Supporting Information

for

Facile Enzymatic Synthesis of Phosphorylated Ketopentoses

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General methods. ^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR spectra were recorded on a Bruker 400-MHz NMR spectrometer (D_2O as the solvent). High resolution electrospray ionization (ESI) mass spectra were obtained using Thermo HPLC-Orbitrap Elite. Thin-layer chromatography (TLC) was performed on silica gel 60 F254 plates (Merck, MA) using *p*-anisaldehyde sugar stain. High performance liquid chromatography (HPLC) was performed on a Shimadzu SPD-20A equipped with evaporative light scattering detector (ELSD) or ultraviolet (UV) detector. The HPLC columns used in this work are HPX-87H (100 cm × 2.5 cm) (Bio-Rad, Hercules, CA), Sugar-Pak 1 column (Waters Corp., Milford, MA) and ZIC®-cHILIC (Merck, Darmstadt, Germany). Gel filtration chromatography was performed using a column packed with Bio-Gel P-2 fine resins (45–90μm) (Bio-Rad, Hercules, CA).

Bacteria strains, plasmids and chemicals. *Pfx*50 DNA Polymerase, Subcloning Efficiency™ DH5α™ competent cells and *E. coli* BL21 (DE3) chemically competent cells were from Invitrogen (Grand Island, NY). Plasmid pET-28a was from Novagen (Madison, WI). Restriction enzymes and T4 DNA ligase were from NEB (Beverly, MA). *Escherichia coli* and *Bacillus subtilis* genomic DNA used for PCR in this work was extracted by using QIAamp DNA Blood Mini Kit (Valencia, CA). ATP was from Carbosynth (San Diego, CA). All other chemicals unless otherwise stated were purchased from Sigma without further purification.

Plasmid constructions and protein purification. L-arabinose isomerase (AraA) from *Bacillus subtilis*,[1] D-xylulose kinase (XylB),[2] L-ribulose kinase (AraB),[3] L-xylulose kinase (LyxK)[4] were amplified from *Escherichia coli* genomic DNA. All genes were cloned into pET-28a vector and the recombinant plasmids were confirmed by restriction mapping and sequencing. The confirmed constructs were subsequently transformed into *E.coli* BL21 (DE3) for protein expression. After being induced by IPTG, bacteria cells were harvested by centrifugation and re-suspended in lysis buffer (50 mM Tris-HCl, 300 mM NaCl, 10 mM imadozle; pH 8.0). Cells were disrupted by a microfluidizer and the lysate was removed by centrifugation (12,000 g, 30 min). The supernatant was loaded onto a Ni-NTA agarose column equilibrated with the lysis buffer (50 mM Tris-HCl, 300 mM NaCl, 10 mM imadozle; pH 8.0). The column was washed with 2 column volumes of the lysis buffer and 2 column volumes of the wash buffer (50 mM Tris-HCl, 300 mM NaCl, 30 mM imadozle; pH 8.0). The proteins were finally eluted with elution buffer (50 mM Tris-HCl, 300 mM NaCl, 300 mM imadozle; pH 8.0). The purified proteins were desalted by filtration (Millipore, 15,000 MWCO). Fructokinase (HK) from humans,[5,6] L-rhamnulose kinase (RhaB) from *Thermotoga maritima* MSB8, D-tagatose 3-epimerase (DTE) from *Pseudomonas* Sp, ST-24,[7] D-xylose isomerase (XylA)[8] and acid phosphatase (AphA)[9] from *Escherichia coli* BL21 were prepared as previously reported.[10] The protein concentration was determined by the Bradford method with bovine serum as a standard and the purity was confirmed by SDS-PAGE.

Substrate specificity of kinases. Substrate specificity of XylB, AraB and LyxK was studied by the reactions that were performed in 50 ul reaction mixture containing a Tris-HCl buffer (100 mM, pH 7.5), 20 mM of sugar standards, 20mM of ATP, 5 mM of Mg^{2+}, and 10 ug of enzymes. The reactions were carried at 37°C for 10

minutes and were stopped by diluting ten times using a cold buffer of acetonitrile/100 mM aqueous ammonium acetate pH 4.5 (60% acetonitrile). The reactions were quantified by analyzing the formation of ADP by HPLC equipped with UV detector at 254 nm using ZIC®-cHILIC column. The column was eluted at 30°C with acetonitrile/100 mM aqueous ammonium acetate pH 4.5 (60% acetonitrile) at a flow rate of 0.6 ml/min.

Preparative scale synthesis of D-xylulose 5-phosphate and L-ribulose 5-phosphate. Reactions were carried in a final volume of 250 ml reaction system containing 25 mM of ATP, 3 mM of Mg^{2+}, 3 mM of Mn^{2+}, 20 mM starting sugars and conversion-related enzymes (Table 1). D-xylose (5.0 mmol) was incubated with 20 mg of XylA and 20 mg of XylB. L-arabinose (5.0 mmol) was incubated with 15 mg of AraA and 20 mg of AraB. The reactions were carefully shaken at pH near 7.5 at 37°C to allow the formation of ketose 5-phosphates. The reactions were monitored by TLC, and HPLC equipped ELSD using HPX-87H column with pure water as mobile phase. Once no detectable starting sugars were found, silver nitrate (1 M) was added to precipitate ATP and ADP until no new precipitate formed. The precipitate was removed by centrifugation (14000 g, 1 min) and washed twice using distilled water. Sodium chloride was added to a final concentration of 200 mM to remove the remnant silver ions. Silver chloride was removed by centrifugation (14000 g, 1 min). The solution from each reaction was concentrated under reduced pressure and purified by using Bio-Gel P-2 column to afford final products.

D-xylulose 5-phosphate (1). (1143 mg, Yield 91%); ^{1}H NMR (D_2O, 400 MHz): δ 4.64 (d, 1 H, J = 19.4 Hz, H-1a), 4.52 (d, 1 H, J = 19.4 Hz, H-1b), 4.50 (d, 1 H, J = 1.8 Hz, H-3), 4.18-4.22 (m, 1 H, H-4), 3.85-3.89 (m, 2 H, H-5); ^{13}C NMR (D_2O, 100 Hz): δ 211.9, 73.9, 69.7, 65.0, 63.6; ^{31}P NMR (D_2O, 133 Hz): δ 2.23. HRMS (ESI) m/z calculated for $[C_5H_{11}O_8P - H]^-$ 229.0119, found 229.0122.

L-ribulose 5-phosphate (5). (1164 mg, Yield 92%); ^{1}H NMR (D_2O, 400 MHz): δ 4.61 (d, 1 H, J = 19.4 Hz, H-1a), 4.54 (d, 1 H, J = 19.4 Hz, H-1b), 4.40 (d, 1 H, J = 5.8 Hz, H-3), 4.02 (dd, J = 10.6, 5.2 Hz, 1 H, H-5a), 3.83-3.91 (m, 2 H, H-4, H-5b); ^{13}C NMR (D_2O, 100 Hz): δ 212.6, 74.9, 71.4 (d, J = 7.0 Hz), 66.3, 63.7; ^{31}P NMR (D_2O, 133 Hz): δ 4.38. HRMS (ESI) m/z calculated for $[C_5H_{11}O_8P - H]^-$ 229.0119, found 229.0122.

Preparative scale synthesis of D-xylulose 1-phosphate and L-ribulose 1-phosphate. Reactions were carried in a final volume of 250 ml reaction system containing 25 mM of ATP, 3 mM of Mg^{2+}, 3 mM of Mn^{2+}, 20 mM starting sugars and conversion-related enzymes (Table 1). D-xylose (5.0 mmol) was incubated with 20 mg of XylA and 15 mg of HK. L-arabinose (5.0 mmol) was incubated with 15 mg of AraA and 25 mg of HK. The reactions were carefully shaken at pH near 7.5 at 37°C to allow the formation of ketose 1-phosphates. All the reactions were allowed to proceed until no detectable starting sugars were found. Silver nitrate precipitation method (as described above) was used to purify D-xylulose 1-phosphate and L-ribulose 1-phosphate. The solution from each reaction was concentrated under reduced pressure and purified by using Bio-Gel P-2 column to afford final products.

D-xylulose 1-phosphate (2). 1159 mg; Yield 92%; ^{1}H NMR (D_2O, 400 MHz): δ 4.29-4.33 (m, 0.7 H), 4.22-4.26 (m, 0.49 H), 4.19-4.23 (m, 0.25 H), 4.12-4.16 (m, 0.80 H), 4.02-4.05 (m, 1.01 H), 3.93 (dd, 1 H, J = 11.3, 8.6 Hz, 0.33 H), 3.87 (dd, J = 9.4, 0.33 Hz), 3.72-3.78 (m, 1.67 H), 3.59-3.63 (m, 0.94 H); ^{13}C NMR (D_2O, 100 Hz): δ 105.9 (d, $J_{C\text{-}P}$ = 6.1 Hz, $\beta_{C\text{-}1}$), 102.3 (d, $J_{C\text{-}P}$ = 7.4 Hz, $\alpha_{C\text{-}1}$), 80.1, 76.9, 75.3, 74.6, 72.7, 69.7, 65.9 (d, $J_{C\text{-}P}$ = 3.1 Hz), 64.4 (d, $J_{C\text{-}P}$ = 4.1 Hz); ^{31}P NMR (D_2O, 133 Hz): δ 5.11 (α), 4.24 (β); α:β=1:2. HRMS (ESI) m/z calculated for $[C_5H_{11}O_8P$ -H]$^-$ 229.0119, found 229.0123.

L-ribulose 1-phosphate (5). 1170 mg; Yield 93%; ^{1}H NMR (D_2O, 400 MHz): δ 4.58 (dd, 0.33 H, J = 12.5, 6.4 Hz), 4.31 (brs, 0.57 H), 4.11-4.17 (m, 0.85 H), 3.99-4.04 (m, 1.12 H), 3.88-3.90 (m, 0.59 H), 3.69-3.75 (m, 1.67 H); ^{13}C NMR (D_2O, 100 Hz): δ 105.9 (d, $J_{C\text{-}P}$ = 6.2 Hz, $\beta_{C\text{-}1}$), 101.9 (d, $J_{C\text{-}P}$ = 8.5 Hz, $\alpha_{C\text{-}1}$), 74.9, 71.5, 71.1, 70.5, 70.0, 69.9, 65.9 (d, $J_{C\text{-}P}$ = 3.3 Hz), 64.7 (d, $J_{C\text{-}P}$ = 4.4 Hz); ^{31}P NMR (D_2O, 133 Hz): δ 5.18 (α), 4.40 (β); α:β= 1:2. HRMS (ESI) m/z calculated for $[C_5H_{11}O_8P$ -H]$^-$ 229.0119, found 229.0123.

Preparative scale synthesis of D-ribulose 1-phosphate and L-xylulose 1-phosphate. Reactions were carried in a final volume of 250 ml reaction system containing 25 mM of ATP, 3 mM of Mg^{2+}, 3 mM of Mn^{2+}, 20 mM starting sugars and conversion-related enzymes (Table 1). D-xylose (5.0 mmol) was incubated with 20 mg of XylA, 35 mg of DTE and 13 mg of RhaB. L-arabinose (5.0 mmol) was incubated with 15 mg of AraA, 35 mg of DTE and 13 mg of RhaB. The reactions were carefully shaken at pH near 7.5 at 45°C to allow the formation of ketose 1-phosphates. Once no detectable starting sugars were found, silver nitrate precipitation method (as described above) was used to purify D-ribulose 1-phosphate and L-xylulose 1-phosphate. The solution from each reaction was concentrated under reduced pressure and desalted by using Bio-Gel P-2 column to afford final products.

D-ribulose-1-phosphate (3). 1183 mg; Yield 94%; ^{1}H NMR (D_2O, 400 MHz): δ 4.31 (s, 1 H), 4.14 (s, 1 H), 4.00-4.02 (m, 1 H), 3.88-3.90 (m, 1 H), 3.69-3.78 (m, 2 H); ^{13}C NMR (D_2O, 100 Hz): δ 105.5 (d, $J_{C\text{-}P}$ = 7.0 Hz, $\beta_{C\text{-}1}$), 101.7 (d, $J_{C\text{-}P}$ = 8.0 Hz, $\alpha_{C\text{-}1}$), 74.9, 71.2, 70.9, 70.5, 70.0 (2 C), 65.9 (2 C), 65.9 (d, $J_{C\text{-}P}$ = 3.0 Hz), 65.1 (d, $J_{C\text{-}P}$ = 5.0 Hz); ^{31}P NMR (D_2O, 133 Hz): δ 3.60 (α), 2.60 (β); α:β= 1:2. HRMS (ESI) m/z calculated for $[C_5H_{11}O_8P$ -H]$^-$ 229.0119, found 229.0123.

L-xylulose-1-phosphate (7). 1204 mg; Yield, 96%; ^{1}H NMR (D_2O, 400 MHz): δ 4.32 (brs, 1 H), 4.22-4.26 (m, 1 H), 4.14-4.16 (m, 1 H), 4.04-4.06 (m, 1 H), 3.79 (brs, 1 H), 3.63 (brs, 1 H); ^{13}C NMR (D_2O, 100 Hz): δ 105.9 (d, $J_{C\text{-}P}$ = 6.0 Hz, $\beta_{C\text{-}1}$), 102.4 (d, $J_{C\text{-}P}$ = 8.0 Hz, $\alpha_{C\text{-}1}$), 80.3, 76.9, 75.3, 74.7, 72.7, 69.7, 66.0 (d, $J_{C\text{-}P}$ = 5.0 Hz), 64.4 (d, $J_{C\text{-}P}$ = 4.0 Hz); ^{31}P NMR (D_2O, 133 Hz): δ 5.28 (α), 4.44 (β); α:β= 1:2. HRMS (ESI) m/z calculated for $[C_5H_{11}O_8P$ -H]$^-$ 229.0119, found 229.0121.

Preparative scale synthesis of D-ribulose 5-phosphate and L-xylulose 5-phosphate. In the first reaction step, D-ribulose 1-phosphate and L-xylulose 1-phosphate were prepared from 6 mmol of D-xylose or L-arabinose as described above. To prepare D-ribulose and L-xylulose, a reaction mixture in total volume of 100

ml containing ketose 1-phosphates, 5 mM of Mg^{2+}, 0.1 mM Zn^{2+} and 10 mg of AphA. The reactions were carefully shaken at 37°C to allow the formation of ketoses. Once no detectable ketose 1-phosphates were found by TLC, The solution from each reaction was concentrated under reduced pressure and desalted by using Bio-Gel P-2 column to afford ketose 1-phosphates. In the second reaction step, reactions were carried in a final volume of 300 ml reaction system containing 25 mM of ATP, 3 mM of Mg^{2+}, 3 mM of Mn^{2+}, ~20 mM of ketoses and kinases (Table 1). D-ribulose was incubated with 15 mg of AraB. L-xylulose was incubated with 20 mg of LyxK. The reactions were carefully shaken at pH near 7.5 at 37°C to allow the formation of ketose 5-phosphates. Once no detectable ketoses were found, silver nitrate precipitation method (as described above) was used to purify D-ribulose 5-phosphate and L-xylulose 5-phosphate. The solution from each reaction was concentrated under reduced pressure and desalted by using Bio-Gel P-2 column to afford final products.

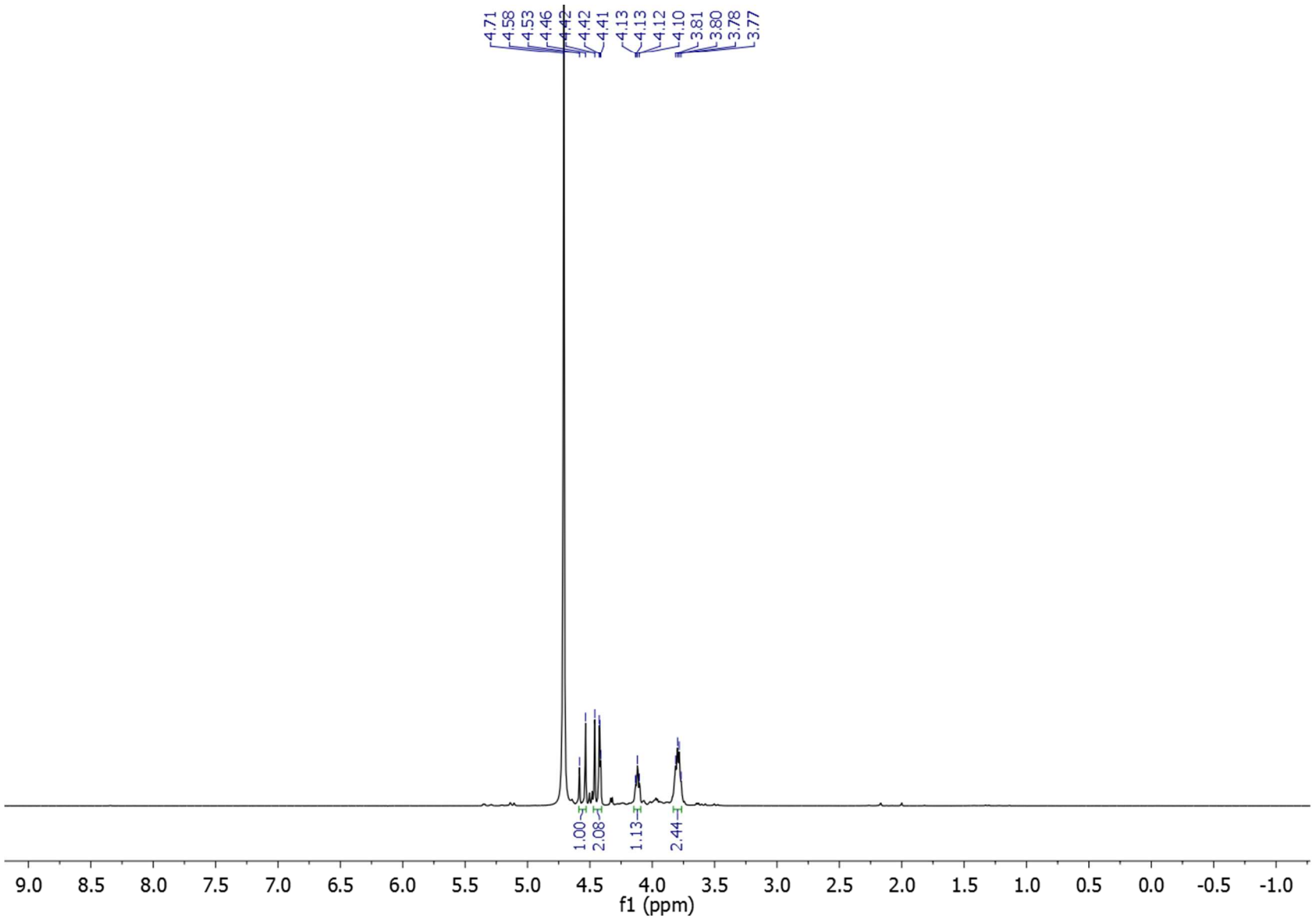
D-ribulose-5-phosphate (4). 1291 mg; Yield, 85%; ^{1}H NMR (D_2O, 400 MHz): δ 4.61 (d, 1 H, J = 19.4 Hz, H-1a), 4.55 (d, 1 H, J = 19.4 Hz, H-1b), 4.41 (d, J = 5.8, H-3), 4.01 (dd, J = 10.2, 4.9 Hz, H-5a), 3.82-3.92 (m, 2 H, H-4, H-5b); ^{13}C NMR (D_2O, 100 Hz): δ 211.4, 73.8, 70.2, 65.4, 63.4; ^{31}P NMR (D_2O, 133 Hz): δ 4.24. HRMS (ESI) m/z calculated for $[C_5H_{11}O_8P - H]^-$ 229.0119, found 229.0099.

L-xylulose-5-phosphate (8). 1265 mg; Yield, 84%; ^{1}H NMR (D_2O, 400 MHz): δ 4.64 (d, 1 H, J = 19.4 Hz, H-1a), 4.52 (d, 1 H, J = 19.4 Hz, H-1b), 4.51 (d, 1 H, J = 1.5 Hz, H-3), 4.17 (dd, 1 H, J = 5.8, 4.4 Hz, H-5a), 3.80-3.83 (m, 2 H, H-4, H-5b); ^{13}C NMR (D_2O, 100 Hz): δ 213.1, 75.2, 71.2 (d, J = 6.9 Hz), 66.0, 63.8 (d, J = 4.6 Hz); ^{31}P NMR (D_2O, 133 Hz): δ 4.35. HRMS (ESI) m/z calculated for $[C_5H_{11}O_8P - H]^-$ 229.0119, found 229.0122.

Purity analysis

To analyze the purity of phosphorylated ketoses, the phosphate group of each sugar phosphate was hydrolyzed by a reaction system (pH 5.5) containing 20 mM of phosphorylated sugars, 3 mM of Mg^{2+} and 10 U of acid phosphatase. The reactions were incubated at 37°C until no sugar phosphates were observed by TLC (EtOAc/MeOH/H2O/HOAc=5:2:1.4:0.4). The mixture was then analyzed by HPLC employing authentic sugar standards as controls as previously reported.[10]

^{1}H-NMR of D-xylulose 5-phosphate



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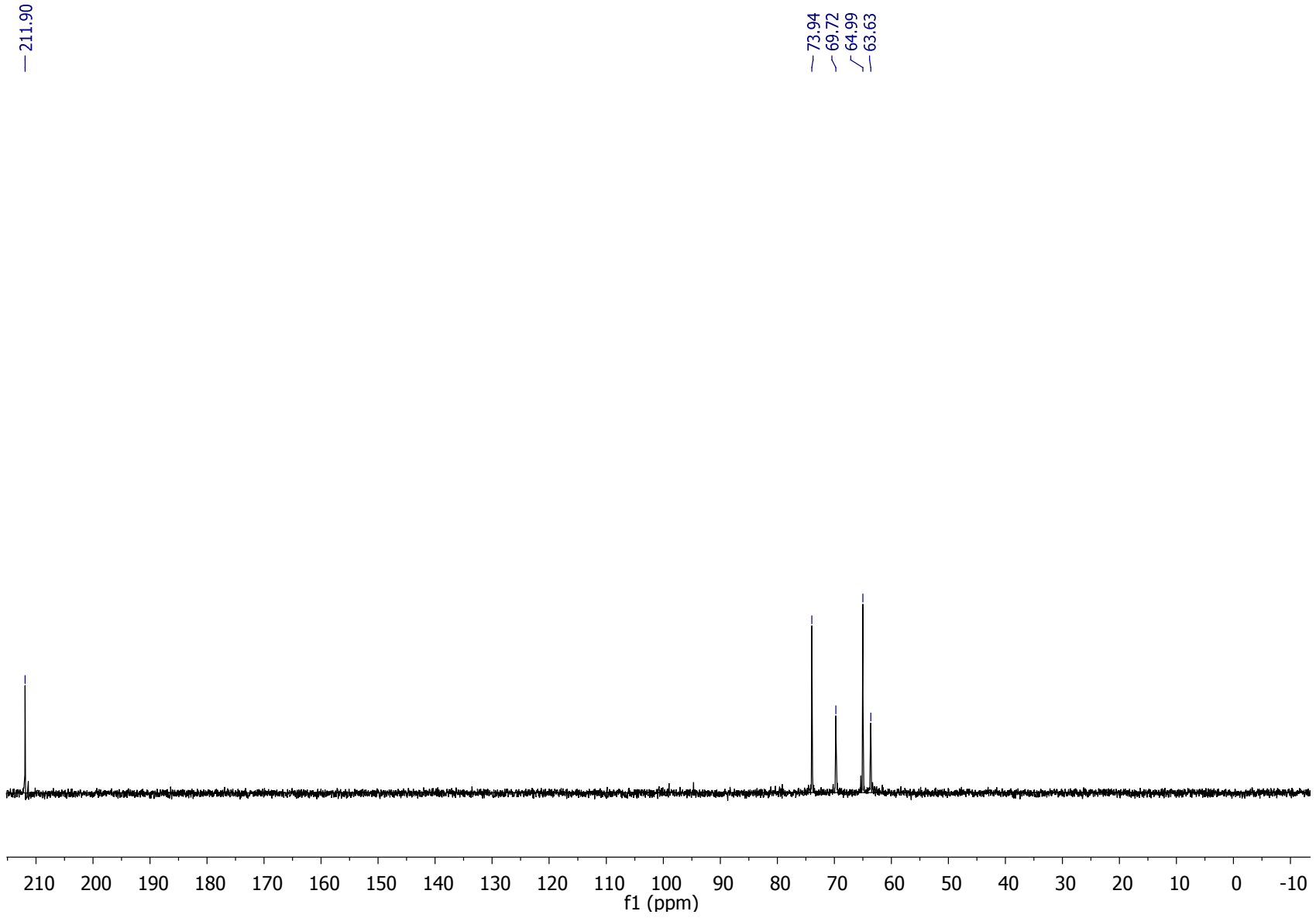

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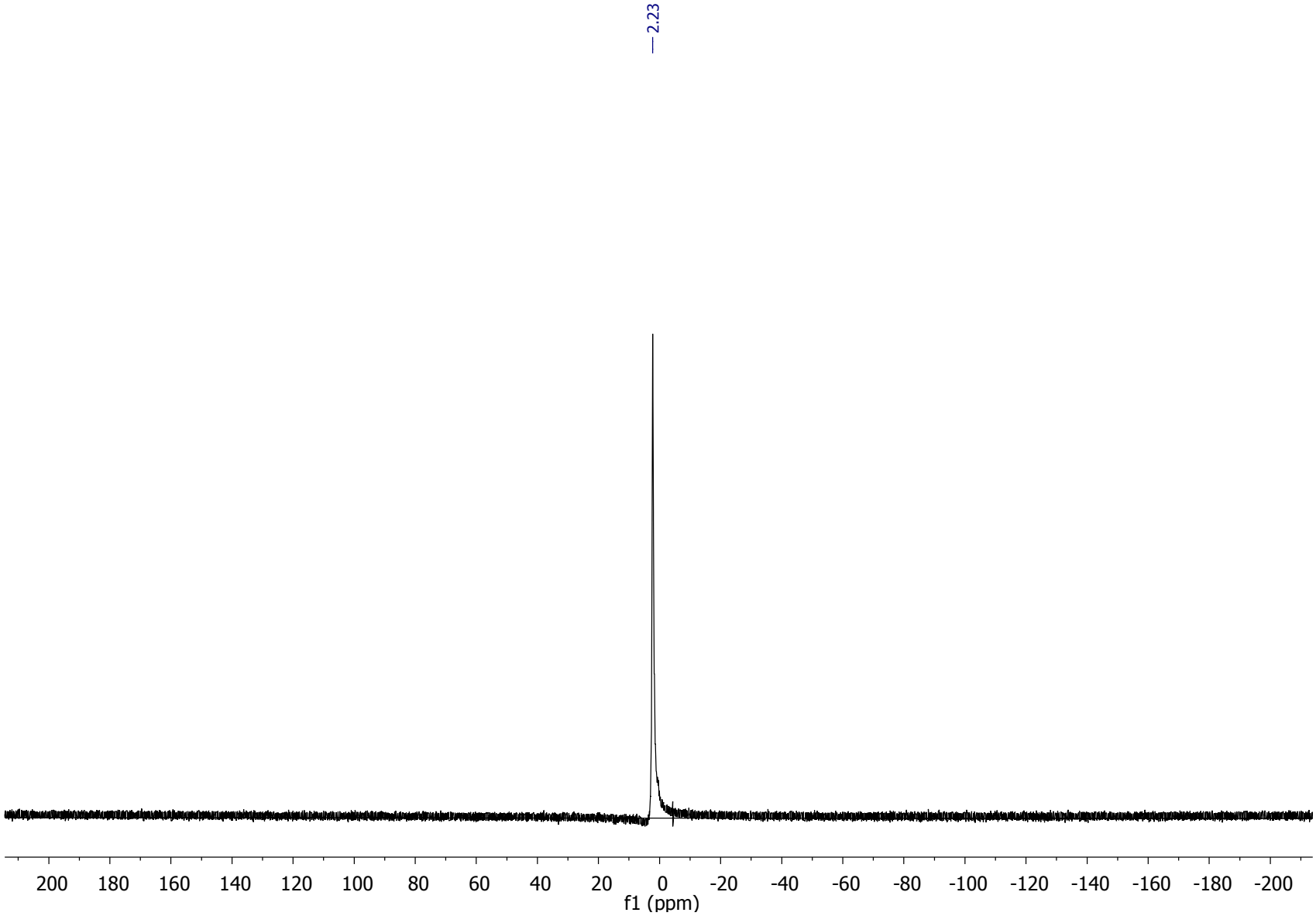

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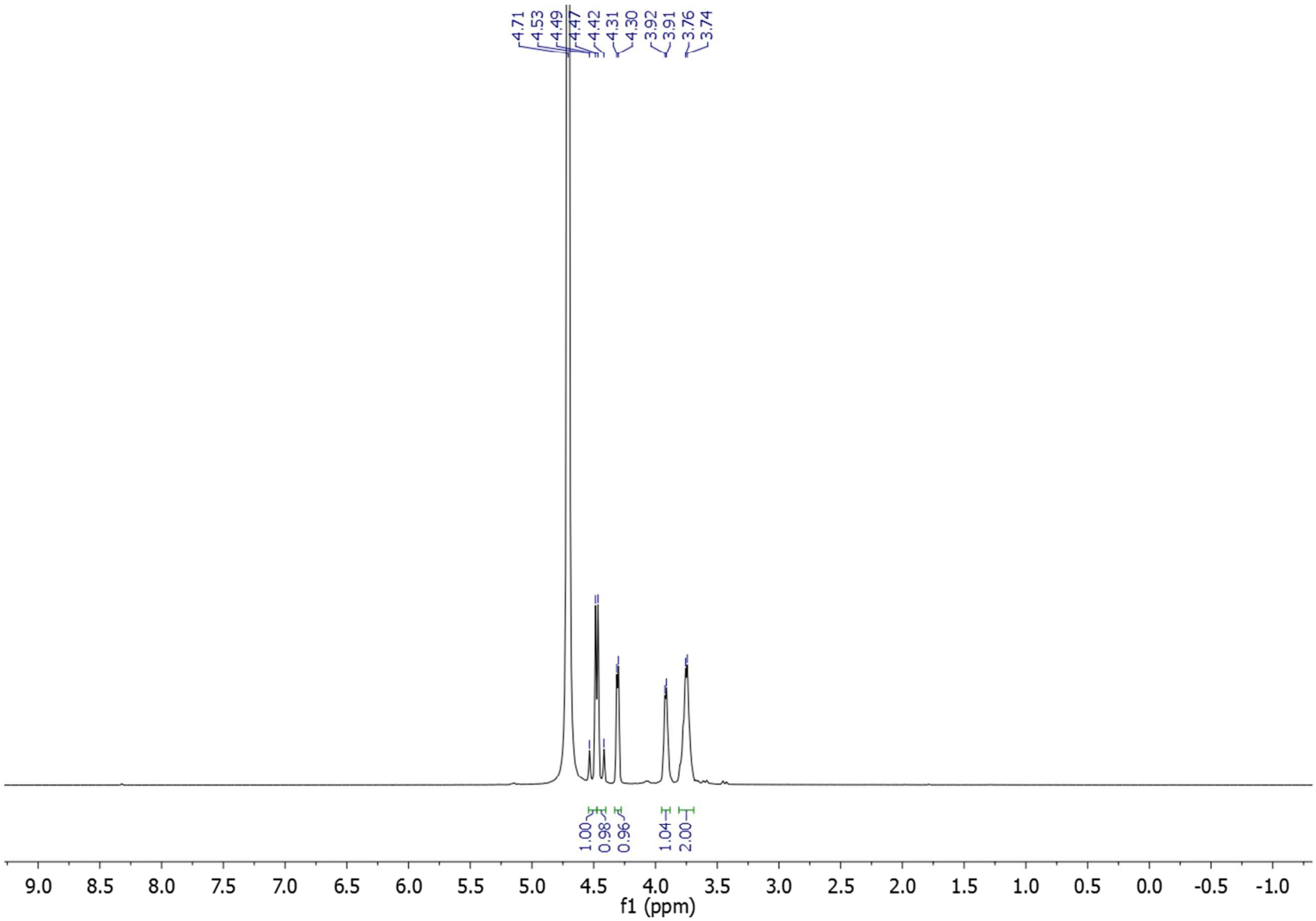

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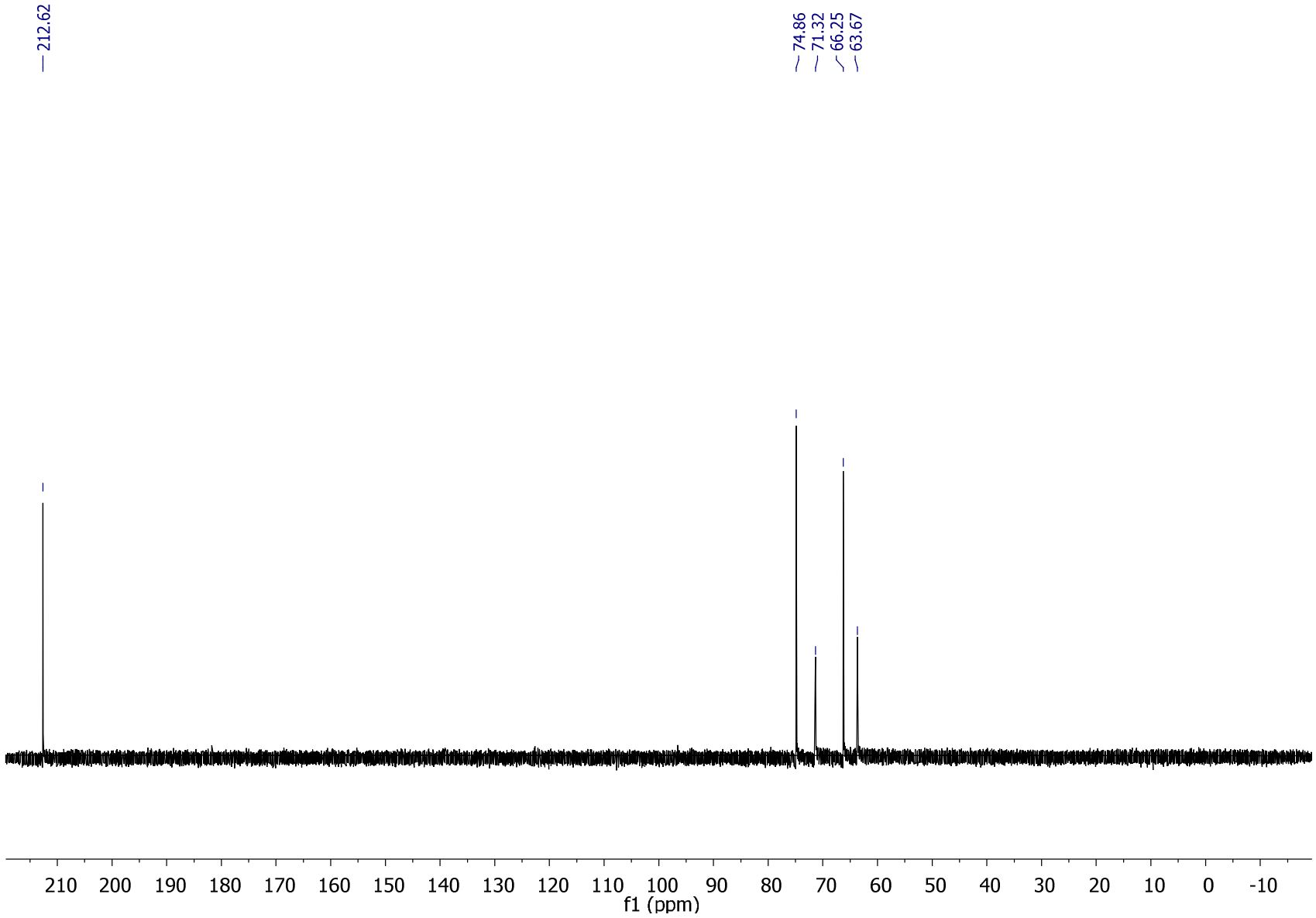

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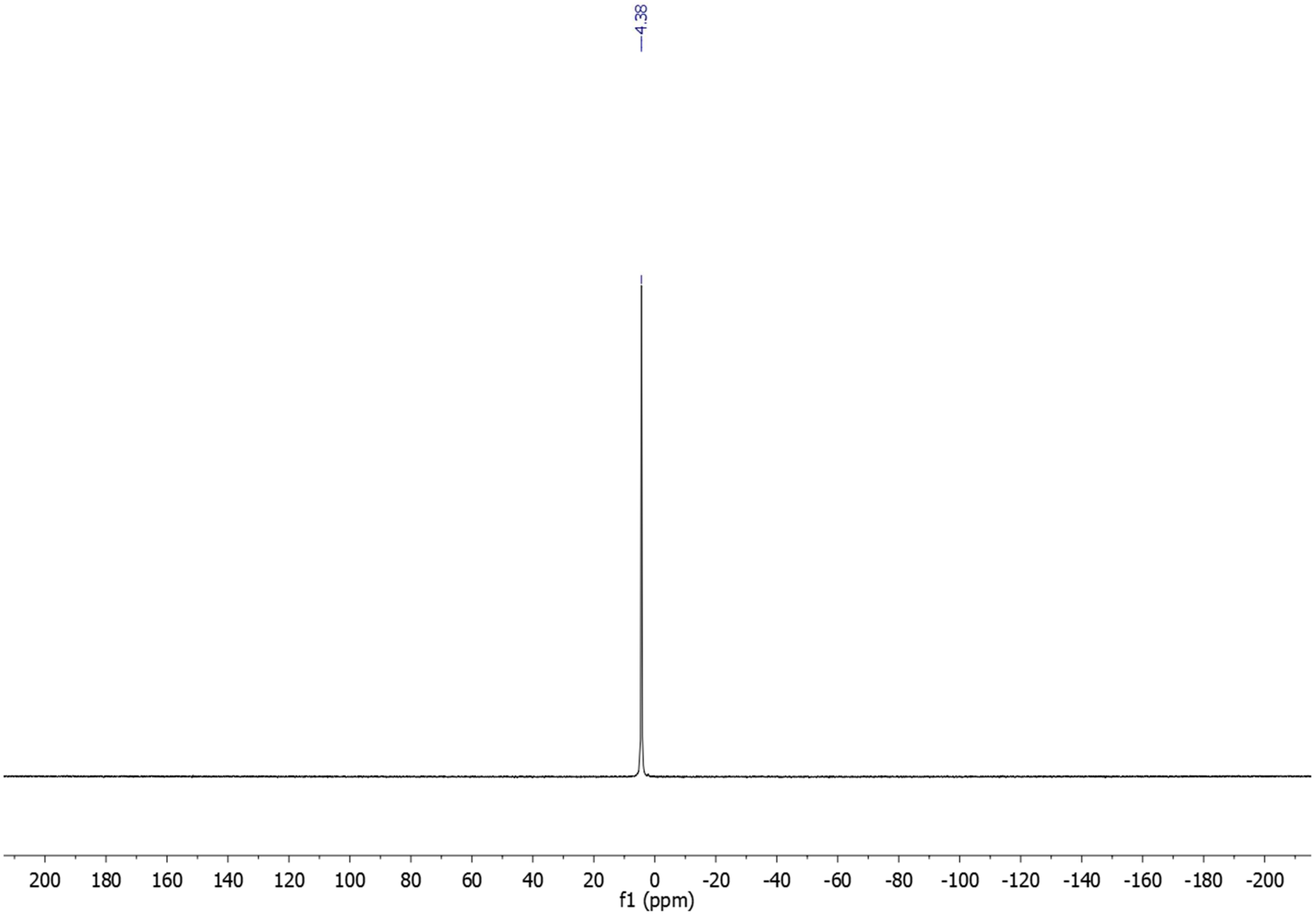

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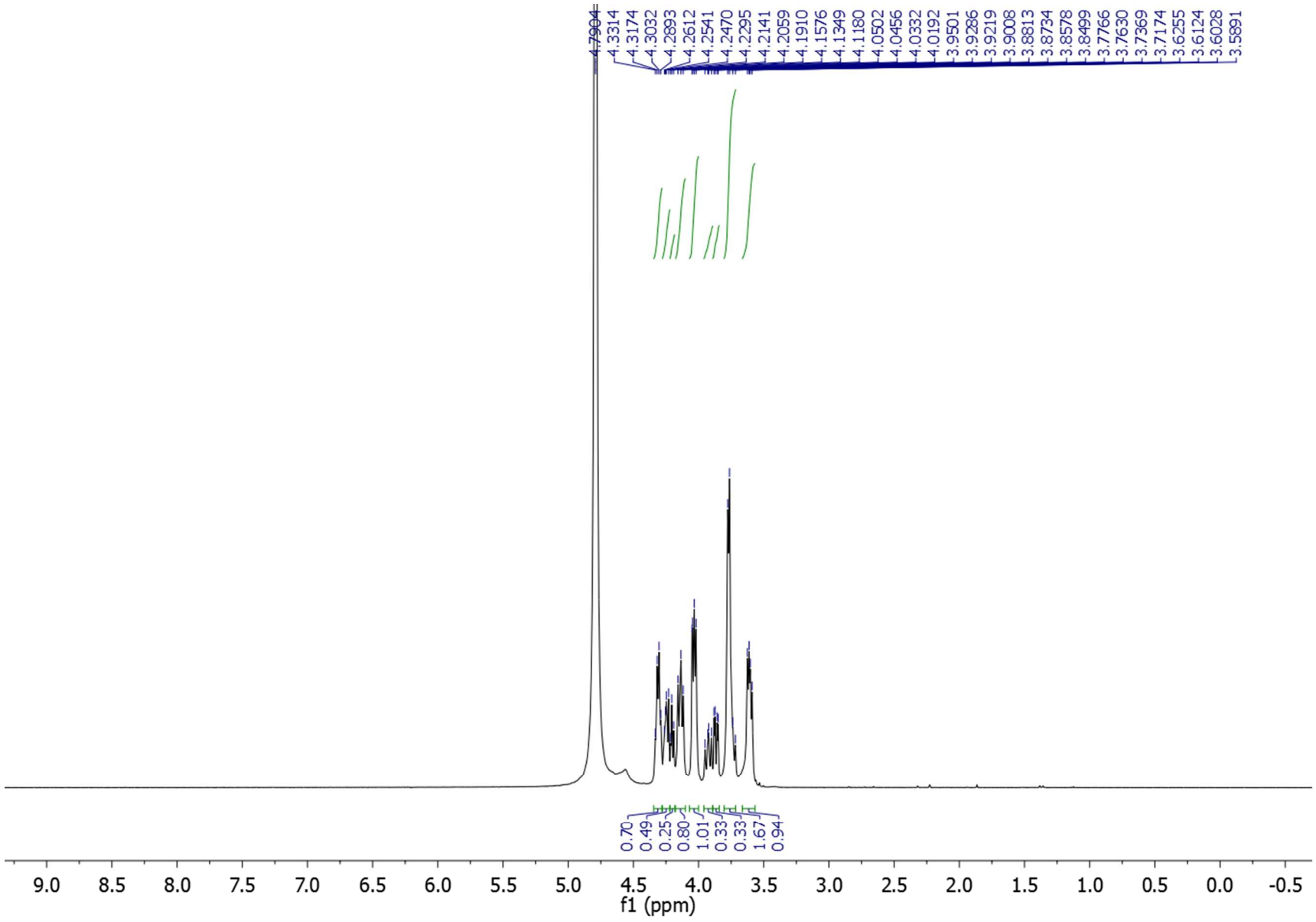

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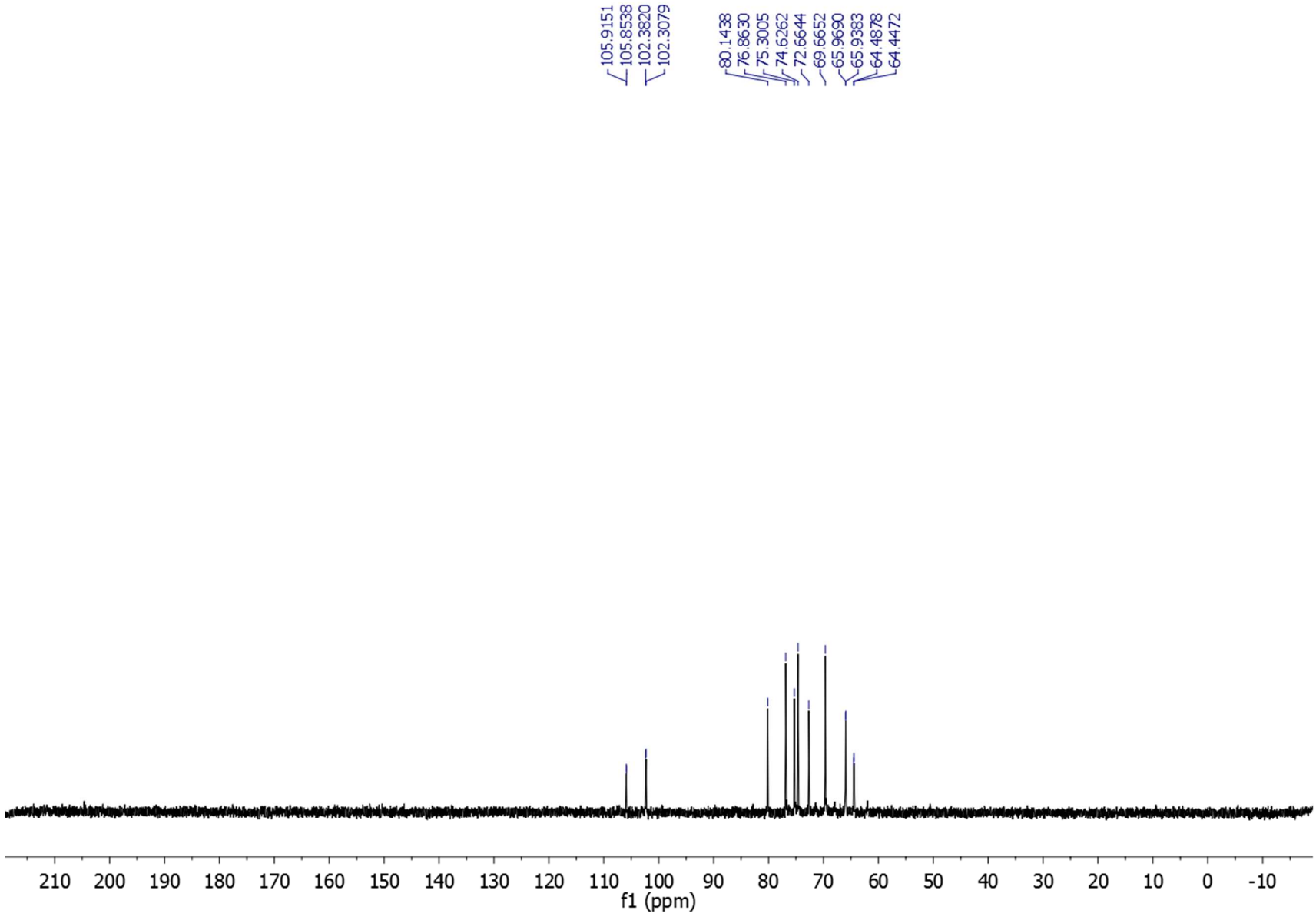

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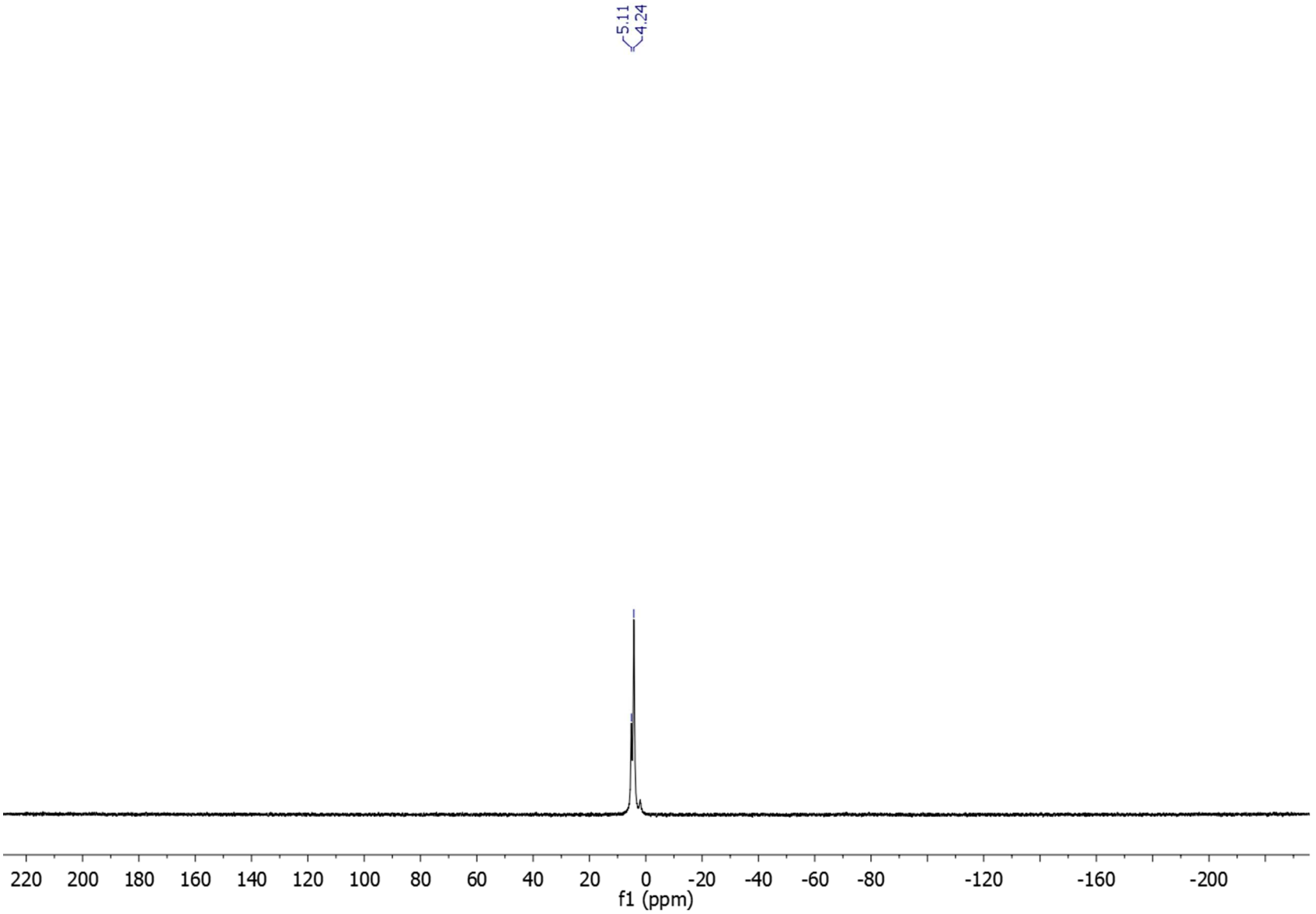

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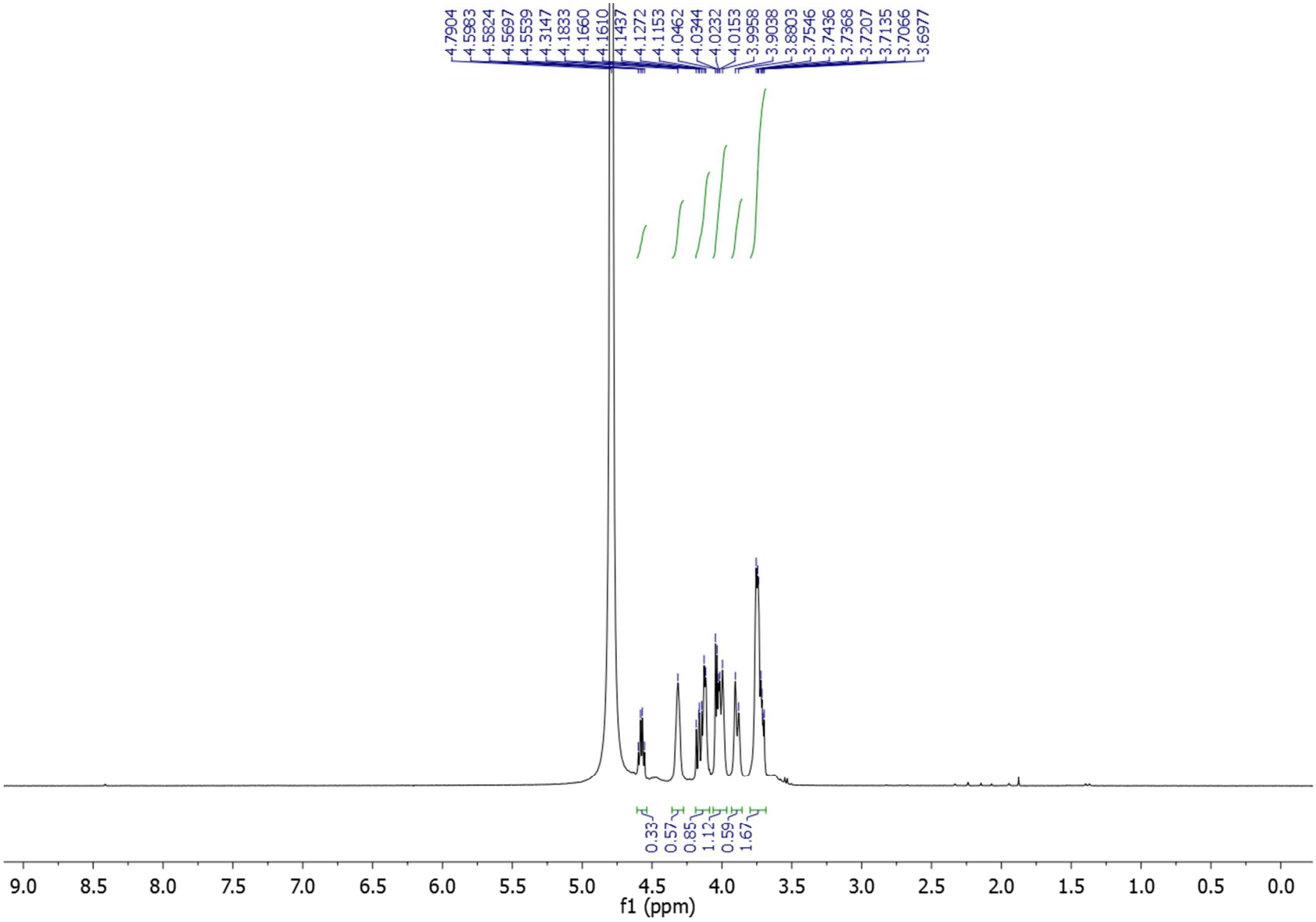

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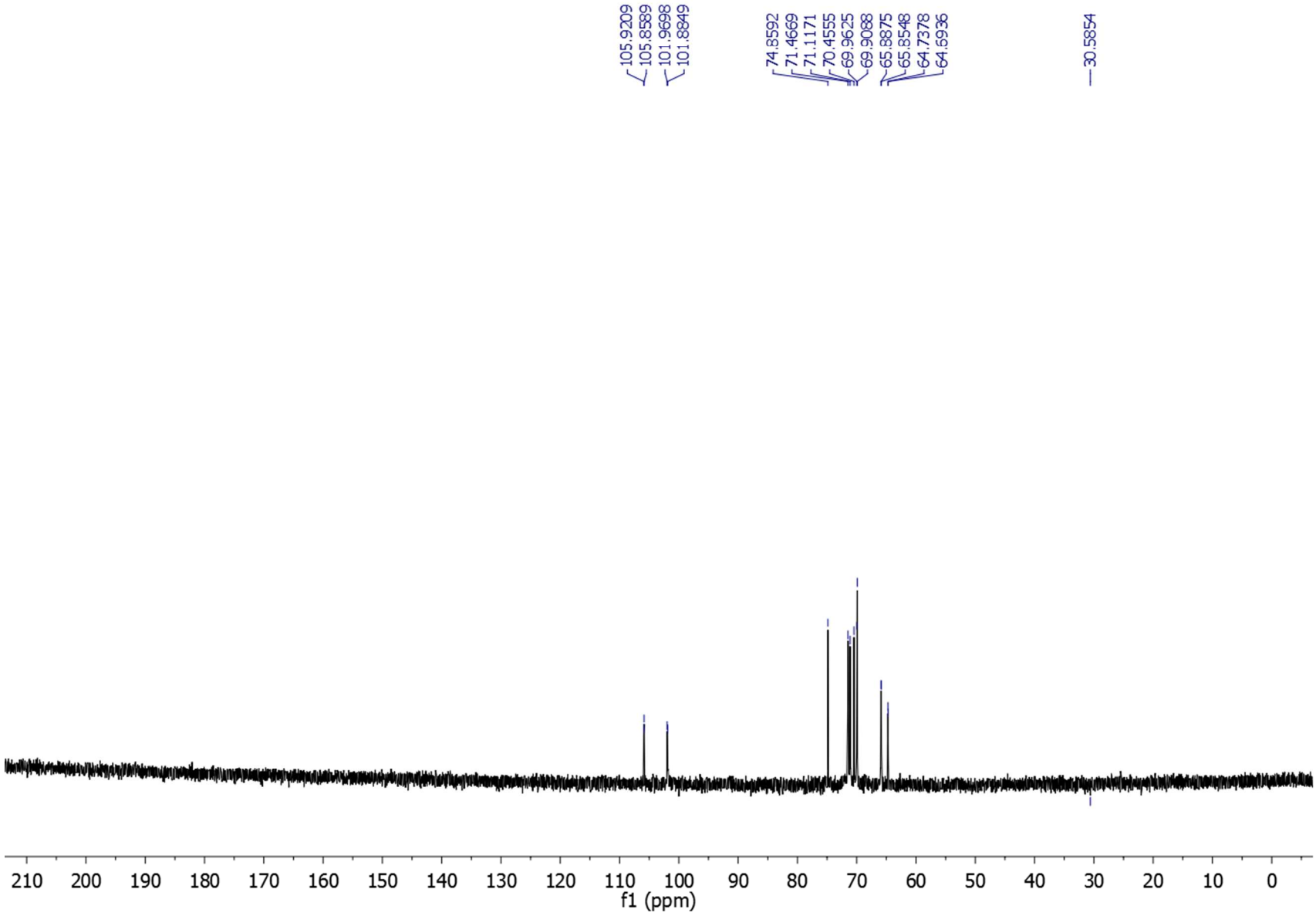

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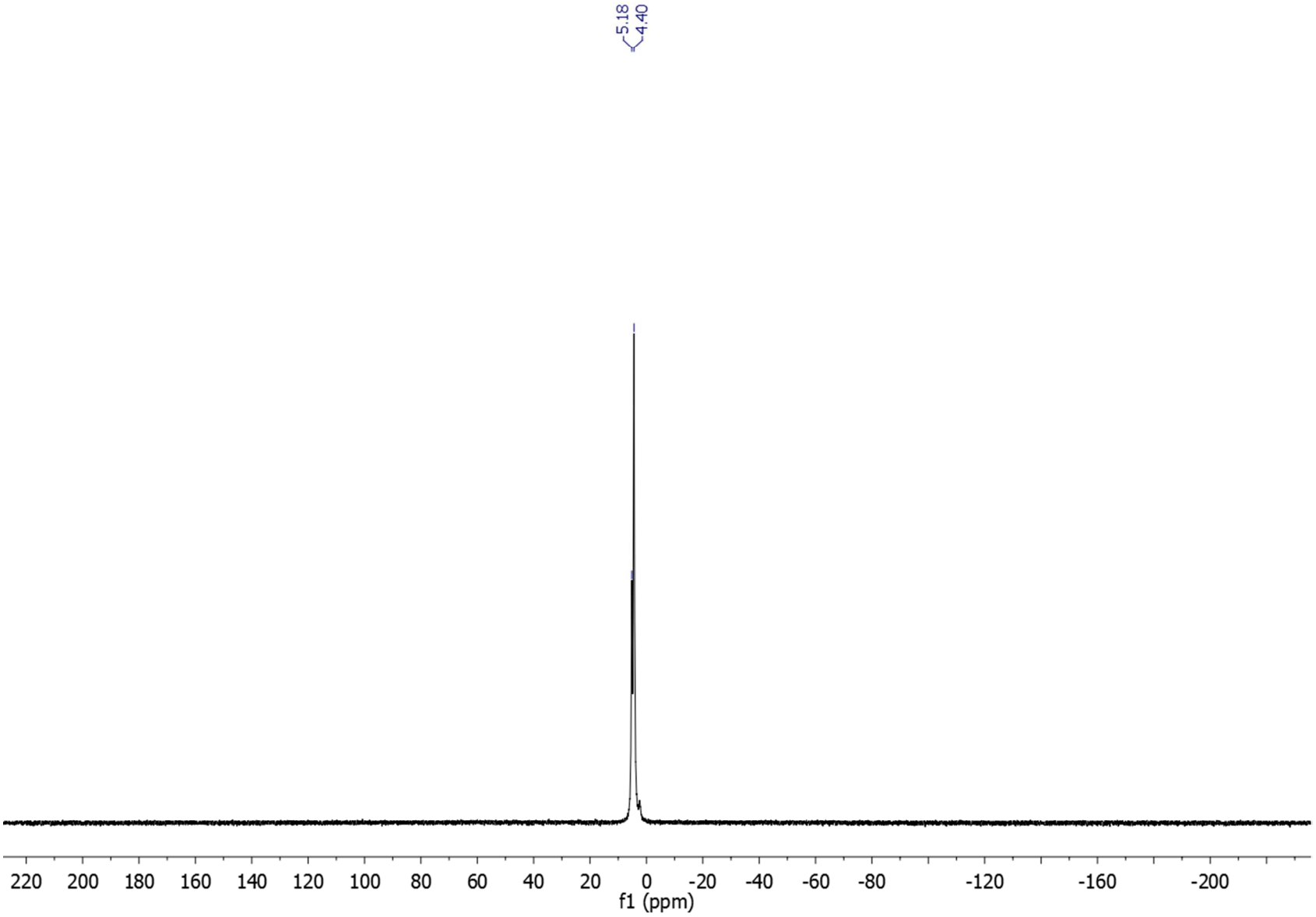

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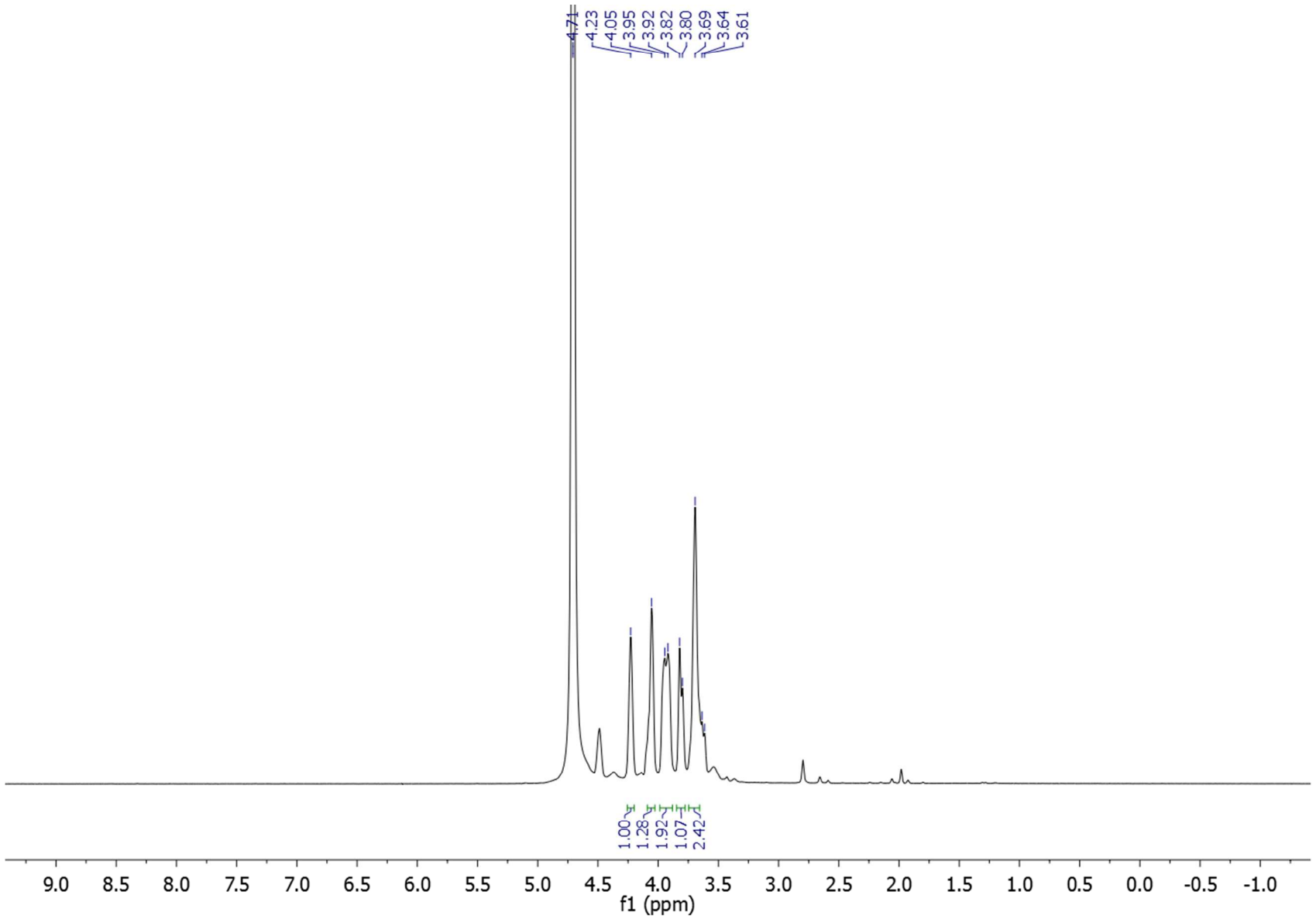

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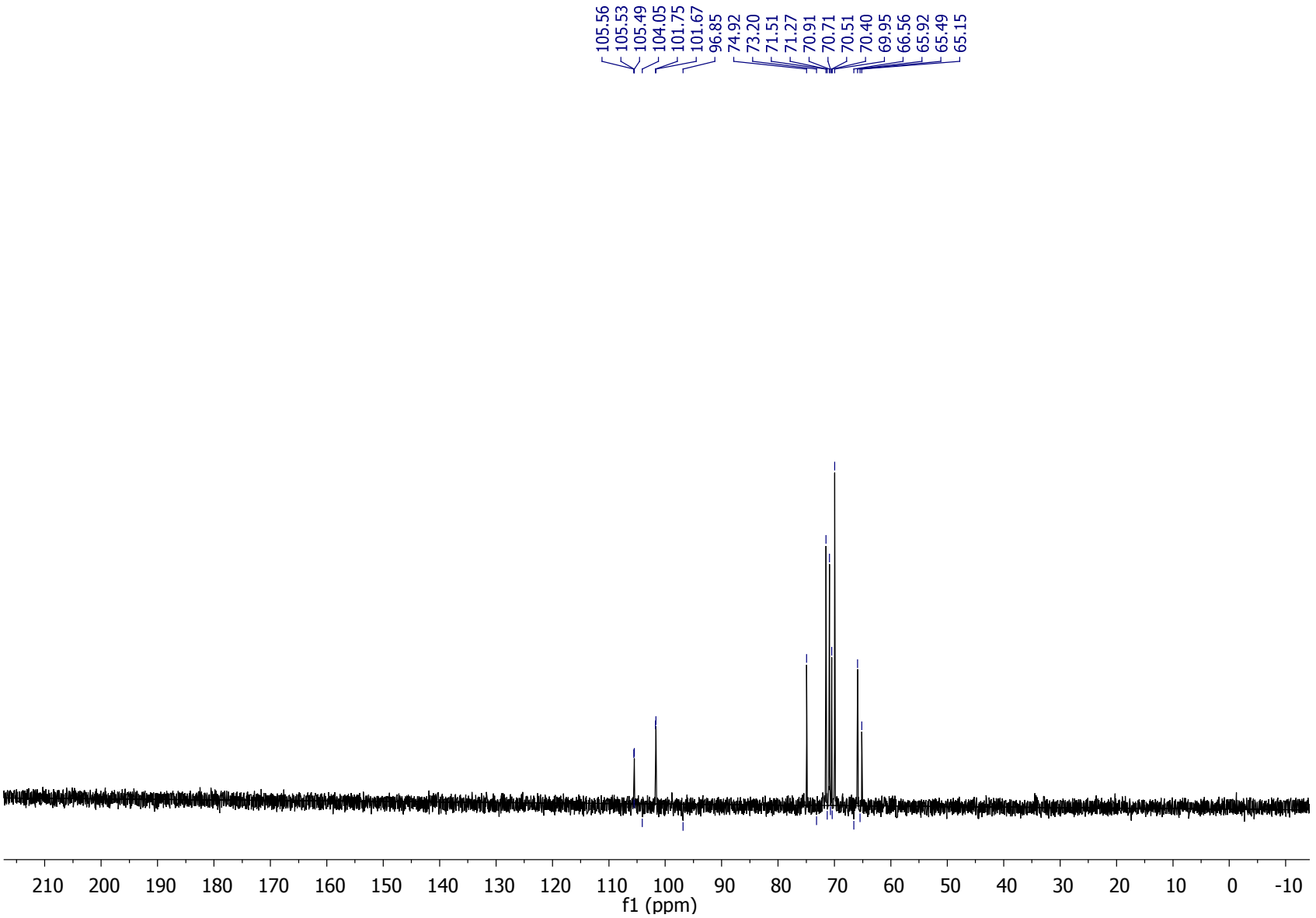
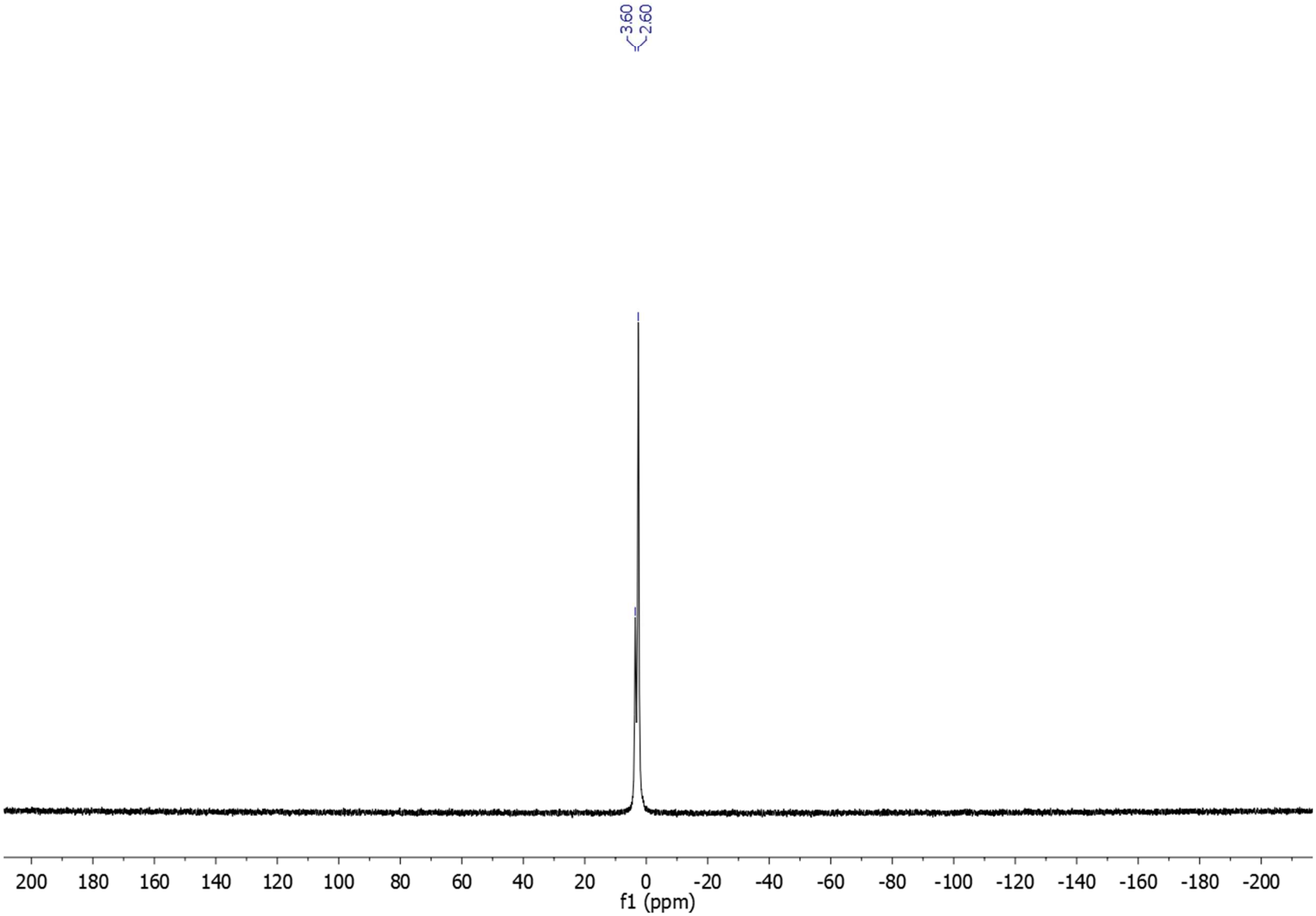


^{31}P-NMR of D-ribulose 1-phosphate



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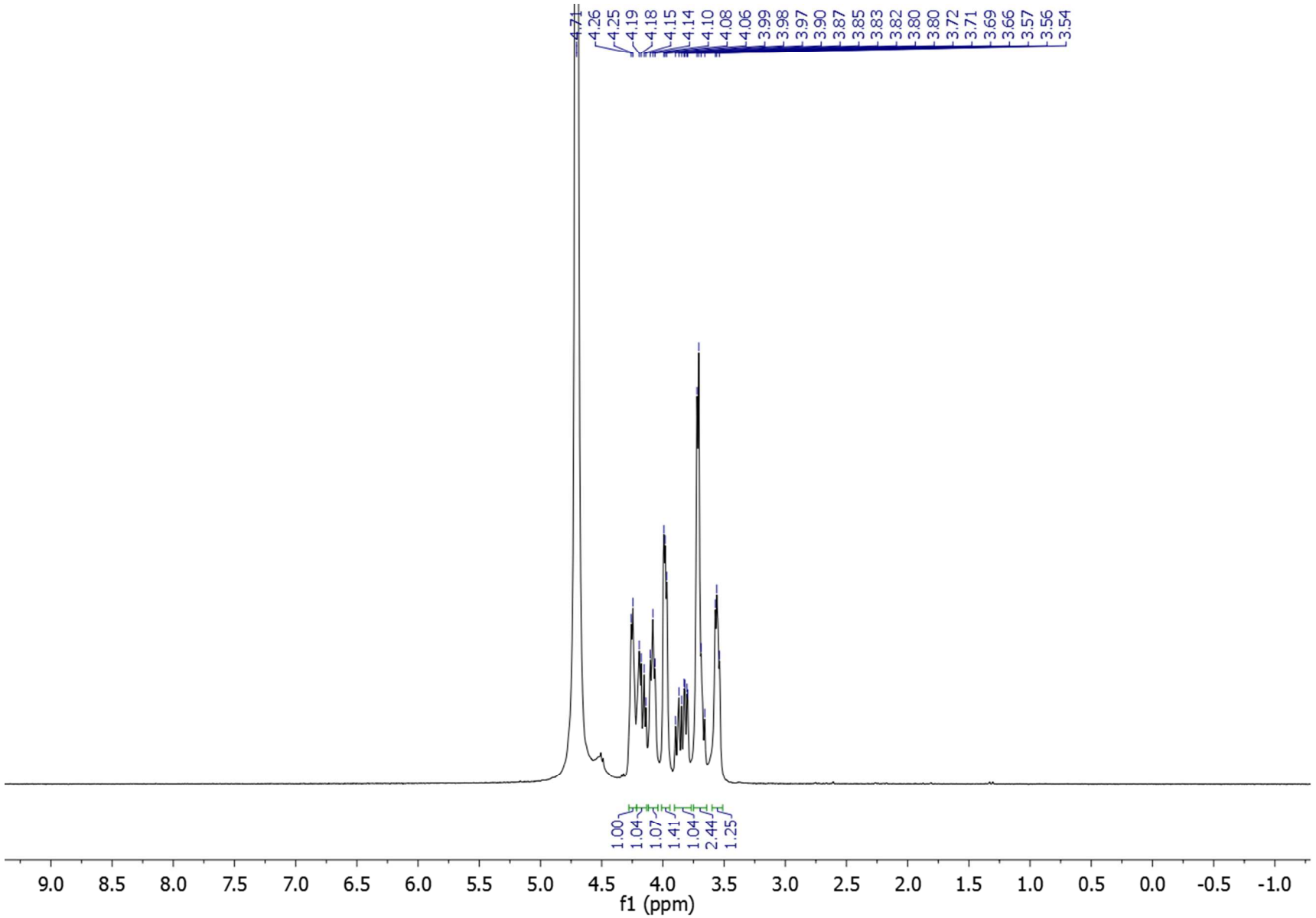

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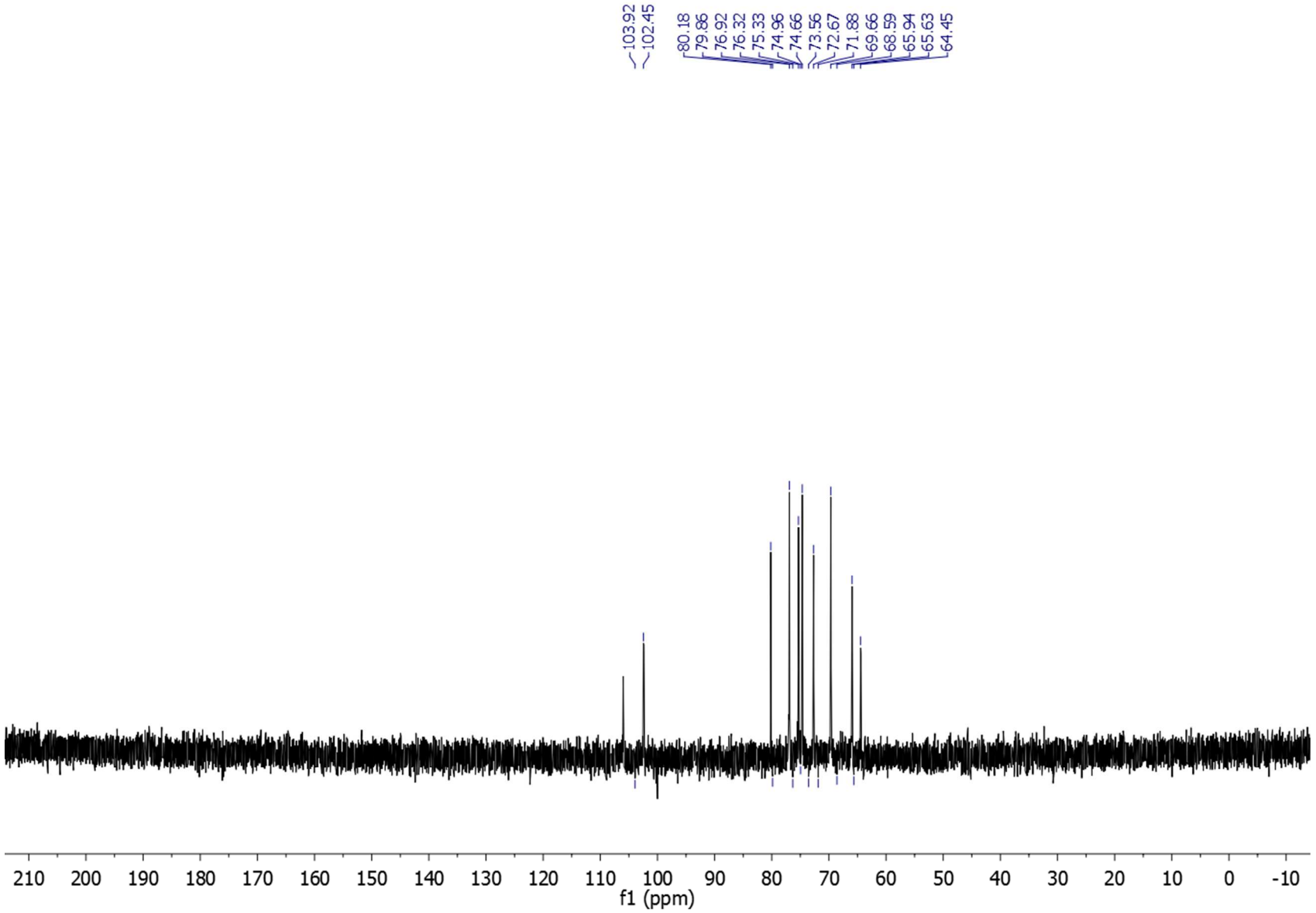

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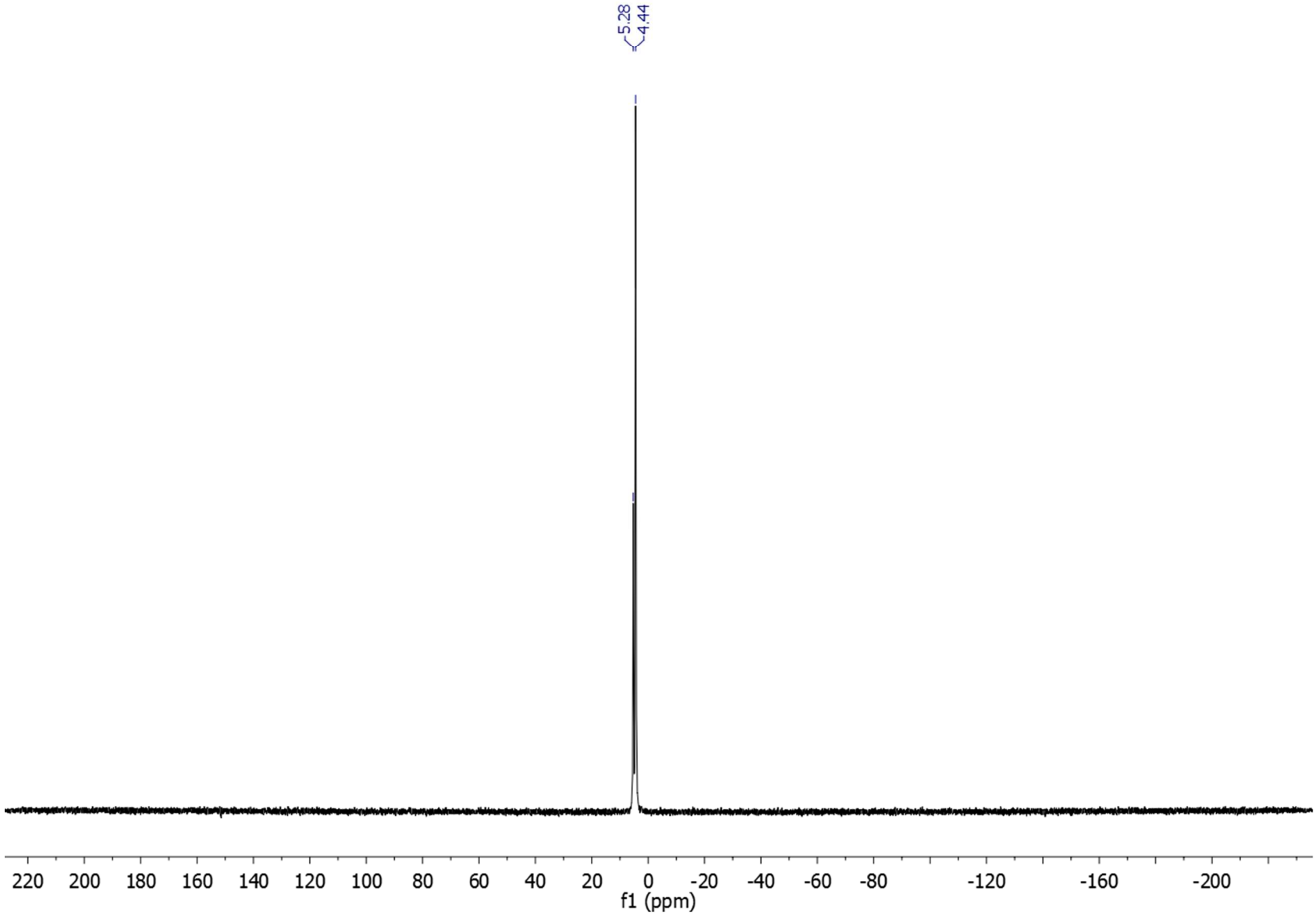

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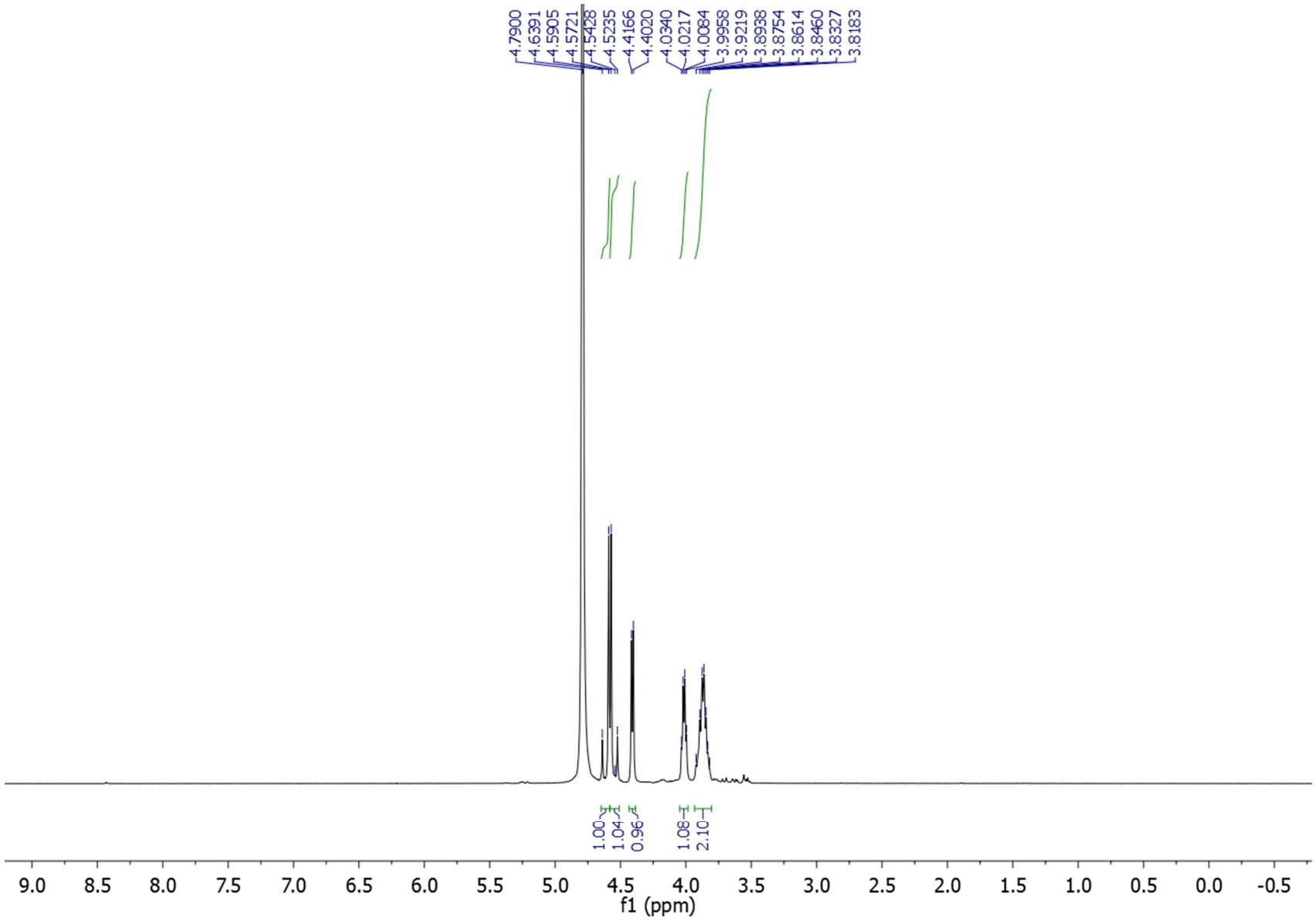

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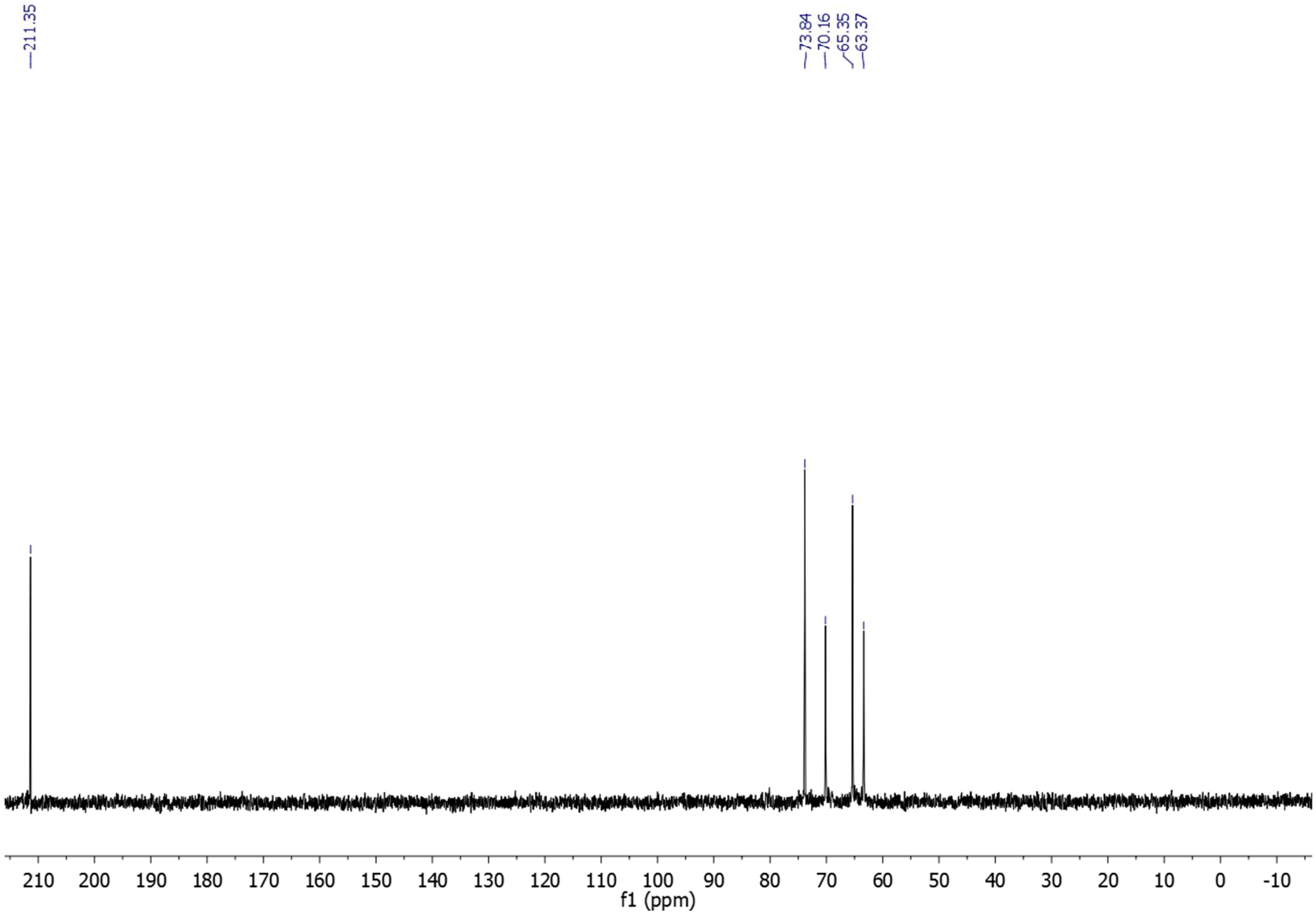

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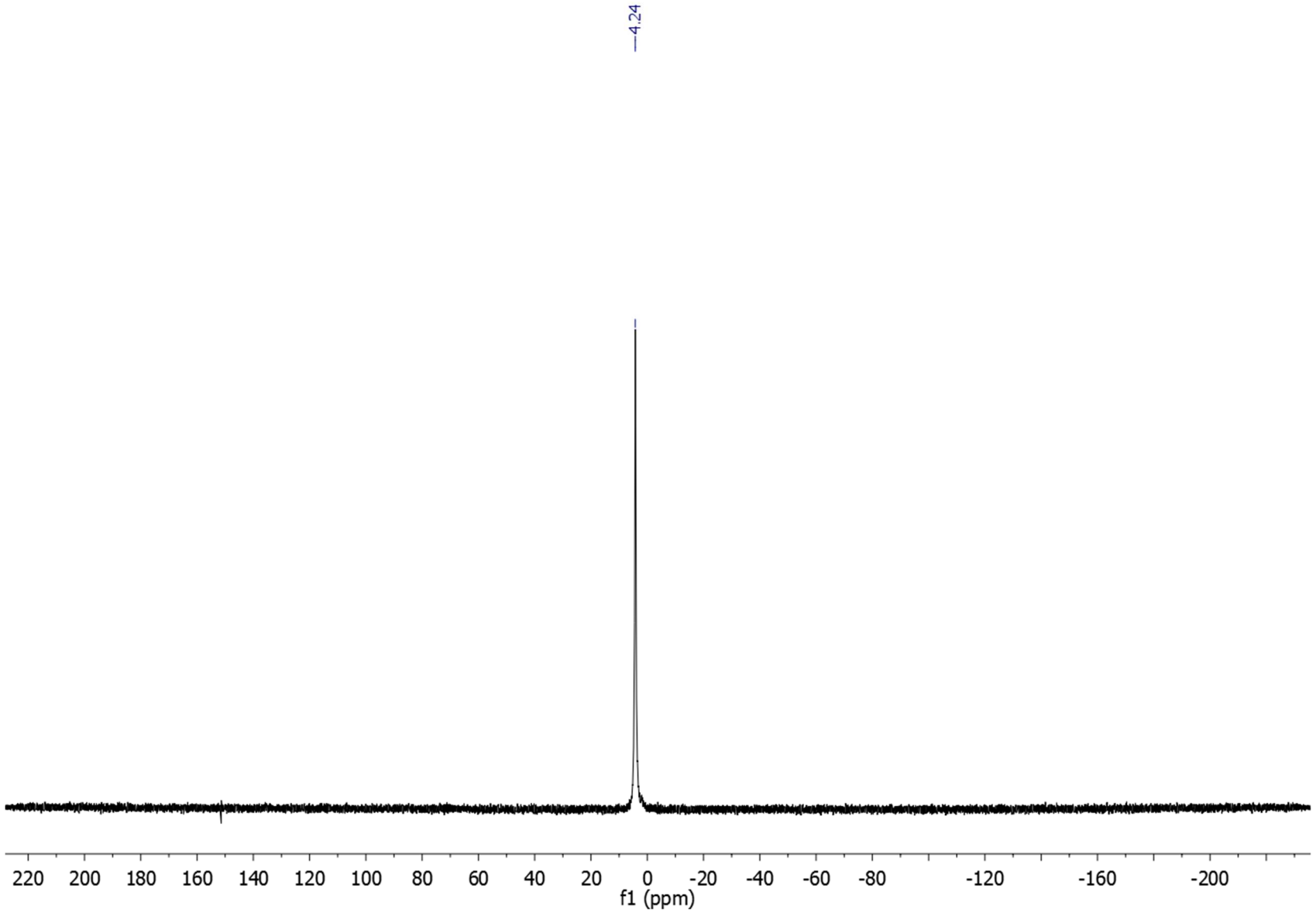

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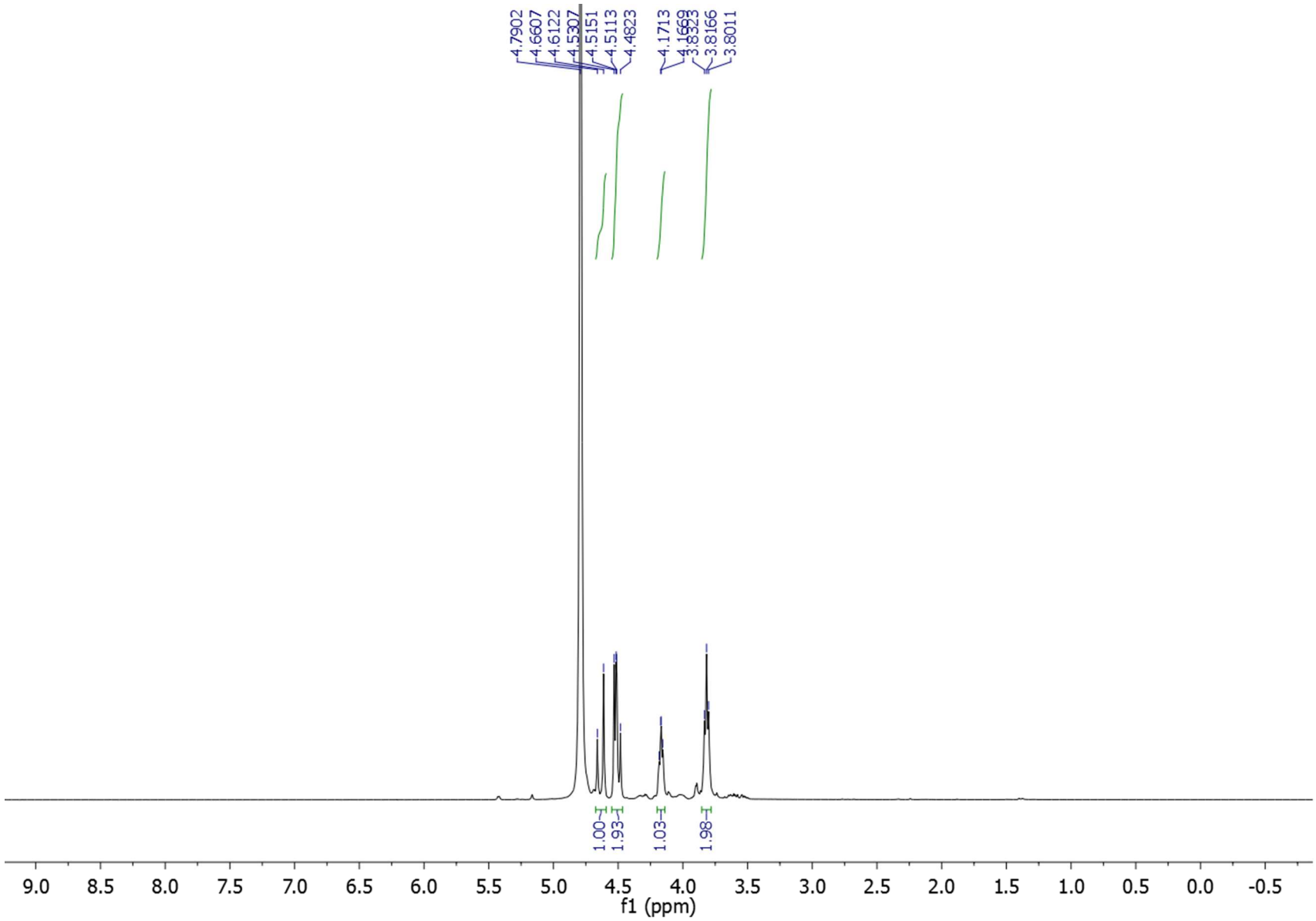

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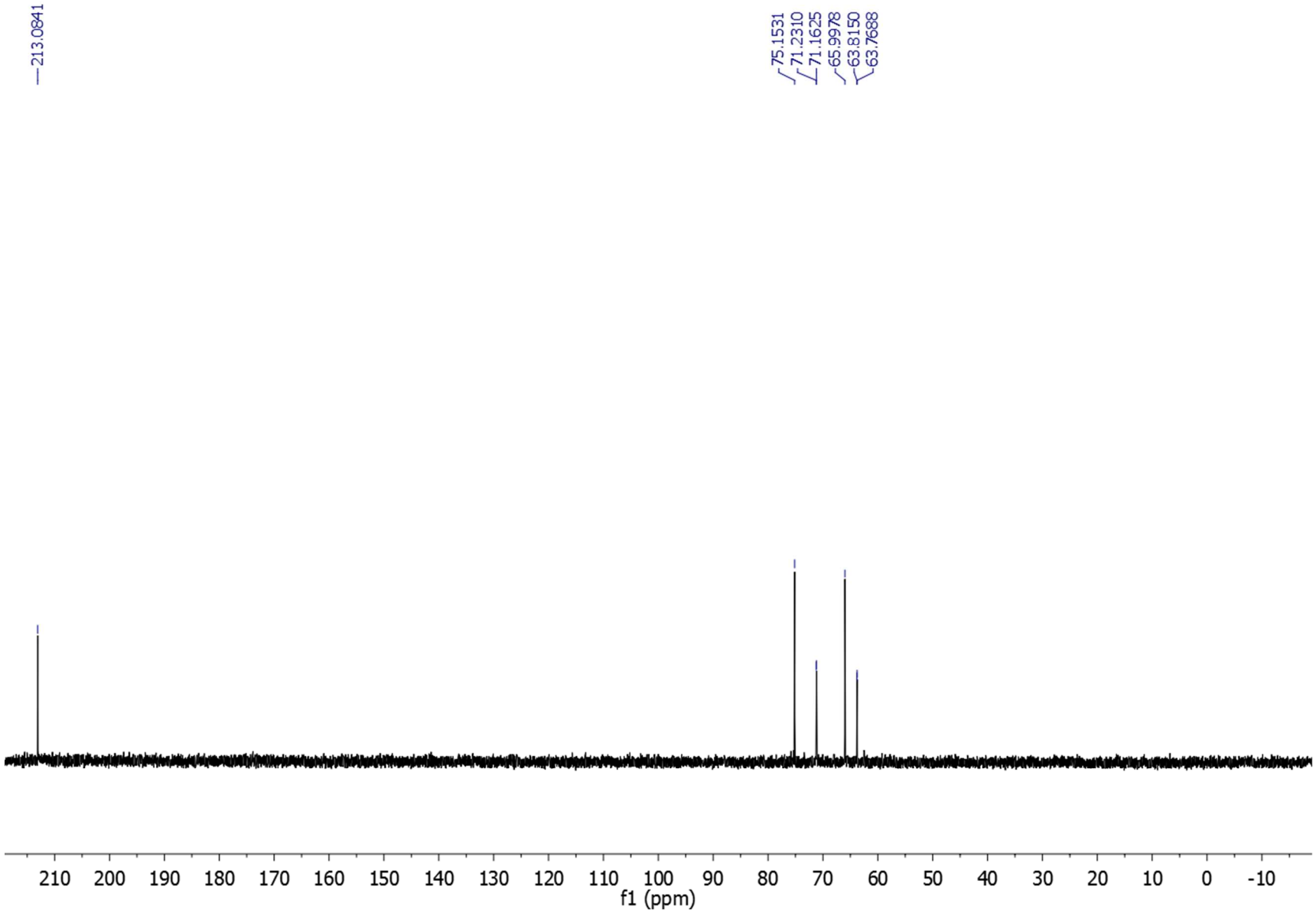
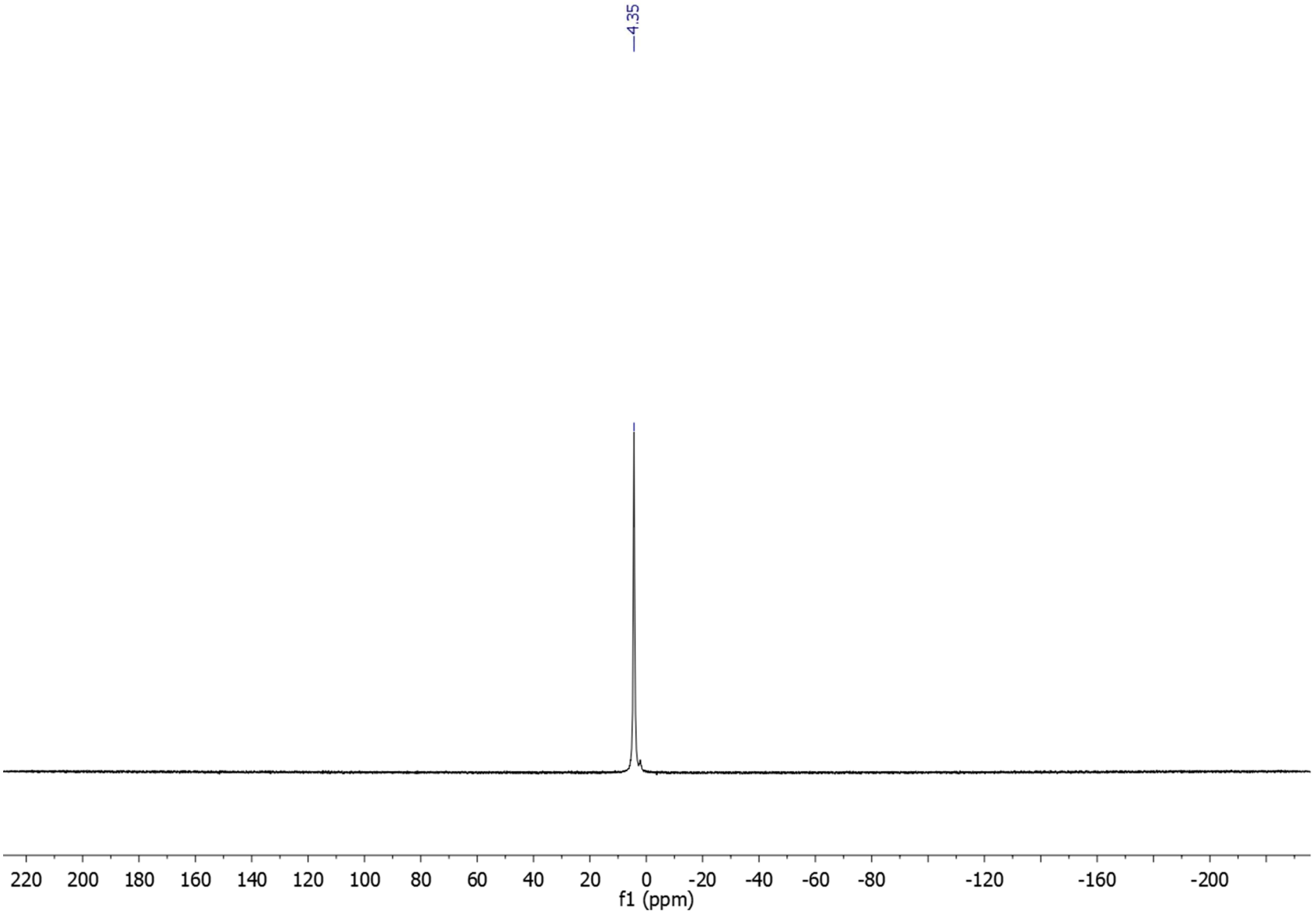


^{31}P-NMR of L-xylulose 5-phosphate



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