Supplementary Material

5-Methoxybenzothiophene-2-carboxamides as inhibitors of Clk1/4: optimization of the selectivity and cellular potency

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Figure S1

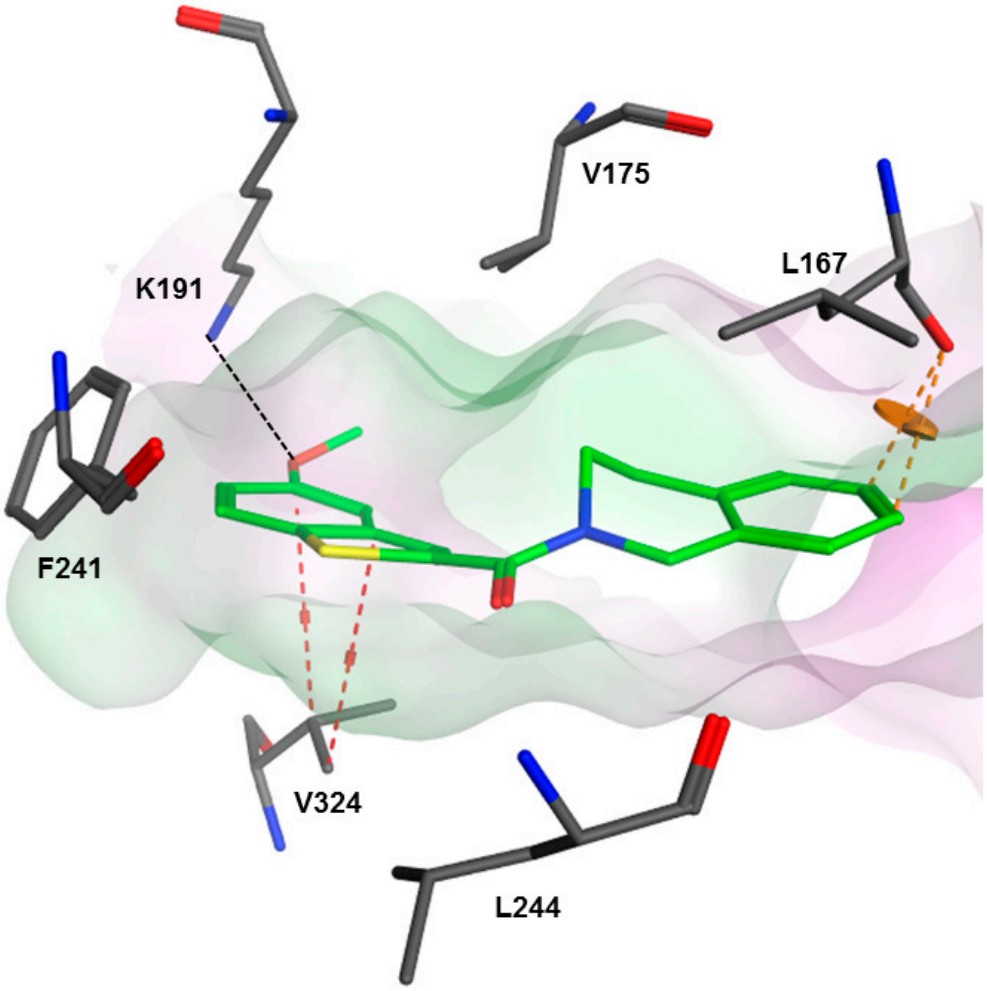


Figure S1: Molecular docking of compound **5a** (green) in the ATP binding pocket of Clk1 (PDB code of the coordinates: 1Z57) using MOE. Depicted is the least impaired potential binding pose. Although H-bonds were predicted with Lys191 (indicated in black), and CH-π interactions between the benzothiophene core and Val324, the steric clash with Leu167 (indicated in orange) is expected to strongly compromise the potential binding affinity. In addition, no H-bond was formed between Leu244 and the carbonyl oxygen of **5a**.

Figure S2

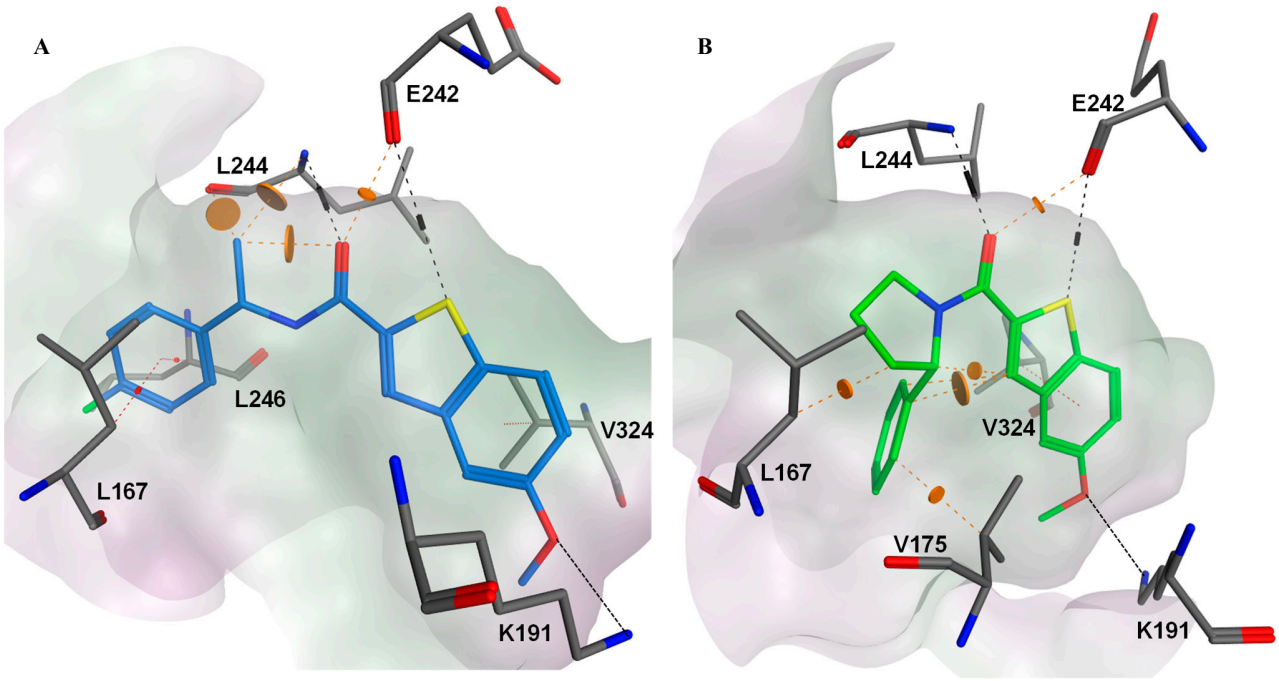


Figure S2: Molecular docking of compounds **3a (*S*)** (blue) and **6a** (green). **3a (*S*)** and **6a** were docked in the ATP binding pocket of Clk1 (PDB code: 1Z57) using MOE, and the binding poses with the lowest steric interferences were selected. (A) **3a (*S*)** (blue) was predicted to form H-bonds with Leu244 and Lys191 (indicated by black dashed lines) and CH-π interactions with Leu167, Leu264 and Val324 (red lines). However, steric clashes (orange) with Leu244 and Glu242 were also inherent to this pose. (B) **6a** (green) was predicted to form H-bonds with Leu244 and Lys191 (black dashed lines), in addition to a CH-π interaction between the benzothiophene core and Val324 (red line). However, steric clashes with Leu167 and Val175 as well as intramolecular steric interference also occurred with this pose (indicated in orange).

Figure S3

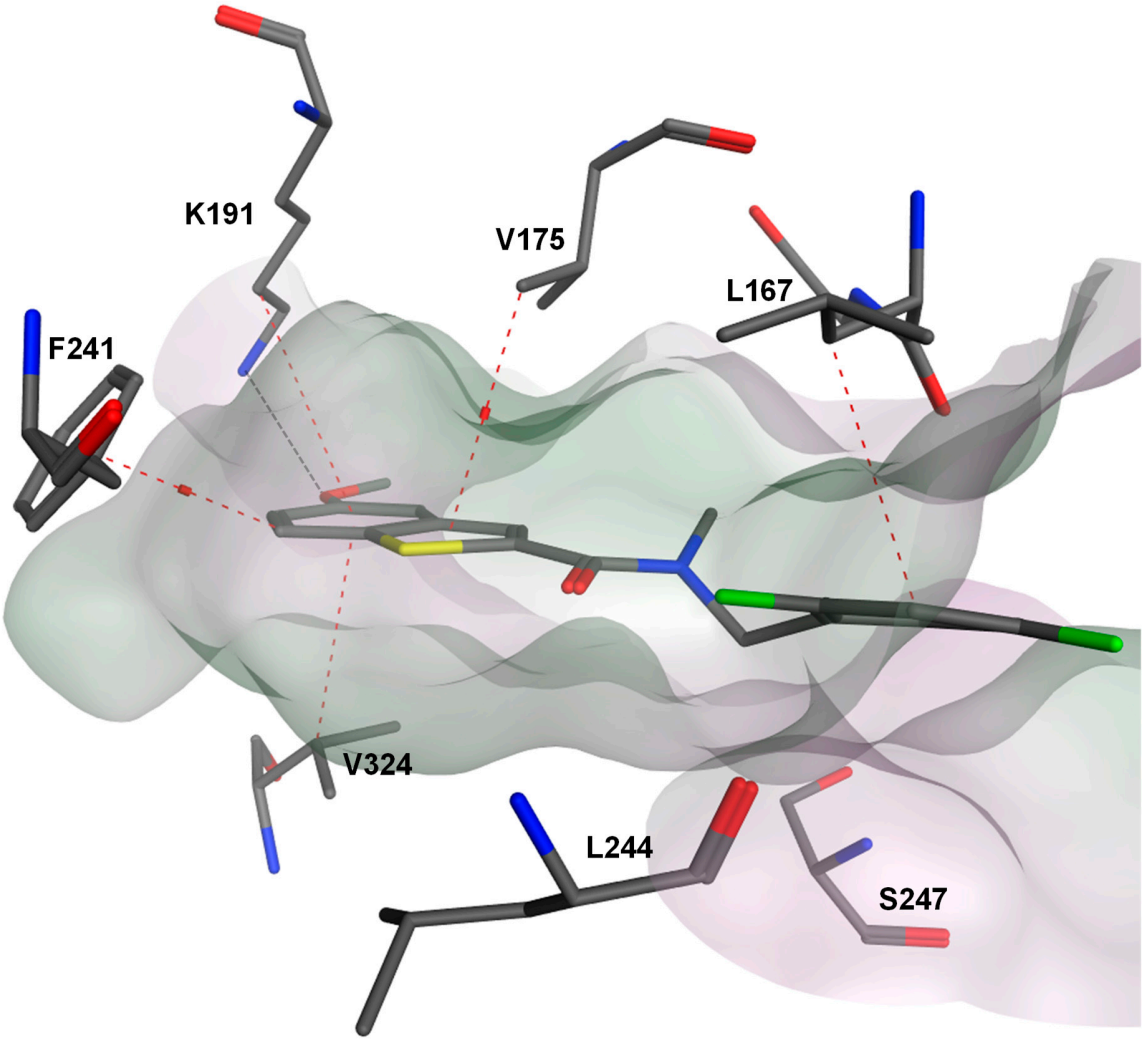
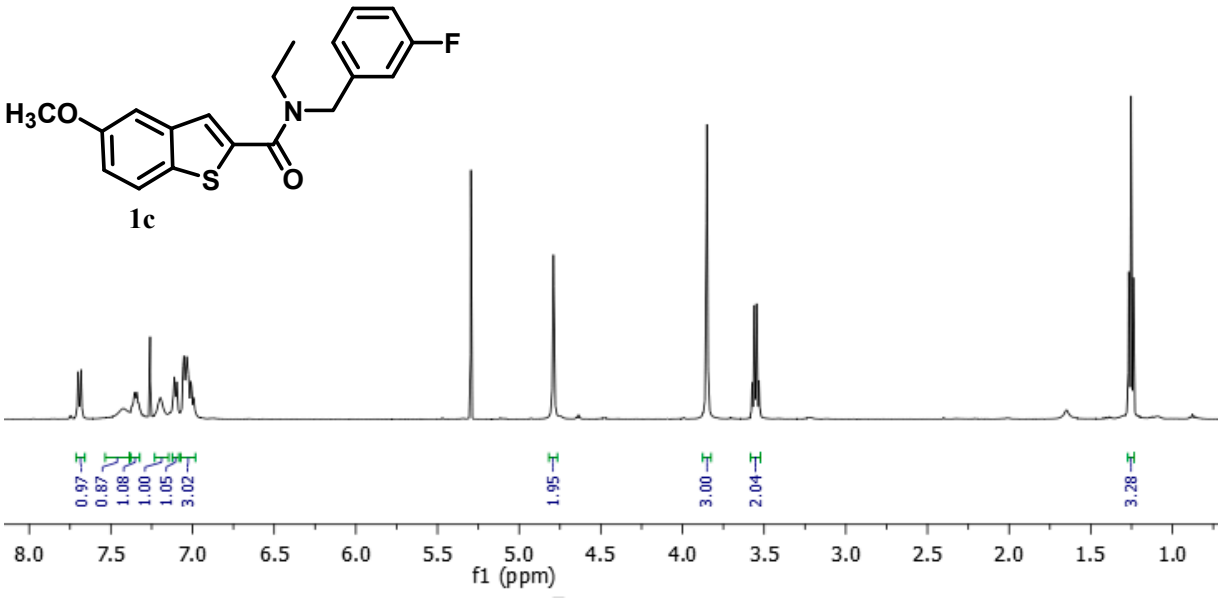


Figure S3: Molecular docking of compound **9b** (dark grey) in the binding pocket of Clk1 (PDB code 1Z57) using MOE. **9b** was predicted to interact through an H-bond with Lys191 (indicated by black dashed lines), CH-π interactions with Leu167, Val175, Lys191 and Val324 residues (red dashed lines), and an edge-to-face CH-π interaction with Phe241. However, the H-bond between Leu244 and the carbonyl oxygen could not form, suggesting a strong reduction of the overall binding affinity.

[1]H-NMR (500 MHz, DMSO) and [13]C-NMR (126 MHz, DMSO) spectra of all synthesized compounds.



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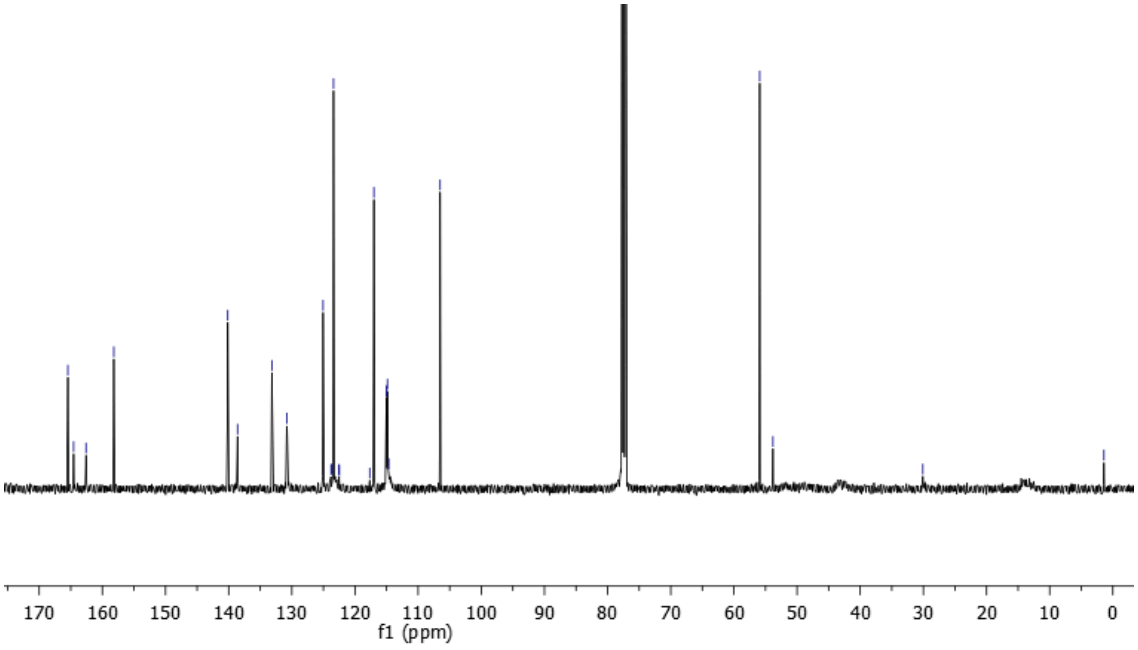

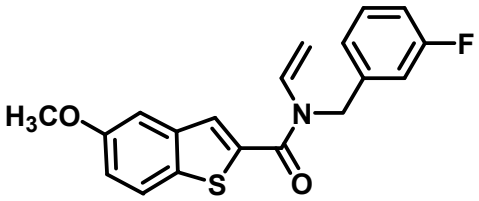
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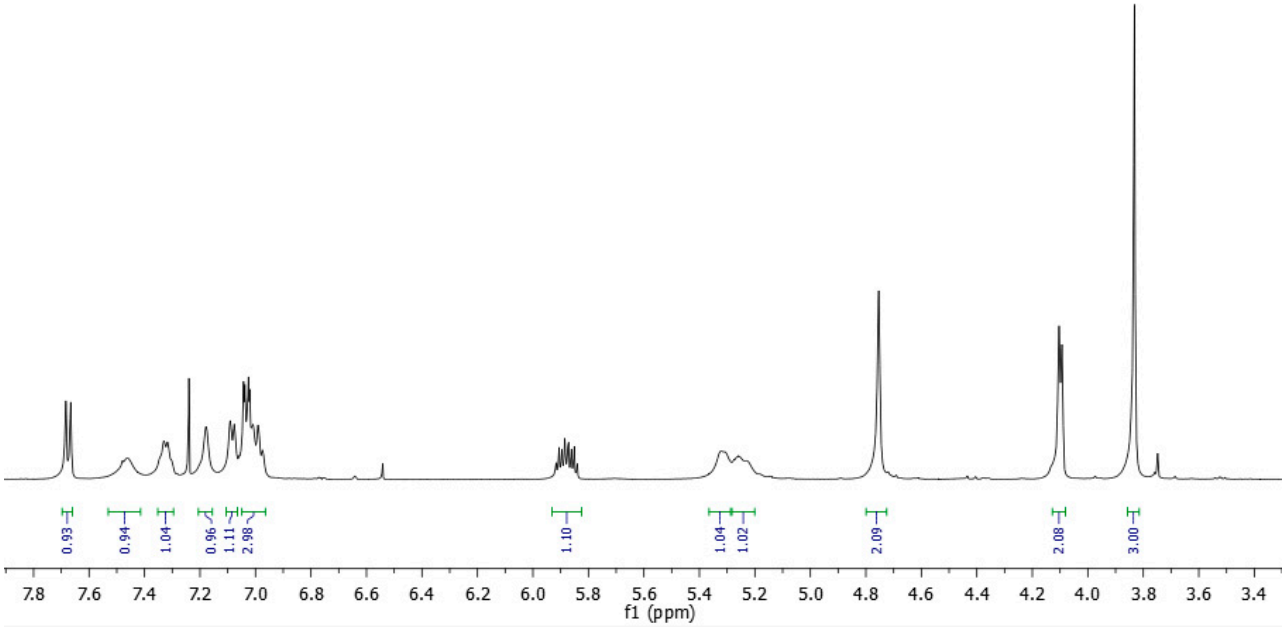
S
F

1d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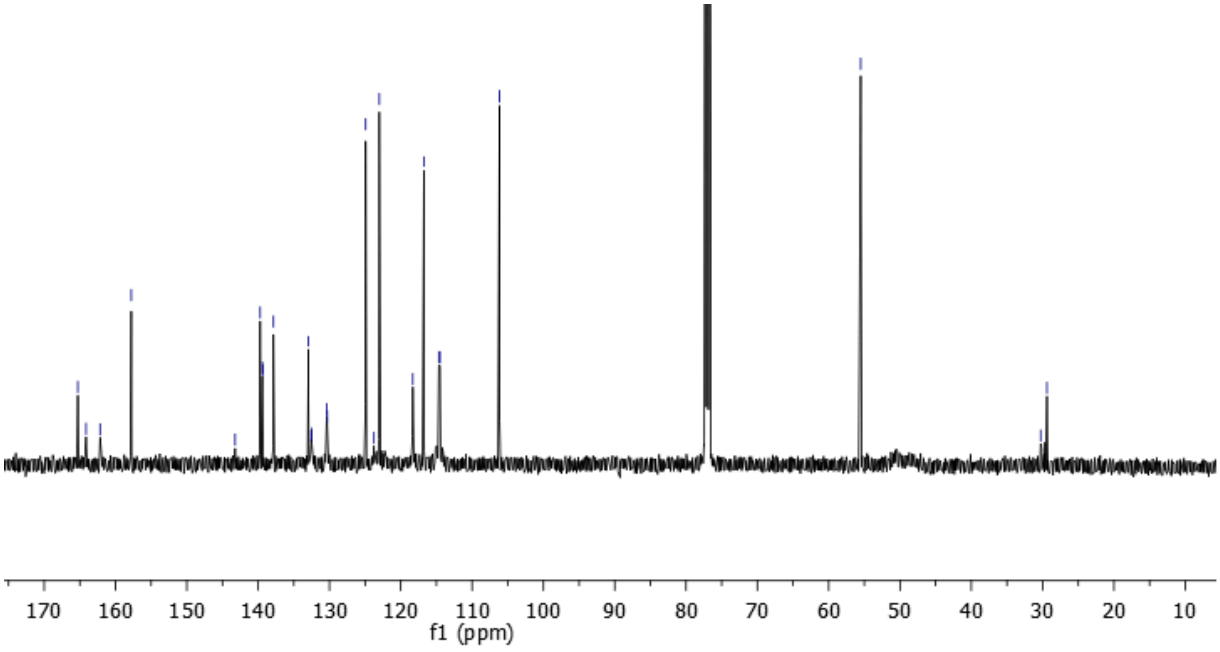
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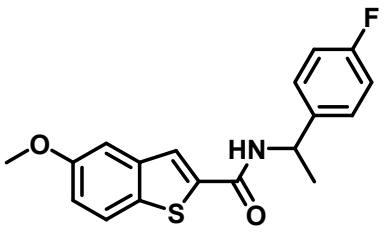
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S
O

3a

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3.03
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8.0
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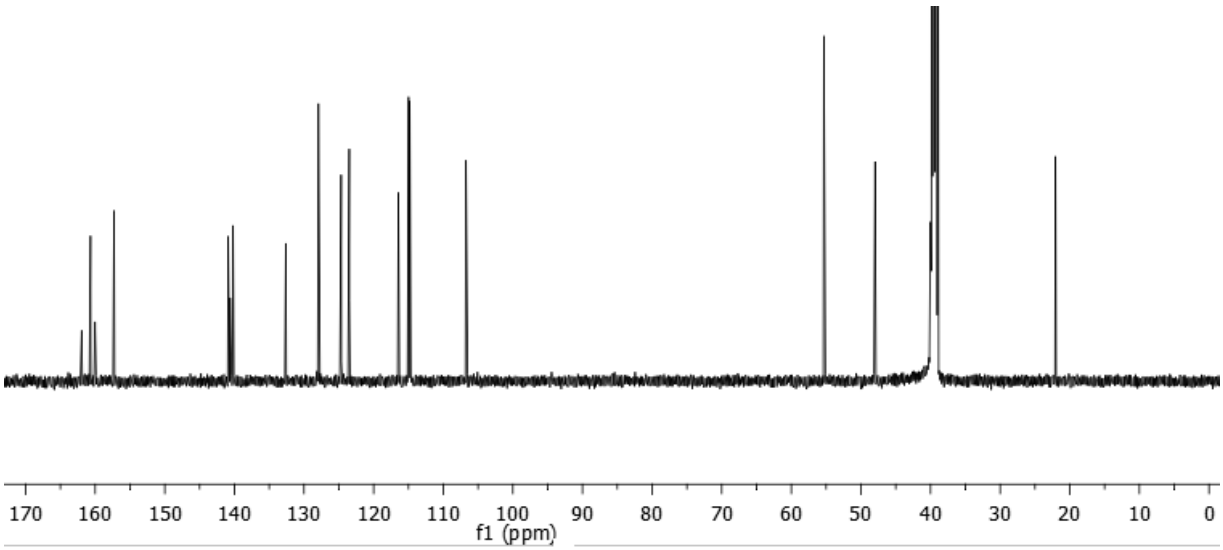
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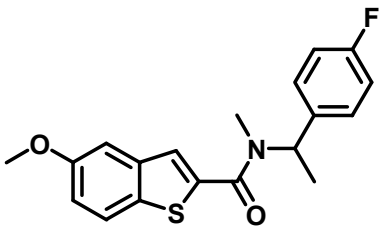
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3b

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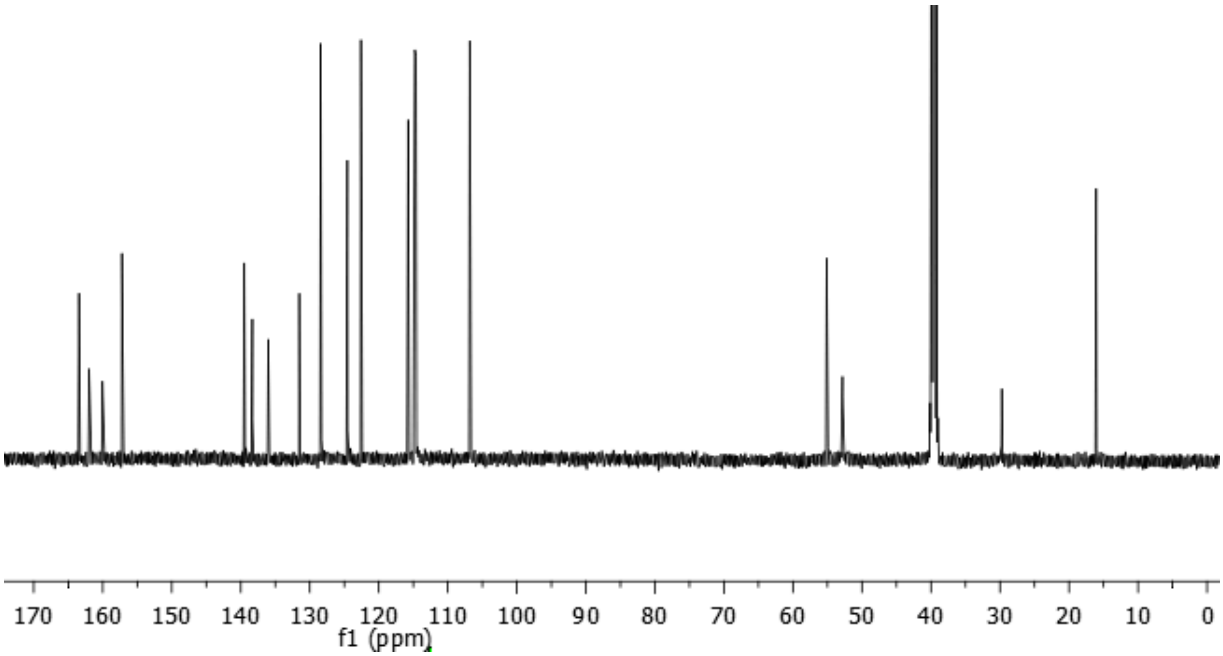
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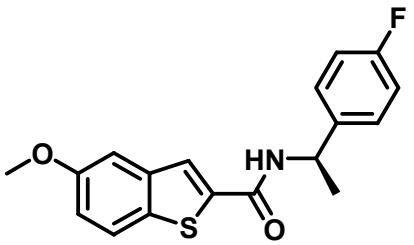
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4a

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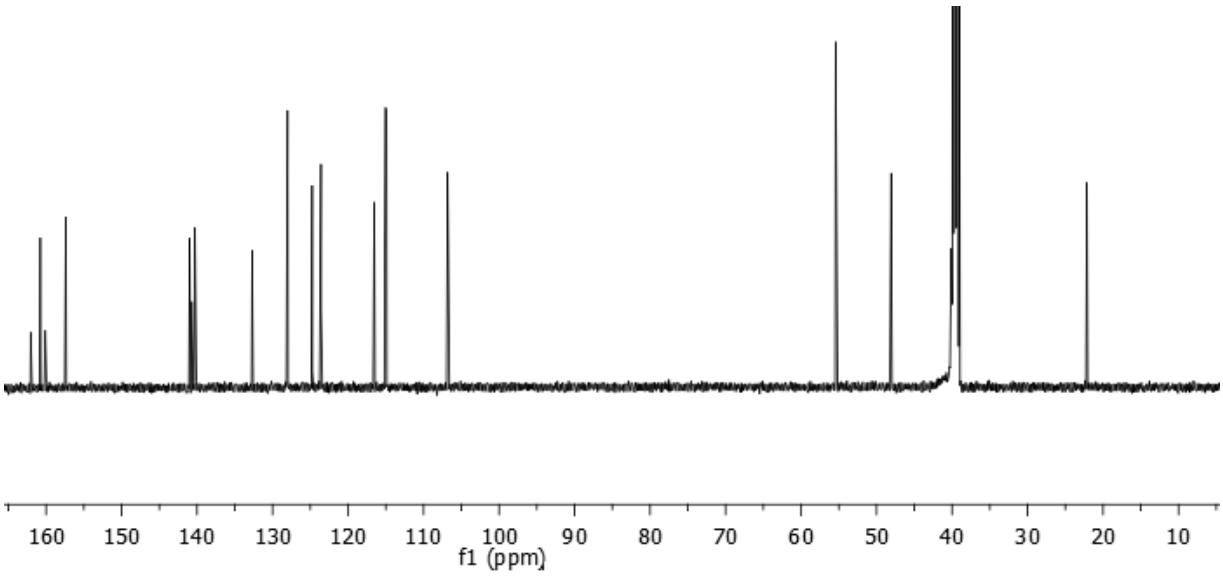
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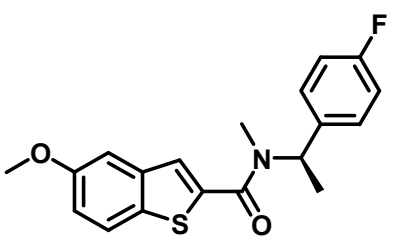
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4b

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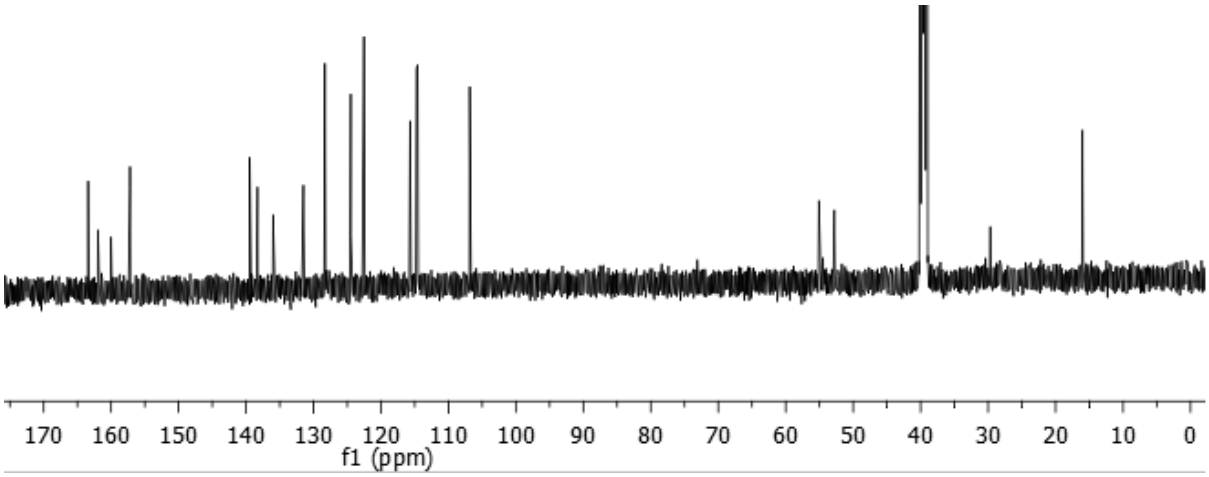
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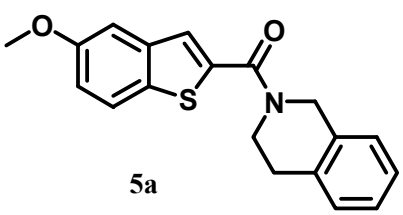
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5a

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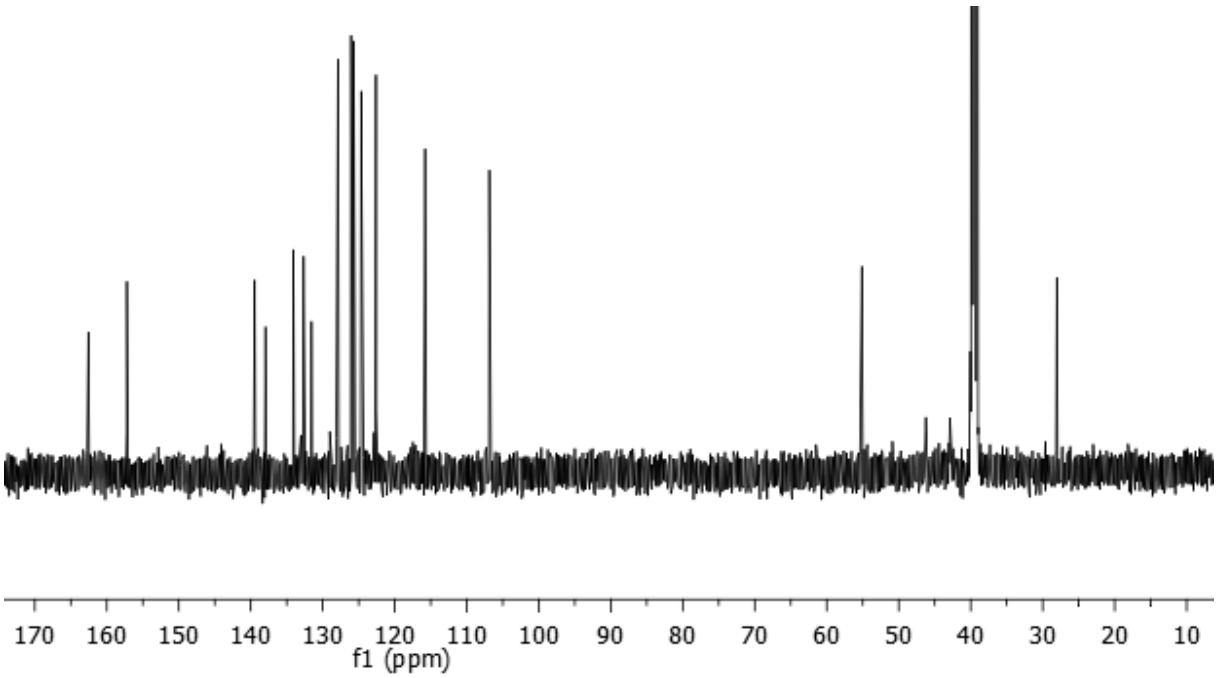
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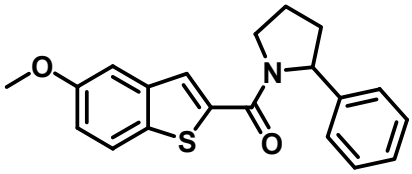
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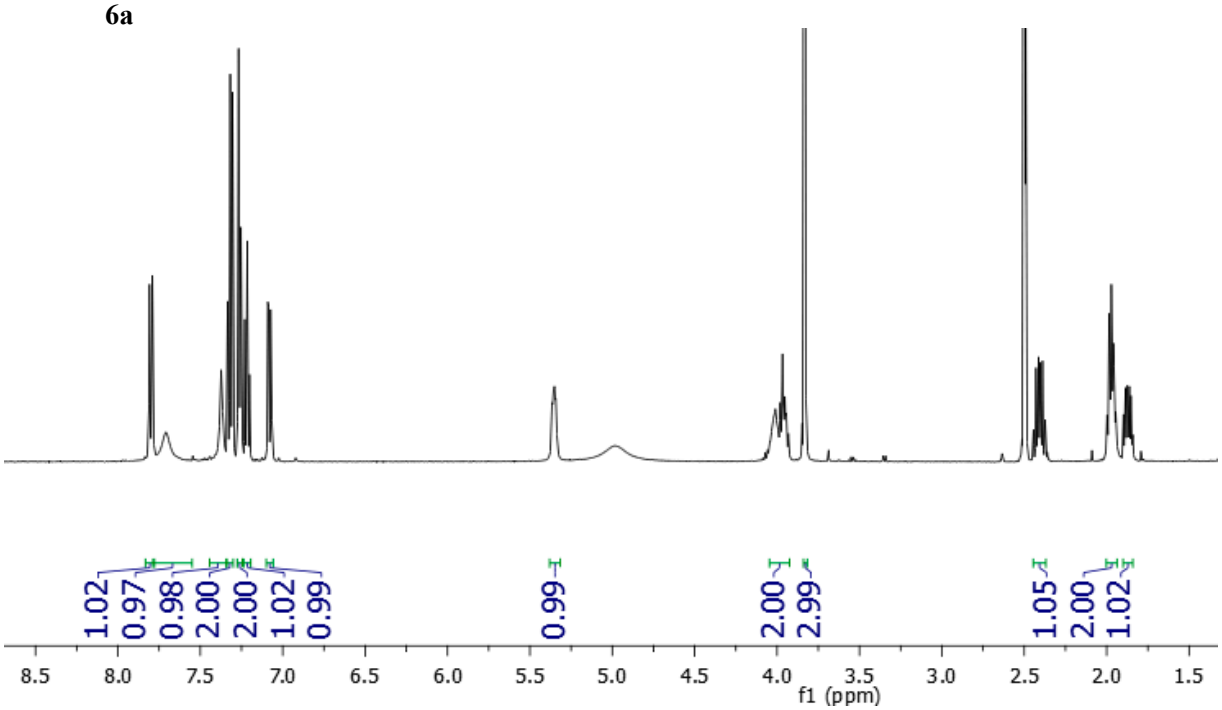
6a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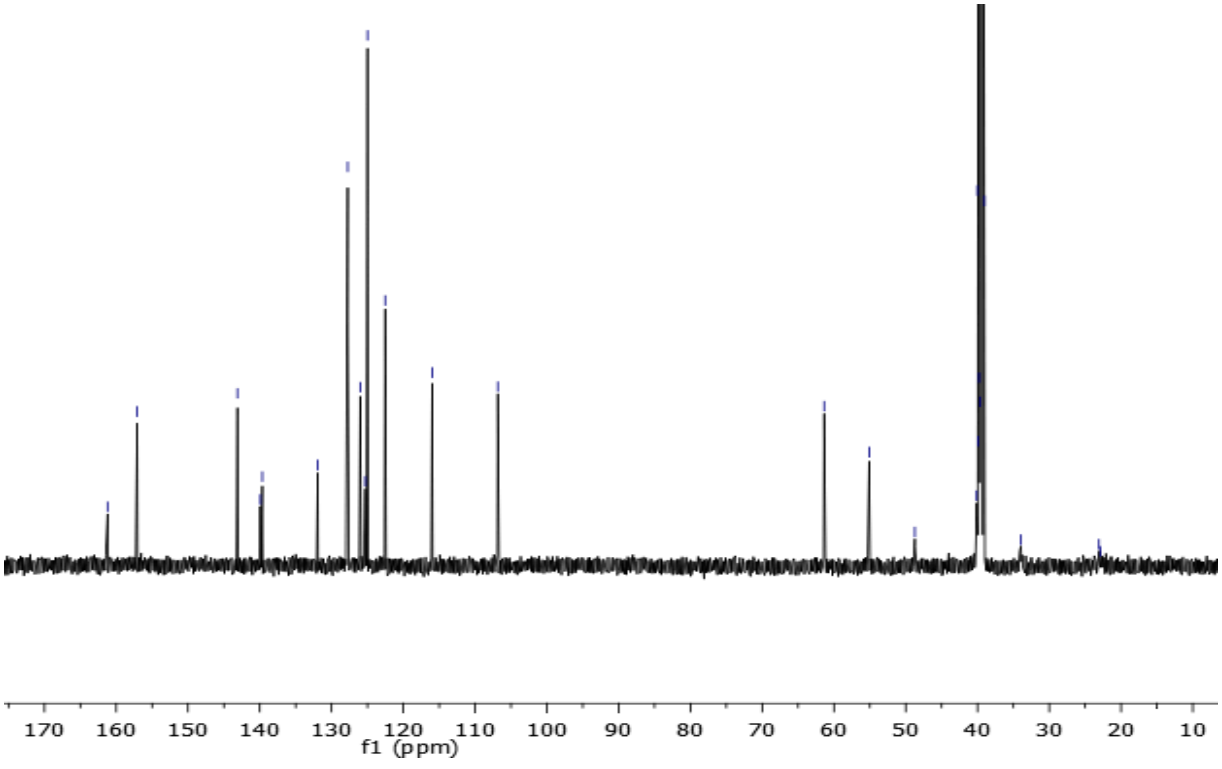
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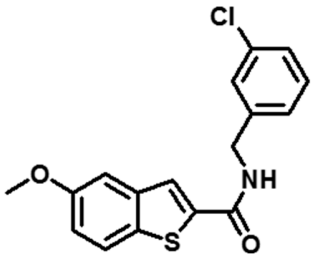
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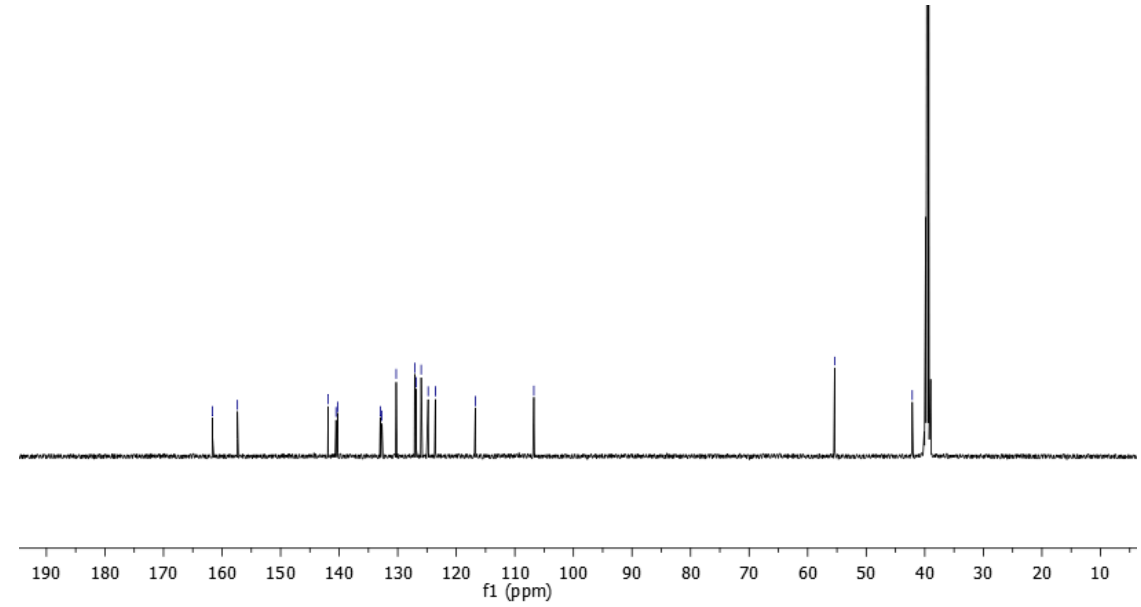
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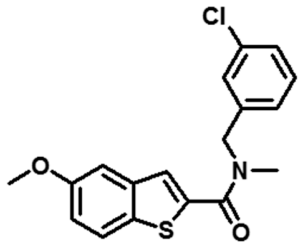
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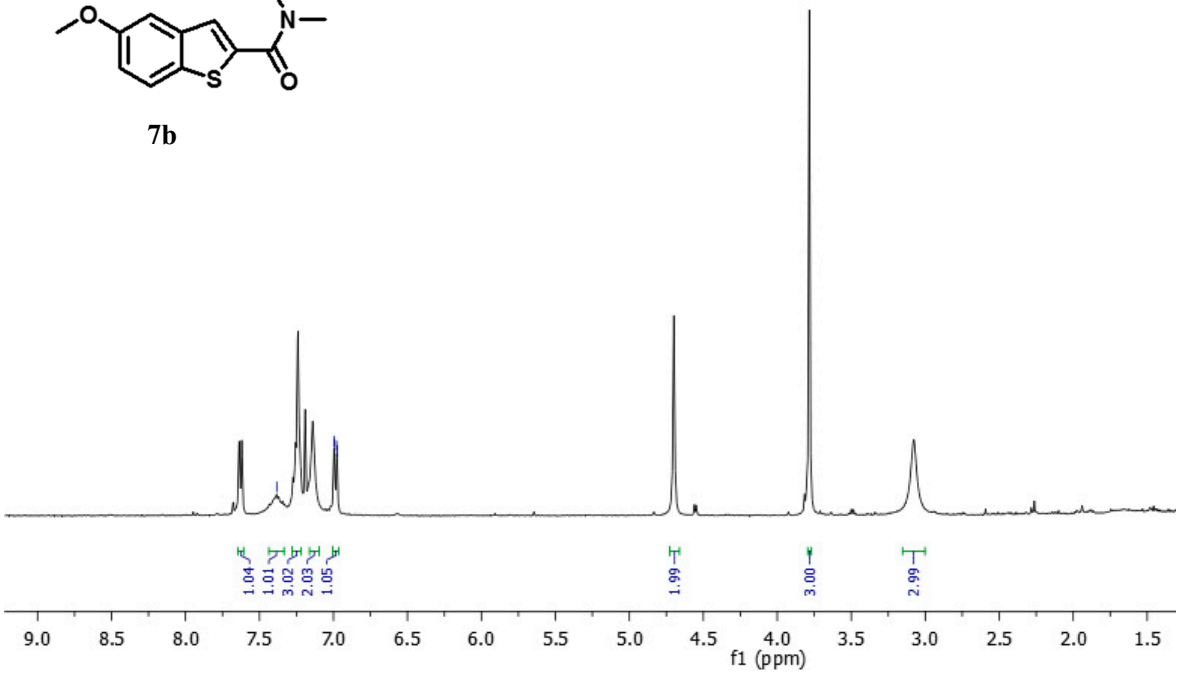
7b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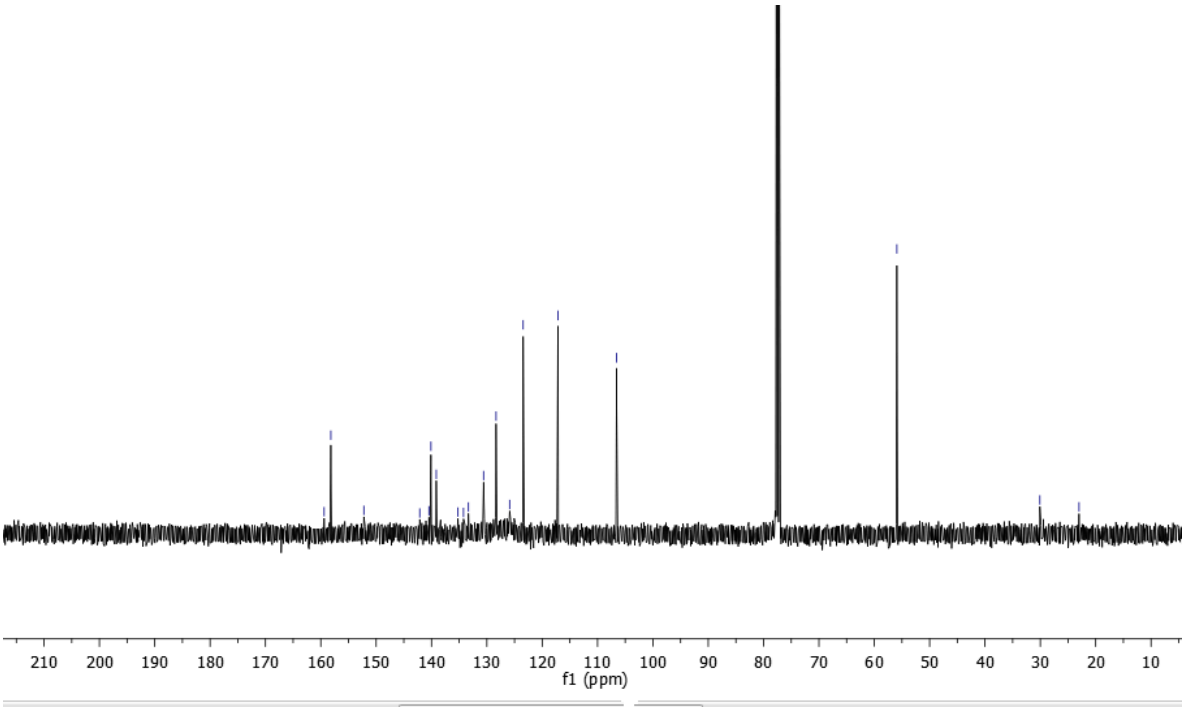
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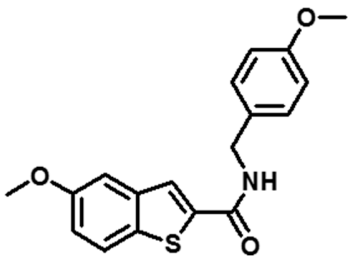
O
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8a

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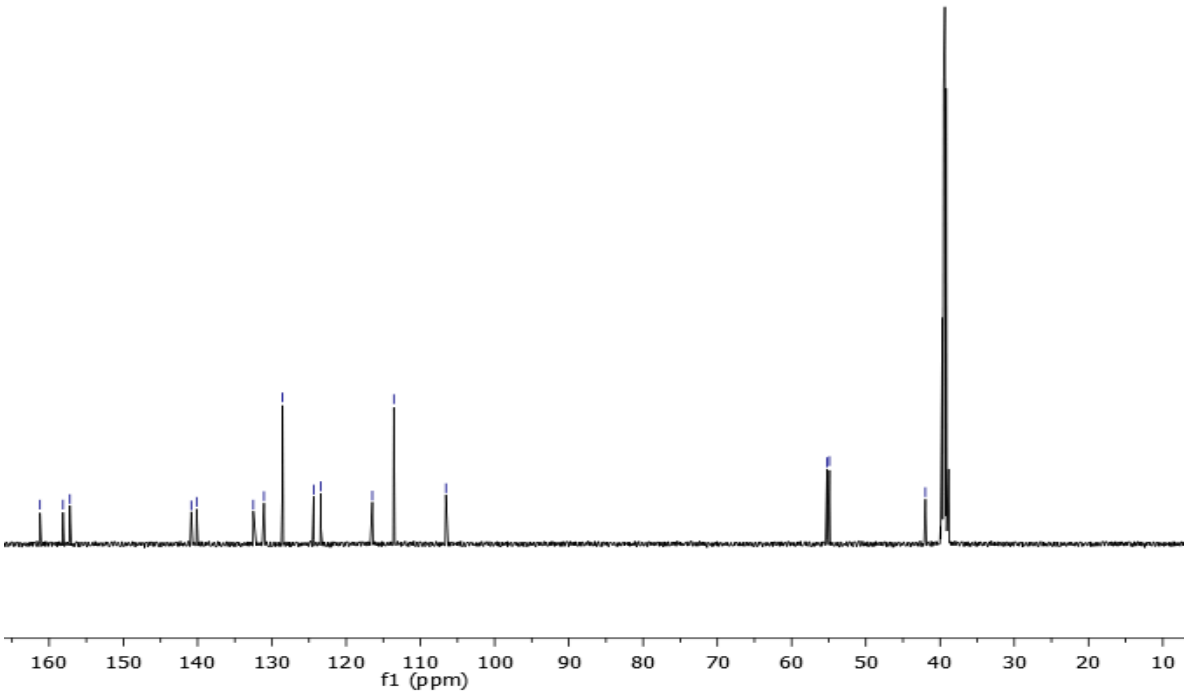
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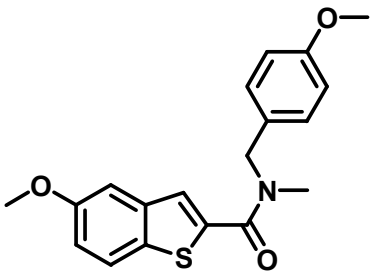
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8b

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3.00
3.18
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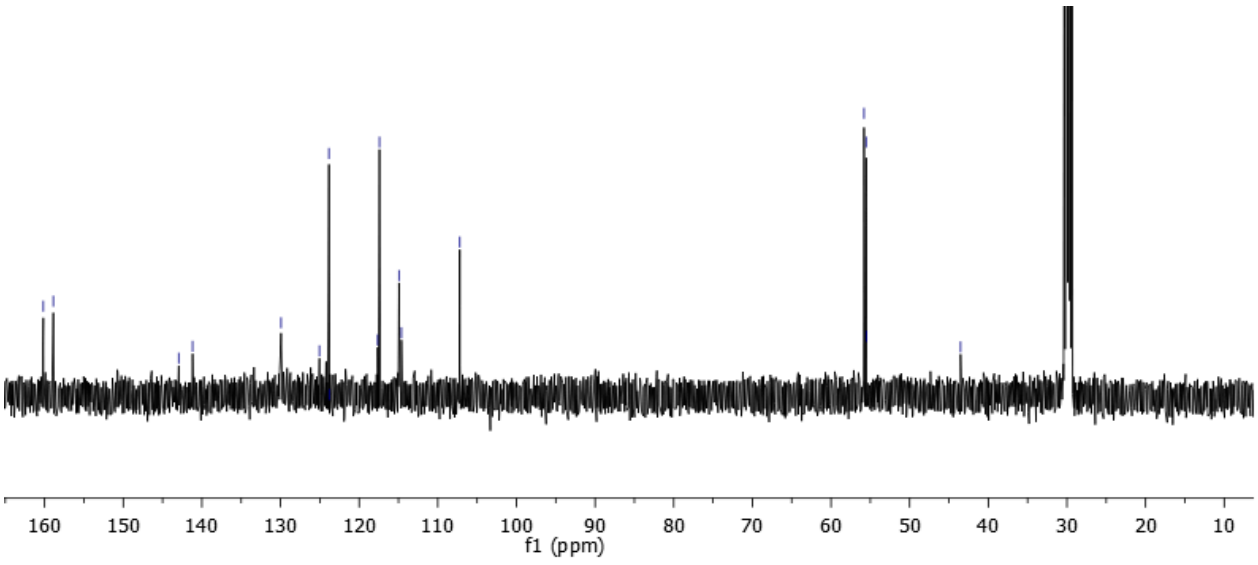
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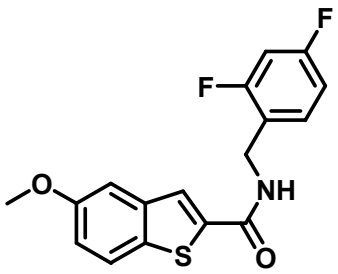
F
F
O
NH
S
O

9a

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1.00
0.93
1.11
1.05
2.12
2.03
3.00
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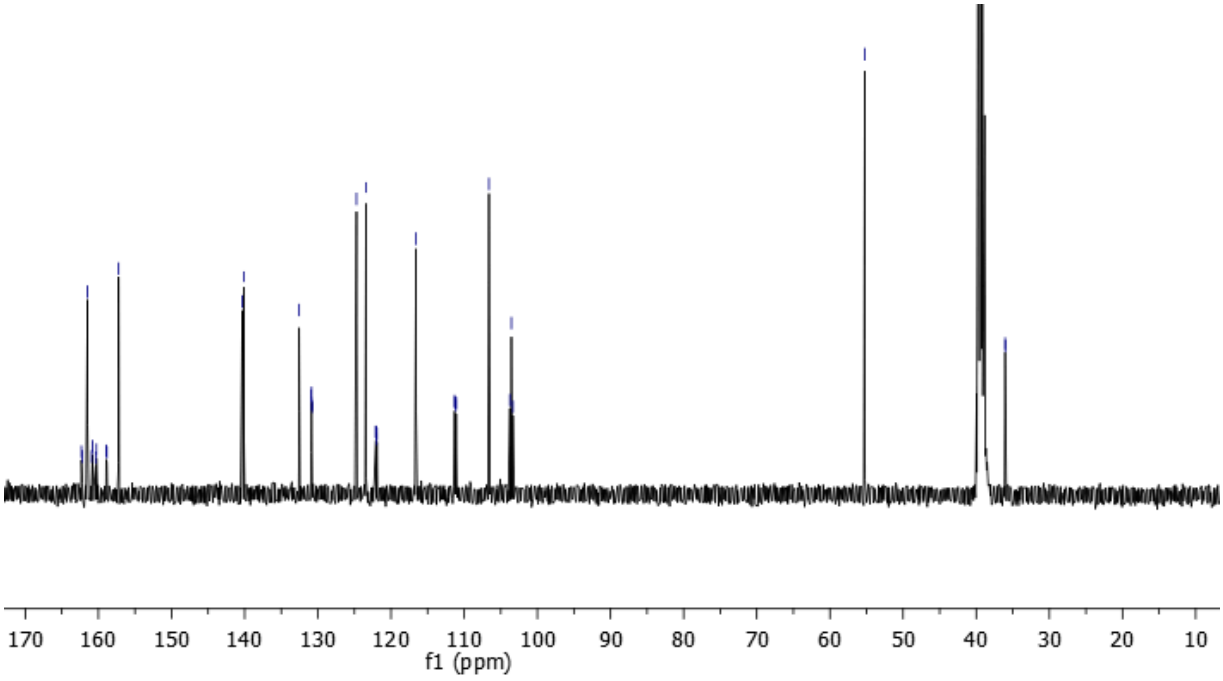
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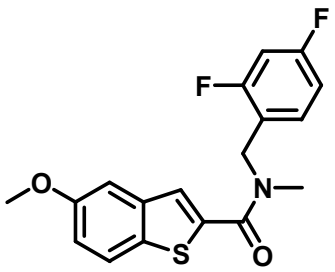
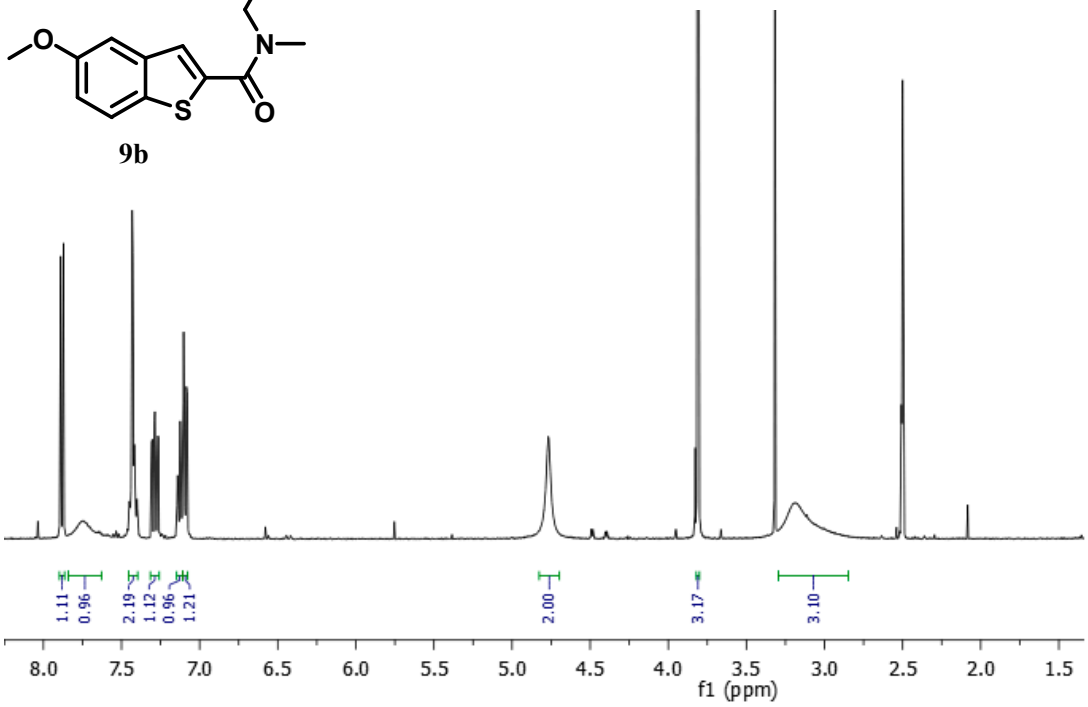
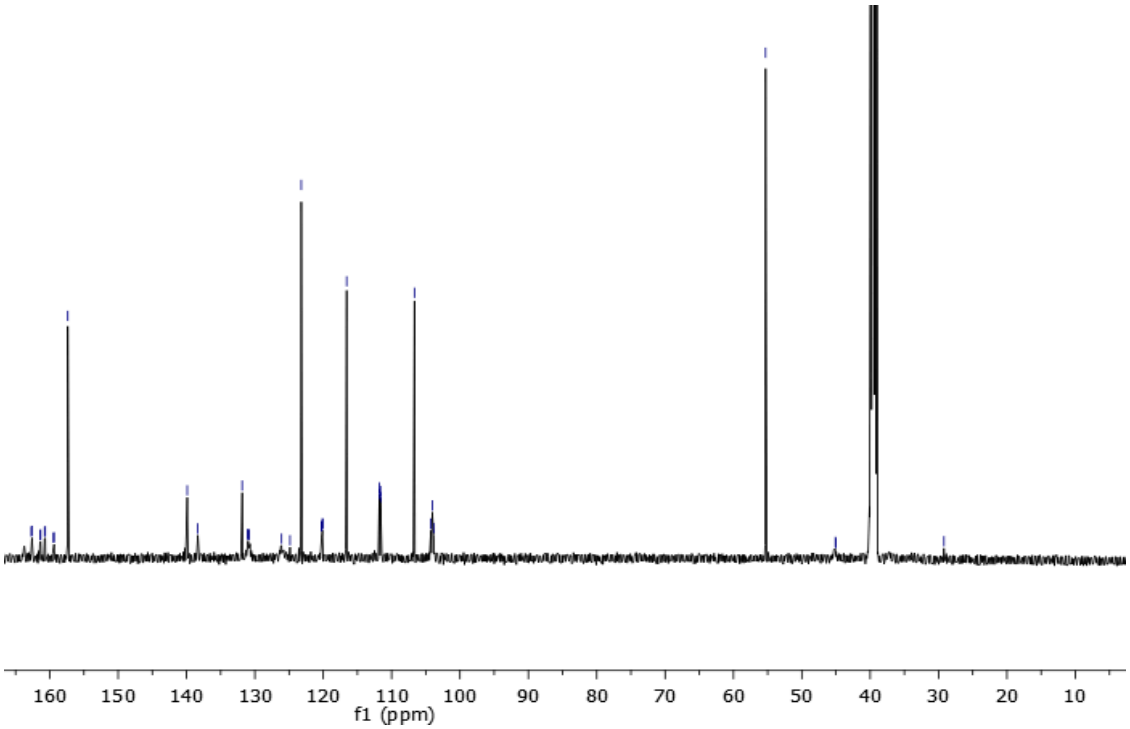
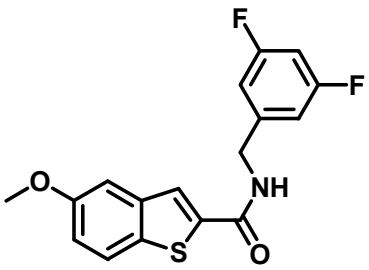

F
F
O
N
S
O

9b

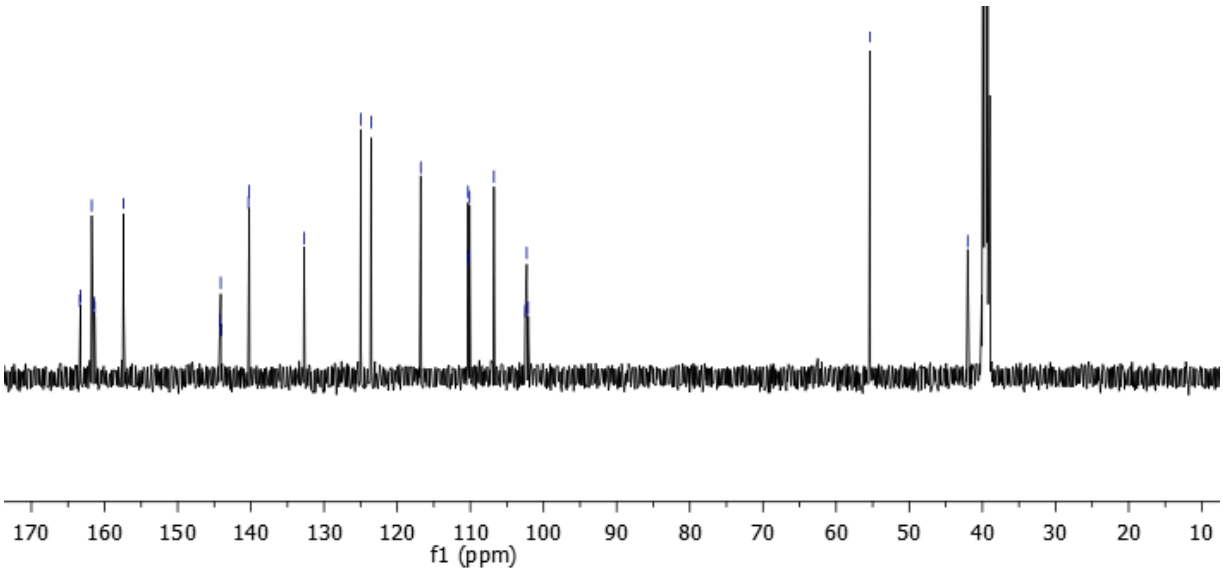

1.11
0.96
2.19
1.12
0.96
1.21
2.00
3.17
3.10
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)

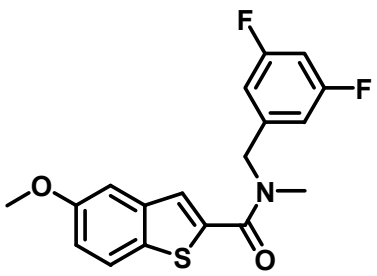

160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


F
F
O
NH
O
S

10a

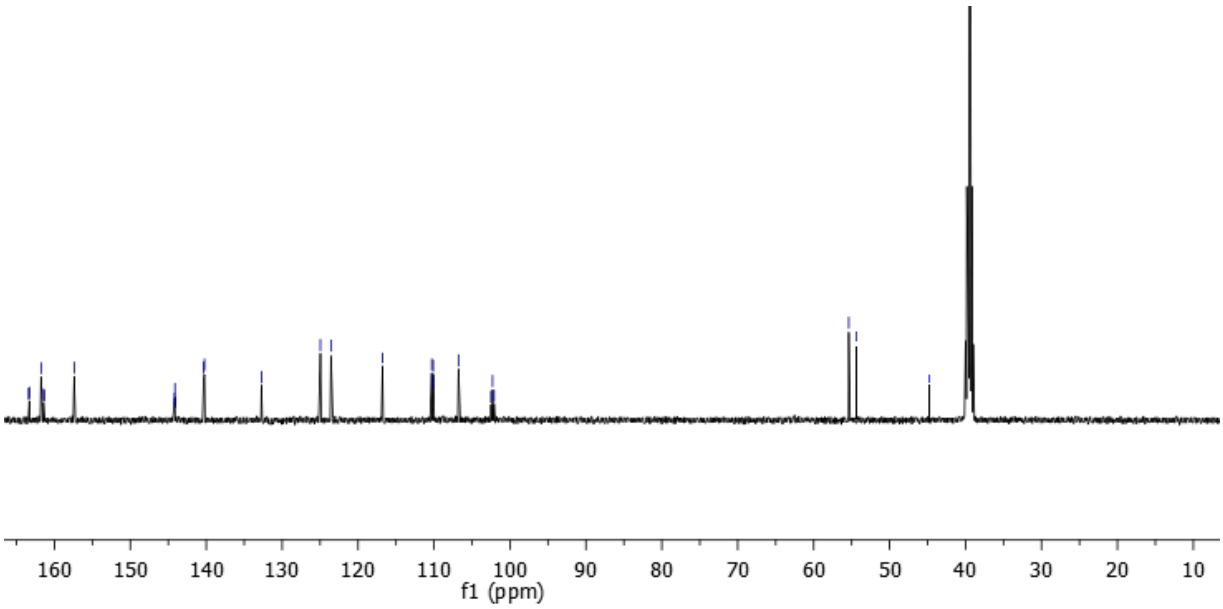
0.99
1.02
1.02
1.04
2.06
2.12
2.00
3.06
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)

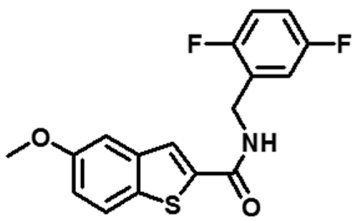

170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


F
F
O
N
S
O

10b

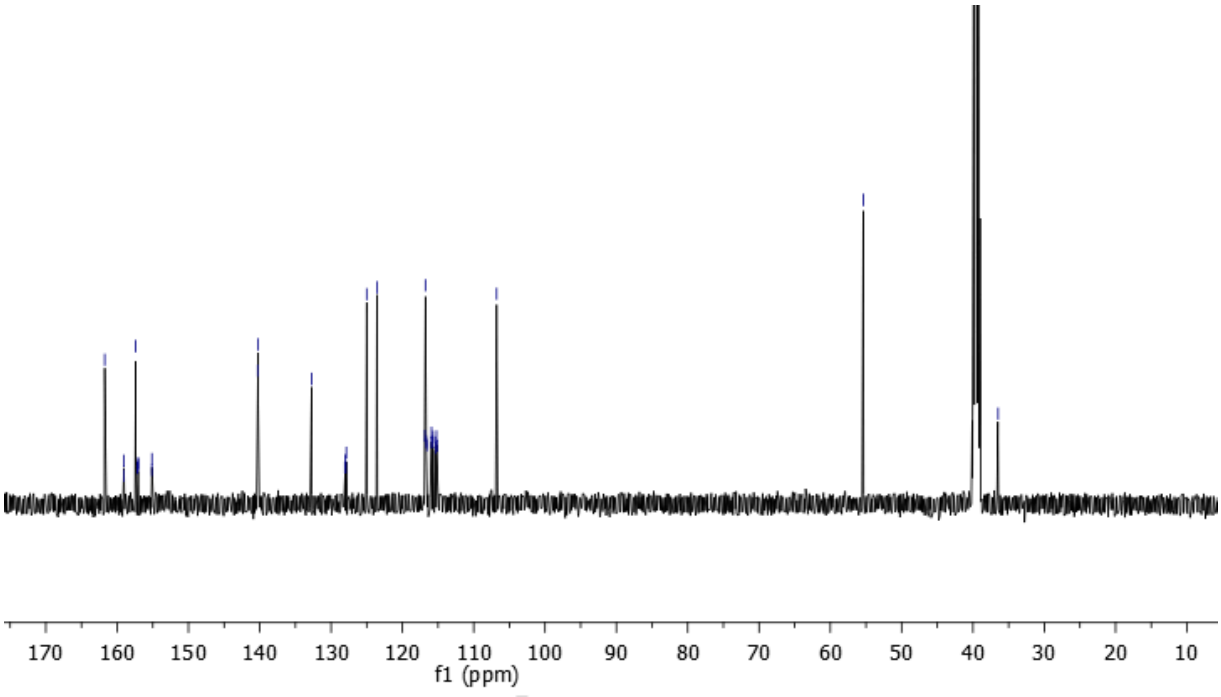
1.16
1.11
1.11
1.16
2.07
2.00
3.13
3.08
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)

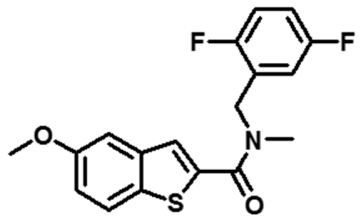

160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


F
F
NH
O
O
S

11a

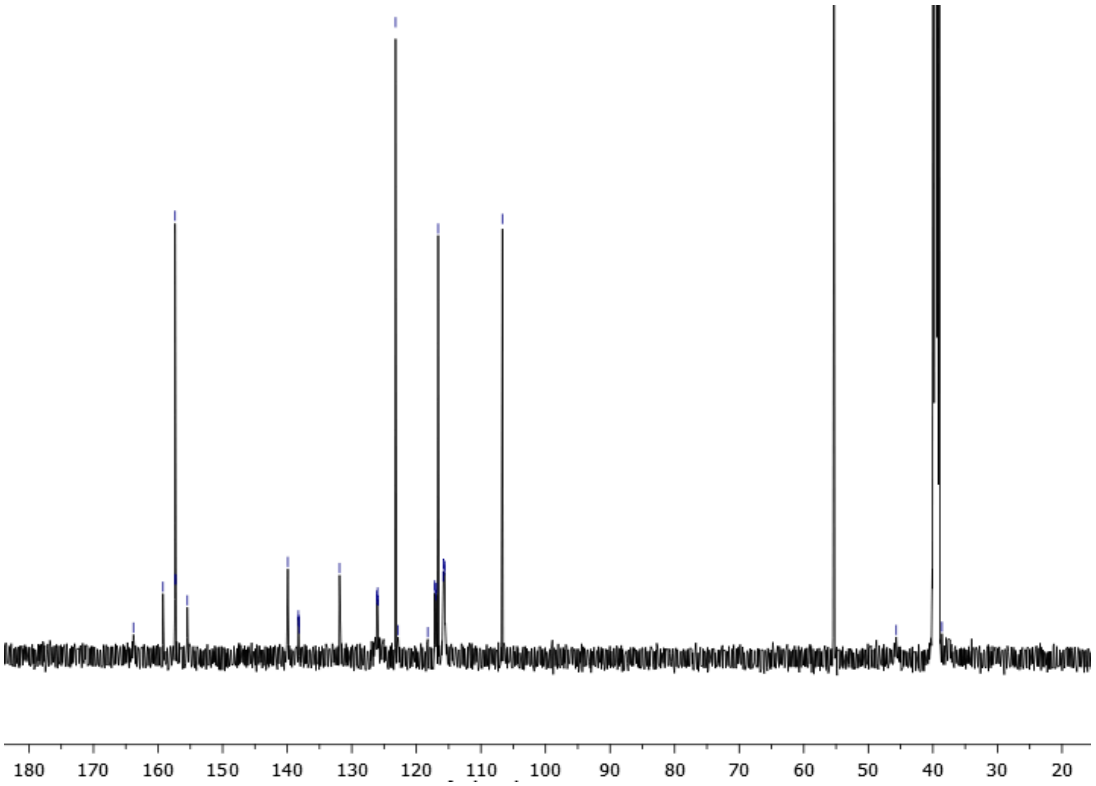
0.98
0.99
1.02
0.99
1.02
2.08
1.02
2.06
3.00
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5
f1 (ppm)

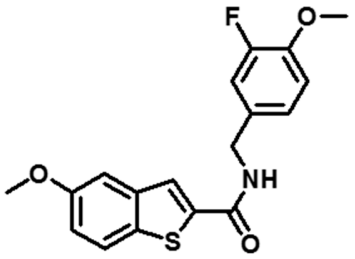

170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)


F
F
O
N
S
O

11b

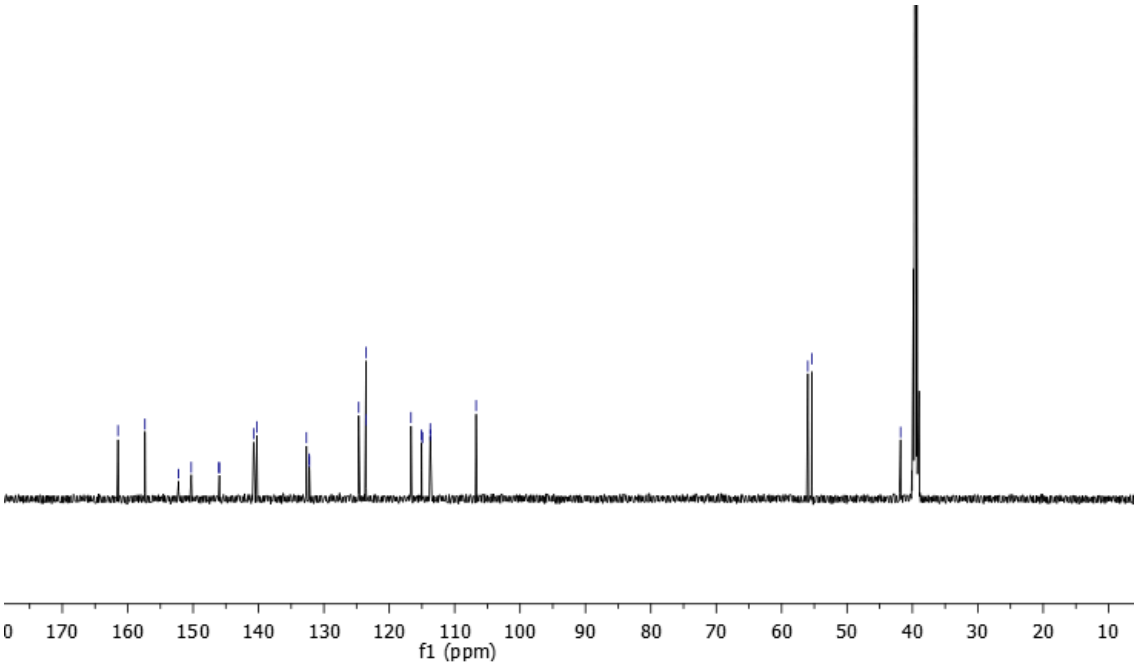
1.11
0.90
1.04
1.10
2.17
1.03
2.00
3.18
3.00
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)

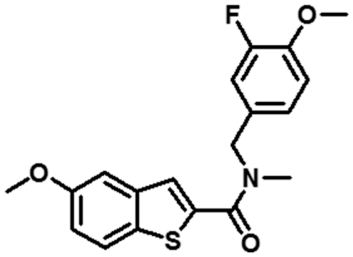

180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20


F
O
NH
O
S
O

12a

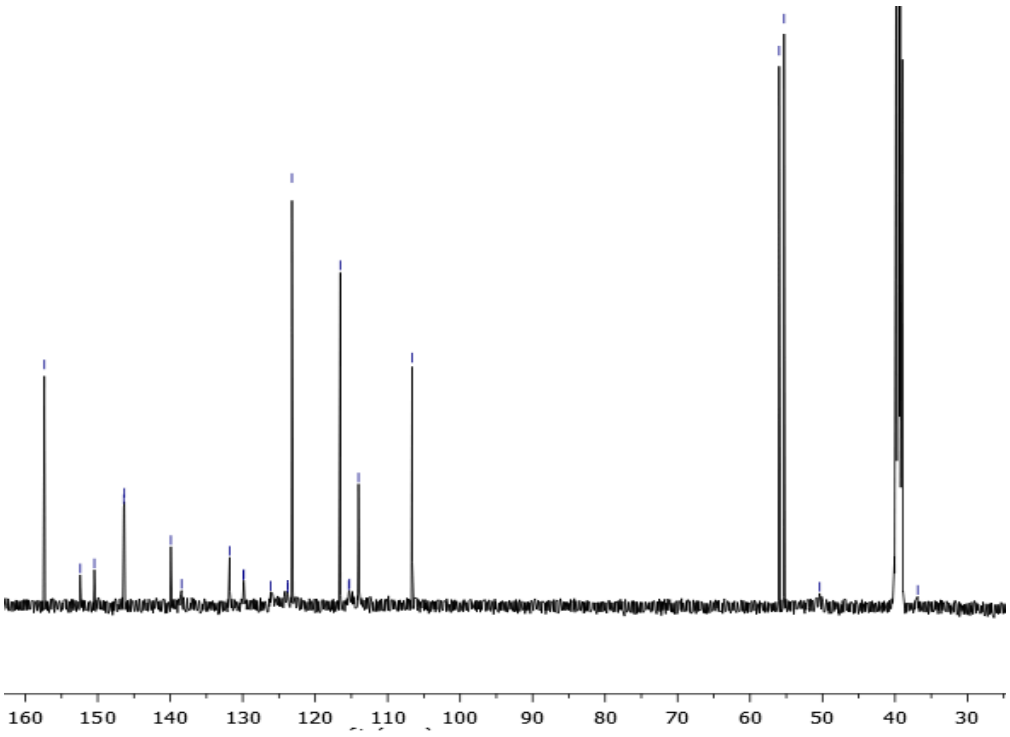
0.99
1.00
1.00
1.00
2.75
1.16
2.00
3.03
3.01
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0
f1 (ppm)

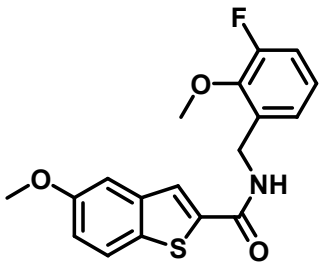

0 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)


F
O
O
N
O
S

12b

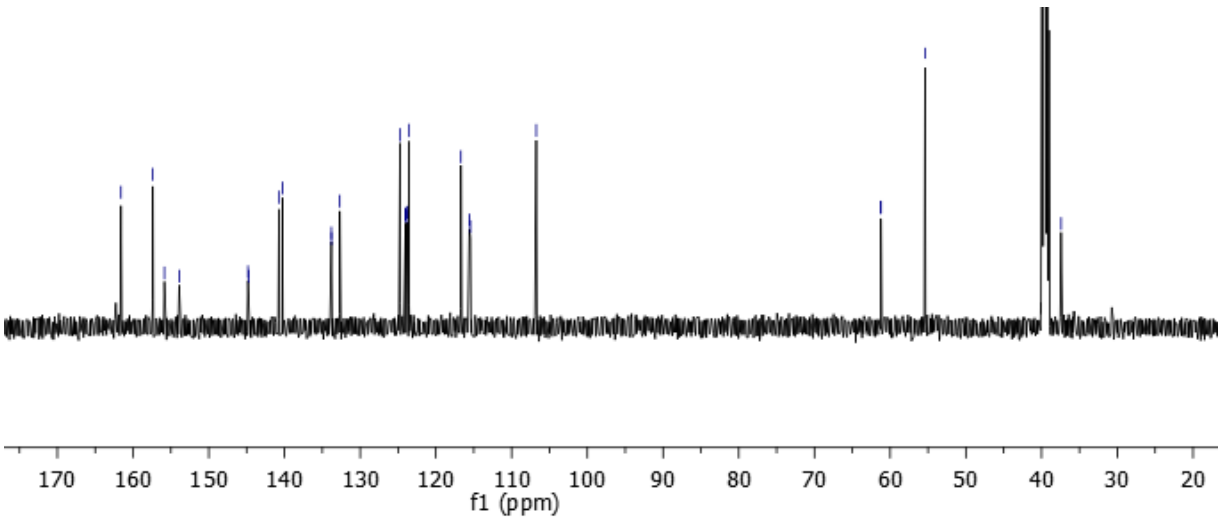
1.00
0.94
1.00
1.97
1.96
1.98
3.10
3.07
3.01
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)

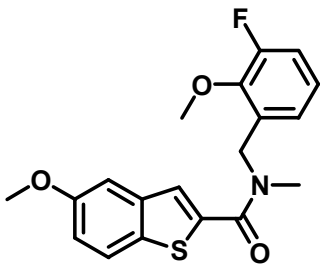

160
150
140
130
120
110
100
90
80
70
60
50
40
30


F
O
NH
O
S
O

13a

1.01
1.03
1.05
1.04
1.07
1.03
2.15
2.00
3.00
3.07
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
f1 (ppm)


170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)


F
O
O
N
S
O

13b

1.00
0.90
1.00
0.99
1.06
1.80
2.02
2.90
3.23
2.91
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
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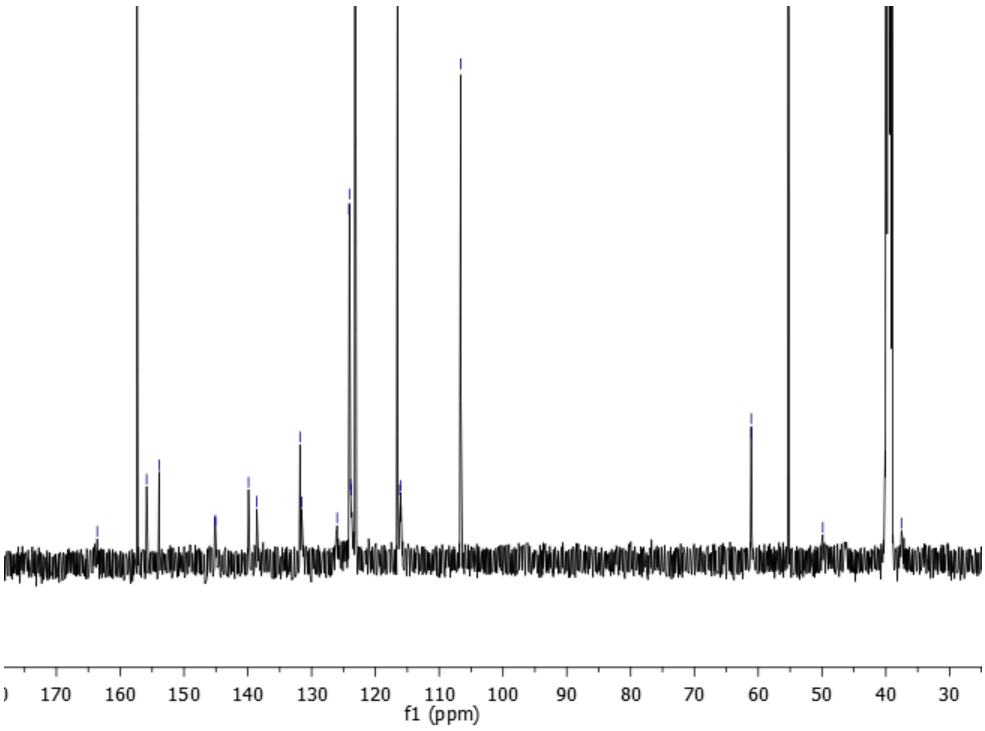
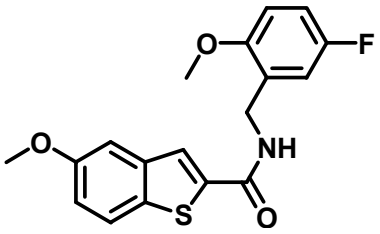
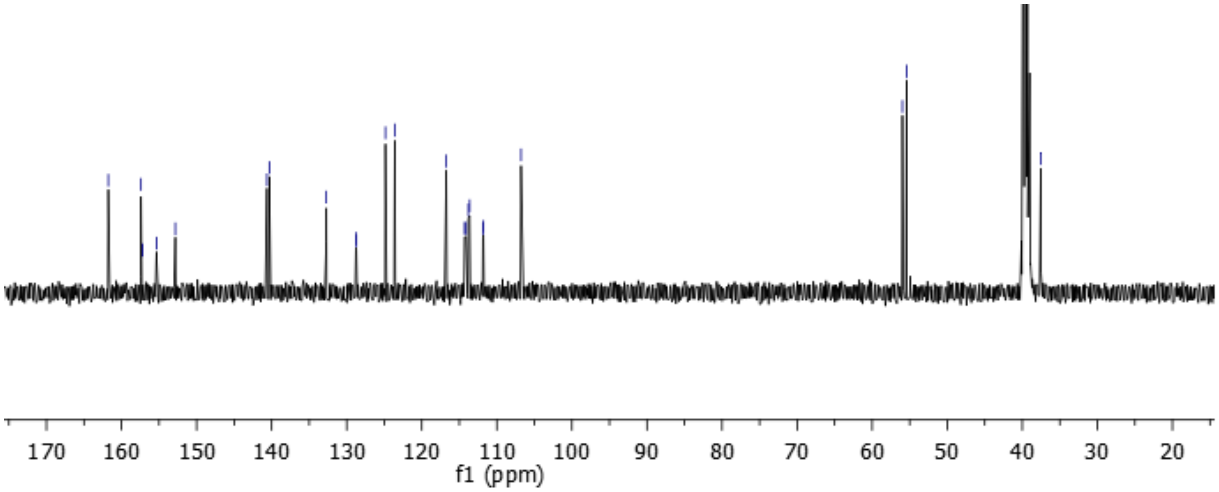
3.5
3.0
2.5
2.0
1.5
f1 (ppm)


170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
f1 (ppm)


O
F
NH
O
O
S

14a

1.02
1.02
1.03
0.99
1.10
0.88
2.00
2.00
2.92
3.24
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)


170
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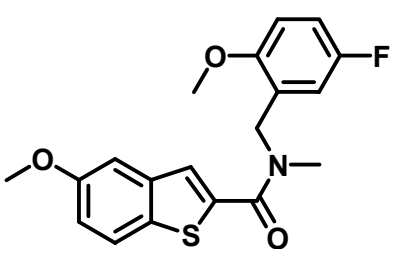
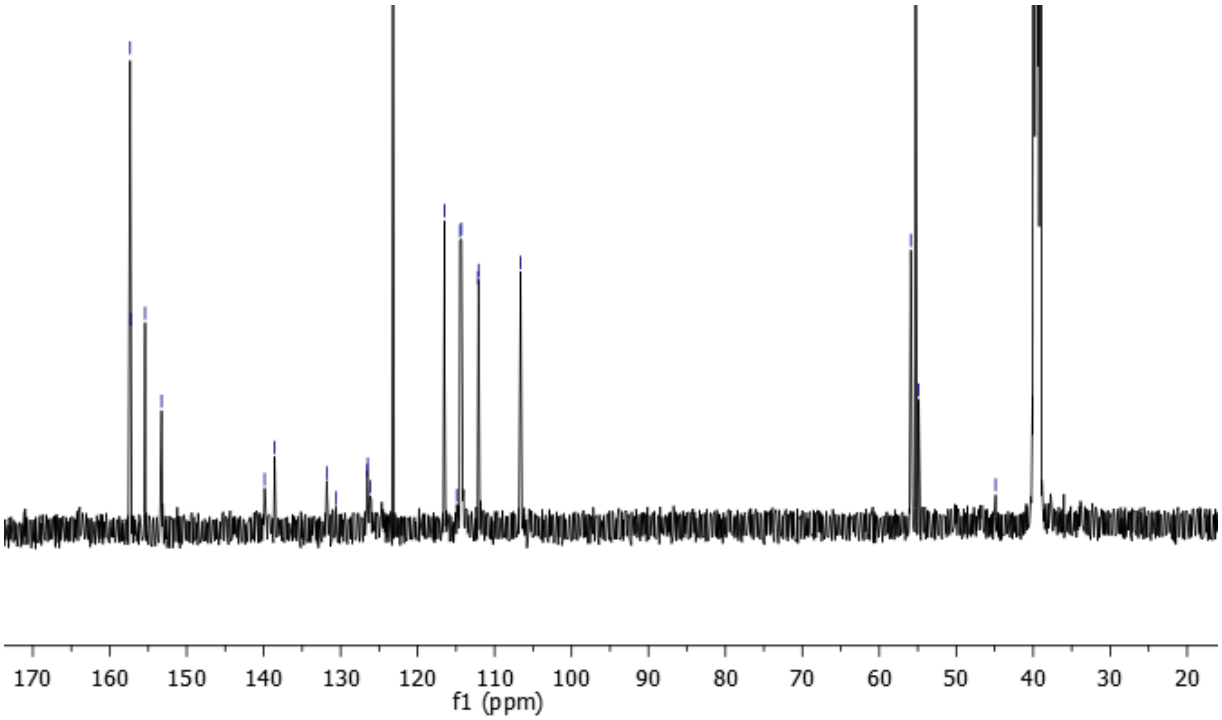
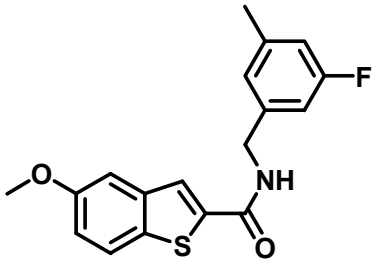
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)


O
F
O
N
S
O

14b

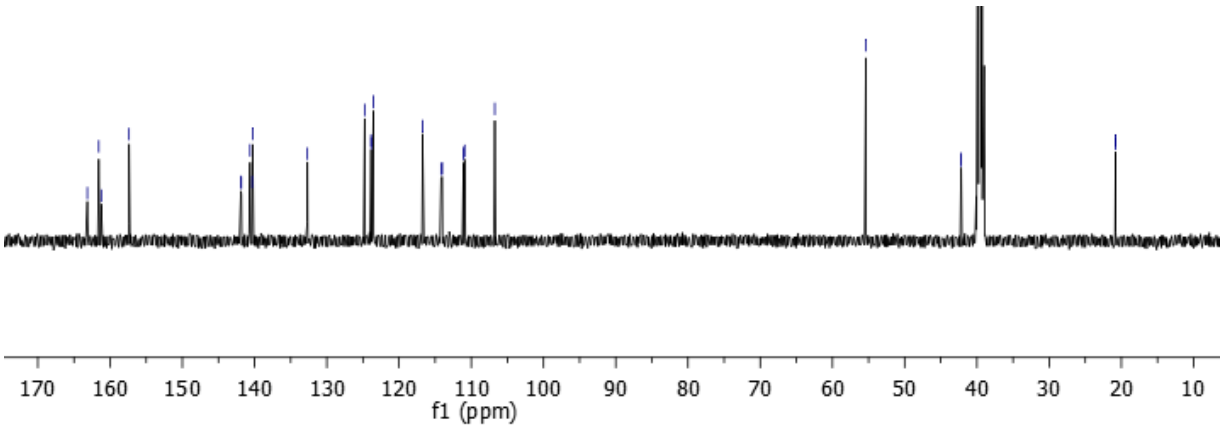
1.03
0.64
0.93
2.15
2.05
2.00
6.01
2.83
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f1 (ppm)


170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)


F
NH
O
O
S

15a

1.00
1.02
1.03
1.03
1.04
1.02
2.06
2.00
3.07
2.95
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)


170
160
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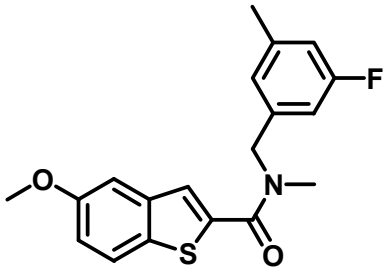
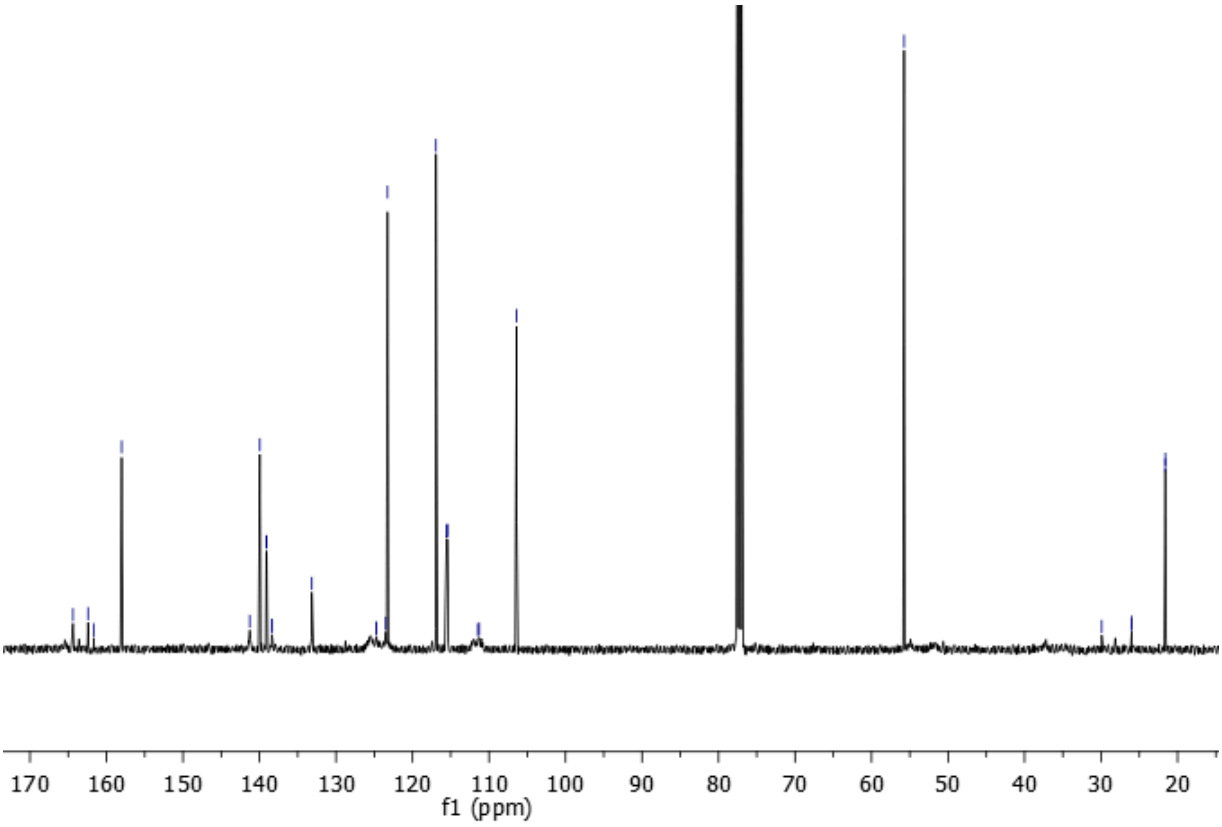
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


F
O
N
O
S

15b

1.00
0.95
0.99
1.20
1.28
2.23
2.12
3.15
3.09
3.17
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)


170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)