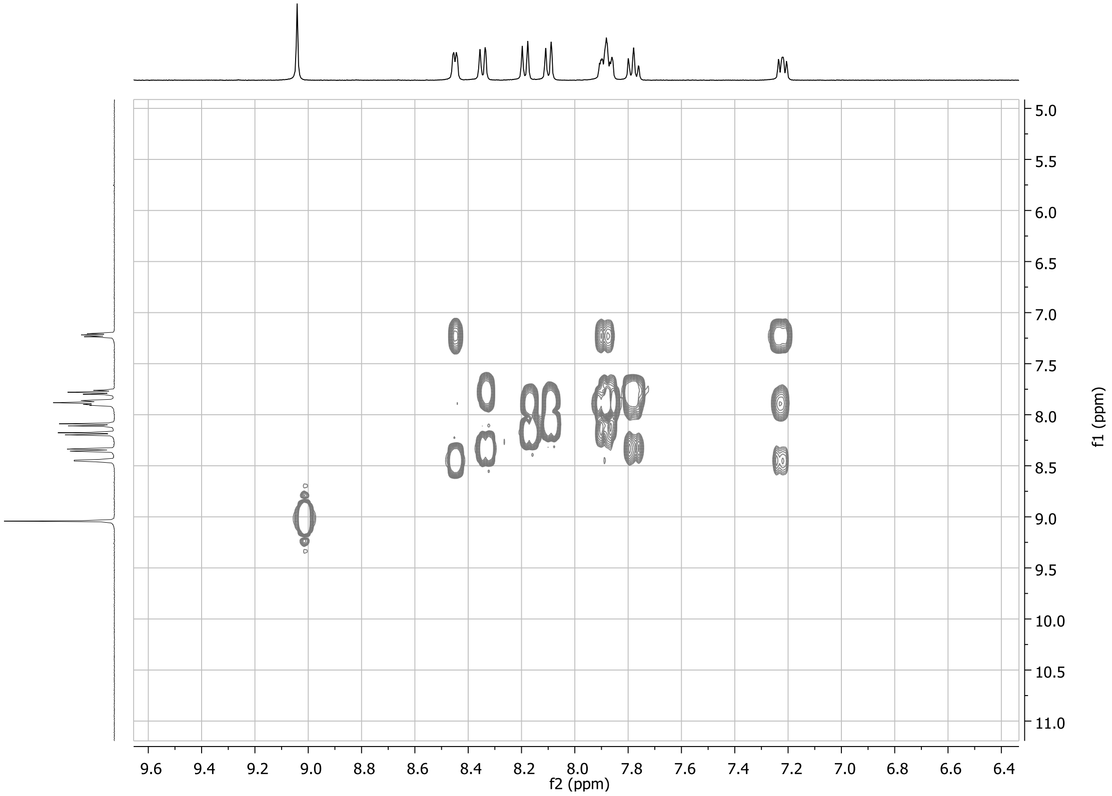


HPLC scan of 25v

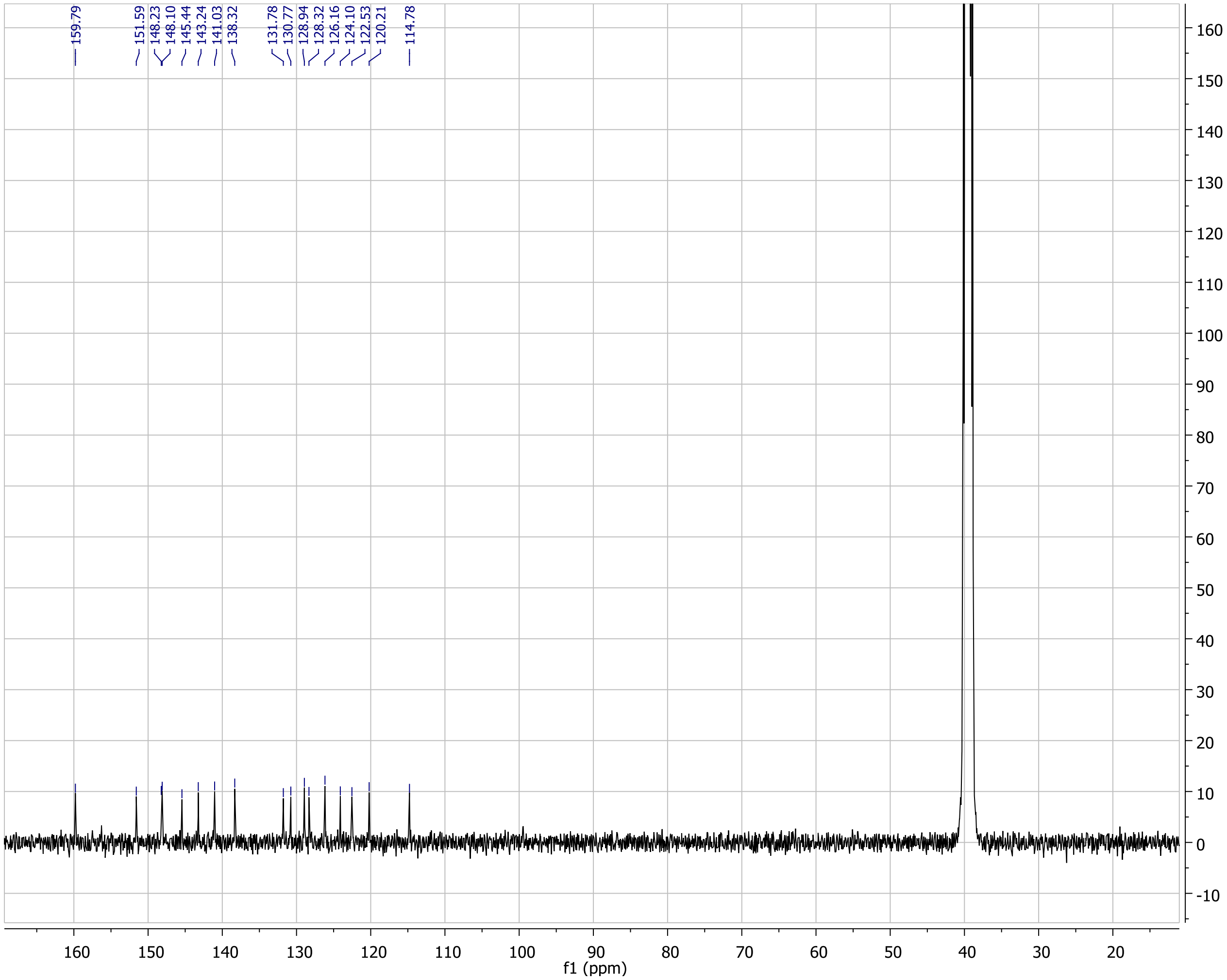
Retention time (min)	**% Area**
11.518	100



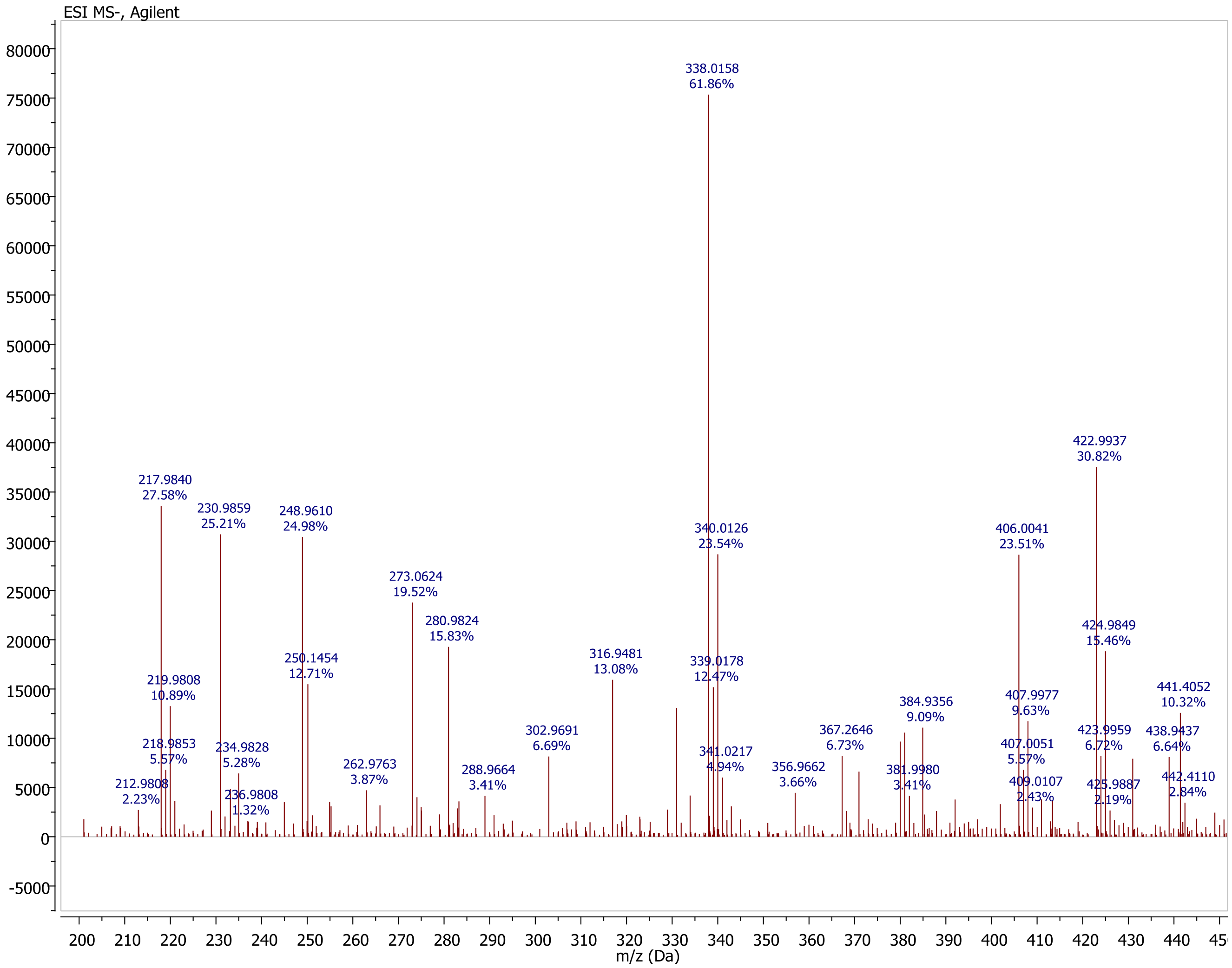
^{1}H NMR spectrum of 25w



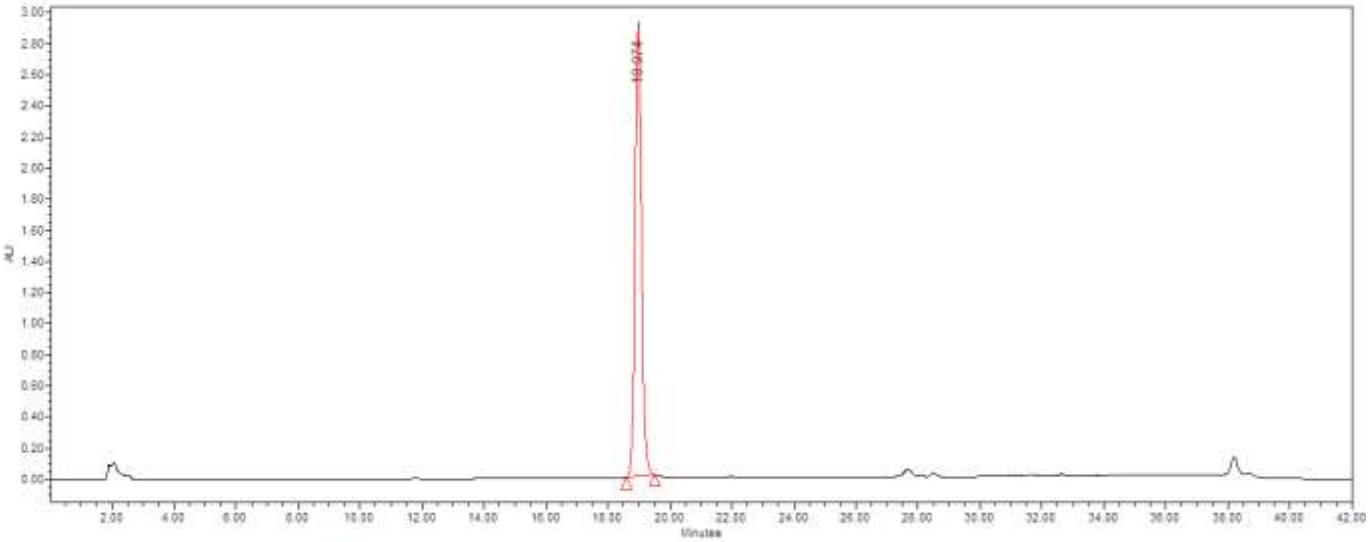
COSY spectrum of 25w



^{13}C NMR spectrum of 25w

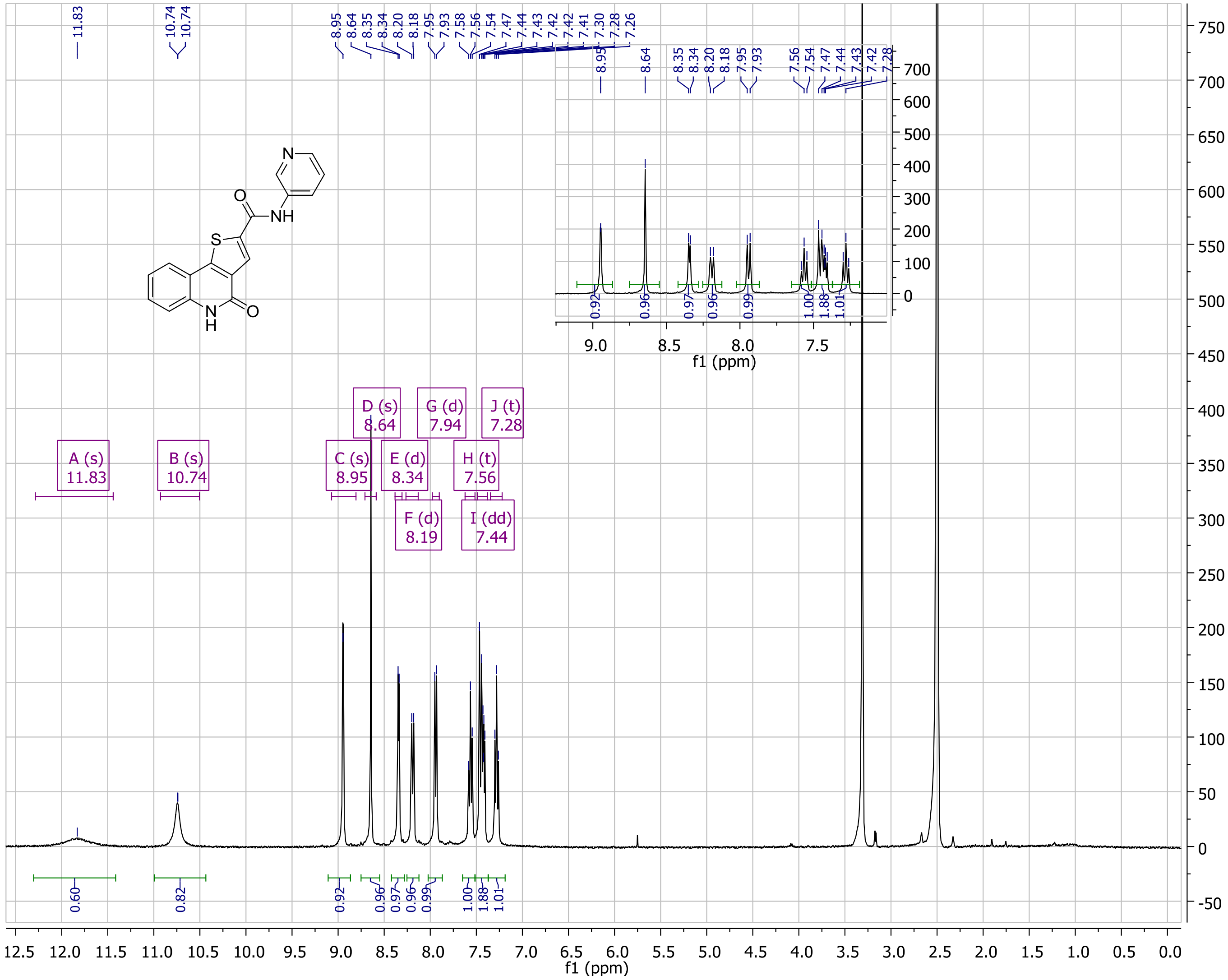


HRMS (TOF) of 25w

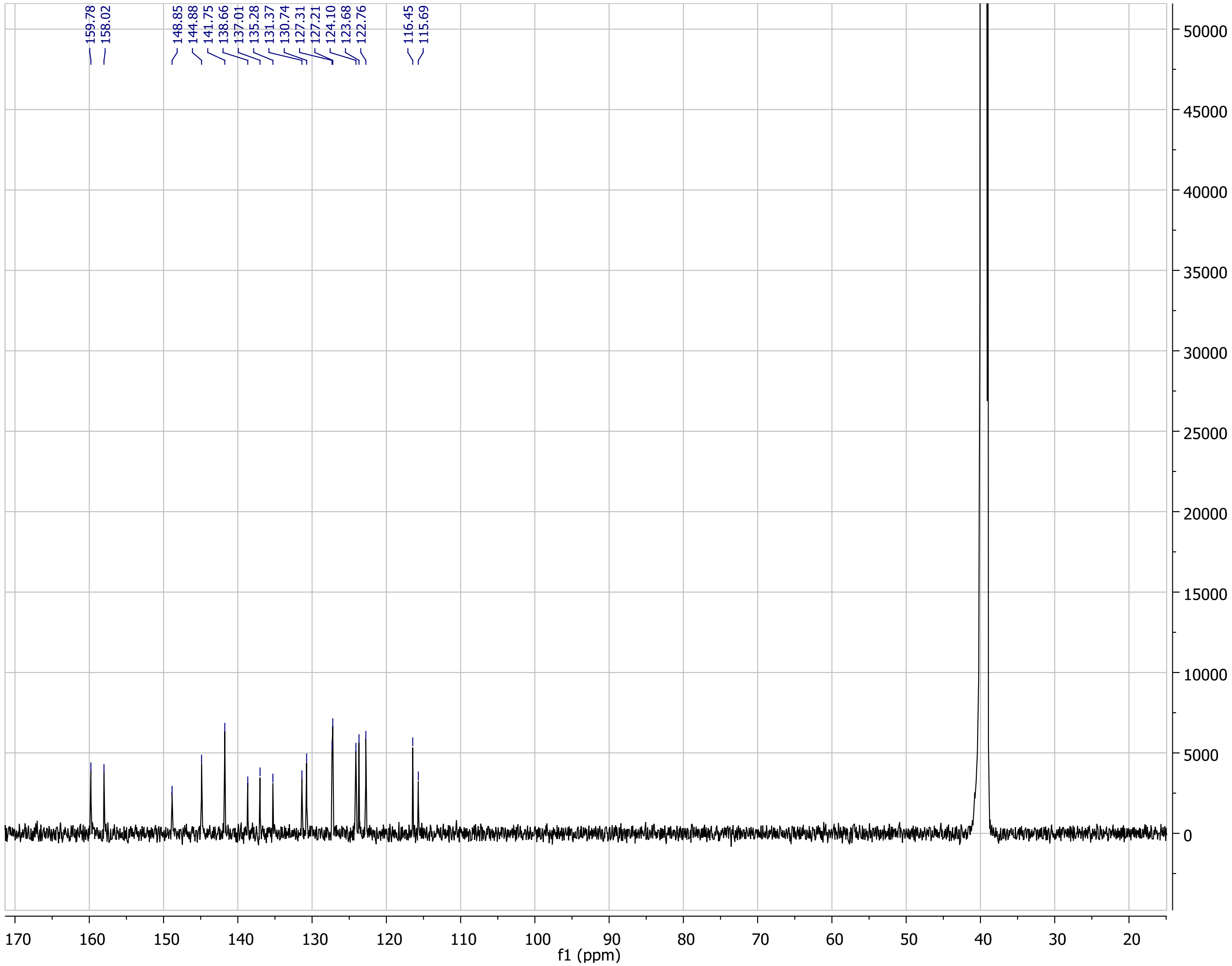


HPLC scan of 25w

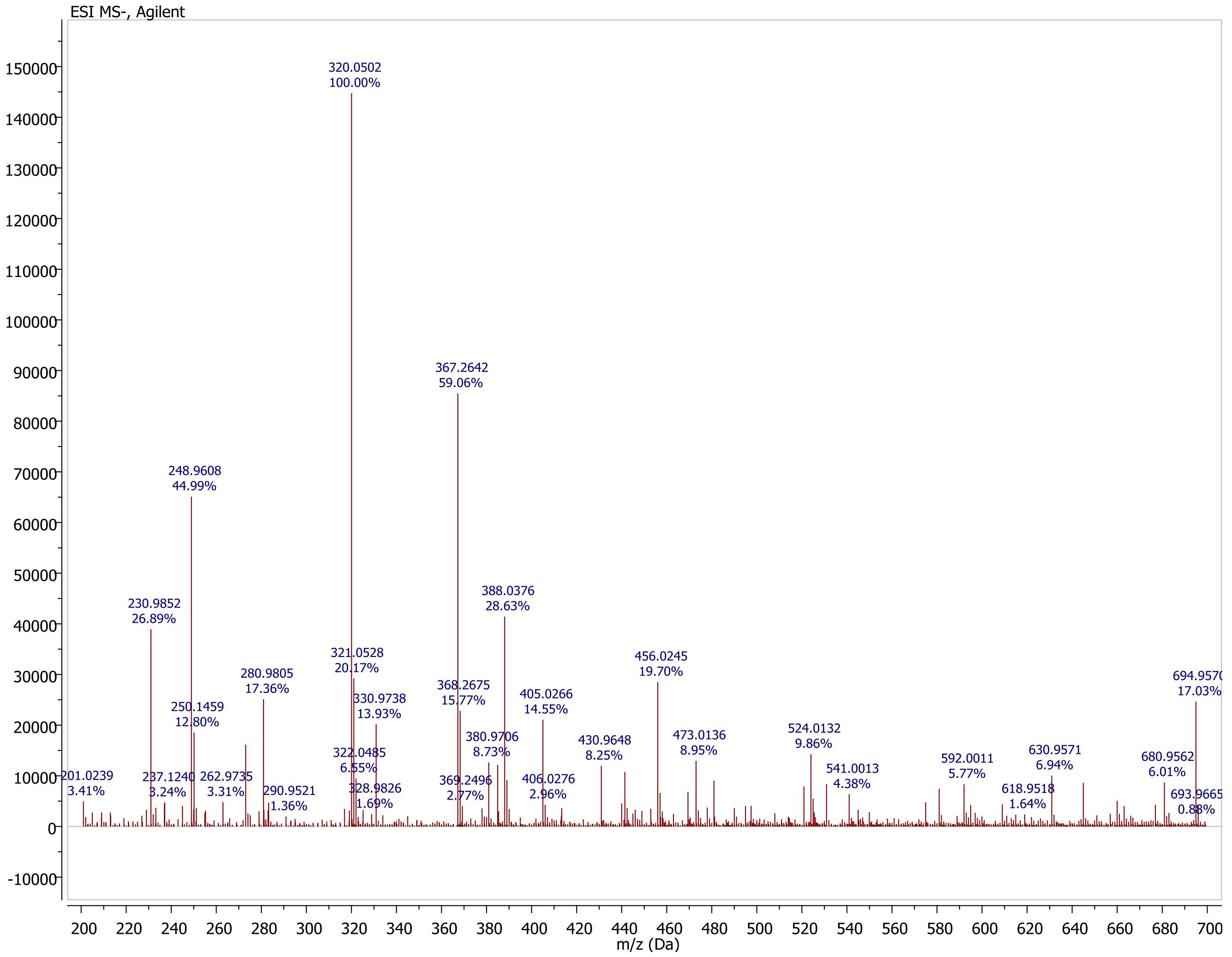
Retention time (min)	% Area
18.974	97.52



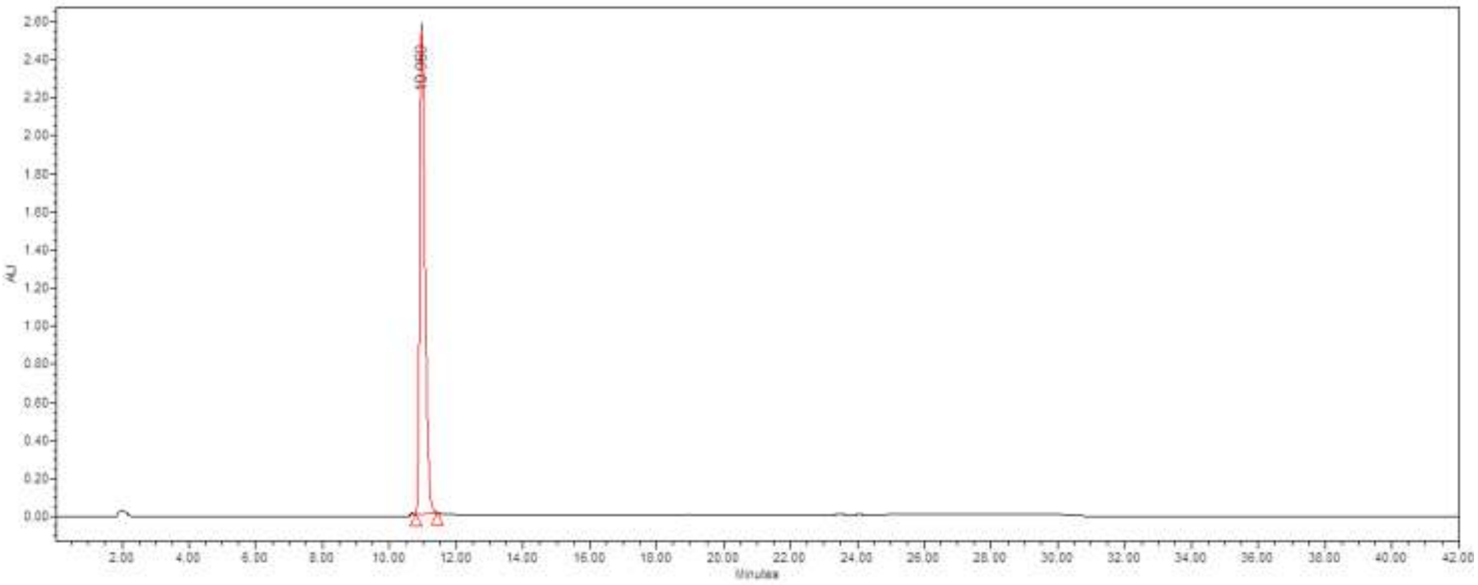
^{1}H NMR spectrum of 25x



^{13}C NMR spectrum of 25x



HRMS (TOF) of 25x



HPLC scan of 25x

Retention time (min)	**% Area**
10.969	100