Supporting Information

(2*S*,4*R*)- and (2*S*,4*S*)-Perfluoro-*tert*-butyl 4-Hydroxyproline: Two Conformationally Distinct Proline Amino Acids for Sensitive Application in ^{19}F NMR

Caitlin M. Tressler and Neal J. Zondlo*
Department of Chemistry and Biochemistry
University of Delaware
Newark, DE 19716

Contents

Materials

COMU was purchased from Sigma-Aldrich. Novagel Rink amide resin was purchased from EMD Millipore. Perfluoro-*tert*-butanol was purchased from Matrix Scientific (Columbia, SC). Tetrahydrofuran (THF) was dried prior to use. Thin layer chromatography was conducted using glass-backed plates (silica gel, 250 μm, 60 Å, F254). Flash chromatography was performed using 230-400 mesh (32-63 μm, 60 Å) silica gel. NMR spectra were recorded on a 400 MHz NMR spectrometer equipped with a cryogenic probe or a 600 MHz NMR spectrometer equipped with a 5-mm SMART probe. Peptides were characterized by ESI-MS in positive ion mode.

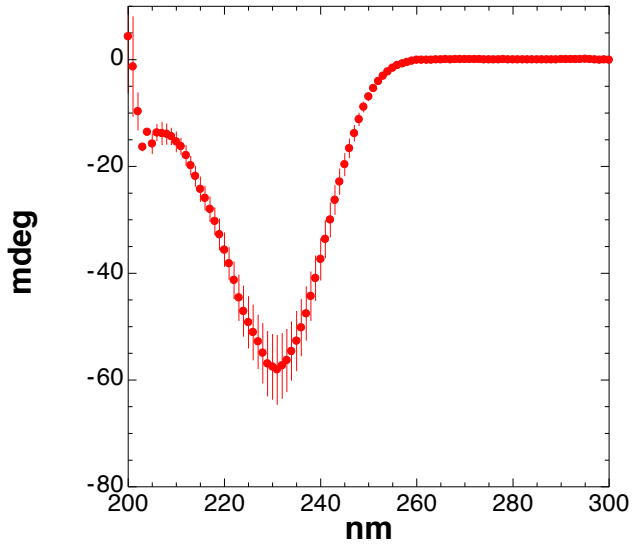


Figure S1. CD spectrum of compound **4**, Boc-(2*S*,4*R*)-perfluoro-*tert*-butyl-4-hydroxyproline methyl ester. Error bars indicate standard error.

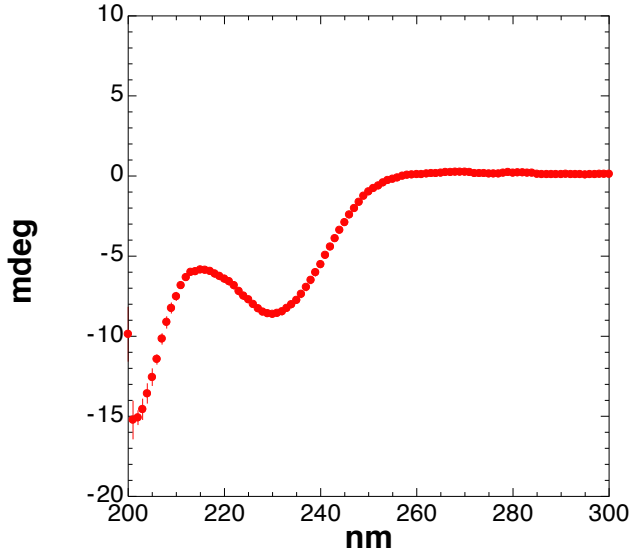


Figure S2. CD spectrum of compound **5**, Boc-(2*S*,4*R*)-perfluoro-*tert*-butyl-4-hydroxyproline. Error bars indicate standard error.

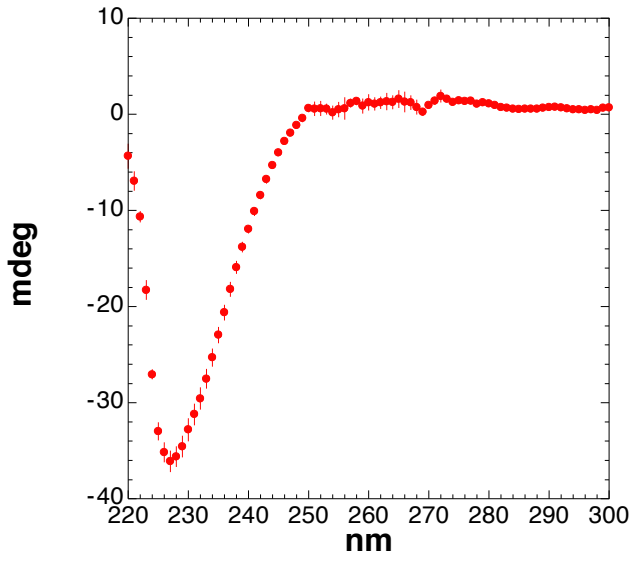


Figure S3. CD spectrum of compound **7**, Fmoc-(2*S*,4*R*)-perfluoro-*tert*-butyl-4-hydroxyproline. Error bars indicate standard error. Data below 220 nm are not reported due to high dynode voltage.

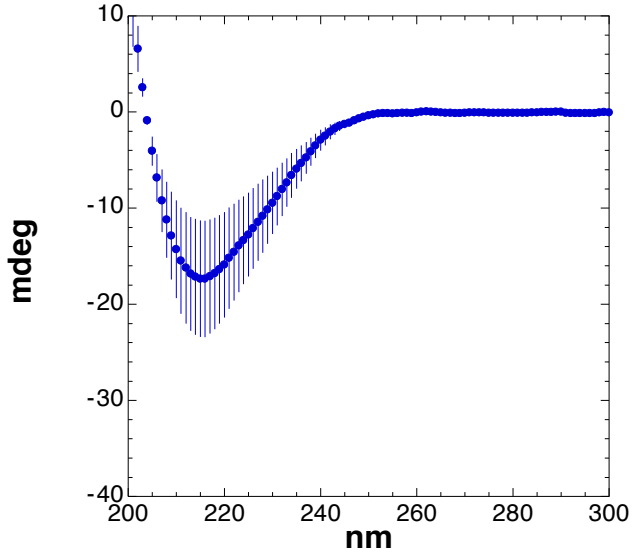


Figure S4. CD spectrum of compound **8**, Boc-(2*S*,4*S*)-perfluoro-*tert*-butyl-4-hydroxyproline methyl ester. Error bars indicate standard error.

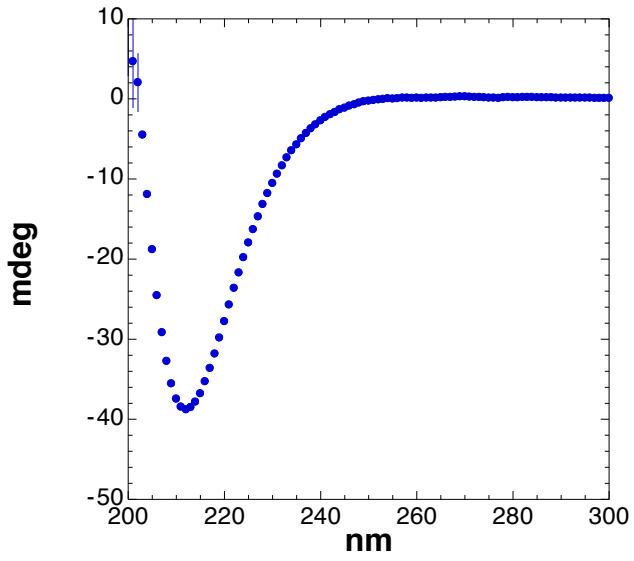


Figure S5. CD spectrum of compound **9**, Boc-(2*S*,4*S*)-perfluoro-*tert*-butyl-4-hydroxyproline. Error bars indicate standard error.

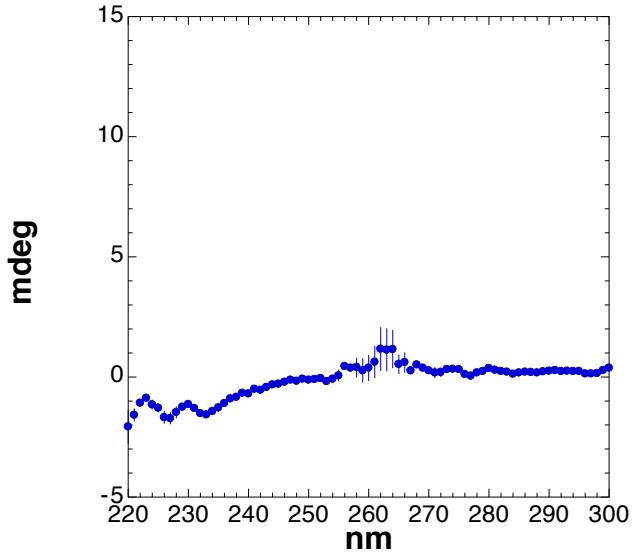


Figure S6: CD spectrum of compound **10**, Fmoc-(2*S*,4*S*)-perfluoro-*tert*-butyl-4-hydroxyproline. Error bars indicate standard error. Data below 220 nm are not reported due to high dynode voltage.

Circular Dichroism

All data were collected using a 0.1 cm cell. All data represent at least three independent trials. Data were background corrected but were not smoothed. Error bars indicate standard error.

Small molecule CD experiments were performed in methanol. All small molecule CD data were collected at 25 °C. Data for compounds **4** and **8** were collected using 2.31 mM compound. Data for compounds **5** and **9** were collected using 2.24 mM compound. Data for compounds **7** and **11** were collected using 1.75 mM compound.

NMR Characterization of Peptides

Peptide NMR experiments were performed at 298 K in 90% H_2O/10% D_2O with 5 mM phosphate buffer (pH 4) and 25 mM NaCl. ^{19}F NMR experiments were performed on a 600 MHz (^{19}F 564.5 MHz) NMR spectrometer equipped with a 5-mm SMART probe. Residual TFA was used as a standard for ^{19}F NMR. ^{19}F peptide NMR spectra were collected using a 20 ppm sweep width without decoupling and a 1.5 second relaxation delay. ^{1}H 1-D and TOCSY NMR specta were performed on a 600 MHz NMR spectrometer equipped with a triple resonance cryoprobe. TSP was used as a standard for ^{1}H NMR. All 1-D NMR spectra were collected with a w5 watergate pulse sequence and a 2 to 3 second relaxation delay. TOCSY spectra were collected using a watergate TOCSY pulse sequence.

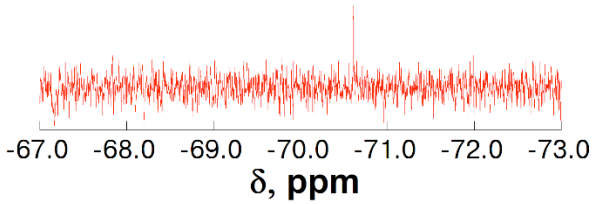


Figure S7. ^{19}F NMR spectrum of 200 nM Ac-((**2*S*,4*R*)-Hyp(C_4F_9)**)KAAAAKAAAAKAAGY-NH_2 in D_2O with 5 mM phosphate buffer (pH 4) and 25 mM NaCl. Data were collected using the NMR parameters indicated above but with a 7 ppm sweep width. Data were collected in 5 minutes and 23 seconds with 128 scans. 7228 data points were collected (acquisition time = 0.91 seconds). Data were processed by zero filling to 8192 points, exponential multiplication, and a line broadening of 2 Hz. The signal to noise ratio was calculated by the SNR peak calculator in MestReNova version 8.

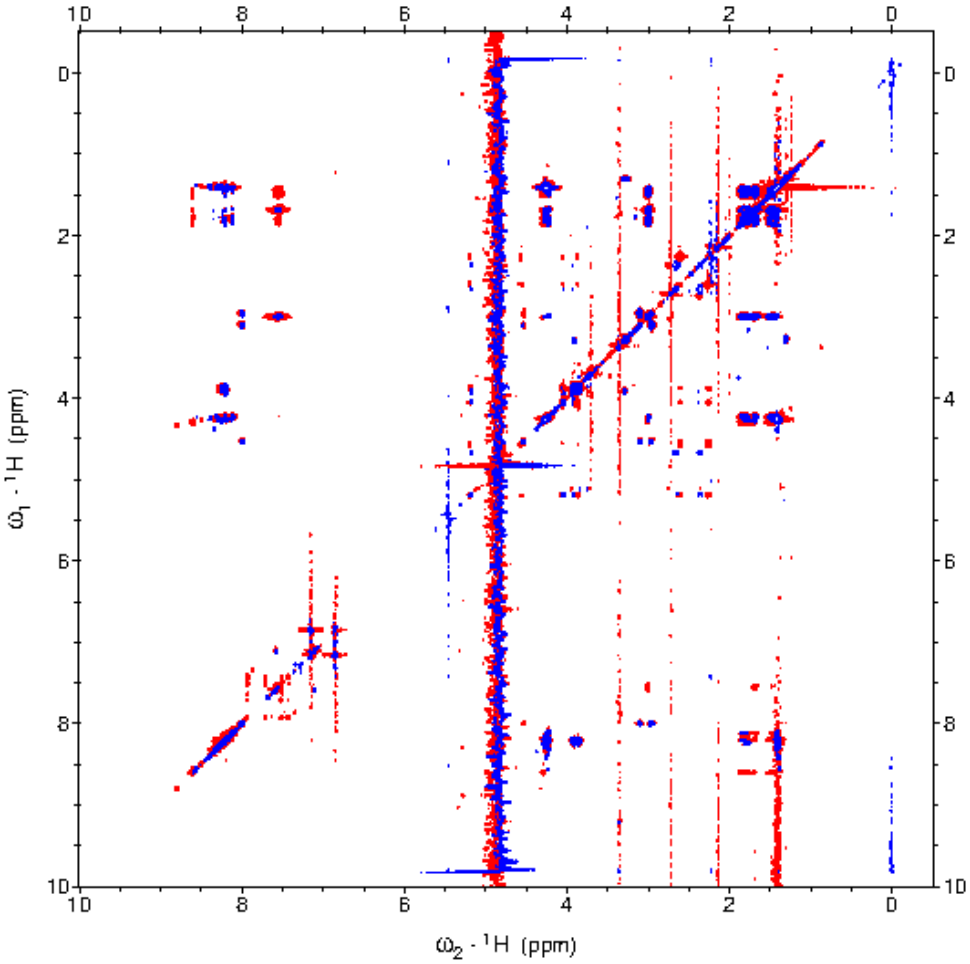


Figure S8. Superposition of the TOCSY spectra of the peptides Ac-((**2*S*,4*R*)-Hyp(C_4F_9)**)KAAAAKAAAAKAAGY-NH_2 (red) and Ac-((**2*S*,4*S*)-hyp(C_4F_9)**)KAAAAKAAAAKAAGY-NH_2 (blue). NMR data on other Ac-XKAAAAKAAAAKAAGY-NH_2 peptides and related peptides may be found in Elbaum, M. B.; Zondlo, N. J. *Biochemistry* **2014**, *53*, 2242-2260.

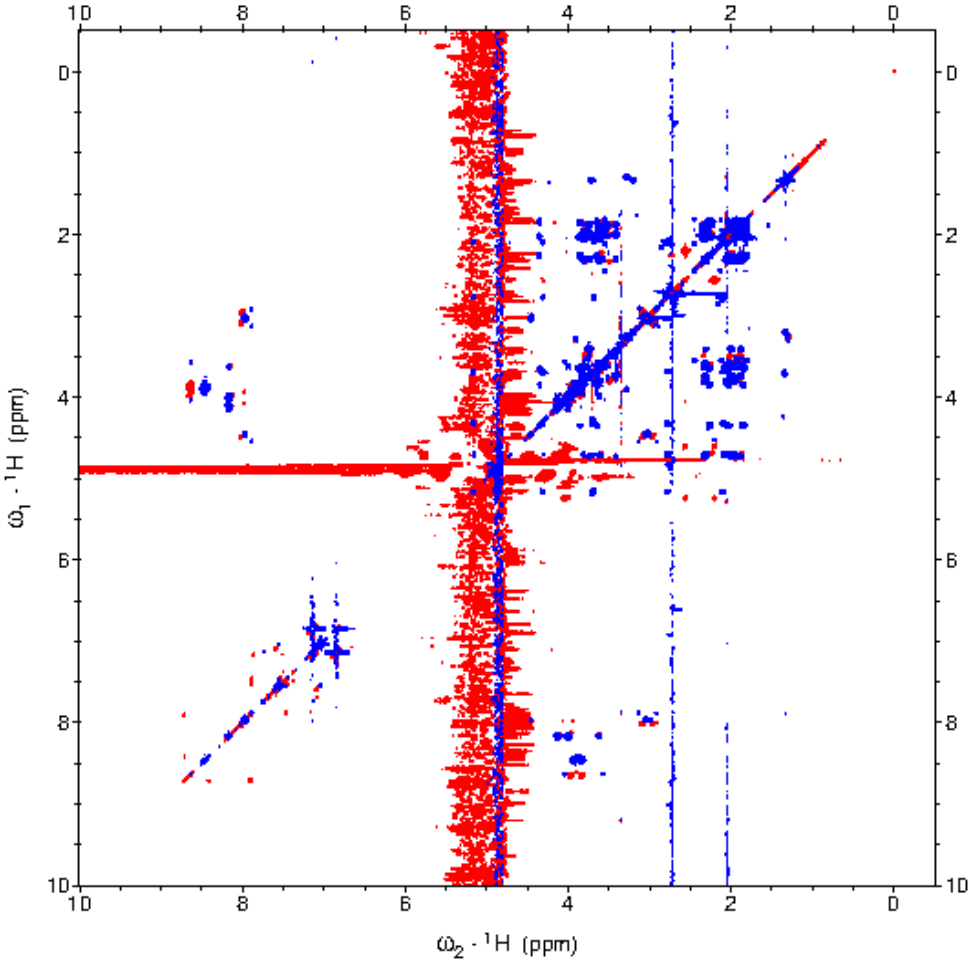
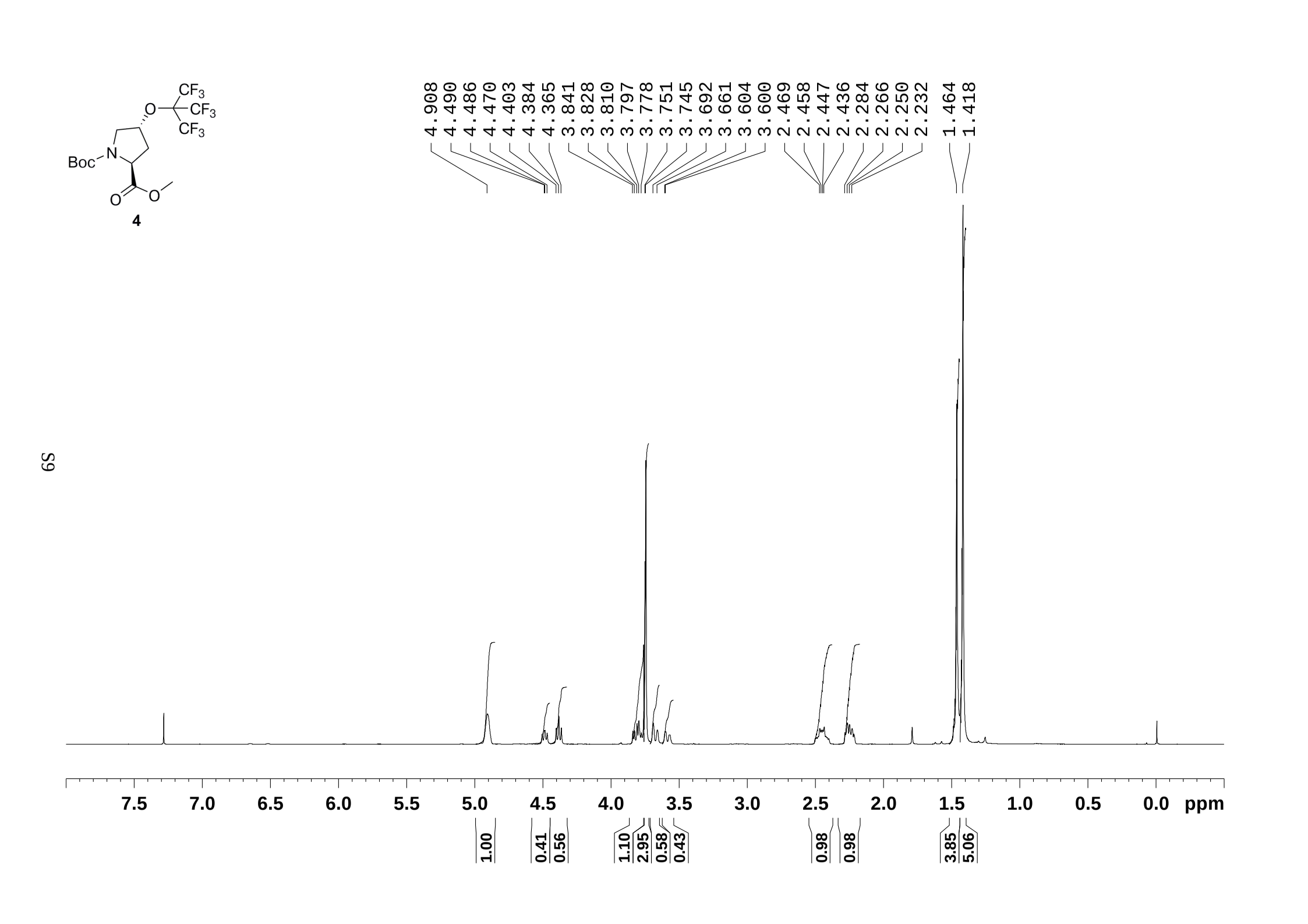


Figure S9. Superposition of the TOCSY spectra of the peptides Ac-GPP((**2*S*,4*R*)-Hyp(C_4F_9)**)PPGY-NH_2 (red) and Ac-GPP((**2*S*,4*S*)-hyp(C_4F_9)**)PPGY-NH_2 (blue). NMR data on other Ac-GPPXPPGY-NH_2 peptides and assignments of the amide hydrogens may be found in Brown, A. M.; Zondlo, N. J. *Biochemistry* **2012**, *51*, 5041-5051.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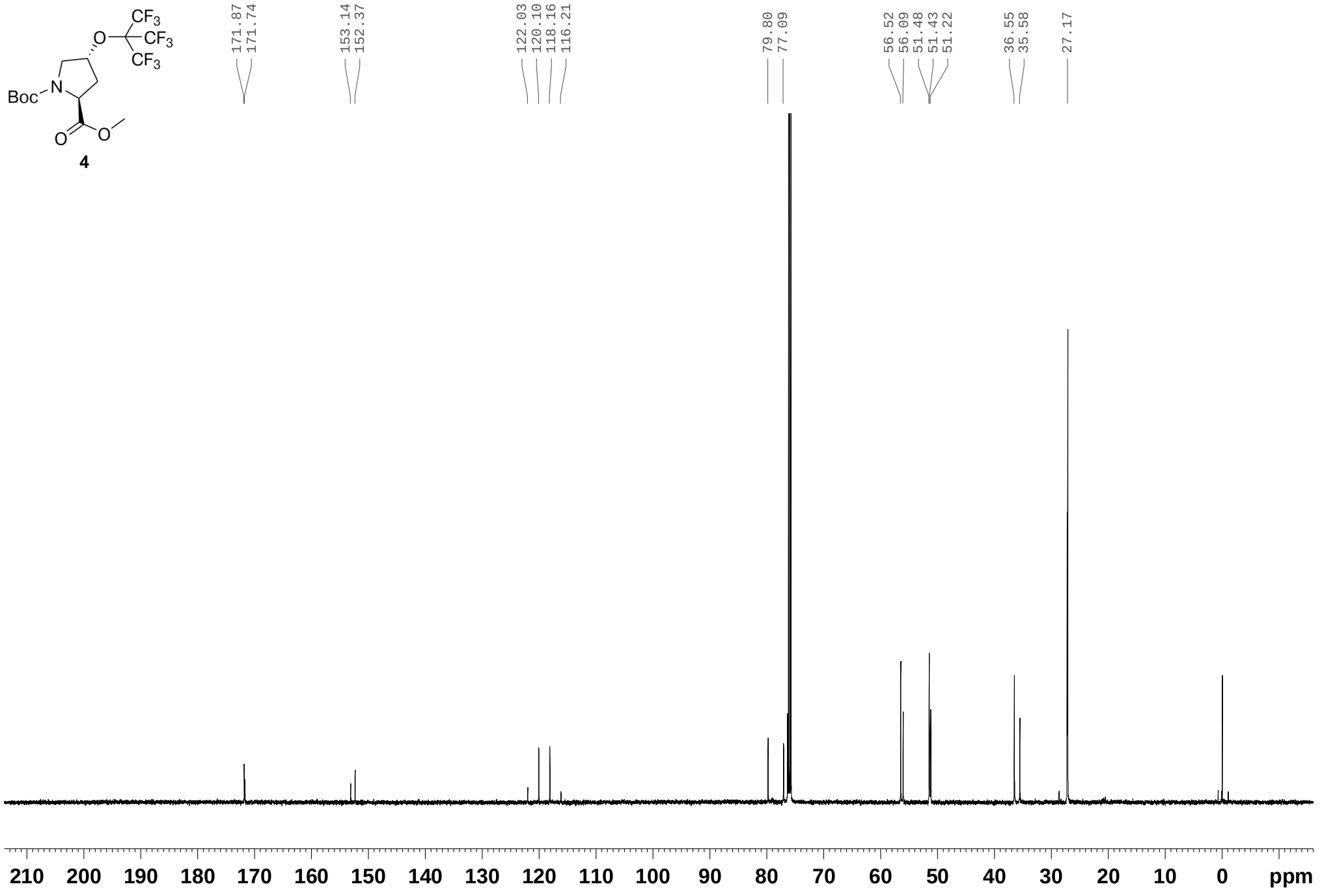
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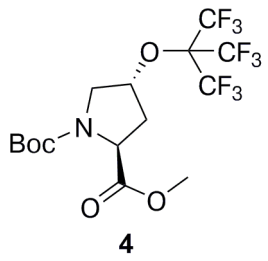

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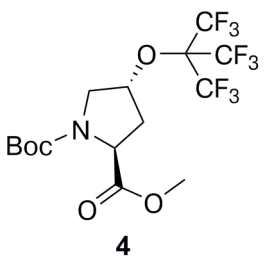
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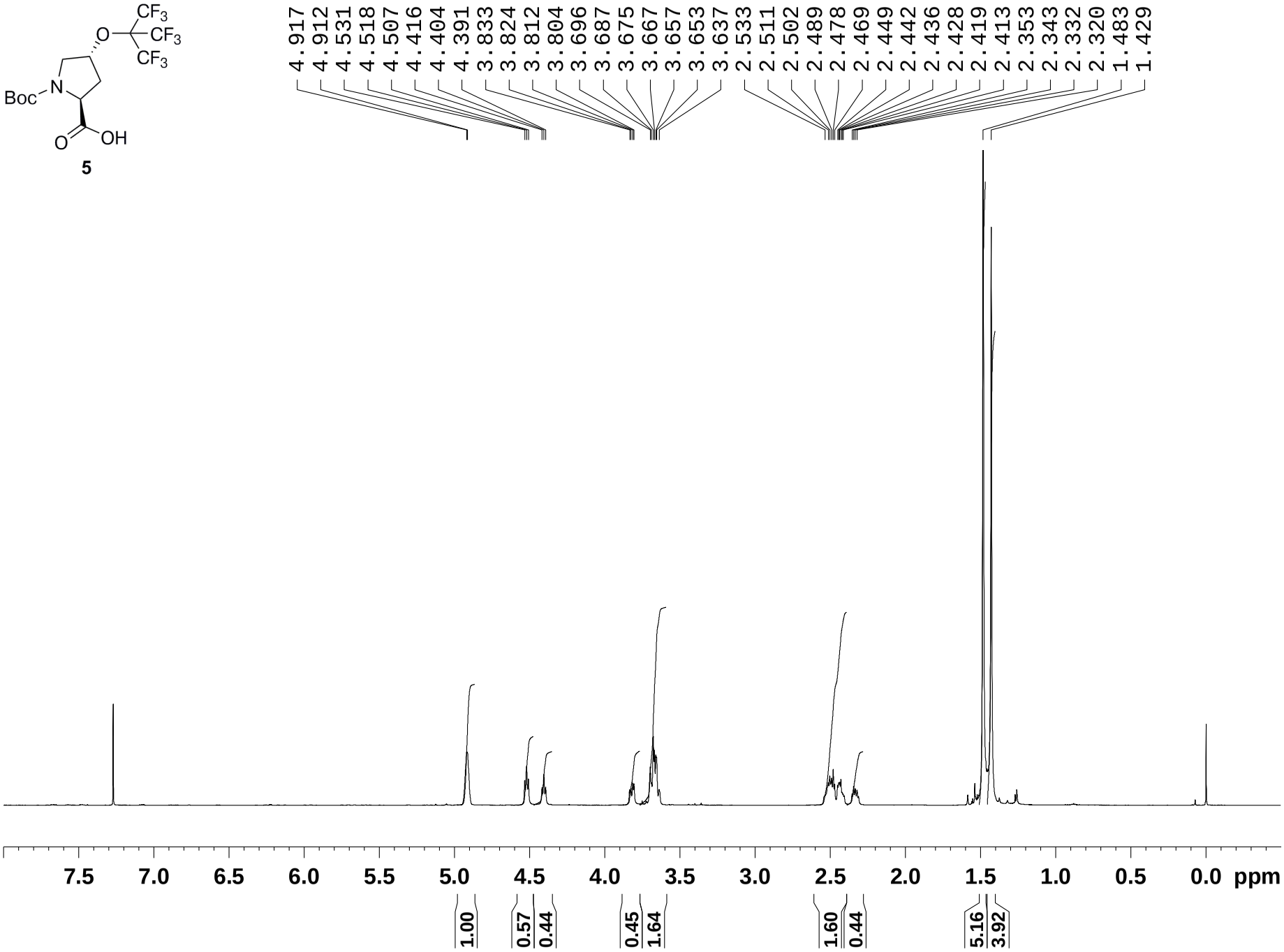
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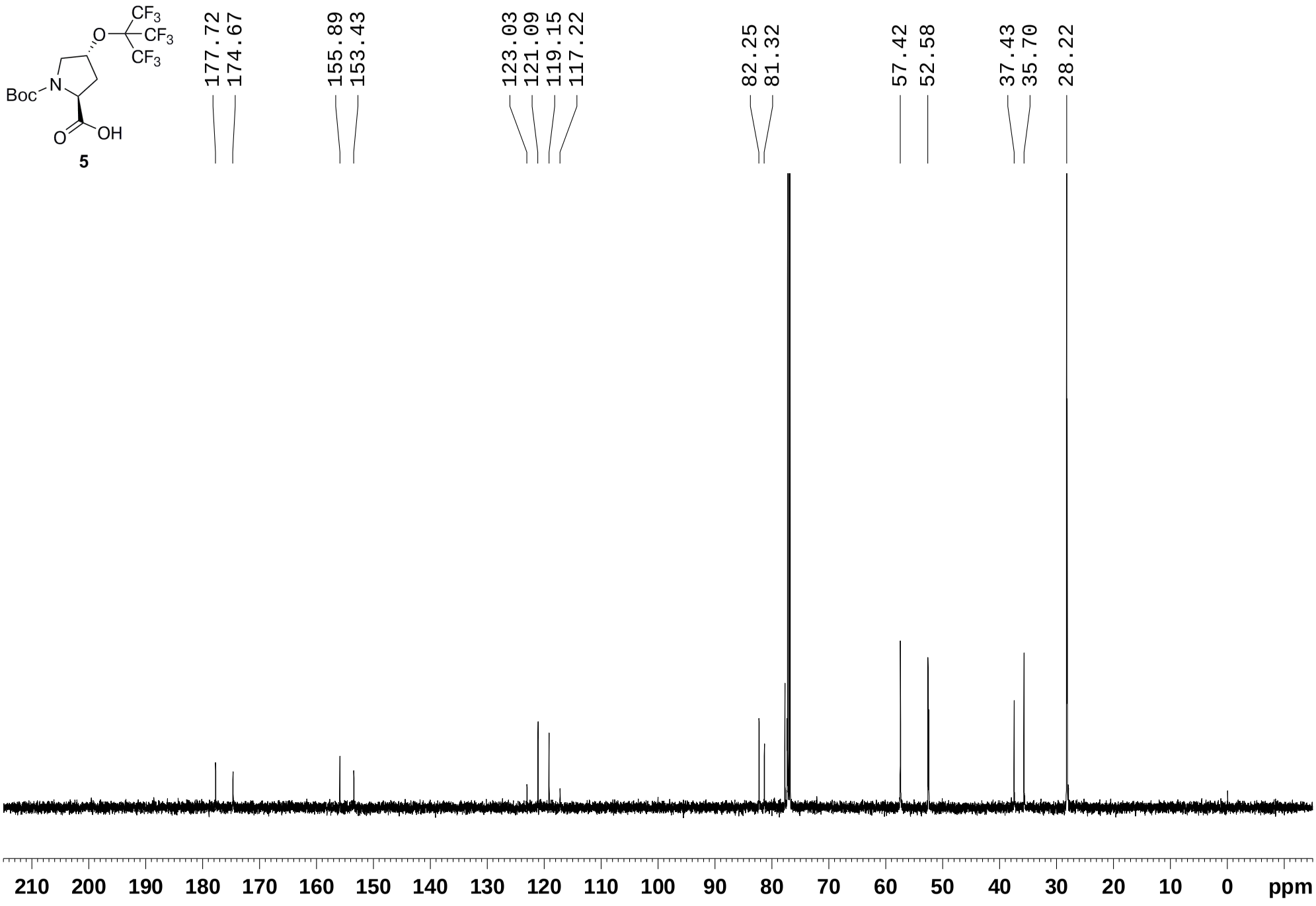
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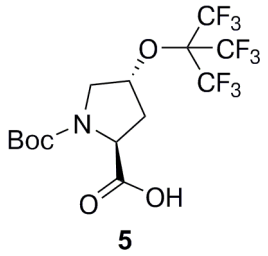

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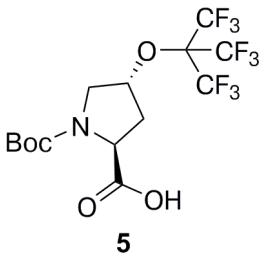
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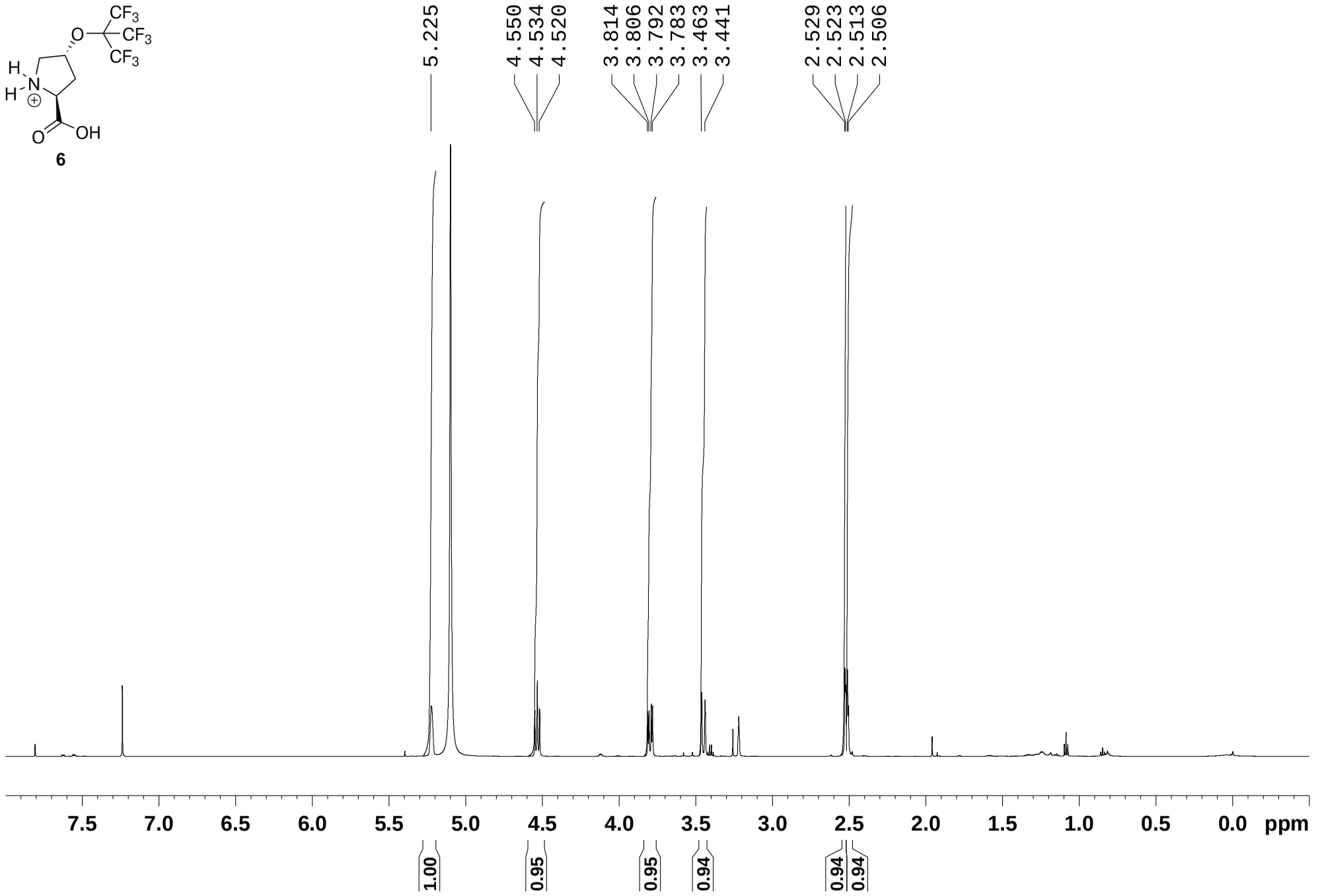
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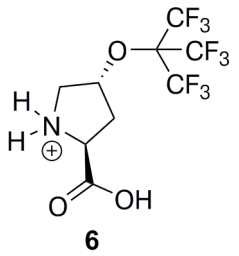

6

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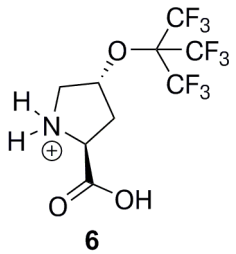

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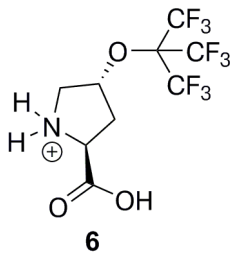
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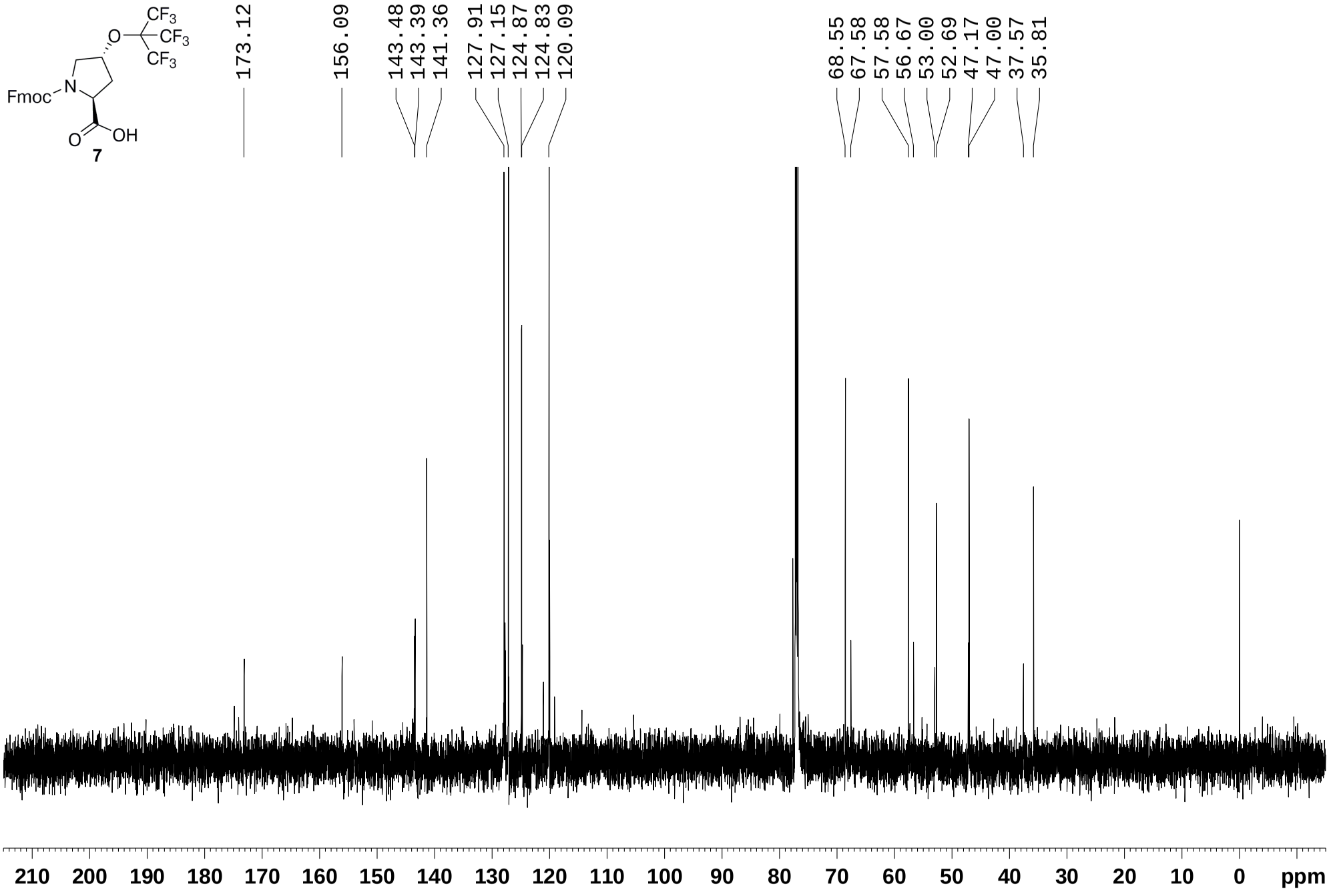
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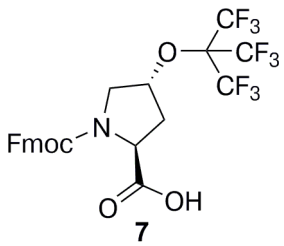

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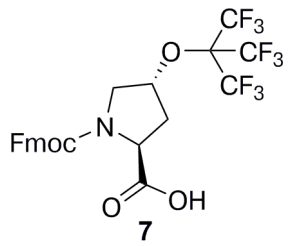

7

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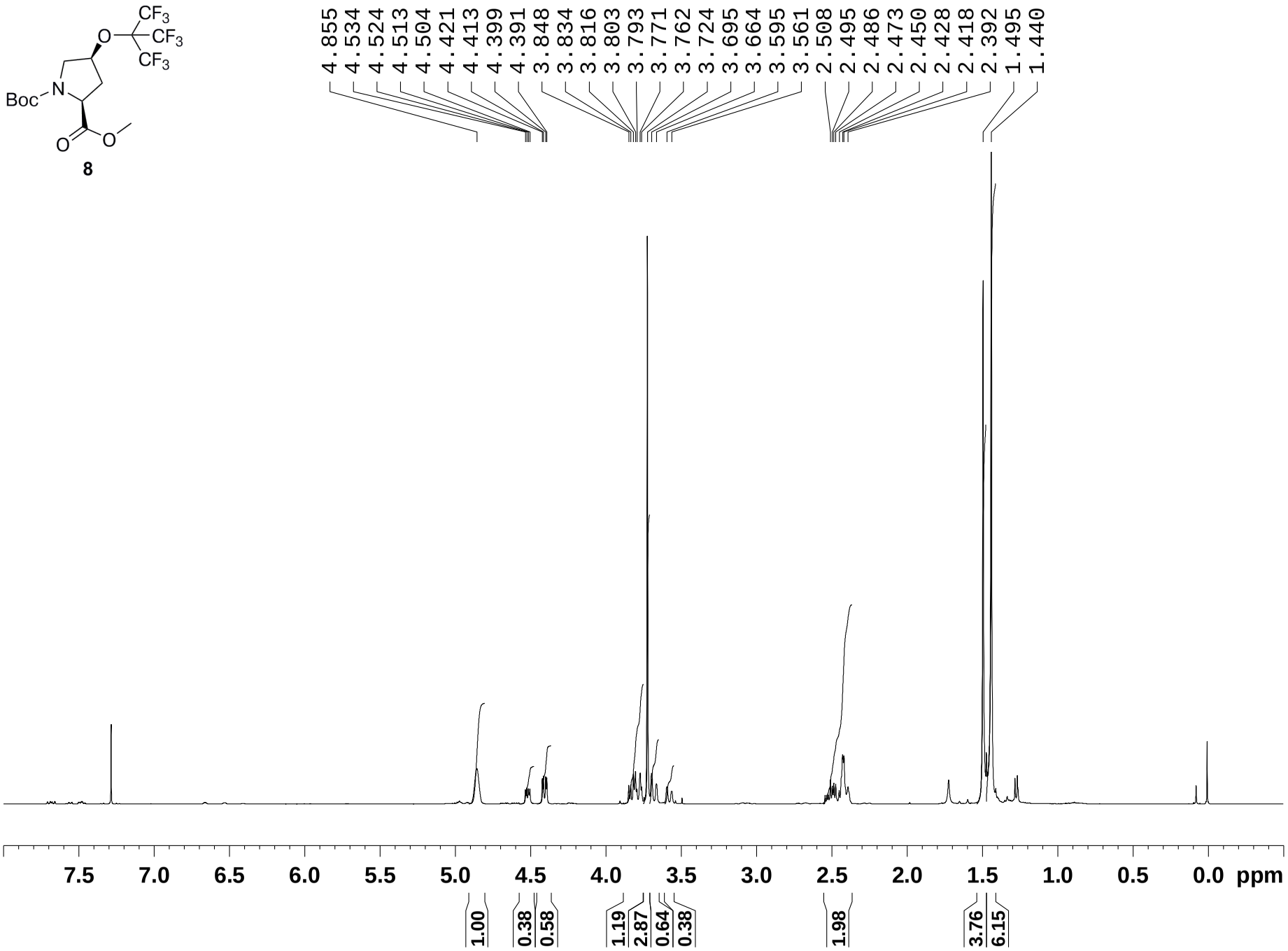
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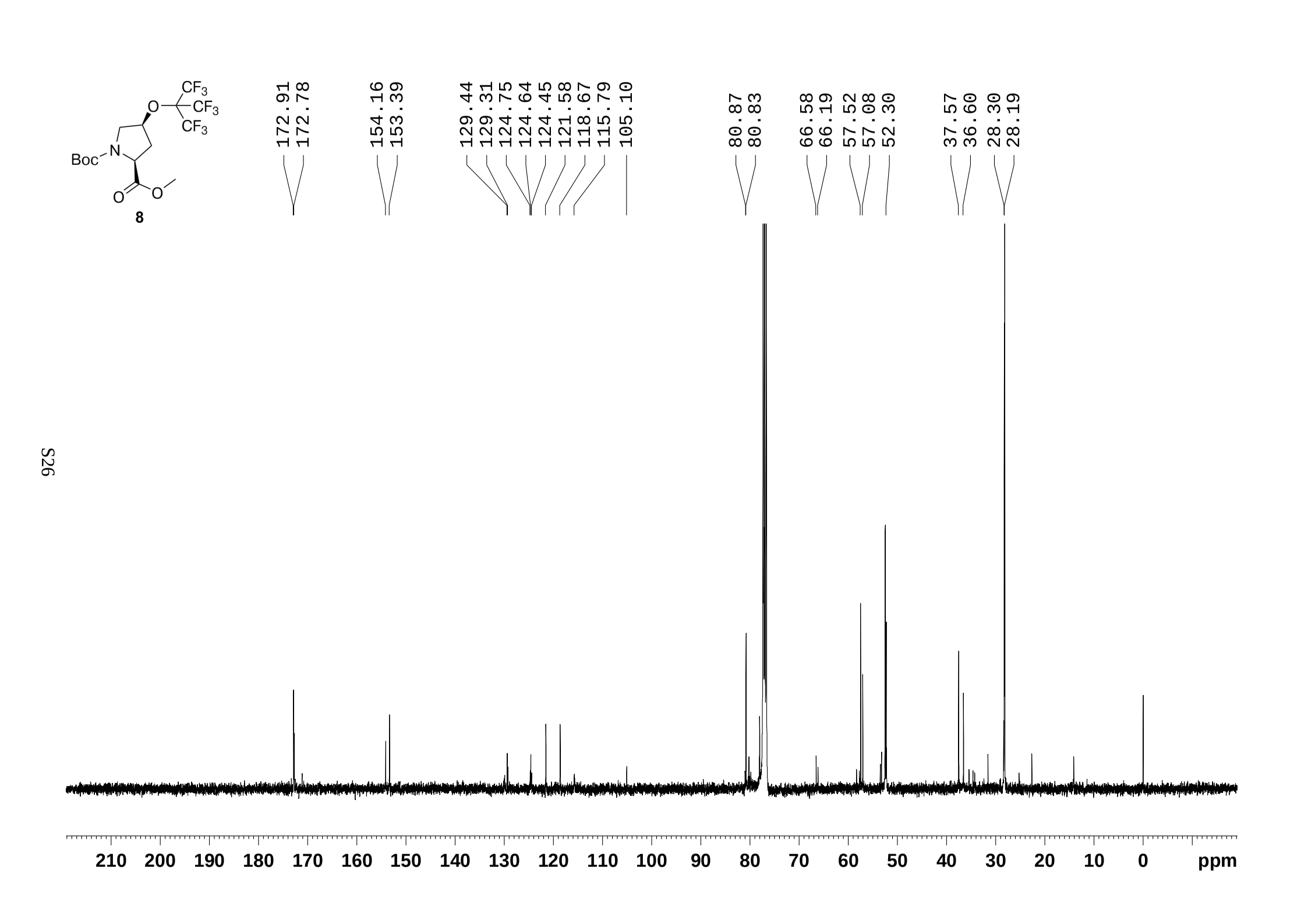
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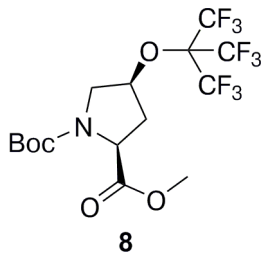

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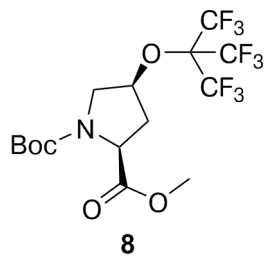
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8


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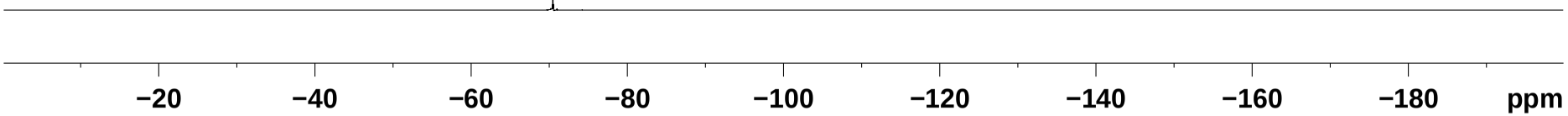
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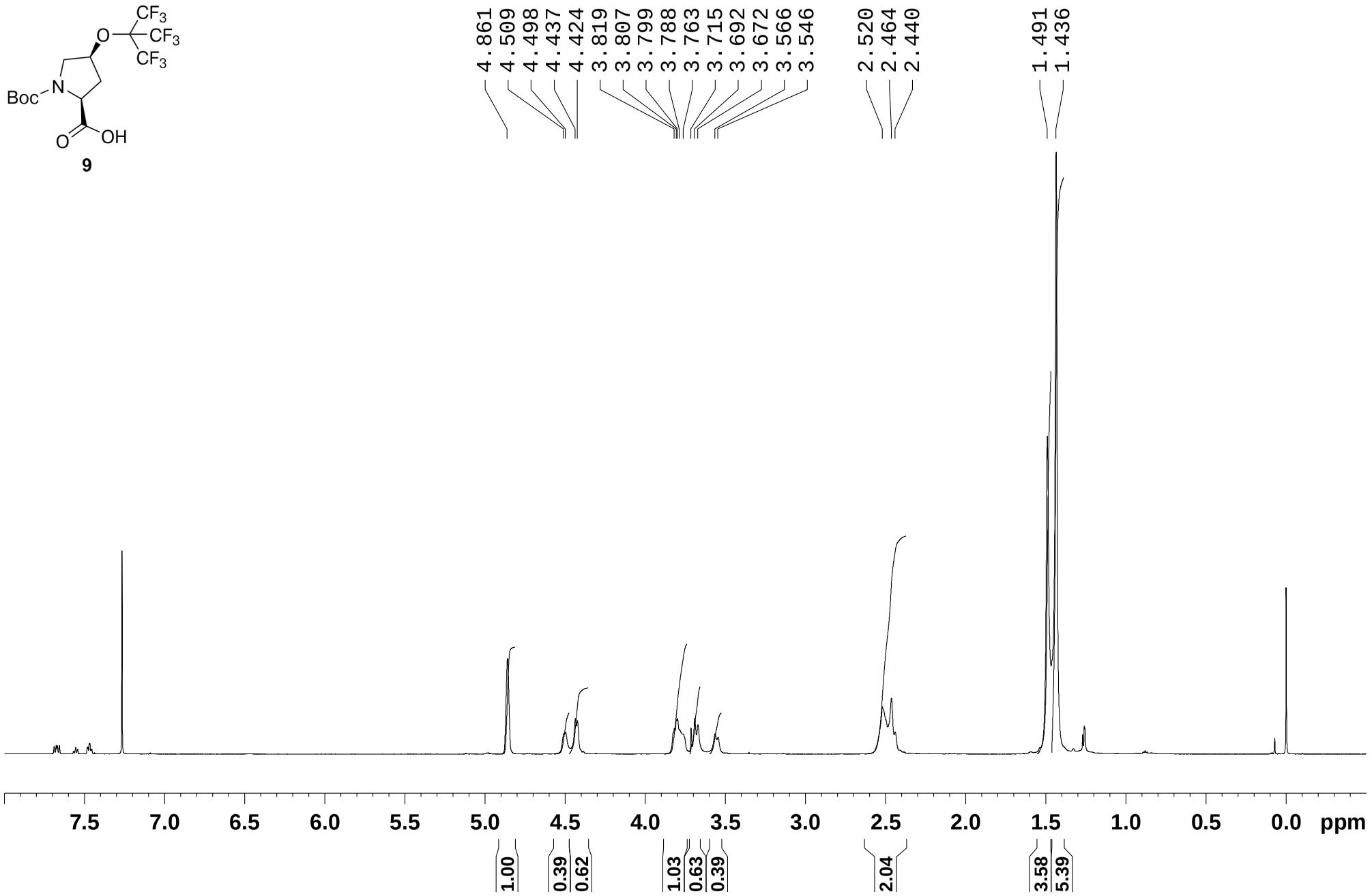
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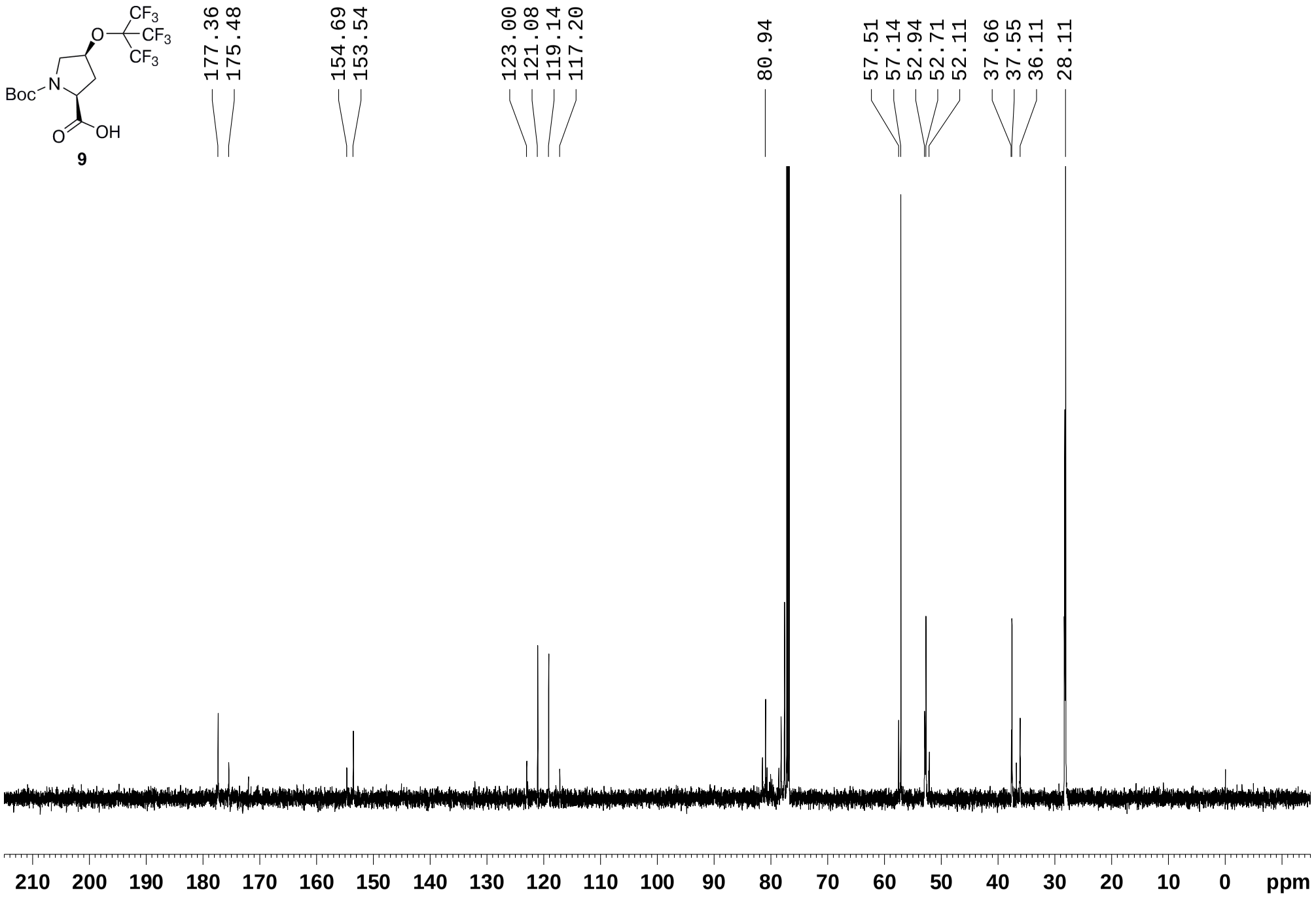
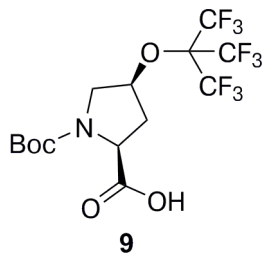
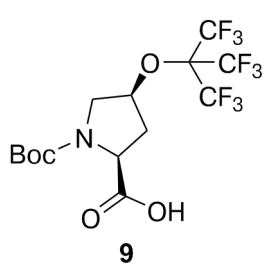
9
4.861
4.509
4.498
4.437
4.424
3.819
3.807
3.799
3.788
3.763
3.715
3.692
3.672
3.566
3.546
2.520
2.464
2.440
1.491
1.436
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
1.00
0.39
0.62
1.03
0.63
0.39
2.04
3.58
5.39


CF3
CF3
CF3
O
N
Boc
O
OH
9
177.36
175.48
154.69
153.54
123.00
121.08
119.14
117.20
80.94
57.51
57.14
52.94
52.71
52.11
37.66
37.55
36.11
28.11
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

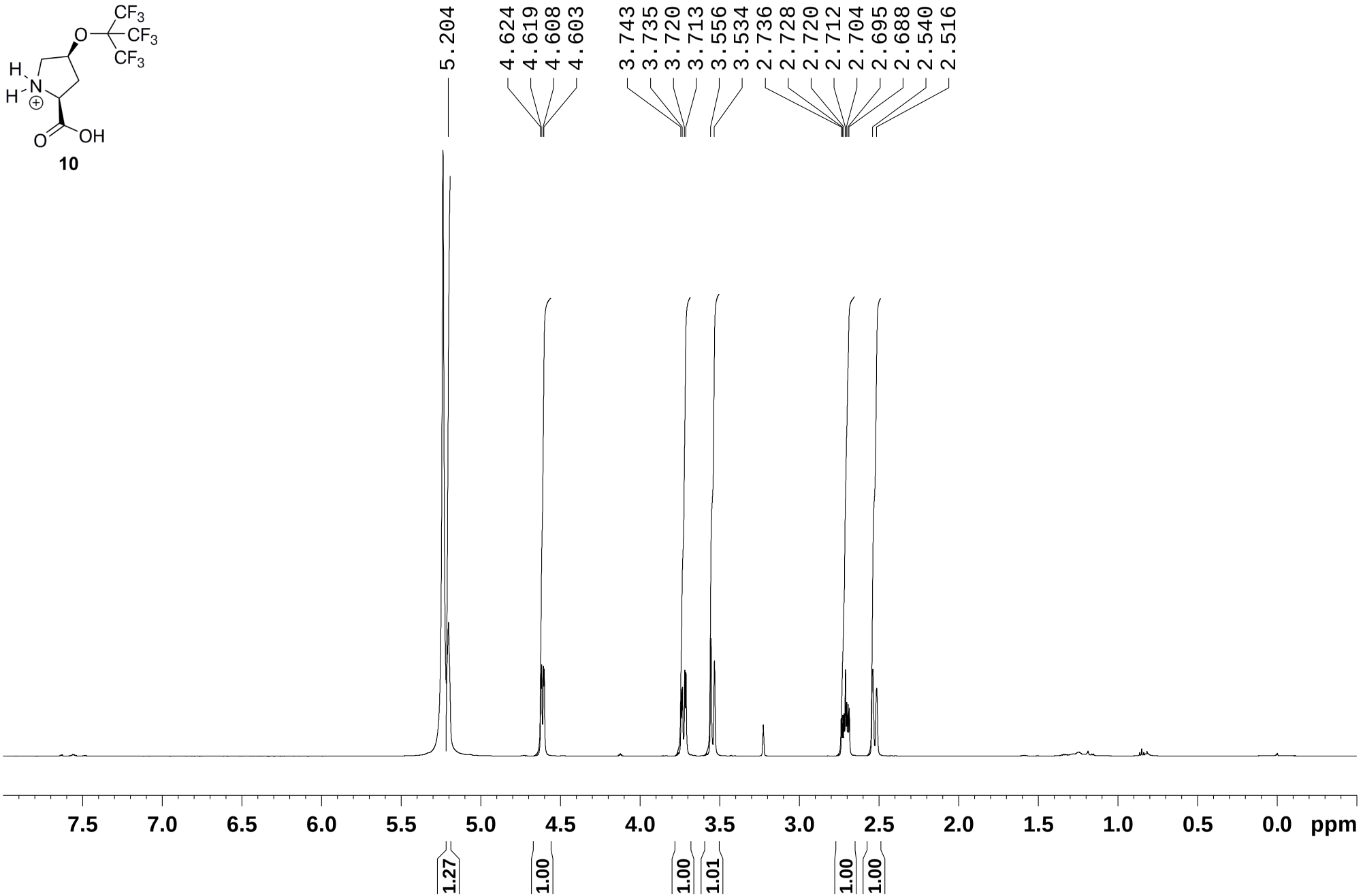


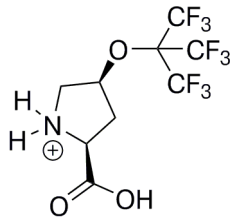
9


CF3
O
CF3
CF3
N
Boc
O
OH

9

-70.38
-70.44
0
−20
−40
−60
−80
−100
−120
−140
−160
−180
−200
ppm

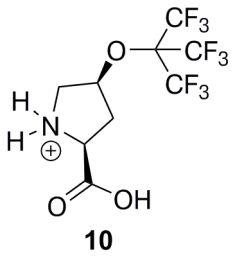

CF3
CF3
CF3
O
H
N
H
OH
O
10
5.204
4.624
4.619
4.608
4.603
3.743
3.735
3.720
3.713
3.556
3.534
2.736
2.728
2.720
2.712
2.704
2.695
2.688
2.540
2.516
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
1.27
1.00
1.00
1.01
1.00
1.00



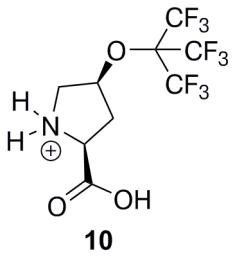
10

169.26
122.92
120.99
119.05
117.11
78.90
58.30
52.12
36.05

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm



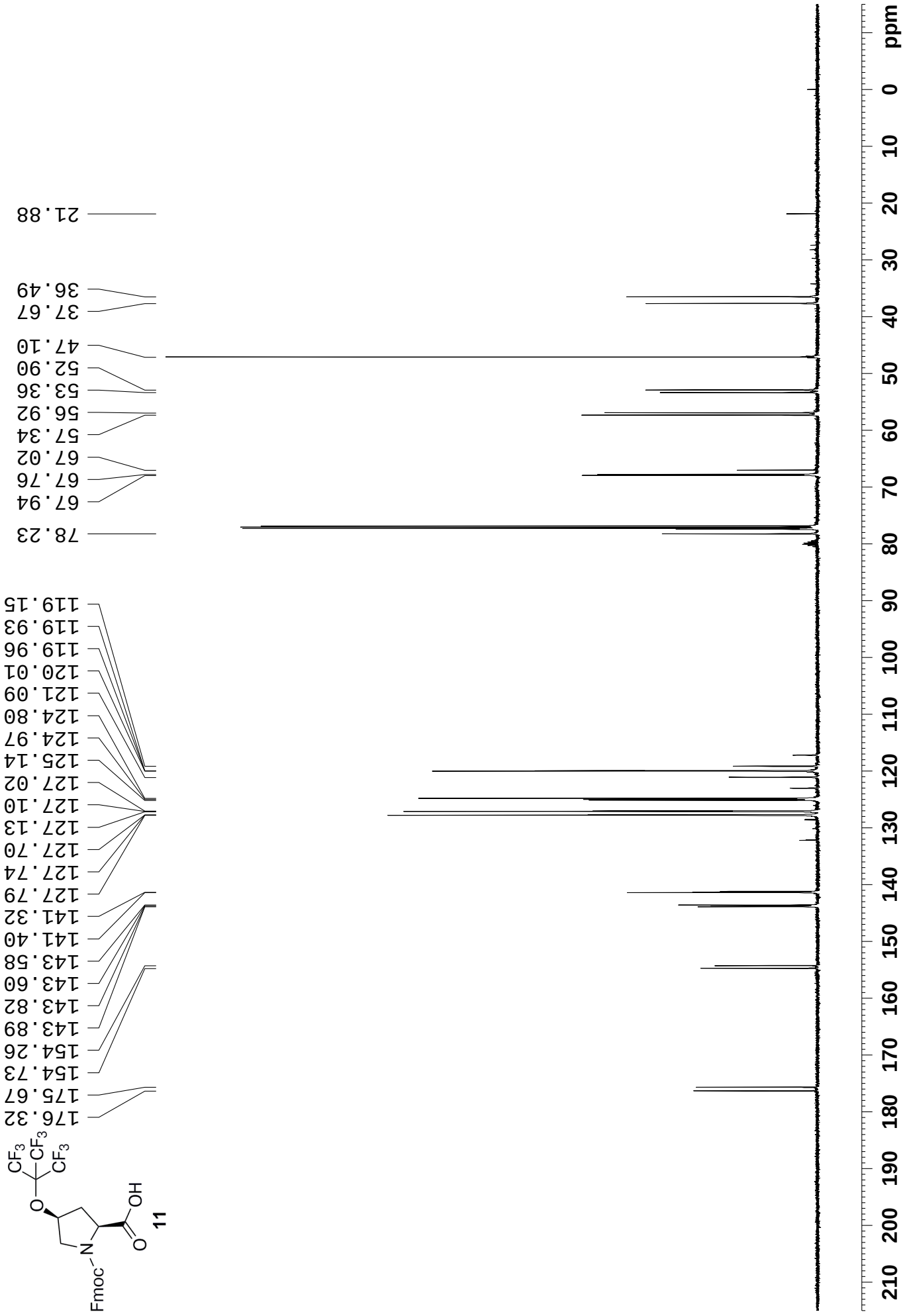
10

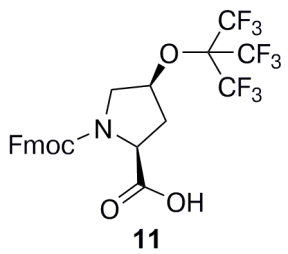

CF3
CF3
CF3
O
H
N
H
OH
O

10

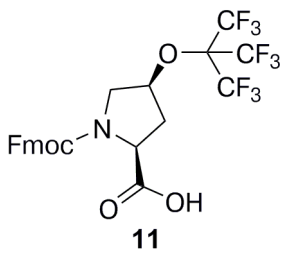
-71.65
0
−20
−40
−60
−80
−100
−120
−140
−160
−180
−200
ppm

7.520
7.447
7.410
7.386
7.369
7.296
7.269
4.917
4.877
4.605
4.590
4.579
4.555
4.529
4.513
4.503
4.487
4.434
4.418
4.408
4.392
4.370
4.350
4.334
4.311
4.165
4.149
4.134
3.901
3.888
3.869
3.855
3.840
3.822
3.809
3.766
3.710
3.646
3.635
2.574
2.565
2.553
2.540
2.436
Fmoc
N
O
O
OH
O
CF3
CF3
CF3
11
0.94
1.06
0.90
1.04
2.95
0.97
0.94
0.49
1.97
0.45
0.94
1.76
0.18
1.98
ppm


21.88
36.49
37.67
47.10
52.90
53.36
56.92
57.34
67.02
67.76
67.94
78.23
119.15
119.93
119.96
120.01
121.09
124.80
124.97
125.14
127.02
127.10
127.13
127.70
127.74
127.79
141.32
141.40
143.58
143.60
143.82
143.89
154.26
154.73
175.67
176.32
CF3
CF3
CF3
O
N
Fmoc
OH
O
11
ppm
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210



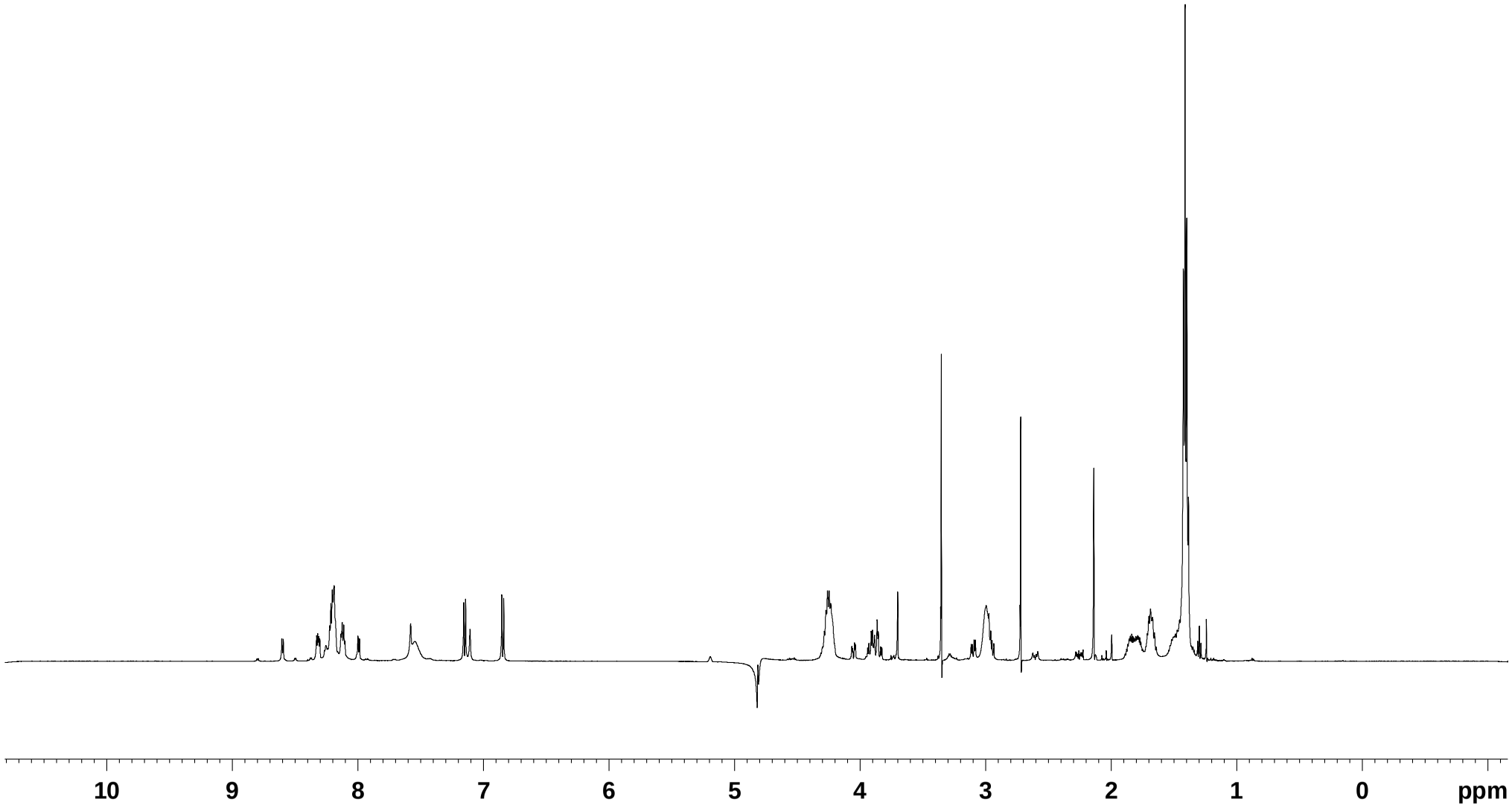
11


CF3
CF3
CF3
O
N
Fmoc
O
OH

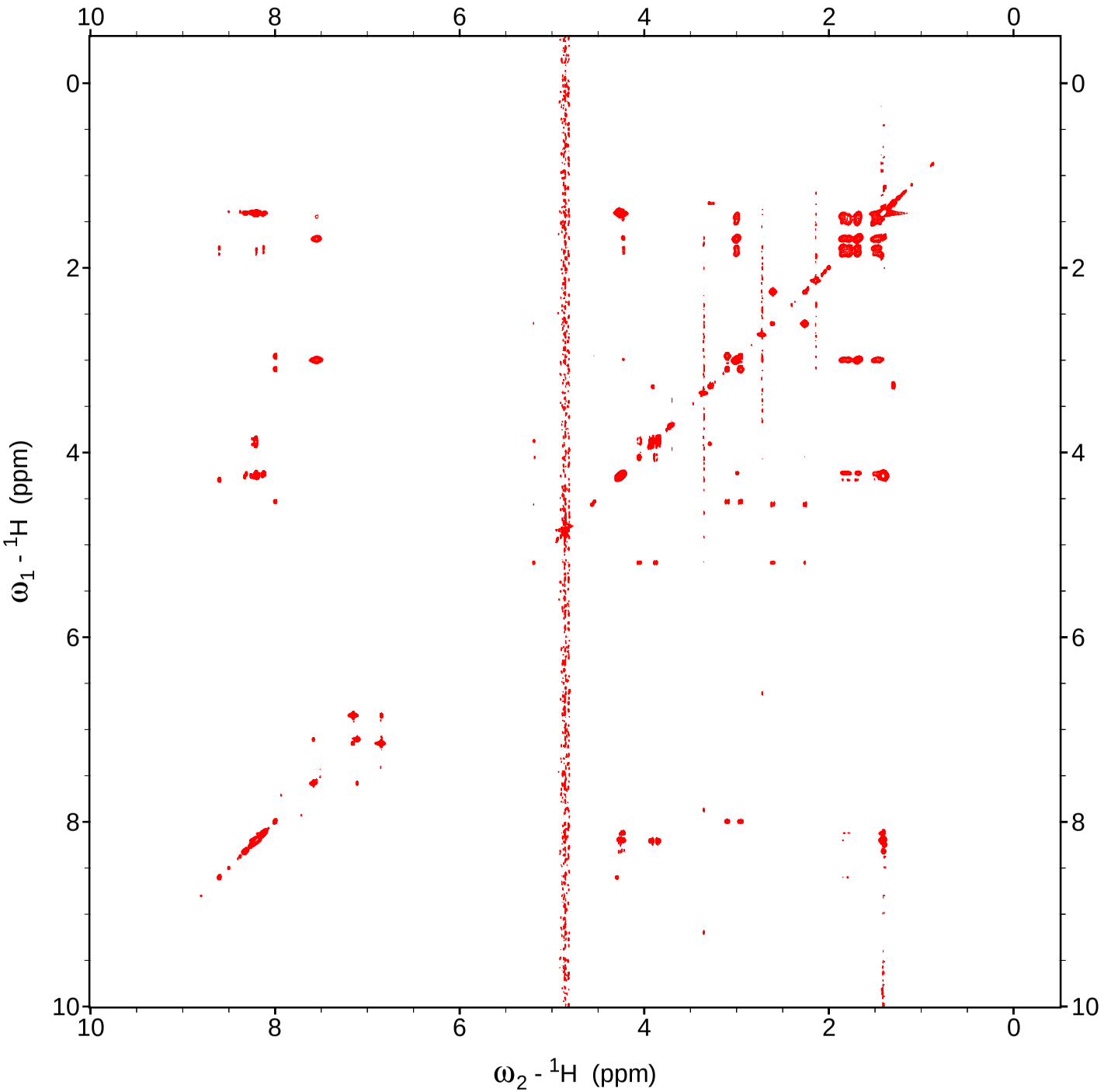
11

-70.42
-70.49
0
−20
−40
−60
−80
−100
−120
−140
−160
−180
−200
ppm

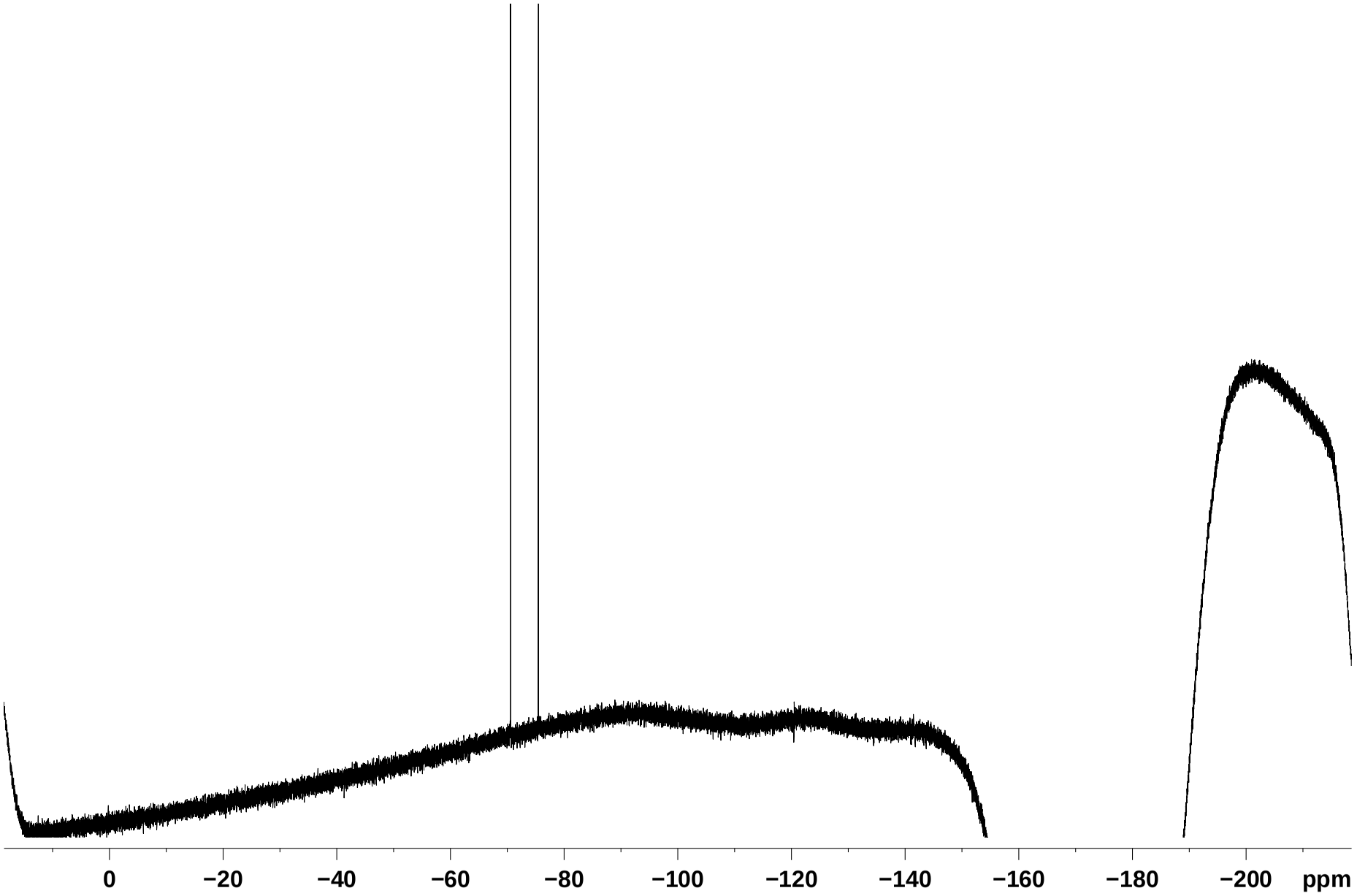
Ac-((**2*S*,4*R*)-Hyp(C_4F_9)**)KAAAAKAAAAKAAGY-NH_2



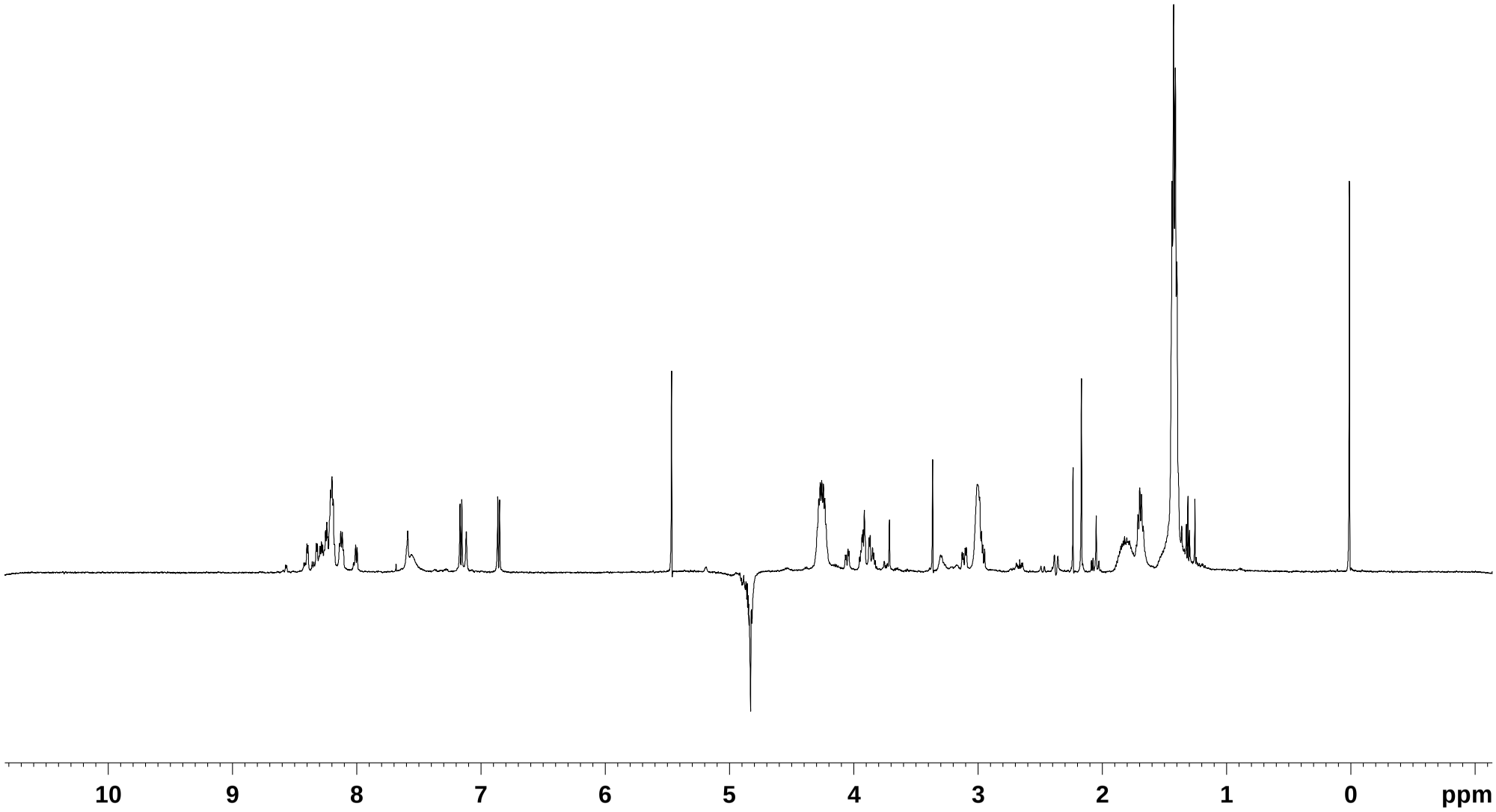
Ac-((**2*S*,4*R***)-**Hyp(C$_4$F$_9$)**)KAAAAKAAAAKAAGY-NH$_2$
TOCSY



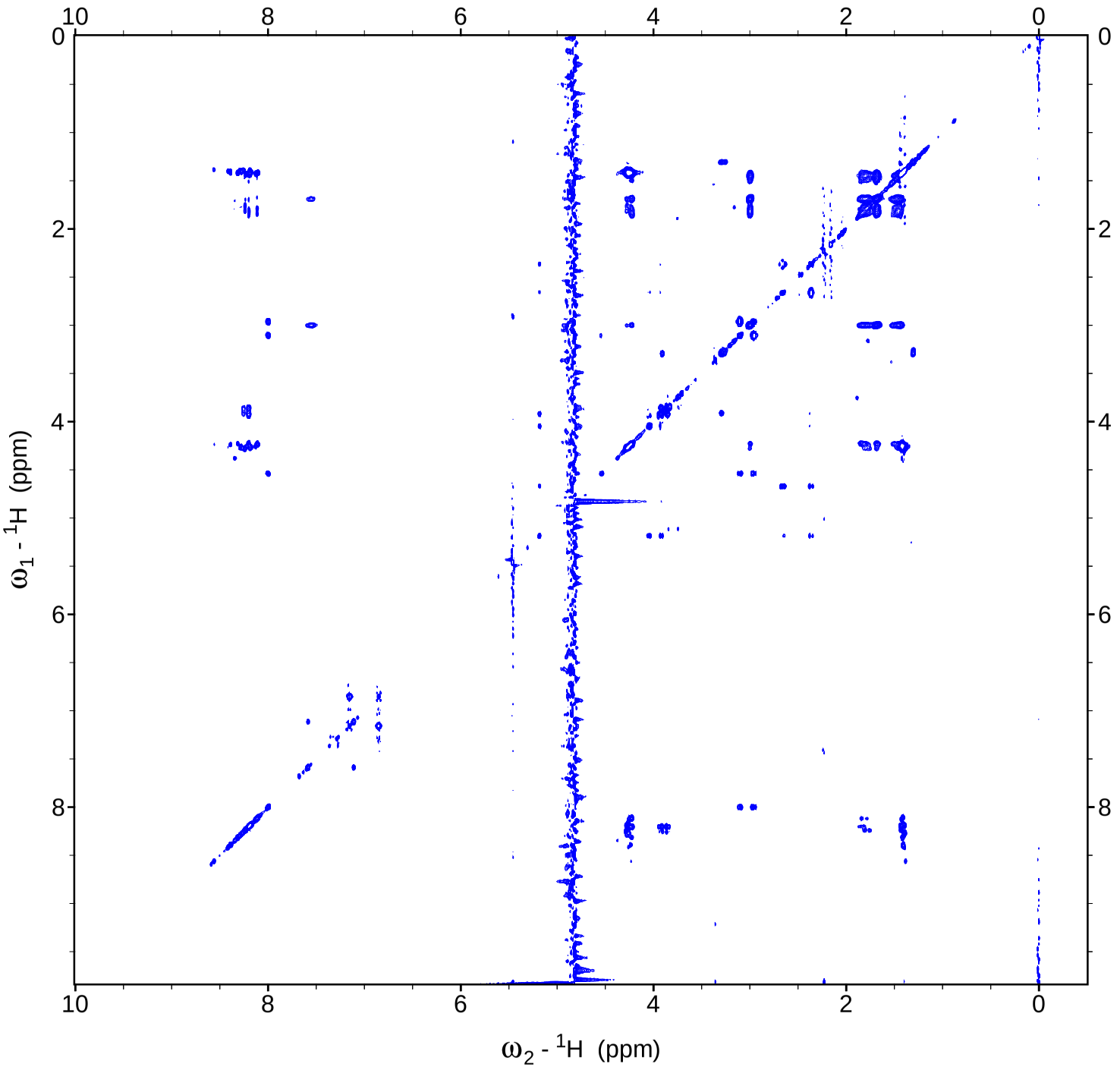
Ac-((**2*S*,4*R*-Hyp(C_4F_9)**)KAAAAKAAAAKAAGY-NH_2



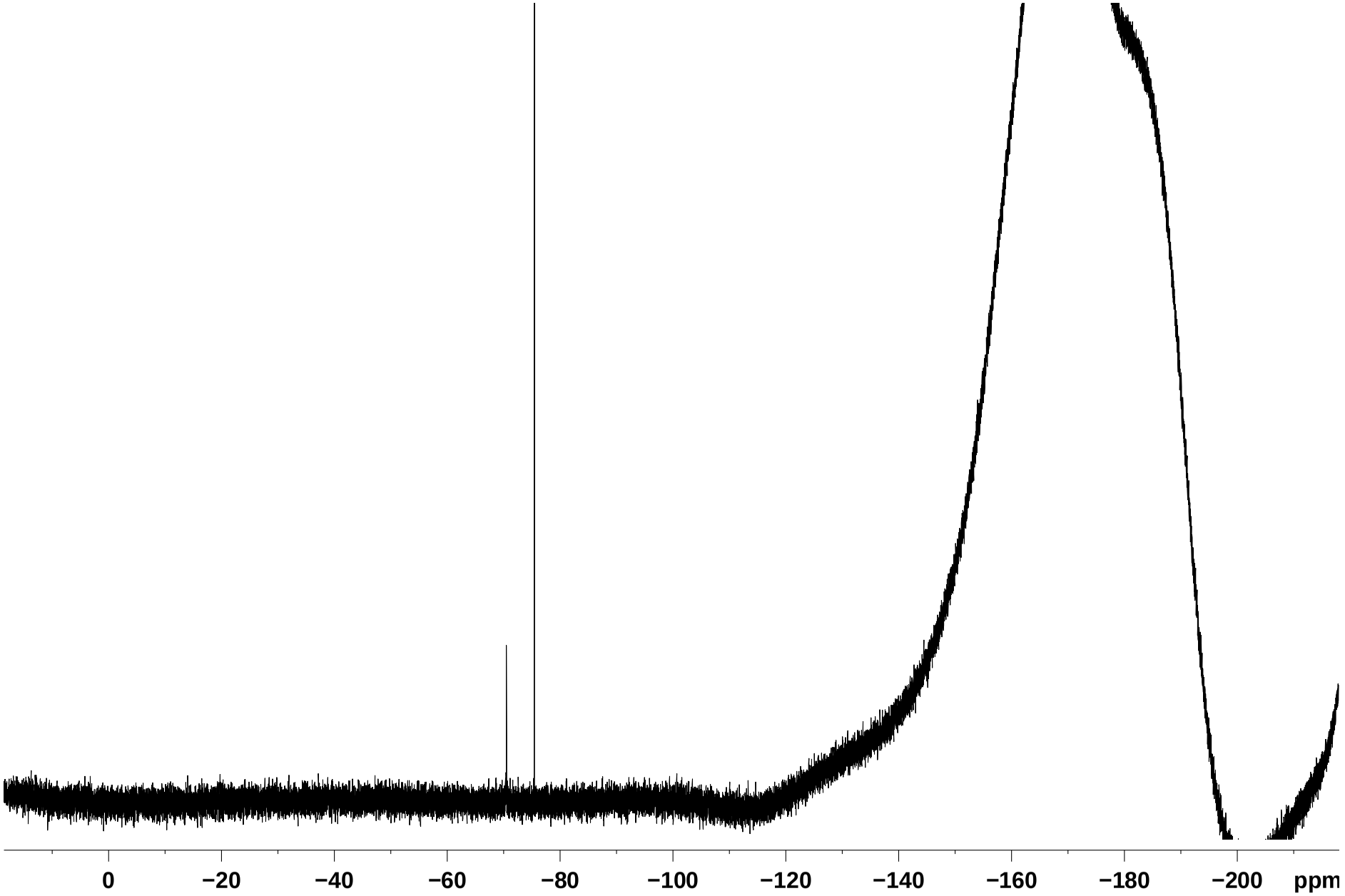
Ac-((**2*S*,4*S*)-Hyp(C_4F_9)**)KAAAAKAAAAKAAGY-NH_2



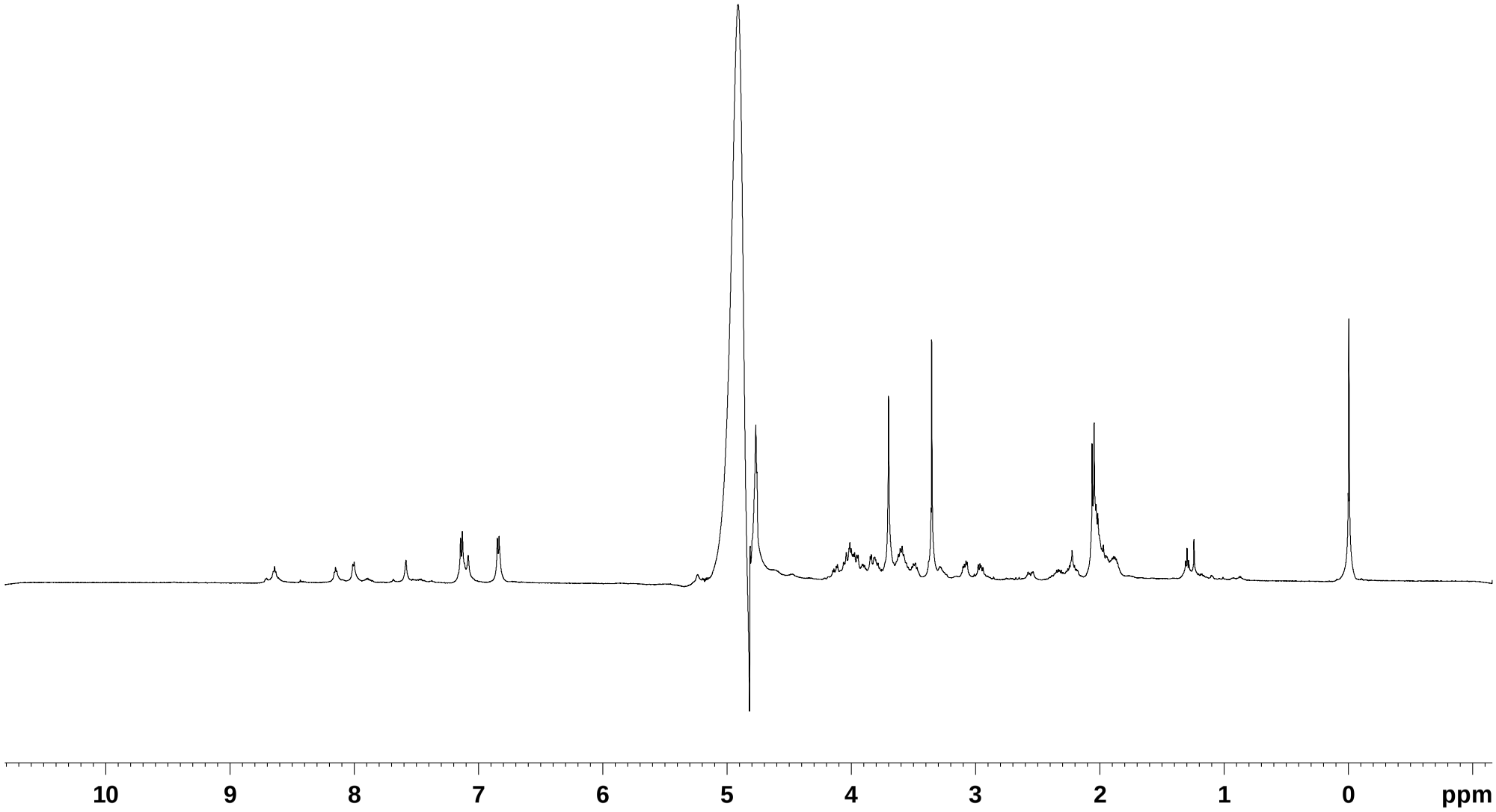
Ac-((**2*S*,4*S*)-Hyp(C_4F_9)**)KAAAAKAAAAKAAGY-NH_2
TOCSY



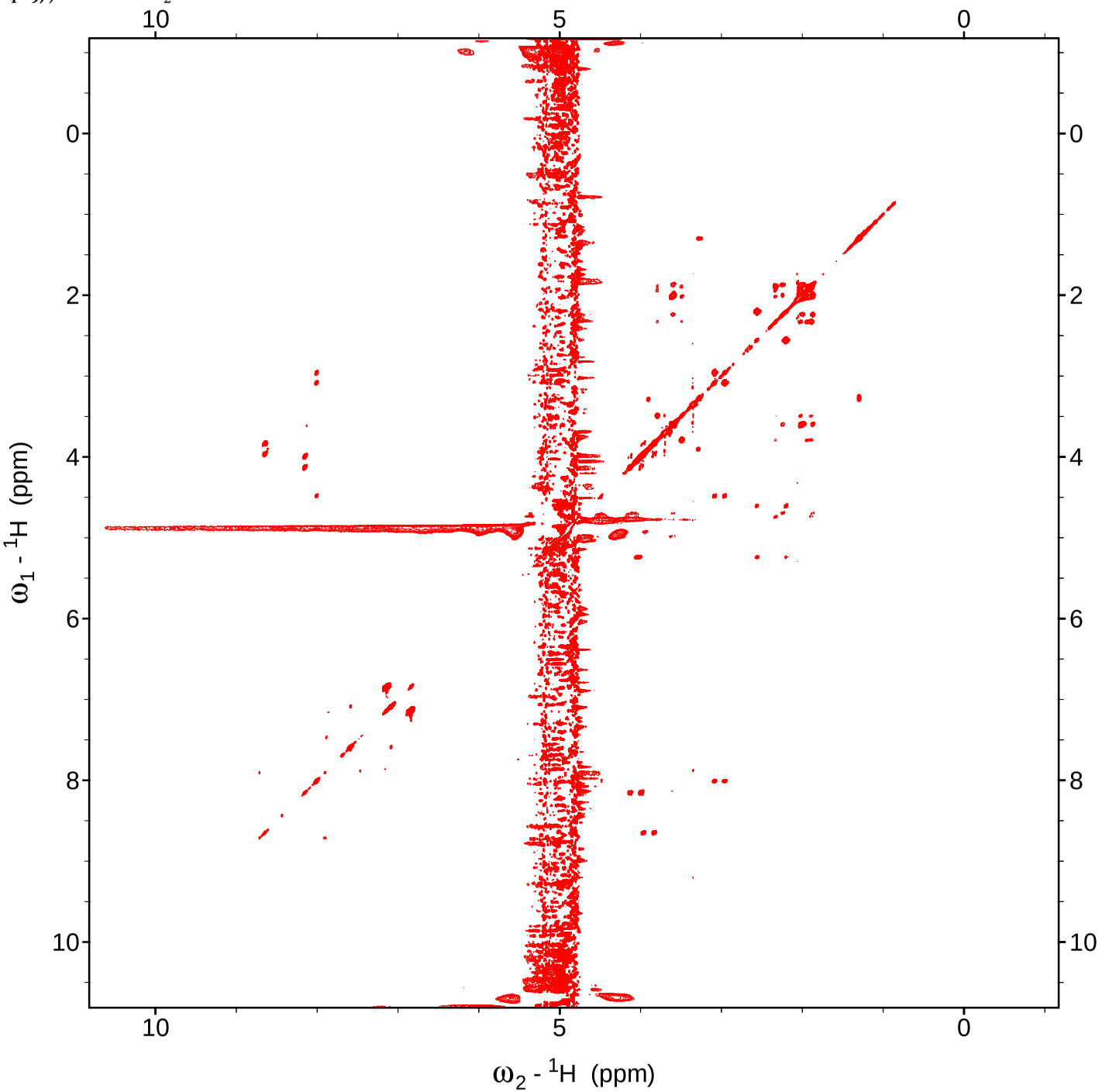
Ac-((**2*S*,4*S*-Hyp(C_4F_9)**)KAAAAKAAAAKAAGY-NH_2



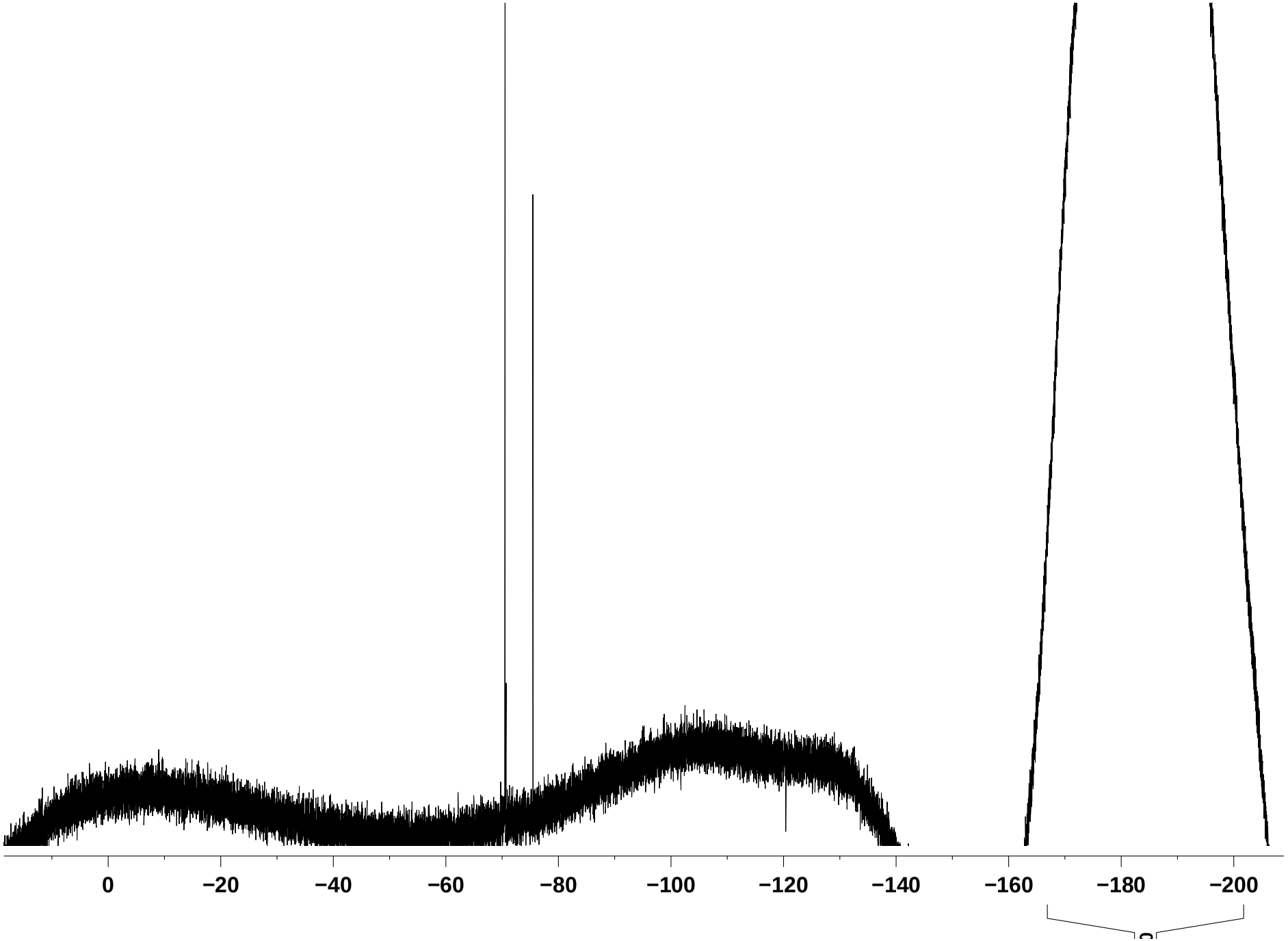
Ac-GPP((**2*S*,4*R*-Hyp(C_4F_9)**)PPGY-NH_2



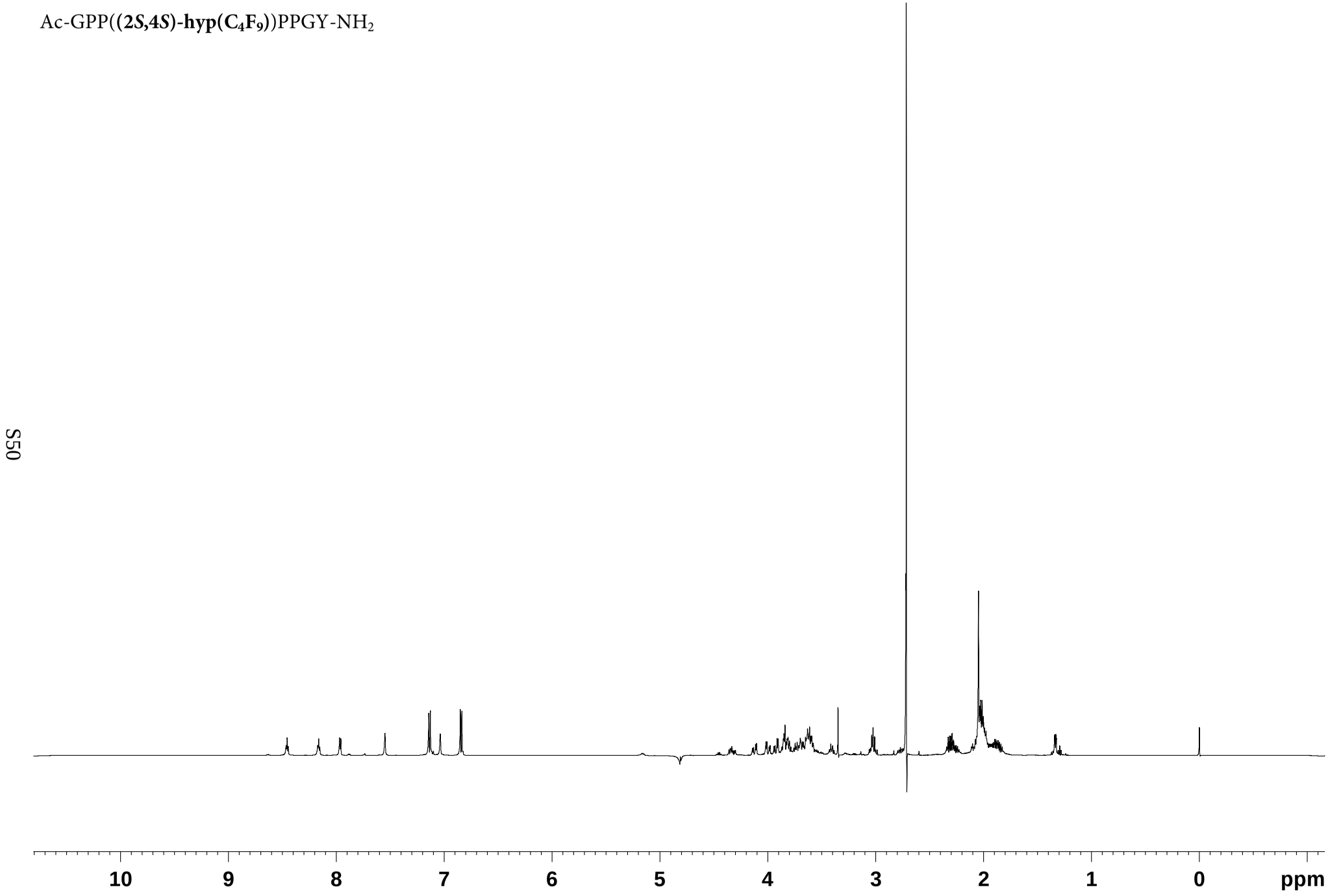
Ac-GPP((**2*S*,4*R*-Hyp(C$_4$F$_9$)**)PPGY-NH$_2$
TOCSY



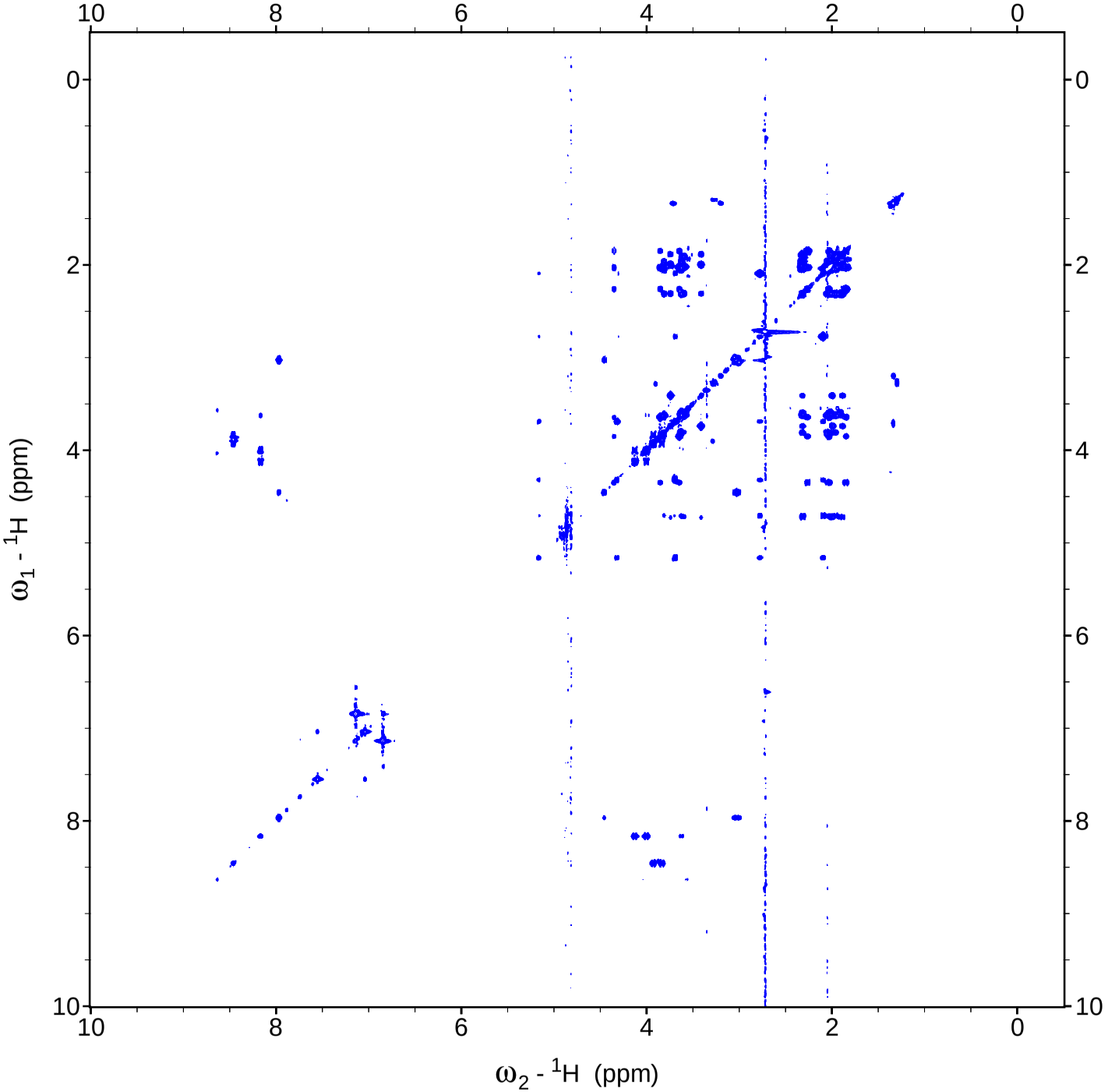
Ac-GPP((**2*S*,4*R*-Hyp(C_4F_9)**)PPGY-NH_2



Ac-GPP((**2*S*,4*S*-hyp(C_4F_9)**)PPGY-NH_2



Ac-GPP((**2*S*,4*S*-hyp(C$_4$F$_9$)**)PPGY-NH$_2$
TOCSY



Ac-GPP((**2*S*,4*S*-hyp(C_4F_9)**)PPGY-NH_2