New fluorinated fructose analogs as selective probes of the hexose transporter protein GLUT5

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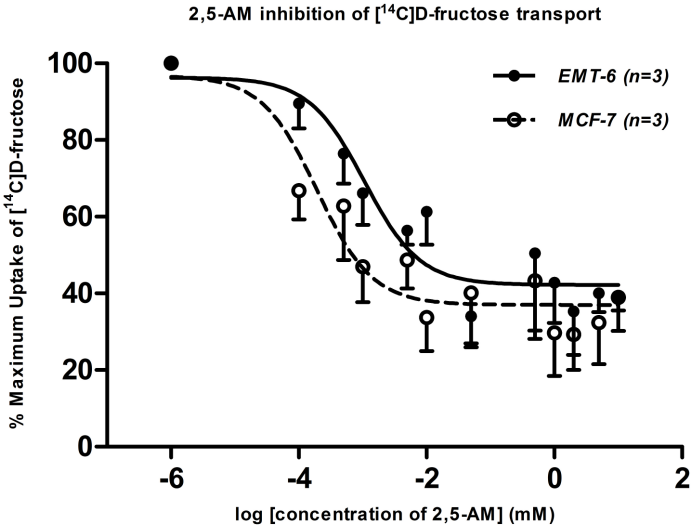
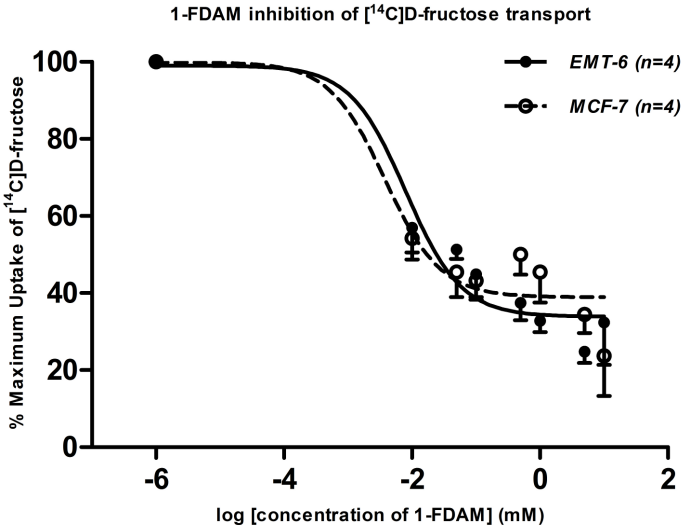
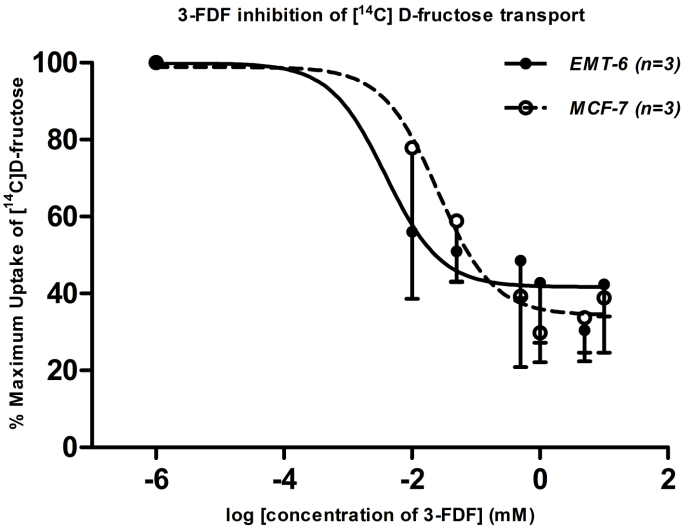
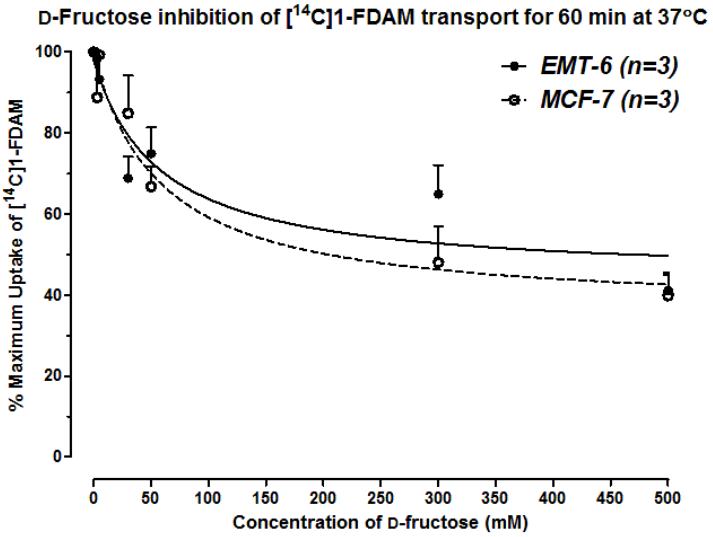
Supporting information:

- Inhibition experiments of [^{14}C]D-fructose transport by increasing concentration of 2,5-AM, 1-FDAM and 3-FDF.
- Inhibition experiments of [^{14}C]1-FDAM transport by D-fructose.
- Flux experiments of [^{14}C]-3FDF and [^{14}C]D-fructose in oocytes expressing GLUT5.
- Modified procedure for the synthesis of 2,5-anhydro-D-mannitol (**1**).
- ^{1}H, ^{13}C and ^{19}F NMR for all new compounds (**4**, **5**, **7**, **8**, **9**, **10**, **11** and **12**) are included in this section (S2-S28).

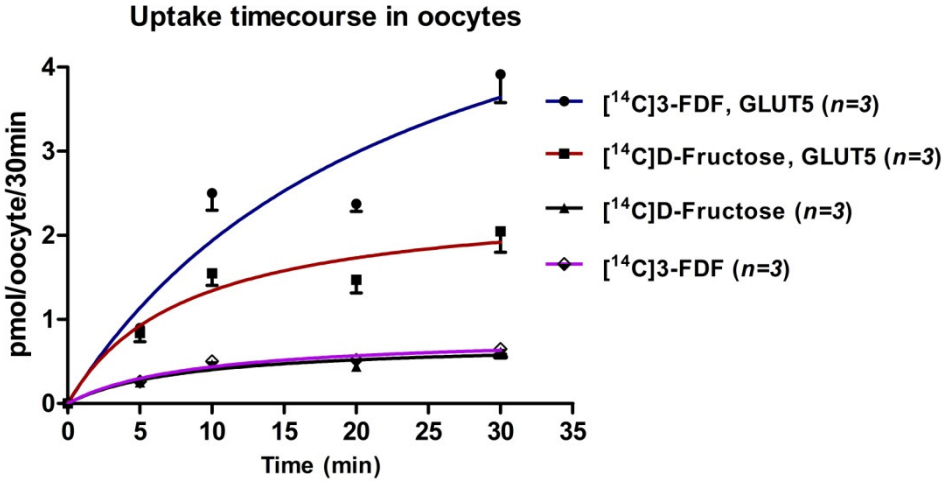

2,5-AM inhibition of [14C]D-fructose transport
EMT-6 (n=3)
MCF-7 (n=3)
% Maximum Uptake of [14C]D-fructose
log [concentration of 2,5-AM] (mM)


1-FDAM inhibition of [14C]D-fructose transport
EMT-6 (n=4)
MCF-7 (n=4)
% Maximum Uptake of [14C]D-fructose
log [concentration of 1-FDAM] (mM)


3-FDF inhibition of [14C] D-fructose transport
EMT-6 (n=3)
MCF-7 (n=3)
% Maximum Uptake of [14C]D-fructose
log [concentration of 3-FDF] (mM)



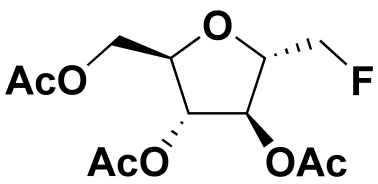
Inhibition experiments: Fructose inhibition of [^{14}C]1-FDAM transport after a 60 min incubation at 37 °C with both EMT-6 and MCF-7 using increasing concentrations of D-Fructose. [^{14}C]1-FDAM transport was inhibited by increasing concentrations of D-Fructose. At the highest examined concentration (500 mM), the uptake of [^{14}C]1-FDAM into EMT-6 was inhibited 59% (*n=3*) and into MCF-7 by 60% (*n=3*) of control values. Error bars represent the SEM.



Uptake experiments in oocytes. [^{14}C]-3FDF and [^{14}C]D-fructose 30 minute time courses in oocytes injected with GLUT5 mRNA or with water as a control experiment. The uptake of [^{14}C]D-fructose in oocytes injected with mRNA-GLUT5 (red curve) was about four times higher than the [^{14}C]D-fructose uptake in oocytes injected with water (black curve). On the other hand, the transport [^{14}C]-3FDF into oocytes injected with GLUT5 mRNA was about two times higher than [^{14}C]D-fructose and eight times higher than the uptake of [^{14}C]-3FDF in oocytes injected with water. These results indicate clearly that the uptake of fructose is GLUT5 dependent and demonstrate that 3-FDF is selectively mediated by GLUT5. Each data point represents the average of 10 oocytes. Error bars indicate SEM.

Modified procedure for the synthesis of 2,5-anhydro-D-mannitol (1). D-Glucosamine (1.03 g, 4.78 mmol) was dissolved in distilled water (14 mL) and mixed at room temperature for 3 hours to achieve mutarotational equilibrium. Solid sodium nitrite (1.00 g, 14.5 mmol) was added and the solution was cooled to 0 ^{o}C. Glacial acetic acid (0.54 mL) was added dropwise which caused the evolution of nitrogen gas. After mixing at 0 ^{o}C for two hours, the solution was warmed to room temperature and argon gas was bubbled through the solution for 30 minutes. To the resulting yellow solution was re-cooled to 0 ^{o}C and solid $NaBH_4$ (0.900 g, 23.9 mmol) was added in small portions. After completion of gas evolution, the solution was warmed to room temperature and mixed for 18 hours. The mixture was filtered off and then quenched with Amberlite IR120 (H^+). The resin was filtered off and the resulting filtrate was concentrated *in vacuo* to give a white solid. As previously reported,[1] it was convenient to peracetylate crude 2,5-anhydro-D-mannitol **1** for a convenient purification. Therefore, crude 2,5-anhydro-D-mannitol was dissolved in pyridine (5 mL). Acetic anhydride (5 mL) was added and the solution was cooled to 0 °C. Then, 4-dimethylaminopyridine (0.06 g, 0.48 mmol) was carefully added in a small portion at 0 ^{o}C and the solution was warmed up to room temperature. After 18 hours, the solution was cooled to 0 ^{o}C and H_2O was added (10 mL). The solution extracted with CH_2Cl_2 (3 x 15 mL) and the combined organic layers were washed with 10% aqueous H_2SO_4. (20 mL) and H_2O (20 mL). The solution was dried over anhydrous $MgSO_4$, filtered and concentrated to a viscous off yellow oil. The crude mixture was then purified *via* column chromatography (9:1 to 1:1 Hexane: EtOAc) to afford the desired compound as a clear colourless oil that matched previously reported data [24] (1.19 g, 3.59 mmol, 75% yield from D-glucosamine): ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.18-5.14 (m, 2H), 4.30-4.15 (m, 6H), 2.09 (s, 9H). Peracetylated 2,5-anhydro-D-mannitol (1.19 g, 3.59 mmol) was dissolved in methanol (10 mL) and 1.5 M NaOMe in methanol (0.68 mL) was added. The solution was mixed at room temperature for 1 h and subsequently neutralized with Amberlite IR-120 (H^+). The resin was filtered off and the filtrate concentrated to afford 2,5-anhydromannitol as a viscous, slightly yellow oil (0.56 g, 3.4 mmol, 95 % yield). The crude mixture was then crystallized with (MeCN/MeOH) to afford the 2,5-anhydro-D-mannitol **1** as clear crystals that matched previously reported characterization data. ^{1}H NMR (300 MHz, D_2O): δ 4.15-4.05 (m, 2H), 3.95-3.85 (m, 2H), 3.79 (dd, J = 3.1,12.2 Hz, 2H), 3.70 (dd, J = 5.6, 12.6 Hz, 2H).

[1]S. Cassel, C. Debaig, T. Benvegnu, P. Chaimbault, M. Lafosse, D. Plusquellec and P. Rollin, *Eur. J. Org. Chem.,* 2001, 875.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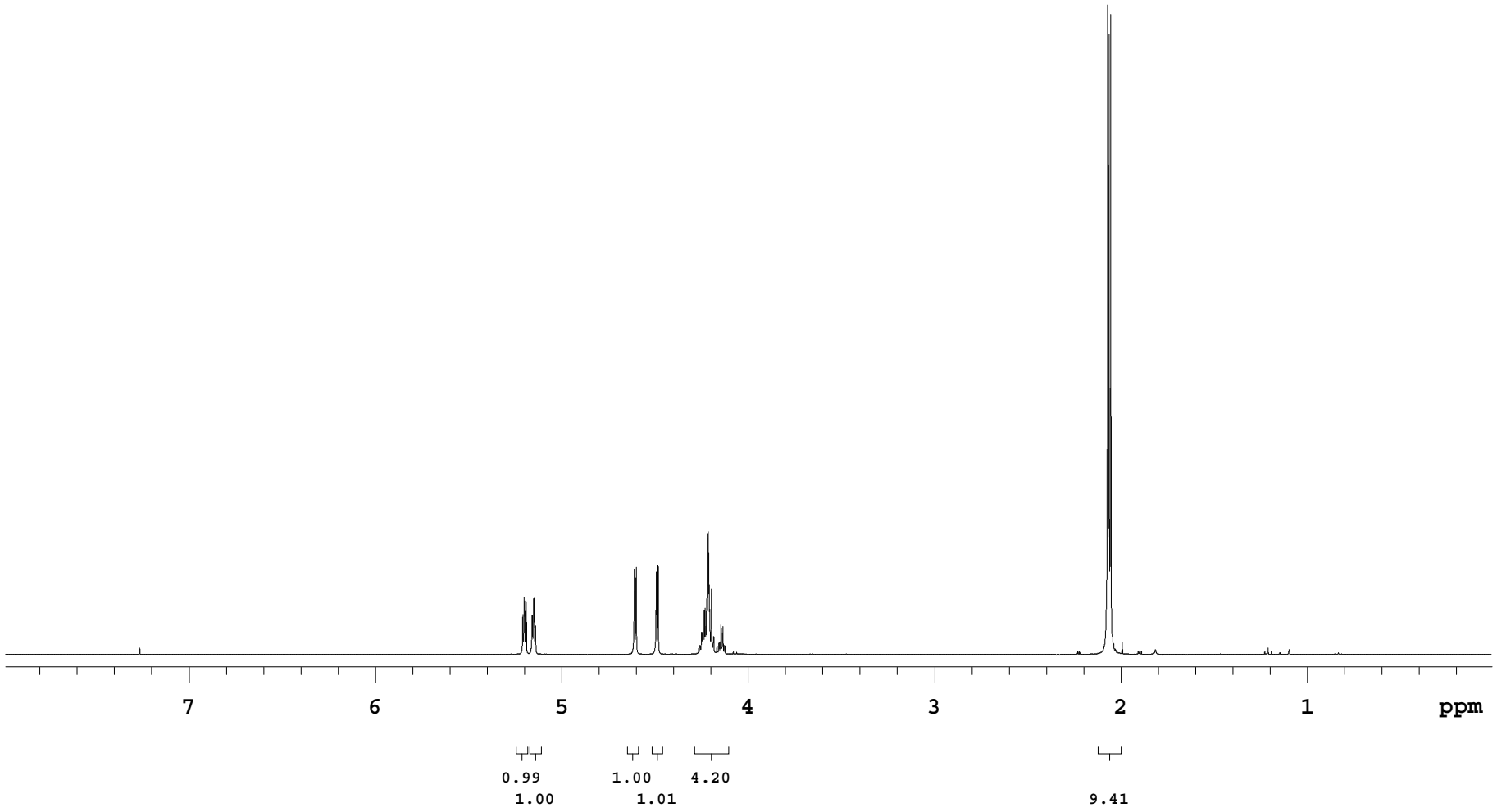
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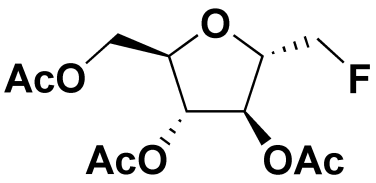
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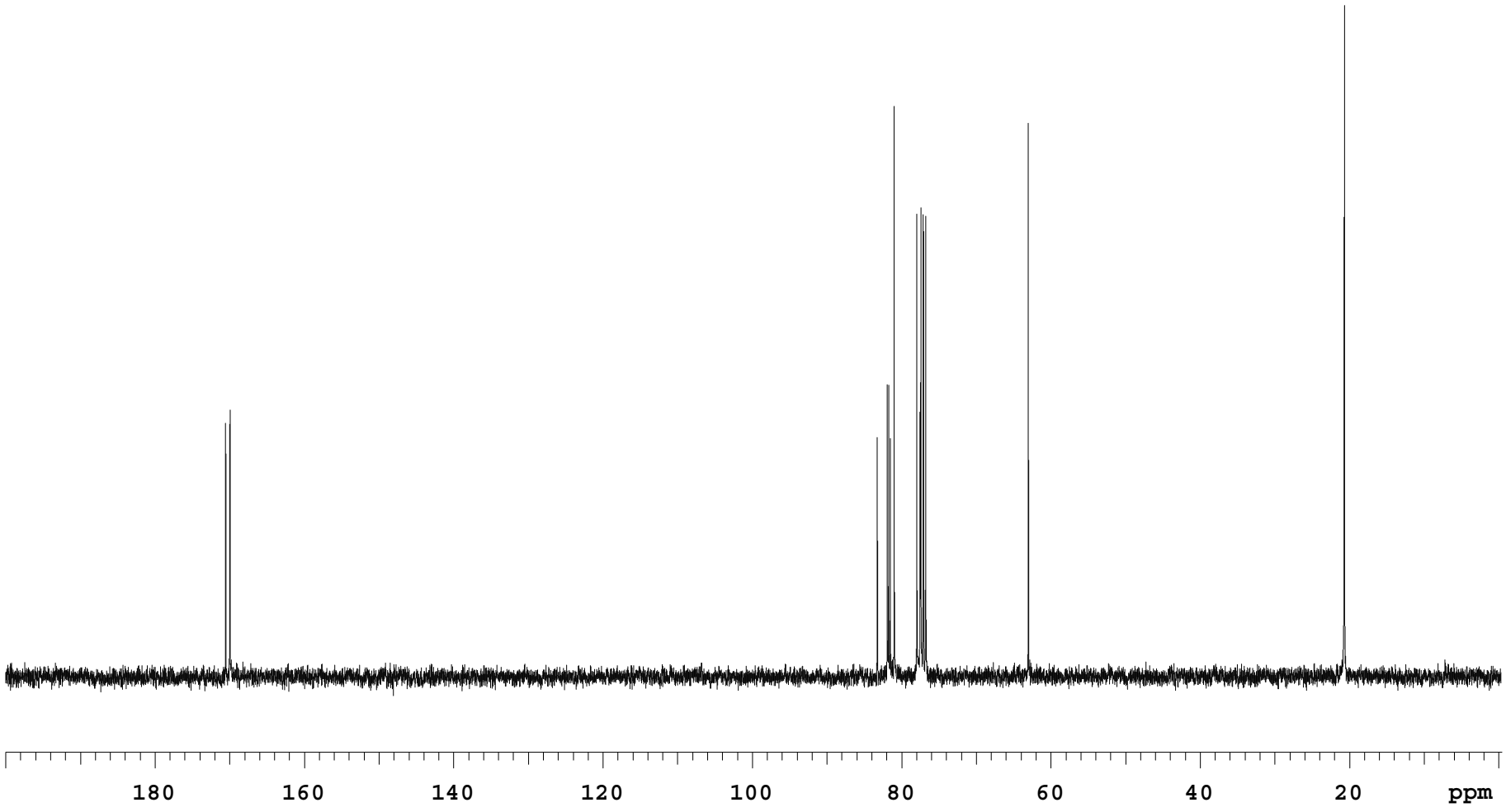
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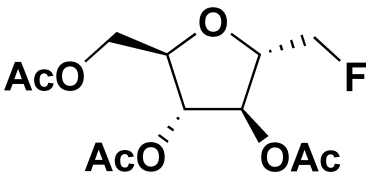
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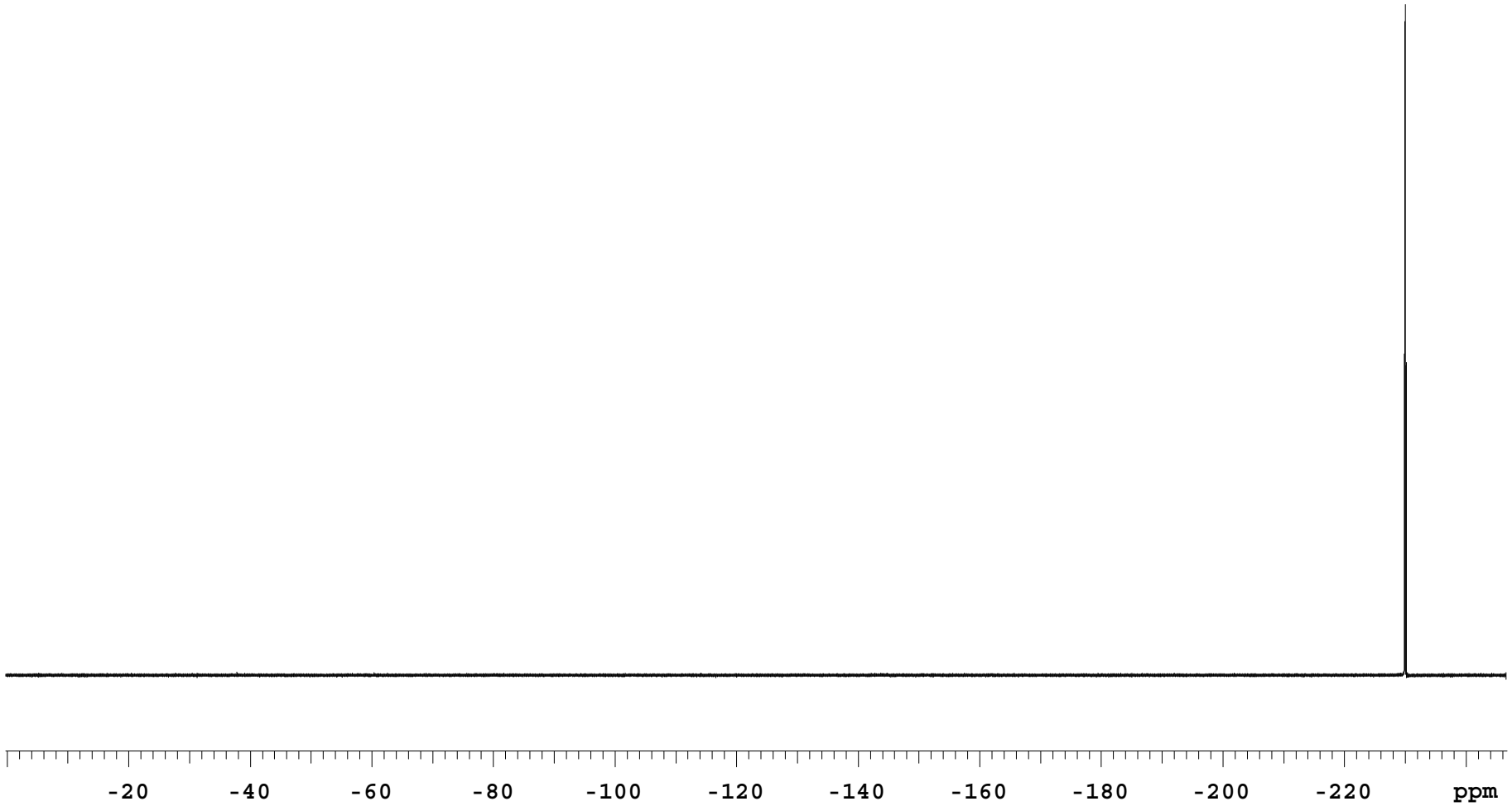
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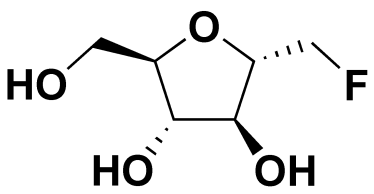
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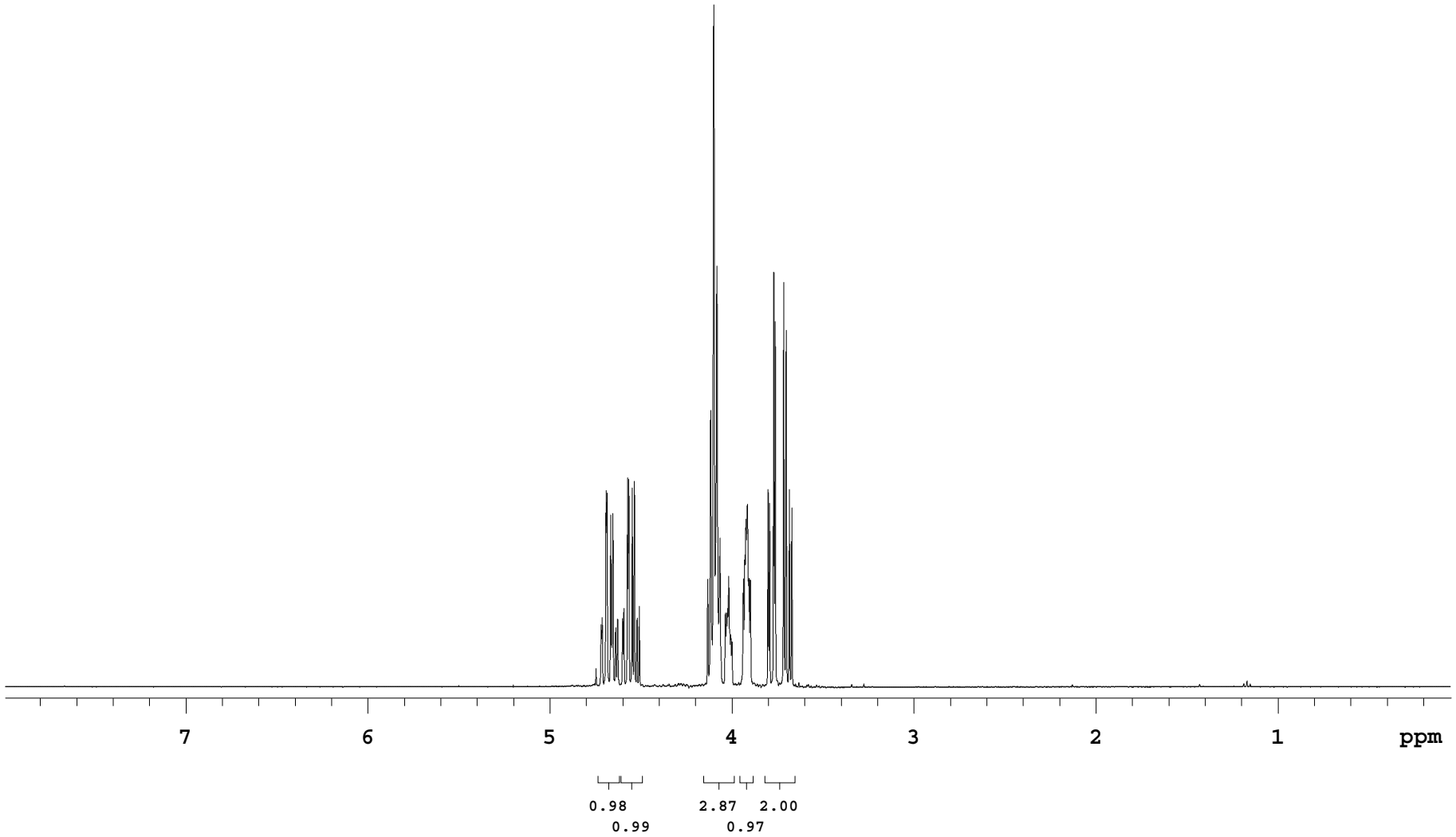
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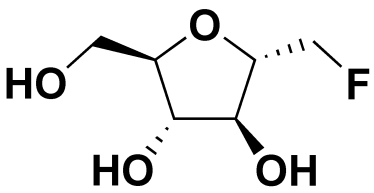
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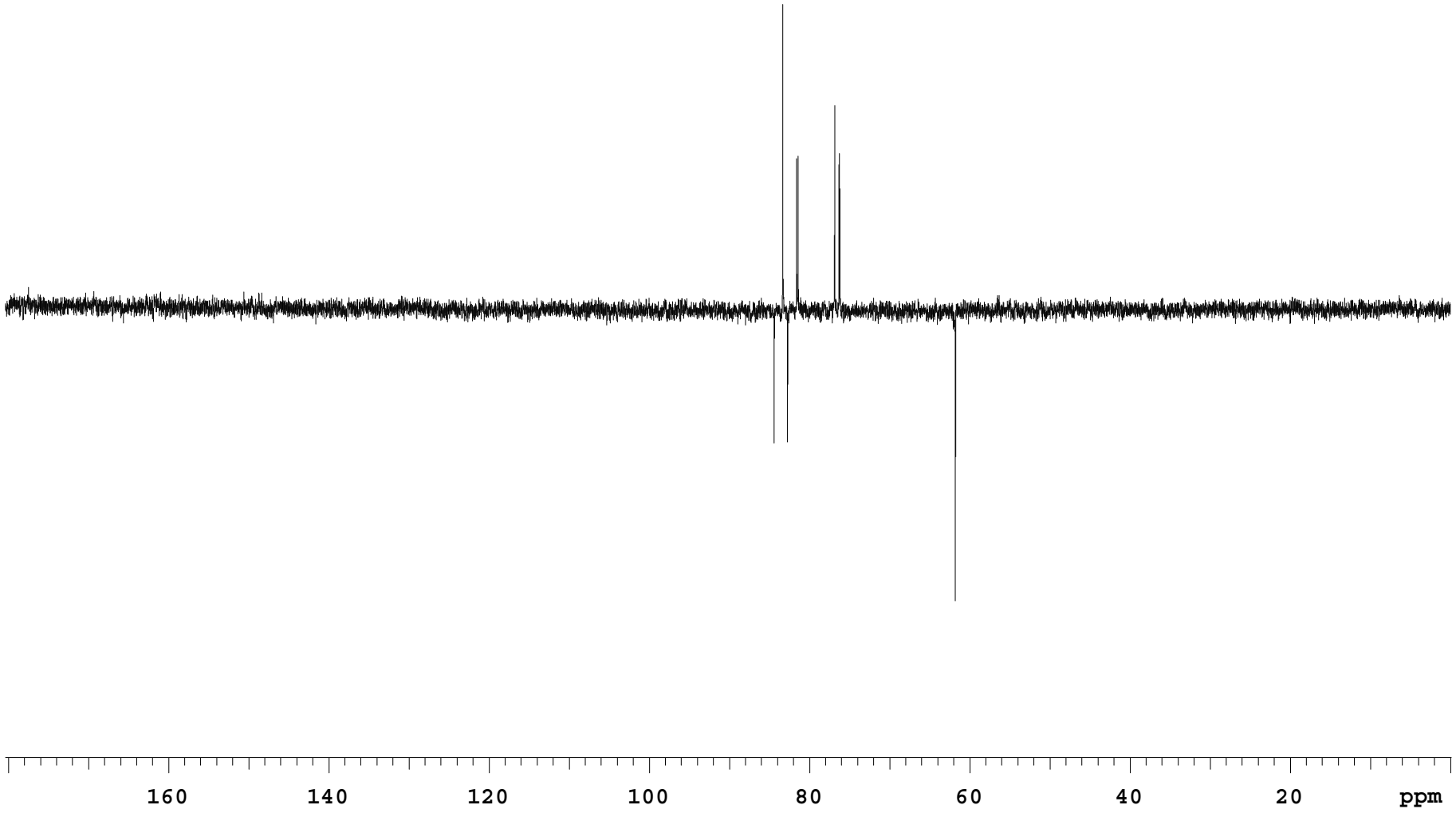
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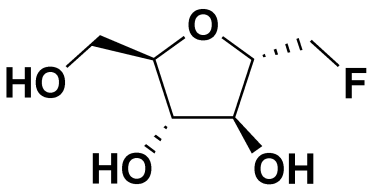
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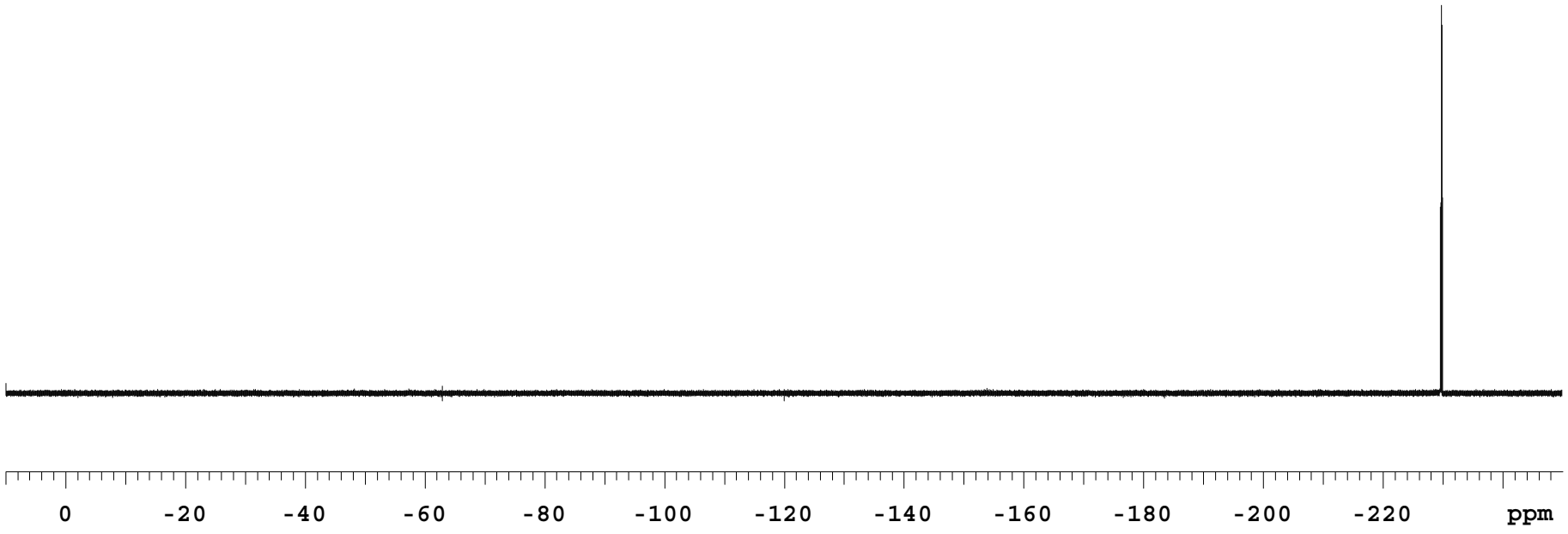
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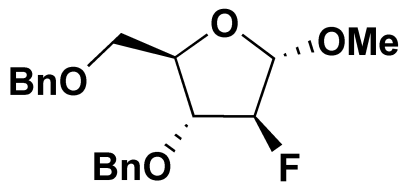
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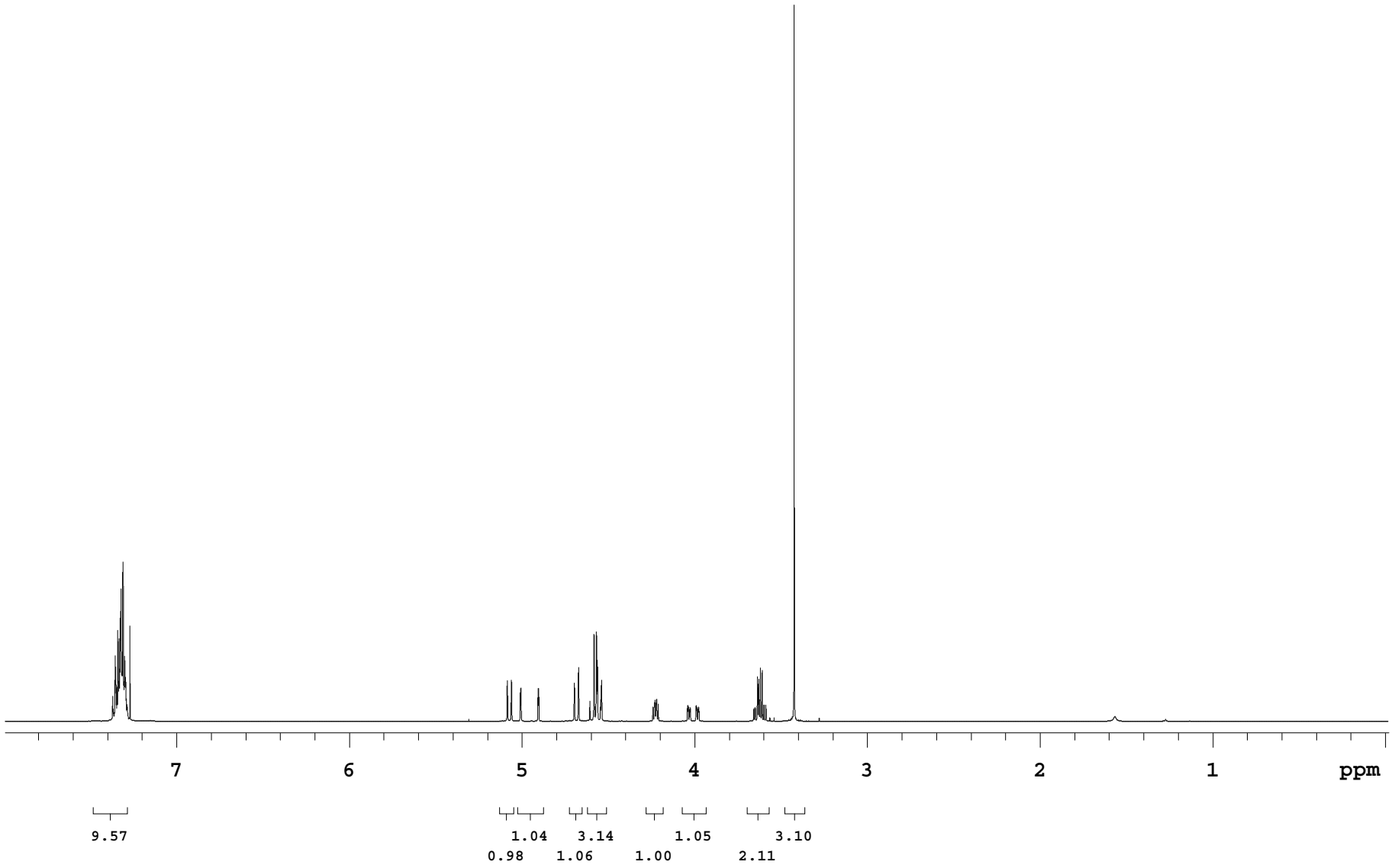
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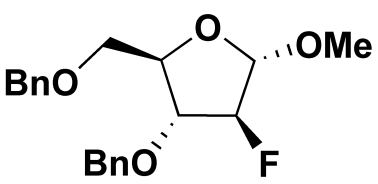
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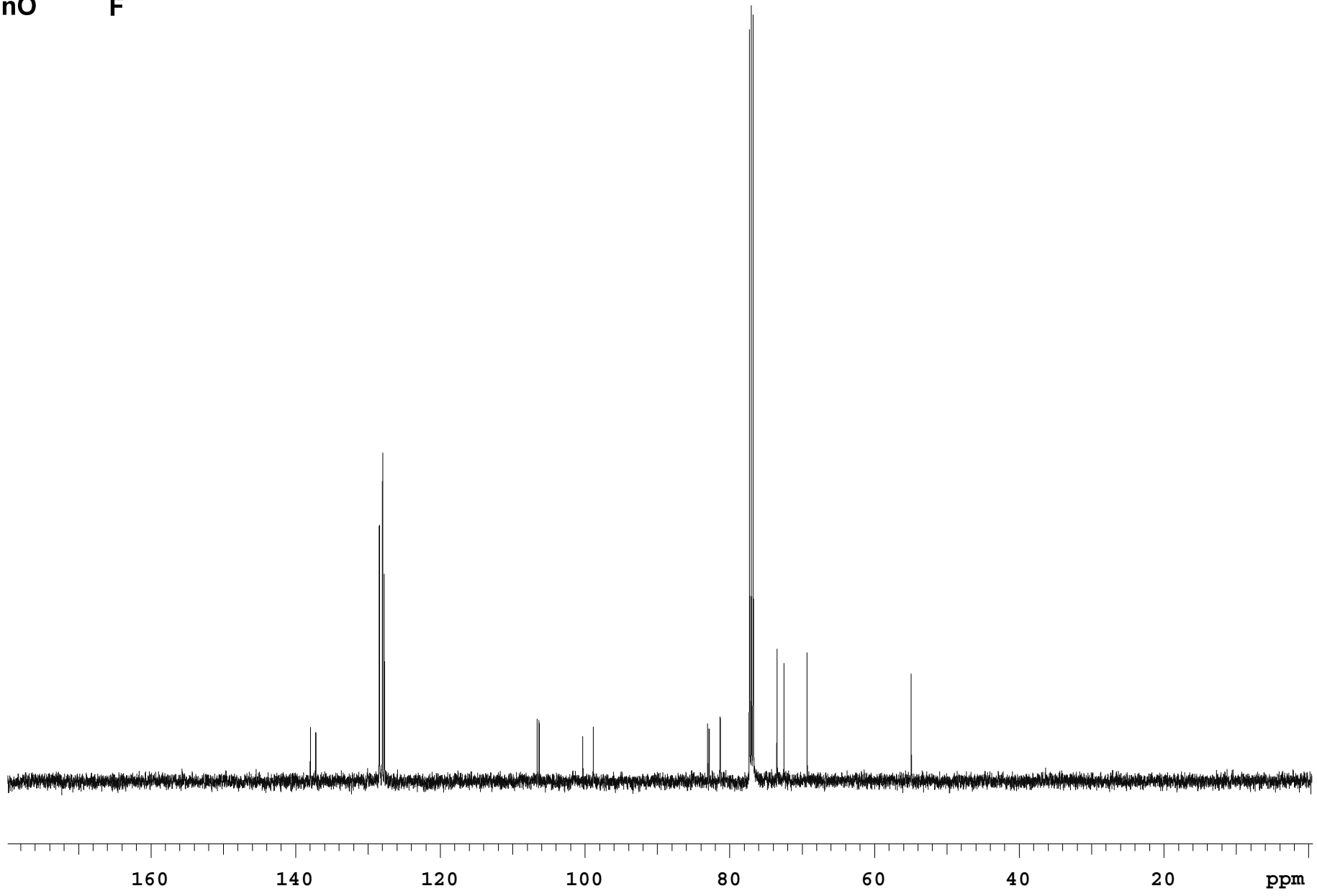
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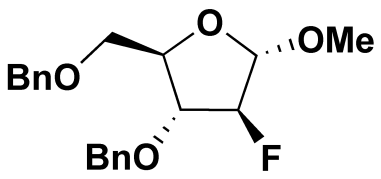
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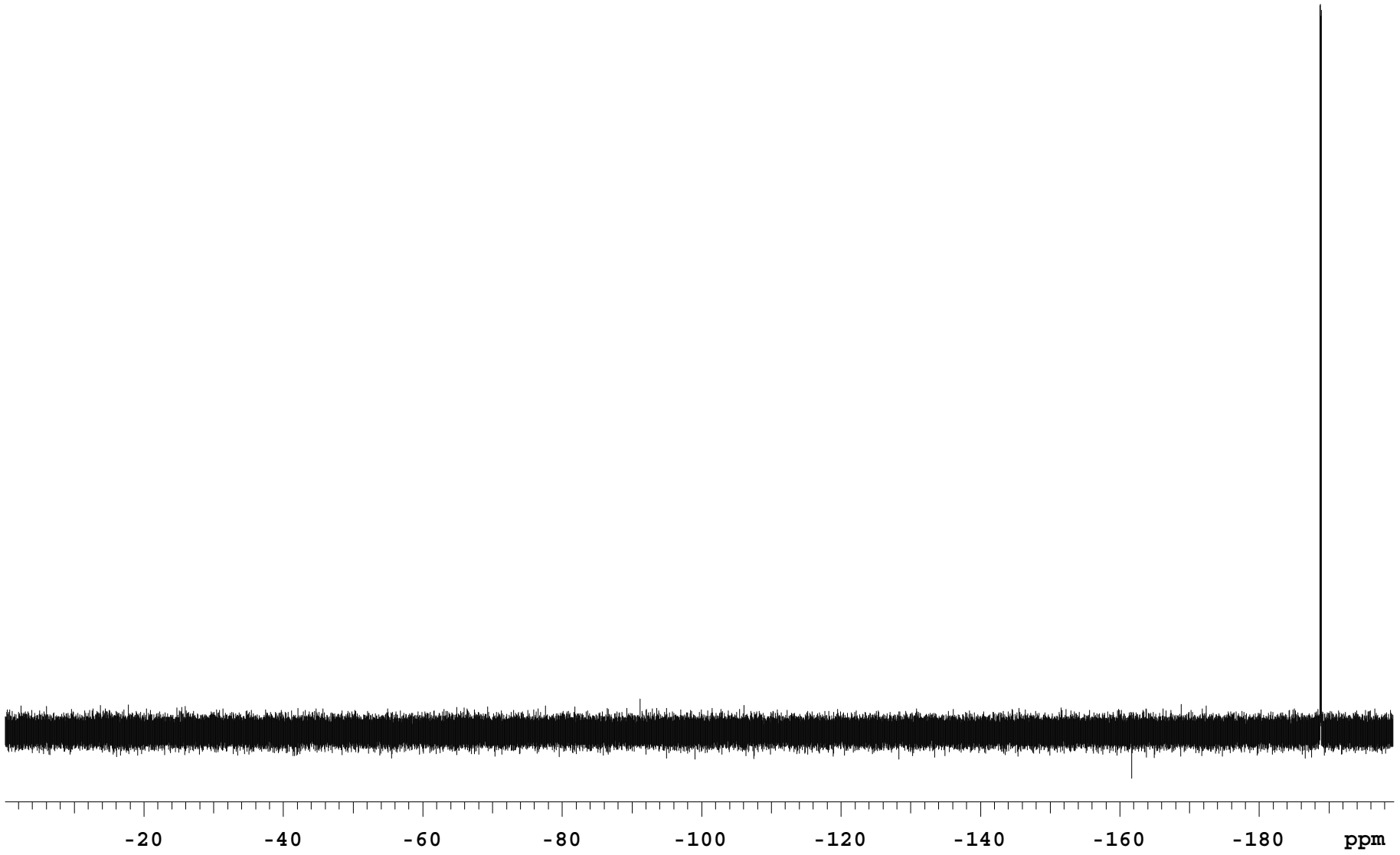
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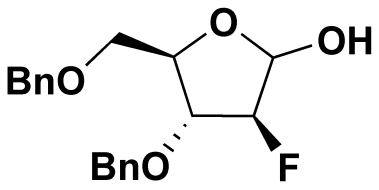
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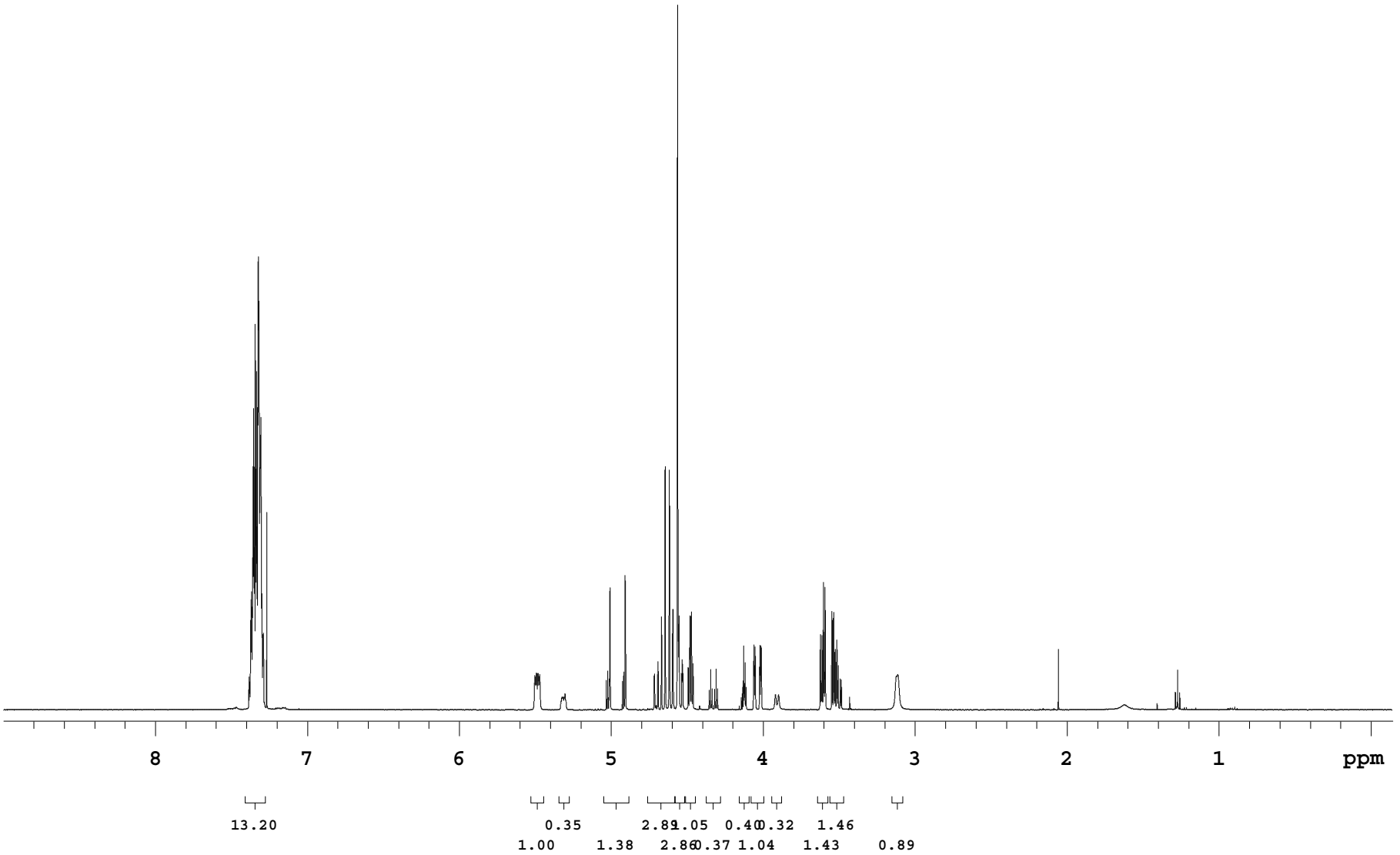
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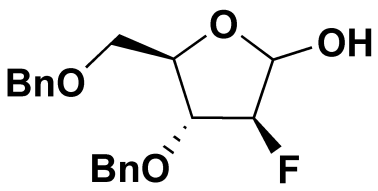
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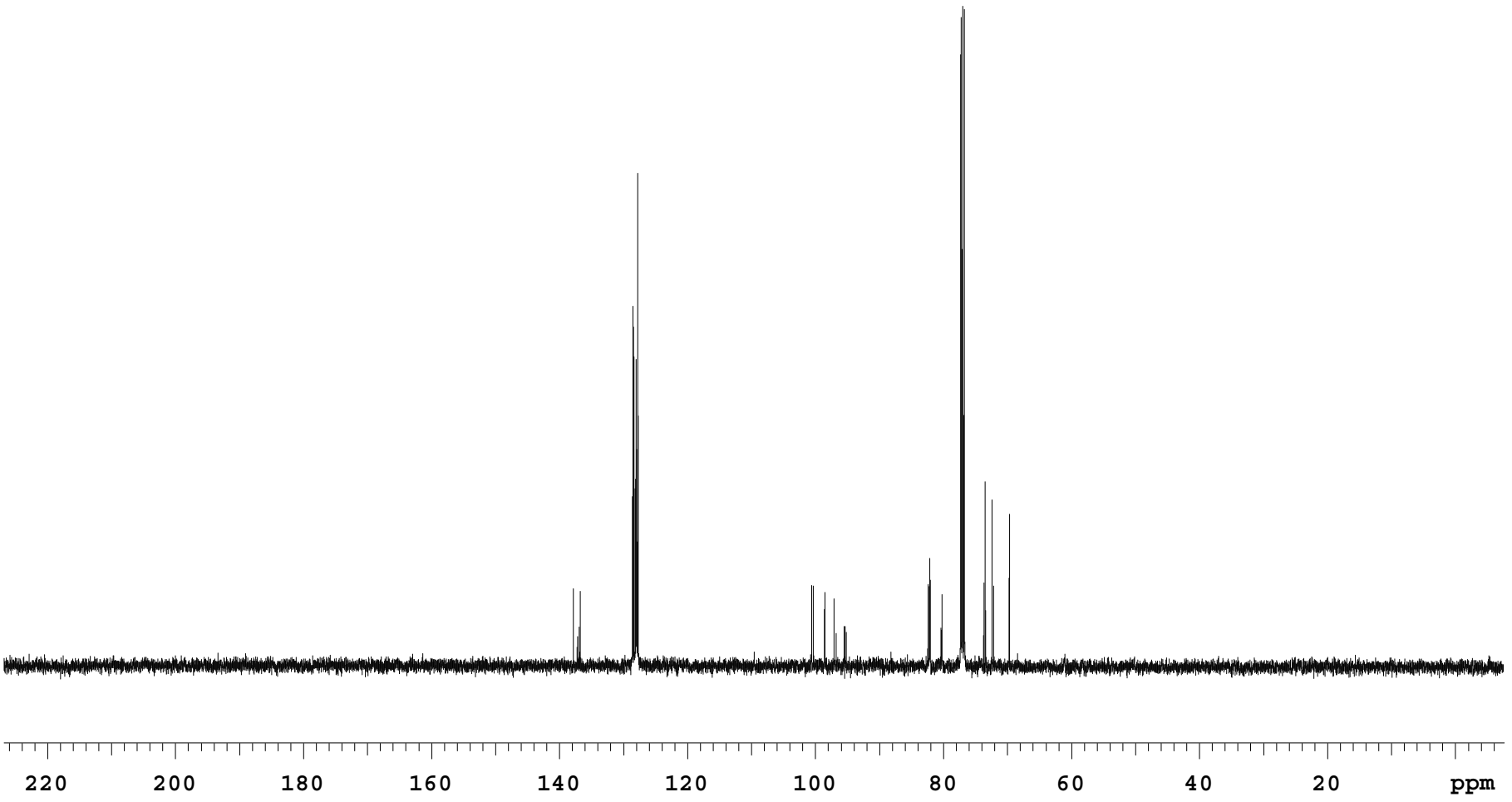
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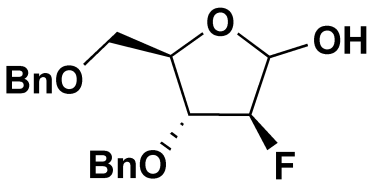
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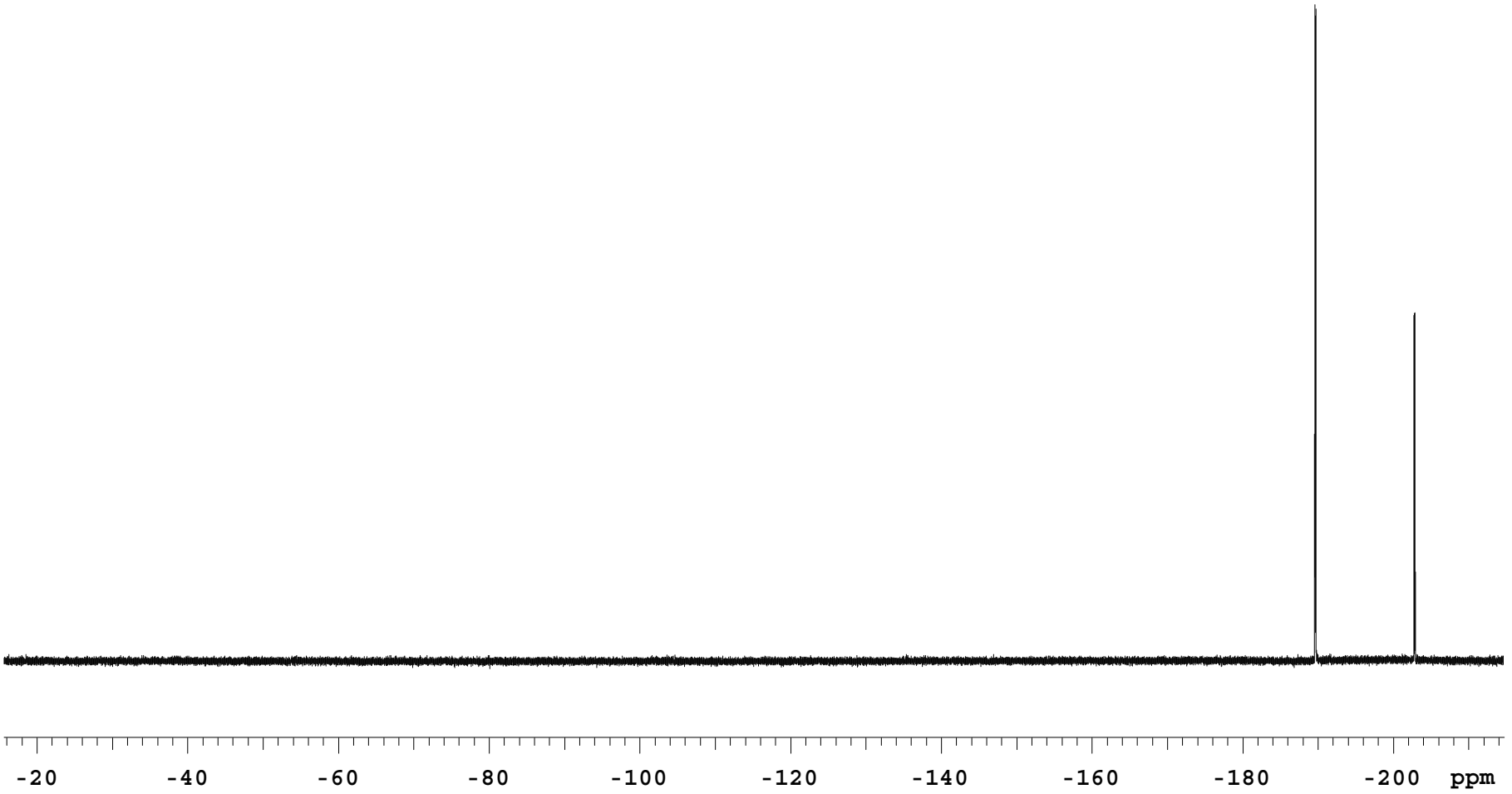
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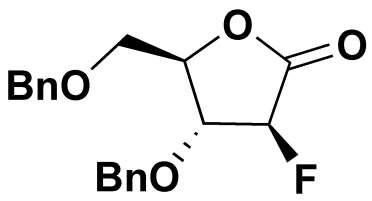
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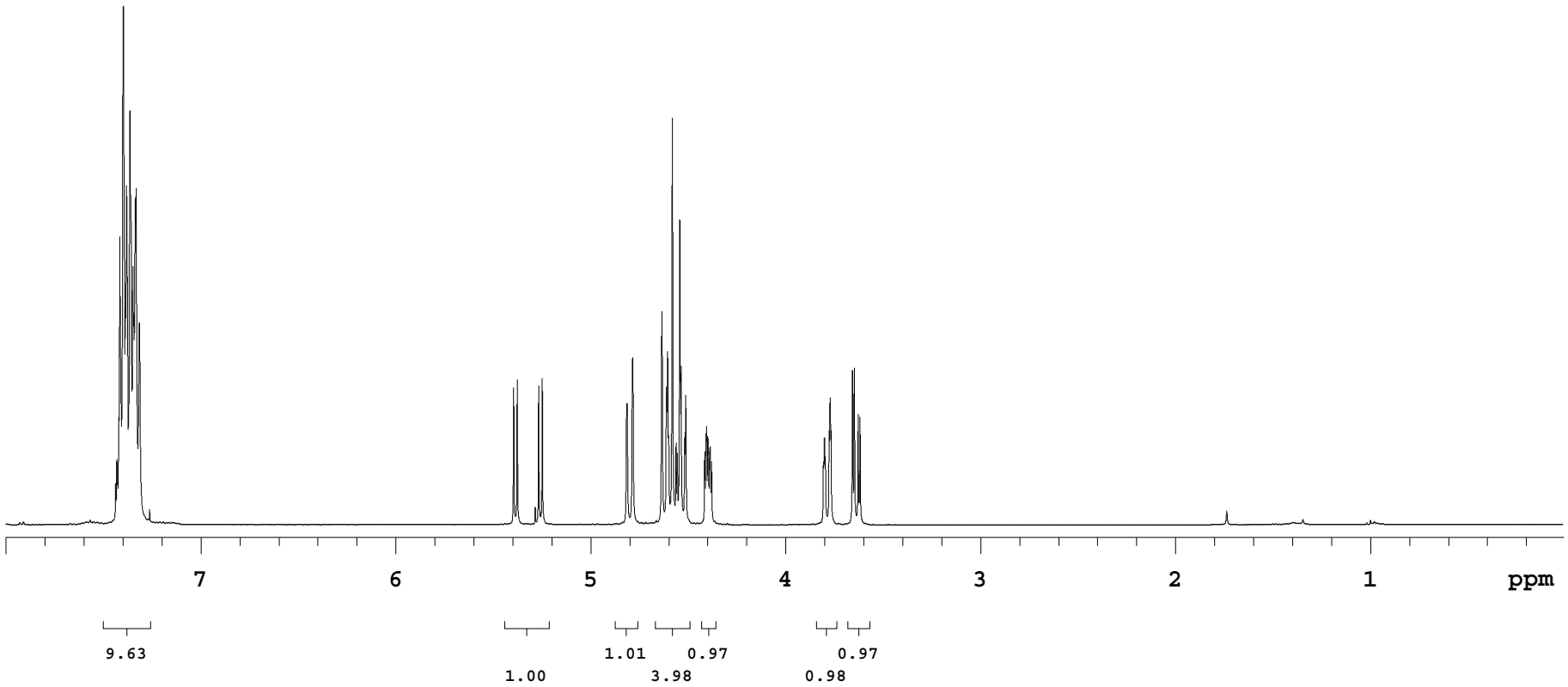
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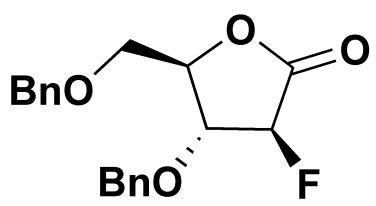
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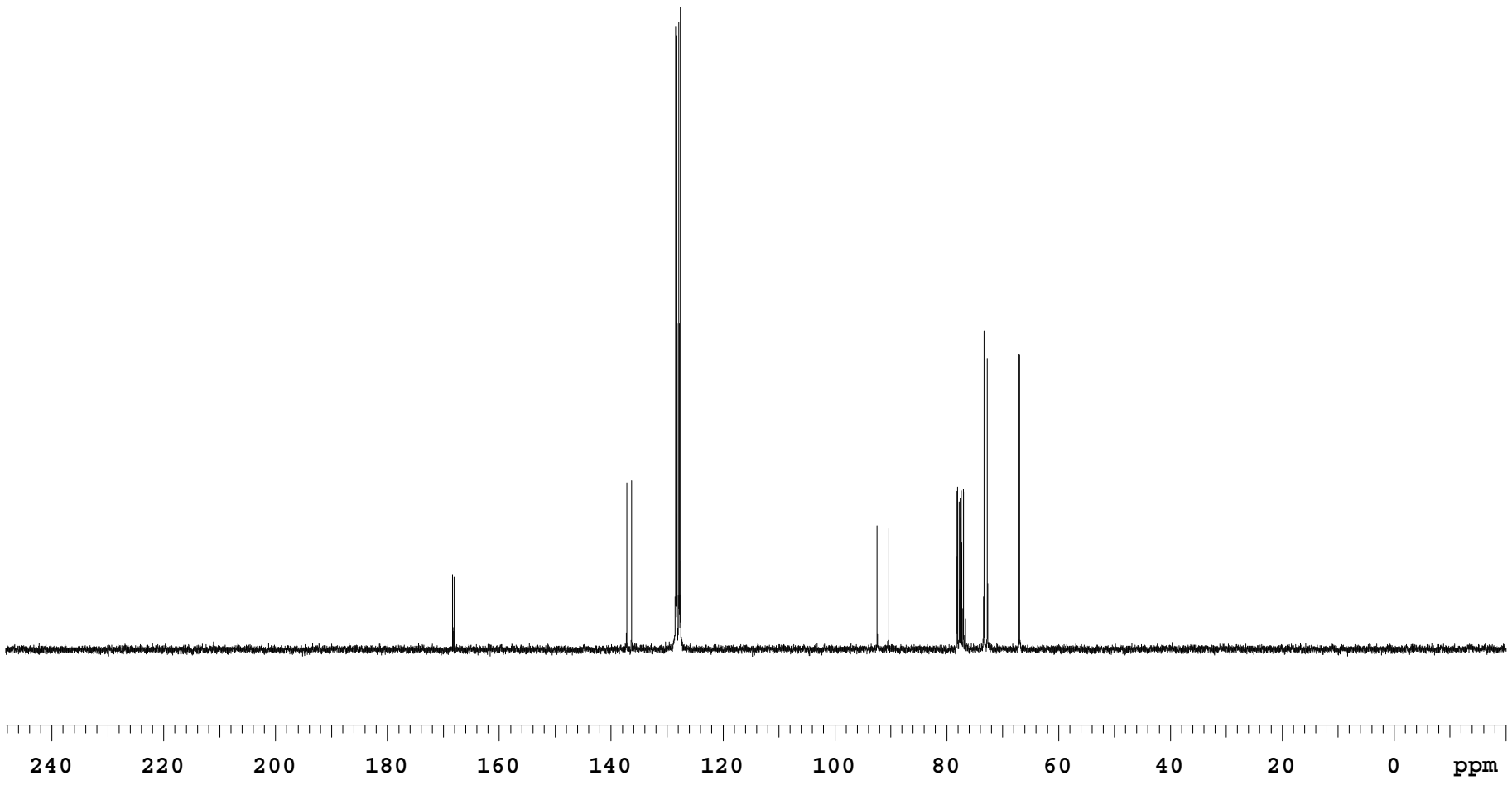
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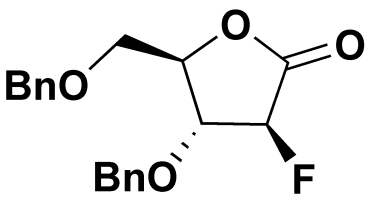
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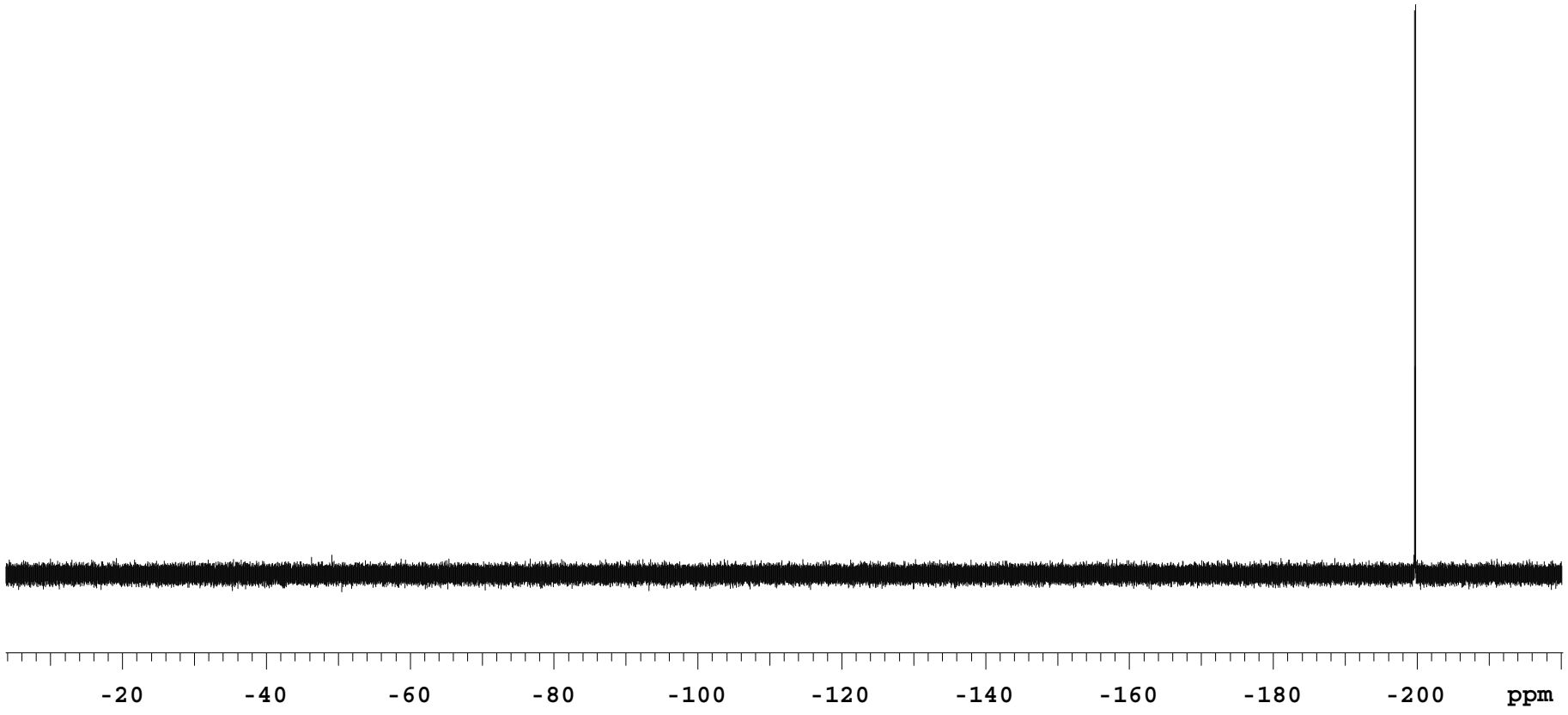
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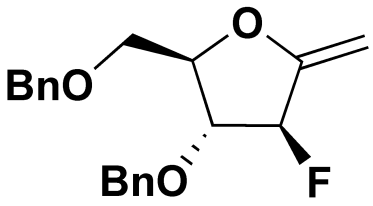
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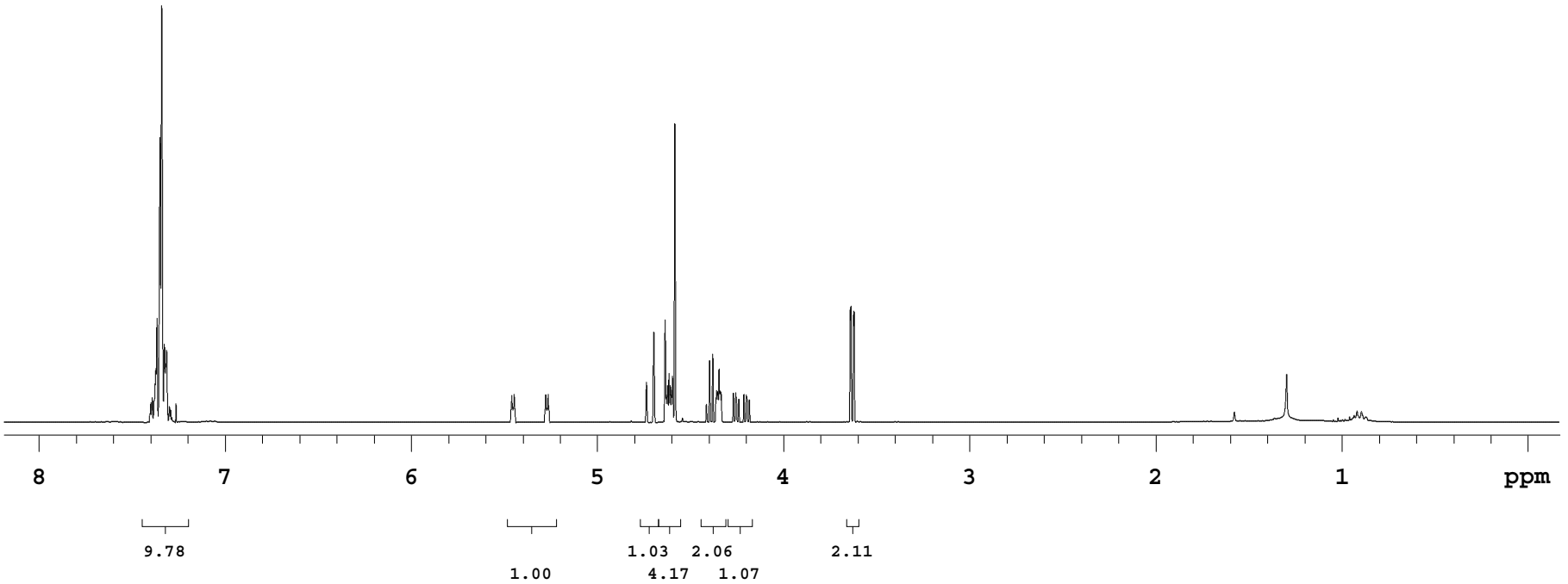
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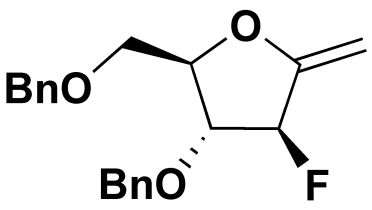
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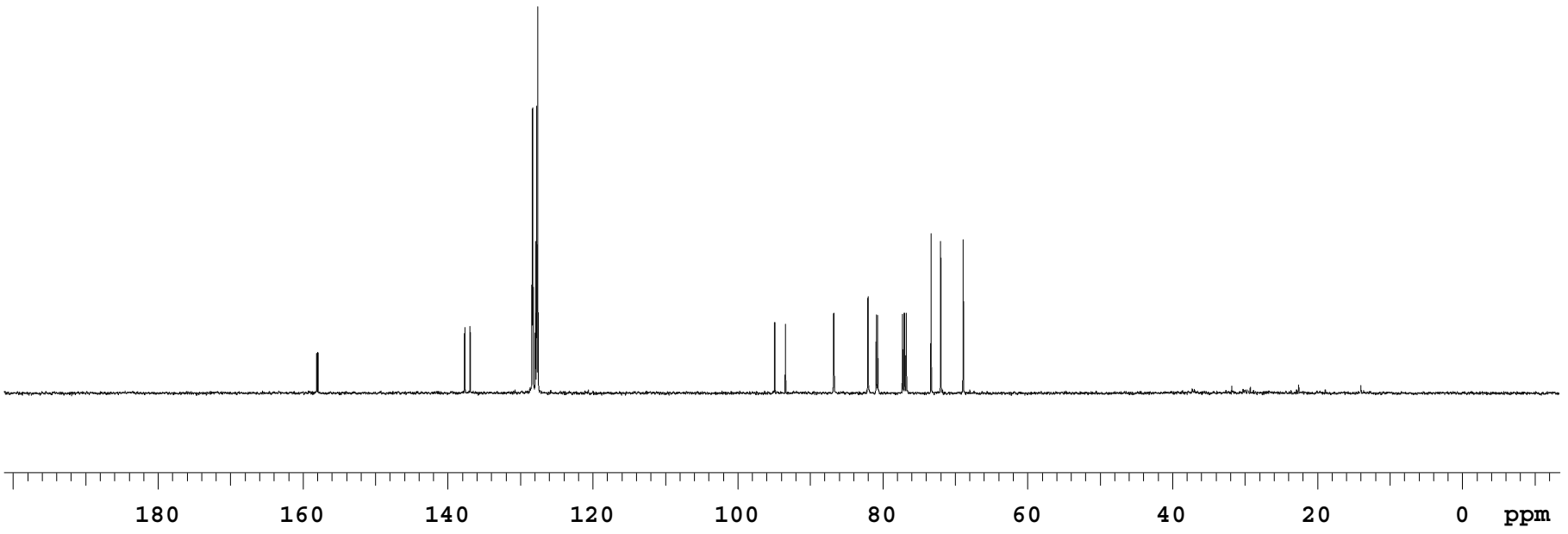
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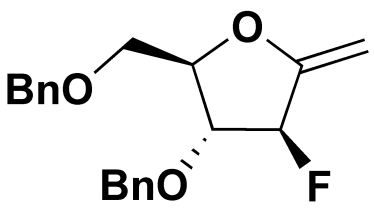
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BnO
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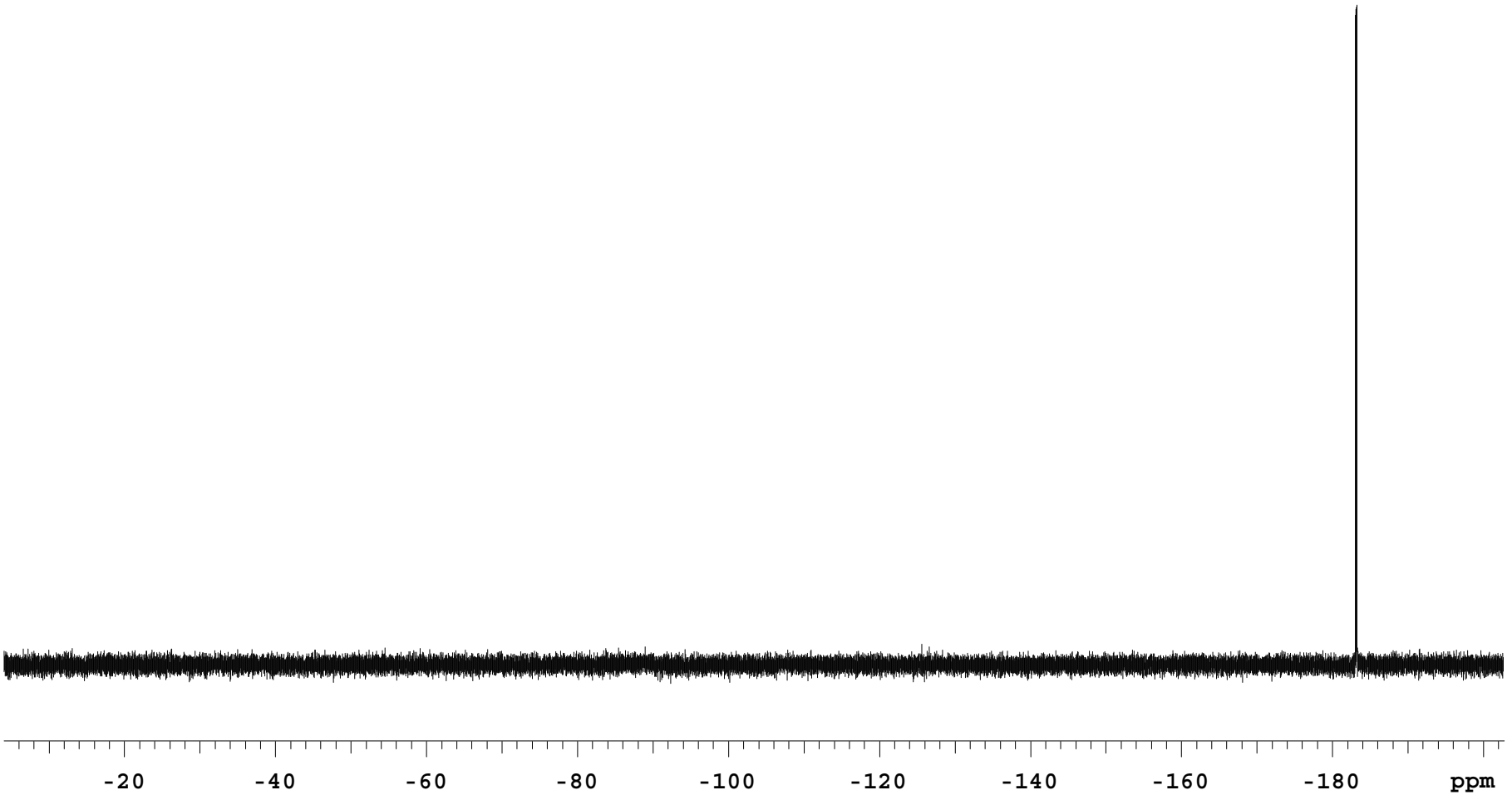
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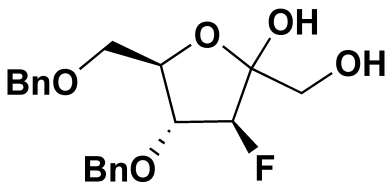
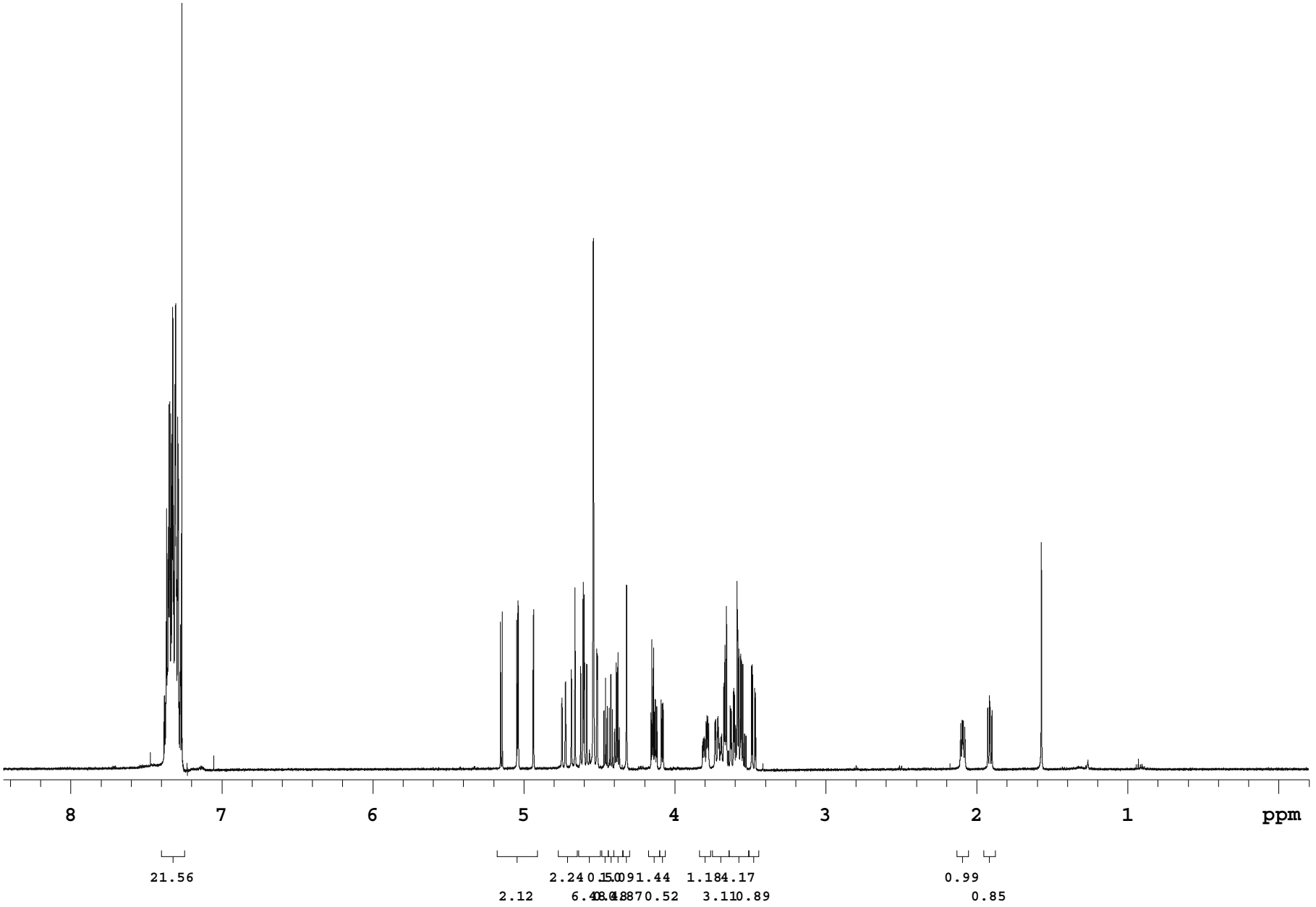
-180
ppm


BnO
O
OH
OH
BnO
F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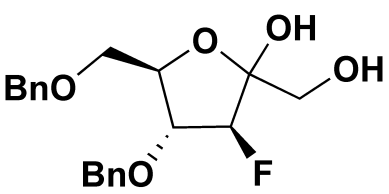
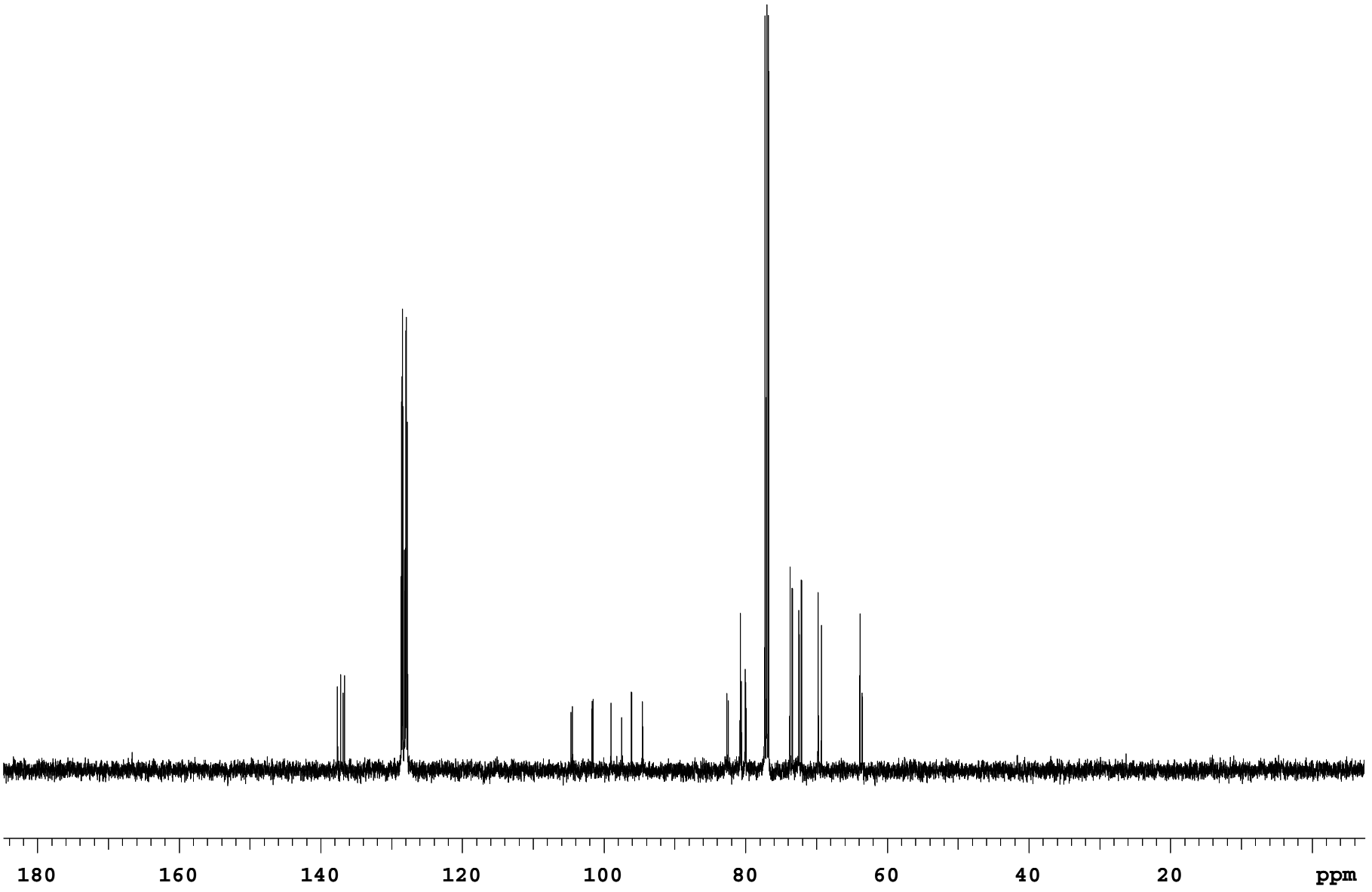
7
6
5
4
3
2
1
ppm
21.56
2.12
2.24
0.15
0.09
1.44
1.18
4.17
0.99
0.85
0.52
3.11
0.89


BnO
O
OH
OH
BnO
F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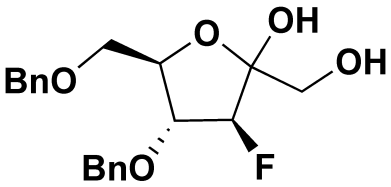
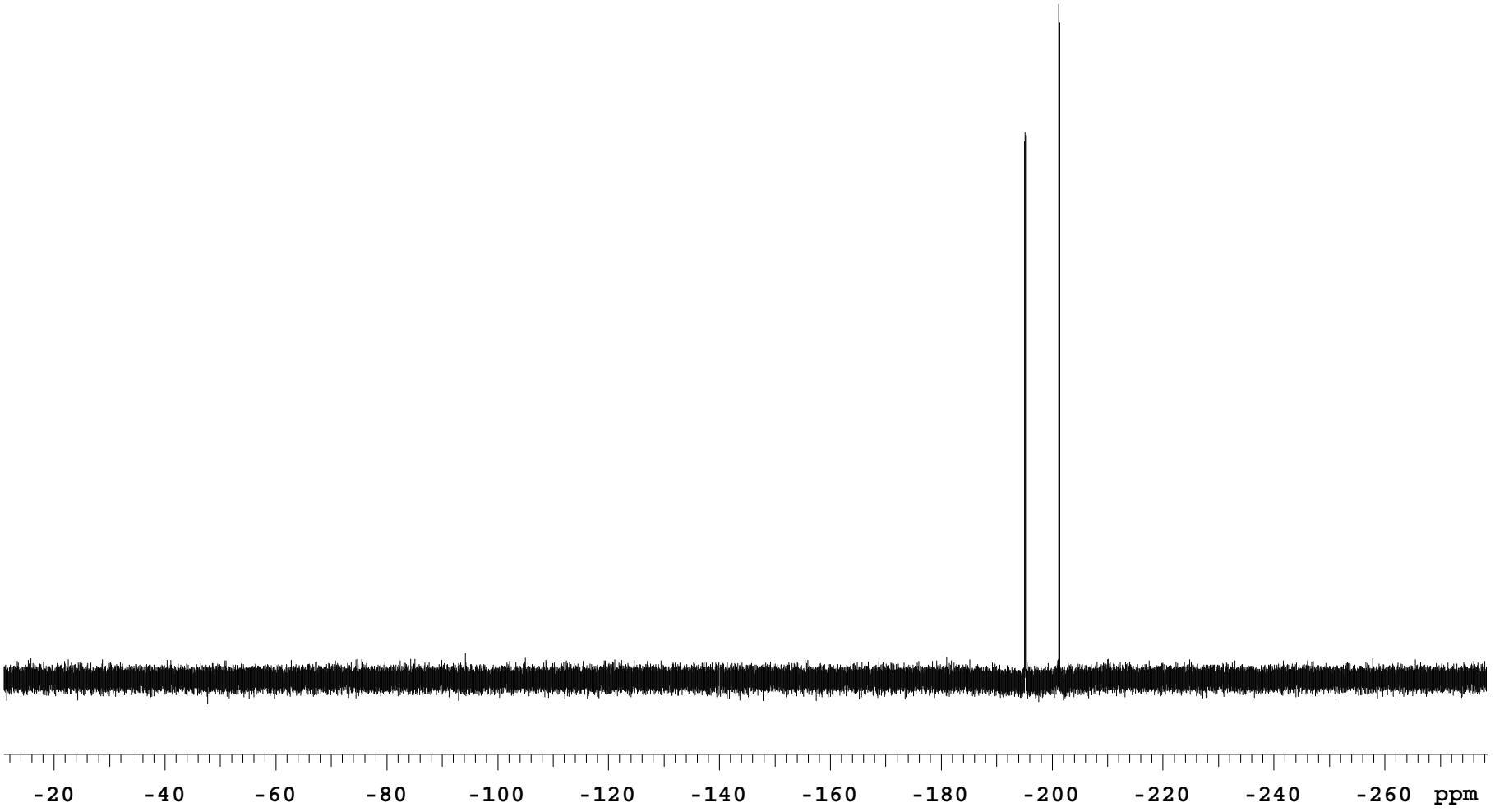
100
80
60
40
20
ppm


BnO
O
OH
OH
BnO
F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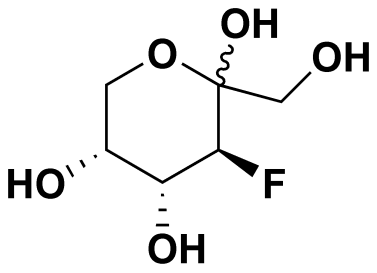
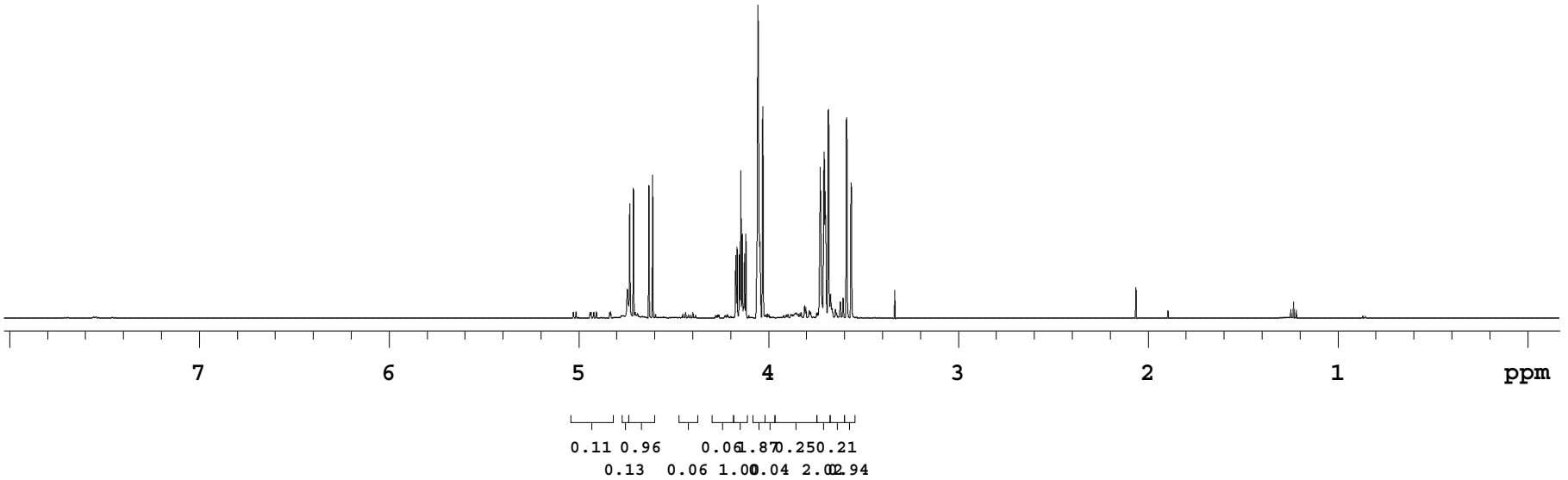
ppm


OH
O
OH
HO
F
OH


7
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5
4
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2
1
ppm
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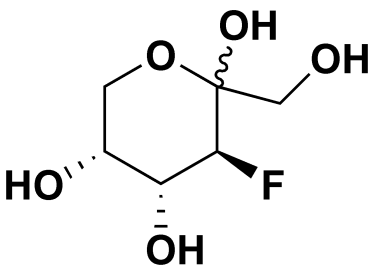
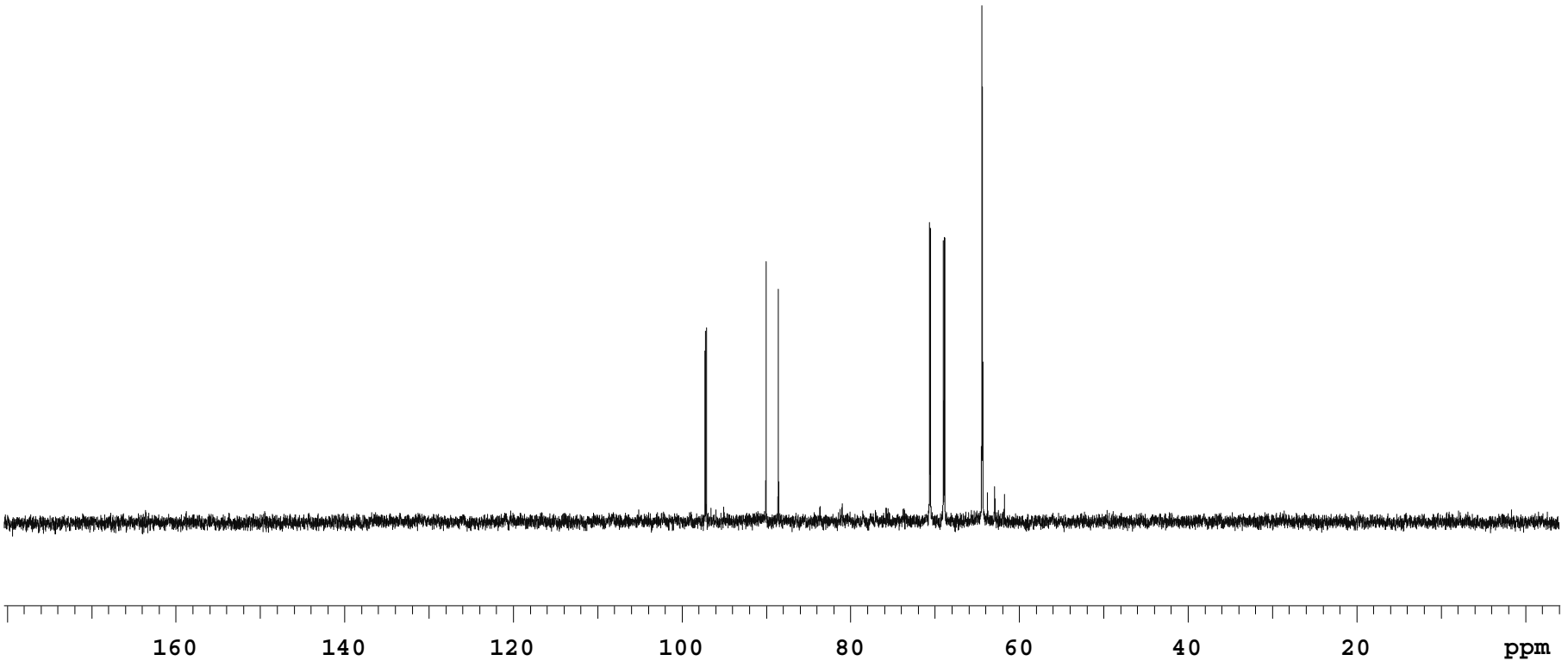
0.13 0.06 1.00 0.04 2.00 2.94


OH
O
OH
HO
F
OH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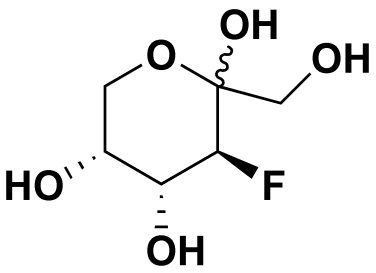
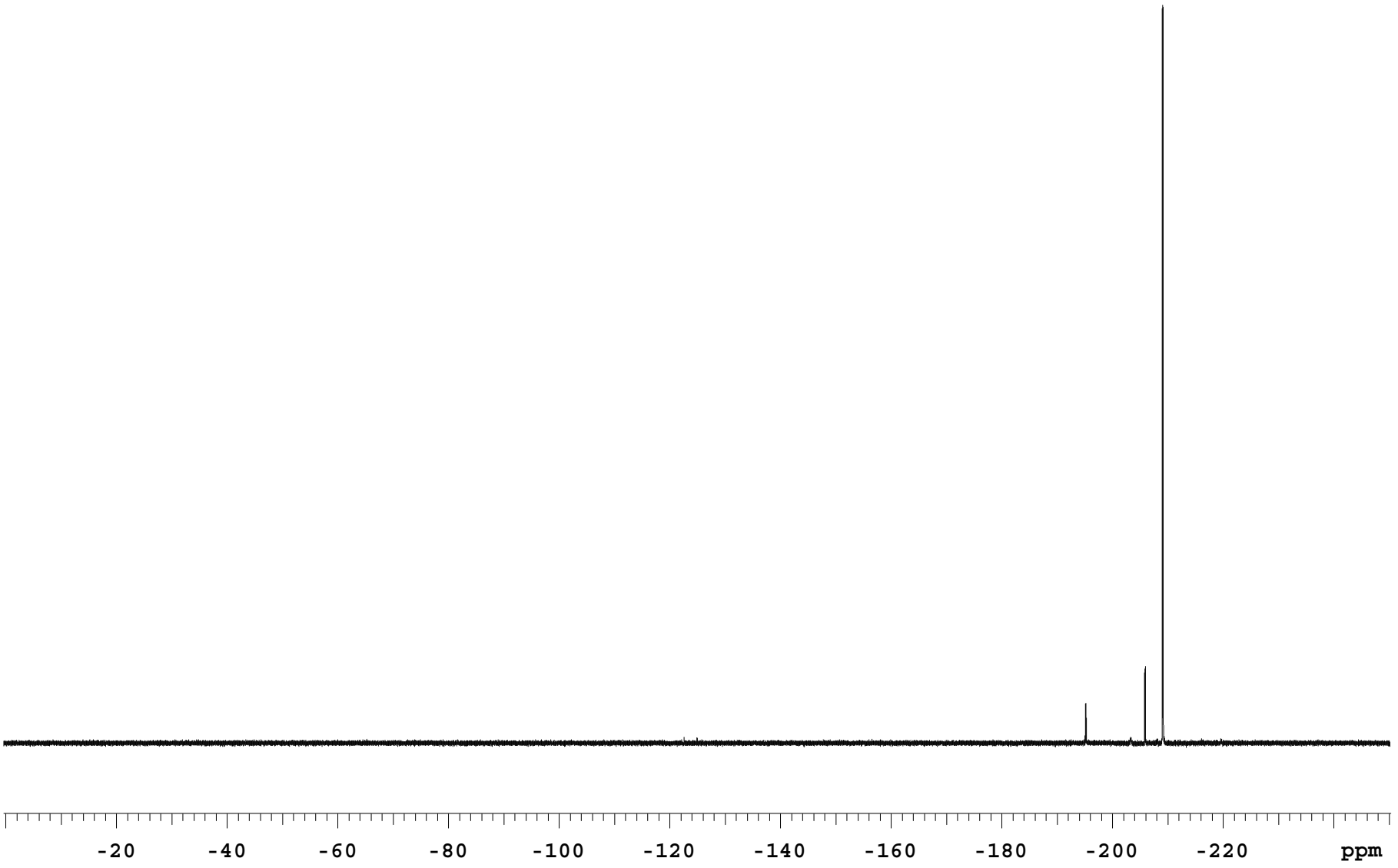
80
60
40
20
ppm


OH
O
OH
HO
F
OH


-20
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-100
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-160
-180
-200
-220
ppm