Supplementary Information

Highly selective PI4K IIIβ inhibitors and structural insight into their mode of action

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Contents

Supplementary Results

Elemental analyses

Supplementary Table 1.

Compound	Formula	Calculated (%)			Found (%)		
		C	**H**	**N**	**C**	**H**	**N**
2a	$C_{22}H_{29}N_5O_3$	64.21	7.10	17.02	64.28	7.23	16.85
2b	$C_{23}H_{31}N_5O_3$	64.92	7.34	16.46	64.73	7.29	16.64
2c	$C_{20}H_{25}N_5O_3 \cdot 0.25H_2O$	61.92	6.63	18.05	61.92	6.62	17.74
2d	$C_{18}H_{21}N_5O_3 \cdot 0.33H_2O$	59.82	6.04	19.38	59.76	6.04	19.18
2e	$C_{26}H_{29}N_5O_3$	67.95	6.36	15.24	67.77	6.36	15.00
2f	$C_{18}H_{22}N_4O_3$	63.14	6.48	16.36	63.05	6.34	16.12
2g	$C_{20}H_{24}N_4O_3$	65.20	6.57	15.21	65.10	6.60	14.88
2h	$C_{20}H_{24}N_4O_3 \cdot 0.33EtOAc$	73.24	6.74	12.44	73.48	6.74	12.64
2i	$C_{16}H_{18}N_4O_2$	64.41	6.08	18.78	64.17	6.20	18.49
2j	$C_{18}H_{23}N_5O_2$	63.32	6.79	20.51	63.01	6.50	20.19
2k	$C_{19}H_{25}N_5O_2$	64.20	7.09	19.70	63.90	7.41	19.35
2l	$C_{22}H_{27}N_5O_3$	64.53	6.65	17.10	64.13	6.75	16.73
3	$C_{22}H_{29}N_5O_3$	64.21	7.10	17.02	64.13	7.30	16.67
4	$C_{25}H_{33}N_5O_3$	66.50	7.37	15.51	66.20	7.48	15.23
5	$C_{27}H_{31}N_5O_3$	68.48	6.60	14.79	68.23	6.72	14.53
6	$C_{19}H_{24}N_6O_3 \cdot 0.5CH_3COCH_3$	59.55	6.58	20.33	59.39	6.36	20.71
7	$C_{25}H_{48}N_6O_3 \cdot 0.66EtOAc$	63.99	6.47	16.18	63.75	6.33	16.68
8	$C_{21}H_{27}N_5O_3$	61.00	6.58	16.94	60.74	6.59	16.54
9	$C_{19}H_{25}N_5SO_3$	54.40	6.01	16.69	54.34	6.06	16.25
10	$C_{20}H_{25}ClN_4O_4 \cdot H_2O$	54.73	6.20	12.97	54.59	6.34	12.65
11	$C_{21}H_{28}ClN_5O_3$	58.13	6.50	16.14	57.75	6.45	15.90
12	$C_{16}H_{17}N_3SO_2$	60.93	5.43	13.32	60.66	6.01	13.15
13	$C_{22}H_{28}N_4SO_3$	61.66	6.59	13.07	61.39	6.61	12.76
14	$C_{26}H_{28}N_4SO_3$	65.52	5.92	11.76	65.24	5.92	11.46
15	$C_{22}H_{28}N_4O_4 \cdot H_2O$	61.38	7.02	13.01	61.50	6.78	12.65
16	$C_{17}H_{17}BrN_2O_2$	56.52	4.74	7.75	56.29	4.89	7.57
17	$C_{22}H_{29}N_5O_3$	64.21	7.10	17.02	64.08	7.01	17.27
18	$C_{21}H_{28}N_6O_3$	61.15	6.84	20.37	60.88	6.75	20.15
23	$C_{13}H_{18}ClN_5O$	52.79	6.13	23.68	52.45	6.26	23.75
24	$C_{14}H_{21}N_5O$	61.07	7.69	25.43	60.50	7.82	25.21
25	$C_{14}H_{20}IN_5O$	41.91	5.02	17.45	42.16	5.40	17.18
26	$C_{13}H_{19}N_5O$	59.75	7.33	26.80	59.62	7.18	26.91
27	$C_{13}H_{18}IN_5O$	40.32	4.69	18.09	40.50	4.57	17.95
28	$C_{21}H_{27}N_5O_3$	63.46	6.85	17.62	63.15	6.98	17.77
29	$C_{13}H_{17}ClIN_5O$	37.03	4.06	16.61	36.75	4.18	16.42
30a	$C_{21}H_{17}ClIN_5O_3$	58.40	6.07	16.21	58.66	5.91	16.47
30b	$C_{20}H_{23}ClFN_5O_3$	57.21	5.52	16.68	56.89	5.63	16.43
30c	$C_{18}H_{21}ClN_6O$	57.98	5.68	22.54	58.12	5.39	22.50
33	$C_{20}H_{25}N_5O_3$	59.36	6.29	21.86	58.98	6.36	21.52

32	$C_{15}H_{15}N_4O_3$	59.99	5.37	18.66	59.86	5.41	18.66
34	$C_7H_4Cl_2N_3I$	25.64	1.23	12.81	25.40	1.21	12.64
36a	$C_{19}H_{22}ClN_5O_3$	56.51	5.49	17.34	56.34	5.53	17.02
36b	$C_{16}H_{17}ClN_6O$	55.74	4.97	24.37	55.87	5.06	24.35
36c	$C_{15}H_{16}ClN_5OS$	51.50	4.61	20.02	51.62	4.86	19.93
36d	$C_{19}H_{22}ClN_5O_3$	56.51	5.49	17.34	56.35	5.58	17.12
36e	$C_{18}H_{18}ClN_5O_2$	58.15	4.88	18.84	57.92	4.95	18.71
36f	$C_{20}H_{24}ClN_5O_4$	55.36	5.58	16.14	55.55	5.33	16.27
36g	$C_{18}H_{20}ClN_5O_2$	57.83	5.39	18.73	57.70	5.01	18.40
36h	$C_{18}H_{17}ClN_6O$	58.62	4.65	22.79	58.97	4.28	22.47
36i	$C_{18}H_{18}ClN_5O_2$	58.15	4.88	18.84	57.87	4.95	18.49
36j	$C_{18}H_{20}ClN_5O_2$	57.83	5.39	18.73	57.57	5.41	18.54
36k	$C_{17}H_{17}Cl_2N_5O$	53.98	4.53	18.52	54.19	4.30	18.21
36l	$C_{19}H_{21}O_3N_5Cl$	56.79	5.02	17.43	56.64	4.90	17.17
36m	$C_{18}H_{18}ClN_5O_3 \cdot 0.66MeOH$	54.79	5.09	17.12	55.25	4.69	16.84
36n	$C_{18}H_{19}O_2N_6Cl$	55.89	4.95	21.73	55.32	5.00	21.11
36o	$C_{20}H_{23}O_2N_6Cl$	57.90	5.59	20.26	57.04	5.45	19.50
36p	$C_{18}H_{18}O_2N_5ClF$	55.46	4.40	17.97	55.37	4.49	17.08
36q	$C_{18}H_{17}ON_6ClF$	55.89	4.17	21.73	55.81	4.11	21.52
36r	$C_{18}H_{17}O_3N_5ClF \cdot H_2O$	51.01	4.52	16.52	51.23	4.55	16.14
36s	$C_{18}H_{20}O_3N_5ClS$	51.24	4.78	16.60	51.11	4.72	16.38
36t	$C_{19}H_{23}ClN_6O_3S$	50.61	5.14	18.64	50.26	5.06	18.09
37	$C_{20}H_{23}ClN_6O_3$	55.75	5.38	19.50	55.18	5.51	19.47
38	$C_{25}H_{31}ClN_6O_3 \cdot 0.5H_2O$	59.11	6.35	16.54	59.12	6.43	16.25
39	$C_{23}H_{29}ClN_6O_3 \cdot 0.75EtOAc$	57.93	6.54	15.59	57.55	6.41	15.81
40	$C_{22}H_{28}ClN_7O_3$	55.75	5.95	20.69	55.18	6.11	20.35
41	$C_{21}H_{27}ClN_6O_2$	58.53	6.32	19.50	58.05	6.33	19.14
42	$C_{21}H_{28}ClN_7O_4S \cdot 0.5EtOAc$	49.86	5.82	17.70	49.65	5.71	17.68
44	$C_{18}H_{20}ClN_5O_2$	57.83	5.39	18.73	57.47	5.66	18.44
45	$C_{18}H_{20}ClN_5O_2$	57.83	5.39	18.73	57.67	5.45	18.48
46	$C_{18}H_{22}Cl_2N_6O$	52.82	5.42	20.53	52.99	5.16	20.44
47	$C_{19}H_{23}ClN_5O_3$	55.89	4.95	21.73	55.22	4.78	21.47
48	$C_7H_7ClN_4I$	27.25	1.96	18.16	27.38	1.94	17.81
49	$C_{15}H_{15}ClN_4O_2 \cdot 0.17CHCl_3$	53.79	4.51	16.54	53.29	4.77	16.28

Detailed SAR study

The ultimate goal of our study was to determine the importance of all three parts of the molecule - both side chains and central core. Primarily, we measured the residual enzymatic activity at 10 μM concentration of each compound and determined the IC_{50} values for compounds when it was lower than 30%. On the enzymatic level, we were also interested in selectivity to PI4K IIIβ.Since the selectivity towards PI4K IIα is generally excellent and none of the most potent compounds inhibited PI4K IIα with IC_{50} lower than 100 μM, we routinely screened only the selectivity towards PI4K IIIα.

The resuts of our enzymatic assays and antiviral cell-based assays are summarized in the tables S7 and S8, respectively.

We observed several trends in inhibitory activity regarding the amino side chain:

1) Derivatives with diaminoethane or aminoethanol side chain substituted with small acyl group, such as acetyl or isopropanoyl, exerted the highest activity against PI4K IIIβ. The compounds with larger substituents, e.g. **4** or **5**, are significantly less potent.
2) Absence of the acyl group on the side chain results in a drop or even loss of activity. Except for mesyl group, various other substitutions of the acyl moiety resulted in decrease of the inhibition.
3) Derivative **2i** bearing only amino group instead of this side chain exerts surprisingly high potency comparable to the parent compound.
4) Rigidity introduced into the structure by incorporation of aromatic ring in compound **2e** let to diminished inhibition of the enzyme.
5) Cyclic derivatives **2g**, **2h** and **2l** were rather inactive.
6) Prolongation of the linker between acyl group and the central core resulted in reduction of inhibitory potency.
7) The substitution of the nitrogen atom adjacent to central core by sulfur or oxygen atom led to decrease in activity. In case of sulfur derivative we also observed significant loss of selectivity.

The alteration of the central core led us to the following conclusions:

1) Pyrazolo[1,5-a]pyrimidine central core can be substituted by imidazo[1,2-b]pyridazine without loss of activity. Removal or addition of a nitrogen atom from the structure of the central core in case of compounds **16**, **18** and **33** resulted in significant drop of activity.

2) The influence of the substituent at the positon 6 of the imidazo[1,2-b]pyridazine core is intriguing, since the activity of the compounds with hydrogen, chlorine or methyl group seems to fluctuate depending on the amino side chain. However, the selectivity towards PI4K IIIβ in comparison with PI4K IIIα seems to decrease in the line Cl>CH_3>H.

Our detailed investigation of the aromatic side chain unveiled potential directions in modification of this part:

1) Most of the modification of the dimethoxyphenyl moiety resulted in decrease or even complete loss of inhibitory activity.
2) The only exception are compounds bearing benzene ring substituted with either aldehyde or sulfonyl group in *meta* position in respect to the central core. The derivative **36t** with the sulfonamide group seems to be the most active compound of the series.
3) Interestingly, we observed complete reverse of the selectivity when carboxylic group was present at this position. Thus, derivative **36r** is rather inhibitor of PI4K IIIα.

Results of biochemical assays and crystalography

Supplementary Table 2.

Compound	PI4KIIIβ % ra[a]	PI4KIIIα % ra[a]	PI4KIIIβ IC_{50} (μM)
1	6±2	57±4	0,37±0.04
2b	4±1	66±3	0,25±0.06
2d	7±3	69±5	0,21±0.04
2e	23±6	84±8	0,88±0.07
2f	6±0	73±8	0,47±0.01
2g	61±20	98±7	ND
2h	56±15	99±11	ND
2i	1±0	59±5	0,17±0.01
2j	52±2	93±4	ND
2k	33±9	100±12	ND
2l	84±4	108±3	ND
4	7±4	113±5	0,46±0.01
5	6±2	87±11	0,42±0.02
6	24±8	69±7	0,41±0.05
7	18±5	88±10	1,11±0.16
8	4±2	66±1	0,32±0.02
11	8±2	71±4	0,27±0.02
12	51±16	83±6	ND
13	5±0	41±3	0,39±0.05
14	107±18	85±6	ND
15	35±4	80±8	ND
16	29±2	89±11	10,10±1.2
17	5±1	87±5	0,15±0.02
30a	3±2	72±4	0,15±0.01
30b	8±2	83±4	0,46±0.01
30c	17±1	69±4	0,65±0.10
18	5±1	61±2	0,41±0.08
33	3±1	77±24	0,53±0.10
36b	21±3	75±2	8,11±1.00
36d	120±5	111±4	ND
36e	5±0	99±1	0,13
36f	87±9	108±7	ND
36g	41±7	97±1	ND
36h	45±10	121±7	ND
36i	15±8	107±7	1,84±0.07
36j	20±2	127±14	3,16±0.20
36k	34±8	105±8	ND
36l	35±6	78±8	ND
36m	34±9	81±3	ND
36n	37±16	90±1	ND

36o	6±1	87±4	0,24±0.03
36q	62±0	93±3	ND
36r	51±16	23±4	ND
36s	5±3	60±1	0,25±0.05
36u	35±5	81±26	ND
37	63±8	88±31	ND
38	120±29	80±16	ND
39	60±12	93±7	ND
40	53±4	92±3	ND
41	46±8	85±28	ND
42	17±4	73±7	2,16±0.12
43	3±1	74±4	0,26±0.43
44	18±2	101±4	3,28±0.17
45	15±2	115±12	2,32±0.19
46	58±10	96±9	ND

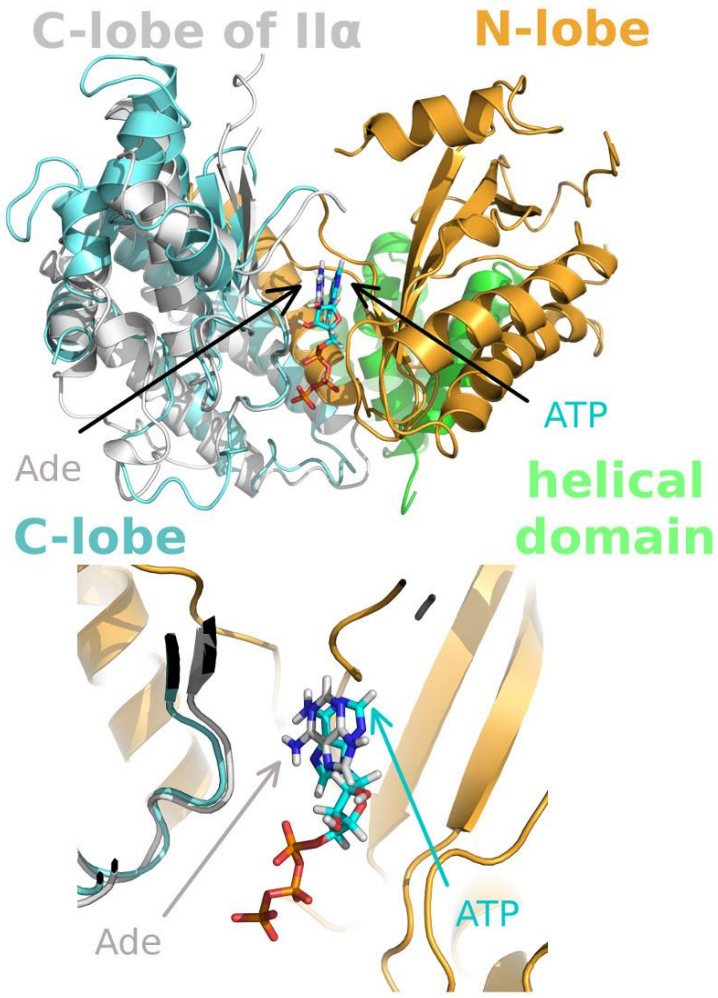
[a] r.a. – residual activity of enzyme at 10 μM of the inhibitor

Supplementary Table 3. Activity of PI4K IIIβ inhibitors against selected viruses.

Compound	CVB3 EC_{50}(μM)	HRV10 EC_{50}(μM)	Hela[a] CC_{50} (μM)	HCV 1b EC_{50}(μM)	CC_{50} (μM)	HCV 2a EC_{50}(μM)	CC_{50} (μM)
1	>50	ND	28.5	0.991	2.31	1.69	2.44
2b	1.55	2.79	>50	0.688	>44	11.6	>44
2d	1.50	7.35	>50	1.10	>44	>44	>44
2e	0.142	9.43	>50	11.3	>44	>44	>44
2f	2.08	3.70	>50	1.15	27.9	>44	>44
2g	>50	ND	ND	6.52	>44	>44	>44
2h	>50	>50	ND	>44	>44	>44	>44
2i	0.221	0.370	>50	0.067	>44	12.1	>44
2j	>50	0.939	>50	8.24	>44	>44	>44
2k	6.69	8.39	>50	1.79	>44	38.5	>44
2l	>50	>50	>50	26.6	>44	>44	>44
4	1.40	3.35	>50	2.39	>44	37.1	>44
5	1.24	2.95	>50	1.32	>44	25.1	>44
6	3.47	39.7	>50	2.60	>44	>44	>44
7	1.09	3.07	>50	2.48	>44	11.9	>44
8	0.509	0.960	>50	0.408	>44	25.9	>44
11	4.18	4.02	>50	1.63	>44	>44	>44
12	>50	>50	15.9	1.51	7.19	2.30	4.86
13	14.2	2.91	>50	0.80	>44	5,23	>44
14	ND	>50	>50	>44	>44	>44	>44
15	39.3	>50	>50	>44	>44	>44	>44
16	>50	3.14	>50	ND	ND	ND	ND
17	0.255	0.878	>50	0.362	>44	18.0	>44

30a	0.406	0.880	43.35	0.349	>44	18.0	>44
30b	0.945	1.26	>50	0.590	>44	17.9	35.5
30c	17.5	27.7	>50	4.27	>44	>44	>44
18	0.430	3.19	>50	2.39	>44	>44	>44
33	1.39	3.27	>50	3.79	38.2	35.5	43.0
36b	> 50	>50	>50	7.06	>44	>44	>44
36d	ND	>50	>50	22.5	>44	>44	>44
36e	14.1	33.5	>50	21.5	>44	>44	>44
36f	> 50	>50	>50	38.8	>44	>44	>44
36g	25.3	20.7	>50	6.00	>44	>44	>44
36h	48.9	>50	>50	16.3	>44	>44	>44
36i	2.83	12.3	>50	11.9	27.2	29.8	35.4
36j	7.93	7.39	>50	2.63	>44	34.9	>44
36k	12.5	14.6	>50	3.24	>44	21.5	>44
36l	4.9	30.2	47.6	5.37	15.7	>44	>44
36m	1.7	10.0	26.6	3.15	>44	34.9	>44
36n	> 50	>50	>50	23.6	>44	>44	>44
36o	5.57	30.8	>50	3.37	>44	>44	>44
36q	> 50	>50	>50	3.95	>44	>44	>44
36r	> 50	>50	>50	>44	>44	>44	>44
36s	1.17	9.74	>50	0.824	>44	>44	>44
36u	3.51	>50	>50	>44	>44	>44	>44
37	> 50	25,5	>50	18,0	>44	26,2	35,0
38	> 50	>50	>50	17,6	>44	>44	>44
39	> 50	31,0	>50	30,9	>44	>44	>44
40	> 50	10,2	>50	4,90	>44	17,2	36,9
41	32.6	>50	>50	33,65	>44	>44	>44
42	13.0	>50	>50	>44	>44	>44	>44
43	0.513	0.981	>50	1.54	>44	>44	>44
44	13.3	38.6	>50	18.7	>44	>44	>44
45	6.24	14.6	>50	7.42	>44	>44	>44
46	ND	>50	>50	>44	>44	>44	>44

Supplementary figures

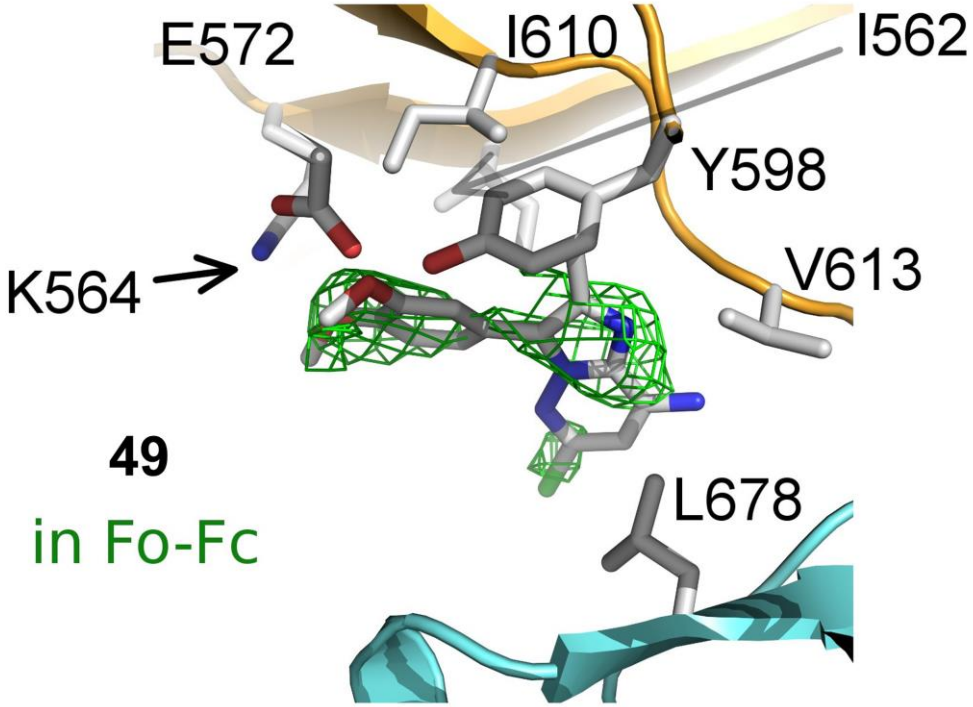


Supplementary Figure 1

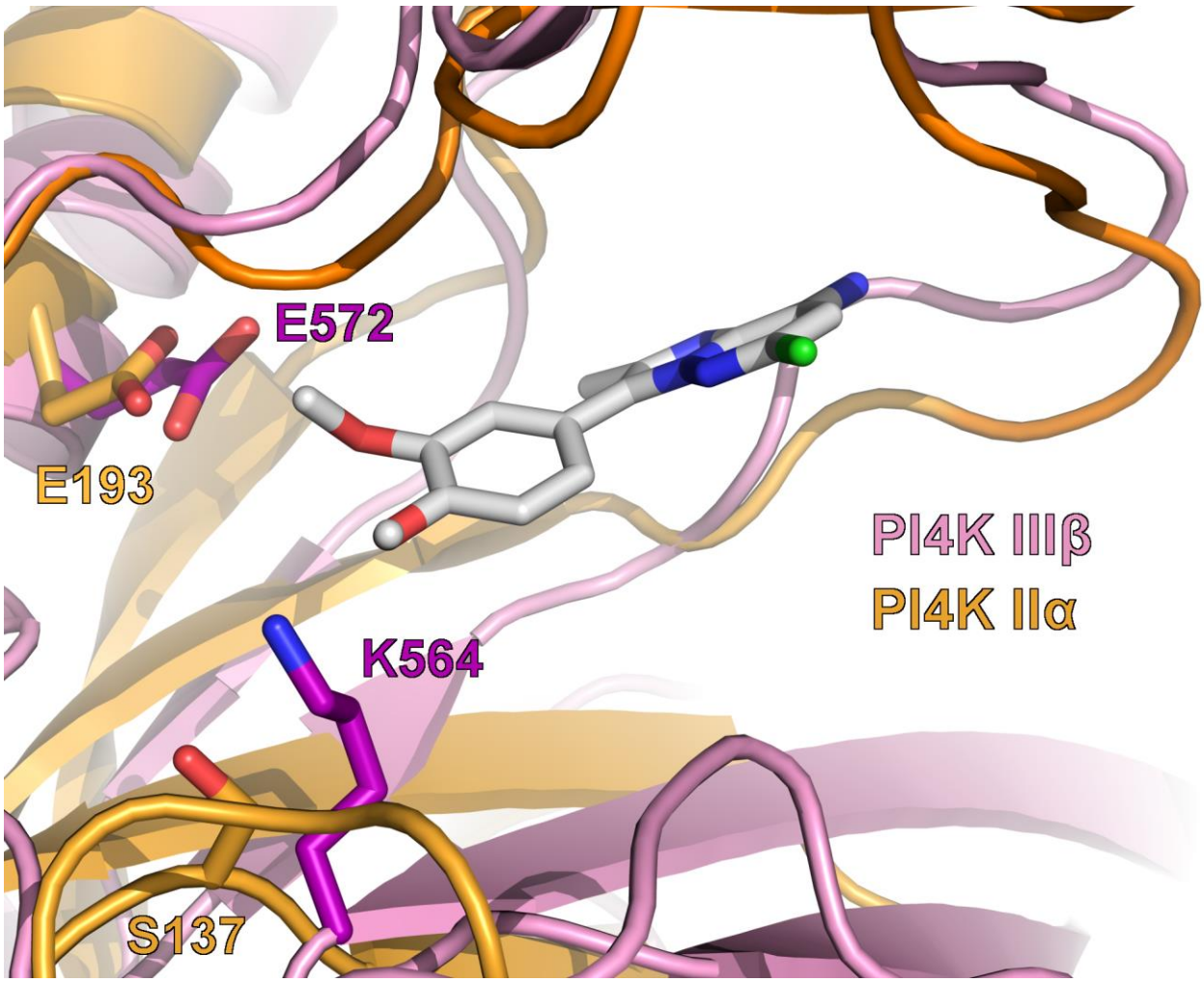
Superposition of PI4K IIIβ and PI4K IIα

Superposition was performed using only the identical C-lobes. Top – the overall view of the superposed enzymes. Bottom – detailed view of the ATP binding site reveals that the adenine ring in the crystal structure of PI4K IIIβ and PI4K IIα is in the same relative orientation.

The C-lobe of PI4K IIα is colored in silver. The N-lobe, C-lobe and the helical domain of PI4K IIIβ are in orange, cyan and green. In the detailed views carbon atoms of ATP of PI4K IIα are colored in cyan, carbon atoms of the adenine ring of PI4K IIIβ are colored silver, nitrogen in blue, phosphorus in orange, and oxygen in red.



Supplementary Figure 2



Supplementary Figure 3

Results of the docking study

The docking study was performed fot the top 5 most active PI4K IIIβ inhibitors. The mode of binding of **36t** with the lowest energy (mode 1, affinity -8.9 kcal/mol) is presented in the main text as Figure 4d.

The results of the docking study are ordered according to PI4K IIIβ potency:

Results for the compound **36t**

```
Output will be I35MI2001_out.pdbqt
Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 154776648
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -8.9      0.000      0.000
   2         -8.9      2.666      7.371
   3         -8.8      2.891      7.809
   4         -8.4      1.189      2.025
   5         -8.3      2.018      2.913
   6         -8.1      1.798      3.270
   7         -8.1      2.671      7.022
   8         -8.1      1.721      2.742
   9         -8.0      2.283      3.400
Writing output ... done.
```

Results for the compound **10**

```
Output will be I35MI2002_out.pdbqt
Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1337419820
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -7.7      0.000      0.000
   2         -7.6      0.954      1.647
   3         -7.4      1.699      2.182
   4         -7.0      2.069      7.074
   5         -7.0      2.052      7.240
   6         -7.0      2.076      6.632
   7         -6.9      2.867      5.066
   8         -6.9      1.436      6.626
```

```
   9         -6.9      1.970      7.424
Writing output ... done.
```

Results for the compound **2c**

```
Output will be I35MI2003_out.pdbqt
Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -788120288
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -8.5      0.000      0.000
   2         -7.9      2.436      7.454
   3         -7.8      2.248      7.533
   4         -7.8      2.180      7.973
   5         -7.8      2.465      4.425
   6         -7.8      2.402      7.359
   7         -7.7      2.150      3.711
   8         -7.7      1.435      2.160
   9         -7.7      3.130      8.061
Writing output ... done.
```

Results for the compound **49**

```
Output will be I35MI2004_out.pdbqt
Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 917762412
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -7.7      0.000      0.000
   2         -7.5      1.321      1.640
   3         -7.2      2.822      4.744
   4         -7.2      3.014      5.451
   5         -7.1      2.794      5.117
   6         -7.0      2.095      3.094
   7         -6.9      3.099      5.708
   8         -6.9      3.274      5.444
   9         -6.9      1.875      2.832
Writing output ... done.
```

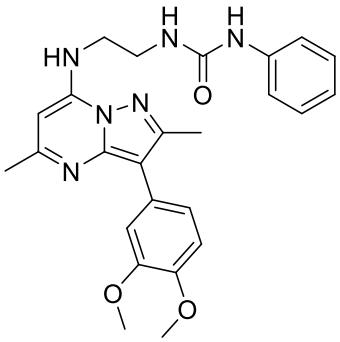
Results for the compound **36p**

```
Output will be I35MI2004_out.pdbqt
Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1213118736
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -7.7      0.000      0.000
   2         -7.7      3.238      7.249
   3         -7.6      3.473      7.754
   4         -7.5      3.195      7.289
   5         -7.2      1.204      2.080
   6         -7.0      3.154      7.458
   7         -7.0      3.010      7.069
   8         -7.0      3.032      4.689
   9         -6.9      1.979      2.978
Writing output ... done.
```

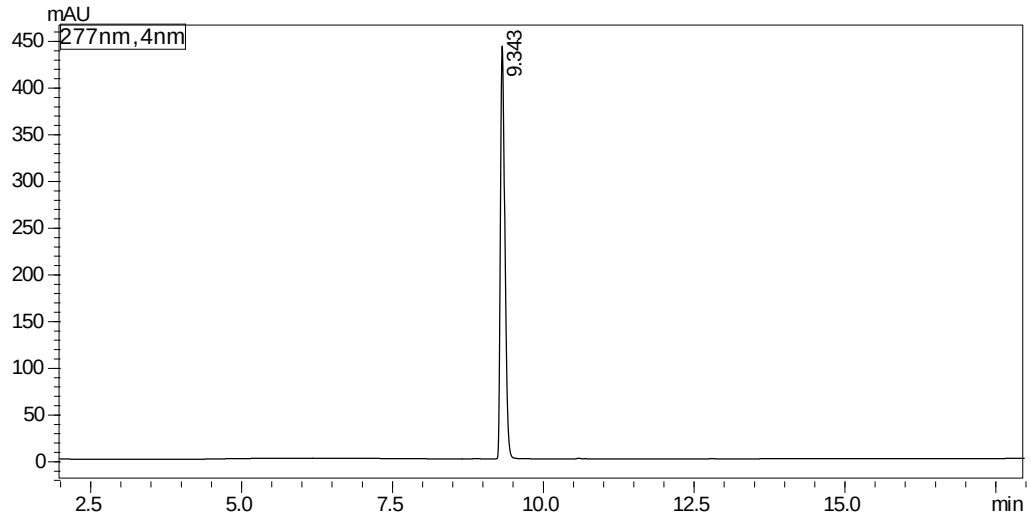
Selected analytical data

HPLC-MS, ^{1}H-NMR and ^{13}C-NMR analysis of the compound **7**

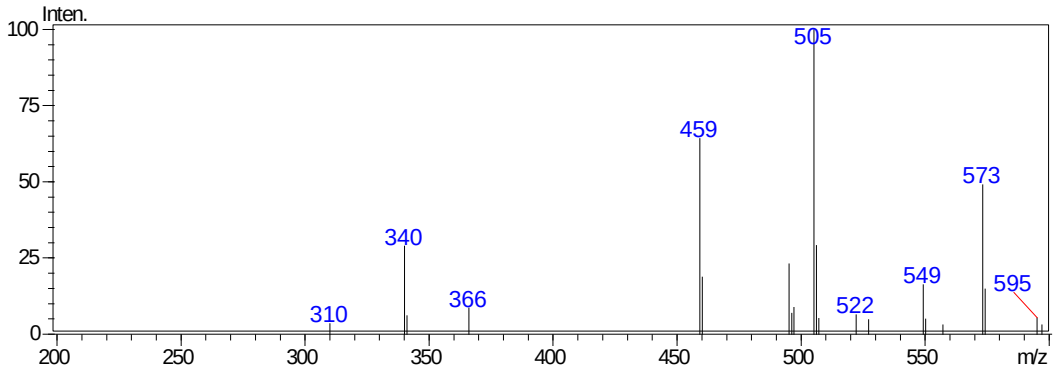


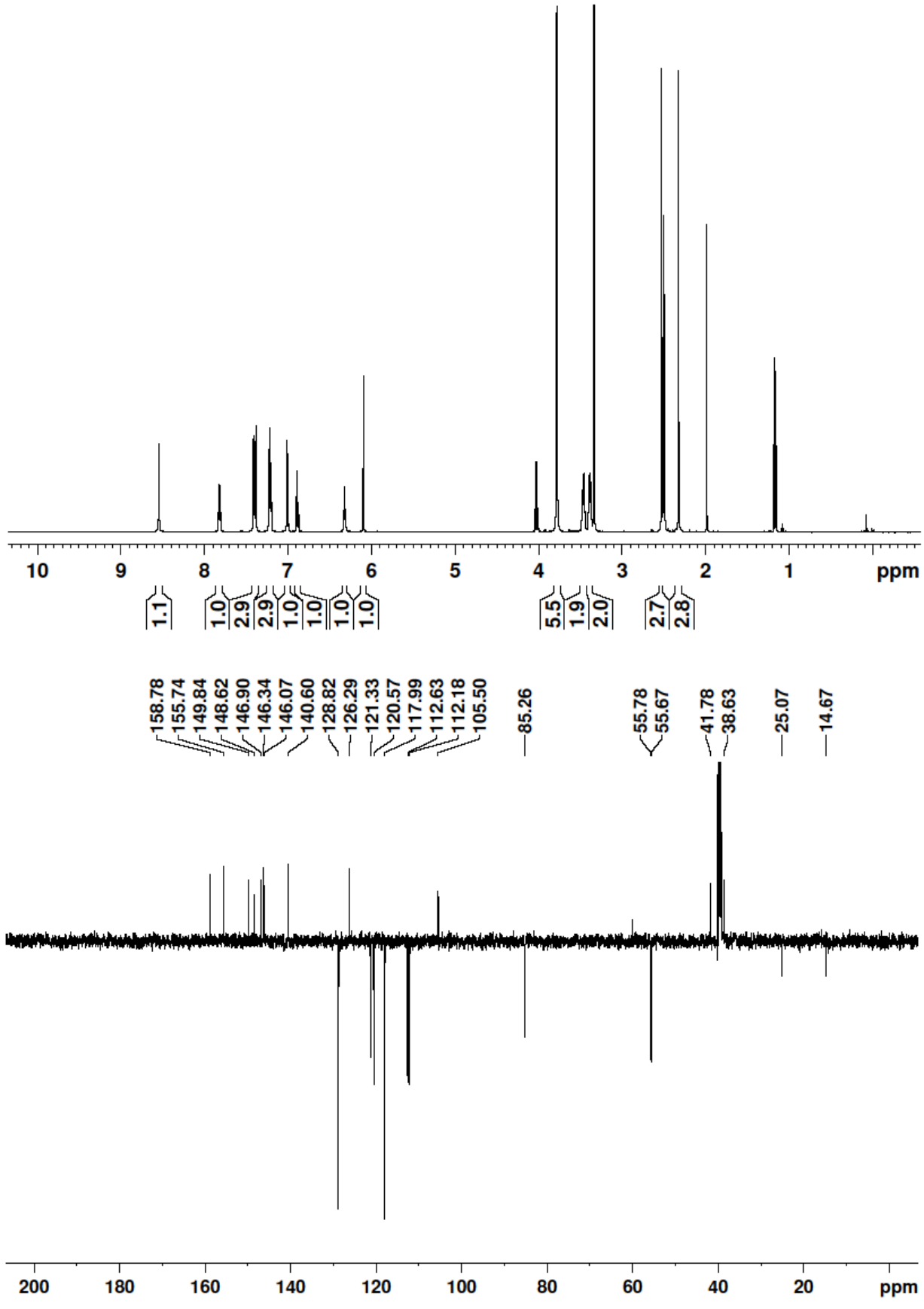
7

Exact Mass: 460,222
Molecular Weight: 460,538

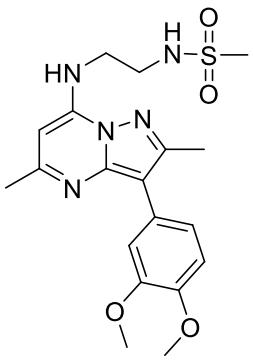


ESI-



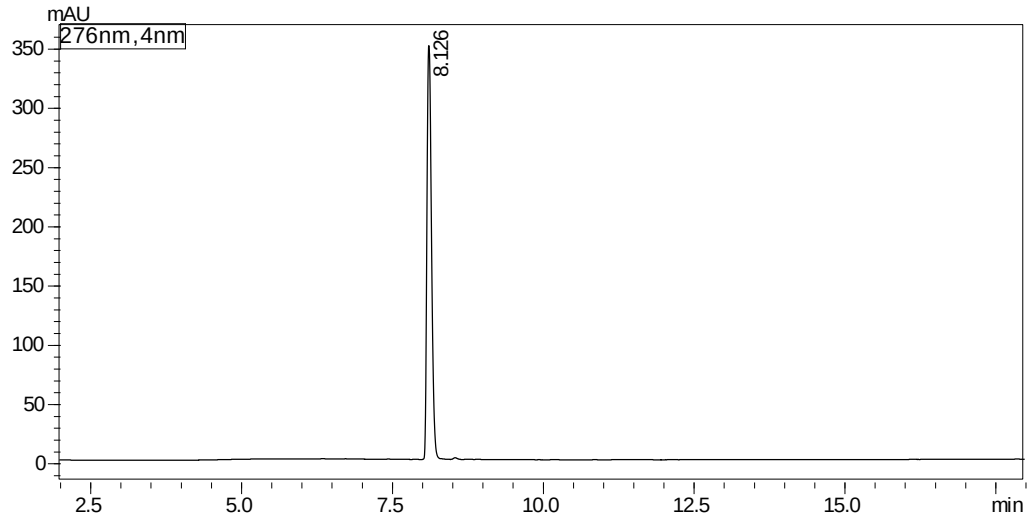

10
9
8
7
6
5
4
3
2
1
ppm
1.1
1.0
2.9
2.9
1.0
1.0
1.0
1.0
5.5
1.9
2.0
2.7
2.8
158.78
155.74
149.84
148.62
146.90
146.34
146.07
140.60
128.82
126.29
121.33
120.57
117.99
112.63
112.18
105.50
85.26
55.78
55.67
41.78
38.63
25.07
14.67
200
180
160
140
120
100
80
60
40
20
ppm

HPLC-MS, ^{1}H-NMR and ^{13}C-NMR analysis of the compound **9**

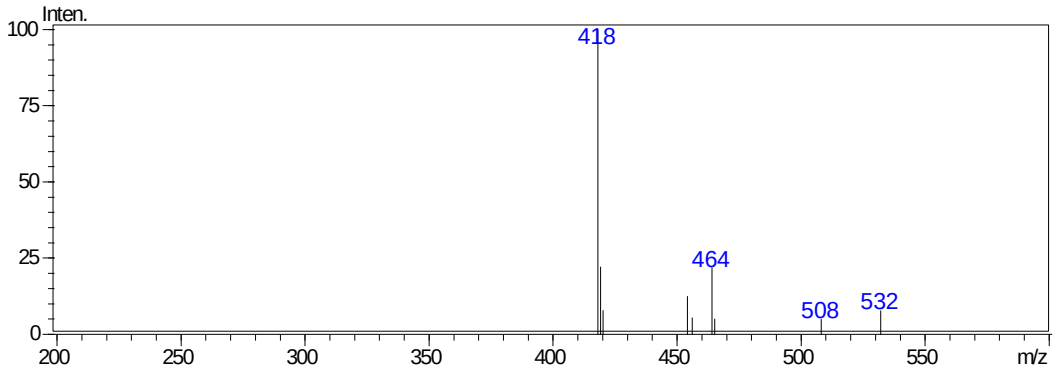


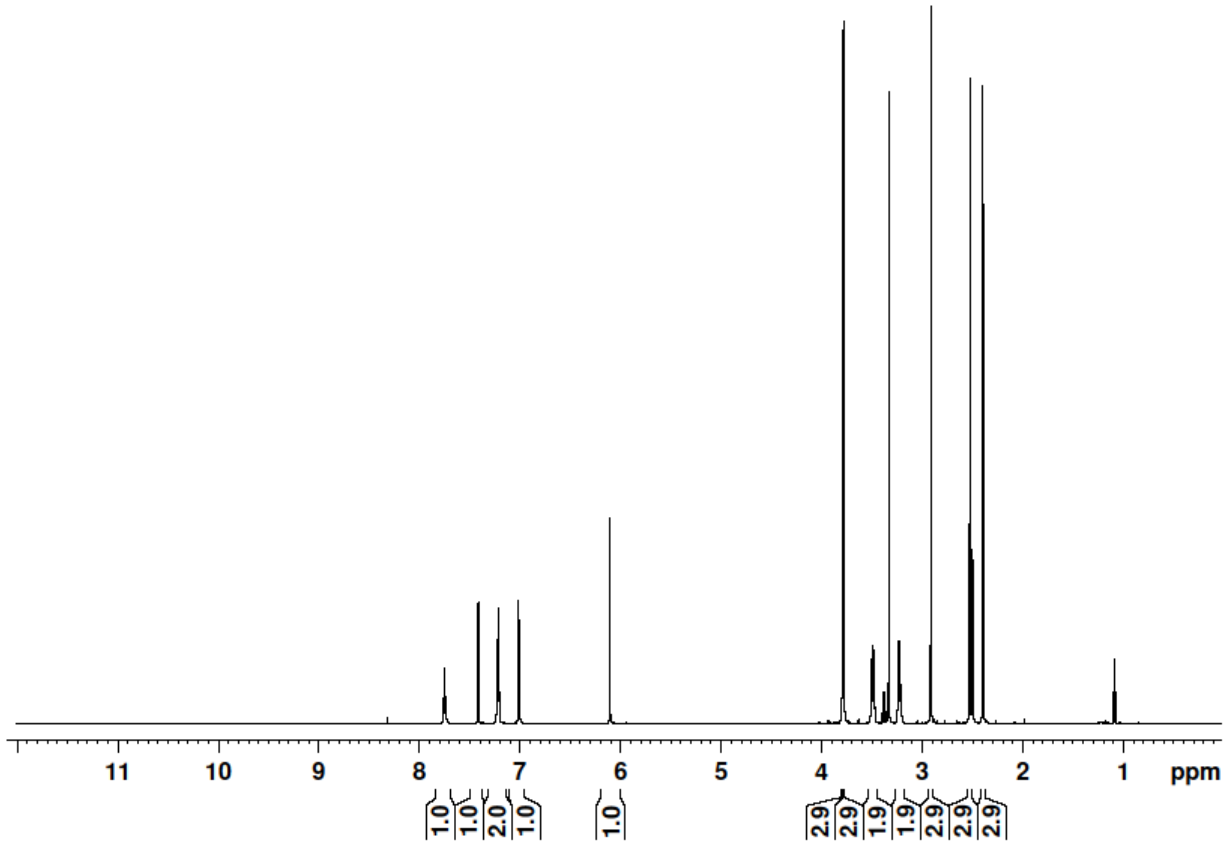
9

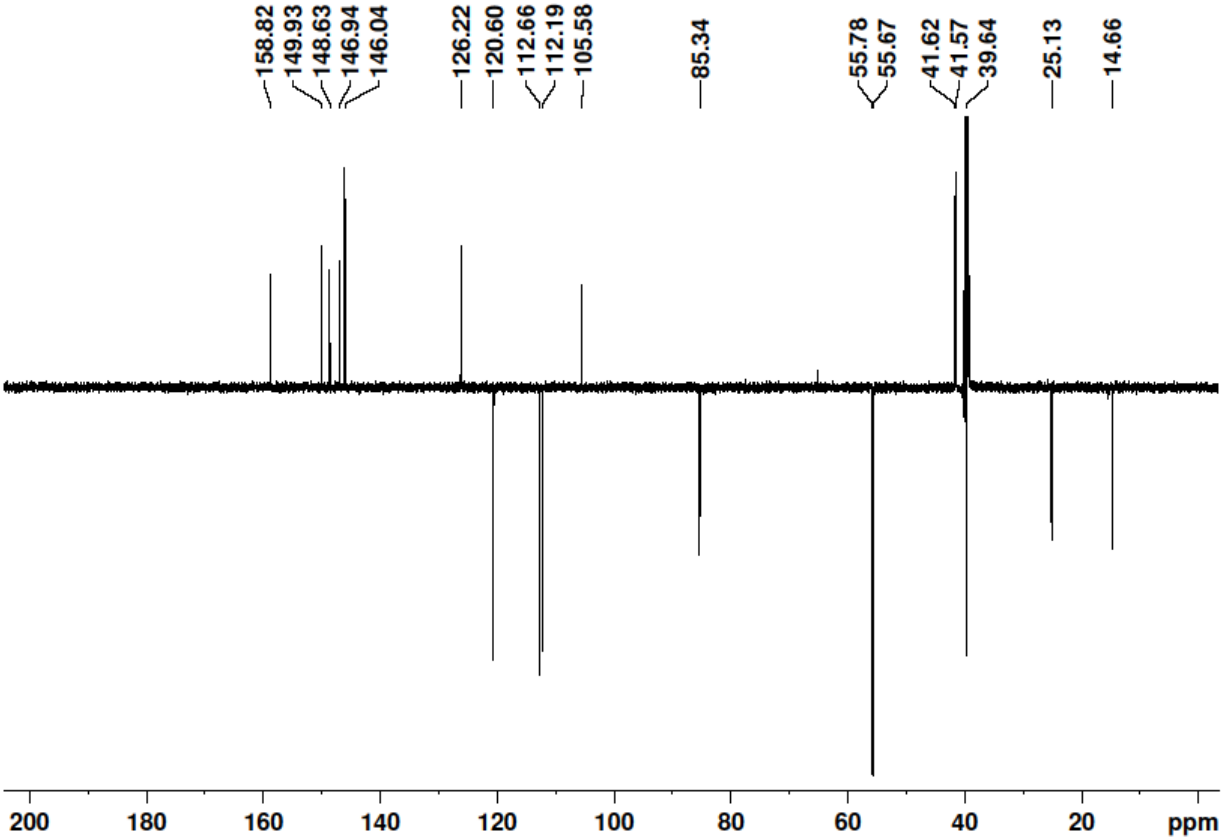
Exact Mass: 419,163
Molecular Weight: 419,500



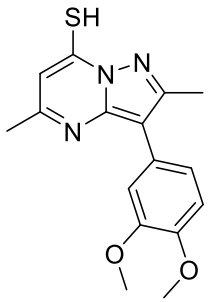
ESI-




11
10
9
8
7
6
5
4
3
2
1
ppm
1.0
1.0
2.0
1.0
1.0
2.9
2.9
1.9
1.9
2.9
2.9
2.9

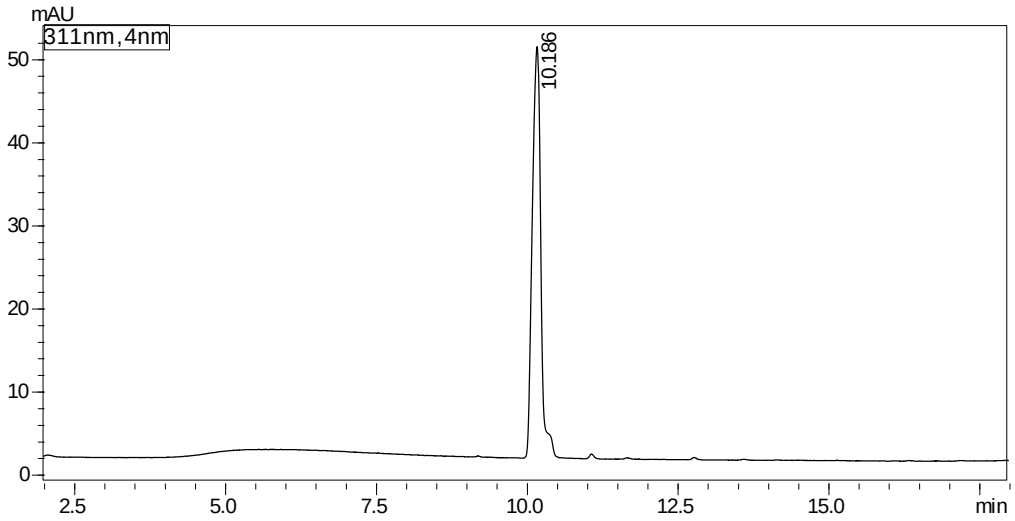

158.82
149.93
148.63
146.94
146.04
126.22
120.60
112.66
112.19
105.58
85.34
55.78
55.67
41.62
41.57
39.64
25.13
14.66
200
180
160
140
120
100
80
60
40
20
ppm

HPLC-MS, ^{1}H-NMR and ^{13}C-NMR analysis of the compound **12**

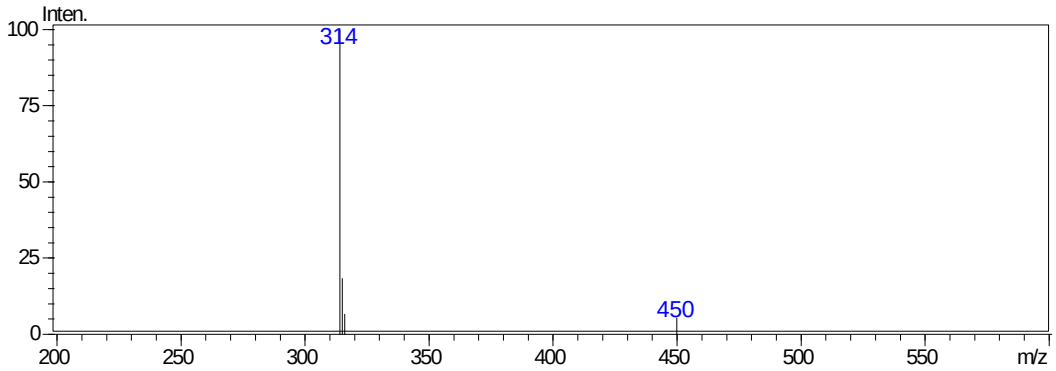


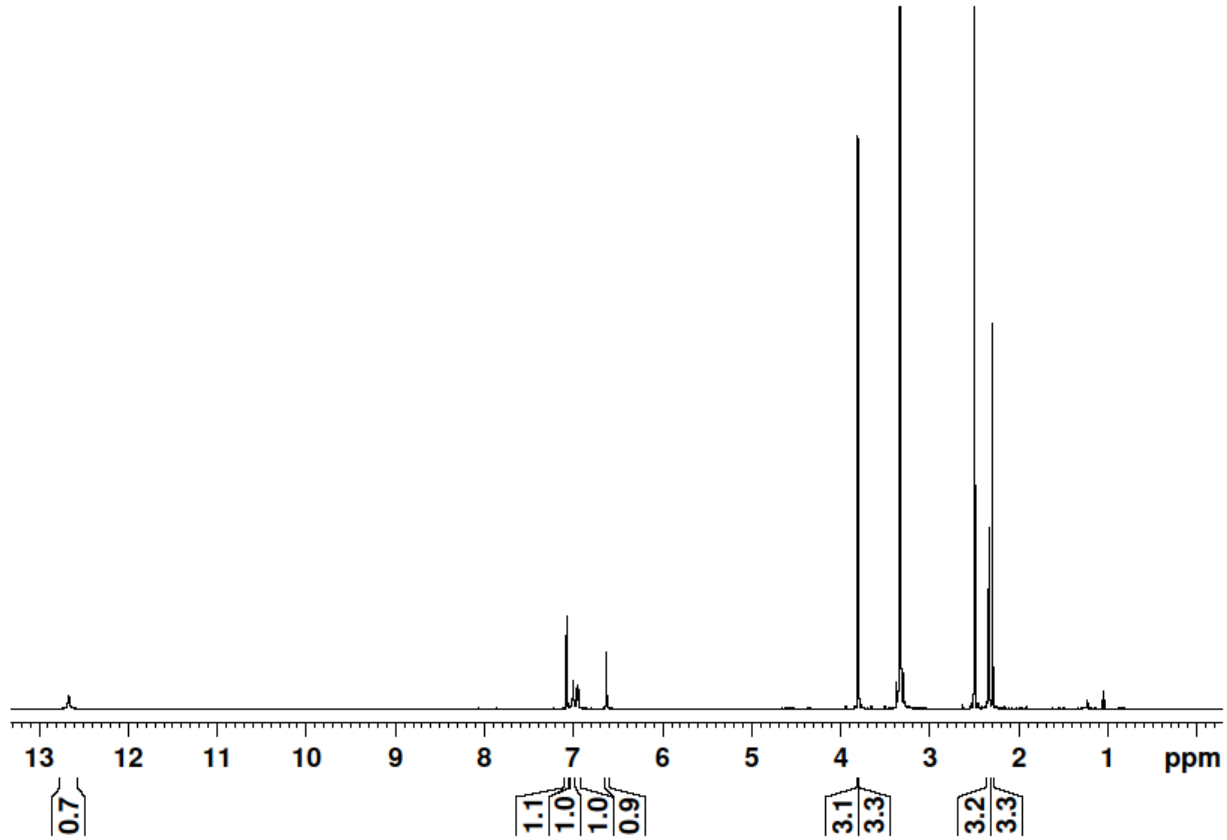
12

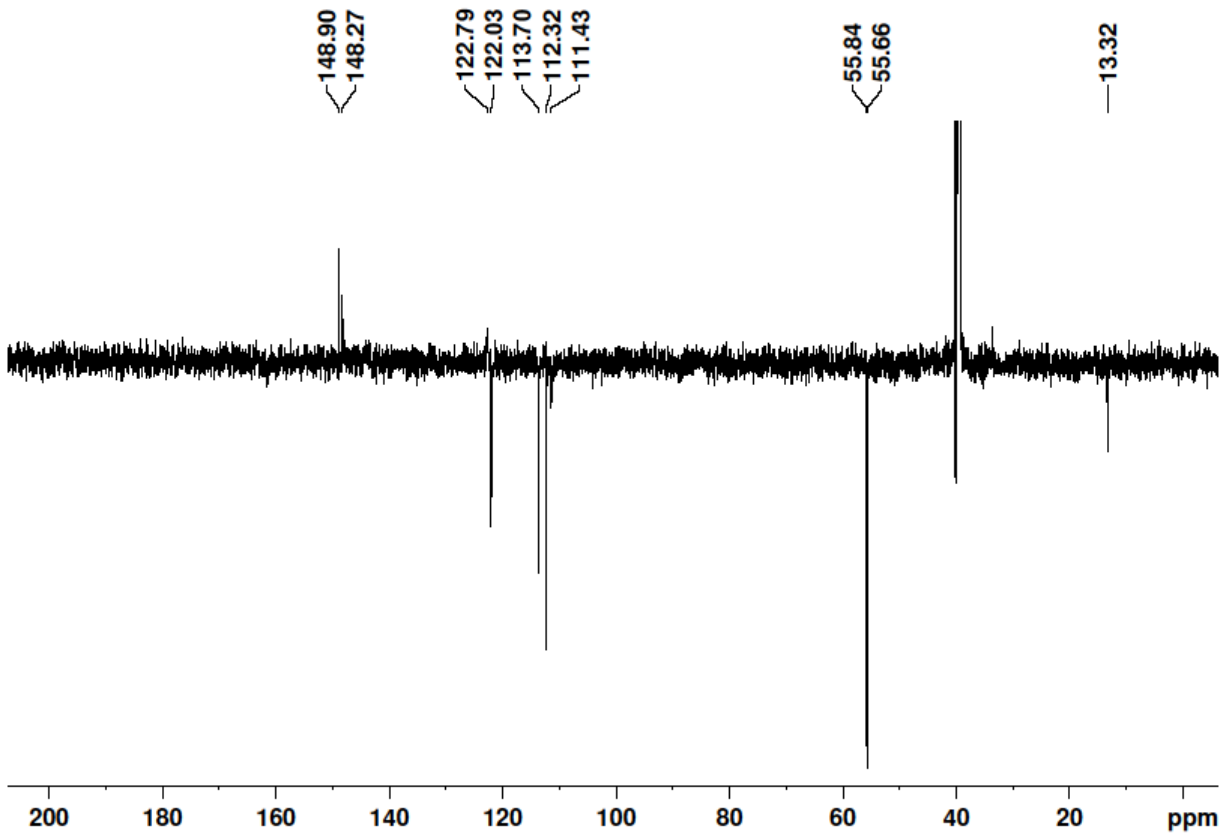
Exact Mass: 315,104
Molecular Weight: 315,391



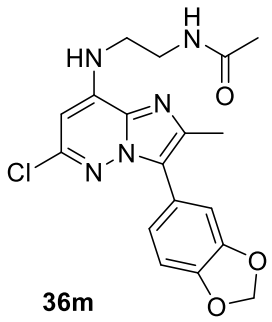
ESI-




13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.7
1.1
1.0
1.0
0.9
3.1
3.3
3.2
3.3

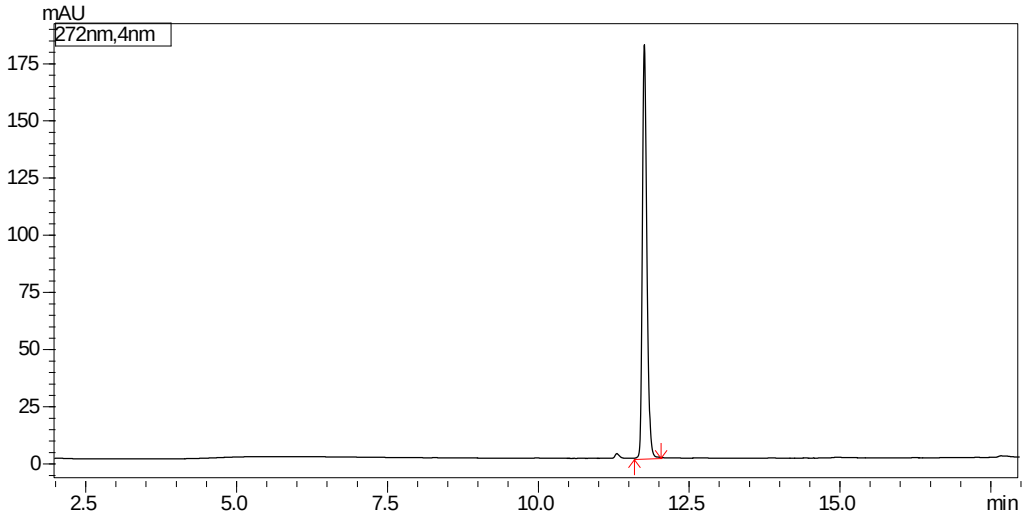

148.90
148.27
122.79
122.03
113.70
112.32
111.43
55.84
55.66
13.32
200
180
160
140
120
100
80
60
40
20
ppm

HPLC-MS, ^{1}H-NMR and ^{13}C-NMR analysis of the compound **36m**

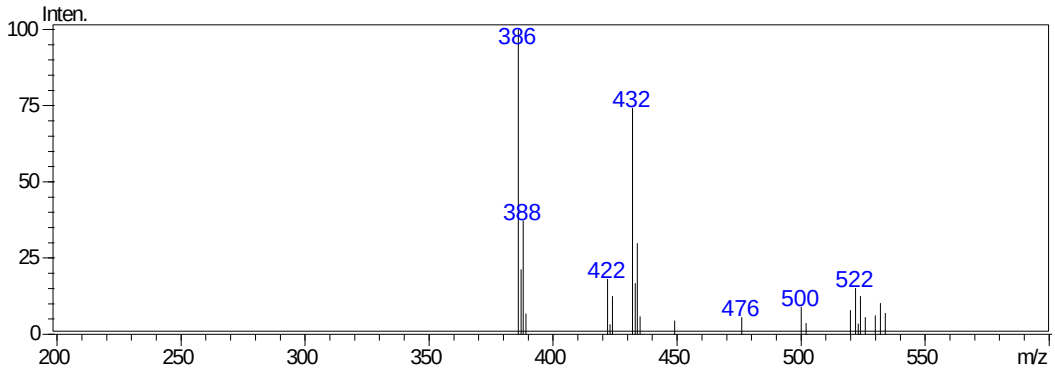


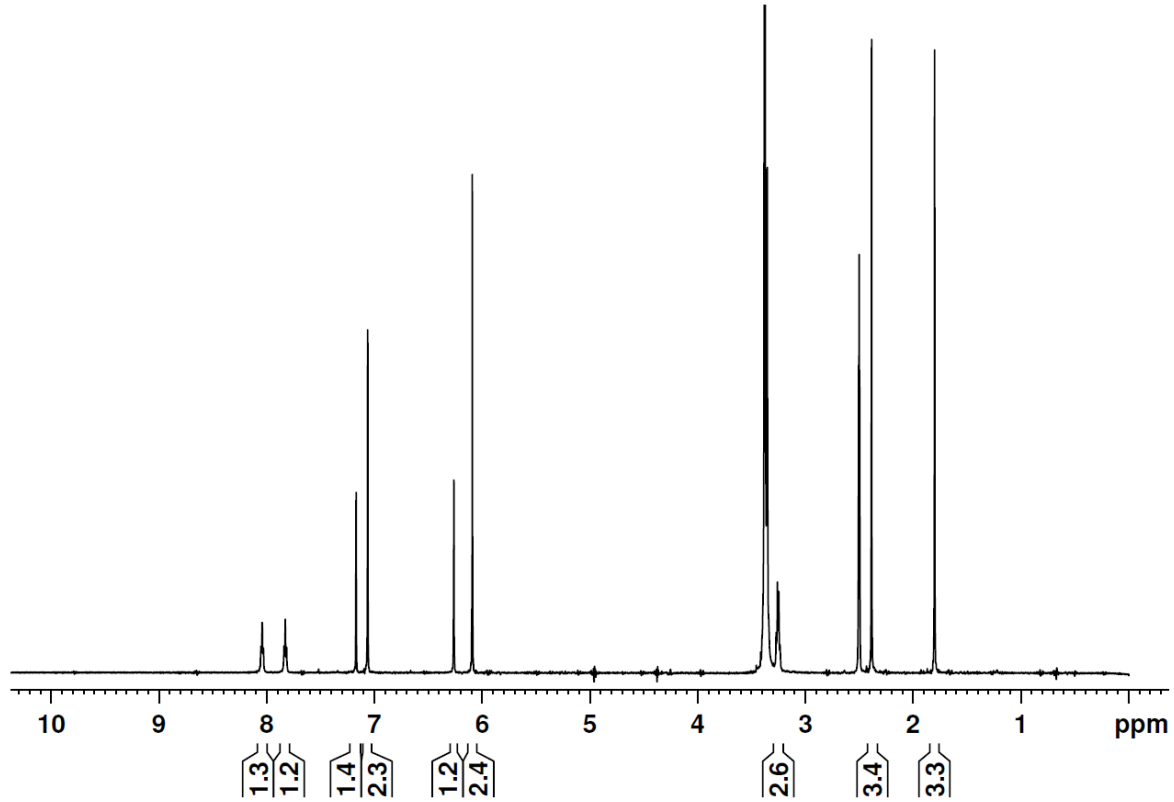
36m

Exact Mass: 387,110
Molecular Weight: 387,824



ESI-




10
9
8
7
6
5
4
3
2
1
ppm
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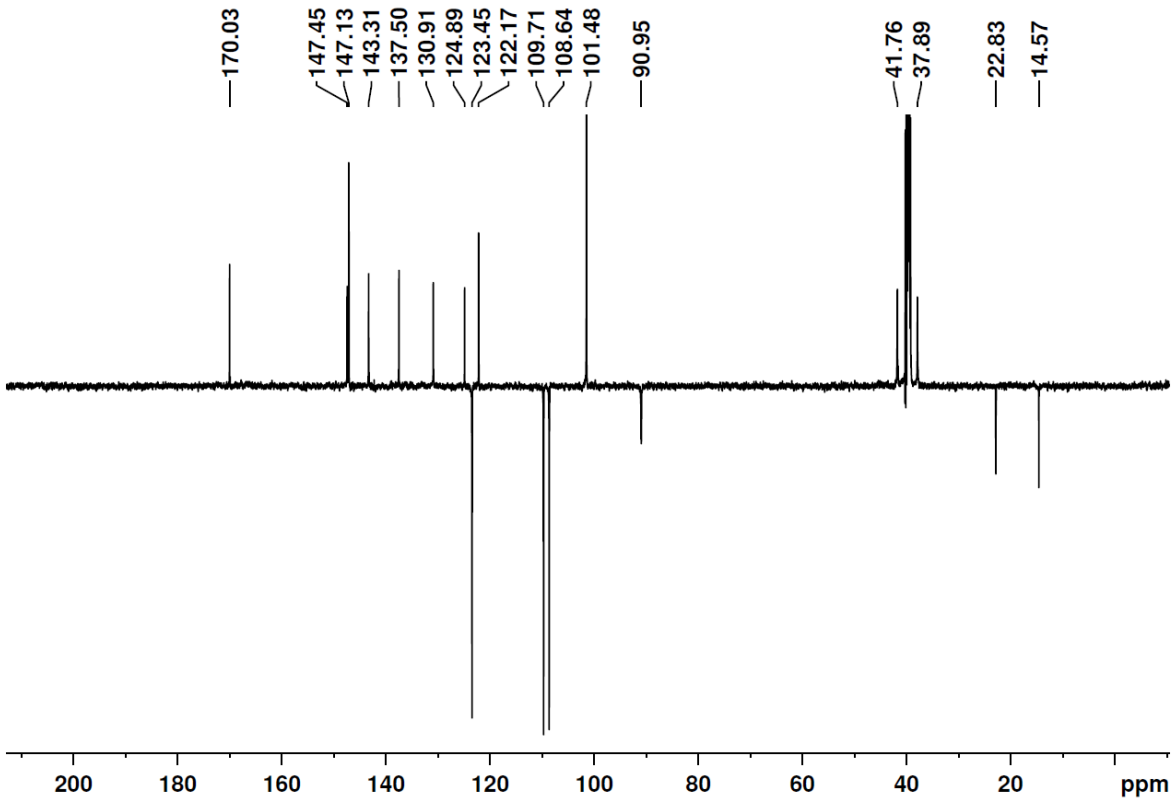
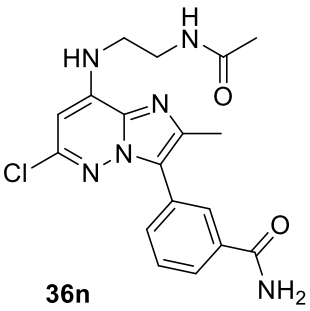
1.2
1.4
2.3
1.2
2.4
2.6
3.4
3.3

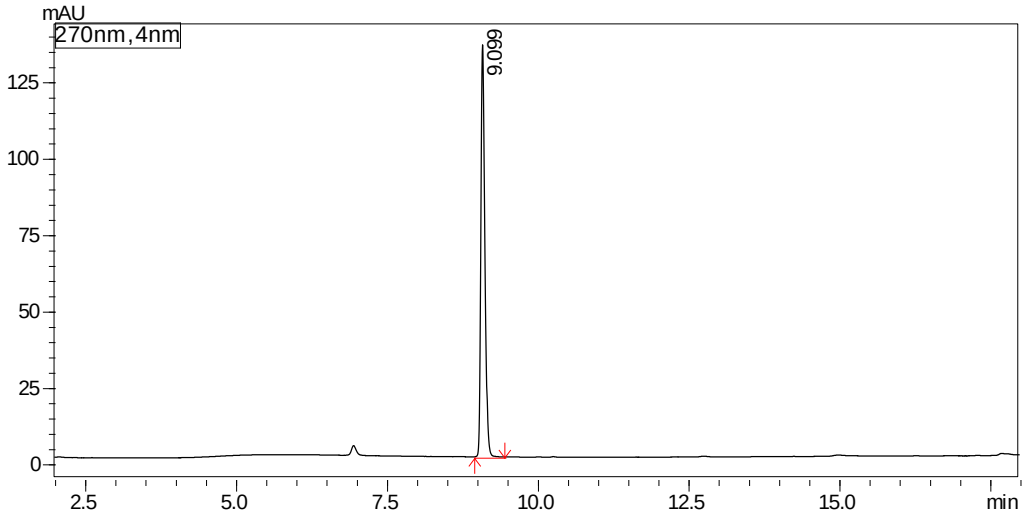

170.03
147.45
147.13
143.31
137.50
130.91
124.89
123.45
122.17
109.71
108.64
101.48
90.95
41.76
37.89
22.83
14.57
200
180
160
140
120
100
80
60
40
20
ppm

HPLC-MS, ^{1}H-NMR and ^{13}C-NMR analysis of the compound **36n**

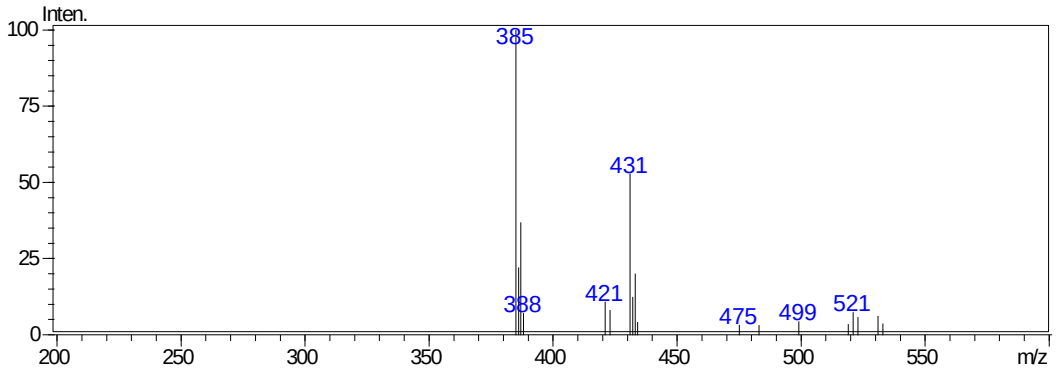


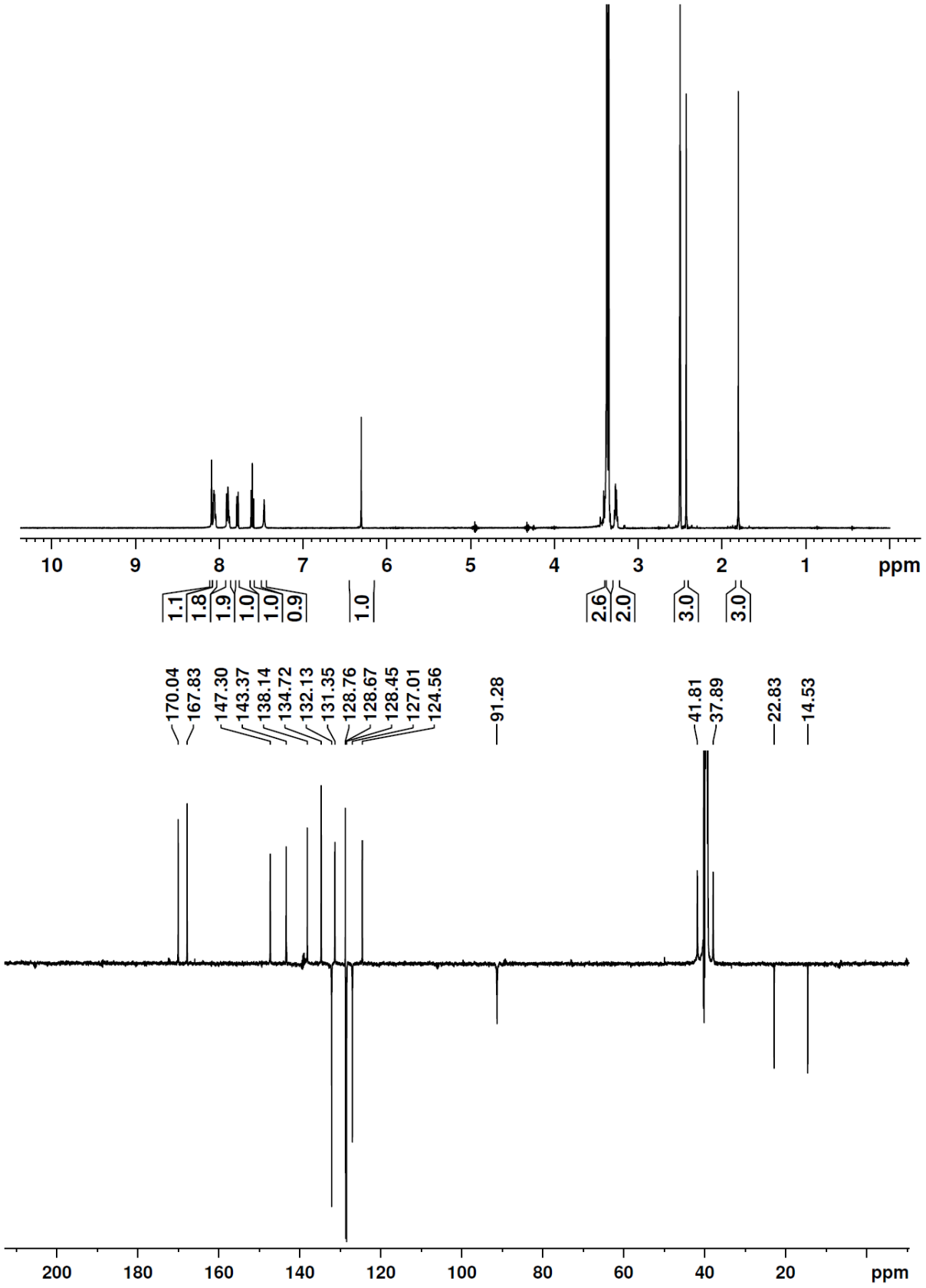
36n

Exact Mass: 386,126
Molecular Weight: 386,840



ESI-




10
9
8
7
6
5
4
3
2
1
ppm
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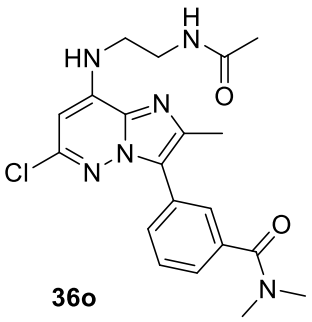
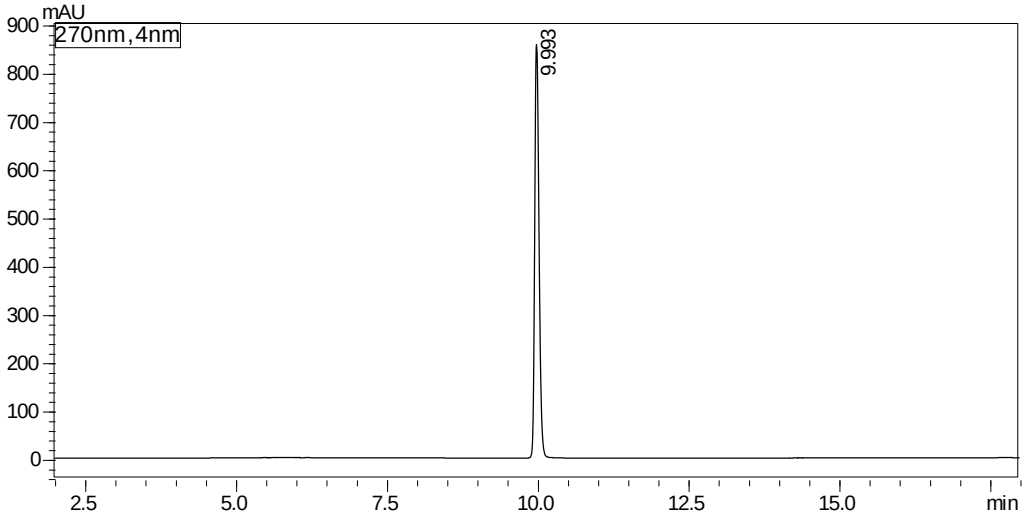
1.8
1.9
1.0
1.0
0.9
1.0
2.6
2.0
3.0
3.0
170.04
167.83
147.30
143.37
138.14
134.72
132.13
131.35
128.76
128.67
128.45
127.01
124.56
91.28
41.81
37.89
22.83
14.53
200
180
160
140
120
100
80
60
40
20
ppm

HPLC-MS, ^{1}H-NMR and ^{13}C-NMR analysis of the compound **36o**

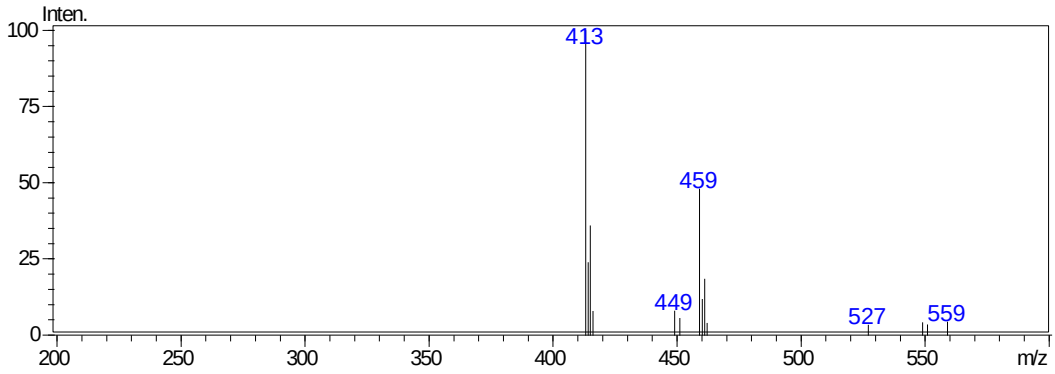


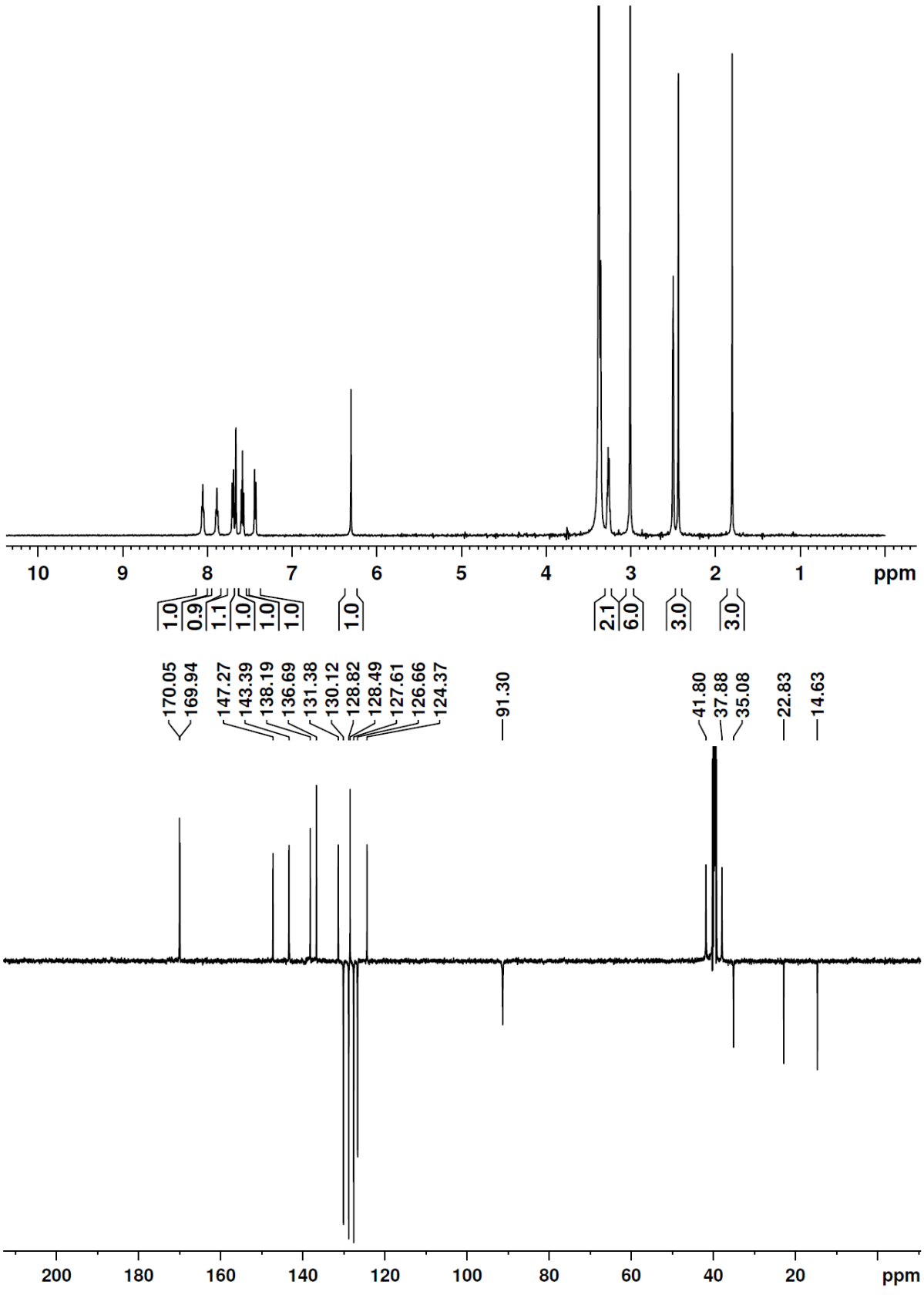
36o

Exact Mass: 414,157
Molecular Weight: 414,894

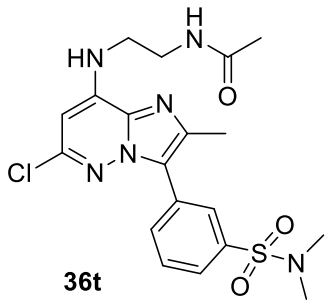


ESI-



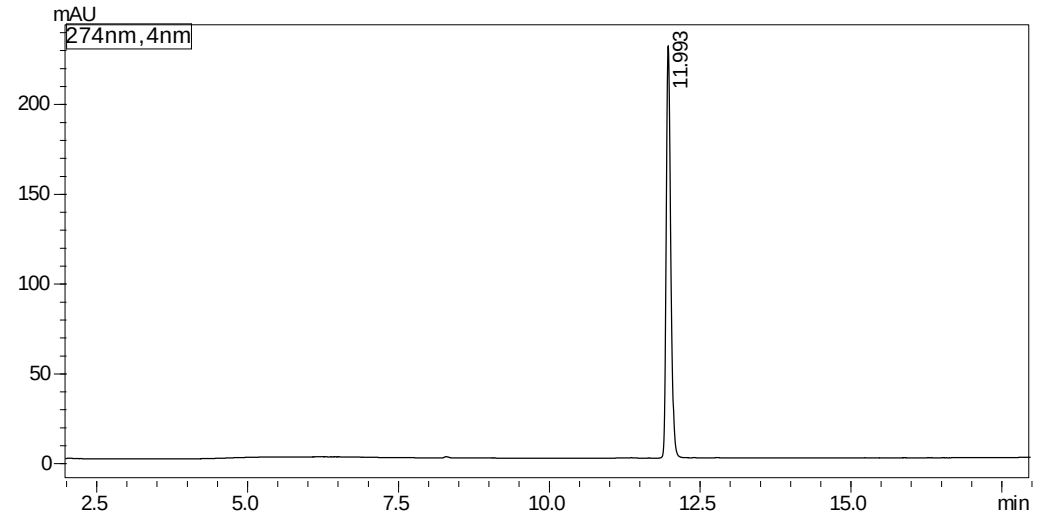

10
9
8
7
6
5
4
3
2
1
ppm
1.0
0.9
1.1
1.0
1.0
1.0
1.0
2.1
6.0
3.0
3.0
170.05
169.94
147.27
143.39
138.19
136.69
131.38
130.12
128.82
128.49
127.61
126.66
124.37
91.30
41.80
37.88
35.08
22.83
14.63
200
180
160
140
120
100
80
60
40
20
ppm

HPLC-MS, ^{1}H-NMR and ^{13}C-NMR analysis of the compound **36t**

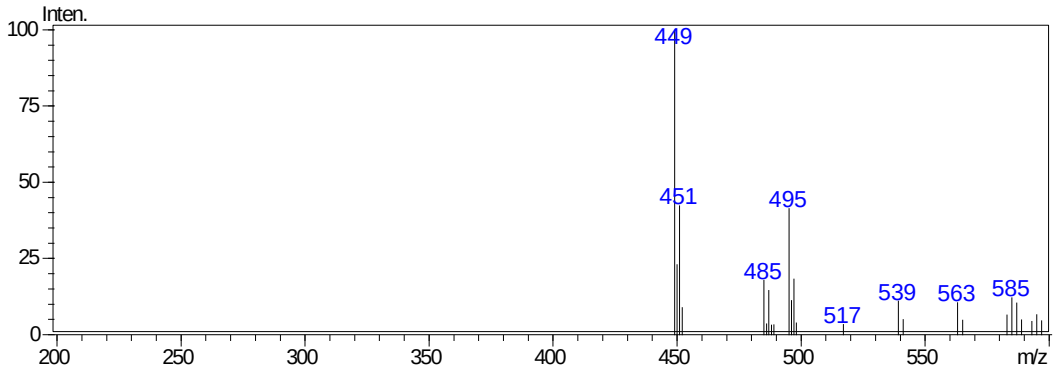


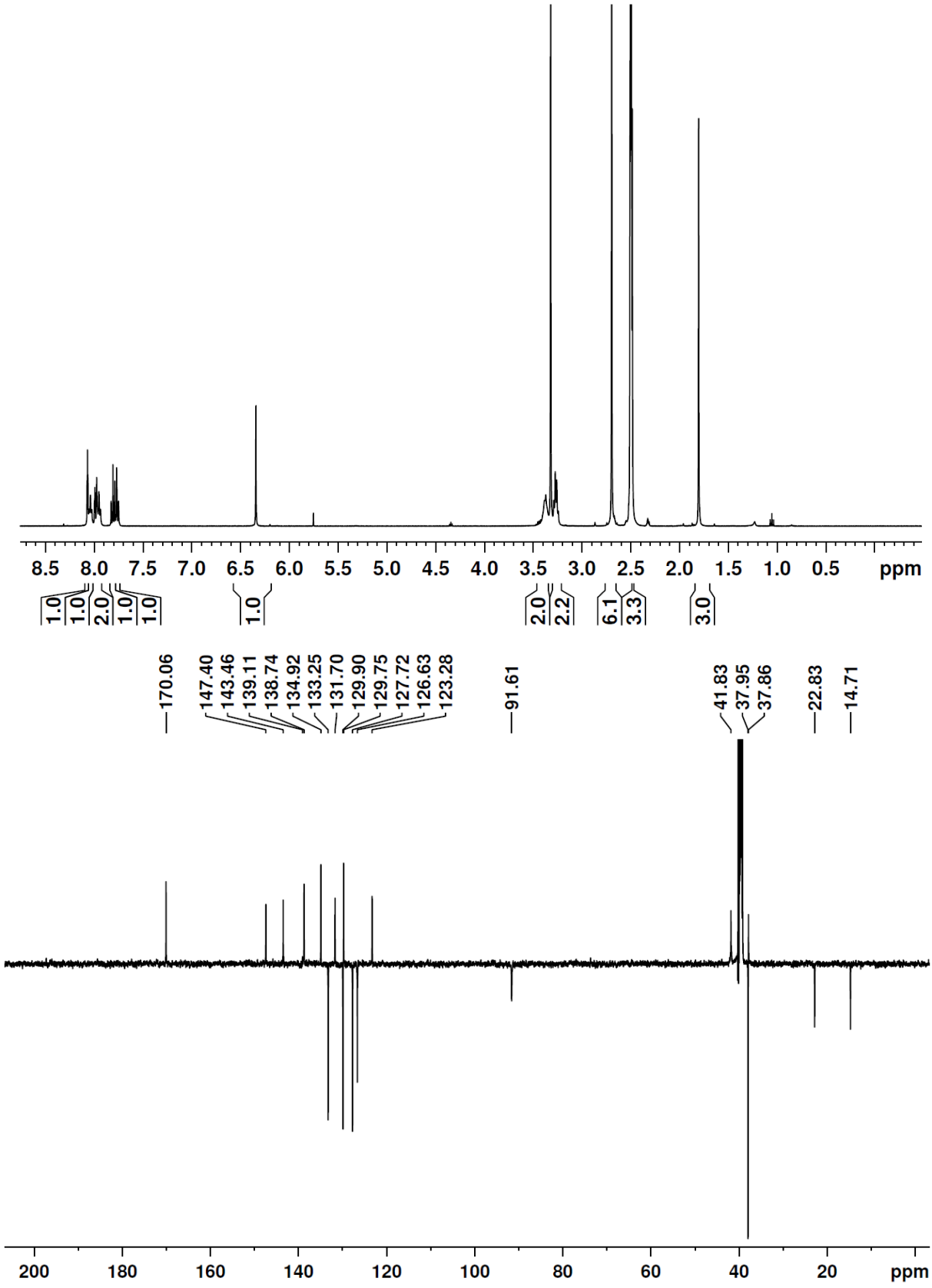
36t

Exact Mass: 450,124
Molecular Weight: 450,942

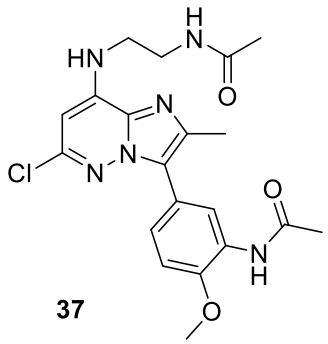


ESI-



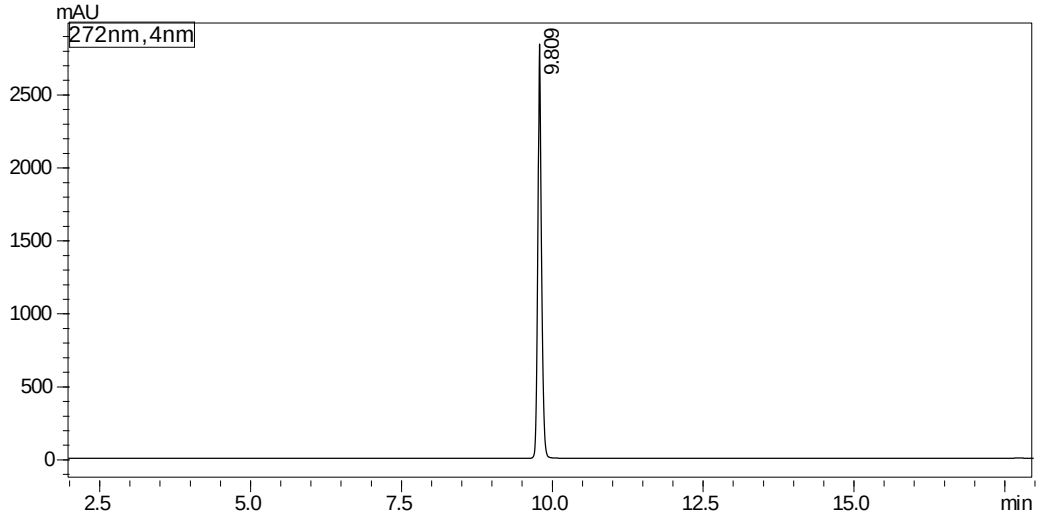

8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.0 1.0 2.0 1.0 1.0 1.0 2.0 2.2 6.1 3.3 3.0
170.06 147.40 143.46 139.11 138.74 134.92 133.25 131.70 129.90 129.75 127.72 126.63 123.28 91.61 41.83 37.95 37.86 22.83 14.71
200 180 160 140 120 100 80 60 40 20 ppm

HPLC-MS, ^{1}H-NMR and ^{13}C-NMR analysis of the compound **37**

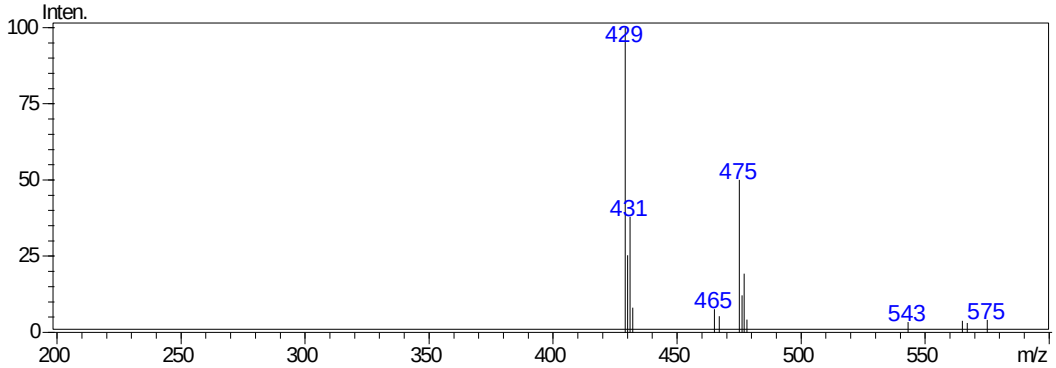


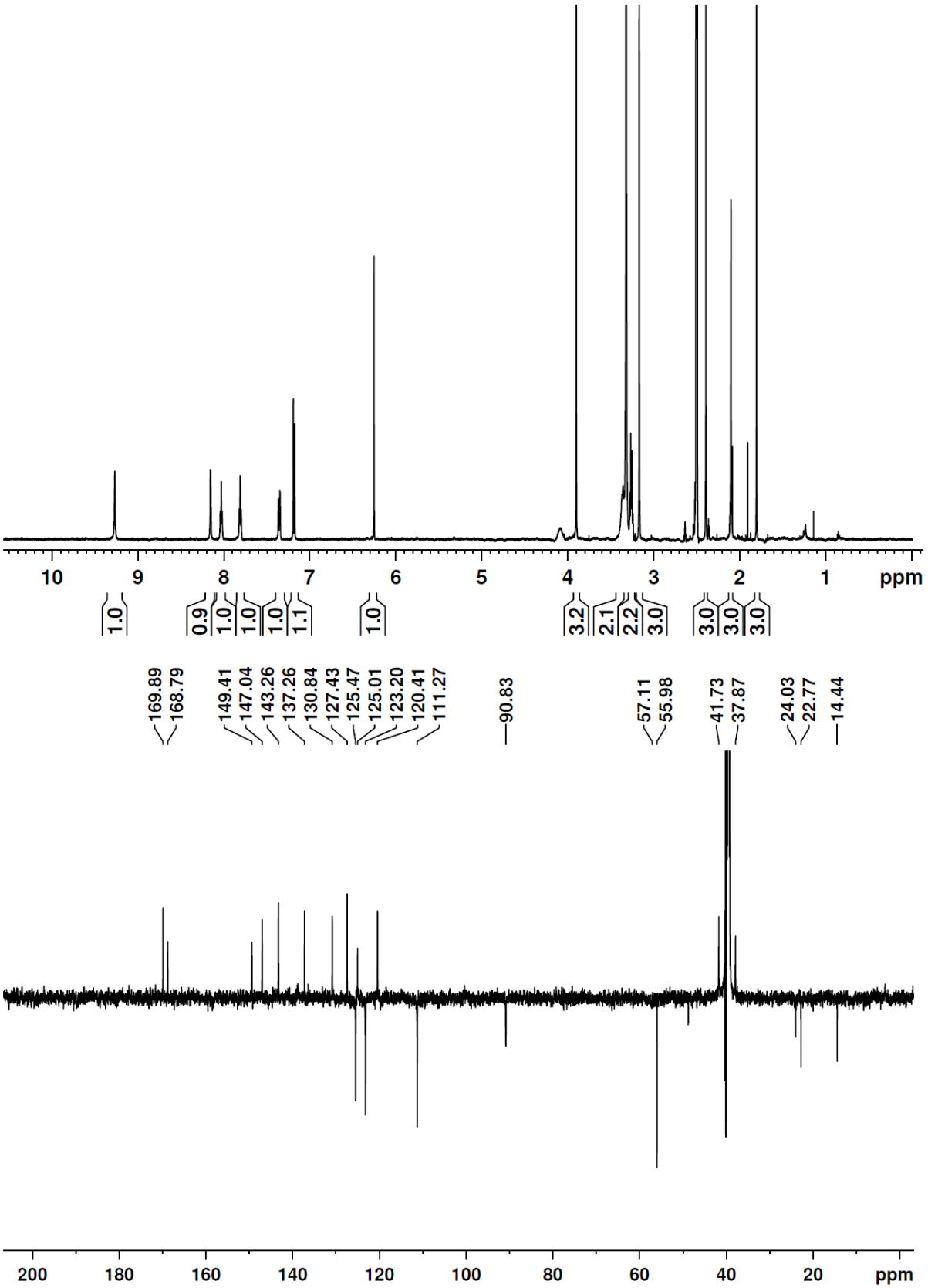
37

Exact Mass: 430,152
Molecular Weight: 430,893

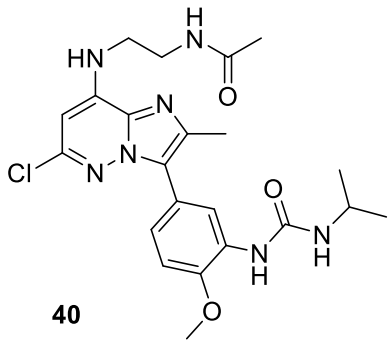


ESI-



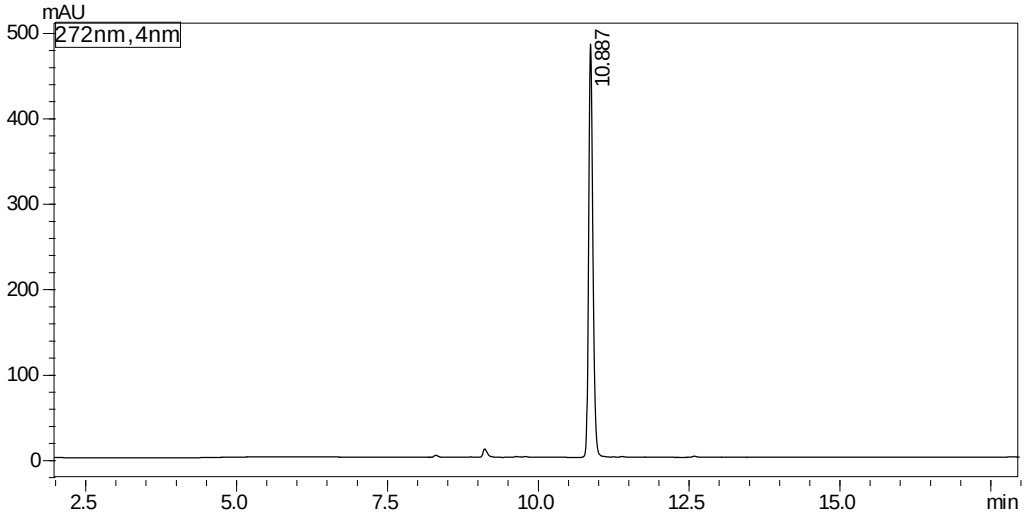

10
9
8
7
6
5
4
3
2
1
ppm
1.0
0.9
1.0
1.0
1.0
1.1
1.0
3.2
2.1
2.2
3.0
3.0
3.0
3.0
169.89
168.79
149.41
147.04
143.26
137.26
130.84
127.43
125.47
125.01
123.20
120.41
111.27
90.83
57.11
55.98
41.73
37.87
24.03
22.77
14.44
200
180
160
140
120
100
80
60
40
20
ppm

HPLC-MS, ^{1}H-NMR and ^{13}C-NMR analysis of the compound **40**

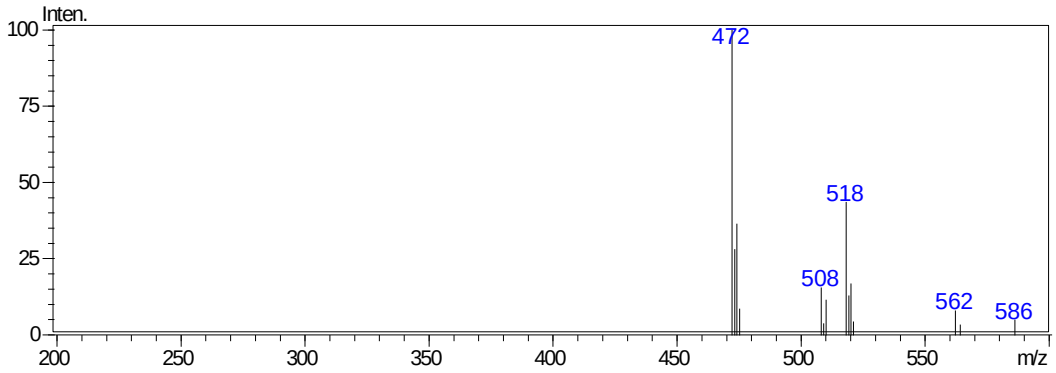


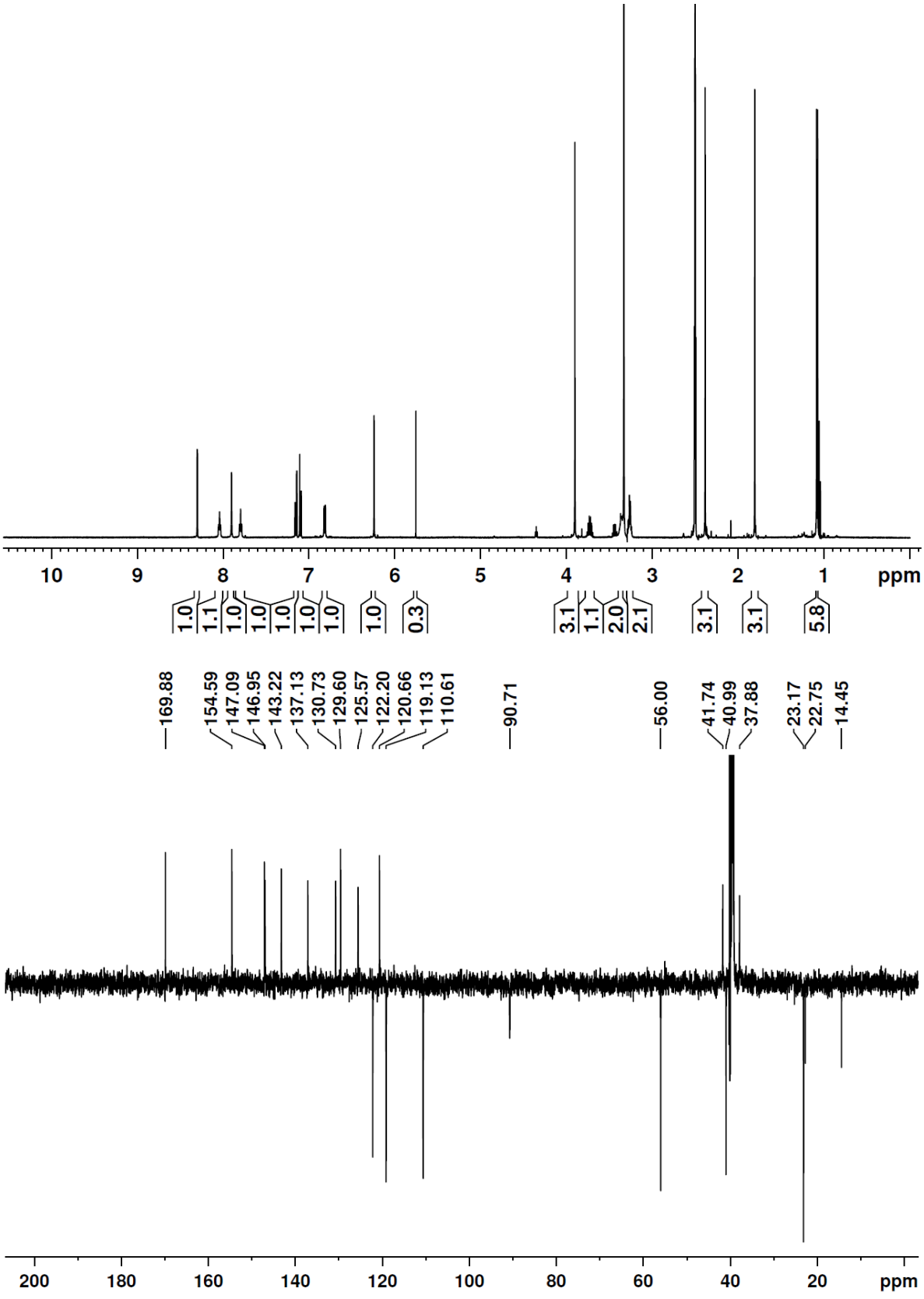
40

Exact Mass: 473,194
Molecular Weight: 473,962



ESI-

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9
8
7
6
5
4
3
2
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ppm
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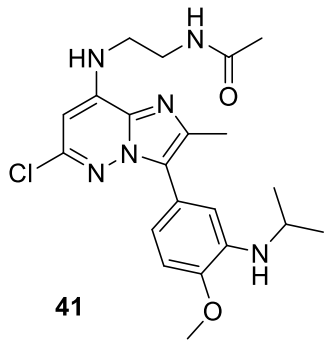
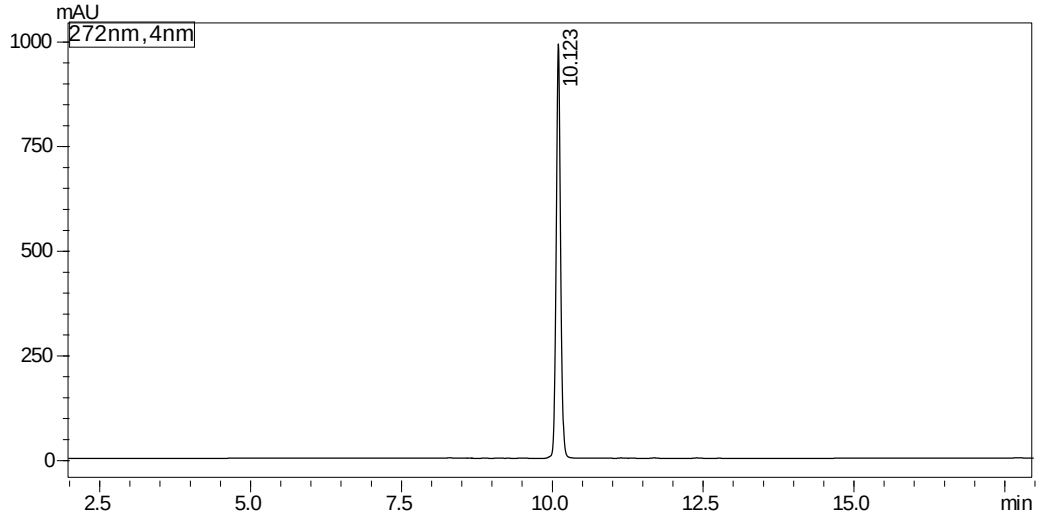
1.0
1.0
0.3
3.1
1.1
2.0
2.1
3.1
3.1
5.8
169.88
154.59
147.09
146.95
143.22
137.13
130.73
129.60
125.57
122.20
120.66
119.13
110.61
90.71
56.00
41.74
40.99
37.88
23.17
22.75
14.45
200
180
160
140
120
100
80
60
40
20
ppm

HPLC-MS, ^{1}H-NMR and ^{13}C-NMR analysis of the compound **41**



41

Exact Mass: 430,188
Molecular Weight: 430,937



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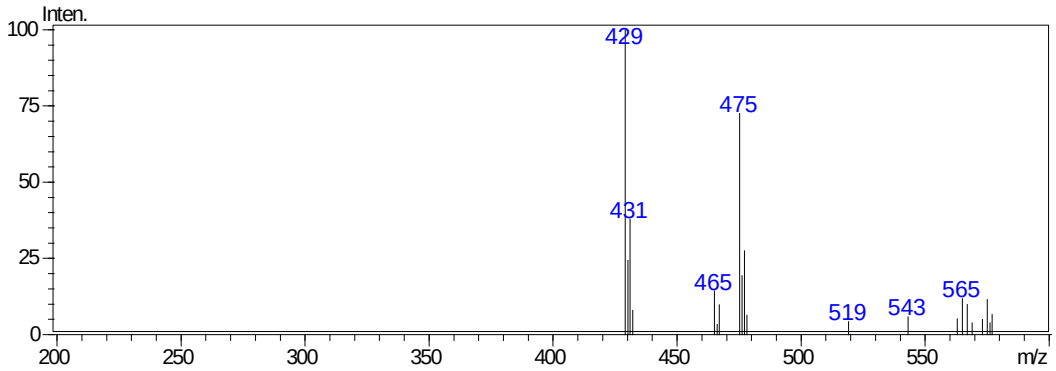

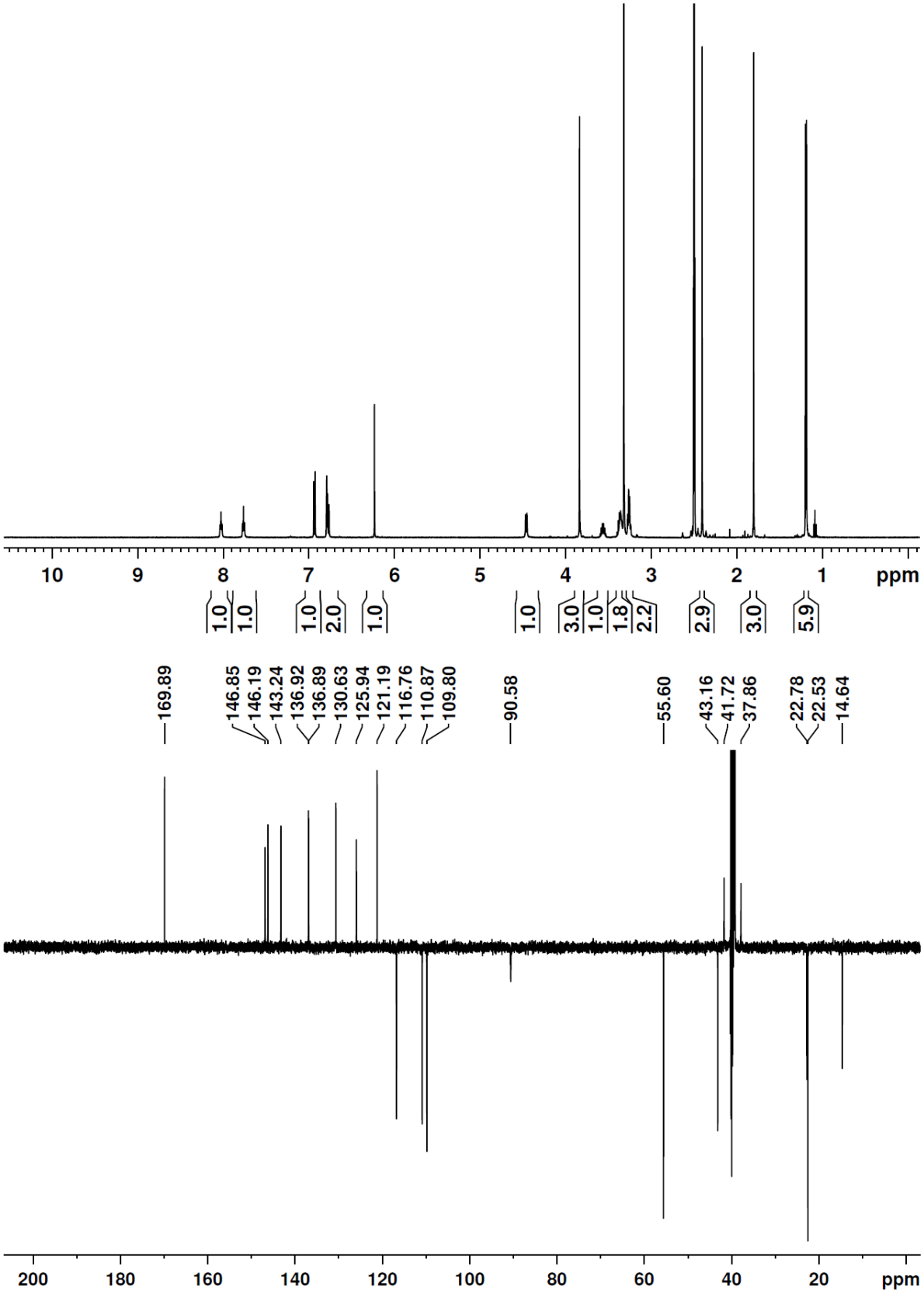
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6
5
4
3
2
1
ppm
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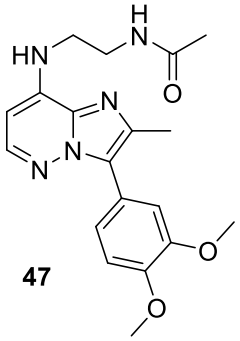
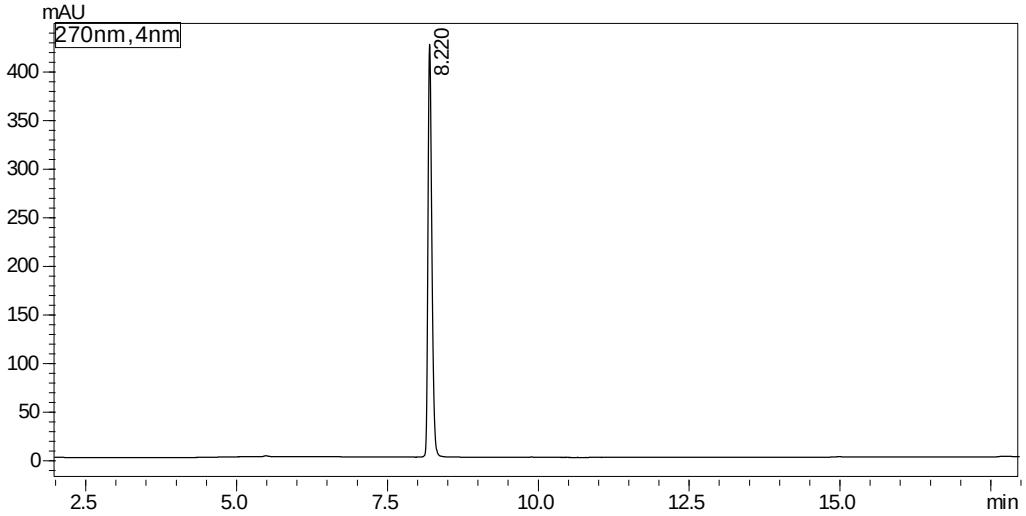
1.0
2.0
1.0
1.0
3.0
1.0
1.8
2.2
2.9
3.0
5.9
169.89
146.85
146.19
143.24
136.92
136.89
130.63
125.94
121.19
116.76
110.87
109.80
90.58
55.60
43.16
41.72
37.86
22.78
22.53
14.64
200
180
160
140
120
100
80
60
40
20
ppm

HPLC-MS, ^{1}H-NMR and ^{13}C-NMR analysis of the compound **47**

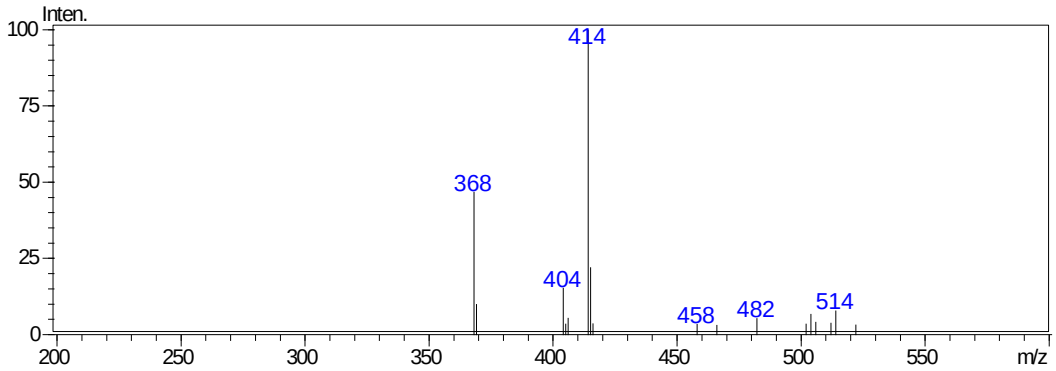


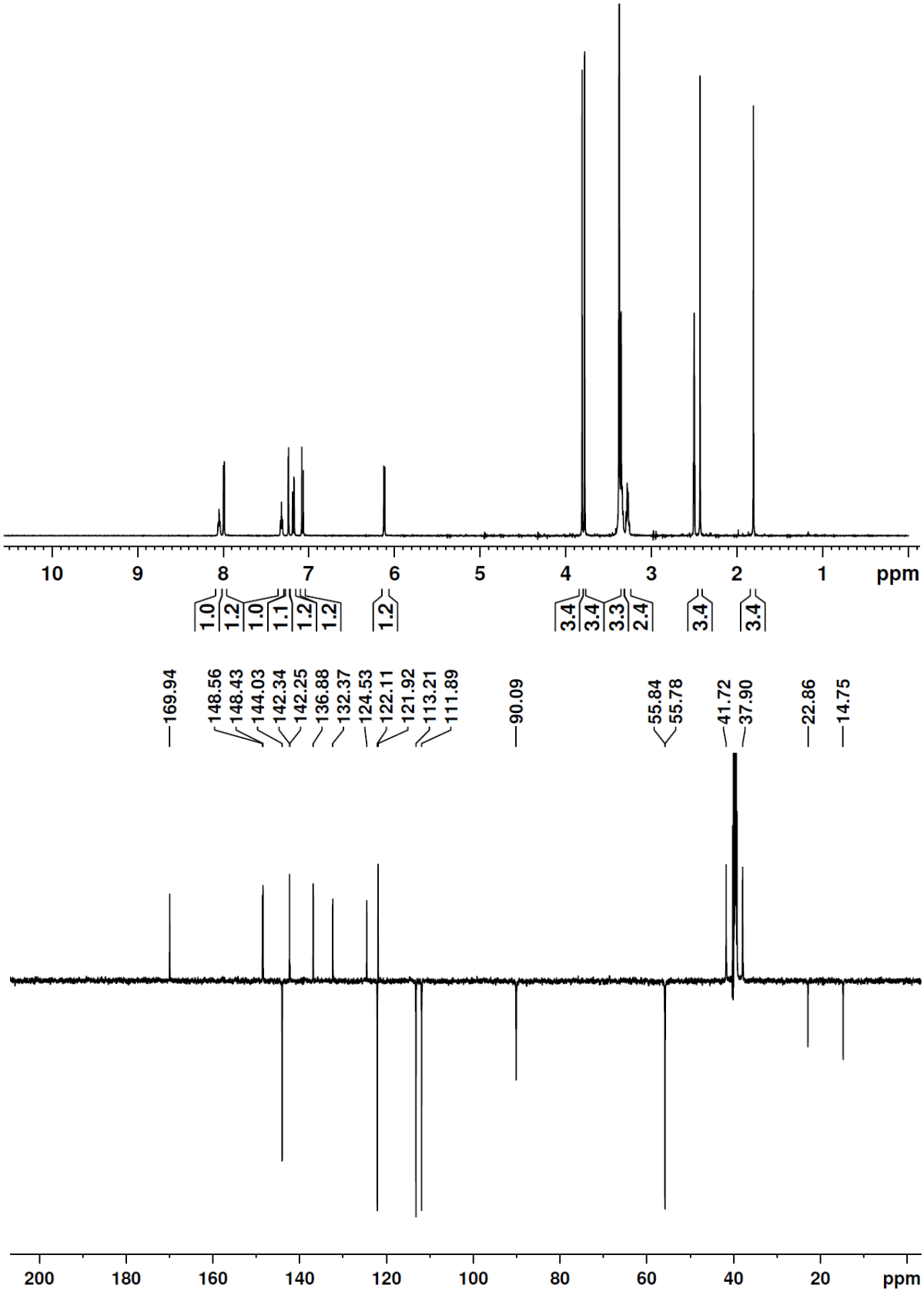
47

Exact Mass: 369,180
Molecular Weight: 369,425



ESI-

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5
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2
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ppm
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1.2
1.0
1.1
1.2
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1.2
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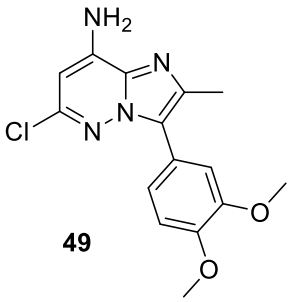
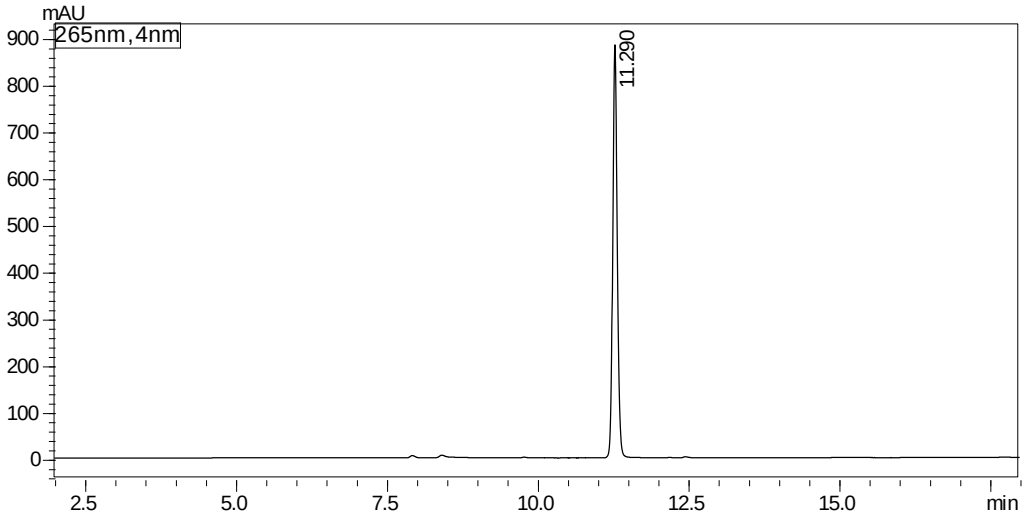
3.4
3.3
2.4
3.4
3.4
169.94
148.56
148.43
144.03
142.34
142.25
136.88
132.37
124.53
122.11
121.92
113.21
111.89
90.09
55.84
55.78
41.72
37.90
22.86
14.75
200
180
160
140
120
100
80
60
40
20
ppm

HPLC-MS, ^{1}H-NMR and ^{13}C-NMR analysis of the compound **49**

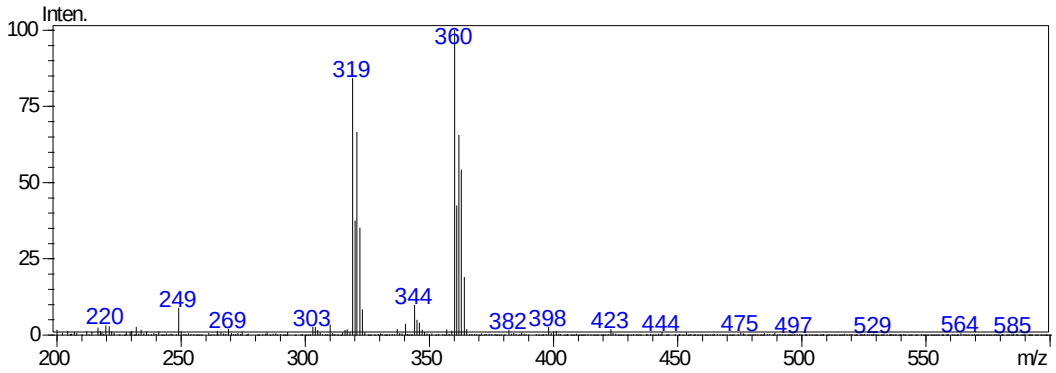


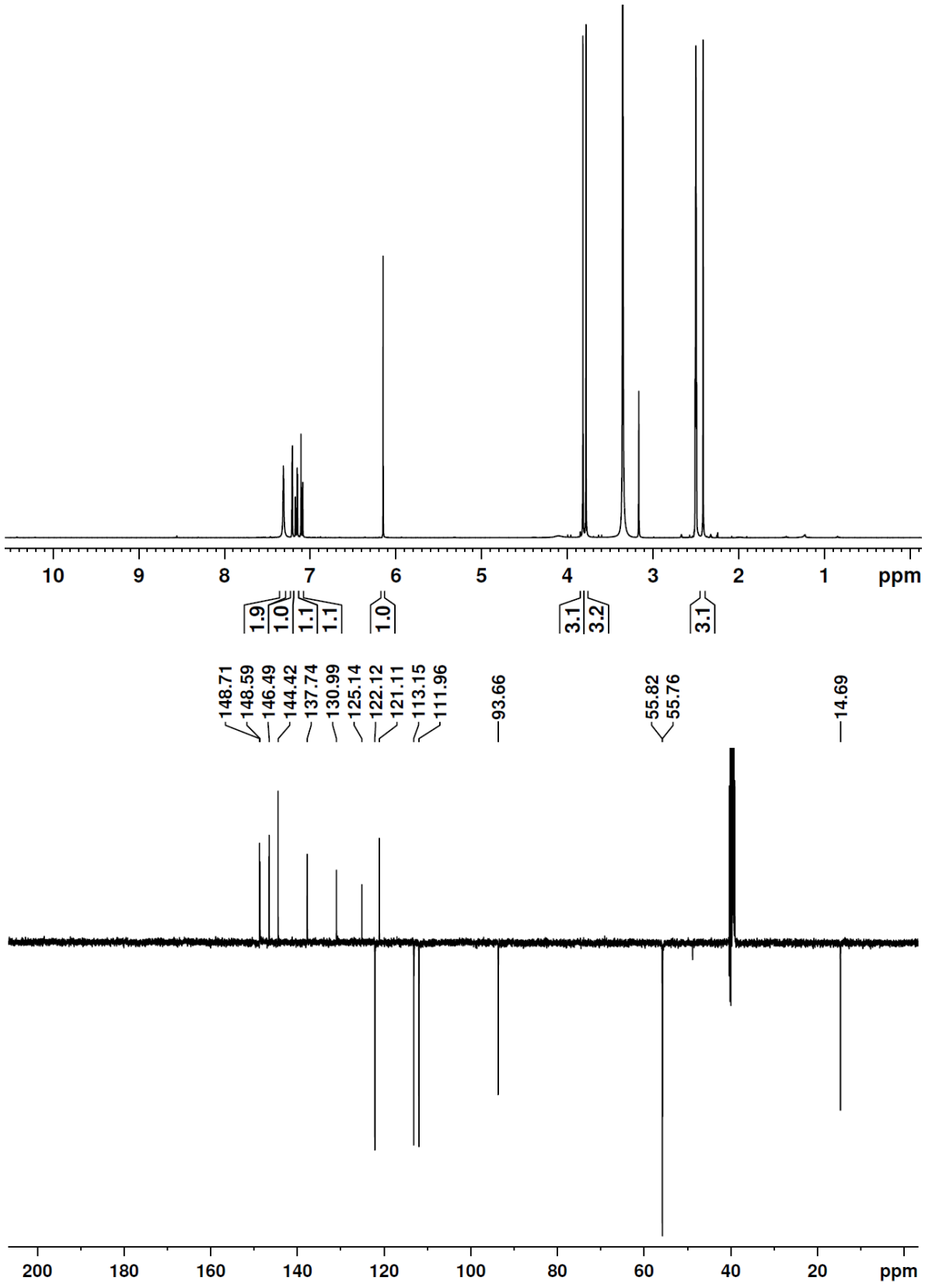
49

Exact Mass: 318,088
Molecular Weight: 318,761



ESI+

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ppm
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1.1
1.1
1.0
3.1
3.2
3.1
148.71
148.59
146.49
144.42
137.74
130.99
125.14
122.12
121.11
113.15
111.96
93.66
55.82
55.76
14.69
200
180
160
140
120
100
80
60
40
20
ppm