Supplemental Information

Hinge Binder Scaffold Hopping Identifies Potent Calcium/Calmodulin-Dependent Protein Kinase Kinase 2 (CAMKK2) Inhibitor Chemotypes

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⊥ UNC Lineberger Comprehensive Cancer Center, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599, USA

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Supporting Information

Content

1. Mass spectrometry method

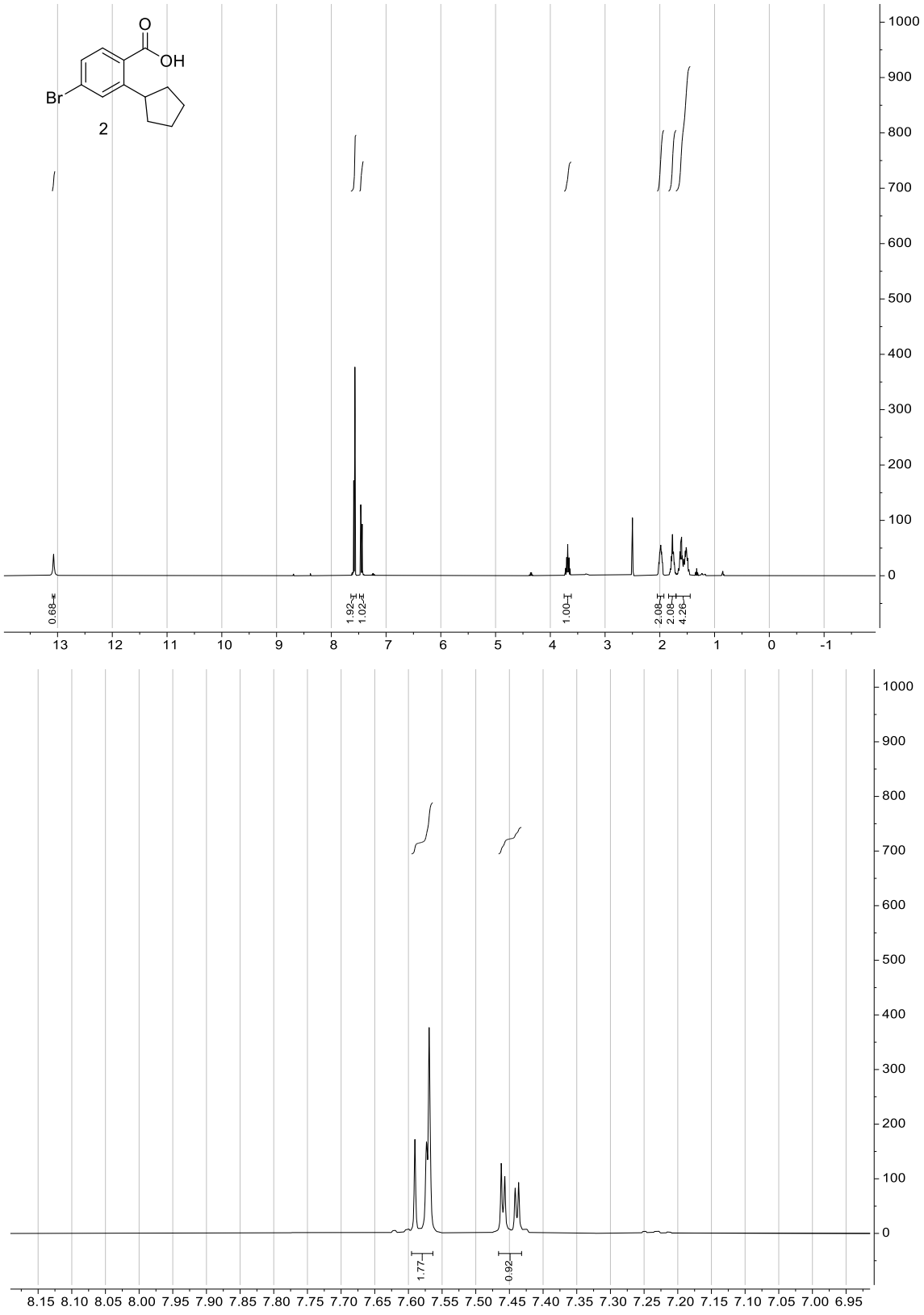
Samples were analyzed with a ThermoFisher Q Exactive HF-X (ThermoFisher, Bremen, Germany) mass spectrometer coupled with a Waters Acquity H-class liquid chromatograph system. Samples were introduced via a heated electrospray source (HESI) at a flow rate of 0.6 mL/min. Electrospray source conditions were set as: spray voltage 3.0 kV, sheath gas (nitrogen) 60 arb, auxiliary gas (nitrogen) 20 arb, sweep gas (nitrogen) 0 arb, nebulizer temperature 375 degrees C, capillary temperature 380 °C, RF funnel 45 V. The mass range was set to 150-2,000 m/z. All measurements were recorded at a resolution setting of 120,000.

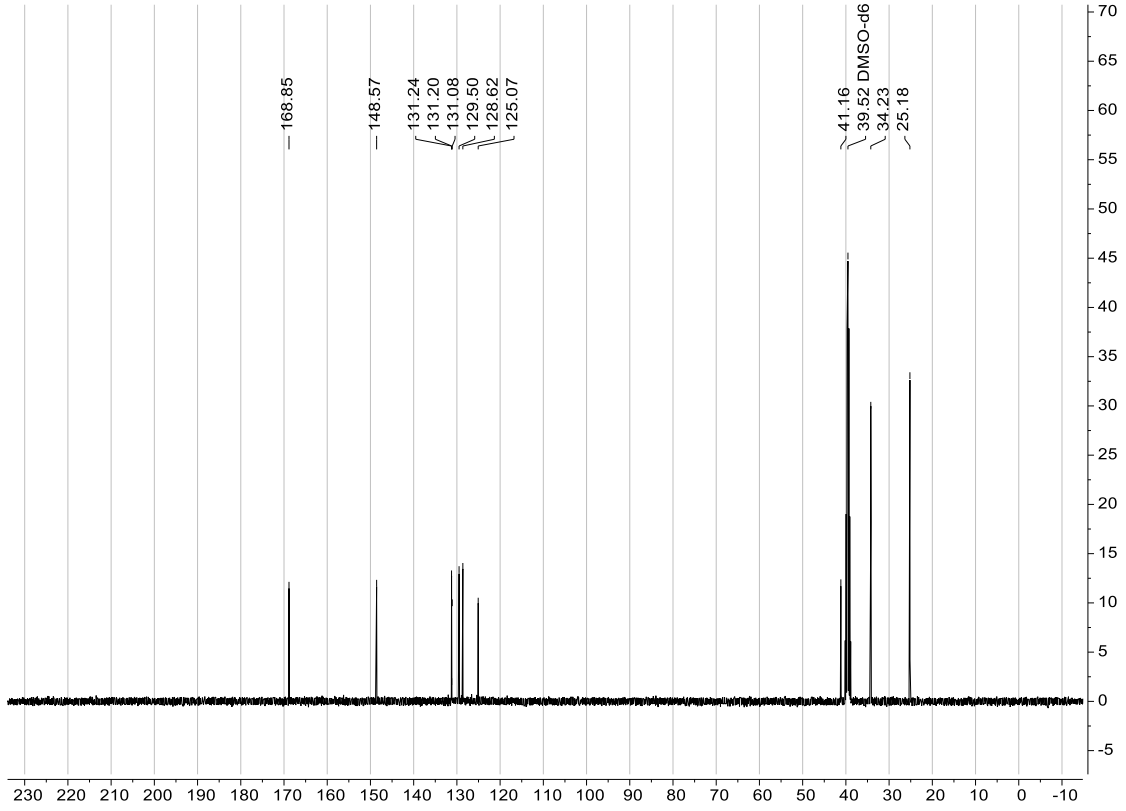
Separations were conducted on a Waters Acquity UPLC BEH C18 column (2.1 x 50 mM, 1.7 μM particle size). LC conditions were set at 100 % water with 0.1 % formic acid (A) ramped linearly over 9.8 min to 95 % acetonitrile with 0.1 % formic acid (B) and held until 10.2 min. At 10.21 min the gradient was switched back to 100 % A and allowed to re-equilibrate until 11.25 min. Injection volume for all samples was 3 μL.

Xcalibur (ThermoFisher, Breman, Germany) was used to analyze the data. Solutions were analyzed at 0.1 mg/mL or less based on responsiveness to the ESI mechanism. Molecular formula assignments were determined with Molecular Formula Calculator (v 1.2.3). All observed species were singly charged, as verified by unit m/z separation between mass spectral peaks corresponding to the ^{12}C and $^{13}C^{12}C_{c-1}$ isotope for each elemental composition.

2. Compound Characterization Spectra

4-Bromo-2-cyclopentylbenzoic acid (2)

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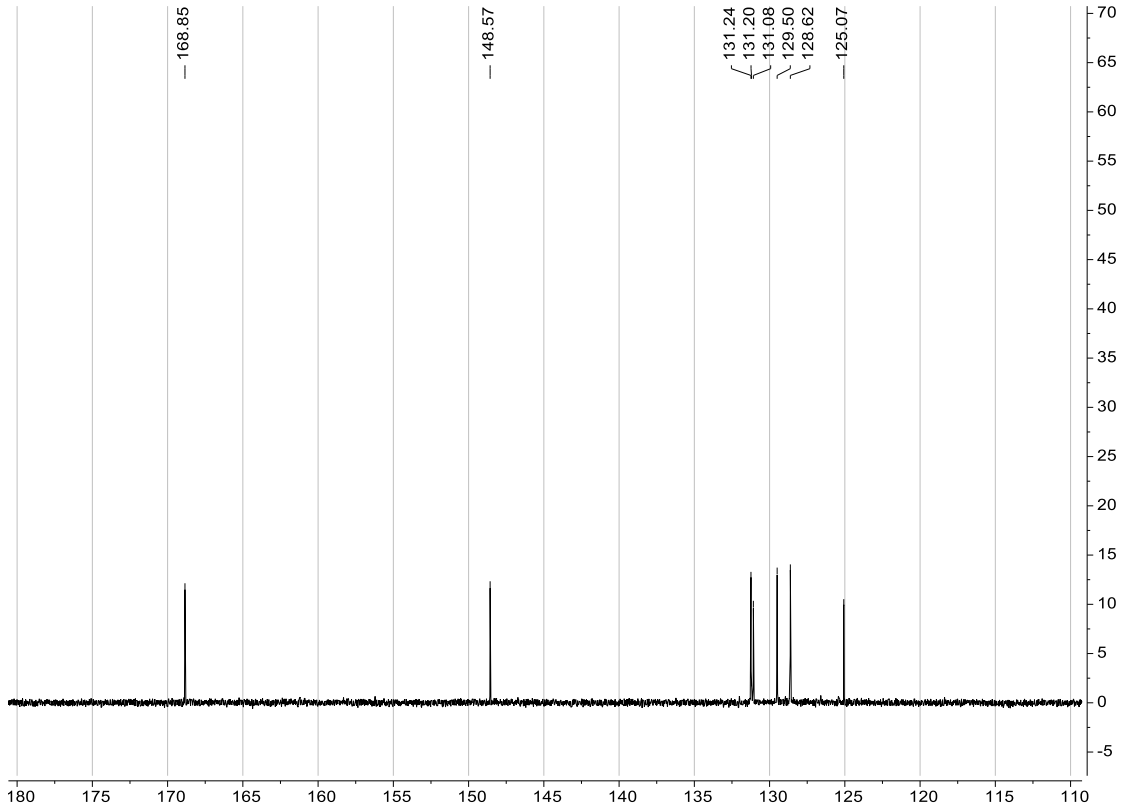
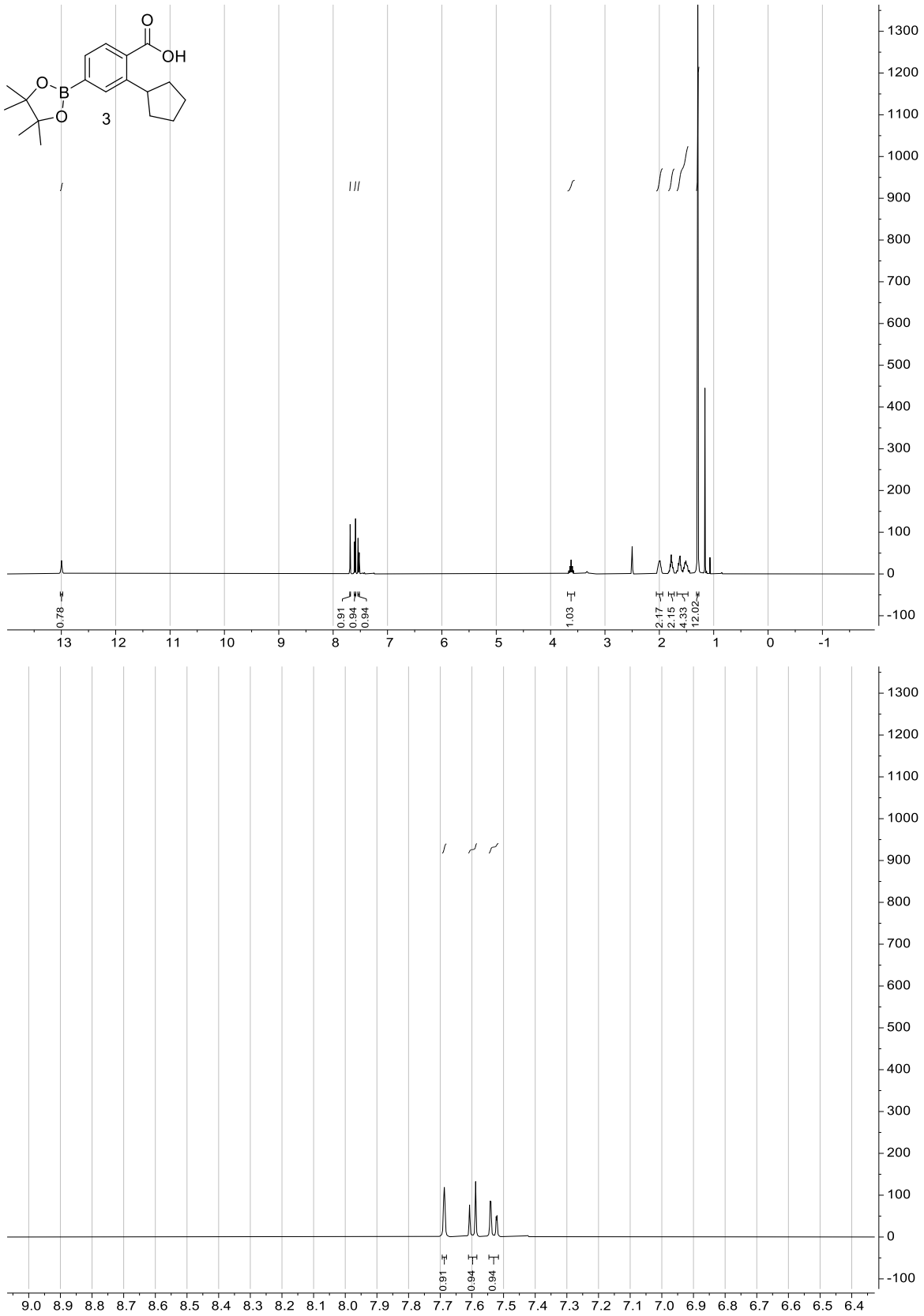
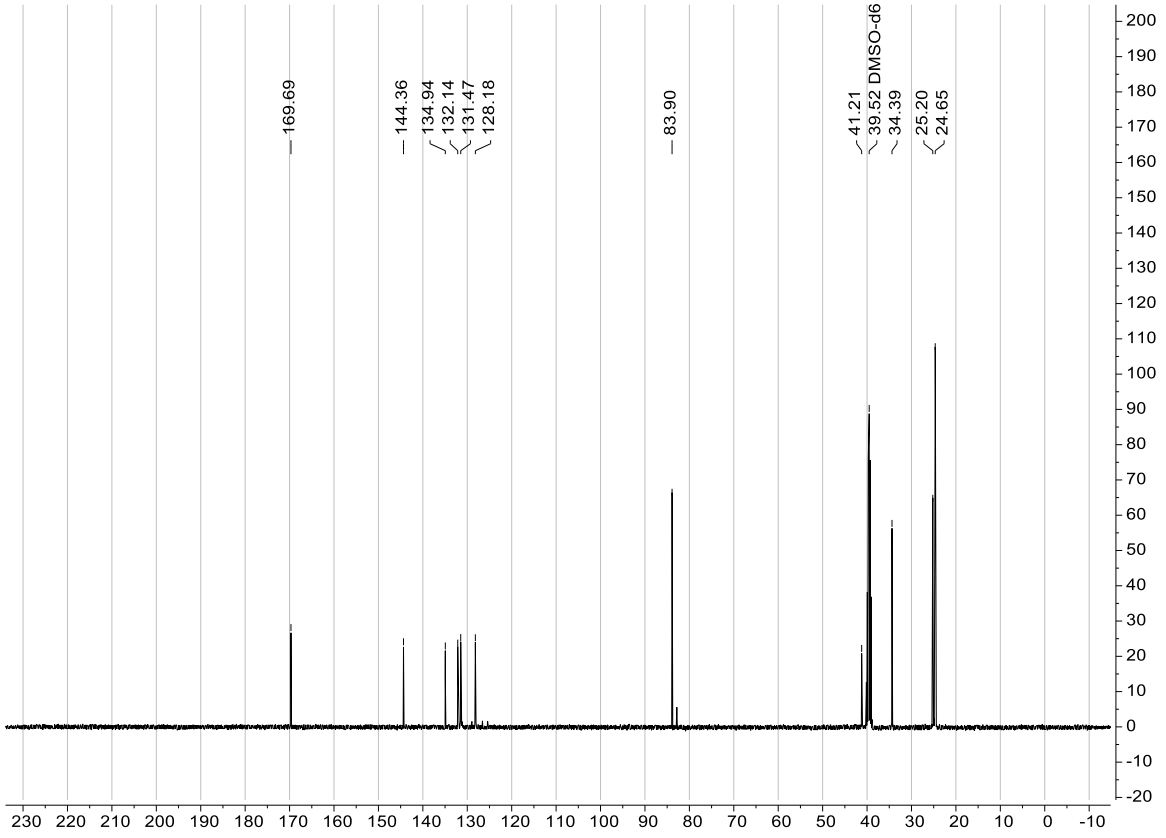
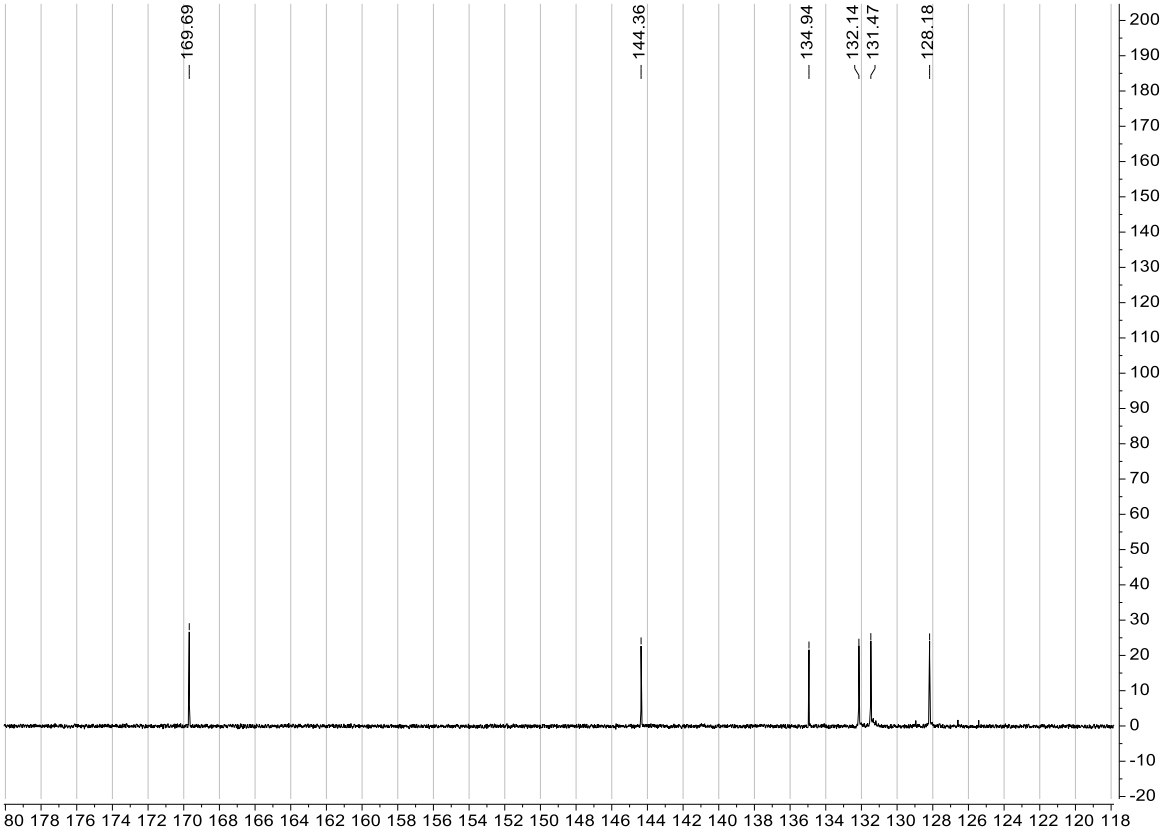
131.20
131.08
129.50
128.62
125.07
41.16
39.52 DMSO-d6
34.23
25.18


168.85
148.57
131.24
131.20
131.08
129.50
128.62
125.07

2-Cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid (3)




169.69
144.36
134.94
132.14
131.47
128.18
83.90
41.21
39.52 DMSO-d6
34.39
25.20
24.65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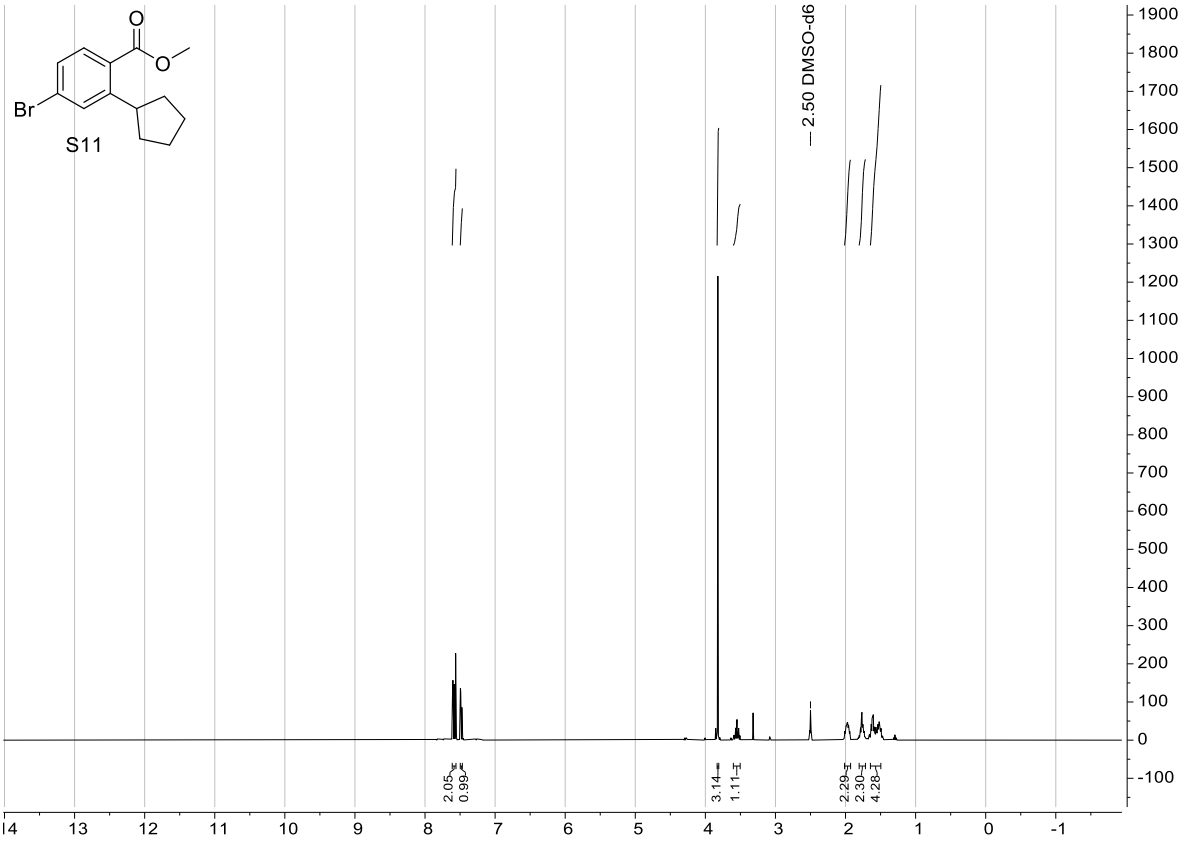
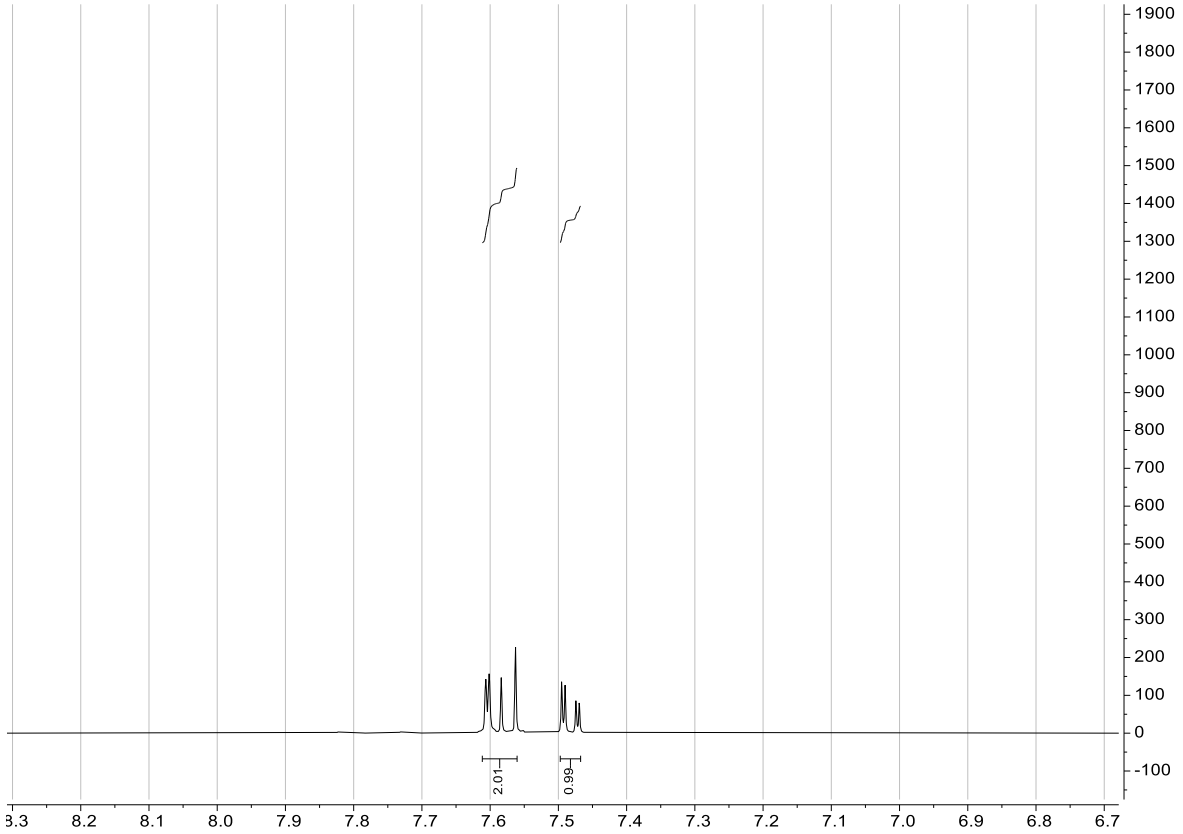
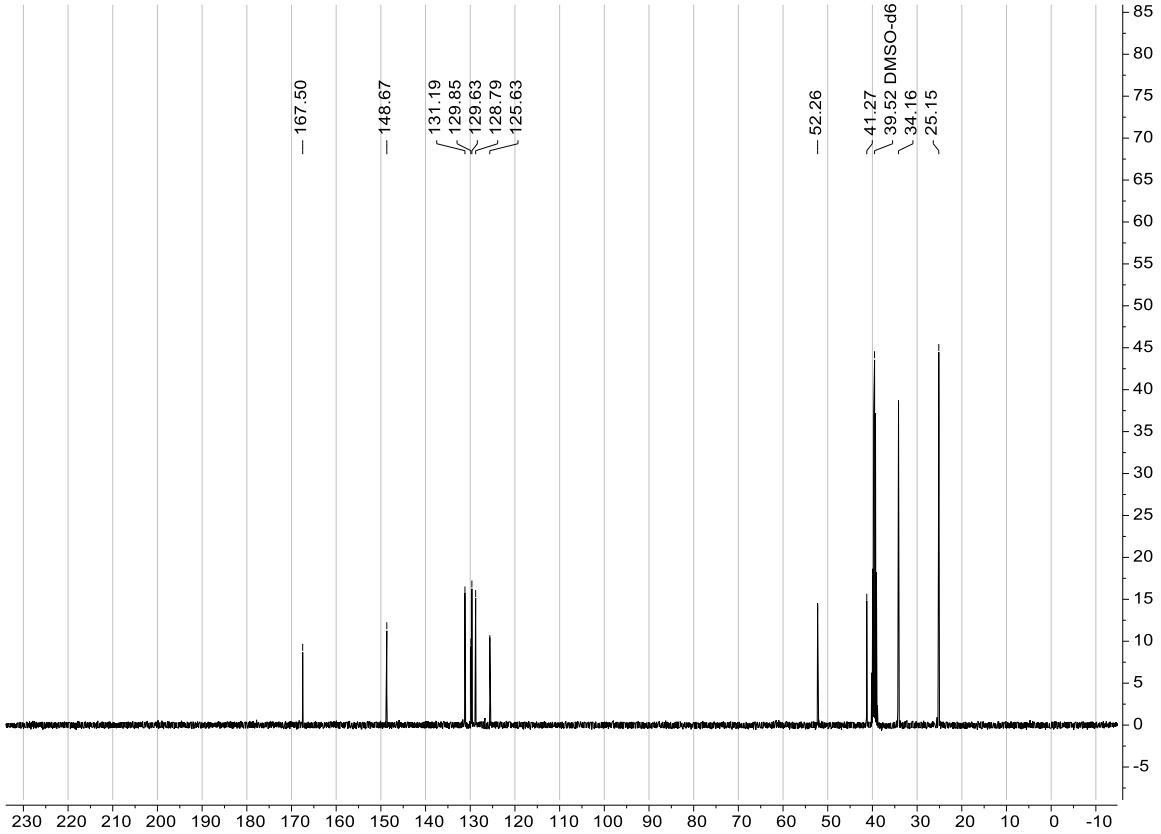
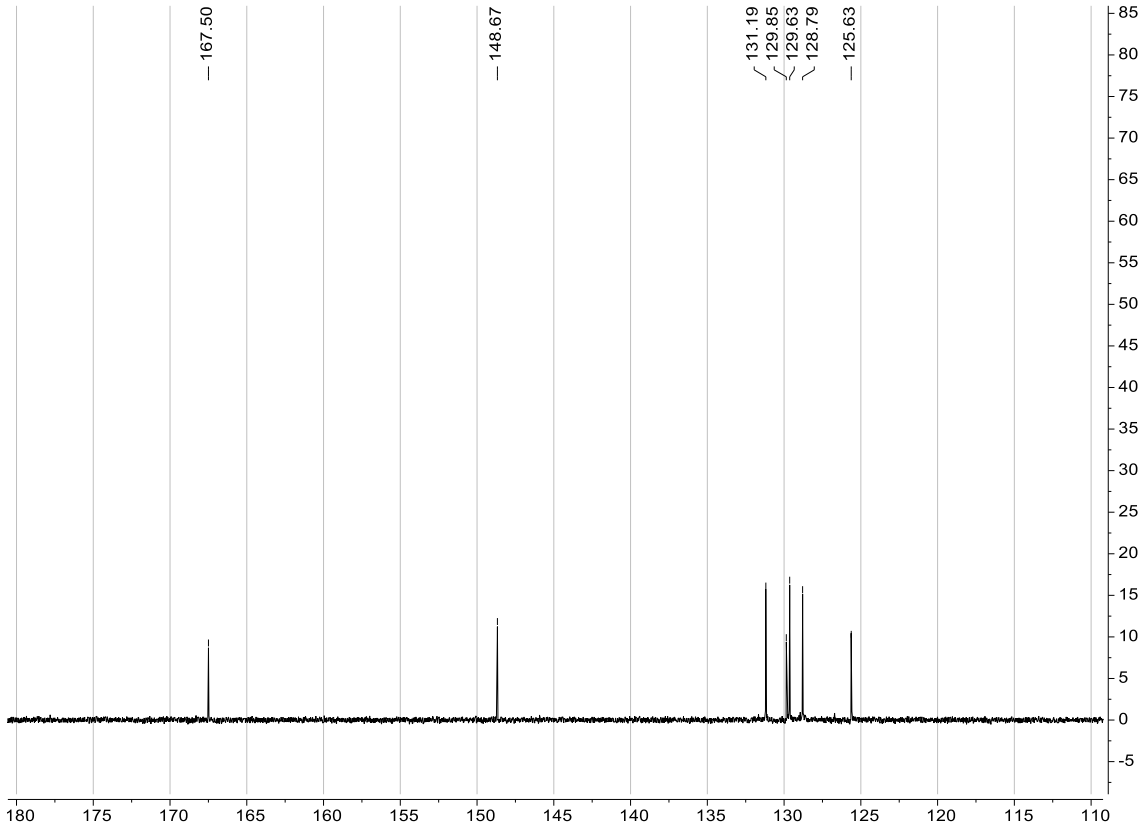
144.36
134.94
132.14
131.47
128.18

Methyl 4-bromo-2-cyclopentylbenzoate (SI1)

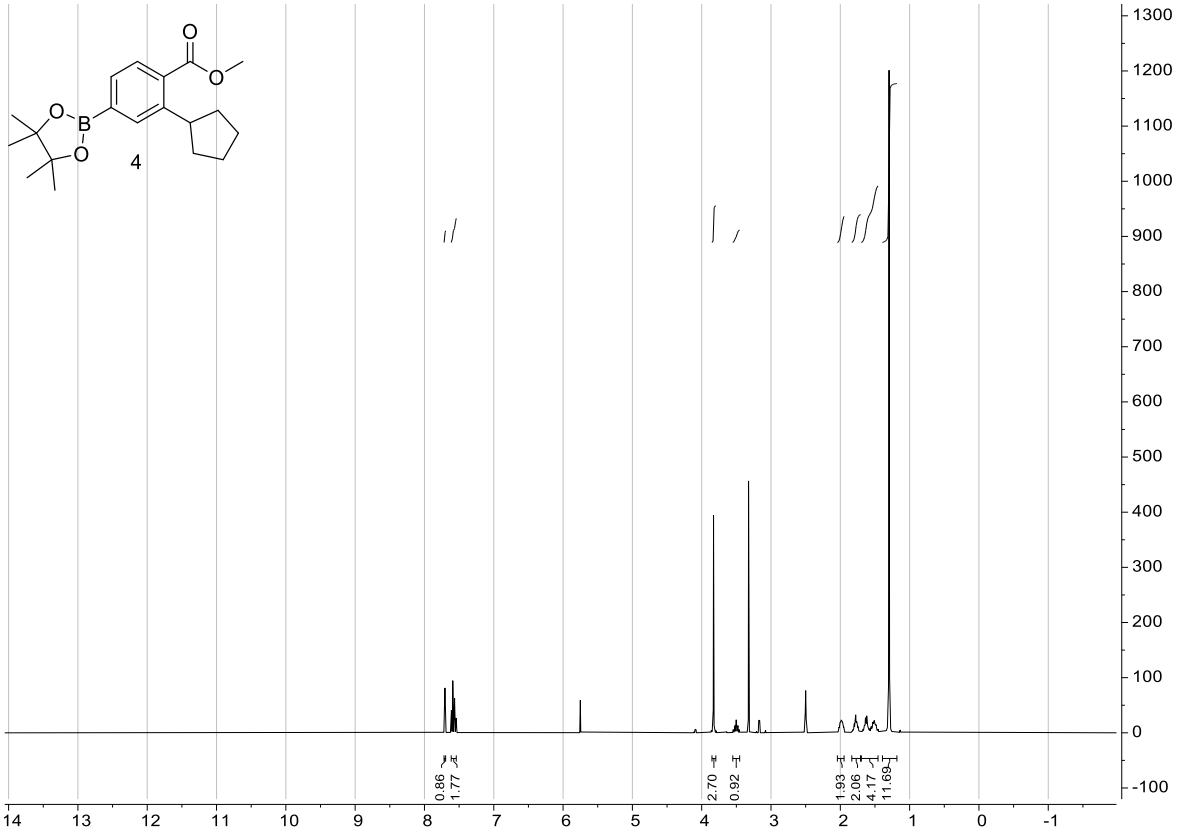


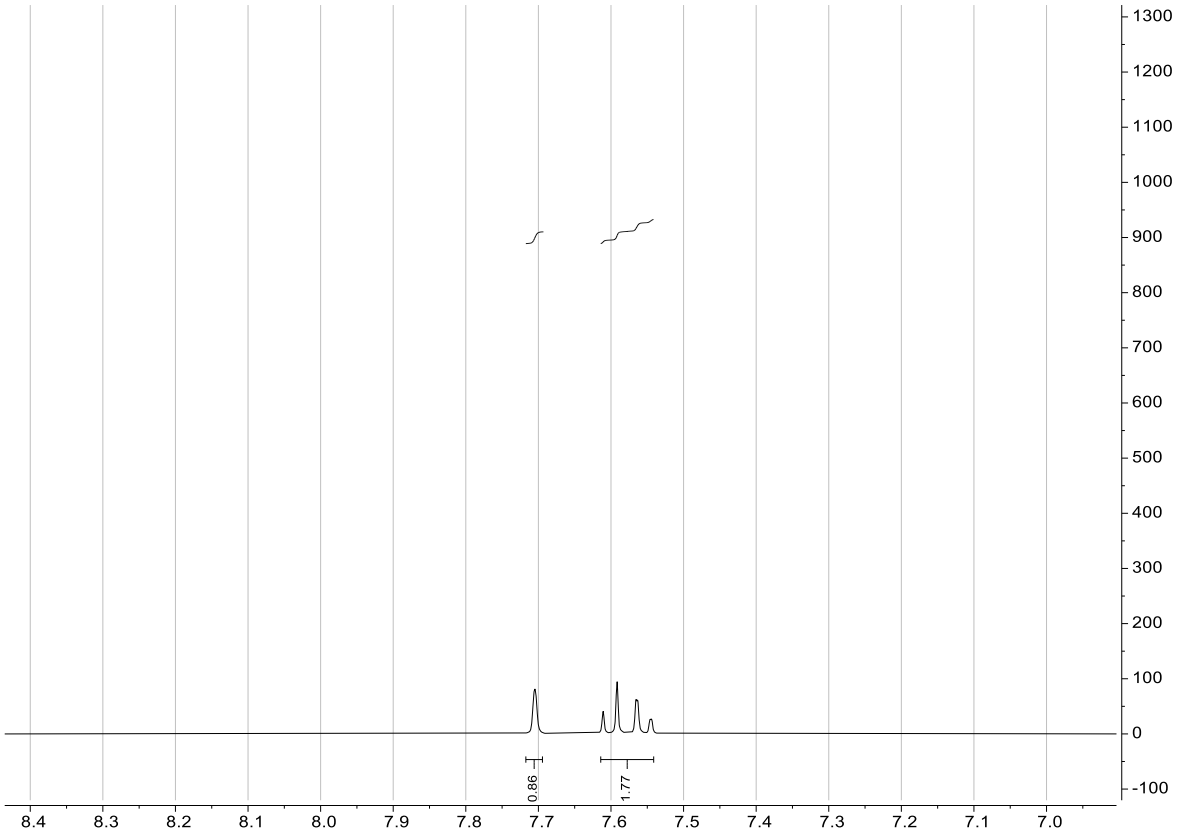



167.50
148.67
131.19
129.85
129.63
128.79
125.63
52.26
41.27
39.52 DMSO-d6
34.16
25.15

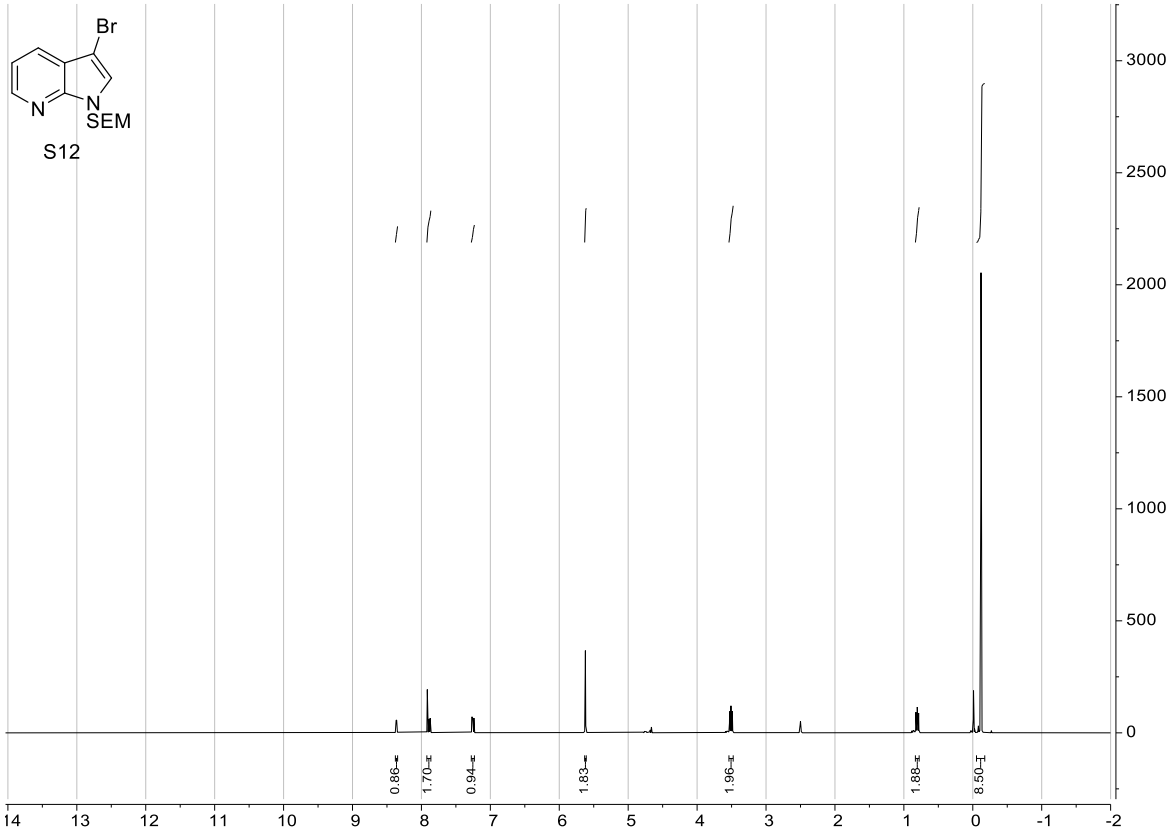

167.50
148.67
131.19
129.85
129.63
128.79
125.63

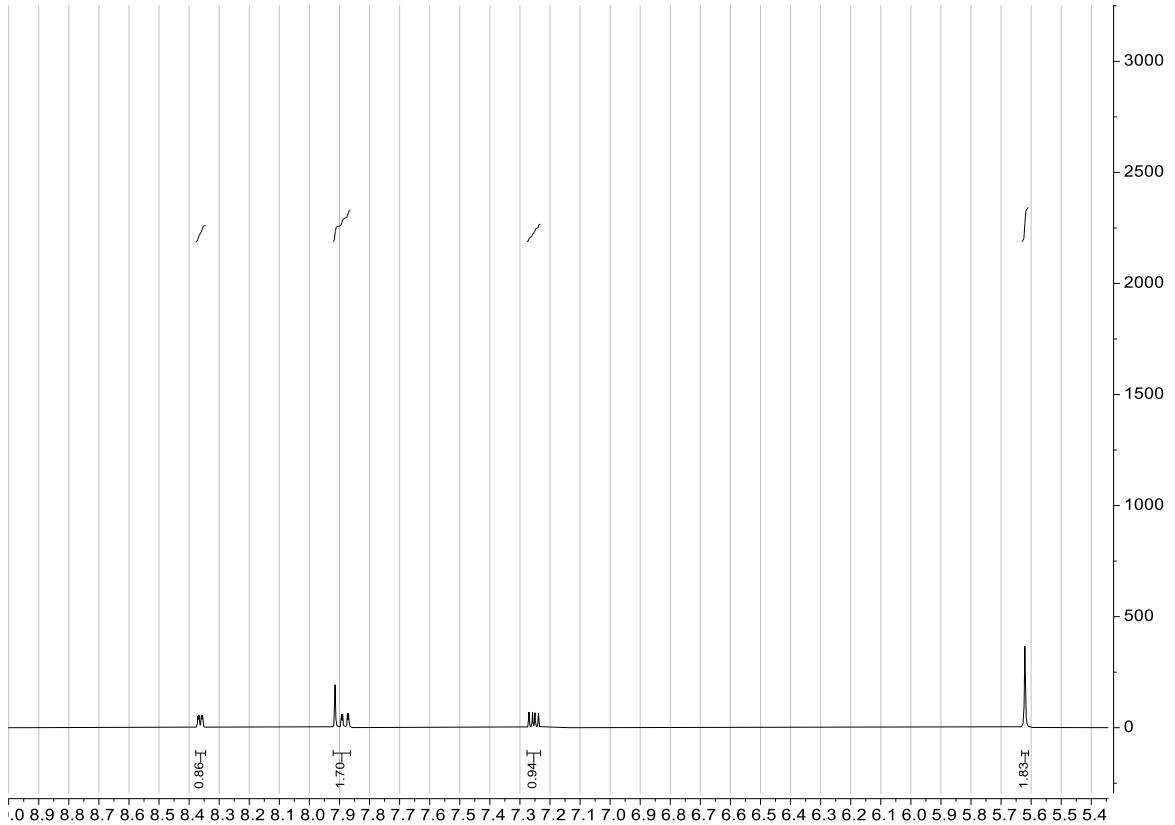
Methyl 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (4)

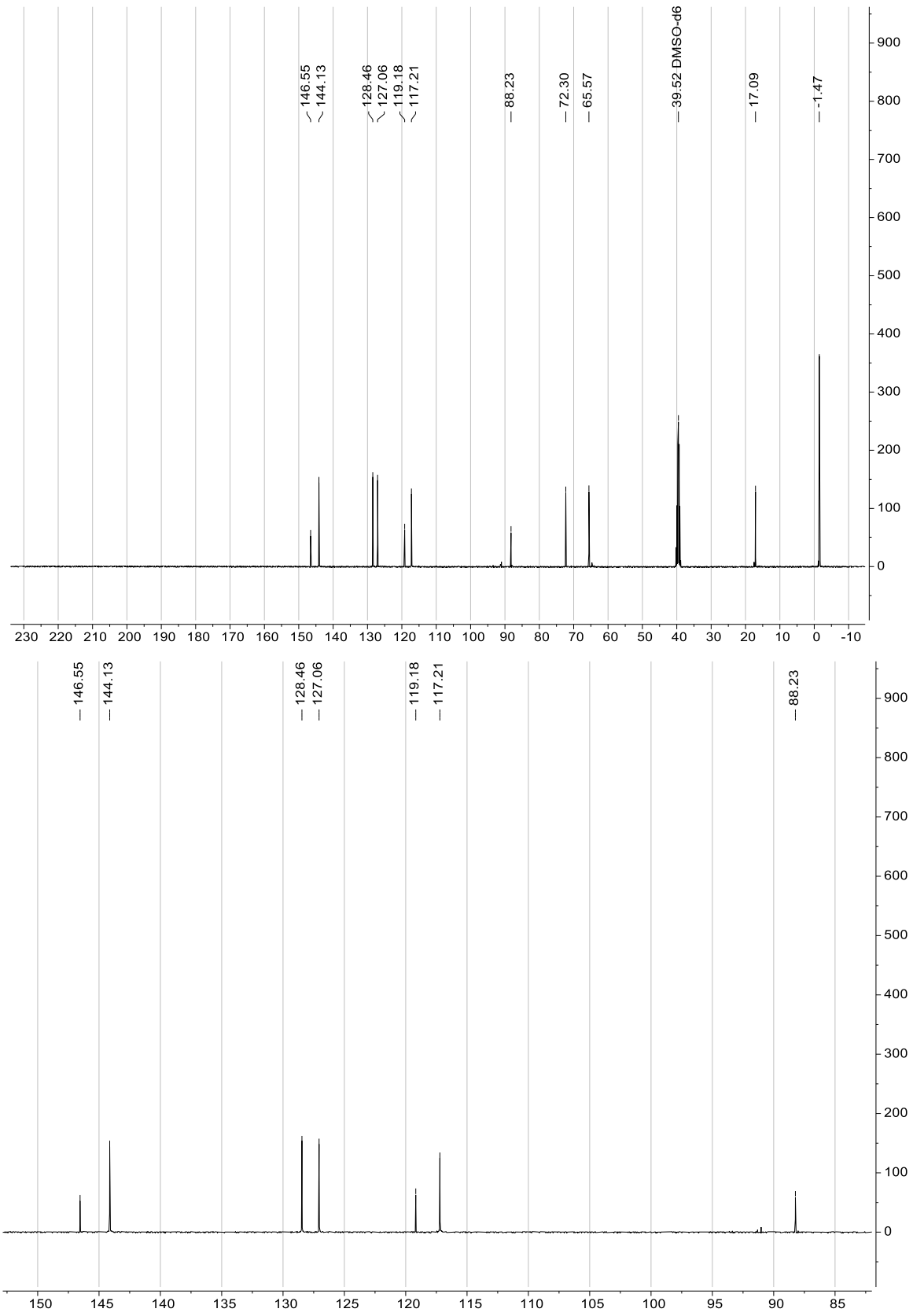




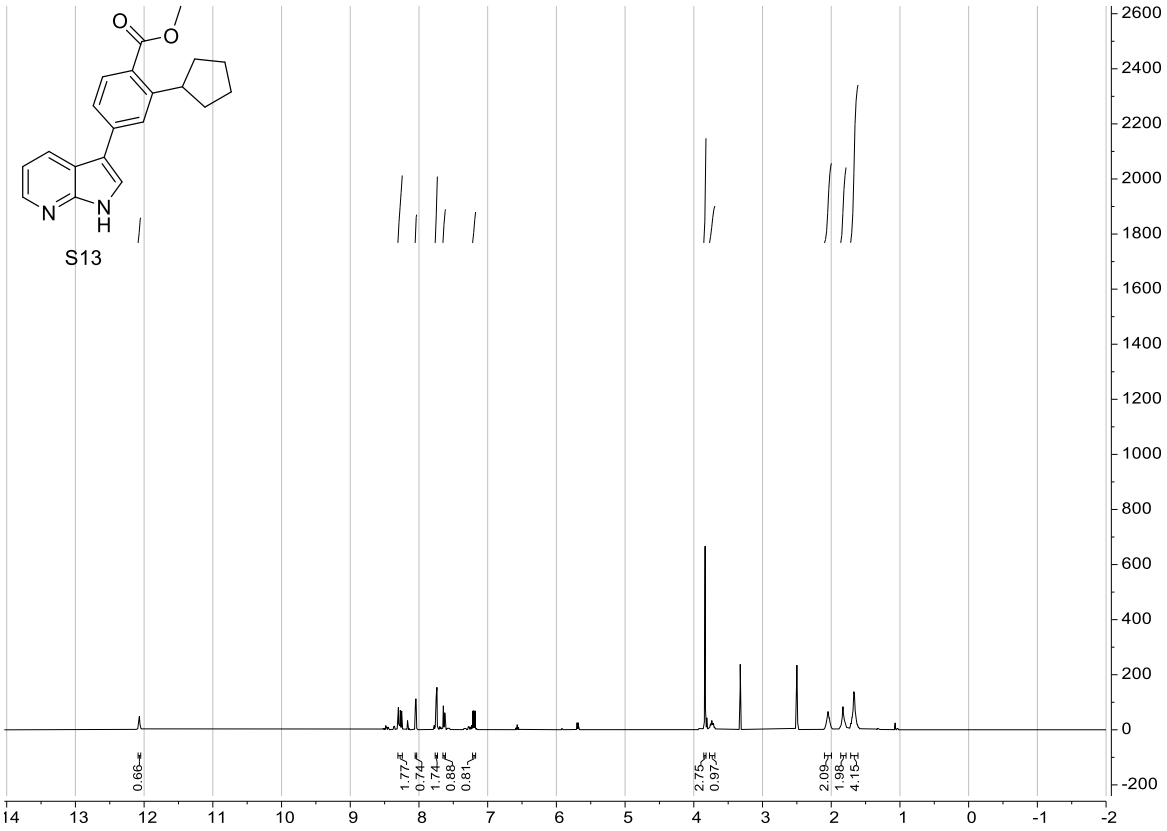
3-bromo-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrrolo[2,3-*b*]pyridine (SI2)

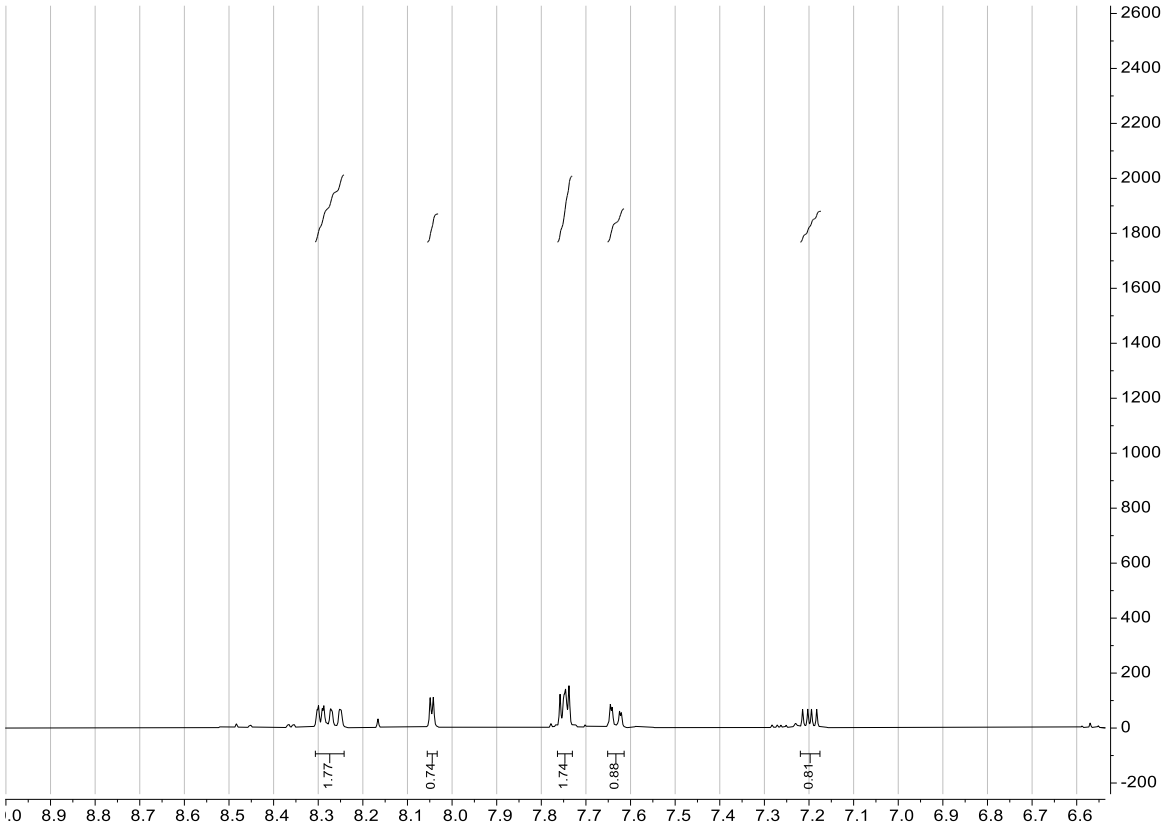


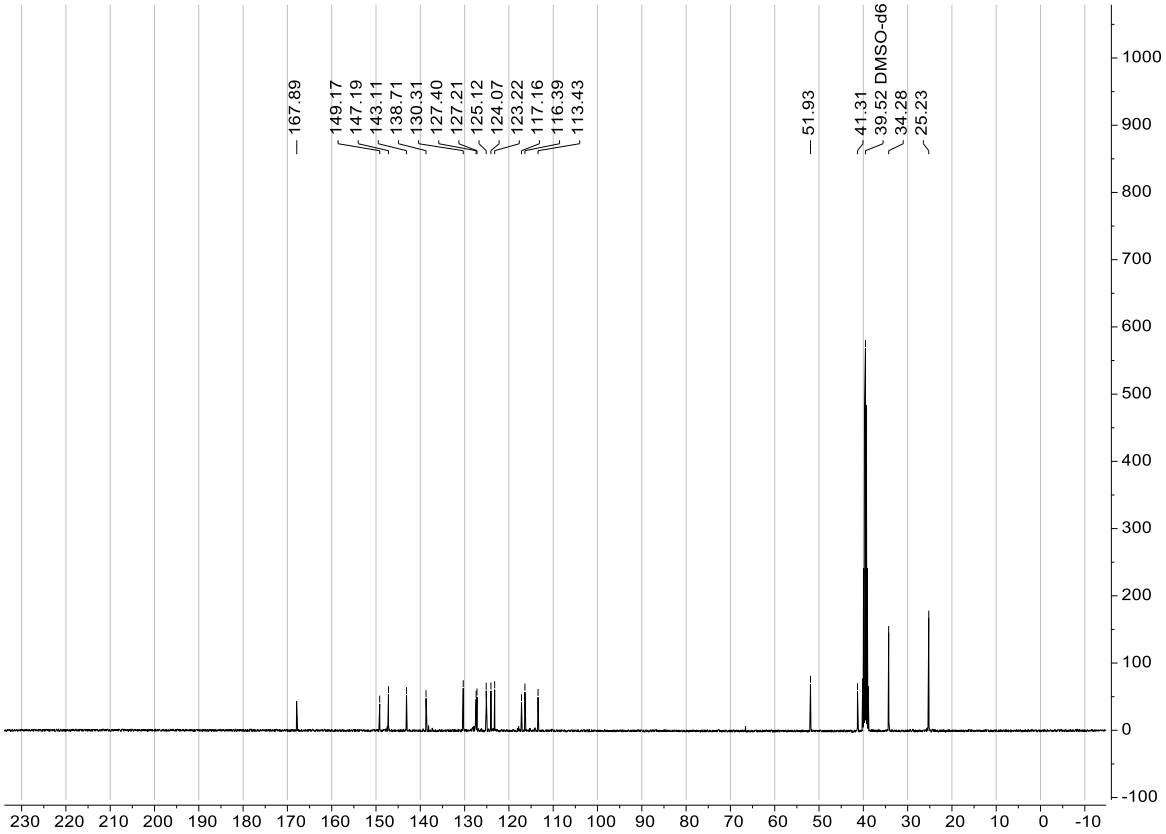



146.55
144.13
128.46
127.06
119.18
117.21
88.23
72.30
65.57
39.52 DMSO-d6
17.09
-1.47
146.55
144.13
128.46
127.06
119.18
117.21
88.23

Methyl 2-cyclopentyl-4-(1H-pyrrolo[2,3-*b*]pyridin-3-yl)benzoate (SI3)



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130.31
127.40
127.21
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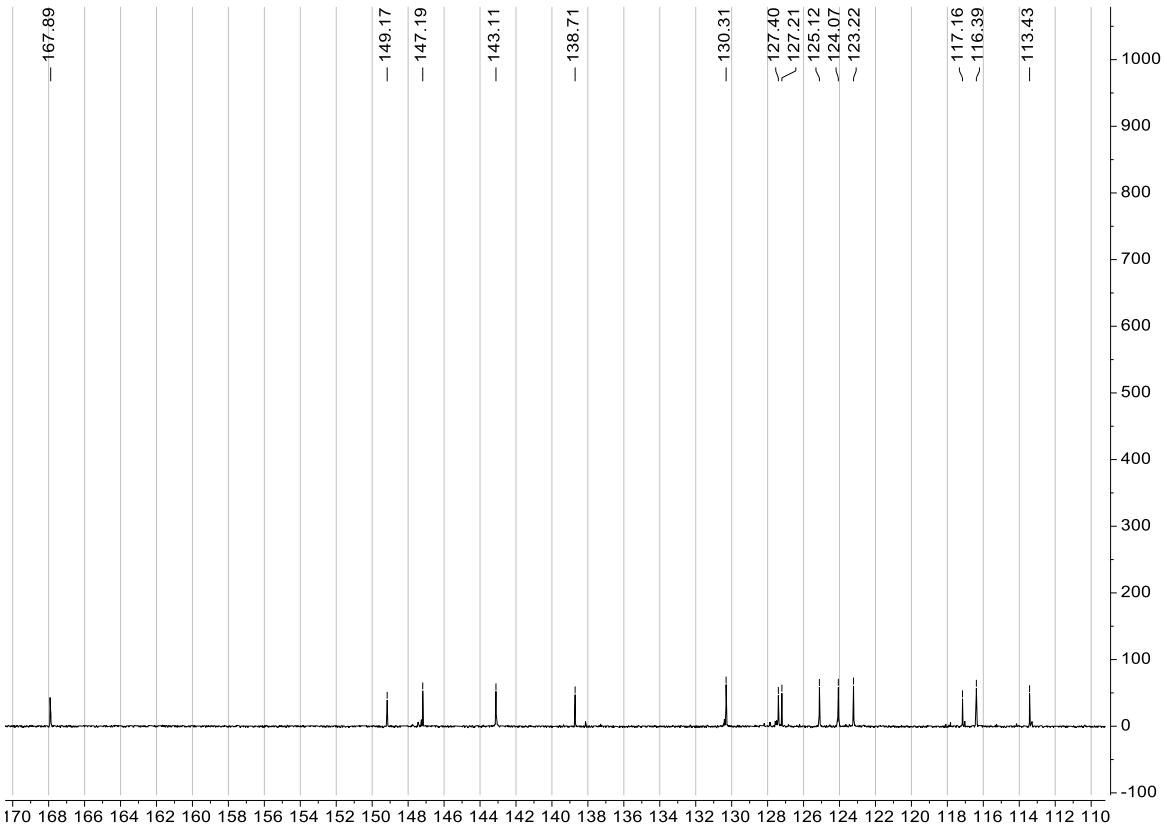
51.93
41.31
39.52 DMSO-d6
34.28
25.23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149.17
147.19
143.11
138.71
130.31
127.40
127.21
125.12
124.07
123.22
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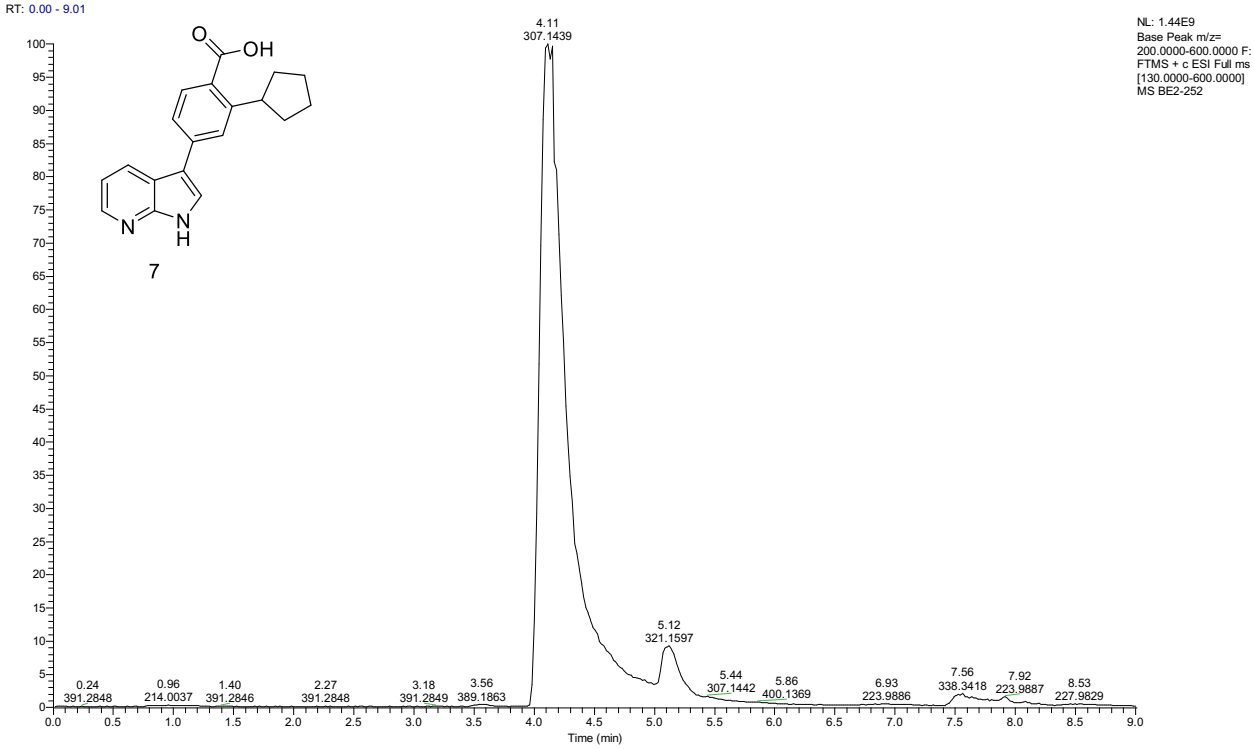
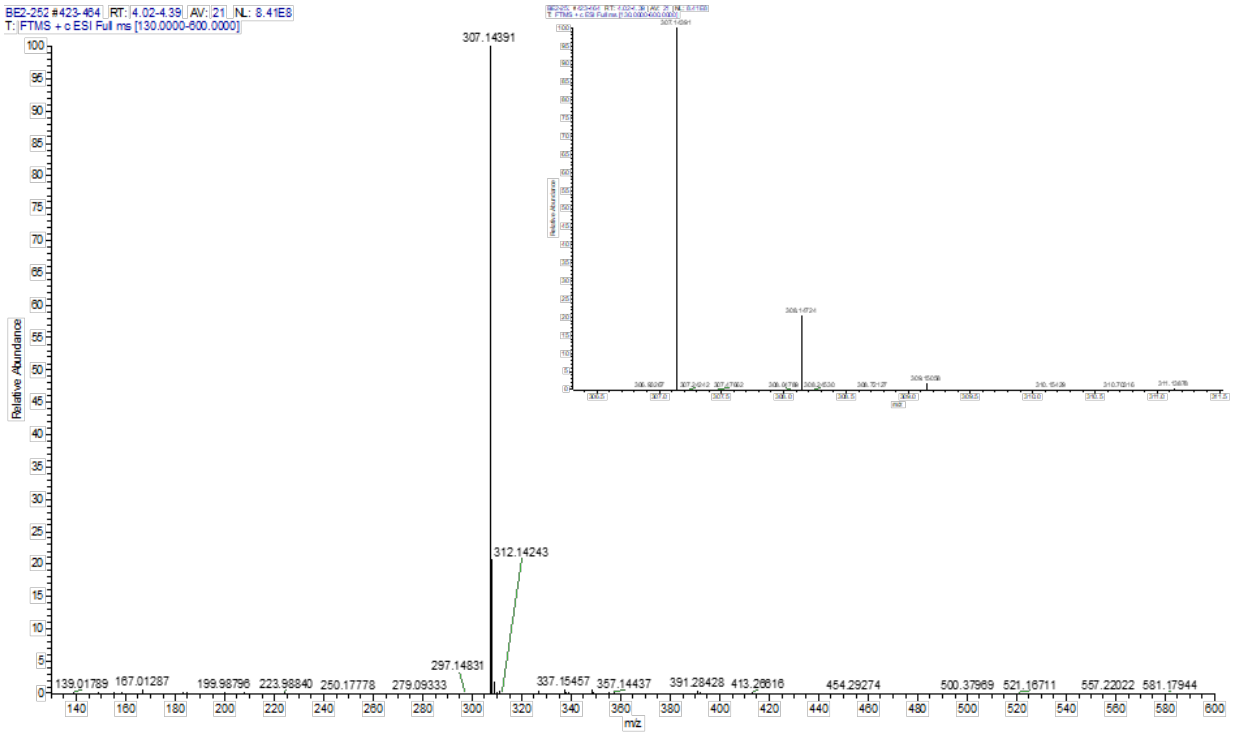
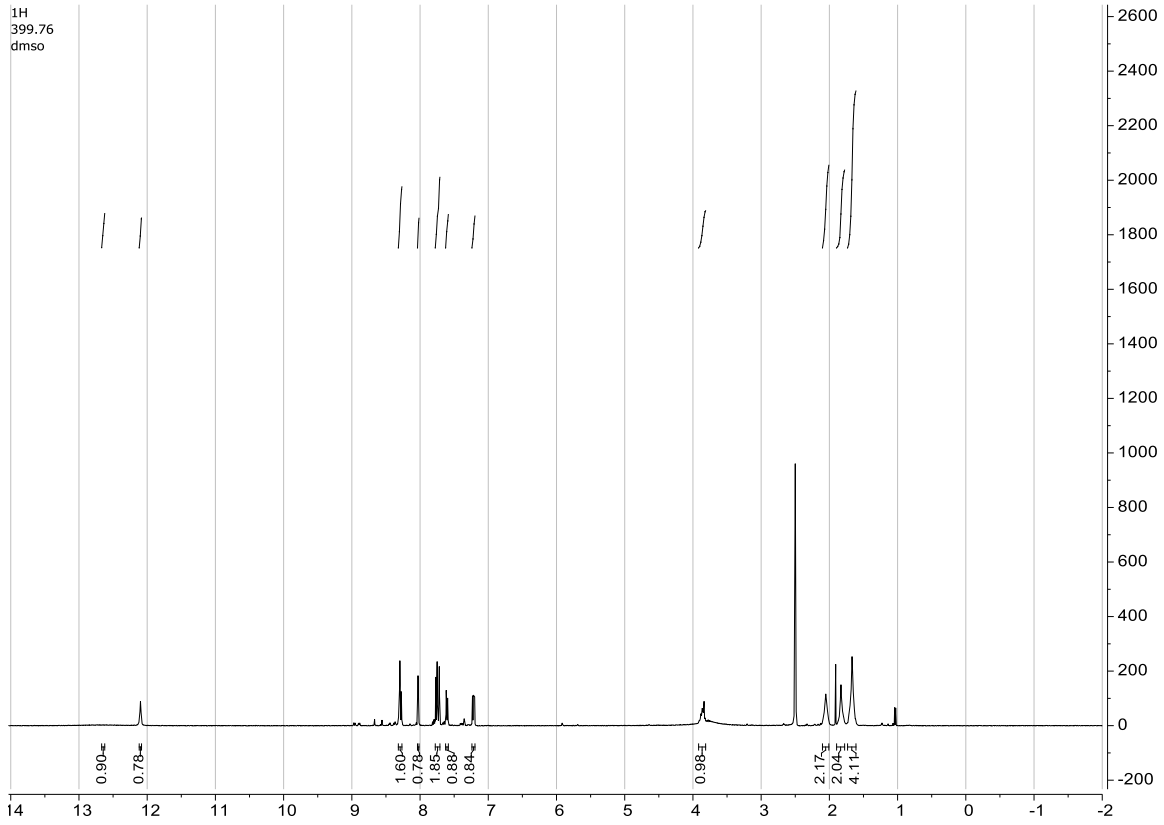
116.39
113.43

2-Cyclopentyl-4-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)benzoic acid (7)



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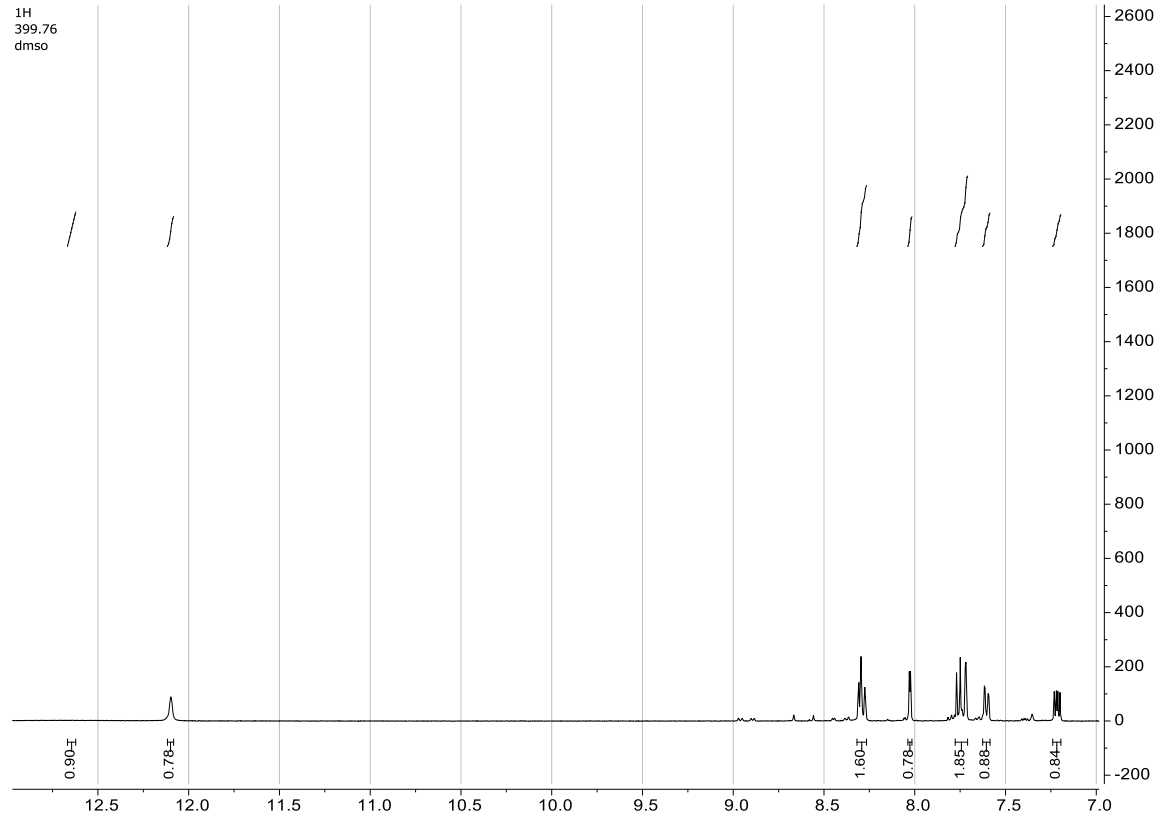
1.85
0.88
0.84
0.98
2.17
2.04
4.11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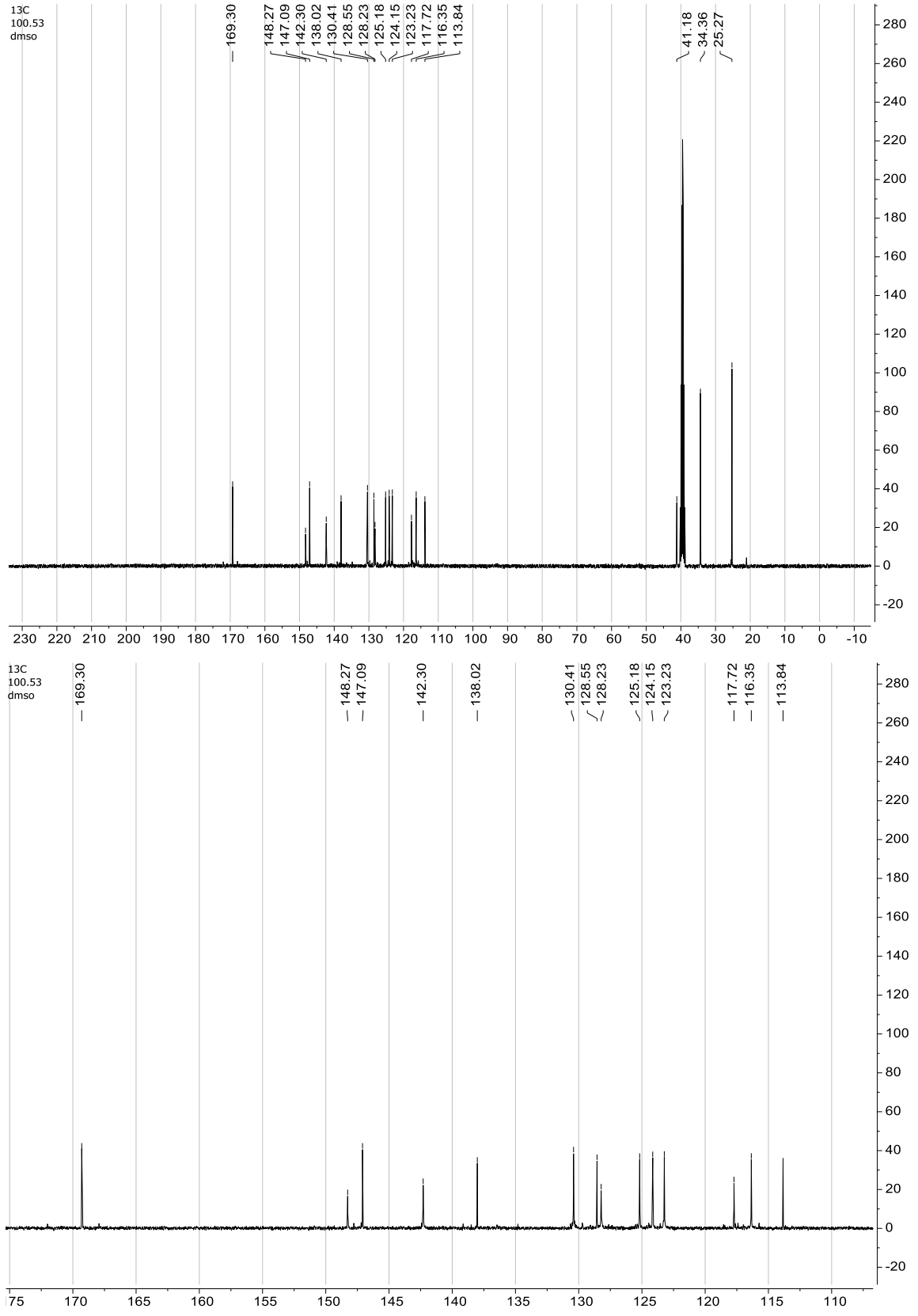
1.85
0.88
0.84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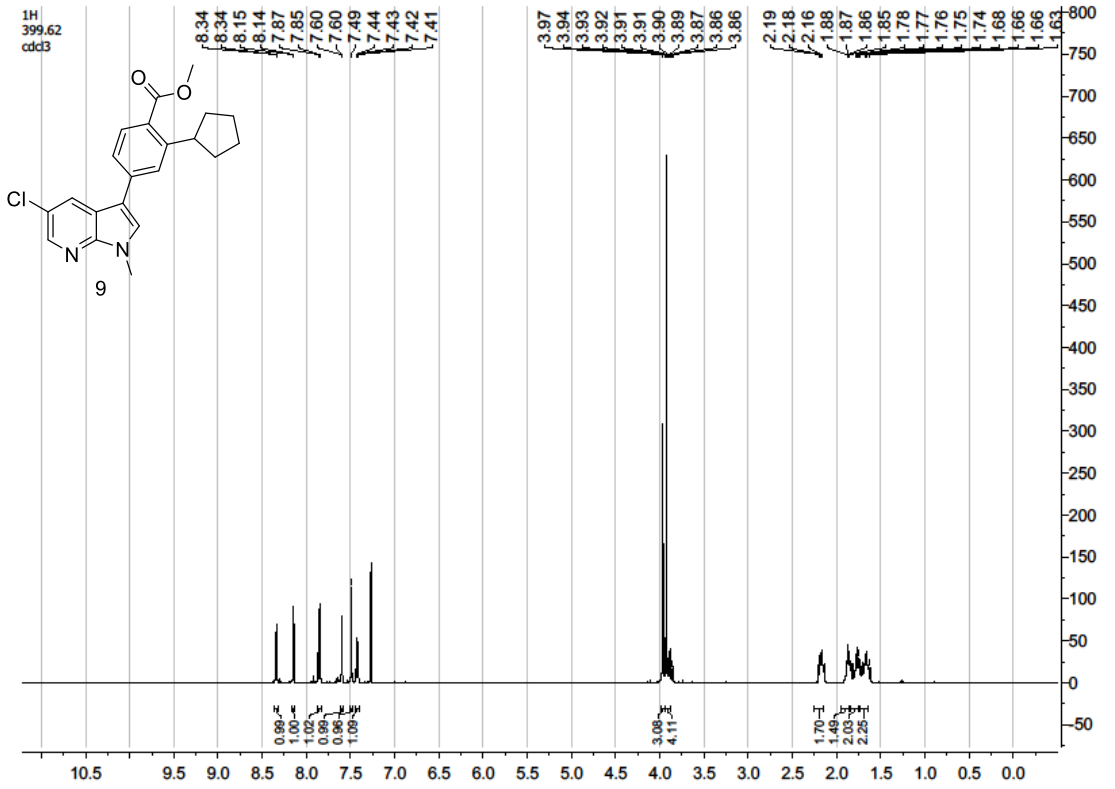
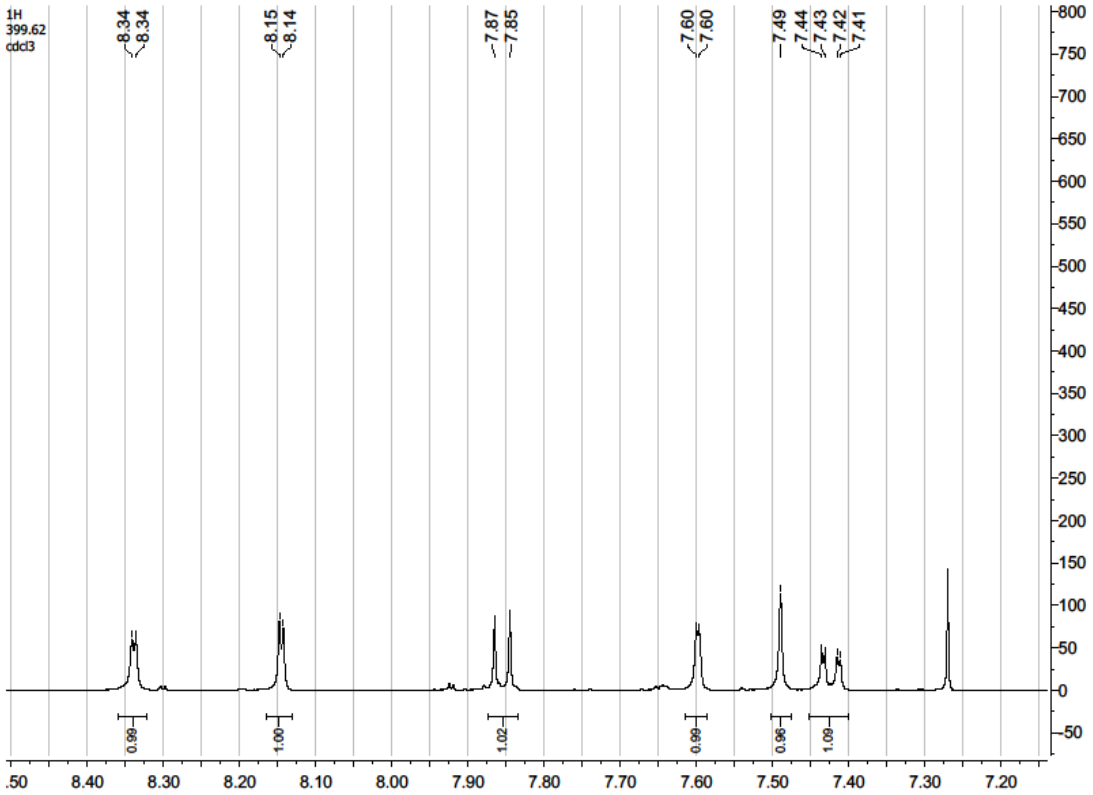
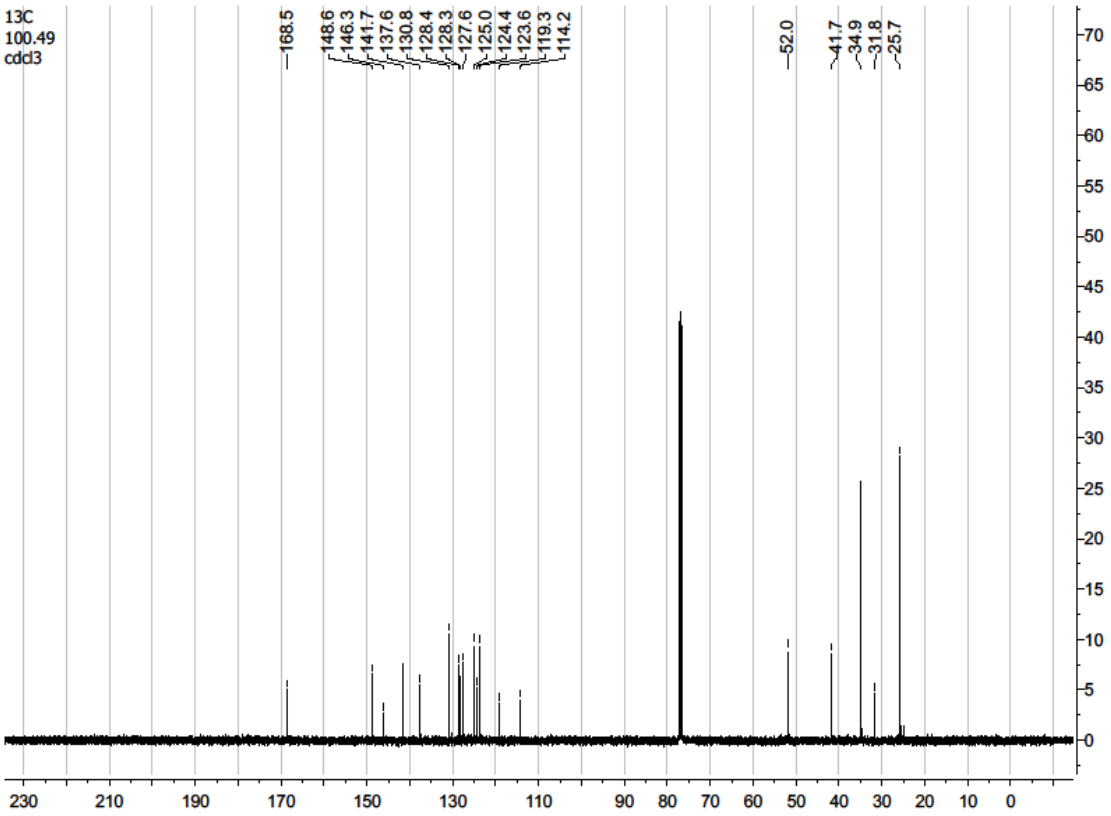
124.15
123.23
117.72
116.35
113.84
41.18
34.36
25.27

Methyl 4-(5-chloro-1-methyl-1H-pyrrolo[2,3-*b*]pyridin-3-yl)-2-cyclopentylbenzoate (9)



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141.7
137.6
130.8
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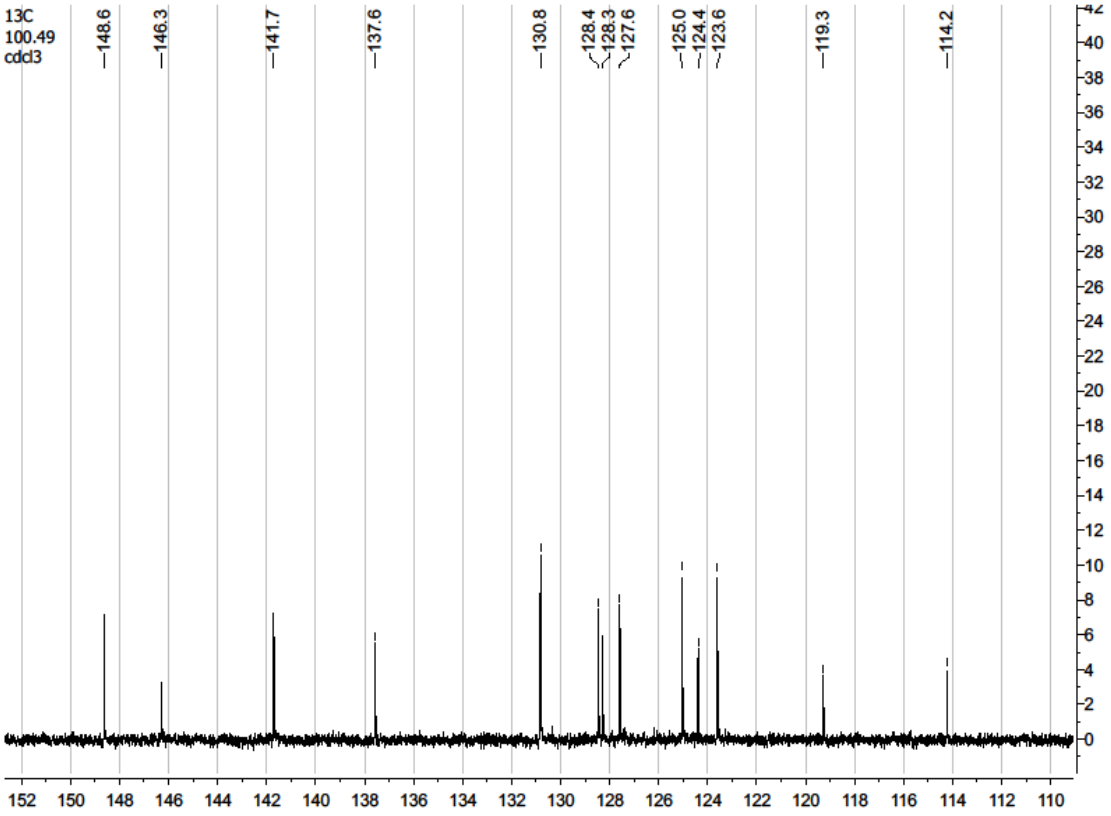
124.4
123.6
119.3
114.2
52.0
41.7
34.9
31.8
25.7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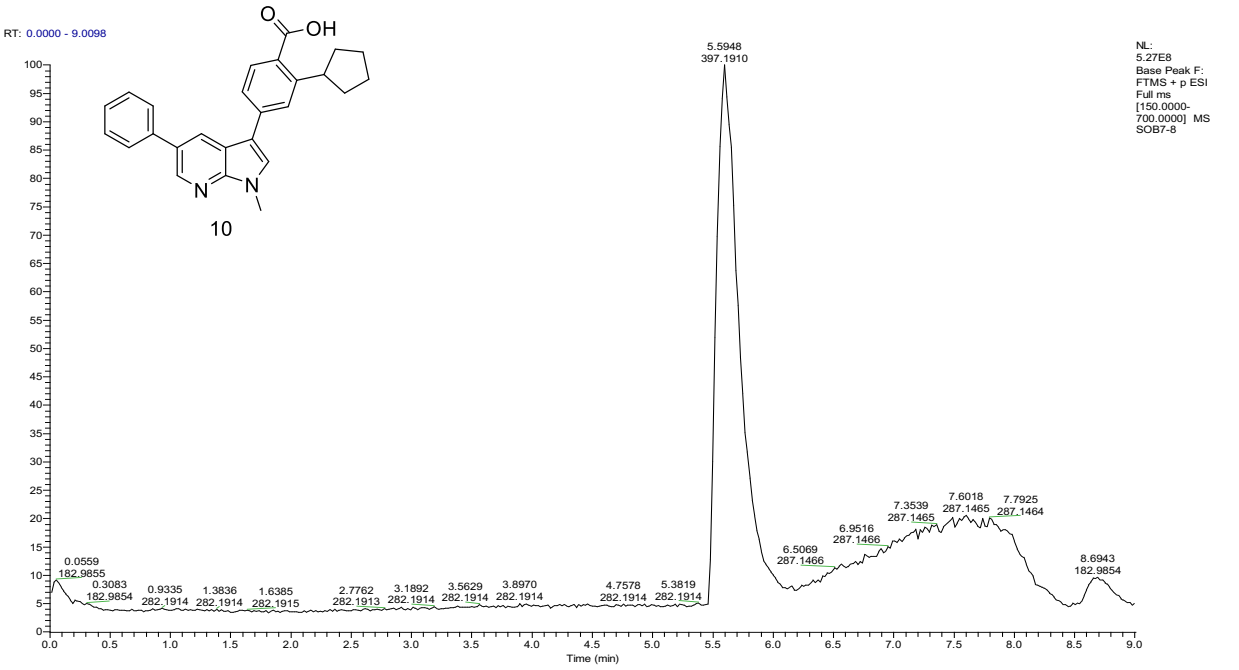
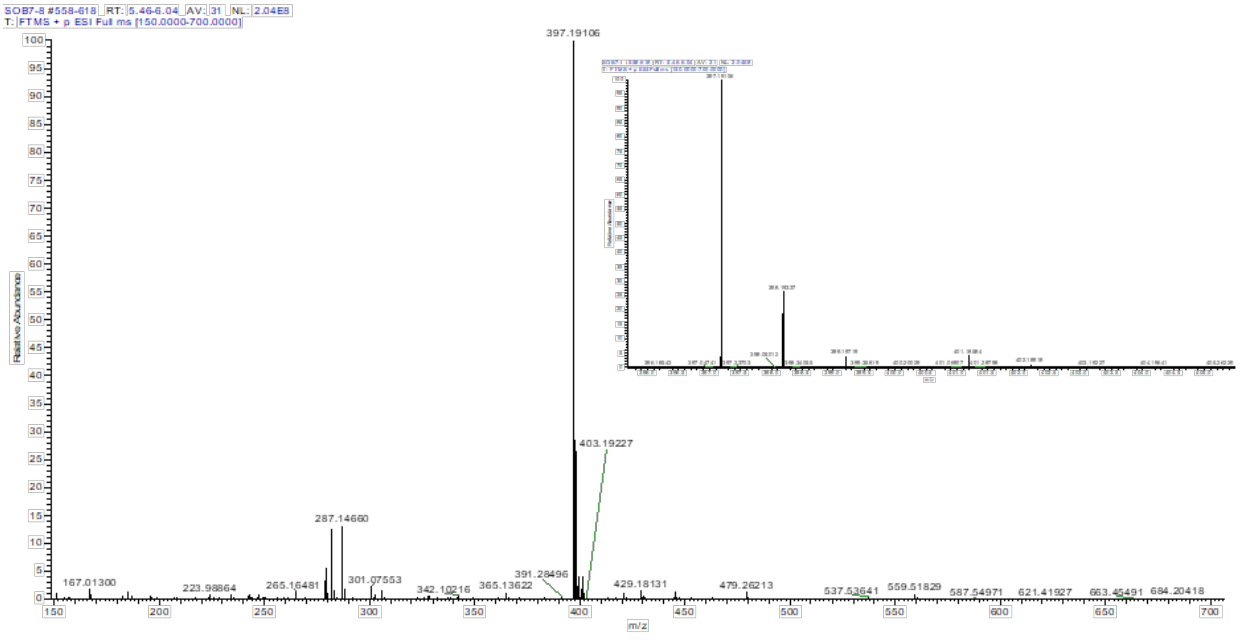
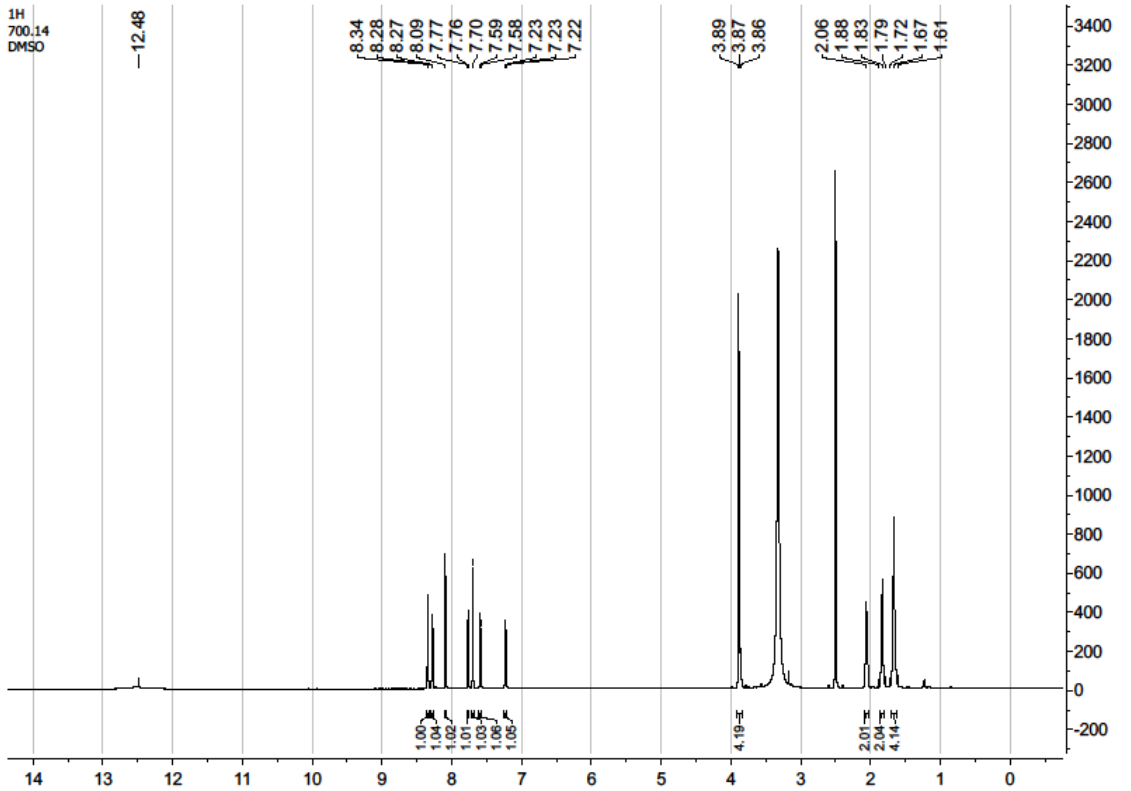
124.4
123.6
119.3
114.2

2-Cyclopentyl-4-(1-methyl-5-phenyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)benzoic acid (**10**)



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DMSO
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8.27
8.09
7.77
7.76
7.70
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7.23
7.23
7.22
3.89
3.87
3.86
2.06
1.88
1.83
1.79
1.72
1.67
1.61
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1.03
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1.05
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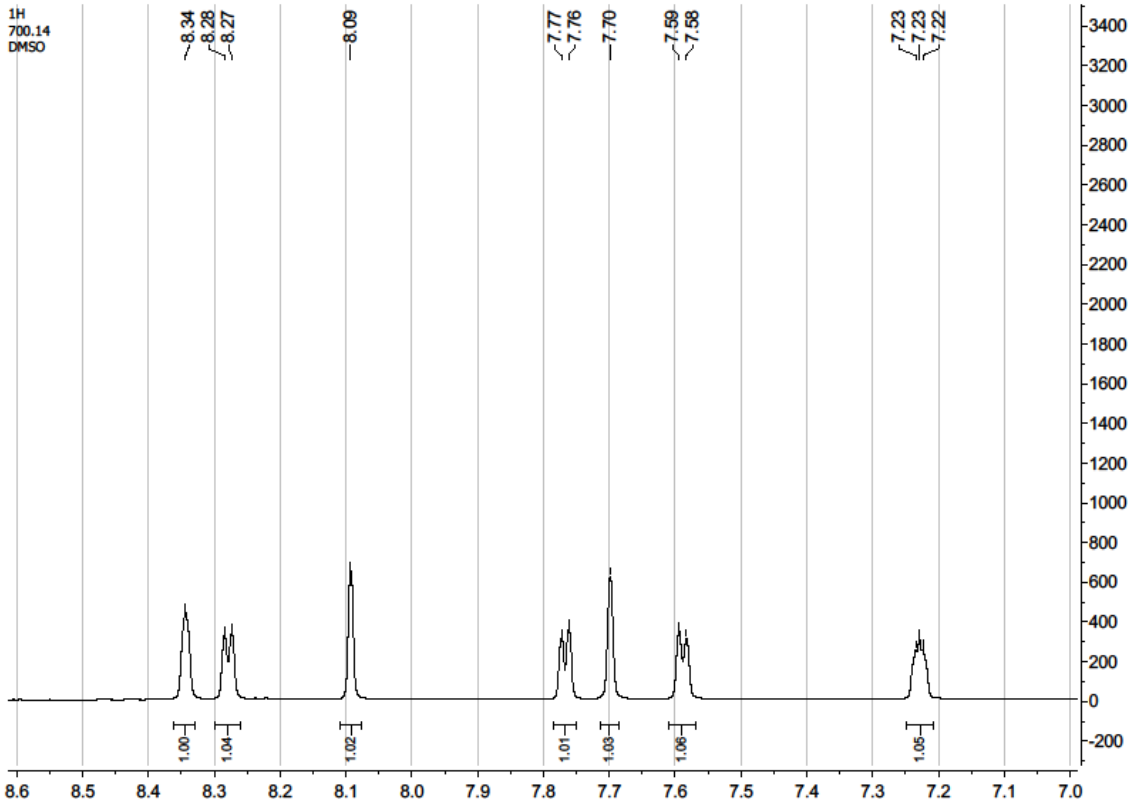
2.04
4.14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8.28
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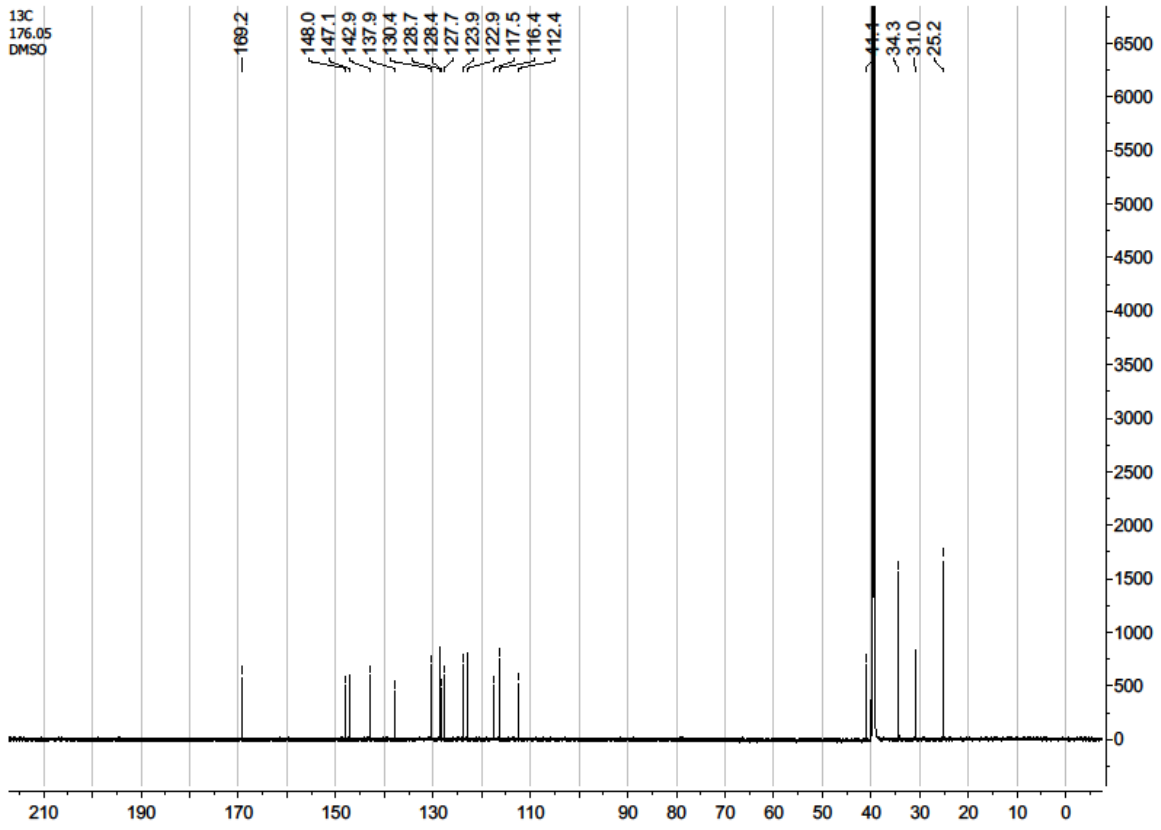
1.04
1.02
1.01
1.03
1.06
1.05


13C
176.05
DMSO
169.2
148.0
147.1
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137.9
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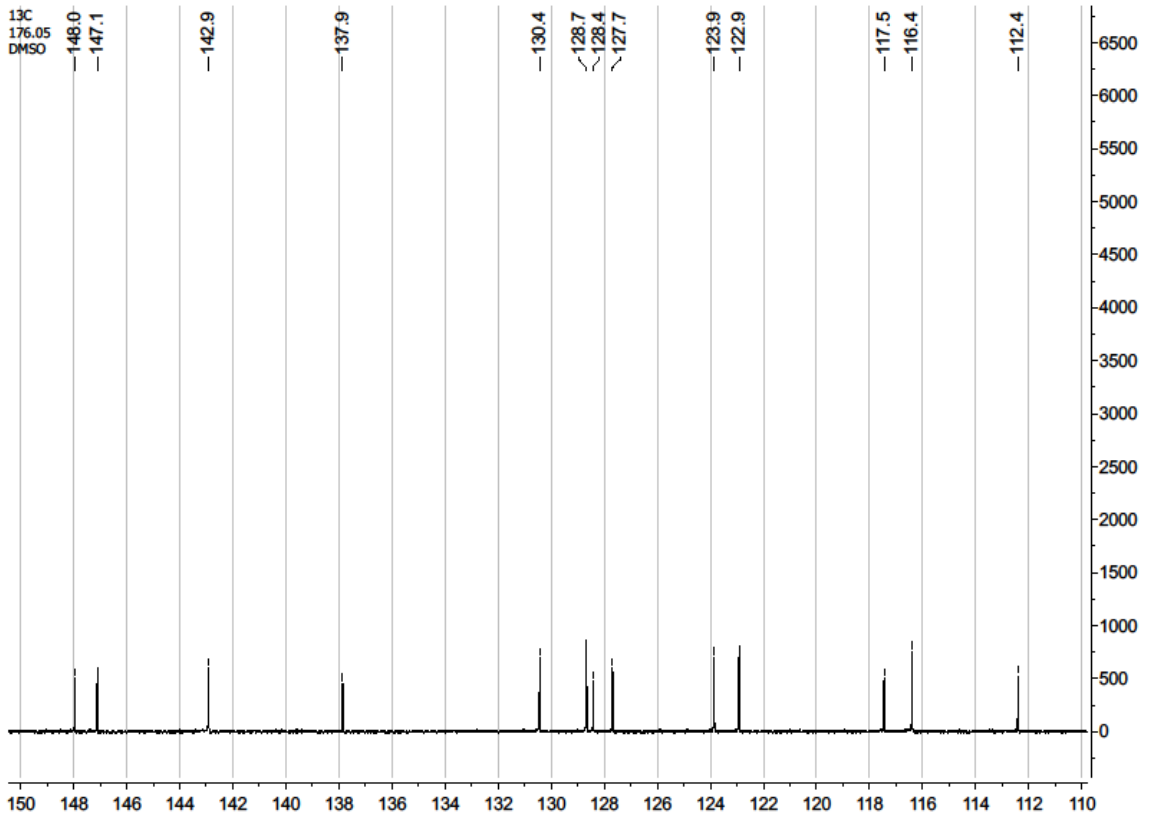
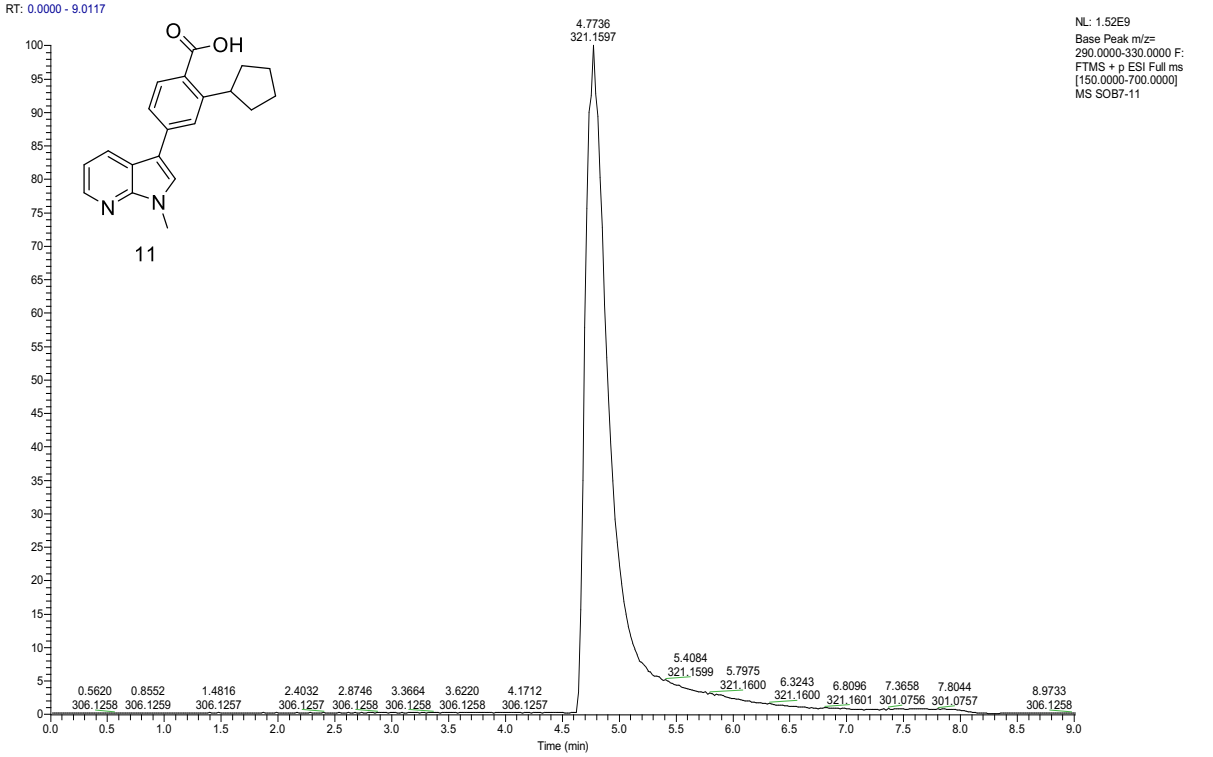
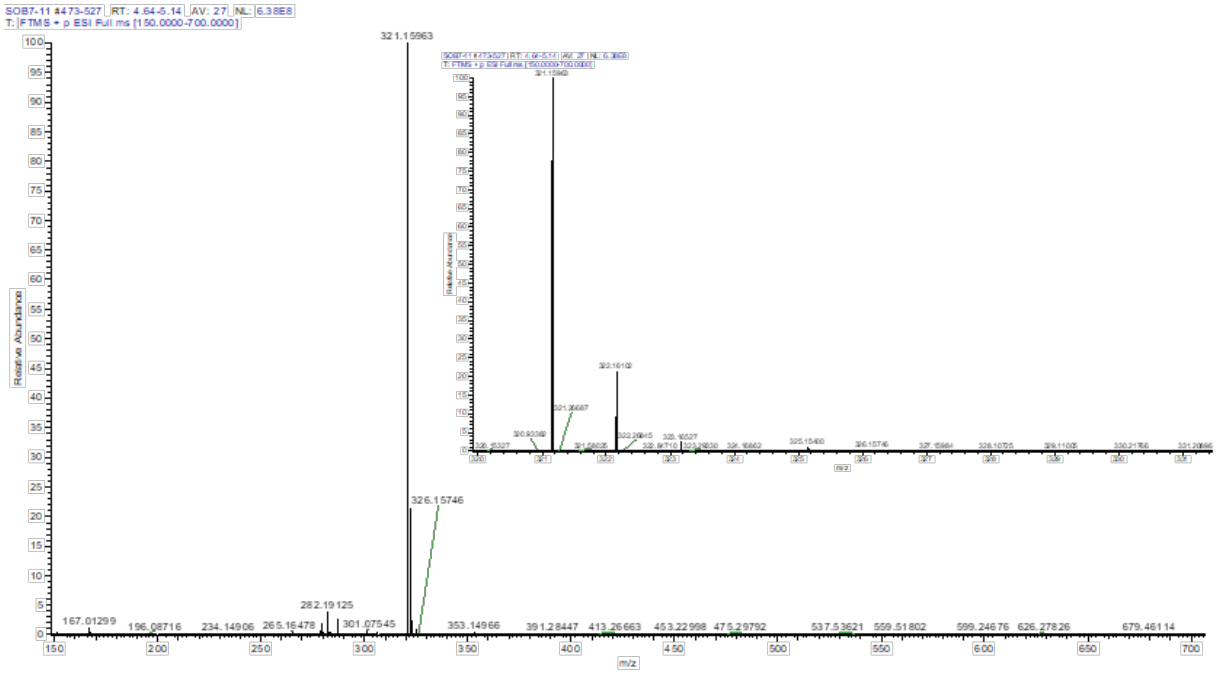
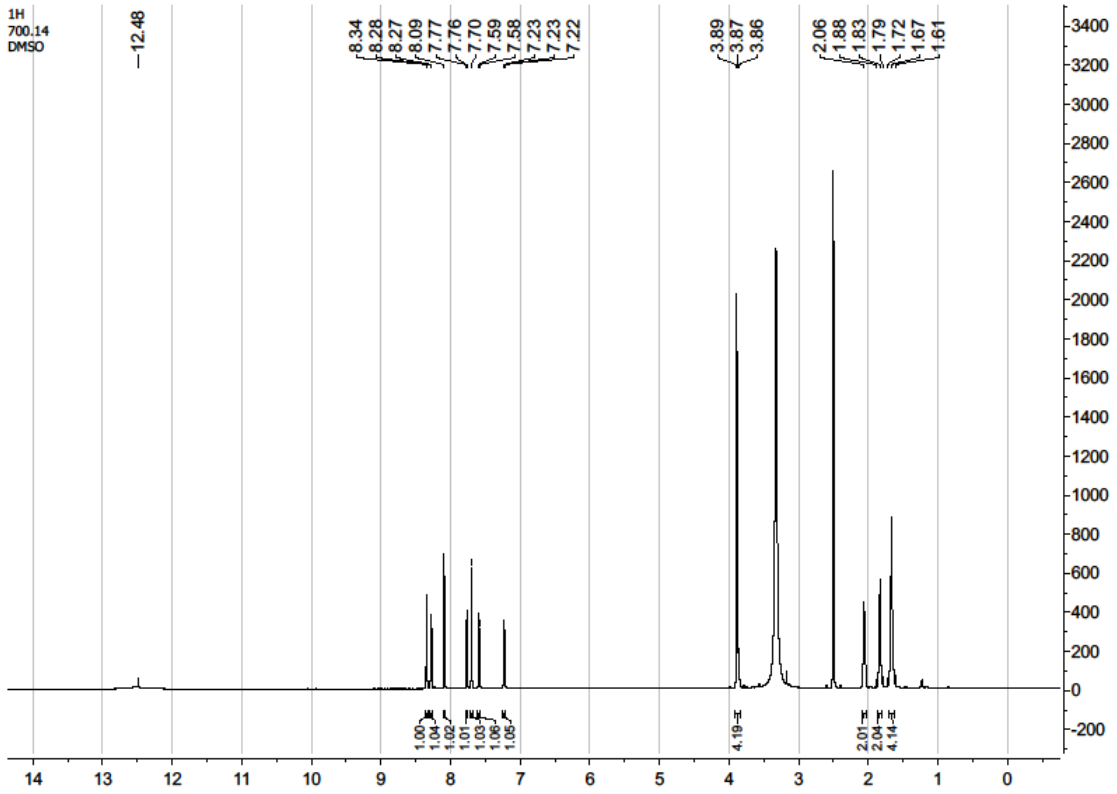
34.3
31.0
25.2

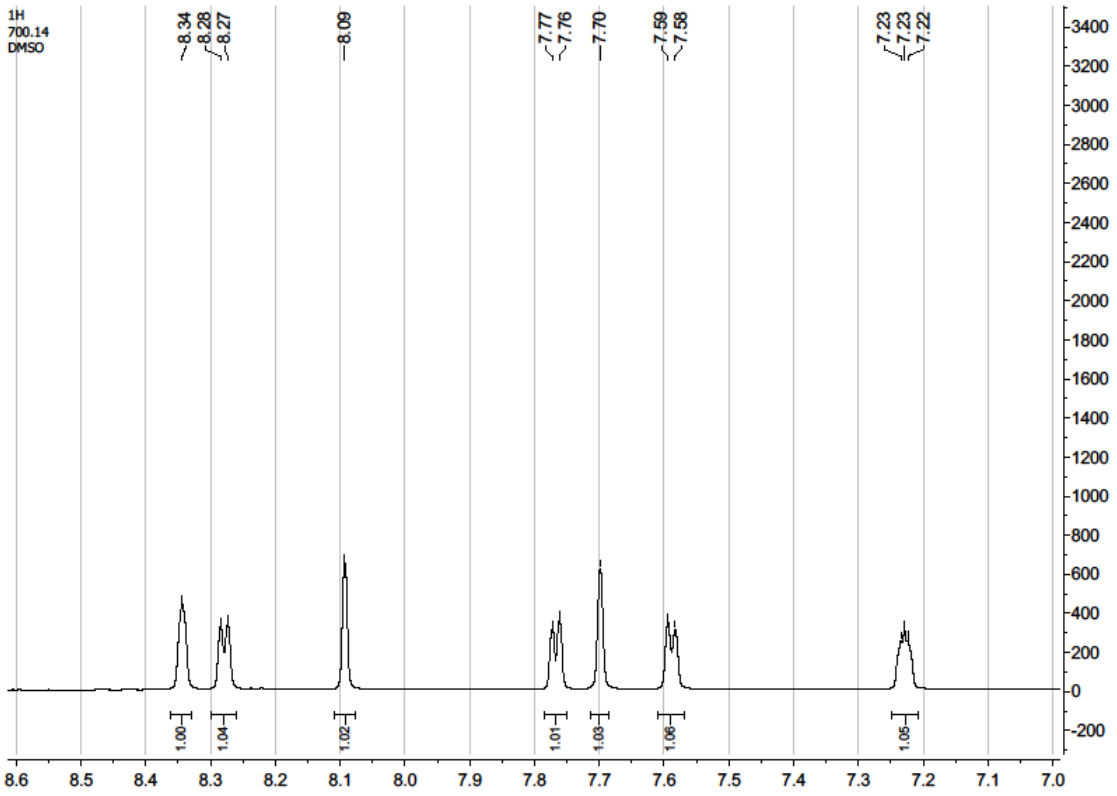

13C
176.05
DMSO
148.0
147.1
142.9
137.9
130.4
128.7
128.4
127.7
123.9
122.9
117.5
116.4
112.4

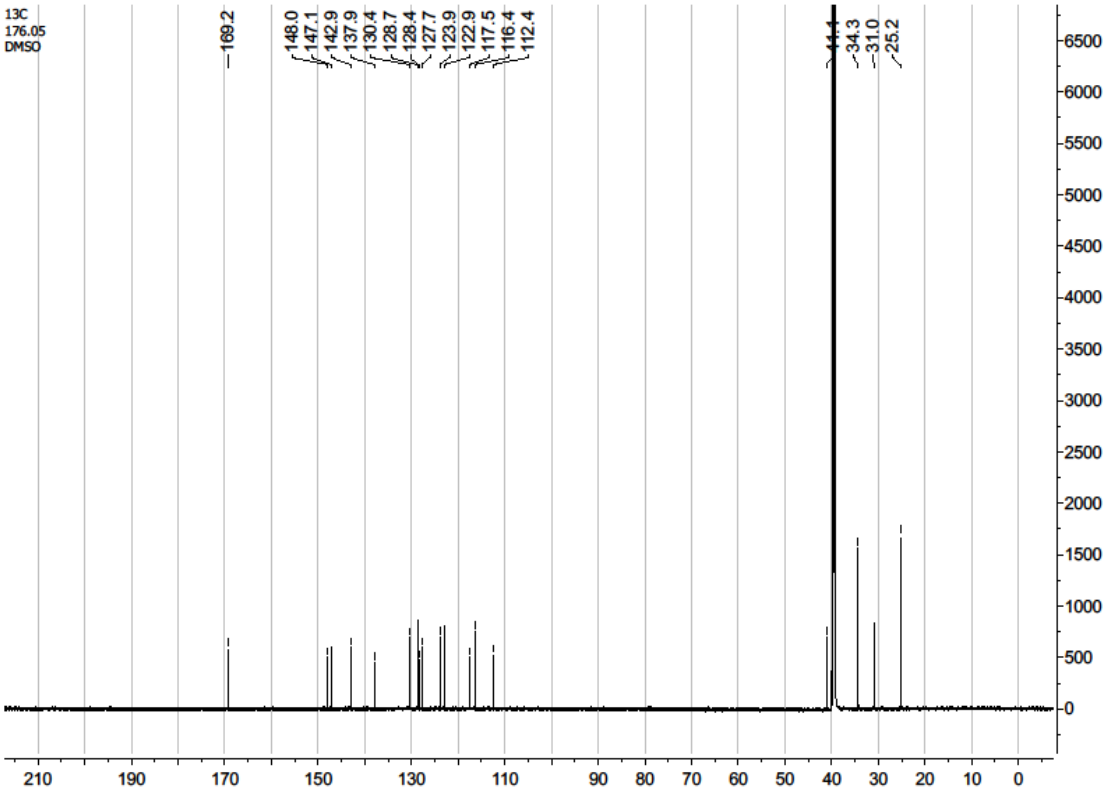
2-Cyclopentyl-4-(1-methyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)benzoic acid (11)

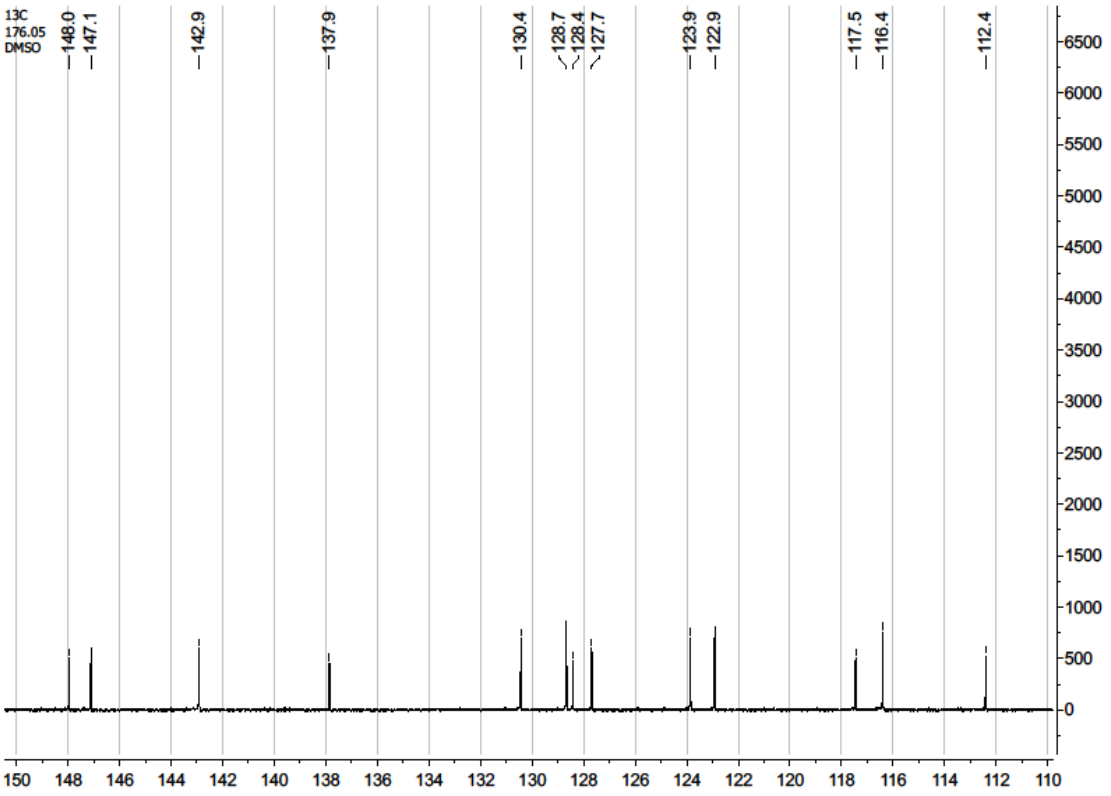




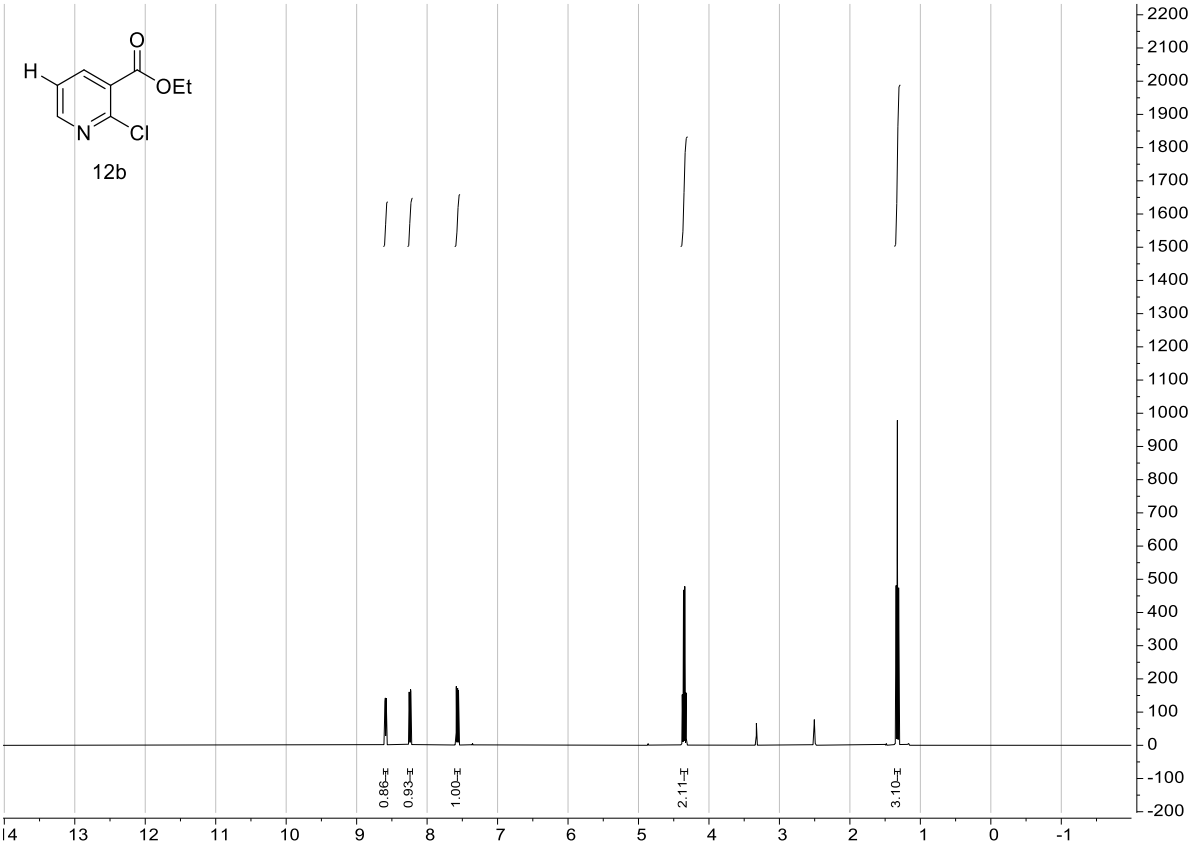

1H
700.14
DMSO
12.48
8.34
8.28
8.27
8.09
7.77
7.76
7.70
7.59
7.58
7.23
7.23
7.22
3.89
3.87
3.86
2.06
1.88
1.83
1.79
1.72
1.67
1.61
1.00
1.04
1.02
1.01
1.03
1.06
1.05
4.19
2.01
2.04
4.14

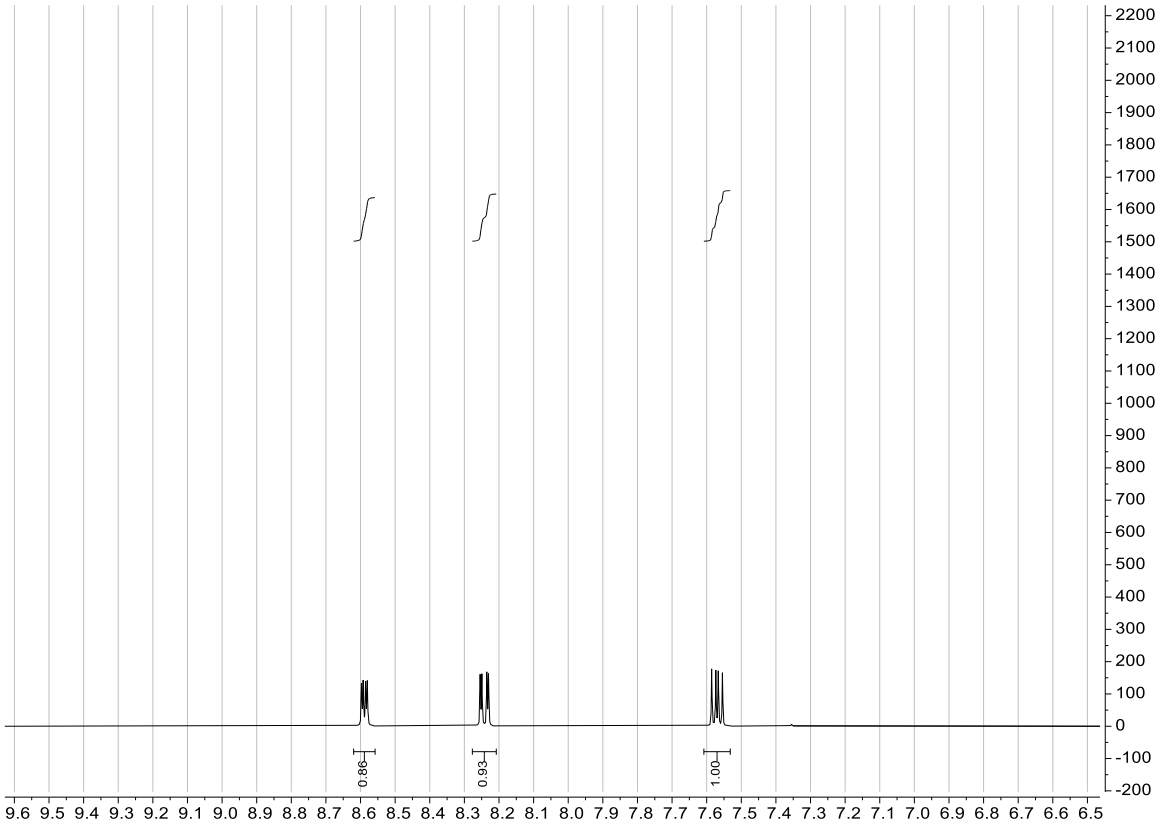

1H
700.14
DMSO
8.34
8.28
8.27
8.09
7.77
7.76
7.70
7.59
7.58
7.23
7.23
7.22
1.00
1.04
1.02
1.01
1.03
1.06
1.05

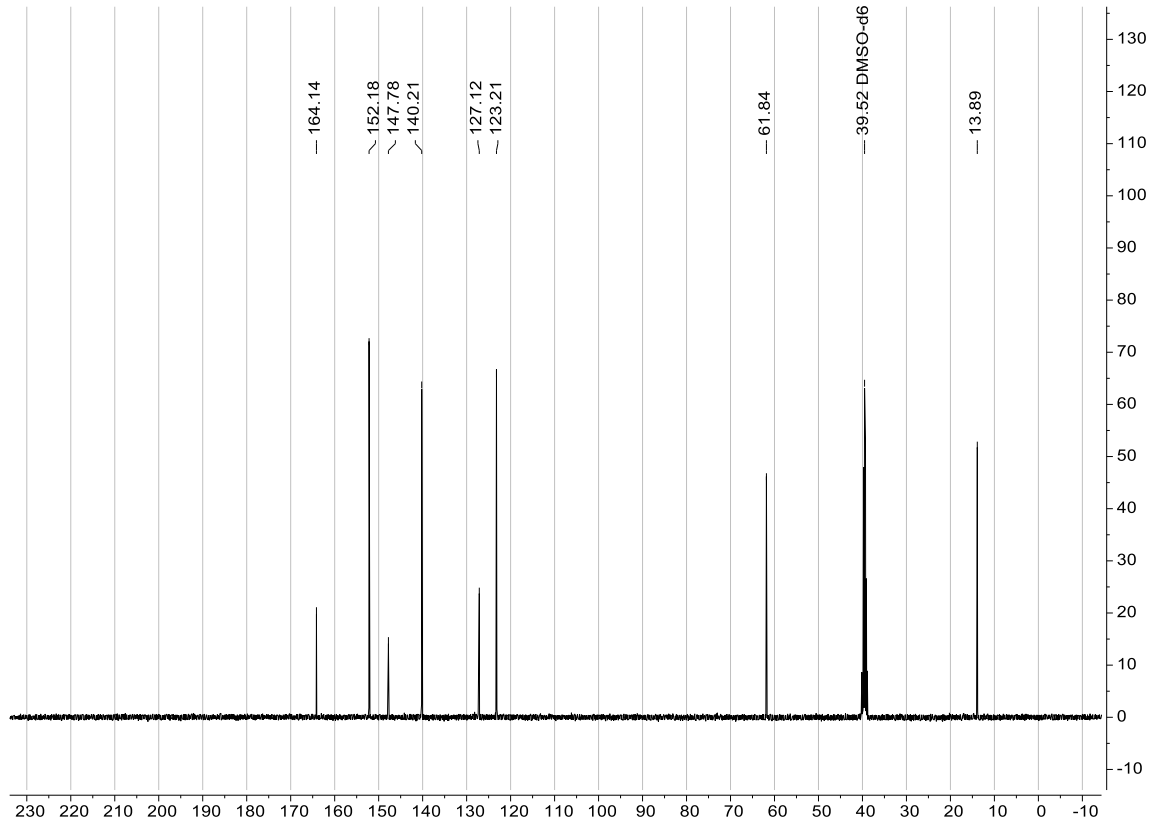

13C
176.05
DMSO
169.2
148.0
147.1
142.9
137.9
130.4
128.7
128.4
127.7
123.9
122.9
117.5
116.4
112.4
44.1
34.3
31.0
25.2

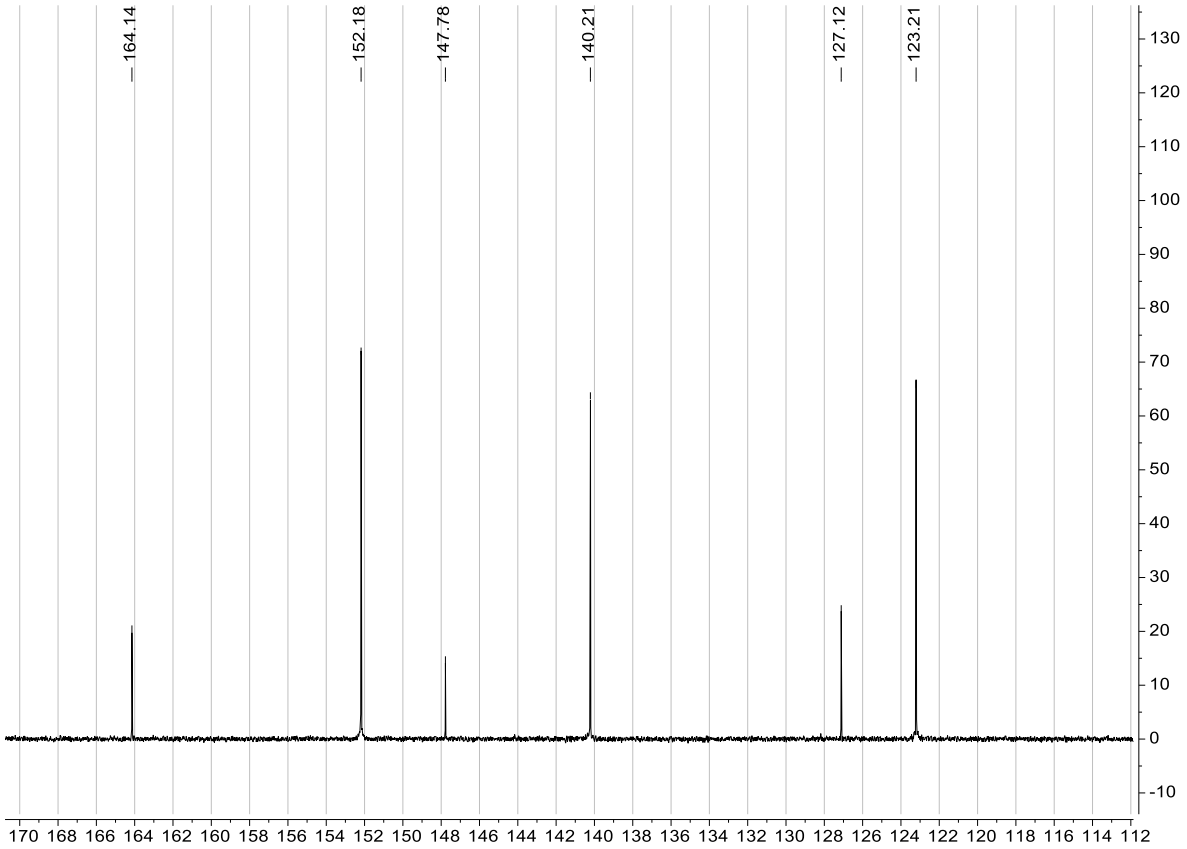

13C
176.05
DMSO
148.0
147.1
142.9
137.9
130.4
128.7
128.4
127.7
123.9
122.9
117.5
116.4
112.4

Ethyl 2-chloronicotinate (12b)

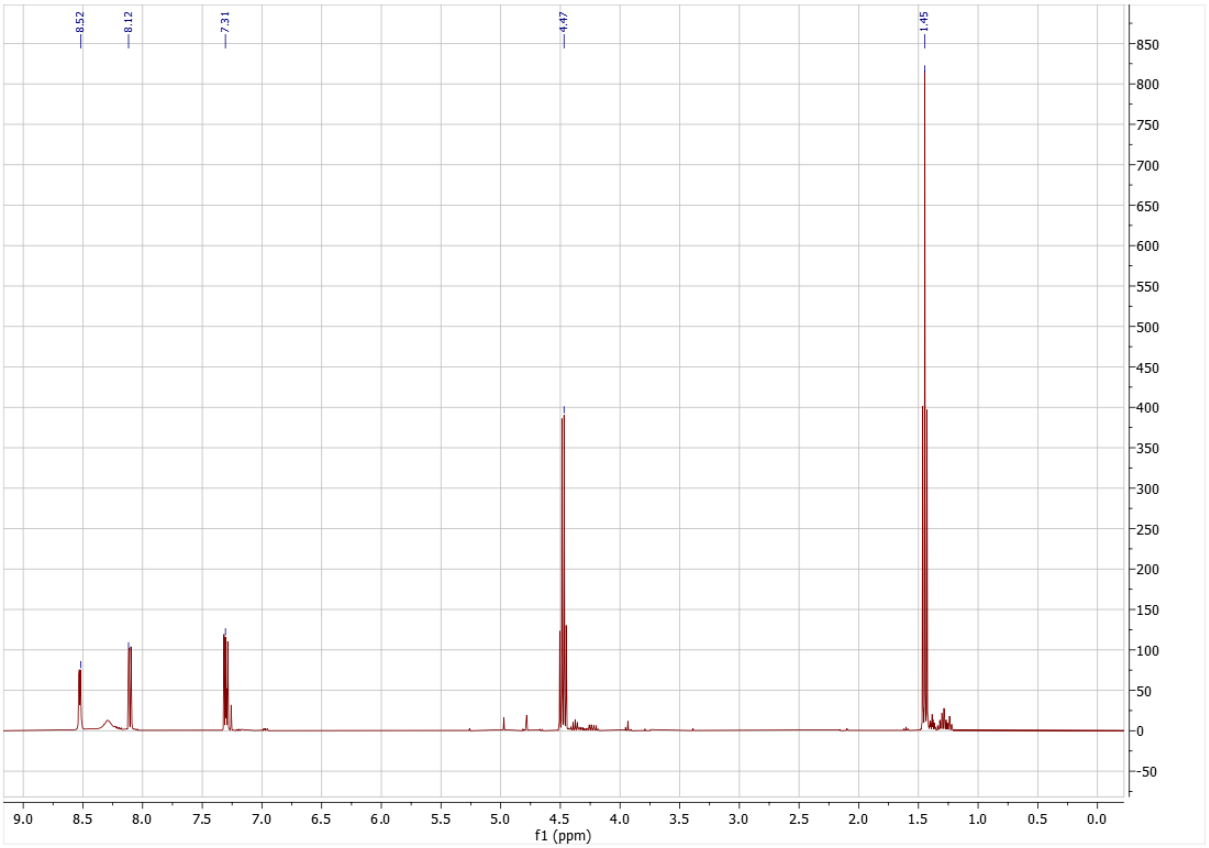




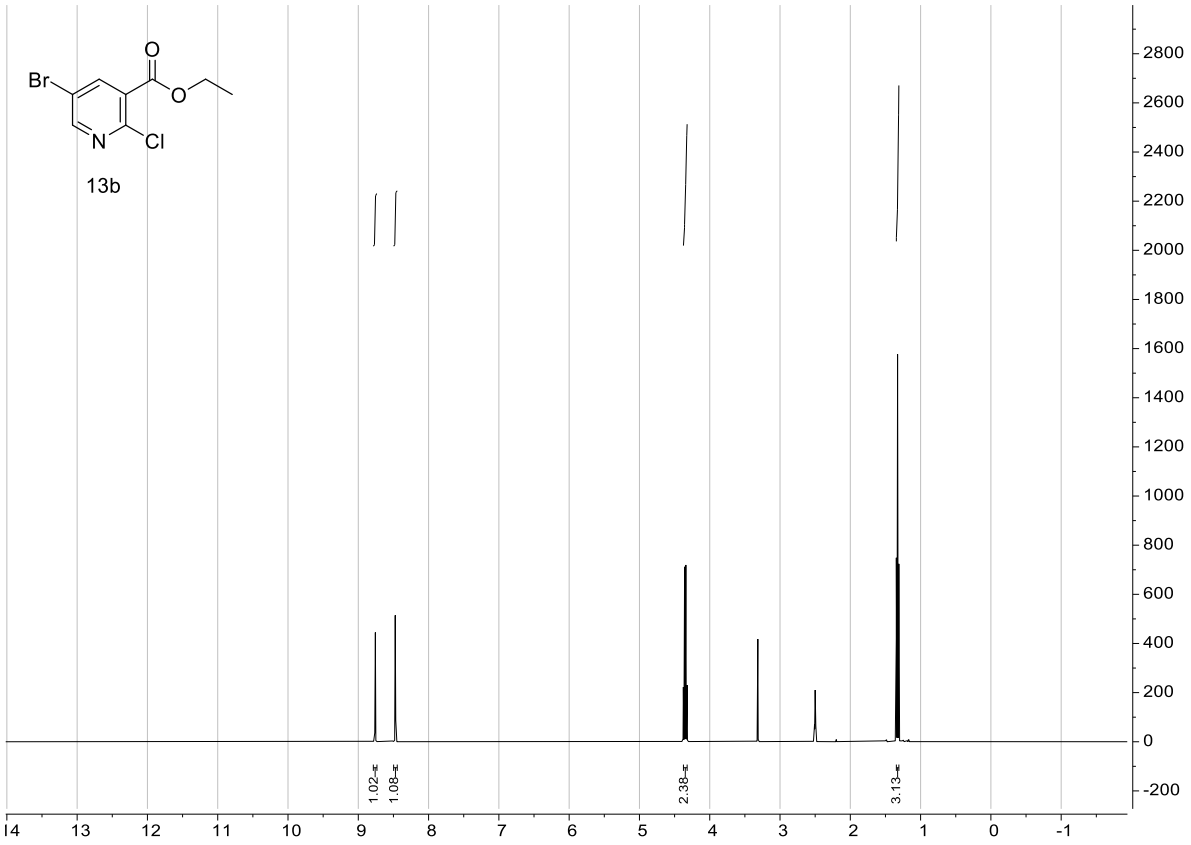

164.14
152.18
147.78
140.21
127.12
123.21
61.84
39.52 DMSO-d6
13.89

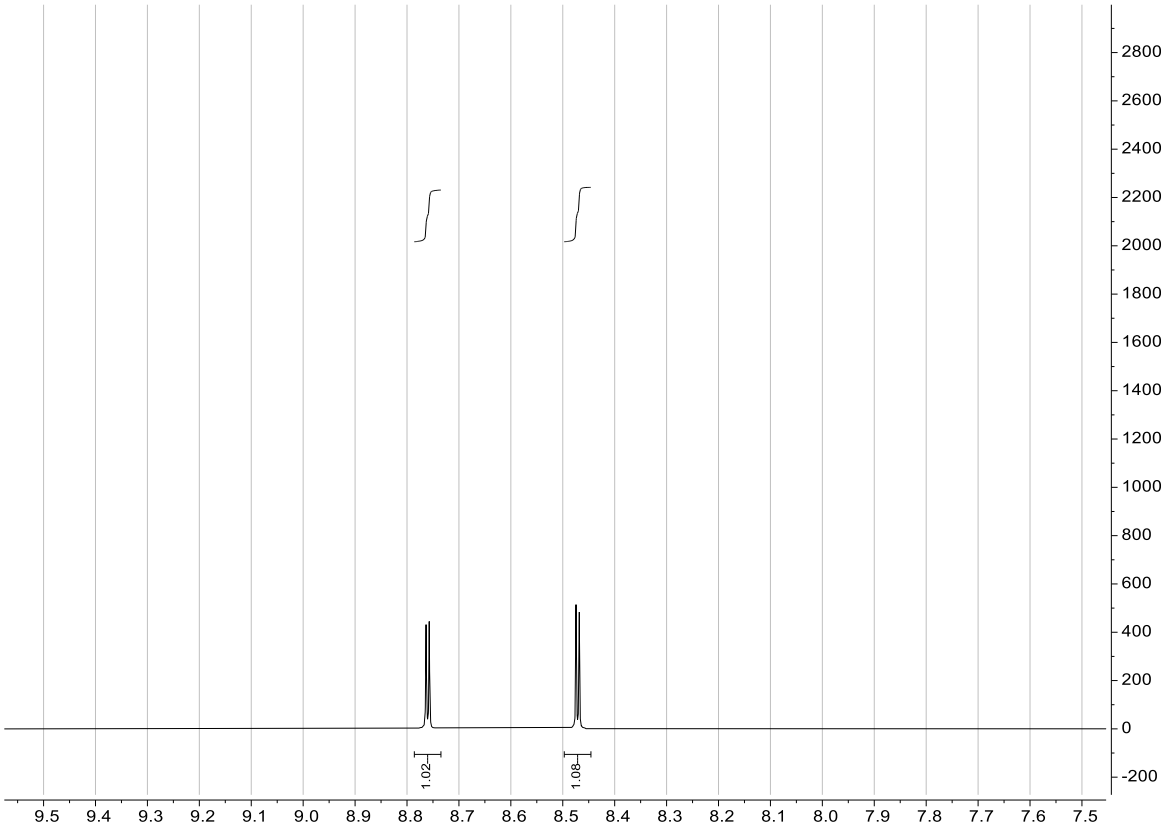

164.14
152.18
147.78
140.21
127.12
123.21

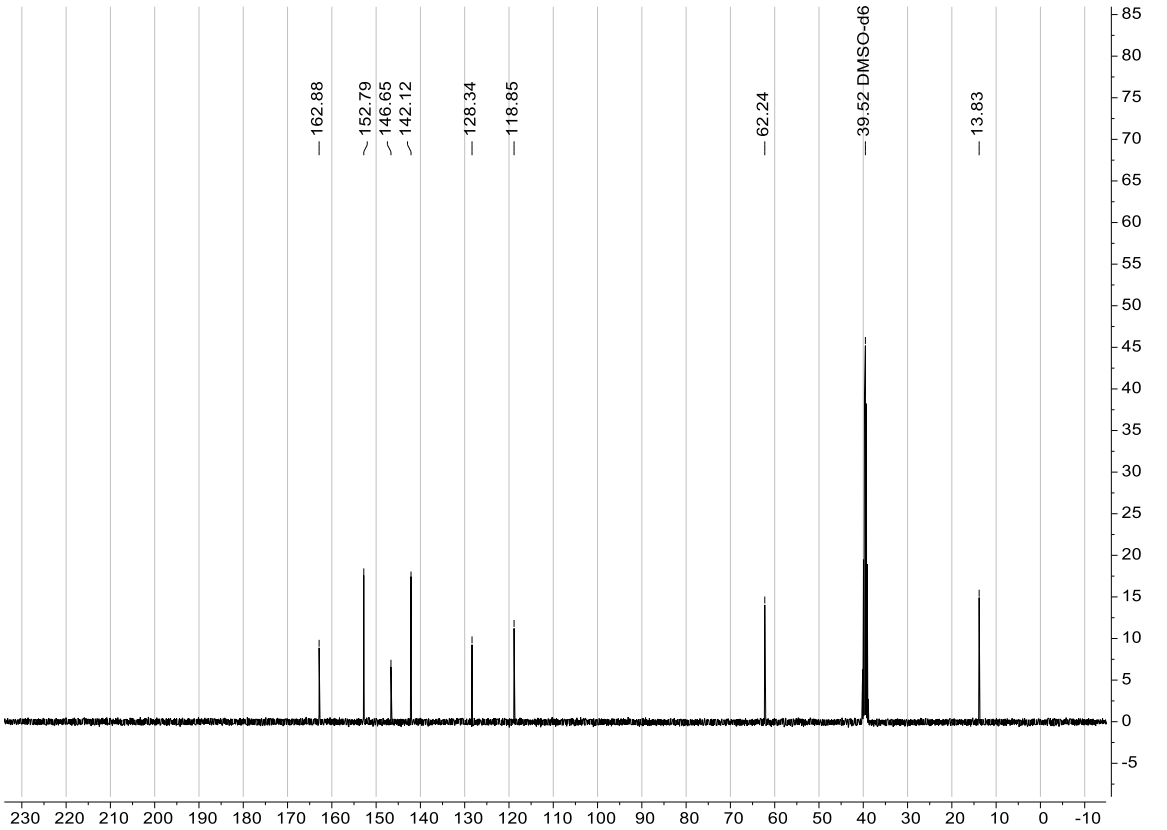
Ethyl 3-hydroxyfuro[2,3-*b*]pyridine-2-carboxylate (12c)

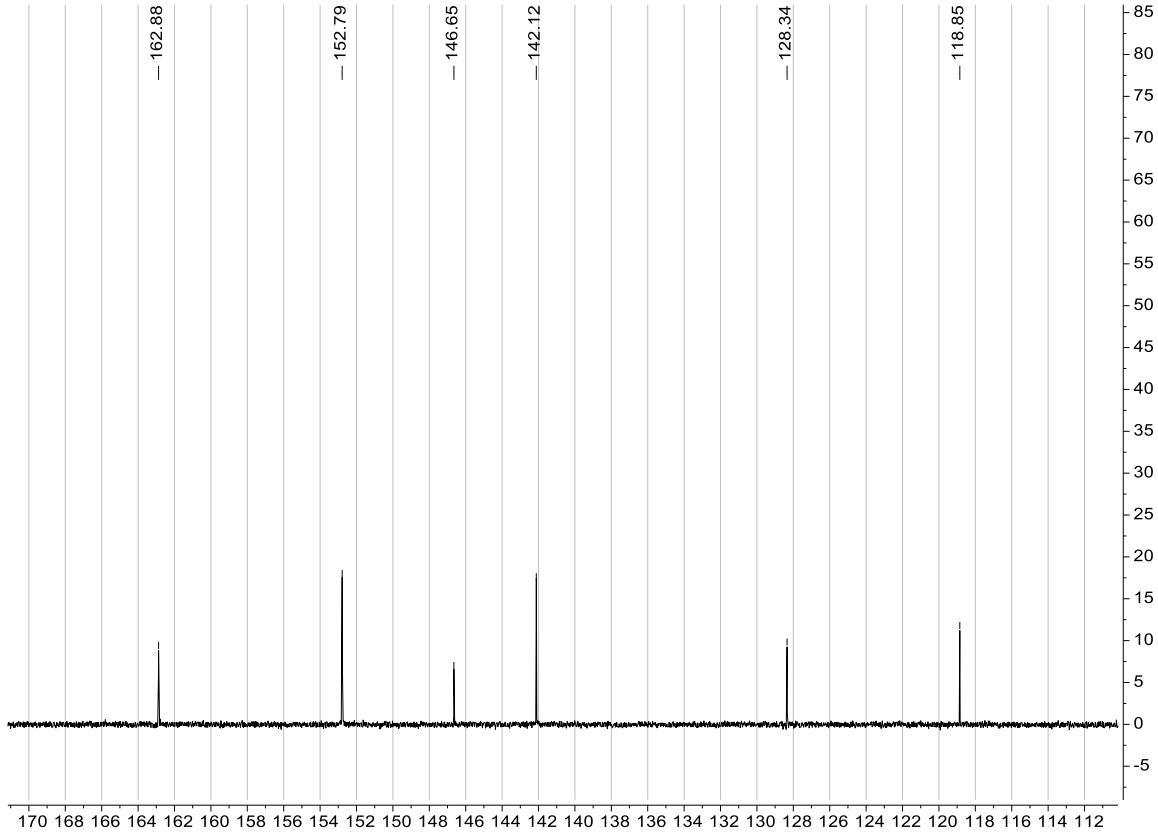


Ethyl 5-bromo-2-chloronicotinate (13b)

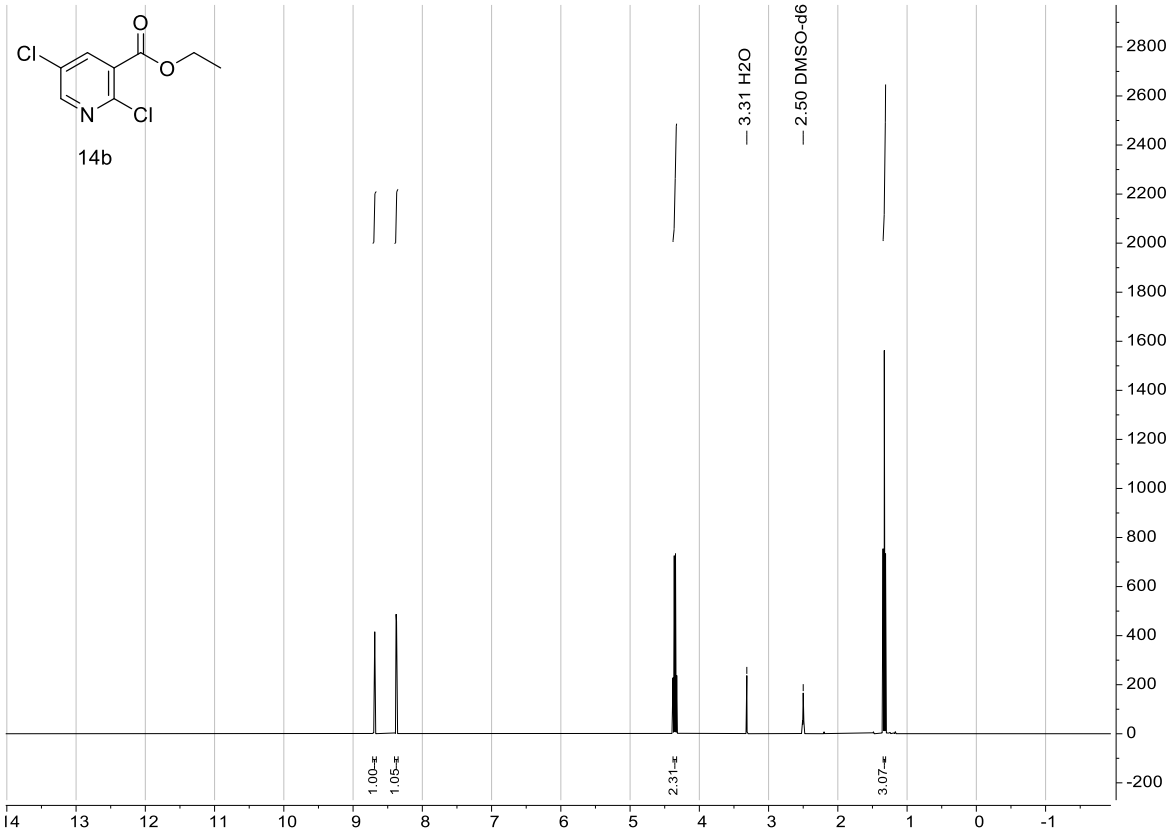


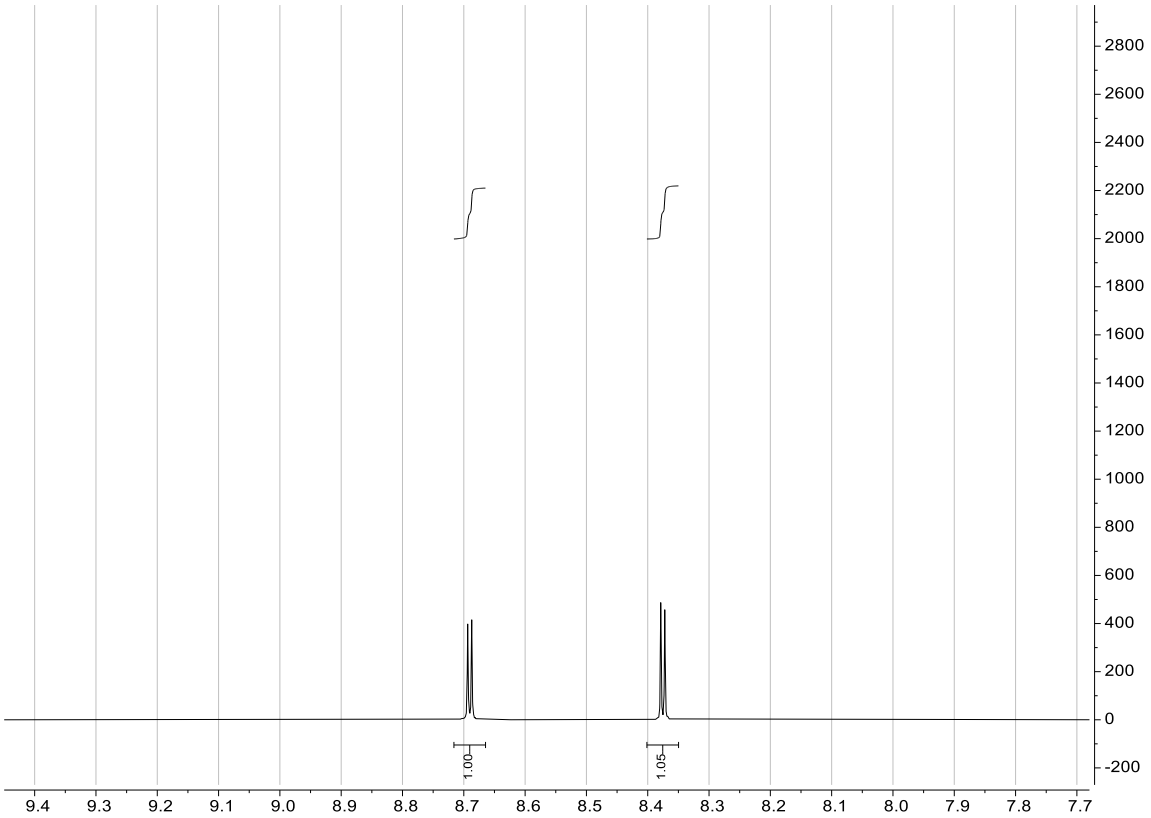

1.02
1.08


162.88
152.79
146.65
142.12
128.34
118.85
62.24
39.52 DMSO-d6
13.83

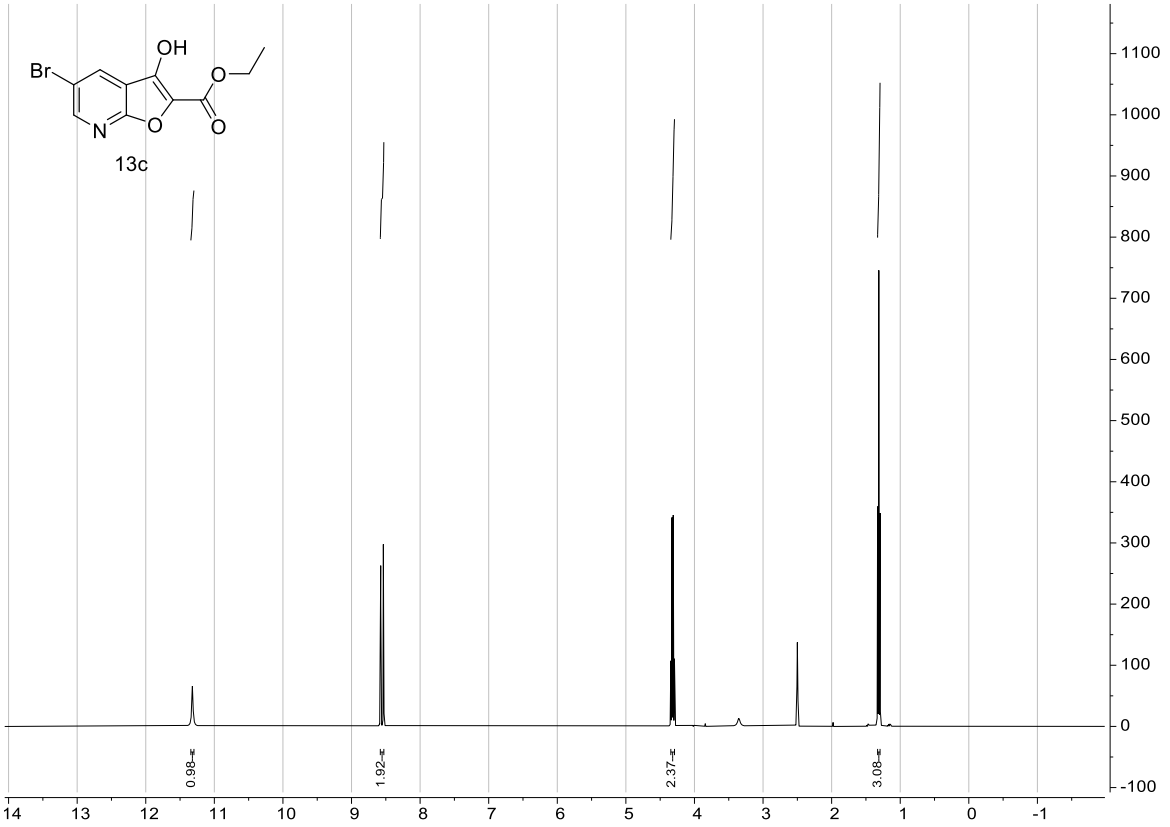

162.88
152.79
146.65
142.12
128.34
118.85
85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 -5
170 168 166 164 162 160 158 156 154 152 150 148 146 144 142 140 138 136 134 132 130 128 126 124 122 120 118 116 114 112

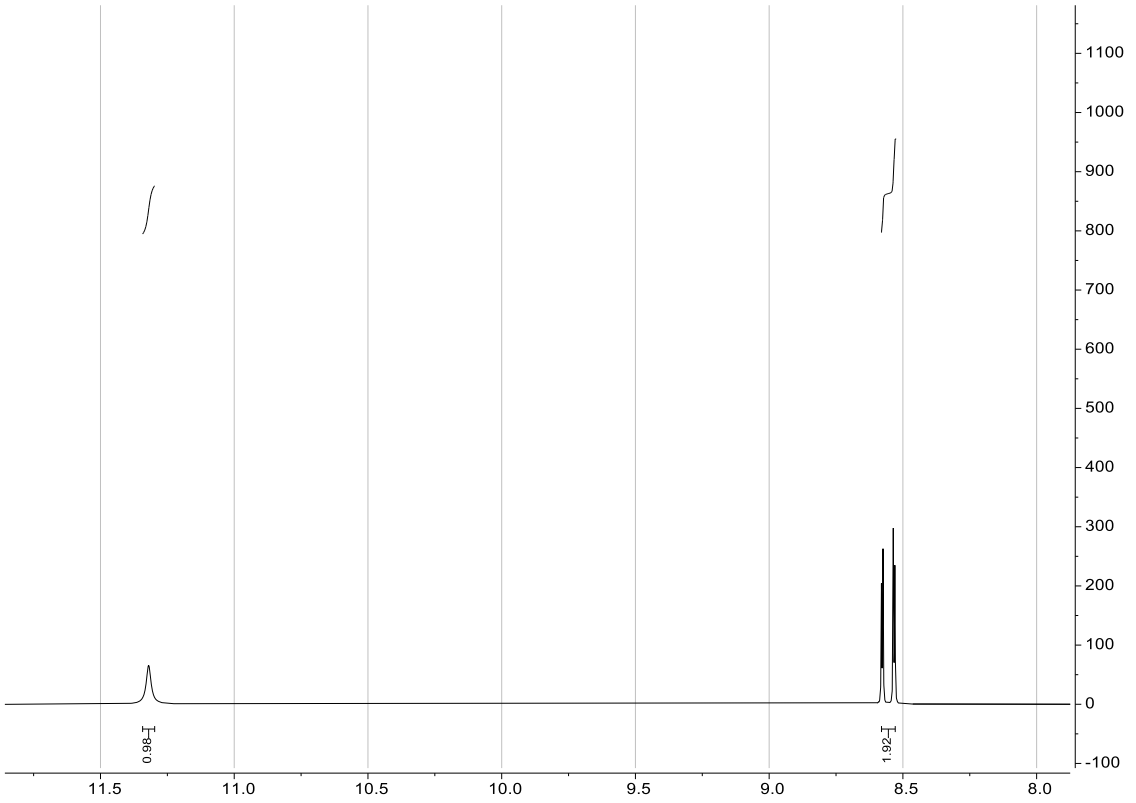
Ethyl 2,5-dichloronicotinate (14b)

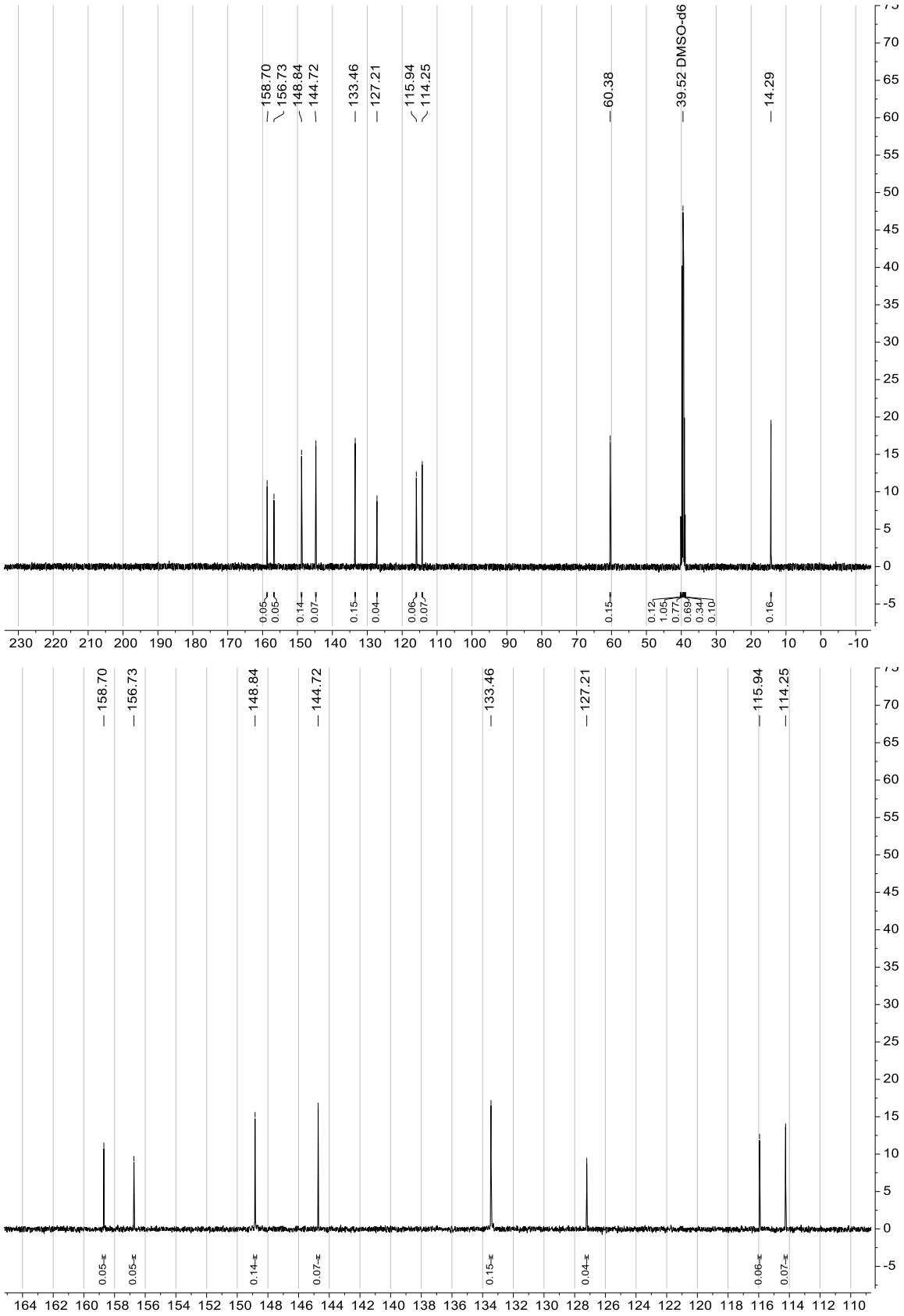




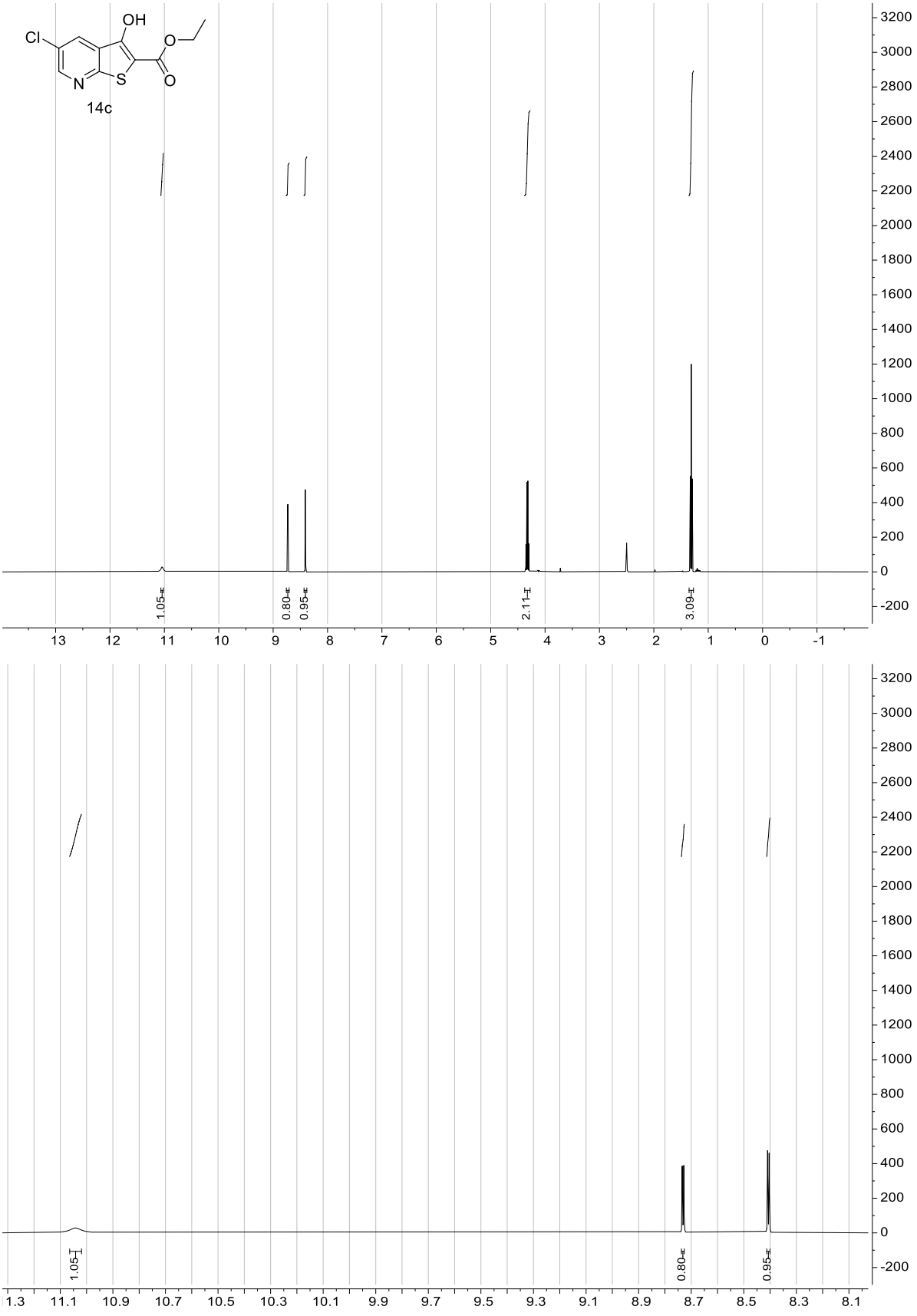
Ethyl 5-bromo-3-hydroxyfuro[2,3-*b*]pyridine-2-carboxylate (13c)

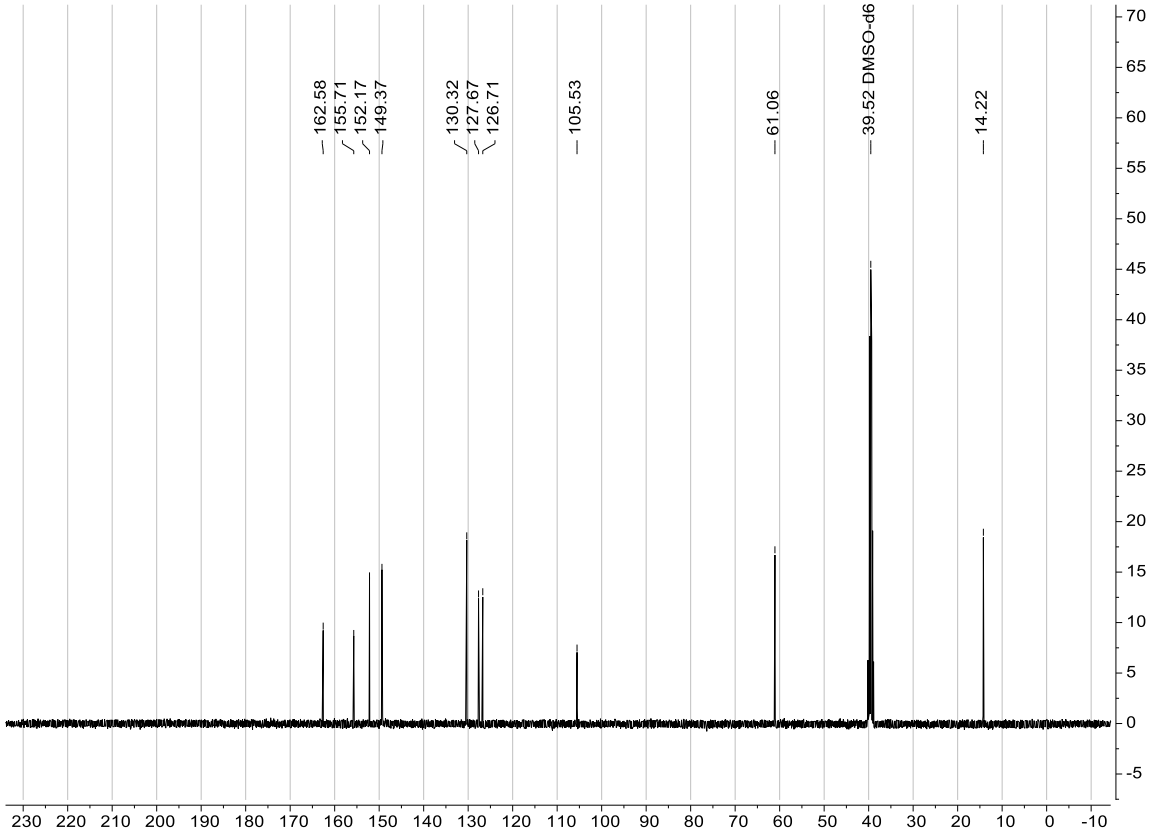


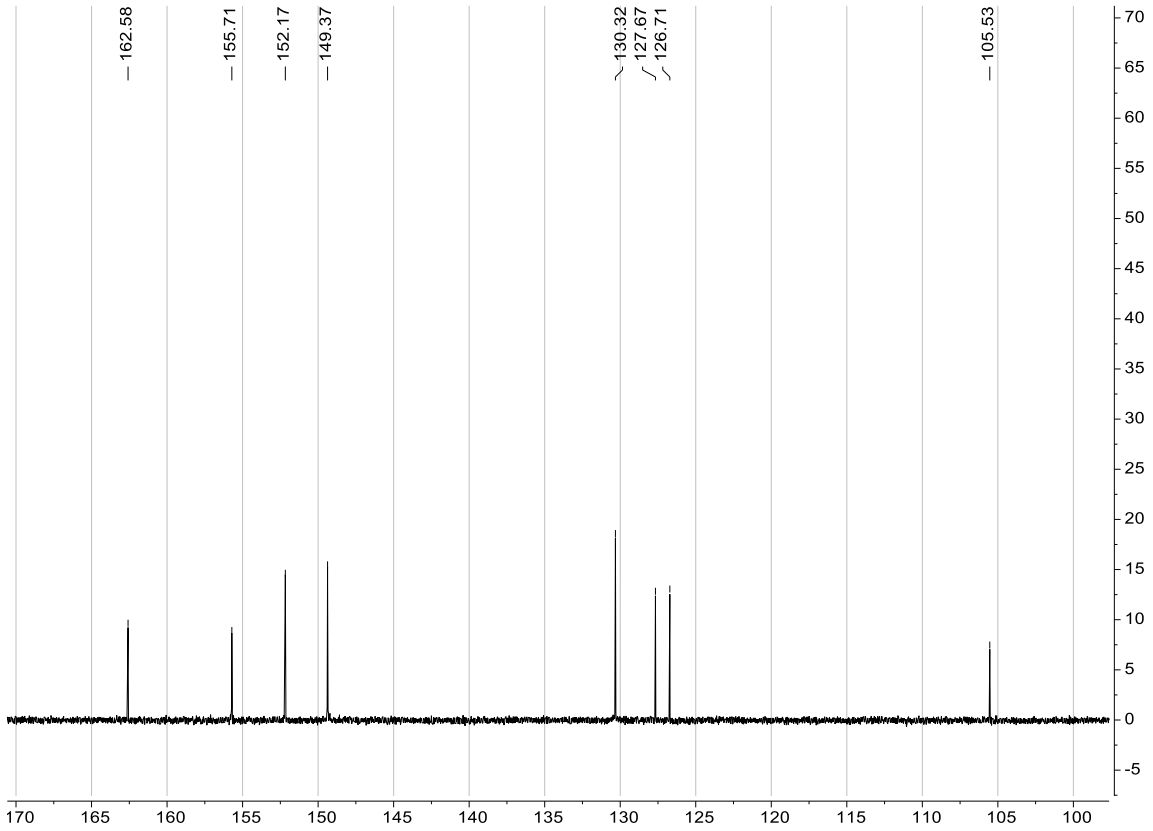



158.70
156.73
148.84
144.72
133.46
127.21
115.94
114.25
60.38
39.52 DMSO-d6
14.29
158.70
156.73
148.84
144.72
133.46
127.21
115.94
114.25

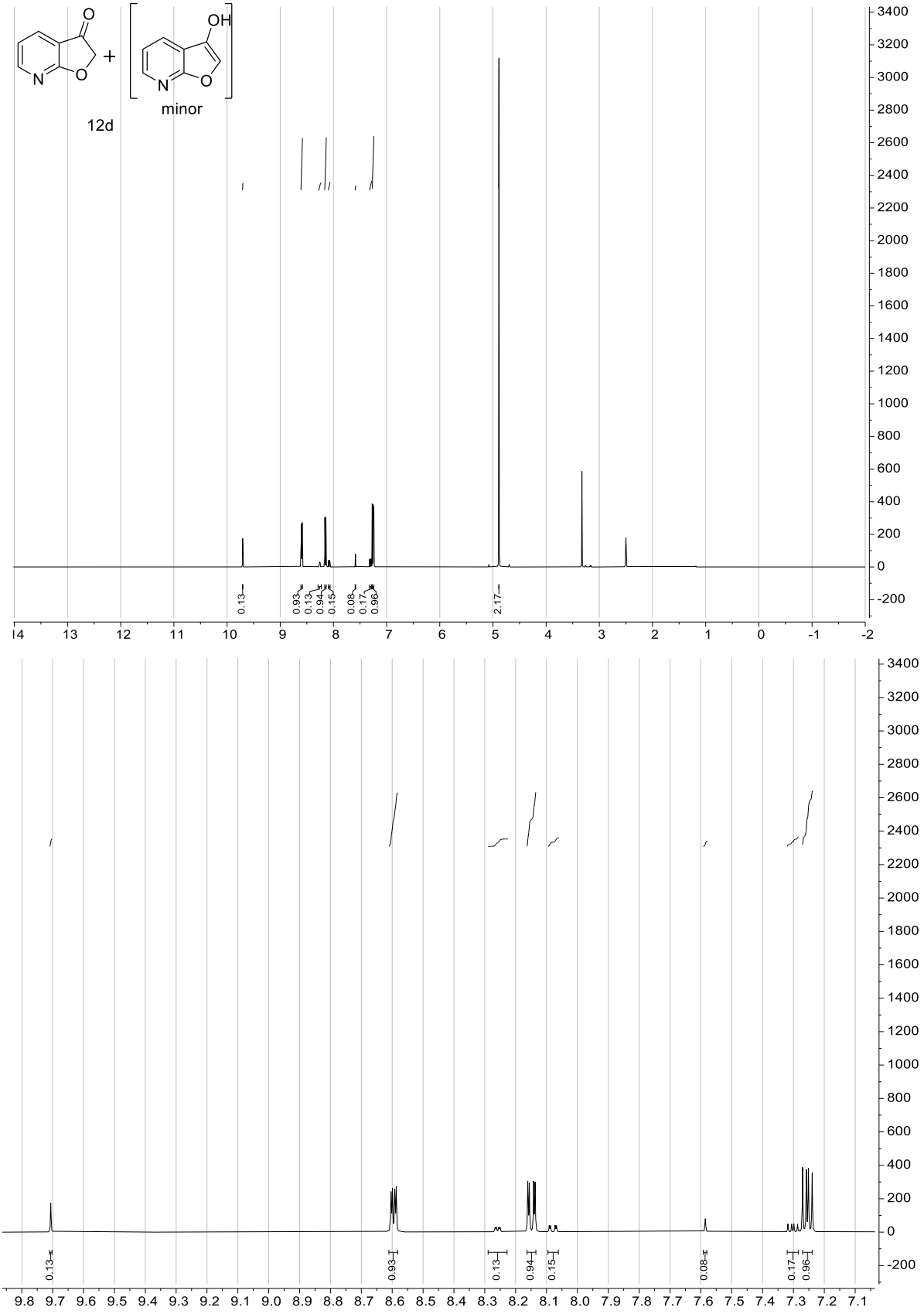
Ethyl 5-chloro-3-hydroxythieno[2,3-*b*]pyridine-2-carboxylate (14c)

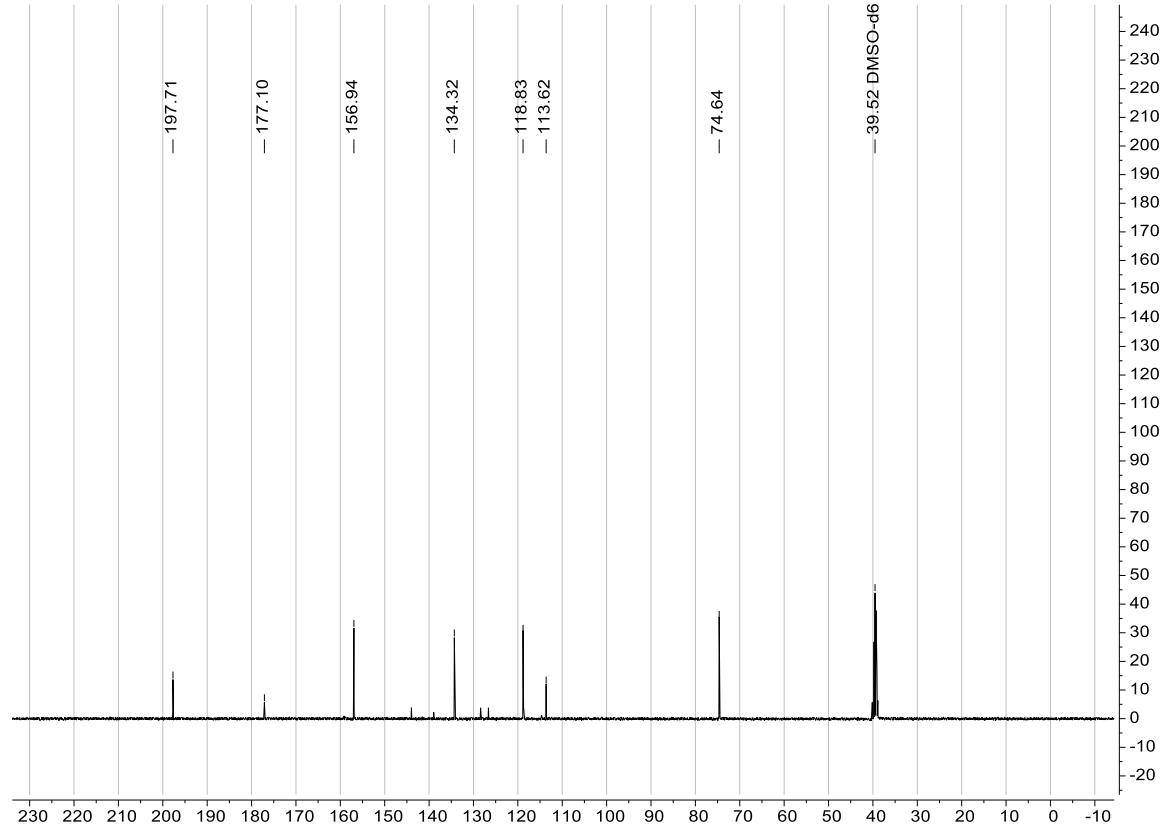


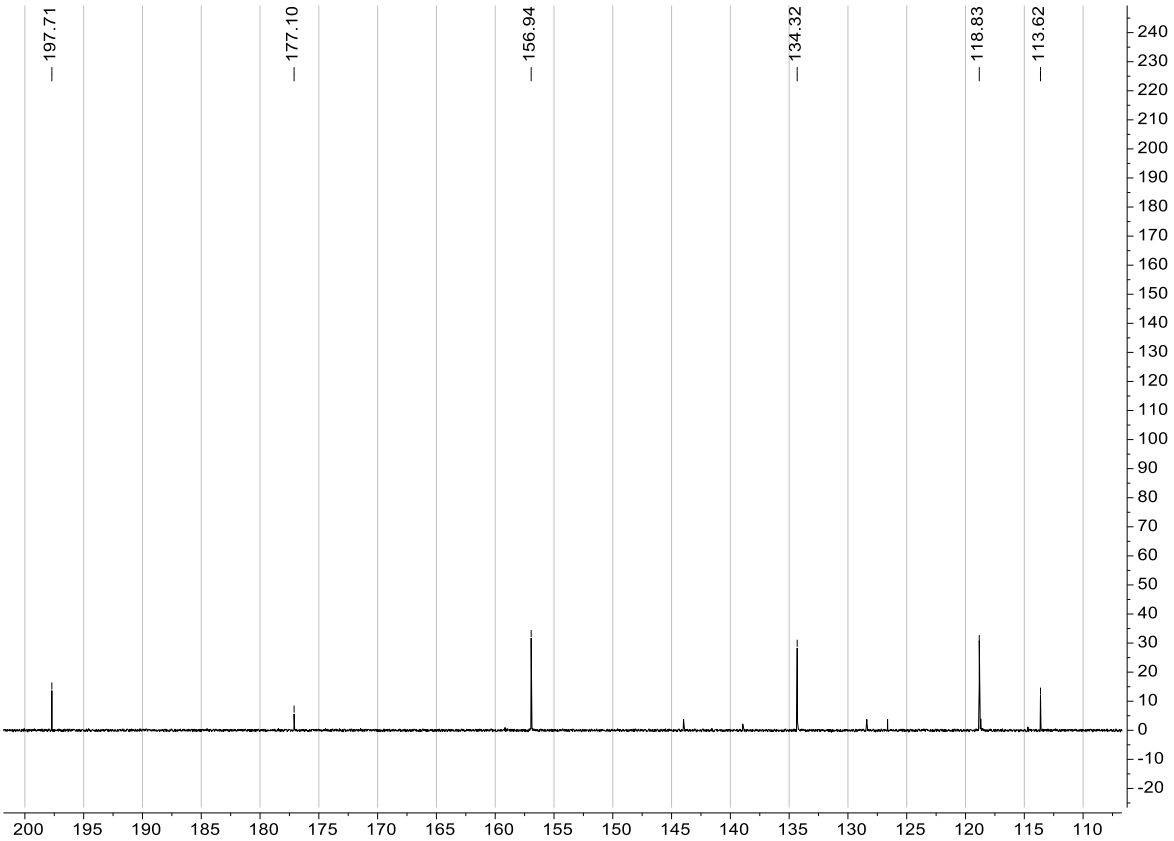

162.58
155.71
152.17
149.37
130.32
127.67
126.71
105.53
61.06
39.52 DMSO-d6
14.22


162.58
155.71
152.17
149.37
130.32
127.67
126.71
105.53

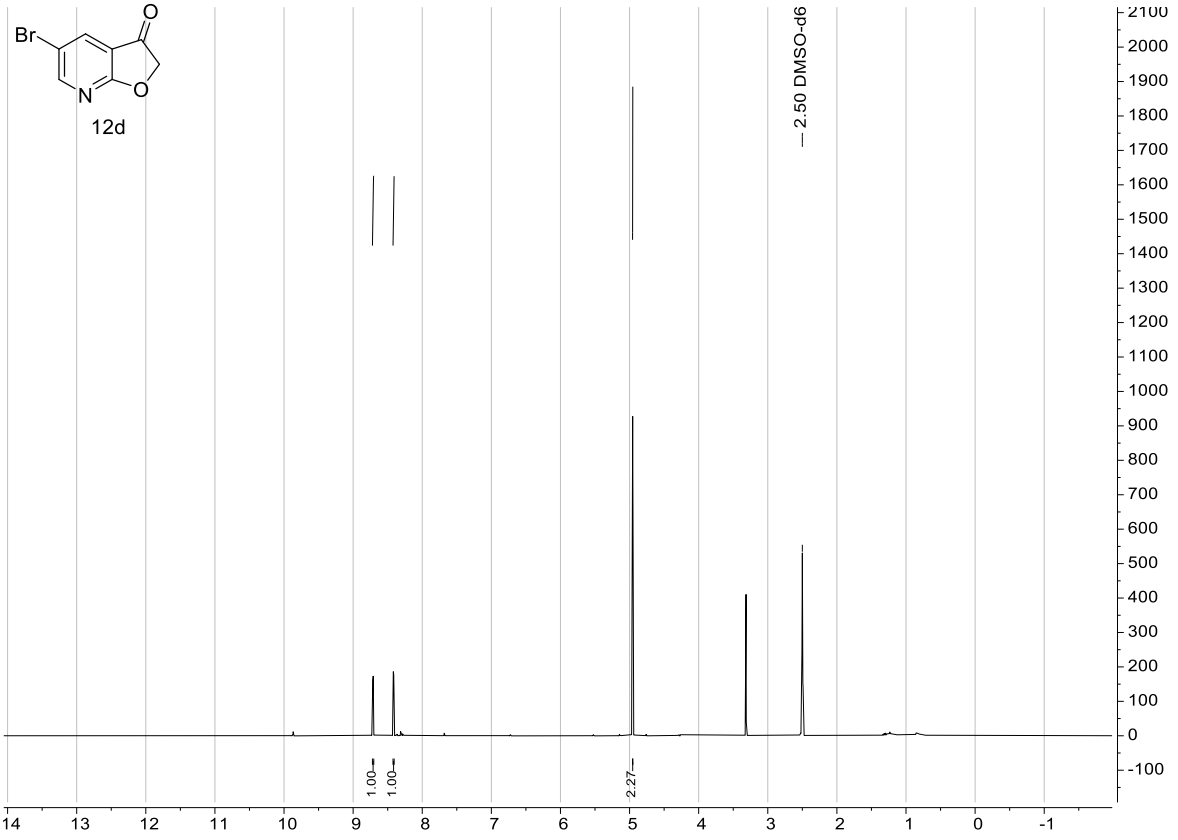
Furo[2,3-*b*]pyridin-3(2*H*)-one (12d)

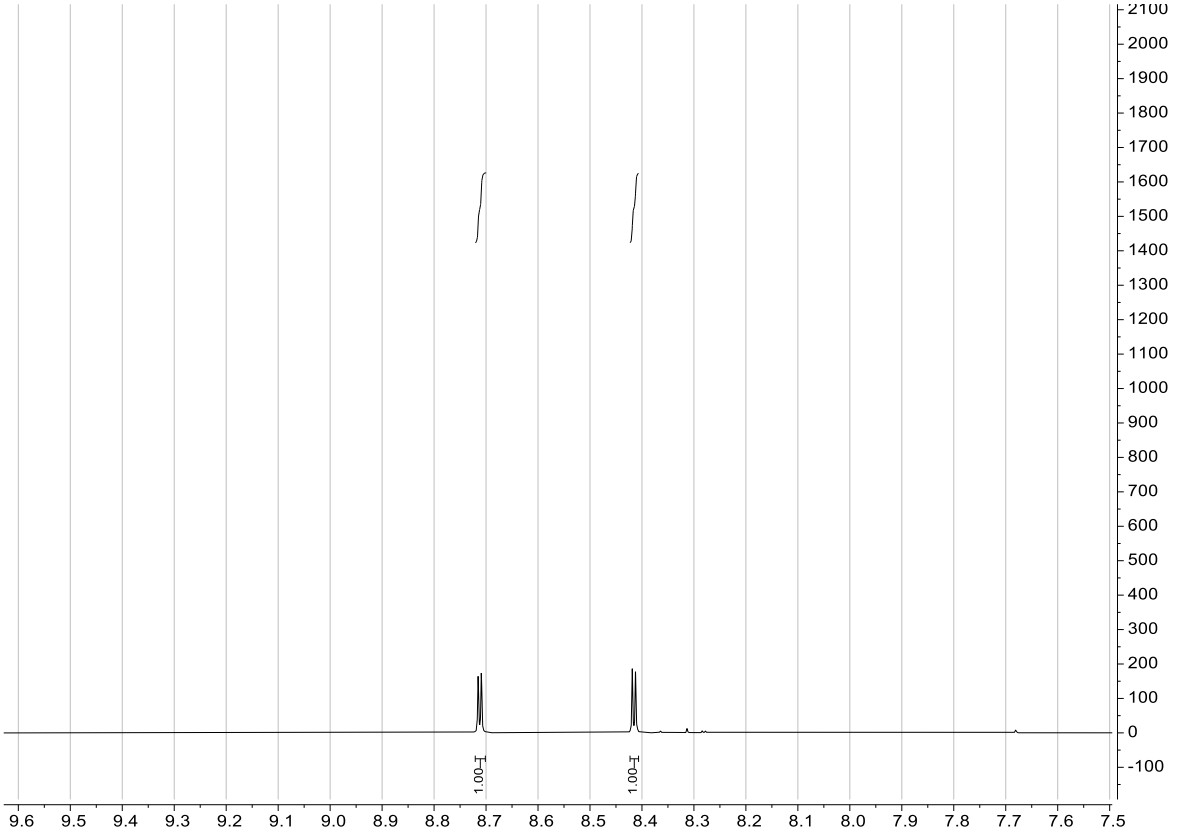


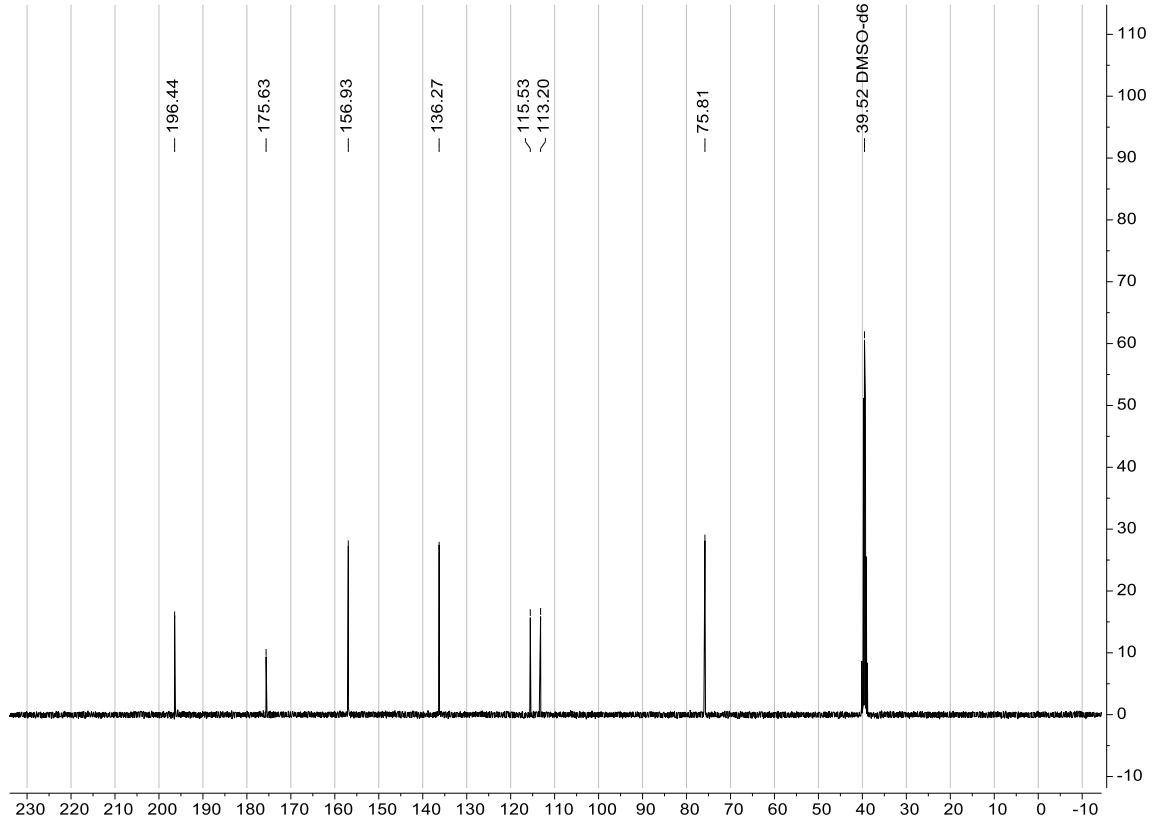

197.71
177.10
156.94
134.32
118.83
113.62
74.64
39.52 DMSO-d6

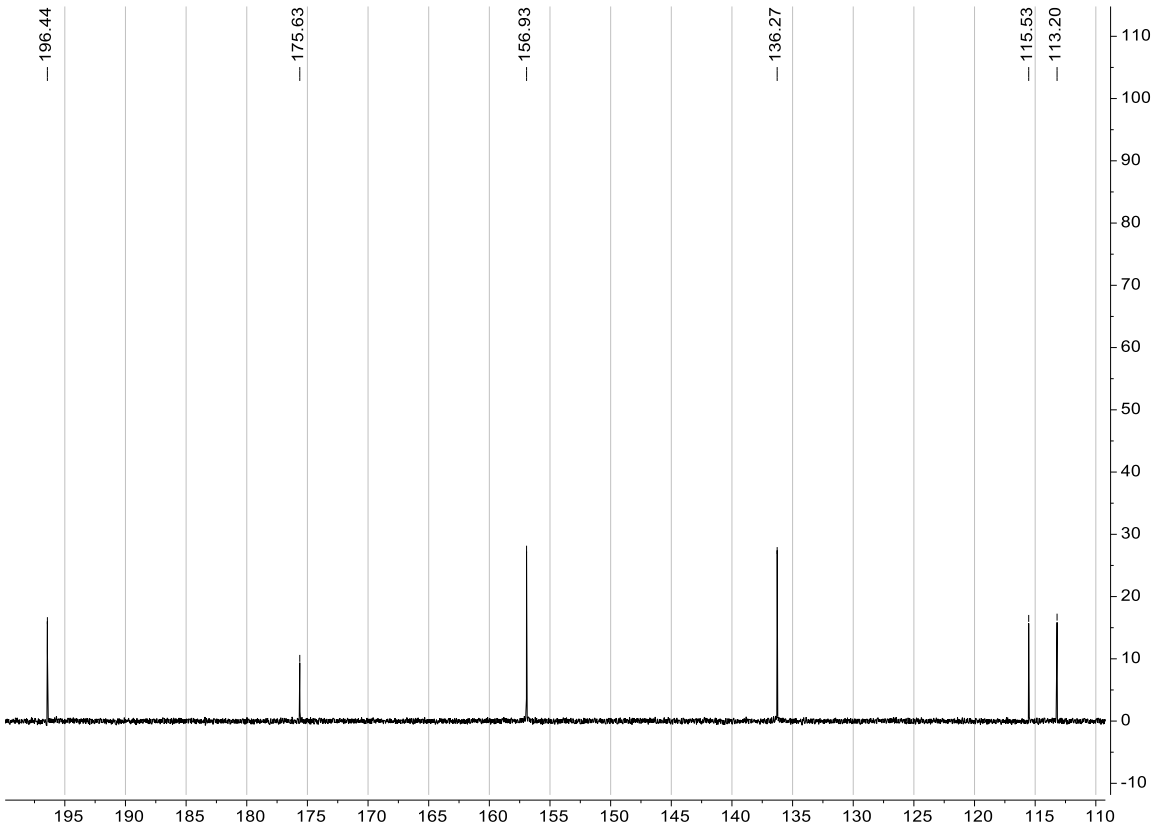

197.71
177.10
156.94
134.32
118.83
113.62

5-Bromofuro[2,3-*b*]pyridin-3(2*H*)-one (13d)

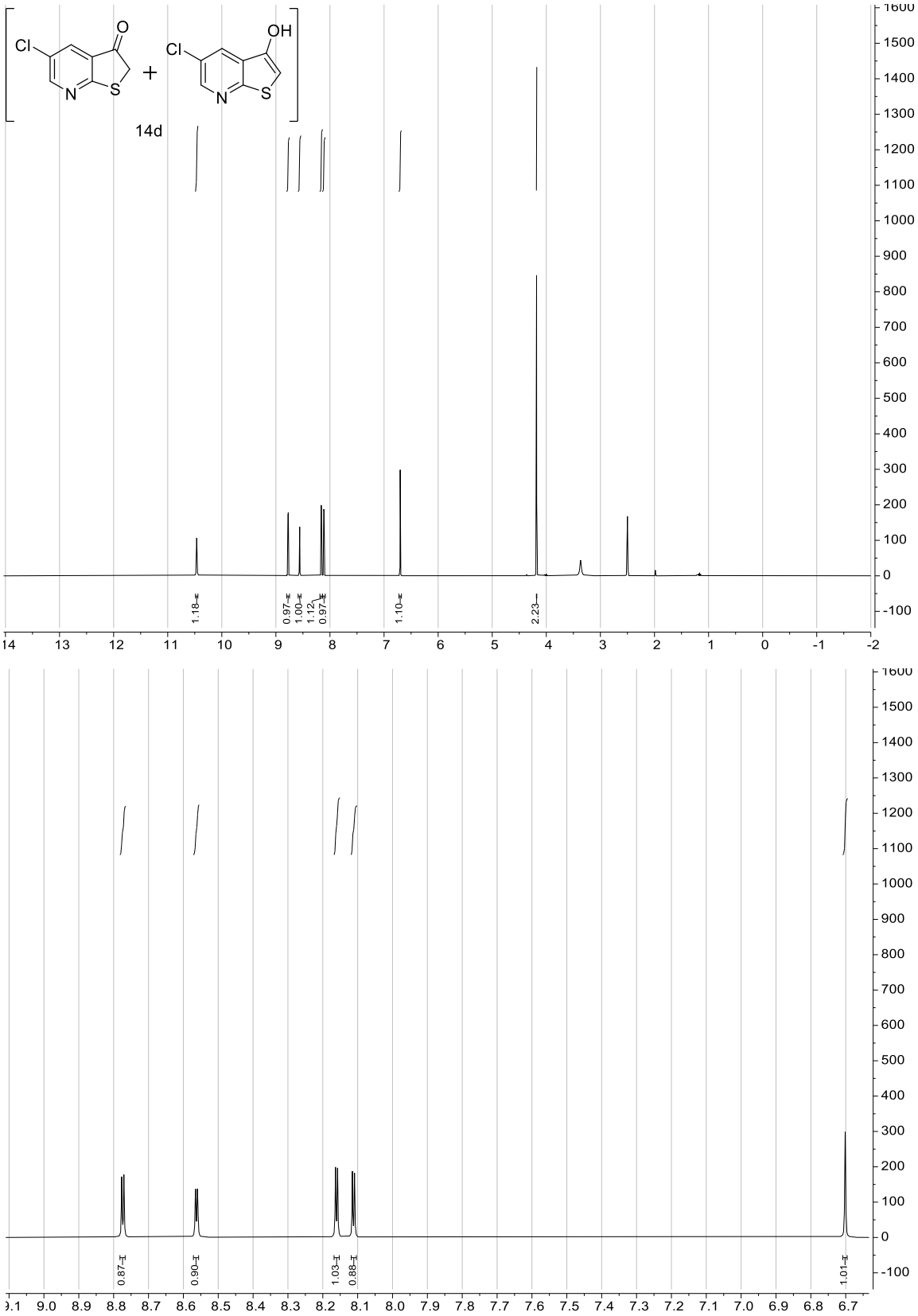


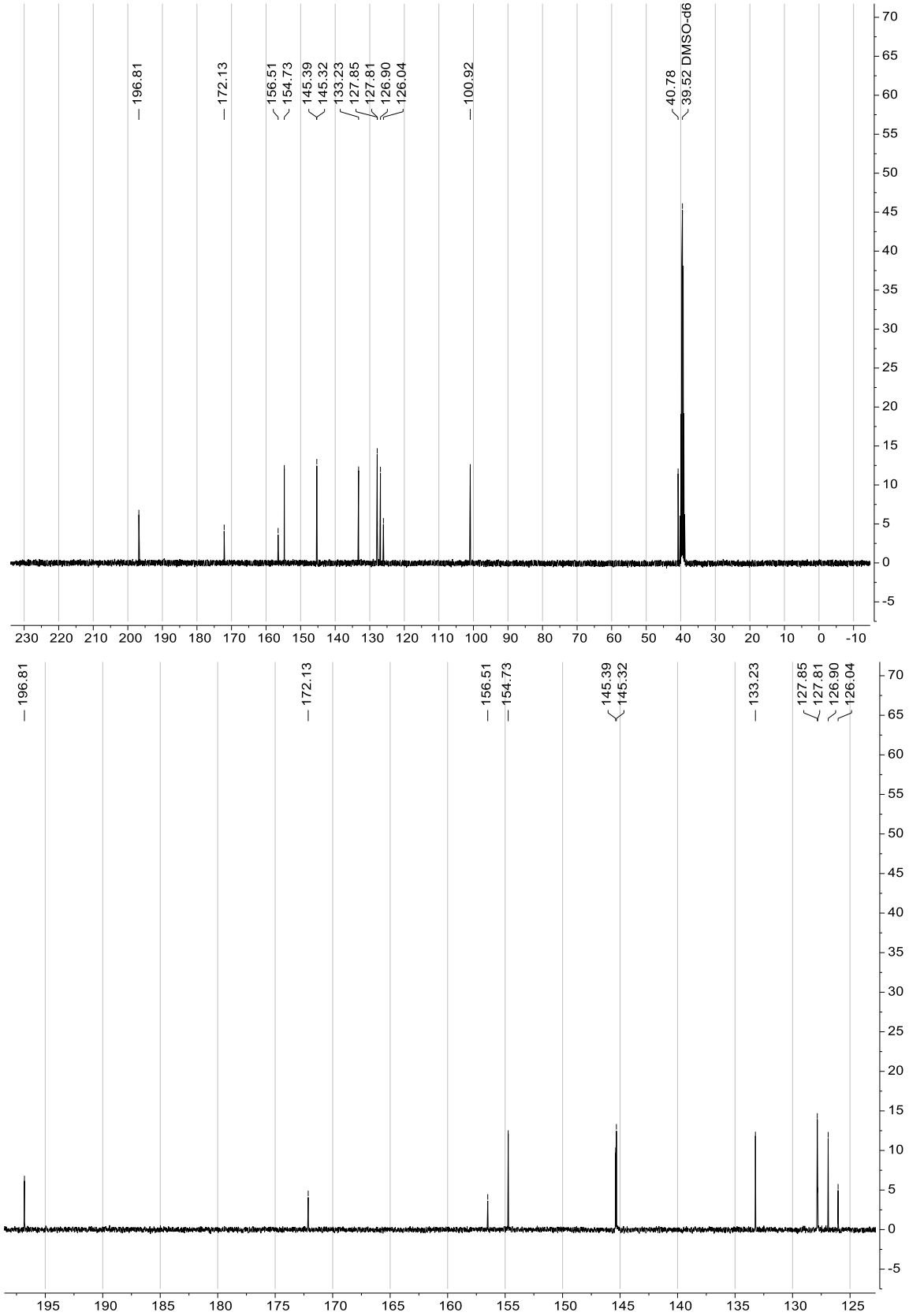



196.44
175.63
156.93
136.27
115.53
113.20
75.81
39.52 DMSO-d6

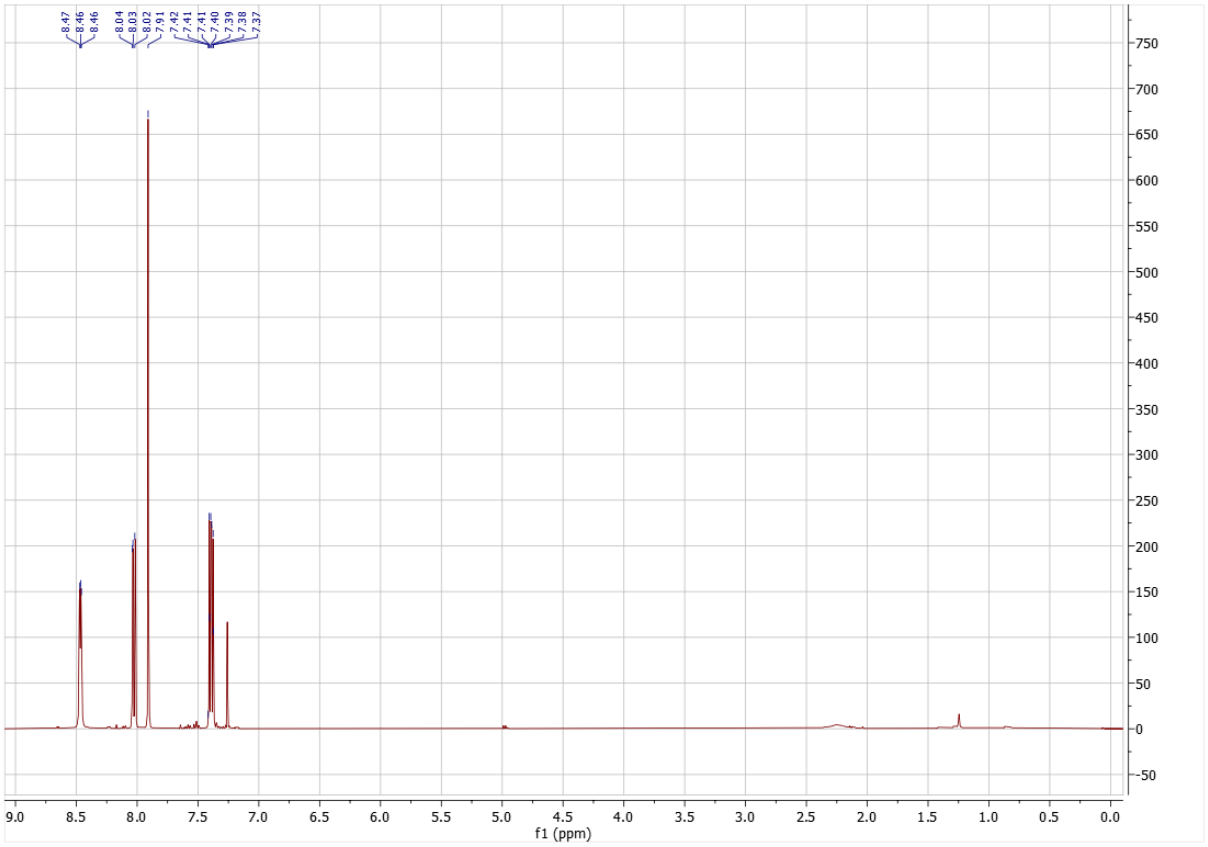

196.44
175.63
156.93
136.27
115.53
113.20

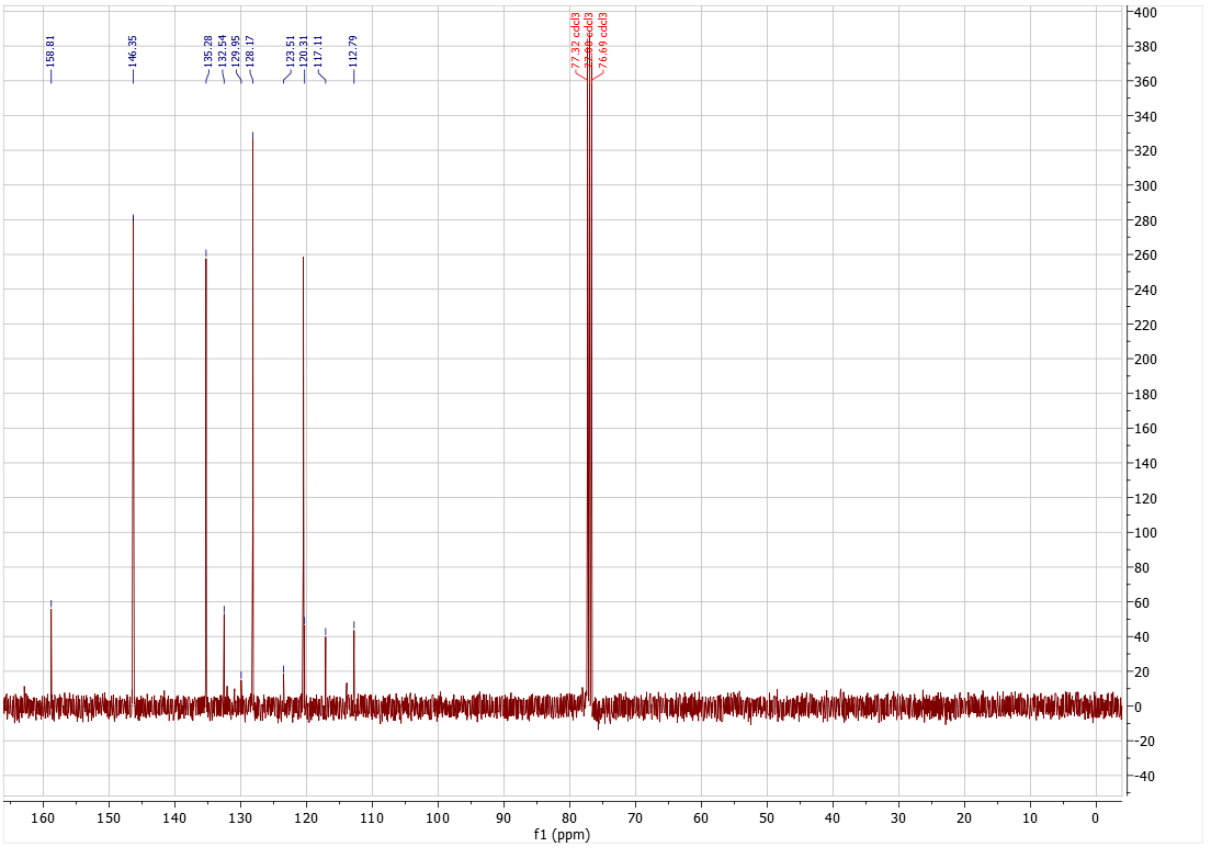
5-Chlorothieno[2,3-*b*]pyridin-3(2*H*)-one (14d)



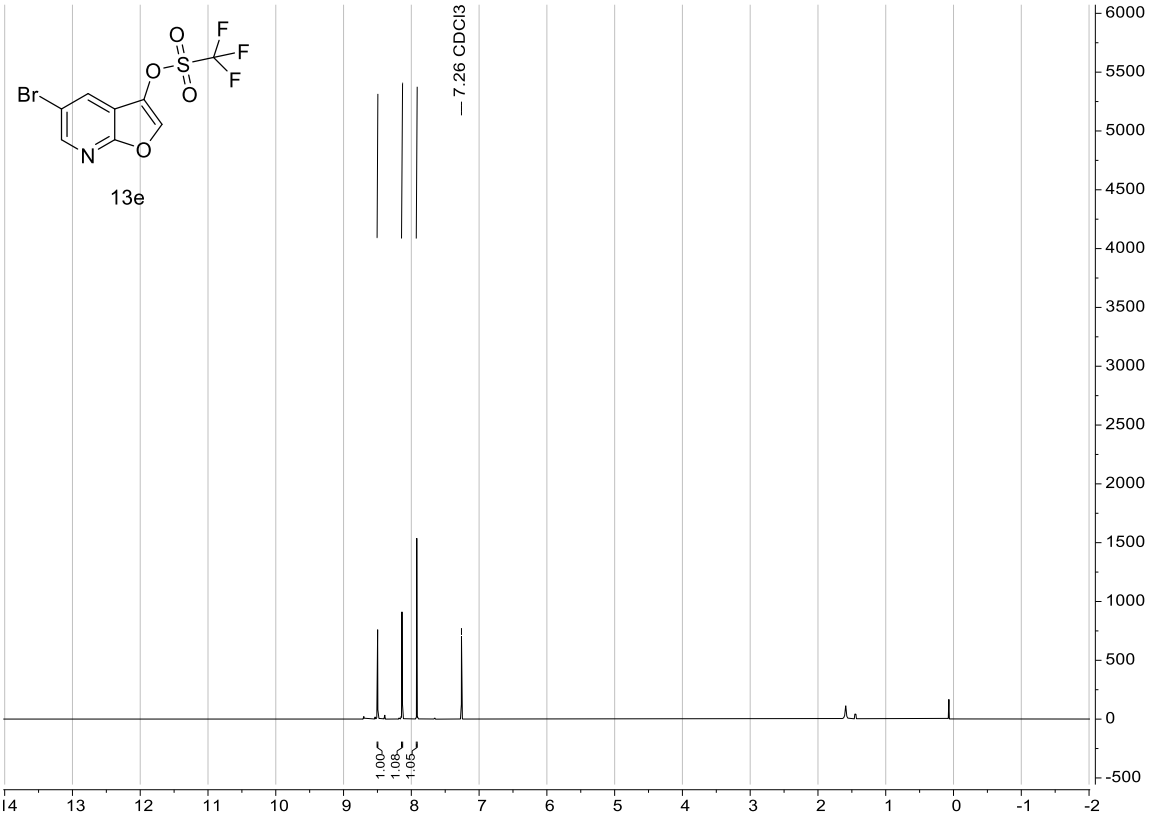

196.81
172.13
156.51
154.73
145.39
145.32
133.23
127.85
127.81
126.90
126.04
100.92
40.78
39.52 DMSO-d6

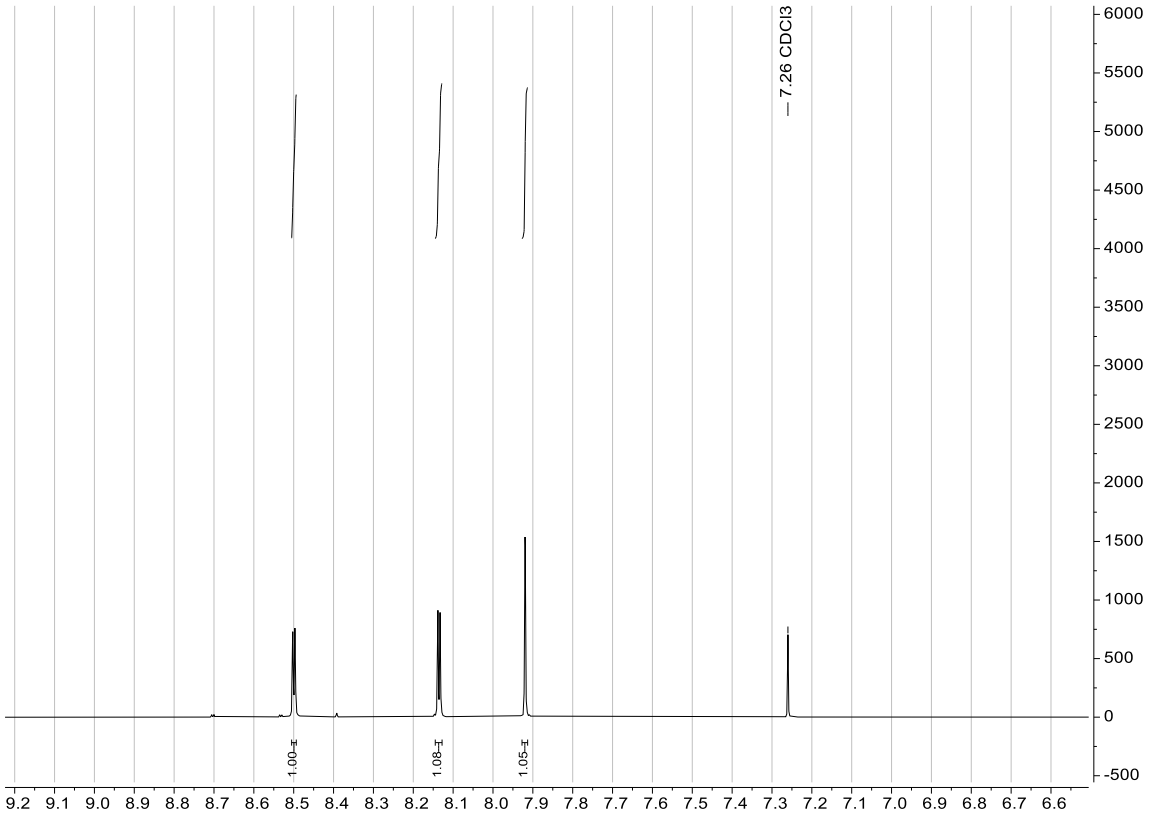
Furo[2,3-*b*]pyridin-3-yl trifluoromethanesulfonate (12e)



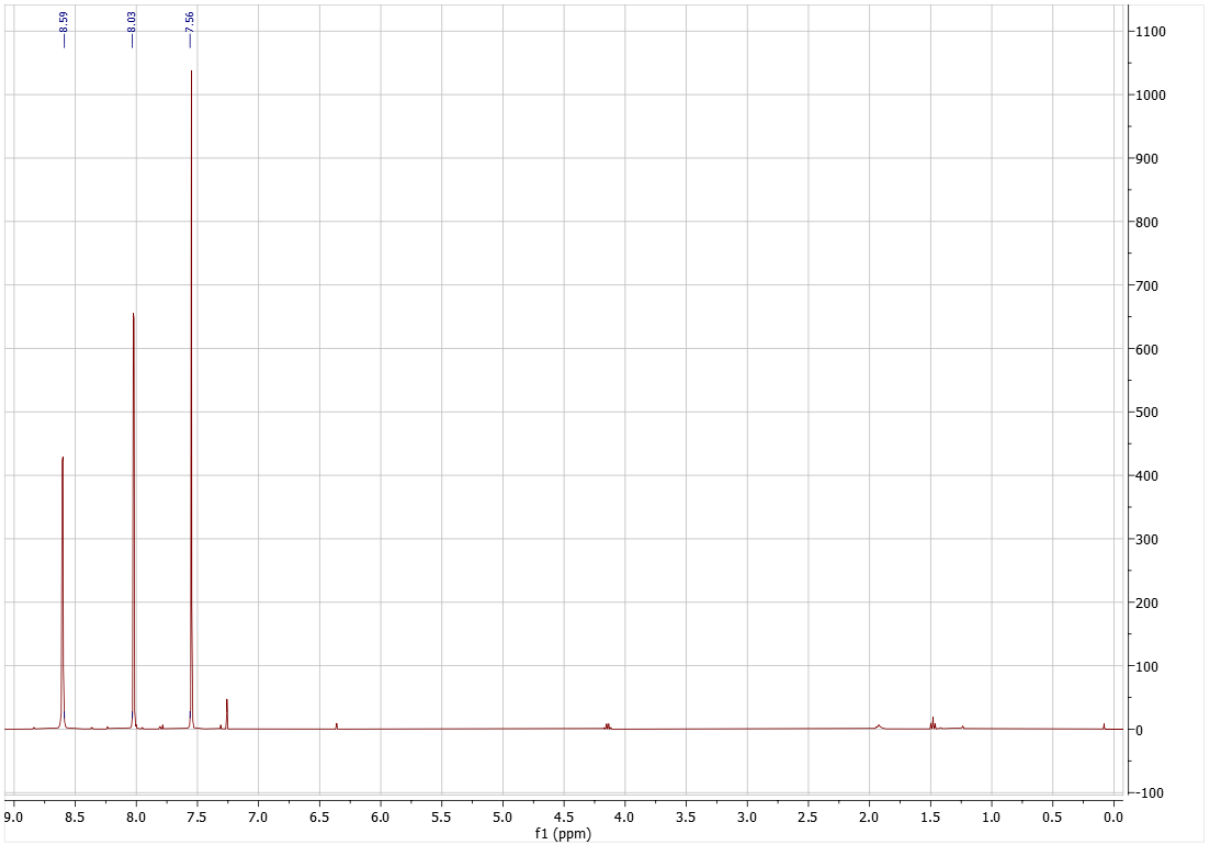


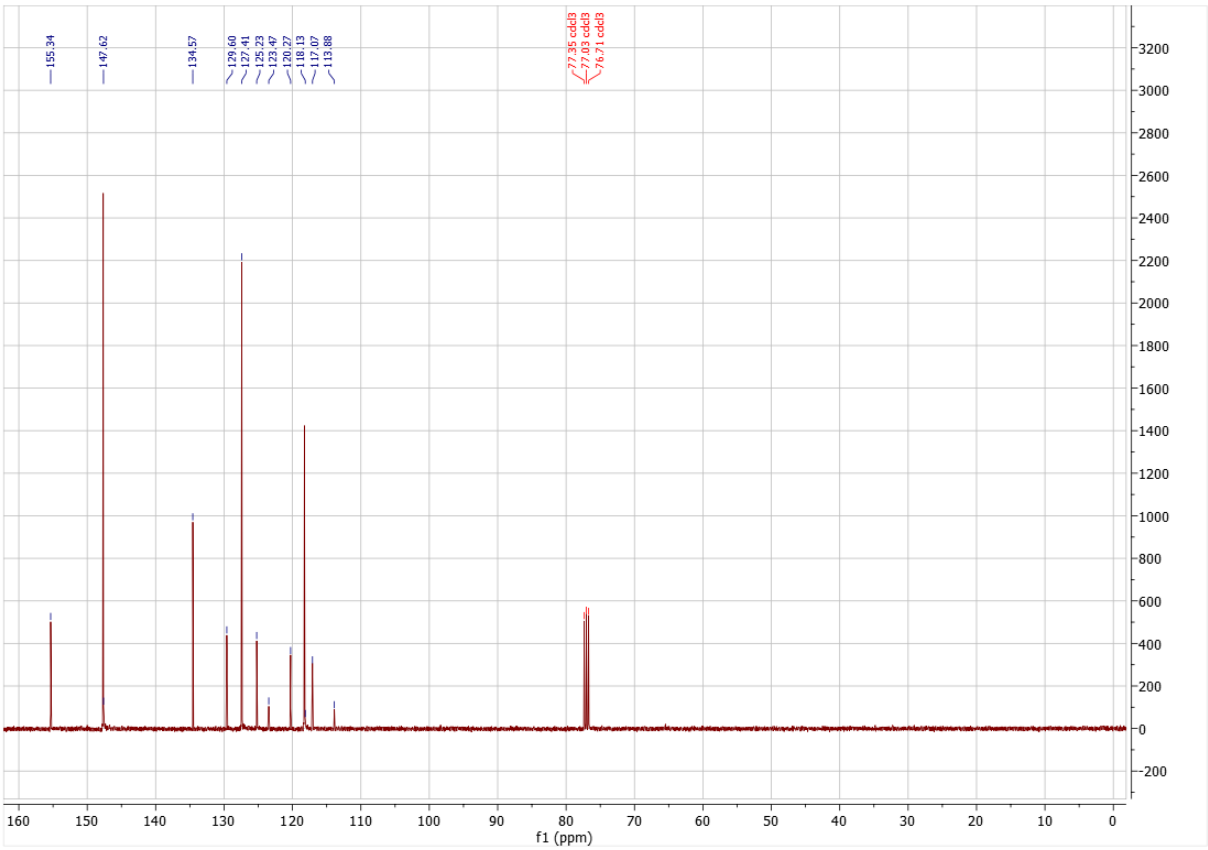
5-Bromofuro[2,3-*b*]pyridin-3-yl trifluoromethanesulfonate (13e)


Br
O
S
O
O
F
F
F
N
O
13e
7.26 CDCl3
1.00
1.08
1.05

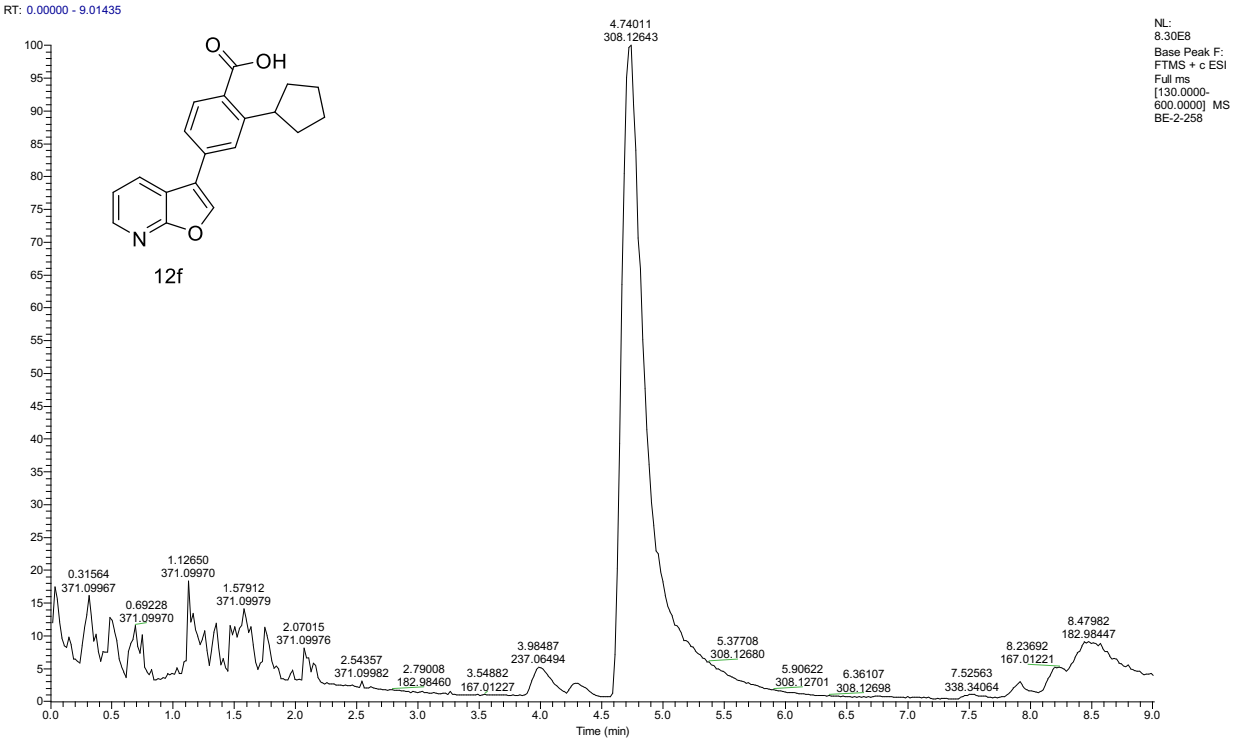

7.26 CDCl3
1.00
1.08
1.05

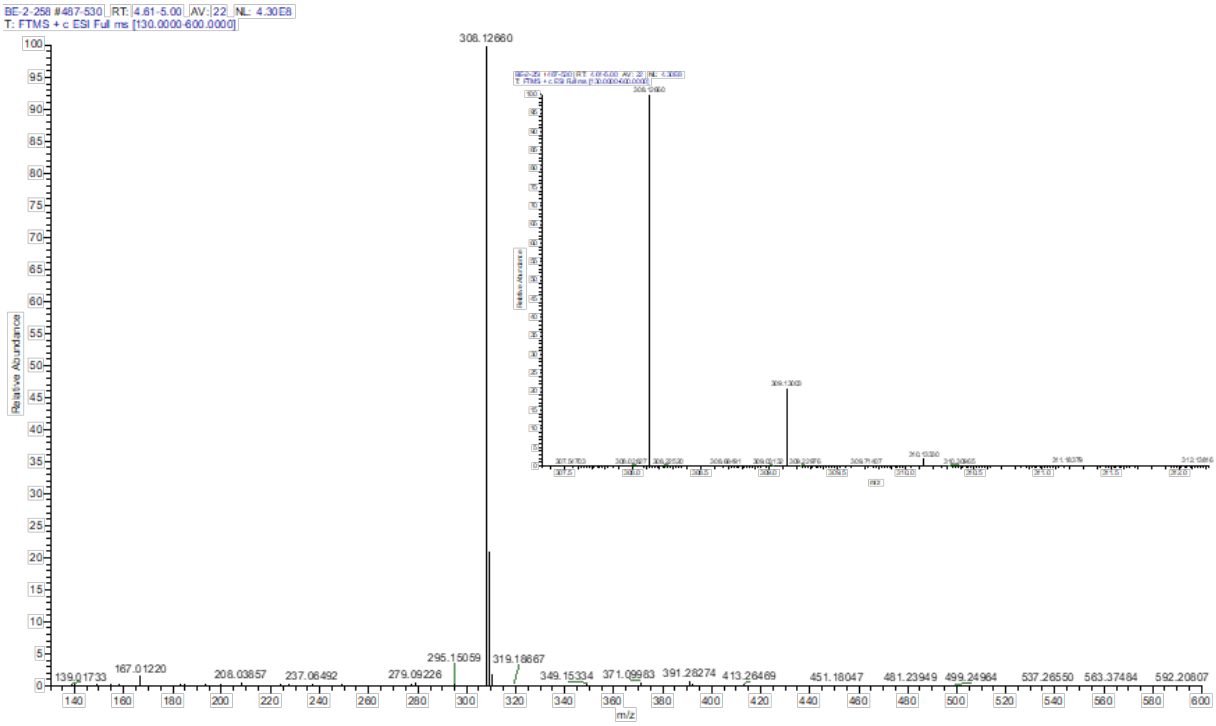
5-Chlorothieno[2,3-*b*]pyridin-3-yl trifluoromethanesulfonate (14e)

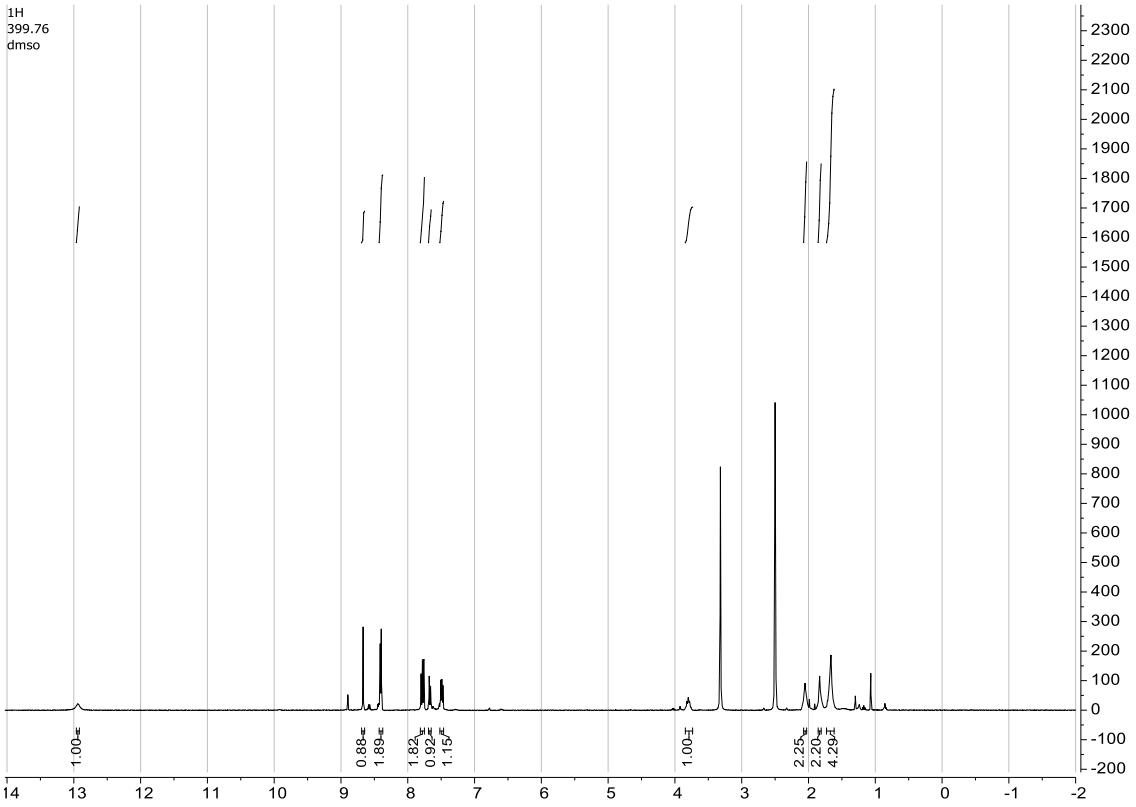


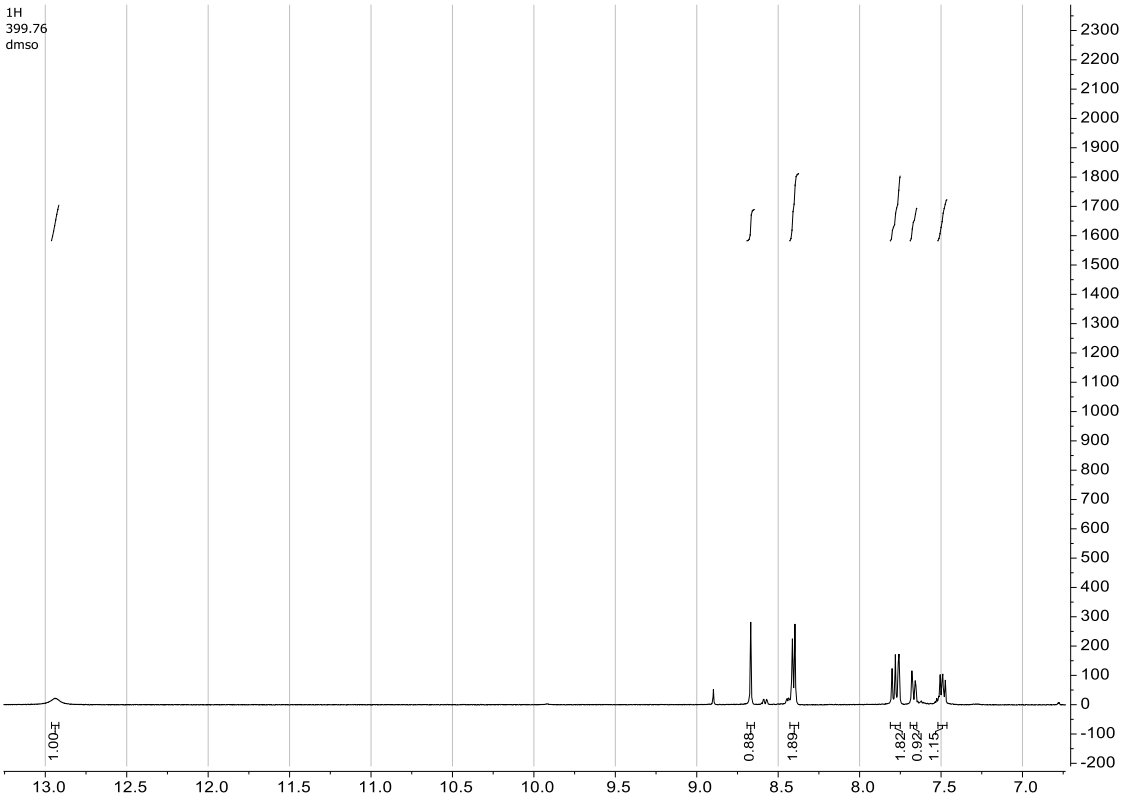


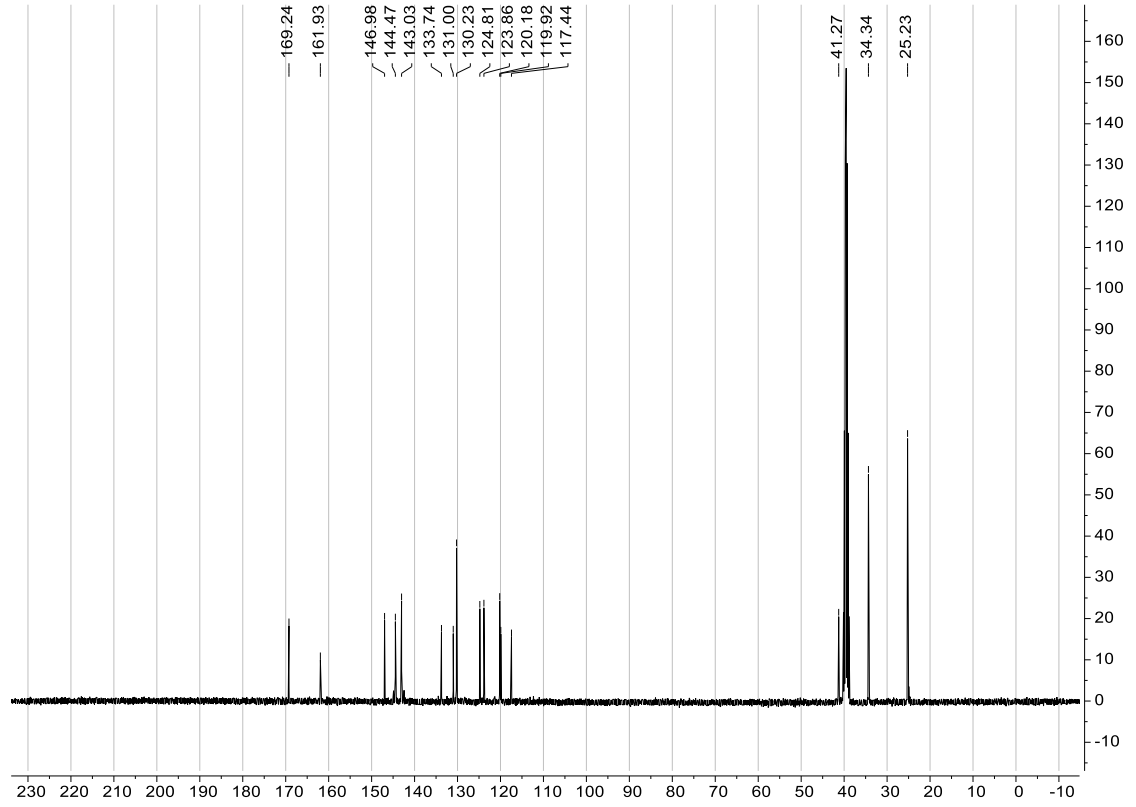
2-Cyclopentyl-4-(furo[2,3-*b*]pyridin-3-yl)benzoic acid (12f)

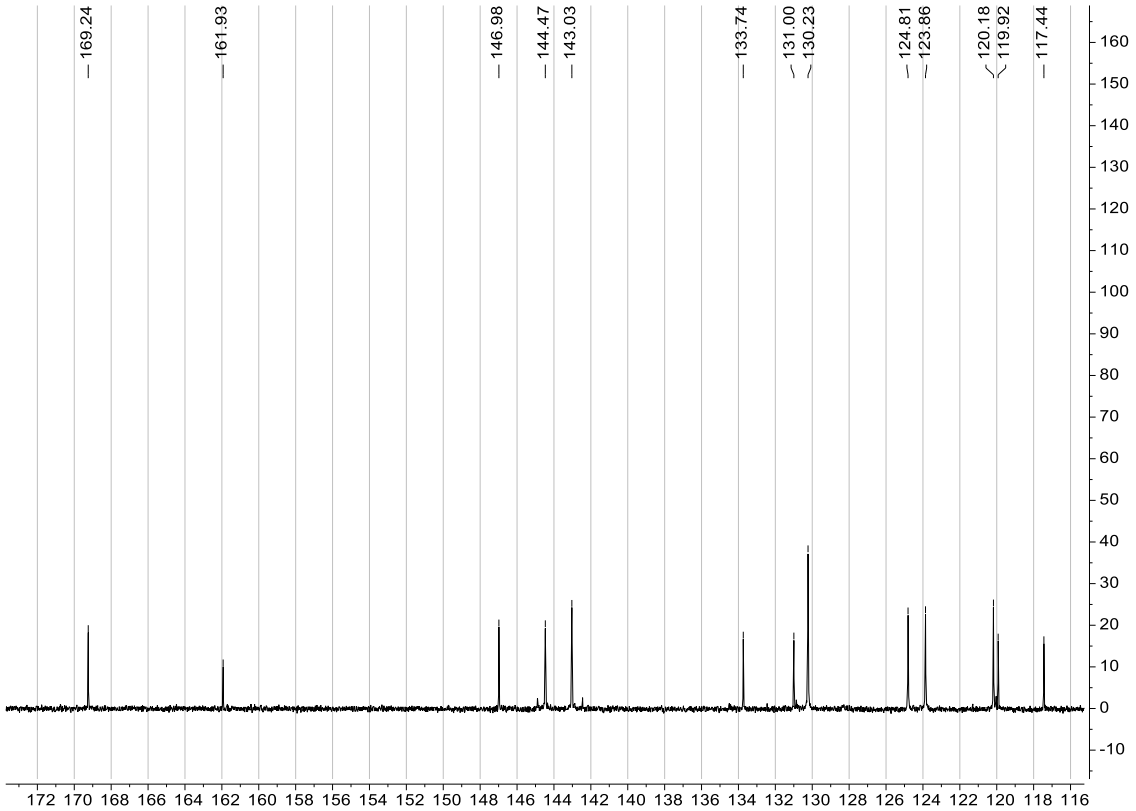

RT: 0.00000 - 9.01435
NL:
8.30E8
Base Peak F:
FTMS + c ESI
Full ms
[130.0000-
600.0000] MS
BE-2-258
12f
4.74011
308.12643
0.31564
371.09967
0.69228
371.09970
1.12650
371.09970
1.57912
371.09979
2.07015
371.09976
2.54357
371.09982
2.79008
182.98460
3.54882
167.01227
3.98487
237.06494
5.37708
308.12680
5.90622
308.12701
6.36107
308.12698
7.52563
338.34064
8.23692
167.01221
8.47982
182.98447
Time (min)


BE-2-258 #487-530 RT: 4.61-5.00 AV: 22 NL: 4.30E8
T: FTMS + c ESI Full ms [130.0000-600.0000]
308.12660
295.15059
319.18667
Relative Abundance
m/z

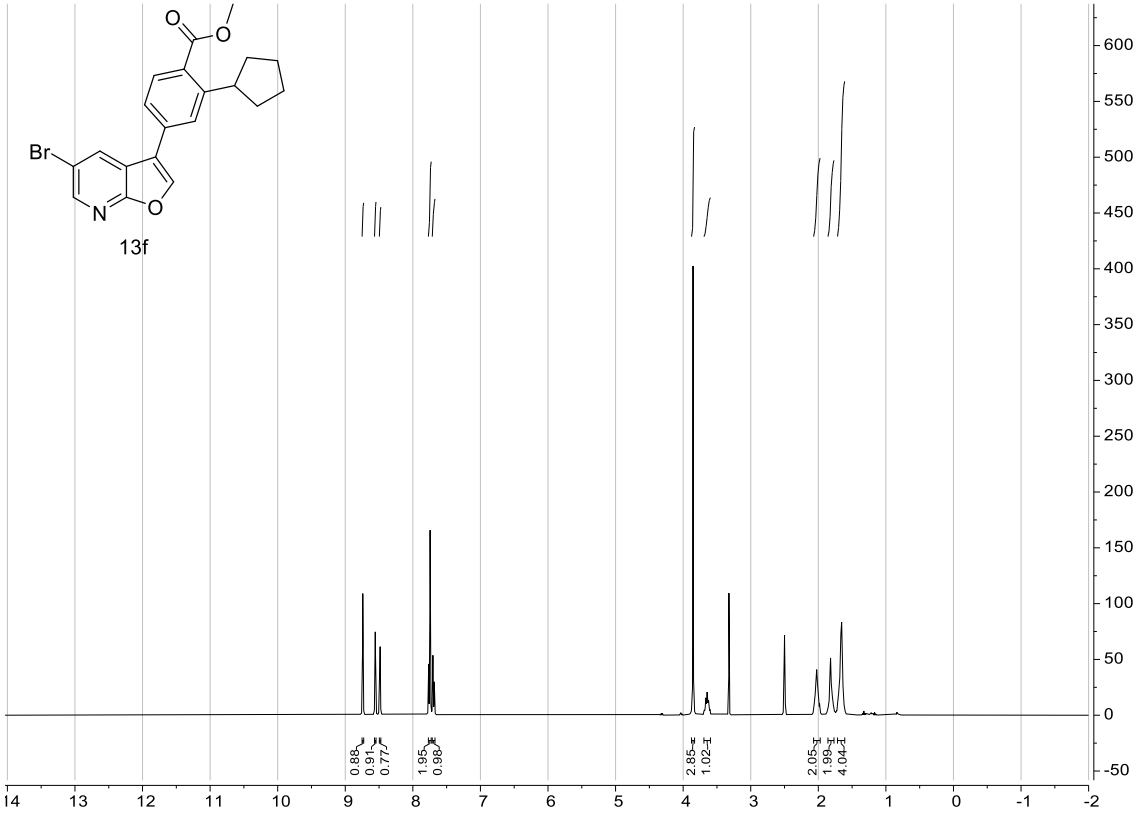

1H
399.76
dmso
1.00
0.88
1.89
1.82
0.92
1.15
1.00
2.25
2.20
4.29

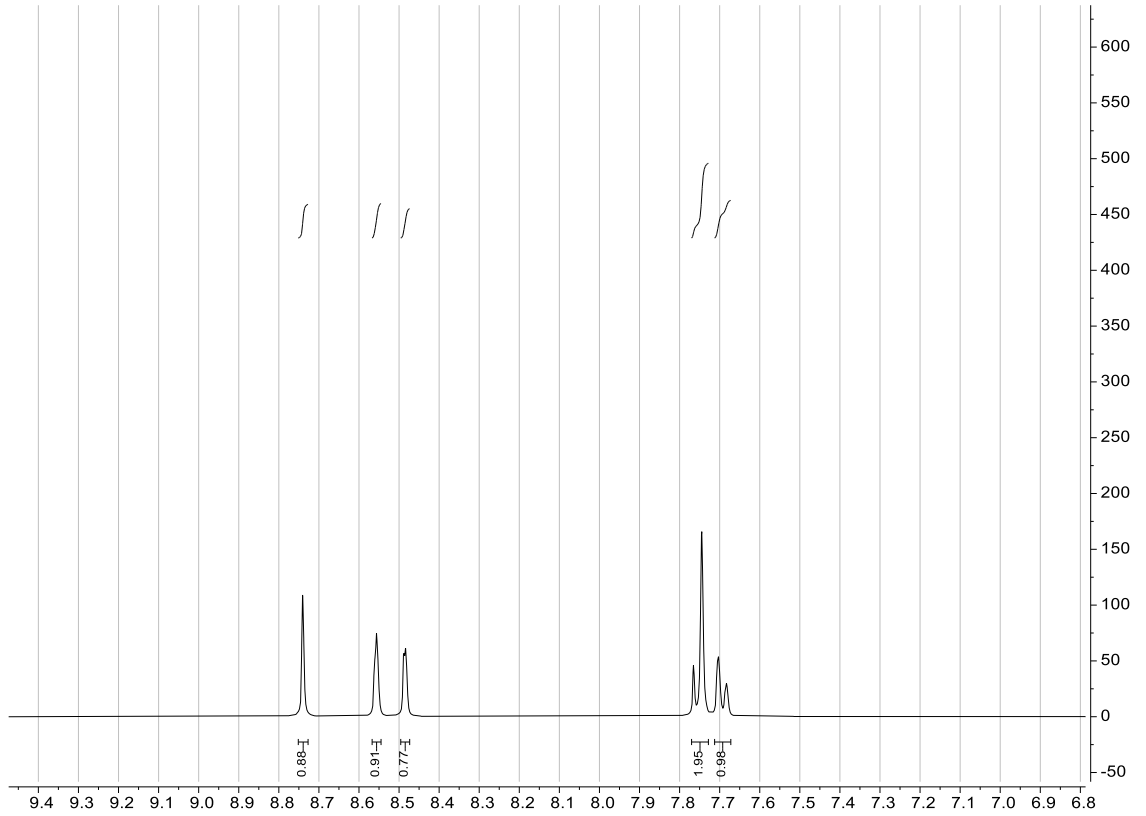

1H
399.76
dmso
1.00
0.88
1.89
1.82
0.92
1.15

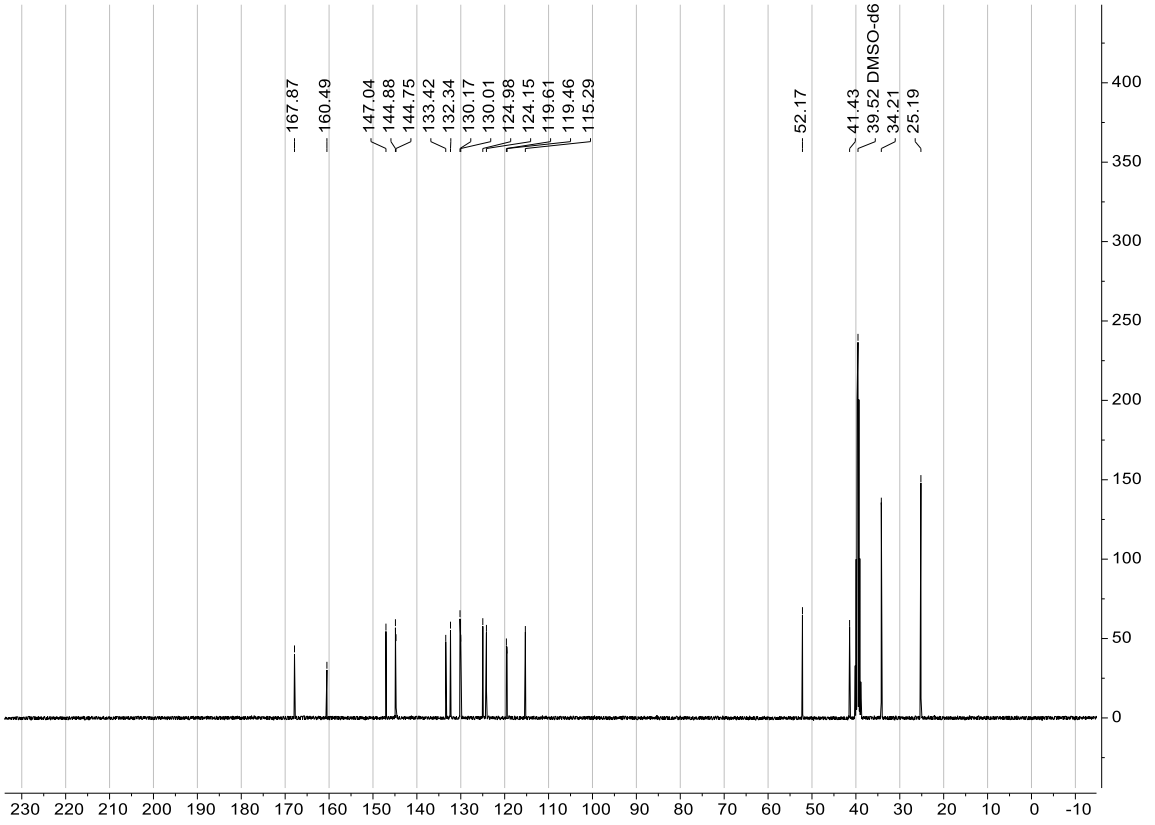

169.24
161.93
146.98
144.47
143.03
133.74
131.00
130.23
124.81
123.86
120.18
119.92
117.44
41.27
34.34
25.23

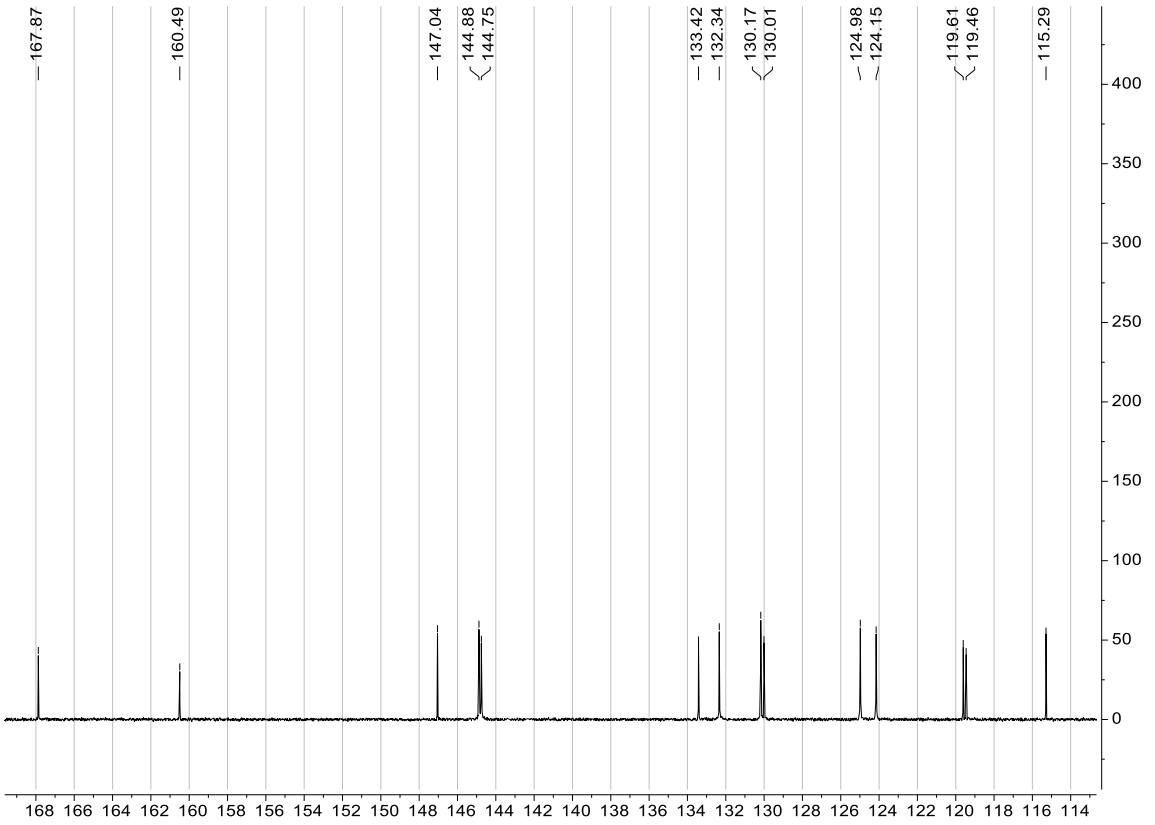

169.24
161.93
146.98
144.47
143.03
133.74
131.00
130.23
124.81
123.86
120.18
119.92
117.44

Methyl 4-(5-bromofuro[2,3-*b*]pyridin-3-yl)-2-cyclopentylbenzoate (13f)

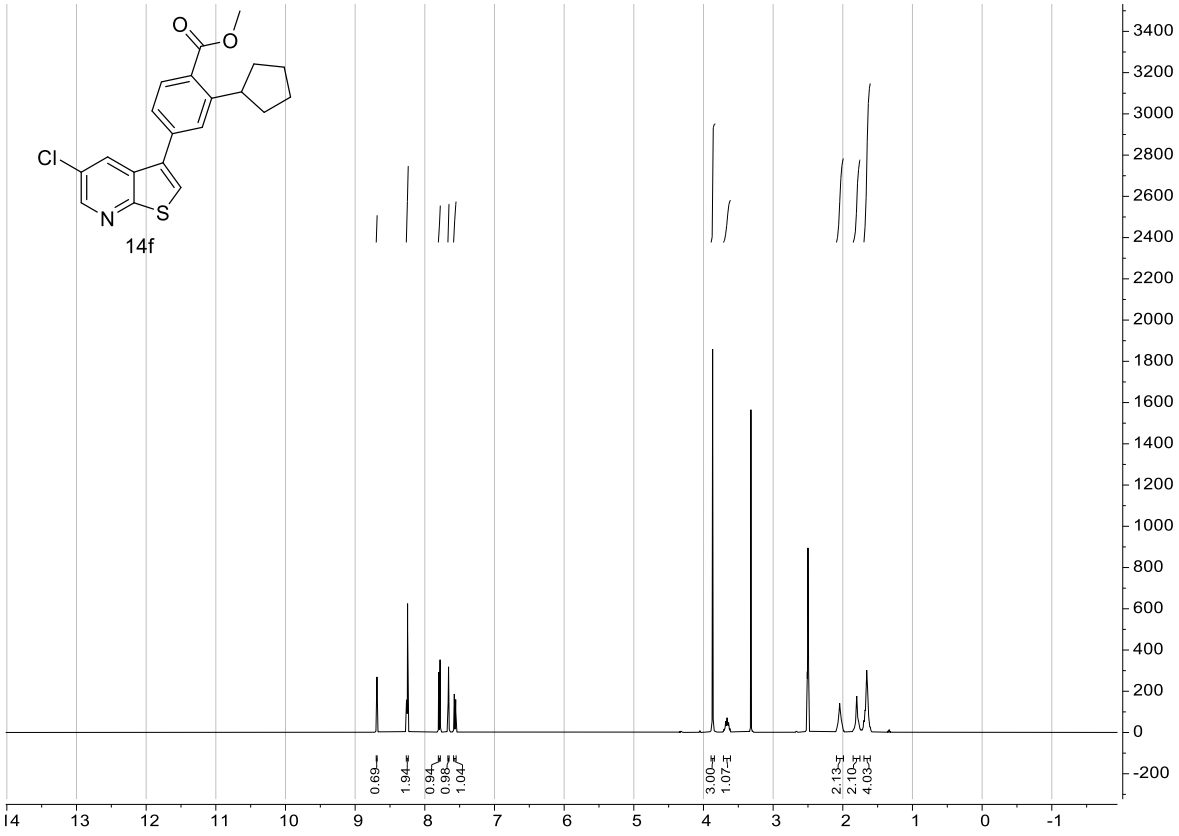


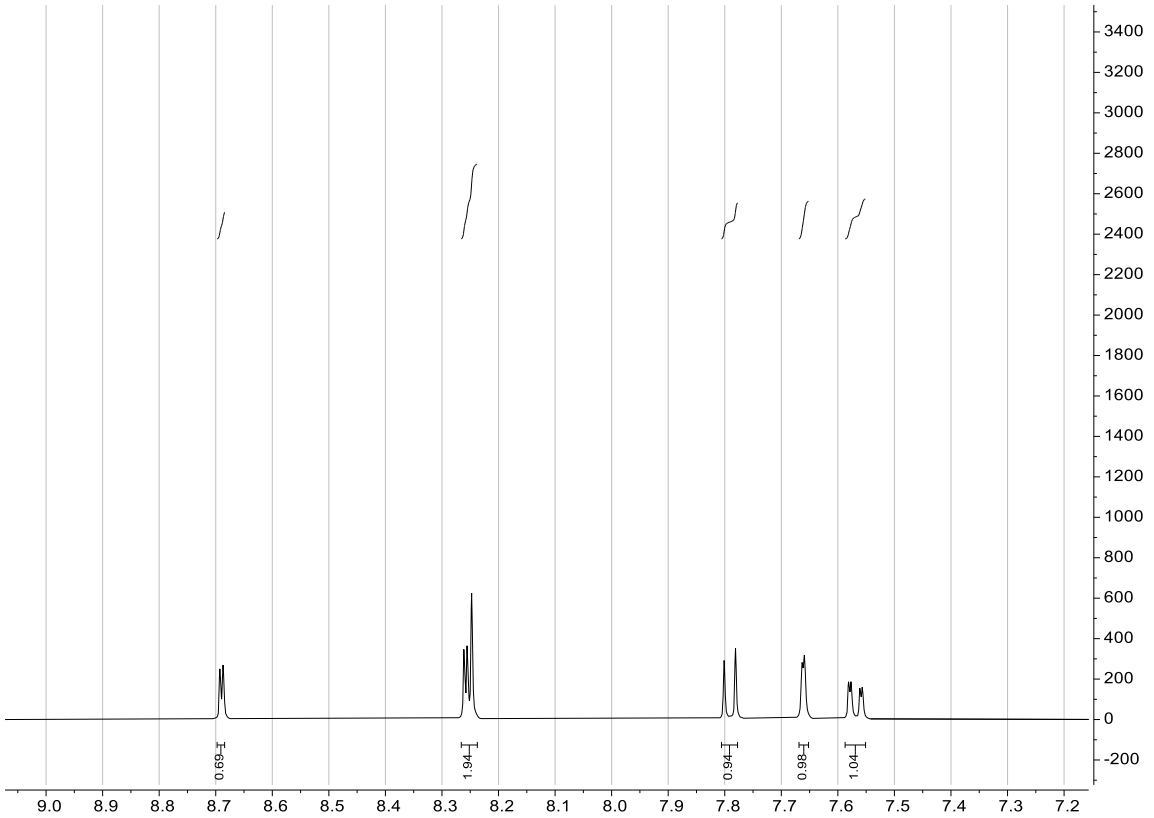


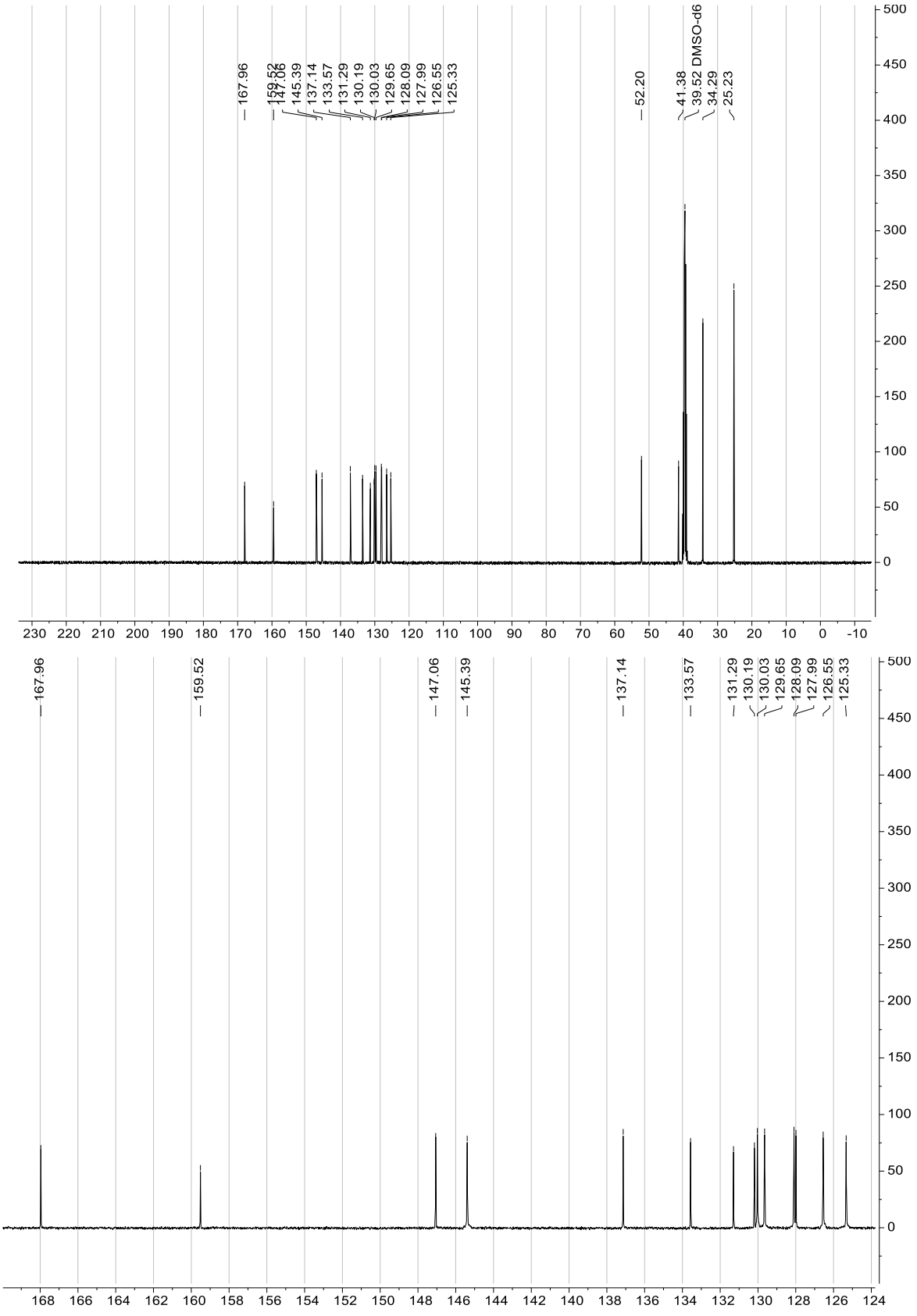

167.87
160.49
147.04
144.88
144.75
133.42
132.34
130.17
130.01
124.98
124.15
119.61
119.46
115.29
52.17
41.43
39.52 DMSO-d6
34.21
25.19


167.87
160.49
147.04
144.88
144.75
133.42
132.34
130.17
130.01
124.98
124.15
119.61
119.46
115.29

Methyl 4-(5-chlorothieno[2,3-*b*]pyridin-3-yl)-2-cyclopentylbenzoate, (14f)



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159.52
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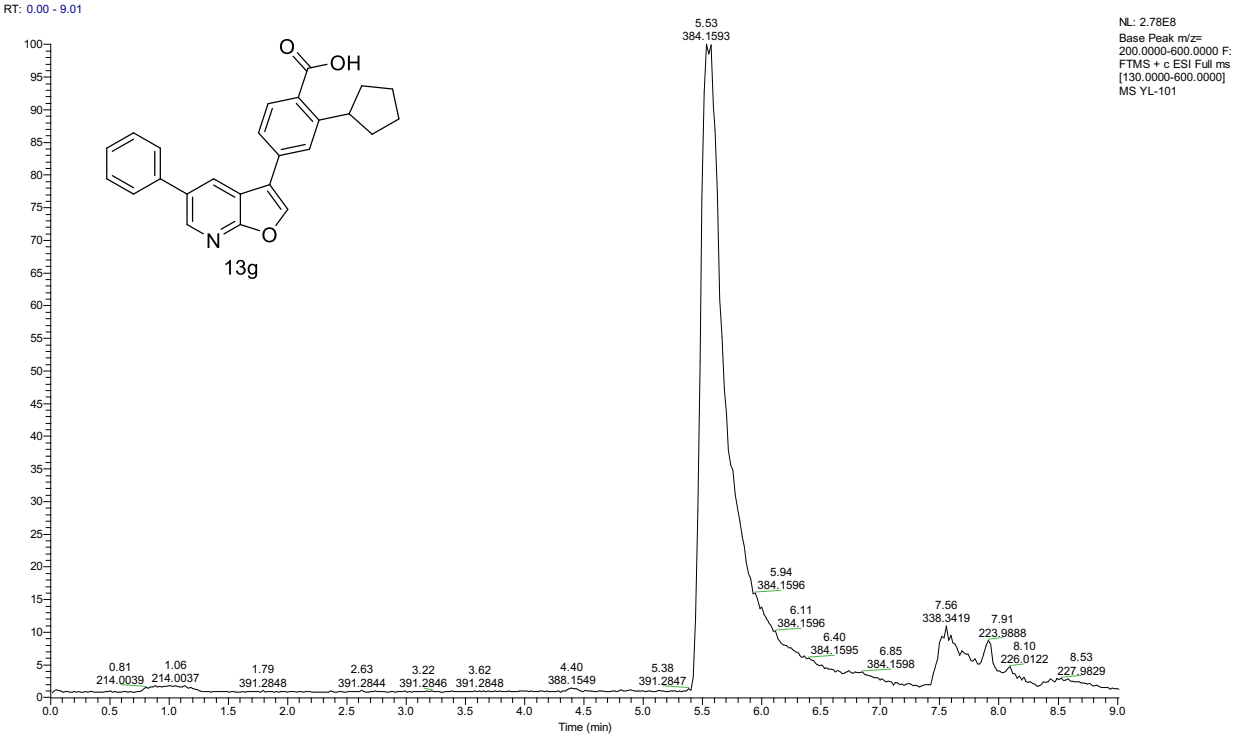
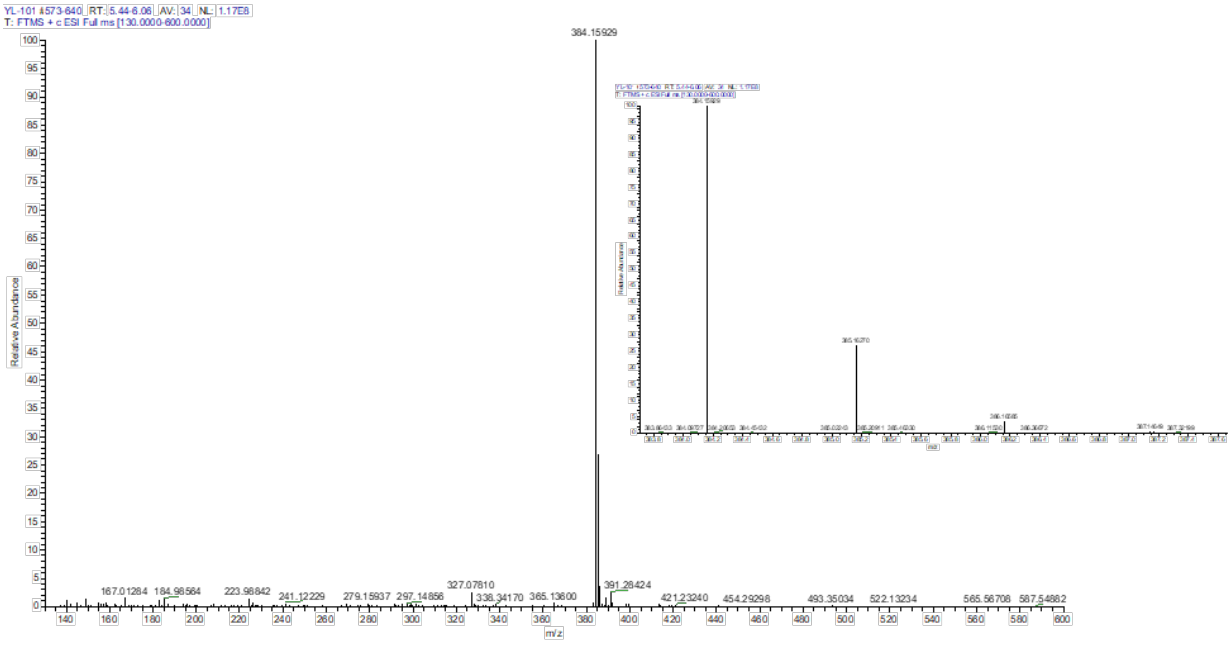
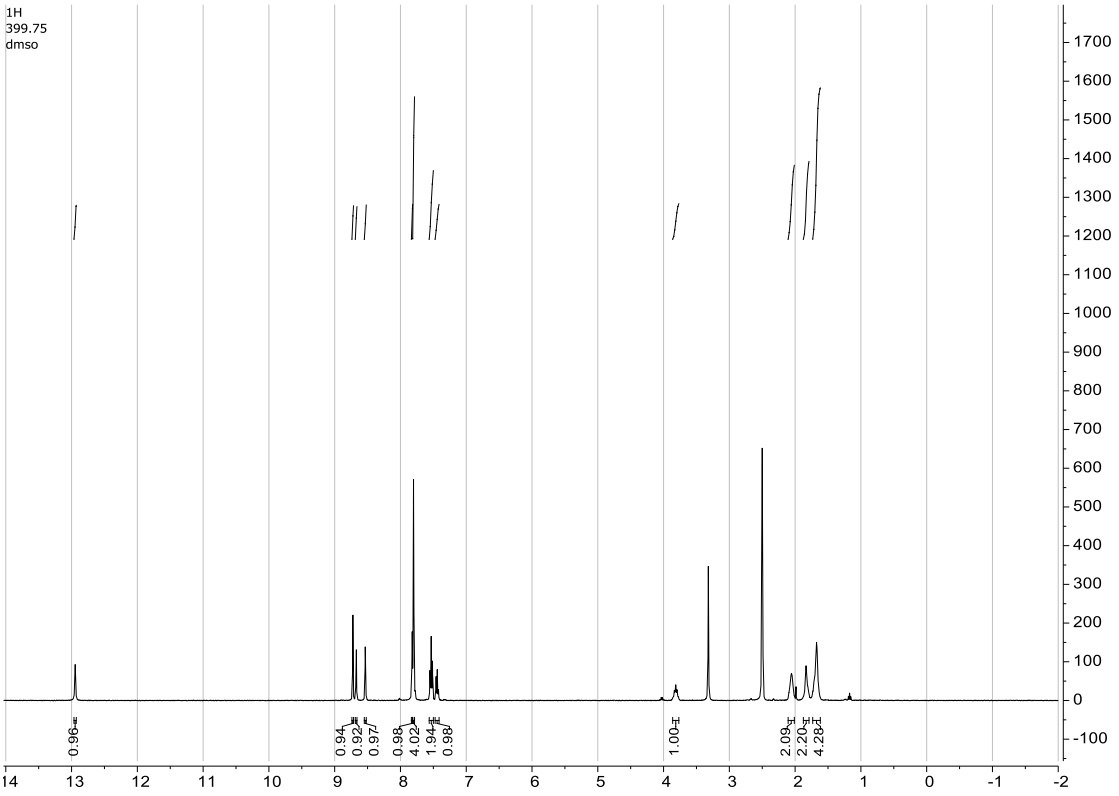
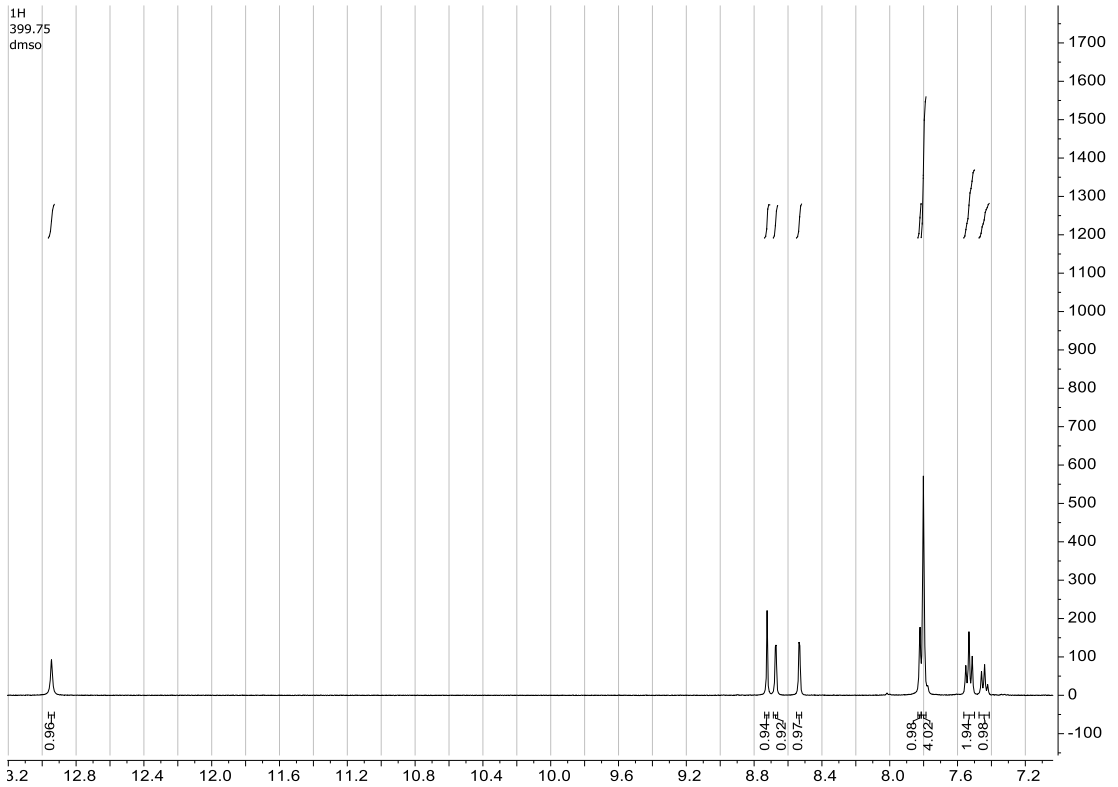
145.39
137.14
133.57
131.29
130.19
130.03
129.65
128.09
127.99
126.55
125.33
52.20
41.38
39.52 DMSO-d6
34.29
25.23

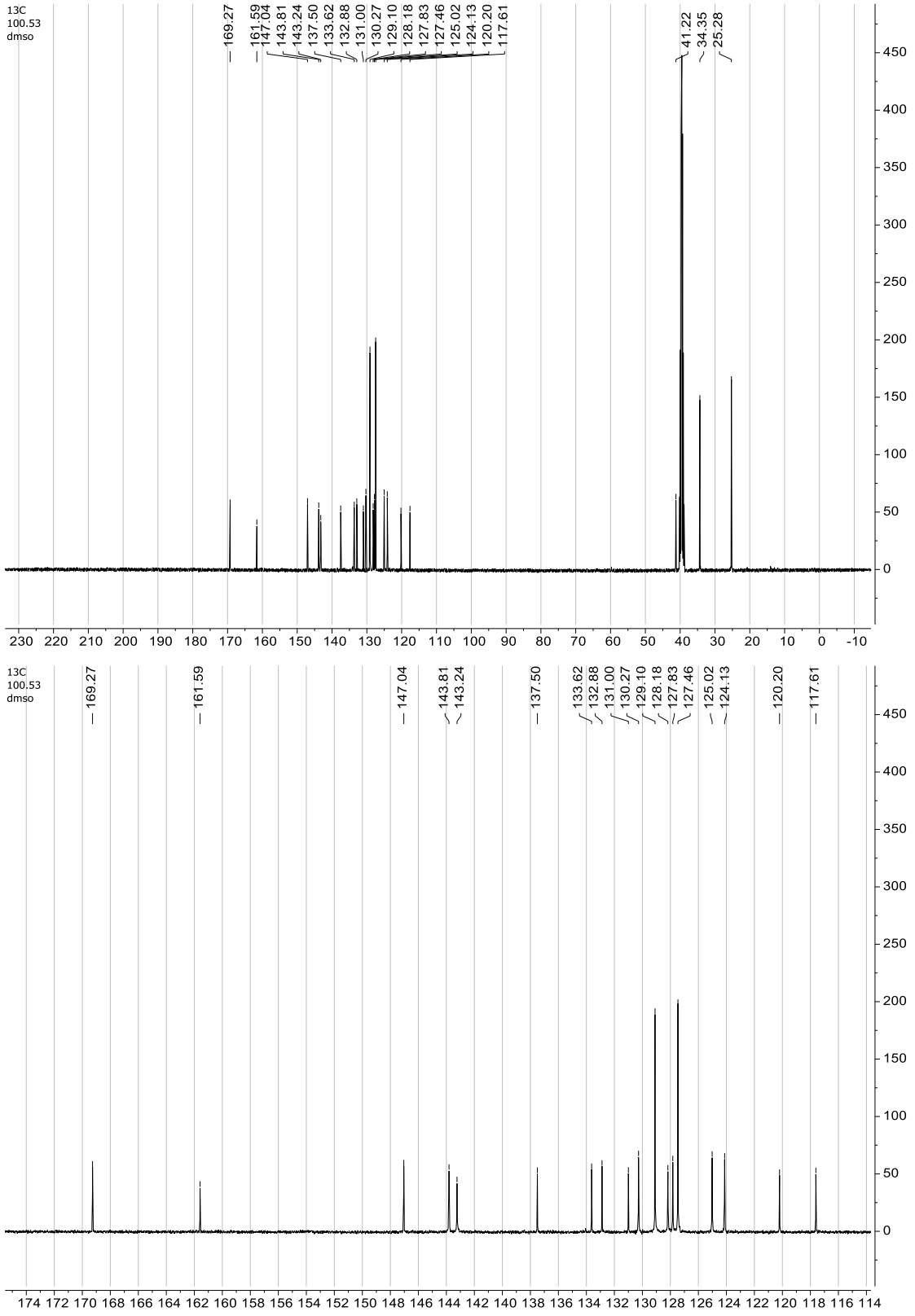
2-Cyclopentyl-4-(5-phenylfuro[2,3-*b*]pyridin-3-yl)benzoic acid (13g)



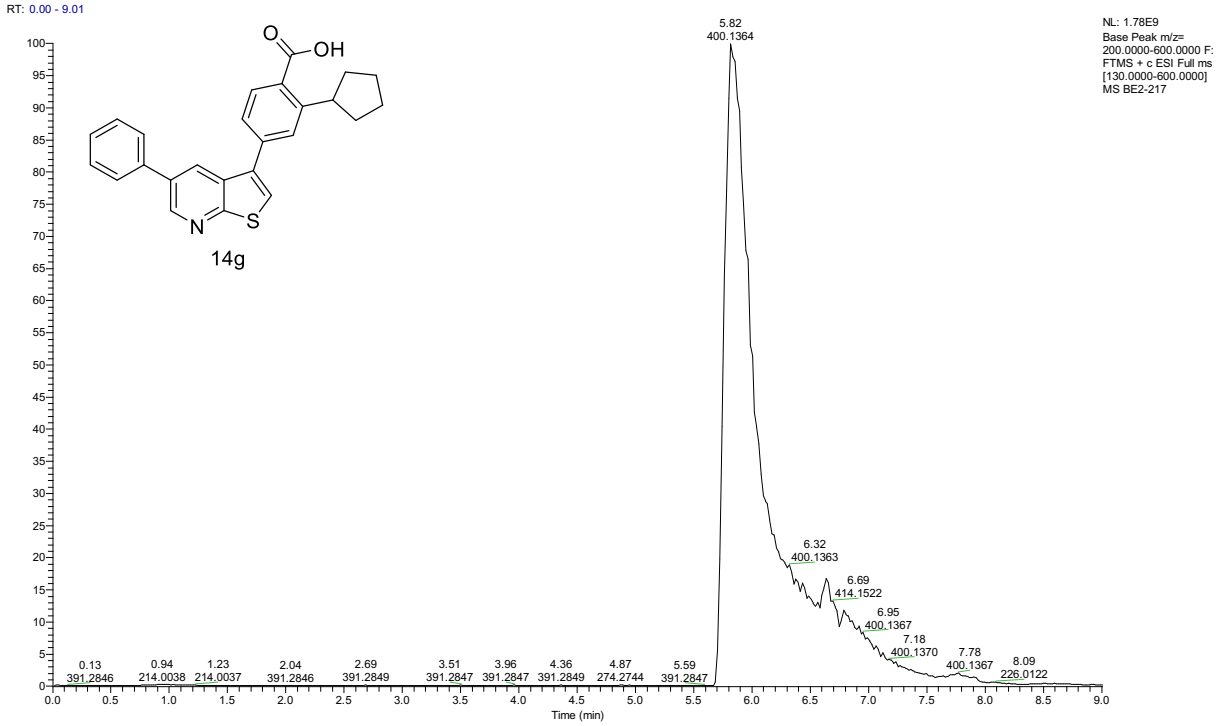


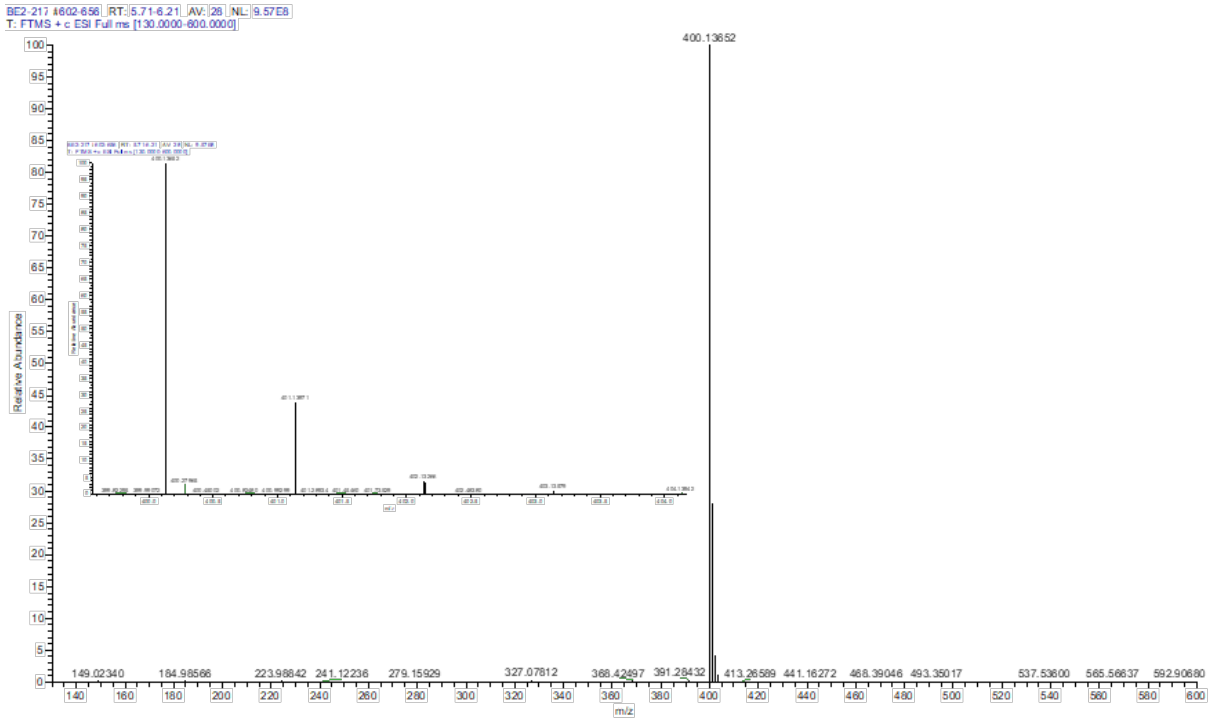

1H
399.75
dmso
0.96
0.94
0.92
0.97
0.98
4.02
1.94
0.98
1.00
2.09
2.20
4.28

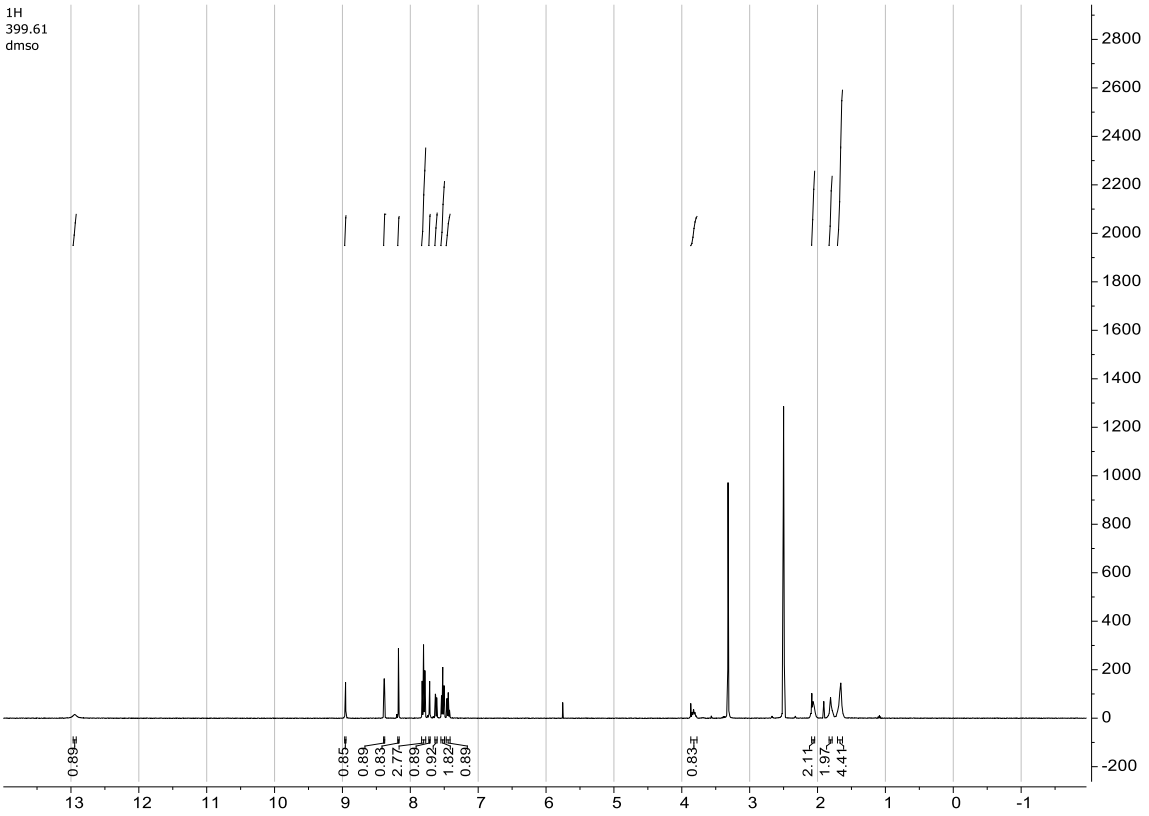

1H
399.75
dmso
0.96
0.94
0.92
0.97
0.98
4.02
1.94
0.98

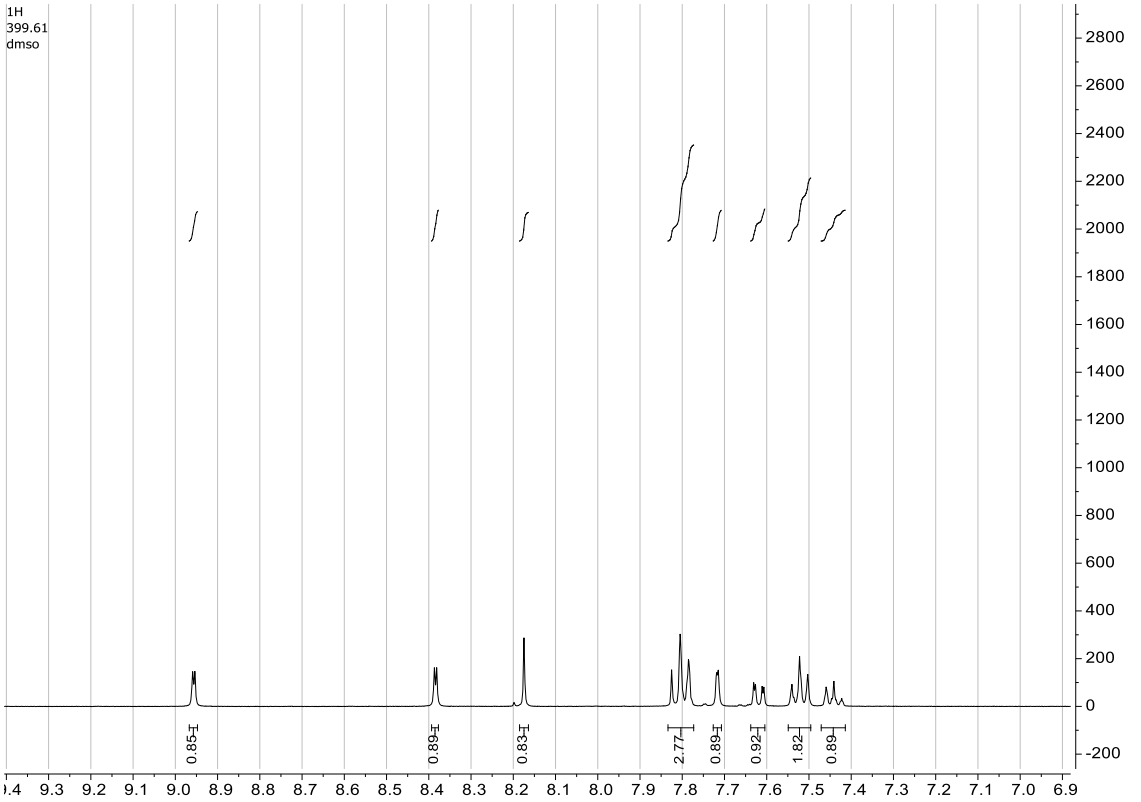

13C
100.53
dmso
169.27
161.59
147.04
143.81
143.24
137.50
133.62
132.88
131.00
130.27
129.10
128.18
127.83
127.46
125.02
124.13
120.20
117.61
41.22
34.35
25.28
13C
100.53
dmso

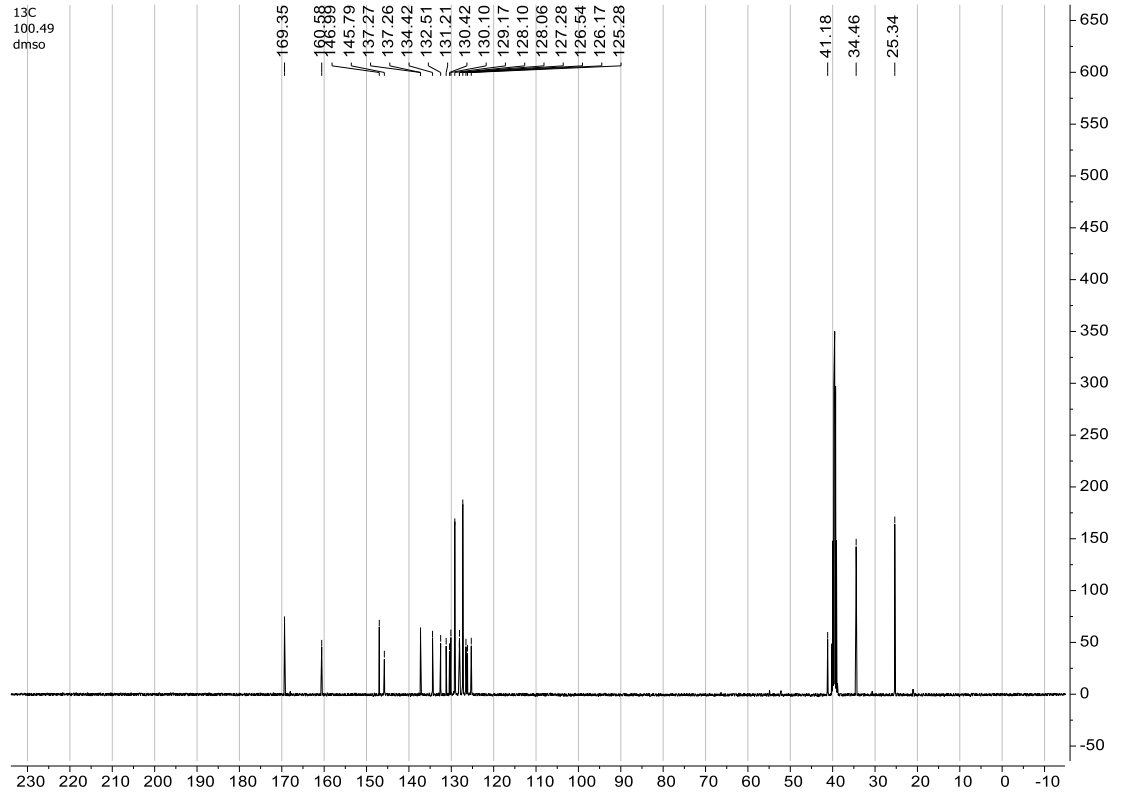
2-Cyclopentyl-4-(5-phenylthieno[2,3-b]pyridin-3-yl)benzoic acid, BE2-217 (14g)






1H
399.61
dmso
0.89
0.85
0.89
0.83
2.77
0.89
0.92
1.82
0.89
0.83
2.11
1.97
4.41


1H
399.61
dmso
0.85
0.89
0.83
2.77
0.89
0.92
1.82
0.89


13C
100.49
dmso
169.35
160.58
146.99
145.79
137.27
137.26
134.42
132.51
131.21
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130.10
129.17
128.10
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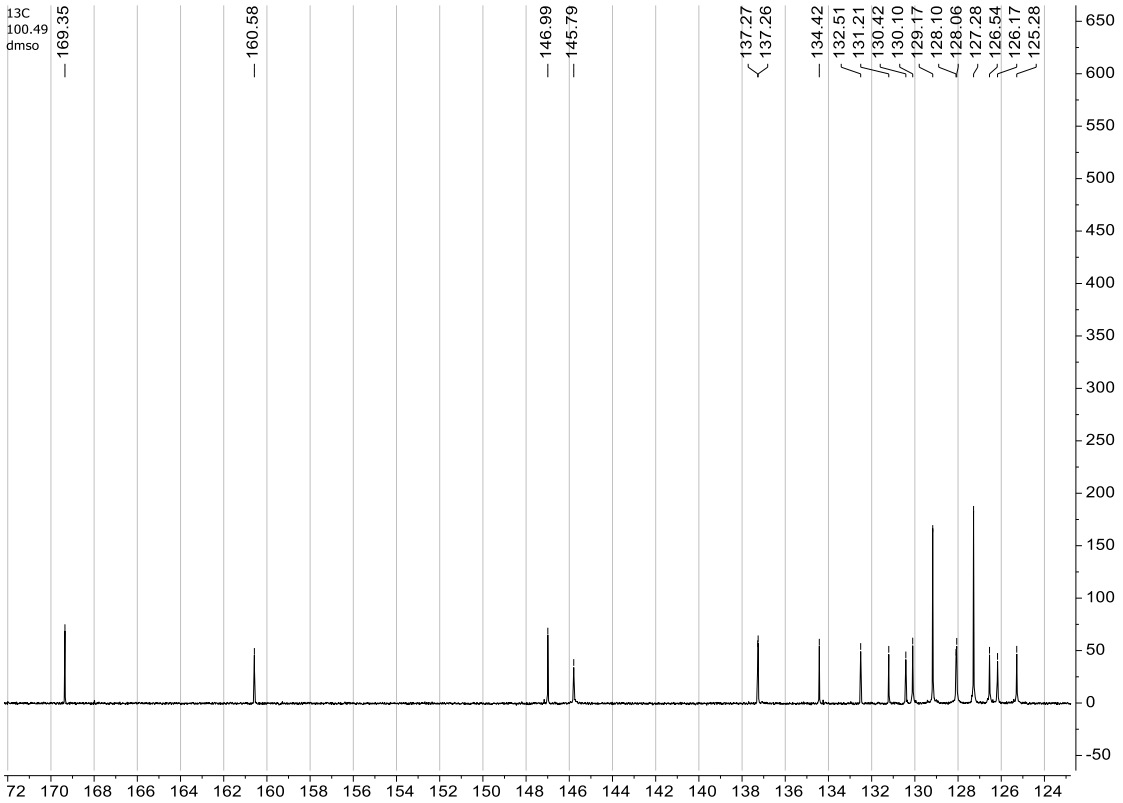
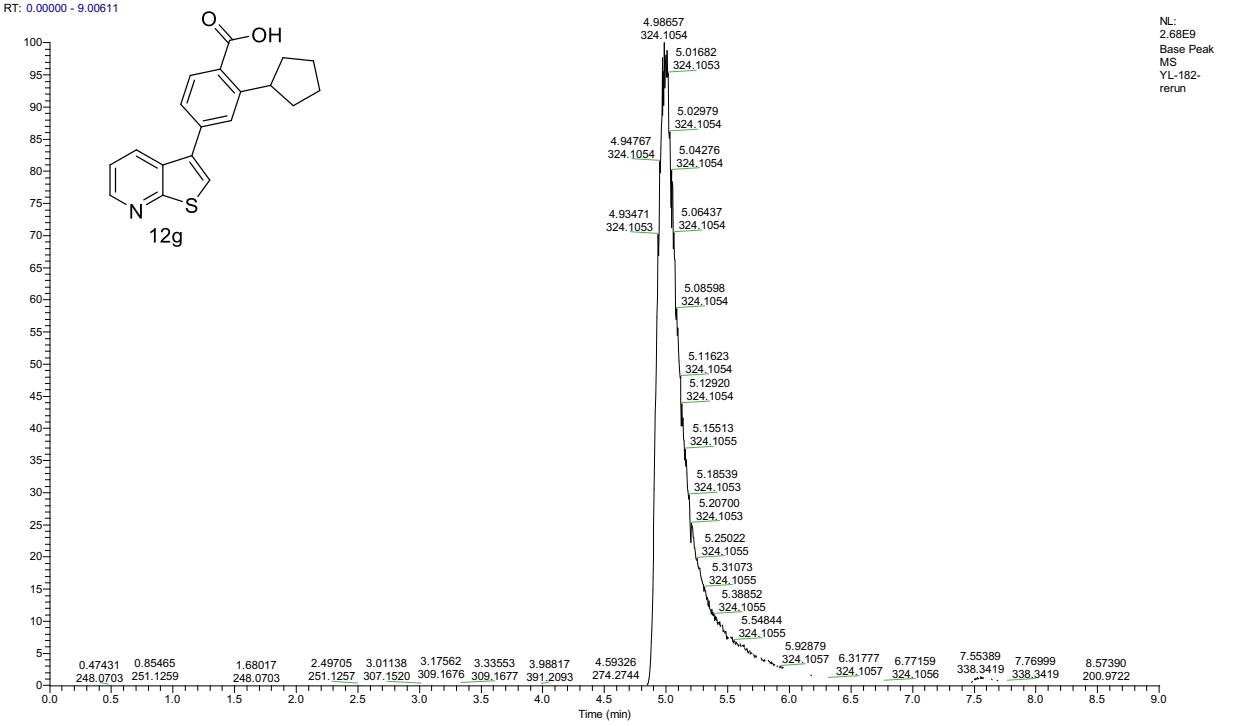
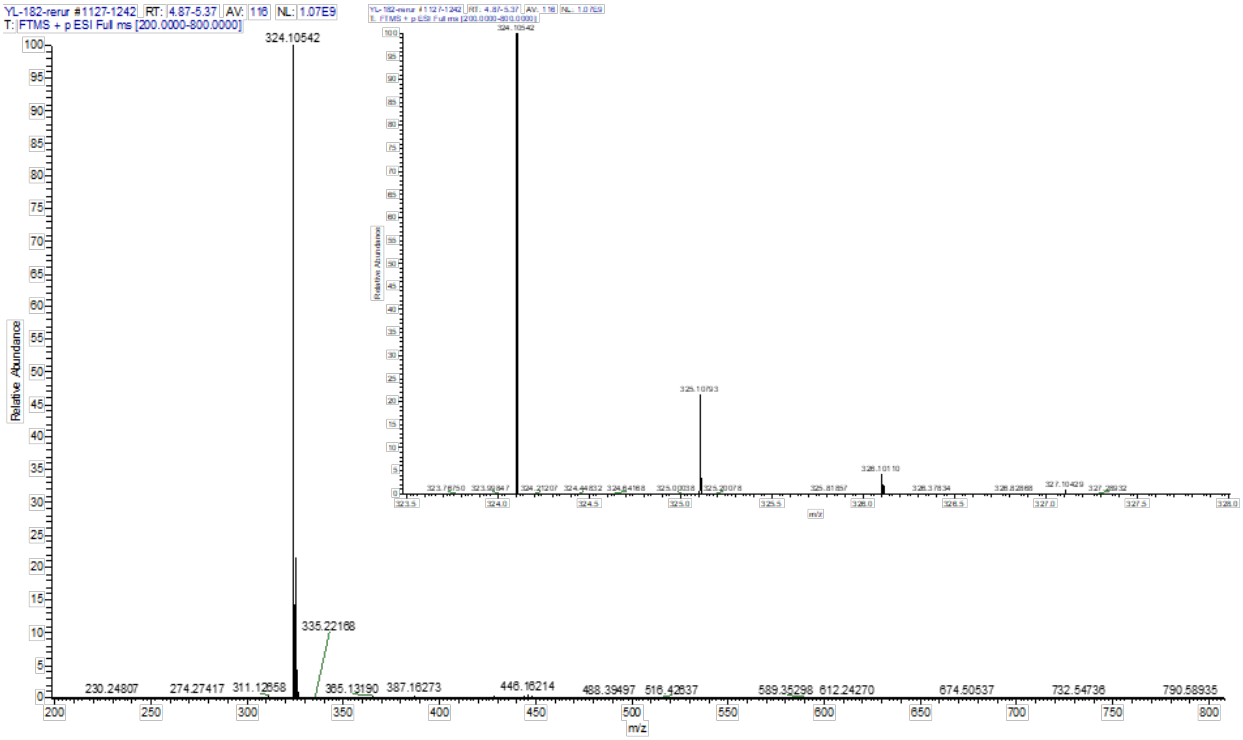
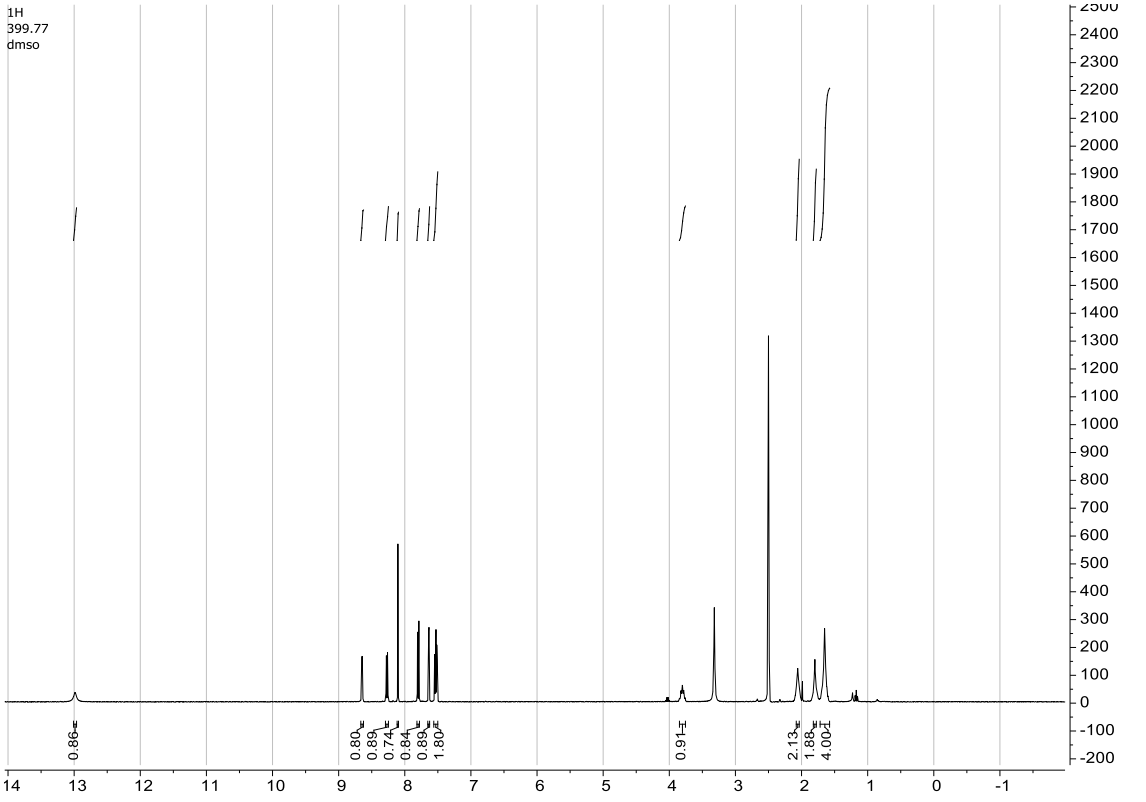
126.54
126.17
125.28
41.18
34.46
25.34

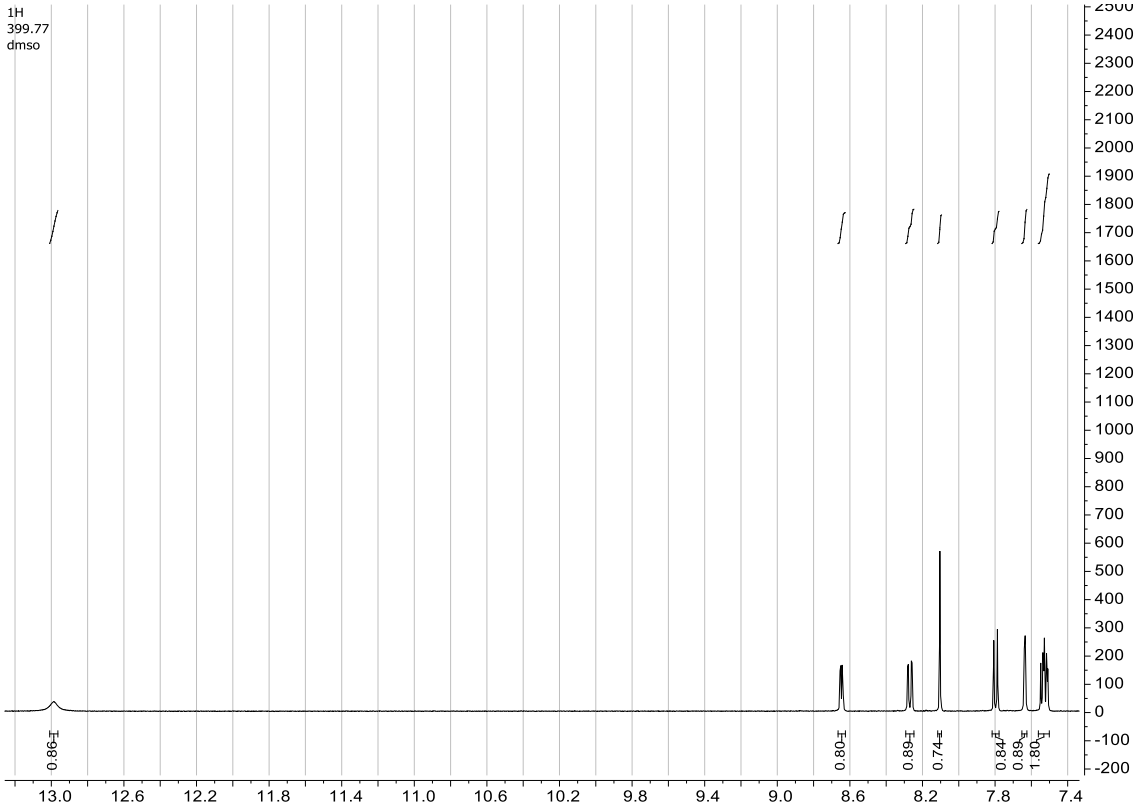

13C
100.49
dmso
169.35
160.58
146.99
145.79
137.27
137.26
134.42
132.51
131.21
130.42
130.10
129.17
128.10
128.06
127.28
126.54
126.17
125.28

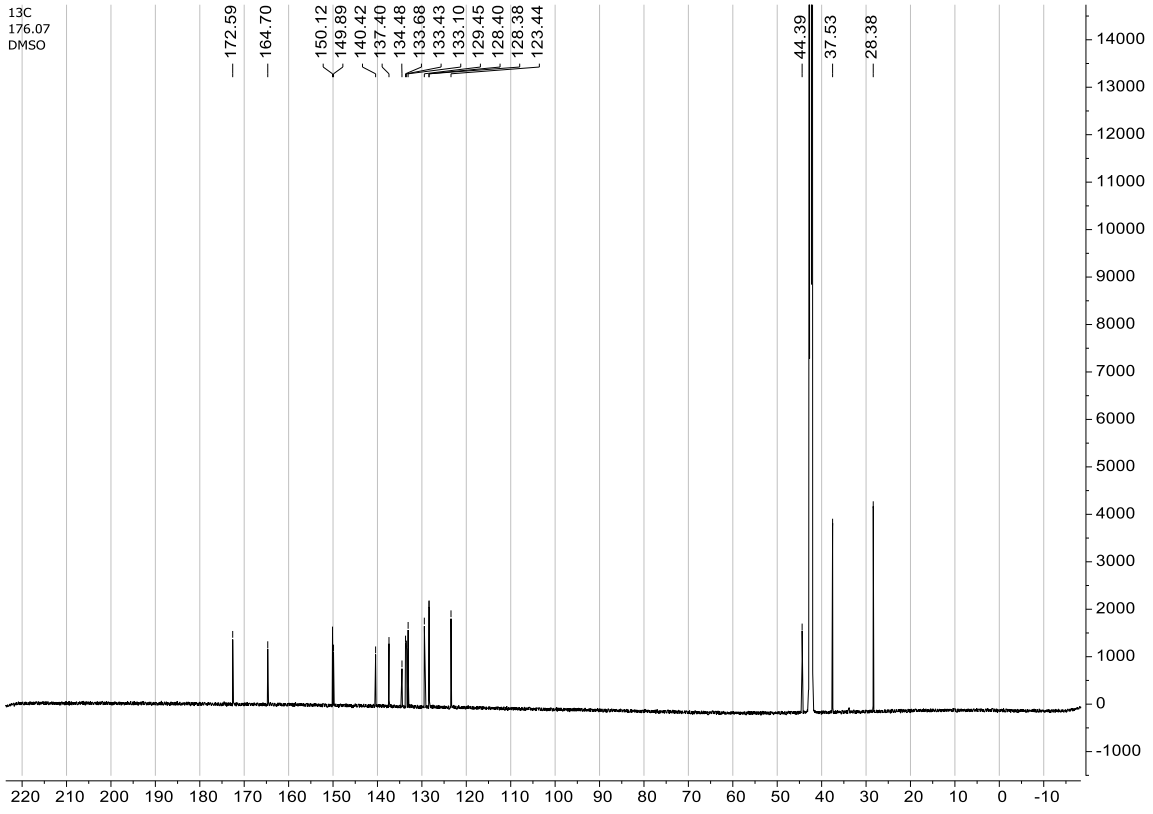
2-Cyclopentyl-4-(thieno[2,3-*b*]pyridin-3-yl)benzoic acid (12g)

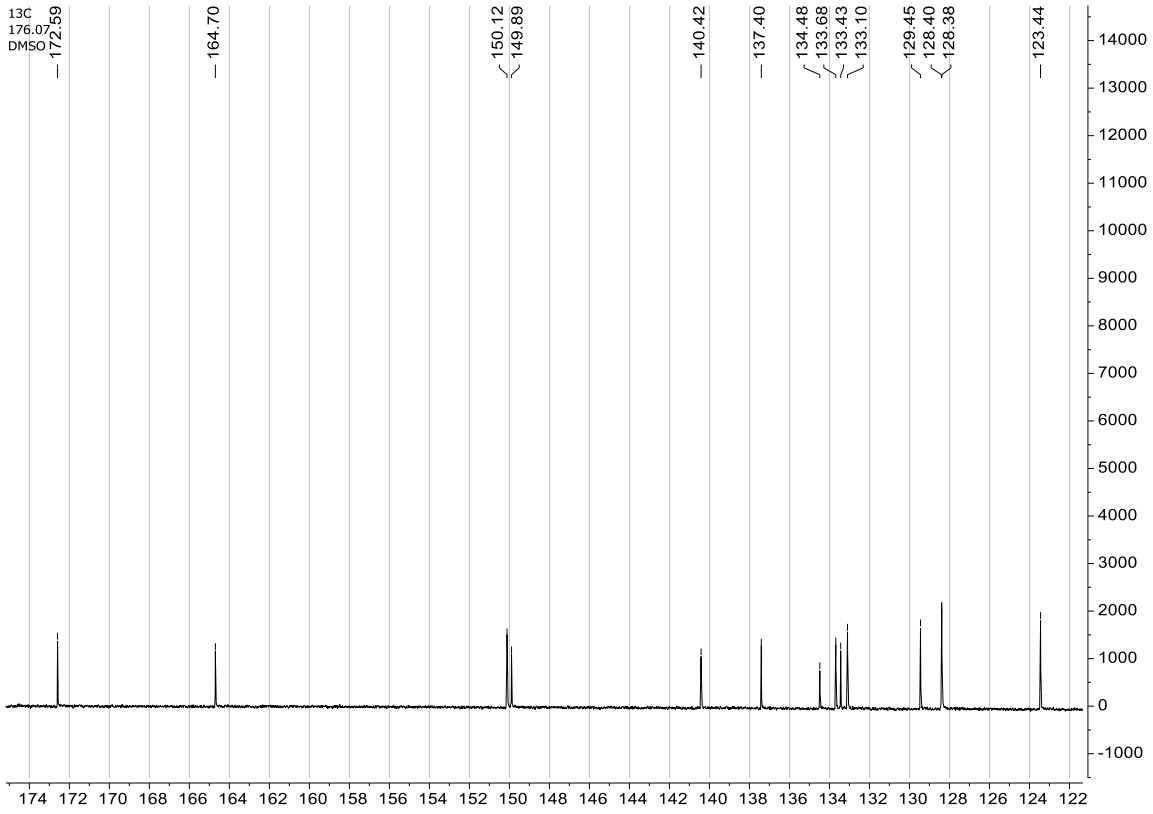




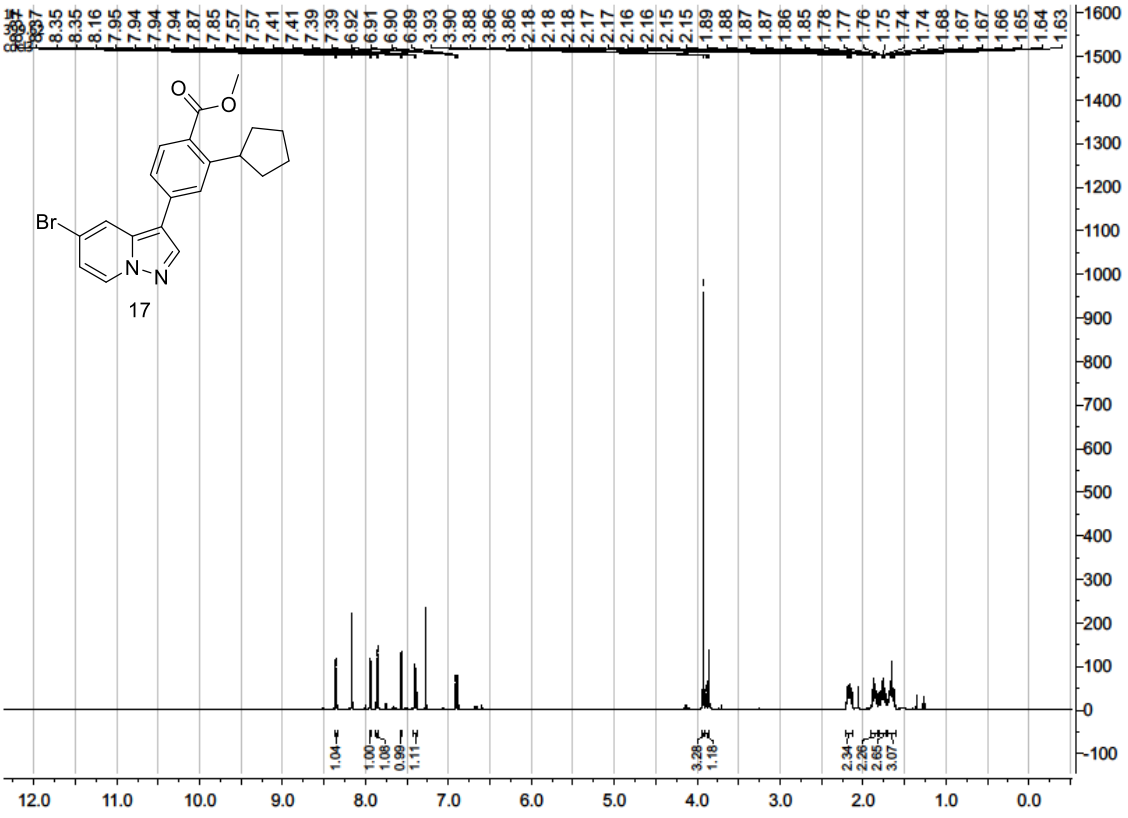

1H
399.77
dmso
0.86
0.80
0.89
0.74
0.84
0.89
1.80
0.91
2.13
1.88
4.00

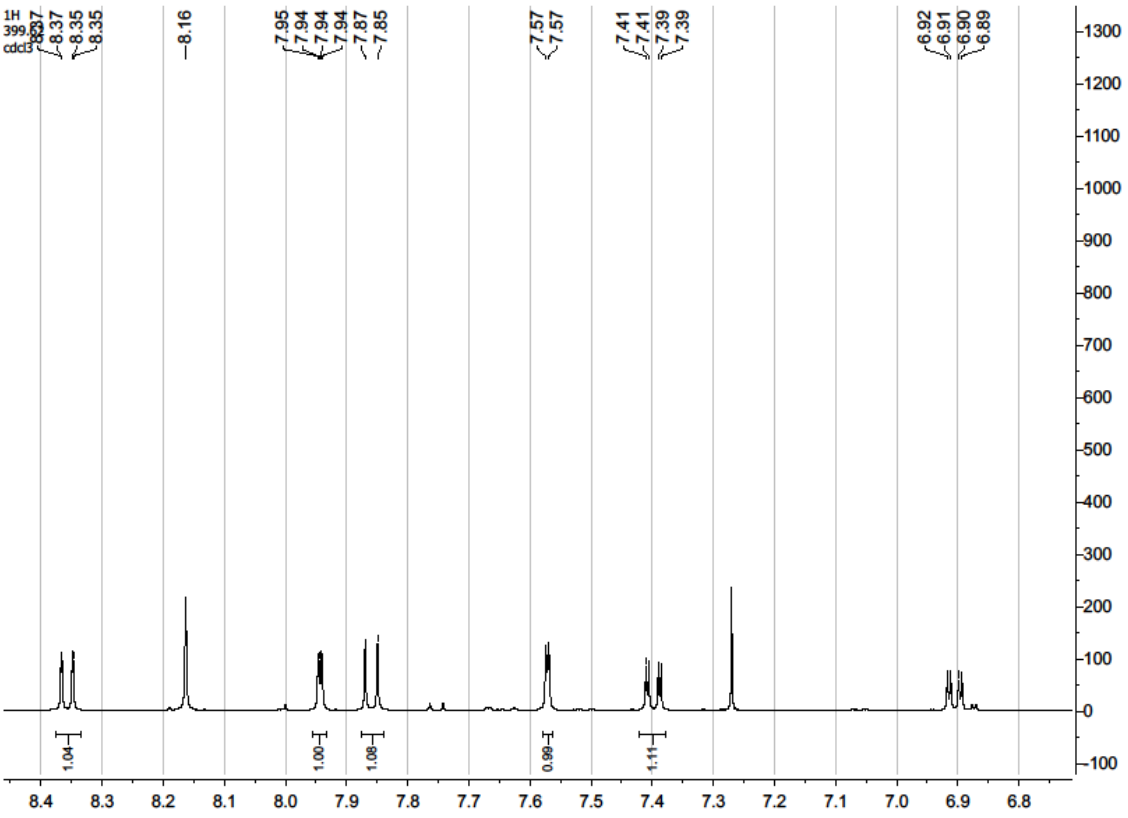

1H
399.77
dmso
0.86
0.80
0.89
0.74
0.84
0.89
1.80

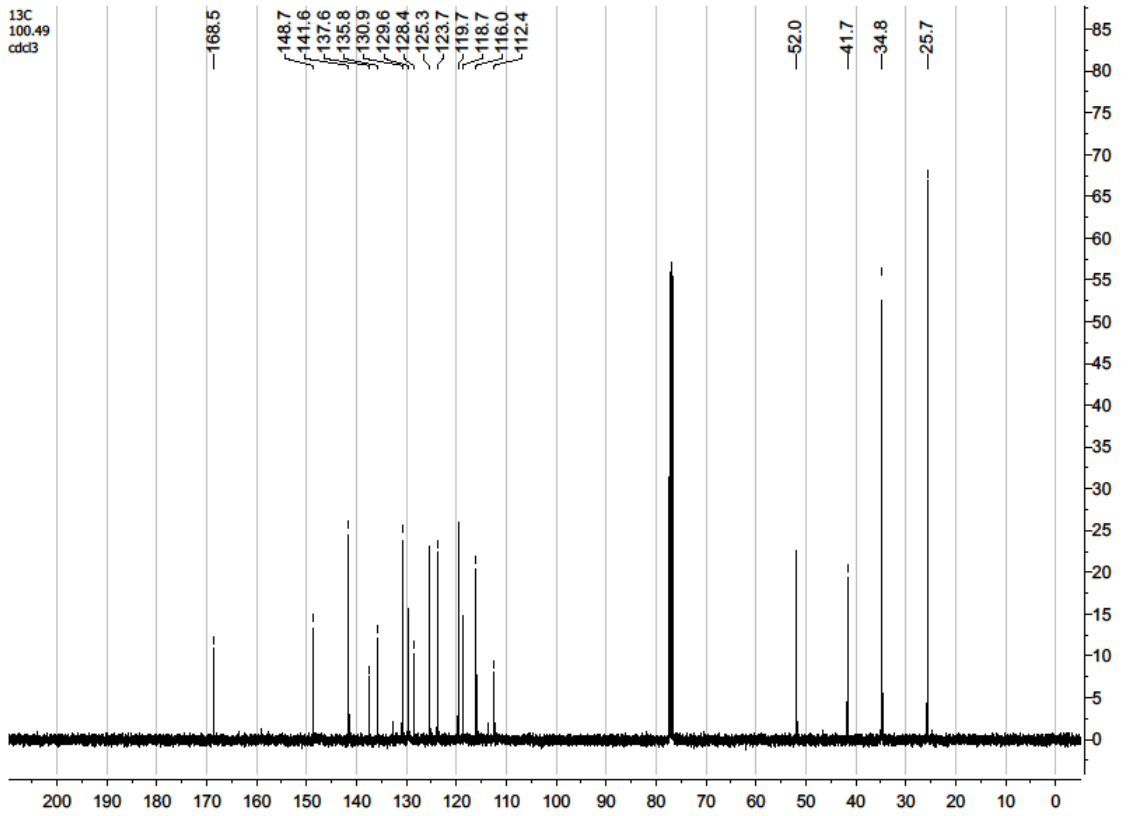

13C
176.07
DMSO
172.59
164.70
150.12
149.89
140.42
137.40
134.48
133.68
133.43
133.10
129.45
128.40
128.38
123.44
44.39
37.53
28.38


13C
176.07
DMSO
172.59
164.70
150.12
149.89
140.42
137.40
134.48
133.68
133.43
133.10
129.45
128.40
128.38
123.44

Methyl 4-(5-bromopyrazolo[1,5-a]pyridin-3-yl)-2-cyclopentylbenzoate (17)






13C
100.49
cdcl3
168.5
148.7
141.6
137.6
135.8
130.9
129.6
128.4
125.3
123.7
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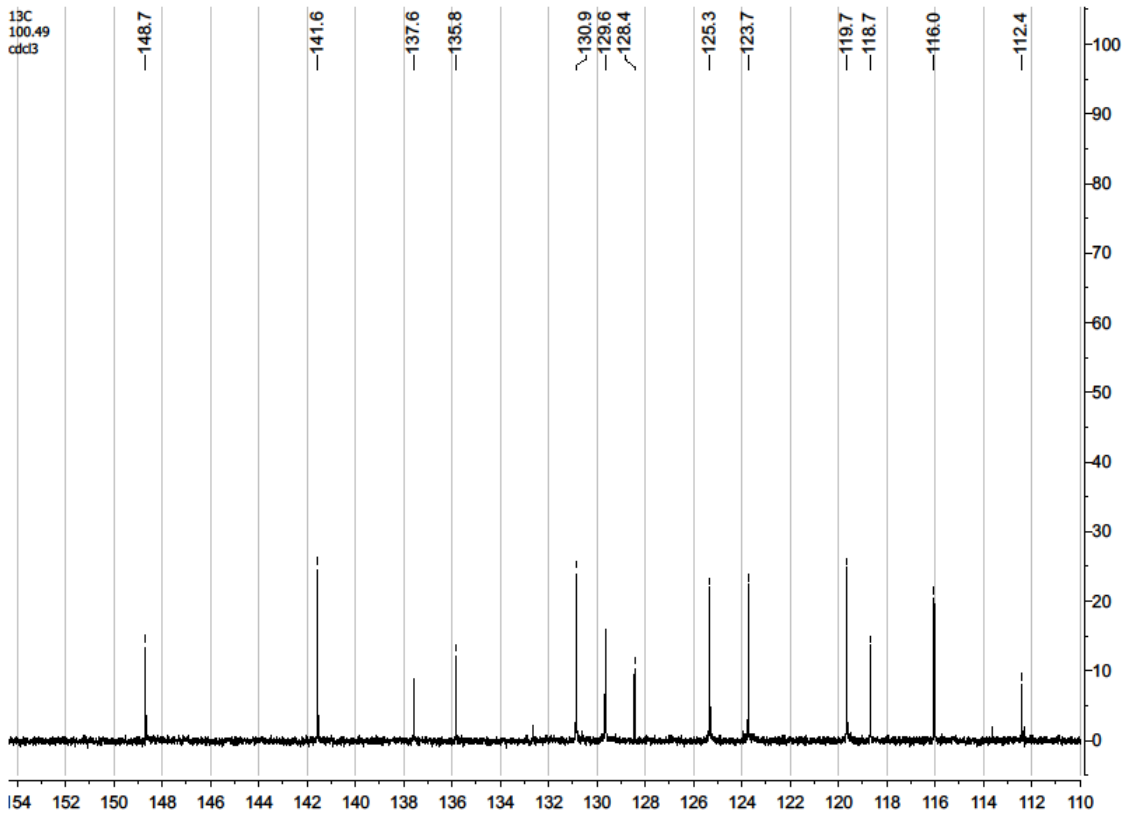
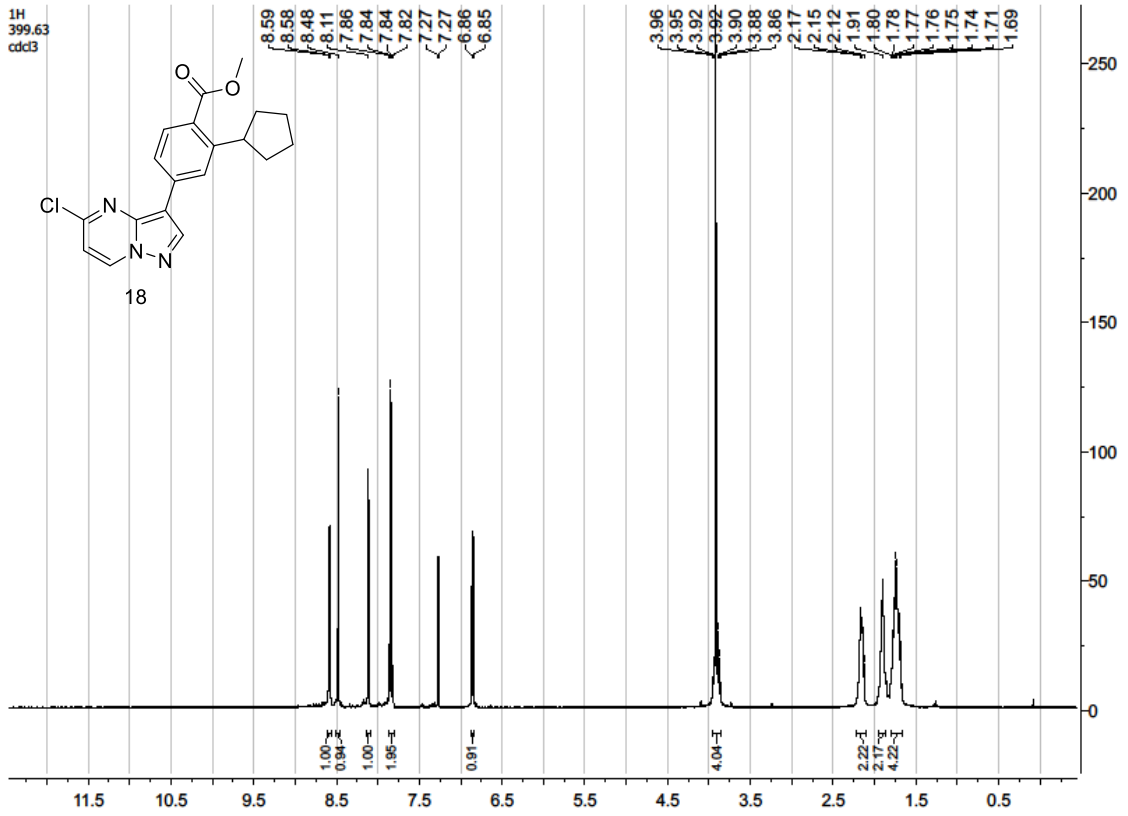
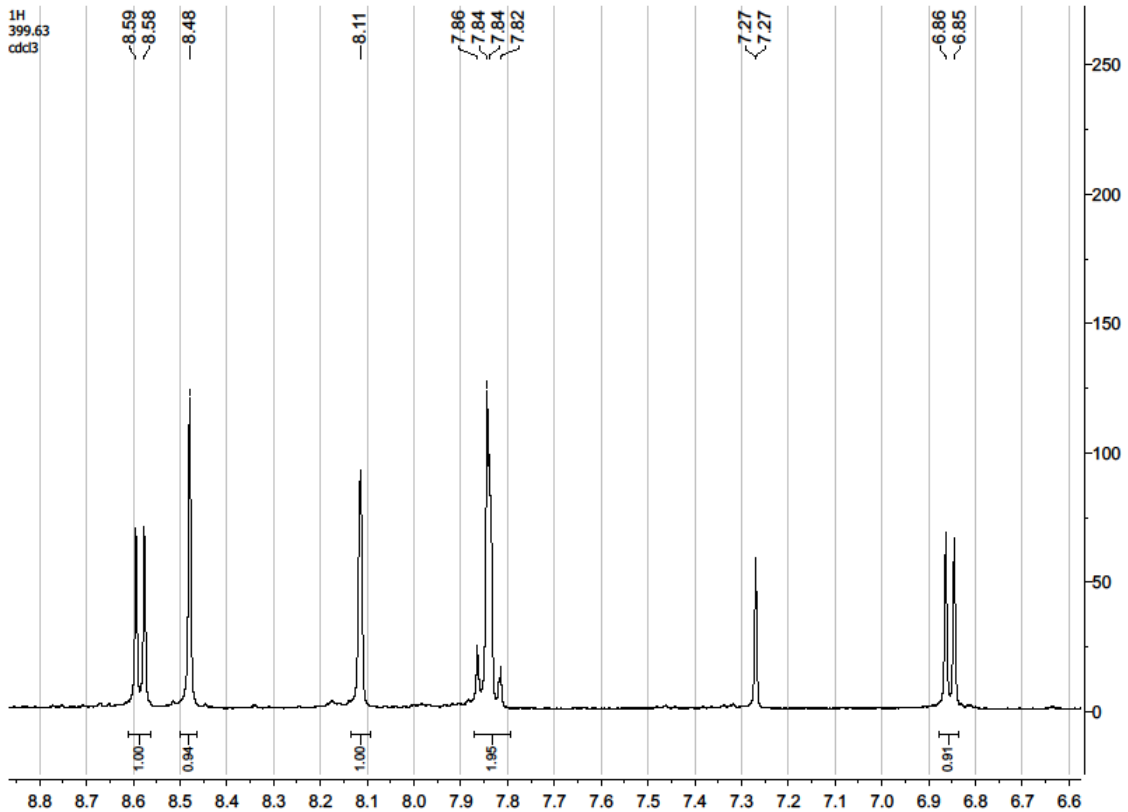
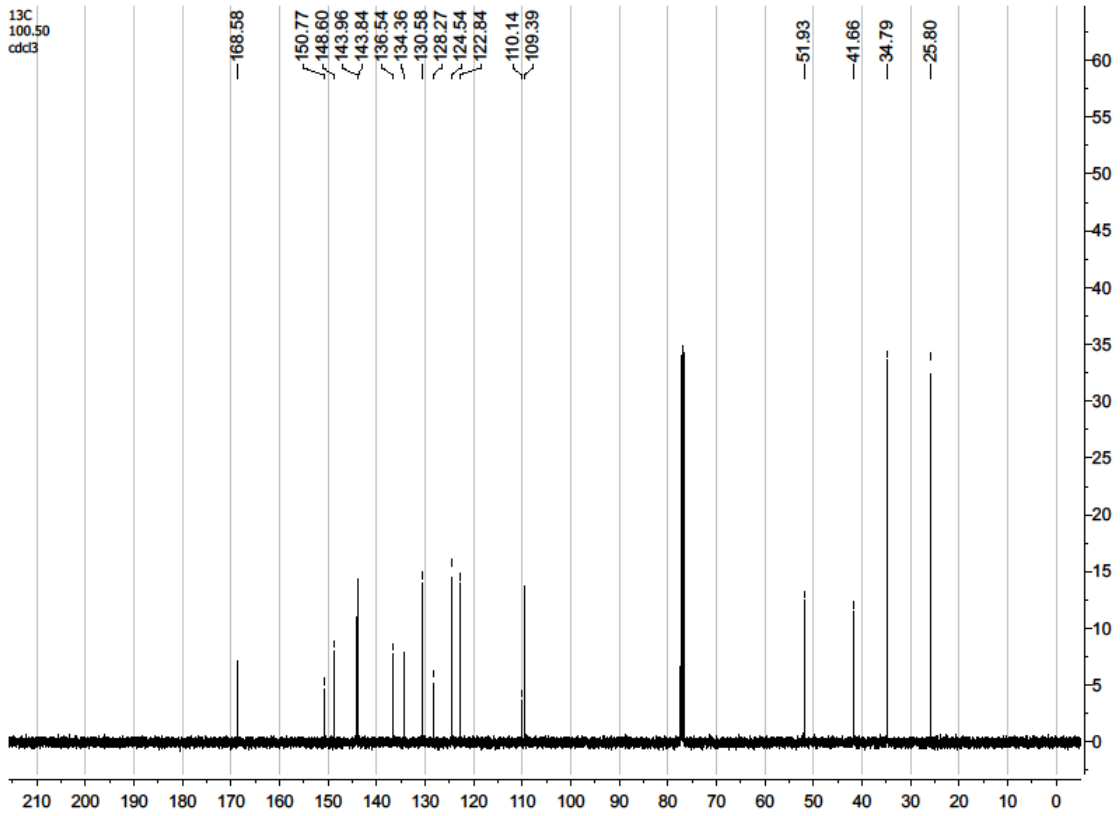
116.0
112.4
52.0
41.7
34.8
25.7


13C
100.49
cdcl3
148.7
141.6
137.6
135.8
130.9
129.6
128.4
125.3
123.7
119.7
118.7
116.0
112.4

Methyl 4-(5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)-2-cyclopentylbenzoate (18)






13C
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cdcl3
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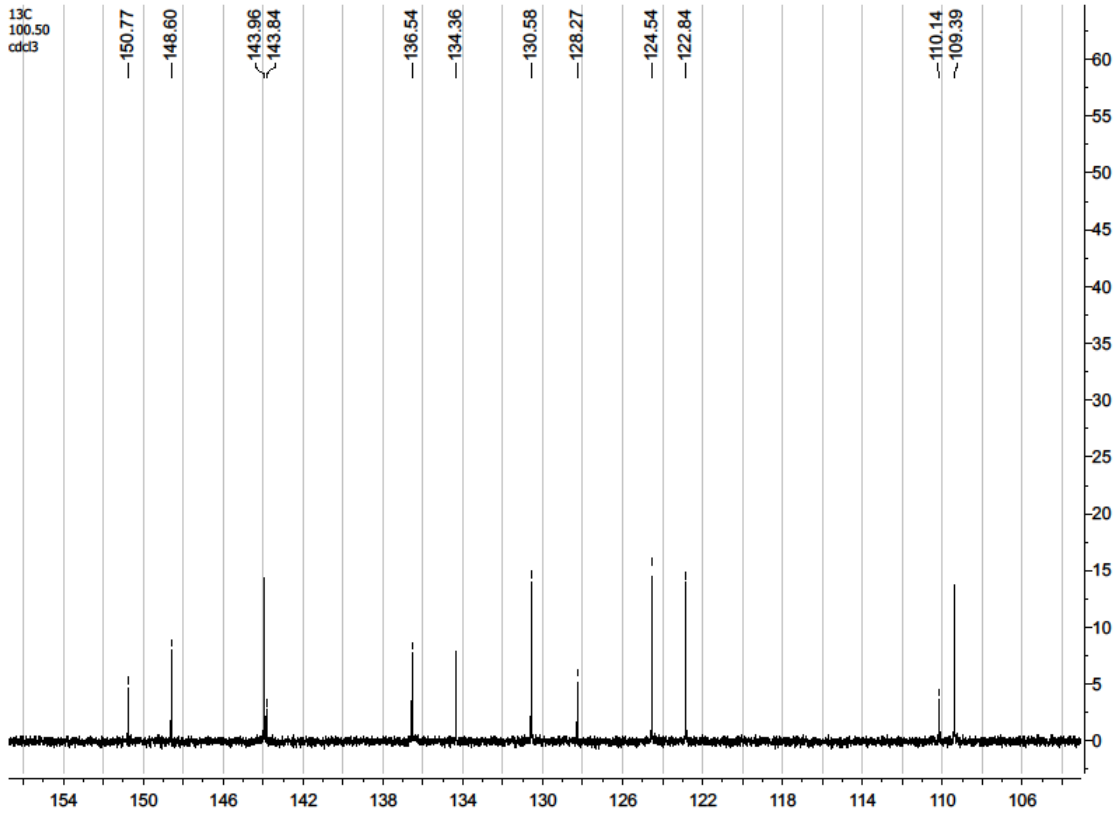
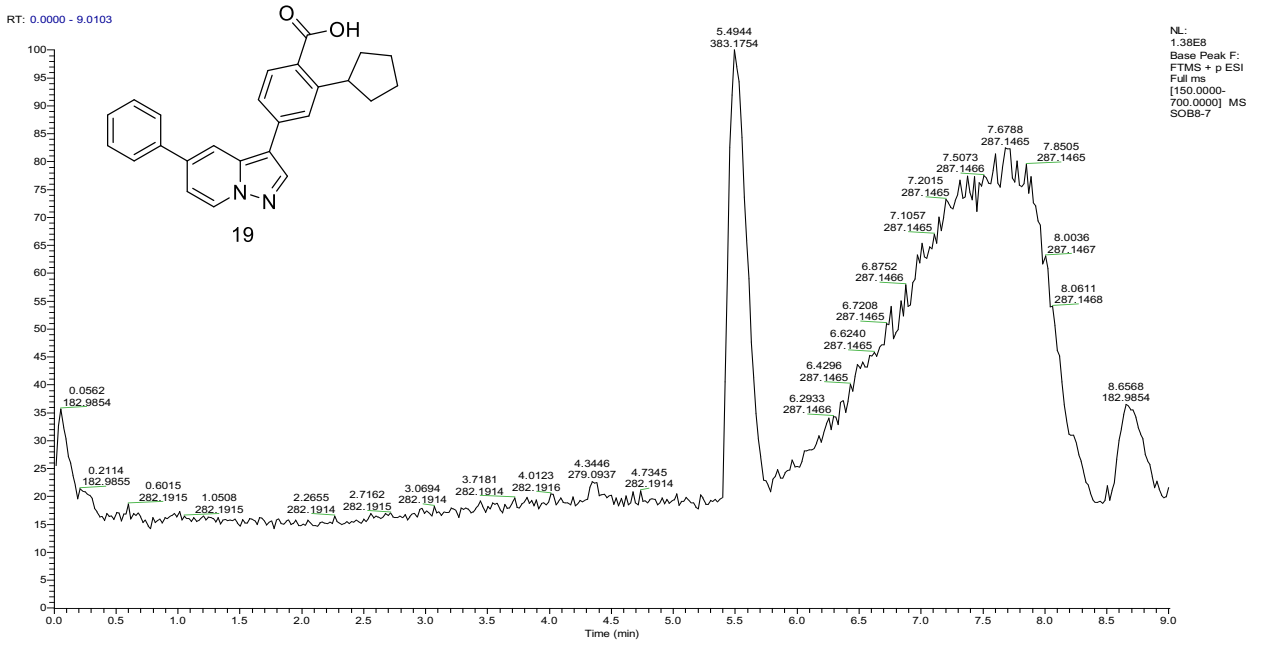
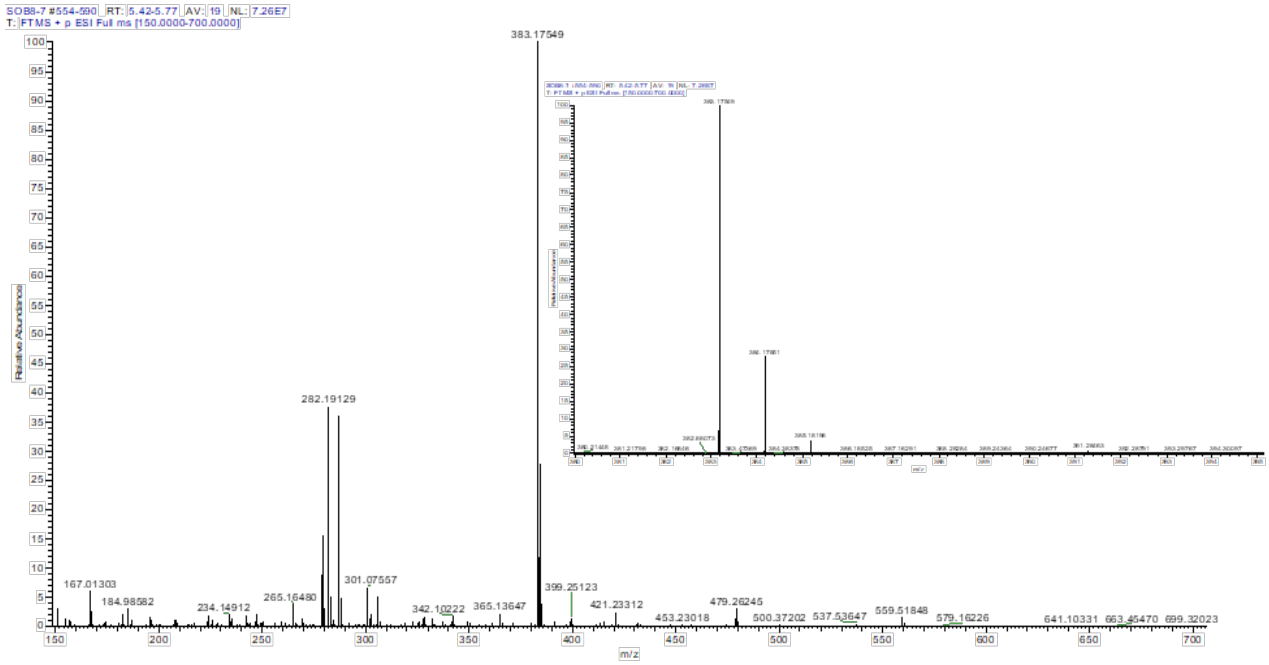
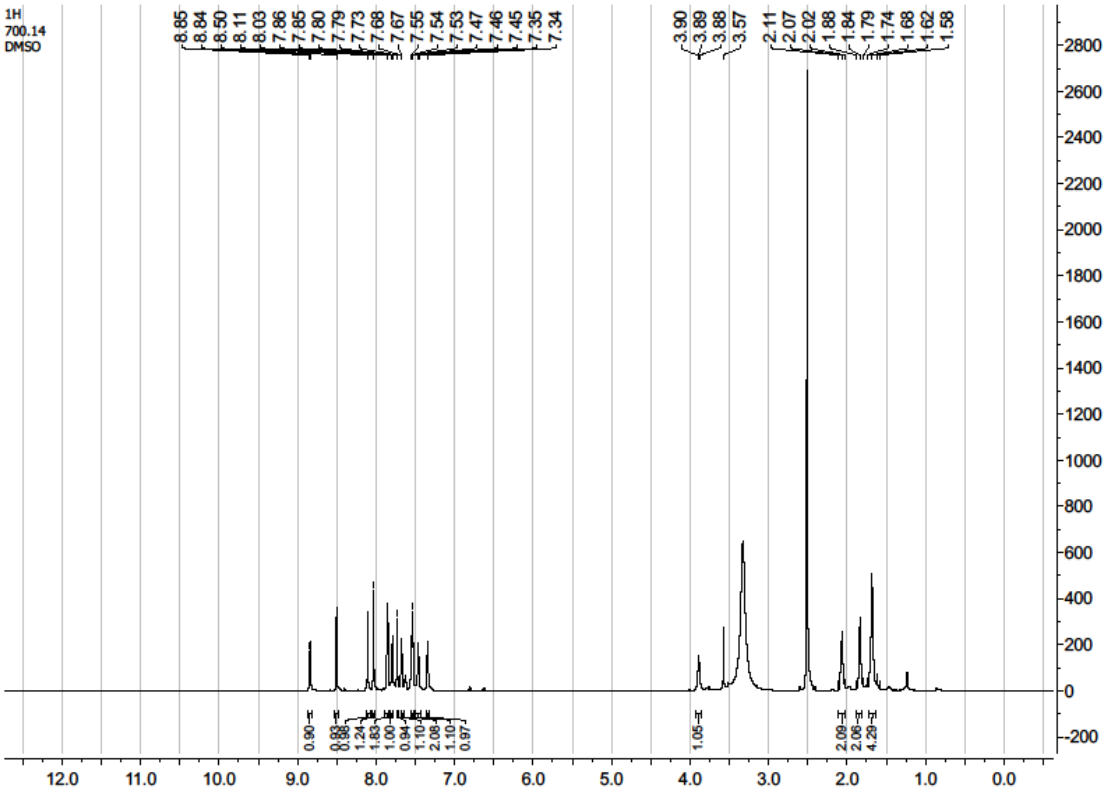
150.77
148.60
143.96
143.84
136.54
134.36
130.58
128.27
124.54
122.84
110.14
109.39
51.93
41.66
34.79
25.80


13C
100.50
cdcl3
150.77
148.60
143.96
143.84
136.54
134.36
130.58
128.27
124.54
122.84
110.14
109.39

2-Cyclopentyl-4-(5-phenylpyrazolo[1,5-*a*]pyridin-3-yl)benzoic acid (19)






1H
700.14
DMSO
8.85
8.84
8.50
8.11
8.03
7.86
7.85
7.80
7.79
7.73
7.68
7.67
7.55
7.54
7.53
7.47
7.46
7.45
7.35
7.34
3.90
3.89
3.88
3.57
2.11
2.07
2.02
1.88
1.84
1.79
1.74
1.68
1.62
1.58
0.90
0.83
0.88
1.24
1.83
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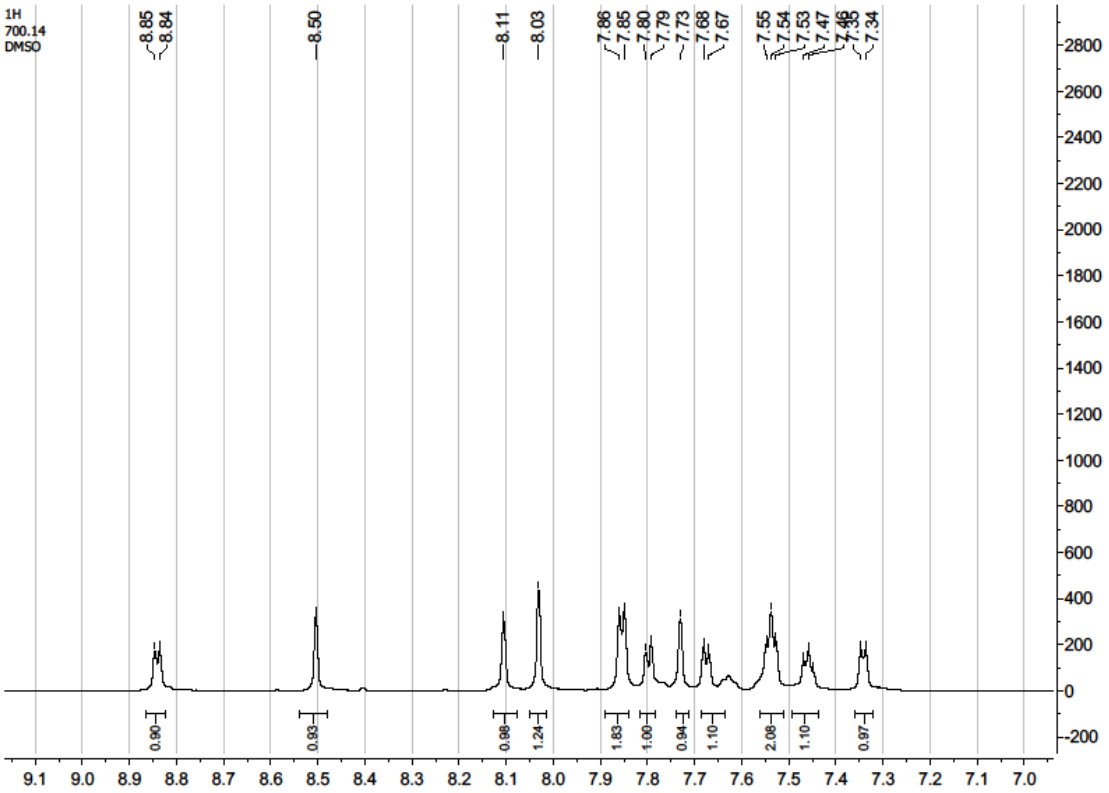
1.10
2.08
1.10
0.97
1.05
2.09
2.06
4.29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700.14
DMSO
8.85
8.84
8.50
8.11
8.03
7.86
7.85
7.80
7.79
7.73
7.68
7.67
7.55
7.54
7.53
7.47
7.46
7.35
7.34
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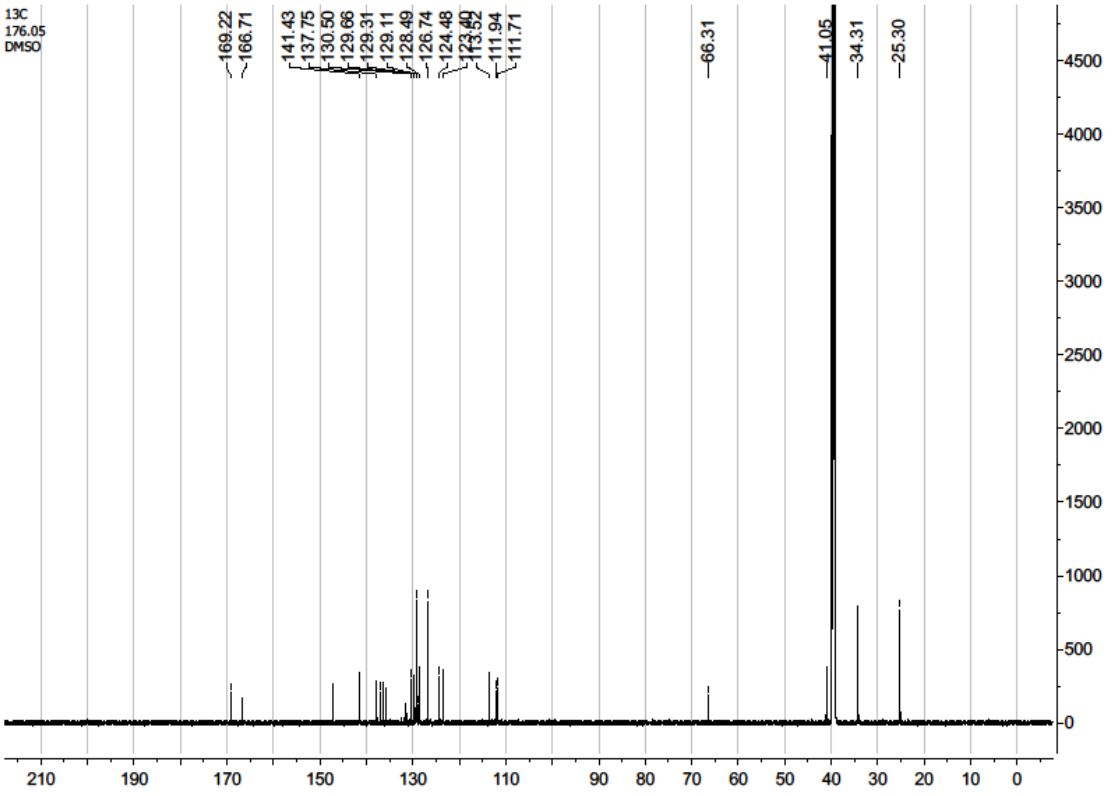
0.93
0.98
1.24
1.83
1.00
0.94
1.10
2.08
1.10
0.97


13C
176.05
DMSO
169.22
166.71
141.43
137.75
130.50
129.66
129.31
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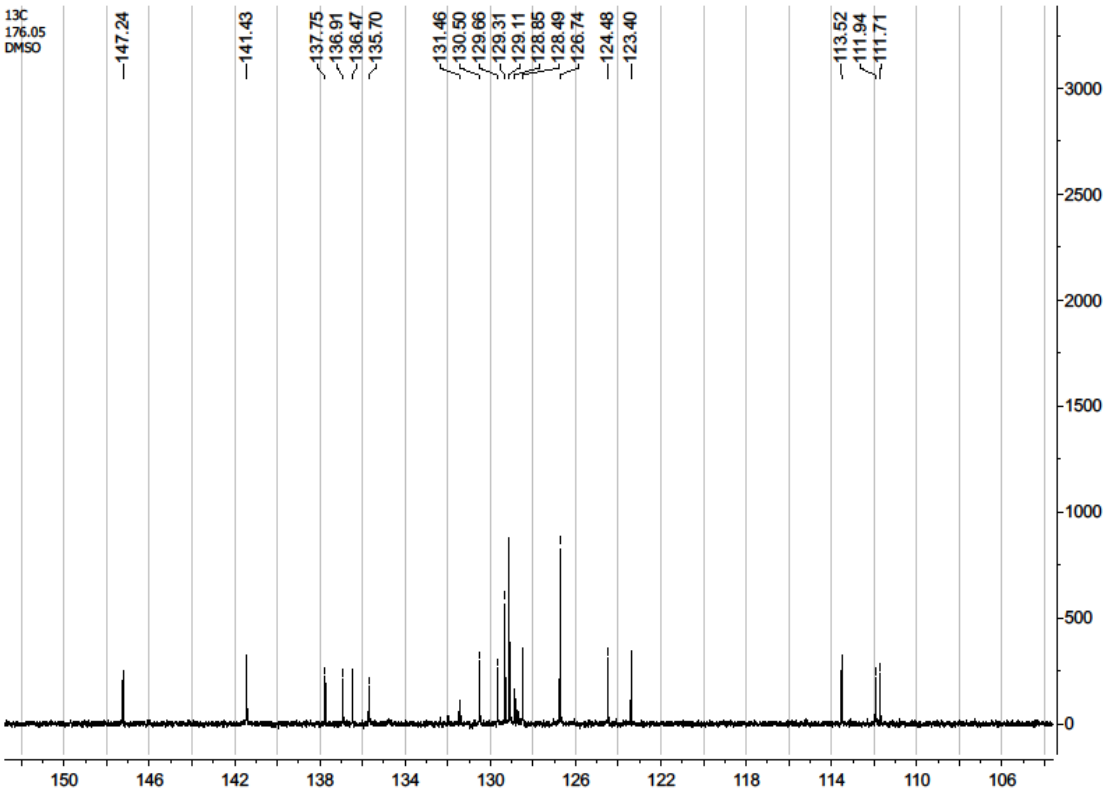
126.74
124.48
123.40
113.52
111.94
111.71
66.31
41.05
34.31
25.30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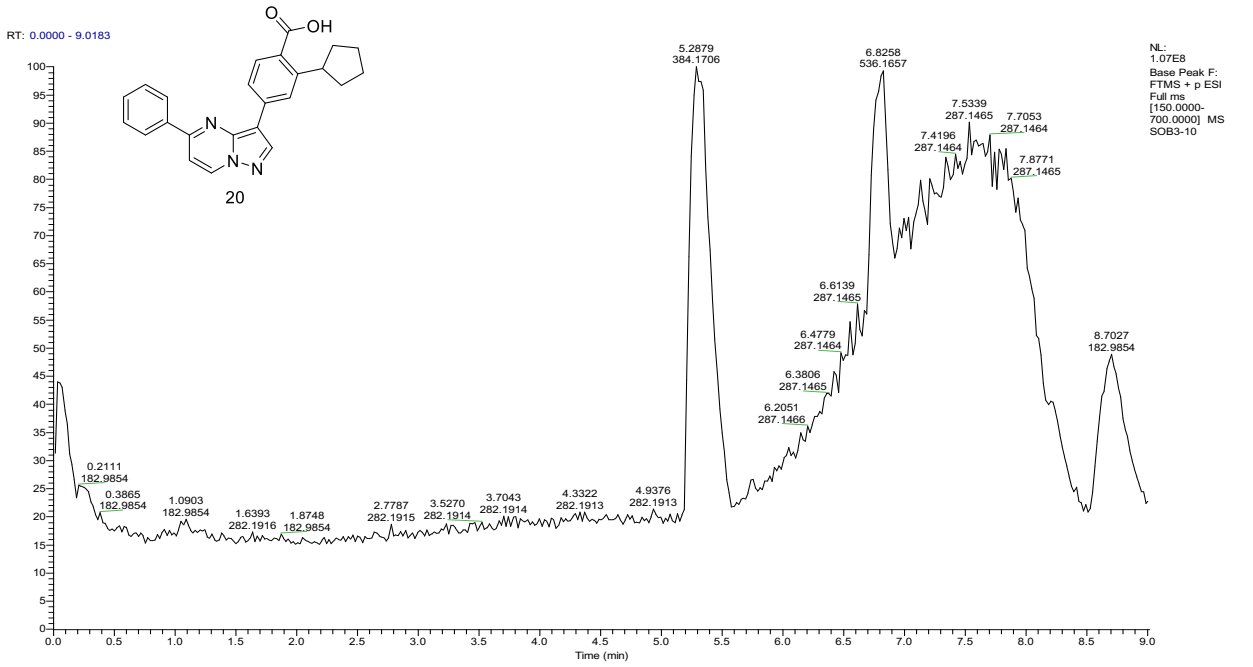
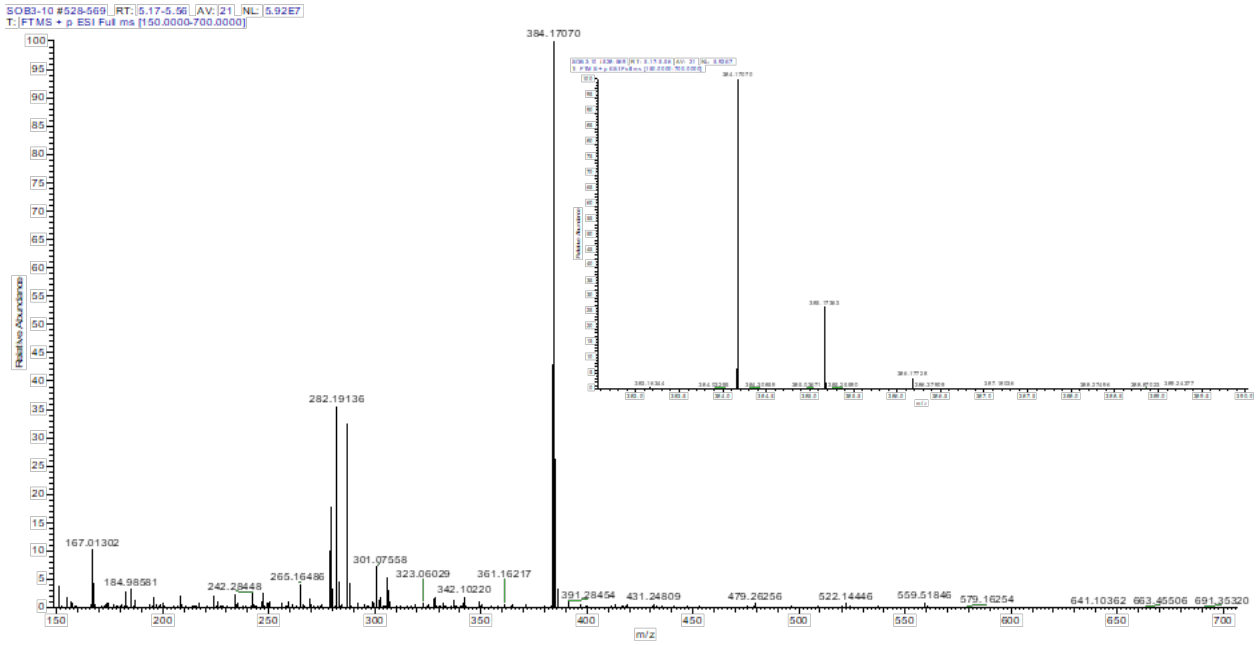
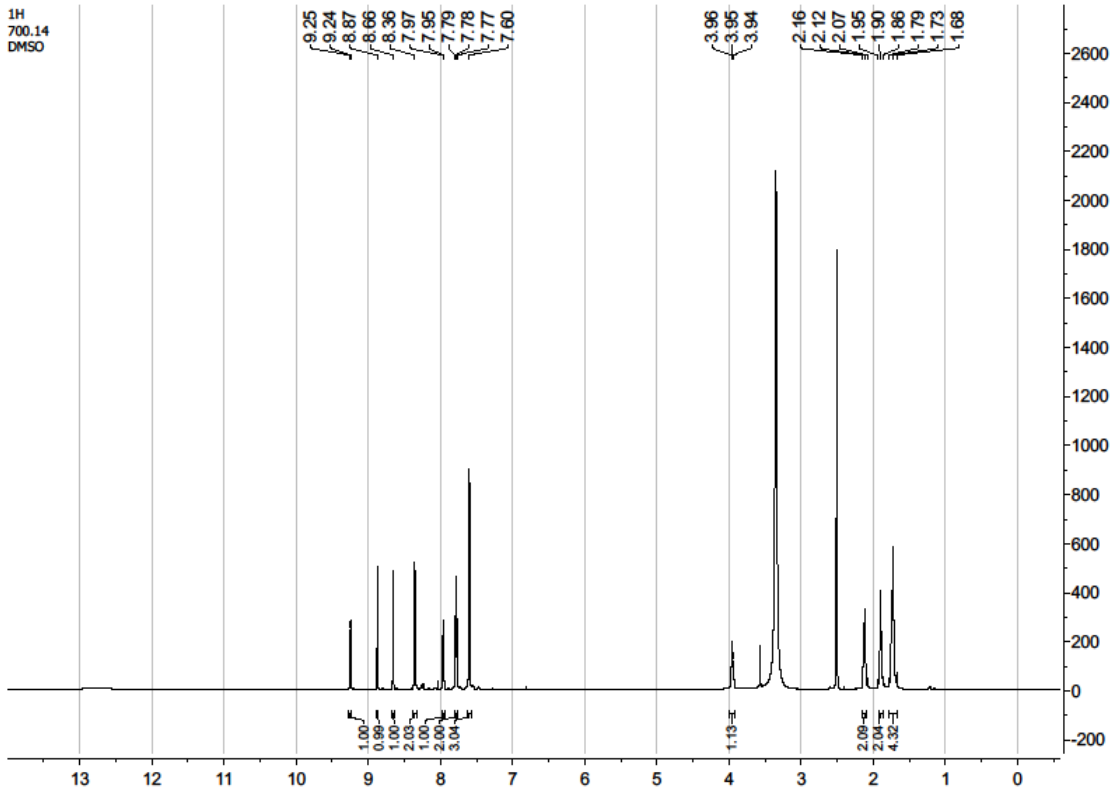
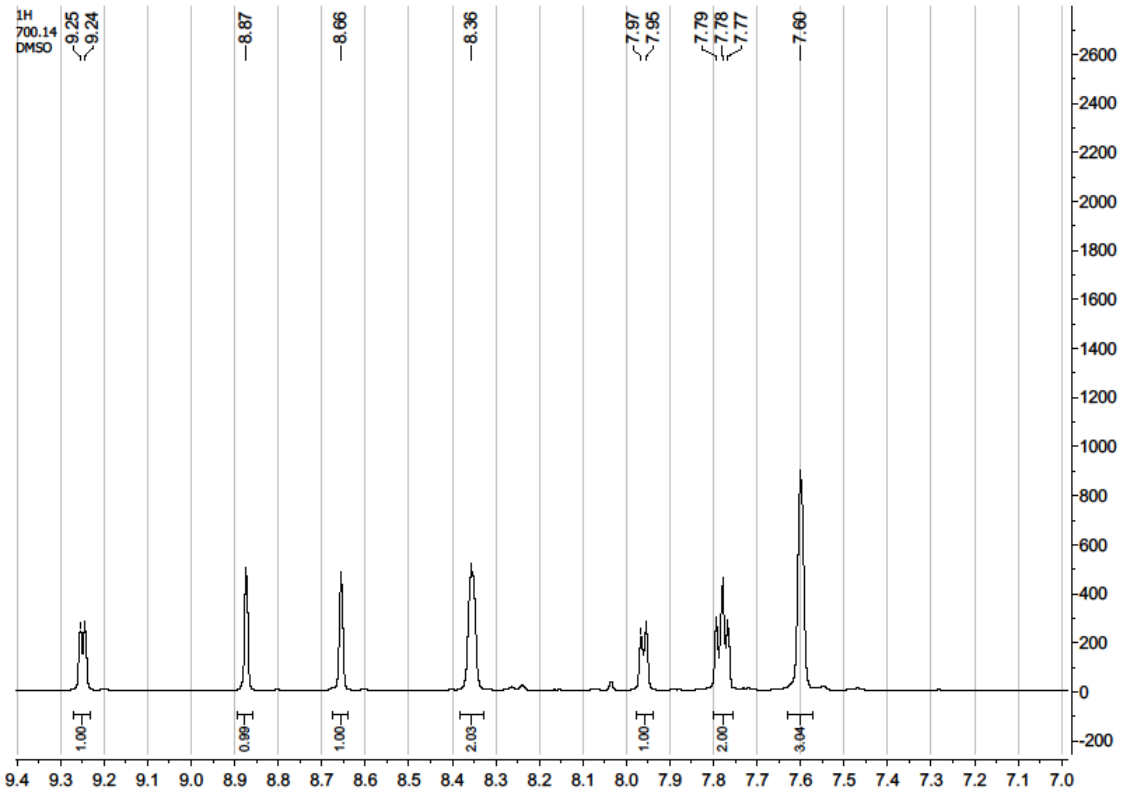
176.05
DMSO
147.24
141.43
137.75
136.91
136.47
135.70
131.46
130.50
129.66
129.31
129.11
128.85
128.49
126.74
124.48
123.40
113.52
111.94
111.71

2-Cyclopentyl-4-(5-phenylpyrazolo[1,5-*a*]pyrimidin-3-yl)benzoic acid (20)






1H
700.14
DMSO
9.25
9.24
8.87
8.66
8.36
7.97
7.95
7.79
7.78
7.77
7.60
3.96
3.95
3.94
2.16
2.12
2.07
1.95
1.90
1.86
1.79
1.73
1.68
1.00
0.99
1.00
2.03
1.00
2.00
3.04
1.13
2.09
2.04
4.32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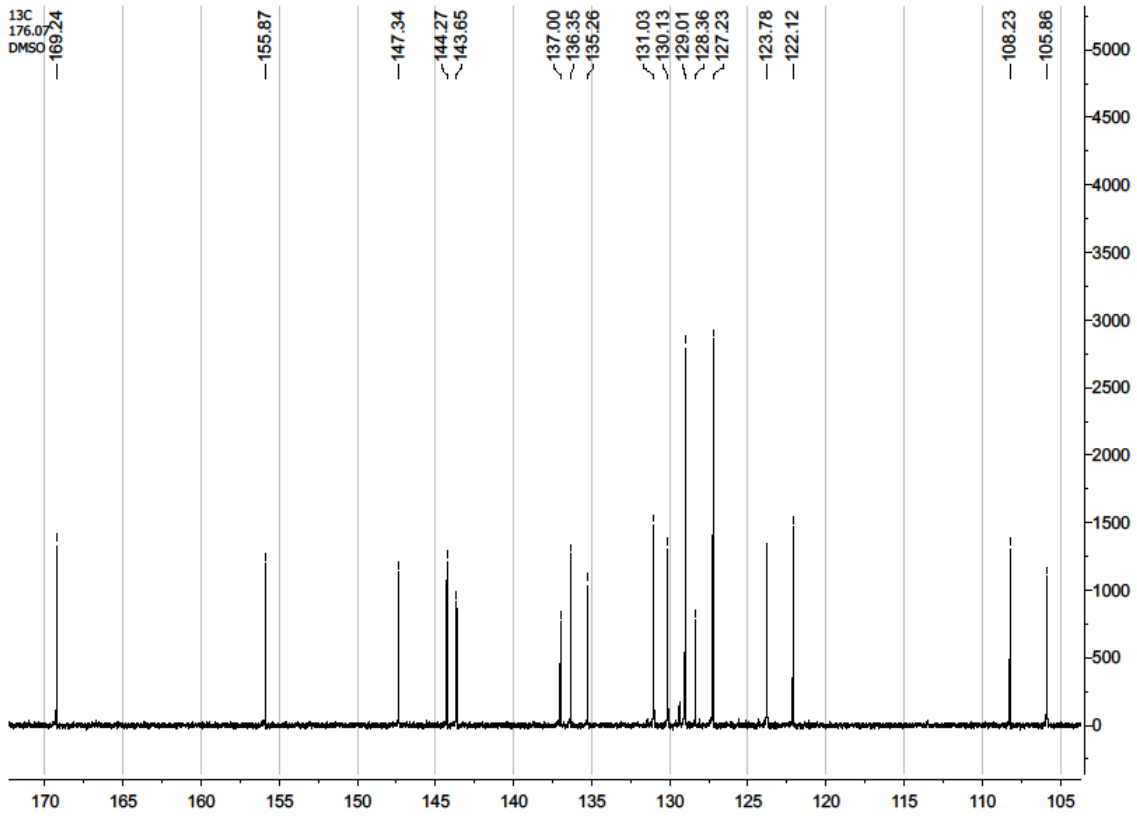
700.14
DMSO
9.25
9.24
8.87
8.66
8.36
7.97
7.95
7.79
7.78
7.77
7.60
1.00
0.99
1.00
2.03
1.00
2.00
3.04


13C
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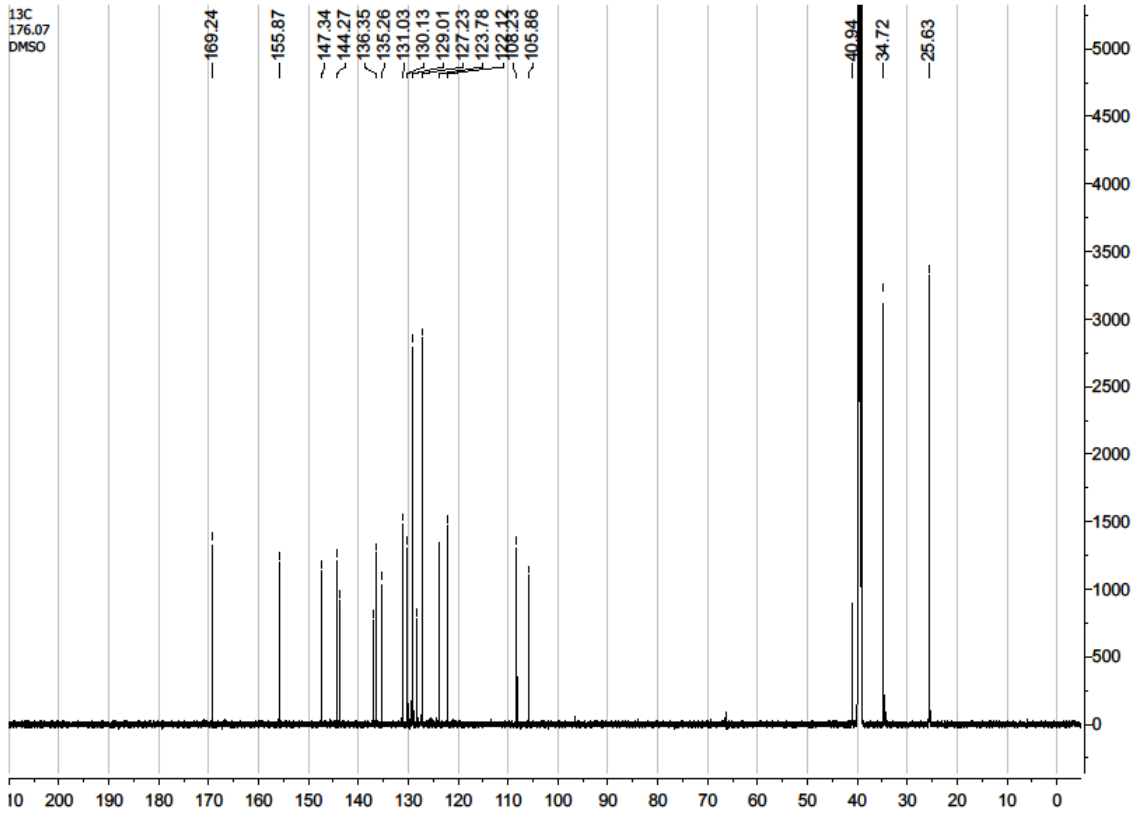
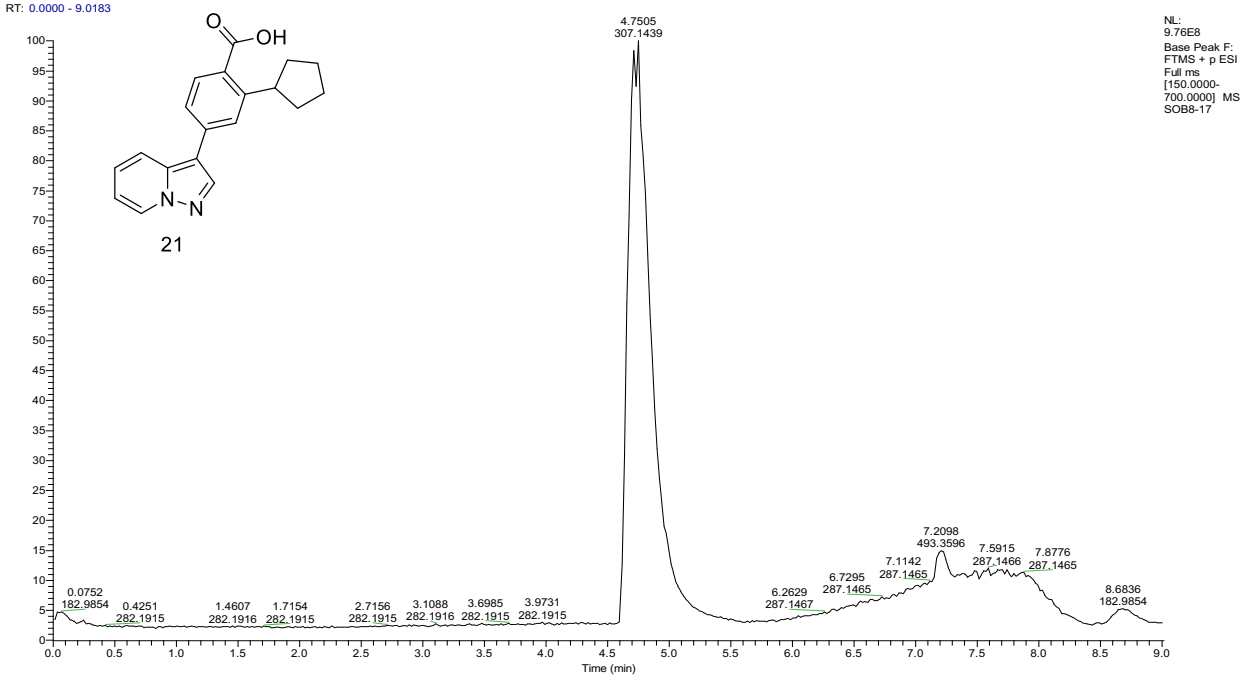
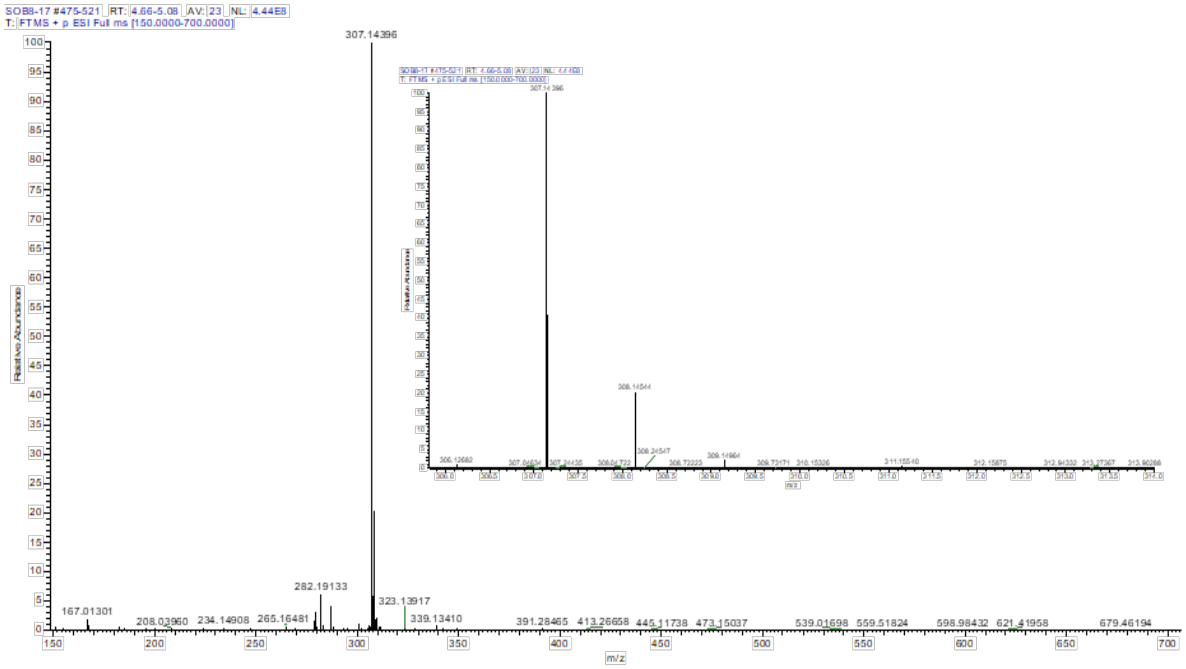
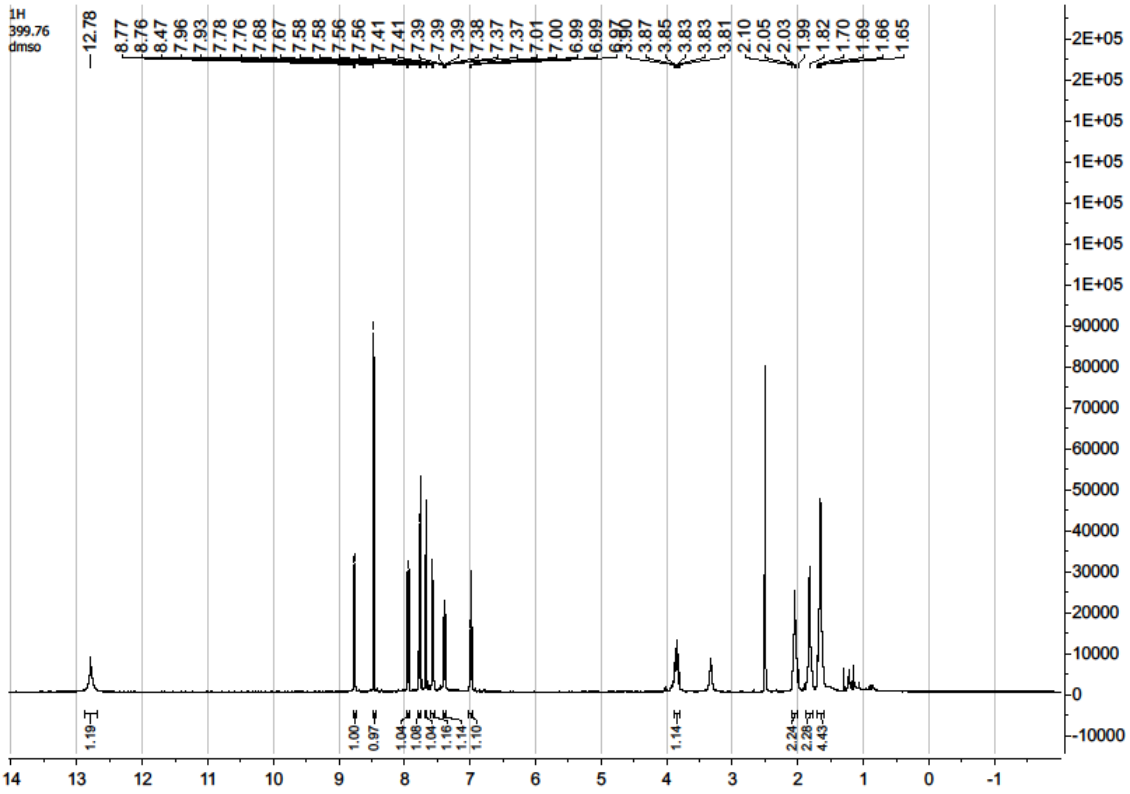
DMSO
169.24
155.87
147.34
144.27
143.65
137.00
136.35
135.26
131.03
130.13
129.01
128.36
127.23
123.78
122.12
108.23
105.86


13C
176.07
DMSO
169.24
155.87
147.34
144.27
136.35
135.26
131.03
130.13
129.01
127.23
123.78
122.12
108.23
105.86
40.94
34.72
25.63

2-Cyclopentyl-4-(pyrazolo[1,5-*a*]pyridin-3-yl)benzoic acid (21)



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dmso
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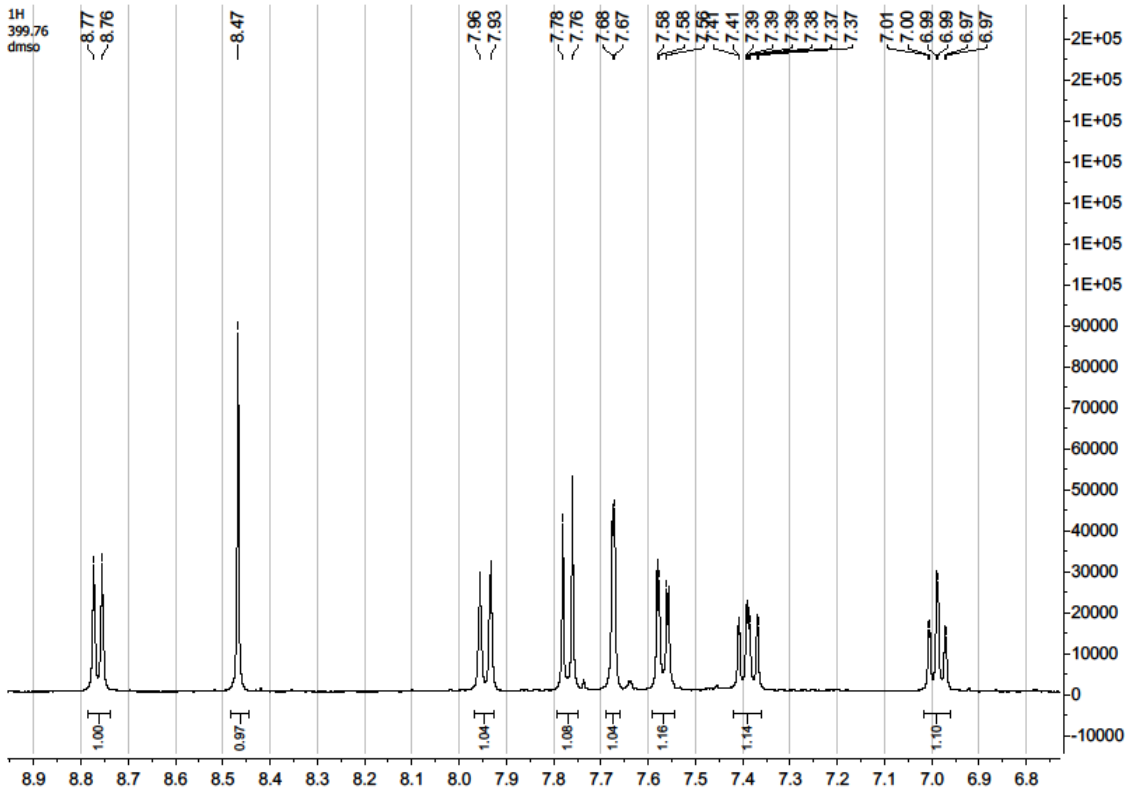
8.77
8.76
8.47
7.96
7.93
7.78
7.76
7.68
7.67
7.58
7.58
7.56
7.56
7.41
7.41
7.39
7.39
7.39
7.38
7.37
7.37
7.01
7.00
6.99
6.99
3.90
3.87
3.85
3.83
3.83
3.81
2.10
2.05
2.03
1.99
1.82
1.70
1.69
1.66
1.65
1.19
1.00
0.97
1.04
1.08
1.04
1.16
1.14
1.10
1.14
2.24
2.28
4.43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399.76
dmso
8.77
8.76
8.47
7.96
7.93
7.78
7.76
7.68
7.67
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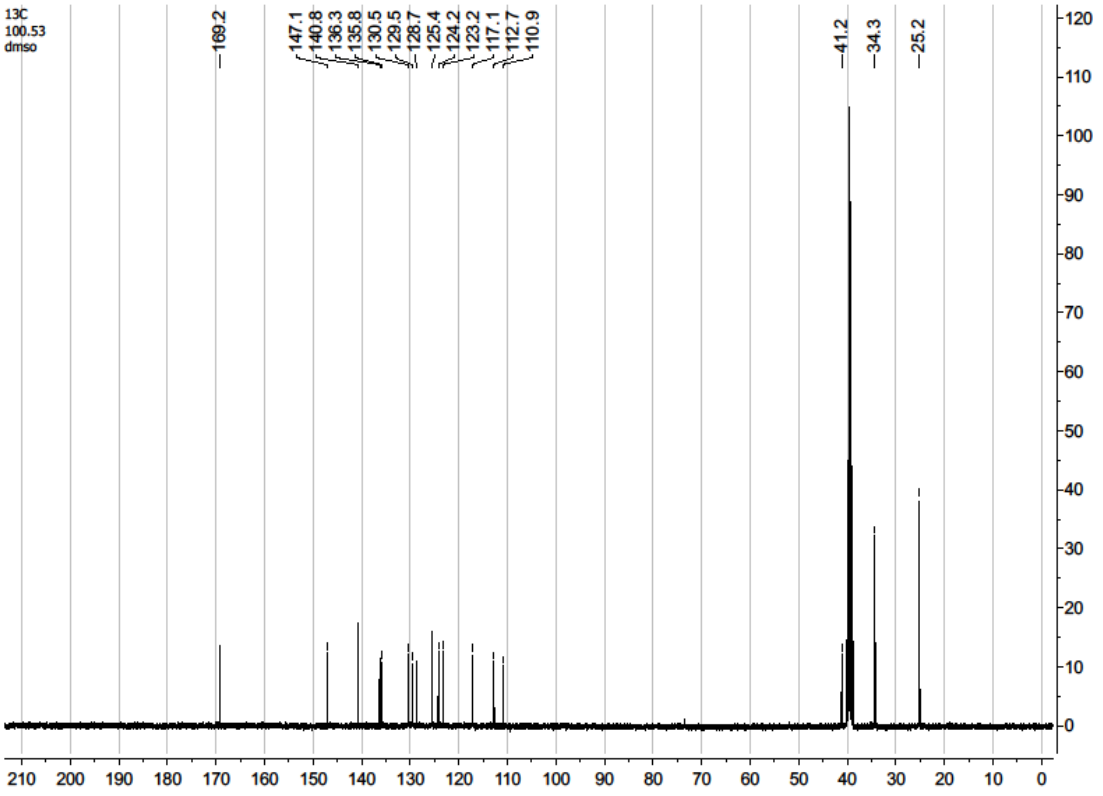
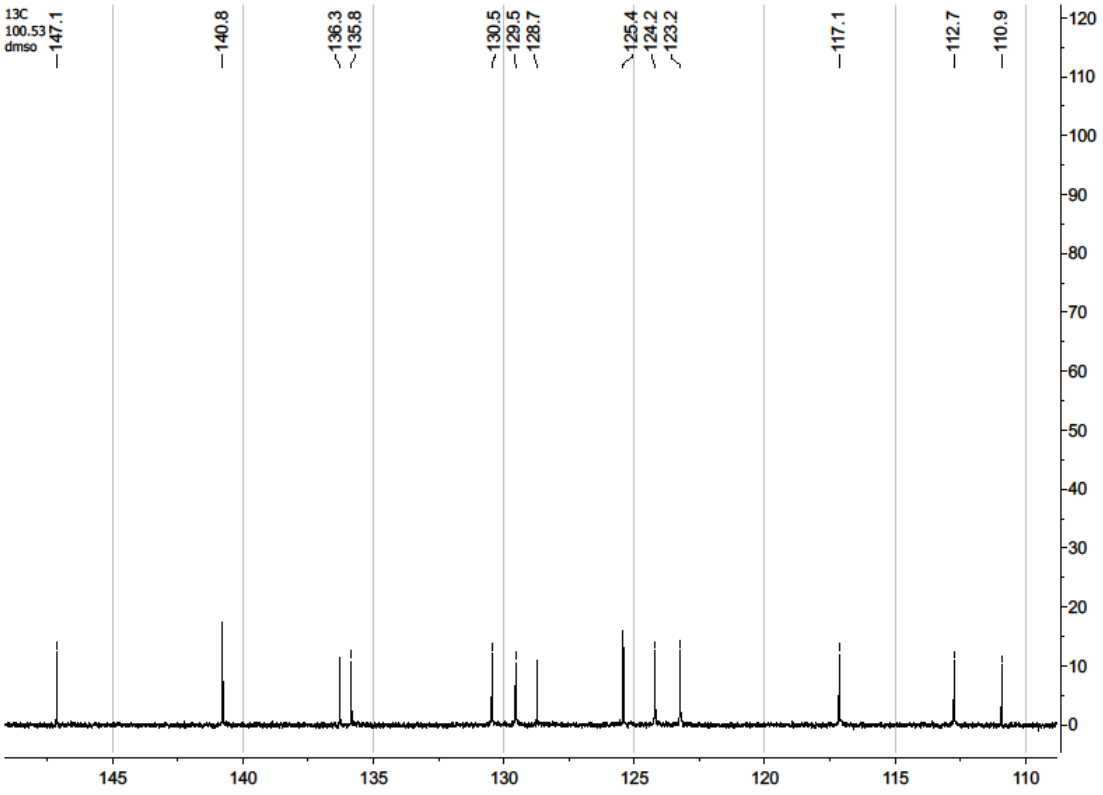
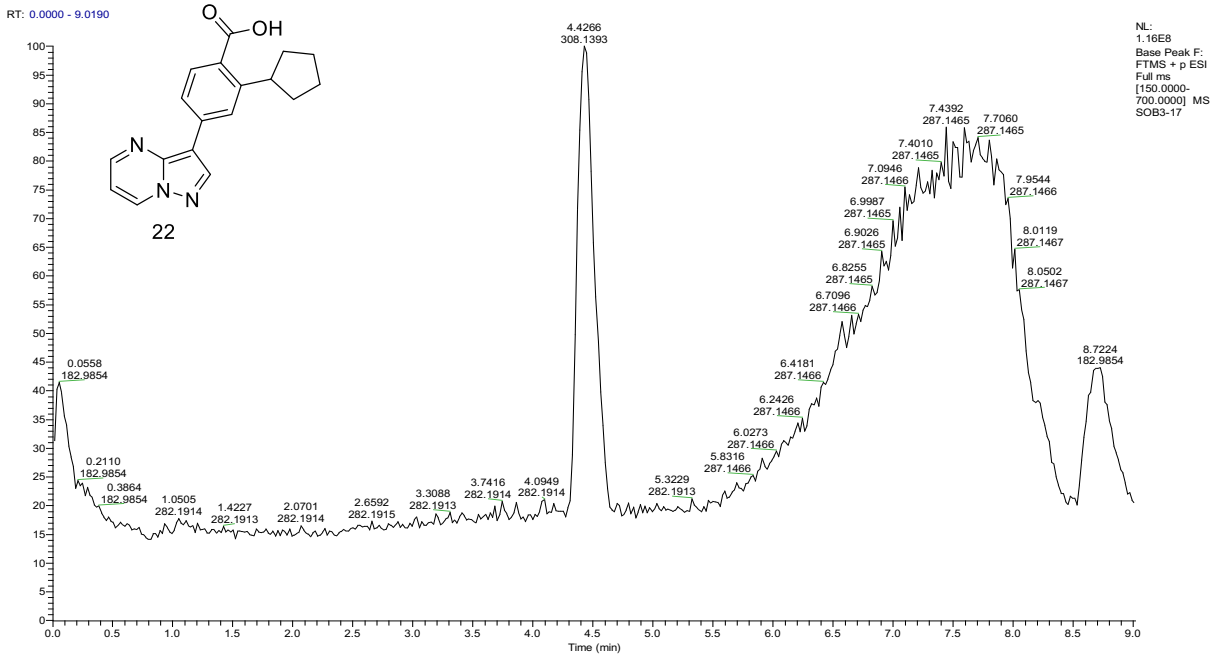
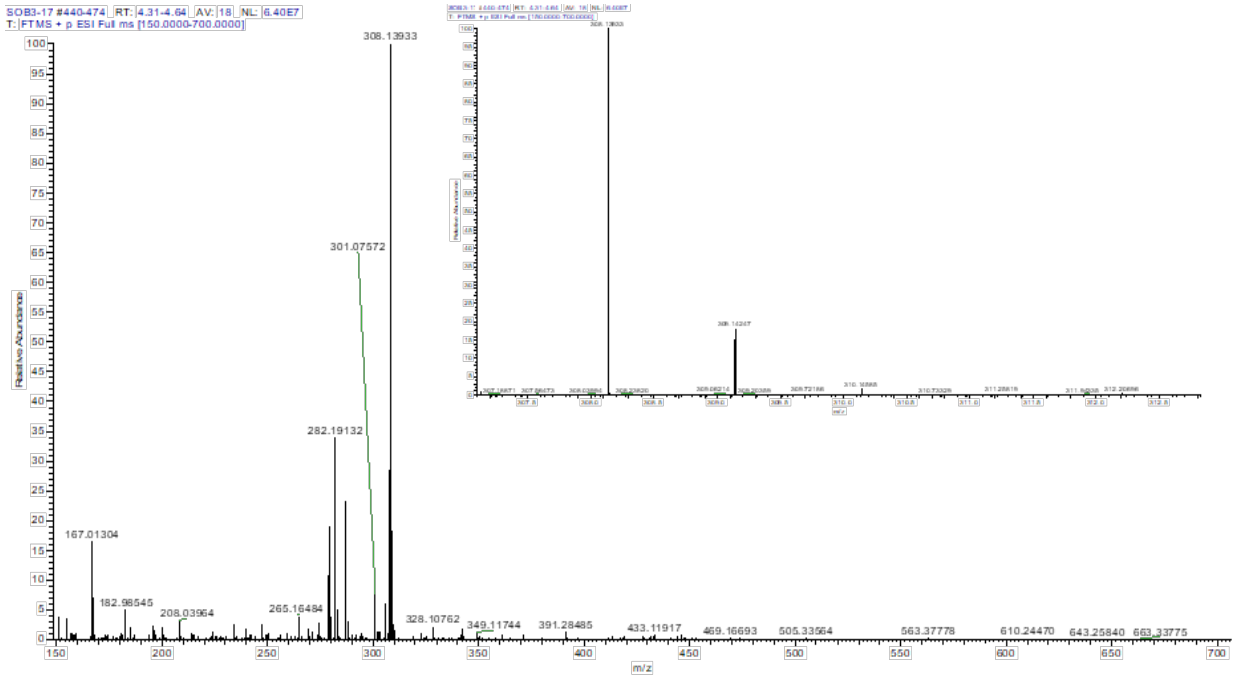
7.58
7.56
7.56
7.41
7.41
7.39
7.39
7.39
7.38
7.37
7.37
7.01
7.00
6.99
6.99
6.97
6.97
1.00
0.97
1.04
1.08
1.04
1.16
1.14
1.10

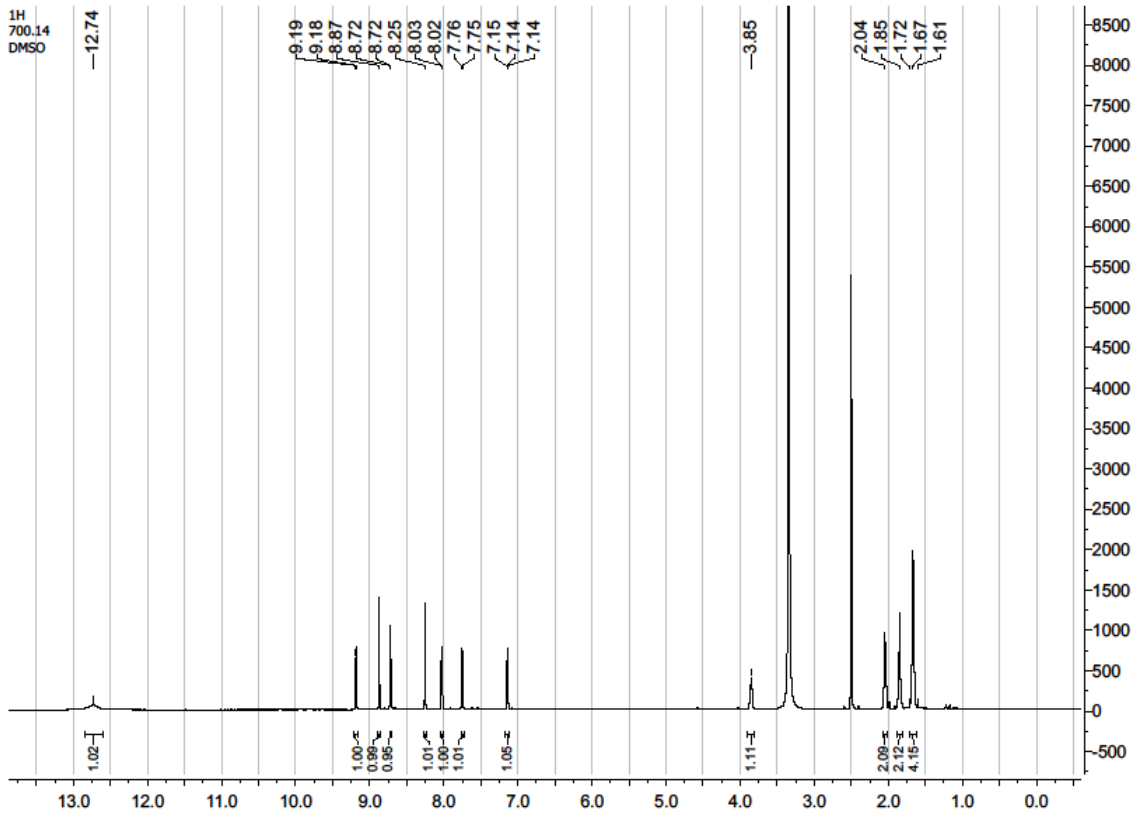

13C
100.53
dmso
169.2
147.1
140.8
136.3
135.8
130.5
129.5
128.7
125.4
124.2
123.2
117.1
112.7
110.9
41.2
34.3
25.2

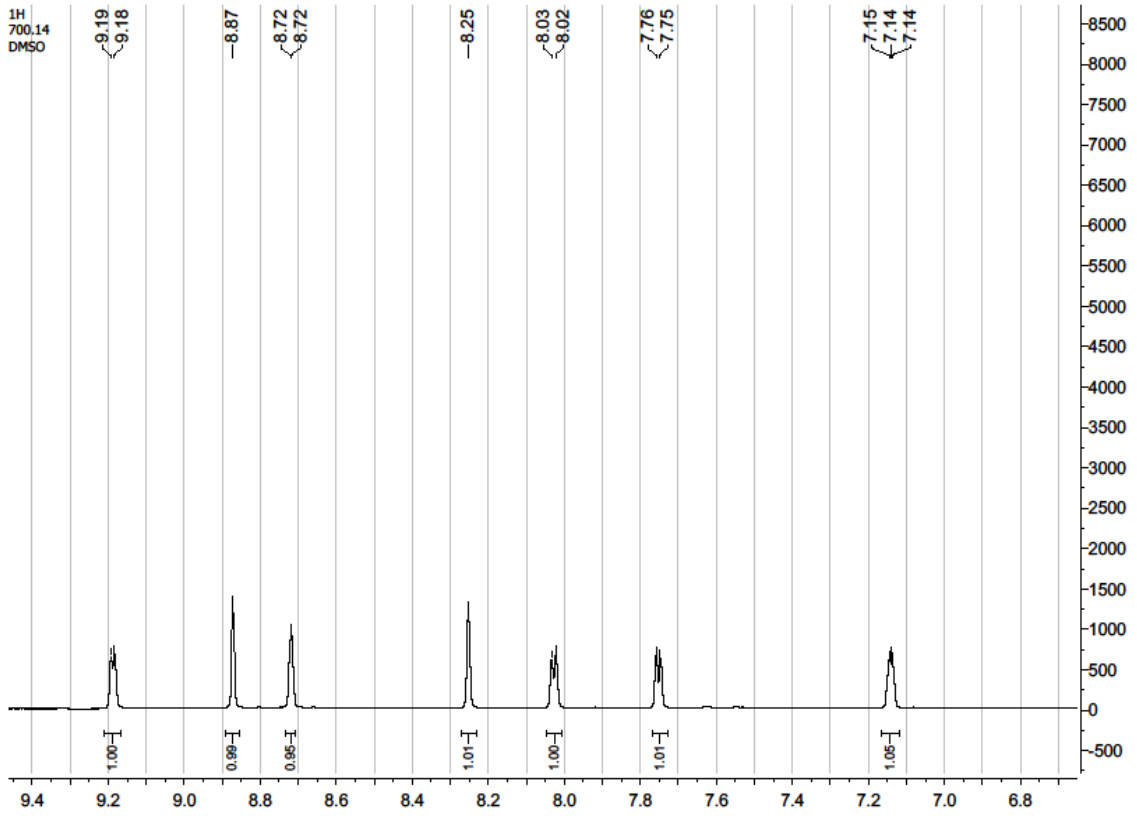

13C
100.53
dmso
147.1
140.8
136.3
135.8
130.5
129.5
128.7
125.4
124.2
123.2
117.1
112.7
110.9

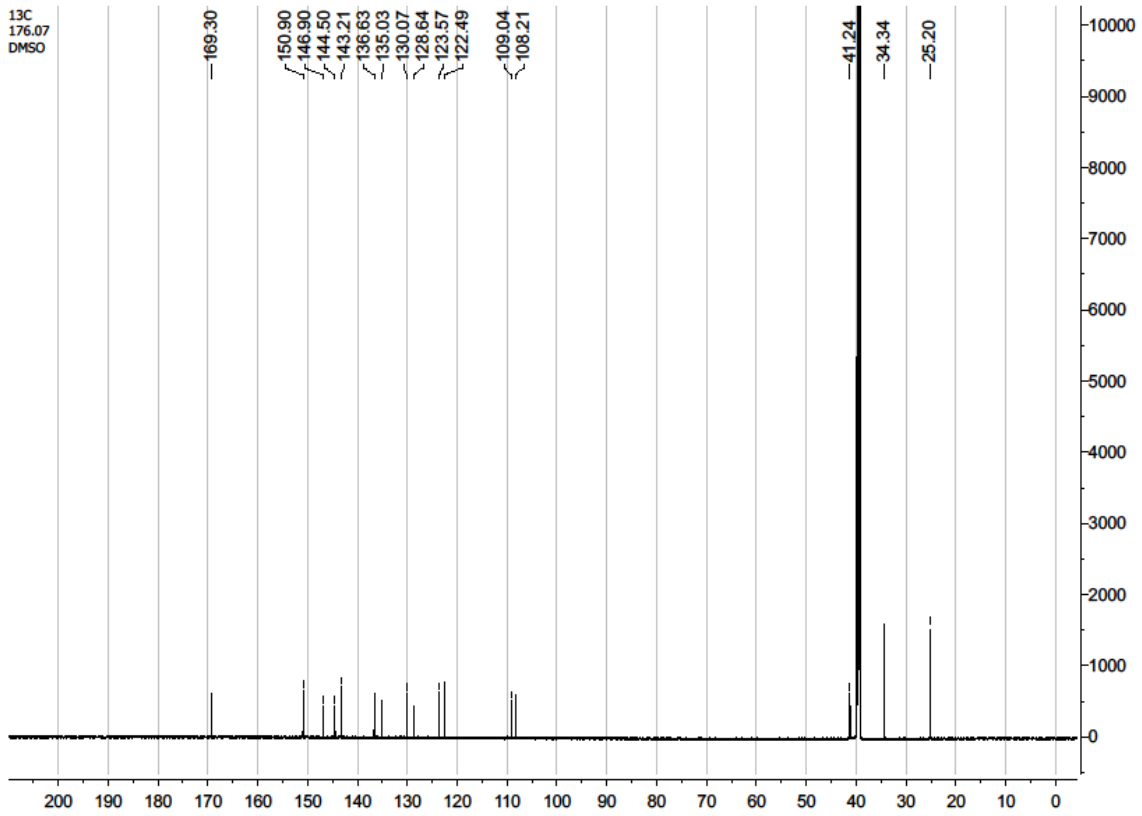
2-Cyclopentyl-4-(pyrazolo[1,5-*a*]pyrimidin-3-yl)benzoic acid (22)

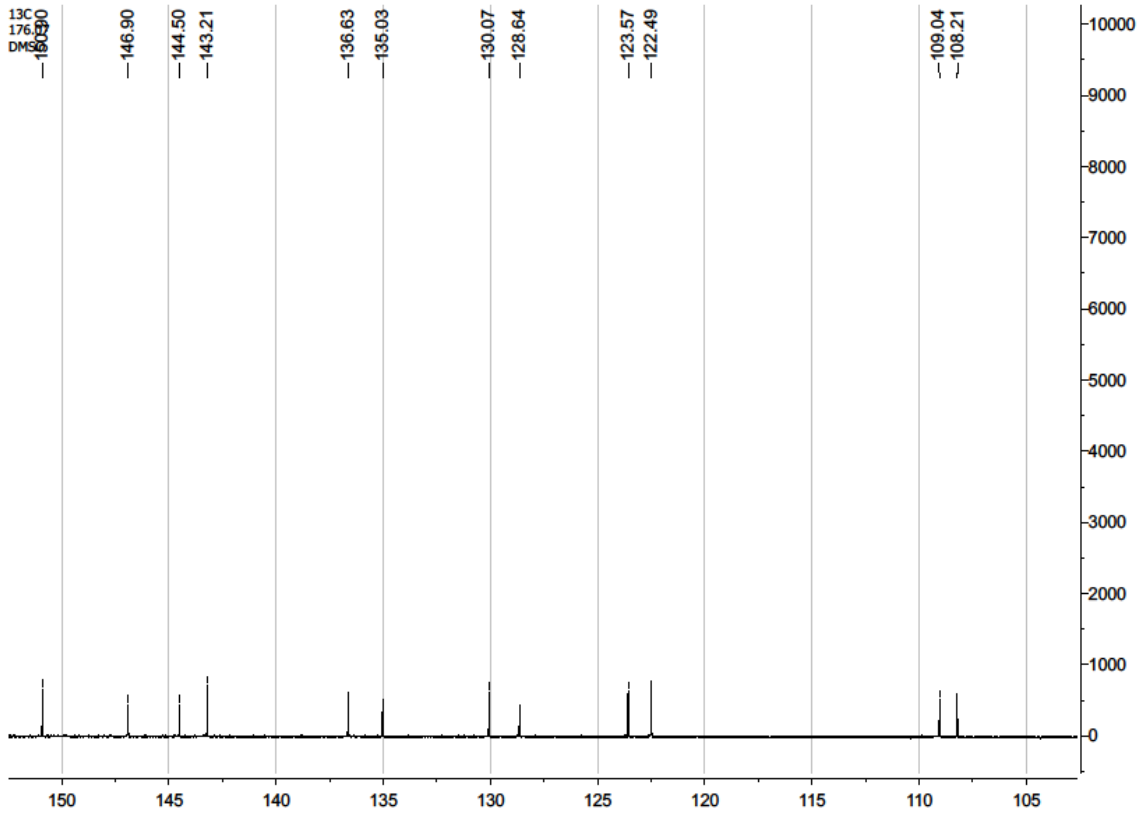




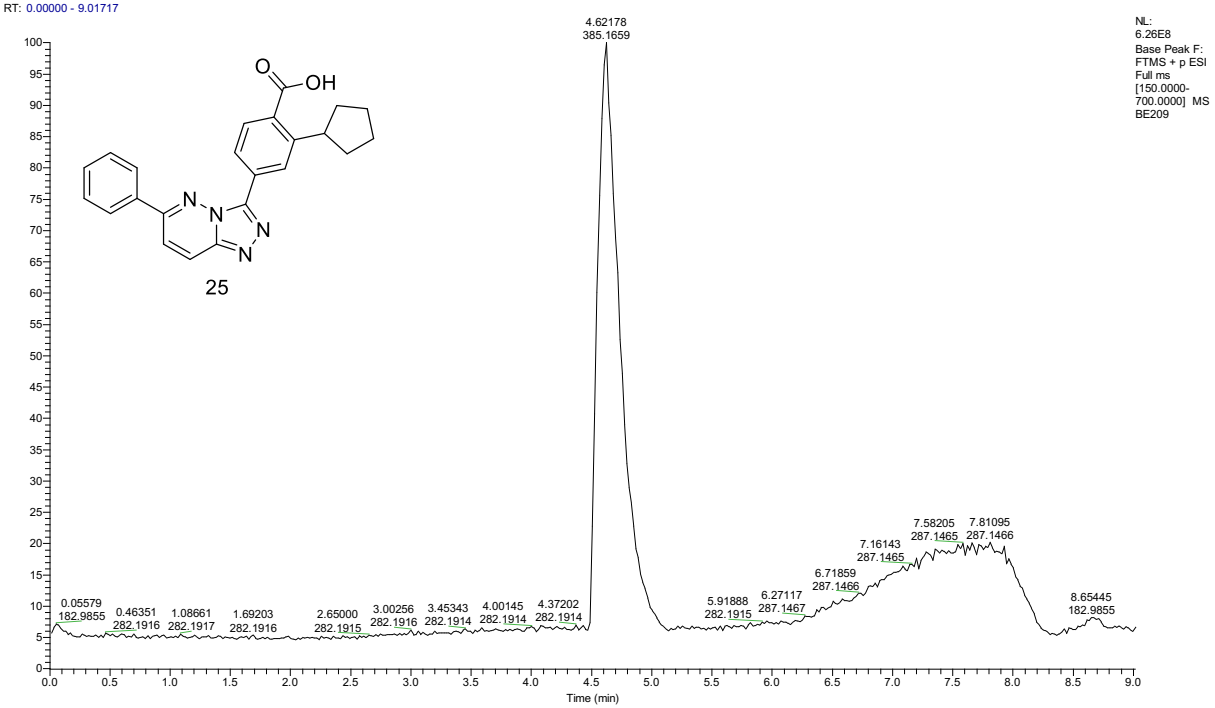

1H
700.14
DMSO
12.74
9.19
9.18
8.87
8.72
8.72
8.25
8.03
8.02
7.76
7.75
7.15
7.14
7.14
3.85
2.04
1.85
1.72
1.67
1.61
1.02
1.00
0.99
0.95
1.01
1.00
1.01
1.05
1.11
2.09
2.12
4.15

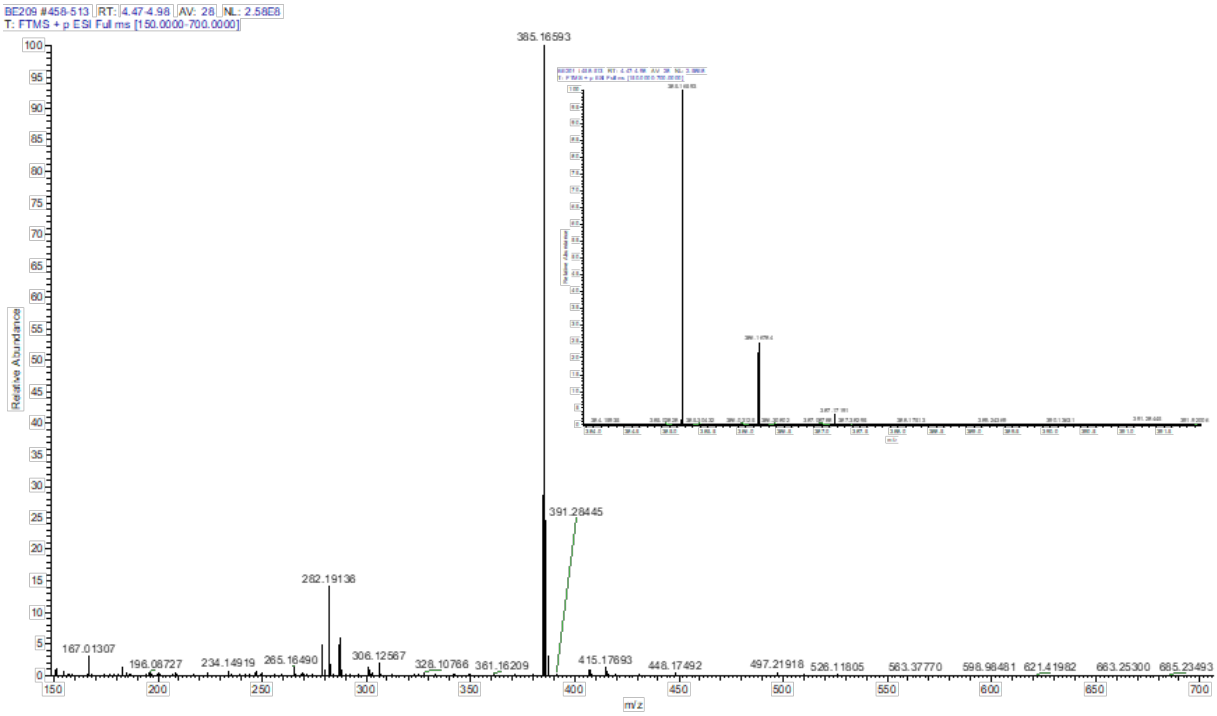

1H
700.14
DMSO
9.19
9.18
8.87
8.72
8.72
8.25
8.03
8.02
7.76
7.75
7.15
7.14
7.14
1.00
0.99
0.95
1.01
1.00
1.01
1.05

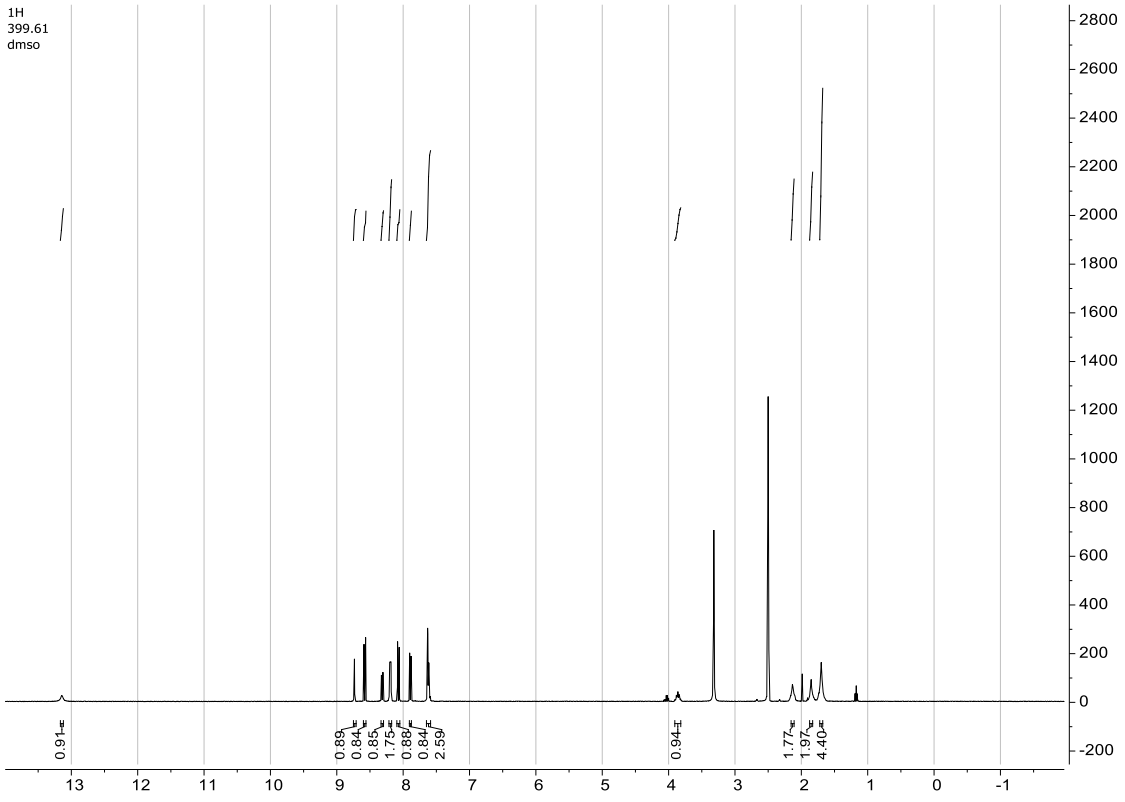

13C
176.07
DMSO
169.30
150.90
146.90
144.50
143.21
136.63
135.03
130.07
128.64
123.57
122.49
109.04
108.21
41.24
34.34
25.20

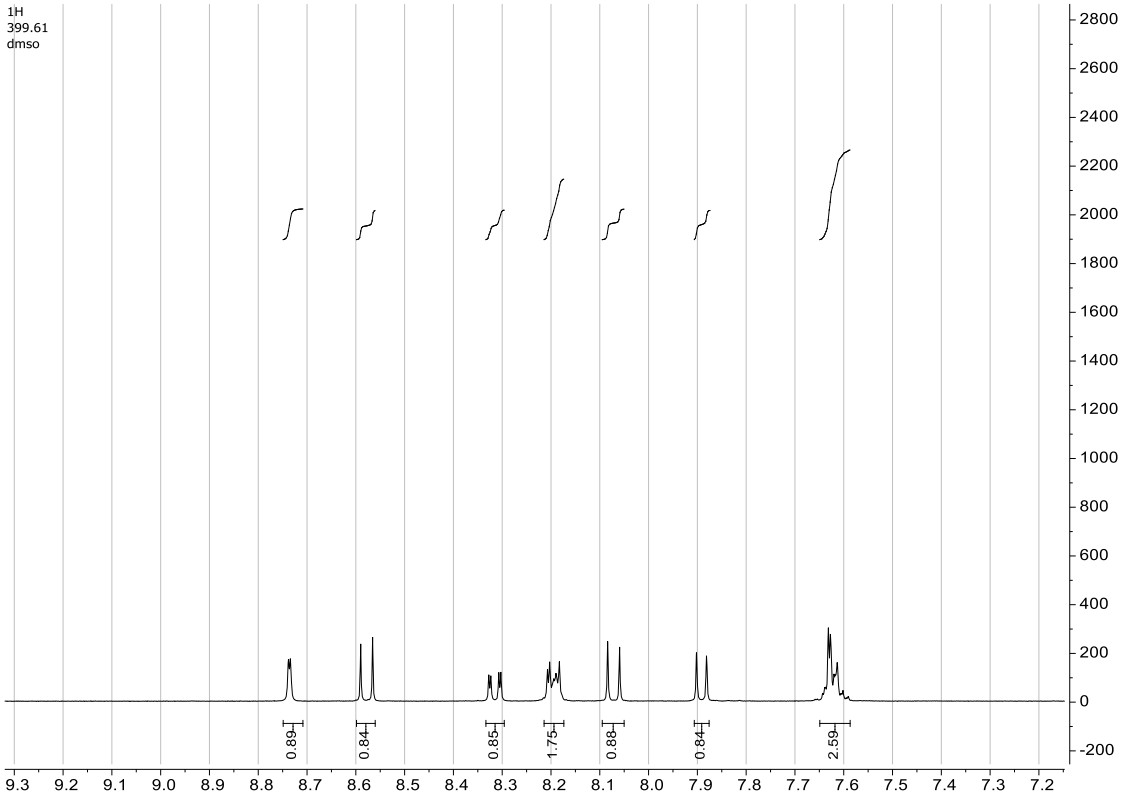

13C
DMSO
146.90
144.50
143.21
136.63
135.03
130.07
128.64
123.57
122.49
109.04
108.21

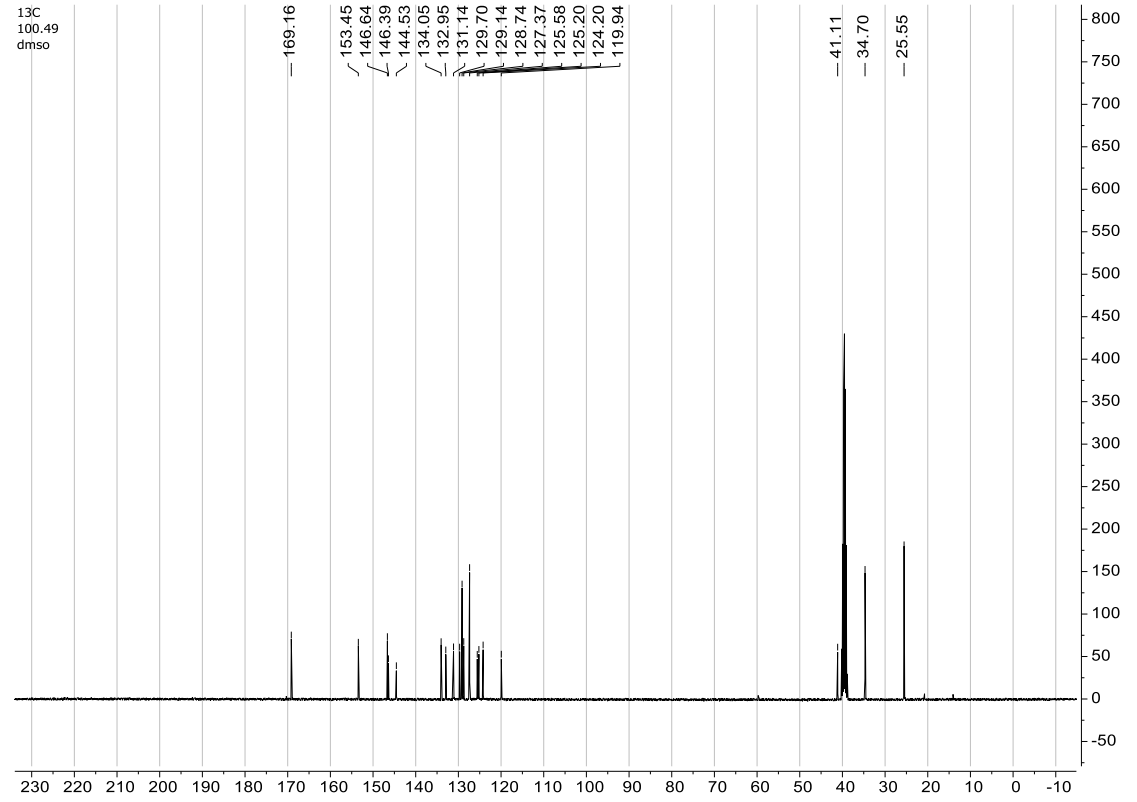
2-cyclopentyl-4-(6-phenyl-[1,2,4]triazolo[4,3-*b*]pyridazin-3-yl)benzoic acid (**25**)

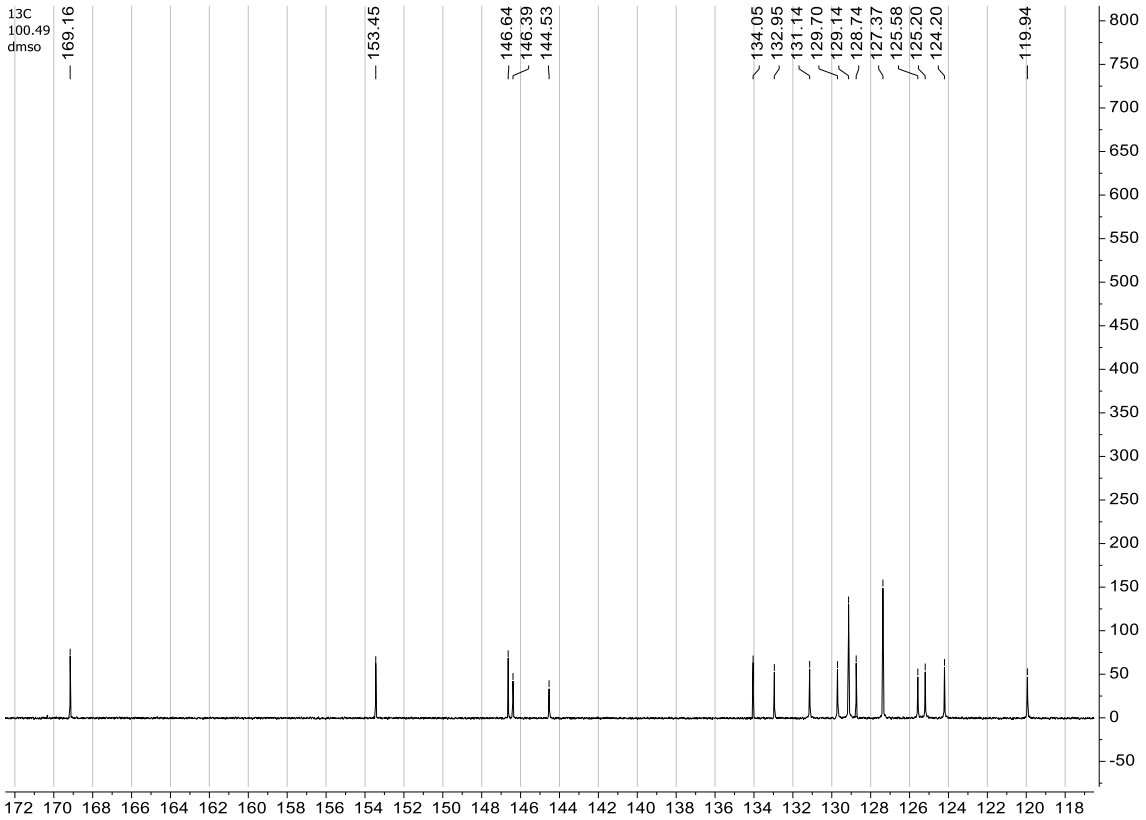




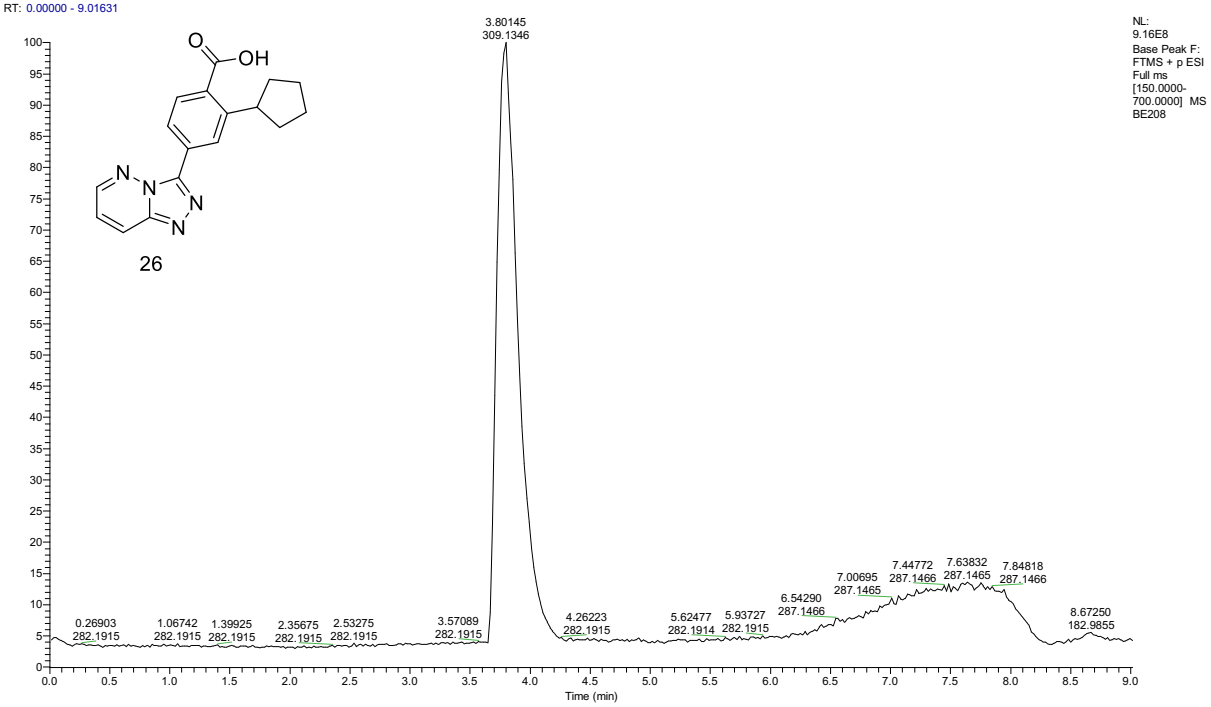

1H
399.61
dmso
0.91
0.89
0.84
0.85
1.75
0.88
0.84
2.59
0.94
1.77
1.97
4.40

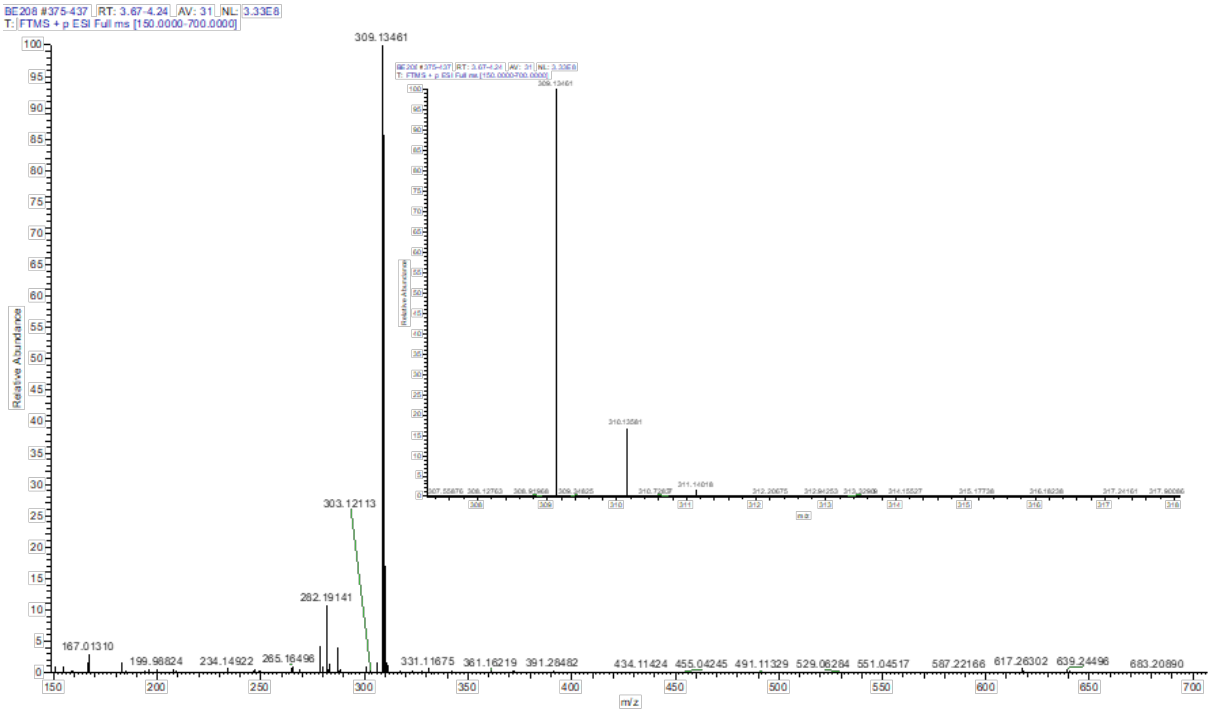

1H
399.61
dmso
0.89
0.84
0.85
1.75
0.88
0.84
2.59

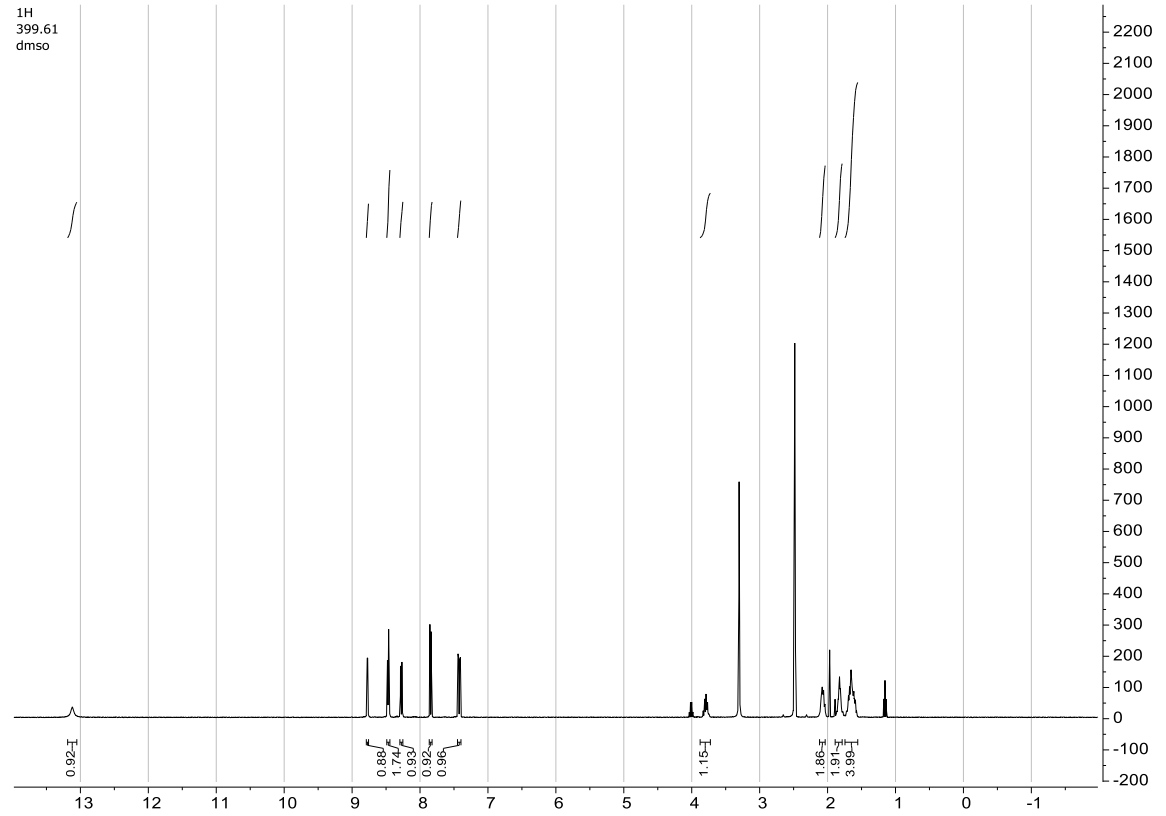

13C
100.49
dmso
169.16
153.45
146.64
146.39
144.53
134.05
132.95
131.14
129.70
129.14
128.74
127.37
125.58
125.20
124.20
119.94
41.11
34.70
25.55

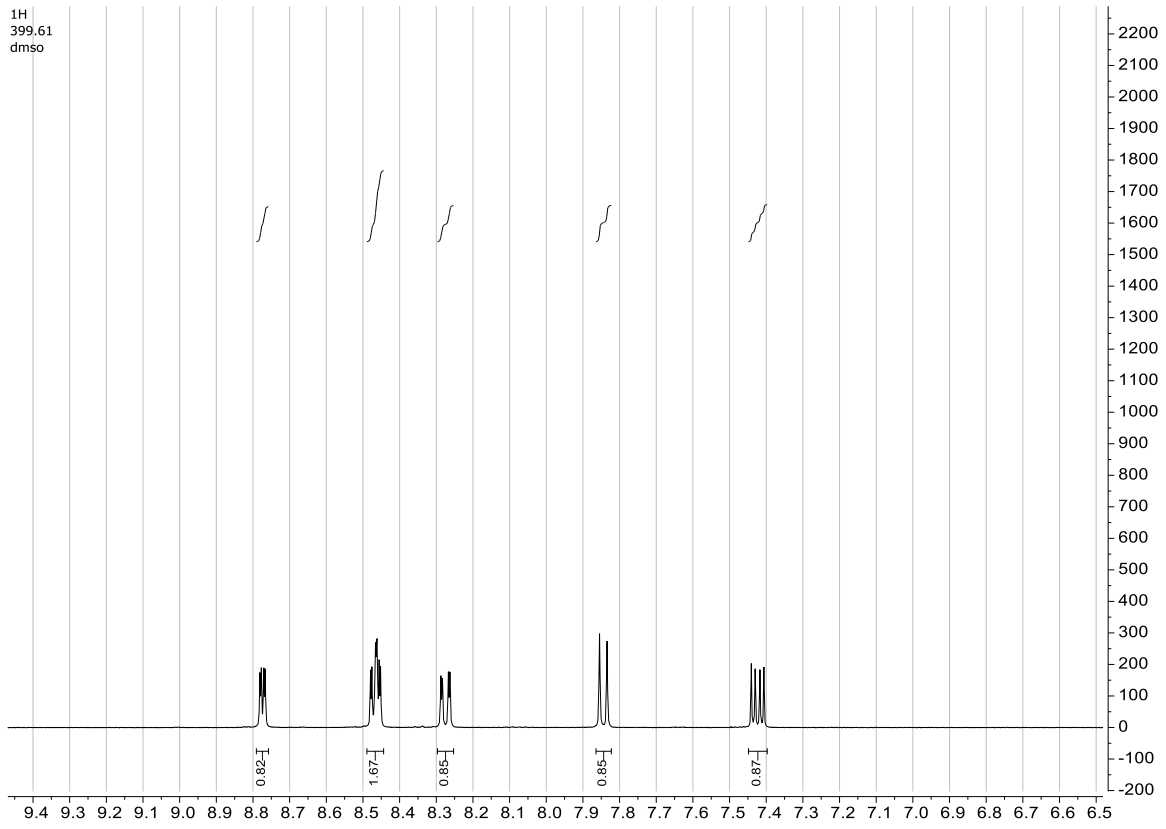

13C
100.49
dmso
169.16
153.45
146.64
146.39
144.53
134.05
132.95
131.14
129.70
129.14
128.74
127.37
125.58
125.20
124.20
119.94

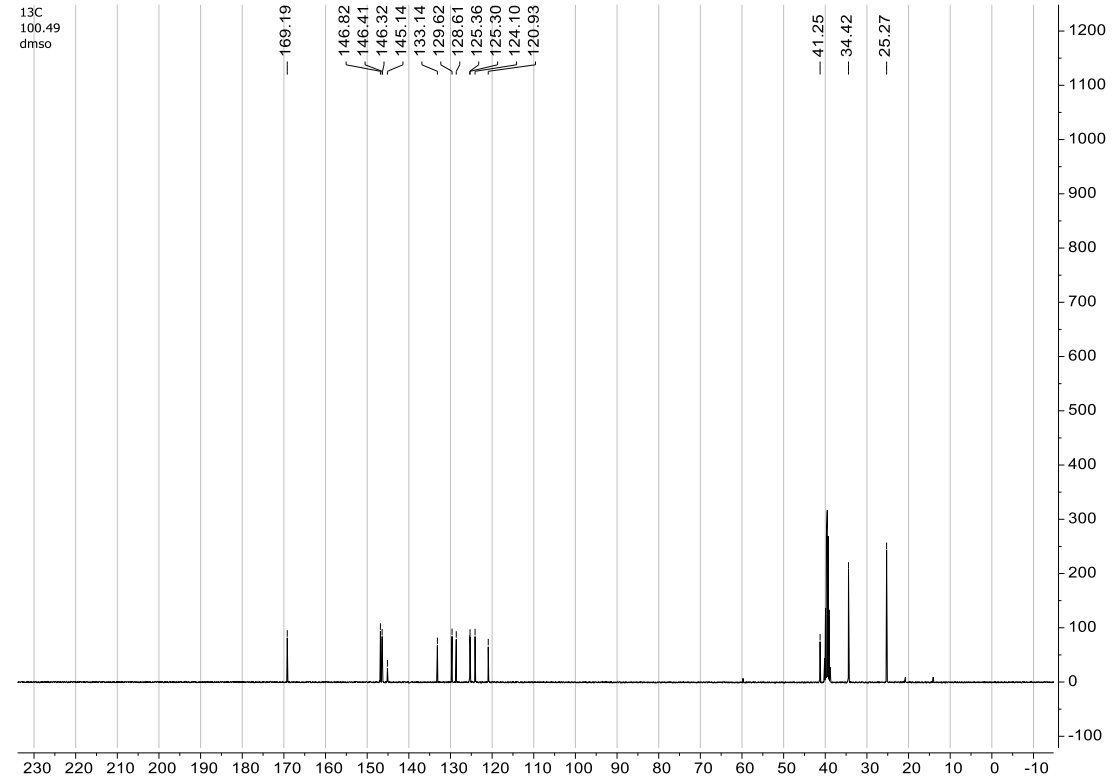
4-([1,2,4]Triazolo[4,3-*b*]pyridazin-3-yl)-2-cyclopentylbenzoic acid (26)

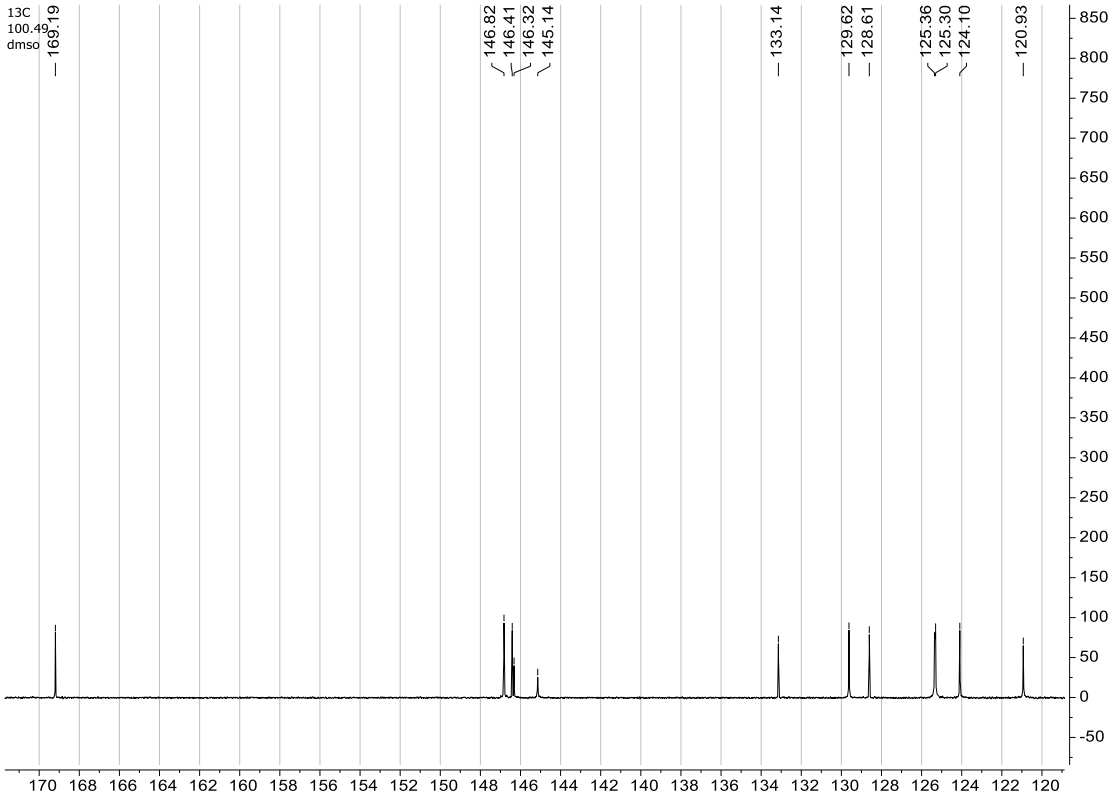




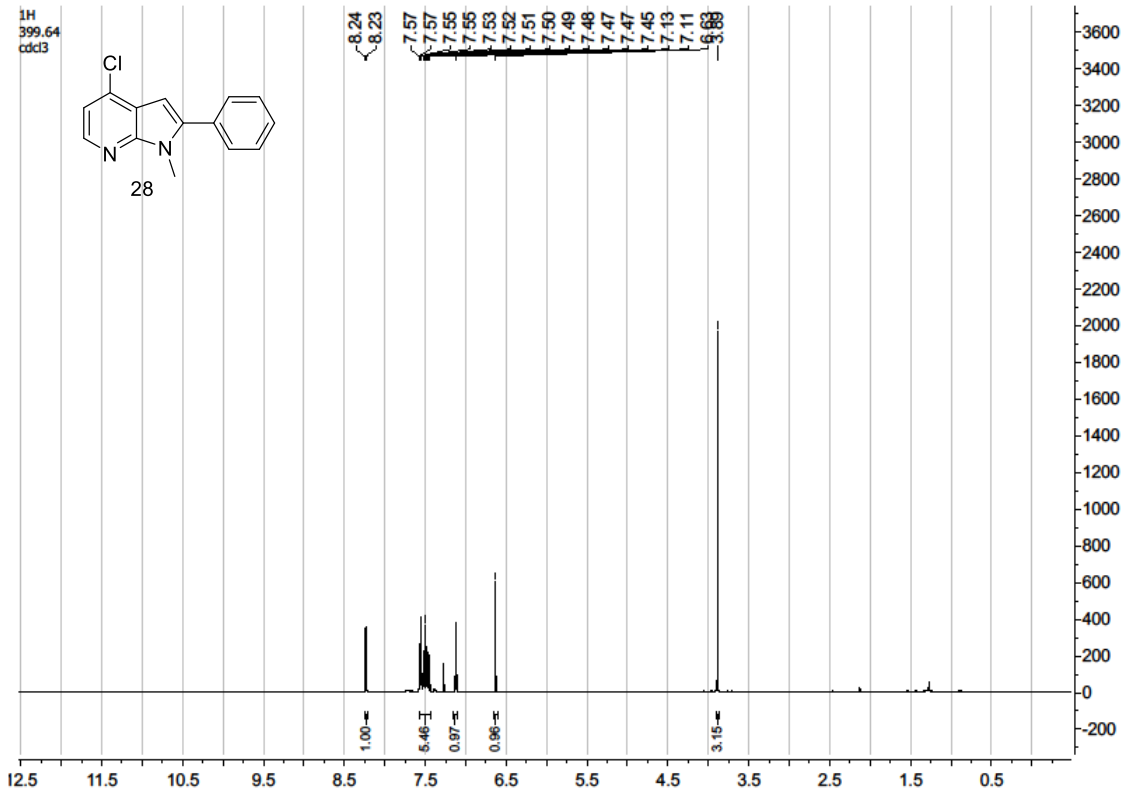

1H
399.61
dmso
0.92
0.88
1.74
0.93
0.92
0.96
1.15
1.86
1.91
3.99

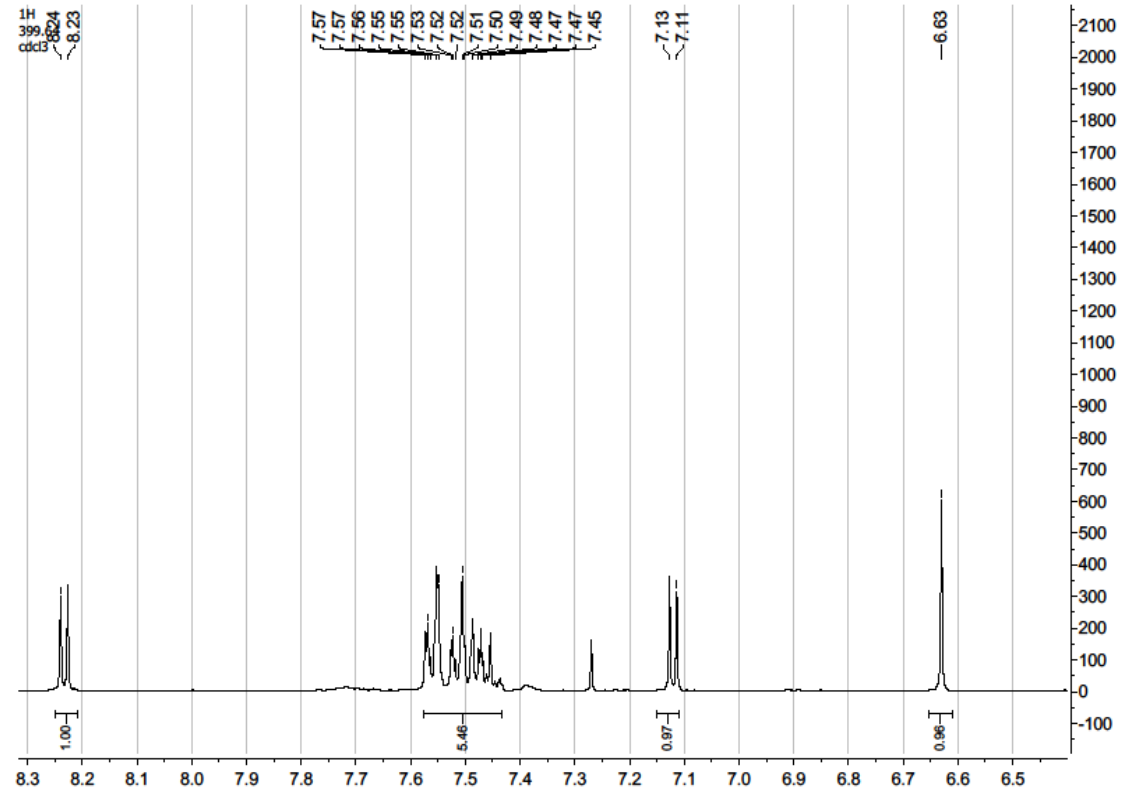

1H
399.61
dmso
0.82
1.67
0.85
0.85
0.87

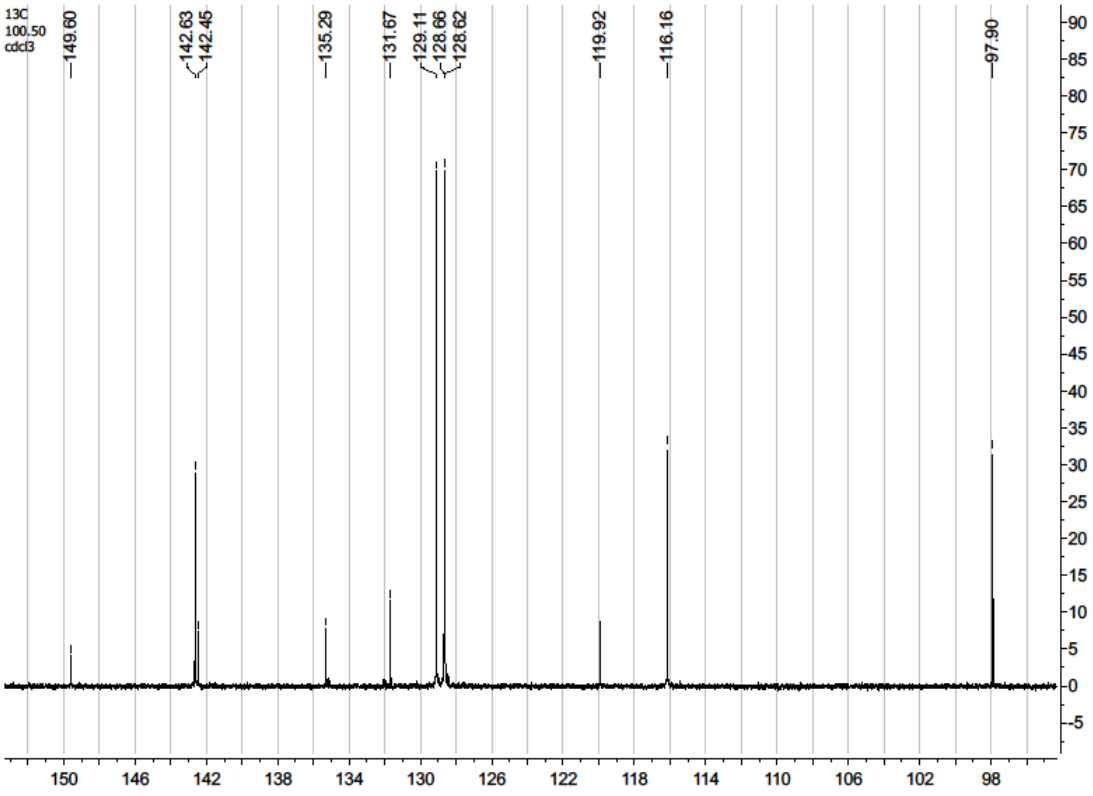

13C
100.49
dmso
169.19
146.82
146.41
146.32
145.14
133.14
129.62
128.61
125.36
125.30
124.10
120.93
41.25
34.42
25.27

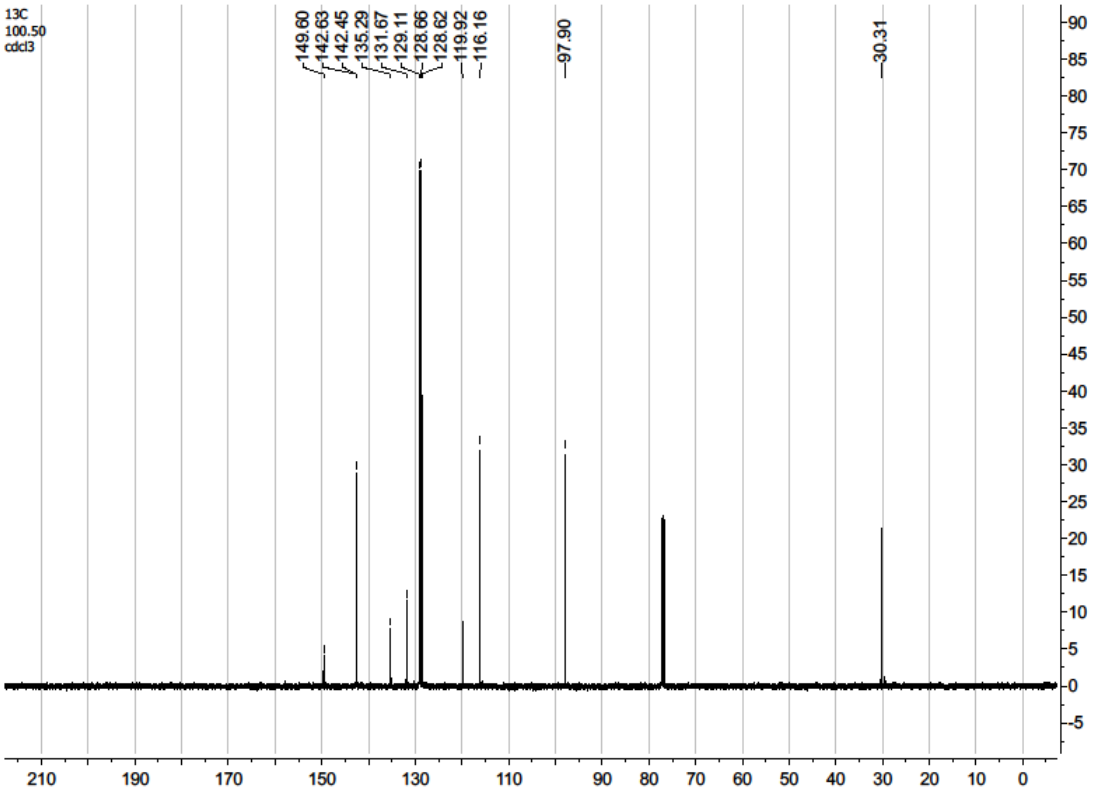

13C
100.49
dmso
169.19
146.82
146.41
146.32
145.14
133.14
129.62
128.61
125.36
125.30
124.10
120.93

4-Chloro-1-methyl-2-phenyl-1*H*-pyrrolo[2,3-*b*]pyridine (28)

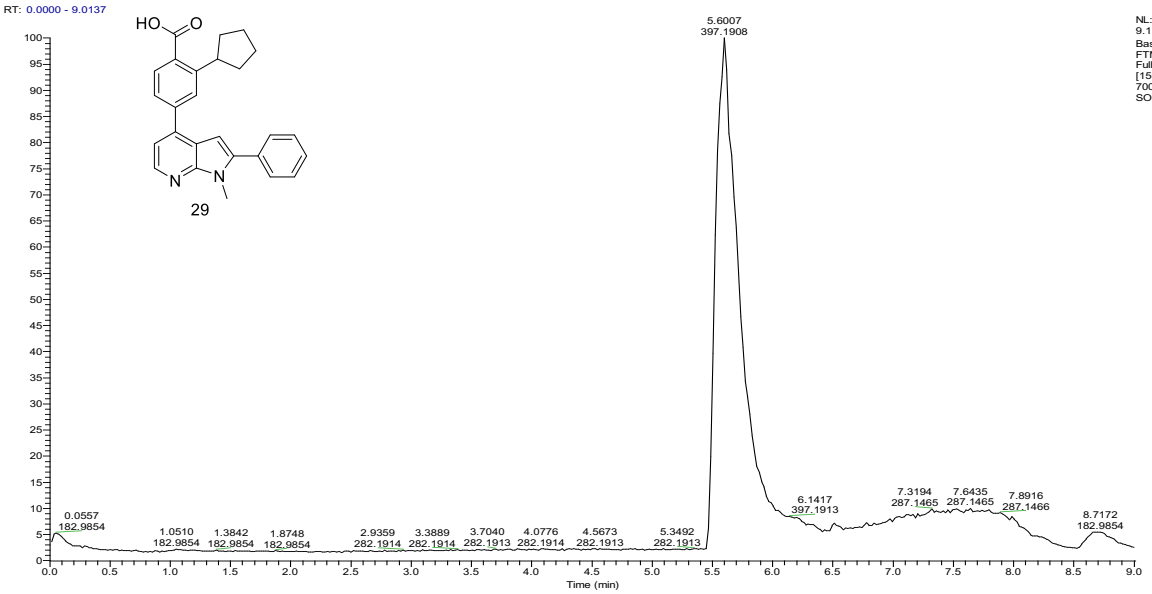


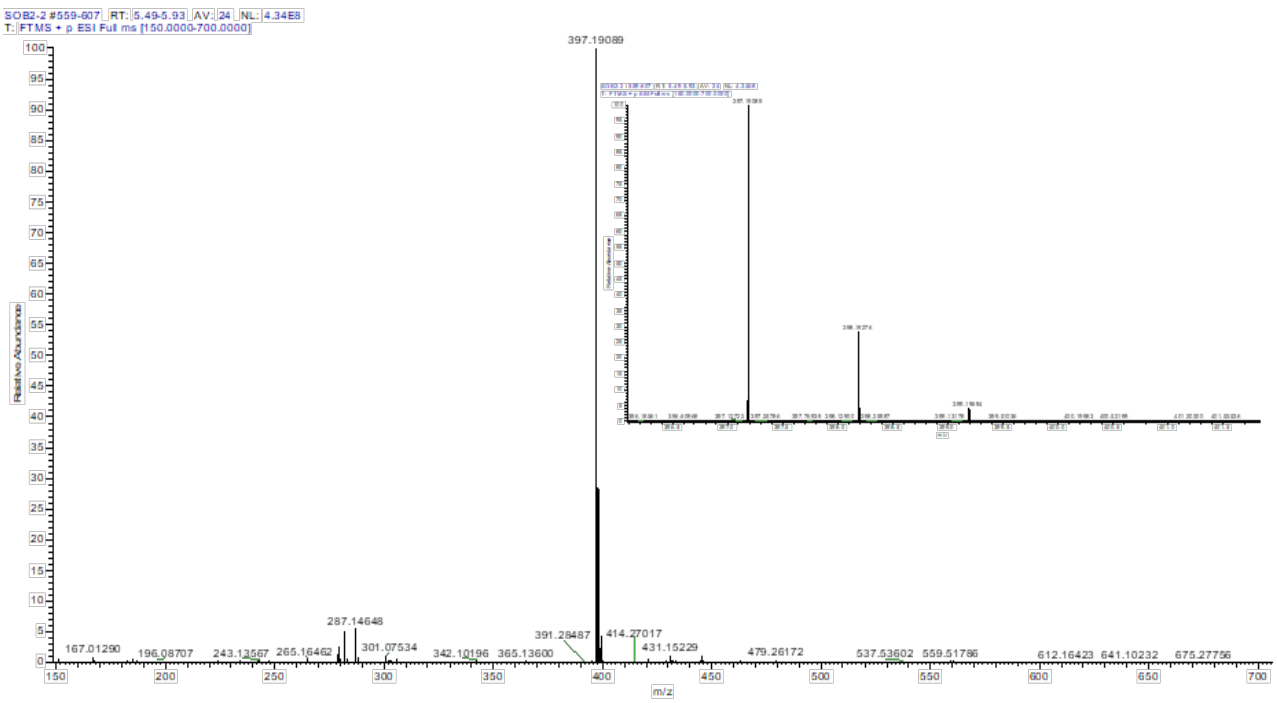


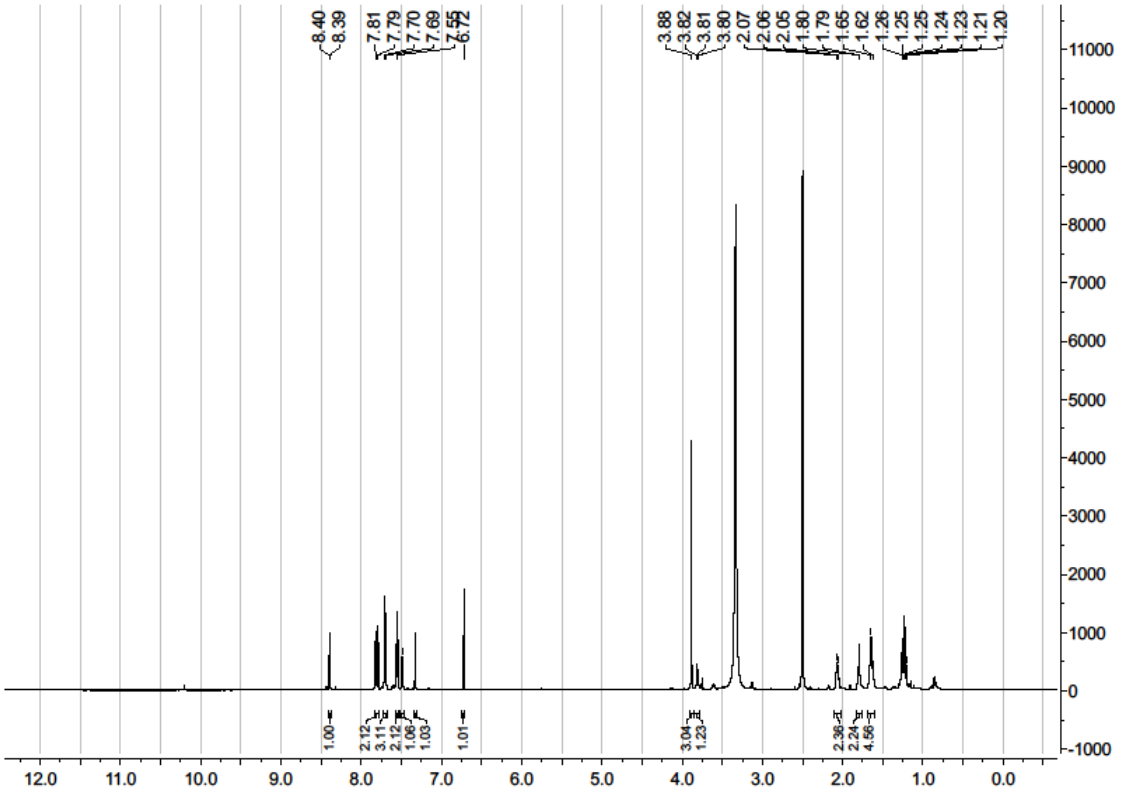

13C
100.50
cdcl3
149.60
142.63
142.45
135.29
131.67
129.11
128.66
128.62
119.92
116.16
97.90

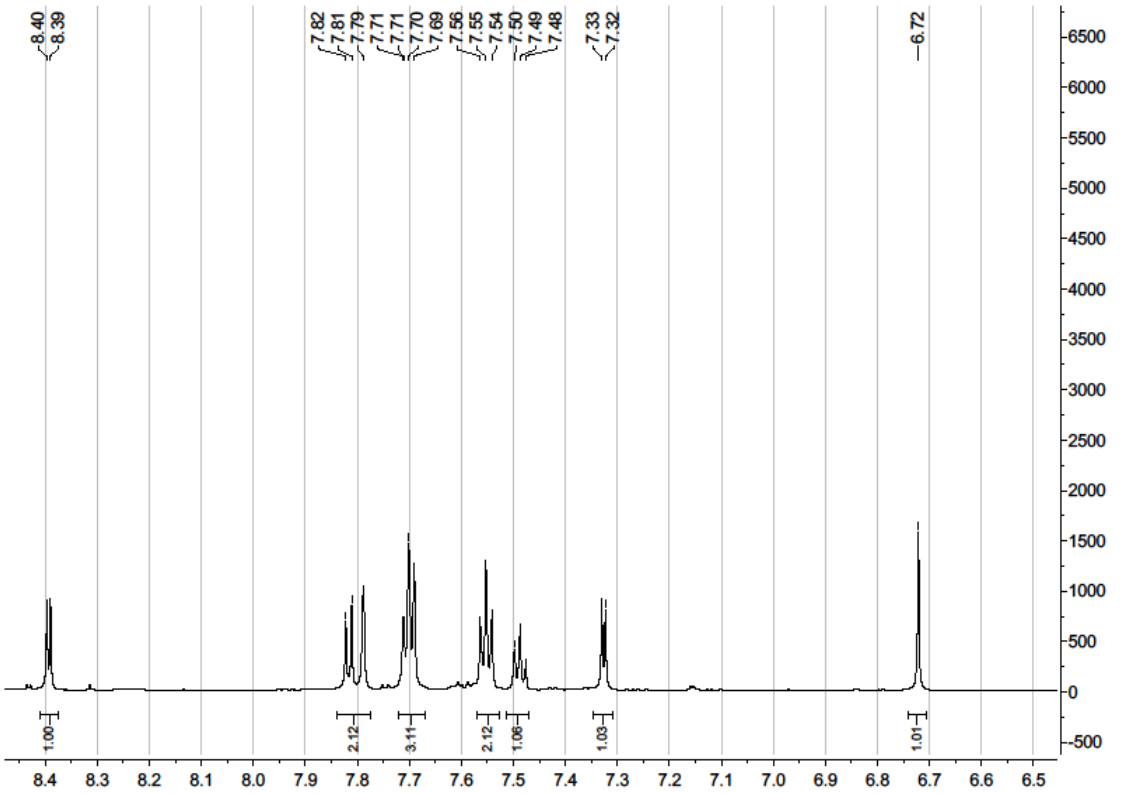

13C
100.50
cdcl3
149.60
142.63
142.45
135.29
131.67
129.11
128.66
128.62
119.92
116.16
97.90
30.31

2-Cyclopentyl-4-(1-methyl-2-phenyl-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)benzoic acid (**29**)






8.40
8.39
7.81
7.79
7.70
7.69
7.55
6.72
3.88
3.82
3.81
3.80
2.07
2.06
2.05
1.80
1.79
1.65
1.62
1.26
1.25
1.25
1.24
1.23
1.21
1.20
1.00
2.12
3.11
2.12
1.06
1.03
1.01
3.04
1.23
2.36
2.24
4.56
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0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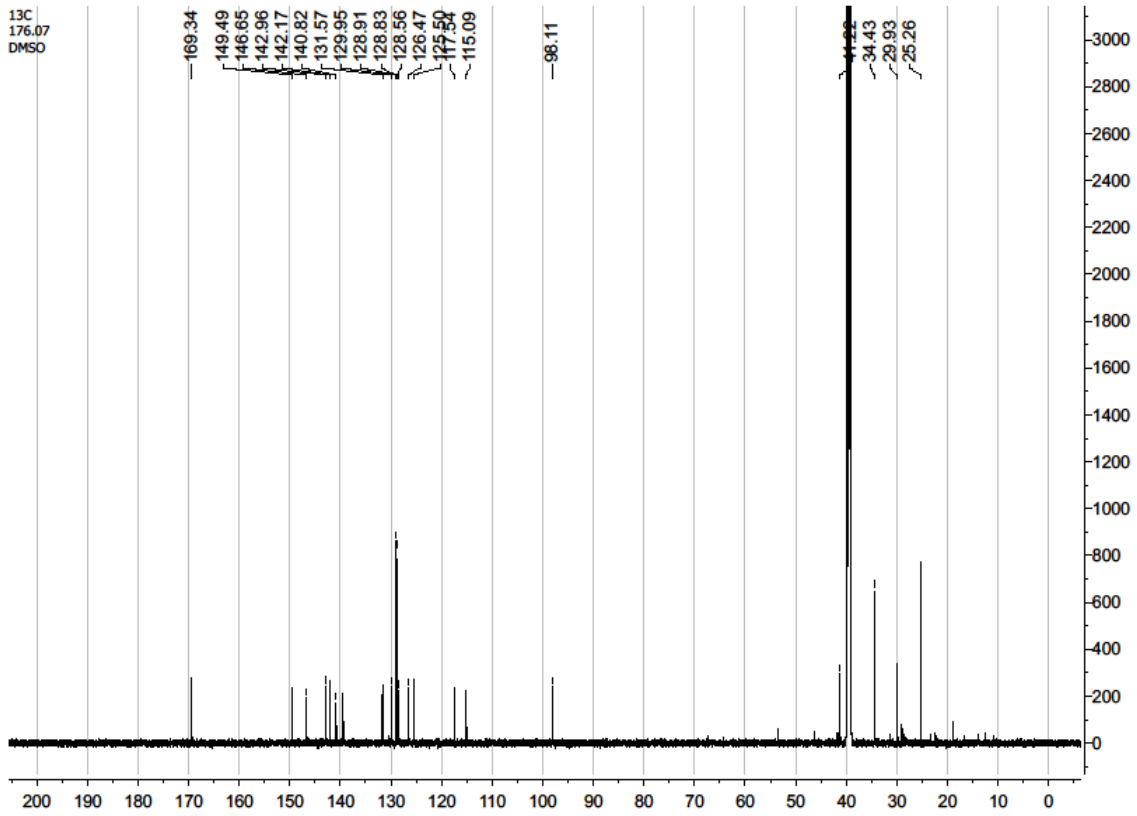
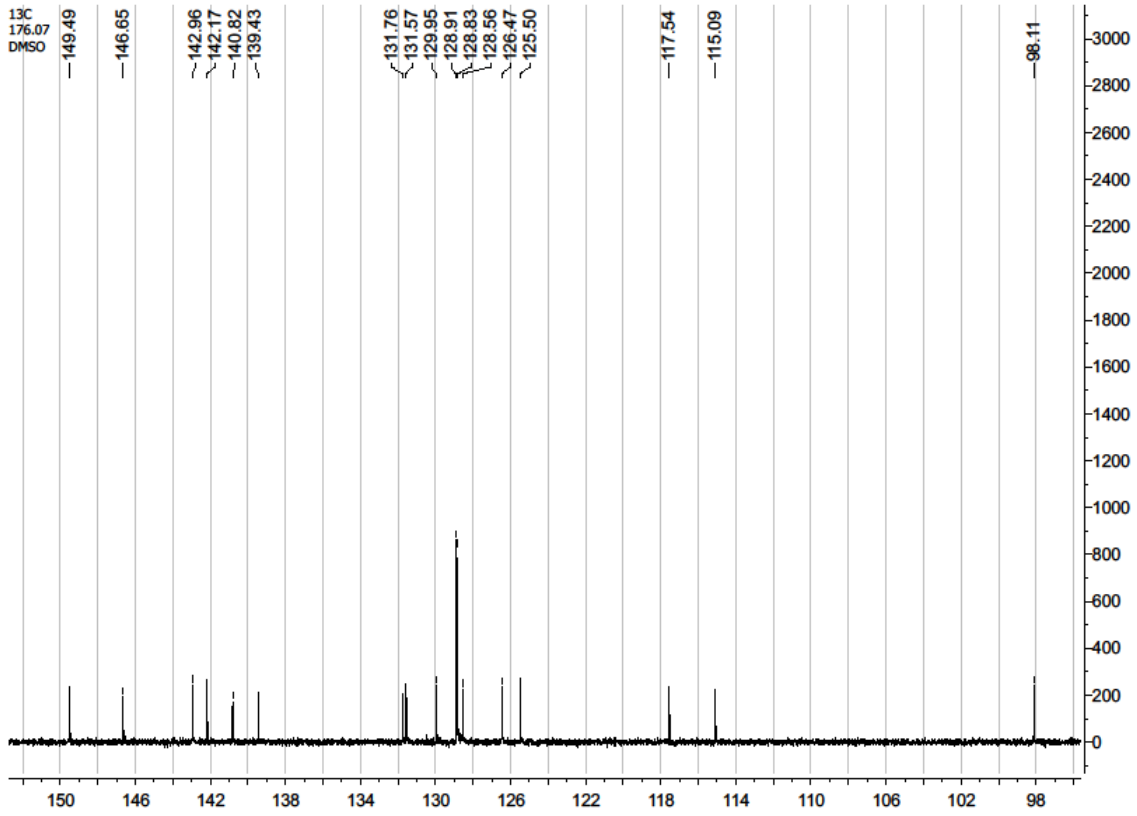
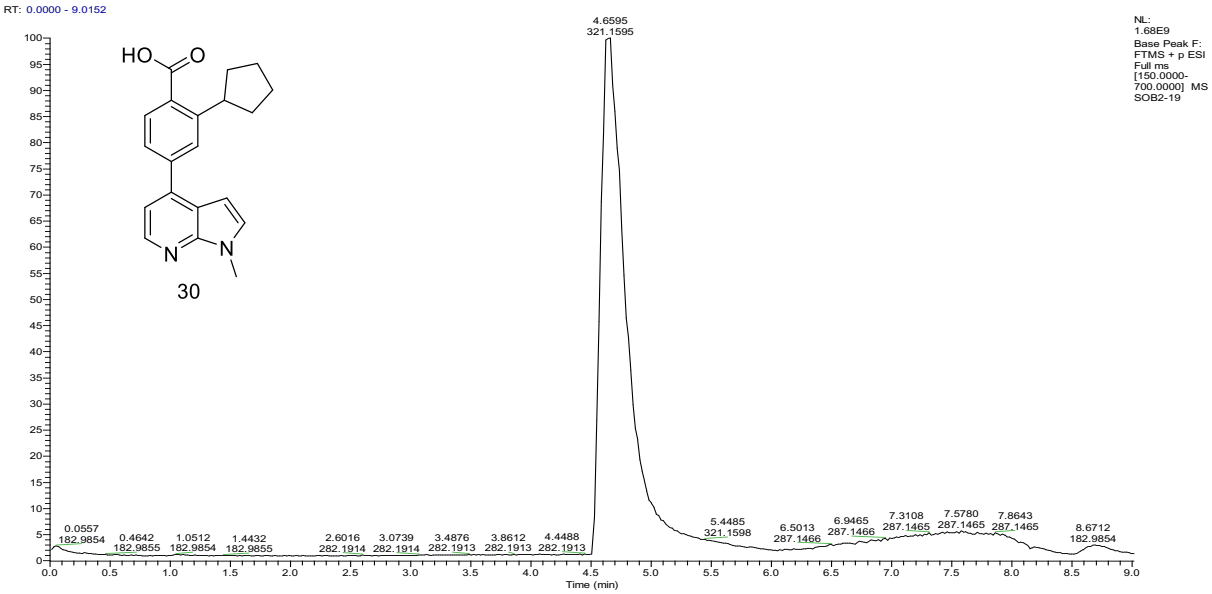
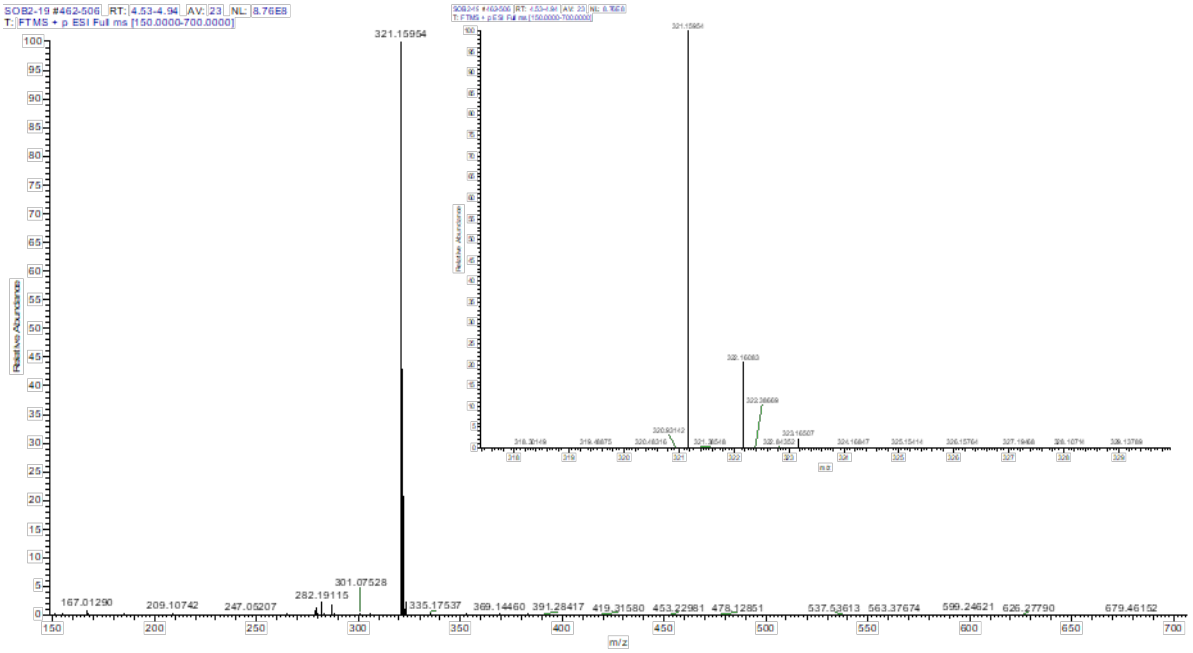
8.39
7.82
7.81
7.79
7.71
7.71
7.70
7.69
7.56
7.55
7.54
7.50
7.49
7.48
7.33
7.32
6.72
1.00
2.12
3.11
2.12
1.06
1.03
1.01
8.4
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
6.7
6.6
6.5

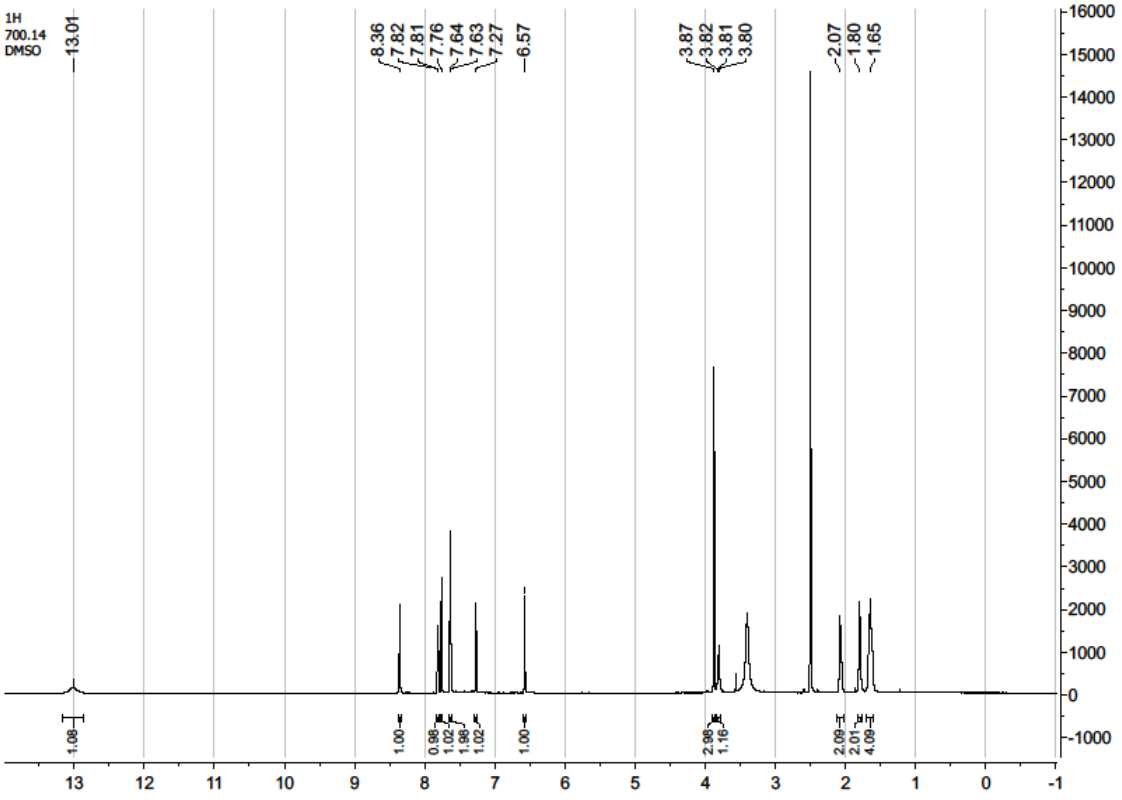

13C
176.07
DMSO
169.34
149.49
146.65
142.96
142.17
140.82
131.57
129.95
128.91
128.83
128.56
126.47
125.50
117.54
115.09
98.11
41.22
34.43
29.93
25.26

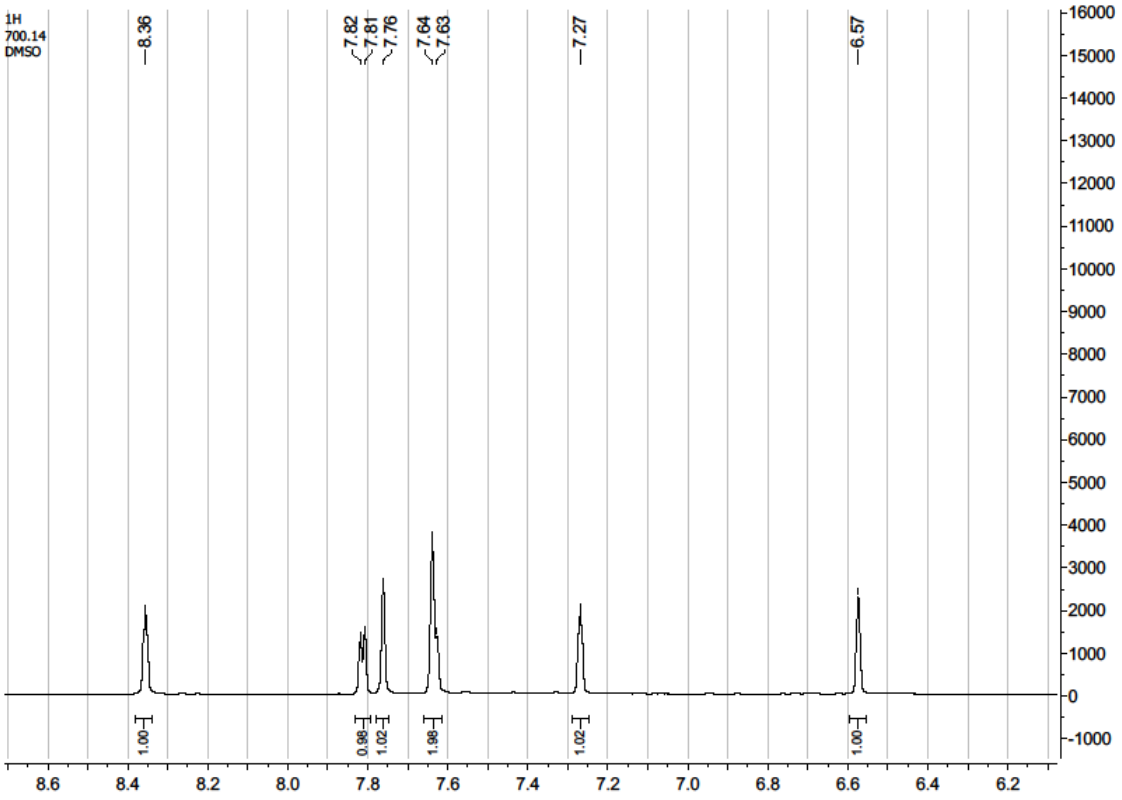

13C
176.07
DMSO
149.49
146.65
142.96
142.17
140.82
139.43
131.76
131.57
129.95
128.91
128.83
128.56
126.47
125.50
117.54
115.09
98.11

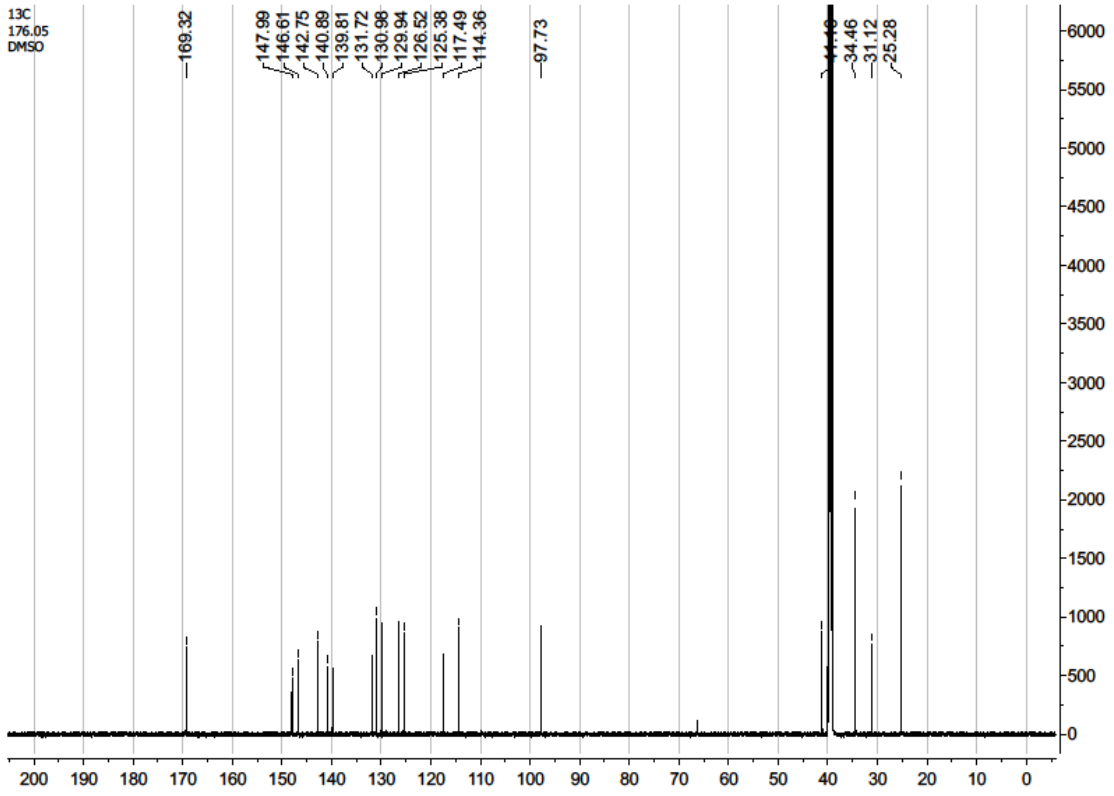
2-Cyclopentyl-4-(1-methyl-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)benzoic acid (30)

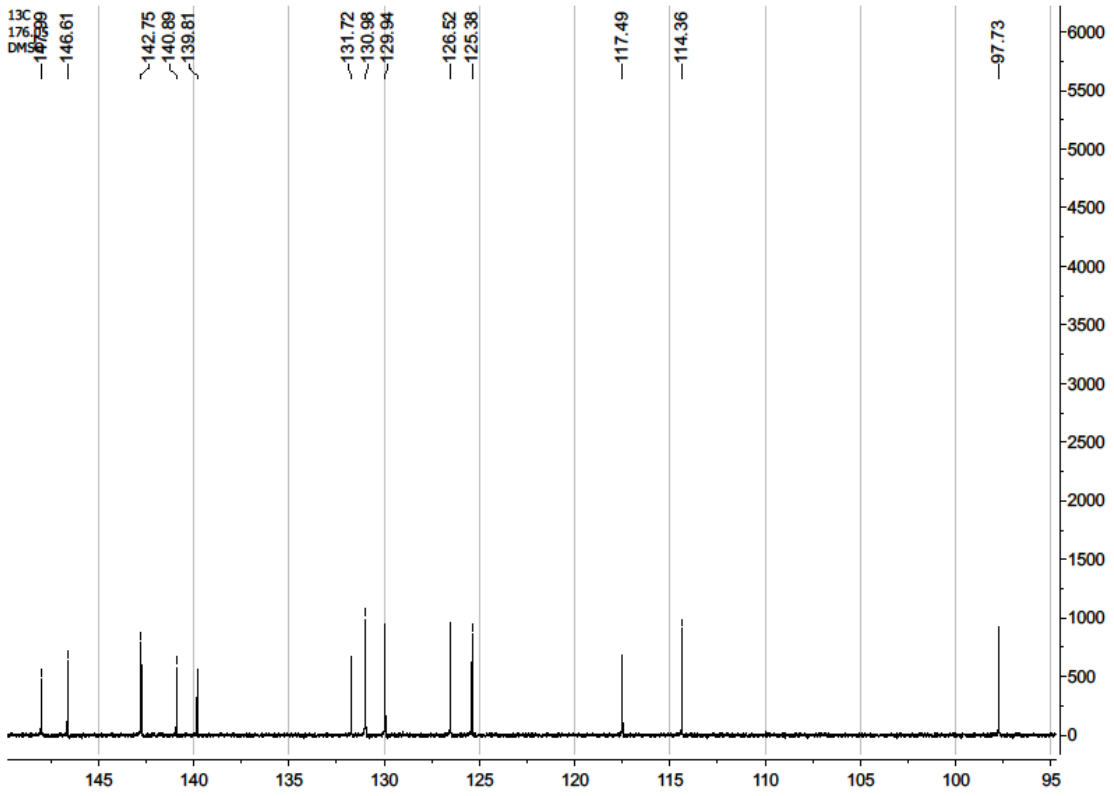




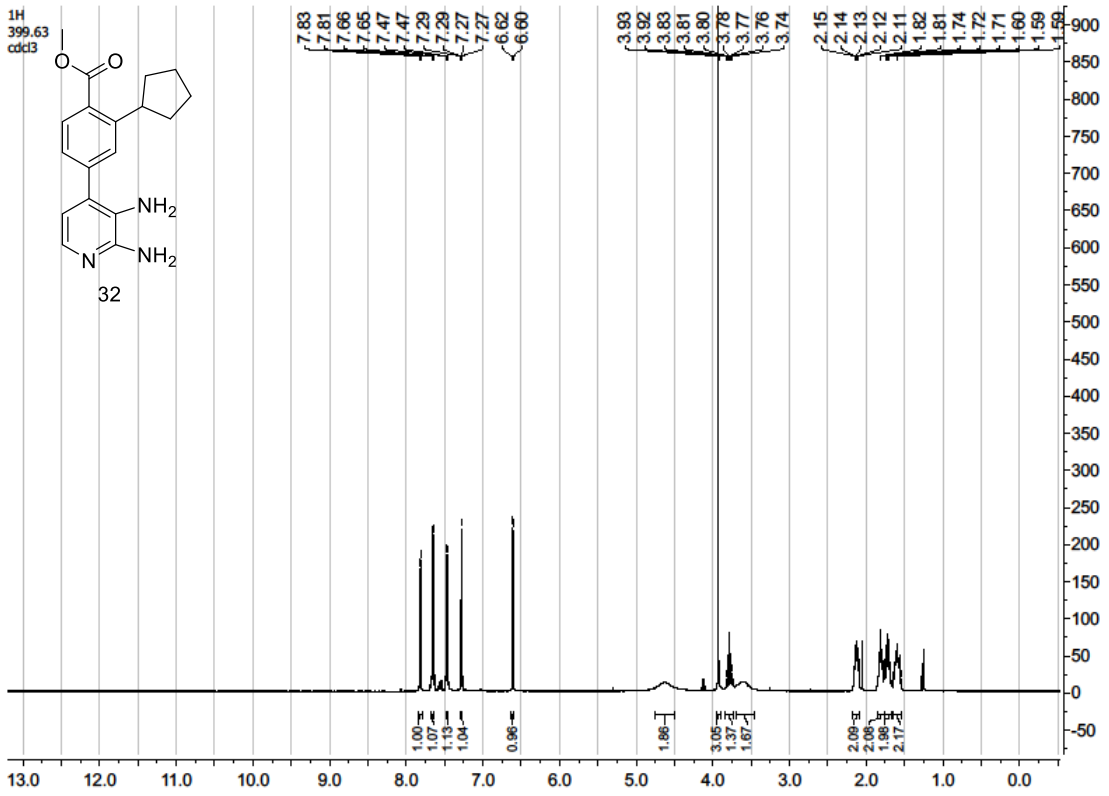

1H
700.14
DMSO
13.01
8.36
7.82
7.81
7.76
7.64
7.63
7.27
6.57
3.87
3.82
3.81
3.80
2.07
1.80
1.65
1.08
1.00
0.98
1.02
1.98
1.02
1.00
2.98
1.16
2.09
2.01
4.09

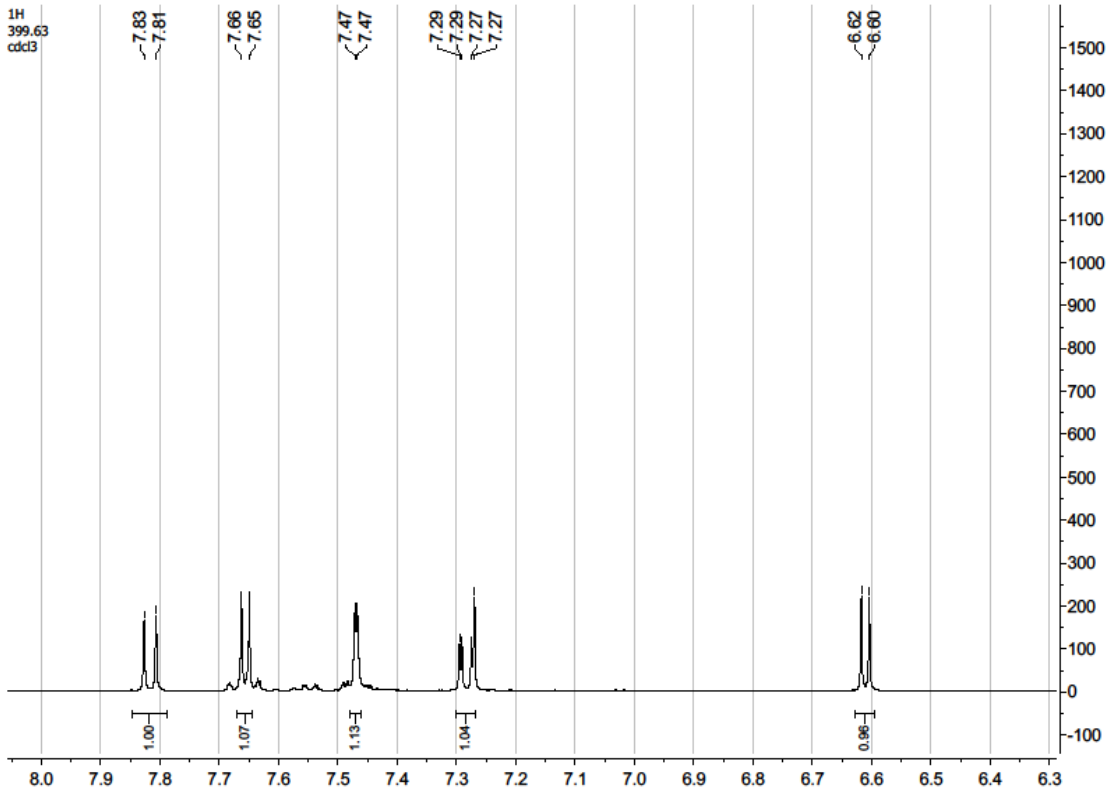

1H
700.14
DMSO
8.36
7.82
7.81
7.76
7.64
7.63
7.27
6.57
1.00
0.98
1.02
1.98
1.02
1.00

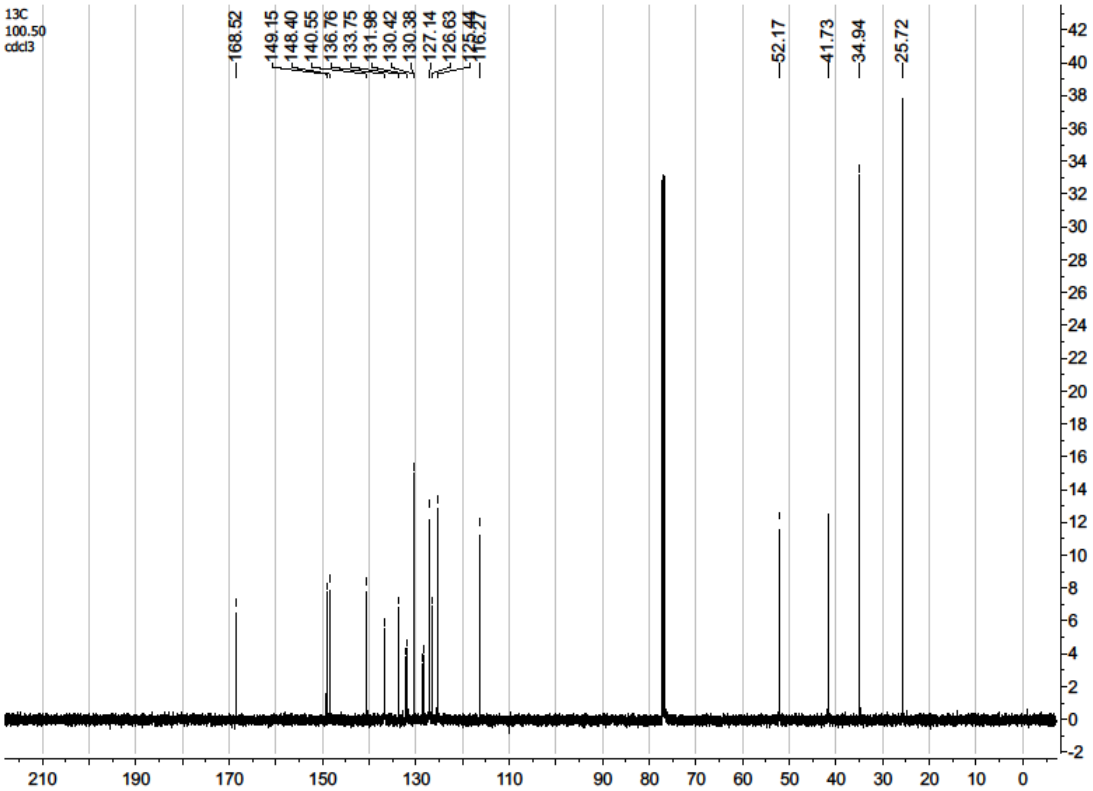

13C
176.05
DMSO
169.32
147.99
146.61
142.75
140.89
139.81
131.72
130.98
129.94
126.52
125.38
117.49
114.36
97.73
34.46
31.12
25.28

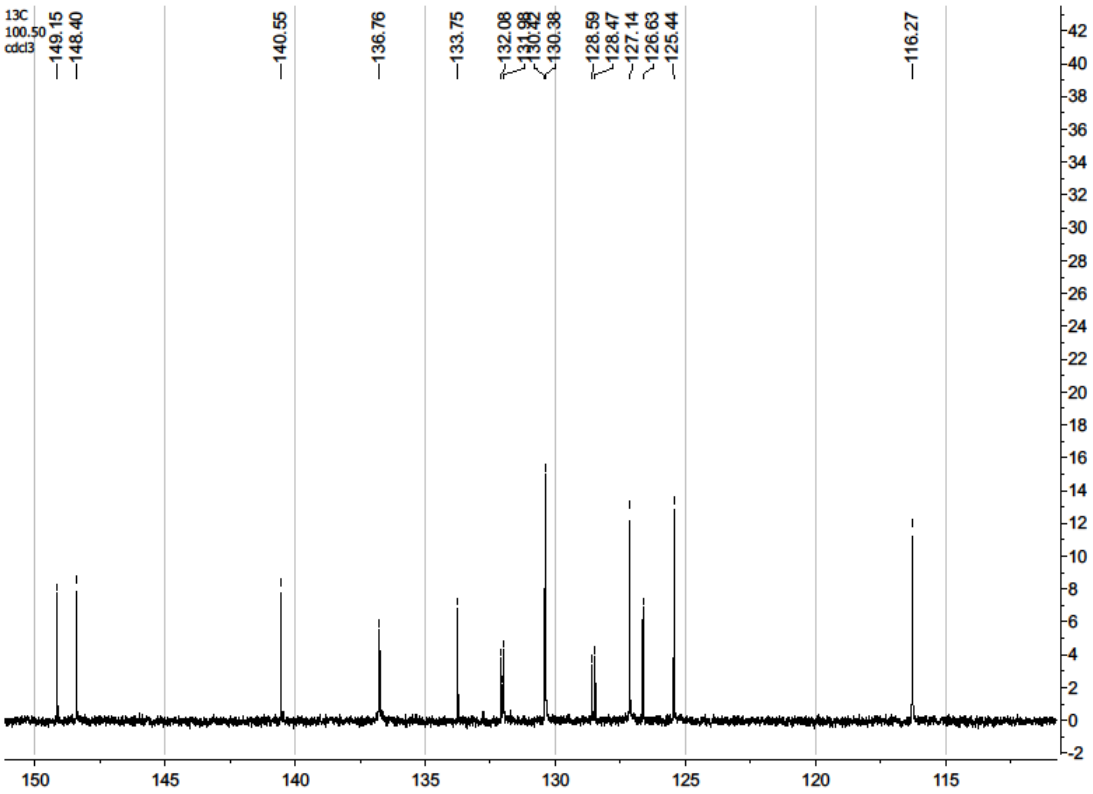

13C
DMSO
146.61
142.75
140.89
139.81
131.72
130.98
129.94
126.52
125.38
117.49
114.36
97.73

Methyl 2-cyclopentyl-4-(2,3-diaminopyridin-4-yl)benzoate (32)

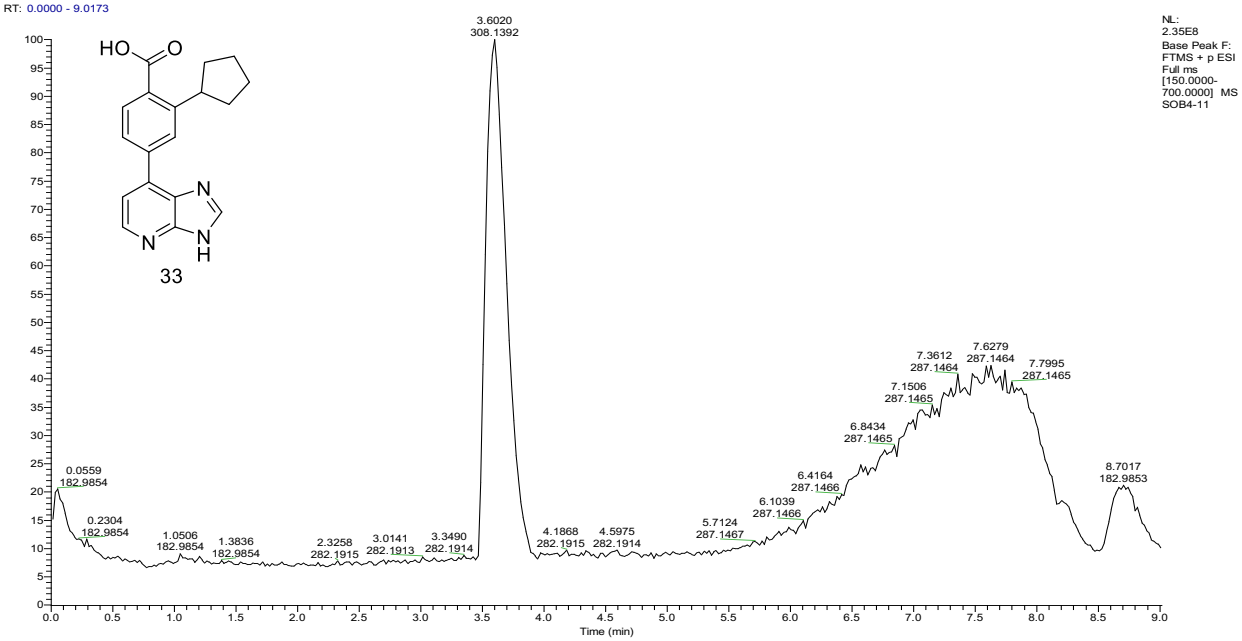


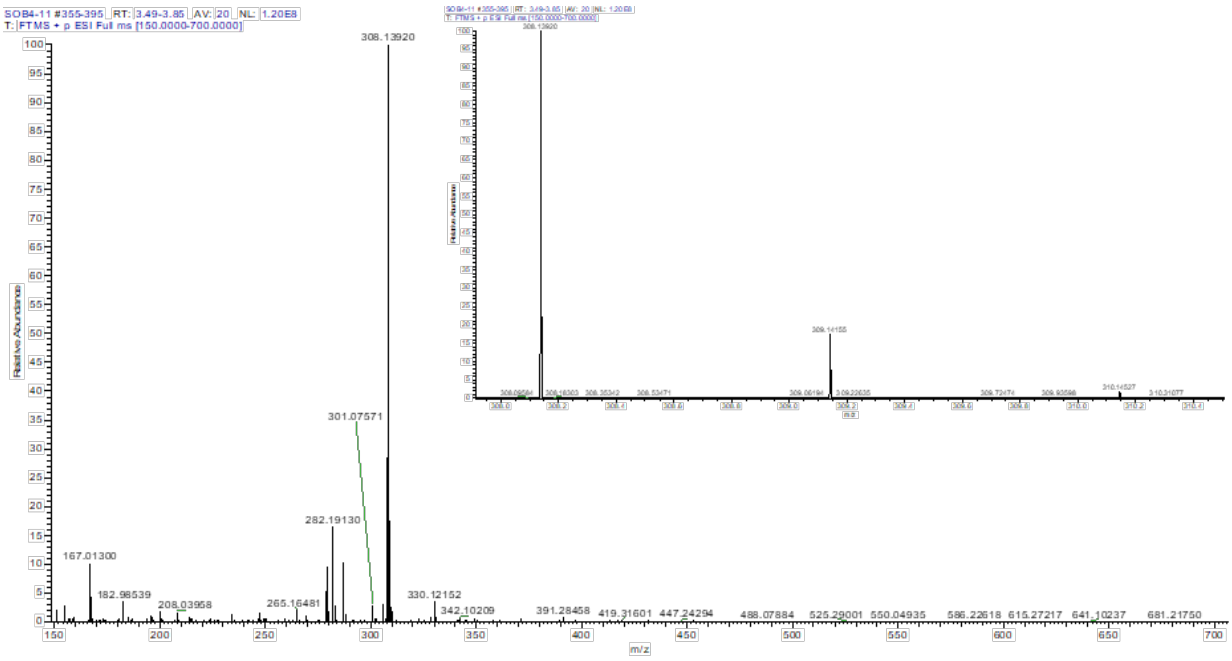


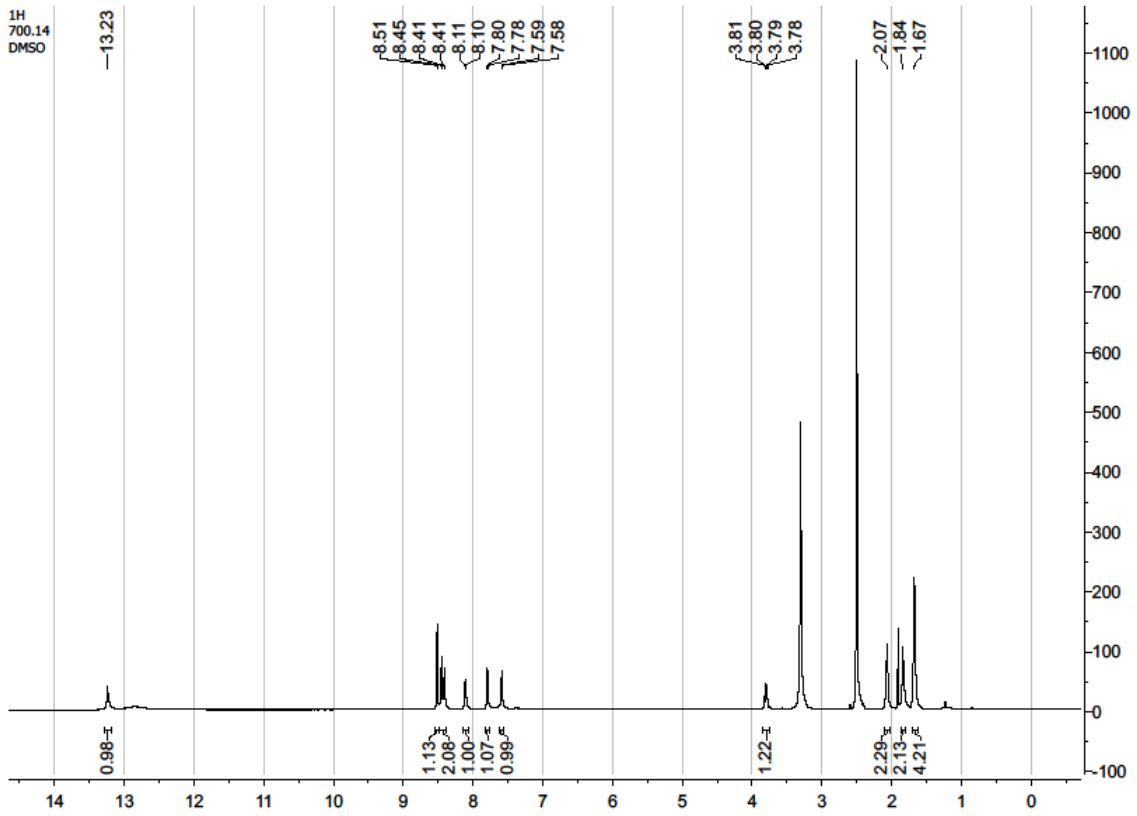

13C
100.50
cdcl3
168.52
149.15
148.40
140.55
136.76
133.75
131.98
130.42
130.38
127.14
126.63
125.44
116.27
52.17
41.73
34.94
25.72

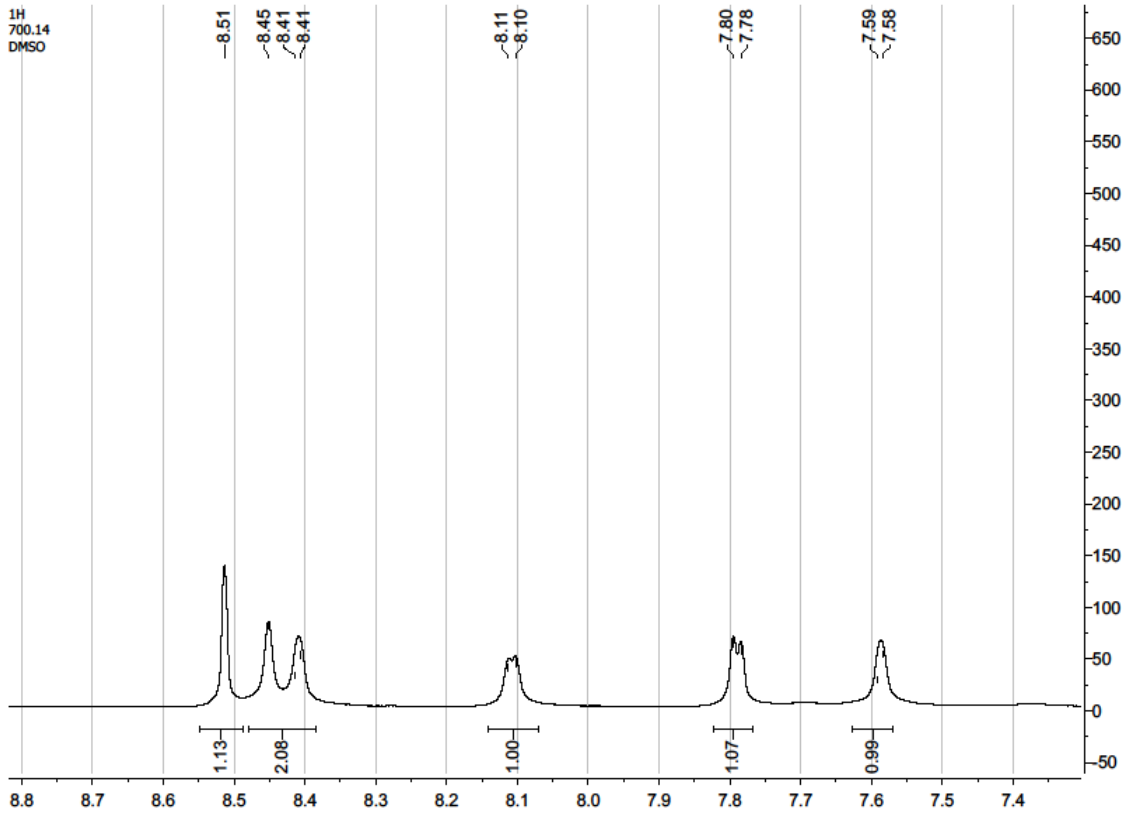

13C
100.50
cdcl3
149.15
148.40
140.55
136.76
133.75
132.08
131.98
130.42
130.38
128.59
128.47
127.14
126.63
125.44
116.27

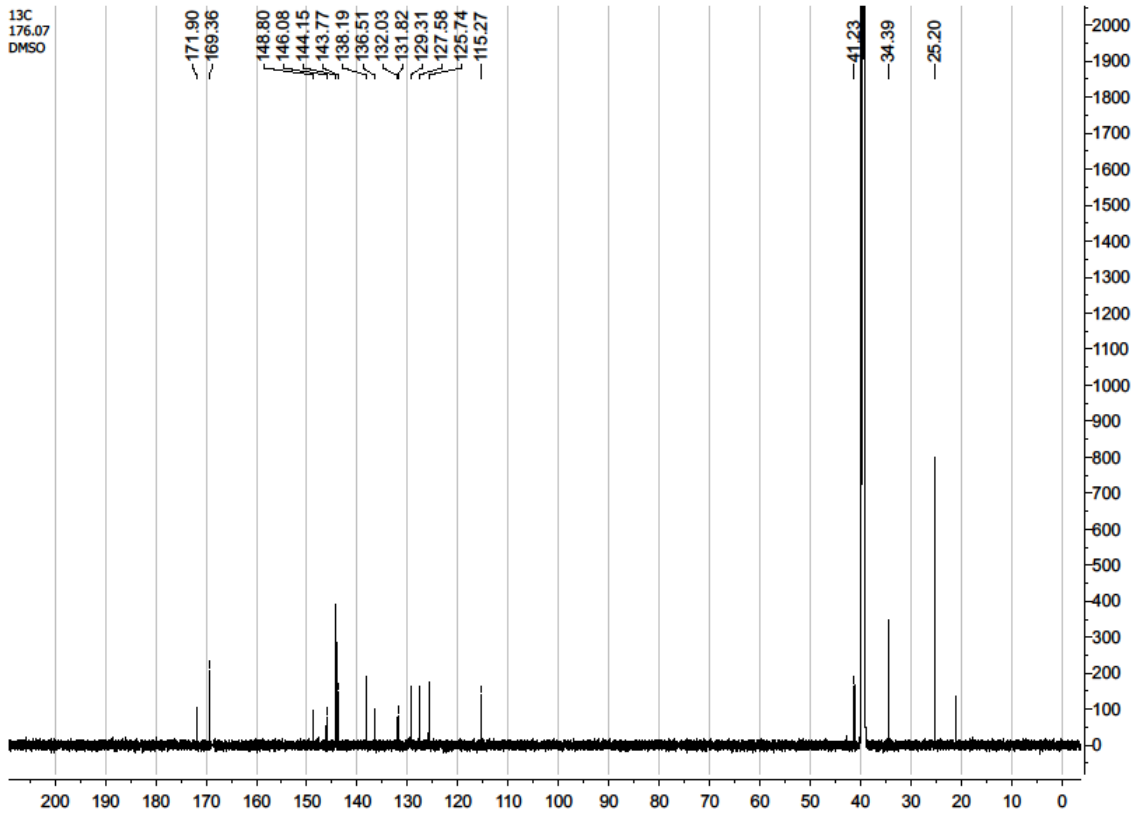
2-Cyclopentyl-4-(3H-imidazo[4,5-*b*]pyridin-7-yl)benzoic acid (**33**)

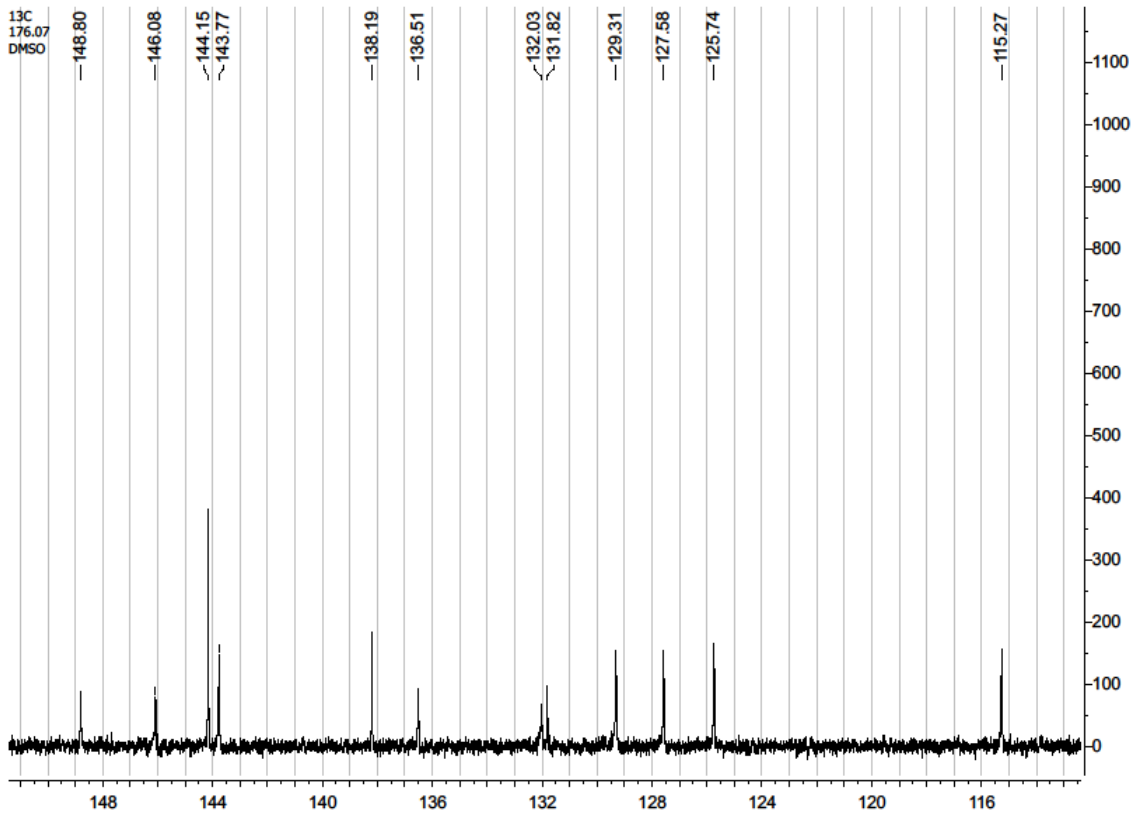




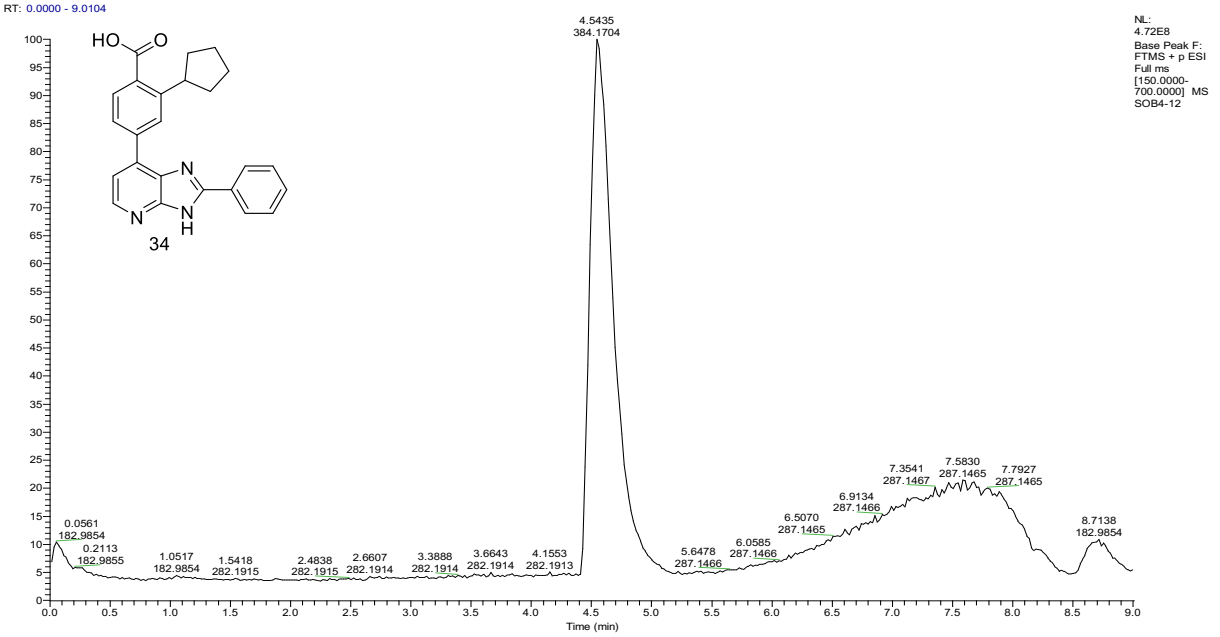

1H
700.14
DMSO
13.23
8.51
8.45
8.41
8.41
8.11
8.10
7.80
7.78
7.59
7.58
3.81
3.80
3.79
3.78
2.07
1.84
1.67
0.98
1.13
2.08
1.00
1.07
0.99
1.22
2.29
2.13
4.21

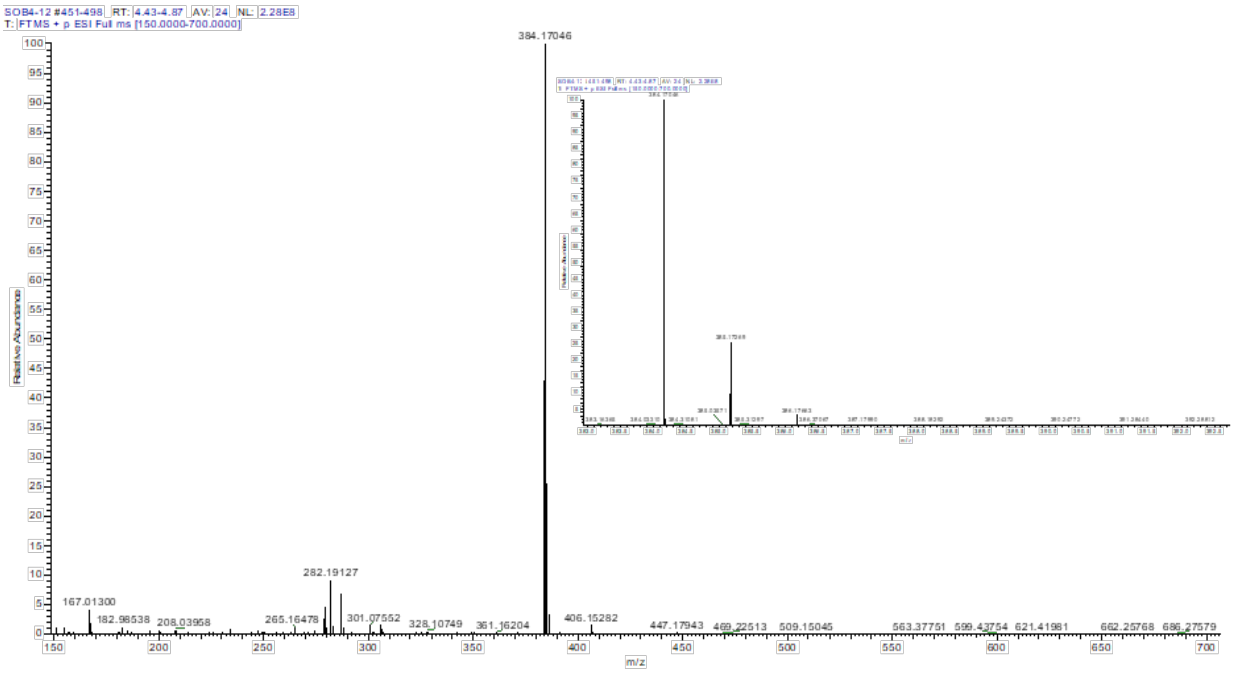

1H
700.14
DMSO
8.51
8.45
8.41
8.41
8.11
8.10
7.80
7.78
7.59
7.58
1.13
2.08
1.00
1.07
0.99

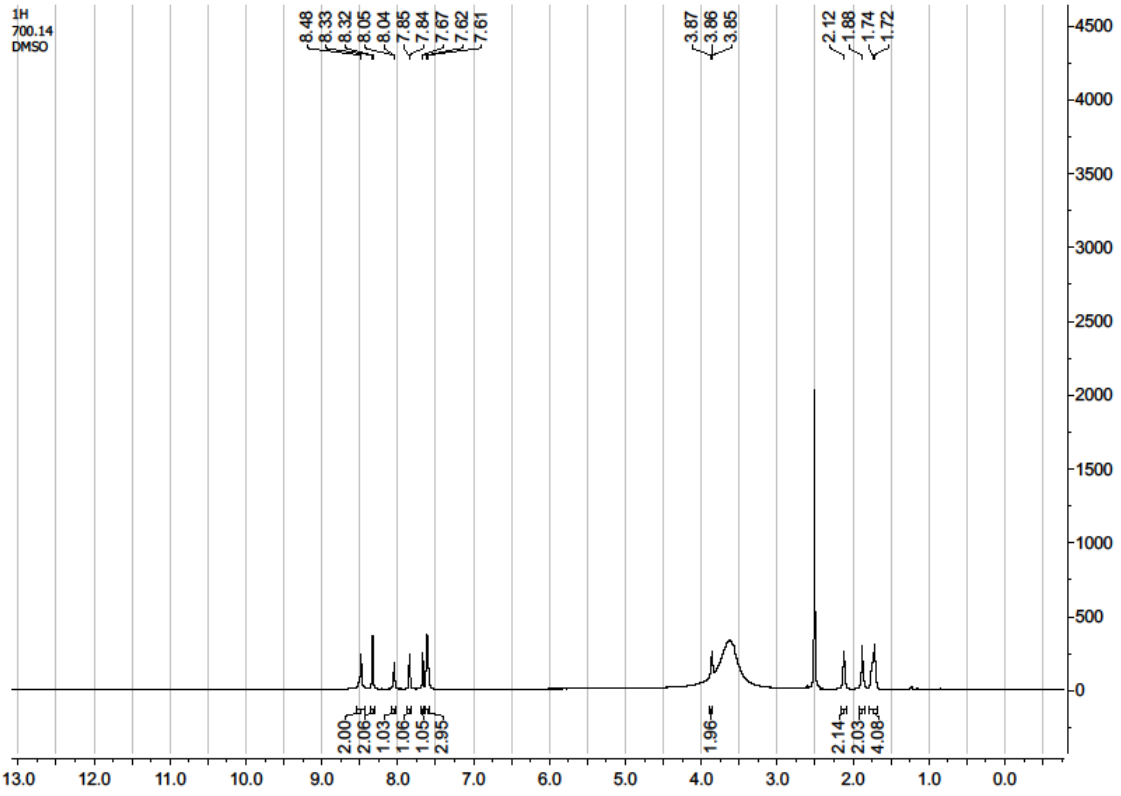

13C
176.07
DMSO
171.90
169.36
148.80
146.08
144.15
143.77
138.19
136.51
132.03
131.82
129.31
127.58
125.74
115.27
41.23
34.39
25.20

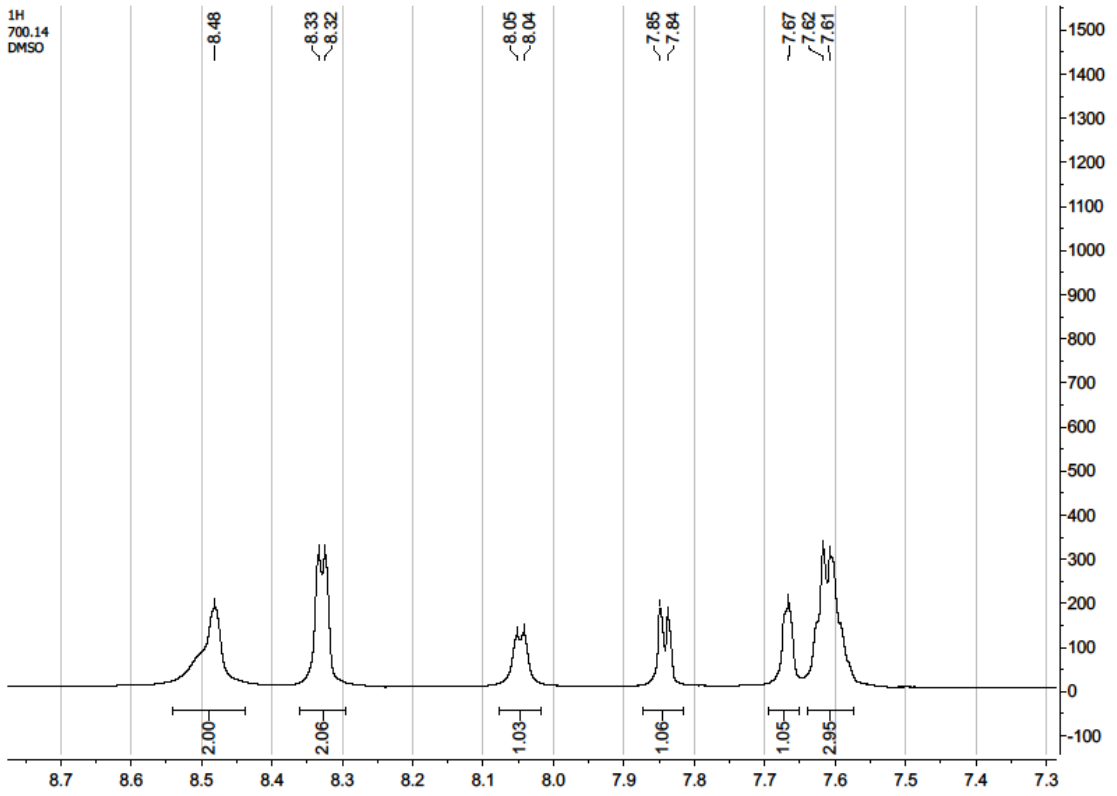

13C
176.07
DMSO
148.80
146.08
144.15
143.77
138.19
136.51
132.03
131.82
129.31
127.58
125.74
115.27

2-Cyclopentyl-4-(2-phenyl-3H-imidazo[4,5-*b*]pyridin-7-yl)benzoic acid (34)

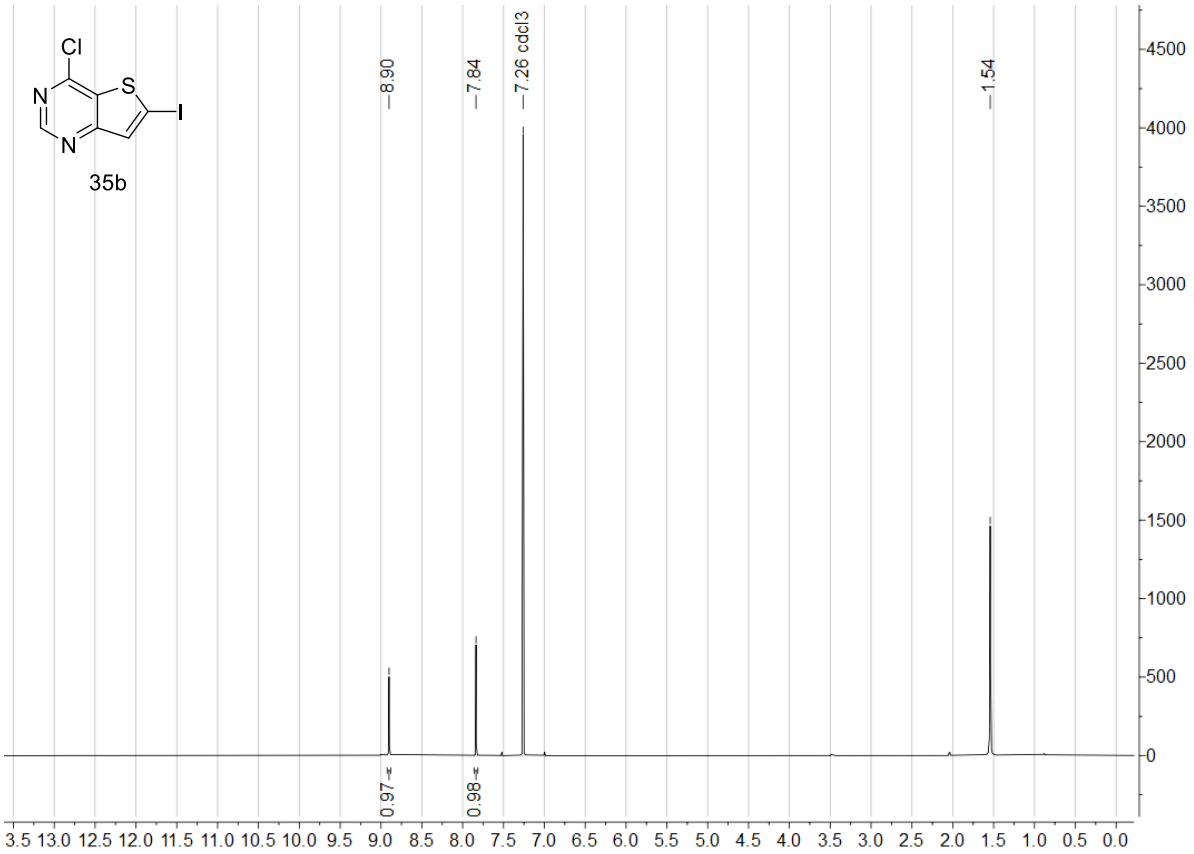


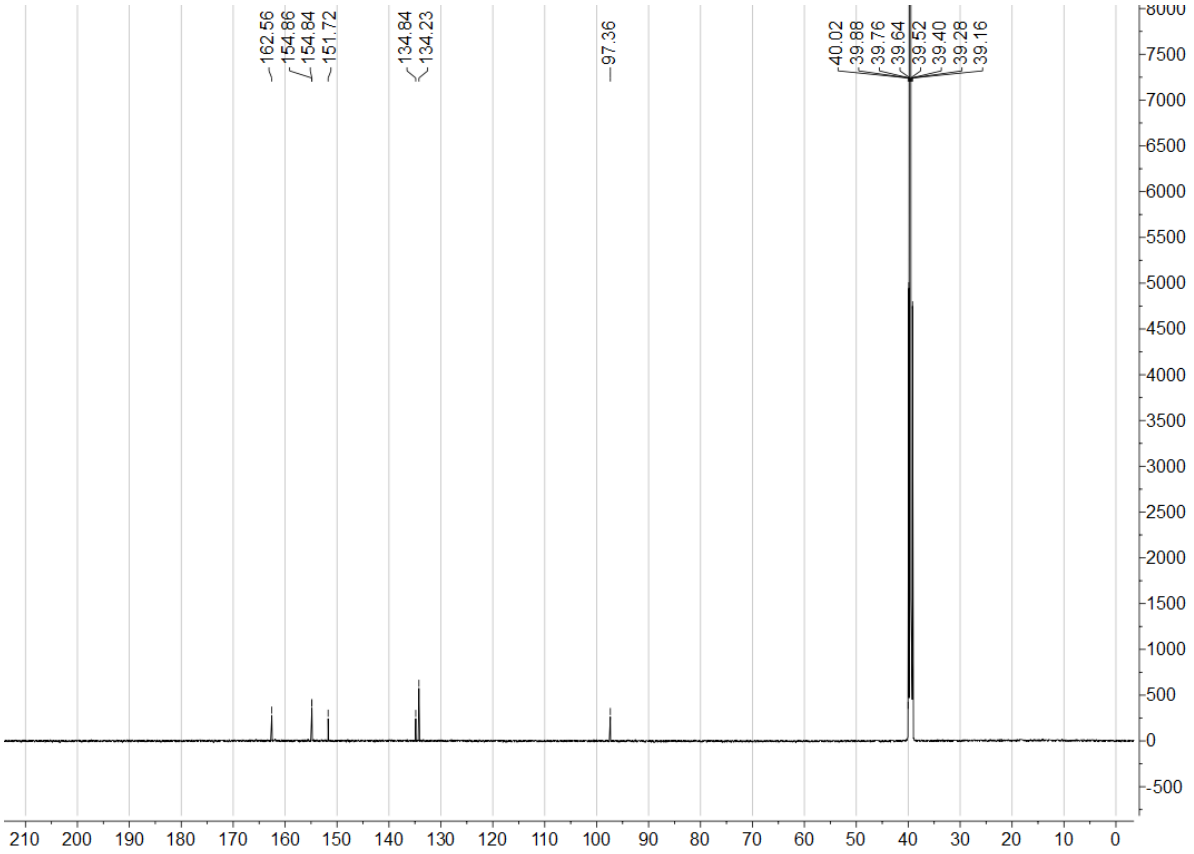



1H
700.14
DMSO
8.48
8.33
8.32
8.05
8.04
7.85
7.84
7.67
7.62
7.61
3.87
3.86
3.85
2.12
1.88
1.74
1.72
2.00
2.06
1.03
1.06
1.05
2.95
1.96
2.14
2.03
4.08

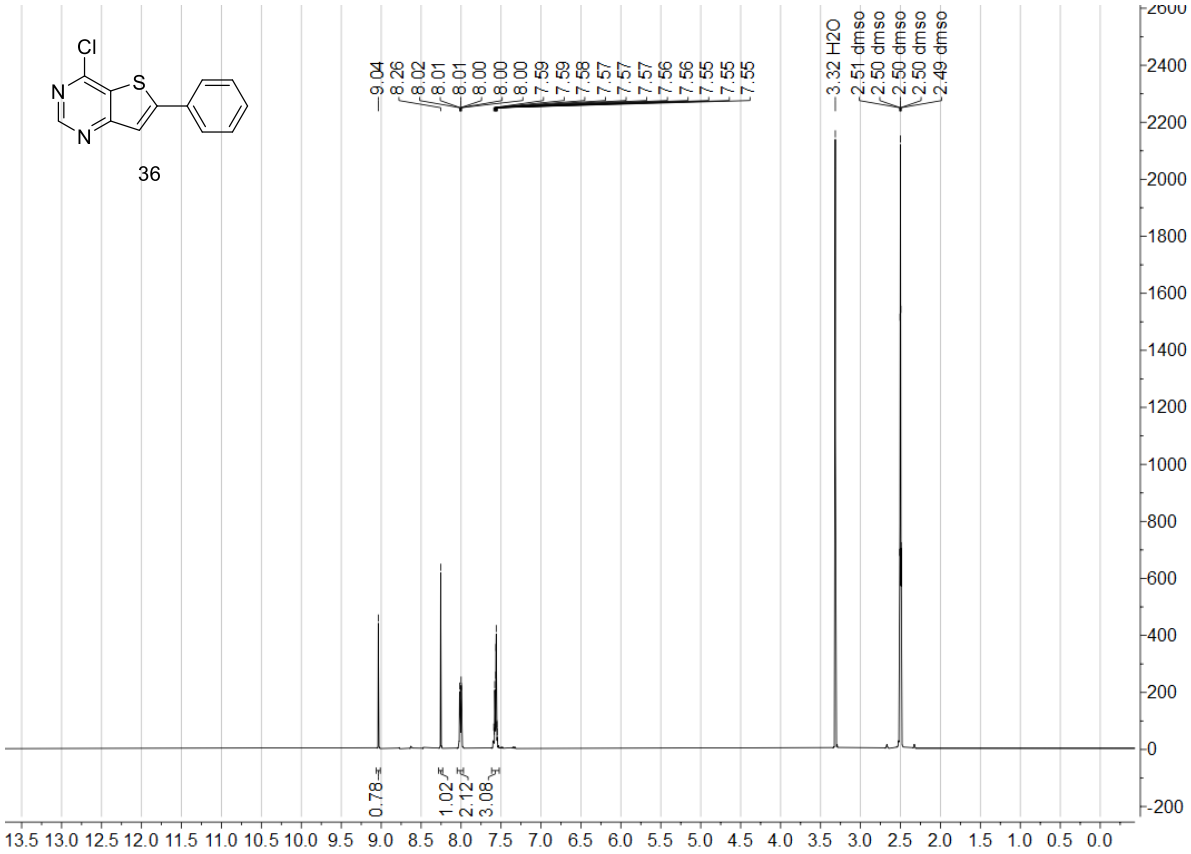

1H
700.14
DMSO
8.48
8.33
8.32
8.05
8.04
7.85
7.84
7.67
7.62
7.61
2.00
2.06
1.03
1.06
1.05
2.95

4-Chloro-6-iodothieno[3,2-d]pyrimidine (35b)

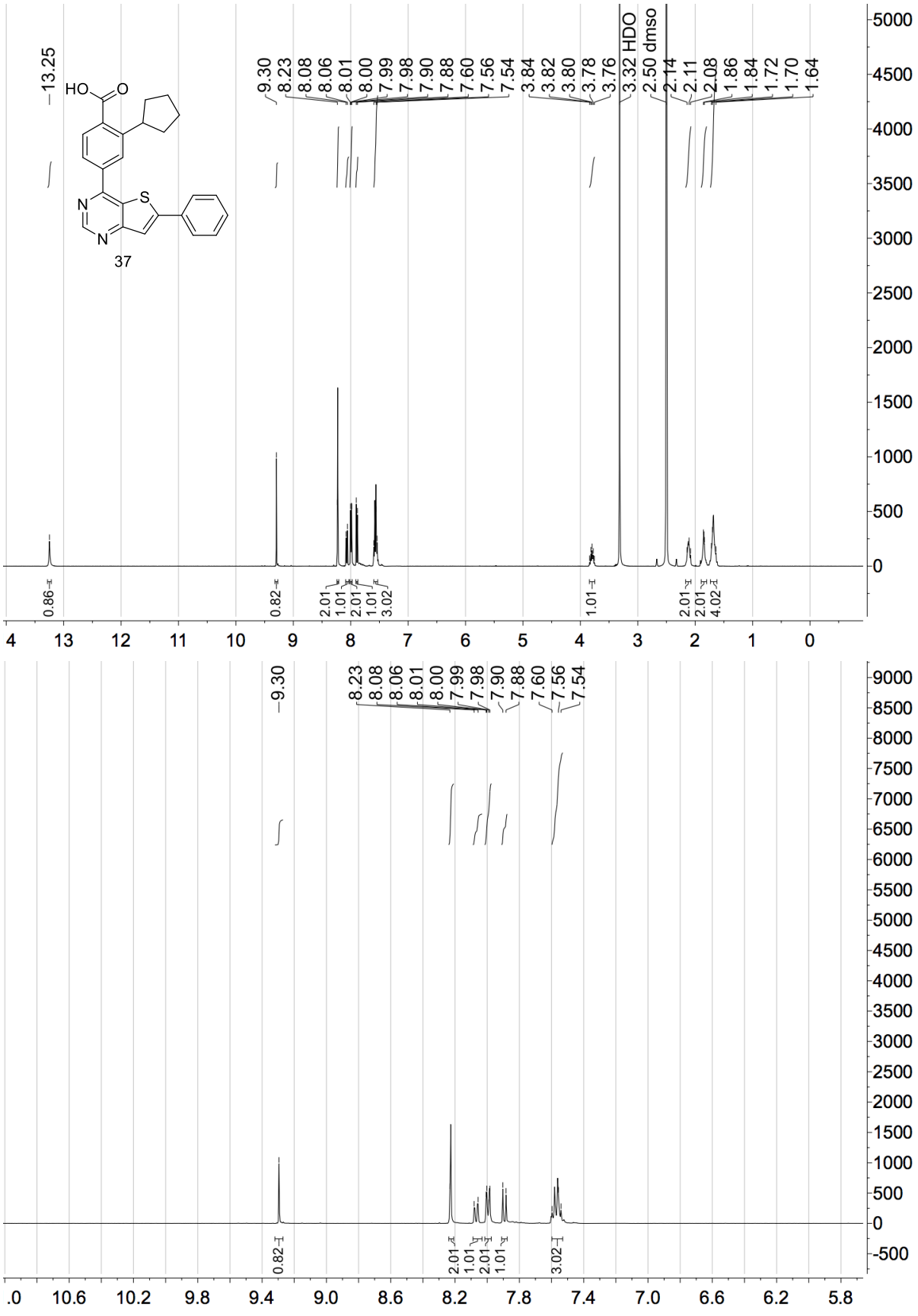


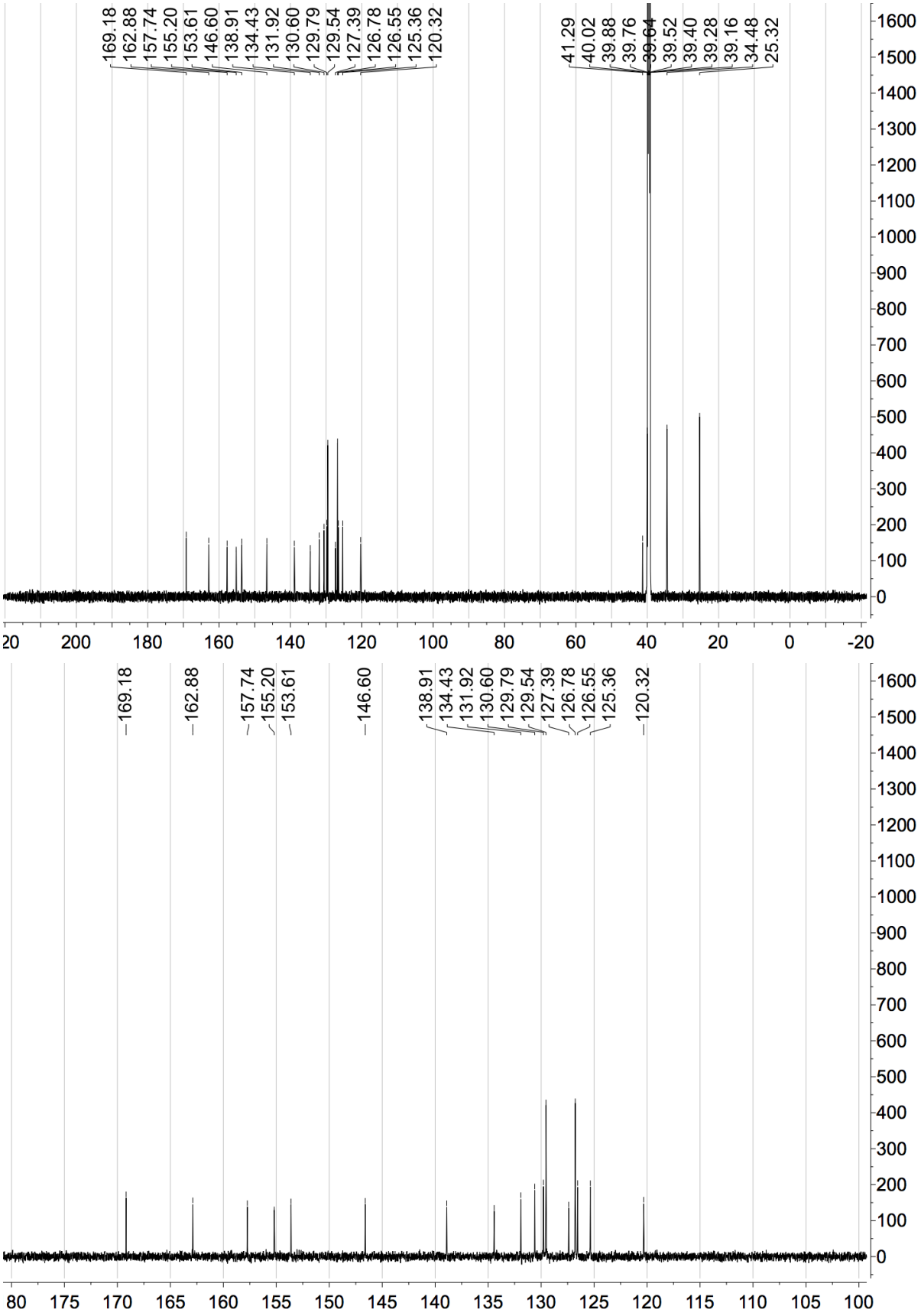


4-Chloro-6-phenylthieno[3,2-*d*]pyrimidine (36)

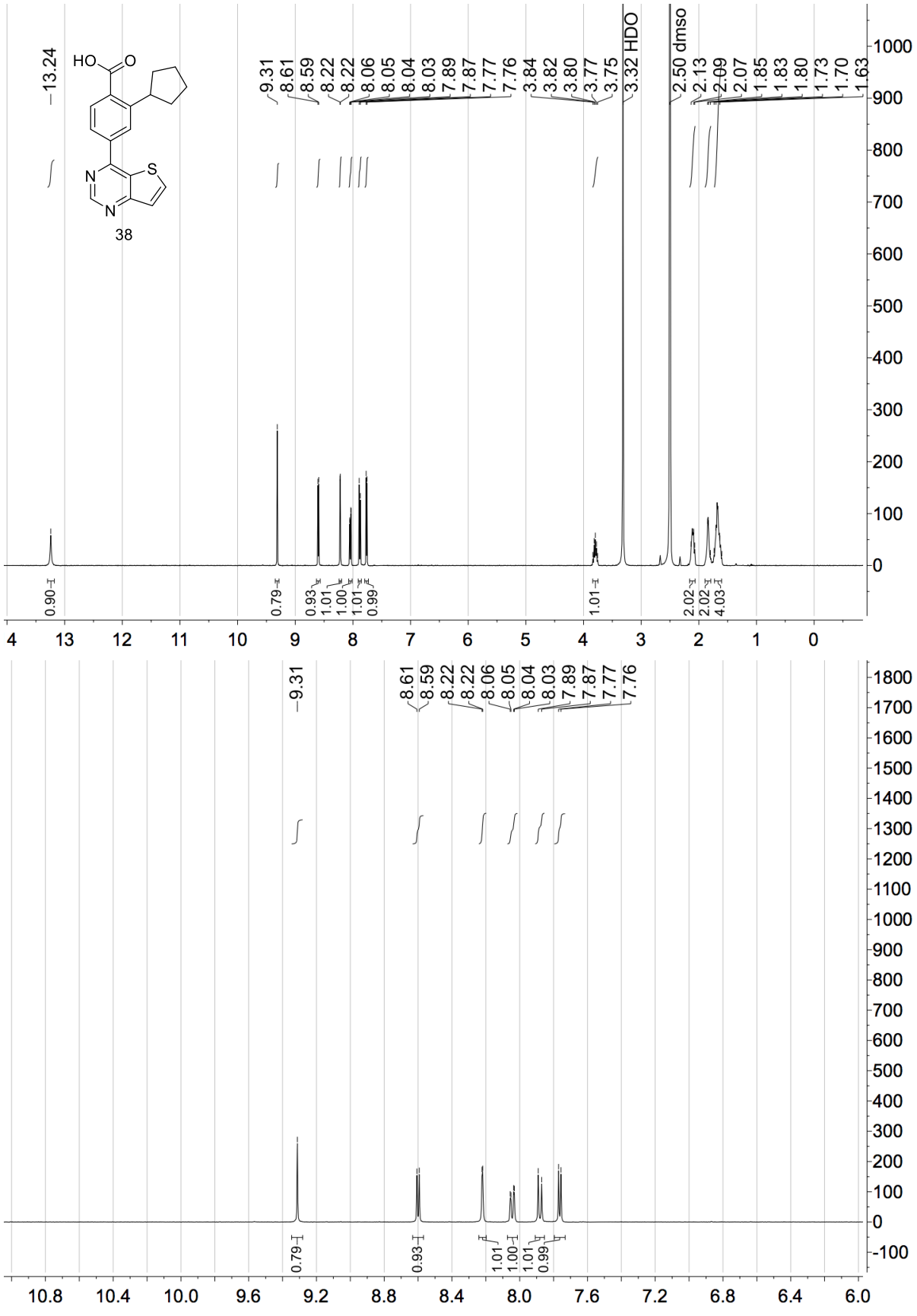


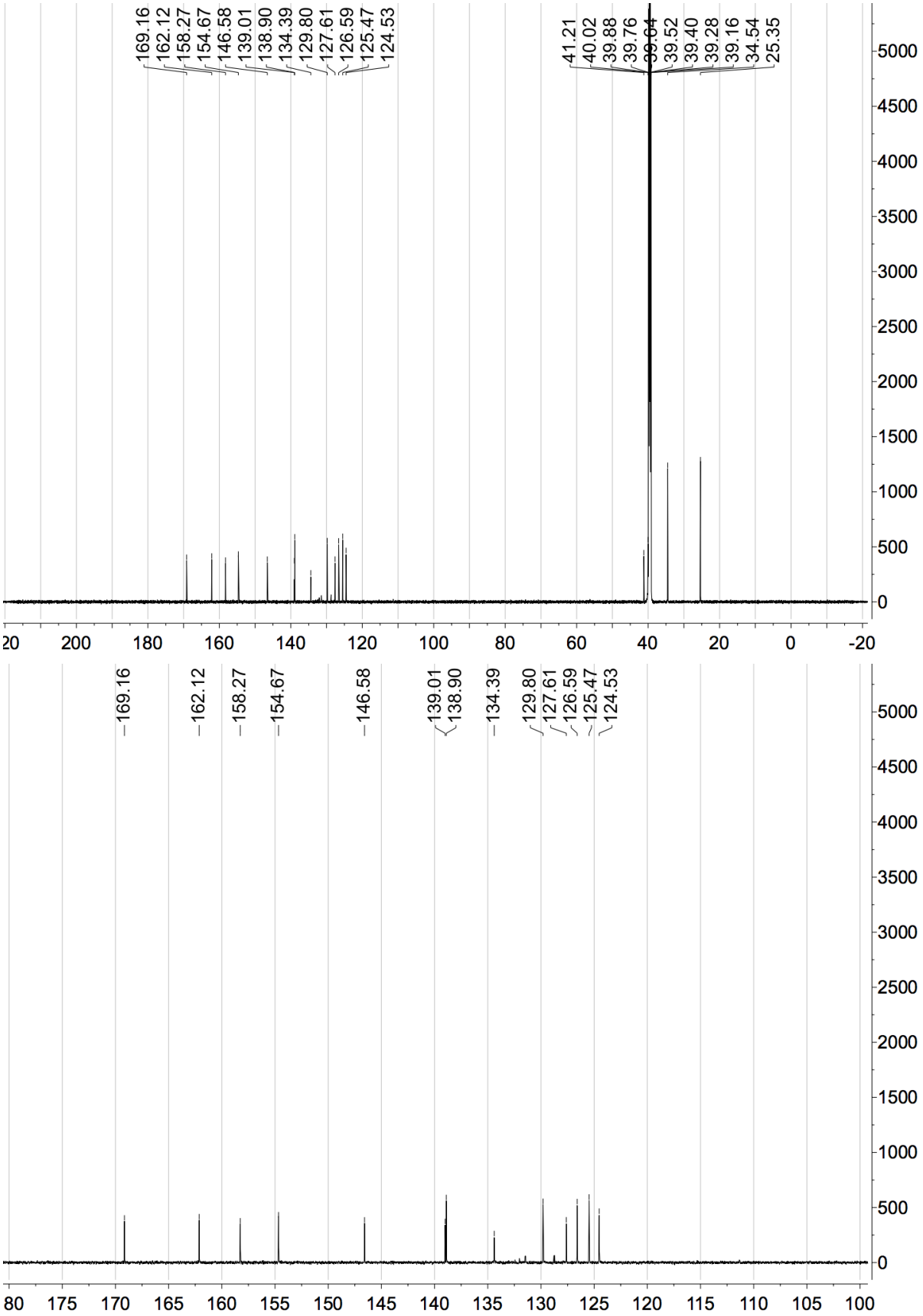
2-Cyclopentyl-4-(6-phenylthieno[3,2-*d*]pyrimidin-4-yl)benzoic acid (37)



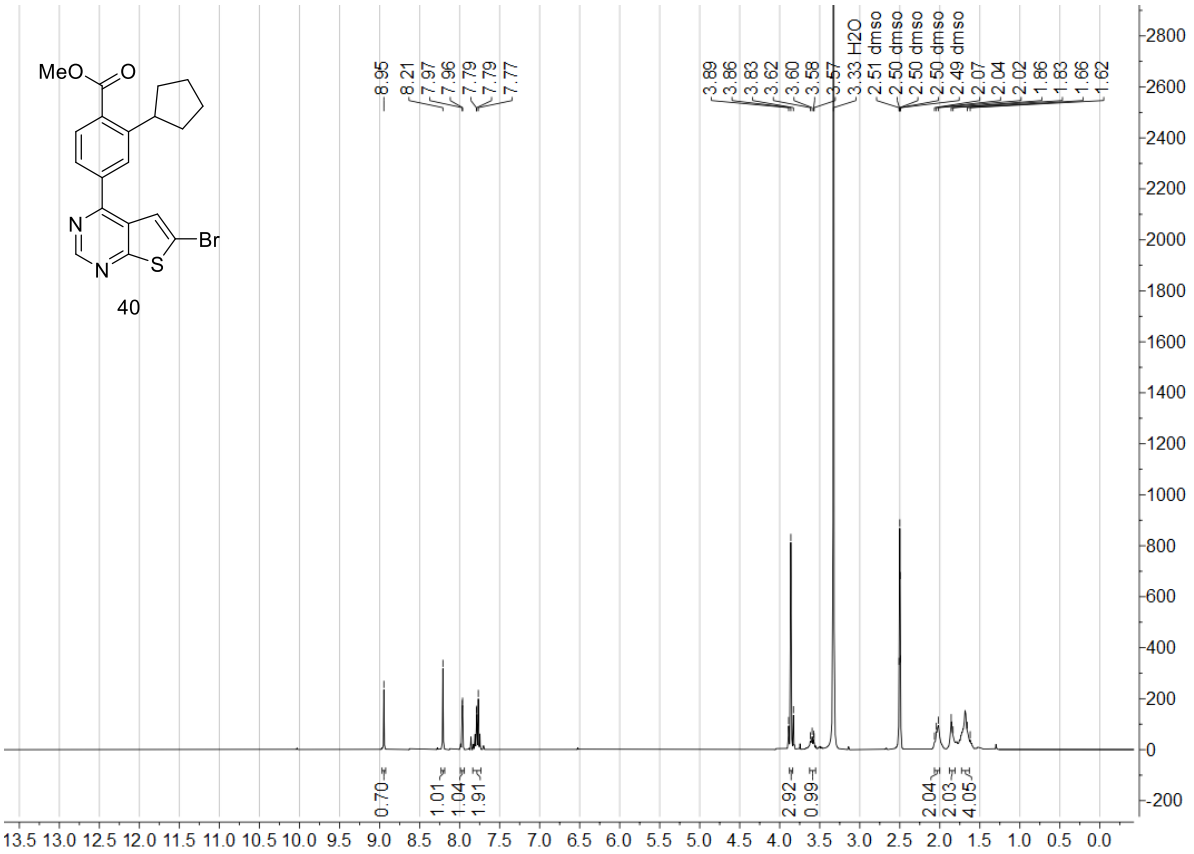

169.18
162.88
157.74
155.20
153.61
146.60
138.91
134.43
131.92
130.60
129.79
129.54
127.39
126.78
126.55
125.36
120.32
41.29
40.02
39.88
39.76
39.64
39.52
39.40
39.28
39.16
34.48
25.32

2-Cyclopentyl-4-(thieno[3,2-*d*]pyrimidin-4-yl)benzoic acid (38)

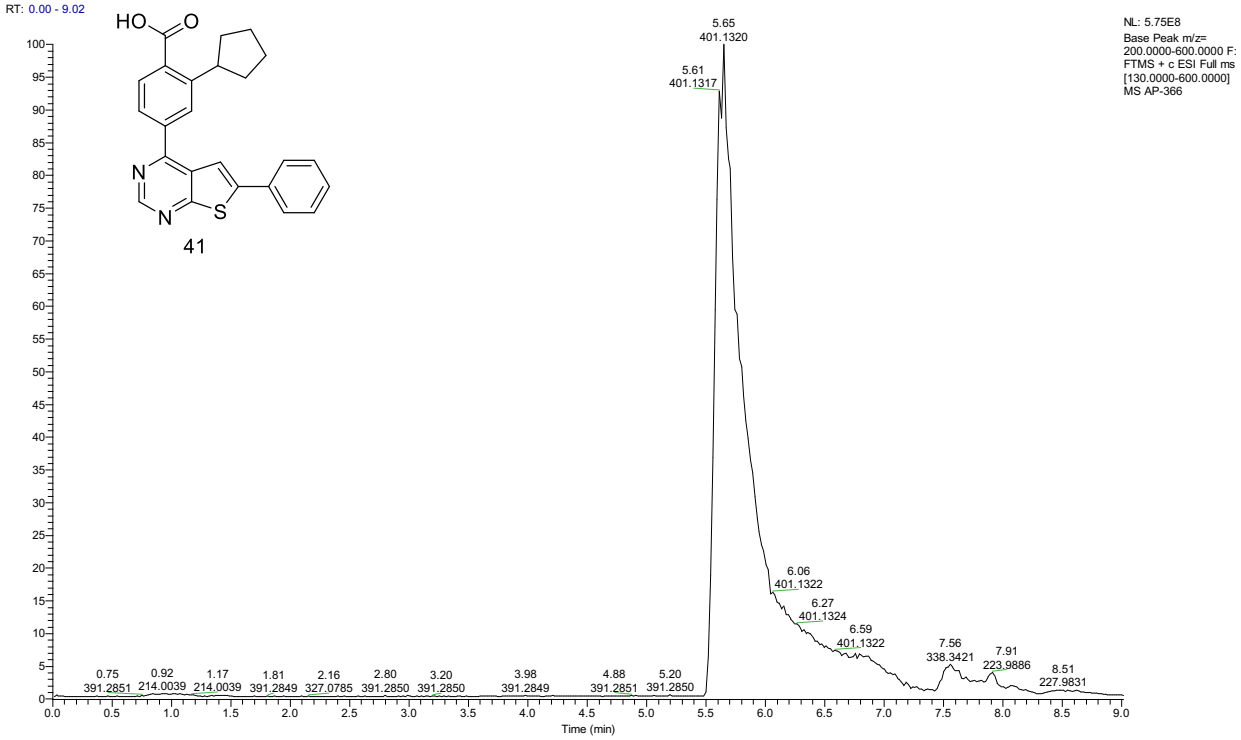


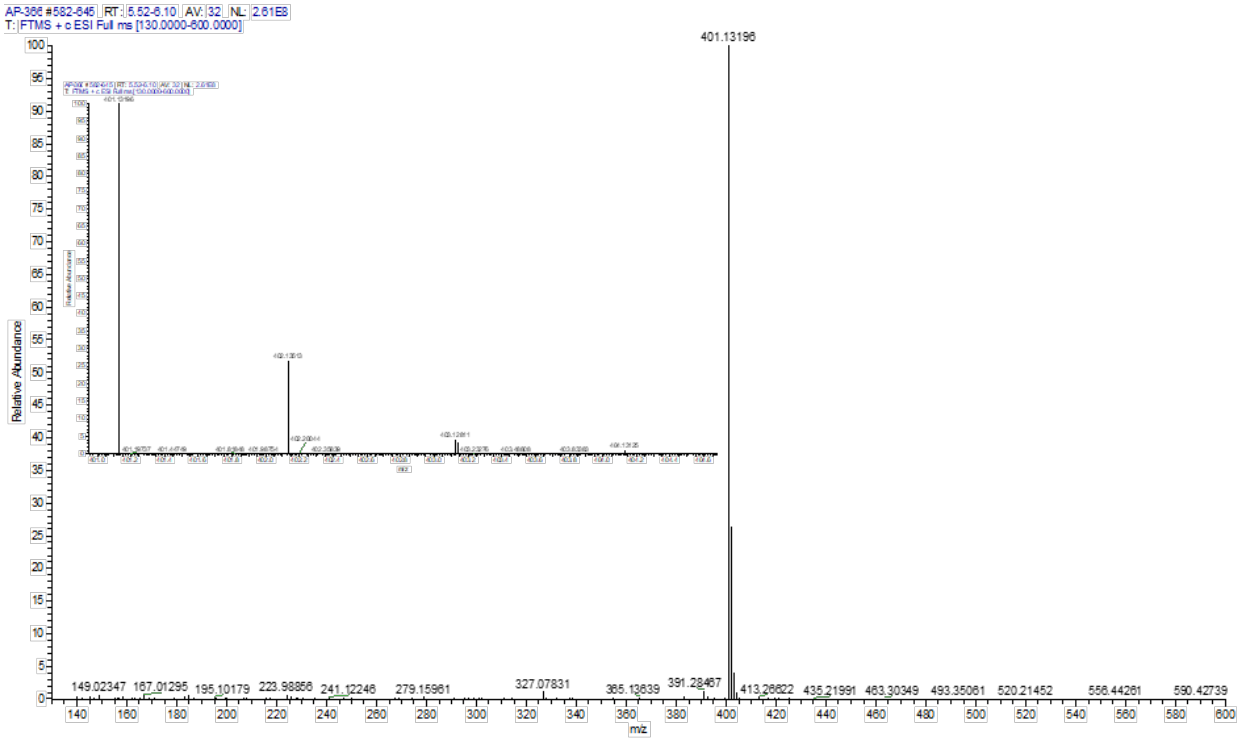

169.16
162.12
158.27
154.67
146.58
139.01
138.90
134.39
129.80
127.61
126.59
125.47
124.53
41.21
40.02
39.88
39.76
39.64
39.52
39.40
39.28
39.16
34.54
25.35
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
200
180
160
140
120
100
80
60
40
20
0
-20
169.16
162.12
158.27
154.67
146.58
139.01
138.90
134.39
129.80
127.61
126.59
125.47
124.53
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
175
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100

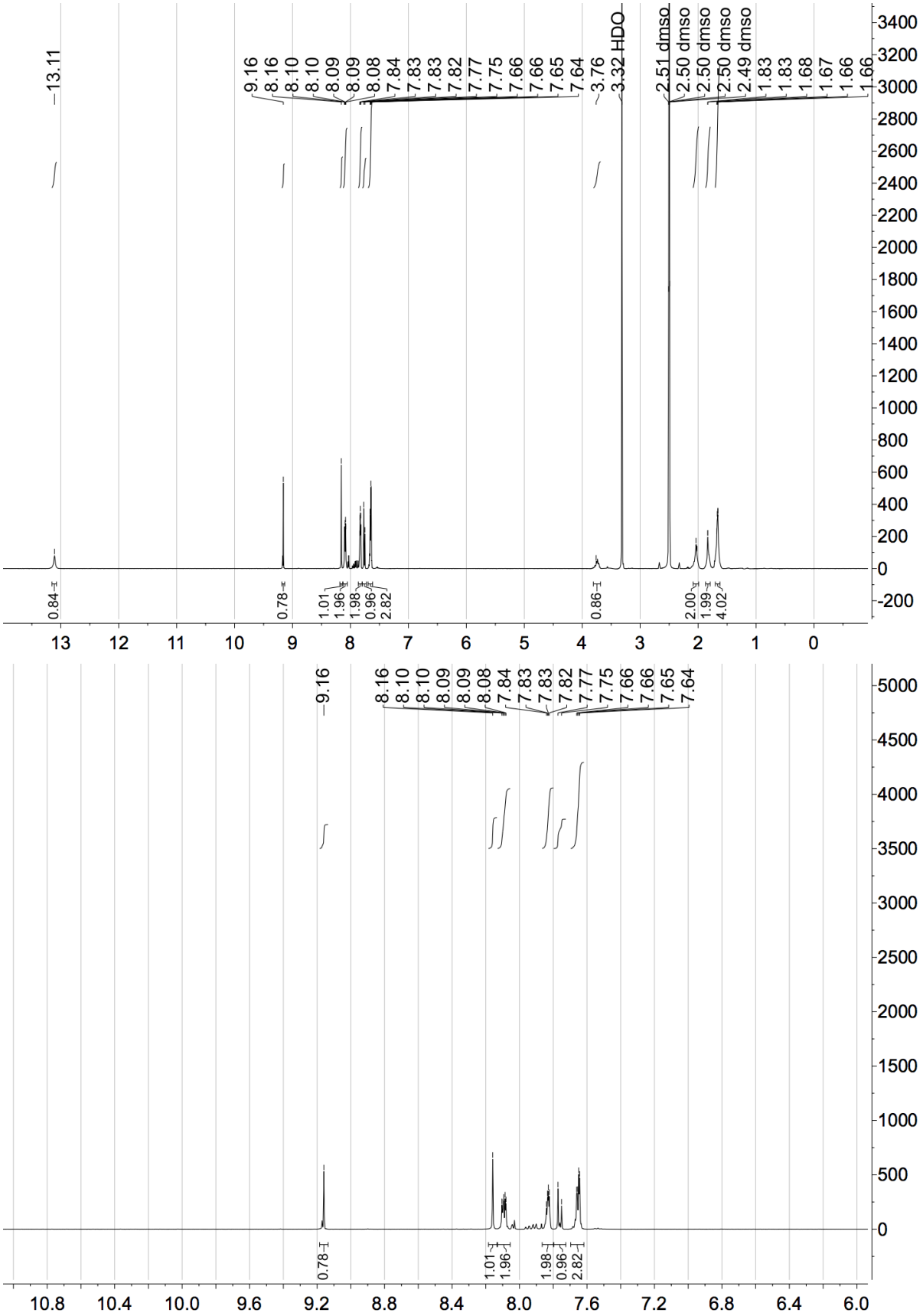
Methyl 4-(6-bromothieno[2,3-*d*]pyrimidin-4-yl)-2-cyclopentylbenzoate (40)

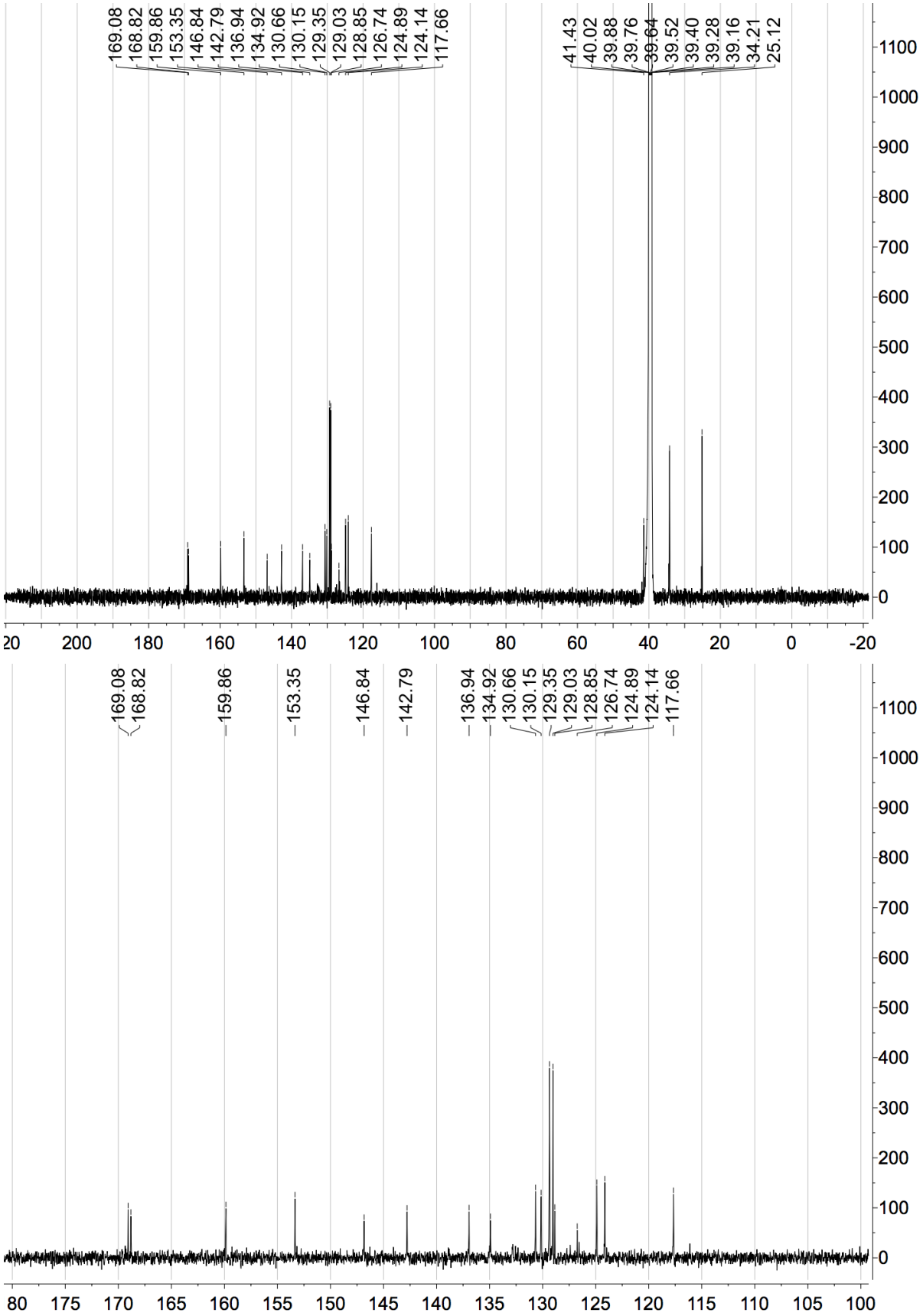


2-Cyclopentyl-4-(6-phenylthieno[2,3-*d*]pyrimidin-4-yl)benzoic acid (41)

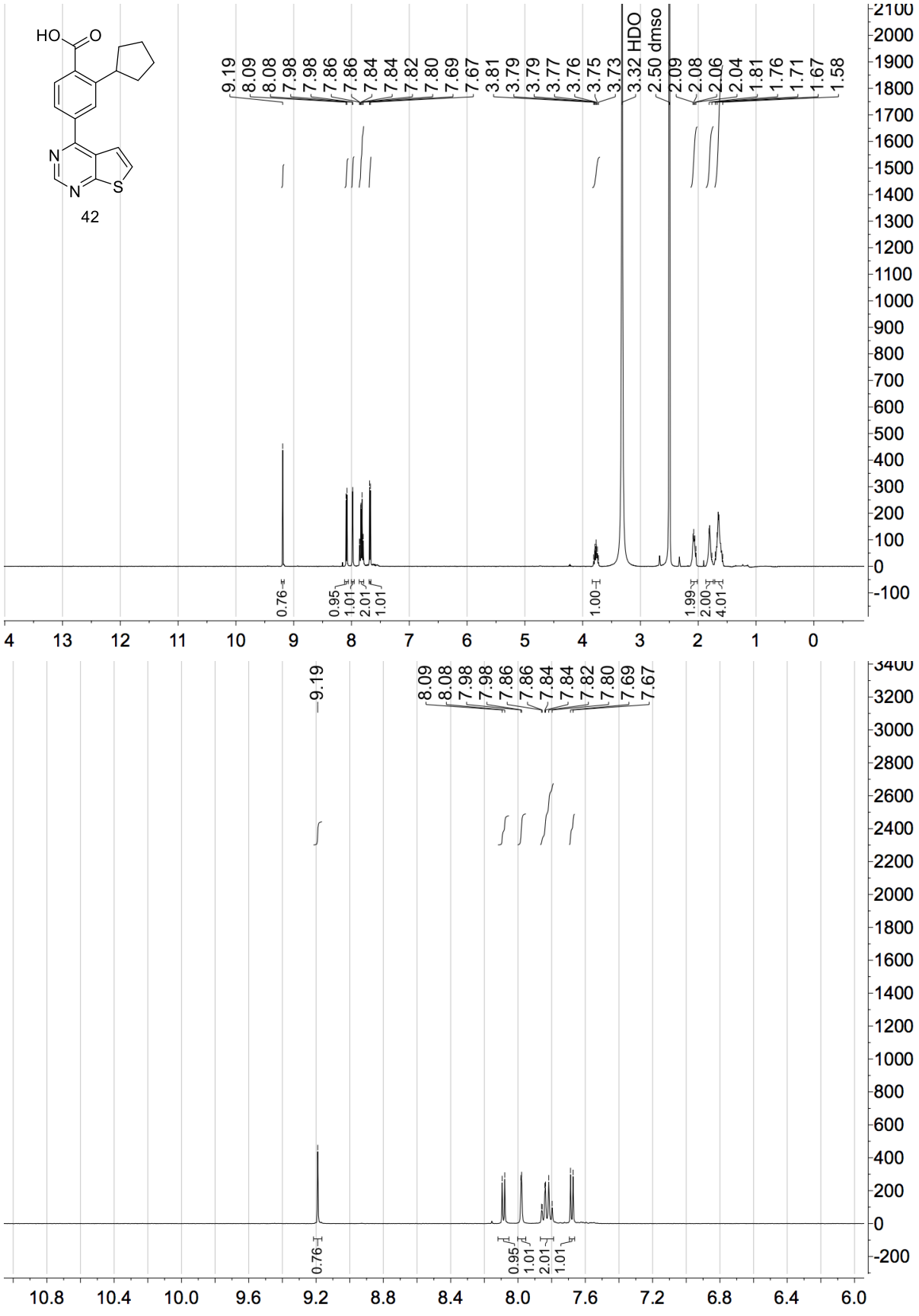


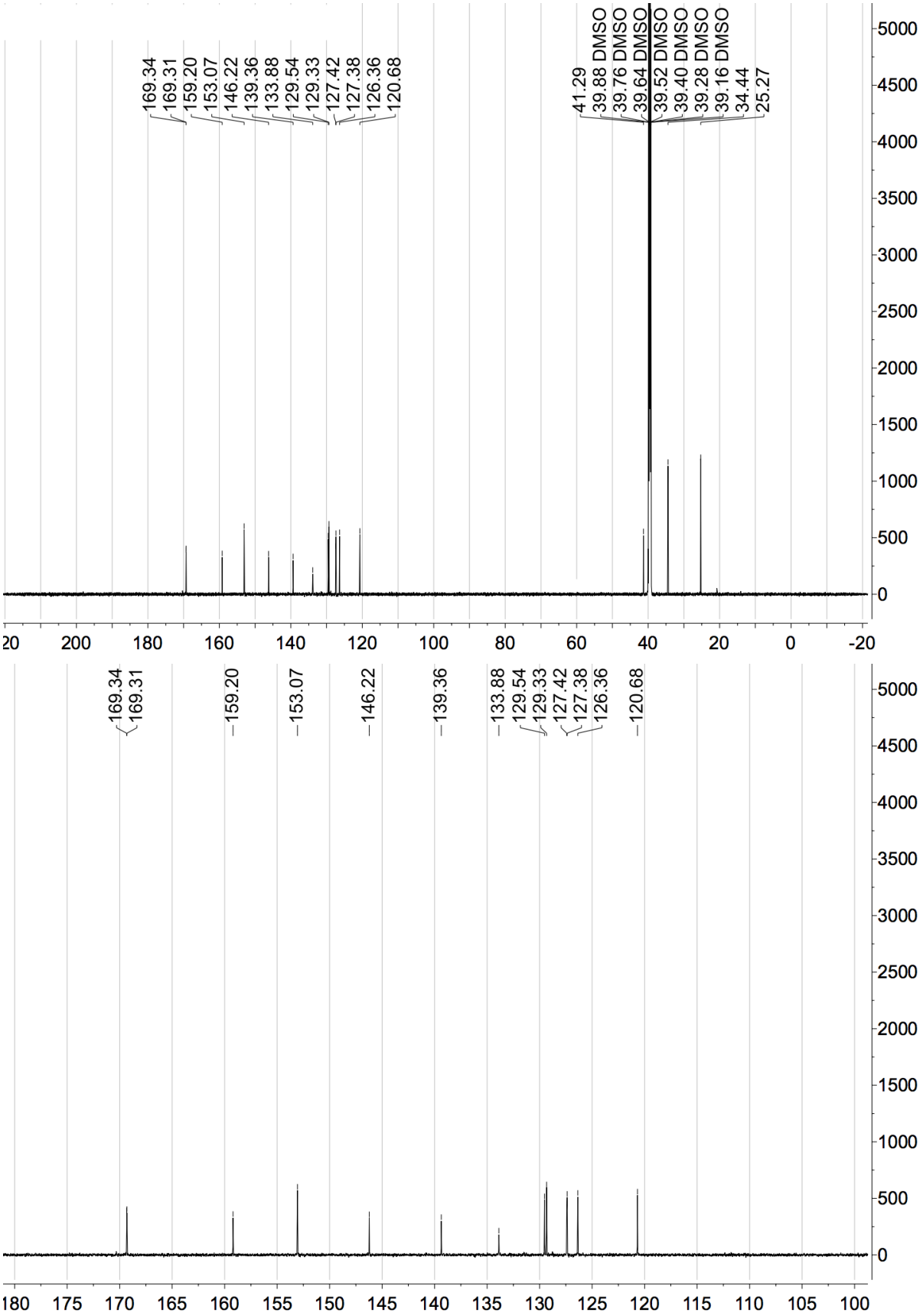



13.11
9.16
8.16
8.10
8.10
8.09
8.09
8.08
7.84
7.83
7.83
7.82
7.77
7.75
7.66
7.66
7.65
7.64
3.76
3.32 HDO
2.51 dmso
2.50 dmso
2.50 dmso
2.50 dmso
2.49 dmso
1.83
1.83
1.68
1.67
1.66
1.66
0.84
0.78
1.01
1.96
1.98
0.96
2.82
0.86
2.00
1.99
4.02

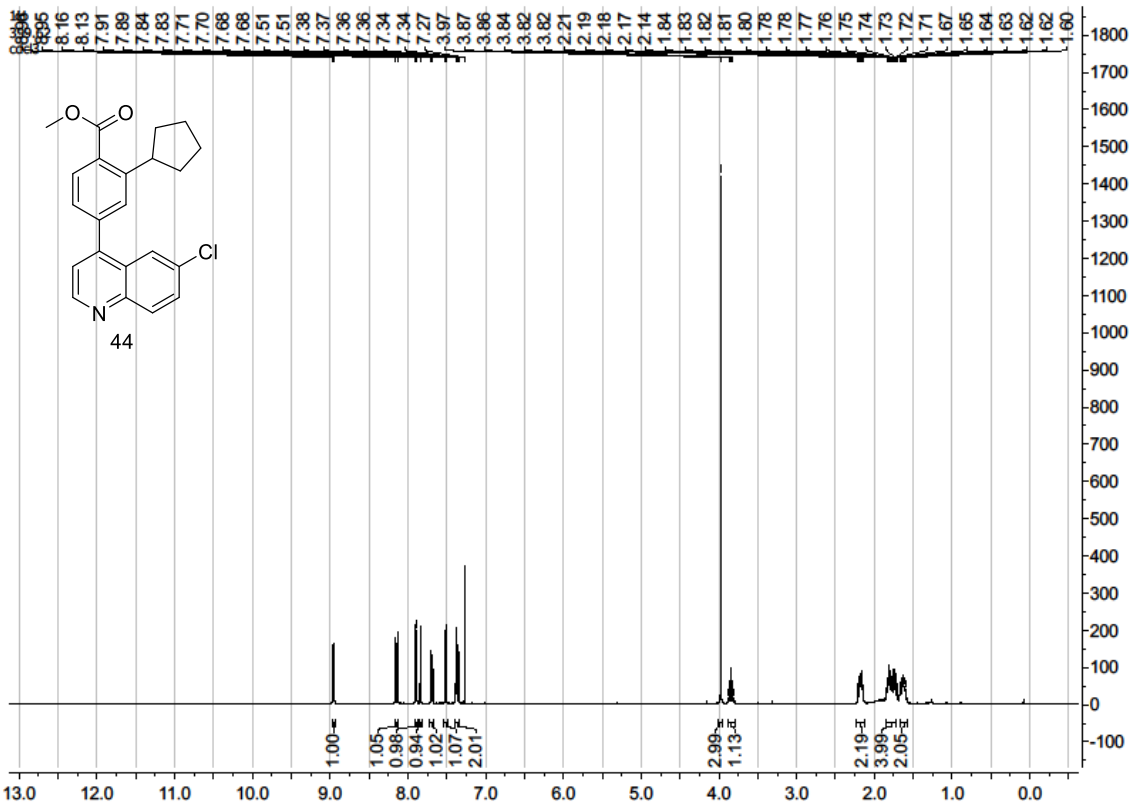

169.08
168.82
159.86
153.35
146.84
142.79
136.94
134.92
130.66
130.15
129.35
129.03
128.85
126.74
124.89
124.14
117.66
41.43
40.02
39.88
39.76
39.64
39.52
39.40
39.28
39.16
34.21
25.12
1100
1000
900
800
700
600
500
400
300
200
100
0
20
200
180
160
140
120
100
80
60
40
20
0
-20
169.08
168.82
159.86
153.35
146.84
142.79
136.94
134.92
130.66
130.15
129.35
129.03
128.85
126.74
124.89
124.14
117.66
1100
1000
900
800
700
600
500
400
300
200
100
0
80
175
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100

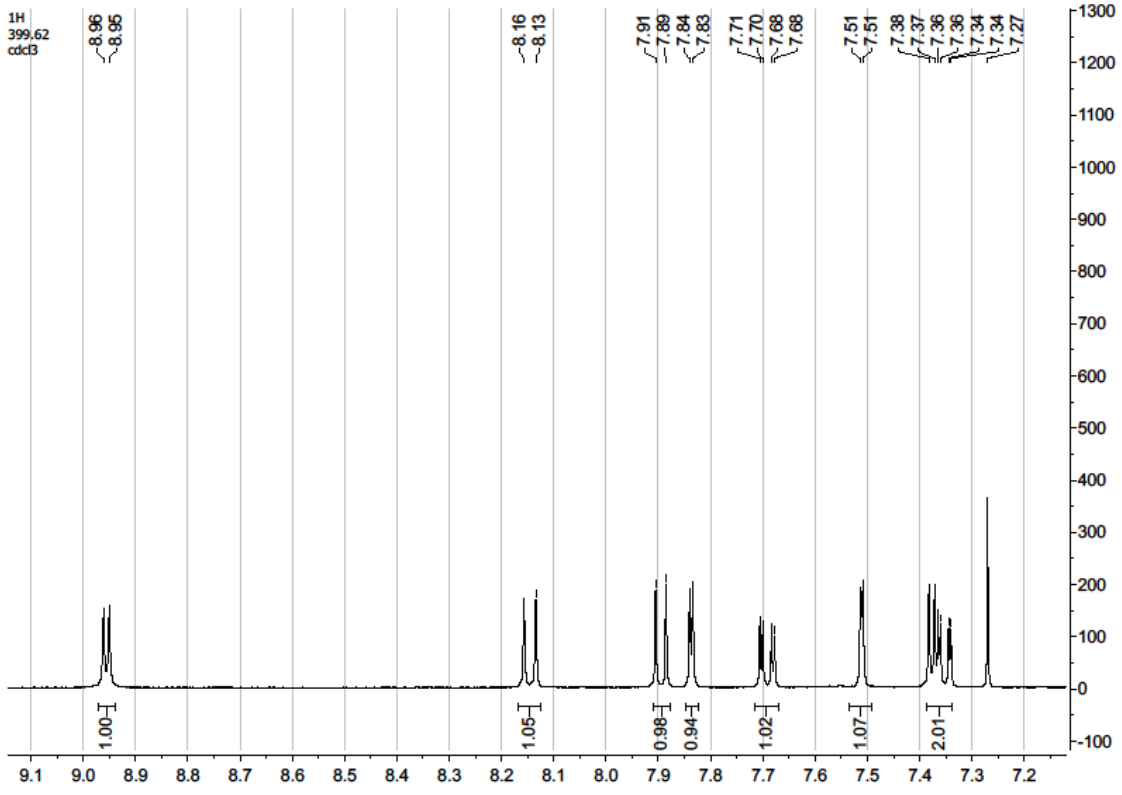
2-Cyclopentyl-4-(thieno[2,3-*d*]pyrimidin-4-yl)benzoic acid (42)

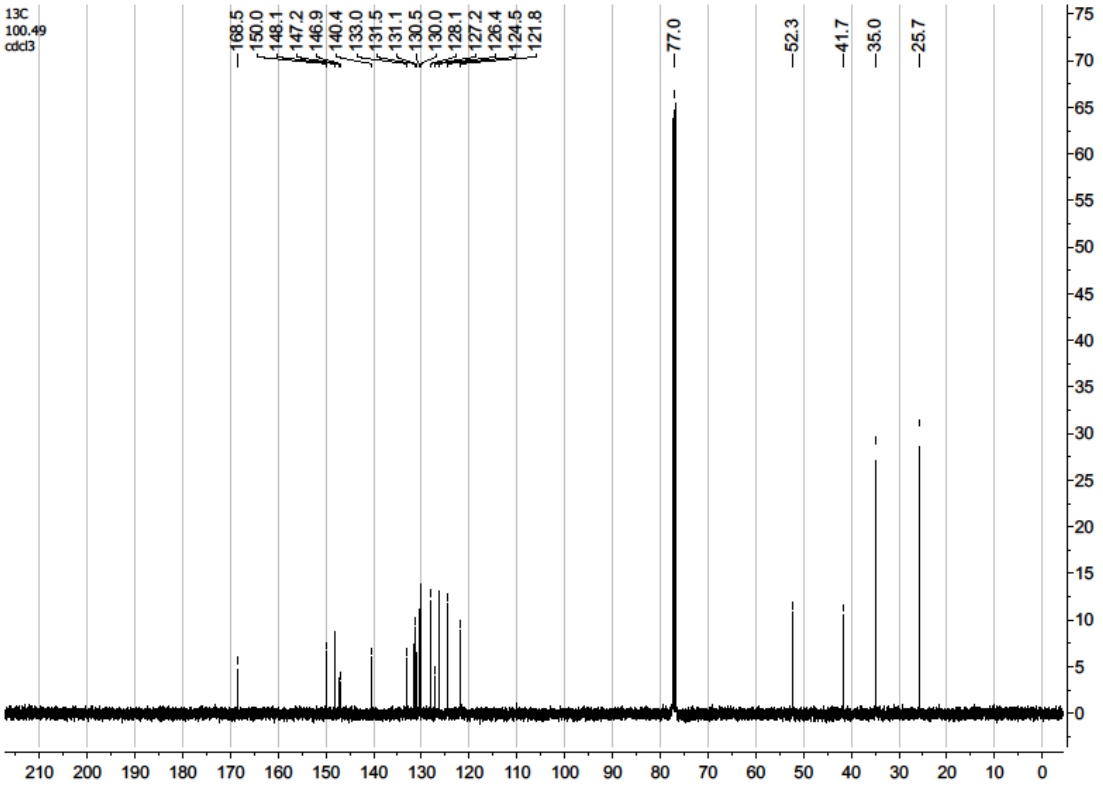


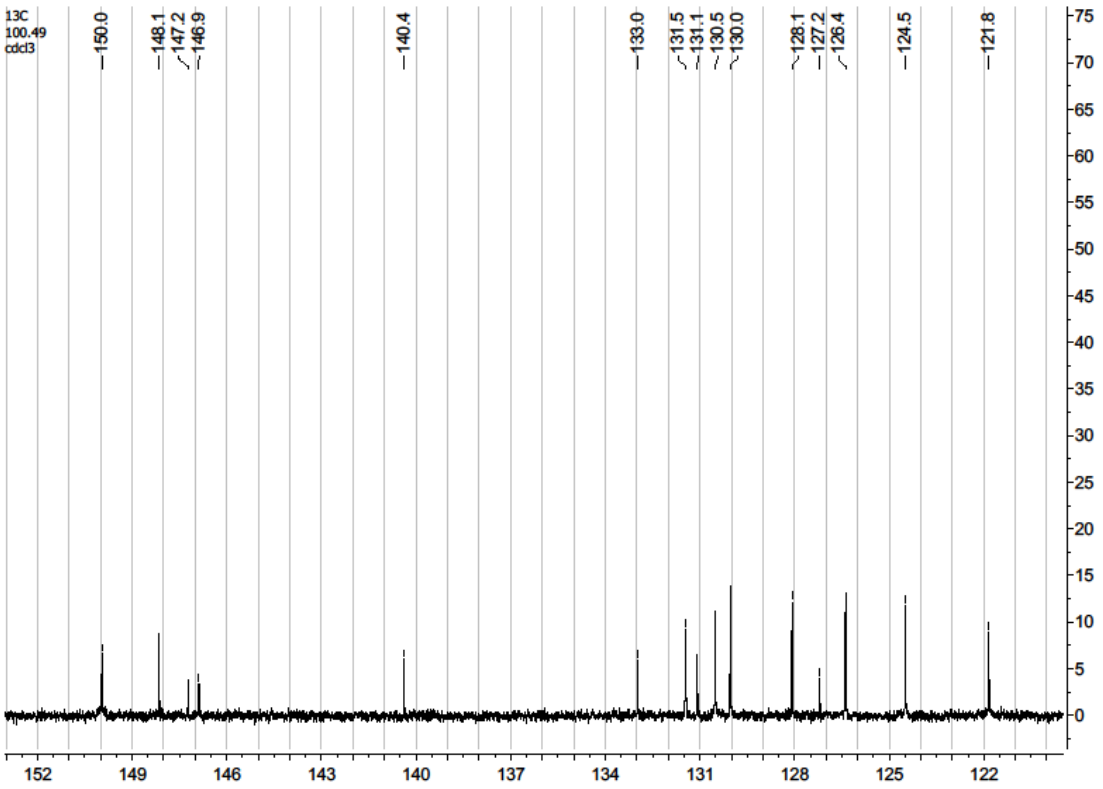

169.34
169.31
159.20
153.07
146.22
139.36
133.88
129.54
129.33
127.42
127.38
126.36
120.68
41.29
39.88 DMSO
39.76 DMSO
39.64 DMSO
39.52 DMSO
39.40 DMSO
39.28 DMSO
39.16 DMSO
34.44
25.27
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
20 200 180 160 140 120 100 80 60 40 20 0 -20
169.34
169.31
159.20
153.07
146.22
139.36
133.88
129.54
129.33
127.42
127.38
126.36
120.68
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100

Methyl 4-(6-chloroquinolin-4-yl)-2-cyclopentylbenzoate (44)

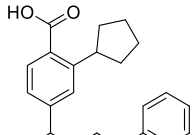


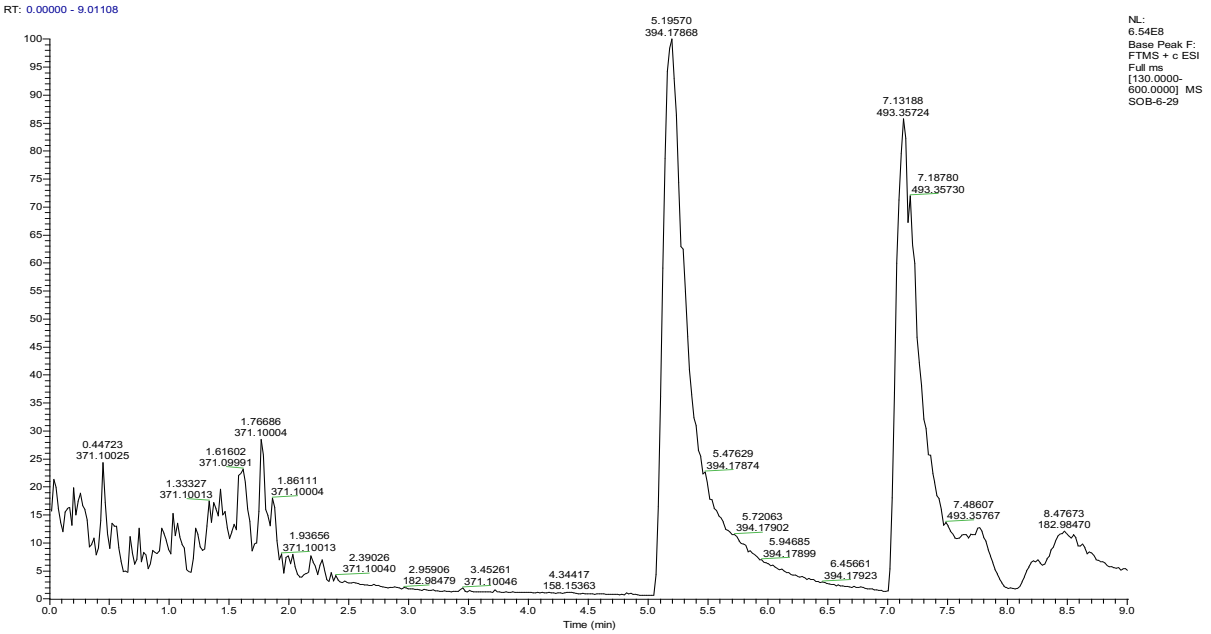






2-Cyclopentyl-4-(6-phenylquinolin-4-yl)benzoic acid (45)

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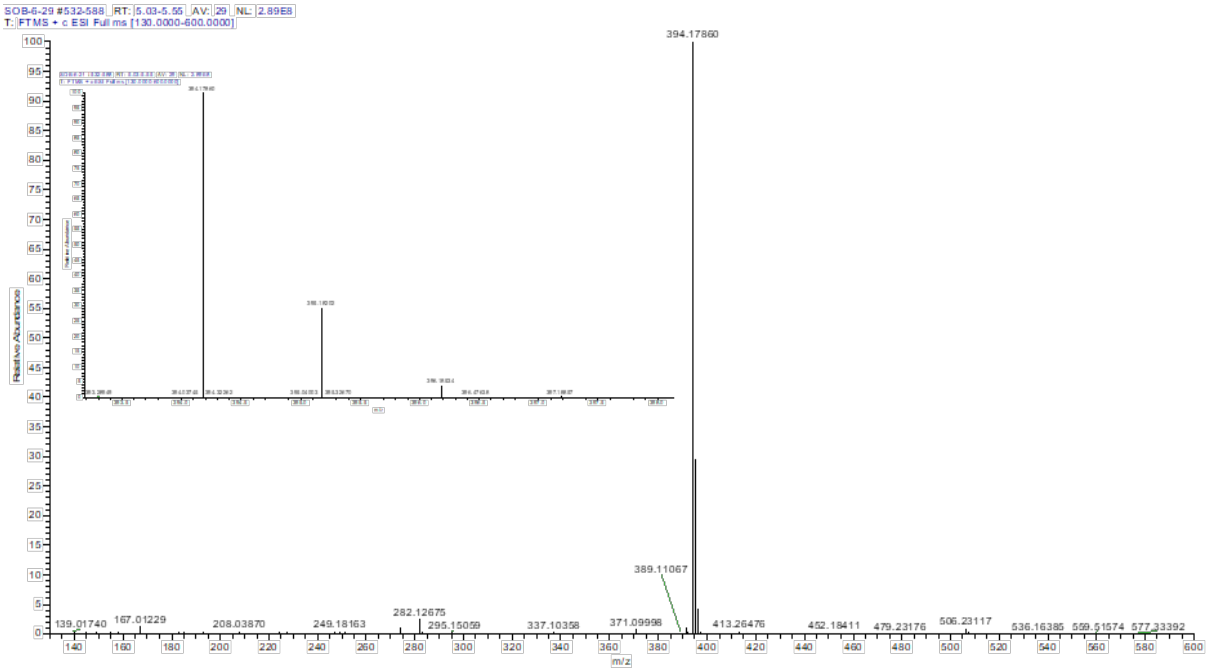
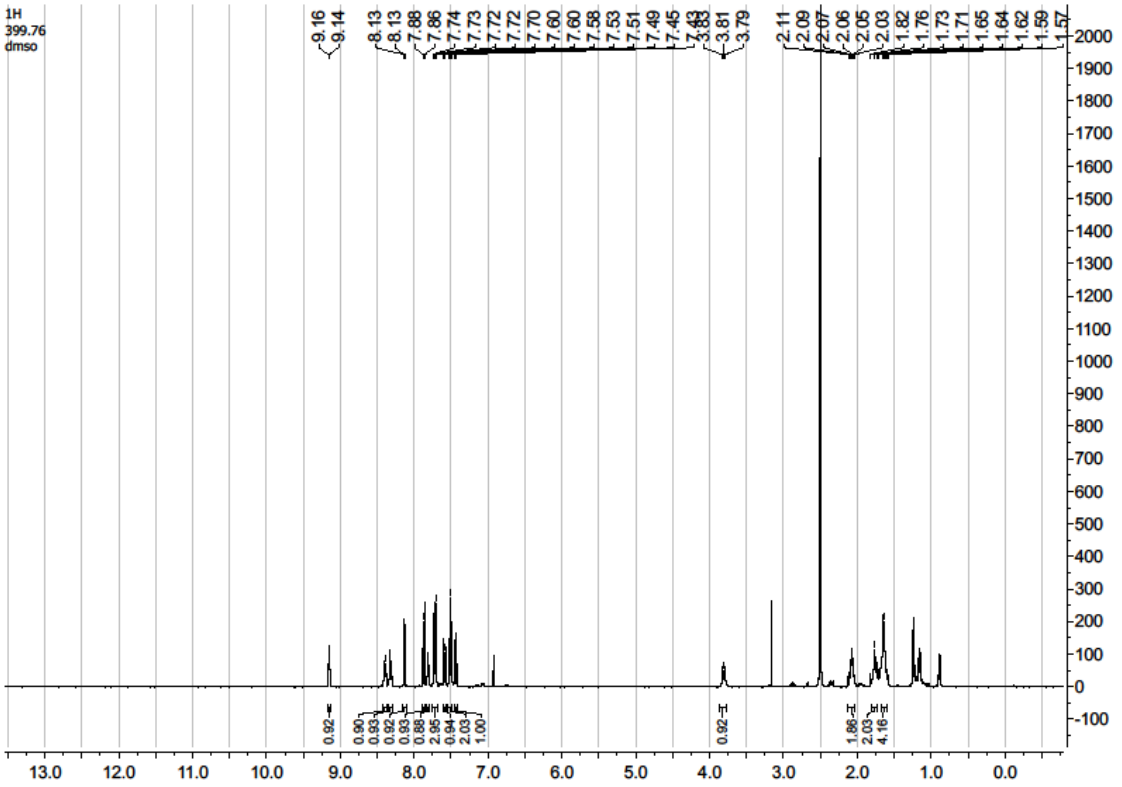
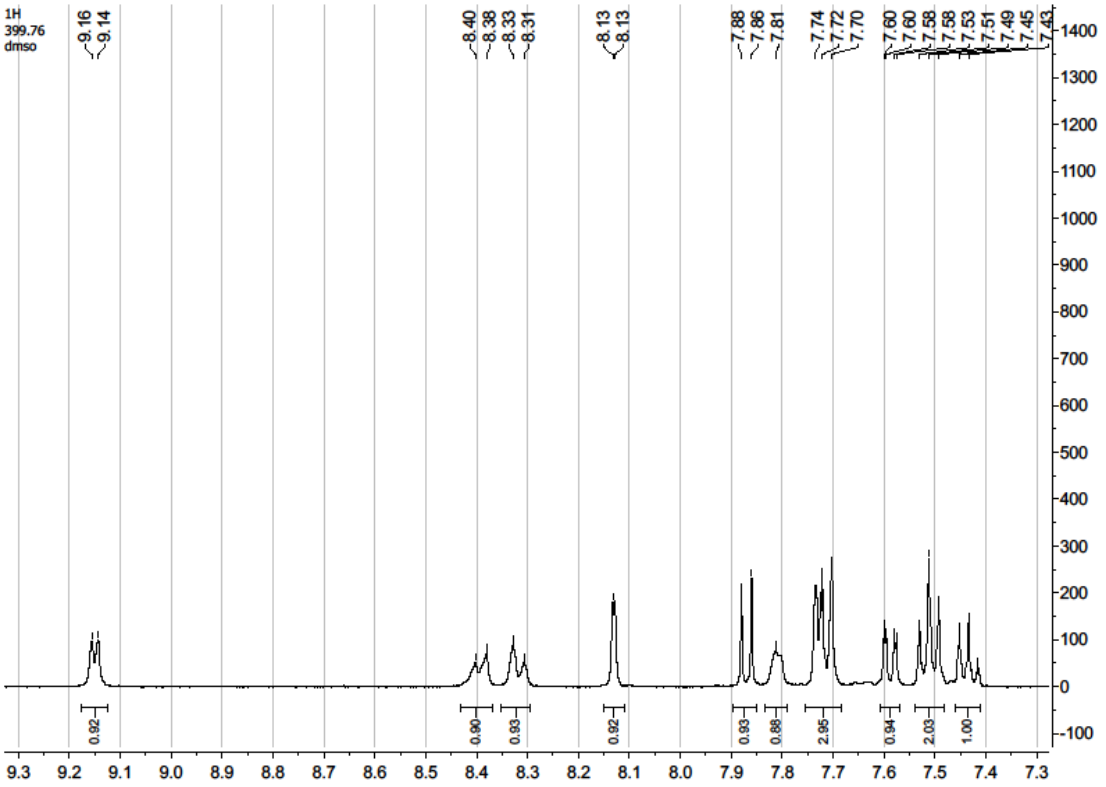
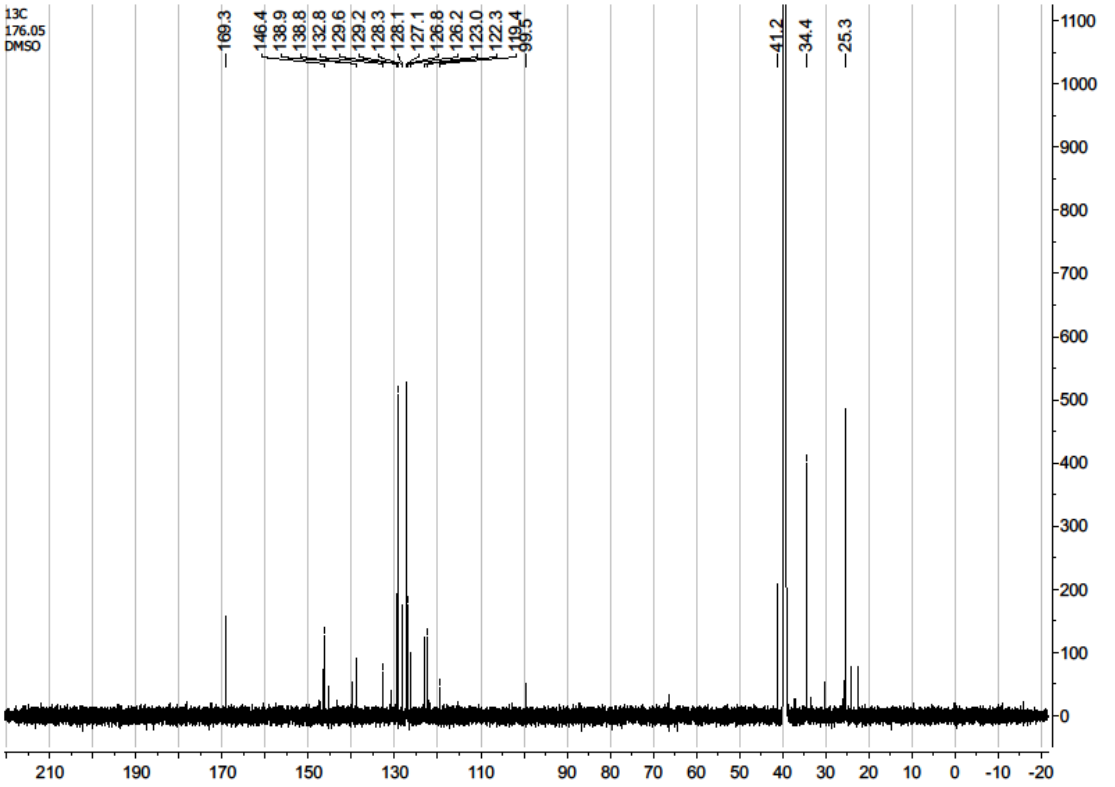
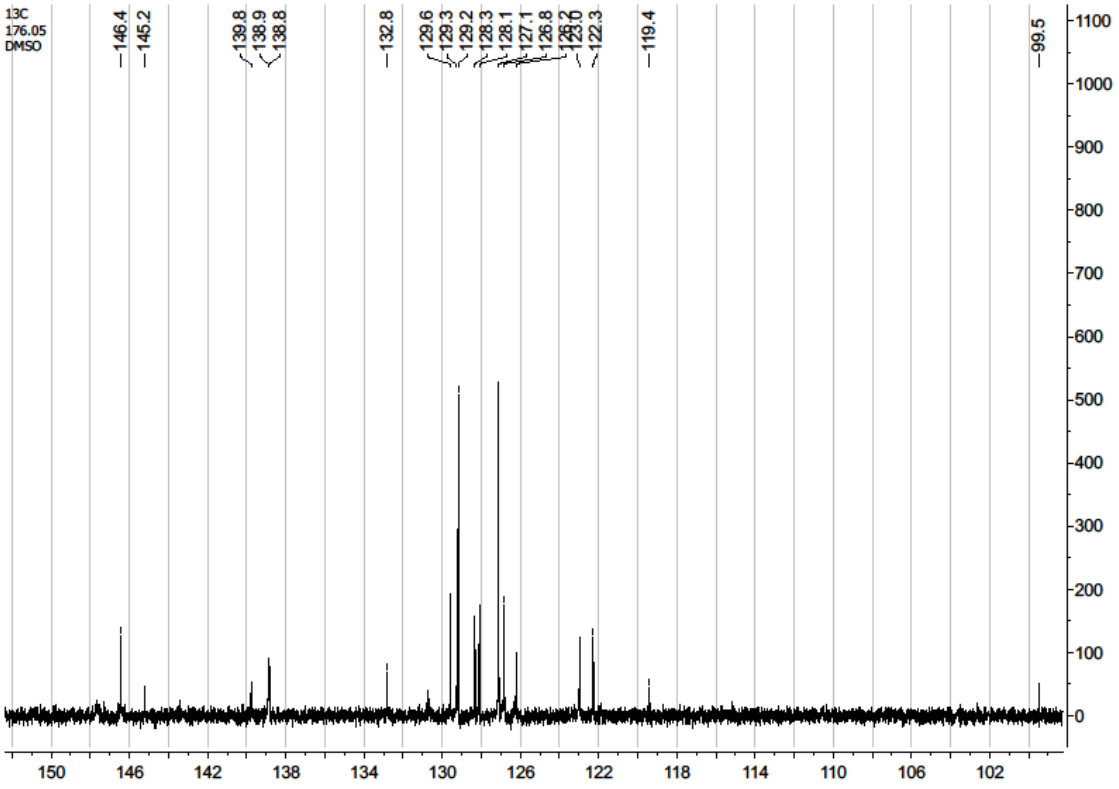
NL:
6.54E8
Base Peak F:
FTMS + c ESI
Full ms
[130.0000-
600.0000] MS
SOB-6-29
5.19570
394.17868
7.13188
493.35724
7.18780
493.35730
0.44723
371.10025
1.61602
371.09991
1.76686
371.10004
1.33327
371.10013
1.86111
371.10004
1.93656
371.10013
2.39026
371.10040
2.95906
182.98479
3.45261
371.10046
4.34417
158.15363
5.47629
394.17874
5.72063
394.17902
5.94685
394.17899
6.45661
394.17923
7.48607
493.35767
8.47673
182.98470
Time (min)


SOB-6-29 #532-588 RT: 5.03-5.55 AV: 29 NL: 2.89E8
T: FTMS + c ESI Full ms [130.0000-600.0000]
Relative Abundance
394.17860
389.11067
139.01740
167.01229
208.03870
249.18163
282.12675
295.15059
337.10358
371.09998
413.26476
452.18411
479.23176
506.23117
536.16385
559.51574
577.33392
m/z

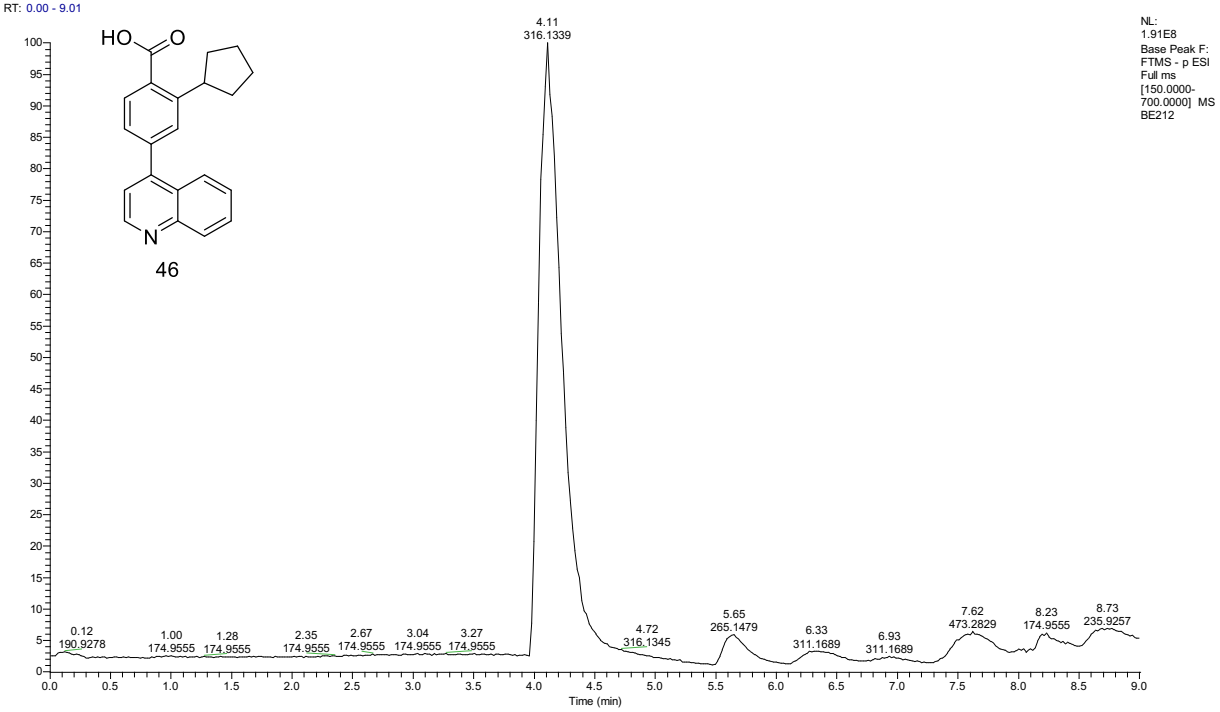

1H
399.76
dmso

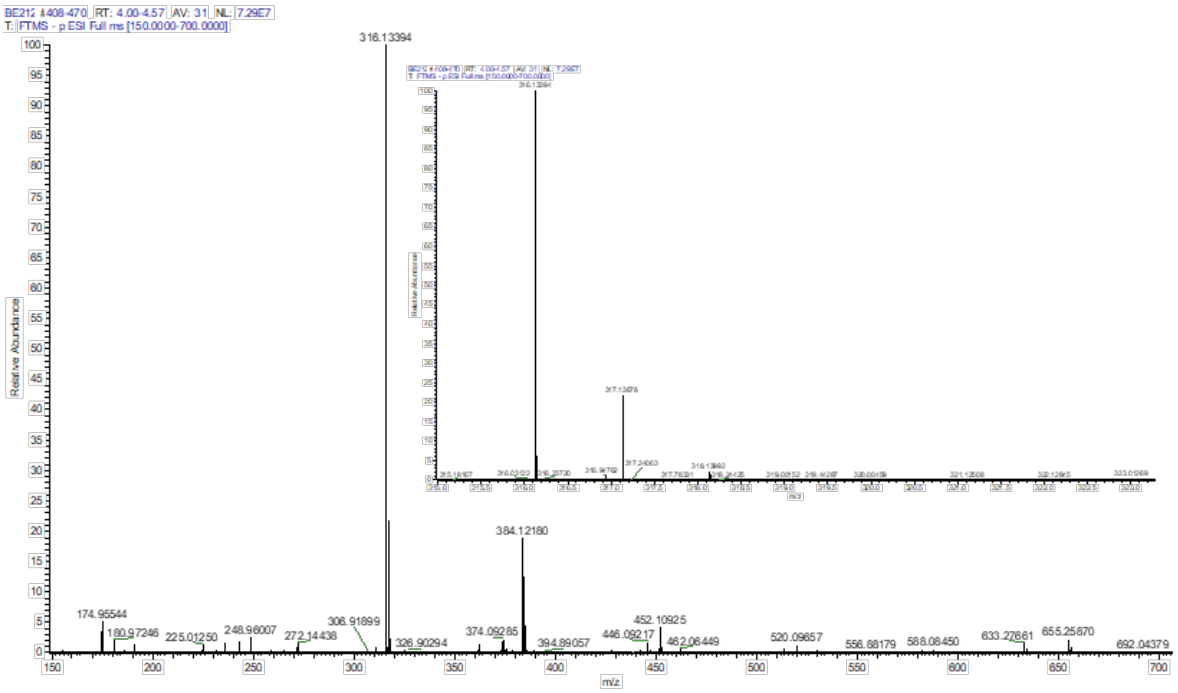

1H
399.76
dmso

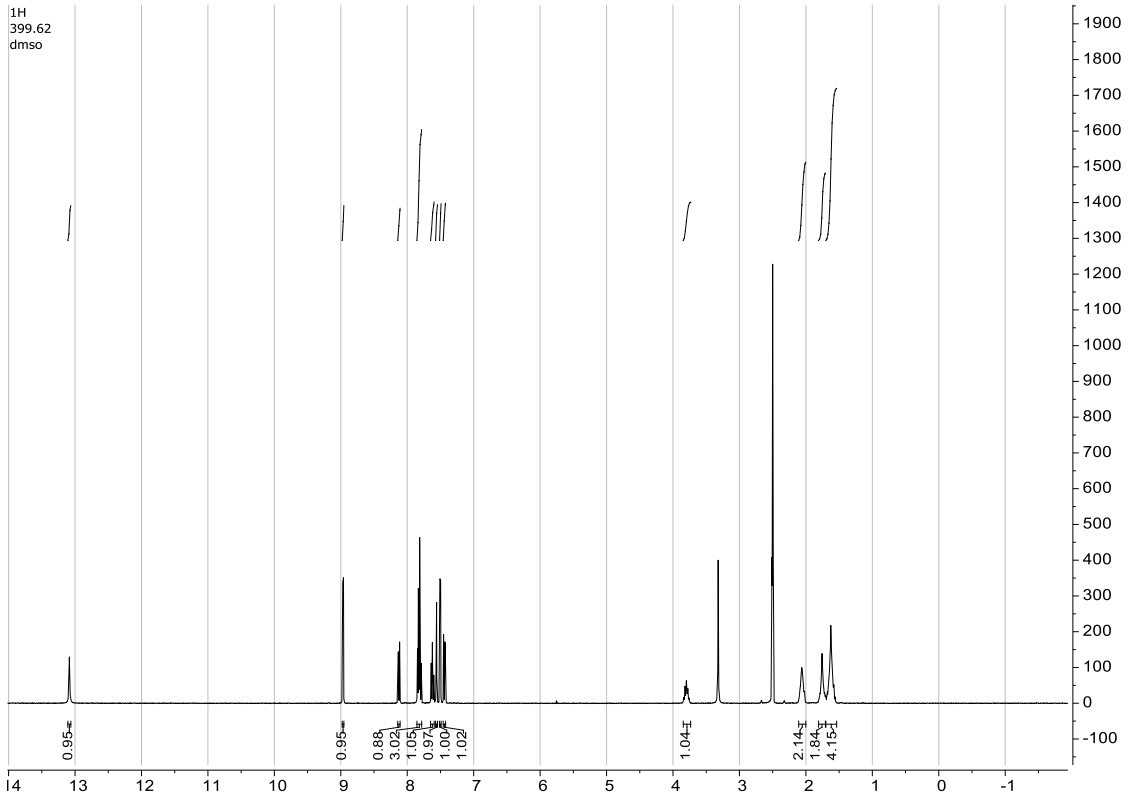

13C
176.05
DMSO
169.3
146.4
138.9
138.8
132.8
129.6
129.2
128.3
128.1
127.1
126.8
126.2
123.0
122.3
119.4
99.5
41.2
34.4
25.3

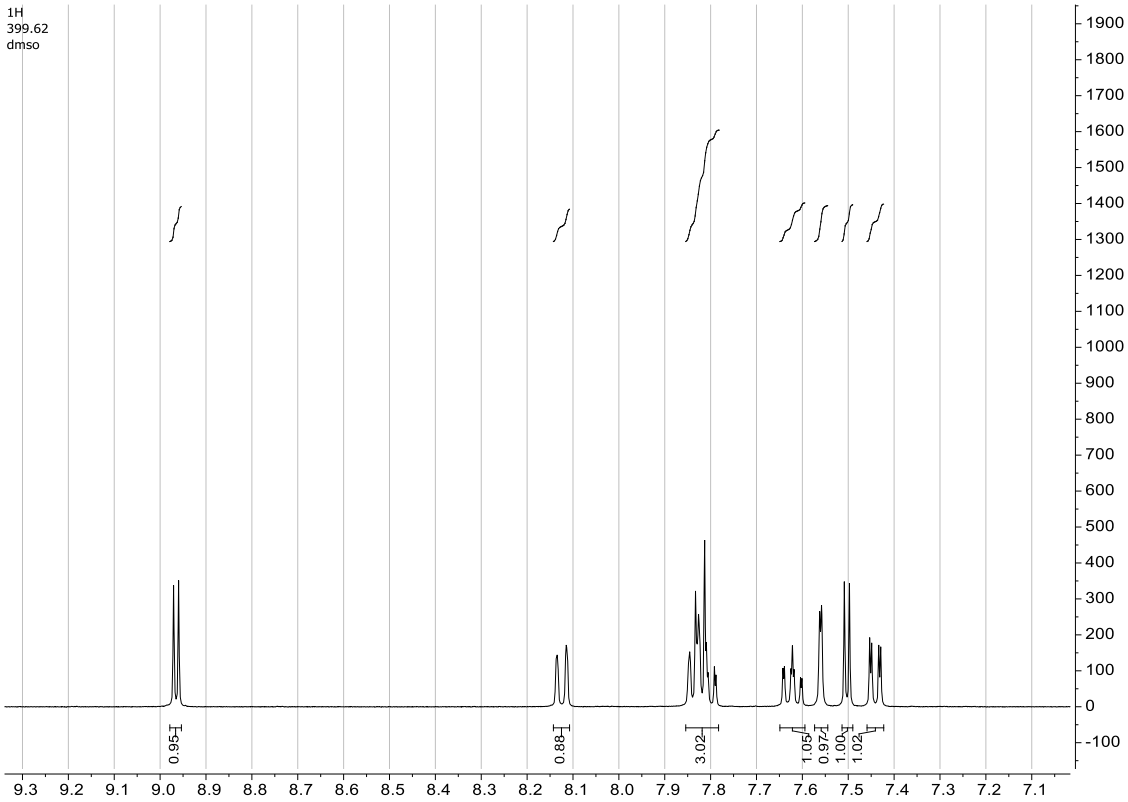

13C
176.05
DMSO
146.4
145.2
139.8
138.9
138.8
132.8
129.6
129.3
129.2
128.3
128.1
127.1
126.8
122.3
119.4
99.5

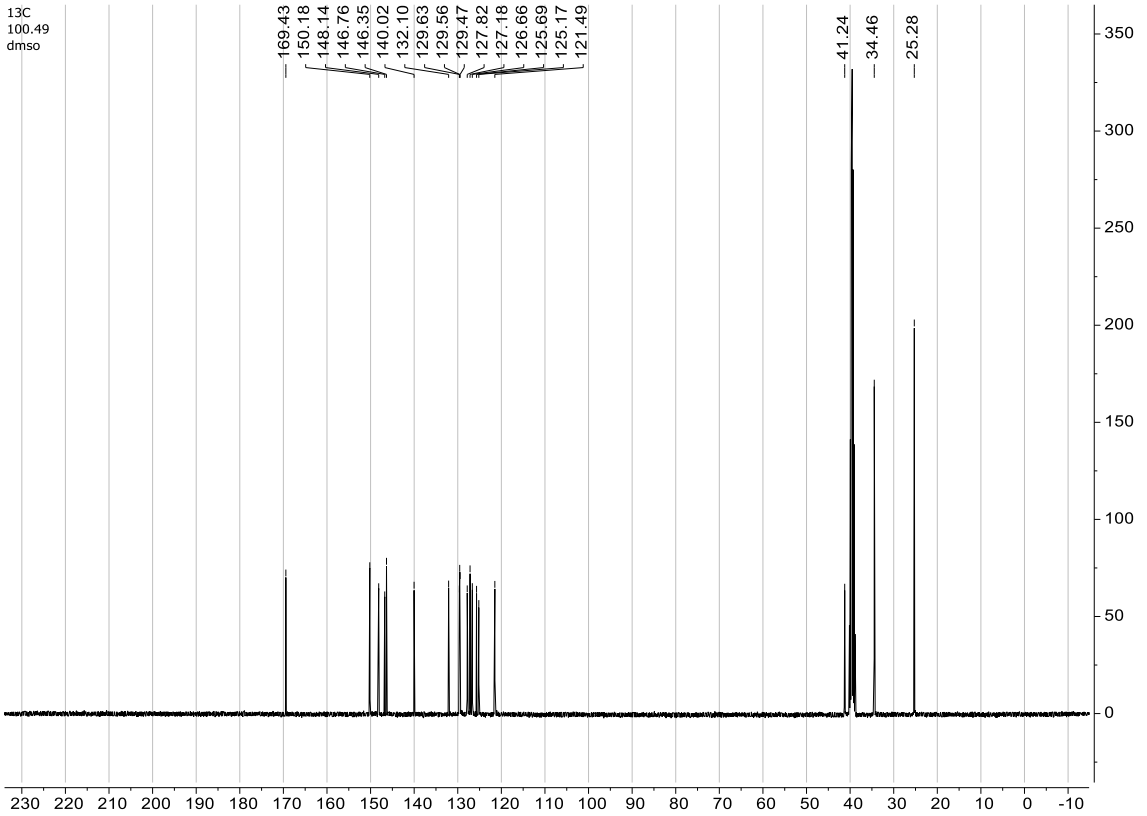
2-Cyclopentyl-4-(quinolin-4-yl)benzoic acid (46)

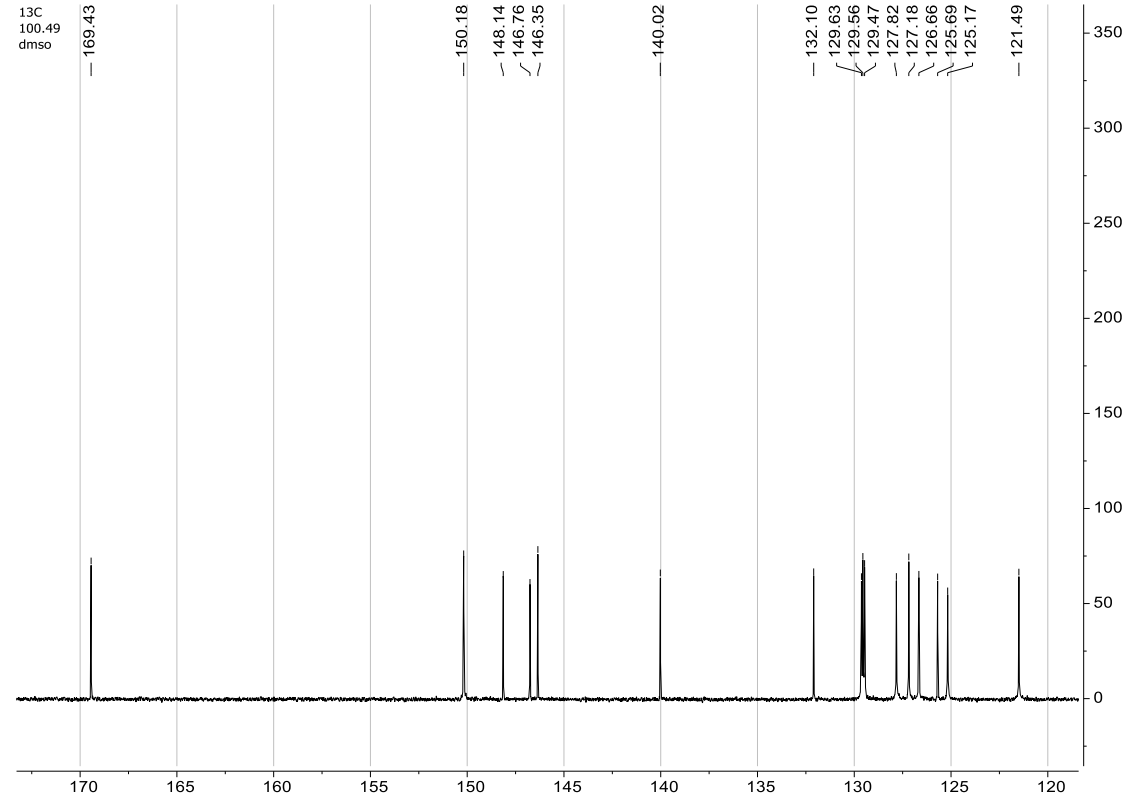




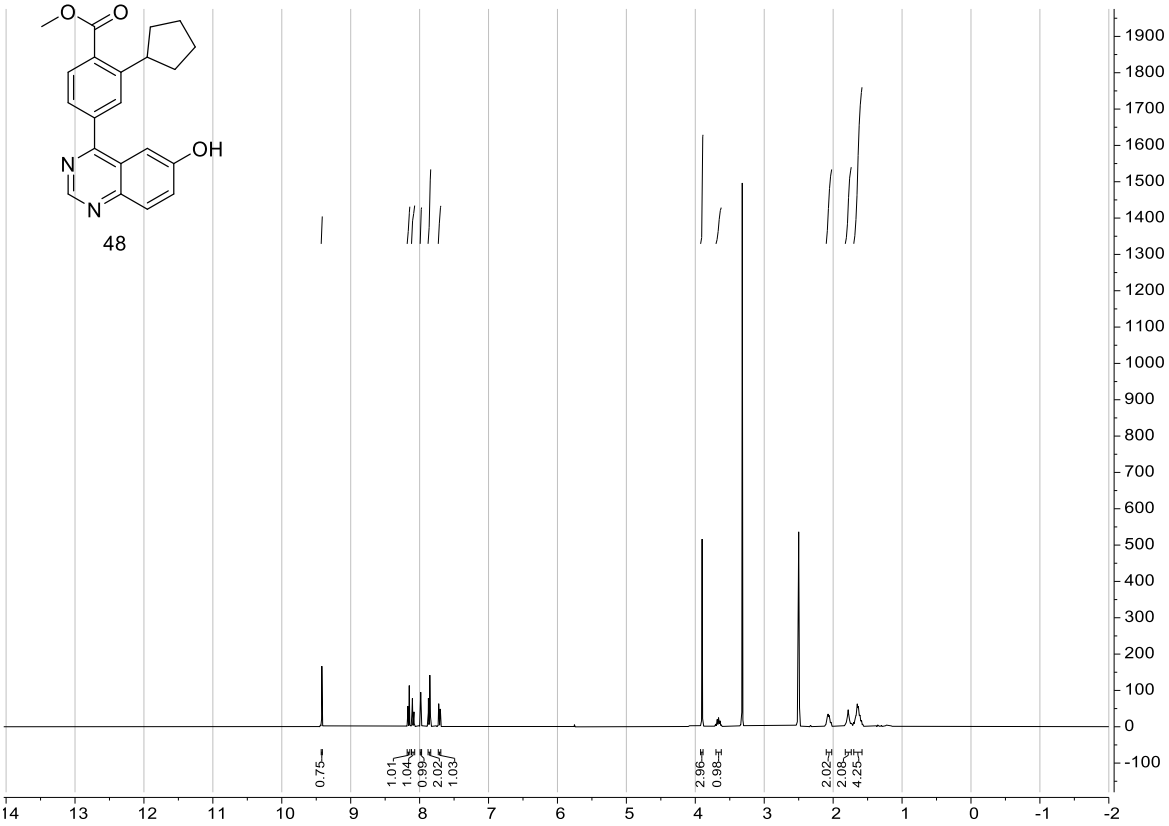

1H
399.62
dmso
0.95
0.95
0.88
3.02
1.05
0.97
1.00
1.02
1.04
2.14
1.84
4.15

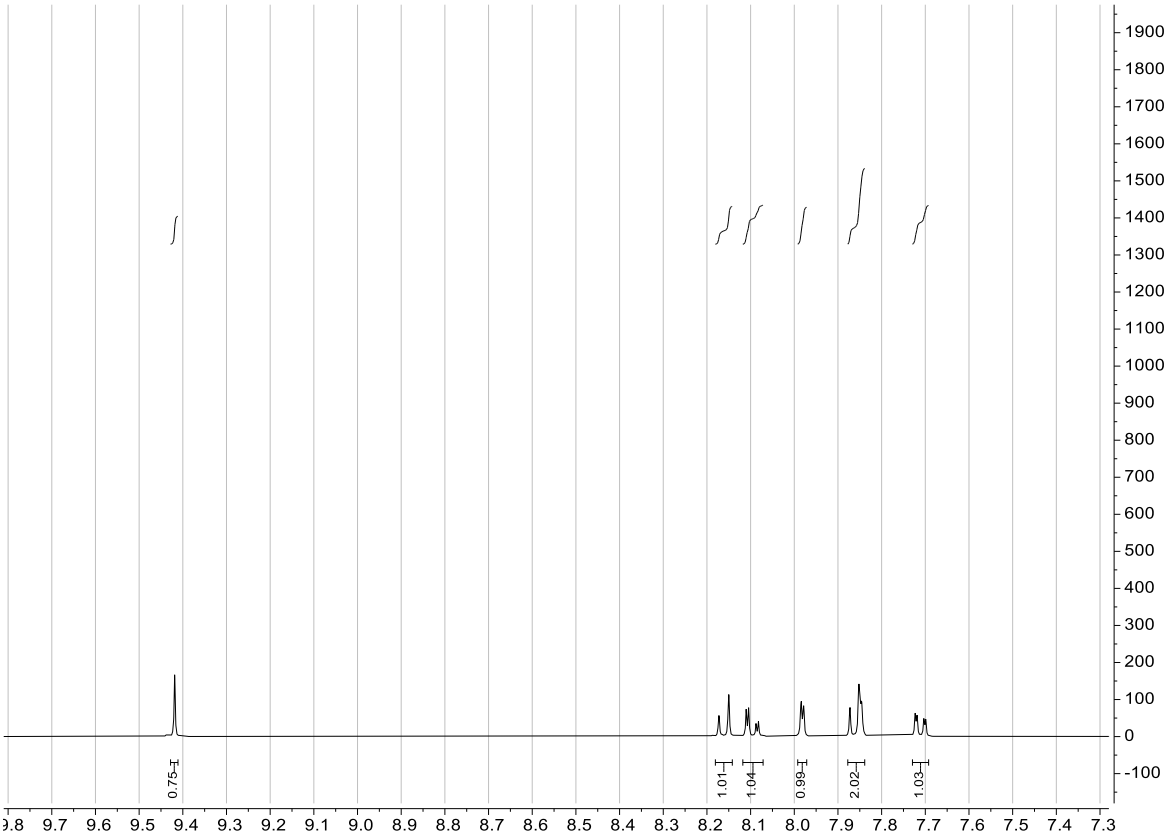

1H
399.62
dmso
0.95
0.88
3.02
1.05
0.97
1.00
1.02

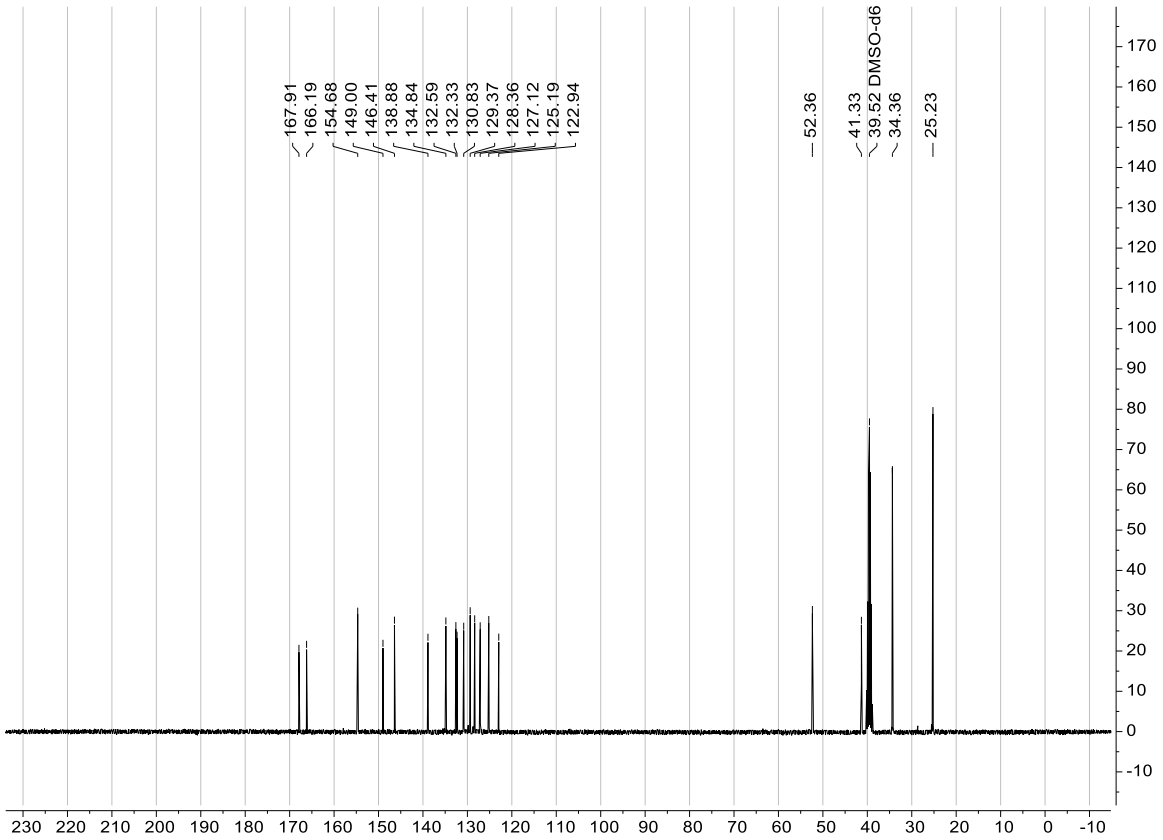

13C
100.49
dmso
169.43
150.18
148.14
146.76
146.35
140.02
132.10
129.63
129.56
129.47
127.82
127.18
126.66
125.69
125.17
121.49
41.24
34.46
25.28

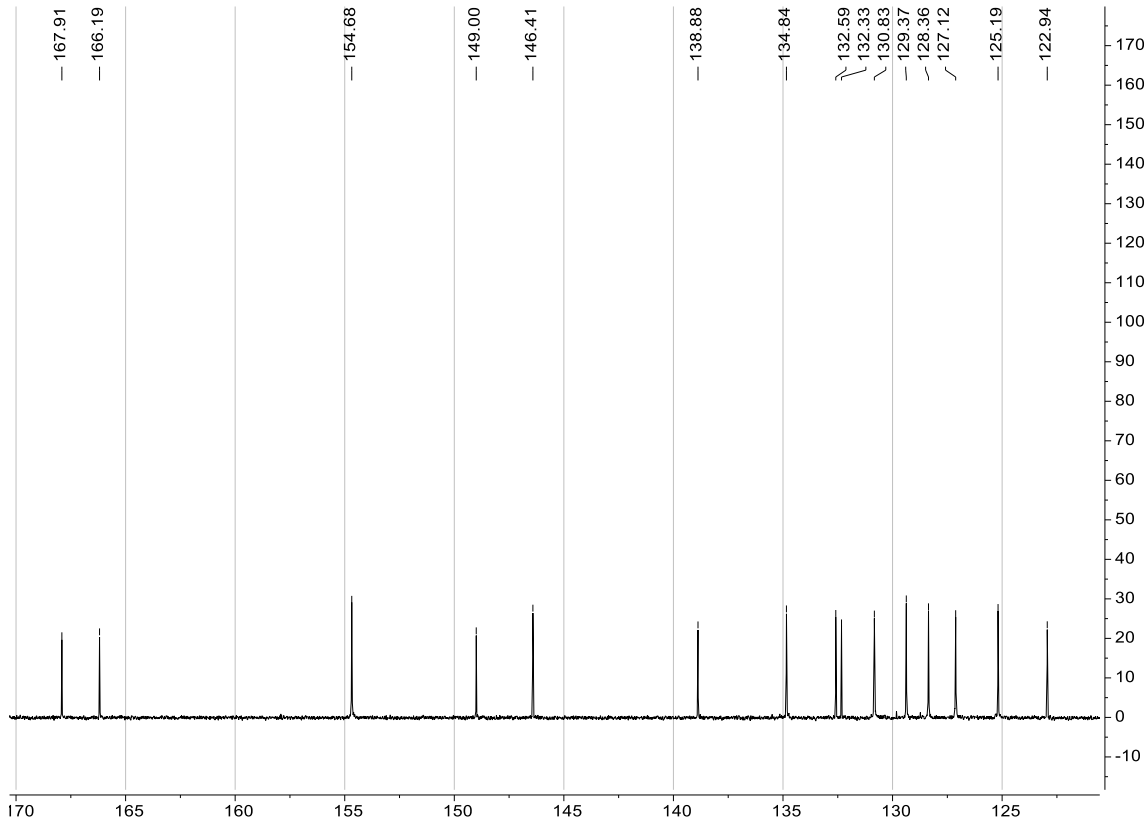

13C
100.49
dmso
169.43
150.18
148.14
146.76
146.35
140.02
132.10
129.63
129.56
129.47
127.82
127.18
126.66
125.69
125.17
121.49

Methyl 2-cyclopentyl-4-(6-hydroxyquinazolin-4-yl)benzoate (48)

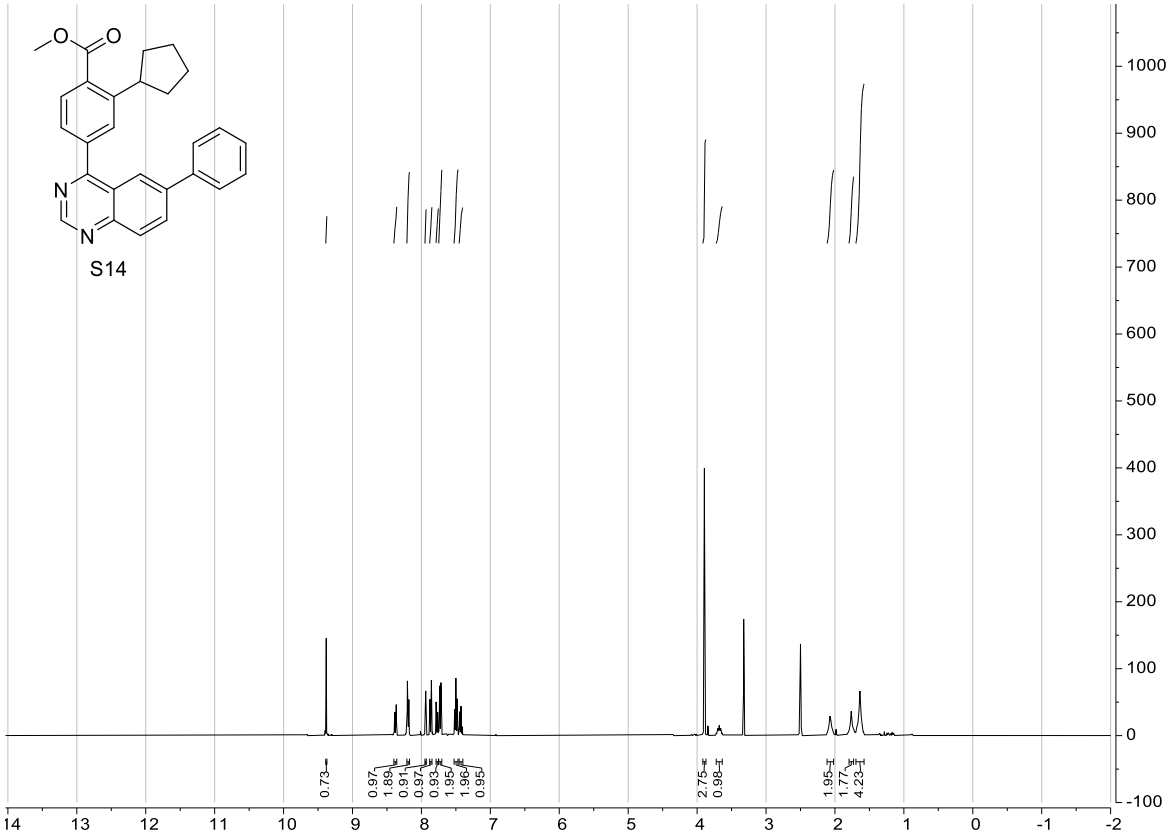


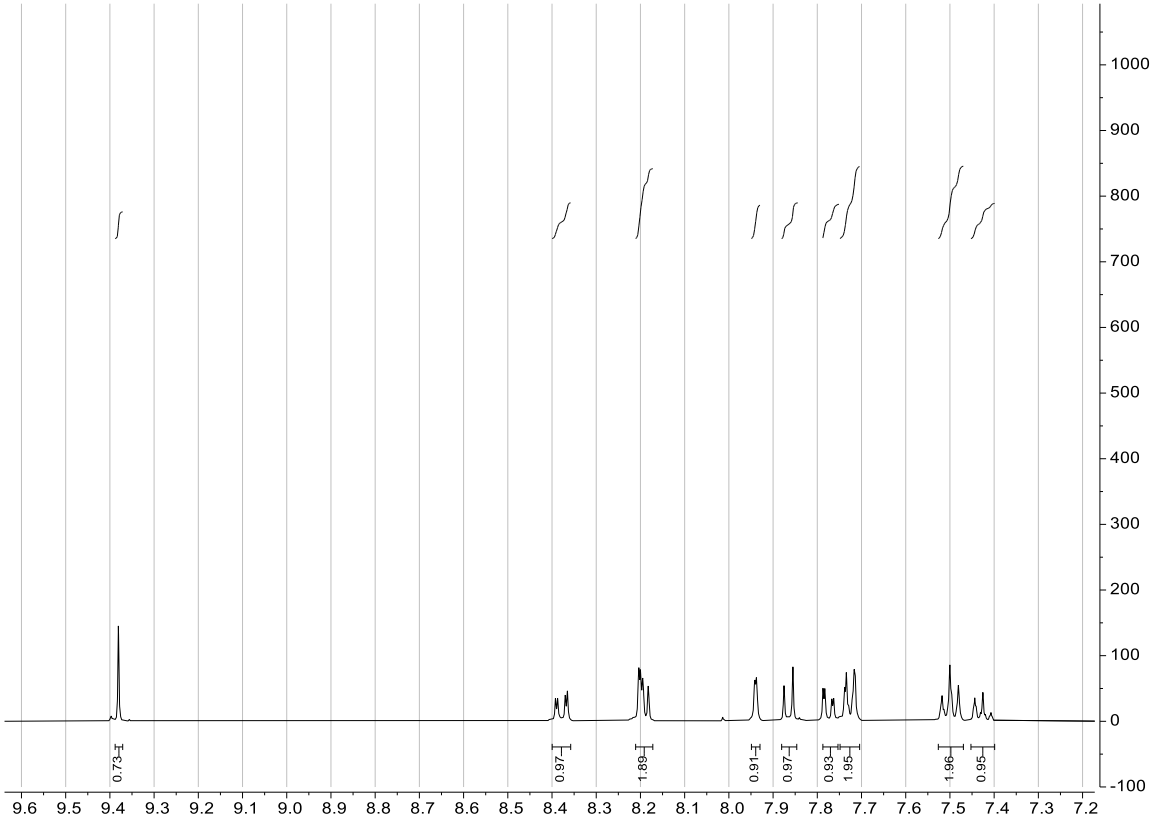


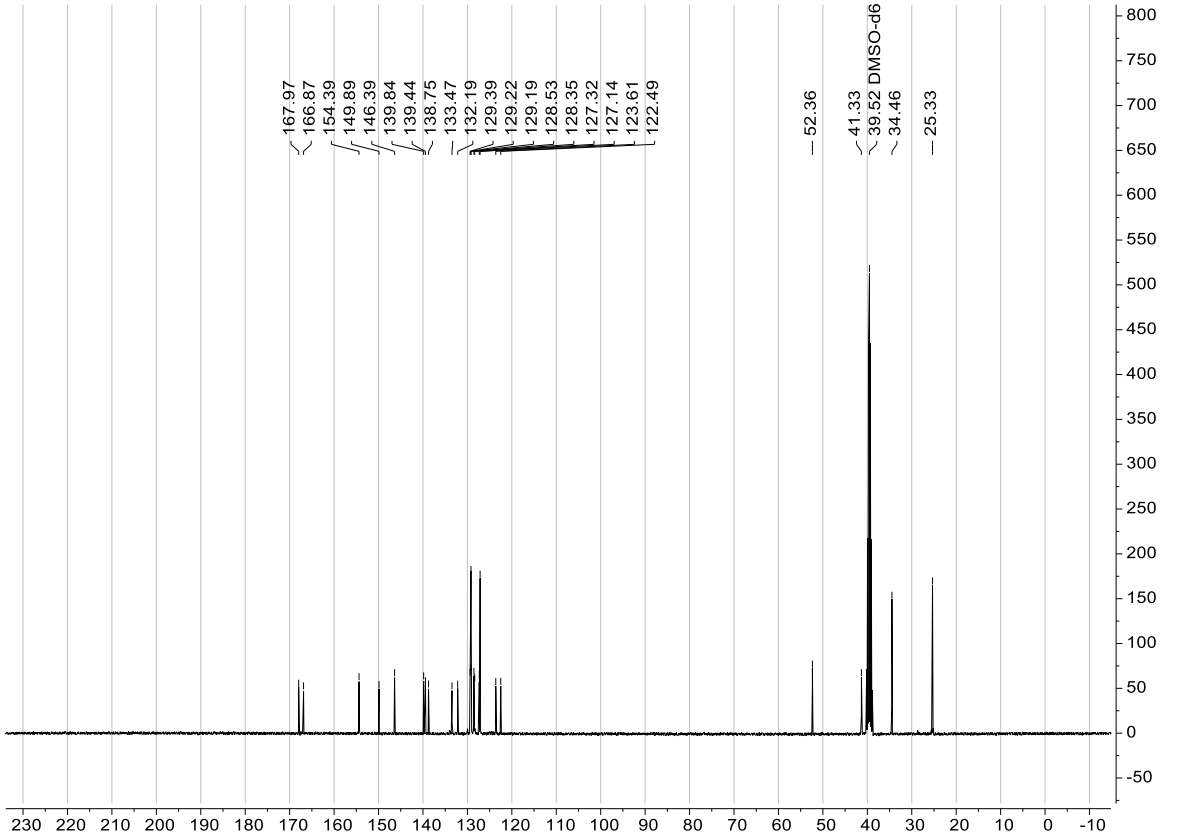

167.91
166.19
154.68
149.00
146.41
138.88
134.84
132.59
132.33
130.83
129.37
128.36
127.12
125.19
122.94
52.36
41.33
39.52 DMSO-d6
34.36
25.23

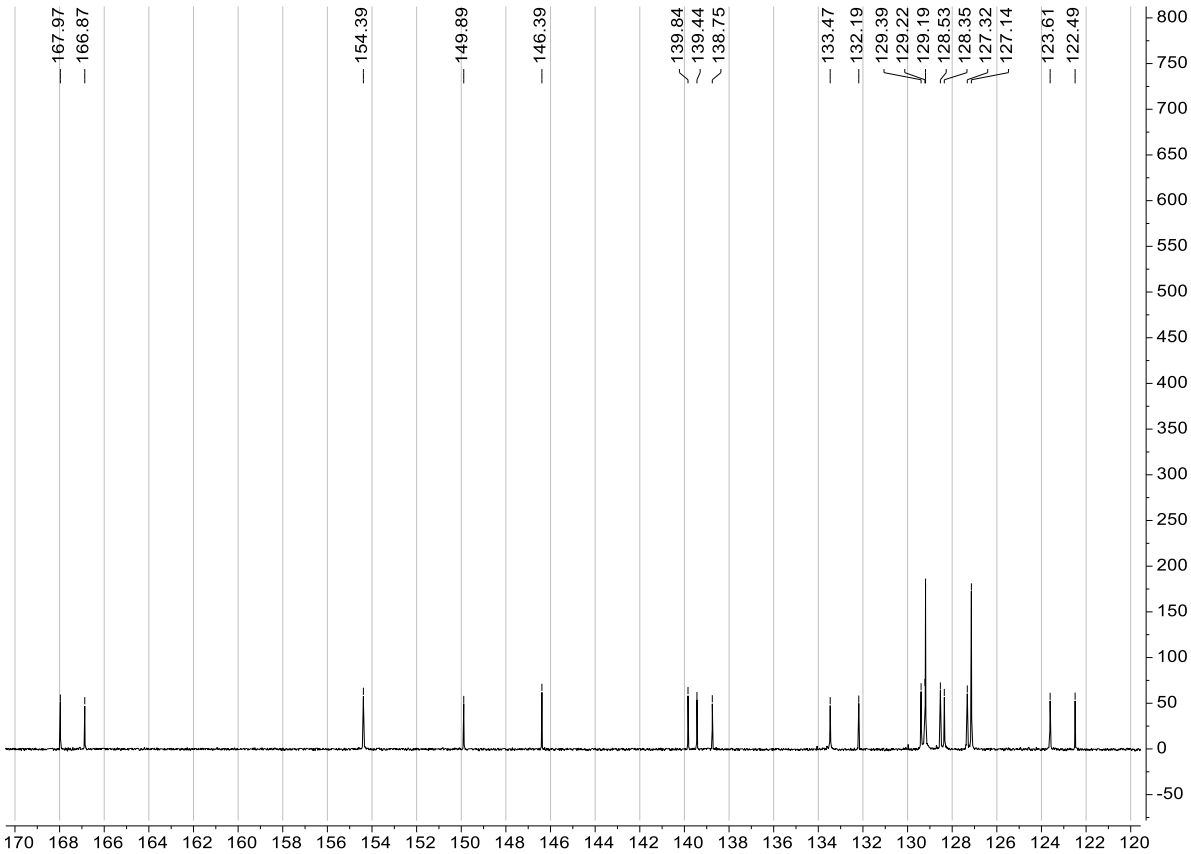

167.91
166.19
154.68
149.00
146.41
138.88
134.84
132.59
132.33
130.83
129.37
128.36
127.12
125.19
122.94

Methyl 2-cyclopentyl-4-(6-phenylquinazolin-4-yl)benzoate (SI4)

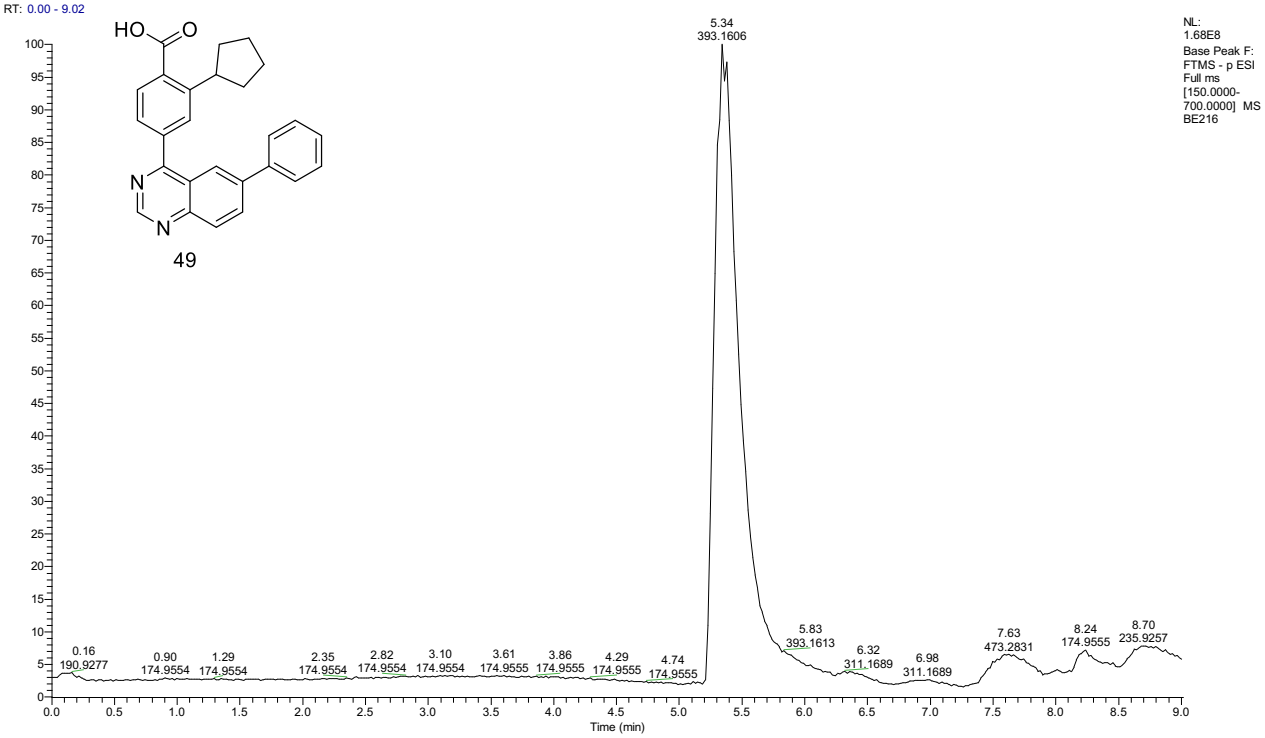


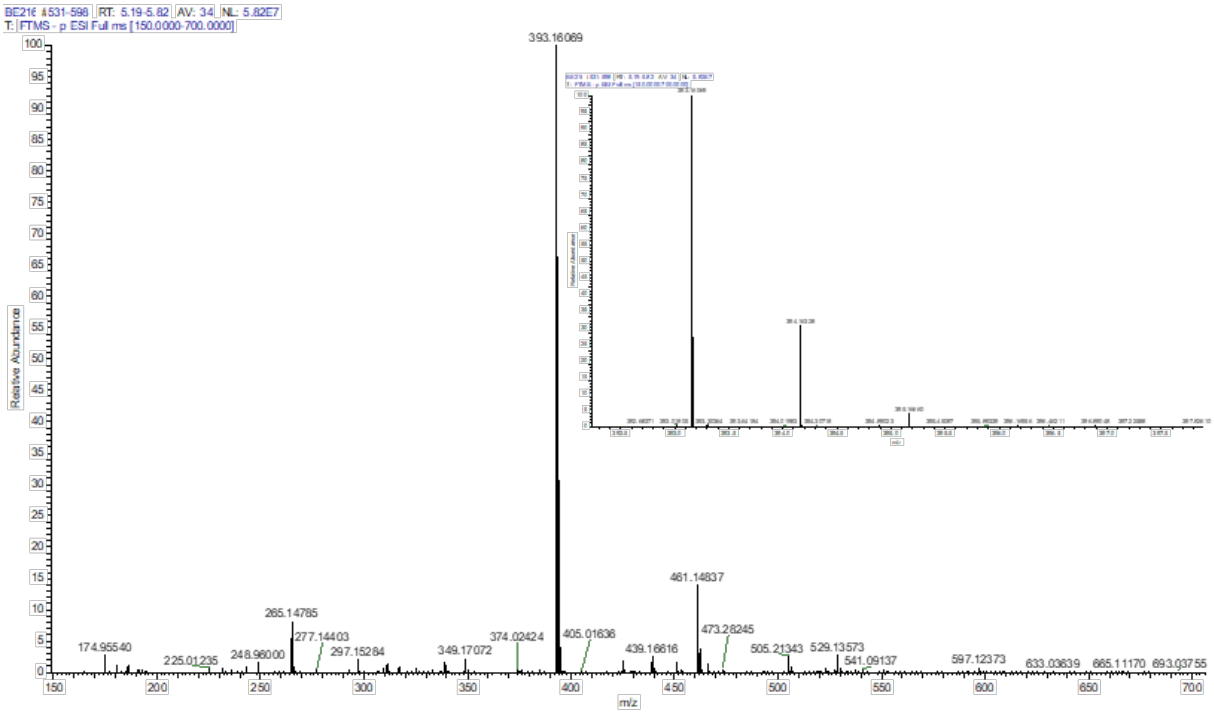


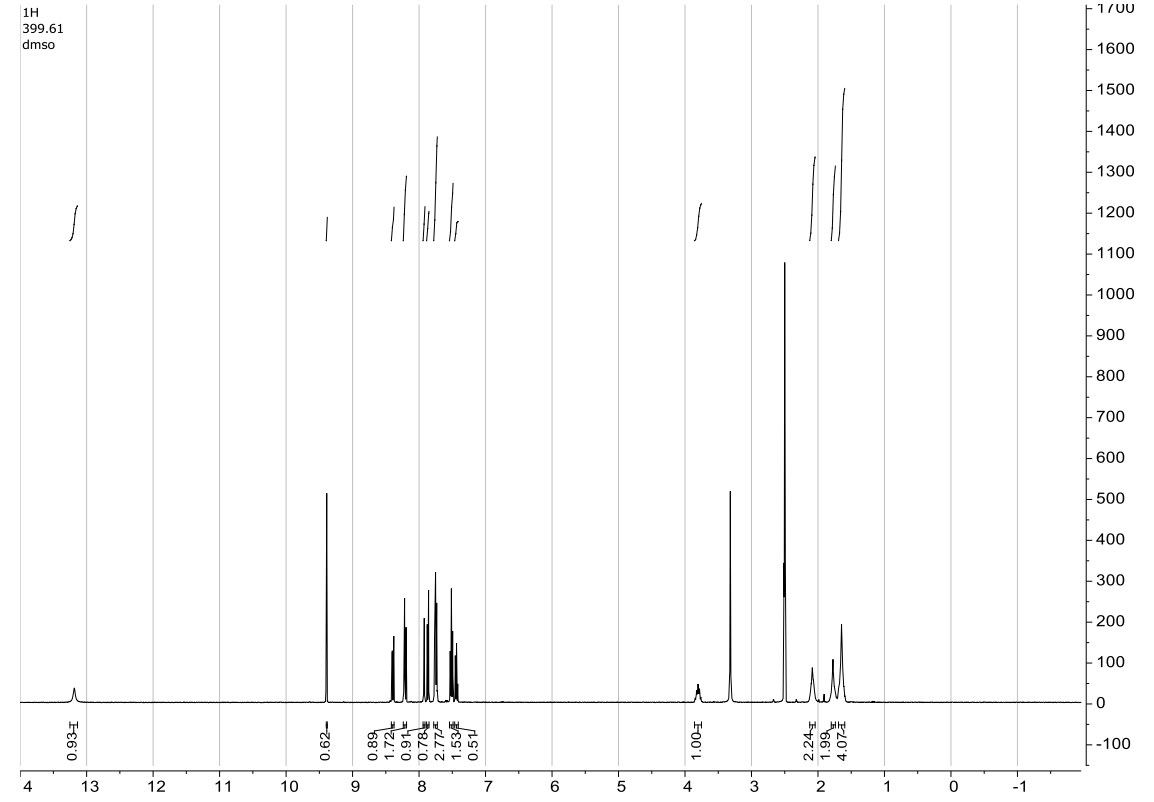

167.97
166.87
154.39
149.89
146.39
139.84
139.44
138.75
133.47
132.19
129.39
129.22
129.19
128.53
128.35
127.32
127.14
123.61
122.49
52.36
41.33
39.52 DMSO-d6
34.46
25.33

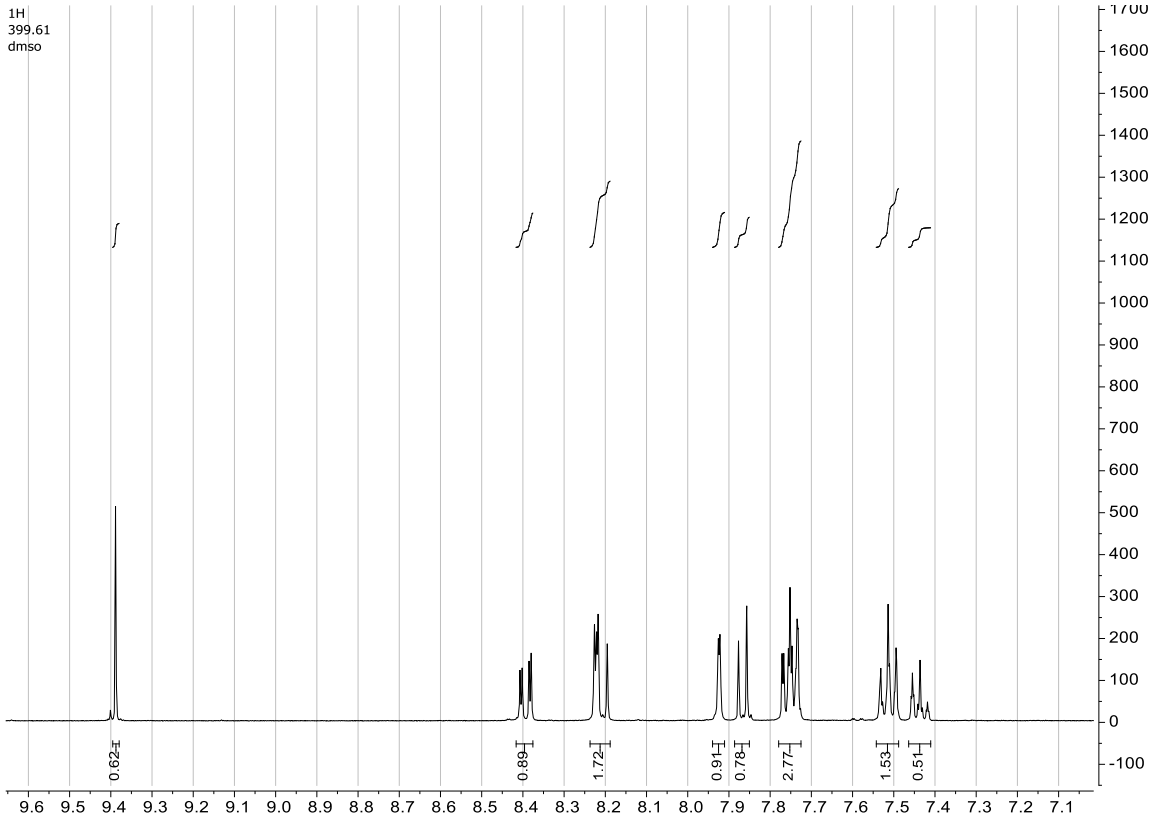

167.97
166.87
154.39
149.89
146.39
139.84
139.44
138.75
133.47
132.19
129.39
129.22
129.19
128.53
128.35
127.32
127.14
123.61
122.49

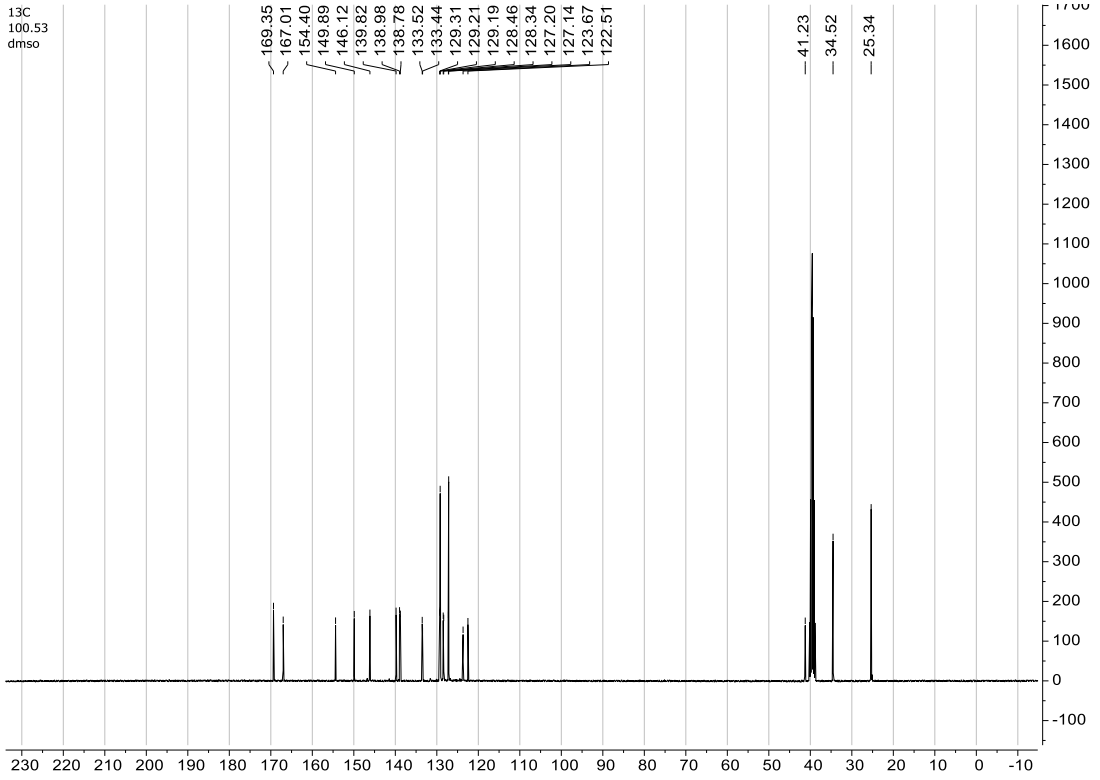
2-Cyclopentyl-4-(6-phenylquinazolin-4-yl)benzoic acid (49)

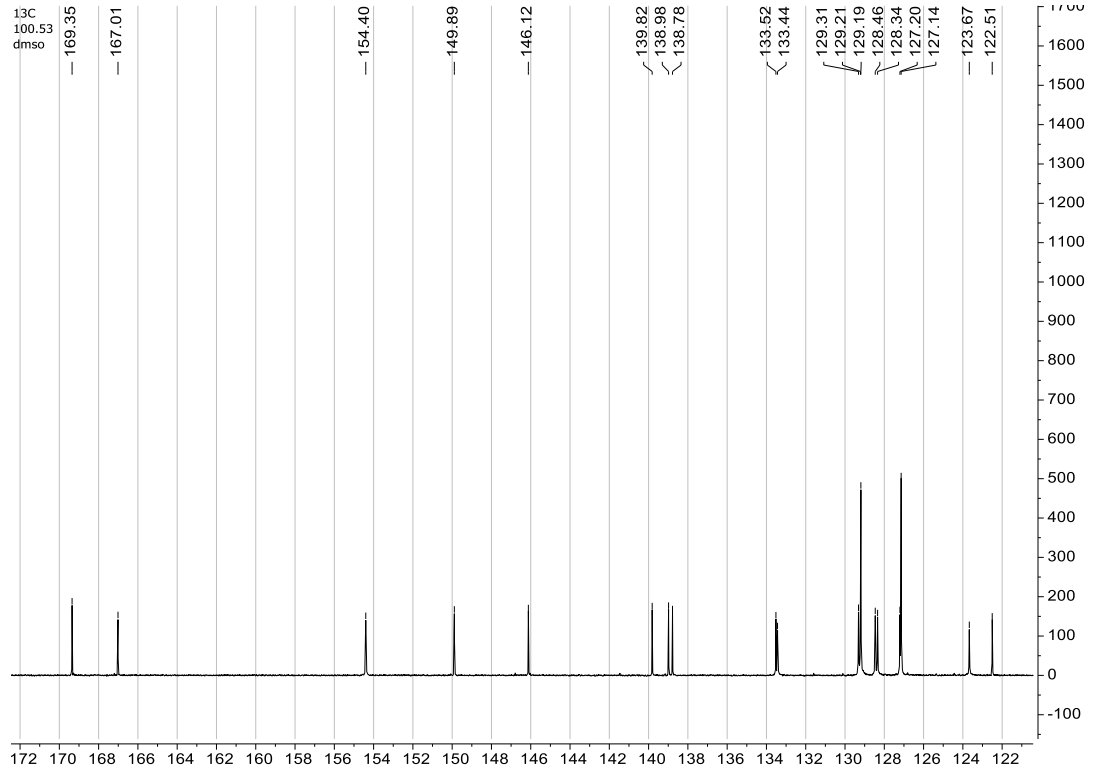




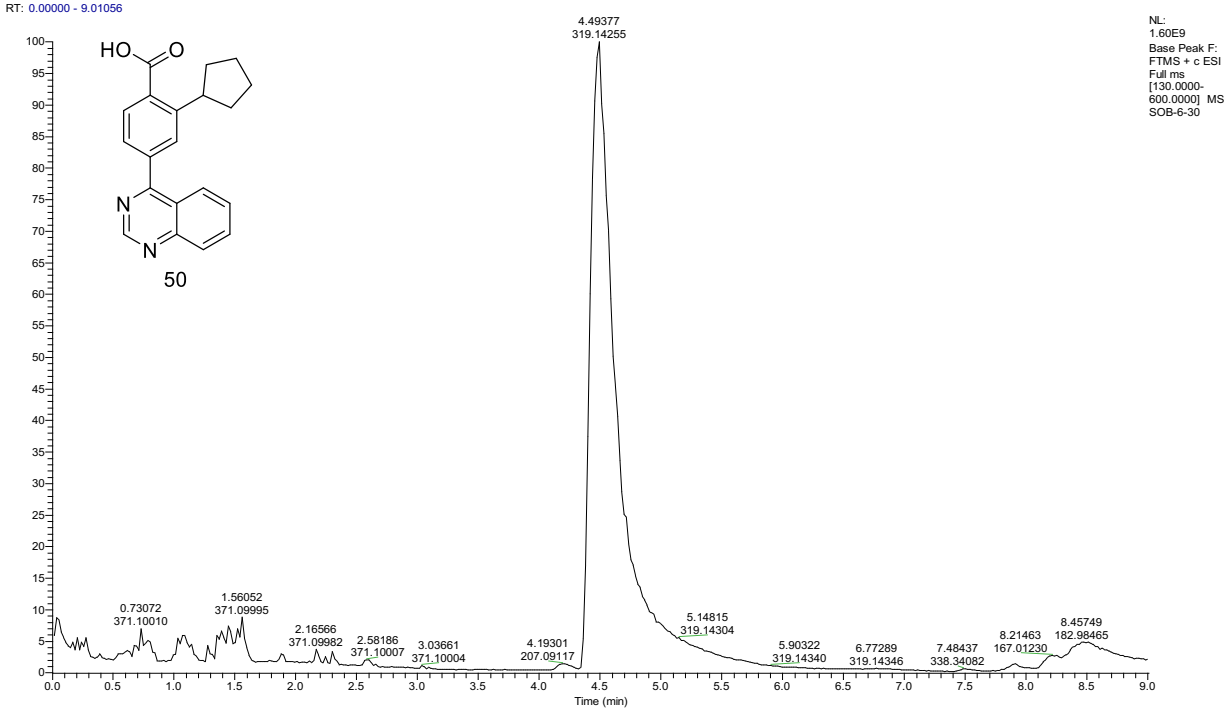

1H
399.61
dmso
0.93
0.62
0.89
1.72
0.91
0.78
2.77
1.53
0.51
1.00
2.24
1.99
4.07

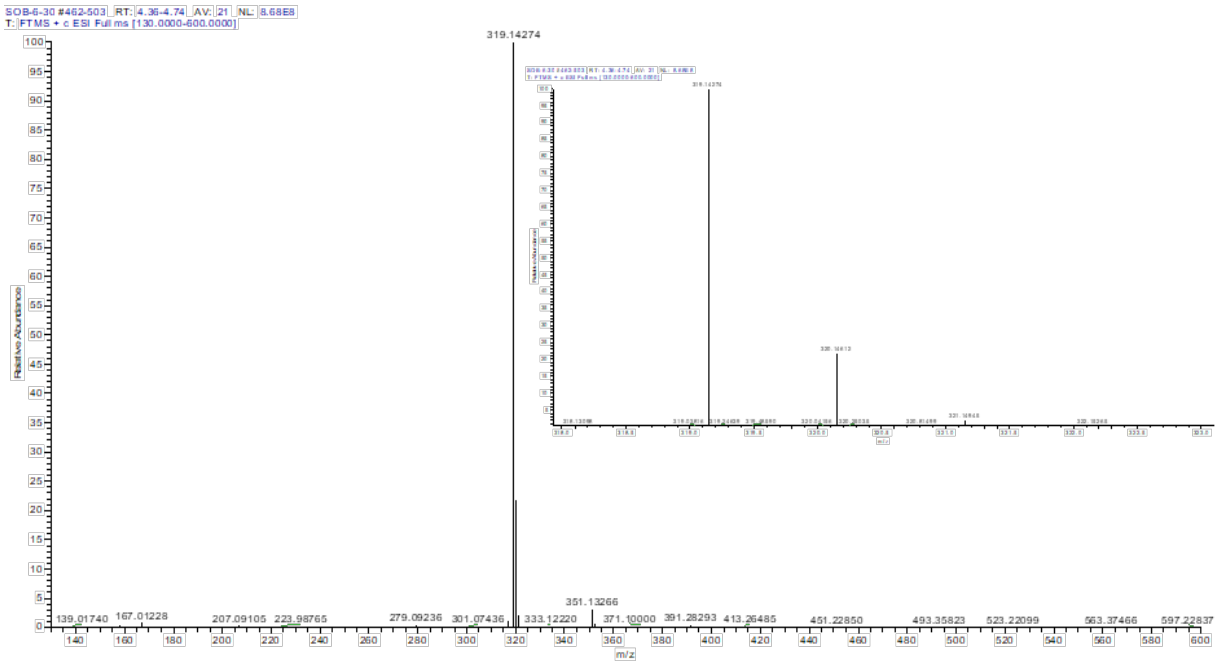

1H
399.61
dmso
0.62
0.89
1.72
0.91
0.78
2.77
1.53
0.51

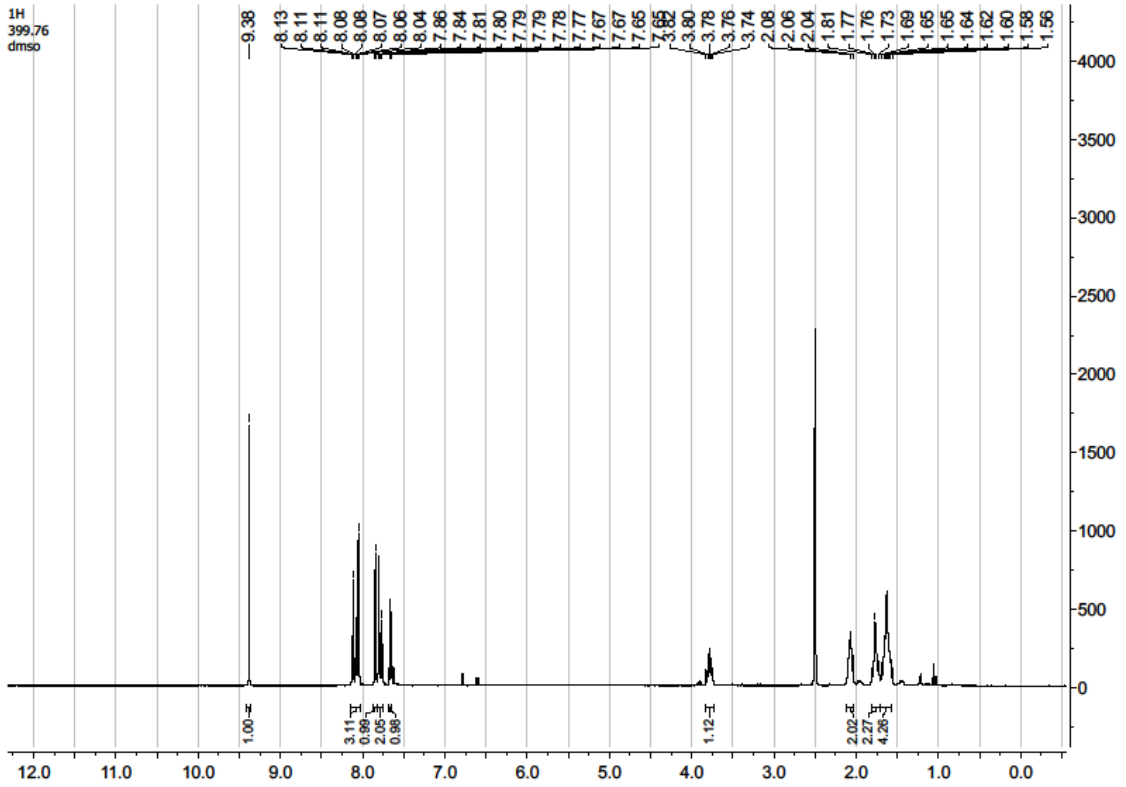

13C
100.53
dmso
169.35
167.01
154.40
149.89
146.12
139.82
138.98
138.78
133.52
133.44
129.31
129.21
129.19
128.46
128.34
127.20
127.14
123.67
122.51
41.23
34.52
25.34

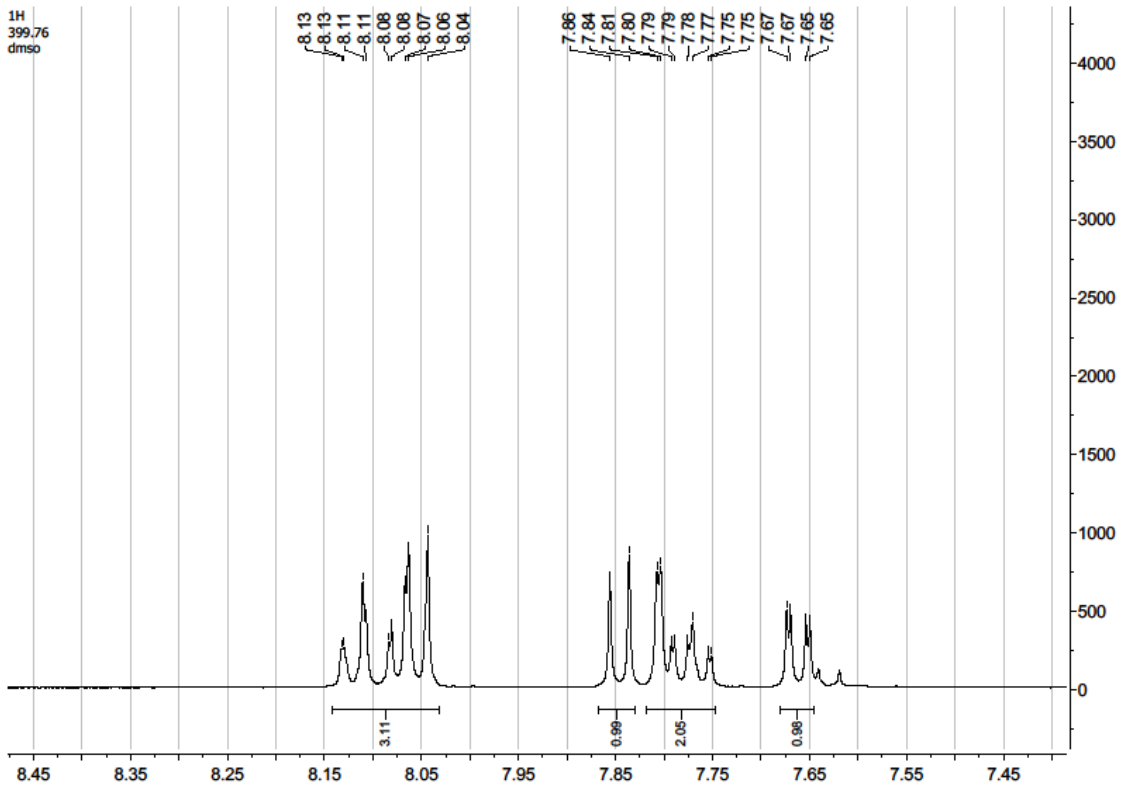

13C
100.53
dmso
169.35
167.01
154.40
149.89
146.12
139.82
138.98
138.78
133.52
133.44
129.31
129.21
129.19
128.46
128.34
127.20
127.14
123.67
122.51

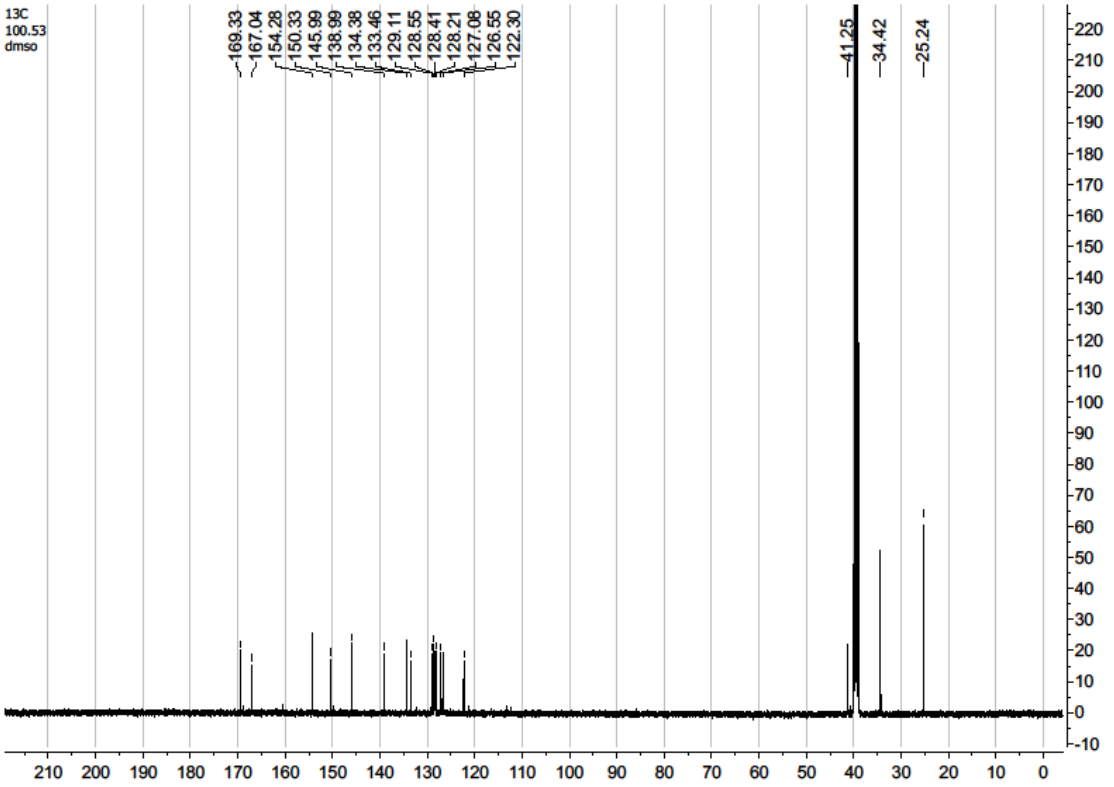
2-Cyclopentyl-4-(quinazolin-4-yl)benzoic acid (50)

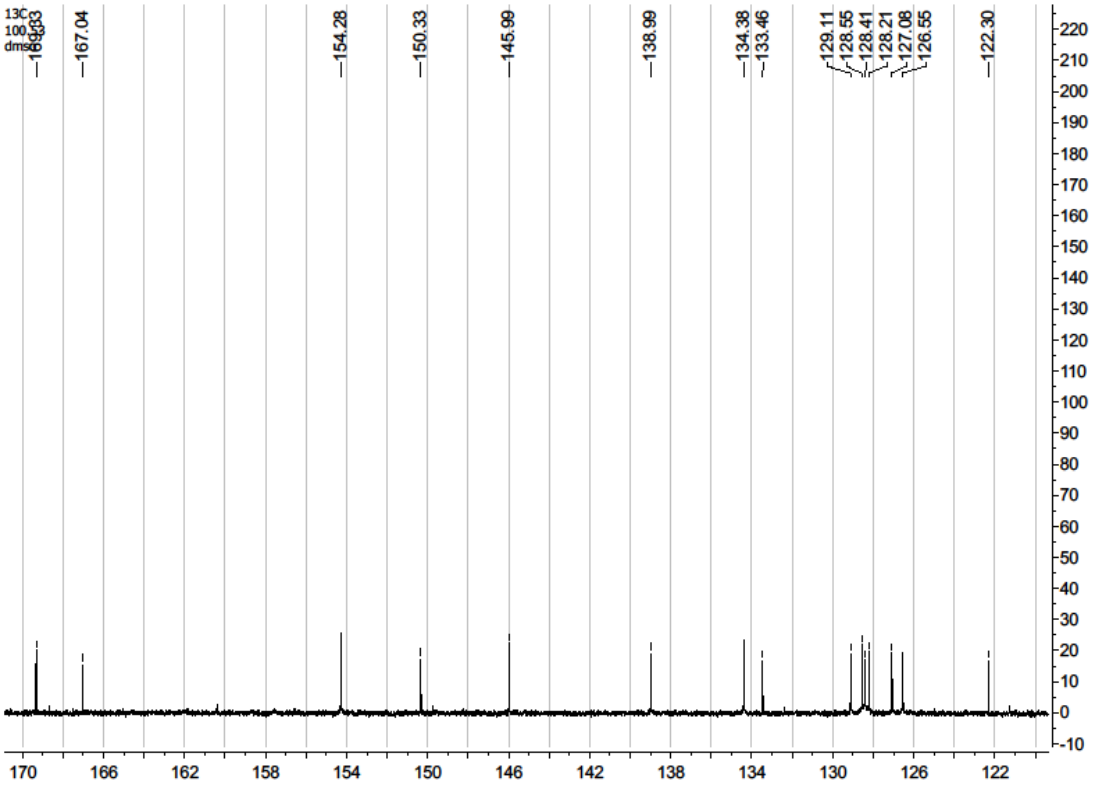




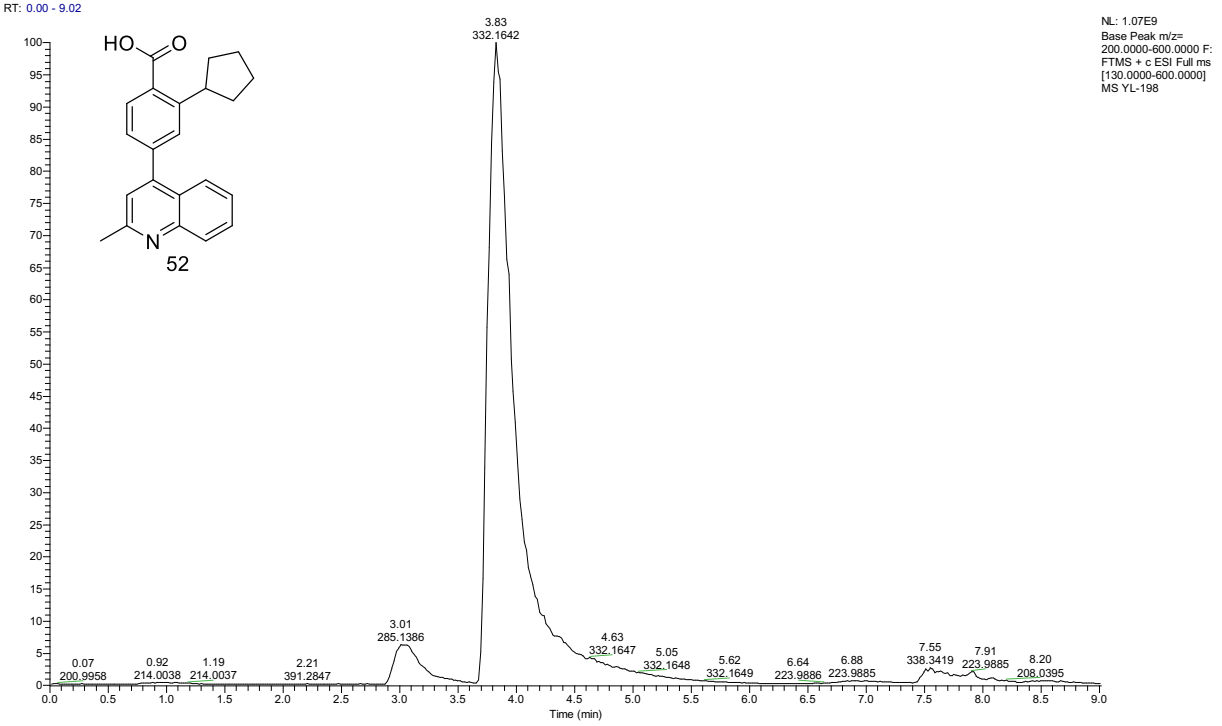

1H
399.76
dmso
9.38
8.13
8.11
8.11
8.08
8.08
8.07
8.06
8.04
7.86
7.84
7.81
7.80
7.79
7.79
7.78
7.77
7.67
7.67
7.65
3.82
3.80
3.78
3.76
3.74
2.08
2.06
2.04
1.81
1.77
1.76
1.73
1.69
1.65
1.65
1.64
1.62
1.60
1.58
1.56
1.00
3.11
0.99
2.05
0.98
1.12
2.02
2.27
4.26
4000
3500
3000
2500
2000
1500
1000
500
0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

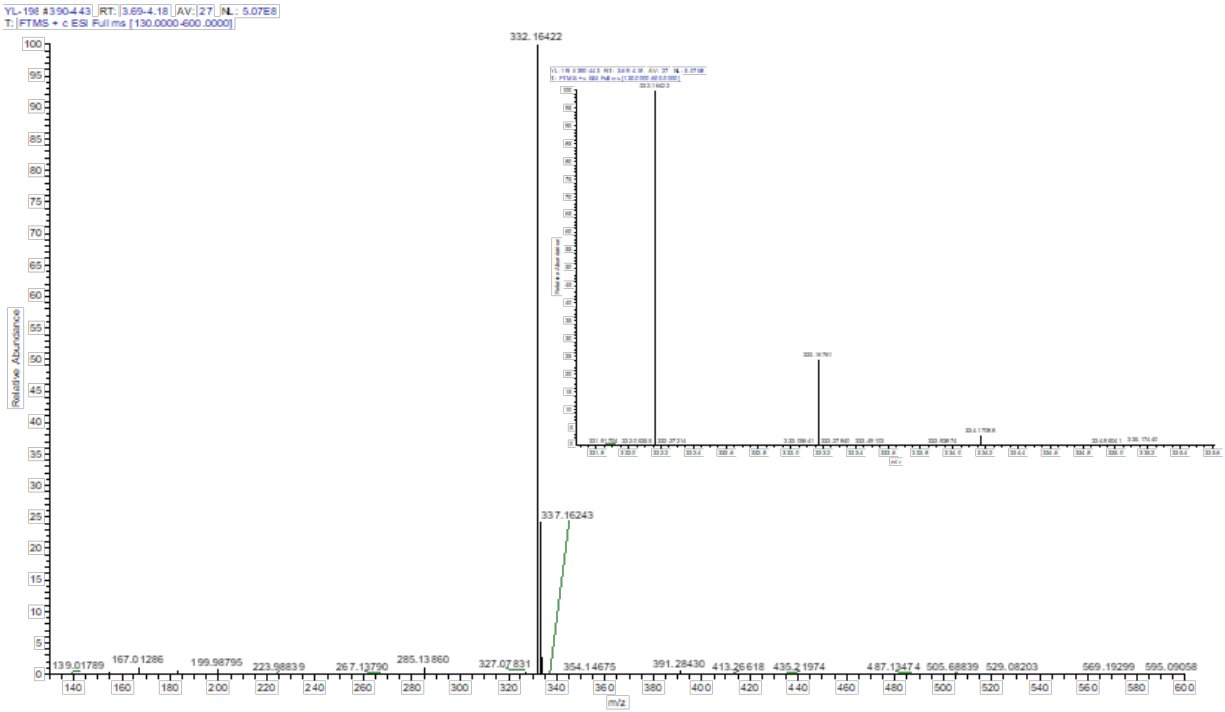

1H
399.76
dmso
8.13
8.13
8.11
8.11
8.08
8.08
8.07
8.06
8.04
7.86
7.84
7.81
7.80
7.79
7.79
7.78
7.77
7.75
7.75
7.67
7.67
7.65
7.65
3.11
0.99
2.05
0.98
4000
3500
3000
2500
2000
1500
1000
500
0
8.45
8.35
8.25
8.15
8.05
7.95
7.85
7.75
7.65
7.55
7.45

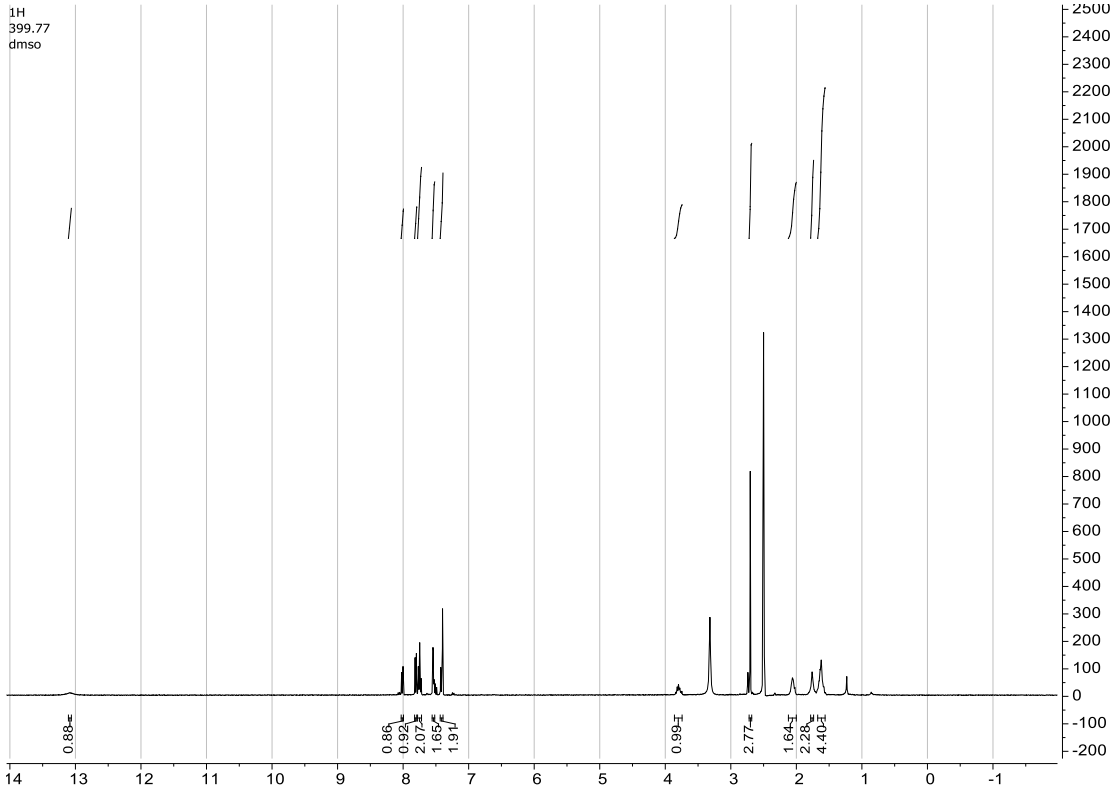

13C
100.53
dmso
169.33
167.04
154.28
150.33
145.99
138.99
134.38
133.46
129.11
128.55
128.41
128.21
127.08
126.55
122.30
41.25
34.42
25.24

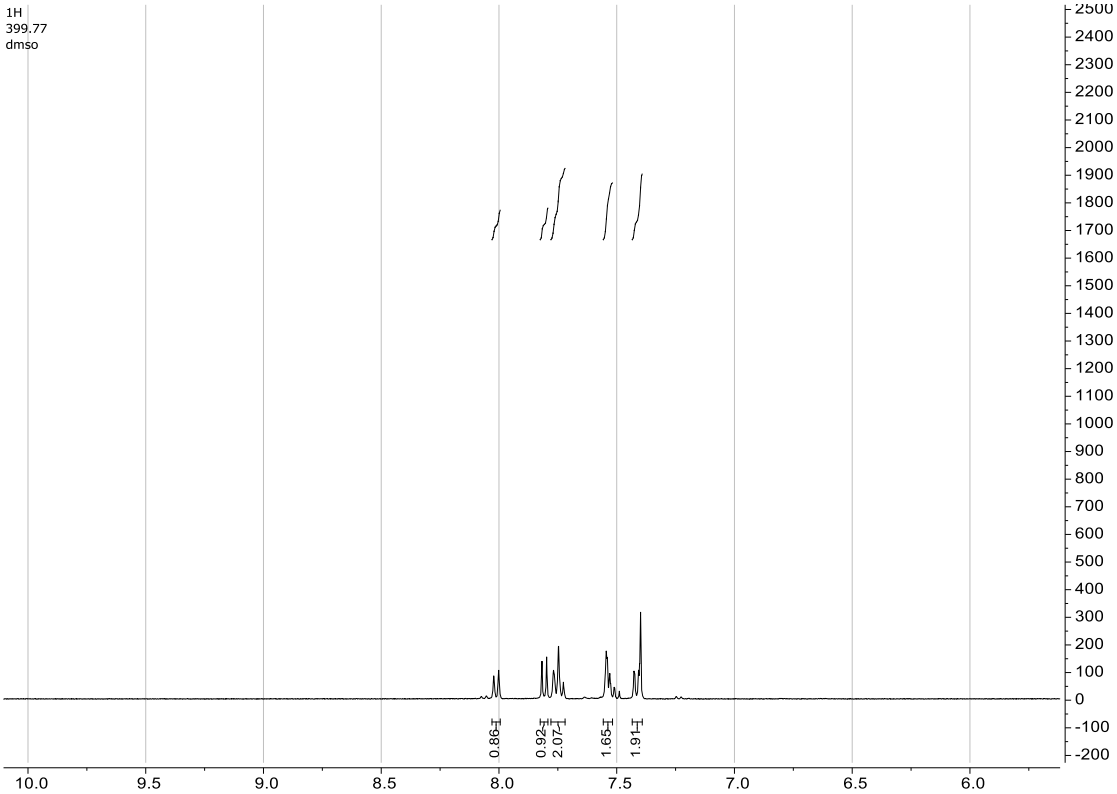

13C
100.53
dmso
169.33
167.04
154.28
150.33
145.99
138.99
134.38
133.46
129.11
128.55
128.41
128.21
127.08
126.55
122.30

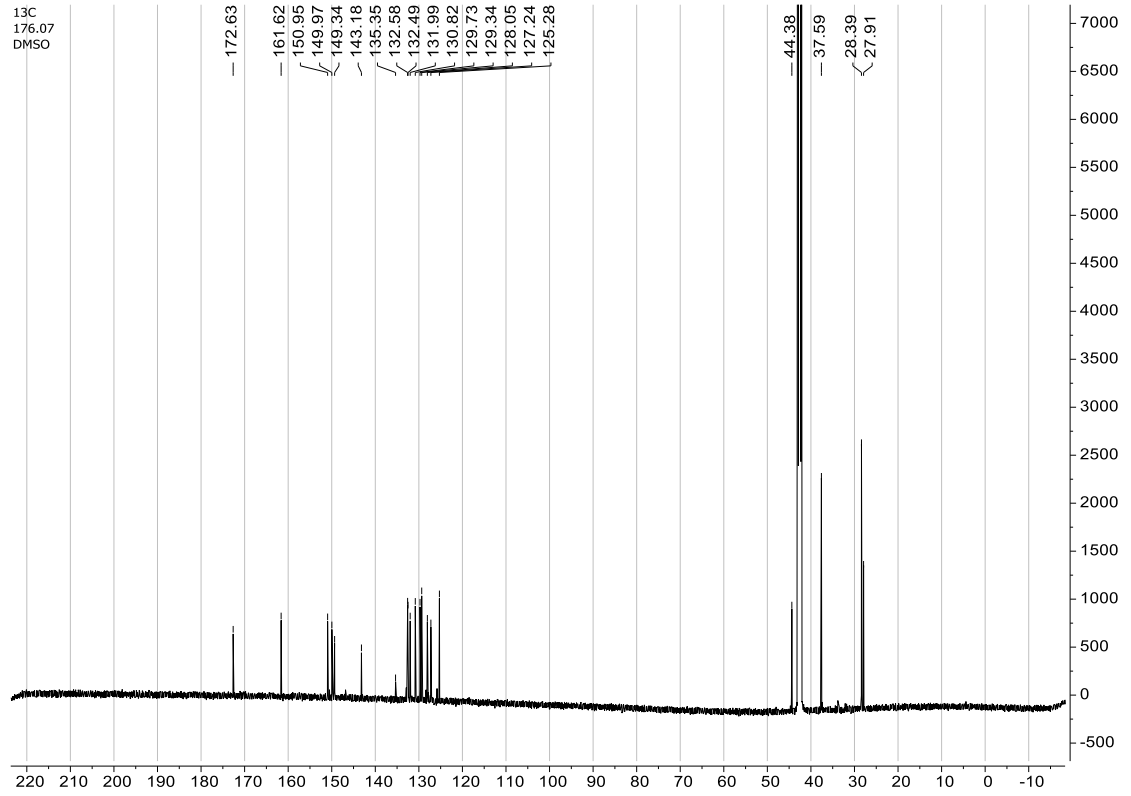
2-Cyclopentyl-4-(2-methylquinolin-4-yl)benzoic acid (52)

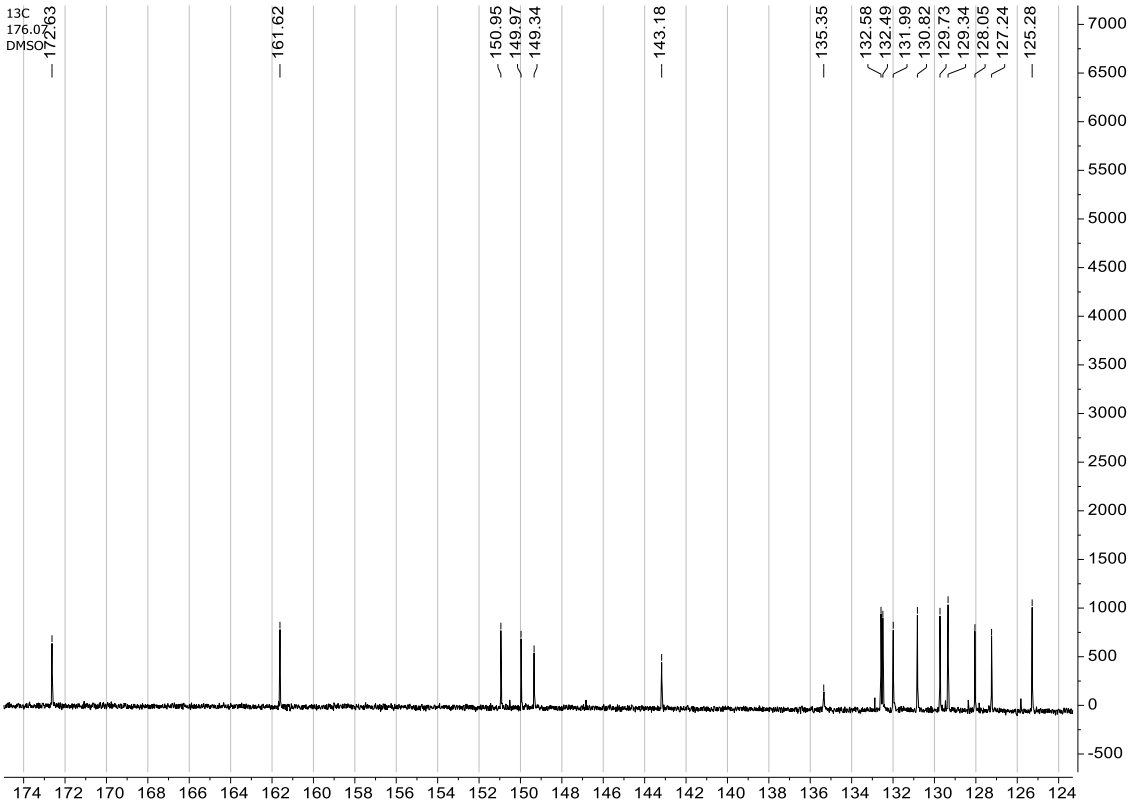




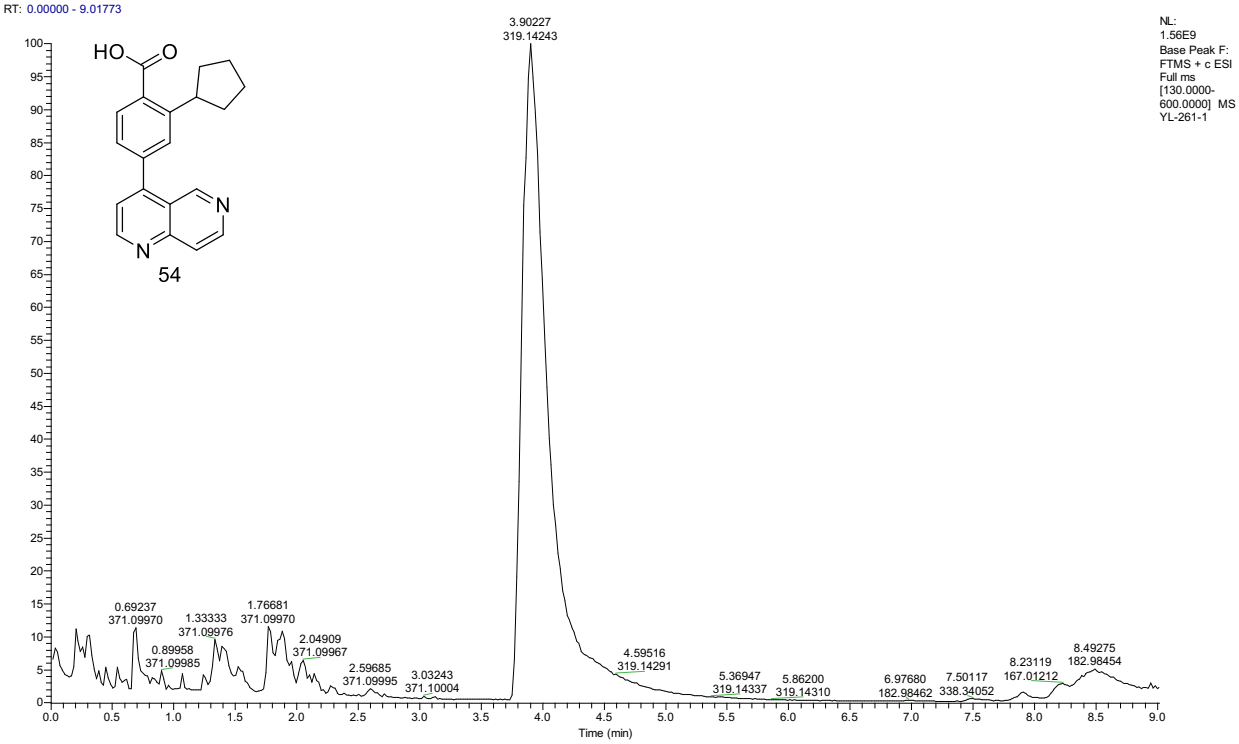

1H
399.77
dmso
0.88
0.86
0.92
2.07
1.65
1.91
0.99
2.77
1.64
2.28
4.40

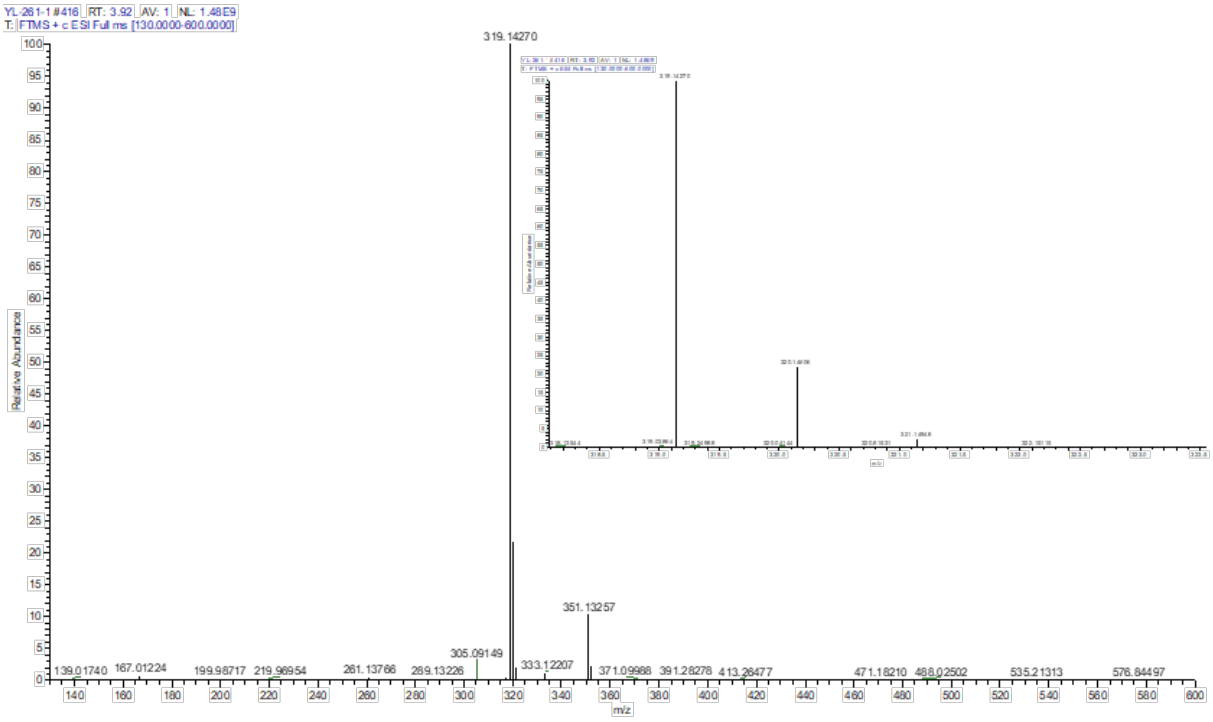

1H
399.77
dmso
0.86
0.92
2.07
1.65
1.91

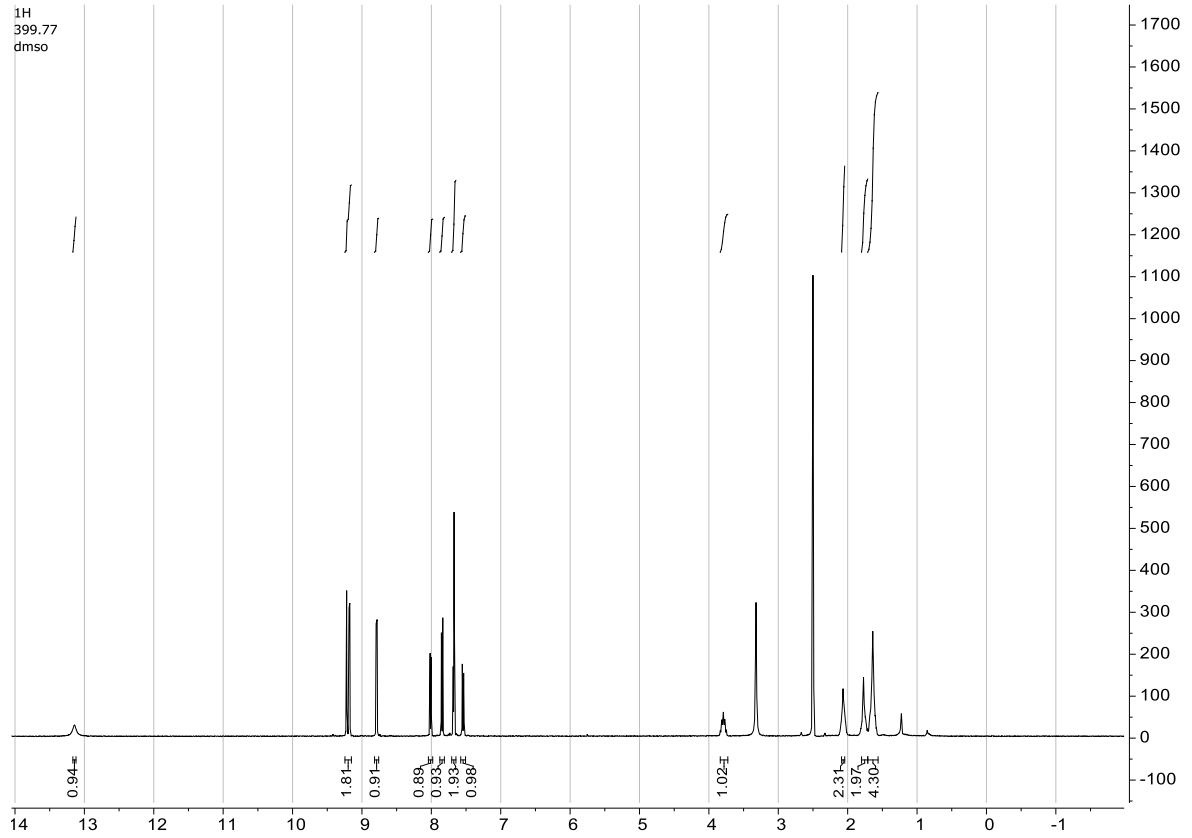

13C
176.07
DMSO
172.63
161.62
150.95
149.97
149.34
143.18
135.35
132.58
132.49
131.99
130.82
129.73
129.34
128.05
127.24
125.28
44.38
37.59
28.39
27.91

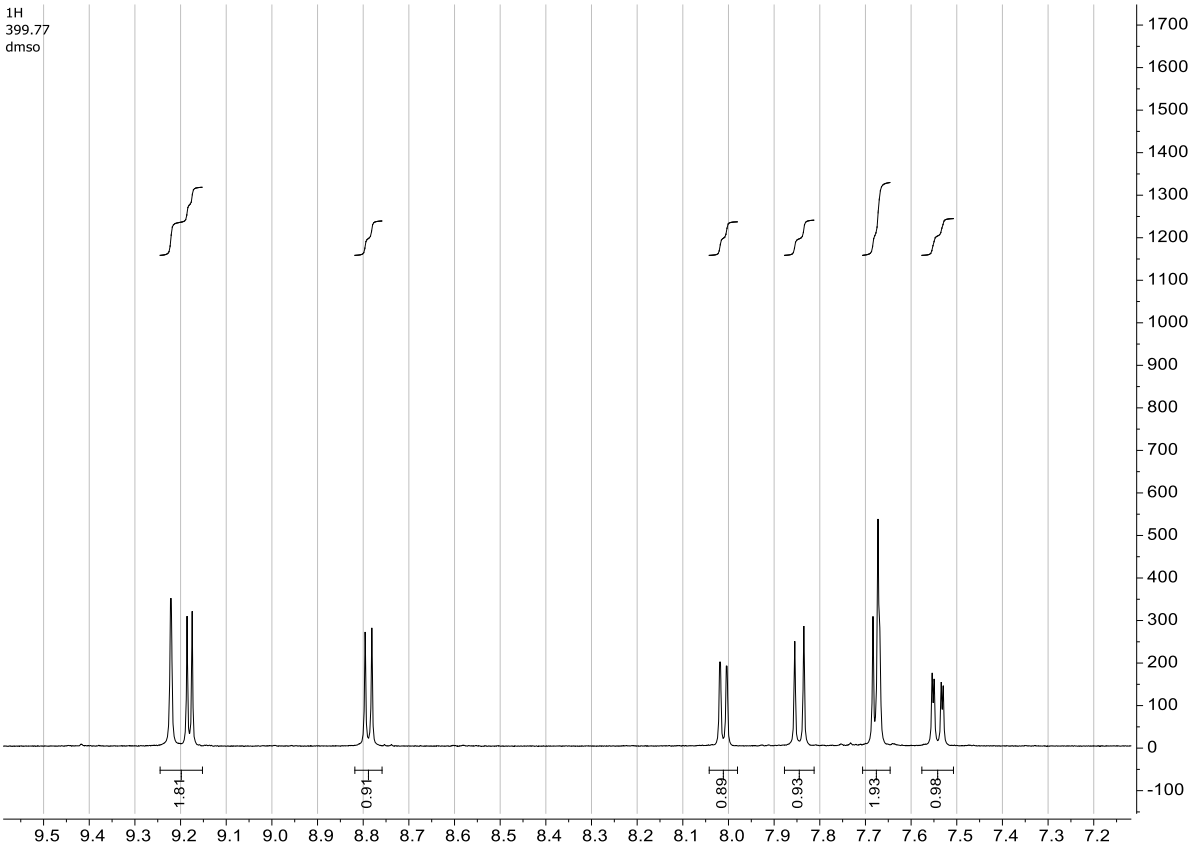

13C
176.07
DMSO
172.63
161.62
150.95
149.97
149.34
143.18
135.35
132.58
132.49
131.99
130.82
129.73
129.34
128.05
127.24
125.28

2-Cyclopentyl-4-(1,6-naphthyridin-4-yl)benzoic acid (54)






1H
399.77
dmso
0.94
1.81
0.91
0.89
0.93
1.93
0.98
1.02
2.31
1.97
4.30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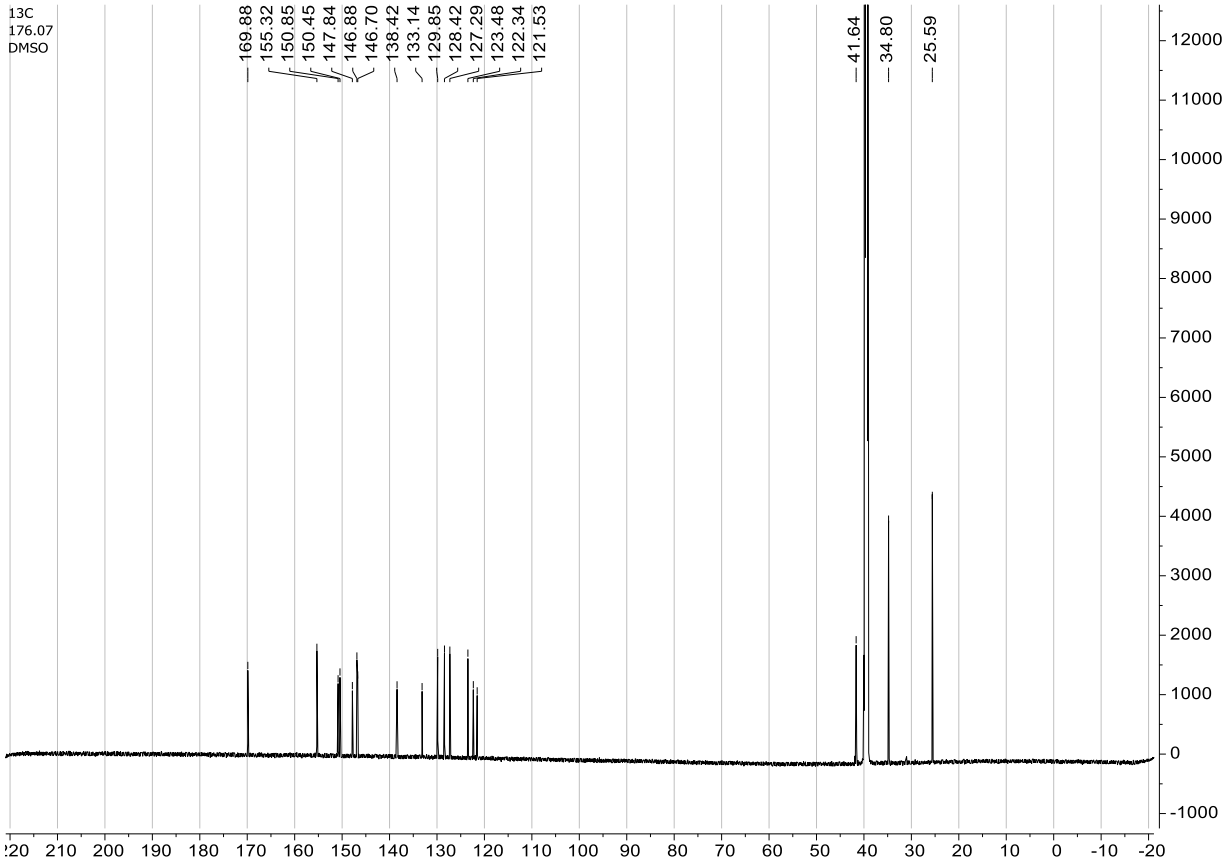
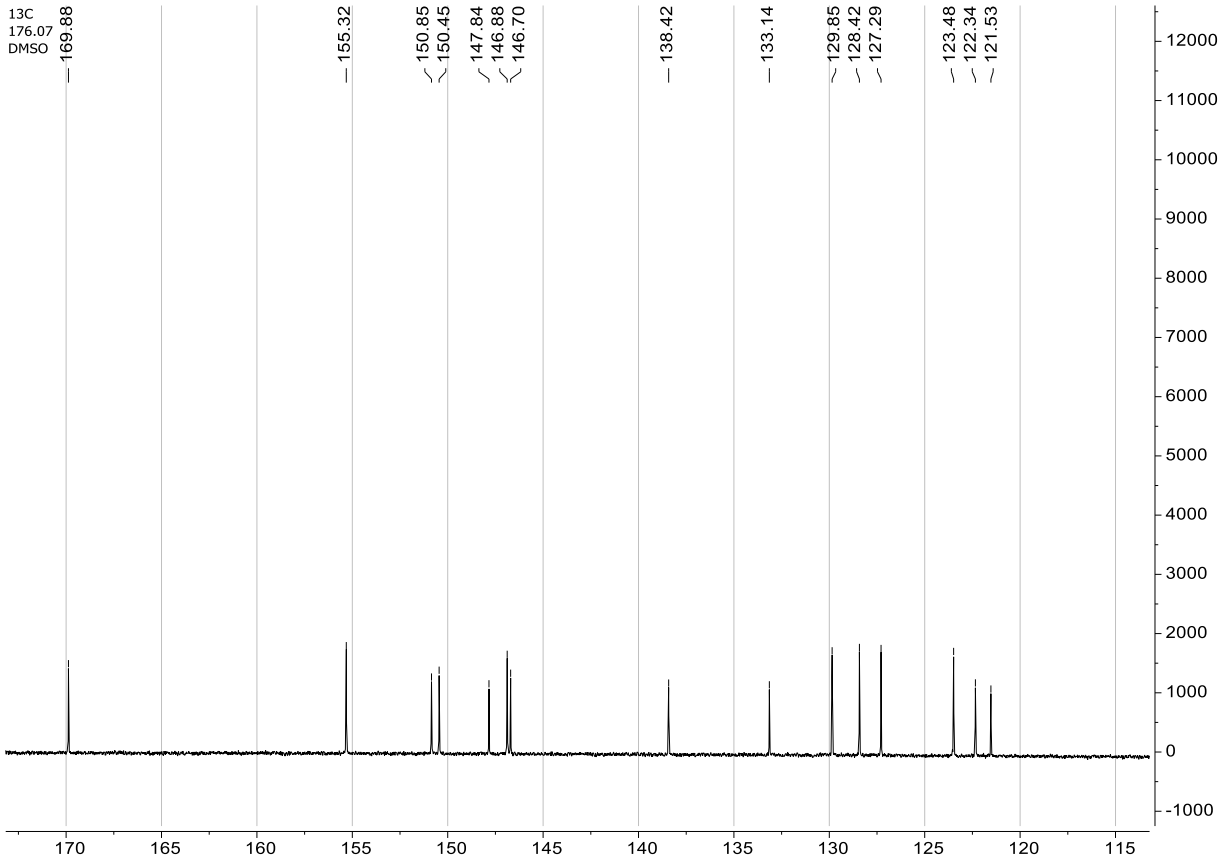
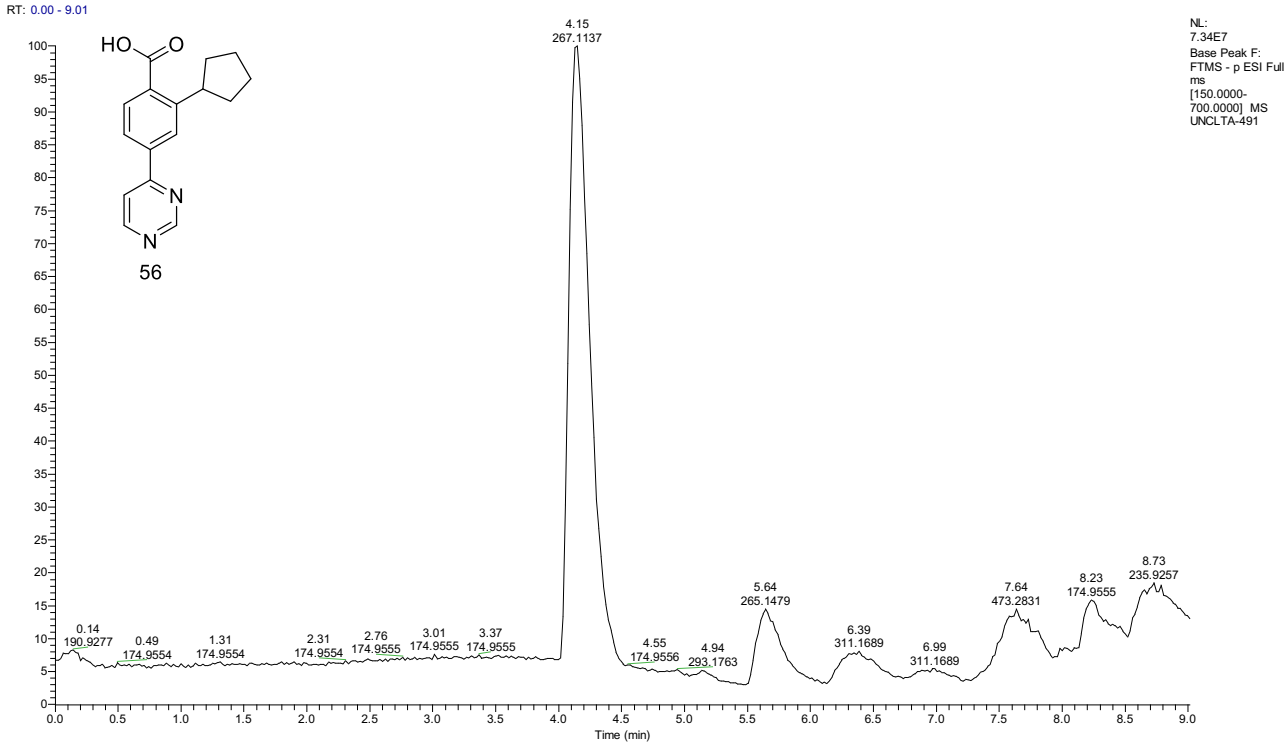
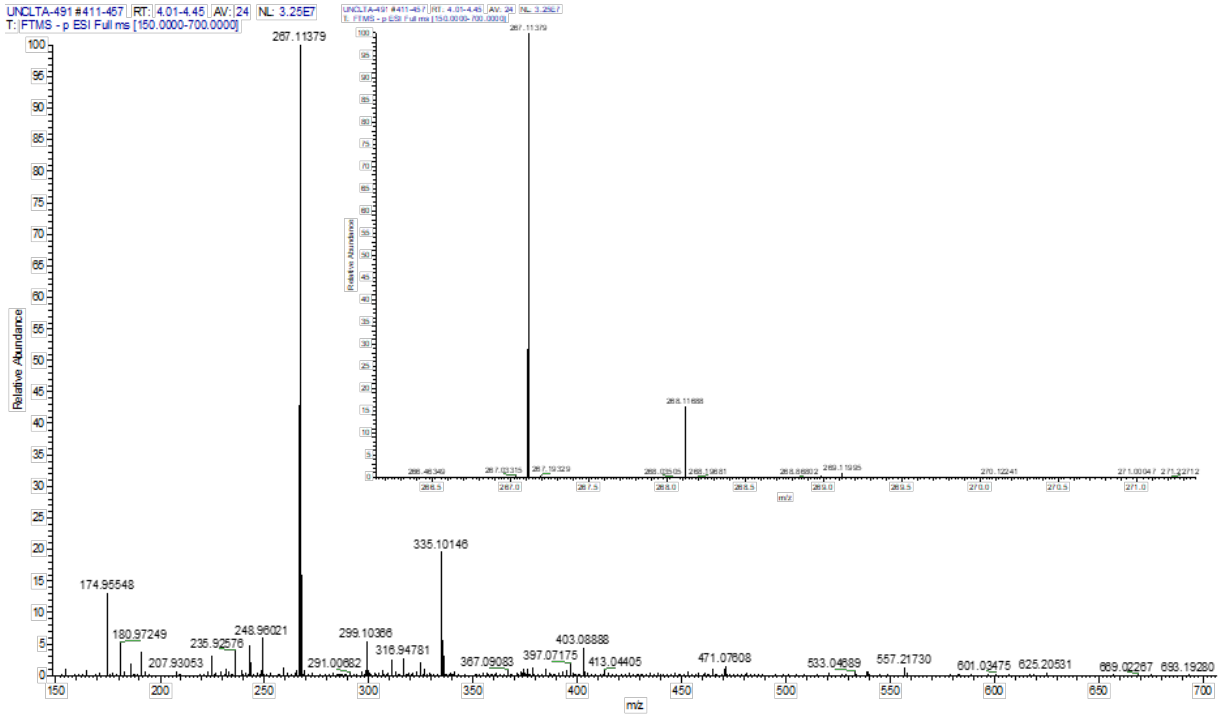
399.77
dmso
1.81
0.91
0.89
0.93
1.93
0.98

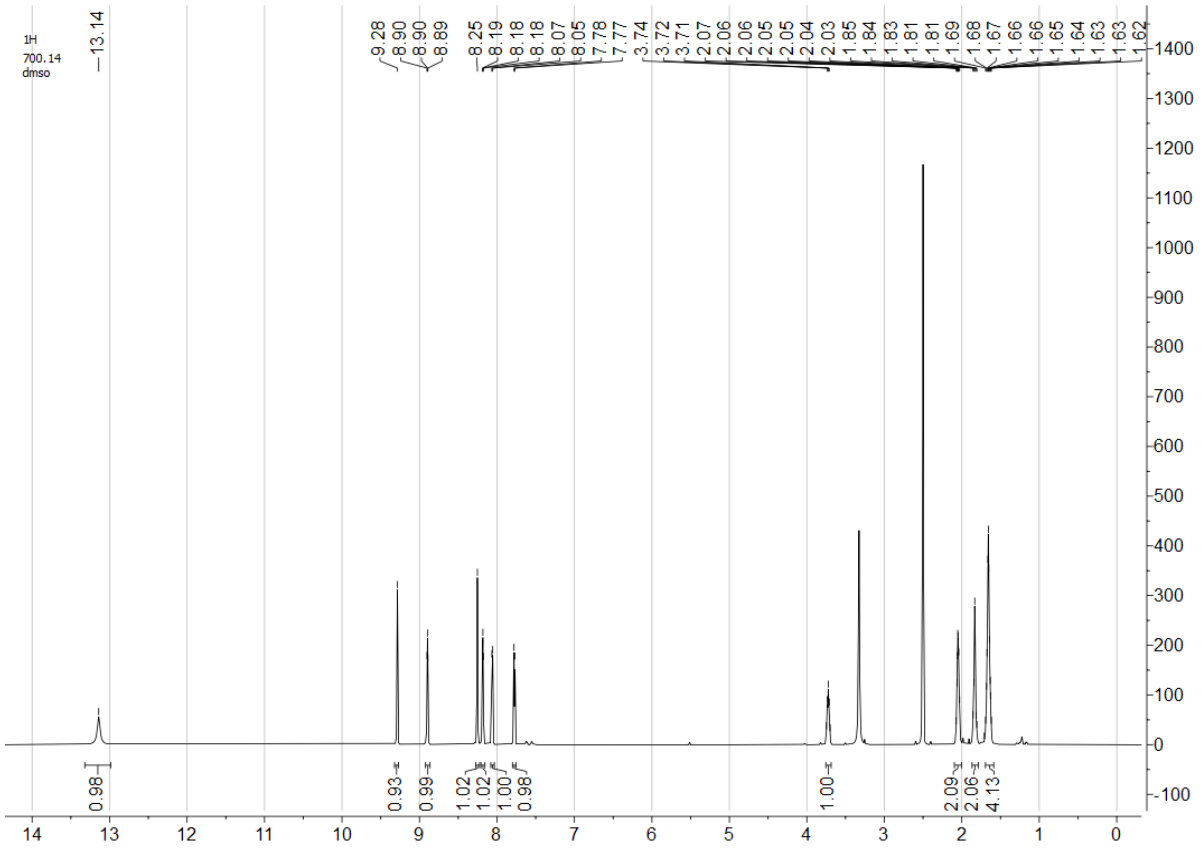

13C
176.07
DMSO
169.88
155.32
150.85
150.45
147.84
146.88
146.70
138.42
133.14
129.85
128.42
127.29
123.48
122.34
121.53
41.64
34.80
25.59


13C
176.07
DMSO
169.88
155.32
150.85
150.45
147.84
146.88
146.70
138.42
133.14
129.85
128.42
127.29
123.48
122.34
121.53

2-Cyclopentyl-4-(pyrimidin-4-yl)benzoic acid (56)






1H
700.14
dmso
13.14
9.28
8.90
8.90
8.89
8.25
8.19
8.18
8.18
8.07
8.05
7.78
7.77
3.74
3.72
3.71
2.07
2.06
2.06
2.05
2.05
2.04
2.03
1.85
1.84
1.83
1.81
1.81
1.69
1.68
1.67
1.66
1.66
1.65
1.64
1.63
1.63
1.62
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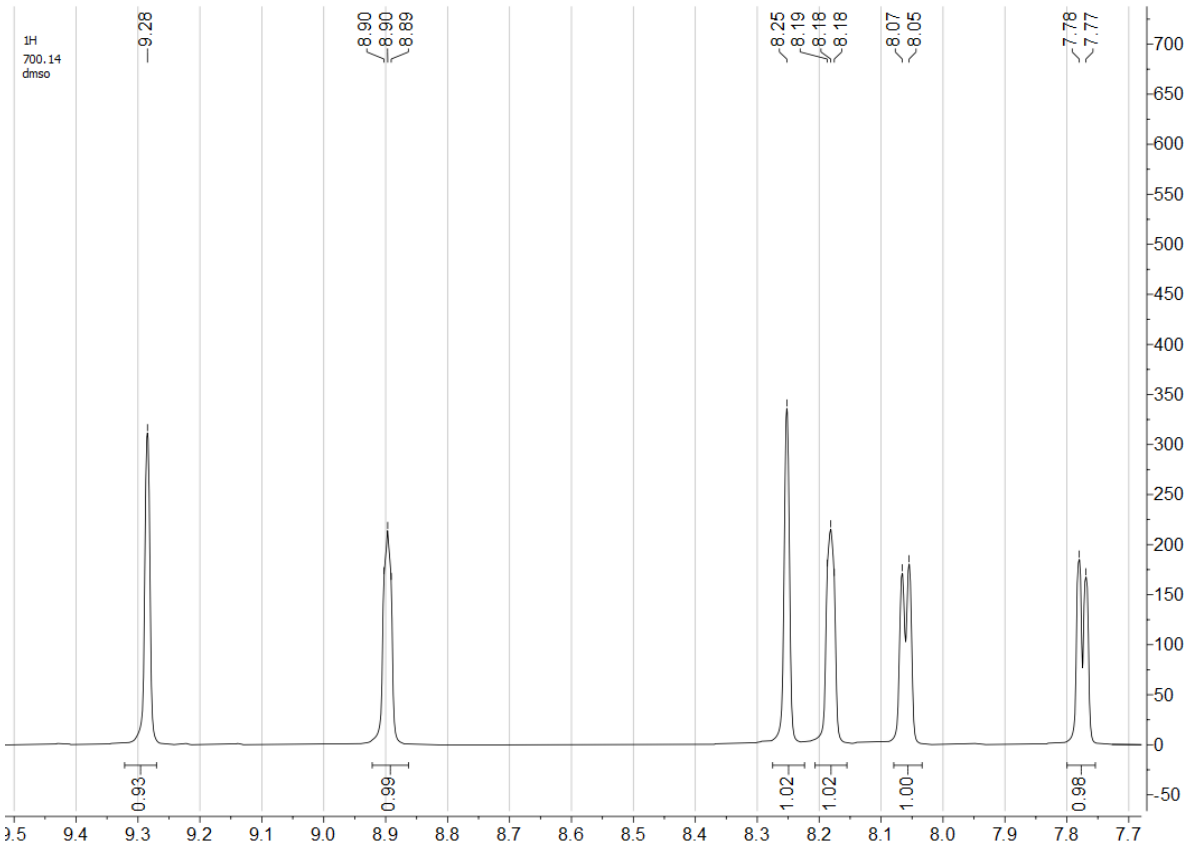
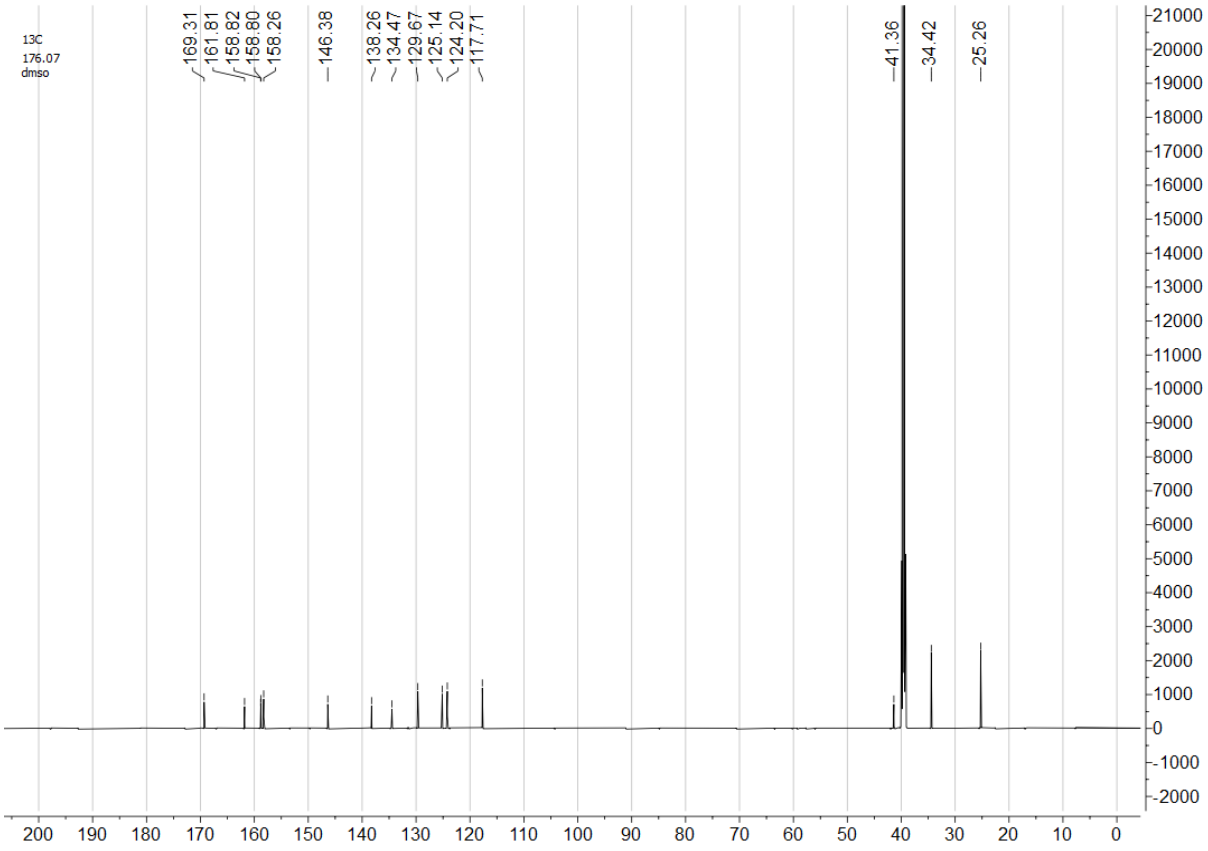
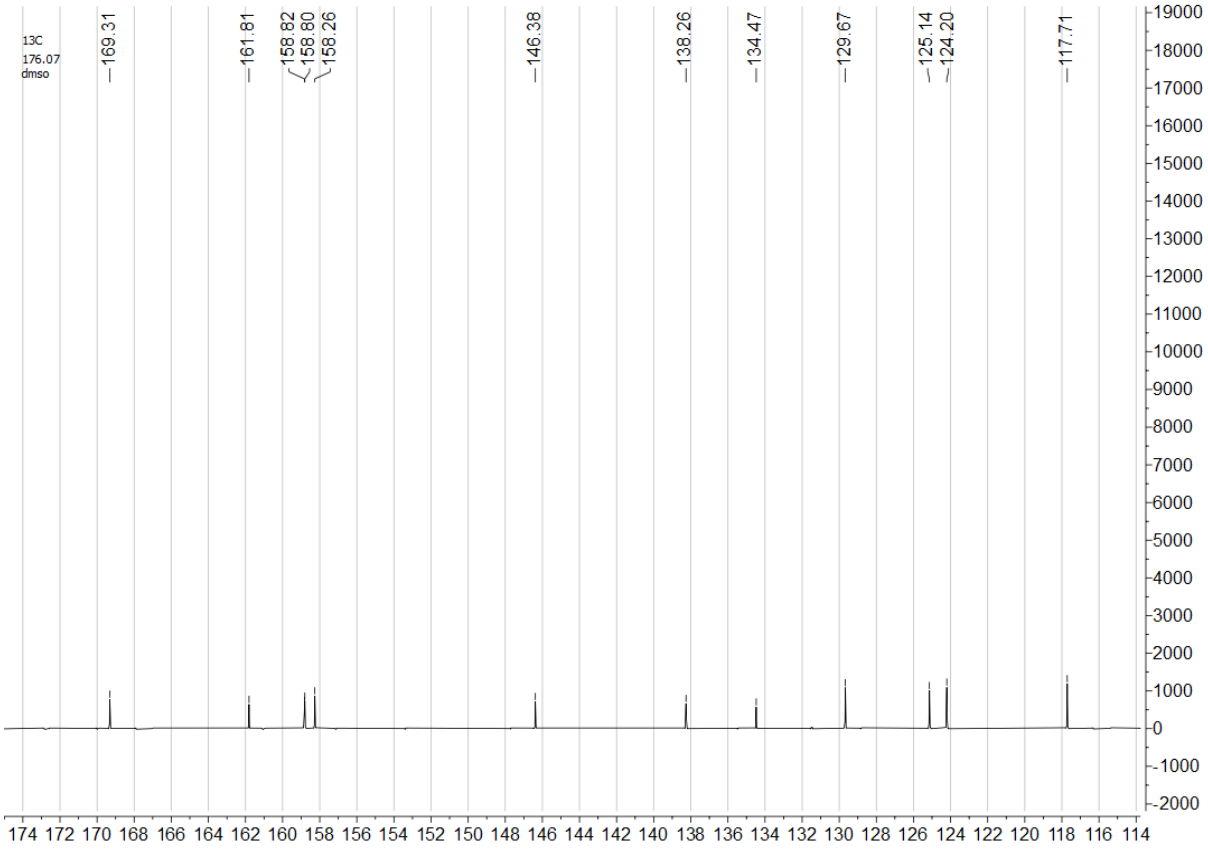
0.93
0.99
1.02
1.02
1.00
0.98
1.00
2.09
2.06
4.13


1H
700.14
dmso
9.28
8.90
8.90
8.89
8.25
8.19
8.18
8.18
8.07
8.05
7.78
7.77
0.93
0.99
1.02
1.02
1.00
0.98


13C
176.07
dmso
169.31
161.81
158.82
158.80
158.26
146.38
138.26
134.47
129.67
125.14
124.20
117.71
41.36
34.42
25.26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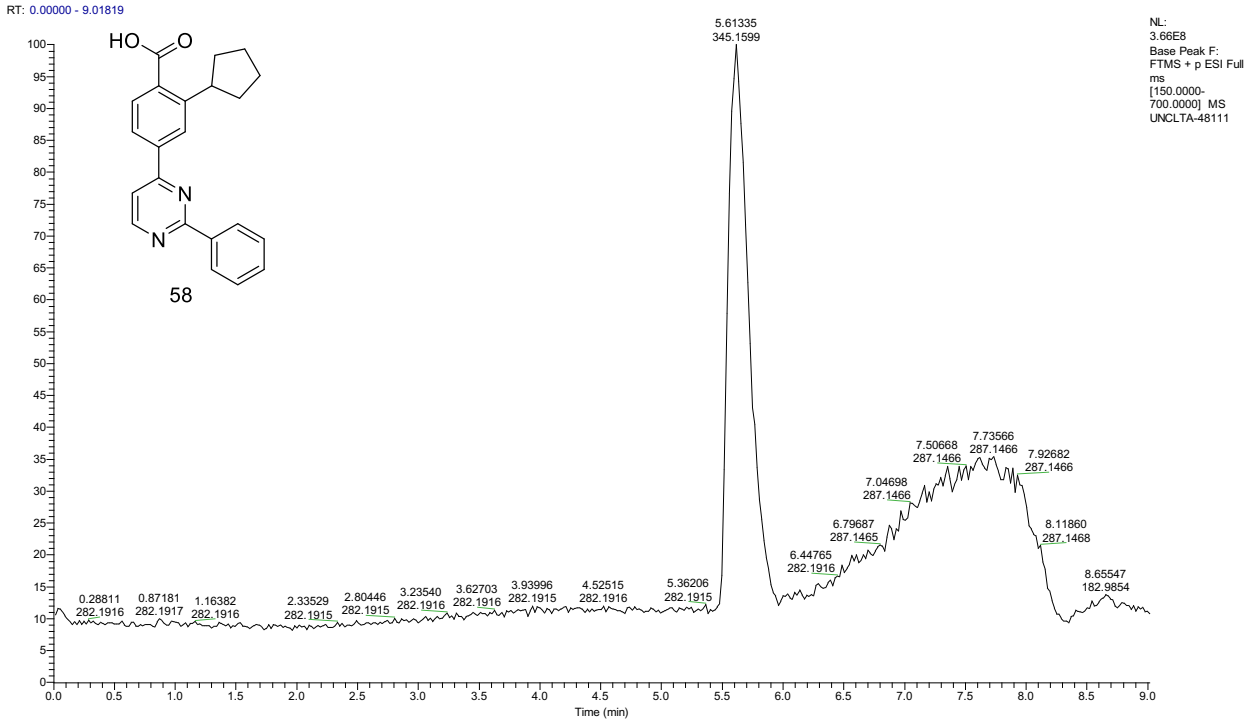
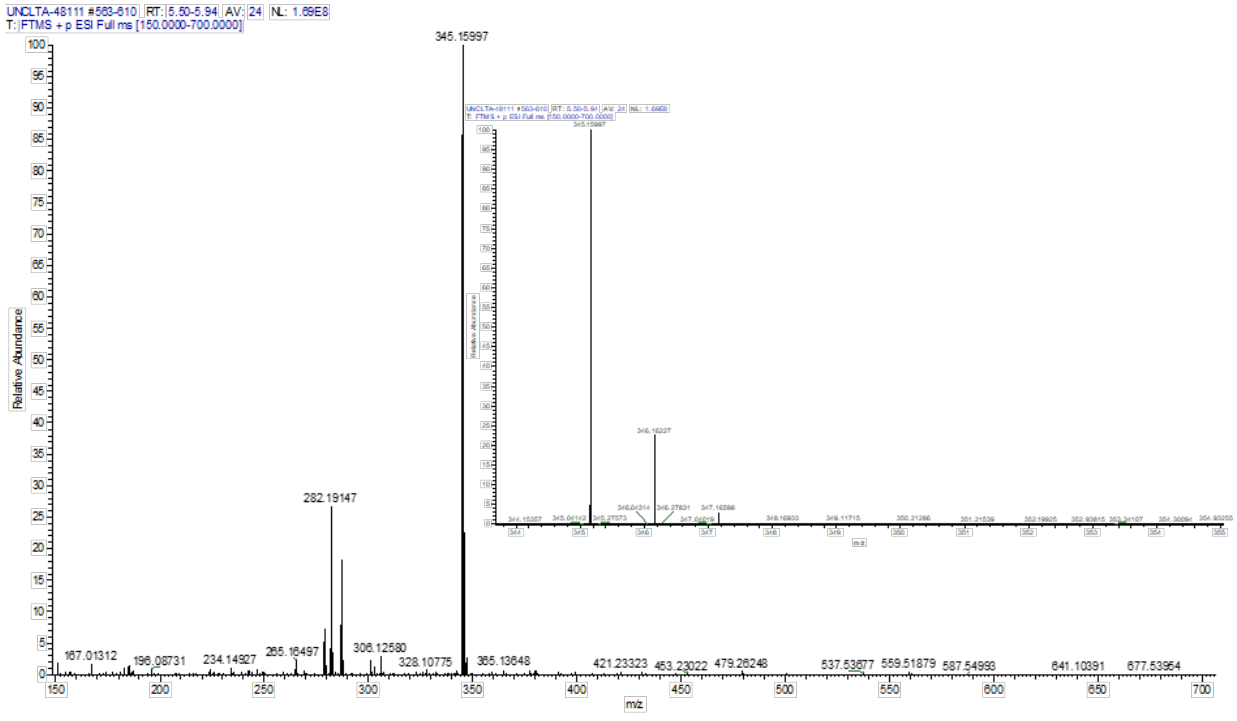
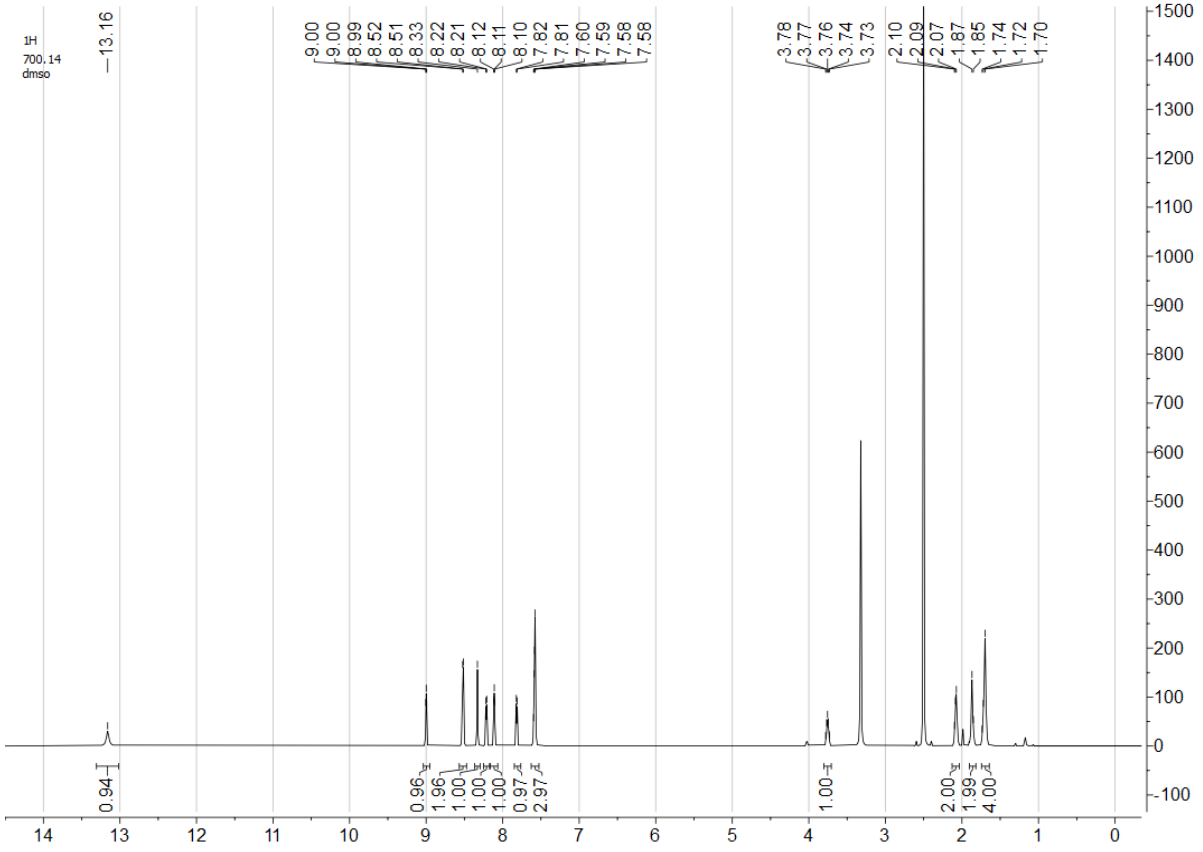
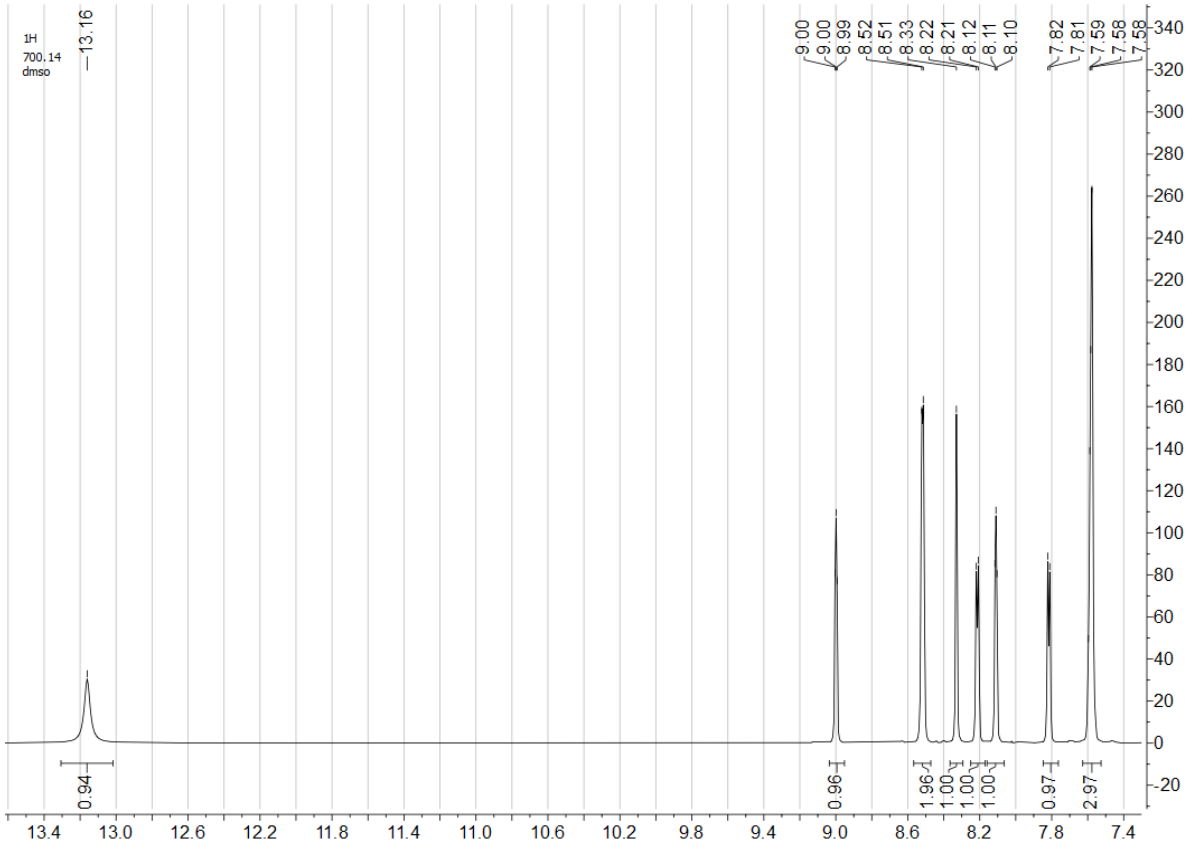
176.07
dmso
169.31
161.81
158.82
158.80
158.26
146.38
138.26
134.47
129.67
125.14
124.20
117.71

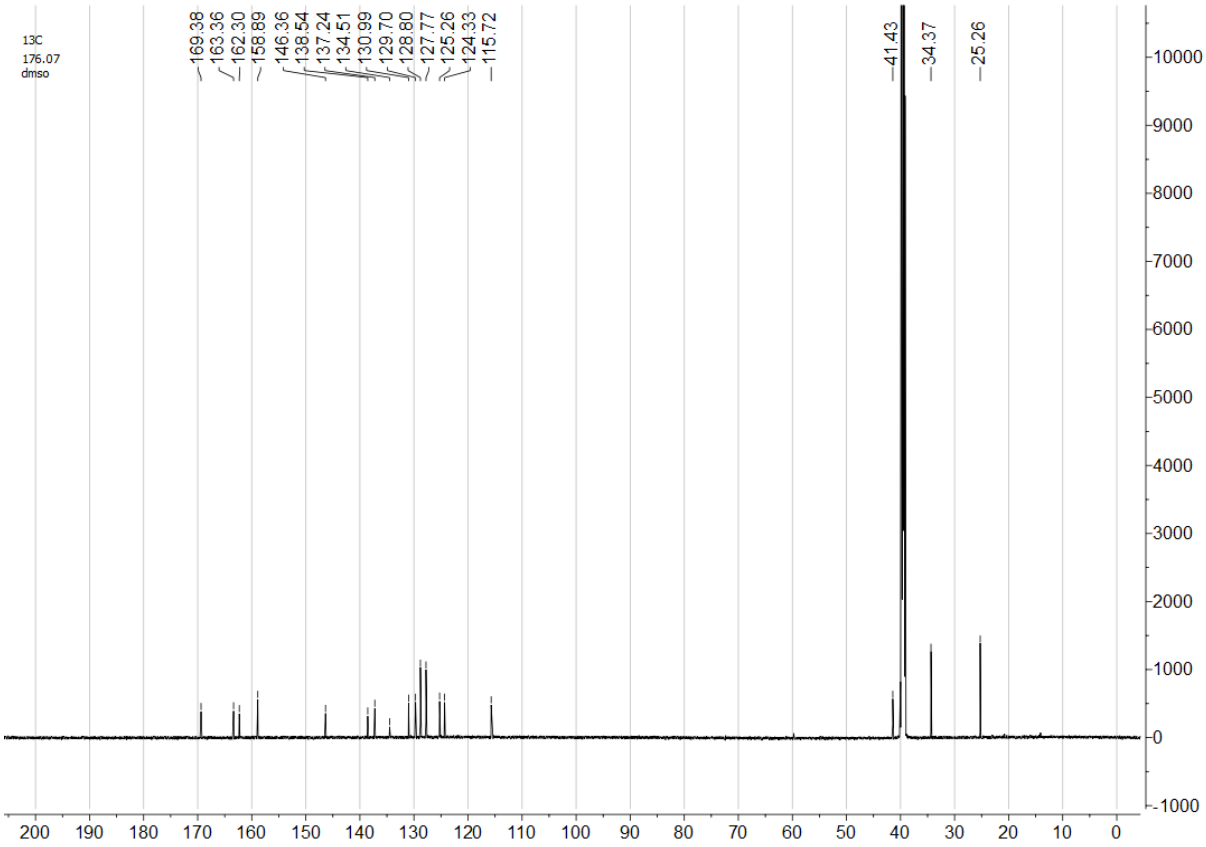
2-Cyclopentyl-4-(2-phenylpyrimidin-4-yl)benzoic acid (58)






1H
700.14
dmso
13.16
9.00
9.00
8.99
8.52
8.51
8.33
8.22
8.21
8.12
8.11
8.10
7.82
7.81
7.60
7.59
7.58
7.58
3.78
3.77
3.76
3.74
3.73
2.10
2.09
2.07
1.87
1.85
1.74
1.72
1.70
0.94
0.96
1.96
1.00
1.00
0.97
2.97
1.00
2.00
1.99
4.00


1H
700.14
dmso
13.16
9.00
9.00
8.99
8.52
8.51
8.33
8.22
8.21
8.12
8.11
8.10
7.82
7.81
7.59
7.58
7.58
0.94
0.96
1.96
1.00
1.00
0.97
2.97


13C
176.07
dmso
169.38
163.36
162.30
158.89
146.36
138.54
137.24
134.51
130.99
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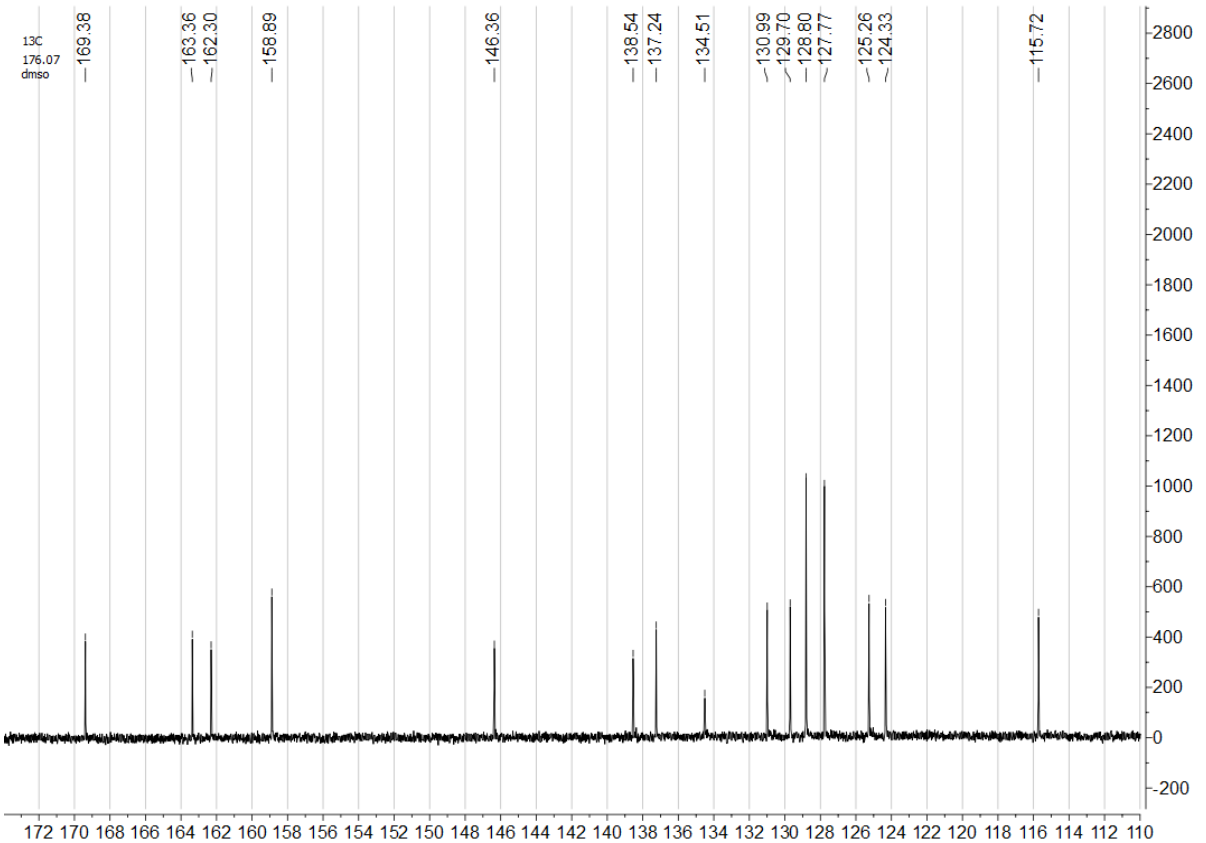
125.26
124.33
115.72
41.43
34.37
25.26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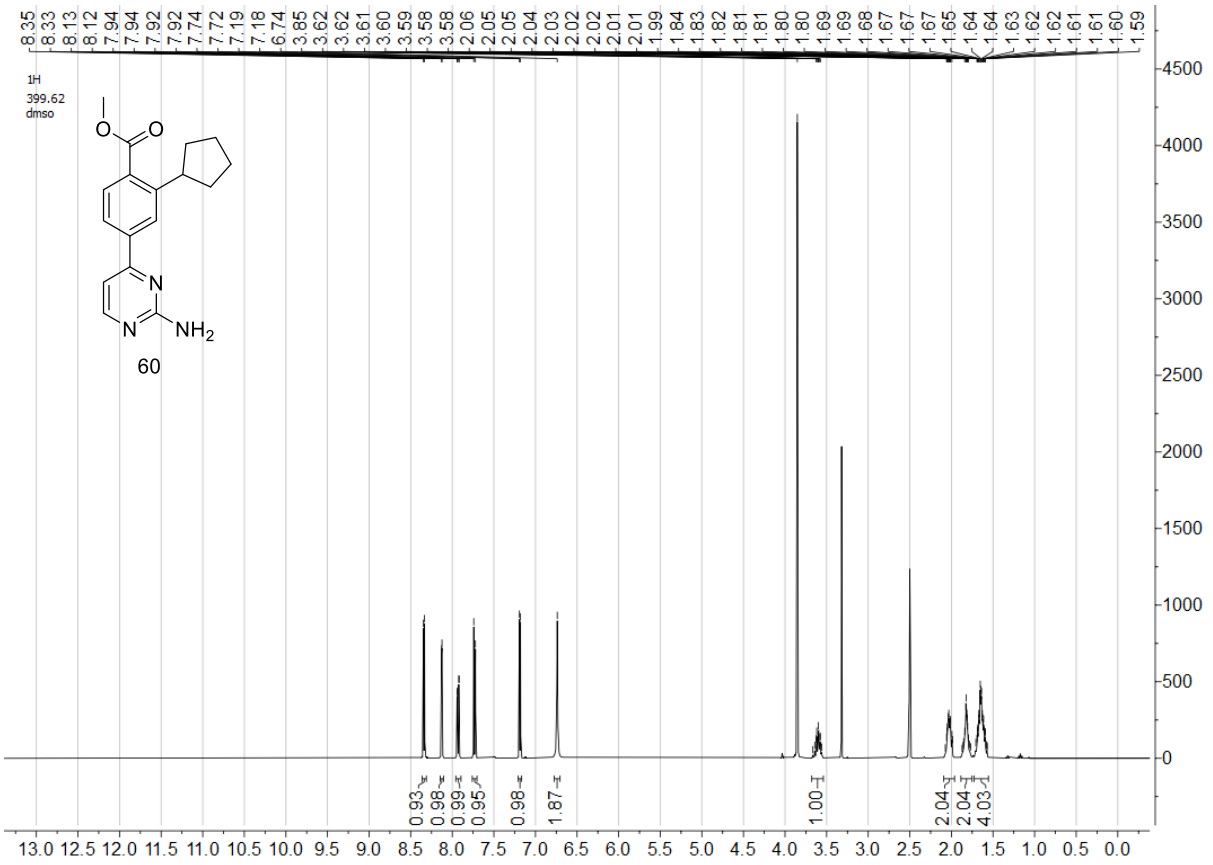
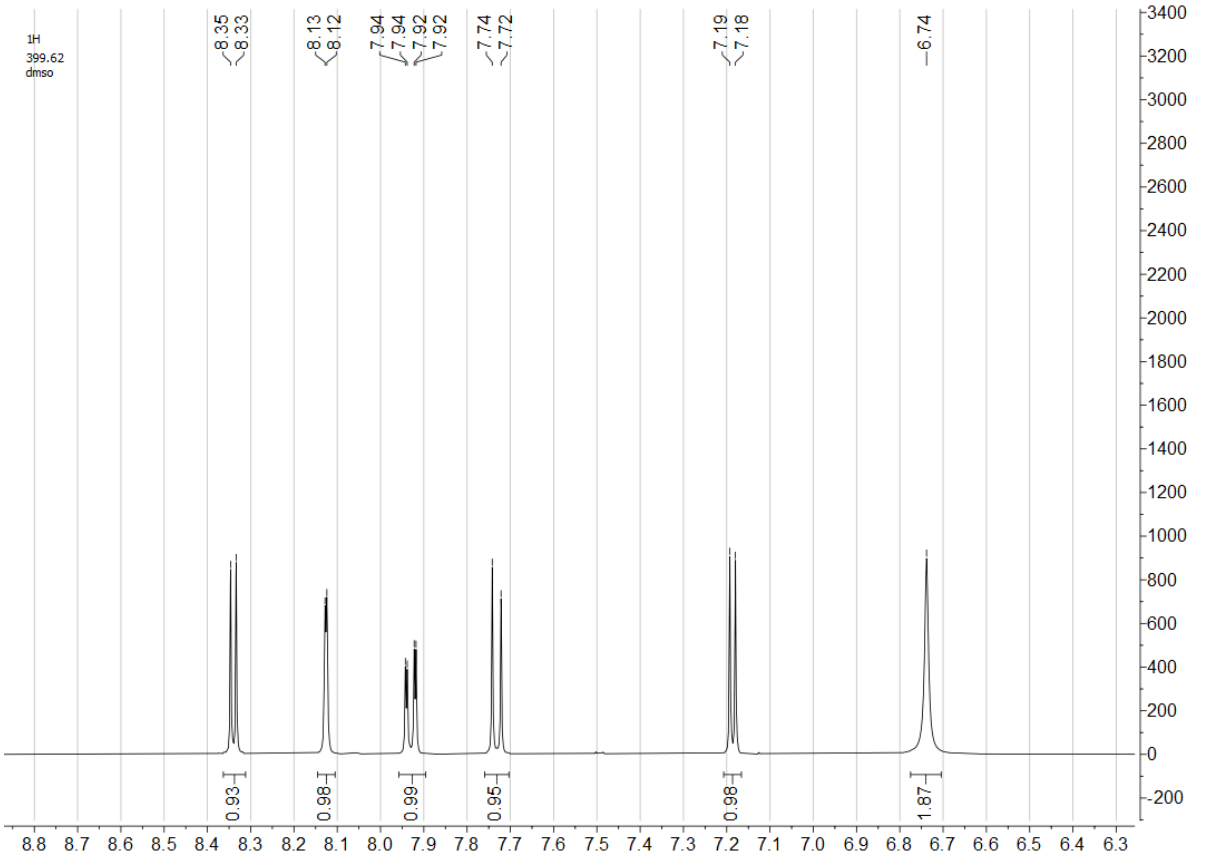
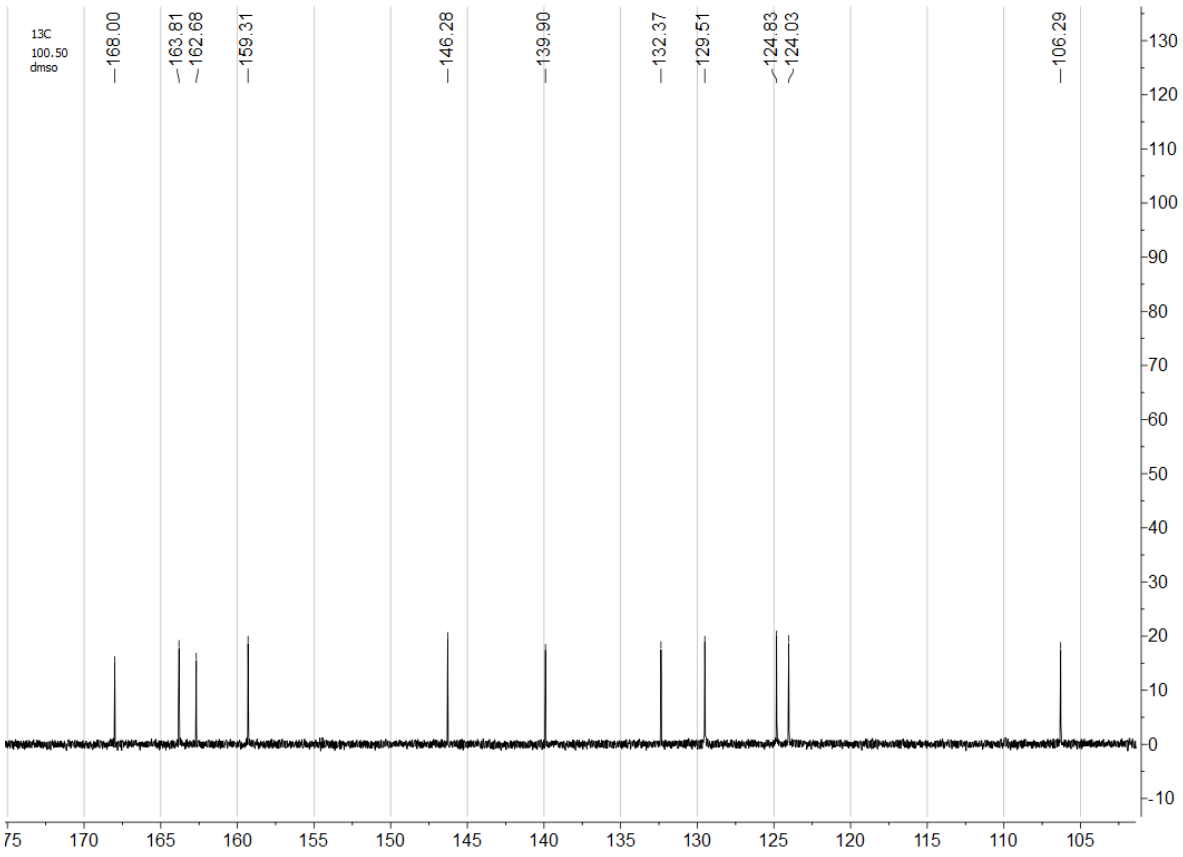
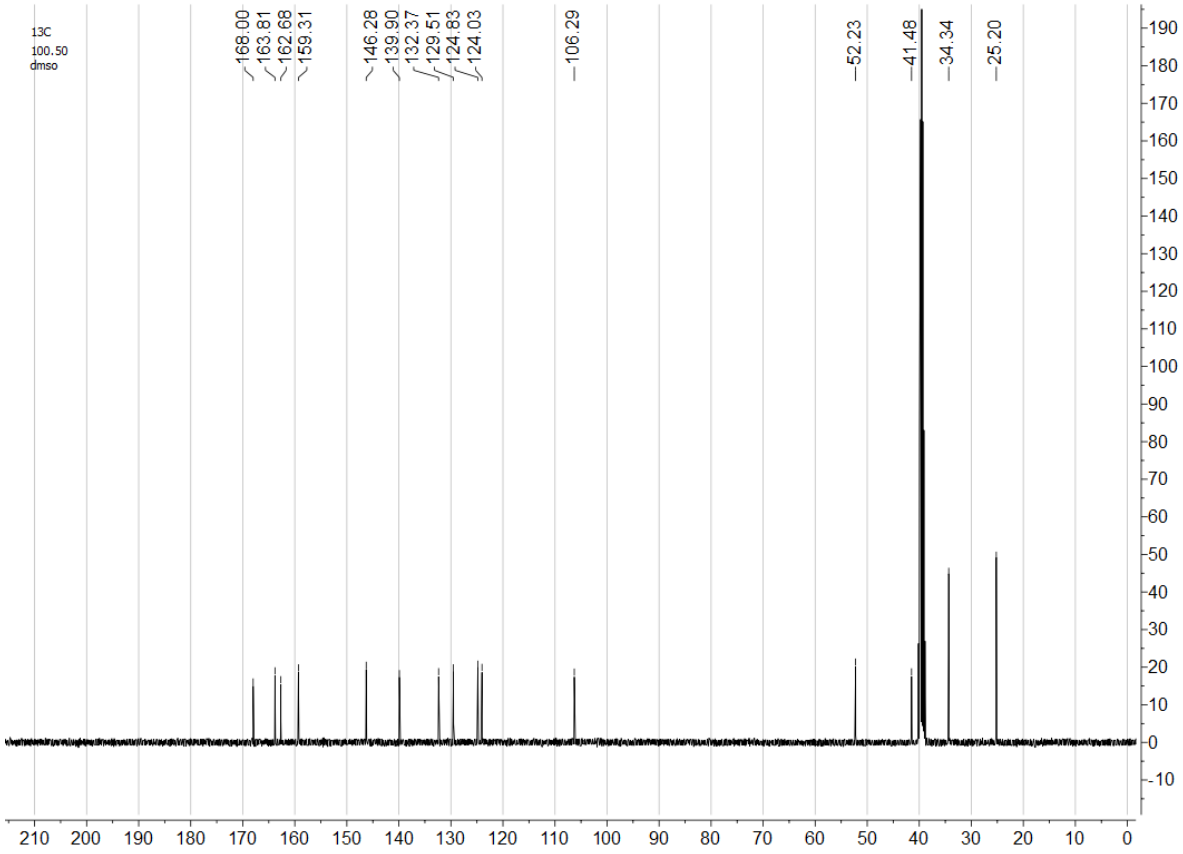
176.07
dmso
169.38
163.36
162.30
158.89
146.36
138.54
137.24
134.51
130.99
129.70
128.80
127.77
125.26
124.33
115.72

Methyl 4-(2-aminopyrimidin-4-yl)-2-cyclopentylbenzoate (60)

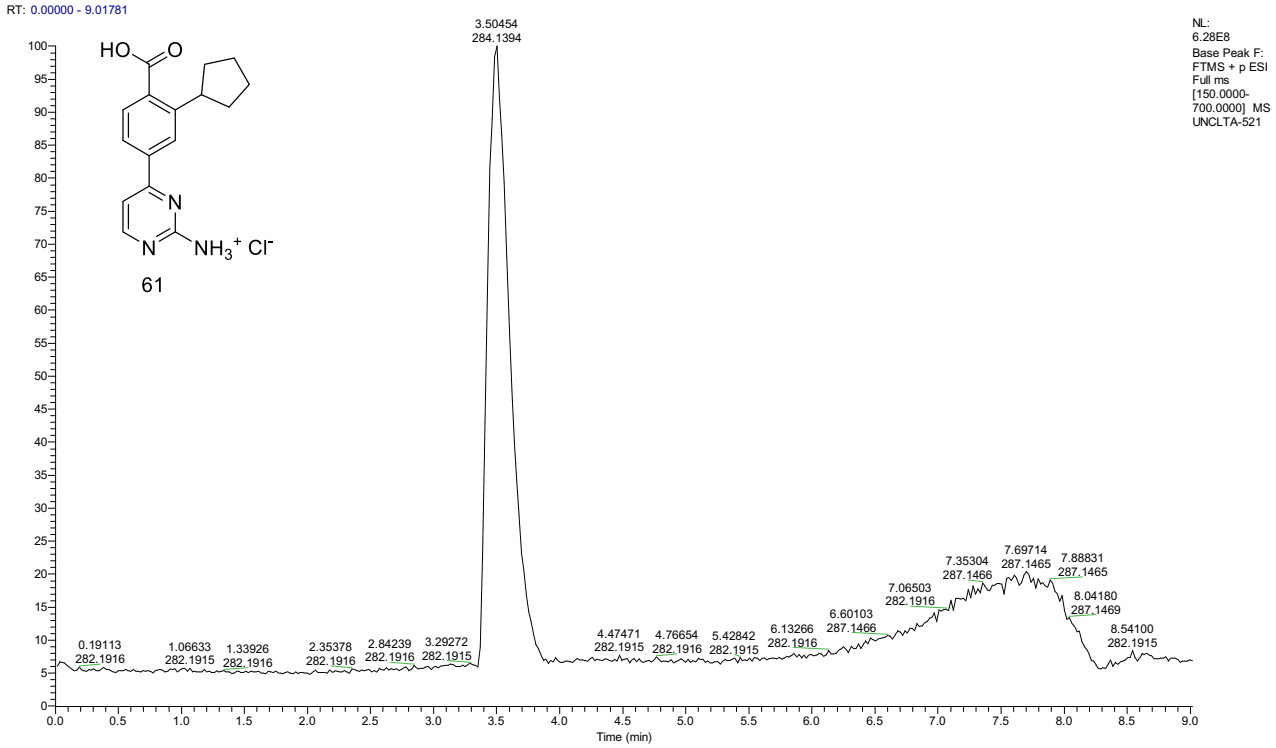


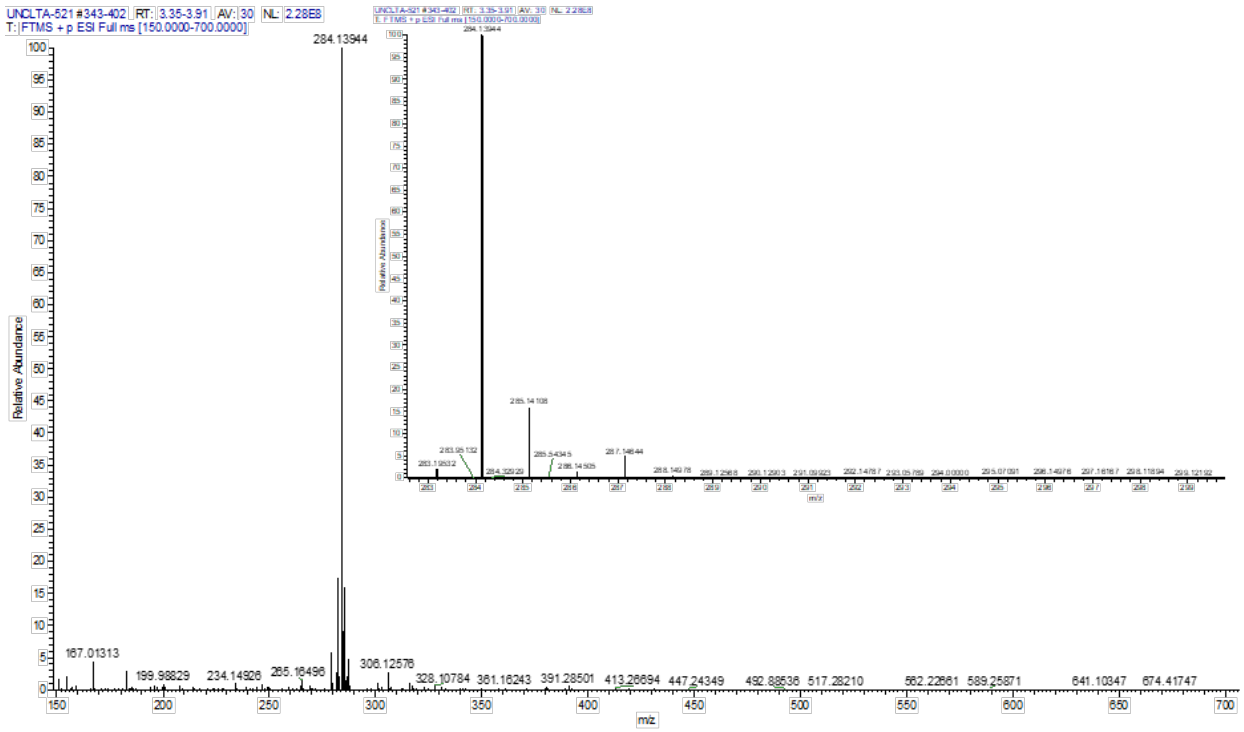


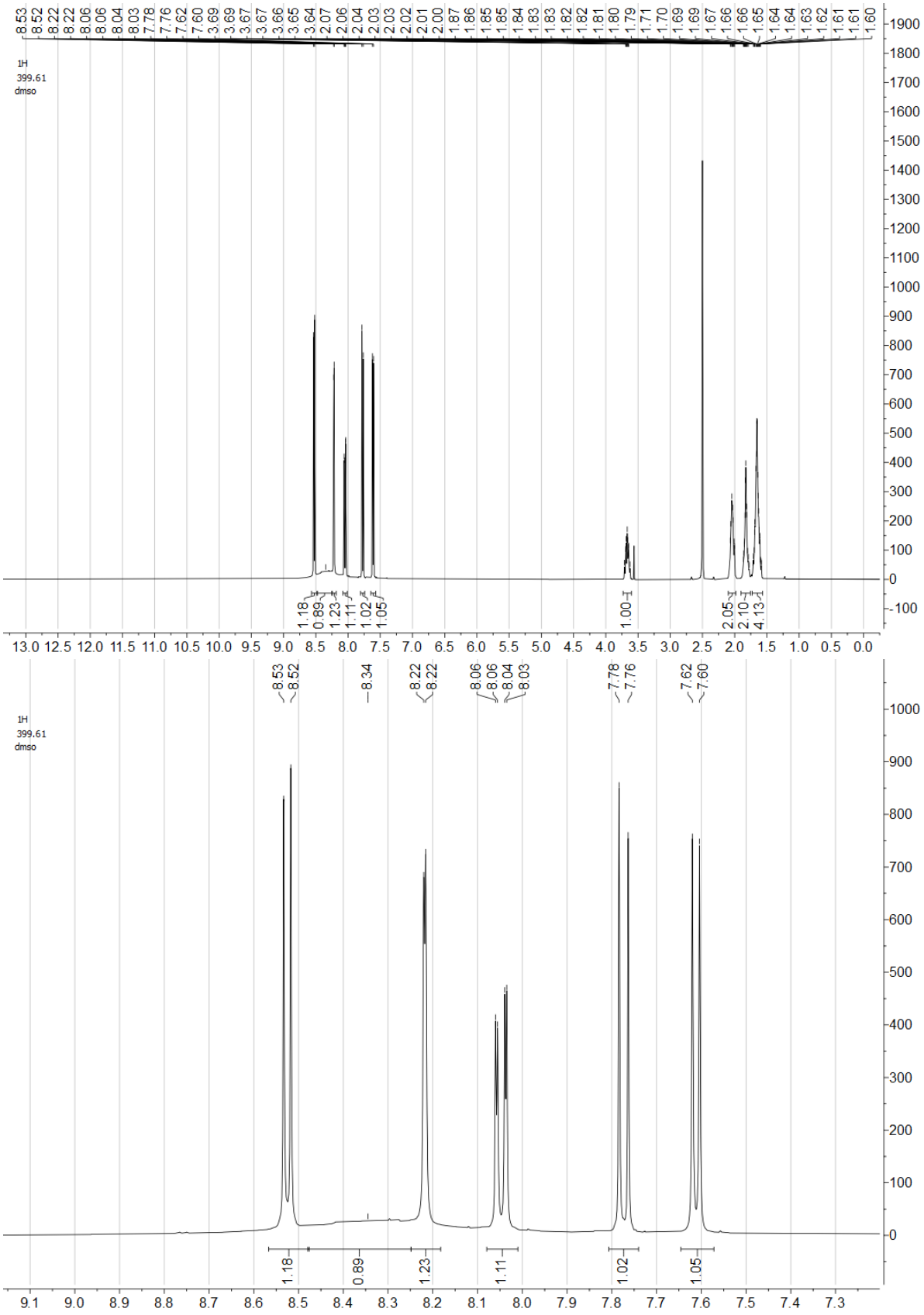

13C
100.50
dmso
168.00
163.81
162.68
159.31
146.28
139.90
132.37
129.51
124.83
124.03
106.29


13C
100.50
dmso
168.00
163.81
162.68
159.31
146.28
139.90
132.37
129.51
124.83
124.03
106.29
52.23
41.48
34.34
25.20

4-(2-Aminopyrimidin-4-yl)-2-cyclopentylbenzoic acid hydrochloride (**61**·HCl)



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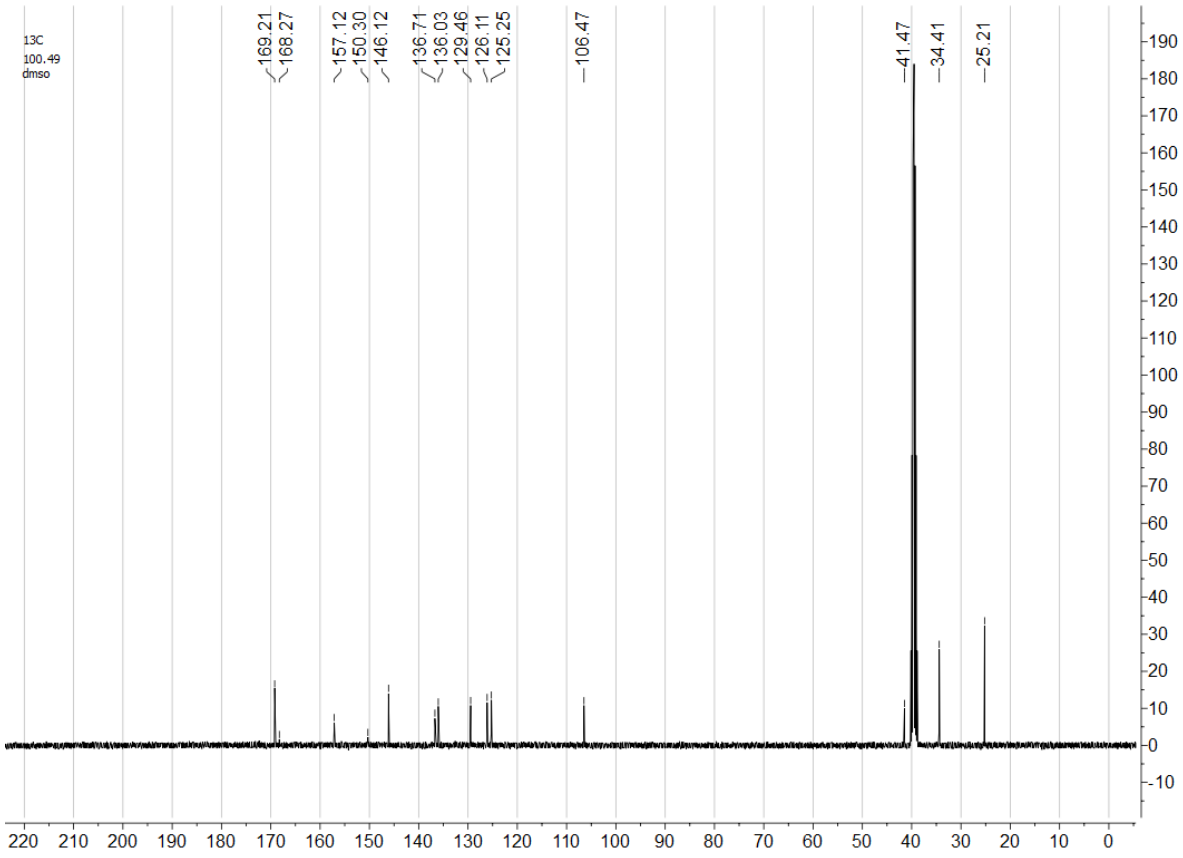
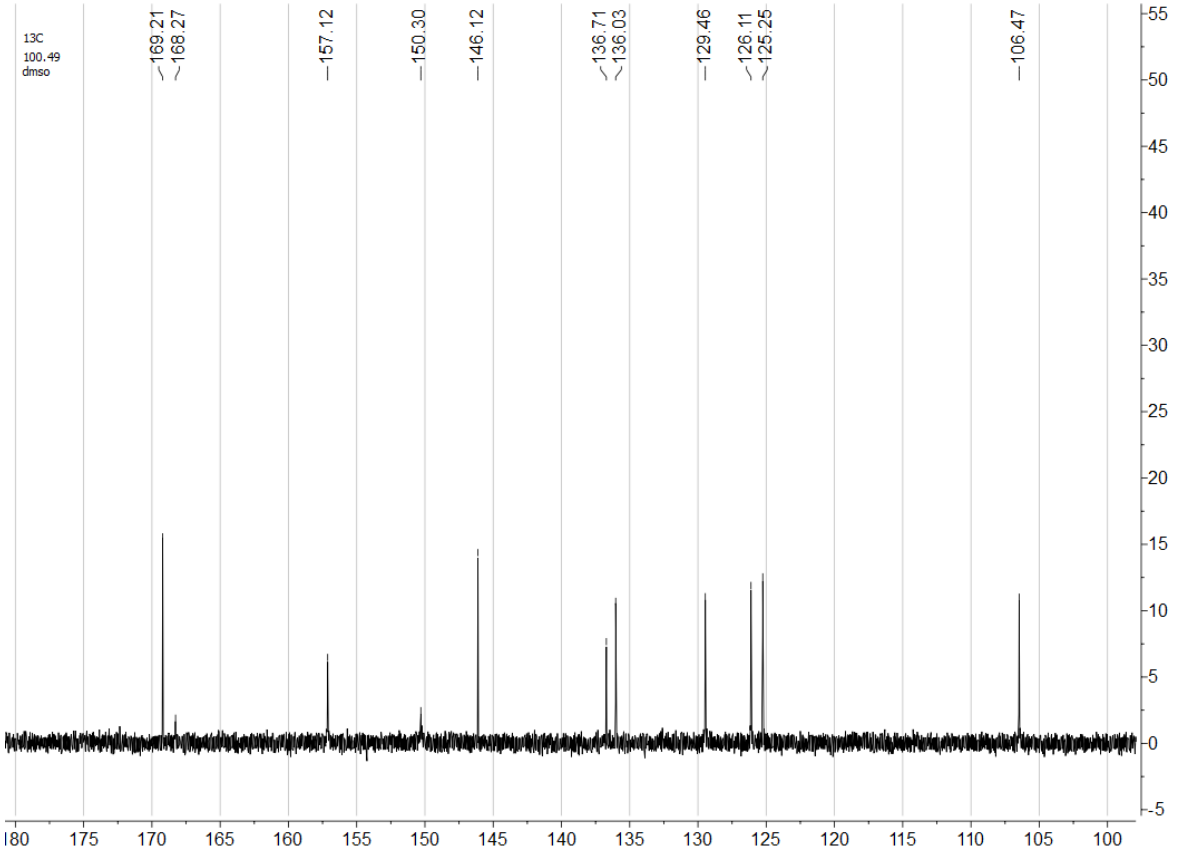
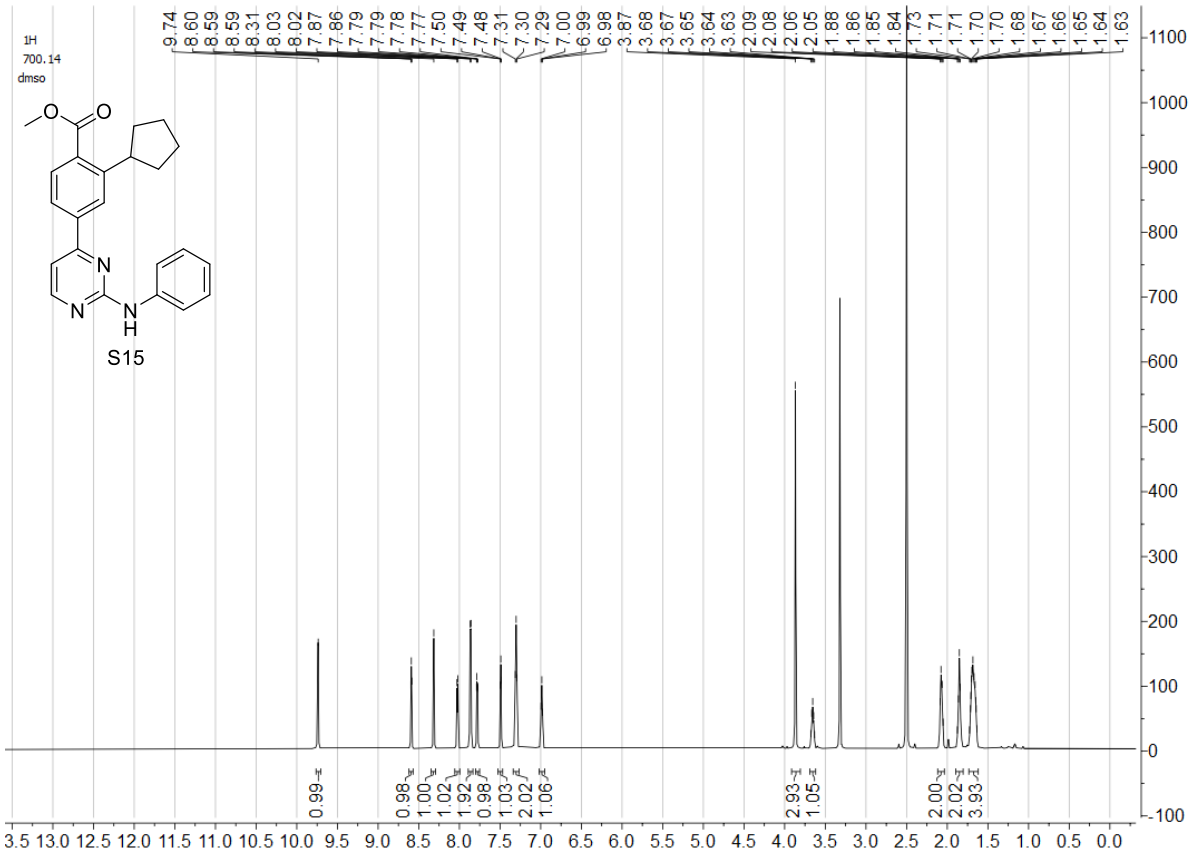
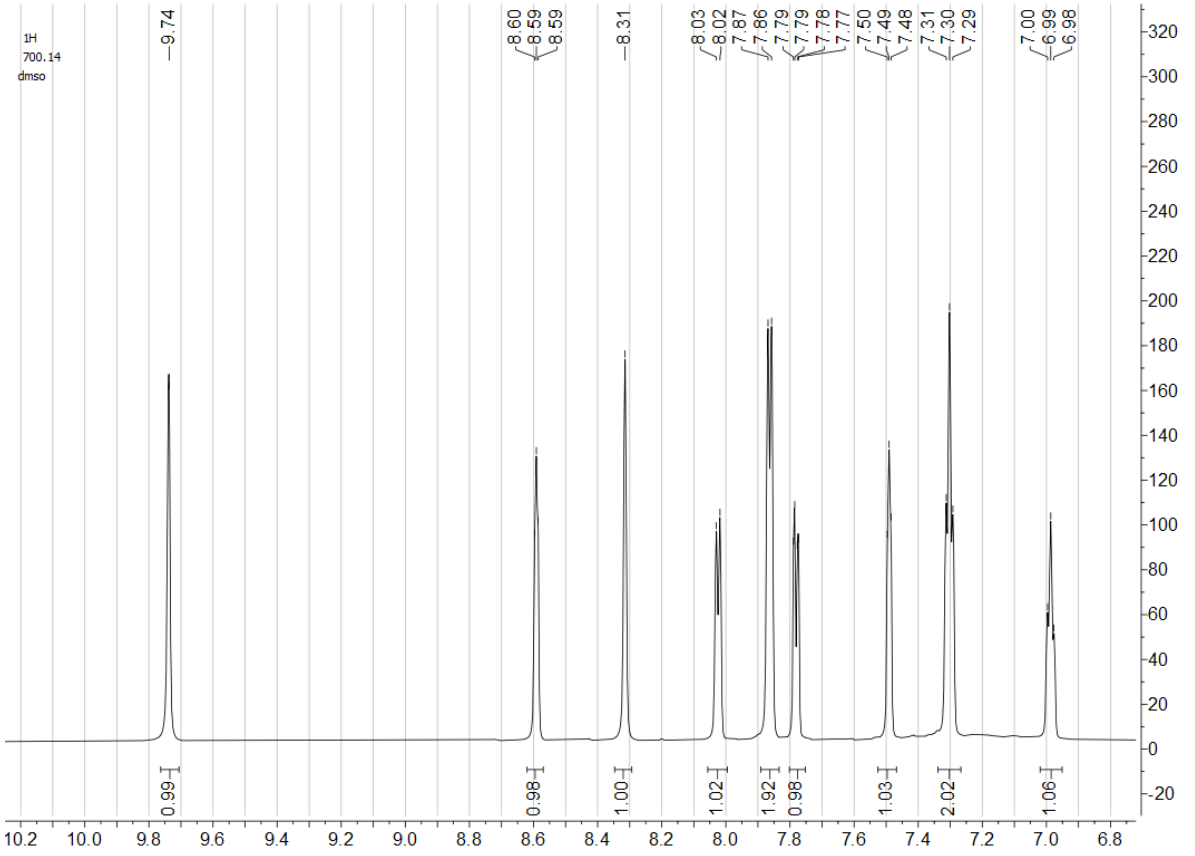
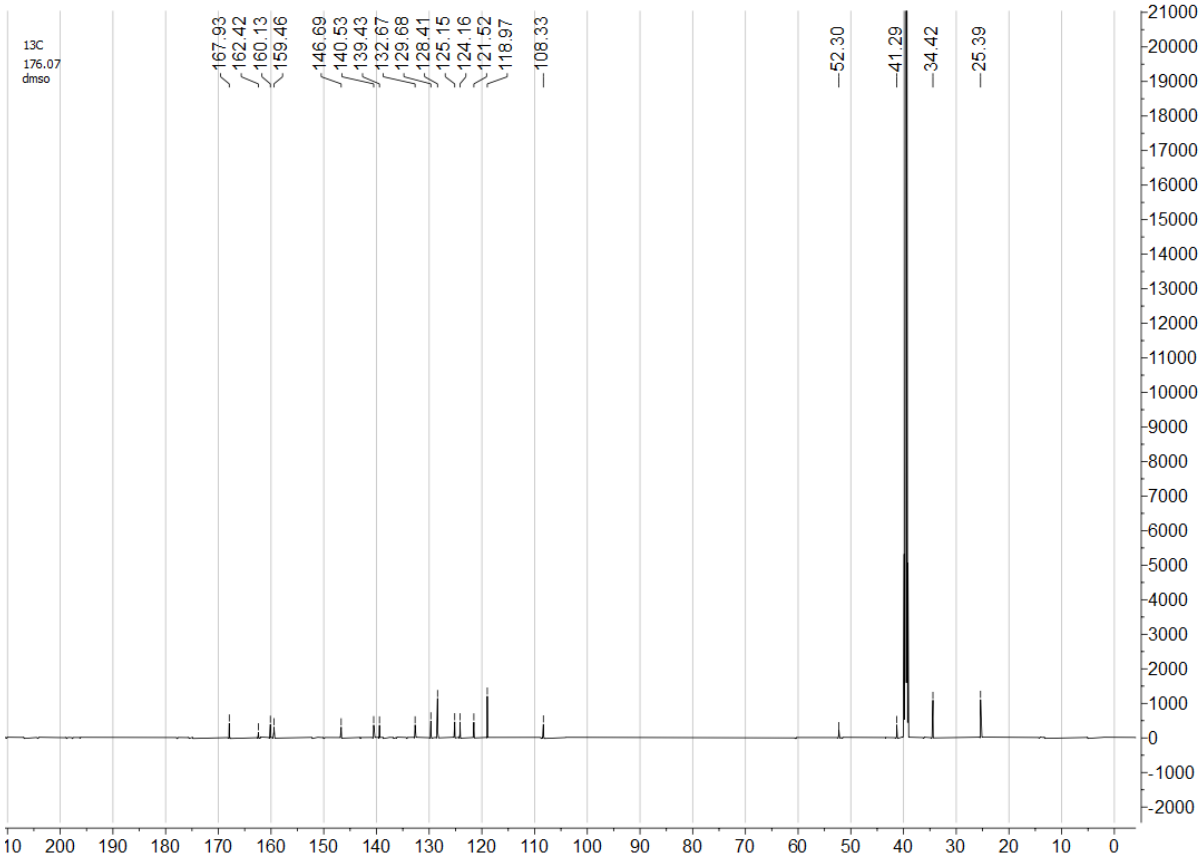
8.52
8.22
8.06
8.04
8.03
7.78
7.76
7.62
7.60
1.18
0.89
1.23
1.11
1.02
1.05
1.00
2.05
2.10
4.13
1H
399.61
dmso
8.34
8.06
7.78
7.76
7.62
7.60

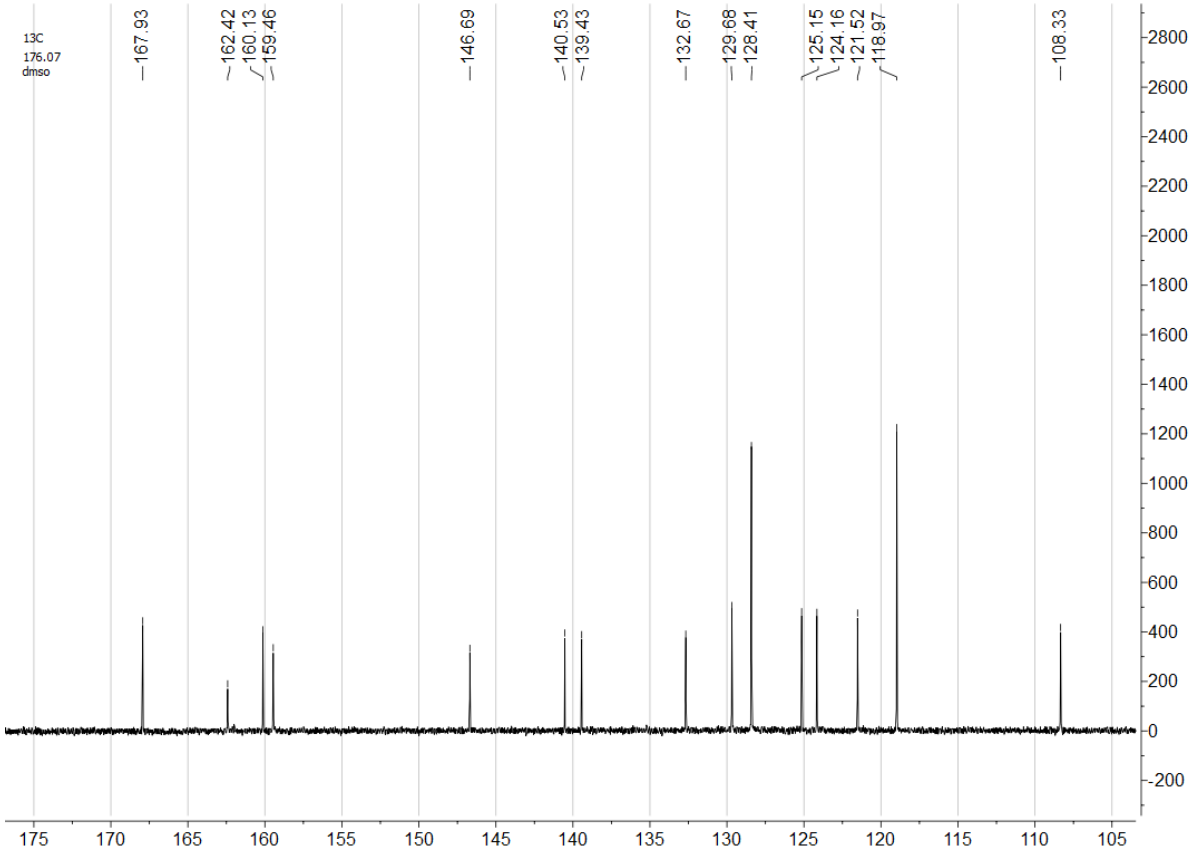

13C
100.49
dmso
169.21
168.27
157.12
150.30
146.12
136.71
136.03
129.46
126.11
125.25
106.47
41.47
34.41
25.21


13C
100.49
dmso
169.21
168.27
157.12
150.30
146.12
136.71
136.03
129.46
126.11
125.25
106.47

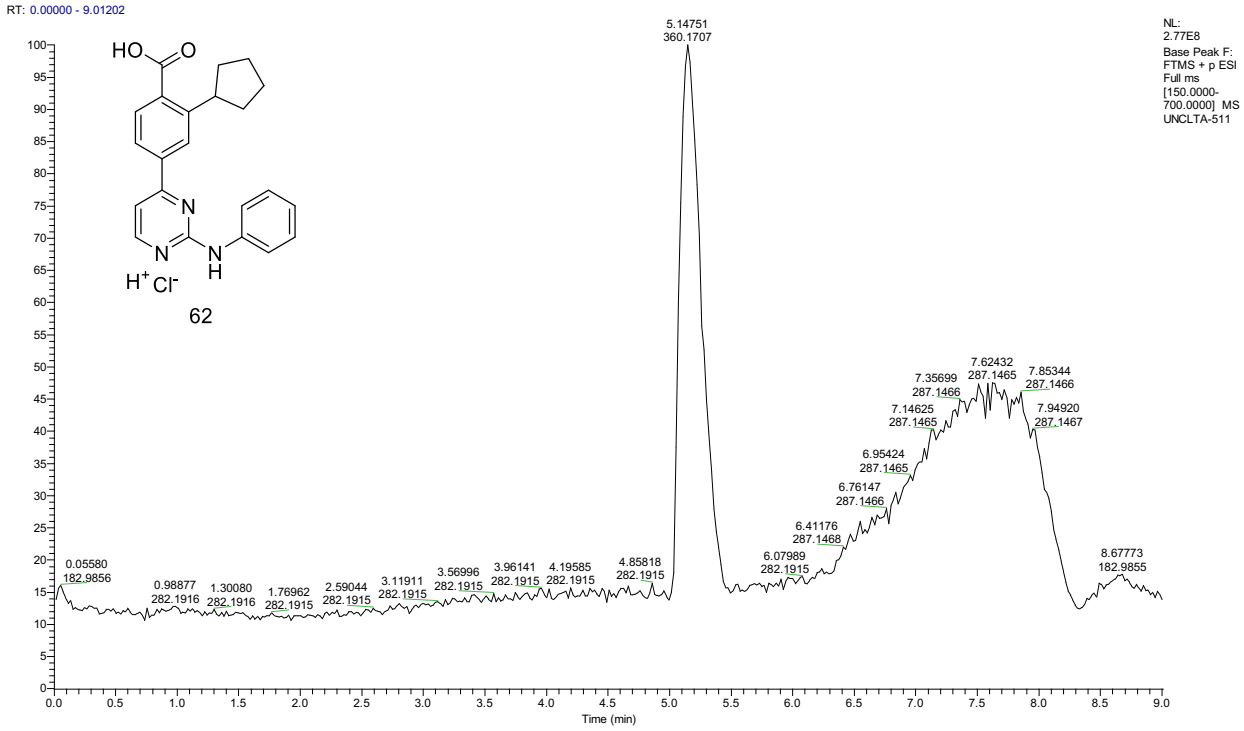

1H
700.14
dmso
S15

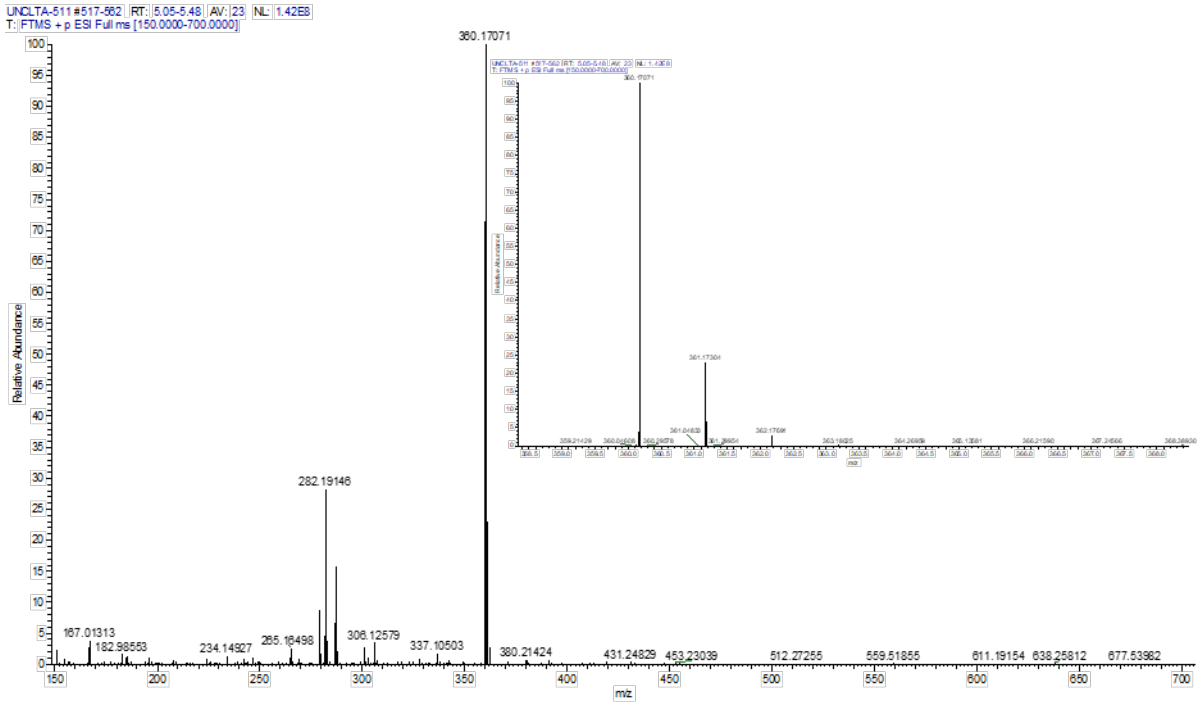

1H
700.14
dmso

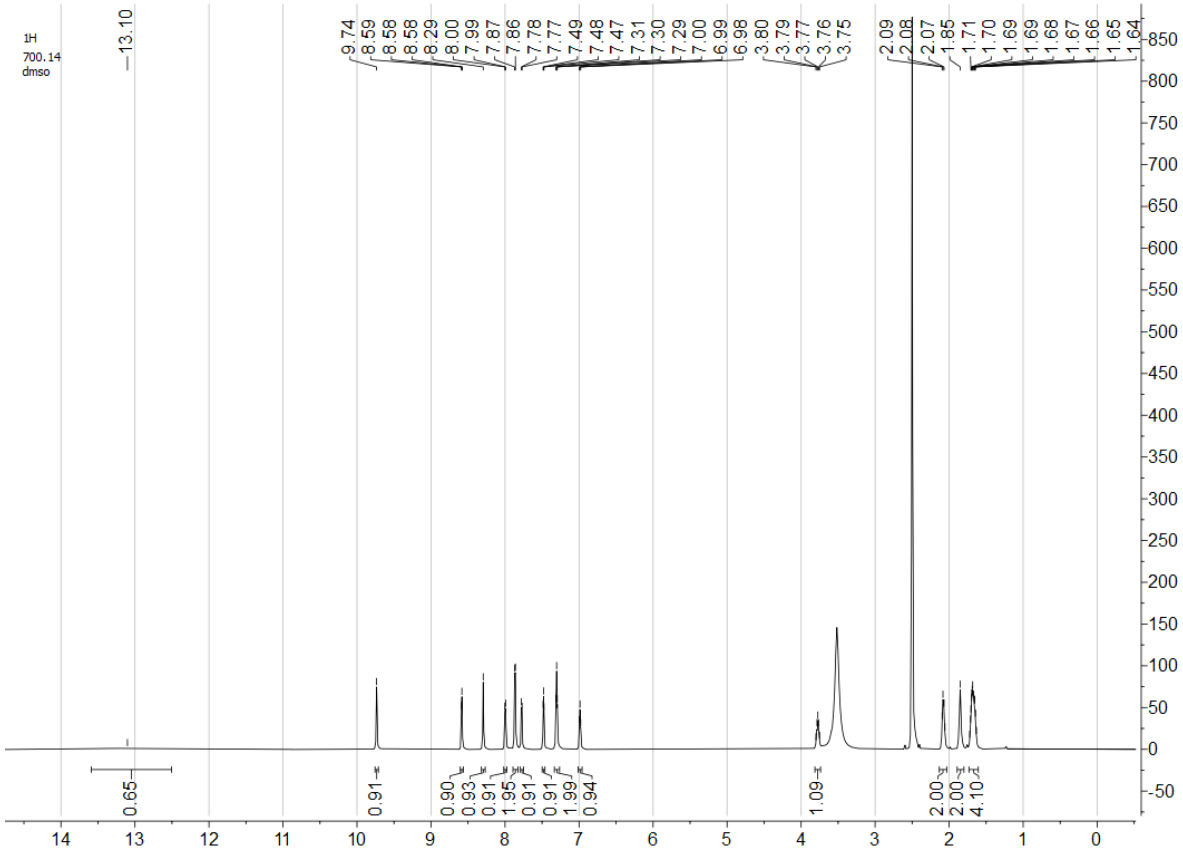

13C
176.07
dmso
167.93
162.42
160.13
159.46
146.69
140.53
139.43
132.67
129.68
128.41
125.15
124.16
121.52
118.97
108.33
52.30
41.29
34.42
25.39


13C
176.07
dmso
167.93
162.42
160.13
159.46
146.69
140.53
139.43
132.67
129.68
128.41
125.15
124.16
121.52
118.97
108.33

2-Cyclopentyl-4-(2-(phenylamino)pyrimidin-4-yl)benzoic acid hydrochloride (**62**·HCl)






1H
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dmso
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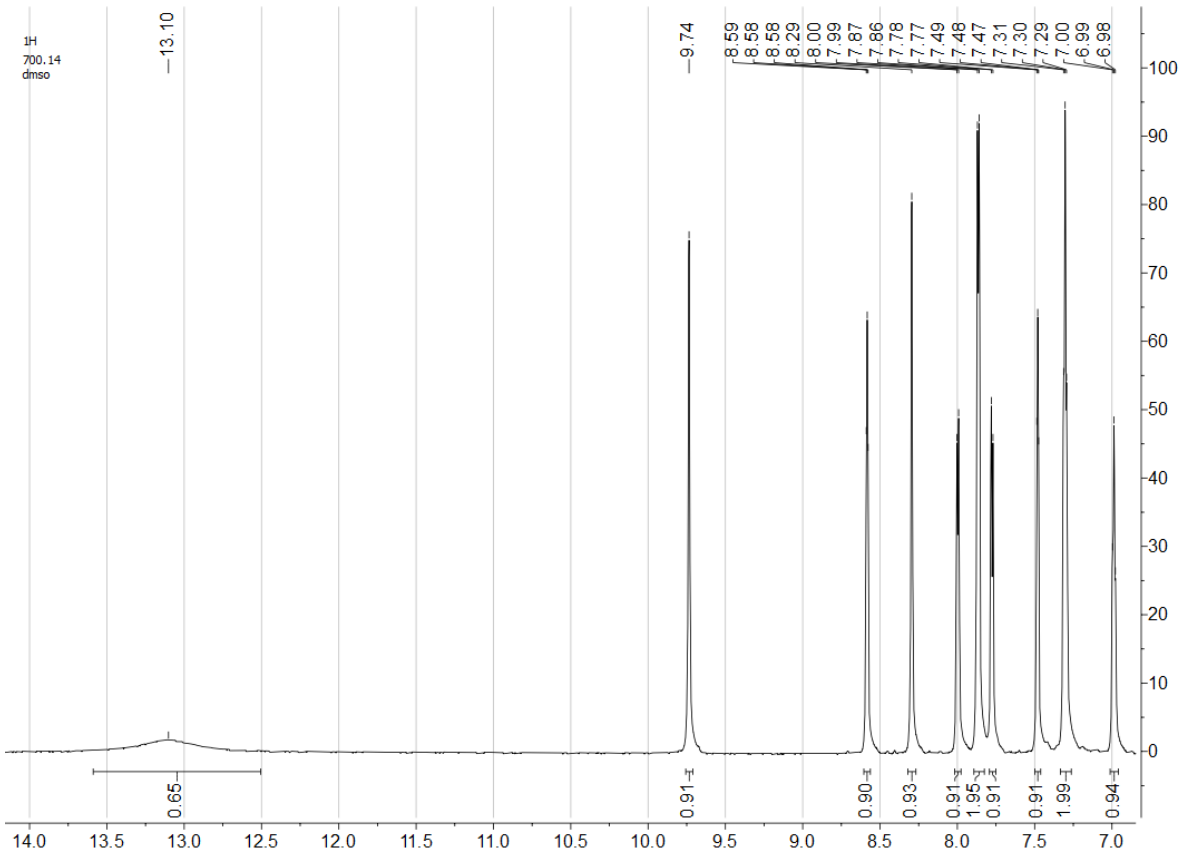
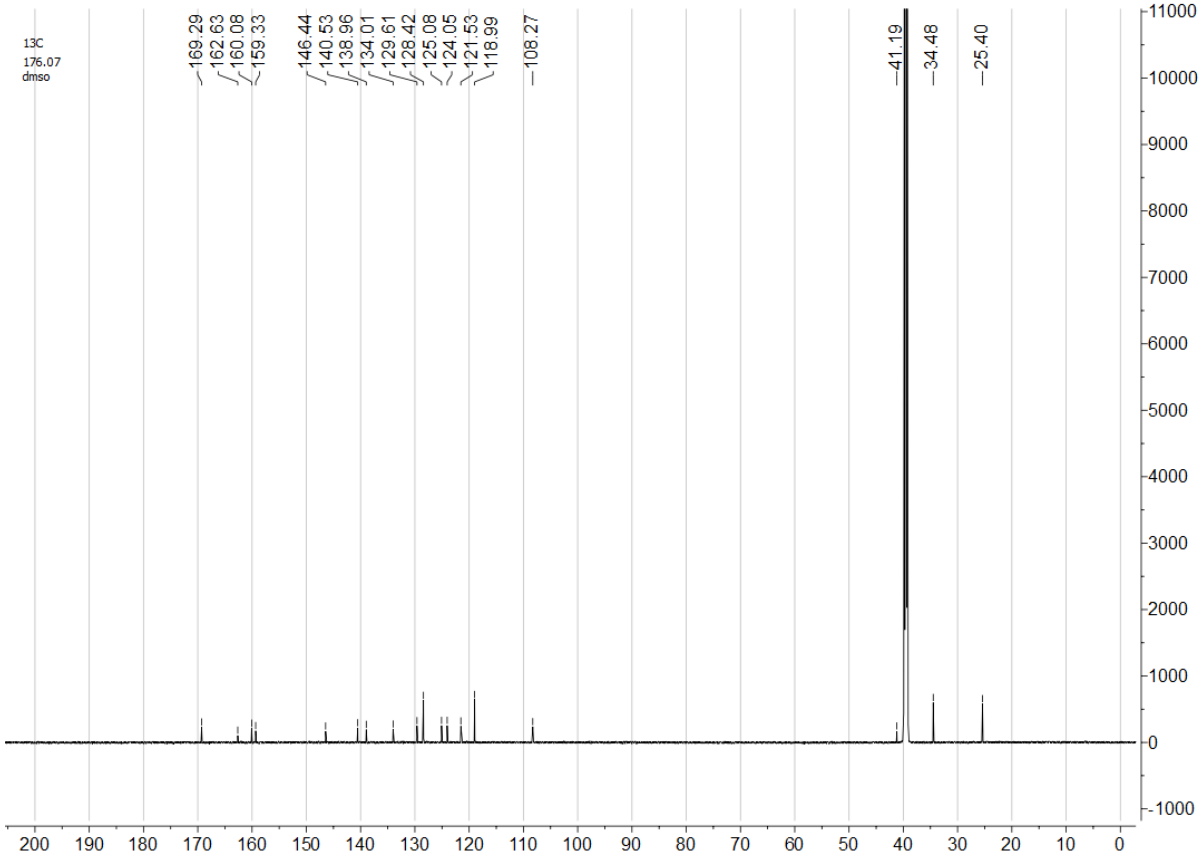
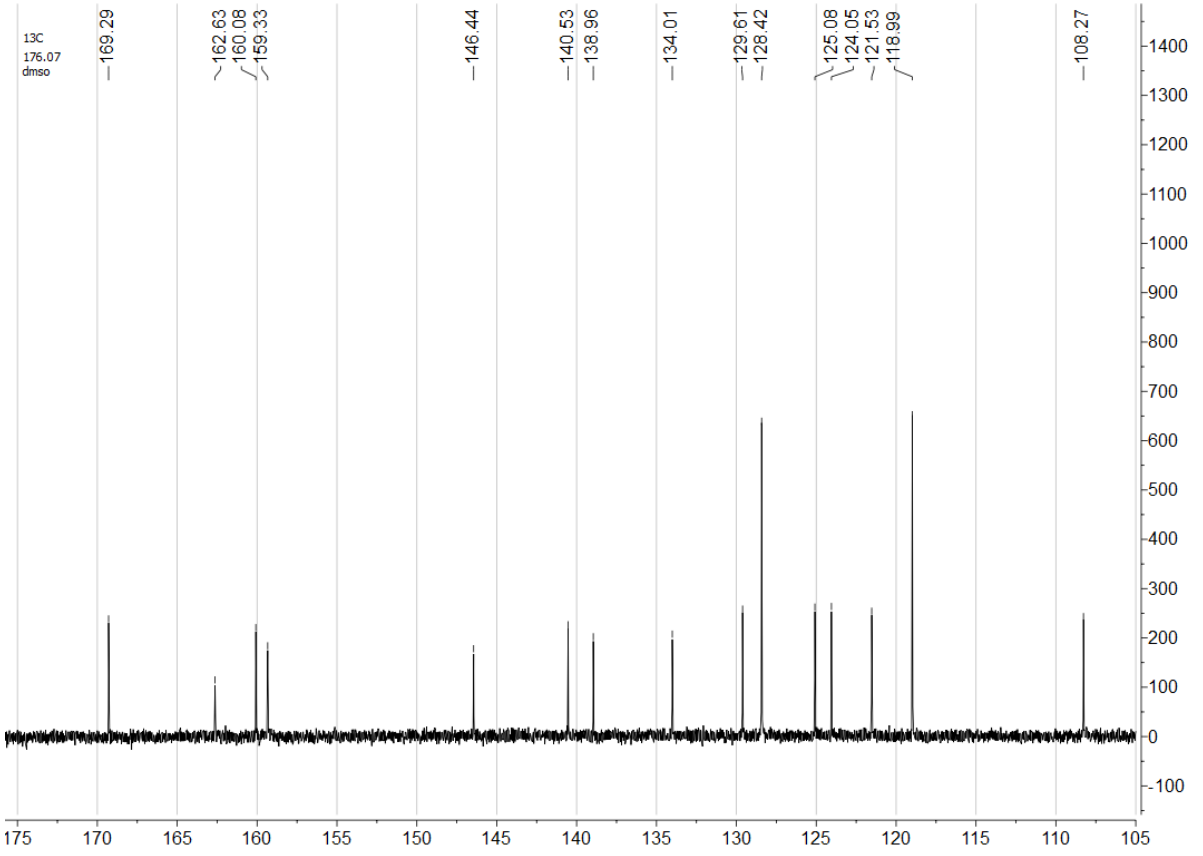
9.74
8.59
8.58
8.58
8.29
8.00
7.99
7.87
7.86
7.78
7.77
7.49
7.48
7.47
7.31
7.30
7.29
7.00
6.99
6.98
3.80
3.79
3.77
3.76
3.75
2.09
2.08
2.07
1.85
1.71
1.70
1.69
1.69
1.68
1.67
1.66
1.65
1.64
0.65
0.91
0.90
0.93
0.91
1.95
0.91
0.91
1.99
0.94
1.09
2.00
2.00
4.10


1H
700.14
dmso
13.10
9.74
8.59
8.58
8.58
8.29
8.00
7.99
7.87
7.86
7.78
7.77
7.49
7.48
7.47
7.31
7.30
7.29
7.00
6.99
6.98
0.65
0.91
0.90
0.93
0.91
1.95
0.91
0.91
1.99
0.94


13C
176.07
dmso
169.29
162.63
160.08
159.33
146.44
140.53
138.96
134.01
129.61
128.42
125.08
124.05
121.53
118.99
108.27
41.19
34.48
25.40


13C
176.07
dmso
169.29
162.63
160.08
159.33
146.44
140.53
138.96
134.01
129.61
128.42
125.08
124.05
121.53
118.99
108.27

3. NanoBRET assay

3.1. CAMKK2 NanoLuciferase construct

N- terminal NanoLuc/CAMKK2 Protein Kinase (PK) fusion was encoded in pFN31K expression vectors (Promega), including flexible Gly-Ser-Ser-Gly linkers between Nluc and the CAMKK2 PK domain. The PK domain is predicted (pfam) as Tyr165-Val446.

3.2. Cell Culture

HEK-293 cells were cultured in DMEM supplemented with 10 % FBS. Cells were incubated in 5 % CO_2 at 37˚C. Cells lines were passaged every 72 hours with trypsin and not allowed to reach confluency.

3.3. NanoBRET measurements

The *N*-terminal Nano Luciferase/CAMKK2 PK fusion (NL-CAMKK2(PK)) was encoded in pFN31K expression vector, including flexible Gly-Ser-Ser-Gly linkers between NanoLuc and CAMKK2. For cellular NanoBRET target engagement experiments, a 10 µg/mL solution of DNA in Opti-MEM without serum was made containing 9 µg/mL of Carrier DNA (Promega) and 1 µg/mL of NL-CAMKK2 for a total volume of 1.05 mL. To this solution was then added 31.5 µL of FuGENE HD (Promega) to form a lipid:DNA complex. The solution was then mixed by inversion 8 times and incubated at room temperature for 20 min. The resulting transfection complex (1.082 mL) was then gently mixed with 21 mL of HEK-293 cells (ATCC) suspended at a density of 2 x 10^5 cells/mL in DMEM (Gibco) + 10% FBS (Corning). This solution was then dispensed (100 µL) into 96-well tissue culture treated plates (Corning #3917) followed by incubation (37 °C / 5 % CO_2) for 24 h.

After 24 hours the media was removed and replaced with 85 µL of room temperature Opti-MEM without phenol red. NanoBRET Tracer K5 (Promega) was used at a final concentration of 1.0 µM as previously determined to be the optimal concentration in a titration experiment. A total of 5 µL per well (20x working stock of NanoBRET Tracer K5 [20 µM] in Tracer Dilution Buffer (Promega N291B) was added to all wells, except the “no tracer” control wells. All test compounds were prepared initially as concentrated (10 mM) stock solutions in 100% DMSO (Sigma), then diluted in Opti-MEM media (99 %) to prepare 1 % DMSO working stock solutions. A total of 10 µL per well of the 10-fold test compound stock solutions (final assay concentration 0.1 % DMSO) were added. For “no compound” and “no tracer” control wells, a total of 10 µL per well of Opti-MEM plus DMSO (9 µL Opti-MEM with 1 µL DMSO) was added, final concentration 1 % DMSO). 96-well plates containing cells with NanoBRET Tracer K5 and test compounds (100 µL total volume per well) were equilibrated (37 °C / 5 % CO_2) for 2 h.

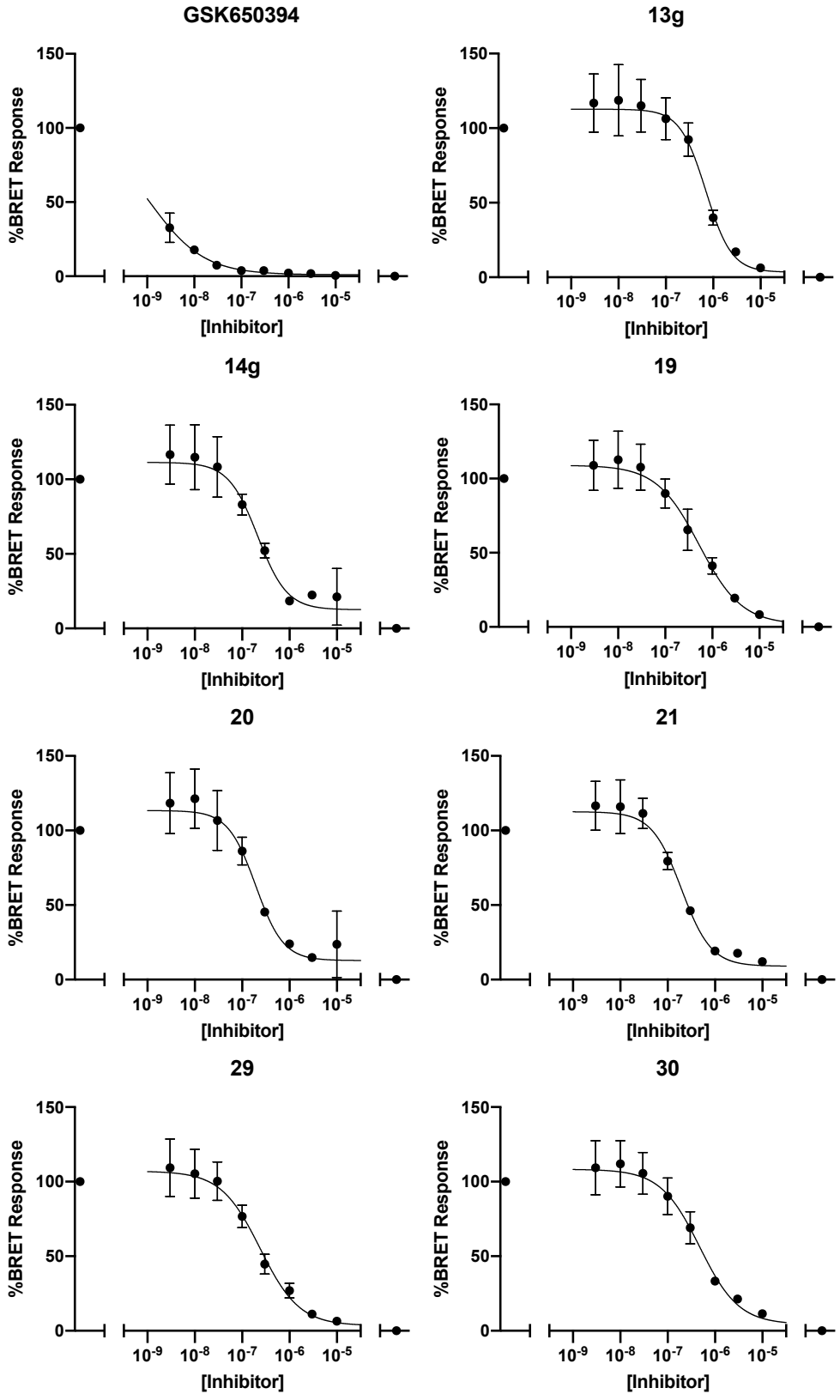
After 2 hours the plates were cooled to room temperature for 15 min. To measure NanoBRET signal, NanoBRET NanoGlo substrate (Promega) at a ratio of 1:166 to Opti-MEM media in combination with extracellular NanoLuc Inhibitor (Promega) diluted 1:500 (10 µL [30 mM stock] per 5 mL Opti-MEM plus substrate) were combined to create a 3X stock solution. A total of 50 µL of the 3X substrate/extracellular NanoLuc inhibitor were added to each well. The plates were read within 10 min on a GloMax Discover luminometer (Promega, Madison, WI, USA) equipped with 450 nm BP filter (donor) and 600 nm LP filter (acceptor), using 0.3 s integration time utilizing the “NanoBRET 618” protocol.

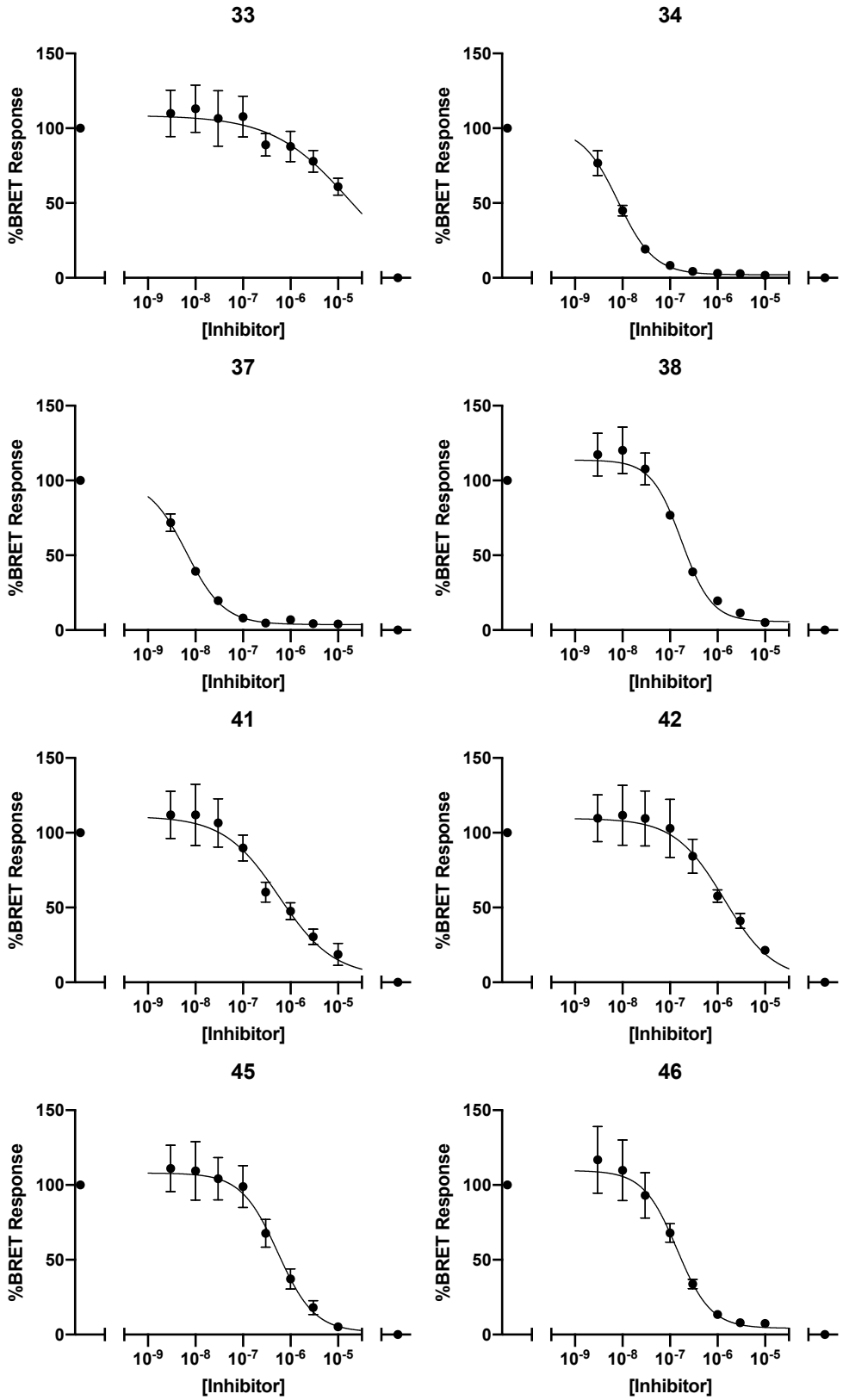
Test compounds were evaluated at eight concentrations in competition with NanoBRET Tracer K5 in HEK293 cells transiently expressing the CAMKK2 fusion protein. Raw milliBRET (mBRET) values were obtained by dividing the acceptor emission values (600

nm) by the donor emission values (450 nm) then multiplying by 1000. Averaged control values were used to represent complete inhibition (no tracer control: Opti-MEM + DMSO only) and no inhibition (tracer only control: no compound, Opti-MEM + DMSO + Tracer K5 only) and were plotted alongside the raw mBRET values. The data with n=3 biological replicates was first normalized and then fit using Sigmoidal, 4PL binding curve in Prism Software (version 8, GraphPad, La Jolla, CA, USA). All error bars are based on n=3 and are +/- standard error (SE).

3.4. NanoBRET assay results – Binding curves and IC_{50} values

Figure S1: NanoBRET concentration response




33
34
37
38
41
42
45
46
%BRET Response
150
100
50
0
10-9
10-8
10-7
10-6
10-5
[Inhibitor]

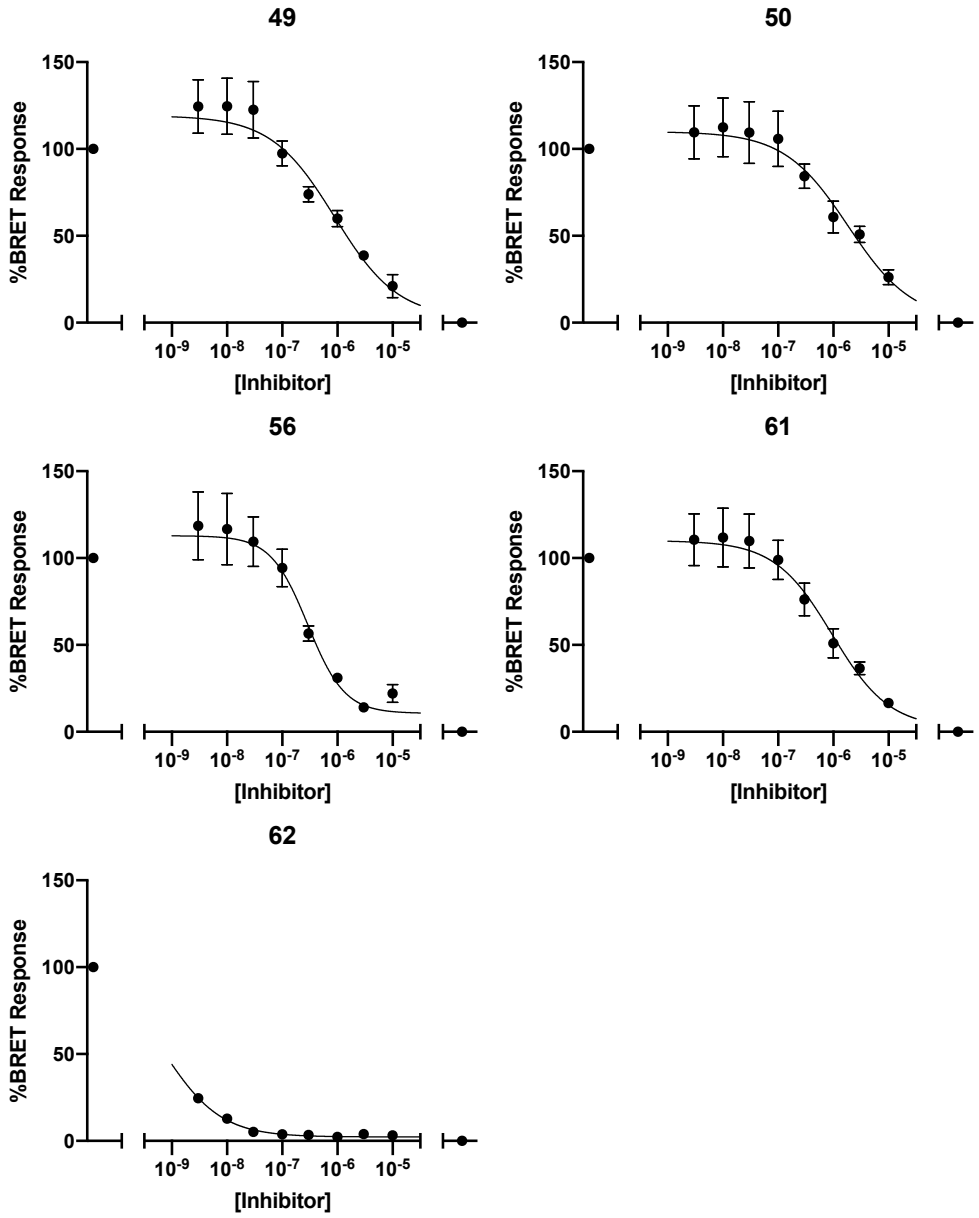


Table S1: NanoBRET IC50 values

Cmpd	IC_{50} ± SEM [nM]
GSK650394	<3
13g	700 ± 9.3
14g	210 ± 24
19	530 ± 95
20	190 ± 19
21	200 ± 11
29	240 ± 7
30	470 ± 44
33	>10000
34	8.1 ± 0.8
37	6.4 ± 0.5
38	170 ± 18
41	570 ± 130
42	1400 ± 250
45	540 ± 62

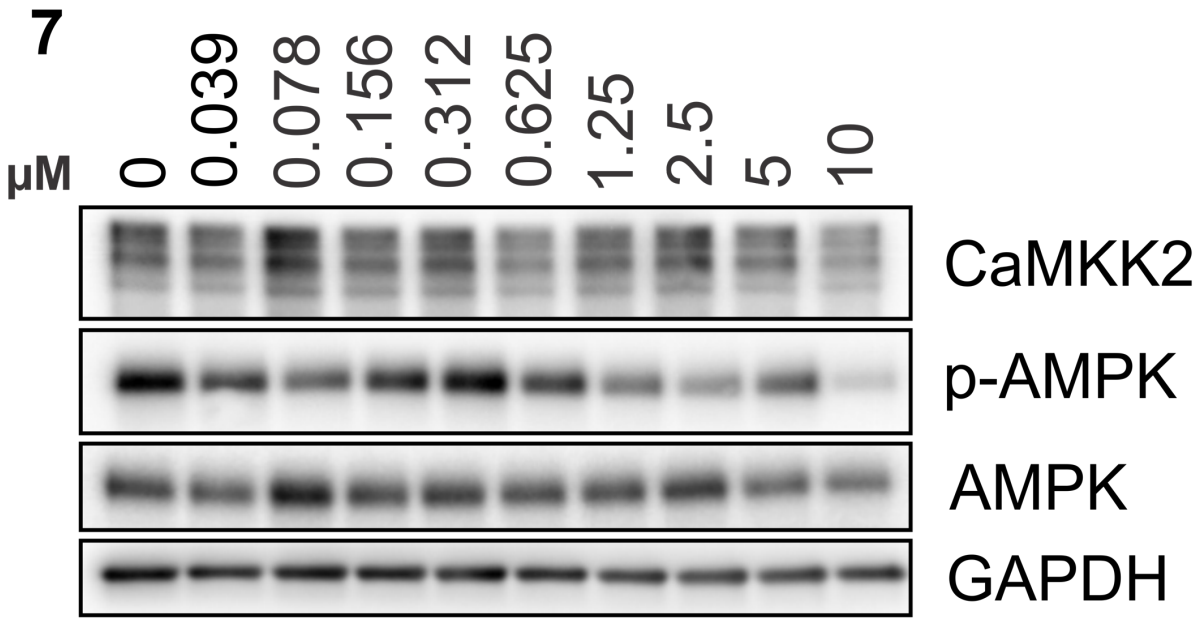
46	140 ± 12
49	890 ± 71
50	1900 ± 200
56	290 ± 23
61	950 ± 110
62	<3

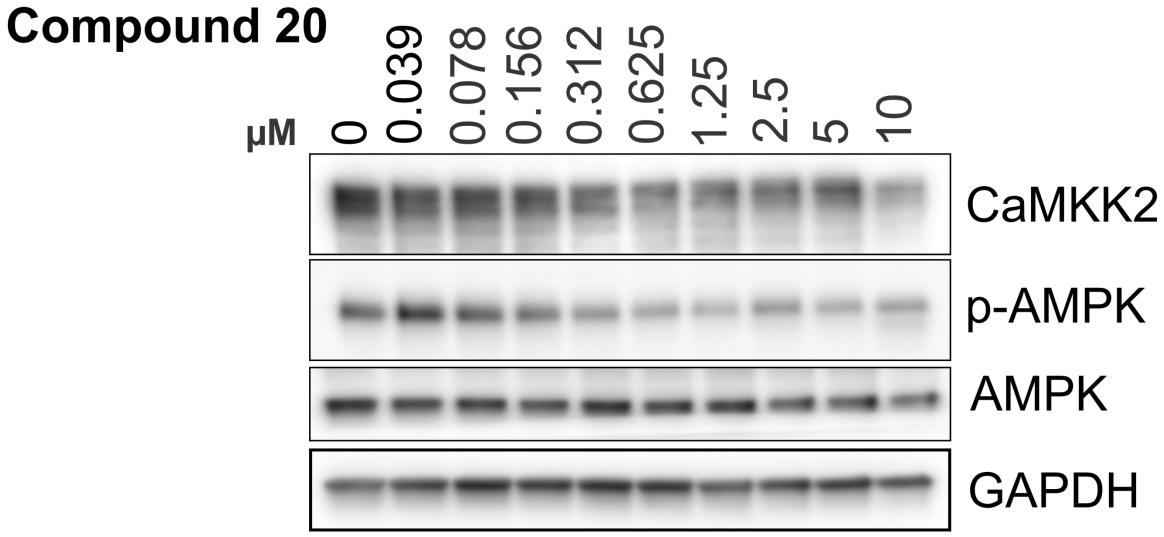
4. Western blots

Western blot analysis: C4-2 cells, a castration resistant prostate cancer cell line, were plated in 6-well plates at 500,000 cells/well in IMEM medium containing 0.5% FBS to mimic starvation. After 72 hours, the cells were then treated with the compounds for 24 hours before the media was aspirated, and wells were washed twice in ice cold PBS. Cells were lysed using RIPA buffer containing phosphatase and protease inhibitor cocktail while rotating for 30 minutes at 4 °C. In each lane, 30 μg/well of protein lysate was loaded into a 10% SDS-PAGE gel and run at 110V for 1 hour and 30 minutes. Gels were then transferred at 30V overnight in a TRIS-glycine/methanol transfer buffer onto a PVDF membrane at 4 °C. Membranes were blocked, incubated with primary overnight at 4 °C, washed, incubated with secondary at room temperature for 1 hour, washed, and then developed on an Azure Biosystems C-600 imager. Densitometry was performed by quantifying p-AMPK and AMPK expression levels using ImageJ. Each p-AMPK blot was normalized to the expression of corresponding AMPK blot.
[Cell Signaling: Phospho-AMPKα (Thr172) (40H9) Rabbit mAb: Cat#: 2535; AMPKα (D5A2) Rabbit mAb Cat#: 5831; BD Bioscience: CAMKK mouse mAb Cat# 610544; Sigma: GAPDH rabbit pAb: Cat# G9545; Secondary antibody: Goat Anti-Rabbit IgG (H + L)-HRP Conjugate was from Bio-Rad (Cat#:1706515).]

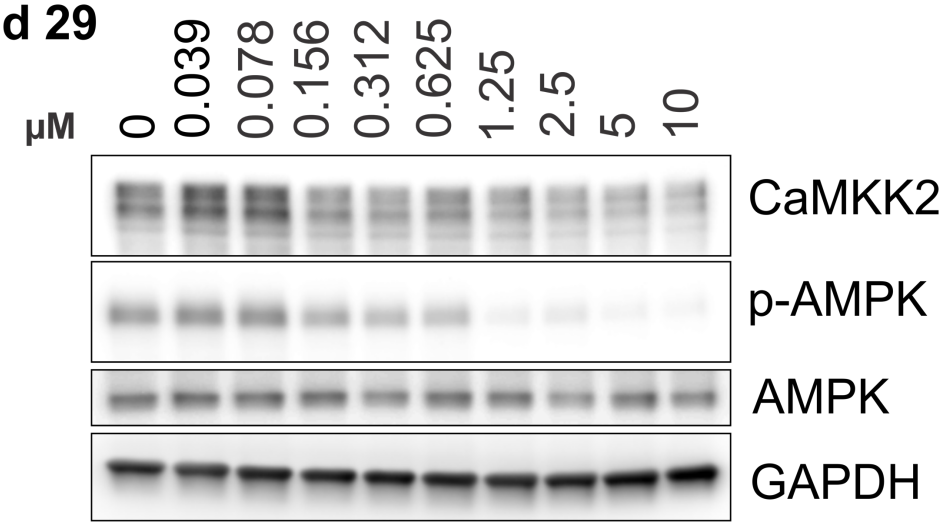
Figure S2: Western blot dose response experiments

Dose response for compounds 7, 20, 29, 34, 38, 46, 56, 62


7
μM
0
0.039
0.078
0.156
0.312
0.625
1.25
2.5
5
10
CaMKK2
p-AMPK
AMPK
GAPDH


Compound 20
μM
0
0.039
0.078
0.156
0.312
0.625
1.25
2.5
5
10
CaMKK2
p-AMPK
AMPK
GAPDH

Compound 29



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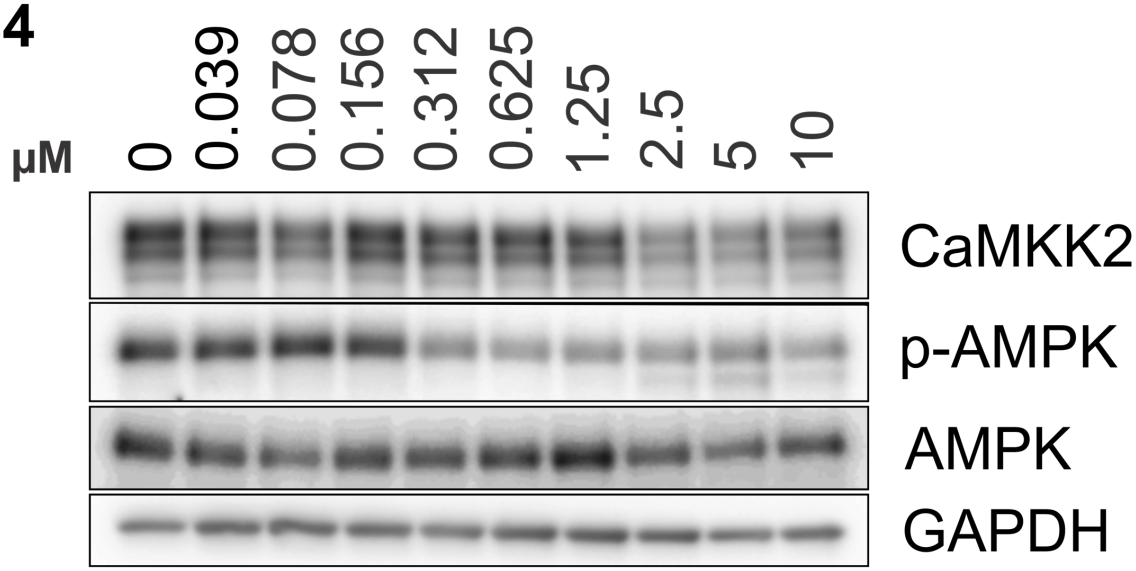

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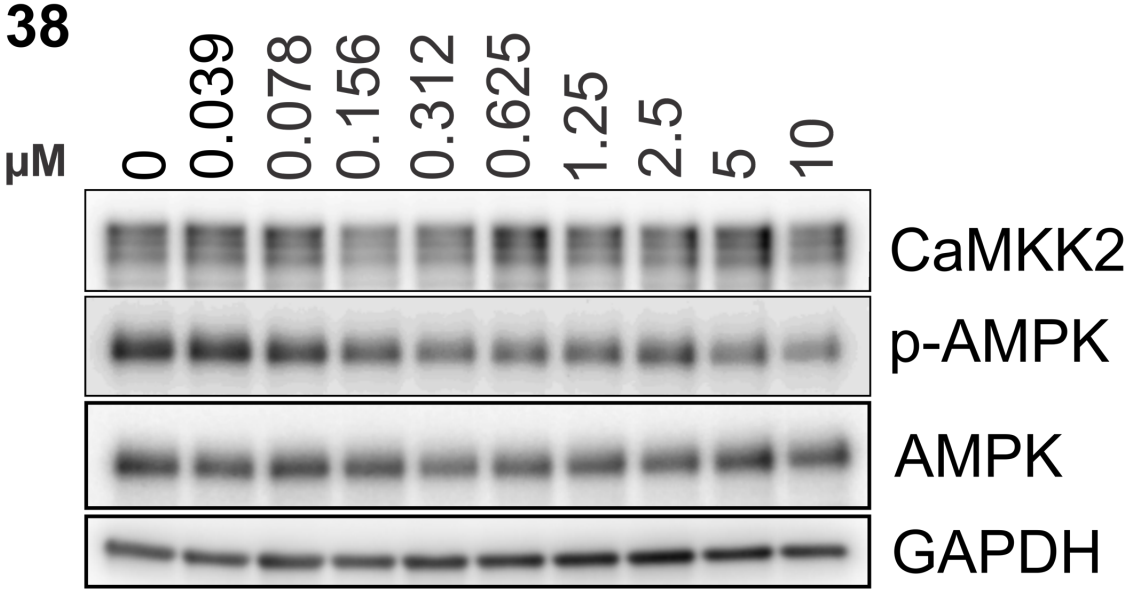
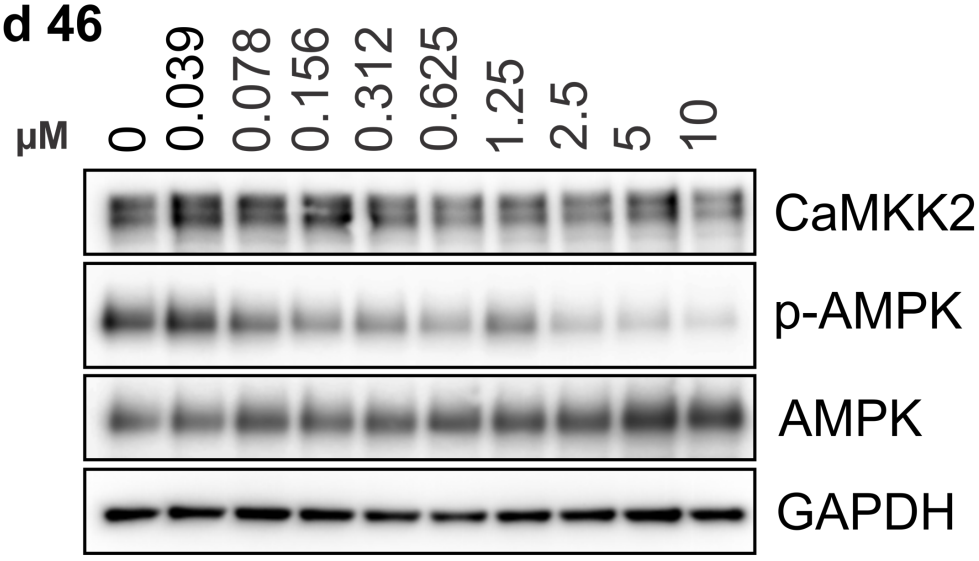
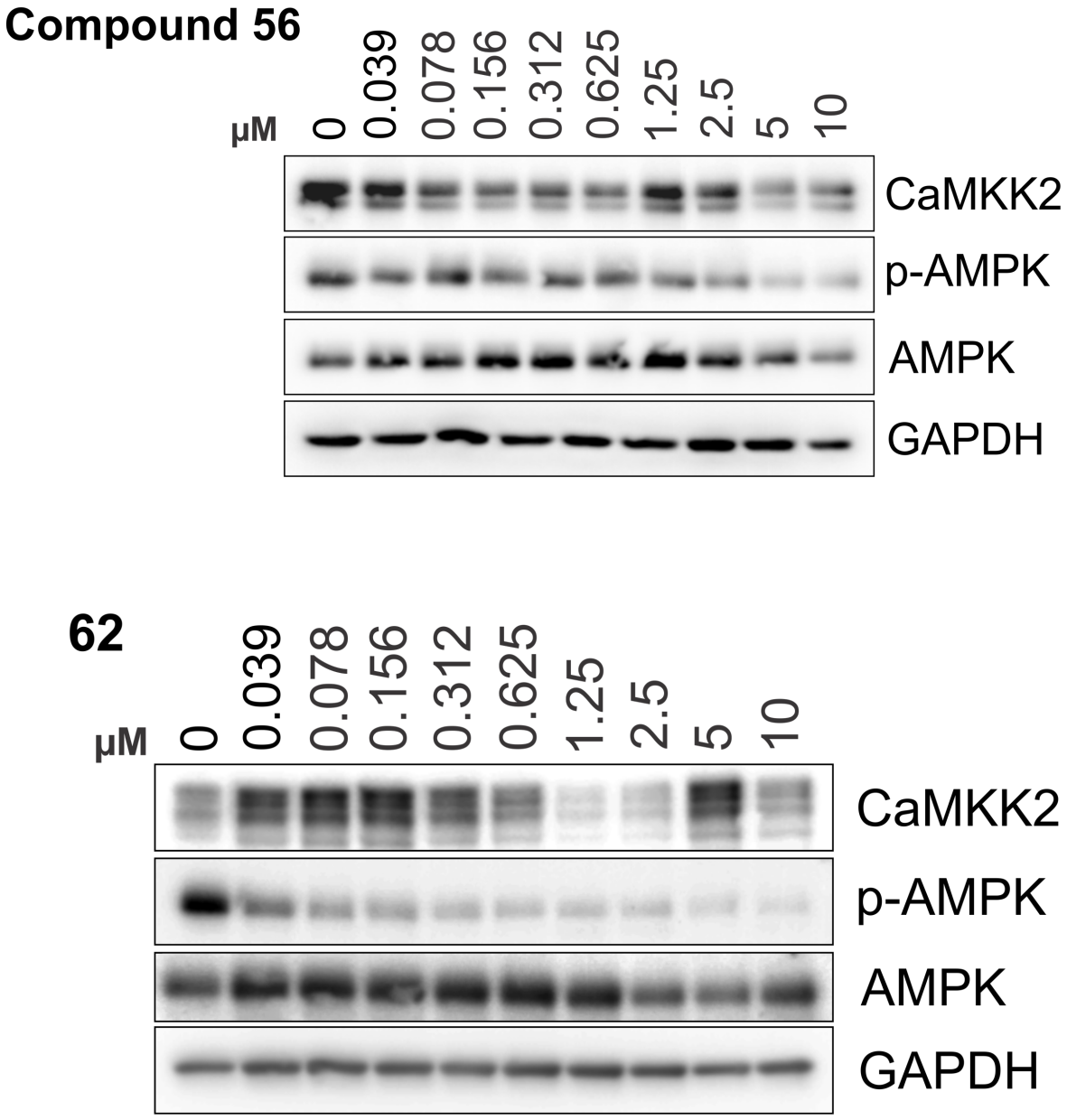
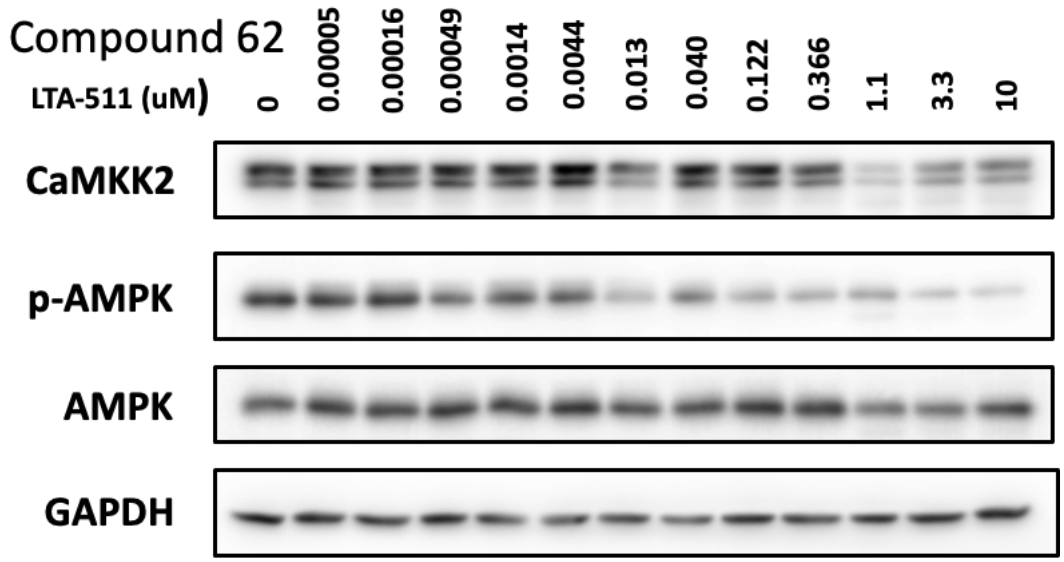


Compound 46





Compound 62 Western blot data at lower compound concentrations:


Compound 62
LTA-511 (uM)
0
0.00005
0.00016
0.00049
0.0014
0.0044
0.013
0.040
0.122
0.366
1.1
3.3
10
CaMKK2
p-AMPK
AMPK
GAPDH

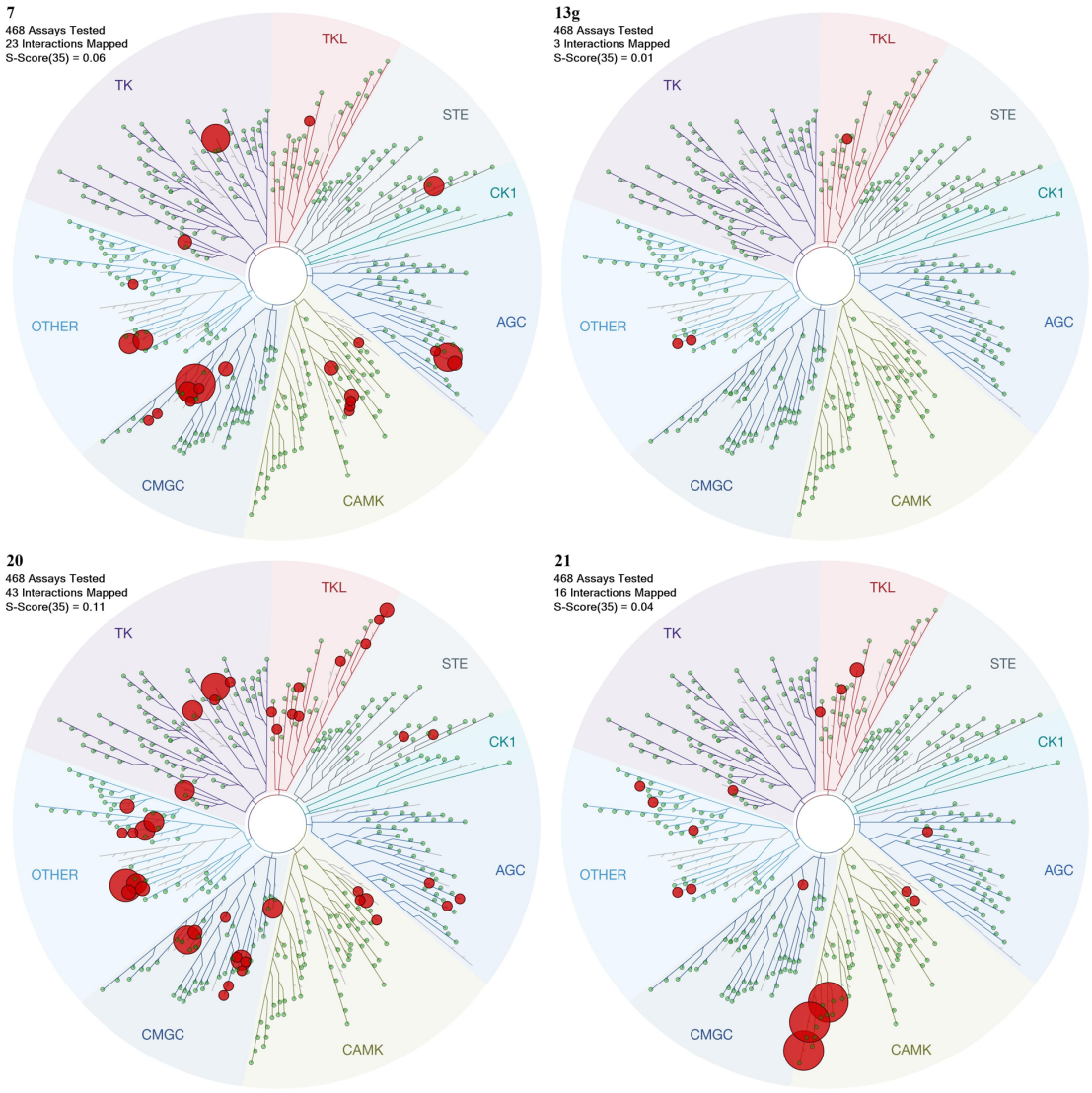
5. Docking studies

The crystal structures of CaMKK2 in complex with **13g** (PDB ID: 5UY6), UNC10244803 (PDB ID: 5UYJ) and GSK650394 (PDB ID: 6BKU) were taken from the Protein Data Bank (PDB; www.rcsb.org).
All water molecules, except of the conserved water molecule 605 (6BKU), 629 (5UY6) and 631 (5UYJ) respectively, were removed from the binding pocket. Compounds GSK650394 and **22** were docked in X-ray structure 6BKU, compound **14g** were docked in X-ray structure 5UY6 and compounds **29**, **38**, **46** and **56** were docked in X-ray structure 5UYJ. The following docking procedure applies to docking of all compounds.
The protein structure with the conserved water molecule and without the co-crystallized ligand was prepared using Protein Preparation Wizard[1] from Maestro (Schrödinger Release 2018-4: Protein Preparation Wizard, Schrödinger, LLC, New York, NY, 2018). Hydrogen atoms were added, protonation states were assigned as default, and a restrained minimization was performed (0.3 Å, OPLS3e). The Flare tool provided by Cresset Software (Flare 2.0, Cresset Inc., Cambridgeshire, UK) was used to generate the molecular structures of all compounds at the physiological pH. The ligands were subsequently prepared for docking using the LigPreptool[3] as implemented in Schrödinger's software, where all possible tautomeric forms as well as stereoisomers were generated, and energy minimized using the OPLS forcefield. The receptor grid preparation for the docking procedure was carried out by assigning the co-crystallized ligand as the centroid of the grid box while keeping the conserved water molecule (605, 629 or 631, respectively) in the binding pocket. The prepared ligand structures were docked into the binding pocket using the program Glide (Schrödinger Release 2018-4: Glide, Schrödinger, LLC, New York, NY, 2018)[4] in the Standard Precision mode. A total of 20 poses per ligand conformer were included in the post-docking minimization step and a maximum of two docking poses were output for each ligand conformer. Using this setup, the co-crystallized inhibitors could be correctly docked (RMSD below 1 Å) into the CaMKK2 binding pocket.

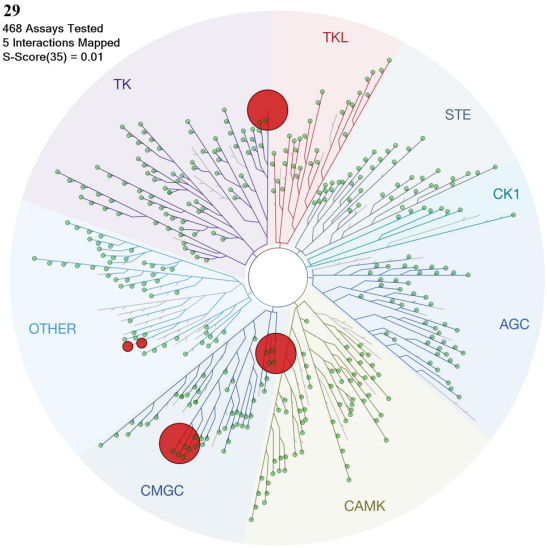
References:

1. Madhavi Sastry, G.; Adzhigirey, M.; Day, T.; Annabhimoju, R.; Sherman, W. Protein and ligand preparation: Parameters, protocols, and influence on virtual screening enrichments. *J. Comput. Aided. Mol. Des.* **2013**, *27*, 221–234.
2. Greenwood, J.R.; Calkins, D.; Sullivan, A.P.; Shelley, J.C. Towards the comprehensive, rapid, and accurate prediction of the favorable tautomeric states of drug-like molecules in aqueous solution. *J. Comput. Aided. Mol. Des.* **2010**, *24*, 591–604.
3. Friesner, R.A.; Banks, J.L.; Murphy, R.B.; Halgren, T.A.; Klicic, J.J.; Mainz, D.T.; Repasky, M.P.; Knoll, E.H.; Shelley, M.; Perry, J.K.; et al. Glide: A New Approach for Rapid, Accurate Docking and Scoring. 1. Method and Assessment of Docking Accuracy. *J. Med. Chem.* **2004**, *47*, 1739–1749.
4. Friesner, R.A.; Murphy, R.B.; Repasky, M.P.; Frye, L.L.; Greenwood, J.R.; Halgren, T.A.; Sanschagrin, P.C.; Mainz, D.T. Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. *J. Med. Chem.* **2006**, *49*, 6177–6196.

Figure S3. KINOME*scan* treespots depicting selectivity of selected CAMKK2 inhibitors. Red circles indicate kinase affinity less than or equal to 35% of control treated at 1 μM.



29
468 Assays Tested
5 Interactions Mapped
S-Score(35) = 0.01



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29 Interactions Mapped
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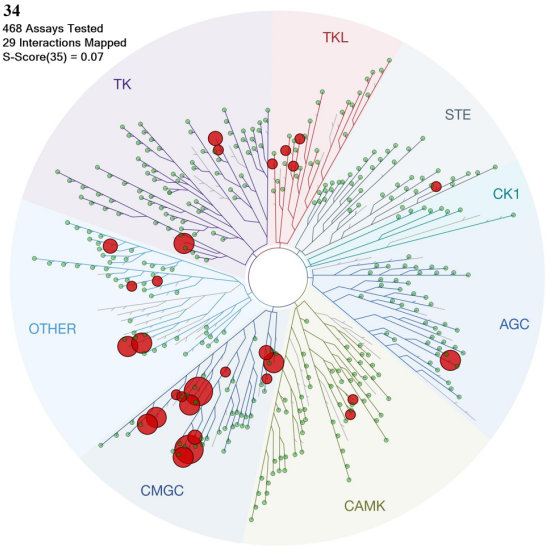

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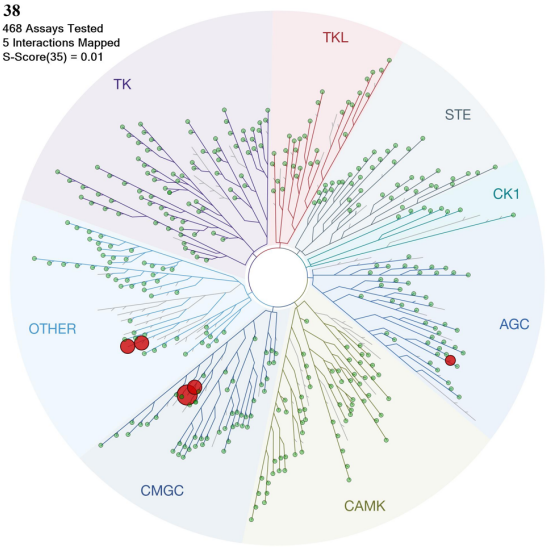
468 Assays Tested
5 Interactions Mapped
S-Score(35) = 0.01



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7 Interactions Mapped
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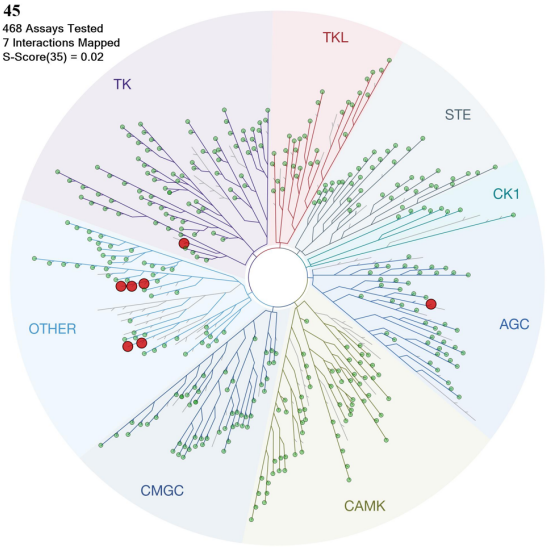

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936 Assays Tested
8 Interactions Mapped
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