Supporting Information

Design, synthesis and biological evaluation of naphthalene-1,4-dione analogues as anticancer agents

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Contents of Supporting Information

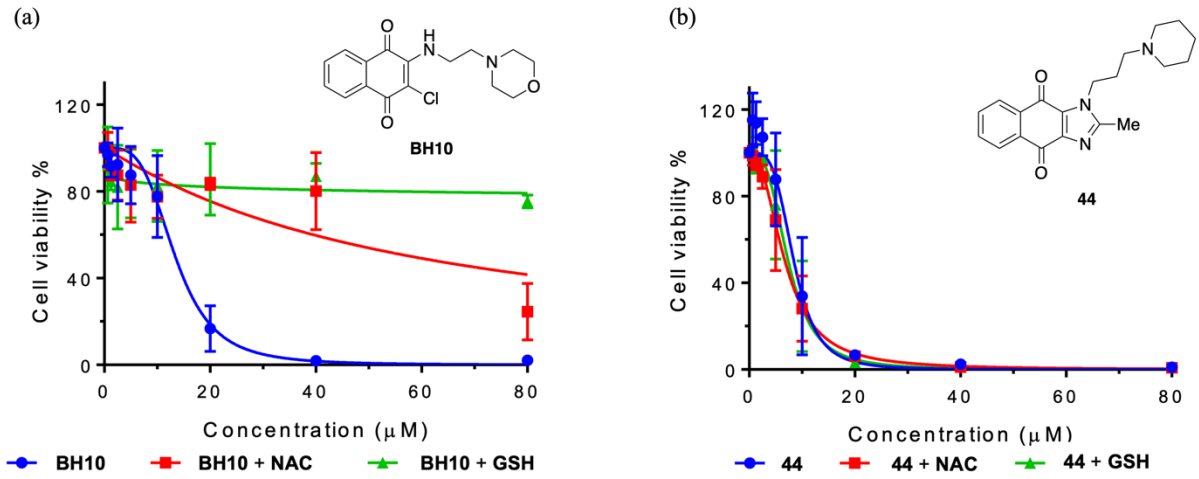

Fig. S1. Effect of thiol-containing compounds on cytotoxicity of compound (a) **BH10** and (b) compound **44**. The thiol rescue experiment was conducted following the standard MTT assay protocol with an additional step. On Day 1, HEC1A cancer cells were seeded as per the standard protocol. On Day 2, cells were pre-treated with NAC or GSH (2 mM) for 40 minutes, followed by the addition of test compounds. The cells were then incubated for 48 hours according to the standard MTT assay protocol. (a) HEC1A cancer cells treated with BH10 were rescued by the thiol-containing compounds NAC and GSH as BH10 lost its cytotoxicity as demonstrated by the increased IC_{50} value of 58.00 μM and greater than 80.00 μM with NAC and GSH pre-treatment, respectively. (b) Pretreating cancer cells with either NAC or GSH did not rescue the cytotoxicity of compound **44** as no significant change in IC_{50} values from the control group were observed.

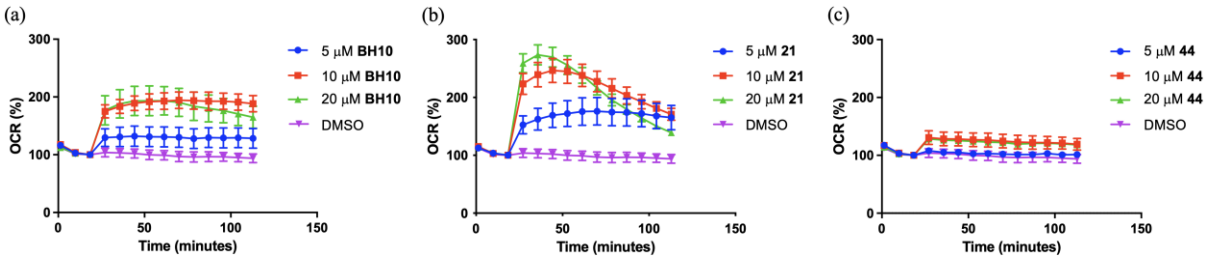

Fig. S2. Effect of **BH10** (a) and its analogues **21** (b) and **44** (c) on the percentage of oxygen consumption rate (OCR) in HEC1A cancer cells. HEC1A cells were treated with **BH10**, **21**, and **44** at concentrations of 5 μM, 10 μM, and 20 μM, with DMSO as the vehicle control. OCR, normalized to basal levels (defined as 100%), was measured over a 120-minute period.

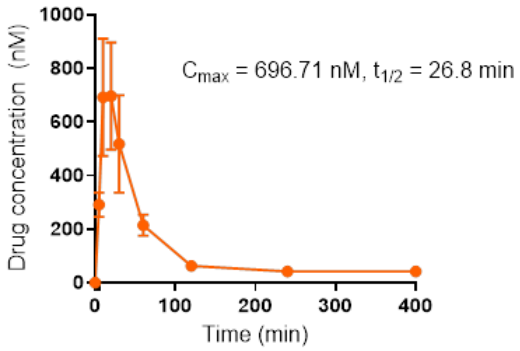

Fig. S3. *In vivo* pharmacokinetic profiling of compound **44**. Mice were administered the compound via oral gavage, and blood samples were collected from the tail at regular intervals. The collected blood was centrifuged to obtain plasma, which was then treated with 10% methanol in acetonitrile to precipitate proteins. Quantification of the compound in plasma was performed using mass spectrometry analysis of each sample and corresponding compound standards, enabling the generation of pharmacokinetic profiles over time. Compound **44** achieved a maximum plasma concentration of 696.71 nM with a half-life of 26.8 min.

NMR spectrum:

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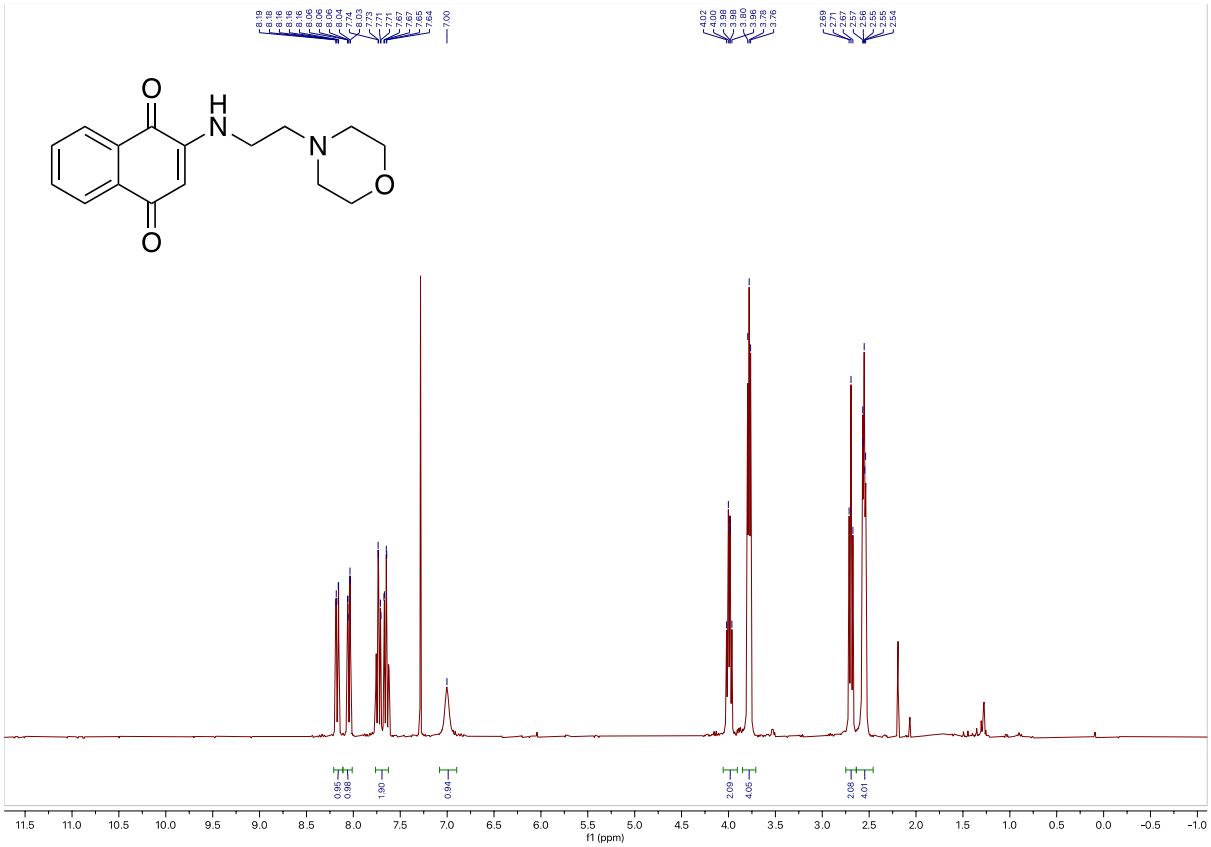

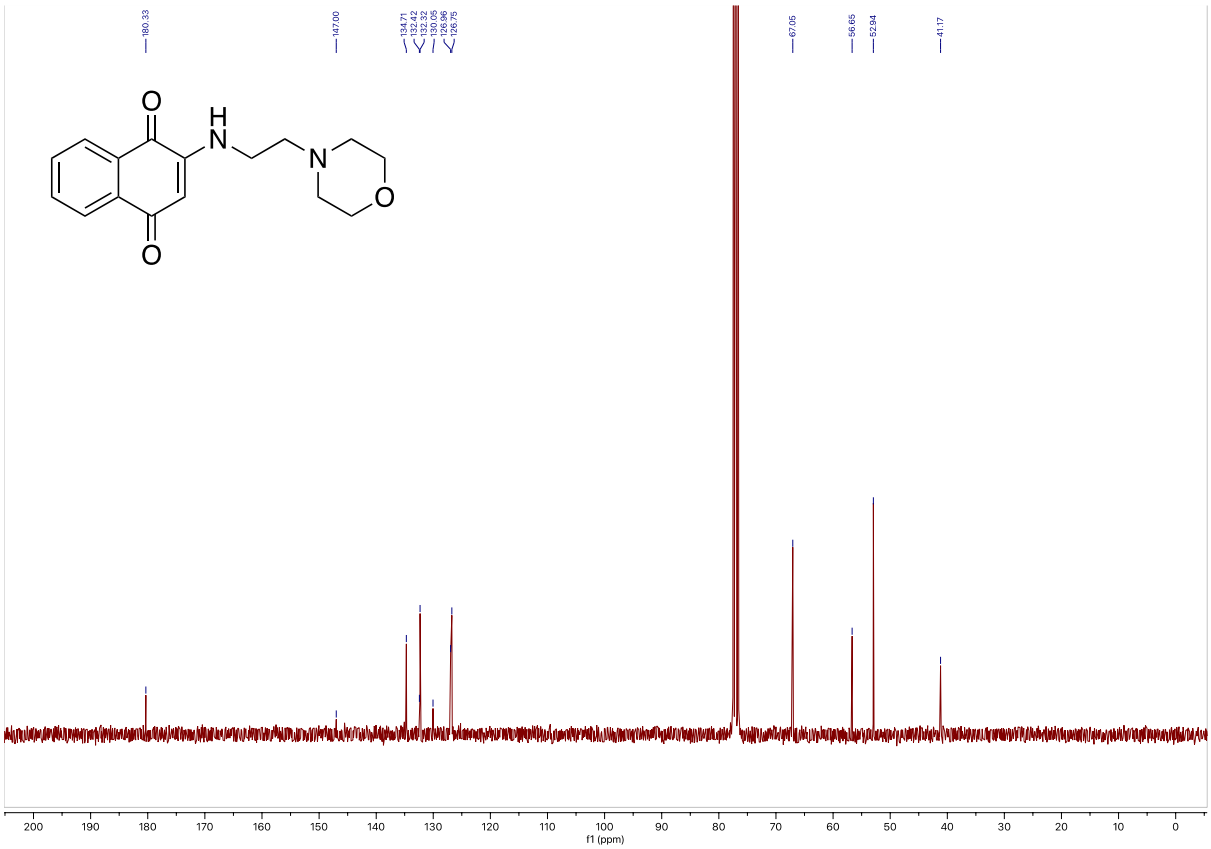

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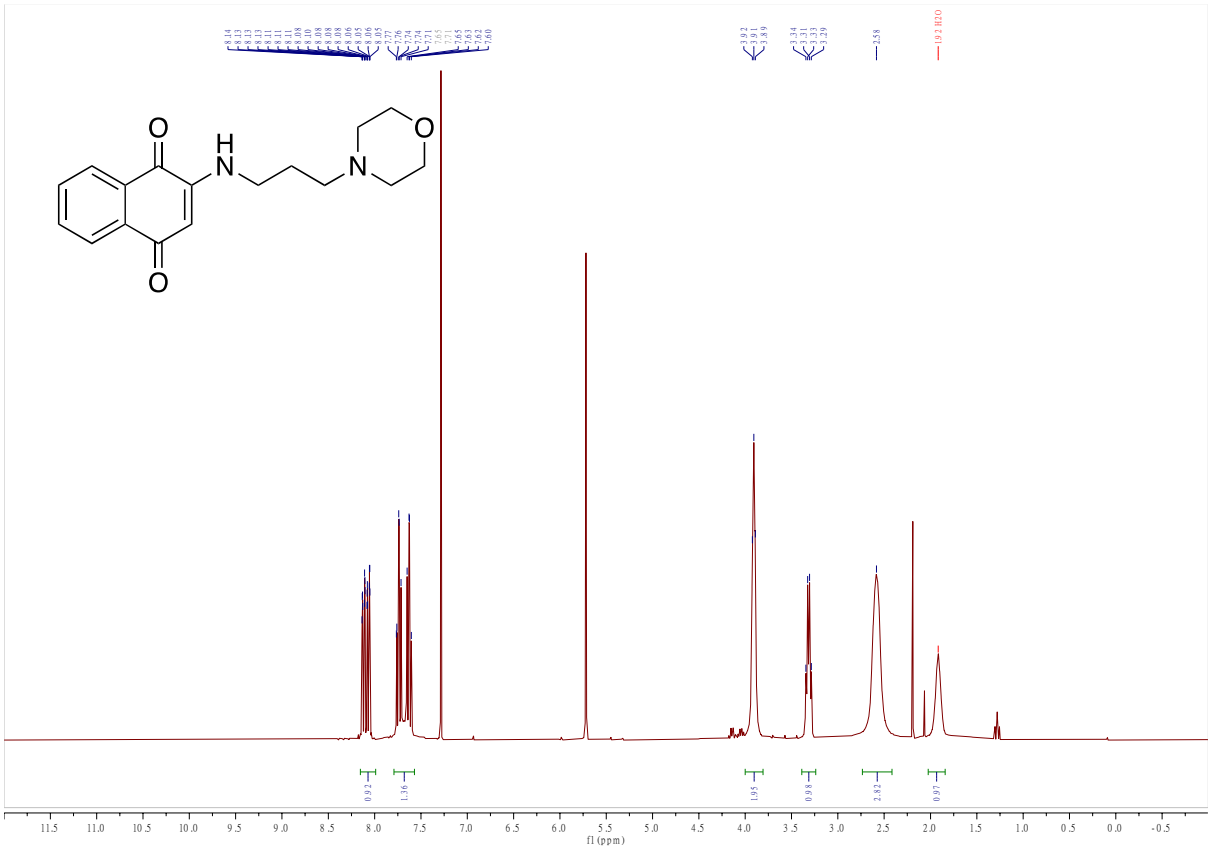

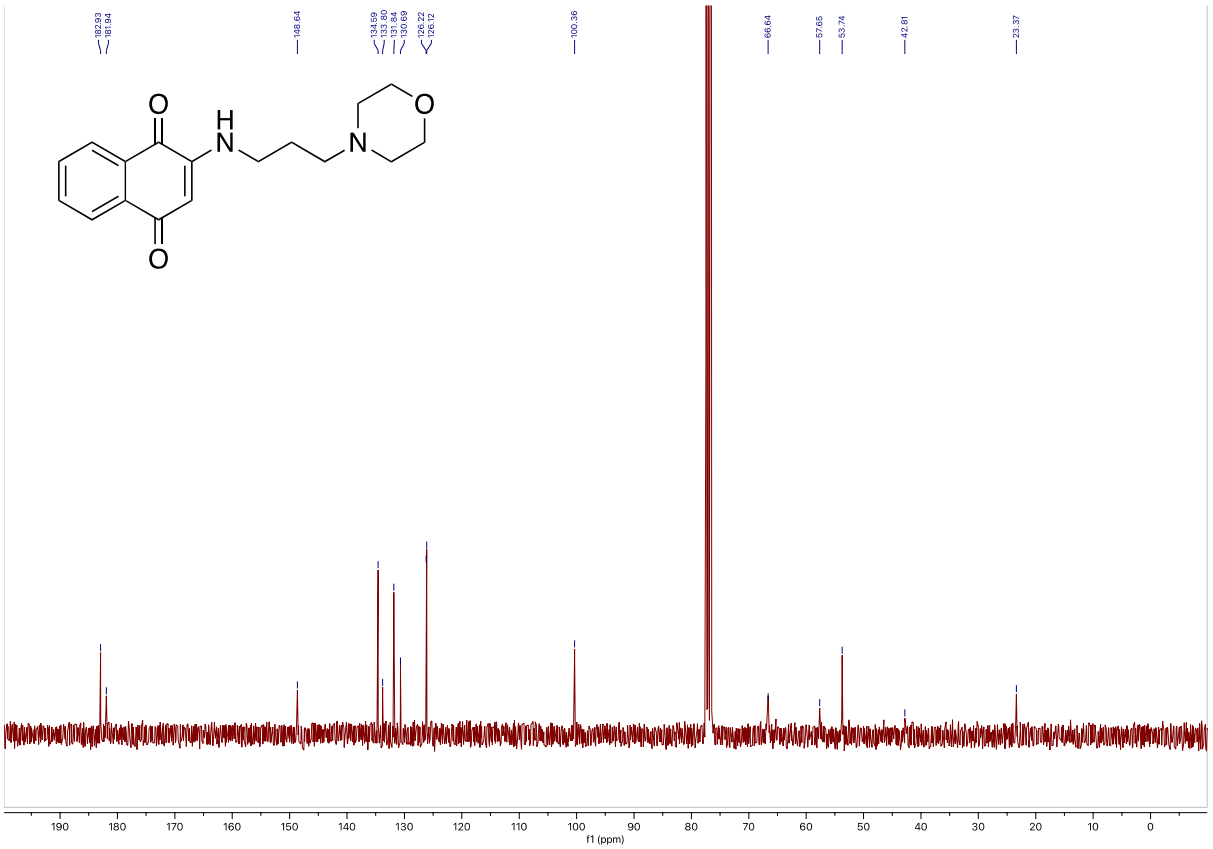

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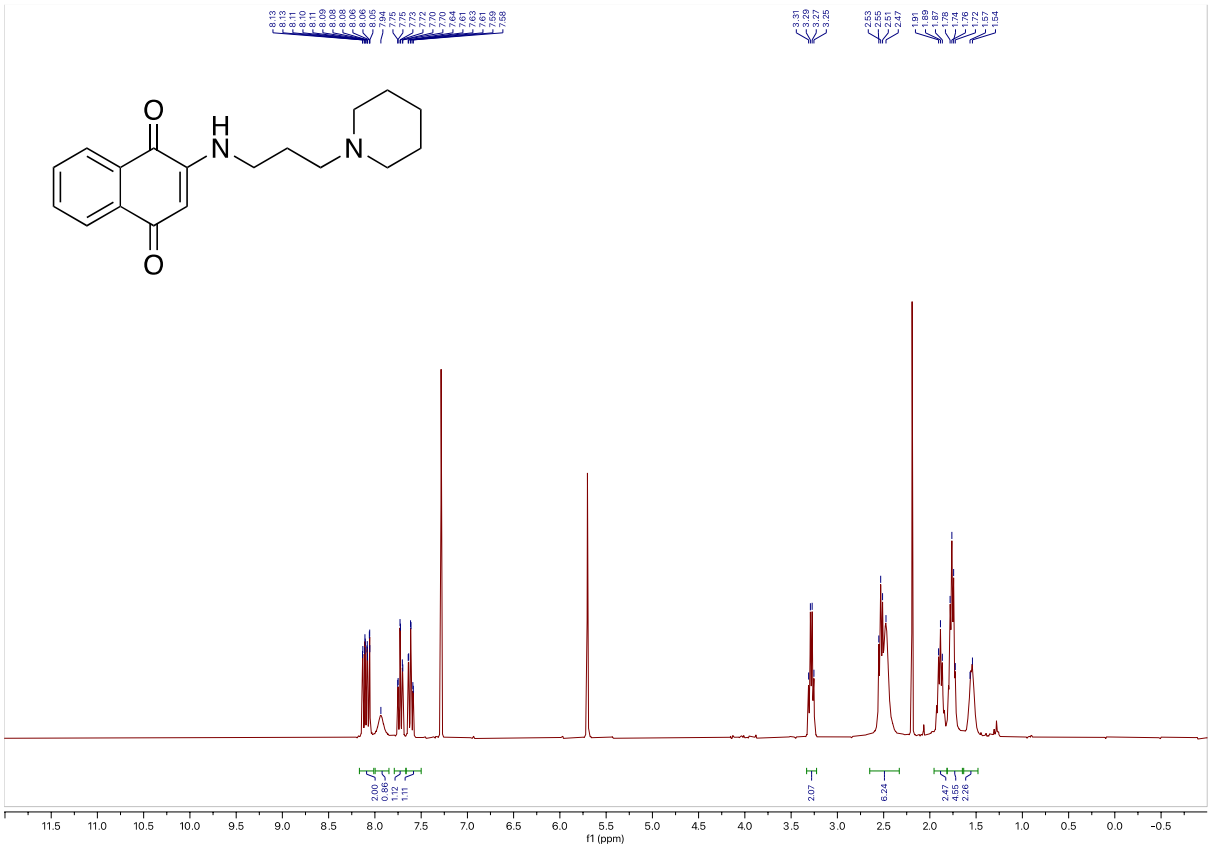

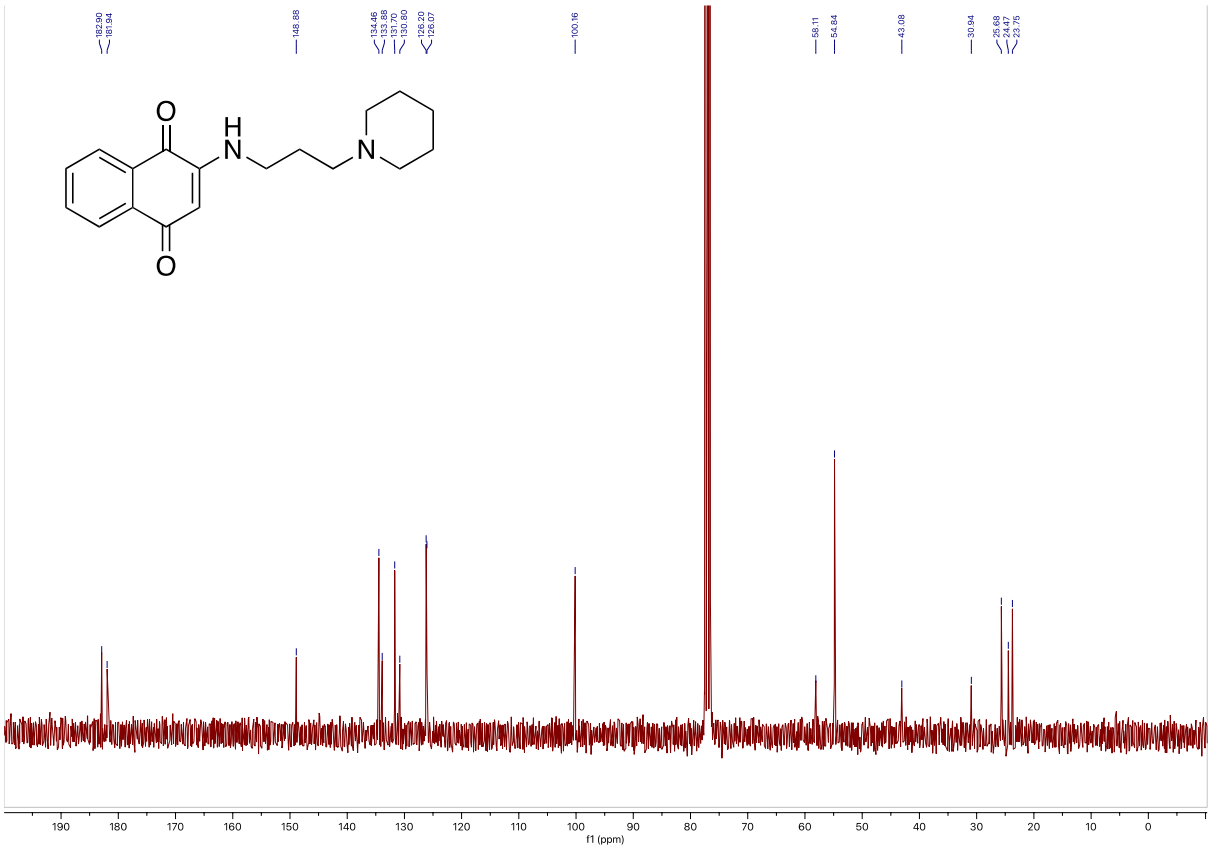

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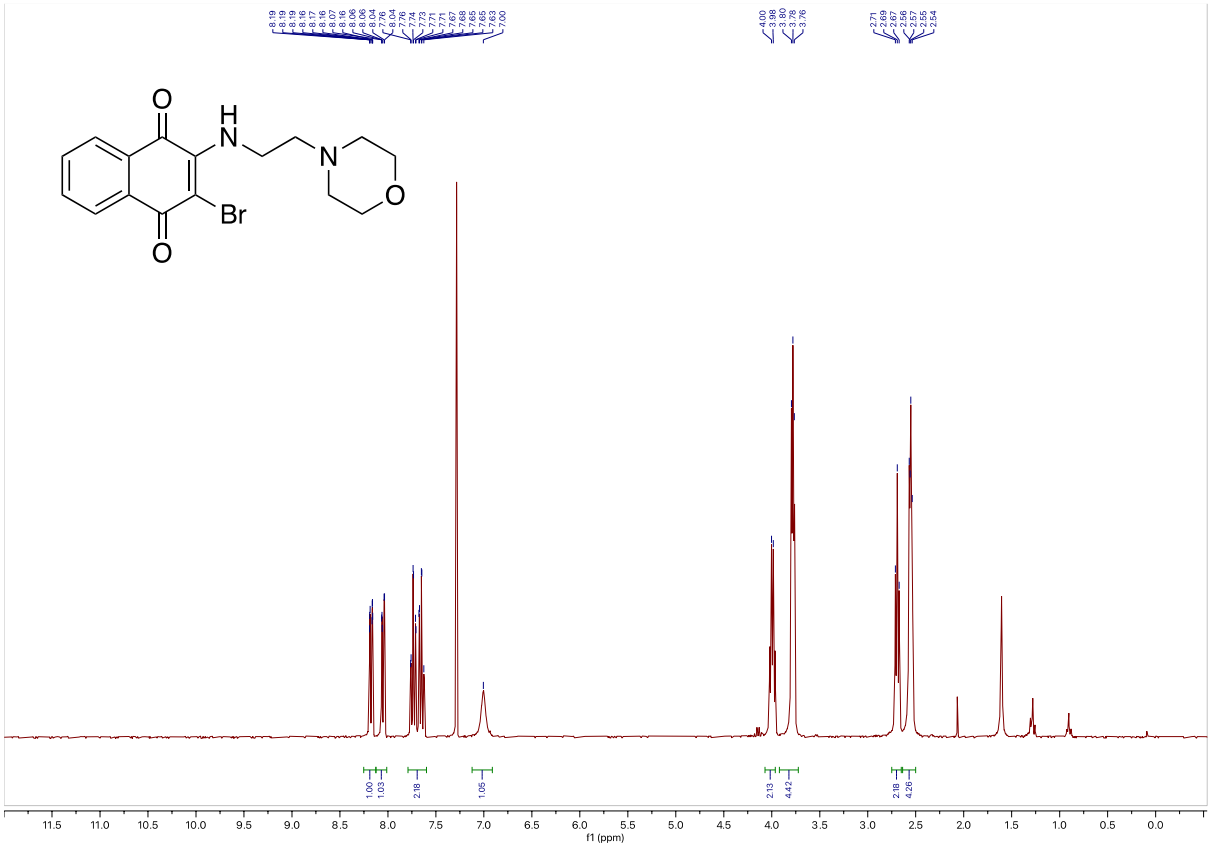
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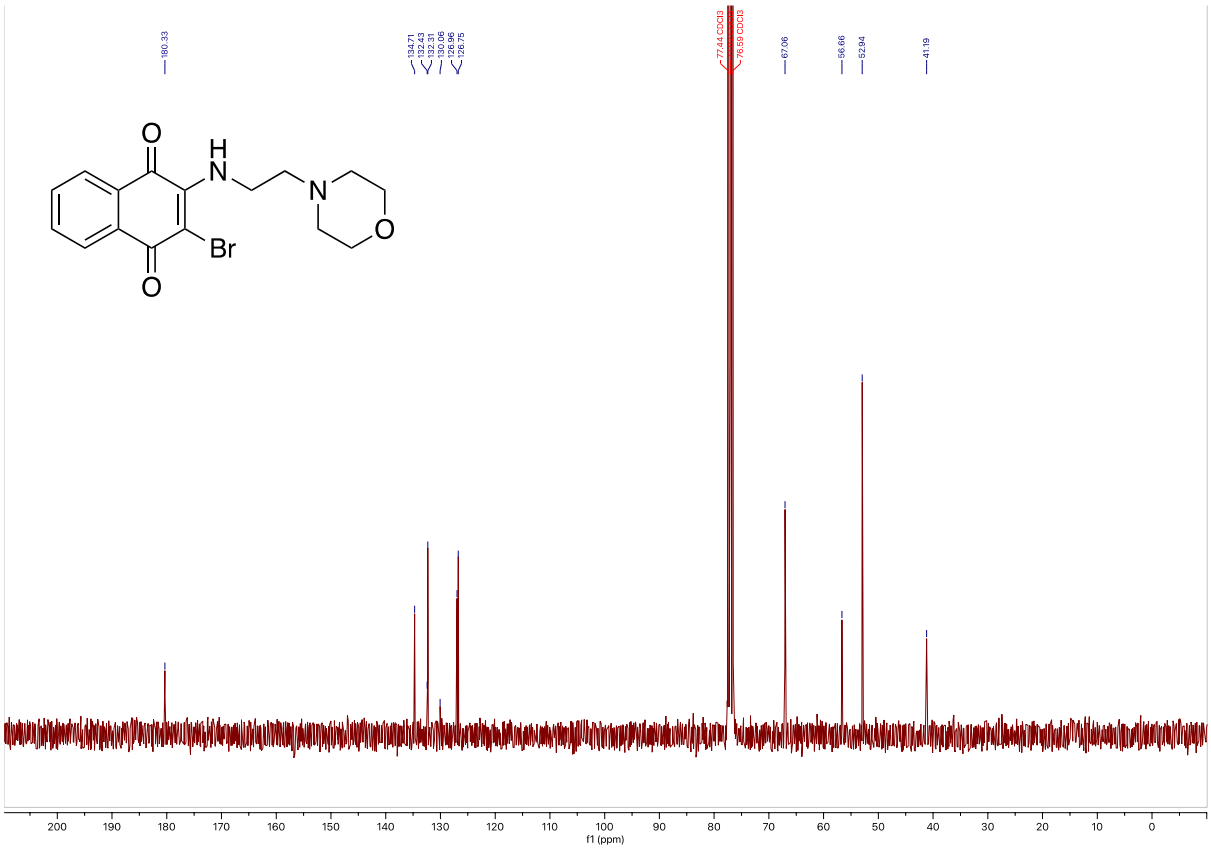
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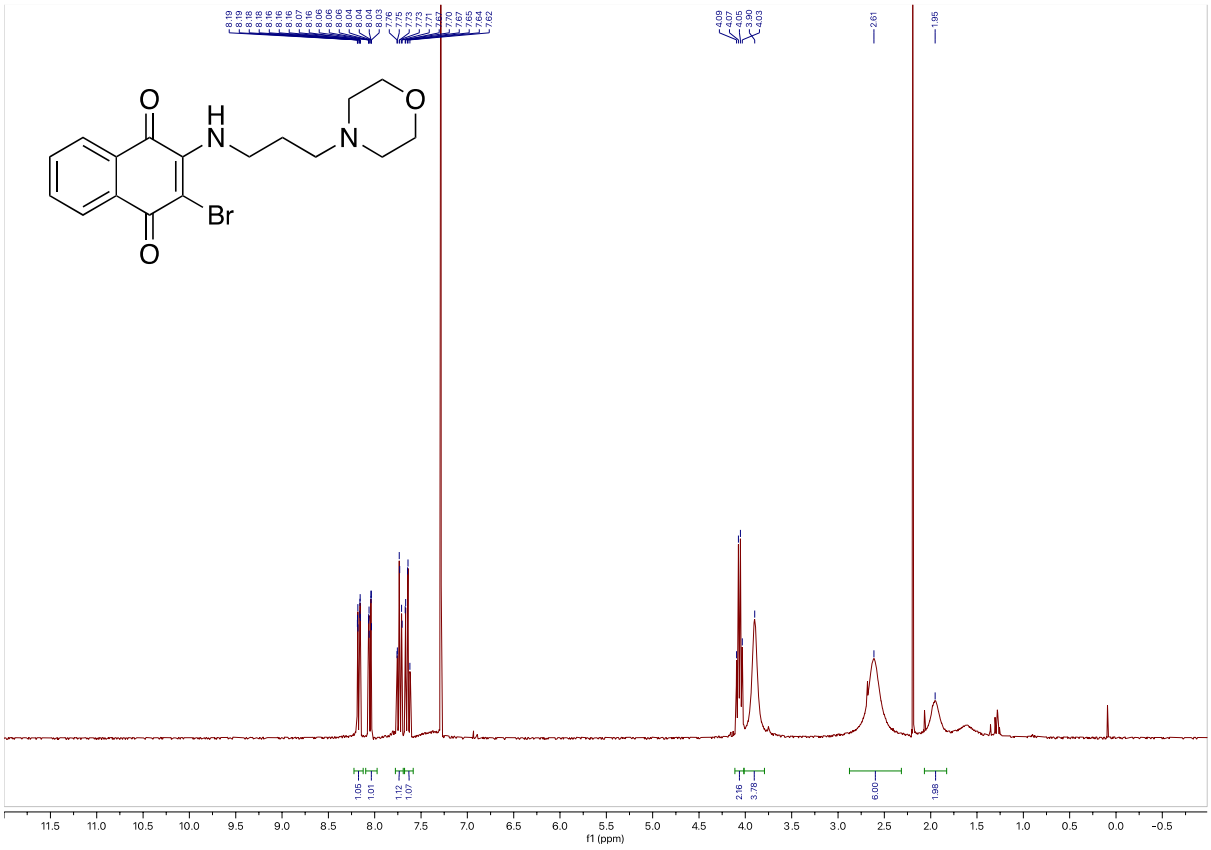

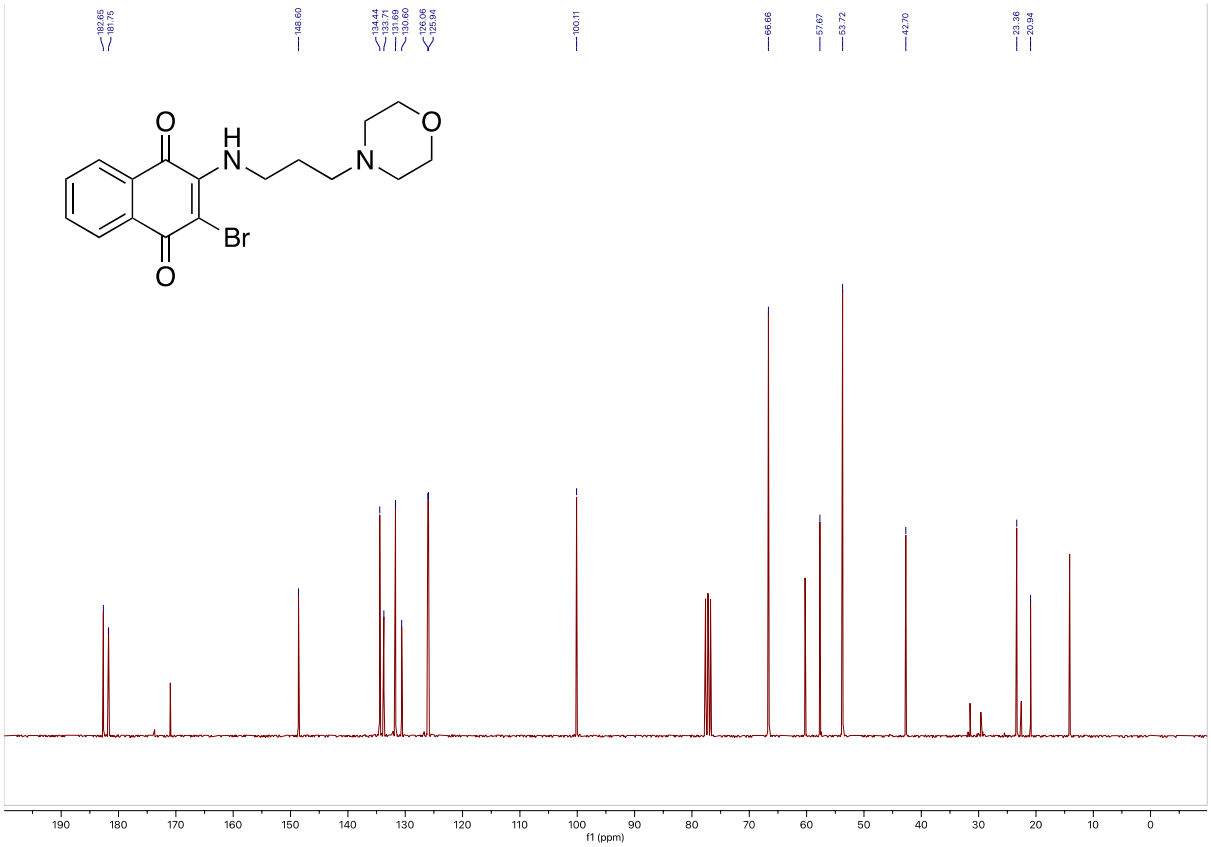

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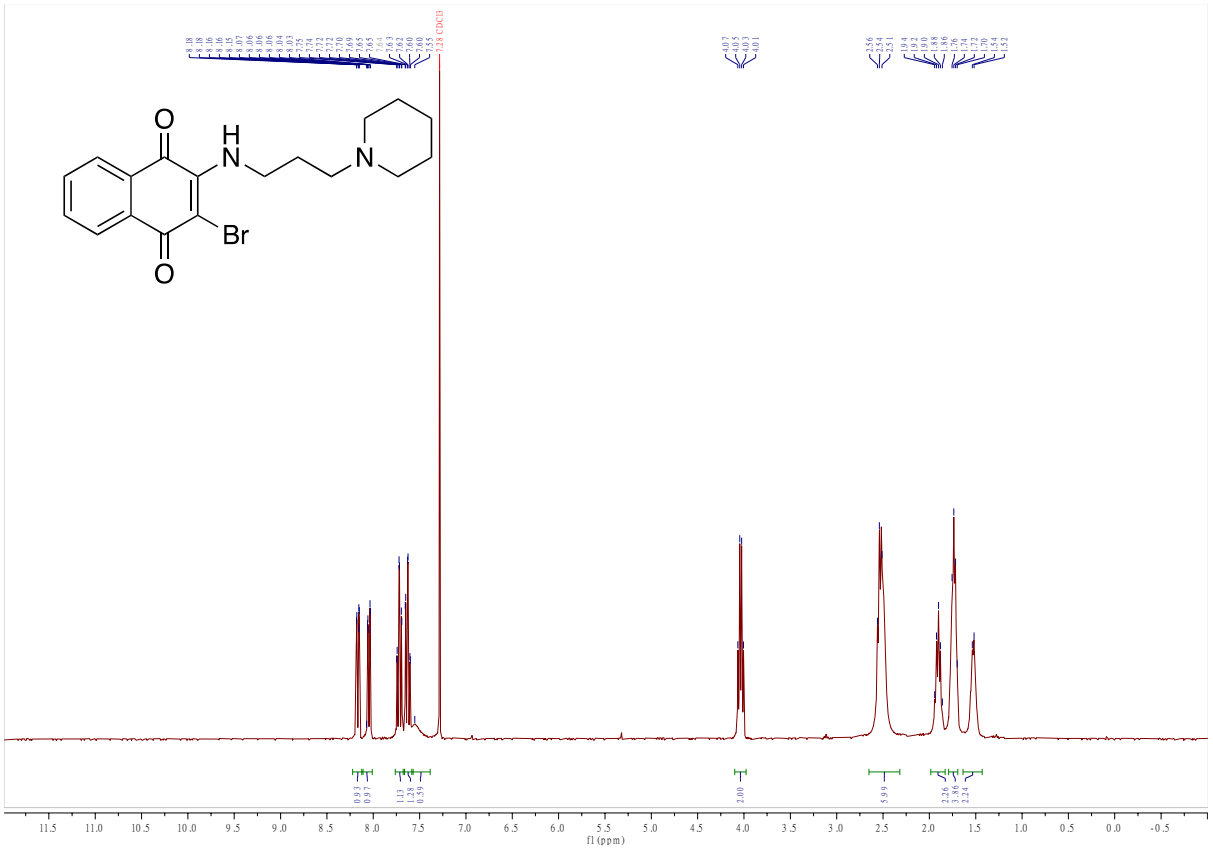

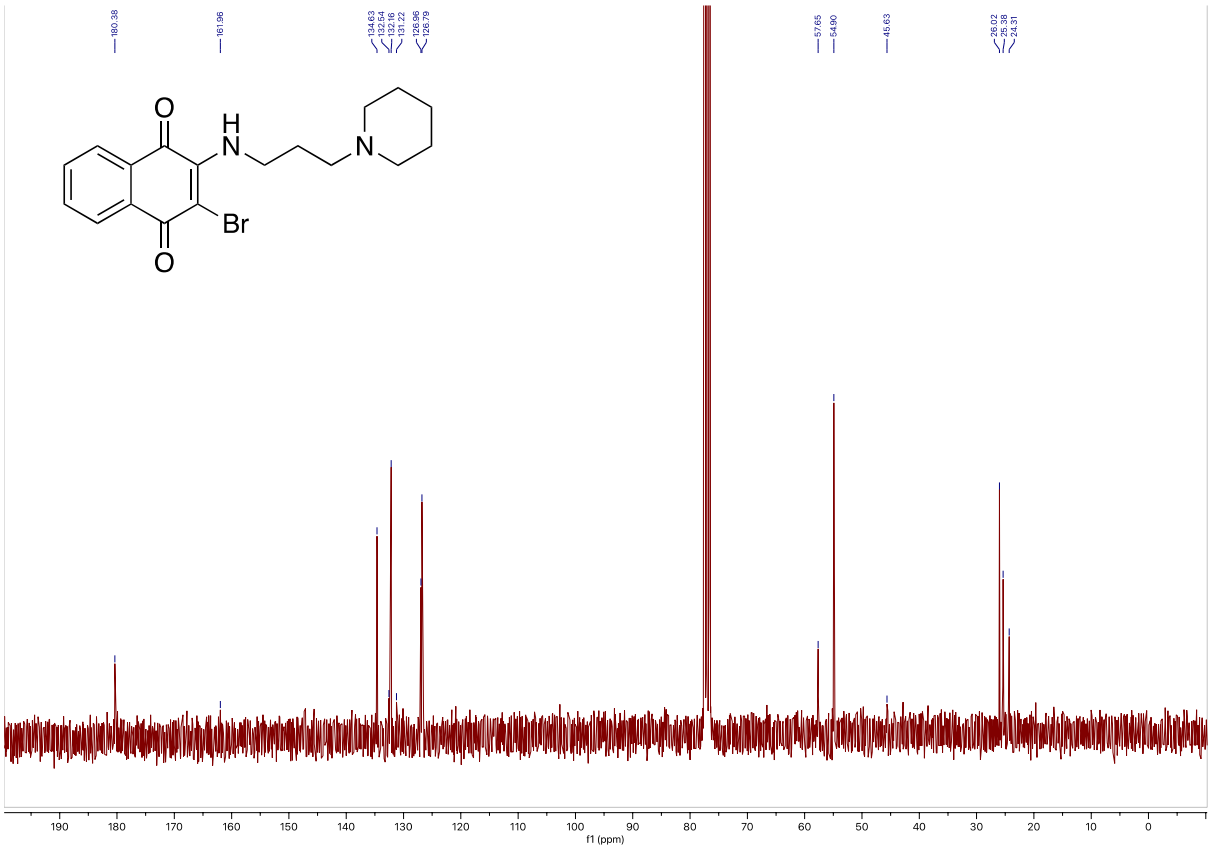

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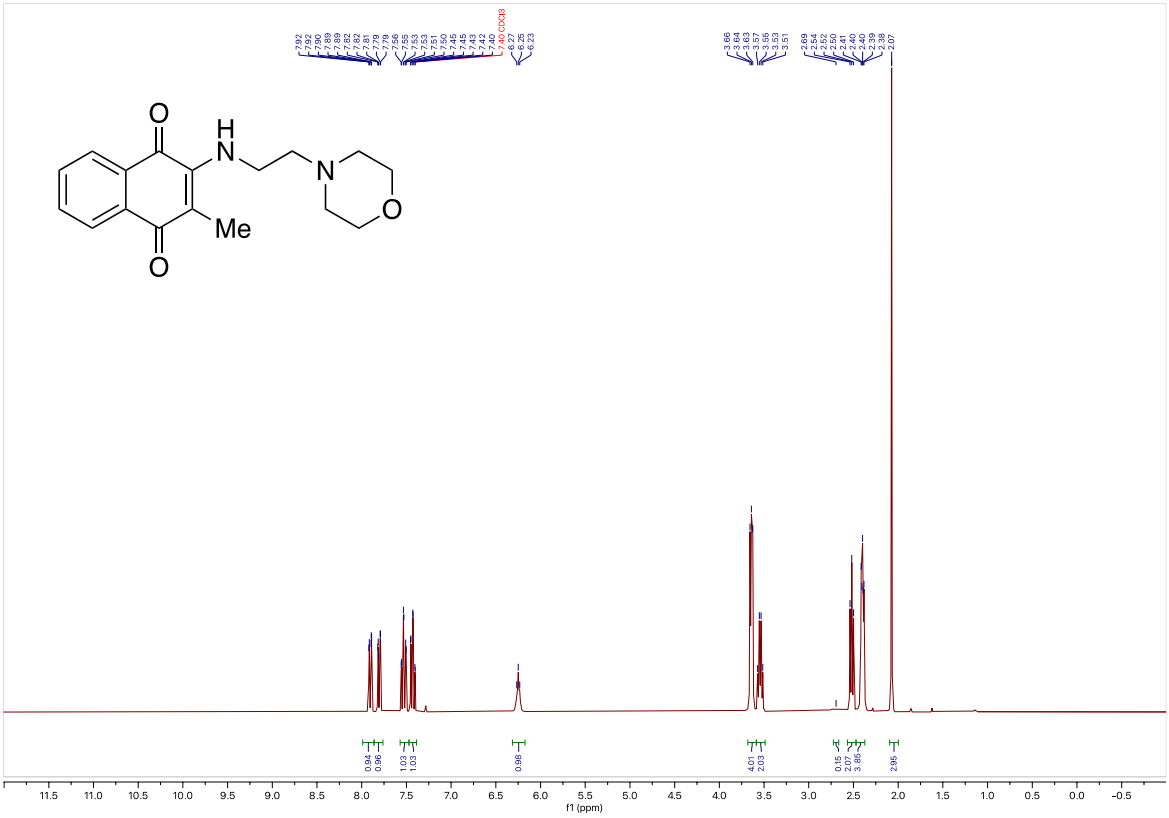

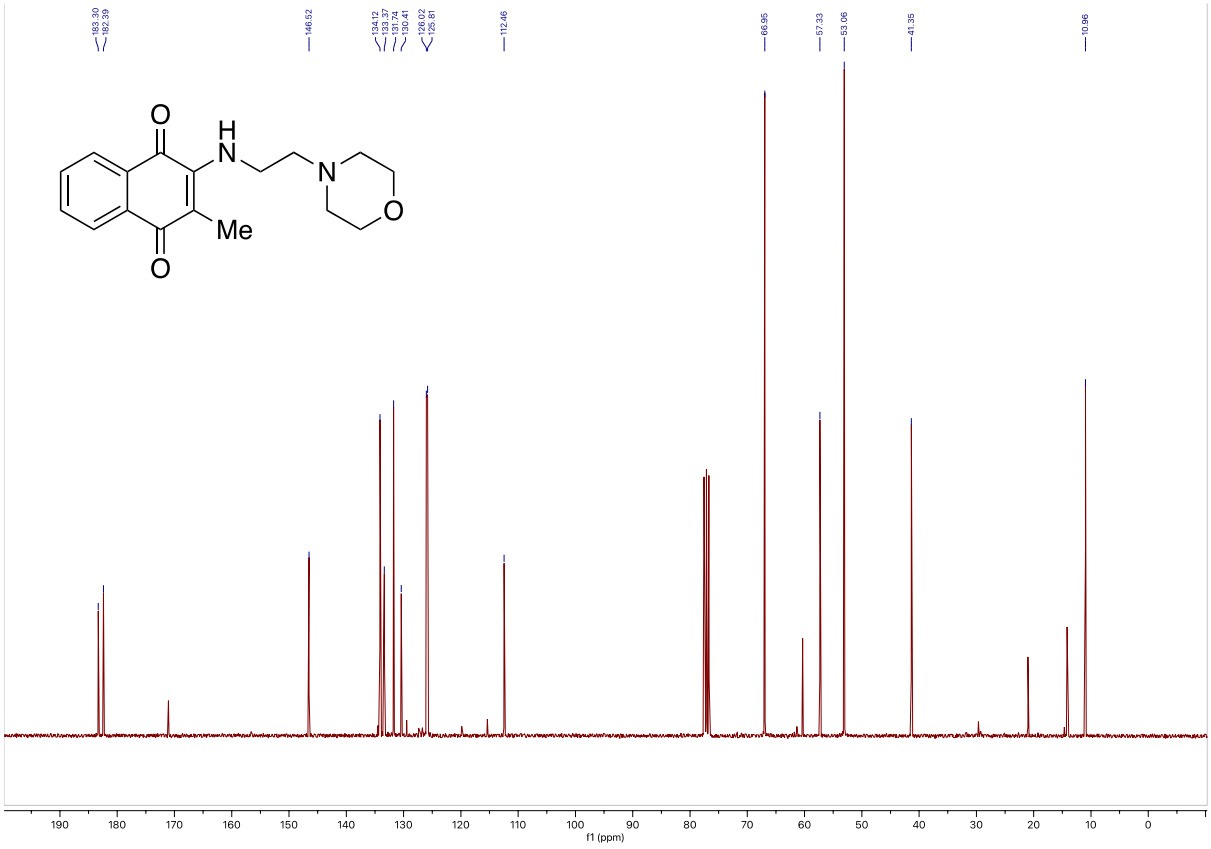

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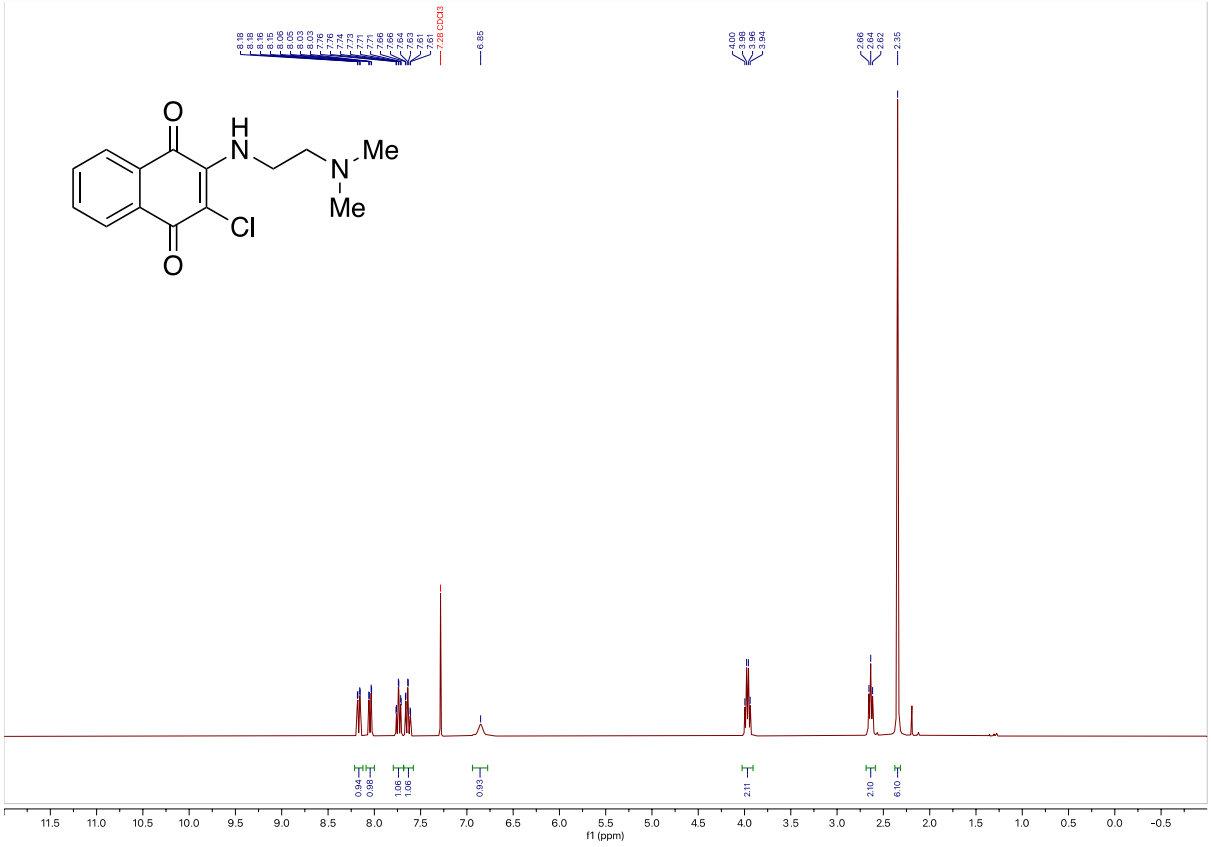
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N
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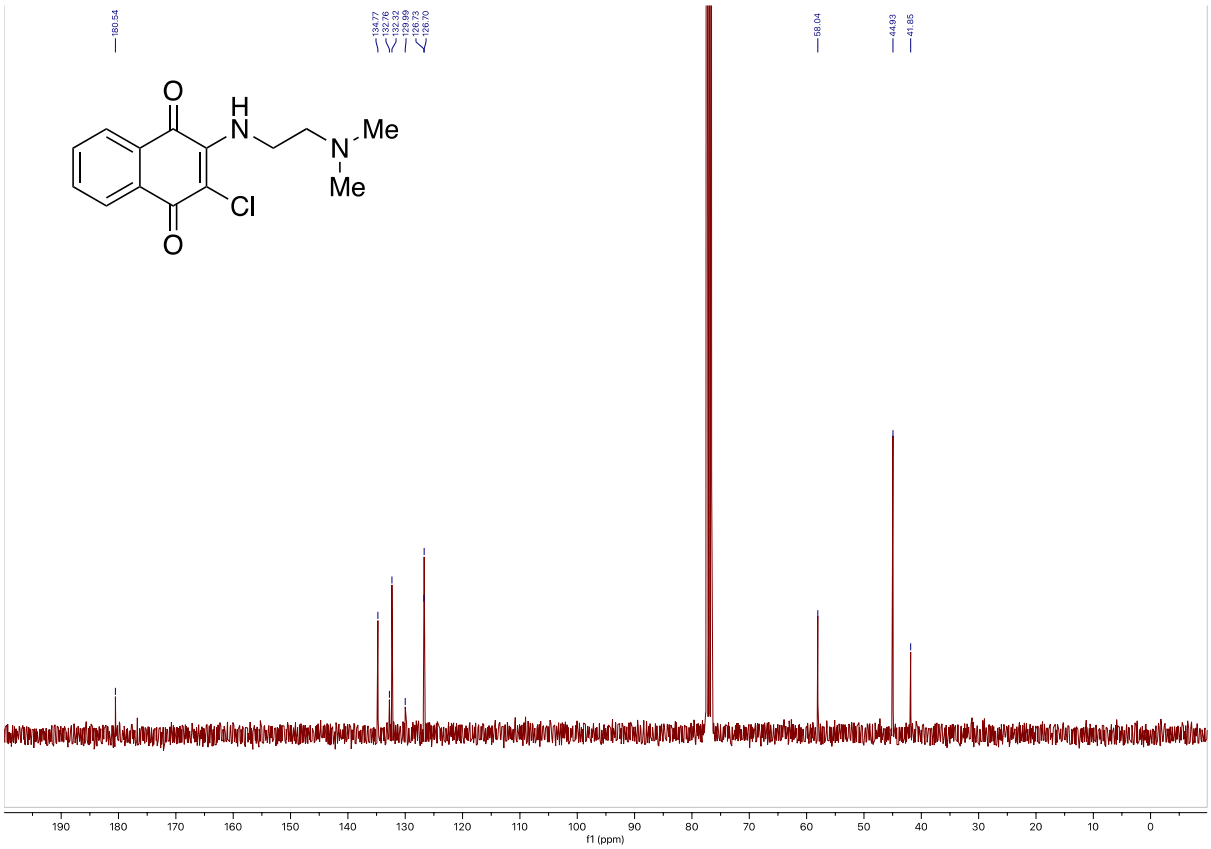
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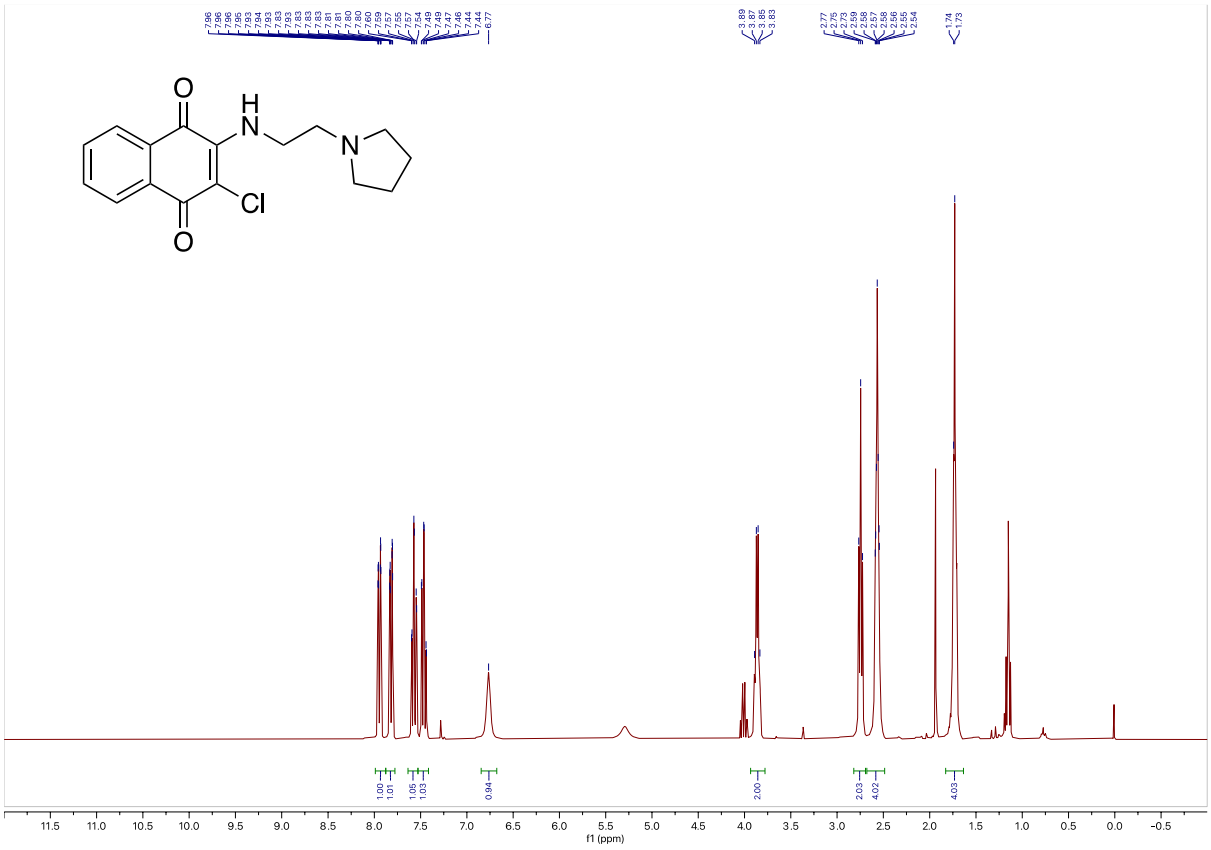

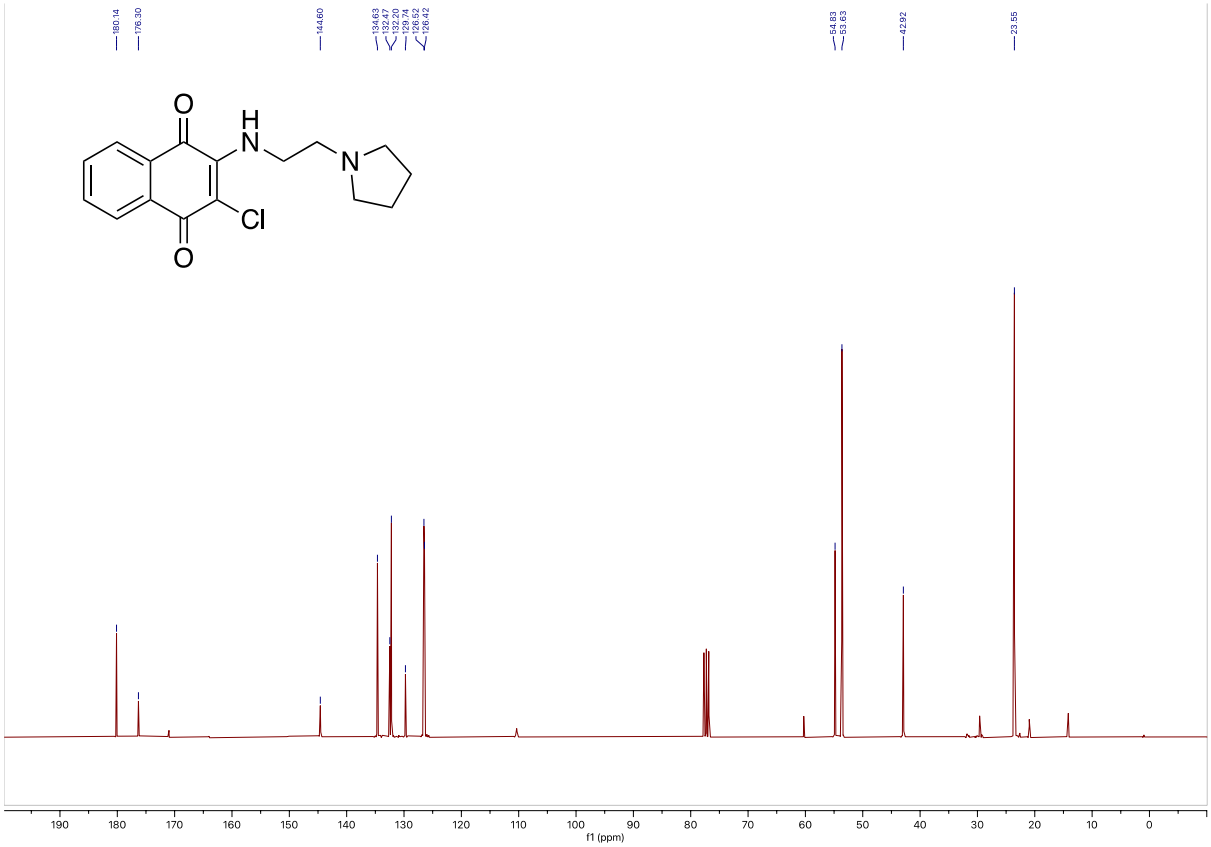

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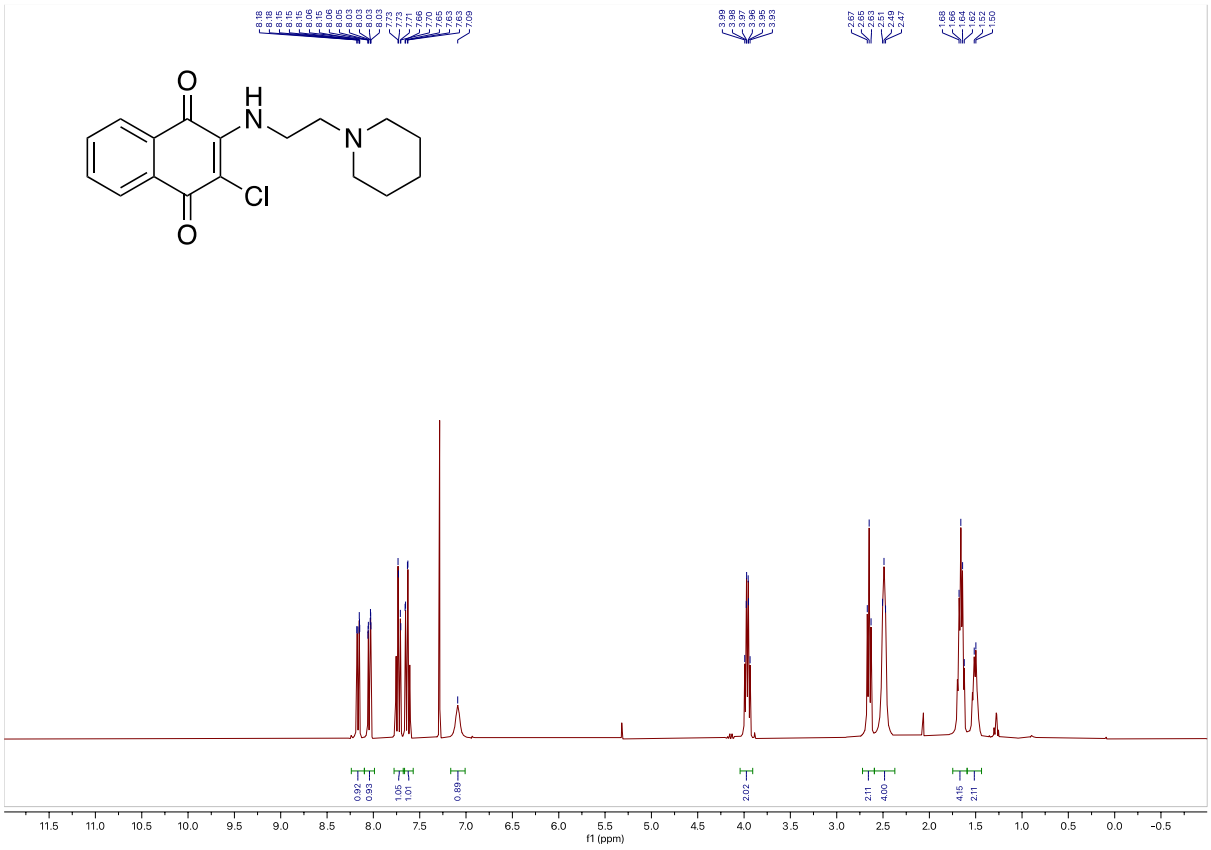

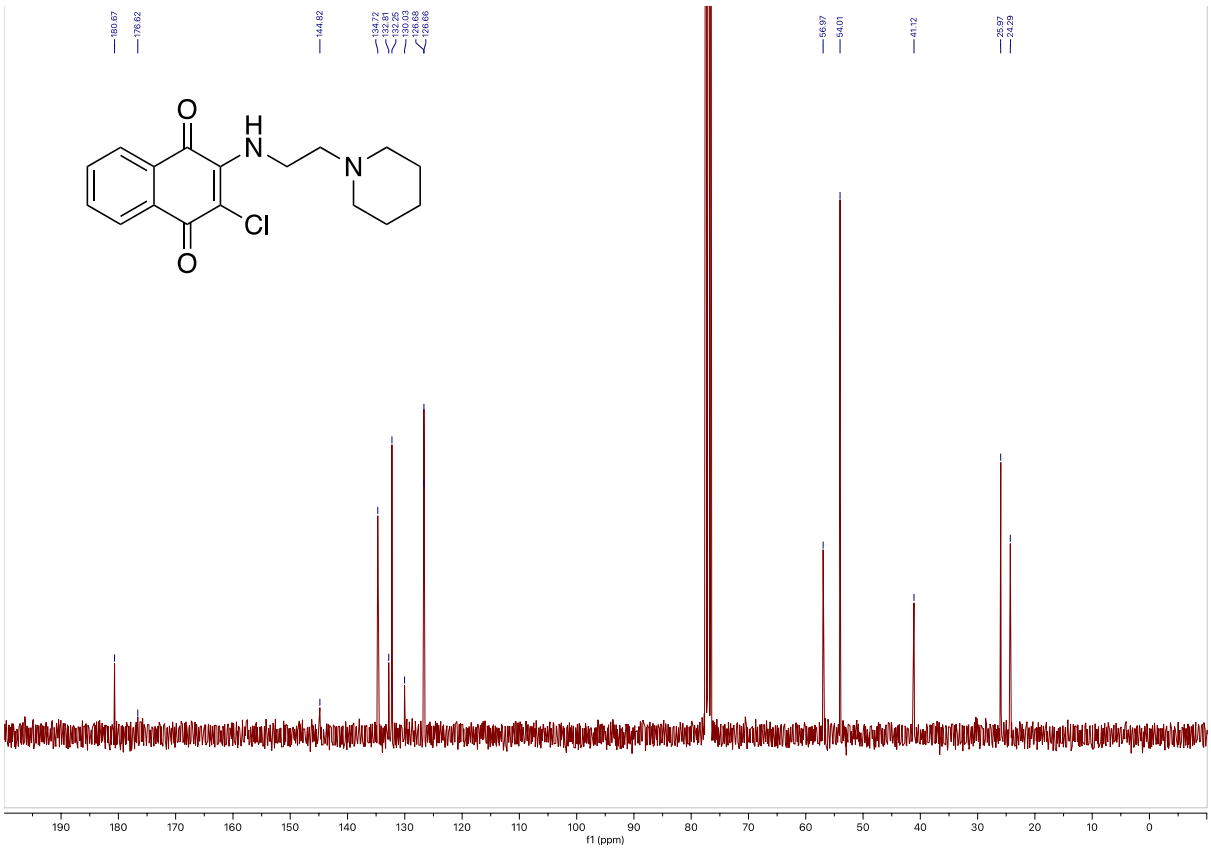

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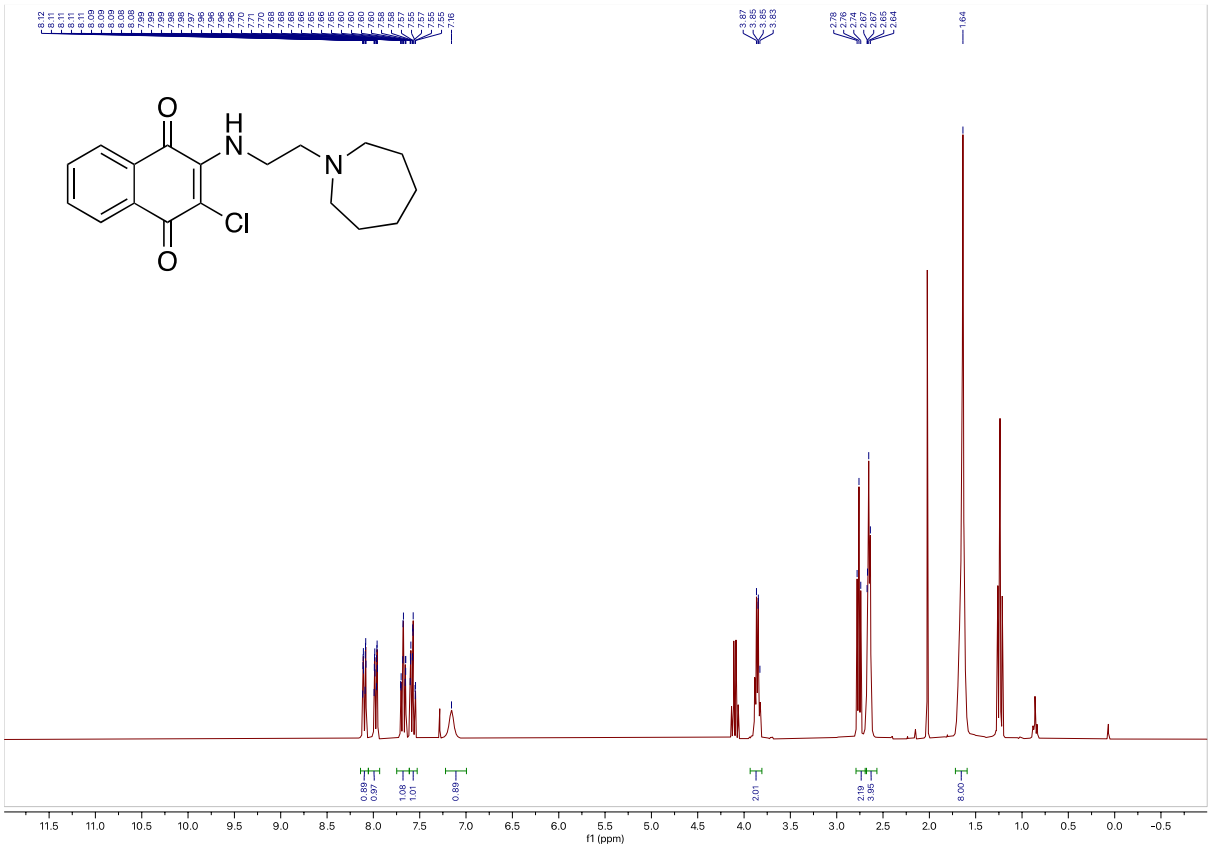

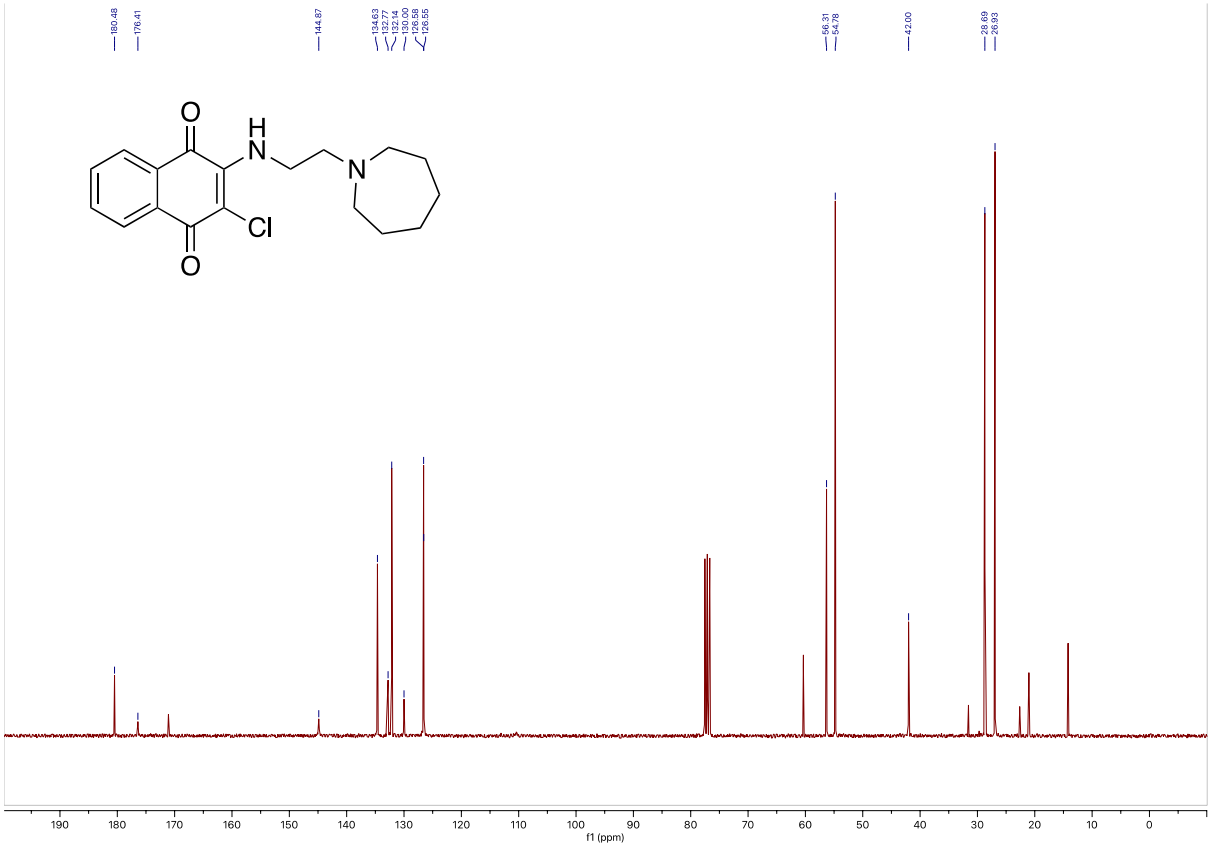

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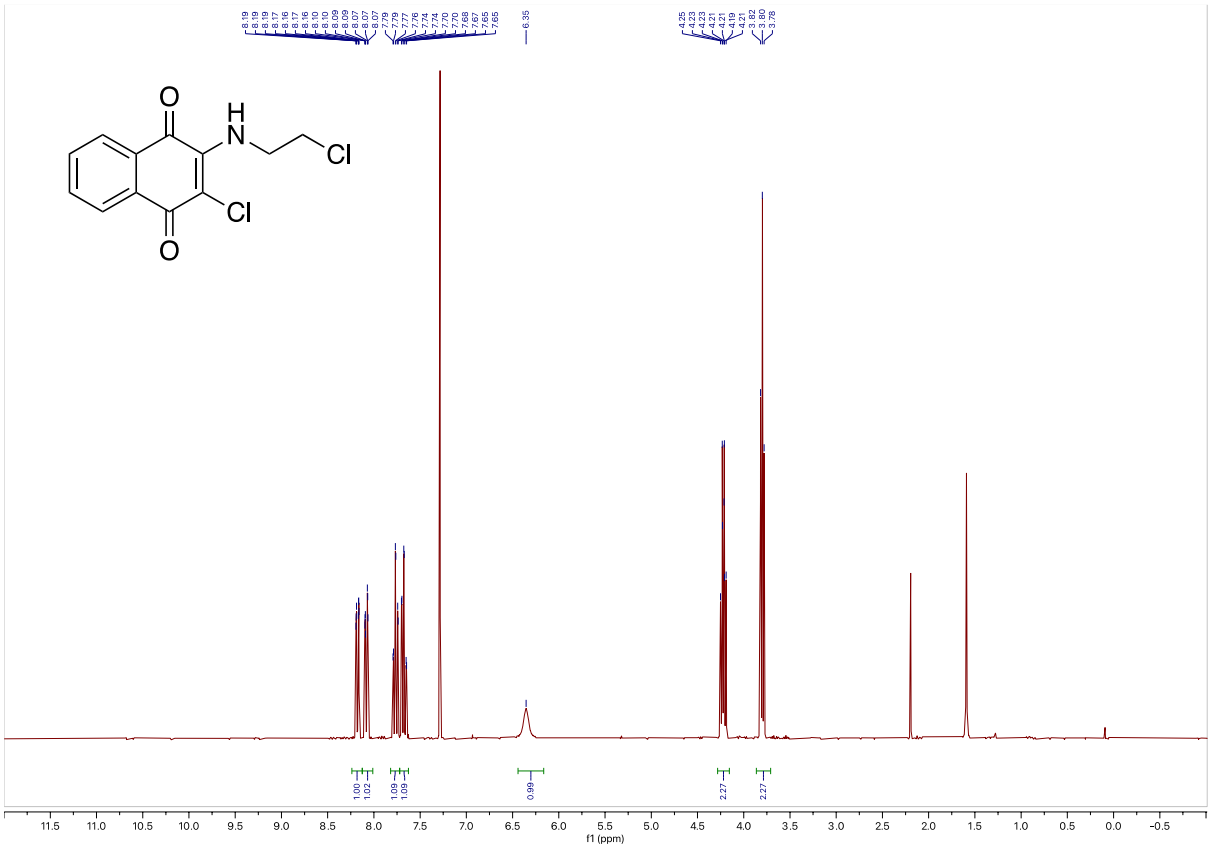
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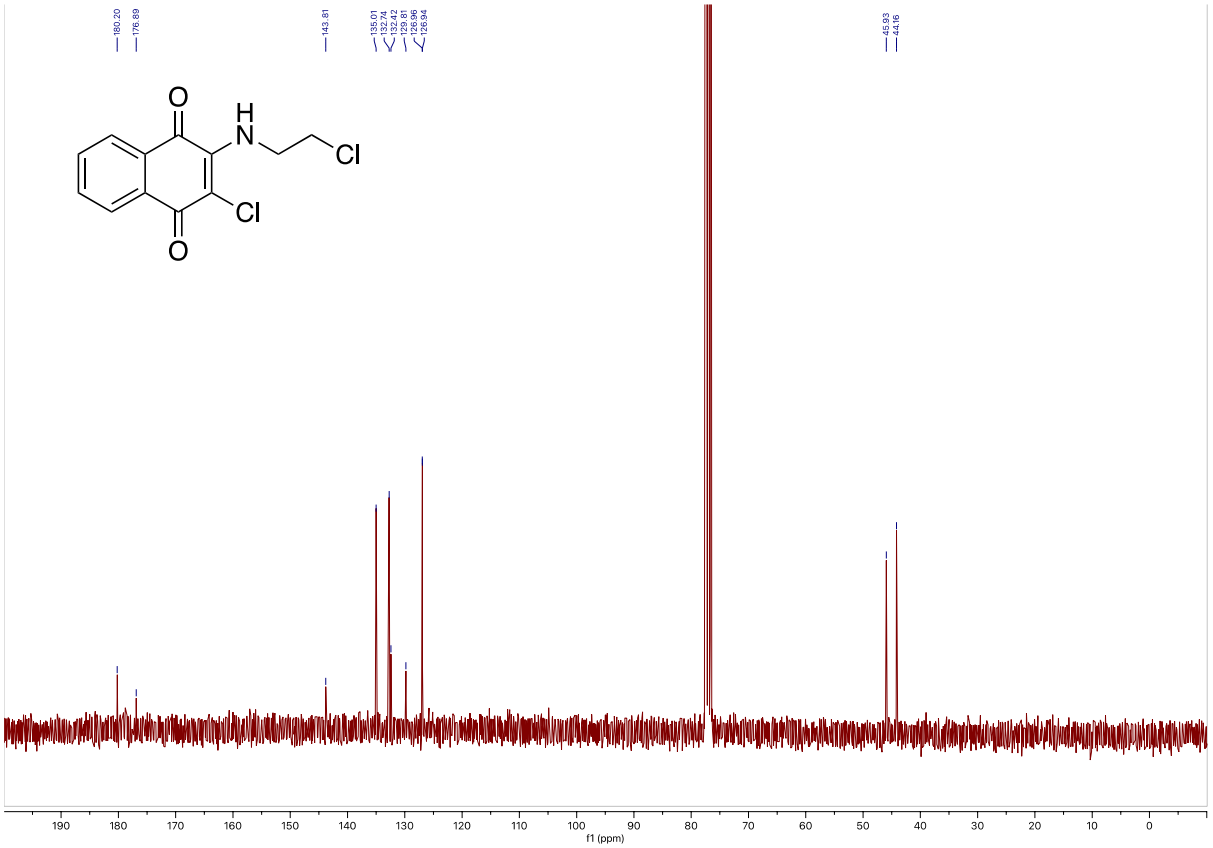
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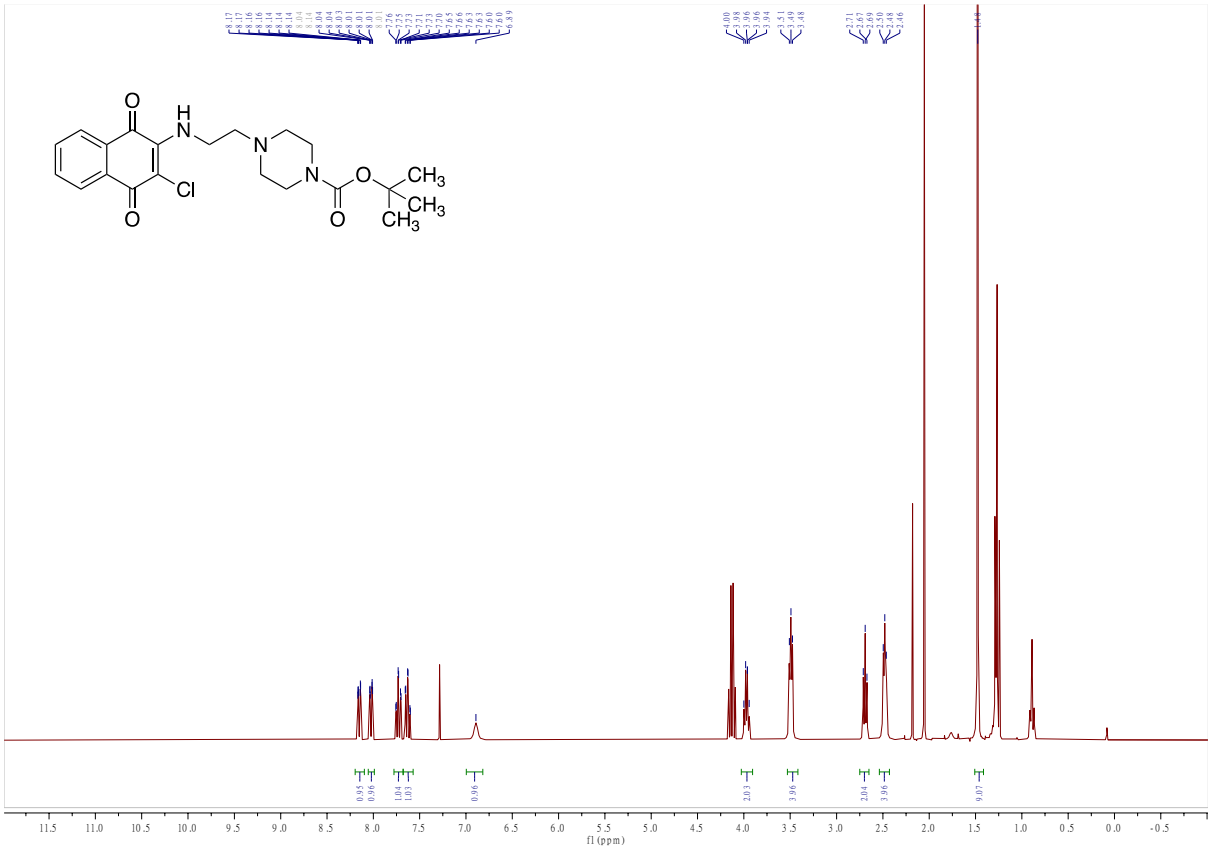
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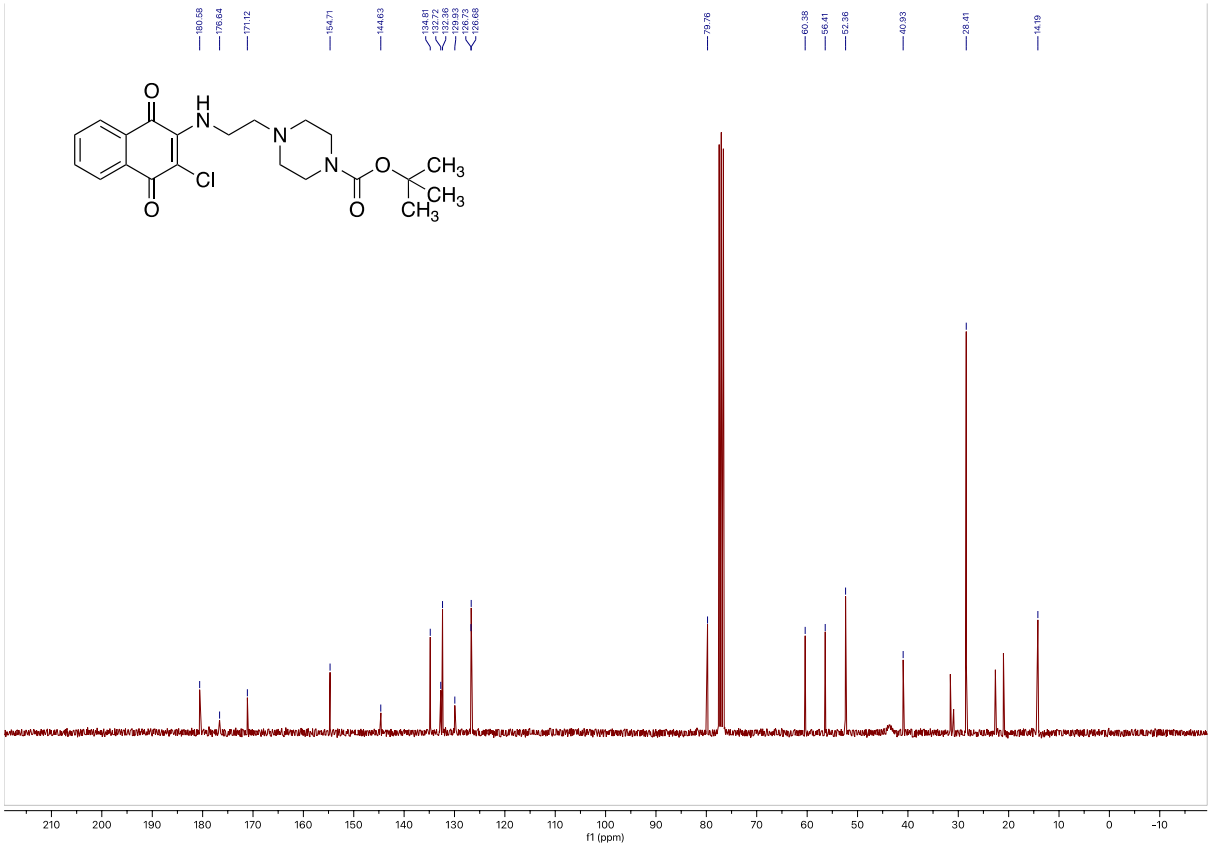
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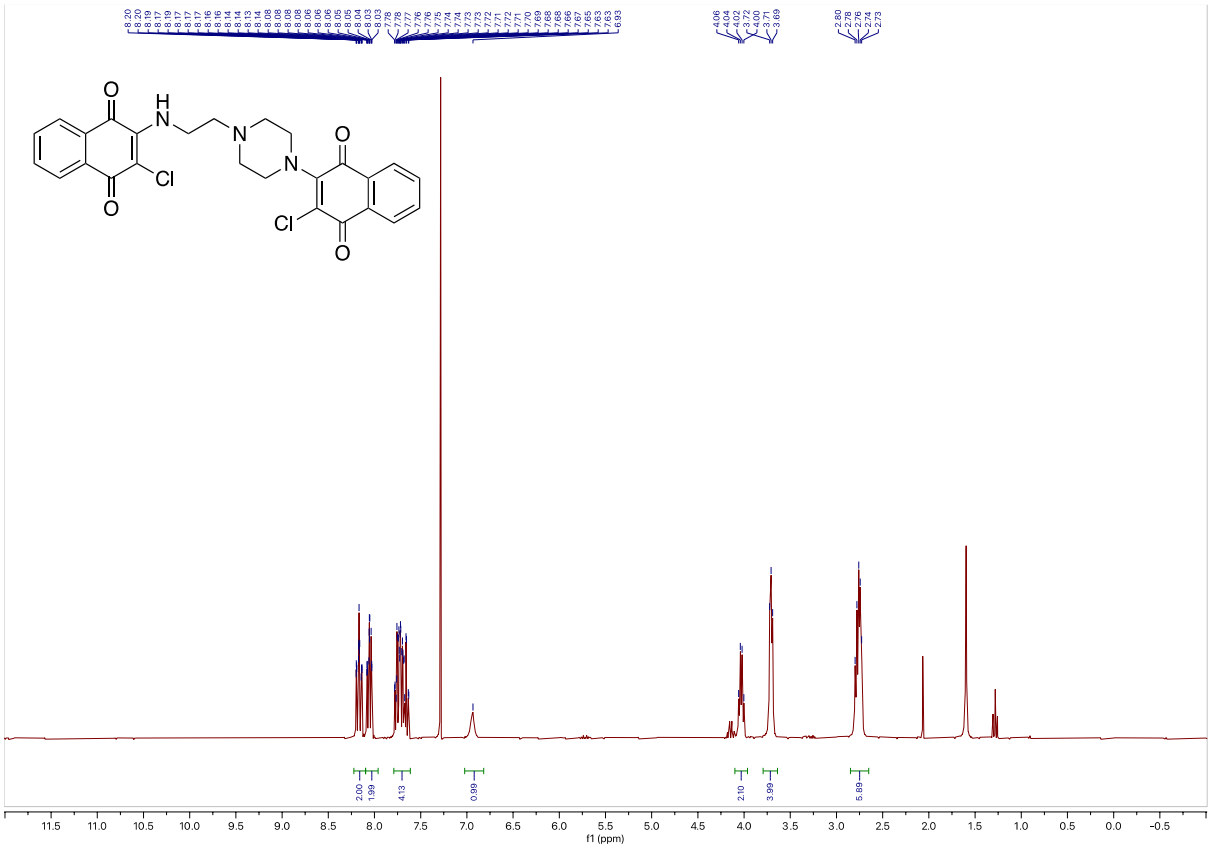
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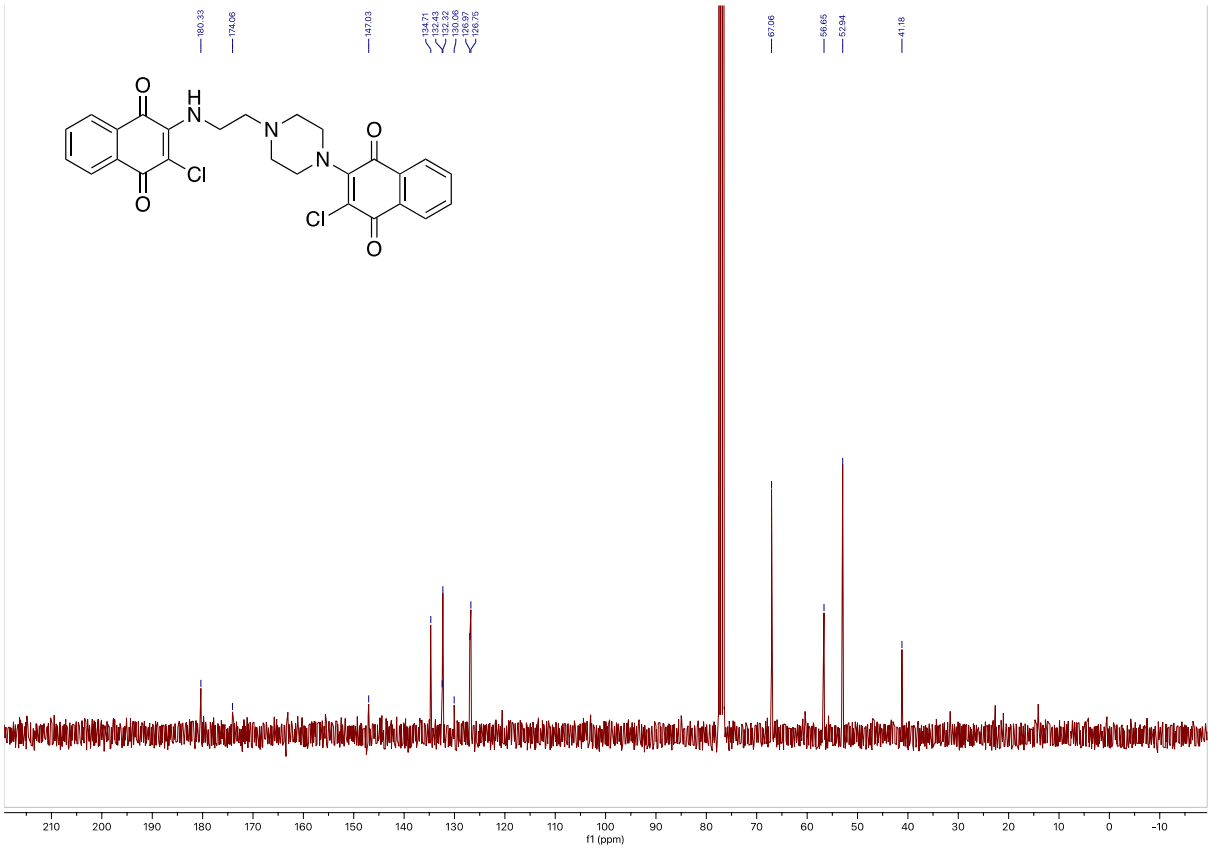
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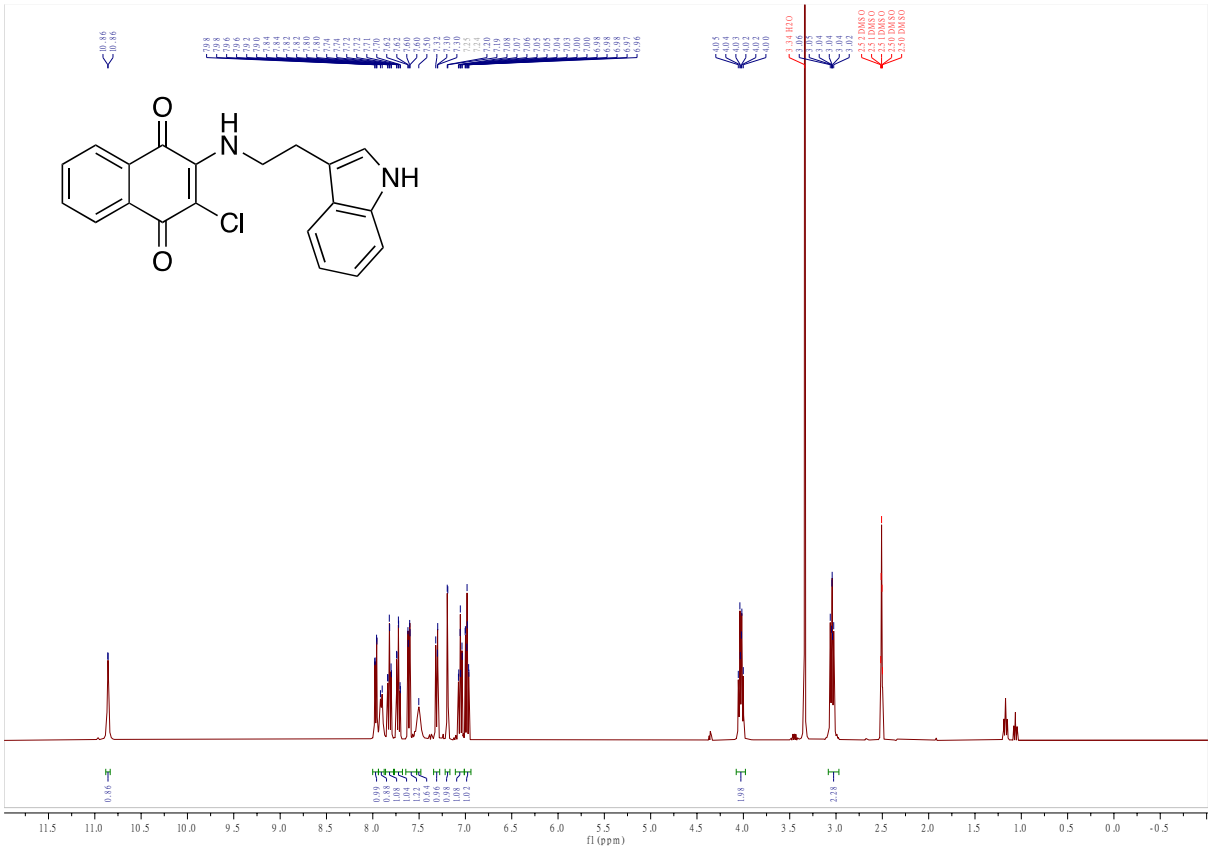

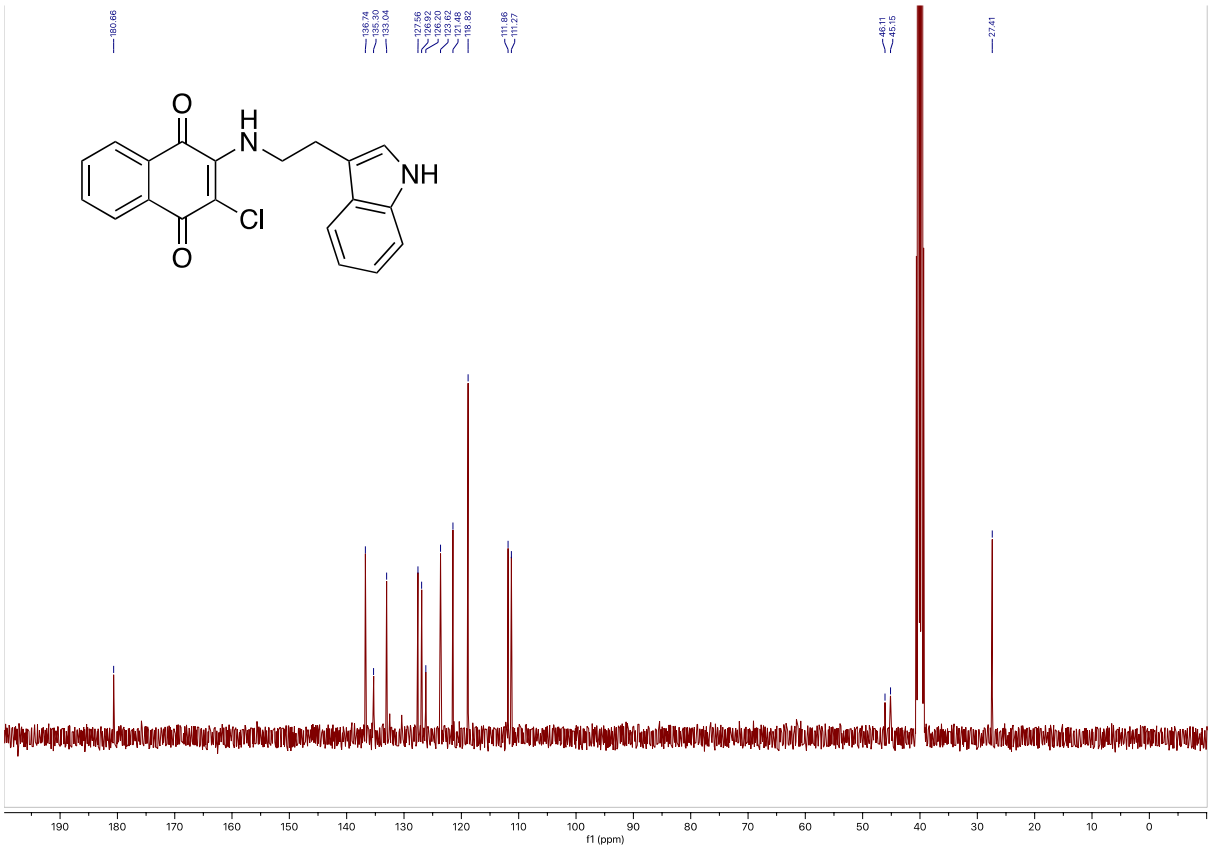

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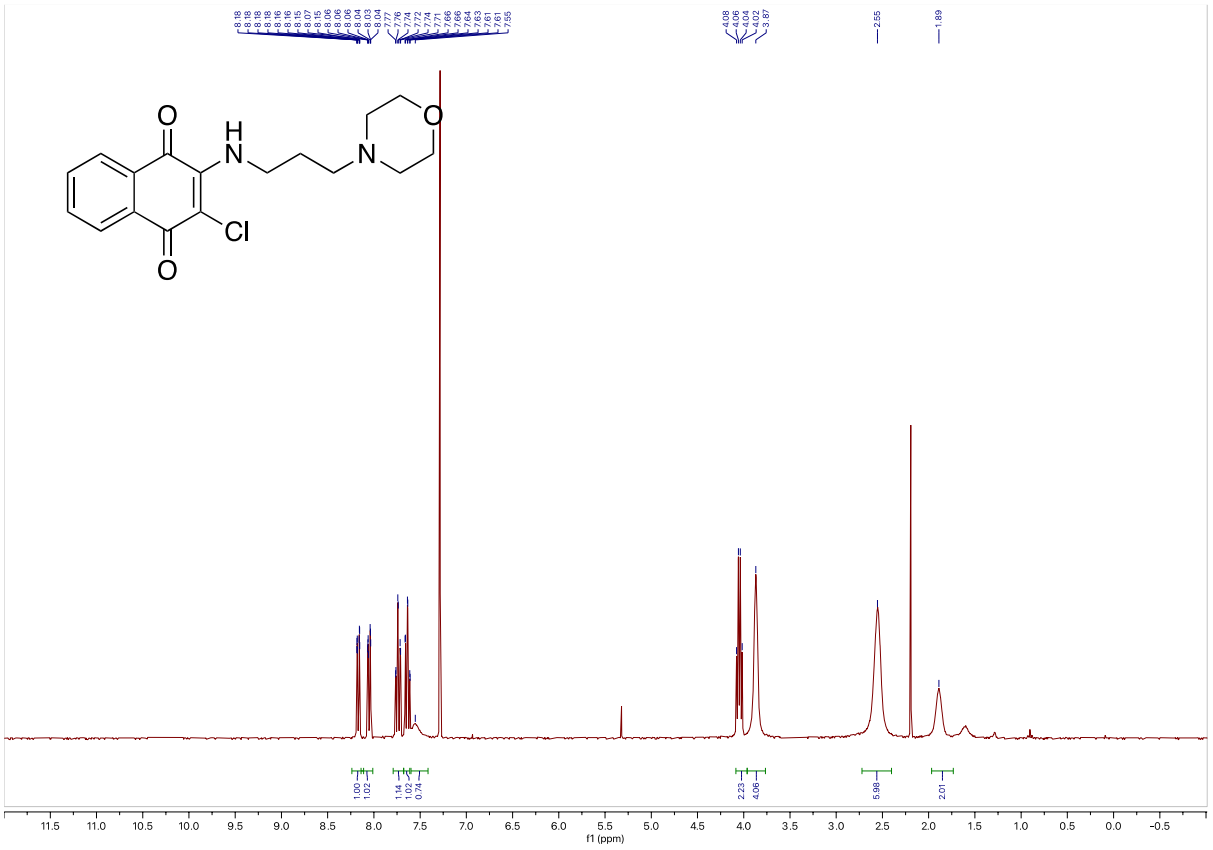

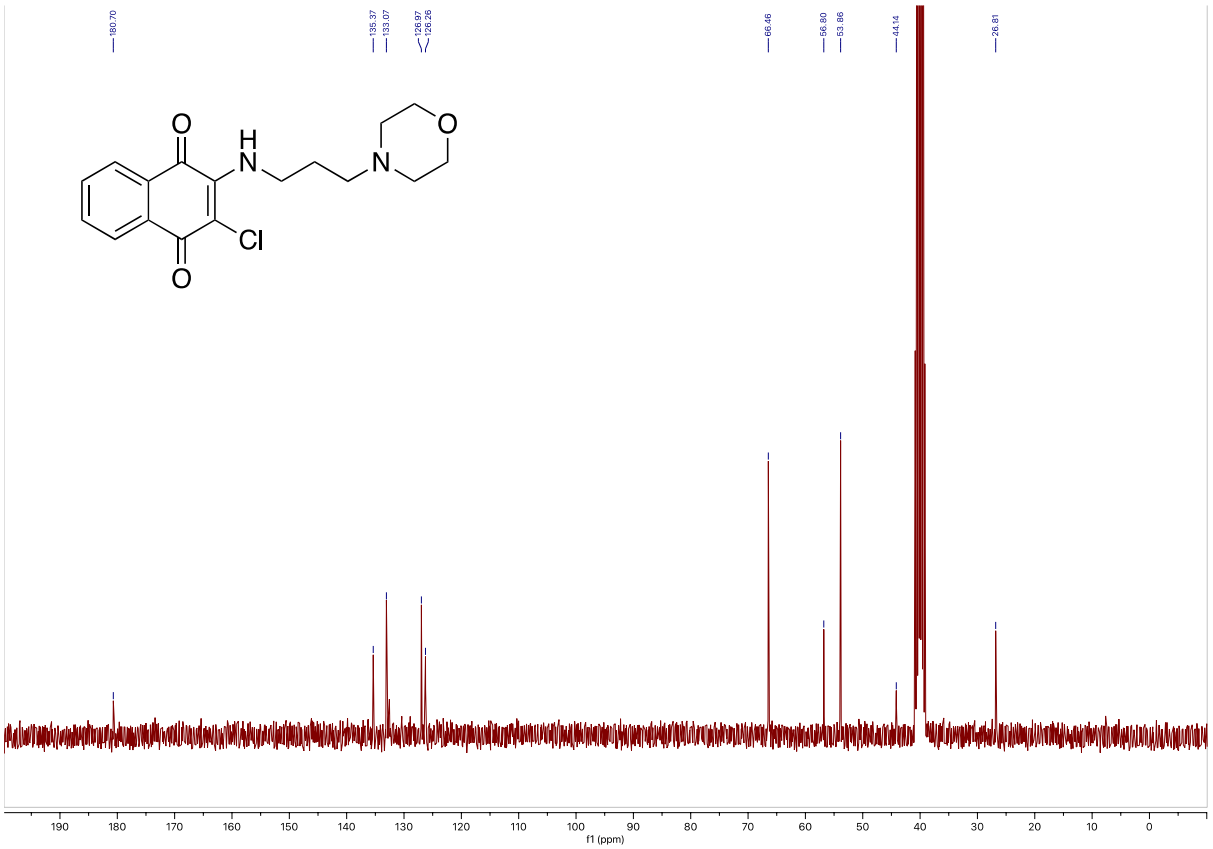

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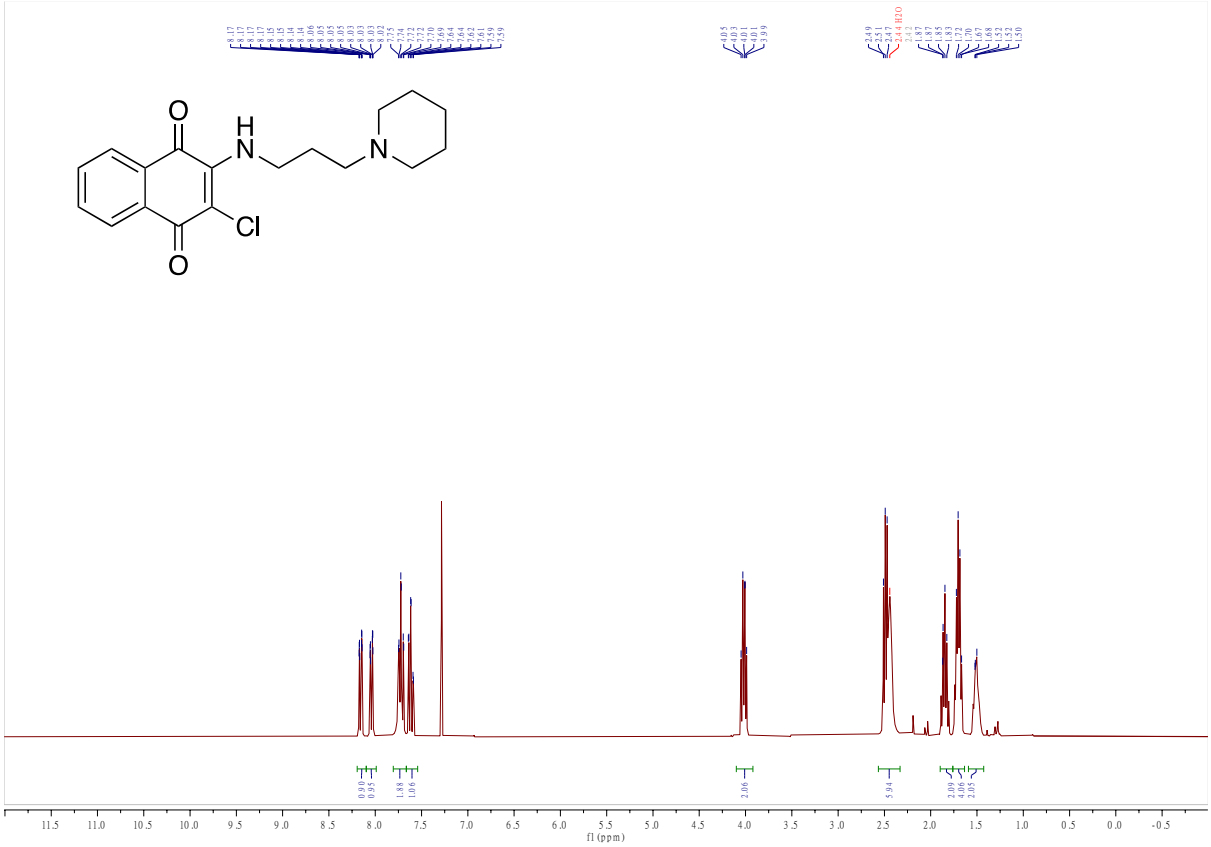
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Cl
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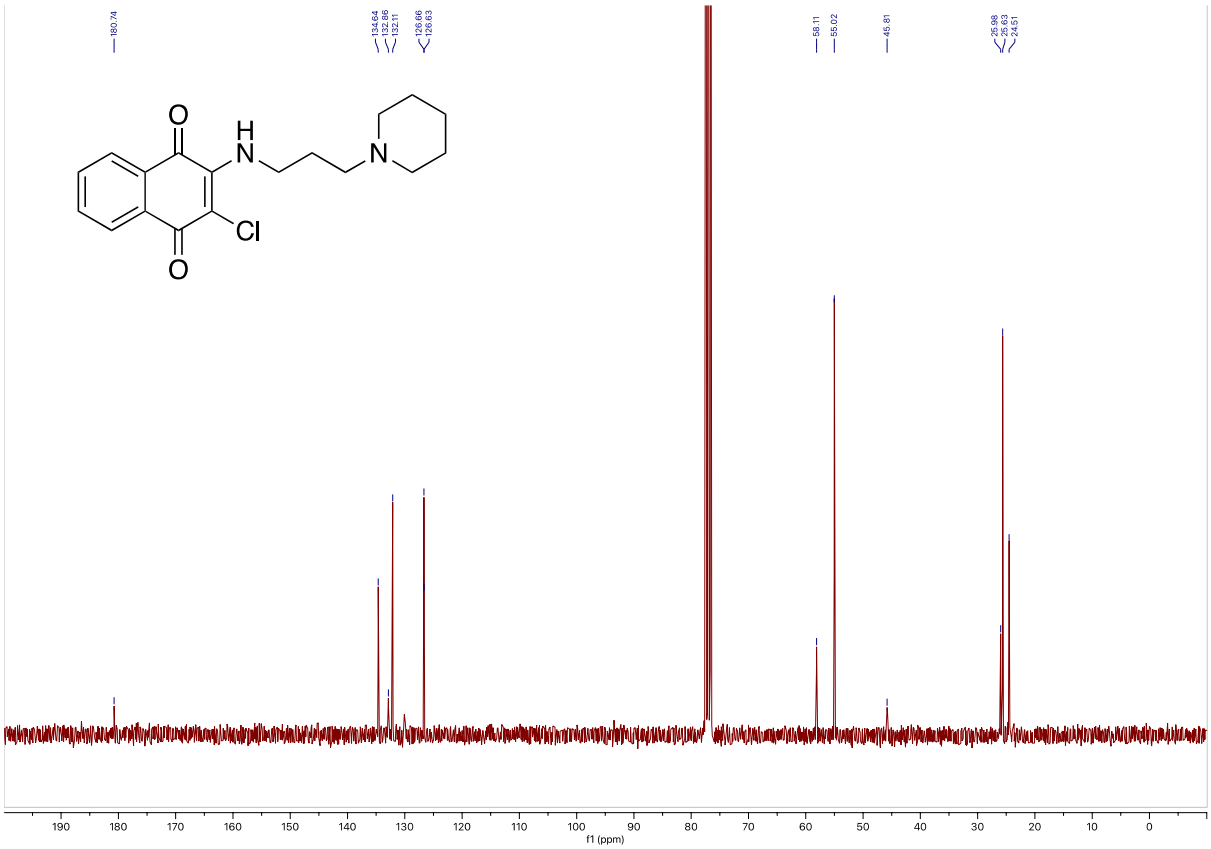
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O
f1 (ppm)

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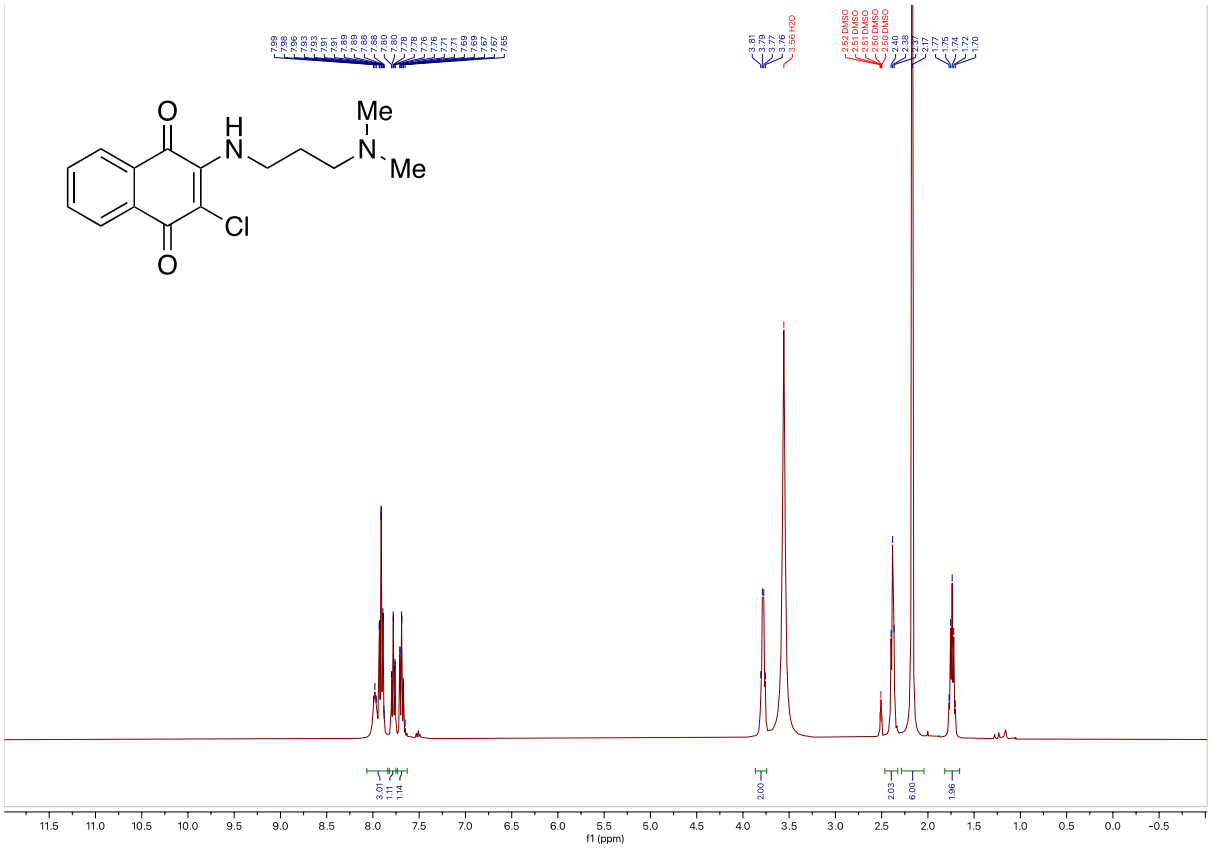

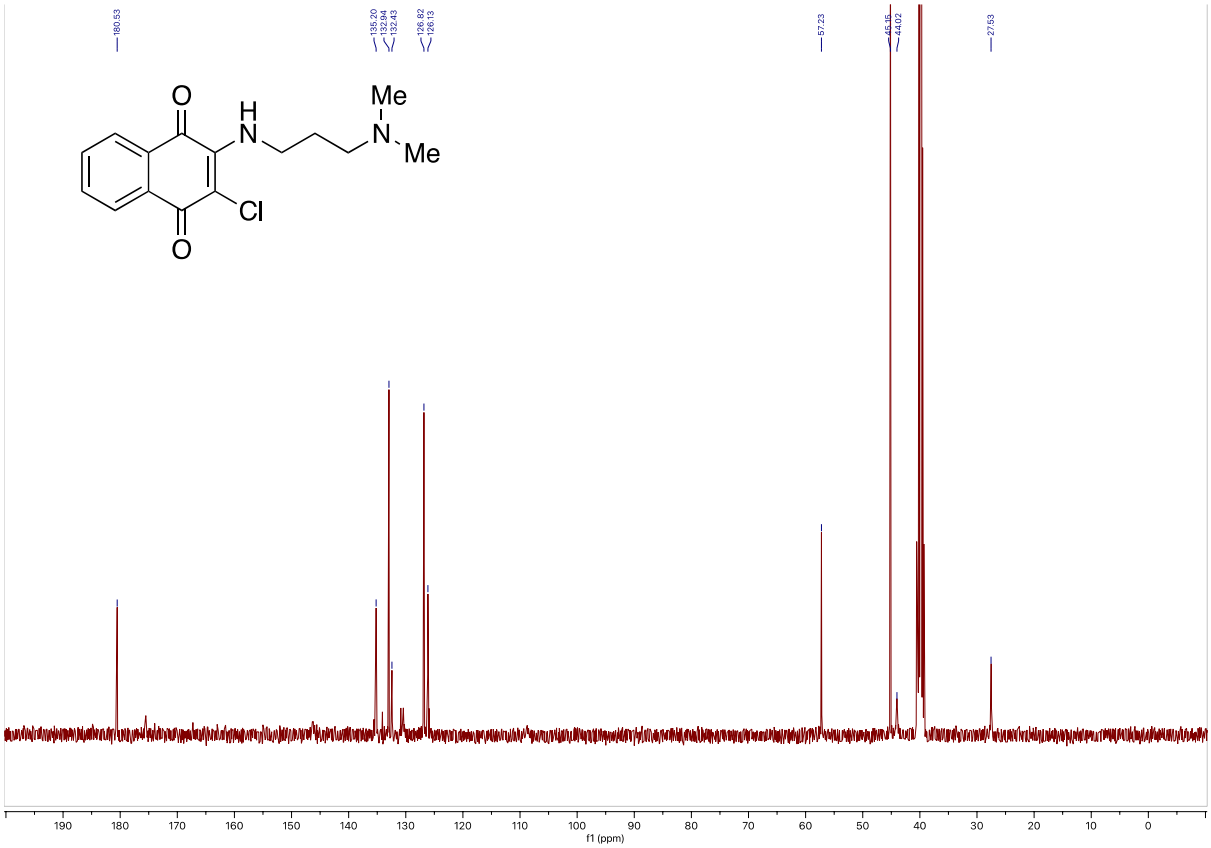

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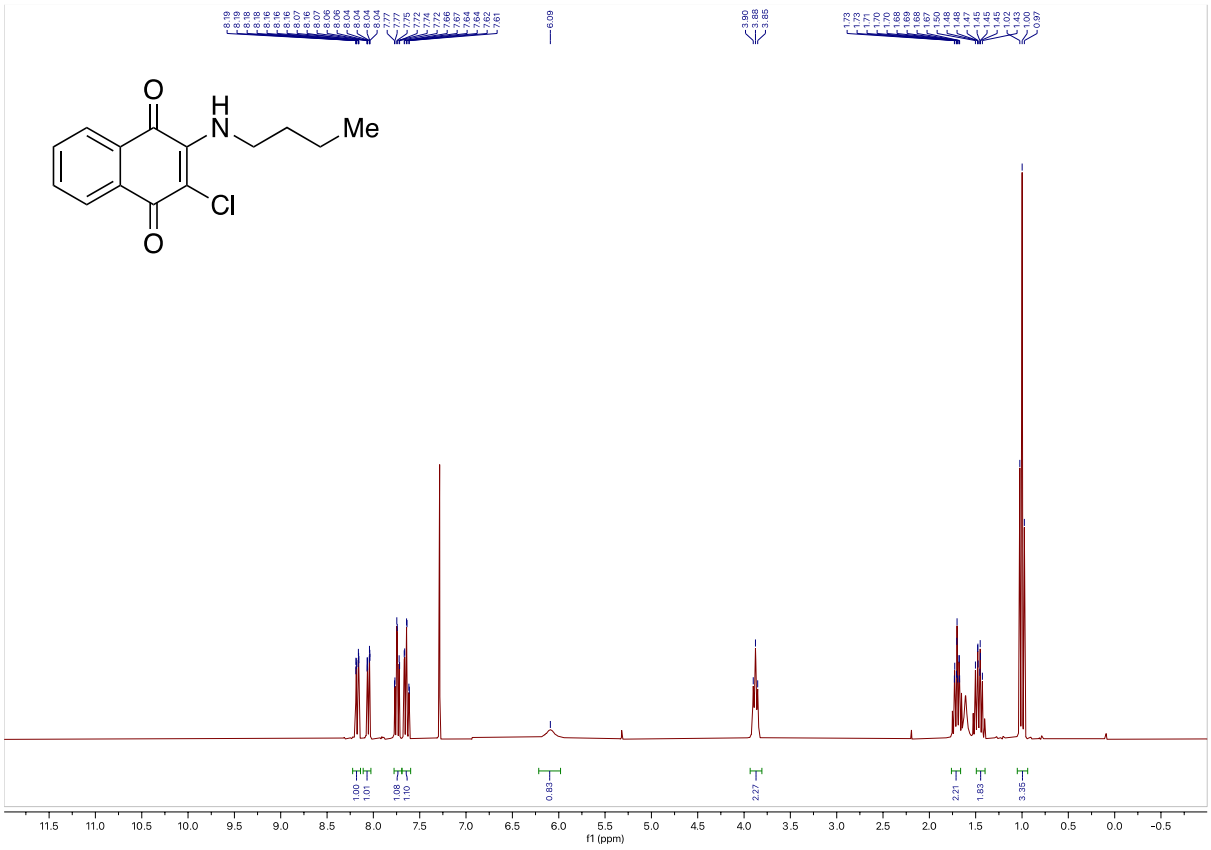

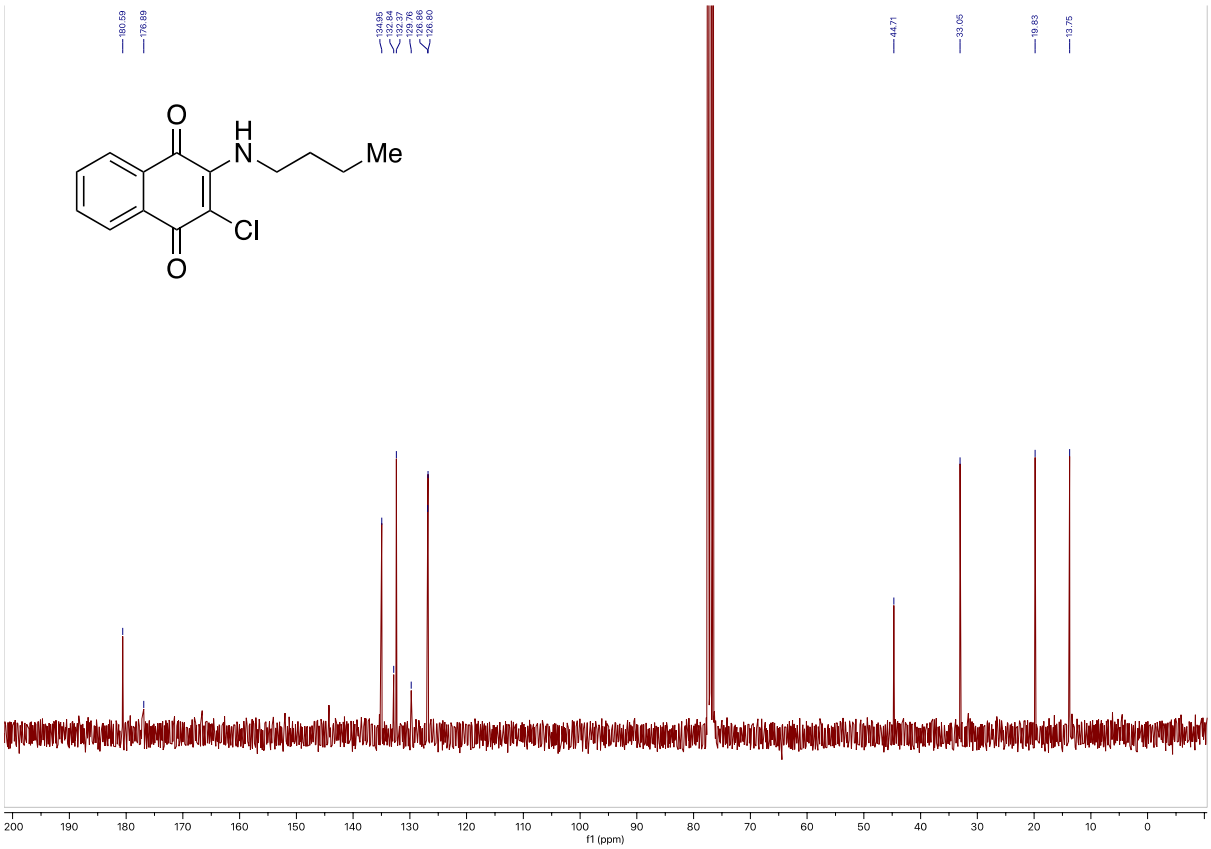

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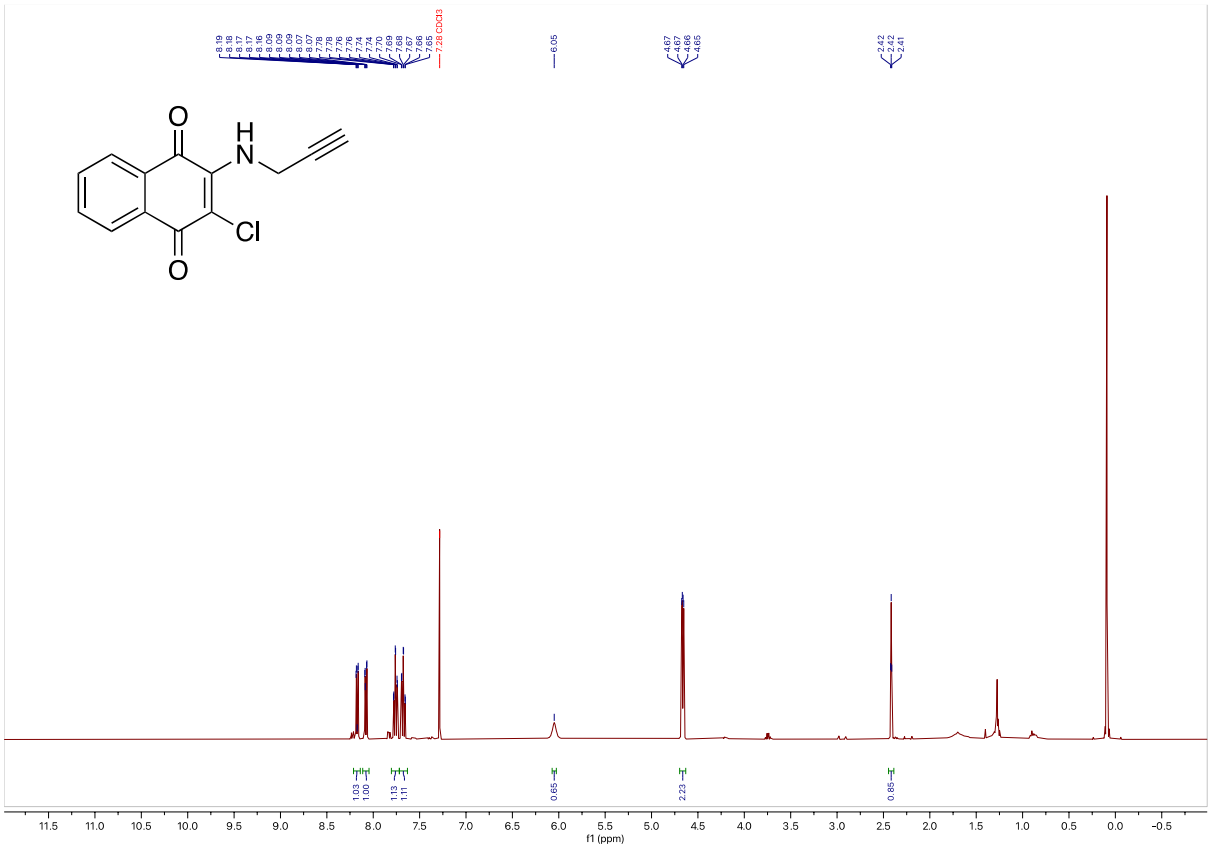
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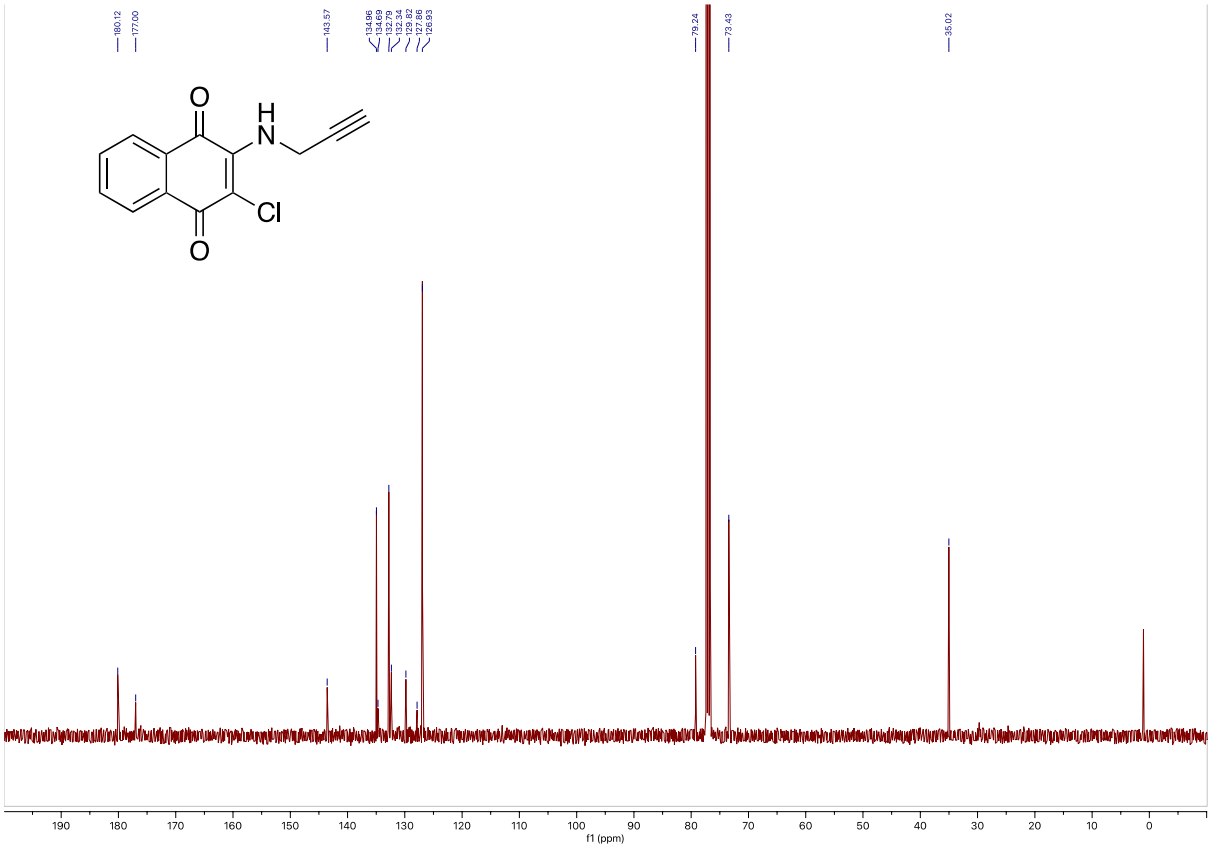
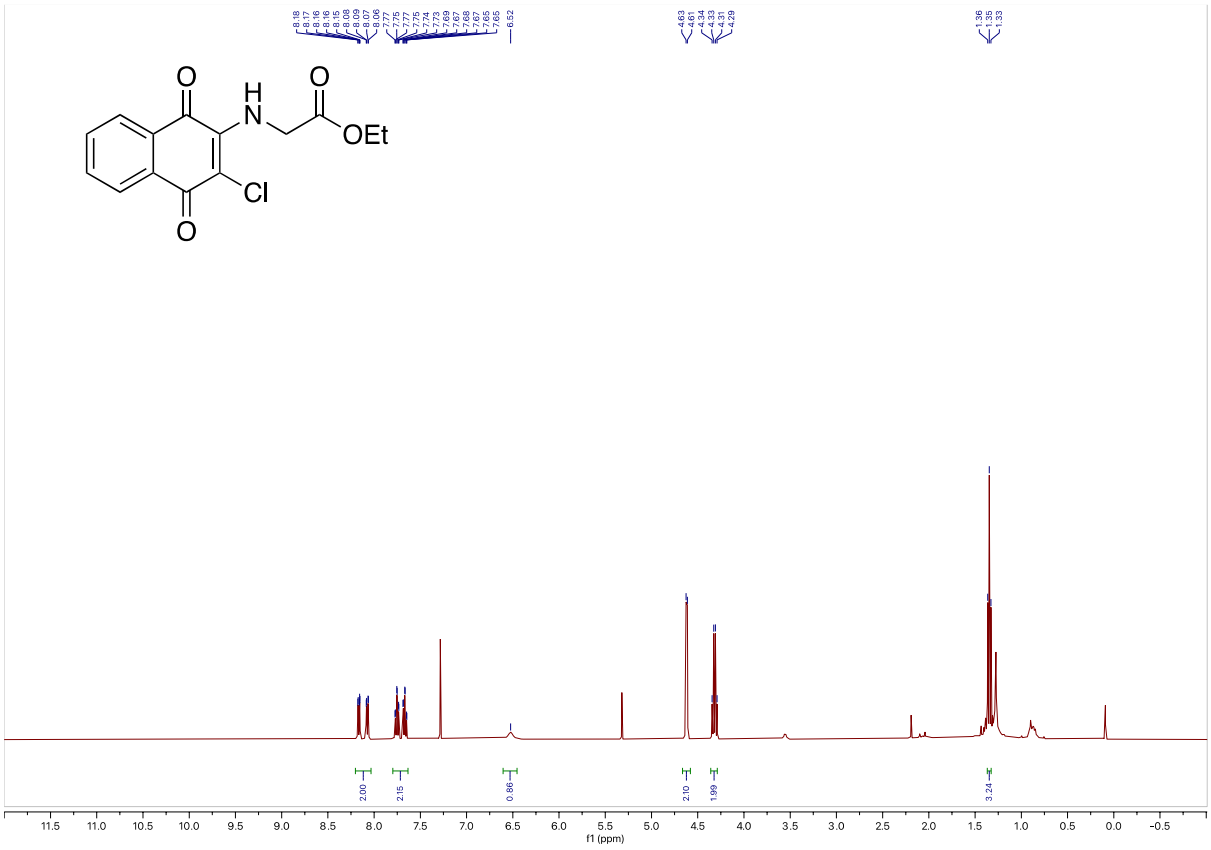
8.18
8.17
8.16
8.09
8.09
8.09
8.07
7.78
7.78
7.76
7.76
7.74
7.74
7.70
7.69
7.68
7.67
7.66
7.65
7.28 CDCl3
6.05
4.67
4.67
4.66
4.65
2.42
2.42
2.41
O
H
N
Cl
O
1.03
1.00
1.13
1.11
0.65
2.23
0.85
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


180.12
177.00
143.57
134.96
134.69
132.79
132.34
129.82
127.86
126.93
79.24
73.43
35.02
O
H
N
Cl
O
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

25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8.17
8.16
8.16
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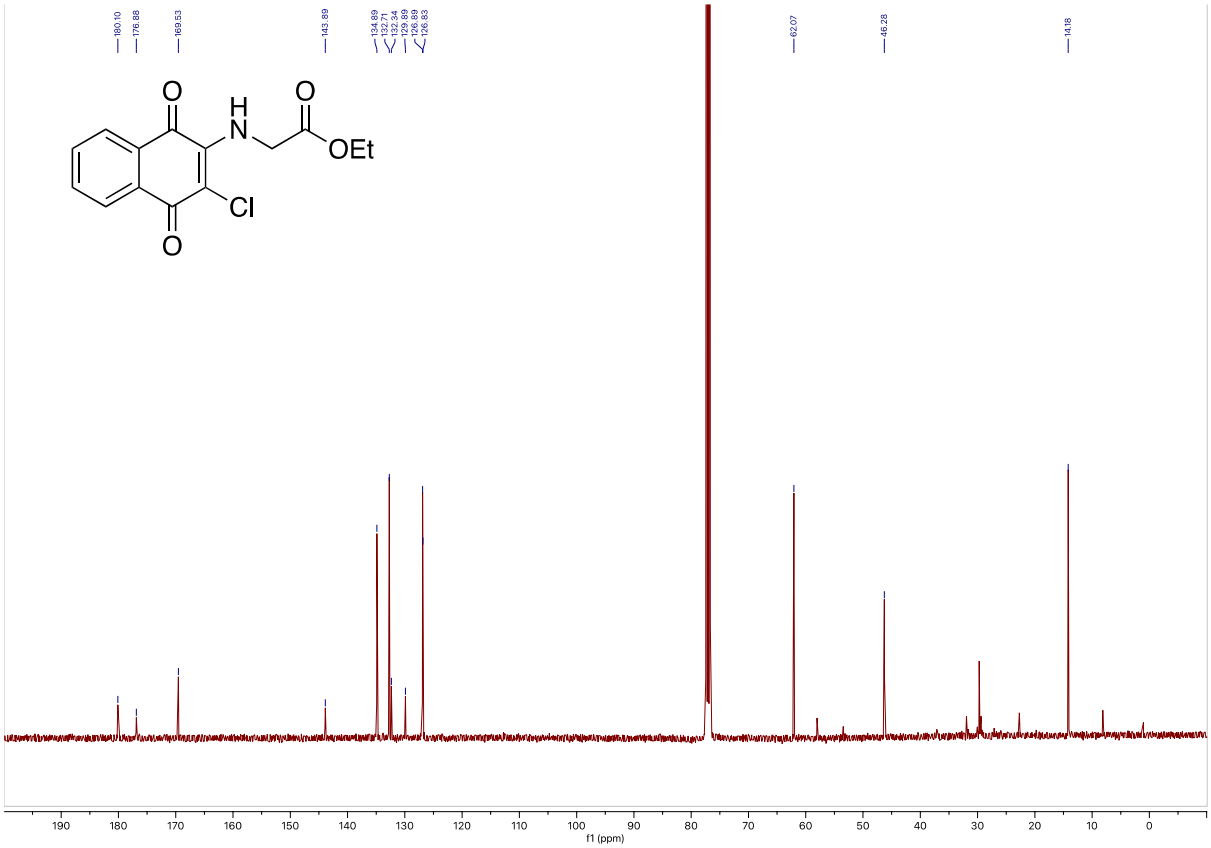
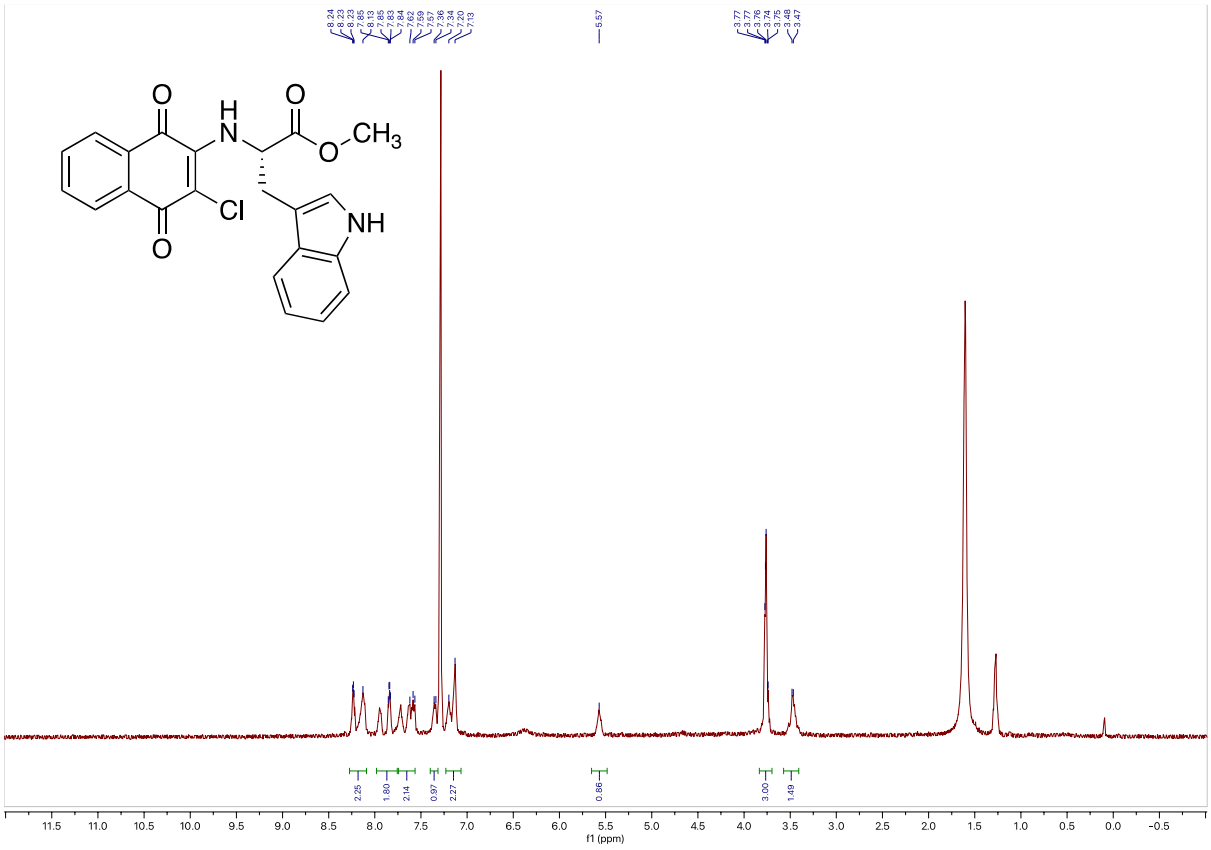
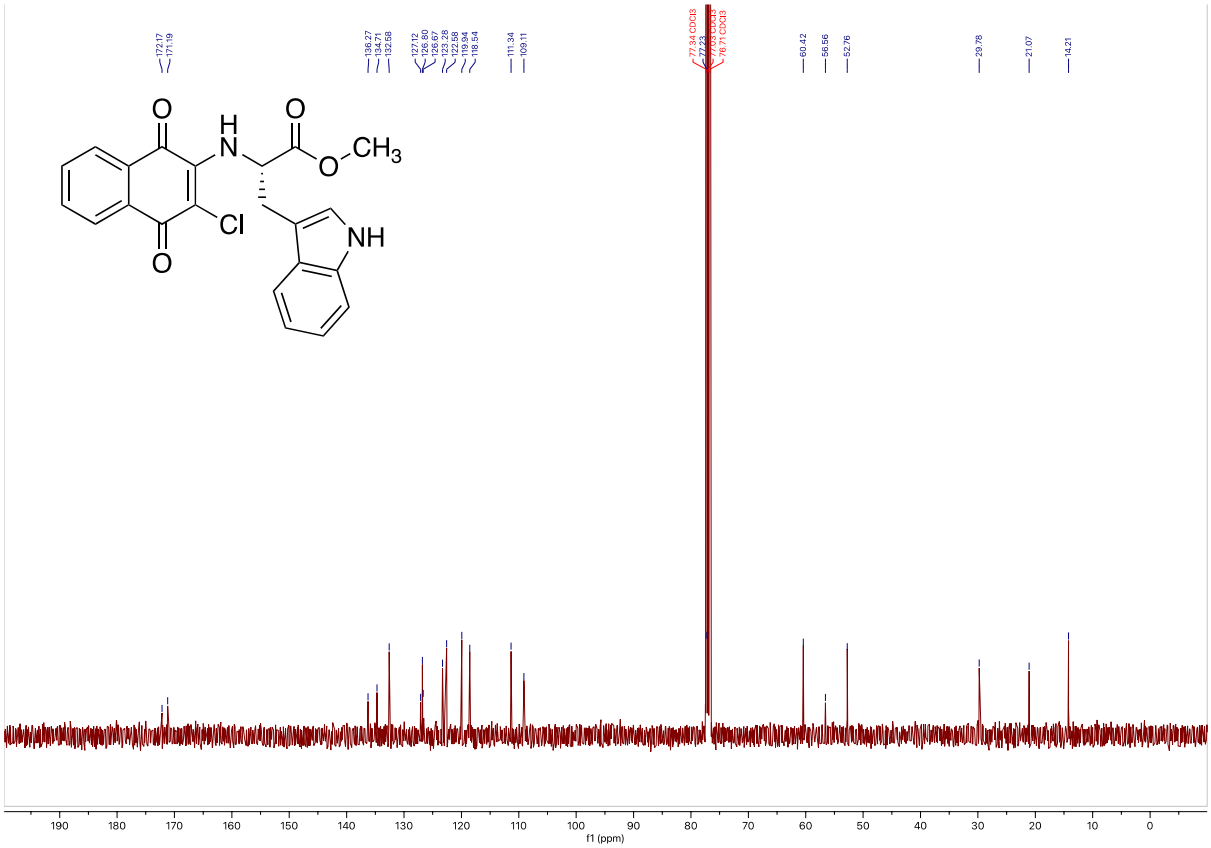
8.15
8.08
8.08
8.07
8.06
7.77
7.75
7.77
7.75
7.74
7.73
7.69
7.67
7.68
7.67
7.65
7.65
6.52
4.63
4.61
4.34
4.33
4.31
4.29
1.36
1.35
1.33
O
H
N
O
OEt
Cl
O
2.00
2.15
0.86
2.10
1.99
3.24
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


180.10
176.88
169.53
143.89
134.89
132.71
132.34
129.89
128.69
126.83
62.07
46.28
14.18
O
H
N
O
OEt
Cl
O
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

26


8.24
8.23
8.23
7.85
7.83
7.85
7.83
7.84
7.82
7.59
7.57
7.36
7.34
7.20
7.13
5.57
3.77
3.77
3.76
3.74
3.75
3.48
3.47
O
H
N
O
O
CH3
Cl
NH
O
2.25
1.80
2.14
0.97
2.27
0.86
3.00
1.49
11.5
11.0
10.5
10.0
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
1.0
0.5
0.0
-0.5
f1 (ppm)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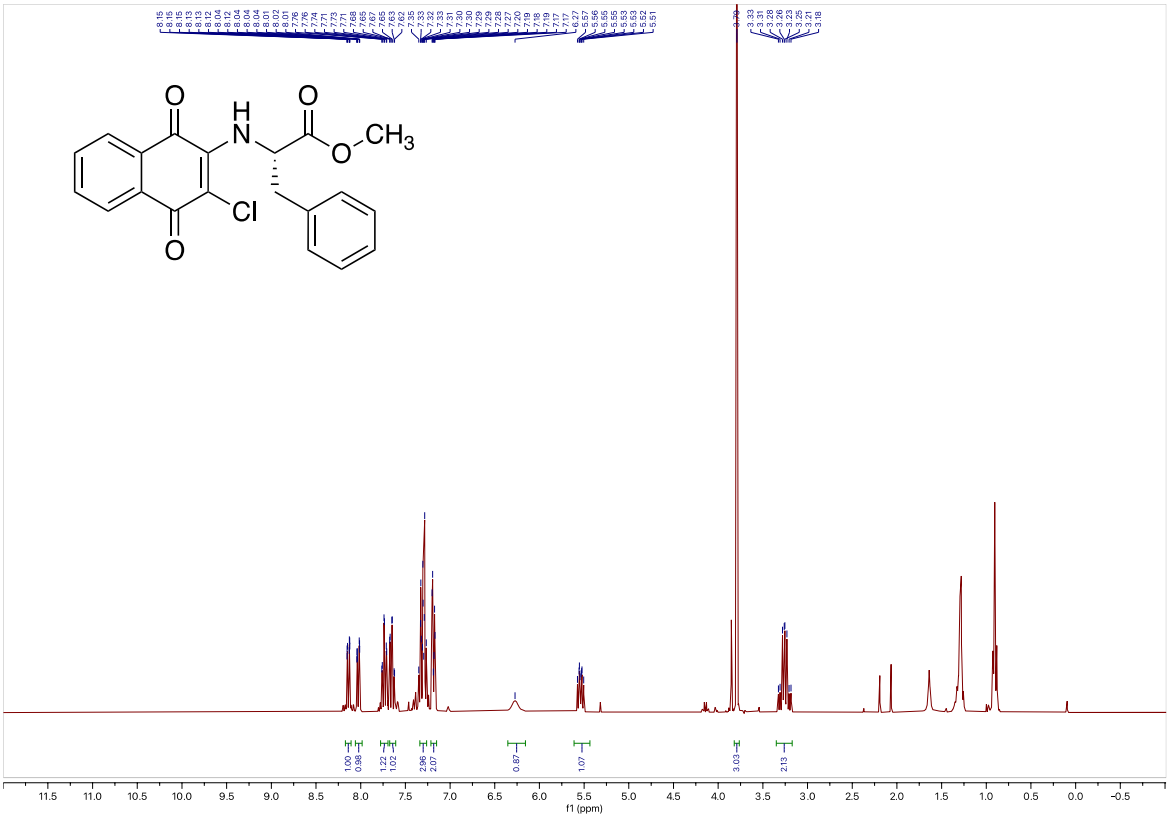
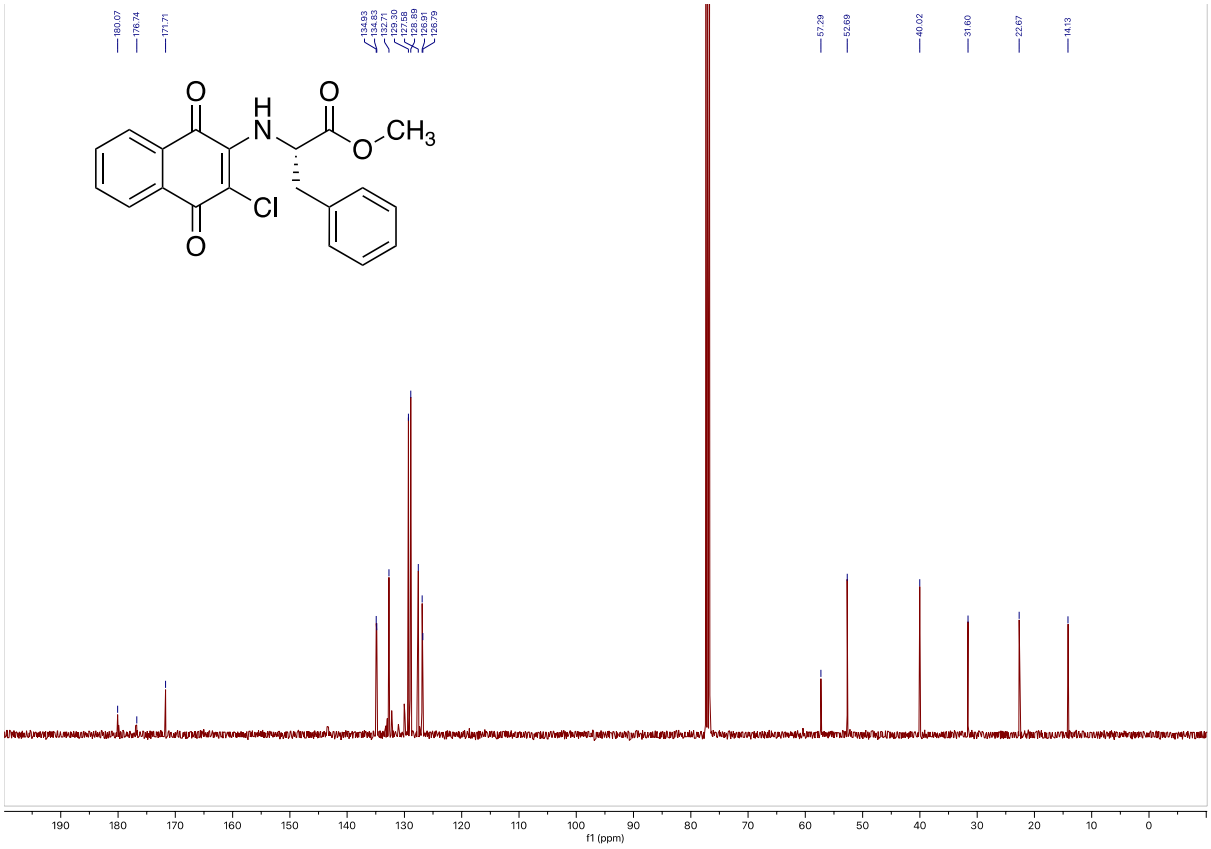
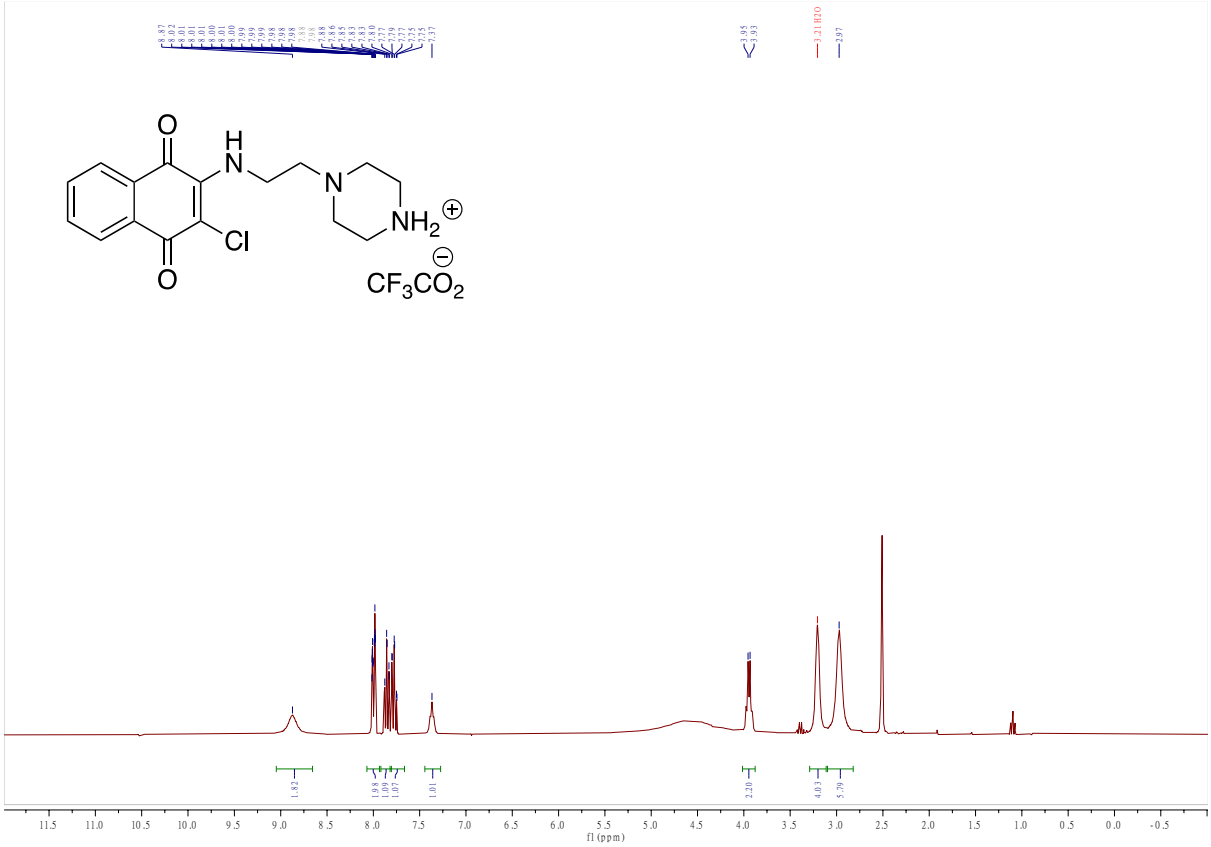
171.19
136.27
134.71
132.58
127.12
126.80
126.67
123.28
122.56
119.94
118.54
111.34
109.11
77.34 CDCl3
77.23
77.03 CDCl3
76.71 CDCl3
60.42
56.66
52.76
29.78
21.07
14.21
O
H
N
O
O
CH3
Cl
NH
O
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

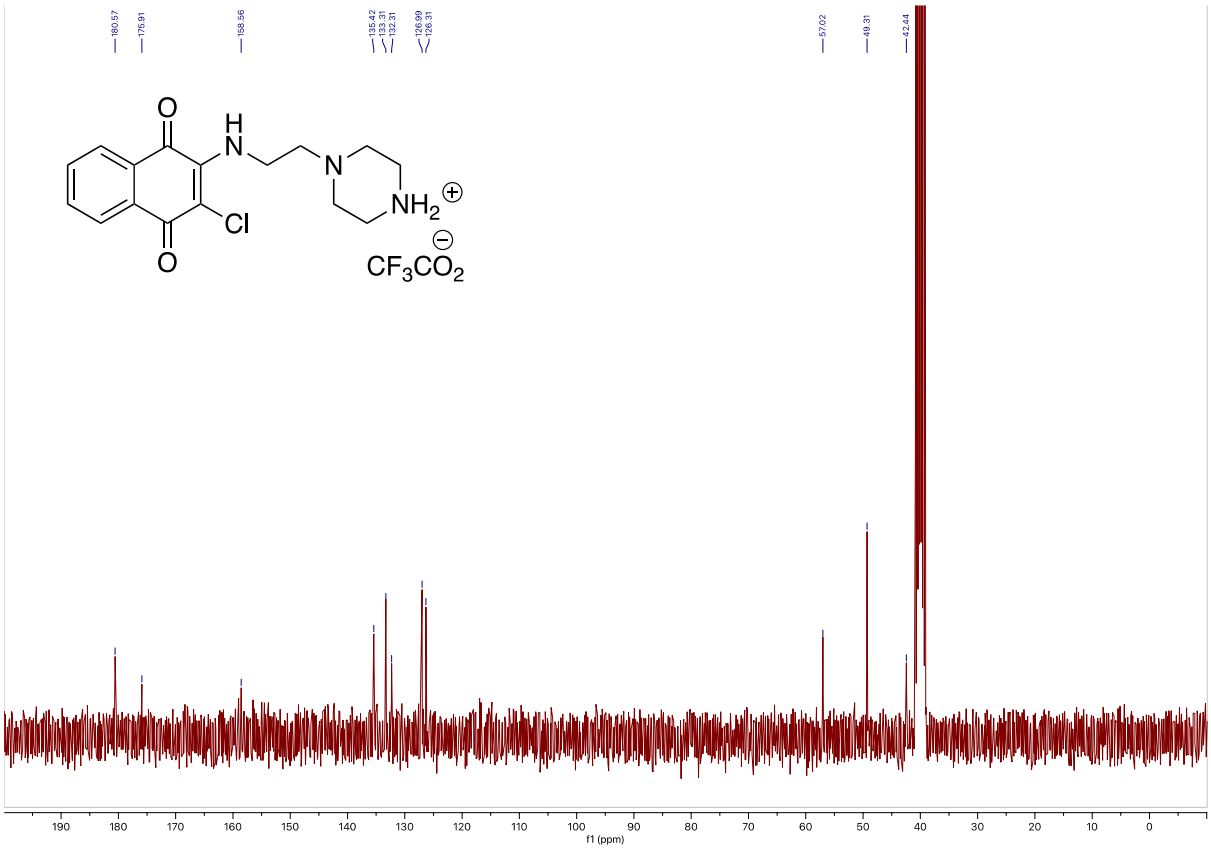
27





29





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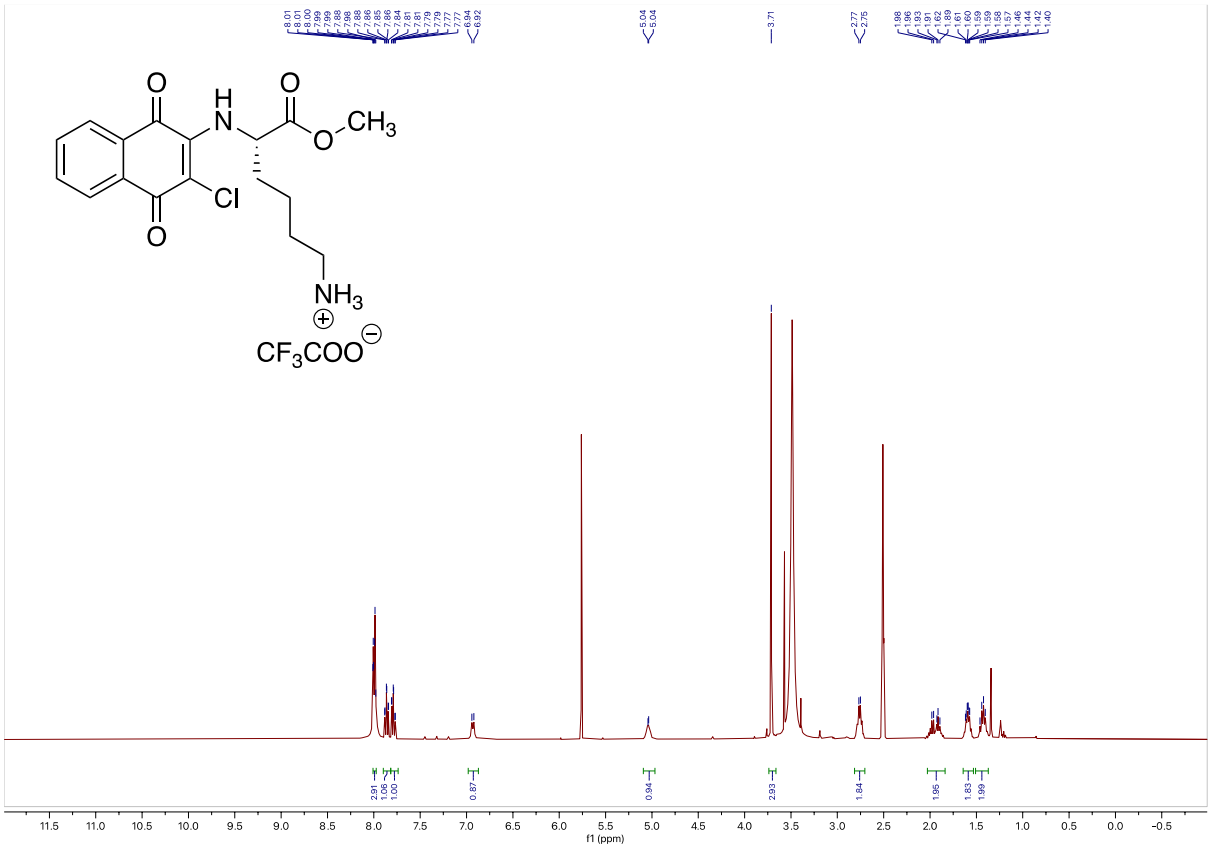
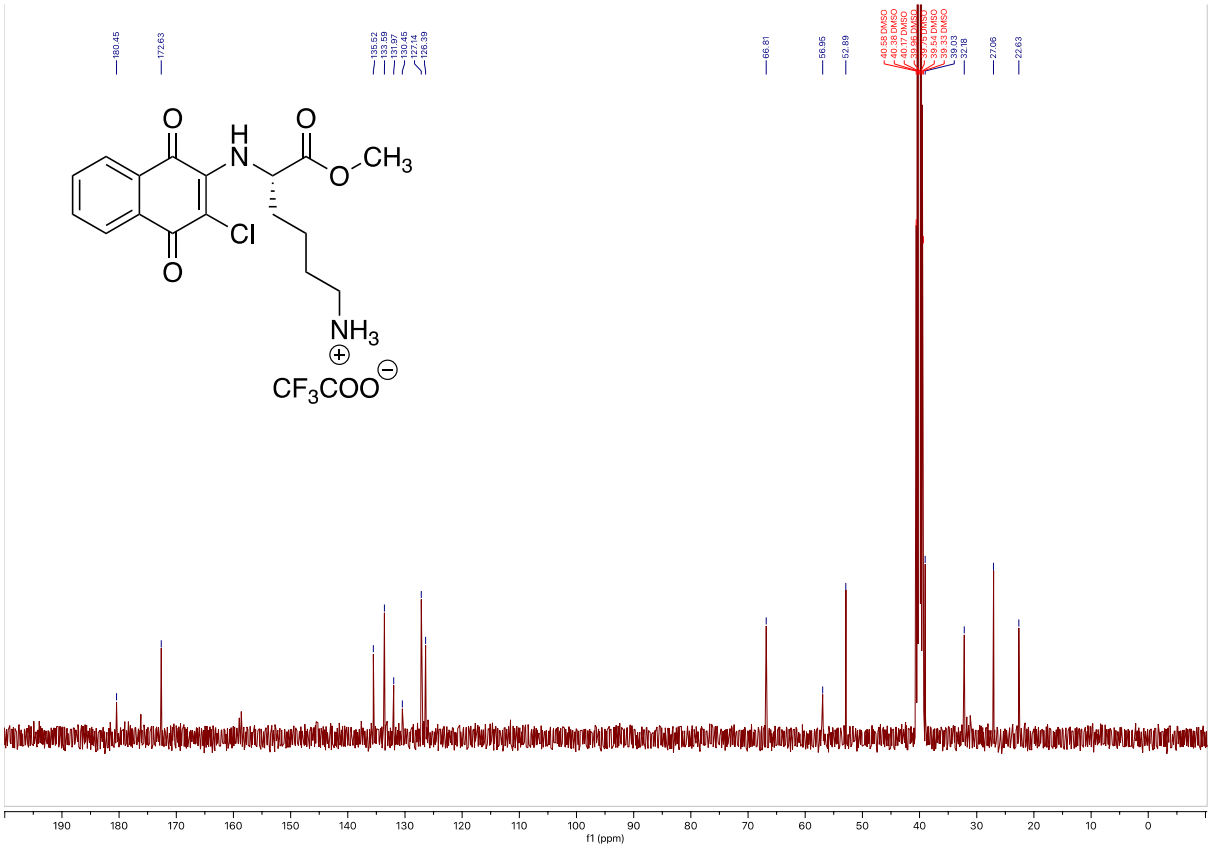
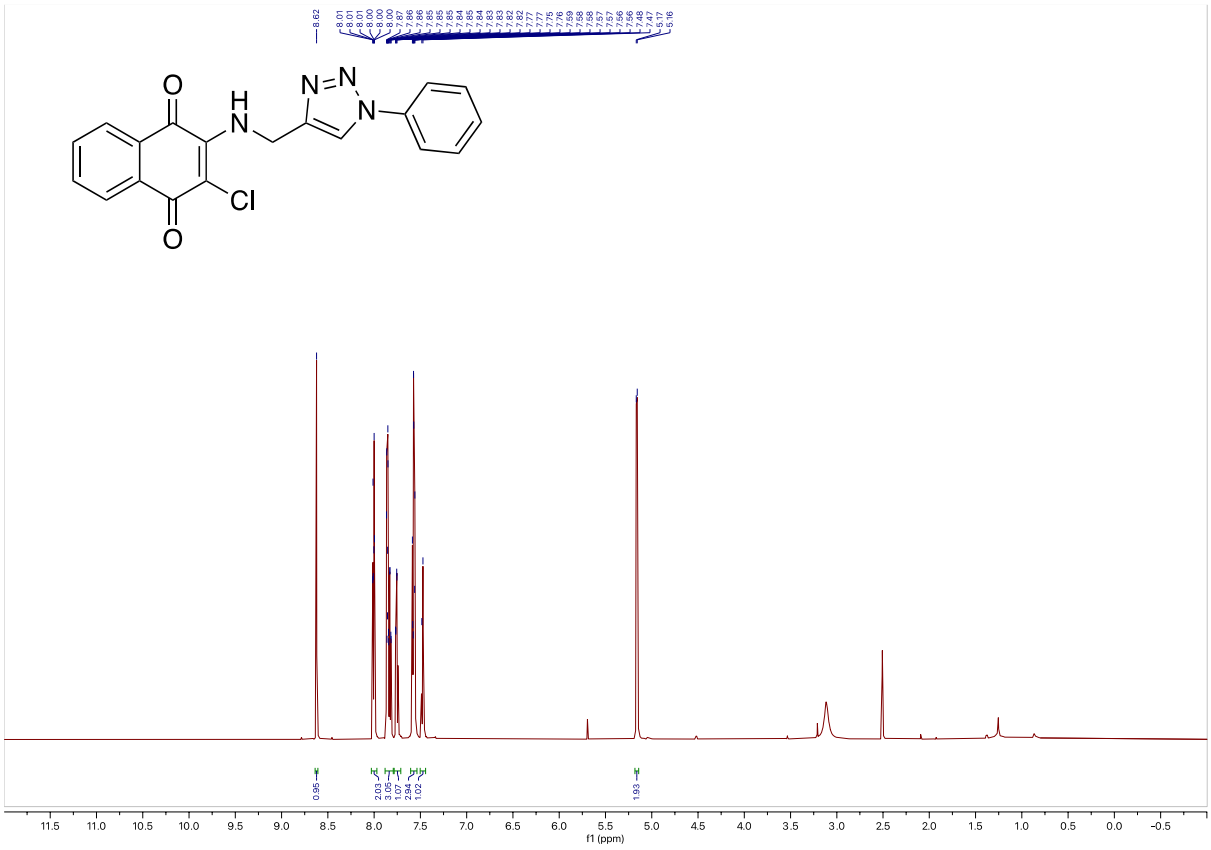
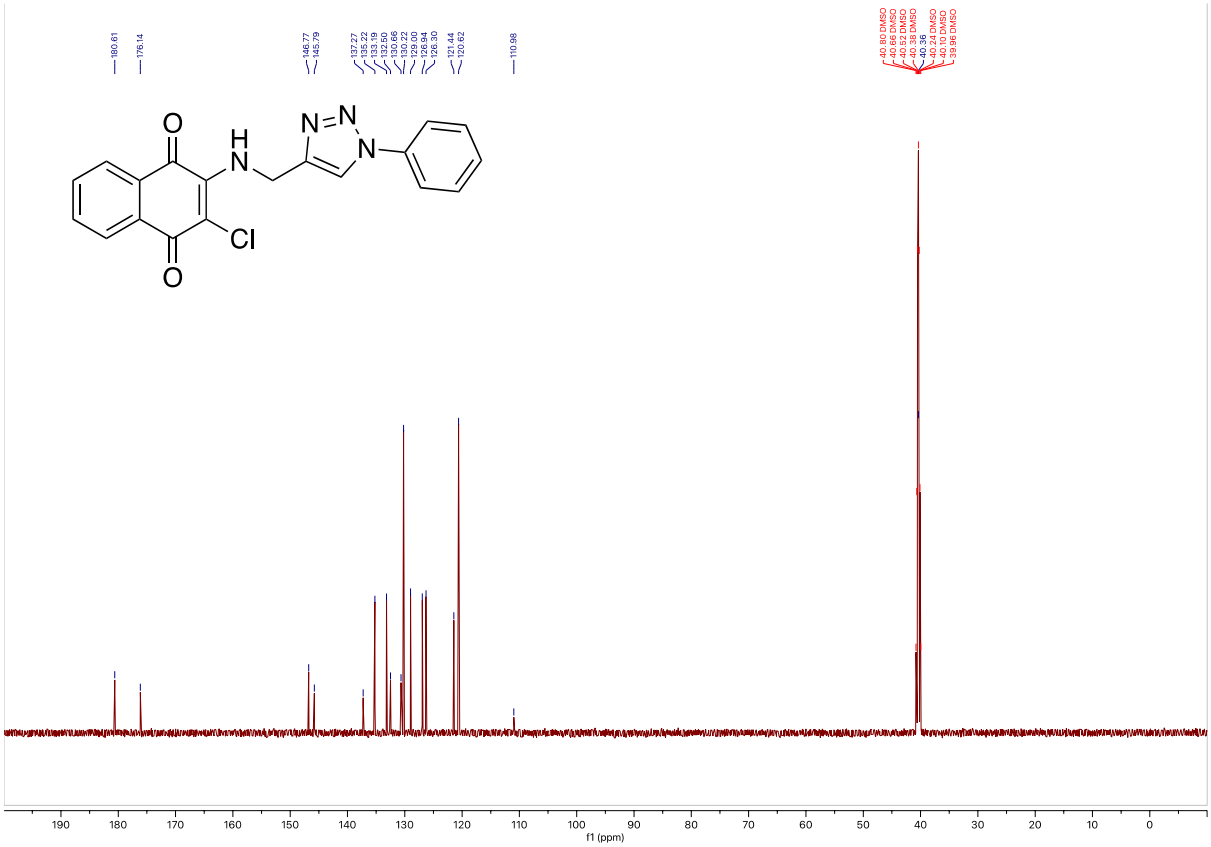

O
H
N
O
O
CH3
Cl
O
NH3
CF3COO
f1 (ppm)

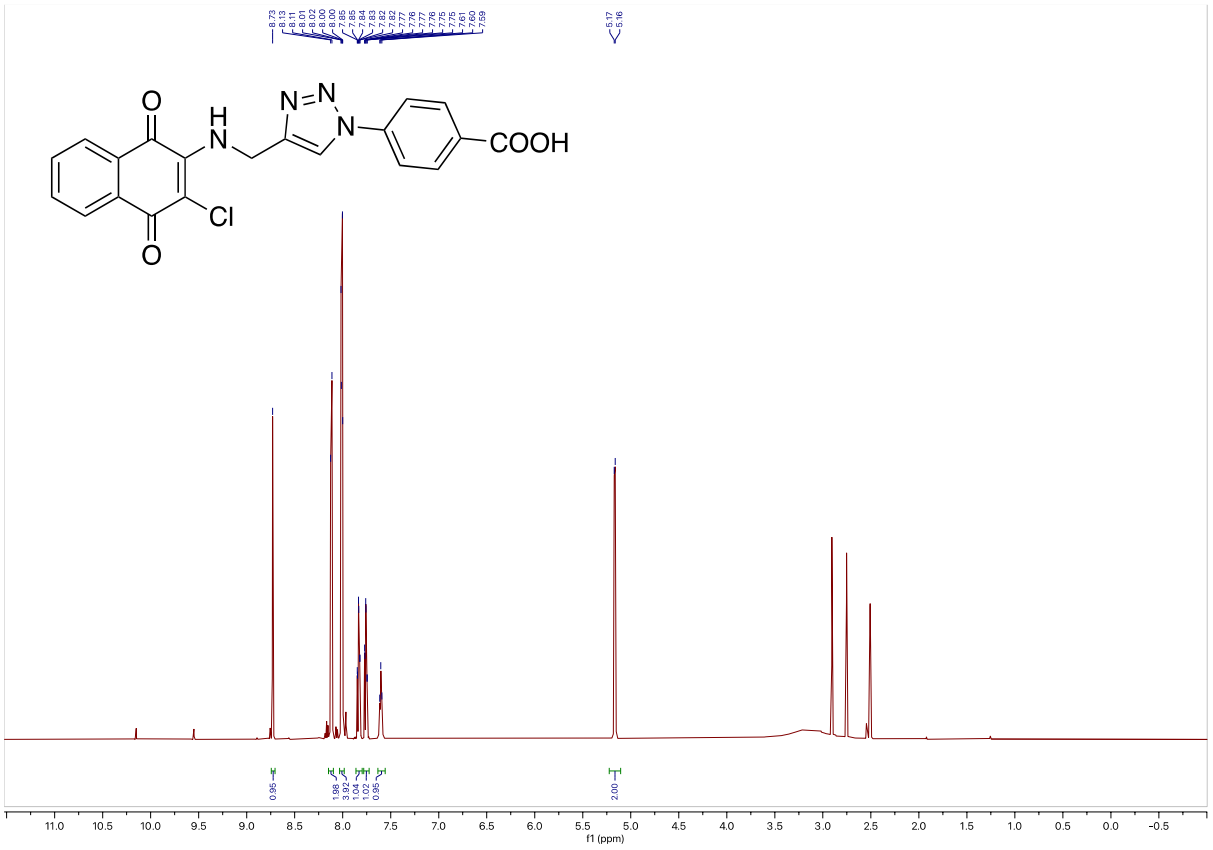

O
H
N
O
O
CH3
Cl
O
NH3
CF3COO
f1 (ppm)

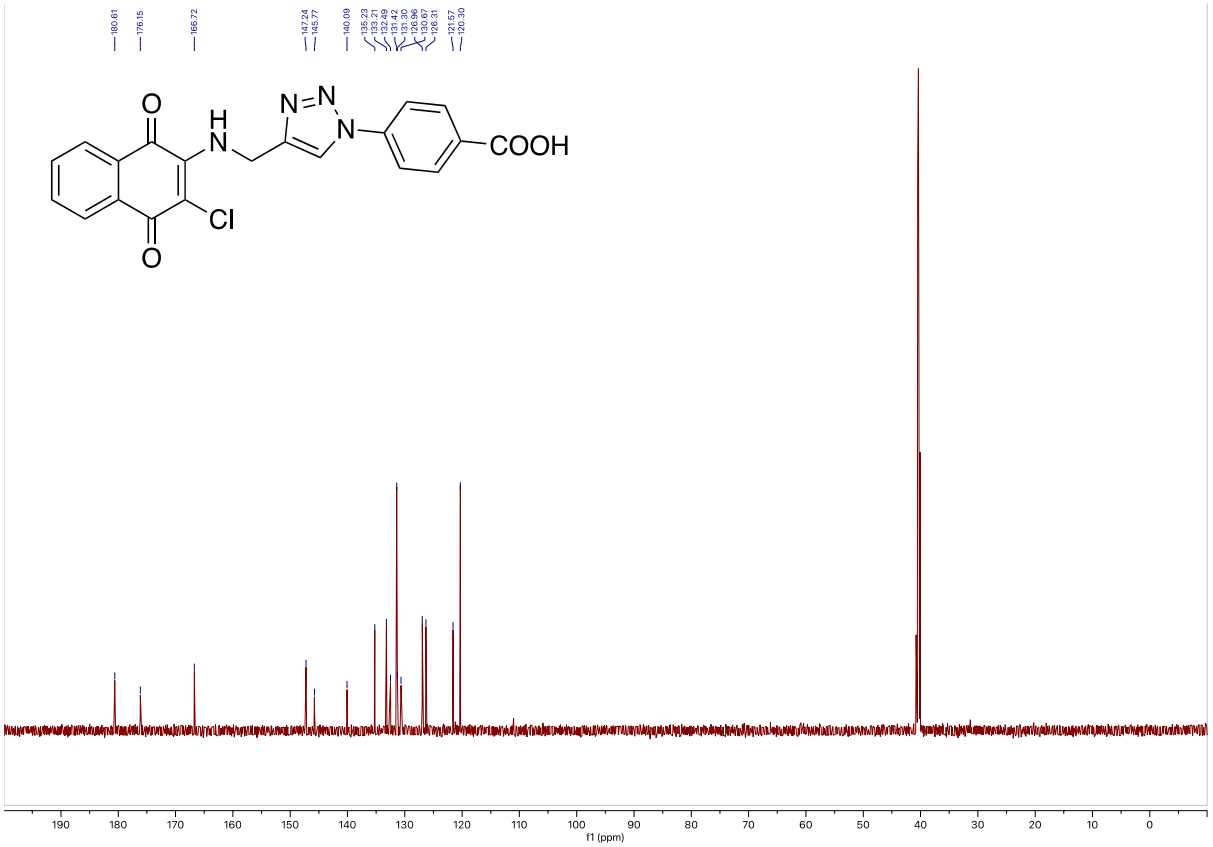
31



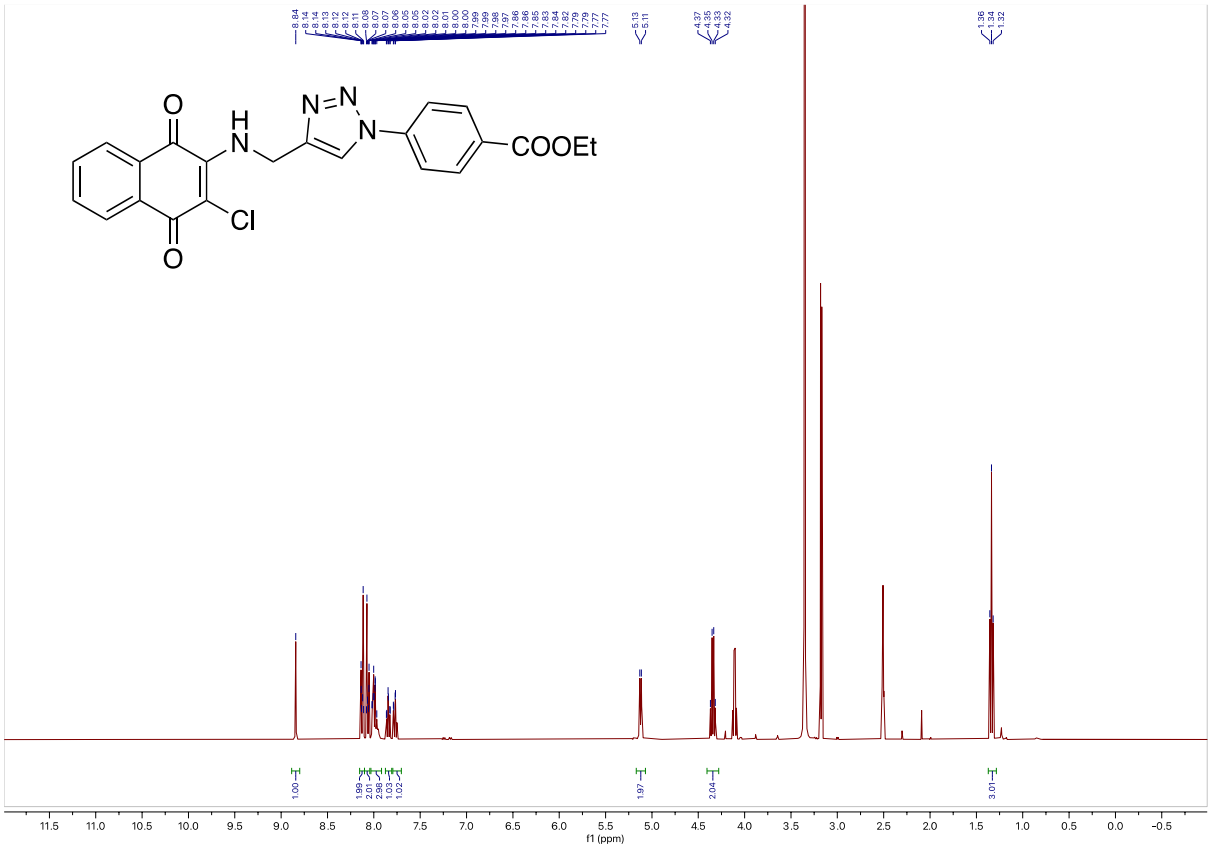


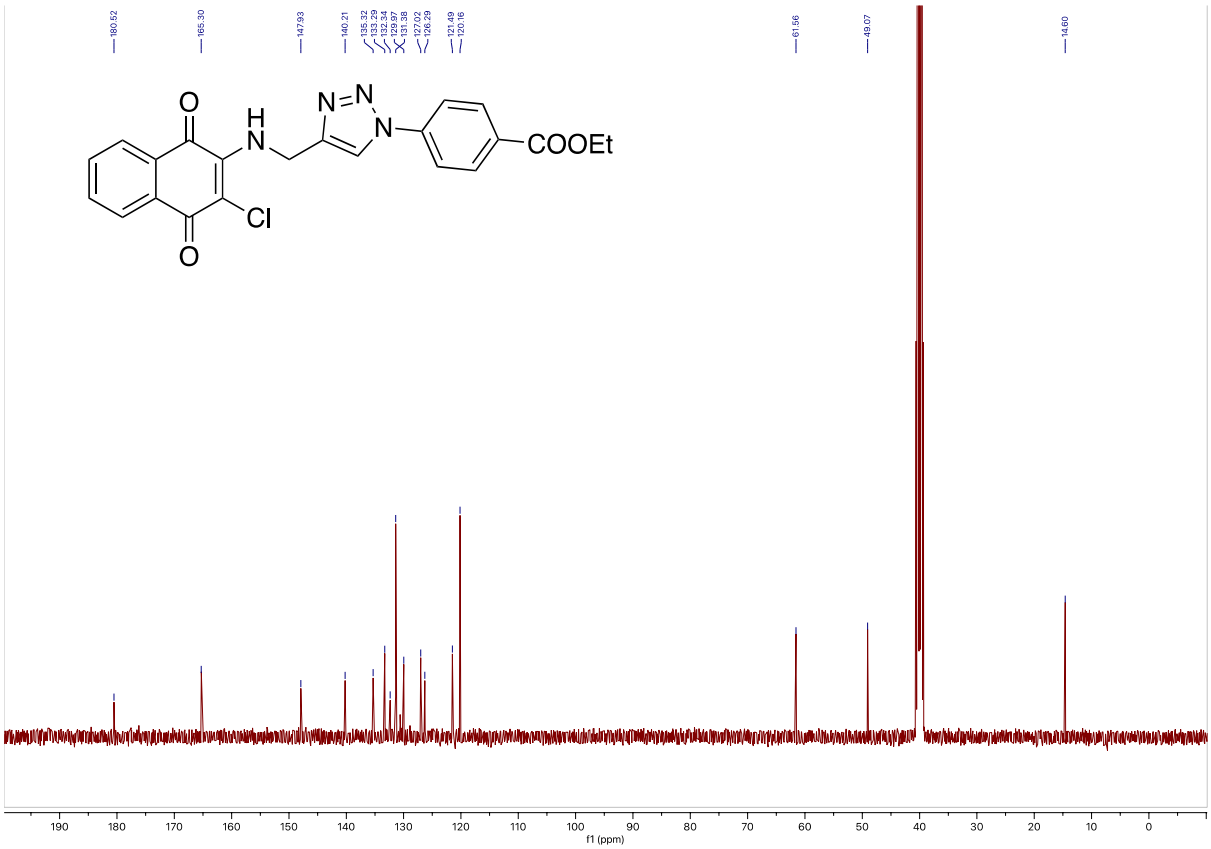
32


8.73
8.13
8.11
8.01
8.02
8.00
7.86
7.85
7.84
7.83
7.82
7.77
7.76
7.76
7.75
7.61
7.60
7.59
5.17
5.16
O
H
N
N=N
N
COOH
Cl
O
0.96
1.98
3.92
1.94
1.02
0.96
2.00
11.0
10.5
10.0
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
1.0
0.5
0.0
-0.5
f1 (ppm)


180.61
176.15
166.72
147.24
145.77
140.09
135.23
133.21
132.49
131.42
131.30
129.66
130.67
126.31
121.57
120.30
O
H
N
N=N
N
COOH
Cl
O
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

33





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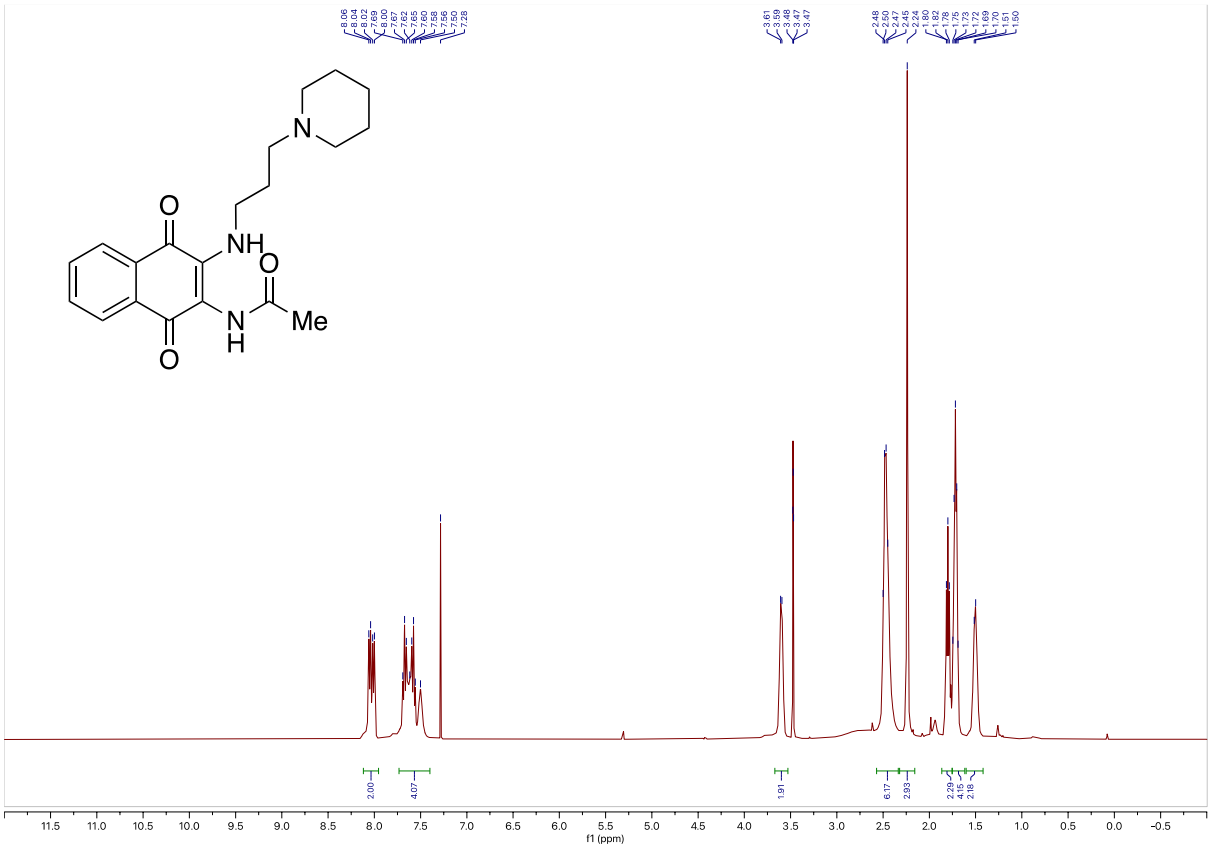
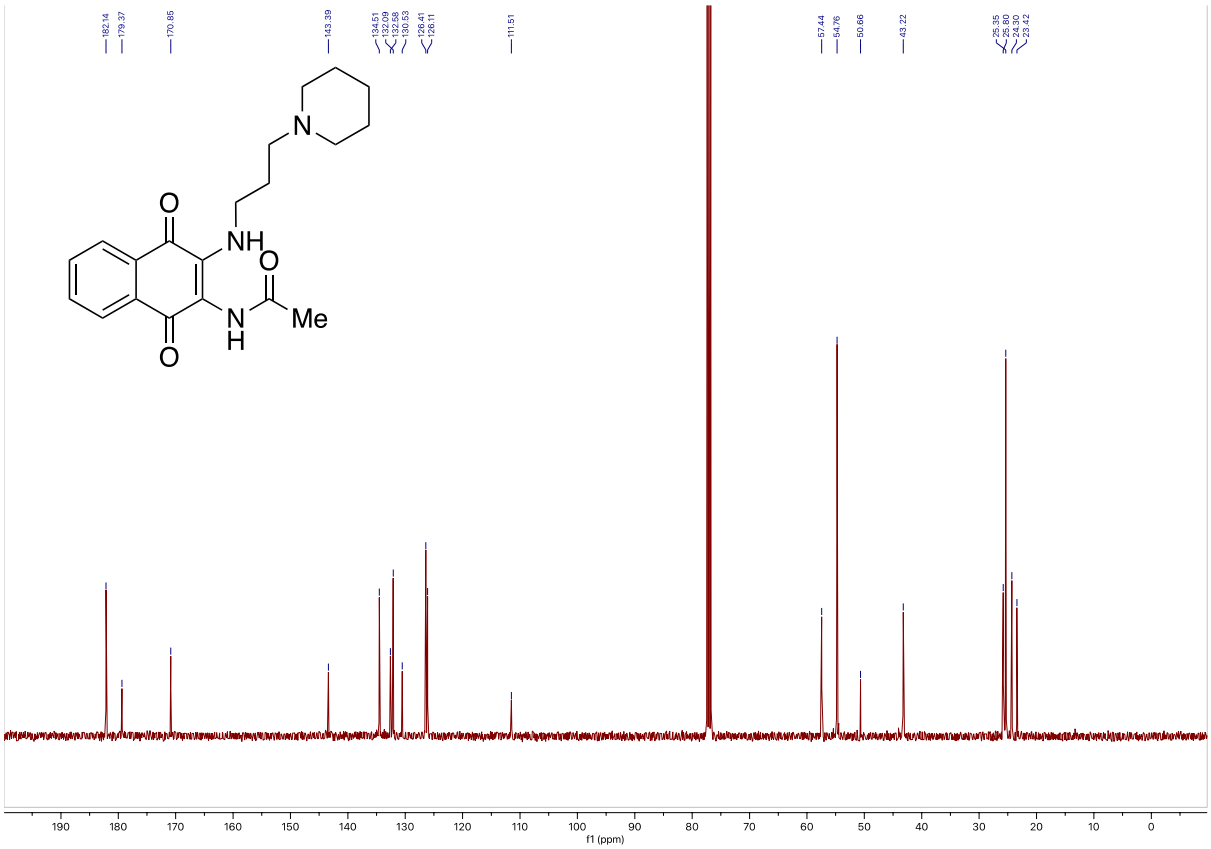
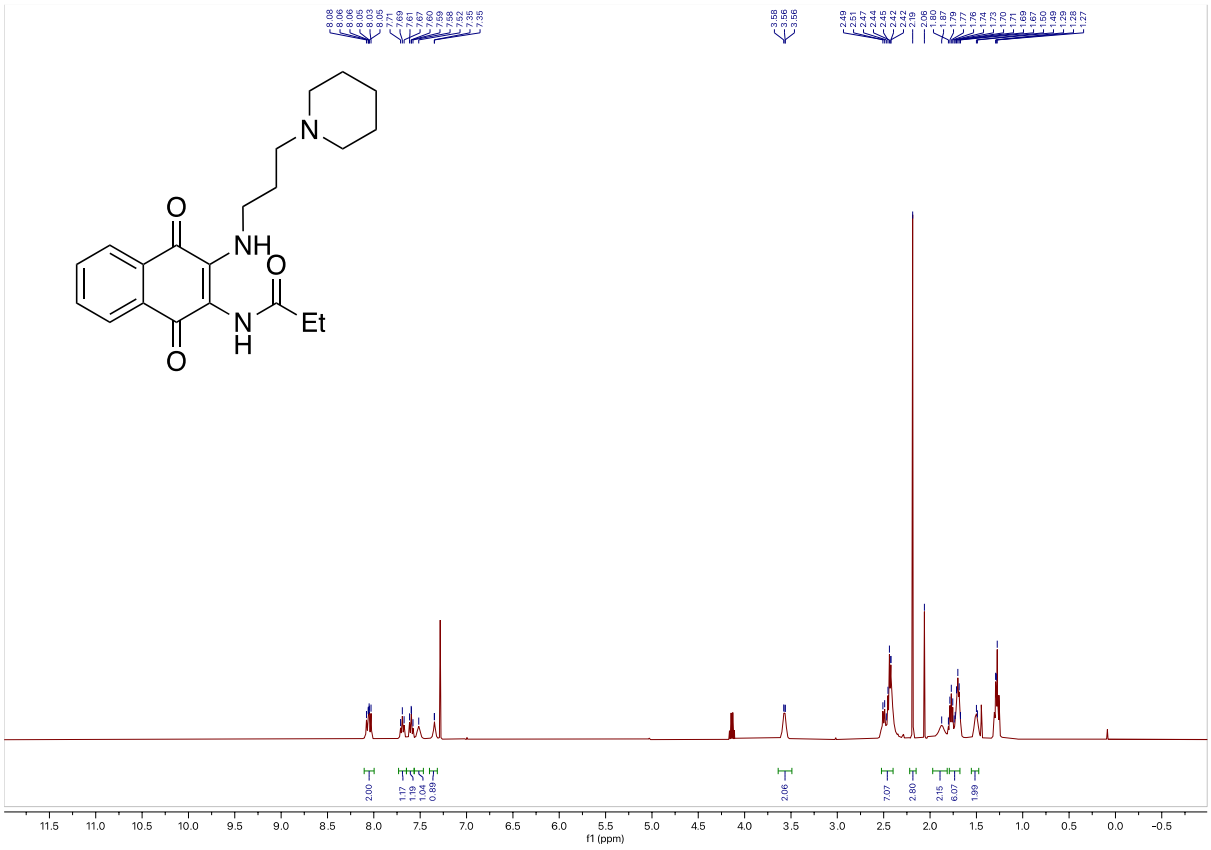
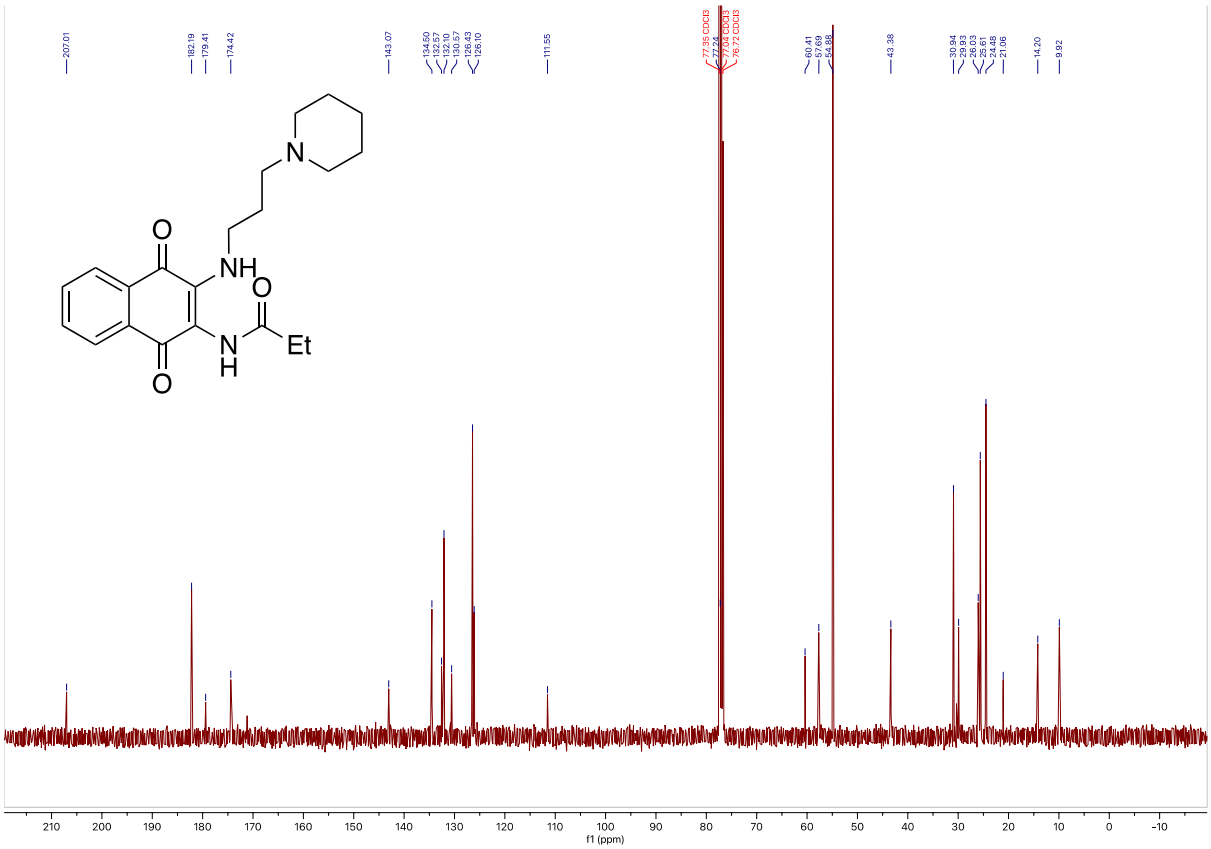

8.06
8.04
8.02
7.69
7.67
7.67
7.67
7.62
7.60
7.60
7.58
7.56
7.30
7.28
3.61
3.59
3.48
3.47
3.47
2.48
2.50
2.47
2.45
2.44
1.80
1.82
1.78
1.75
1.73
1.72
1.69
1.70
1.51
1.50
O
NH
O
N
H
N
Me
O
2.00
4.07
1.91
6.17
2.93
2.29
4.45
2.18
f1 (ppm)


182.14
179.37
170.85
143.39
134.51
132.69
132.58
130.53
126.41
126.11
111.51
57.44
54.76
50.66
43.22
25.35
25.80
24.70
23.42
O
NH
O
N
H
N
Me
O
f1 (ppm)

40





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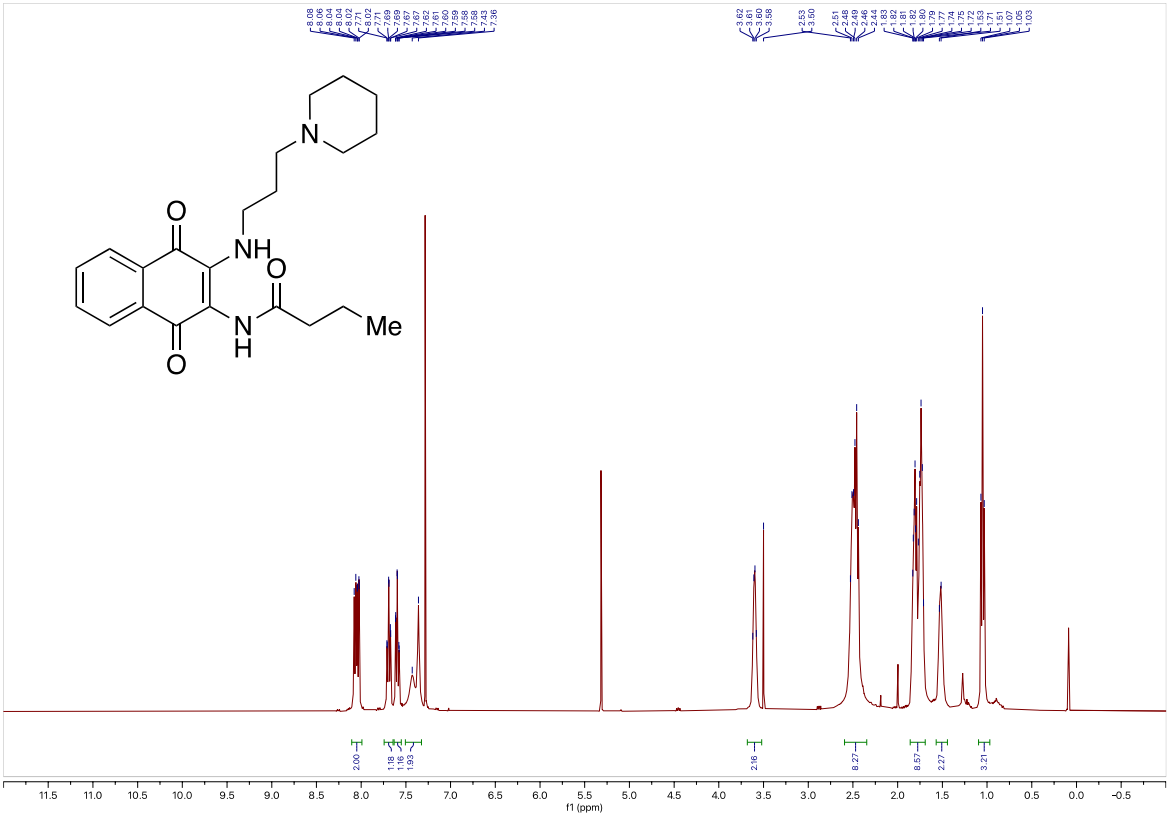

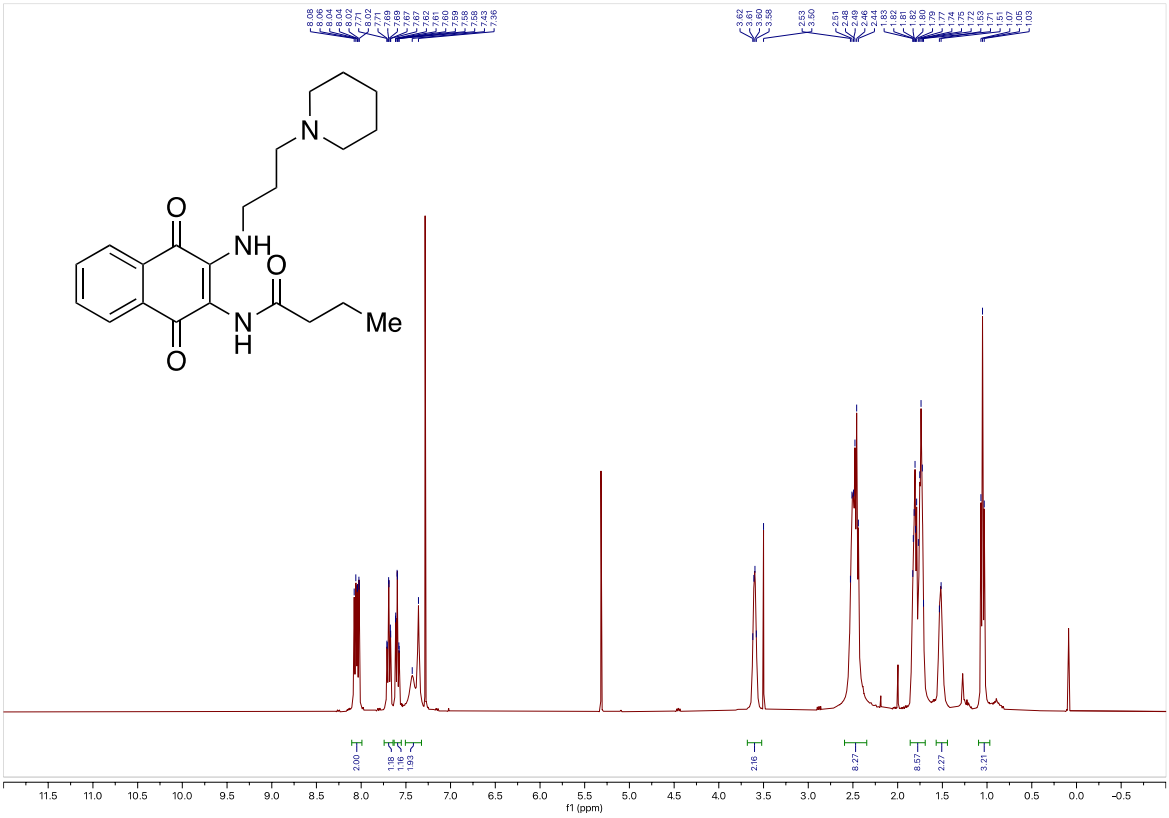

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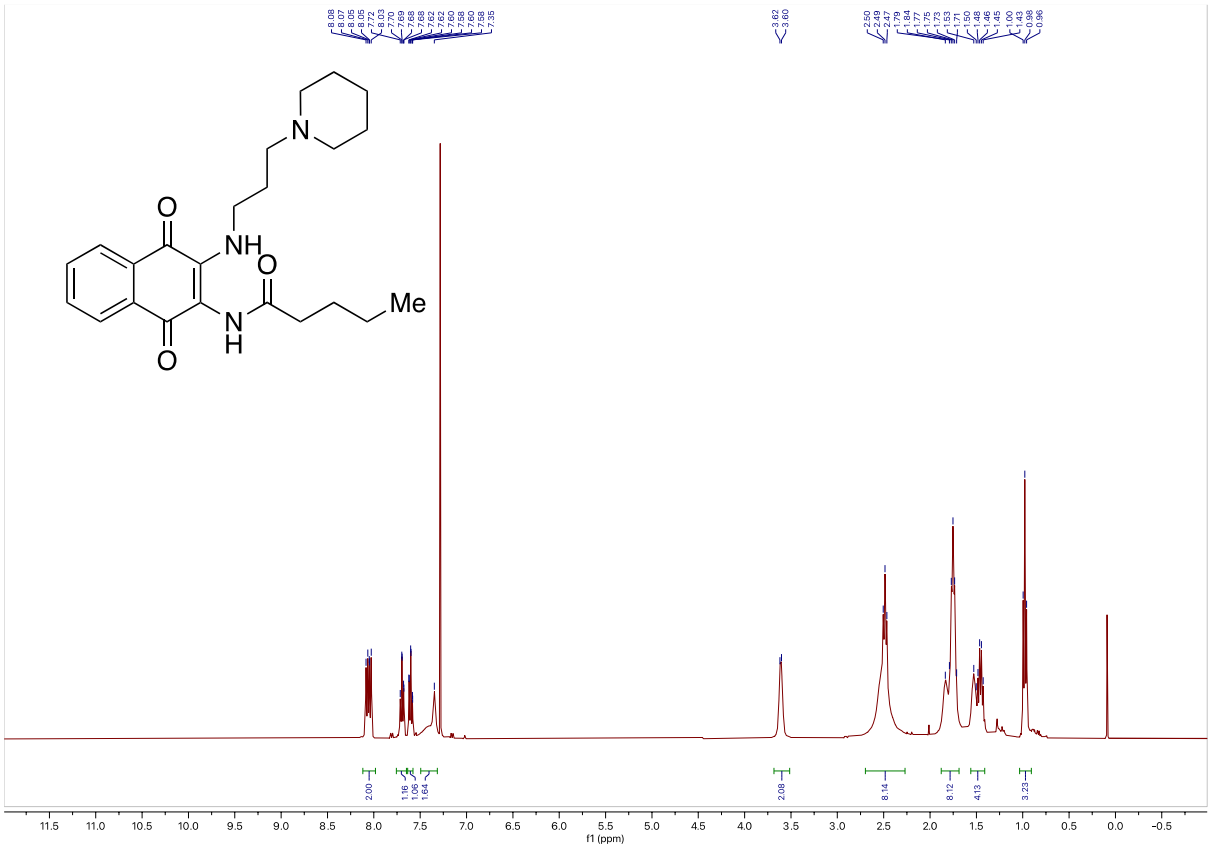
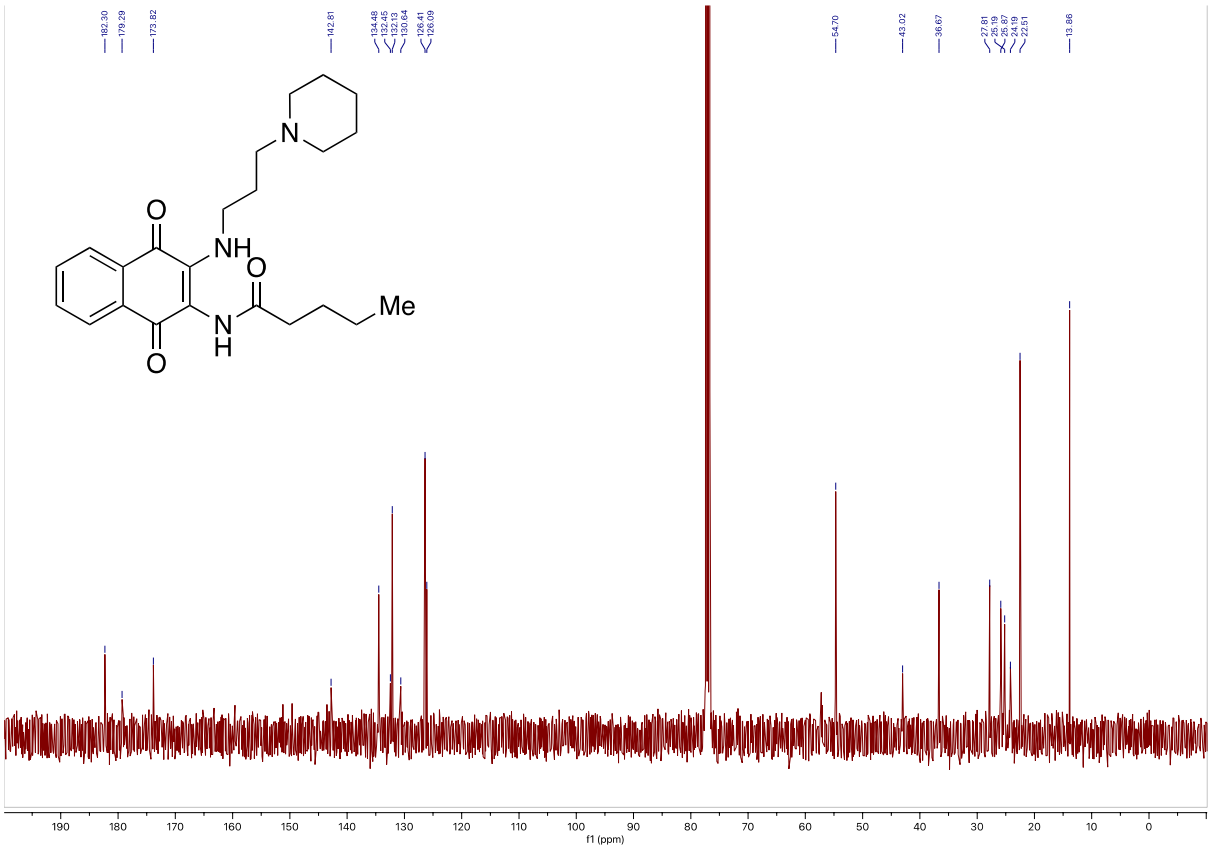
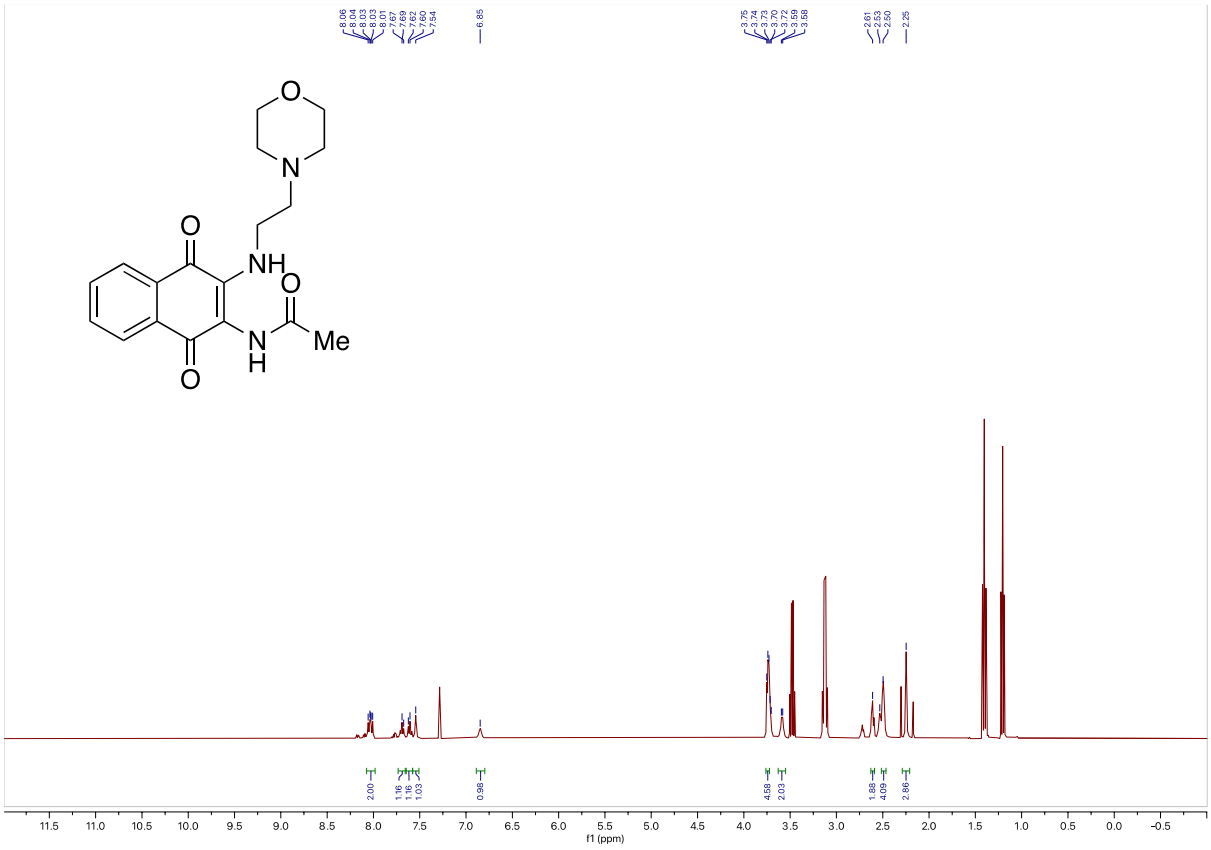




43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8.04
8.03
8.03
8.01
7.67
7.89
7.66
7.60
7.54
6.85
3.75
3.74
3.73
3.70
3.72
3.69
3.68
2.61
2.53
2.50
2.25
O
N
O
NH
O
N
H
Me
O
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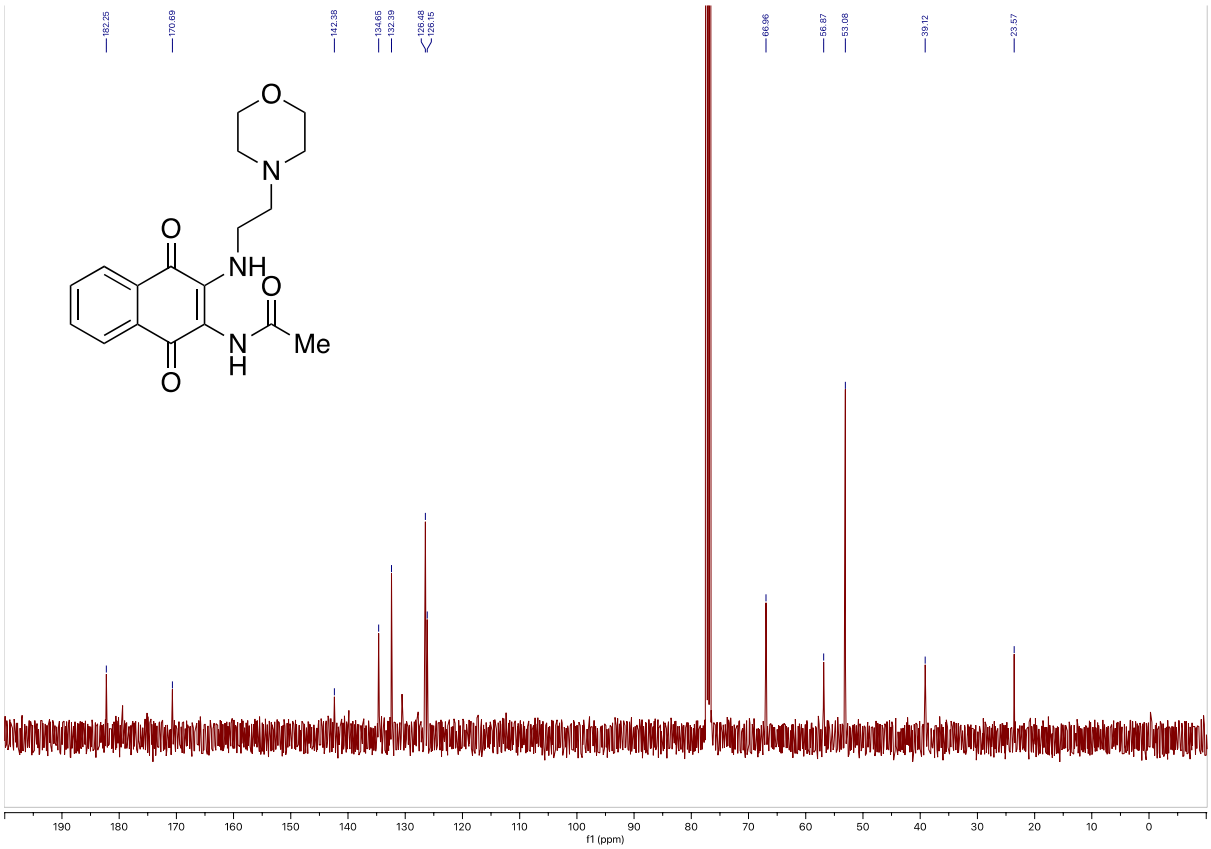
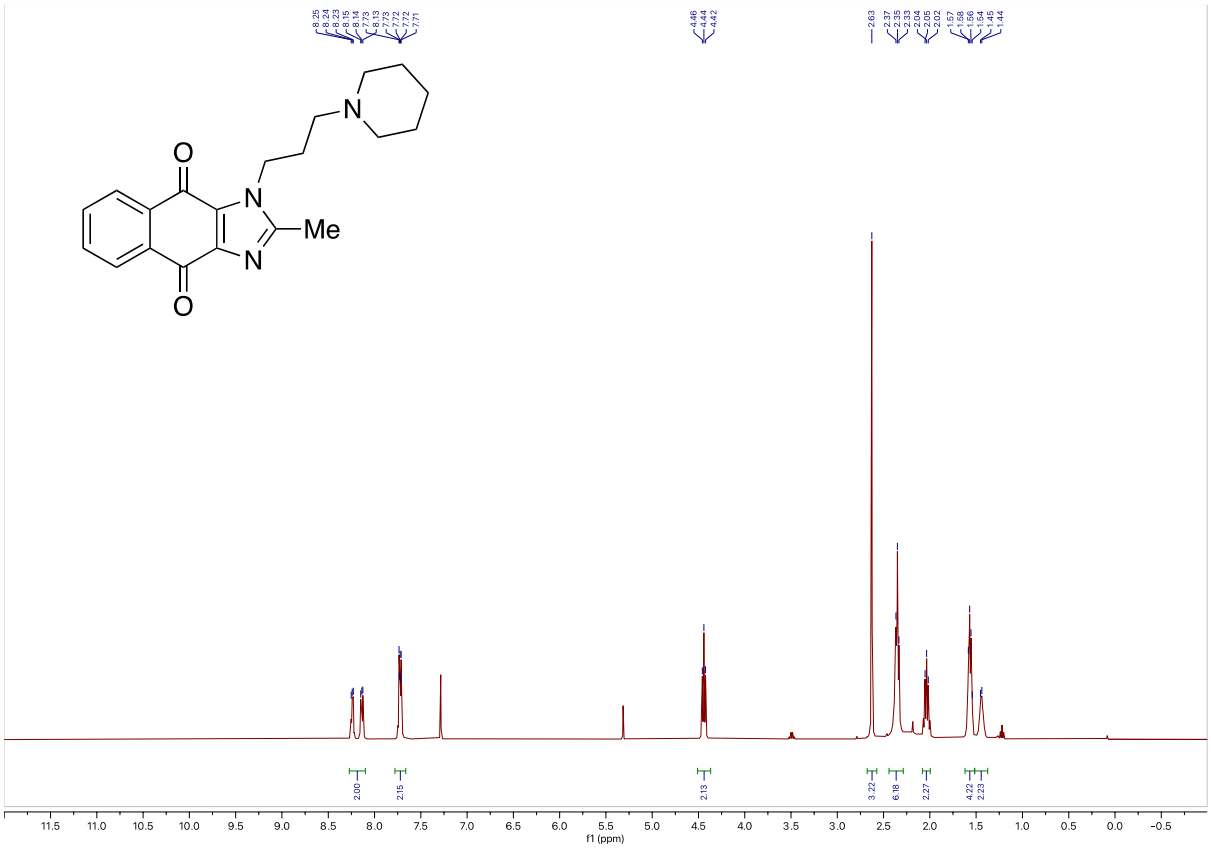
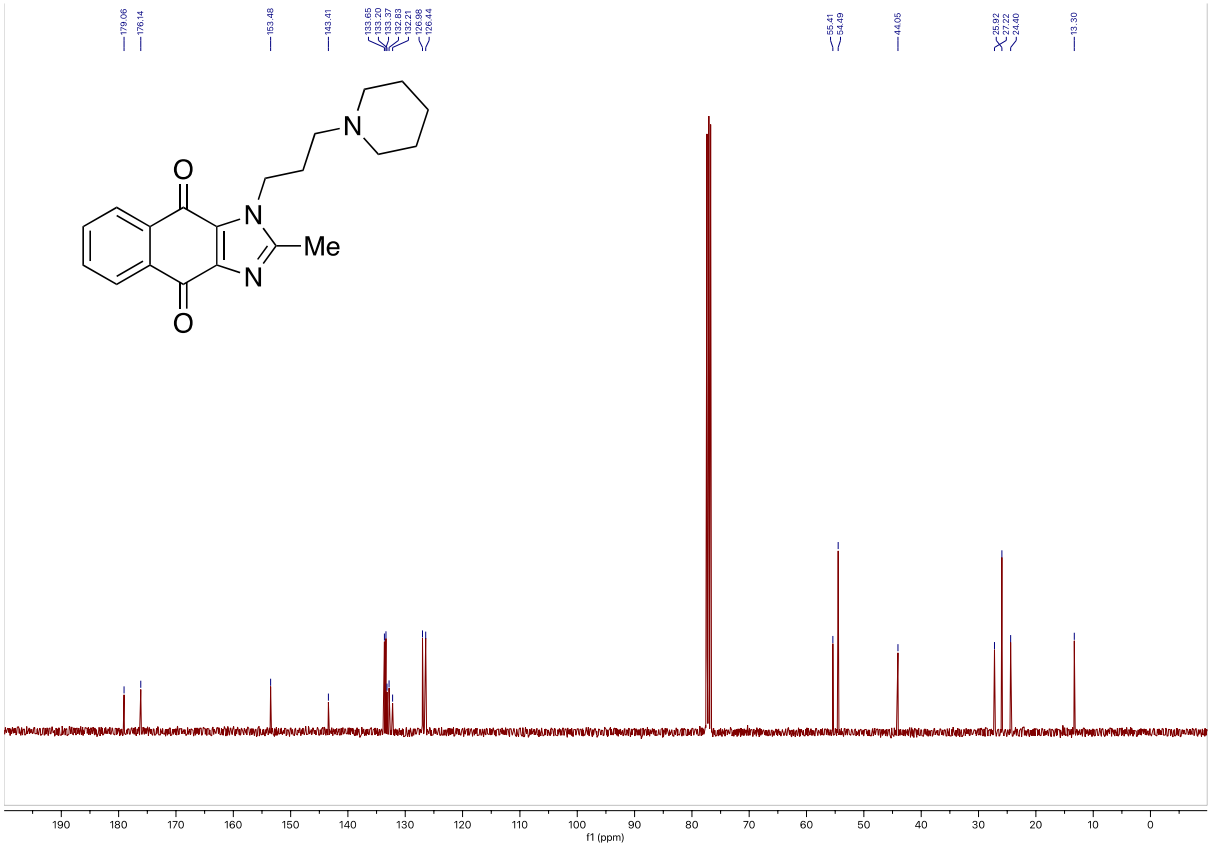
1.16
1.16
1.03
0.98
4.58
2.03
1.88
4.09
2.86
f1 (ppm)


182.25
170.69
142.38
134.65
132.39
126.48
126.15
66.96
56.87
53.08
39.12
23.57
O
N
O
NH
O
N
H
Me
O
f1 (ppm)

44


8.25
8.24
8.23
8.15
8.14
7.73
8.13
7.72
7.72
7.72
7.71
4.46
4.44
4.42
2.63
2.37
2.35
2.33
2.04
2.05
2.02
1.57
1.56
1.56
1.54
1.45
1.44
2.00
2.15
2.13
3.22
6.18
2.27
4.22
2.23
11.5 11.0 10.5 10.0 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 1.0 0.5 0.0 -0.5
f1 (ppm)
O
N
N
Me
N
O
O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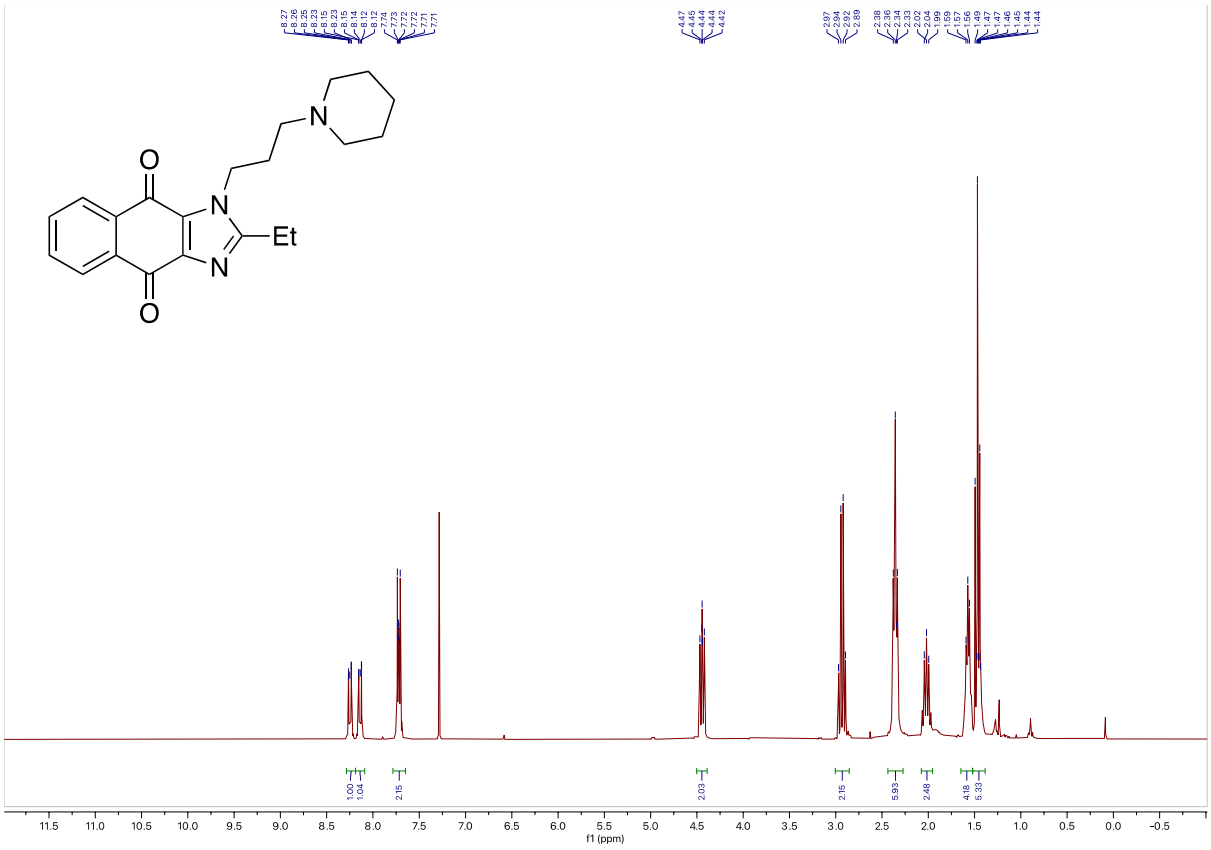
176.14
153.48
143.41
133.65
133.20
133.37
132.69
132.21
126.98
126.44
55.41
54.49
44.05
26.92
27.22
24.40
13.30
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)
O
N
N
Me
N
O
O

45


8.27
8.26
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8.23
8.23
8.15
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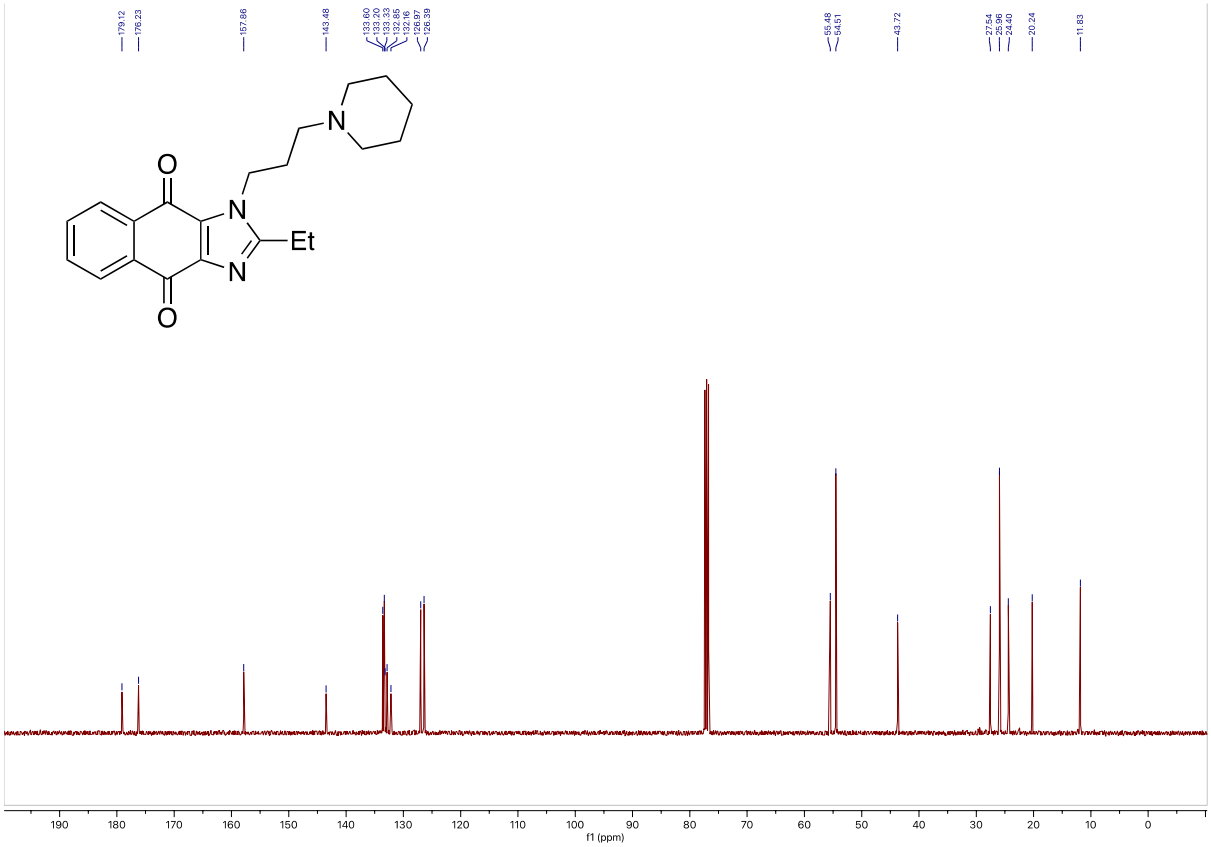
8.12
8.12
7.73
7.72
7.72
7.71
7.71
4.47
4.46
4.44
4.44
4.42
2.97
2.94
2.92
2.89
2.38
2.36
2.34
2.33
2.02
2.02
2.04
1.99
1.59
1.57
1.56
1.49
1.47
1.47
1.46
1.45
1.44
1.44
O
N
N
Et
N
O
1.00
1.04
2.15
2.03
2.15
5.93
2.48
4.18
5.33
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)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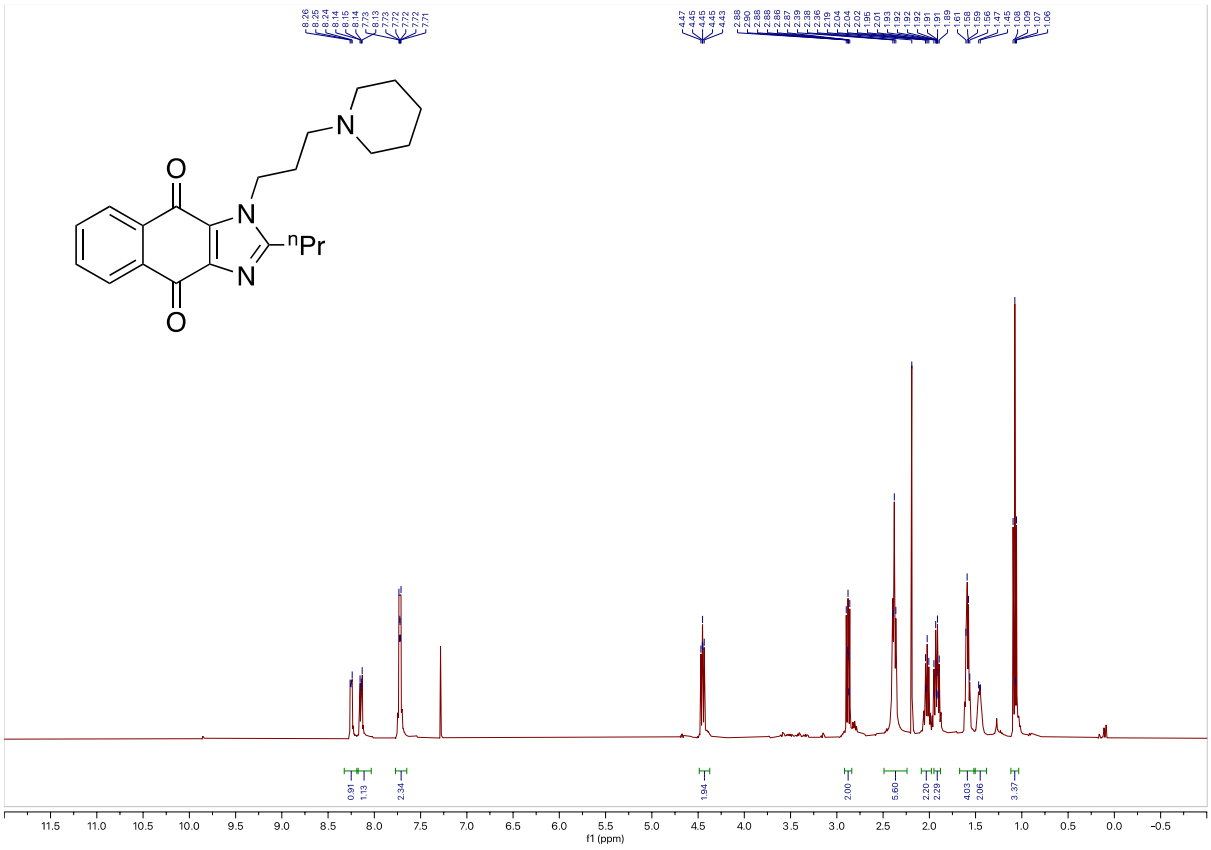
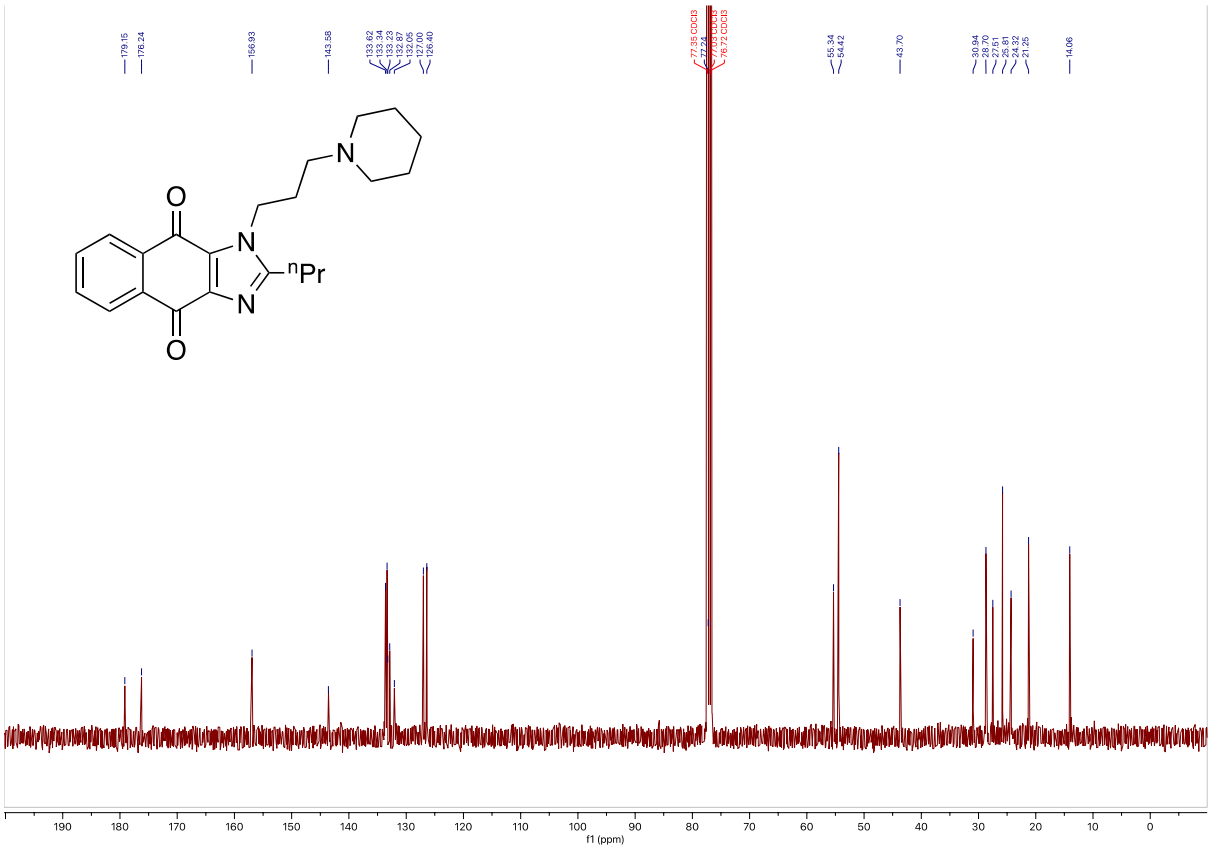
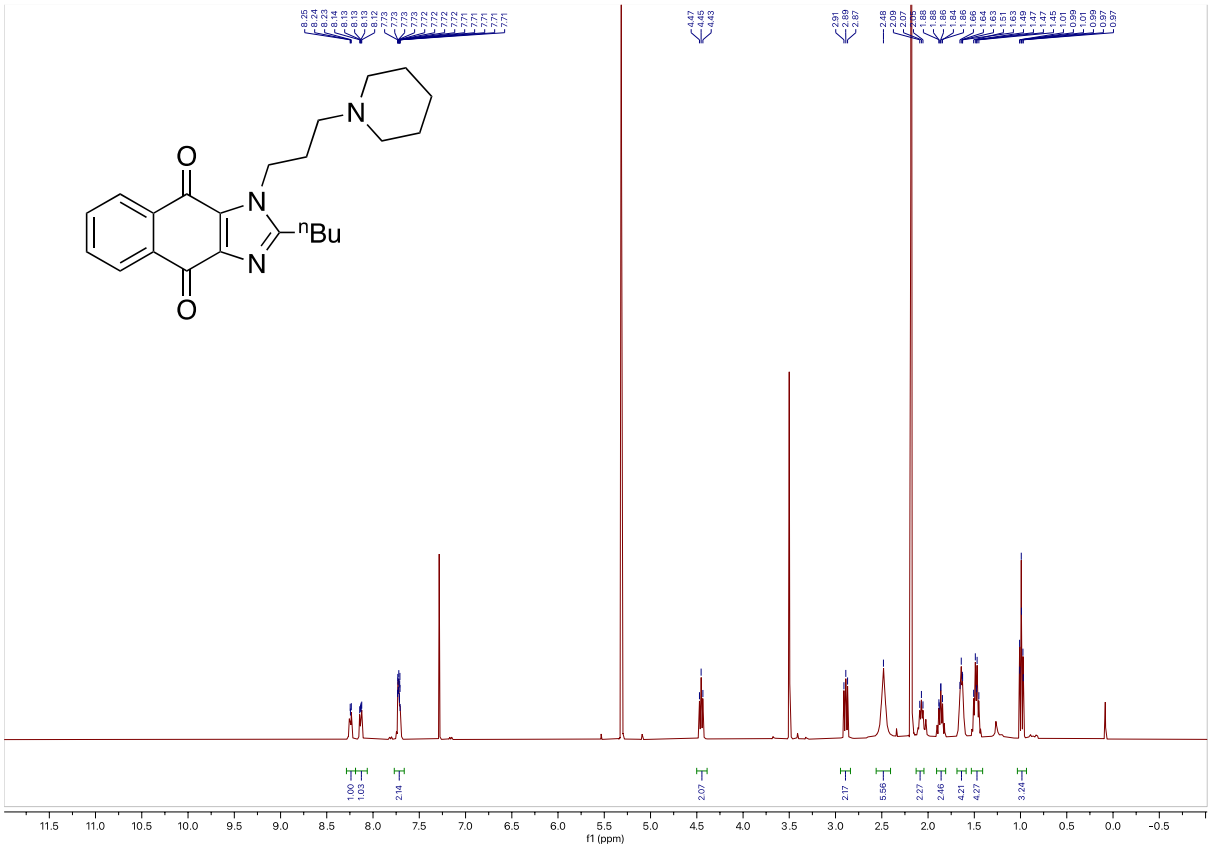
176.23
157.86
143.48
133.60
133.20
132.93
132.85
132.16
126.97
126.39
55.48
54.51
43.72
27.54
25.96
24.40
20.24
11.83
N
O
N
Et
N
O
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

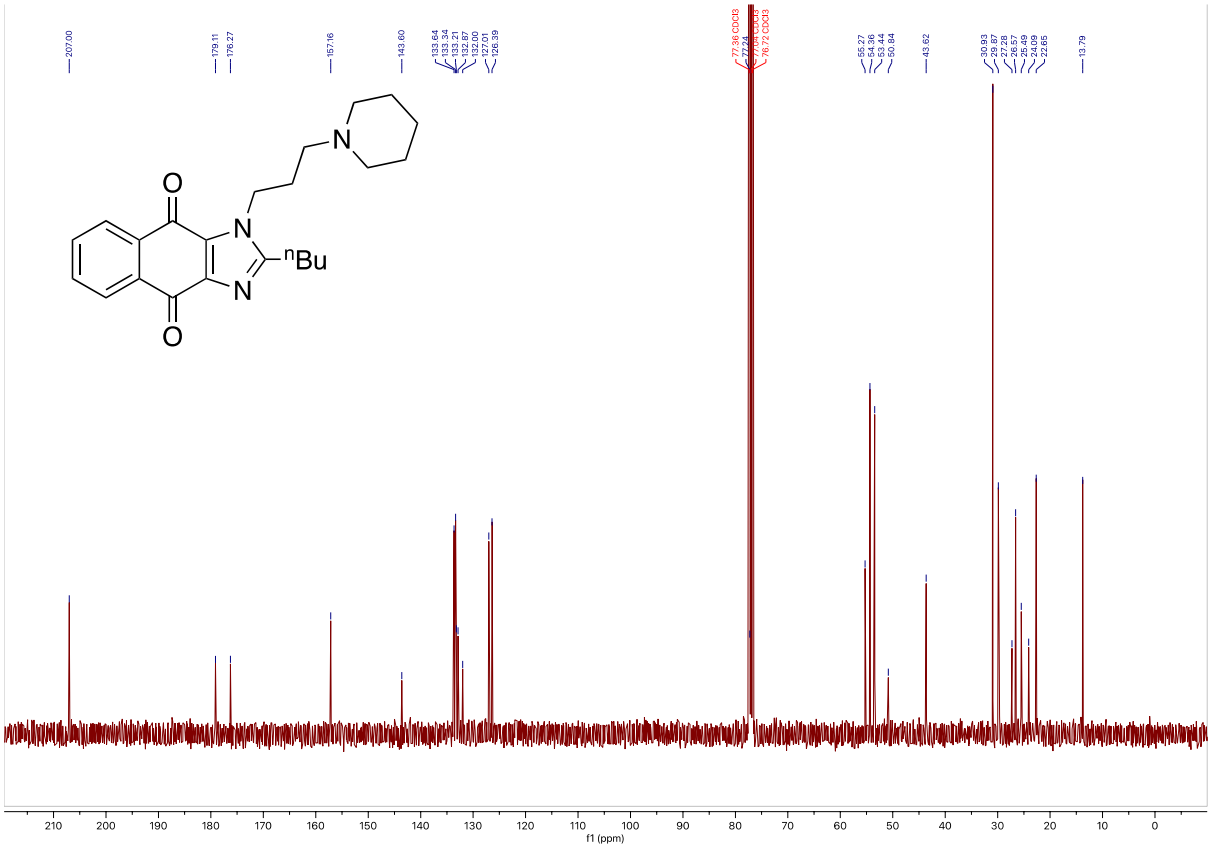
46



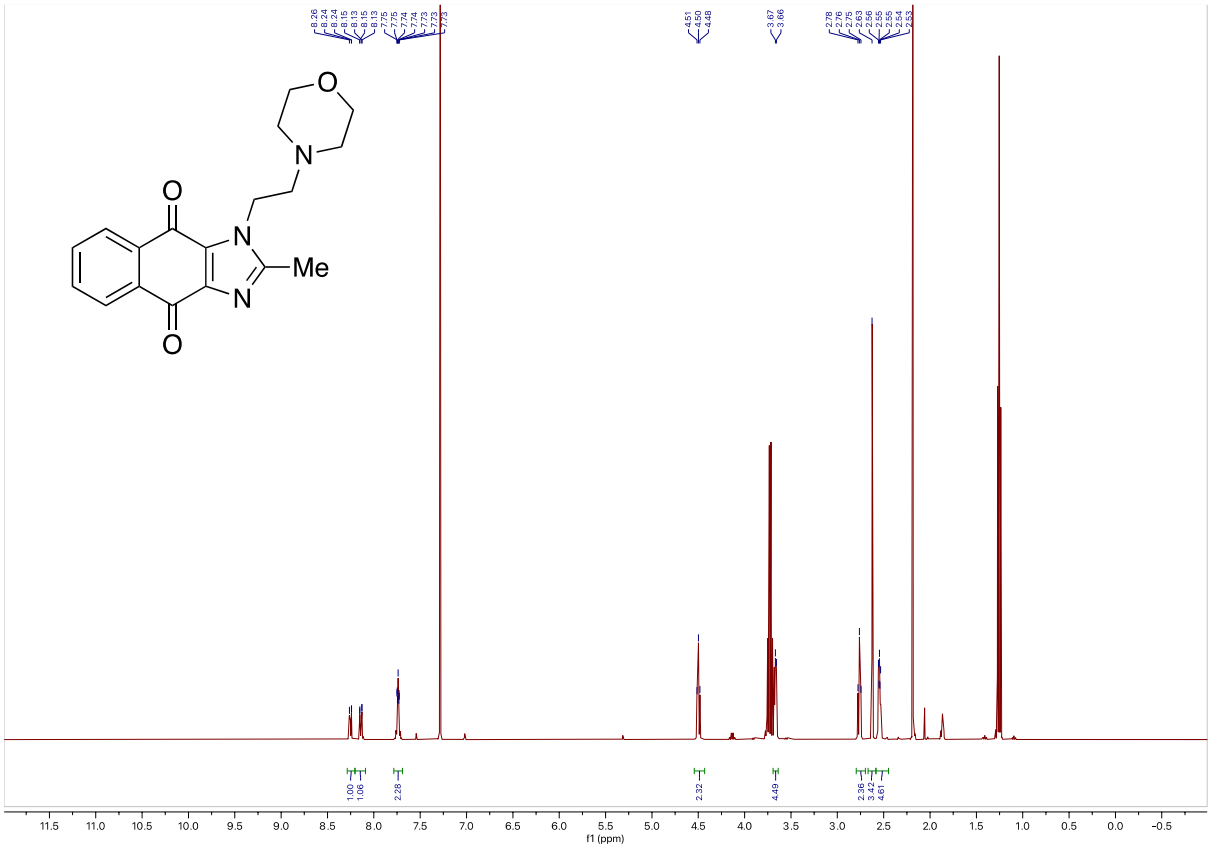


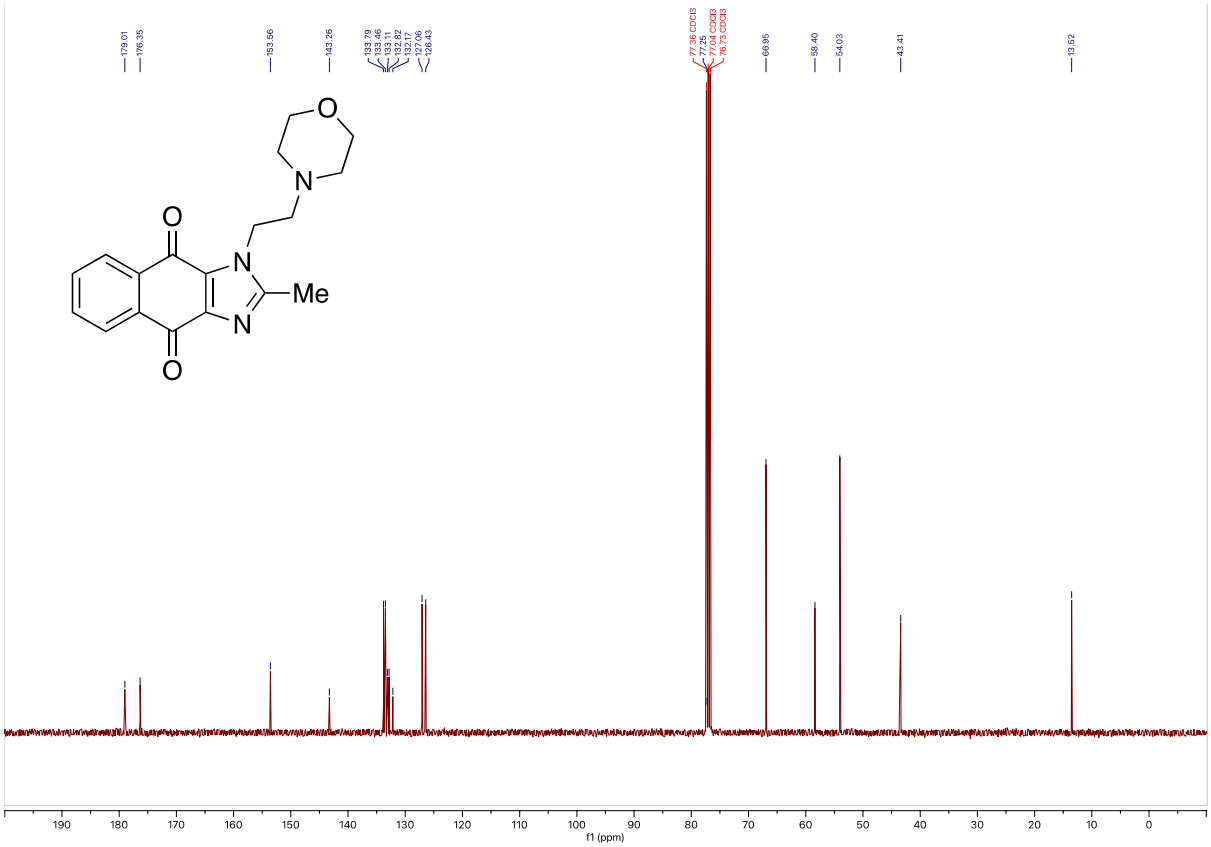
47





48





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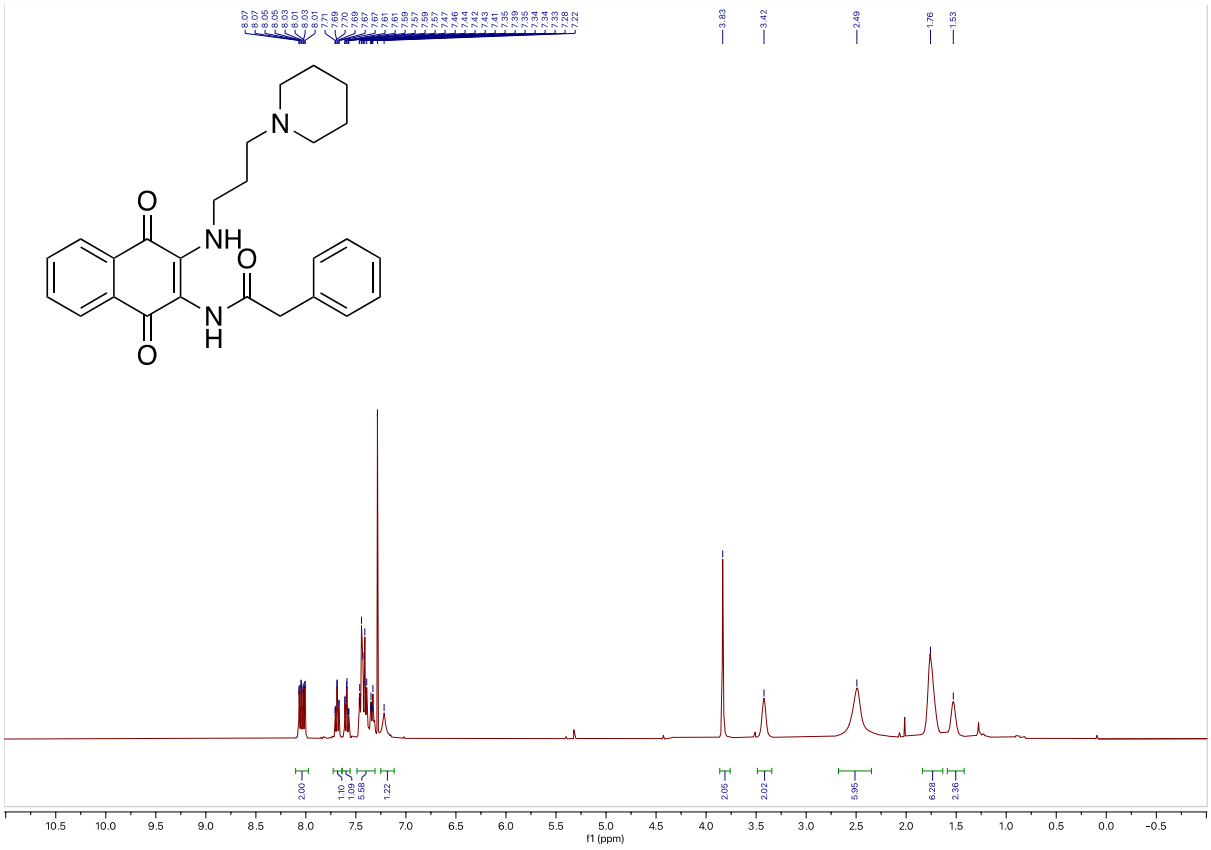
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8.07
8.05
8.05
8.03
8.03
8.01
8.01
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7.67
7.67
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7.61
7.61
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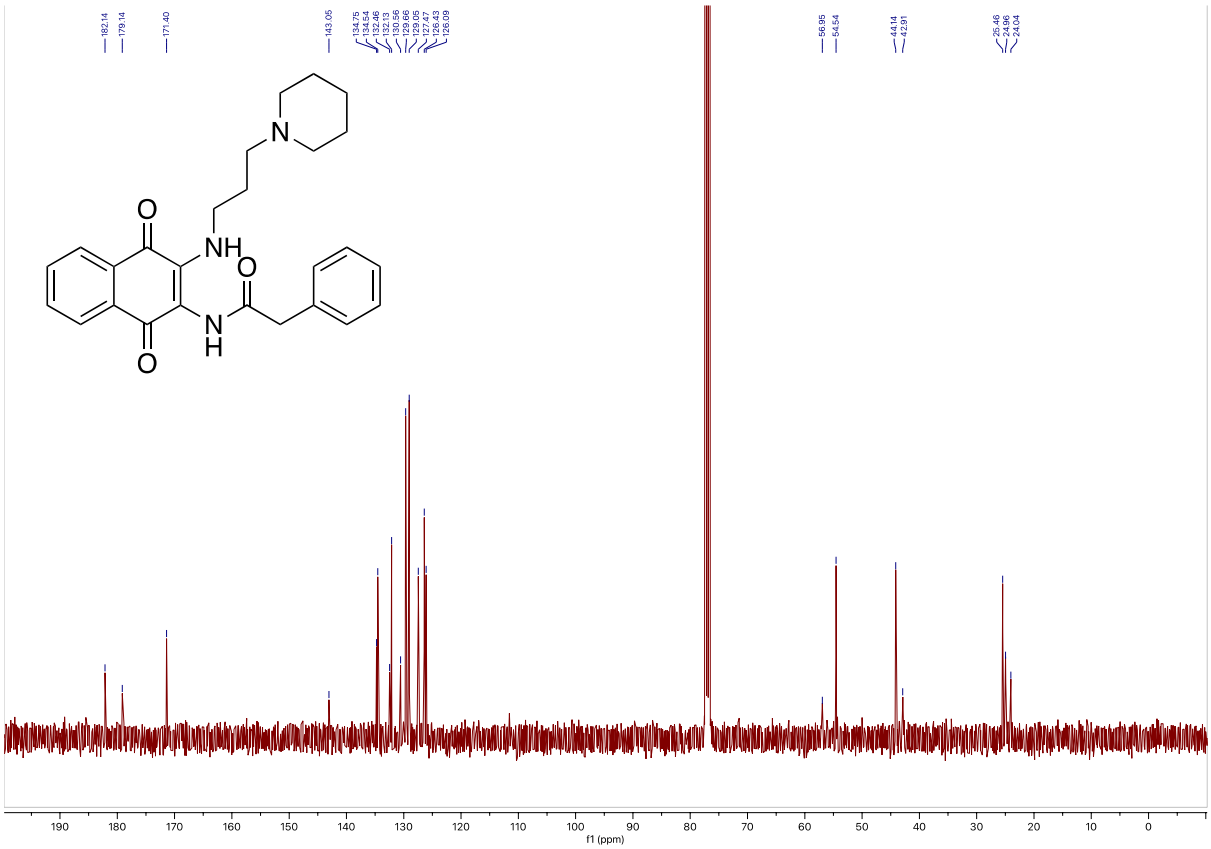
7.57
7.57
7.47
7.46
7.44
7.42
7.43
7.41
7.35
7.39
7.35
7.34
7.33
7.33
7.28
7.22
3.83
3.42
2.49
1.76
1.53
2.00
1.10
1.09
5.58
1.22
2.05
2.02
5.95
6.28
2.36
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)
O
NH
O
N
H
O
N
O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171.40
143.06
134.75
134.54
132.46
132.33
130.56
129.66
129.05
127.47
126.43
126.09
56.95
54.54
44.14
42.91
25.46
24.96
24.04
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)
O
NH
O
N
H
O
N
O

53