Supporting Information for

Characterization of autoinducer-3 structure and biosynthesis in *E. coli*.

Chung Sub Kim,[†,‡] Alexandra Gatsios,[†,‡] Santiago Cuesta,[§,⊥] Yick Chong Lam,[†,‡] Zheng Wei,[‡,∥] Haiwei Chen,[∥] Regan M. Russell,[§,⊥] Emilee E. Shine,[‡,▽] Rurun Wang,[#] Thomas P. Wyche,[#] Grazia Piizzi,[#] Richard A. Flavell,[∥,$] Noah W. Palm,[∥] Vanessa Sperandio,[§,⊥] and Jason M. Crawford,[†,‡,▽,*]

[†]Department of Chemistry, Yale University, New Haven, CT 06520, USA.

[‡]Chemical Biology Institute, Yale University, West Haven, CT 06516, USA.

[§]Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA.

[⊥]Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA.

[∥]Department of Immunobiology, Yale School of Medicine, New Haven, CT 06520, USA.

[▽]Department of Microbial Pathogenesis, Yale School of Medicine, New Haven, CT 06536, USA.

[#]Merck Exploratory Science Center, Merck & Co., Inc., Cambridge, MA, USA.

[$]Howard Hughes Medical Institute, Yale School of Medicine, New Haven, CT 06519, USA.

*Correspondence: jason.crawford@yale.edu

MATERIALS AND METHODS

No unexpected or unusually high safety hazards were encountered during the course of the study. Pathogenic bacteria were handled in accordance with biosafety-level 2 procedures.

Instrumentation

Low-resolution LC-MS data were obtained using an Agilent 6120 single quadrupole LC-MS system. High-resolution electrospray ionization mass spectrometry (ESI-MS) data were obtained using an Agilent iFunnel 6550 quadrupole time-of-flight (QTOF) instrument fitted with an ESI source coupled to an Agilent 1290 Infinity HPLC system and a Kinetex 5µ C18 100 Å column (250 × 4.6 mm) with a water:acetonitrile gradient containing 0.1% formic acid at 0.7 mL/min: 0-30 min, 5 to 100% acetonitrile. The mass spectra were recorded in positive ionization mode with a mass range from *m/z* 100 to 1,700. For tandem MS, a mass range from *m/z* 50 to 1,700 and 20-50 eV collision energies were used. 10 ppm mass windows around calculated exact masses were used to generate extracted ion chromatogram (EIC) graphs (**1**, *m/z* 213.1056; **2**, *m/z* 185.0743; **3**, *m/z* 213.1056; **4** and **8**, *m/z* 167.1179; **5**, *m/z* 201.1022; **6** and **7**, *m/z* 125.0709; **9**, *m/z* 217.0972; **10**, *m/z* 153.1022; linear-**1**, *m/z* 233.1318; linear-**2**, *m/z* 205.1005; linear-**4**, *m/z* 184.1441; linear-**5**, *m/z* 221.1285). Isolation of metabolites was performed using an Agilent Prepstar high-performance liquid chromatography (HPLC) system with an Agilent Polaris C18-A 5 µm (250 × 21.2 mm^2) column and a Phenomenex Luna C18 (2) 100 Å (250 × 10 mm) column. 1D (^1H and ^{13}C) and 2D (gCOSY, gHSQCAD and gHMBCAD) NMR spectral data were measured on an Agilent 600 or 400 MHz NMR spectrometer equipped with a cold probe, and the chemical shifts were recorded as δ values (ppm) referenced to solvent residual signals.

Cultivation of *E. coli* strains and detection of pyrazinones 1-10

E. coli Nissle 1917 (ArdeyPharm) was grown overnight at 37 °C under aerobic conditions (250 rpm) in 5 mL of lysogeny broth (LB). Overnight cultures were used to inoculate three replicates of 5 mL of LB or LB + erythromycin (0.4, 1.6, 6.3, 25, or 100 μg/mL, Acros Organics) at a 1:200 dilution. Cultures were incubated at 37 °C with 250 rpm shaking for 48 h. After OD_{600} values were measured, 6.0 mL of ethyl acetate was added to each culture, which was vortexed for 10 seconds and centrifuged for 10 mins at 1500 × g. The top 5 mL of the organic layer was transferred and dried *in vacuo*. The dried extracts were dissolved in 200 μL of methanol for LC-MS analysis. *E. coli* O157:H7 (obtained from the Stavroula Hatzios Lab, Yale University), BW25113 (CGSC, see below), MG1655 (CGSC), and LF82 were individually grown and extracted with the same methods as described above in the absence of erythromycin.

E. coli BW25113 and its single-gene knockout strains were obtained from the Coli Genetic Stock Center (CGSC, Yale University, New Haven, CT, USA): JW1474-1 (Δ*adhP771::kan*), JW0342-1 (Δ*mhpF720::kan*), and JW3591-4 (Δ*tdh-728::kan*).[1] For ethanol metabolism mutant experiments, *E. coli* BW25113, JW1474-1 (Δ*adhP*), and JW0342-1 (Δ*mhpF*) were grown in LB with and without ethanol supplementation (100 mM). For threonine dehydrogenase mutant experiments, *E. coli* BW25113 and JW3591-4 (Δ*tdh*) were incubated in LB + 3*R*-hydroxy-L-Leu (10 mM, for **1**) or L-Thr (10 mM, for **2**, **4**, and **5**) or M9 minimal medium (Difco) + L-Thr (10 mM, for **6** and **7**). Detailed LC-MS sample preparation procedures were the same as described above.

Isolation, synthesis, and characterization of the metabolites

Overnight culture of *E. coli* Nissle 1917 in eighteen 5 mL aliquots of LB were used to inoculate eighteen 1 L cultures of LB + erythromycin (25 μg/mL) in 4 L Elenmeyer flasks. These cultures were incubated at 37 °C with shaking (250 rpm) for 48 h before pooling and extracting with 36 L (2 × 18 L) of ethyl acetate. Several rounds of HPLC on this organic extract afforded metabolites **1**, **2**, **3**, **6**, and **7** (see below for detailed isolation procedures). The chemical structures of **1-3** and **6** were elucidated through 1D- and 2D-NMR (^{1}H, COSY, HSQC, and HMBC, Table S1–S5, see NMR catalog). Trace peaks from **7** (2.5% of **6**) were observed in the ^{1}H NMR spectrum of major **6** (Figure S3). Chemical synthesis of **1-5** were carried out, and the structures of natural **4** and **5** were confirmed by comparison of their retention times with synthetic standards (Figure S2).

Detailed metabolite isolation procedure

The dried crude material from 18 L of *E. coli* Nissle 1917 cultures was subjected to a Biotage® SNAP Cartridge (KP-C18-HS, 30 g) and separated using a step gradient with the following solvent composition (each 100 mL): Fraction 1, 20% aqueous methanol; Fraction 2, 40% aqueous methanol; Fraction 3, 60% aqueous methanol; Fraction 4, 80% aqueous methanol; Fraction 5, 100% methanol. Fraction 4 containing compound **1** was fractionated by an Agilent Prepstar HPLC system with an Agilent Polaris C18-A 5 μm (250 × 21.2 mm^2) column with a gradient elution from 10 to 100% aqueous acetonitrile with 0.01% trifluoroacetic acid over 60 mins and flow rate of 8 mL/min using a 1 min fraction collection time window. Fractions 4-32 were further separated using a Phenomenex Luna C18 (2) 100 Å column (250 × 10 mm, flow rate 4.0 mL/min, an isocratic elution with 20% aqueous acetonitrile with 0.01% trifluoroacetic acid over 60 mins) to give compound **1** (0.5 mg) at t_R = 32.1 min. Fractions 2 and 3 containing compounds **2** and **3**, respectively, were individually fractionated by an Agilent Prepstar HPLC system with an Agilent

Polaris C18-A 5 μm (250 × 21.2 mm^2) column with a gradient elution from 10 to 100% aqueous acetonitrile with 0.01% trifluoroacetic acid over 60 mins and flow rate of 8 mL/min using a 1 min fraction collection time window. Fractions 2-21 and 3-27 were separated using a Phenomenex Luna C18 (2) 100 Å column (250 × 10 mm, flow rate 4.0 mL/min, an isocratic elution with 15% aqueous acetonitrile with 0.01% trifluoroacetic acid over 60 mins) to yield compounds **2** (2 mg, t_R = 13.5 min) and **3** (0.5 mg, t_R = 29.5 min), respectively. Fraction 1 was separated by an Agilent Prepstar HPLC system with an Agilent Polaris C18-A 5 μm (250 × 21.2 mm^2) column with an isocratic elution of 4% aqueous acetonitrile with 0.01% trifluoroacetic acid over 60 mins and flow rate 8 mL/min. The compound **6** with 2.5% of **7** (0.4 mg, t_R = 11.4 min) was obtained from the combined fraction 1-26 and 1-27 by a Phenomenex Luna C18 (2) 100 Å column (250 × 10 mm, flow rate 4.0 mL/min, a gradient elution from 5 to 15% aqueous acetonitrile with 0.01% trifluoroacetic acid over 30 mins).

Isotope labeling experiments

Overnight cultures of *E. coli* Nissle 1917 in LB (5 mL) were used to inoculate two biological replicates of 1 mL of LB at a 1:200 dilution containing individual universally ^{13}C-labeled (Cambridge Isotope Laboratories) or normal amino acid supplements (L-Met, L-Leu, L-Thr, L-Phe, and L-Ala (10 mM)). These cultures were incubated at 37 °C with 250 rpm shaking and extracted with ethyl acetate after 24 h (for linear-**1**, **2**, **4**, **5**) or 48 h (for **1**-**7**). Detailed LC-MS sample preparation procedures were the same as described above.

Feeding experiments with 3*R*- and 3*S*- hydroxy-L-Leu, L-Leu, AMB, and AA

LB medium was supplemented with 3*R*-hydroxy-L-Leu (0.01, 0.03, or 0.1 mM, Santa Cruz Biotechnology), 3*S*- hydroxy-L-Leu (10, 30, or 100 mM, Santa Cruz Biotechnology), L-Leu (10 or 100 mM), AMB (0.1 or 1 mM, Enamine), or AA (0.1 or 1 mM, Cayman Chemical). Overnight cultures of *E. coli* Nissle 1917 in LB (5 mL) were used to inoculate three biological replicates of 5 mL of these media at a 1:200 dilution, and they were grown at 37 °C for 48 h before extraction. Detailed LC-MS sample preparation procedures were the same as described above.

Time-course analysis of linear-1, 2, 4, and 5

Overnight cultures of *E. coli* Nissle 1917 in LB (5 mL) were used to inoculate three replicates of 5 mL of LB at a 1:200 dilution, which were extracted with ethyl acetate after 12, 24, or 48 h. Detailed LC-MS sample preparation procedures were the same as described above.

***In vitro* synthesis of linear-1, 2, 4, 5, and pyrazinone 6 using PURExpress® *in vitro* protein synthesis technologies**

The *in vitro* protein synthesis reaction mixtures were prepared according to the manufacturer's protocol (PURExpress® Δ ribosome Kit #E3313 and Δ (aa, tRNA) Kit #E6840, NEB) with slight modifications. For Δ ribosome Kit, 2 μL of control template was added into each reaction mixture. Among a total of ten reaction mixtures, 4.5 μL of control ribosomes was supplemented into the two reaction mixtures, one of which was also treated with AA (final concentration 100 μM). The eight non-ribosome-treated reaction mixtures were added with different concentrations of AA (final concentration 0, 10, 25, 50, 100, 250, or 500 μM) or AMB (final concentration 100 μM). After 3 h of incubation at 37 °C without shaking, each reaction mixture was diluted with 475 μL of Milli-Q water and extracted with 500 μL of ethyl acetate. The dried organic materials were

dissolved with 200 μL of methanol for LC-MS analysis. For Δ (aa, tRNA) Kit, 1 μL of control template and 500 μM (final concentration) of AA or AMB were added into each reaction mixture.

Expression and purification of MetRS

The plasmid used for MetRS expression and purification is B1-2 (Addgene, Plasmid no. 111464), which harbors the MetRS from *E. coli*. The plasmid was transformed into electrocompetent *E. coli* BL21(DE3) (NEB) cells and cultivated on LB agar plates supplemented with 100 μg/mL ampicillin at 37 °C in a static incubator. 5 mL of LB medium aliquots supplemented with 100 μg/mL ampicillin were inoculated with single colonies and grown overnight at 37 °C under aerobic conditions (250 rpm). For protein expression, 1 L of fresh LB medium aliquots were supplemented with 100 μg/mL ampicillin and inoculated at 1:200 dilution from overnight cultures and further cultivated at 37 °C and 250 rpm. At OD_{600} = 0.5, the cultures were induced with 1 mM IPTG. Temperature was reduced, and MetRS protein expression occurred at 25 °C and 250 rpm for 24 h. MetRS was purified using Ni-NTA Agarose (Qiagen) according to the manufacturer's protocol and purity was assessed by SDS-PAGE analysis. Protein concentrations were measured using the Bradford Protein Assay Kit (Amresco, Inc.) according to the manufacturer's protocol.

***In vitro* synthesis of linear-1 and 2 with isolated MetRS**

To 500 μL of aminoacylation buffer (100 mM HEPES, pH 7.2, 30 mM KCl, and 10 mM $MgCl_2$), the following components were added: 1 μM MetRS (final), 1 mM AMB (for linear-**1**) or AA (for linear-**2**), 1 mM L-Met, 10 mM ATP (pH 7.5), and 1 mM DTT. The reaction mixtures were incubated for 4 h at 37 °C in a standing incubator before extraction with 500 μL of ethyl acetate. After centrifugation at 18000 × g for 5 mins, 400 μL of the ethyl acetate extracts were transferred

and dried *in vacuo*. The dried materials were dissolved in 200 μL of methanol for LC-MS analysis. For time-course analysis, the reaction mixtures were extracted with ethyl acetate after 0.25, 0.5, 1, 2, and 4 h incubations.

Synthesis of 6 and 11 from AA

0.3 mg of AA was individually incubated in 1 mL of PBS, aminoacylation buffer, or water at 25 °C and 250 rpm. After 3, 24, and 48 h incubation, 100 μL of each sample was transferred to a LC-MS vial, and 10 μL of each sample was directly injected onto the LC-MS instrument for analysis. Standard **11** (Acros Organics) was purchased for retention time comparisons.

RNA isolation and quantitative real-time PCR

Overnight cultures of WT EHEC and Δ*qseC* EHEC were grown in LB with or without 5 nM of each compound (**1**-7) dissolved in low-glucose Dulbecco's modified Eagle medium (DMEM, Gibco). Overnight cultures were diluted 1:100 and compound was added at final concentration of 5 nM and grown aerobically in low-glucose DMEM (Gibco) at 250 rpm to late exponential growth phase (OD_{600} = 0.9-1.0). Bacterial RNA was extracted using the RiboPure bacterial isolation kit (Ambion) according to the manufacturer's instructions. cDNA was synthesized using SuperScript II reverse transcriptase (ThermoFisher Scientific). qPCR was performed in a QuantStudio 6 Flex Instrument (Life Technologies) with Power SYBR Green (Applied Biosystems) using the following PCR conditions: a single hold at 50 °C for 2 mins and at 95 °C for 10 mins, followed by 40 cycles at 95 °C for 15 seconds and 60 °C for 1 min. Each PCR was performed in 10 μL reactions and contained 1× SYBR green mix and 0.25 μM of each primer. All data were normalized to a *rpoA* (RNA polymerase subunit A) endogenous control and analyzed using the comparative

critical threshold (*CT*) method. Virulence gene expression was presented as fold changes over the expression level of WT EHEC grown in the absence of AI-3 analog. Error bars indicate the standard deviations of the fold change values. The Student unpaired *t* test was used to determine statistical significance.

PRESTO-Tango Assay

HTLA cells, a HEK293 cell line that stably expresses β-arrestin-TEV and tTA-Luciferase (a kind gift from Gilad Barnea, Brown University), were seeded in 96-well tissue culture plates (Eppendorf) in DMEM containing 10% FBS and 1% penicillin/streptomycin. One day after plating (after reaching approximately 90% confluence), 200 ng per well GPCR-Tango plasmids[2] in 20 μl DMEM were mixed with 400 ng polyethylenimine (Polysciences) in an equal volume of DMEM and incubated for 20 mins at room temperature before adding the transfection mixture to the HTLA cells. 16-24 h after transfection, medium was replaced with 180 μL fresh DMEM containing 1% penicillin/streptomycin and 10 mM HEPES and 20 μL indicated concentration of molecules were added into the supernatants. Supernatants were aspirated 16-24 h after stimulation and 50 μL per well of Bright-Glo solution (Promega) diluted 20-fold with PBS containing 20 mM HEPES was added into each well. After 20 min incubation at room temperature, luminescence was quantified using a Spectramax i3x (Molecular Devices). Activation fold for each sample was calculated by dividing relative luminescence units (RLU) for each condition by RLUs from media alone controls.

BioMAP® Phenotypic Profiling Assay

BioMAP® Diversity PLUS assay was performed by Eurofins DiscoverX. Human primary cells in BioMAP systems were used at early passage (passage 4 or earlier) to minimize adaptation to cell culture conditions and preserve physiological signaling responses. All cells were from a pool of multiple donors (n = 2 – 6), commercially purchased and handled according to the recommendations of the manufacturers. Human blood derived CD14+ monocytes are differentiated into macrophages *in vitro* before being added to the /Mphg system. Abbreviations are used as follows: Human umbilical vein endothelial cells (HUVEC), Peripheral blood mononuclear cells (PBMC), Human neonatal dermal fibroblasts (HDFn), B cell receptor (BCR), T cell receptor (TCR) and Toll-like receptor (TLR). Cell types and stimuli used in each system are as follows: 3C system [HUVEC + (IL-1β, TNFα and IFNγ)], 4H system [HUVEC + (IL-4 and histamine)], LPS system [PBMC and HUVEC + LPS (TLR4 ligand)], SAg system [PBMC and HUVEC + TCR ligands (1×)], BT system [CD19+ B cells and PBMC + (α-IgM and TCR ligands (0.001×))], BF4T system [bronchial epithelial cells and HDFn + (TNFα and IL-4)], BE3C system [bronchial epithelial cells + (IL-1β, TNFα and IFNγ)], CASM3C system [coronary artery smooth muscle cells + (IL-1β, TNFα and IFNγ)], HDF3CGF system [HDFn + (IL-1β, TNFα, IFNγ, EGF, bFGF and PDGF-BB)], KF3CT system [keratinocytes and HDFn + (IL-1β, TNFα and IFNγ)], MyoF system [differentiated lung myofibroblasts + (TNFα and TGFβ)] and /Mphg system [HUVEC and M1 macrophages + Zymosan (TLR2 ligand)]. Systems are derived from either single cell types or co-culture systems. Adherent cell types were cultured in 96 or 384-well plates until confluence, followed by the addition of PBMC (SAg and LPS systems). The BT system consists of CD19+ B cells co-cultured with PBMC and stimulated with a BCR activator and low levels of TCR stimulation. Metabolites **1-5** and **7** were prepared in DMSO (final concentration $\leqslant$ 0.1%) and added at a final concentration of 36 µM, 1 h before stimulation and remained in culture for 24

h or as otherwise indicated (48 h: MyoF system; 72-h: BT system (soluble readouts); 168 h: BT system (secreted IgG)). Each plate contained drug controls, negative controls (*e.g.*, non-stimulated conditions) and vehicle controls (e.g., 0.1% DMSO) appropriate for each system. Direct ELISA was used to measure biomarker levels of cell-associated and cell membrane targets. Soluble factors from supernatants were quantified using either HTRF® detection, bead-based multiplex immunoassay or capture ELISA. Overt adverse effects of test agents on cell proliferation and viability (cytotoxicity) were detected by sulforhodamine B (SRB) staining, for adherent cells, and alamarBlue® reduction for cells in suspension. For proliferation assays, individual cell types were cultured at subconfluence and measured at time points optimized for each system (48 h: 3C and CASM3C systems; 72 h: BT and HDF3CGF systems; 96 h: SAg system). Cytotoxicity for adherent cells was measured by SRB (24 h: 3C, 4H, LPS, SAg, BF4T, BE3C, CASM3C, HDF3CGF, KF3CT, /Mphg systems; 48 h: MyoF system), and by alamarBlue staining for cells in suspension (24 h: SAg system; 42 h: BT system) at the time points indicated.

IL-8 analysis

THP-1 (ATCC TIB-202) cells cultured in RPMI medium (Gibco 11875093) with 10% FBS (Sigma F8192-500ML) and 1% penicillin-streptomycin (Gibco 15070063) were pretreated and differentiated into macrophage-like cells by incubation in the presence of 50 nM PMA (Sigma P1585-1MG). Compound **2** (17 μM, 1 μM, 0.1 μM, 0.01 μM, 0.001 μM in DMSO) or DMSO (Santa Cruz 202581) were added 1 h prior to differentiation. 100 ng/mL LPS (Invivogen tlrl-peklps) were added after 16 h PMA incubation. After 24 h LPS treatment, culture supernatants were collected for IL-8 ELISA assay (R&D DY208-05) according to the manufacturer's instructions.

Metabolite profiling in Gram-negative and Gram-positive bacteria

All Gram-negative [*E. coli* Nissle 1917, *V. cholerea* El Tor N16961 Δ*ctxAB*, *V. parahaemolyticus*, *V. vulnificus*, *K. pneumoniae* ATCC 700603, *K. pneumonia* ATCC BAA 1705, *P. aeruginosa* PA01, and *P. aeruginosa* PA14], and Gram-positive [*S.* Typhimurium, *E. faecalis*, *E. gallinarum*,[3] and *S. aureus* ATCC BAA-1717] bacteria with the exception of *B. subtilis* were grown in triplicates for 48 h at 37 °C under aerobic conditions (250 rpm) in 5 mL of LB alongside a 5 mL LB control. *B. subtilis* and corresponding LB controls were grown at 30 °C. Ethyl acetate (6 mL) was added to each culture, inverted several times, and centrifuged for 5–10 mins at 3000 × g. The top 5 mL of organic layer was transferred and dried *in vacuo*. The dried extracts were dissolved in 200 μL of methanol for LC-MS analysis.

Metabolites detection from mouse fecal samples. Before colonization, 8-10-week-old C57BL/6 mice were fasted for 4 hrs followed by gavage of kanamycin (20 mg). After 20 hrs, mice were fasted again for 4 hrs and administrated with 1×10^4 kanamycin resistant *E. coli* (BW25113 WT carrying pET-28a(+) plasmid). Fecal pellets were collected 7 days post colonization and sonicated in methanol (HPLC grade) followed by centrifugation. Supernatants were dried *in vacuo* and resuspended in 100 μL of methanol and analyzed by LC-MS. All mouse studies were performed in compliance with Yale Institutional Animal Care and Use Committee protocols.

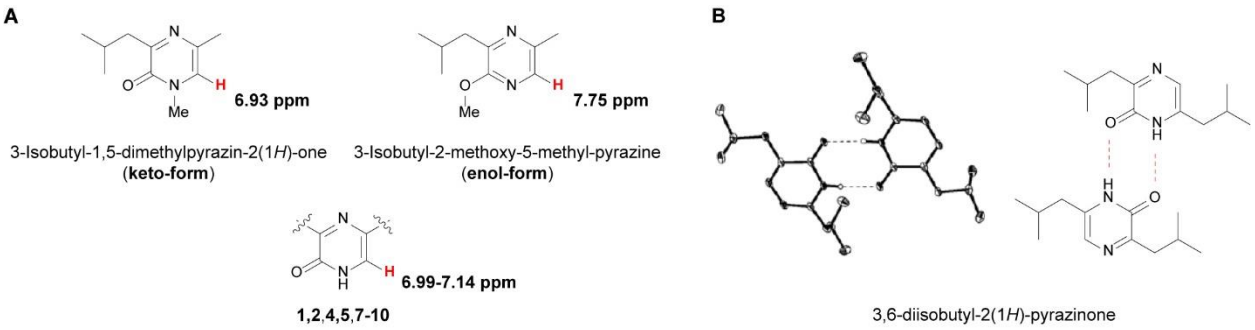


Figure S1. Determination of keto-form favored structures. (A) The olefinic ^{1}H NMR signal of 3-isobutyl-1,5-dimethylpyrazin-2(1*H*)-one (keto-form) and 3-isobutyl-2-methoxy-5-methyl-pyrazine (enol-form) were previously observed at 6.93 ppm and 7.75 ppm in chloroform-*d*, respectively.[4] The same proton signals of **1**, **2**, **4**, **5**, and **7**–**10** were observed at 6.99 – 7.14 ppm (chloroform-*d* or methanol-d_4), which are closer to that of keto-form (6.93 ppm) than that of the enol-form (7.75 ppm). (B) The crystal structure of 3,6-diisobutyl-2(1*H*)-pyrazinone showed that this molecule exists as a dimer of two keto-form-favored pyrazinones with intermolecular hydrogen bonds[5] suggesting the same phenomenon would occur in **1**, **2**, **4**, **5**, and **7**–**10**. Collectively, these data support that **1**, **2**, **4**, **5**, and **7**–**10** possess a keto-form favored pyrazinone core structure, which is broadly consistent with natural product literature.

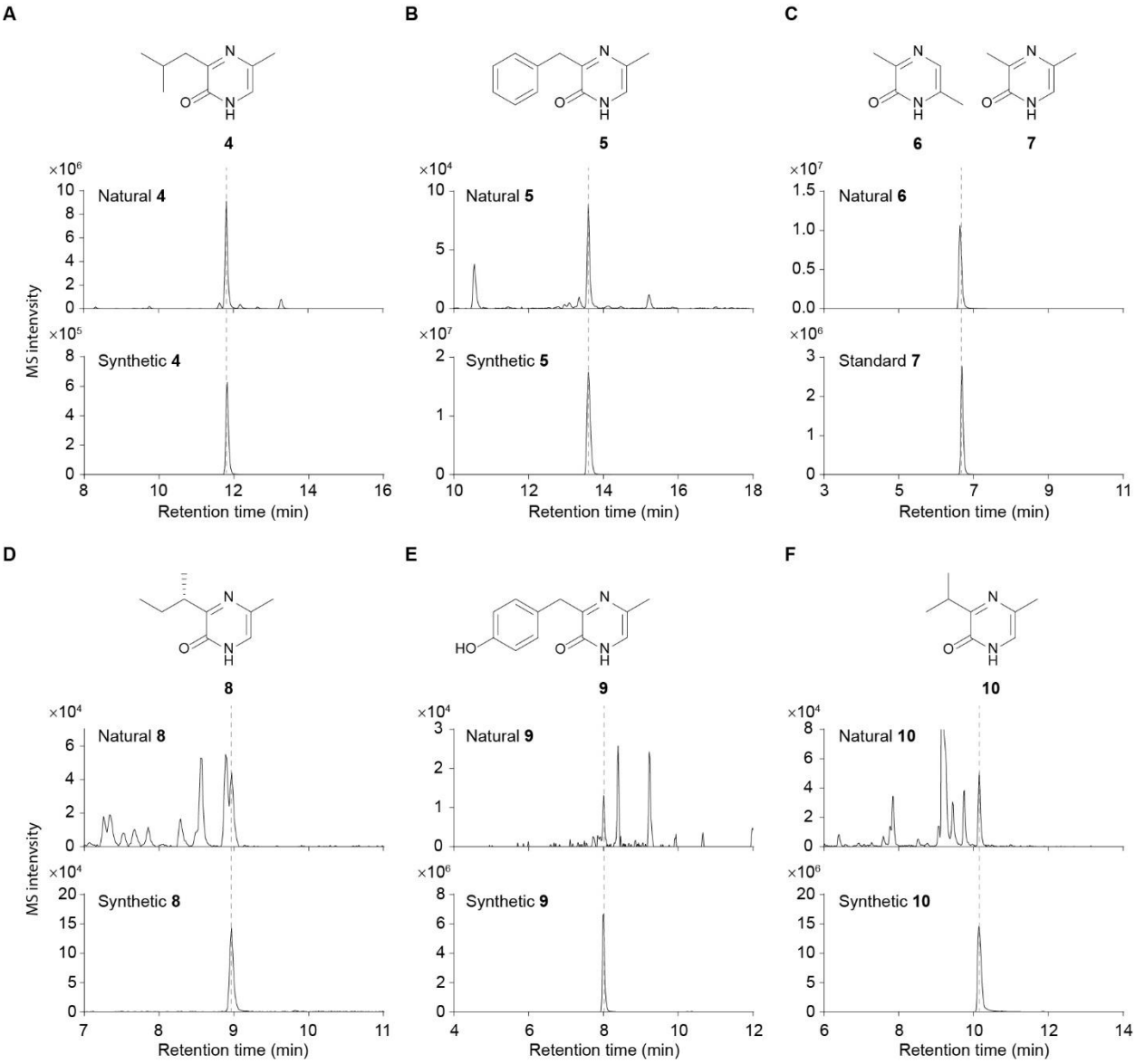


Figure S2. Structural identification of 4–10. (A), (B), and (D)–(F) The EICs of natural and synthetic **4**, **5**, and **8**–**10**, respectively. (C) The EICs of natural **6** and standard **7**. These two compounds co-eluted and were non-separable in standard chromatographic conditions.

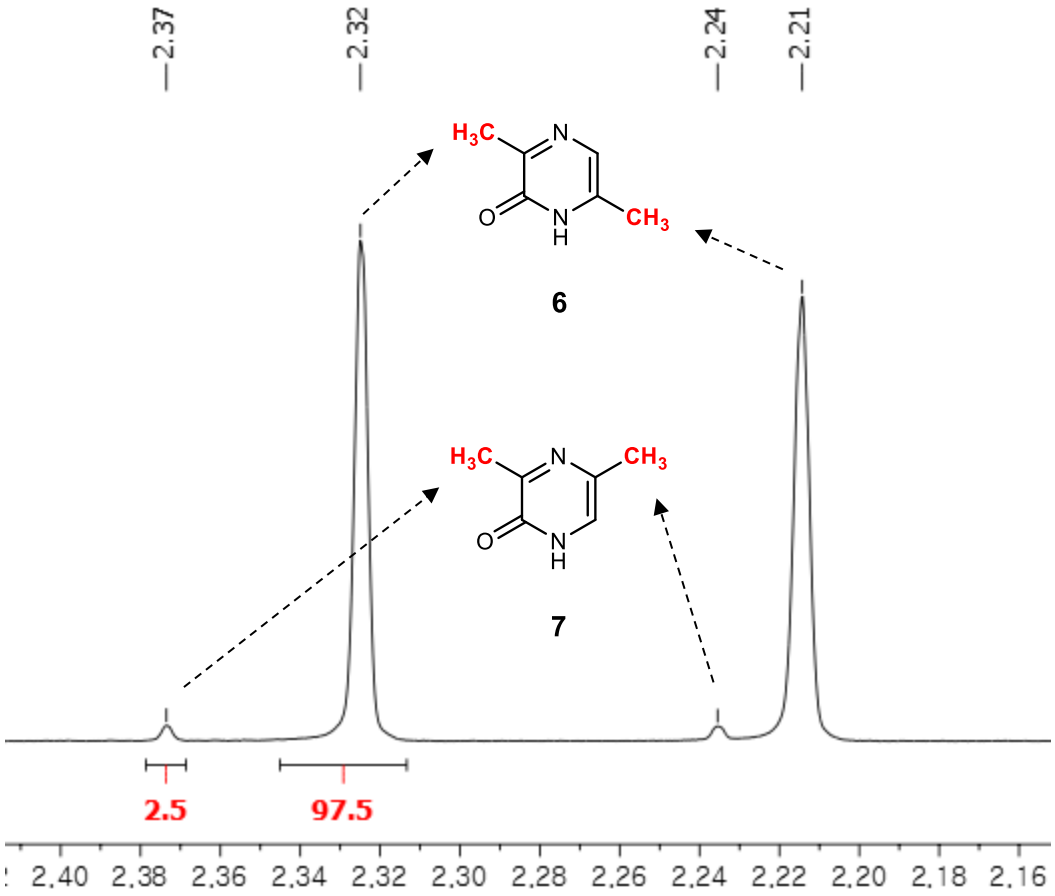


Figure S3. Zoomed-in region of ^{1}H NMR spectrum of natural *E. coli* metabolite 6 measured in methanol-d_4 showing that 7 was co-isolated (6 : 7 = 97.5 : 2.5). See NMR catalog below for full NMR spectra.

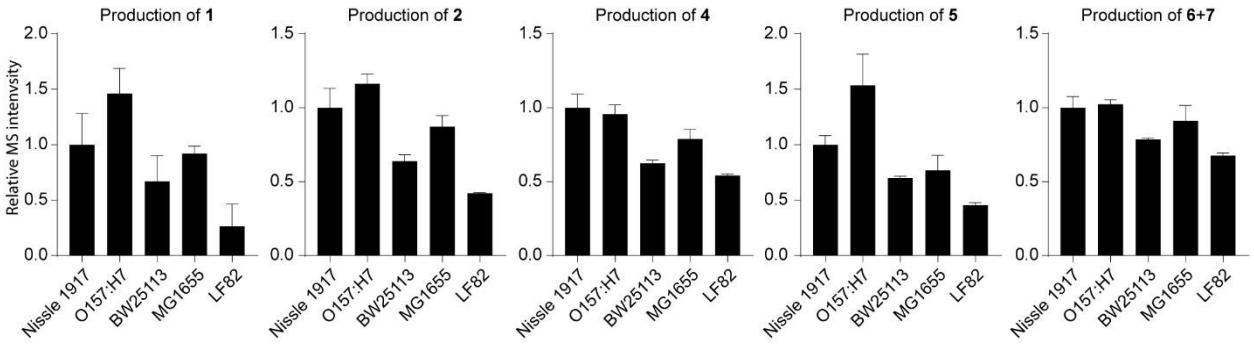


Figure S4. Production of 1, 2, and 4–7 from selected *E. coli* strains. These molecules were detected in selected probiotic (Nissle 1917), pathogenic (O157:H7 and LF82), and commensalistic (BW25113 and MG1655) *E. coli* strains. n = 3 biological replicates. Data are mean ± s.d. The metabolites have been detected in all *E. coli* strains that we have analyzed to date.

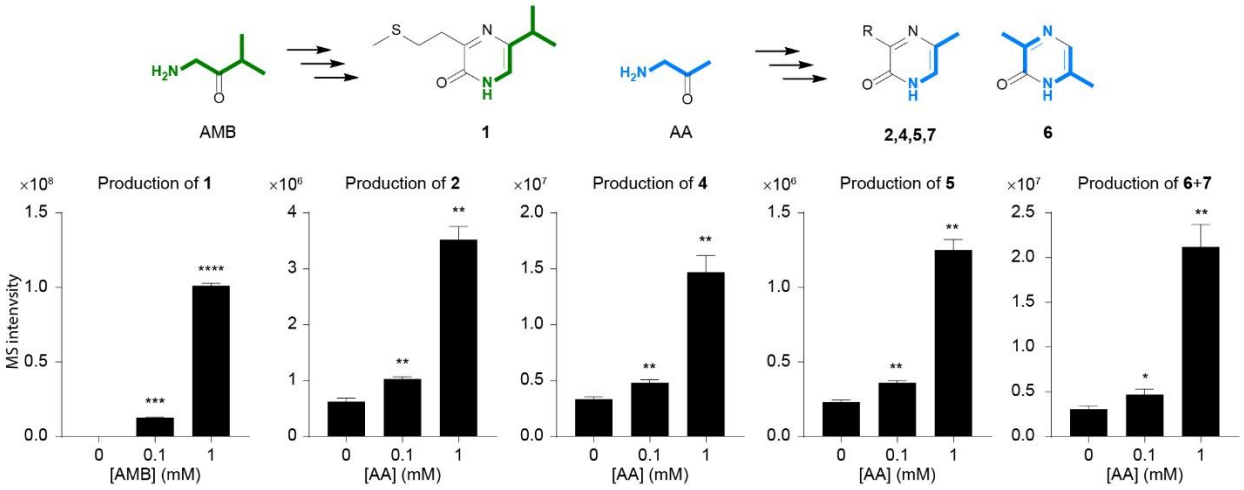


Figure S5. AMB/AA dose-dependent production of 1, 2, and 4–7. *n* = 3 biological replicates. Data are mean ± s.d. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001; two-tailed t-test.

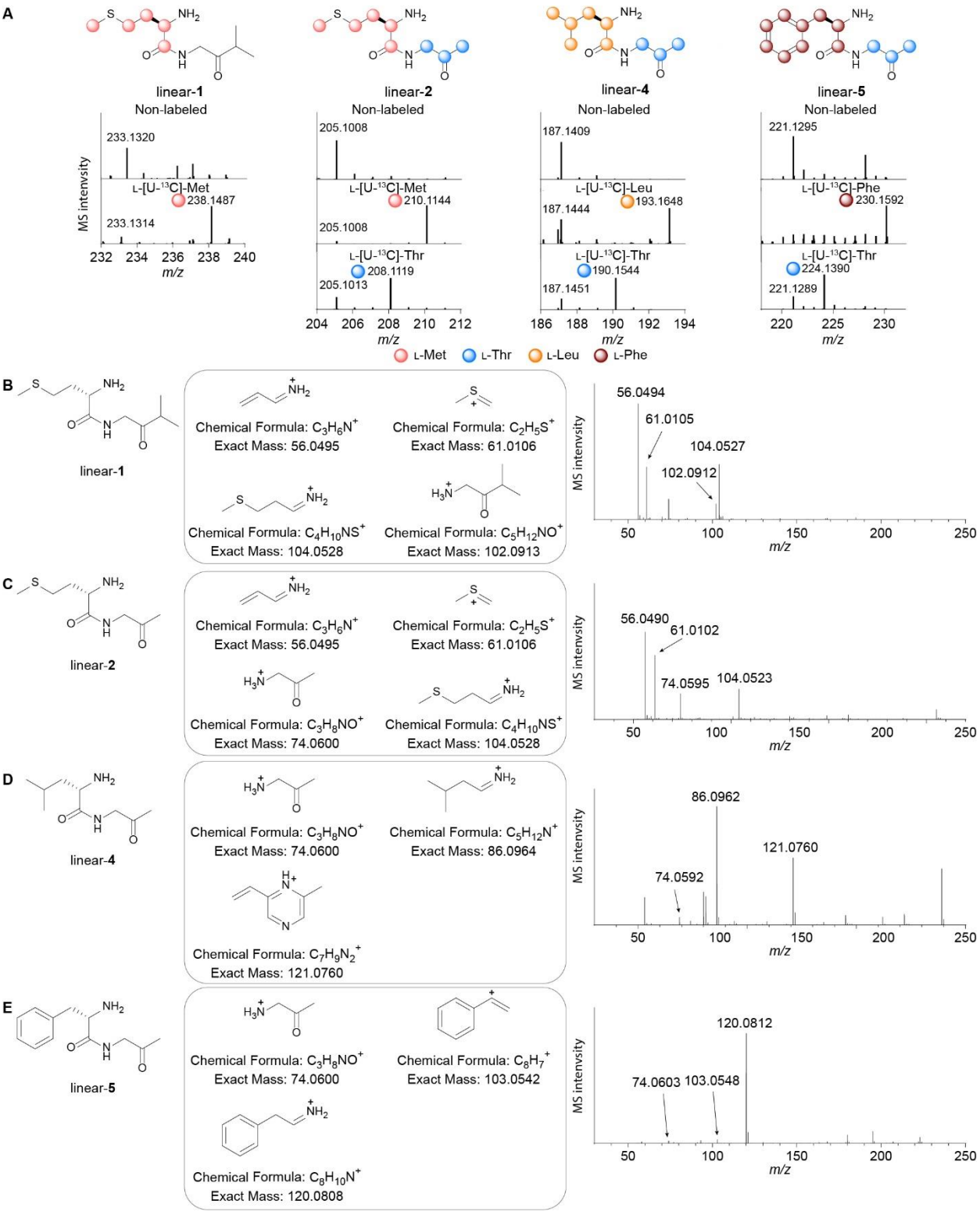


Figure S6. Structural confirmation of linear-1, 2, 4, and 5. (A) Proposed ^{13}C-isotopic labeling patterns of linear-**1**, **2**, **4**, and **5** and their HRMS spectra from organic extracts of *E. coli* Nissle 1917 individually cultivated with universal ^{13}C-isotopic amino acids. (B)–(E), Tandem MS spectra of linear-**1**, **2**, **4**, and **5** and their fragment ions confirmed their structures.

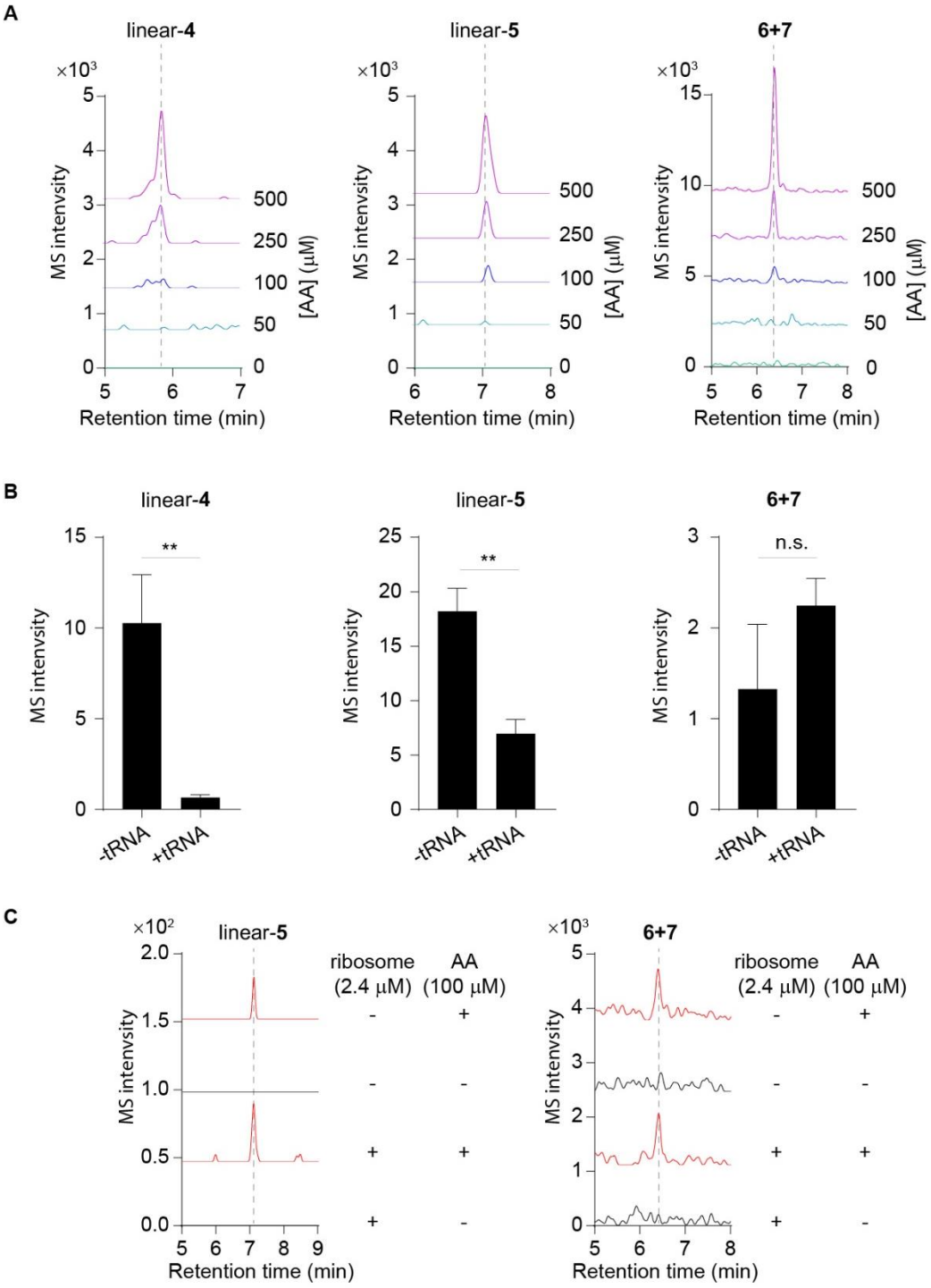


Figure S7. *In vitro* synthesis of linear-4 and 5 and 6 + 7 using PURExpress® technologies. (A) AA dose-dependent production of linear-**4** and **5** and **6** + **7** was observed. (B) Presence of tRNA decreased the production of linear-**4** and **5** but not **6** + **7**. *n* = 3 biological replicates. Data are mean ± s.d. ***P* < 0.01. n.s., not significant. Two-tailed t-test. (C) No significant change in the production of linear-**5**, **6**, and **7** was observed between the samples with and without ribosomes. The EICs were generated within a 10 ppm window.

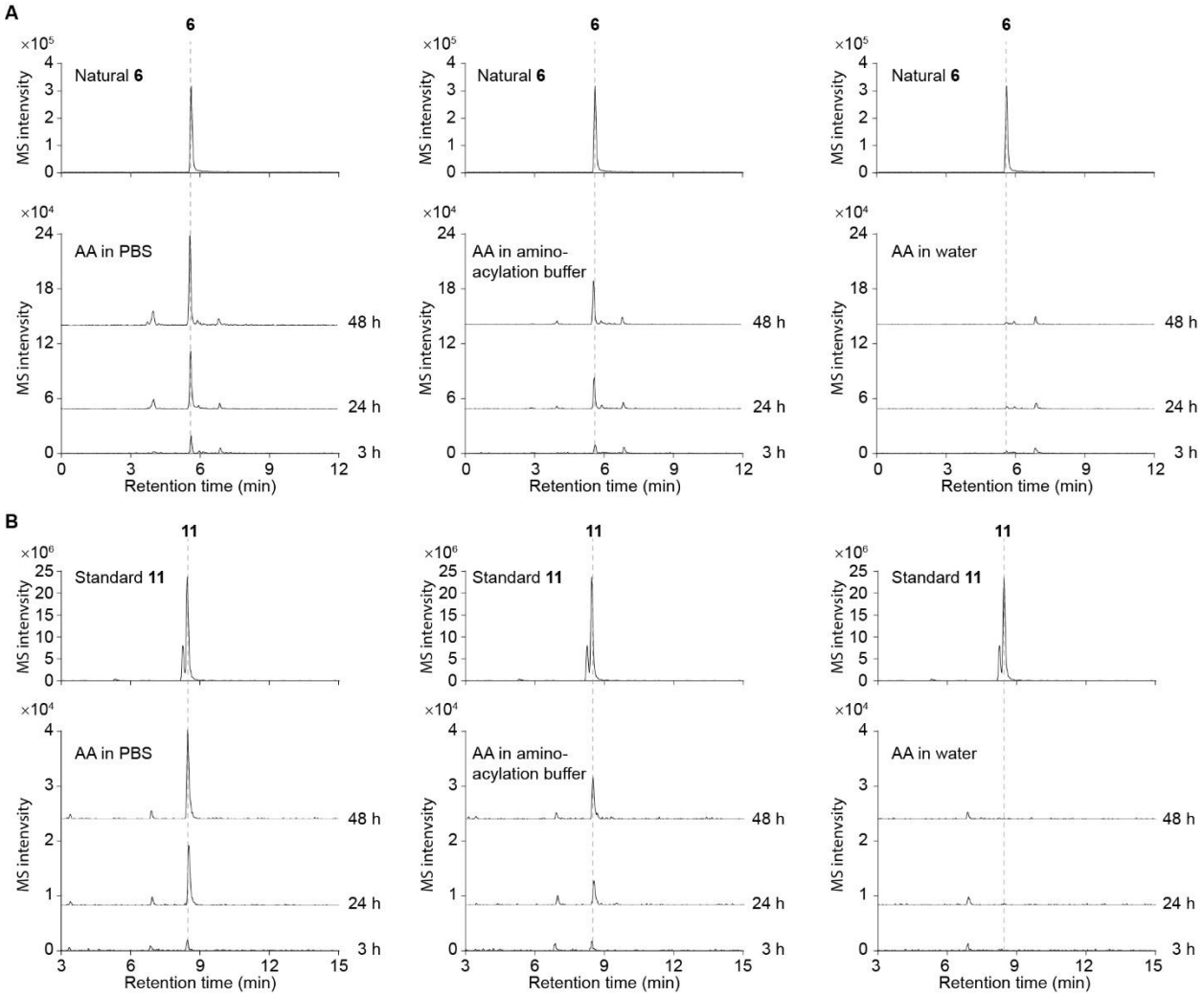


Figure S8. Non-enzymatic synthesis of pyrazinone 6 and pyrazine 11. (A) EICs of pyrazinone **6**, which was spontaneosly synthesized from AA in PBS (left), aminoacylation buffer (middle, see Material and Methods above for the recipe of aminoacylation buffer), and water (right). The production of **6** increased over time until 48 h in PBS and aminoacylation buffer. (B) Similar production patterns were observed for **11**.

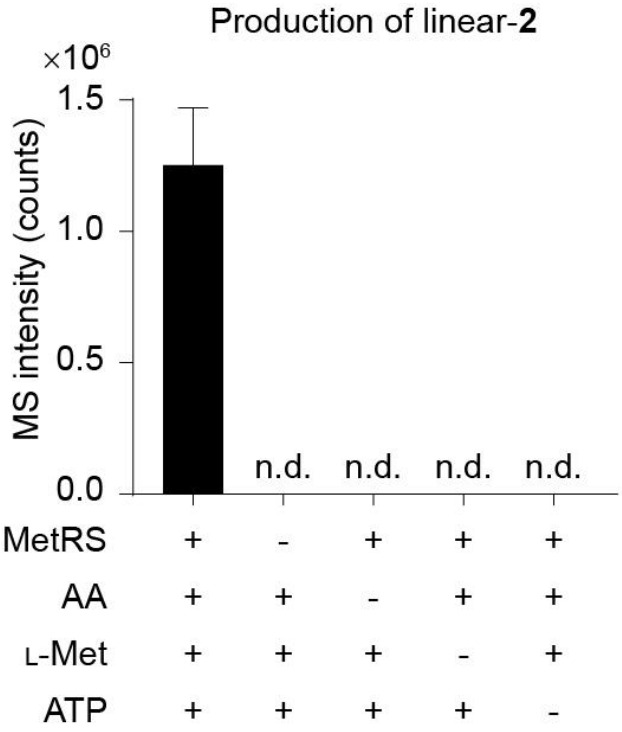


Figure S9. Results for *in vitro* synthesis of linear-2 with isolated MetRS. MetRS, AA, L-Met, and ATP are required for the *in vitro* synthesis of linear-**2**. *n* = 3 biological replicates. Data are mean ± s.d. n.s., not significant. n.d., not detected.

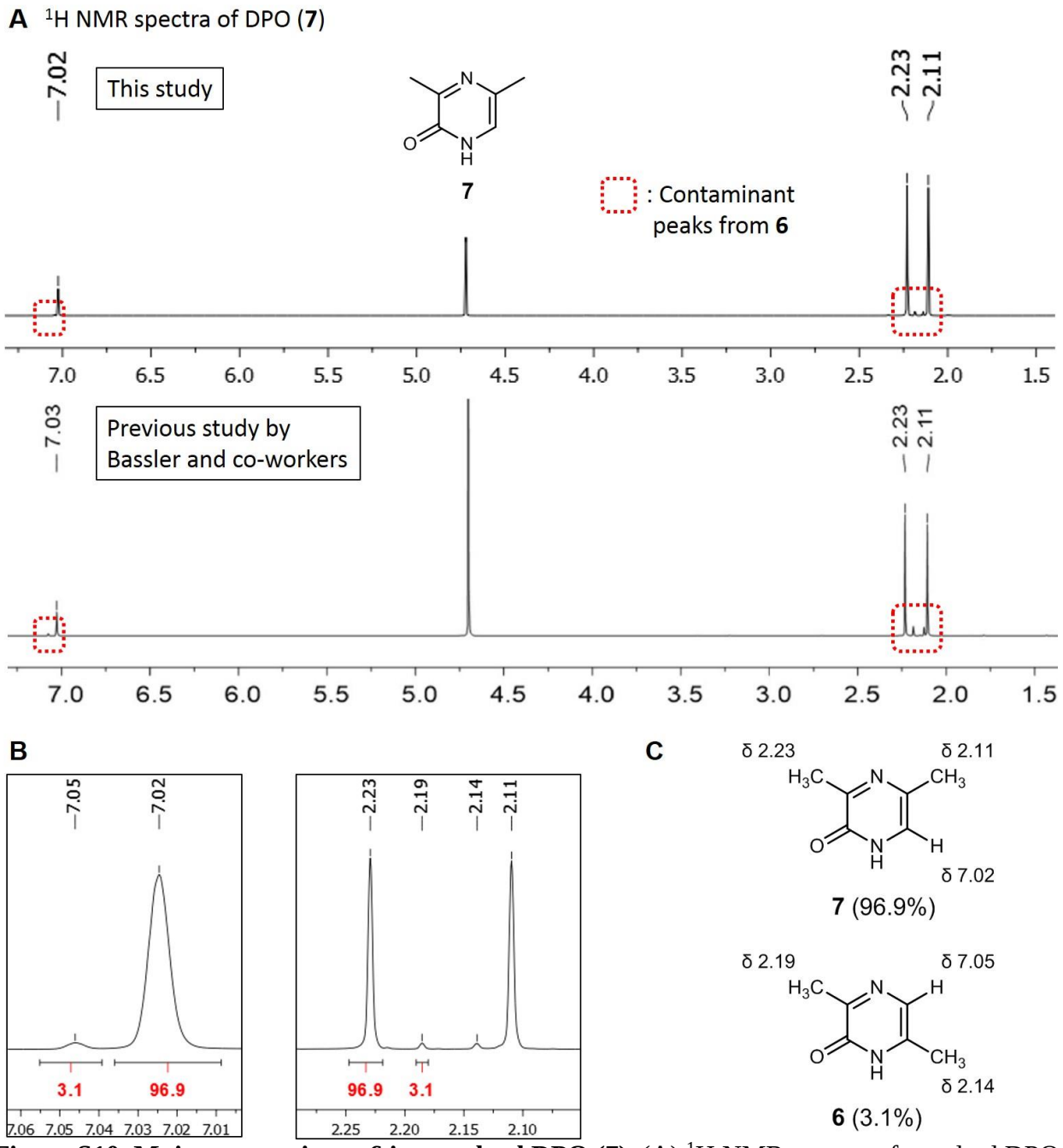


Figure S10. **Major contaminant 6 in standard DPO (7).** (A) ^{1}H NMR spectra of standard DPO (**7**) from this study (top) and from the previous study by Bassler and co-workers[6] (bottom) measured in deuterium oxide. The contaminant peaks from **6** can be found in the red dotted rectangles. (B) Zoomed-in regions of ^{1}H NMR spectrum of **7** from this study indicating that **6** is a major contaminant (**7** : **6** = 96.9 : 3.1). **C**. Observed ^{1}H NMR chemical shifts of two methyl groups and one olefinic proton in **7** and **6**.

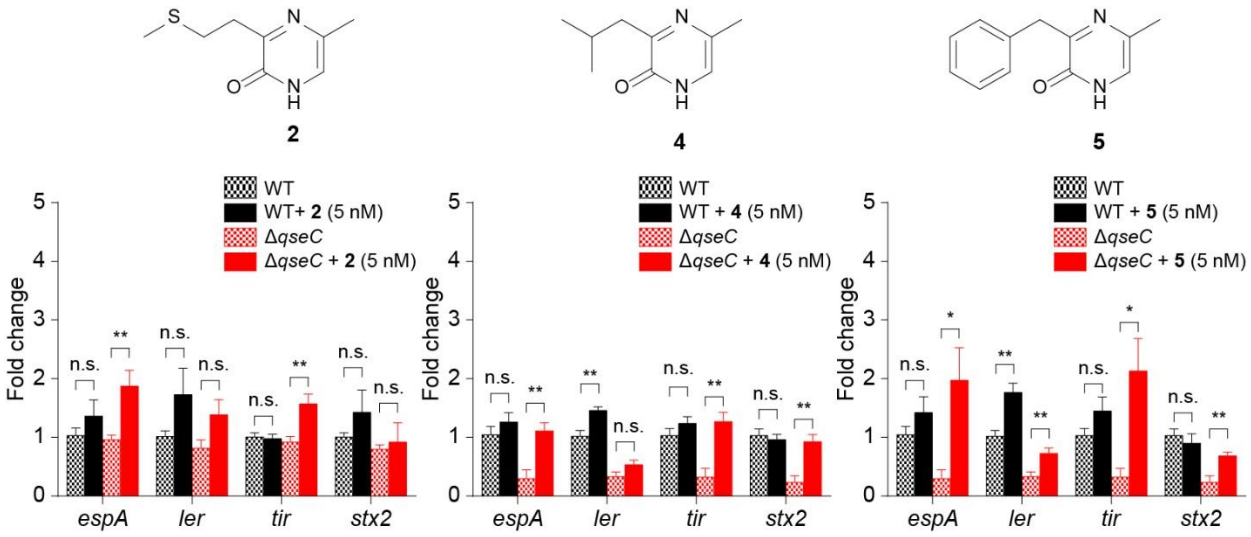


Figure S11. **qRT-PCR analysis for the expression of virulence genes from WT EHEC and Δ*qseC* EHEC with pyrazinones 2, 4, and 5 (5 nM).** *n* = 6 (three biological replicates and two technical replicates). Fold change were calculated relative to *rpoA* as an internal control. Data are mean ± s.d. **P* < 0.05, ***P* < 0.01; n.s., not significant; two-tailed *t*-test.

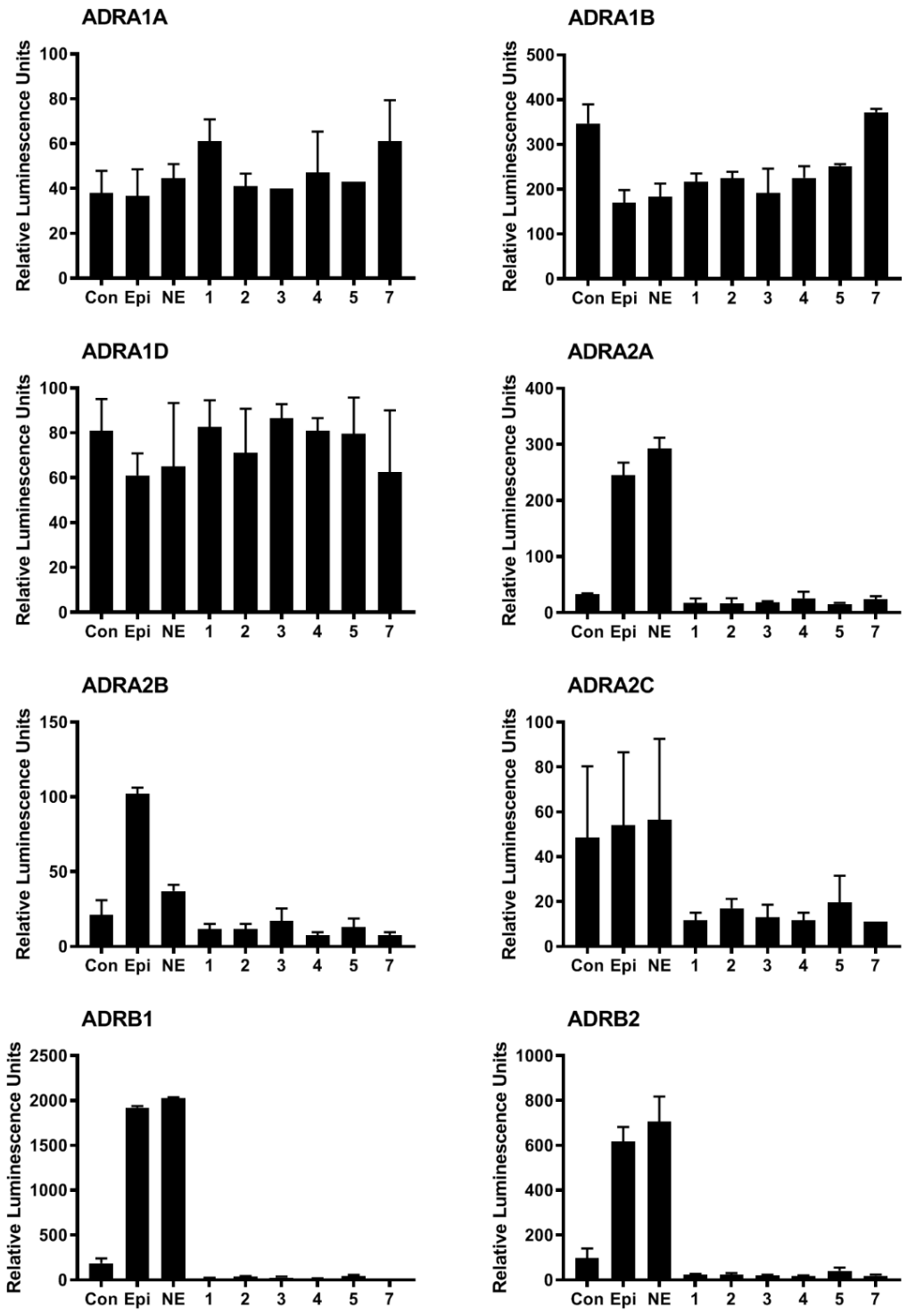


Figure S12. Evaluation of human adrenergic signaling activities of pyrazinones 1-5 and 7 (10 μM) via PRESTO-Tango assays. *n* = 2 biological replicates. Epi, epinephrine; NE, norepinephrine.

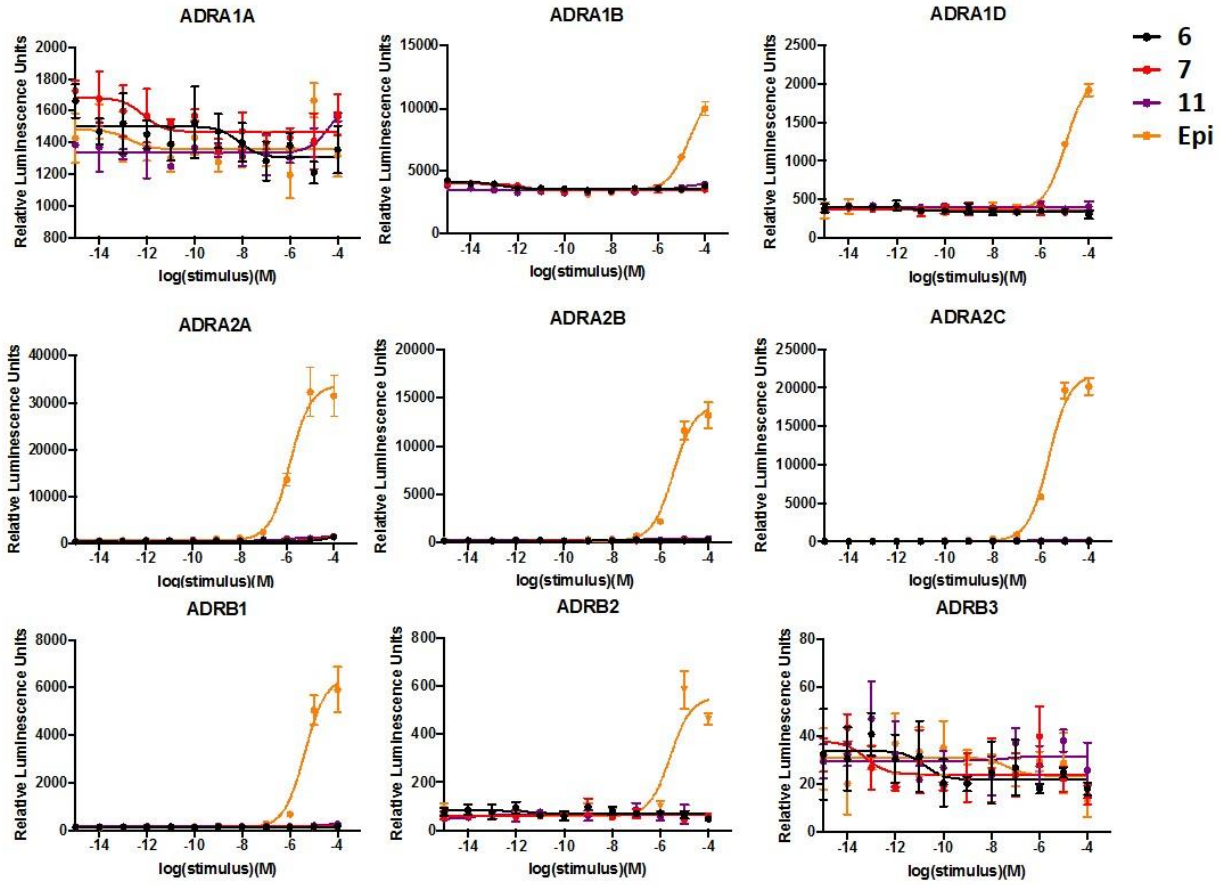


Figure S13. Evaluation of human adrenergic signaling activities of 6, 7, and 11 via PRESTO-Tango assays. *n* = 3 biological replicates. Epi, epinephrine.

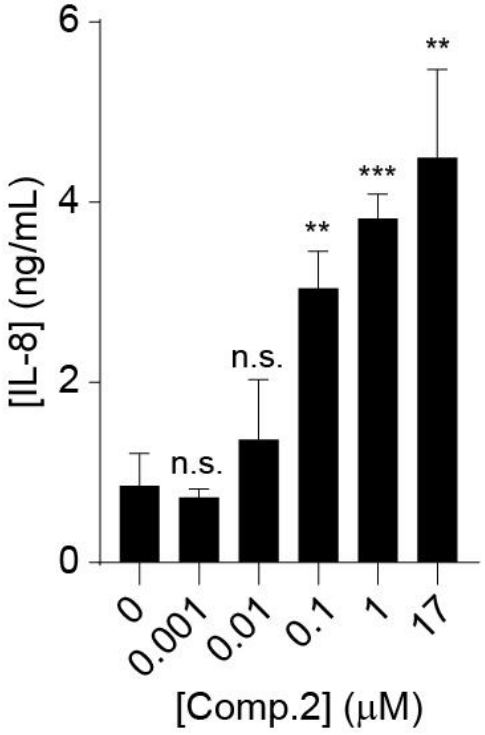
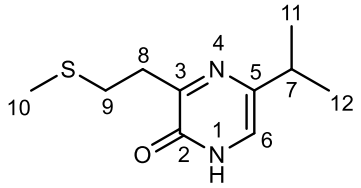


Figure S14. **Dose-dependent increase of IL-8 secretion by 2 in macrophages differentiated from THP-1 cells.** n = 3 biological replicates. Data are mean ± s.d. $^{**}P < 0.01$, $^{***}P < 0.001$; two-tailed t-test; n.s., not significant.

Table S1. ^{1}H [ppm, mult., (*J* in Hz)] and ^{13}C NMR data of natural and synthetic **1** measured in methanol-d_4.

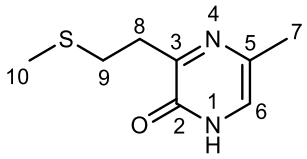
pos.	**1** (nat)		**1** (syn)	
	δ_H	δ_C	δ_H	δ_C
2		157.1		157.2
3		158.7		158.7
5		142.5		142.6
6	7.00, s	n.d.	7.00, s	120.8
7	2.82, m	33.2	2.82, hept (6.9)	33.3
8	3.05, t (7.4)	33.8	3.05, t (7.3)	33.8
9	2.88, overlap	31.6	2.88, t (7.3)	31.7
10	2.12, s	15.2	2.12, s	15.3
11	1.22, d (6.8)	22.2	1.22, d (6.9)	22.3
12	1.22, d (6.8)	22.2	1.22, d (6.9)	22.3



1

Table S2. ^{1}H [ppm, mult., (*J* in Hz)] and ^{13}C NMR data of natural and synthetic **2** measured in methanol-d_4.

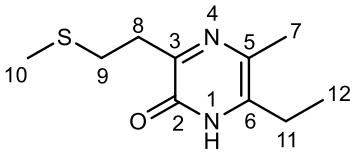
pos.	**2** (nat)		**2** (syn)	
	δ_H	δ_C	δ_H	δ_C
2		156.7		157.0
3		158.5		158.7
5		133.1		133.4
6	7.07, s	123.0	7.07, s	123.3
7	2.25, s	19.1	2.25, s	19.3
8	3.03, t (7.5)	33.7	3.03, t (7.5)	33.9
9	2.86, t (7.5)	31.6	2.85, t (7.5)	31.9
10	2.12, s	15.0	2.12, s	15.3



2

Table S3. ^{1}H [ppm, mult., (*J* in Hz)] and ^{13}C NMR data of natural and synthetic **3** measured in methanol-d_4.

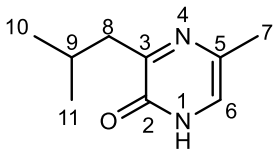
pos.	**3** (nat)		**3** (syn)	
	δ_H	δ_C	δ_H	δ_C
2		157.9		157.9
3		155.0		155.1
5		130.0		129.9
6		138.9		138.8
7	2.28, s	17.9	2.27, s	18.0
8	2.99, dd (8.5, 6.6)	33.4	2.99, dd (8.5, 6.5)	33.5
9	2.84, dd (8.5, 6.6)	32.0	2.83, dd (8.5, 6.5)	32.1
10	2.12, s	15.2	2.12, s	15.3
11	2.55, q (7.5)	24.3	2.55, q (7.6)	24.3
12	1.20, t (7.5)	13.2	1.20, t (7.6)	13.3



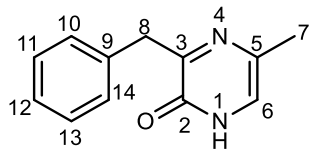
3

Table S4. ^{1}H [ppm, mult., (*J* in Hz)] and ^{13}C NMR data of synthetic **4** and **5** measured in methanol-d_4.

pos.	**4** (syn)		**5** (syn)	
	δ_H	δ_C	δ_H	δ_C
2		157.3		157.1
3		159.7		159.6
5		132.9		133.7
6	7.14, s	124.2	7.07, s	123.5
7	2.27, s	19.0	2.25, s	19.3
8	2.63, d (7.2)	42.5	4.05, s	40.1
9	2.18, m	28.3		138.9
10	0.95, d (6.7)	22.9	7.31, d (7.5)	130.1
11	0.95, d (6.7)	22.9	7.24, t (7.5)	129.4
12			7.17, t (7.5)	127.5
13			7.24, t (7.5)	130.1
14			7.31, d (7.5)	130.1



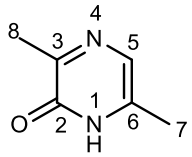
4



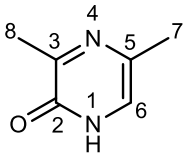
5

Table S5. ^{1}H (ppm, mult.) and ^{13}C NMR data of natural **6** and synthetic **7** measured in methanol-d_4.

pos.	**6** (nat)		**7** (syn)	
	δ_H	δ_C	δ_H	δ_C
2		158.4		157.3
3		154.7		157.8
5	7.08, s	122.2		133.0
6		137.0	7.06, s	122.9
7	2.21, s	15.4	2.23, s	19.2
8	2.32, s	19.2	2.37, s	20.2



6

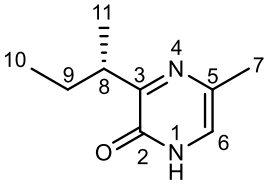


7

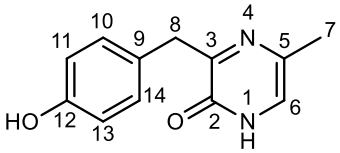
Table S6. ^{1}H [ppm, mult., (J in Hz)] and ^{13}C NMR data of synthetic **8**–**10**.

pos.	**8** (syn)[a]		**9** (syn)[b]		**10** (syn)[b]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2		155.4		156.9		156.4
3		162.4		159.5		164.6
5		137.1		133.2		133.5
6	7.03, s	120.0	7.07, s	123.7	6.99, s	122.1
7	2.37, s	20.2	2.25, s	19.2	2.24, s	19.5
8	3.27, h (6.9)	37.2	3.95, s	39.2	3.39, hept (6.9)	31.3
9a	1.83, m	27.6		129.5	1.20, d (6.9)	20.4
9b	1.57, m					
10	0.89, t (7.4)	12.1	7.13, d (8.5)	131.1	1.20, d (6.9)	20.4
11	1.23, d (6.8)	17.9	6.68, d (8.5)	116.1		
12				157.1		
13			6.68, d (8.5)	116.1		
14			7.13, d (8.5)	131.1		

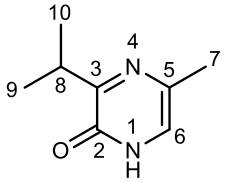
[a]Measured in chloroform-*d*
[b]Measured in methanol-d_4



8



9



10

Table S7. qPCR oligonucleotides used in this study.

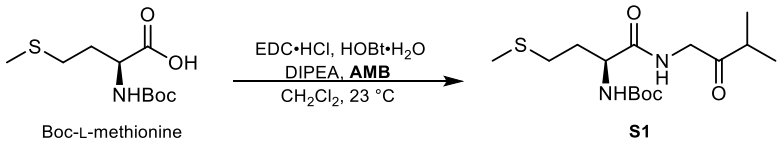
Primer	**Sequence**	**Description**
rpoA F	GTGACCCTTGAGCCTTTAGAG	EHEC (Endogenous ctrl)
rpoA R	ACACCATCAATCTCAACCTCG	EHEC
ler F	CGAGAGCAGGAAGTTCAAAGTG	EHEC (*LEE1*)
ler R	ACACCTTTCGAATGAGTTCCG	EHEC
tir F	GAGGGAGTCAAATAGCGGTG	EHEC (*LEE5)*
tir R	ATCTGAACGAAGGCTGGAAG	EHEC
espA F	AGCTATTTGAGGAACTCGGTG	EHEC (*LEE4*)
espA R	CATCTTTTGTGCCGTGGTTG	EHEC
stx2a F	TGTCGTGAAACTGCTCCTGTG	EHEC
stx2a R	GTATTCTCCCCACTCTGACAC	EHEC

Synthetic procedures

General Experimental Procedures. All reactions were performed in single-neck, flame-dried, round-bottomed flasks fitted with rubber septa under a positive pressure of nitrogen unless otherwise noted. Air- and moisture-sensitive liquids were transferred via syringe or stainless steel cannula). Organic solutions were concentrated by rotary evaporation at 23–32 °C. Flash-column chromatography was performed as using an Isolera™ Biotage system.

*Synthesis of pyrazinone **1***

*Step 1: Synthesis of dipeptide **S1***

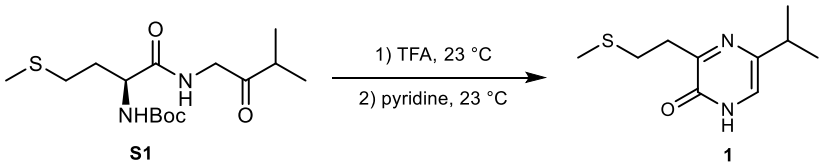


Boc-L-methionine **S1**

To a stirring solution of Boc-L-methionine (150 mg, 0.60 mmol), EDC hydrochloride (138 mg, 0.72 mmol, 1.2 equiv) and 1-hydroxybenzotriazole monohydrate (110 mg, 0.72 mmol, 1.2 equiv) in dimethylformamide (8 mL) at 0 °C was added *N*,*N*-diisopropylethylamine (Hünig's base, 366 μL, 2.11 mmol, 3.5 equiv). 1-Amino-3-methylbutan-2-one hydrochloride (**AMB**) (99 mg, 0.72 mmol, 1.2 equiv) dissolved in dimethylformamide (1.2 mL) was then added to the reaction. The flask was removed from the ice bath and allowed to warm to room temperature. After 2 h, the reaction mixture was cooled to 0 °C and treated with an aqueous hydrochloride solution (50 mL, 0.3 M) and extracted with ethyl acetate (4 × 50 mL). The organic layers were combined and washed with saturated aqueous sodium bicarbonate, followed by saturated sodium chloride, dried over sodium sulfate, filtered and then concentrated. The residue obtained was purified by flask-column chromatography (eluting with dichloromethane initially, grading to 5% methanol) to provide dipeptide **S1** as a white solid (80 mg, 41%).

^{1}H NMR (600 MHz, chloroform-*d*) δ 7.17 (1H, brs), 5.61 (1H, d, *J* = 8.4 Hz), 4.27 (1H, q, *J* = 7.5 Hz), 4.12 (1H, dd, *J* = 19.6, 5.0 Hz), 4.05 (1H, dd, *J* = 19.6, 4.7 Hz), 2.55 (1H, hept, *J* = 7.0 Hz), 2.44 (2H, t, *J* = 7.6 Hz), 1.98 (1H, overlapped), 1.96 (3H, s), 1.80 (1H, dq, *J* = 14.7, 7.6 Hz), 1.29 (9H, s), 1.00 (6H, d, *J* = 7.2 Hz) ^{13}C NMR (150 MHz, chloroform-*d*) δ 208.69, 171.93, 155.47, 79.63, 53.31, 47.10, 38.50, 32.01, 29.96, 28.15, 17.96, 17.95, 15.09.

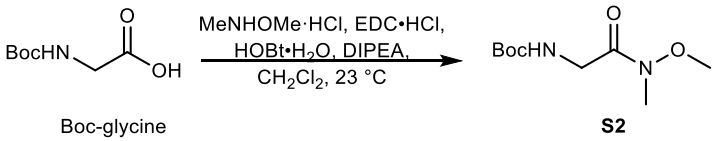
*Step 2: Synthesis of pyrazinone **1***



Dipeptide **S1** (30 mg, 0.09 mmol) was treated with trifluoroacetic acid (1 mL) at room temperature for 1 h. The reaction mixture was concentrated and treated with pyridine (2 mL) and stirred vigorously for 40 min, open to atmosphere. The reaction mixture was then concentrated resulting in a red residue. The product mixture was directly injected onto a semipreparative reverse phase HPLC system equipped with a Phenomenex Luna C18 (2) 100 Å column (250 × 10 mm, flow rate 4.0 mL/min, a gradient elution from 10 to 100% acetonitrile in water with 0.01% trifluoroacetic acid over 30 min) to yield pyrazinone **1** as a red solid (t_R = 16.6 min, 4.5 mg, 24%). See Table S1 for ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data.

*Synthesis of pyrazinone **2***

*Step 1: Synthesis of tert-butyl (2-(methoxy(methyl)amino)-2-oxoethyl)carbamate (or Weinreb amide **S2**)*

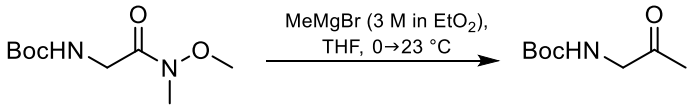


Boc-glycine **S2**

Boc-glycine (10 g, 57.08 mmol), *N*,*O*-dimethylhydroxylamine hydrochloride (5.57 g, 57.08 mmol, 1 eq), EDC hydrochloride (13.13 g, 68.50 mmol, 1.2 equiv), and 1-hydroxybenzotriazole monohydrate (25.82 mg, 68.50 mmol, 1.2 equiv) were dissolved in dichloromethane (200 mL), then cooled to 0 °C. Following the addition of *N*,*N*-diisopropylethylamine (Hünig's base, 34.8 mL, 199.78 mmol, 3.5 equiv), the reaction flask was removed from the ice bath and stirred at 23 °C. After 2 h, the reaction mixture was cooled to 0 °C and treated with an aqueous hydrochloride solution (50 mL, 0.3 M) and extracted with dichloromethane (3 × 50 mL). The organic layers were combined and washed with saturated aqueous sodium bicarbonate (100 mL), followed by saturated sodium chloride, dried over sodium sulfate, filtered and then concentrated. The crude residue was purified by flash column chromatography (eluting with hexane initially, grading to 100% ethyl acetate) to yield Weinreb amide **S2** as a clear oil (496 mg, 82%).

^{1}H NMR (400 MHz, chloroform-*d*) δ 5.25 (1H, brs), 4.08 (2H, d, *J* = 4.0 Hz), 3.71 (3H, s), 3.20 (3H, s), 1.46 (9H, s). ^{13}C NMR (100 MHz, chloroform-*d*) δ 170.40, 156.05, 79.76, 61.58, 41.86, 32.55, 28.48.

*Step 2: Synthesis of tert-butyl (2-oxopropyl)carbamate (or Boc-aminoacetone, **S3**)*

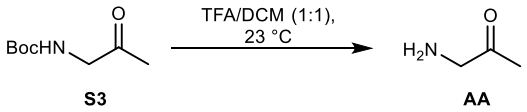


S2 **S3**

A 3.0 M solution of methylmagnesium bromide in diethyl ether (11.46 mL, 34.38 mmol, 3 equiv) was added dropwise at 0 °C to Weinreb amide **S2** (2.5 g, 11.46 mmol) in 46 mL of tetrahydrofuran, maintaining the internal temperature below 5 °C. The reaction mixture was stirred for 2–4 h with monitoring by ninhydrin stained TLC. Once completed the reaction was quenched by slow addition of saturated ammonium chloride, extracted with diethyl ether (3 × 50 mL), dried over sodium sulfate, filtered, and concentrated to give **S3** (1.88 g, 94%). No further purification was used prior to deprotection.

^{1}H NMR (400 MHz, chloroform-*d*) δ 5.19 (1H, brs), 4.02 (2H, brd, *J* = 4.4 Hz), 2.18 (3H, s), 1.45 (9H, s). ^{13}C NMR (100 MHz, chloroform-*d*) δ 203.45, 155.72, 79.98, 51.07, 28.45, 27.19.

*Step 3: Synthesis of 1-aminopropan-2-one (or aminoacetone, **AA**)*

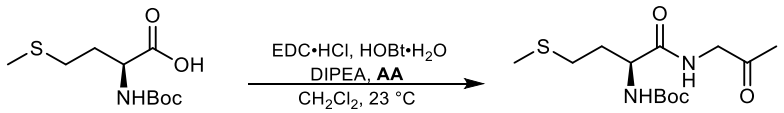


S3 **AA**

To a 0 °C solution of Boc-aminoacetone **S3** (1.88 mg, 10.85 mmol) in dichloromethane (30 mL) was added trifluoroacetic acid (30 mL). The mixture was stirred at 23 °C for 20 min, then concentrated to yield **AA** trifluoracetate salt (2.33 g, 98%).

^{1}H NMR (400 MHz, methanol-d_4) δ 3.96 (2H, s), 2.24 (3H, s). ^{13}C NMR (100 MHz, methanol-d_4) δ 201.79, 48.57, 26.93.

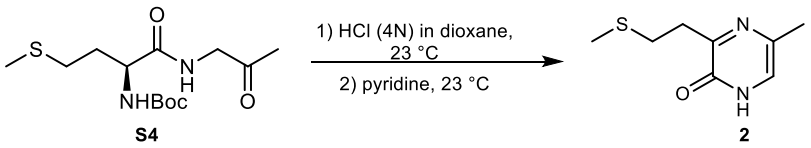
*Step 4: Synthesis of dipeptide **S4***



To a stirring solution of Boc-L-methionine (500 mg, 2.01 mmol), EDC hydrochloride (462 mg, 2.41 mmol, 1.2 equiv) and 1-hydroxybenzotriazole monohydrate (368 mg, 2.41 mmol, 1.2 equiv) in dimethylformamide (8 mL) at 0 °C was added *N*,*N*-diisopropylethylamine (Hünig's base, 1.22 mL, 7.01 mmol, 3.5 equiv). Aminoacetone (**AA**) hydrochloride (264 mg, 2.41 mmol, 1.2 equiv) dissolved in dimethylformamide (1.2 mL) was then added to the reaction. The flask was removed from the ice bath and allowed to warm to room temperature. After 2 h, the reaction mixture was cooled to 0 °C and treated with an aqueous hydrochloride solution (50 mL, 0.3 M) and extracted with ethyl acetate (4 × 50 mL). The organic layers were combined and washed with saturated aqueous sodium bicarbonate, followed by saturated sodium chloride, dried over sodium sulfate, filtered and then concentrated. The residue obtained was purified by flask-column chromatography (eluting with dichloromethane initially, grading to 5% methanol) to provide dipeptide **S4** as a white solid (282 mg, 46%).

^{1}H NMR (400 MHz, chloroform-*d*) δ 6.89 (1H, brs), 5.25 (1H, brd, *J* = 7.6 Hz), 4.31 (1H, m), 4.14 (2H, m), 2.57 (2H, t, *J* = 7.2 Hz), 2.19 (3H, s), 2.15–2.06 (1H, m), 1.97–1.88 (1H, m), 1.44 (9H, s). ^{13}C NMR (100 MHz, chloroform-*d*) δ 202.48, 171.90, 155.68, 80.48, 53.65, 49.85, 31.84, 30.65, 28.44, 27.39, 15.45.

*Step 5: Synthesis of pyrazinone **2***

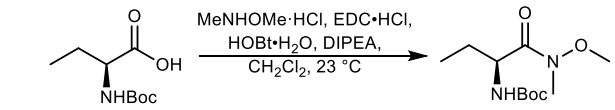


S4 **2**

Dipeptide **S4** (23.8 mg, 0.08 mmol) was treated with hydrochloric acid in dioxane (4 N, 1 mL) at room temperature for 2 h. The reaction mixture was concentrated and treated with pyridine (2 mL) and stirred vigorously for 40 min, open to atmosphere. The reaction was then diluted with water (20 mL) and concentrated via lyophilization resulting in a red residue. The product mixture was directly injected onto a semipreparative reverse phase HPLC system equipped with a Phenomenex Luna C8 (2) 100 Å column (250 × 10 mm, flow rate 2.0 mL/min, a gradient elution from 10 to 40% acetonitrile in water with 0.01% trifluoroacetic acid over 21 min, then 40-100% acetonitrile for 5 min) using a 1 min fraction collection time window. Fraction 21 was concentrated under nitrogen to remove some of the acetonitrile and then fully concentrated via lyophilization to yield pyrazinone **2** as an orange solid (t_R = 20 min, 4.9 mg, 34%). See Table S2 for ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data.

*Synthesis of pyrazinone **3***

*Step 1: Synthesis of tert-butyl (1-(methoxy(methyl)amino)-1-oxobutan-2-yl)carbamate (or Weinreb amide **S5**)*

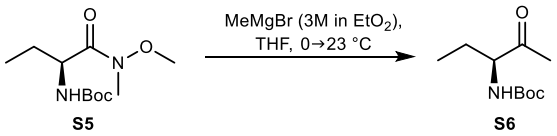


(*S*)-2-(Boc-amino)butyric acid **S5**

(*S*)-2-(Boc-amino)butyric acid (500 mg, 2.46 mmol), *N*,*O*-dimethylhydroxylamine hydrochloride (288 mg, 2.95 mmol, 1.2 eq), EDC hydrochloride (566 mg, 2.95 mmol, 1.2 equiv), and 1-hydroxybenzotriazole monohydrate (452 mg, 2.95 mmol, 1.2 equiv) were dissolved in dichloromethane (8 mL) and then the reaction flask was cooled to 0 °C. *N*,*N*-diisopropylethylamine (Hünig's base, 1.5 mL, 8.61 mmol, 3.5 equiv) was added and then the reaction flask was removed from the ice bath and left to warm to room temperature. After 2 h, the reaction mixture was cooled to 0 °C and treated with an aqueous hydrochloride solution (300 mL, 0.3 M) and extracted with dichloromethane (3 × 200 mL). The organic layers were combined and washed with saturated aqueous sodium bicarbonate (500 mL), followed by saturated sodium chloride, dried over sodium sulfate, filtered and then concentrated to yield Weinreb amide **S5**. The solids where used in the next step without further purification (10.738 g, 86%).

^{1}H NMR (400 MHz, chloroform-*d*) δ 5.17 (1H, d, *J* = 7.2 Hz), 4.61 (1H, brs), 3.76 (3H, s), 3.19 (3H, s), 1.80-1.70 (1H, m), 1.61–1.52 (1H, m), 1.42 (9H, s), 0.93 (3H, t, *J* = 7.5 Hz). ^{13}C NMR (100 MHz, chloroform-*d*) δ 173.32, 155.72, 79.58, 61.70, 51.66, 28.49, 26.23, 9.90.

*Step 2: Synthesis of tert-butyl (2-oxopentan-3-yl)carbamate (**S6**)*

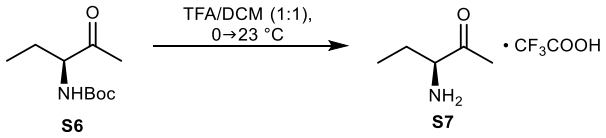


S5 **S6**

A 3.0 M solution of methylmagnesium bromide in diethyl ether (2.33 mL, 6.98 mmol, 4 equiv) was added dropwise to the Weinreb amide **S5** (430 mg, 1.75 mmol) in 5.8 mL of tetrahydrofuran at 0 °C. The reaction mixture removed from the ice bath and stirred for 2–5 h with monitoring by TLC. Once completed the reaction was quenched by slow addition of saturated ammonium chloride, extracted with diethyl ether (3 × 40 mL), dried over sodium sulfate, filtered, and concentrated. The residue was purified by flash-column chromatography (eluting with 0% ethyl acetate–hexanes initially, grading to 30% ethyl acetate–hexanes) to provide **S6** as a white or yellow solid (252 mg, 76%).

^{1}H NMR (400 MHz, chloroform-*d*) δ 5.21 (1H, brs), 4.29 (1H, brs), 2.18 (3H, s), 2.00–1.87 (1H, m), 1.66–1.55 (1H, m), 1.44 (9H, s), 0.89 (3H, t, *J* = 7.4 Hz). ^{13}C NMR (100 MHz, chloroform-*d*) δ 207.20, 155.62, 79.81, 60.98, 28.47, 27.18, 24.69, 9.27.

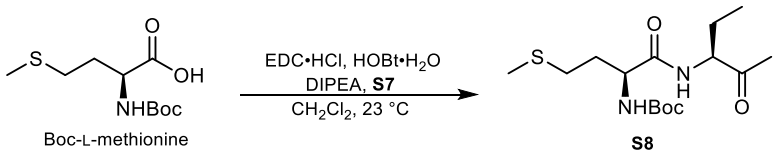
*Step 3: Synthesis of 3-aminopentan-2-one (**S7**)*



To a 0 °C solution of **S6** (97 mg, 0.48 mmol) in dichloromethane (1.5 mL) was added trifluoroacetic acid (1.5 mL). The mixture was stirred at 23 °C for 20 min, then concentrated, diluted in water, and concentrated again by lyophilization to yield **S7** trifluoracetate salt (102 mg, 98%).

^{1}H NMR (400 MHz, methanol-d_4) δ 4.14 (1H, dd, J = 7.1, 4.4 Hz), 2.27 (3H, s), 2.08 (1H, dqd, J = 15.2, 7.6, 4.4 Hz), 1.91 (1H, dp, J = 14.8, 7.4 Hz), 1.02 (3H, t, J = 7.5 Hz). ^{13}C NMR (100 MHz, methanol-d_4) δ 204.75, 61.43, 26.46, 23.69, 9.18.

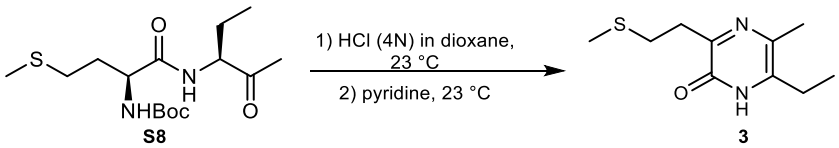
*Step 4: Synthesis of dipeptide **S8***



To a stirring solution of Boc-L-methionine (83 mg, 0.33 mmol), EDC hydrochloride (77 mg, 0.40 mmol, 1.2 equiv) and 1-hydroxybenzotriazole monohydrate (61 mg, 0.40 mmol, 1.2 equiv) in dimethylformamide (2 mL) at 0 °C was added *N*,*N*-diisopropylethylamine (Hünig's base, 204 μL, 1.17 mmol, 3.5 equiv). 3-Aminopentan-2-one (**S7**) trifluoroacetate (40 mg, 0.40 mmol, 1.2 equiv) dissolved in dimethylformamide (1 mL) was then added to the reaction. The flask was removed from the ice bath and allowed to warm to room temperature. After 2 h, the reaction mixture was cooled to 0 °C and treated with an aqueous potassium bisulfate solution (30 mL, 5%) and extracted with ethyl acetate (4 × 40 mL). The organic layers were combined and washed with saturated aqueous sodium bicarbonate, followed by saturated sodium chloride, dried over sodium sulfate, filtered and then concentrated. The residue obtained was purified by flask-column chromatography (eluting with dichloromethane initially, grading to 5% methanol) to provide dipeptide **S8** as a white solid or yellow oil (87 mg, 82%).

^{1}H NMR (400 MHz, chloroform-*d*) δ 6.95 (1H, m), 5.35 (1H, brs), 4.52 (1H, m), 4.25 (1H, m), 2.52 (t, *J* = 7.2 Hz), 2.15 (3H, s), 2.06 (3H, s), 1.90 (1H, m), 1.62 (1H, m), 1.39 (9H, s), 0.82 (3H, t, *J* = 7.5 Hz). ^{13}C NMR (100 MHz, chloroform-*d*) δ 206.20, 171.56, 155.57, 80.09, 59.77, 53.55, 31.71, 30.23, 28.33, 27.10, 24.09, 15.29, 9.19.

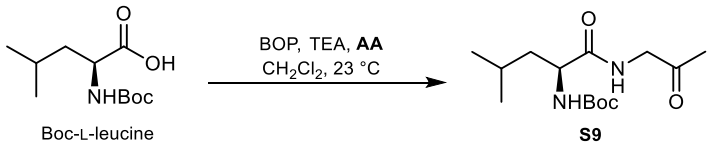
*Step 5: Synthesis of pyrazinone **3***



Dipeptide **S8** (30 mg, 0.09 mmol) was treated with hydrochloric acid in dioxane (4 N, 1 mL) at room temperature for 4 h. The reaction mixture was then diluted with water (20 mL) and concentrated via lyophilization resulting in an orange residue. The deprotected compound was then treated with pyridine (2 mL) and stirred vigorously for 40 min, open to atmosphere. The reaction mixture was then diluted with water (20 mL) and concentrated via lyophilization resulting in an orange residue. The product was directly injected onto a semipreparative reverse phase HPLC system equipped with a Phenomenex Luna C8 (2) 100 Å column (250 × 10 mm, flow rate 2.0 mL/min, a gradient elution from 10 to 33% acetonitrile in water with 0.01% trifluoroacetic acid over 17 min, then 33-100% acetonitrile for 1 min, and 100% acetonitrile isocratic for 5 min) using a 1 min fraction collection time window. Fraction 20 was concentrated under nitrogen to remove some of the acetonitrile and then fully concentrated via lyophilization to yield pyazinone **3** as an orange solid (t_R = 20 min, 5.7 mg, 30%). See Table S3 for ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data.

*Synthesis of pyrazinone **4***

*Step 1: Synthesis of dipeptide **S9***

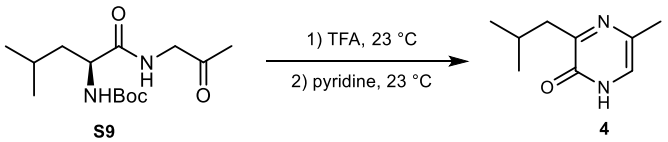


Boc-L-leucine **S9**

To a stirring solution of Boc-L-leucine (260 mg, 1.12 mmol) and BOP reagent (597 mg, 1.35 mmol, 1.2 equiv) in dimethylformamide (6 mL) at 0 °C was added triethylamine (470 µL, 3.37 mmol, 3 equiv). Aminoacetone (**AA**) hydrochloride (148 mg, 1.35 mmol, 1.2 equiv) dissolved in dimethylformamide (2 mL) was then added to the reaction. The flask was removed from the ice bath and allowed to warm to room temperature. After 2 h, the reaction mixture was cooled to 0 °C and treated with an aqueous potassium bisulfate solution (50 mL, 5%) and extracted with ethyl acetate (4 × 50 mL). The organic layers were combined and washed with saturated aqueous sodium bicarbonate, followed by saturated aqueous sodium chloride, dried over anhydrous sodium sulfate, filtered and then concentrated. The residue obtained was purified by flask-column chromatography (eluting with dichloromethane initially, grading to 5% methanol) to provide dipeptide **S9** as a white solid (261 mg, 81 %).

^{1}H NMR (600 MHz, chloroform-*d*) δ 6.83 (1H, brs), 4.93 (1H, d, *J* = 8.2 Hz), 4.16 (1H, m), 4.13 (1H, brd, *J* = 3.9 Hz), 2.19 (3H, s), 1.67 (2H, m), 1.47 (1H, m), 1.43 (9H, s), 0.93 (3H, d, *J* = 6.4 Hz), 0.92 (3H, d, *J* = 6.4 Hz). ^{13}C NMR (150 MHz, chloroform-*d*) δ 202.78, 172.85, 155.76, 80.27, 53.15, 49.83, 41.47, 28.42, 27.43, 24.88, 23.10, 21.97.

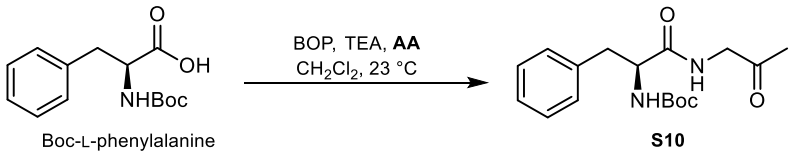
*Step 2: Synthesis of pyrazinone **4***



Dipeptide **S9** (83 mg, 0.29 mmol) was treated with trifluoroacetic acid (1 mL) at room temperature for 20 min. The reaction mixture was concentrated, treated with pyridine (2 mL) and stirred vigorously for 1 hr, open to atmosphere. The reaction mixture was concentrated and then directly injected onto a semipreparative reverse phase HPLC system equipped with a Phenomenex Luna C18 (2) 100 Å column (250 × 10 mm, flow rate 4.0 mL/min, a gradient elution from 10 to 100% acetonitrile in water with 0.01% trifluoroacetic acid over 30 min) to yield pyrazinone **4** as a red solid (t_R = 22 min, 14.8 mg, 31%). See Table S4 for ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data.

*Synthesis of pyrazinone **5***

*Step 1: Synthesis of dipeptide **S10***

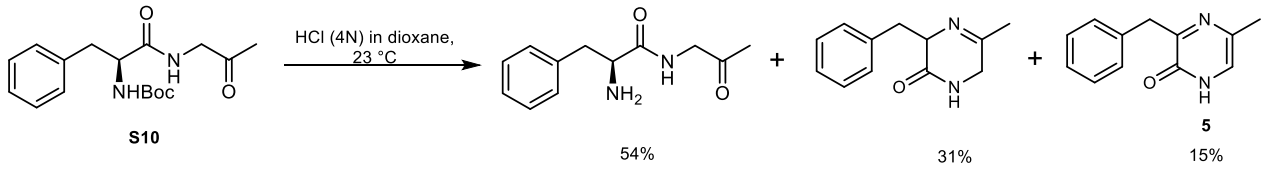


Boc-L-phenylalanine **S10**

To a stirring solution of Boc-L-phenylalanine (395 mg, 1.49 mmol) and BOP reagent (792 mg, 1.79 mmol, 1.2 equiv) in dimethylformamide (6 mL) at 0 °C was added triethylamine (623 µL, 4.47 mmol, 3 equiv). Aminoacetone (**AA**) hydrochloride (196 mg, 1.79 mmol, 1.2 equiv) dissolved in dimethylformamide (2 mL) was then added to the reaction. The flask was removed from the ice bath and allowed to warm to room temperature. After 2 h, the reaction mixture was cooled to 0 °C and treated with an aqueous potassium bisulfate solution (50 mL, 5%) and extracted with ethyl acetate (4 × 50 mL). The organic layers were combined and washed with saturated aqueous sodium bicarbonate, followed by saturated aqueous sodium chloride, dried over anhydrous sodium sulfate, filtered and then concentrated. The residue obtained was purified by flask-column chromatography (eluting with dichloromethane initially, grading to 5% methanol) to provide dipeptide **S10** as a white solid (413 mg, 86 %).

^{1}H NMR (600 MHz, methanol-d_4) δ 7.26 (4H, overlapped), 7.20 (1H, m), 4.36 (1H, dd, J = 9.5, 5.3), 4.01 (2H, s), 3.16 (1H, dd, J = 13.9, 5.3 Hz), 2.83 (1H, dd, J = 13.9, 9.5 Hz), 2.11 (3H, s), 1.35 (9H, s). ^{13}C NMR (150 MHz, methanol-d_4) δ 205.61, 147.70, 157.57, 138.68, 130.31, 129.36, 127.63, 80.61, 57.34, 50.26, 39.14, 28.62, 26.99.

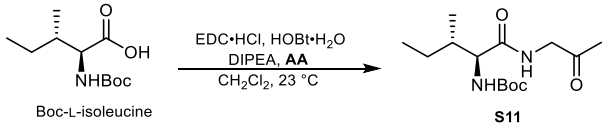
*Step 2: Synthesis of pyrazinone **5***



Dipeptide **S10** (224 mg, 0.70 equiv) was treated with hydrochloric acid in dioxane (4 N) at room temperature for 24 h, then heated at 50 °C and stirred for another 10 h. Conversion to the desired pyrazine was slow. The reaction mixture was concentrated and purified using preparative reverse phase HPLC equipped with a Polaris 5 C18-A column (250 × 21.2 mm, flow rate 8.0 mL/min, a gradient elution from 10-100% acetonitrile in water with 0.01% trifluoroacetic acid over 35 min) to obtain pyrazinone **5** (t_R = 22 min, 10.8 mg, 7.7%). See Table S4 for ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data.

*Synthesis of pyrazinone **8***

*Step 1: Synthesis of dipeptide **S11***



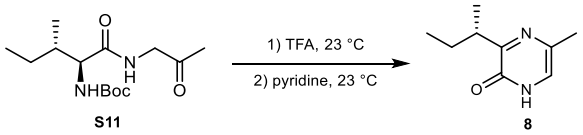
Boc-L-isoleucine

S11

To a stirring solution of Boc-L-isoleucine (500 mg, 2.16 mmol), EDC hydrochloride (539 mg, 2.81 mmol, 1.2 equiv) and 1-hydroxybenzotriazole monohydrate (430.7 mg, 2.81 mmol, 1.2 equiv) in dimethylformamide (6.2 mL) at 0 °C was added *N*,*N*-diisopropylethylamine (Hünig's base, 1.32 mL, 7.57 mmol, 3.5 equiv). Aminoacetone (**AA**) hydrochloride (356 mg, 3.25 mmol, 1.5 equiv) dissolved in dimethylformamide (1 mL) was then added to the reaction. The flask was removed from the ice bath and allowed to warm to room temperature. After 2 h, the reaction mixture was cooled to 0 °C and treated with an aqueous potassium bisulfate solution (50 mL, 5%) and extracted with ethyl acetate (4 × 50 mL). The organic layers were combined and washed with saturated aqueous sodium bicarbonate, followed by saturated sodium chloride, dried over sodium sulfate, filtered and then concentrated. The residue obtained was purified by flask-column chromatography (eluting with dichloromethane initially, grading to 5% methanol) to provide dipeptide **S11** as a white solid (315.4 mg, 51%).

^{1}H NMR (400 MHz, chloroform-*d*) δ 6.78 (1H, brt, *J* = 5.1 Hz), 5.09 (1H, d, *J* = 8.7 Hz), 4.12 (2H, d, *J* = 4.7 Hz), 4.02 (1H, m), 2.17 (3H, s), 1.87 (1H, m), 1.46 (1H, m), 1.41 (9H, s), 1.11 (1H, m), 0.91 (3H, d, *J* = 6.8 Hz), 0.88 (3H, t, *J* = 7.4 Hz). ^{13}C NMR (100 MHz, chloroform-*d*) δ 202.57, 171.73, 155.72, 79.85, 59.19, 49.60, 37.24, 28.47, 27.23, 24.62, 15.55, 11.43.

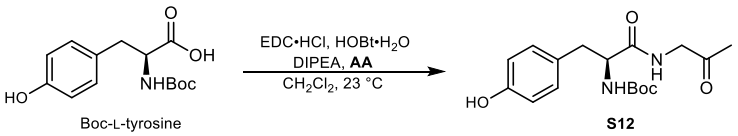
*Step 2: Synthesis of pyrazinone **8***



Dipeptide **S11** (53 mg, 0.19 mmol) was treated with trifluoroacetic acid (1 mL) at room temperature for 1 h. The reaction mixture was concentrated and treated with pyridine (2 mL) and stirred vigorously for 40 min, open to atmosphere. The reaction mixture was then concentrated resulting in a red residue. The product mixture was directly injected onto preparative reverse phase HPLC system equipped with an Agilent Polaris 5 C18-A column (250 × 21.2 mm, flow rate 8.0 mL/min, a gradient elution from 10 to 100% acetonitrile in water with 0.01% trifluoroacetic acid over 40 min) to yield red pyrazinone **8** (t_R = 30 min, 16.7 mg, 54%). See Table S6 for ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data.

*Synthesis of pyrazinone **9***

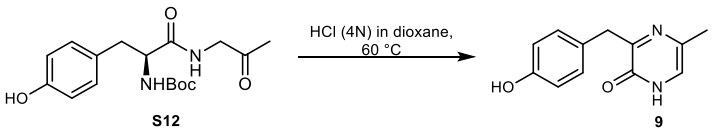
*Synthesis of dipeptide **S12***



To a stirring solution of Boc-L-tyrosine (200 mg, 0.711 mmol), EDC hydrochloride (164 mg, 0.853 mmol, 1.2 equiv) and 1-hydroxybenzotriazole monohydrate (131 mg, 0.853 mmol, 1.2 equiv) in dimethylformamide (3.5 mL) at 0 °C was added *N*,*N*-diisopropylethylamine (Hünig's base, mL, 7.57 mmol, 3.5 equiv). Aminoacetone (**AA**) hydrochloride (94 mg, 0.853 mmol, 1.2 equiv) dissolved in dimethylformamide (1 mL) was then added to the reaction. The flask was removed from the ice bath and allowed to warm to room temperature. After 2 h, the reaction mixture was cooled to 0 °C and treated with an aqueous potassium bisulfate solution (40 mL, 5%) and extracted with ethyl acetate (4 × 40 mL). The organic layers were combined and washed with saturated aqueous sodium bicarbonate, followed by saturated sodium chloride, dried over sodium sulfate, filtered and then concentrated. The residue obtained was purified by flask-column chromatography (eluting with dichloromethane initially, grading to 5% methanol) to provide dipeptide **S11** as a white solid or yellow oil (104 mg, 43%).

^{1}H NMR (400 MHz, chloroform-*d*) δ 7.05 (2H, d, *J* = 8.5 Hz), 6.75 (2H, d, *J* = 8.5 Hz), 6.61 (1H, brs), 5.03 (1H, brs), 4.35 (1H, m), 4.13 (1H, dd, *J* = 19.8, 4.7 Hz), 4.05 (1H, dd, *J* = 19.8, 4.3 Hz), 3.00 (1H, d, *J* = 6.2 Hz), 2.17 (3H, s), 1.42 (9H, s). ^{13}C NMR (100 MHz, chloroform-*d*) δ 202.24, 171.86, 155.65, 154.91, 130.59, 128.43, 115.76, 77.36, 49.86, 37.69, 28.41, 27.41.

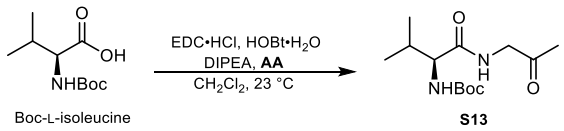
*Step 2: Synthesis of pyrazinone **9***



Dipeptide **S12** (25 mg, 0.07 mmol) was treated with with hydrochloric acid in dioxane (4 N) at 60 °C for 2 days. The reaction mixture was concentrated and treated with pyridine (2 mL) and stirred vigorously for 40 min, open to atmosphere. The reaction mixture was then concentrated resulting in a red residue. The product mixture was directly injected onto preparative reverse phase HPLC system equipped with an Agilent Polaris 5 C18-A column (250 × 21.2 mm, flow rate 8.0 mL/min, a gradient elution from 2 to 50% acetonitrile in water with 0.01% trifluoroacetic acid over 30 min) to yield orange pyrazinone **9** (t_R = 18 min, 4 mg, 25%). See Table S6 for ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data.

*Synthesis of pyrazinone **10***

*Step 1: Synthesis of dipeptide **S13***

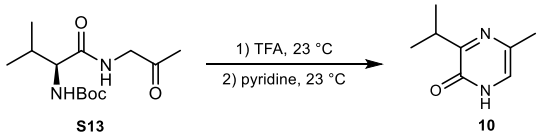


Boc-L-isoleucine **S13**

To a stirring solution of Boc-L-valine (500 mg, 2.30 mmol), EDC hydrochloride (574 mg, 2.99 mmol, 1.3 equiv) and 1-hydroxybenzotriazole monohydrate (458 mg, 2.99 mmol, 1.3 equiv) in dimethylformamide (7.7 mL) at 0 °C was added *N*,*N*-diisopropylethylamine (Hünig's base, 1.40 mL, 8.06 mmol, 3.5 equiv). Aminoacetone (**AA**) hydrochloride (378 mg, 3.45 mmol, 1.5 equiv) dissolved in dimethylformamide (1 mL) was then added to the reaction. The flask was removed from the ice bath and allowed to warm to room temperature. After 2 h, the reaction mixture was cooled to 0 °C and treated with an aqueous potassium bisulfate solution (50 mL, 5%) and extracted with ethyl acetate (4 × 50 mL). The organic layers were combined and washed with saturated aqueous sodium bicarbonate, followed by saturated sodium chloride, dried over sodium sulfate, filtered and then concentrated. The residue obtained was purified by flask-column chromatography (eluting with dichloromethane initially, grading to 5% methanol) to provide dipeptide **S13** as a white or yellow solid (340.65 mg, 54%).

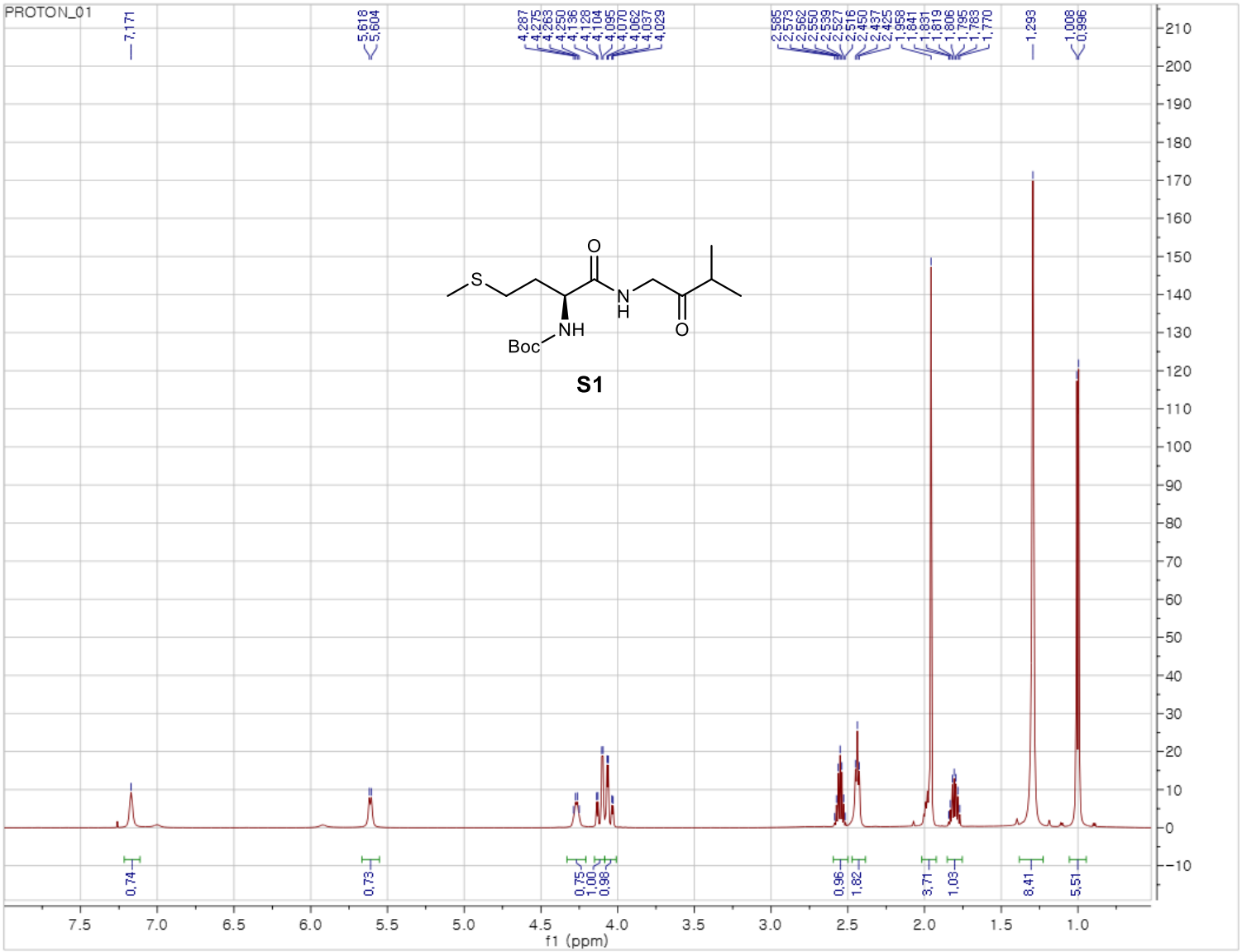
^{1}H NMR (400 MHz, chloroform-*d*) δ 6.88 (1H, brt, *J* = 4.9 Hz), 5.18 (1H, brd, *J* = 6.3 Hz), 4.11 (2H, m), 3.99 (1H, brs), 2.16 (3H, s), 2.11 (1H, m), 1.40 (9H, s), 0.93 (3H, d, *J* = 6.9 Hz), 0.88 (3H, d, *J* = 6.9 Hz). ^{13}C NMR (100 MHz, chloroform-*d*) δ 202.83, 172.07, 155.98, 79.98, 59.89, 49.72, 30.95, 28.38, 27.34, 19.34, 17.74.

*Step 2: Synthesis of pyrazinone **10***

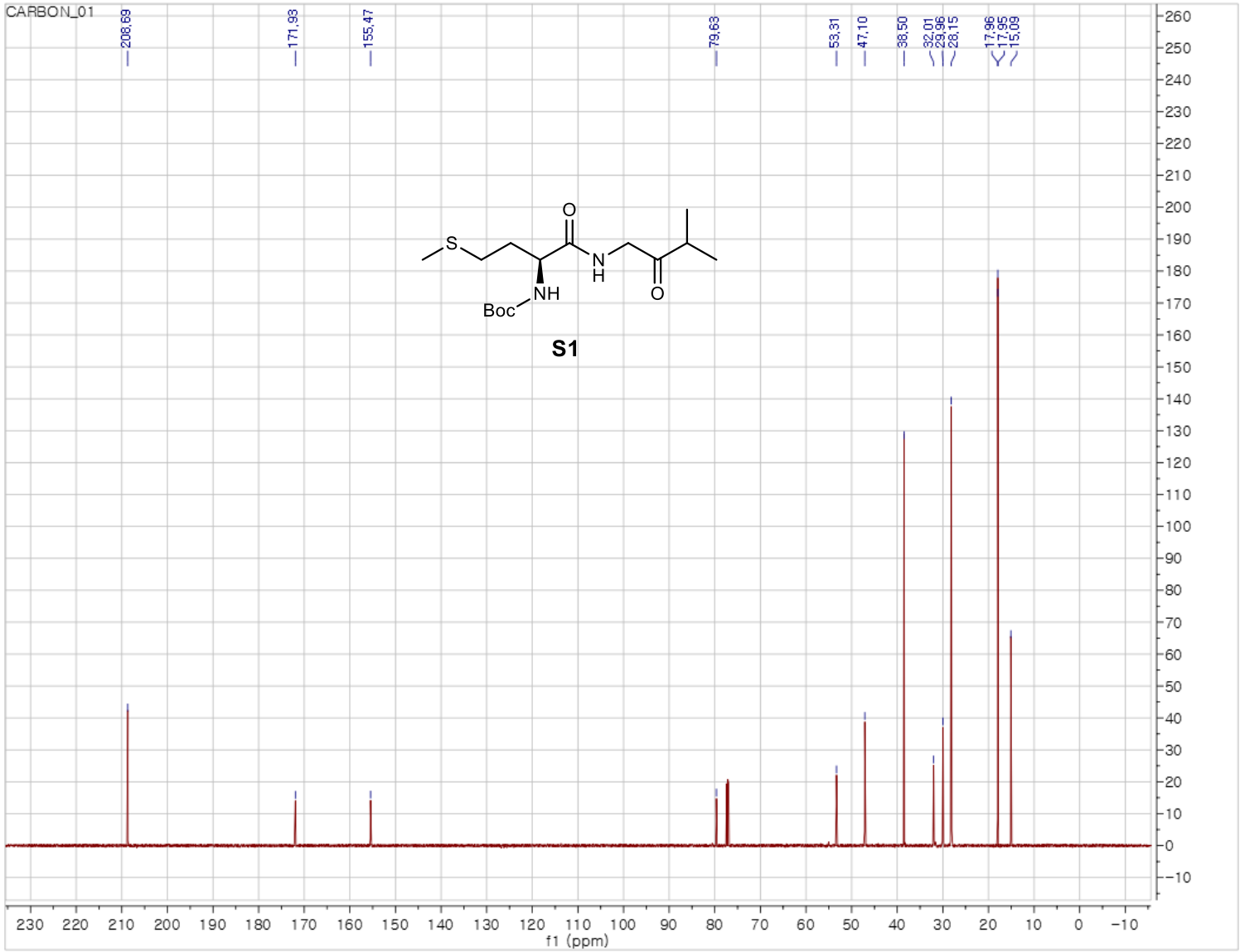


Dipeptide **S13** (50 mg, 0.18 mmol) was treated with trifluoroacetic acid (1 mL) at room temperature for 1 h. The reaction mixture was concentrated and treated with pyridine (2 mL) and stirred vigorously for 40 min, open to atmosphere. The reaction mixture was then concentrated resulting in a red residue. The product mixture was directly injected onto a preparative reverse phase HPLC system equipped with an Agilent Polaris 5 C18-A column (250 × 21.2 mm, flow rate 8.0 mL/min, a gradient elution from 10 to 100% acetonitrile in water with 0.01% trifluoroacetic acid over 40 min) to yield yellow pyrazinone **10** (t_R = 21 min, 6.8 mg, 24%). See Table S6 for ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data.

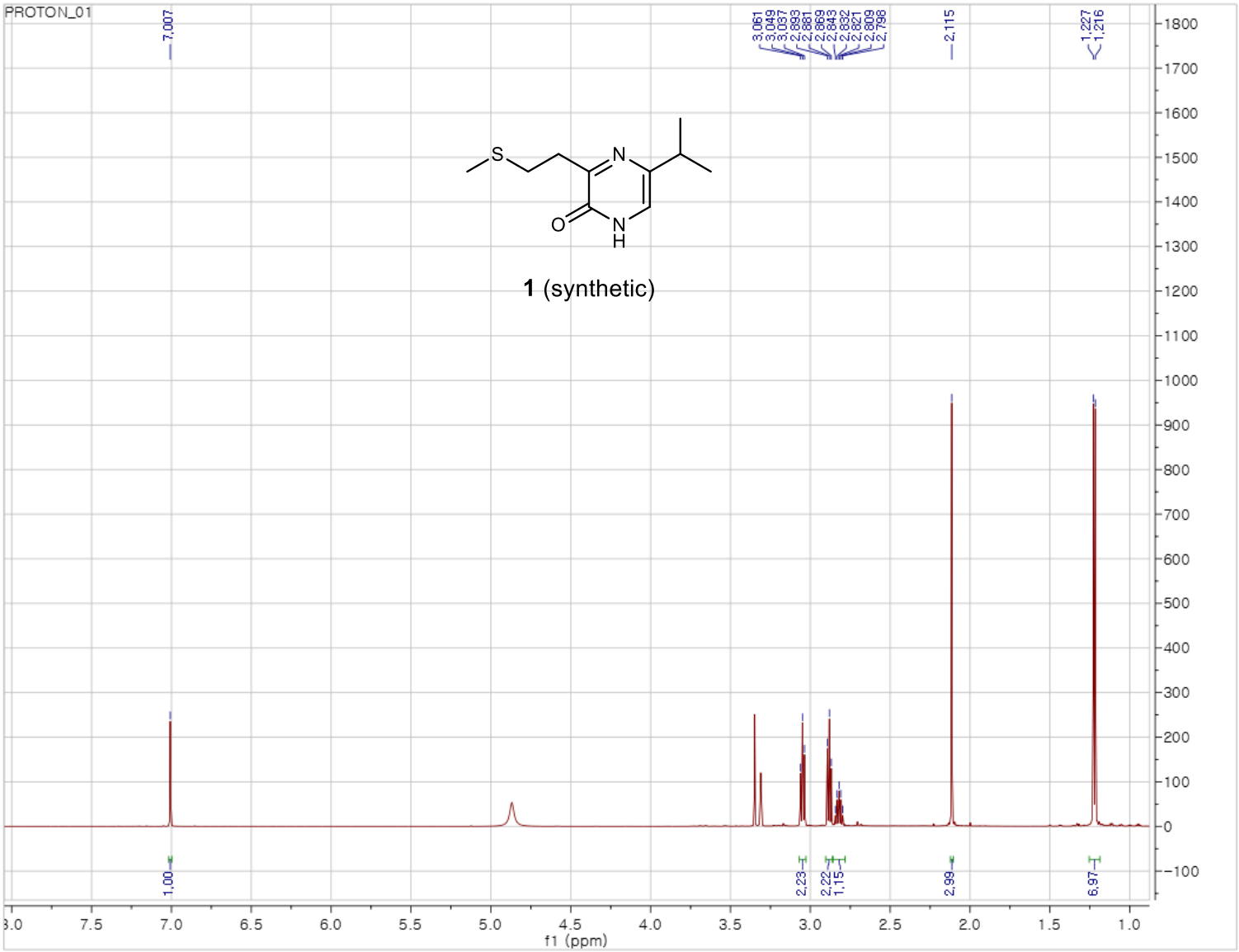
^{1}H NMR, 600 MHz, chloroform-*d*



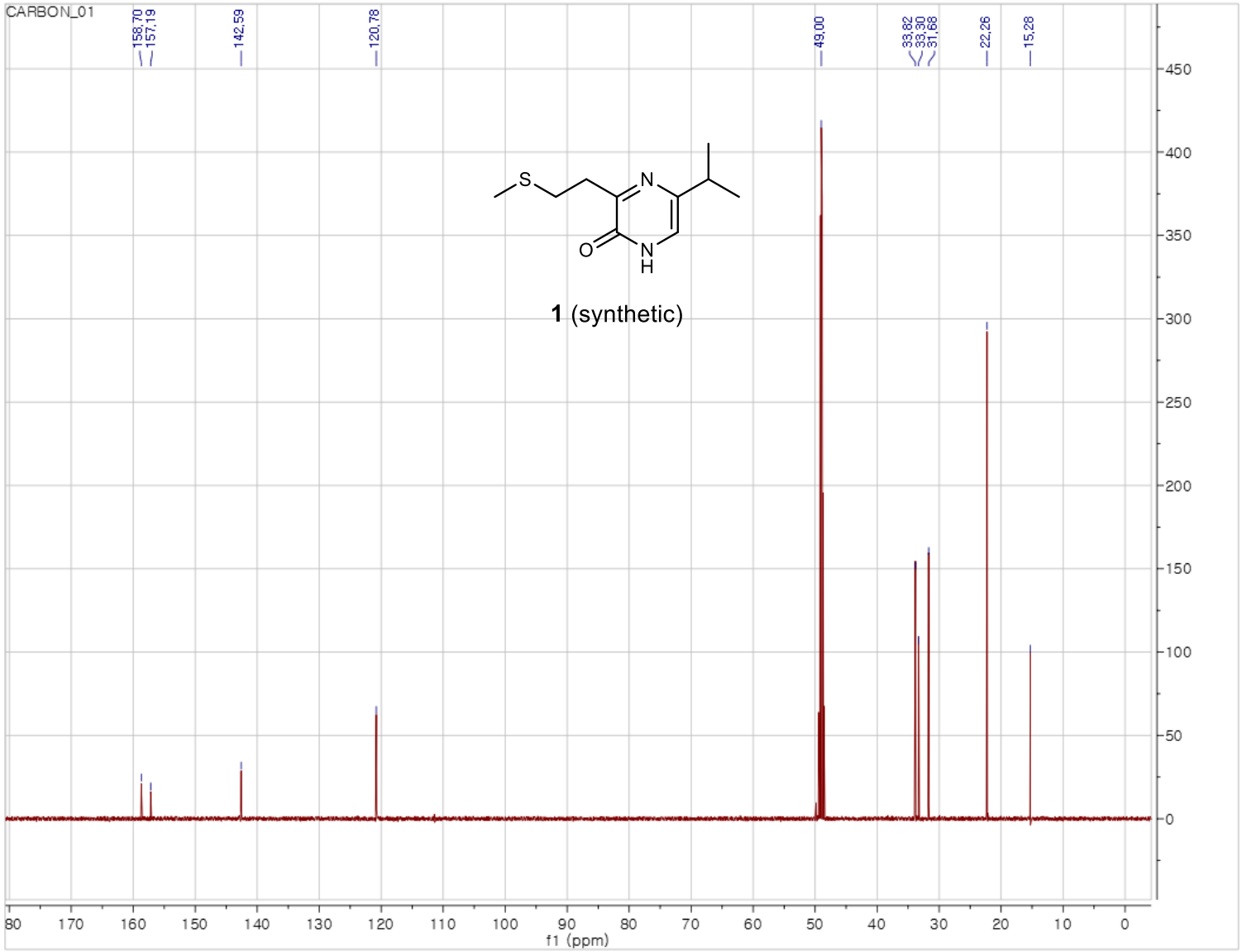
^{13}C NMR, 150 MHz, chloroform-*d*



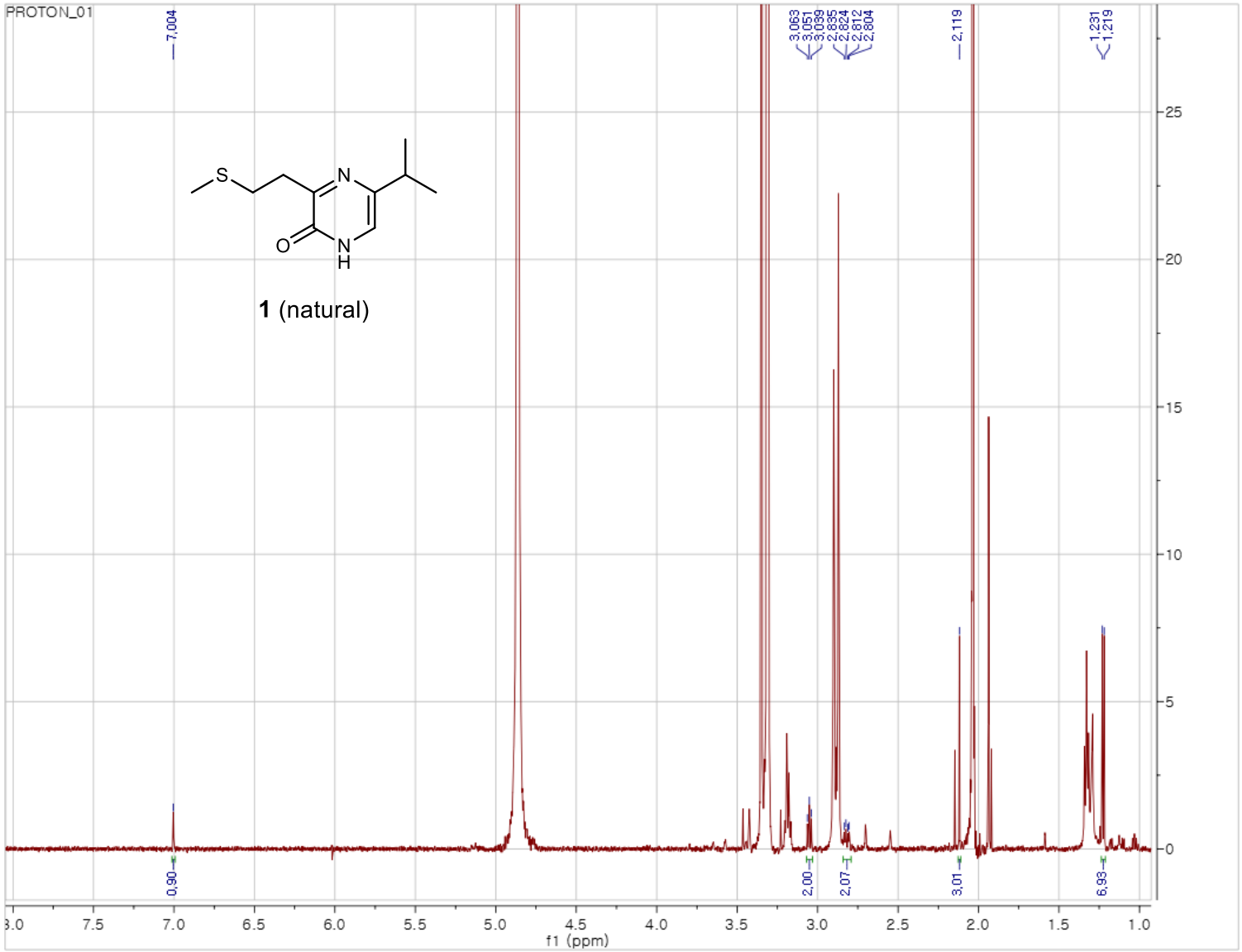
^{1}H NMR, 600 MHz, methanol-d_4



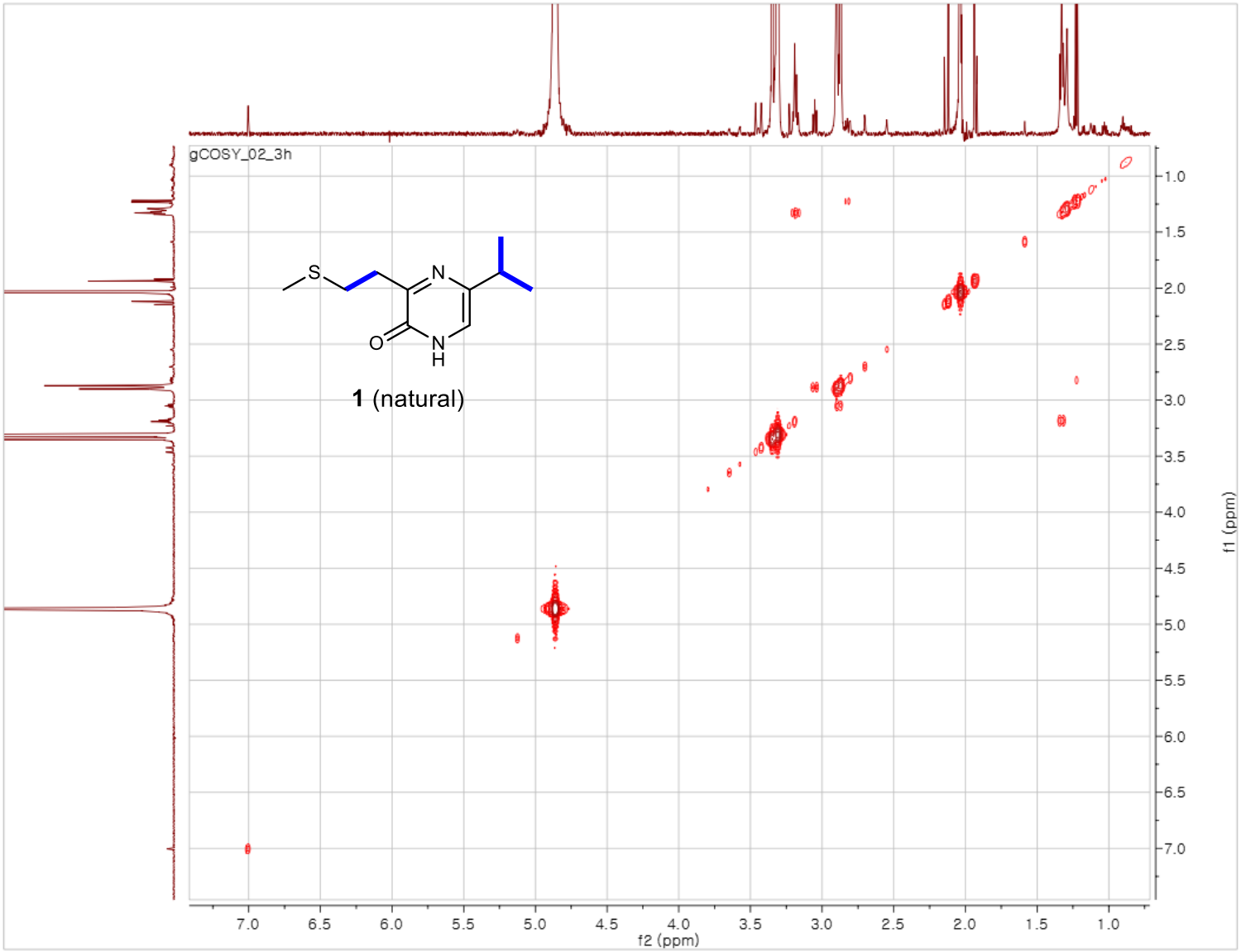
^{13}C NMR, 150 MHz, methanol-d_4



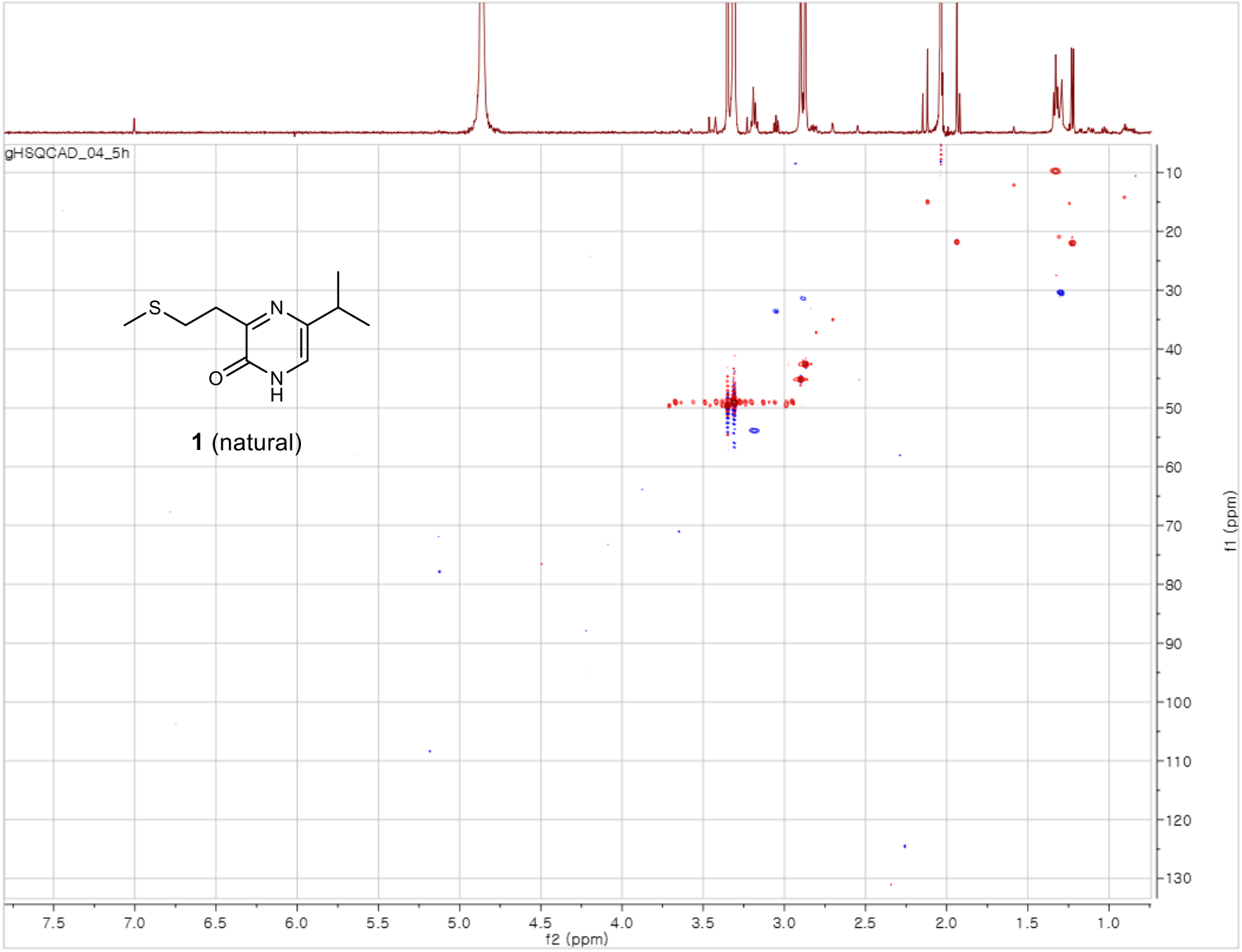
^{1}H NMR, 600 MHz, methanol-d_4



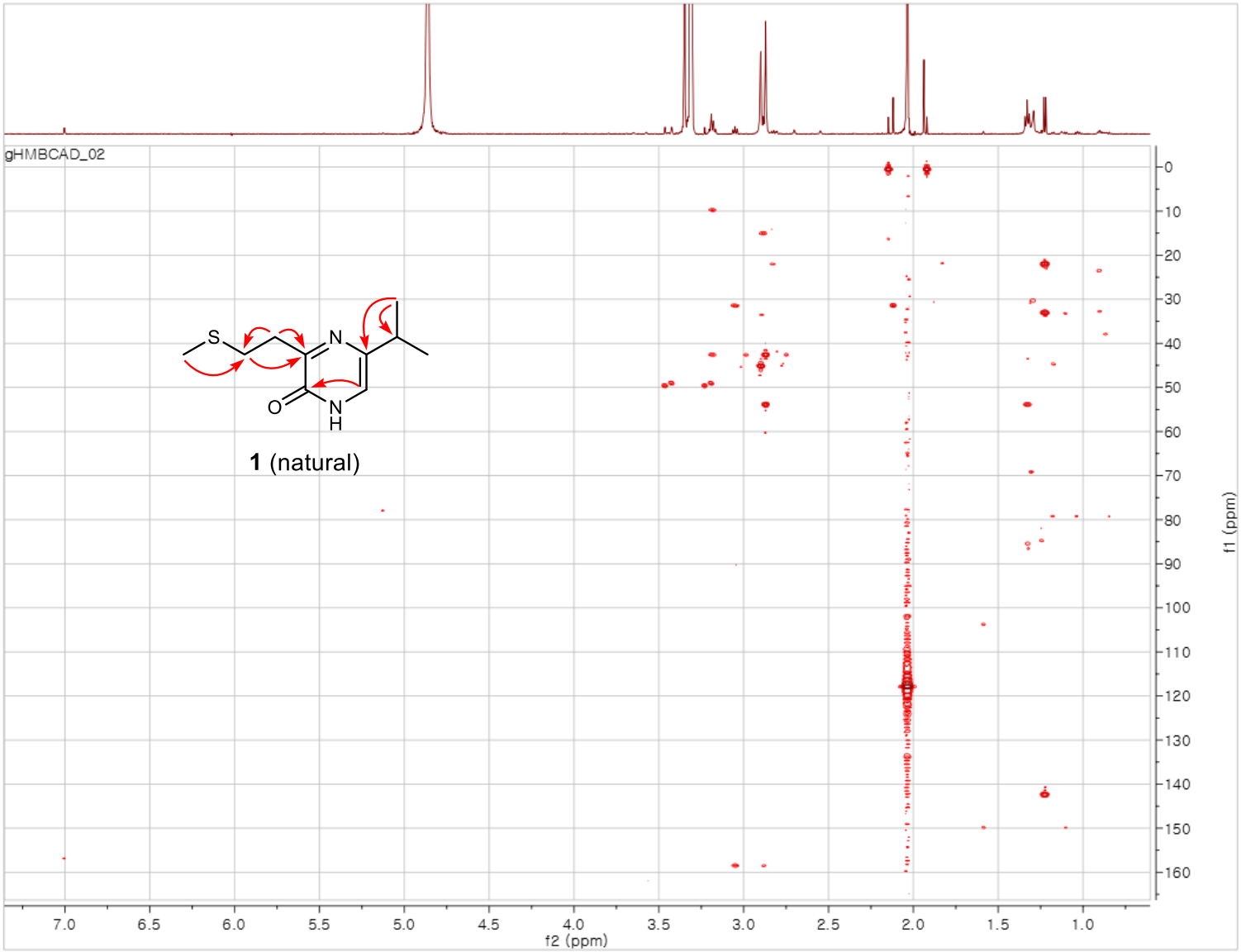
^{1}H-^{1}H COSY, 600 MHz, methanol-d_4



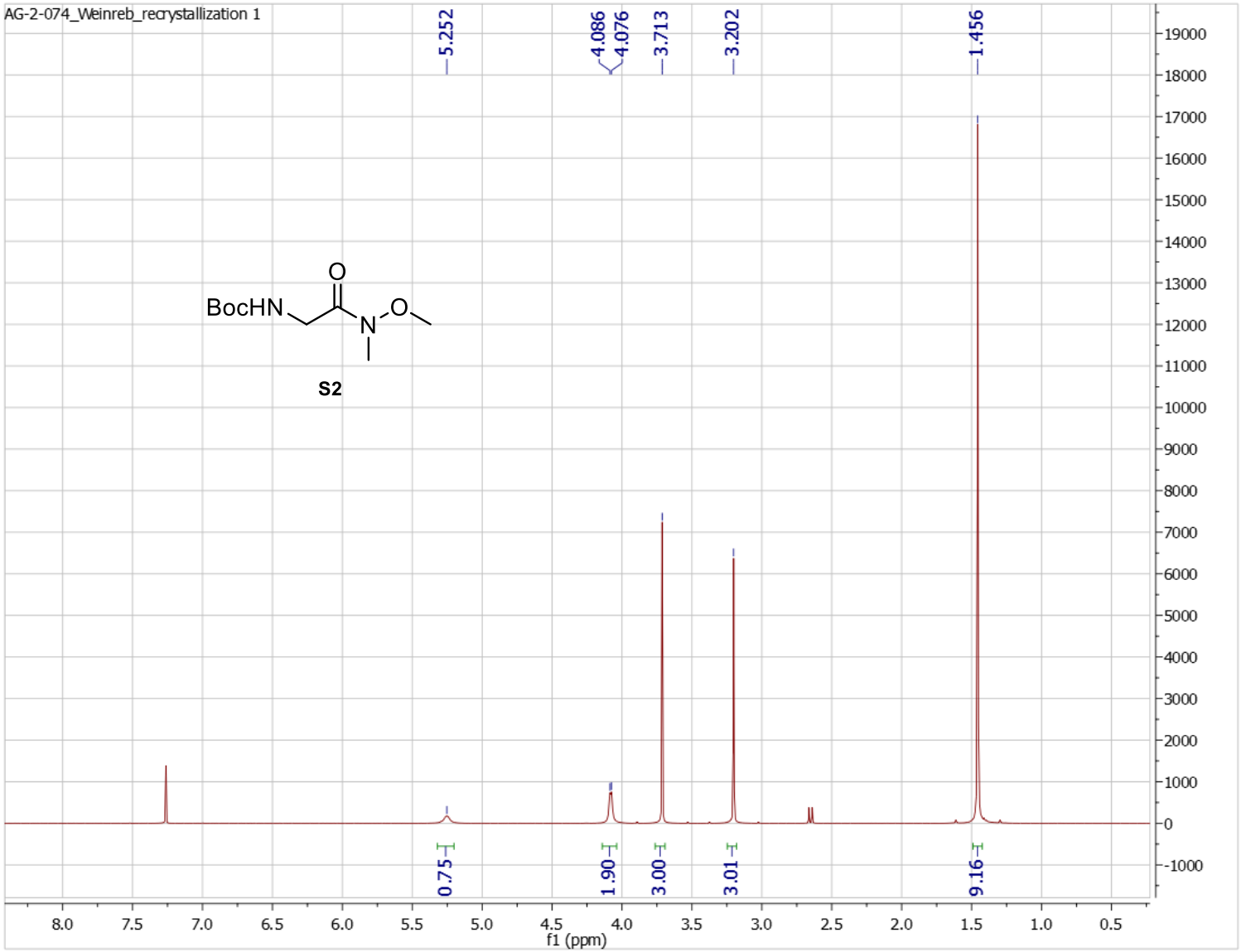
^{1}H-^{13}C HSQCAD, 600 MHz, methanol-d_4



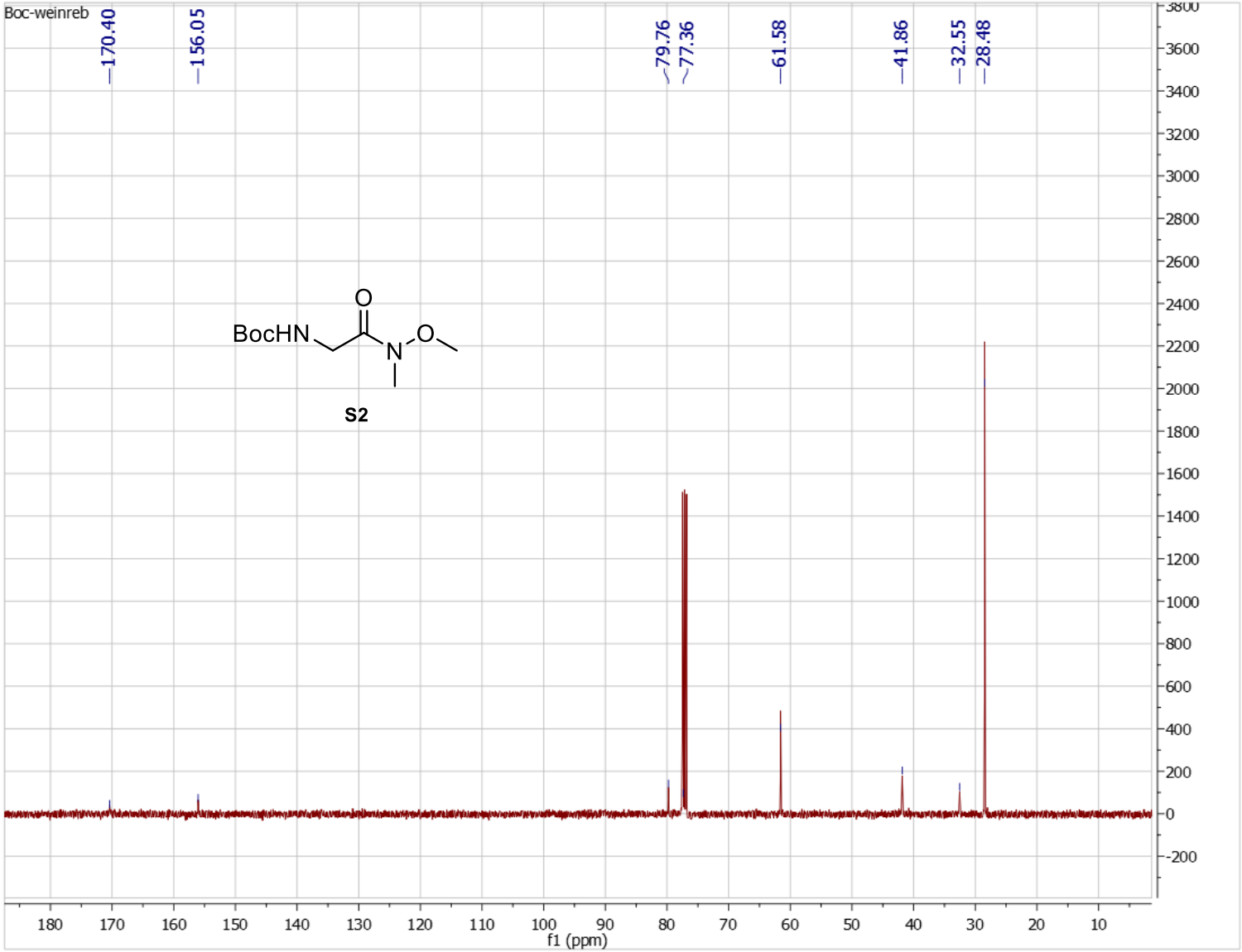
^{1}H-^{13}C HMBCAD, 600 MHz, methanol-d_4



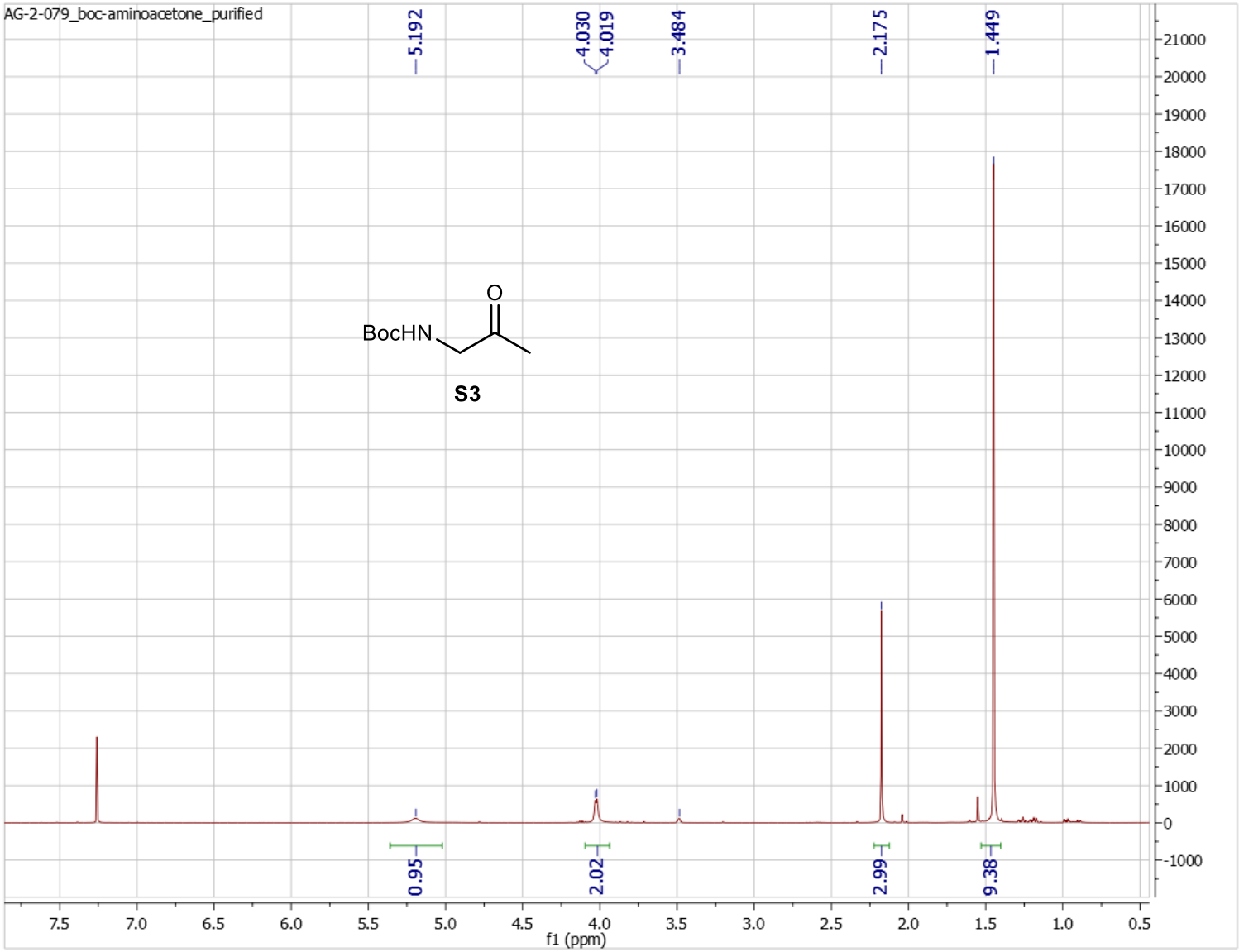
^{1}H NMR, 400 MHz, chloroform-*d*



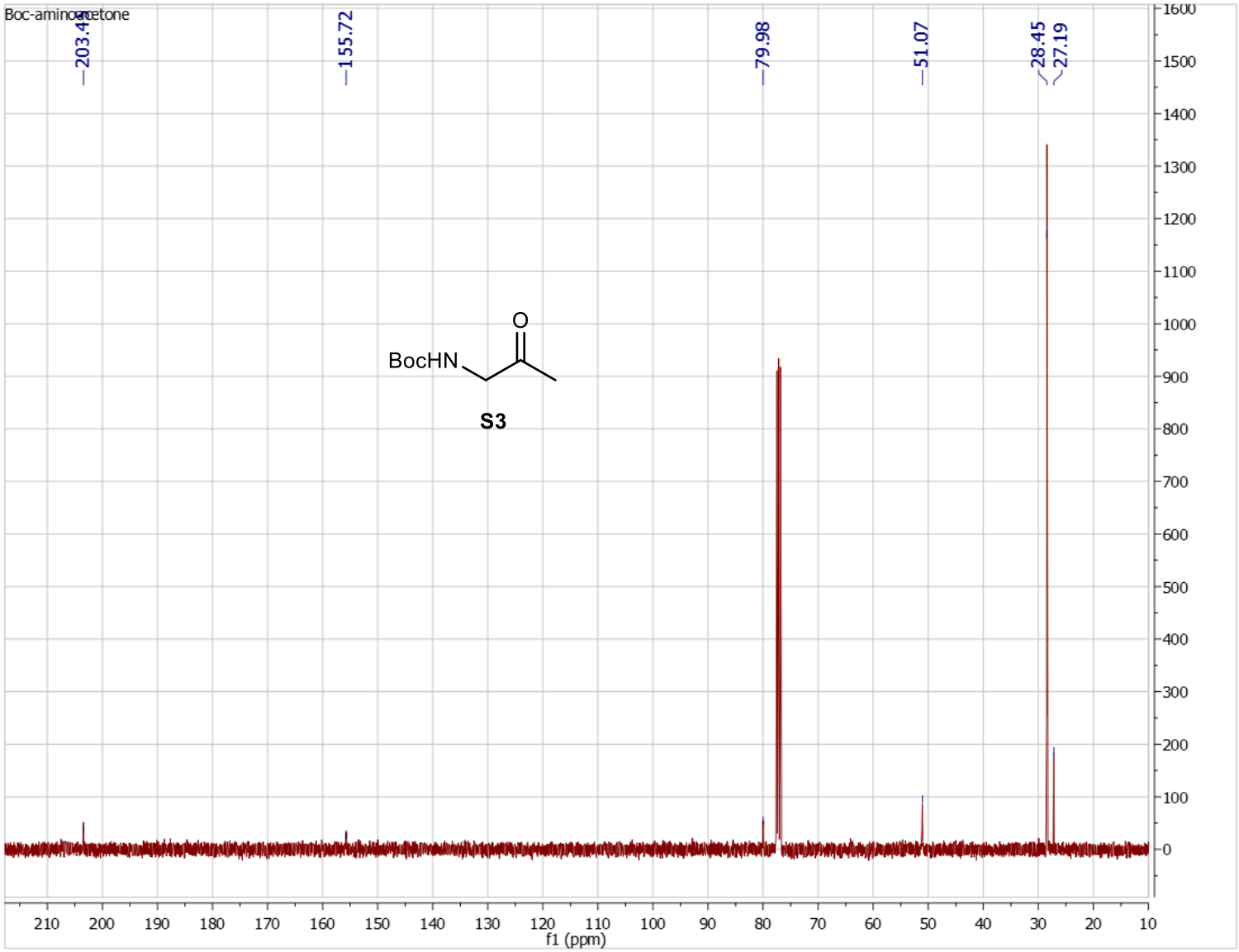
^{13}C NMR, 100 MHz, chloroform-*d*



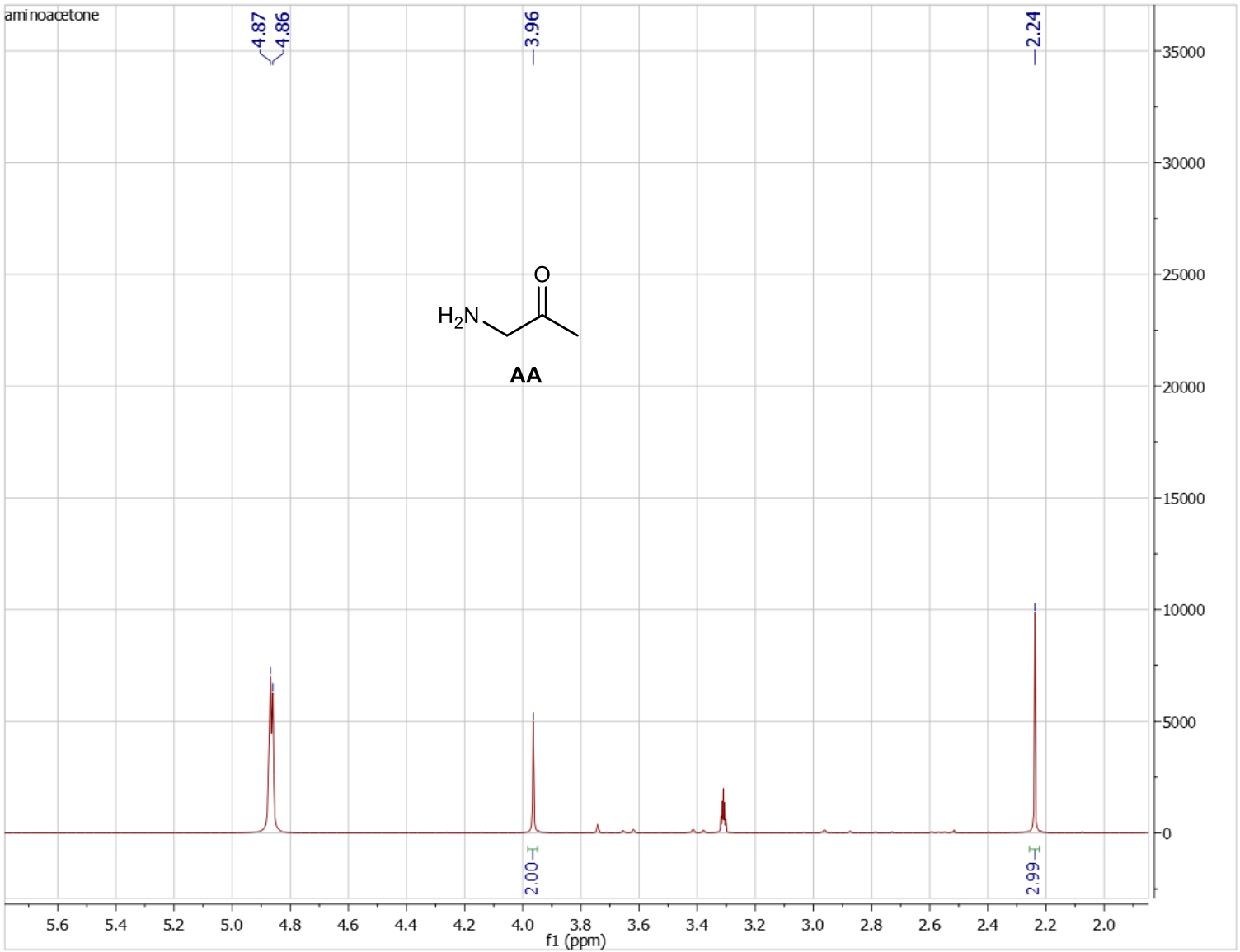
^{1}H NMR, 400 MHz, chloroform-*d*



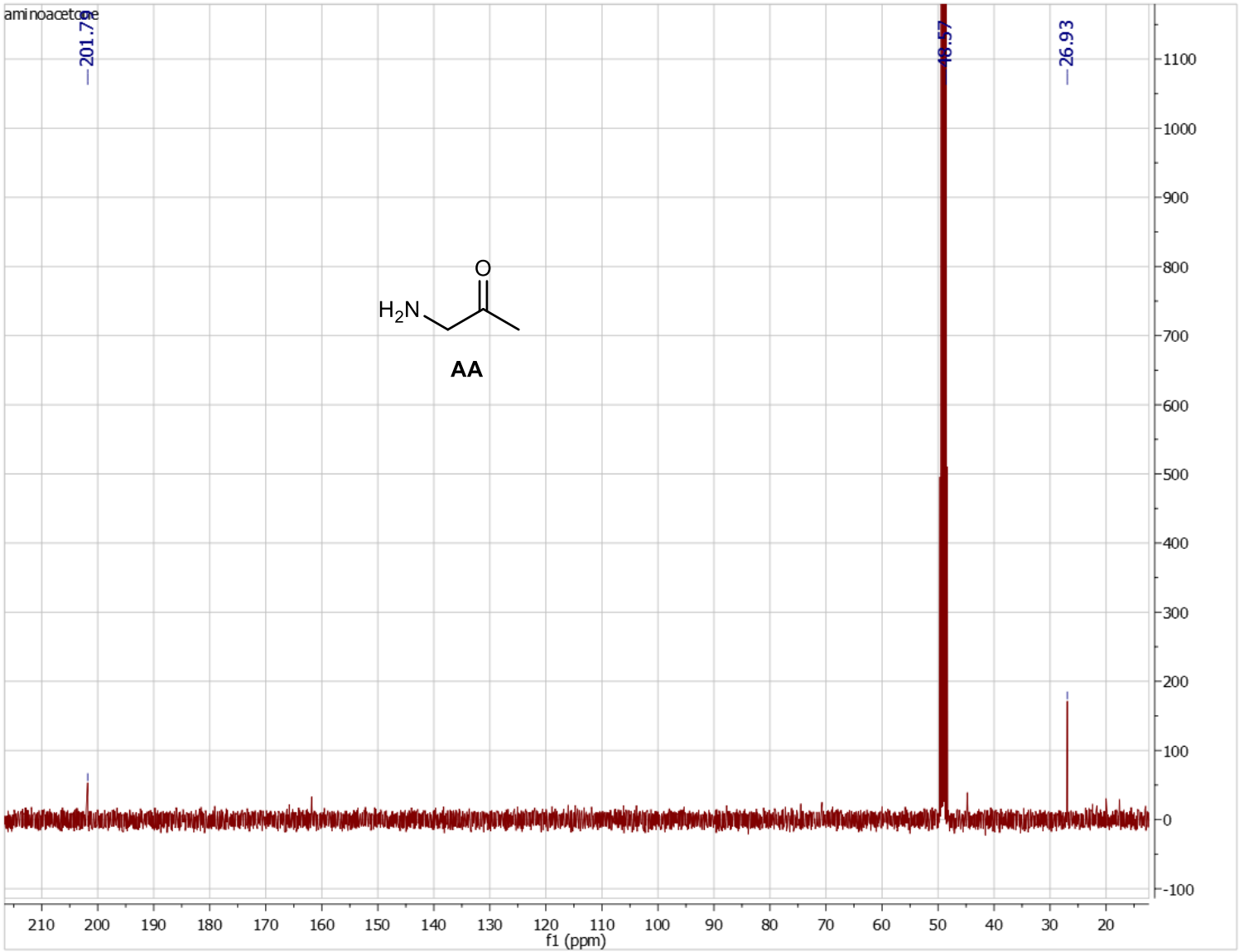
^{13}C NMR, 100 MHz, chloroform-*d*



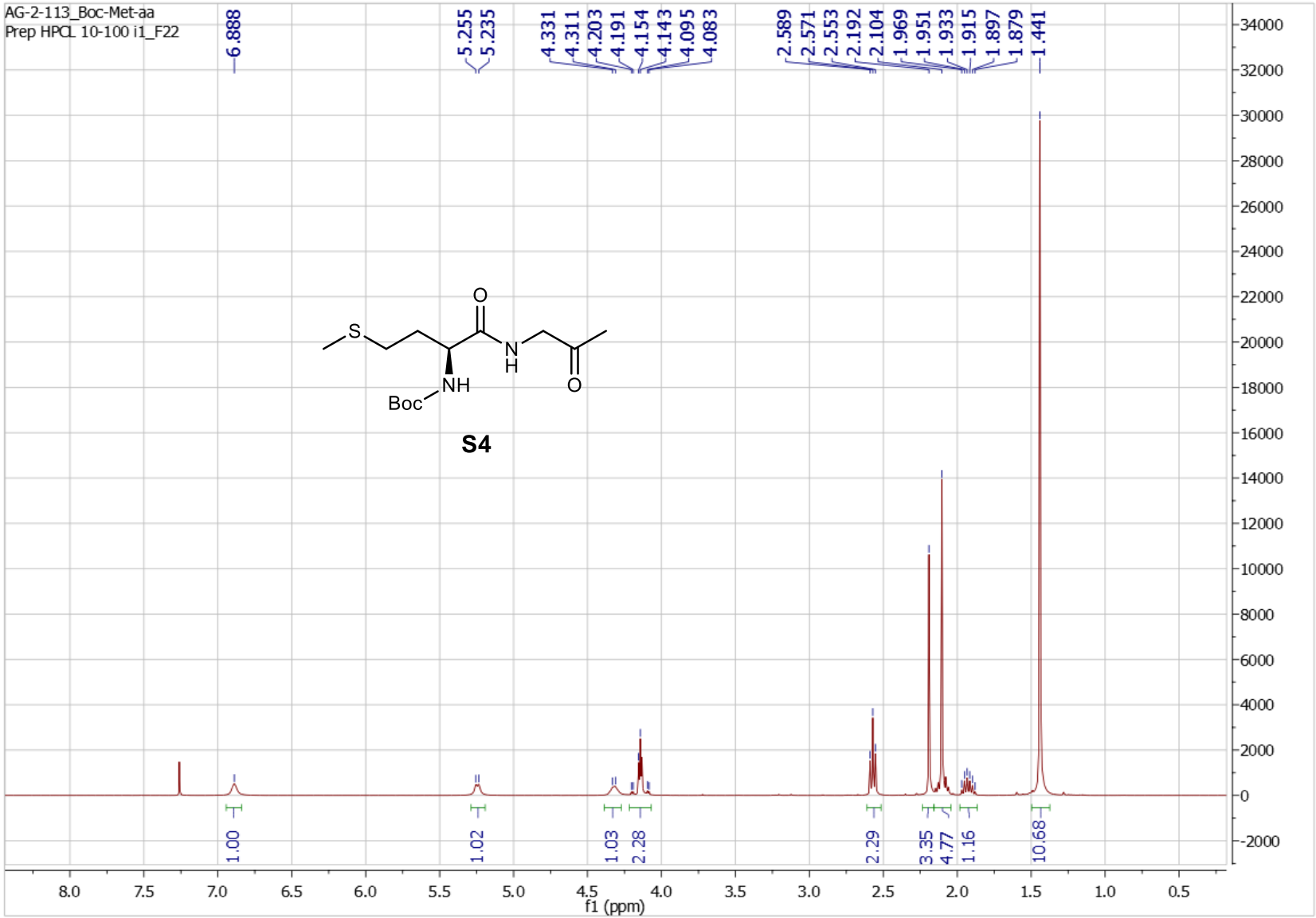
^{1}H NMR, 400 MHz, methanol-d_4



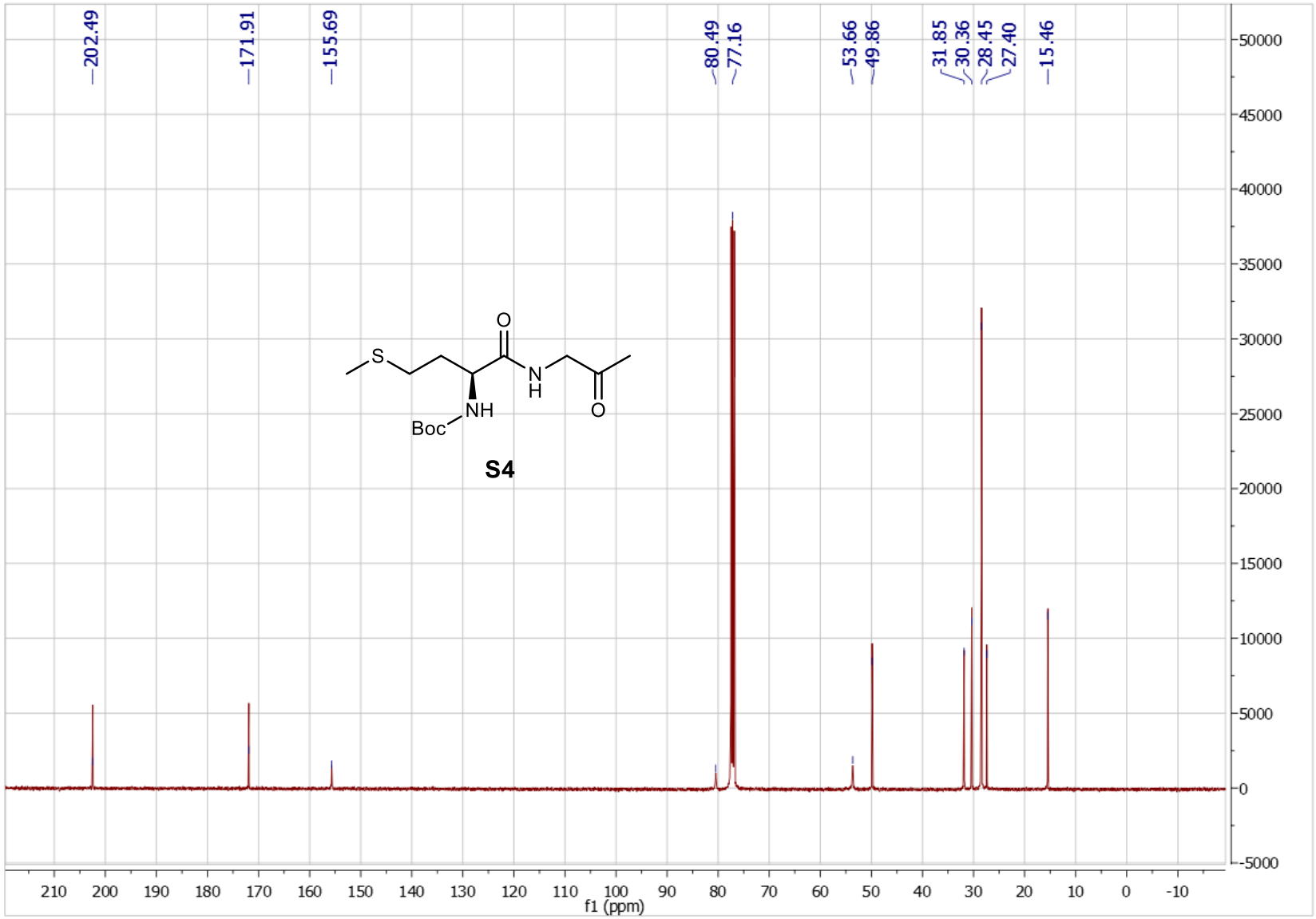
^{13}C NMR, 100 MHz, methanol-d_4



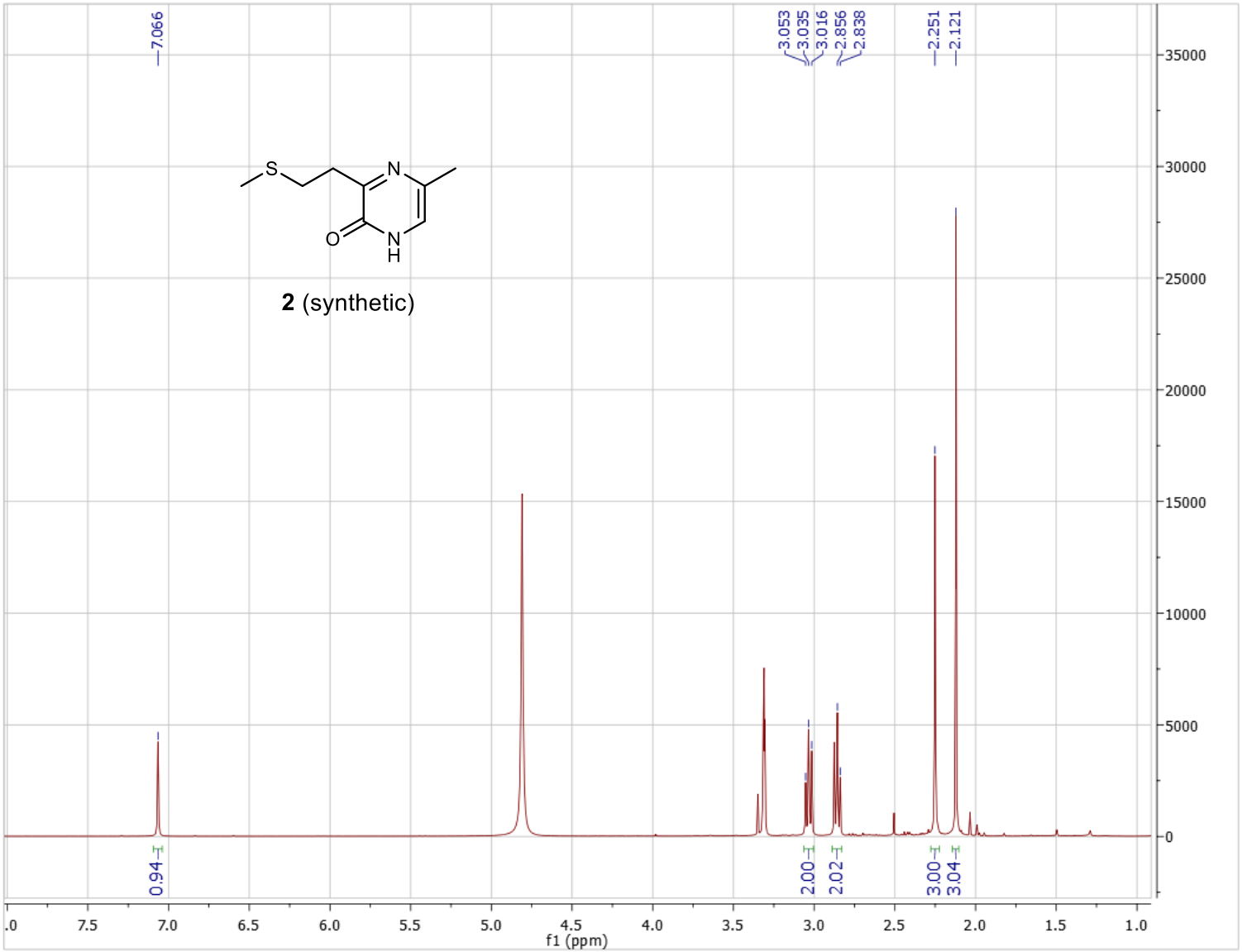
^{1}H NMR, 400 MHz, chloroform-*d*



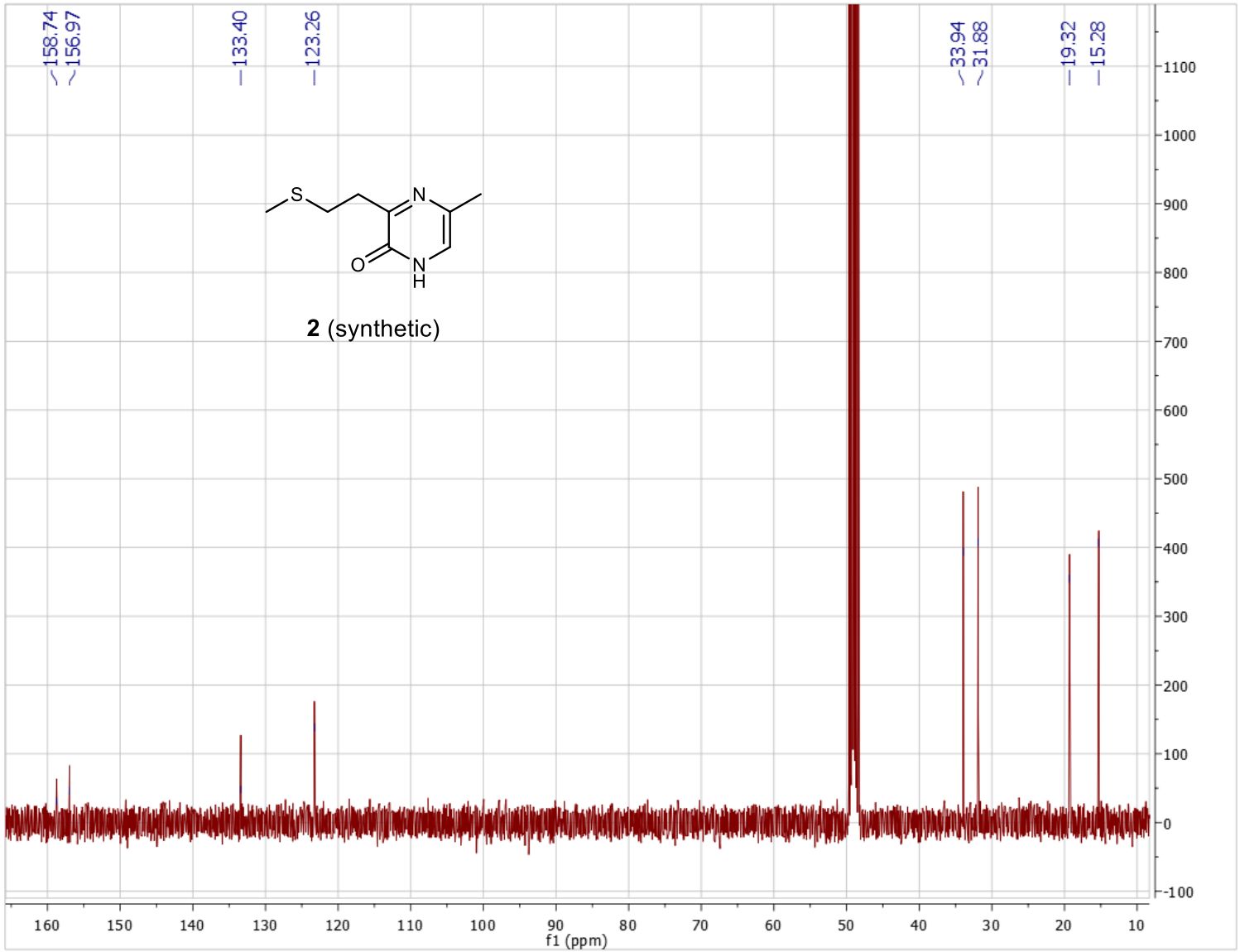
^{13}C NMR, 100 MHz, chloroform-*d*



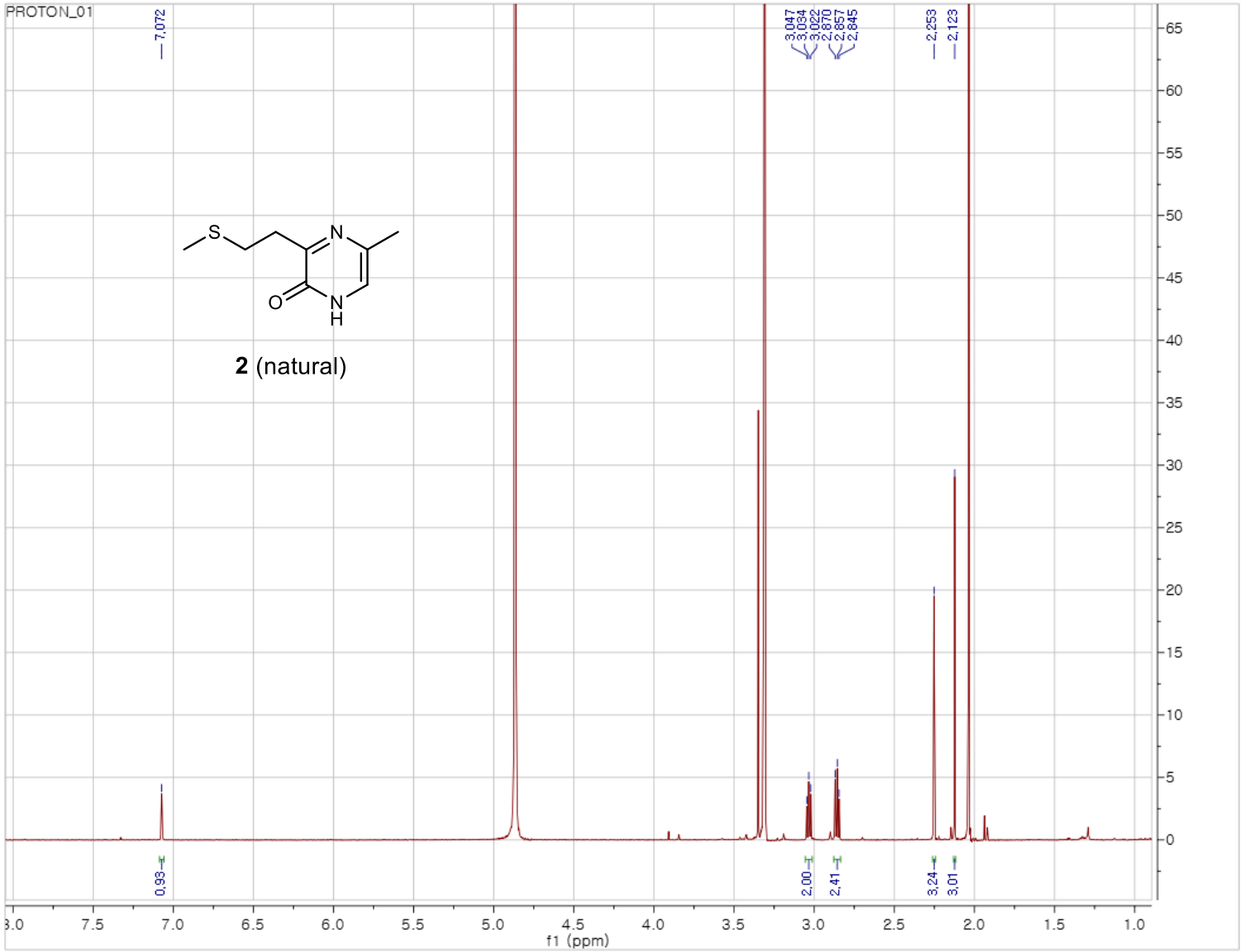
^{1}H NMR, 400 MHz, methanol-d_4



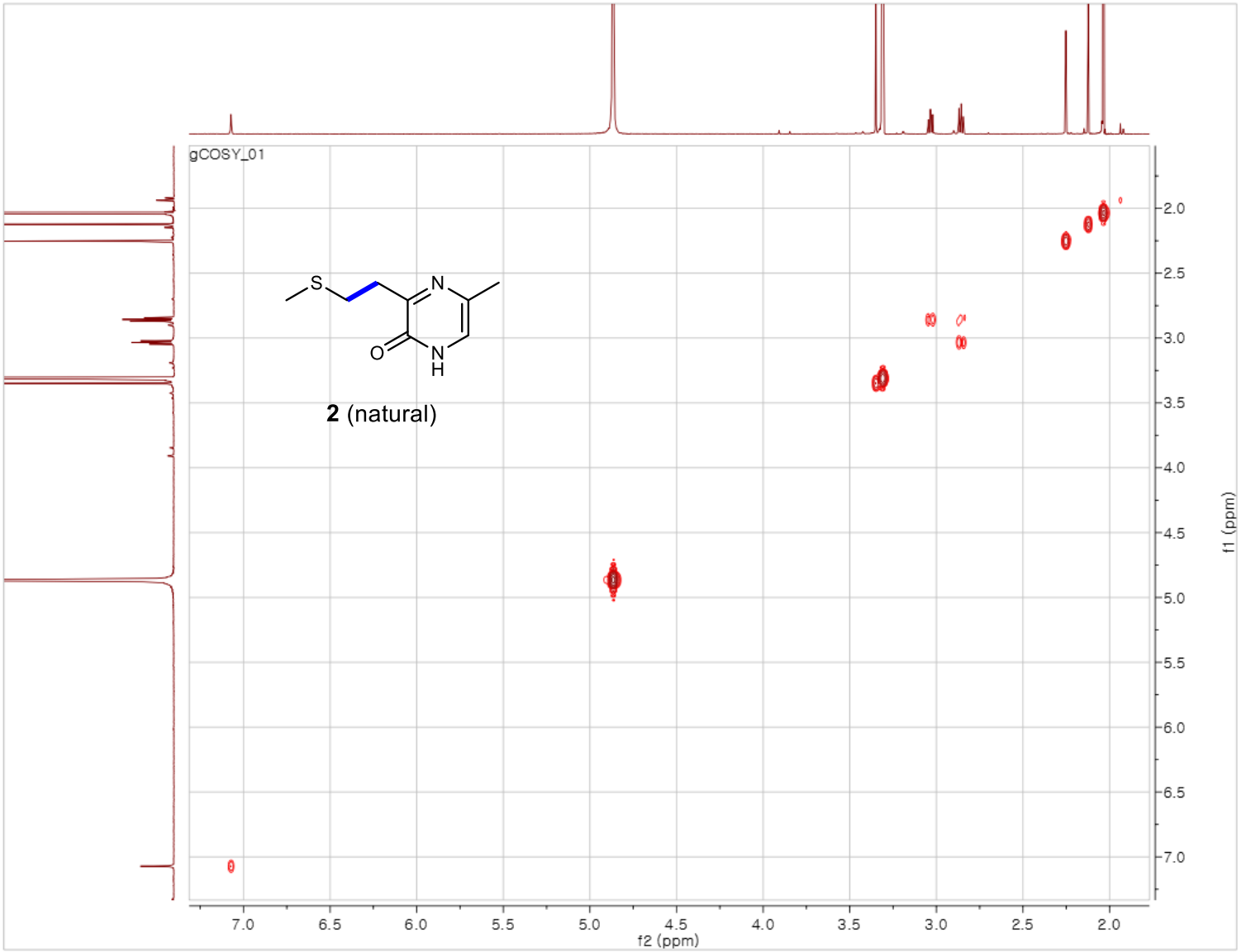
^{13}C NMR, 100 MHz, methanol-d_4



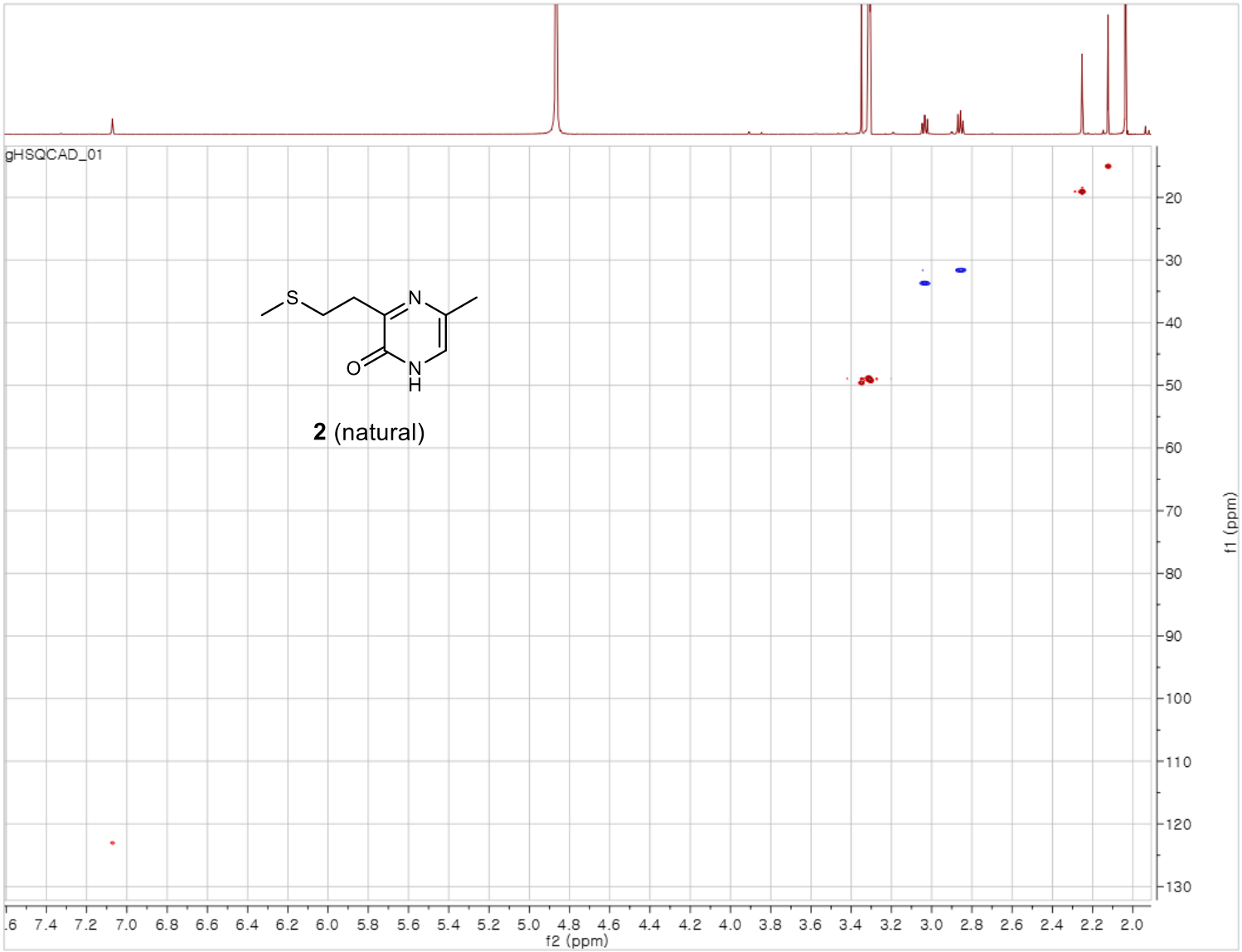
^{1}H NMR, 600 MHz, methanol-d_4



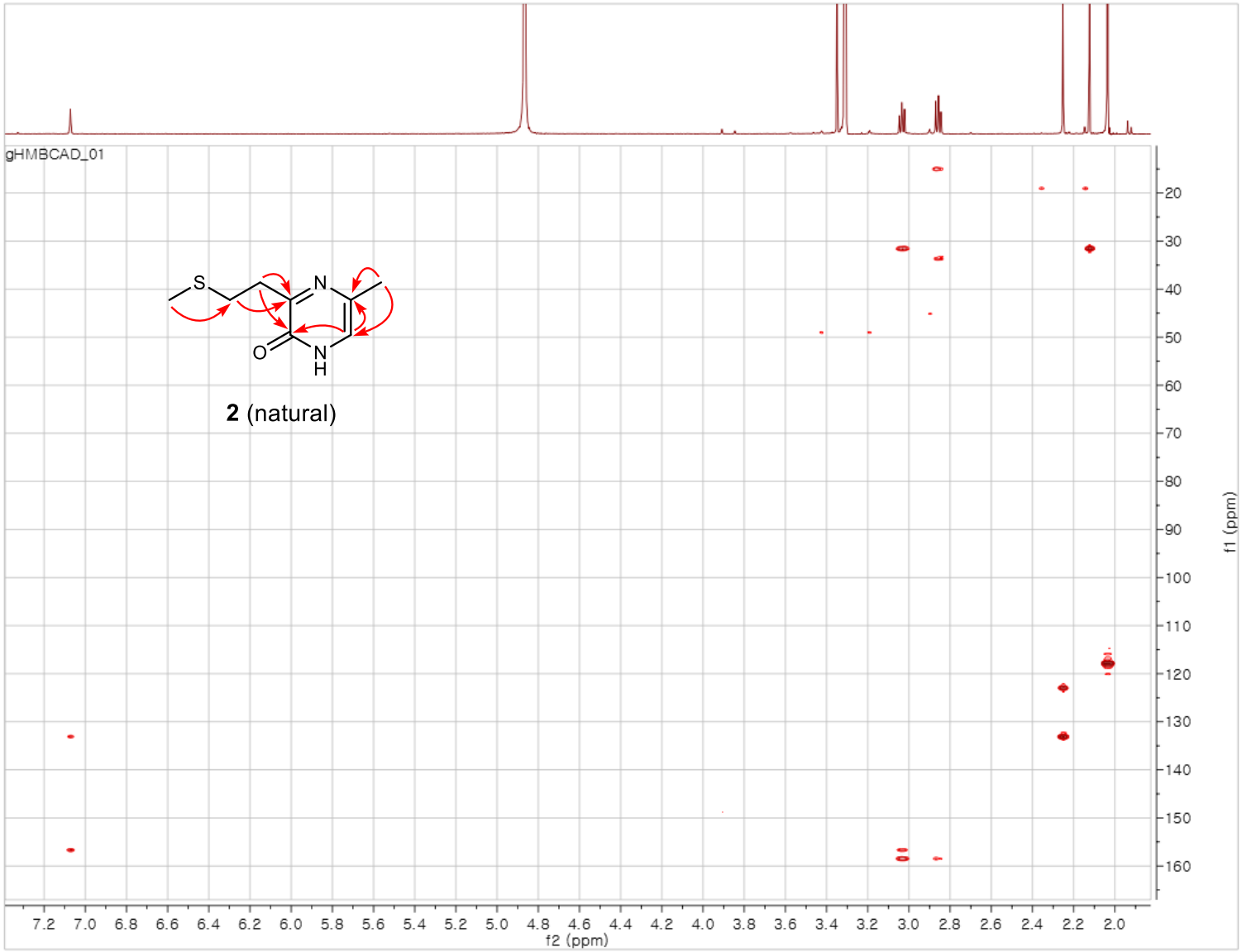
^{1}H-^{1}H COSY, 600 MHz, methanol-d_4



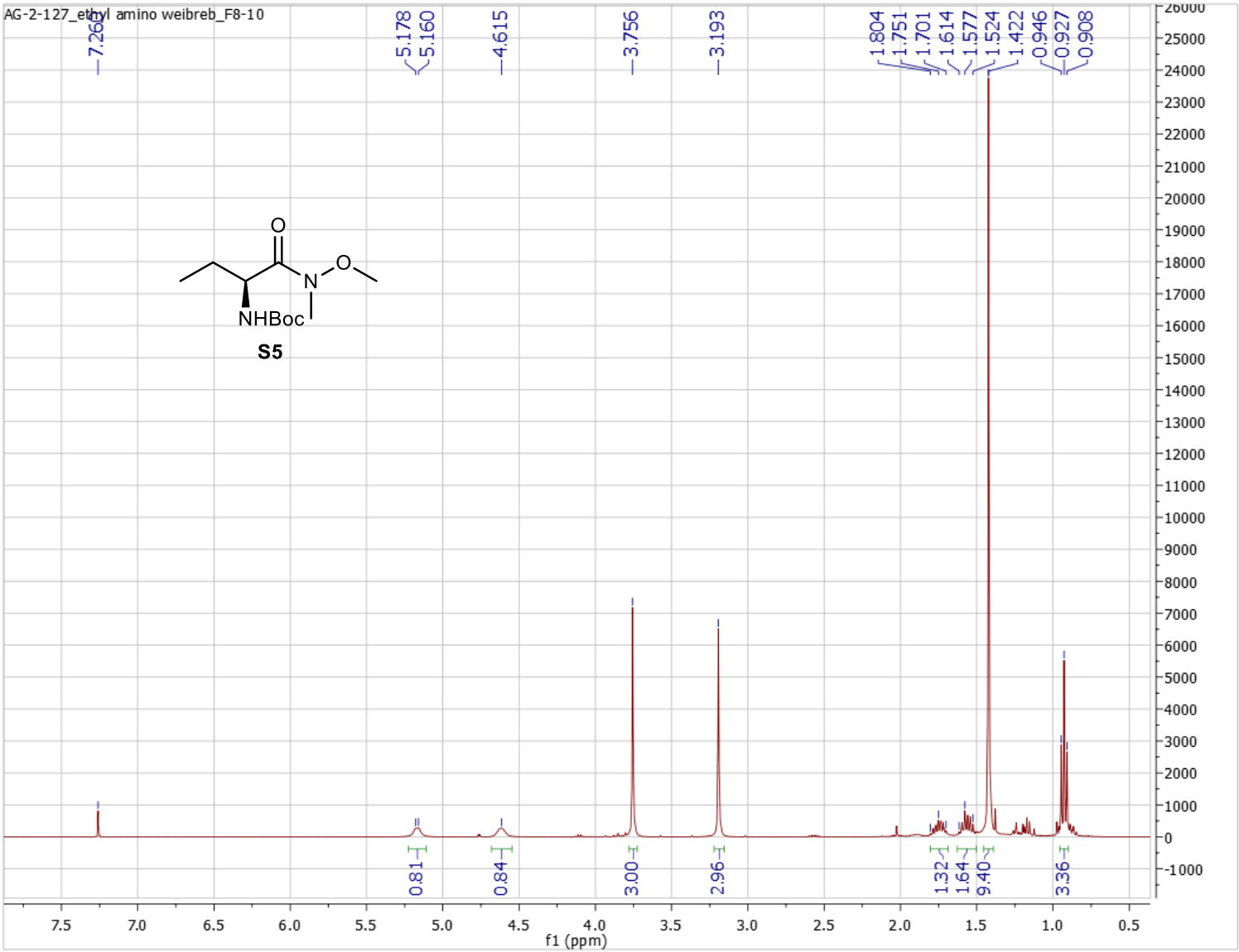
^{1}H-^{13}C HSQCAD, 600 MHz, methanol-d_4



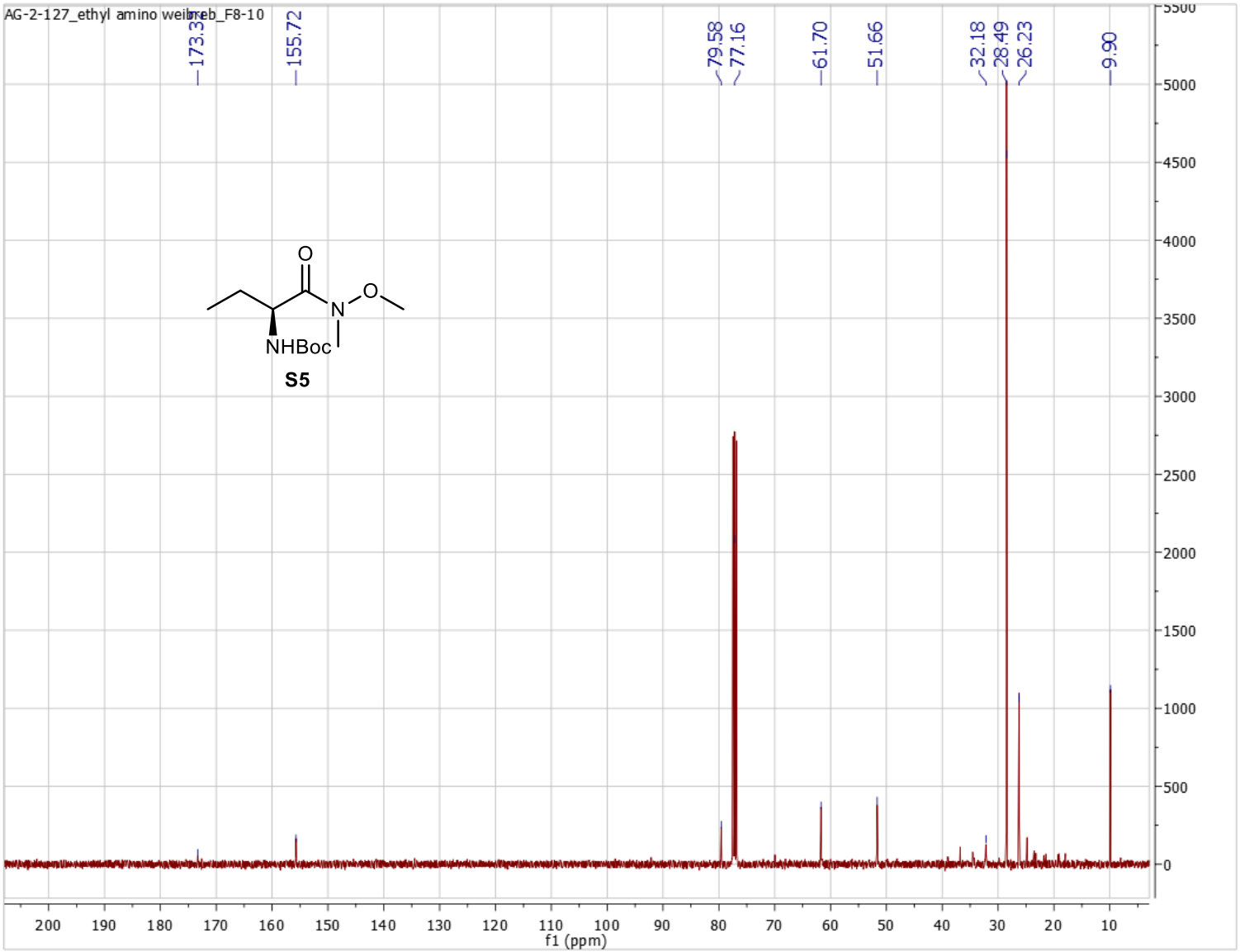
^{1}H-^{13}C HMBCAD, 600 MHz, methanol-d_4



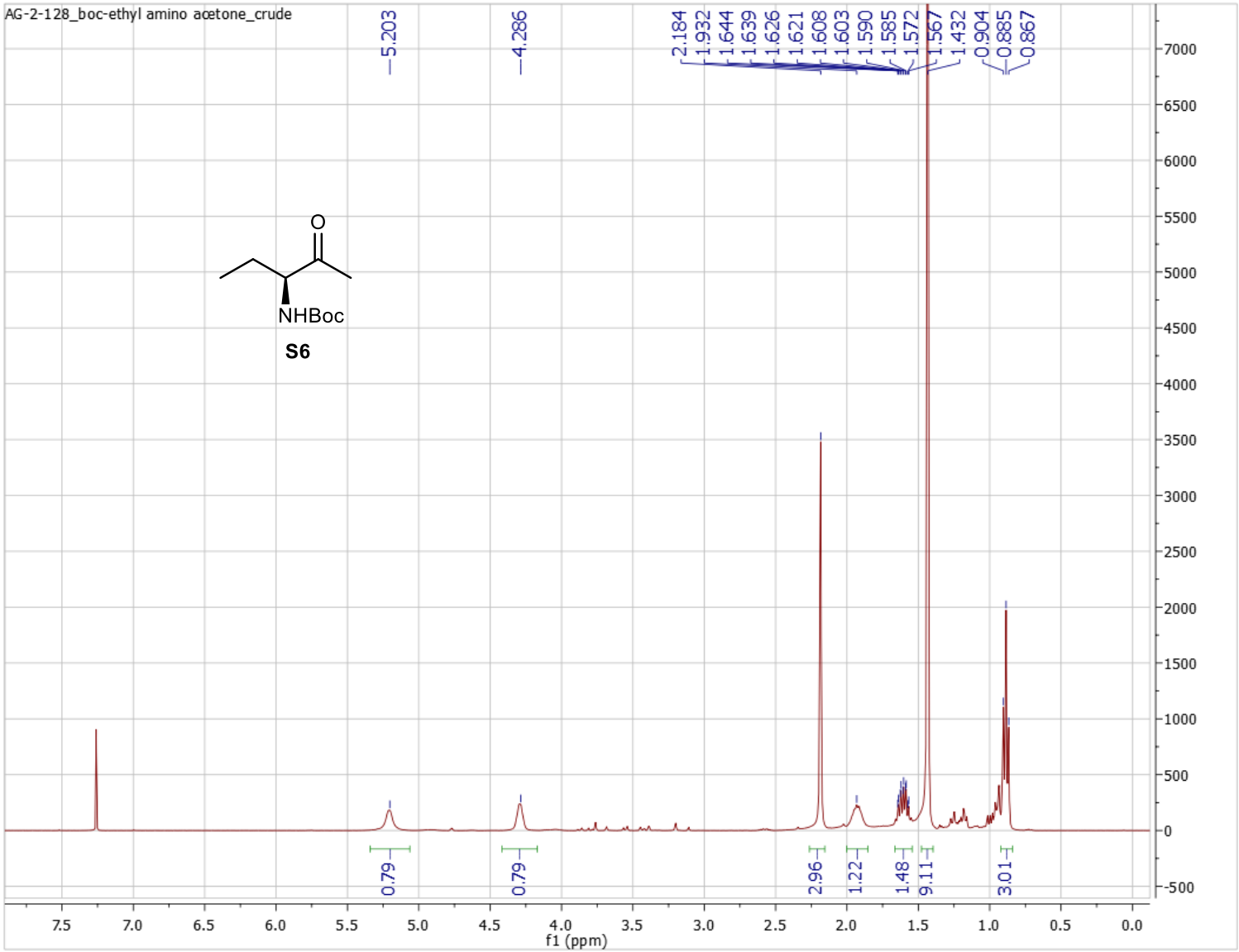
^{1}H NMR, 400 MHz, chloroform-*d*



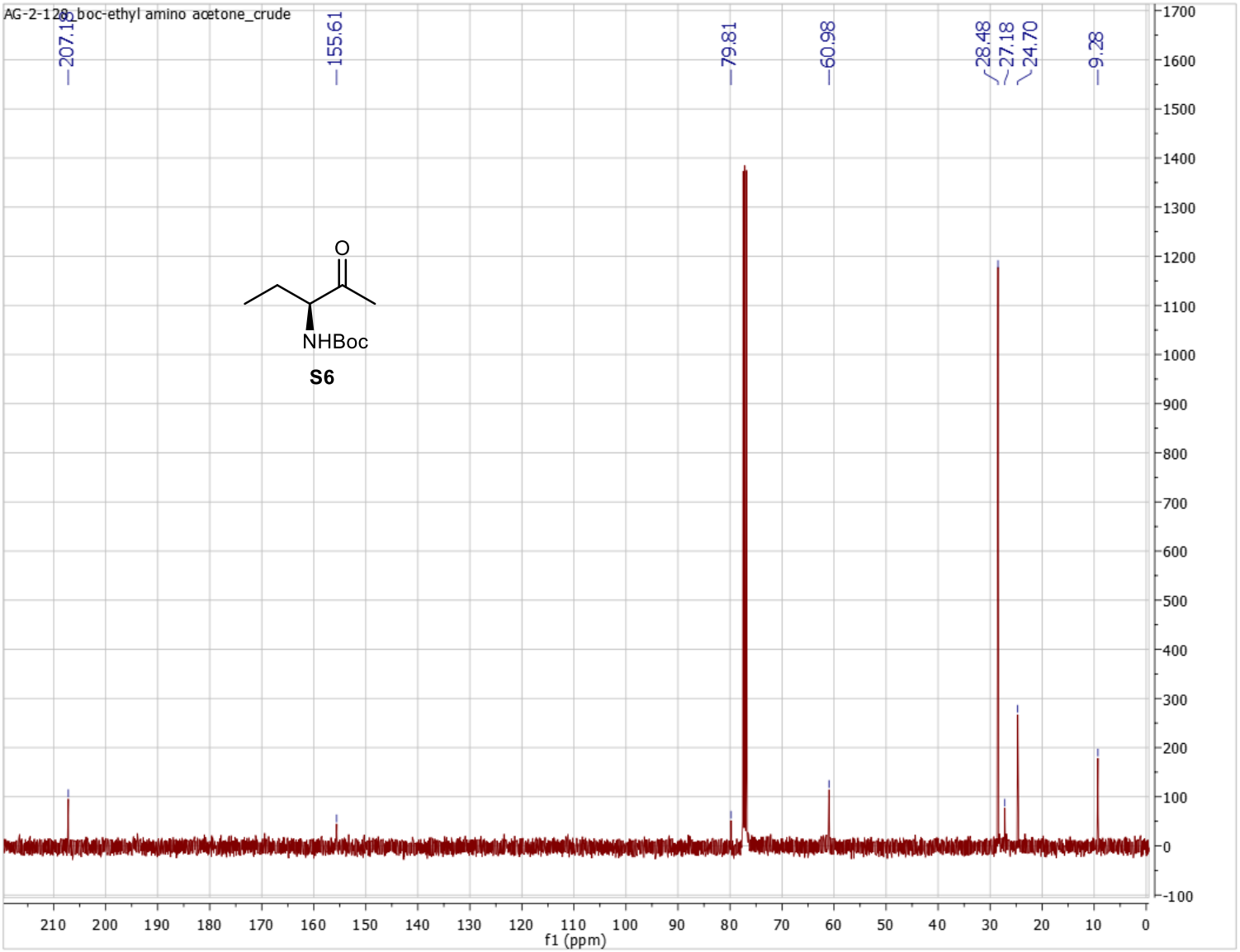
^{13}C NMR, 100 MHz, chloroform-*d*



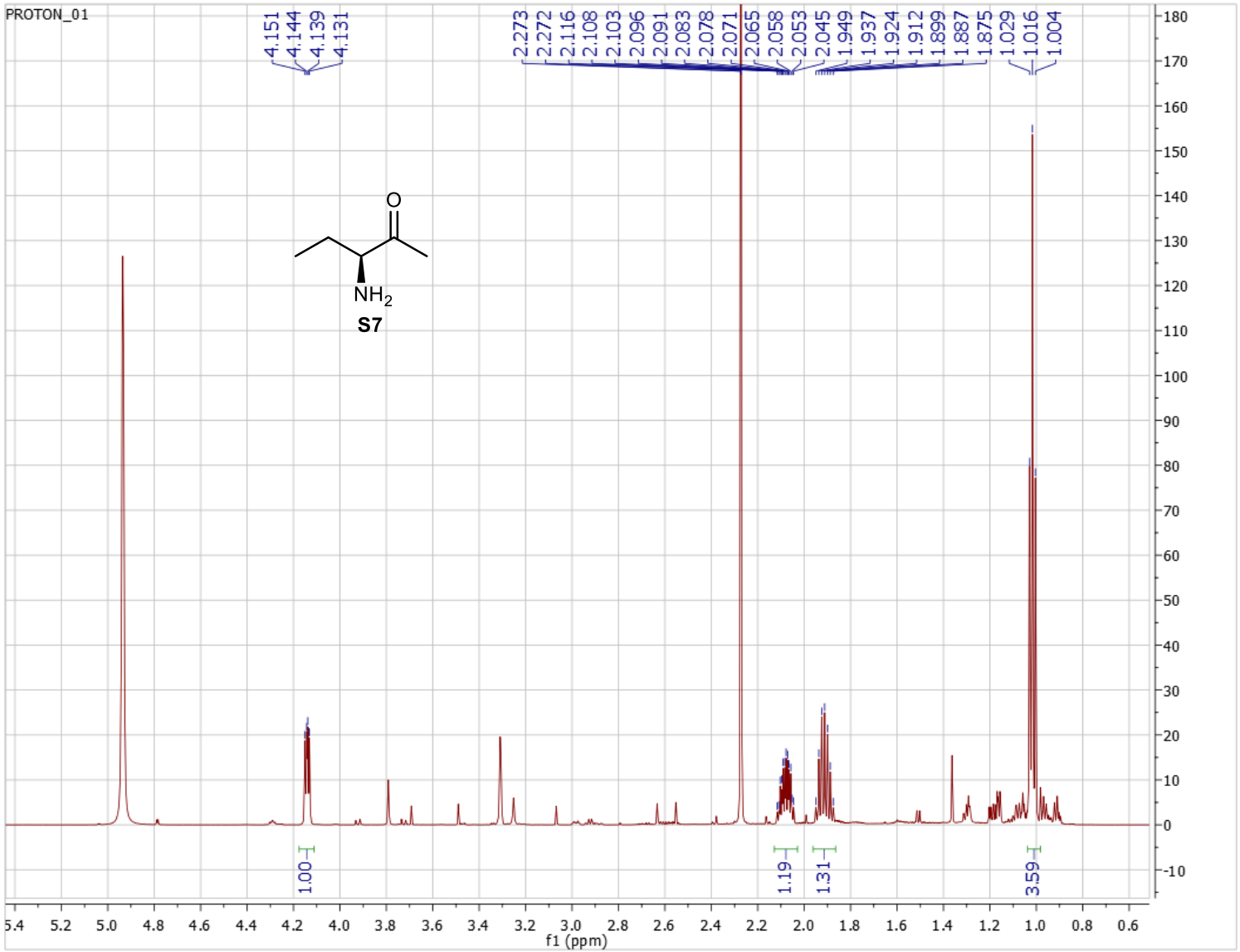
^{1}H NMR, 400 MHz, chloroform-*d*



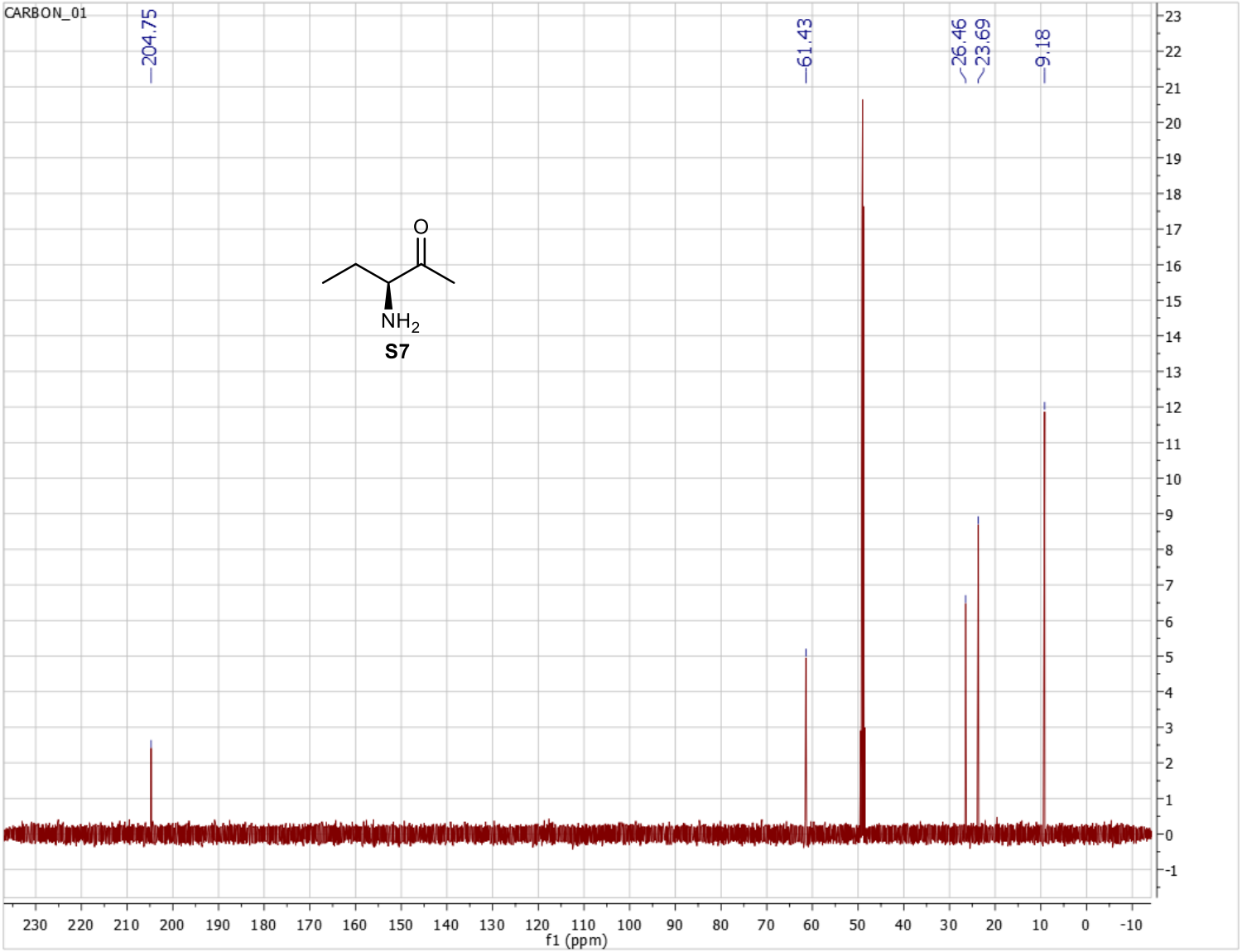
^{13}C NMR, 100 MHz, chloroform-*d*



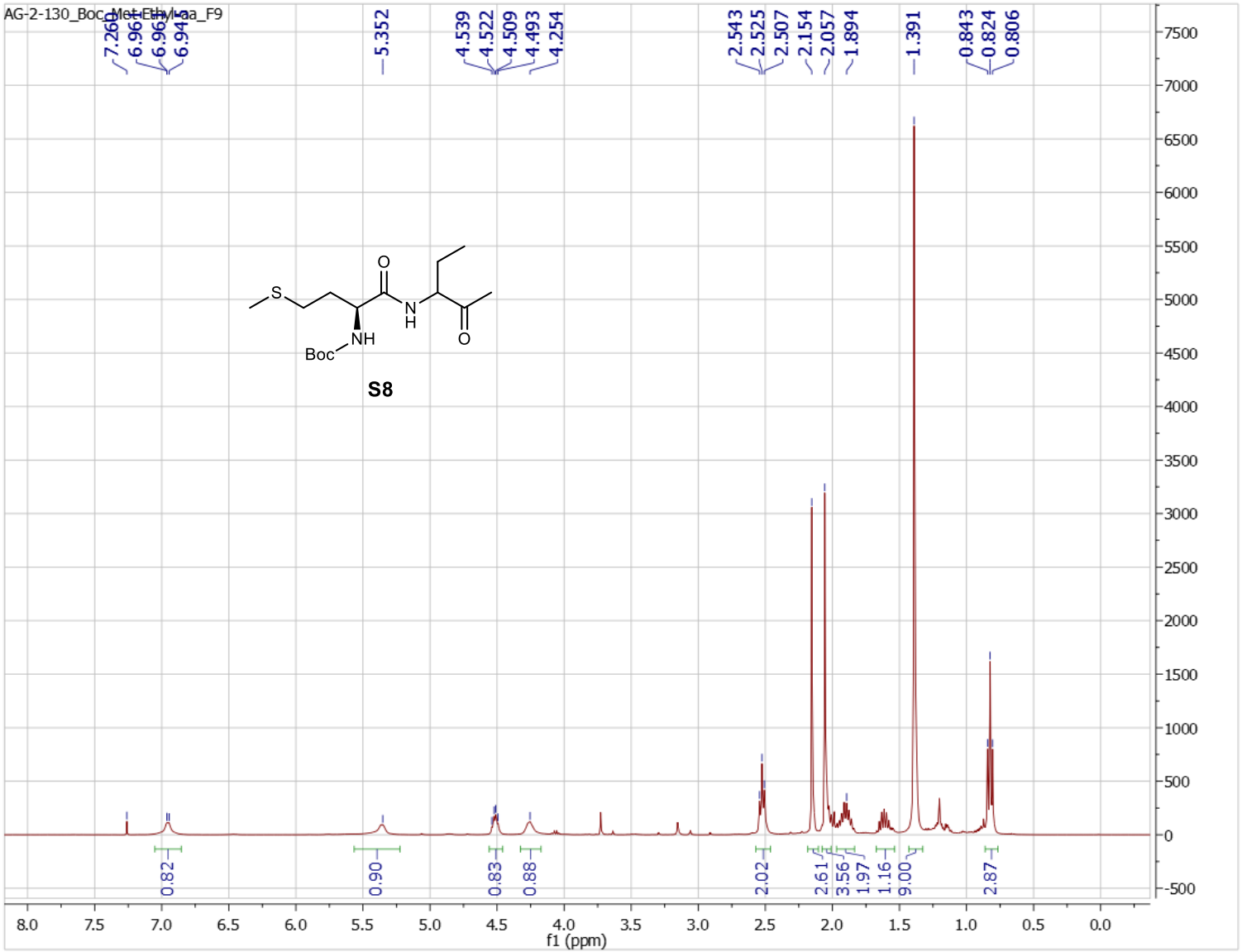
^{1}H NMR, 400 MHz, methanol-d_4



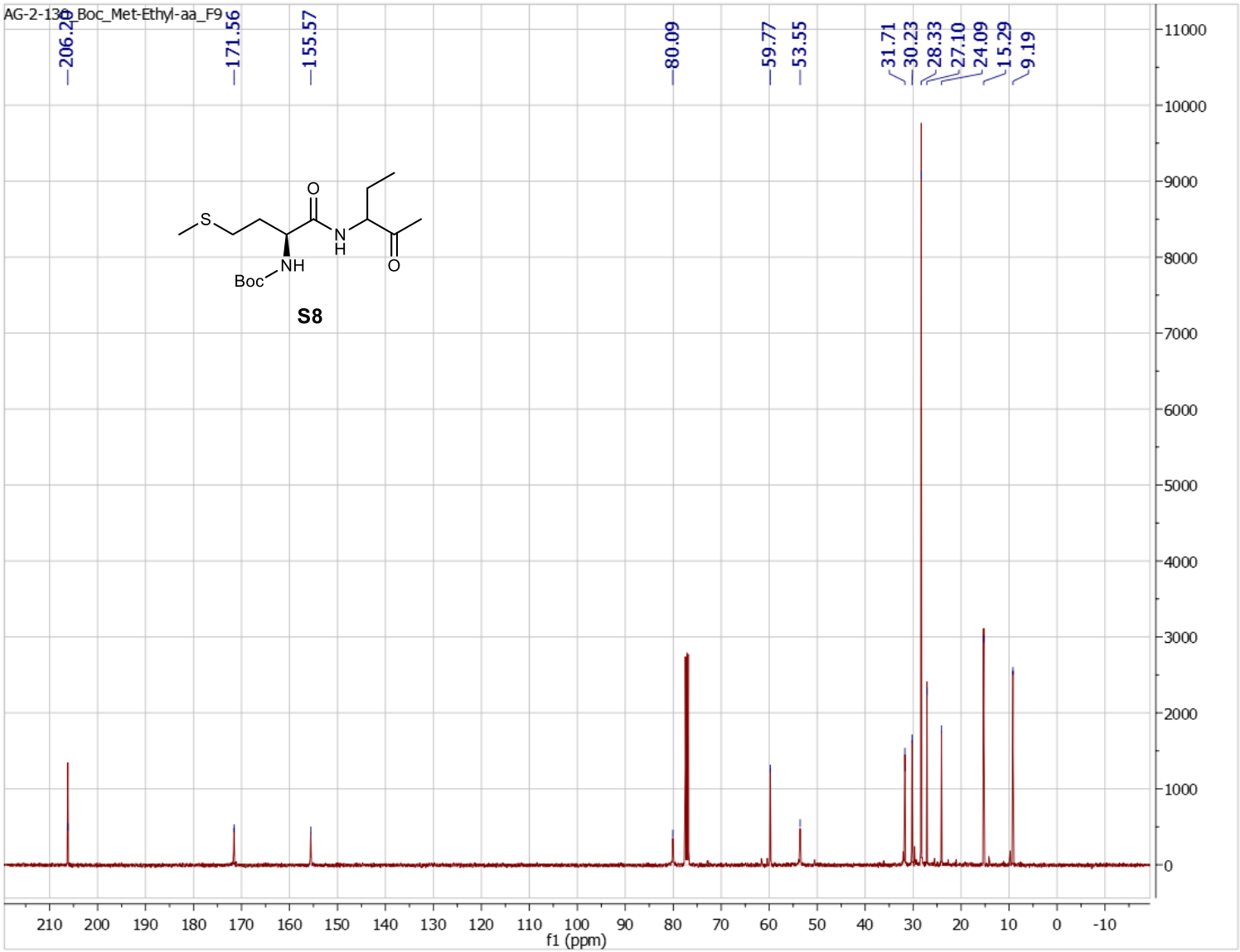
^{13}C NMR, 100 MHz, methanol-d_4



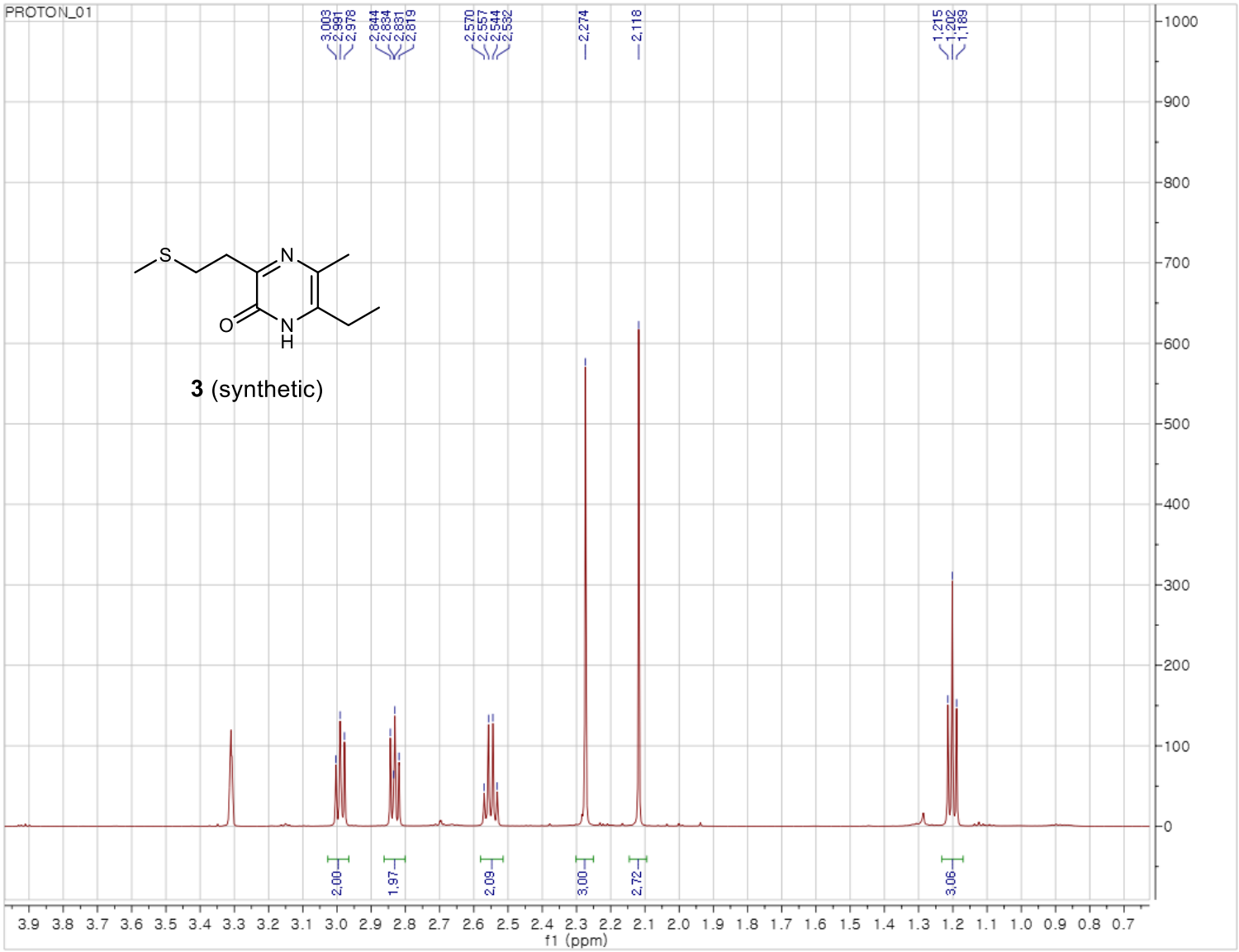
^{1}H NMR, 400 MHz, chloroform-*d*



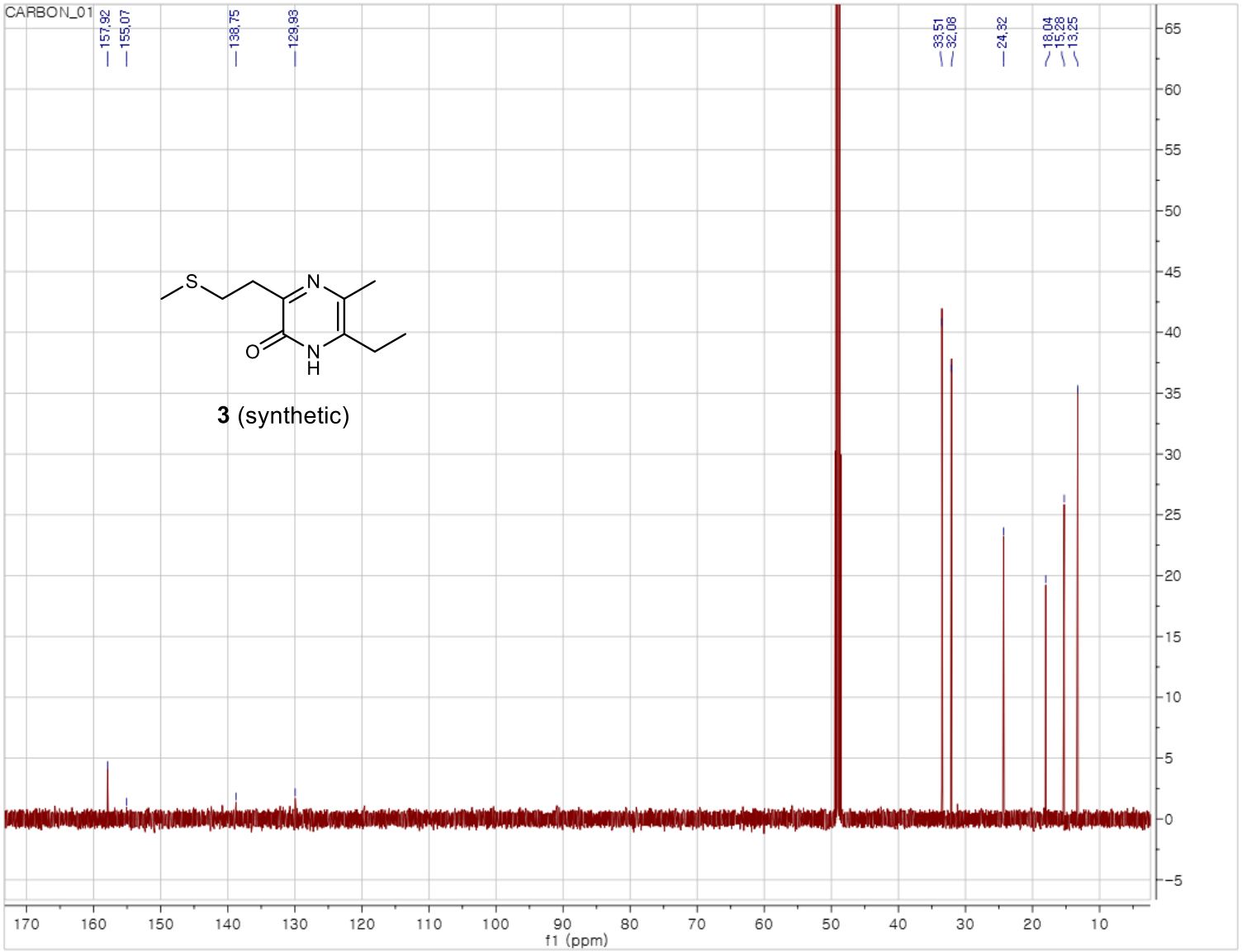
^{13}C NMR, 100 MHz, chloroform-*d*



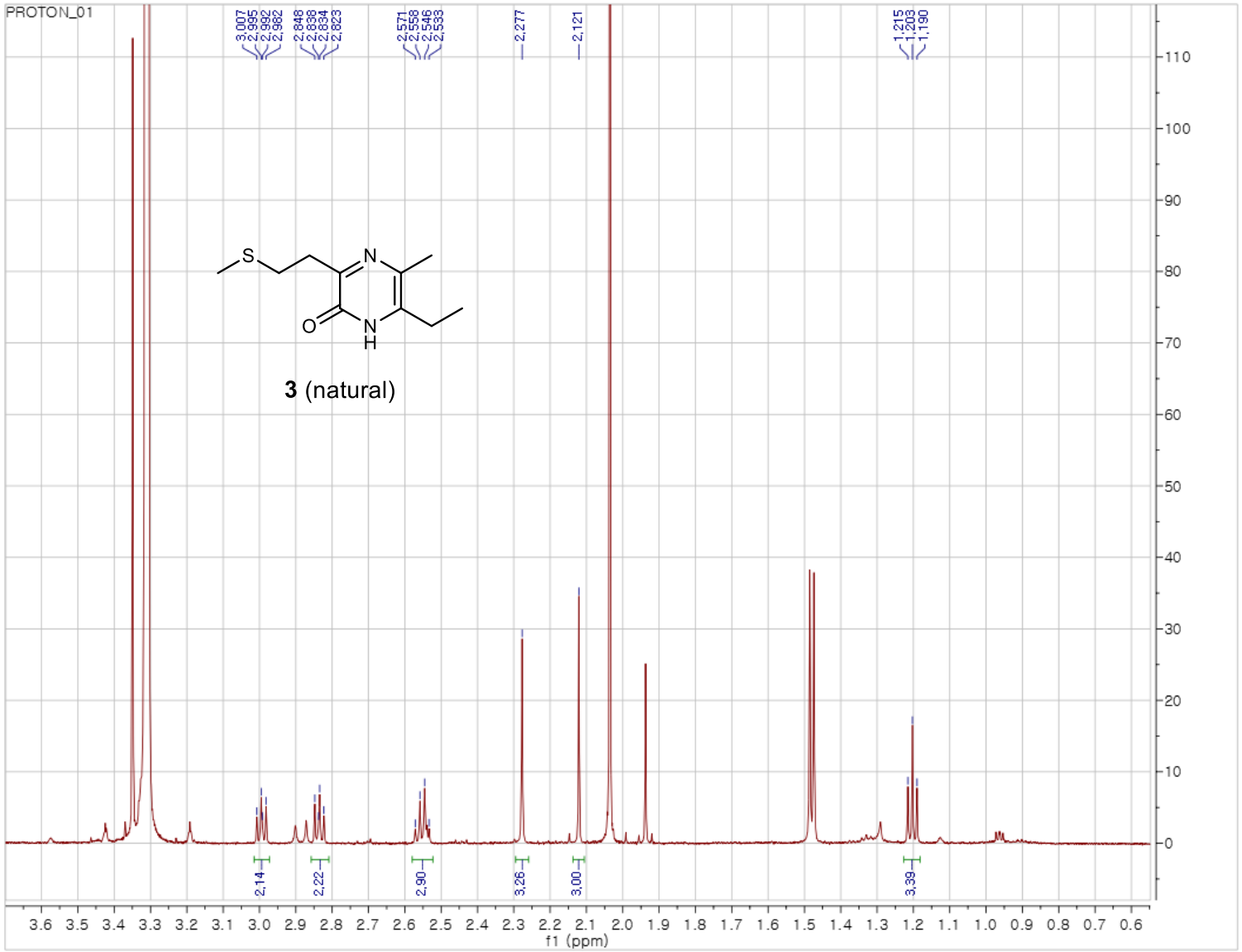
^{1}H NMR, 600 MHz, methanol-d_4



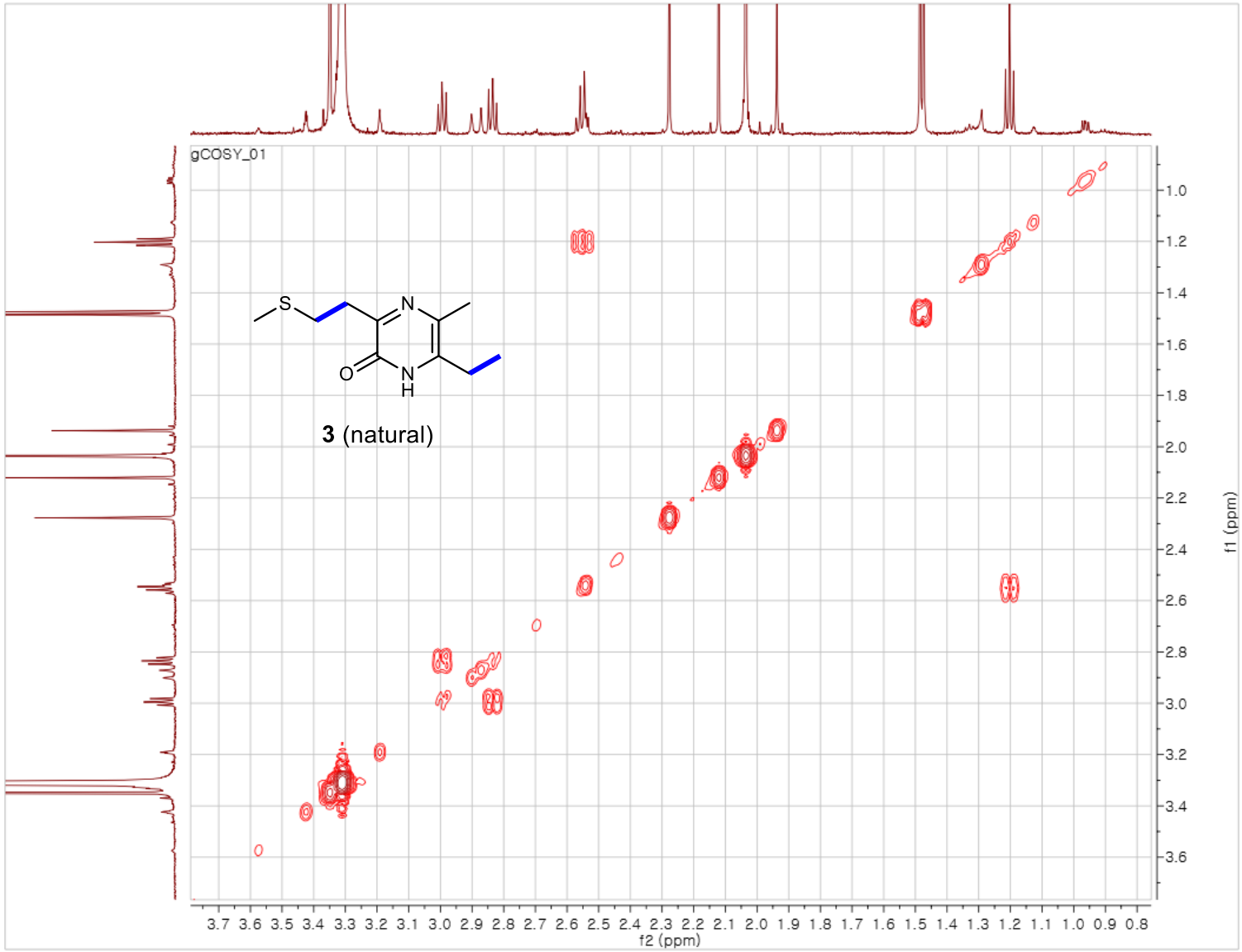
^{13}C NMR, 150 MHz, methanol-d_4



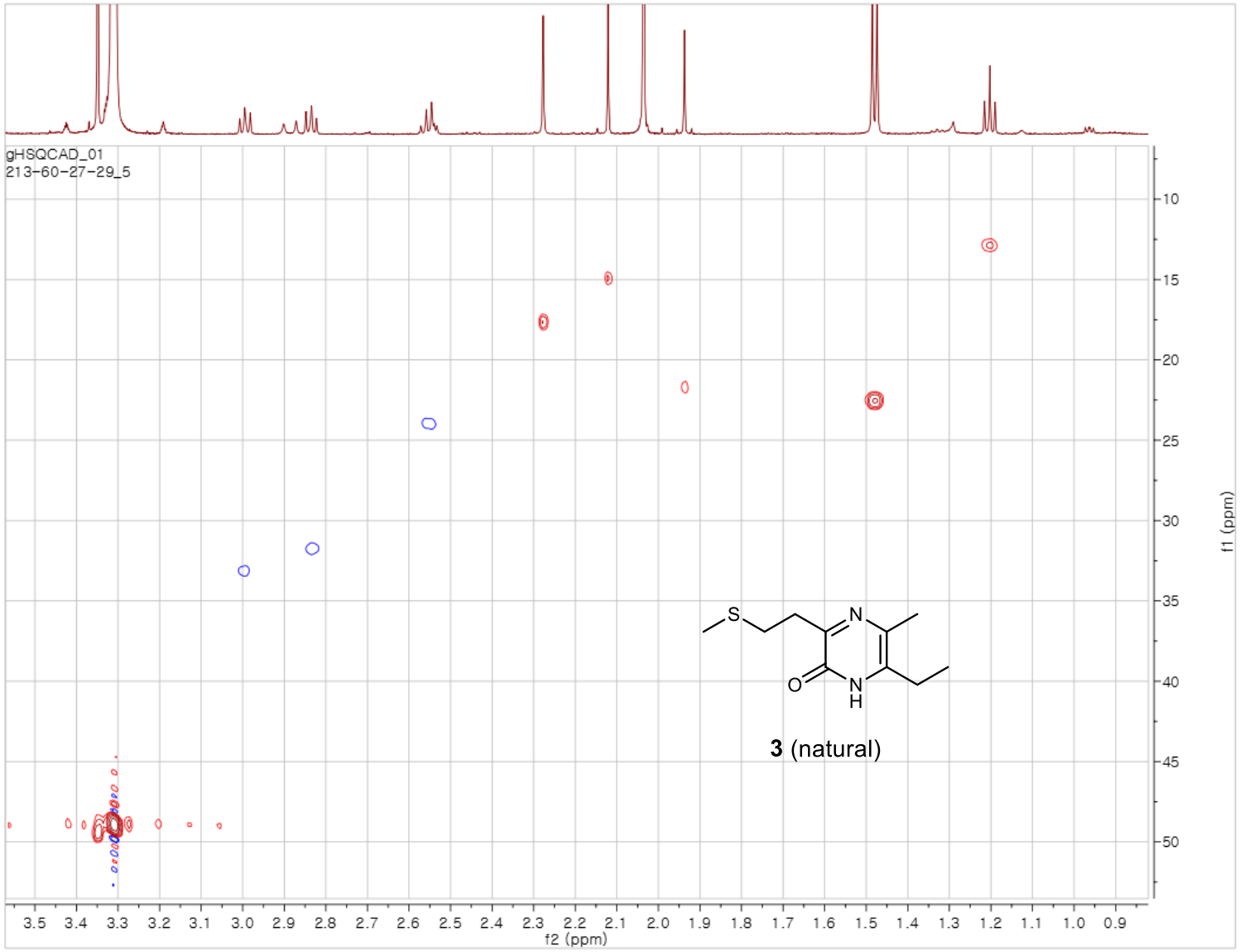
^{1}H NMR, 600 MHz, methanol-d_4



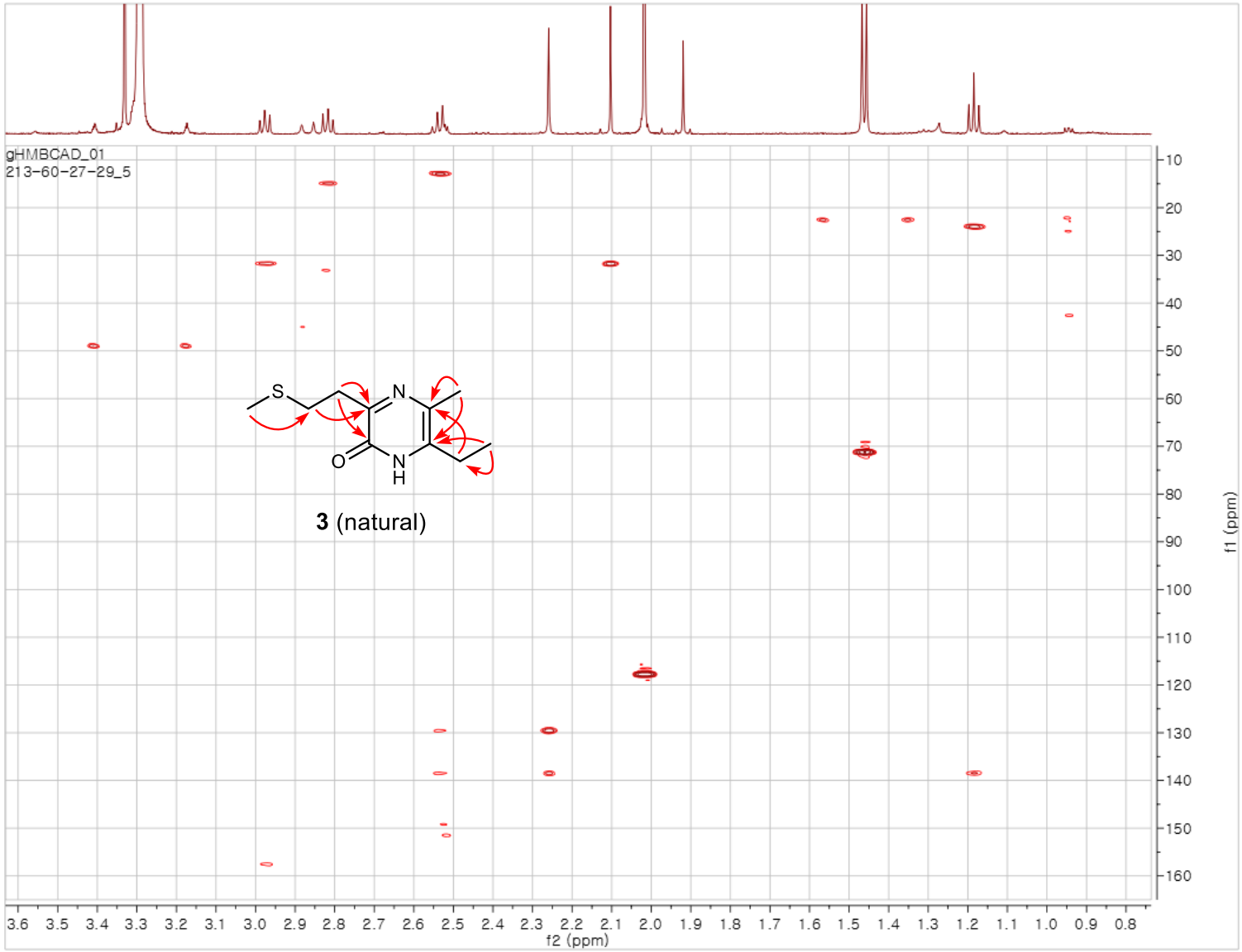
^{1}H-^{1}H COSY, 600 MHz, methanol-d_4



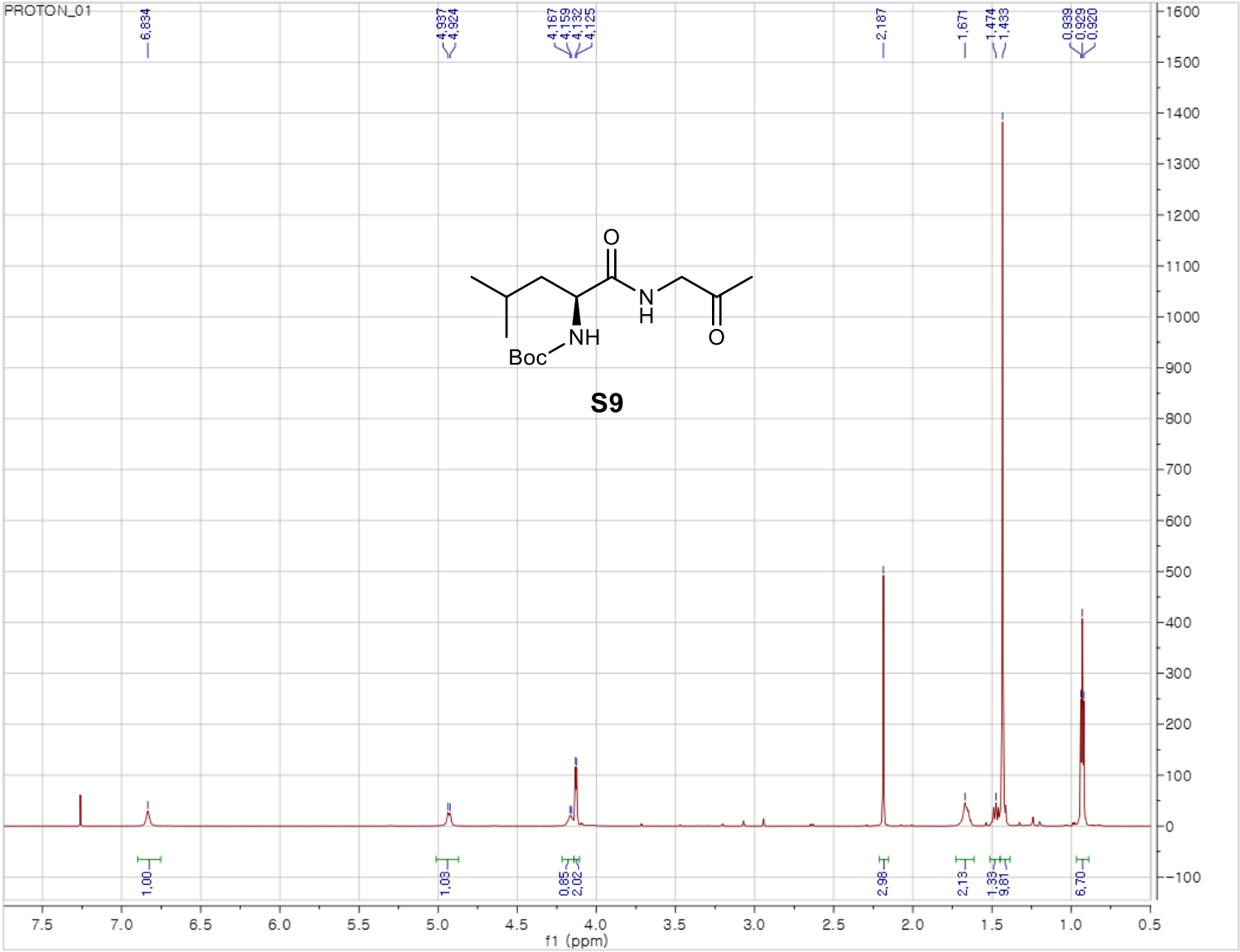
^{1}H-^{13}C HSQCAD, 600 MHz, methanol-d_4



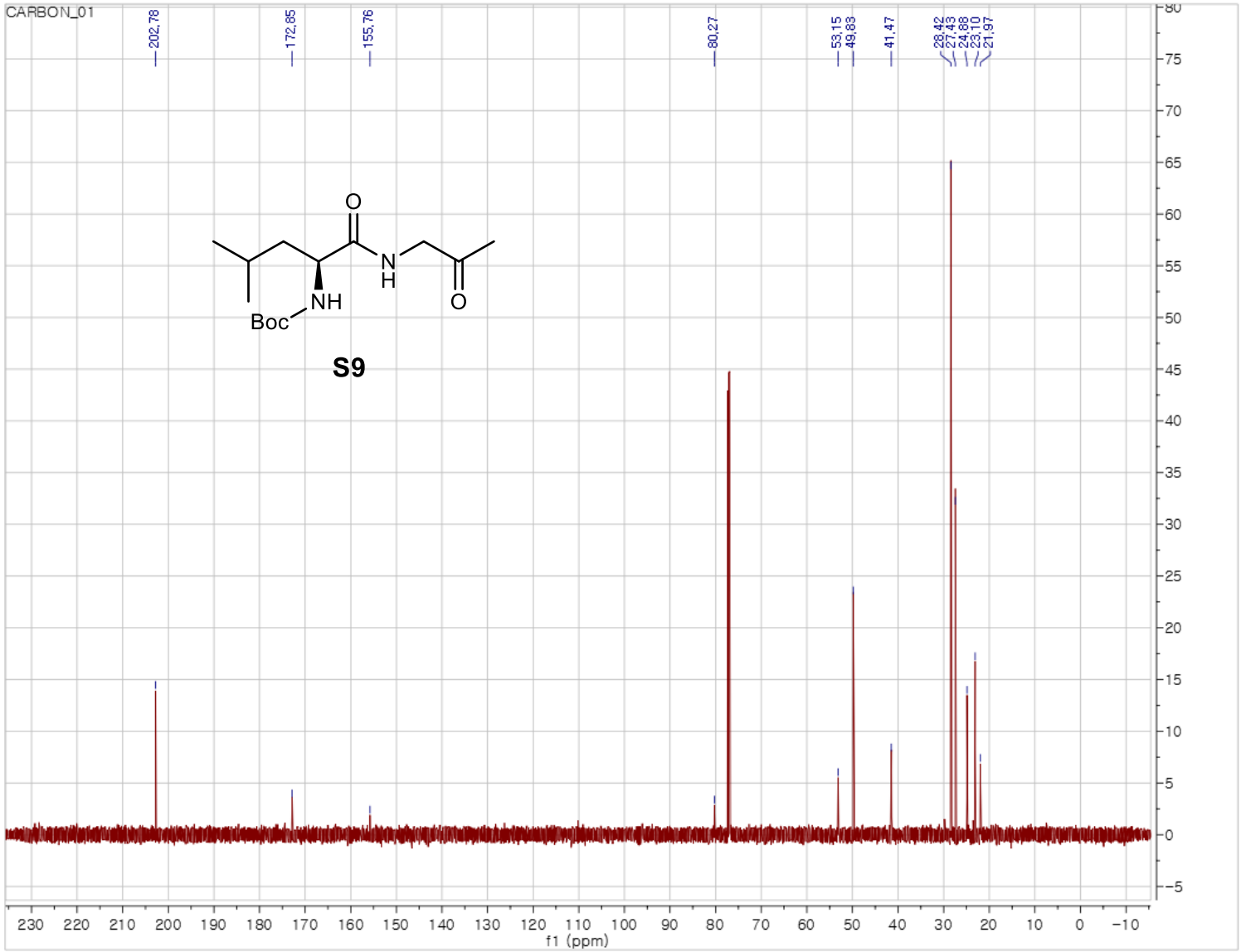
^{1}H-^{13}C HMBCAD, 600 MHz, methanol-d_4



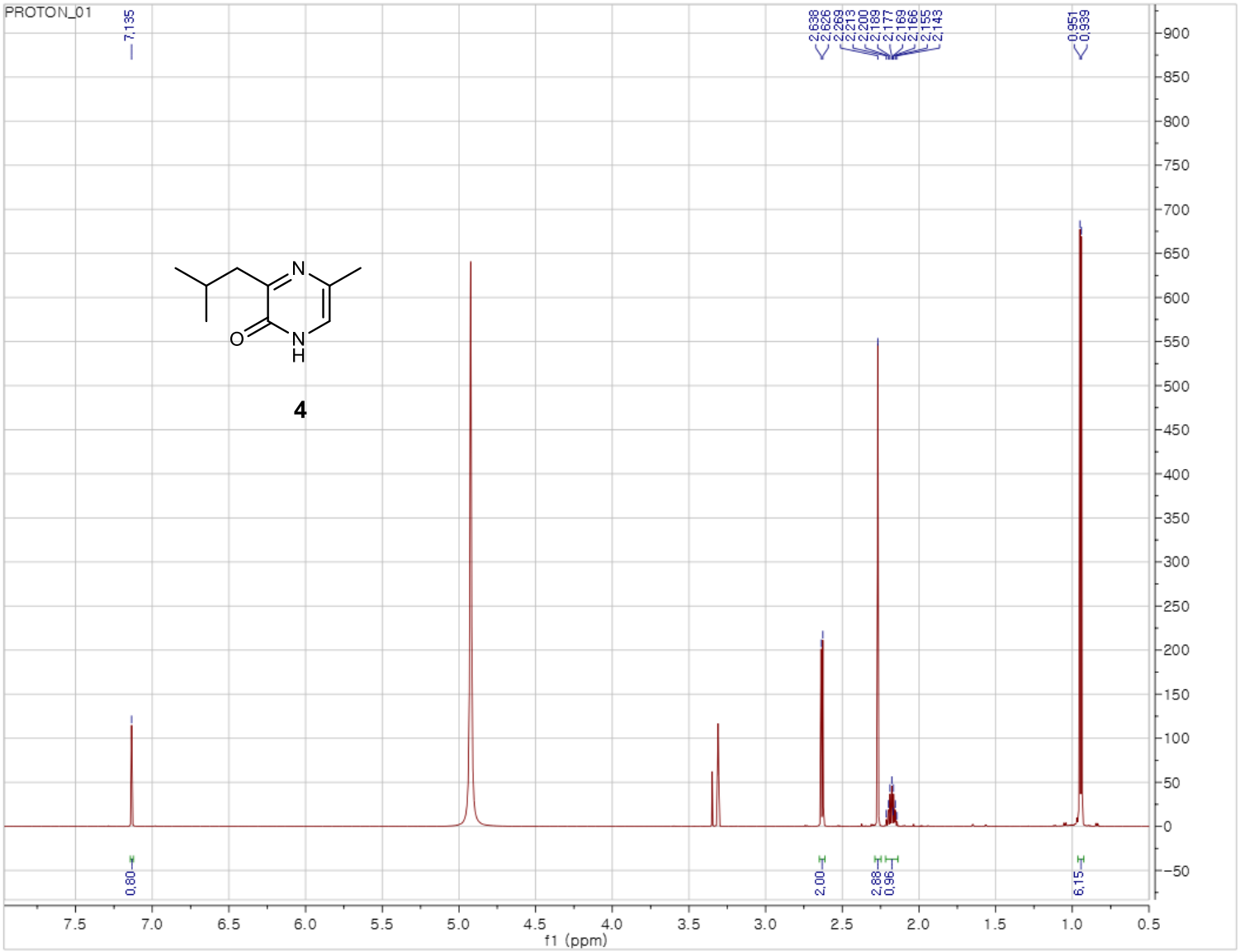
^{1}H NMR, 600 MHz, chloroform-*d*



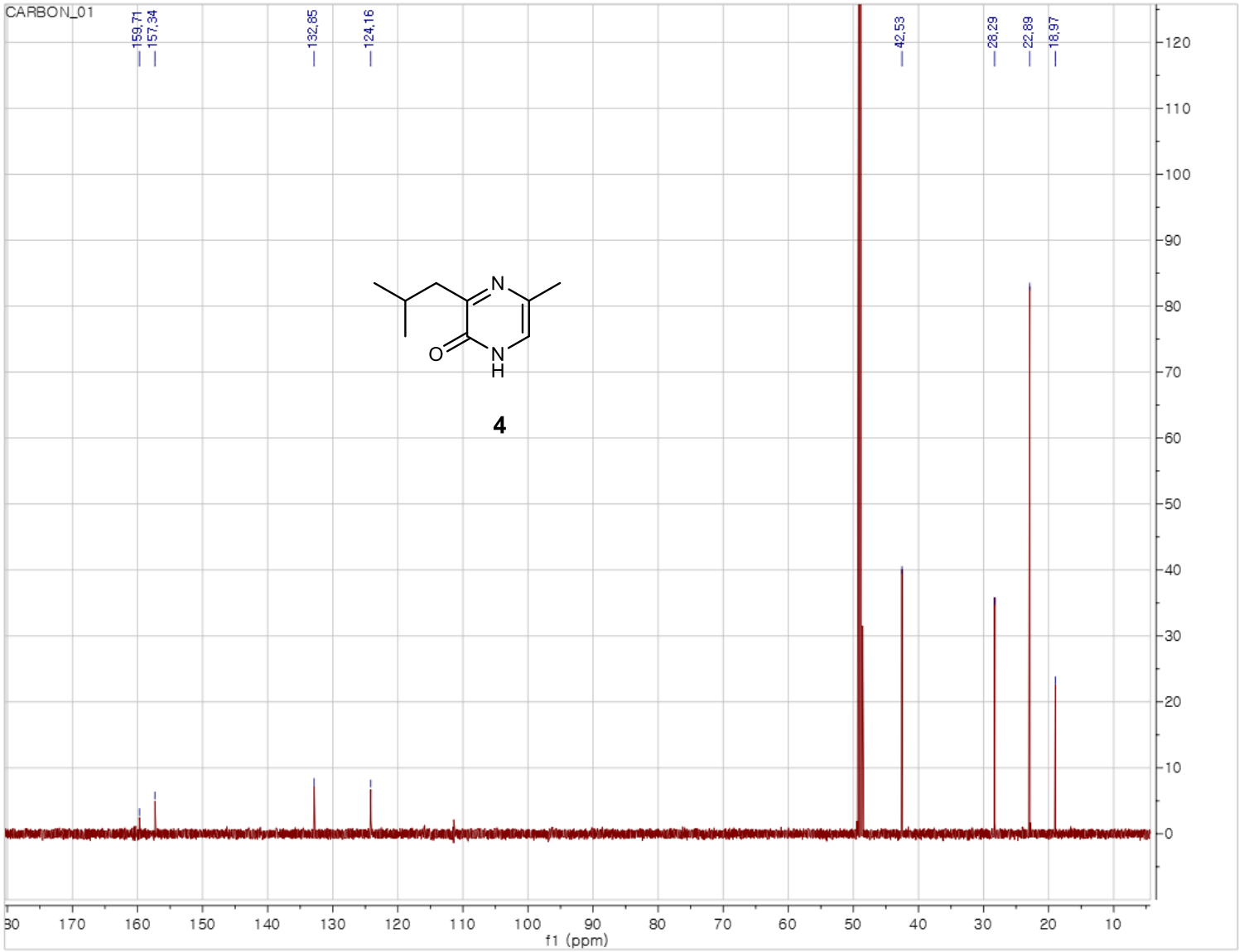
^{13}C NMR, 150 MHz, chloroform-*d*



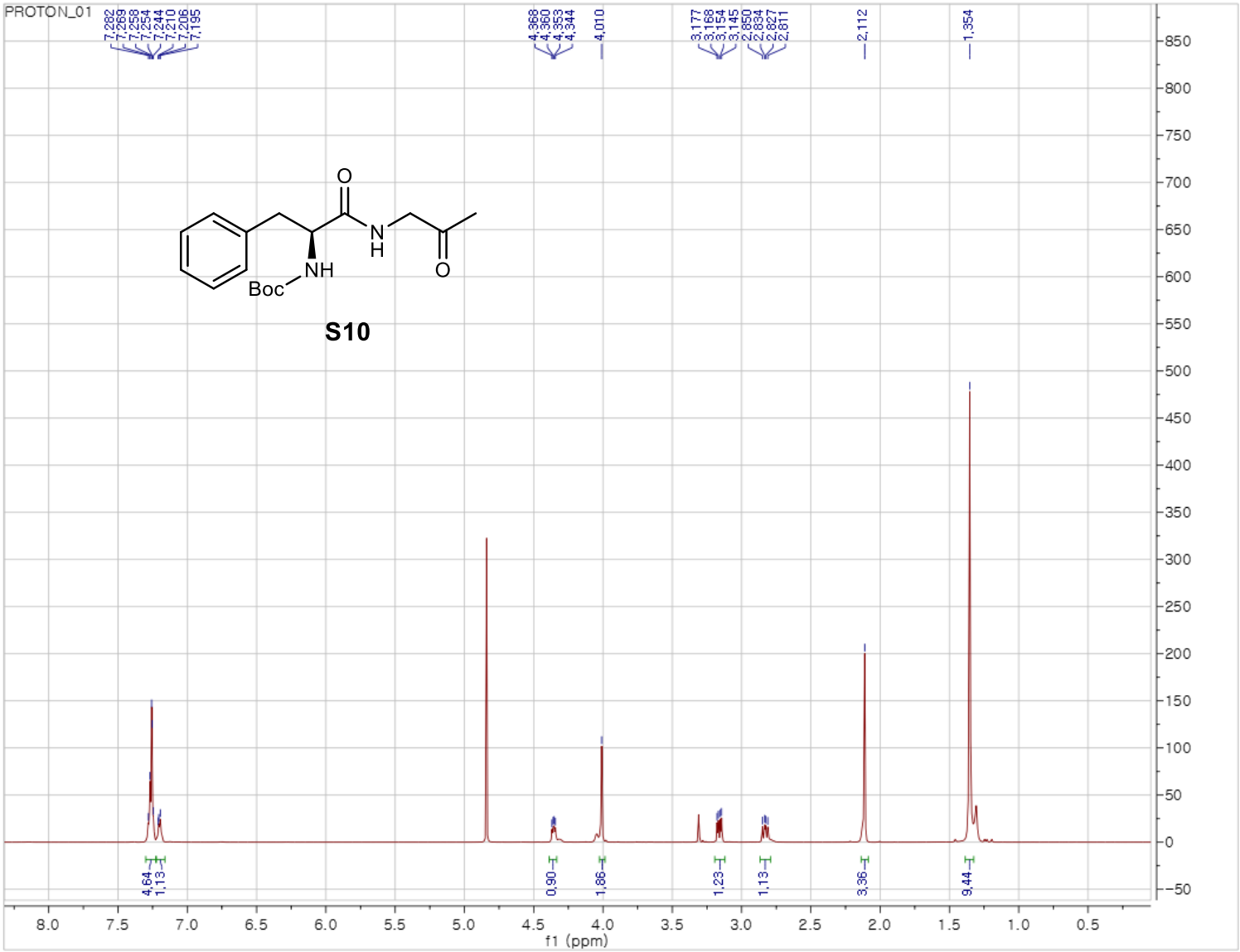
^{1}H NMR, 600 MHz, methanol-d_4



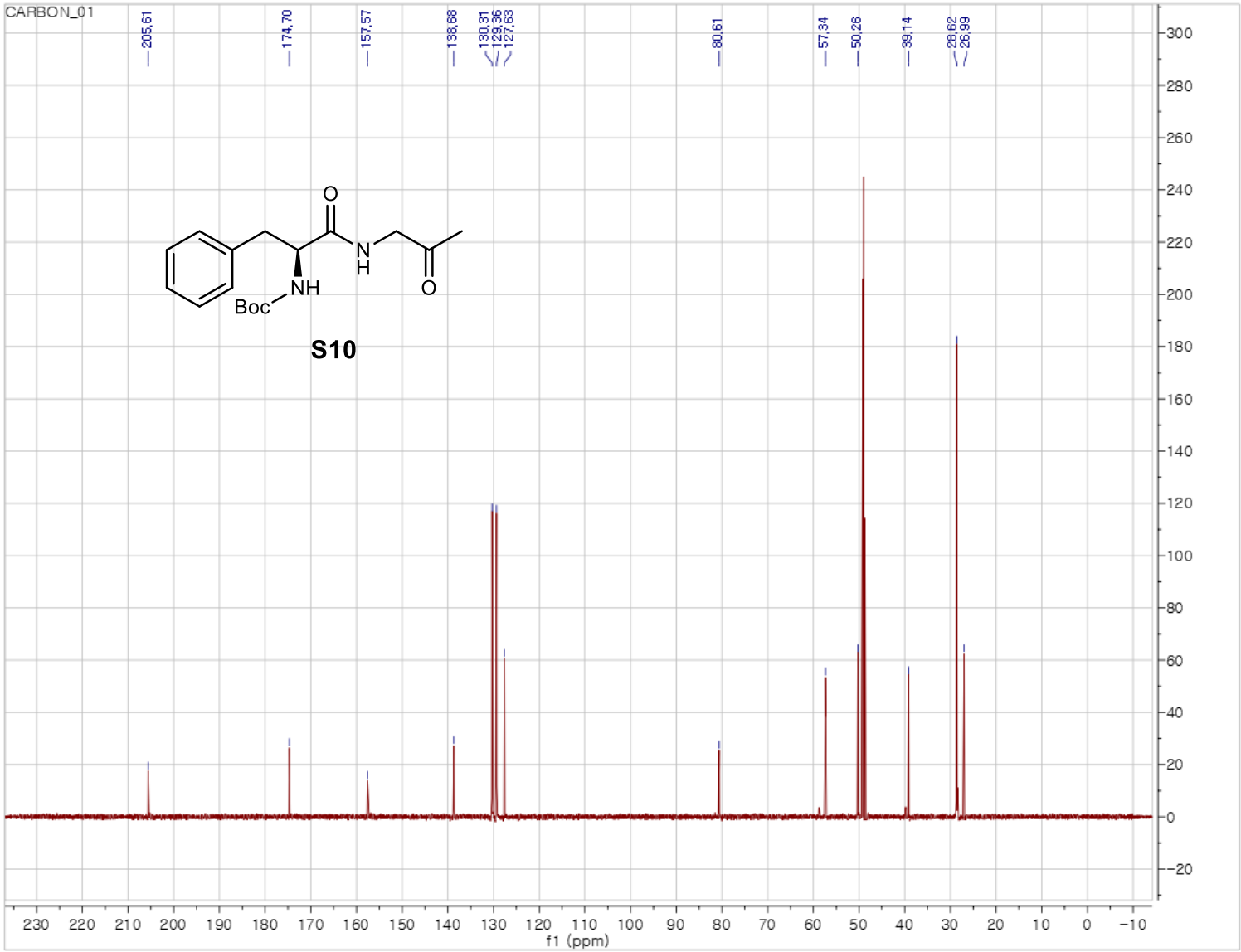
^{13}C NMR, 150 MHz, methanol-d_4



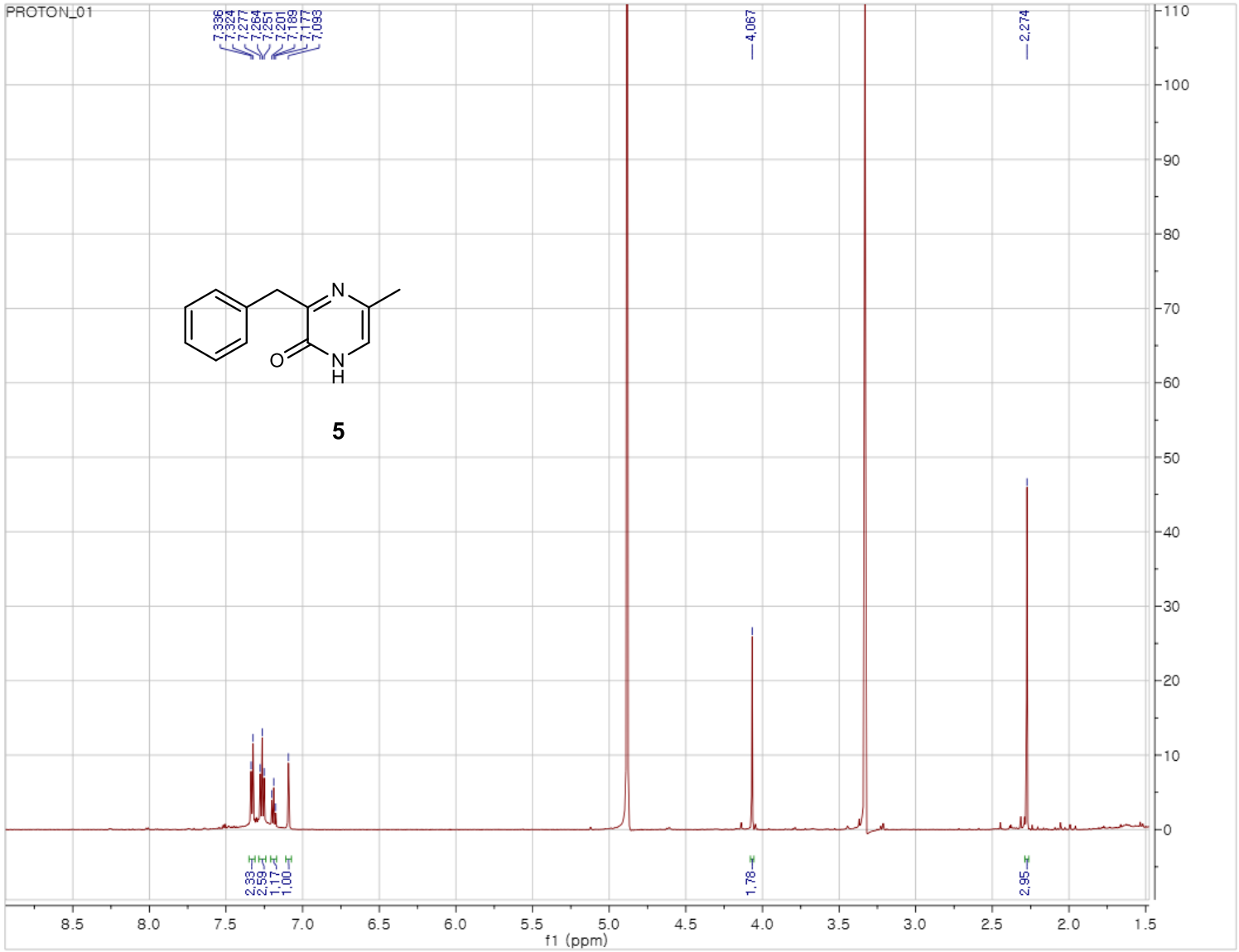
^{1}H NMR, 600 MHz, methanol-d_4



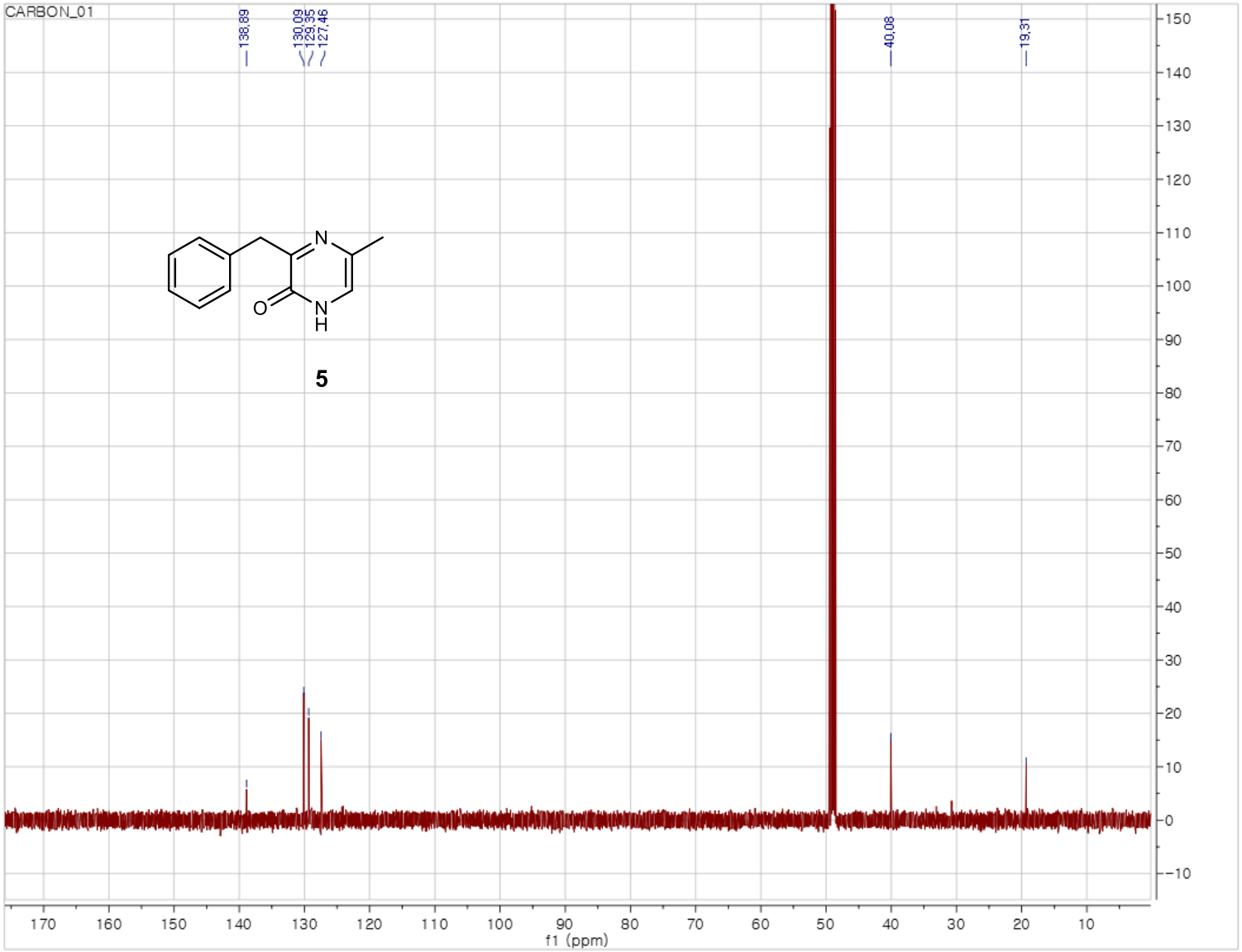
^{13}C NMR, 150 MHz, methanol-d_4



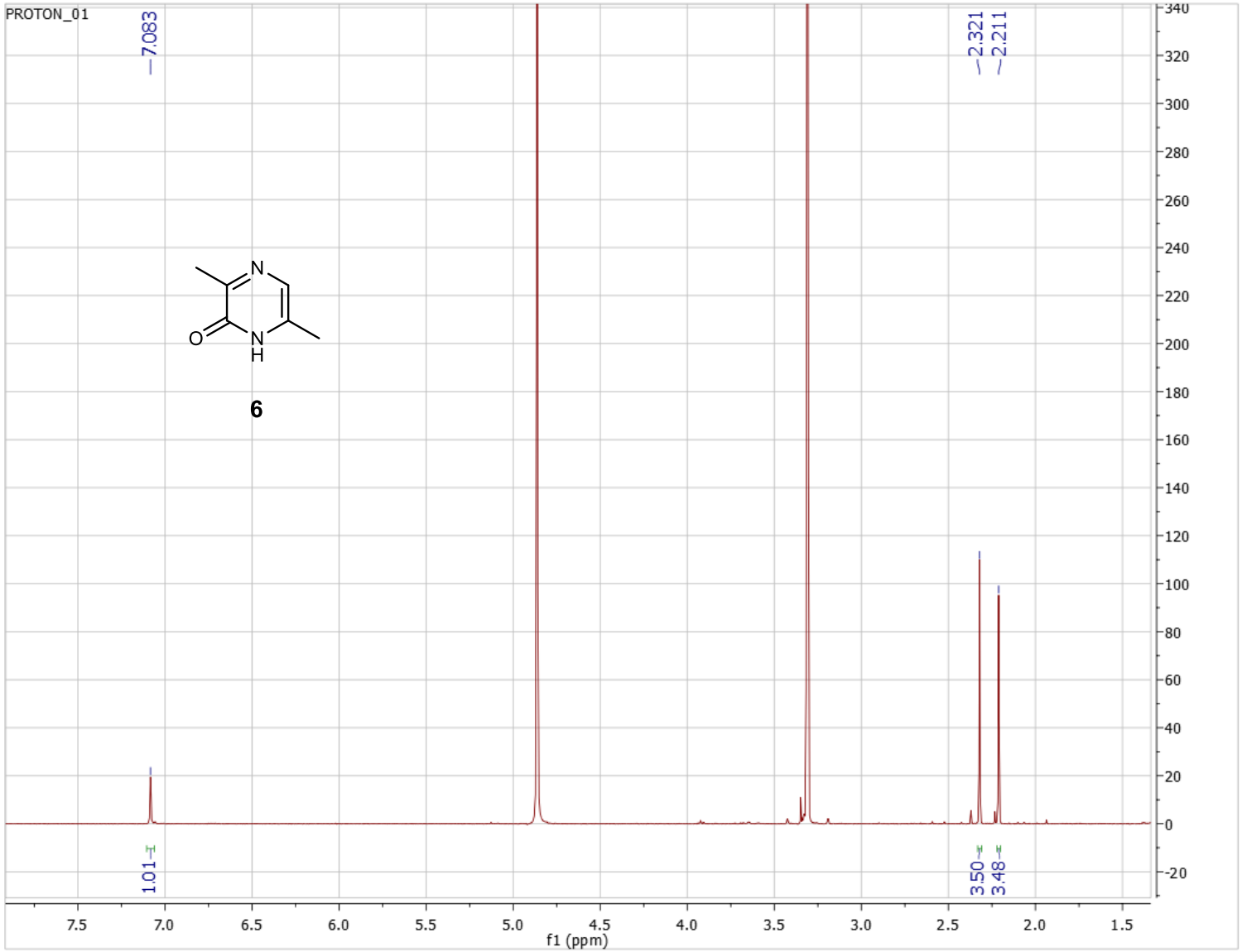
^{1}H NMR, 600 MHz, methanol-d_4



^{13}C NMR, 150 MHz, methanol-d_4

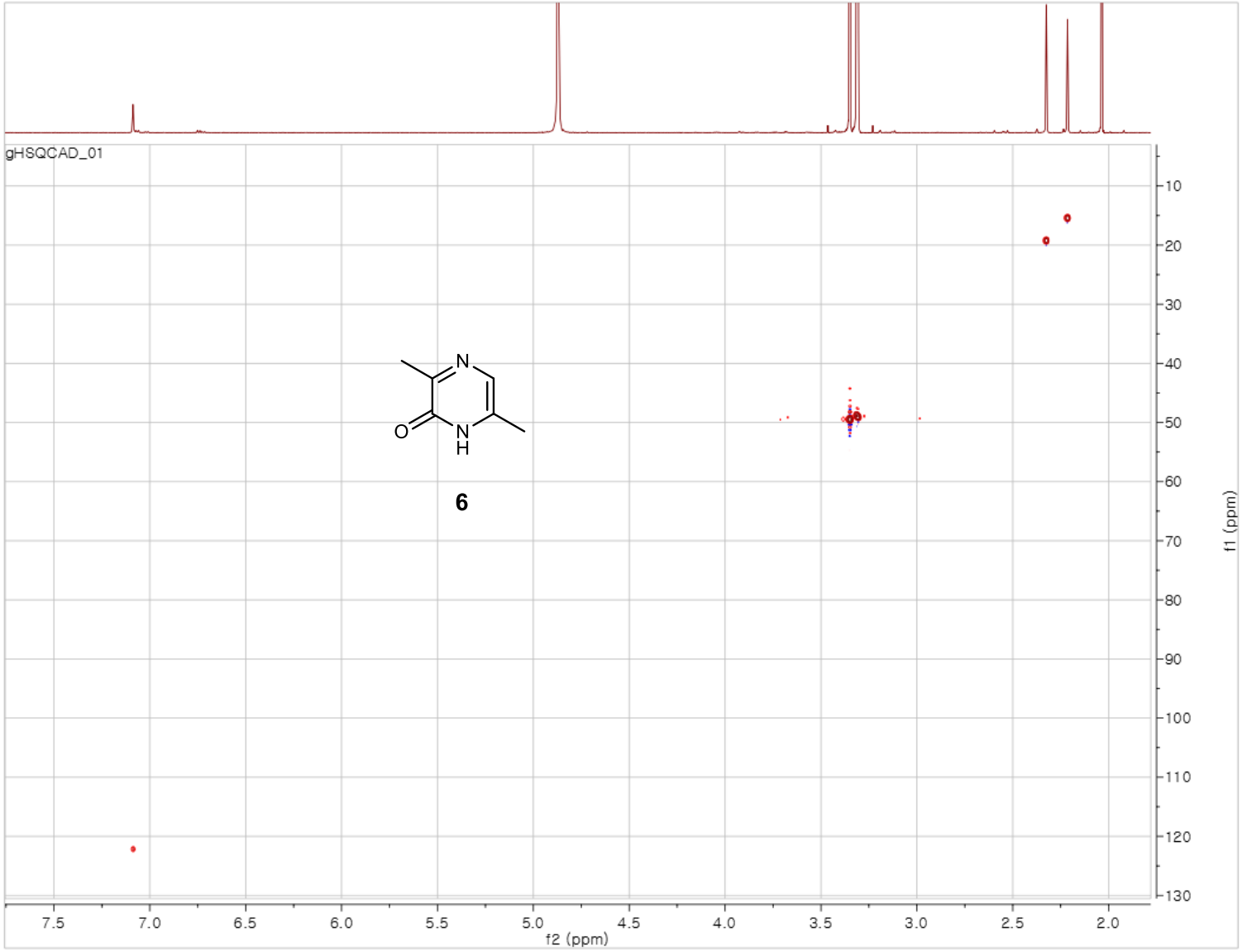


^{1}H NMR, 600 MHz, methanol-d_4

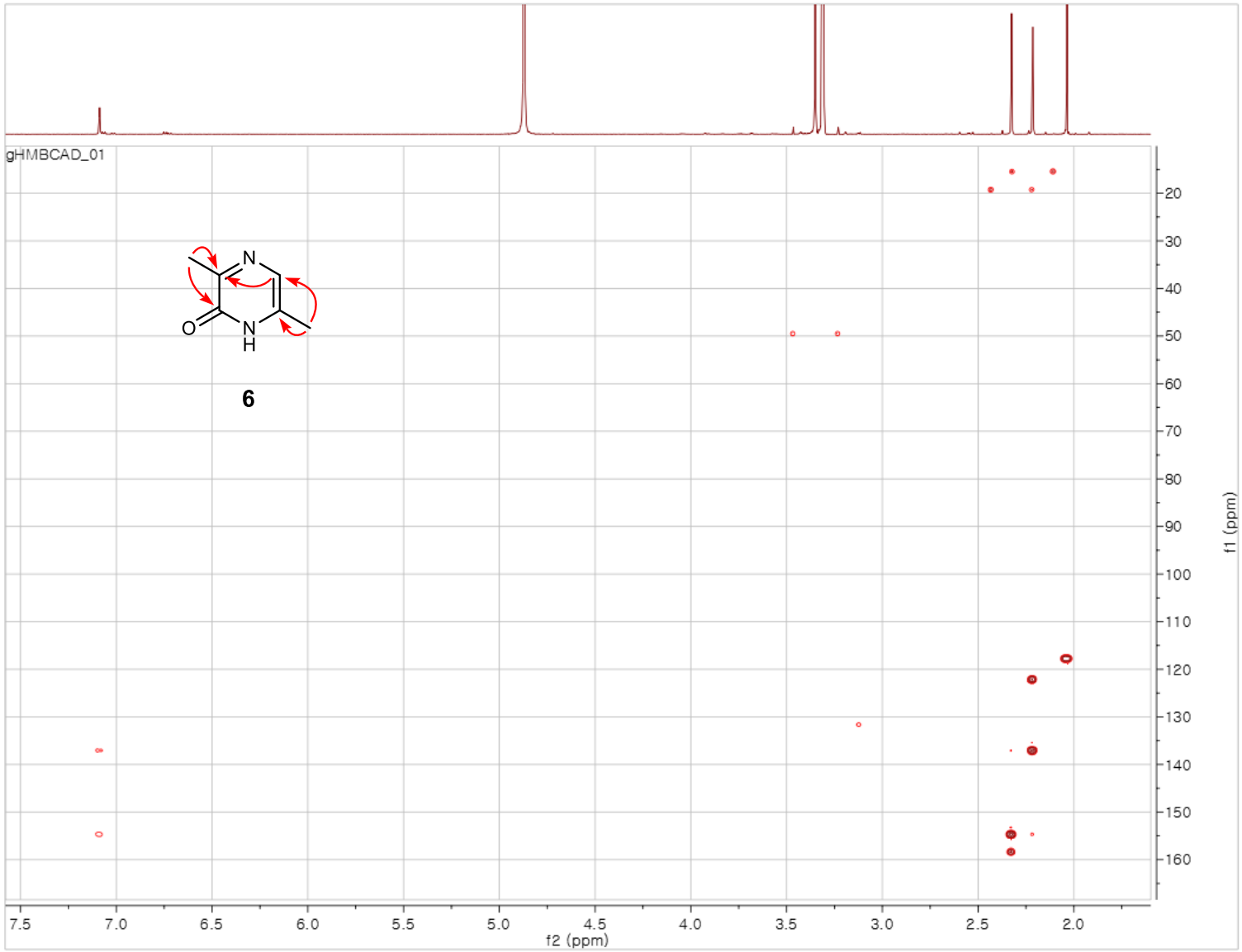


6

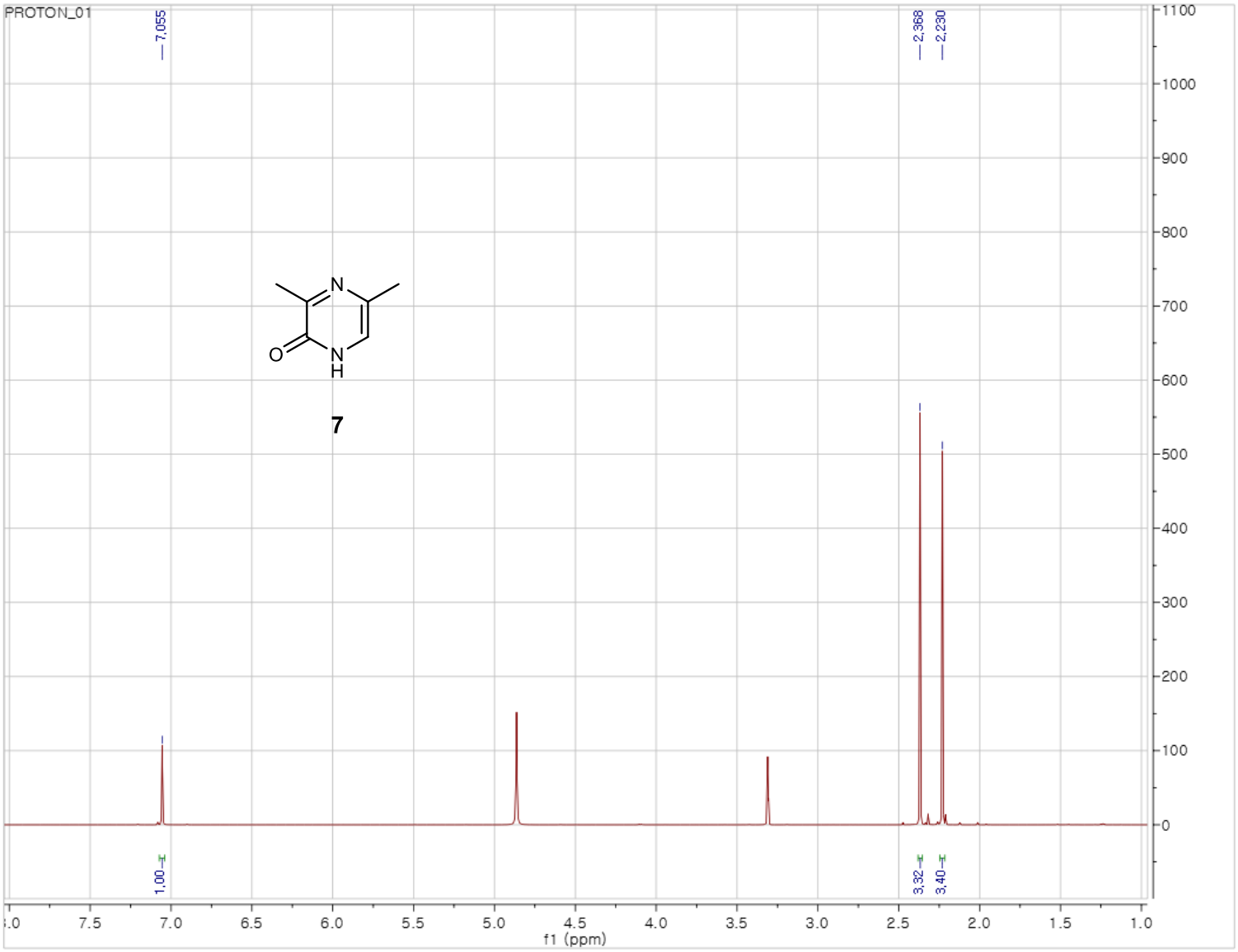
^{1}H-^{13}C HSQCAD, 600 MHz, methanol-d_4



^{1}H-^{13}C HMBCAD, 600 MHz, methanol-d_4

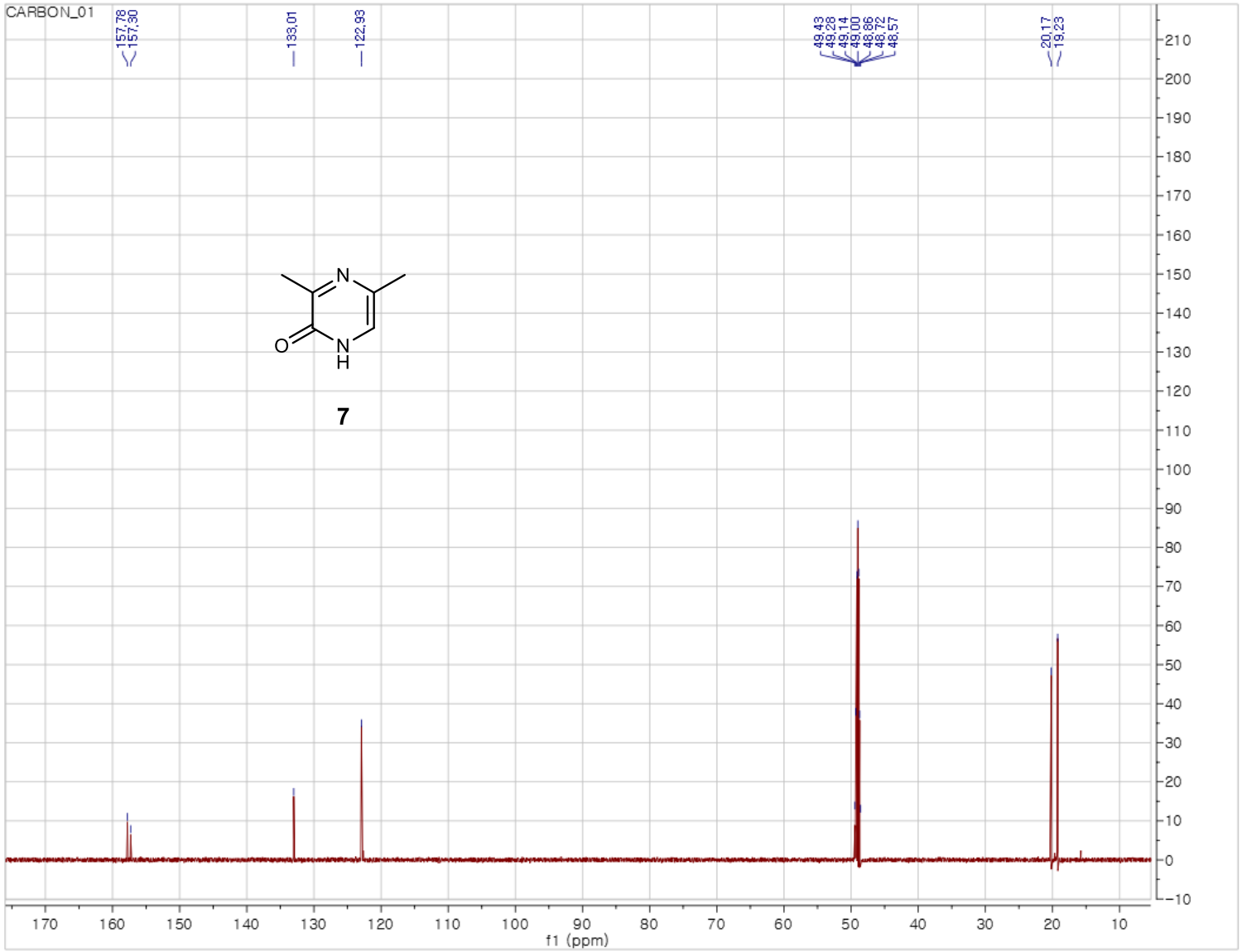


^{1}H NMR, 600 MHz, methanol-d_4

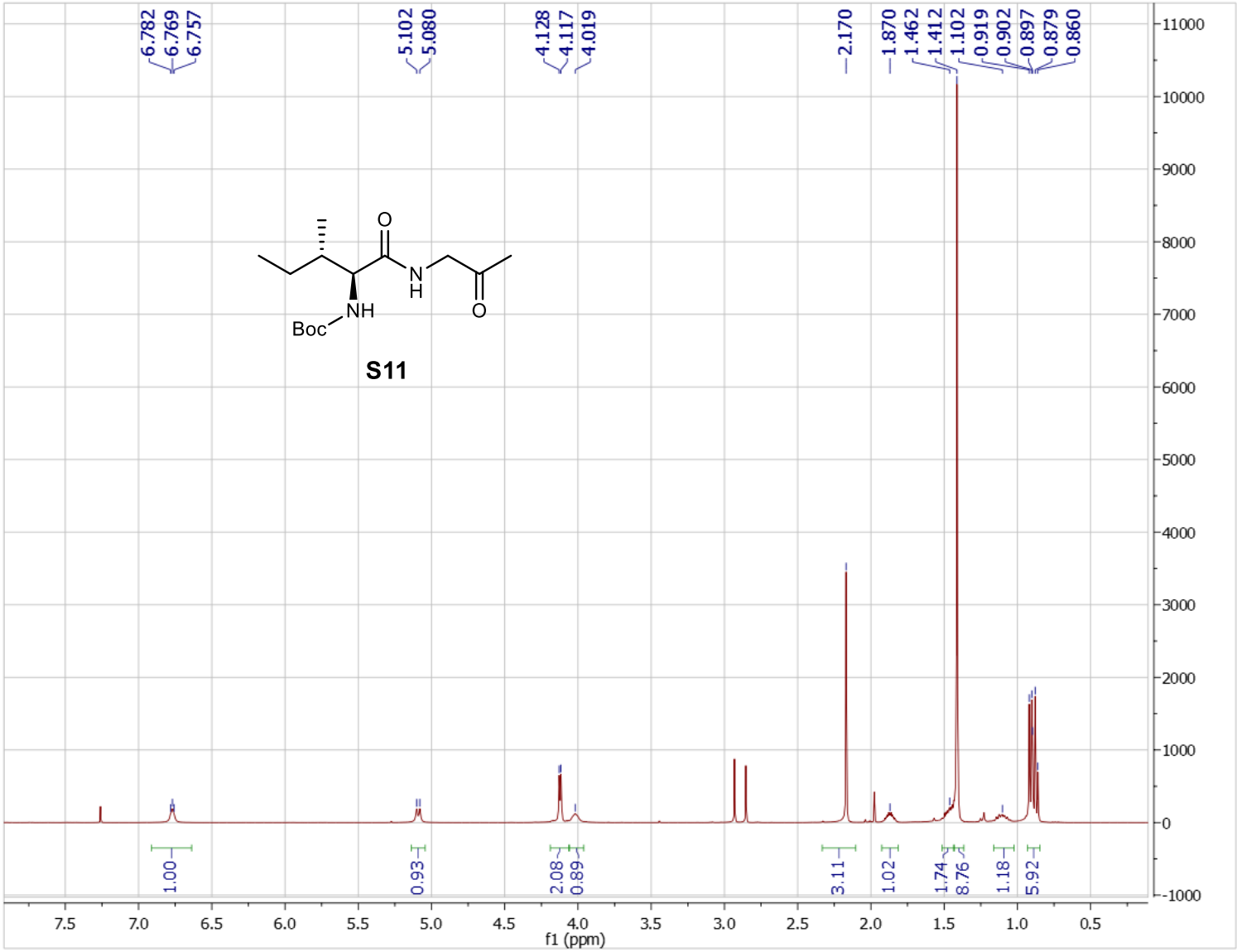


7

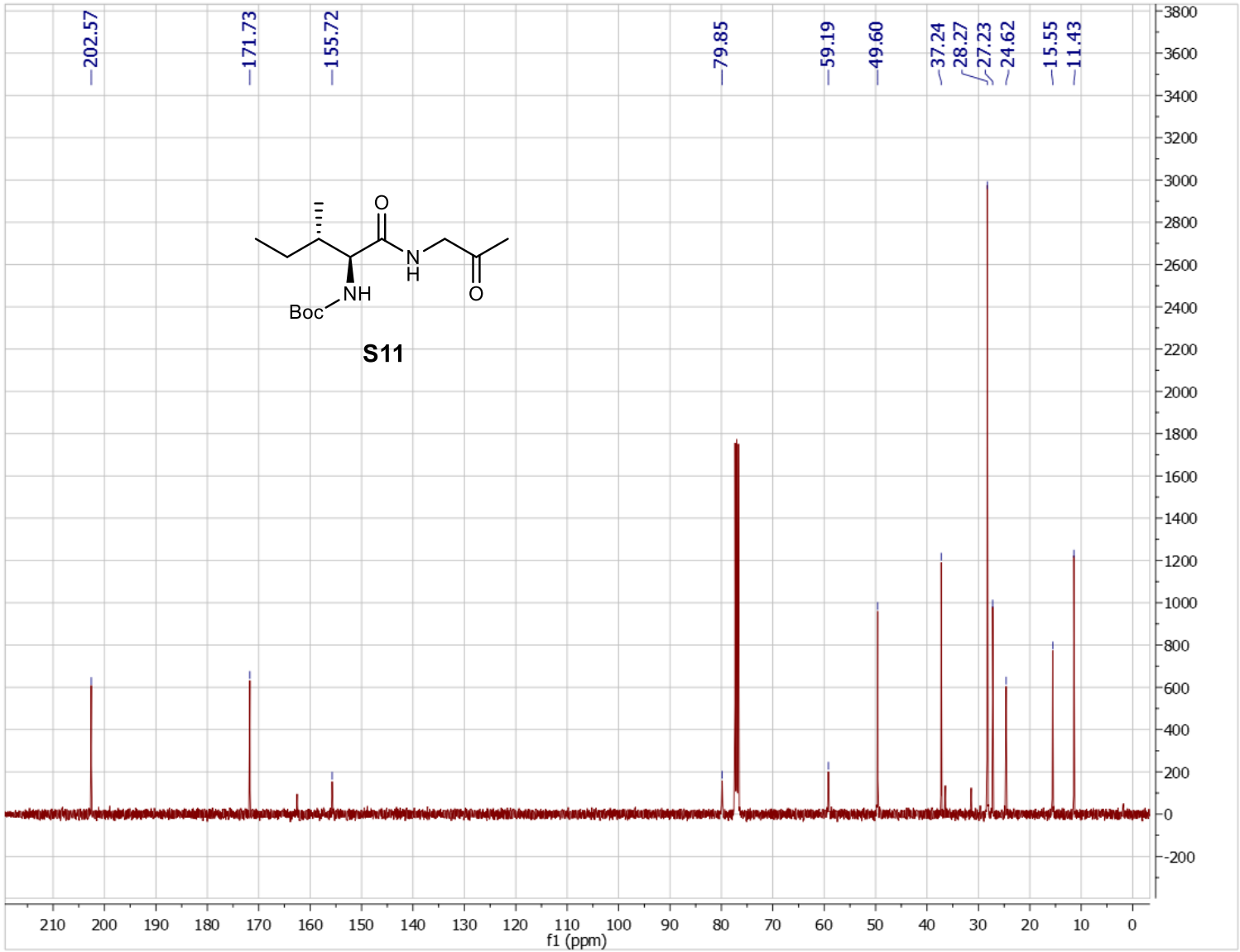
^{13}C NMR, 150 MHz, methanol-d_4



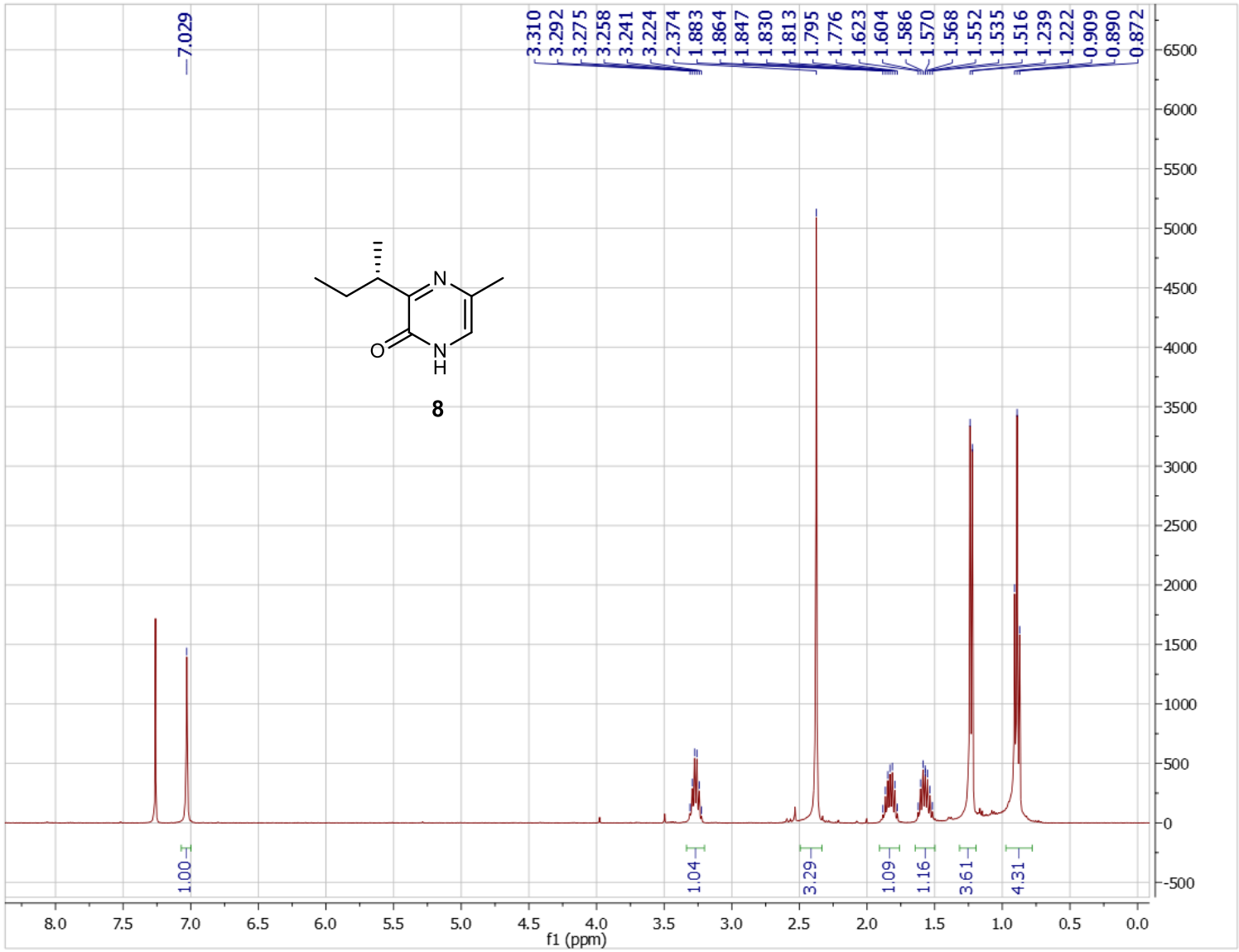
^{1}H NMR, 400 MHz, chloroform-*d*



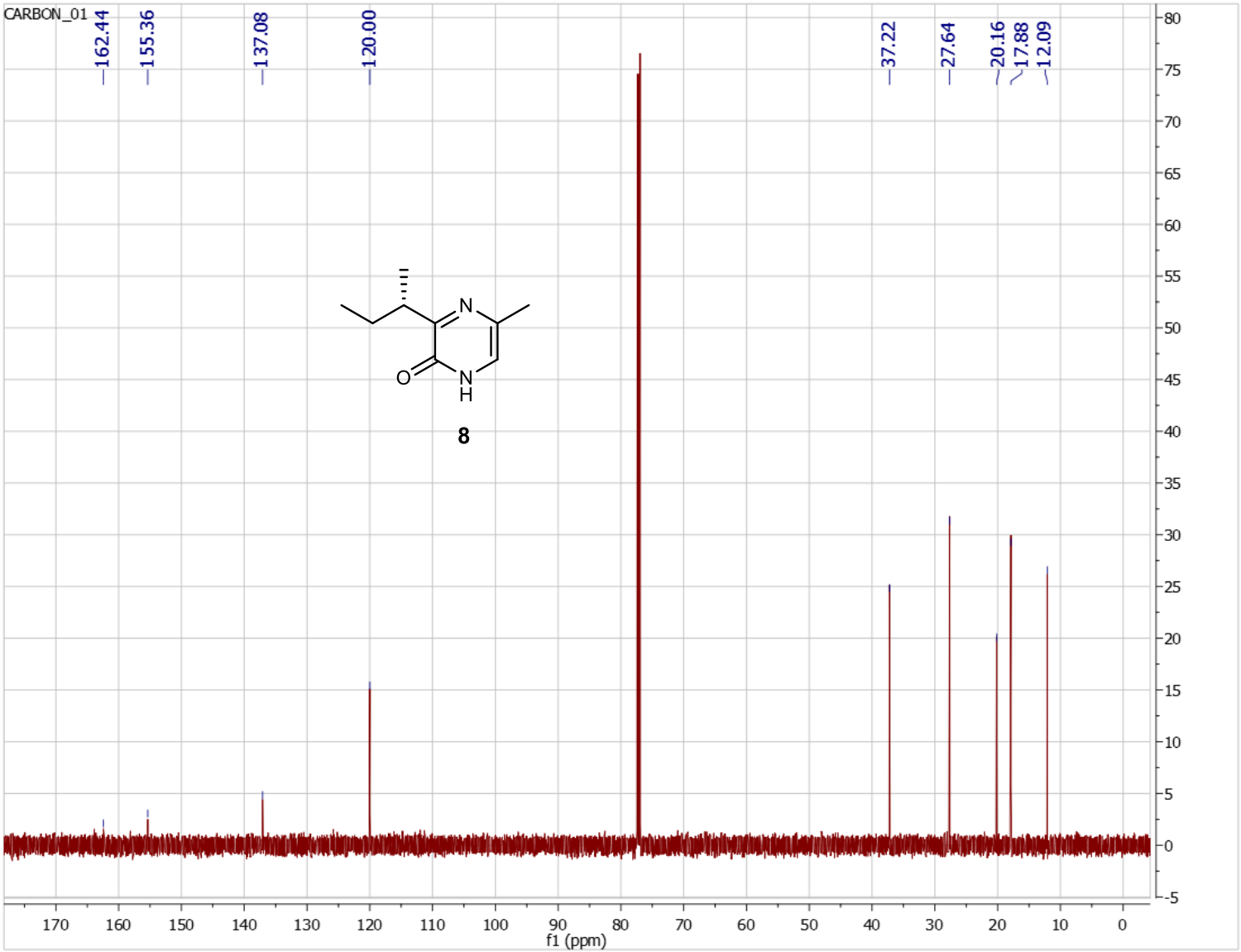
^{13}C NMR, 100 MHz, chloroform-*d*



^{1}H NMR, 400 MHz, chloroform-*d*

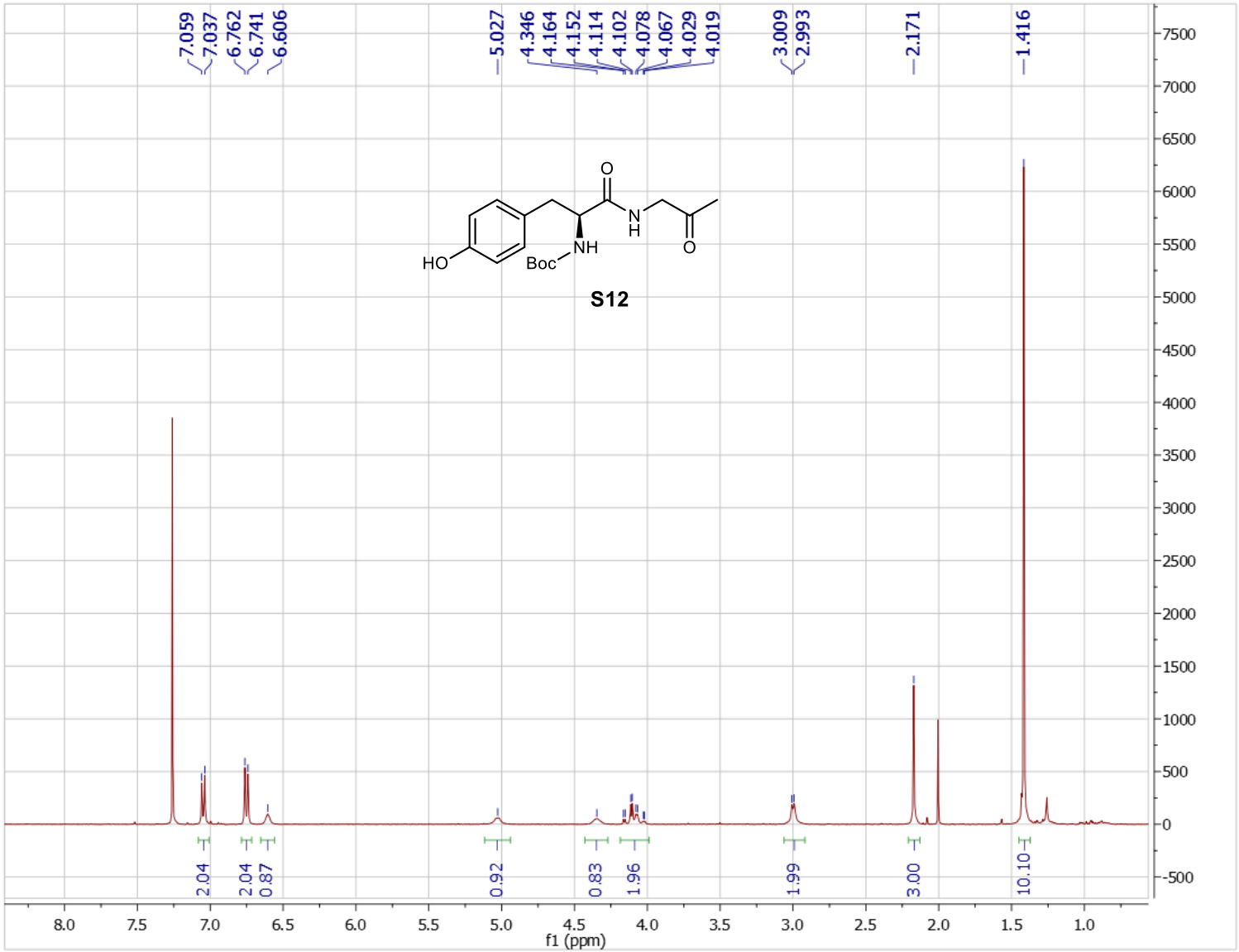


^{13}C NMR, 100 MHz, chloroform-*d*

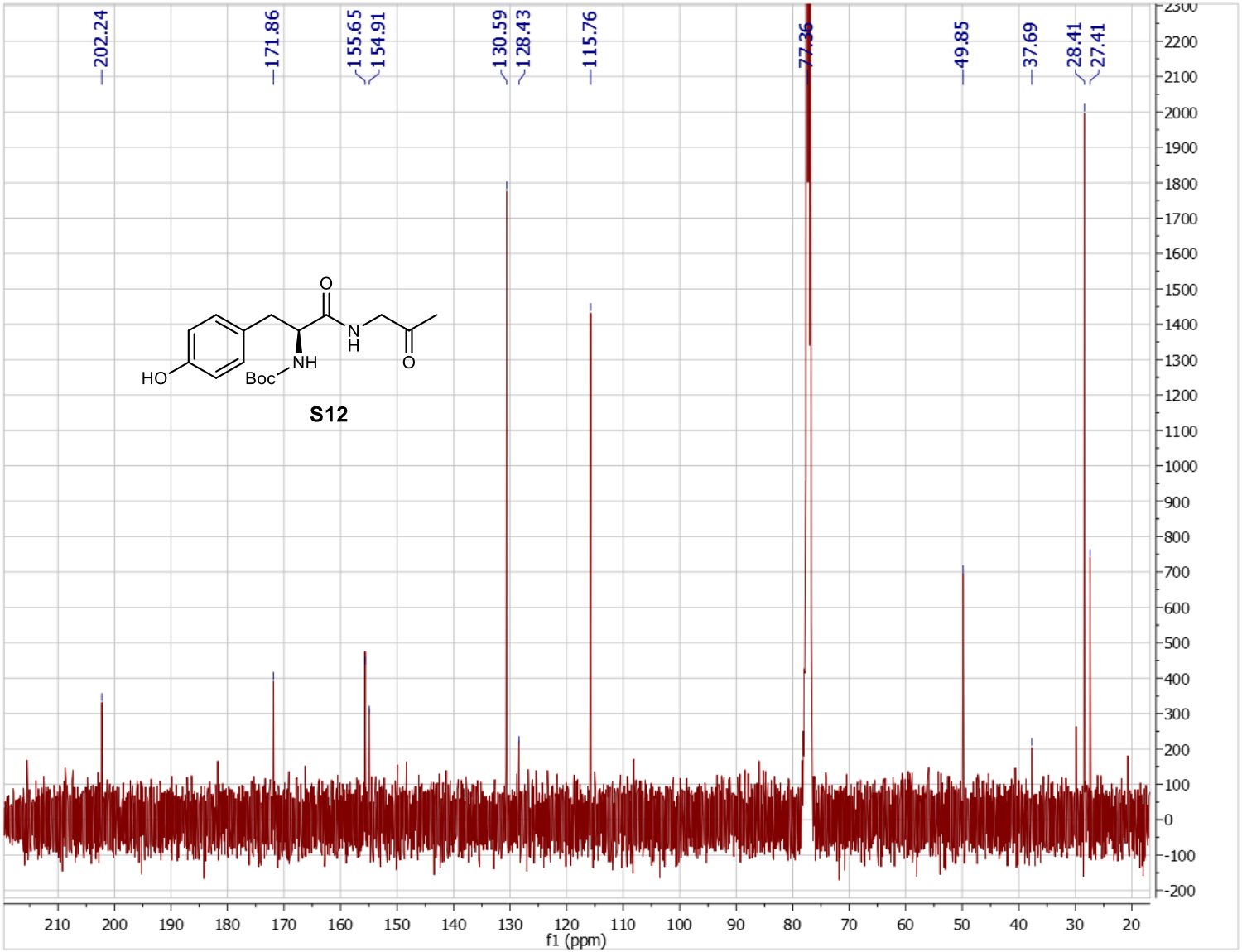


8

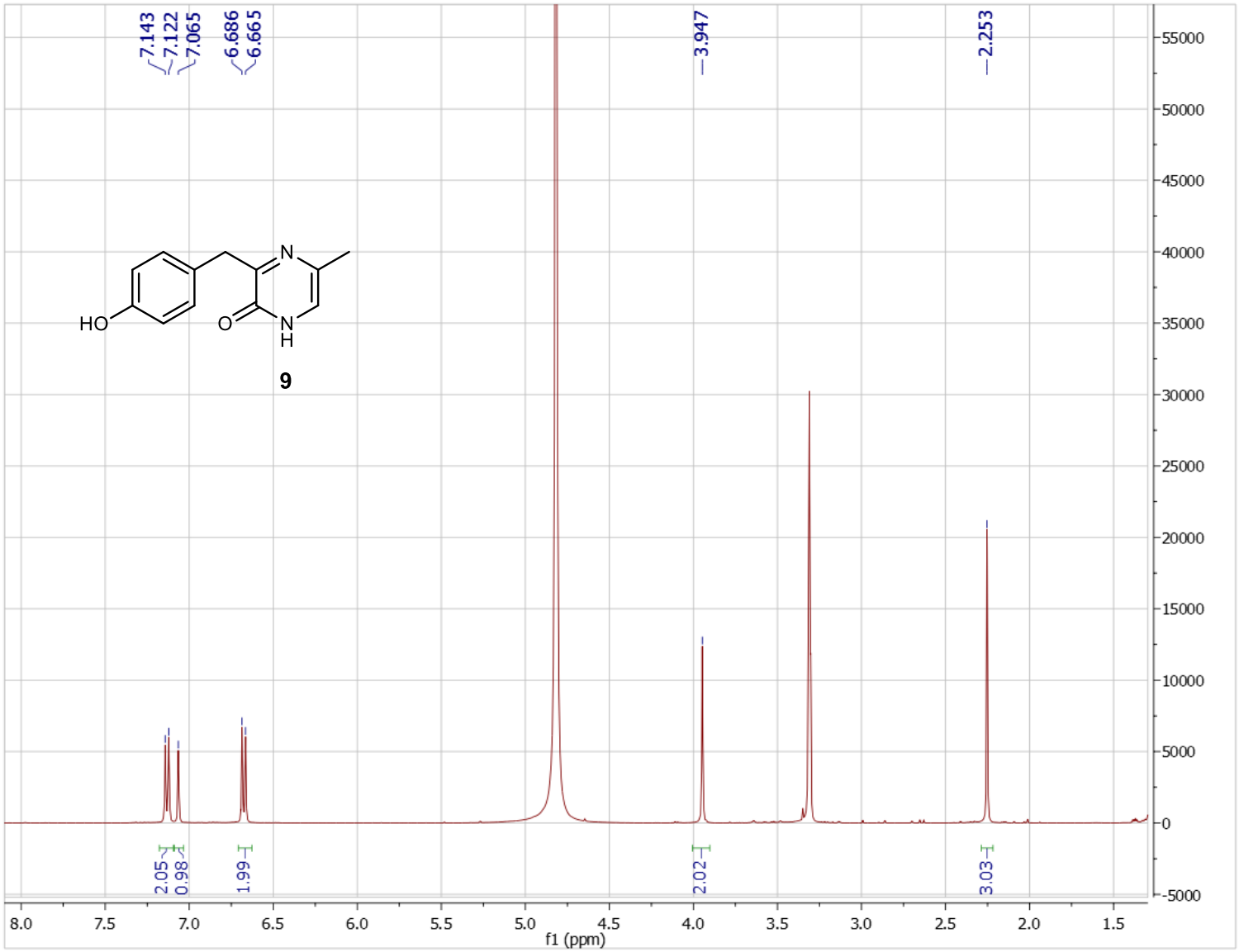
^{1}H NMR, 400 MHz, chloroform-*d*



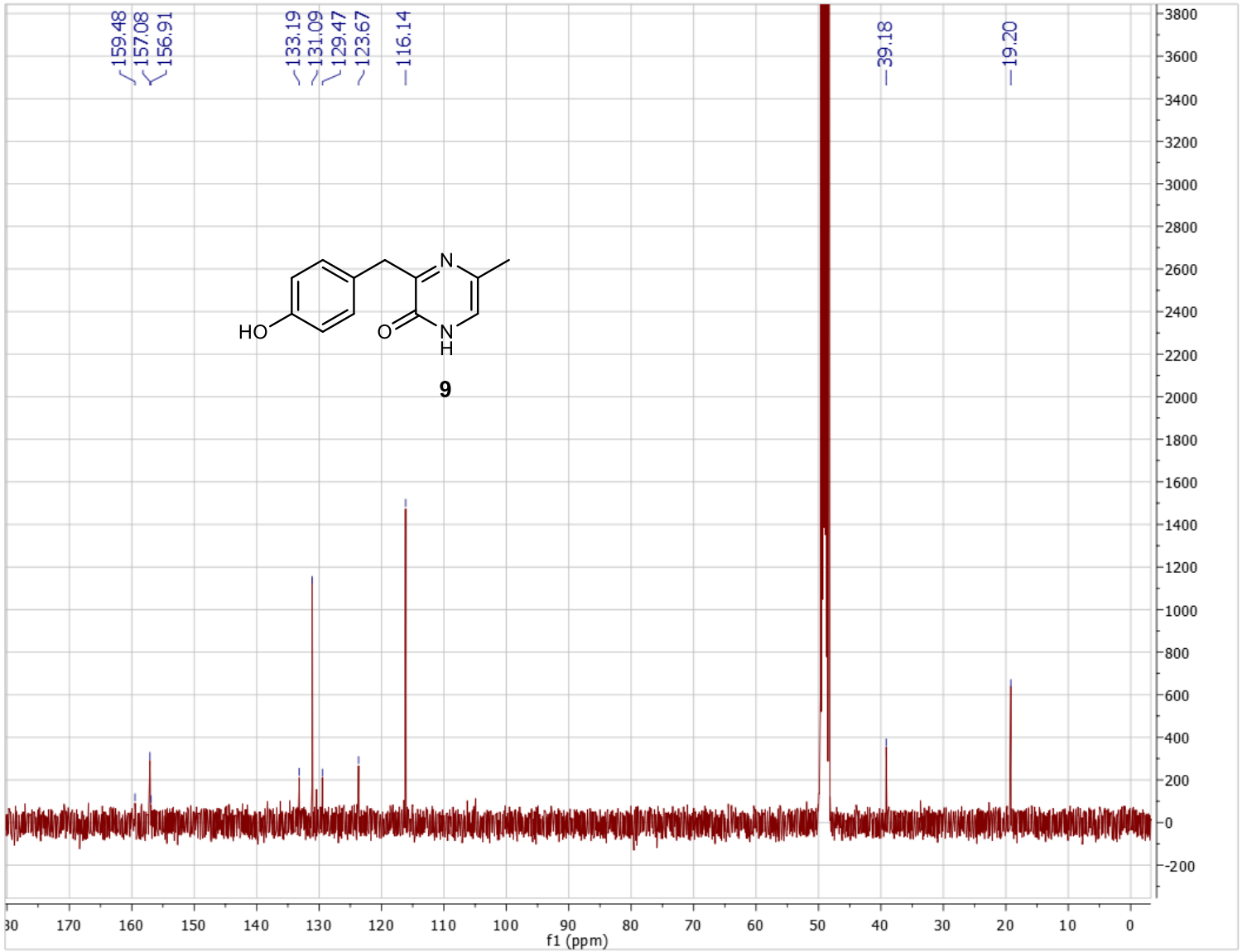
^{13}C NMR, 100 MHz, chloroform-*d*



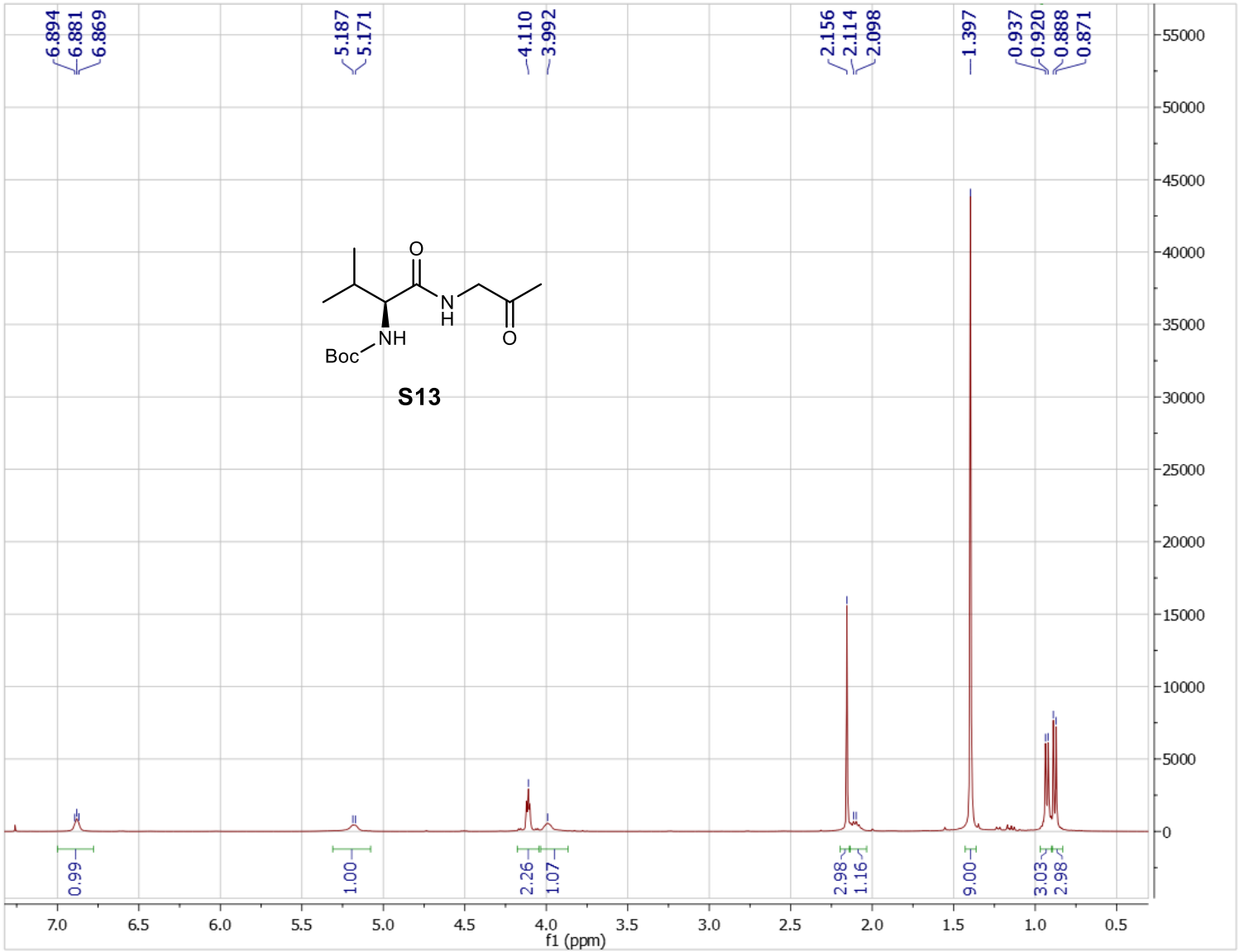
^{1}H NMR, 400 MHz, methanol-d_4



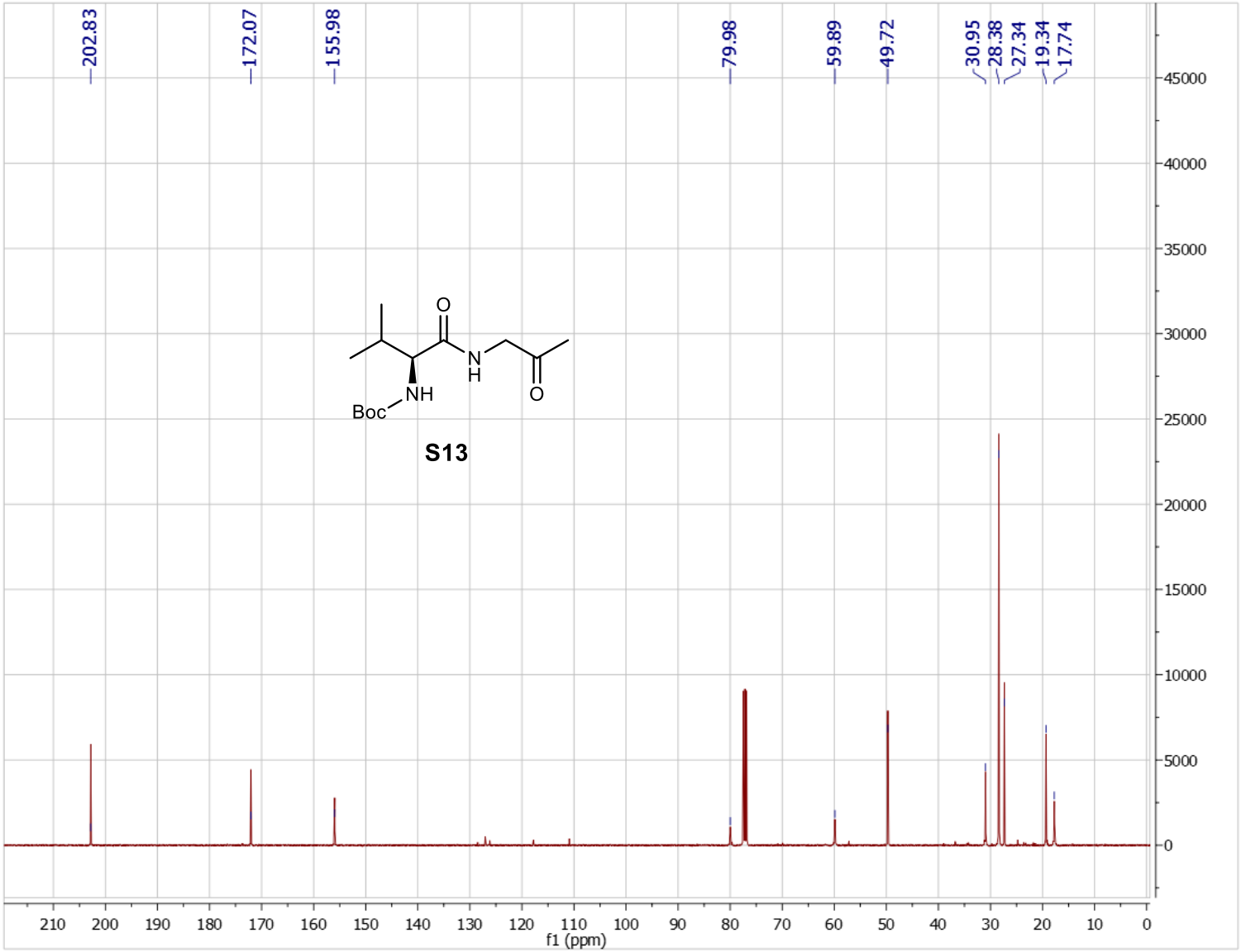
^{13}C NMR, 100 MHz, methanol-d_4



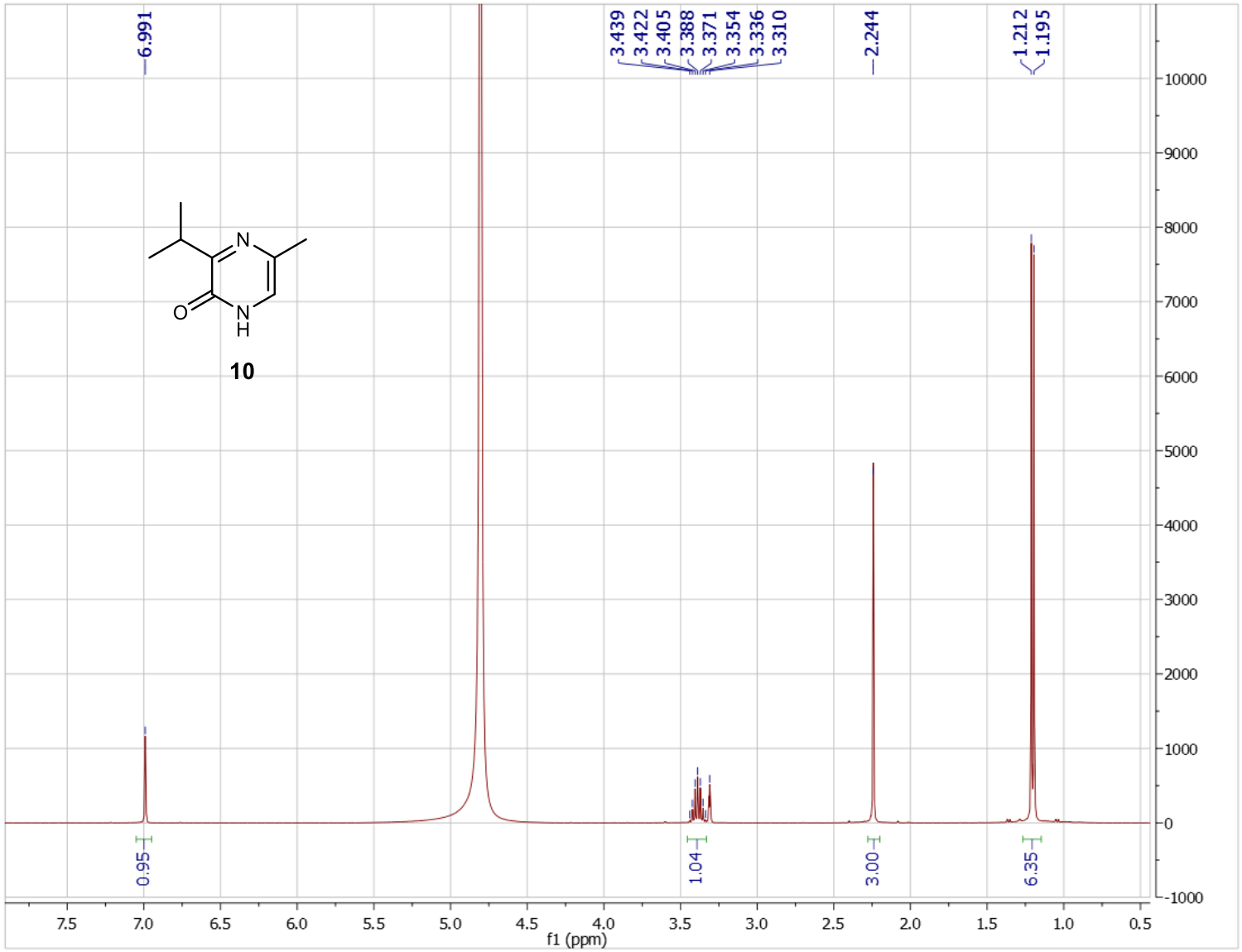
^{1}H NMR, 400 MHz, chloroform-*d*



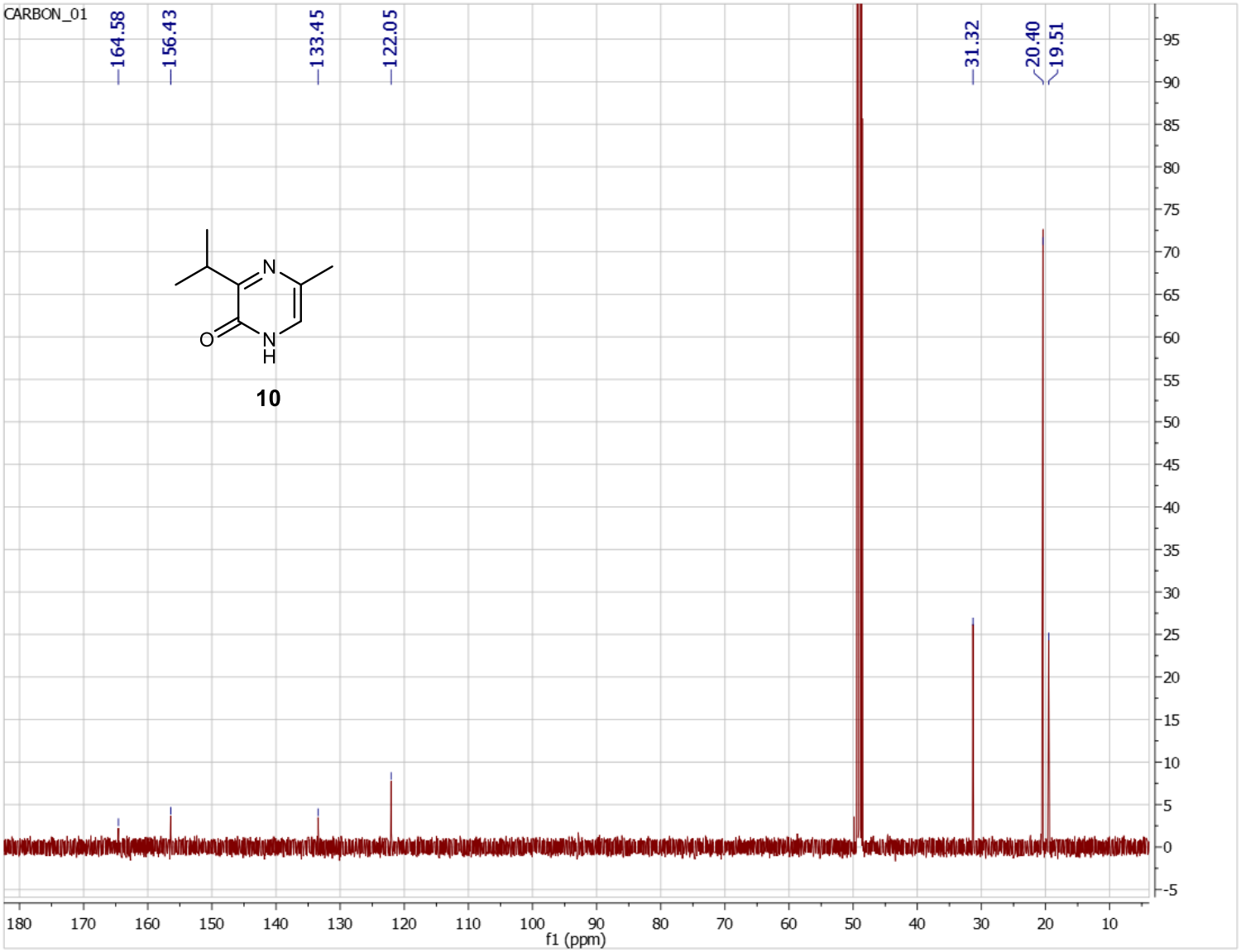
^{13}C NMR, 100 MHz, chloroform-*d*



^{1}H NMR, 400 MHz, methanol-d_4



^{13}C NMR, 100 MHz, methanol-d_4



Supplemental References

(1) Baba, T.; Ara, T.; Hasegawa, M.; Takai, Y.; Okumura, Y.; Baba, M.; Datsenko, K. A.; Tomita, M.; Wanner, B. L.; Mori, H., Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol. Syst. Biol.* **2006,** *2* (1), 2006.0008.

(2) Kroeze, W. K.; Sassano, M. F.; Huang, X.-P.; Lansu, K.; McCorvy, J. D.; Giguère, P. M.; Sciaky, N.; Roth, B. L., PRESTO-Tango as an open-source resource for interrogation of the druggable human GPCRome. *Nat. Struct. Mol. Biol.* **2015,** *22* (5), 362.

(3) Vieira, S. M.; Hiltensperger, M.; Kumar, V.; Zegarra-Ruiz, D.; Dehner, C.; Khan, N.; Costa, F.; Tiniakou, E.; Greiling, T.; Ruff, W.; Barbieri, A.; Kriegel, C.; Mehta, S. S.; Knight, J. R.; Jain, D.; Goodman, A. L.; Kriegel, M. A., Translocation of a gut pathobiont drives autoimmunity in mice and humans. *Science* **2018,** *359* (6380), 1156-1161.

(4) Candelon, N.; Shinkaruk, S.; Bennetau, B.; Bennetau-Pelissero, C.; Dumartin, M.-L.; Degueil, M.; Babin, P., New approach to asymmetrically substituted methoxypyrazines, derivatives of wine flavors. *Tetrahedron* **2010,** *66* (13), 2463-2469.

(5) Li, H.-J.; Cai, Y.-T.; Chen, Y.-Y.; Lam, C.-K.; Lan, W.-J., Metabolites of marine fungus *Aspergillus* sp. collected from soft coral *Sarcophyton tortuosum*. *Chem. Res. Chin. Univ.* **2010,** *26*, 415-419.

(6) Papenfort, K.; Silpe, J. E.; Schramma, K. R.; Cong, J.-P.; Seyedsayamdost, M. R.; Bassler, B. L., A *Vibrio cholerae* autoinducer–receptor pair that controls biofilm formation. *Nat. Chem. Biol.* **2017,** *13* (5), 551-557.