

Supporting Information

Synthesis and preliminary studies of ¹¹C-labeled tetrahydro-1,7-naphthyridine-2-carboxamides for PET imaging of metabotropic glutamate receptor 2

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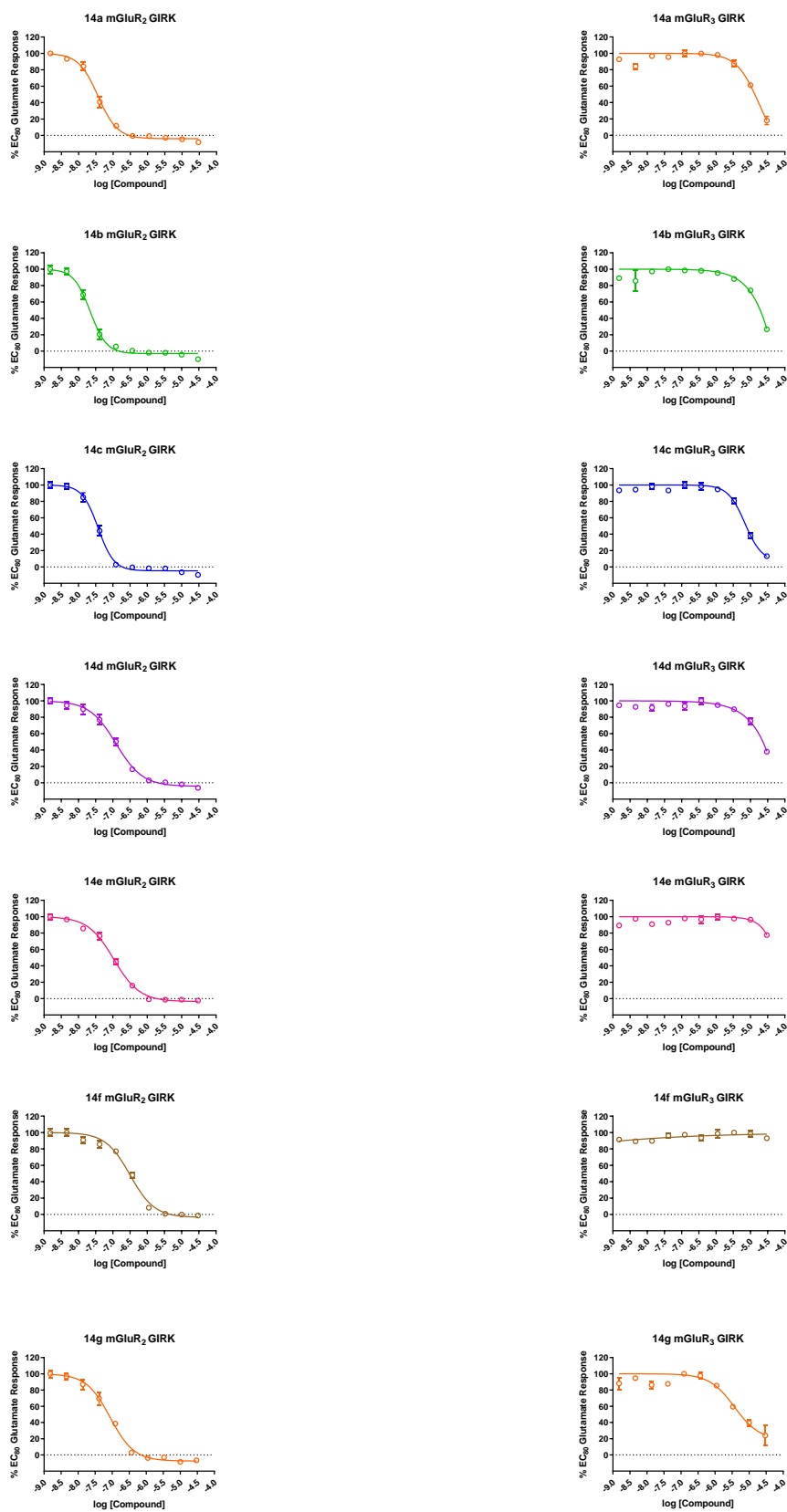
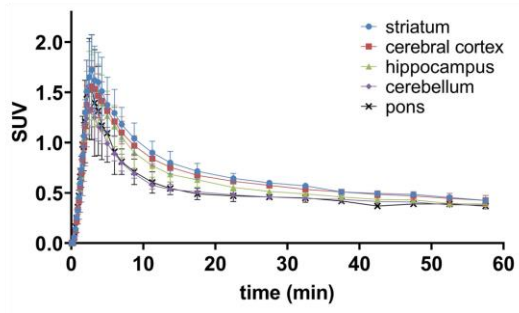


Figure S1. Concentration–response curves of mGlu₂ NAMs compounds **14a-14g** in mGlu₂ GIRK or mGlu₃ GIRK functional assays.

A. Baseline studies of [¹¹C]14a



B. Baseline studies with pretreatment of 14a

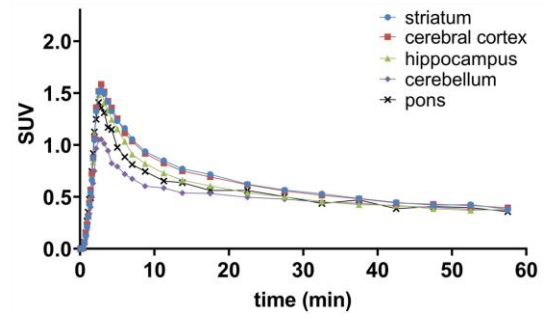


Figure S2. Time-activity curves of [¹¹C]14a in rat brains. “Blocking conditions: 14a (1 mg/kg), 30 min *i.v.* before radioligand injection.

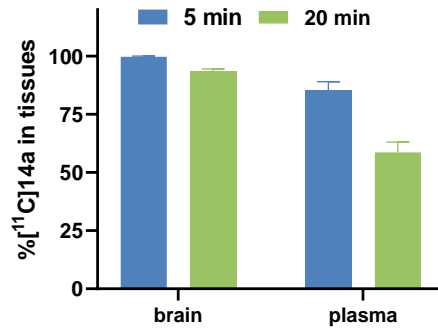


Figure S3. Radiometabolite analysis of [¹¹C]14a in rat brain (average two runs)

Table S1. Radiometabolite and parent (unchanged) fraction of [¹¹C]14a in rat brain and plasma

	Metabolite (%)	Unchanged (%)
Plasma 5 mins-1	11.96	88.04
Plasma 5 mins-2	17.06	82.94
Plasma 20 mins-1	44.51	55.49
Plasma 20 mins-2	38.26	61.74
Brain 5 mins-1	0.06	99.94
Brain 5 mins-2	0.49	99.51
Brain 20 mins-1	7.3	92.7
Brain 20 mins-2	5.76	94.24

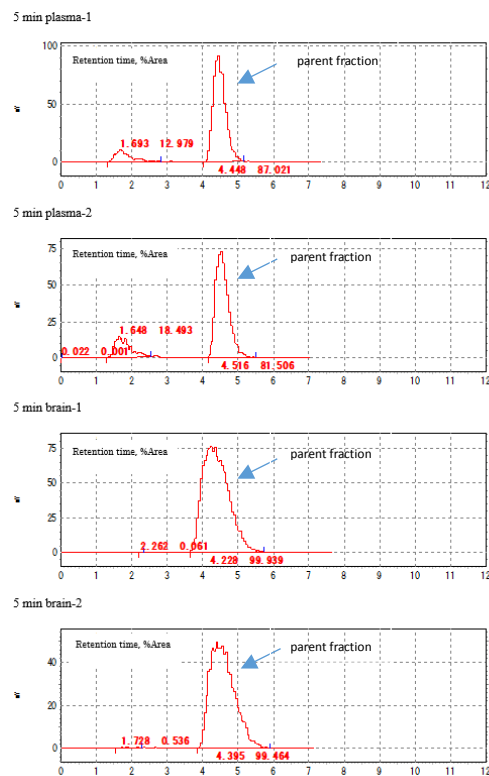


Figure S4. RadioHPLC chromatogram in the brain and plasma 5 min post injection of [¹¹C]14a

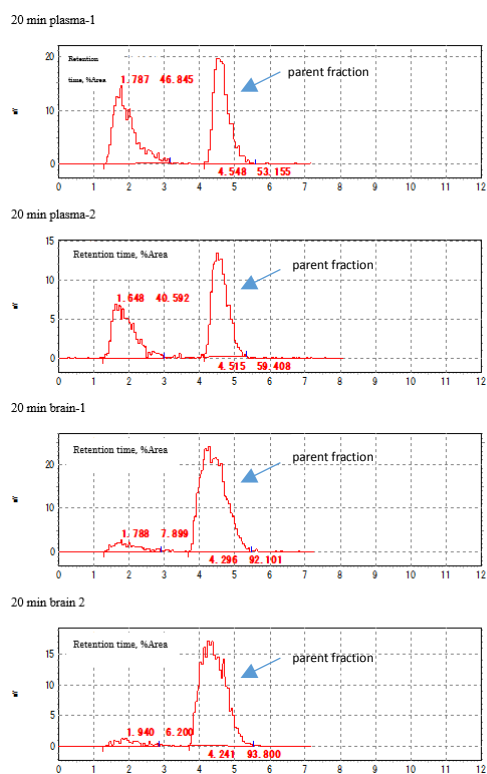


Figure S5. RadioHPLC chromatogram in the brain and plasma 20 min post injection of $[^{11}\text{C}]\mathbf{14a}$

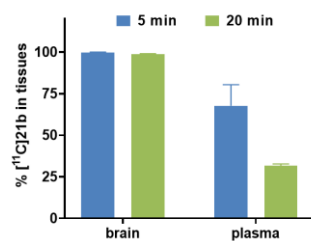
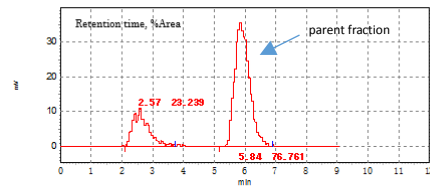


Figure S6. Radiometabolite analysis of $[^{11}\text{C}]\mathbf{14b}$ in rat brain (average two runs)

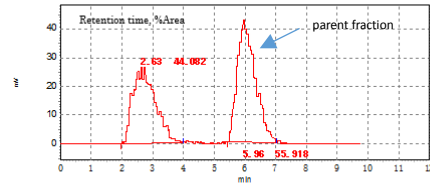
Table S2. Radiometabolite and parent (unchanged) fraction of $[^{11}\text{C}]\mathbf{14b}$ in rat brain and plasma

	Metabolite(%)	Unchanged (%)
Plasma 5 mins-1	21.32	76.68
Plasma 5 mins-2	41.31	58.69
Plasma 20 mins-1	69.22	30.78
Plasma 20 mins-2	67.60	32.40
Brain 5 mins-1	0.28	99.72
Brain 5 mins-2	0.04	99.96
Brain 20 mins-1	0.97	99.03
Brain 20 mins-2	1.22	98.78

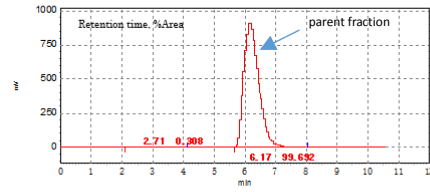
5 min plasma-1



5 min plasma-2



5 min brain-1



5 min brain-2

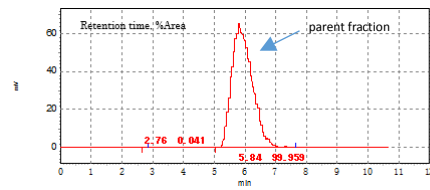
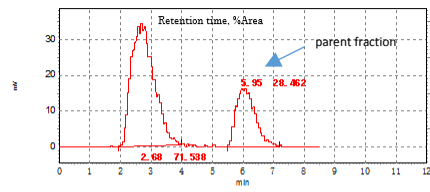
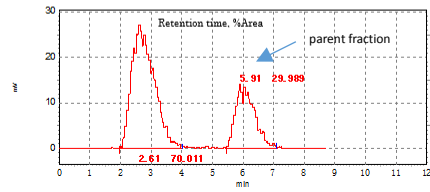


Figure S7. RadioHPLC chromatogram in the brain and plasma 5 min post injection of [^{11}C]14b

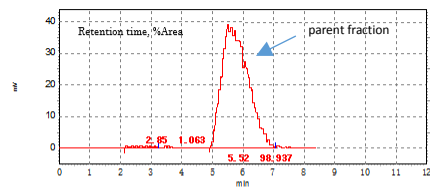
20 min plasma-1



20 min plasma-2



20 min brain-1



20 min brain 2

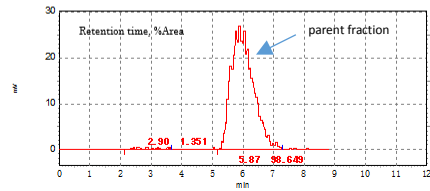


Figure S8. RadioHPLC chromatogram in the brain and plasma 20 min post injection of [¹¹C]14b

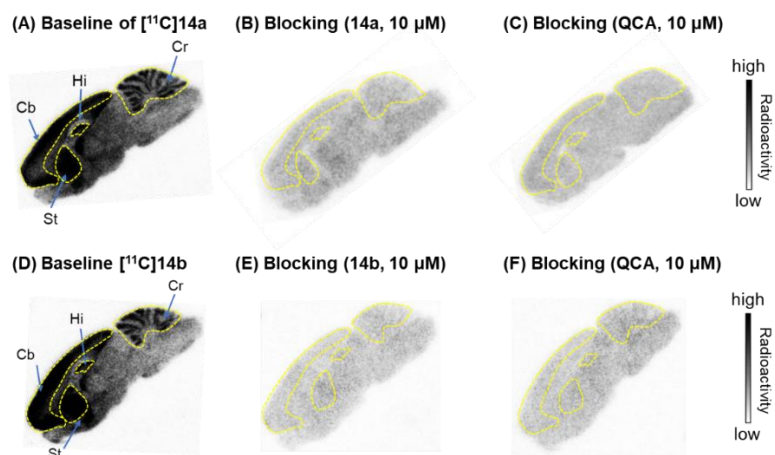


Figure S9. Image of the ROIs used for quantification of in vitro autoradiography. (A) Brain sections were treated with [¹¹C]**14a**; (B) Brain sections were pre-treated with **14a** (10 μM), followed by [¹¹C]**14a**; (C) Brain sections were pre-treated with **QCA** (10 μM), followed by [¹¹C]**14a**; (A) Brain sections were treated with [¹¹C]**14b**; (B) Brain sections were pre-treated with **14b** (10 μM), followed by [¹¹C]**14b**; (C) Brain sections were pre-treated with **QCA** (10 μM), followed by [¹¹C]**14b**.

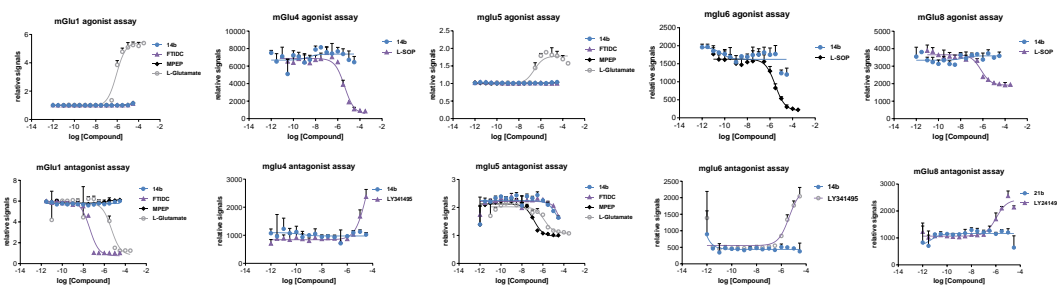


Figure S10. Functional (agonist and antagonist) assays of compound **14b** towards mGlu receptors, including mGlu₁, mGlu₄, mGlu₅, mGlu₆ and mGlu₈.

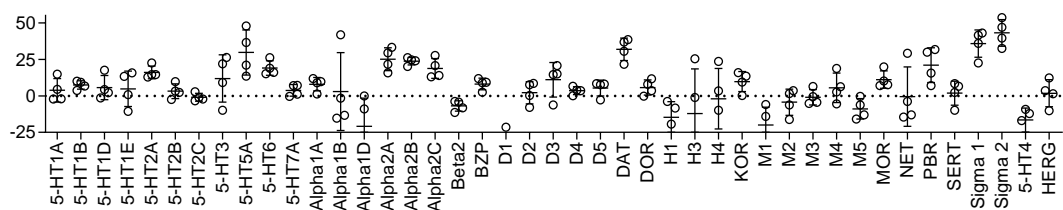


Figure S11. Off-target pharmacological evaluation of compound **14b** at a concentration of 10 μ M against major CNS targets, including common GPCRs, enzymes, ion channels and transporters: Initial screening at a concentration of 10 μ M. All data are mean \pm SD (n = 4). No significant off-target binding (>50%) was observed at 10 μ M compound testing concentration.

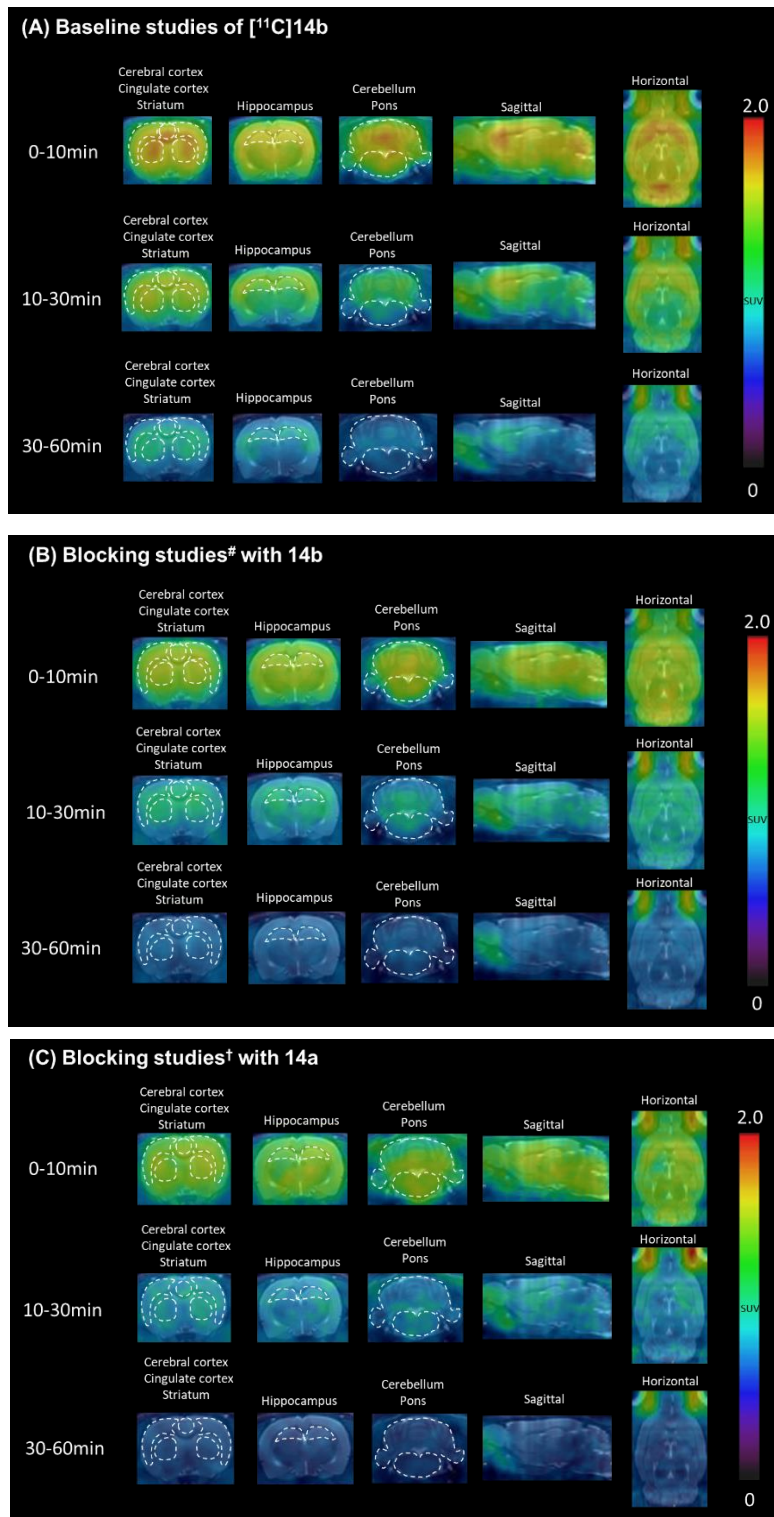
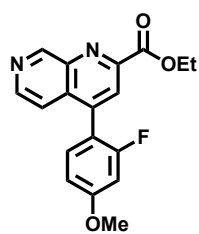


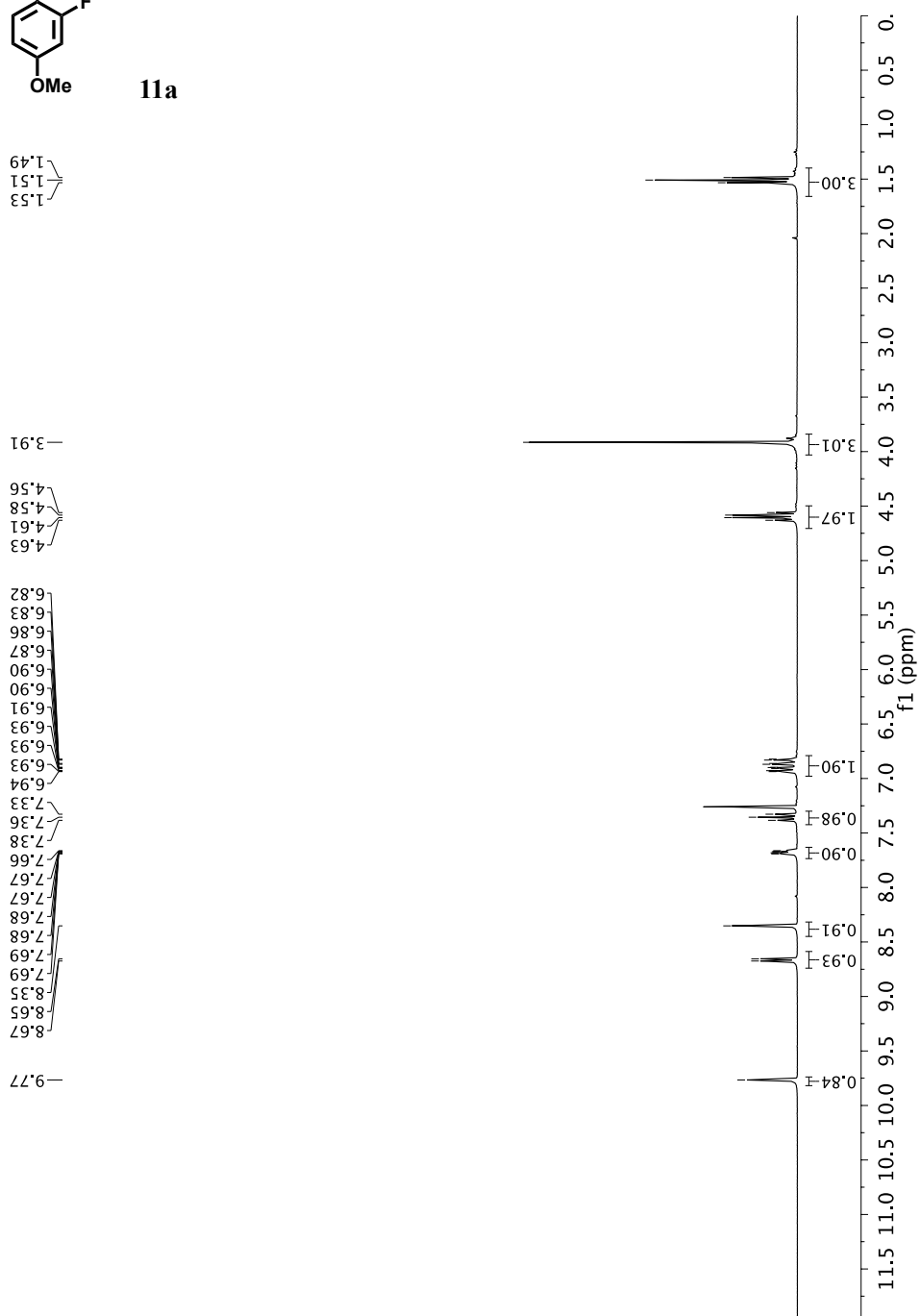
Figure S12. Representative PET/MRI fused images (summed at 0-10 min, 10-30 min and 30-60 min) and time-activity curves of [¹¹C]14b under baseline and blocking conditions in SD rat brain. [#]Blocking conditions: **14b** (1 mg/kg), 30 min *i.v.* before radioligand injection; [†]Blocking conditions: **14a** (3 mg/kg), 30 min *i.v.* before radioligand injection. Data are presented as mean ± SEM (n = 3).

NMR spectra of synthesized compounds

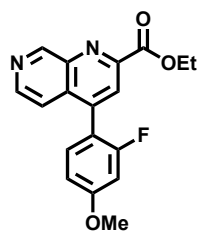
¹H spectrum of 11a



11a



¹³C spectrum of 11a



14.36

55.76

62.59

99.58

99.84

107.56

107.78

118.25

120.58

125.75

128.43

128.55

131.60

132.00

132.10

142.68

144.97

145.76

149.40

155.63

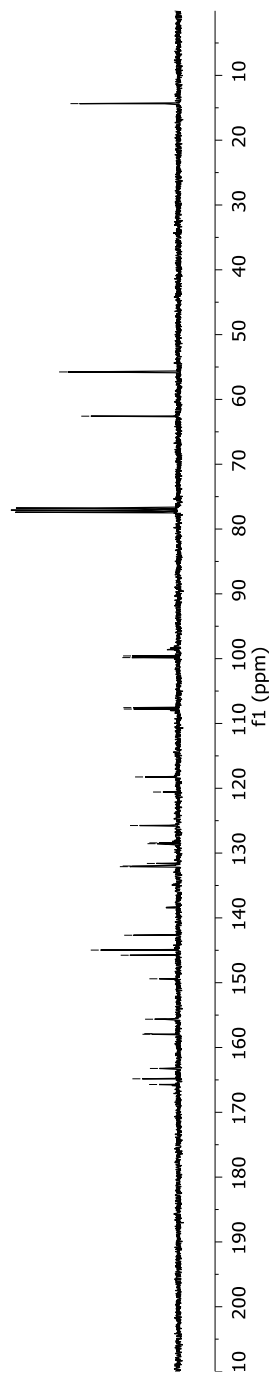
157.86

157.96

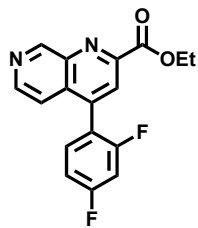
163.23

164.83

165.71



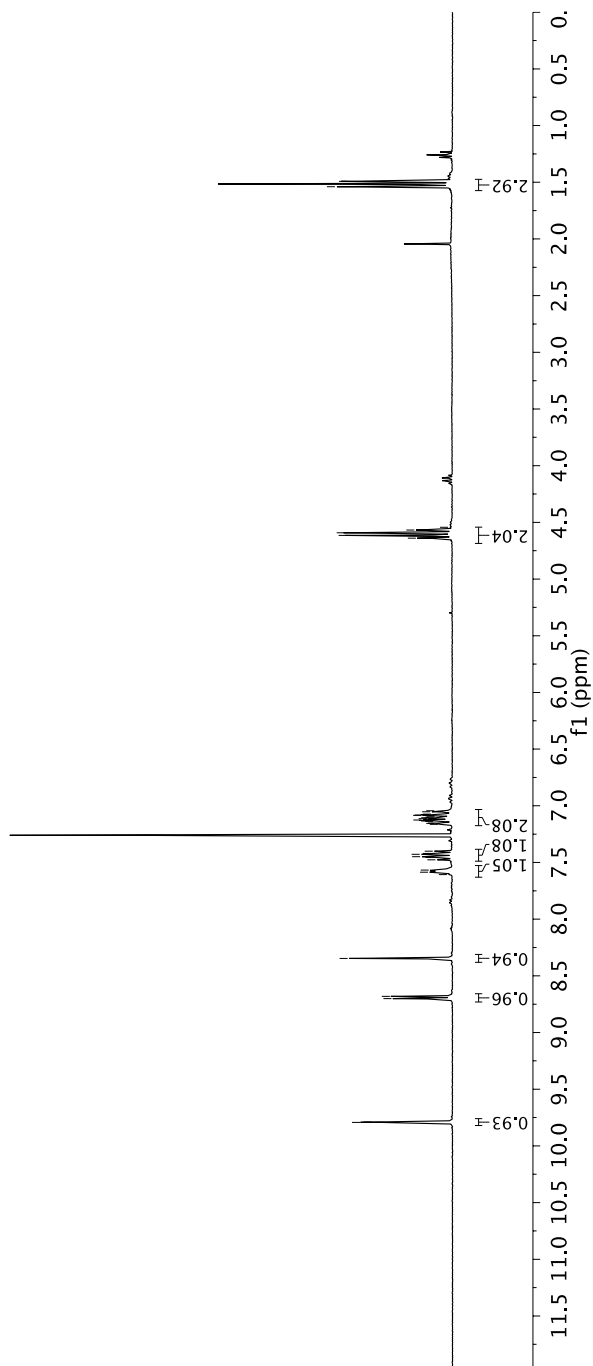
¹H spectrum of 11b



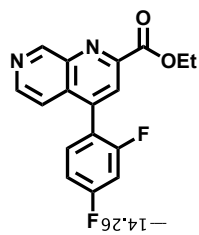
1.54
1.52
1.49

4.54
4.57
4.59
4.62
4.64
7.04
7.05
7.07
7.08
7.08
7.10
7.10
7.11
7.11
7.11
7.13
7.13
7.13
7.14
7.15
7.15
7.16
7.16
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7.59
7.61
8.35
8.68
8.70

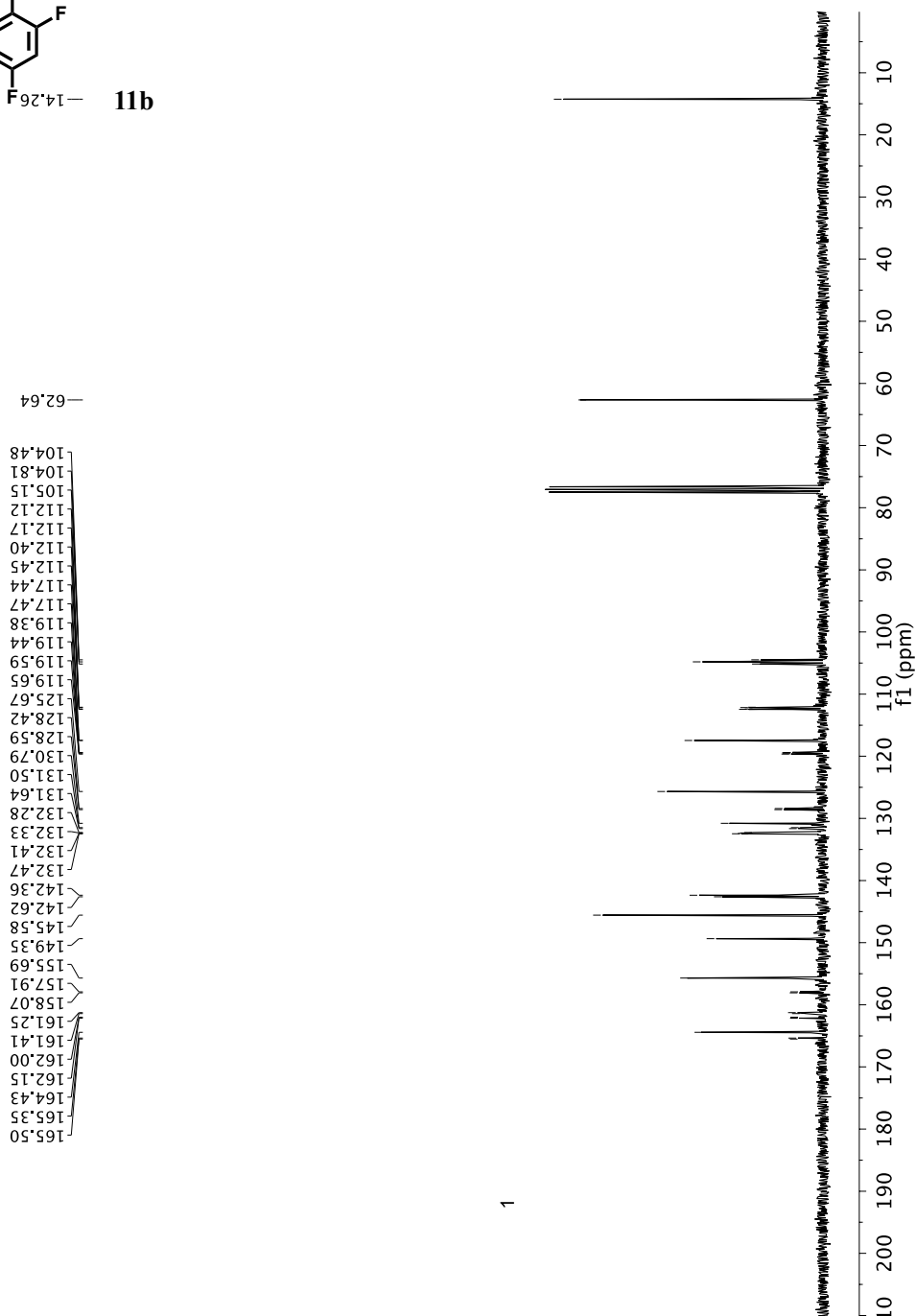
9.79



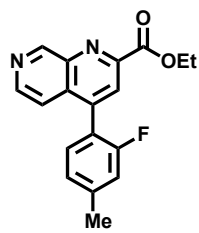
¹³C spectrum of 11b



11b



¹H spectrum of 11c



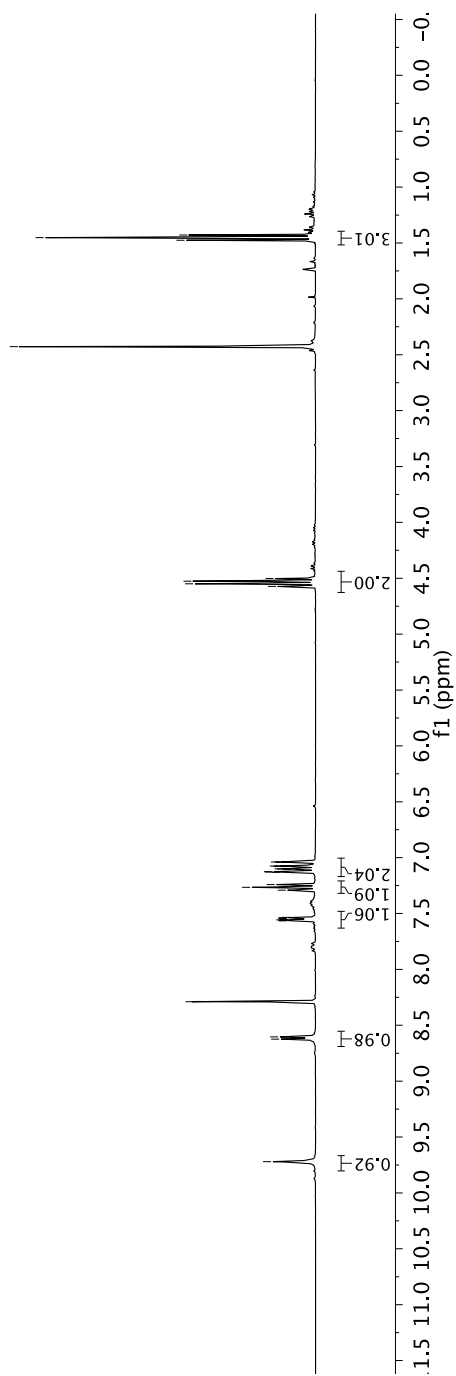
11c

1.43
1.45
1.48

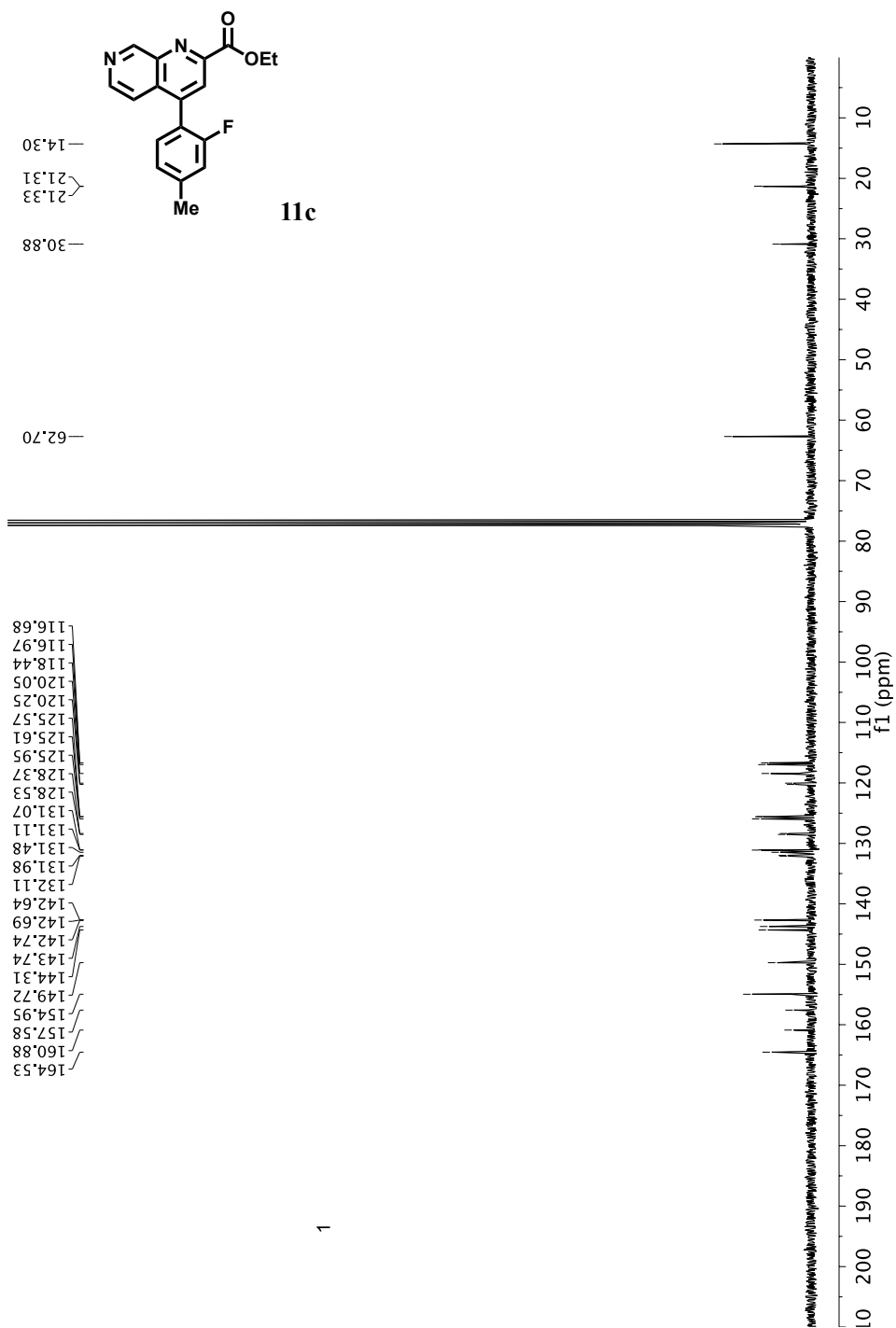
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4.55
4.57
7.03
7.04
7.04
7.04
7.07
7.07
7.08
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7.10
7.10
7.10
7.11
7.12
7.13
7.13
7.13
7.24
7.26
7.27
7.29
7.53
7.54
7.54
7.55
7.55
7.56
7.56
7.57

8.29
8.29
8.60
8.62

9.72

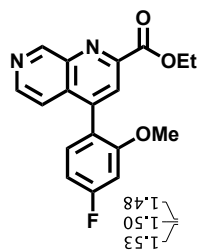


¹³C spectrum of 11c



1

¹H spectrum of 11d



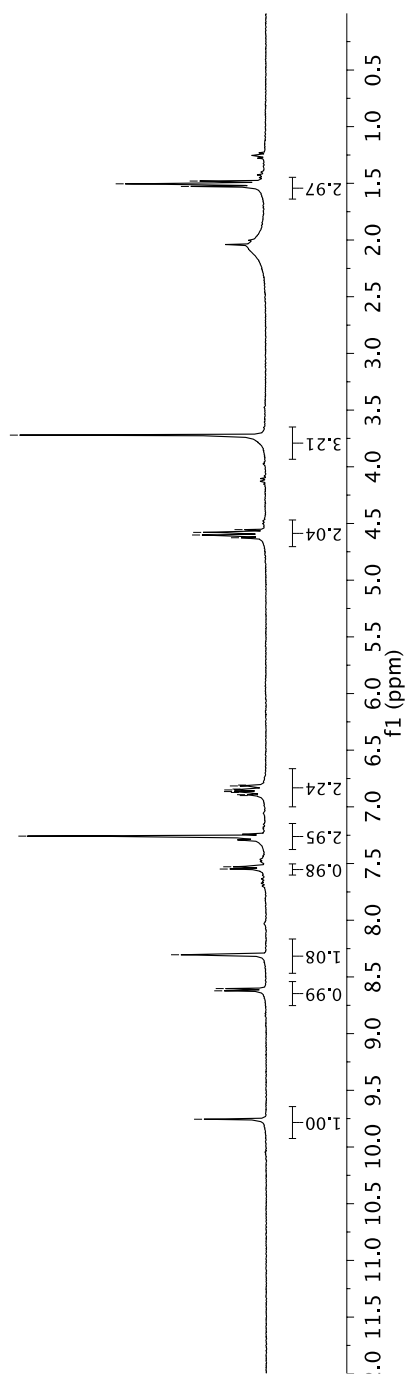
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4.55
4.58
4.60
4.63

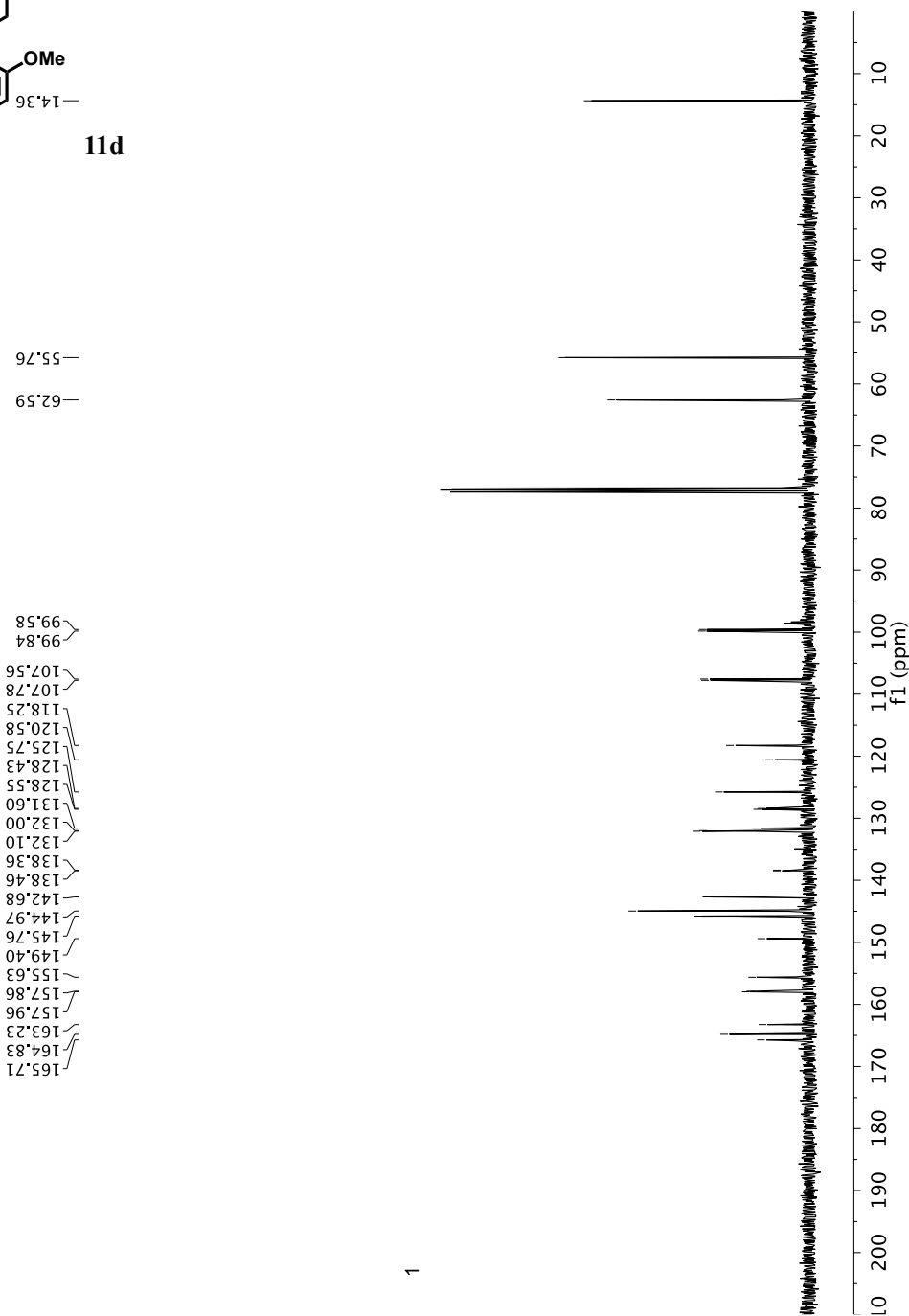
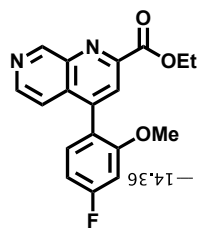
6.81
6.81
6.84
6.85
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6.87
6.89
6.90
7.26
7.53
7.55

8.30
8.60
8.62

9.75

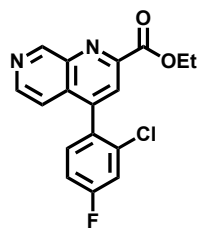


¹³C spectrum of 11d



1

¹H spectrum of 11g



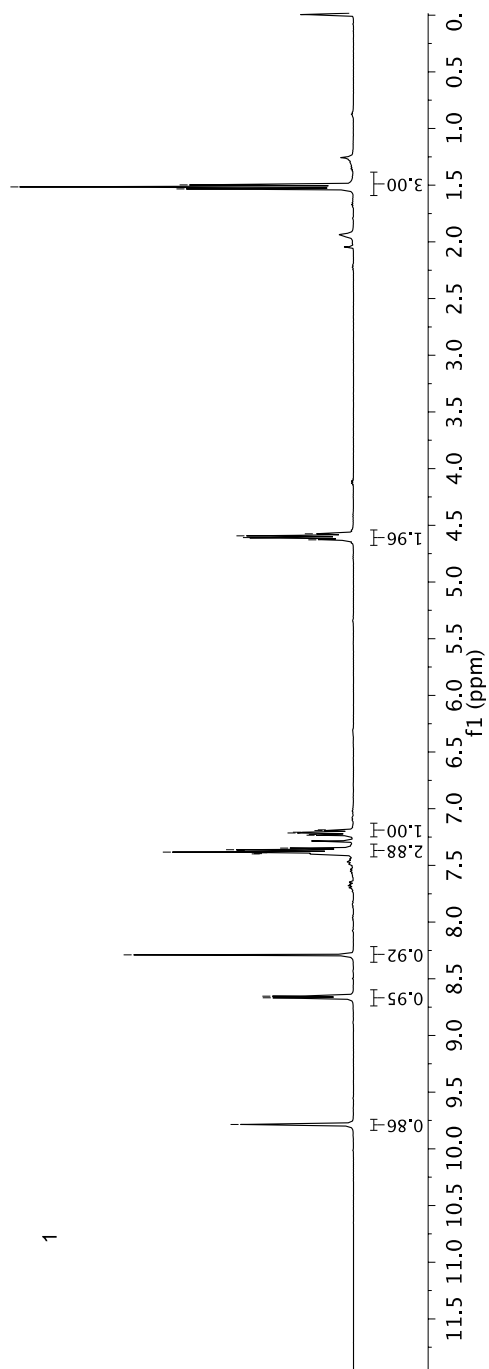
11g

1.53
1.51
1.50

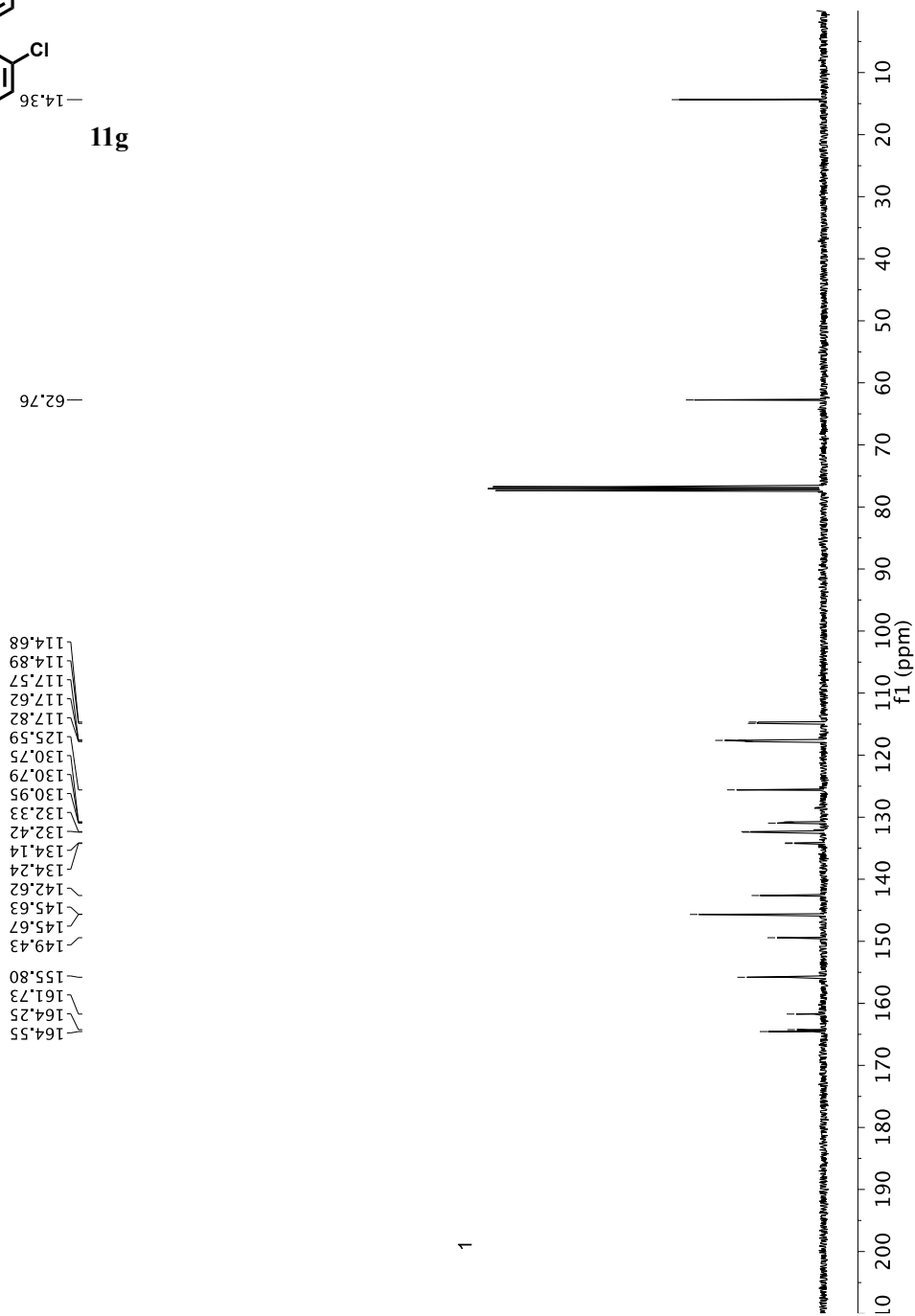
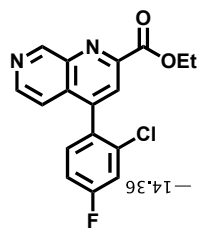
4.63
4.61
4.59
4.58

7.19
7.19
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7.21
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9.79

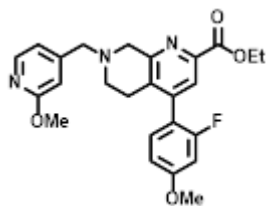


¹³C spectrum of 11g

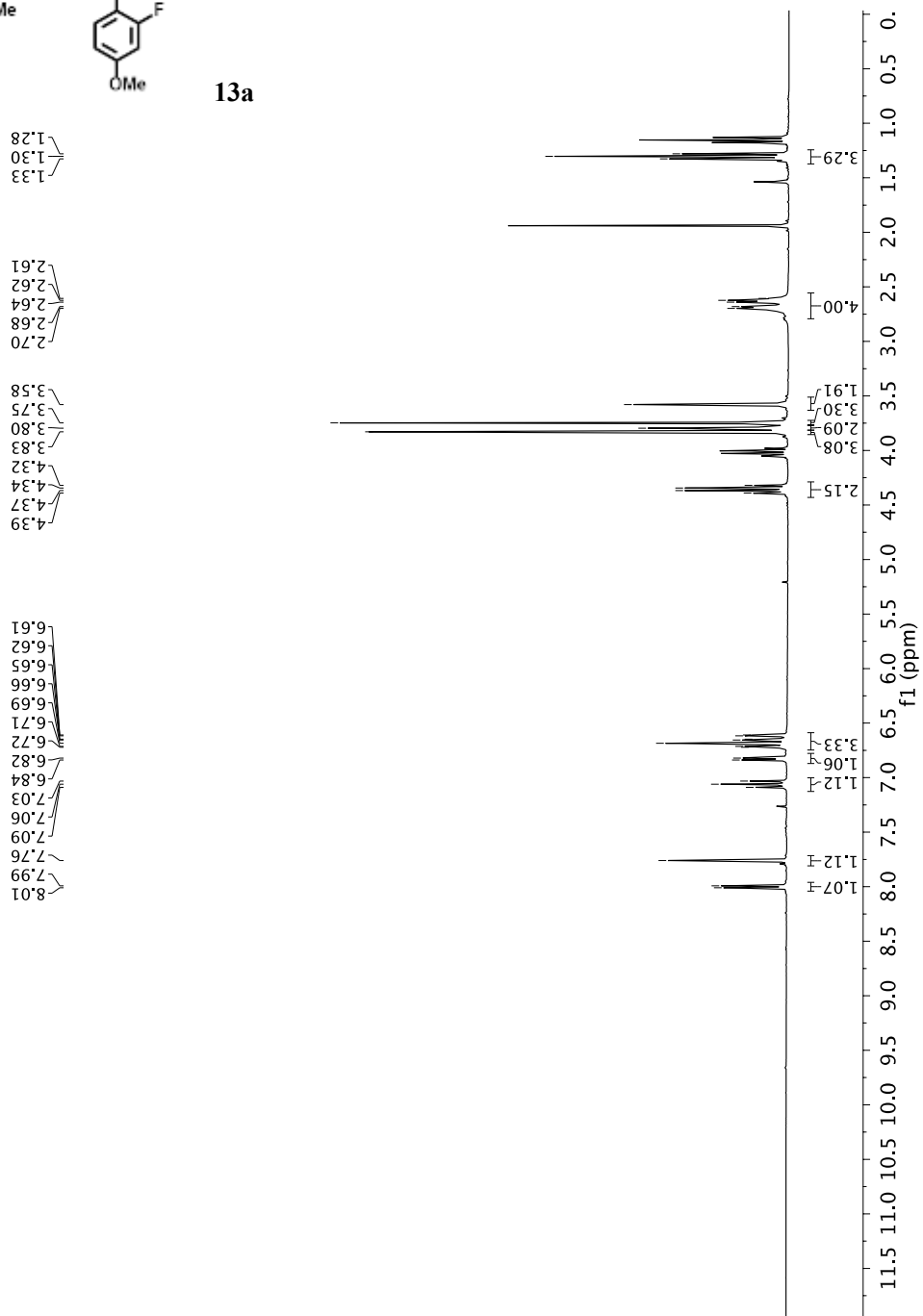


1

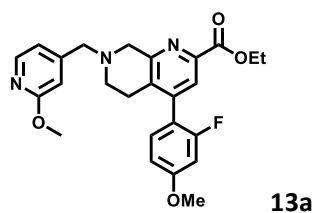
¹H spectrum of 13a



13a

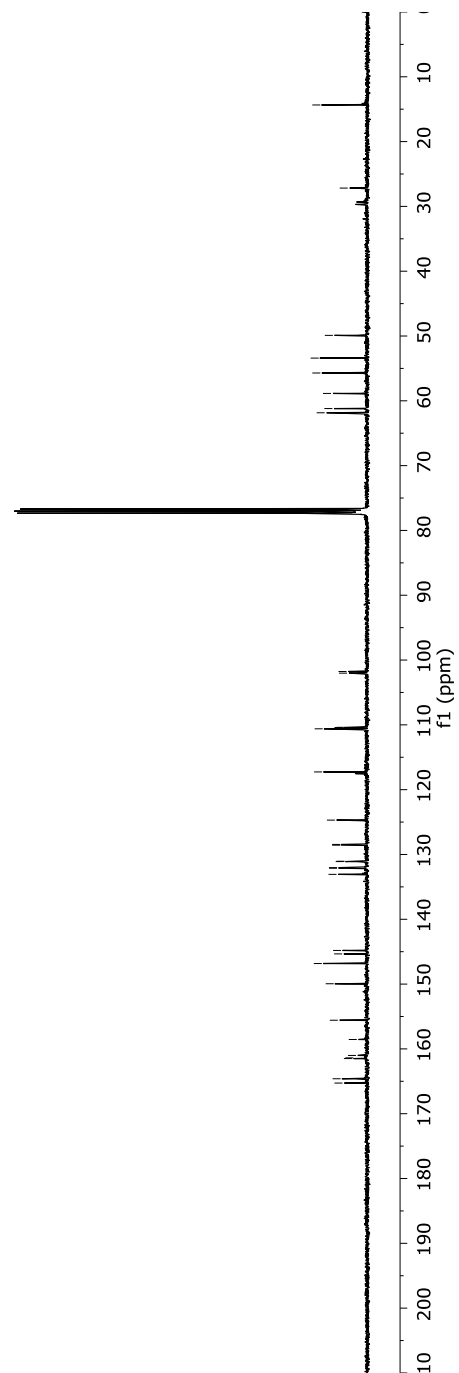


¹³C spectrum of 13a

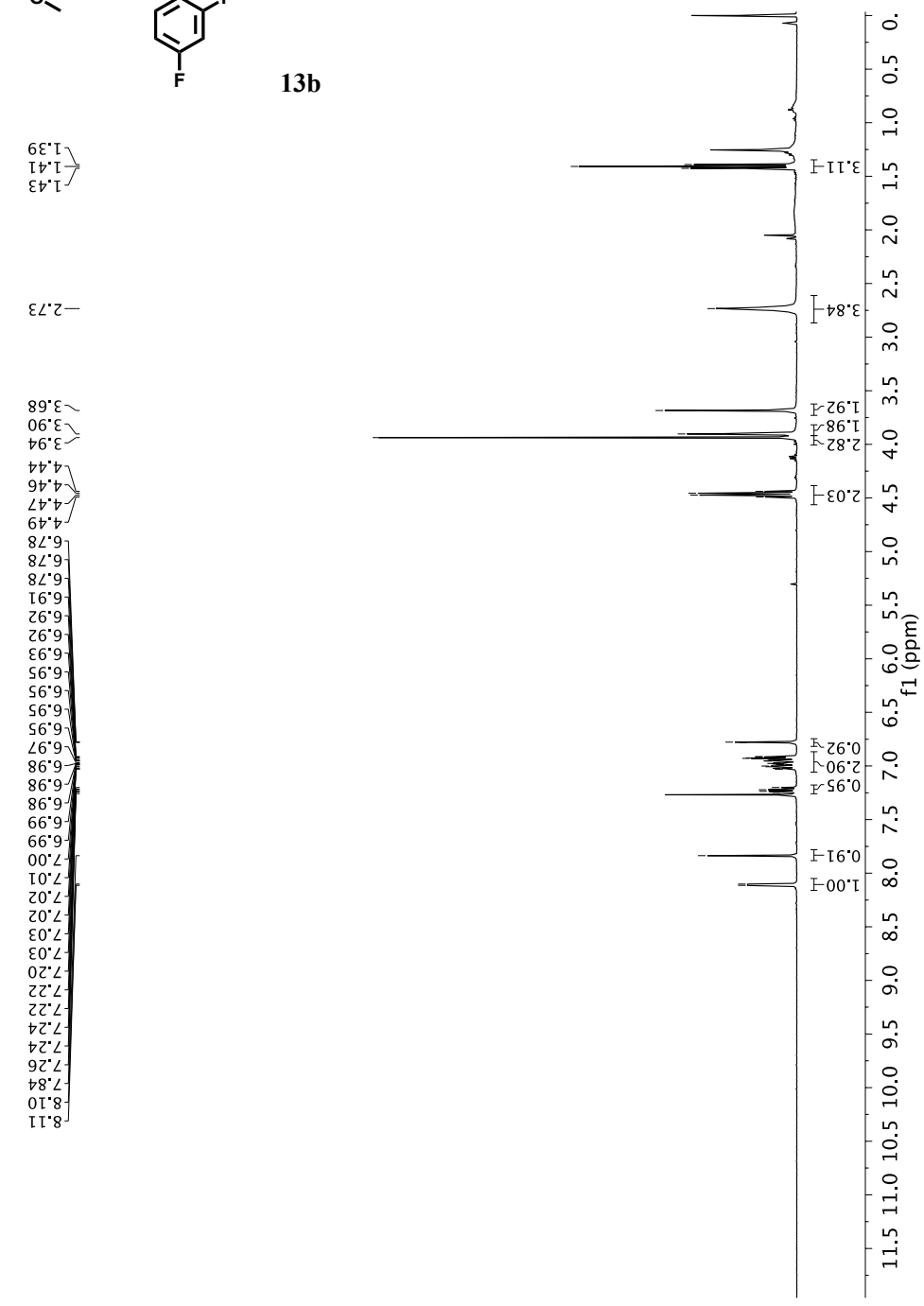
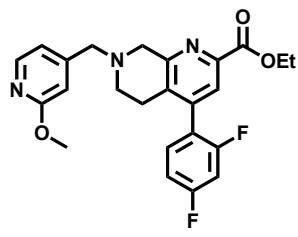


14.35
27.17
49.90
53.41
55.71
58.86
61.19
61.84

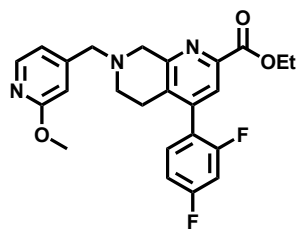
101.77
102.02
110.38
110.41
110.61
117.26
124.70
128.45
128.57
131.04
131.10
132.03
132.12
133.07
144.81
145.34
146.83
149.95
155.58
158.55
161.01
161.38
161.49
164.61
165.27



¹H spectrum of 13b



¹³C spectrum of 13b



13b

14.34

26.99

49.78

53.43

58.67

61.11

61.95

104.14

104.48

104.81

110.67

111.79

112.07

117.26

121.40

124.49

124.91

131.39

131.46

131.52

131.59

131.64

132.83

143.95

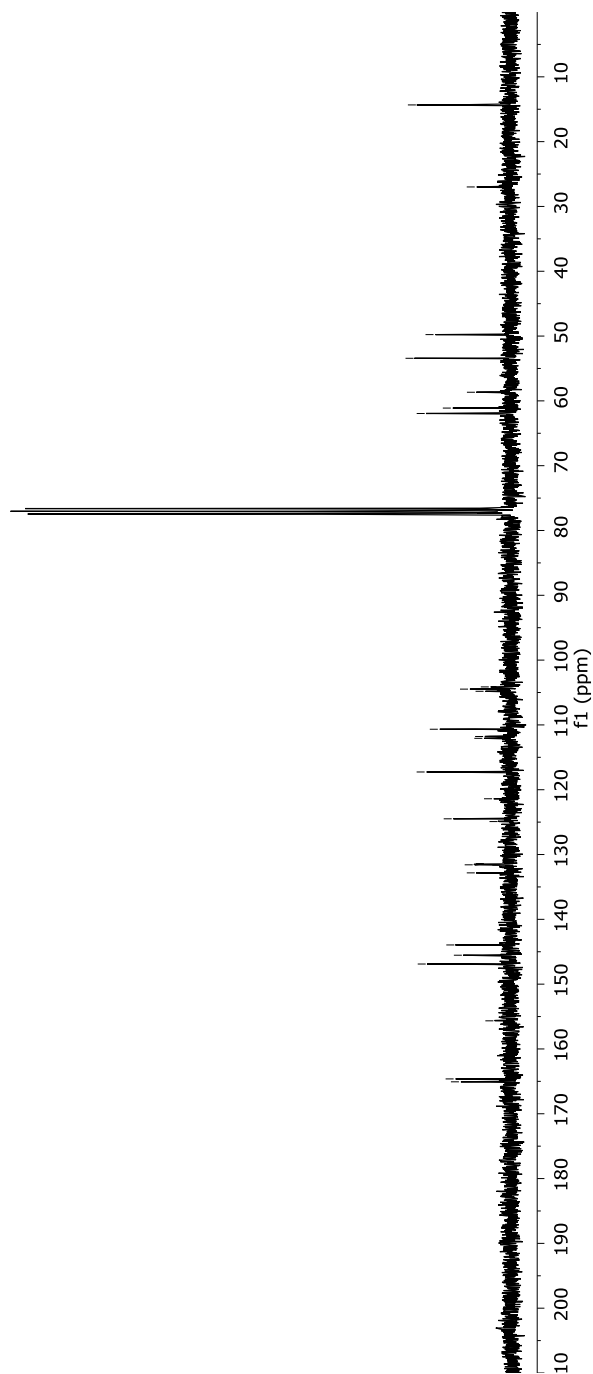
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146.90

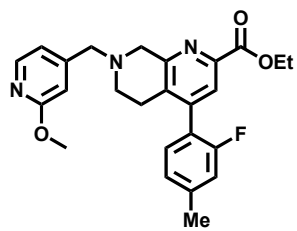
155.66

164.62

165.07



¹H spectrum of 13c



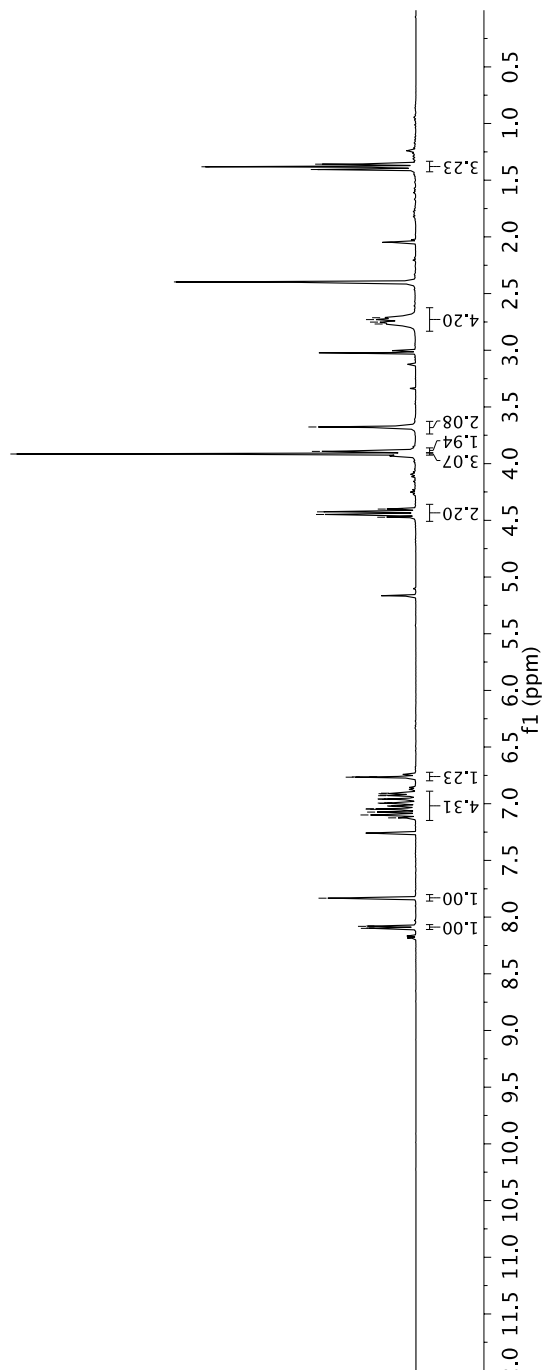
13c

1.36
1.38
1.41

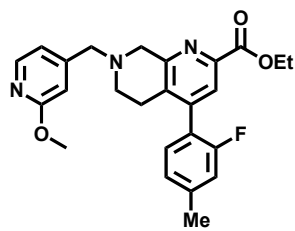
2.40
2.40
2.71
2.73
2.75
2.77

3.68
3.89
3.92
4.40
4.42
4.45
4.47

6.76
6.76
6.77
6.77
6.91
6.91
6.92
6.93
6.95
6.96
6.96
6.96
6.99
6.99
7.00
7.02
7.02
7.02
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7.07
7.10
7.12
7.83
8.08
8.08
8.10

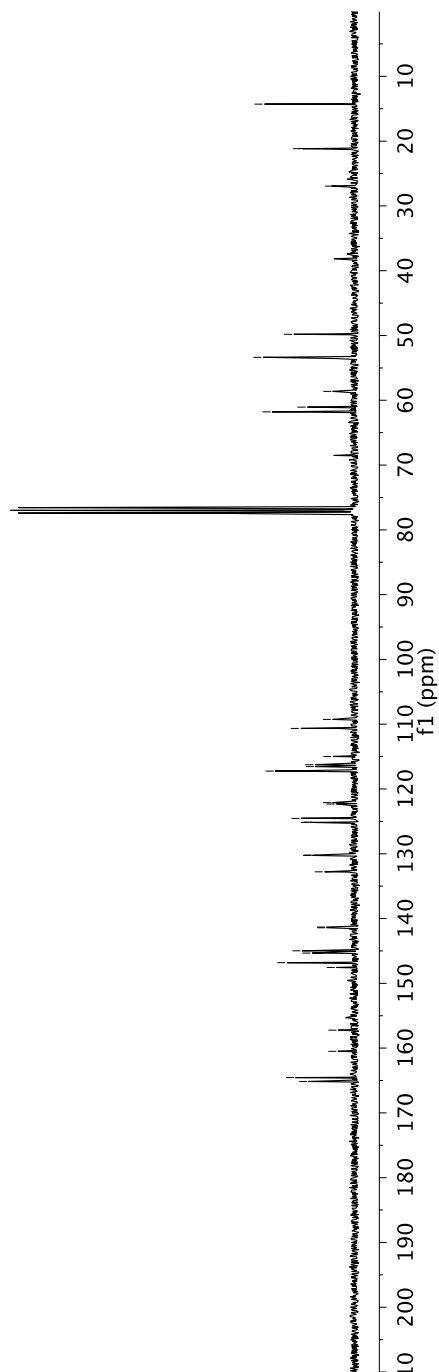


¹³C spectrum of 13c

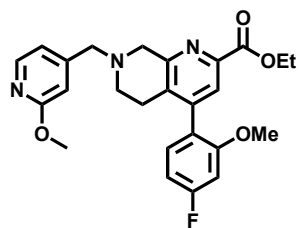


13c

14.29
21.14
21.16
26.93
49.81
53.37
58.62
61.05
61.80
109.26
110.64
114.98
116.25
116.53
117.24
122.10
122.32
124.53
125.12
125.17
130.21
130.26
132.80
141.32
141.42
144.97
145.32
146.81
147.55
157.22
160.50
164.55
165.14



¹H spectrum of 13d



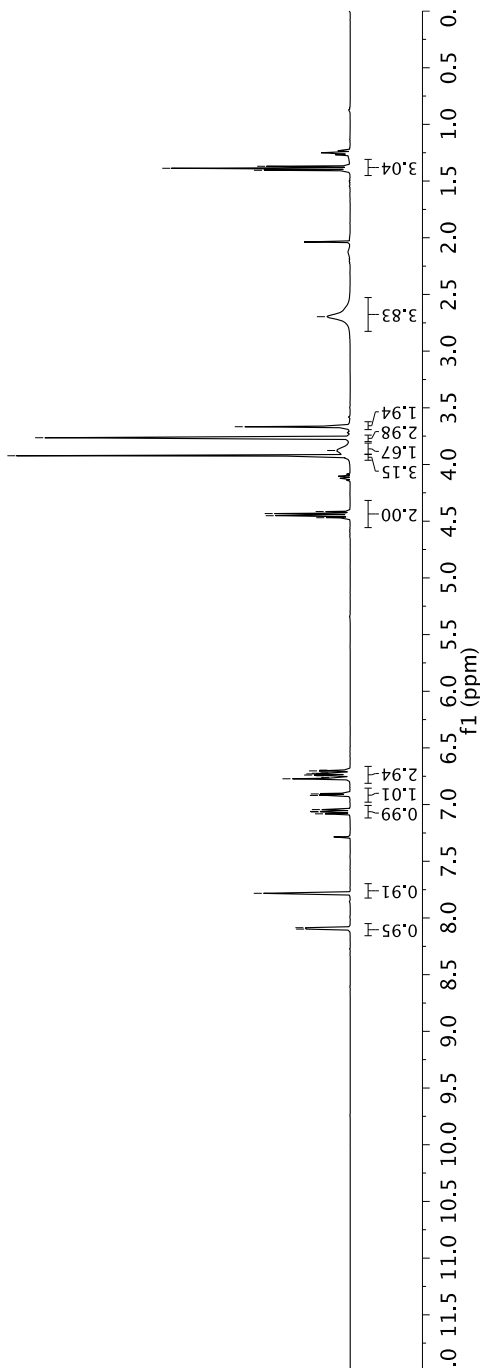
13d

1.37
1.39
1.40

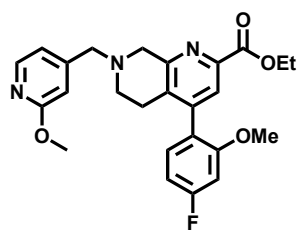
2.70

3.67
3.76
3.88
3.92
4.42
4.43
4.45
4.47

6.70
6.70
6.72
6.72
6.73
6.74
6.75
6.76
6.77
6.91
6.92
7.04
7.06
7.06
7.08
7.78
8.09
8.10

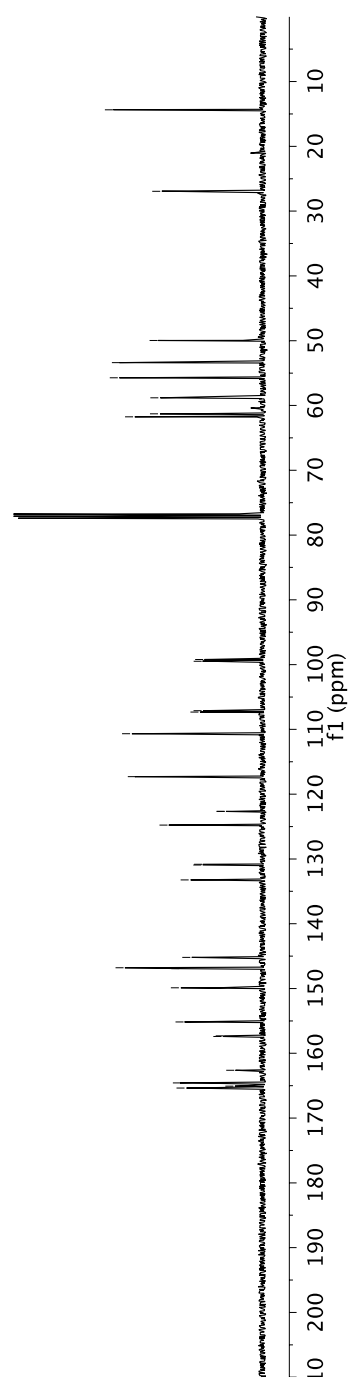


¹³C spectrum of 13d

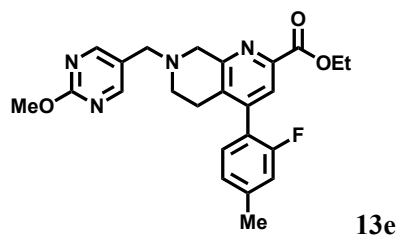


13d

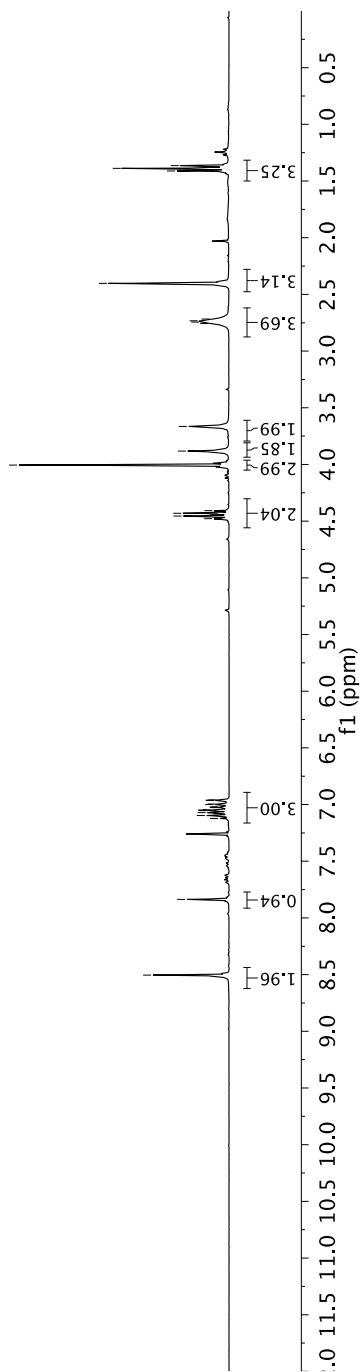
- 165.36
- 165.08
- 164.57
- 162.62
- 157.41
- 157.31
- 155.15
- 149.89
- 146.93
- 146.79
- 145.19
- 133.23
- 130.93
- 130.83
- 124.76
- 122.68
- 122.64
- 117.30
- 110.66
- 107.33
- 107.12
- 99.47
- 99.22
- 61.75
- 61.28
- 58.82
- 55.73
- 53.38
- 49.97
- 26.93
- 14.35



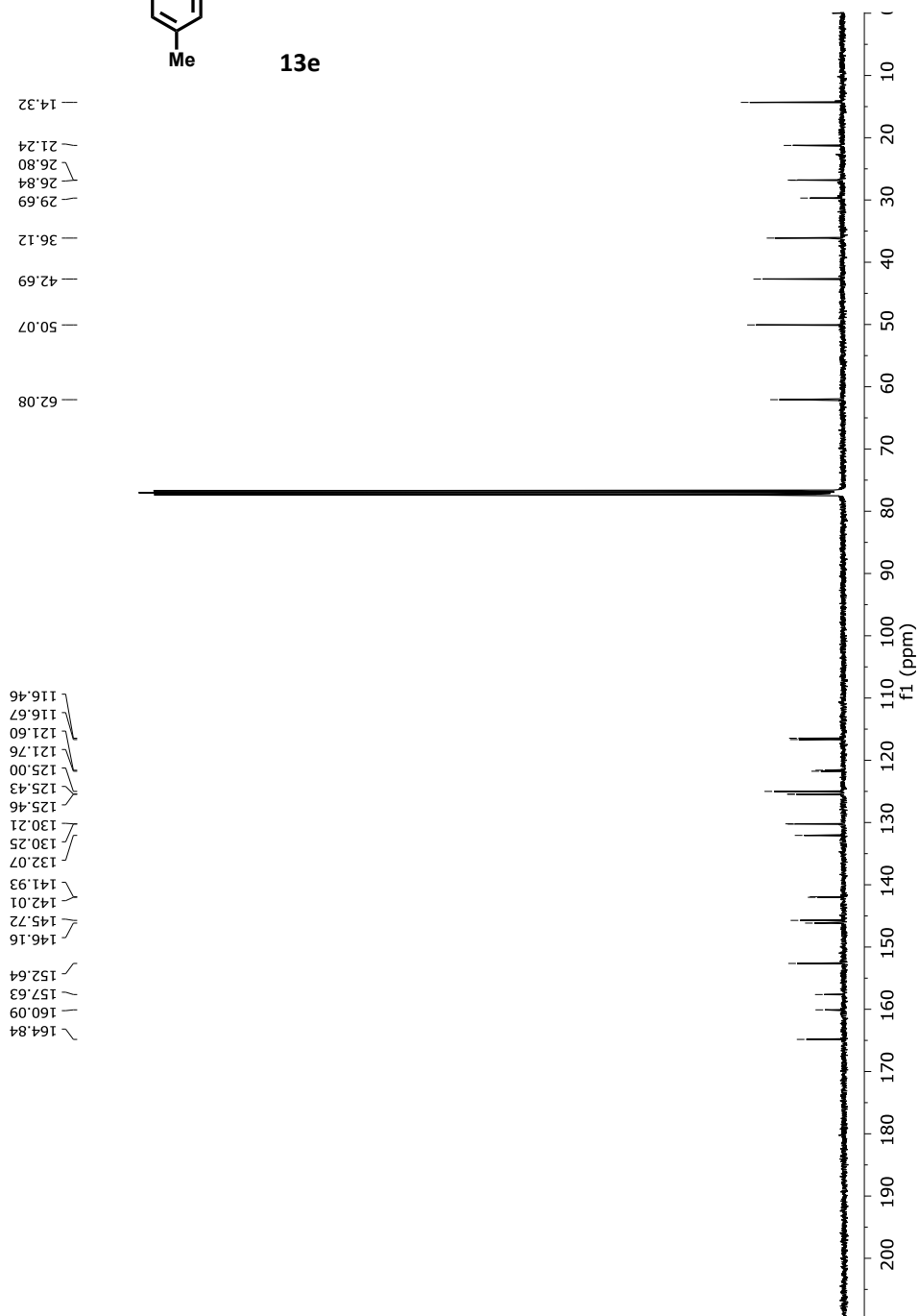
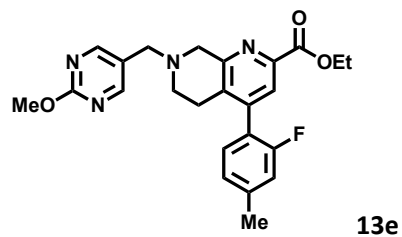
¹H spectrum of 13e



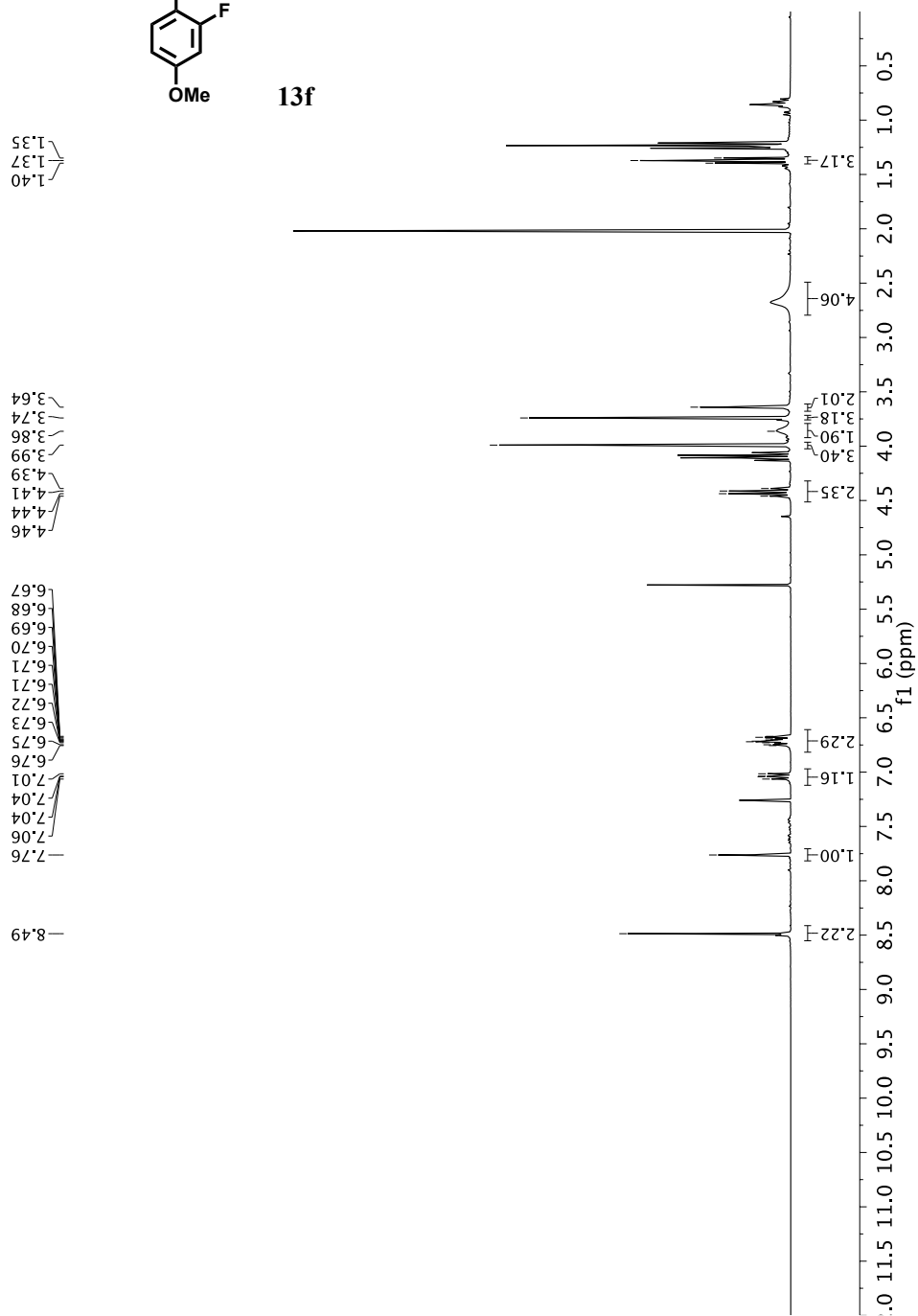
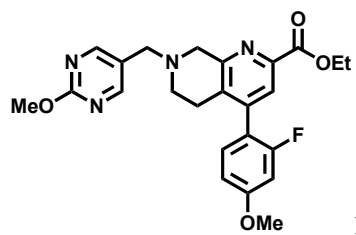
1.36
1.39
1.41
2.40
2.72
2.73
2.74
2.76
3.66
3.88
4.01
4.41
4.42
4.43
4.44
4.45
4.45
4.47
4.48
4.48
6.96
6.96
6.96
7.00
7.00
7.02
7.02
7.03
7.03
7.05
7.05
7.05
7.05
7.07
7.08
7.10
7.12
7.84
8.50



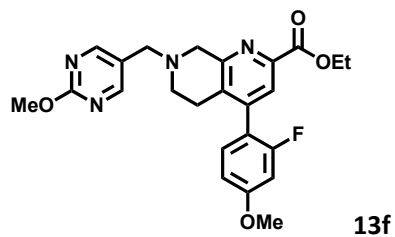
¹³C spectrum of 13e



¹H spectrum of 13f



¹³C spectrum of 13f



14.33

27.08

49.59

54.95

55.72

56.57

58.58

61.95

101.78

102.04

110.42

110.45

117.40

117.23

124.09

124.80

128.47

128.59

131.02

131.07

132.02

132.12

133.12

144.97

145.28

155.31

158.53

159.91

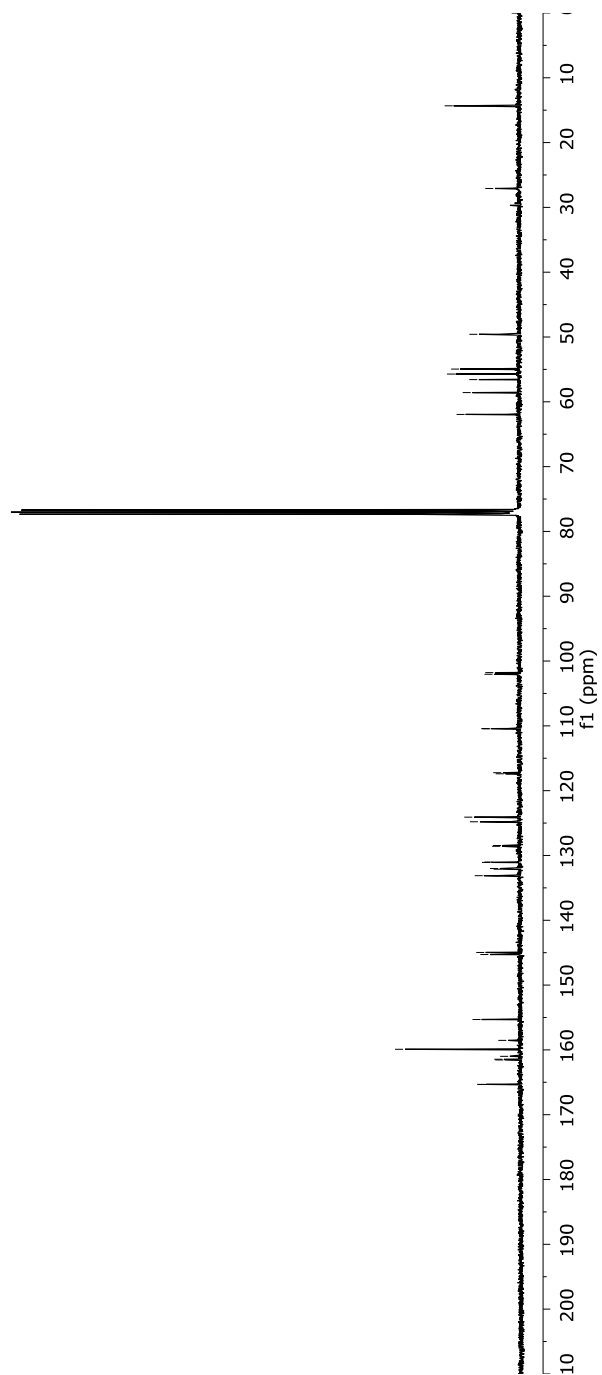
160.99

161.44

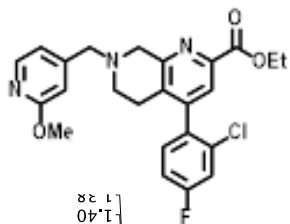
161.55

165.32

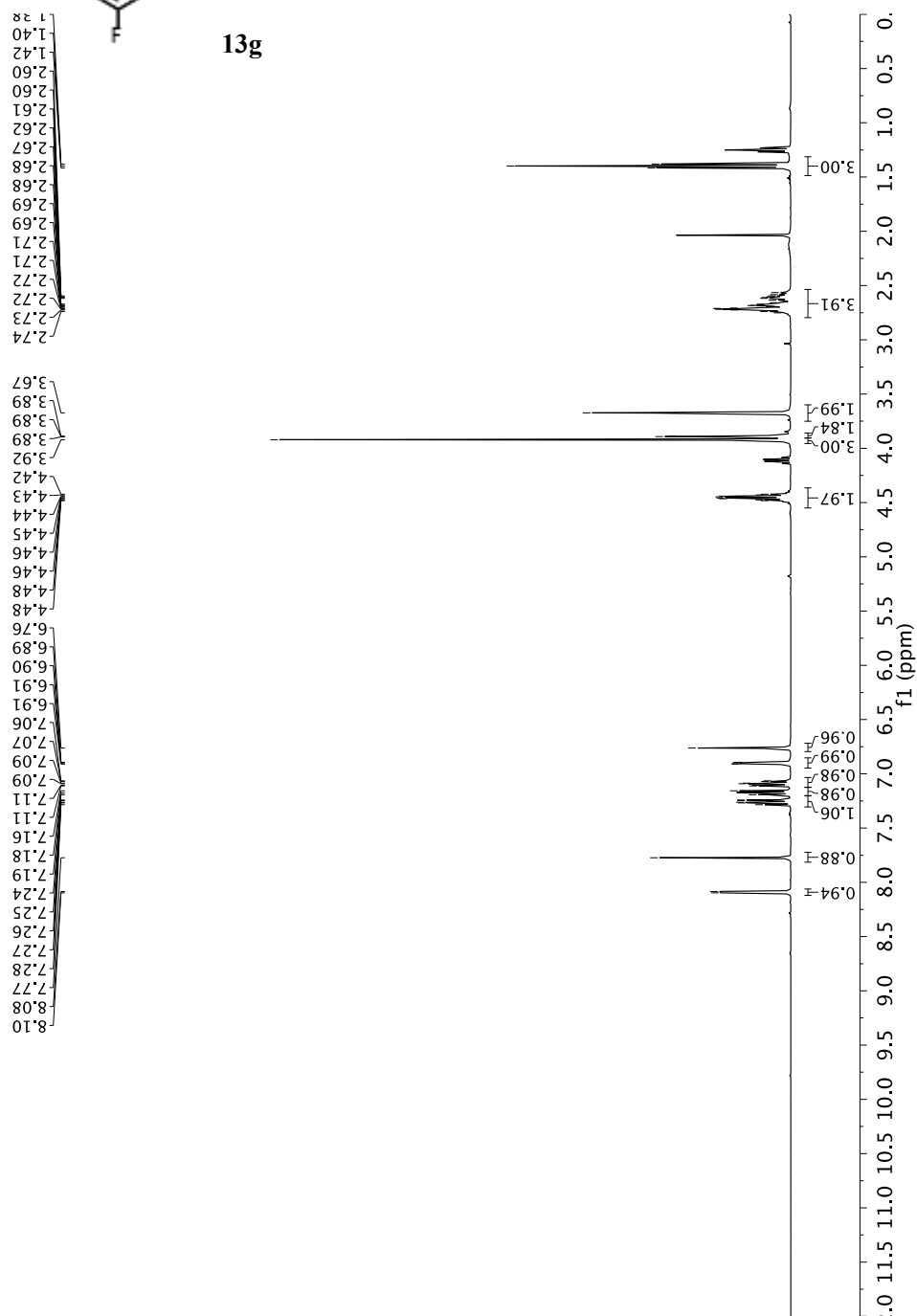
165.34



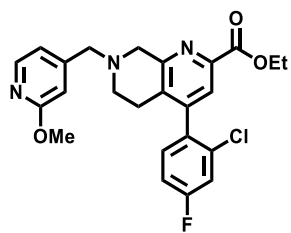
¹H spectrum of 13g



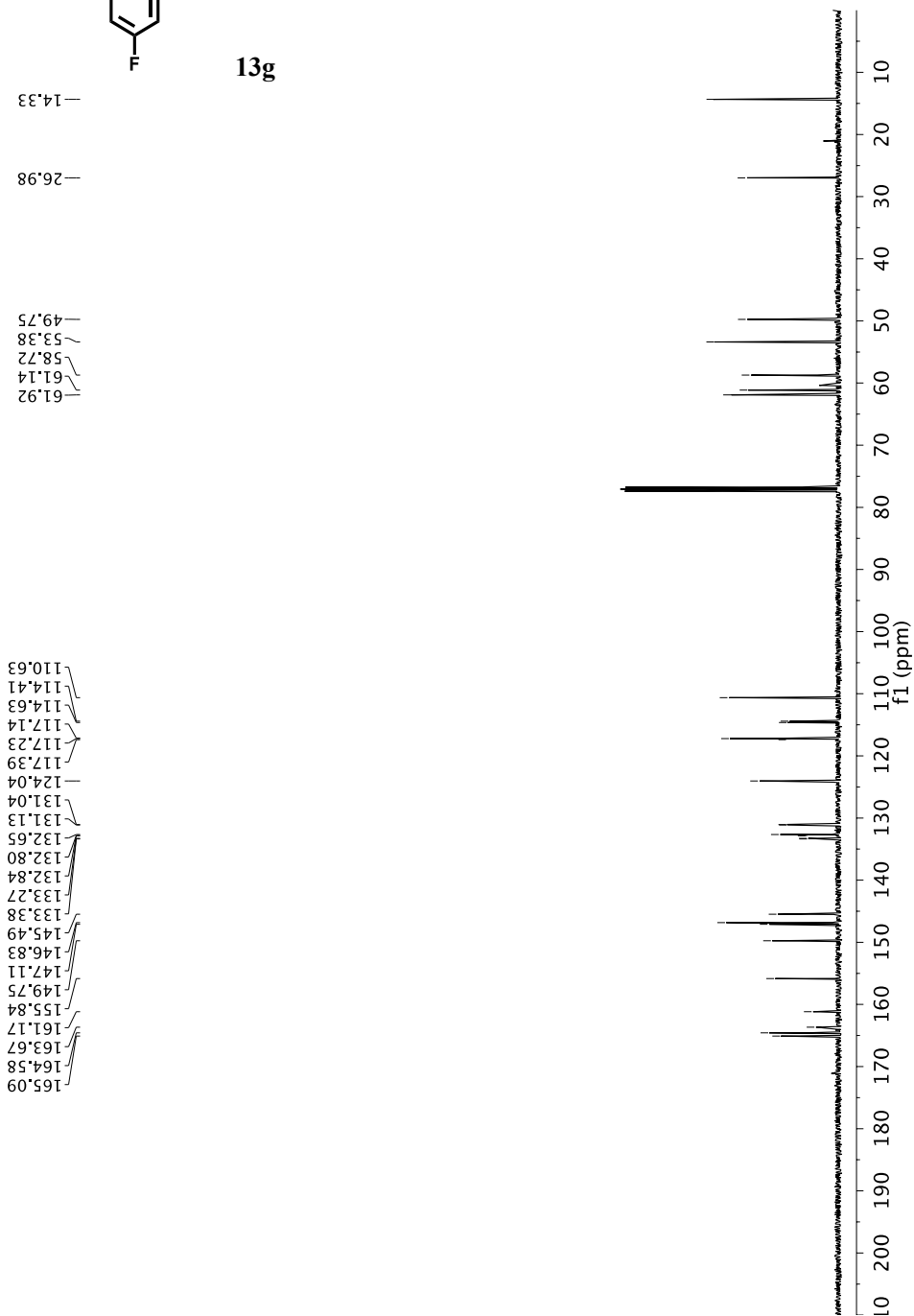
13g



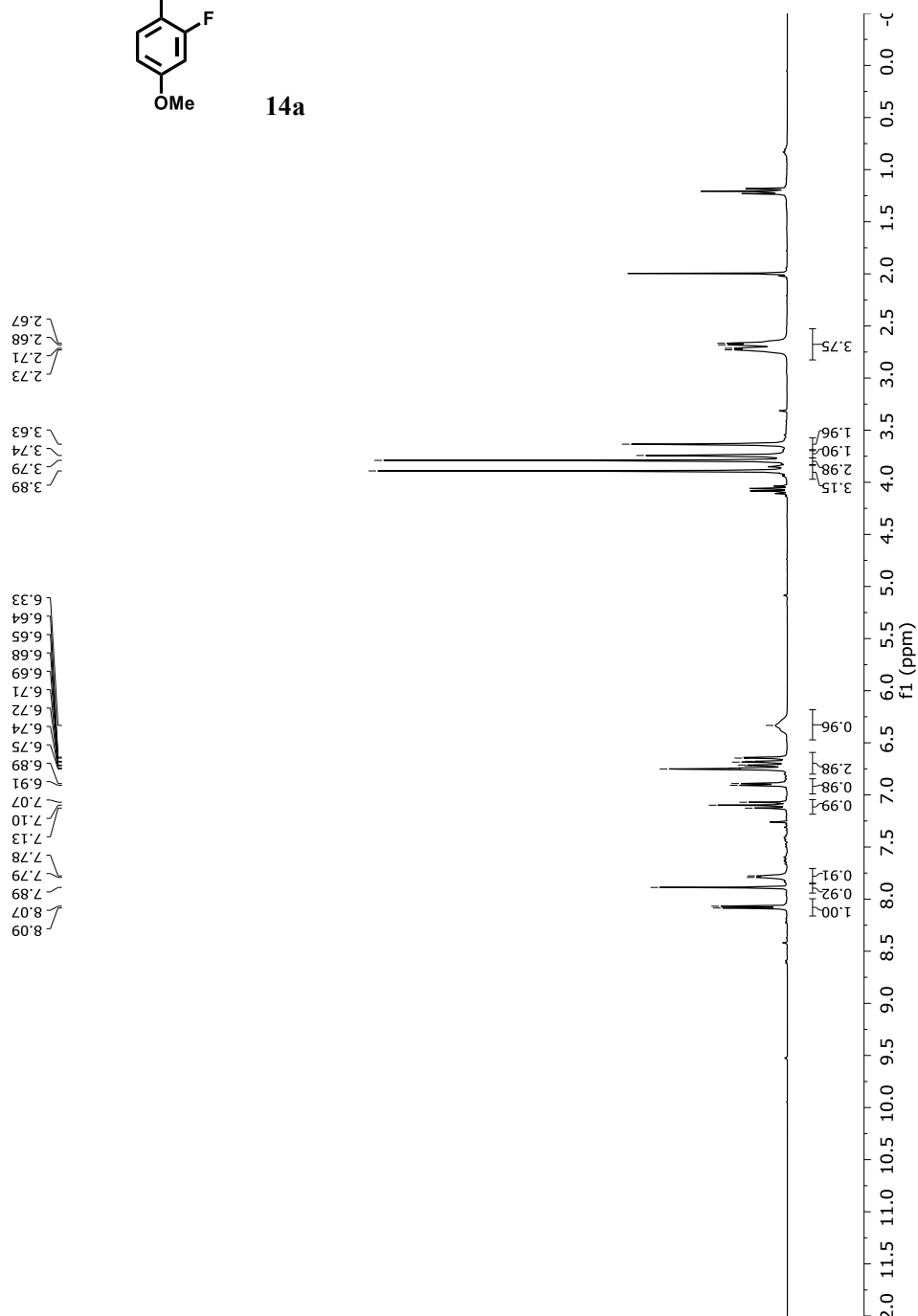
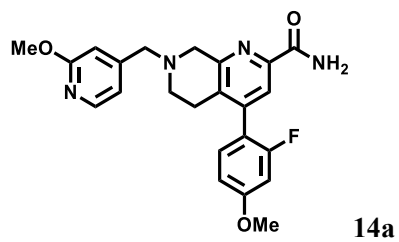
¹³C spectrum of 13g



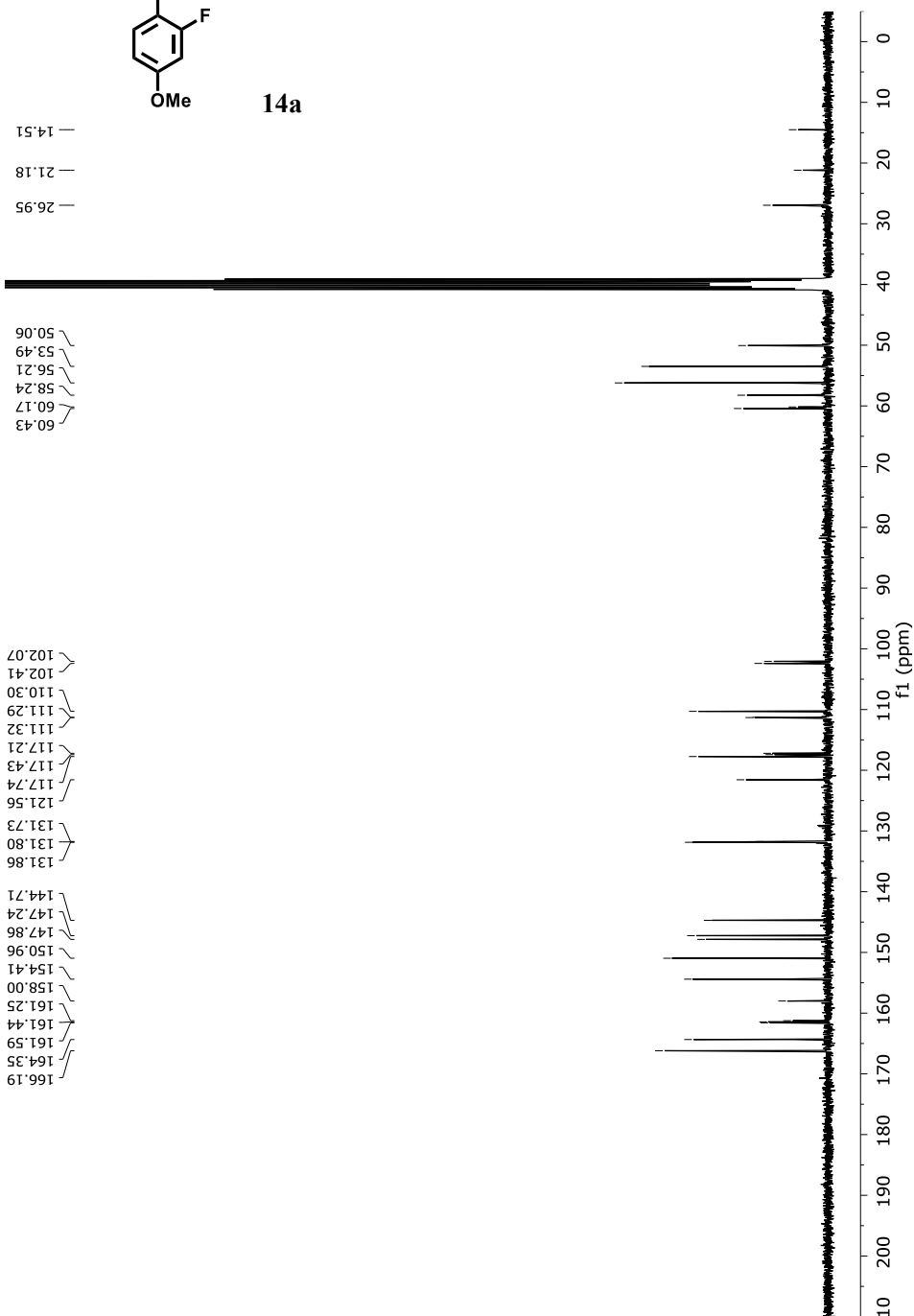
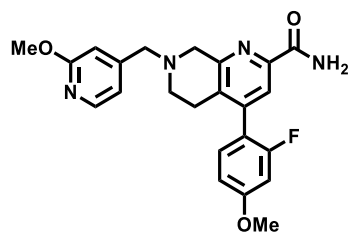
13g



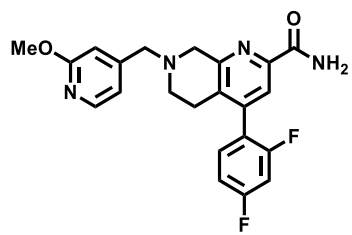
¹H spectrum of 14a



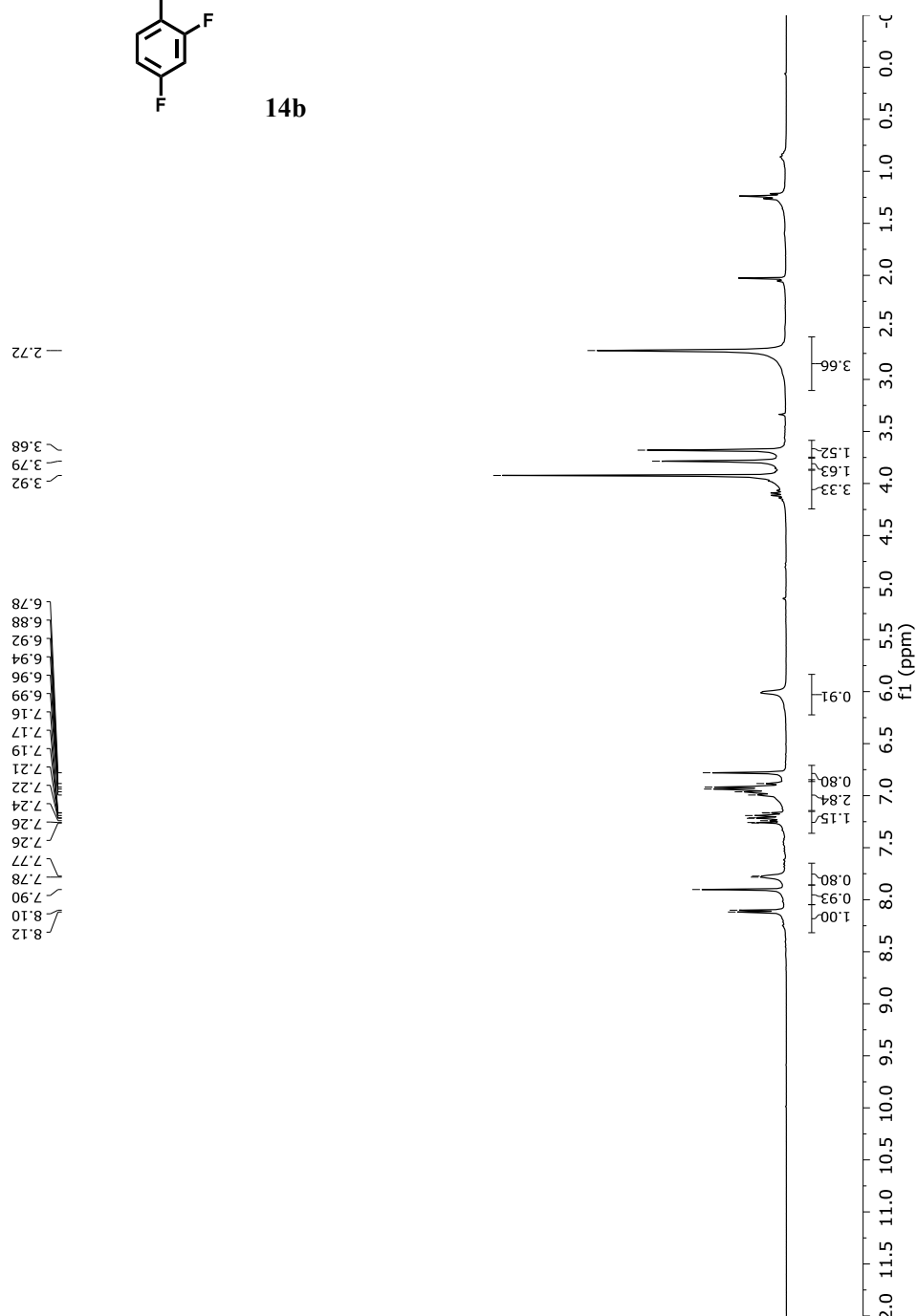
¹³C spectrum of 14a



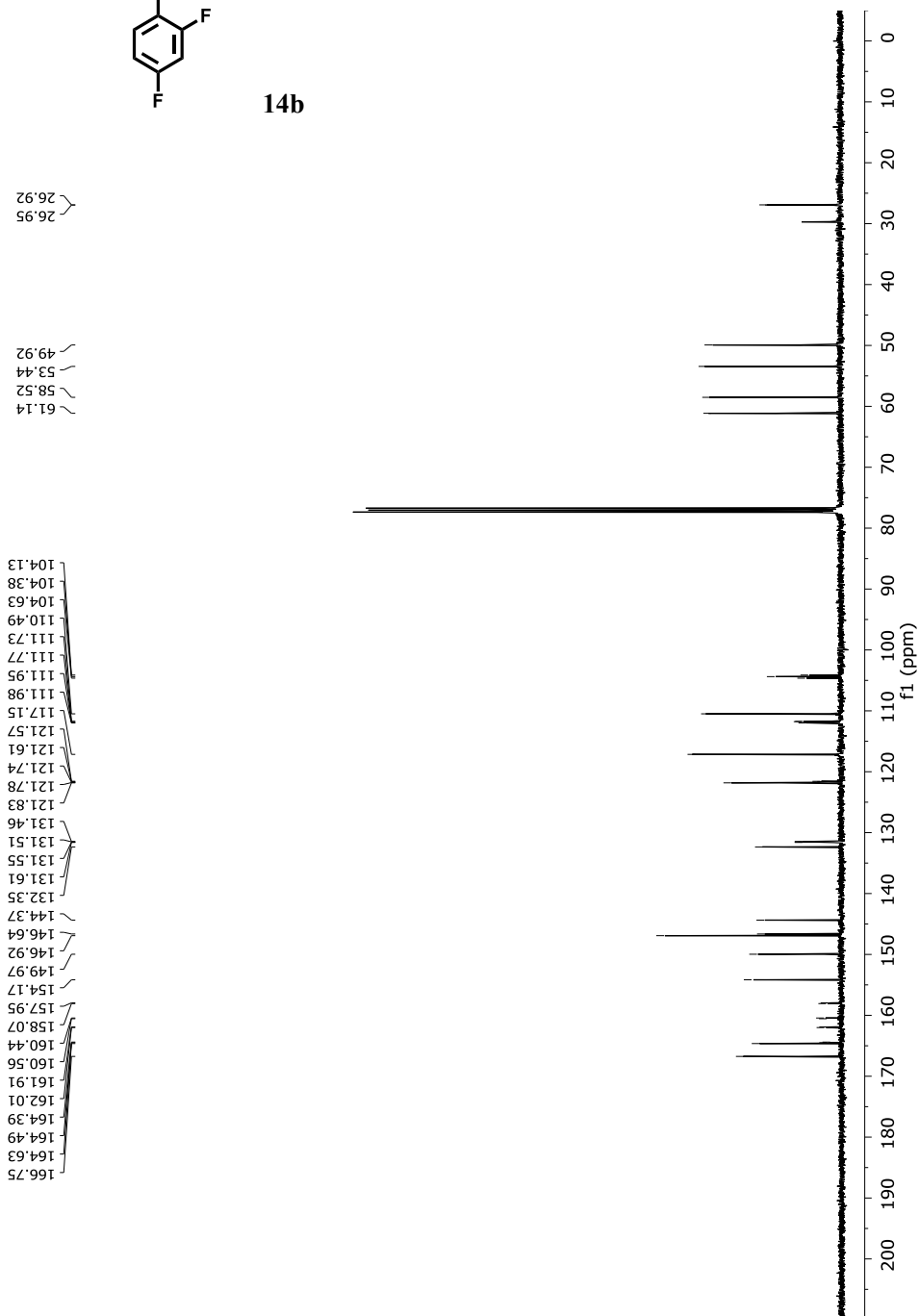
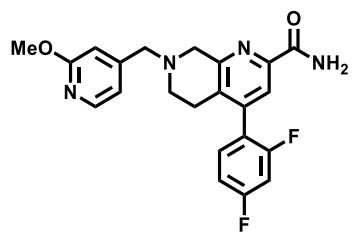
¹H spectrum of 14b



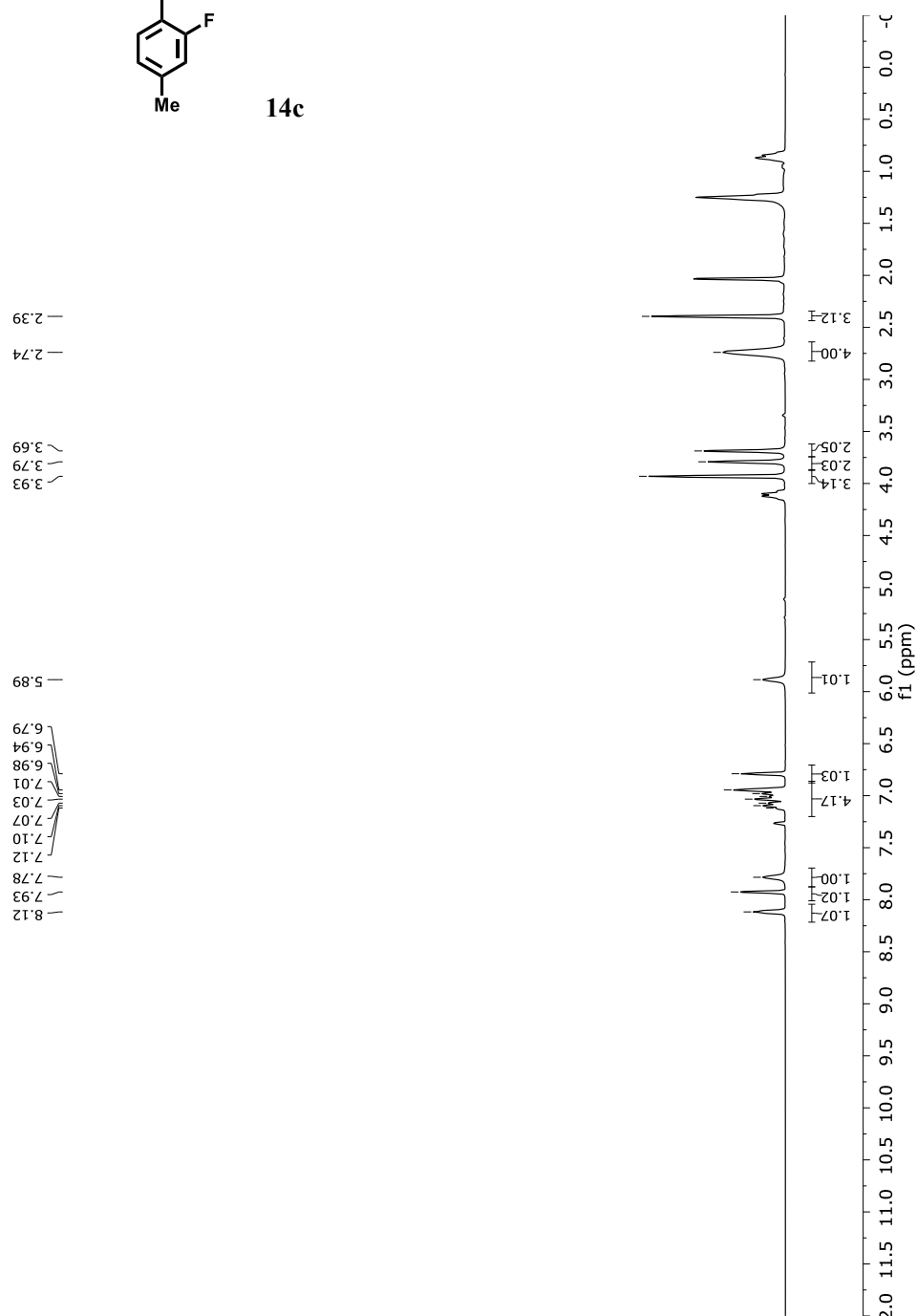
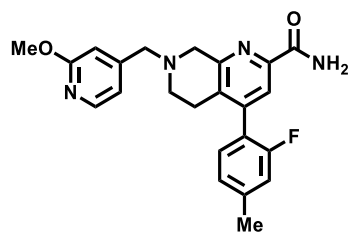
14b



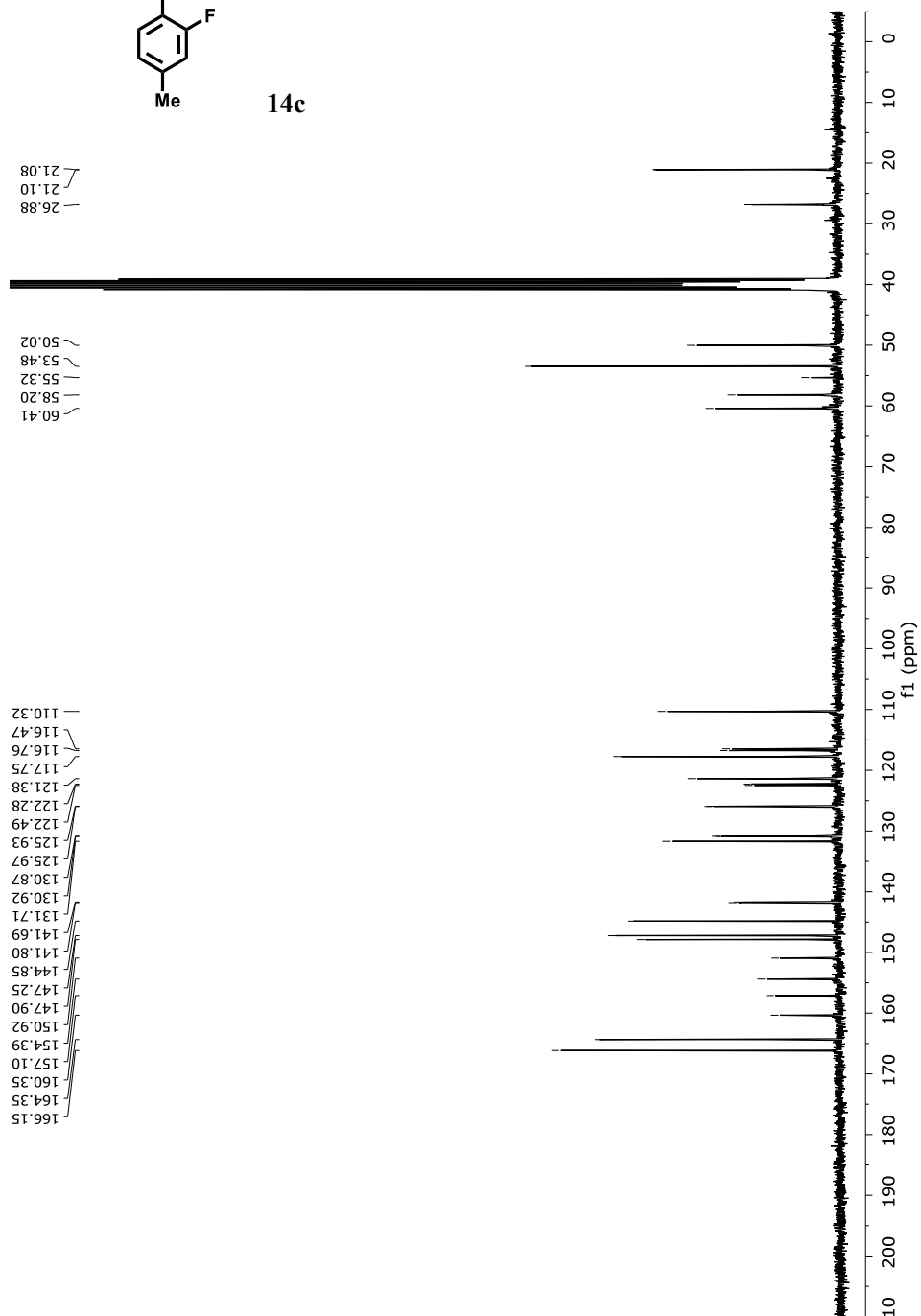
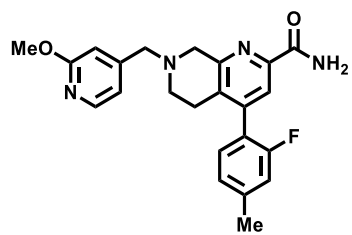
¹³C spectrum of 14b



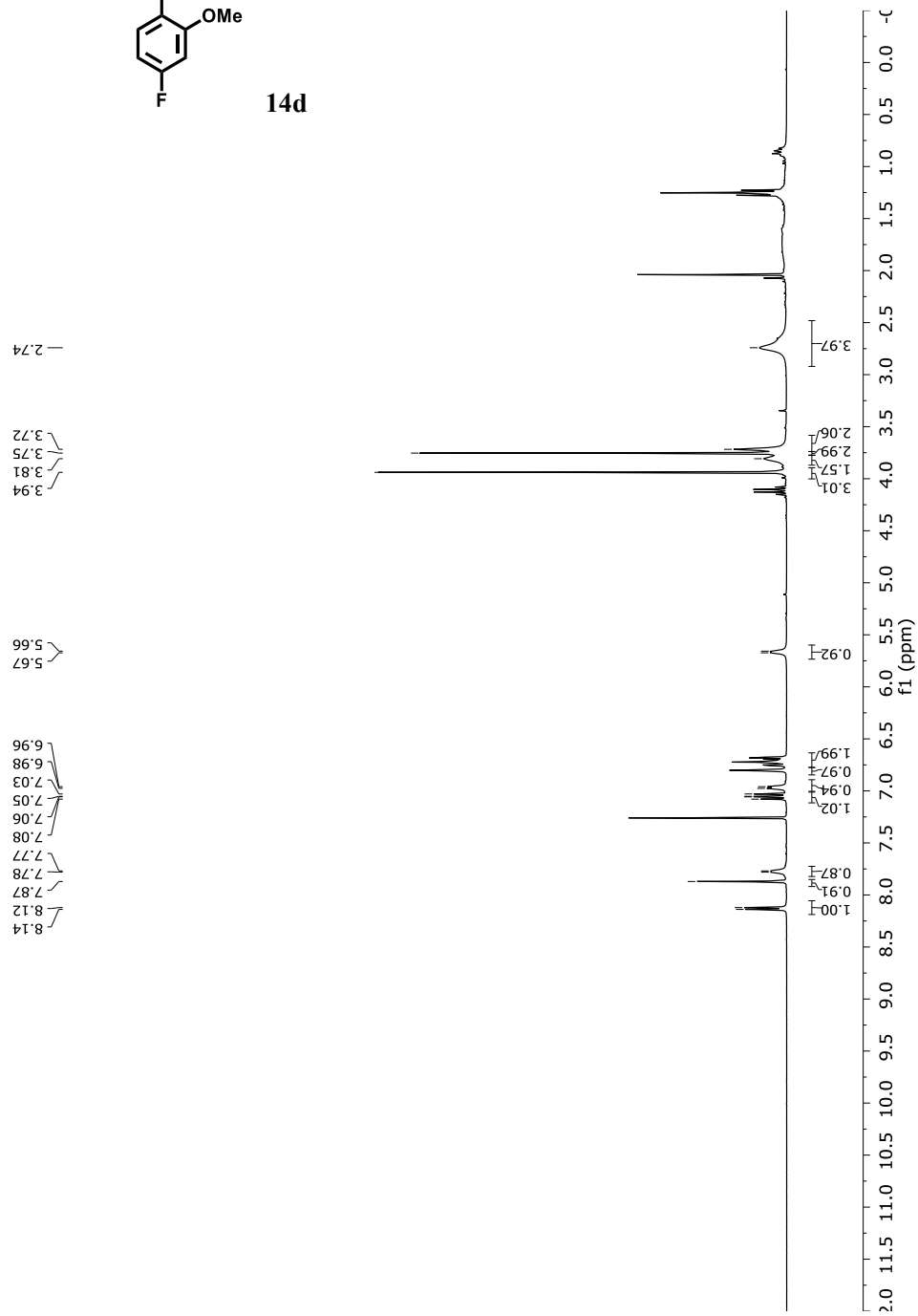
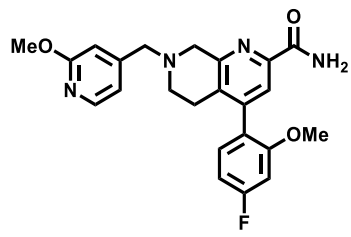
¹H spectrum of 14c



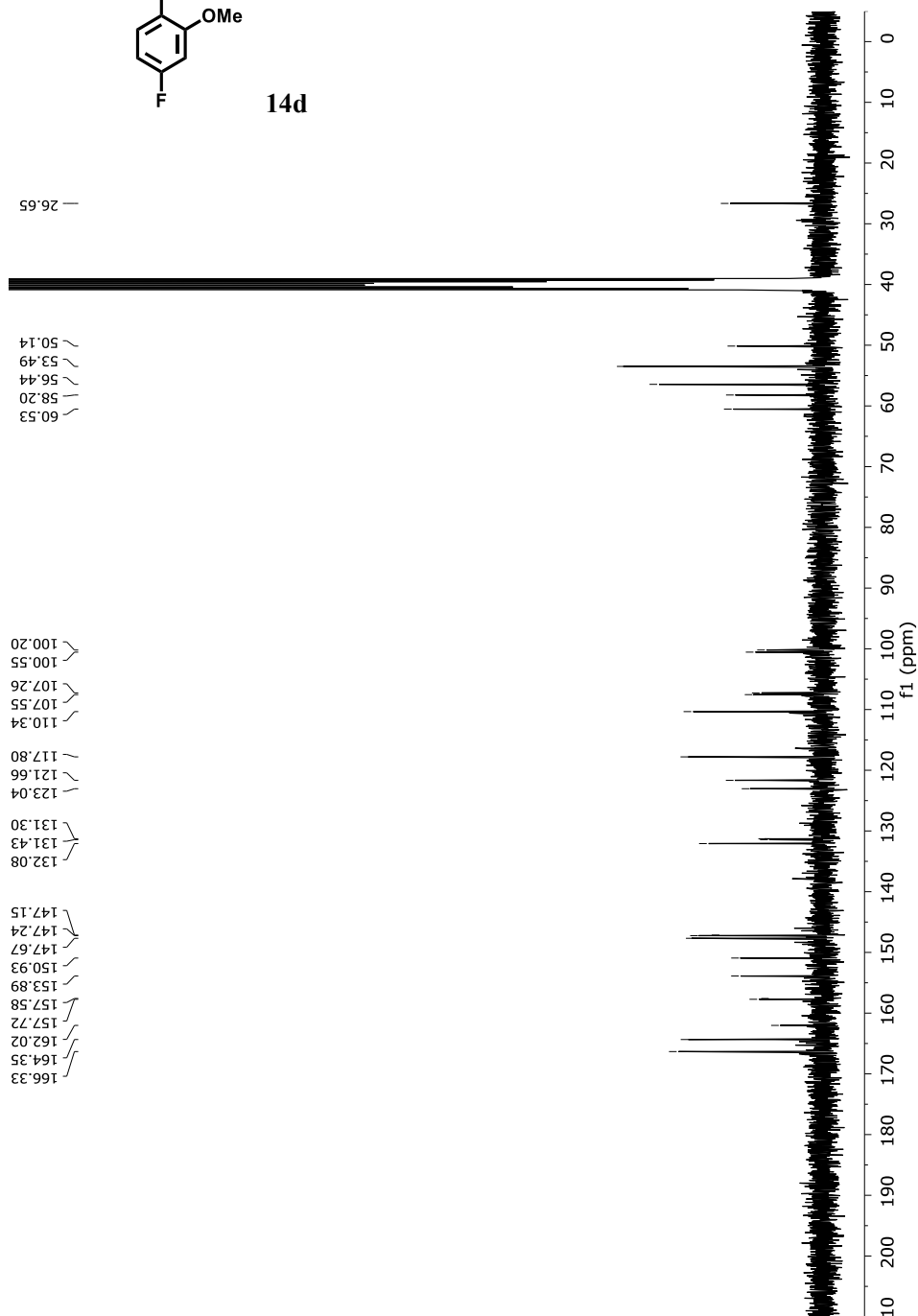
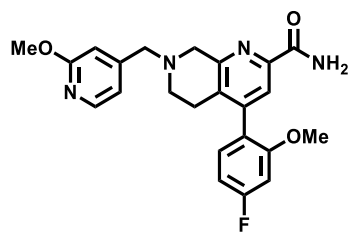
¹³C spectrum of 14c



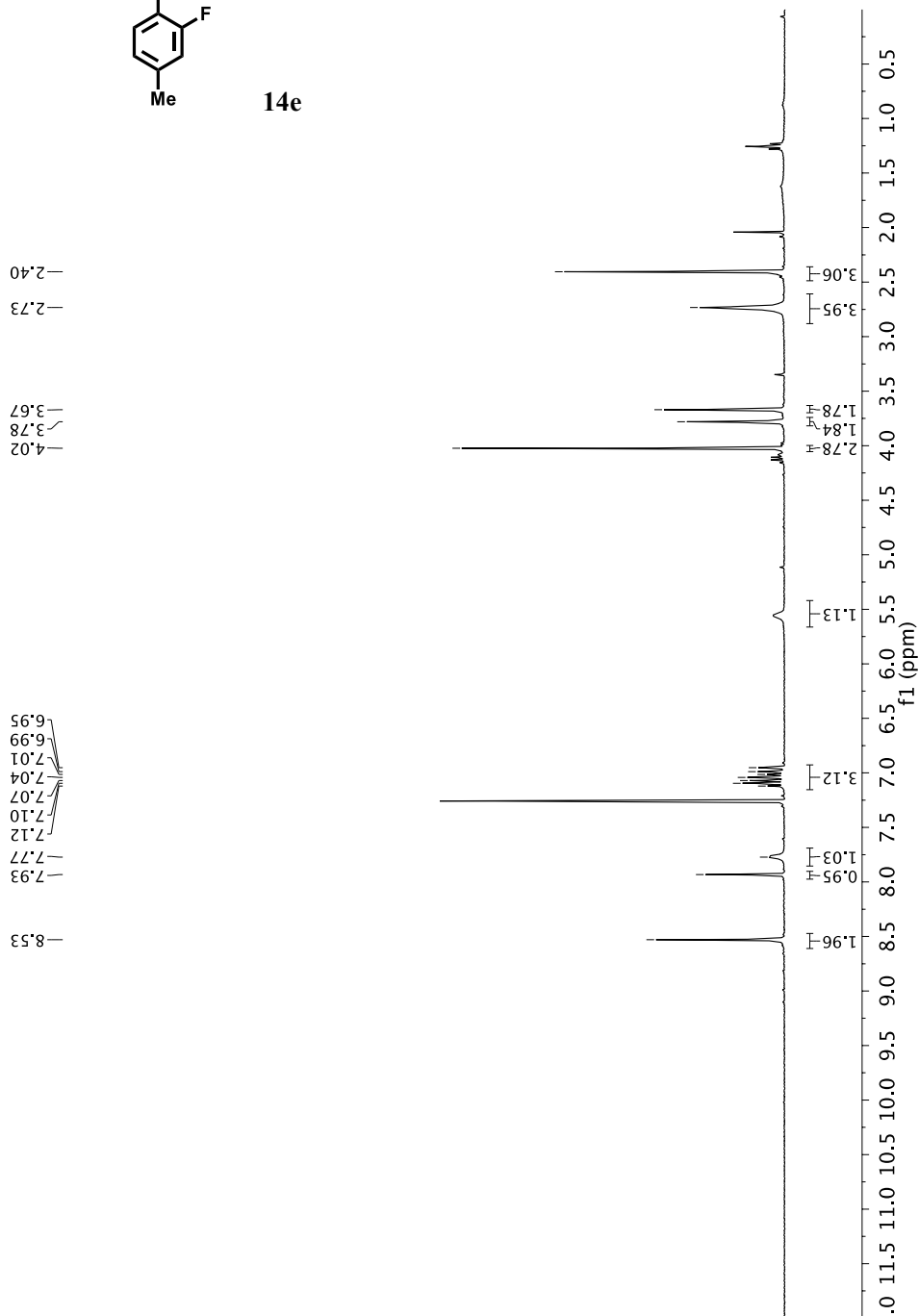
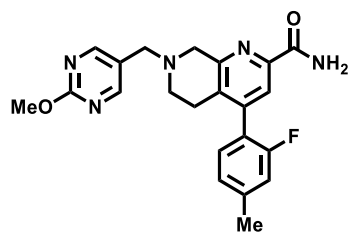
¹H spectrum of 14d



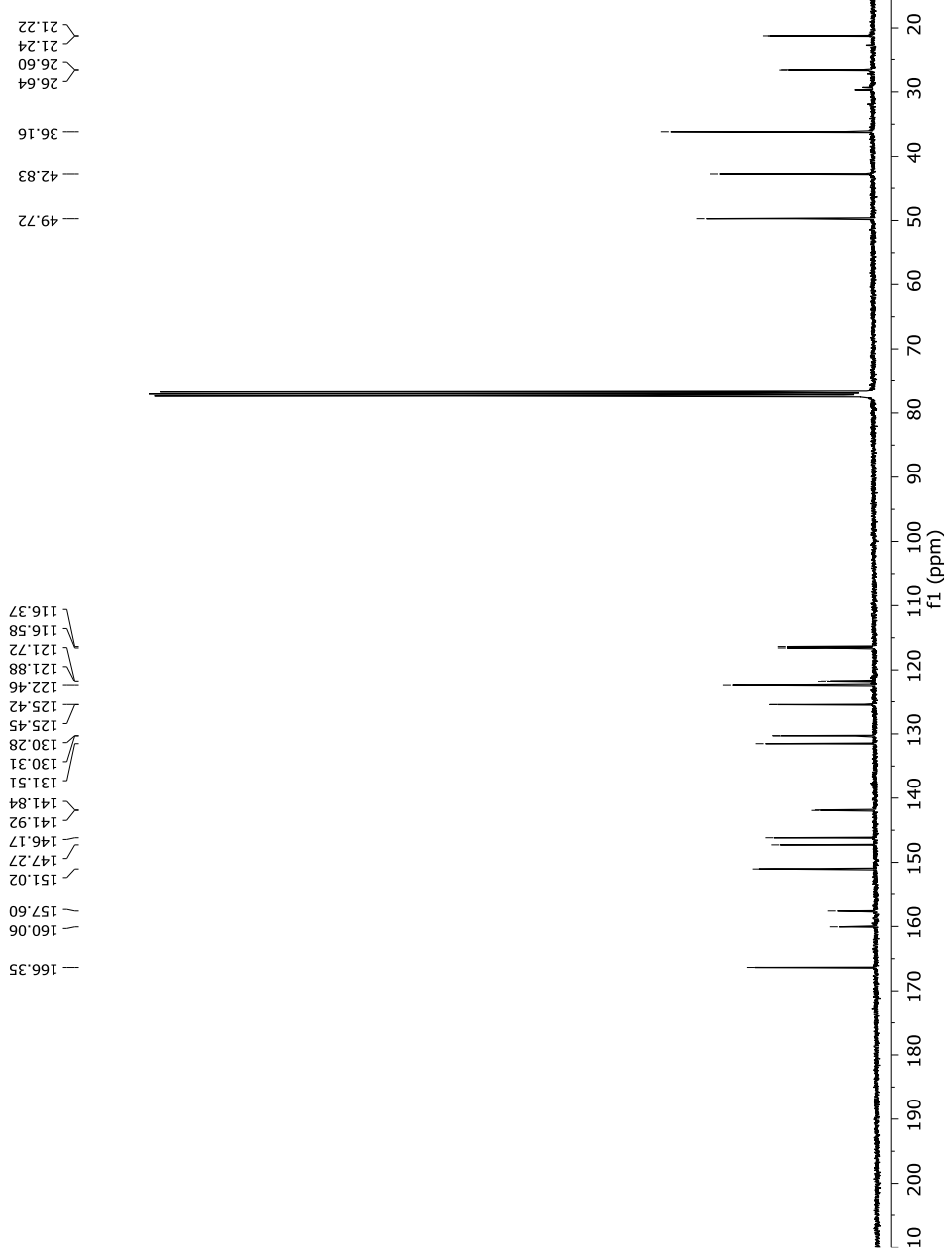
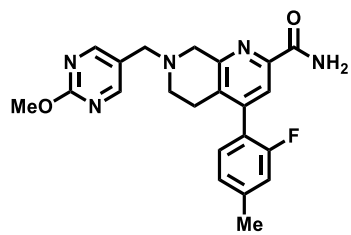
¹³C spectrum of 14d



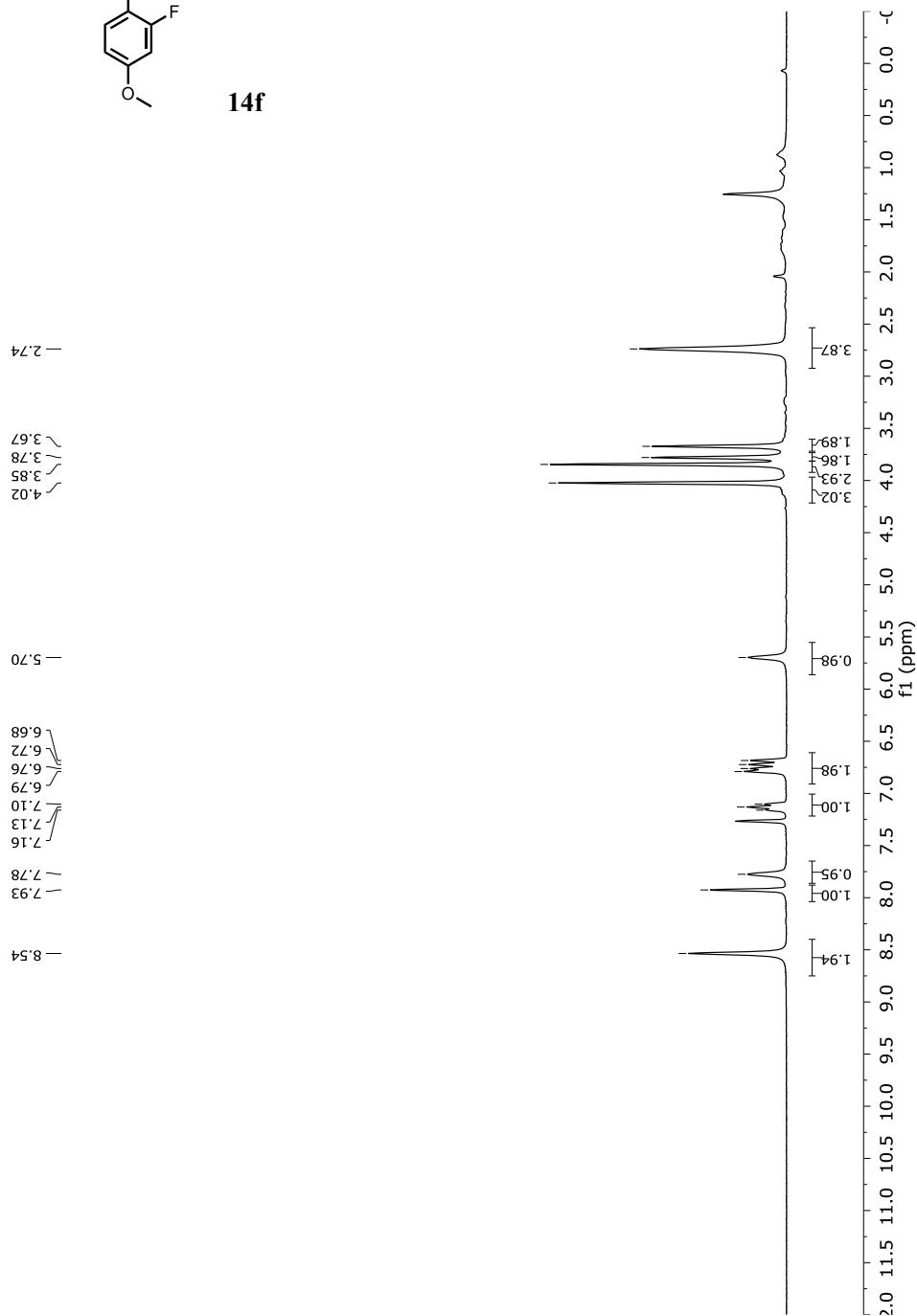
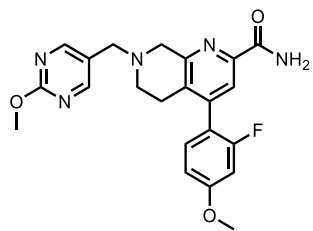
¹H spectrum of 14e



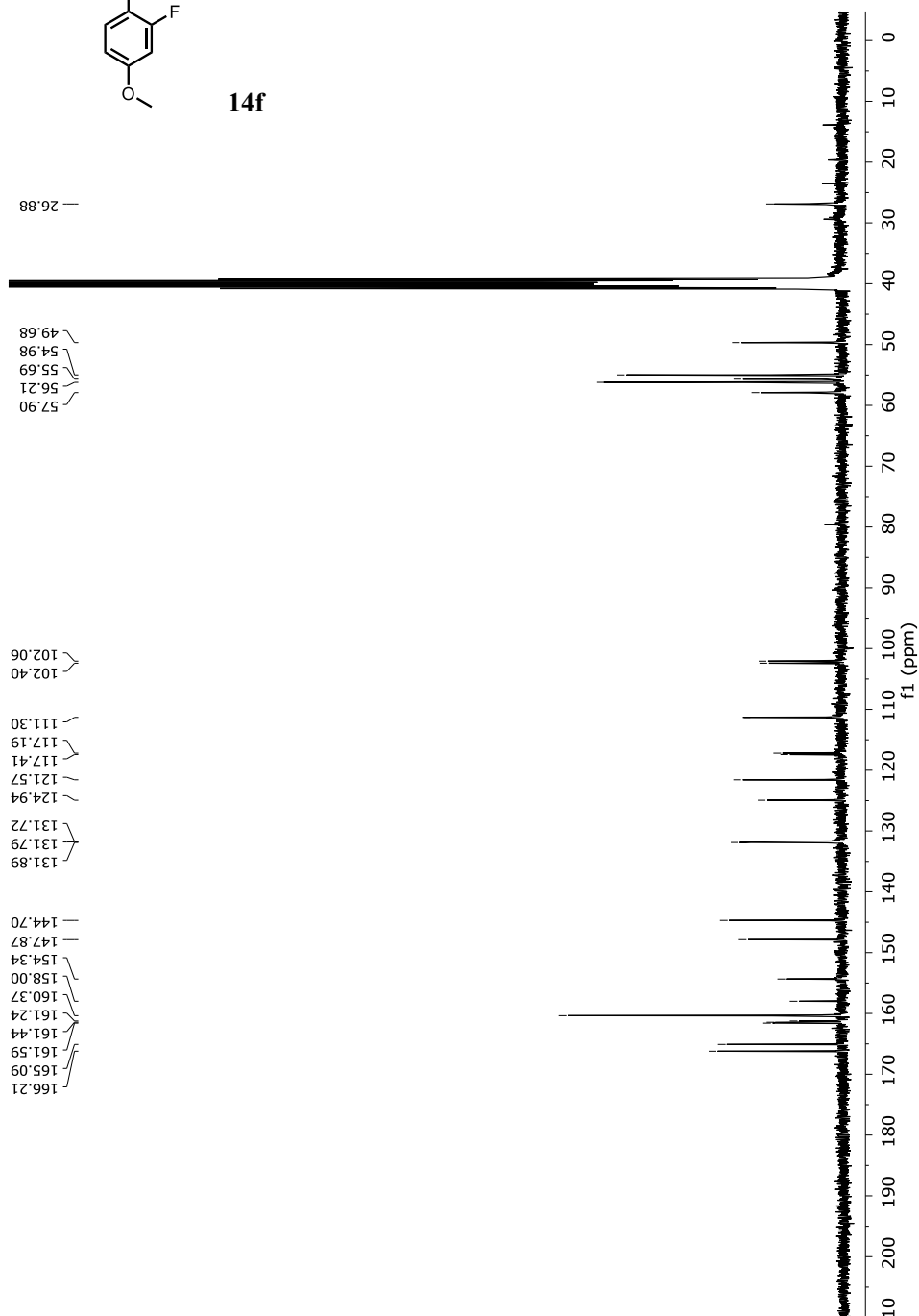
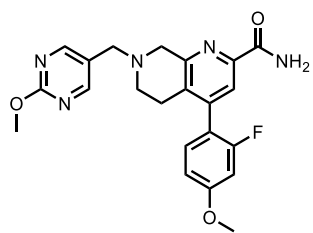
¹³C spectrum of 14e



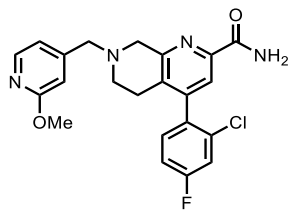
¹H spectrum of 14f



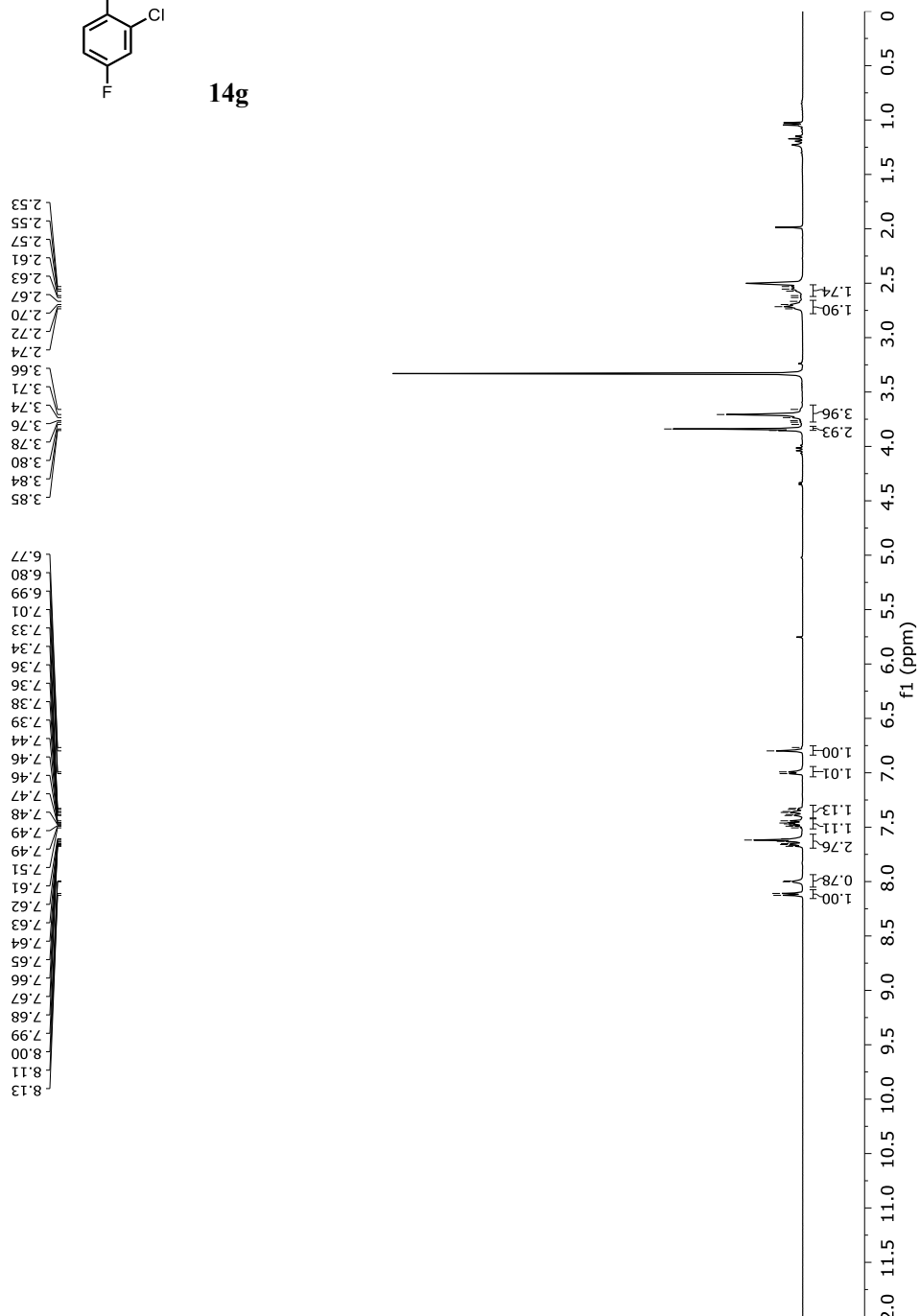
¹³C spectrum of 14f



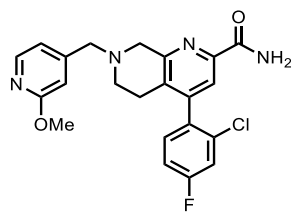
¹H spectrum of 14g



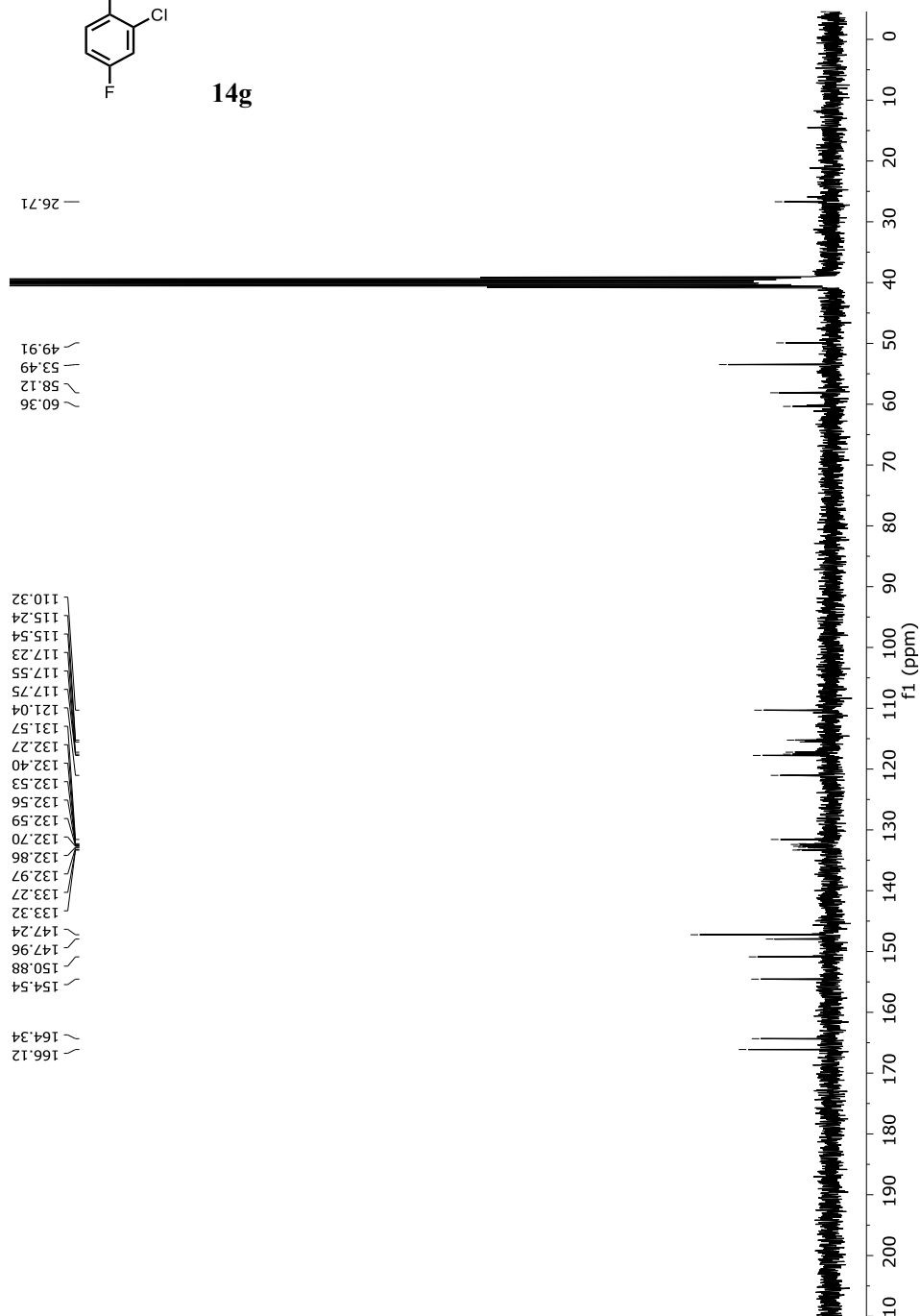
14g



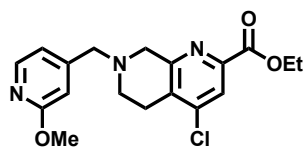
¹³C spectrum of 14g



14g



¹H spectrum of 16



16

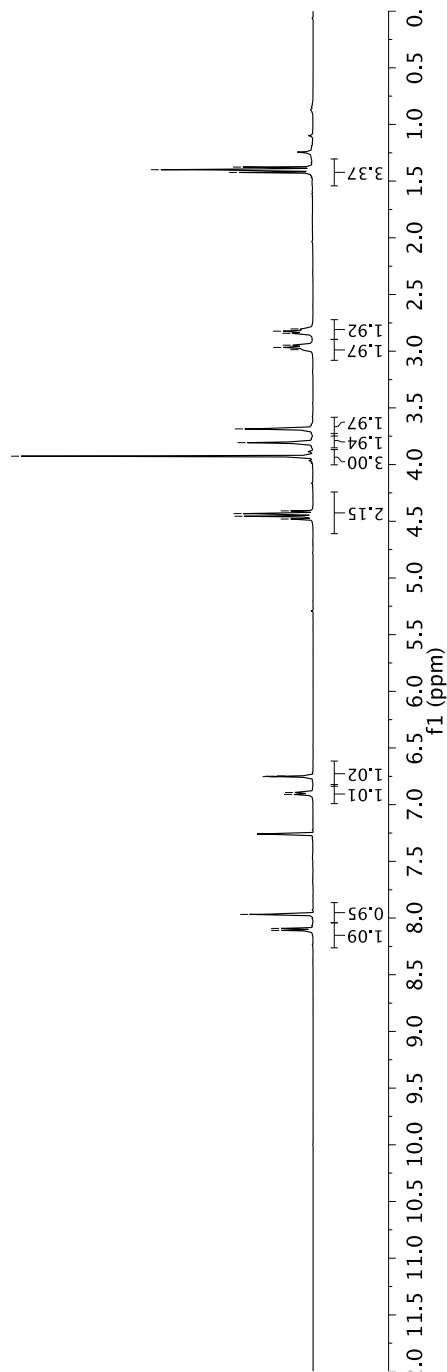
1.42
1.40
1.38

2.80
2.82
2.84
2.95
2.97
2.99

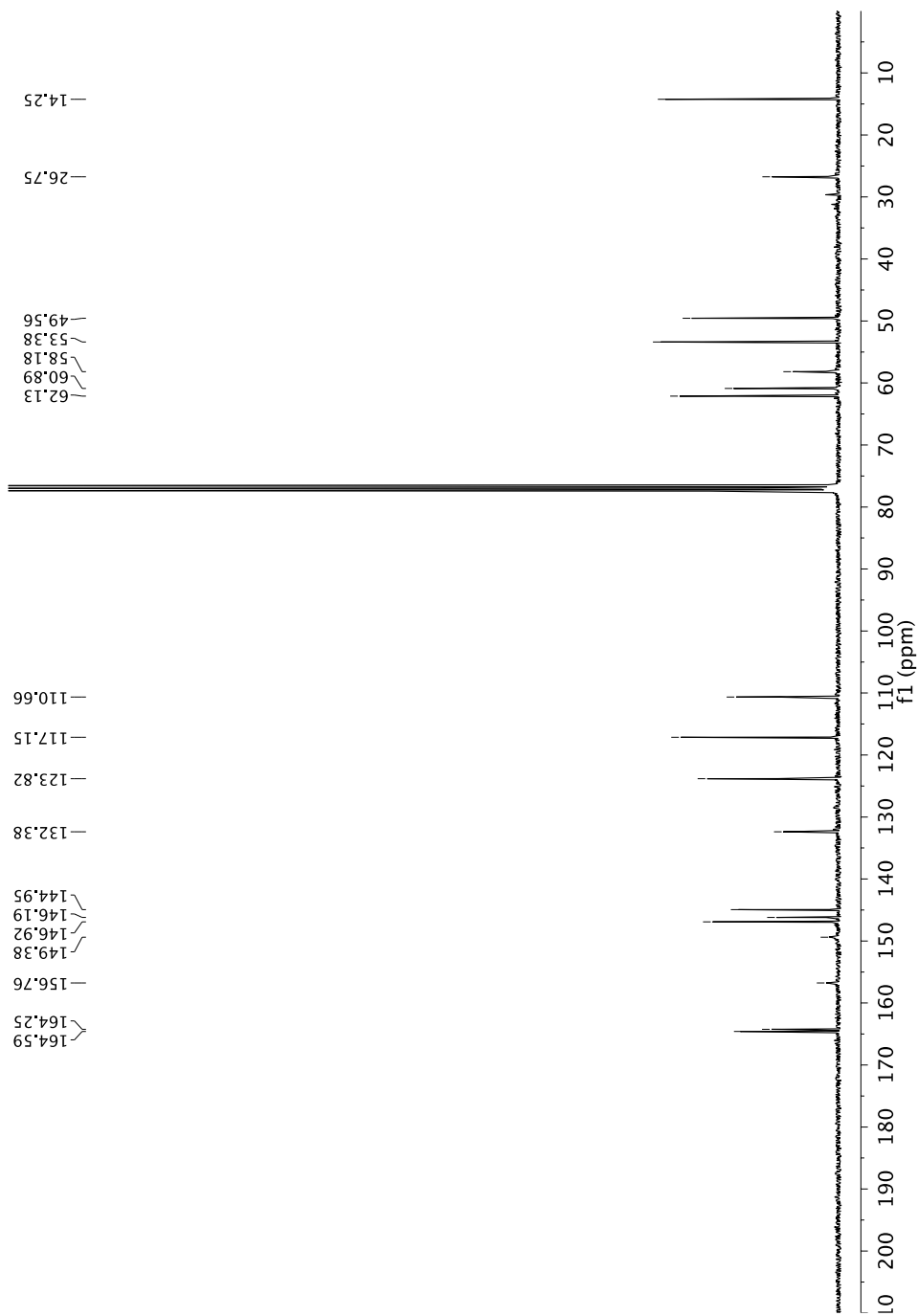
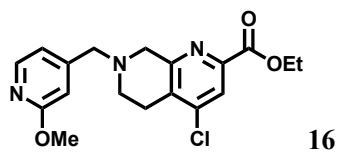
3.69
3.81
3.93
4.41
4.43
4.46
4.48

6.75
6.75
6.75
6.75
6.89
6.91

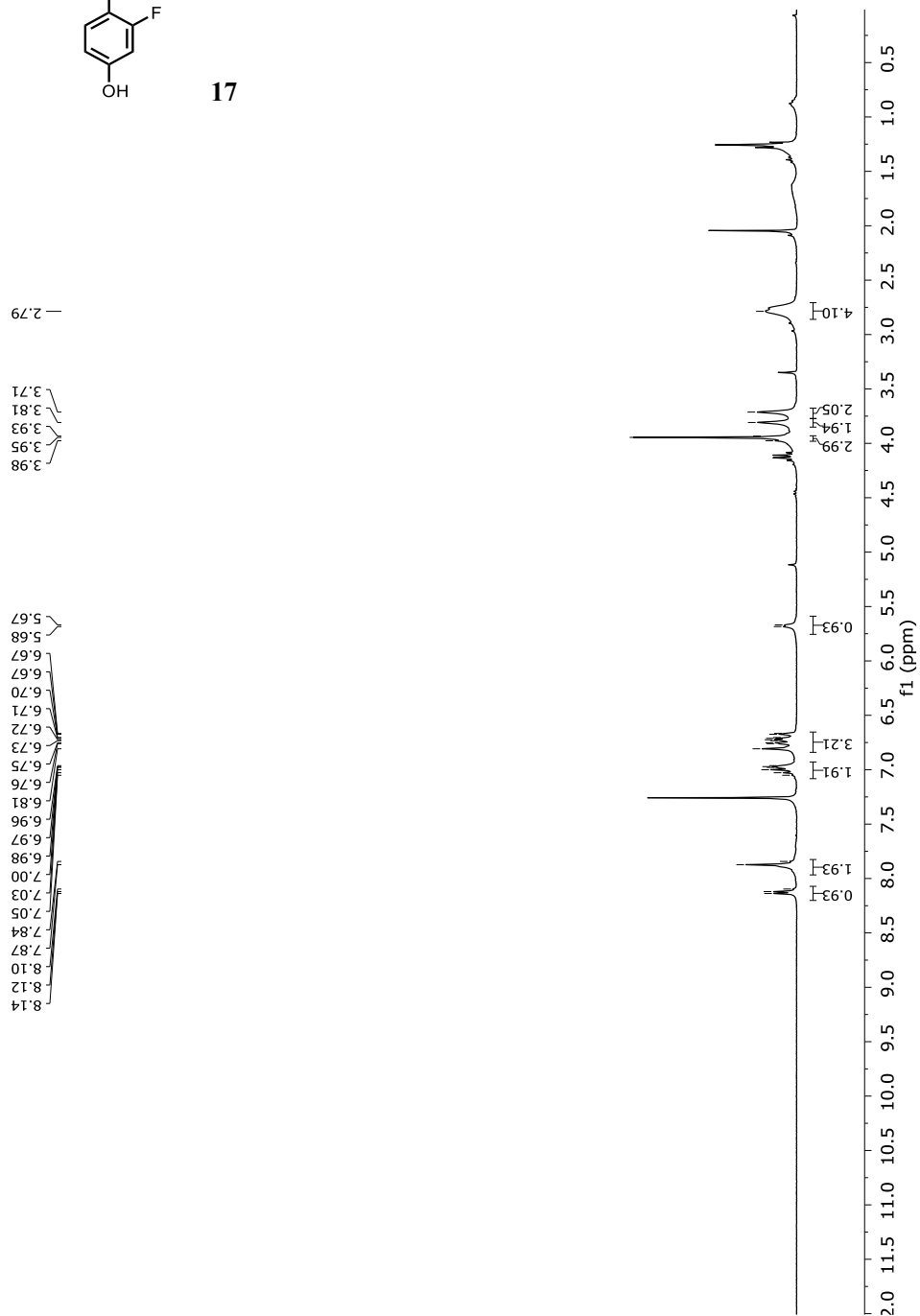
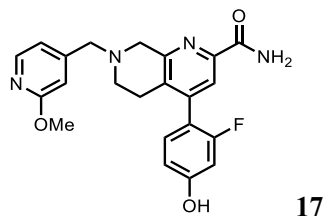
7.97
8.09
8.11



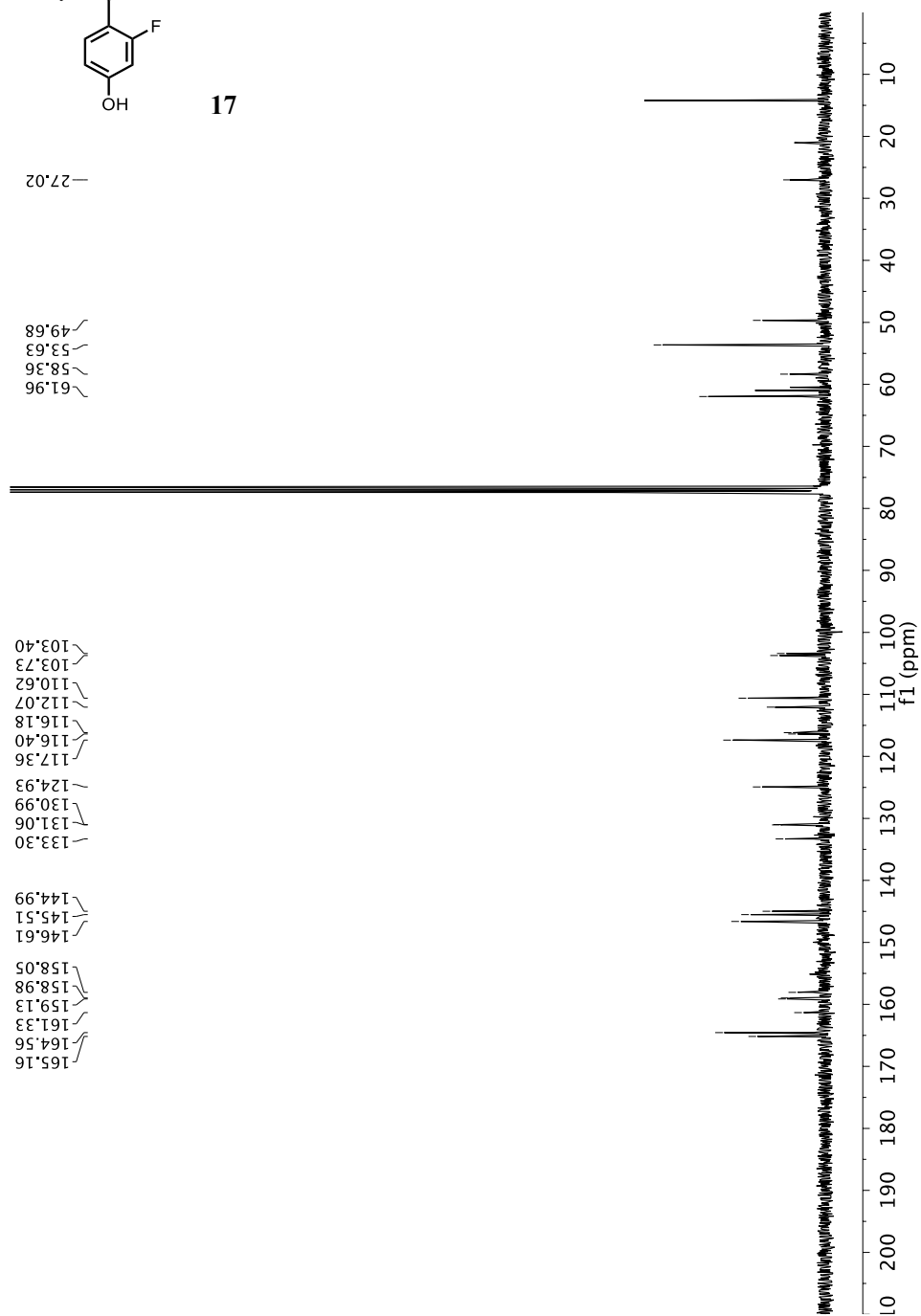
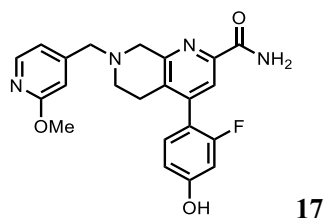
¹³C spectrum of 16



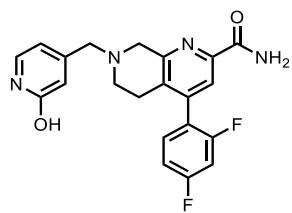
¹H spectrum of 17



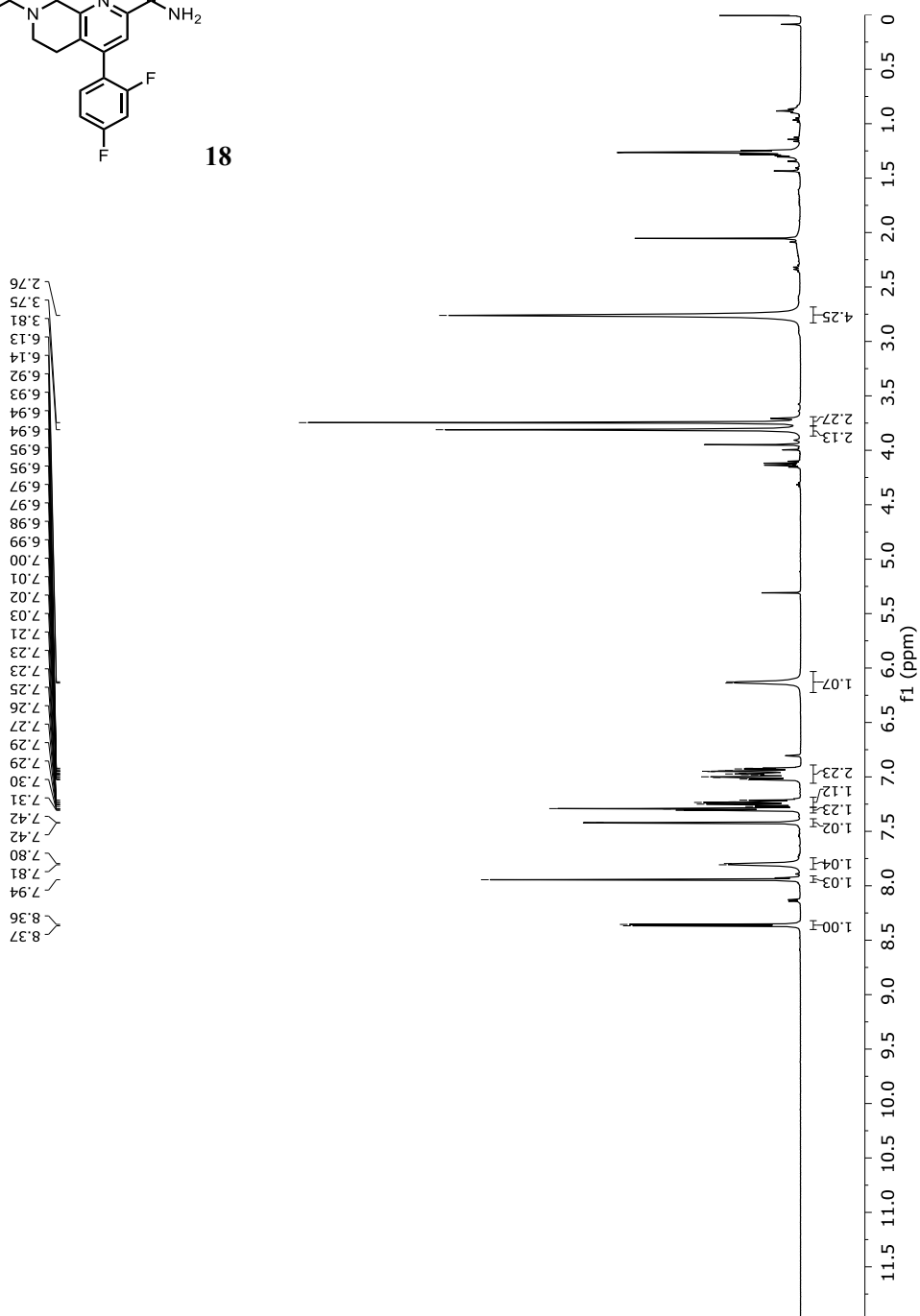
¹³C spectrum of 17



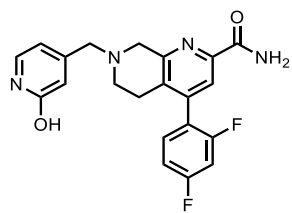
¹H spectrum of 18



18



¹³C spectrum of 18



18

