## Table of Contents

## Experimental Procedures

### S1. General notes

#### A. Materials

The bacterial strain *Streptomyces showdoensis* ATCC 15227 was obtained from the American Type Culture Collection. Kits for isolation of genomic DNA and plasmid DNA were products of Life Technologies (Carlsbad, CA) and Qiagen (Hilden, Germany), respectively. Q5® High-Fidelity DNA polymerase and restriction enzymes were acquired from New England Biolabs (Ipswich, MA). Econo-Pac® 10DG columns and reagents for denaturing (sodium dodecyl sulfate) polyacrylamide gel electrophoresis (SDS-PAGE) were purchased from Bio-Rad (Hercules, CA). Amicon® YM-10 ultrafiltration membranes were products of Millipore (Billerica, MA). The ion-exchange resins Dowex® 50W X8 (hydrogen form, 200-400 mesh) and AG® 1-X8 (formate form, 100-200 mesh) were obtained from Sigma-Aldrich (St. Louis, MO) and Bio-Rad Laboratories (Hercules, CA), respectively. All other chemicals were purchased from Fisher Scientific (Pittsburgh, PA) or Sigma-Aldrich (St. Louis, MO) and were used without further purification unless otherwise specified.

#### B. Instrumentation

NMR spectra were recorded on a Bruker AVANCE III HD 500 MHz instrument equipped with CryoProbe Prodigy and Varian DirectDrive 400 MHz spectrometers at the NMR Facility in the Department of Chemistry, University of Texas at Austin. $^{13}C$ NMR spectra were recorded with proton broadband decoupling. Deuterated solvents were used as internal standards during acquisition of the NMR spectra unless stated otherwise. LC-ESI-HRMS analysis was performed using an Agilent Technologies 1260 Infinity HPLC system with a 6230 ToF mass spectrometer (G6230B) equipped with an electrospray ionization (ESI) source and an inline diode array multiple wavelength UV-vis absorbance detector (G1315D). All protein samples were analyzed with the following ToF settings: 4000V Vcap, 1000V nozzle voltage, 310V fragmentor, 65V skimmer and *m/z* range 100-3000. High-performance liquid chromatography (HPLC) without MS detection was performed using an Agilent Technologies 1260 Infinity HPLC System equipped with a diode array multiple wavelength UV-vis absorbance detector (G1315D). DNA and protein concentrations were measured using a Thermo Fisher Scientific NanoDrop ND-1000 UV-visible spectrophotometer. UV-visible absorbance spectra were acquired on an Agilent 8453 UV-visible spectrophotometer.

### S2. Cloning and Expression of *N*-$His_6$-tagged Enzymes

The *sdmA*, *sdmB*, *sdmC*, *sdmD*, *sdmE*, *sdmF* and *sdmG* genes were individually amplified by polymerase chain reaction (PCR) from the genomic DNA of *S. showdoensis* ATCC 15227 and cloned into the pET28b(+) vector. The primers used in the cloning are shown in Table S1. The resulting plasmids were used to transform *E. coli* BL21 Star (DE3) cells. The overnight culture of each recombinant strain grown at 37 ℃ in Luria broth (LB) medium containing kanamycin (50 μg/mL) was used to inoculate 1 L of the same medium in a 200-fold dilution. These cultures were incubated at 37 ℃ until the OD 600 reached between 0.4-0.6. Protein expression was then induced by the addition of isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.1 mM. After overnight incubation at 18 ℃, the cells were harvested by centrifugation at 4000 × *g* for 15 min and resuspended in 30 mL of 50 mM tris(hydroxymethyl)-aminomethane (Tris) buffer (pH 8.0) containing 300 mM NaCl, 10 mM imidazole and 10% glycerol. Cells were subjected to 6 × 10 s ultrasonic burst with a 20 s cooling interval between each burst. Cell debris was removed by centrifugation at 20000 × *g* for 20 min, and the supernatant was mixed by slow agitation with 2 mL of nickel-nitrilotriacetic acid (Ni-NTA) resin for 60 min at 4 ℃. The slurry was transferred to a column and washed with 50 mM Tris buffer (pH 8.0) containing 300 mM NaCl, 20 mM imidazole and 10% glycerol. The protein was eluted with 50 mM Tris buffer (pH 8.0) containing 300 mM NaCl and 250 mM imidazole. The pooled protein isolates were dialyzed three times against 1 L of 50 mM Tris buffer (pH 8.0) containing 300 mM NaCl and 10% glycerol. Proteins were flash-frozen and stored at -80 ℃ before use. The SDS-PAGE of purified enzymes are shown in Figure S1.

### S3. Enzymatic Assays

#### S3.1 SdmE activity assays

L-Glutamine (**9**, 5 mM) was incubated with or without 10 μM SdmE in Tris·HCl buffer (50 mM, pH 8.0) at room temperature for 24 h. The mixture was then filtered using a YM-10 centrifugal filter to remove protein. A total volume of 450 μL filtrate, which contained SdmE product **10**, was mixed with 50 μL $D_2O$ and then analyzed by $^1H$-NMR spectroscopy. Synthetic standard of compound **10** (1 mM) was dissolved in 450 μL Tris·HCl buffer (50 mM, pH 8.0) and 50 μL $D_2O$, and analyzed by NMR (see Figure 3C).

#### S3.2 SdmC/SdmD and SdmF activity assays

Substrate **10** (1 mM) was incubated with 50 μM *apo*-SdmD, 2 μM SdmC, 1 μM SdmF, 2 μM 4'-phosphopantetheinyl transferase (Sfp) from *Bacillus subtilis*, 75 μM CoA, 1 mM ATP, 10 mM $MgCl_2$, and 5 mM tris-(2-carboxyethyl)phosphine (TCEP) in Tris·HCl buffer (100 mM, pH 8.0) in a total volume of 200 μL. The reaction was assembled on ice and incubated at room temperature for 2 h before quenched with equal volume of acetonitrile. The precipitate was removed by centrifugation and the supernatant was subjected to HPLC or LC-HRMS analysis (see Figures 3D and S2). HPLC and LC-HRMS separations were performed using an AdvanceBio Peptide Mapping column (Agilent, 2.7 μm, 2.1 × 150 mm). The mobile phase was 0.1% trifluoroacetic acid (TFA) in $H_2O$ as solvent A and 0.08% trifluoroacetic acid (TFA) in acetonitrile as solvent B. The flow rate was 0.25 mL/min and the elution gradients were as follows: 0–2 min 30% solvent B, 2–20 min 30% to 70% solvent B, 20–25 min 70% solvent B, 25–27 min 70% to 30% solvent B. The temperature was set to 40 °C and the UV detector was set at 220 nm, 280 nm or 352 nm.

**S3.3 Large-scale preparation of compound 12 and determination of its extinction coefficient**
Substrate **10** (1 mM) in a 1.5 mL Eppendorf tube was incubated with 100 μM *apo*-SdmD, 2 μM SdmC, 0.5 μM SdmF, 0.5 μM Sfp, 150 μM CoA, 1 mM ATP, 10 mM $MgCl_2$, and 5 mM TCEP in Tris·HCl buffer (100 mM, pH 8.0) in a total volume of 0.5 mL. After 24 h, the reaction mixture was centrifuged, and the supernatant was loaded onto an Econ-Pac® 10DG desalting column (Bio-Rad). The elution was performed using 50 mM Tris·HCl (pH 8.0), 50 mM NaCl and 10% (v/v) glycerol. The fractions containing desire product were combined and concentrated using an Amicon® Ultra Centrifugal Filter (10k cut-off). The obtained product was checked by LC-HRMS to be free of *apo*-SdmD, *holo*-SdmD and compound **11**, but contained a small amount (< 5%) of other proteins including SdmC, SdmF or Sfp. The protein concentration was determined with Bradford's reagent and the UV-Vis absorption at 352 nm was recorded on an UV-Vis spectrometer (see Figure S3). The extinction coefficient at 352 nm was calculated to be 26,950 $M^{-1}$ $cm^{-1}$.

**S3.4 Alternative substrate of SdmF**
$^1$H-NMR spectroscopy was used to determine whether compound **10** is an alternative substrate for SdmF. The assay consisted of 5 mM **10** and 1 μM SdmF in 500 μL Tris·HCl buffer (50 mM, pH 8.0). After incubating at room temperature for 24 h, the mixture was filtered using a YM-10 centrifugal filter to remove protein. A total volume of 450 μL filtrate was mixed with 50 μL $D_2O$ and then analyzed by $^1$H-NMR spectroscopy (see Figure S4).

**S3.5 SdmG activity assays by HPLC, LC-HRMS and UV-Vis spectroscopy analysis**
Compound **12** (50 μM) was incubated with 1 μM SdmG in Tris·HCl buffer (50 mM, pH 8.0) aerobically at room temperature (200 μL total volume). After 5 min or 3 h, an equal volume of acetonitrile was added, and the resulting mixture was centrifuged. The supernatant was analyzed by HPLC and LC-HRMS as described in Section S3.2.
The SdmG activity was also studied using UV-Vis spectroscopy. Compound **12** (30 μM) was incubated with 50 nM SdmG in Tris·HCl buffer (50 mM, pH 8.0) aerobically in a 1 cm pathlength quartz cuvette at room temperature (200 μL total volume). The time-course consumption of **12** was monitored accordingly (see Figure 4A).
To explore the decomposition of SdmG product **13**, compound **12** (30 μM) was incubated with 1 μM SdmG in Tris·HCl buffer (50 mM, pH 8.0) aerobically at room temperature (400 μL total volume). After 1 min, the mixture was immediately filtered using a YM-10 centrifugal filter (14,000 RPM × 4 min) to remove protein and the filtrate (containing ca. 30 μM **13**) was characterized by UV-Vis spectroscopy in a 1 cm pathlength quartz cuvette. The decomposition of **13** was monitored over 24 h (see Figure 4B).

**S3.6 Incubation of *holo*-SdmD with maleimide or showdomycin**
A solution of 100 μM *apo*-SdmD was incubated with 2 μM Sfp, 150 μM CoA, 10 mM $MgCl_2$, and 5 mM TCEP in a total volume of 100 μL Tris·HCl buffer (100 mM, pH 8.0). After 3 h, 1 μL of maleimide or showdomycin (100 mM in DMSO, 10 eq.) was added and incubation was continued for another 1 h. The resulting mixture was diluted with 100 μL acetonitrile and centrifuged. The supernatant was then subjected to HPLC and LC-MS analysis as described in Section S3.2 and the results are shown in Figures 4D and S14.

**S3.7 Preparation of $^{15}$N-labeled compound 12 and SdmG activity assays using 12 as substrate**
A solution of 4 mM L-glutamine, L-glutamine-(*amide*-$^{15}$N), or L-glutamine-($^{15}N_2$) was incubated with 50 μM *apo*-SdmD, 2 μM SdmC, 5 μM SdmE, 1 μM SdmF, 2 μM Sfp, 75 μM CoA, 4 mM ATP, 10 mM $MgCl_2$, and 5 mM TCEP in a total volume of 1 mL Tris·HCl buffer (100 mM, pH 8.0). The reaction was assembled on ice and incubated at room temperature. After 24 h, the reaction was subjected to centrifugation and the supernatant was buffer exchanged with 50 mM Tris·HCl (pH 8.0), 50 mM NaCl and 10% (v/v) glycerol using an Amicon® Ultra Centrifugal Filter (10k cut-off). This process was repeated at least six times to remove small molecules. LC-HRMS analysis of the obtained product indicated the presence of *holo*-SdmD as major side product (ranging from 30% to 50%). The concentration of compound **12** was determined based on its UV-Vis absorbance at 352 nm. The samples were flash frozen and stored at -80 °C prior to activity assays.
In SdmG activity assay, each sample of unlabeled and labeled **12** (50 μM) was incubated with 1 μM SdmG in Tris·HCl buffer (50 mM, pH 8.0) under aerobic condition at room temperature (100 μL total volume). After 3 h, the reaction was quenched with an equal volume of acetonitrile and centrifuged. The supernatant was analyzed by LC-HRMS as described in Section S3.2 and the results are shown in Figure S7.

**S3.8 SdmG activity assays in the presence of $^{18}O_2$ or $H_2{}^{18}O$**
In an anaerobic chamber under an atmosphere of > 98% $N_2$ and < 2% $H_2$, a 200 μL solution containing 1 μM SdmG, 50 μM **12** and 50 mM Tris·HCl (pH 8.0) was prepared in a 4 mL glass vial. The vial was then capped with a rubber septum and removed from the anaerobic chamber. The headspace was evacuated under vacuum and refilled with $^{18}O_2$ (97 atom%) using a balloon. After 3 h incubation, the reaction mixture was quenched with 200 μL acetonitrile and centrifuged under aerobic condition. The supernatant was analyzed by LC-HRMS within one hour after the reaction mixture was exposed to natural abundance $O_2$.
The same experiment was also performed under aerobic condition with natural abundance $O_2$ and $H_2{}^{18}O$. The final reaction mixture contained approximately 94% $H_2{}^{18}O$. After 3 h incubation, the reaction was quenched with 200 μL acetonitrile and centrifuged. The supernatant was then analyzed by LC-HRMS as described in Section S3.2. Results are shown in Figures 5A and 5B.

**S3.9 Incubation of SdmG product with 0.1% TFA buffer**
Compound **12** (50 μM) was incubated with 1 μM SdmG aerobically in a total volume of 100 μL Tris·HCl buffer (5 mM, pH 8.0) at room temperature. After 3 h incubation, an aliquot of 100 μL 0.1% TFA in $ddH_2O$ and 200 μL acetonitrile was added to this

reaction mixture. The final pH of the solution was estimated using pH paper to be between 2 and 3. The solution was incubated at room temperature for 3 h and analyzed by LC-HRMS using 50% acetonitrile in $ddH_2O$ as elution solution (0.1 mL/min) without column separation. Ppant ejection spectra of SdmG reaction mixture with and without acid treatment were shown in Figures 5D and 5C, respectively. Due to the absence of column separation, **23**, **24** and *holo*-SdmD (product of SdmG assay) were eluted as a single peak.

**S3.10 SdmA and SdmB activity assays**

Aerobic coupled assays:
Compound **12** (50 μM) was incubated with 200 nM SdmG, 10 μM SdmA, 2 μM SdmB, 0.5 mM ribose 5-phosphate (R5P) or phosphoribosyl pyrophosphate (PRPP), 5 mM $MgCl_2$ in a total volume of 100 μL Tris·HCl buffer (50 mM, pH 8.0). After 4 h, the reaction was quenched with 100 μL acetonitrile and chilled on ice for 5 min to facilitate protein precipitation. The mixture was then centrifuged, and the supernatant was subjected to HPLC and LC-HRMS analysis as described in Section S3.2 and the results are shown in Figures 6C, S13 and S14.

Anaerobic assays:
In an anaerobic chamber under an atmosphere of > 98% $N_2$ and < 2% $H_2$, **13** was freshly prepared by incubating 2 μM SdmG with 100 μM compound **12** in a total volume of 400 μL Tris·HCl buffer (25 mM, pH 8.0). After 2 min, the reaction was filtered using a YM-10 centrifugal filter (13,000 RPM × 5 min) to remove protein. To an 80 μL aliquot of the filtrate (containing ca. 100 μM **13**) was added R5P or PRPP, $MgCl_2$, SdmA, SdmB and $ddH_2O$ to a total volume of 100 μL. The final reaction mixture contained ca. 80 μM **13**, 0.8 mM R5P or PRPP, 5 mM $MgCl_2$, 10 μM SdmA, 2 μM SdmB and Tris·HCl buffer (20 mM, pH 8.0). After 1 h incubation, the reaction was filtered using a YM-10 centrifugal filter to remove proteins and the filtrate was transferred to a sealed 2.0 mL screw thread mass vial equipped with an insert. The LC-HRMS analysis was conducted immediately after the mass vial was exposed to air. The LC separation was performed using a Capcell PAK C18 MGIII column (Osaka Soda, 5 μm, 4.6 × 250 mm). The mobile phase was 5 mM ammonium formate (pH 8.0) in $H_2O$ as solvent A and acetonitrile as solvent B. The flow rate was 0.50 mL/min and the elution gradients were as following: 0–4 min 1% solvent B, 4–10 min 1% to 5% solvent B, 10–13 min 5% to 50% solvent B, 13–20 min 50% solvent B, 20–21 min 50% to 1% solvent B. The temperature was set to 30 °C and UV absorbance was monitored at 288 nm. Results are shown in Figures 6B, S9 and S10.

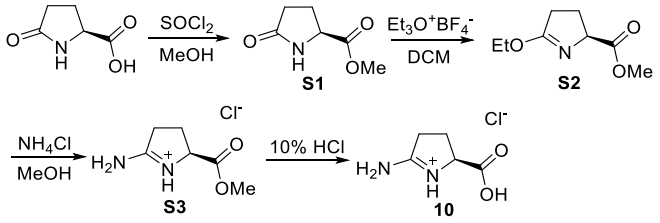
**S3.11 Preparation of [4-$^2$H]-13 and SdmA activity assays**
3,3-Dideutero-L-glutamine (5 mM) was incubated with 5 μM SdmE in a total volume of 1 mL Tris·HCl buffer (25 mM, pH 8.0). The reaction mixture was incubated for 24 h and then centrifuged. To an 800 μL aliquot of the supernatant was added 50 μM *apo*-SdmD, 2 μM SdmC, 1 μM SdmF, 2 μM Sfp, 75 μM CoA, 2 mM ATP, 5 mM $MgCl_2$, 5 mM TCEP and $ddH_2O$ to a final volume of 1 mL. After 24 h incubation at room temperature, the reaction mixture was centrifuged, and the supernatant was desalted as described in Section S3.3. The desired product [4-$^2$H]-**12** was collected, concentrated using an Amicon® Ultra Centrifugal Filter (10k cut-off), flash-frozen, and stored at -80 ℃ before use.

In an anaerobic chamber under an atmosphere of > 98% $N_2$ and < 2% $H_2$, 100 μM [4-$^2$H]-**12** was incubated with 2 μM SdmG in a total volume of 100 μL Tris·HCl buffer (25 mM, pH 8.0). After 2 min, the reaction was filtered using a YM-10 centrifugal filter (13,000 RPM × 5 min) to remove protein. To an 80 μL aliquot of the filtrate (containing ca. 100 μM [4-$^2$H]-**13**) was added R5P, $MgCl_2$, SdmA and $ddH_2O$ to a final volume of 100 μL. The final reaction mixture contained ca. 80 μM [4-$^2$H]-**13**, 0.8 mM R5P, 5 mM $MgCl_2$, 10 μM SdmA and Tris·HCl buffer (20 mM, pH 8.0). After 1 h incubation, the reaction was filtered using a YM-10 centrifugal filter to remove proteins and the filtrate was transferred to a sealed 2.0 mL screw thread mass vial equipped with an insert. The LC-HRMS analysis was conducted immediately after the mass vial was exposed to air. The results are shown in Figure S11.

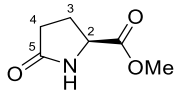
**S3.12 Autoxidation of 16**
A solution of **16** (ca. 80 μM in 20 mM Tris·HCl buffer pH 8.0) was enzymatically prepared and deproteinized via ultrafiltration anaerobically as described in Section S3.10. The filtrate was removed from the anaerobic chamber and exposed to air for 3 h at room temperature. A 10 μL aliquot was then diluted with 100 μL 0.1% formic acid (FA) and incubated at room temperature for another 2 h. The final pH of the solution was estimated using pH paper to be approximately 3. The samples before and after acid treatment were subjected to LC-MS analysis and compared with synthetic standards of showdomycin (**1**) and 1'-*epi*-showdomycin (1'-*epi*-**1**). LC-HRMS separations were performed using a Poroshell 120 EC-C18 column (2.7 μm, 4.6 × 100 mm) equipped with an Eclipse Plus C18 guard column (1.8 μm, 2.1 × 5 mm). The mobile phase was 0.1% formic acid (FA) in $H_2O$ as solvent A and acetonitrile as solvent B. The flow rate was 0.4 mL/min and the elution gradients were as following: 0–2 min 2% solvent B, 2–5 min 2% to 10% solvent B, 5–10 min 10% to 50% solvent B, 10–13 min 50% solvent B, 13–14 min 50% to 2% solvent B. The results are shown in Figure 6D.

**S4. Chemical Synthesis**

**S4.1 Synthesis of 2-amino-1-pyrroline-5-carboxylic acid (10)**




**Scheme S1**. Synthesis of **10**. Compound **10** was synthesized according to a reported procedure with some modifications.[1]

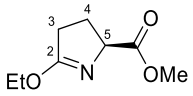


***Methyl (S)-5-oxopyrrolidine-2-carboxylate*** (**S1**): L-Pyroglutamic acid (2.00 g, 15.5 mmol) was suspended in 50 mL anhydrous MeOH and to this solution was added $SOCl_2$ (0.22 mL, 3.0 mmol) dropwise. After stirring at room temperature for 24 h, the clear solution was concentrated under reduced pressure and purified by silica gel chromatography (ethyl acetate to ethyl acetate:MeOH = 95:5) to give **S1** (2.04 g, 92%) as a colorless oil.

$^1$H NMR (400 MHz, $CDCl_3$) δ 6.71 (br, 1H), 4.29 – 4.21 (m, 1H, H-2), 3.76 (s, 3H, $OCH_3$), 2.54 – 2.28 (m, 3H, H-3 & H-4), 2.26 – 2.13 (m, 1H, H-3).

$^{13}$C NMR (126 MHz, $CDCl_3$) δ 178.22, 172.62, 55.49, 52.69, 29.33, 24.88.

ESI-HRMS calcd. for $C_6H_{10}NO_3^+$ $[M+H]^+$ 144.0661, found 144.0664.

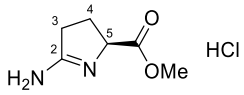


***Methyl (S)-2-ethoxy-1-pyrroline-5-carboxylate*** (**S2**): **S1** (1.01 g, 7.1 mmol) was dissolved in 10 mL anhydrous dichloromethane (DCM) and triethyloxonium tetrafluoroborate (10 mL, 1 M in DCM, 1.4 eq.) was added dropwise via a syringe under argon protection. After stirring at room temperature for 24 h, the reaction was quenched with 10 mL sat. $NaHCO_3$. The organic layer was separated, and the aqueous phase was extracted with DCM twice. The combined organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated under vacuo. The crude product was purified by silica gel chromatography (hexanes:ethyl acetate = 1:1) to afford **S2** (905 mg, 75%) as a pale-yellow oil.

$^1$H NMR (500 MHz, $CDCl_3$) δ 4.54 (ddt, *J* = 8.4, 5.7, 1.1 Hz, 1H, H-5), 4.26 (dq, *J* = 10.5, 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 2.61 (dddd, *J* = 16.4, 10.1, 6.0, 1.4 Hz, 1H, H-3), 2.49 (dddd, *J* = 16.7, 10.0, 6.7, 1.0 Hz, 1H, H-3), 2.31 (dddd, *J* = 13.1, 10.0, 8.6, 6.0 Hz, 1H, H-4), 2.16 (dddd, *J* = 12.9, 10.2, 6.7, 5.7 Hz, 1H, H-4), 1.32 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$).

$^{13}$C NMR (126 MHz, $CDCl_3$) δ 175.38, 174.48, 68.32, 64.75, 52.27, 31.40, 26.96, 14.48.

ESI-HRMS calcd. for $C_8H_{14}NO_3^+$ $[M+H]^+$ 172.0974, found 172.0977.

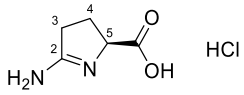


***Methyl (S)-2-amino-1-pyrroline-5-carboxylate hydrochloride*** (**S3**): **S2** (140 mg, 0.82 mmol) was dissolved in 3 mL anhydrous MeOH and to this solution was added anhydrous $NH_4Cl$ (46 mg, 0.86 mmol) in one portion. The resulting mixture was refluxed at 70 °C under argon atmosphere for 6 h and then cooled to room temperature. The solvent was removed in vacuo to give the crude product as a white solid. Recrystallization from DCM afforded **S3** (87 mg, 60%) as white powder.

$^1$H NMR (500 MHz, $D_2O$) δ 4.74 (dd, *J* = 9.4, 5.1 Hz, 1H, H-5), 3.04 – 2.90 (m, 2H, H-3), 2.63 (dddd, *J* = 13.4, 9.1, 9.1, 7.8 Hz, 1H, H-4), 2.33 (dddd, *J* = 13.5, 8.5, 6.7, 5.1 Hz, 1H, H-4).

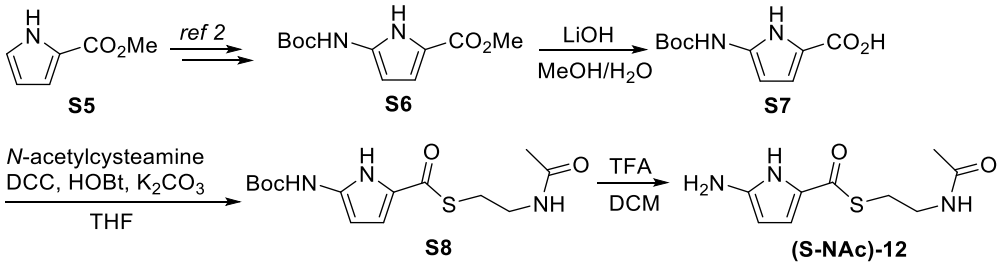
$^{13}$C NMR (126 MHz, $D_2O$) δ 173.03, 172.22, 60.60, 53.24, 29.28, 24.46.

ESI-HRMS calcd. for $C_6H_{11}N_2O_2^+$ $[M+H]^+$ 143.0821, found 143.0814.

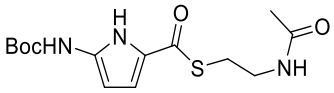


***(S)-2-amino-1-pyrroline-5-carboxylic acid hydrochloride*** (**10**): **S3** (45 mg, 0.25 mmol) was dissolved in 2 mL 10% hydrochloric acid and the solution was heated at 50 °C for 3 h. The reaction was cooled down and the volatiles were removed in vacuo. The residual was co-evaporated with 5 mL toluene twice to yield **10** (40 mg, 96%) as white crystalline solid.
$^1$H NMR (500 MHz, $D_2O$) δ 4.66 (dd, *J* = 9.3, 5.3 Hz, 1H, H-5), 3.02 – 2.89 (m, 2H, H-3), 2.69 – 2.57 (m, 1H, H-4), 2.30 (dddd, *J* = 13.6, 8.4, 6.9, 5.4 Hz, 1H, H-4).
$^{13}$C NMR (126 MHz, $D_2O$) δ 175.01, 172.07, 60.89, 29.34, 24.81.
ESI-HRMS calcd. for $C_5H_9N_2O_2^+$ $[M+H]^+$ 129.0664, found 129.0659.

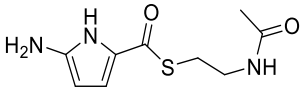
**S4.2 Synthesis of *N*-acetylcysteamine (S-NAc) derivative of 12**




**Scheme S2**. Synthesis of *N*-acetylcysteamine (S-NAc) derivative of **12**. Compound **S6** was synthesized according to a reported procedure.[2]

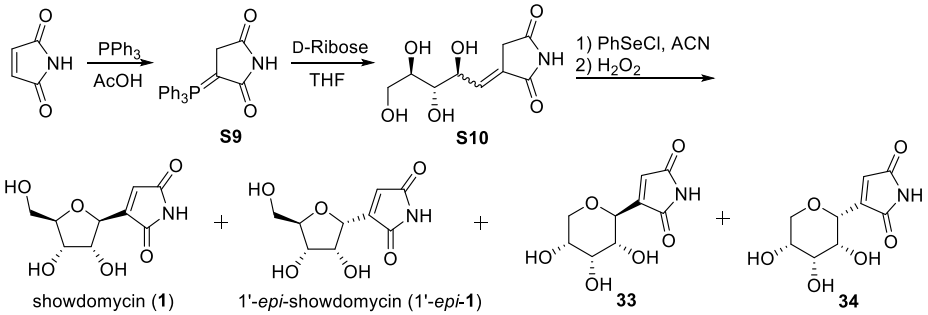


***S-(2-acetamidoethyl) 2-((tert-butoxycarbonyl)amino)-1H-pyrrole-5-carbothioate*** (**S8**): **S6** was chemically synthesized from **S5** as reported.[2] To a stirring solution of **S6** (190 mg, 0.79 mmol) in MeOH (5 mL) and $H_2O$ (5 mL) was added LiOH·$H_2O$ (330 mg, 7.8 mmol, 10 eq.). The solution was stirred at 45 °C for 24 h under an argon atmosphere. After complete consumption of the substrate as indicated by TLC (ethyl acetate/hexanes = 1:3), the volatiles were removed in vacuo. The remaining residue was diluted with water, neutralized to pH ~3 with 1 M HCl, and extracted with ethyl acetate. The organic layers were combined, dried over $Na_2SO_4$ and concentrated to a small volume. The residual solvent was removed by blowing argon to give the crude acid **S7** as red to purple solid. The crude product was immediately used for the next step without purification to prevent degradation.
To a solution of crude acid **S7** in anhydrous THF (20 mL) was added dicyclohexylcarbodiimide (DCC) (180 mg, 0.87 mmol, 1.1 eq.), hydroxybenzotriazole (HOBt) (120 mg, 0.88 mmol, 1.1 eq.) and *N*-acetylcysteamine (0.42 mL, 3.95 mmol, 5 eq.) under an argon atmosphere. After stirring the solution for 45 min, $K_2CO_3$ (66 mg, 0.48 mmol, 0.6 eq.) was added and the reaction stirred overnight. The mixture was then filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes/ethyl acetate/methanol = 50:50:0 to 50:50:5). The fractions containing the desired product were combined, concentrated and repurified by silica gel column chromatography to remove residual dicyclohexylurea (DCU). The purified product **S8** was obtained as a pale-yellow oil (68 mg, 26% two steps).
$^1$H NMR (500 MHz, $CDCl_3$) δ 10.51 (s, 1H, $NH_{pyrrole}$), 7.09 (s, 1H, $NH_{amide}$), 6.93 (dd, *J* = 4.1, 2.2 Hz, 1H), 6.20 (s, 1H, $NH_{amide}$), 5.66 – 5.57 (m, 1H), 3.50 (m, 2H), 3.16 (m, 2H), 1.97 (s, 3H, $COCH_3$), 1.52 (s, 9H, $C(CH_3)_3$).
$^{13}$C NMR (126 MHz, $CDCl_3$) δ 179.54, 170.60, 152.37, 135.15, 123.89, 117.08, 95.59, 82.54, 40.80, 28.33, 27.64, 23.43.
ESI-HRMS calcd. for $C_{14}H_{22}N_3O_4S^+$ $[M+H]^+$ 328.1331, found 328.1333.

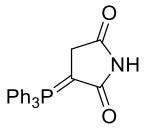


***S-(2-acetamidoethyl) 2-amino-1H-pyrrole-5-carbothioate*** (S-NAc-**12**): To a stirring solution of **S8** (12.5 mg, 0.038 mmol) in DCM (1 mL) was added trifluoroacetic acid (TFA) (1 mL) dropwise in an ice-water bath. After 1 h, the solvent was removed, and the residue was purified by silica gel column chromatography (hexanes/ethyl acetate/methanol = 50:50:5) to give a crude mixture of (S-NAc)-**12** and an unknown compound. This mixture was further purified by HPLC using a Capcell PAK C18 MGIII column (Osaka Soda, 5 μm, 10 × 250 mm) with water as solvent A and acetonitrile as solvent B. Gradient elution was performed with a flow rate of 4 mL/min and UV absorbance monitored at 352 nm using the following conditions: 0–2 min 10% solvent B, 2–15 min 10% to 50% solvent B, 15–17 min 50% to 90% solvent B, 17–20 min 90% solvent B, 20–21 min 90% to 10% solvent B. The typical retention time of (S-NAc)-**12** is 11.5 min.
$^1$H NMR (400 MHz, 10% $D_2O$) δ 8.05 (s, 1H, $NH_{amide}$), 7.11 (d, *J* = 4.2 Hz, 1H), 5.57 (d, *J* = 4.1 Hz, 1H), 3.39 (m, 2H), 3.11 (t, *J* = 6.3 Hz, 2H), 1.93 (s, 3H).
$^{13}$C NMR (126 MHz, 10% $D_2O$) δ 178.13, 174.35, 147.19, 122.02, 121.88, 96.12, 39.57, 27.09, 21.90.
ESI-HRMS calcd. for $C_9H_{14}N_3O_2S^+$ $[M+H]^+$ 228.0807, found 228.0788.

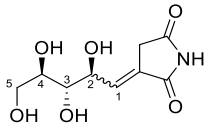
**S4.3 Synthesis of showdomycin (1), 1'-*epi*-showdomycin (1'-*epi*-1) and pyranose isomers 33, 34**



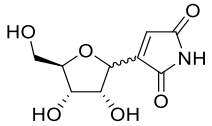

**Scheme S3.** Synthesis of showdomycin (**1**) and 1'-*epi*-showdomycin (1'-*epi*-**1**) were performed by following a known procedure with some modifications.[3] Compounds **33** and **34** were obtained as very minor isomers from the last step.



***3-(triphenyl-λ⁵-phosphaneylidene)pyrrolidine-2,5-dione*** (**S9**): Triphenylphosphine (6.50 g, 24.7 mmol) and maleimide (2.50 g, 25.7 mmol) were dissolved in 50 mL glacier acetic acid and the solution was heated to 100 °C with stirring. After 1 h, the orange solution was cooled to room temperature and the majority of solvent was removed in vacuo. To the residue was added 100 mL acetone and the resulting mixture was stirred vigorously until white precipitate formed. This was filtered, and the filter cake was washed with cold acetone and then diethyl ether to yield **S9** (7.53 g, 85%) as white amorphous powder.
$^1$H NMR (500 MHz, $CDCl_3$) δ 7.66 – 7.58 (m, 9H, Ph), 7.56 – 7.49 (m, 6H, Ph), 3.03 (s, 2H, $CH_2$).
$^{13}$C NMR (126 MHz, $CDCl_3$) δ 177.88 (d, *J* = 16.8 Hz), 170.89 (d, *J* = 15.0 Hz), 133.49 (d, *J* = 10.6 Hz), 132.92 (d, *J* = 2.9 Hz), 129.35 (d, *J* = 12.6 Hz), 125.56 (d, *J* = 92.4 Hz), 38.47 (d, *J* = 10.3 Hz), 36.82 (d, *J* = 137.1 Hz).
ESI-HRMS calcd. for $C_{22}H_{19}NO_2P^+$ $[M+H]^+$ 360.1153, found 360.1171.



***3-((3S,4R)-2,3,4,5-tetrahydroxypentylidene)pyrrolidine-2,5-dione*** (**S10**): D-Ribose (0.85 g, 5.7 mmol) and **S9** (4.00 g, 11.1 mmol) were suspended in 40 mL anhydrous THF and the mixture was refluxed at 80 °C for 8 days under an argon atmosphere. After the solvent was removed in vacuo, the residue was partitioned between 35 mL $H_2O$ and 35 mL DCM. The organic layer was back extracted with 10 mL $H_2O$ and the aqueous layers were combined and concentrated to small volume. The residue was passed through a mixture of C18 reverse-phase silica gel (40 g) and normal silica gel (0.4 g), and eluted with 300 mL $H_2O$. The elution was concentrated and purified by silica gel chromatography (ethyl acetate:acetone:MeOH = 100:0:0 to 75:20:5). The purified product was recrystallized from ethyl acetate/ethanol to give 0.92 g **S10** (70%, *d.r.* = 20:1) as colorless needles.
$^1$H NMR (400 MHz, $D_2O$) δ 6.73 (dt, *J* = 8.3, 2.4 Hz, 1H), 4.59 (dd, *J* = 8.3, 4.2 Hz, 1H), 3.81 (dd, *J* = 8.2, 4.2 Hz, 1H), 3.78 (dd, *J* = 11.8, 2.8 Hz, 1H) 3.63 (dd, *J* = 11.8, 6.2 Hz, 1H), 3.57 (ddd, *J* = 8.2, 6.2, 2.8 Hz, 1H), 3.50 (ddd, *J* = 5.1, 2.4, 0.7 Hz, 2H).
$^{13}$C NMR (126 MHz, $D_2O$) δ 178.97, 173.15, 134.34, 129.88, 73.56, 71.76, 69.67, 62.73, 33.22.
ESI-HRMS calcd. for $C_9H_{14}NO_6^+$ $[M+H]^+$ 232.0821, found 232.0827.



***Showdomycin*** (**1**) and ***1'-epi-showdomycin*** (1'-*epi*-**1**): To a solution of **S10** (150 mg, 0.65 mmol) in 20 mL anhydrous acetonitrile was added PhSeCl (125 mg, 0.65 mmol). The suspension was stirred and heated at 65 °C for 21 h. It was then

cooled down to room temperature and mixed with 1 mL 30% $H_2O_2$ which was added dropwise over five minutes. The resulting solution was stirred for another 30 min. At the end of the reaction, the solvent was removed in vacuo and the residual material was purified by silica gel column chromatography (ethyl acetate/ethanol = 20:1) to give a crude mixture of showdomycin and 1′-*epi*-showdomycin as white solid. This mixture was dissolved in a small amount of $ddH_2O$ and purified by HPLC using a C18 reversed-phase column (Agilent, ZORBAX, ODS, 5 μm, 9.4 mm x 250 mm) with water as solvent A and acetonitrile as solvent B. Gradient elution was performed with a flow rate of 4 mL/min and UV absorbance monitored at 254 nm using the following conditions: 0–7 min 0.5% to 2% solvent B, 7–9 min 2% to 50% solvent B, 9–14 min 50% solvent B, 14–15 min 50% to 0.5% solvent B. The typical retention time of showdomycin and 1'-*epi*-showdomycin were 7.6 min and 6.8 min, respectively. Lyophilization of desired fractions afforded showdomycin **1** (20 mg, 14%) and 1'-*epi*-showdomycin 1'-*epi*-**1** (35 mg, 24%) as fluffy white solids.

Showdomycin (**1**)

$^1$H NMR (500 MHz, $D_2O$) δ 6.74 (d, *J* = 1.7 Hz, 1H, maleimide), 4.83 (dd, *J* = 4.6, 1.7 Hz, 1H, H-1'), 4.30 (dd, *J* = 4.9, 4.9 Hz, 1H, H-2'), 4.13 (dd, *J* = 6.1, 5.2 Hz, 1H, H-3'), 4.07 (ddd, *J* = 6.1, 5.2, 3.1 Hz, 1H, H-4'), 3.87 (dd, *J* = 12.6, 3.1 Hz, 1H, H-5'), 3.73 (dd, *J* = 12.6, 5.2 Hz, 1H, H-5').

$^{13}$C NMR (126 MHz, $D_2O$) δ 173.05, 172.44, 147.07, 129.17, 83.23, 77.49, 74.45, 70.81, 61.34.

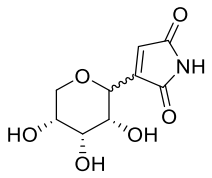
ESI-HRMS calcd. for $C_9H_{12}NO_6^+$ $[M+H]^+$ 230.0665, found 230.0653.

1′-*epi*-Showdomycin (1'-*epi*-**1**)

$^1$H NMR (500 MHz, $D_2O$) δ 6.69 (d, *J* = 2.1 Hz, 1H, maleimide), 5.08 (dd, *J* = 3.6, 2.2 Hz, 1H, H-1'), 4.51 (dd, *J* = 4.0, 4.0 Hz, 1H, H-2'), 4.34 (dd, *J* = 8.6, 4.4 Hz, 1H, H-3'), 4.04 (ddd, *J* = 8.4, 5.0, 2.6 Hz, 1H, H-4'), 3.92 (dd, *J* = 12.5, 2.6 Hz, 1H, H-5') 3.73 (dd, *J* = 12.5, 5.0 Hz, 1H, H-5').

$^{13}$C NMR (126 MHz, $D_2O$) δ 173.15, 172.42, 146.90, 129.35, 81.09, 76.44, 72.33, 71.90, 61.09.

ESI-HRMS calcd. for $C_9H_{12}NO_6^+$ $[M+H]^+$ 230.0665, found 230.0649.




***D*-Ribopyranosylmaleimide** (**33** and **34**): Compounds **33** and **34** were synthesized from **S10** (117 mg) and PhSeCl (100 mg) in 7 mL anhydrous acetonitrile followed by treatment with $H_2O_2$ (30%, 0.8 mL) as described above. The resulting reaction mixture was concentrated under vacuo and diluted with 15 mL $ddH_2O$. The aqueous phase was washed with ethyl acetate three times and purified by HPLC using a Capcell PAK C18 MGIII column (Osaka Soda, 5 μm, 10 × 250 mm) with 0.1% trifluoroacetic acid in water as solvent A and acetonitrile as solvent B. Gradient elution was performed with a flow rate of 4 mL/min and UV absorbance monitored at 254 nm using the following conditions: 0–2 min 3% solvent B, 2–10 min 3% to 10% solvent B, 10–12 min 10% to 50% solvent B, 12–18 min 50% solvent B, 18–19 min 50% to 3% solvent B. The typical retention times of **33** and **34** are 8.3 min and 10.5 min, respectively. Lyophilization of the desired fractions afforded **33** (1.5 mg, 1%) and **34** (<1 mg, <1%) as pale-yellow solids.

*β*-D-Ribopyranosylmaleimide (**33**)

$^1$H NMR (500 MHz, $D_2O$) δ 6.83 (s, 1H, maleimide), 4.53 (d, *J* = 9.8 Hz, 1H, H-1'), 4.25 (dd, *J* = 2.9, 2.9 Hz, 1H, H-3'), 3.95 (ddd, *J* = 10.8, 5.3, 2.8 Hz, 1H, H-4'), 3.90 (dd, *J* = 9.8, 2.7 Hz, 1H, H-2'), 3.83 (dd, *J* = 10.9, 5.4 Hz, 1H, H-5'), 3.66 (dd, *J* = 10.9, 10.9 Hz, 1H, H-5').

$^{13}$C NMR (126 MHz, $D_2O$) δ 172.85, 172.53, 145.61, 131.87, 70.29, 69.97, 68.68, 66.32, 64.86.

ESI-HRMS calcd. for $C_9H_{10}NO_6^-$ $[M-H]^-$ 228.0508, found 228.0520.

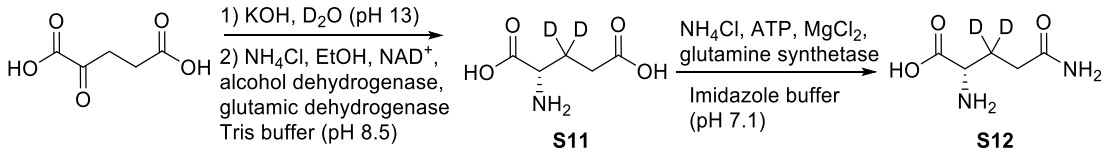
*α*-D-Ribopyranosylmaleimide (**34**)

$^1$H NMR (400 MHz, $D_2O$) δ 6.69 (d, *J* = 2.0 Hz, 1H, maleimide), 4.57 (dd, *J* = 1.7, 1.7 Hz, 1H, H-1'), 4.16 (dt, *J* = 3.2, 1.5 Hz, 1H, H-2'), 4.11 (dd, *J* = 12.8, 2.1 Hz, 1H, H-5'), 3.99 – 3.96 (m, 1H, H-4'), 3.92 (dd, *J* = 3.4, 3.4 Hz, 1H, H-3'), 3.79 (dd, *J* = 12.9, 1.3 Hz, 1H, H-5').

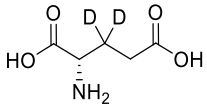
$^{13}$C NMR (126 MHz, $D_2O$) δ 173.02, 172.23, 146.02, 130.14, 74.47, 71.03, 70.01, 68.60, 67.78.

ESI-HRMS calcd. for $C_9H_{10}NO_6^-$ $[M-H]^-$ 228.0508, found 228.0515.

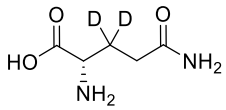
### S4.4 Synthesis of 3,3-dideutero-L-glutamine (S12)




**Scheme S4.** The enzymatic synthesis of 3,3-dideutero-L-glutamine was carried out according to a known protocol with some modifications.[4]



***3,3-Dideutero-L-glutamic acid*** (**S11**): A solution of 0.70 g α-ketoglutaric acid sodium salt dissolved in 15 mL $D_2O$ was prepared with pH adjusted to 13 by the addition of 4 M KOH in $D_2O$. The solution was stirred for 4 h at 37 °C and lyophilized to dryness. The crude 3,3-dideutero-α-ketoglutaric acid and 0.44 g $NH_4Cl$ were dissolved in 20 mL 25 mM Tris buffer in $D_2O$. The pH was adjusted to 8.5 with 35 wt% DCl in $D_2O$. To the solution was added 0.5 mL EtOH, 66 mg nicotinamide adenine dinucleotide ($NAD^+$), 168 units glutamic dehydrogenase from bovine liver and 1500 units alcohol dehydrogenase from yeast. After incubation at 37 °C for 5 h, the solution was brought to 90 °C for 10 min and then cooled down. The pH was adjusted to 3.0 using conc. HCl. After centrifugation, the supernatant was passed through an anion-exchange column (diameter 2 cm, length 15 cm) packed with AG 1-X8 resin (acetate form). The column was washed with water and the desired product was eluted with 1 M acetic acid. The elution was monitored by TLC and the fractions containing the desired product were combined and evaporated to dryness. To the residue was added a small amount of water and the pH was adjusted to 3.2. The suspension was heated until all solid was dissolved. After cooling of the solution, the white precipitate was filtered, washed with ethanol and diethyl ether to give 3,3-dideutero-L-glutamic acid (**S11**) as off-white solid (380 mg, 54%, >98% D).
$^1$H NMR (400 MHz, $D_2O$) δ 3.78 (s, 1H), 2.52 (d, *J* = 17.1 Hz, 2H).
$^{13}$C NMR (126 MHz, $D_2O$) δ 177.08, 173.81, 53.71, 29.85, 24.90 (m).
ESI-HRMS calcd. for $C_5H_8D_2NO_4^+$ $[M+H]^+$ 150.0730, found 150.0741.



***3,3-Dideutero-L-glutamine*** (**S12**): A mixture of 80 mg 3,3-dideutero-L-glutamic acid (**S11**), 56 mg $NH_4Cl$, 1.40 g adenosine triphosphate (ATP) disodium salt hydrate and 840 mg $MgCl_2{\cdot}6H_2O$ were dissolved in 50 mL 80 mM imidazole buffer (pH 7.1). To this solution was added 1.86 mg glutamine synthetase (GS) from *E. coli* K-12. The reaction was gently stirred at 30 °C for 12 h upon which additional 1.86 mg GS was added. After 12 h, the reaction mixture was centrifuged, and the supernatant was passed through a cation-exchange column (diameter 5 cm, length 8 cm) packed with Dowex 50W X8 resin (ammonium form). The column was eluted with water and the eluate containing the desired product was subsequently passed through an anion-exchange column (diameter 5 cm, length 8 cm) packed with AG 1-X8 resin (acetate form). The column was eluted with water and the eluate containing the desired product was combined and lyophilized to give 3,3-dideutero-L-glutamine **S12** (108 mg containing 63 mg glycerol, 56%, >96% D).
$^1$H NMR (400 MHz, $D_2O$) δ 3.75 (s, 1H), 2.43 (d, *J* = 15.6 Hz, 2H).
$^{13}$C NMR (126 MHz, $D_2O$) δ 177.62, 173.94, 53.98, 30.61, 25.53 (m).
ESI-HRMS calcd. for $C_5H_9D_2N_2O_3^+$ $[M+H]^+$ 149.0895, found 149.0886.

## Supplementary Tables

**Table S1.** Primers used in PCR amplification of the target genes.

| Target Gene | Primer Direction | Primer Sequence | Restriction Site |
|---|---|---|---|
| *sdmA* | Forward | 5'- TCCCATATGACCACCTCCTCGCACCTTCGTGCG -3' | *NdeI* |
| *sdmA* | Reverse | 5'- GTCAAGCTTTCACCGCTGCTCCTCCTTGGCGAG -3' | *HindIII* |
| *sdmB* | Forward | 5'- GTACATATGAGCGCGGTCCTCTTCGACCTGGACG -3' | *NdeI* |
| *sdmB* | Reverse | 5'- GACAAGCTTCTAGTGCGGTGCGGACAGCG -3' | *HindIII* |
| *sdmC* | Forward | 5'- GTACATATGCCATCGCCCAGCCTTGCC -3' | *NdeI* |
| *sdmC* | Reverse | 5'- CGGAAGCTTTCATGCGCTGATCTCCTCCTTG -3' | *HindIII* |
| *sdmD* | Forward | 5'- TAGCATATGAGCGCCCCCACCCGTGACAT -3' | *NdeI* |
| *sdmD* | Reverse | 5'- TCGAAGCTTTCAGAGGGACGTGTTGTCCGTGATGG -3' | *HindIII* |
| *sdmE* | Forward | 5'- TCTCATATGTTCACGCGCACGCTCGAGGACGT-3' | *NdeI* |
| *sdmE* | Reverse | 5'- TGGAAGCTTTCAGTACTGGGAGAAGCCGTCCTCGG -3' | *HindIII* |
| *sdmF* | Forward | 5'- AAGCATATGACCGCCGATGTACTGCCGCCC -3' | *NdeI* |
| *sdmF* | Reverse | 5'- GCTAAGCTTTCAGAGTCCGAGGAGGGCGGC -3' | *HindIII* |
| *sdmG* | Forward | 5'- CCCCATATGAGCATCGCCCTCGACGAACTGCAC -3' | *NdeI* |
| *sdmG* | Reverse | 5'- TCTAAGCTTCTTATCTGCCGGAGTCGGCGGGGT -3' | *HindIII* |

## Supplementary Figures

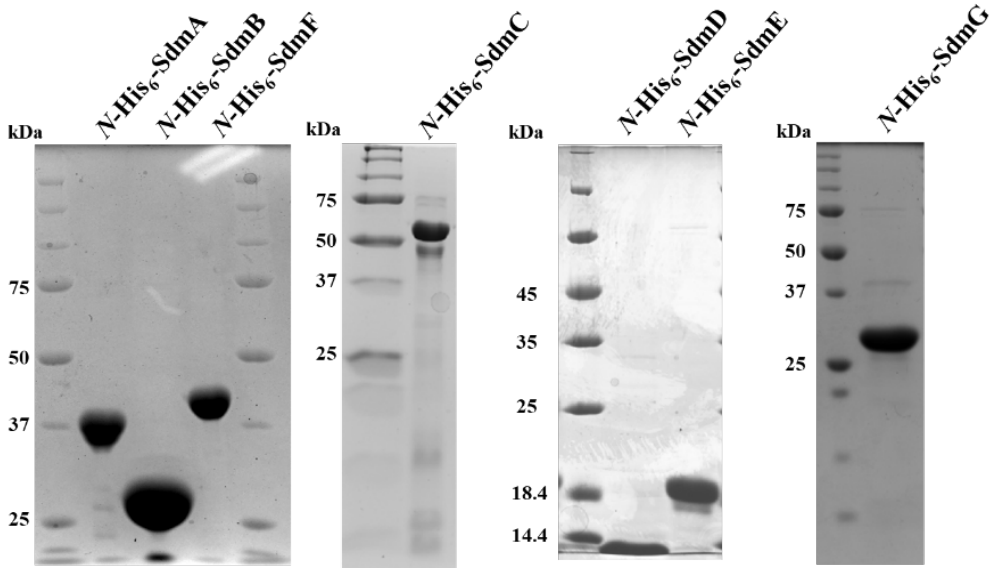



**Figure S1.** SDS-PAGE of *N*-His$_6$-SdmA (35.4 kDa), *N*-His$_6$-SdmB (27.9 kDa) and *N*-His$_6$-SdmF (43.4 kDa), *N*-His$_6$-SdmC (53.8 kDa *N*-His$_6$-SdmD (11.8 kDa) and *N*-His$_6$-SdmE (16.1 kDa), *N*-His$_6$-SdmG (28.2 kDa).

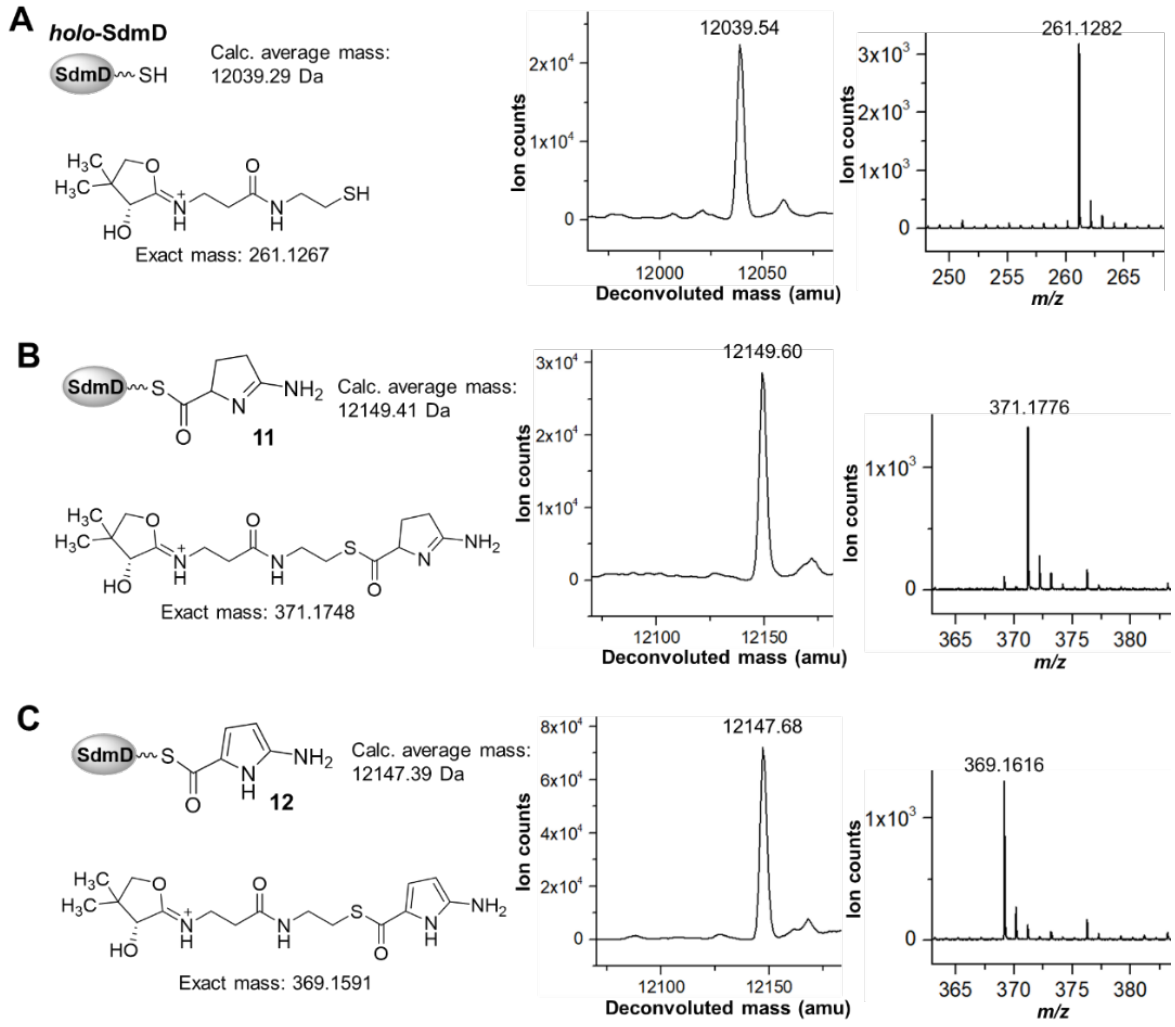



**Figure S2.** LC-HRMS analysis of SdmC/SdmD and SdmF activity assays. (A) Deconvoluted protein mass spectrum and Ppant ejection mass spectrum of *holo*-SdmD. (B) Deconvoluted protein mass spectrum and Ppant ejection mass spectrum of **11**. (C) Deconvoluted protein mass spectrum and Ppant ejection mass spectrum of **12**.

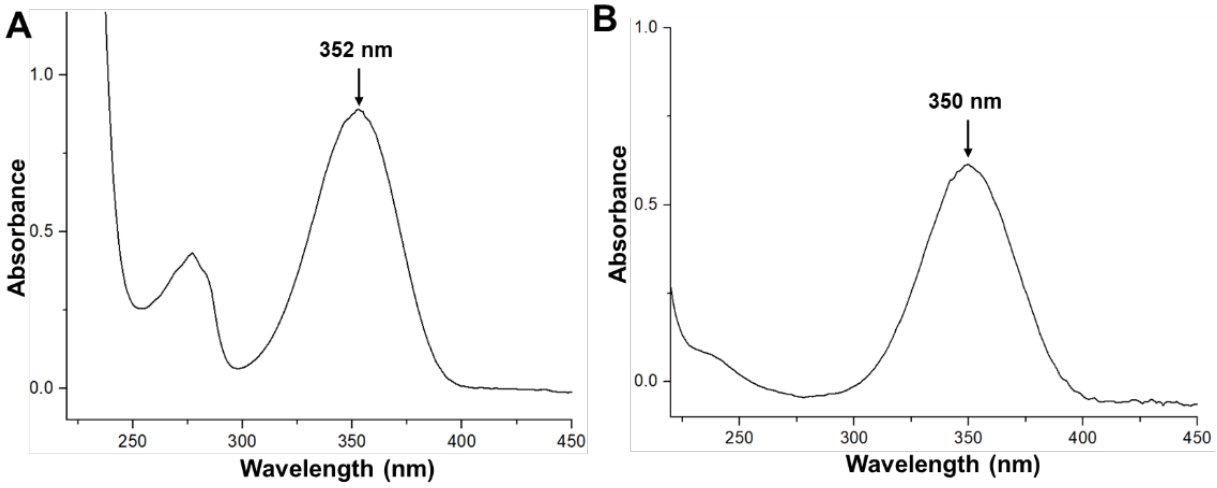



**Figure S3.** (A) UV-Vis spectrum of purified **12** at pH 8.0. (B) UV-Vis spectrum of synthesized *N*-acetylcysteamine (S-NAc) derivative of **12** at pH 8.0.

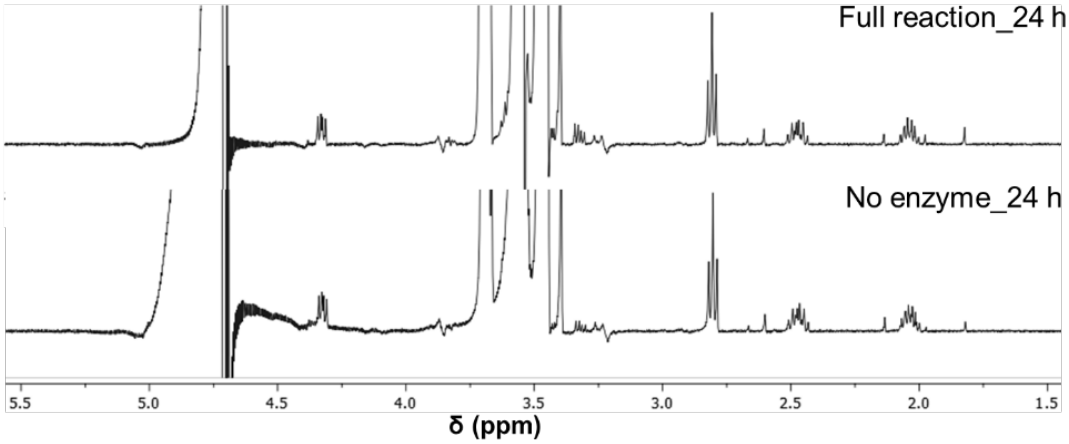



**Figure S4.** $^{1}$H-NMR spectroscopy analysis of SdmF activity assay using **10** as substrate. No significant consumption of substrate was detected after 24 h.

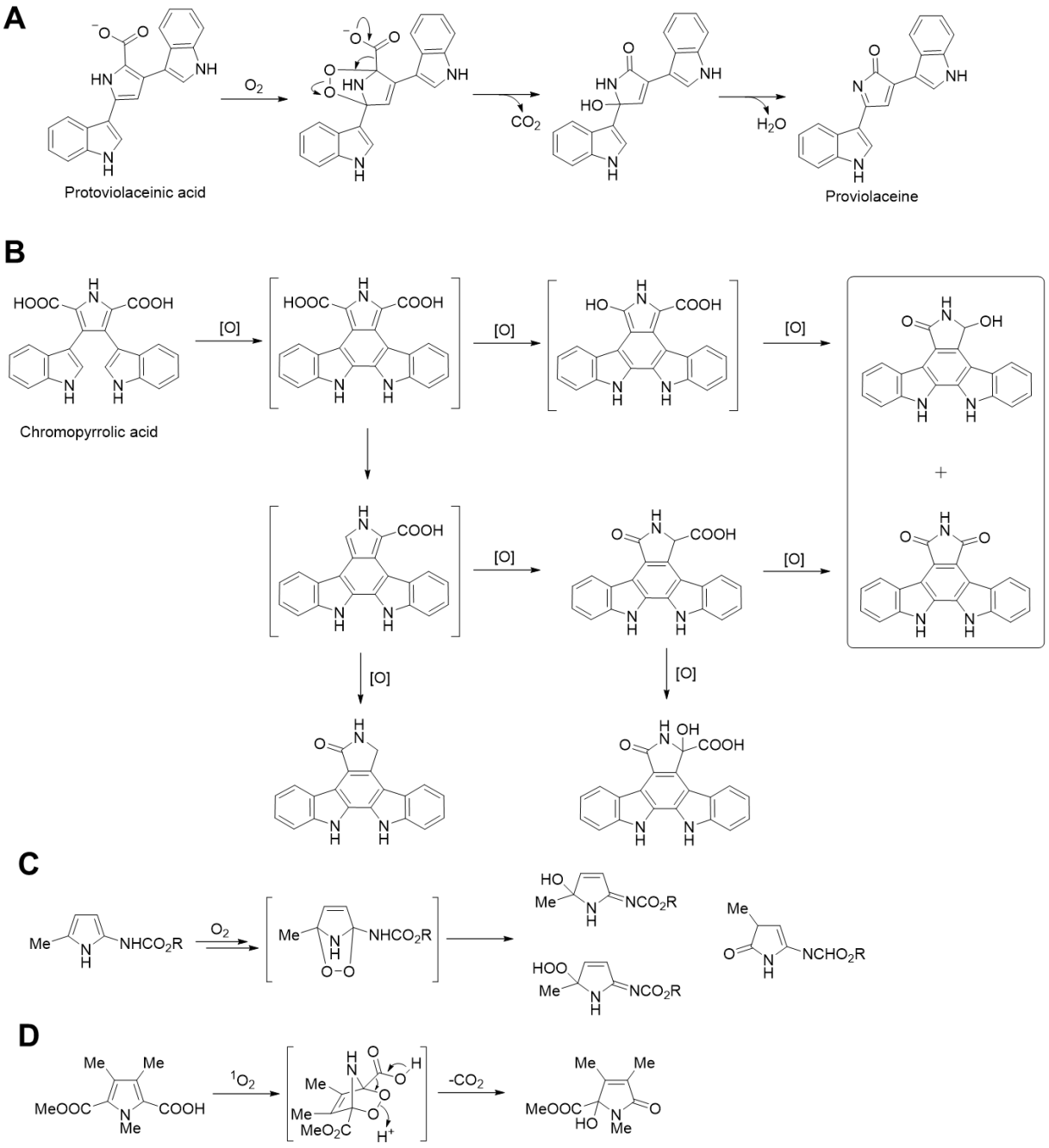



**Figure S5.** Examples of auto-oxidation of pyrroles. (A) Non-enzymatic auto-oxidation of protoviolaceinic acid to proviolaceine in violacein biosynthesis.[5] (B) Non-enzymatic auto-oxidation of chromopyrrolic acid in rebeccamycin biosynthesis.[6,7] (C) Air oxidation of pyrrolylurethanes.[8] (D) Singlet oxygen mediated oxidative decarboxylation of pyrrole-2-carboxylic acids.[9,10]

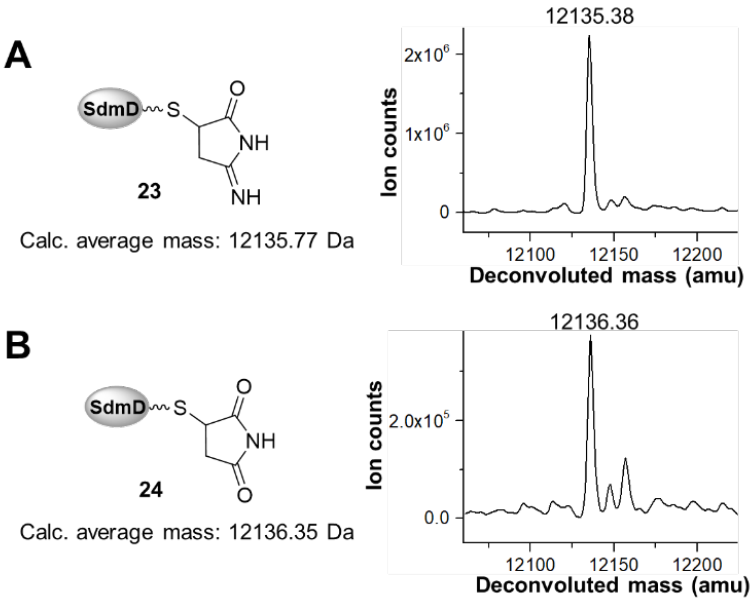



**Figure S6.** Deconvoluted protein mass spectra of (A) **23** and (B) **24**.

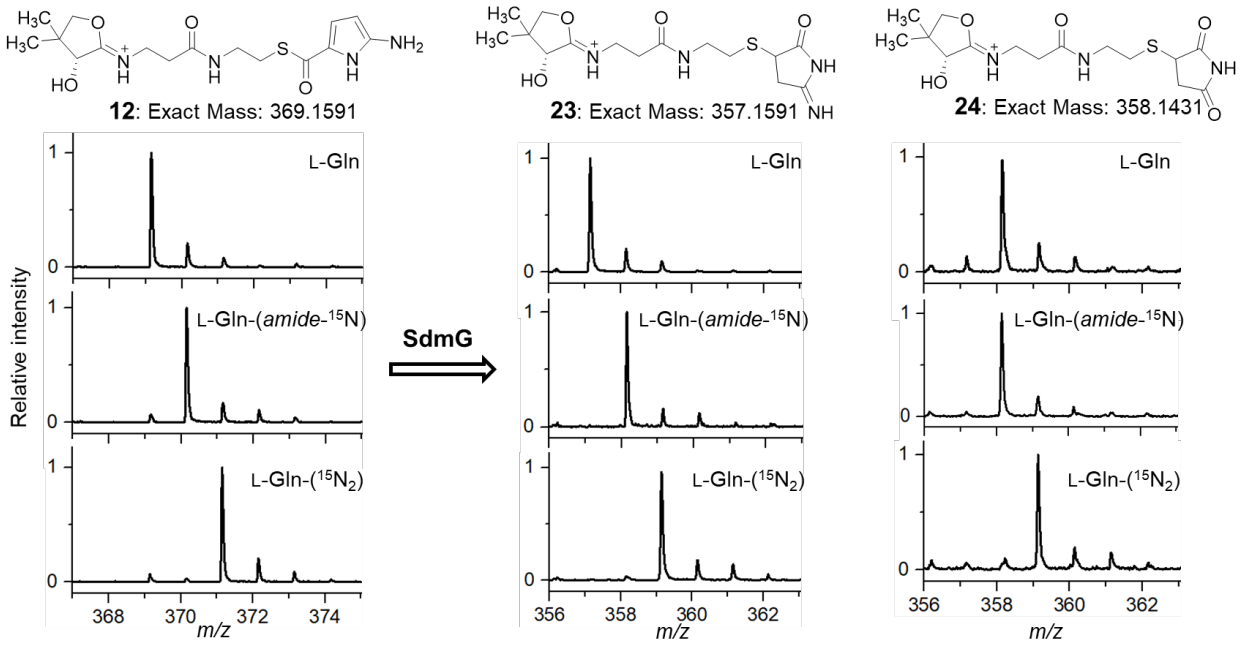



**Figure S7.** Ppant ejection mass spectra of **12**, **23** and **24** using L-glutamine, L-glutamine-(*amide*-$^{15}$N) or L-glutamine-($^{15}N_2$) as initial substrates.

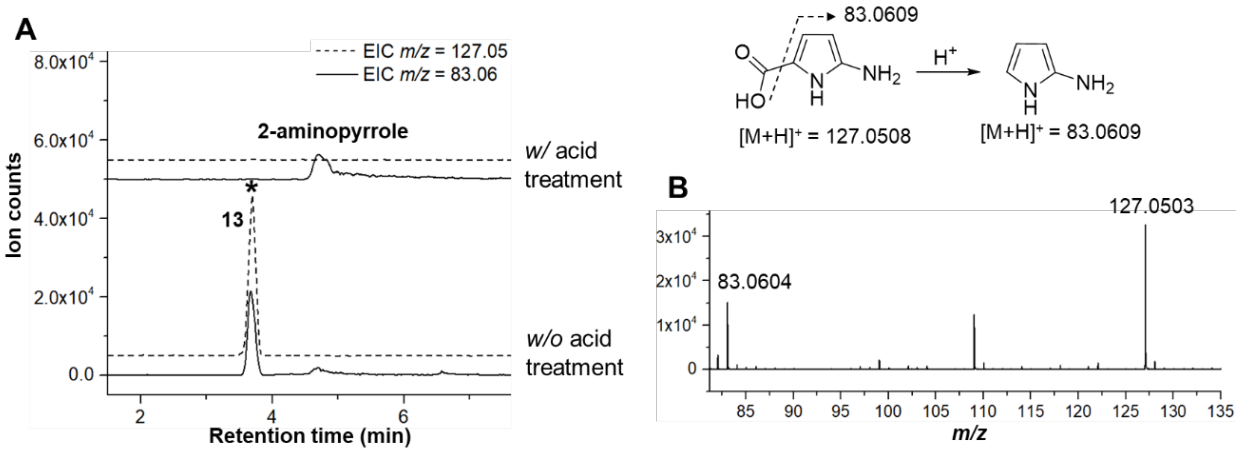



**Figure S8.** LC-HRMS characterization of **13**, which undergoes rapid decarboxylation under acidic condition. (A) LC-HRMS analysis of **13** before and after acid treatment. Extracted ion chromatogram (EIC) traces corresponding to the $[M+H]^+$ signal of **13** and 2-aminopyrrole. A sample of 50 µM **13** was prepared in 5 mM Tris·HCl buffer (pH 8.0) anaerobically as described in Section S3.10. To a 100 µL aliquot of this solution was added 100 µL 0.1% TFA to a final pH = 2-3. Both samples with (*w*) or without (*w/o*) acid treatment were analyzed by LC-HRMS using 5 mM ammonium formate (pH 8.0) and acetonitrile as elution solvents. The LC separation was performed as described in Section S3.10. (B) Mass spectrum of peak labeled with * revealing the in-source decarboxylation of **13**.

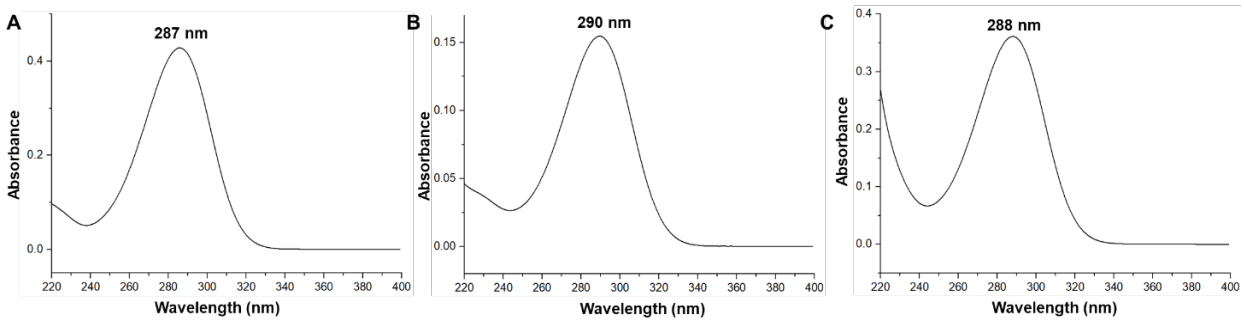



**Figure S9.** UV-Vis spectra of (A) compound **13**, (B) compound **15**, and (C) compound **16**. The spectra were recorded by the diode-array detector (DAD) during HPLC analysis.

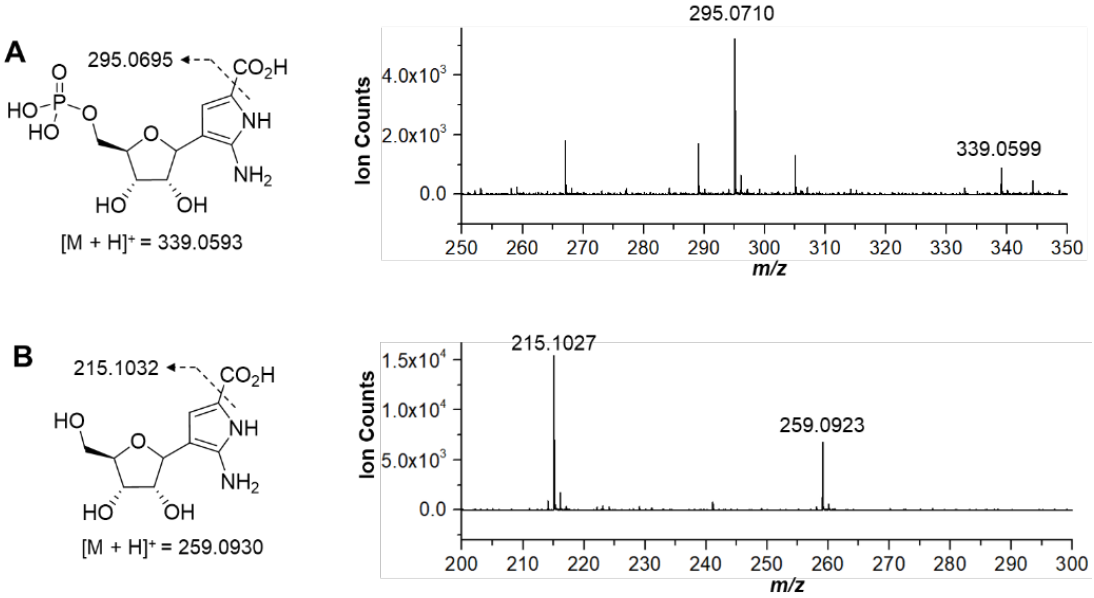



**Figure S10.** Mass spectra of (A) compound **15** and (B) compound **16**. In-source decarboxylation was detected in both **15** and **16** indicating the presence of a carboxylic acid functional group in their structures.

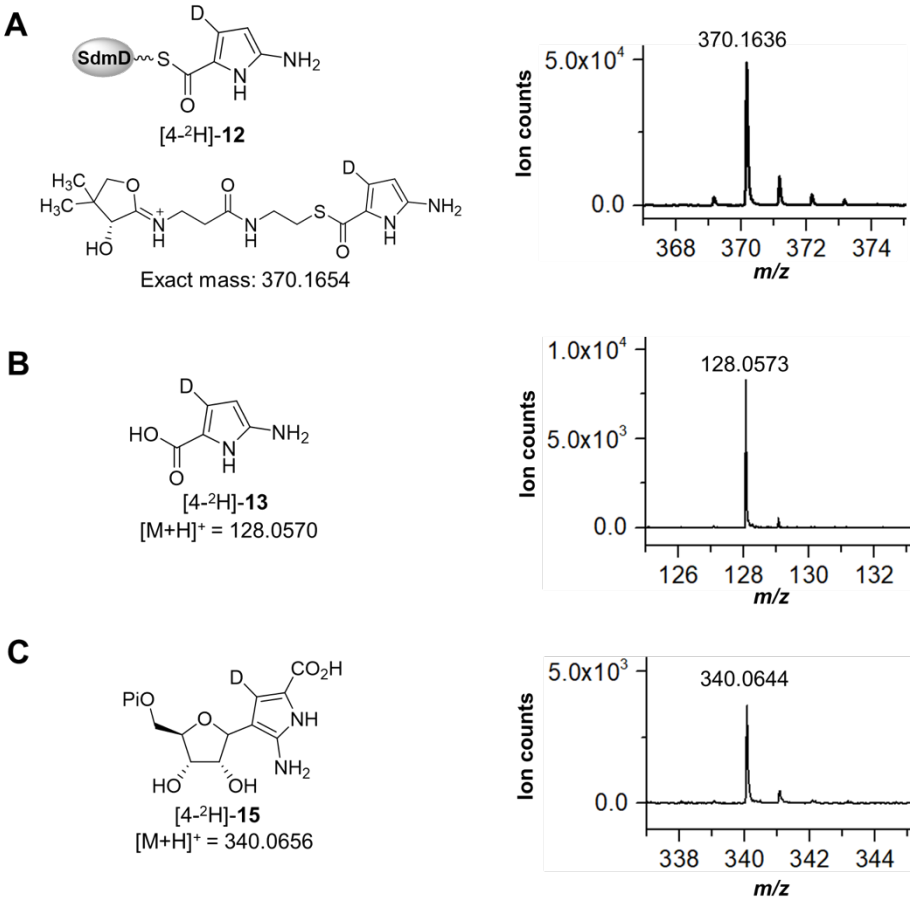



**Figure S11.** Mass spectra of (A) [4-$^2$H]-**12** prepared enzymatically from 3,3-dideutero-L-glutamine, (B) [4-$^2$H]-**13**, and (C) [4-$^2$H]-**15**.

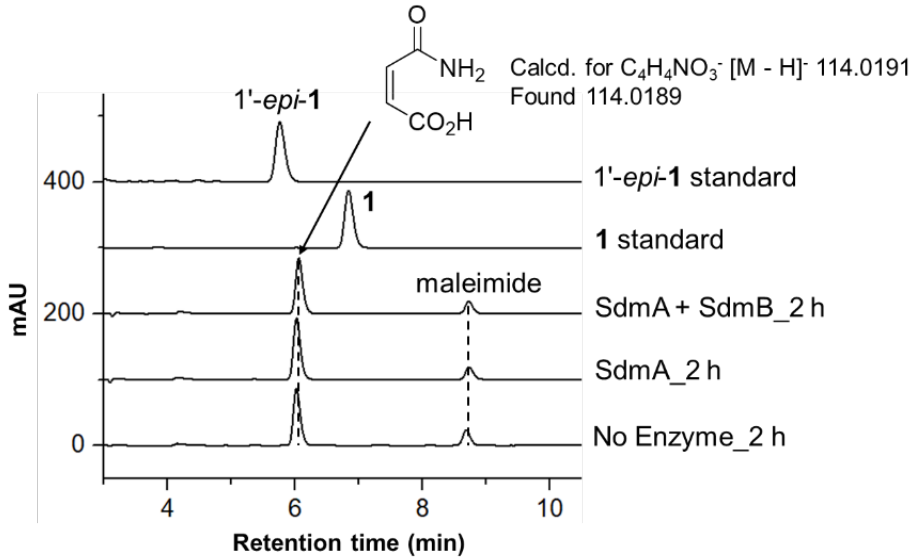



**Figure S12.** HPLC analysis of SdmA/SdmB activity assays using maleimide and ribose 5-phosphate as substrates. Maleimide (1 mM) was incubated with 10 mM R5P, 2.5 mM $MgCl_2$, 10 μM SdmA and 2 μM SdmB in Tris·HCl buffer (50 mM, pH 8.0) in a total volume of 200 μL. After 2 h incubation, the reaction was filtered using a YM-10 centrifugal filter to remove proteins and the filtrate was analyzed by HPLC using a Capcell PAK C18 MGIII column (Osaka Soda, 5 μm, 4.6 × 250 mm). The mobile phase was 0.1% TFA in $H_2O$ as solvent A and acetonitrile as solvent B. The flow rate was 1 mL/min and the elution gradients were as following: 0–2 min 1% solvent B, 2–10 min 1% to 10% solvent B, 10–15 min 10% to 50% solvent B, 15–19 min 50% solvent B, 19–20 min 50% to 1% solvent B. UV absorbance was monitored at 250 nm. Only non-enzymatic hydrolysis of maleimide was observed.

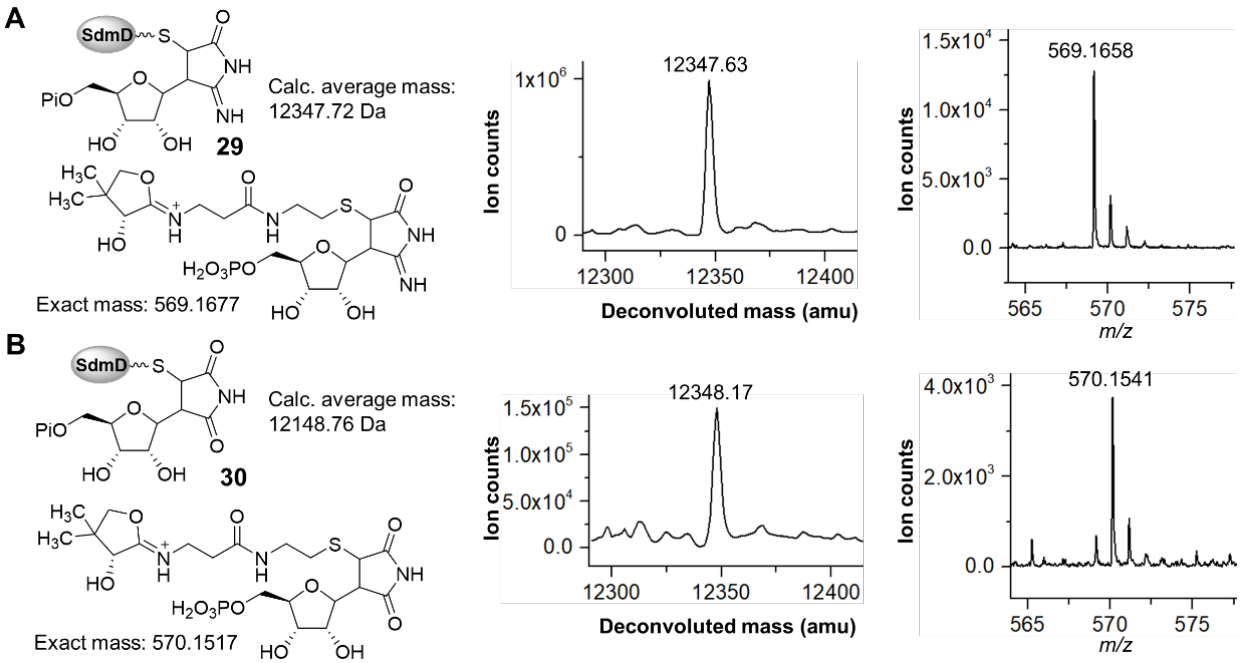



**Figure S13.** LC-HRMS analysis of the SdmA activity assays under aerobic condition. (A) Deconvoluted protein mass spectrum and Ppant ejection mass spectrum of **29**. (B) Deconvoluted protein mass spectrum and Ppant ejection mass spectrum of **30**.

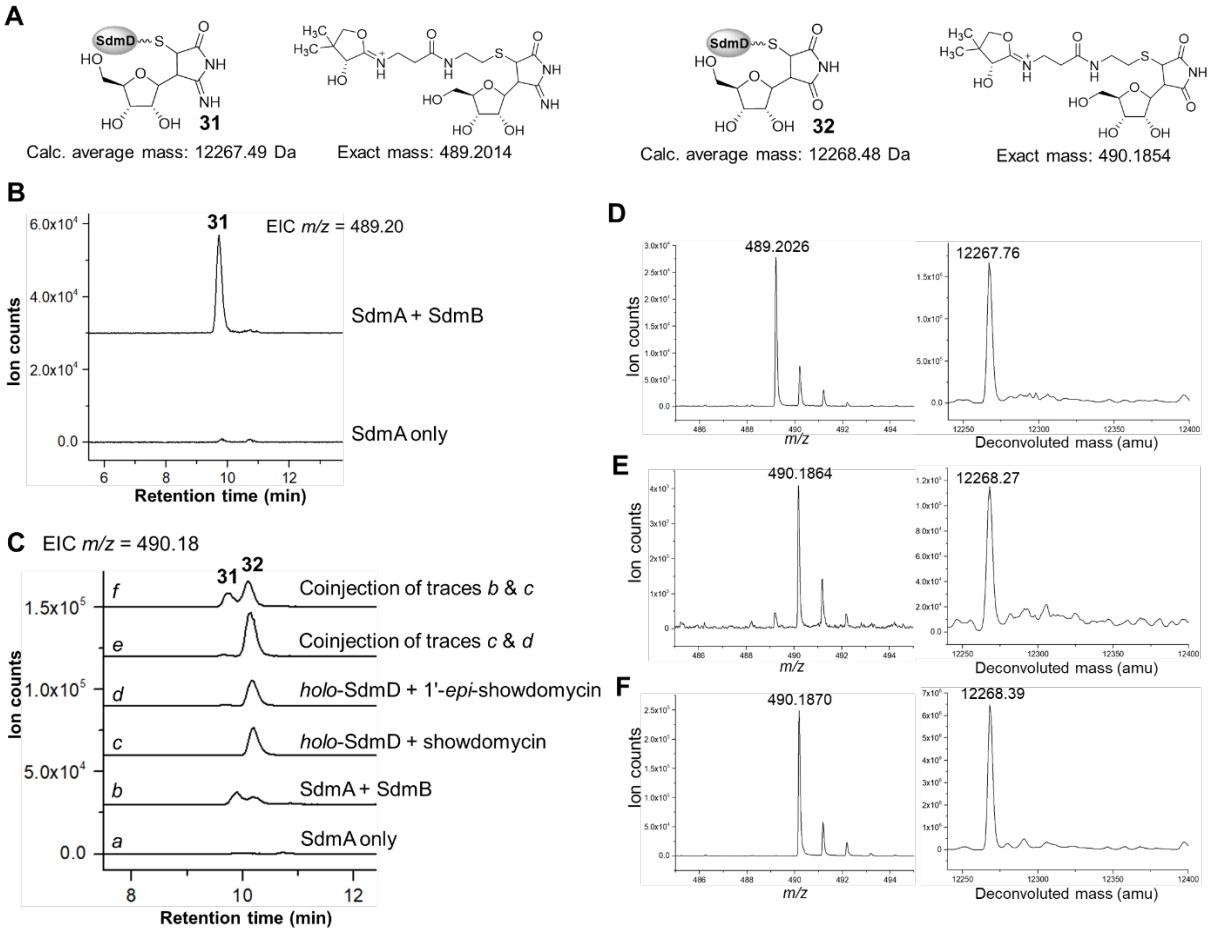



**Figure S14.** LC-HRMS characterization of the SdmA/SdmB activity assays under aerobic condition. (A) Structures of **31**, **32** and their corresponding Ppant ejection ions. (B) LC-HRMS analysis of the SdmA/SdmB reactions. Extracted ion chromatogram (EIC) traces corresponding to the $[M+H]^+$ signal of Ppant ejection ion of **31**. (C) LC-HRMS analysis of the SdmA/SdmB reactions. Extracted ion chromatogram (EIC) traces corresponding to the $[M+H]^+$ signal of Ppant ejection ion of **32**. The co-appearance of **31** is resulted from its +1 isotope. The adduct prepared from *holo*-SdmD and 1'-*epi*-showdomycin coeluted with the standard prepared from *holo*-SdmD and showdomycin. Thus, the stereochemistry of the enzymatic adduct could not be determined using this method. (D) Ppant ejection mass spectrum and deconvoluted protein mass spectrum of **31**. (E) Ppant ejection mass spectrum and deconvoluted protein mass spectrum of **32**. (F) Ppant ejection mass spectrum and deconvoluted protein mass spectrum of chemically prepared showdomycin adduct with *holo*-SdmD.

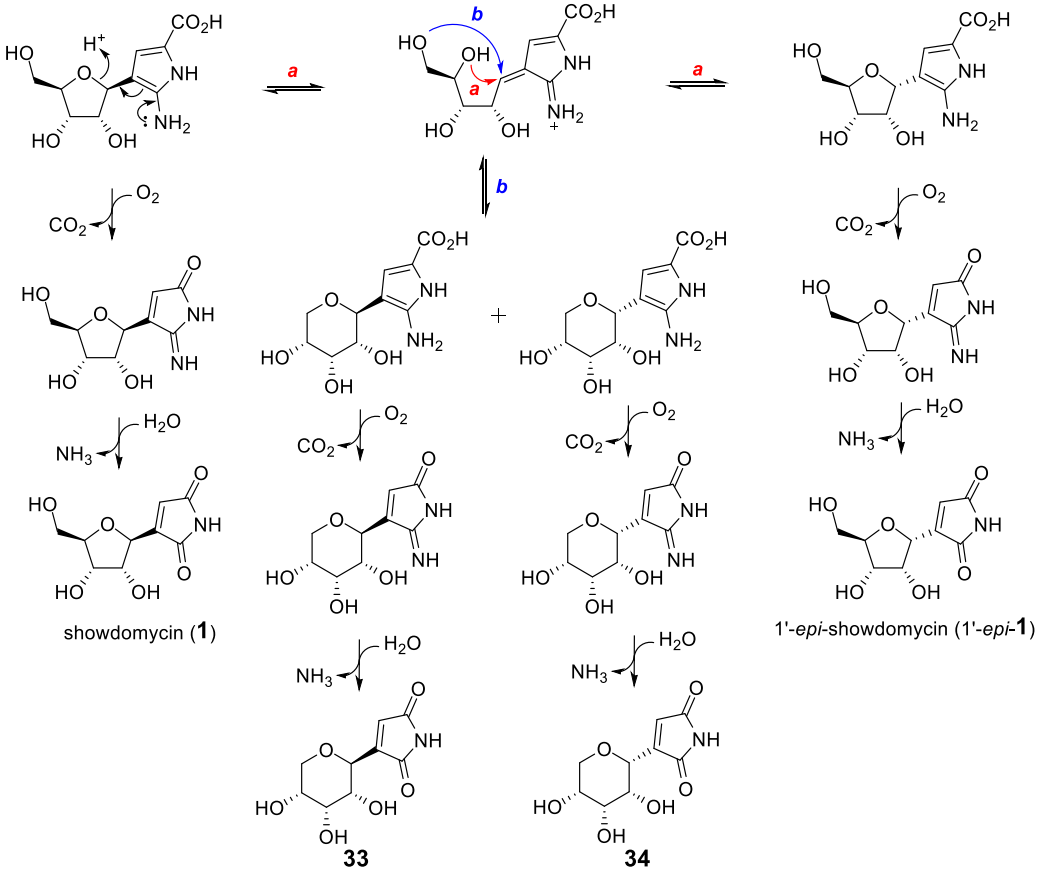



**Figure S15.** Proposed mechanism of C1' epimerization and ring expansion according to previous reports.[11,12] The isomerization or ring expansion likely occurs prior to autoxidation and deamination because no isomerization of showdomycin (**1**), 1'-*epi*-showdomycin (1'-*epi*-**1**), **33** or **34** was observed within 16 h when incubated in 0.1% TFA buffer (pH ~2).

## References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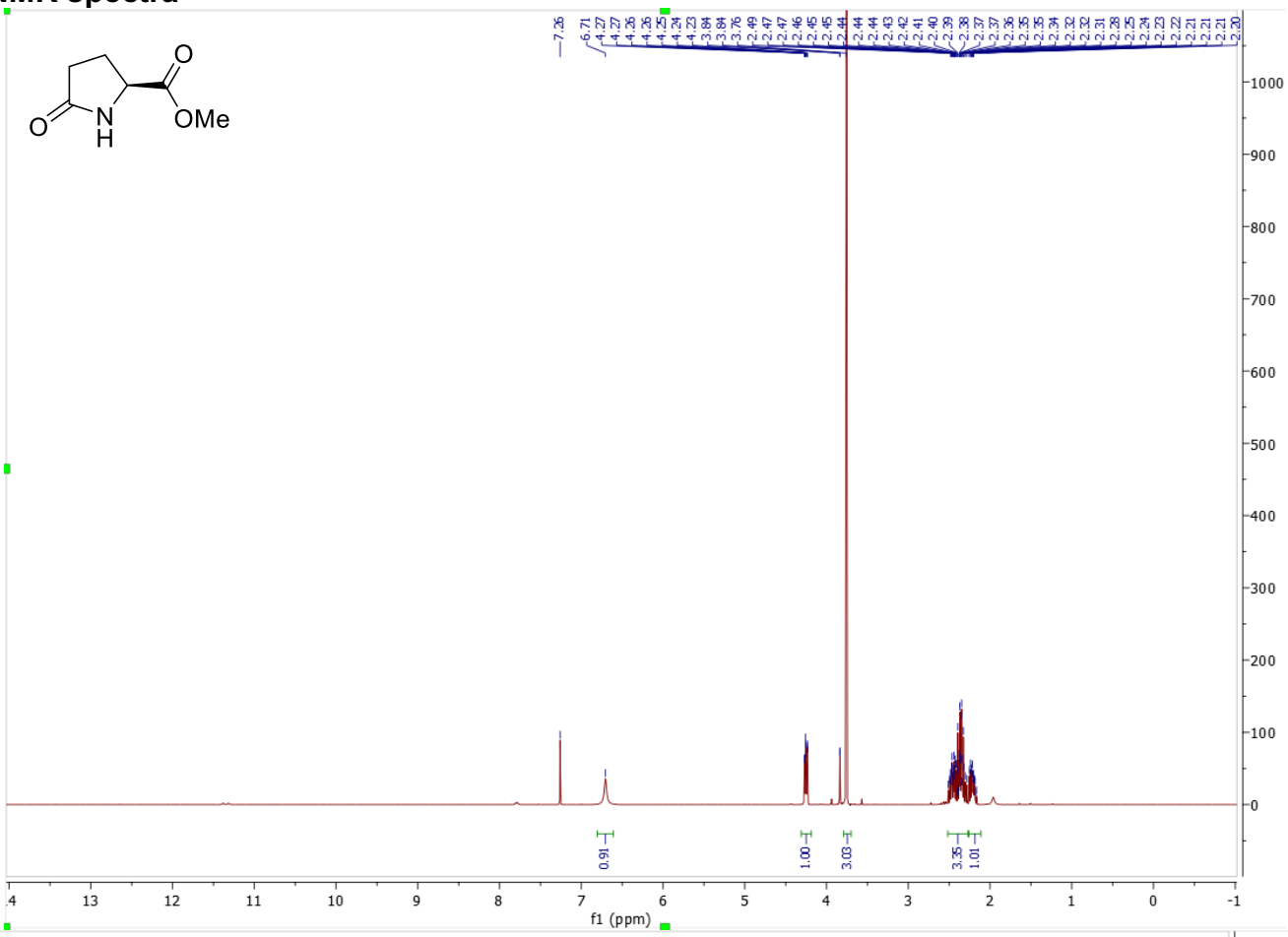
## Author Contributions

D. R., S.-A. W. and H.-w. L. designed research; D. R. and M. K. performed research; D. R. and H.-w. L. analyzed data; and D. R. and H.-w. L. wrote the paper.

**NMR spectra**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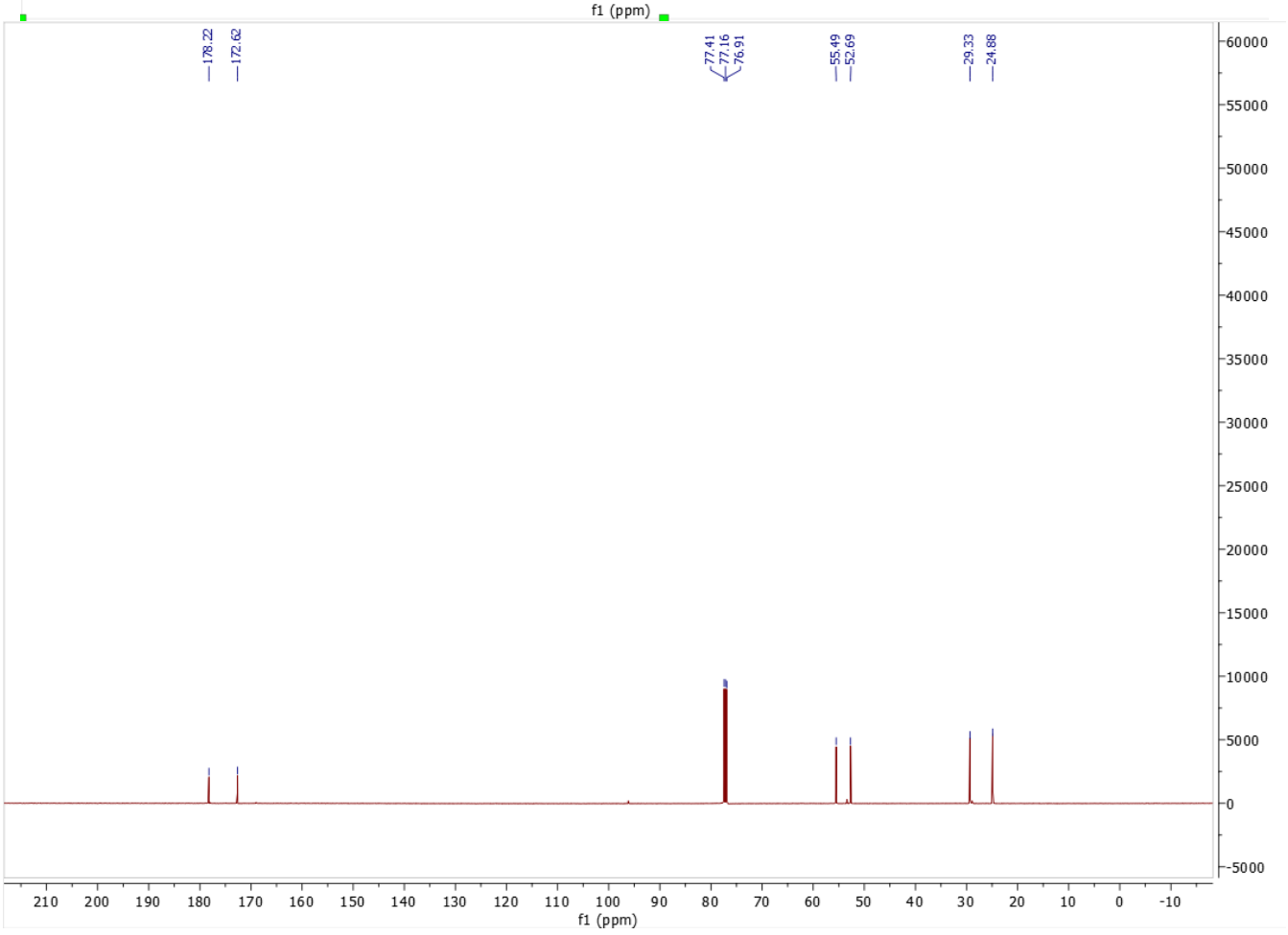
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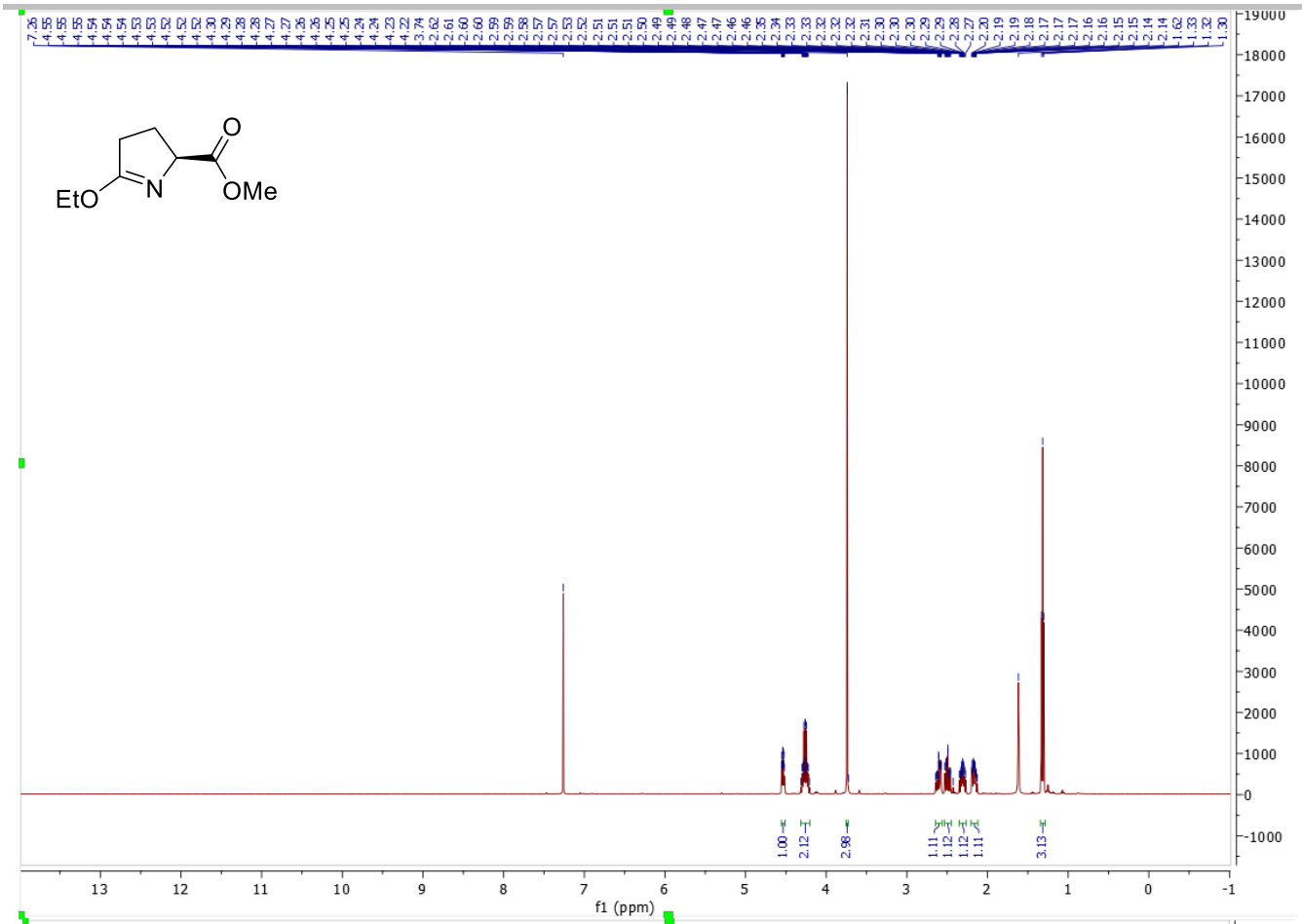
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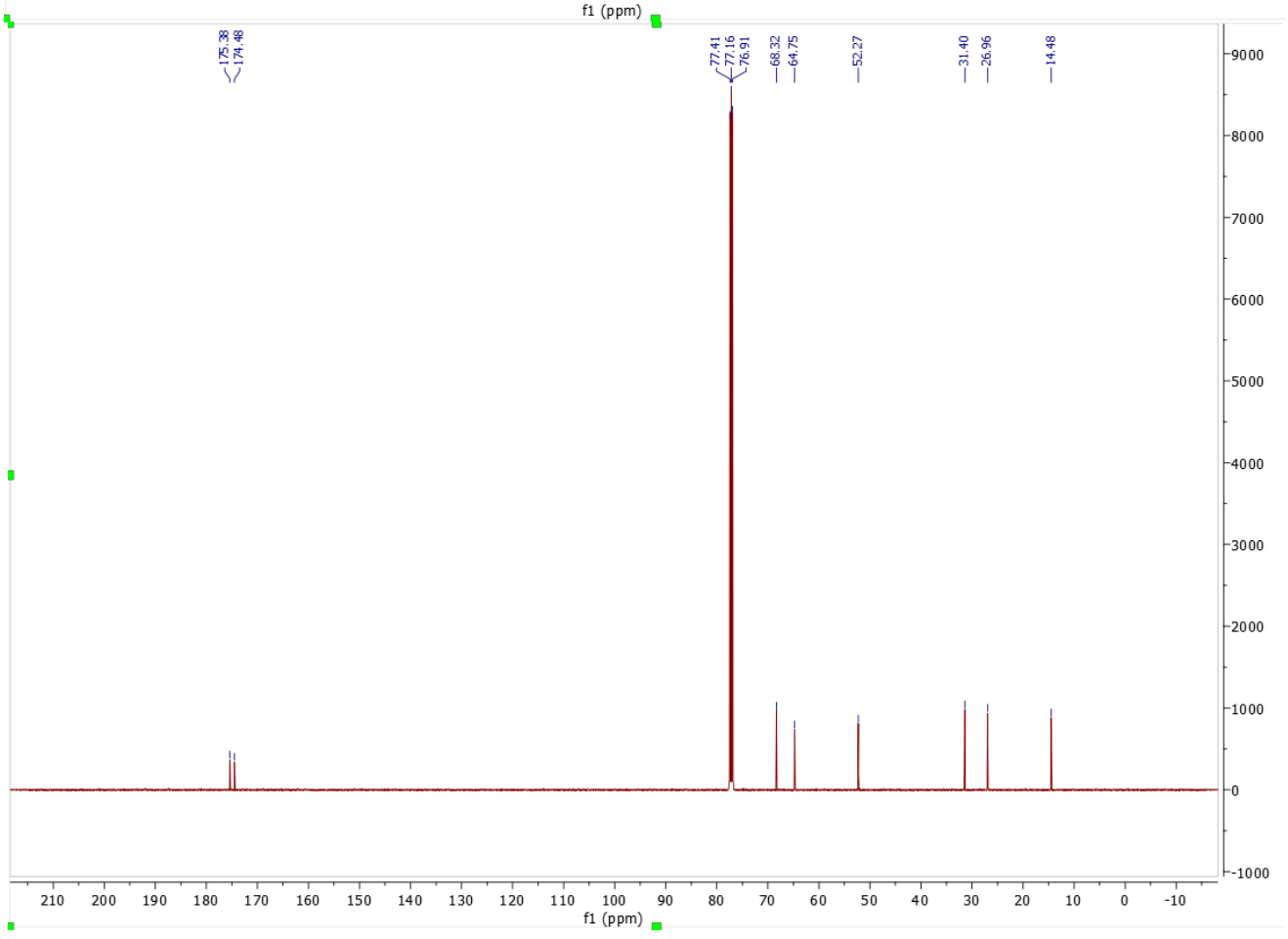
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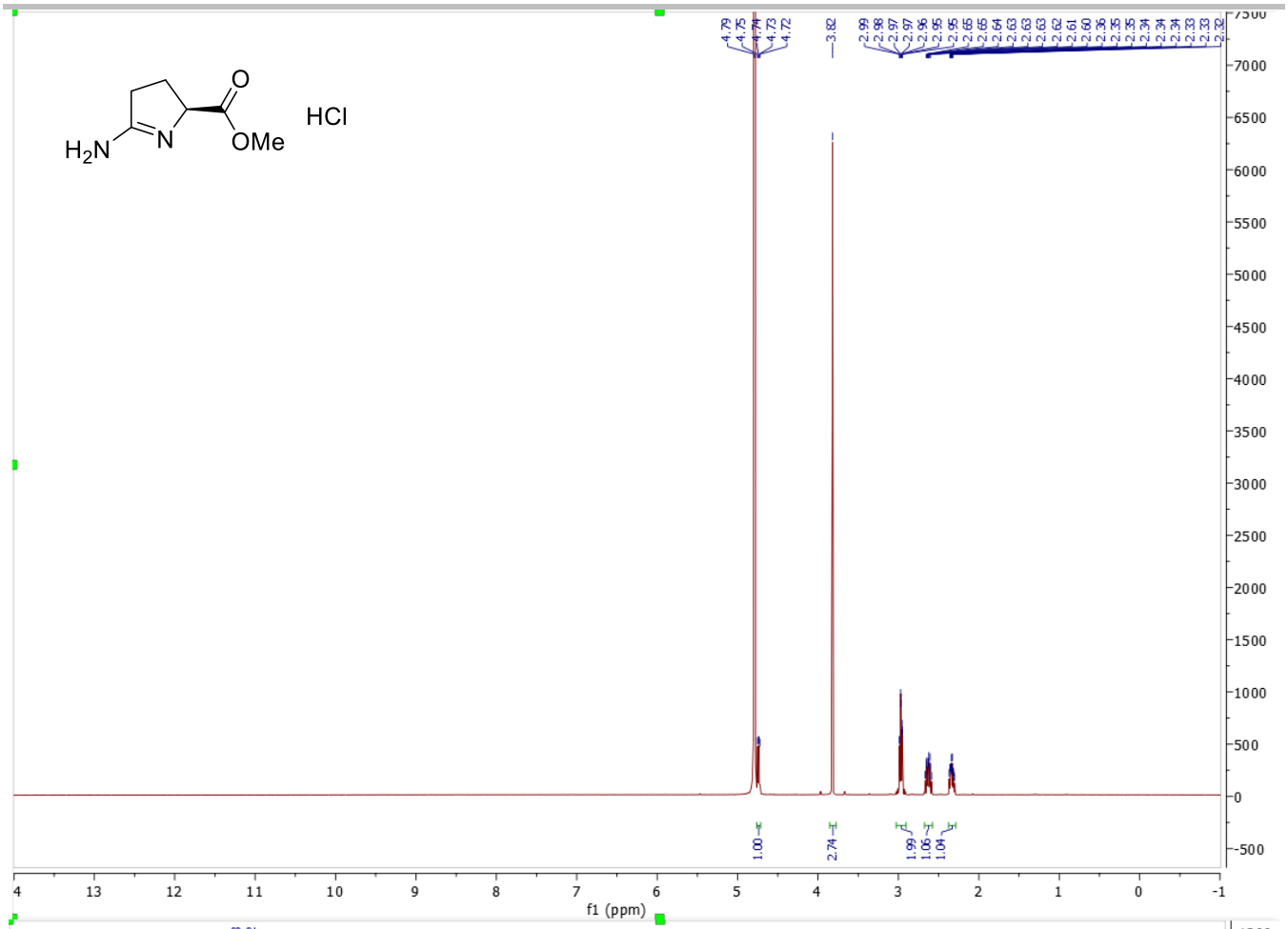
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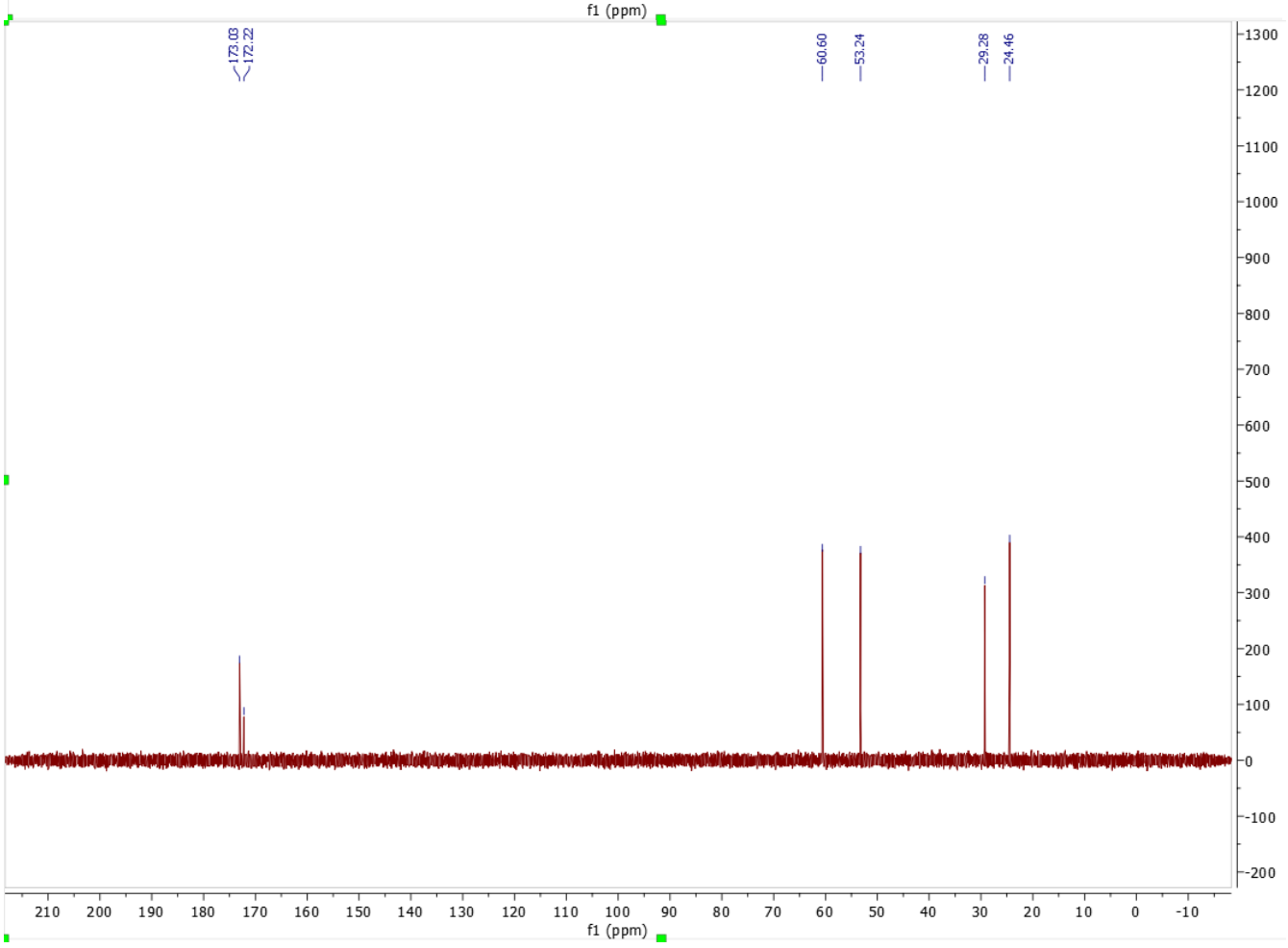
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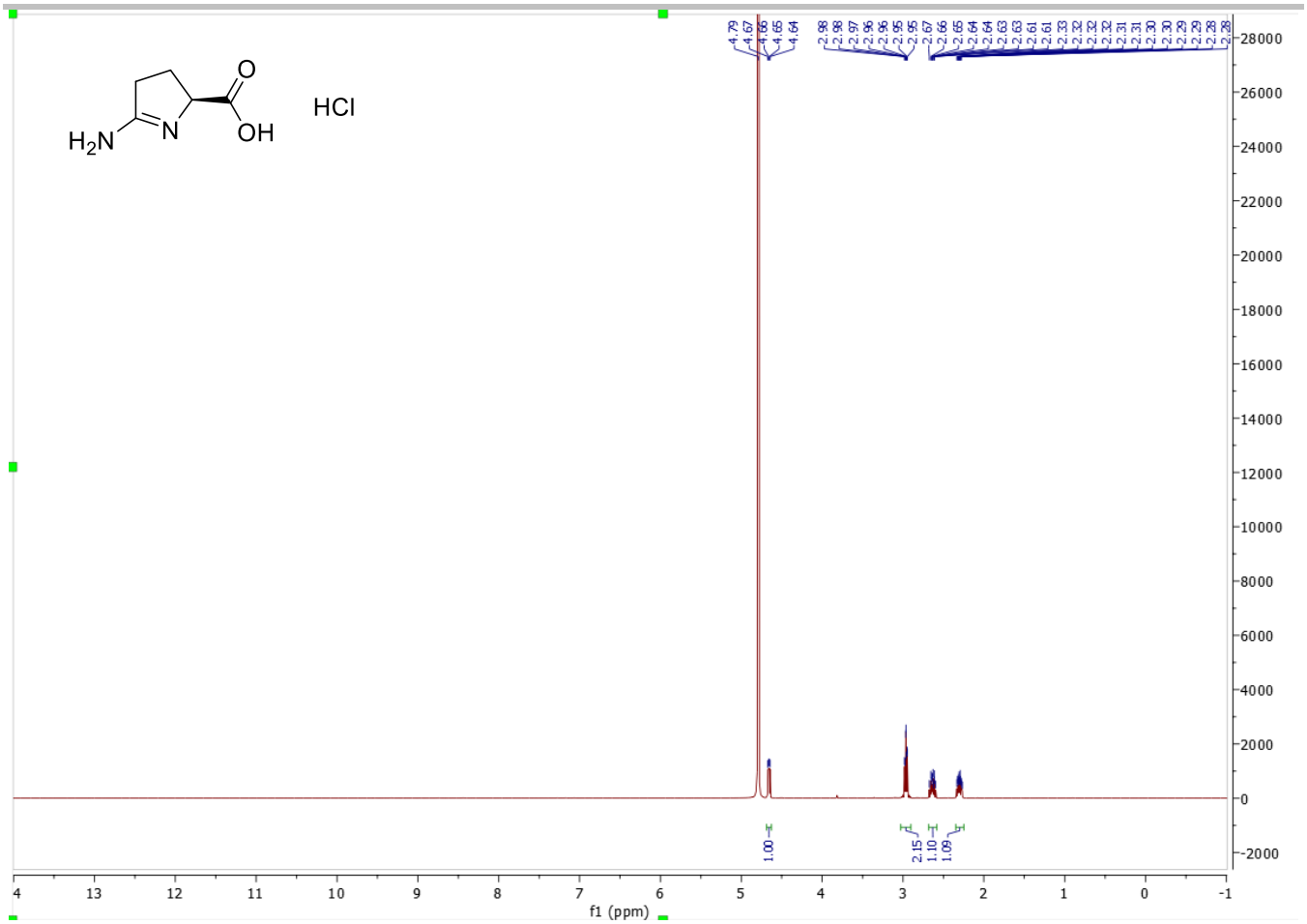
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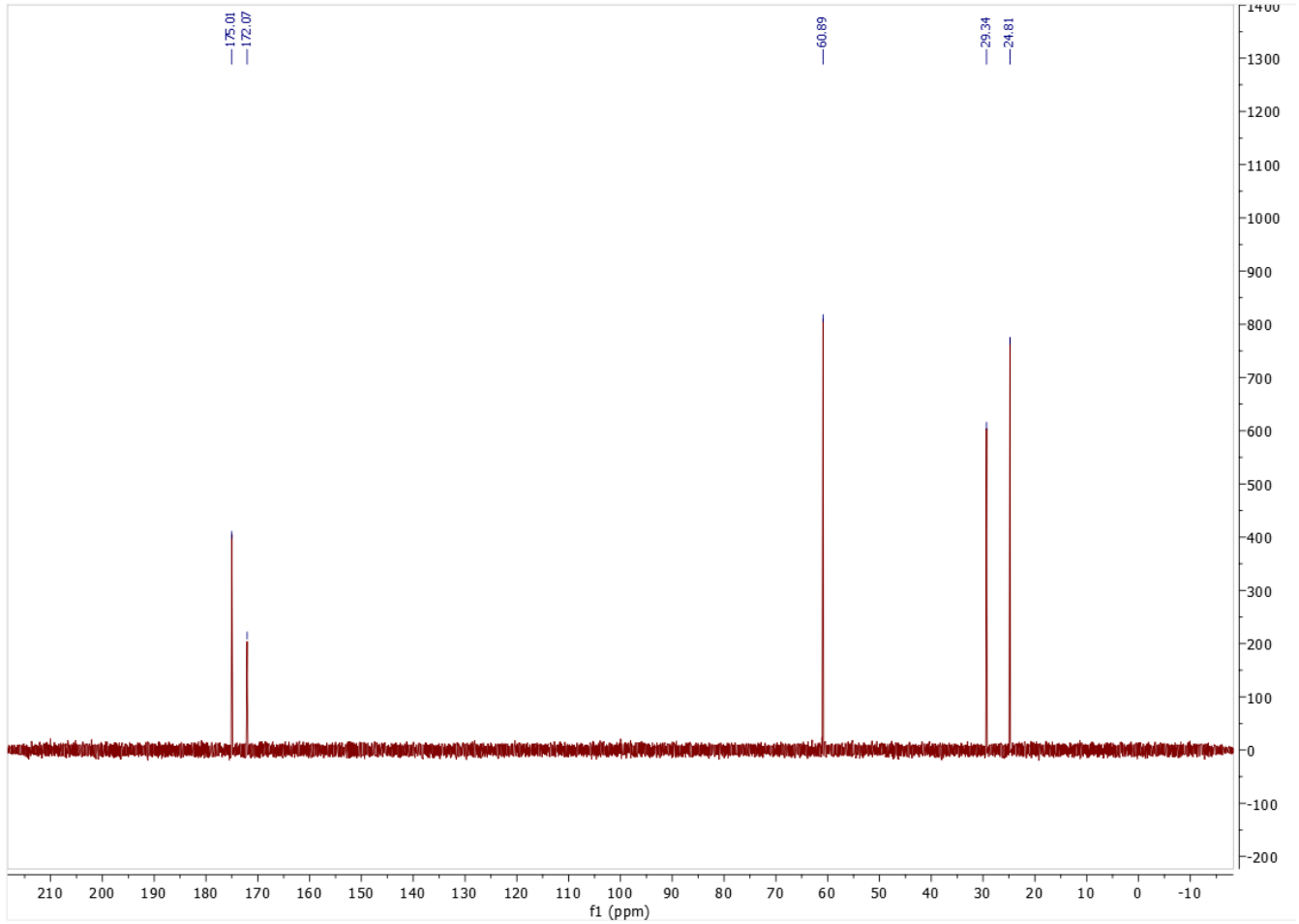
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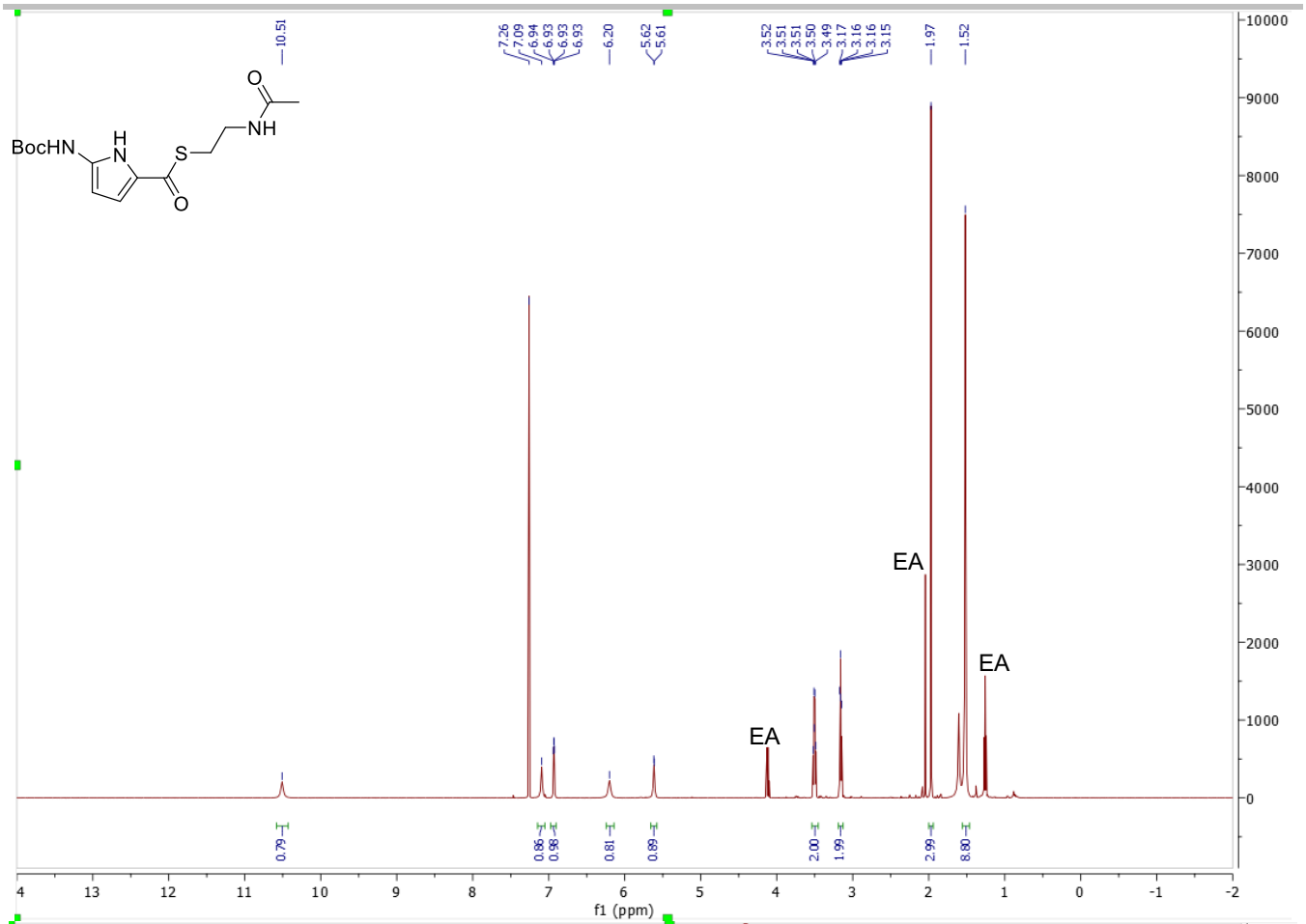
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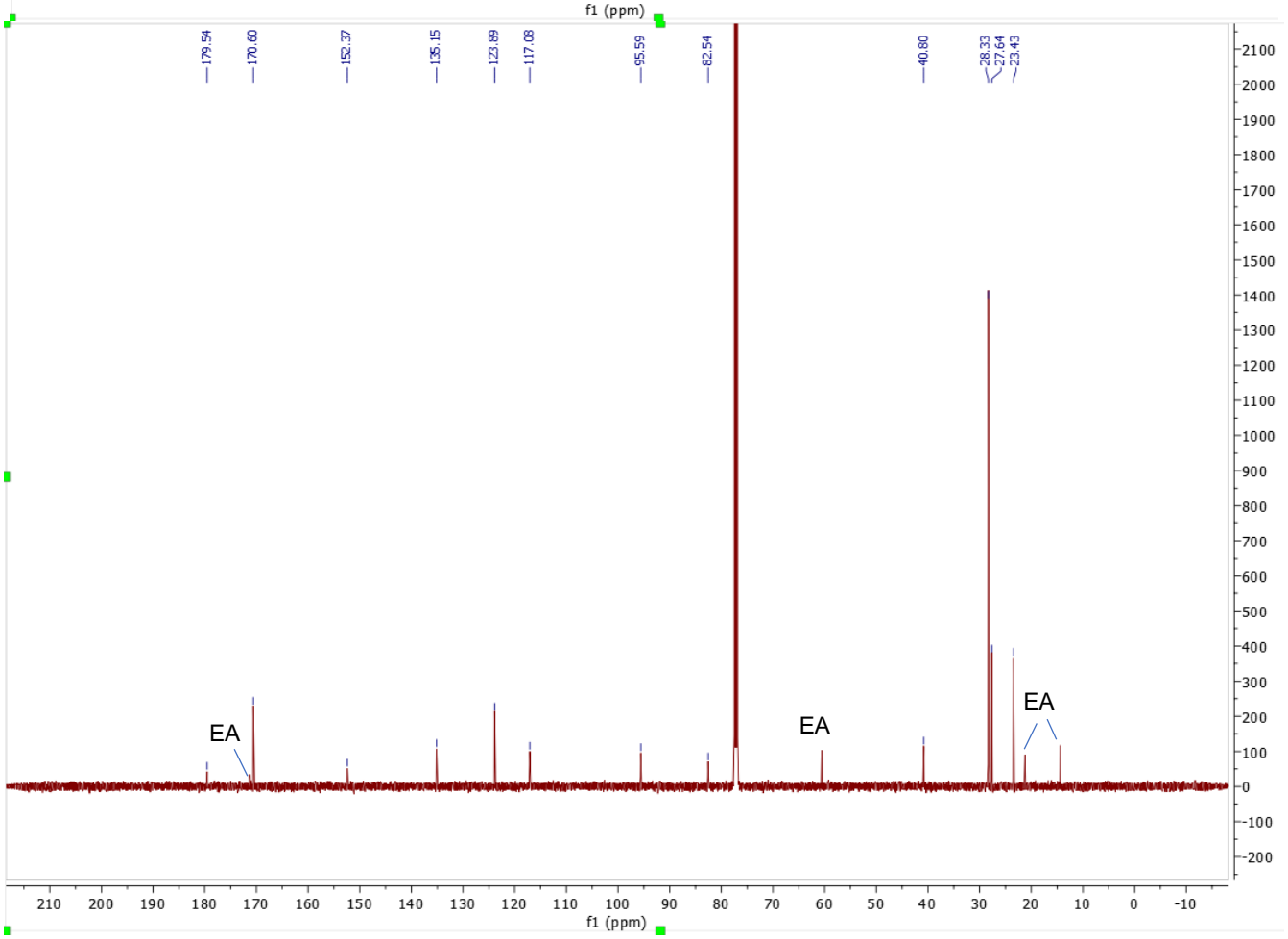
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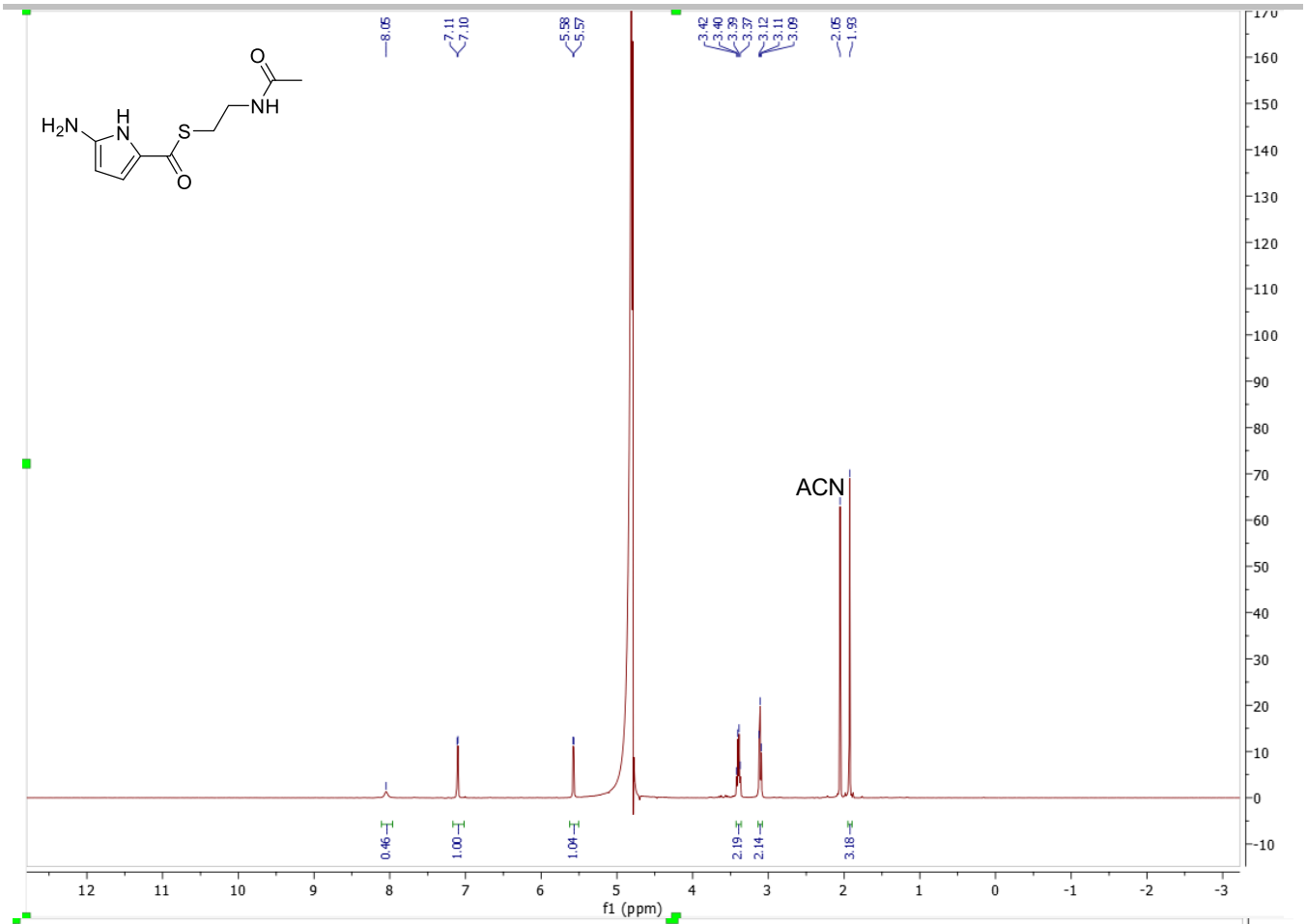
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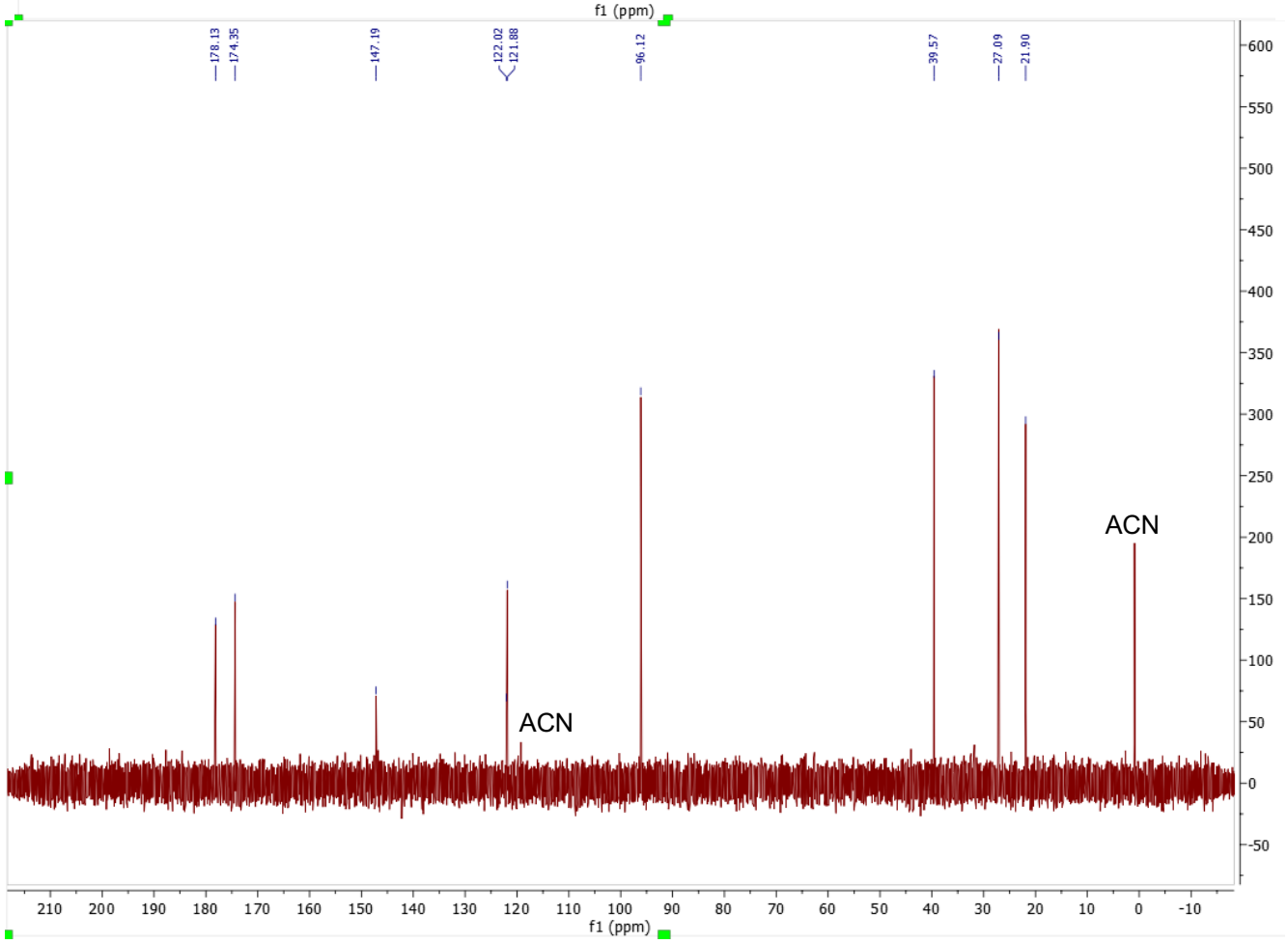
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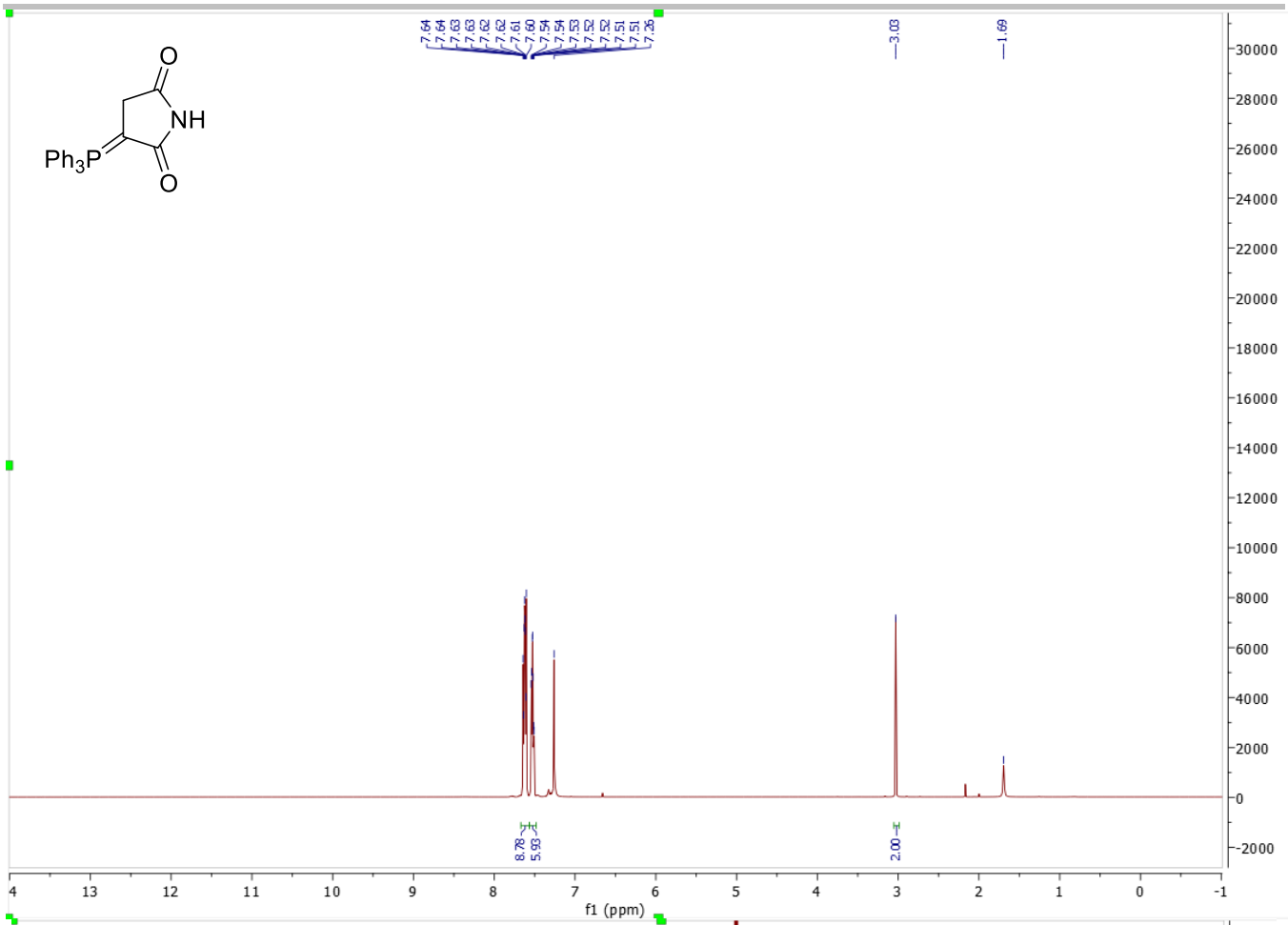
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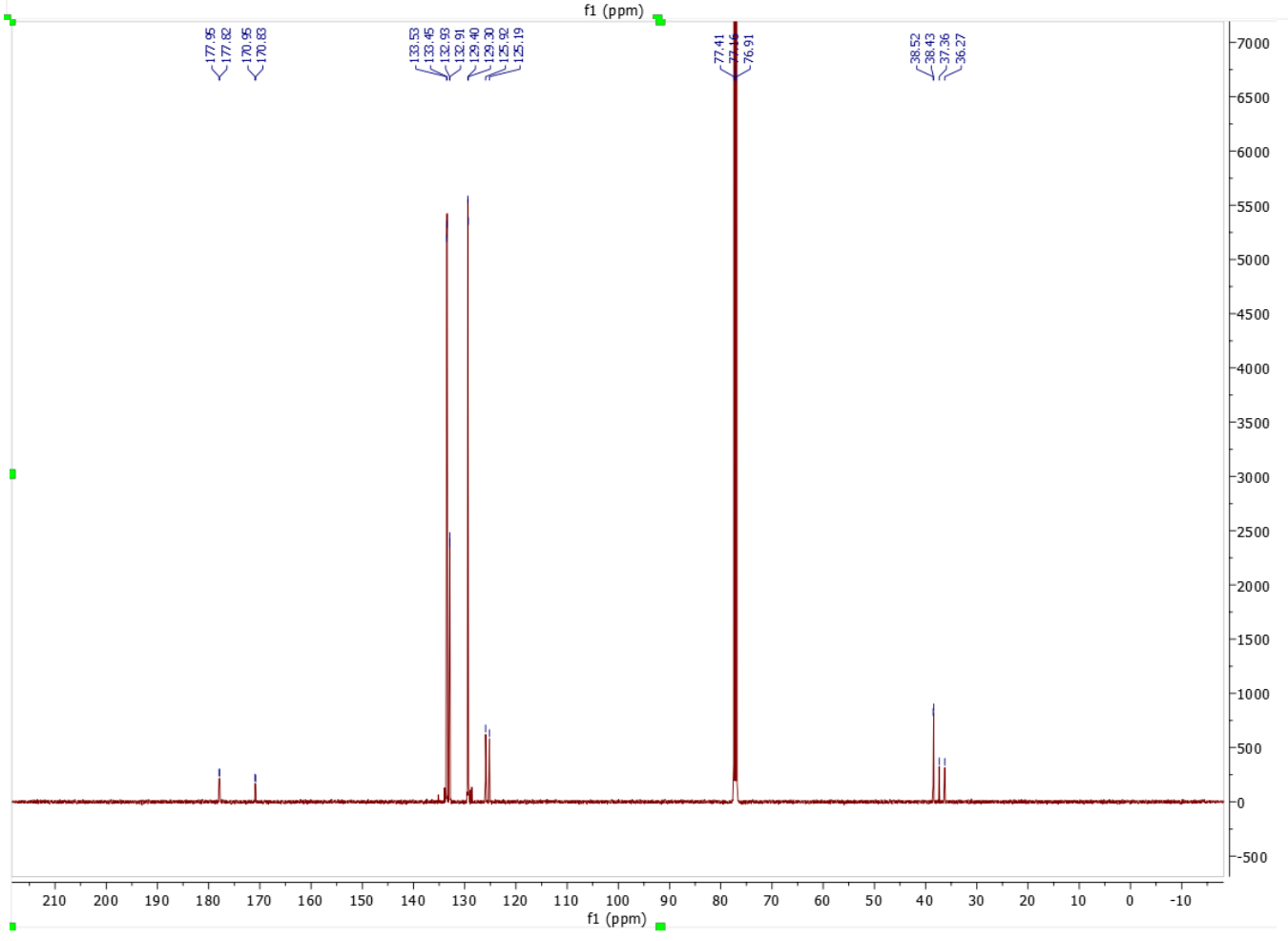
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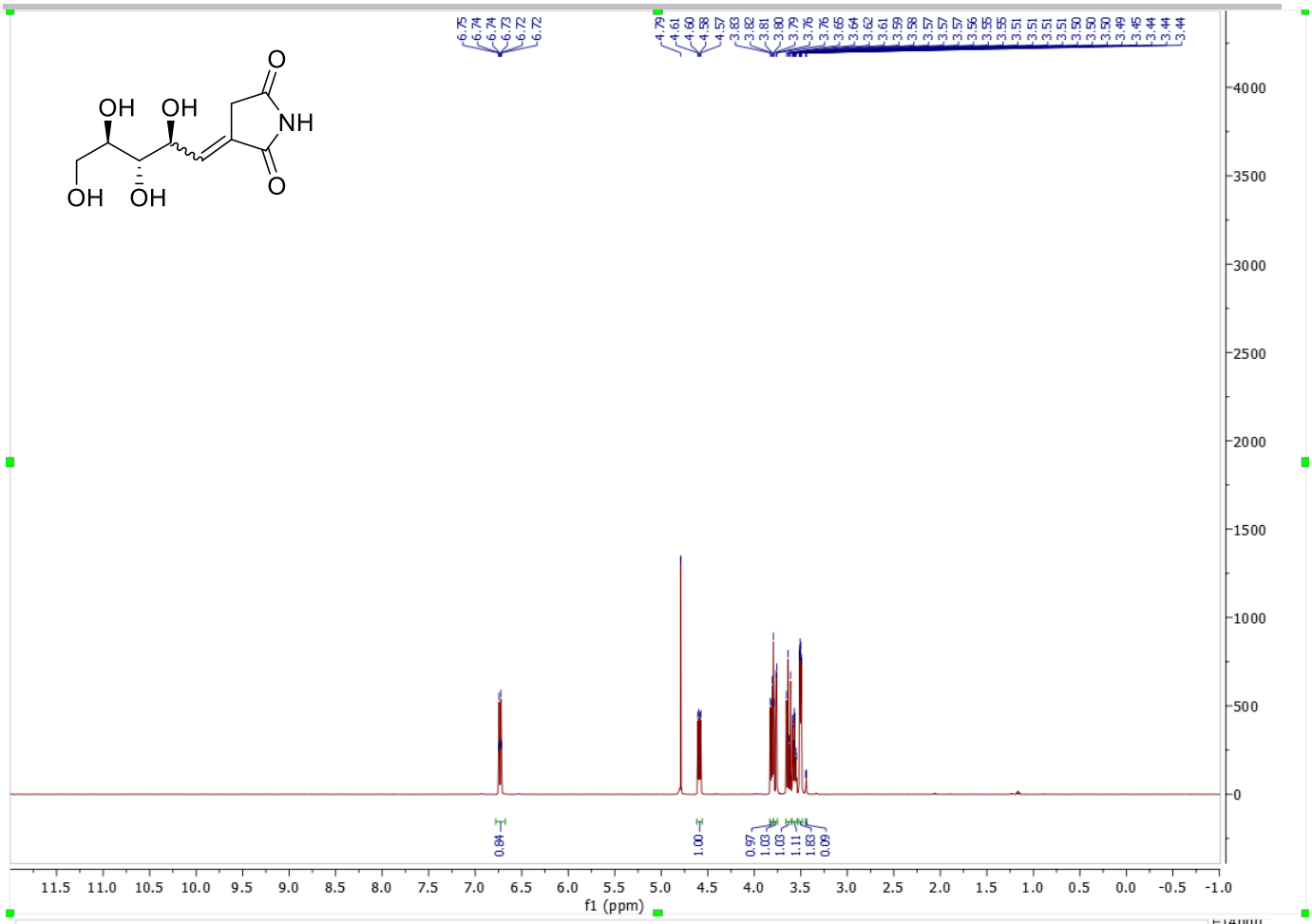
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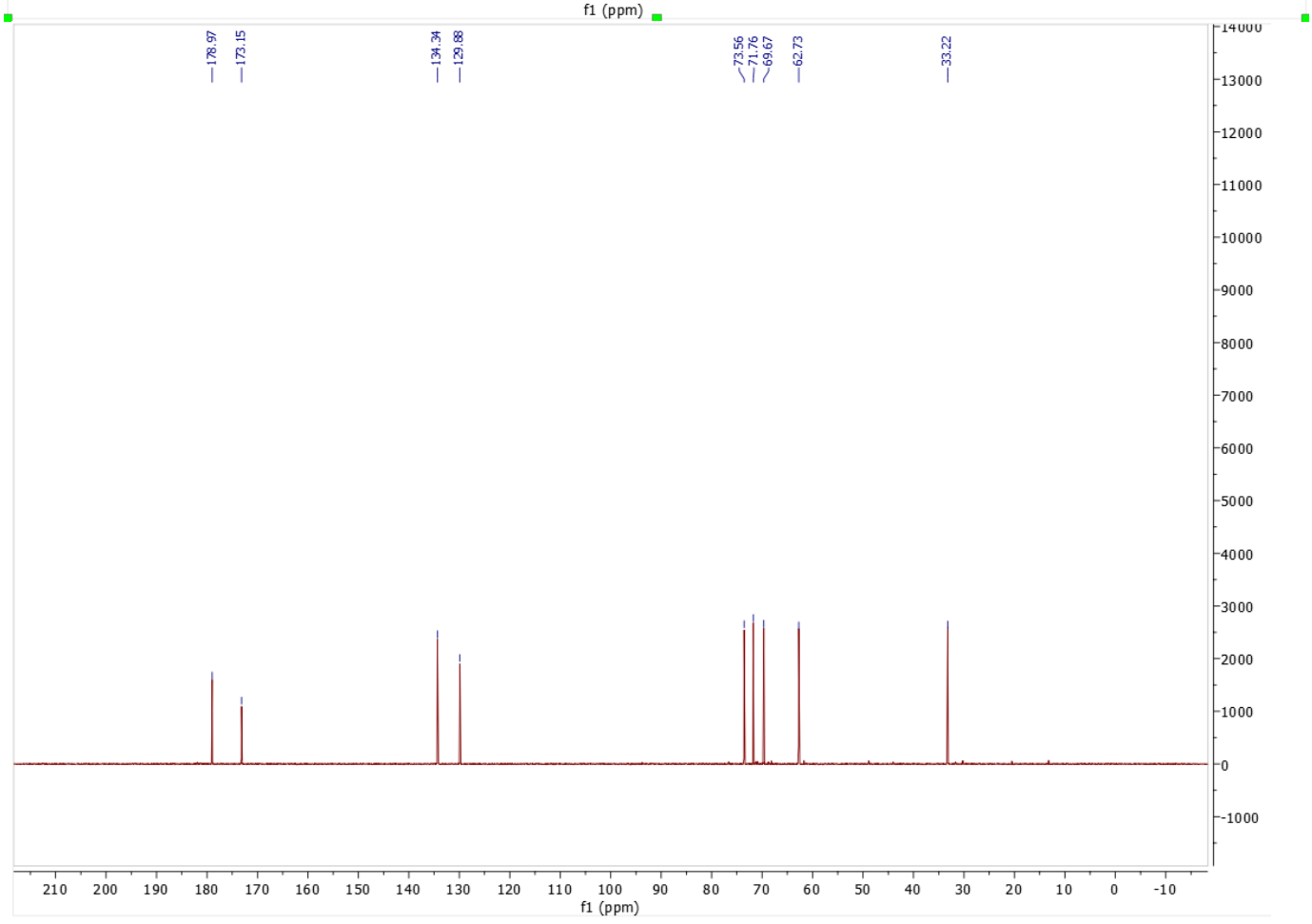
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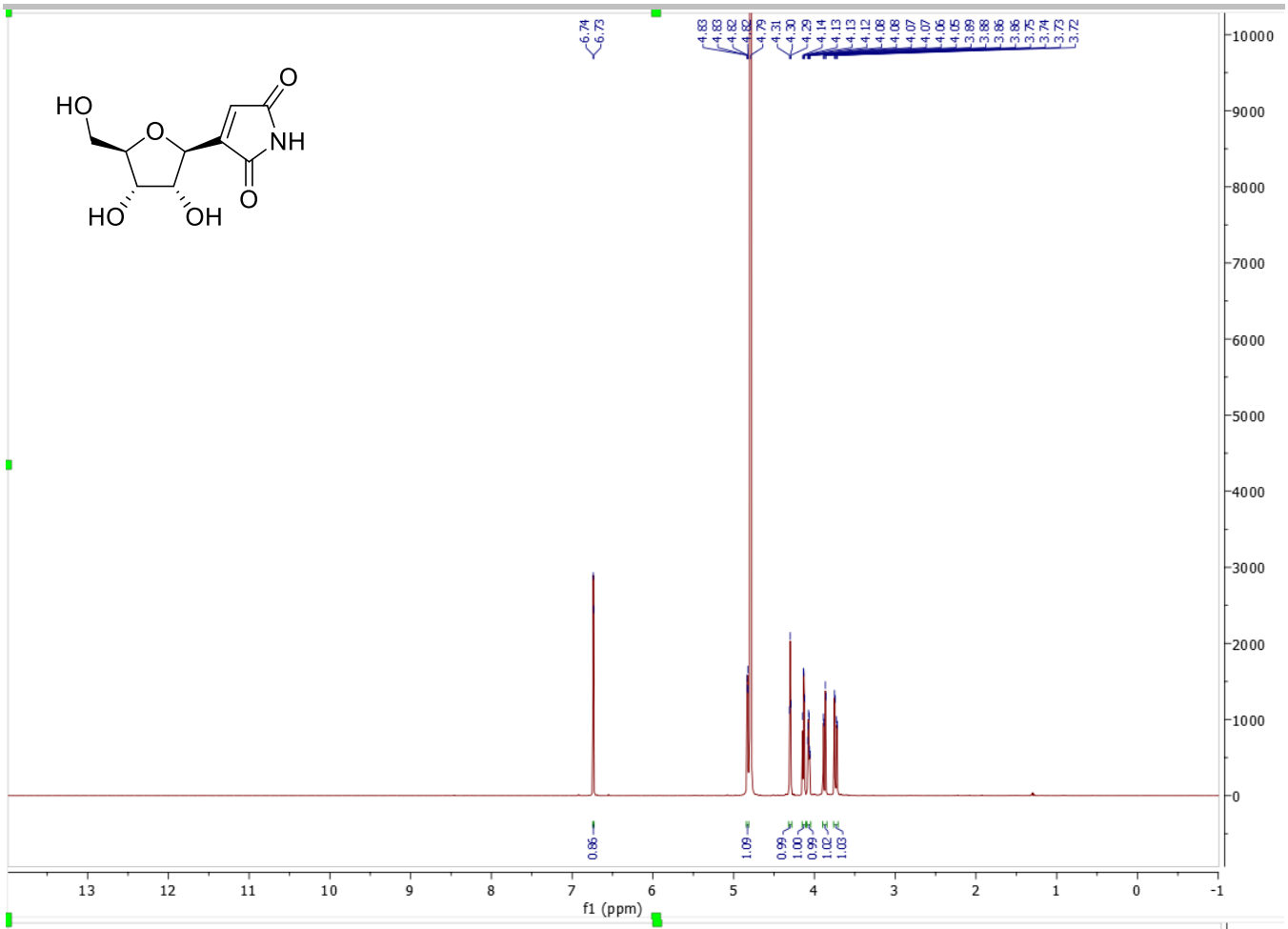
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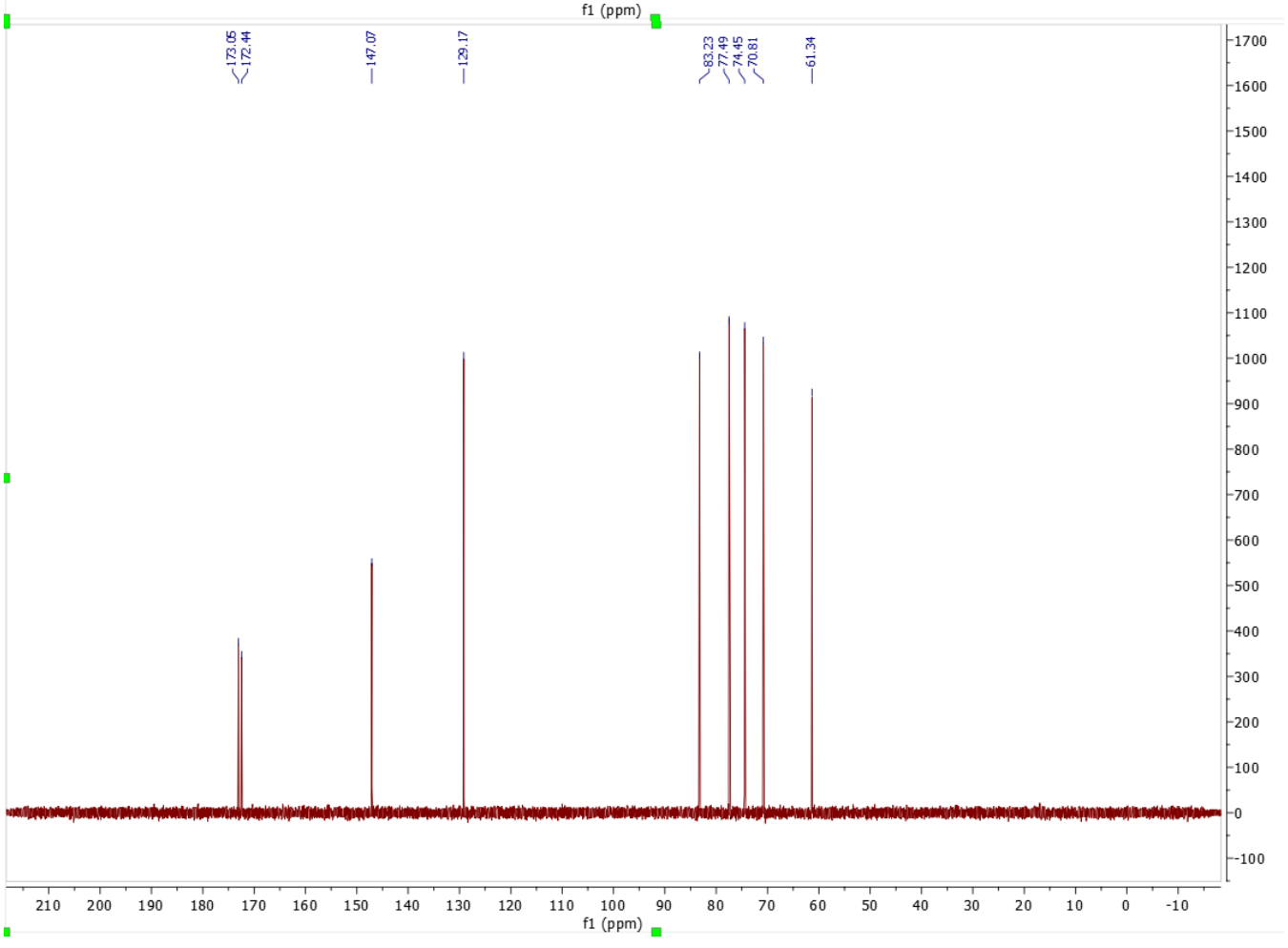
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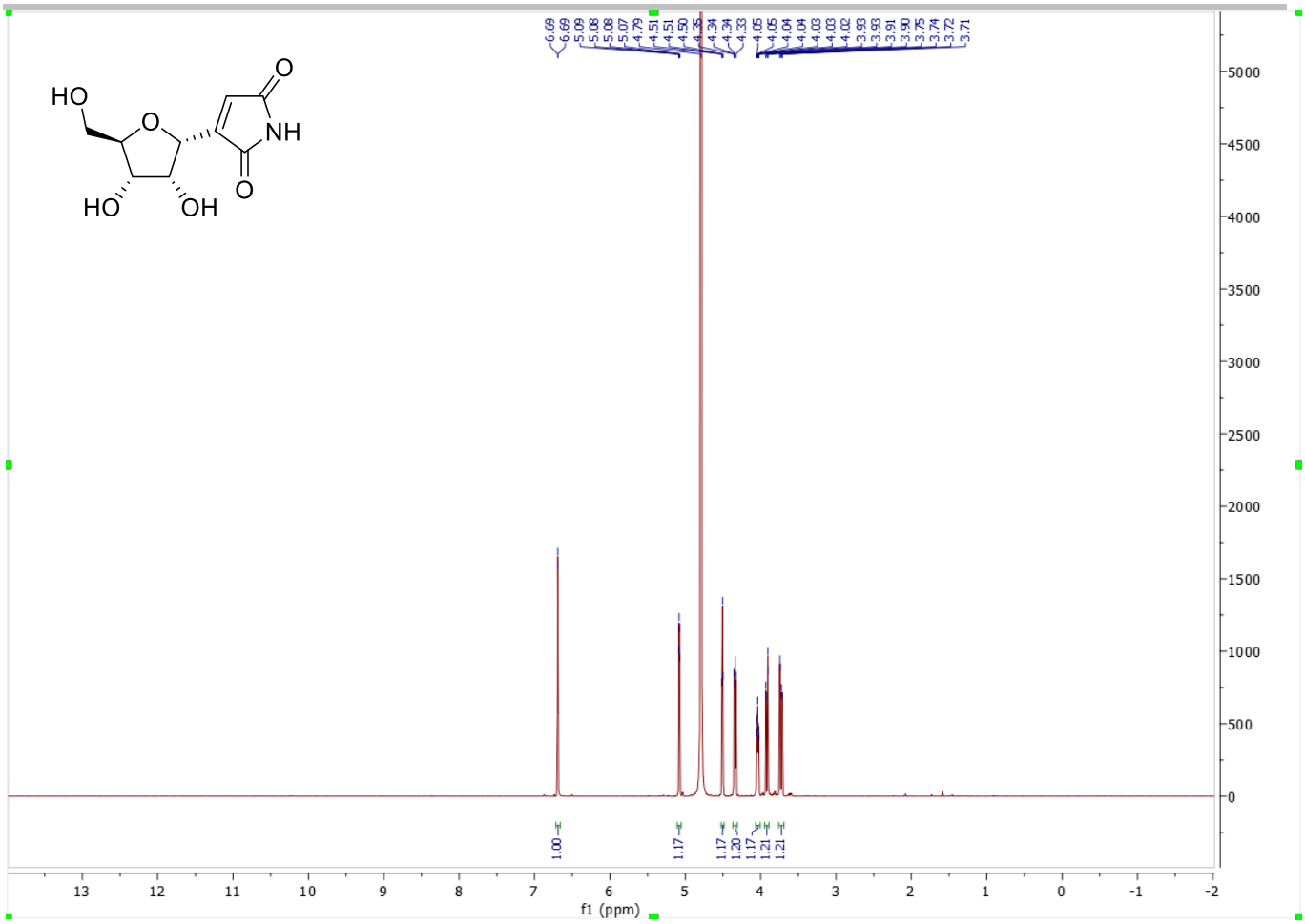
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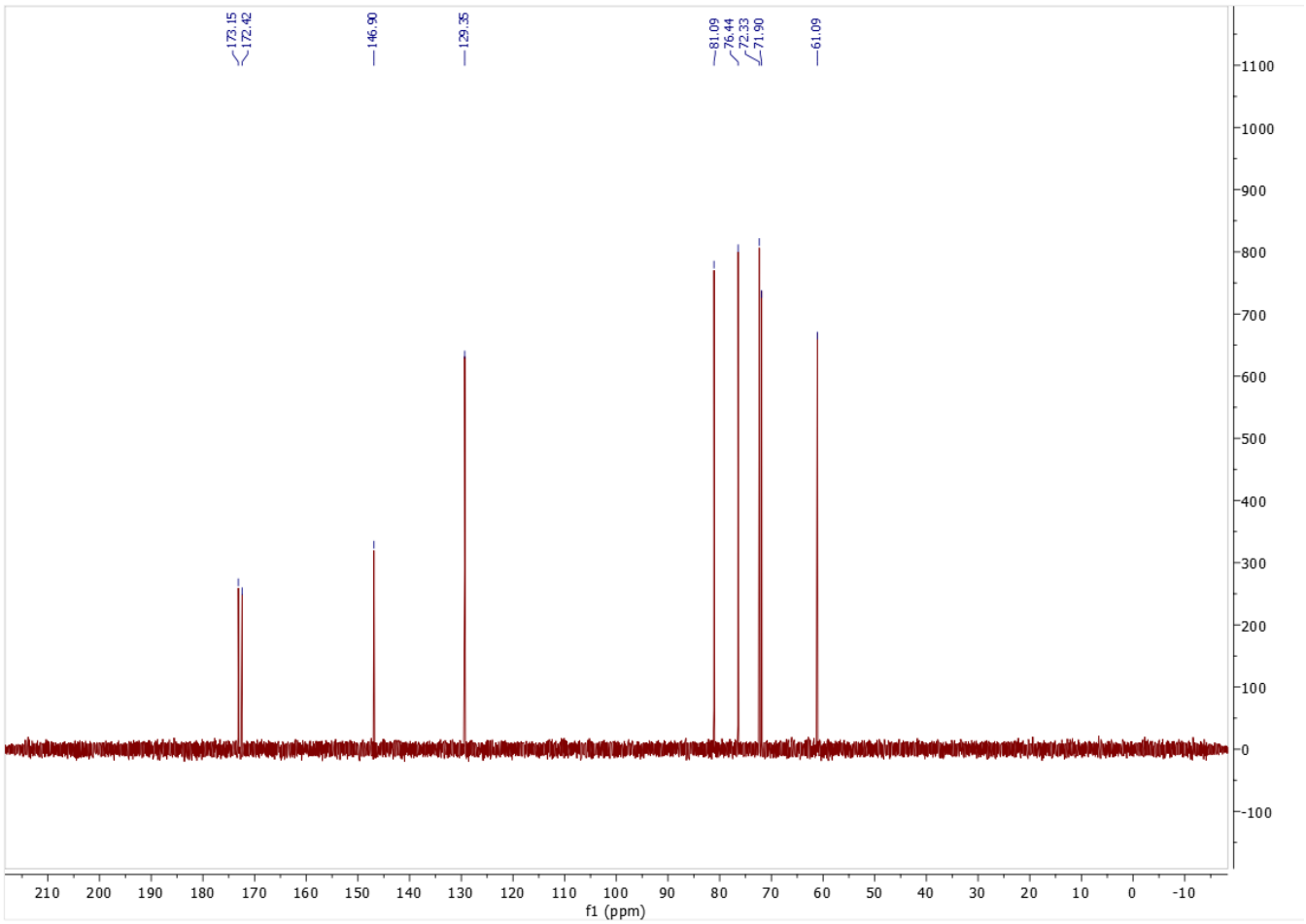
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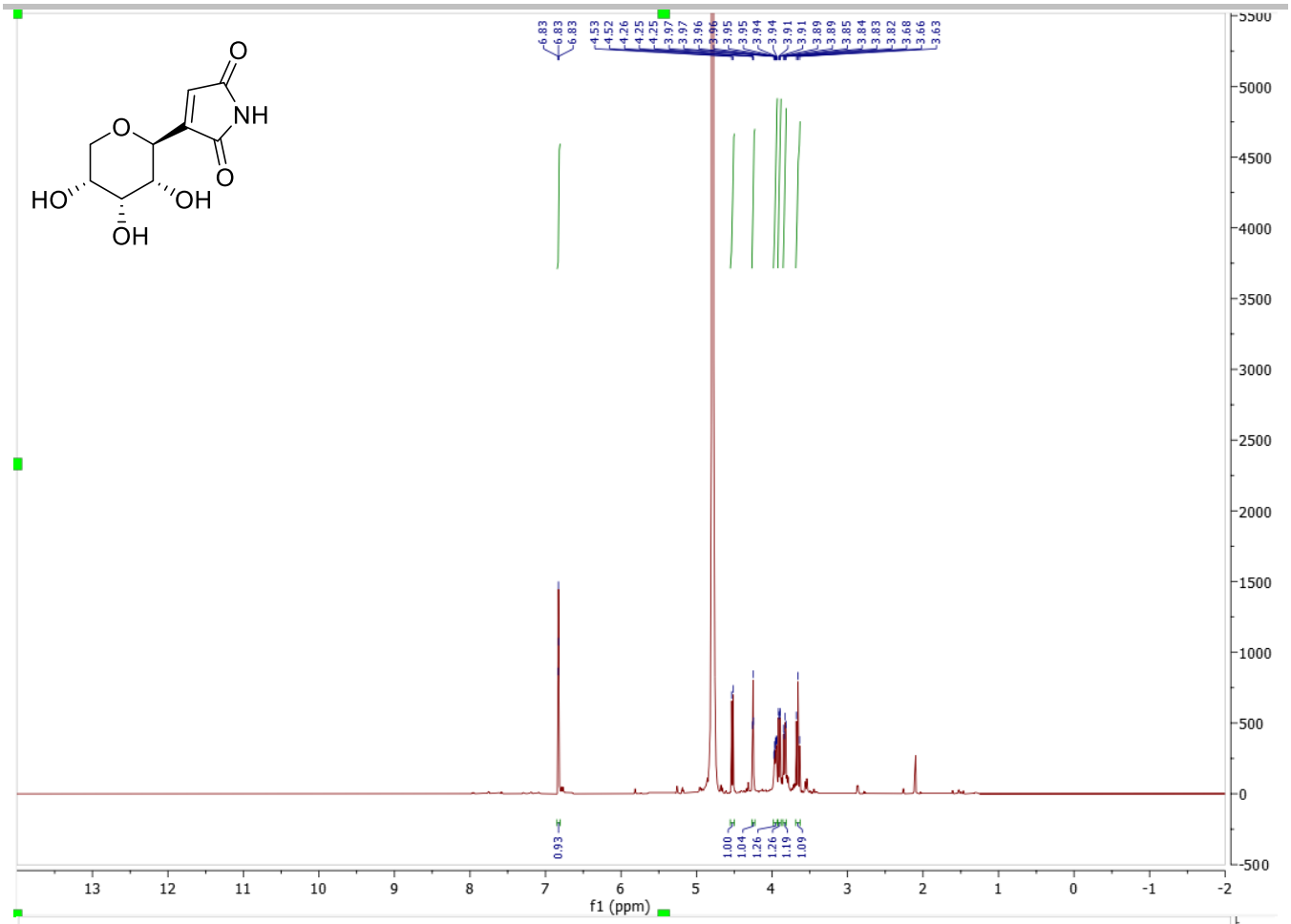
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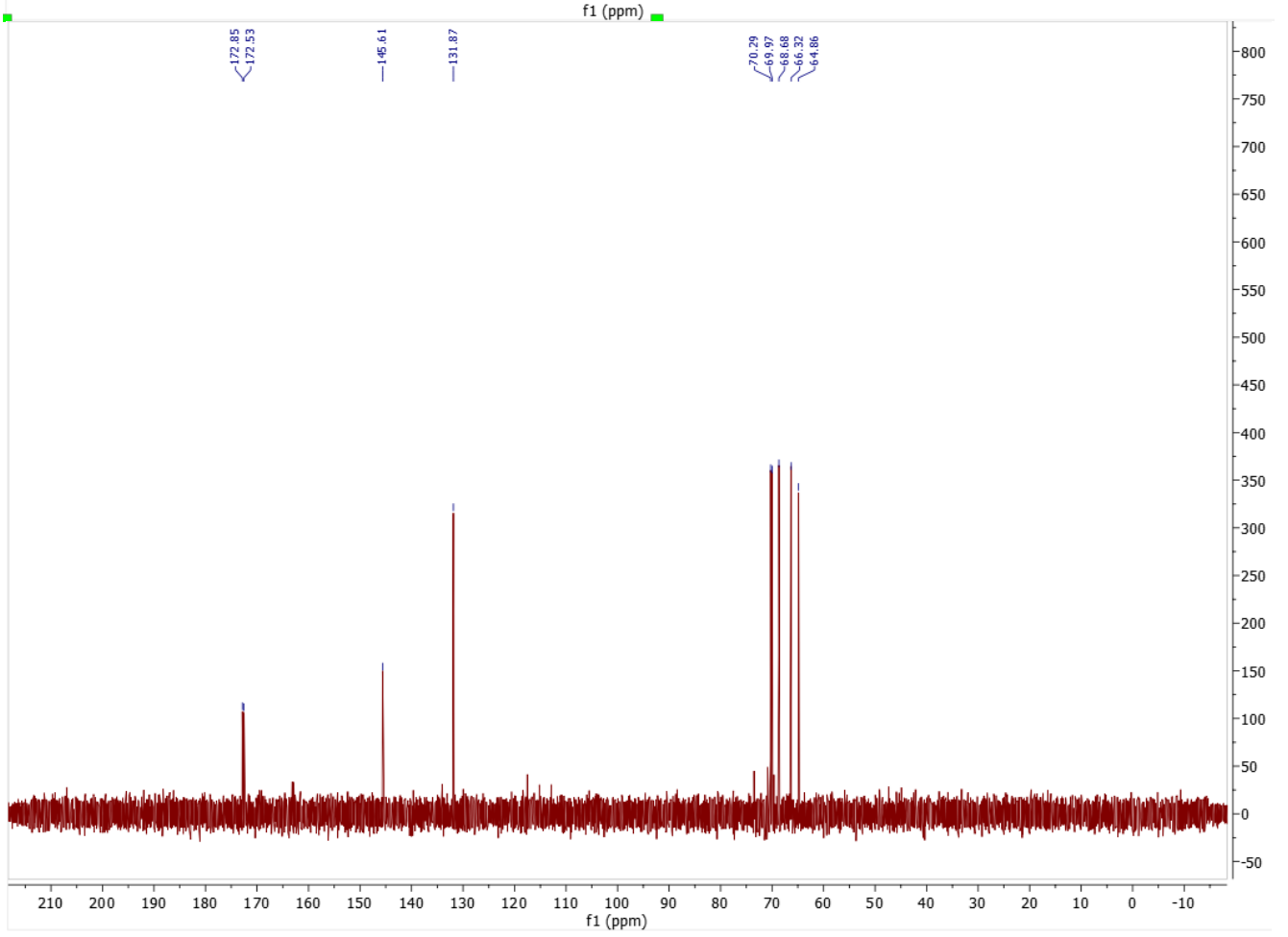
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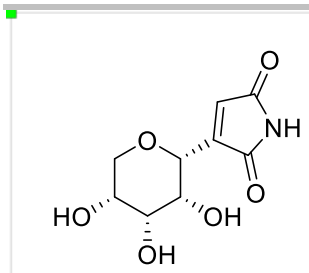
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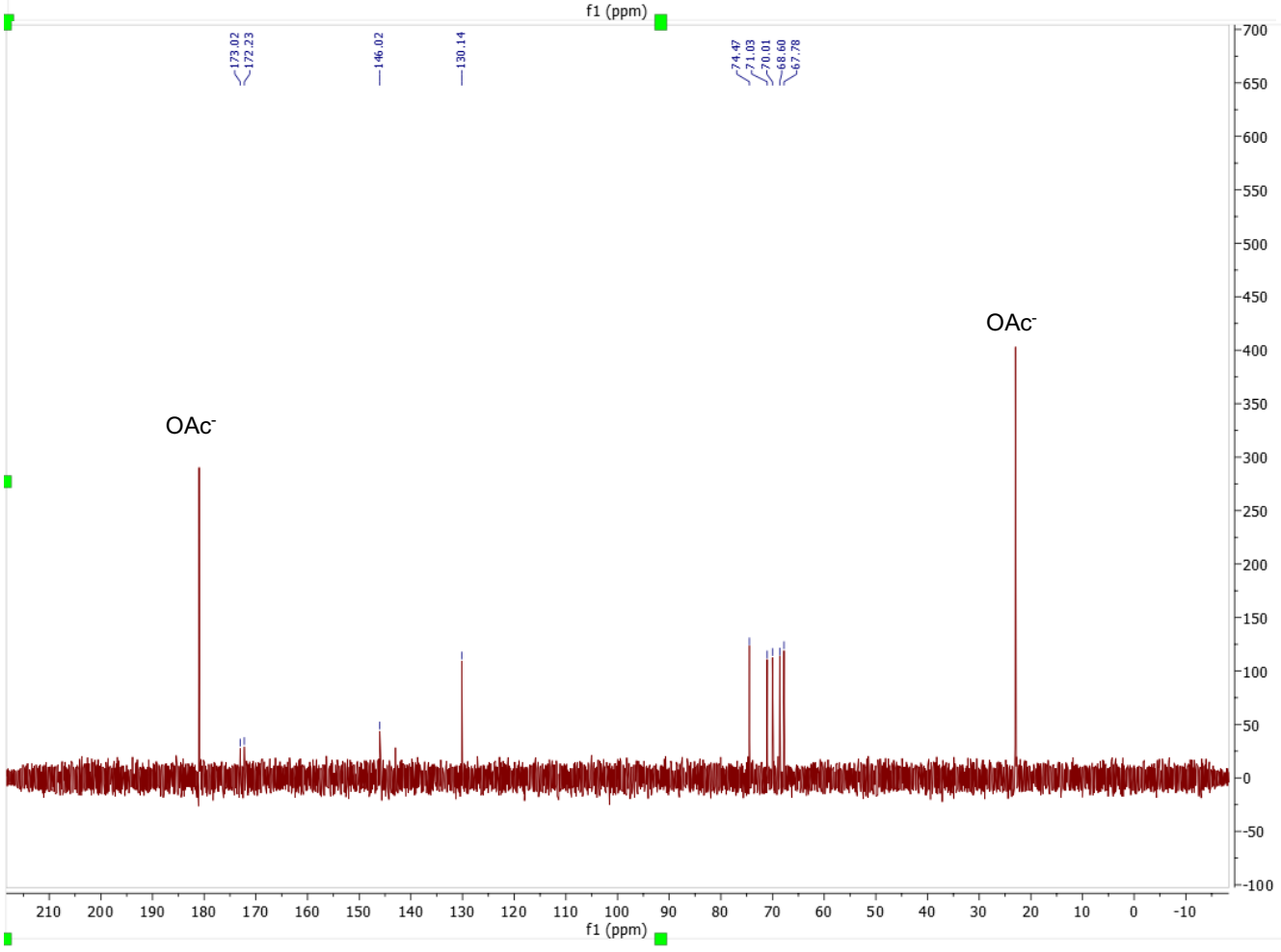
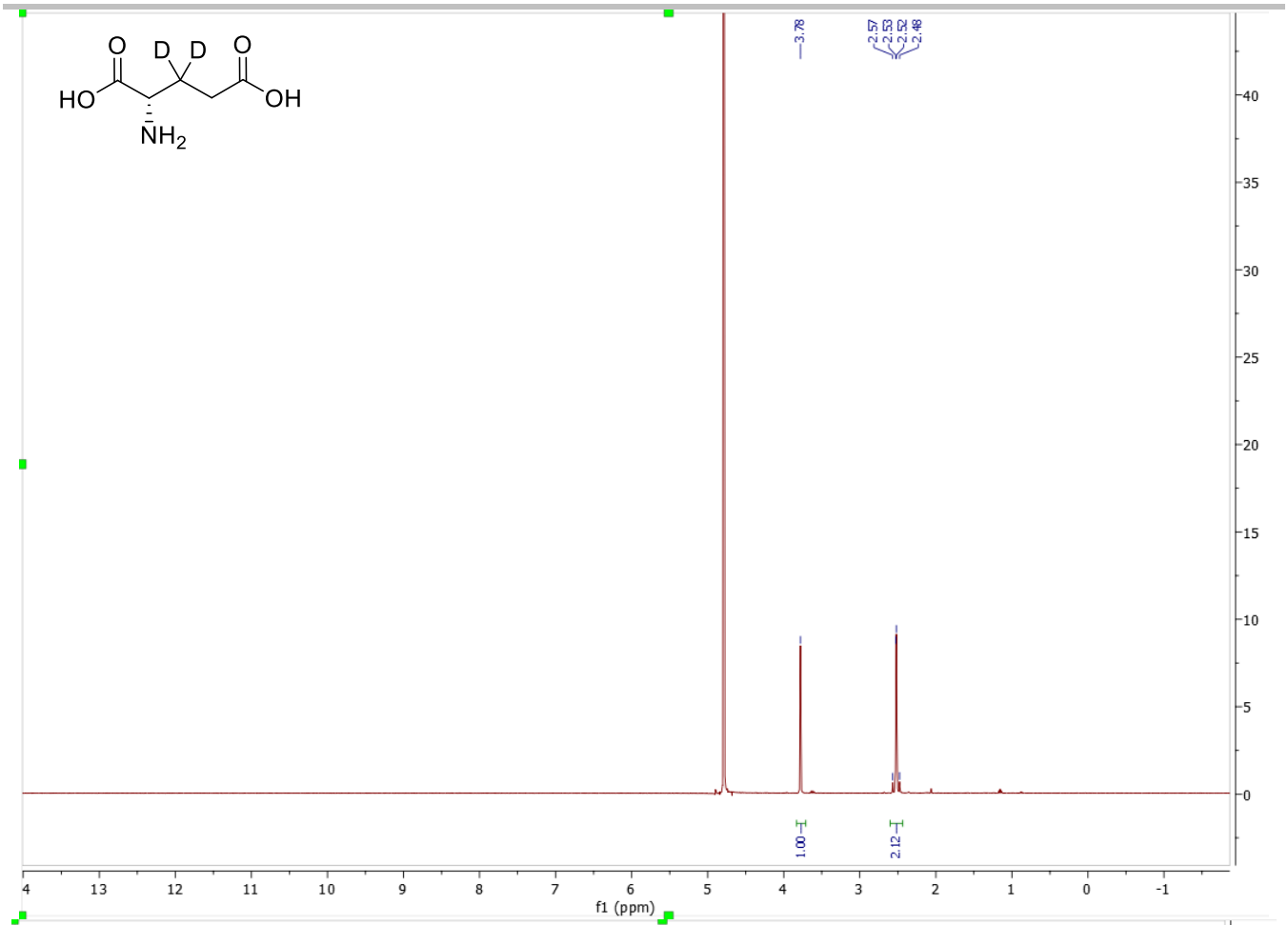
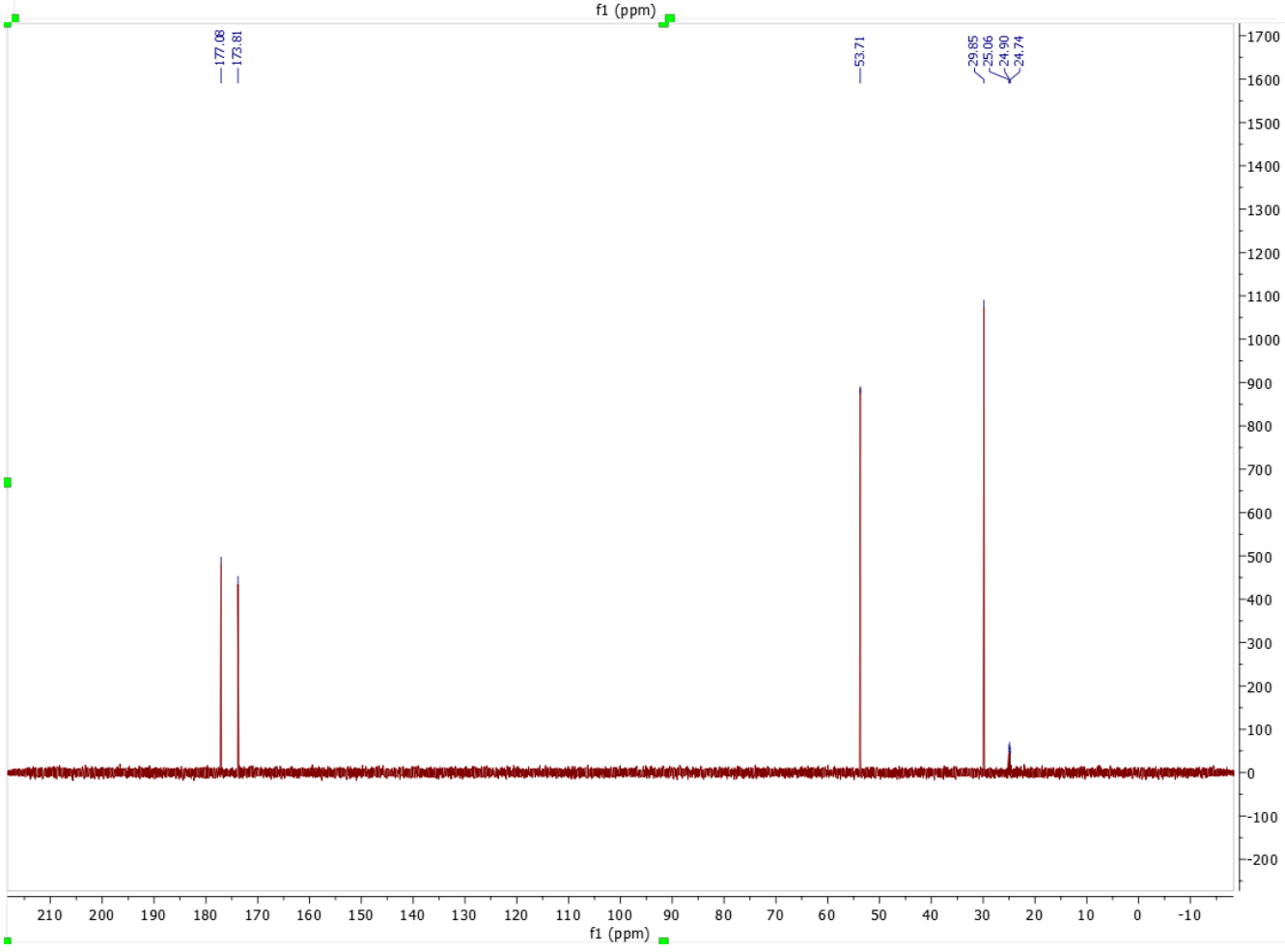
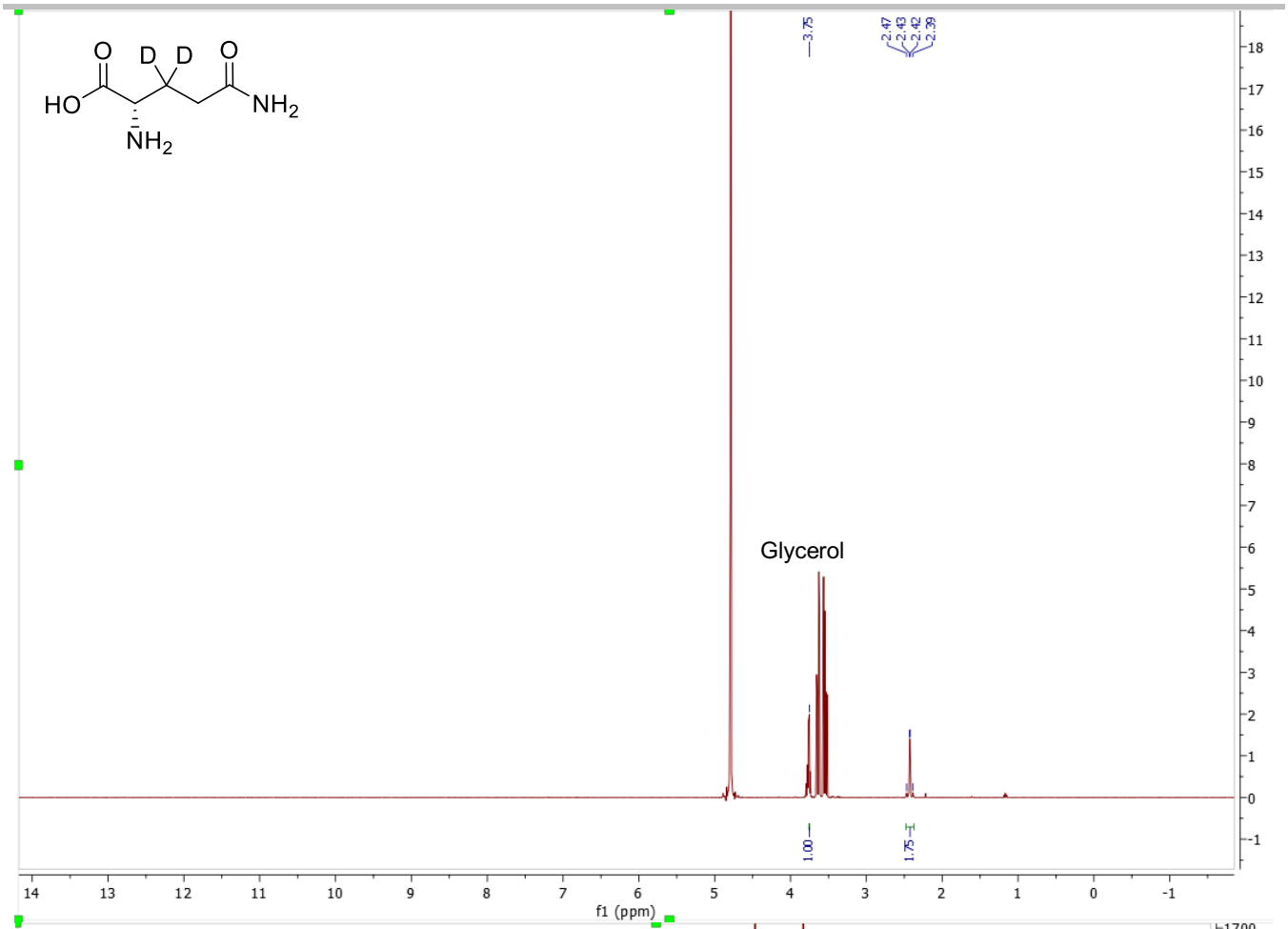
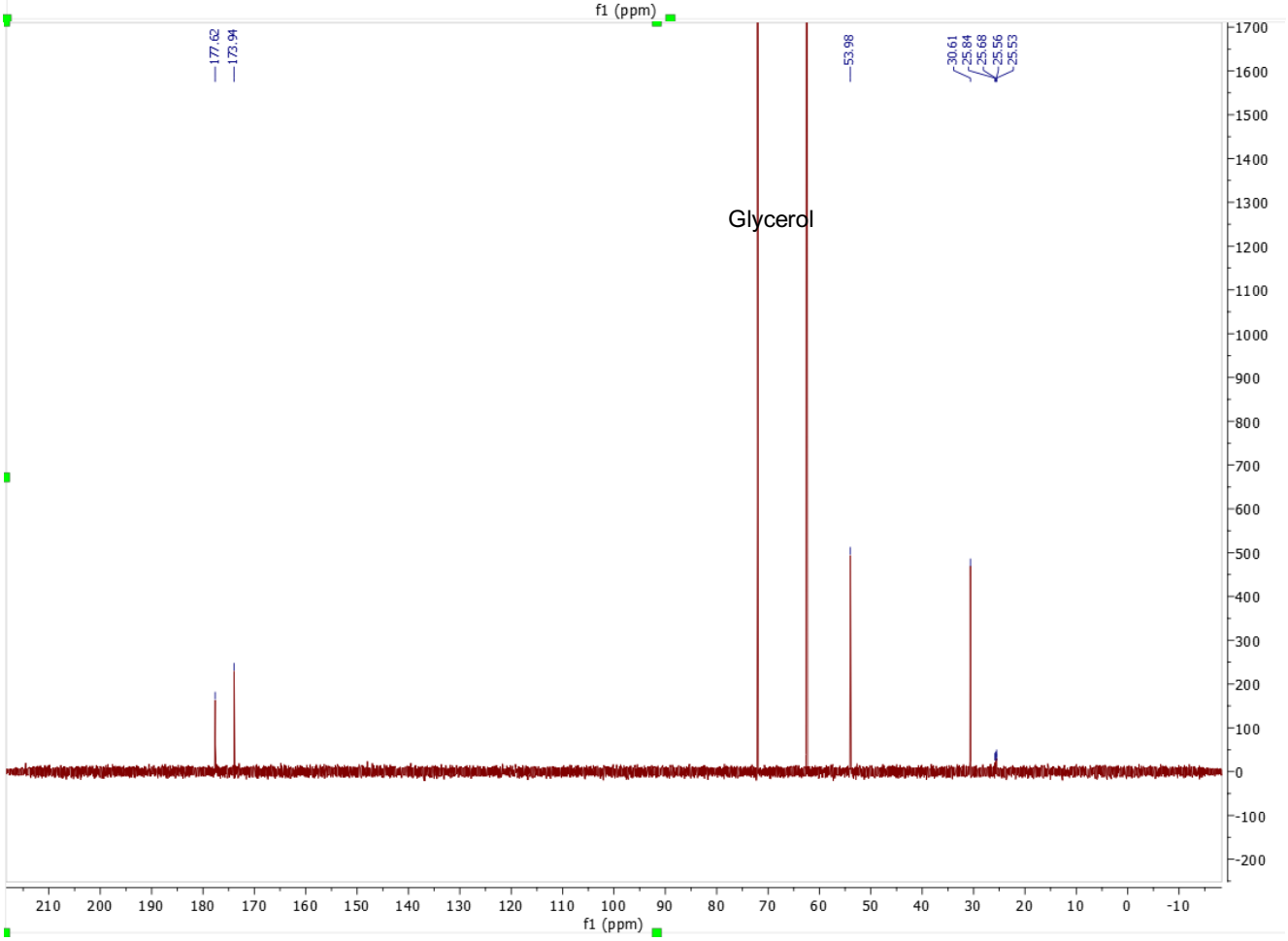
OAc-
f1 (ppm)


OAc-
OAc-
f1 (ppm)


O
D
D
O
HO
OH
NH2
3.78
2.57
2.53
2.52
2.48
1.00
2.12
f1 (ppm)


177.08
173.81
53.71
29.85
25.06
24.90
24.74
f1 (ppm)


Glycerol
f1 (ppm)


Glycerol
f1 (ppm)