# Supporting Information

## Synthesis of Functionalized 2,3-Diaminopropionates and Their Potential for Directed Monobactam Biosynthesis

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## General Materials and Methods

All biological reagents, media components, DNA modifying enzymes and cloning reagents were purchased from Becton Dickson (Franklin Lakes, NJ), Sigma-Aldrich (St. Louis, MO), VWR (Radnor, PA), New England Biolabs (Ipswich, MA) or Thermo Fisher Scientific (Waltham, MA). Supelclean™ ENVI-Carb™ SPE Tubes were purchased from Sigma-Aldrich (St. Louis, MO). All reagents were purchased and used without further purification unless indicated otherwise. Anhydrous solvents were either purchased or dried using an LC Technology Solutions (Salisbury, MA) SPBT-1 solvent purification system. Silica gel chromatography was performed using Sorbtech Silica Gel (60 Å, 40-75mm particle size). Thin layer chromatography was performed on Analtech Silica Gel GHLF UV254 250 μm glass plates. All NMR spectra were recorded on a Bruker (Billerica, MA) UltraShield 300 or 400 MHz Avance spectrometer. $^{1}$H-NMR spectra were referenced according to the solvent peaks: $CHCl_3$ δ = 7.24 ppm, DMSO-$d_5$ δ = 2.50 ppm, HOD δ = 4.80 ppm. $^{13}$C-NMR spectra were referenced according to the solvent peaks: $CDCl_3$ δ = 77.23 ppm, DMSO-$d_6$ δ = 39.51 ppm. Ultra-performance liquid chromatography-high resolution mass spectrometry (UPLC-HRMS) analyses were done using a Waters (Milford, MA) Acquity/Xevo-G2 at the Johns Hopkins University Department of Chemistry Mass Spectrometry Facility. Chromatographic separations were carried out on a Waters Acquity BEH UPLC column (ethylene-bridged hybrid C18 stationary phase, 2.1 mm × 350 mm, 1.7 μm) with HRMS detection using an electrospray ionization (ESI) ion source in positive mode or a Waters ACQUITY UPLC BEH Amide Column (130 Å, 1.7 μm, 2.1 mm × 100 mm) with HRMS detection using an electrospray ionization (ESI) ion source in either negative or positive mode. Data were analyzed using MassLynx (Waters) software.

## Adenylation Domain *in vitro* Activity Assays and Michaelis-Menten Kinetics of $A_3$

His-tagged $A_3$-$PCP_3$ didomain was expressed and purified by standard gravity flow Ni-NTA affinity chromatography as described previously.[1,2] For the MesG/hydroxylamine coupled assay His-tagged $A_3$-$PCP_3$ didomain and inorganic pyrophosphatase (PPase) were purified using Tris-based buffers. All *in vitro* assays were performed in triplicate; error bars denote standard deviation.

Pyrophosphate-detection assay: A-domain activity was measured through colorimetric detection of released pyrophosphate using an *in vitro* assay modified from Maruyama *et al*.[3] Each reaction was performed on a 100 μL scale containing 50 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl, pH 8.0), 5 mM magnesium chloride ($MgCl_2$), 5 mM adenosine 5'-triphosphate (ATP), 3 mM amino acid substrate, 40 mM hydroxylamine and $A_3$-$PCP_3$ to a final concentration of 130 μg/mL (approx. 1.8 μM). Reactions were initiated by addition of enzyme and incubated for 70 min at 30 °C. 1 mL Mo(VI) solution (20 mM sodium molybdate ($NaMoO_4$) in 0.6 M hydrochloric acid and 60% acetonitrile) was added, vortexed briefly, and samples were incubated for 5 min at room temperature. 20 μL of bis(triphenylphosphoranylidene)ammonium chloride (BTPPACl, 50 mM in acetonitrile) were added, vortexed briefly, and samples were incubated for 5 min at room temperature. The mixture was then centrifugated for 20 min at 16,000 × g. After removal of supernatant, the pellet was dissolved in 200 μL of acetonitrile. 20 μL ascorbic acid (0.5 M in 2 M hydrochloric acid and 60% acetonitrile) was added and the mixture was incubated at room temperature for 10 min. 200 μL acetonitrile were added and the absorbance at 620 nm was measured using a Varian (Palo Alto, CA) Cary 50 UV-Vis spectrophotometer. Each amino acid substrate was blanked against an identical sample with no added enzyme.

Hydroxamate-detection assay: A-domain activity was measured through colorimetric detection of released acyl-hydroxamates using an *in vitro* assay modified from Kadi *et al*. and Hara *et al*.[4,5] Each reaction was performed on a 100 μL scale containing 50 mM tris(hydroxymethyl)aminomethane (Tris-HCl, pH 8.5), 10 mM magnesium chloride ($MgCl_2$), 10 mM adenosine 5'-triphosphate (ATP), 6 mM amino acid substrate,

200 mM hydroxylamine (2 M stock solution with pH adjusted to 7.5) and $A_3$-$PCP_3$ to a final concentration of 390 µg/mL (approx. 5.5 µM). Reactions were initiated by addition of enzyme and incubated for 18 h at 30 °C. After incubation, reactions were quenched with 100 µL of a 10% (w/v) iron (III) chloride ($FeCl_3$) solution in 0.7 M hydrochloric acid with 3.3% (w/v) trichloroacetic acid added. Samples were centrifuged at 14,500 × g for 4 min and the absorbance of the supernatant at 490 nm was measured using a Varian Cary 50 UV-Vis spectrophotometer. Each amino acid substrate was blanked against an identical sample with no added enzyme.

MesG/hydroxylamine coupled assay: Michaelis-Menten parameters of $A_3$-$PCP_3$ for the adenylation reaction of three different substrates (L-Dap **1**, (2S,3R)-MeDap **6**, and F-MeDap **39**) were determined based on kinetic data recorded using the continuous MesG/hydroxylamine coupled assay with minor modifications.[6,7] Reactions contained 50 mM Tris-HCl (pH 8), 5 mM magnesium chloride, 3 mM ATP, 1 mM Tris(2-carboxyethyl)phosphine (TCEP), 150 mM hydroxylamine (pH 7.5), 0.1 U nucleoside phosphorylase (PNP, N2415 from Sigma-Aldrich), 2.5 µM PPase, 0.2 mM 6-methyl-7-thioguanosine (MesG) and varying amounts of $A_3$-$PCP_3$ (150 – 1000 nM) and amino acid substrates. In 96-well plates (BRAND, Wertheim, Germany) reactions (total volume 100 µL) were initiated by addition of substrate and the phosphate-dependent cleavage of MesG was monitored on a Tecan Spark plate reader at 360 nm. Background activity recorded in wells without substrates was subsequently subtracted from the obtained slopes. Initial velocities $V_0/E_0$ were fit to the Michaelis-Menten equation by nonlinear regression using the method of least squares.

## Fermentation, Chemical Complementation and Monobactam Detection Procedures

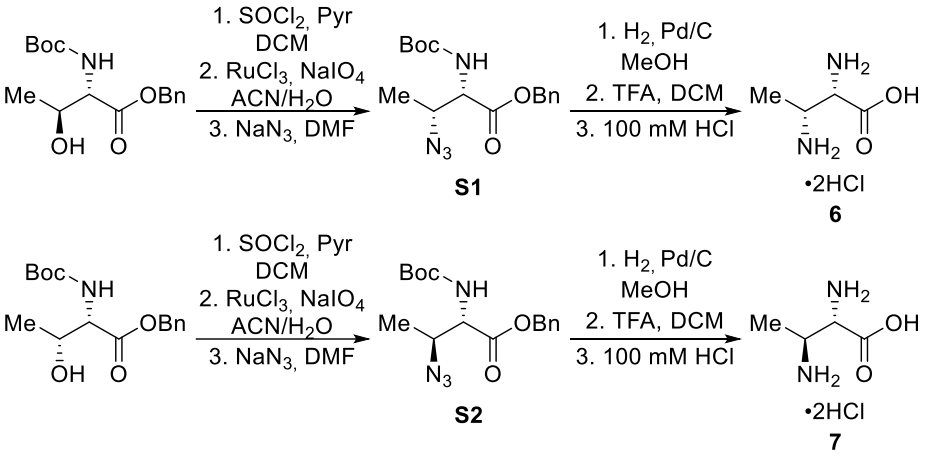
Fermentation and chemical complementation: 50 mL sulfazecin seed medium[8] containing 50 µg/mL gentamicin (Gold Biotechnology, St. Louis, MO) was inoculated with *P. acidophila* Δ*sulG* mutant from a cell stock or agar plate and grown at 28 °C for 48-72 h and shaken at 280 rpm. Fermentation cultures were grown at 28 °C after transferring 2.5 mL seed culture to 50 mL sulfazecin fermentation medium.[8] The selected amino acid substrate was weighed and added directly into the 24-h fermentation cultures. The final concentration of substrates was 2 mM or 3 mM. Fermentation was continued for an additional 96 h after feeding.

Bioassays for monobactam detection: 2 mL fermentation broth was withdrawn and centrifuged for 3 min at 16,000 × g. 250 µL cell-free supernatant (CFS) was assayed against *E. coli* ESS,[9] *K. rhizophila* ATCC 9341 (ATCC, Manassas, VA) (in nutrient agar) and *B. licheniformis* ATCC 14580 (in BA2)[10] for antibacterial activities. The latter was also used to screen for induction of β-lactamase activity. Induction of β-lactamase was visualized by overlaying the plate with 1.5 mL of 200 µg/mL nitrocefin solution in phosphate buffer (pH 7.0) (Toku-E, Inc. Bellingham, WA). To analyze the antibacterial and β-lactamase-inducing activities from concentrated samples, 7 mL of fermentation supernatant was lyophilized and resuspended in 1 mL of water prior to addition to bioassay and nitrocefin assay plates as described above.

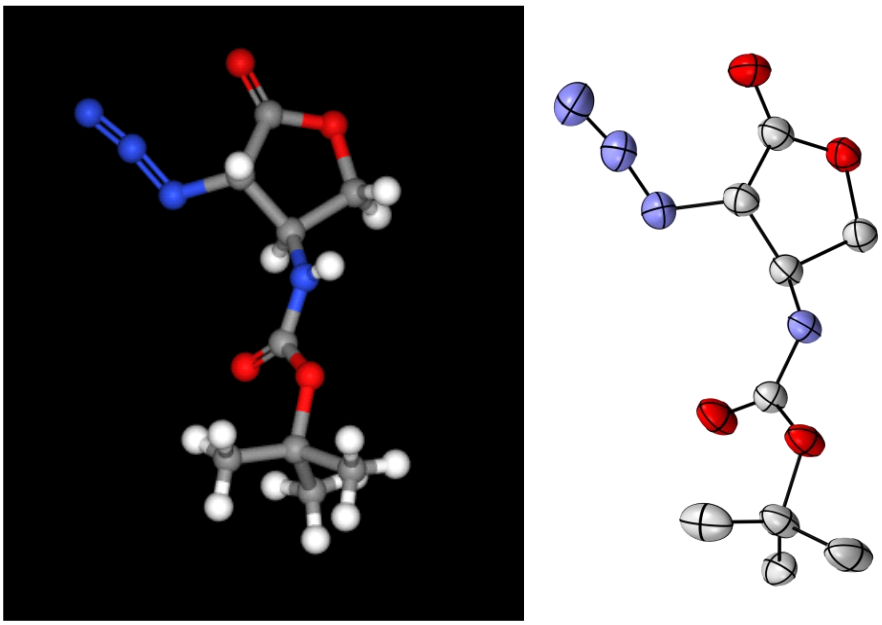
Semi-purification of CFS and UPLC-HRMS analysis protocol: A Supelclean™ ENVI-Carb™ SPE Tube (250 mg bed wt.) was conditioned with 2 mL of water and 2 mL of 50% acetonitrile, then equilibrated with 3 mL of water by gravity flow. A 500 µL sample of CFS was loaded on to the column, then washed with 2.5 mL of water by gravity flow. Products were eluted in 400 µL fractions of 50% acetonitrile by gravity flow, which were collected separately. Each elution fraction was directly analyzed by UPLC-HRMS method A. Monobactam products were detected between fractions two and four.

UPLC–HRMS method A: ES– [binary gradient: water (solvent A), acetonitrile (solvent B), 0.3 mL/min]: 0–1 min isocratic 20% A; 1–7.5 min gradient 20% to 100% A; 7.5–8.4 min isocratic 100% A; 8.4–8.5 min gradient 100% to 20% A; 8.5–10 min isocratic 20% A. Waters ACQUITY UPLC BEH Amide Column, 130 Å, 1.7 µm, 2.1 mm × 100 mm.

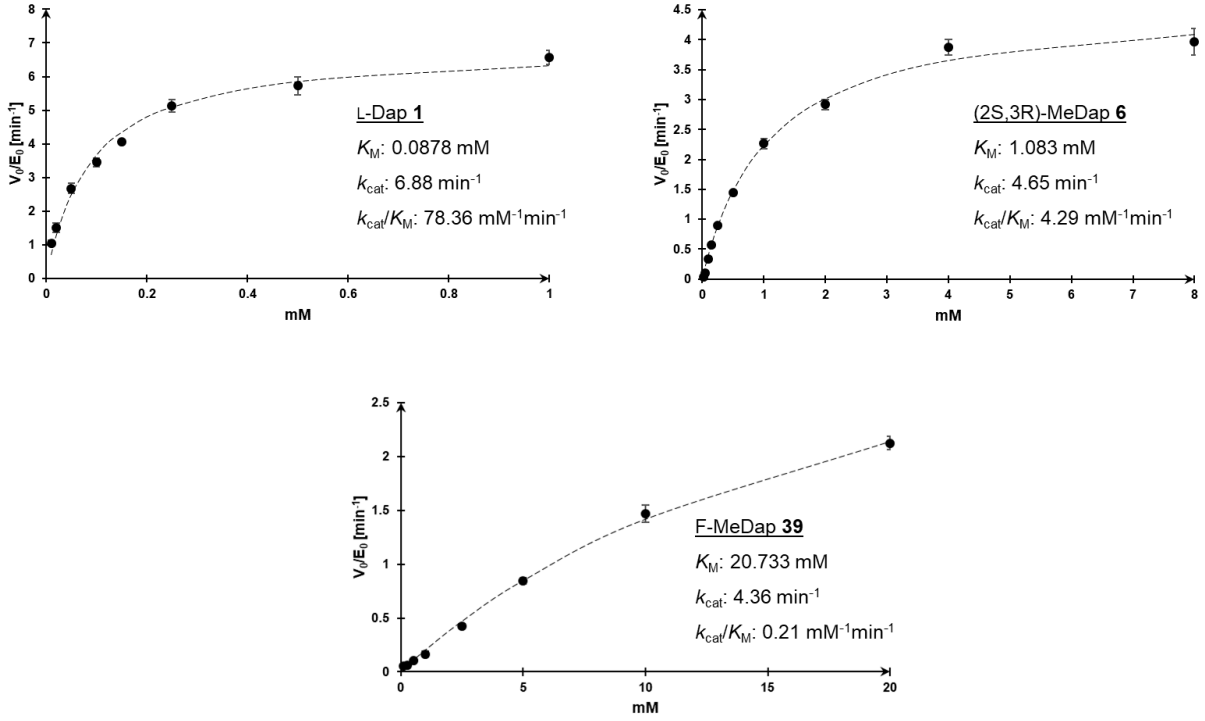
## Supplemental Schemes and Figures



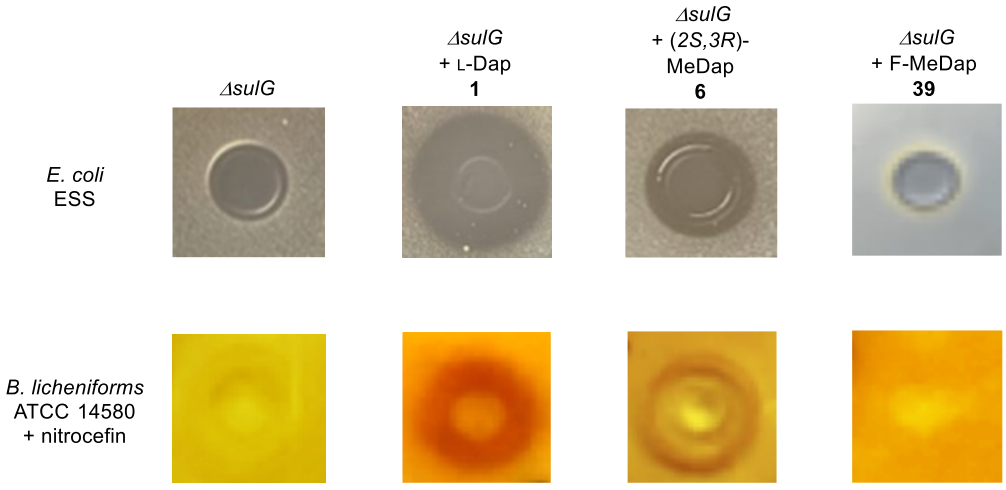

**Supplemental Scheme S1.** Synthesis of MeDap Derivatives **6** and **7**.



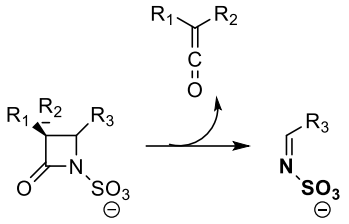
**Supplemental Figure S1.** Ball and stick structure and displacement ellipsoid plot (50% probability level) of butyrolactone **11** at 173(2) K. Disorder of the *tert*-butyl group and H atoms have been omitted for clarity.




**Supplemental Figure S2.** Michaelis-Menten kinetics of A3 based on the MesG/hydroxylamine continuous assay.

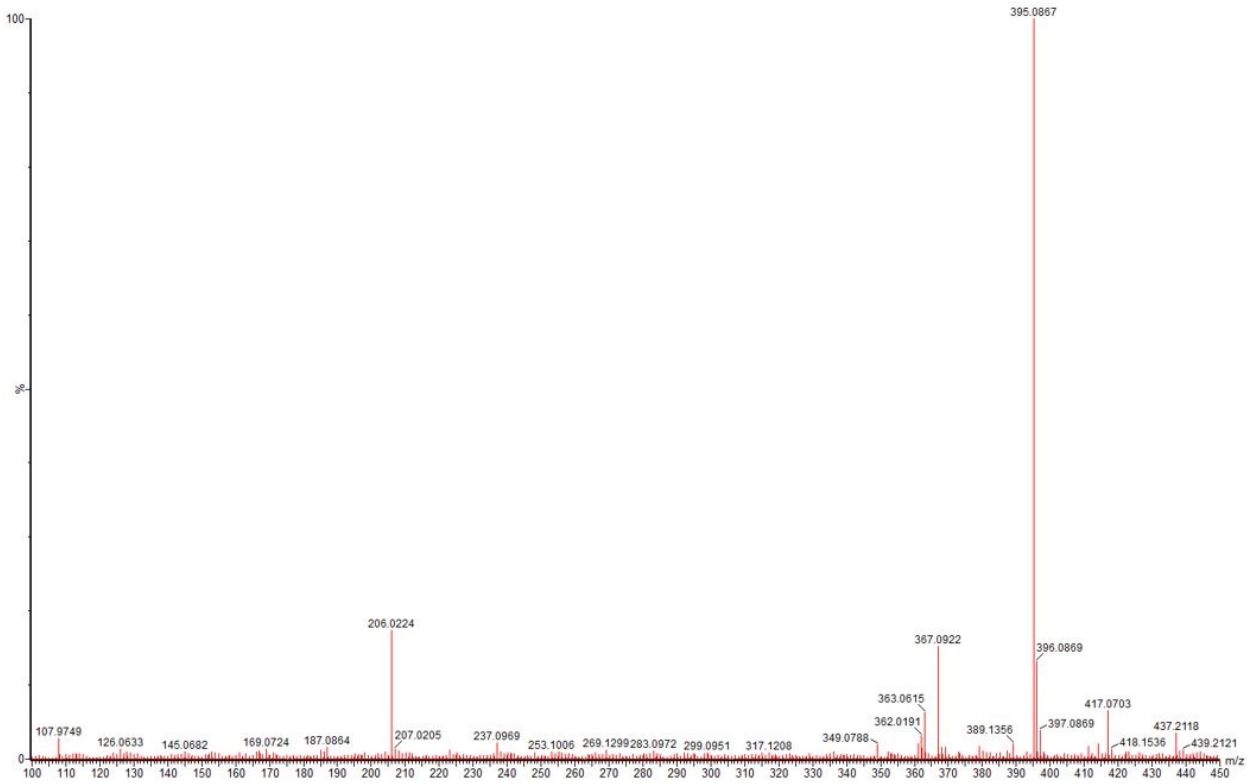



**Supplemental Figure S3.** Bioassay-based detection of monobactams from unconcentrated CFS.

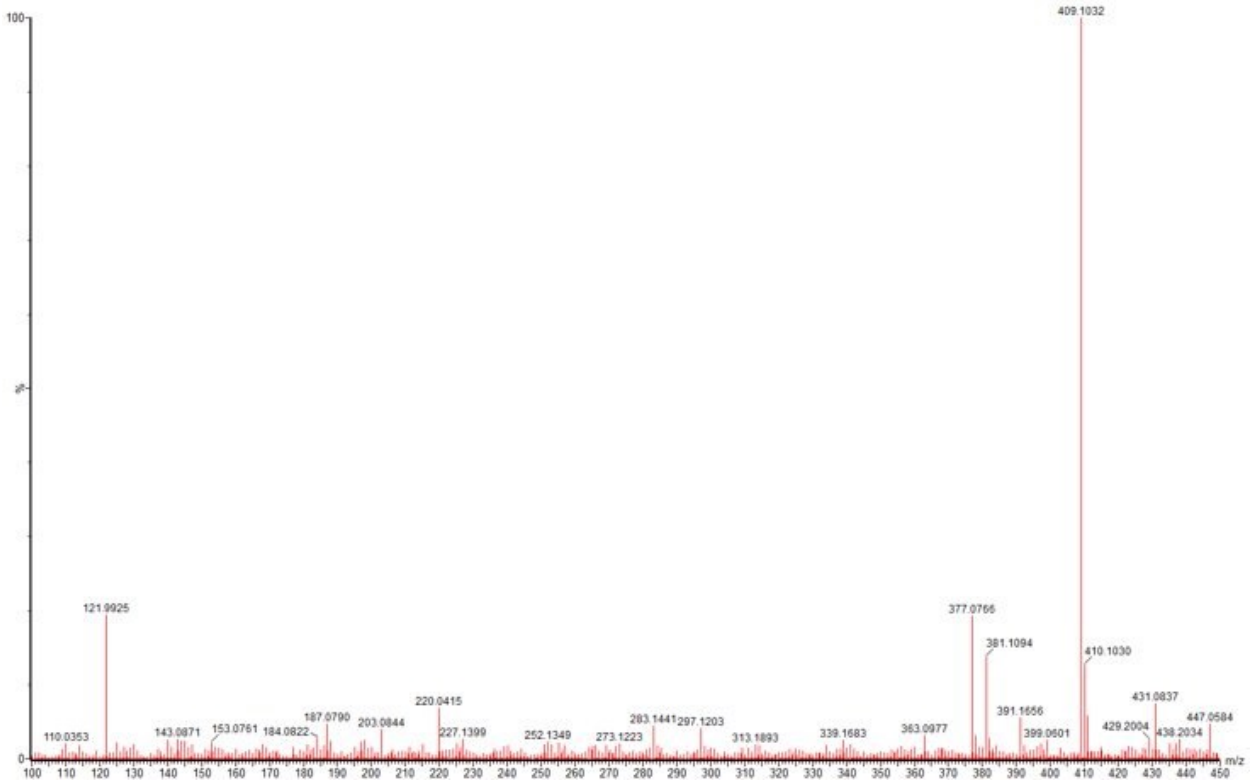



| Compound Name | Compound Formula | Parent Mass (Calculated) | Parent Mass (Found) | Formal [2+2]-Cycloreversion Fragment (Calculated) | Formal [2+2]-Cycloreversion Fragment (Found) |
|---|---|---|---|---|---|
| **Sulfazecin** | $C_{12}H_{19}N_4O_9S$ | 395.0878 | 395.08867 | 107.9761 | 107.9749 |
| **Methyl-Sulfazecin** | $C_{13}H_{21}N_4O_9S$ | 409.1035 | 409.1032 | 121.9917 | 121.9925 |
| **Fluoromethyl-Sulfazecin** | $C_{13}H_{20}FN_4O_9S$ | 427.0941 | 427.0922 | 139.9823 | 139.9831 |
| **Aztreonam** | $C_{13}H_{16}N_5O_8S_2$ | 434.0446 | 434.0445 | 121.9917 | 121.9920 |

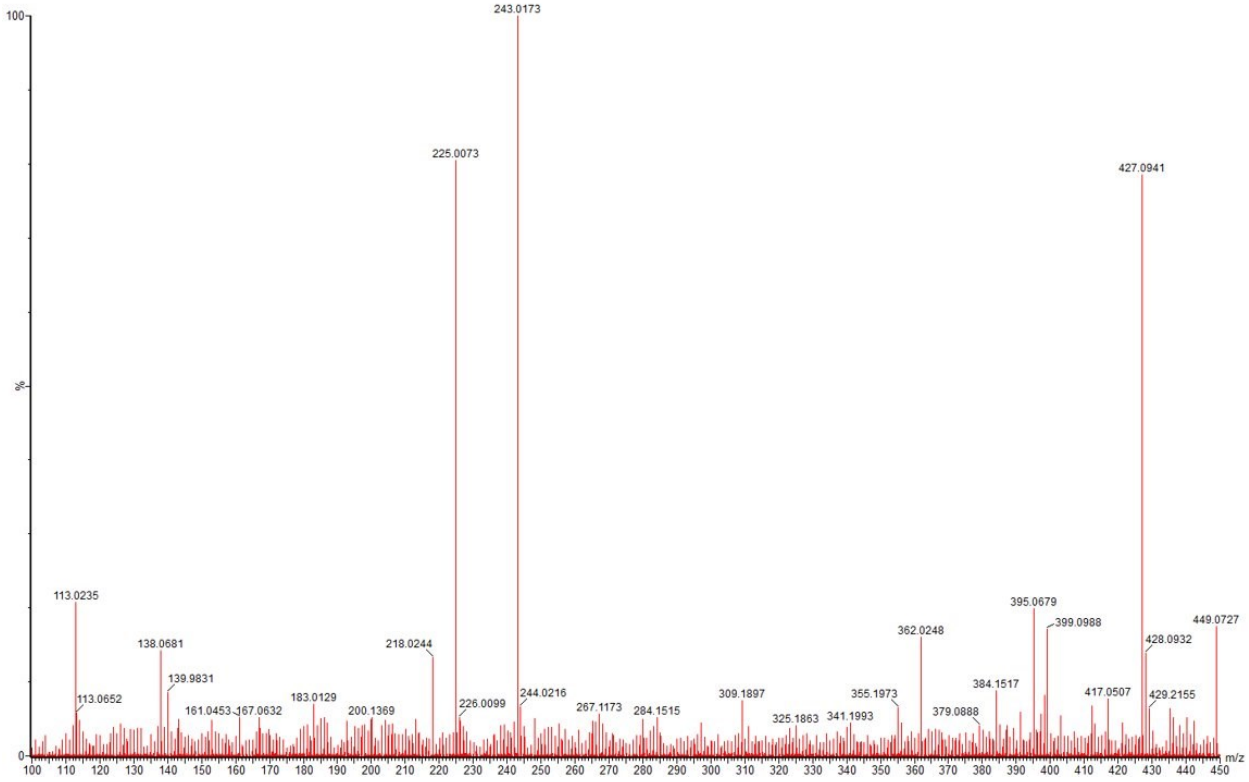
**Supplemental Figure S4.** Masses of monobactams and formal [2+2]-cycloreversion fragments found by tandem ESI (-) MS/MS ($MS^e$).



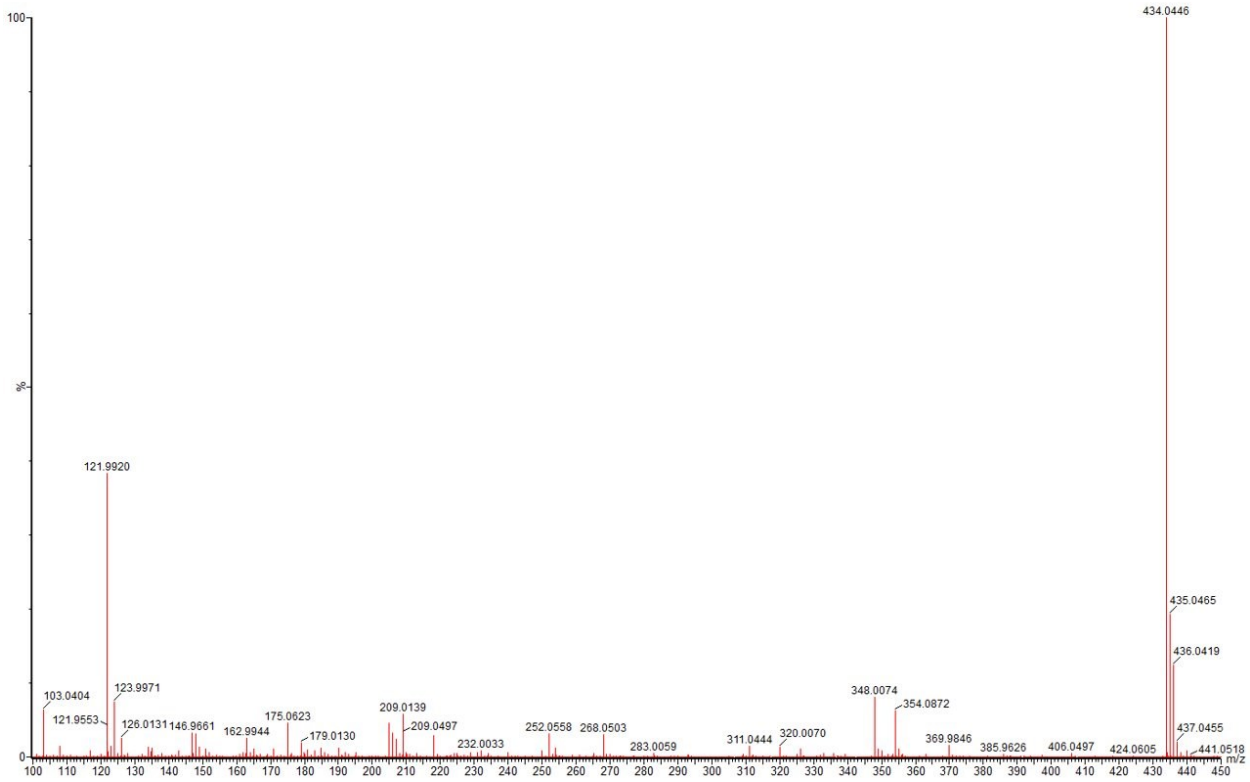

**Supplemental Figure S5.** ESI (-) MS/MS ($MS^e$) of Δ*sulG* + L-Dap (**1**) fermentation semi-purified fraction of sulfazecin after background subtraction of Δ*sulG* negative control; *m/z*: $[M]^-$ calculated for $C_{12}H_{19}N_4O_9S$ 395.0878




**Supplemental Figure S6.** ESI (-) MS/MS ($MS^e$) of Δ*sulG* + (2*S*,3*R*)-MeDap (**6**) fermentation semi-purified fraction of methyl-sulfazecin after background subtraction of Δ*sulG* negative control; *m/z*: $[M]^-$ calculated for $C_{13}H_{21}N_4O_9S$ 409.1035




**Supplemental Figure S7.** ESI (-) MS/MS ($MS^e$) of Δ*sulG* + F-MeDap (**39**) fermentation semi-purified fraction of fluoromethyl-sulfazecin after background substraction of Δ*sulG* negative control; *m/z*: $[M]^-$ calculated for $C_{13}H_{20}FN_4O_9S$ 427.0941




**Supplemental Figure S8.** ESI (-) MS/MS ($MS^e$) of Aztreonam (**3**); *m/z*: $[M]^-$ calculated for $C_{13}H_{16}N_5O_8S_2$ 434.0446

## Single Crystal X-ray Crystallography

All reflection intensities were measured at 173(2) K using a SuperNova diffractometer (equipped with Atlas detector) with Cu *K*α radiation (λ = 1.54178 Å) under the program CrysAlisPro (Version CrysAlisPro 1.171.39.29c, Rigaku OD, 2017). The same program was used to refine the cell dimensions and for data reduction. The structure was solved with the program SHELXS-2018/3[11] and was refined on $F^2$ with SHELXL-2018/3.[11] Analytical numeric absorption correction using a multifaceted crystal model was applied using CrysAlisPro. The temperature of the data collection was controlled using the system Cryojet (manufactured by Oxford Instruments). The H atoms were placed at calculated positions (unless otherwise specified) using the instructions AFIX 13, AFIX 23 or AFIX 137 with isotropic displacement parameters having values 1.2 or 1.5 $U_{eq}$ of the attached C atoms. The H atom attached to N4 was found from difference Fourier map, and its coordinates and isotropic temperature factor were refined freely.

The structure is mostly ordered. The *tert*-butyl group was found to be disordered over two orientations, and the occupancy factor of the major component of the disorder refines to 0.515(17).

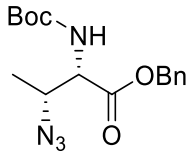
The absolute configuration has been established by anomalous-dispersion effects in diffraction measurements on the crystal, and the Flack and Hooft parameters refine to 0.03(7) and 0.01(6), respectively.

**Table S1. Single crystal X-ray crystallography experimental details**

| | |
|---|---|
| **Crystal data** | |
| Chemical formula | $C_9H_{14}N_4O_4$ |
| $M_r$ | 242.24 |
| Crystal system, space group | Orthorhombic, $P2_12_12_1$ |
| Temperature (K) | 173 |
| $a$, $b$, $c$ (Å) | 5.17882 (11), 9.5846 (2), 23.5161 (6) |
| $V$ (Å$^3$) | 1167.27 (5) |
| $Z$ | 4 |
| Radiation type | Cu $K\alpha$ |
| μ (mm$^{-1}$) | 0.93 |
| Crystal size (mm) | 0.66 × 0.07 × 0.06 |
| | |
| **Data collection** | |
| Diffractometer | SuperNova, Dual, Cu at zero, Atlas |
| Absorption correction | Analytical<br>*CrysAlis PRO* 1.171.41.93a (Rigaku Oxford Diffraction, 2020) Analytical numeric absorption correction using a multifaceted crystal model based on expressions derived by R.C. Clark & J.S. Reid.[12] Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm. |
| $T_{min}$, $T_{max}$ | 0.675, 0.953 |
| No. of measured, independent and observed [$I > 2\sigma(I)$] reflections | 9129, 2299, 2240 |
| $R_{int}$ | 0.028 |
| $(\sin \theta/\lambda)_{max}$ (Å$^{-1}$) | 0.616 |
| | |
| **Refinement** | |
| $R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, $S$ | 0.029, 0.078, 1.04 |
| No. of reflections | 2299 |
| No. of parameters | 192 |
| No. of restraints | 102 |
| H-atom treatment | H atoms treated by a mixture of independent and constrained refinement |
| $\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å$^{-3}$) | 0.13, -0.18 |
| Absolute structure | Flack x determined using 888 quotients [(I+)-(I-)]/[(I+)+(I-)][13] |
| Absolute structure parameter | 0.03 (7) |

Computer programs: *CrysAlis PRO* 1.171.39.29c (Rigaku OD, 2017), *SHELXS2018*/3,[11] *SHELXL2018*/3,[11] *SHELXTL* v6.10.[8]

## Synthetic Procedures




**Benzyl (3*R*)-azido-(2*S*)-((*tert*-butoxycarbonyl)amino)butanoate (S1).**

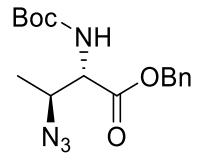
Compound **S1** was prepared following a modified protocol.[14]

Boc-L-*allo*-Thr-OBn (1.88 g, 6.08 mmol) was placed under Argon and dissolved in 15 mL of anhydrous dichloromethane. This solution was added dropwise to a mixture of thionyl chloride (0.88 mL, 12.2 mmol) in 8 mL of anhydrous dichloromethane at -40 °C, followed by the rapid addition of pyridine (2.45 mL, 30.4 mmol). The reaction was stirred at this temperature for 1 h then warmed to room temperature, diluted with 20 mL of dichloromethane and washed with 30 mL of a 0.5 N hydrochloric acid solution and 30 mL of saturated brine. The organic phase was dried over anhydrous sodium sulfate and concentrated.

The crude material was dissolved in 20 mL of acetonitrile and cooled to 0 °C. Catalytic ruthenium (III) chloride trihydrate (8 mg, 0.5 mol %) was added, followed by sodium periodate (1.95 g, 9.12 mmol) and 20 mL of water. The reaction was stirred for 1 h, then diluted with 30 mL of water and extracted 3 × 30 mL of diethyl ether. The combined organics were washed with 30 mL of a saturated aqueous solution of sodium bicarbonate, 30 mL of a 10% aqueous solution of sodium thiosulfate, and 30 mL of saturated brine. The organic phase was dried over anhydrous sodium sulfate, concentrated, and used without further purification.

The crude product was placed under Argon, dissolved in 12 mL of anhydrous dimethylformamide and cooled to -40 °C. Sodium azide (538 mg, 8.28 mmol) was added in a single portion, the reaction was warmed to room temperature and stirred for 3 h. Diethyl ether (60 mL) and a 1 N solution of hydrochloric acid (60 mL) were added, and the mixture was stirred vigorously at room temperature for 3 h. The phases were separated, and the aqueous layer was extracted 2 × 30 mL of diethyl ether. The combined organics were washed with 30 mL of a saturated aqueous solution of sodium bicarbonate and 30 mL of saturated brine, dried over anhydrous sodium sulfate and concentrated. Purification by flash chromatography (9/1 → 8/2 hexanes/ethyl acetate) yielded **S1** (960 mg, 47%) as a colorless oil.

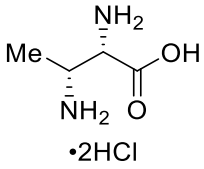
**$^1$H NMR** (400 MHz; $CDCl_3$): δ 7.40-7.28 (m, 5H), 5.19 (ABq, $J$ = 12.1 Hz, 2H), 5.16-5.08 (m, 1H), 4.41-4.33 (m, 1H), 4.20-4.10 (m, 1H), 1.43 (s, 9H), 1.32 (d, $J$ = 6.7 Hz, 3H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 170.3, 156.1, 135.2, 128.9, 128.8, 128.6, 80.5, 67.8, 58.9, 57.5, 28.4, 16.3; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{16}H_{22}N_4O_4Na$ 357.1533, found 357.1529




**Benzyl (3*S*)-azido-(2*S*)-((*tert*-butoxycarbonyl)amino)butanoate (S2).**

An identical procedure to the above was performed using Boc-L-Thr-OBn (3.08 g, 9.73 mmol) to afford **S2** (2.05 g, 63 %) as a colorless oil.

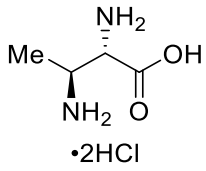
**$^1$H NMR** (400 MHz; $CDCl_3$): δ 7.39-7.27 (m, 5H), 5.38 (br. d, *J* = 7.8 Hz, 1H), 5.18 (ABq, *J* = 12.1 Hz, 2H), 4.52-4.41 (m, 1H), 3.86-3.74 (m, 1H), 1.42 (s, 9H), 1.26 (d, *J* = 6.8 Hz, 3H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 169.6, 155.2, 135.0, 128.8, 128.7, 128.6, 80.5, 67.7, 58.8, 57.6, 28.3, 15.5; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{16}H_{22}N_4O_4Na$ 357.1533, found 357.1531.




**(2*S*,3*R*)-Diaminobutanoic acid dihydrochloride (6).**

Compound **S1** (260 mg, 0.78 mmol) was dissolved in 10 mL of methanol and 10% palladium on carbon (26 mg) was added. The mixture was placed in a pressure tube and shaken on a Parr apparatus under 50 psi of hydrogen gas for 24 h at room temperature. The suspension was filtered over Celite and rinsed with methanol, then concentrated to afford a white solid residue. This product was dissolved in 6 mL of a 1:1 mixture of trifluoroacetic acid and dichloromethane and stirred at room temperature for 1 h. The reaction mixture was concentrated and the crude residue was re-dissolved in 15 mL of a 100 mM solution of hydrochloric acid which was stirred at room temperature for 5 min. The solution was concentrated, and this procedure was repeated three times. The product was then dissolved in water, frozen and lyophilized to dryness to afford **6** (135 mg, 91%) as an off-white solid. The spectral data were consistent with those previously reported.[15]

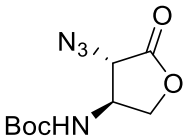
**$^1$H NMR** (400 MHz; $D_2O$): δ 4.40 (d, *J* = 3.5 Hz, 1H), 3.99 (qd, *J* = 6.8, 3.5 Hz, 1H), 1.45 (d, *J* = 6.8 Hz, 3H); **$^{13}$C NMR** (100 MHz; $D_2O$): δ 168.7, 53.5, 46.0, 12.6; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_4H_{11}N_2O_2$ 119.0815, found 119.0818




**(2*S*,3*S*)-Diaminobutanoic acid dihydrochloride (7).**

An identical procedure to the above was performed using compound **S2** (360 mg, 1.05 mmol) to afford **7** (180 mg, 90%) as an off-white solid. The spectral data were consistent with those previously reported.[15]

**$^1$H NMR** (400 MHz; $D_2O$): δ 4.23 (d, *J* = 7.0 Hz, 1H), 4.01 (dq, *J* = 7.0, 6.8 Hz, 1H), 1.53 (d, *J* = 6.8 H, 3H); **$^{13}$C NMR** (100 MHz; $D_2O$): δ 169.2, 54.6, 46.8, 14.8; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_4H_{11}N_2O_2$ 119.0815, found 119.0821

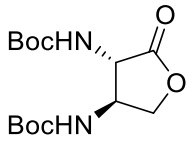



***tert*-Butyl ((3*S*,4*S*)-4-azido-5-oxotetrahydrofuran-3-yl)carbamate (11).**

The procedure of Hanessian *et al.* was used with minor modifications.[16] (3*S)*-Boc-amino butyrolactone **10** was either prepared from L-Aspartate according to known procedures[16] or purchased from Combi-Blocks (San Diego, CA).

To a dry flask under Argon was added 40 mL of anhydrous tetrahydrofuran which was cooled to -78 °C, followed by the addition of lithium hexamethyldisilazide (37.2 mL, 37.2 mmol, 1.0 M in tetrahydrofuran). A solution of (3*S*)-Boc-amino butyrolactone **10** (3.40 g, 16.9 mol) in 80 mL of anhydrous tetrahydrofuran was added dropwise via cannula over 20 min, and the mixture was stirred at -78 °C for 30 min. A solution of triisopropylbenzenesulfonyl azide (6.27 g, 20.3 mmol) in 40 mL of anhydrous tetrahydrofuran was added quickly and the reaction was stirred for 5 min before being quenched by the rapid addition of trimethylsilyl chloride (6.46 mL, 50.7 mmol). The flask was allowed to warm to room temperature and the mixture was stirred for 3 h. A 1 N solution of hydrochloric acid (100 mL) was added and the mixture was extracted 2 × 100 mL of ethyl acetate. The combined organic phases were washed with 100 mL of a saturated solution of sodium bicarbonate, 100 mL of saturated brine, dried over anhydrous sodium sulfate and concentrated. The crude product was purified by flash chromatography (8/2 hexanes/ethyl acetate) and then recrystallized from hexanes/ethyl acetate to afford **11** (2.1 g, 51%) as fine, white needles.

**$^1$H NMR** (400 MHz; $CDCl_3$): δ 4.95-4.83 (m, 1H), 4.52 (ABq, *J* = 7.5 Hz, 1H), 4.48-4.38 (m, 1H), 4.20-4.11 (m, 1H), 4.12-4.03 (m, 1H), 1.44 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 171.3, 155.1, 81.4, 68.9, 60.7, 53.5, 28.4; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_9H_{14}N_4O_4Na$ 265.0907, found 265.0910




**Di-*tert*-butyl ((3*S*,4*S*)-2-oxotetrahydrofuran-3,4-diyl)dicarbamate (12).**

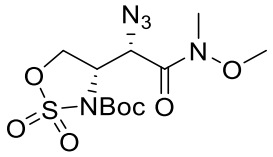
Lactone **11** (1.27 g, 5.24 mmol) was dissolved in 25 mL of ethyl acetate. Di-*tert*-butyl dicarbonate (2.28 g, 10.48 mmol) was added, followed by 10% palladium on carbon (70 mg). The mixture was placed in a pressure tube and shaken on a Parr apparatus under 40 psi of hydrogen gas for 16 hours at room temperature. The solution was filtered over Celite and rinsed with ethyl acetate, then concentrated. Purification by flash chromatography (7/3 hexanes/ethyl acetate) yielded **12** (1.23 g, 74%) as a white solid.

**$^1$H NMR** (400 MHz; $CDCl_3$): δ 6.06-5.95 (m, 1H), 5.23 (br. d, $J$ = 5.6 Hz, 1H), 4.84-4.72 (m, 1H), 4.40-4.29 (m, 1H), 4.15-4.04 (m, 1H), 3.98 (t, $J$ = 9.2 Hz, 1H), 1.44 (s, 9H), 1.41 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 172.5, 156.8, 156.1, 81.6, 80.6, 70.3, 55.5, 55.0, 28.5, 28.4; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{14}H_{24}N_2O_6Na$ 339.1527, found 339.1525

**General procedure A for ring-opening of lactones 11 and 12.**

The procedure of Joullié *et al*. was used with slight modifications.[17]

*N,O*-Dimethyl hydroxylamine hydrochloride (3.0 equiv) was added to a dry flask under Argon and suspended in anhydrous dichloromethane (10 mL/mmol lactone). Pyridine (2.2 equiv) was added, followed by the dropwise addition of trimethyl aluminium (3 equiv, 1.0 M in heptane). **CAUTION:** Vigorous gas evolution. The mixture was stirred at room temperature for 15 min, then a solution of lactone **11** or **12** (1.0 equiv) in anhydrous dichloromethane (5 mL/mmol lactone) was added dropwise. The solution was stirred at room temperature for 2 h and reaction quenched by the careful addition of a 10% aqueous solution of sodium potassium tartrate (Rochelle salt, 20 mL/mmol lactone). Ethyl acetate (20 mL/mmol lactone) was added, and the mixture was stirred vigorously for 30 min. The phases were separated and the aqueous layer was extracted twice with ethyl acetate (10 mL/mmol lactone), the combined organic extracts were washed with saturated brine (20 mL/mmol lactone), dried over anhydrous sodium sulfate and concentrated. The crude product **13** or **14** was used without further purification due to spontaneous re-lactonization.




***tert*-Butyl-(*S*)-4-((*S*)-1-azido-2-(methoxy(methyl)amino)-2-oxoethyl)-1,2,3-oxathiazolidine-3-carboxylate 2,2-dioxide (15).**

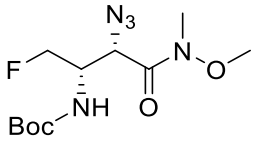
Lactone **11** (1.22 g, 5.04 mmol) was converted to **13** following general procedure A. The crude product was used without further purification.

Crude **13** was dissolved in 25 mL of anhydrous dichloromethane and added slowly to a solution of thionyl chloride (1.1 mL, 15.2 mmol) in anhydrous dichloromethane (10 mL) at -40 °C, followed by the rapid addition of pyridine (2.0 mL, 25.2 mmol). The solution was stirred at this temperature for 2 h, then warmed

to room temperature. Dichloromethane (30 mL) was added and the solution was washed with 30 mL of a 0.5 N solution of hydrochloric acid and 30 mL of saturated brine. The organic phase was dried over anhydrous sodium sulfate and concentrated.

The crude residue was dissolved in 30 mL of acetonitrile and cooled to 0 °C. Catalytic ruthenium (III) chloride trihydrate (6 mg, 0.5 mol%) was added, followed by sodium periodate (1.62 g, 7.56 mmol) and water (30 mL). The reaction mixture was stirred at 0 °C for 1 h, then warmed to room temperature and diluted with 30 mL of water. The mixture was extracted 3 × 30 mL of diethyl ether, then the combined organic extracts were washed with 30 mL of a saturated aqueous solution of sodium bicarbonate, 30 mL of a 10% aqueous solution of sodium thiosulfate and 30 mL of saturated brine. The organic phase was dried over anhydrous sodium sulfate, concentrated, and purified by flash chromatography (7/3 → 6/4 hexanes/ethyl acetate) to afford **15** (1.20 g, 65%) as a white solid.

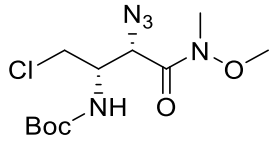
**$^1$H NMR** (400 MHz; $CDCl_3$): δ 4.76 (d, *J* = 5.5 Hz, 1H), 4.70-4.64 (m, 1H), 4.63-4.56 (m, 2H), 3.70 (s, 3H), 3.24 (s, 3H), 1.53 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 166.5, 148.8, 86.2, 66.8, 61.9, 57.7, 57.5, 32.6, 28.0; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{11}H_{19}N_5SO_7Na$ 388.0897, found 388.0903




***tert*-Butyl ((2*S*,3*S*)-3-azido-1-fluoro-4-(methoxy(methyl)amino)-4-oxobutan-2-yl)carbamate (16a).**

To a dry flask under Argon was added **15** (400 mg, 1.1 mmol) and 20 mL of anhydrous tetrahydrofuran. Tetrabutylammonium fluoride (1.4 mL, 1.4 mmol, 1.0 M in tetrahydrofuran) was added and the reaction was stirred at room temperature for 30 min. Diethyl ether (40 mL) and a 1 *N* solution of hydrochloric acid (40 mL) were added and the mixture was stirred vigorously for 4 h. The layers were separated and the aqueous phase was extracted 2 × 20 mL of diethyl ether. The organic phases were combined, dried over anhydrous sodium sulfate and concentrated. Purification by flash chromatography (8/2 hexanes/ethyl acetate) yielded **16a** (314 mg, 94%) as a white solid.

**$^1$H NMR** (400 MHz; $CDCl_3$): δ 4.78 (br. d, *J* = 9.0 Hz, 1H), 4.64-4.58 (m, 1H), 4.54-4.40 (m, 2H), 4.35 (m, 1H), 3.74 (s, 3H), 3.21 (s, 3H), 1.40 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 168.2, 155.4, 81.6 (d, *J* = 173.0 Hz), 80.5, 61.9, 59.9, 50.4 (d, *J* = 22.1 Hz), 39.9, 28.4; **$^{19}$F NMR** (282 MHz; $CDCl_3$): δ -225.8 (td, *J* = 46.2, 15.2 Hz); **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{11}H_{20}FN_5O_4Na$ 328.1392, found 328.1387

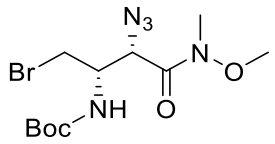



***tert*-Butyl ((2*S*,3*S*)-3-azido-1-chloro-4-(methoxy(methyl)amino)-4-oxobutan-2-yl)carbamate (17a).**

To a dry flask under Argon was added **15** (250 mg, 0.68 mmol) and 10 mL of anhydrous tetrahydrofuran. Lithium chloride (80 mg, 1.9 mmol) was added and the reaction was stirred at room temperature for 2 h until the starting material was consumed as assessed by thin layer chromatography. Water (20 mL) and diethyl ether (20 mL) were added and the mixture was stirred vigorously for 15 min.

The layers were separated and the aqueous phase was extracted 2 × 20 mL of diethyl ether. The organic phases were combined, dried over anhydrous sodium sulfate and concentrated to afford **17a** (205 mg, 92%) as a white solid which did not require further purification.

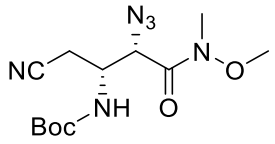
**$^1$H NMR** (400 MHz; $CDCl_3$): δ 4.88 (br. d, $J$ = 9.5 Hz, 1H), 4.82 (d, $J$ = 3.0 Hz, 1H), 4.42 (m, 1H), 3.75 (s, 3H), 3.56 (ABX, $J_{AB}$ = 11.0 Hz, $J_{AX}$ = 8.6 Hz, $J_{BX}$ = 4.9 Hz, 2H), 3.19 (s, 3H), 1.39 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 168.2, 155.2, 80.6, 61.9, 60.3, 51.9, 43.5, 33.0, 28.4; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{11}H_{20}ClN_5O_4Na$ 344.1096, found 344.1091




***tert*-Butyl ((2*S*,3*S*)-3-azido-1-bromo-4-(methoxy(methyl)amino)-4-oxobutan-2-yl)carbamate (18a).**

To a dry flask under Argon was added **15** (240 mg, 0.66 mmol) and dissolved in 10 mL of anhydrous tetrahydrofuran. Lithium bromide (90 mg, 1.03 mmol) was added and the reaction was stirred at room temperature for 2 h until the starting material was consumed as observed by thin layer chromatography. Water (20 mL) and diethyl ether (20 mL) were added and the mixture was stirred vigorously for 15 min. The layers were separated and the aqueous phase was extracted 2 × 20 mL of diethyl ether. The organic phases were combined, dried over anhydrous sodium sulfate and concentrated to afford **18a** (236 mg, 98%) as a white solid, which did not require further purification.

**$^1$H NMR** (400 MHz; $CDCl_3$): δ 4.95-4.83 (m, 2H), 4.44 (m, 1H), 3.77 (s, 3H), 3.44 (ABX, $J_{AB}$ = 10.1 Hz, $J_{AX}$ = 9.0 Hz, $J_{BX}$ = 4.9 Hz, 2H), 3.21 (s, 3H), 1.40 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 168.2, 155.1, 80.6, 61.9, 61.0, 51.7, 33.0, 31.9, 28.4; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{11}H_{20}BrN_5O_4Na$ 388.0591, found 388.0589

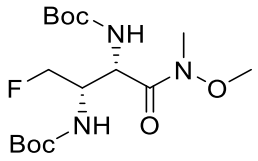



***tert*-Butyl ((2*R*,3*S*)-3-azido-1-cyano-4-(methoxy(methyl)amino)-4-oxobutan-2-yl)carbamate (19a).**

To a dry flask under Argon was added 1**5** (350 mg, 0.96 mmol) and 12 mL of anhydrous tetrahydrofuran. Potassium cyanide (93 mg, 1.43 mmol) and 18-crown-6 (26 mg, 0.1 mmol) were added, and the reaction was stirred at room temperature for 18 h until the starting material was consumed as monitored by thin layer chromatography. A 1 *N* solution of hydrochloric acid (20 mL) and diethyl ether (20 mL) were added, and the mixture was stirred vigorously for 15 min. The layers were separated, and the aqueous phase was extracted 2 × 20 mL of diethyl ether. The organic phases were combined, dried over anhydrous sodium sulfate, concentrated, and purified by flash chromatography (6/4 hexanes/ethyl acetate) to afford **19a** (200 mg, 66%) as a white solid.

**$^1$H NMR** (400 MHz; $CDCl_3$): δ 4.91 (br. d, $J$ = 9.0 Hz, 1H), 4.69 (d, $J$ = 3.5 Hz, 1H), 4.54-4.44 (m, 1H), 3.78 (s, 3H), 3.22 (s, 3H), 2.70 (ABX, $J_{AB}$ = 16.8 Hz, $J_{AX}$ = 8.2 Hz, $J_{BX}$ = 6.2 Hz, 2H), 1.41 (s, 9H); **$^{13}$C**

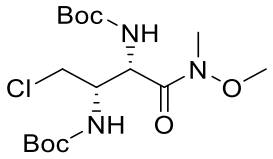
**NMR** (100 MHz; $CDCl_3$): δ 167.3, 155.0, 116.9, 80.9, 61.9, 61.3, 48.1, 32.9, 28.3, 21.1; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{12}H_{20}N_6O_4Na$ 335.1438, found 335.1434




**Di-*tert*-butyl ((2*S*,3*S*)-4-fluoro-1-(methoxy(methyl)amino)-1-oxobutane-2,3-diyl)dicarbamate (16b).**

Compound **16a** (320 mg, 1.05 mmol) was dissolved in 20 mL of ethyl acetate. Di-*tert*-butyl dicarbonate (915 mg, 4.2 mmol) was added, followed by 10% palladium on carbon (30 mg) and the mixture was placed in a pressure tube and shaken on a Parr apparatus under 40 psi of hydrogen gas for 24 h at room temperature. The solution was filtered over Celite and rinsed with ethyl acetate, then concentrated. Purification by flash chromatography (7/3 hexanes/ethyl acetate) yielded **16b** (262 mg, 66%) as a white foam.

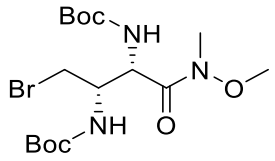
**$^1$H NMR** (400 MHz; $CDCl_3$): δ 5.52 (br. d, $J$ = 7.8 Hz, 1H), 4.86-4.75 (m, 2H), 4.55-4.25 (m, 3H), 3.76 (s, 3H), 3.18 (s, 3H), 1.41 (s, 9H), 1.39 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 170.3, 156.0, 155.6, 82.6 (d, $J$ = 170.1 Hz), 80.3, 80.1, 61.9, 51.9 (d, $J$ = 19.7 Hz), 51.5, 32.9, 28.5, 28.4; **$^{19}$F NMR** (282 MHz; $CDCl_3$): δ -227.2 (td, $J$ = 46.9, 18.0 Hz) ; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{16}H_{30}FN_3O_6Na$ 402.2011, found 402.2018




**Di-*tert*-butyl ((2*S*,3*S*)-4-chloro-1-(methoxy(methyl)amino)-1-oxobutane-2,3-diyl)dicarbamate (17b).**

Compound **17a** (205 mg, 0.62 mmol) was dissolved in 10 mL of ethyl acetate. Di-*tert*-butyl dicarbonate (540 mg, 2.4 mmol) was added, followed by 10% palladium on carbon (20 mg) and the mixture was placed in a pressure tube and shaken on a Parr apparatus under 40 psi of hydrogen gas for 24 h at room temperature. The suspension was filtered over Celite and rinsed with ethyl acetate, then concentrated. Purification by flash chromatography (7/3 hexanes/ethyl acetate) yielded **17b** (97 mg, 36%) as a white foam.

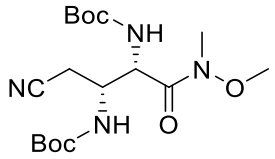
**$^1$H NMR** (400 MHz; $CDCl_3$): δ 5.52 (br. d, $J$ = 8.5 Hz, 1H), 4.89 (dd, $J$ = 8.1, 3.0 Hz, 1H), 4.85-4.74 (m, 1H), 4.43-4.32 (m, 1H), 3.76 (s, 3H), 3.54 (ABX, $J_{AB}$ = 11.2, $J_{AX}$ = 6.7, $J_{BX}$ = 5.9 Hz, 2H), 3.18 (s, 3H), 1.42 (s, 9H), 1.39 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 170.4, 156.1, 155.5, 80.5, 80.2, 61.9, 53.3, 52.1, 44.7, 33.0, 28.5, 28.4; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{16}H_{30}ClN_3O_6Na$ 418.1715, found 418.1716




***tert*-Butyl (2*S*,3*S*)-4-bromo-2,3-bis((*tert*-butoxycarbonyl)amino)butanoate (18b).**

Compound **18a** (236 mg, 0.65 mmol) was dissolved in 10 mL of ethyl acetate. Di-*tert*-butyl dicarbonate (566 mg, 2.6 mmol) was added, followed by 10% palladium on carbon (20 mg) and the mixture was placed in a pressure tube and shaken on a Parr apparatus under 40 psi of hydrogen gas for 24 h at room temperature. The mixture was filtered over Celite and rinsed with ethyl acetate, then concentrated. Purification by flash chromatography (7/3 hexanes/ethyl acetate) yielded **18b** (102 mg, 35%) as a white foam.

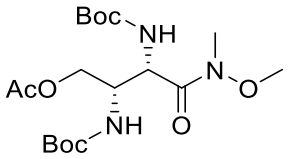
**$^1$H NMR** (400 MHz; $CDCl_3$): δ 5.54 (br. d, $J$ = 8.2 Hz, 1H), 4.94-4.85 (m, 1H), 4.84-4.73 (m, 1H), 4.46-4.34 (m, 1H), 3.77 (s, 3H), 3.40 (ABX, $J_{AB}$ = 10.2, $J_{AX}$ = 6.6, $J_{BX}$ = 6.4 Hz, 2H) 3.18 (s, 3H), 1.42 (s, 9H), 1.39 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 170.2, 156.2, 155.4, 80.5, 80.2, 62.0, 53.2, 52.7, 33.2, 33.0, 28.5, 28.5; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{16}H_{30}BrN_3O_6Na$ 462.1210, found 462.1208




**Di-*tert*-butyl ((2*S*,3*R*)-4-cyano-1-(methoxy(methyl)amino)-1-oxobutane-2,3-diyl)dicarbamate (19b).**

Compound **19a** (200 mg, 0.64 mmol) was dissolved in 3 mL of ethyl acetate and 3 mL of methanol. Di-*tert*-butyl dicarbonate (280 mg, 1.28 mmol) was added, followed by 10% palladium on carbon (20 mg). The reaction flask was affixed with a balloon of $H_2$ and the mixture stirred for 2 h at room temperature, then filtered through a pad of Celite, rinsed with ethyl acetate and concentrated. Purification by flash chromatography yielded **19b** (82 mg, 33%) as a white foam.

**$^1$H NMR** (400 MHz; $CDCl_3$): δ 5.62 (d, $J$ = 7.7 Hz, 1H), 4.85 (br. d, $J$ = 7.6 Hz, 1H), 4.72 (dd, $J$ = 7.7, 2.5 Hz, 1H), 4.54-4.44 (m, 1H), 3.78 (s, 3H), 3.19 (s, 3H), 2.61 (ABX, $J_{AB}$ = 16.8 Hz, $J_{AX}$ = 6.9 Hz, $J_{BX}$ = 6.2 Hz, 2H), 1.43 (s, 9H), 1.38 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 169.5, 156.3, 155.1, 117.2, 80.8, 80.5, 62.0, 53.7, 48.8, 33.0, 28.4, 28.4, 22.0; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{17}H_{30}N_4O_6Na$ 409.2058, found 409.2061

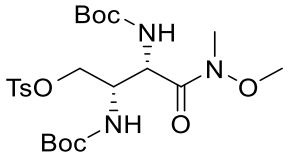



**(2*S*,3*S*)-2,3-Bis((*tert*-butoxycarbonyl)amino)-4-(methoxy(methyl)amino)-4-oxobutyl acetate (20).**

Lactone **12** (477 mg, 1.51 mmol) was converted to **14** following general procedure A. The crude product was used without further purification.

Crude **14** was dissolved in 15 mL of dichloromethane and cooled to 0 °C. Acetic anhydride (0.57 mL, 6.04 mmol) and pyridine (0.49 mL, 6.04 mmol) were added, followed by a catalytic amount of dimethylaminopyridine (18 mg. 0.15 mmol). The reaction was stirred at this temperature for 3 h, then diluted with 30 mL of ethyl acetate and washed with 30 mL of a saturated solution of ammonium chloride, 30 mL of a saturated solution of sodium bicarbonate and 30 mL of saturated brine. The organic phase was dried over anhydrous sodium sulfate, concentrated and purified by flash chromatography (7/3 → 6/4 hexanes/ethyl acetate) to afford **20** (456 mg, 77%) as a white foam.

**$^1$H NMR** (400 MHz; $CDCl_3$): δ 5.54-5.44 (m, 1H), 4.83-4.73 (m, 1H), 4.68 (d, *J* = 10.0 Hz, 1H), 4.43-4.32 (m, 1H), 4.10-4.04 (m, 2H), 3.76 (s, 3H), 3.18 (s, 3H), 2.05 (s, 3H), 1.41 (s, 9H), 1.39 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 170.9, 170.5, 156.0, 155.6, 80.2, 80.0, 63.9, 61.9, 51.9, 51.1, 32.9, 28.5, 28.4, 21.0; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{18}H_{34}N_3O_8$ 420.2340, found 420.2343

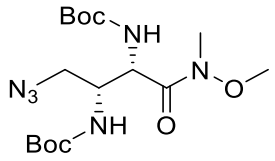



**(2*S*,3*S*)-2,3-Bis((*tert*-butoxycarbonyl)amino)-4-(methoxy(methyl)amino)-4-oxobutyl 4-methylbenzenesulfonate (21).**

Lactone **12** (840 mg, 2.66 mmol) was converted to **14** following general procedure A. The crude product was used without further purification.

Crude **14**, *p*-toluenesulfonyl chloride (2.03 g, 10.6 mmol) and 4-dimethylaminopyridine (65 mg, 0.53 mmol) were dissolved in 30 mL of dichloromethane. Triethylamine (1.85 mL, 13.3 mmol) was added and the reaction was stirred at room temperature for 1.5 h. The solution was diluted with 30 mL dichloromethane and washed with 60 mL of a 1 N solution of hydrochloric acid. The phases were separated and the aqueous fraction was extracted with 30 mL dichloromethane. The combined organics were dried over anhydrous sodium sulfate and concentrated. Purification by flash chromatography (7/3 → 6/4 hexanes/ethyl acetate) yielded **21** (1.1 g, 78%) as a white foam.

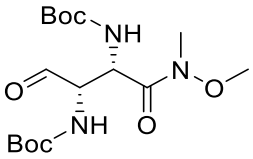
**$^1$H NMR** (400 MHz; $CDCl_3$): δ 7.76 (d, *J* = 8.2 Hz, 2H), 7.31 (d, *J* = 8.2 Hz, 2H), 5.45 (br. d, *J* = 6.5 Hz, 1H), 4.77-4.66 (m, 2H), 4.42-4.31 (m, 1H), 4.08-4.01 (m, 1H), 3.98-3.90 (m, 1H), 3.72 (s, 3H), 3.14 (s, 3H), 2.42 (s, 3H), 1.39 (s, 9H), 1.36 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 169.8, 155.9, 155.4, 145.1, 132.8, 130.0, 128.2, 80.4, 80.2, 69.1, 61.9, 51.7, 51.3, 32.9, 28.4, 28.4 21.8; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{23}H_{38}N_3SO_9$ 532.2323, found 532.2327




**Di-*tert*-butyl ((2*S*,3*R*)-4-azido-1-(methoxy(methyl)amino)-1-oxobutane-2,3-diyl)dicarbamate (22).**

Tosylate **21** (450 mg, 0.85 mmol) was placed under Argon and dissolved in 12 mL of anhydrous toluene. A solution of tetra-*n*-butylammonium azide (3.4 mL, 1.7 mmol, 0.5 M in toluene) was added, the reaction was heated to 50 °C and stirred for 2 h. After cooling to room temperature, the reaction was quenched by the addition of 35 mL of a saturated solution of ammonium chloride. The mixture was extracted 3 × 20 mL of ethyl acetate, the combined organic phases were washed with 30 mL of saturated brine, dried over anhydrous sodium sulfate and concentrated. Purification by flash chromatography (8/2 hexanes/acetone) yielded **22** (257 mg, 75%) as a white foam.

**$^1$H NMR** (400 MHz; $CDCl_3$): δ 5.54 (d, *J* = 8.2 Hz, 1H), 4.81-4.67 (m, 2H), 4.33-4.22 (m, 1H), 3.77 (s, 3H), 3.40-3.29 (m, 2H), 3.18 (s, 3H), 1.42 (s, 9H), 1.39 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 170.3, 156.1, 155.5, 80.5, 80.2, 61.9, 52.3, 51.6, 33.0, 28.5, 28.5, 28.4; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{16}H_{30}N_6O_6Na$ 425.2119, found 425.2120 (MSL-7182)

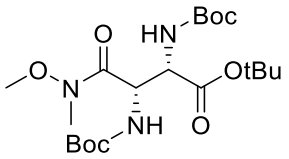



**Di-*tert*-butyl ((2*S*,3*S*)-1-(methoxy(methyl)amino)-1,4-dioxobutane-2,3-diyl)dicarbamate (23).**

Lactone **12** (638 mg, 2.02 mmol) was converted to **14** by general procedure A and used without further purification.

Crude **14** was dissolved in 25 mL of dichloromethane and Dess-Martin periodinane (1.28 g, 3.03 mmol) was added in one portion. The reaction was stirred at room temperature for 3.5 h, diluted with 25 mL of dichloromethane and washed with 25 mL of a 10% solution of sodium thiosulfate and 25 mL of a saturated solution of sodium bicarbonate. The organic phase was dried over anhydrous sodium sulfate and concentrated. Purification by flash chromatography (6/4 →1/1 hexanes/ethyl acetate) yielded the product aldehyde **23** (695 mg, 92%) as a white foam.

**$^1$H NMR** (400 MHz; $CDCl_3$): δ 9.68 (s, 1H), 5.58 (br. d, *J* = 7.5 Hz, 1H), 5.28 (br. d, *J* = 7.2 Hz, 1H), 5.12 (br. d, *J* = 7.2 Hz, 1H), 4.91 (br. d, *J* = 7.7 Hz, 1H), 3.81 (s, 3H), 3.21 (s, 3H), 1.39 (s, 9H), 1.38 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 195.6, 169.2, 155.4, 80.7, 80.5, 62.0, 61.7, 51.4, 33.4, 28.4; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{16}H_{29}N_3O_7Na$ 398.1898, found 398.1896




**(2*S*,3*S*)-2,3-Bis((*tert*-butoxycarbonyl)amino)-4-(methoxy(methyl)amino)-4-oxobutanoic acid (24).**

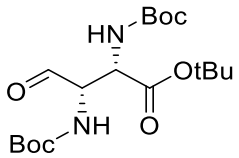
Lactone **12** (895 mg, 2.83 mmol) was converted to **14** by general procedure A and used without further purification.

Crude **14** was dissolved in 30 mL of dichloromethane then Dess-Martin periodinane (1.80 g, 4.25 mmol) was added in a single portion and the reaction was stirred at room temperature for 2 h. Diethyl ether (60 mL) and a 10% aqueous solution to sodium thiosulfate (60 mL) were added and the mixture was stirred vigorously for 30 min. The phases were separated and the aqueous layer was extracted with 30 mL of diethyl ether. The combined organics were washed with 45 mL of a saturated aqueous solution of sodium bicarbonate and 45 mL of saturated brine, dried over anhydrous sodium sulfate and concentrated to afford the crude aldehyde **23** which was used directly in the next reaction.

Crude **23** was dissolved in *tert*-butanol (30 mL) and water (20 mL) and cooled to 0 °C. Amylene (6.0 mL, 56.6 mmol)) and sodium dihydrogen phosphate monohydrate (1.25 g, 9.06 mmol) were added, followed by the addition of sodium chlorite (917 mg, 10.2 mmol). The reaction mixture was stirred for 18 h before being quenched with a saturated aqueous solution of ammonium chloride (70 mL) and extracted 3 × 35 mL of ethyl acetate. The combined organic phases were dried over anhydrous sodium sulfate, concentrated and carried forward without purification.

The crude carboxylic acid was placed under Argon and dissolved in anhydrous dichloromethane (15 mL). *Tert*-butyl 2,2,2-trichloroacetamidate (1.0 mL, 5.66 mmol) was added and the reaction was stirred at room temperature for 18 h. The reaction was then diluted with 60 mL of diethyl ether, washed with a 45 mL of a 2.0 M solution of sodium hydroxide and 45 mL of saturated brine, dried over anhydrous sodium sulfate, and concentrated to afford the crude ester. Purification by flash chromatography (8/2 → 7/3 hexanes/ethyl acetate) gave **24** (620 mg, 49% over four steps) as a white foam.

**$^1$H NMR** (400 MHz; $CDCl_3$): δ 5.43 (br. d, *J* = 9.5 Hz, 1H), 5.20 (d, *J* = 8.3 Hz, 1H), 5.07 (br. d, *J* = 9.5 Hz, 1H), 4.81 (br. d, *J* = 8.3 Hz, 1H), 3.78 (s, 3H), 3.16 (s, 3H), 1.45 (s, 9H), 1.37 (s, 18H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 170.3, 168.6, 155.3, 155.3, 83.1, 80.2, 80.0, 61.8, 54.9, 53.4, 33.4, 28.4, 28.4, 27.9; **HRMS** (ESI) *m/z*: $[M+Na]^+$ $C_{20}H_{37}N_3O_8Na$ calculated 470.2473, found 470.2479

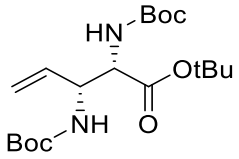



**(2*S*,3*S*)-2,3-Bis((*tert*-butoxycarbonyl)amino)-4-oxobutanoic acid (25).**

Weinreb amide **24 (**420 mg, 0.94 mmol) was placed under Argon, dissolved in 5 mL of anhydrous tetrahydrofuran and cooled to -78 °C. Diisobutylaluminum hydride (1.88 mL, 1.88 mmol 1.0 M in hexanes) was added dropwise and the reaction mixture was stirred at this temperature for 3 h. A 10% aqueous solution of sodium potassium tartrate (30 mL) and ethyl acetate (30 mL) were added and the mixture was warmed

to room temperature and stirred vigorously for 1 h. The phases were separated, and the aqueous layer was extracted 2 × 20 mL of ethyl acetate. The combined organic phases were washed with 20 mL of saturated brine, dried over anhydrous sodium sulfate and concentrated. Purification by flash chromatography yielded **25** (330 mg, 90%) as a white foam.

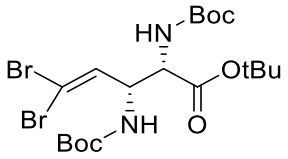
**$^1$H NMR** (400 MHz; $CDCl_3$): δ 9.65 (s, 1H), 5.28 (br. d, *J* = 6.4 Hz, 1H), 5.23 (br. d, *J* = 8.9 Hz, 1H), 4.82, (br. d, *J* = 8.9 Hz, 1H), 4.76-4.67 (m, 1H), 1.47 (s, 9H), 1.40 (s, 9H), 1.39 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 196.6, 168.5, 155.4, 155.3, 84.0, 80.8, 80.5, 62.4, 53.8, 28.4, 28.4, 28.0; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{18}H_{32}N_2O_7Na$ 411.2102, found 411.2104




**(2*S*,3*R*)-2,3-Bis((*tert*-butoxycarbonyl)amino)pent-4-enoic acid (26).**

To a dry flask under Argon was added methyltriphenylphosphonium bromide (180 mg, 0.5 mmol) which was suspended in 5 mL of anhydrous tetrahydrofuran and cooled to 0 °C. Potassium *tert*-butoxide (56 mg, 0.5 mmol) was added and the mixture was stirred at this temperature for 30 min. Aldehyde **25** (130 mg, 0.335 mmol) was then added dropwise as a solution in 3 mL of anhydrous tetrahydrofuran and the reaction mixture was stirred at 0 °C for 2 h A saturated aqueous solution of ammonium chloride (15 mL) was added and mixture was extracted 3 × 15 mL of ethyl acetate. The combined organics were dried over anhydrous sodium sulfate and concentrated. Purification by flash chromatography (9/1 → 8/2 hexanes/ethyl acetate) yielded **26 (**70 mg, 54%) as a white solid. This product could be further purified by precipitation from hot cyclohexane.

**$^1$H NMR** (400 MHz; $CDCl_3$): δ : 5.85-5.72 (m, 1H), 5.26-5.12 (m, 3H), 4.94-4.82 (m, 1H), 4.64-4.52 (m, 1H), 4.37-4.27 (m, 1H), 1.45 (s, 9H), 1.40 (s, 18H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 169.5, 155.7, 155.2, 135.1, 116.7, 83.2, 80.2, 79.8, 57.4, 55.3, 28.5, 28.5, 28.1; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{19}H_{35}N_2O_6$ 387.2490, found 387.2493

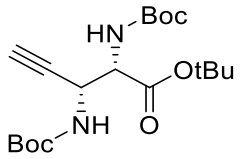



**(2*S*,3*R*)-5,5-Dibromo-2,3-bis((*tert*-butoxycarbonyl)amino)pent-4-enoic acid (S3).**

Carbon tetrabromide (349 mg, 1.05 mmol) was placed in a dry flask under Argon and dissolved in 8 mL of anhydrous dichloromethane. The solution was cooled to 0 °C and triphenylphosphine (348 mg, 2.1 mmol) was added. The mixture was stirred at this temperature for 15 min, then added dropwise to a solution of aldehyde **25** (200 mg, 0.52 mmol) in 8 mL of anhydrous dichloromethane at 0 °C. The reaction was stirred for 30 min then quenched by the addition of 20 mL of a saturated aqueous solution of ammonium chloride. The phases were separated and the aqueous layer was extracted with 20 mL of dichloromethane.

The combined organics were dried over anhydrous sodium sulfate and concentrated. Purification by flash chromatography (9/1 → 8/2 hexanes/ethyl acetate) yielded **S3** (170 mg, 60%) as a white solid

**$^1$H NMR** (400 MHz; $CDCl_3$): δ 6.33 (br. d, *J* = 8.2 Hz, 1H), 5.42-55.25 (m, 1H), 5.17-4.96 (m, 1H), 4.83-4.63 (m, 1H), 4.35-4.25 (m, 1H), 1.47 (s, 9H), 1.43 (s, 9H), 1.40 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 168.7, 155.7, 154.9, 135.7, 83.6, 80.7, 80.3, 56.9, 55.7, 28.5, 28.5, 28.1; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{19}H_{32}Br_2N_2O_6Na$ 565.0519, found 565.0513




**(2*S*,3*R*)-2,3-Bis((*tert*-butoxycarbonyl)amino)pent-4-ynoic acid (27).**

Compound **25** (170 mg, 0.31 mmol) was placed under Argon and dissolved in 10 mL of anhydrous THF, then cooled to -78 °C. A solution of *n*-butyllithium (0.50 mL, 1.25 mmol, 2.5M in hexanes) was added dropwise and the mixture was stirred at this temperature for 2 h. The reaction was quenched by the addition of 10 mL of a saturated solution of ammonium chloride and warmed to room temperature. The mixture was extracted 3 × 10 mL of ethyl acetate, the combined organics were washed with 10 mL of saturated brine and dried over anhydrous sodium sulfate. Purification by flash chromatography (9/1 hexanes/ethyl acetate) afforded **27** (40 mg, 33%) as a white solid.

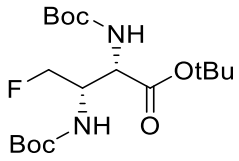
**$^1$H NMR** (400 MHz; $CDCl_3$): δ 5.29-5.14 (m, 2H), 4.86-4.74 (m, 1H), 4.46-4.36 (m, 1H), 2.27 (d, *J* = 2.2 Hz, 1H), 1.48 (s, 9H), 1.43 (s, 9H), 1.42 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 168.5, 155.3, 154.7, 83.6, 80.4, 80.1, 72.8, 57.4, 45.3, 28.5, 28.4, 28.1; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{19}H_{32}N_2O_6Na$ 407.2153, found 407.2156

**General procedure B for Weinreb amide cleavage and re-protection.**

Weinreb amide (**16b-19b, 20, 22**) (1.0 equiv) was placed under Argon, dissolved in anhydrous tetrahydrofuran (10 mL/mmol) and cooled to -78 °C. Diisobutylaluminum hydride (2.0 equiv, 1.0 M in hexanes) was added dropwise and the reaction was stirred at this temperature for 3 h. A 10% aqueous solution of sodium potassium tartrate (30 mL/mmol) and ethyl acetate (30 mL/mmol) were added and the mixture was warmed to room temperature and stirred vigorously for 1 h. The phases were separated, and the aqueous layer was extracted twice with ethyl acetate (20 mL/mmol). The combined organic phases were washed with saturated brine (30 mL/mmol), dried over anhydrous sodium sulfate and concentrated.

The crude aldehyde product was dissolved in *tert*-butanol (10 mL/mmol) and water (6 mL/mmol) and cooled to 0 °C. Amylene (20 equiv) and sodium dihydrogen phosphate monohydrate (3.2 equiv) were added, followed by the addition of sodium chlorite (3.6 equiv). The reaction was stirred for 18 h before being quenched with a saturated aqueous solution of ammonium chloride (25 mL/mmol) and extracted three times with ethyl acetate (15 mL/mmol). The combined organic phases were dried over anhydrous sodium sulfate and concentrated.

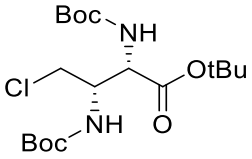
The crude carboxylic acid was placed under Argon and dissolved in anhydrous dichloromethane (5 mL/mmol). *Tert*-butyl 2,2,2-trichloroacetamidate (2.0 equiv) was added and the reaction was stirred at room temperature for 18 hours. The reaction was then concentrated and purified directly.




***tert*-Butyl (2*S*,3*S*)-2,3-bis((*tert*-butoxycarbonyl)amino)-4-fluorobutanoate (28).**

Compound **16b** (260 mg, 0.68 mmol) was subjected to general procedure B. Purification of the crude material by flash chromatography (9/1 hexanes/ethyl acetate) afforded **28** (192 mg, 72%) as a white solid.

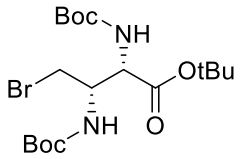
**$^{1}$H NMR** (400 MHz; $CDCl_3$): δ 5.34-5.25 (m, 1H), 4.93 (d, $J$ = 8.4 Hz, 1H), 4.51-4.45 (m, 1H), 4.40-4.26 (m, 3H), 1.46 (s, 9H), 1.43 (s, 9H), 1.40 (s, 9H) ; **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 169.4, 155.8, 155.3, 83.5, 81.1 (d, $J$ = 130.6 Hz), 80.1, 54.6, 52.6 (d, $J$ = 19.9 Hz), 28.5, 28.4, 28.0; **$^{19}$F NMR** (282 MHz; $CDCl_3$): δ -224.7 - -225.4 (m, 0.25 F, minor rotamer) -227.2 (td, $J$ = 46.9, 19.8 Hz, 0.75 F, major rotamer); **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{18}H_{33}FN_2O_6Na$ 415.2215, found 415.2219




***tert*-Butyl (2*S*,3*S*)-2,3-bis((*tert*-butoxycarbonyl)amino)-4-chlorobutanoate (29).**

Compound **17b** (210 mg. 0.53 mmol) was subjected to general procedure B. Purification of the crude material by flash chromatography (9/1 hexanes/ethyl acetate) afforded **29** (113 mg, 52%) as a white solid. This material could be further purified by precipitation from hot cyclohexane.

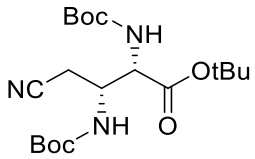
**$^{1}$H NMR** (400 MHz; $CDCl_3$): δ 5.34-5.23 (m, 1H), 4.87 (d, $J$ = 10.0 Hz, 1H), 4.47-4.39 (m, 1H), 4.38-4.29 (m, 1H), 3.57 (ABX, $J_{AB}$ = 11.2 Hz, $J_{AX}$ = 6.2 Hz, $J_{BX}$ = 5.8 Hz, 2H), 1.46 (s, 9H), 1.43 (s, 9H), 1.40 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 169.3, 156.2, 155.3, 83.4, 80.5, 80.1, 55.4, 54.2, 45.2, 28.4, 28.0; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{18}H_{34}ClN_2O_6$ 409.2100, found 409.2095




***tert*-Butyl (2*S*,3*R*)-4-bromo-2,3-bis((*tert*-butoxycarbonyl)amino)butanoate (30).**

Compound **18b** (150 mg, 0.34 mmol) was subjected to general procedure B with minor modifications: saturated sodium bromide solution was used in place of saturated brine. Purification of the crude material by flash chromatograph (9/1 hexanes/ethyl acetate) afforded **30** (40 mg, 26%) as a white solid.

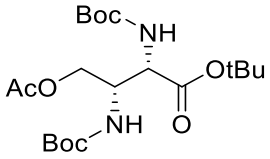
**$^1$H NMR** (400 MHz; $CHCl_3$): δ 5.34-5.23 (m, 1H), 4.85 (d, $J$ = 9.2 Hz, 1H), 4.49-4.40 (m, 1H), 4.39-4.29 (m, 1H), 3.44 (ABX, $J_{AB}$ = 10.7 Hz, $J_{AX}$ = 6.9 Hz, $J_{BX}$ = 5.6 Hz, 2H), 1.46 (s, 9H), 1.43 (s, 9H), 1.40 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 169.2, 156.2, 155.2, 83.5, 80.6, 80.1, 56.1, 54.0, 34.0, 28.5, 28.0; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{18}H_{33}BrN_2O_6Na$ 475.1414, found 475.1412




***tert*-Butyl (2*S*,3*S*)-2,3-bis((*tert*-butoxycarbonyl)amino)-4-cyanobutanoate (31).**

Compound **19b** (80 mg, 0.2 mmol) was subjected to general procedure B. Purification of the crude material by flash chromatography (9/1 hexanes/ethyl acetate) afford **31** (30 mg, 38%) as a white solid.

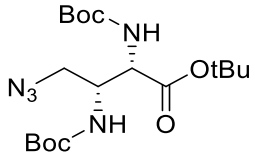
**$^1$H NMR** (400 MHz; $CDCl_3$): δ 5.36 (br. d, $J$ = 6.5 Hz, 1H), 4.92 (d, $J$ = 9.8 Hz, 1H), 4.54-4.43 (m, 1H), 4.37-4.28 (m, 1H), 2.64 (ABX, $J_{AB}$ = 16.7 Hz, $J_{AX}$ = 6.5 Hz, $J_{BX}$ = 6.0 Hz, 2H), 1.46 (s, 9H), 1.44 (s, 9H), 1.39 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 168.6, 156.3, 154.8, 117.1, 84.1, 81.1, 80.6, 56.9, 49.7, 28.4, 28.4, 28.0; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{19}H_{33}N_3O_6Na$ 422.2262, found 422.2261




***tert*-Butyl (2*S*,3*S*)-4-acetoxy-2,3-bis((*tert*-butoxycarbonyl)amino)butanoate (32).**

Compound **20** (197 mg. 0.47 mmol) was subjected to general procedure B. Purification of the crude material by flash chromatography (9/1 hexanes/ethyl acetate) afforded **32** (82 mg, 40%) as a white solid.

**$^1$H NMR** (400 MHz; $CDCl_3$): δ 5.27 (d, $J$ = 7.8 Hz, 1H), 4.75 (br. d, $J$ = 9.1 Hz, 1H), 4.41-4.31 (m, 2H), 4.08 (ABX, $J_{AB}$ = 11.5, $J_{AX}$ = 6.8, $J_{BX}$ = 5.2 H, 2H), 2.04 (s, 3H), 1.45 (s, 9H), 1.41 (s, 9H), 1.39 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 170.9, 169.5, 155.9, 155.4, 83.3, 80.4, 79.9, 64.1, 54.9, 52.0, 28.4, 28.0, 21.0; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{20}H_{37}N_2O_8$ 433.2544, found 433.2549



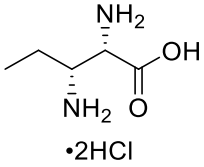

***tert*-Butyl (2*S*,3*R*)-4-azido-2,3-bis((*tert*-butoxycarbonyl)amino)butanoate (33).**

Compound **22** (257 mg, 0.64 mmol) was subjected to general procedure B. Purification of the crude material by flash chromatography (9/1 hexanes/ethyl acetate) afforded **33** (167 mg, 63%) as a white foam.

**$^1$H NMR** (400 MHz; $CDCl_3$): δ 5.35-5.25 (m, 1H), 4.82 (d, $J$ = 9.6 Hz, 1H), 4.36-4.28 (m, 1H), 4.27-4.17 (m, 1H), 3.40 (ABX, $J_{AB}$ = 12.4, $J_{AX}$ = 6.7, $J_{BX}$ = 5.7 Hz, 2H), 1.46 (s, 9H), 1.43 (s, 9H), 1.40 (s, 9H); **$^{13}$C NMR** (100 MHz; $CDCl_3$): δ 169.4, 156.0, 155.2, 83.6, 80.6, 80.2, 55.5, 52.8, 28.4, 28.0; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{18}H_{34}N_5O_6$ 416.2504, found 416.2506

**General procedure C for global acid deprotection and anion exchange.**

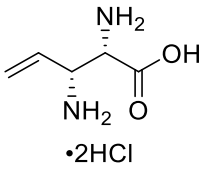
Protected amino acid (**26-33**) (1.0 equiv) was dissolved in a 1:1 mixture of dichloromethane and trifluoroacetic acid (10 mL/mmol) and stirred at room temperature for 3 h. The reaction mixture was concentrated, then a 100 mM aqueous solution of hydrochloric acid (10 mL/mmol) was added and the solution was stirred at room temperature for 5 min. The water was removed under reduced pressure and this procedure was repeated three times. After the final repetition, the residue was dissolved in water (10 mL/mmol), frozen and lyophilized to dryness to afford the product amino acids as the hydrochloride salt.




**(2*S*,3*R*)-2,3-Diaminopentanoic acid (36).**

Compound **26** (18 mg, 0.05 mmol) was dissolved in 3 mL of ethyl acetate and 10% palladium on carbon (2 mg) was added. The suspension was placed under a balloon of hydrogen gas and stirred at room temperature for 16 h. The mixture was filtered over Celite and rinsed with ethyl acetate, then concentrated. The crude residue was then subjected to general procedure C to afford **36** (5 mg, 50%) as an off-white, hygroscopic solid.

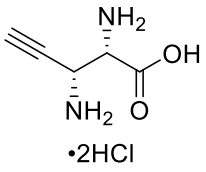
**$^1$H NMR** (400 MHz; $D_2O$): δ 4.26 (d, $J$ = 3.5 Hz, 1H), 3.70 (dt, $J$ = 10.2, 3.5 Hz, 1H), 1.93-1.79 (m, 1H), 1.69-1.55 (m, 1H), 1.07 (t, $J$ = 7.5 Hz, 3H); **$^{13}$C NMR** (100 MHz; $D_2O$): δ 169.2, 53.4, 51.7, 20.5, 9.0; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_5H_{13}N_2O_2$ 133.0972, found 133.0968




**(2*S*,3*R*)-2,3-Diaminopent-4-enoic acid dihydrochloride (37).**

Compound **26** (49 mg, 0.13 mmol) was subjected to general procedure C to afford **37** (20 mg, 76%) as an off-white, hygroscopic solid.

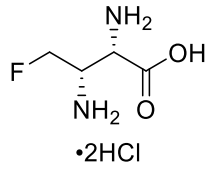
**$^1$H NMR** (400 MHz; $D_2O$): δ 5.84-5.67 (m, 3H), 4.37 (d, *J* = 3.8 Hz, 1H), 4.29 (dd, *J* = 8.4, 3.8 Hz, 1H); **$^{13}$C NMR** (100 MHz; $D_2O$): δ 168.5, 127.5, 125.8, 52.6, 52.4; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_5H_{11}N_2O_2$ 131.0815, found 131.0819




**(2*S*,3*R*)-2,3-Diaminopent-4-ynoic acid dihydrochloride (38).**

Compound **27** (40 mg, 0.10 mmol) was subjected to general procedure C to afford **38** (15 mg, 75%) as an off-white, hygroscopic solid.

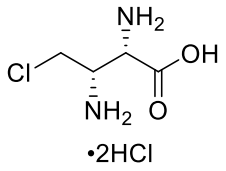
**$^1$H NMR** (400 MHz; $D_2O$): δ 4.72 (dd, *J* = 3.8, 2.2 Hz, 1H), 3.34 (d, *J* = 3.8 Hz, 1H), 3.22 (d, *J* = 2.2 Hz, 1H); **$^{13}$C NMR** (100 MHz; $D_2O$): δ 168.3, 80.7, 71.8, 52.5, 41.8; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_5H_9N_2O_2$ 129.0659, found 129.0656




**(2*S*,3*S*)-2,3-Diamino-4-fluorobutanoic acid dihydrochloride (39).**

Compound **28** (130 mg, 0.3 mmol) was subjected to general procedure C to afford **39** (62 mg, 90%) as an off-white, hygroscopic solid.

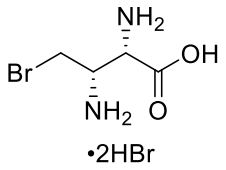
**$^1$H NMR** (400 MHz; DMSO-$d_6$): δ 4.85 (dd, *J* = 46.3, 3.8 Hz, 2H), 4.41 (d, *J* = 3.8 Hz, 1H), 4.09 (dq, *J* = 26.2, 3.8 Hz, 1H); **$^{13}$C NMR** (100 MHz; DMSO-$d_6$): δ 167.4, 79.4 (d, *J* = 169.5 Hz), 49.9 (d, *J* = 3.6 Hz), 49.3 (d, *J* = 19.3 Hz); **$^{19}$F NMR** (282 MHz; DMSO-$d_6$): δ -230.3 (td, *J* = 46.3, 26.2 Hz); **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_4H_{10}FN_2O_2$ 137.0721, found 137.0723




**(2*S*,3*S*)-2,3-Diamino-4-chlorobutanoic acid dihydrochloride (40).**

Compound **29** (103 mg, 0.25 mmol) was subjected to general procedure C to afford **40** (53 mg, 93%) as an off-white, hygroscopic solid.

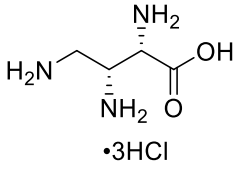
**$^1$H NMR** (400 MHz; $D_2O$): δ 4.40 (d, $J$ = 4.0 Hz, 1H), 4.18 (td, $J$ = 7.5, 4.0 Hz, 1H), 3.95 (ABX, $J_{AB}$ = 13.0, $J_{AX}$ = 7.5, $J_{BX}$ = 3.7 Hz, 2H); **$^{13}$C NMR** (100 MHz; $D_2O$): δ 168.1, 51.9, 51.0, 39.7; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_4H_{10}ClN_2O_2$ 153.0425, found 131.0431




**(2*S*,3*S*)-2,3-Diamino-4-bromobutanoic acid dihydrochloride (41).**

Compound **30** (40 mg, 0.09 mmol) was subjected to general procedure C with slight modifications: a 100 mM aqueous solution of hydrobromic acid was used in place of hydrochloric acid. Compound **41** (29 mg, 93%) was obtained as an off-white, hygroscopic solid with a trace amount of lactone (<10%).

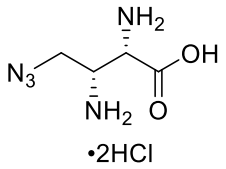
**$^1$H NMR** (400 MHz; $D_2O$): δ 4.34 (d, $J$ = 3.8 Hz, 1H), 4.15 (dt, $J$ = 8.8, 3.8 Hz, 1H), 3.73 (ABX, $J_{AB}$ = 12.0, $J_{AX}$ = 8.8, $J_{BX}$ = 3.6 Hz, 2H); **$^{13}$C NMR** (100 MHz; $D_2O$): δ 167.9, 52.5, 51.0, 26.1; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_4H_{10}BrN_2O_2$ 196.9920, found 196.9919




**(2*S*,3*R*)-2,3,4-Triaminobutanoic acid trihydrochloride (42).**

Compound **33** (45 mg, 0.11 mmol) was dissolved in 3 mL of ethyl acetate and 10% palladium on carbon (4 mg) was added. The suspension was placed under a balloon of hydrogen gas and stirred at room temperature for 16 h. The mixture was filtered over Celite and rinsed with ethyl acetate, then concentrated. The crude residue was then subjected to general procedure C to afford **42** (26 mg, 98%) as an off-white, hygroscopic solid.

**$^1$H NMR** (400 MHz; $D_2O$): δ 4.35 (d, $J$ = 3.5 Hz, 1H), 4.23-4.17 (m, 1H), 3.49 (ABX, $J_{AB}$ = 15.0, $J_{AX}$ = 9.2, $J_{BX}$ = 2.4 Hz, 2H); **$^{13}$C NMR** (100 MHz; $D_2O$): δ 168.0, 52.1, 48.5, 37.6; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_4H_{12}N_3O_2$ 134.0924, found 134.0923

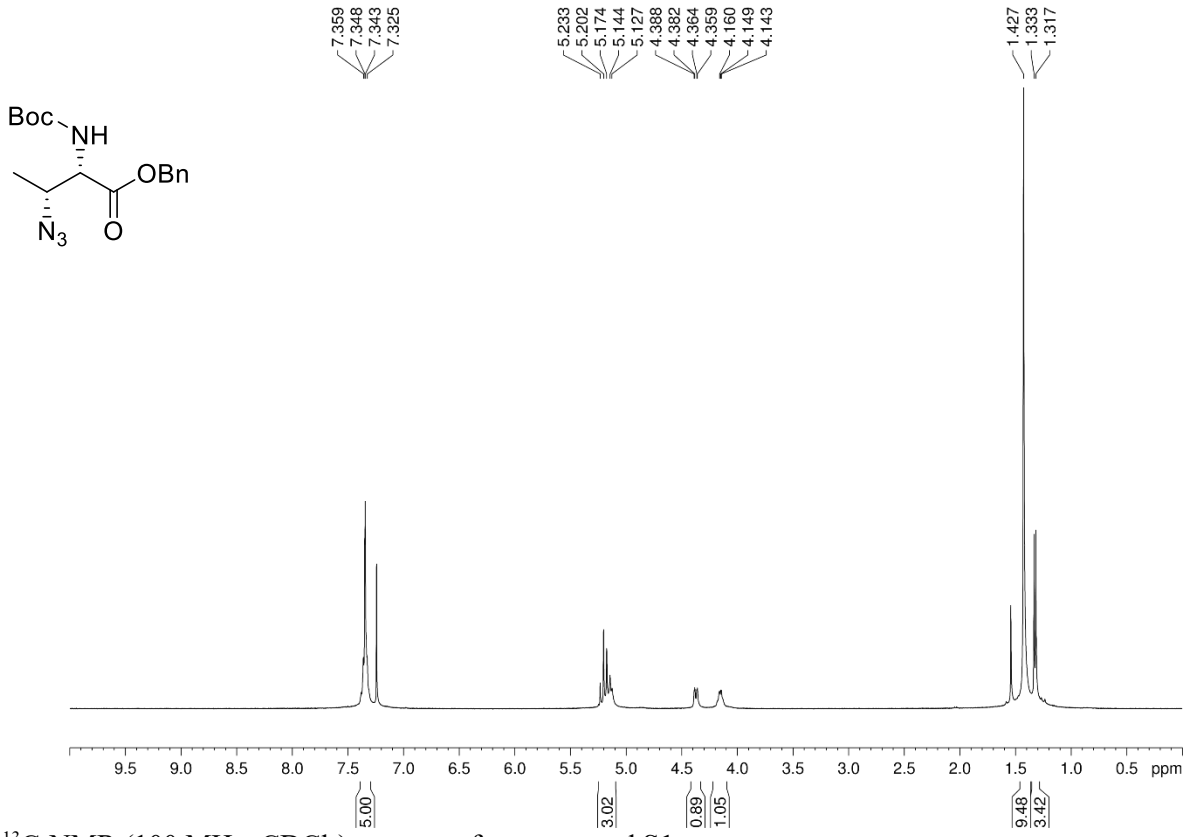



**(2*S*,3*R*)-2,3-Diamino-4-azidobutanoic acid dihydrochloride (43).**

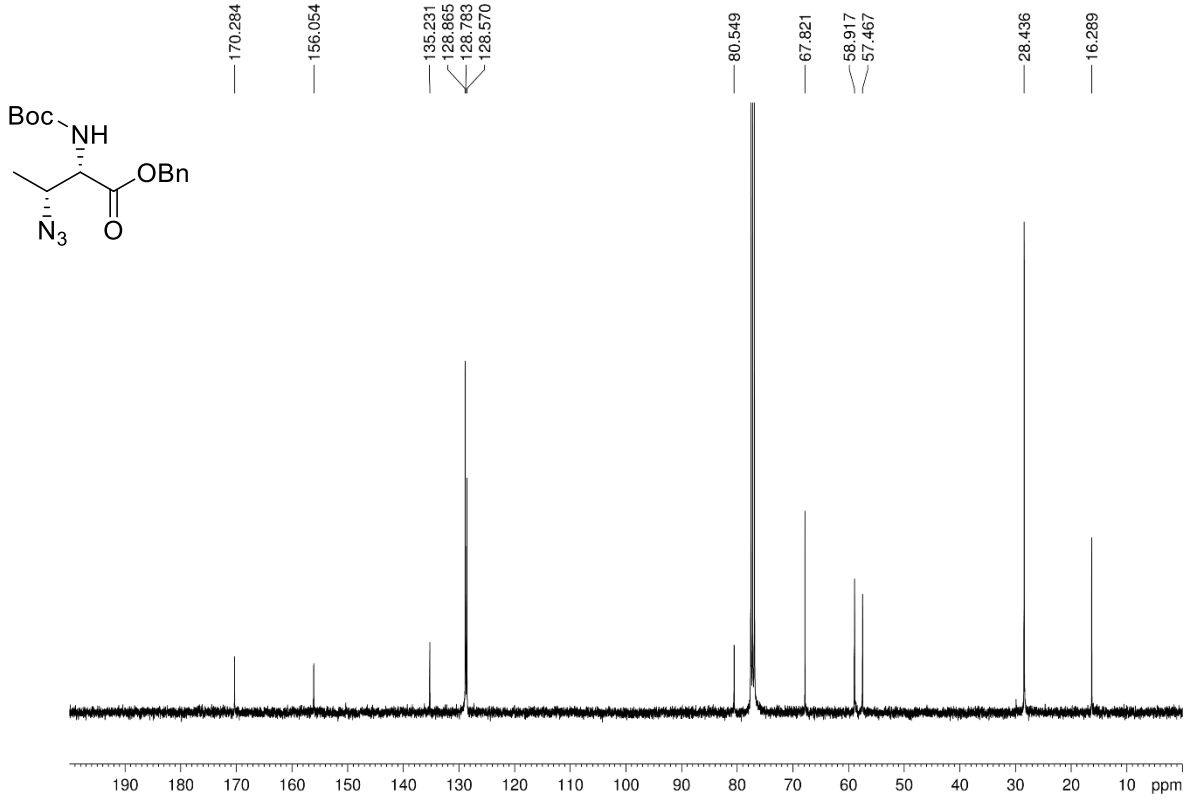
Compound **33** (74 mg, 0.18 mmol) was subjected to general procedure C to afford **43** (35 mg, 84%) as an off-white, hygroscopic solid.

**$^1$H NMR** (400 MHz; $D_2O$): δ 4.36 (d, $J$ = 3.8 Hz, 1H), 3.99-3.93 (m, 1H), 3.85 (ABX, $J_{AB}$ = 13.8, $J_{AX}$ = 6.0, $J_{BX}$ = 4.6 Hz, 2H); **$^{13}$C NMR** (100 MHz; $D_2O$): δ 168.6, 51.8, 48.9, 47.7; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_4H_{10}N_5O_2$ 160.0829, found 160.0833

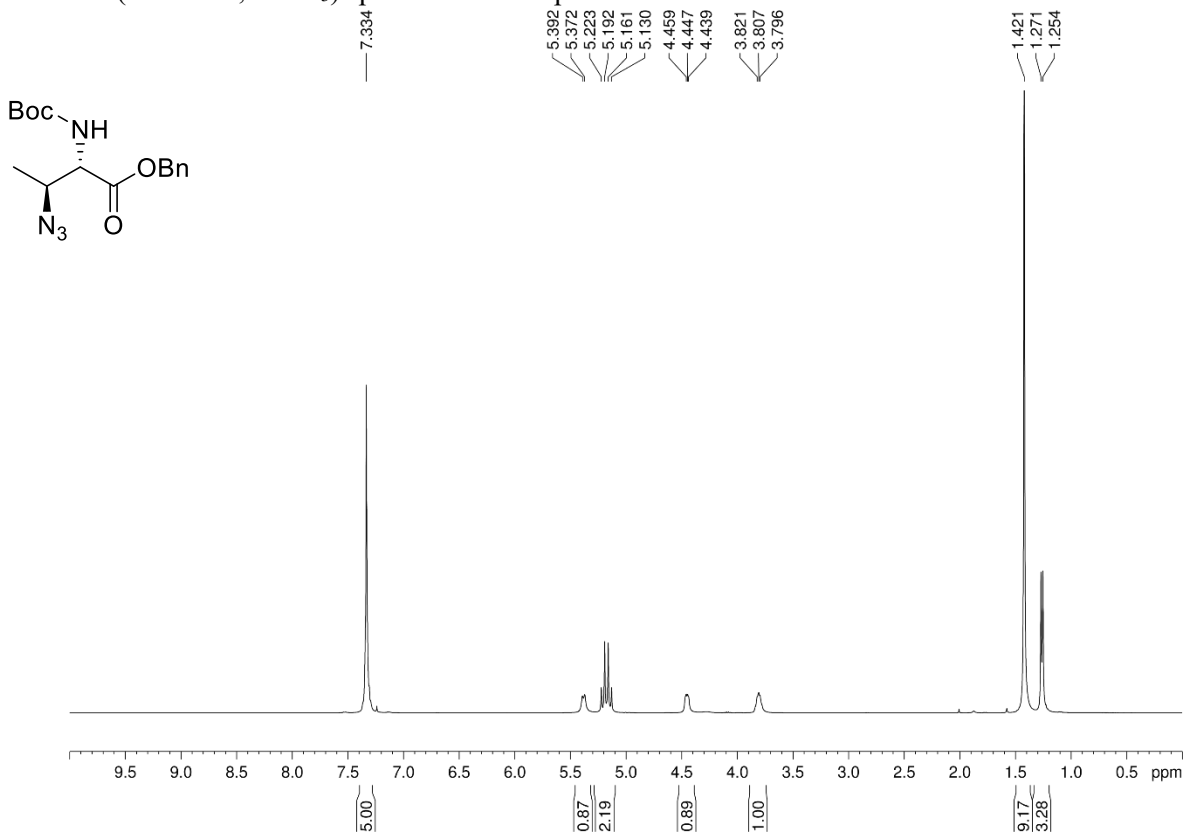
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **S1**



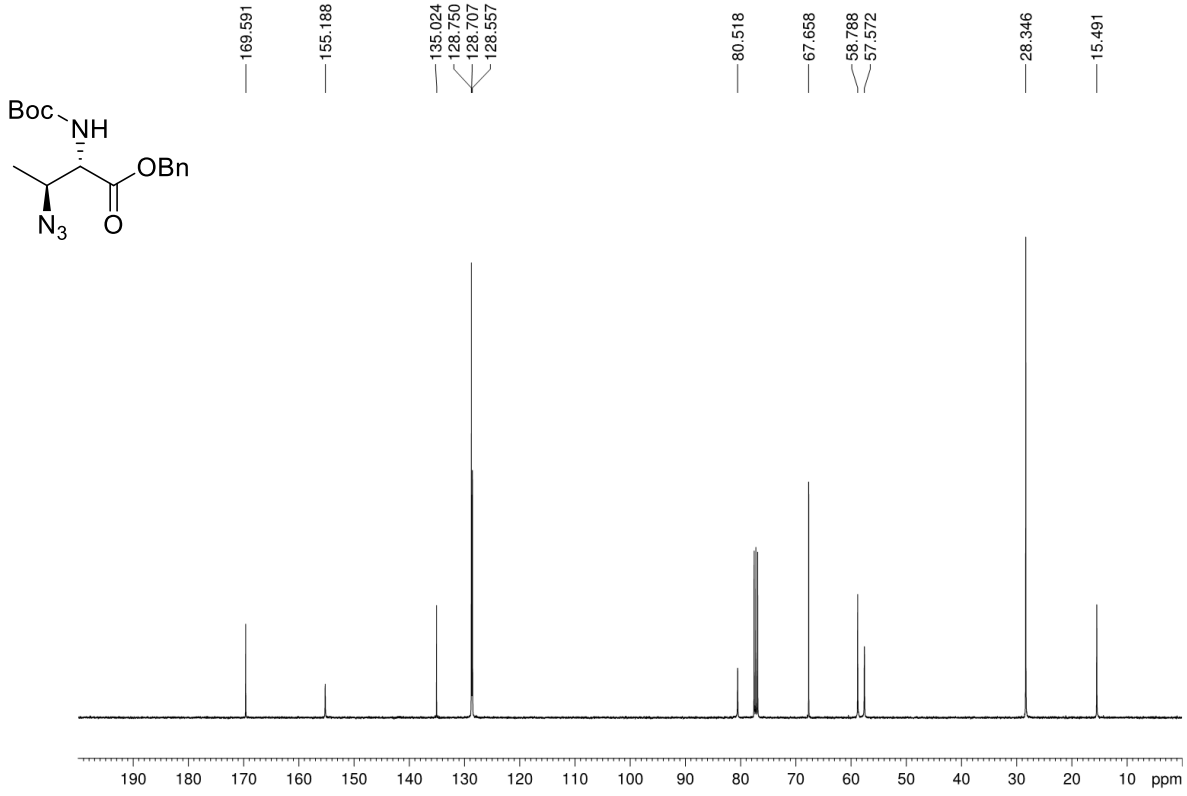

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **S1**



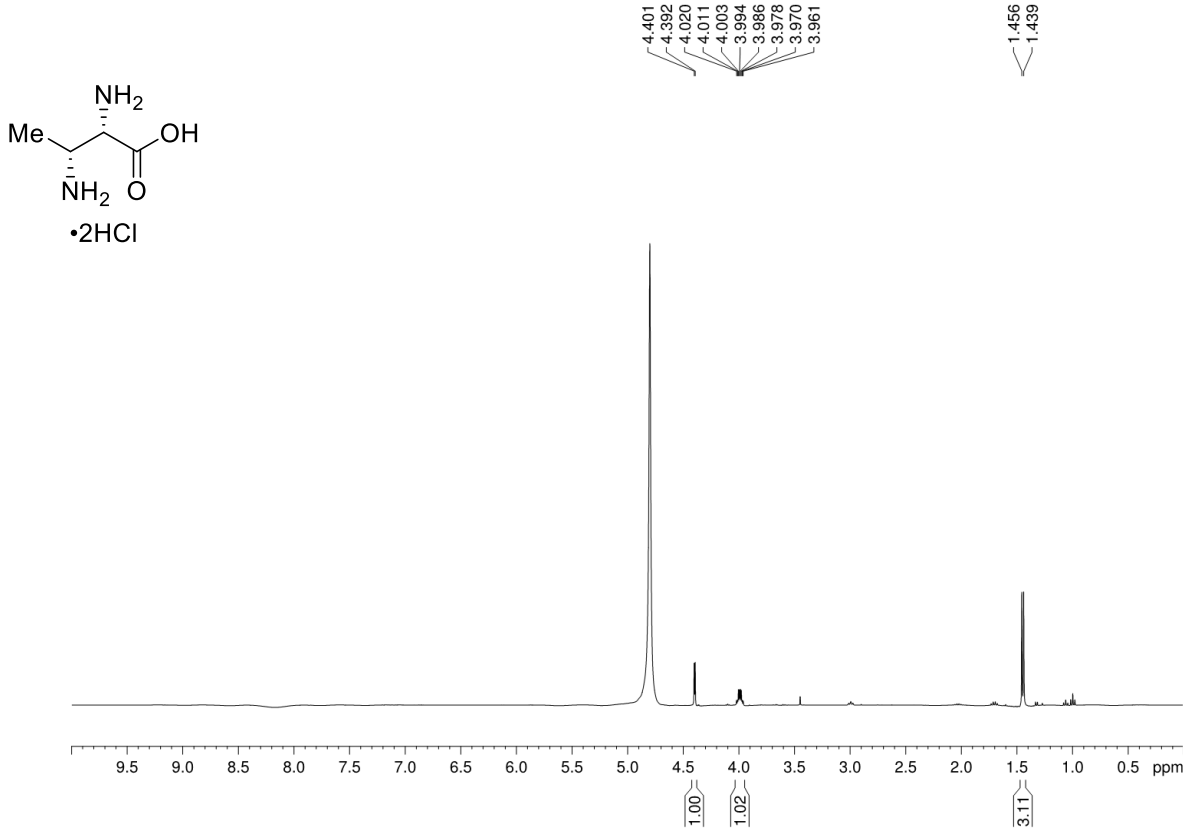
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **S2**



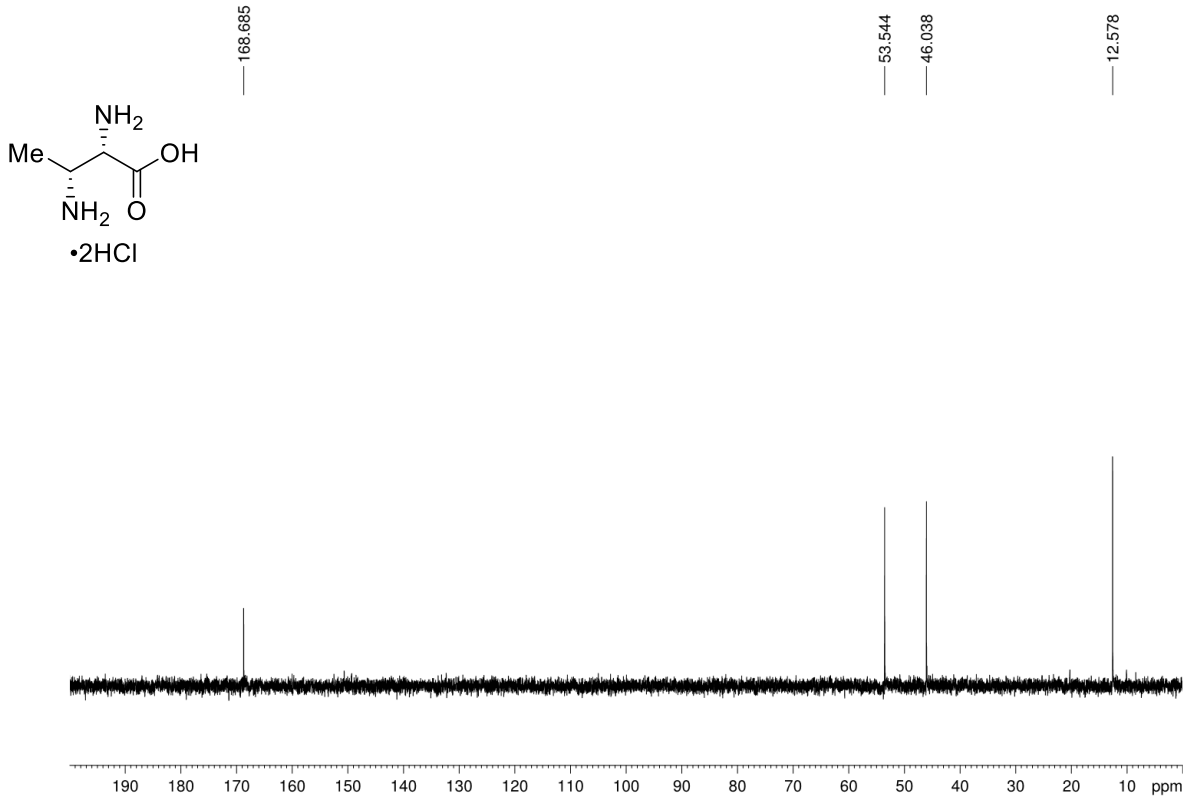

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **S2**



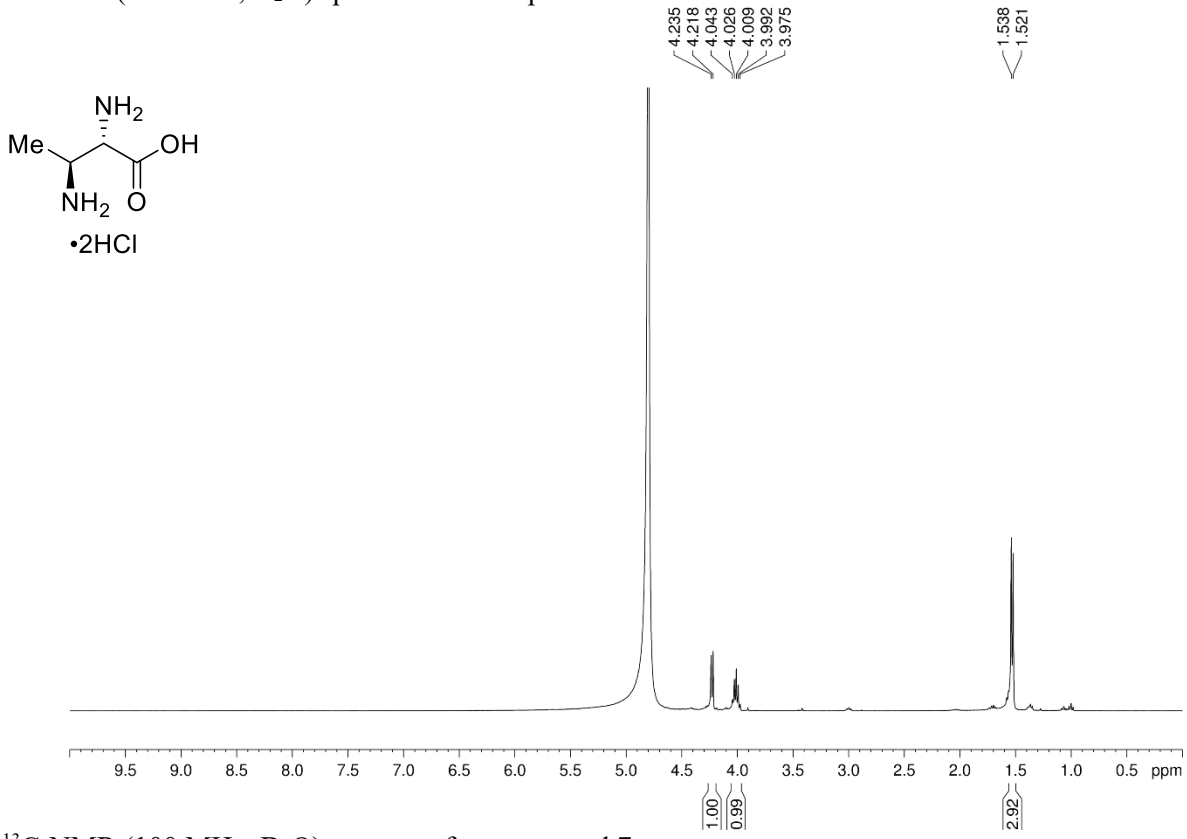
$^{1}$H-NMR (400 MHz, $D_2O$) spectrum for compound **6**



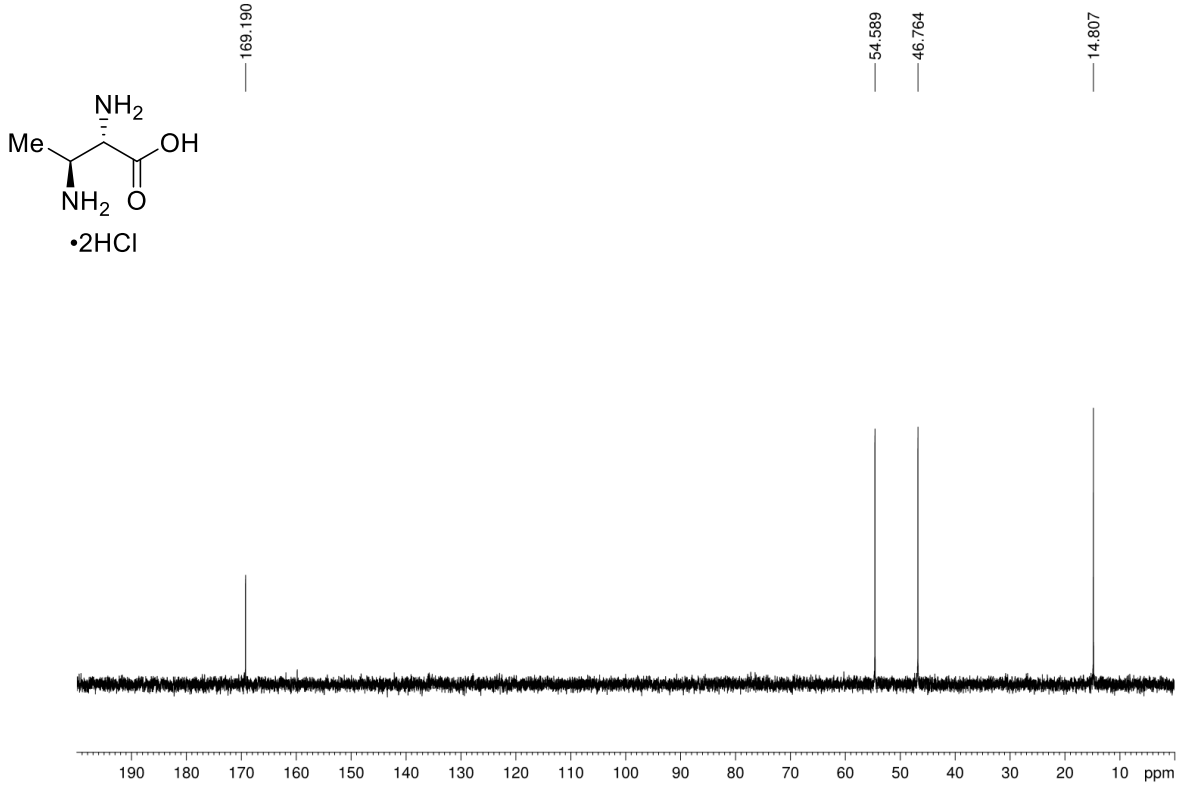

$^{13}$C-NMR (100 MHz, $D_2O$) spectrum for compound **6**



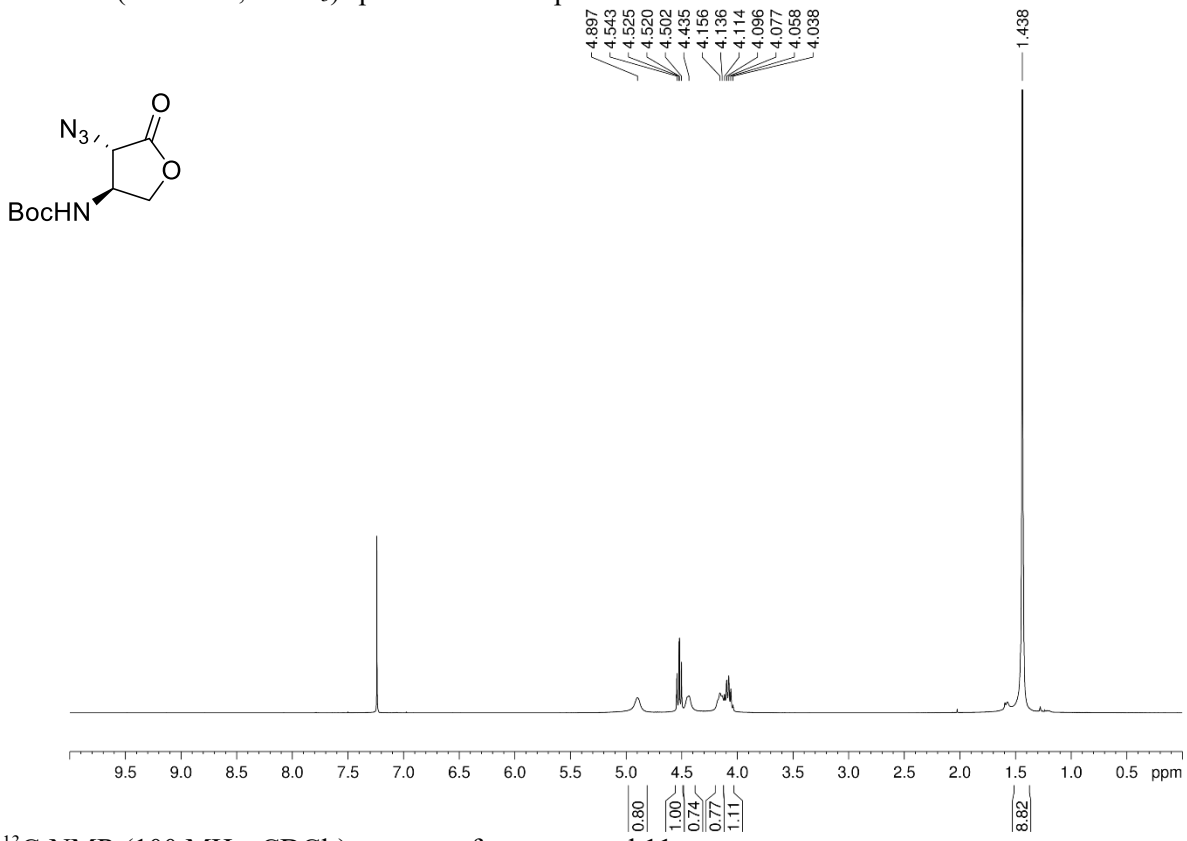
$^{1}$H-NMR (400 MHz, $D_2O$) spectrum for compound **7**



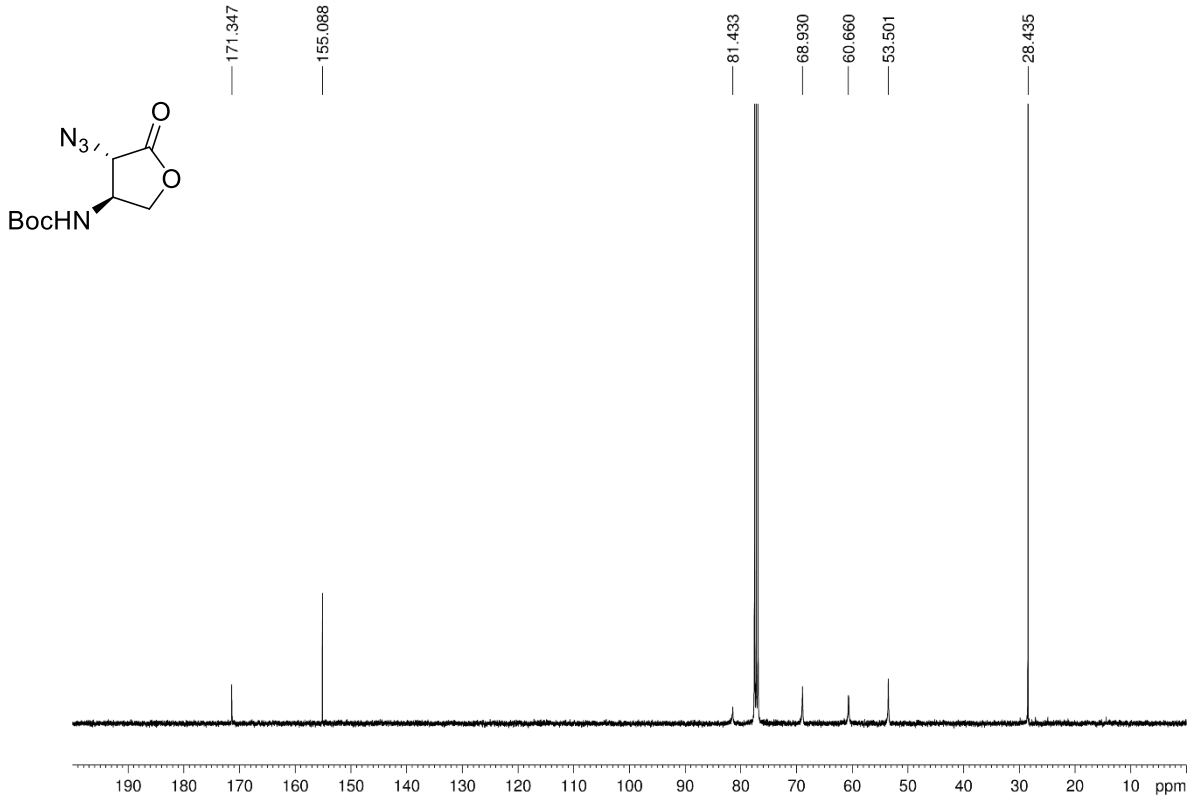

$^{13}$C-NMR (100 MHz, $D_2O$) spectrum for compound **7**



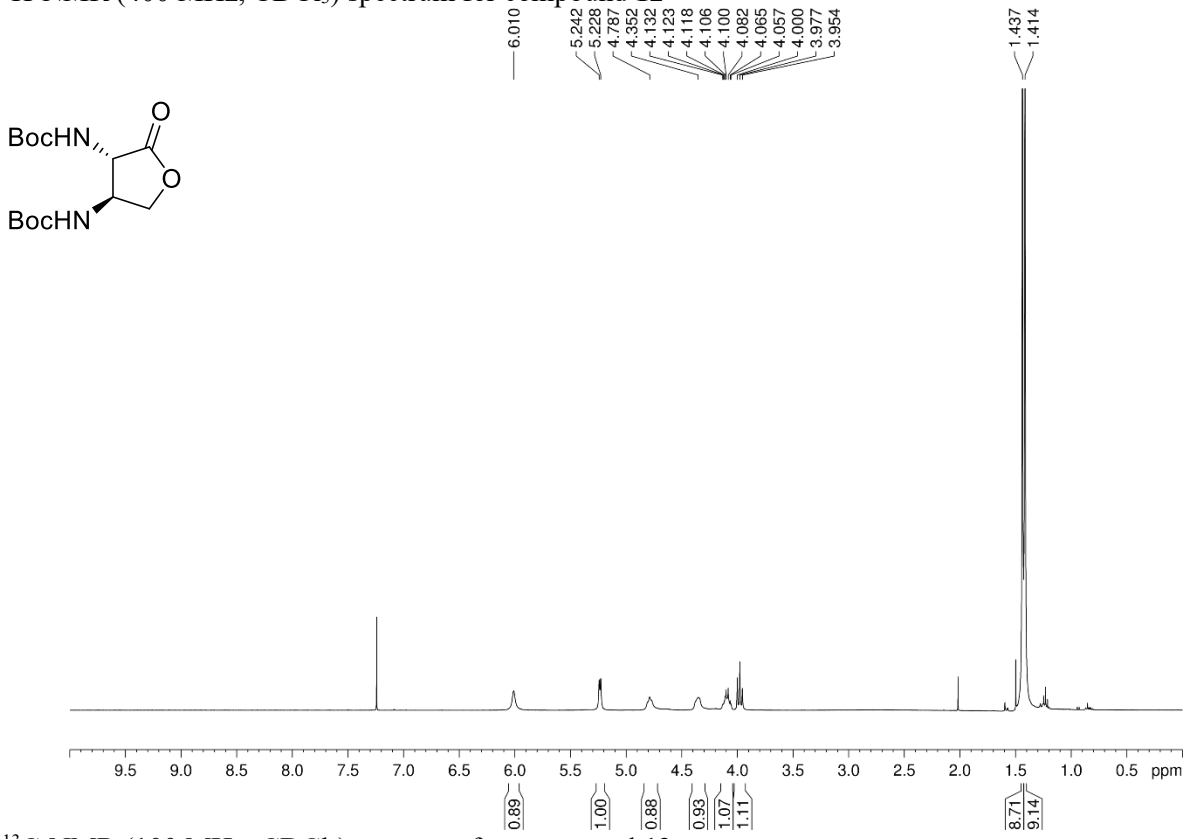
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **11**



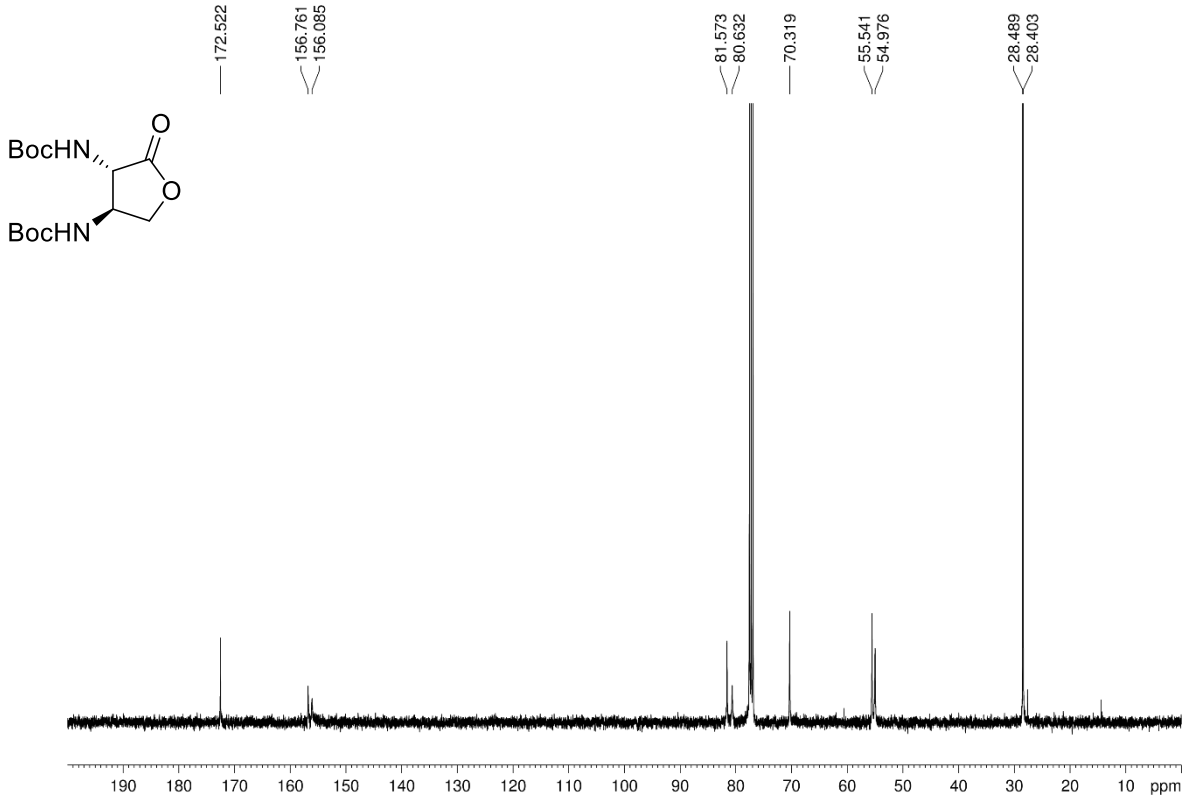

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **11**



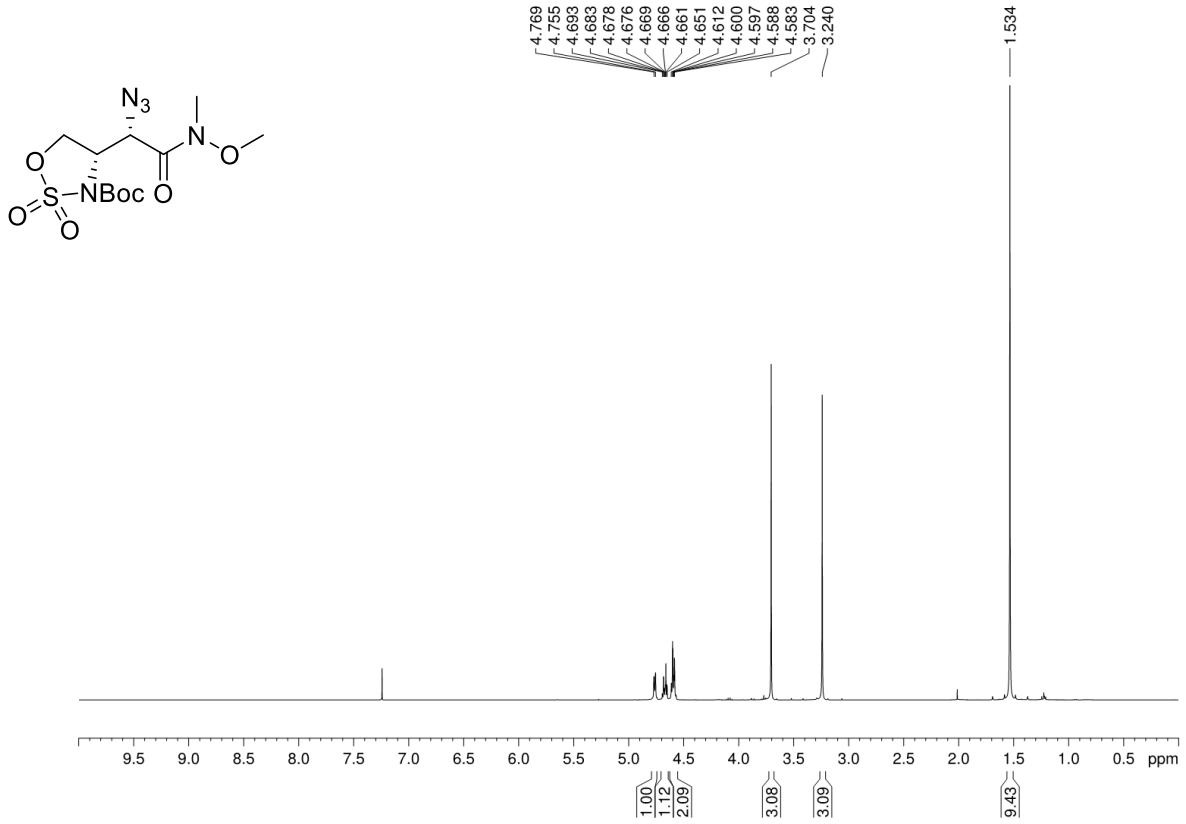
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **12**



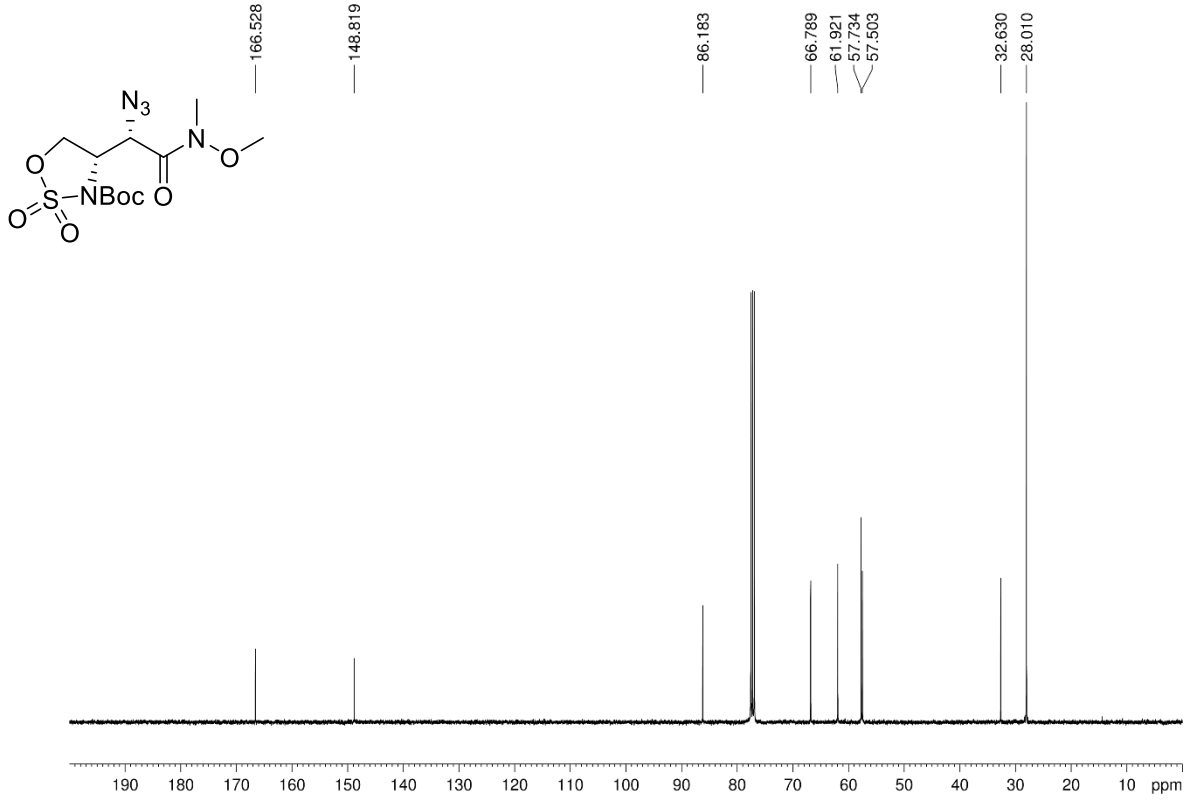

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **12**



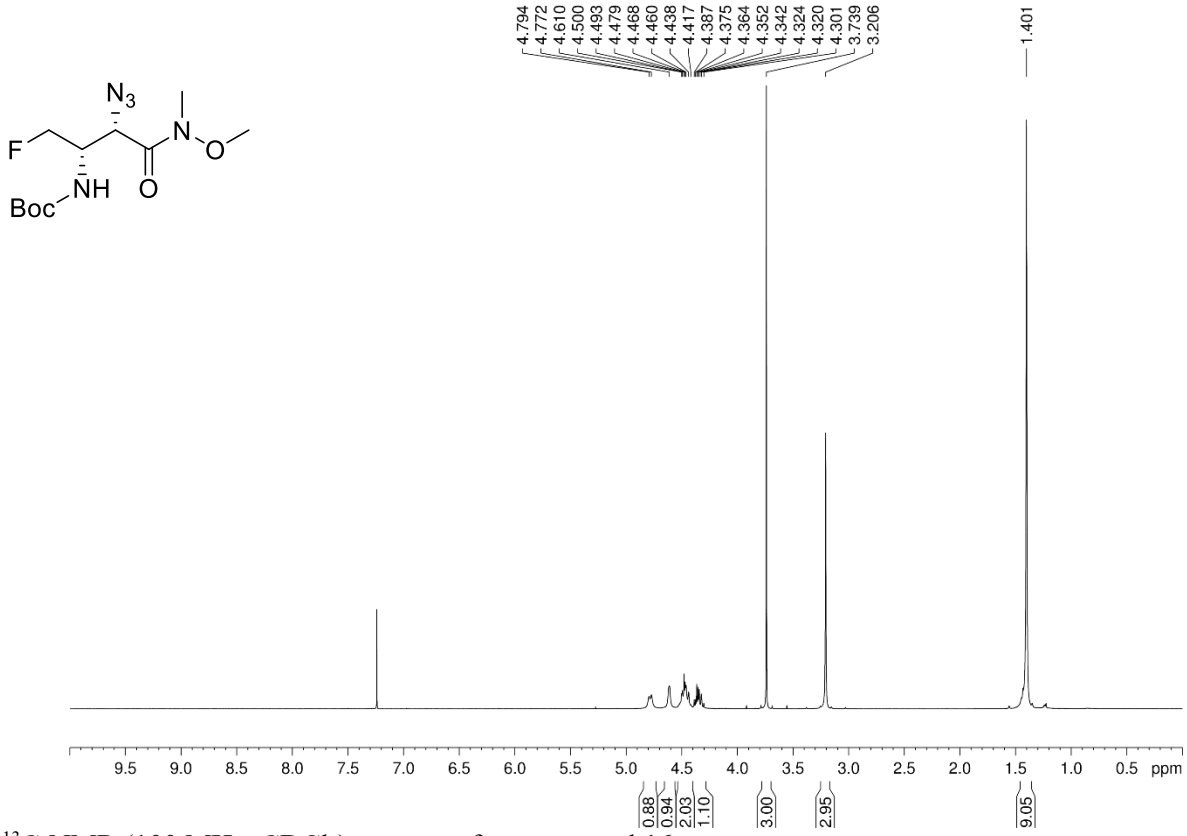
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **15**



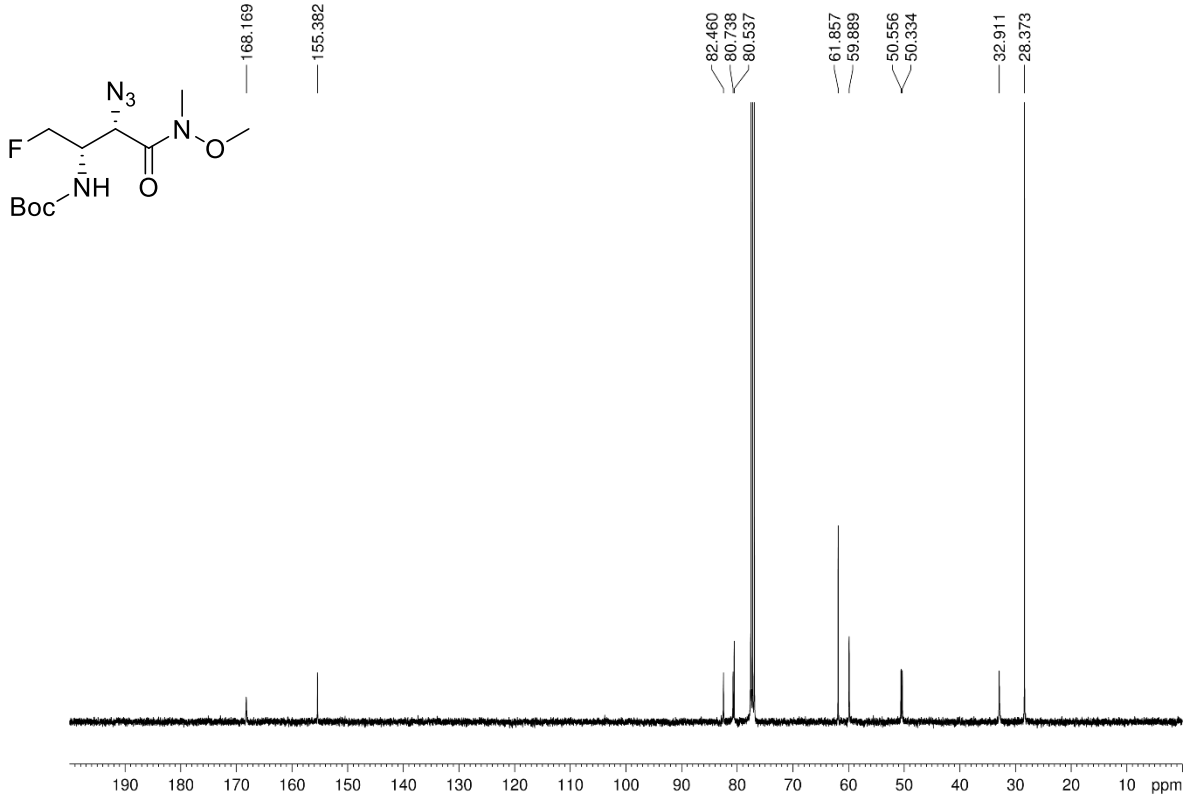

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **15**



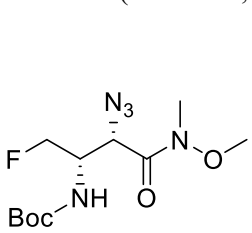
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **16a**



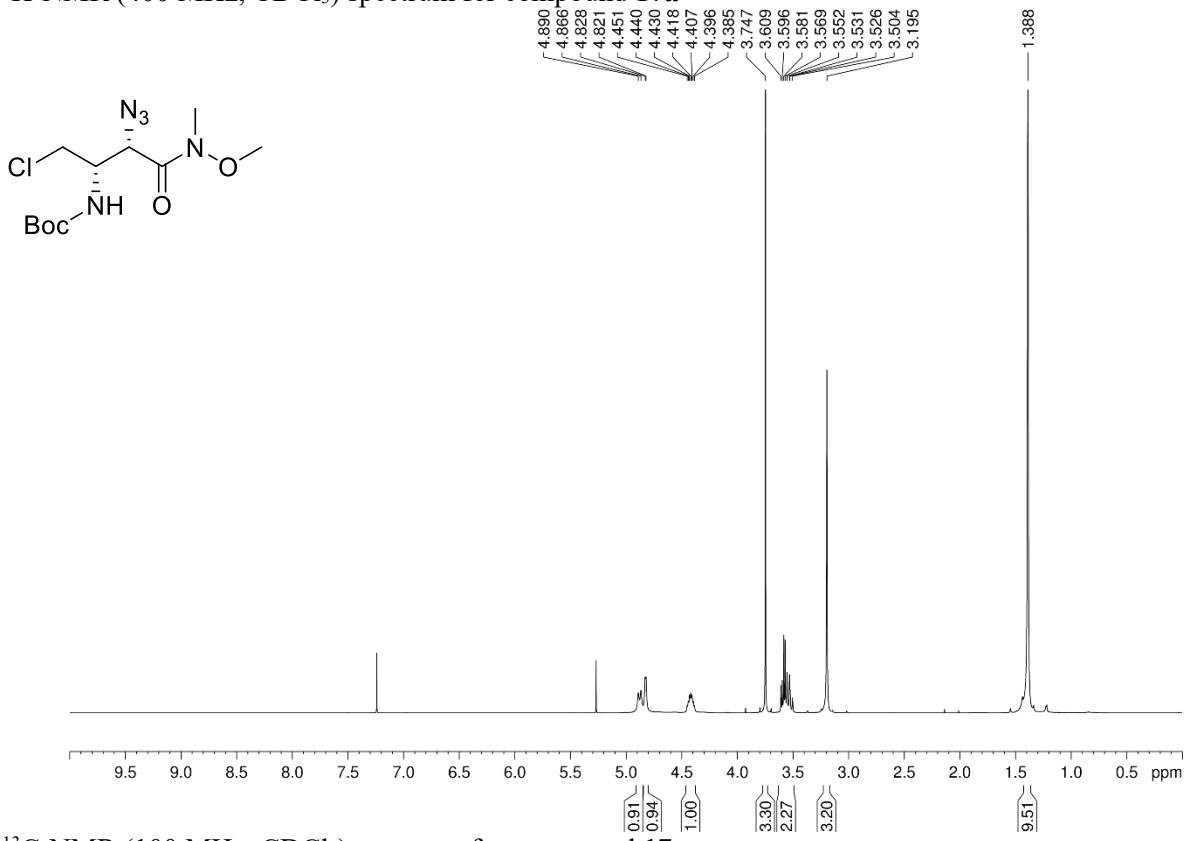

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **16a**



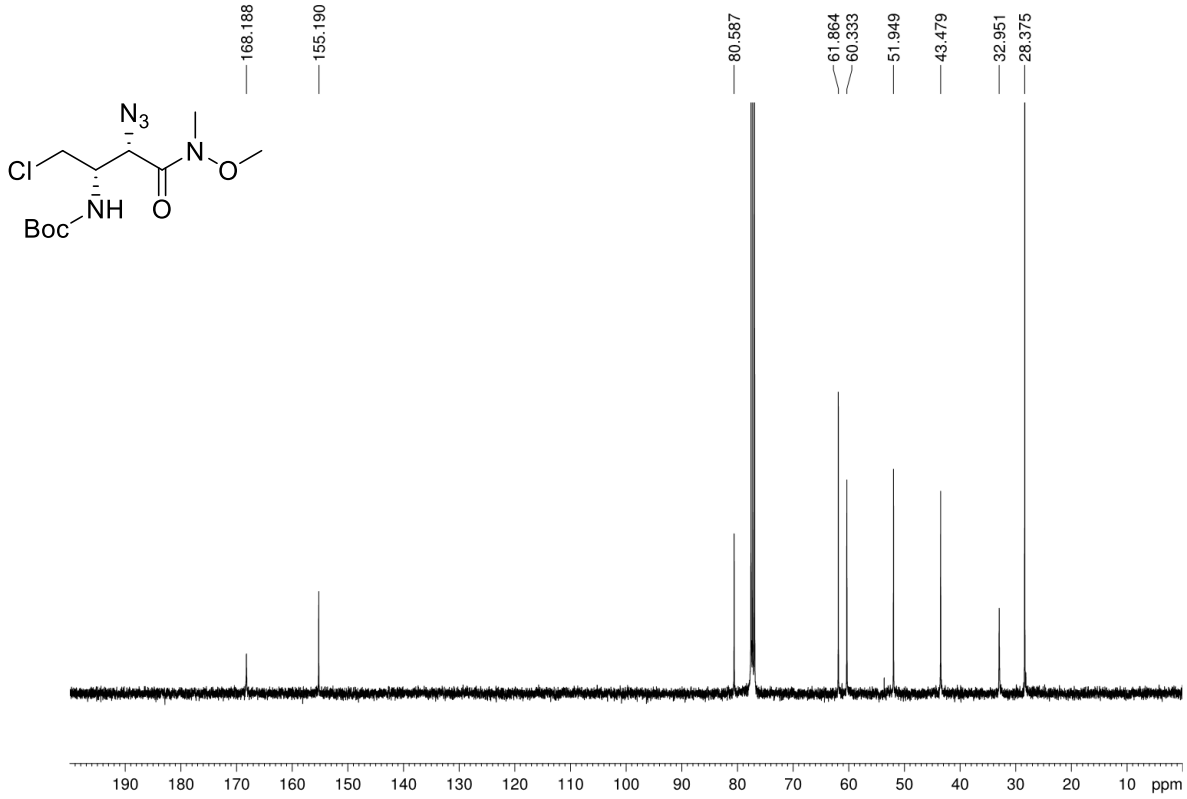
$^{19}$F-NMR (282 MHz, $CDCl_3$) spectrum for compound **16a**



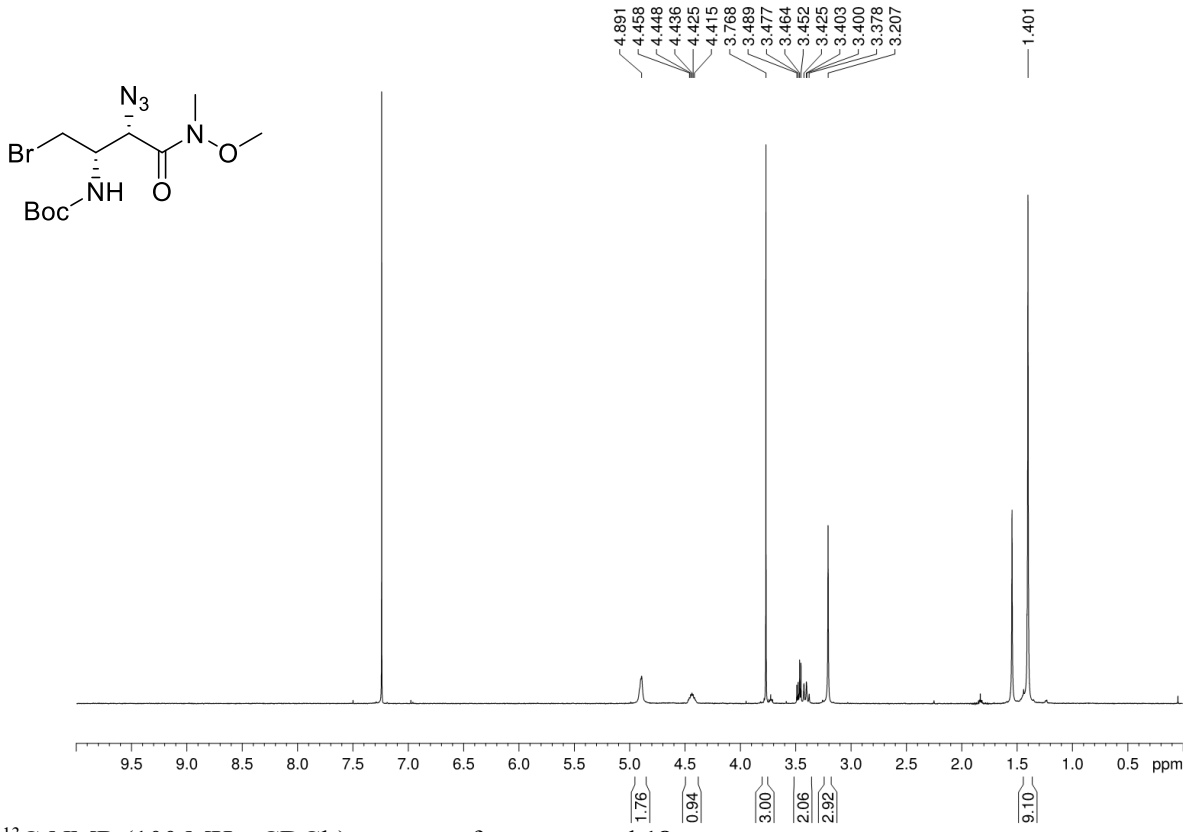
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **17a**



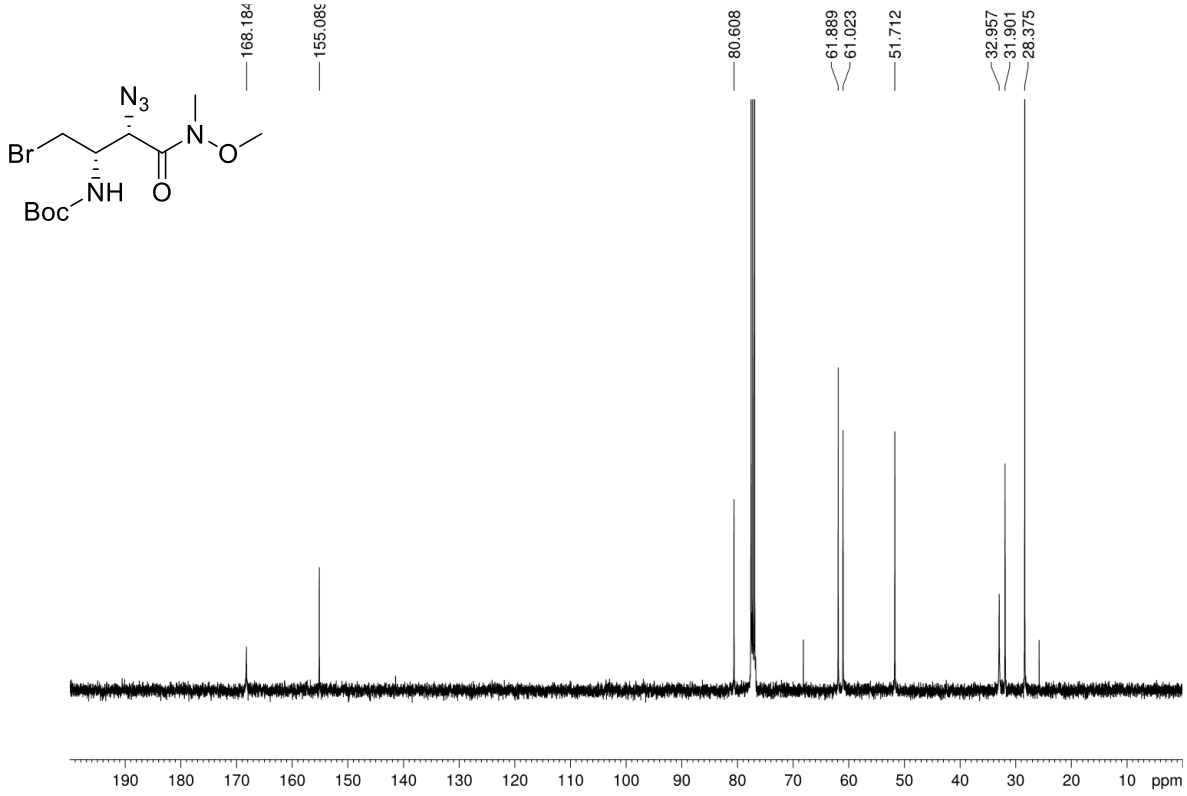

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **17a**



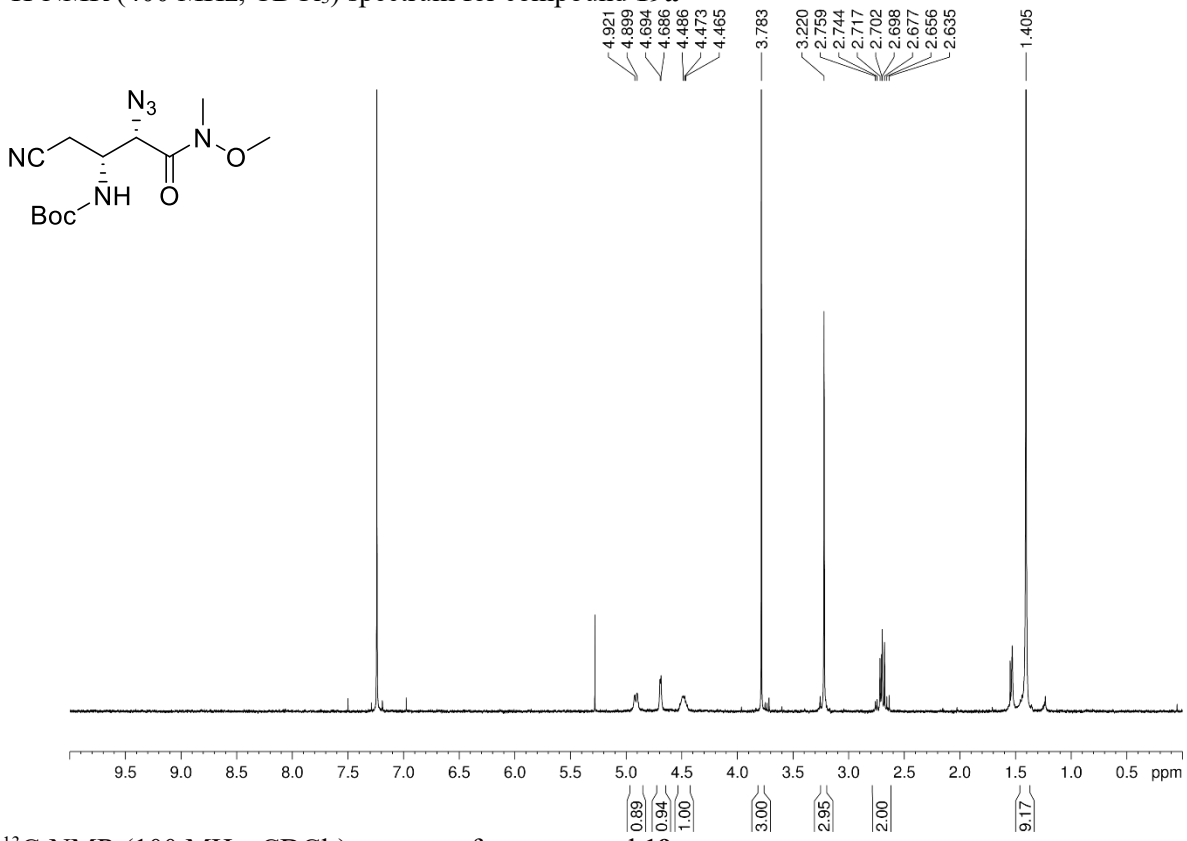
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **18a**



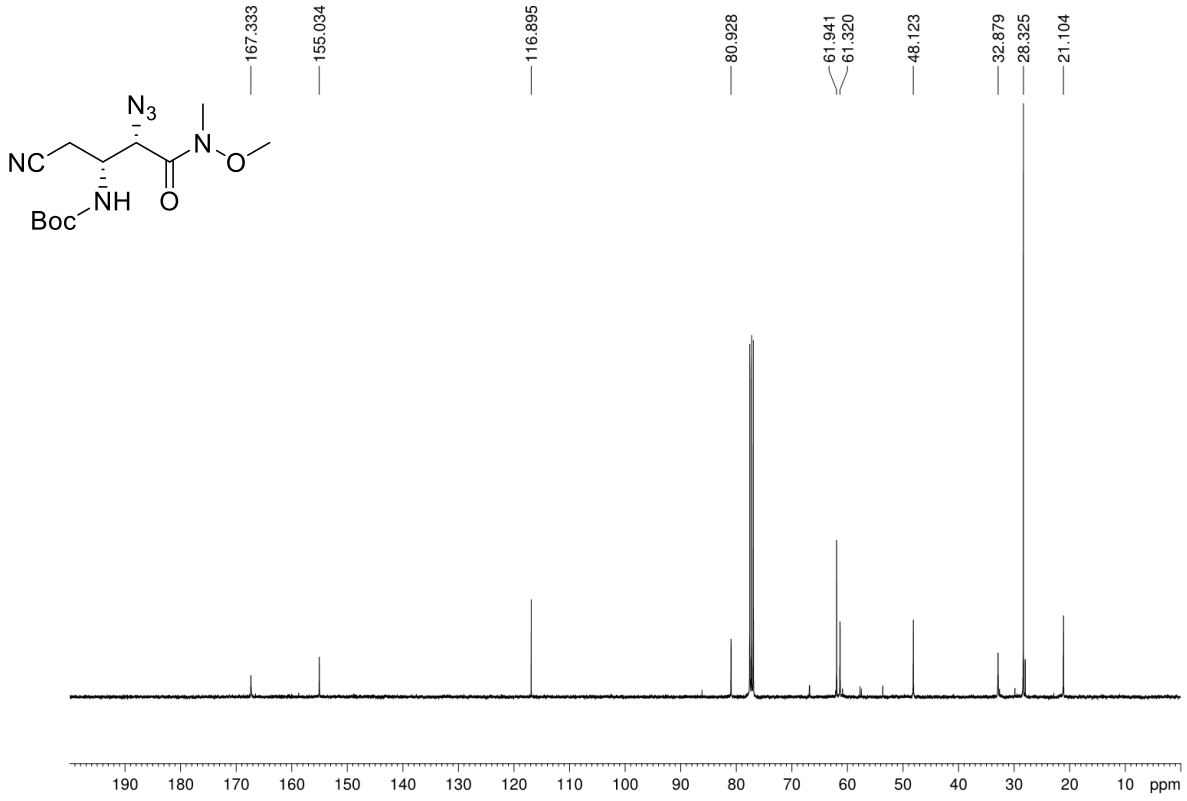

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **18a**



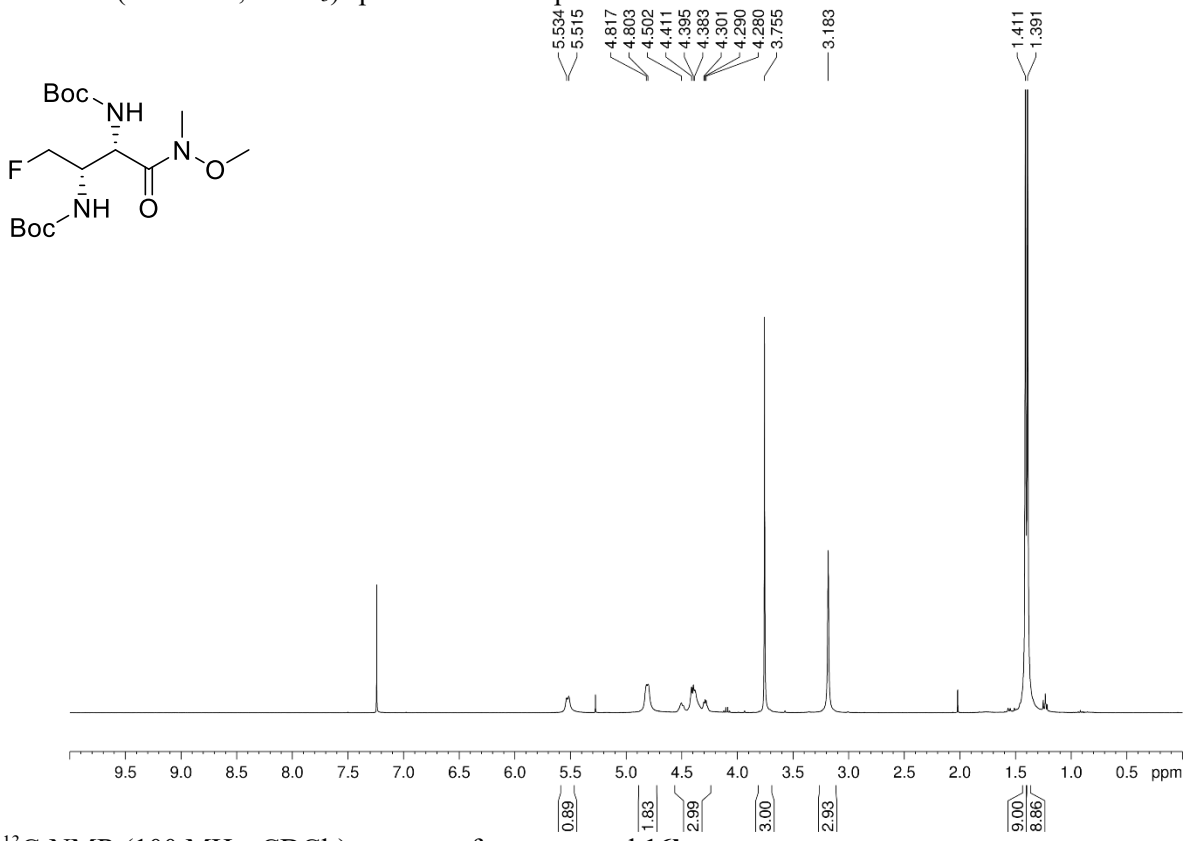
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **19a**



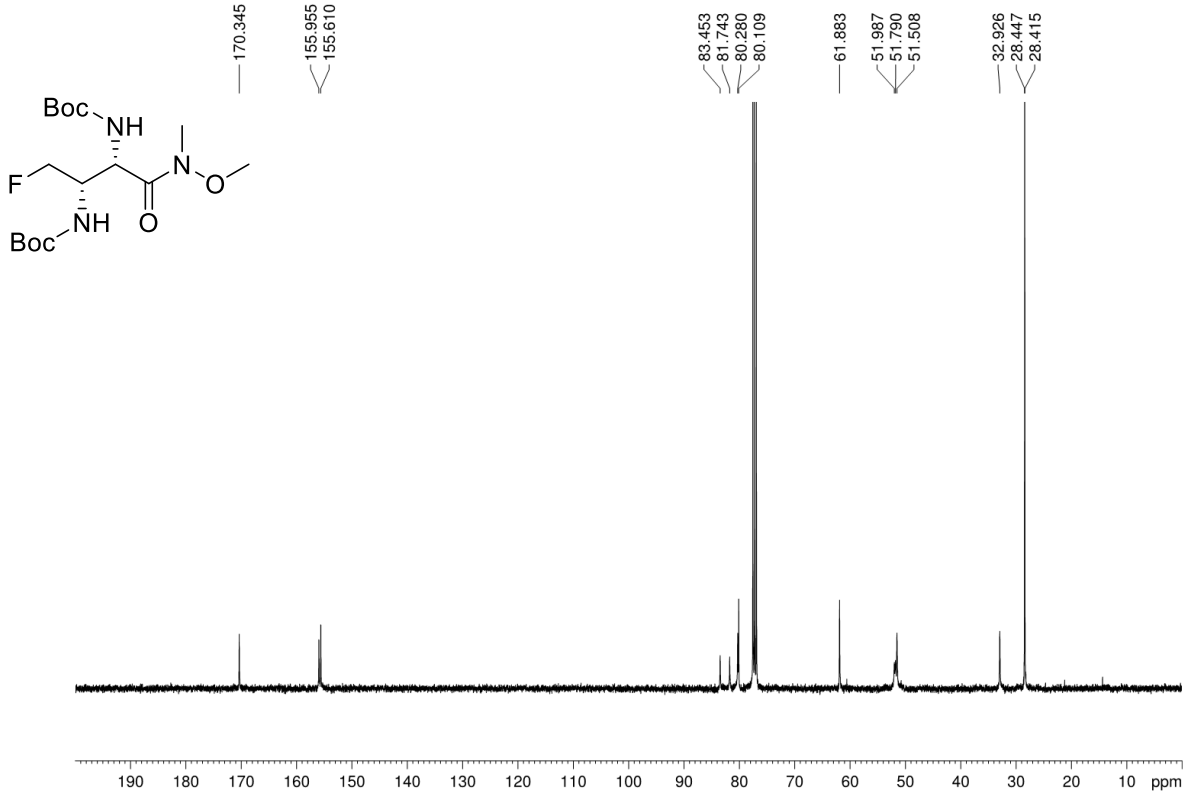

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **19a**



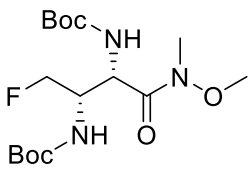
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **16b**



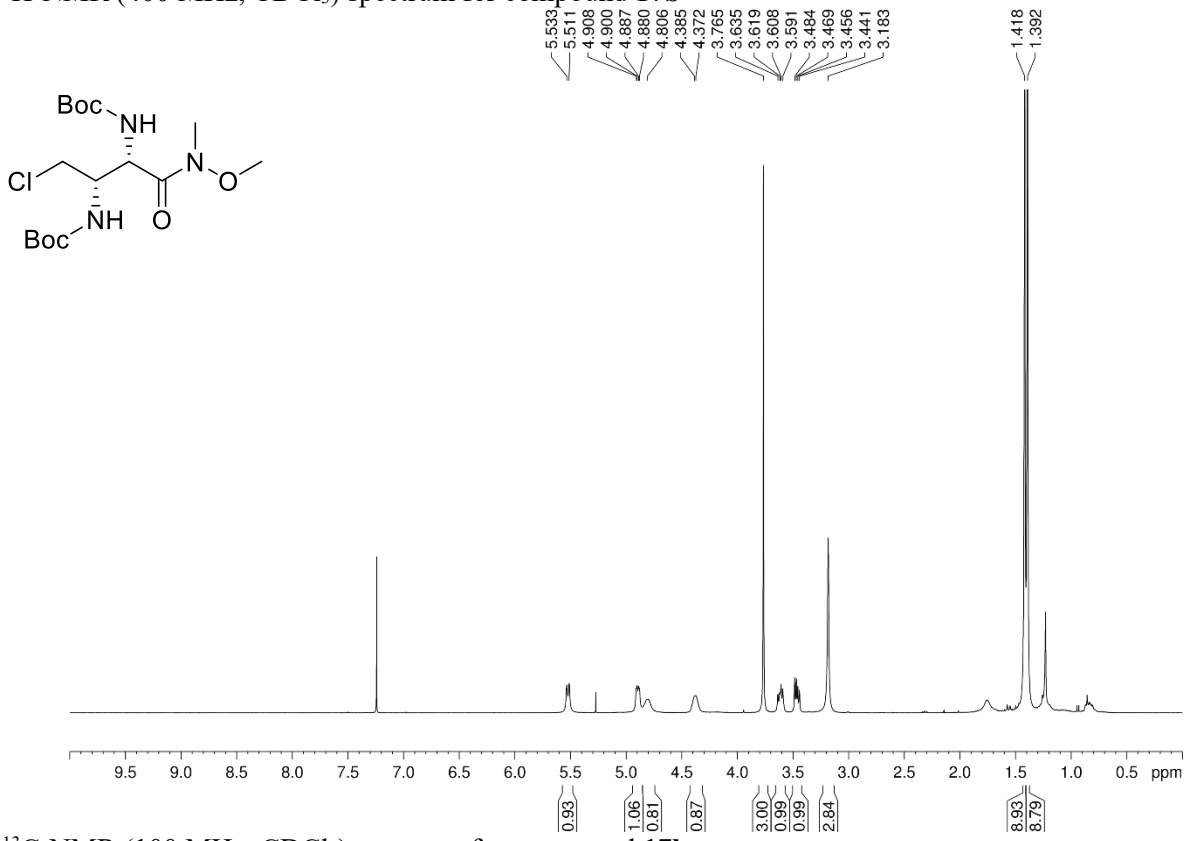

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **16b**



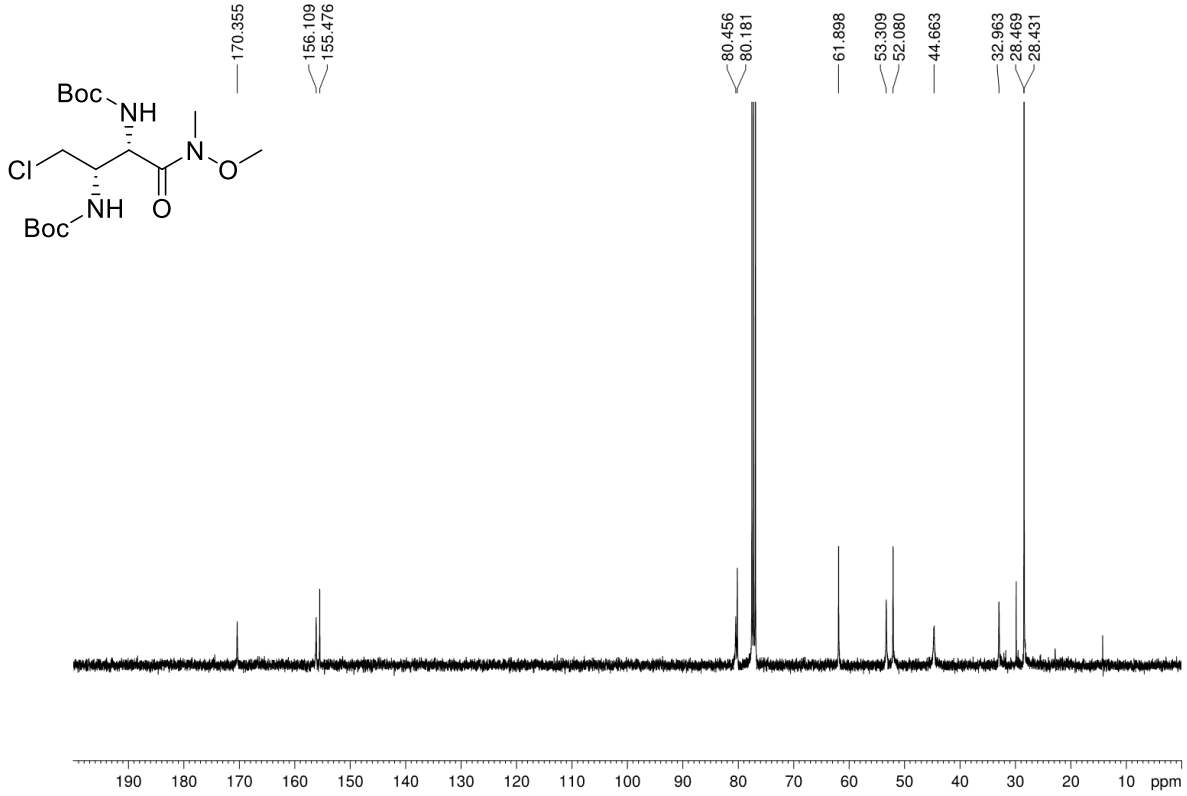
$^{19}$F-NMR (282 MHz, $CDCl_3$) spectrum for compound **16b**



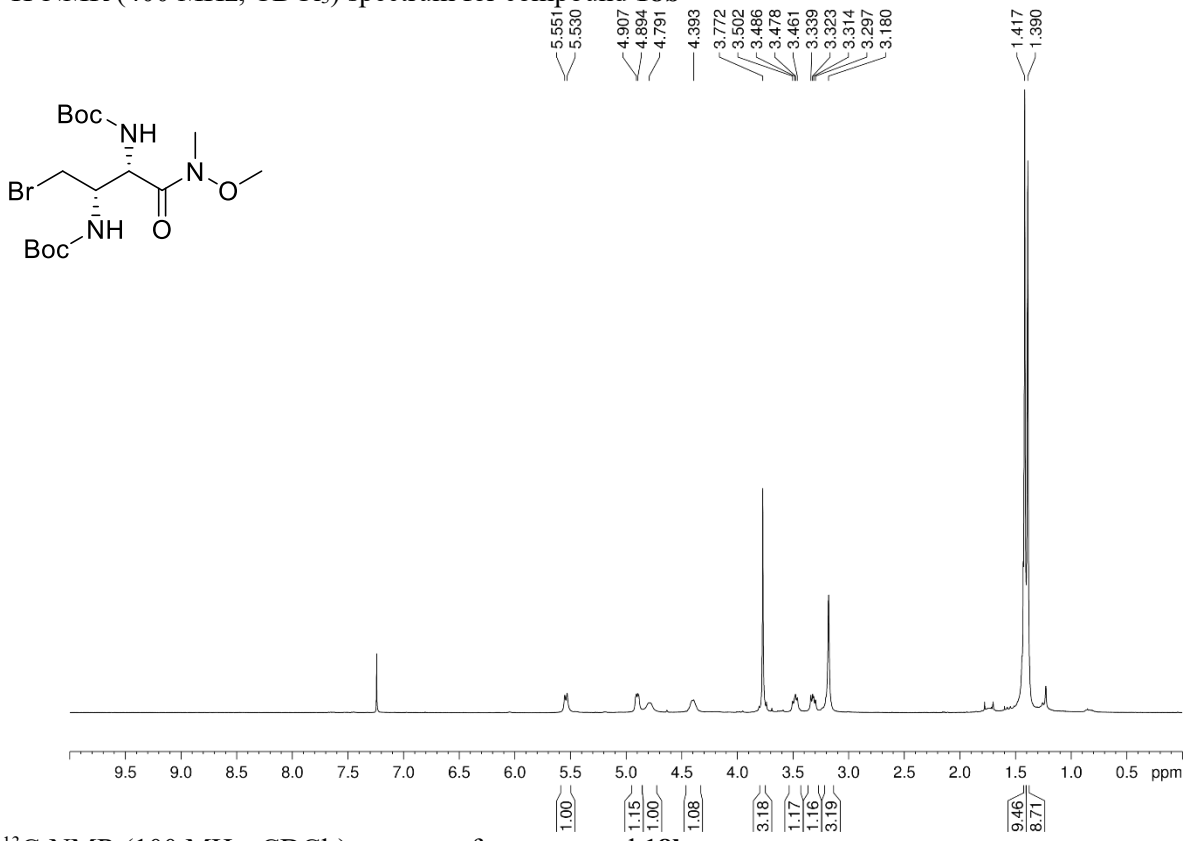
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **17b**



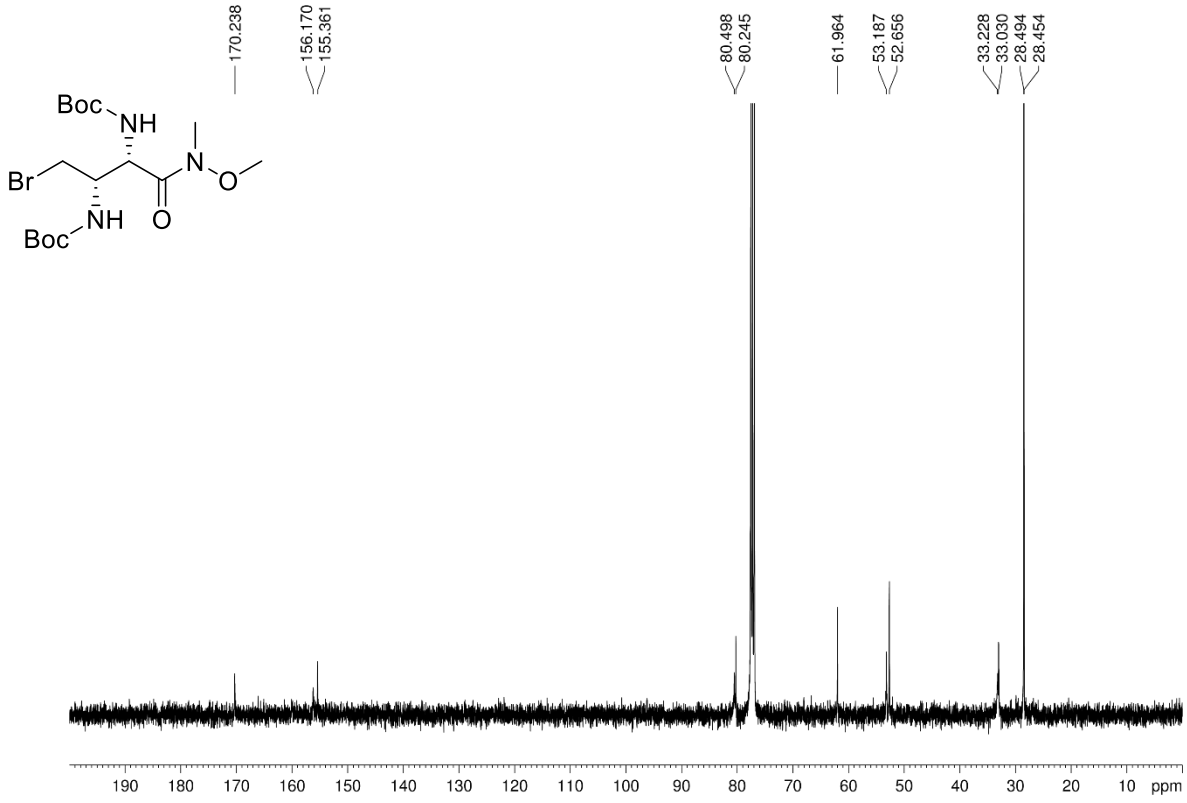

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **17b**



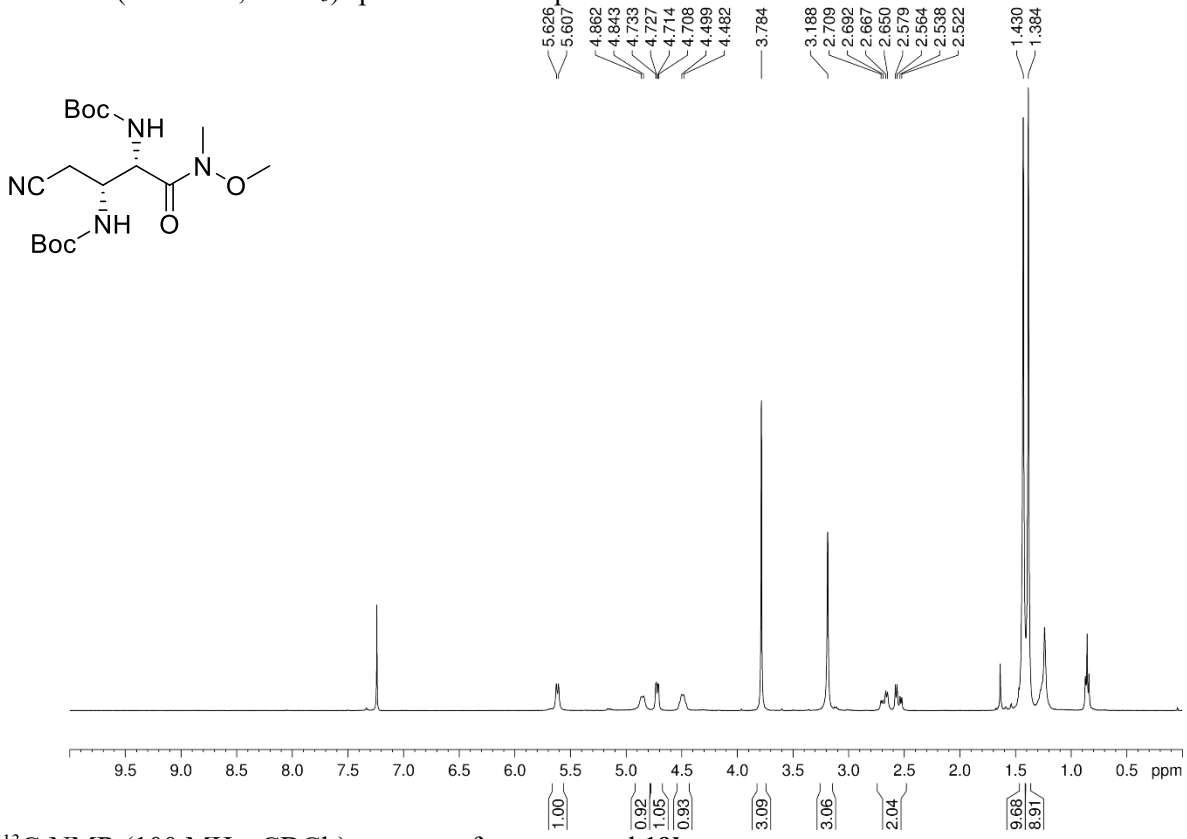
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **18b**



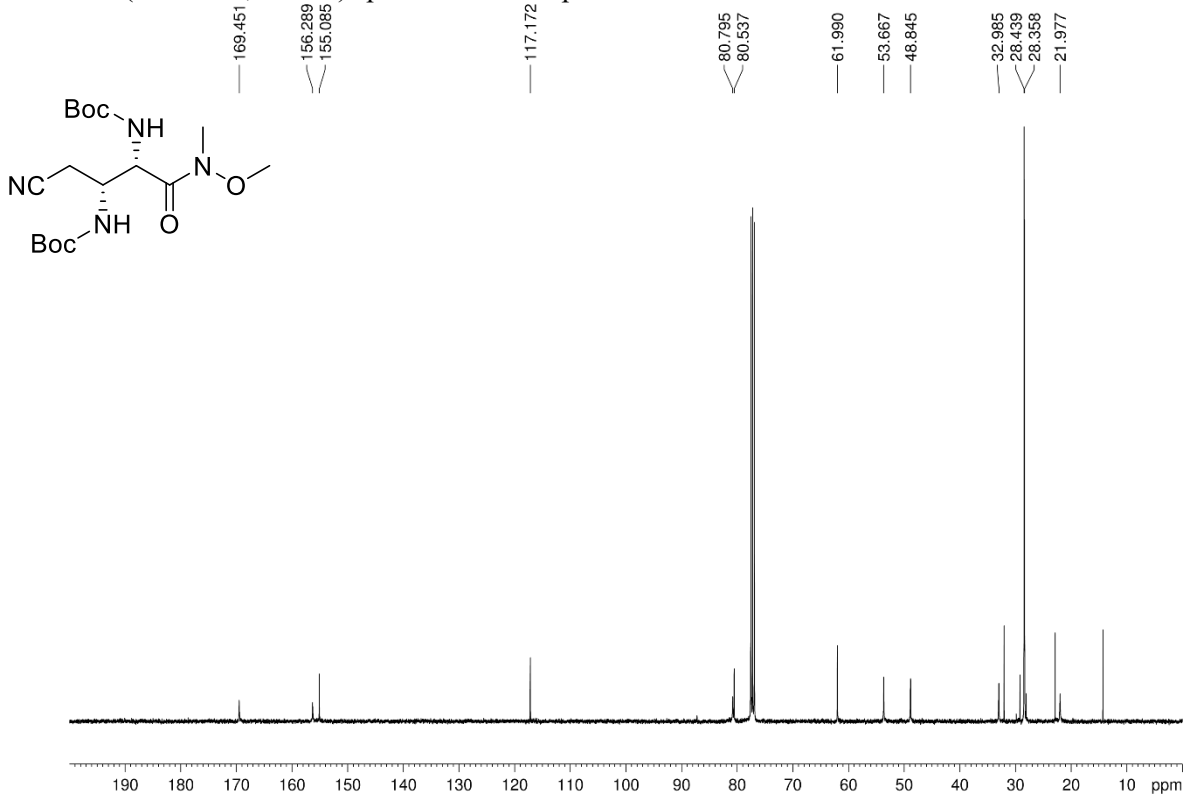

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **18b**



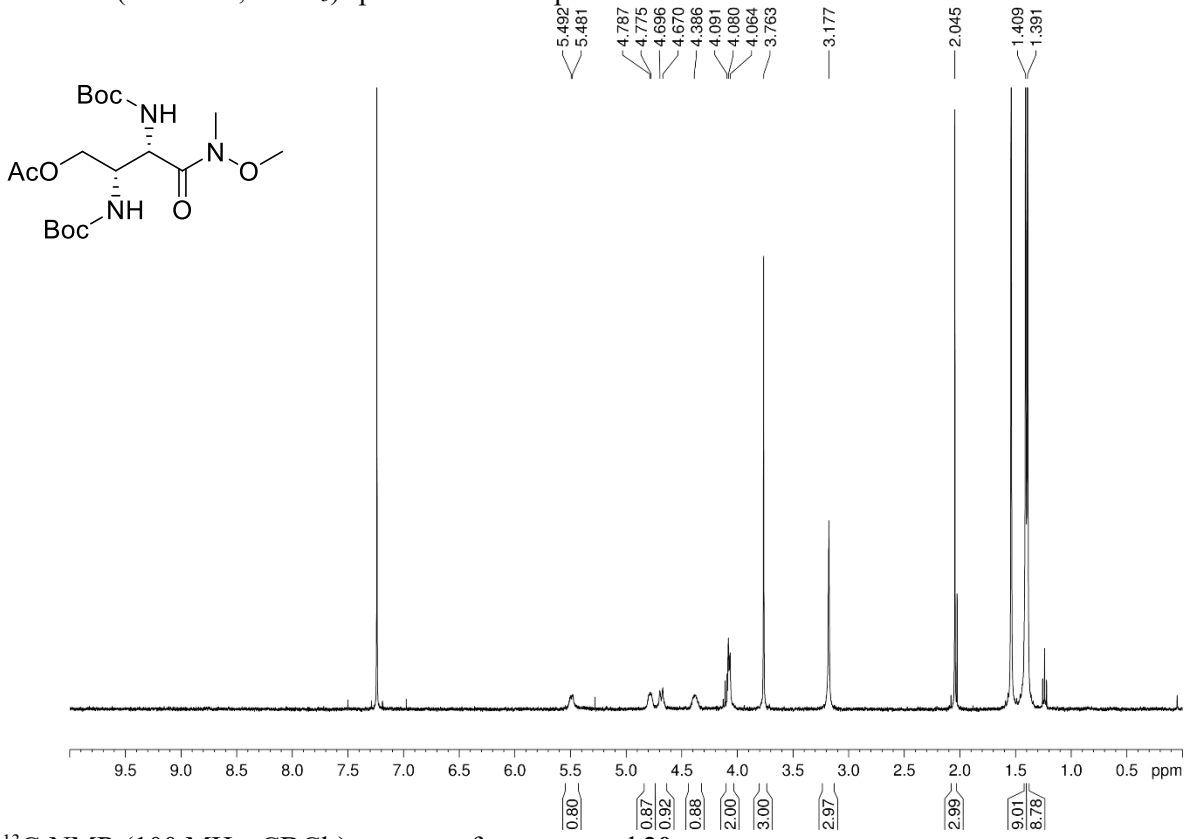
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **19b**



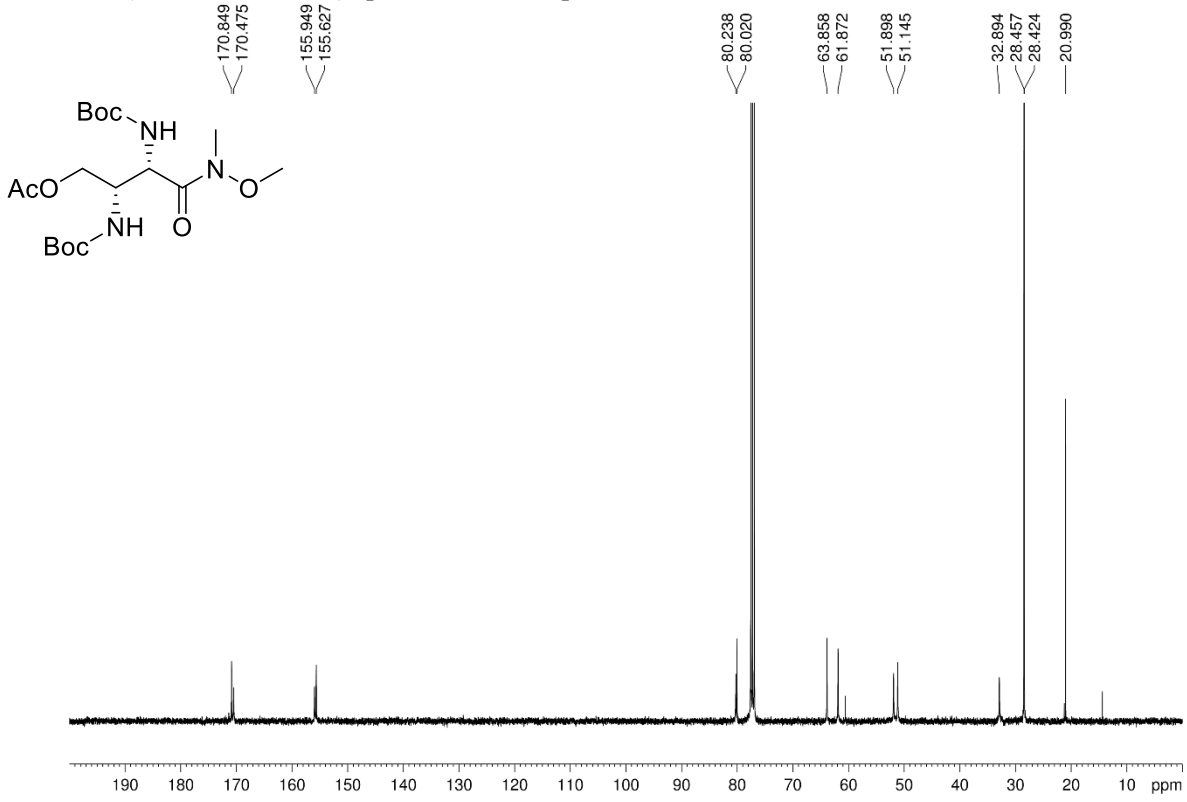

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **19b**



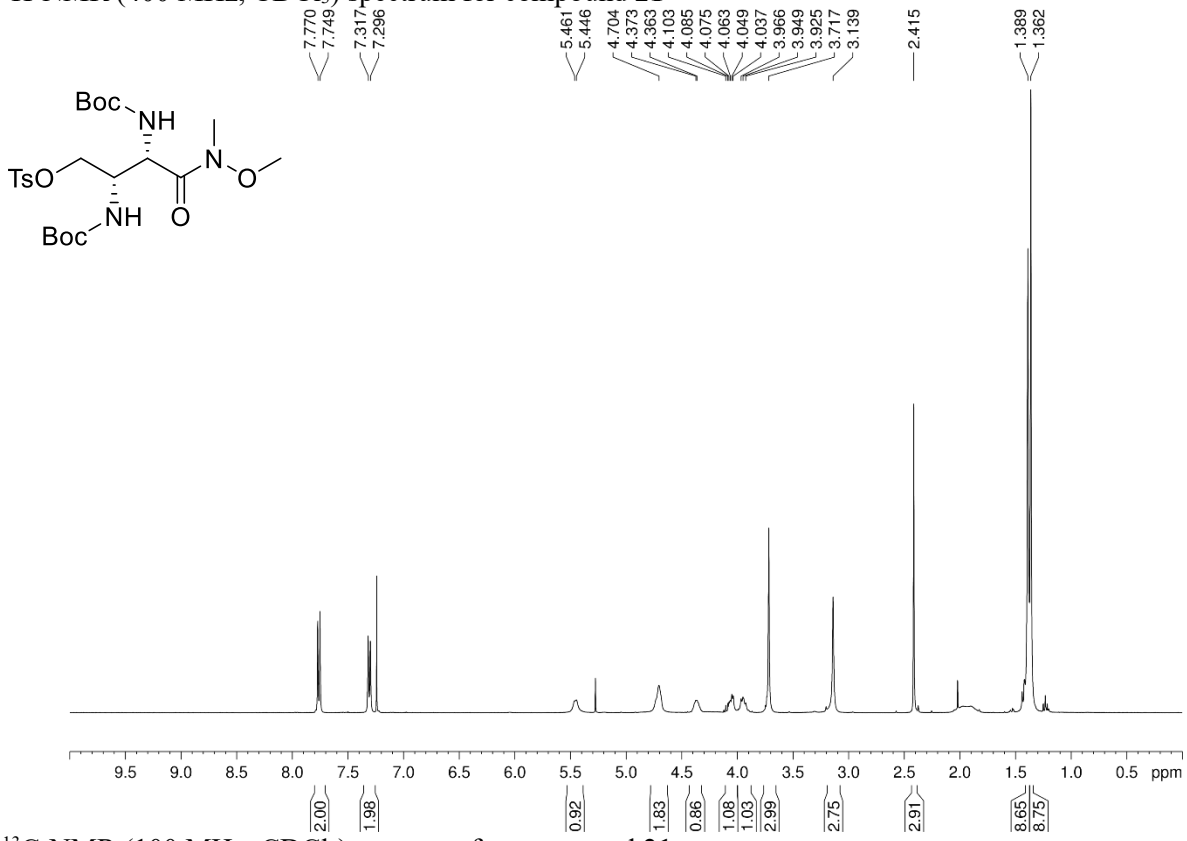
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **20**



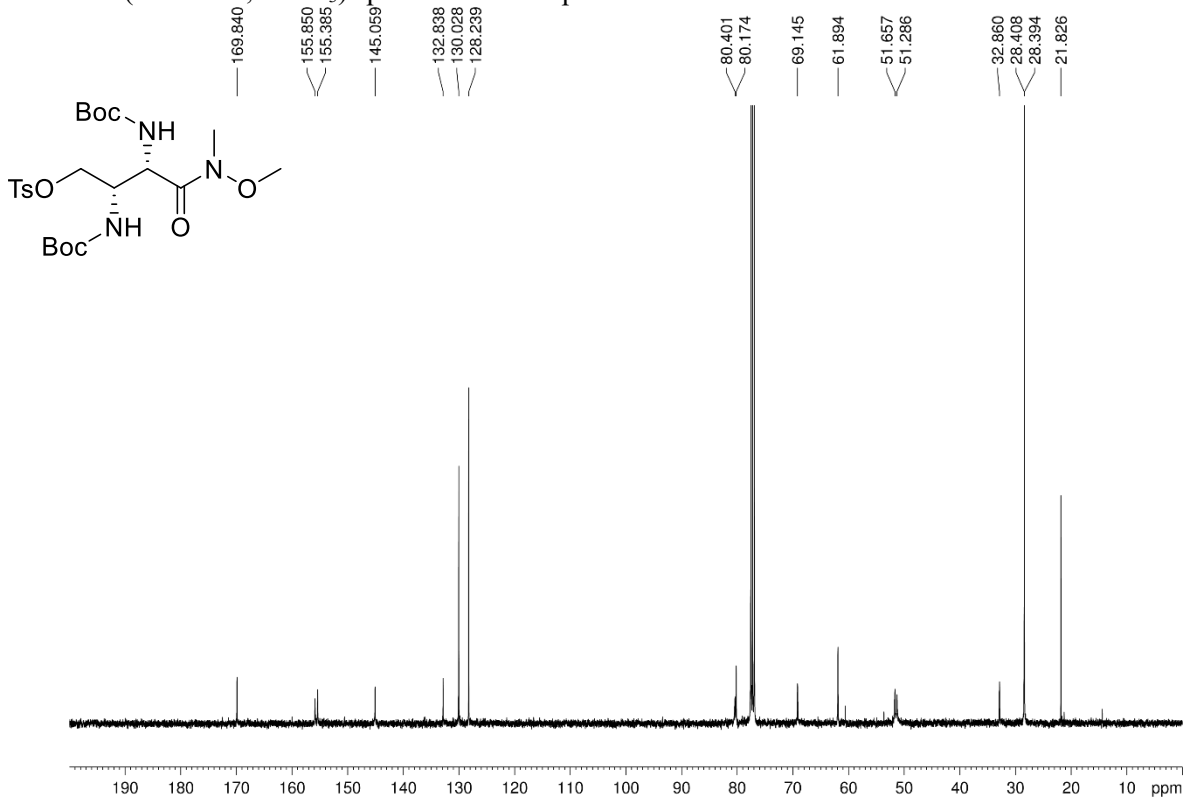

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **20**



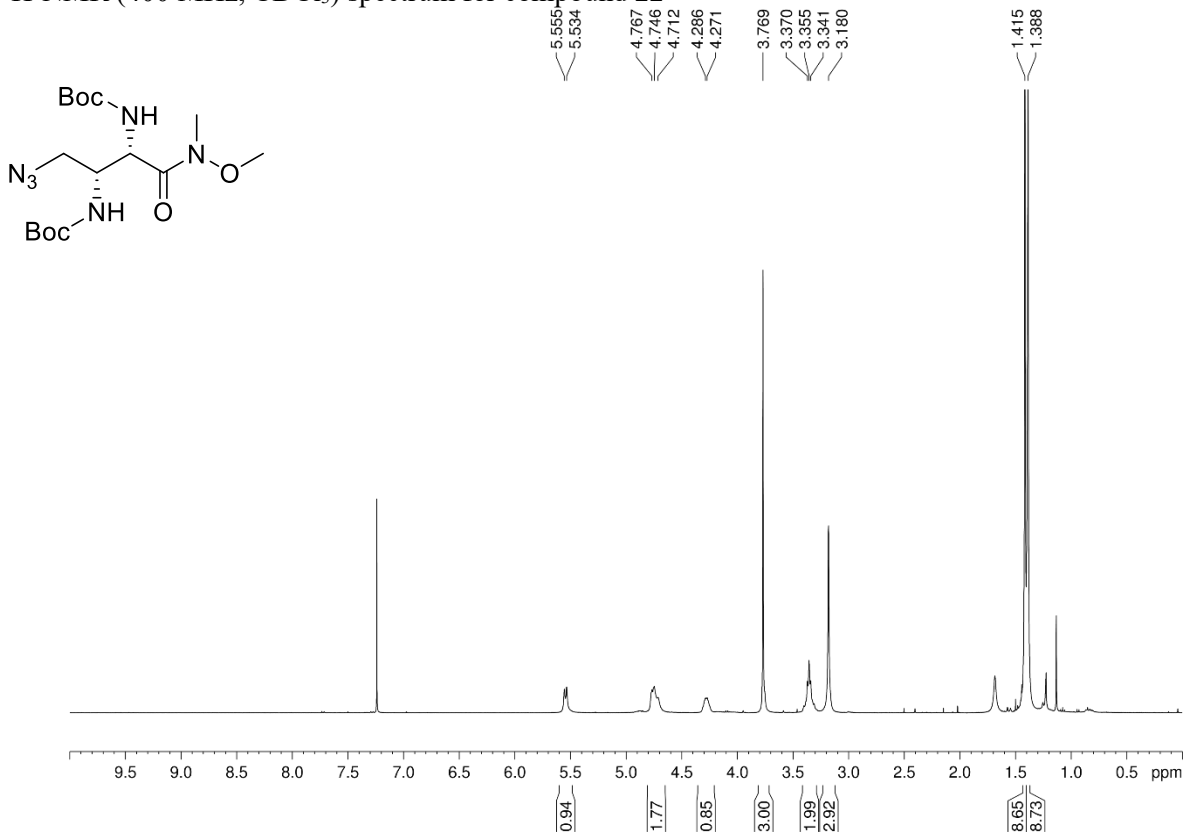
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **21**



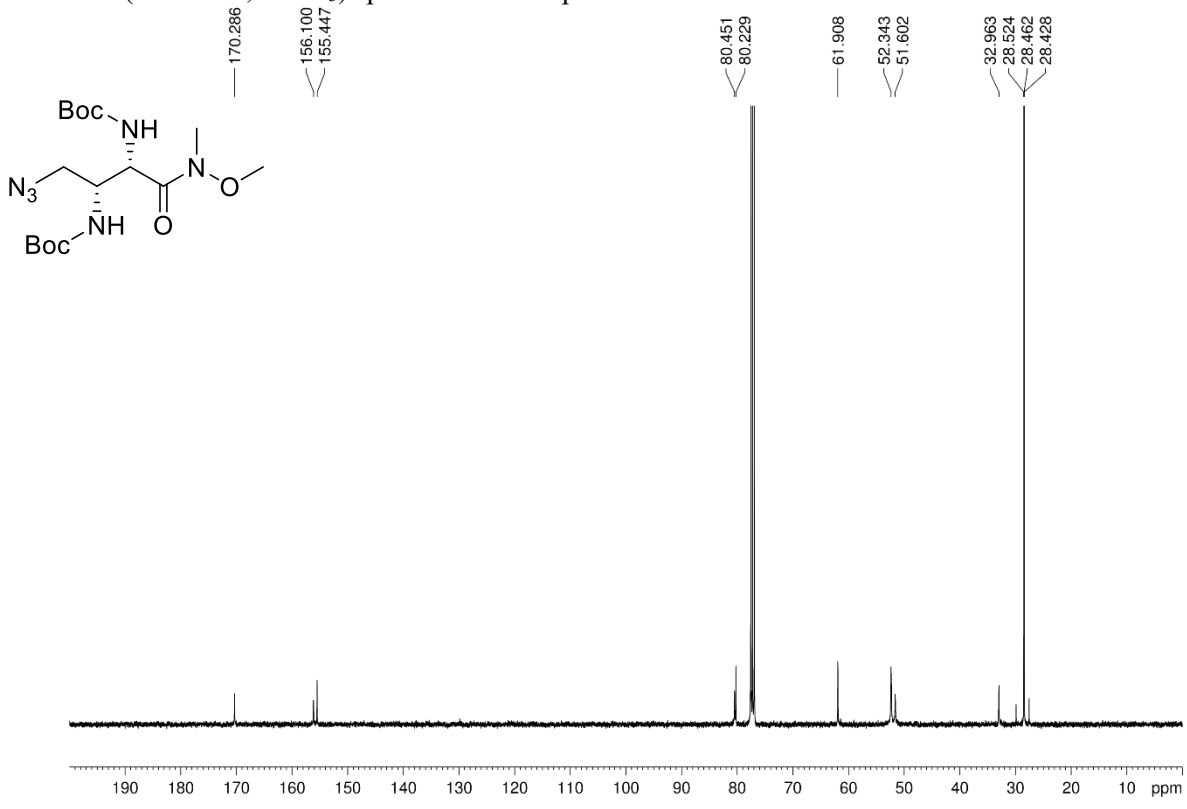

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **21**



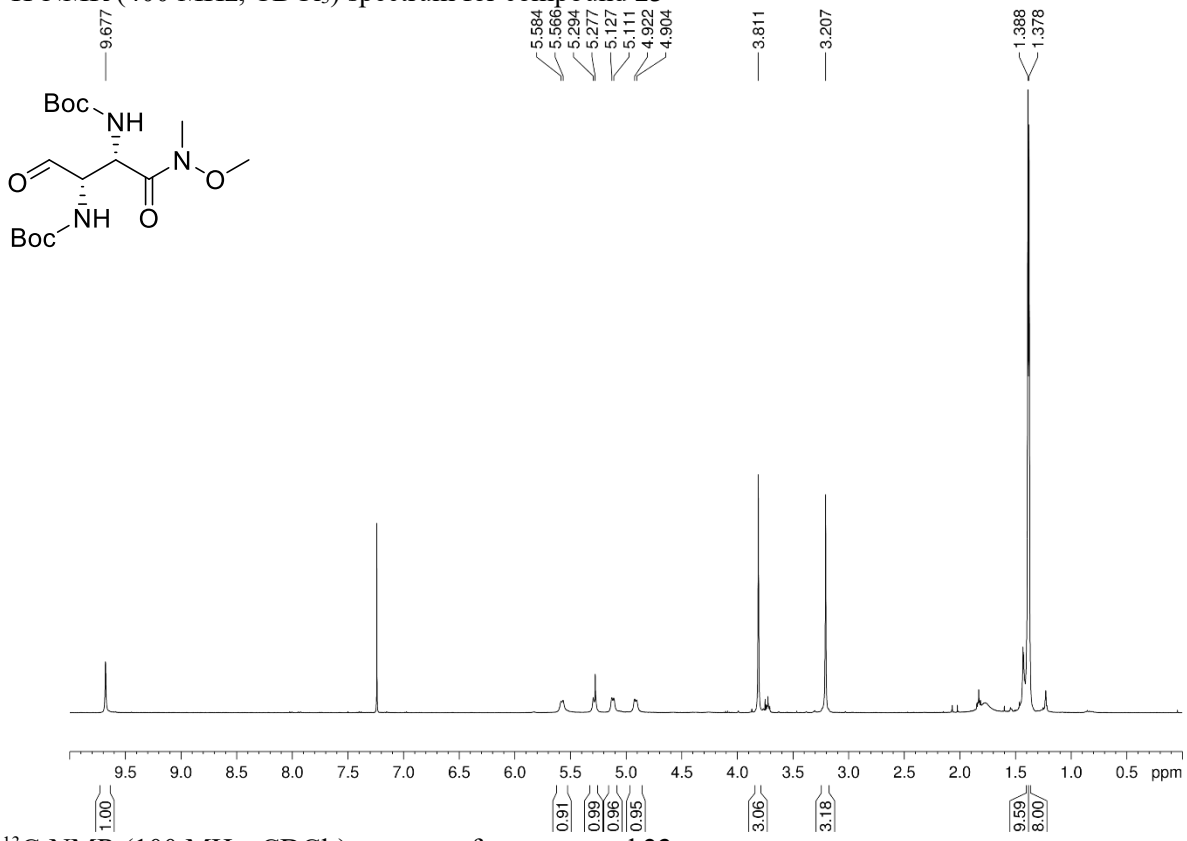
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **22**



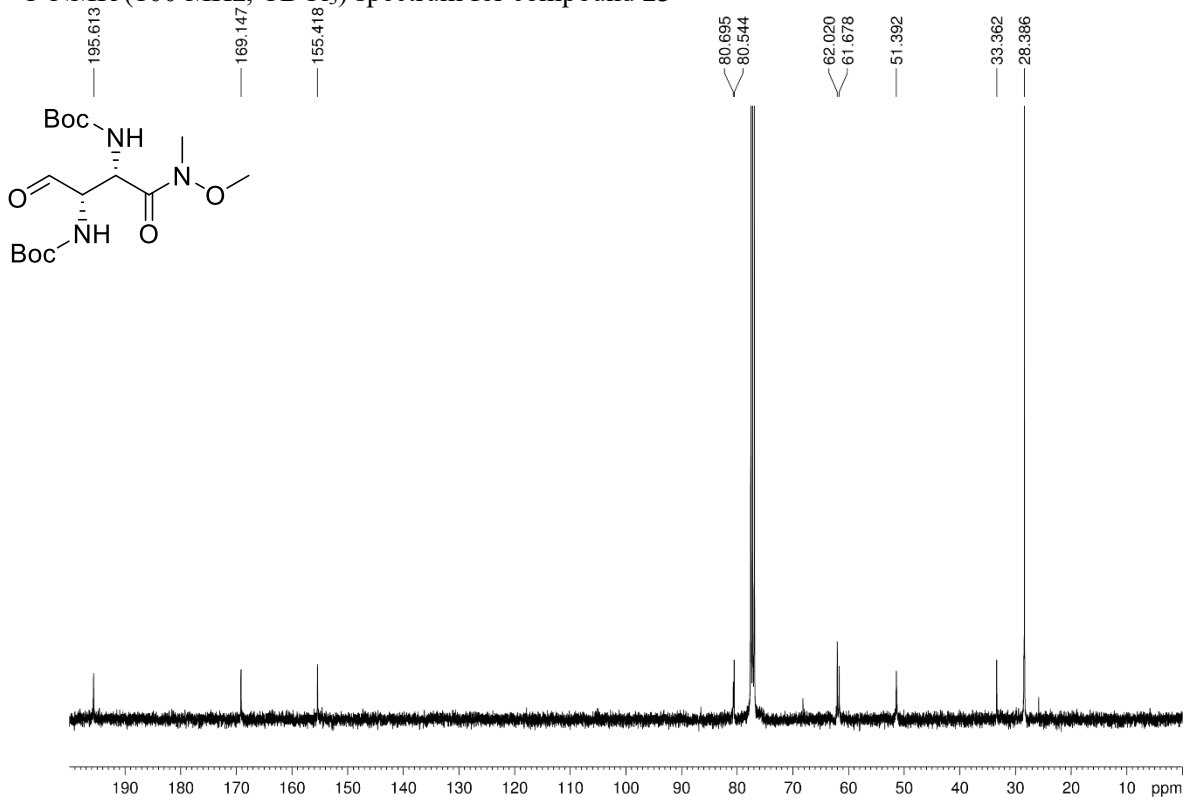

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **22**



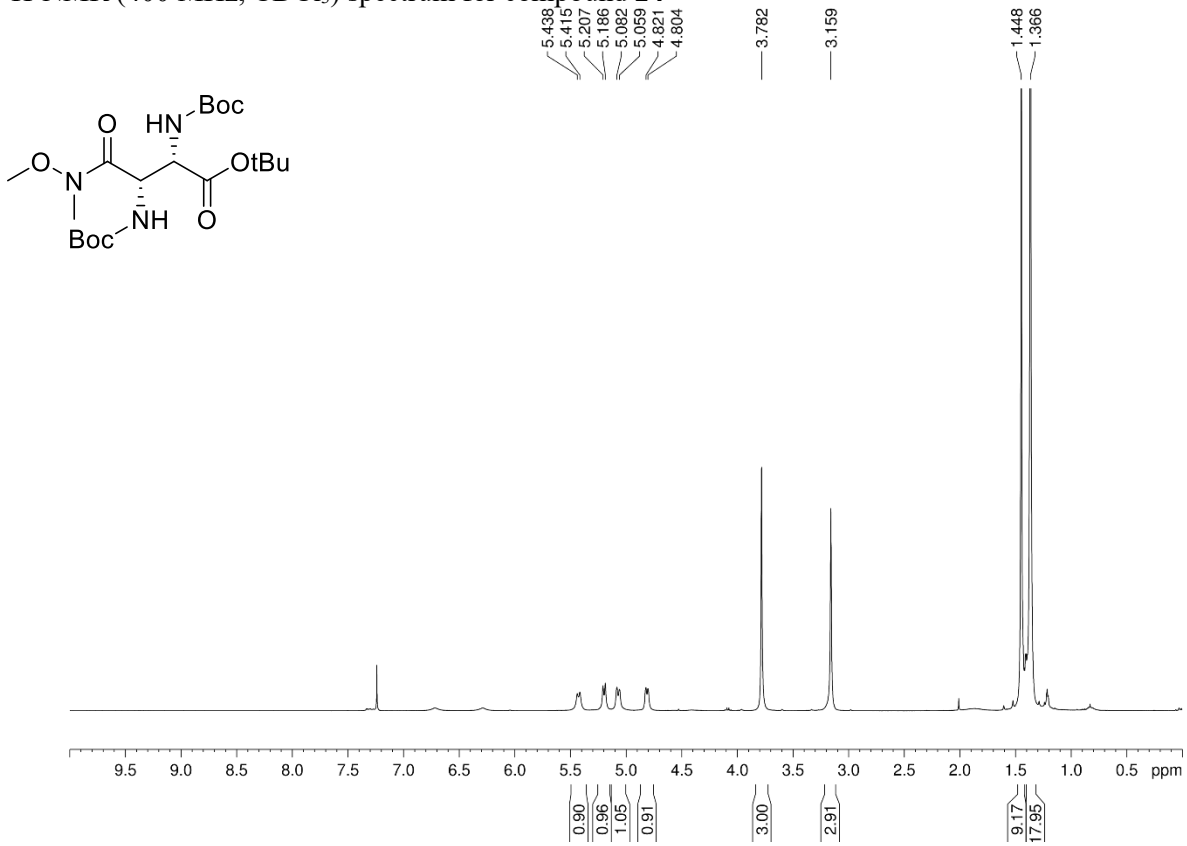
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **23**



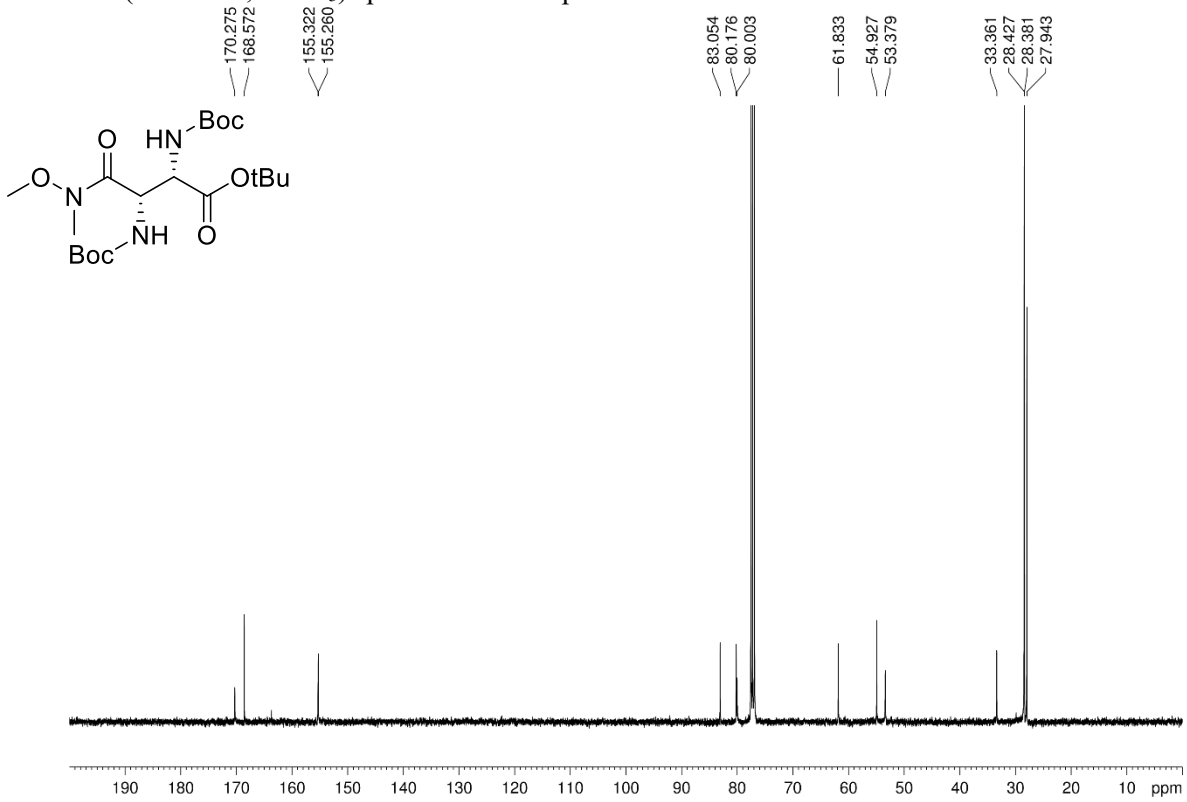

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **23**



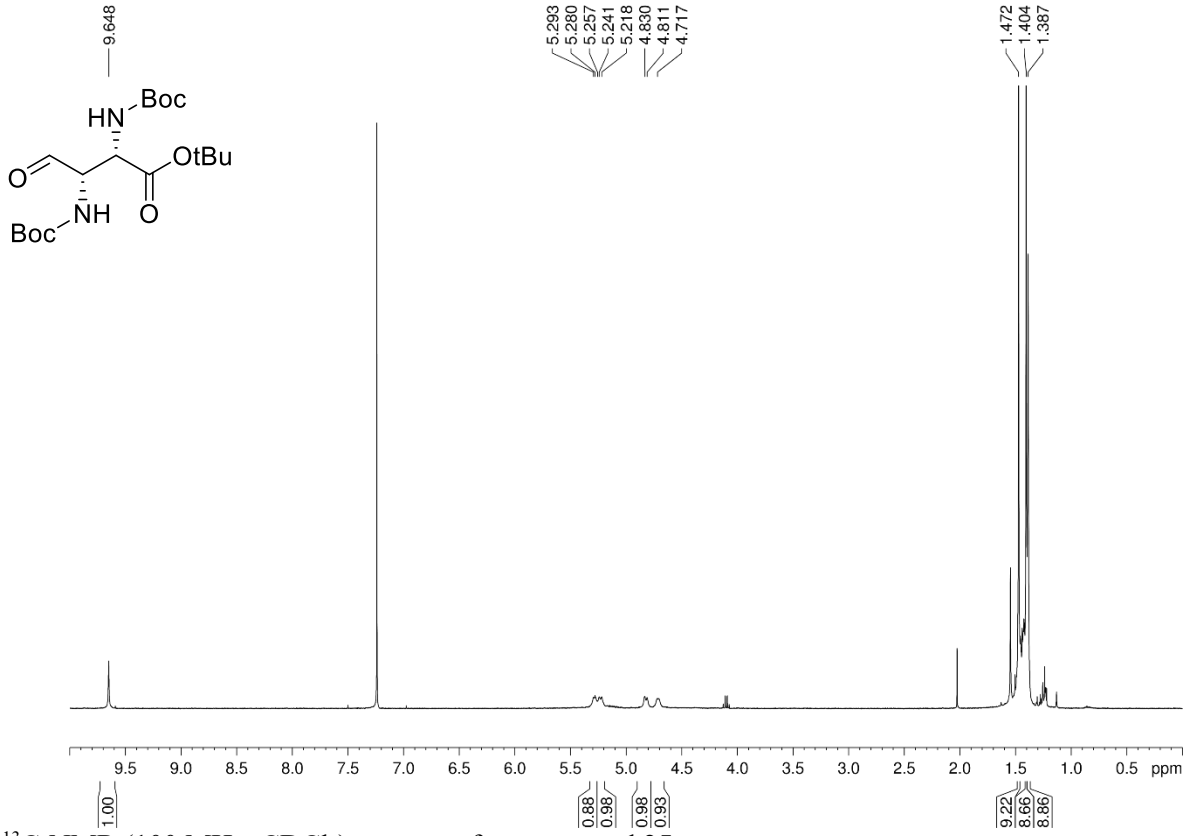
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **24**



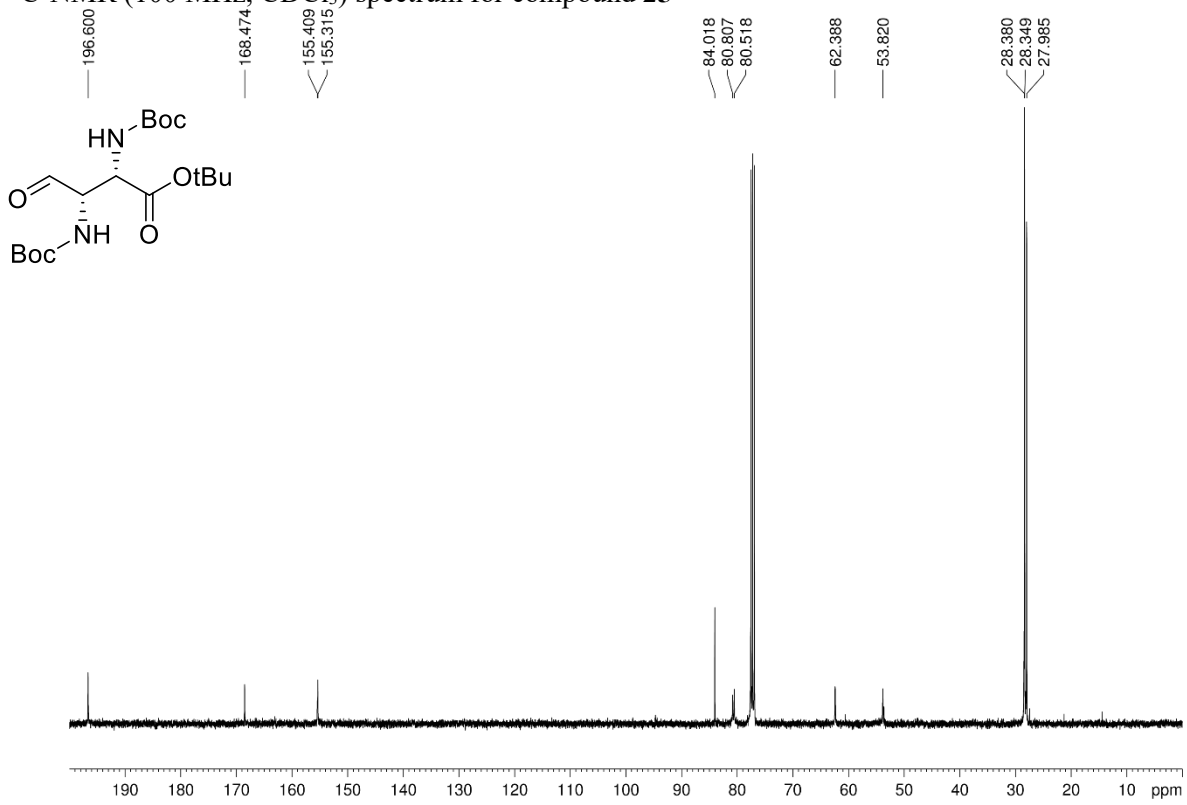

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **24**



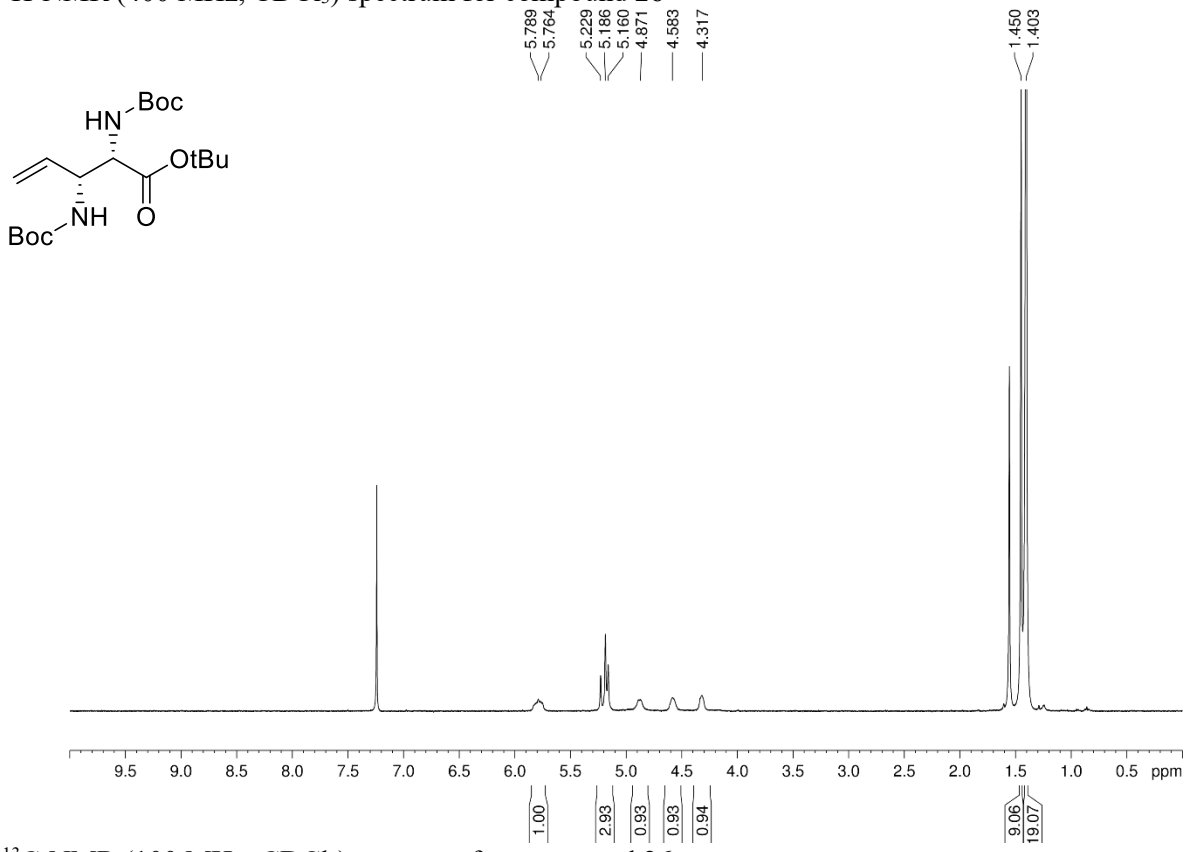
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **25**



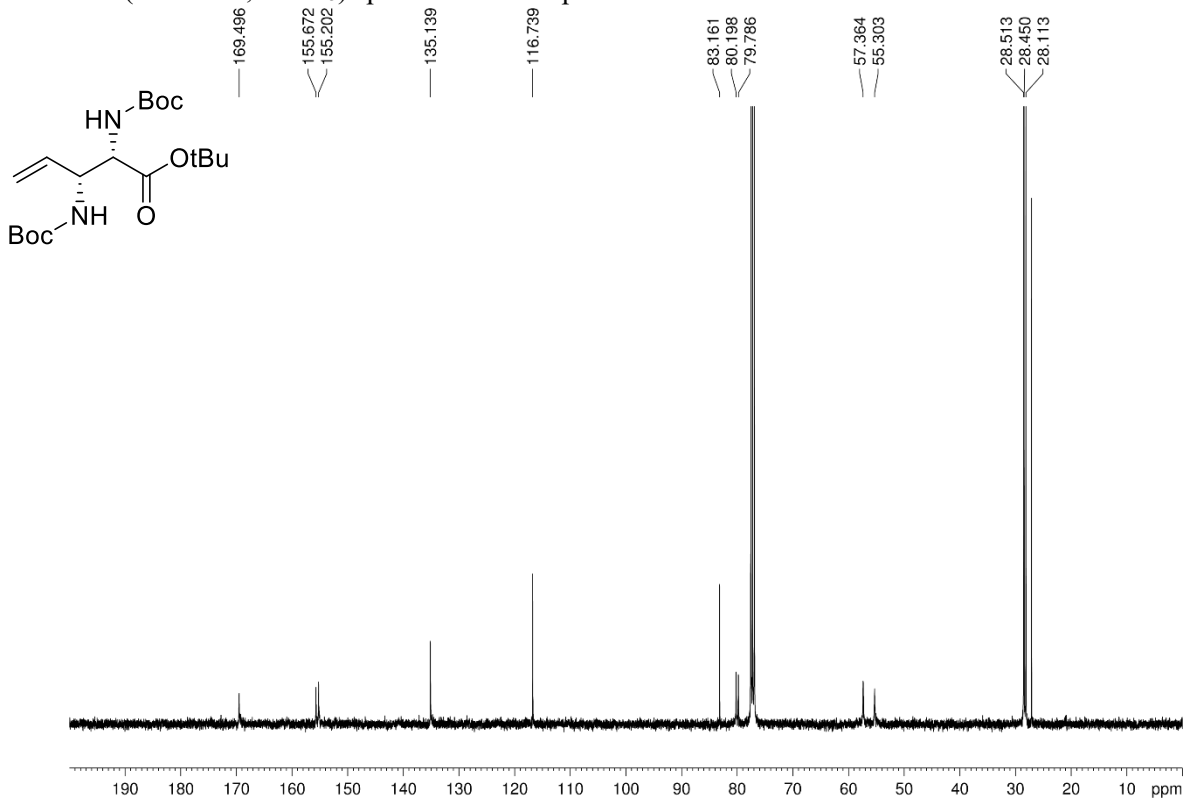

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **25**



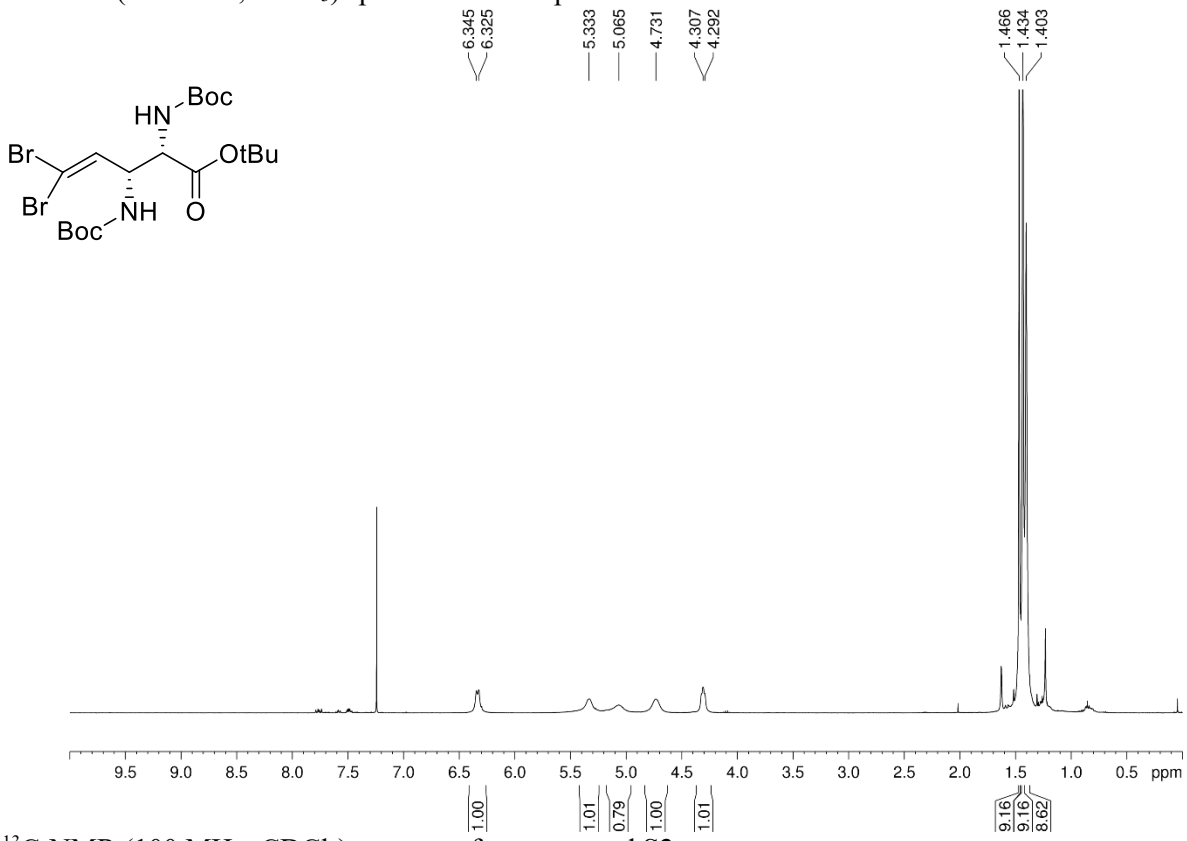
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **26**



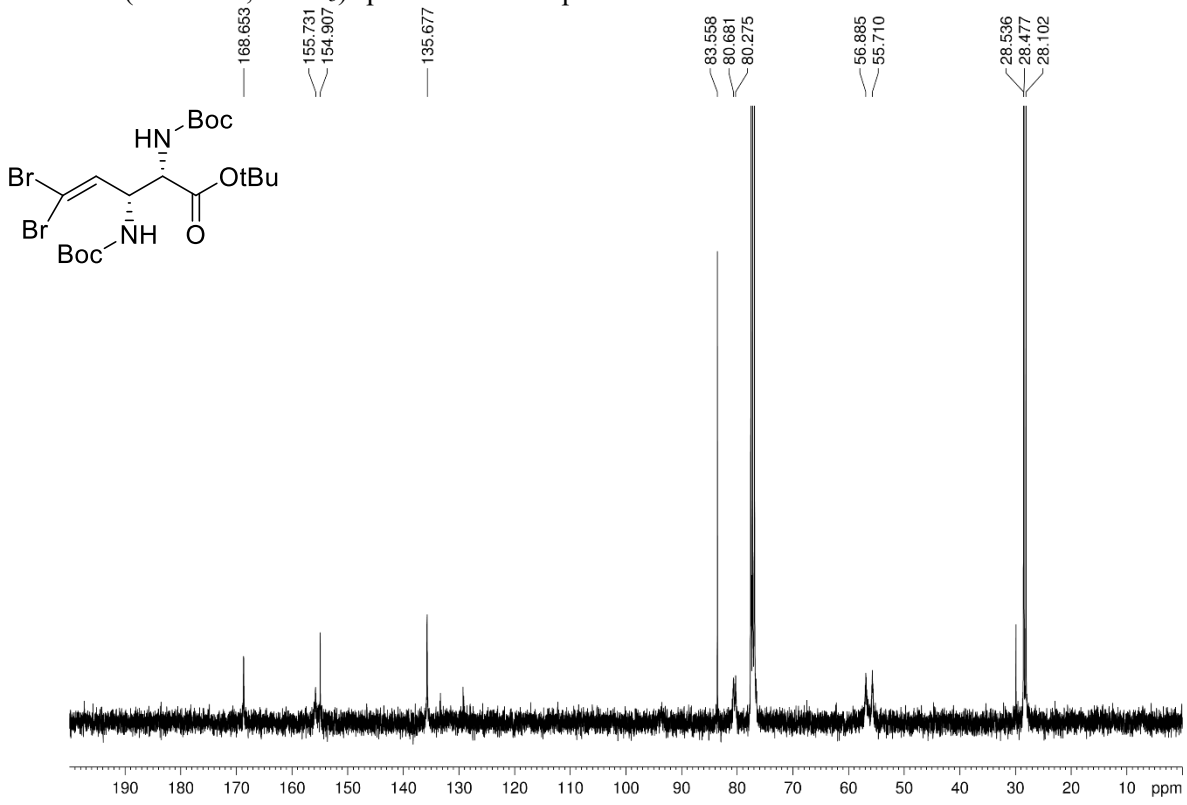

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **26**



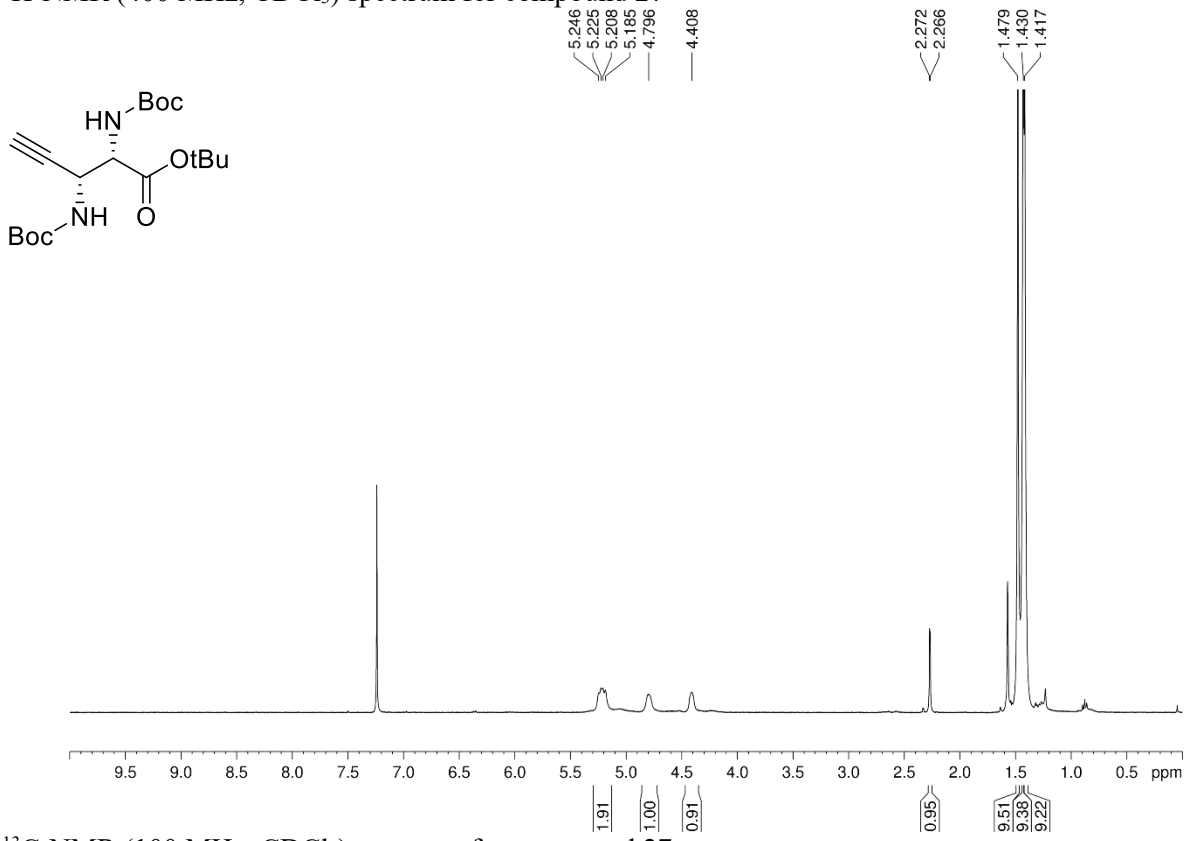
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **S3**



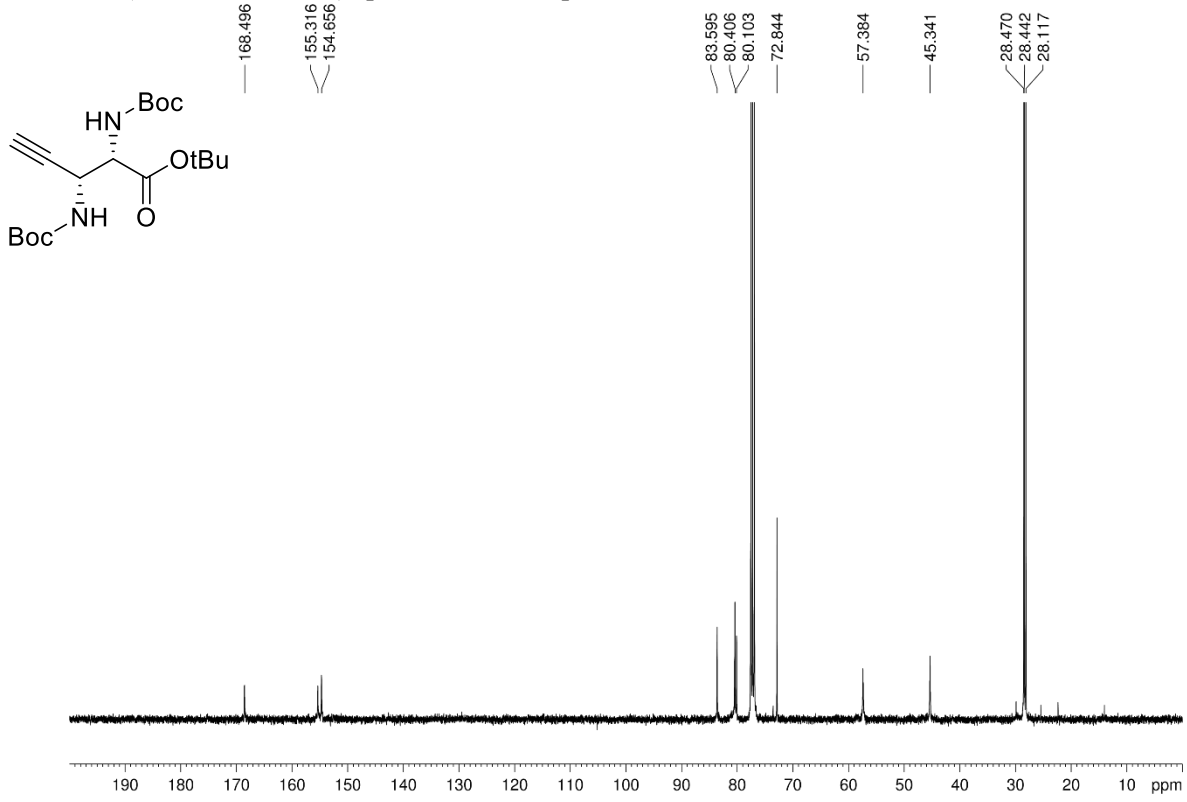

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **S3**



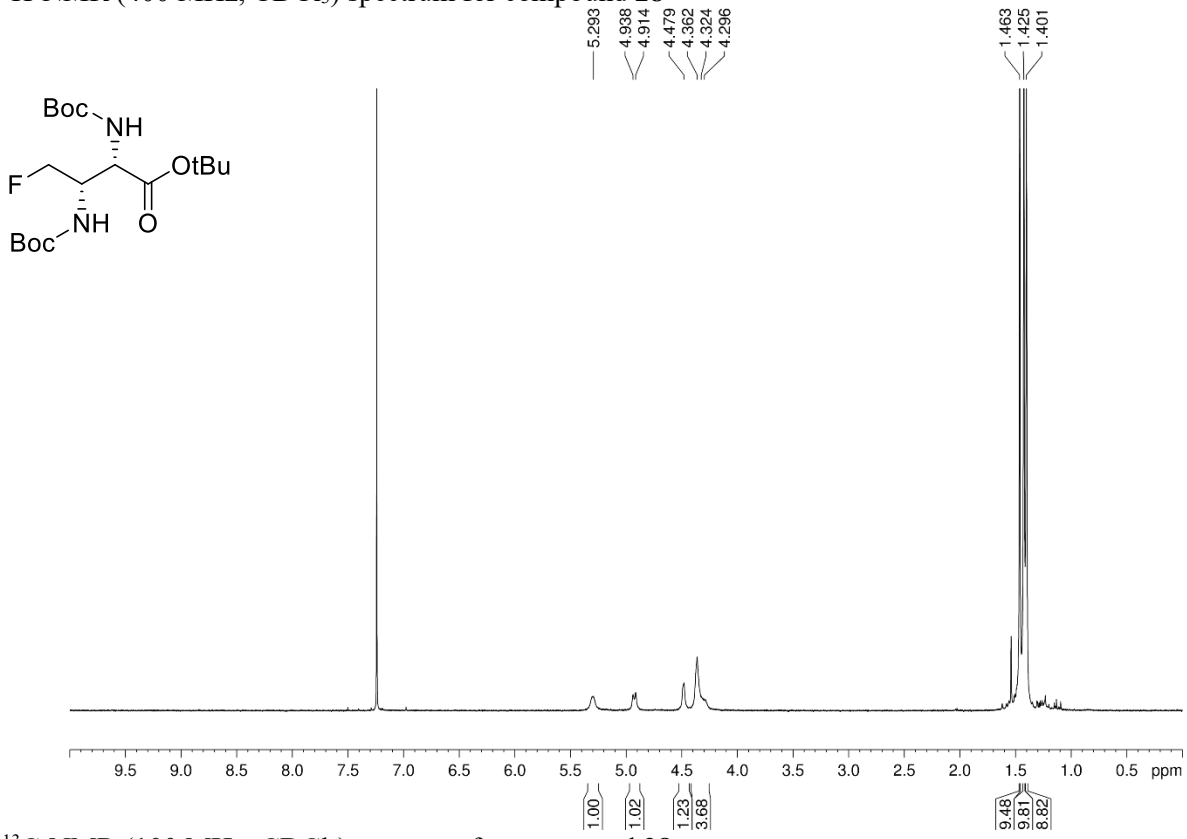
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **27**



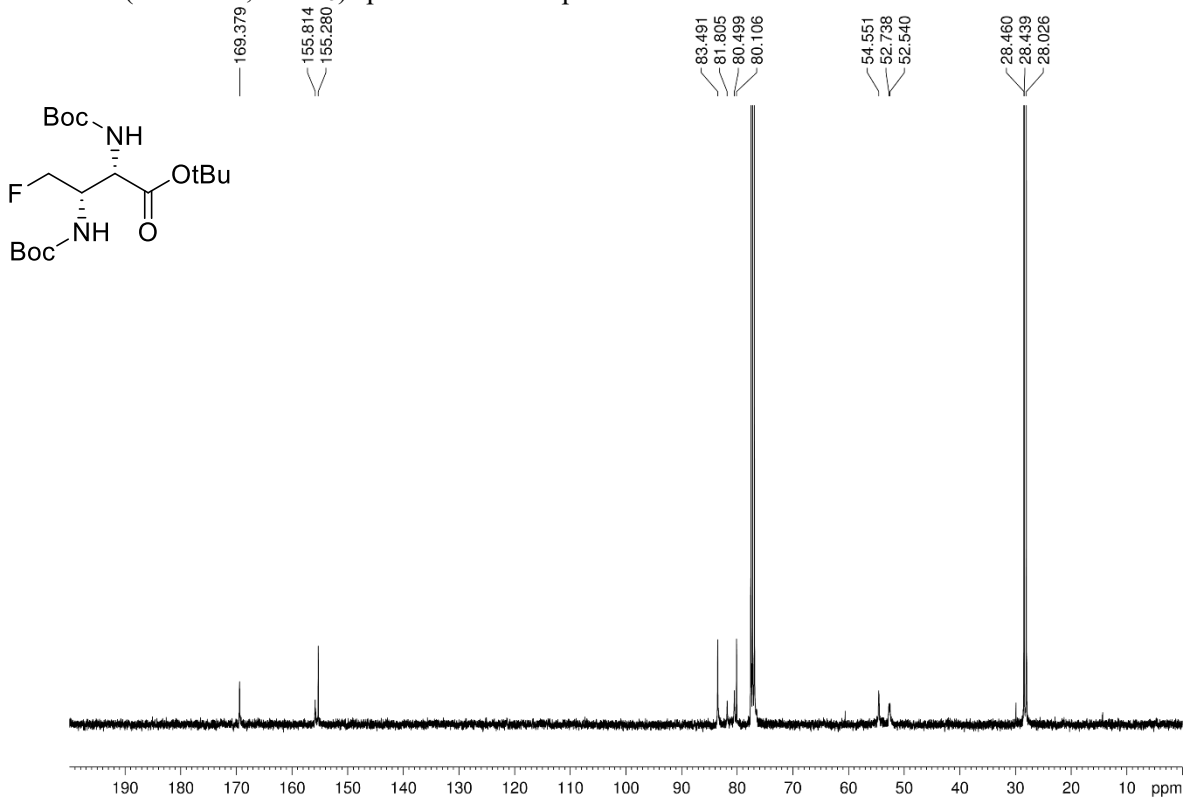

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **27**



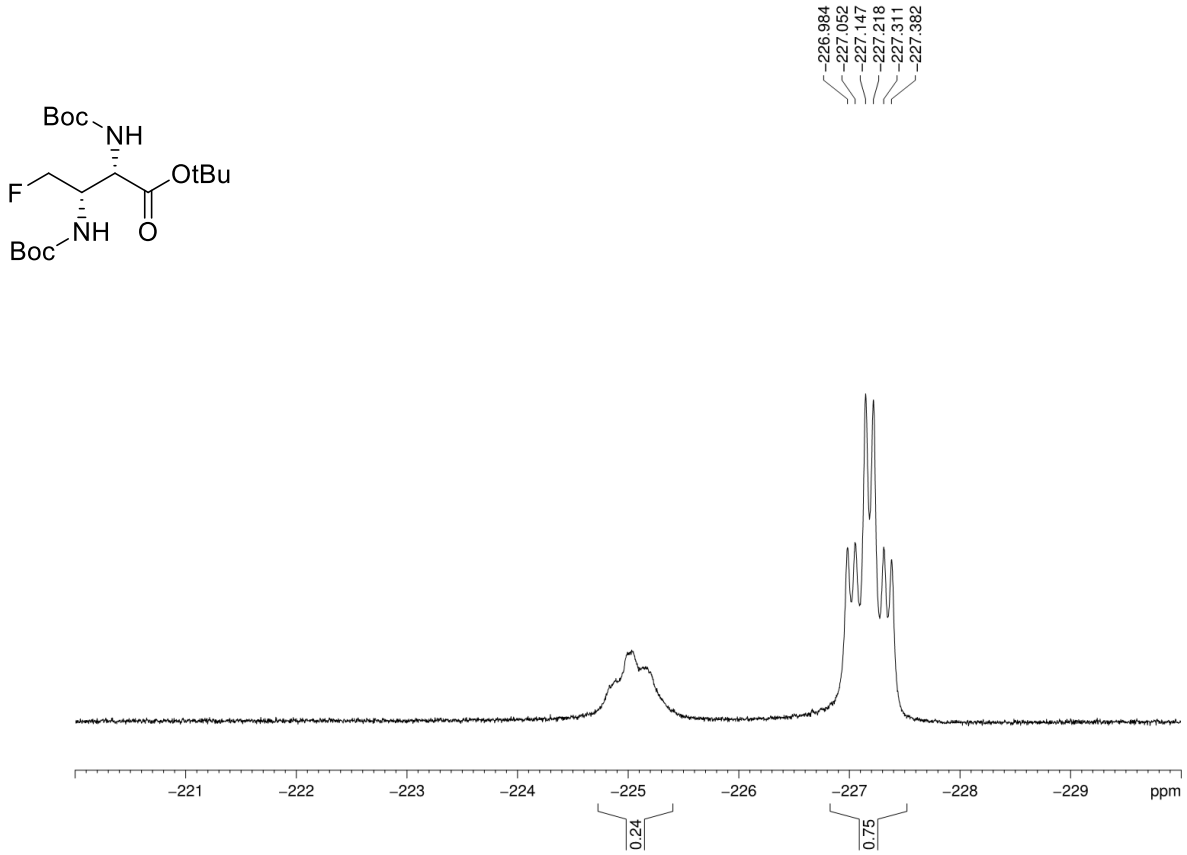
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **28**



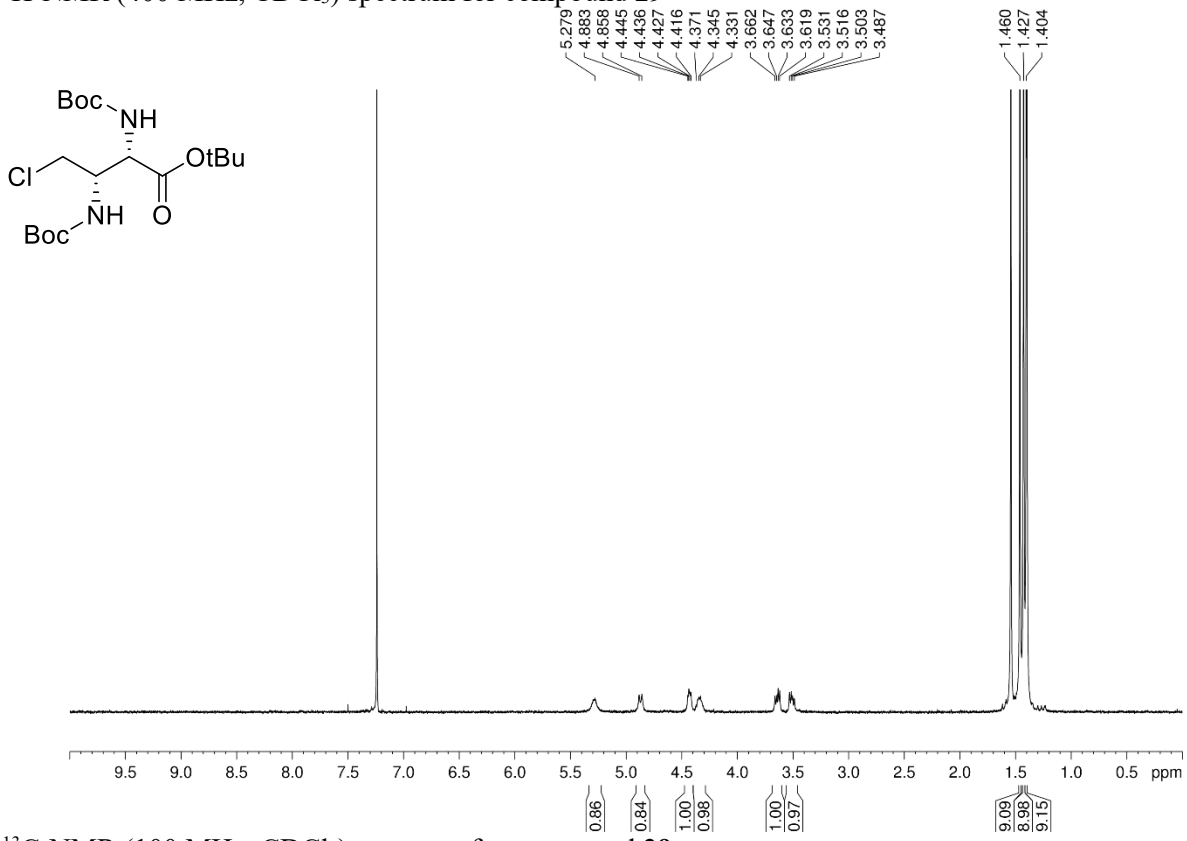

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **28**



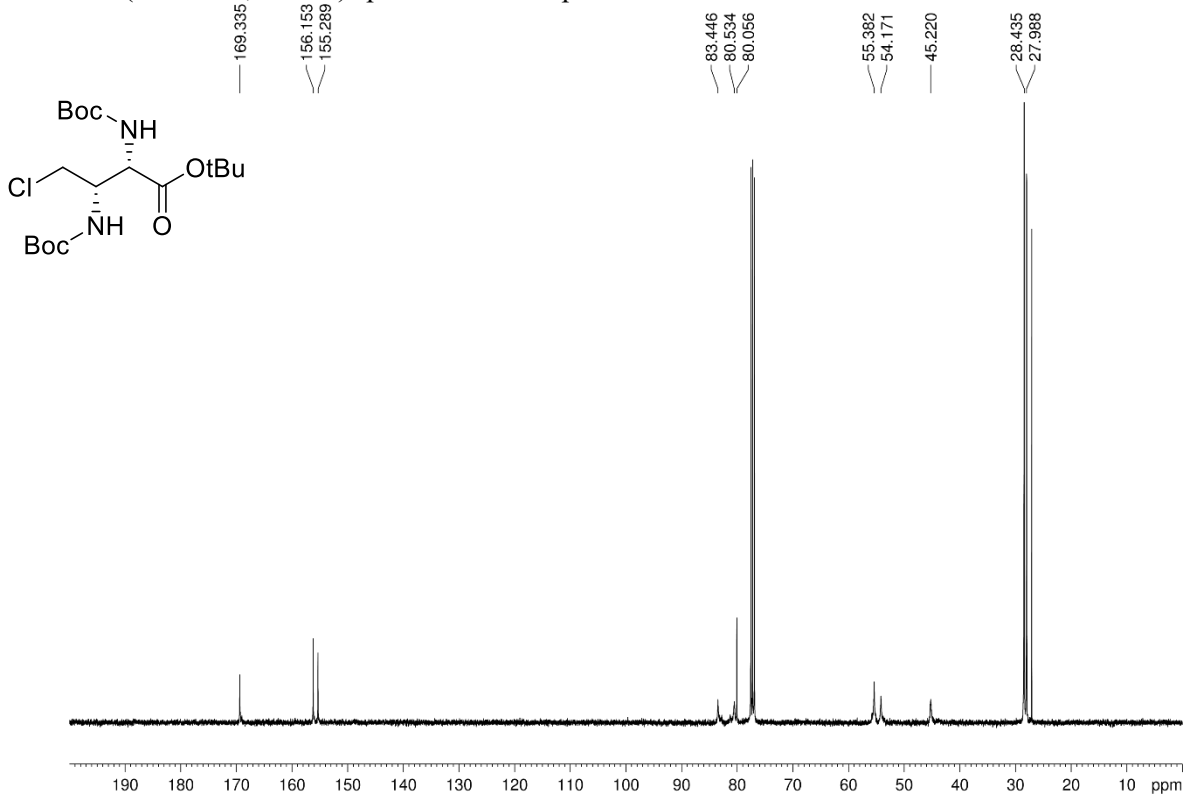
$^{19}$F-NMR (282 MHz, $CDCl_3$) spectrum for compound **28**



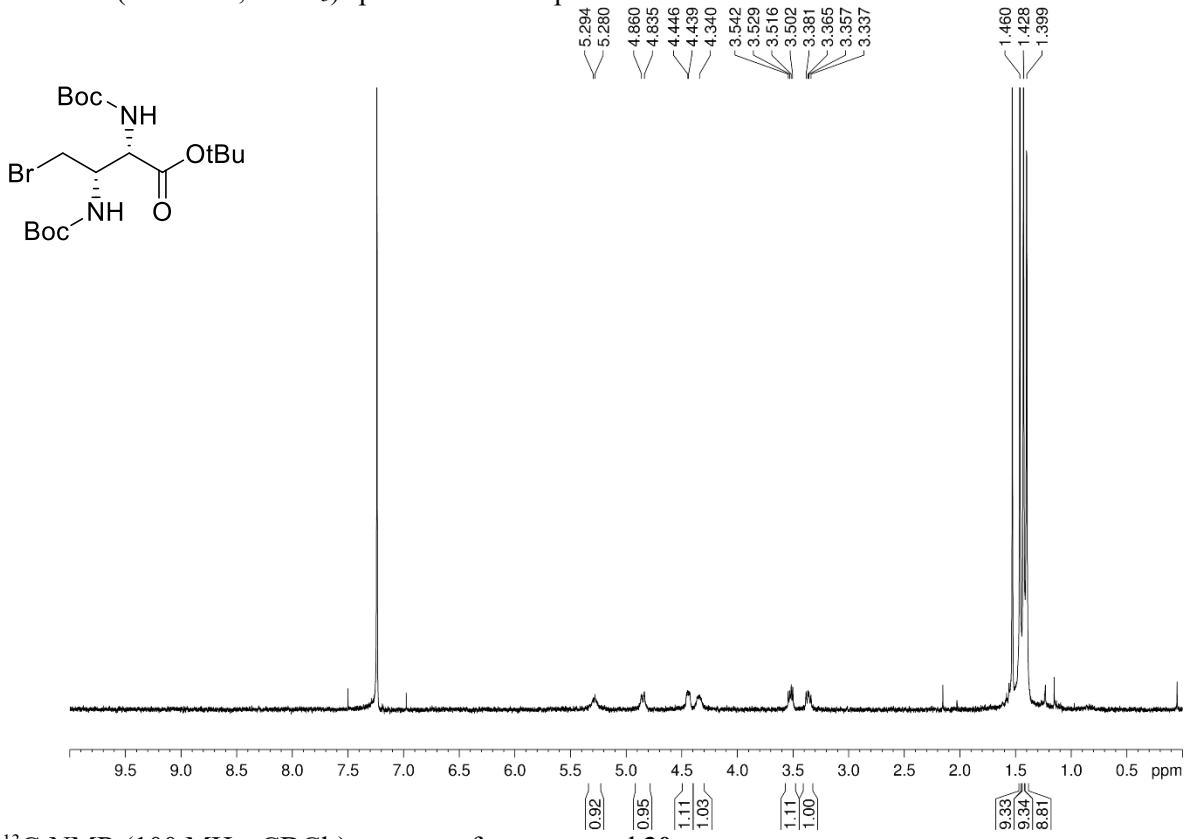
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **29**



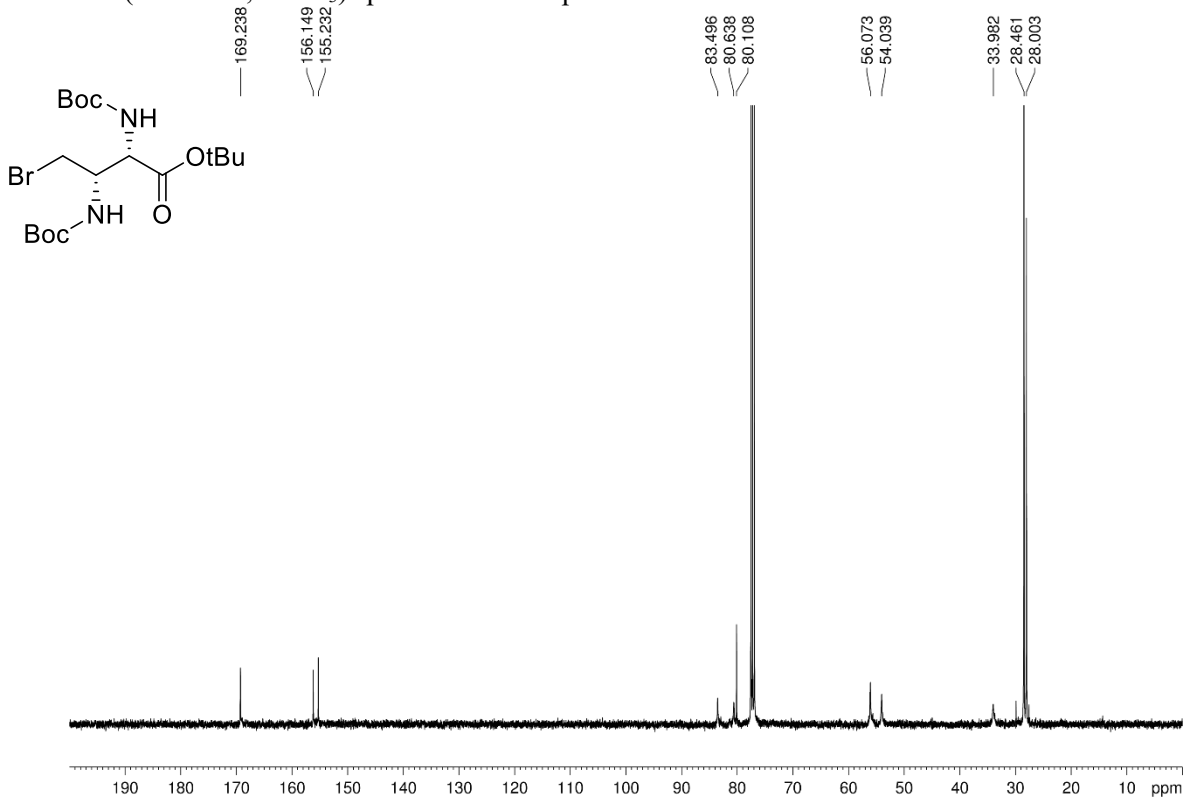

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **29**



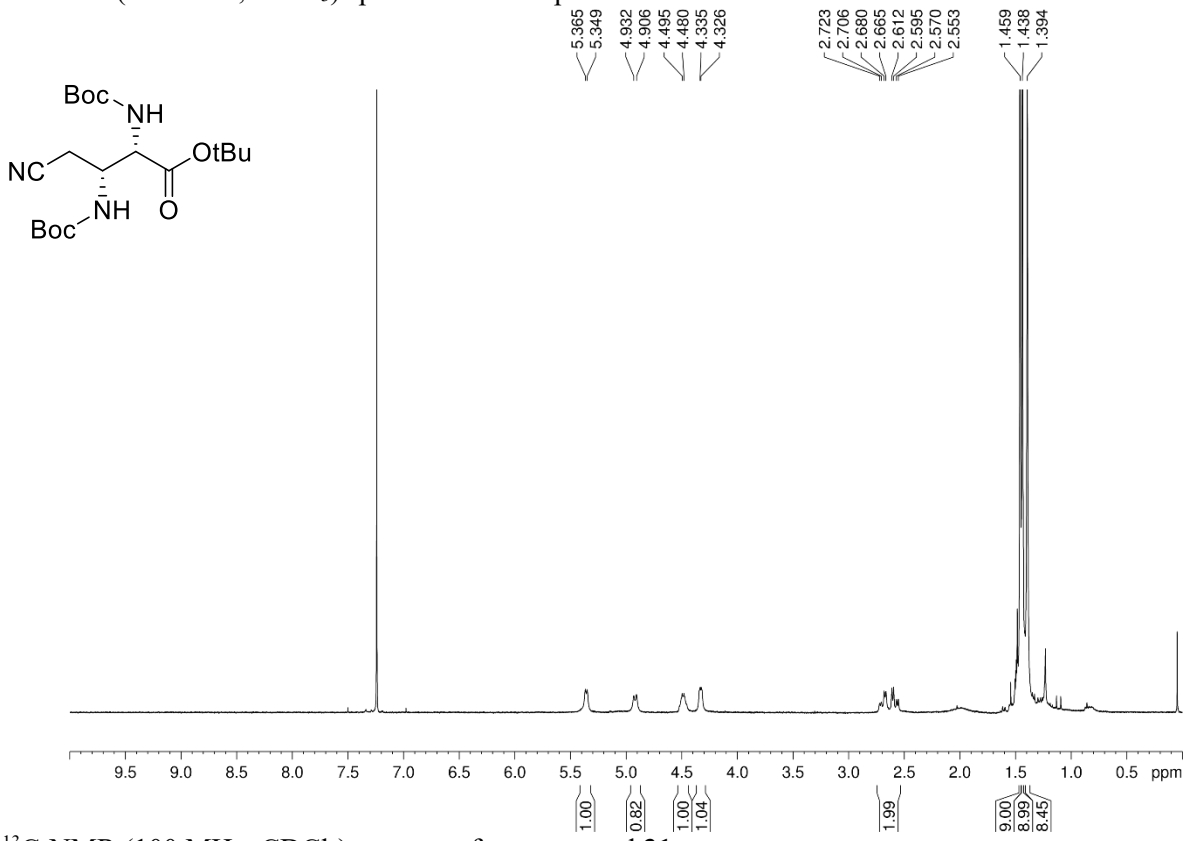
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **30**



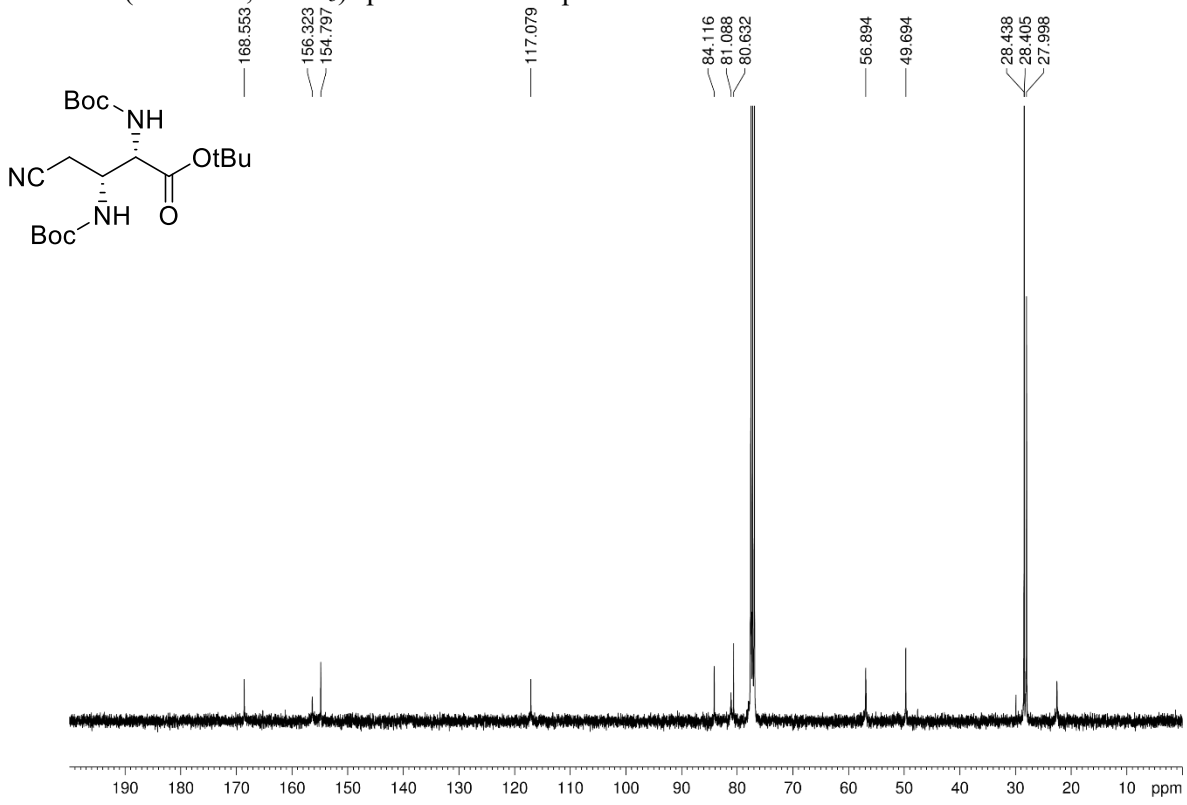

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **30**



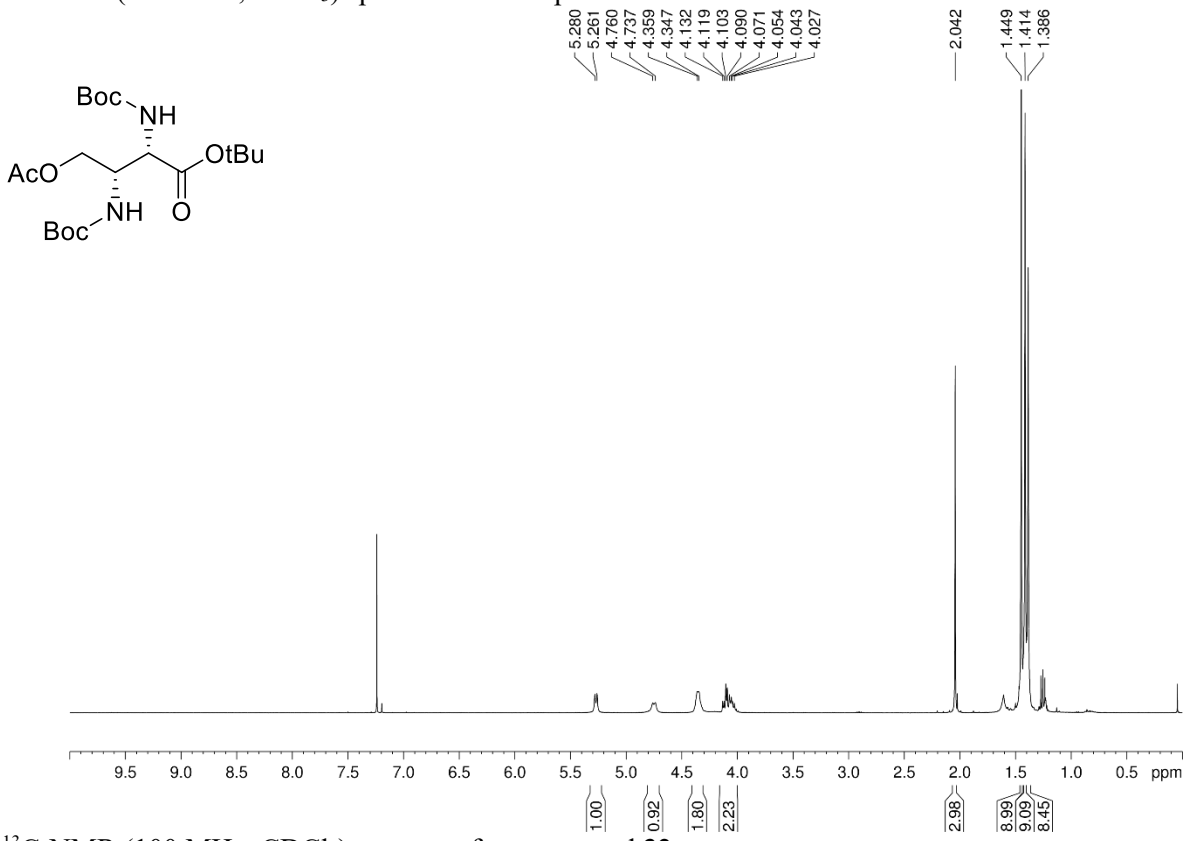
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **31**



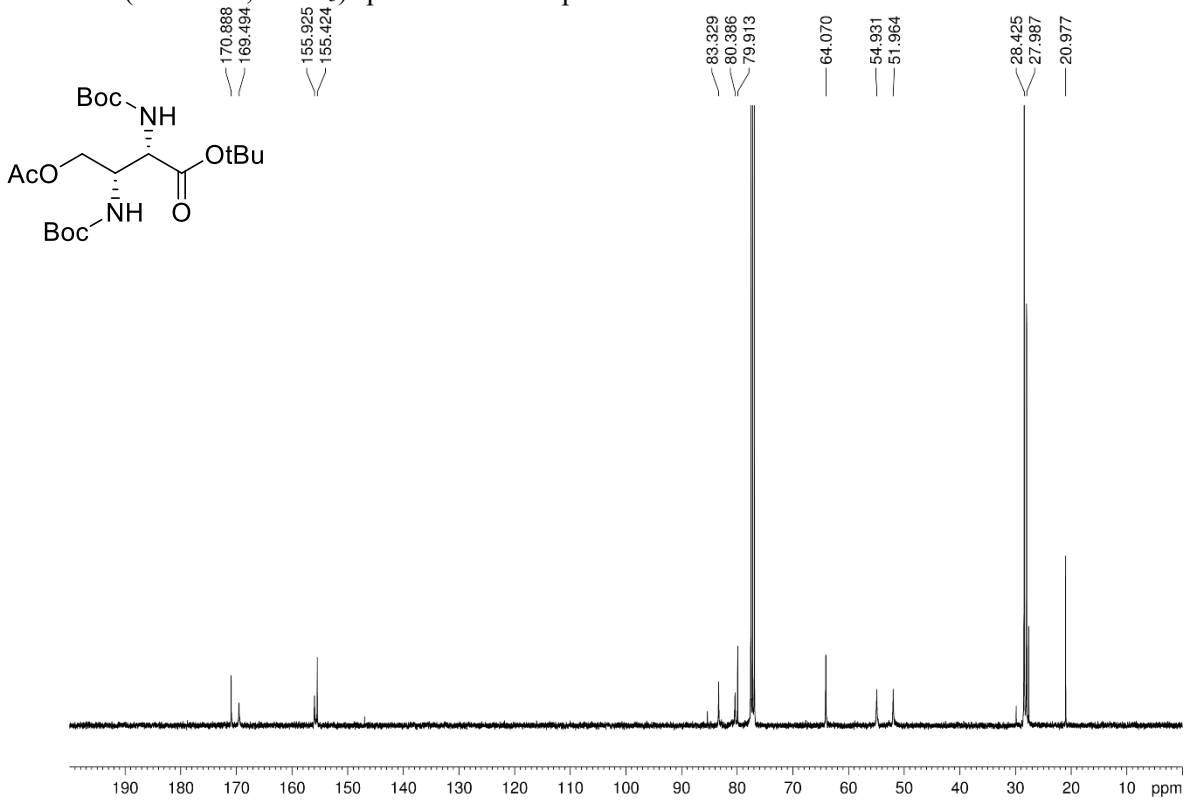

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **31**



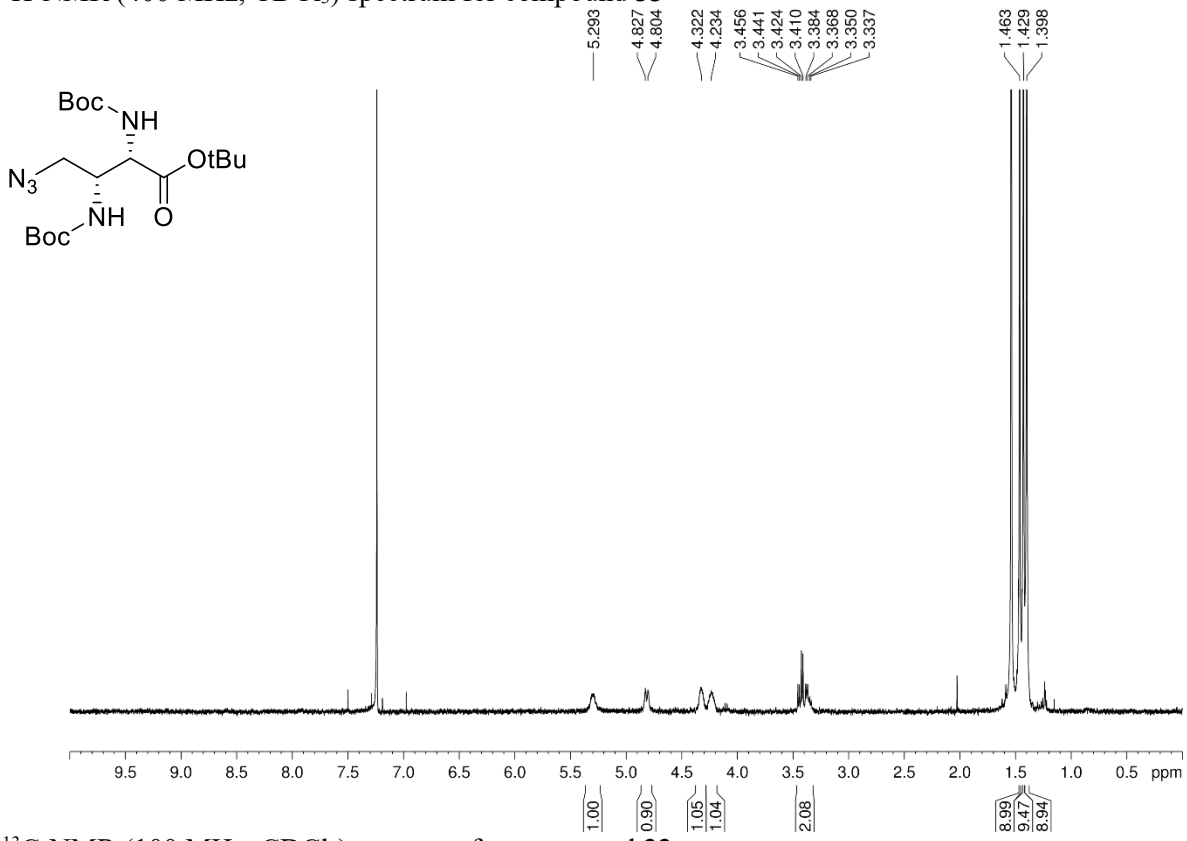
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **32**



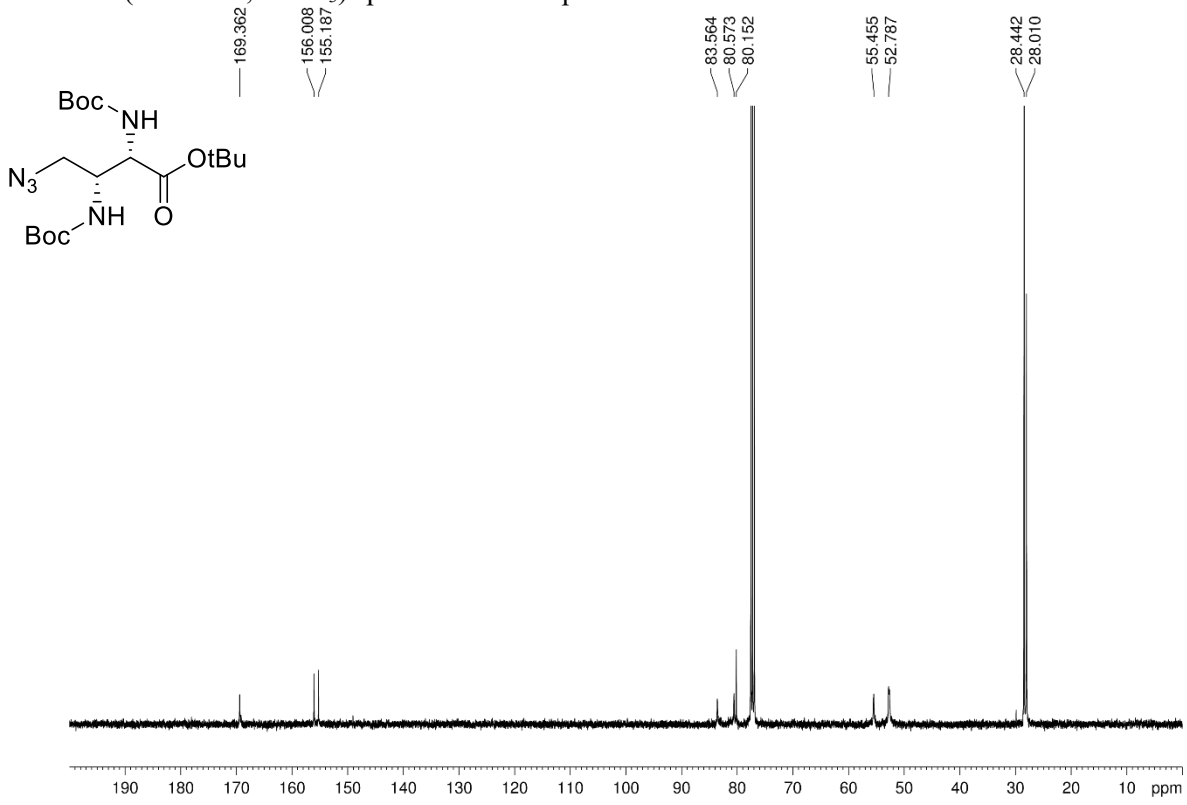

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **32**



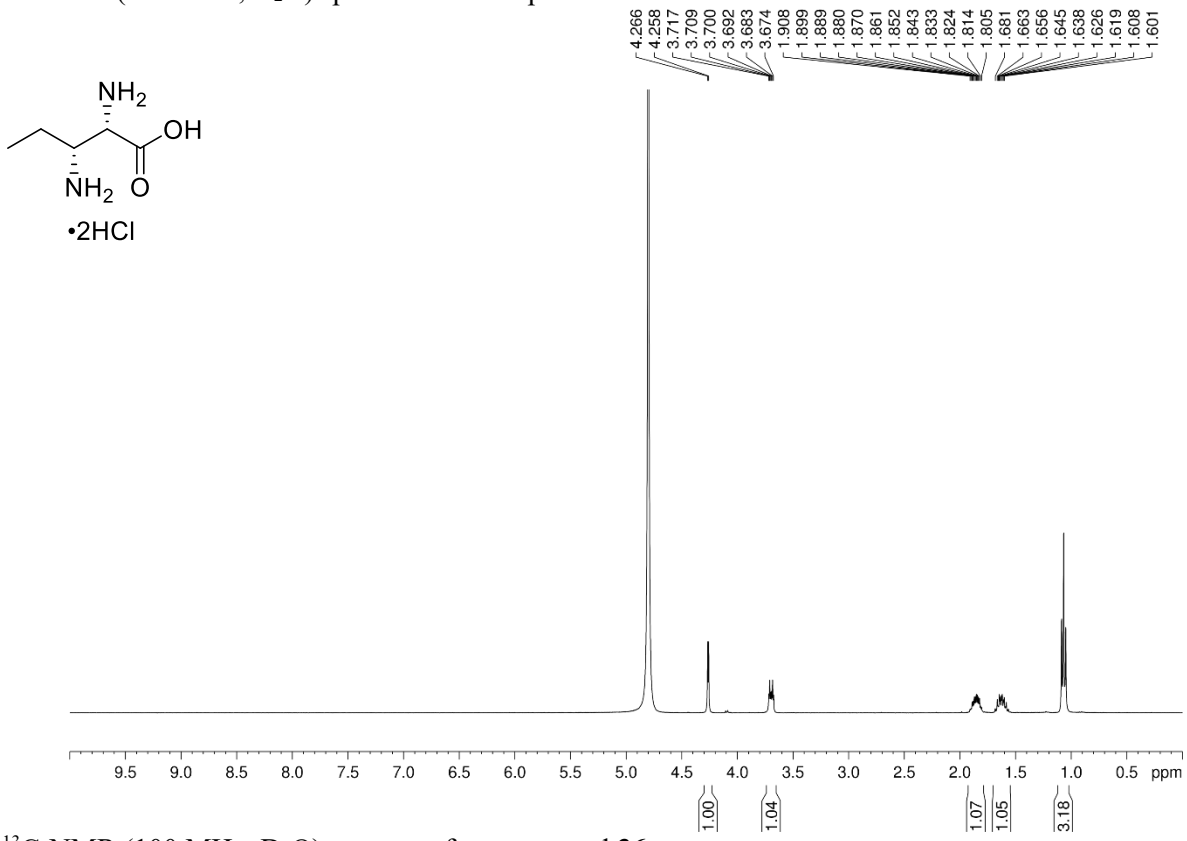
$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum for compound **33**



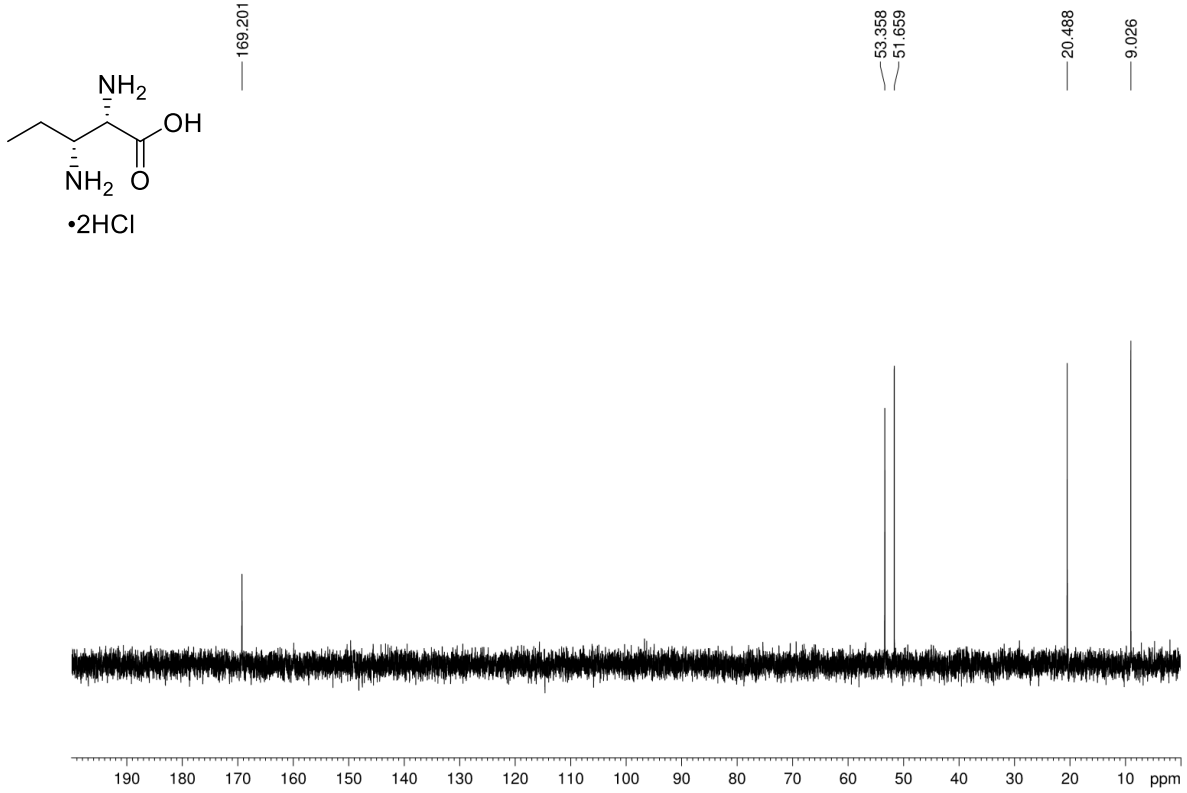

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum for compound **33**



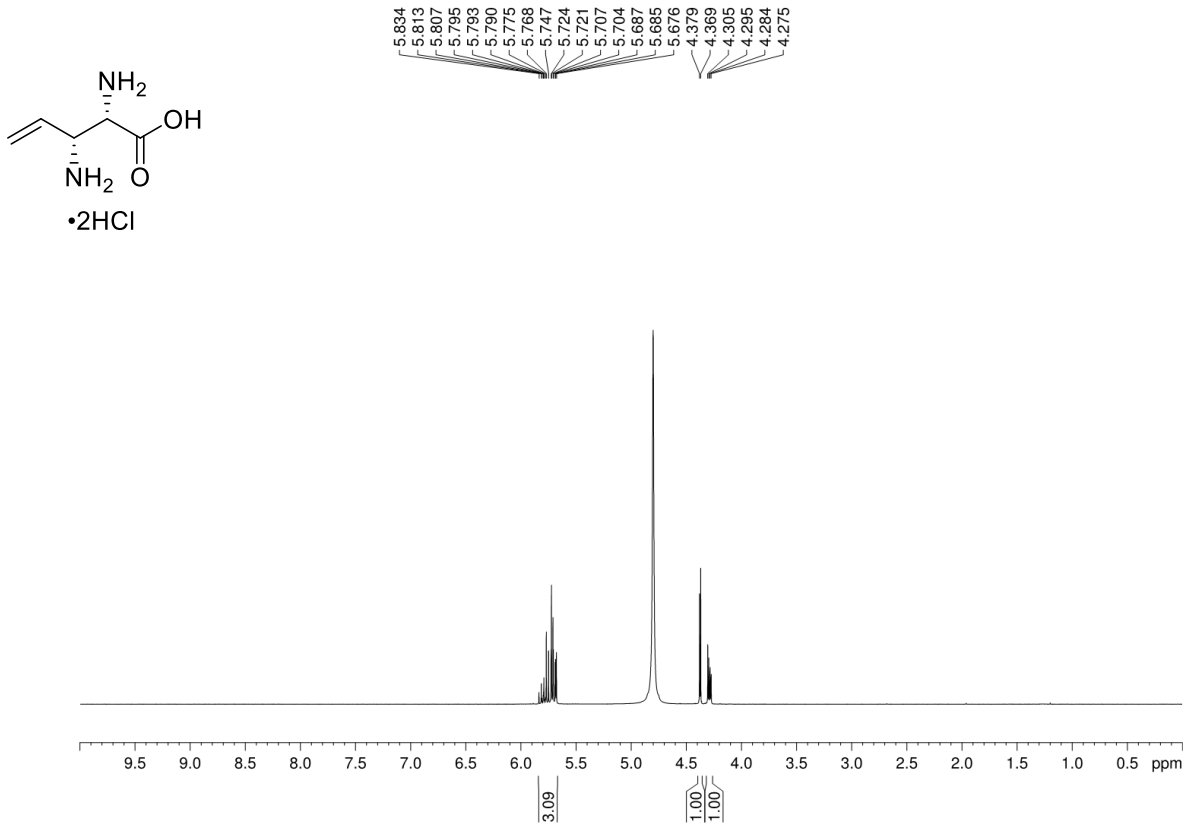
$^{1}$H-NMR (400 MHz, $D_2O$) spectrum for compound **36**



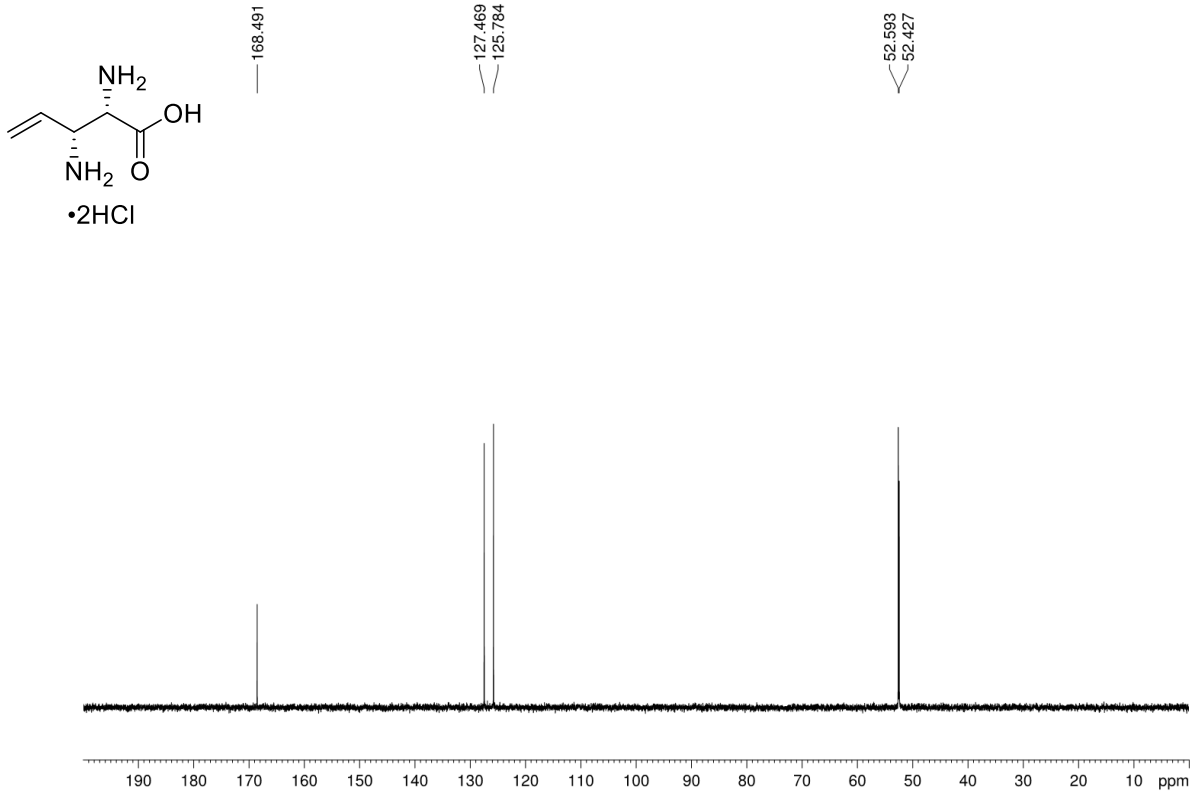

$^{13}$C-NMR (100 MHz, $D_2O$) spectrum for compound **36**



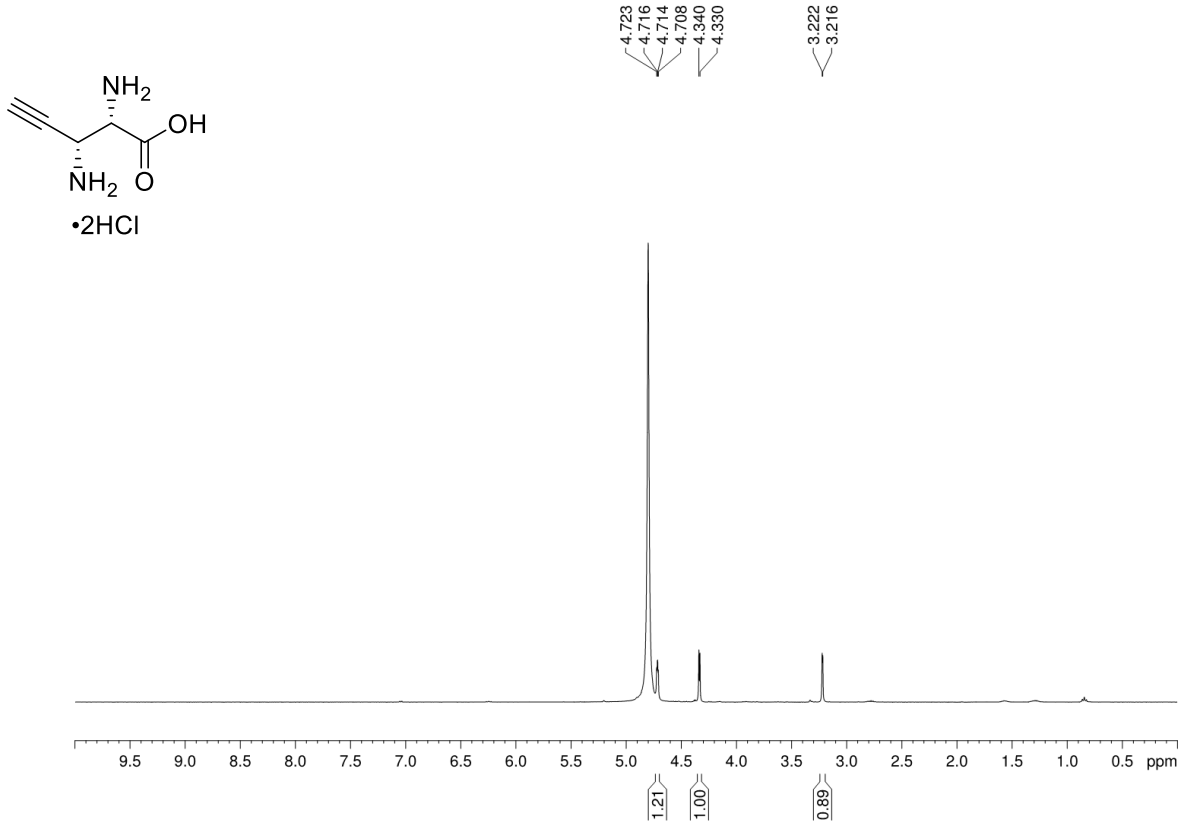
$^{1}$H-NMR (400 MHz, $D_2O$) spectrum for compound **37**



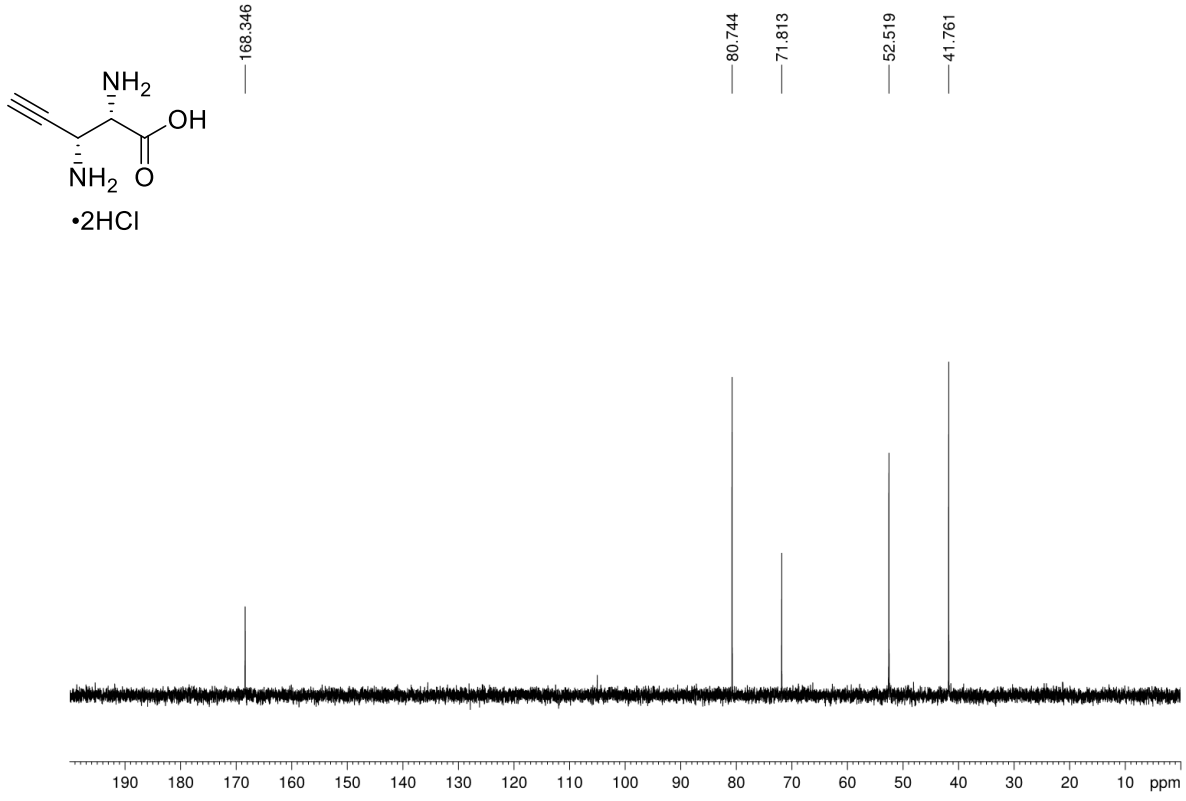

$^{13}$C-NMR (100 MHz, $D_2O$) spectrum for compound **37**



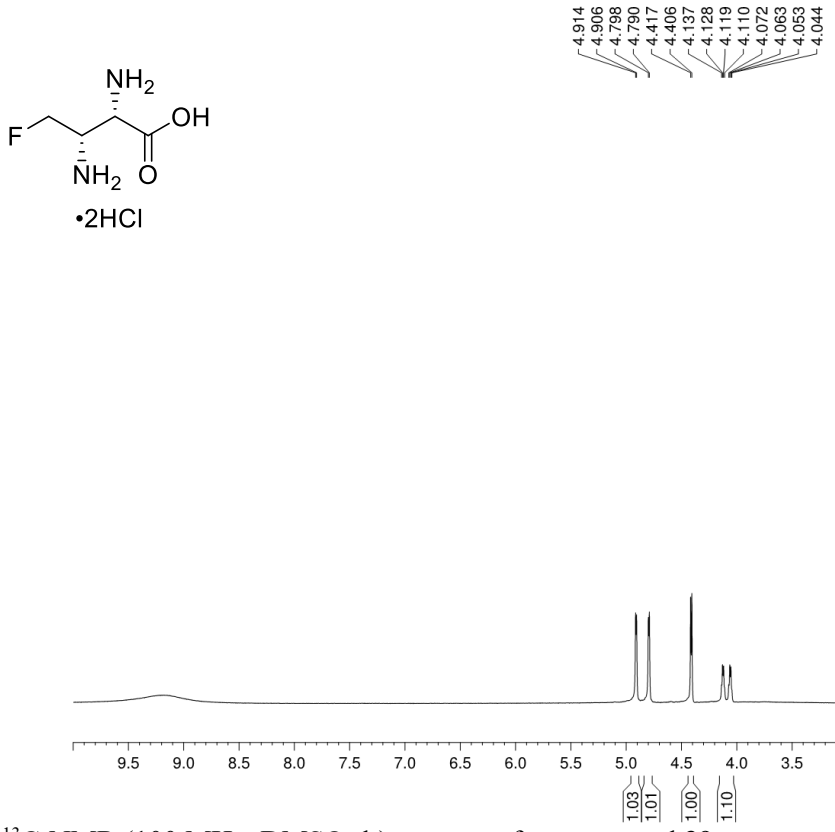
$^{1}$H-NMR (400 MHz, $D_2O$) spectrum for compound **38**



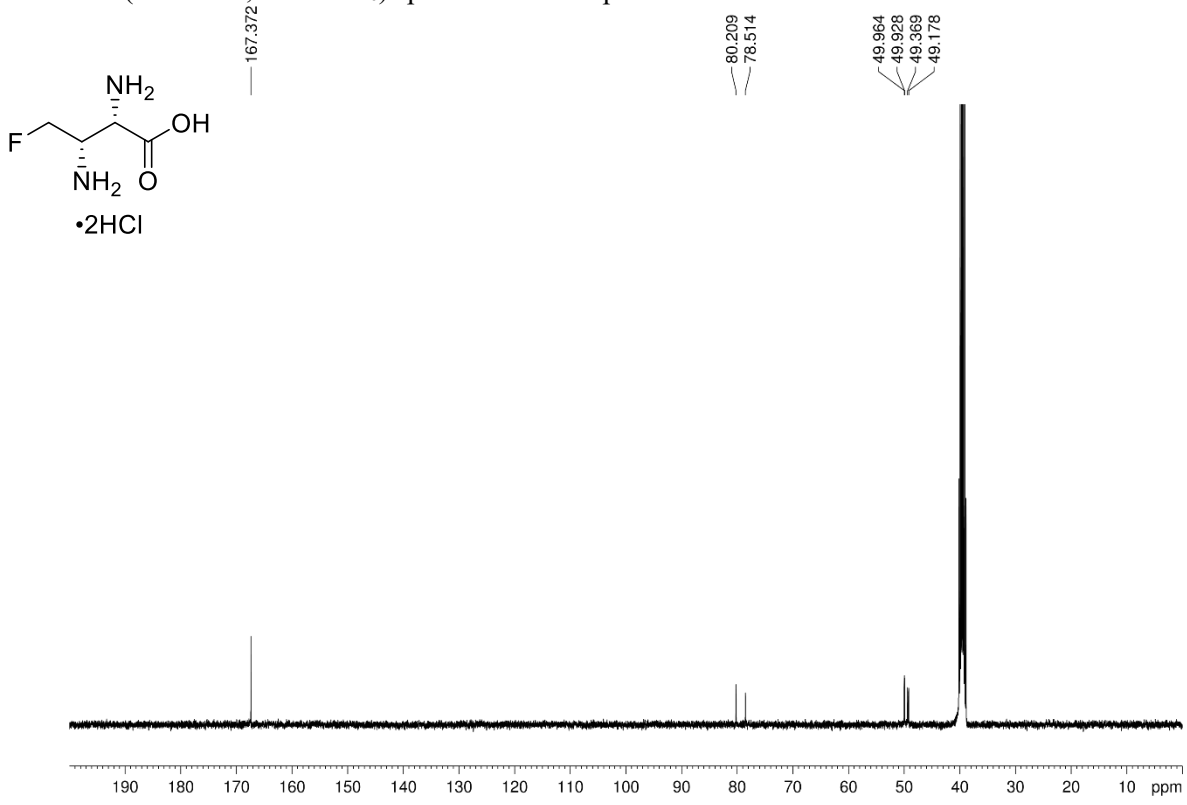

$^{13}$C-NMR (100 MHz, $D_2O$) spectrum for compound **38**



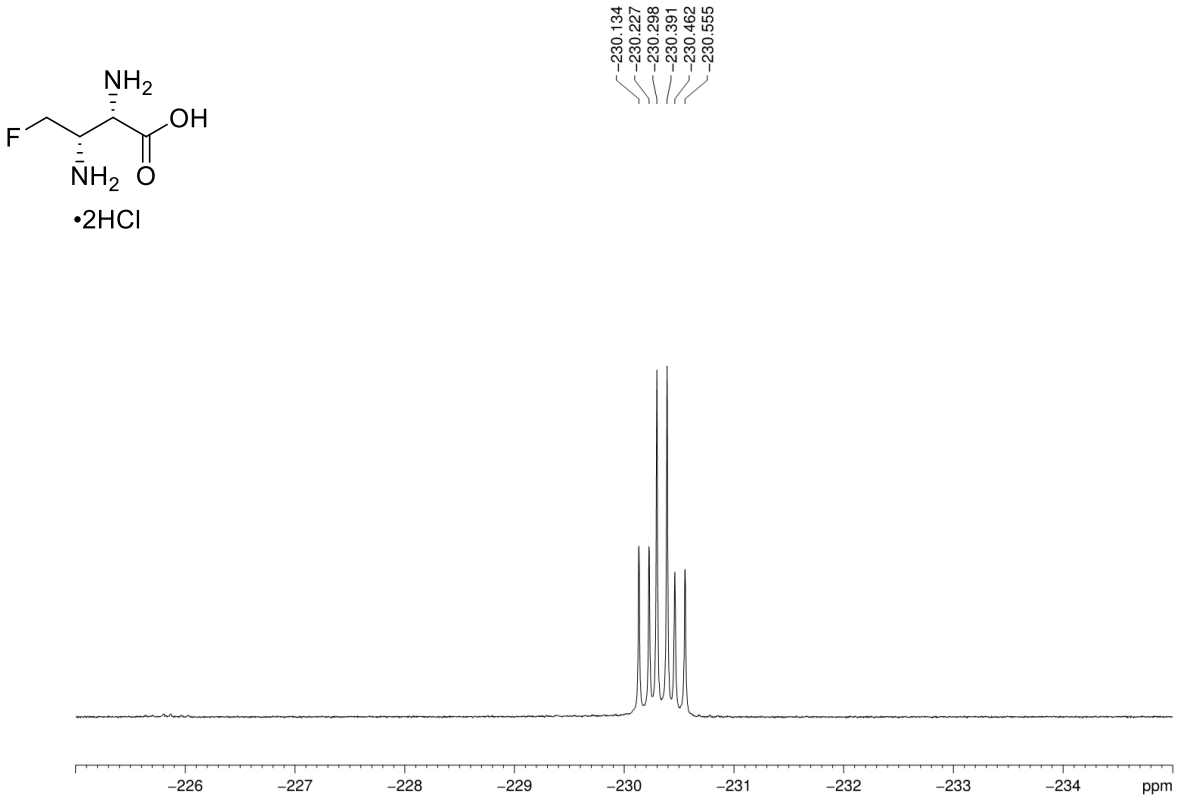
$^{1}$H-NMR (400 MHz, DMSO-$d_6$) spectrum for compound **39**



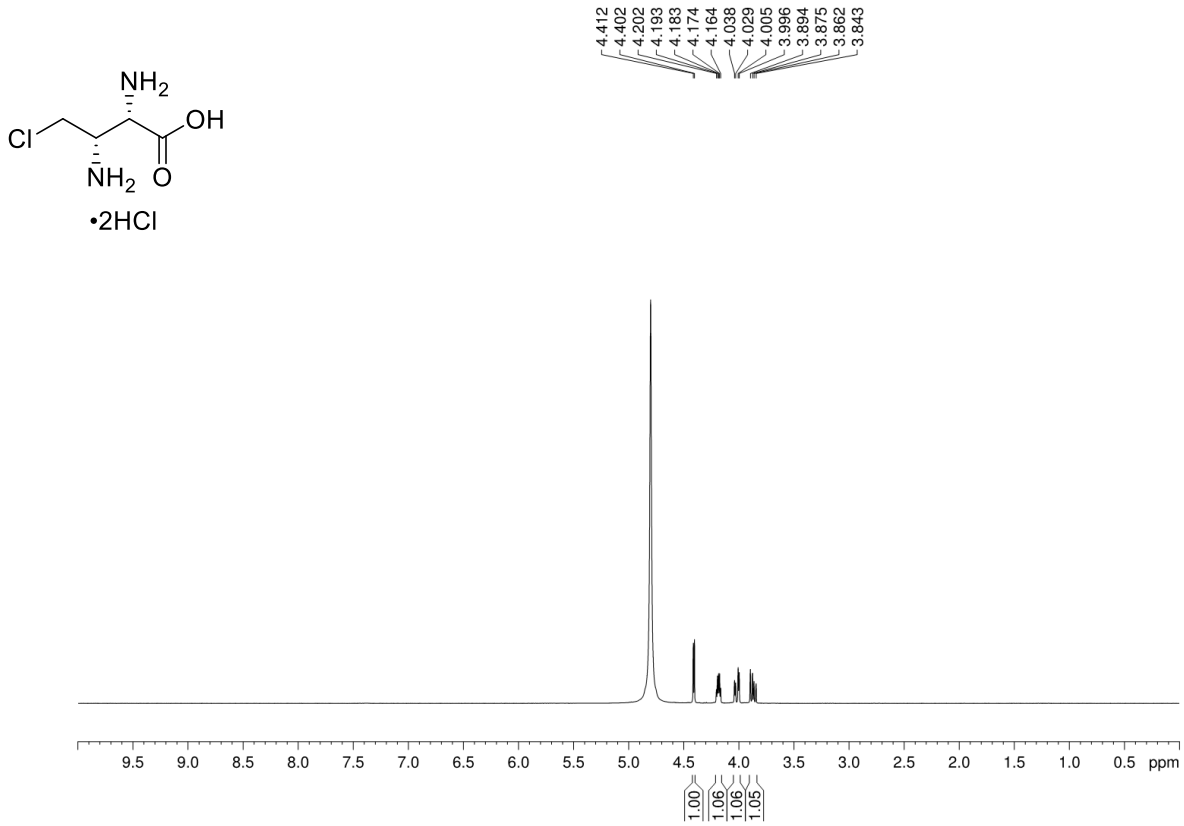

$^{13}$C-NMR (100 MHz, DMSO-$d_6$) spectrum for compound **39**



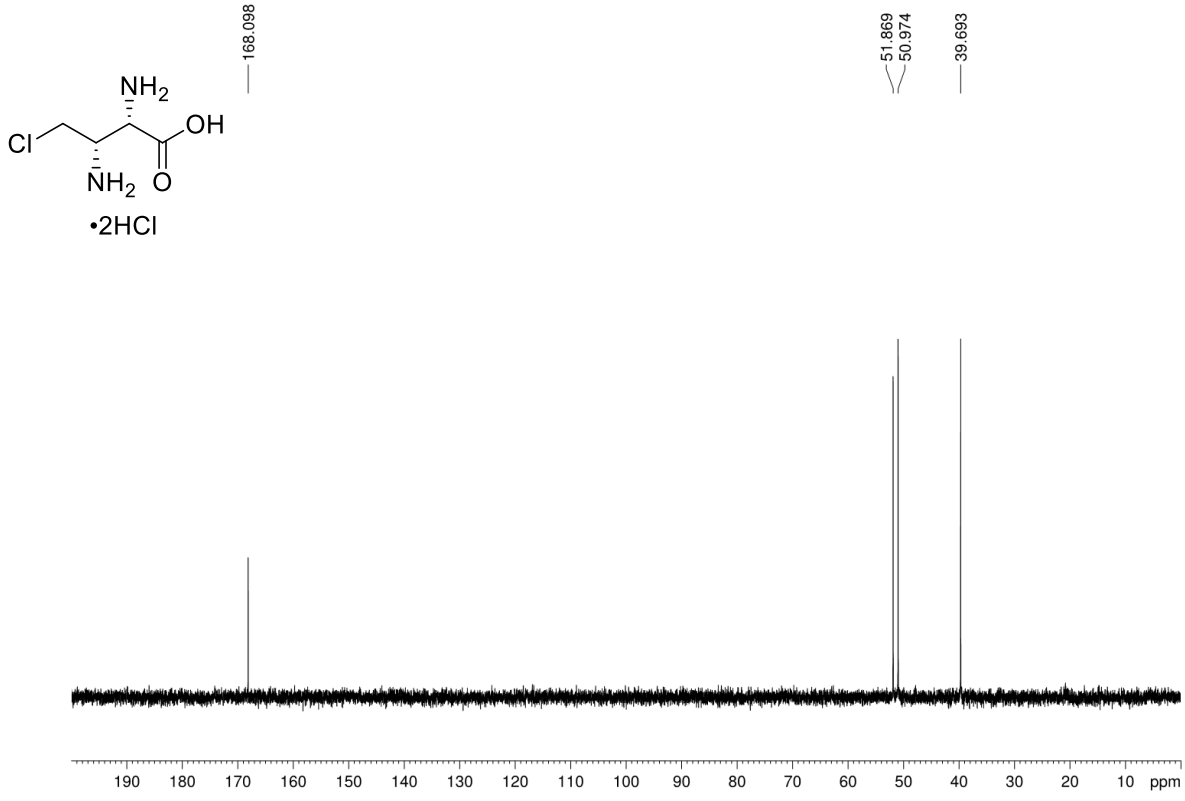
$^{19}$F-NMR (282 MHz, DMSO-$d_6$) spectrum for compound **39**



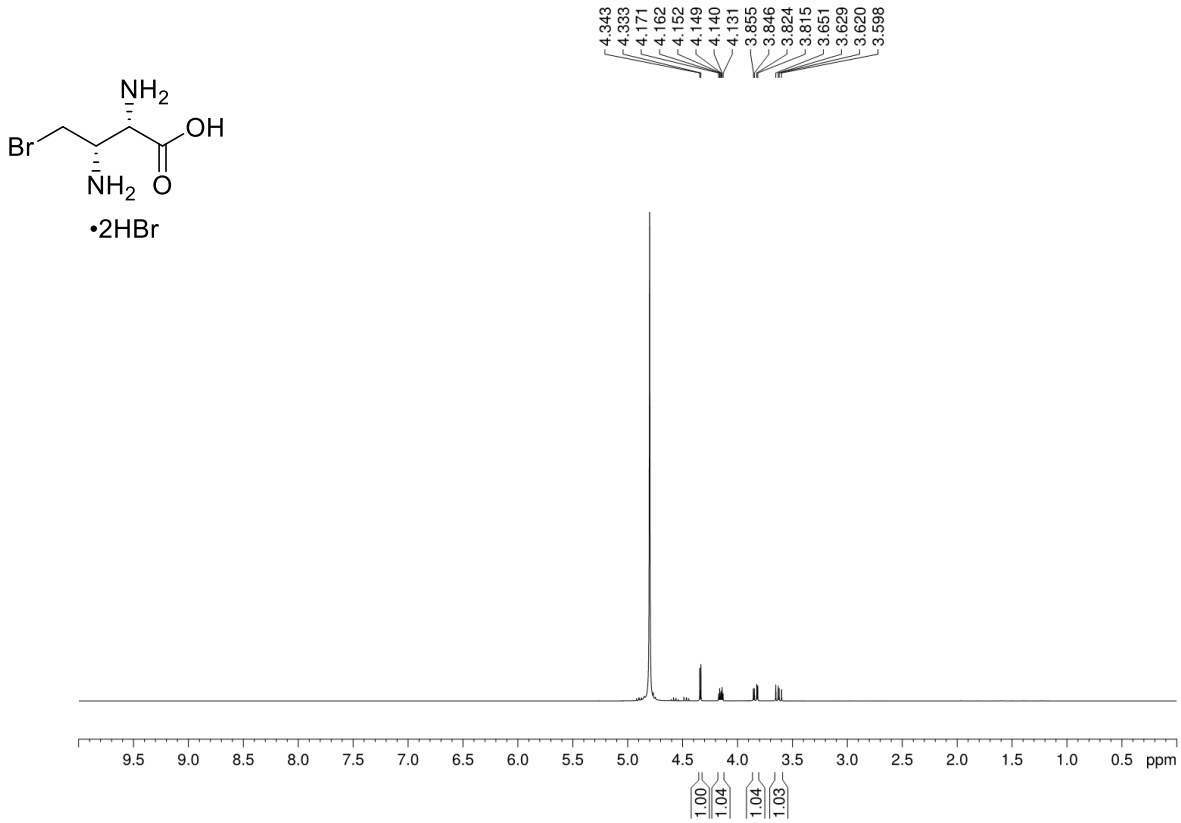
$^{1}$H-NMR (400 MHz, $D_2O$) spectrum for compound **40**



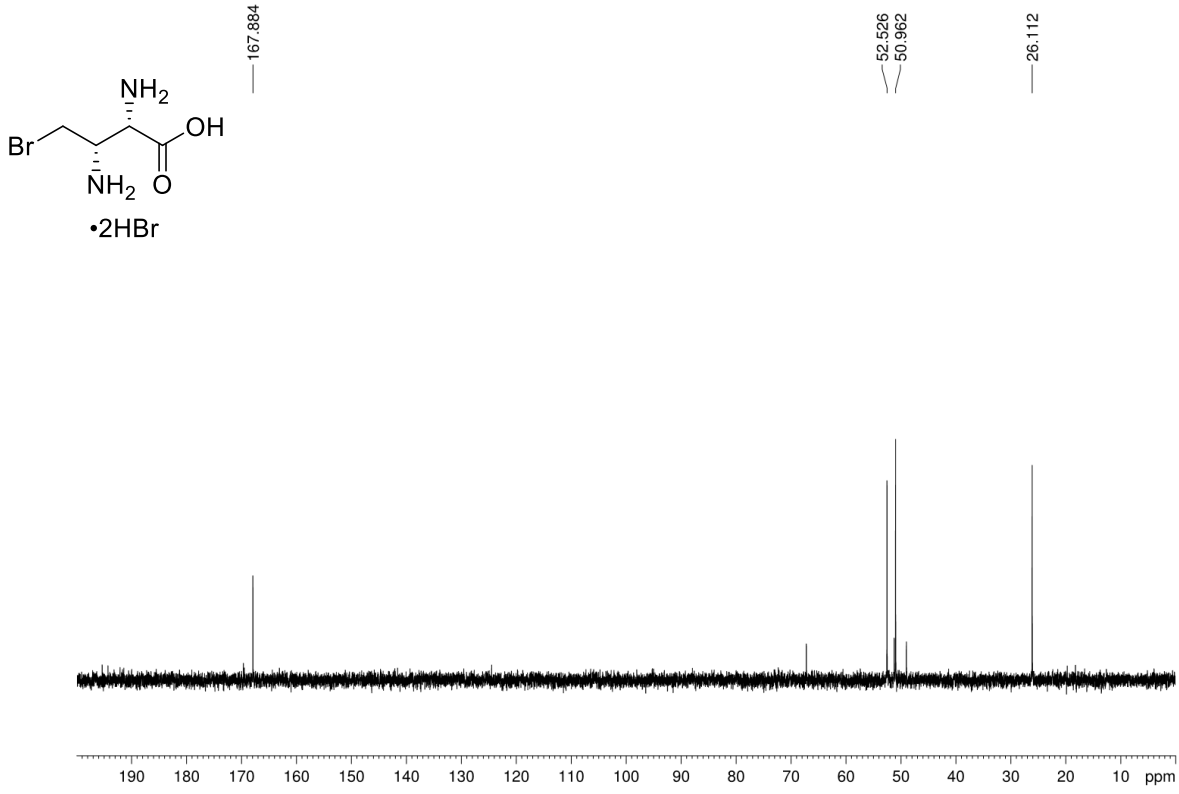

$^{13}$C-NMR (100 MHz, $D_2O$) spectrum for compound **40**



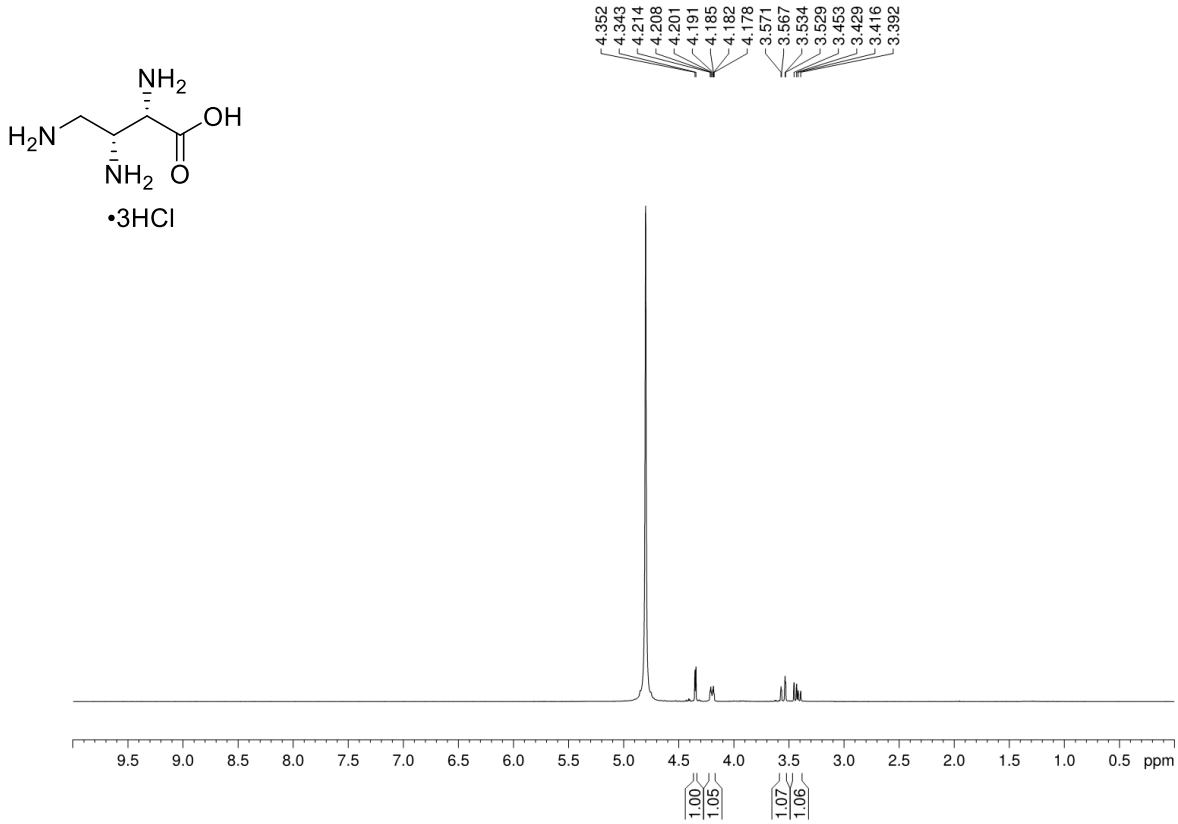
$^{1}$H-NMR (400 MHz, $D_2O$) spectrum for compound **41**



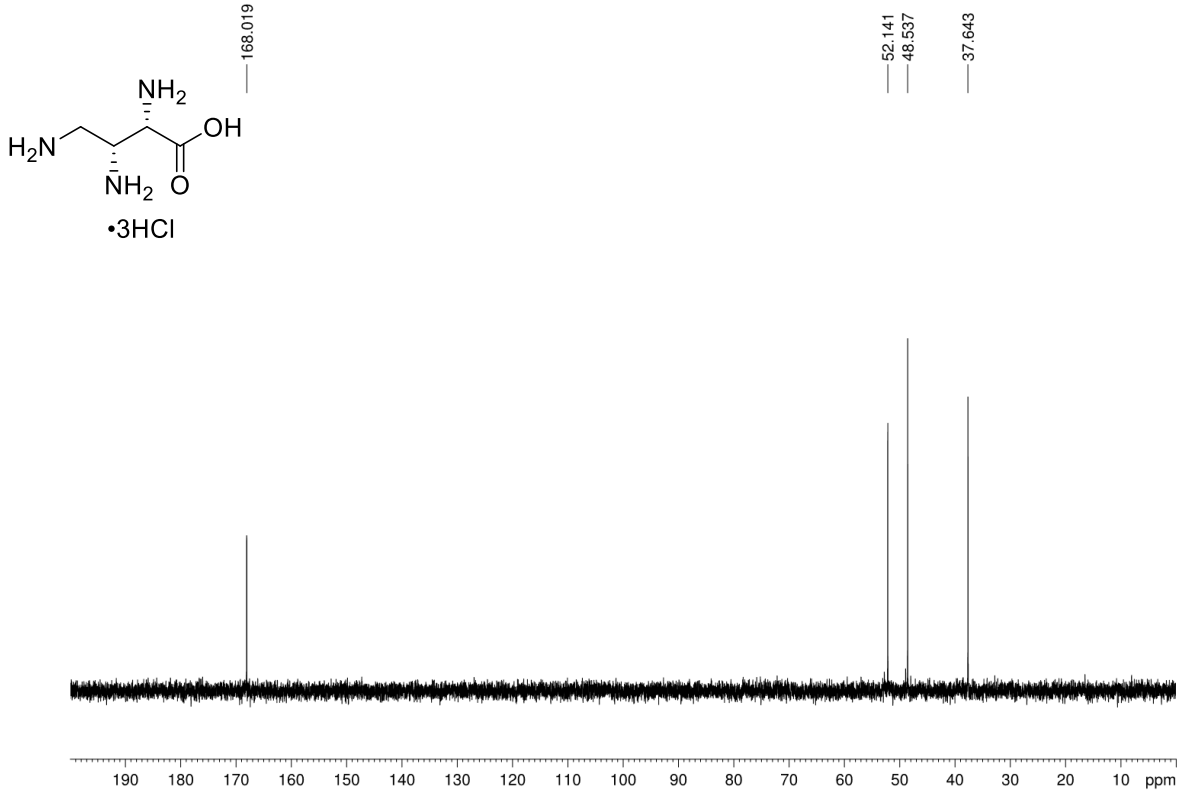

$^{13}$C-NMR (100 MHz, $D_2O$) spectrum for compound **41**



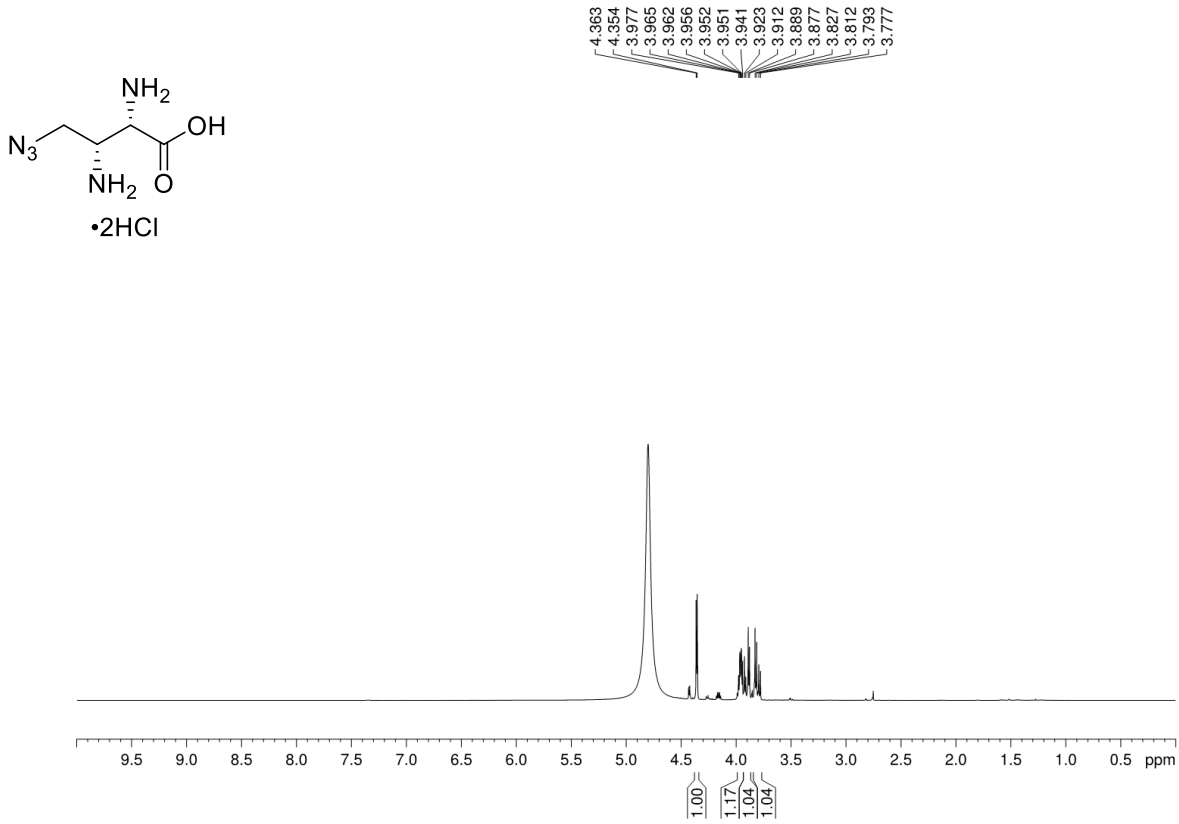
$^{1}$H-NMR (400 MHz, $D_2O$) spectrum for compound **42**



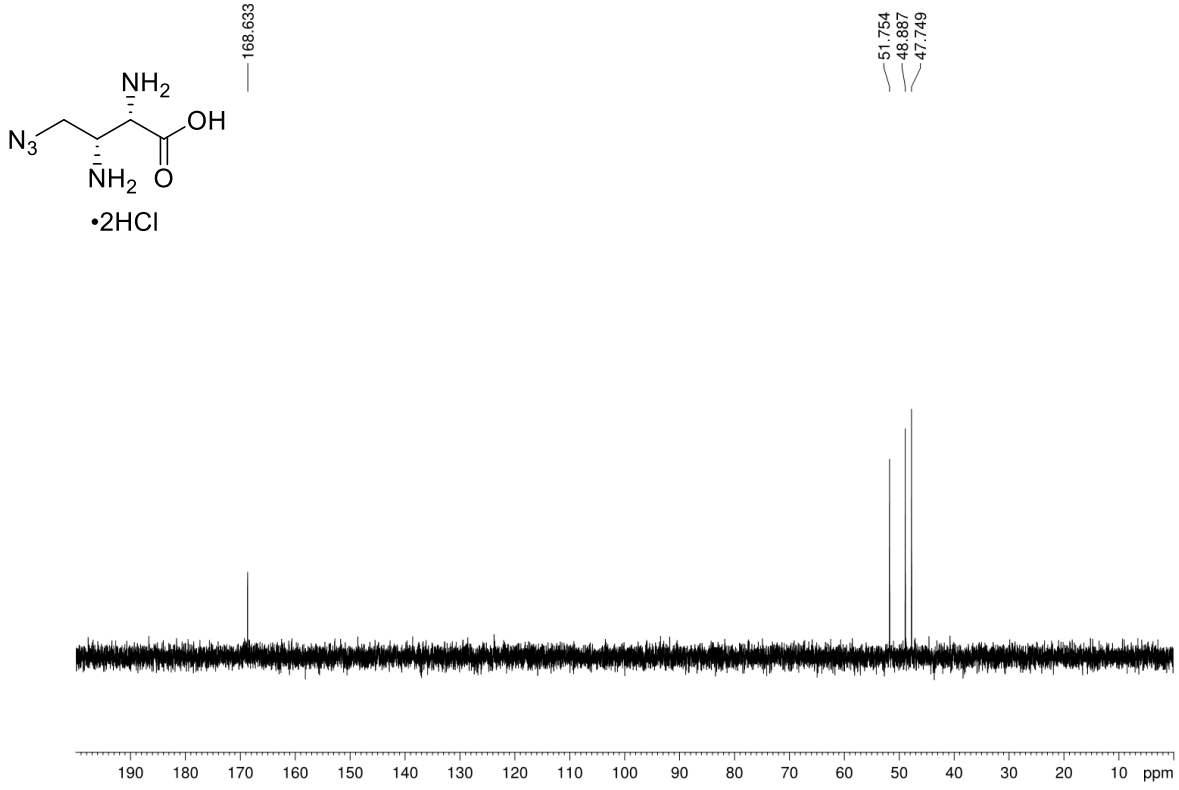

$^{13}$C-NMR (100 MHz, $D_2O$) spectrum for compound **42**



$^{1}$H-NMR (400 MHz, $D_2O$) spectrum for compound **43**




$^{13}$C-NMR (100 MHz, $D_2O$) spectrum for compound **43**



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