Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Isotope-coded, Fluorous Photoaffinity Labeling Reagents

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Experimental

All the solvents were purchased from suppliers as anhydrous grade. The NMR spectra were recorded at room temperature on an Inova-400 MHz spectrometer. Mass spectra and tandem MS/MS spectra were obtained from Applied Biosystems 4700 Proteomics Analyzer.

4-(Methoxycarbonyl)-2,3,5,6-d4-benzoic acid (3)

Compound **3** was prepared according to the literature protocol. Terephthalic- 2,3,5,6-d4 acid (1.94 g, 11.40 mmol) was used to afford **3** (1.81 g, 86 %) as white solid. ^{1}H NMR (CD_3OD, 400 MHz) δ 3.93 (s, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 52.88, 130.37 (t, $J_{C\text{-}D}$ = 25.6 Hz), 130.09 (t, $J_{C\text{-}D}$ = 25.3 Hz), 134.93, 135.91, 167.65, 168.69. HRMS for (M – H) calcd: 183.0596; found 183.0593.

Methyl 4-(hydroxymethyl)-2,3,5,6-d4-benzoate (4)

Compound **4** was prepared from compound **3** by following the literature protocol. To a stirred solution of compound **3** (1.81 g, 9.83 mmol) in THF (30 mL) was added CDI (2.07 g, 12.80 mmol) at room temperature. After stirring for 10 min the solution was cooled to 0 ^{o}C by an ice bath. A solution of sodium borohydride (0.60 g, 5.70 mmol) in water (15 mL) was added in one portion. The reaction mixture was stirred for 30 min at the same temperature. The organic solvent was then removed under reduced pressure and the resulting solution was diluted by brine and extracted with ethyl acetate twice. The combined organic layers were dried over sodium sulfate and concentrated under vacuum. The residue was purified through flash chromatography (Hex : EA = 3 : 2) over silica gel to yield compound **4** as white solid (1.41 g, 84 %). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.45 (s, 1H), 3.90 (s, 3H), 4.74 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.05, 64.44, 125.95 (t, J = 24.2 Hz), 128.96, 129.34 (t, J = 24.7 Hz), 145.90, 166.99. HRMS for (M-H) calcd: 169.0803; found 169.0796.

(4-(3-(Perfluorohexyl)-3*H*-diazirin-3-yl)-2,3,5,6-d4-phenyl)methanol (1-D_4)

Compound **1-D_4** was prepared from by following the literature protocol. compound **4** (347.2 mg, 2.04 mmol) was used to afford **1-D_4** (249.0 mg, 26 %) as a light yellow oil. ^{1}H NMR

($CDCl_3$, 400 MHz) δ 1.86 (s, 1H), 4.69 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 27.53 (t, *J* = 28.4 Hz), 64.40, 126.82 (t, *J* = 24.2 Hz), 127.99 (t, *J* = 24.3Hz), 128.24, 142.79; ^{19}F NMR ($CDCl_3$, 376 MHz) δ -80.91, -109.54, -120.26, -122.05, -122.89, -126.20.

2,5-Dioxopyrrolidin-1-yl 4-(3-(perfluorohexyl)-3*H*-diazirin-3-yl)-2,3,5,6-d4-benzyl carbonate (5-D_4)

This was prepared in a similar manner to **5-H_4.** Compound **1-D_4** (10.0 mg, 21.2 mmol) was used to afford **5-D_4** (5.7 mg, 44%) as white solid. ^1H NMR ($CDCl_3$, 400 MHz) δ 2.84 (s, 4H), 5.31 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.45, 27.42 (t, *J* = 29.2 Hz), 71.44, 128.01-128.59 (m), 120.01, 135.18, 151.56, 168.41; ^{19}F NMR ($CDCl_3$, 376 MHz) -80.79, -109.42, -120.18, -121.97, -122.78, -126.12. HRMS for $C_{19}H_6D_4N_3O_5F_{13}Na$ $[M + Na]^+$: calcd 634.0558; found 634.0569. HRMS for $C_{19}H_6D_4N_3O_5F_{13}Cs$ $[M + Cs]^+$: calcd 743.9714; found 743.9754.

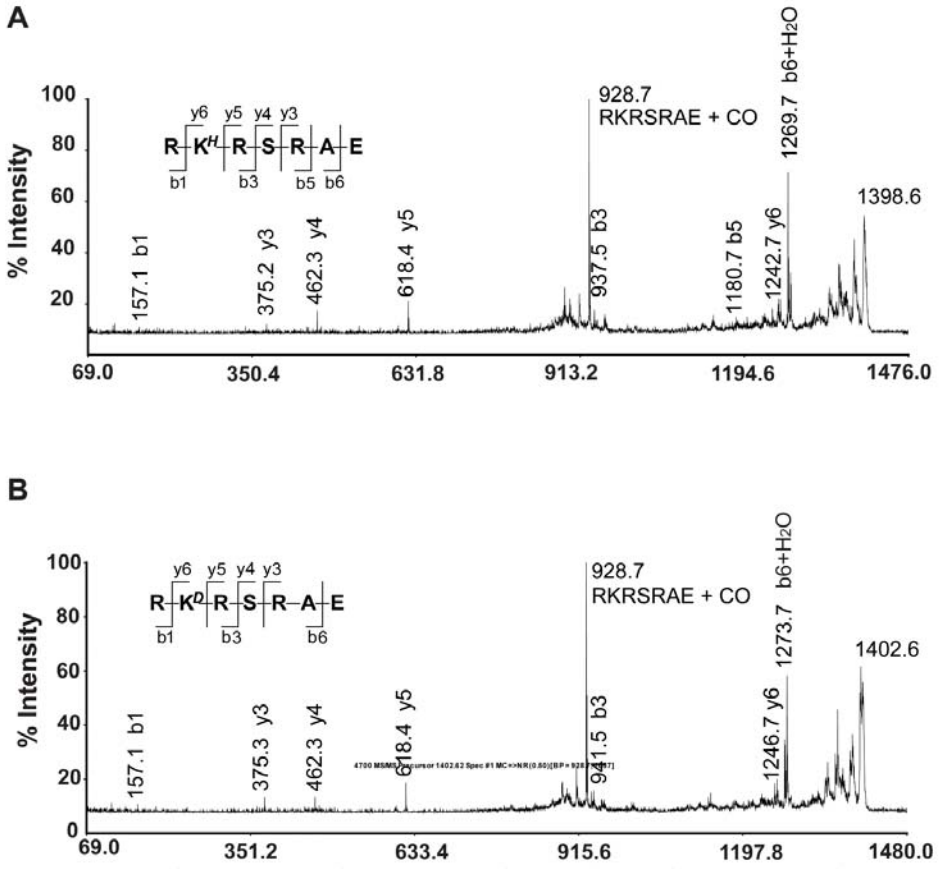
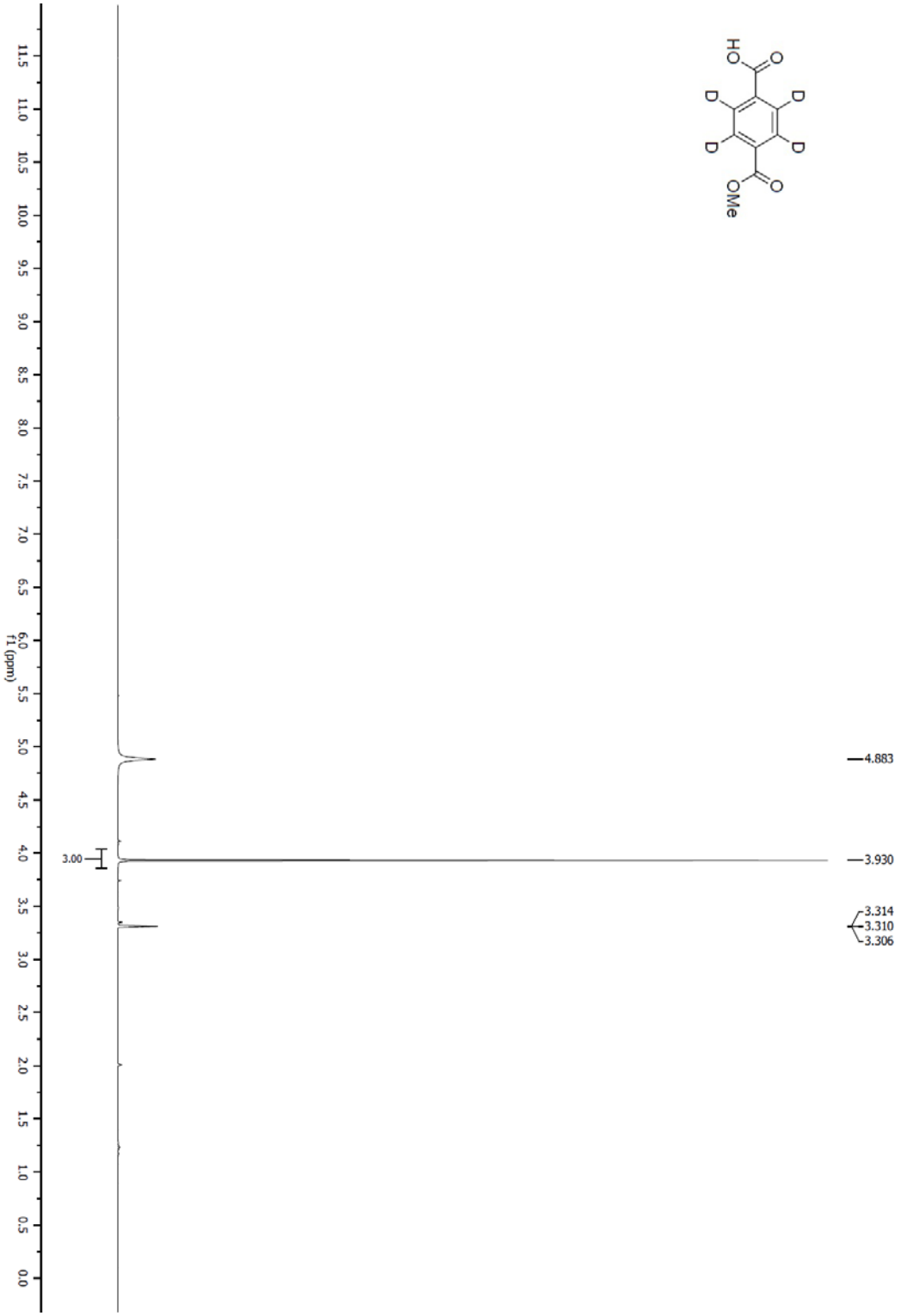


Fig. S1 Tandem MS-MS spectra of the peptides 7 (A) and 8 (B).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D
D
D
O
OMe
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10.5
10.0
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8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
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0.0
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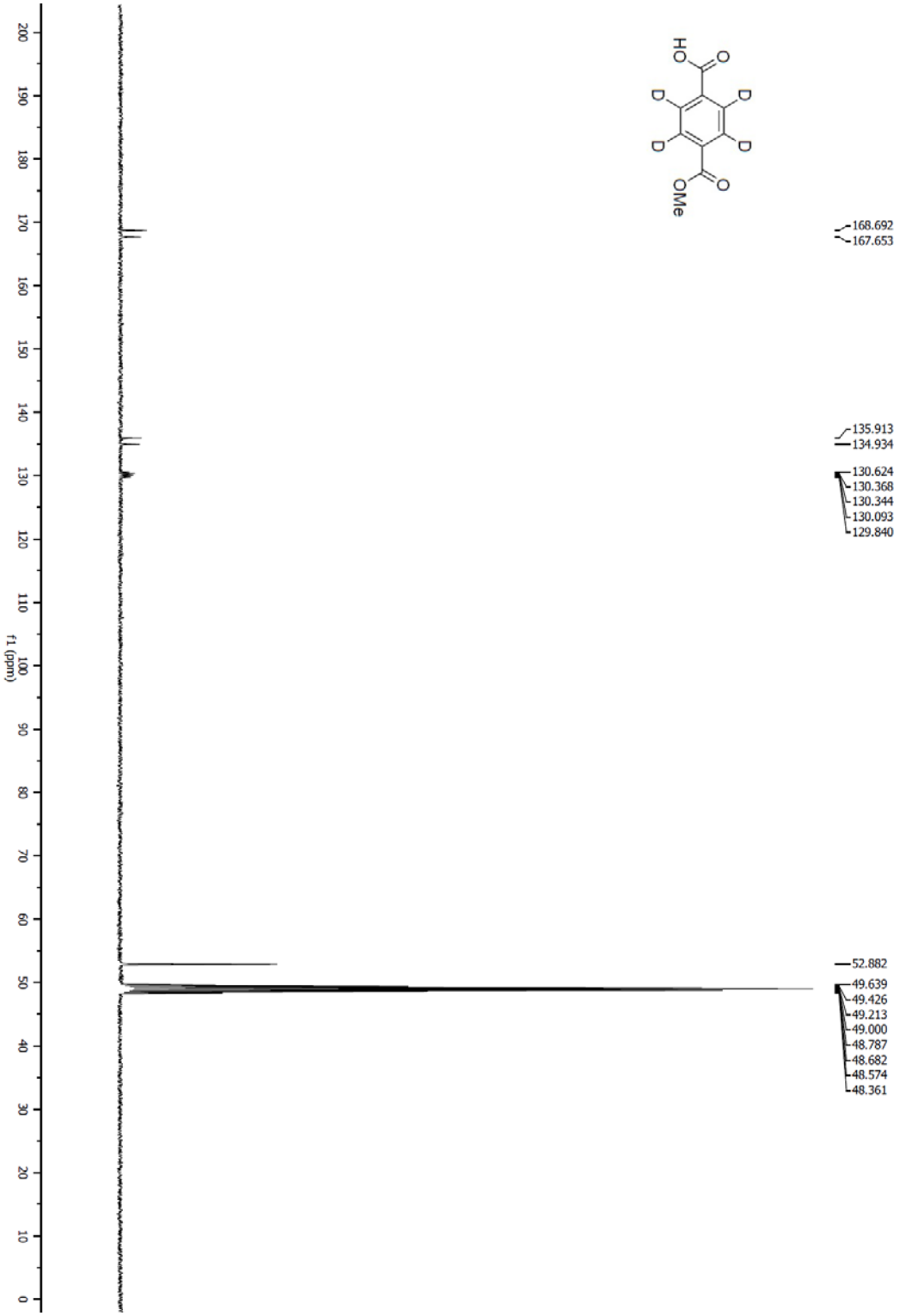
4.883
3.930
3.314
3.310
3.306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O
D
D
D
D
O
OMe
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49.426
49.213
49.000
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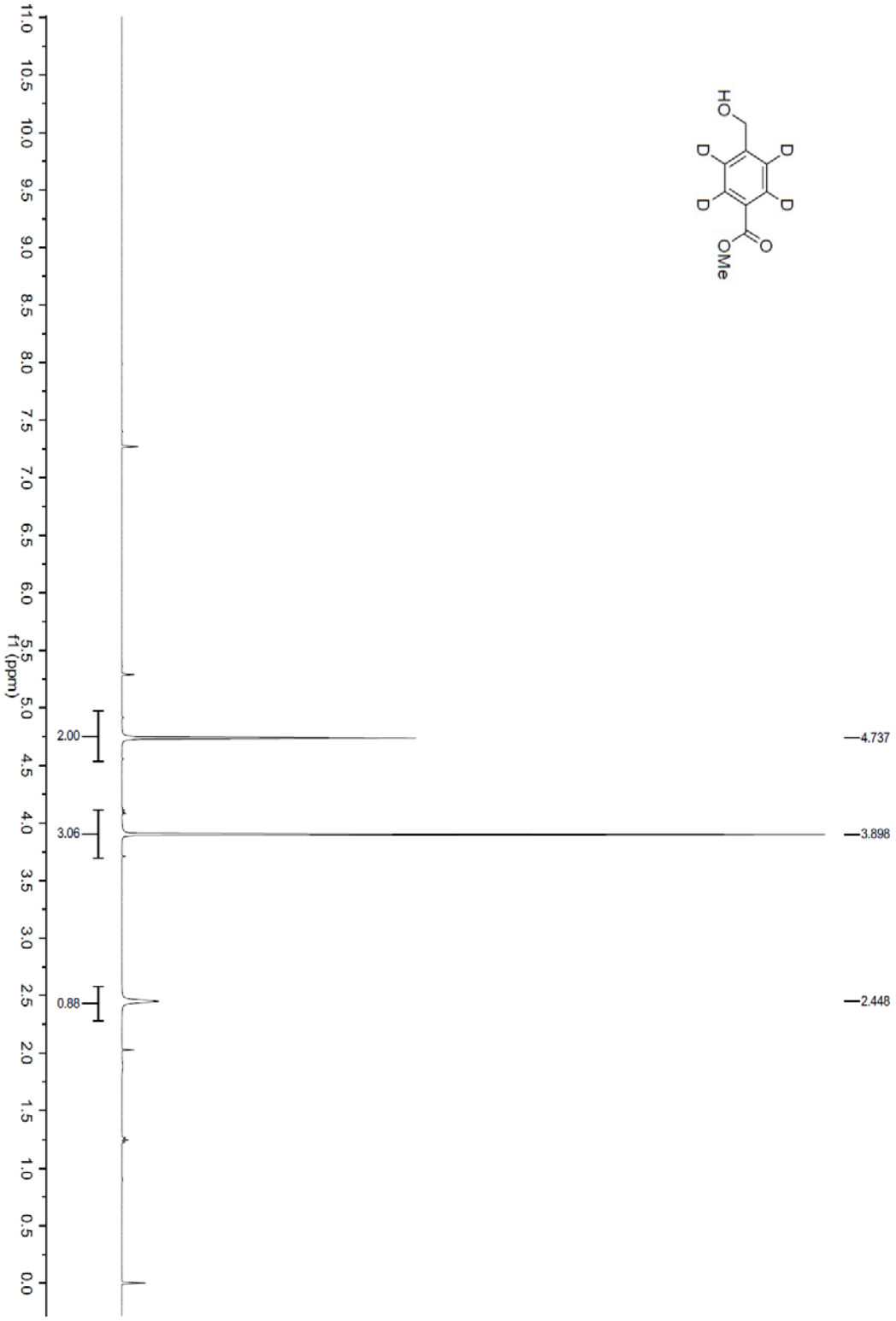
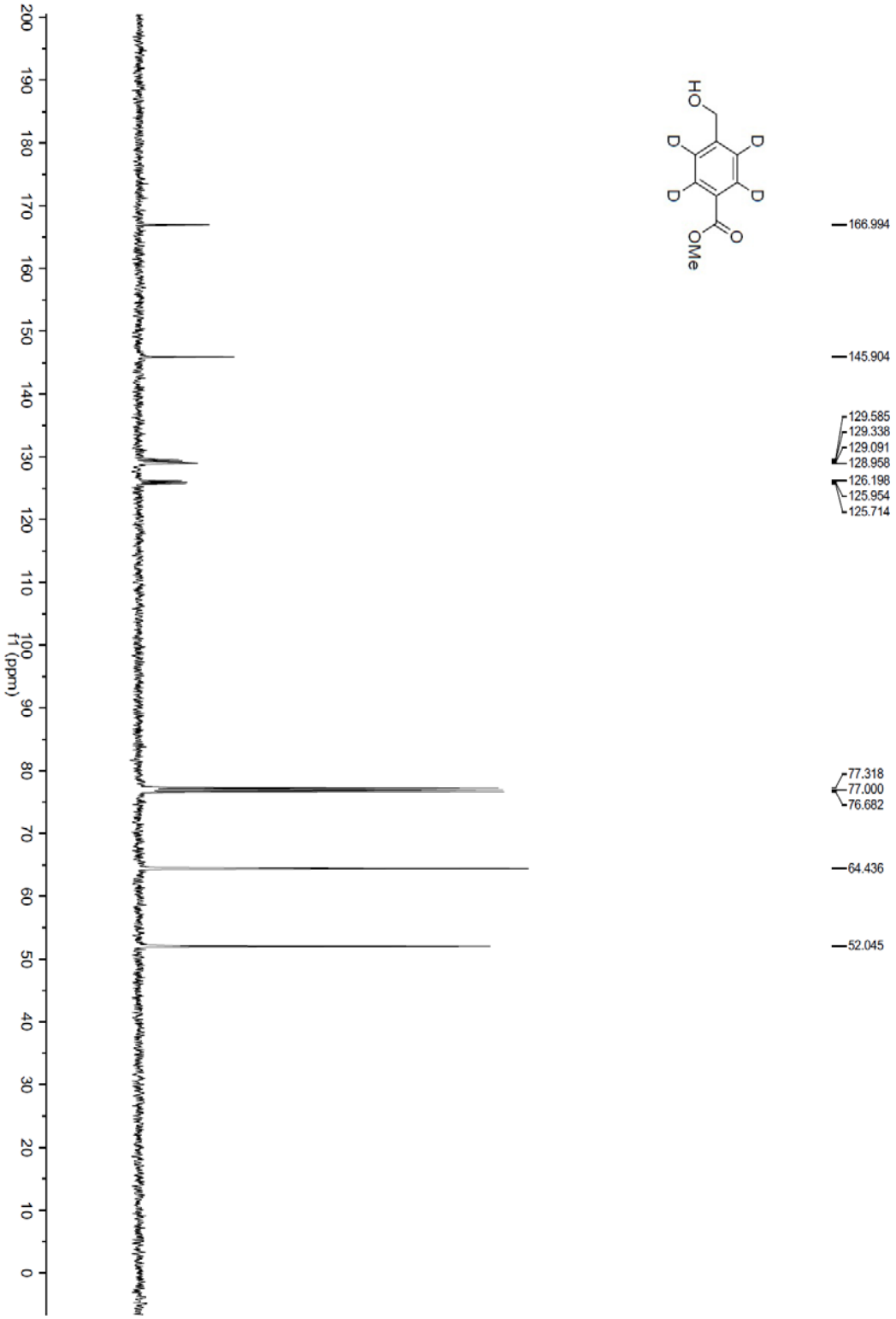
48.682
48.574
48.361
f1 (ppm)

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D
D
D
O
OMe
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125.714
77.318
77.000
76.682
64.436
52.045
f1 (ppm)
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150
140
130
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110
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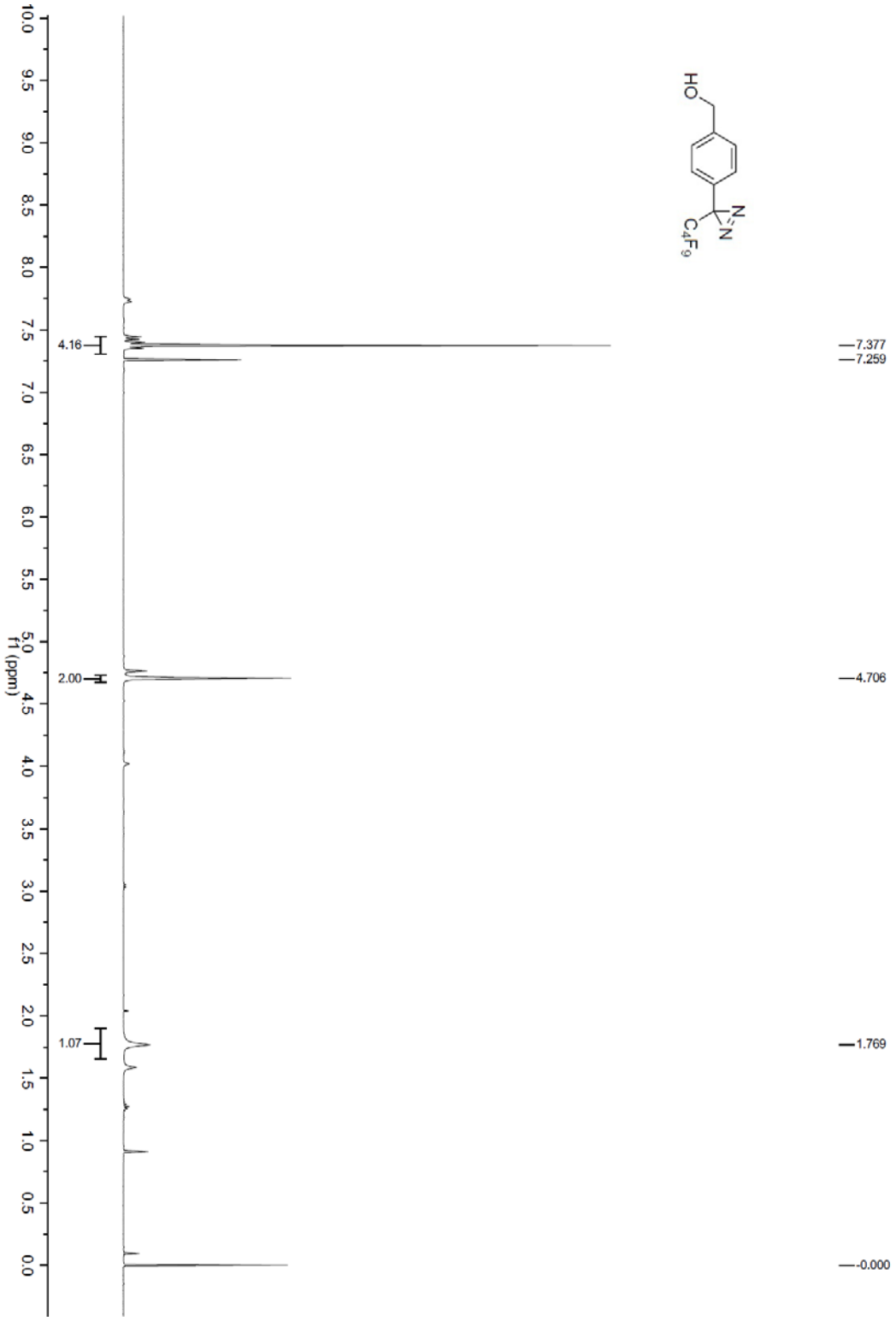
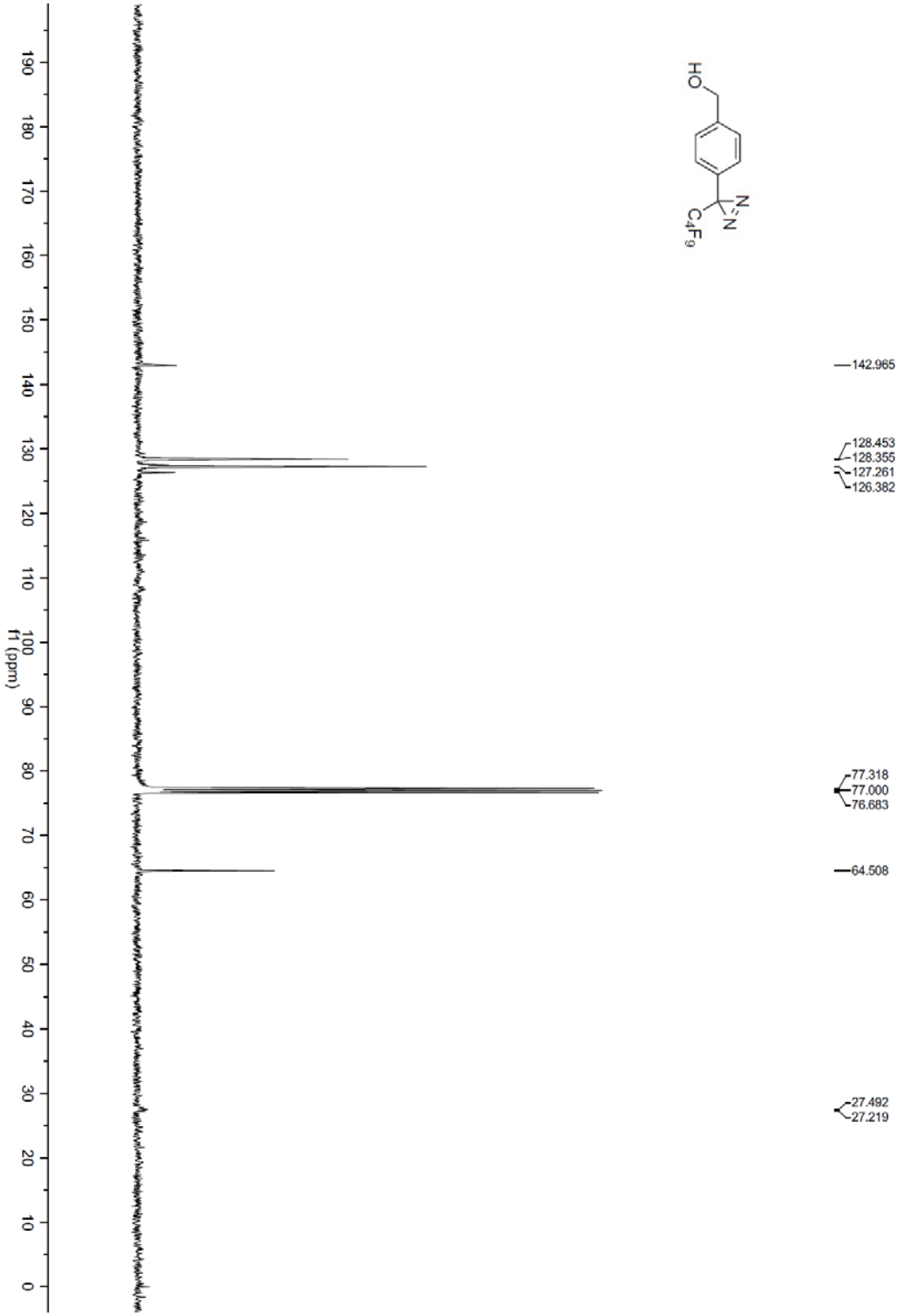
90
80
70
60
50
40
30
20
10
0

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127.261
126.382
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77.000
76.683
64.508
27.492
27.219
f1 (ppm)
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150
140
130
120
110
100
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70
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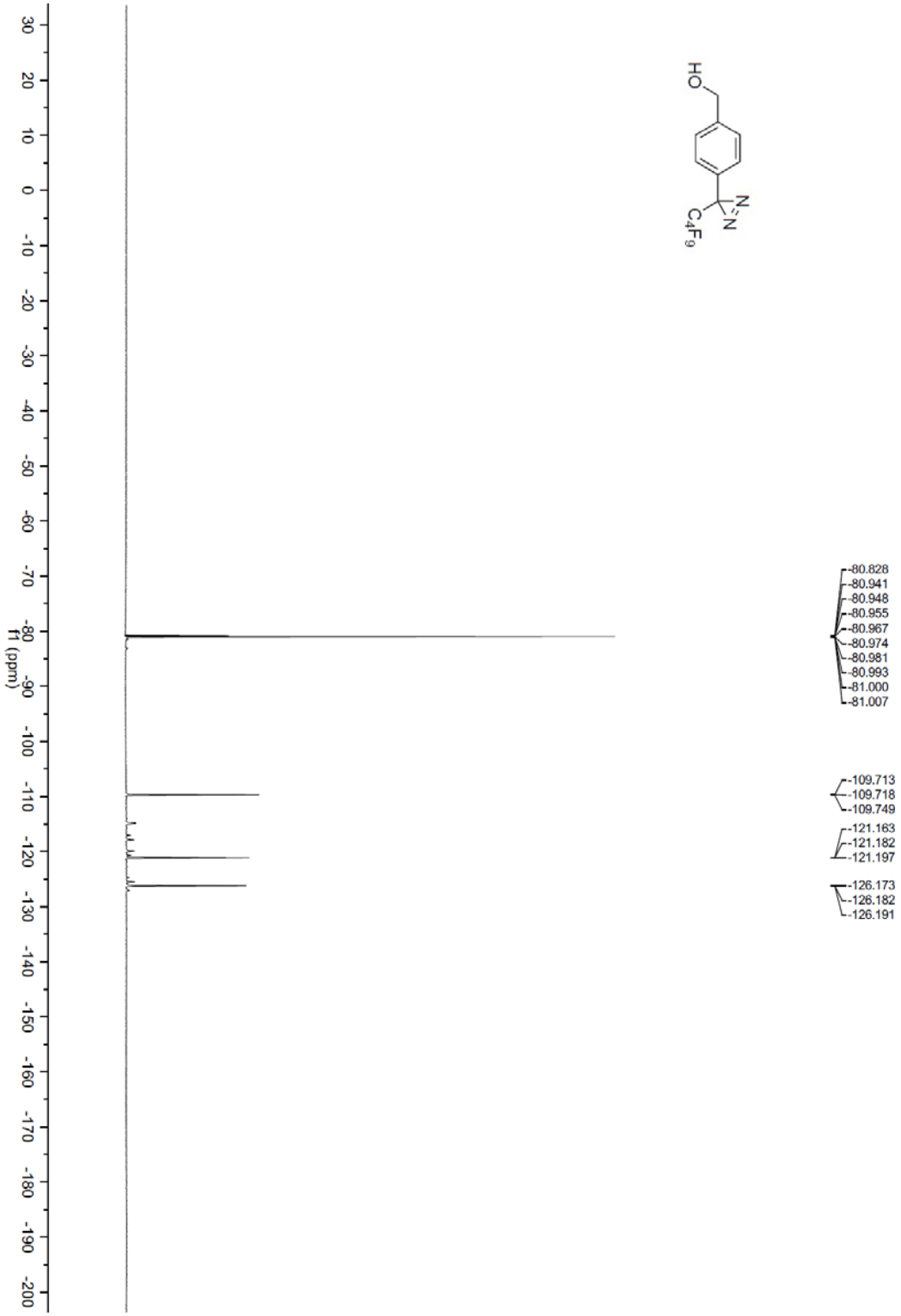
30
20
10
0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N
N
C4F9
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-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
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-80.981
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-81.000
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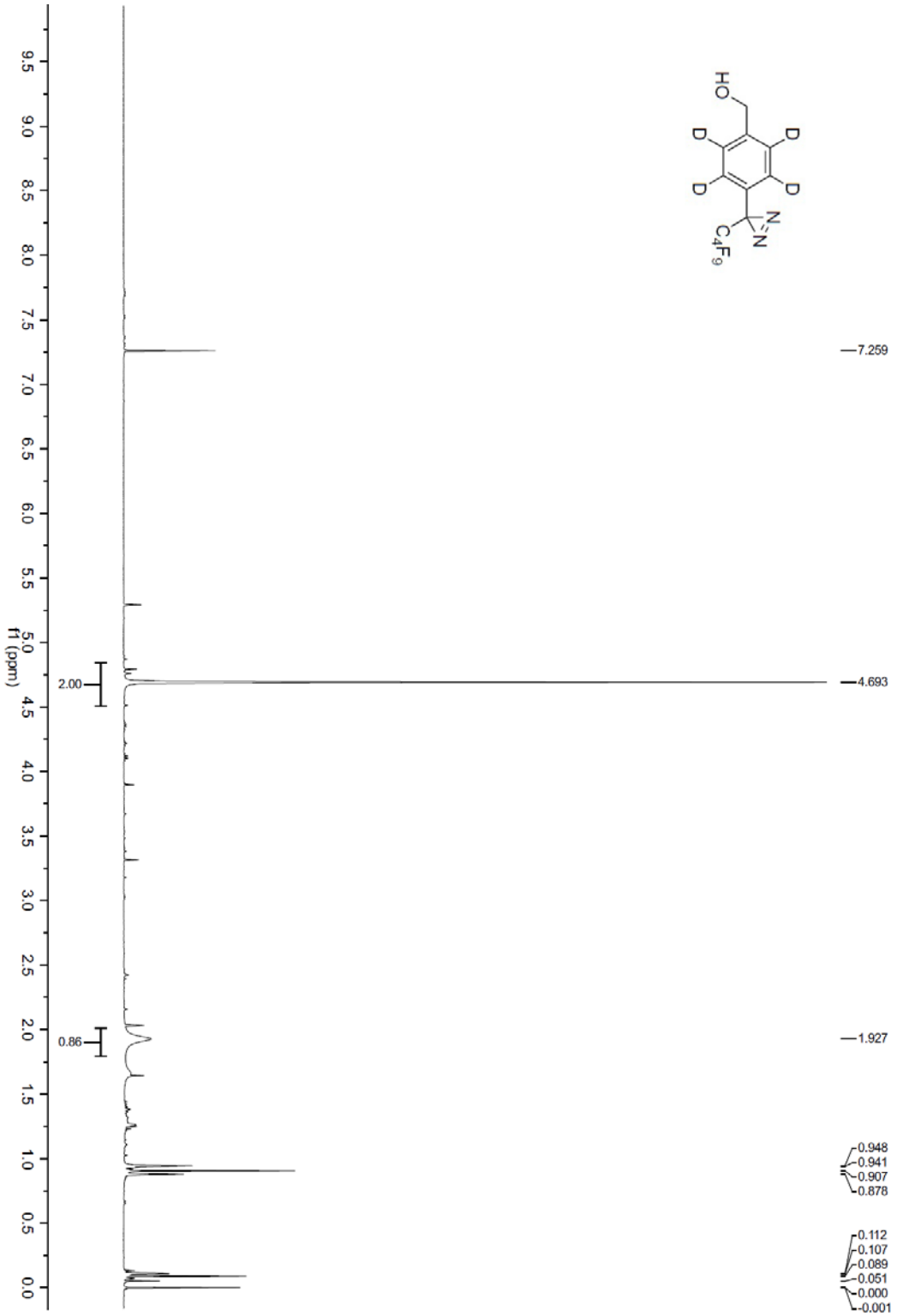
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D
D
D
N
N
C4F9
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6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
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0.0
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4.693
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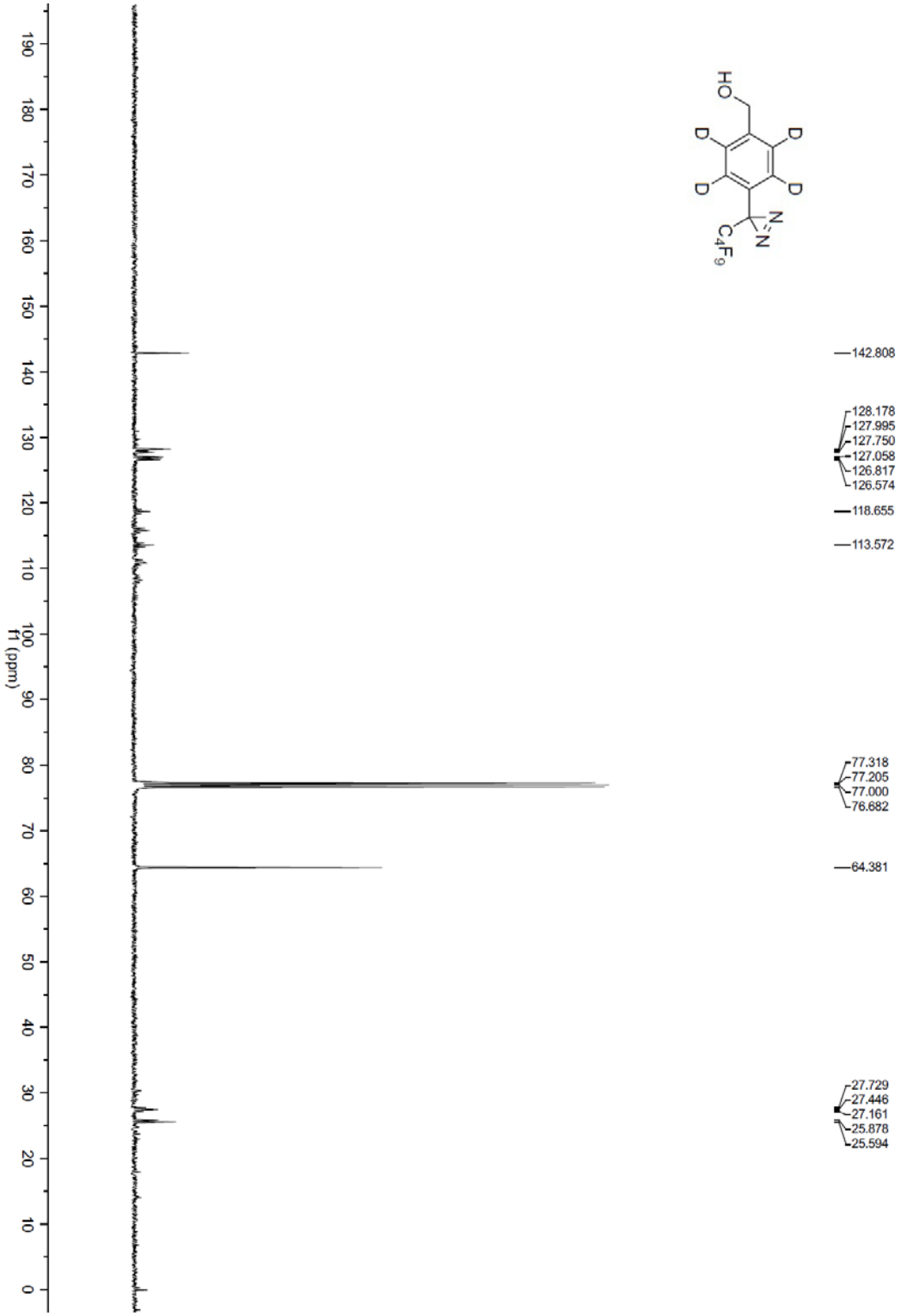
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D
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N
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113.572
77.318
77.205
77.000
76.682
64.381
27.729
27.446
27.161
25.878
25.594
f1 (ppm)
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150
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20
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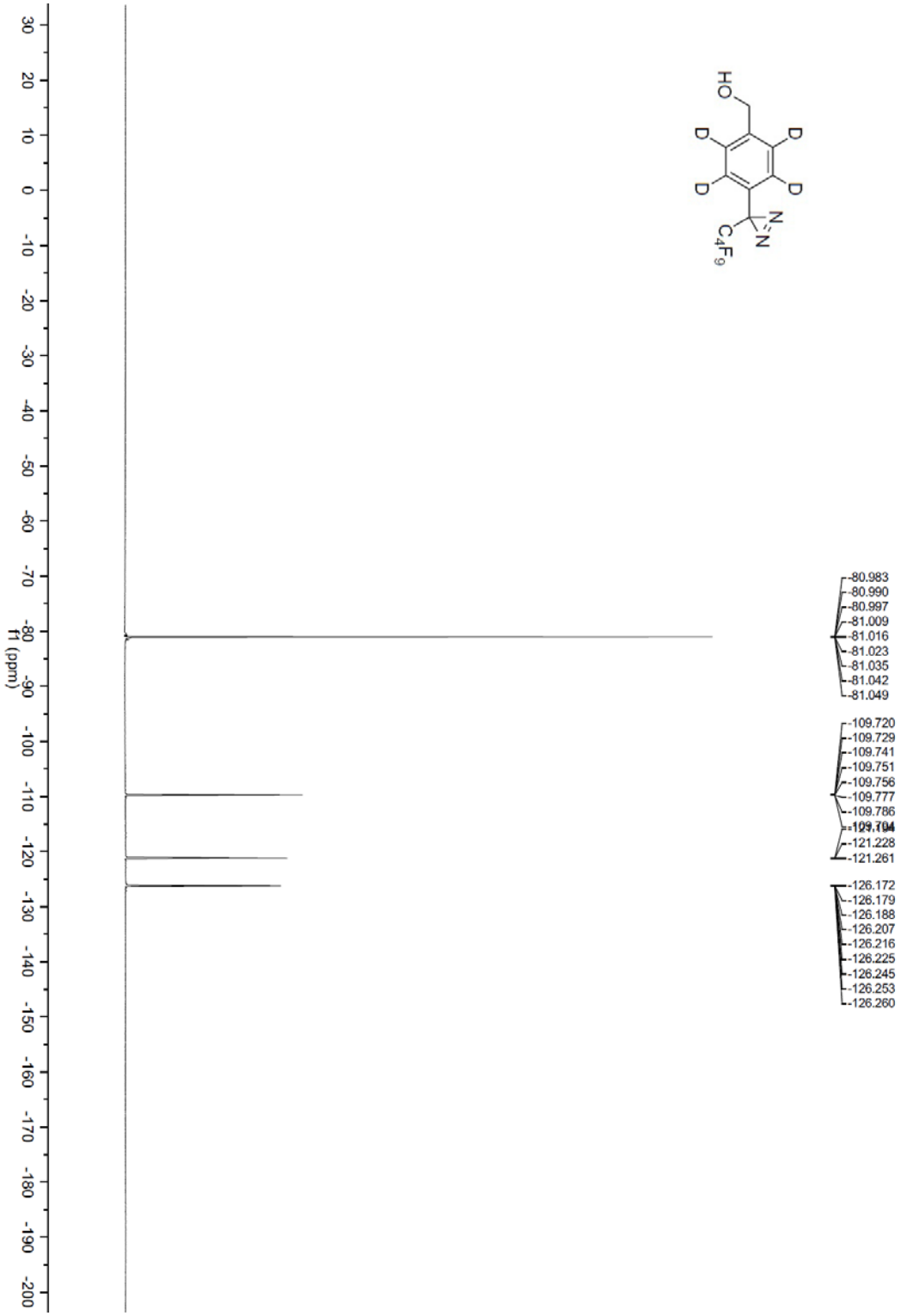
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D
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D
N
N
C4F9
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-10
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-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
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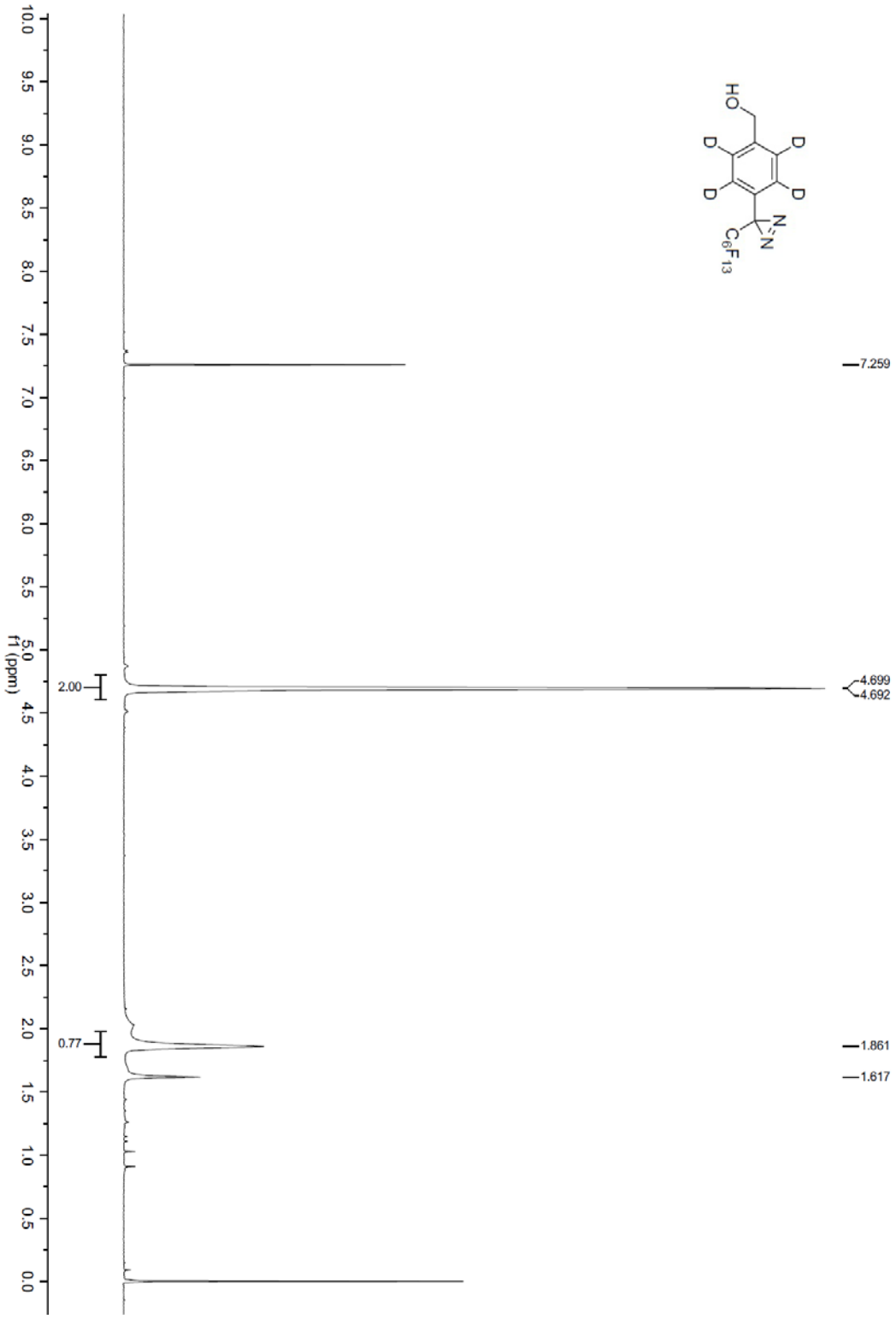
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D
D
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N
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0.77
f1 (ppm)
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9.0
8.5
8.0
7.5
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6.5
6.0
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2.0
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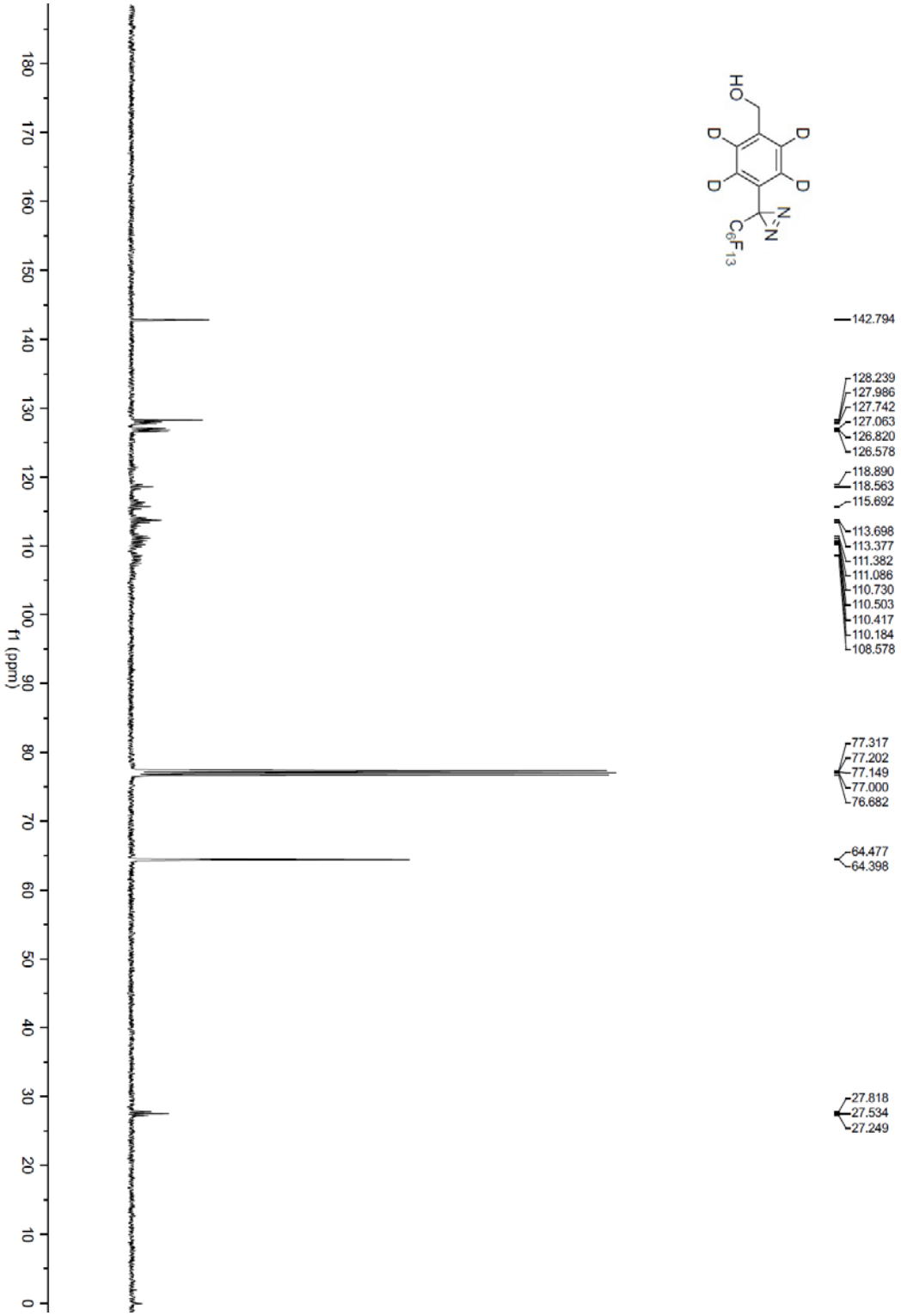
0.0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60
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140
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160
170
180
HO
D
D
D
D
N
N
C6F13
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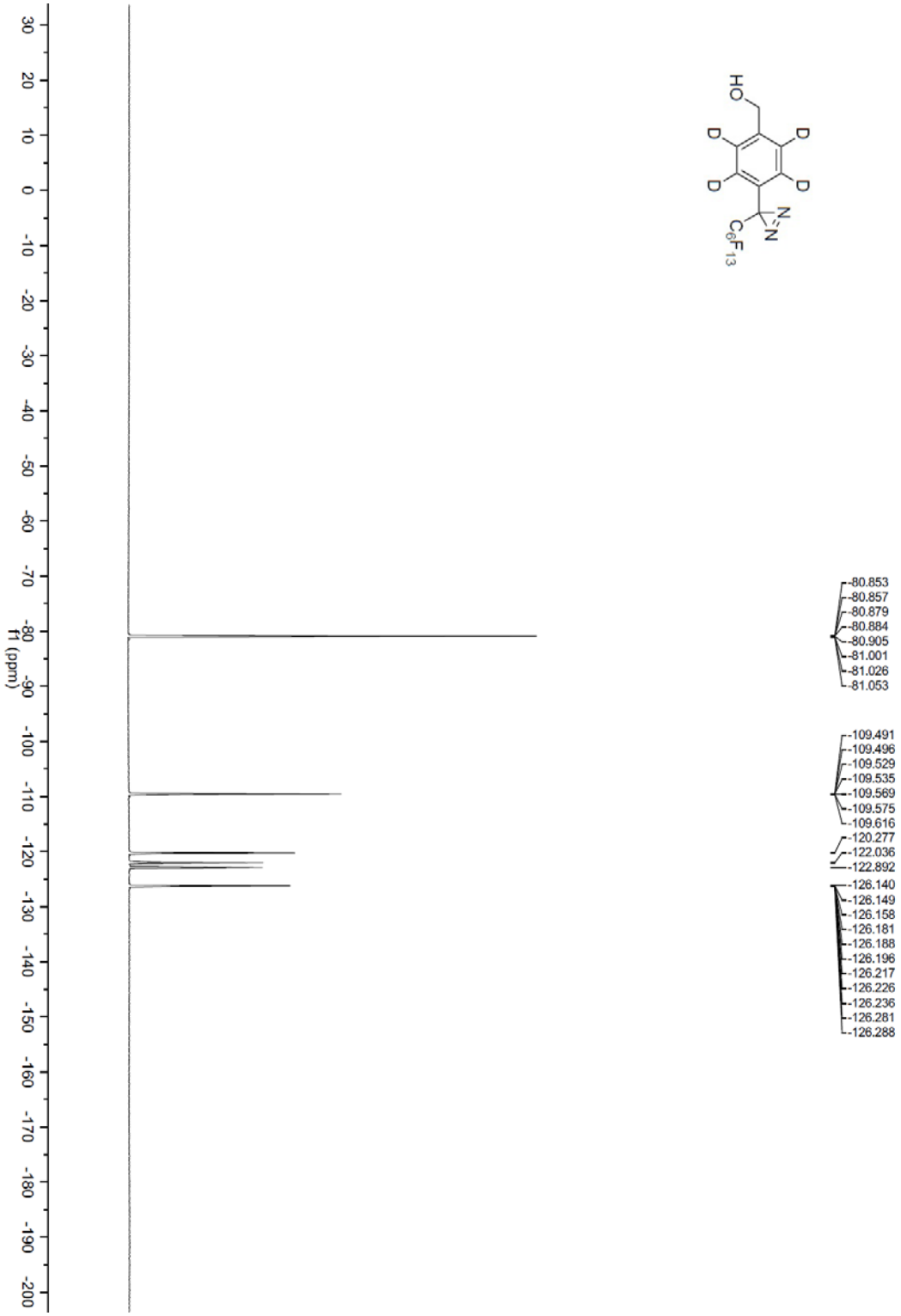
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D
D
D
N
N
C6F13
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-30
-40
-50
-60
-70
-80
-90
-100
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-120
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-140
-150
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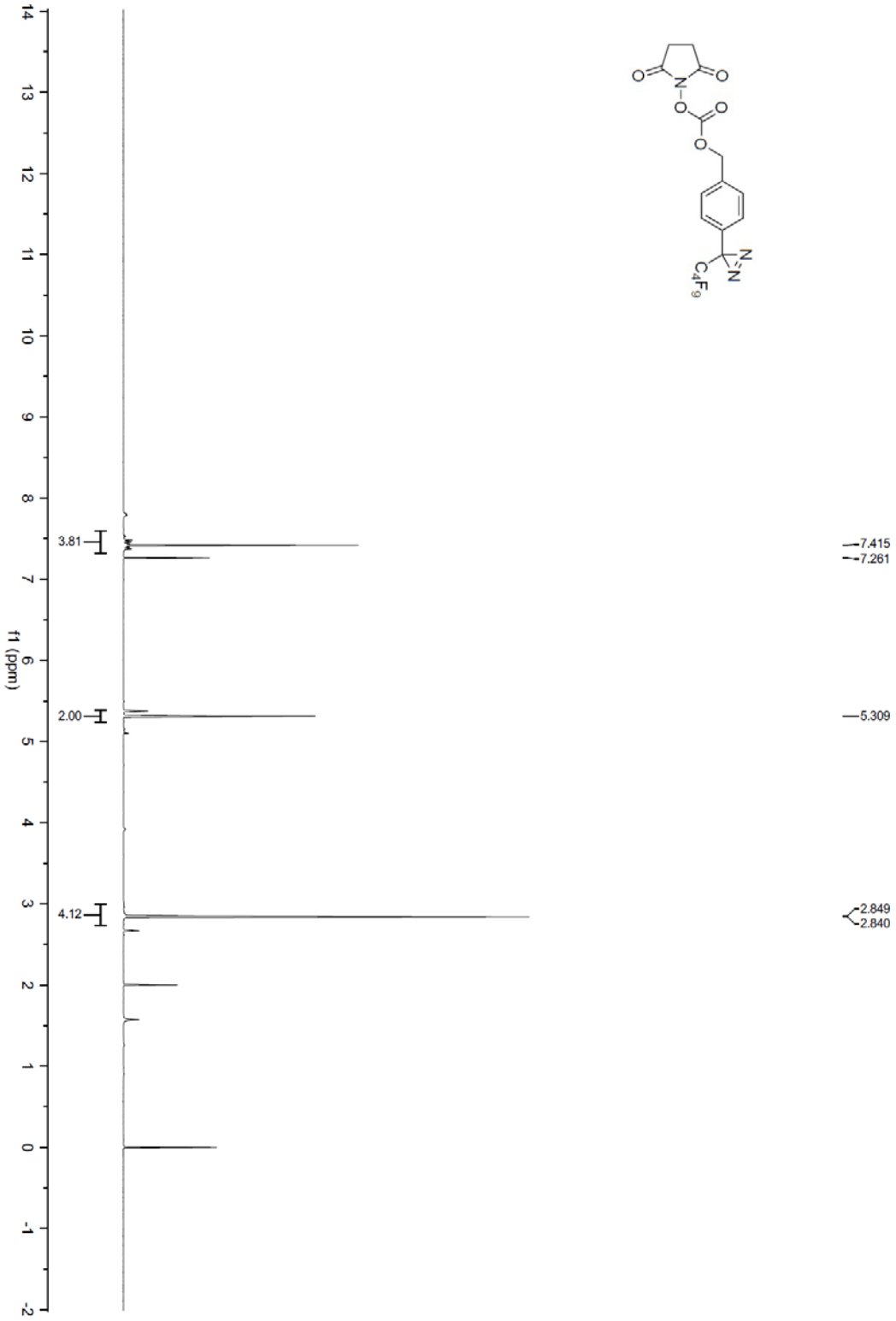
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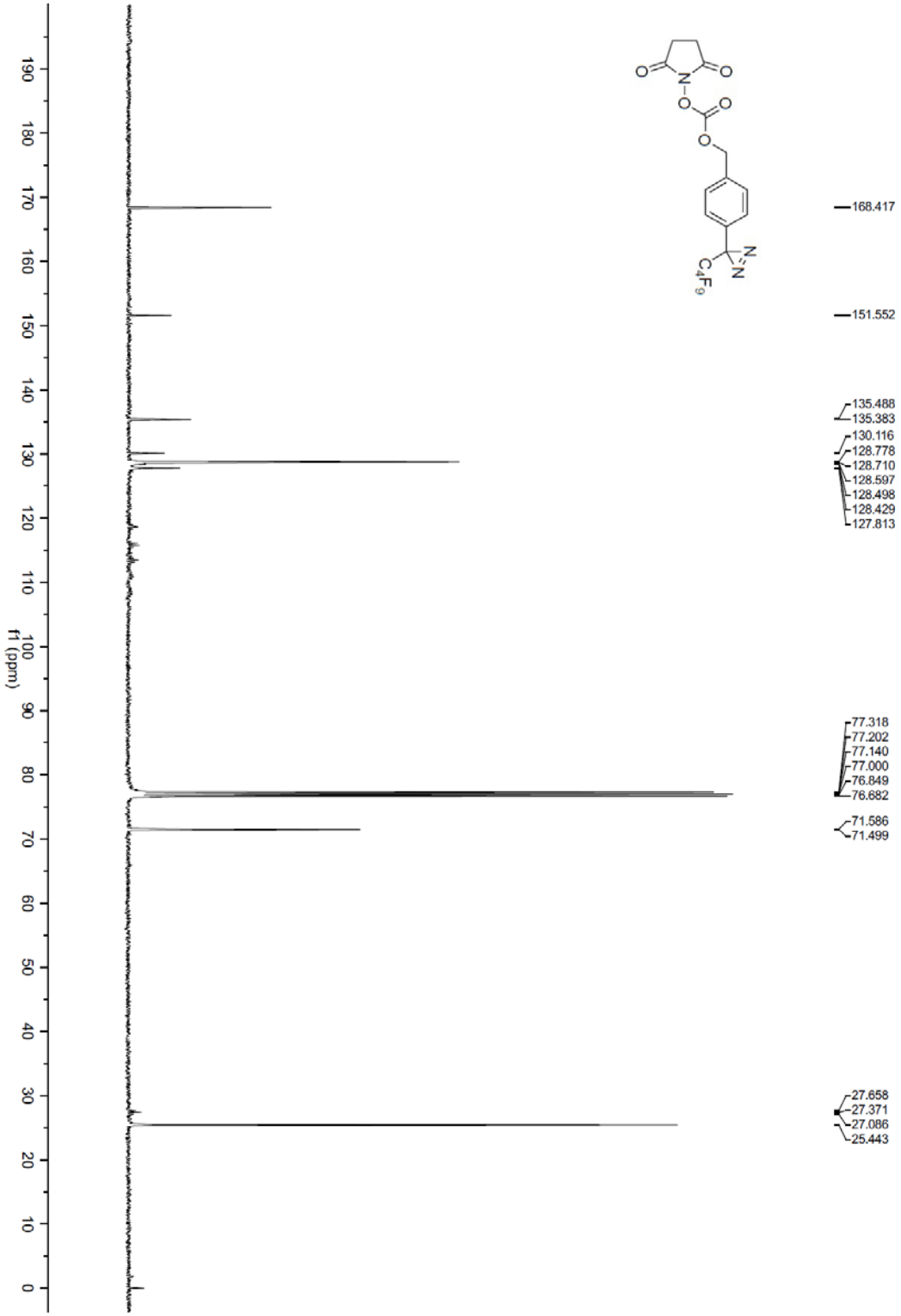
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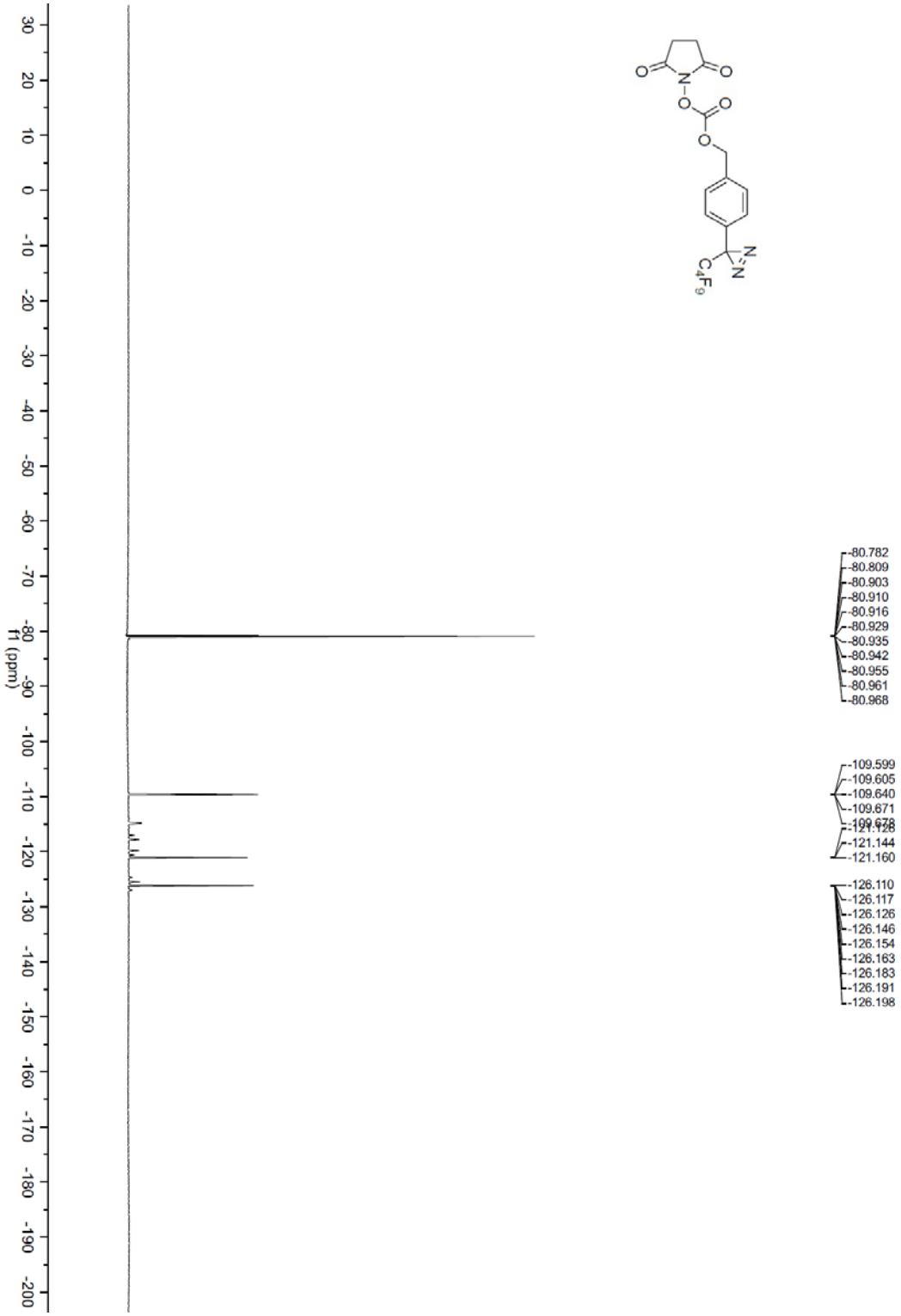
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-130
-140
-150
-160
-170
-180
-190
-200
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-80.910
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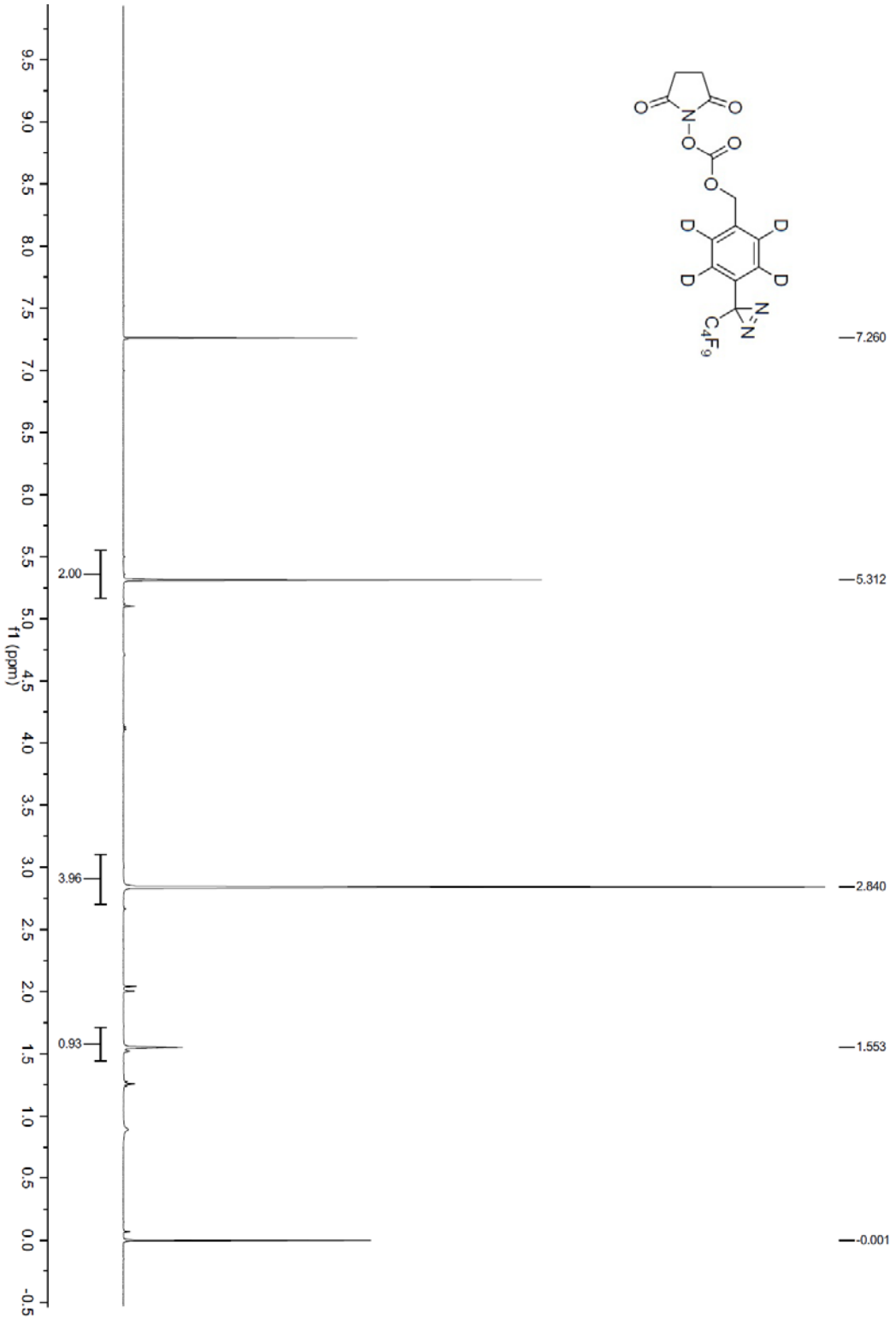
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1.5
1.0
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O
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D
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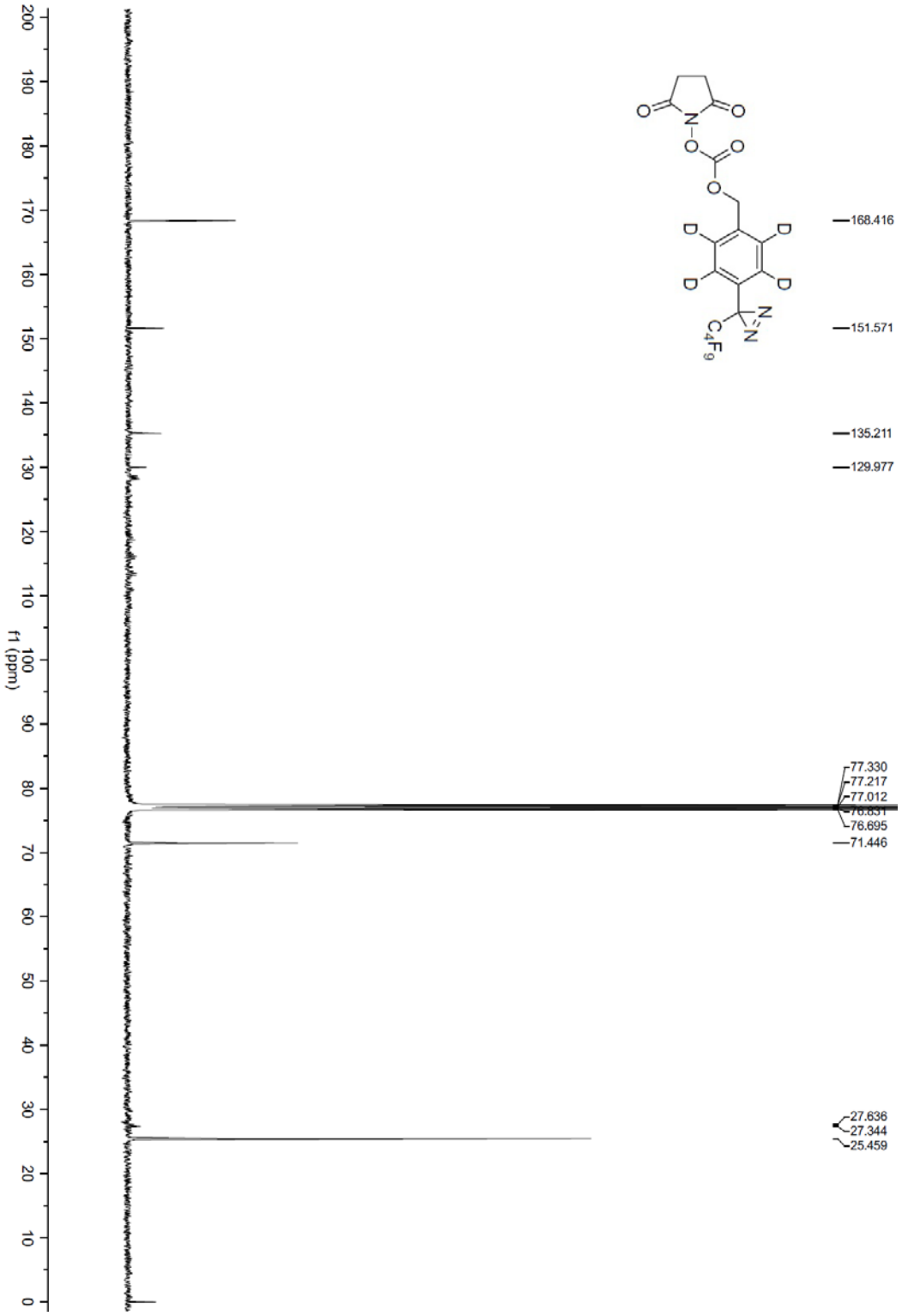
C4F9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30
20
10
0
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151.571
135.211
129.977
77.330
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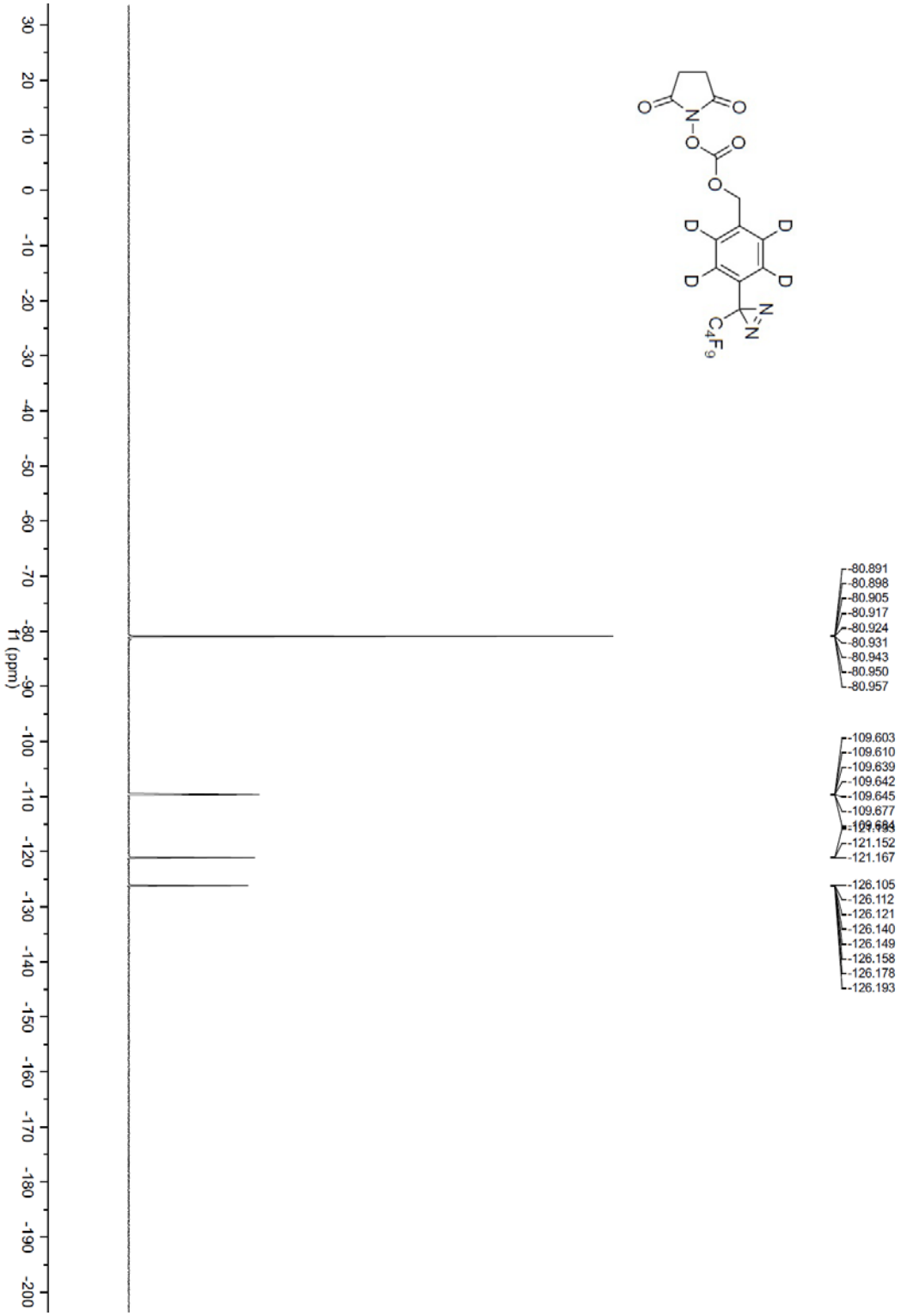
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-120
-130
-140
-150
-160
-170
-180
-190
-200
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O
N
O
O
O
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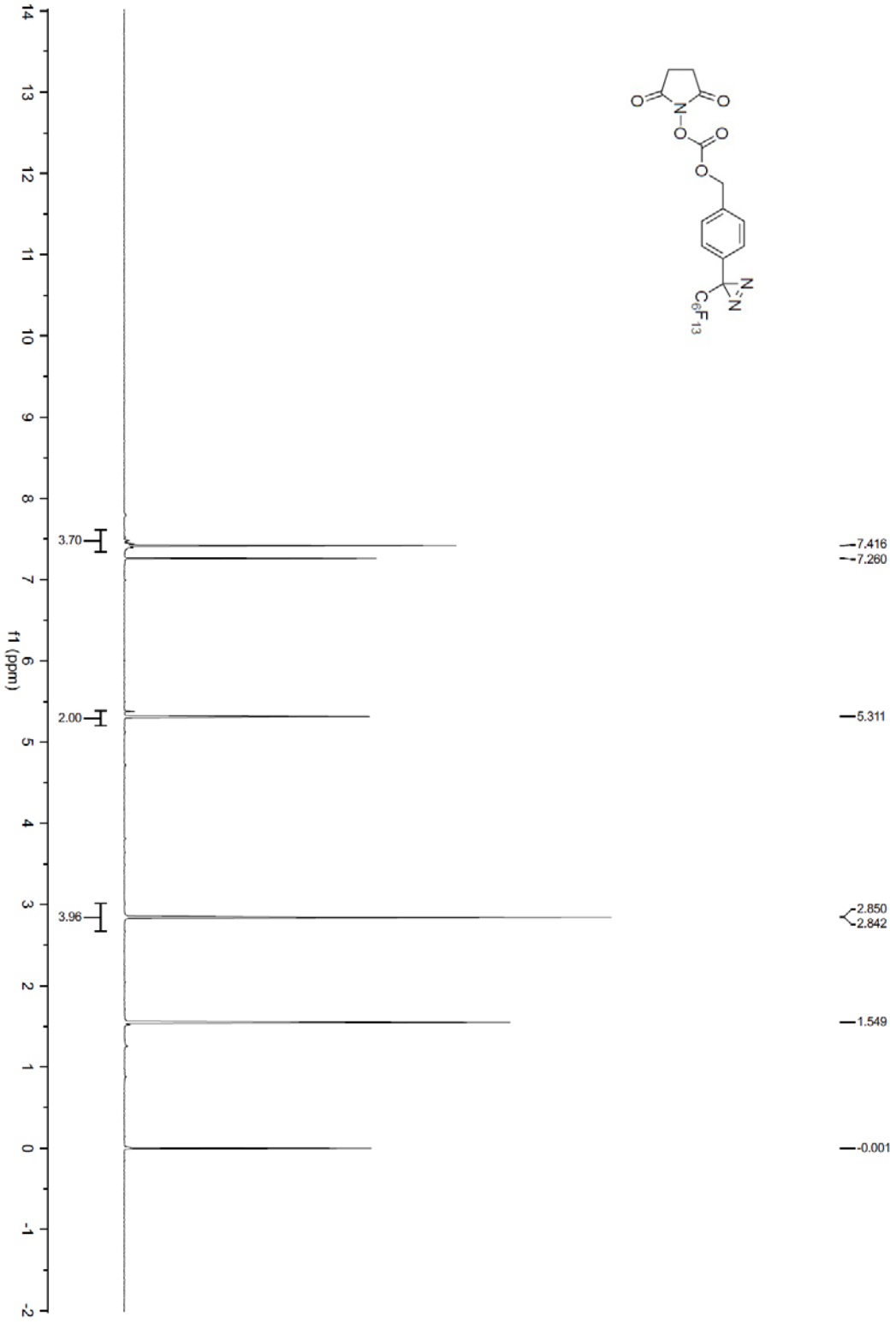
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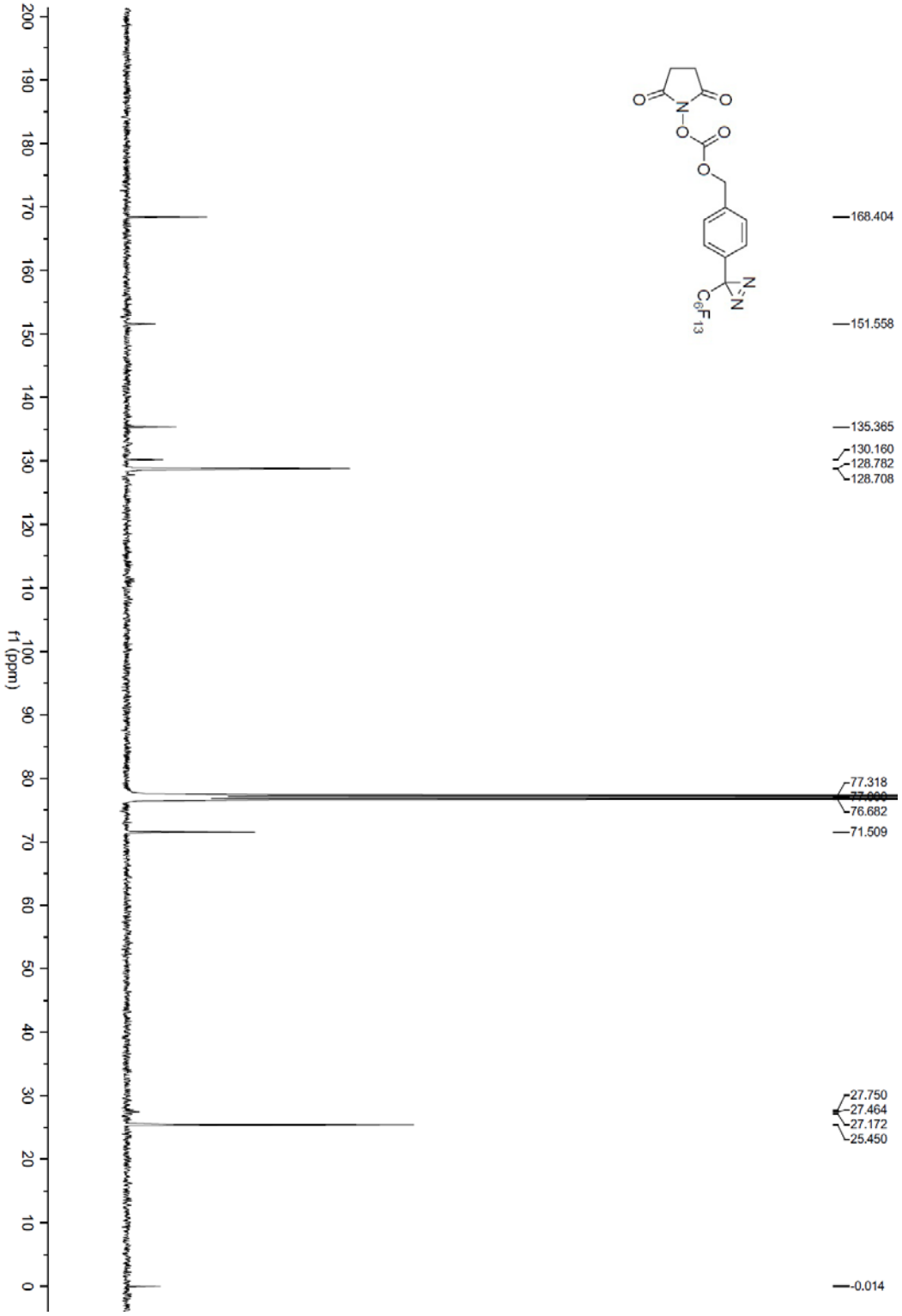
f1 (ppm)
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180
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150
140
130
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100
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30
20
10
0
168.404
151.558
135.365
130.160
128.782
128.708
77.318
76.682
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O
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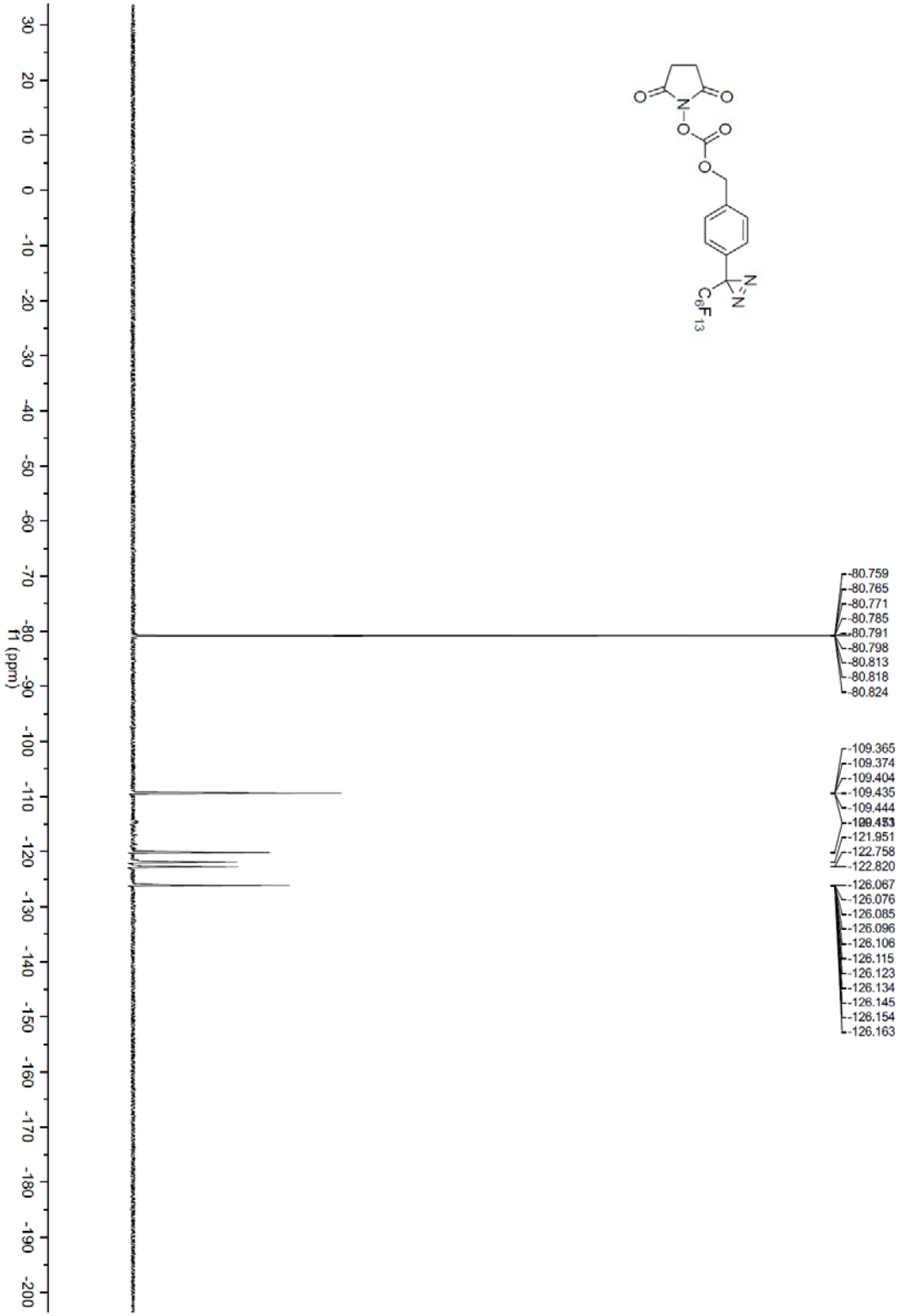
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-10
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-30
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-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
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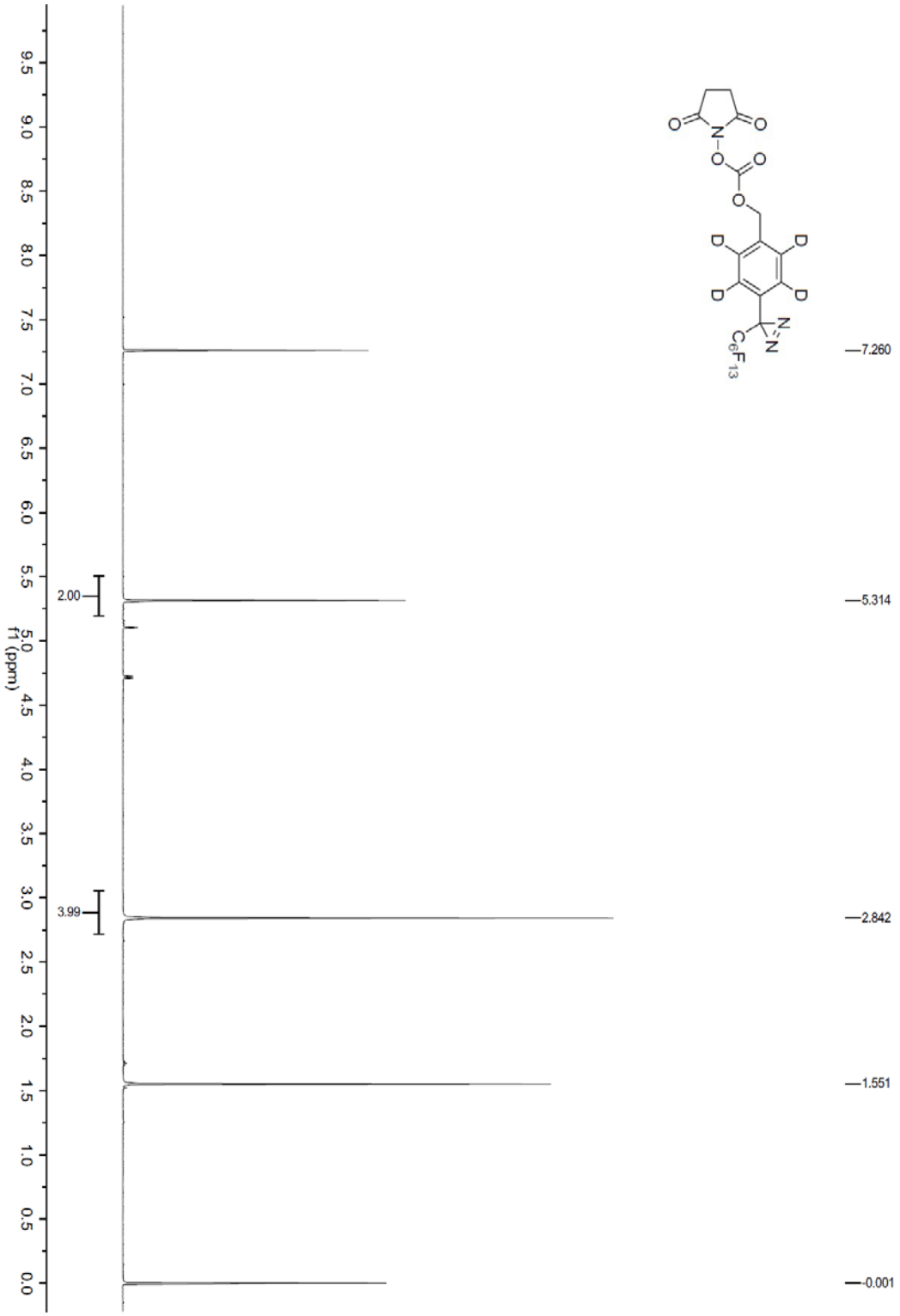
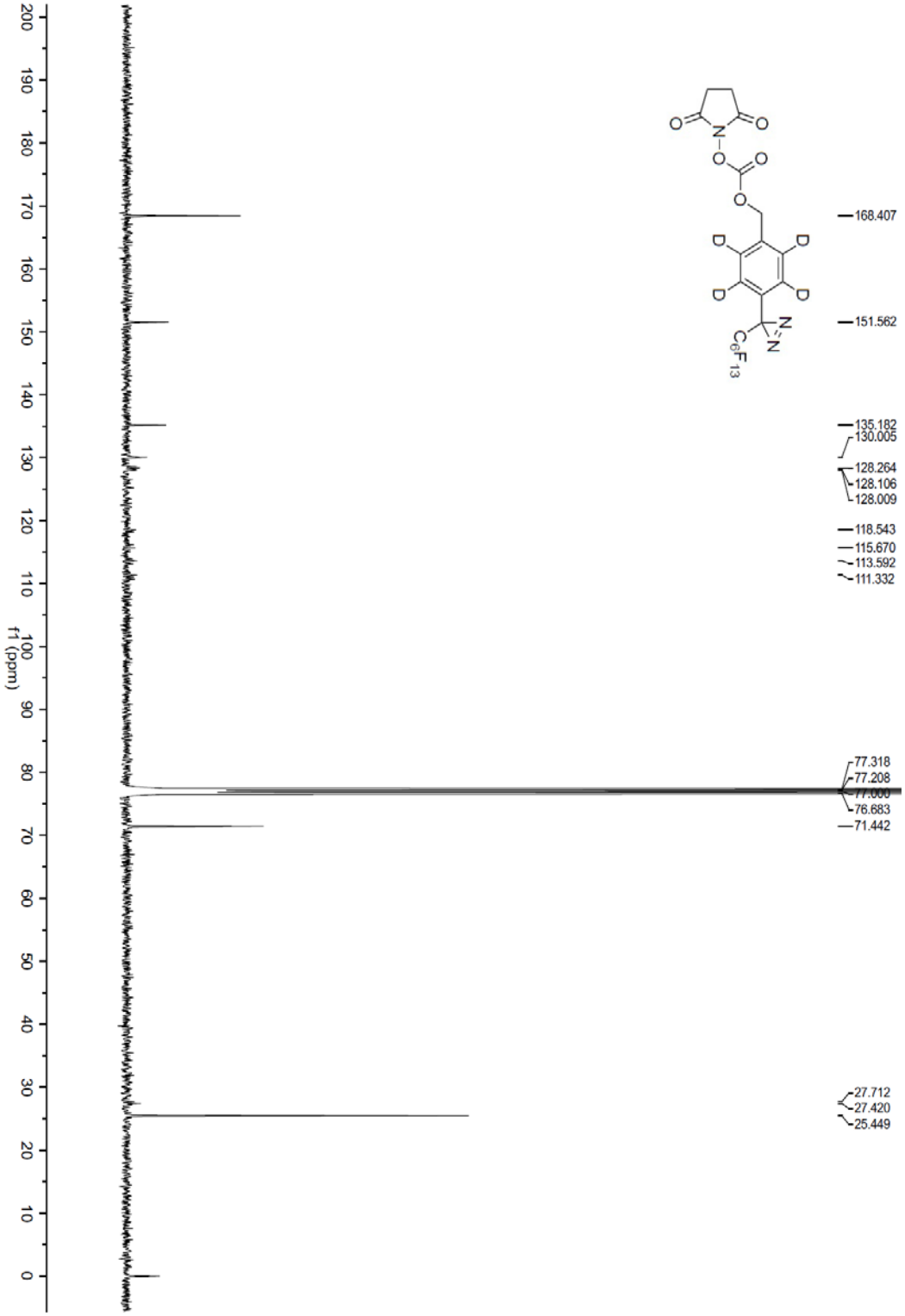
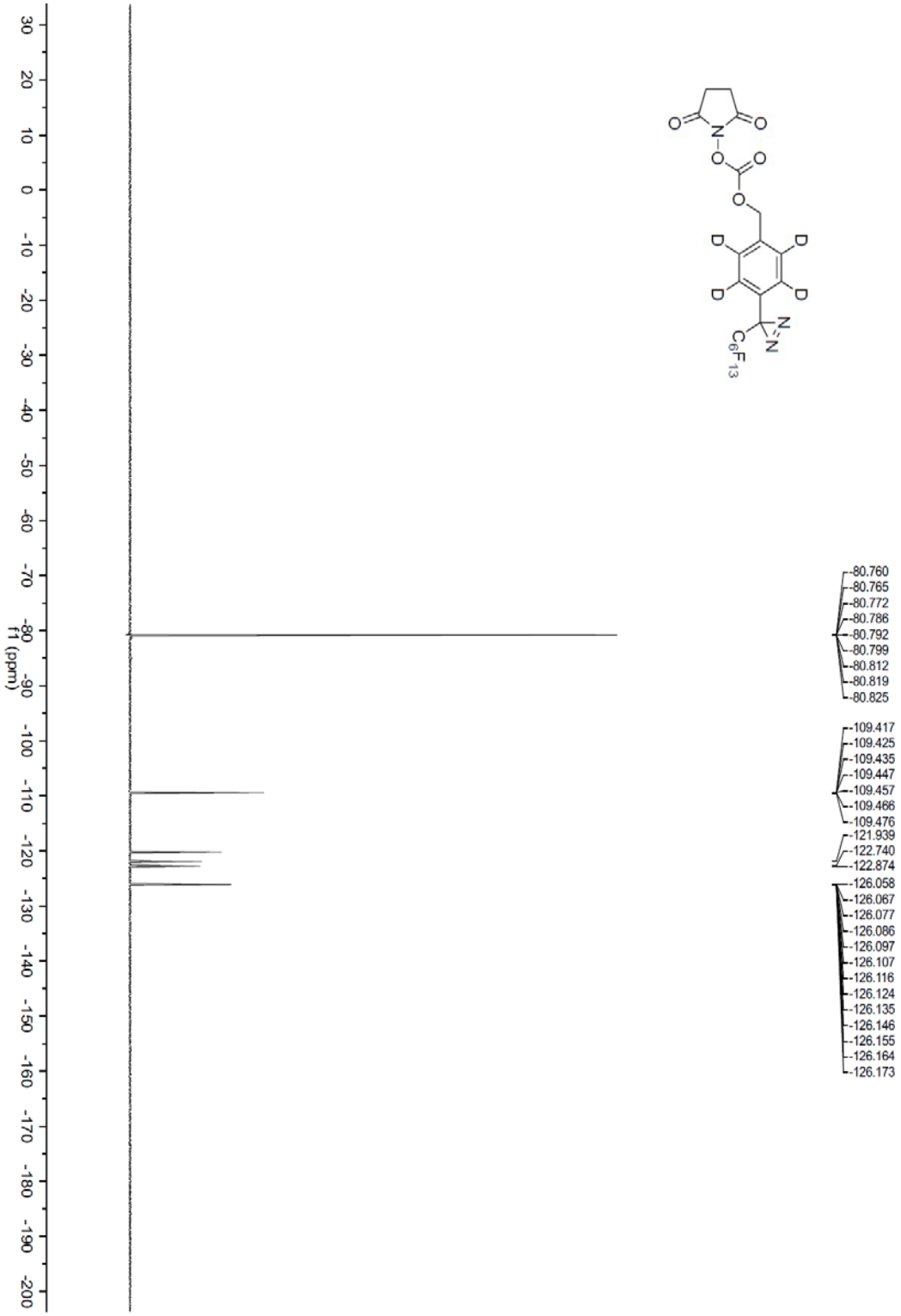
-80.785
-80.791
-80.798
-80.813
-80.818
-80.824
-109.365
-109.374
-109.404
-109.435
-109.444
-121.951
-122.758
-122.820
-126.067
-126.076
-126.085
-126.096
-126.106
-126.115
-126.123
-126.134
-126.145
-126.154
-126.163


f1 (ppm)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
2.00
3.99
7.260
5.314
2.842
1.551
0.001
O
N
O
O
O
O
D
D
D
D
N
N
C6F13


f1 (ppm)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
O
N
O
O
O
O
D
D
D
D
N
N
C6F13
168.407
151.562
135.182
130.005
128.264
128.106
128.009
118.543
115.670
113.592
111.332
77.318
77.208
77.000
76.683
71.442
27.712
27.420
25.449


f1 (ppm)
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
O
O
N
O
O
O
D
D
D
D
N
N
C6F13
-80.760
-80.765
-80.772
-80.786
-80.792
-80.799
-80.812
-80.819
-80.825
-109.417
-109.425
-109.435
-109.447
-109.457
-109.466
-109.476
-121.939
-122.740
-122.874
-126.058
-126.067
-126.077
-126.086
-126.097
-126.107
-126.116
-126.124
-126.135
-126.146
-126.155
-126.164
-126.173