A Facile hybrid 'flow and batch' access to substituted 3,4-dihydro-2*H*-benzo[*b*][1,4]oxazinones

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Electronic supporting information

Contents

N-Ethyl-4-hydroxy-3-nitrobenzamide (6)

Using a two pump equipped Vapourtec R4 system two separate reservoirs were charged with (a) a solution of 4-hydroxy-3-benzoate (**4**, 0.92 mg, 5.0 mmol), COMU (2.6 g, 6.05 mmol) and iPr_2NEt (1.76 mL, 10.1 mmol) in CH_3CN (25 mL); and (b) a solution ethylamine (0.28 mL, 10.1 mmol of a 70% aqueous solution) in DMF (15 mL). These solutions were placed on the reagent shelf and the two pumps promised and set to flow at 0.5 $mL.min^{-1}$ (for a total flow rate of 1.0 $mL.min^{-1}$). A single 10 mL PFA coil reactor was fitted to the Vapourtec R4 and the temperature equilibrated at 60 °C. Once equilibrated, pumping of both solutions commenced at a combined flow rate of 1 $mL.min^{-1}$ (residence time: 10 min) with a backpressure of 5 bar. Reagent pumping was continued, in this instance, for 30 minutes. The resulting product stream was adsorbed on SiO_2 and, then, subjected to column chromatography (SiO_2) with a gradual elution of 0:1 to 1:0 v/v EtOAc/n-Hexane.

Concentration of the relevant fractions (R_f: 0.3 – 1:1 v/v EtOAc/n-Hexane) afforded a yellow solid (340 mg, 32 %). m.p. = 122–124°C;

^1H NMR (400 MHz, $CDCl_3$) δ 10.75 (s, 1H), 8.51 (d, J = 2.2 Hz, 1H), 8.06 (dd, J = 8.8, 2.2 Hz, 1H), 7.22 (d, J = 8.8 Hz, 1H), 6.17 (s, 1H), 3.55 – 3.48 (m, 2H), 1.28 (t, J = 7.3 Hz, 3H);

^{13}C NMR (101 MHz, $CDCl_3$) δ 164.7, 157.2, 136.3, 133.1, 127.3, 123.8, 120.6, 35.4, 15.0;

Mass spectrum (ESI, +ve) m/z 211 [$(M+H)^+$, 100%];

HRMS (ESI) calcd. for $C_9H_{11}N_2O_4$ (M+H) 211.0713, found 211.0714;

IR ν_{max} (cm^{-1}) 3301, 3279, 2996, 1626, 1615, 1520, 1418, 1250, 1240, 1162, 1076, 756, 658, 618, 582, 487, 425.

Ethyl 3-amino-4-hydroxybenzoate (7)

A single reagent bottle was charged with ethyl 4-hydroxy-3-nitrobenzoate (**5**, 130 mg, 0.62 mmol) in CH_3CN (12 mL). This was positioned at the inlet end of the ThalesNano H-Cube Pro® pumping system. A single 30 mm 10% Pd/C CatCart® catalyst installed in line and the flow rate set to 3 $mL.min^{-1}$ (residence time: 4 min) with the catalyst bed equilibrated at 50 °C and the hydrogen pressure set at 50 bar. Once equilibrated, pumping was the CH_3CN/**5** solution commenced at 3 $mL.min^{-1}$ until the reagent reservoir was emptied. The resulting product stream was concentrated under reduced pressure to afforded a creamy white solid (110 mg, 98 %). m.p. 65.5–67.8 °C, R_f 0.15 (1:9 *v/v* CH_3OH/CH_2Cl_2);

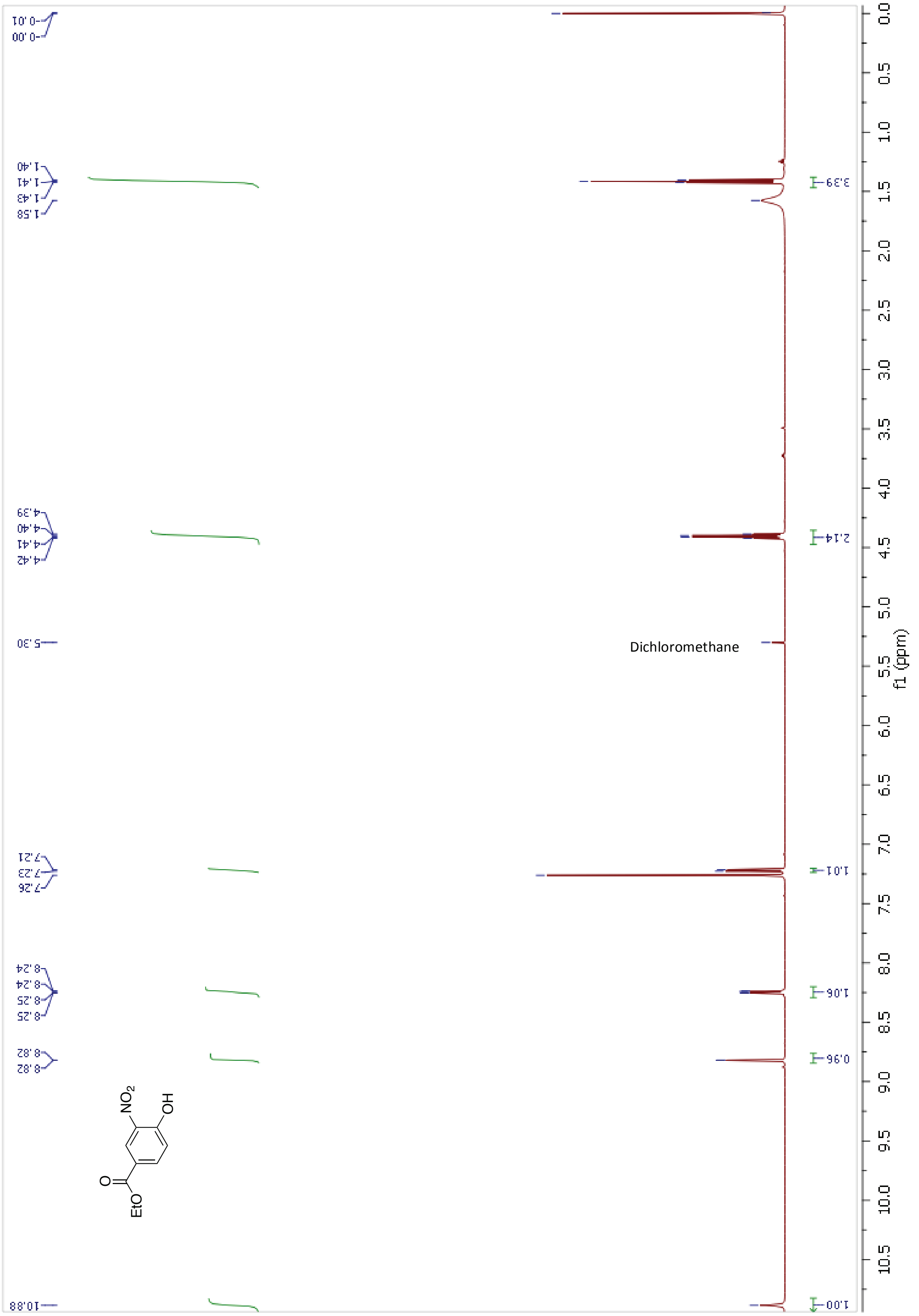
^1H NMR (600 MHz, $CDCl_3$) δ 7.46 (d, J = 1.8 Hz, 1H), 7.43 (dd, J = 8.2, 1.8 Hz, 1H), 6.75 (d, J = 8.2 Hz, 1H), 4.32 (q, J = 7.1 Hz, 2H), 3.74 (broad s, 2H), 1.37 (t, J = 7.1 Hz, 3H);

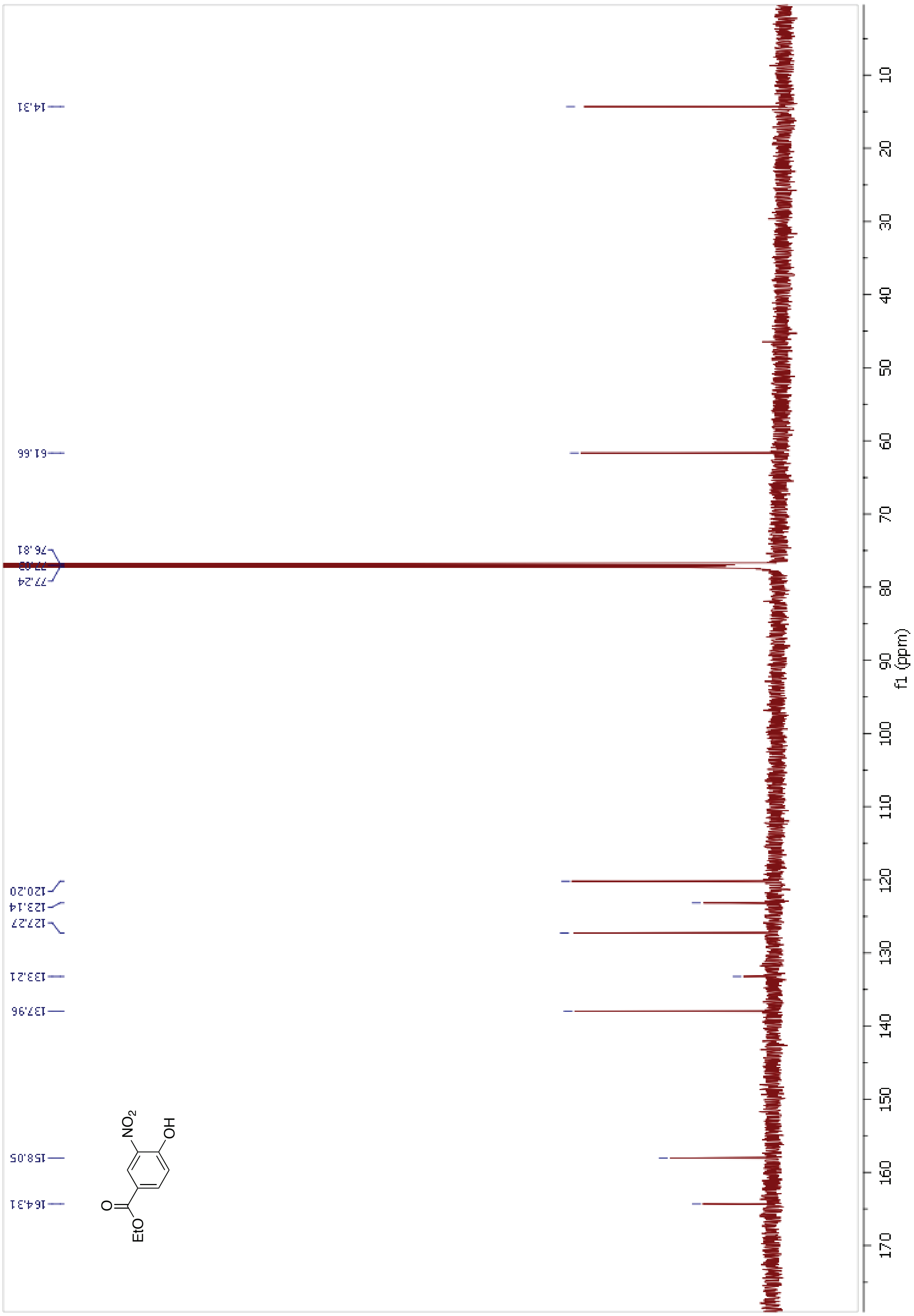
^{13}C NMR (151 MHz, $CDCl_3$) δ 167.2, 149.0, 134.4, 422.7, 122.0, 117.5, 114.5, 60.8, 14.4;

Mass spectrum (ESI, +ve) m/z 265 [$(M+i PrOH+Na+H)^+$, 100%], 182 [$(M+H)^+$, 100%], (ESI, –ve) m/z 180 [$(M–H)^-$, 100%];

HRMS (ESI) calcd. for $C_9H_{10}NO_3$ $(M–H)^–$ 181.1885, found 181.0499; IR ν_{max} (cm^{-1}) 3386, 3100, 2985, 1687, 1602, 1520, 1365, 1302, 1285, 1202, 1151, 1094, 1022, 893, 764, 637, 453.

^{1}H and ^{13}C NMR spectra of product **5**

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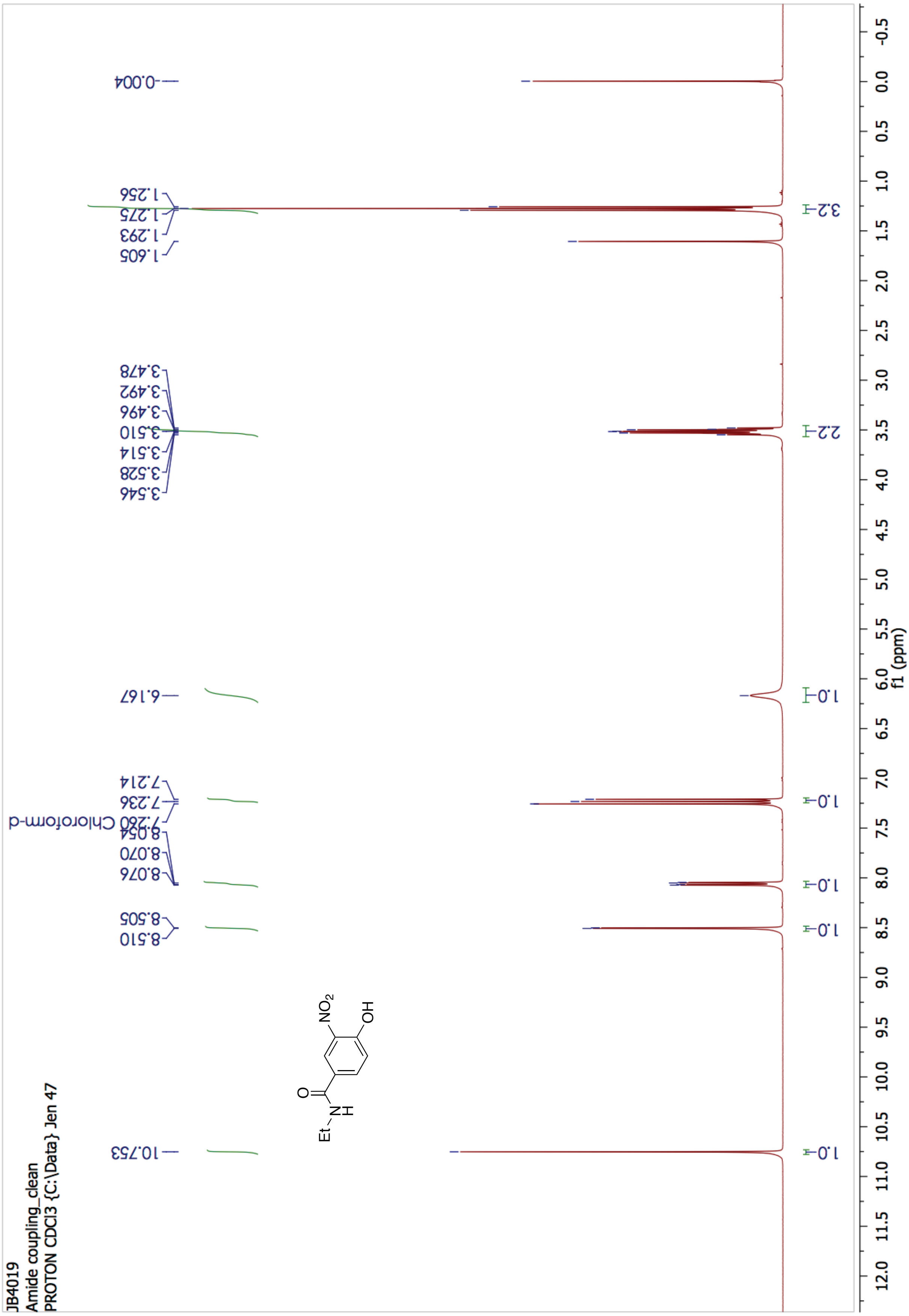
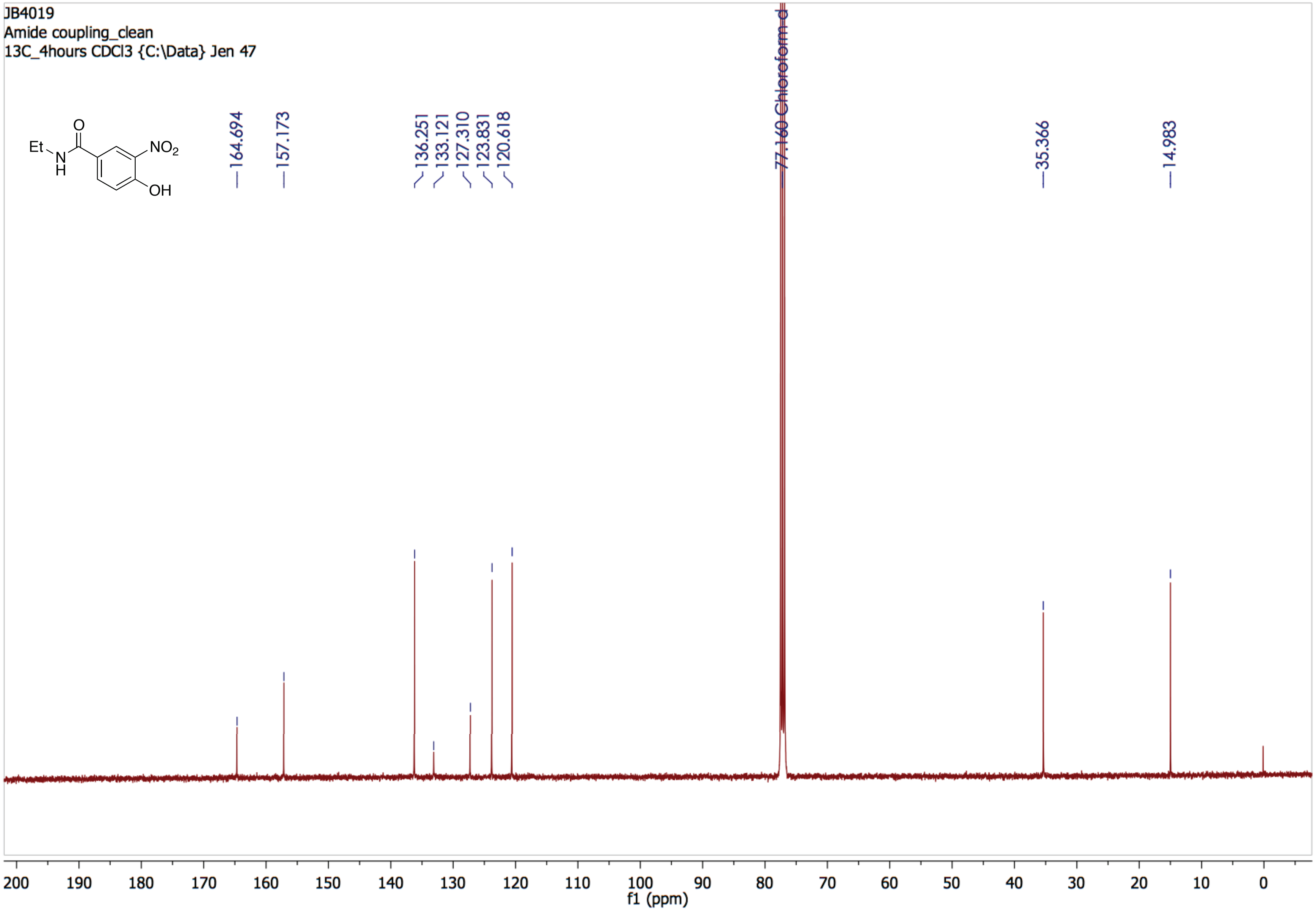
77.03
77.24
120.20
123.14
127.27
133.21
137.96
158.05
164.31
EtO
O
NO_2
OH
f1 (ppm)
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170

[1]H and [13]C NMR spectra of product **6**

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Amide coupling_clean
13C_4hours CDCl3 {C:\Data} Jen 47
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N
H
O
NO2
OH
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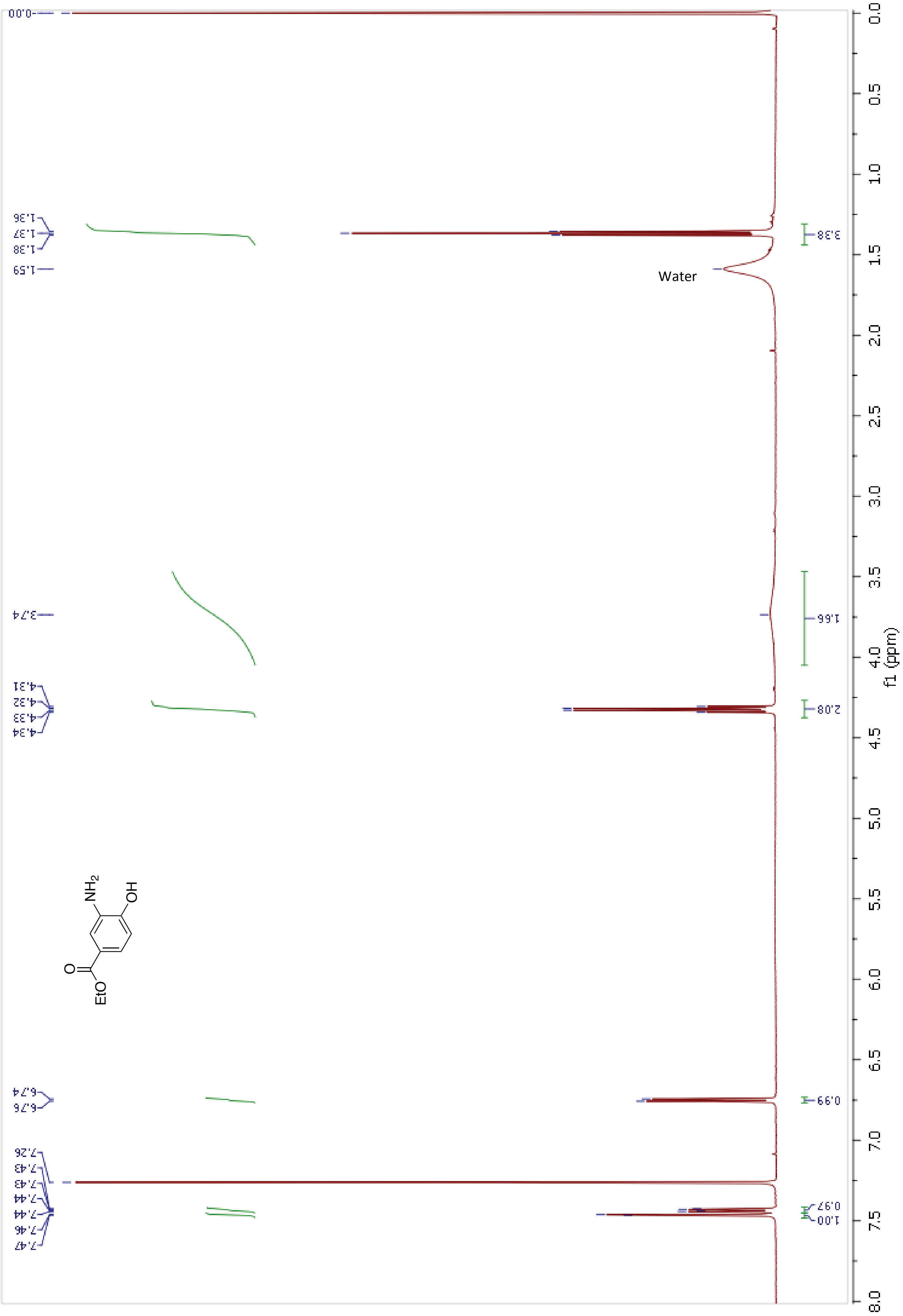
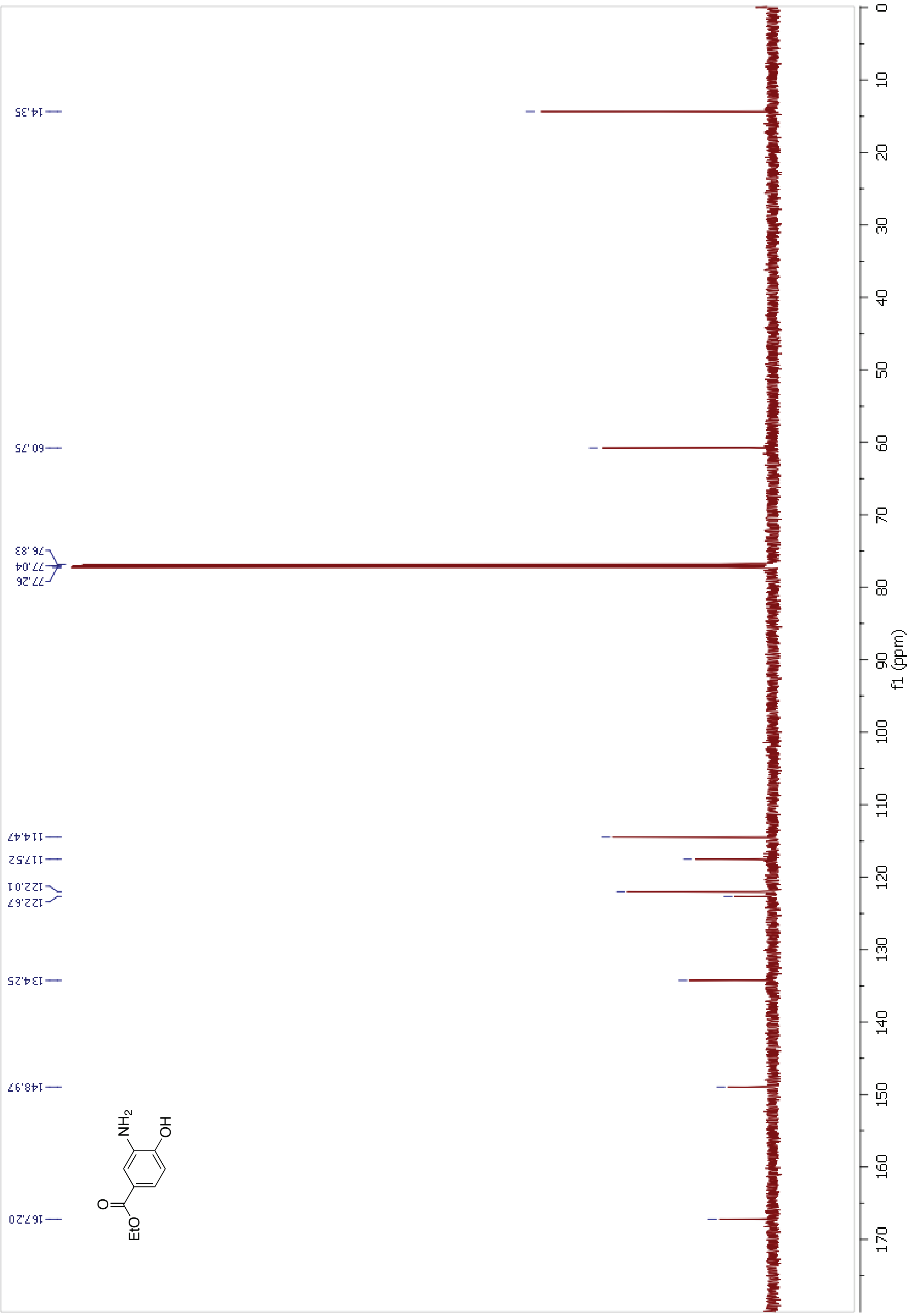
136.251
133.121
127.310
123.831
120.618
77.160 Chloroform-d
35.366
14.983
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

[1]H and [13]C NMR spectra of product **7**

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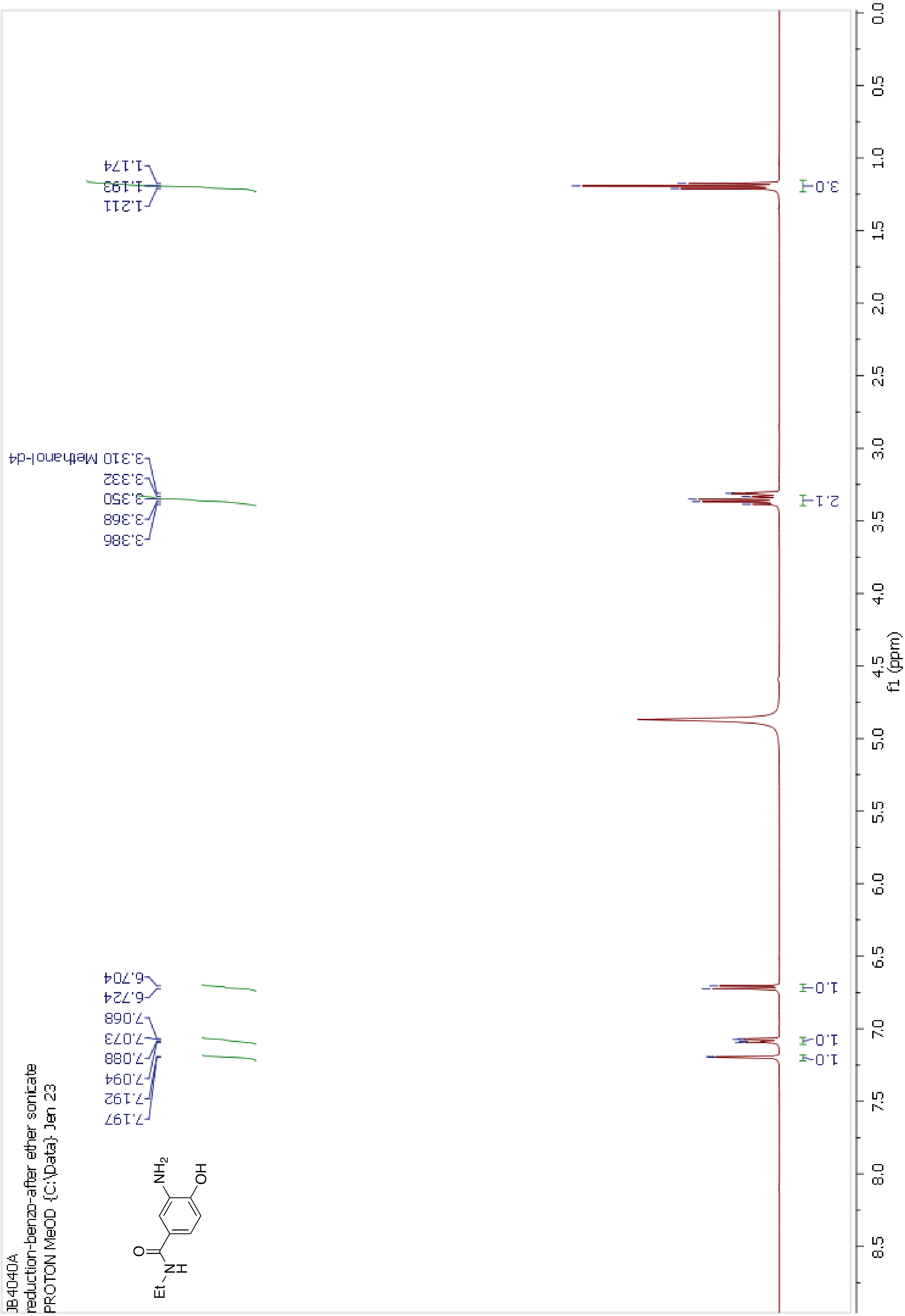
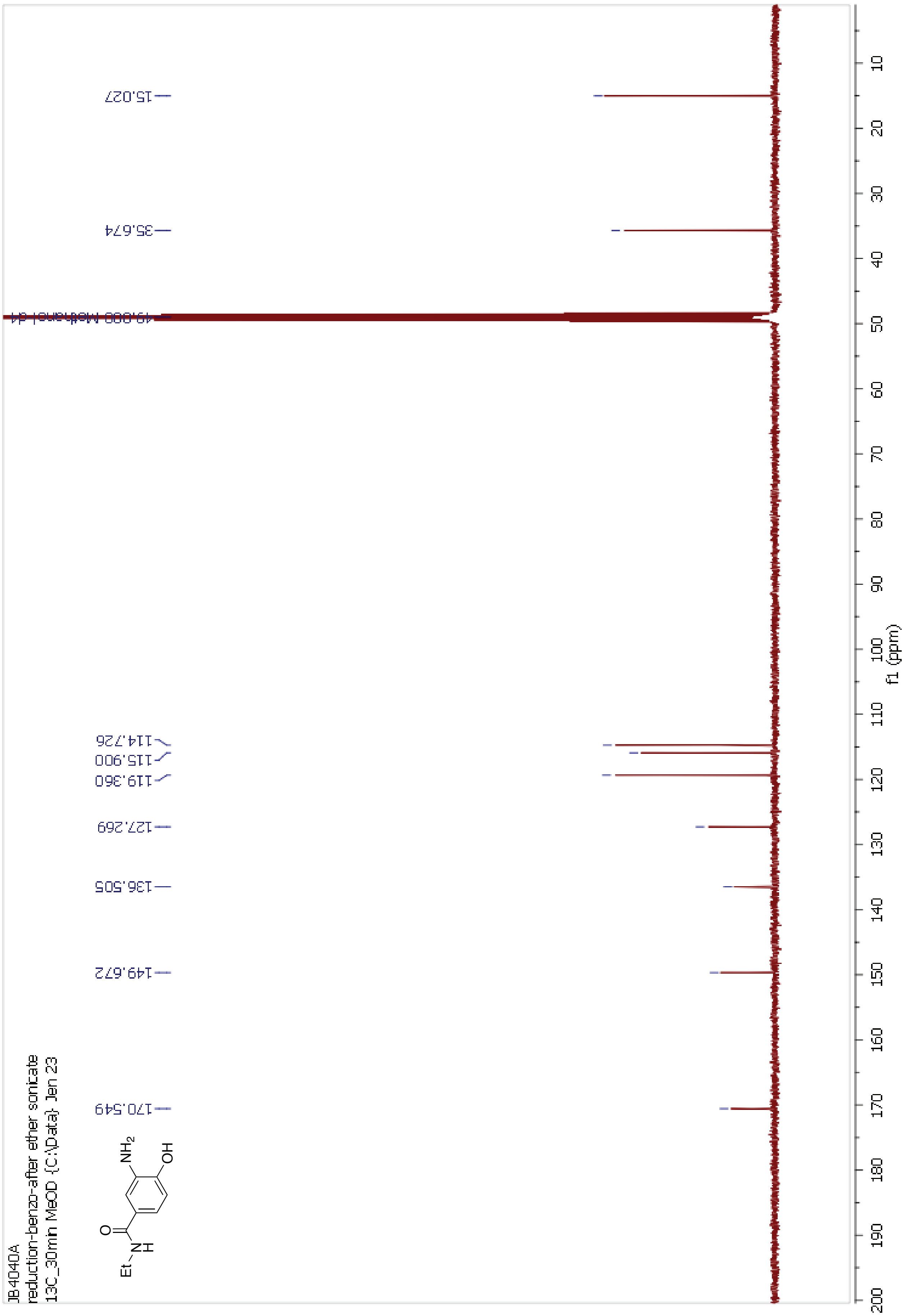
134.25
122.67
122.01
117.52
114.47
77.26
77.04
76.83
60.75
14.35
f1 (ppm)
EtO
O
NH2
OH

[1]H and [13]C NMR spectra of product **8**




JB4040A
reduction-benzo-after ether sonicate
13C_30min MeOD {C:\Data} Jen 23
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N
H
O
NH_2
OH
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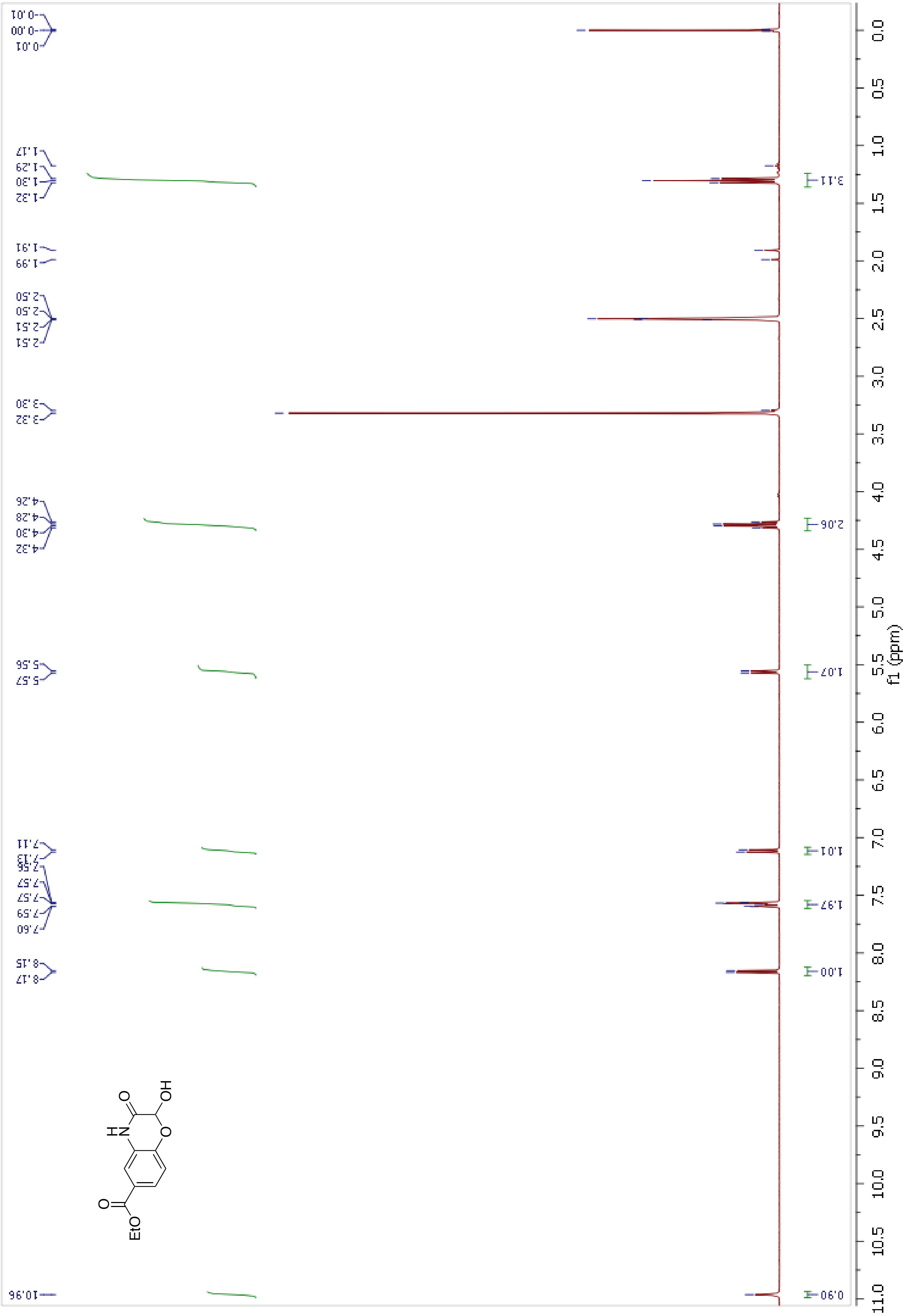
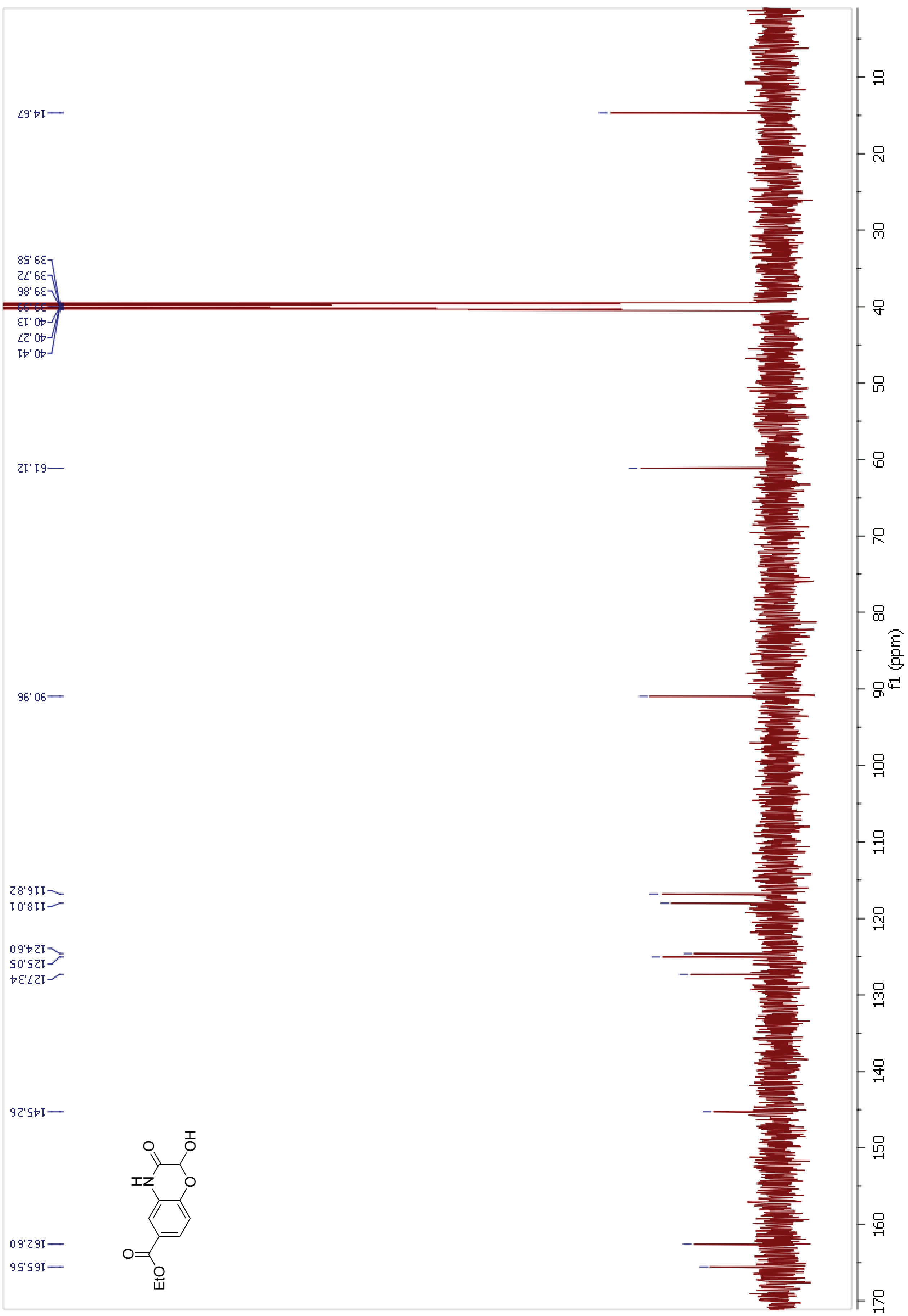
149.672
136.505
127.269
119.360
115.900
114.726
49.000 Methanol-d4
35.674
15.027
f1 (ppm)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

[1]H and [13]C NMR spectra of product **10**





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40.13
40.27
40.41
61.12
90.96
116.82
118.01
124.60
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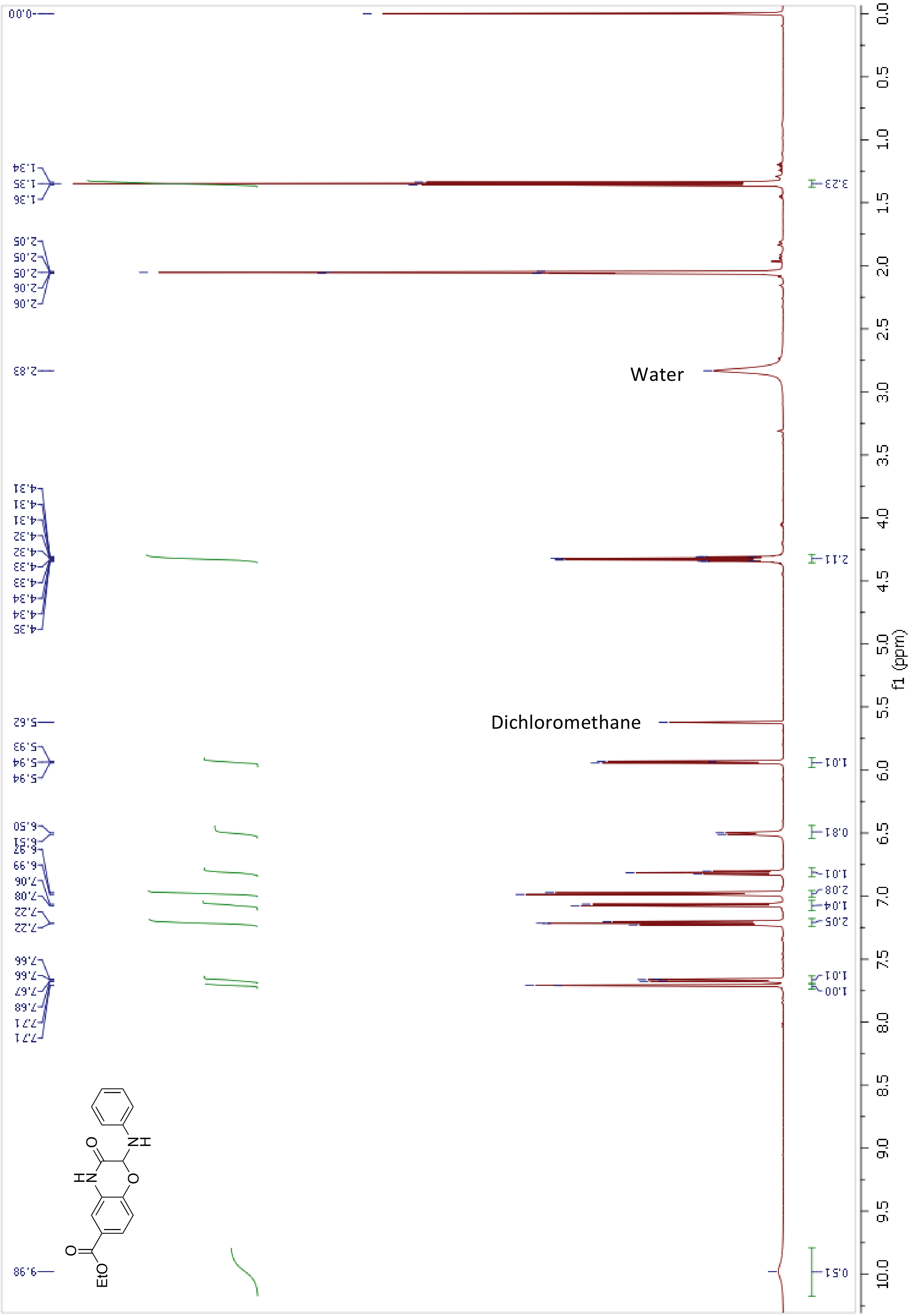
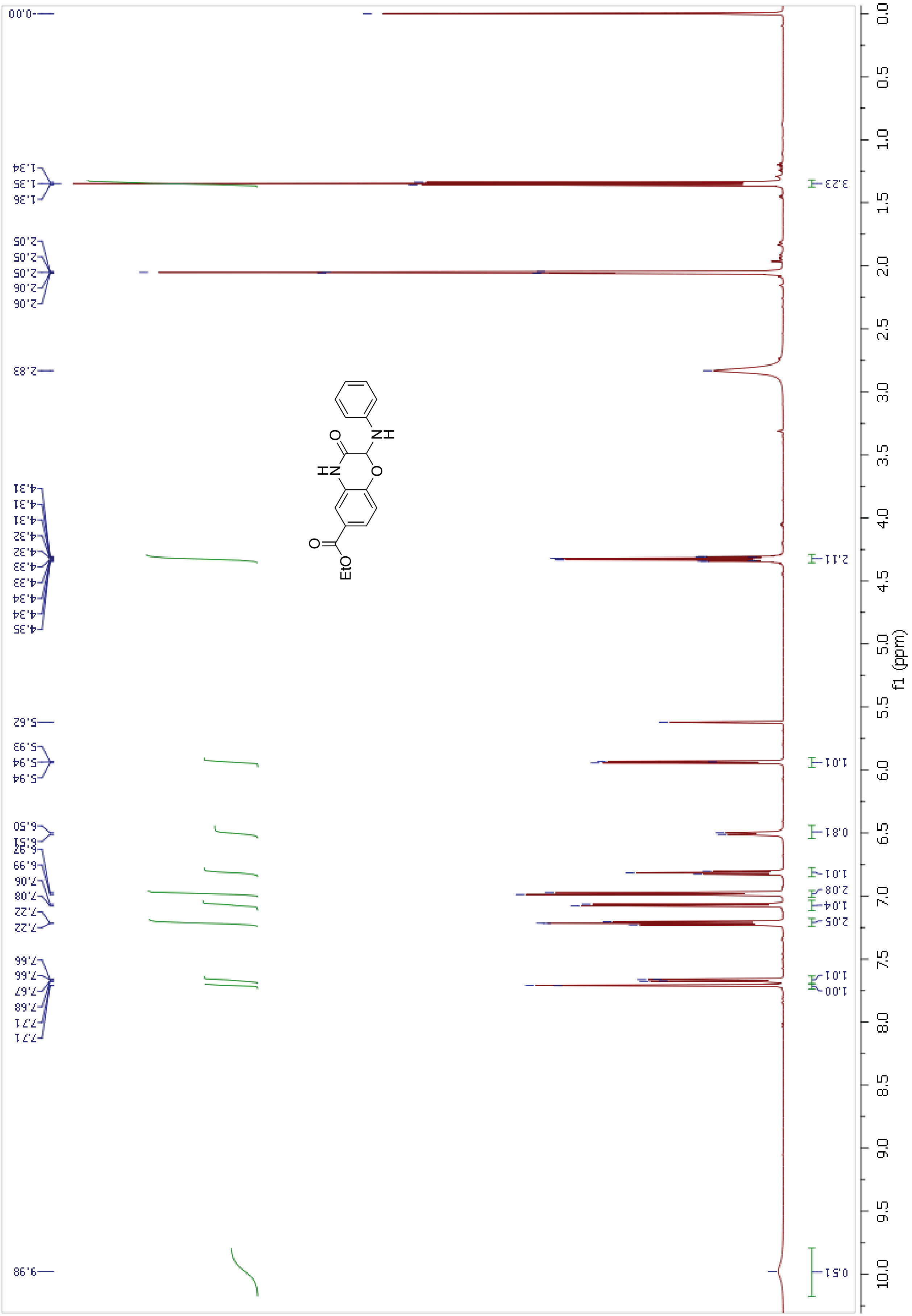
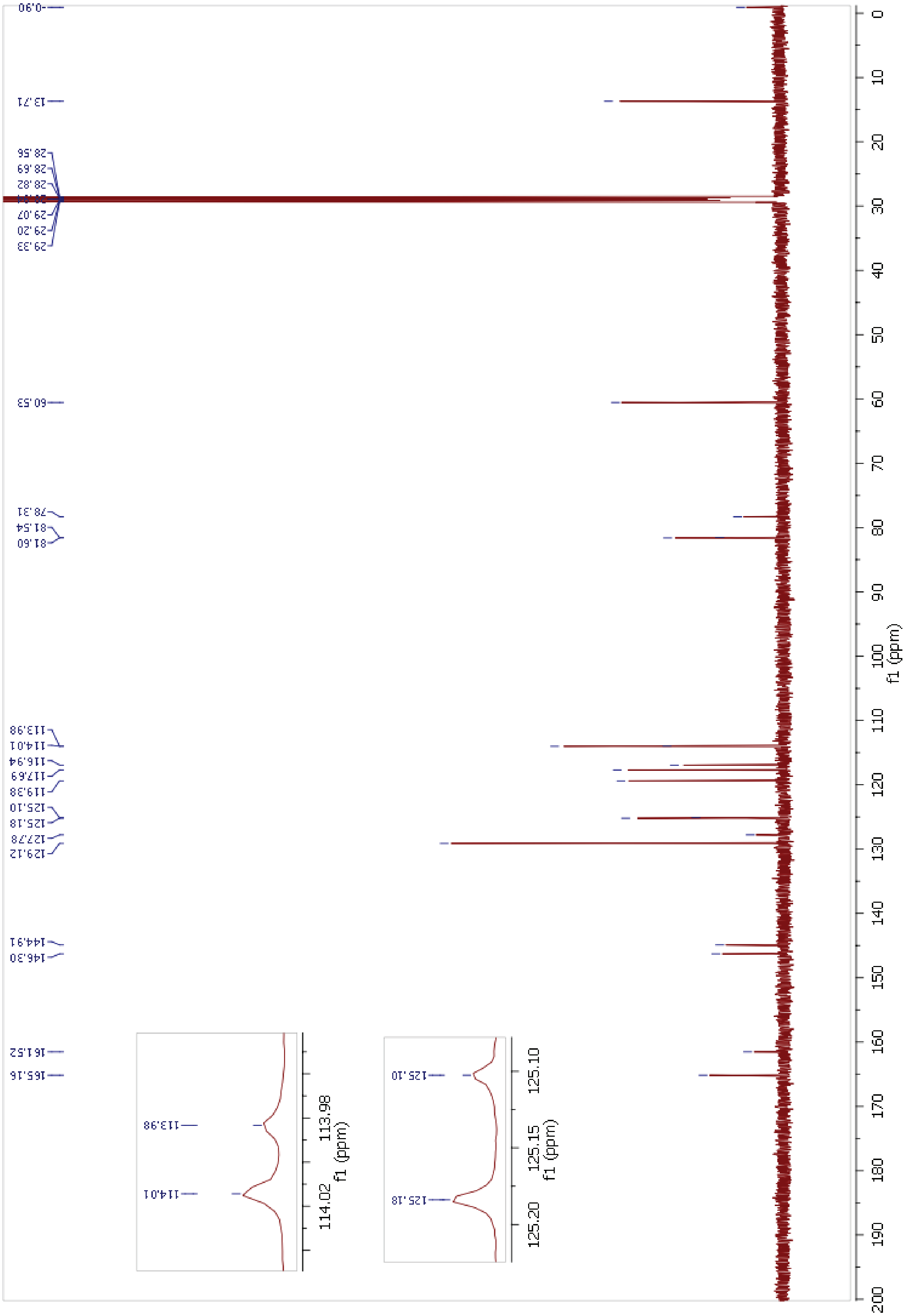
165.56
f1 (ppm)
EtO

^{1}H and ^{13}C NMR spectra of product **12a**



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29.33
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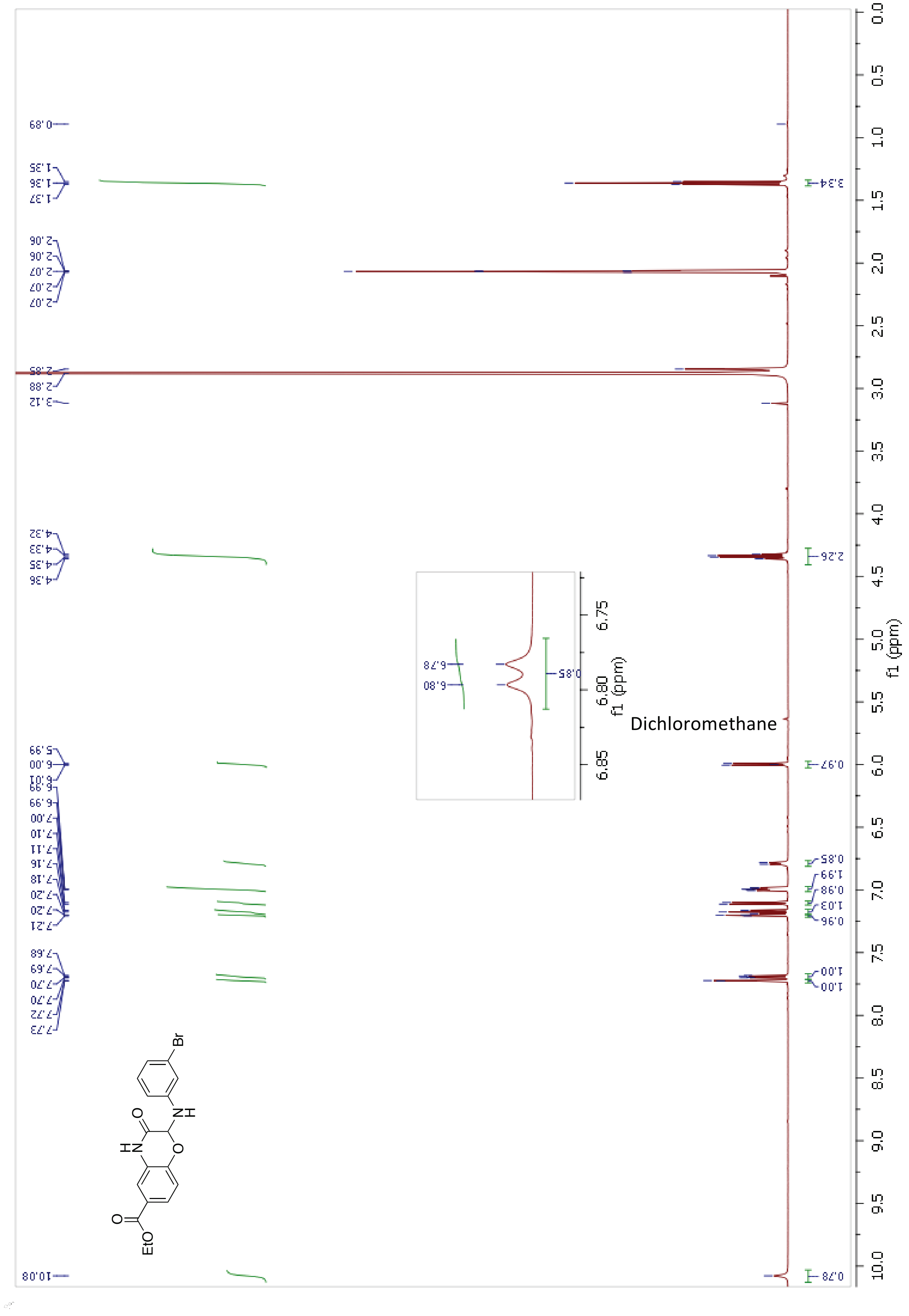
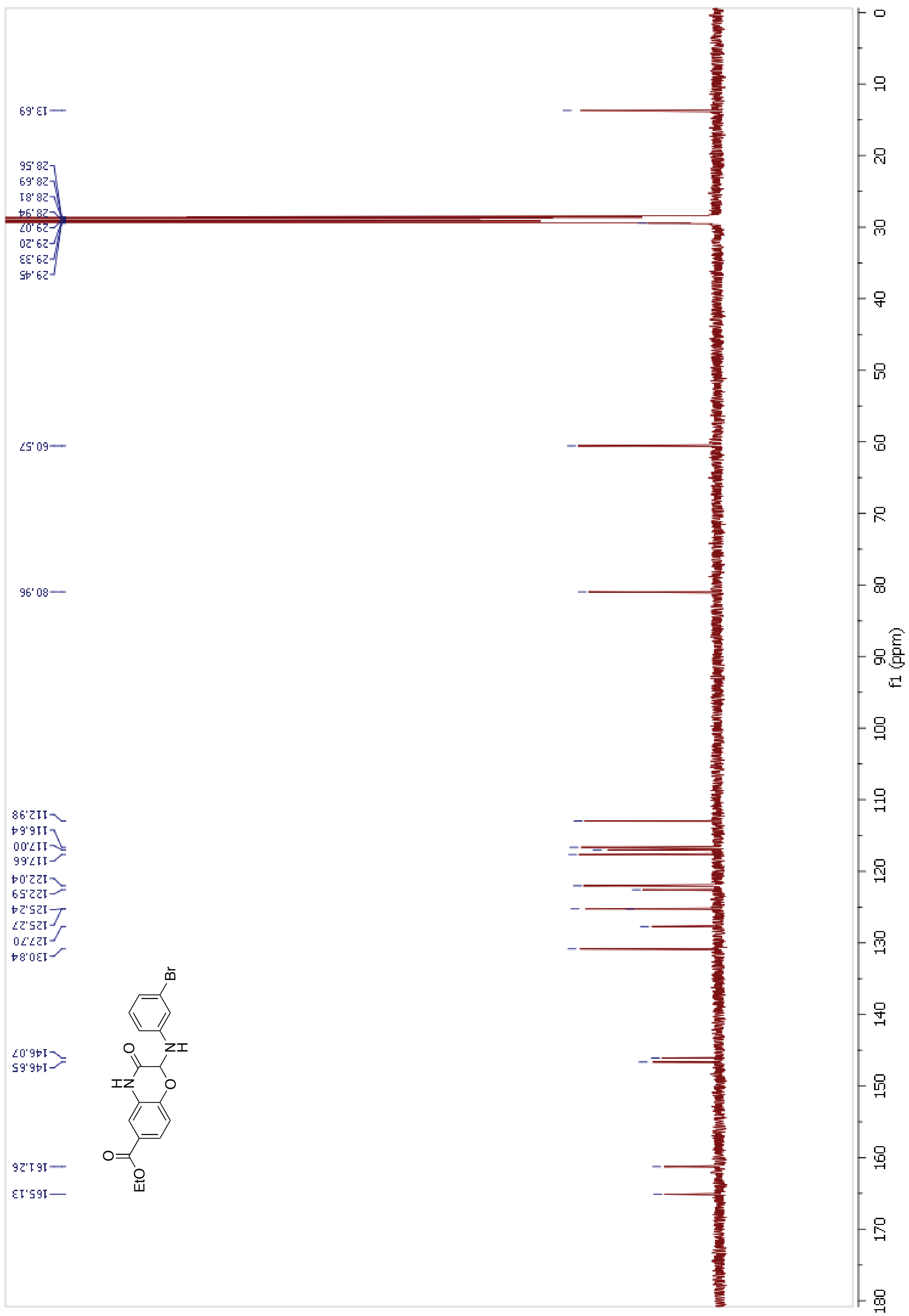
114.01
116.94
117.69
119.38
125.10
125.18
127.78
129.12
144.91
146.30
161.52
165.16
f1 (ppm)
113.98
114.02
125.10
125.15
125.20

^{1}H and ^{13}C NMR spectra of product **12b**

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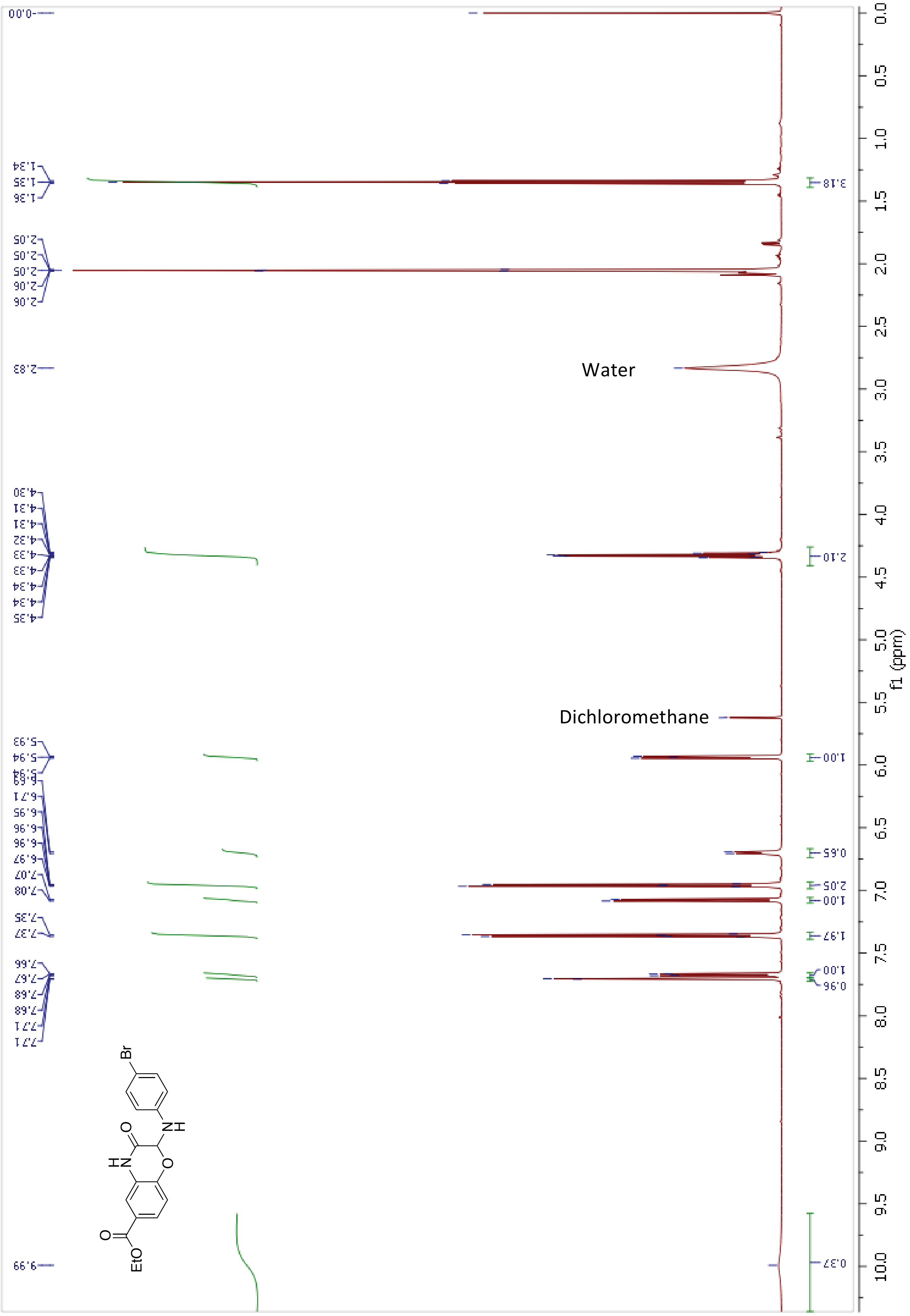
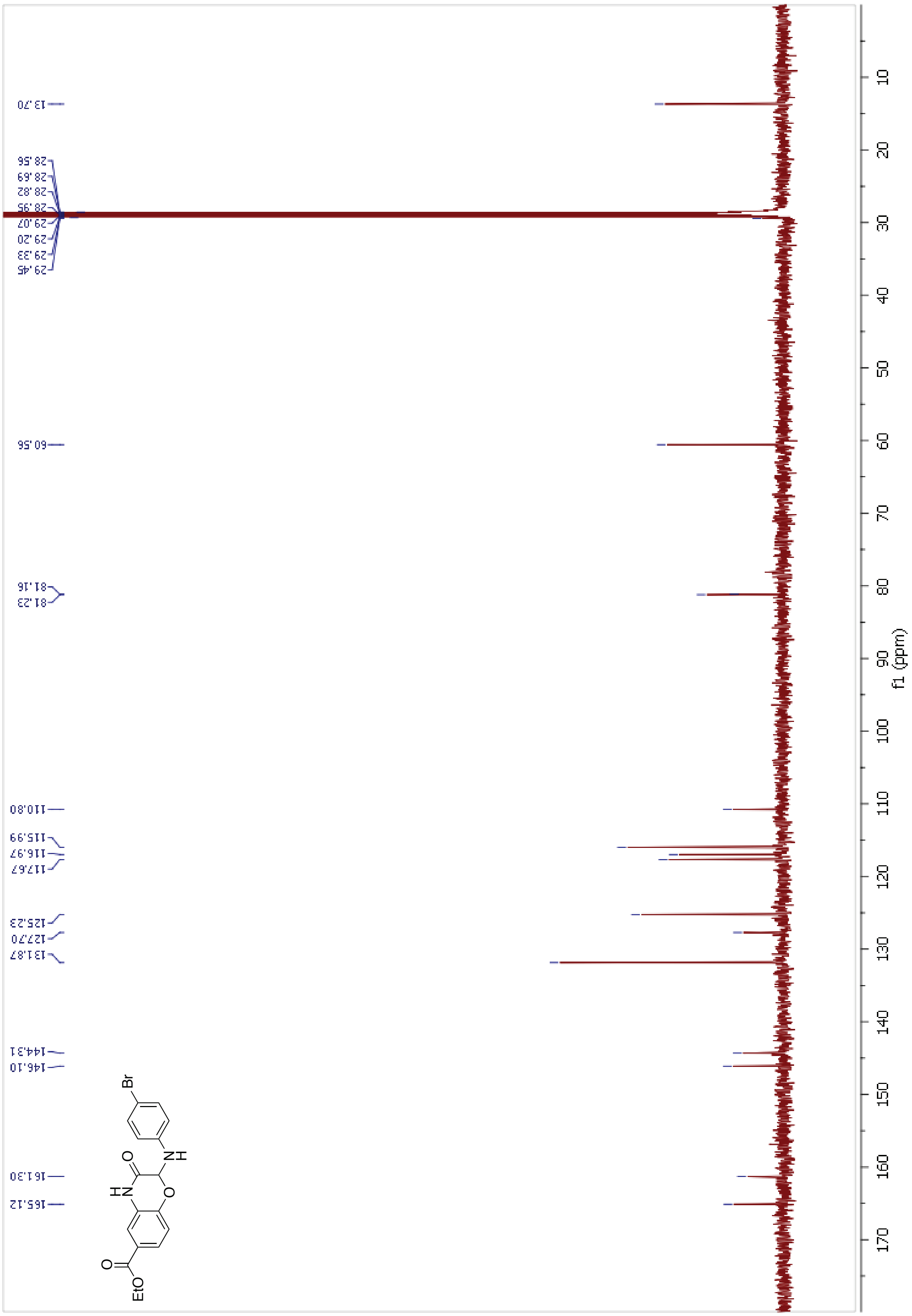
117.66
122.04
122.59
125.24
125.27
127.70
130.84
146.07
146.65
161.26
165.13
Br
NH
O
HN
O
EtO
f1 (ppm)

[1]H and [13]C NMR spectra of product **12c**

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110.80
115.99
116.97
117.67
125.23
127.70
131.87
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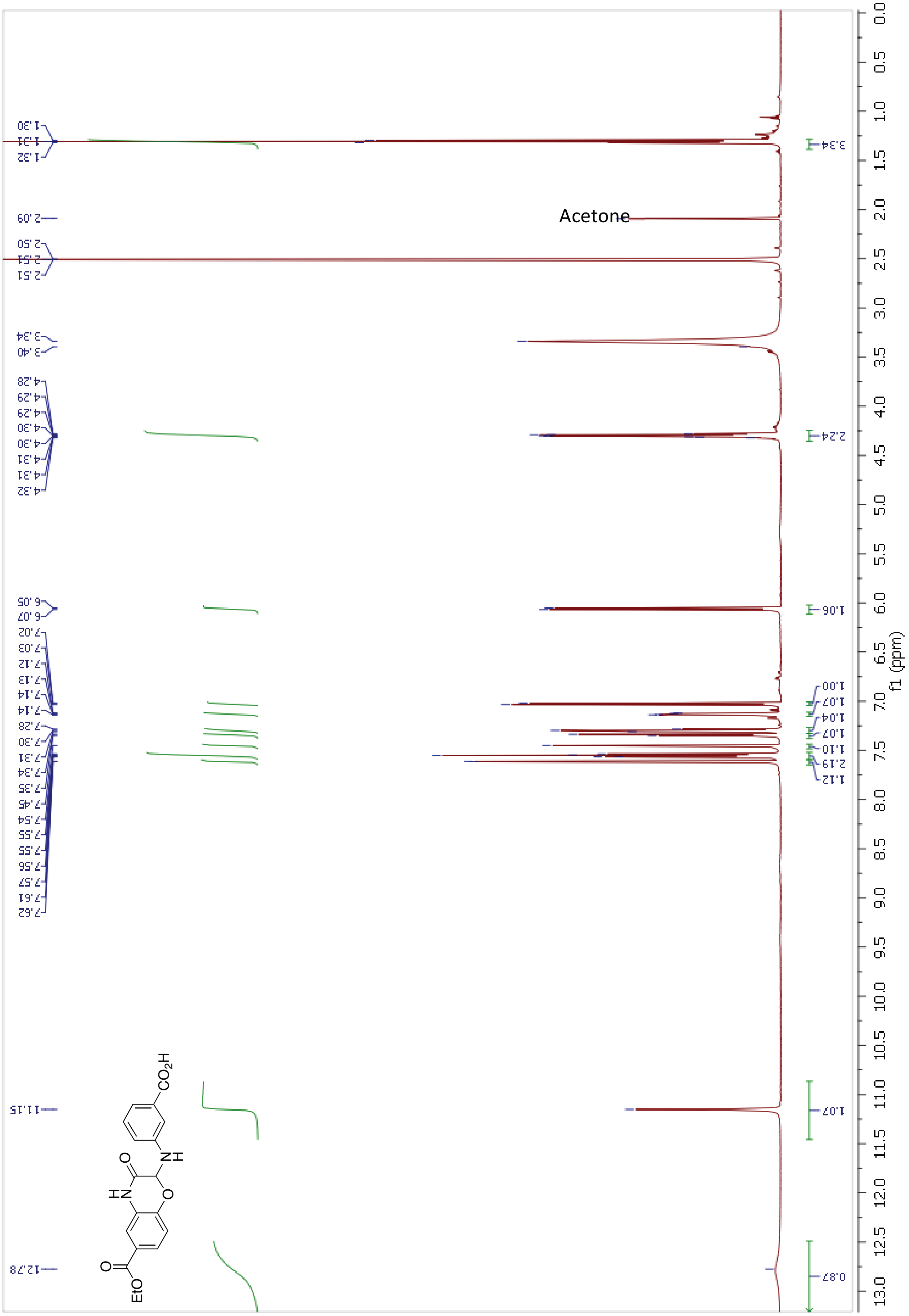
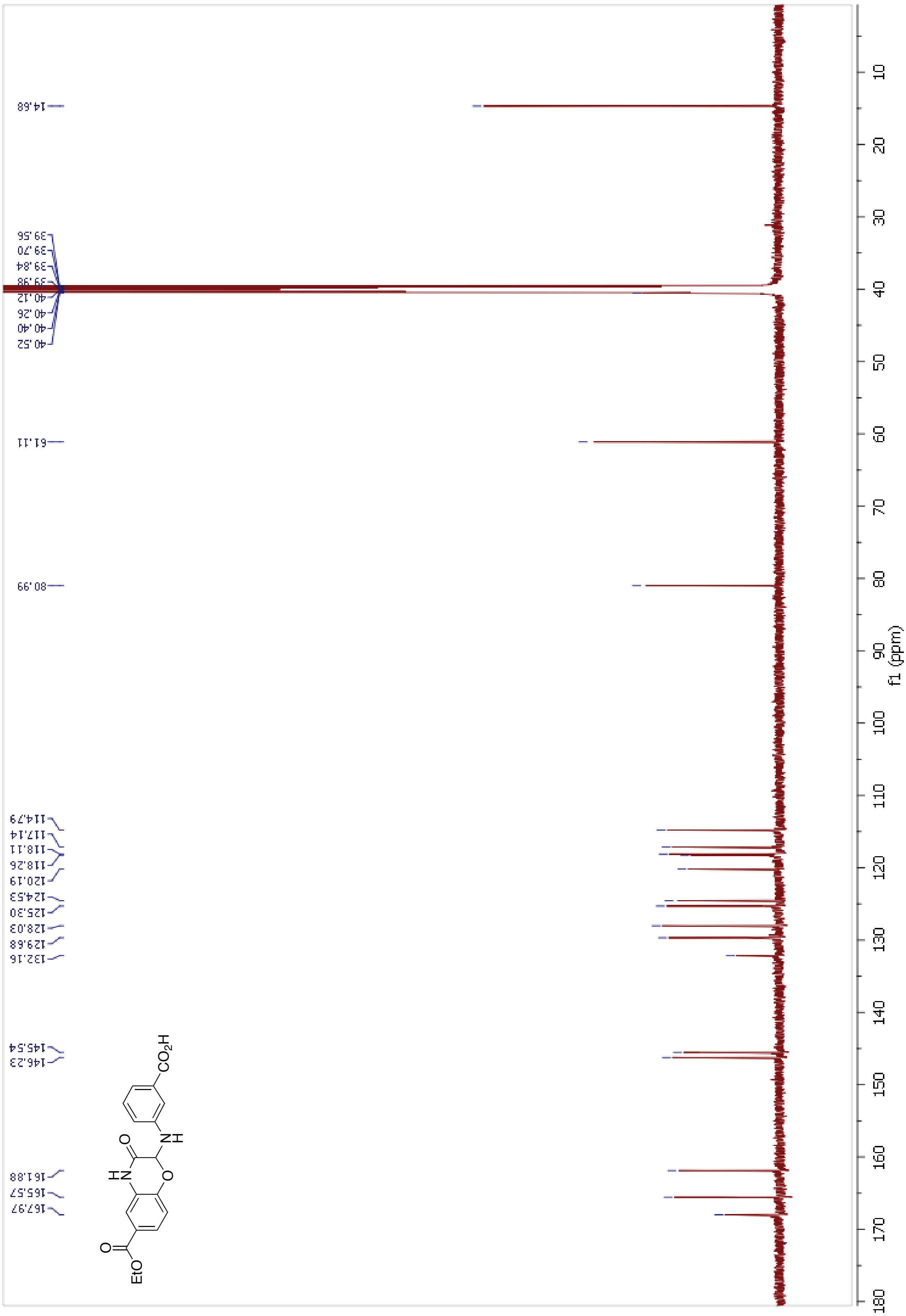
146.10
161.30
165.12
f1 (ppm)
Br
EtO

[1]H and [13]C NMR spectra of product **12d**

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61.11
80.99
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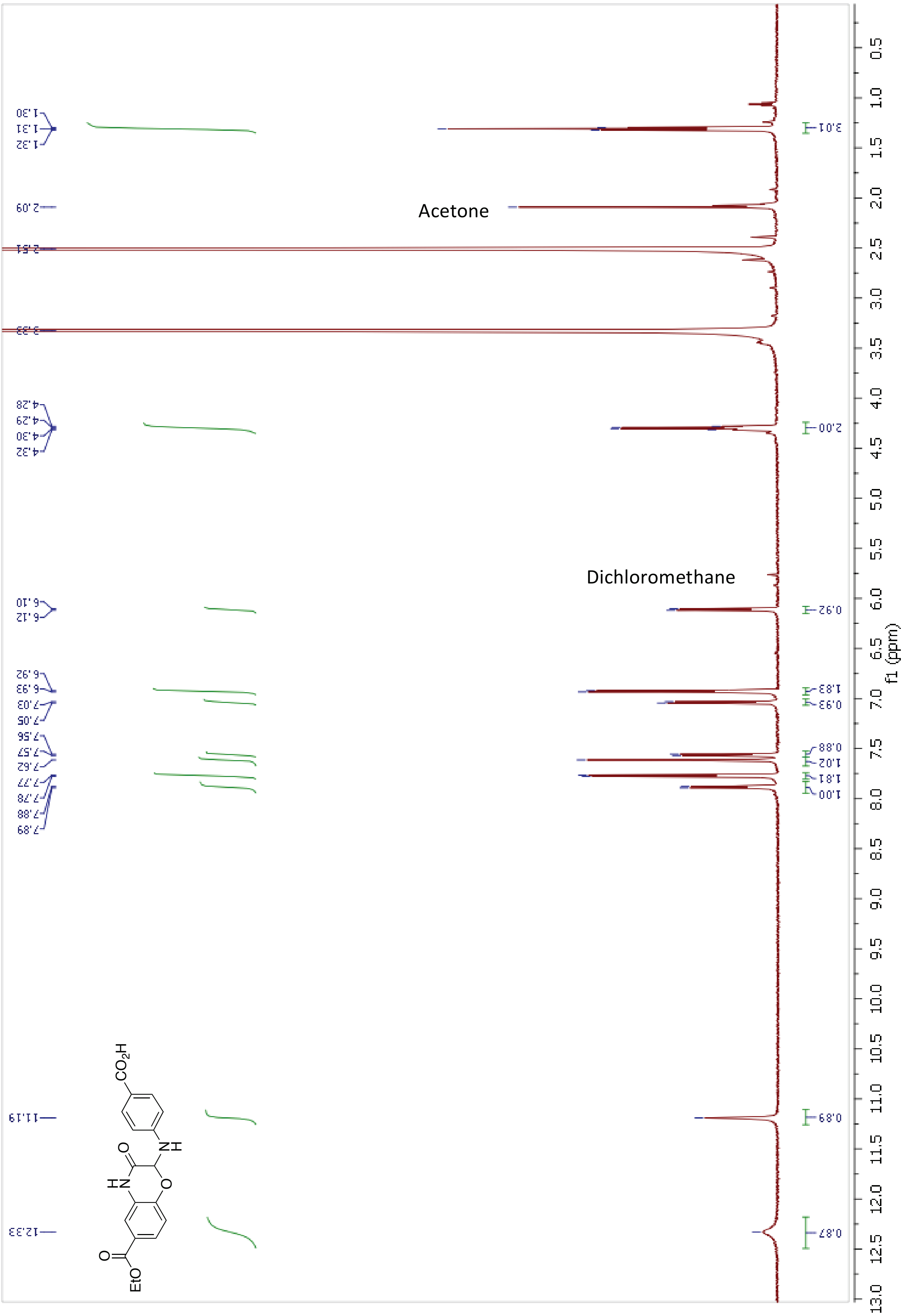
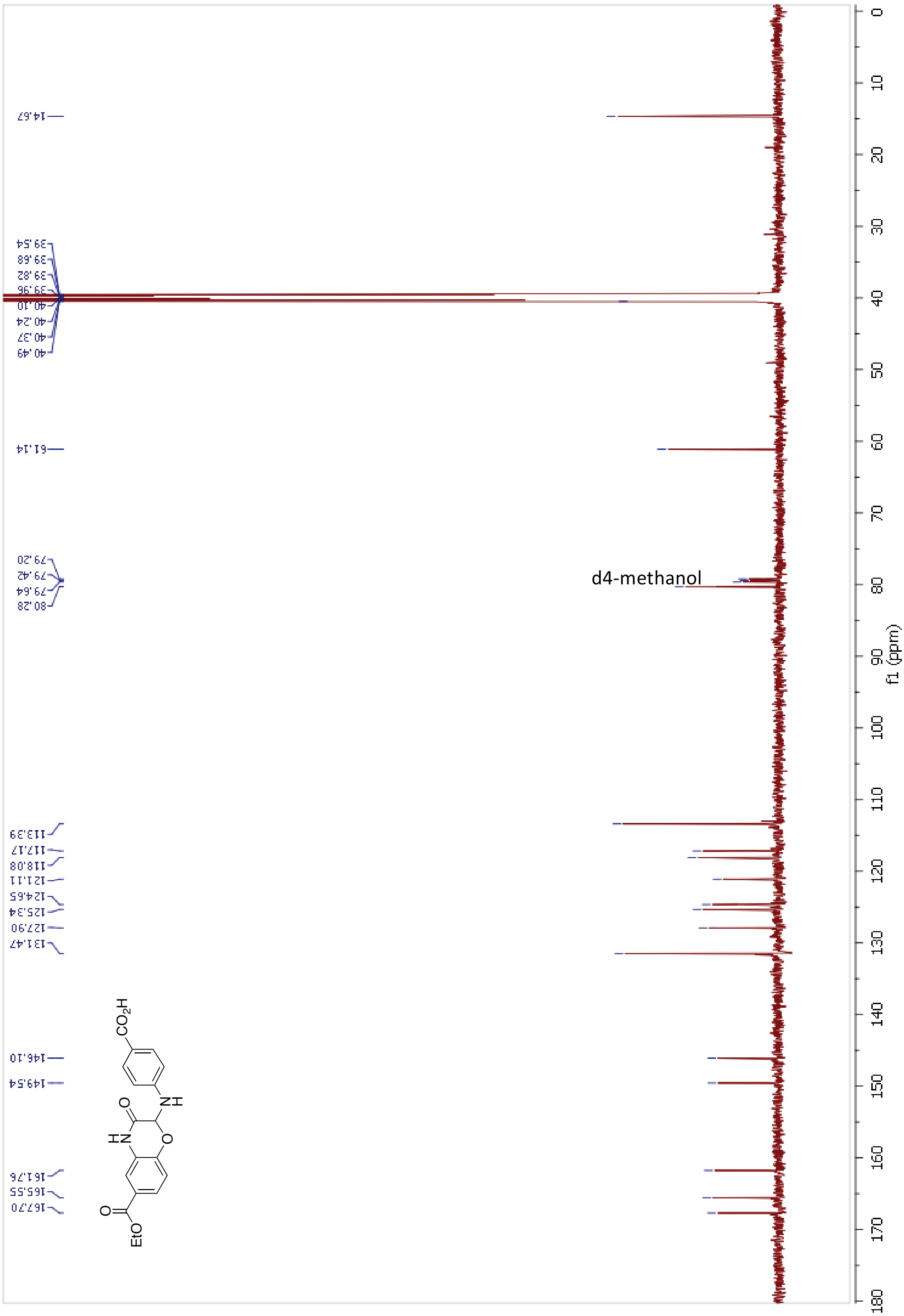
118.11
118.26
120.19
124.53
125.30
128.03
129.68
132.16
145.54
146.23
161.88
165.57
167.97
f1 (ppm)
EtO
HN
NH
CO_2H

[1]H and [13]C NMR spectra of product **12e**





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39.96
40.10
40.24
40.37
40.49
61.14
79.20
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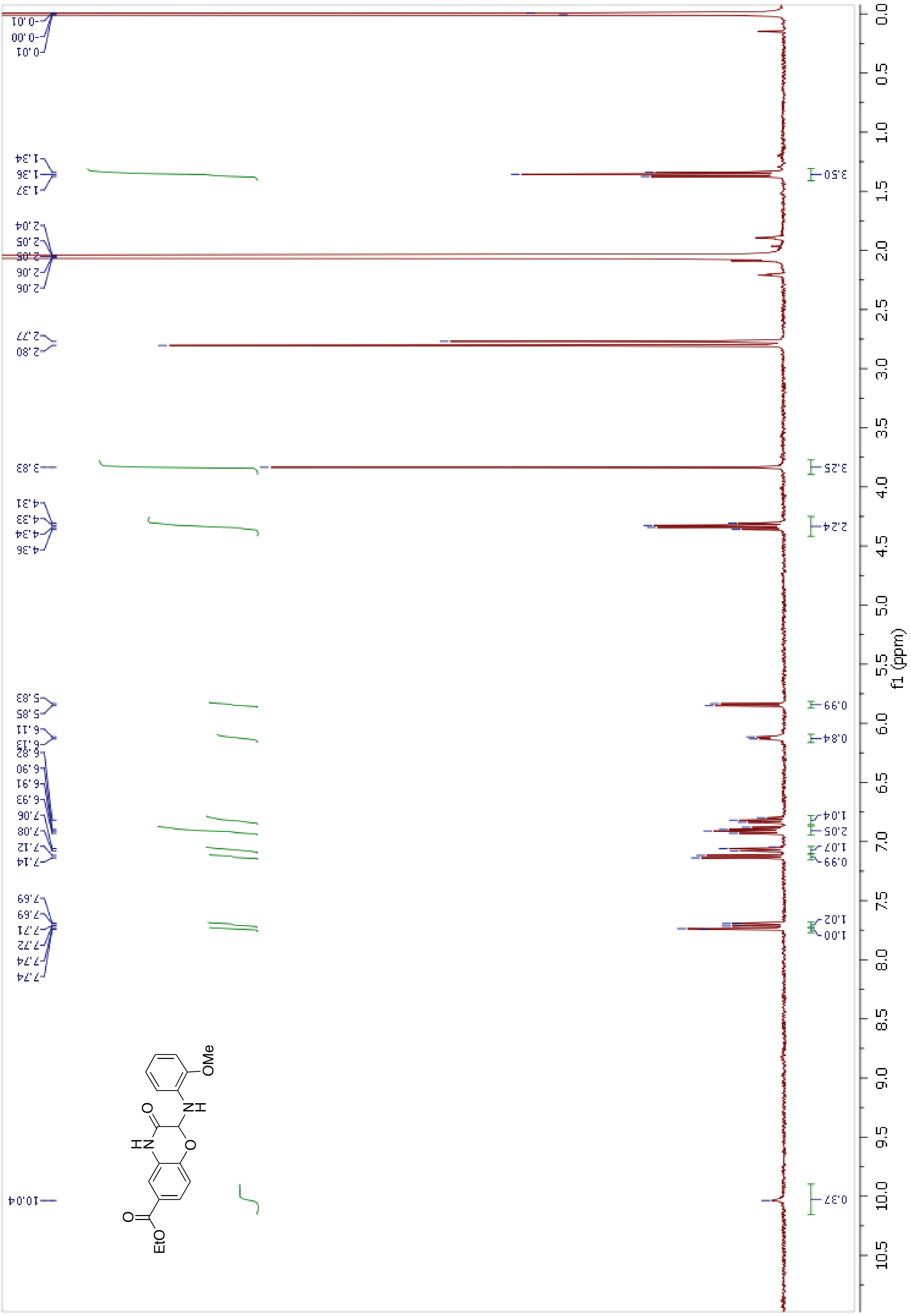
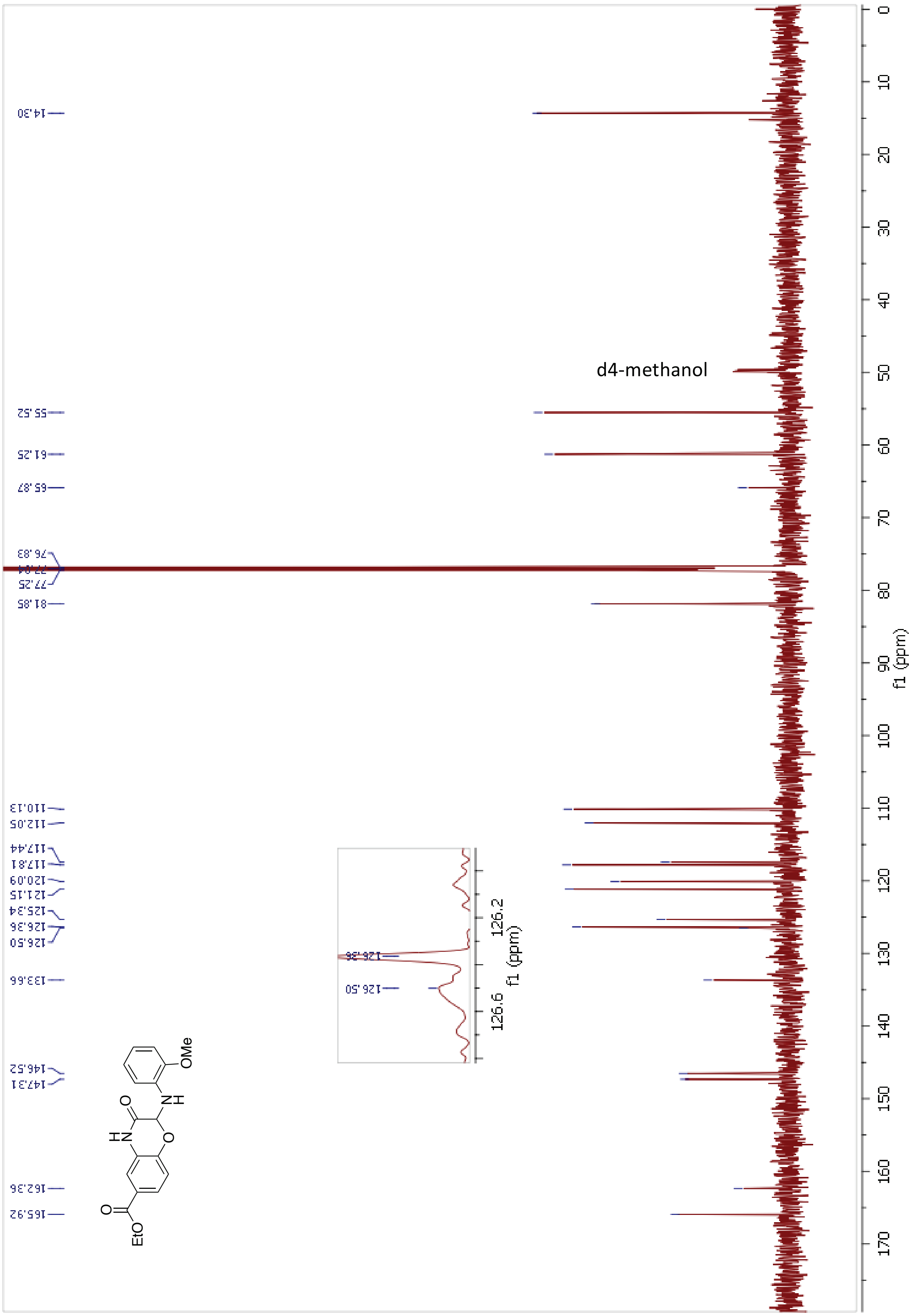
79.64
80.28
113.39
117.17
118.08
121.11
124.65
125.34
127.90
131.47
146.10
149.54
161.76
165.55
167.70
d4-methanol
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
f1 (ppm)
EtO
O
H
N
O
O
N
H
CO_2H

[1]H and [13]C NMR spectra of product **12f**

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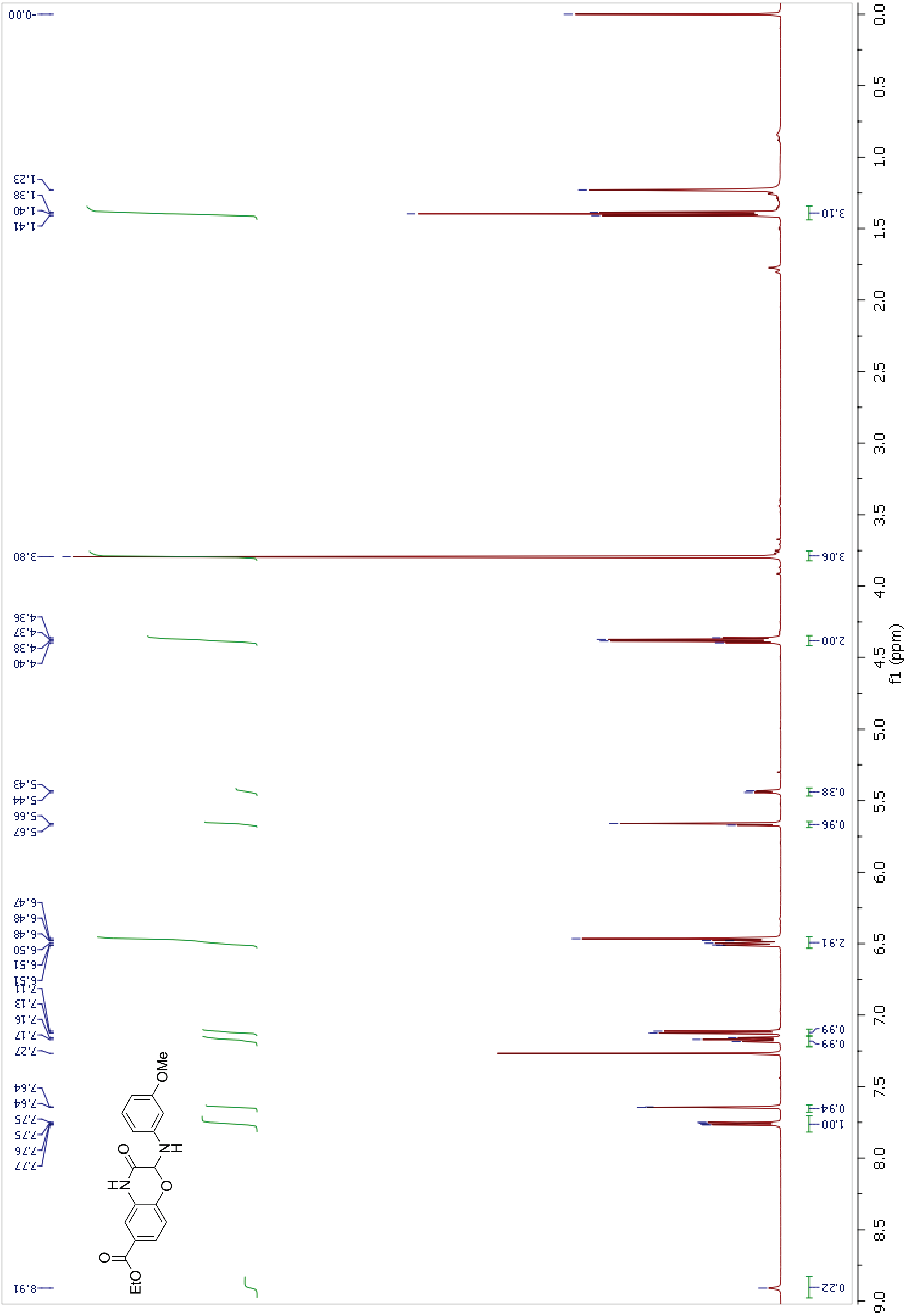
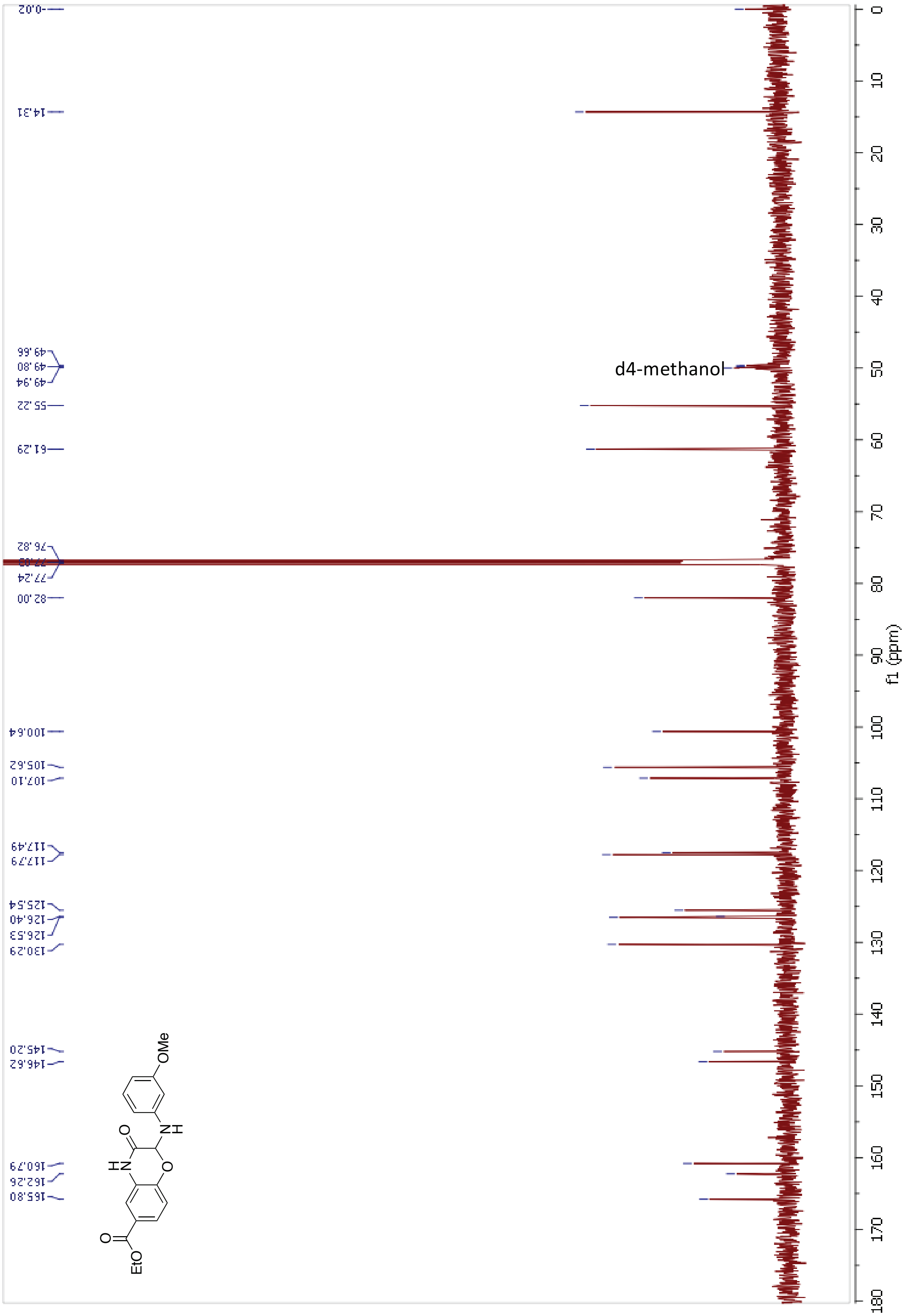
121.15
125.34
126.36
126.50
133.66
146.52
147.31
162.36
165.92
d4-methanol
126.2
126.6
f1 (ppm)
EtO
OMe

^{1}H and ^{13}C NMR spectra of product **12g**





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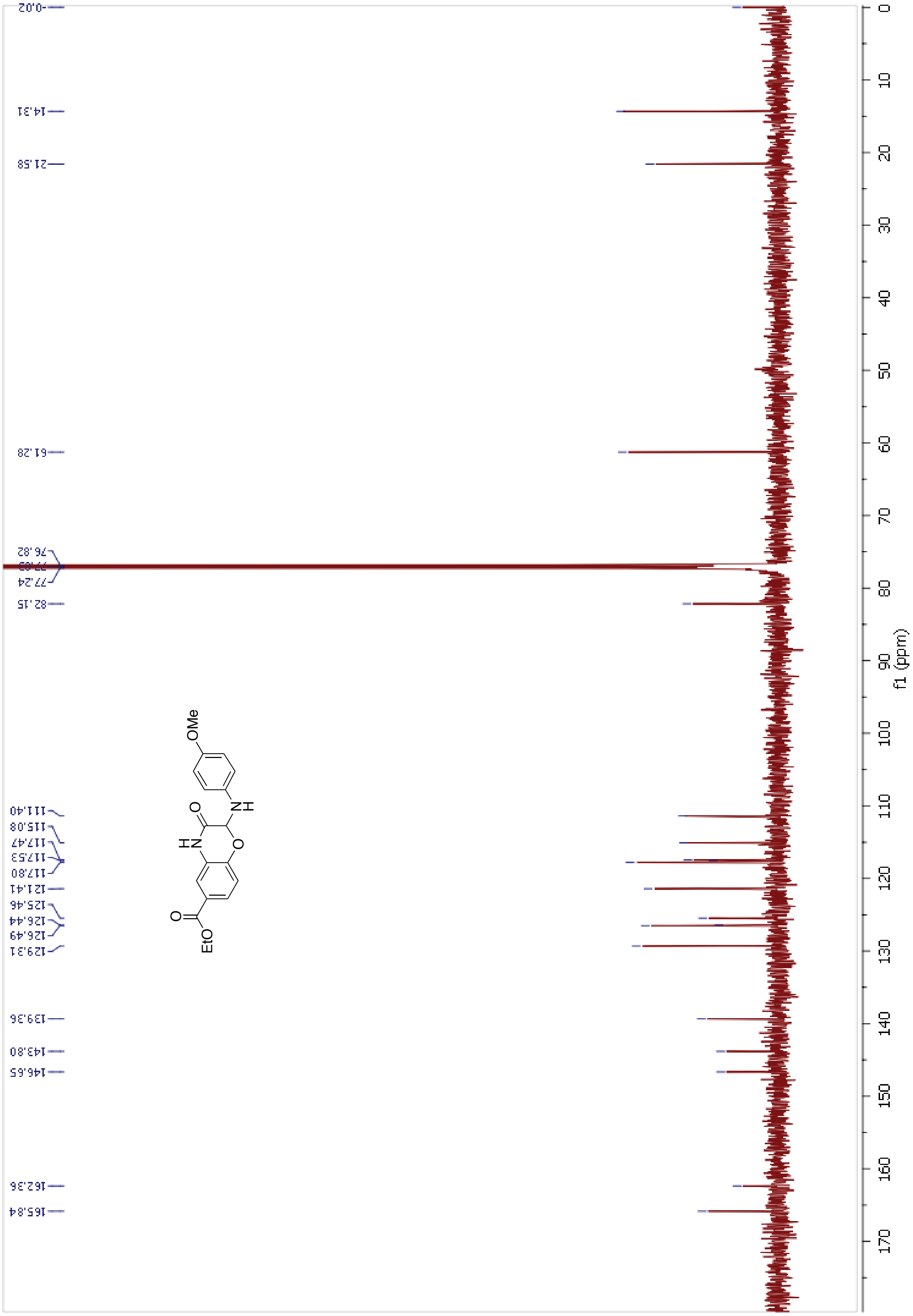
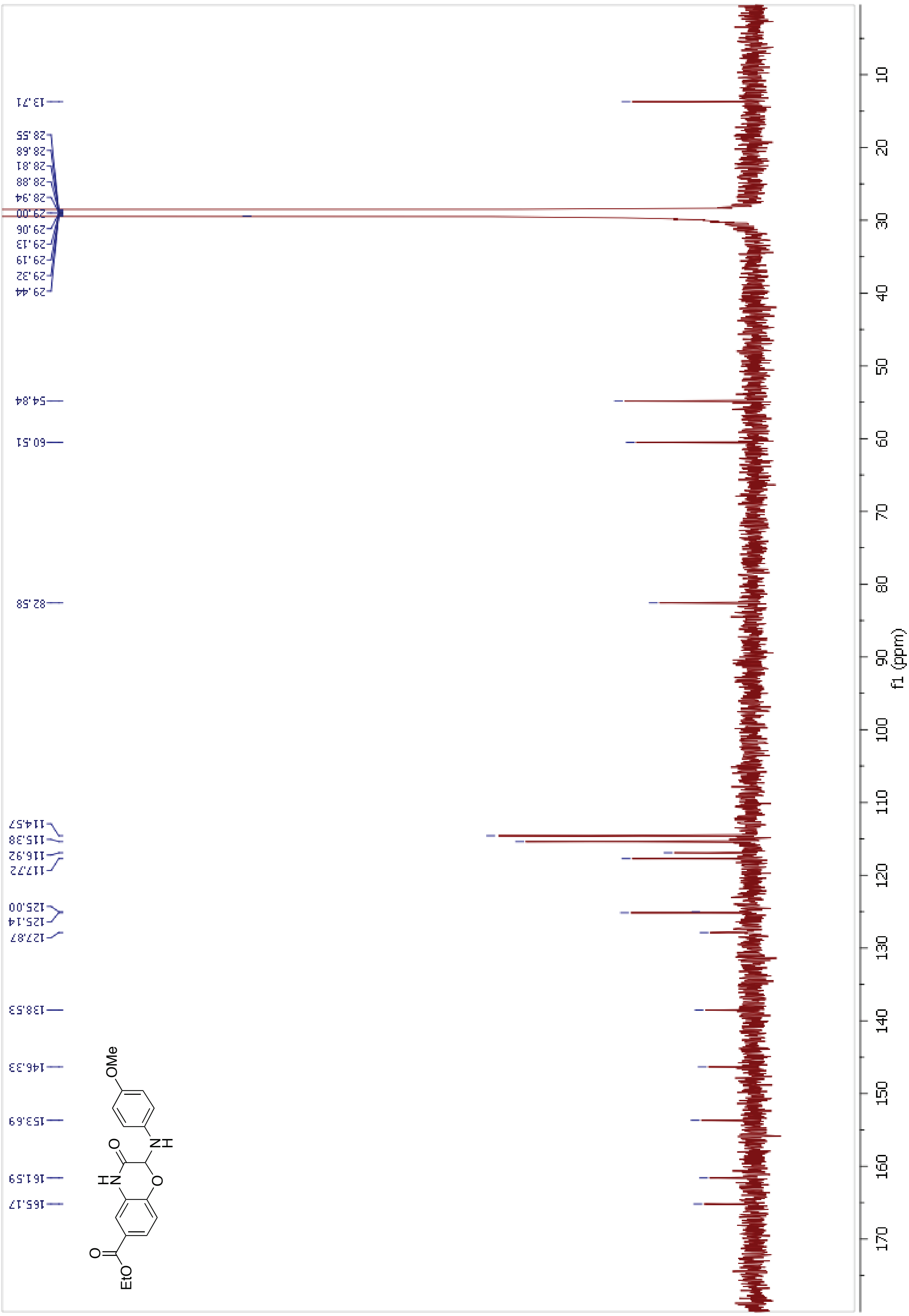
117.79
125.54
126.40
126.53
130.29
145.20
146.62
160.79
162.26
165.80
d4-methanol
f1 (ppm)
EtO
O
NH
OMe

[1]H and [13]C NMR spectra of product **12h**

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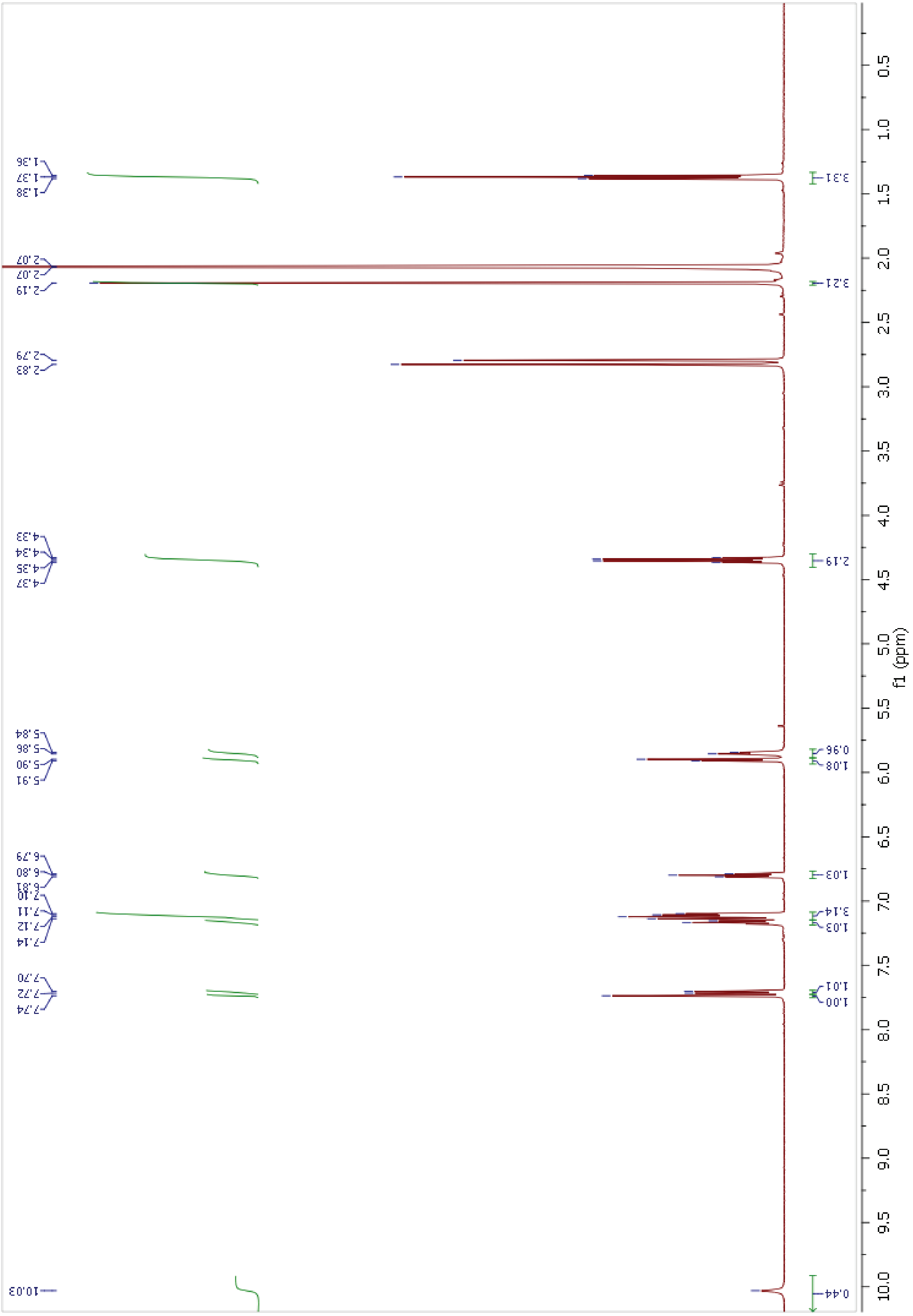
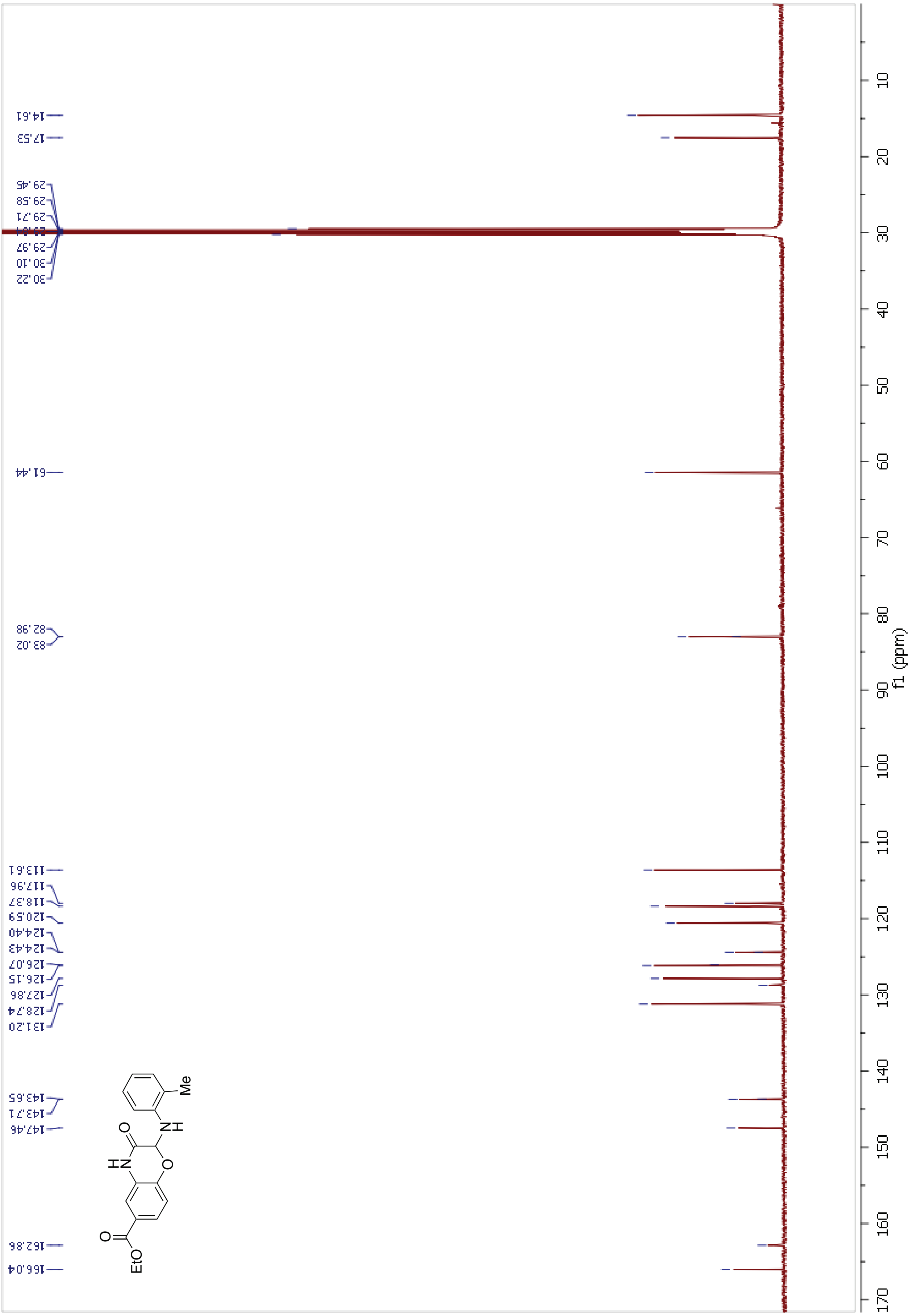
117.72
125.00
125.14
127.87
138.53
146.33
153.69
161.59
165.17
OMe
NH
O
HN
O
EtO
O
f1 (ppm)

[1]H and [13]C NMR spectra of product **12i**

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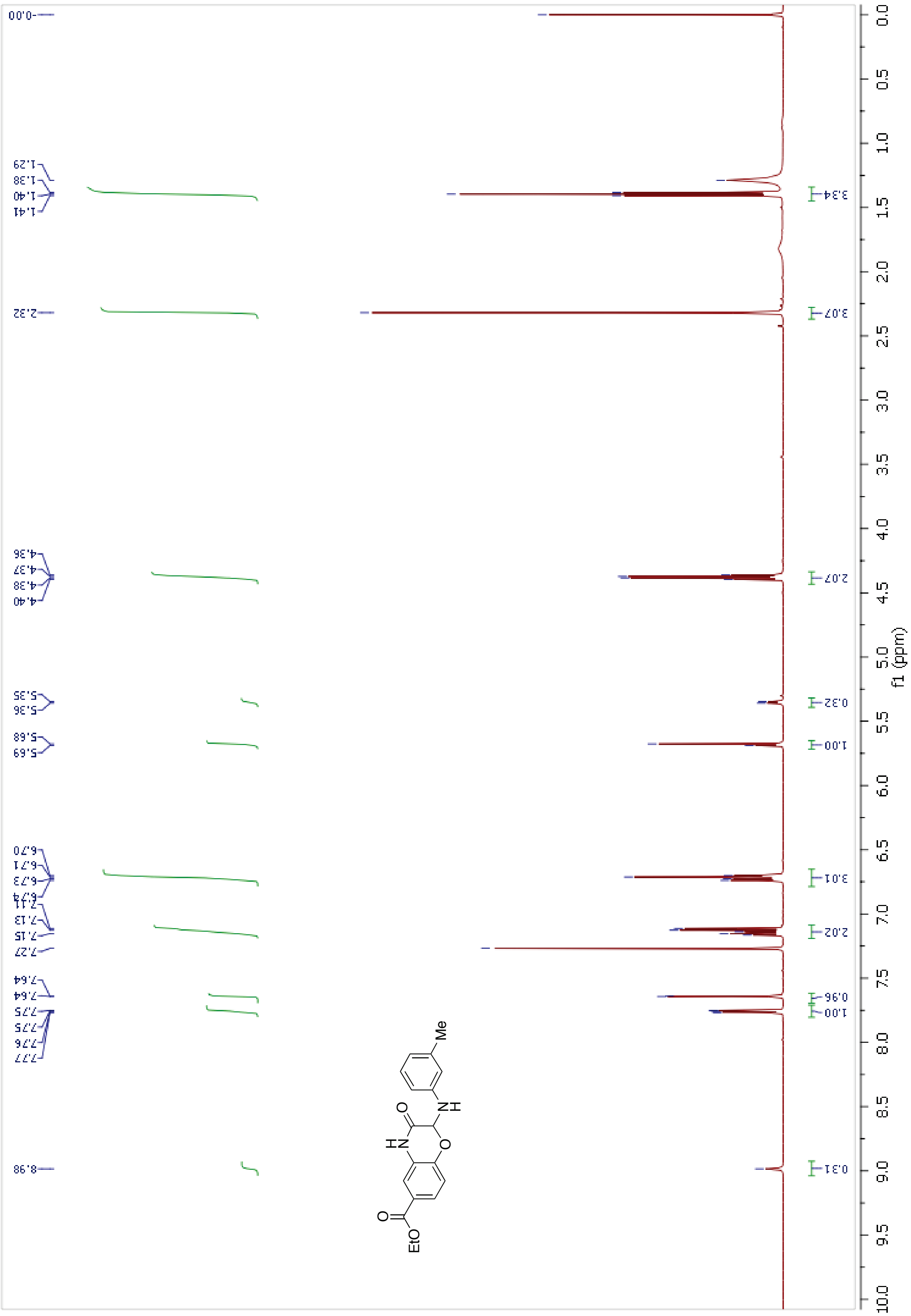
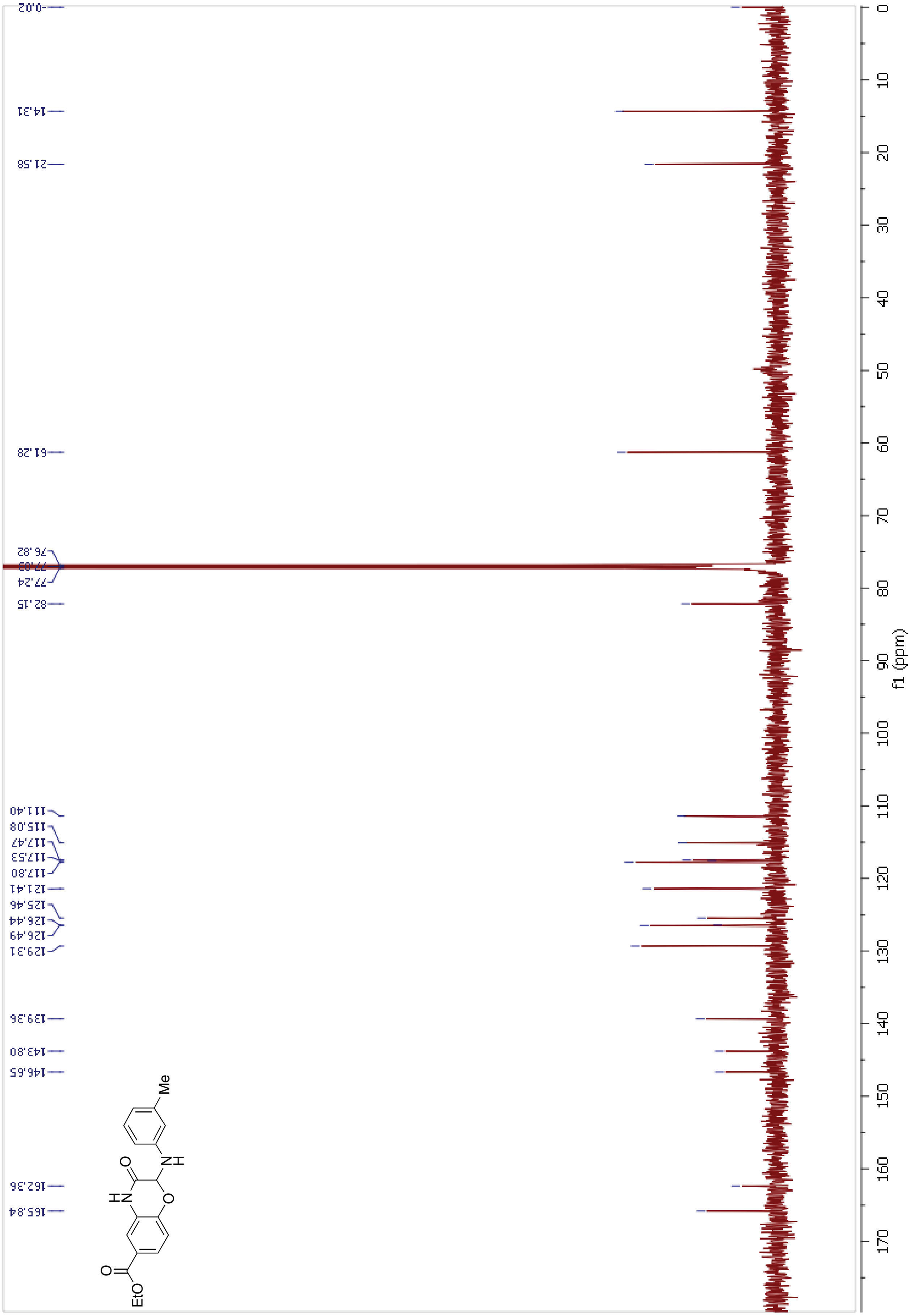
118.37
120.59
124.40
124.43
126.07
126.15
127.86
128.74
131.20
143.65
143.71
147.46
162.86
166.04
EtO
Me
f1 (ppm)

[1]H and [13]C NMR spectra of product **12j**





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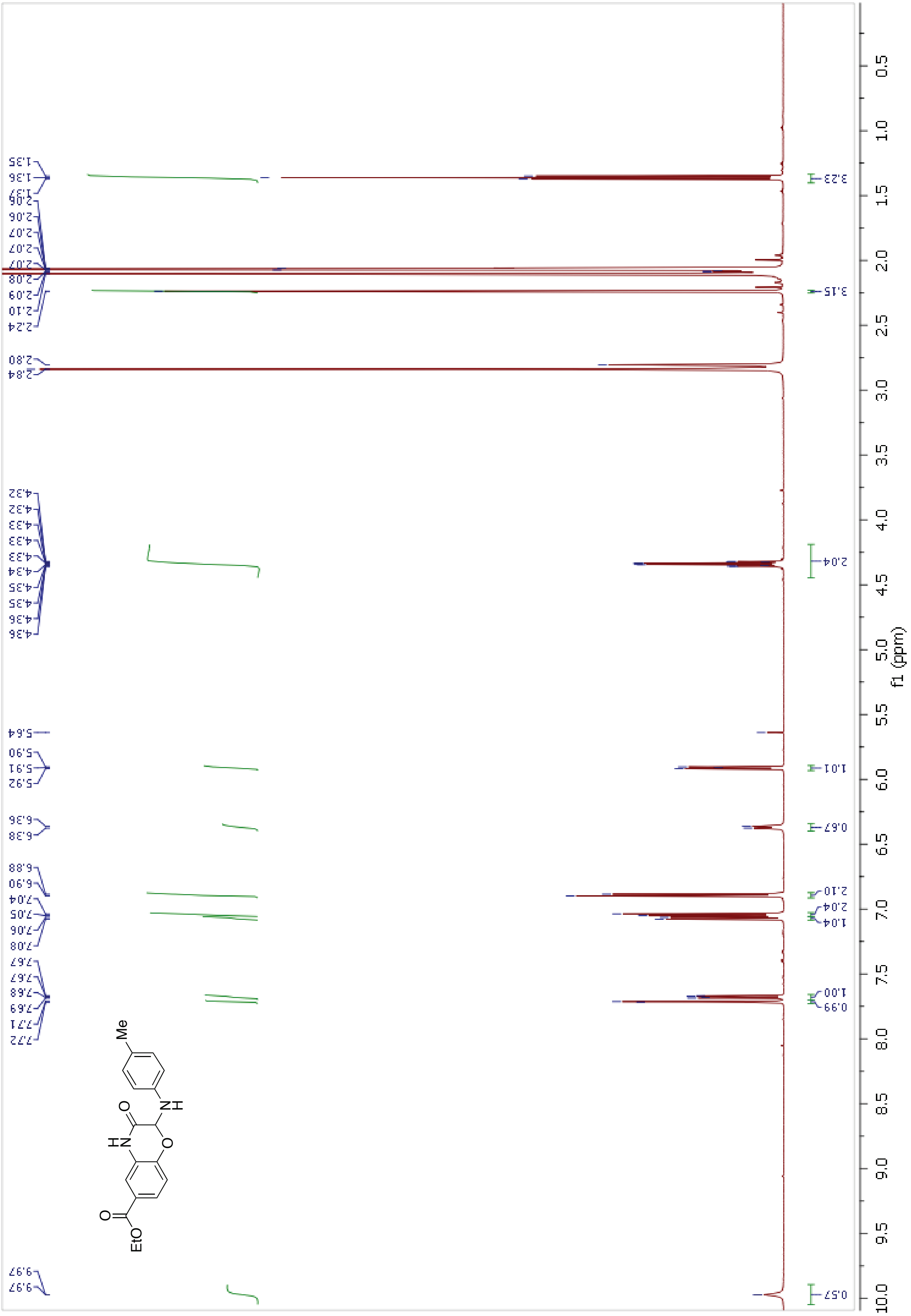
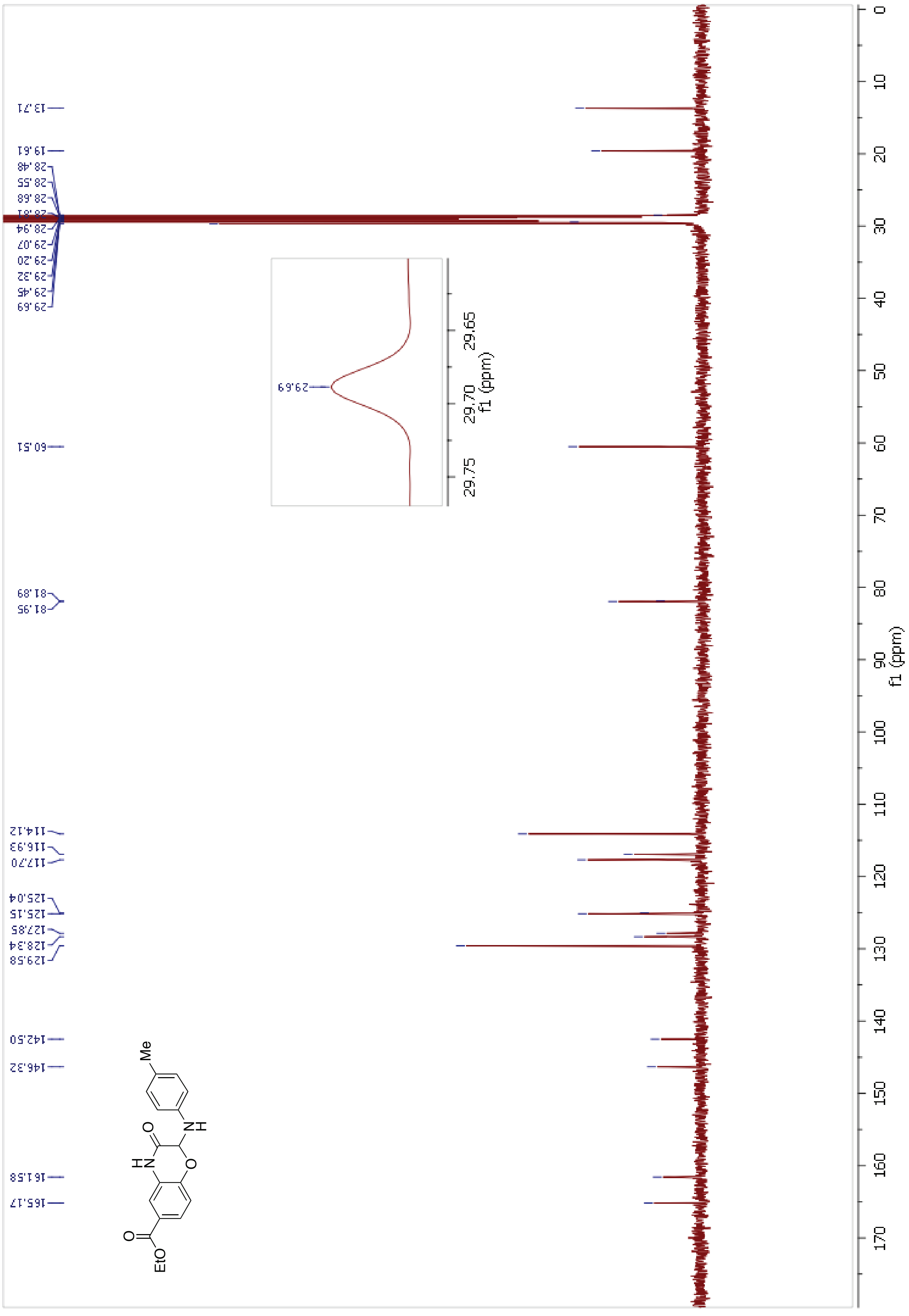
77.03
77.24
82.15
111.40
115.08
117.47
117.53
117.80
121.41
125.46
126.44
126.49
129.31
139.36
143.80
146.65
162.36
165.84
EtO
O
HN
O
NH
Me
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
f1 (ppm)

H and ^{13}C NMR spectra of product **12k**





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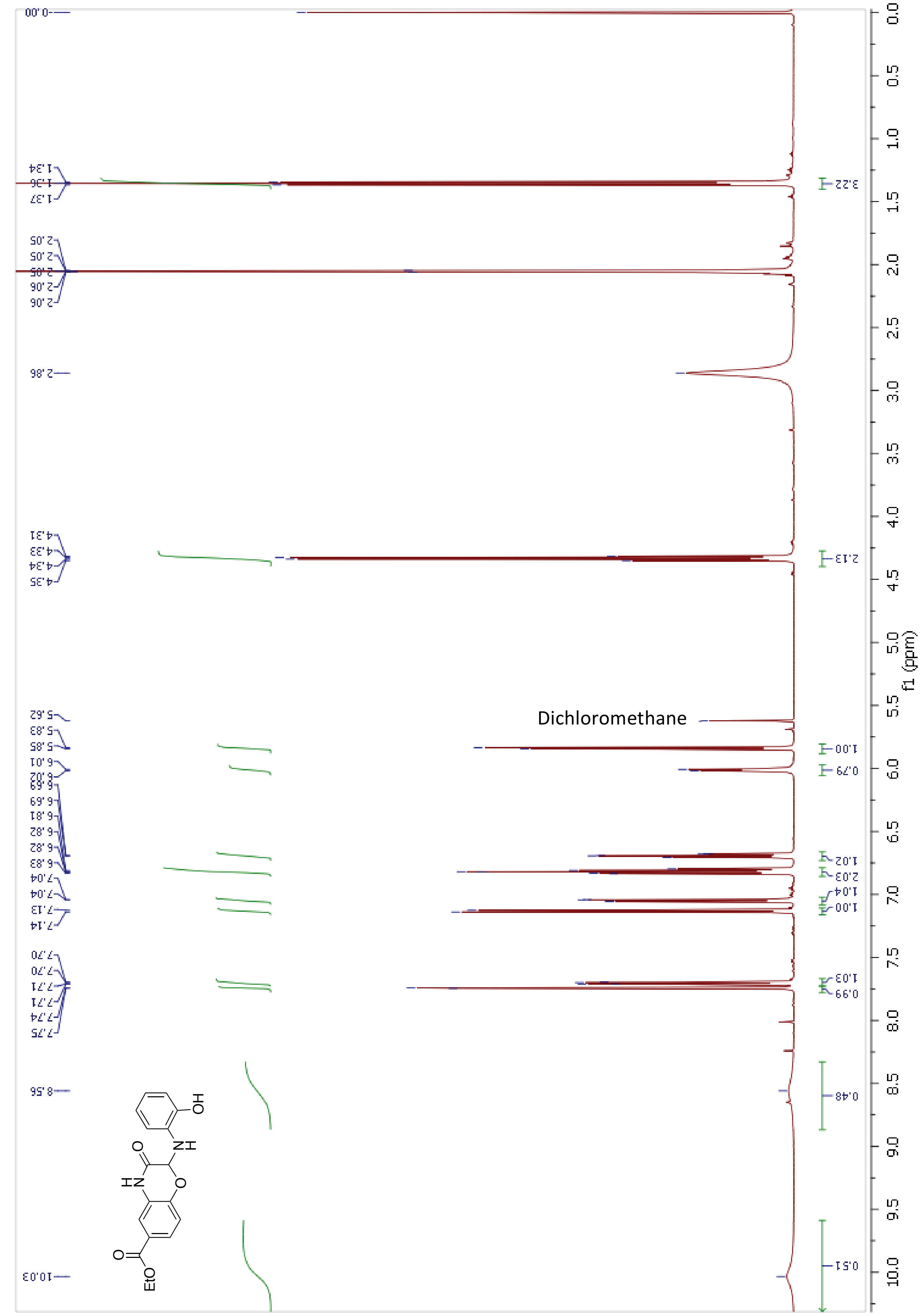
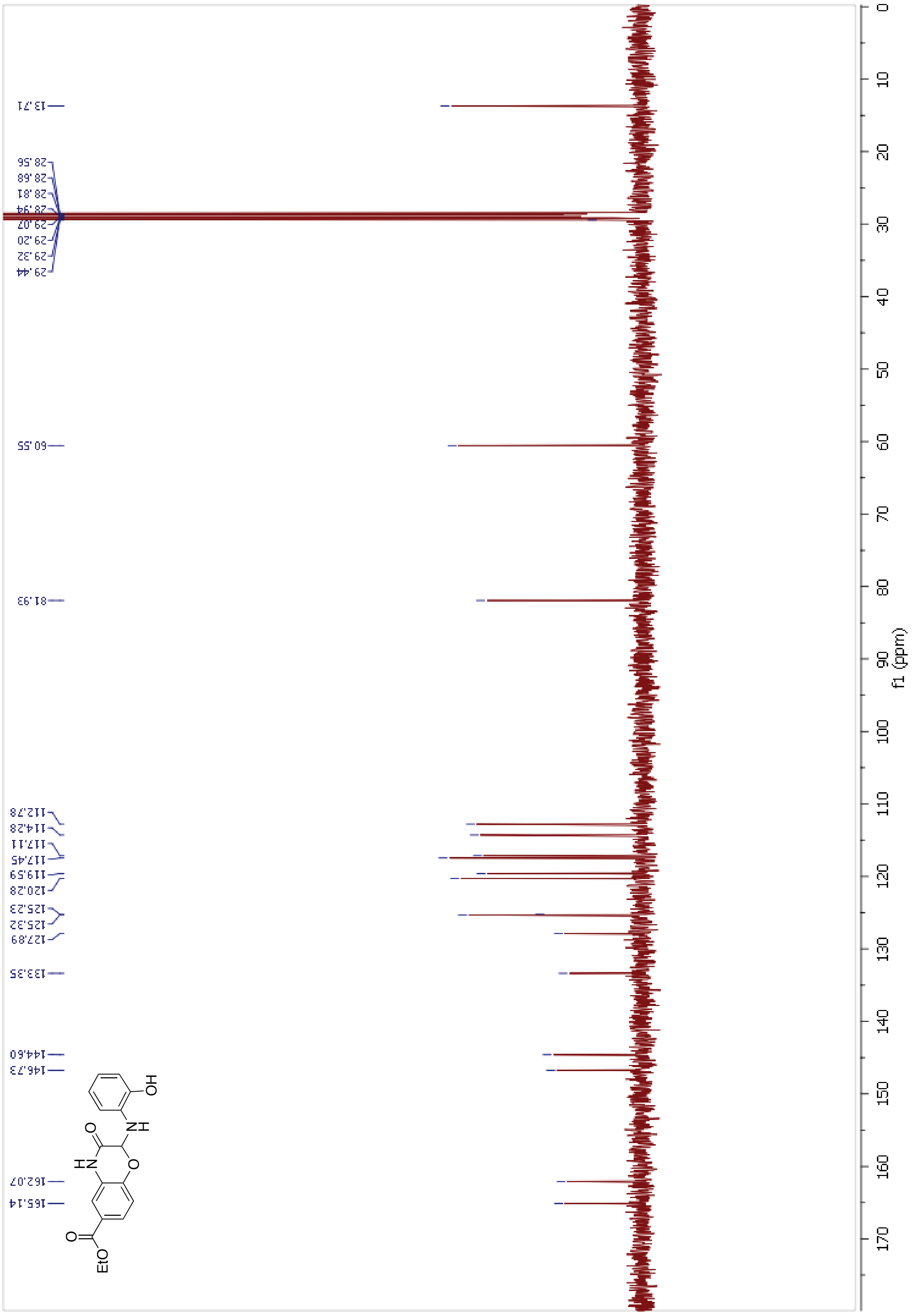
60.51
81.89
81.95
114.12
116.93
117.70
125.04
125.15
127.85
128.34
129.58
142.50
146.32
161.58
165.17
EtO
O
HN
O
NH
O
Me
29.69
29.65
29.70
29.75
f1 (ppm)
f1 (ppm)

^{1}H and ^{13}C NMR spectra of product **12l**

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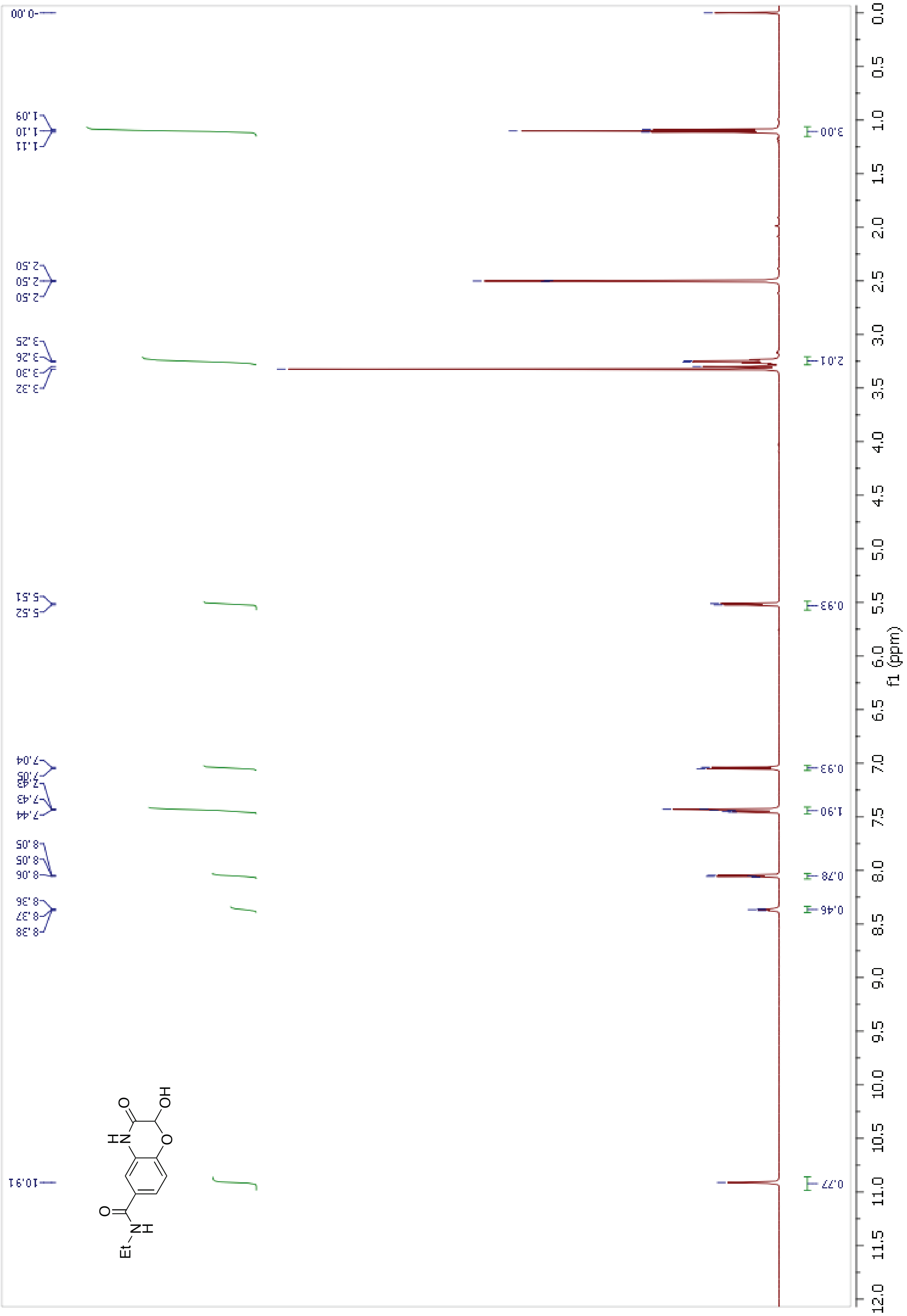
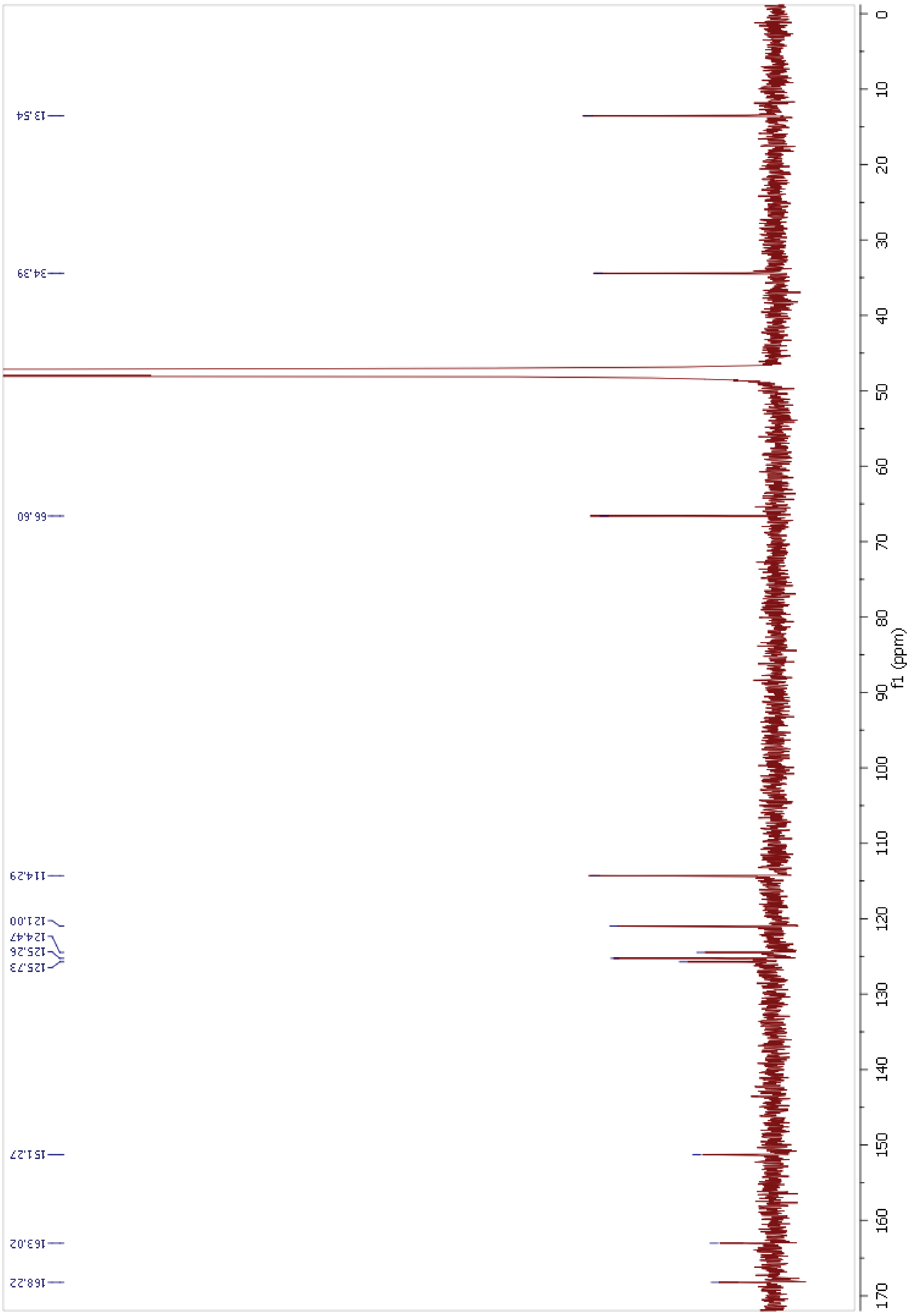
117.11
117.45
119.59
120.28
125.23
125.32
127.89
133.35
144.60
146.73
162.07
165.14
f1 (ppm)
EtO
O
HN
O
O
NH
OH

^{1}H and ^{13}C NMR spectra of product **11**

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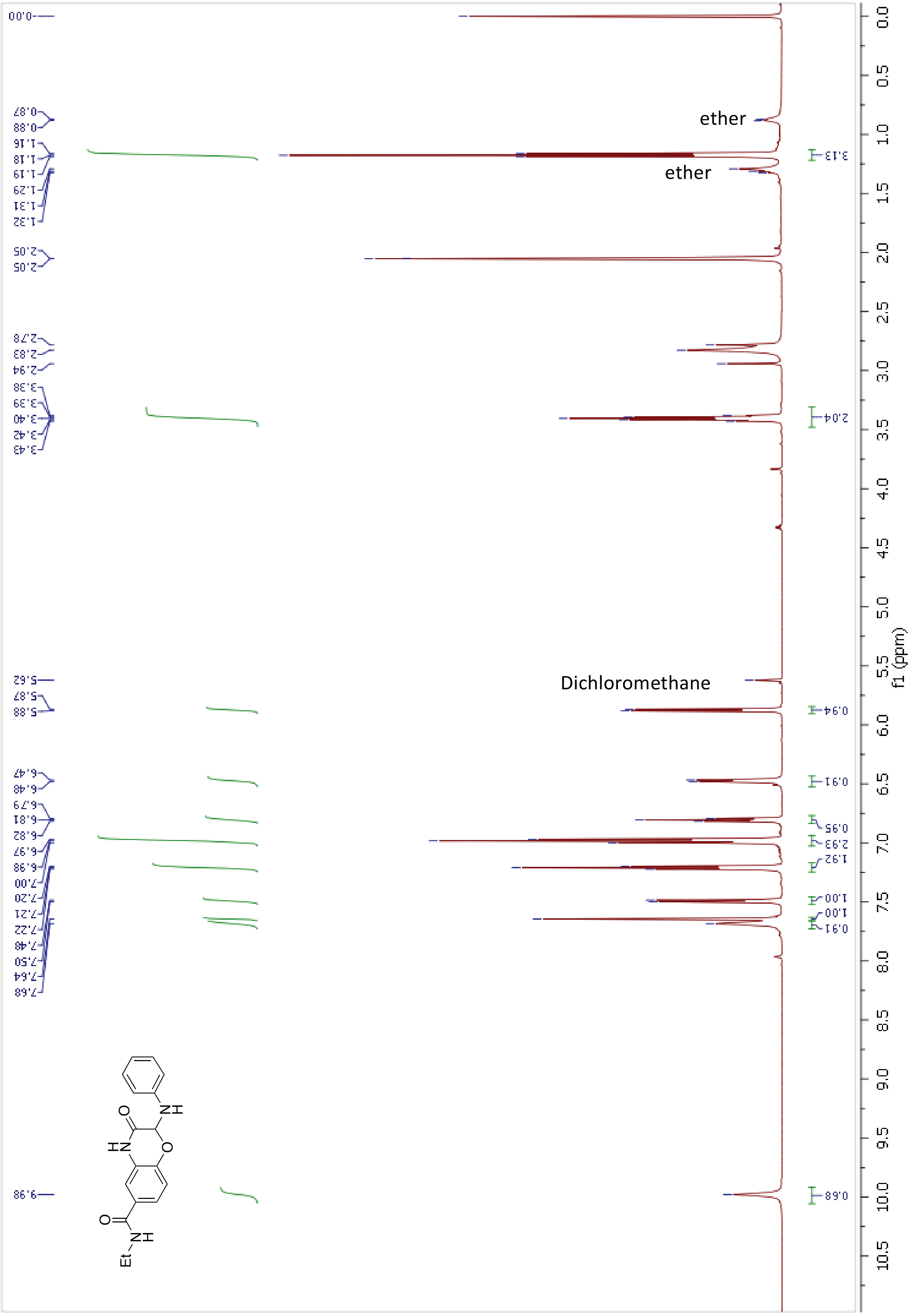
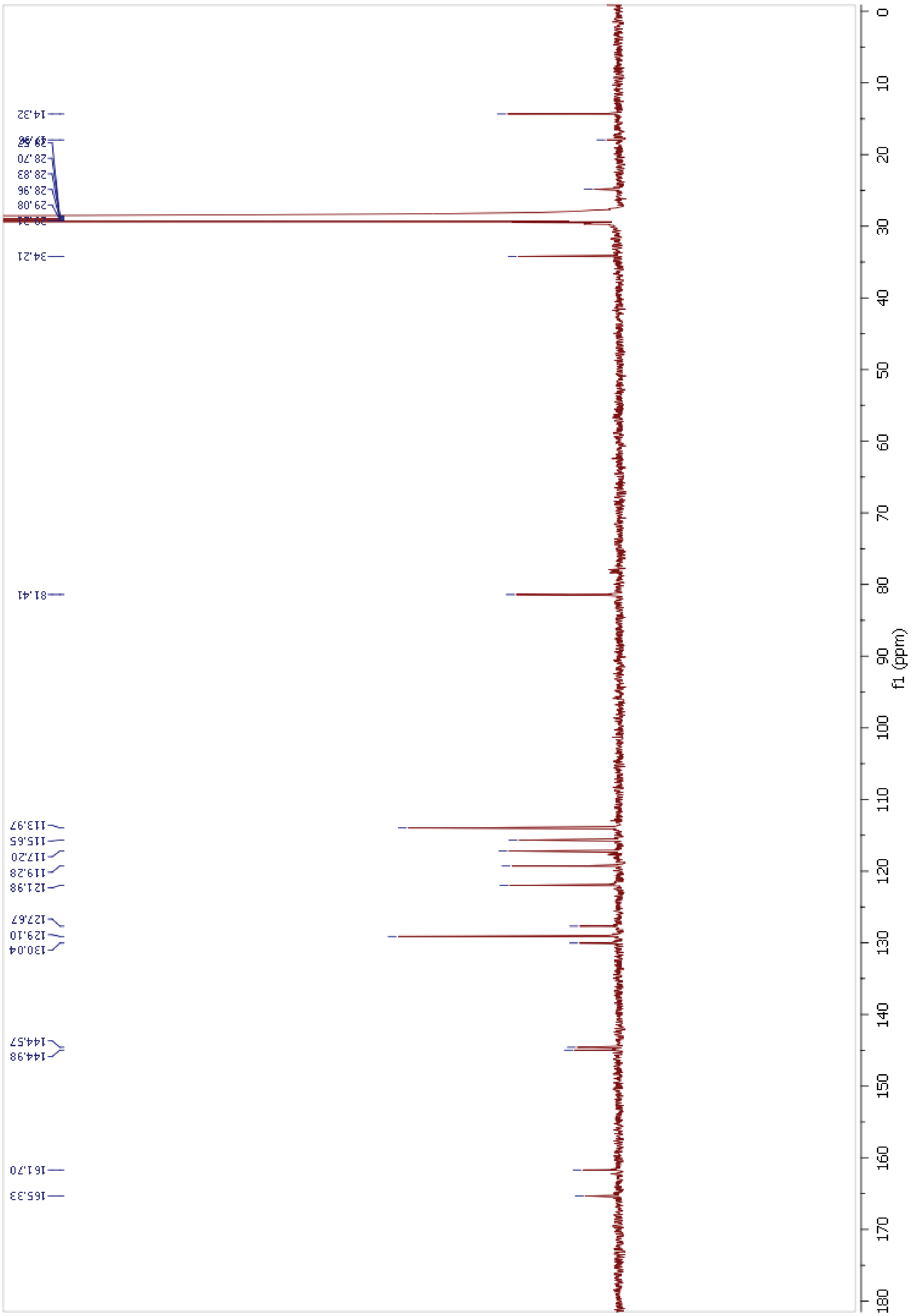
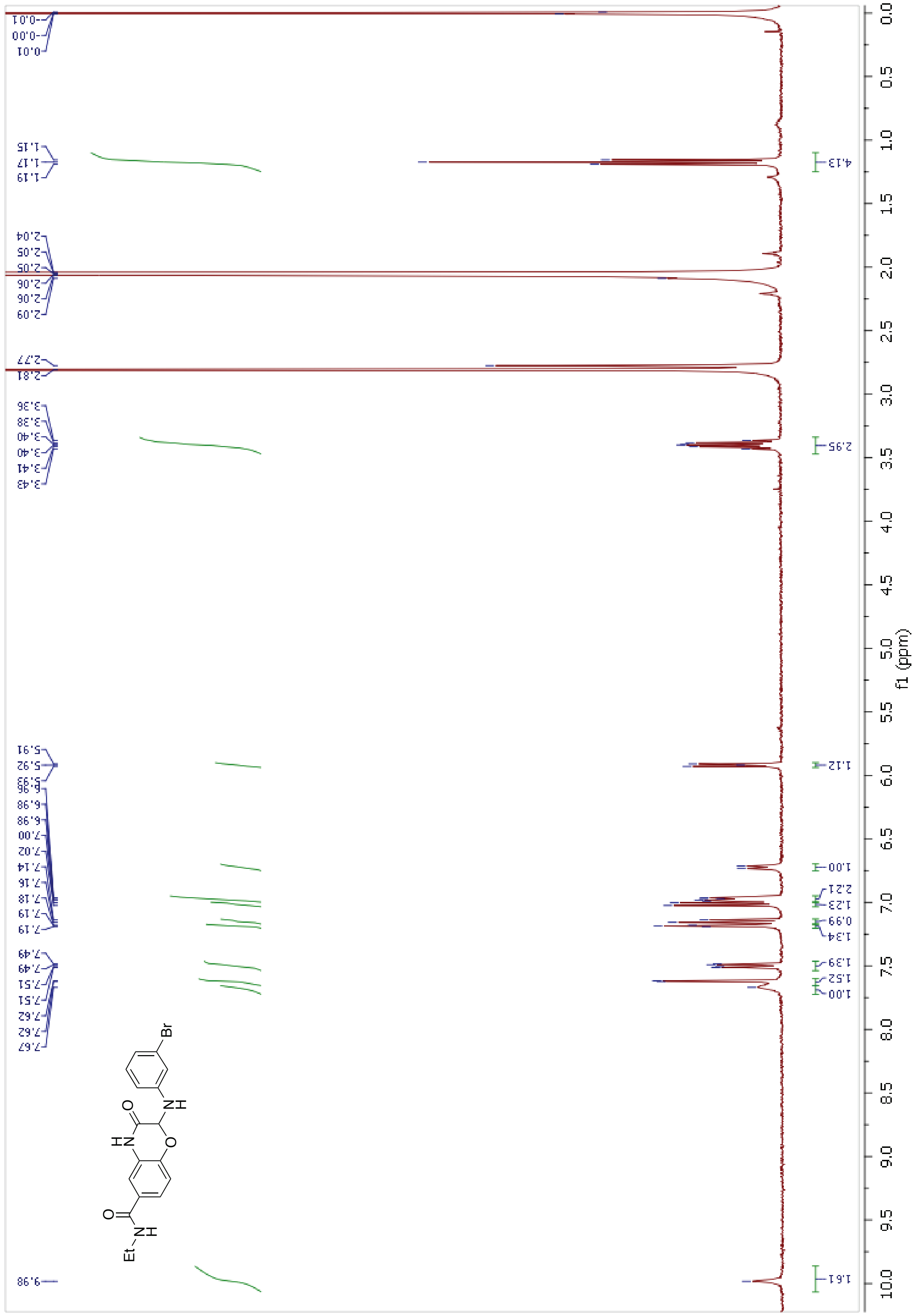
121.00
124.47
125.26
125.73
151.27
163.02
168.22
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
f1 (ppm)

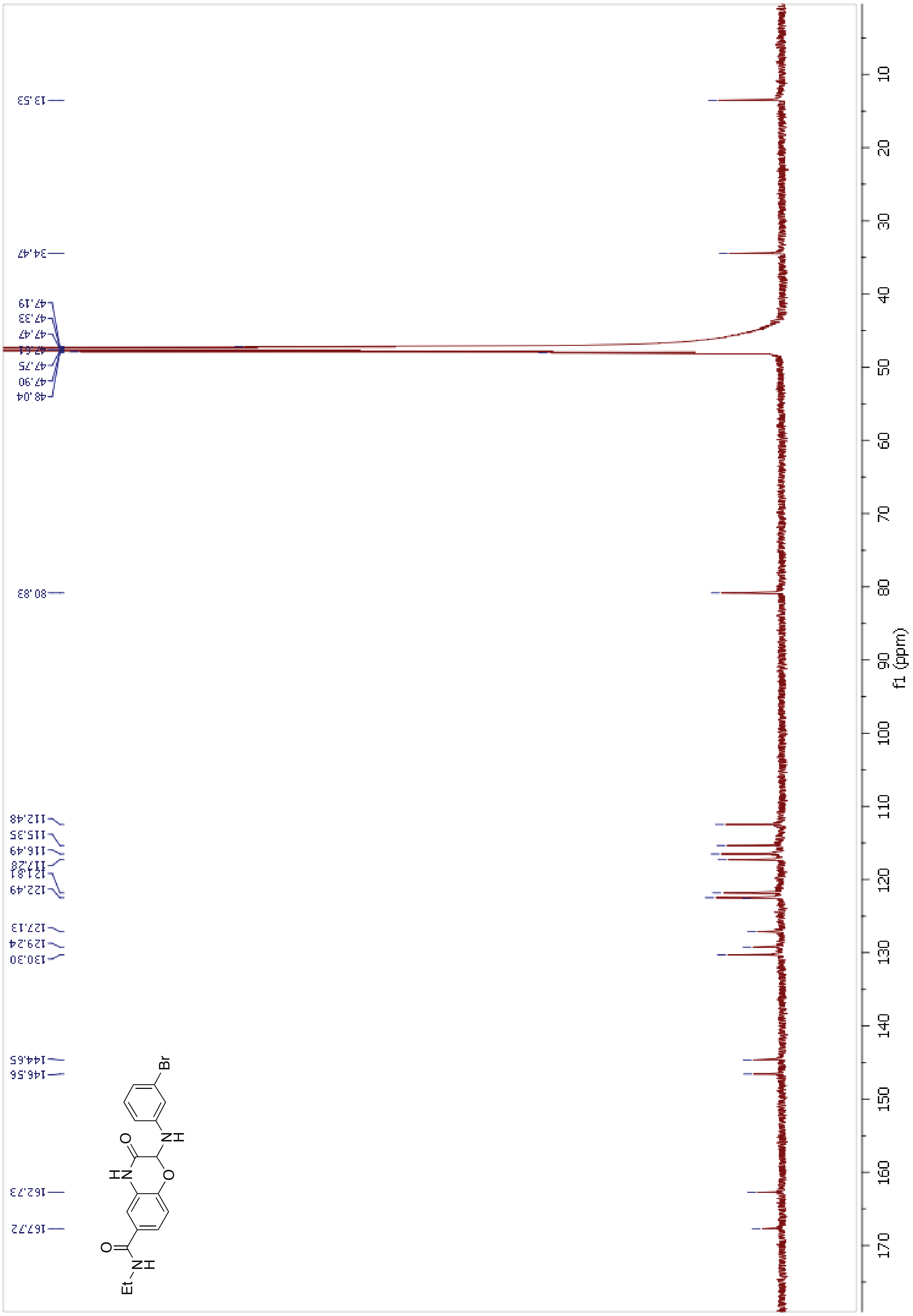
^{1}H and ^{13}C NMR spectra of product **13a**



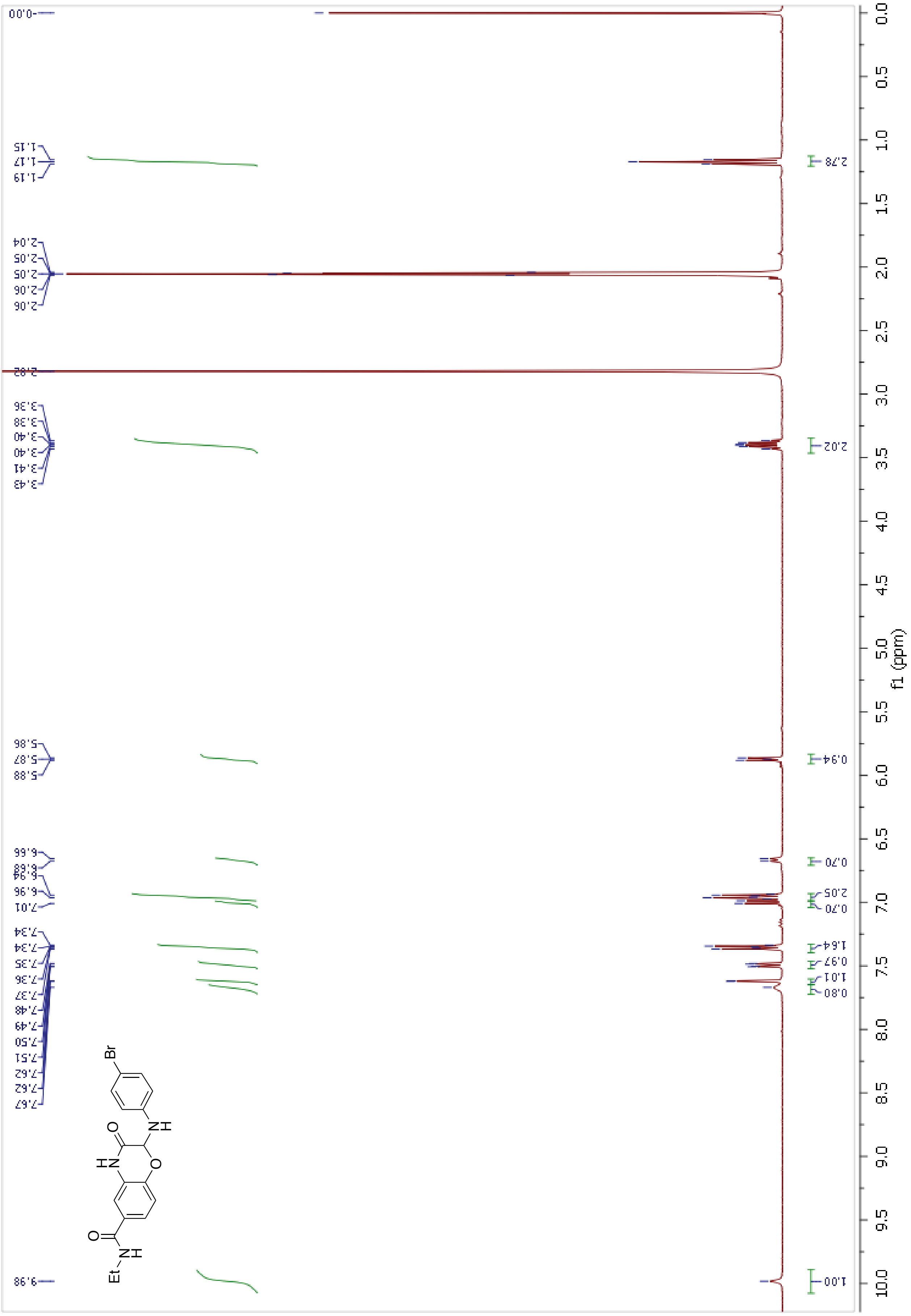

14.32
28.70
28.83
28.96
29.08
34.21
81.41
113.97
115.65
117.20
119.28
121.98
127.67
129.10
130.04
144.57
144.98
161.70
165.33
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
f1 (ppm)

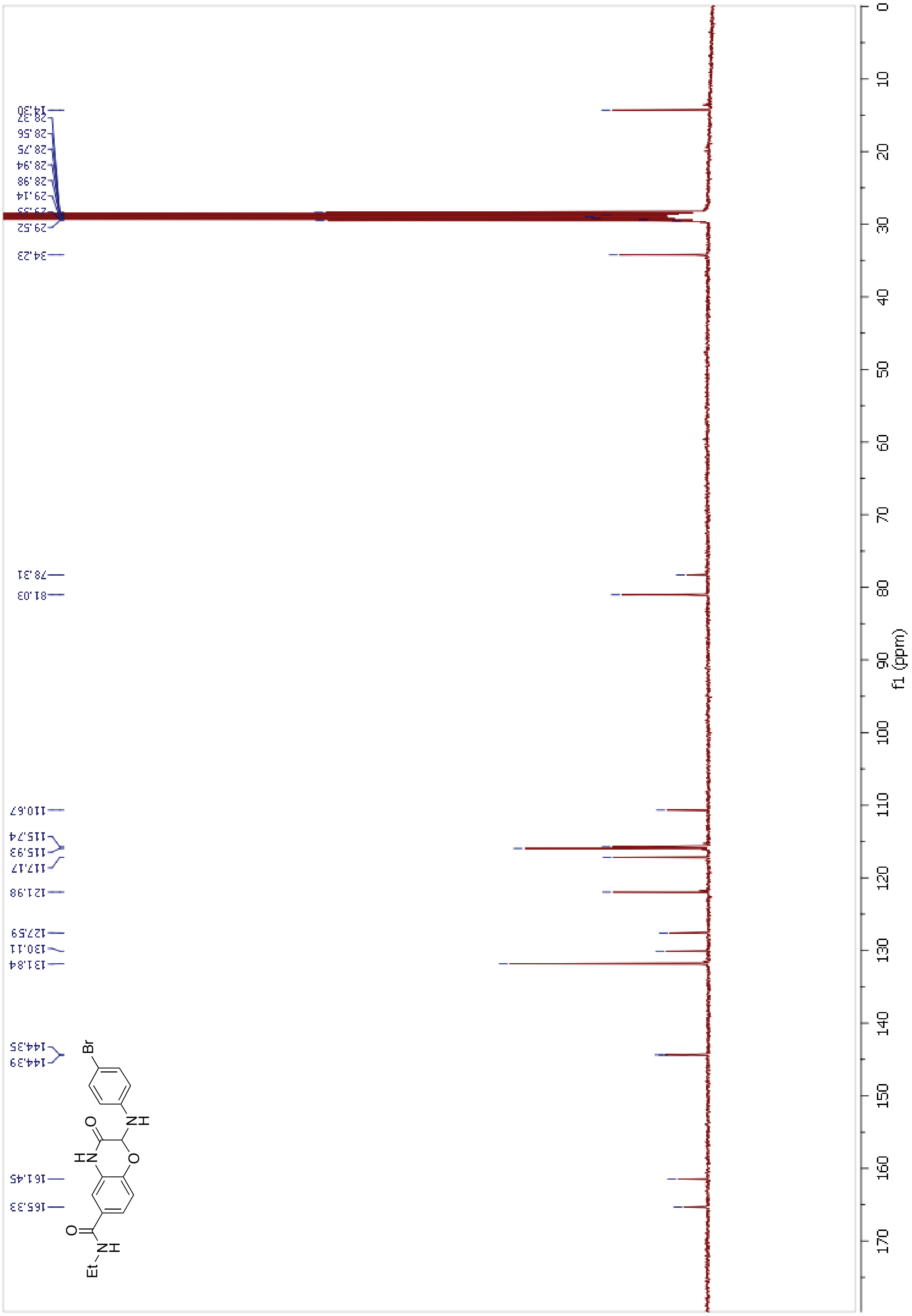
[1]H and [13]C NMR spectra of product **13b**



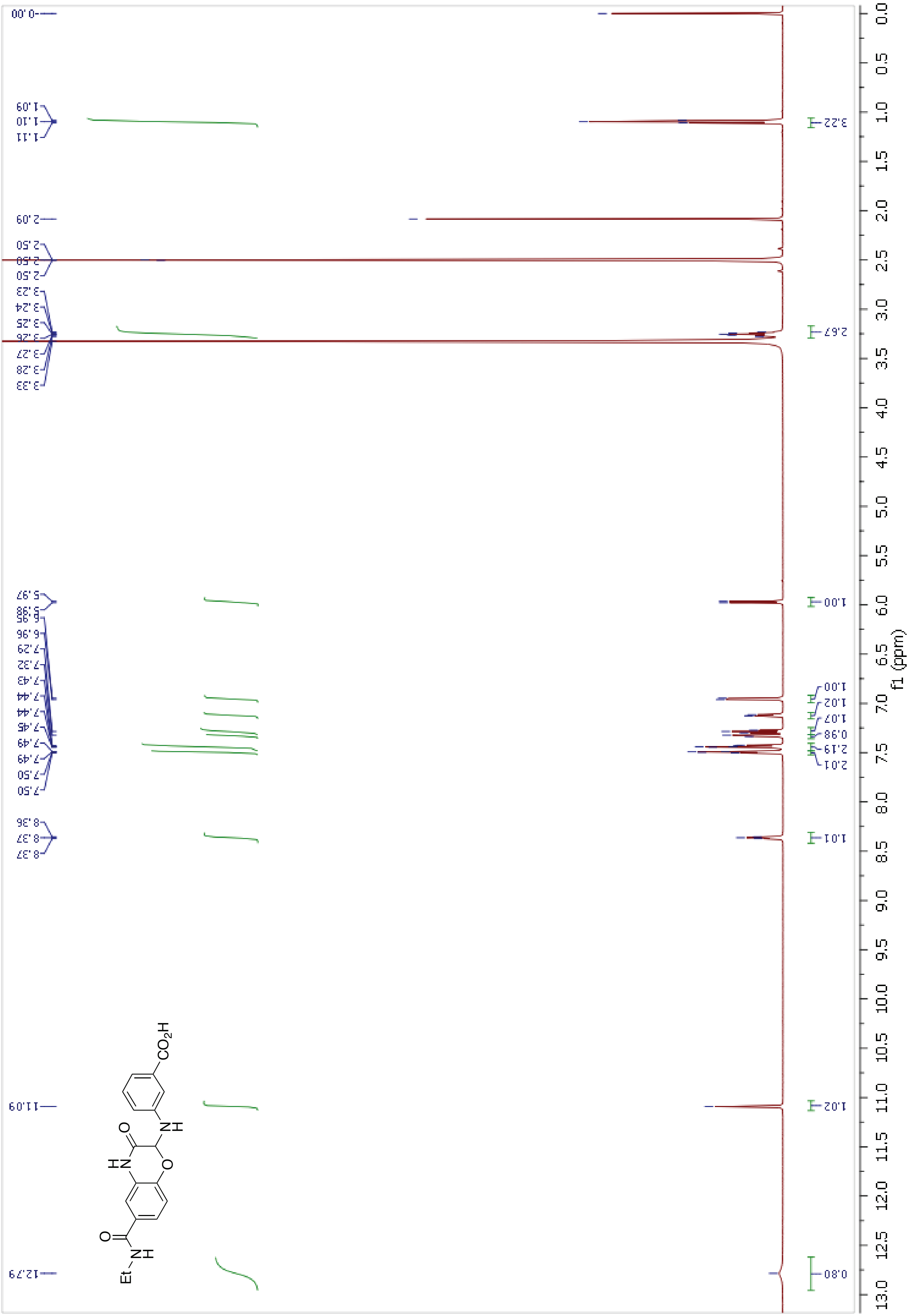

13.53
34.47
47.19
47.33
47.47
47.61
47.75
47.90
48.04
80.83
112.48
115.35
116.49
117.28
121.81
122.49
127.13
129.24
130.30
144.65
146.56
162.73
167.72
Br
NH
O
HN
O
Et
f1 (ppm)

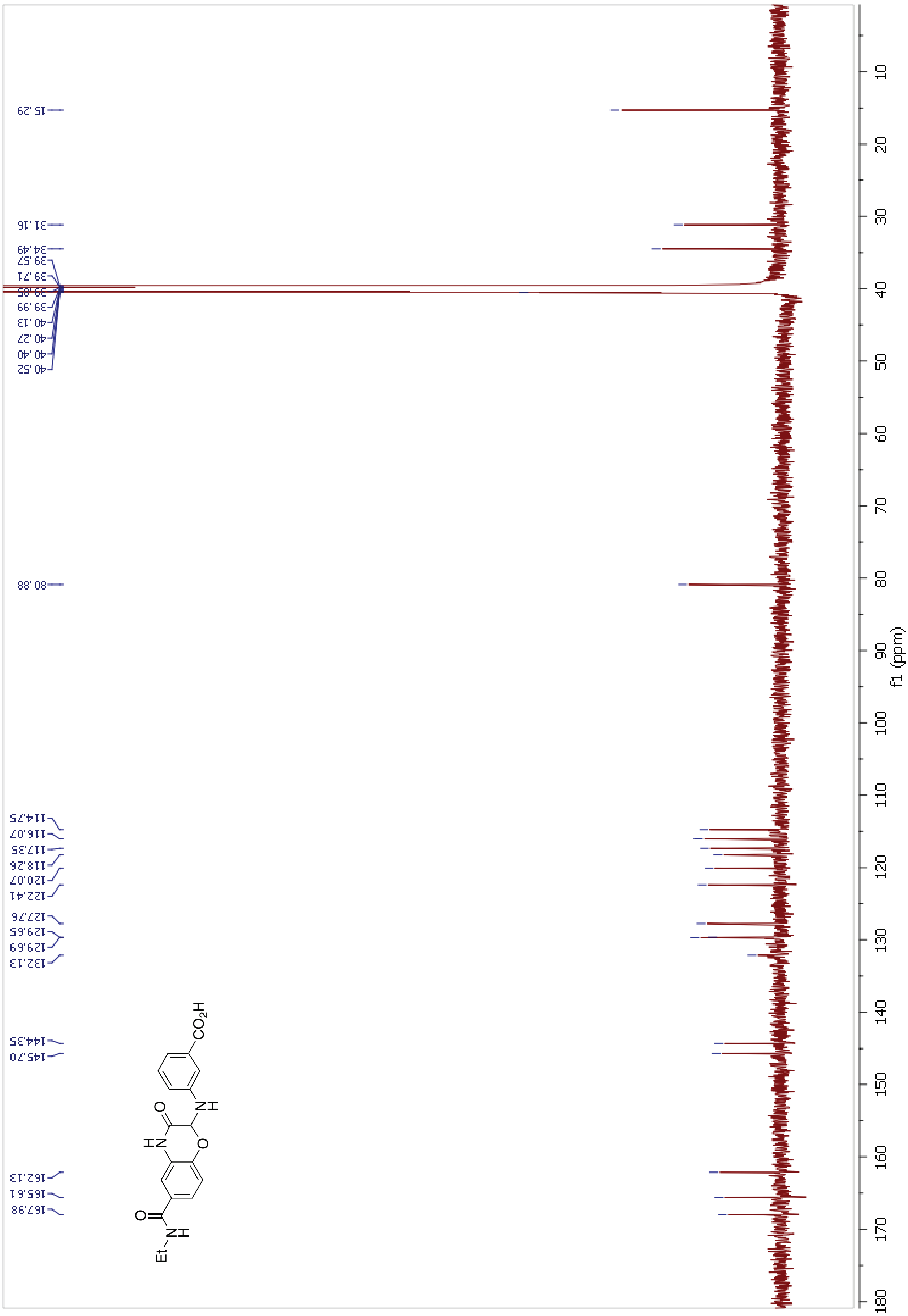
[1]H and [13]C NMR spectra of product **13c**



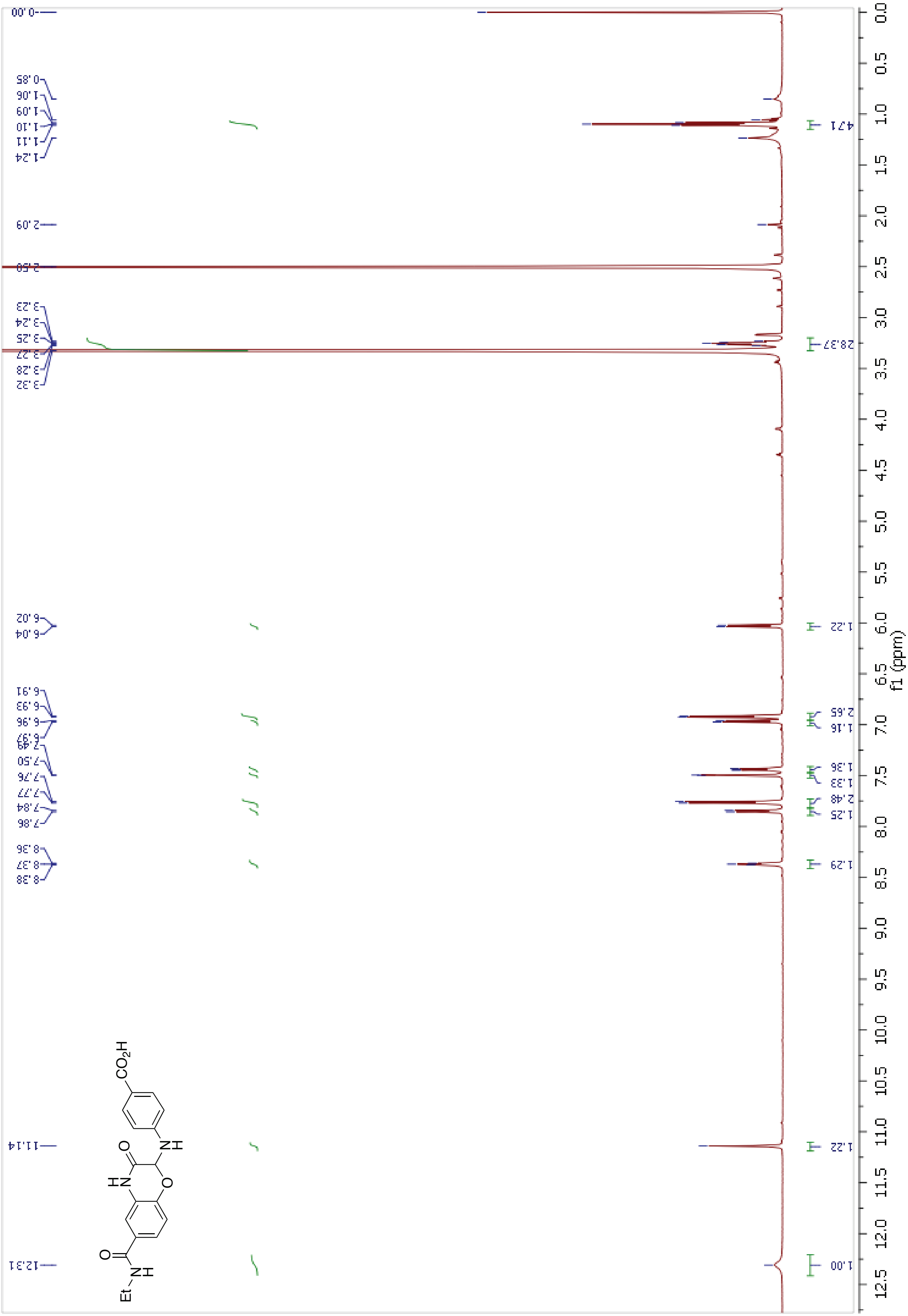

14.30
28.37
28.56
28.75
28.94
28.98
29.14
29.52
34.23
78.31
81.03
110.67
115.74
115.93
117.17
121.98
127.59
130.11
131.84
144.35
144.39
161.45
165.33
Br
NH
O
HN
O
Et
NH
O
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
f1 (ppm)

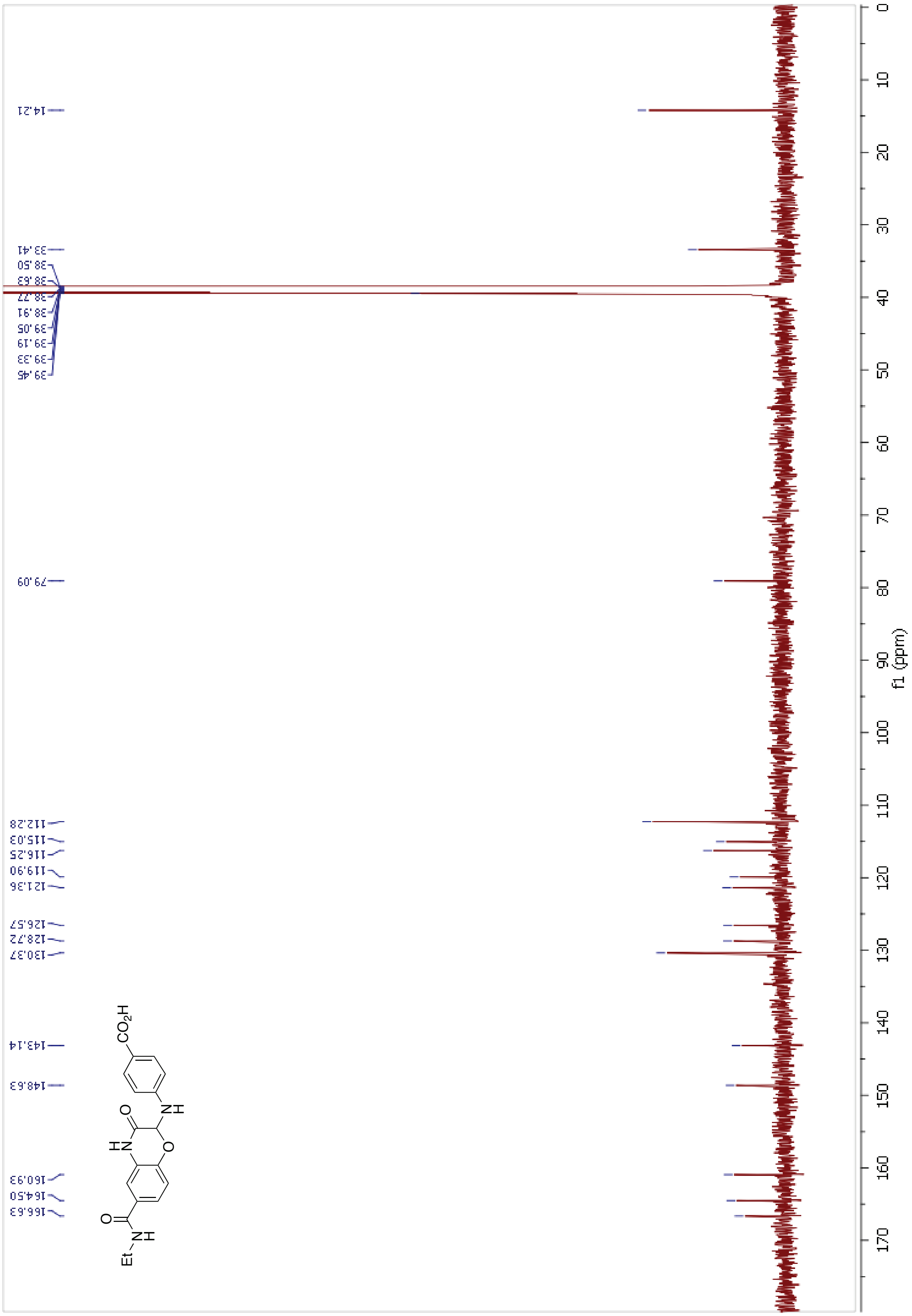
^{1}H and ^{13}C NMR spectra of product **13d**



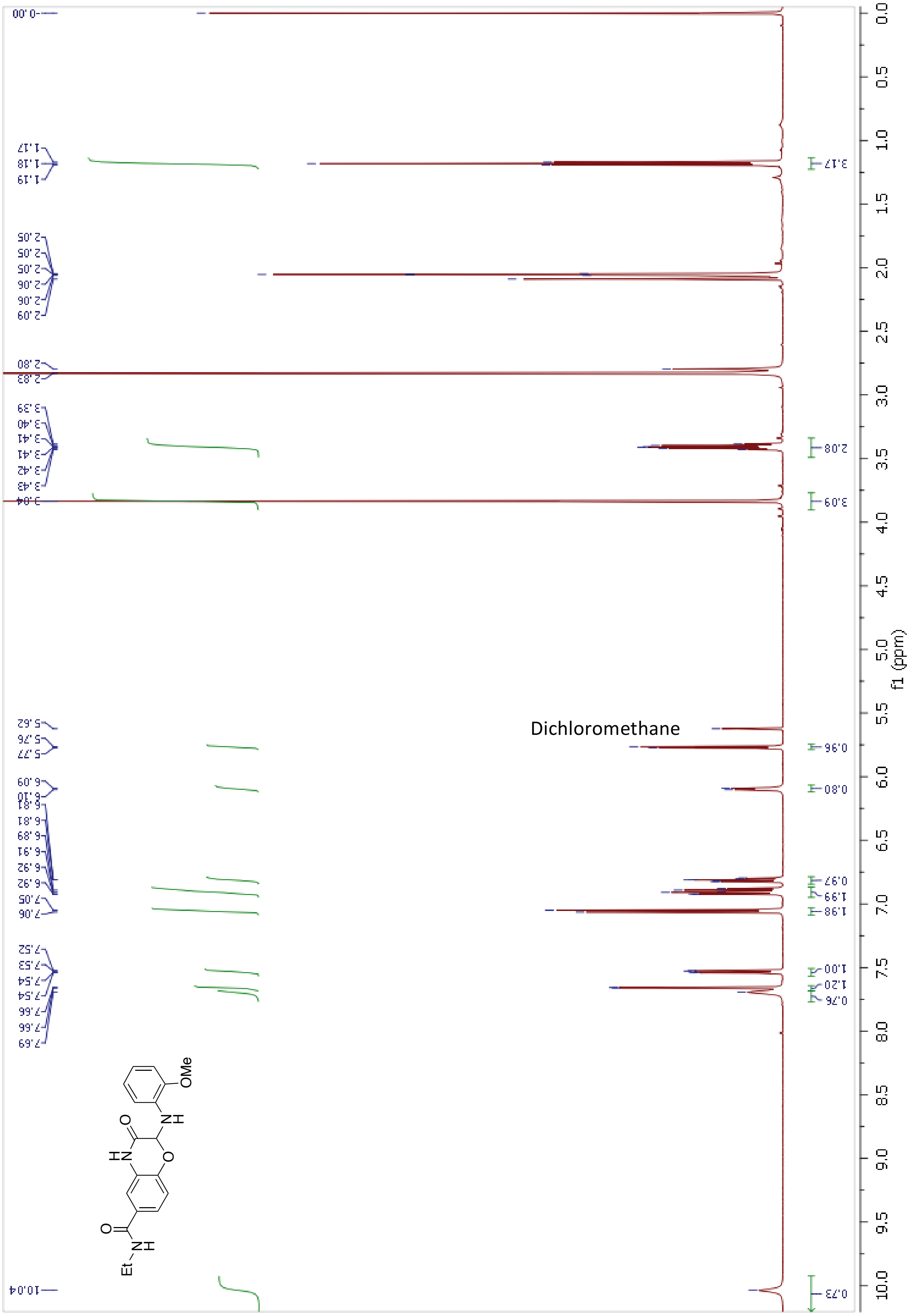

15.29
31.16
34.49
39.57
39.71
39.85
39.99
40.13
40.27
40.40
40.52
80.88
114.75
116.07
117.35
118.26
120.07
122.41
127.76
129.65
129.69
132.13
144.35
145.70
162.13
165.61
167.98
f1 (ppm)
Et
NH
O
CO2H

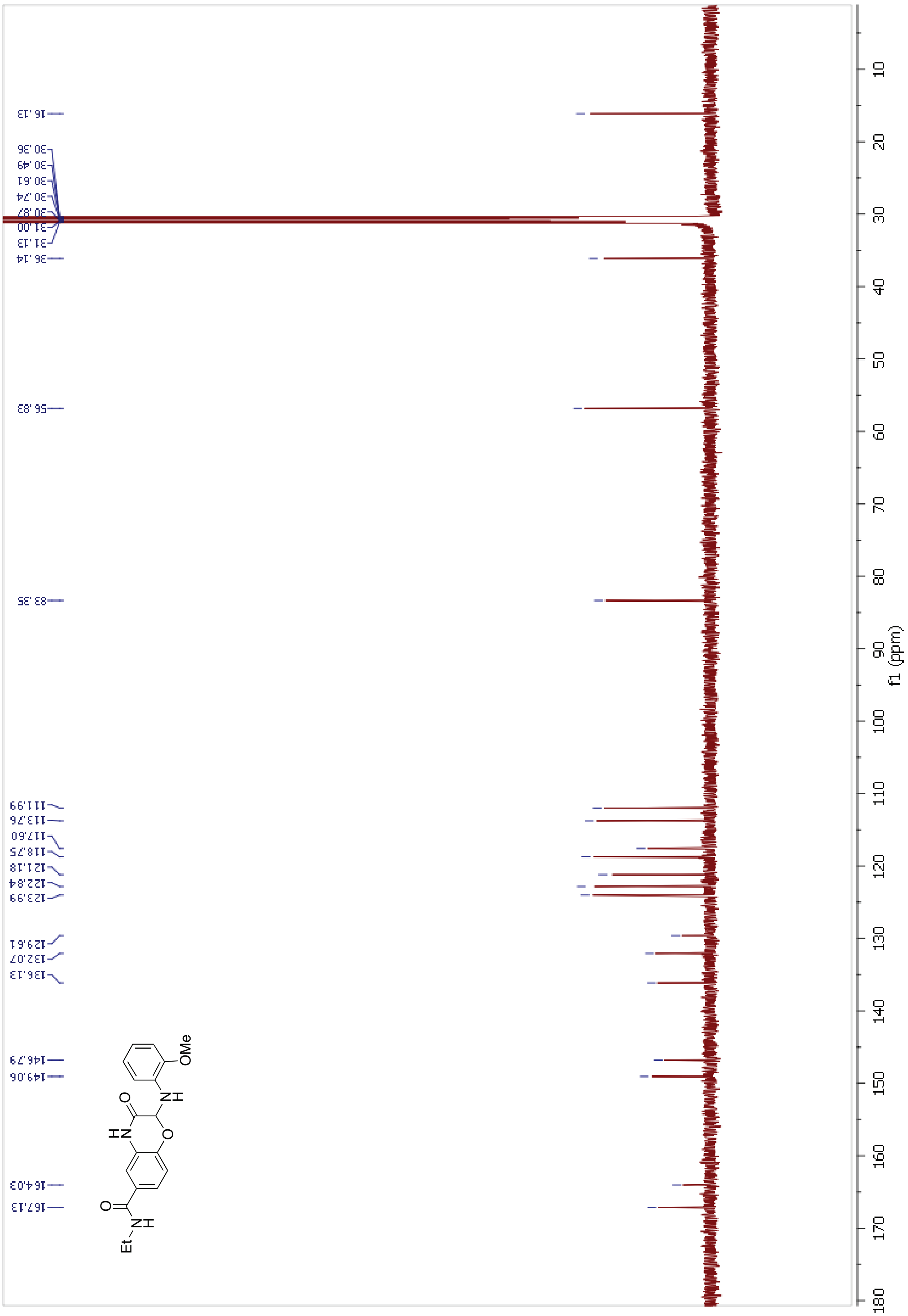
[1]H and [13]C NMR spectra of product **13e**



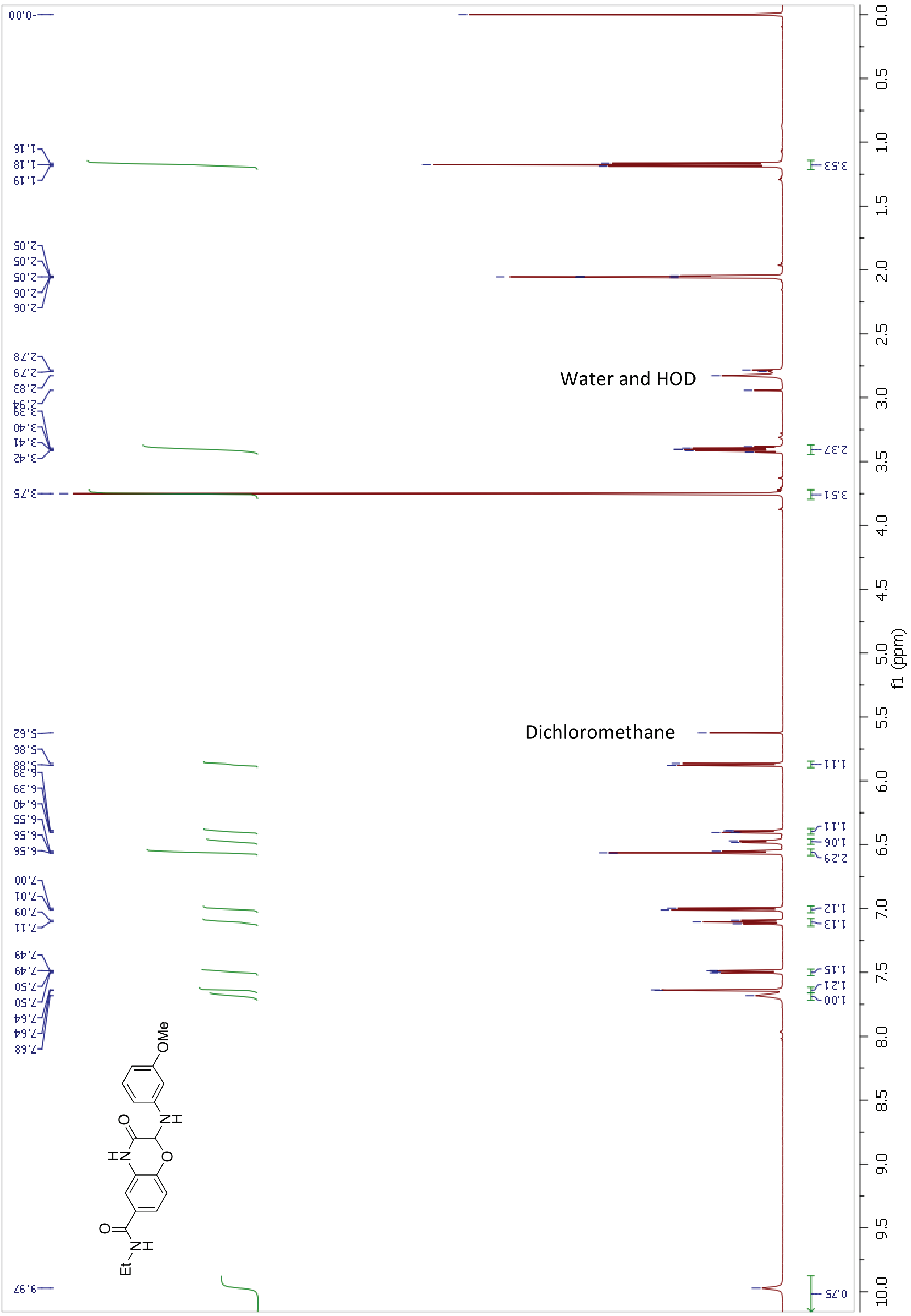

14.21
33.41
38.50
38.63
38.77
38.91
39.05
39.19
39.33
39.45
79.09
112.28
115.03
116.25
119.90
121.36
126.57
128.72
130.37
143.14
148.63
160.93
164.50
166.63
f1 (ppm)

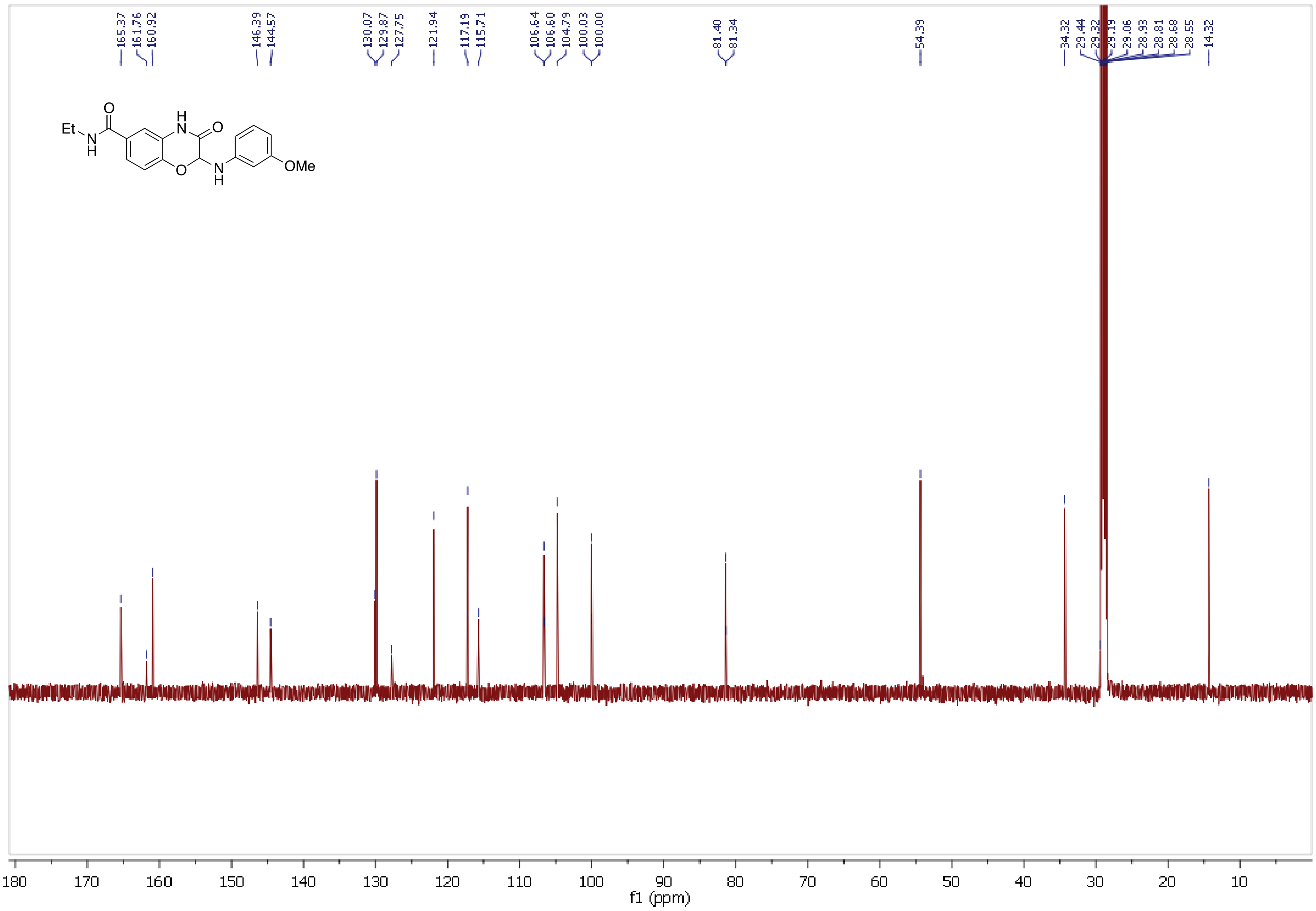
[1]H and [13]C NMR spectra of product **13f**



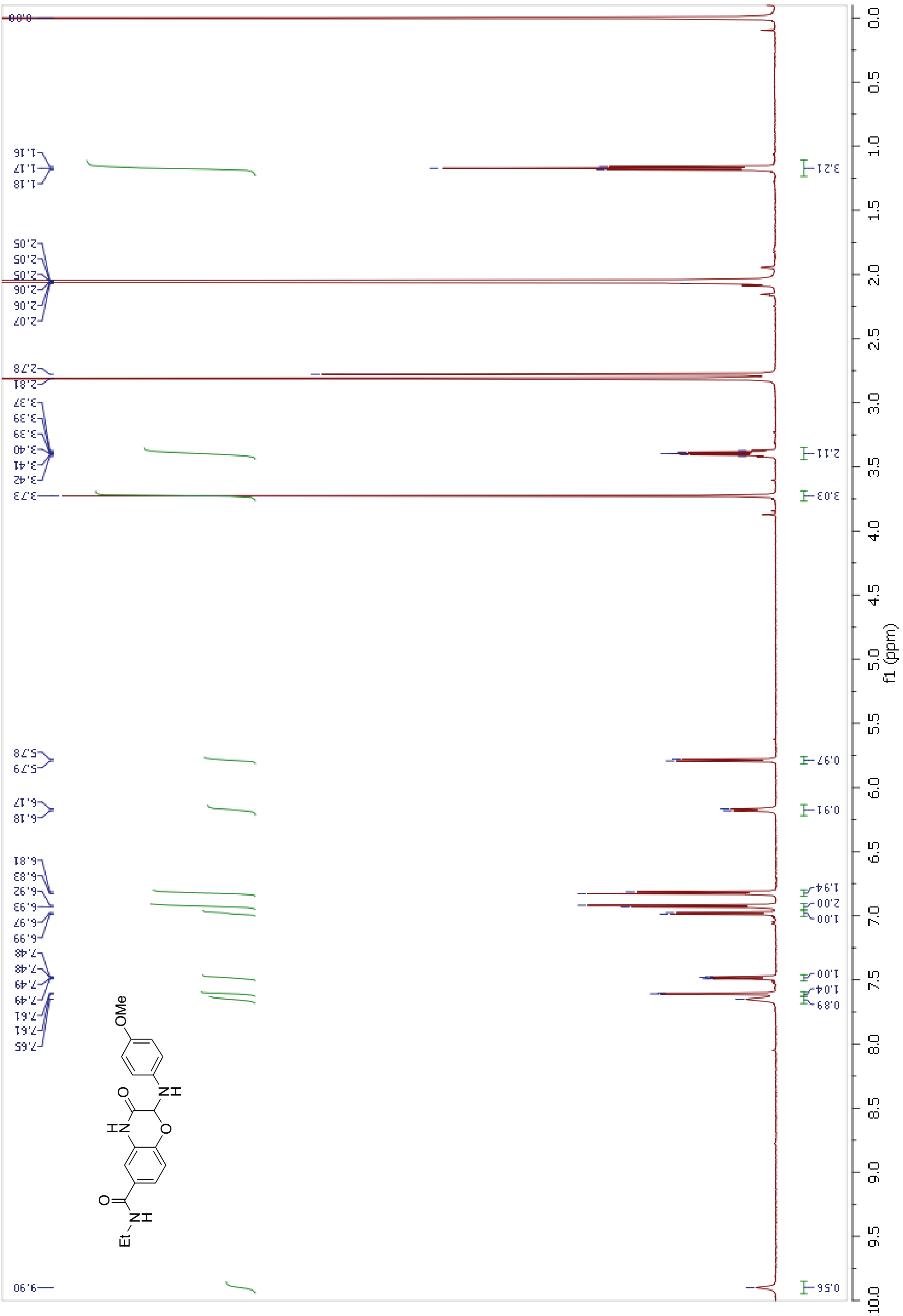

16.13
30.36
30.49
30.61
30.74
30.87
31.00
31.13
36.14
56.83
83.35
111.99
113.76
117.60
118.75
121.18
122.84
123.99
129.61
132.07
136.13
146.79
149.06
164.03
167.13
OMe
f1 (ppm)

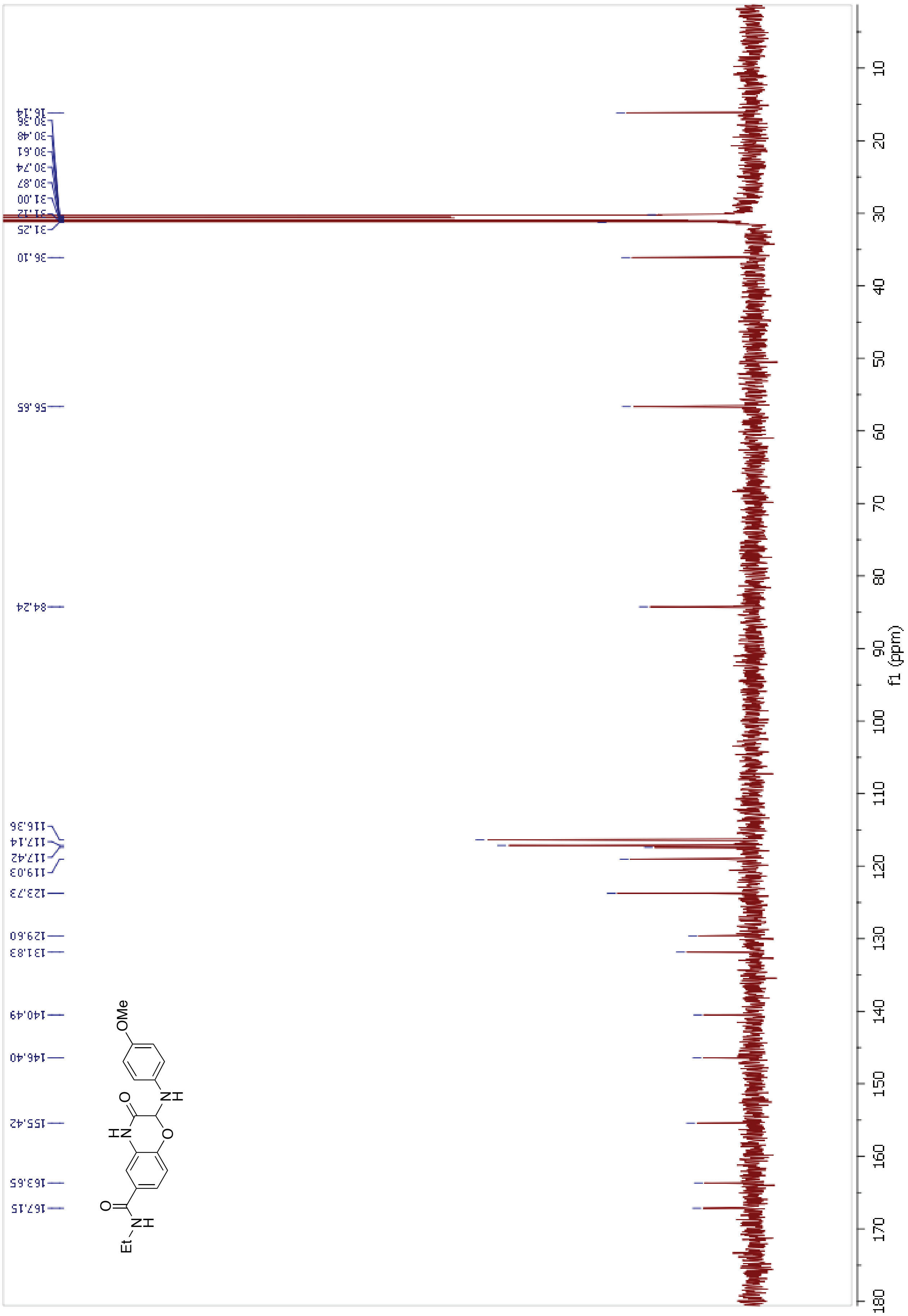
[1]H and [13]C NMR spectra of product **13g**



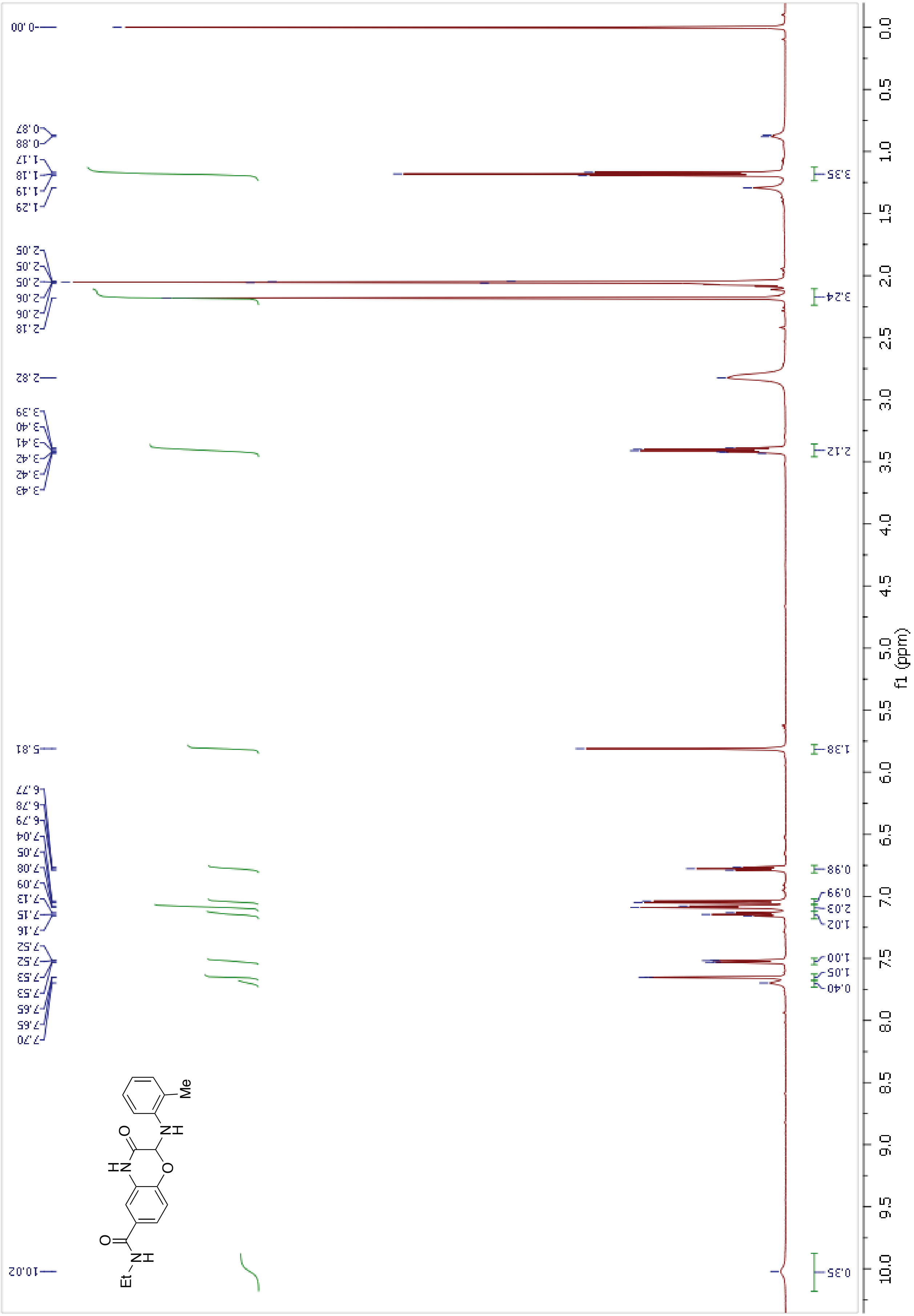

165.37
161.76
160.92
146.39
144.57
130.07
129.87
127.75
121.94
117.19
115.71
106.64
106.60
104.79
100.03
100.00
81.40
81.34
54.39
34.32
29.44
29.32
29.19
29.06
28.93
28.81
28.68
28.55
14.32
Et
N
H
O
H
N
O
O
N
H
OMe
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

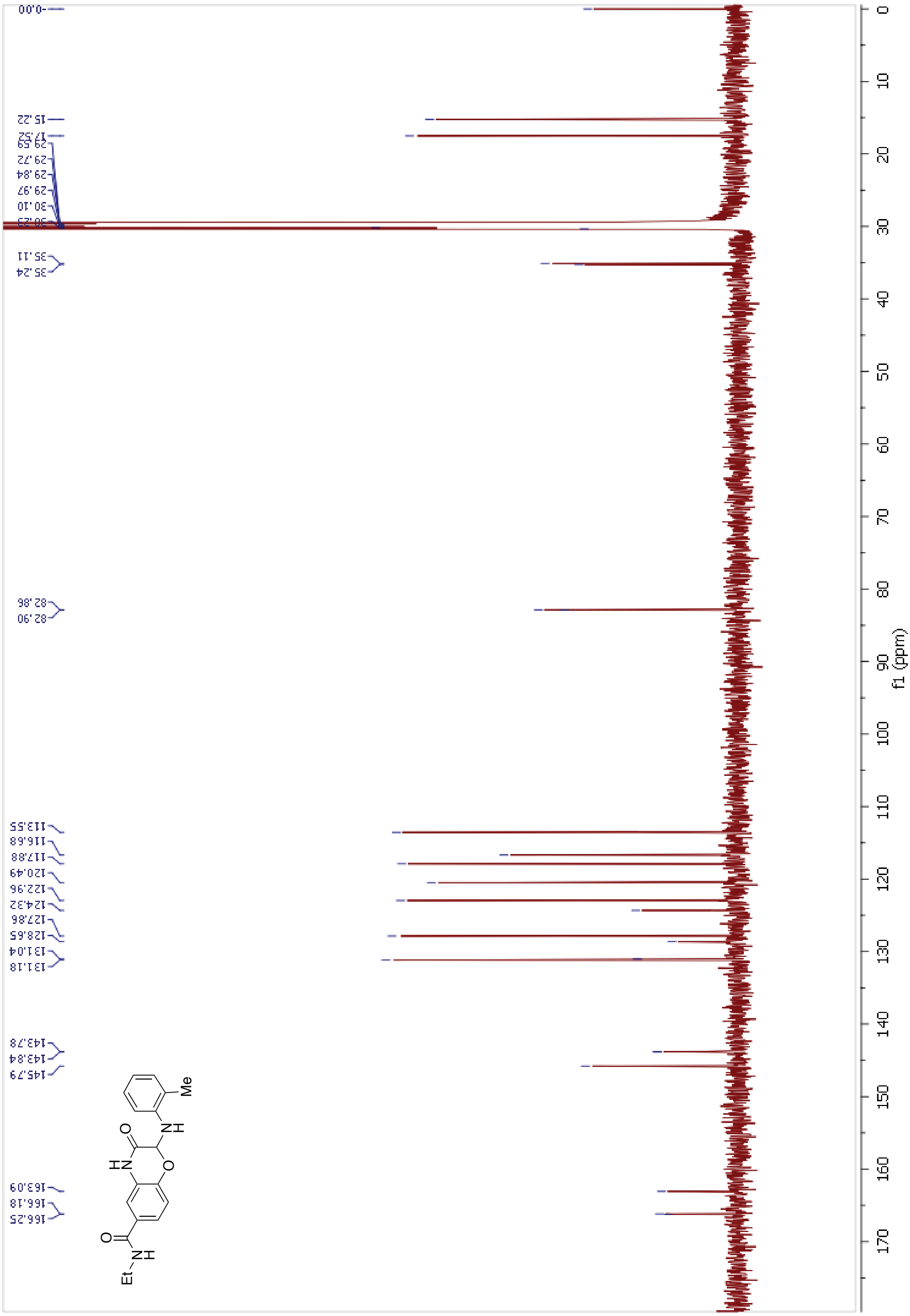
^{1}H and ^{13}C NMR spectra of product **13h**




16.14
30.36
30.48
30.61
30.74
30.87
31.00
31.12
31.25
36.10
56.65
84.24
116.36
117.14
117.42
119.03
123.73
129.60
131.83
140.49
146.40
155.42
163.65
167.15
f1 (ppm)
OMe
NH
O
HN
O
O
Et
NH

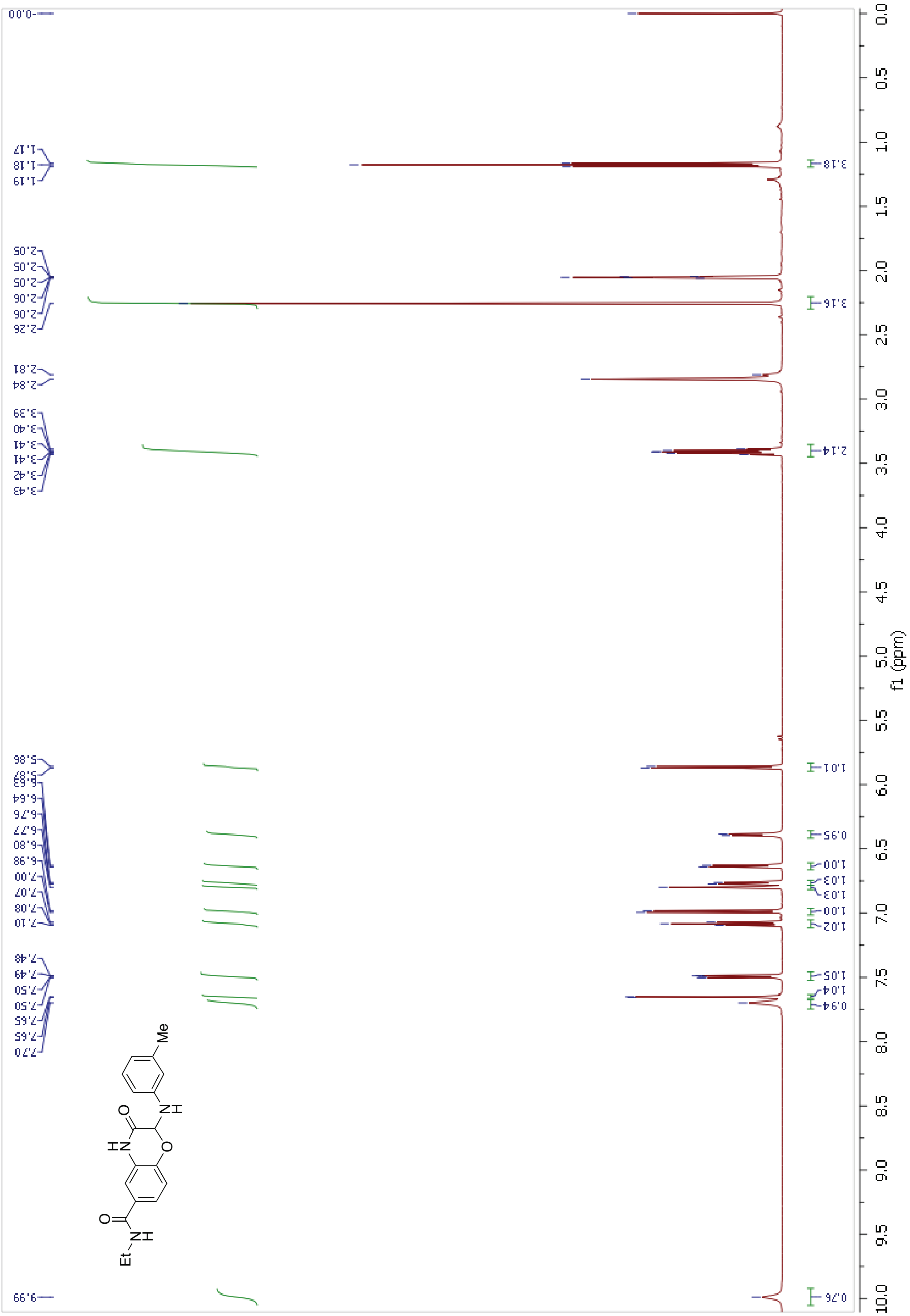
^{1}H and ^{13}C NMR spectra of product **13i**

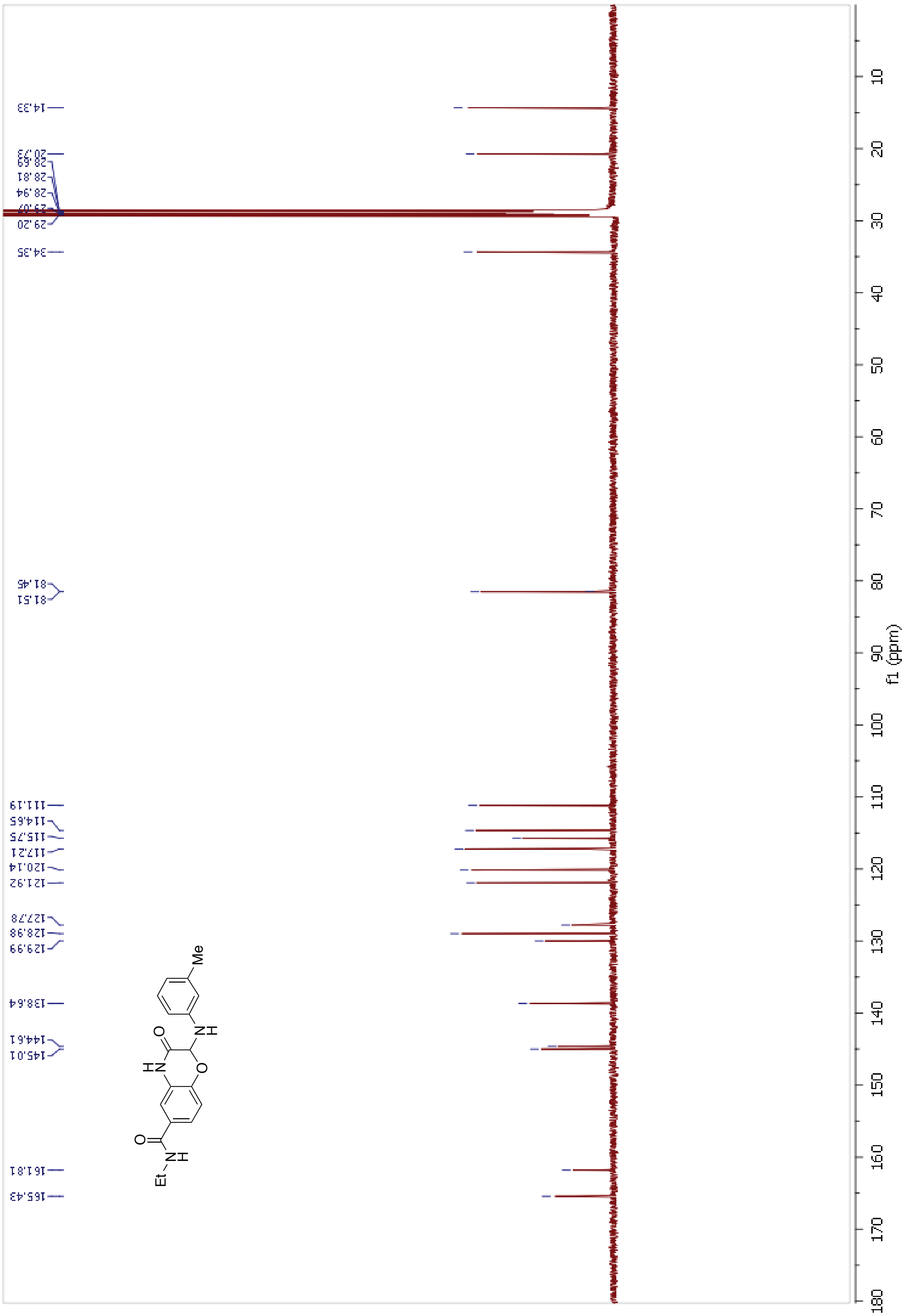




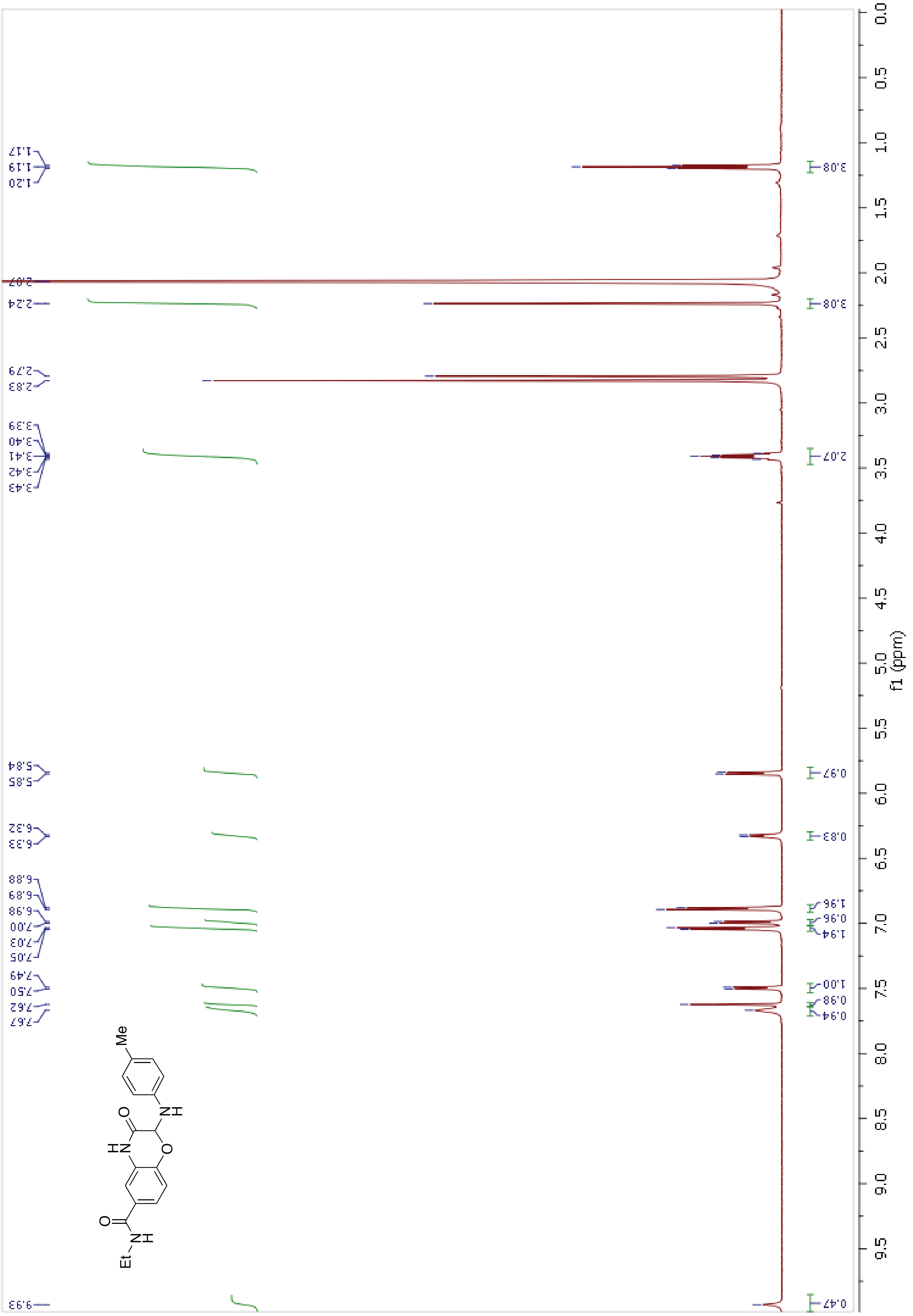
0.00
15.22
17.52
29.59
29.72
29.84
29.97
30.10
30.23
35.11
35.24
82.86
82.90
113.55
116.68
117.88
120.49
122.96
124.32
127.86
128.65
131.04
131.18
143.78
143.84
145.79
163.09
166.18
166.25
Me
NH
O
HN
O
Et
NH
O
f1 (ppm)

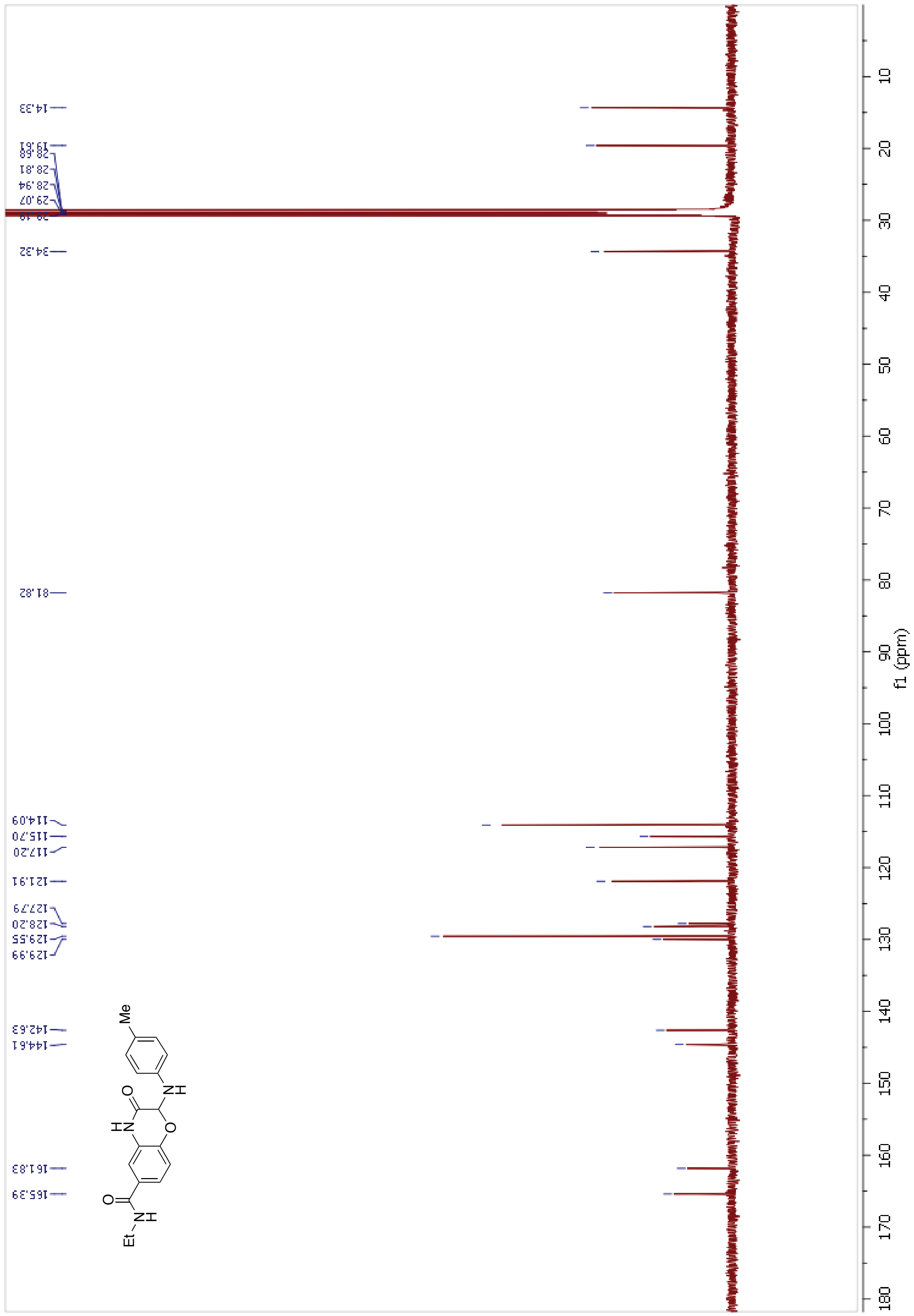
^{1}H and ^{13}C NMR spectra of product **13j**



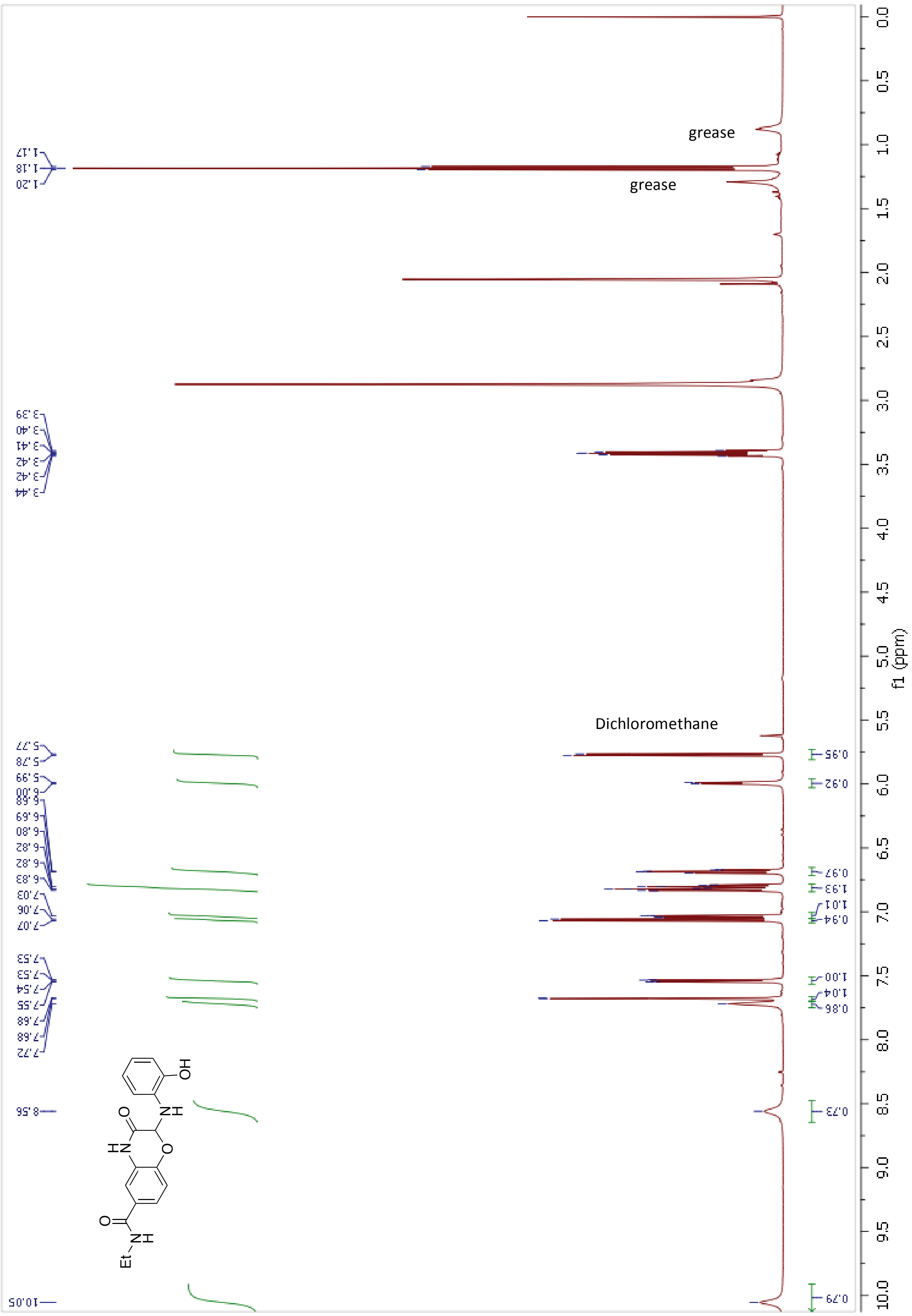

14.33
20.73
28.69
28.81
28.94
29.07
29.20
34.35
81.45
81.51
111.19
114.65
115.75
117.21
120.14
121.92
127.78
128.98
129.99
138.64
144.61
145.01
161.81
165.43
Me
NH
O
HN
Et
f1 (ppm)

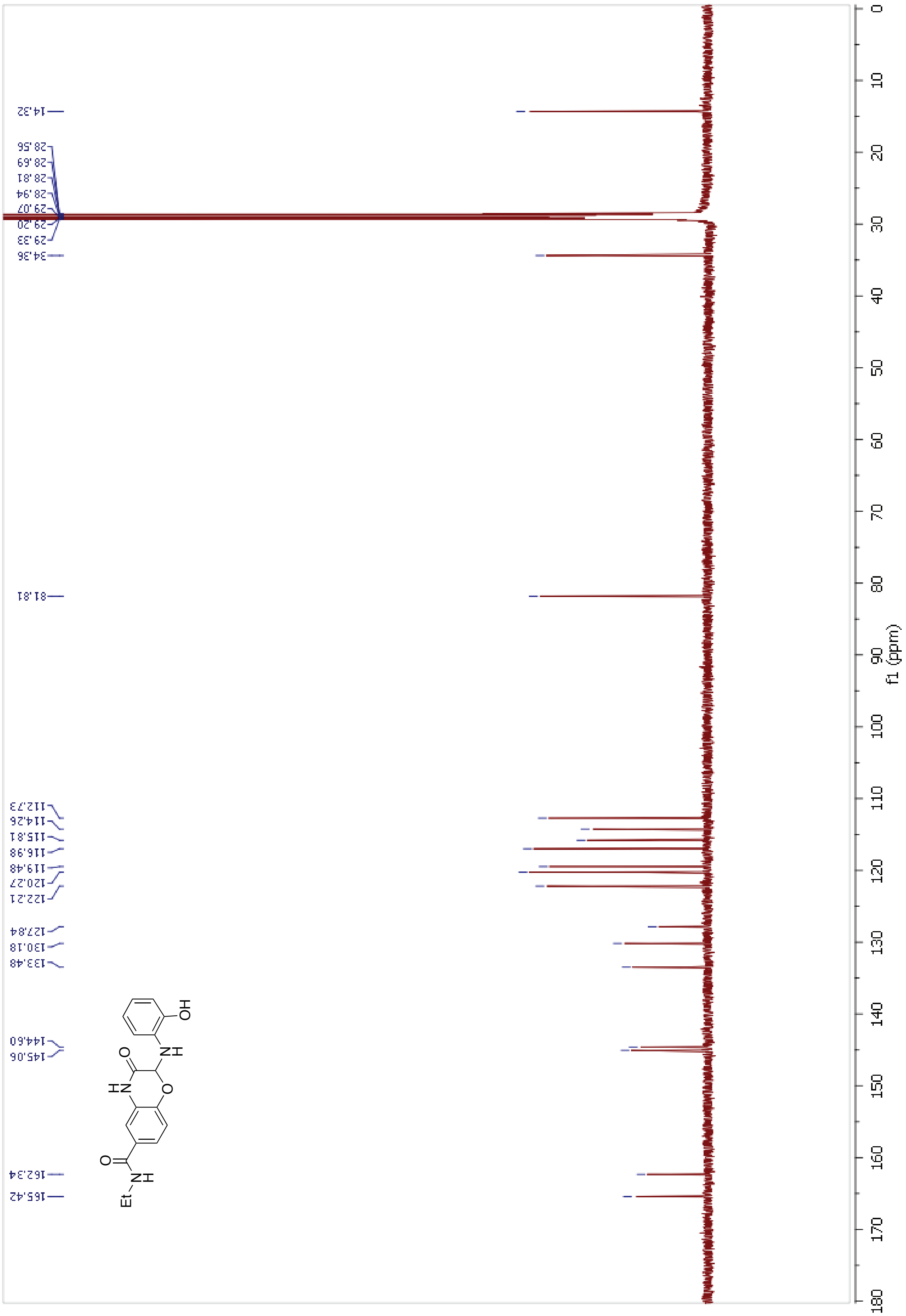
[1]H and [13]C NMR spectra of product **13k**




14.33
19.61
28.68
28.81
28.94
29.07
29.19
34.32
81.82
114.09
115.70
117.20
121.91
127.79
128.20
129.55
129.99
142.63
144.61
161.83
165.39
Me
NH
O
HN
O
O
Et
NH
f1 (ppm)

[1]H and [13]C NMR spectra of product **13l**

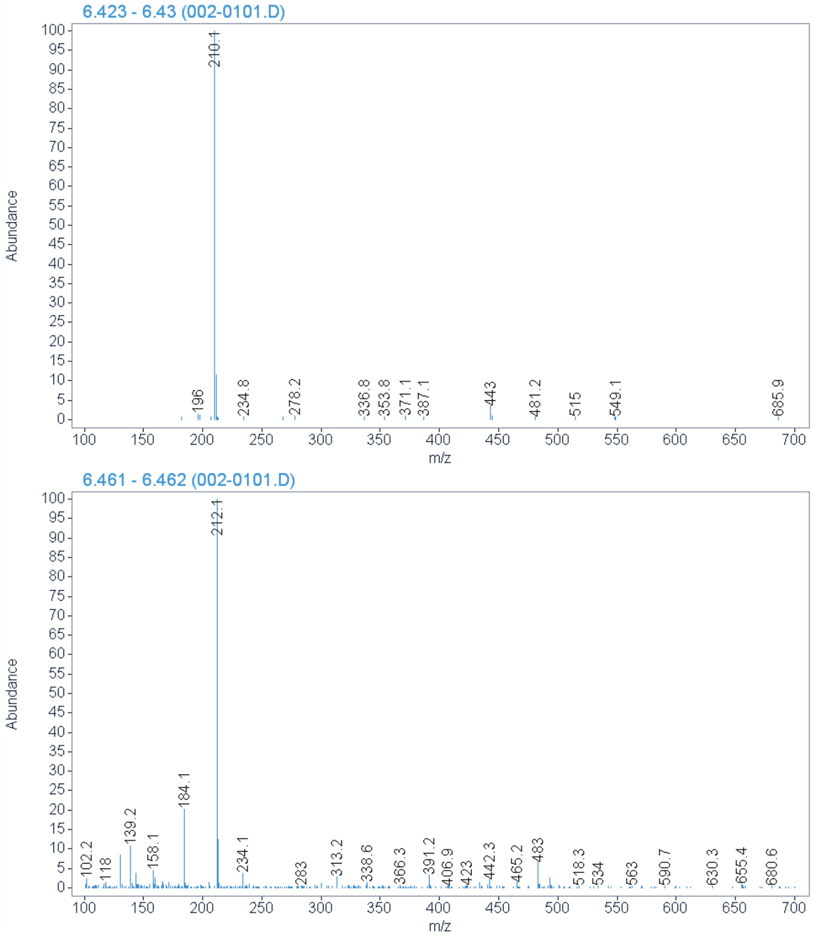



14.32
28.56
28.69
28.81
28.94
29.07
29.20
29.33
34.36
81.81
112.73
114.26
115.81
116.98
119.48
120.27
122.21
127.84
130.18
133.48
144.60
145.06
162.34
165.42
f1 (ppm)

Mass spectrum for product **5**

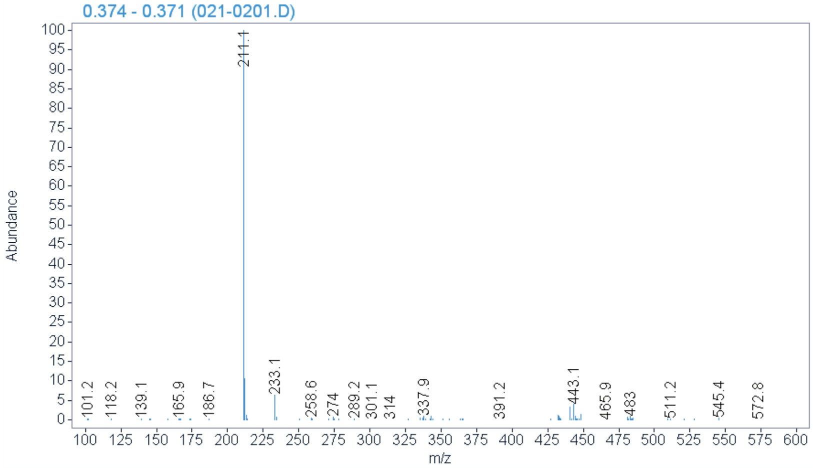
LCMS Report

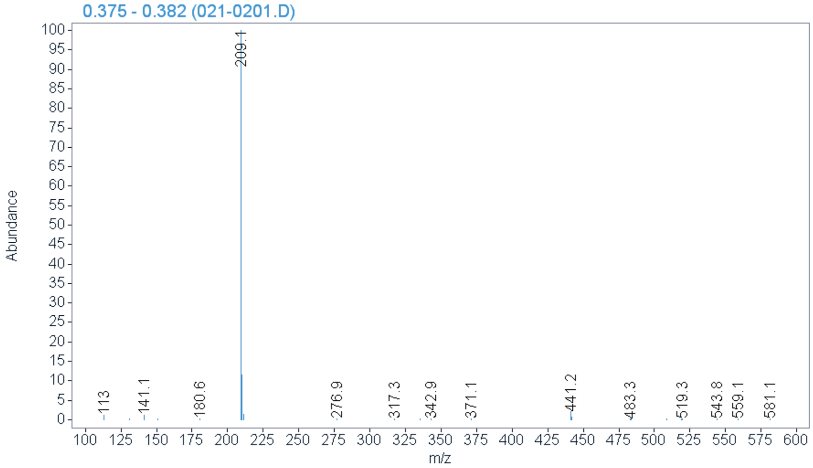
Agilent Technologies



Mass spectrum for product **6**

LCMS Report

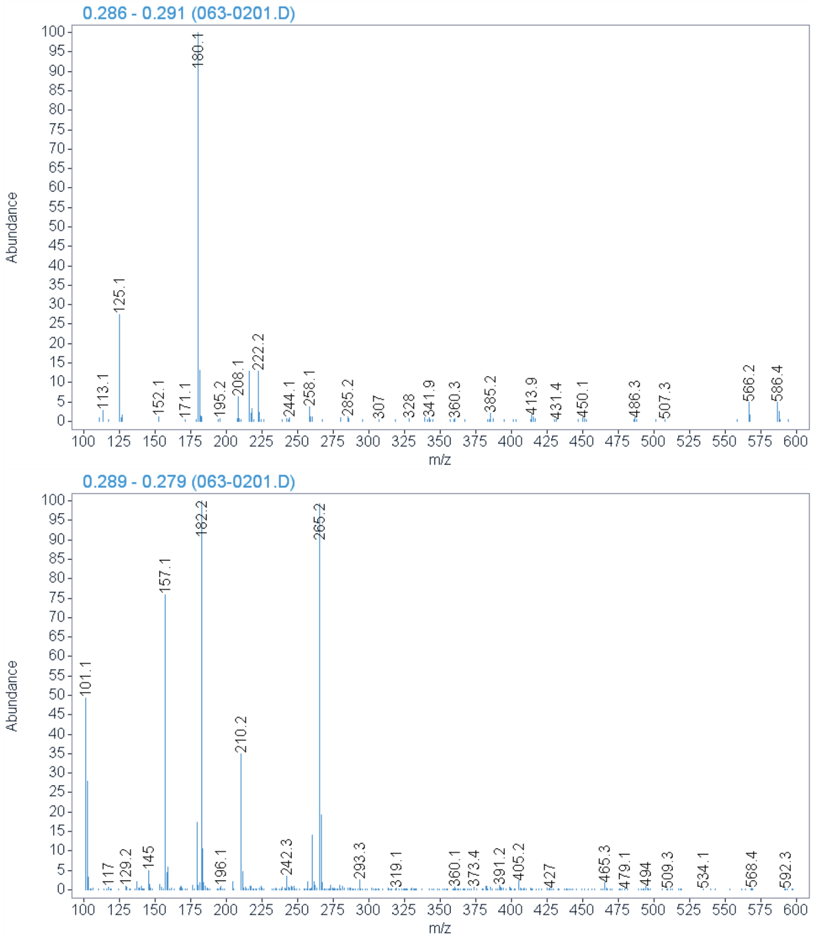




Mass spectrum for product **7**

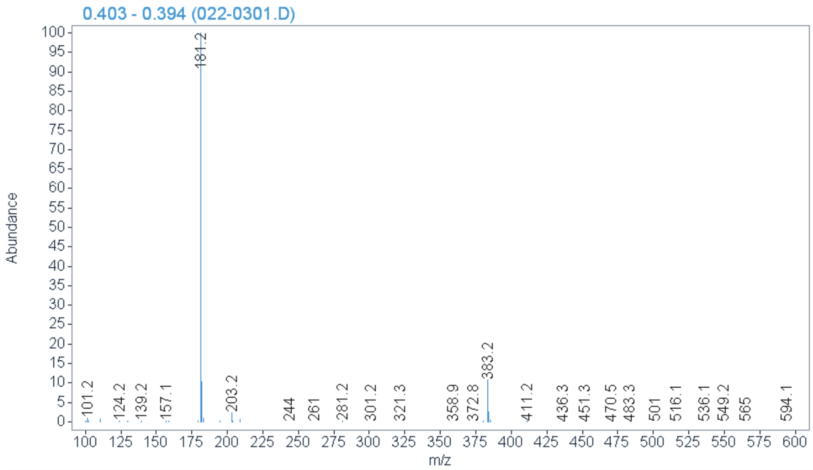
LCMS Report

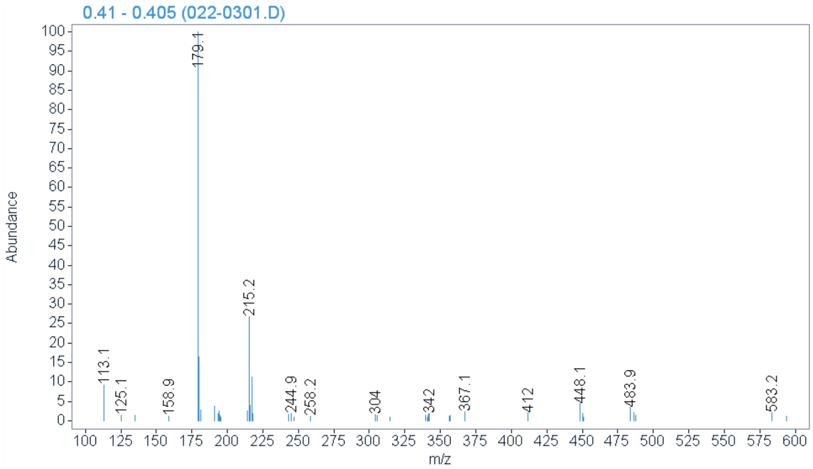
Agilent Technologies



Mass spectrum for product **8**

LCMS Report





Mass spectrum for product **10**

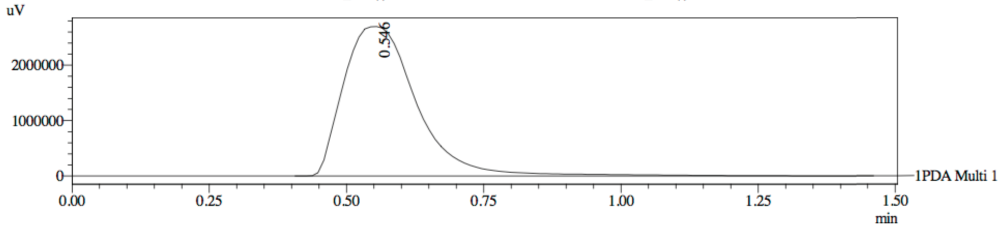
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

Acquired by	: Admin
Date Acquired	: 7/29/2014 11:54:03 AM
Sample Type	: Unknown
Level#	: 0
Sample Name	: AL4148_ESI(-)
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 2
Injection Volume	: 10
Data File	: AL4148_ESI(-).lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Pete\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/29/2014 11:55:34 AM

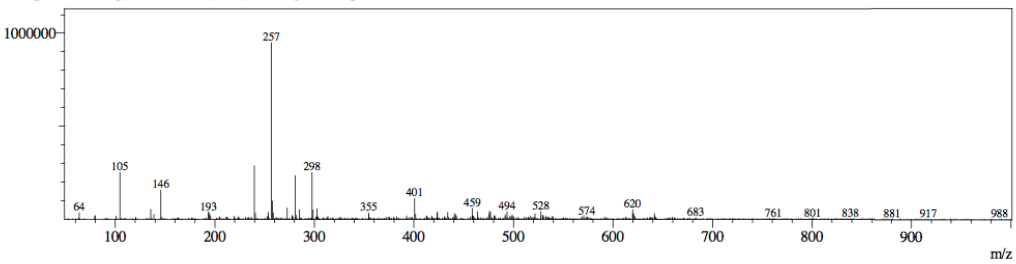
Chromatogram
AL4148_ESI(-) C:\LabSolutions\Data\Andrew Lin\AL4148_ESI(-).lcd



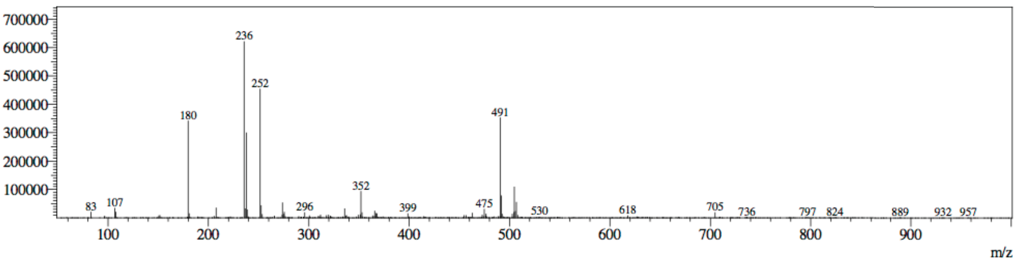
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.600(Scan#:61)
Max Peak:839 Base Peak:256.80(946436)
Spectrum:Averaged 0.480-0.780(49-79)
Background:Averaged 0.000-0.498(1-51) Polarity:Pos Segment1 - Event1



Retention Time:0.610(Scan#:62)
Max Peak:731 Base Peak:235.70(620987)
Spectrum:Averaged 0.490-0.790(50-80)
Background:Averaged 0.010-0.498(2-52) Polarity:Neg Segment1 - Event2



C:¥LabSolutions¥Data¥Andrew Lin¥AL4148_ESI(-).lcd

Mass spectrum for product **12a**

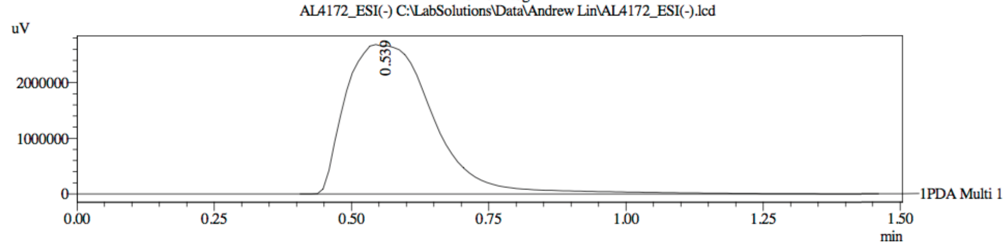
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

Acquired by	: Admin
Date Acquired	: 7/29/2014 12:01:30 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: AL4172_ESI(-)
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 5
Injection Volume	: 10
Data File	: AL4172_ESI(-).lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Pete\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/29/2014 12:03:03 PM

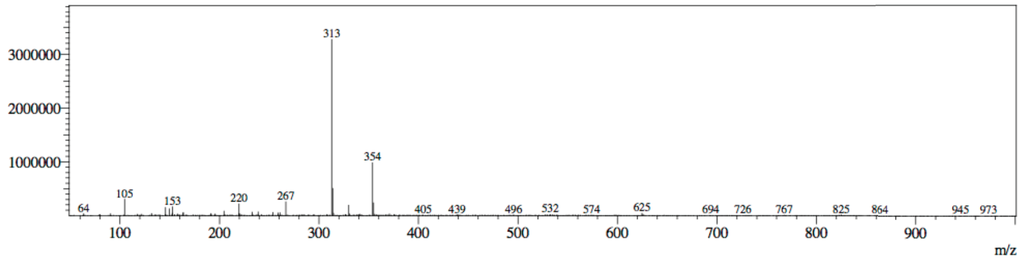
Chromatogram
AL4172_ESI(-) C:\LabSolutions\Data\Andrew Lin\AL4172_ESI(-).lcd



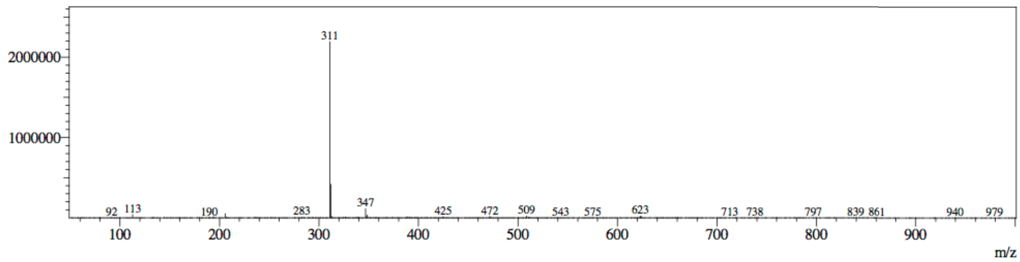
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.600(Scan#:61)
Max Peak:806 Base Peak:312.85(3275173)
Spectrum:Averaged 0.500-0.920(51-93)
Background:Averaged 0.000-0.484(1-49) Polarity:Pos Segment1 - Event1



Retention Time:0.610(Scan#:62)
Max Peak:720 Base Peak:310.90(2195382)
Spectrum:Averaged 0.510-0.930(52-94)
Background:Averaged 0.010-0.484(2-50) Polarity:Neg Segment1 - Event2

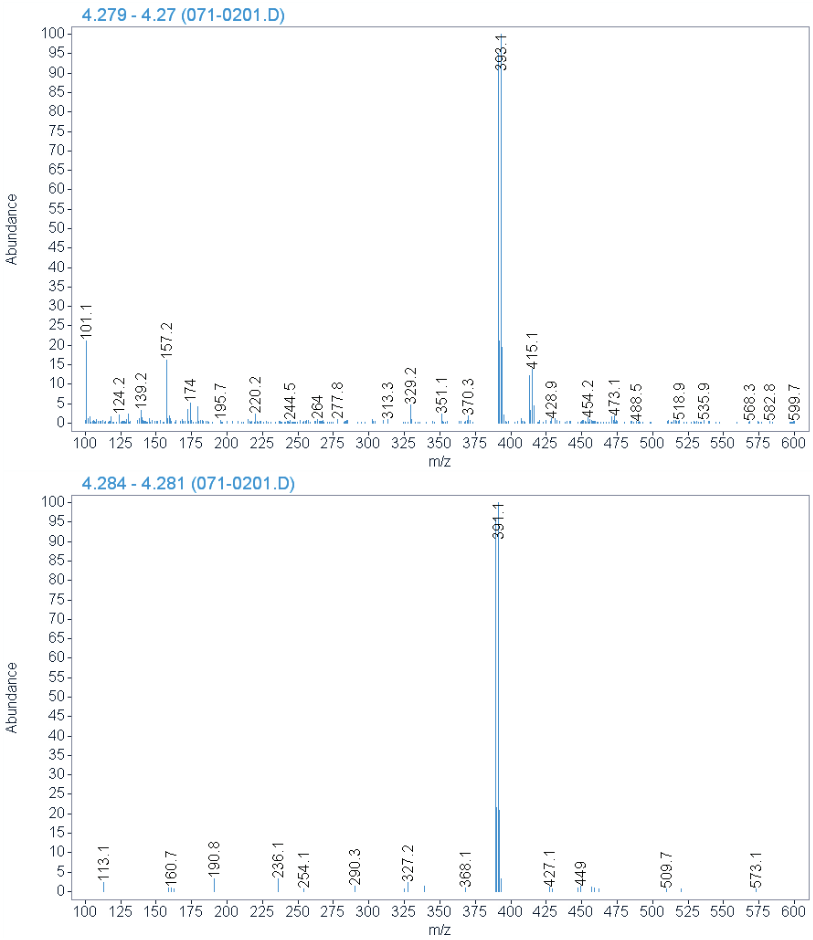


C:¥LabSolutions¥Data¥Andrew Lin¥AL4172_ESI(-).lcd

Mass spectrum of product **12b**

LCMS Report

Agilent Technologies



Mass spectrum of product **12c**

==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

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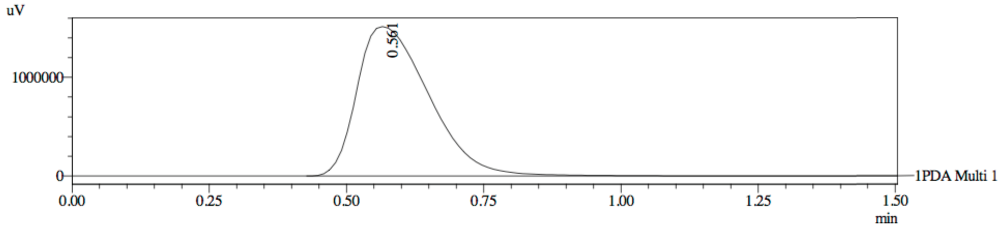
Date Acquired	: 10/13/2015 1:31:54 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: AL4167-ve
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 16
Injection Volume	: 10
Data File	: AL4167-ve.lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Jen Baker\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\Data\AutoTuning\Autotune_ESI_26AUG15.lct
Processed by	: Admin
Modified Date	: 10/13/2015 1:33:25 PM

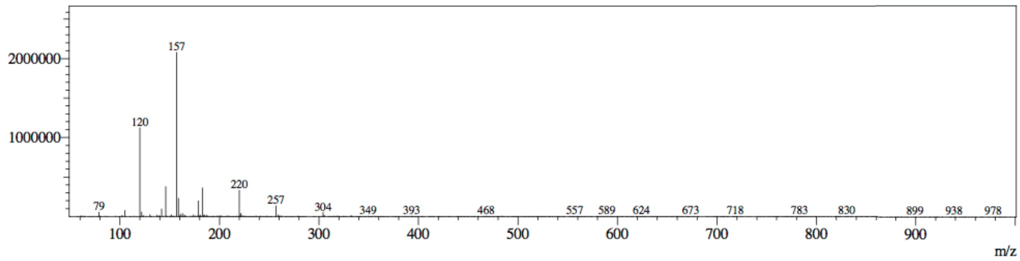
Chromatogram
AL4167-ve C:\LabSolutions\Data\Jen Baker\AL4167-ve.lcd



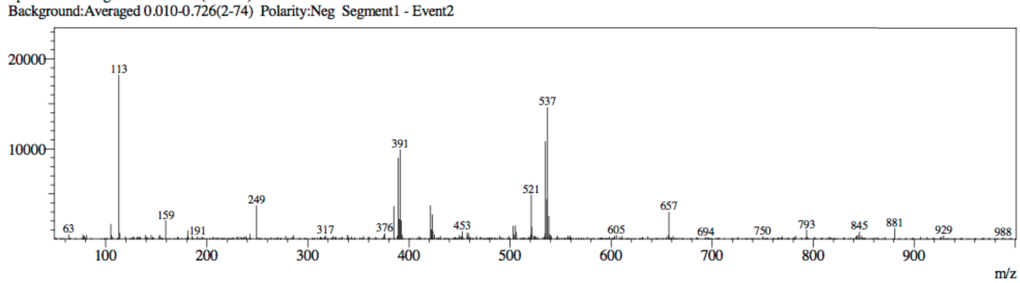
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.860(Scan#:87)
Max Peak:683 Base Peak:156.95(2078115)
Spectrum:Averaged 0.720-1.400(73-141)
Background:Averaged 0.000-0.726(1-73) Polarity:Pos Segment1 - Event1



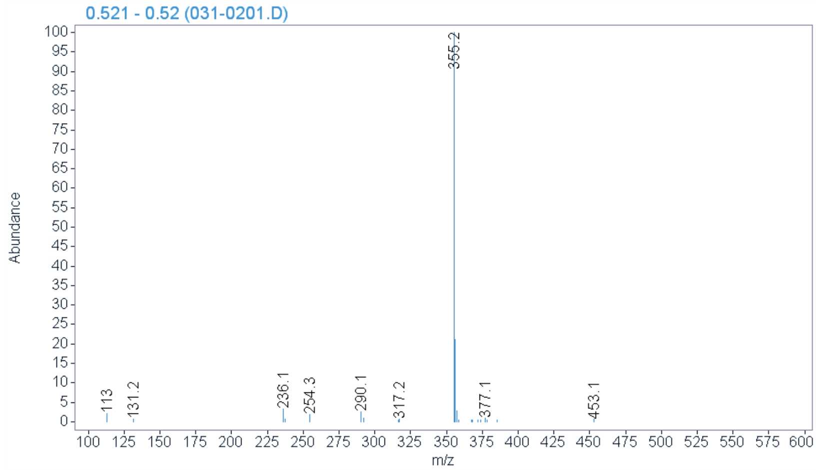
Retention Time:0.850(Scan#:86)
Max Peak:563 Base Peak:113.10(18202)
Spectrum:Averaged 0.730-1.410(74-142)
Background:Averaged 0.010-0.726(2-74) Polarity:Neg Segment1 - Event2

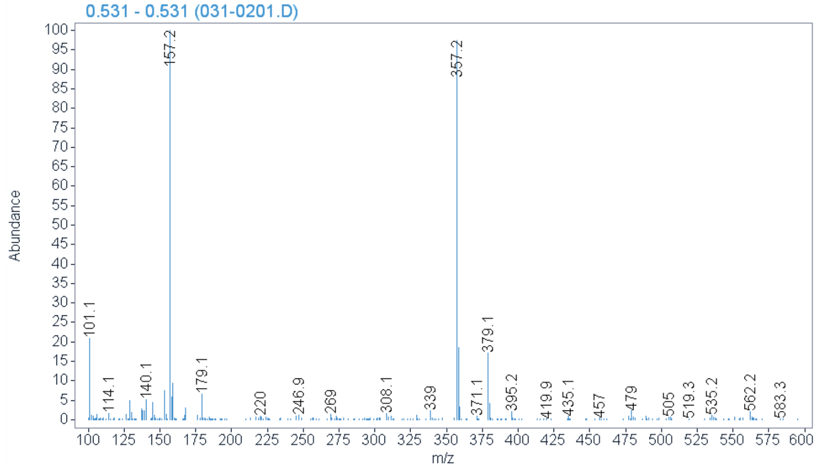


Mass spectrum of product **12d**

LCMS Report

Agilent Technologies





Mass spectrum of product **12e**

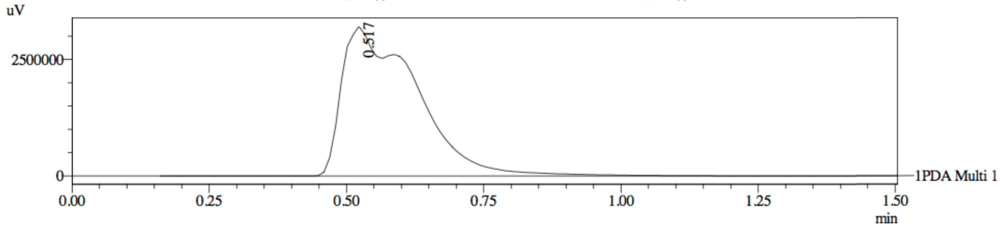
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

Acquired by	: Admin
Date Acquired	: 7/29/2014 12:59:34 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: AL4171_ESI(-)
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 24
Injection Volume	: 10
Data File	: AL4171_ESI(-).lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Pete\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/29/2014 1:01:06 PM

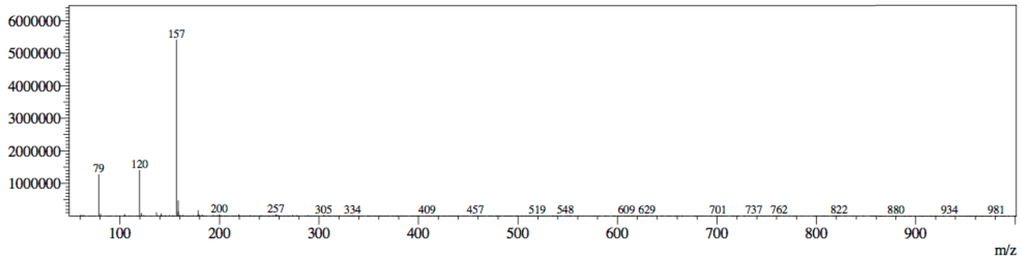
Chromatogram
AL4171_ESI(-) C:\LabSolutions\Data\Andrew Lin\AL4171_ESI(-).lcd



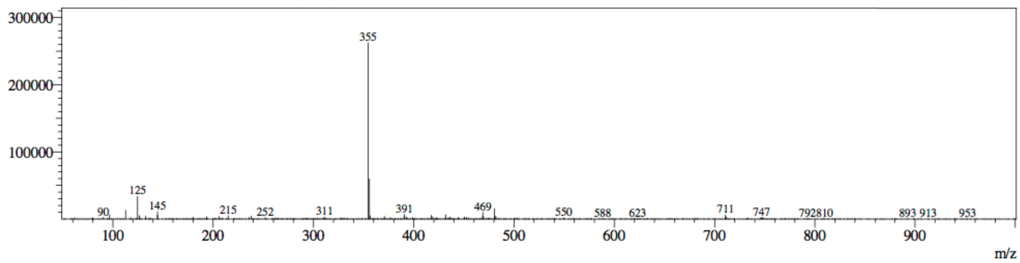
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.860(Scan#:87)
Max Peak:683 Base Peak:156.70(5404445)
Spectrum:Averaged 0.500-1.300(51-131)
Background:Averaged 0.000-0.471(1-49) Polarity:Pos Segment1 - Event1



Retention Time:0.610(Scan#:62)
Max Peak:702 Base Peak:354.60(262352)
Spectrum:Averaged 0.510-1.310(52-132)
Background:Averaged 0.010-0.471(2-50) Polarity:Neg Segment1 - Event2



Mass spectrum of product **12f**

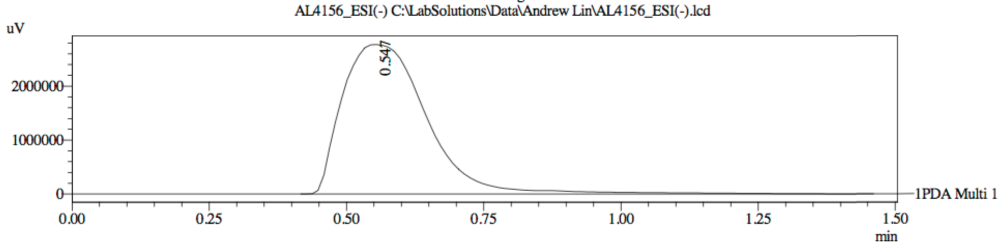
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

Acquired by	: Admin
Date Acquired	: 7/29/2014 11:51:32 AM
Sample Type	: Unknown
Level#	: 0
Sample Name	: AL4156_ESI(-)
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 1
Injection Volume	: 10
Data File	: AL4156_ESI(-).lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Pete\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/29/2014 11:53:05 AM

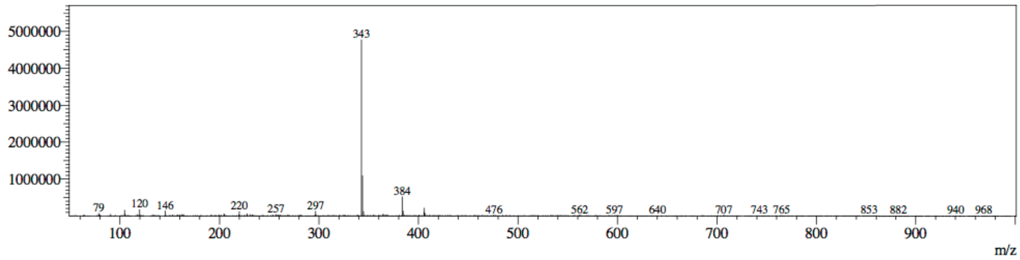
Chromatogram
AL4156_ESI(-) C:\LabSolutions\Data\Andrew Lin\AL4156_ESI(-).lcd



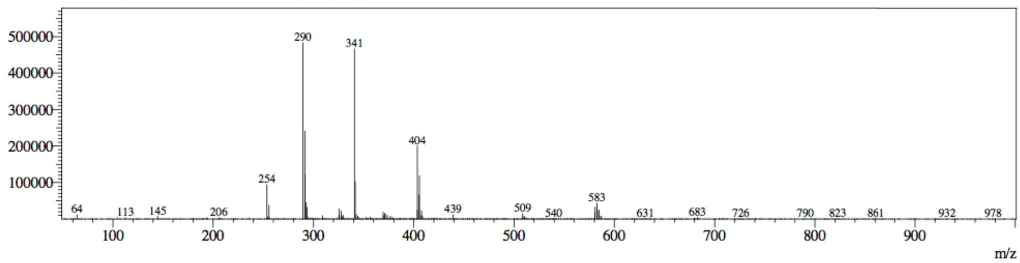
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.600(Scan#:61)
Max Peak:848 Base Peak:342.70(4770539)
Spectrum:Averaged 0.460-0.900(47-91)
Background:Averaged 0.000-0.453(1-47) Polarity:Pos Segment1 - Event1



Retention Time:0.610(Scan#:62)
Max Peak:697 Base Peak:289.60(482937)
Spectrum:Averaged 0.470-0.910(48-92)
Background:Averaged 0.010-0.453(2-48) Polarity:Neg Segment1 - Event2



Mass spectrum of product **12g**

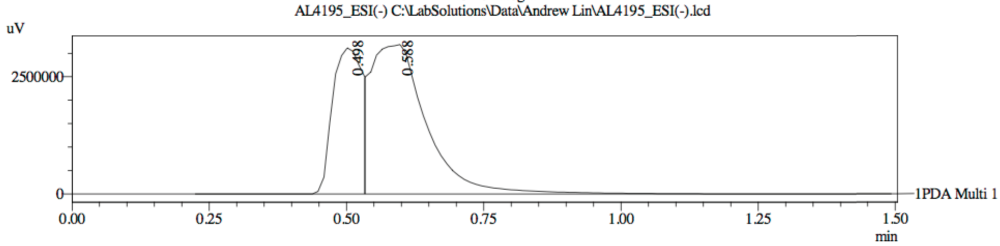
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

Acquired by	: Admin
Date Acquired	: 7/29/2014 1:04:34 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: AL4195_ESI(-)
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 26
Injection Volume	: 10
Data File	: AL4195_ESI(-).lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Pete\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/29/2014 1:06:06 PM

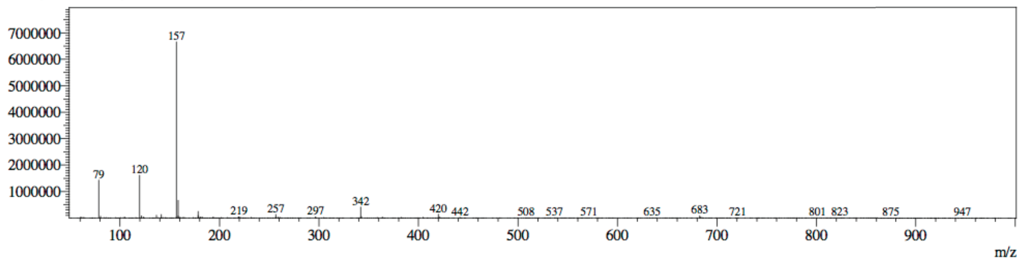
Chromatogram
AL4195_ESI(-) C:\LabSolutions\Data\Andrew Lin\AL4195_ESI(-).lcd



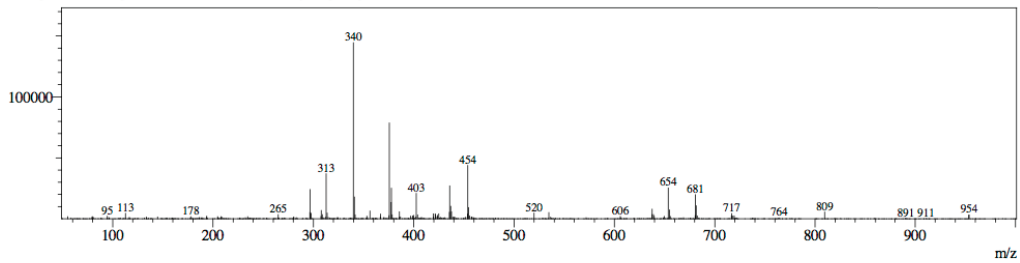
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.780(Scan#:79)
Max Peak:631 Base Peak:156.70(6658120)
Spectrum:Averaged 0.480-1.320(49-133)
Background:Averaged 0.000-0.479(1-49) Polarity:Pos Segment1 - Event1



Retention Time:0.590(Scan#:60)
Max Peak:658 Base Peak:339.95(144620)
Spectrum:Averaged 0.490-1.330(50-134)
Background:Averaged 0.010-0.479(2-50) Polarity:Neg Segment1 - Event2



Mass spectrum of product **12h**

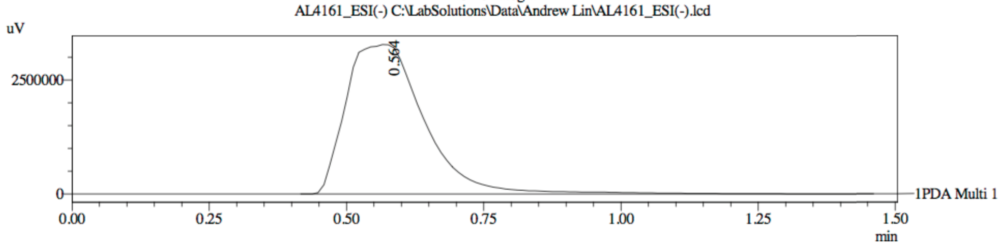
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

Acquired by	: Admin
Date Acquired	: 7/29/2014 12:09:01 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: AL4161_ESI(-)
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 8
Injection Volume	: 10
Data File	: AL4161_ESI(-).lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Pete\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/29/2014 12:10:32 PM

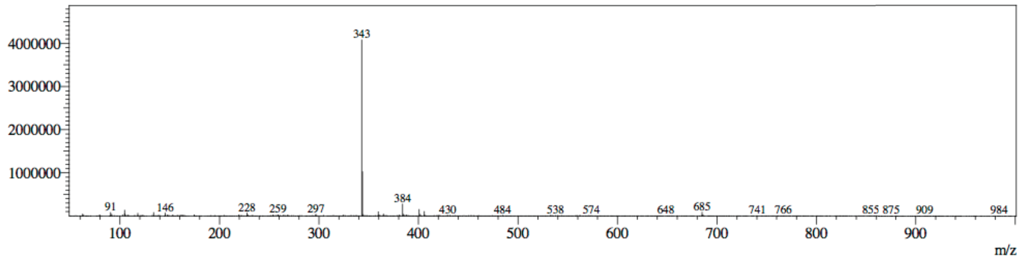
Chromatogram
AL4161_ESI(-) C:\LabSolutions\Data\Andrew Lin\AL4161_ESI(-).lcd



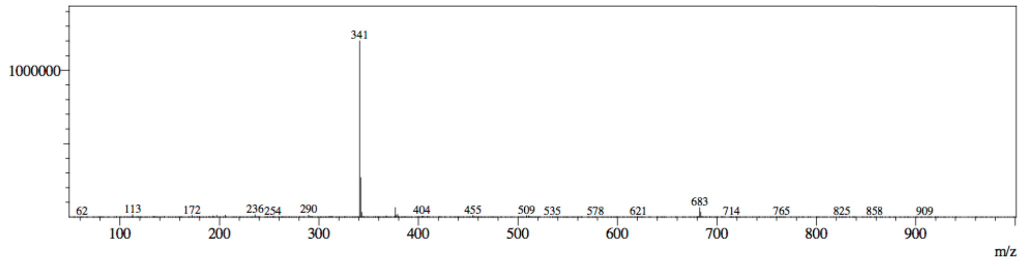
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.620(Scan#:63)
Max Peak:784 Base Peak:342.85(4078820)
Spectrum:Averaged 0.500-0.980(51-99)
Background:Averaged 0.000-0.490(1-51) Polarity:Pos Segment1 - Event1



Retention Time:0.610(Scan#:62)
Max Peak:676 Base Peak:340.75(1200737)
Spectrum:Averaged 0.510-0.990(52-100)
Background:Averaged 0.010-0.490(2-52) Polarity:Neg Segment1 - Event2



C:¥LabSolutions¥Data¥Andrew Lin¥AL4161_ESI(-).lcd

Mass spectrum of product **12i**

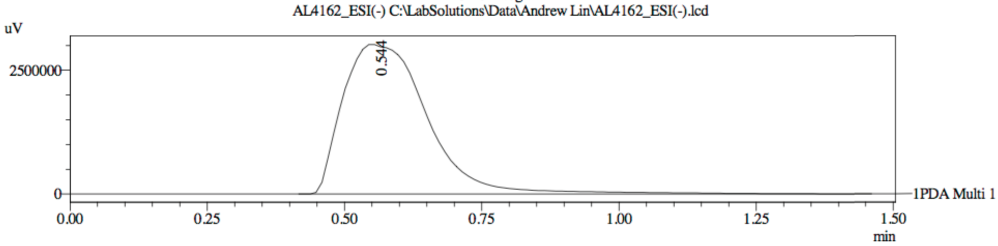
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

Acquired by	: Admin
Date Acquired	: 7/29/2014 12:11:31 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: AL4162_ESI(-)
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 9
Injection Volume	: 10
Data File	: AL4162_ESI(-).lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Pete\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/29/2014 12:13:02 PM

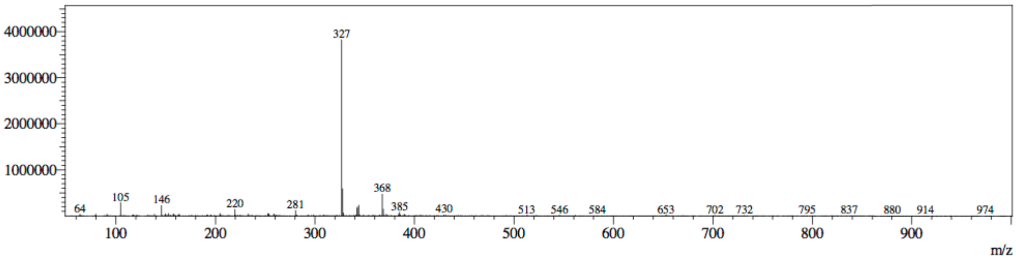
Chromatogram
AL4162_ESI(-) C:\LabSolutions\Data\Andrew Lin\AL4162_ESI(-).lcd



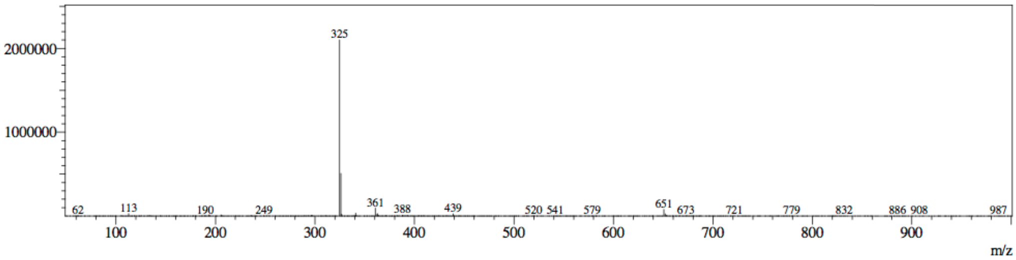
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.640(Scan#:65)
Max Peak:802 Base Peak:326.90(3821296)
Spectrum:Averaged 0.480-0.940(49-95)
Background:Averaged 0.000-0.500(1-51) Polarity:Pos Segment1 - Event1



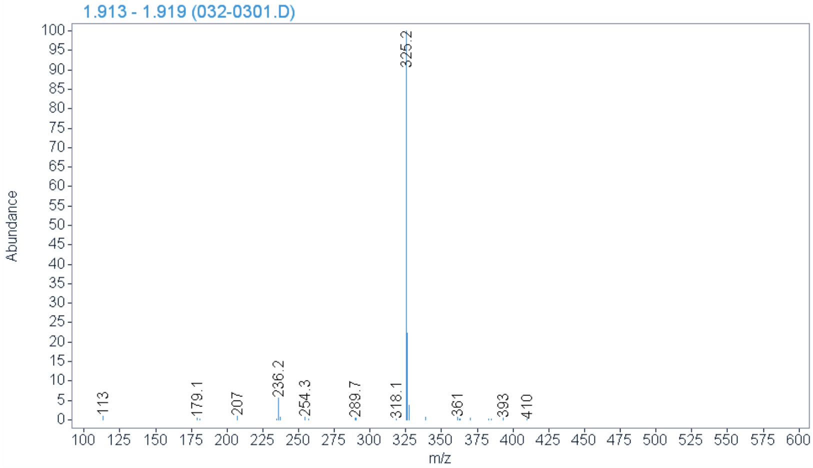
Retention Time:0.610(Scan#:62)
Max Peak:695 Base Peak:324.70(2102673)
Spectrum:Averaged 0.490-0.950(50-96)
Background:Averaged 0.010-0.500(2-52) Polarity:Neg Segment1 - Event2

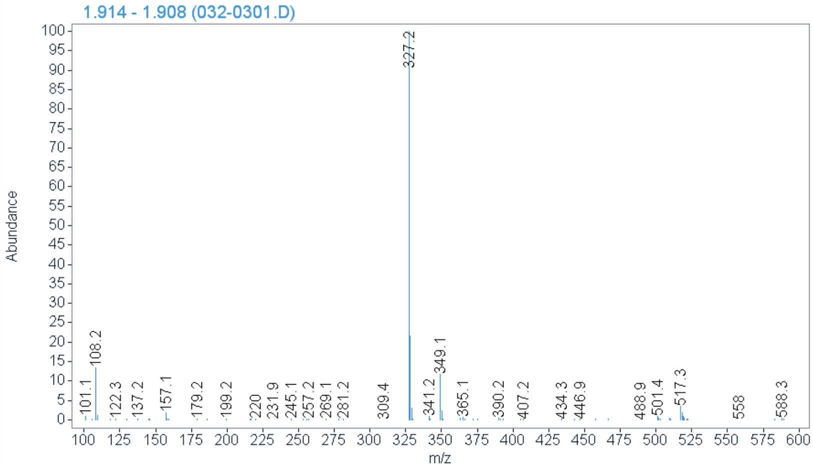


Mass spectrum of product **12j**

LCMS Report

Agilent Technologies





Mass spectrum of product **12k**

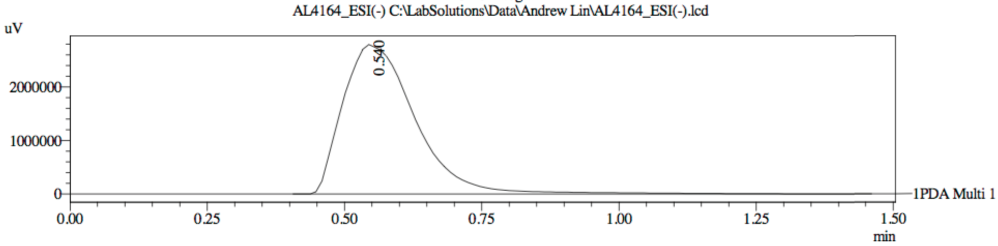
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

Acquired by	: Admin
Date Acquired	: 7/29/2014 12:21:28 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: AL4164_ESI(-)
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 13
Injection Volume	: 10
Data File	: AL4164_ESI(-).lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Pete\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/29/2014 12:22:59 PM

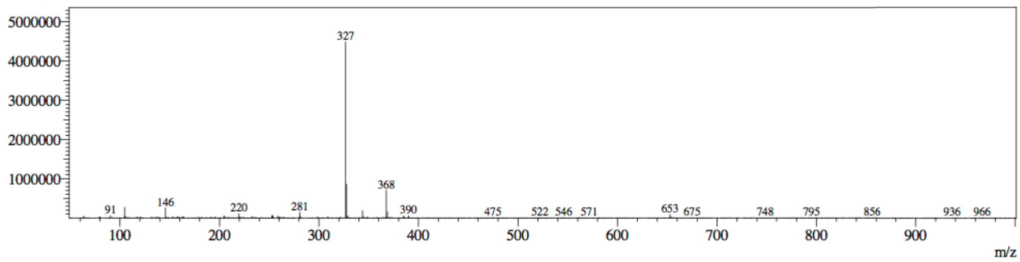
Chromatogram
AL4164_ESI(-) C:\LabSolutions\Data\Andrew Lin\AL4164_ESI(-).lcd



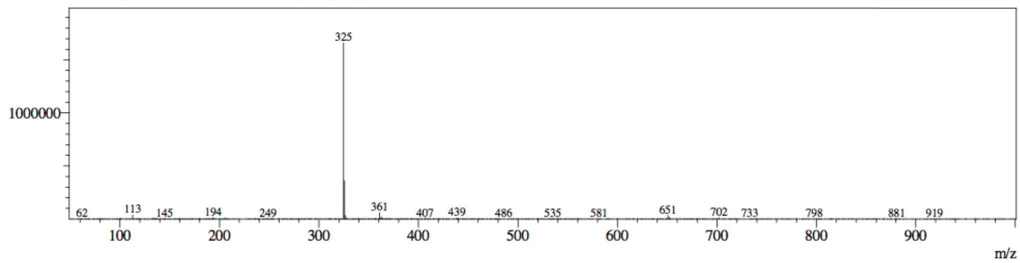
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.600(Scan#:61)
Max Peak:716 Base Peak:326.70(4486207)
Spectrum:Averaged 0.480-0.920(49-93)
Background:Averaged 0.000-0.493(1-51) Polarity:Pos Segment1 - Event1



Retention Time:0.630(Scan#:64)
Max Peak:640 Base Peak:324.70(1660821)
Spectrum:Averaged 0.490-0.930(50-94)
Background:Averaged 0.010-0.493(2-52) Polarity:Neg Segment1 - Event2



Mass spectrum of product **12l**

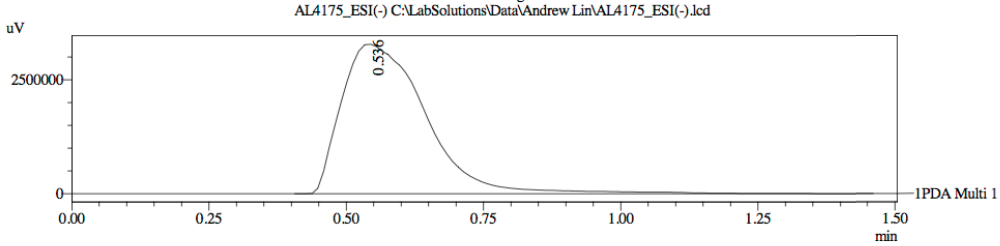
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

Acquired by	: Admin
Date Acquired	: 7/29/2014 12:16:28 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: AL4175_ESI(-)
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 11
Injection Volume	: 10
Data File	: AL4175_ESI(-).lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Pete\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/29/2014 12:18:01 PM

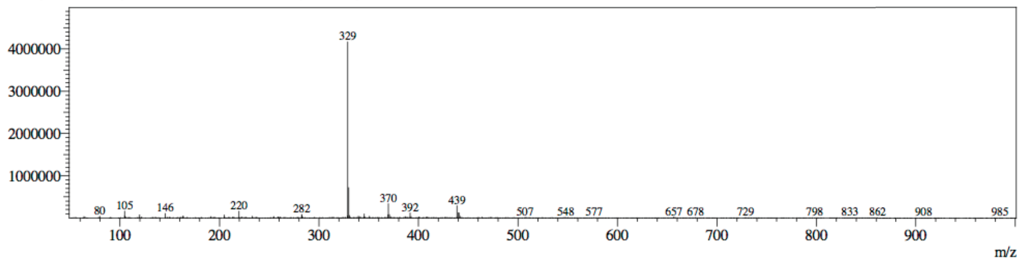
Chromatogram
AL4175_ESI(-) C:\LabSolutions\Data\Andrew Lin\AL4175_ESI(-).lcd



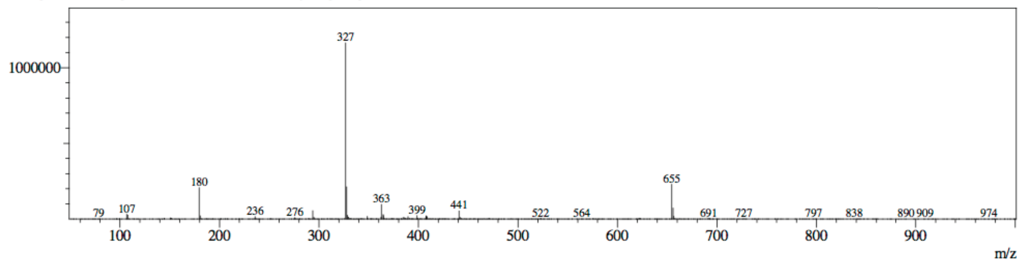
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.580(Scan#:59)
Max Peak:778 Base Peak:328.80(4167136)
Spectrum:Averaged 0.480-0.900(49-91)
Background:Averaged 0.000-0.489(1-49) Polarity:Pos Segment1 - Event1



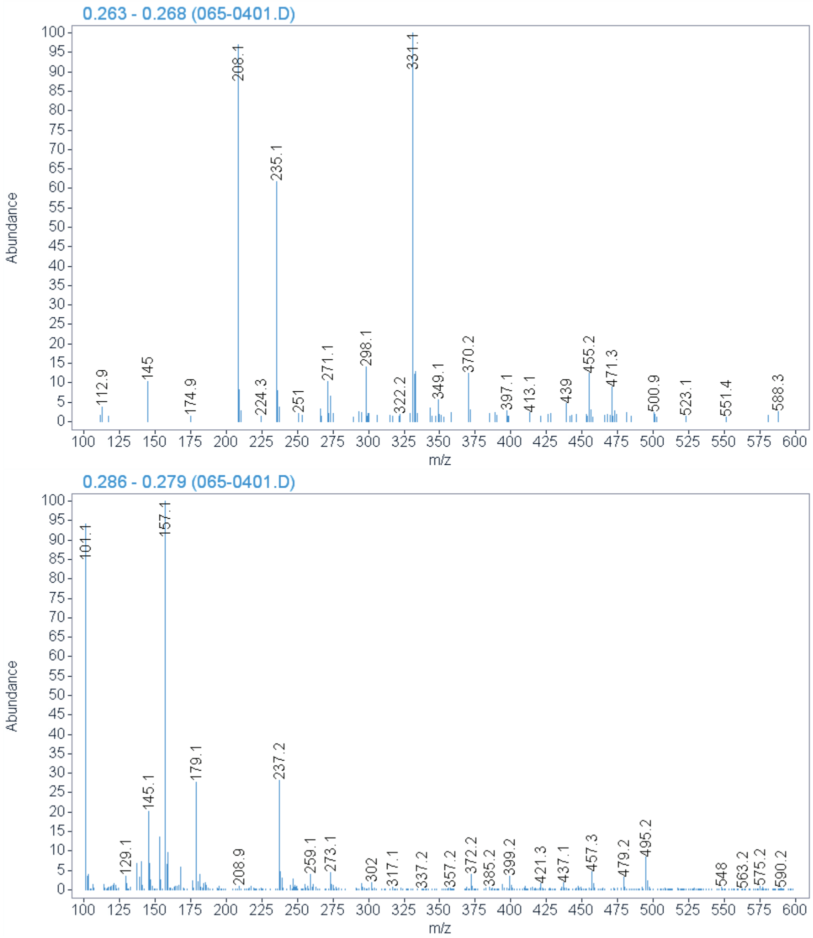
Retention Time:0.630(Scan#:64)
Max Peak:663 Base Peak:326.85(1164430)
Spectrum:Averaged 0.490-0.910(50-92)
Background:Averaged 0.010-0.489(2-50) Polarity:Neg Segment1 - Event2



Mass spectrum of product **11**

LCMS Report

Agilent Technologies



Mass spectrum of product **13a**

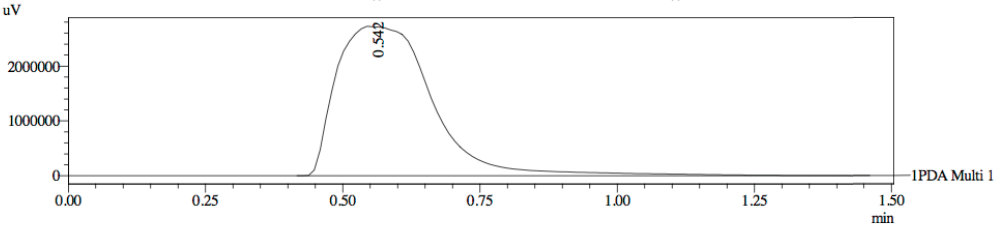
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

Acquired by	: Admin
Date Acquired	: 7/29/2014 12:06:31 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: SF1024_ESI(-)
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 7
Injection Volume	: 10
Data File	: SF1024_ESI(-).lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Pete\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/29/2014 12:08:02 PM

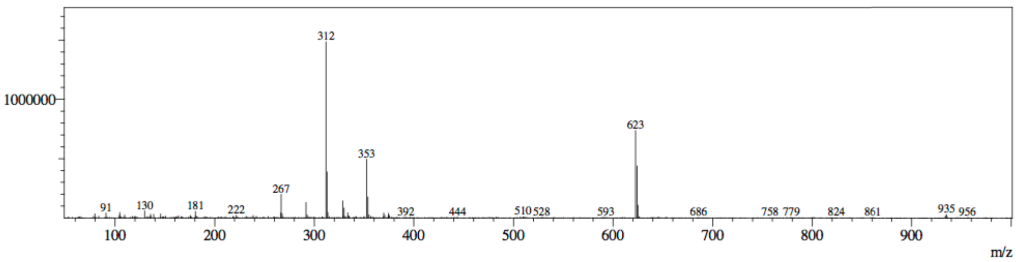
Chromatogram
SF1024_ESI(-) C:\LabSolutions\Data\Andrew Lin\SF1024_ESI(-).lcd



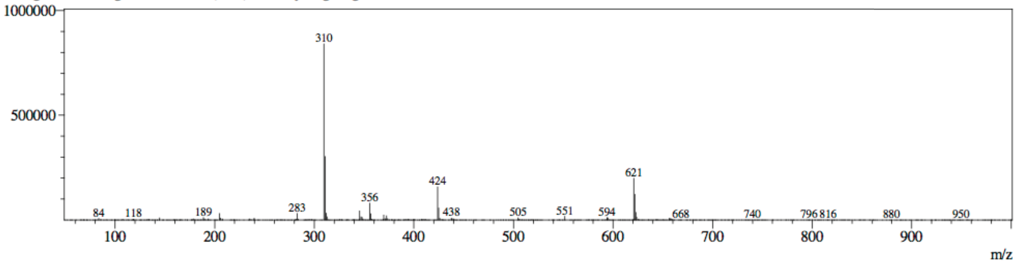
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.600(Scan#:61)
Max Peak:744 Base Peak:311.65(1485286)
Spectrum:Averaged 0.460-0.940(47-95)
Background:Averaged 0.000-0.481(1-49) Polarity:Pos Segment1 - Event1



Retention Time:0.610(Scan#:62)
Max Peak:660 Base Peak:309.70(840982)
Spectrum:Averaged 0.470-0.950(48-96)
Background:Averaged 0.010-0.481(2-50) Polarity:Neg Segment1 - Event2

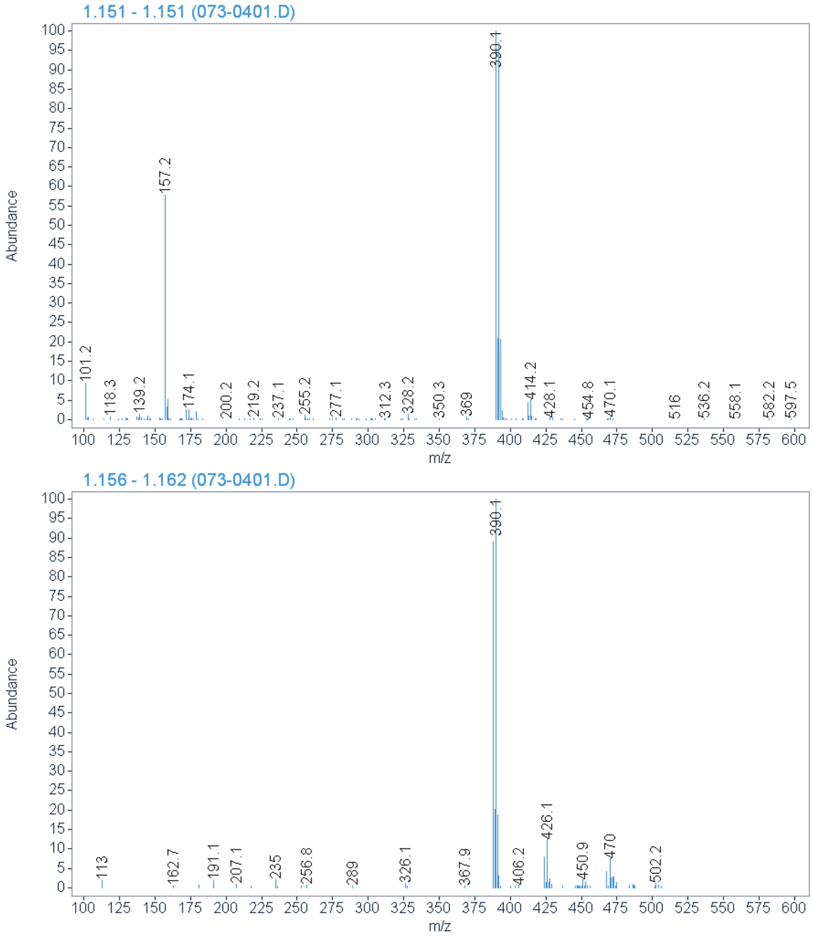


C:¥LabSolutions¥Data¥Andrew Lin¥SF1024_ESI(-).lcd

Mass spectrum of product **13b**

LCMS Report

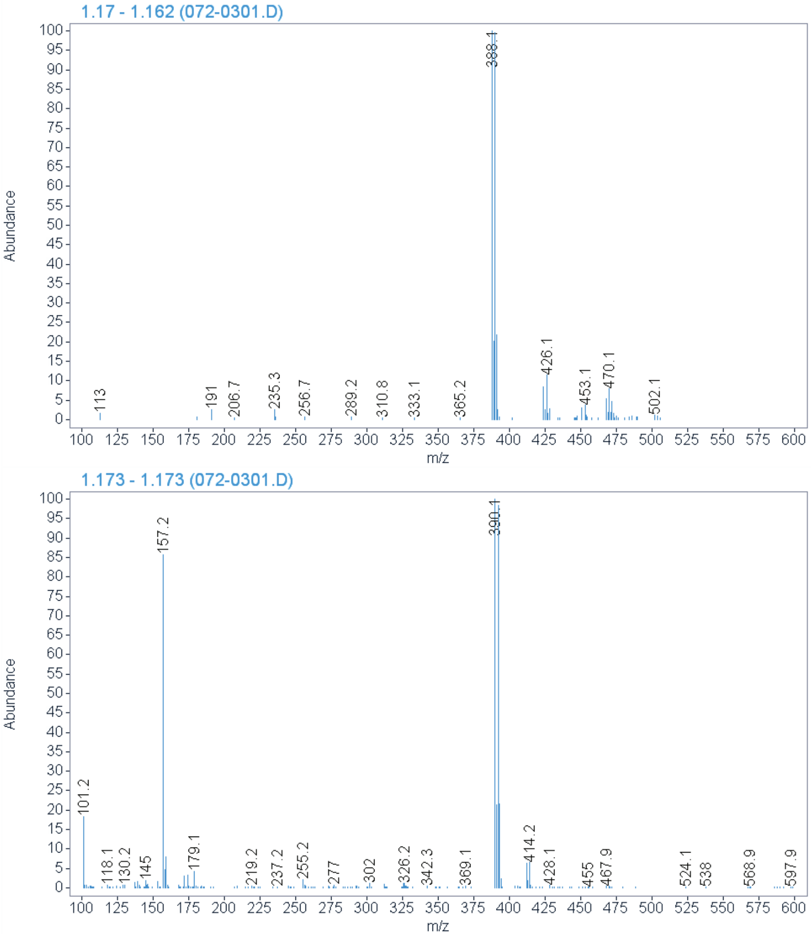
Agilent Technologies



Mass spectrum of product **13c**

LCMS Report

Agilent Technologies



Mass spectrum of product **13d**

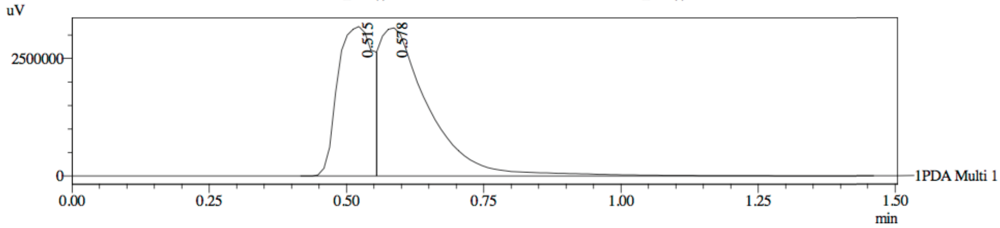
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

Acquired by	: Admin
Date Acquired	: 7/29/2014 12:42:04 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: SF1012_ESI(-)
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 17
Injection Volume	: 10
Data File	: SF1012_ESI(-).lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Pete\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/29/2014 12:43:35 PM

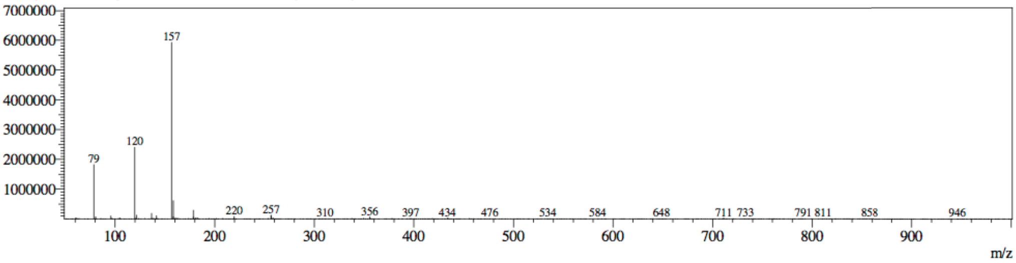
Chromatogram
SF1012_ESI(-) C:\LabSolutions\Data\Andrew Lin\SF1012_ESI(-).lcd



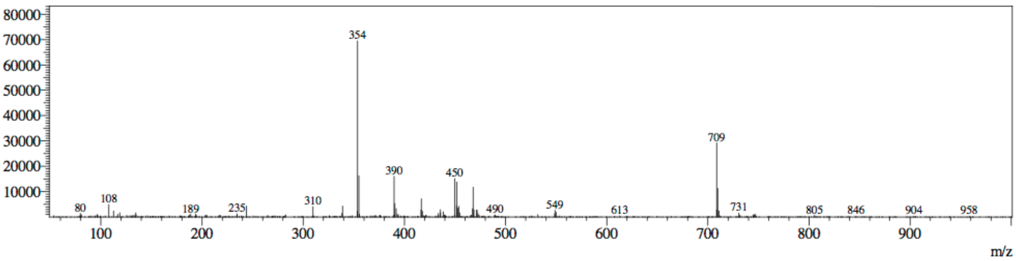
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.820(Scan#:83)
Max Peak:589 Base Peak:156.70(5927498)
Spectrum:Averaged 0.480-1.460(49-147)
Background:Averaged 0.000-0.492(1-51) Polarity:Pos Segment1 - Event1



Retention Time:0.610(Scan#:62)
Max Peak:617 Base Peak:353.70(69495)
Spectrum:Averaged 0.490-1.470(50-148)
Background:Averaged 0.010-0.492(2-52) Polarity:Neg Segment1 - Event2



Mass spectrum of product **13e**

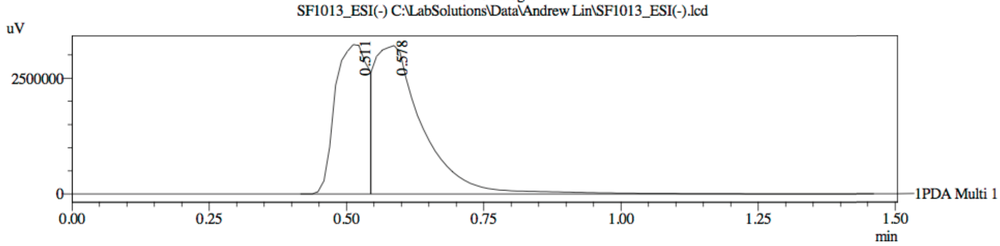
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

Acquired by	: Admin
Date Acquired	: 7/29/2014 12:44:34 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: SF1013_ESI(-)
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 18
Injection Volume	: 10
Data File	: SF1013_ESI(-).lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Pete\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/29/2014 12:46:07 PM

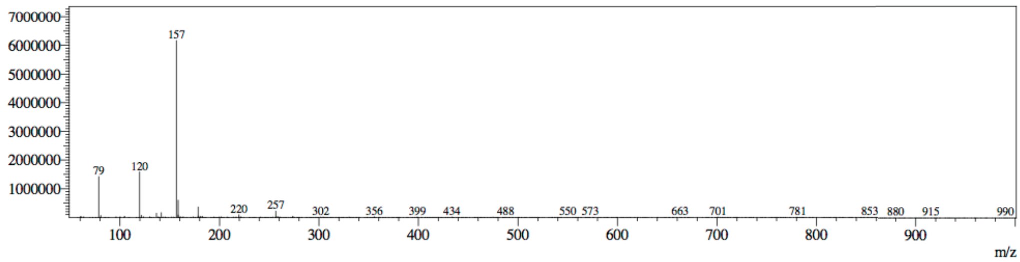
Chromatogram
SF1013_ESI(-) C:\LabSolutions\Data\Andrew Lin\SF1013_ESI(-).lcd



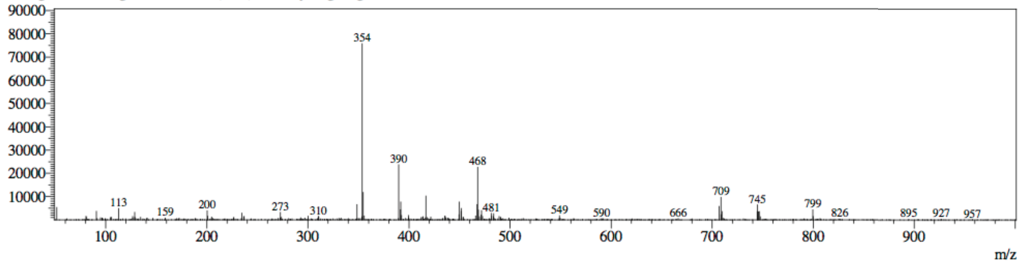
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.760(Scan#:77)
Max Peak:646 Base Peak:156.65(6168329)
Spectrum:Averaged 0.480-1.460(49-147)
Background:Averaged 0.000-0.464(1-47) Polarity:Pos Segment1 - Event1



Retention Time:0.610(Scan#:62)
Max Peak:671 Base Peak:353.60(75790)
Spectrum:Averaged 0.490-1.470(50-148)
Background:Averaged 0.010-0.464(2-48) Polarity:Neg Segment1 - Event2



Mass spectrum of product **13f**

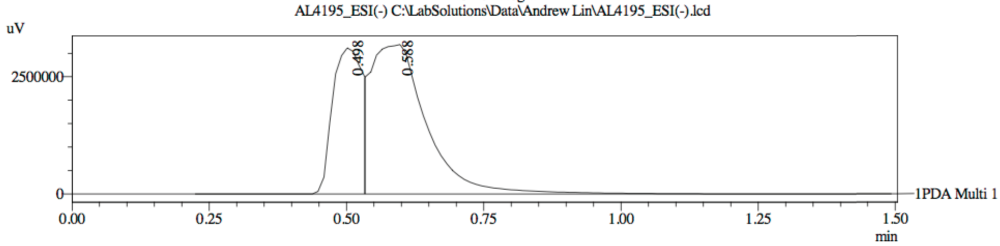
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

Acquired by	: Admin
Date Acquired	: 7/29/2014 1:04:34 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: AL4195_ESI(-)
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 26
Injection Volume	: 10
Data File	: AL4195_ESI(-).lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Pete\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/29/2014 1:06:06 PM

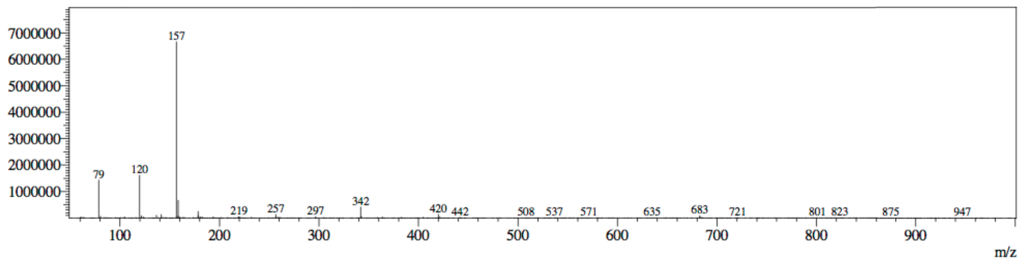
Chromatogram
AL4195_ESI(-) C:\LabSolutions\Data\Andrew Lin\AL4195_ESI(-).lcd



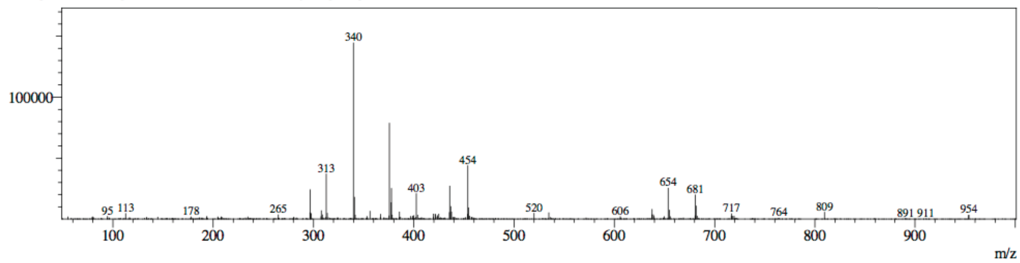
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.780(Scan#:79)
Max Peak:631 Base Peak:156.70(6658120)
Spectrum:Averaged 0.480-1.320(49-133)
Background:Averaged 0.000-0.479(1-49) Polarity:Pos Segment1 - Event1



Retention Time:0.590(Scan#:60)
Max Peak:658 Base Peak:339.95(144620)
Spectrum:Averaged 0.490-1.330(50-134)
Background:Averaged 0.010-0.479(2-50) Polarity:Neg Segment1 - Event2



C:¥LabSolutions¥Data¥Andrew Lin¥AL4195_ESI(-).lcd

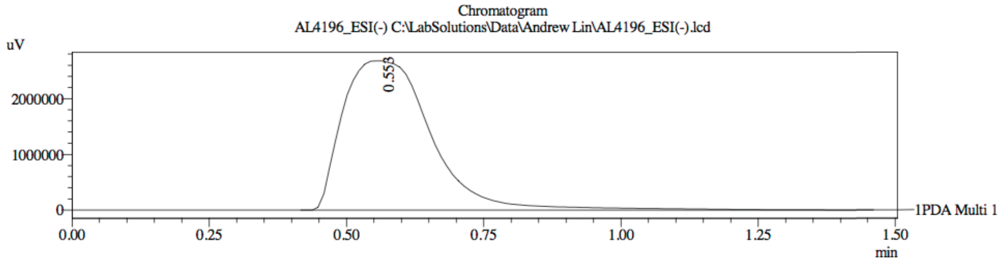
Mass spectrum of product **13g**

==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

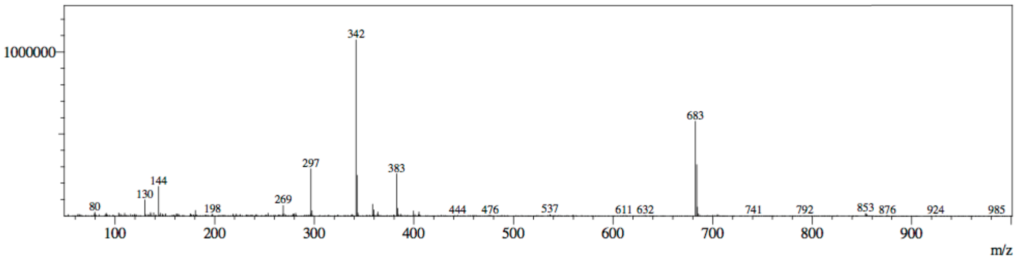
Acquired by	: Admin
Date Acquired	: 7/29/2014 12:04:01 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: AL4196_ESI(-)
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 6
Injection Volume	: 10
Data File	: AL4196_ESI(-).lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Pete\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/29/2014 12:05:33 PM



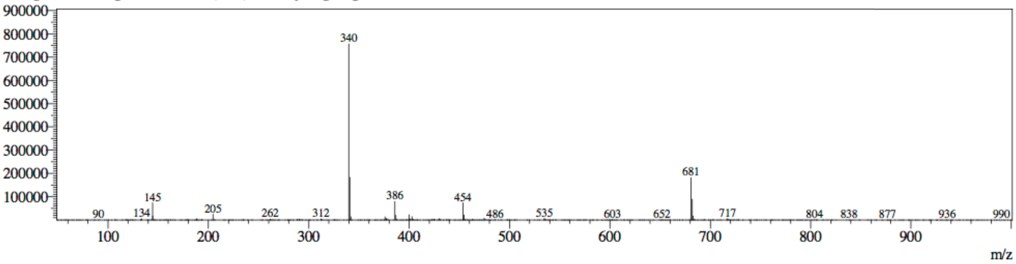
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.600(Scan#:61)
Max Peak:678 Base Peak:341.80(1074308)
Spectrum:Averaged 0.480-0.920(49-93)
Background:Averaged 0.000-0.489(1-49) Polarity:Pos Segment1 - Event1



Retention Time:0.630(Scan#:64)
Max Peak:573 Base Peak:339.95(757065)
Spectrum:Averaged 0.490-0.930(50-94)
Background:Averaged 0.010-0.489(2-50) Polarity:Neg Segment1 - Event2



Mass spectrum of product **13h**

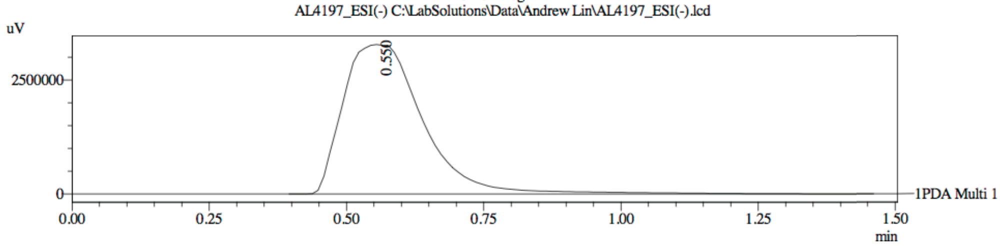
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

Acquired by	: Admin
Date Acquired	: 7/29/2014 11:59:02 AM
Sample Type	: Unknown
Level#	: 0
Sample Name	: AL4197_ESI(-)
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 4
Injection Volume	: 10
Data File	: AL4197_ESI(-).lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Pete\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/29/2014 12:00:32 PM

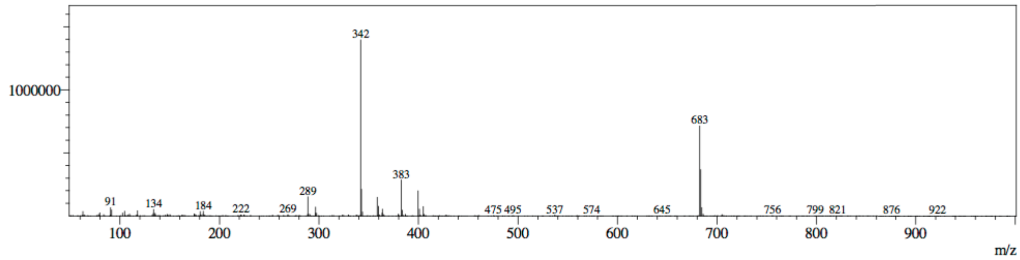
Chromatogram
AL4197_ESI(-) C:\LabSolutions\Data\Andrew Lin\AL4197_ESI(-).lcd



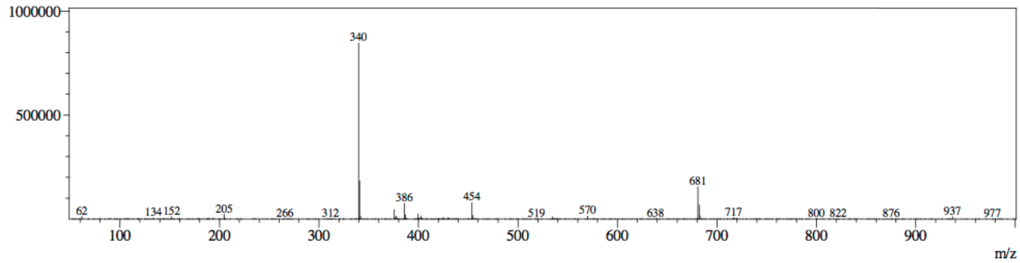
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.600(Scan#:61)
Max Peak:507 Base Peak:341.90(1396290)
Spectrum:Averaged 0.500-0.920(51-93)
Background:Averaged 0.000-0.481(1-49) Polarity:Pos Segment1 - Event1



Retention Time:0.610(Scan#:62)
Max Peak:564 Base Peak:339.75(847526)
Spectrum:Averaged 0.510-0.930(52-94)
Background:Averaged 0.010-0.481(2-50) Polarity:Neg Segment1 - Event2



C:¥LabSolutions¥Data¥Andrew Lin¥AL4197_ESI(-).lcd

Mass spectrum of product **13i**

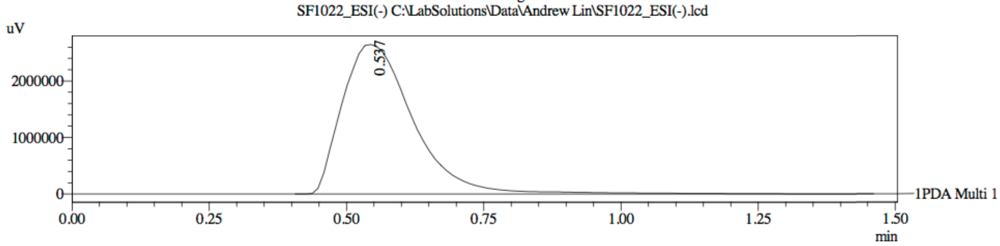
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

Acquired by	: Admin
Date Acquired	: 7/29/2014 12:13:59 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: SF1022_ESI(-)
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 10
Injection Volume	: 10
Data File	: SF1022_ESI(-).lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Pete\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/29/2014 12:15:31 PM

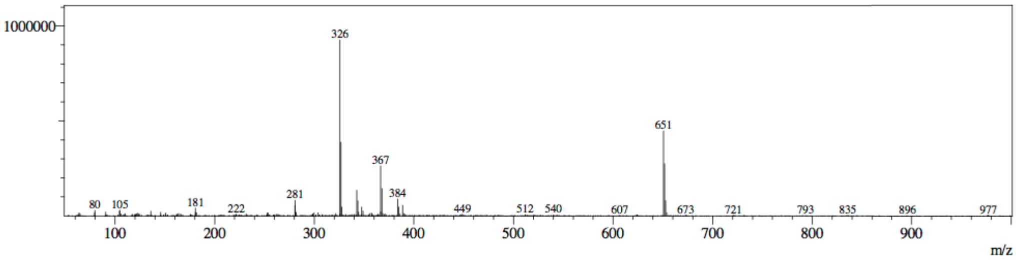
Chromatogram
SF1022_ESI(-) C:\LabSolutions\Data\Andrew Lin\SF1022_ESI(-).lcd



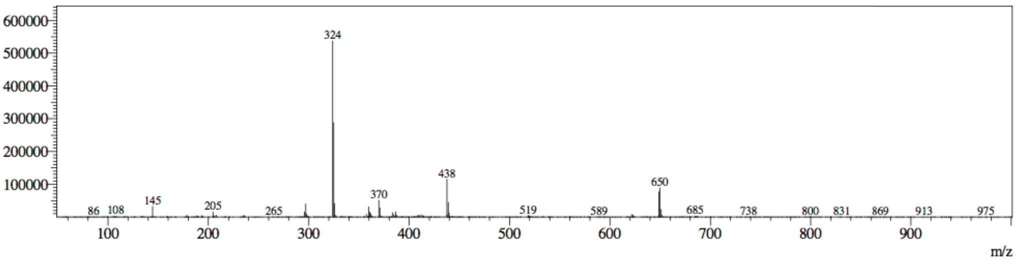
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.600(Scan#:61)
Max Peak:771 Base Peak:325.65(926470)
Spectrum:Averaged 0.480-1.000(49-101)
Background:Averaged 0.000-0.487(1-49) Polarity:Pos Segment1 - Event1



Retention Time:0.610(Scan#:62)
Max Peak:628 Base Peak:323.75(537662)
Spectrum:Averaged 0.490-1.010(50-102)
Background:Averaged 0.010-0.487(2-50) Polarity:Neg Segment1 - Event2



Mass spectrum of product **13j**

==== Shimadzu LCMSsolution Data Report ====

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Sample Information

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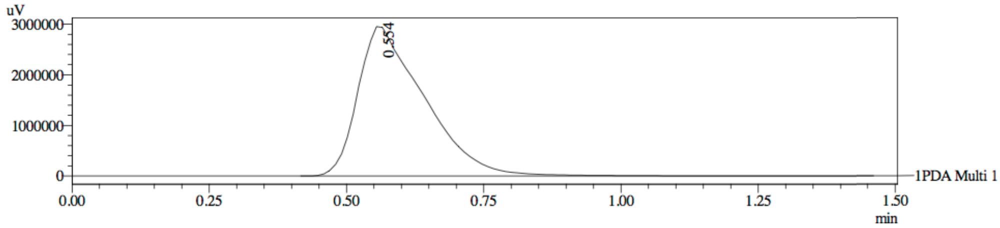
Date Acquired	: 10/13/2015 1:44:22 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: AL5004-ve
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 21
Injection Volume	: 10
Data File	: AL5004-ve.lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Jen Baker\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\Data\AutoTuning\Autotune_ESI_26AUG15.lct
Processed by	: Admin
Modified Date	: 10/13/2015 1:45:54 PM

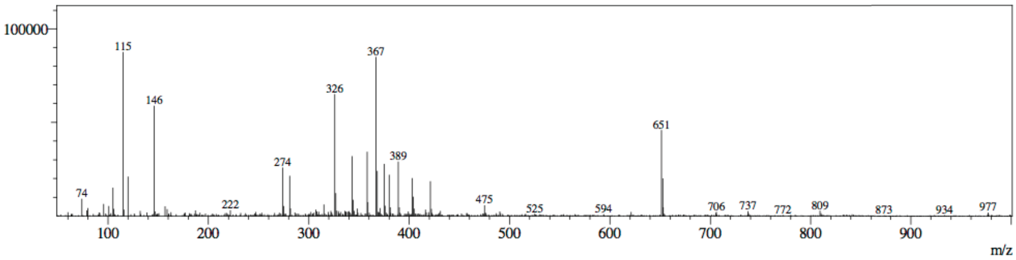
Chromatogram
AL5004-ve C:\LabSolutions\Data\Jen Baker\AL5004-ve.lcd



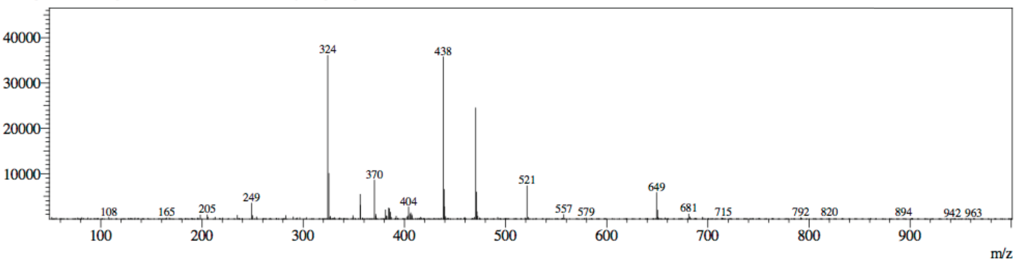
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.760(Scan#:77)
Max Peak:707 Base Peak:115.00(87391)
Spectrum:Averaged 0.660-1.460(67-147)
Background:Averaged 0.020-0.722(3-73) Polarity:Pos Segment1 - Event1



Retention Time:0.870(Scan#:88)
Max Peak:531 Base Peak:324.35(36123)
Spectrum:Averaged 0.670-1.470(68-148)
Background:Averaged 0.030-0.722(4-74) Polarity:Neg Segment1 - Event2



C:¥LabSolutions¥Data¥Jen Baker¥AL5004-ve.lcd

Mass spectrum of product **13k**

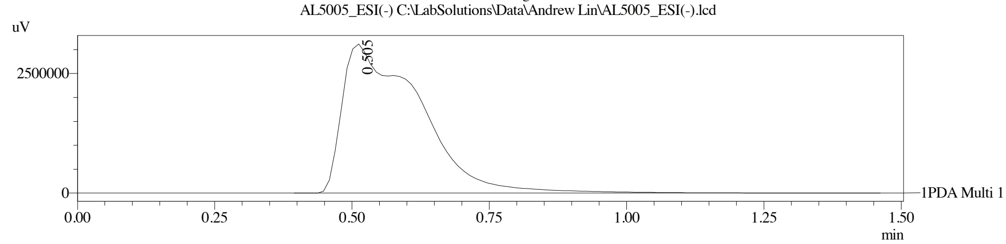
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

Acquired by	: Admin
Date Acquired	: 7/29/2014 1:14:34 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: AL5005_ESI(-)
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 30
Injection Volume	: 10
Data File	: AL5005_ESI(-).lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Pete\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/29/2014 1:16:06 PM

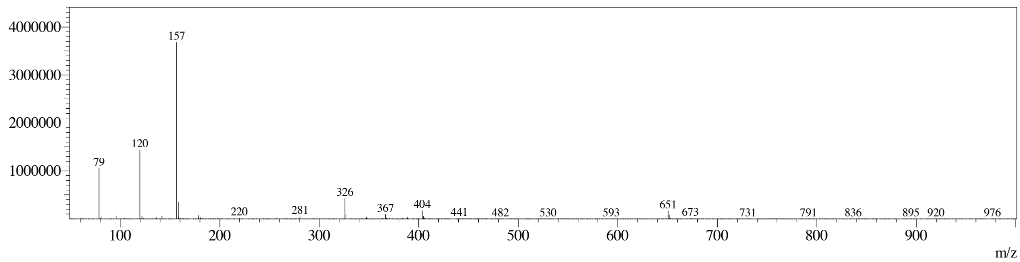
Chromatogram
AL5005_ESI(-) C:\LabSolutions\Data\Andrew Lin\AL5005_ESI(-).lcd



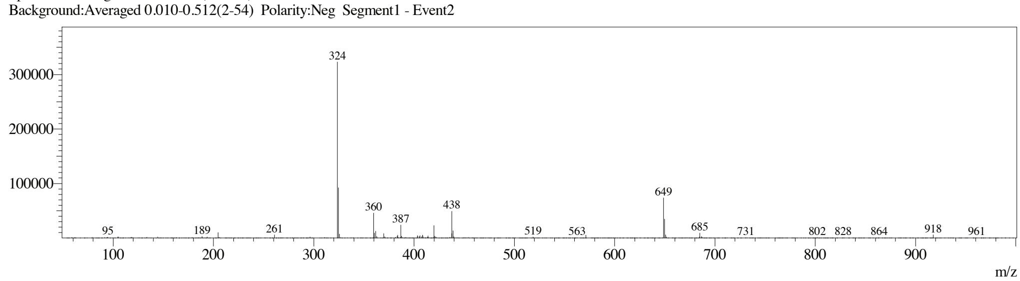
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.740(Scan#:75)
Max Peak:743 Base Peak:156.65(3680975)
Spectrum:Averaged 0.500-1.420(51-143)
Background:Averaged 0.000-0.512(1-53) Polarity:Pos Segment1 - Event1



Retention Time:0.650(Scan#:66)
Max Peak:640 Base Peak:323.70(323248)
Spectrum:Averaged 0.510-1.430(52-144)
Background:Averaged 0.010-0.512(2-54) Polarity:Neg Segment1 - Event2



Mass spectrum of product **13l**

==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

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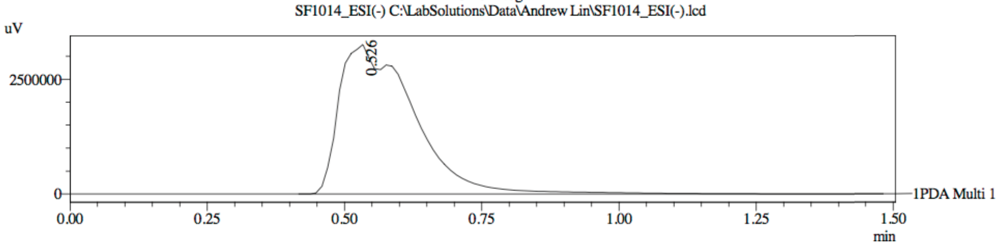
Date Acquired	: 7/29/2014 12:54:35 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: SF1014_ESI(-)
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 22
Injection Volume	: 10
Data File	: SF1014_ESI(-).lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Pete\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/29/2014 12:56:07 PM

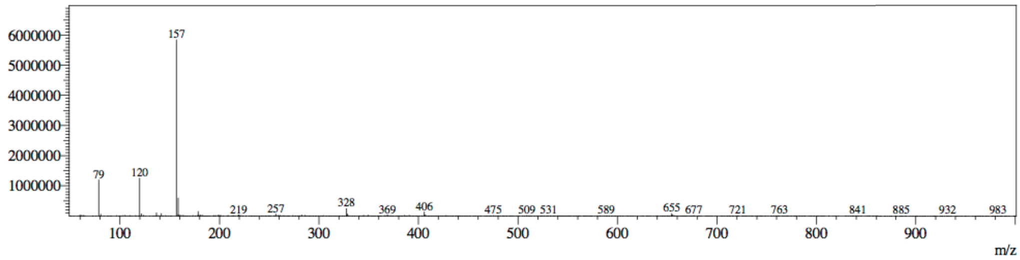
Chromatogram
SF1014_ESI(-) C:\LabSolutions\Data\Andrew Lin\SF1014_ESI(-).lcd



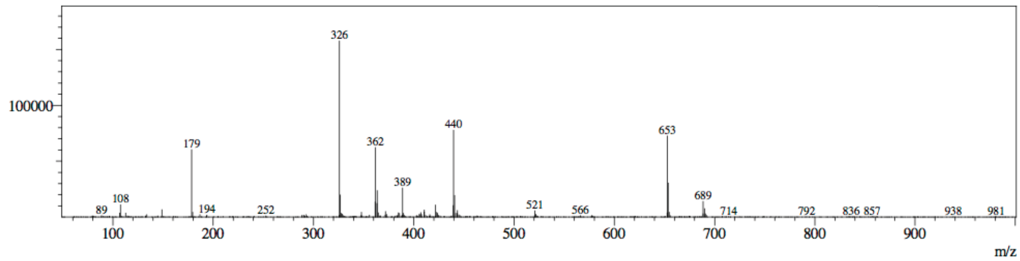
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.740(Scan#:75)
Max Peak:717 Base Peak:156.65(5840799)
Spectrum:Averaged 0.480-1.160(49-117)
Background:Averaged 0.000-0.492(1-51) Polarity:Pos Segment1 - Event1



Retention Time:0.650(Scan#:66)
Max Peak:619 Base Peak:325.85(157792)
Spectrum:Averaged 0.490-1.170(50-118)
Background:Averaged 0.010-0.492(2-52) Polarity:Neg Segment1 - Event2



Cytotoxicity Assay Results

All test agents were prepared as stock solutions (30 mM) in DMSO and stored at -20°C. Cell lines used in the study included HT29 (colorectal carcinoma); U87, SJ-G2, SMA (glioblastoma); MCF-7 (breast carcinoma); A2780 (ovarian carcinoma); H460 (lung carcinoma); A431 (skin carcinoma); Du145 (prostate carcinoma); BE2-C (neuroblastoma); and MiaPaCa-2 (pancreatic carcinoma) together with one non-tumour derived normal breast cell line (MCF10A). All cell lines were incubated in a humidified atmosphere 5% CO_2 at 37°C. The cancer cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM; Trace Biosciences, Australia) supplemented with foetal bovine serum (10 %), sodium bicarbonate (10 mM), penicillin (100 $IUmL^{-1}$), streptomycin (100 $mgmL^{-1}$), and glutamine (4 mM). The non-cancer MCF10A cell line was maintained in DMEM:F12 (1:1) cell culture media, 5% heat inactivated horse serum, supplemented with penicillin (50 $IUmL^{-1}$), streptomycin (50 $mgmL^{-1}$), HEPES (20 mm), l-glutamine (2 mM), epidermal growth factor (20 $ngmL^{-1}$), hydrocortisone (500 $ngmL^{-1}$), cholera toxin (100 $ngmL^{-1}$), and insulin (10 $mgmL^{-1}$). Cytotoxicity was determined by plating cells in duplicate in medium (100 mL) at a density of 2500–4000 cells per well in 96-well plates. On day 0, when the cells were in logarithmic growth (24h after plating), medium (100 mL) with or without the test agent was added to each well. After 72h drug exposure, growth inhibitory effects were evaluated using the MTT (3-[4,5-dimethyltiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay and absorbance read at 540 nm. All analogues were initially tested for percentage growth inhibition at a fixed concentration of 25µM. Those that showed appreciable inhibition underwent further examination to produce an eight-point dose-response curve from which a GI_{50} value was calculated, representing the drug concentration that inhibited cell growth by 50% based on the difference between the optical density values on day 0 and those at the end of drug exposure. All assays were conducted in duplicate and replicated on at least three separate occasions. Values represent the mean ± standard error of the mean.

Table 1. Initial cytotoxicity screening of analogues **10, 11, 12a–12l, 13a–13l**. Values given are percentage inhibition at 25 µM drug concentration.

Compound	HT29	U87	MCF-7	A2780	H460	A431	Du145	BE2-C	SJ-G2	MIA	SMA	MCF10A
5	11 ± 10	21 ± 1	27 ± 4	70 ± 0	26 ± 3	21 ± 3	17 ± 4	74 ± 5	25 ± 3	37 ± 4	31 ± 5	16 ± 6
12a	23 ± 8	35 ± 1	32 ± 4	85 ± 1.2	50 ± 2	25 ± 1	30 ± 4	82 ± 4	31 ± 1	64 ± 3	54 ± 3	33 ± 7
12b	82 ± 2	43 ± 1	42 ± 13	88 ± 1	68 ± 1	>100	59 ± 2	96 ± 3	100 ± 4	68 ± 2	72 ± 1	73 ± 6
12c	20 ± 2	18 ± 3	13 ± 6	84 ± 2	26 ± 6	24 ± 4	26 ± 1	55 ± 7	51 ± 12	33 ± 1	58 ± 2	28 ± 3
12d	7 ± 4	13 ± 2	57 ± 4	42 ± 1	12 ± 2	11 ± 1.4	23 ± 0	50 ± 7	35 ± 7	24 ± 2	30 ± 3	13 ± 4
12e	5 ± 6	13 ± 1	13 ± 6	23 ± 1	19 ± 4	10 ± 4	21 ± 4	44 ± 9	27 ± 3	15 ± 2	18 ± 1	7 ± 4
12f	29 ± 2	26 ± 1	19 ± 7	42 ± 1	25 ± 5	28 ± 3.5	22 ± 3	46 ± 6	31 ± 5	26 ± 2	26 ± 3	14 ± 4
12g	58 ± 2	43 ± 3	60 ± 4	88 ± 1	67 ± 2	>100	55 ± 4	92 ± 4	92 ± 5	67 ± 2	75 ± 2	69 ± 6
12h	6 ± 5	23 ± 2	20 ± 7	68 ± 2	24 ± 5	20 ± 3.0	9 ± 4	50 ± 3	49 ± 10	22 ± 2	43 ± 1	23 ± 3
12i	34 ± 2	15 ± 2	13 ± 6	42 ± 2	14 ± 5	23 ± 3	22 ± 1	49 ± 9	29 ± 3	28 ± 2	27 ± 2	9 ± 4
12j	71 ± 2	41 ± 2	63 ± 4	85 ± 1	65 ± 1	>100	60 ± 1	99 ± 3	97 ± 4	69 ± 2	76 ± 1	73 ± 6
12k	20 ± 2	20 ± 2	13 ± 6	40 ± 2	10 ± 6	15 ± 2	20 ± 2	57 ± 10	32 ± 2	27 ± 3	22 ± 3	3 ± 1
12l	71 ± 3	27 ± 6	ND	86 ± 1	77 ± 2	93 ± 1	56 ± 7	>100	>100	50 ± 4	100 ± 0	79 ± 4
6	10 ± 5	15 ± 1	18 ± 6	25 ± 3	12 ± 2	10 ± 2	17 ± 3	7 ± 5	19 ± 3	19 ± 1	19 ± 4	6 ± 2
13a	10 ± 3	18 ± 1	24 ± 8	28 ± 2	15 ± 2	10 ± 2	16 ± 4	15 ± 5	20 ± 2	21 ± 1	16 ± 3	9 ± 3
13b	1 ± 4	10 ± 3	5 ± 5	43 ± 2	21 ± 2	10 ± 0	20 ± 4	11 ± 3	25 ± 9	24 ± 6	17 ± 3	24 ± 3
13c	9 ± 2	14 ± 3	22 ± 6	86 ± 2	29 ± 2	20 ± 2.0	17 ± 4	28 ± 3	46 ± 8	24 ± 3	57 ± 4	33 ± 3
13d	5 ± 5	10 ± 2	15 ± 3	39 ± 3	16 ± 2	12 ± 1	20 ± 0	11 ± 7	25 ± 9	25 ± 4	18 ± 3	20 ± 1
13e	3 ± 2	11 ± 0	8 ± 6	17 ± 4	16 ± 2	0 ± 4.5	8 ± 5	<0	2 ± 7	14 ± 2	6 ± 4	10 ± 1
13f	<0	10 ± 1	<0	20 ± 3	7 ± 2	4 ± 4	7 ± 3	9 ± 1	22 ± 2	7 ± 2	7 ± 2	1 ± 1
13g	<0	11 ± 5	<0	57 ± 5	28 ± 3	5 ± 3.2	9 ± 5	16 ± 11	32 ± 13	26 ± 5	27 ± 2	21 ± 5
13h	7 ± 2	9 ± 3	8 ± 4	59 ± 3	28 ± 2	14 ± 2	13 ± 1	9 ± 9	33 ± 10	21 ± 1	21 ± 3	27 ± 3
13i	7 ± 2	13 ± 1	19 ± 7	19 ± 4	9 ± 1	9 ± 1.9	17 ± 3	6 ± 2	20 ± 5	17 ± 1	16 ± 2	6 ± 3
13j	17 ± 6	17 ± 2	56 ± 7	31 ± 2	25 ± 4	3 ± 3	27 ± 3	9 ± 2	26 ± 1	24 ± 3	14 ± 1	9 ± 5
13k	<0	13 ± 2	20 ± 7	31 ± 3	10 ± 2	6 ± 3.1	10 ± 4	19 ± 2	23 ± 1	17 ± 3	11 ± 3	6 ± 3
13l	<0	<0	<0	38 ± 2	29 ± 4	<0	<0	30 ± 6	14 ± 19	<0	51 ± 3	30 ± 7