Supporting Information

N-terminal peptidic boronic acids selectively inhibit human ClpXP

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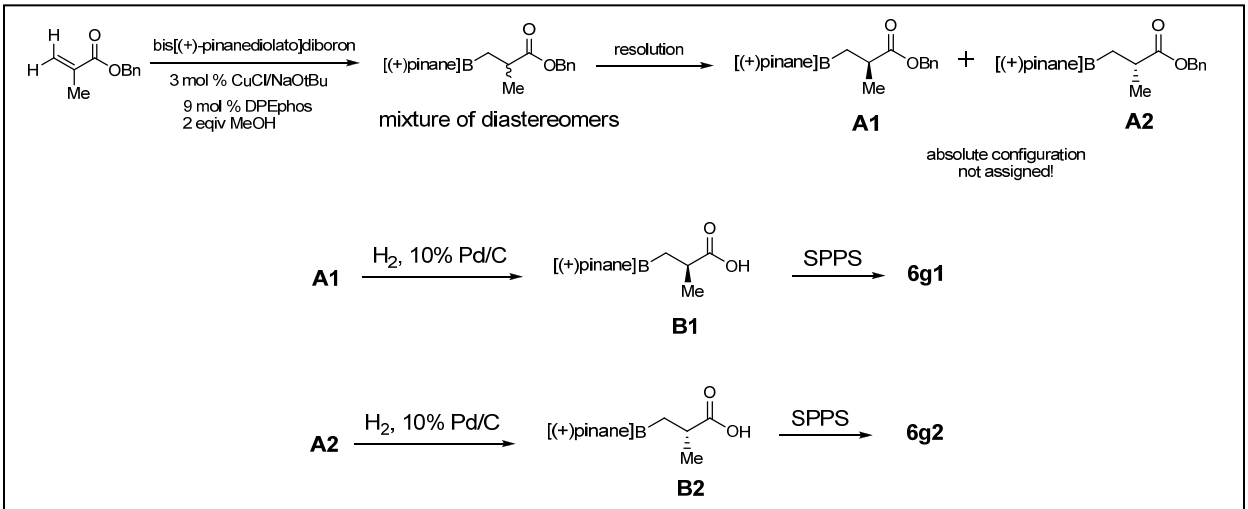
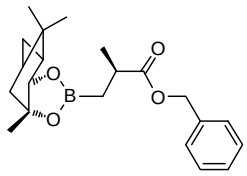
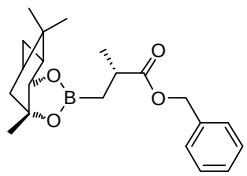


Scheme A. Synthesis of boronic acid monomers **B1** and **B2**.

Benzyl 2-methyl-3-(5,5,7a-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)propanoate (A1, A2). Using general procedure A, beginning with benzyl methacrylate and using bis[(+)-pinanediolato]diboron, the title product was isolated in 75% (403 mg) yield as a colorless oil. The diastereomers (using 150 mg) were resolved using a ChiralPak IA semi-prep column to yield 26mg (**A1**) and 32mg (**A2**). Note that the absolute configurations were not assigned.



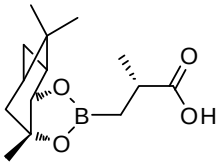
A1: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 – 7.26 (m, 5H), 5.09 (q, *J* = 12.5 Hz, 2H), 4.20 (dd, *J* = 8.8, 2.0 Hz, 1H), 2.78 – 2.68 (m, 1H), 2.31 – 2.23 (m, 1H), 2.20 – 2.11 (m, 1H), 2.01 – 1.97 (m, 1H), 1.85 (ddd, *J* = 8.6, 6.0, 3.0 Hz, 1H), 1.74 (ddd, *J* = 14.6, 3.3, 2.1 Hz, 1H), 1.29 (s, 3H), 1.25 (s, 3H), 1.22 (d, *J* = 7.0 Hz, 3H), 1.19 (dd, *J* = 10.1, 5.9 Hz, 1H), 1.10 (t, *J* = 9.2 Hz, 1H), 1.03 – 0.95 (m, 1H), 0.80 (s, 3H). ^{13}C NMR (126 MHz, CDCl3) δ 177.$_{26}$, 136.66, 128.66, 128.20, 128.17, 85.80, 77.96, 66.23, 51.56, 39.80, 38.34, 35.77, 35.65, 28.79, 27.31, 26.61, 24.19, 19.72, (B-C) 15.98. MS-ESI (*m/z*): $[M+]^+$ calcd for $C_{21}H_{30}[^{11}B]O_4$, 357.22; found, 357.16.



A2: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 – 7.26 (m, 5H), 5.12 – 5.05 (m, 2H), 4.23 – 4.17 (m, 1H), 2.78 – 2.69 (m, 1H), 2.28 (ddt, *J* = 14.5, 8.8, 2.5

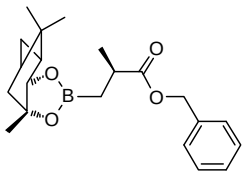
Hz, 1H), 2.18 – 2.11 (m, 1H), 1.99 (dd, *J* = 10.9, 5.6 Hz, 1H), 1.89 – 1.83 (m, 1H), 1.78 (ddd, *J* = 14.6, 3.3, 2.1 Hz, 1H), 1.32 (s, 3H), 1.25 (s, 3H), 1.23 (d, *J* = 7.1 Hz, 3H), 1.20 (d, *J* = 7.1 Hz, 1H), 1.10 (d, *J* = 10.9 Hz, 1H), 0.99 (dd, *J* = 16.0, 7.5 Hz, 1H), 0.80 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.24, 136.67, 128.64, 128.14, 128.11, 85.78, 77.94, 66.18, 51.52, 39.77, 38.32, 35.68, 35.66, 28.82, 27.29, 26.61, 24.17, 19.56, (B-C) 15.87. MS-ESI (*m/z*): $[M+]^+$ calcd for $C_{21}H_{30}[^{11}B]O_4$, 357.22; found, 357.17.

2-methyl-3-((3aR,7aR)-5,5,7a-trimethylhexahydro-4,6-methanobenzo[d] [1,3,2]dioxaborol -2-yl)propanoic acid (B1). **A1** was dissolved in 2 ml MeOH in the presence of catalytic amount of 10% Pd/C and a balloon of hydrogen. After 30 minutes, the mixture was filtered through celite, the solvent dried, and the crude mixture was used without further purification. Note that the absolute configuration was not assigned.



^{1}H NMR (500 MHz, $CDCl_3$) δ 4.22 (d, *J* = 8.2 Hz, 1H), 2.62 (d, *J* = 6.9 Hz, 1H), 2.33 – 2.24 (m, 1H), 2.00 (t, *J* = 5.5 Hz, 1H), 1.86 (s, 1H), 1.78 (d, *J* = 14.8 Hz, 1H), 1.33 (s, 3H), 1.26 (s, 3H), 1.16 (d, *J* = 7.3 Hz, 3H), 1.03 – 0.89 (m, 2H), 0.82 (s, 3H). MS-ESI (*m/z*): $[M-H]^-$ calcd for $C_{21}H_{30}[^{11}B]O_4$, 265.16; found, 265.15.

2-methyl-3-((3aR,7aR)-5,5,7a-trimethylhexahydro-4,6-methanobenzo[d] [1,3,2]dioxaborol -2-yl)propanoic acid (B2). **A2** was dissolved in 2 ml MeOH in the presence of catalytic amount of 10% Pd/C and a balloon of hydrogen. After 30 minutes, the mixture was filtered through celite, the solvent dried, and the crude mixture was used without further purification. Note that the absolute configuration was not assigned.



^{1}H NMR (500 MHz, $CDCl_3$) δ 4.23 (d, *J* = 8.2 Hz, 1H), 2.62 (d, *J* = 5.1 Hz, 1H), 2.34 – 2.25 (m, 1H), 2.20 – 2.12 (m, 1H), 2.00 (t, *J* = 5.5 Hz, 1H), 1.87 (s, 1H), 1.80 (d, *J* = 14.6 Hz, 1H), 1.35 (s, 3H), 1.26 (s, 3H), 1.17 (d, *J* = 7.5 Hz, 3H), 1.09 – 0.91 (m, 2H), 0.82 (s, 3H). MS-ESI (*m/z*): $[M-H]^-$ calcd for $C_{21}H_{30}[^{11}B]O_4$, 265.16; found, 265.15.

Determination of IC_{50} of WLS6a inhibiting ClpXP peptidase activity

Reactions contained 50mM HEPES (pH 8.1), 5mM $Mg(OAc)_2$, 2mM DTT, and 300nM ClpXP. Varying amounts of inhibitor were added and the reaction was incubated at room temperature for 30 min. The reaction was then incubated with 1mM ATP for 1 min at 37°C, then initiated with the addition of 500μM FRETN 89-98. Fluorescent emission of the cleaved peptide was monitored at 420nm for 1200 seconds with excitation at 320nm. All experiments were performed in triplicate. The rate constants for peptidase activity were determined as above and normalized to 1 by dividing by k_{obs} in the absence of inhibitor. These normalized values were plotted against concentration of 6a and fit to Equation 1[1] using Prism 4 (GraphPad)

$$\frac{v_i}{v_0} = \frac{1}{1 + \frac{[I]}{IC_{50}}} \qquad \text{Equation 1}$$

[1] Copeland, R. A. *Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis*, 2nd ed., John Wiley & Sons, Inc., New York, **2000**.

Where [I] is concentration of inhibitor and IC_{50} is the concentration of inhibitor at which cleavage is inhibited by 50%.

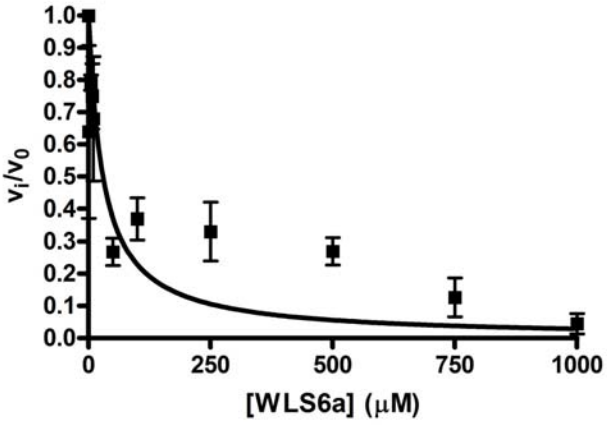
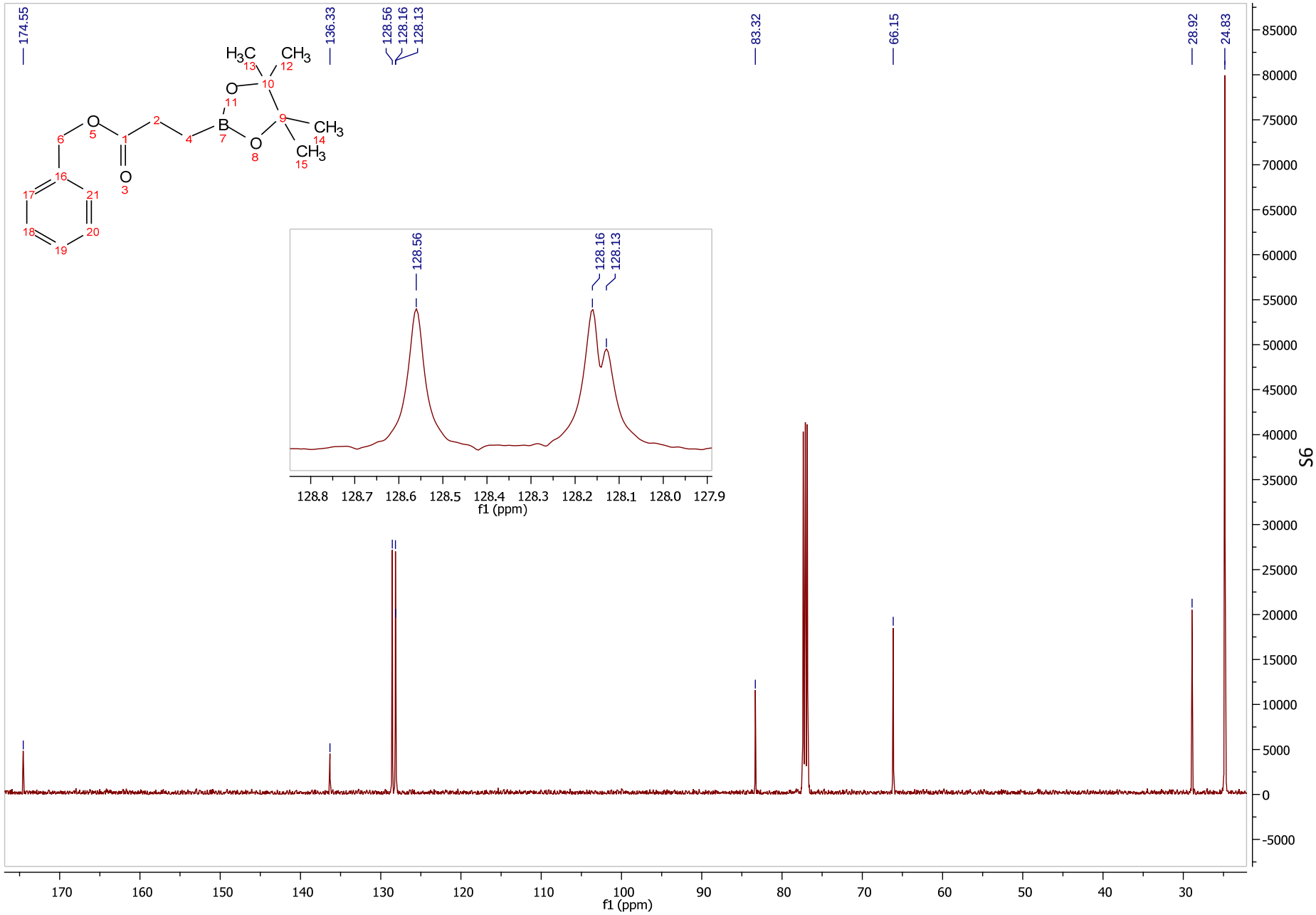


Figure 2: Apparent IC_{50} determination for WLS6a inhibition of ClpXP. Varying amounts of **WLS6a** (0-1000μM) were incubation with 300nM ClpXP at room temperature for 30 minutes. 1mM ATP was added and the reaction was incubated at 37°C for 1 min. The reaction was initiated by the addition of 500μM FRETN 89-98. Normalized average k_{obs} values were fit to Equation 1 (solid line) to reveal an IC_{50} of 29 μM ± 9. All experiments were done in triplicate.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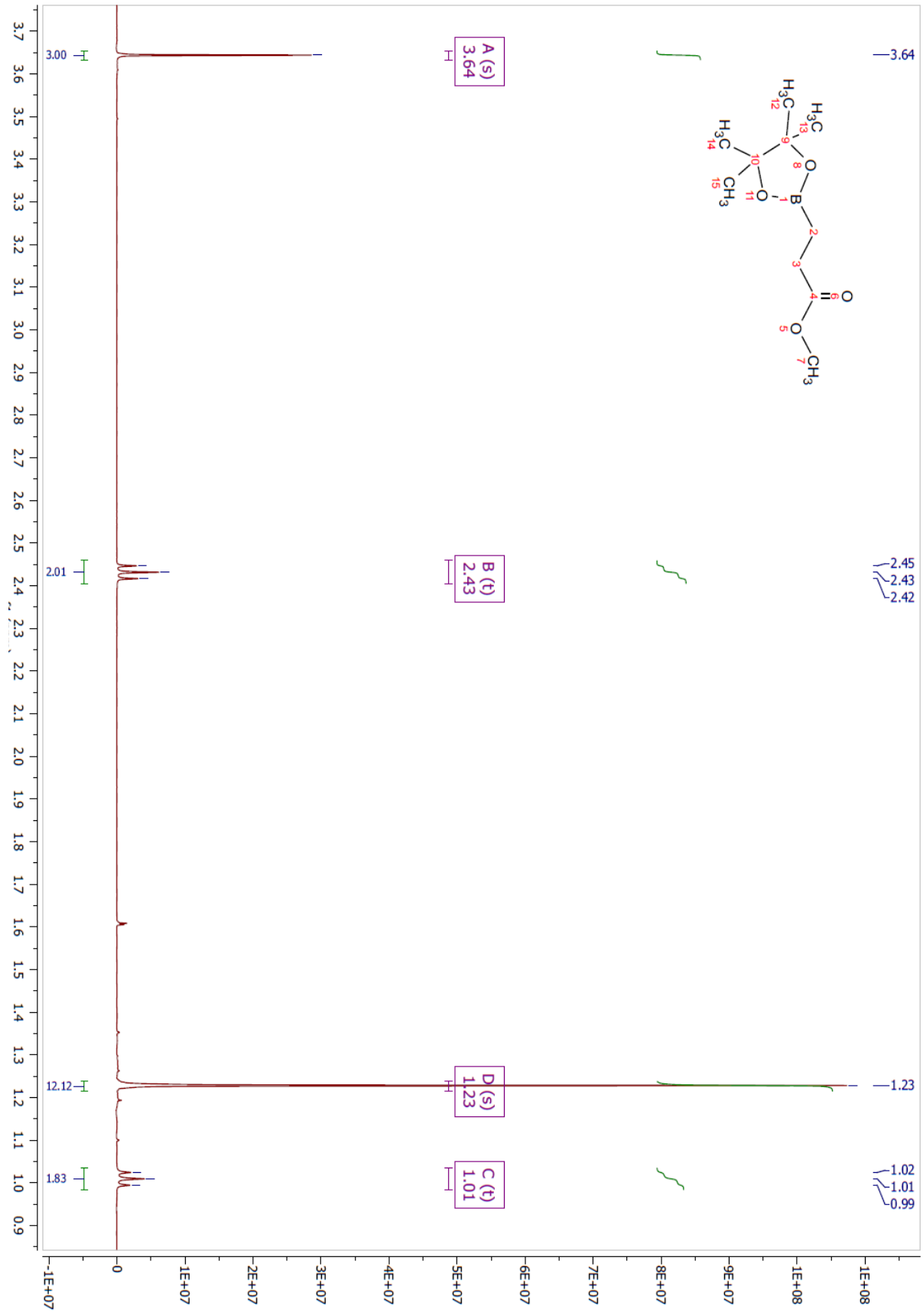
128.56
128.16
128.13
83.32
66.15
28.92
24.83
H3C
CH3
CH3
CH3
O
B
O
O
O
O
1
2
4
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
3
5
128.56
128.16
128.13
128.8 128.7 128.6 128.5 128.4 128.3 128.2 128.1 128.0 127.9
f1 (ppm)
85000
80000
75000
70000
65000
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000
S6
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30
f1 (ppm)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D (s)
1.23
C (t)
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3.00
2.01
12.12
1.83
3.64
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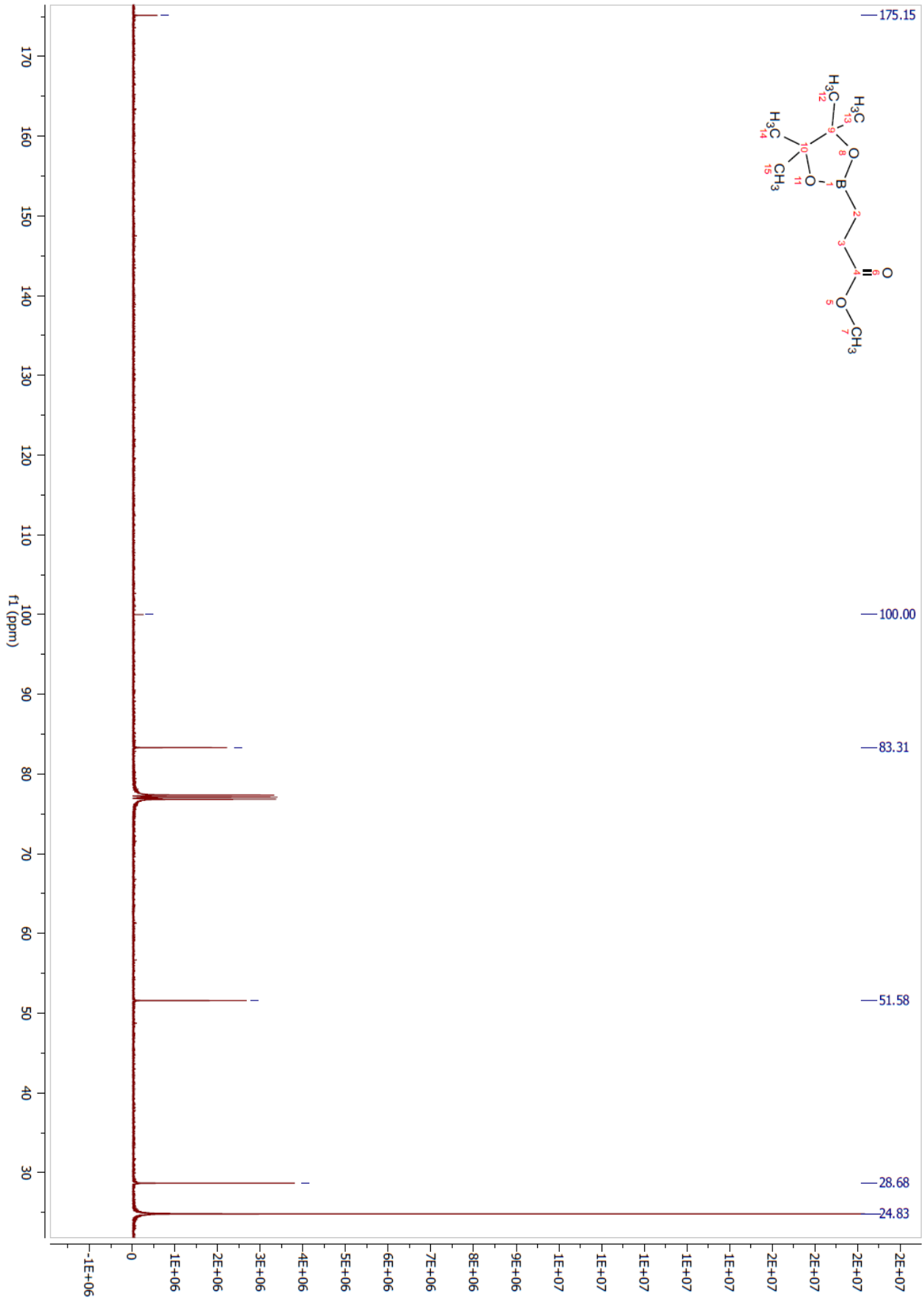
2.43
2.42
1.23
1.02
1.01
0.99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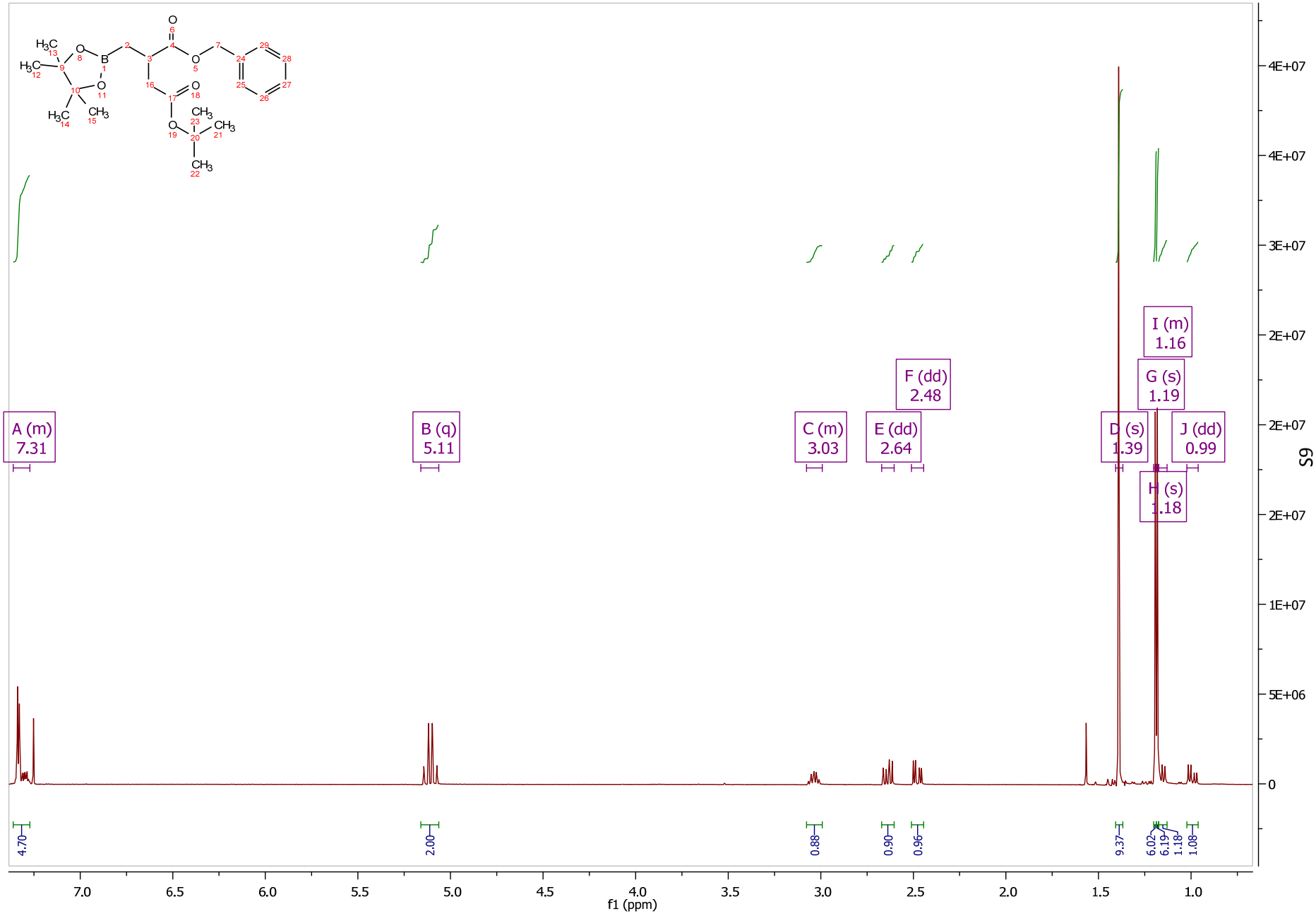
f1 (ppm)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G (s)
1.19
I (m)
1.16
H (s)
1.18
J (dd)
0.99
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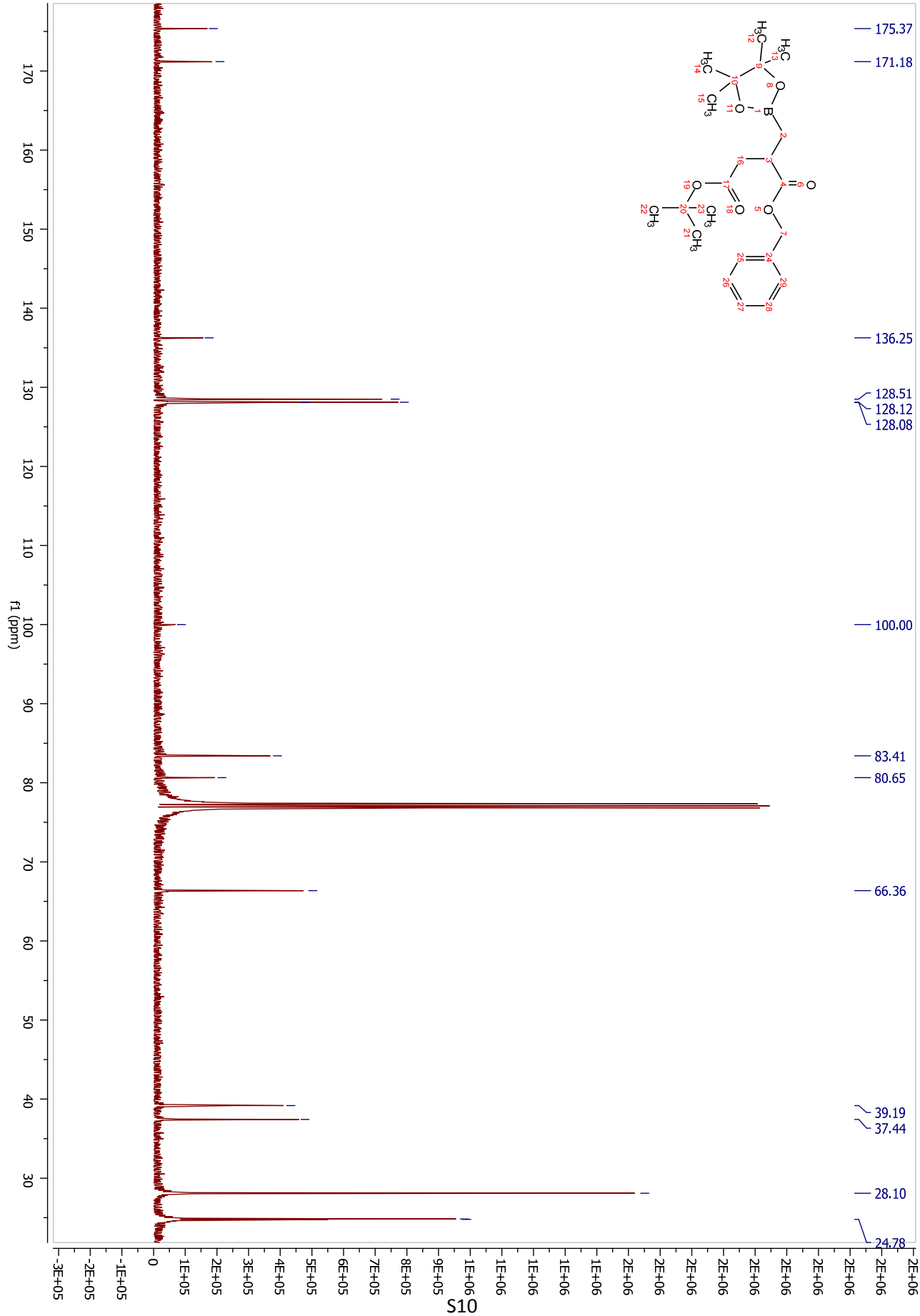
0.90
0.96
9.37
6.02
6.19
1.18
1.08
f1 (ppm)
S9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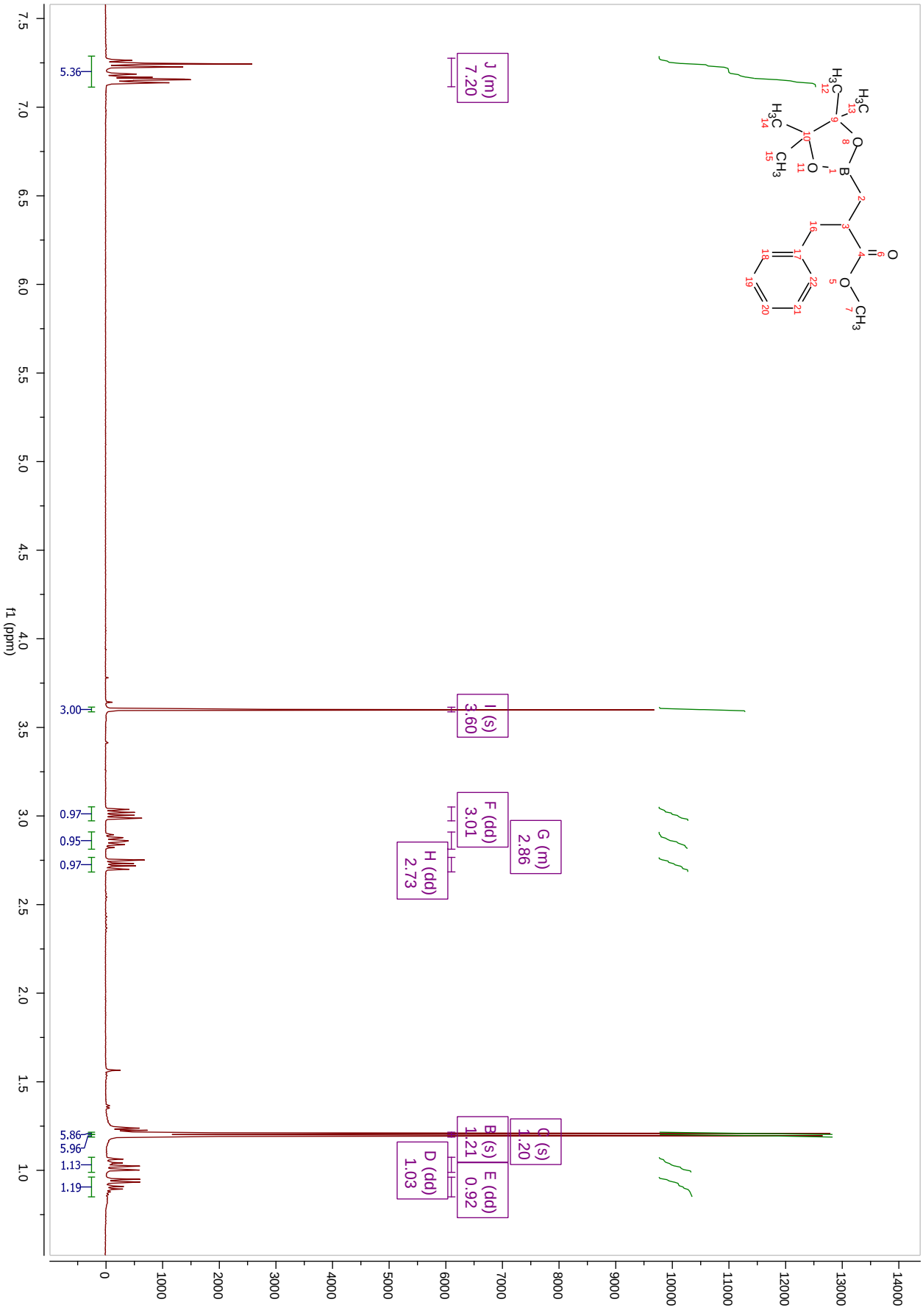
171.18
136.25
128.51
128.12
128.08
100.00
83.41
80.65
66.36
39.19
37.44
28.10
24.78
f1 (ppm)


f1 (ppm)
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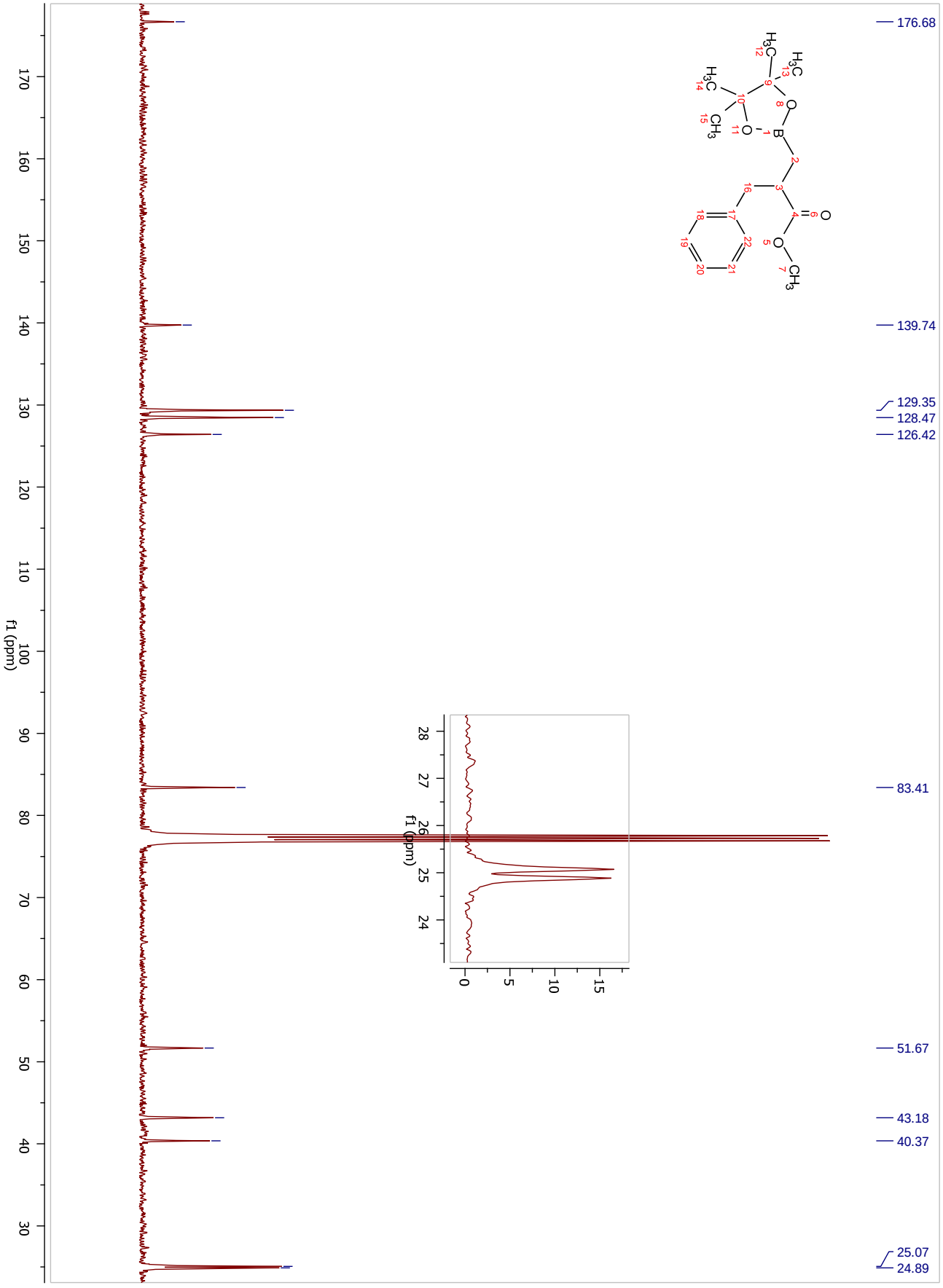
3.00
0.97
0.95
0.97
5.86
5.96
1.13
1.19
J (m) 7.20
I (s) 3.60
F (dd) 3.01
G (m) 2.86
H (dd) 2.73
C (s) 1.20
B (s) 1.21
E (dd) 0.92
D (dd) 1.03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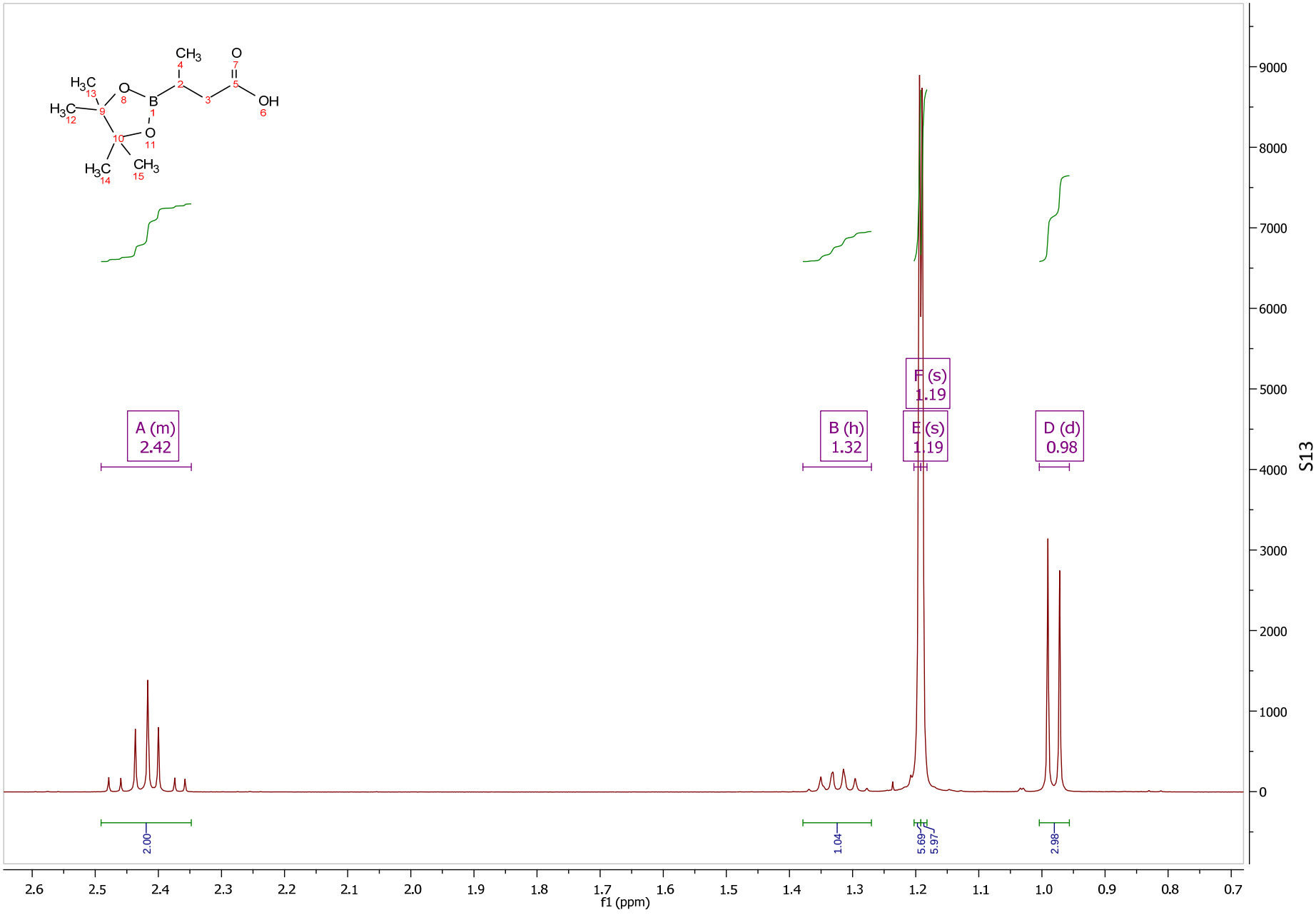
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B (h)
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5.97
2.98
f1 (ppm)
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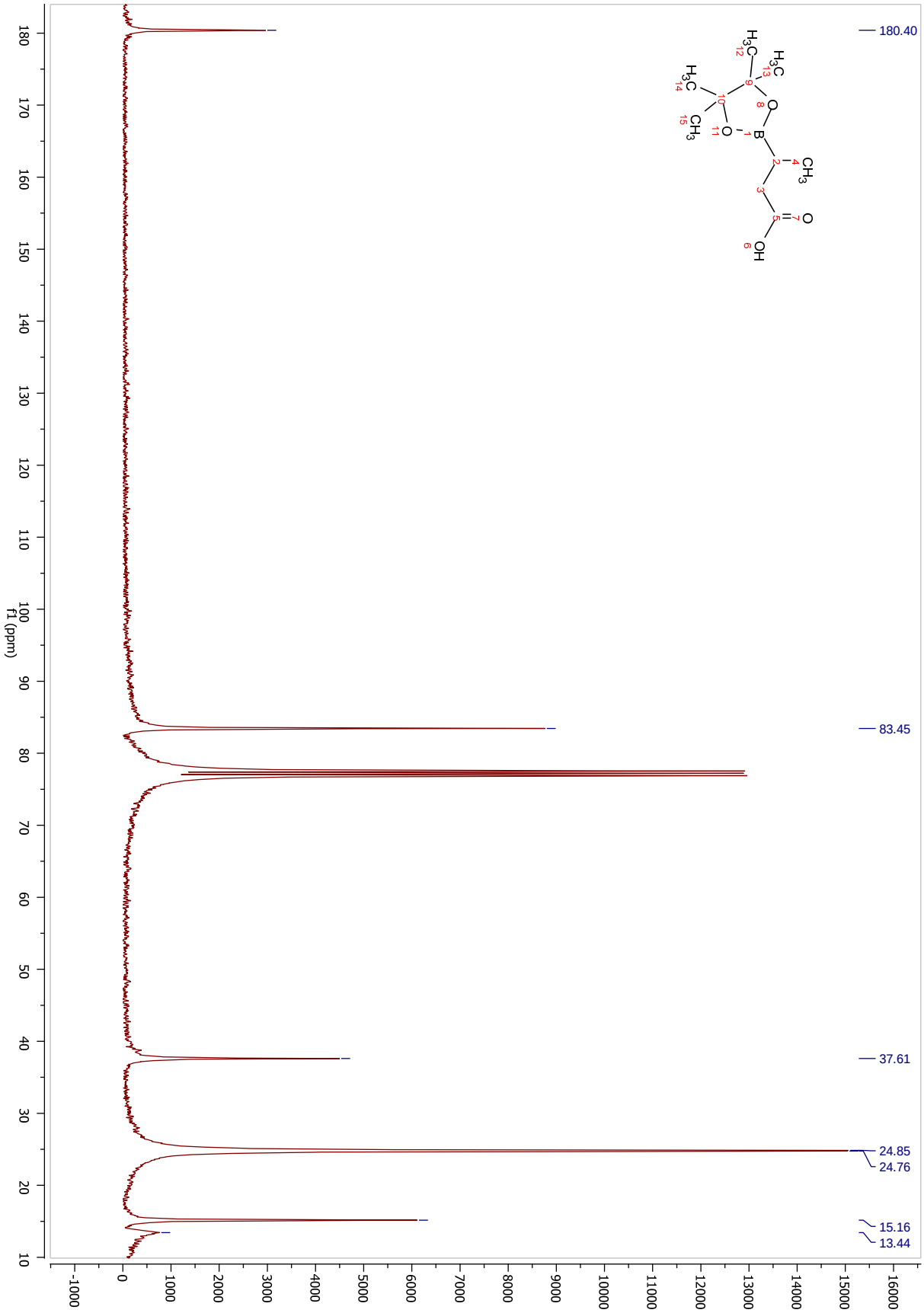
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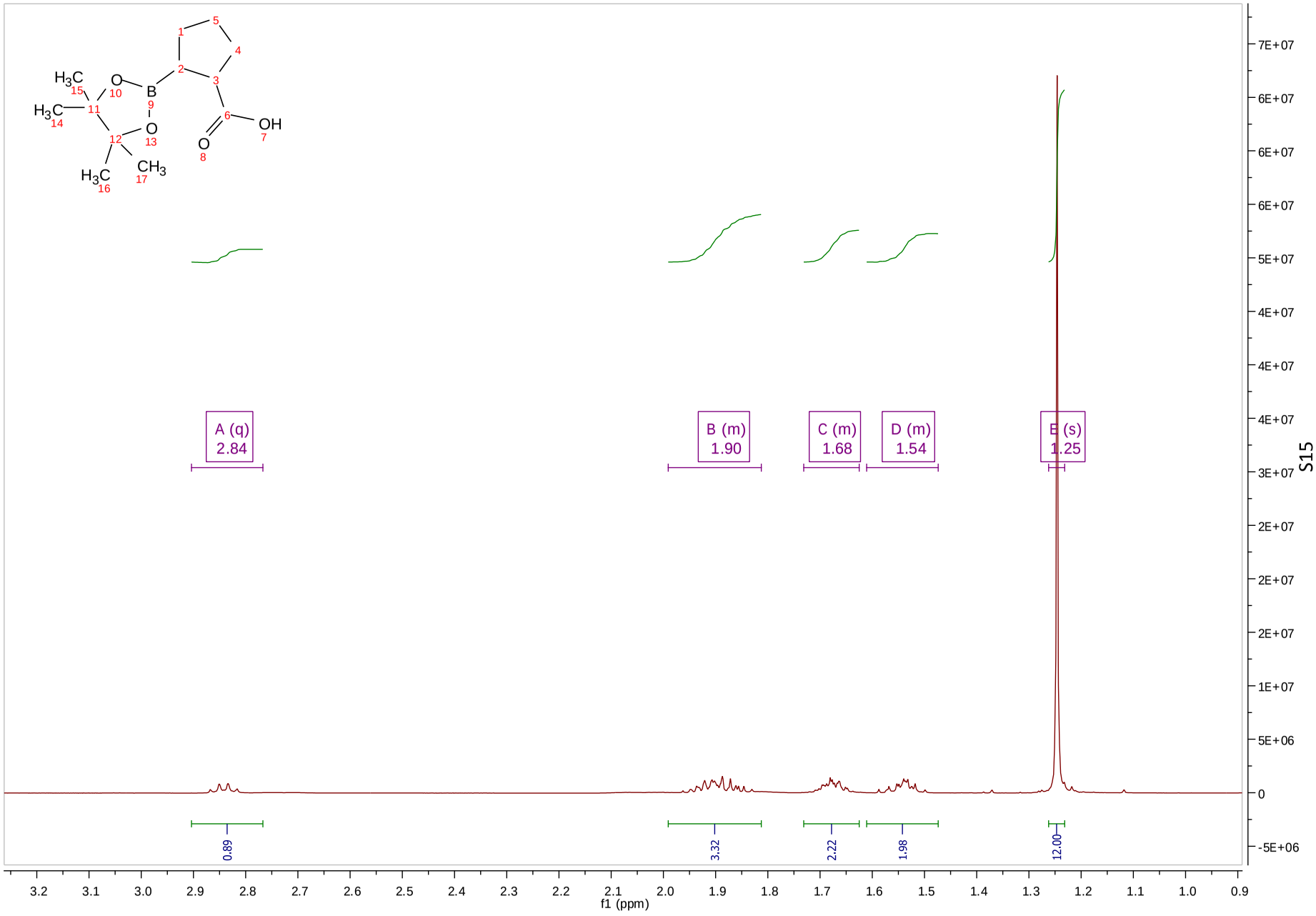
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12.00
f1 (ppm)
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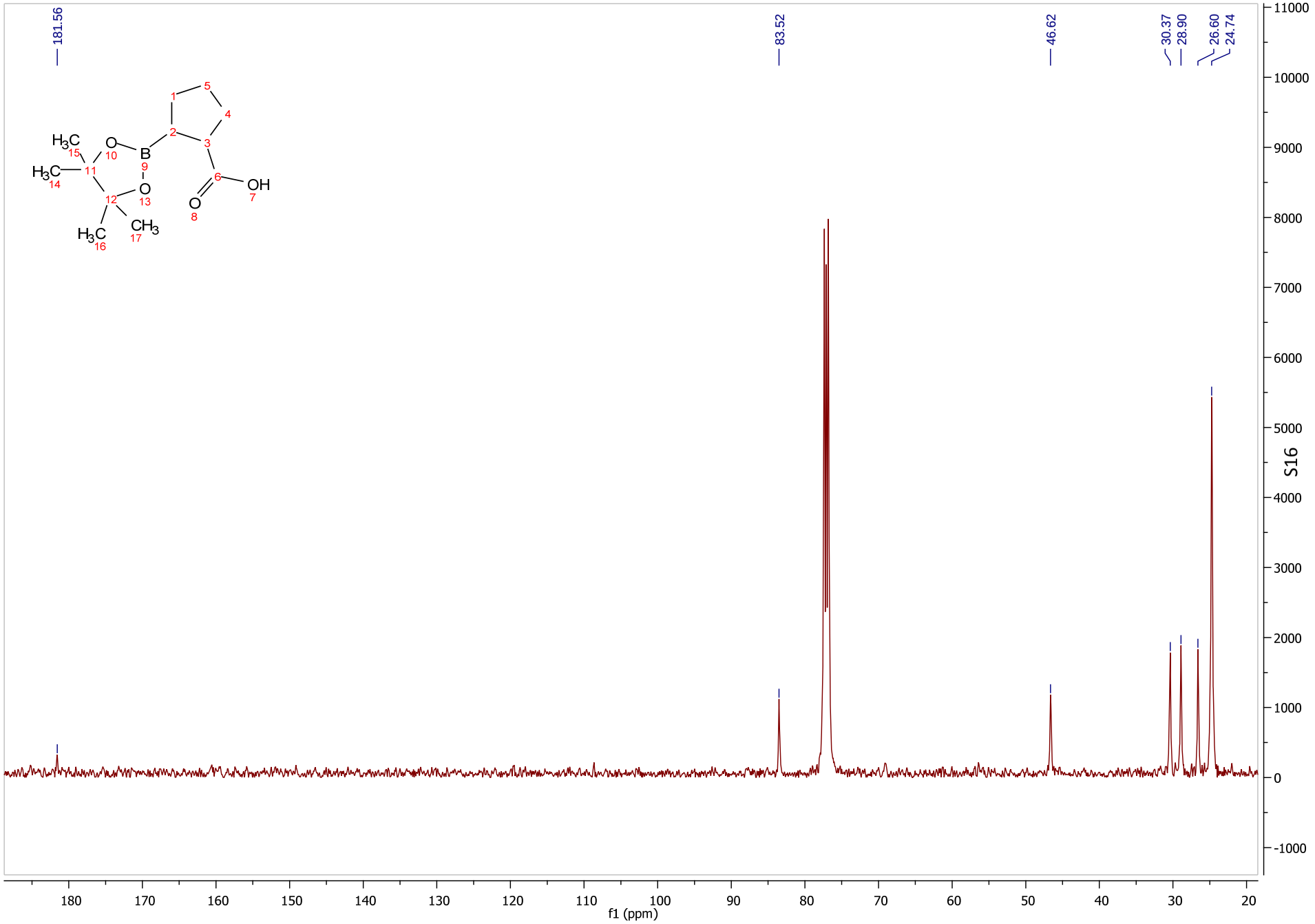
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24.74
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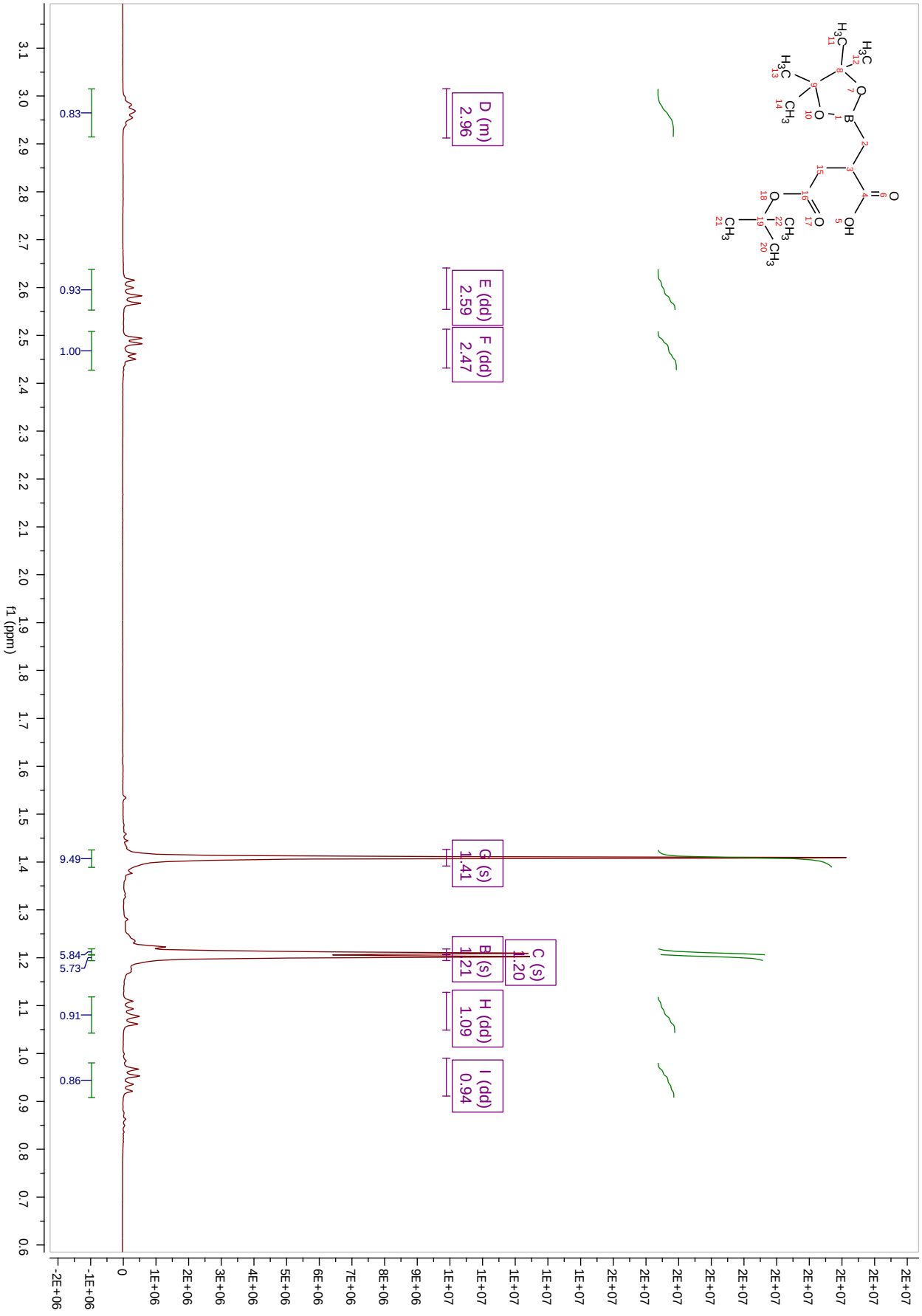
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F (dd)
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1.20
H (dd)
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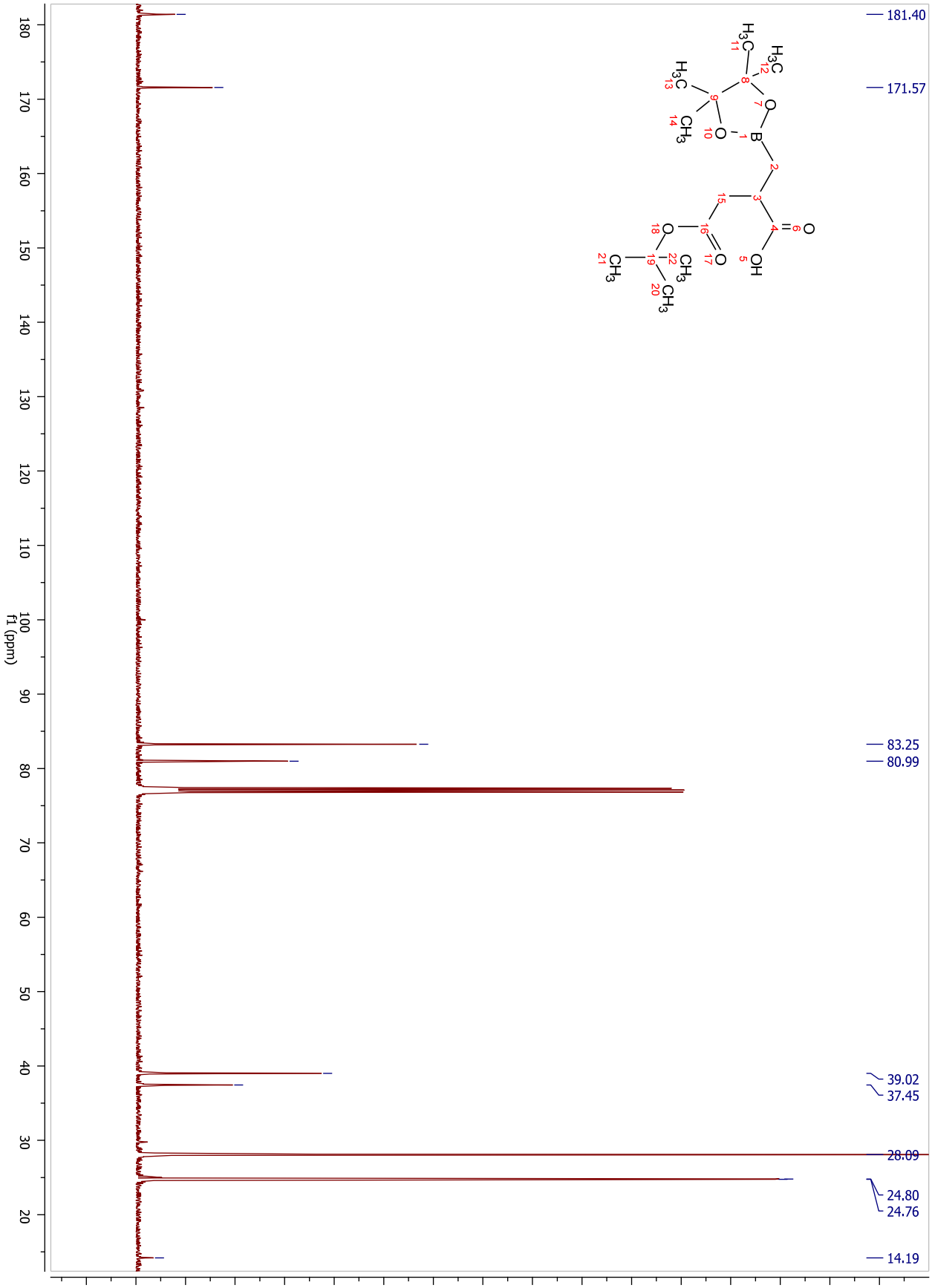
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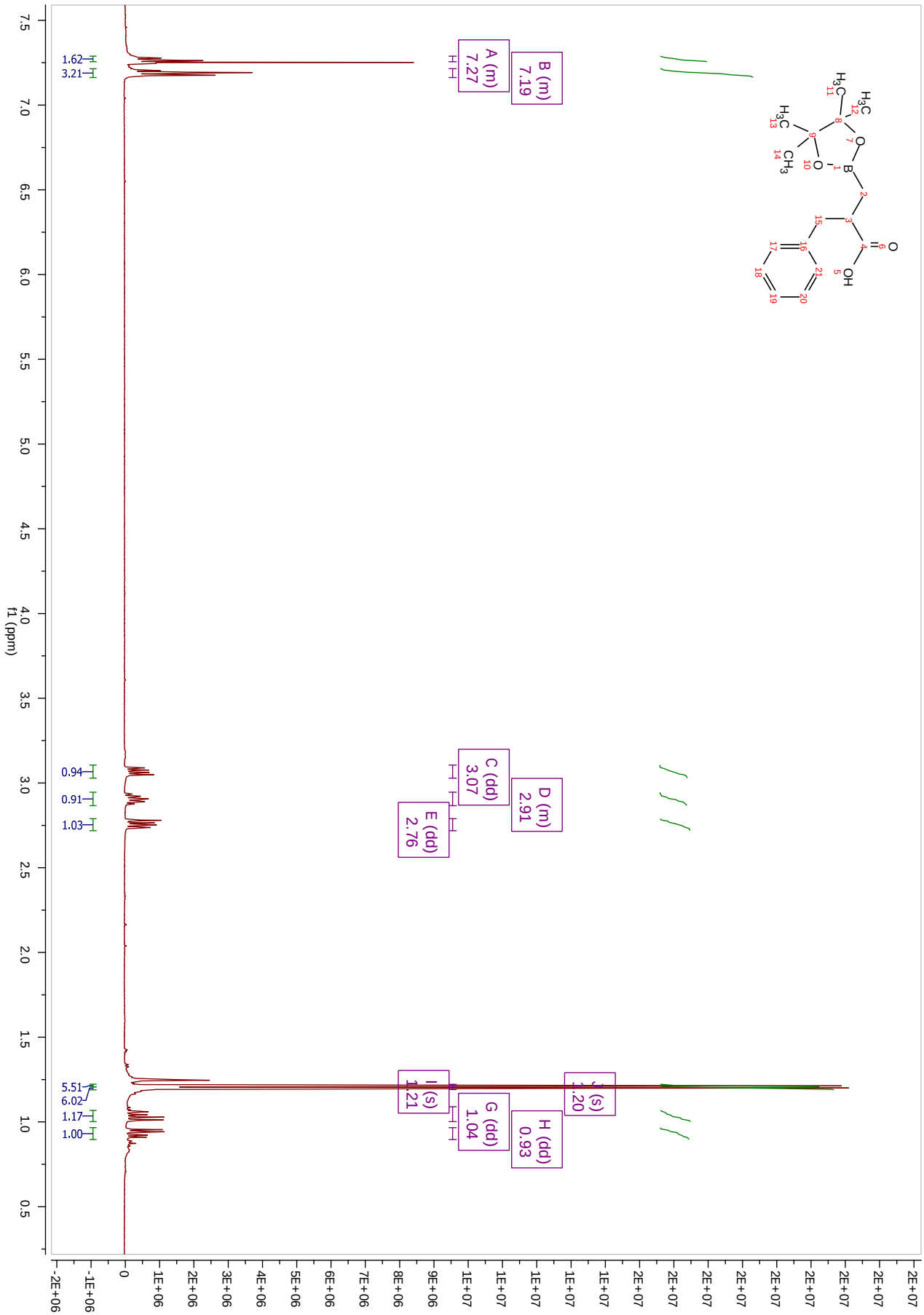
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H (dd) 0.93
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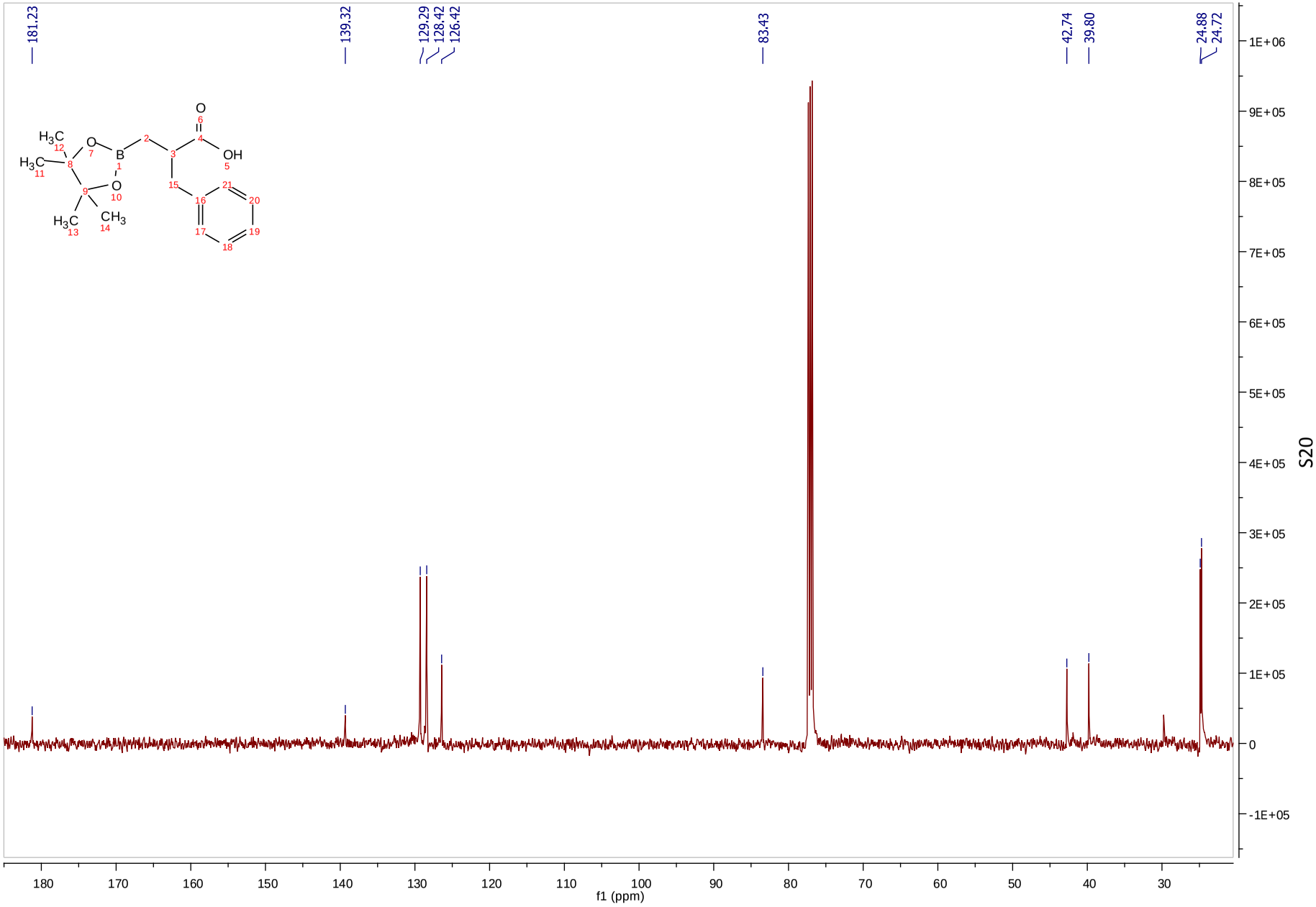
f1 (ppm)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7E+05
6E+05
5E+05
4E+05
3E+05
2E+05
1E+05
0
-1E+05
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150
140
130
120
110
100
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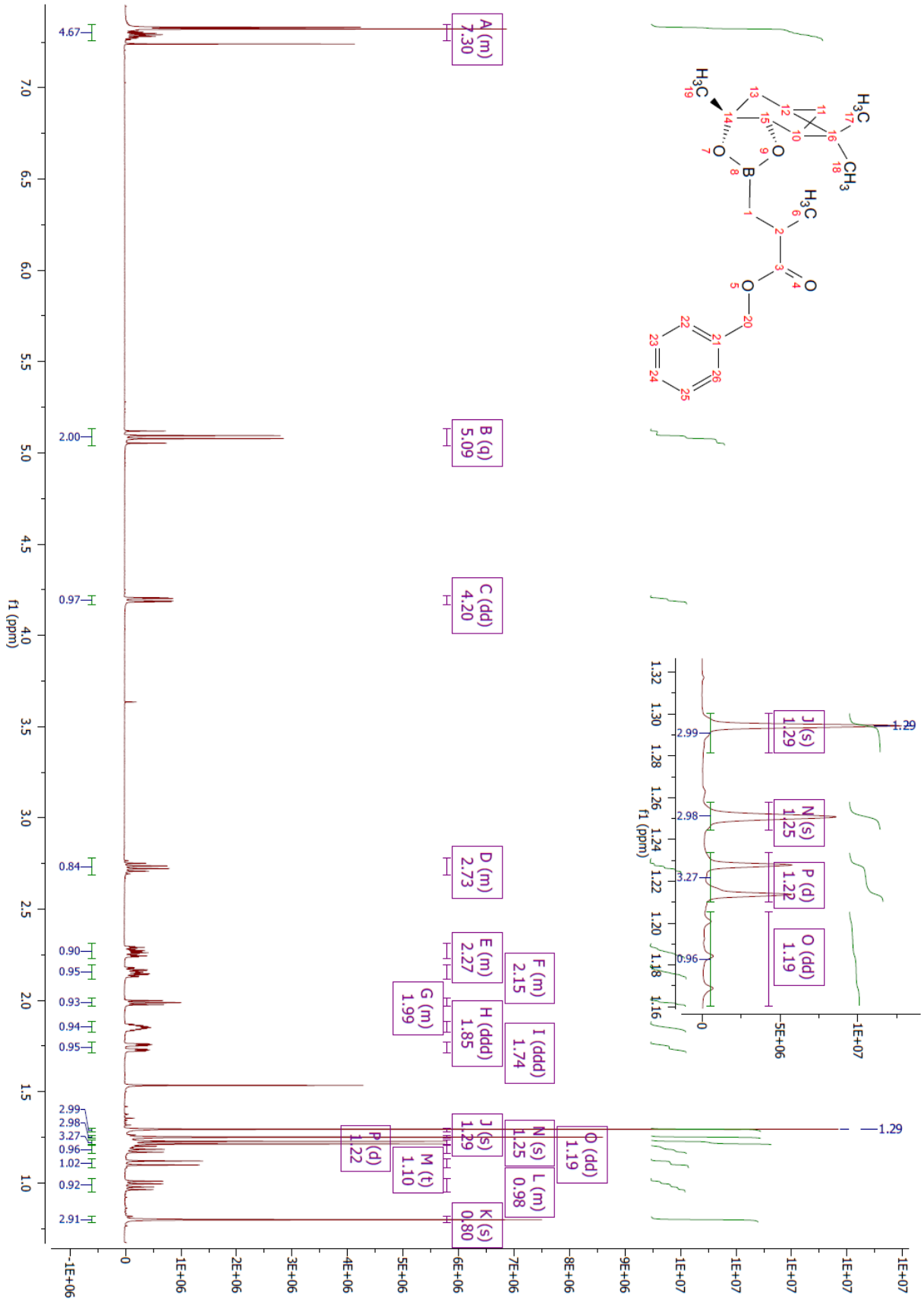
80
70
60
50
40
30
f1 (ppm)
S20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I (ddd) 1.74
J (s) 1.29
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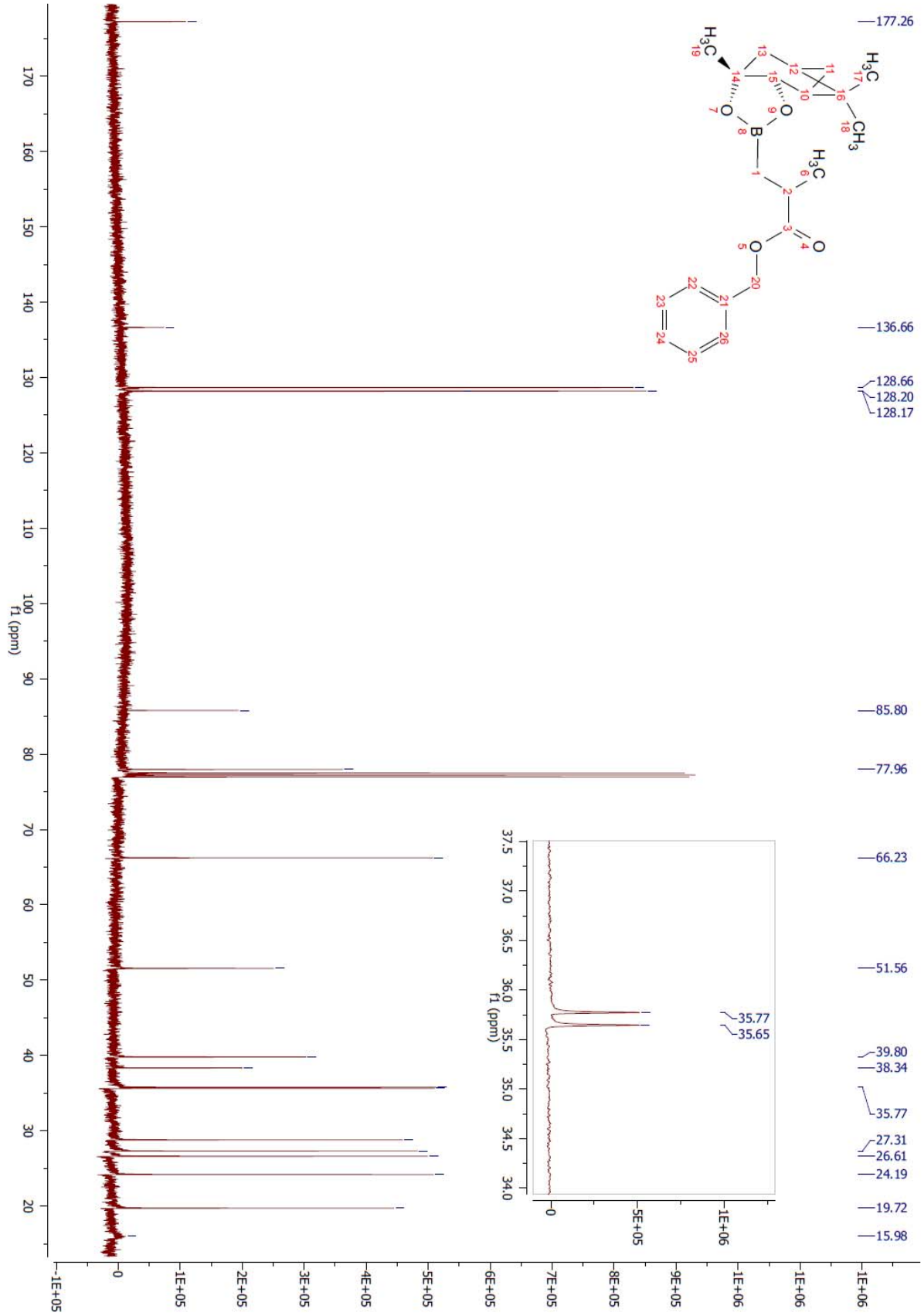
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15.98
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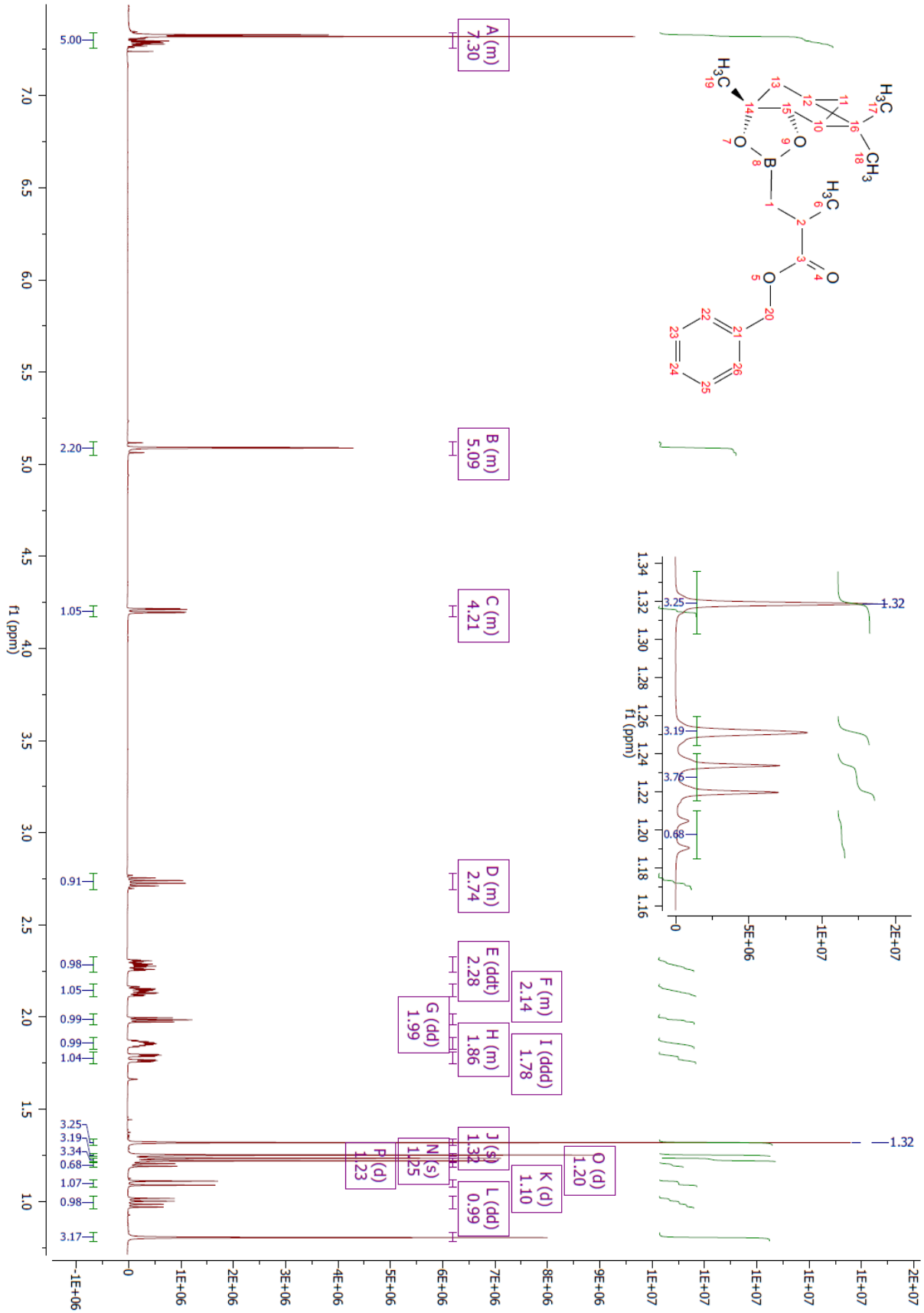
35.65
f1 (ppm)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I (ddd) 1.78
J (s) 1.32
K (d) 1.10
L (dd) 0.99
N (s) 1.25
O (d) 1.20
P (d) 1.23
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2.20
1.05
0.91
0.98
1.05
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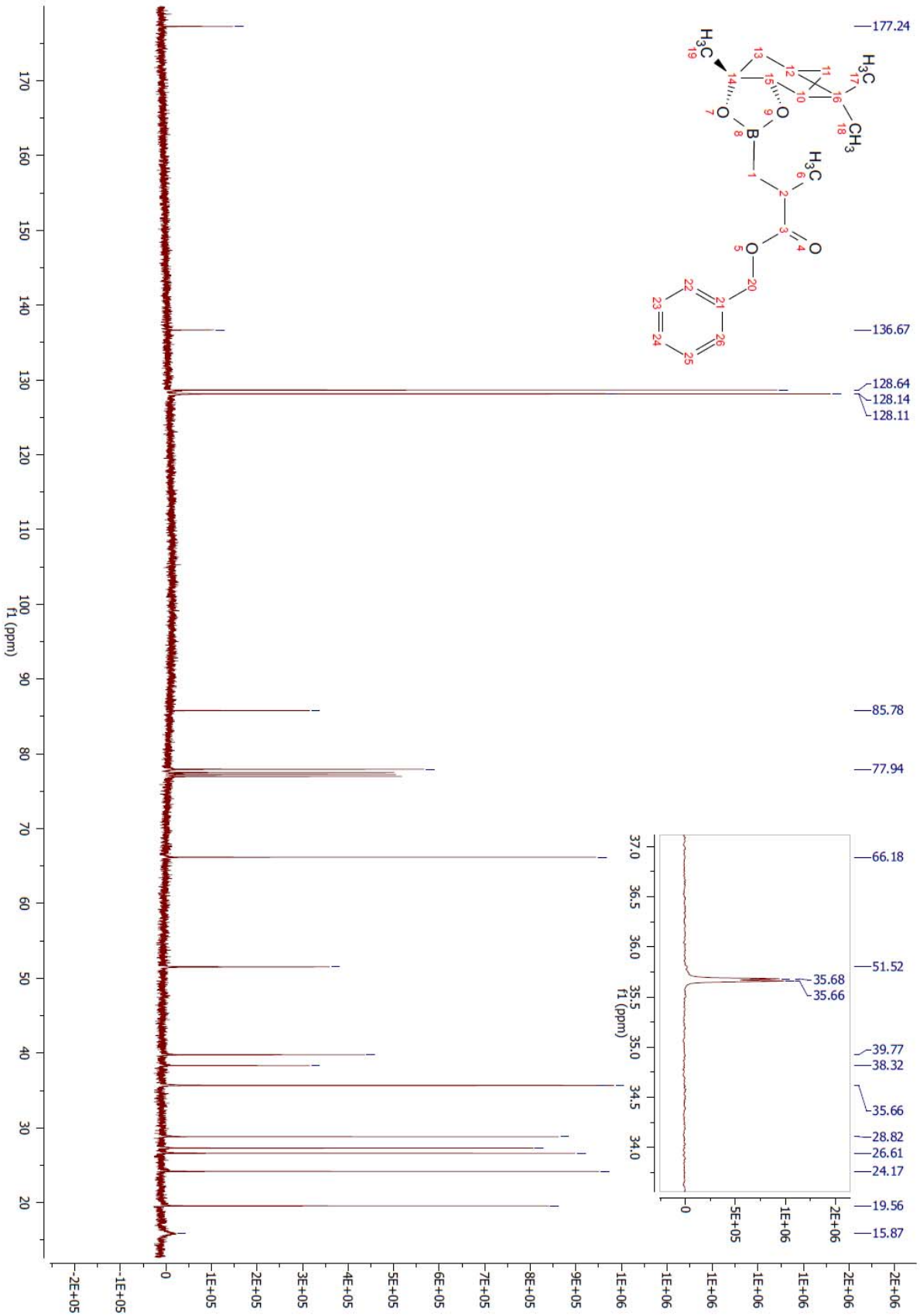
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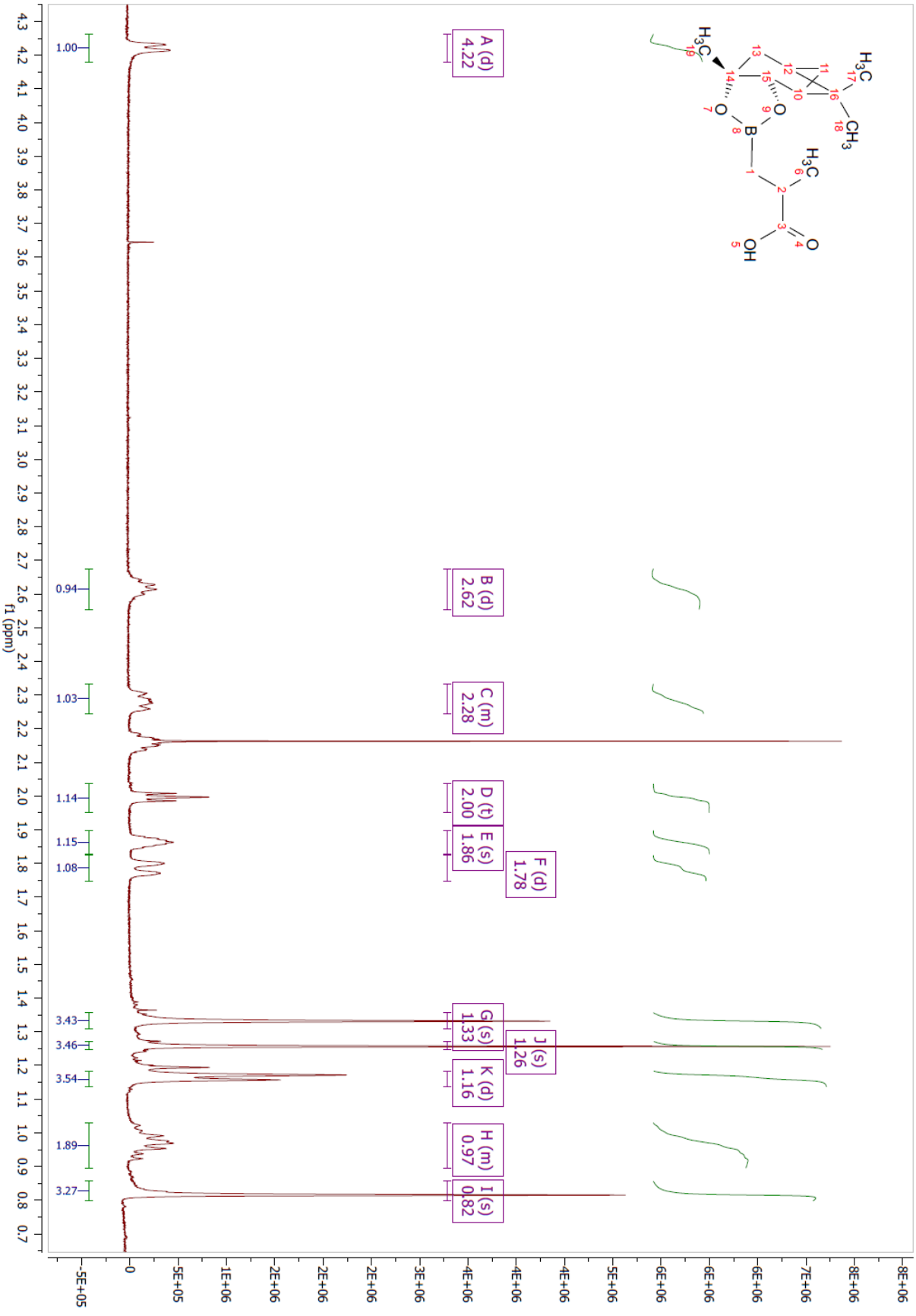
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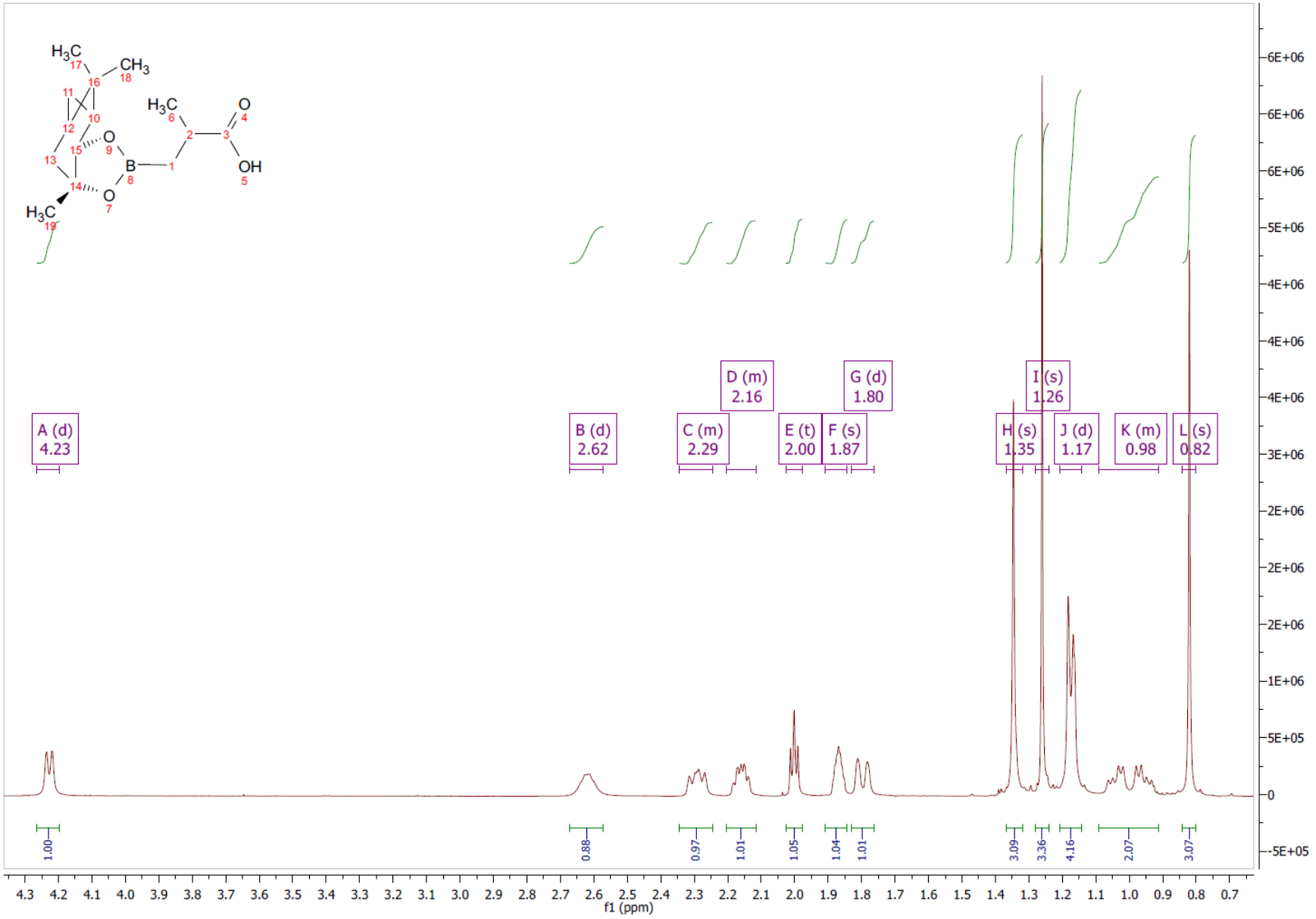
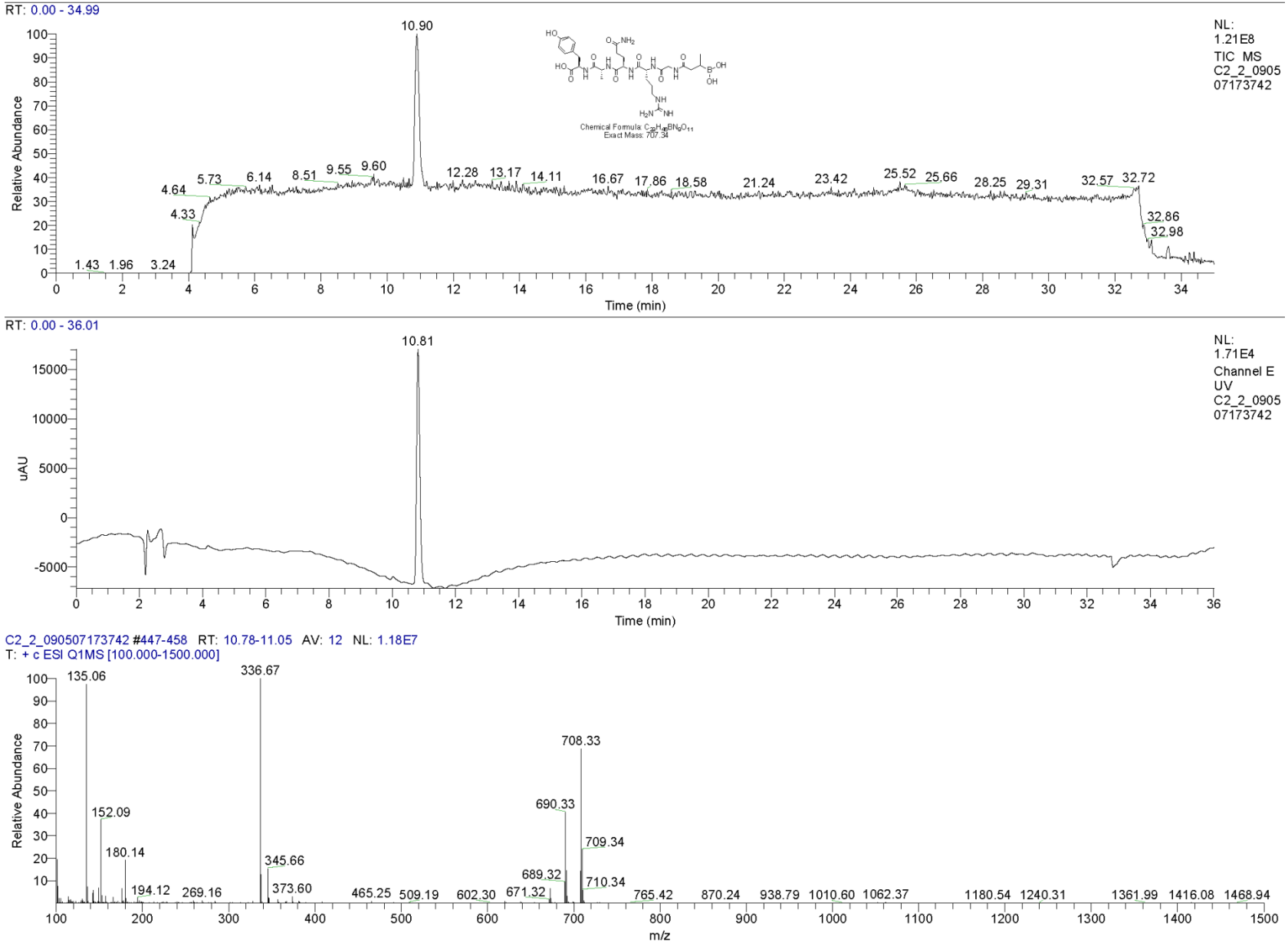
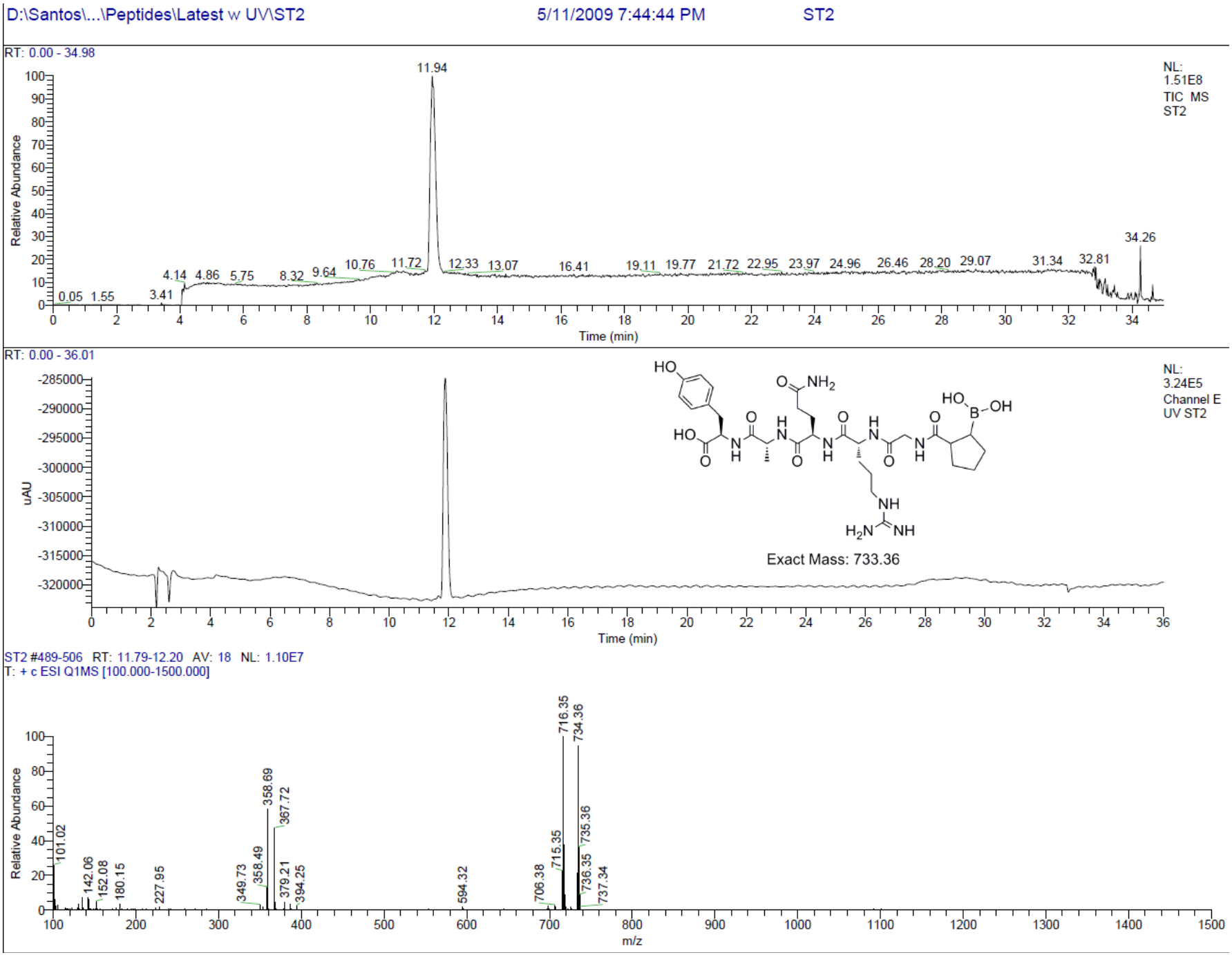
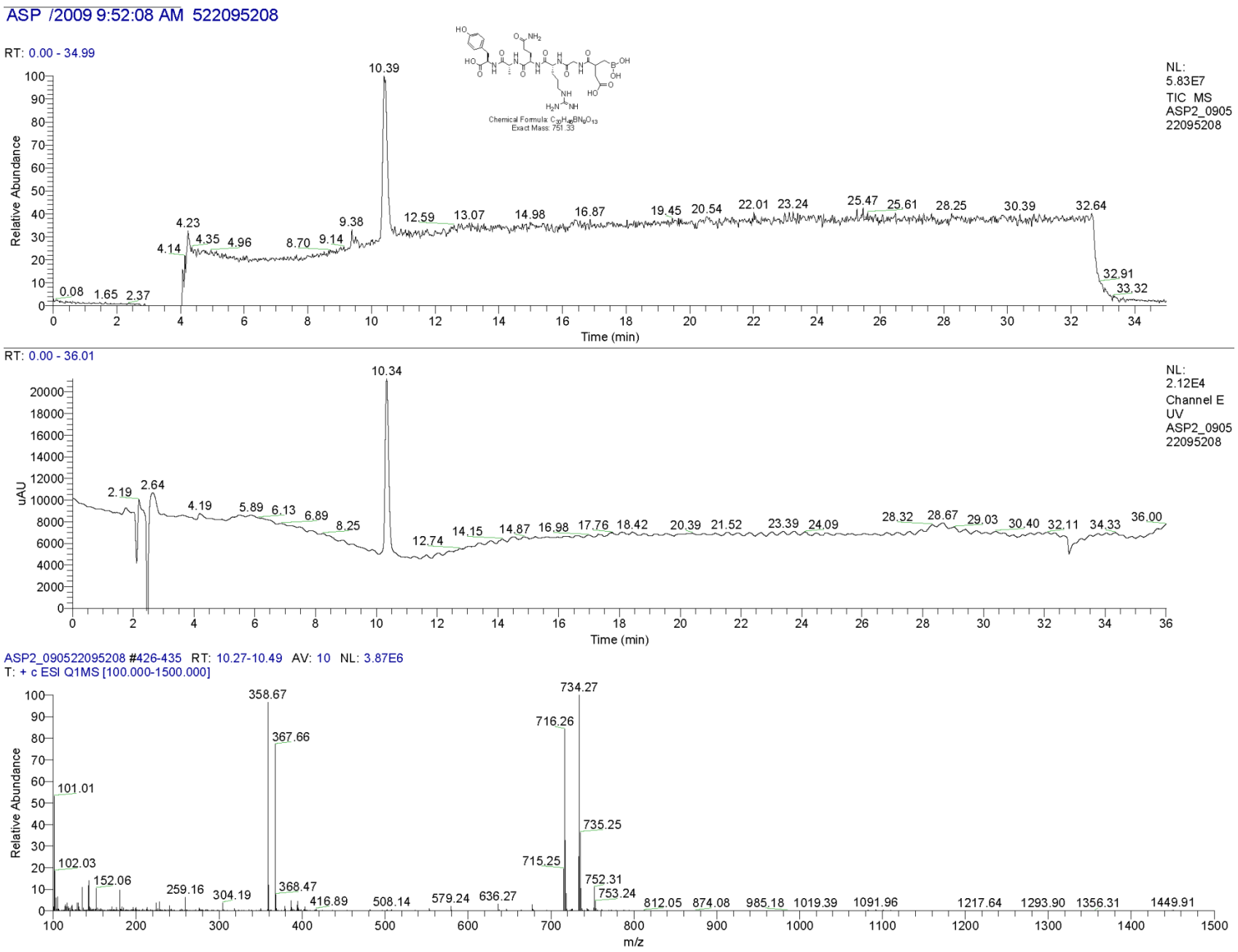
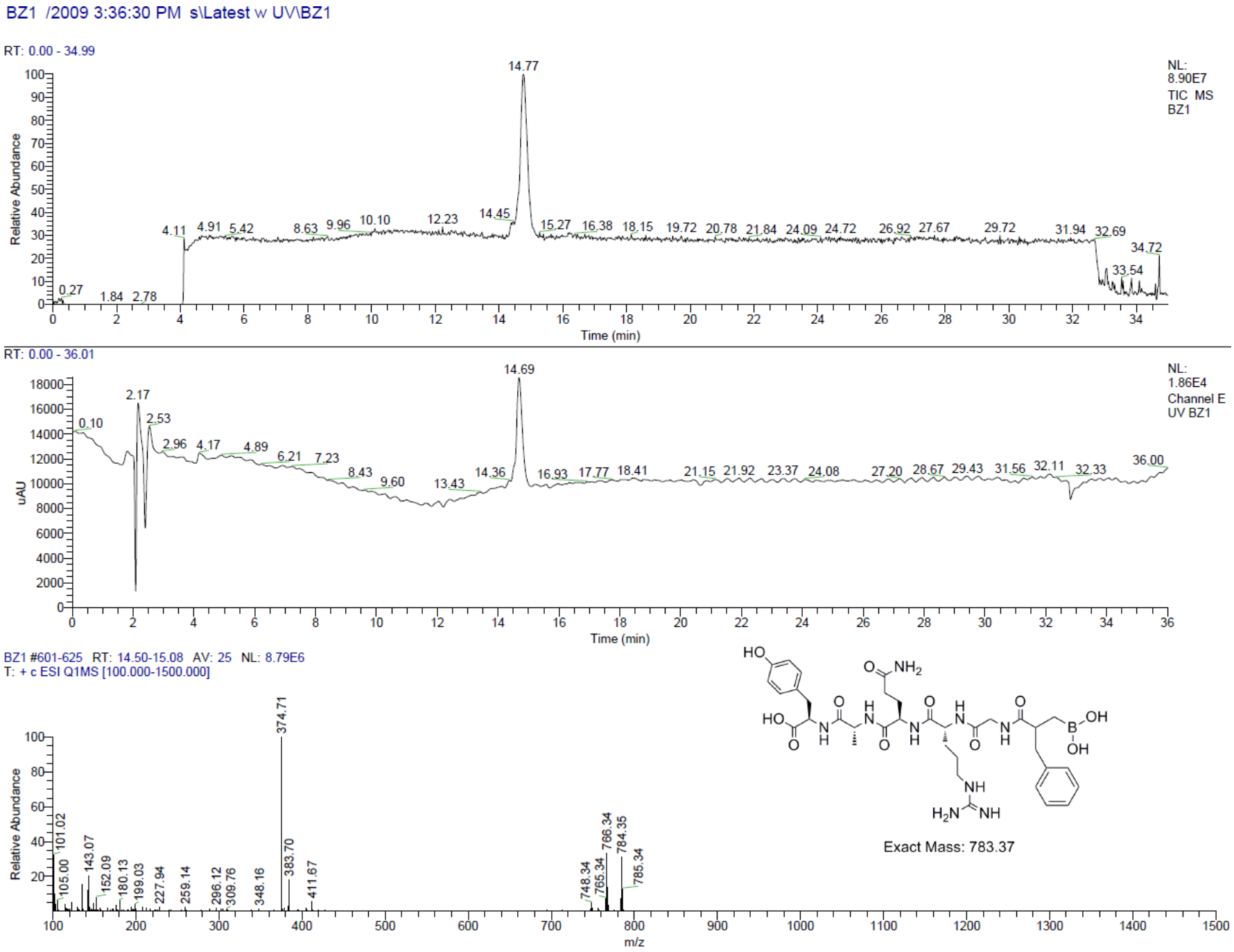
B (d) 2.62
C (m) 2.28
D (t) 2.00
E (s) 1.86
F (d) 1.78
G (s) 1.33
J (s) 1.26
K (d) 1.16
H (m) 0.97
I (s) 0.82
1.00
0.94
1.03
1.14
1.15
1.08
3.43
3.46
3.54
1.89
3.27
f1 (ppm)

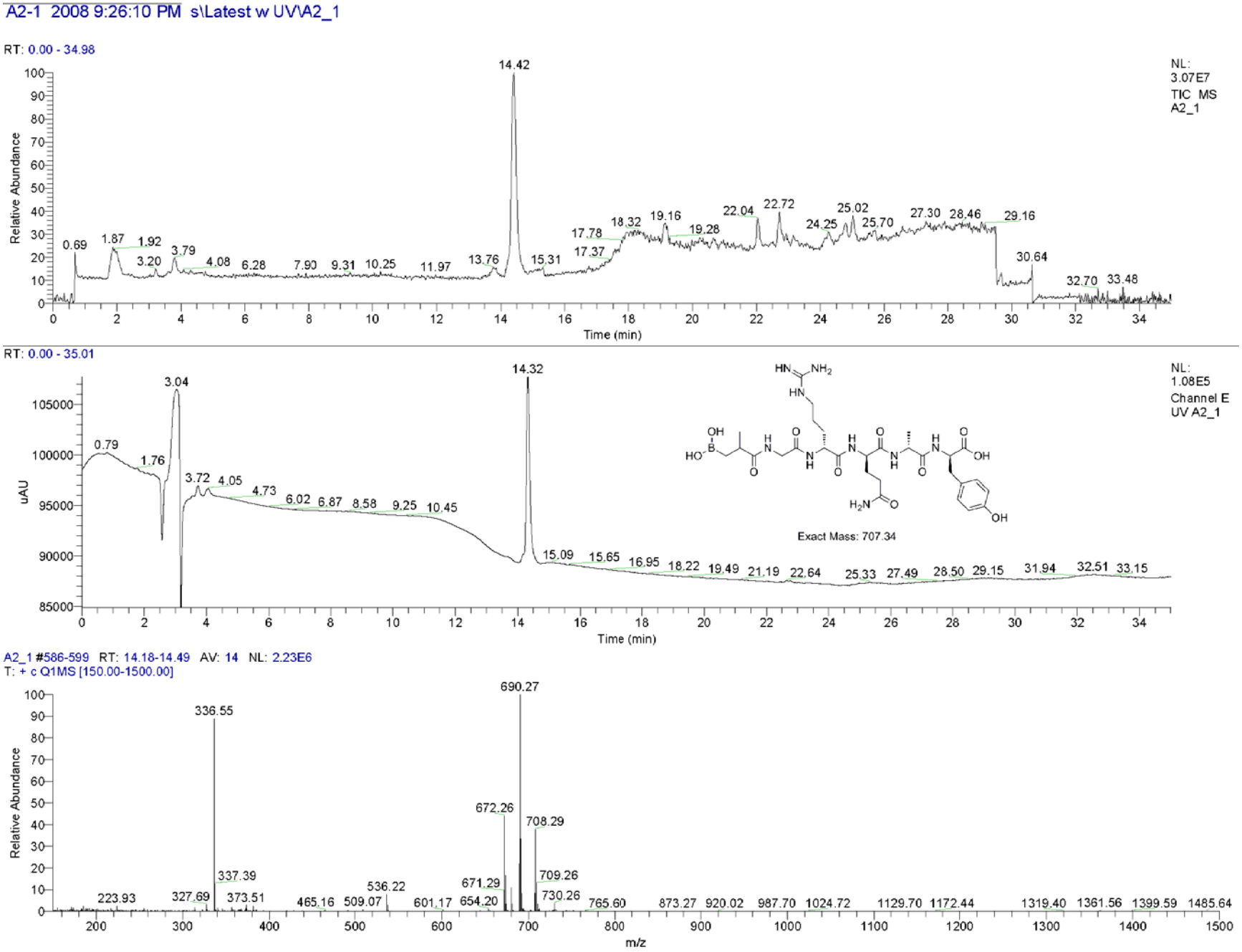

A (d) 4.23
B (d) 2.62
C (m) 2.29
D (m) 2.16
E (t) 2.00
F (s) 1.87
G (d) 1.80
H (s) 1.35
I (s) 1.26
J (d) 1.17
K (m) 0.98
L (s) 0.82
1.00
0.88
0.97
1.01
1.05
1.04
1.01
3.09
3.36
4.16
2.07
3.07
f1 (ppm)


D:\Santos\...\May 7 09\C2_2_090507173742 5/7/2009 5:37:42 PM Cr2
RT: 0.00 - 34.99
NL: 1.21E8 TIC MS C2_2_0905 07173742
Relative Abundance
Time (min)
10.90
4.64
5.73
6.14
8.51
9.55
9.60
4.33
12.28
13.17
14.11
16.67
17.86
18.58
21.24
23.42
25.52
25.66
28.25
29.31
32.57
32.72
32.86
32.98
1.43
1.96
3.24
Chemical Formula: C28H38BN9O11
Exact Mass: 707.34
RT: 0.00 - 36.01
NL: 1.71E4 Channel E UV C2_2_0905 07173742
uAU
Time (min)
10.81
C2_2_090507173742 #447-458 RT: 10.78-11.05 AV: 12 NL: 1.18E7
T: + c ESI Q1MS [100.000-1500.000]
Relative Abundance
m/z
135.06
336.67
708.33
690.33
152.09
709.34
180.14
345.66
689.32
710.34
194.12
269.16
373.60
465.25
509.19
602.30
671.32
765.42
870.24
938.79
1010.60
1062.37
1180.54
1240.31
1361.99
1416.08
1468.94


D:\Santos\...\Peptides\Latest w UV\ST2
5/11/2009 7:44:44 PM
ST2
RT: 0.00 - 34.98
NL:
1.51E8
TIC MS
ST2
Relative Abundance
Time (min)
11.94
34.26
RT: 0.00 - 36.01
NL:
3.24E5
Channel E
UV ST2
uAU
Time (min)
Exact Mass: 733.36
ST2 #489-506 RT: 11.79-12.20 AV: 18 NL: 1.10E7
T: + c ESI Q1MS [100.000-1500.000]
Relative Abundance
m/z
716.35
734.36
358.69
367.72
735.36
715.35
101.02
142.06
152.08
180.15
227.95
349.73
358.49
379.21
394.25
594.32
706.38
736.35
737.34


ASP /2009 9:52:08 AM 522095208
RT: 0.00 - 34.99
Chemical Formula: C30H49BN8O13
Exact Mass: 751.33
NL:
5.83E7
TIC MS
ASP2_0905
22095208
Relative Abundance
Time (min)
RT: 0.00 - 36.01
NL:
2.12E4
Channel E
UV
ASP2_0905
22095208
uAU
Time (min)
ASP2_090522095208 #426-435 RT: 10.27-10.49 AV: 10 NL: 3.87E6
T: + c ESI Q1MS [100.000-1500.000]
Relative Abundance
m/z


BZ1 /2009 3:36:30 PM s\Latest w UV\BZ1
RT: 0.00 - 34.99
NL:
8.90E7
TIC MS
BZ1
Relative Abundance
Time (min)
RT: 0.00 - 36.01
NL:
1.86E4
Channel E
UV BZ1
uAU
Time (min)
BZ1 #601-625 RT: 14.50-15.08 AV: 25 NL: 8.79E6
T: + c ESI Q1MS [100.000-1500.000]
Relative Abundance
m/z
Exact Mass: 783.37


A2-1 2008 9:26:10 PM s\Latest w UV\A2_1
RT: 0.00 - 34.98
NL:
3.07E7
TIC MS
A2_1
Relative Abundance
Time (min)
RT: 0.00 - 35.01
NL:
1.08E5
Channel E
UV A2_1
uAU
Exact Mass: 707.34
A2_1 #586-599 RT: 14.18-14.49 AV: 14 NL: 2.23E6
T: + c Q1MS [150.00-1500.00]
m/z