Supporting Information

Identification of Cyclopropane Formation in the Biosyntheses of Hormaomycins and Belactosins: Sequential Nitration and Cyclopropanation by Metalloenzymes

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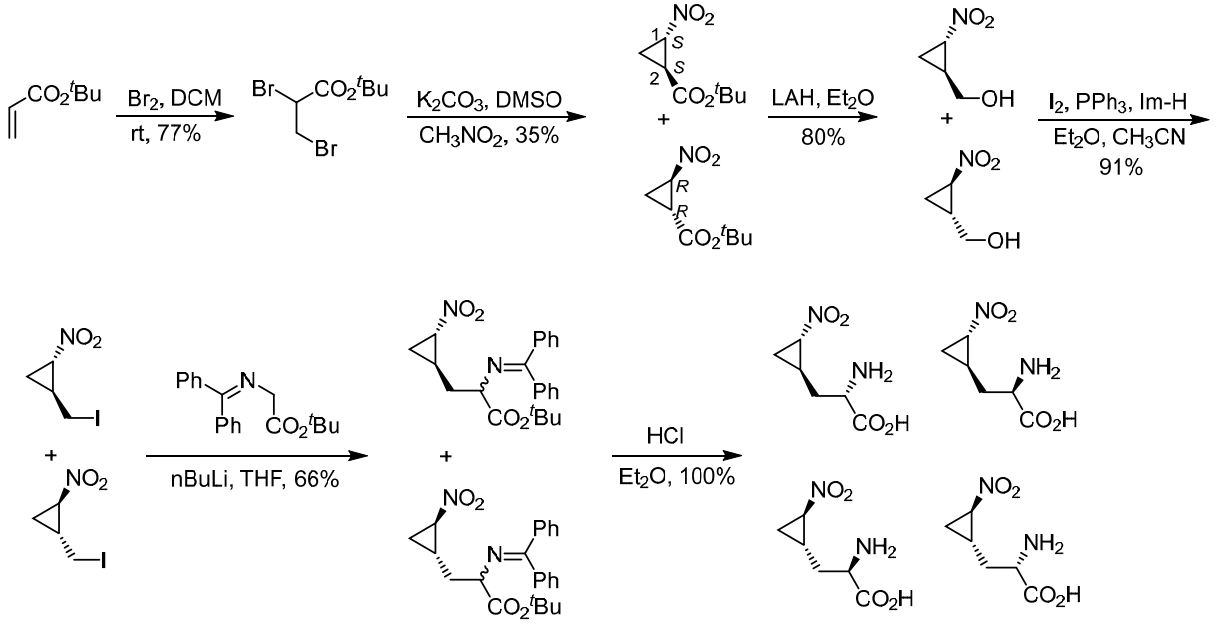
Methods

General chemical procedures

The chemical shift (δ) values are reported in ppm (parts per million) relative to the standard chemical shift for the proton residue peak and ^{13}C peak in the deuterated solvent ($CDCl_3$, D_2O or DMSO-d_6). The coupling constant (*J*) values are expressed in hertz (Hz). Thin-layer chromatography (TLC) was performed on silica gel plate. TLCs were visualized by illumination under UV light (254 nm) or by dipping the TLCs into a $KMnO_4$ solution followed by charring on a hot plate. Silica gel (230 – 400 mesh) was used for flash column chromatography. Evaporations were carried out under reduced pressure (water aspirator or vacuum pump) with the bath temperature below 50 °C unless specified otherwise. Materials obtained from commercial suppliers were used directly without further purification.

Preparation of 2-amino-3-((1S, 2S)-2-nitrocyclopropyl)propanoic acid and 2-amino-3-((1R, 2R)-2-nitrocyclopropyl)propanoic acid (3) [1, 2]



To a solution of *tert*-butyl acrylate (57 mL, 388 mmol, 1.0 equiv.) in dichloromethane (DCM, 300 mL) was added Br_2 (20 mL, 388 mmol, 1.0 equiv.) dropwise at 0 °C. After addition, the reaction mixture was warmed to room temperature and stirred for 22 h. Then the reaction mixture was diluted with DCM (200 mL) and quenched with 10% aq. $Na_2S_2O_3$ (200 mL) and extracted with DCM (2×100 mL). The combined organic layers were washed with brine (2×100 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by vacuum distillation to give *tert*-butyl-2,3-dibromopropionate as a yellow oil (87 g, 77%).[1]

To a solution of CH_3NO_2 (46.2 mL, 862 mmol, 1.08 equiv.) in dimethyl sulfoxide (DMSO, 840 mL) was added K_2CO_3 (319 g, 2.3 mol, 2.89 equiv.) at room temperature. After stirring for 20 mins, *tert*-butyl-2,3-dibromopropionate (230 g, 798 mmol, 1.0 equiv.) was added dropwise. After 72 h stirring, the reaction was quenched with ice water (250 mL) and then extracted with ethyl acetate (EtOAc, 2×200 mL). The combined organic layers were washed with brine (2×100 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by a flash column chromatography on silica gel (15% to 40% ethyl acetate/hexanes (EtOAc/Hex) (v/v)) to give a mixture of *tert*-butyl (*1S*, *2S*)-2-nitrocyclopropane-1-carboxylate and *tert*-butyl (*1R*, *2R*)-2-nitrocyclopropane-1-carboxylate as a yellow oil (53 g, 35%). In below, we will use "(*1S*, *2S*)/(*1R*, *2R*)" to represent the situation that product contains both two enantiomers "(*1S*, *2S*)" and "(*1R*, *2R*)". ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.50 (ddd, *J* = 7.3, 4.3, 2.7 Hz, 1H), 2.60 (ddd, *J* = 10.0, 7.1, 2.7 Hz, 1H), 1.96 (ddd, *J* = 10.1, 5.7, 4.3 Hz, 1H), 1.63 (td, *J* = 7.3, 5.7 Hz, 1H), 1.43 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.8, 82.6, 59.0, 27.8, 26.0, 17.0.[1]

To a solution of *tert*-butyl (*1S*, *2S*)/(*1R*, *2R*)-2-nitrocyclopropane-1-carboxylate (15.8 g, 84 mmol, 1.0 equiv.) in diethyl ether (Et_2O, 84 mL) was added $LiAlH_4$ (2.5 M solution in Et_2O, 11.6 mL, 46.4 mmol, 0.55 equiv.) at −10 °C. After addition, the reaction mixture was warmed to room temperature and kept stirring for 1.5 h. The reaction mixture was quenched sequentially with H_2O (1.89 mL), 15% aq. NaOH (1.89 mL), and H_2O (5.7 mL), and stirred for 1 h. The mixture was filtered and concentrated under reduced pressure. The residue was purified by a flash column chromatography on silica gel (20% to 30% EtOAc/Hex (v/v)) to give ((*1S*, *2S*)/(*1R*, *2R*)-2-nitrocyclopropyl)methanol as a colorless oil (7.9 g, 80%).^{1}H NMR (600 MHz, $CDCl_3$) δ 4.25 (dt, *J* = 6.9, 3.3 Hz, 1H), 3.79 (dd, *J* = 11.7, 4.8 Hz, 1H), 3.51 (dd, *J* = 11.7, 6.2 Hz, 1H), 2.31 (s, 1H), 2.28 – 2.17 (m, 1H), 1.83 (ddd, *J* = 10.2, 5.9, 3.5 Hz, 1H), 1.28 (td, *J* = 7.5, 5.9 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 60.9, 57.6, 27.1, 15.2.[1]

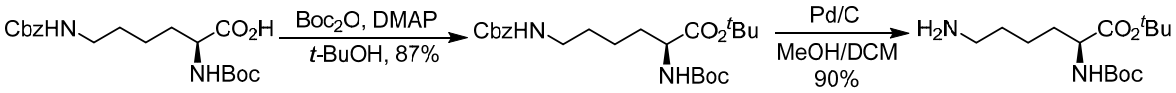
To a solution of ((*1S*, *2S*)/(*1R*, *2R*)-2-nitrocyclopropyl)methanol (210 mg, 1.79 mmol, 1.0 equiv.) in Et_2O/CH_3CN (acetonitrile) (3.9 mL/58 mL) was added imidazole (221 mg, 3.26 mmol, 1.8 equiv.) and triphenylphosphine (PPh_3, 610 mg, 2.32 mmol, 1.3 equiv.) at −5 °C. Iodine (I_2, 440 mg, 3.46 mmol, 1.9 equiv.) was then added in small portions over a period of 40 mins. The reaction mixture was warmed to room temperature and kept stirring for 3 h. It was quenched with 20% aq. $Na_2S_2O_3$ (20 mL) and extracted with EtOAc (2×20 mL). The combined organic layers were washed with brine (2×10 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by a flash column chromatography on silica gel (10% EtOAc/Hex (v/v)) to give (*1S*, *2S*)/(*1R*, *2R*)-1-(iodomethyl)-2-nitrocyclopropane as a light yellow oil (370 mg, 91%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.17 (ddd, *J* = 7.1, 3.8, 2.9 Hz, 1H), 3.31 – 3.00 (m, 2H), 2.47 – 2.40 (m, 1H), 2.07 (ddd, *J* = 10.2, 6.4, 3.8 Hz, 1H), 1.24 (q, *J* = 7.2 Hz, 1H).[2]

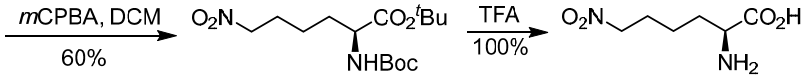
To a solution of *tert*-butyl 2-((diphenylmethylene)amino)acetate (481 mg, 1.63 mmol, 1.0 equiv.) in tetrahydrofuran (THF, 10 mL) was added *n*-BuLi (2.5 M solution in hexane, 0.65 mL, 1.63 mmol, 1.0 equiv.) at −78 °C. After stirring for 1 h, a THF solution (5 mL) of (*1S*, *2S*)/(*1R*, *2R*)-1-

(iodomethyl)-2-nitrocyclopropane (370 mg, 1.63 mmol, 1.0 equiv was added dropwise. The reaction mixture was warmed to room temperature and kept stirring for 13 h. The reaction mixture was quenched with saturated aq. NH_4Cl (10 mL) and extracted with EtOAc (2×10 mL). The combined organic layers were washed with brine (2×10 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by a flash column chromatography on silica gel (5% to 10% EtOAc/Hex (v/v)) to give *tert*-butyl 2-((diphenylmethylene)amino)-3-((*1S*, *2S*)/(*1R*, *2R*)-2-nitrocyclopropyl)propanoate as a colorless oil (427 mg, 66%). The reaction product, *tert*-butyl 2-((diphenylmethylene)amino)-3-((*1S*, *2S*)/(*1R*, *2R*)-2-nitrocyclopropyl) propanoate is a mixture of two set of diastereomers and can only be partially separated on ^1H and ^{13}C NMR spectra. ^1H NMR (600 MHz, $CDCl_3$) δ 7.74 – 7.58 (m, 2H), 7.51 – 7.43 (m, 3H), 7.42 – 7.37 (m, 1H), 7.36 – 7.32 (m, 2H), 7.21 – 7.00 (m, 2H), 4.22 – 3.82 (m, 2H), 2.18 – 1.65 (m, 4H), 1.45 (s, 9H), 1.15 – 0.99 (m, 1H); (Minor product) ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.2, 170.1, 138.9, 136.1, 130.5, 128.7, 128.6(2C), 128.0, 127.3, 81.5, 65.1, 59.3, 34.5, 27.9, 23.2, 18.6; (Major product) ^{13}C NMR (150 MHz, $CDCl_3$) δ171.1, 170.0, 139.0, 136.1, 130.4, 128.7,128.6, 128.5, 128.0, 127.6, 81.5, 65.1, 59.8, 34.6, 27.9, 23.2, 17.7.[1]

To a solution of *tert*-butyl 2-((diphenylmethylene)amino)-3-((*1S*, *2S*)/(*1R*, *2R*)-2-nitrocyclopropyl) propanoate (427 mg, 1.08 mmol, 1.0 equiv.) in Et_2O (10 mL) was added 1 M HCl (15 mL, 15 mmol, 14 equiv.) at room temperature. The reaction mixture was and stirred for 24 h and then diluted with Et_2O (10 mL) and H_2O (10 mL). The reaction mixture was extracted with Et_2O (2×10 mL). Then aqueous lawyer was concentrated under reduced pressure to give 2-amino-3-((*1S*, *2S*)/(*1R*, *2R*)-2-nitrocyclopropyl)propanoic acid as a white solid (188 mg, 100%). 2-amino-3-((*1S*, *2S*)/(*1R*, *2R*)-2-nitrocyclopropyl)propanoic acid gave a mixture of two set of diastereomers, these diastereomers cannot fully separated in ^1H NMR spectra, but are clearly distinguishable in ^{13}C NMR spectra. ^1H NMR (500 MHz, D_2O) δ 4.41 (dt, J = 6.6, 3.1 Hz, 1H), 4.19 (td, J = 6.1, 3.6 Hz, 1H), 2.30 – 1.83 (m, 4H), 1.36 (ddd, J = 13.3, 7.4, 5.8 Hz, 1H); (Minor product) ^{13}C NMR (150 MHz, D_2O) δ171.2, 59.3, 52.1, 30.8, 22.0, 18.1; (Major product) ^{13}C NMR (150 MHz, D_2O) δ 171.3, 59.2, 52.0, 30.6, 21.6, 18.0.[1] (^1H and ^{13}C NMR were recorded with 500 MHz and 600 MHz spectrometers, respectively)

Preparation of compounds (S)-2-amino-6-nitrohexanoic acid (6-NO_2-L-lysine) (2) [3]





To a solution of N^2-*tert*-butoxycarbonyl-N^6-benzyloxycarbonyl lysine (2.0 g, 5.26 mmol, 1.0 equiv.) in *tert*-butanol (*t*-BuOH, 10 mL) was added Boc_2O (1.68 mL, 7.36 mmol, 1.4 equiv.) and 4-dimethylaminopyridine (DMAP, 192 mg, 1.58 mmol, 0.3 equiv.) at room temperature. After

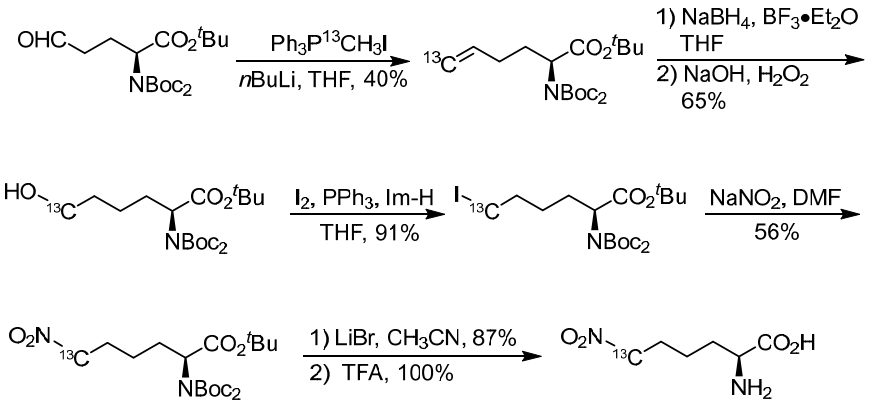
stirring for 21 h, the mixture was concentrated under reduced pressure and the crude product was purified by a flash column chromatography on silica gel (30% EtOAc/Hex (v/v)) to give *tert*-butyl N^6-((benzyloxy)carbonyl)-N^2-(tert-butoxycarbonyl)-L-lysinate as a colorless oil (2.0 g, 87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.26 (m, 5H), 5.19 – 5.01 (m, 3H), 5.01 – 4.89 (m, 1H), 4.13 (t, J = 5.8 Hz, 1H), 3.15 (t, J = 7.0 Hz, 2H), 1.80 – 1.70 (m, 1H), 1.63 – 1.46 (m, 4H), 1.43 (s, 9H), 1.41 (s, 9H), 1.36 – 1.26 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.8, 156.4, 155.4, 136.5, 128.4, 127.9, 81.7, 79.5, 66.5, 53.6, 40.6, 32.5, 29.3, 28.2, 27.9, 22.2.[3]

To a solution of N^6-((benzyloxy)carbonyl)-N^2-(tert-butoxycarbonyl)-L-lysinate (2.0 g, 4.58 mmol, 1.0 equiv.) in DCM/MeOH (methanol) (1:1, 20 mL) was added 10% Pd/C (200 mg) at room temperature, the mixture was subjected to hydrogenation under H_2 balloon for 25 h. The reaction mixture was filtered by celite and concentrated to give *tert*-butyl (*tert*-butoxycarbonyl)-L-lysinate as a colorless oil (1.24 g, 90%). ^{1}H NMR (500 MHz, methanol-d_4) δ 3.96 (dd, J = 9.3, 4.9 Hz, 1H), 2.92 (t, J = 7.3 Hz, 1H), 1.85 – 1.74 (m, 1H), 1.74 – 1.59 (m, 3H), 1.53 – 1.38 (m, 2H), 1.47 (s, 9H), 1.45 (s, 9H); ^{13}C NMR (150 MHz, methanol-d_4) δ 173.4, 158.1, 82.5, 80.4, 55.6, 40.5, 32.0, 28.7, 28.3, 28.0, 23.9.[3] (^{1}H and ^{13}C NMR were recorded with 500 MHz and 600 MHz spectrometers, respectively)

To a solution of *tert*-butyl (*tert*-butoxycarbonyl)-L-lysinate (640 mg, 2.11 mmol, 1.0 equiv.) in 1,2-dichloroethane (DCE, 50 mL) was added *m*-chloroperoxybenzoic acid (*m*CPBA, 2.0 g, 8.1 mmol, 4.0 equiv.) at room temperature. The reaction mixture was heated to reflux and stirred for 2 h. After cooling down, the mixture was diluted with DCM (20 mL) and H_2O (20 mL). The aqueous layer was extracted with DCM (2×20 mL). The combined organic layers were washed with 2 M aq. NaOH (2×10 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by a flash column chromatography on silica gel (10% to 15% EtOAc/Hex (v/v)) to give *tert*-butyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-6-nitrohexanoate as light yellow oil (420 mg, 60%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.07 (d, J = 8.3 Hz, 1H), 4.36 (td, J = 6.9, 2.9 Hz, 2H), 4.15 (q, J = 7.1 Hz, 1H), 2.09 – 1.94 (m, 2H), 1.85 – 1.76 (m, 1H), 1.70 – 1.58 (m, 1H), 1.51 – 1.43 (m, 1H), 1.44 (s, 9H), 1.43 – 1.37 (m, 1H), 1.41 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.4, 155.3, 82.1, 79.7, 75.2, 53.4, 32.2, 28.2, 27.9, 26.8, 21.9.[3]

tert-butyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-6-nitrohexanoate (200 mg, 0.60 mmol, 1.0 equiv.) was dissolved in trifluoroacetic acid (TFA, 7 mL) at room temperature. After stirring for 13 h. The reaction mixture was concentrated under reduced pressure to give (*S*)-2-amino-6-nitrohexanoic acid (**2**) as a light yellow solid (105 mg, 100%). ^{1}H NMR (500 MHz, D_2O) δ 4.50 (t, J = 6.8 Hz, 2H), 4.04 (t, J = 6.4 Hz, 1H), 2.05 – 1.84 (m, 4H), 1.57 – 1.35 (m, 2H); ^{13}C NMR (125 MHz, D_2O) δ 171.7, 75.0, 52.5, 29.0, 26.0, 21.1.[3]

Preparation of compounds [6-^{13}C](S)-2-amino-6-nitrohexanoic acid (6-^{13}C-2)[4]



To a solution of $^{13}CH_3PPh_3I$ (12.59 g, 31 mmol, 1.4 equiv.) in THF (220 mL) was added *n*-BuLi (2.5 M solution in hexane, 12.4 mL, 31 mmol, 1.4 equiv.) at −78 °C. The mixture was warmed to 0 °C and stirred for 1 h. Then, a solution of *tert*-butyl (*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-5-oxopentanoate[4] (8.8 g, 22 mmol, 1.0 equiv.) in THF (55 mL) was added to the resulting orange ylide solution at −78 °C. After stirring for 1 h at 0 °C, the reaction was quenched with saturated aq. NH_4Cl (50 mL). The mixture was diluted with H_2O (50 mL) and extracted with EtOAc (2×50 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by a flash column chromatography on silica gel (5% EtOAc/Hex (v/v)) to give [6-^{13}C]*tert*-butyl (*S*)-2-(bis(*tert*-butoxycarbonyl)amino)hex-5-enoate as a light yellow oil (3.41 g, 40%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.79 (ddt, J = 12.8, 10.1, 6.5 Hz, 1H), 5.15 (dd, J = 28.0, 13.6 Hz, 1H), 4.84 (dd, J = 32.2, 12.9 Hz, 1H), 4.71 (dd, J = 9.5, 4.8 Hz, 1H), 2.21 – 2.03 (m, 3H), 1.97 – 1.85 (m, 1H), 1.49 (s, 18H), 1.43 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8, 152.4, δ 137.6 (d, $J_{C\text{-}C}$ = 69.3 Hz), 115.2 (^{13}C enriched), 82.6, 81.1, 58.3, 30.5, 28.6 (d, $J_{C\text{-}C}$ = 3.8 Hz), 28.0, 27.9.[4]

To a solution of [6-^{13}C]*tert*-butyl (*S*)-2-(bis(*tert*-butoxycarbonyl)amino)hex-5-enoate (3.41 g, 8.8 mmol, 1.0 equiv.) in THF (44 mL) was added $NaBH_4$ (434 mg, 11.5 mmol, 1.3 equiv.) at 0 °C. After stirring for 10 mins at 0 °C, $BF_3{\cdot}Et_2O$ (1.42 mL, 11.5 mmol, 1.3 equiv.) was added. The mixture was warmed to room temperature and kept stirring for 16 h. The solution was cooled to 0 °C and 1 M NaOH (13 mL, 13.1 mmol, 1.5 equiv.) was added which was followed by addition of 30% H_2O_2 (10.8 mL). After stirring for 2 h at 0 °C, the reaction mixture was diluted with H_2O (30 mL) and extracted with EtOAc (2×20 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by a flash column chromatography on silica gel (20% to 30% EtOAc/Hex (v/v)) to give [6-^{13}C]*tert*-butyl (*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-6-hydroxyhexanoate as a colorless oil (2.32 g, 65%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.66 (dd, J = 9.5, 5.2 Hz, 1H), 3.72 (t, J = 6.4 Hz, 1H), 3.44 (t, J = 6.6 Hz, 1H), 2.12 – 1.93 (m, 2H), 1.82 (ddt, J = 14.4, 9.6, 7.6 Hz, 1H), 1.62 – 1.50 (m, 3H), 1.46 (s, 18H), 1.40 (s, 9H), 1.37 – 1.33 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.9, 152.4, 82.7, 81.1, 62.4 (^{13}C enriched), 58.7, 32.2 (d, $J_{C\text{-}C}$ = 37.2 Hz), 28.8 (d, $J_{C\text{-}C}$ = 4.3 Hz), 27.9,

27.8, 22.4.[4]

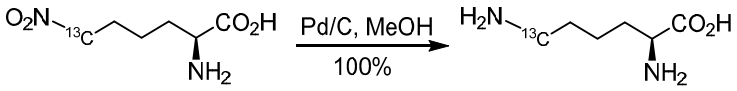
To a solution of [6-^{13}C]*tert*-butyl (*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-6-hydroxyhexanoate (2.32 g, 5.7 mmol, 1.0 equiv.) in THF (57 mL) was added imidazole (775 mg, 11.4 mmol, 2.0 equiv.) and PPh_3 (2.24 g, 8.55 mmol, 1.5 equiv.) at 0 °C. I_2 (2.16 g, 8.55 mmol, 1.5 equiv.) was added in small portions over a period of 15 mins. The reaction mixture was warmed to room temperature and stirred for 20 mins. It was quenched with 20% aq. $Na_2S_2O_3$ (30 mL) and extracted with EtOAc (2×20 mL). The combined organic layers were washed with brine (2×30 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by a flash column chromatography on silica gel (5% EtOAc/Hex (v/v)) to give [6-^{13}C]*tert*-butyl (*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-6-iodohexanoateas a light yellow oil (2.68 g, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.69 (dd, *J* = 9.6, 5.2 Hz, 1H), 3.31 (t, *J* = 7.0 Hz, 1H), 3.01 (t, *J* = 7.0 Hz, 1H), 2.15 – 1.94 (m, 1H), 1.94 – 1.74 (m, 3H), 1.50 (s, 18H), 1.46 – 1.39 (m, 2H),1.43 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.7, 152.4, 82.8, 81.2, 58.6, 33.0 (d, $J_{C\text{-}C}$ = 35.3 Hz), 28.1 (d, $J_{C\text{-}C}$ = 5.1 Hz), 28.0, 27.9, 27.3, <u>6.5</u> (^{13}C enriched).

To a solution of [6-^{13}C]*tert*-butyl (*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-6-iodohexanoateas (2.68 g, 5.2 mmol, 1.0 equiv.) in DMF (52 mL) was added $NaNO_2$ (2.87 g, 41.6 mmol, 8.0 equiv.) at room temperature. After stirring for 36 h at room temperature, the reaction mixture was diluted with H_2O (30 mL) and extracted with EtOAc (2×20 mL). The combined organic layers were washed with brine (2×30 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by a flash column chromatography on silica gel (5% EtOAc/Hex (v/v)) to give [6-^{13}C]*tert*-butyl (*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-6-nitrohexanoateas as a light yellow oil (1.26 g, 56%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.67 (dd, *J* = 9.5, 5.2 Hz, 1H), 4.47 (t, *J* = 7.0 Hz, 1H), 4.23 (t, *J* = 7.0 Hz, 1H), 2.13 – 1.93 (m, 3H), 1.93 – 1.77 (m, 1H), 1.48 (s, 18H), 1.44 – 1.37 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.5, 152.4, 82.9, 81.3, <u>75.3</u> (^{13}C enriched), 58.3, 28.4 (d, $J_{C\text{-}C}$ = 4.4 Hz), 27.9, 27.8, 26.9(d, $J_{C\text{-}C}$ = 34.9 Hz), 23.1.

To a solution of [6-^{13}C]*tert*-butyl (*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-6-nitrohexanoateas (1.26 g, 2.9 mmol, 1.0 equiv.) in CH_3CN (29 mL) was added LiBr (1.0 g, 11.6 mmol, 4.0 equiv.) at room temperature. The reaction mixture was heated to 65 °C and kept stirring for 19 h. The reaction mixture was cooled down and diluted with H_2O (30 mL), the aqueous was extracted with EtOAc (2×20 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by a flash column chromatography on silica gel (10% to 15% EtOAc/Hex (v/v)) to give [6-^{13}C]*tert*-butyl (*S*)-2-((*tert*-butoxycarbonyl)amino)- 6-nitrohexanoateas a light yellow oil (840 mg, 87%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.06 (d, *J* = 8.2 Hz, 1H), 4.48 (td, *J* = 6.9, 2.9 Hz, 1H), 4.24 (td, *J* = 6.9, 3.0 Hz, 1H), 4.16 (q, *J* = 7.2 Hz, 1H), 2.11 – 1.93 (m, 2H), 2.09 – 1.94 (m, 1H), 1.69 – 1.59 (m, 1H), 1.50 – 1.43 (m, 1H), 1.44 (s, 9H), 1.43 – 1.36 (m, 1H), 1.42 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.5, 155.3, 82.1, 79.7, <u>75.2</u> (^{13}C enriched), 53.4, 32.2 (d, $J_{C\text{-}C}$ = 4.4 Hz), 28.2, 27.9, 26.8 (d, $J_{C\text{-}C}$ = 35.2 Hz), 21.9.

[6-^{13}C]*tert*-butyl (*S*)-2-((*tert*-butoxycarbonyl)amino)- 6-nitrohexanoateas (433 mg, 1.1 mmol, 1.0 equiv.) was dissolved in TFA (7 mL) at room temperature. After stirring for 19 h. The reaction mixture was concentrated under reduced pressure to give [6-^{13}C](*S*)-2-amino-6-nitrohexanoic acid as a light yellow solid (178 mg, 100%). ^{1}H NMR (600 MHz, D_2O) δ 4.64 (t, *J* = 6.8 Hz, 1H), 4.39 (t, *J* = 6.8 Hz, 1H), 4.05 (t, *J* = 6.3 Hz, 1H), 2.06 – 1.88 (m, 4H), 1.57 – 1.39 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 171.7, 75.0 (^{13}C enriched), 52.5, 29.0 (d, $J_{C\text{-}C}$ = 4.7 Hz), 26.0 (d, $J_{C\text{-}C}$ = 34.9 Hz), 21.1.

Preparation of compounds [6-^{13}C]L-lysine (6-^{13}C-1)[5]



To a solution of [6-^{13}C](*S*)-2-amino-6-nitrohexanoic acid (40 mg, 0.23 mmol, 1.0 equiv.) in MeOH (10 mL) was added 10% Pd/C (8 mg) at room temperature, the mixture was hydrogenated at 50 psi for 10 h. The reaction mixture was filtered by celite and concentrated to give [6-^{13}C]L-lysine as a colorless oil (33 mg, 100%). ^{1}H NMR (600 MHz, D_2O) δ 3.67 (t, *J* = 6.1 Hz, 1H), 3.13 (t, *J* = 7.6 Hz, 1H), 2.89 (t, *J* = 7.6 Hz, 1H), 1.94 – 1.80 (m, 2H), 1.77 – 1.63 (m, 2H), 1.56 – 1.33 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 170.5, 54.6, 39.1 (^{13}C enriched), 30.5 (d, *J* = 3.3 Hz), 26.4 (d, $J_{C\text{-}C}$ = 35.4 Hz), 21.5.

General procedures for plasmid construction

Polymerase chain reaction (PCR) were carried out using a GeneAmp PCR System 9700 thermal cycler and Tks Gflex DNA polymerase (Takara Bio). Oligonucleotide synthesis and DNA sequencing were performed in Fasmac (Kanagawa, Japan). General genetic manipulations of *E. coli* were performed according to standard protocols. The belactosin-producing bacterium *Streptomyces* sp. KY11780 was kindly provided by Kyowa Hakko Bio Co., Ltd.

Plasmid construction for over-expression of BelK and BelL

The *belK* was amplified by PCR using *Streptomyces* sp. KY11780 genomic DNA as a template with the primer pair 5′- AGCTACATATGACGACCGAGAGGAATCTCG-3′ and 5′-TATAAGCTTCAGGCGGGCAGGGGCTGC-3′. The forward primer 5′- TATATCATATGACTCT CAACGCGCAGC-3′, and the reverse primer, 5′- TATAAAGCTTCATCCGCGGCCCTCGGC-3′, were used in PCR amplification of the *belL* gene. Restriction sites of *Nde*I and *Hin*dIII (underlined) were introduced at the N- and C-terminal regions. The PCR product was cloned into the *Nde*I-*Hin*dIII site of pET28b vector to construct the plasmids.

DNA construct for over-expression of HrmI, HrmJ, WP_052441272 and WP_042425400

The DNA sequences encode the corresponding genes were codon-optimized for over-expression in *E. coli*, synthesized, and inserted into the *Nde*I and *Bam*HI restriction sites of expression vector pET-28a. The codon-optimized gene sequences are shown below.

hrmI

ATGATCCCAGAGAGCTTCAAGATCGACCGCAGCGTGGTAGAGGAATTTCTGGCGCTG
GACCCTGACGCATGGGAACGTCTGAATGCTGACTACACGGCACGTCGCCGTATCGGT
GAAGCATGTCGTGCACTGTCTCGTCATGCATTCGTAGAAGAGGACCCTAGCGCTCTG
GAGGAGCTGCACGATGTACTGGCACTGATCTACCAACAGGACTTCTCCGGTGCTCCA
GTAGAGCTGCTGGGTTGCGAAACTCAGCCAGTTCTGCGTGACATTGCAGCAATCCTG
GAGGGTGCAGTTCTGGCTGCTGAACTGGACTCTATCAGCGAAGAACAGATCAGCGCC
TACCCGCGTTCTGGTAAAGAATACGTGCATTGGCTGAAACGCGTGATCGGCGAACAC
CCGGCAGCTGGTCATCCGTTCTACCGTGACTTCGTGCCGACTCGTGCAACCGAAGGT
GATTTCCGTTTCTACCTGGCTCAGGAAACCAACCTGGACCCGAAATTCGACGACATCC
TGGCGTTCATGCAGATCGGCGCTGCTCCGGATGAAAAGATGGAAATCGCGGGCAACT
ACTGGGACGAAATGGGCAACGGCAAACCGGCTGAAGTCCACACCGCTATGTTCGCTC
ACGCCCTGGATGCGCTGGATGTTAACGACGACTACATCCGTCGTAACCTGCTGCCGGA
AGCGAAAGCGTCTGGTAACCTGGCGTCTTGCCTGGCGATCTCTCGTCGTCATTATTAT
AAATCCGTCGGCTTTTTTGGCGTCACCGAATATCTGGTGCCGCGCCGCTTTAAACTGG
TTGTTGATCGCTGGGCCGATATTGGCCTGCCGCGCGAAGGTATCGCCTATCACGATGC
CCACATTTCCATTGATGCGGTTCACGCGAGCGGCTGGTTCAAAAATGTTATTGCGCCG
GCGGTTGATCGTGATCCGCGCGTTGGTCGCGAAATTGCCGTGGGTGCCCTGATTCGTC
TGAACTCCTCCCAGCGTTATCTGGATTCCCTGCTGATGCACCTGCACCACGATTCTGC
GGCGCATACCTCTTAAGGATCC

hrmJ

ATGCCACTGAACGACCGTGGTTACTCCATCATCGACCTGCCTGAGGTCACTCCTGAGG
TACGTGAGAGCTTCGGTGACCTGAAGTTCGACGAGTACATGGGTGACAACCGCTACC
GTCGTTTCGCACAGTTCCGCATGCACTGGAGCGGTGAGTCTTGGGAACTGGAACGTC
TGGAACACCGTCCGTACGTAACCTTCTCCAAATTCAACCCGGTCGCGGGTGGTATCCG
TCGTCACTATGAACCGATCCTGGCCGACTTCTCCCCACACATCCGTGCAGGTGCAGAA
GGTGTACCGCTGGATACTGCACGTGACTGGCAAGTTAACGTGCACCAGTTTCGCATC
ATTGCCAAACGCCAGGAAACCAGCGGCGTGATCGTGCCGGAAGGTCCGCATTCTGAC
GGCCGTGATTTCGTTCTGATCGCTGTGTTCTCTCGTCACCAGATTACGGGCGCTGAAA
TGACCCTGATGCCGCACGGTGGTGAAGGCGAACCGTTTTTCCGTGCTACTGTTCCGG
CGGGCCAGGGCGCGCTGCTGGCGGATCGCGAAATGTTTCATAATGTTACCGAAATTG
AACCGGTTGGCGATTATGGCCACCGCGATACCCTGATTGTTACCTGGGTTCCGTGGGA
AGATAAATGGCATGGCGACGATTTTGAACAGCGCGCTCTGGCGGAAGGCTAAGGATC
C

WP_052441272

ATGGCGGGTTTCACCGCGGCTCTGACCTCTGTTACCTTCGAAAAATGGCAGGAACTG
GTTGACGATTACACCTACCGTGCTGAACTGGCTCGTGAATGCCGTCGCCTGGCTGACC
TGGCGTTCCACGGTCGTGACGCGGCGGCGCGTGCTCACCTGCACGAAGTTCTGGTTG
TTGTGTACGCCCACGAATTTTCCCAGTCCGCTGCGCGTCGTCCGGACCAGGACCTCC
AGCCGGTTCTGCGCGACGTTACCTCCATCCTGGAAAACGCTATGCTGGATCACGAATT
TCGTCAGGTTCCGGAAGAATCTCTGTCTGGTTACCCGACTGGTGAAAAAGAATACGT

TCGTTGGCTGAAAGCGGTAATCCAGGACCACCCGGCGTCTGCACACCCGCTGTACTG
TGAACACCTCGCGAACTCTGCGACCGTGGAAGATATCCGTCTGCTGCTGGCGCAGGA
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TGGCGCAGAGAAAATGGAAATCGCTTCTAACTACTGGGACGAAATGGGTAACGGTGA
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CAGGATTACGTTGAAACTAACCTGCTGCCGCACTCTAAAGAATGTGGTAACATCAGC
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ACTGAGTTCCTGGCTCCGCGTCGTTTCCGCCAGCTGGTGACCGCGTGGGACCGTCTG
CGTCTGCCGCCGGAAGGTAAAATCTACCACGACATCCACATCGGTGTTGACGCGCAG
CACGCGGCAGGTTGGTACAAAAACGTTATCGGTCCGGTTGTTGAACGTGACCCGGCA
GCGGGCCGTGAAGTTGCACTGGGTACTCTGGTTCGTCTGAACACCTCTGCTCGTTAC
CTGGATCAGGTTCTGGAAGCGTGCCTGAAACAGCCGGTTCCGGCT

WP_042425400

ATGACCCTGAACGCGCAGGGTTTCCAAATCTTCGACCTGCCGGAAACTGGTGAAGAT
GTTCTGAAATCTTTCGACGATCTGGCTTTCGACGAATACATCGGTAACGGTAACCGTT
GGCGTCGTTTCTCTCAGTACCGTCTGGAACACCGTGACGGTGACTGGGAATTTGAAC
GTCTGCCGCACCGTCCGTACGTTACCTACTCTAAATTCAACCCGATCGCTGGCGGTAT
CCGTCGTCACTACCAGCCGATCGAAGTTGACCTGGTTGAACACATCCGTGAAGCGTG
CGCGCAGATCCCGCTGCCGGAAGATGACGTTTGGCAGATCAACGTTCACCAGTACCG
TGTTATCGCGAACAAAGAACTTCAGGGTGTTGTGGTTCCGGAAGGTGTTCACCAGGA
CGGTCACGAGTTCGTTGTTATCTCTGTTTACAACCGTGCTGGTATCACCGGCGCGGAA
CTGACCCTGCGTGCGGCGGACGACAAAGAAACCCCGATCTTCACCGCAACCCTGCC
GGCGGGTCAGGCGATCGCGTTCGACGACCGTGCGCTGTGGCACTACGTTACCGACAT
CGTTCCGGTTGAAGAAGAAGGTCACCGTGACATCACCGTTGTTTCTTTCTCTCGTTGG
AACGAACGTTGGTACGGTGACGCTTTCGAAGAAGATGCTATCGGTGAAGGTCGTGGT

In vivo characterization of hrmI*/*belK* and *hrmJ*/*belL

The plasmid encoding the desired genes described above were individually introduced into *E. coli* BL21 (DE3) cells (New England Biolabs, MA). A liquid culture of the transformant in Lysogeny Broth (LB, 50 mL) supplied with kanamycin (30 μg/mL) was added IPTG (0.5 mM) at $O.D._{600}$ ~ 0.6 to induce protein expression. The cultivation was continued for an additional 17 h at 16°C. Cells were harvested, washed with water two times, and resuspended with water (10 mL). After addition of L-lysine (2.5 mg/mL) into cell suspension, the samples were incubated for 6 h at 37 °C. The cells were then removed by centrifugation and the resulting supernatant was analyzed by Liquid chromatography–mass spectrometry (LC-MS) (Waters ACQUITY UPLC system equipped with a SQ Detector2) under the following conditions: column: Scherzo SS-C18 (Imtakt, Japan, 150 × 2.0 mm, 3 μm); column temperature, 40°C; detection, ESI-positive mode; mobile phase, A: water with 0.1% formic acid, B: 100 mM aq. ammonium acetate/acetonitrile = 60/40, 20% solvent B for 0–3 min and a linear gradient to 95% solvent B for an additional 22 min; flow rate, 0.2 mL/min.

Overexpression and Purification of the target proteins

The above transformants were cultured in Terrific Broth (TB) growth medium with 50 μg/mL kanamycin at 37 °C with shaking at 220 rpm. Upon reaching an OD_{600} of ~ 0.6, IPTG with final concentration of 0.5 mM was added to the cell culture at 18 °C. The cell culture was kept shaking at 220 rpm for 14–16 h. The cells were then harvested by centrifugation at 10 °C and the cell pellets were stored at −80 °C before use. During purification, the cells were thawed and suspended in an ice-chilled buffer with 100 mM Tris-HCl (pH 7.5) and lysed by sonication. The lysate was subjected to centrifugation for 40 minutes at 20,000 ×*g* at 8 °C. The resulting supernatant was loaded onto a Ni-NTA agarose column. The column was washed with 5 volumes of buffer containing 100 mM Tris-HCl and 5 mM imidazole (pH 7.5). The desired protein was then eluted using buffer containing 100 mM Tris-HCl and 250 mM imidazole (pH 7.5). Fractions containing the His-tagged proteins were concentrated to a concentration using Pall® centrifugal 10K filter. The protein solution was then dialyzed against 2 L of buffer (100 mM Tris-HCl, pH 7.5) twice. The purity of protein was shown by SDS-PAGE gel (Figure S3). Protein concentration was determined by UV absorption at 280 nm $^{-1}$ (http://ca.expasy.org). The iron load of purified HrmI and BelK were determined to be 24% and 20%, respectively, by colorimetric ferrozine assay detected at 562 nm.[6] The protein was aliquoted and frozen in −80 °C.

Using LC-MS and ^{13}C-NMR to monitor the enzymatic reactions

LC-MS was conducted on an Agilent Technologies (Santa Clara, CA) 1200 system coupled to an Agilent Technologies 6120 quadrupole mass spectrometer. The associated Agilent MassHunter and OpenLAB software packages were used for data collection and analysis. Assay mixtures were separated on an Agilent InfinityLab Poroshell 120 HILIC column (4.6 x 50 mm, 4.0 μm particle size) with isocratic eluent system of 40% of solvent A (water with 0.1% (v/v) of formic acid) and 60% solvent B (acetonitrile), and with flow rate of 0.4 mL/min. Detection was performed using electrospray ionization in positive mode (ESI^+). The drying gas temperature was 350 °C with a nebulizer pressure of 60 psi and flow rate of 12 L/min. The capillary voltage was set to 3000 V. The fragmentor voltage was set at 130 V.

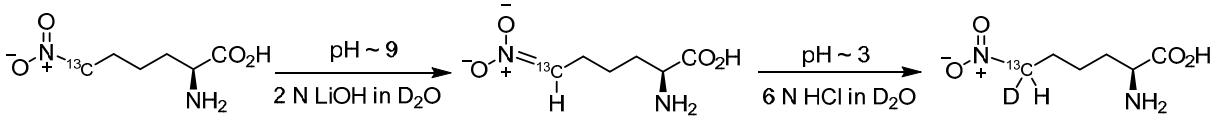
Reactions associated with HrmI, BelK and WP_052441272 were performed as followed. Reaction containing 0.25 mM enzyme, 1.0 mM of the substrate, and 5.0 mM of ascorbate in a total volume of 200 μL (100 mM Tris-HCl, pH 7.5), was initiated by addition of ascorbate to a solution containing enzyme and substrate. After 12 h incubation at 4 °C, the reactions were halted by adding equal volume of acetonitrile. For the reactions associated with HrmJ, BelL and WP_042425400, the reactions containing enzyme substrate and α-KG (α-ketoglutarate) with the final concentration of 0.20 mM enzyme, 1.0 mM of the substrate, and 2.0 mM of α-KG in a total volume of 200 μL (100 mM Tris, pH 7.5) After 12 h at 4 °C, the reactions were quenched using the same method described above. Reactions of HrmI+J, BelK+L and WP_052441272+ WP_042425400 were conducted in a similar manner with the final concentrations of 0.25 mM enzyme, 1.0 mM of the substrate, 5.0 mM of ascorbate and 2.0 mM of α-KG.

For the ^{18}O-isotope experiments, the ^{18}O-gas (98%) and ^{18}O-water (99%) were purchased from

Cambridge Isotope Laboratories. In the ^{18}O gas experiments, ^{18}O gas were purged into the reactions prepared in the glove box. For the ^{18}O-water experiments, ^{18}O-water was mixed with 2 M Tris-HCl stock solution to make 100 mM Tris-HCl in 90% ^{18}O-water. After adding all reagents, the ^{18}O content was ~ 50%.

For those reactions associated with ^{13}C-NMR, the reactions were prepared in a similar manner except that the final volume of each reaction is 550 µL. After the reaction, 30 µL of DMSO-d_6 was added to the reactions. The ^{13}C NMR spectra were recorded using Bruker Avance NEO 700 MHz spectrometers.

In situ ^{13}C-NMR experiments to monitor the C=N formation upon pH change

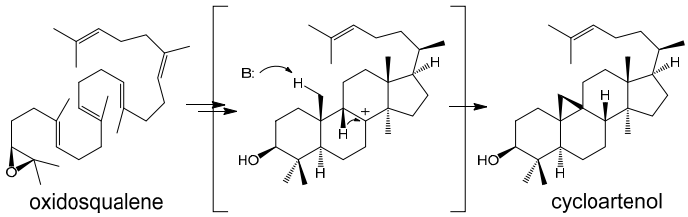


A solution of [6-^{13}C](*S*)-2-amino-6-nitrohexanoic acid (200 mM in D_2O, 50 µL) and D_2O (450 µL) was placed in a NMR tube. After recording the initial NMR spectrum, 2 M LiOH (15 µL, D_2O) was added to the reaction. The resulting mixture (pH ~ 7) was subjected to NMR. Subsequently, another 15 µL of 2 M LiOH was added to the reaction (pH ~ 9). After addition, another NMR spectrum was recorded. The reaction was then acidified using 6 M HCl (7 µL, D_2O) (pH ~ 3) and the NMR spectrum was taken (**Figure 3b)**. The ^{13}C NMR spectra were recorded using Bruker NEO 700 MHz spectrometer under both C–H decoupling and C–H coupling modes.

Supporting Figures

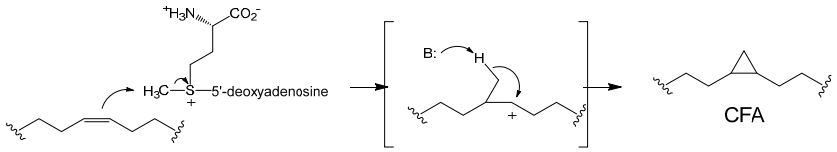
cyclopropanation via cation intermediate

cycloartenol synthase



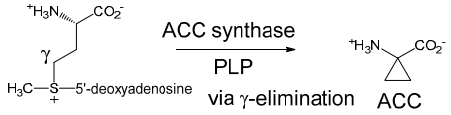
***S*-adenosylmethionine (SAM)-dependent cyclopropanation**

cyclopropane fatty acid (CFA) synthase



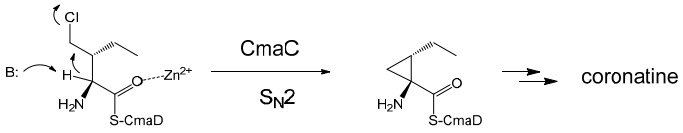
PLP-dependent cyclopropanation

1-aminocyclopropane-1-carboxylate (ACC) synthase



chlorination dependent cyclopropanation

CmaC



radical SAM-dependent cyclopropanation

C10P (radical SAM enzyme) + C10Q

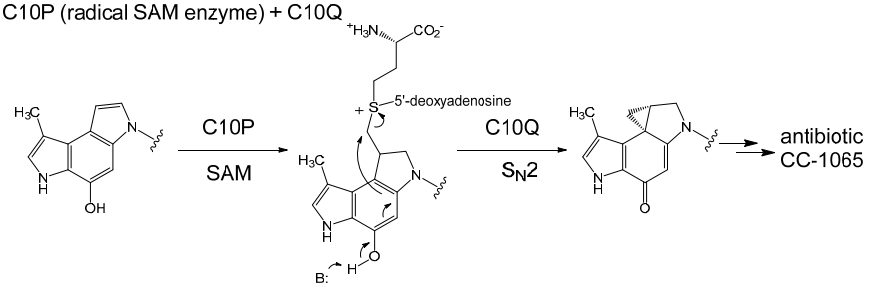


Figure S1. Representative examples of known cyclopropane formations.

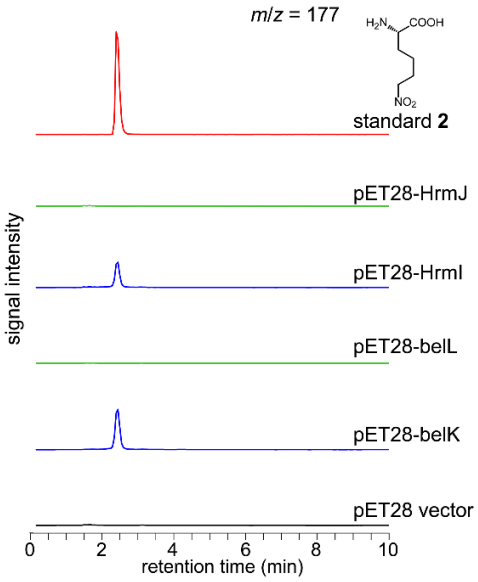


Figure S2. *In vivo* analysis of *hrmI*, *hrmJ*, *belK*, and *belL* reactions monitored with LC-MS.

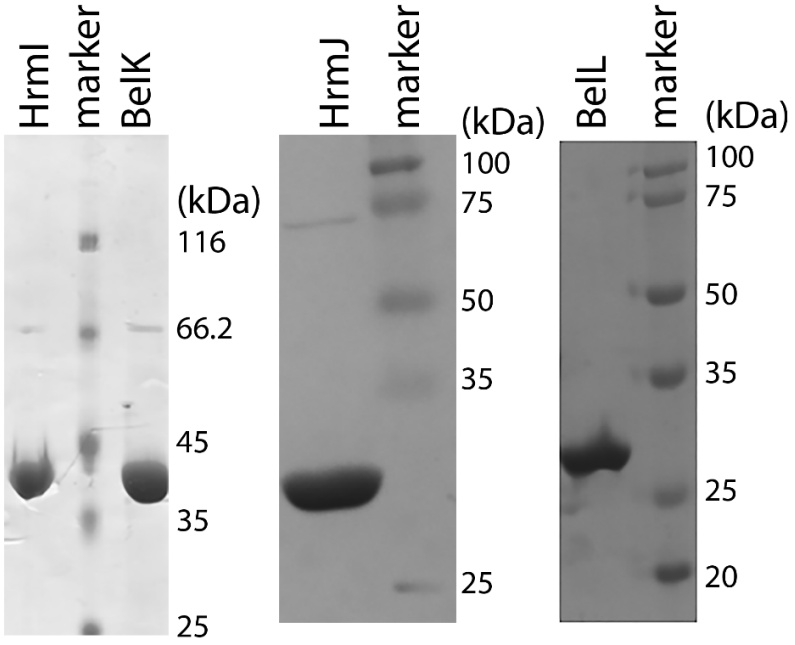


Figure S3. SDS-PAGE analysis of purified HrmI (41.3 kDa), BelK (42.4 kDa), HrmJ (28.2 kDa), and BelL (28.5 kDa).

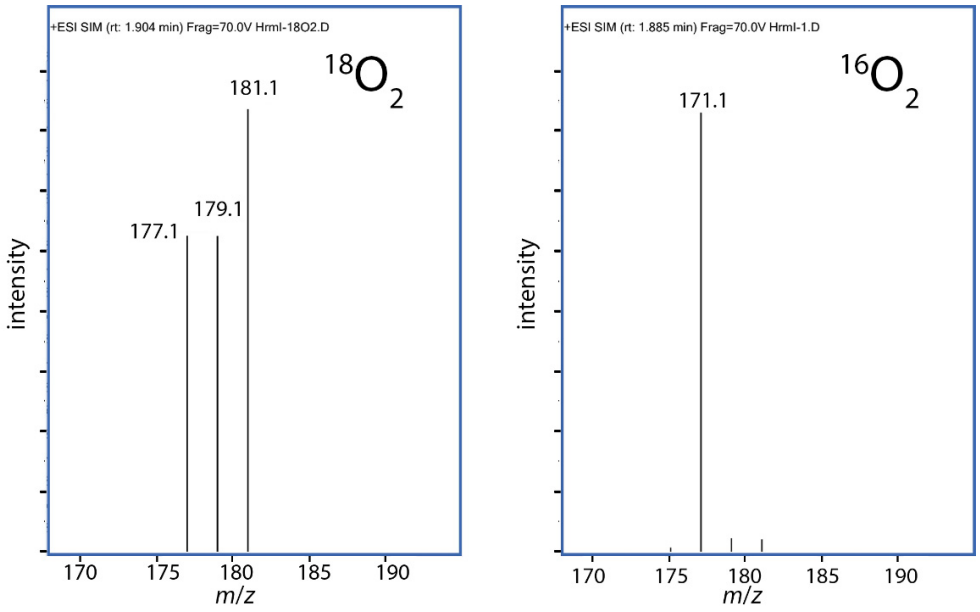


Figure S4. Mass spectra of HrmI product (**2**, $[M+H]^+$) with $^{18}O_2$ (left) and unlabeled O_2 (right).

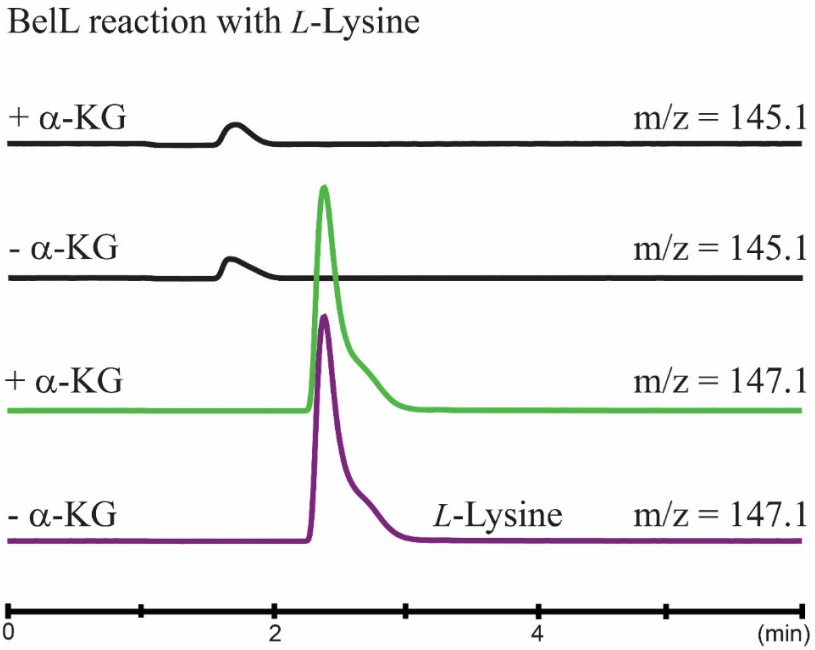


Figure S5. LC-MS chromatograms of BelL reactions using L-lysine (**1**) as the substrate. In comparison with the controlled reaction (without α-KG), no obvious peak with *m/z* value of −2 (147.1 → 145.1) can be detected. The peak which elutes at ~1.7 min with an *m/z* = 145.1 is unlikely associated with the α-KG-dependent reactivity because it was also observed in the absence of α-KG.

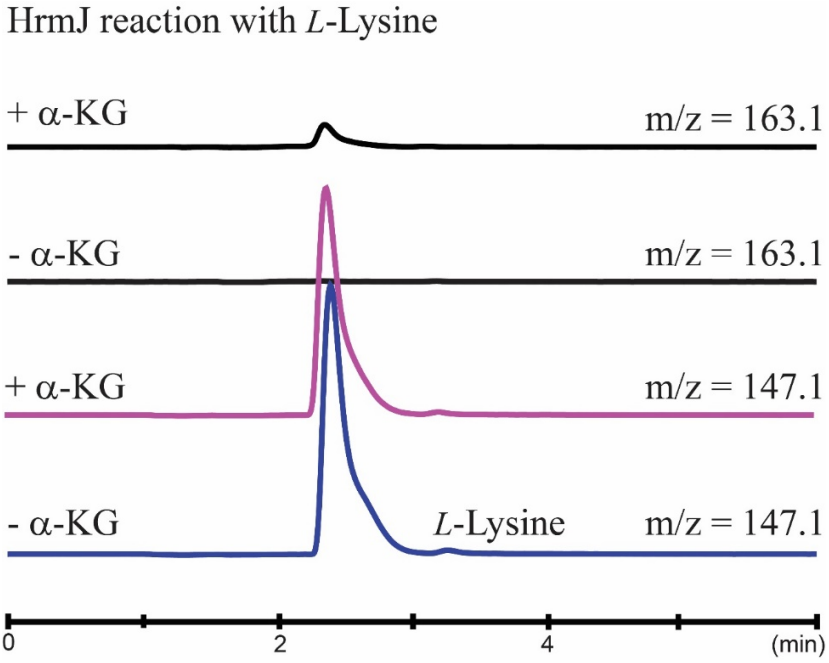


Figure S6. LC-MS chromatograms of HrmJ reactions using L-lysine (**1**) as the substrate. An obvious peak with *m/z* value of +16 (147.1 → 163.1) was detected in the presence of α-KG.

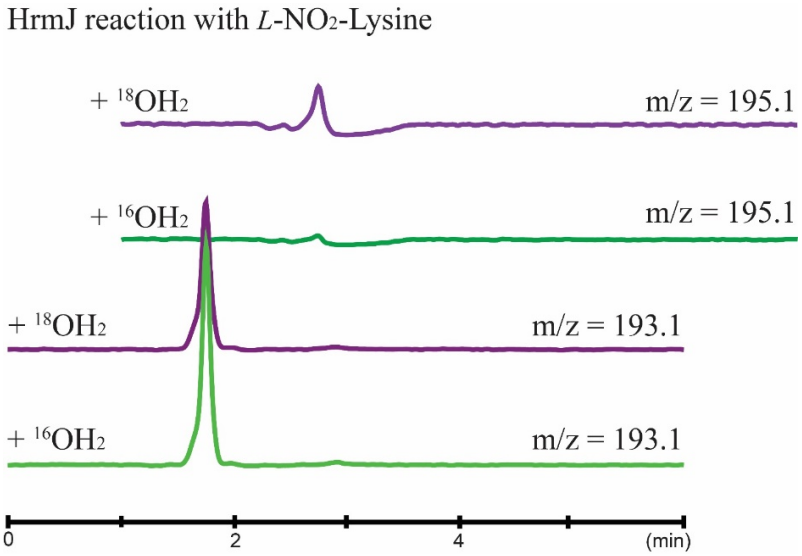


Figure S7. LC-MS chromatograms of HrmJ reactions using L-6-nitronorleucine (**2**) as the substrate. Formation of a peak with *m/z* value of +16 (177.1 → 193.1) can be detected in the presence of α-KG. Under ^{18}O-water conditions, this peak has *m/z* values of 193.1 and 195.1, respectively.

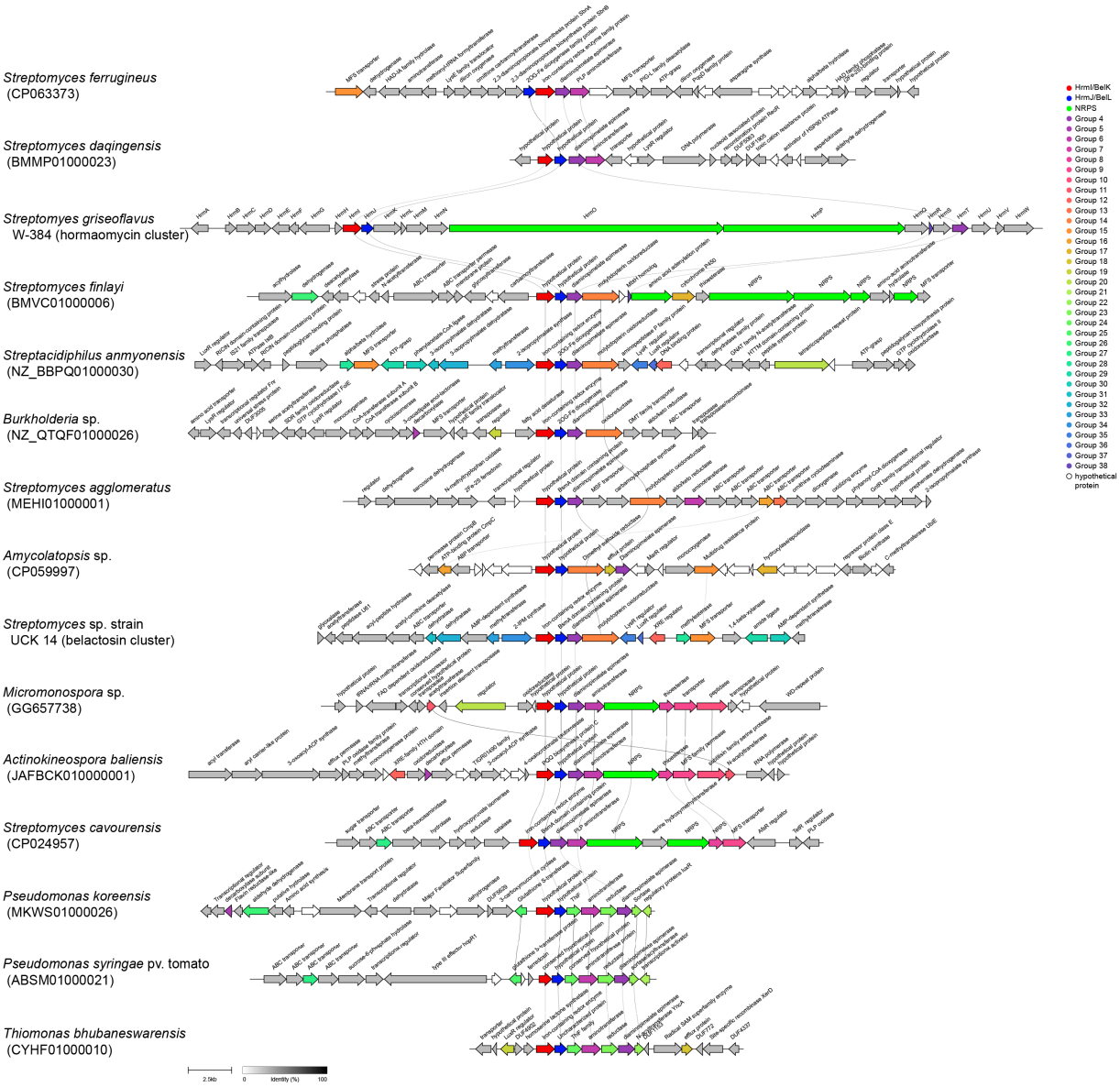


Figure S8. Putative gene clusters containing both HrmI and HrmJ homologs. Graphics was generated with clinker (https://github.com/gamcil/clinker).

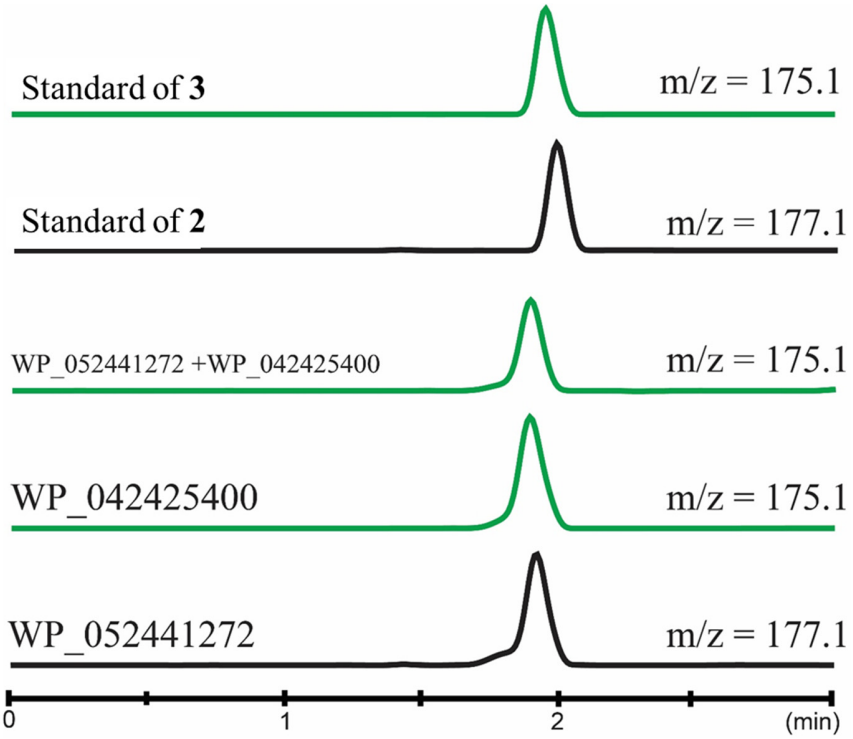


Figure S9. LC-MS chromatograms of WP_052441272 (HrmI ortholog), WP_042425400 (HrmJ ortholog) and WP_052441272+ WP_042425400 reactions using **1** or **2** as the substrate. Formation of **2** was detected in the reaction using WP_052441272. In addition, **3** was detected in the reactions of WP_042425400 and WP_052441272+ WP_042425400.

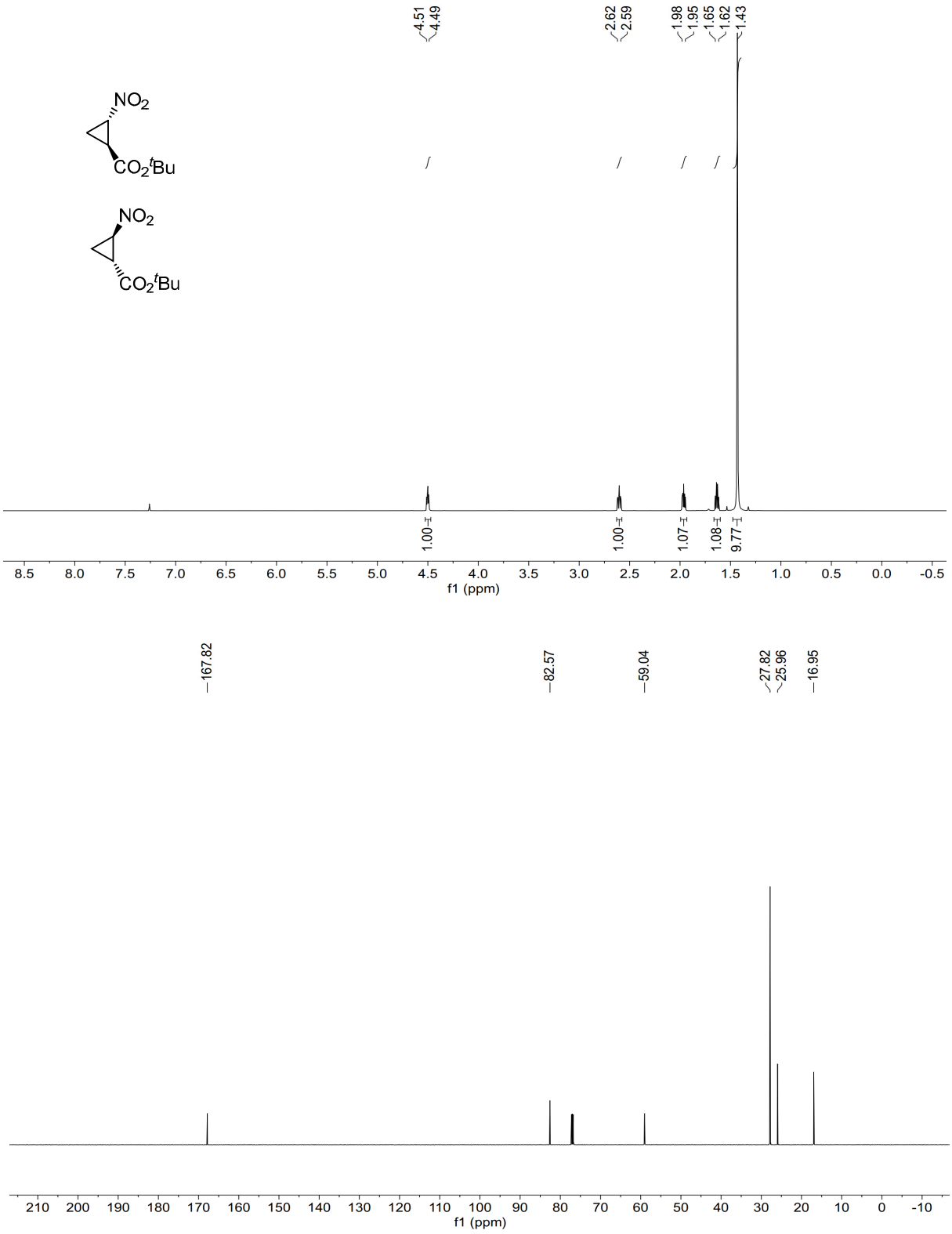


Figure S10. ^{1}H and ^{13}C NMR of *tert*-butyl (*1S*, *2S*)/(*1R*, *2R*)-2-nitrocyclopropane-1-carboxylate.

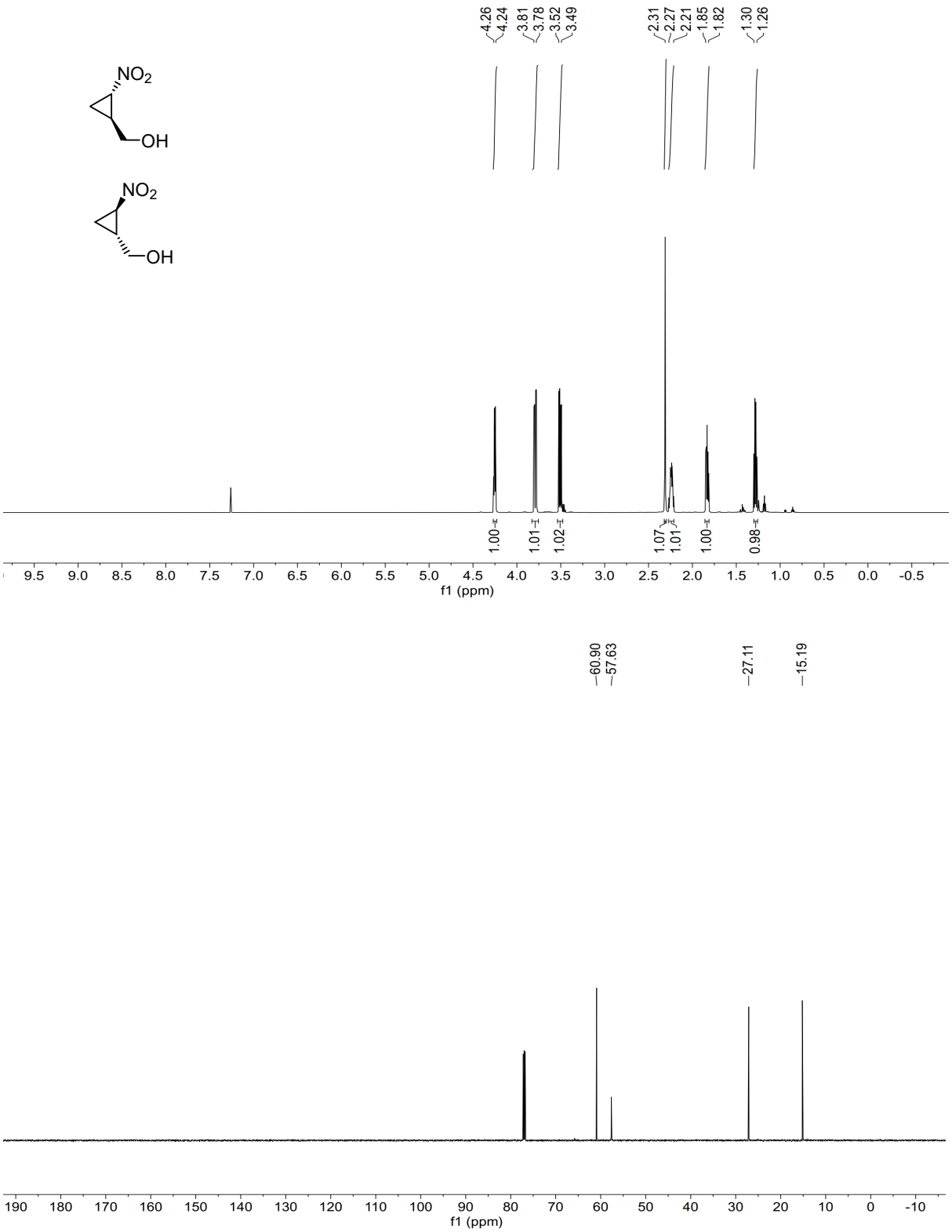


Figure S11. ^{1}H and ^{13}C NMR of ((*1S*, *2S*)/(*1R*, *2R*)-2-nitrocyclopropyl)methanol.

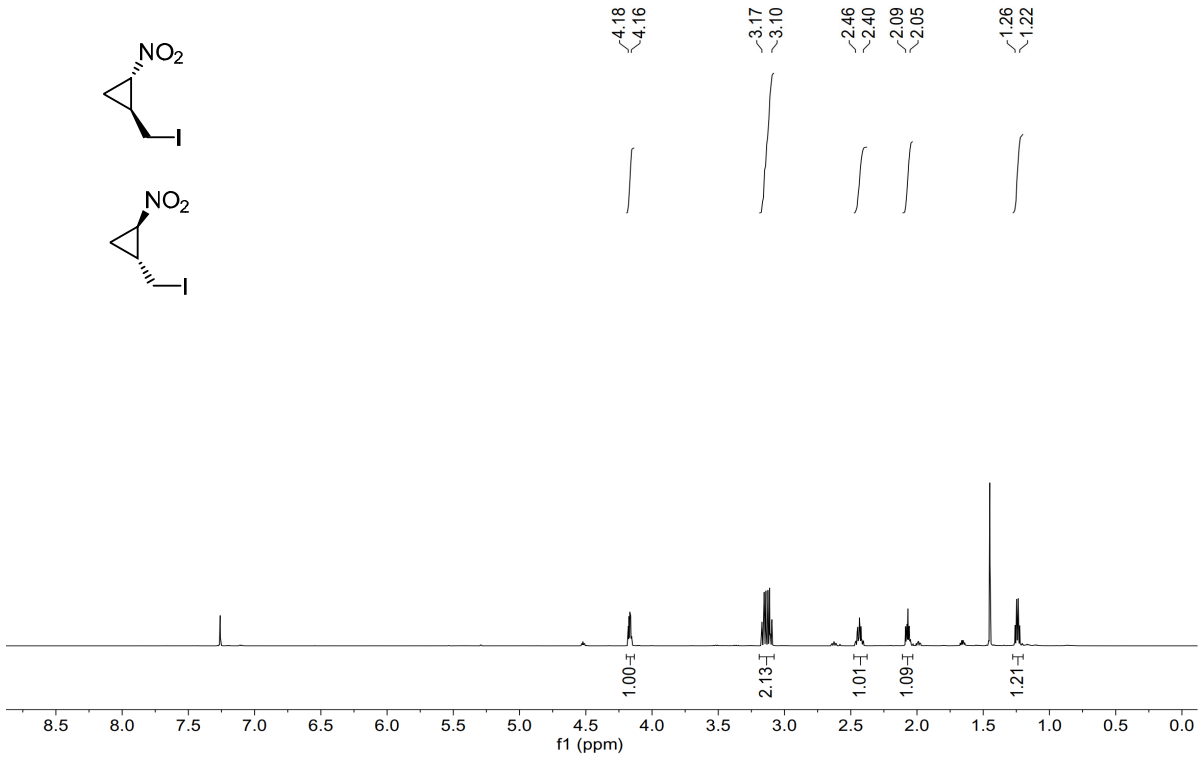


Figure S12. ^{1}H NMR of (*1S*, *2S*)/(*1R*, *2R*)-1-(iodomethyl)-2-nitrocyclopropane.

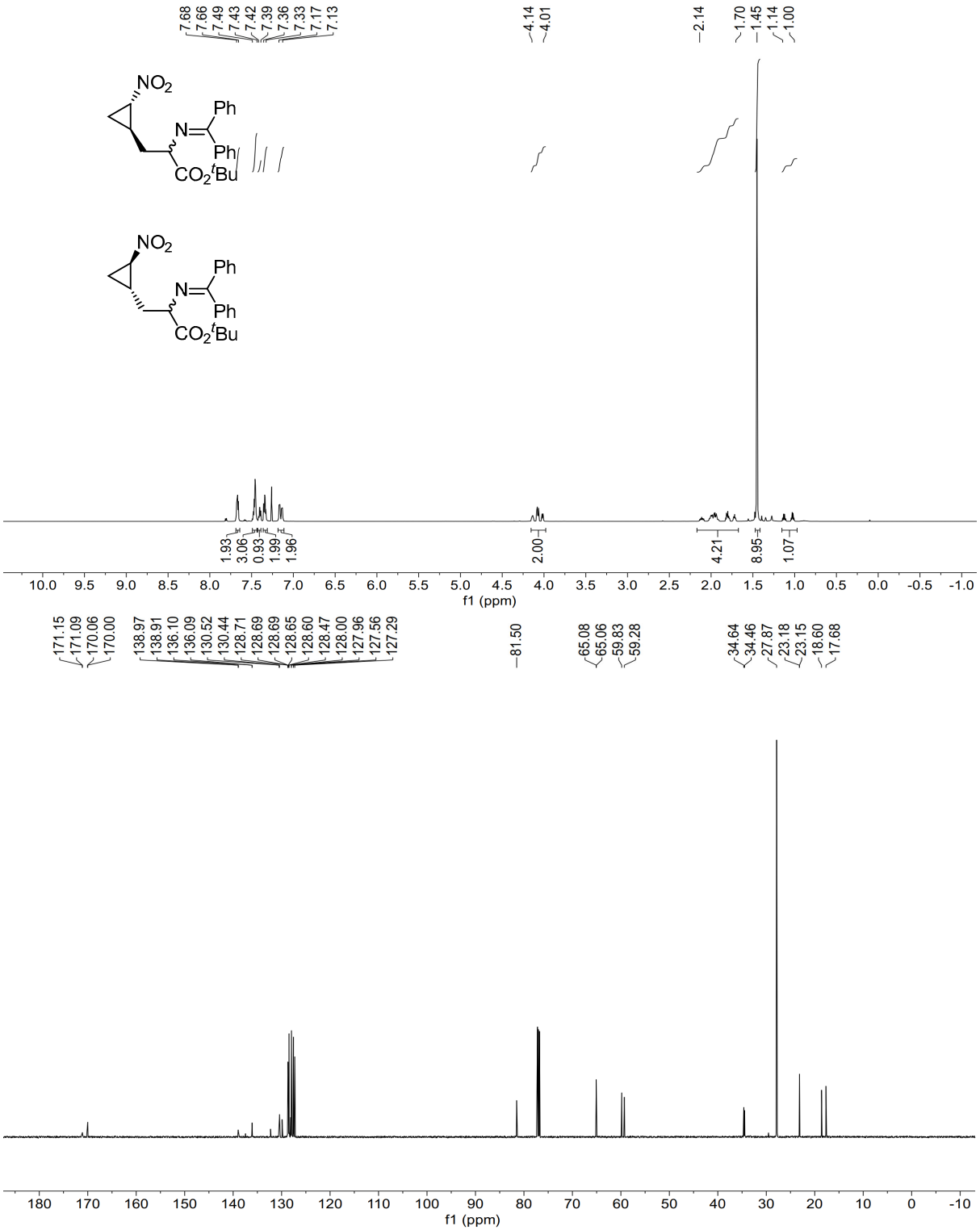


Figure S13. ^{1}H and ^{13}C NMR of *tert*-butyl 2-((diphenylmethylene)amino)-3-((*1S*, *2S*)/(*1R*, *2R*)-2-nitrocyclopropyl) propanoate.

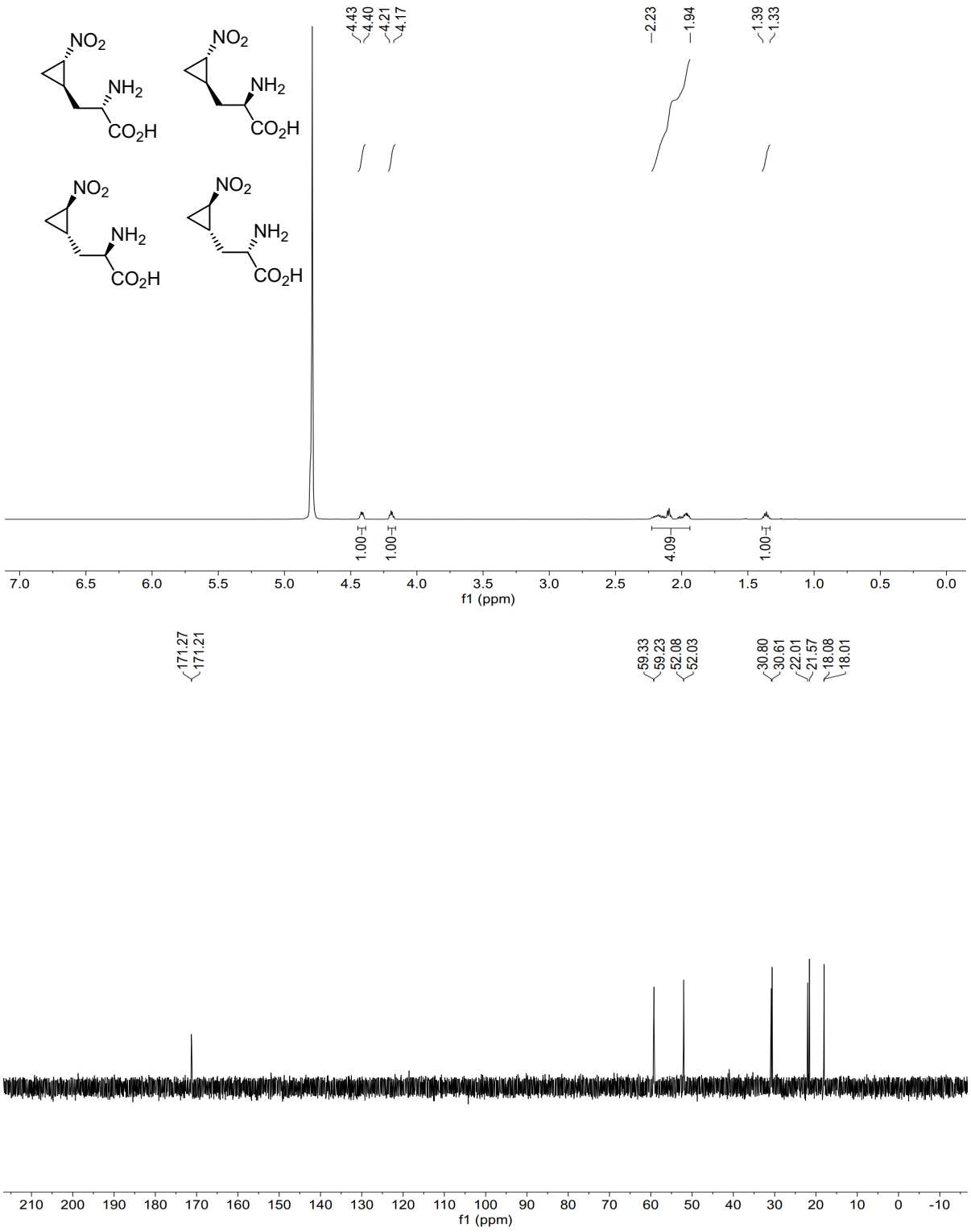


Figure S14. ^{1}H and ^{13}C NMR of 2-amino-3-((*1S*, *2S*)/(*1R*, *2R*)-2-nitrocyclopropyl)propanoic acid.

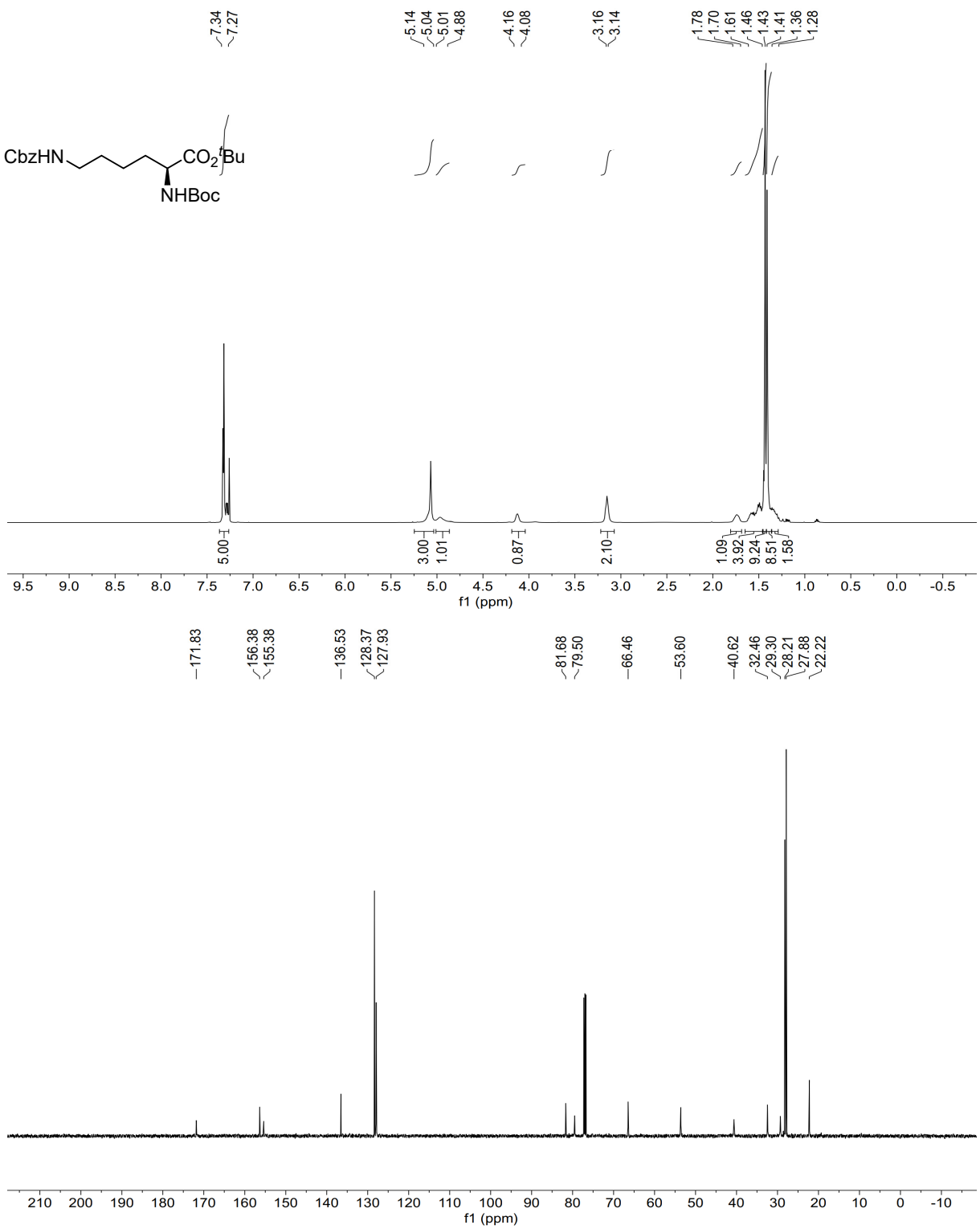


Figure S15. ^{1}H and ^{13}C NMR of *tert*-butyl N^{6}-((benzyloxy)carbonyl)-N^{2}-(tert-butoxycarbonyl)-L-lysinate.

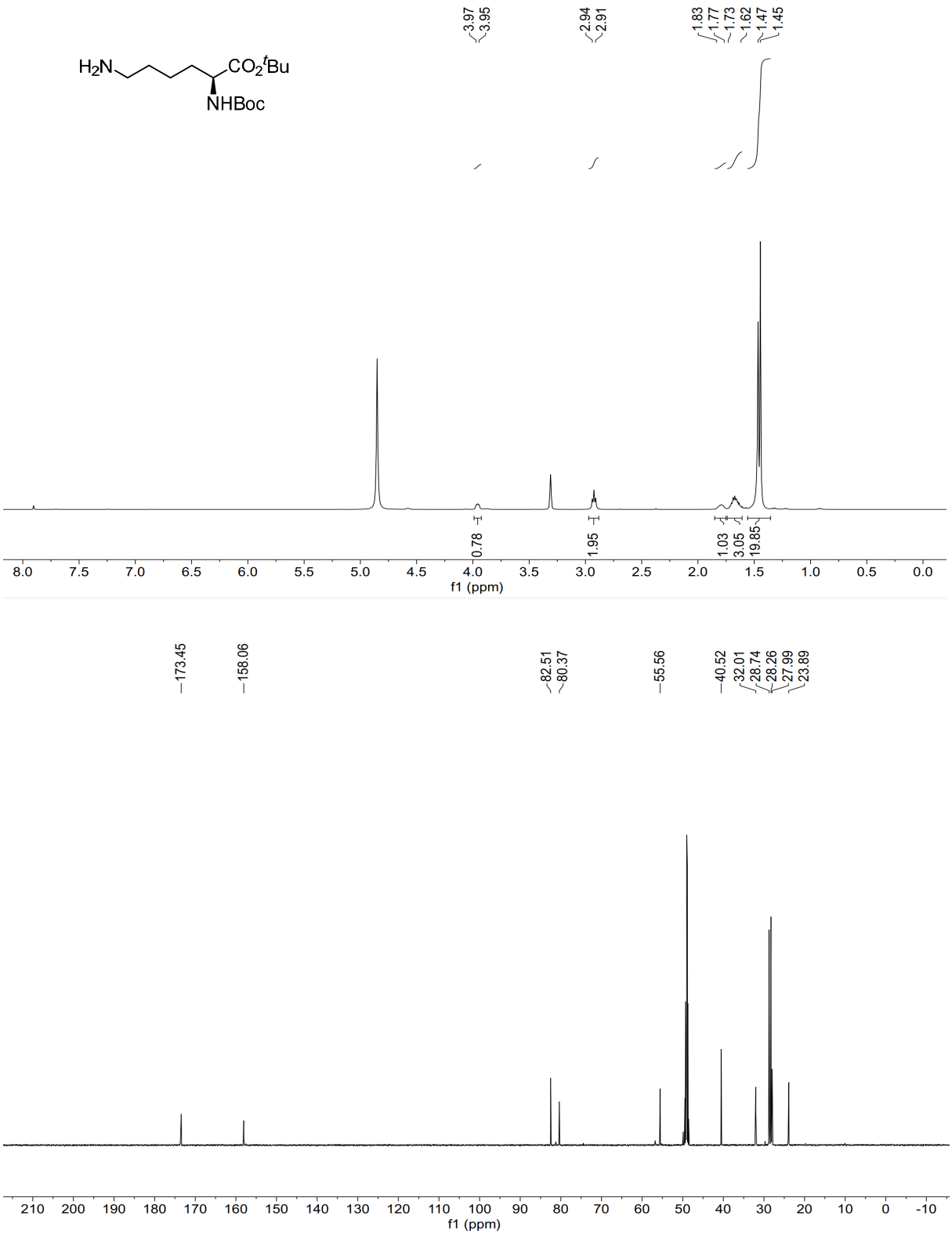


Figure S16. ^{1}H and ^{13}C NMR of *tert*-butyl (*tert*-butoxycarbonyl)-L-lysinate.

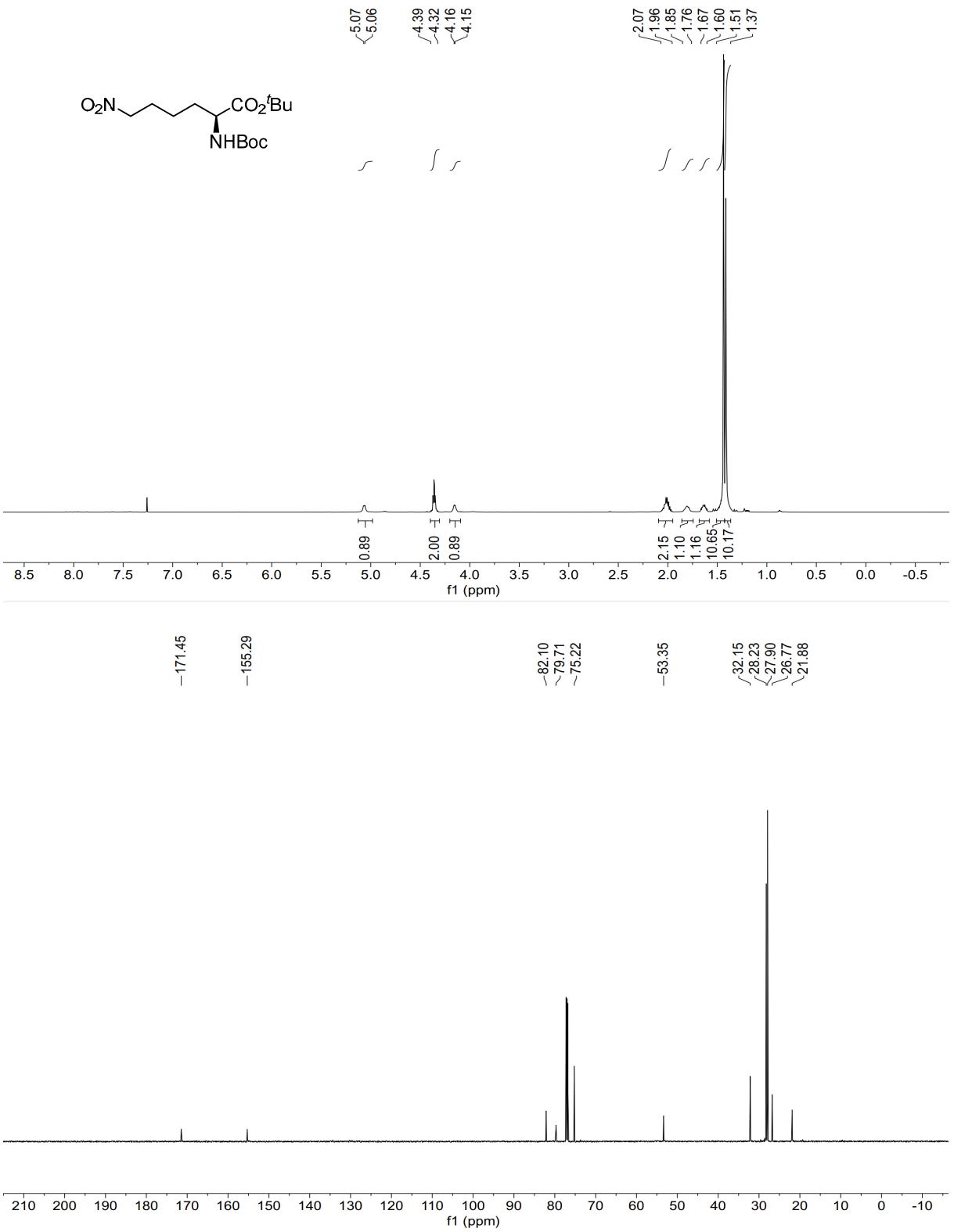


Figure S17. ^{1}H and ^{13}C NMR of *tert*-butyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-6-nitrohexanoate.

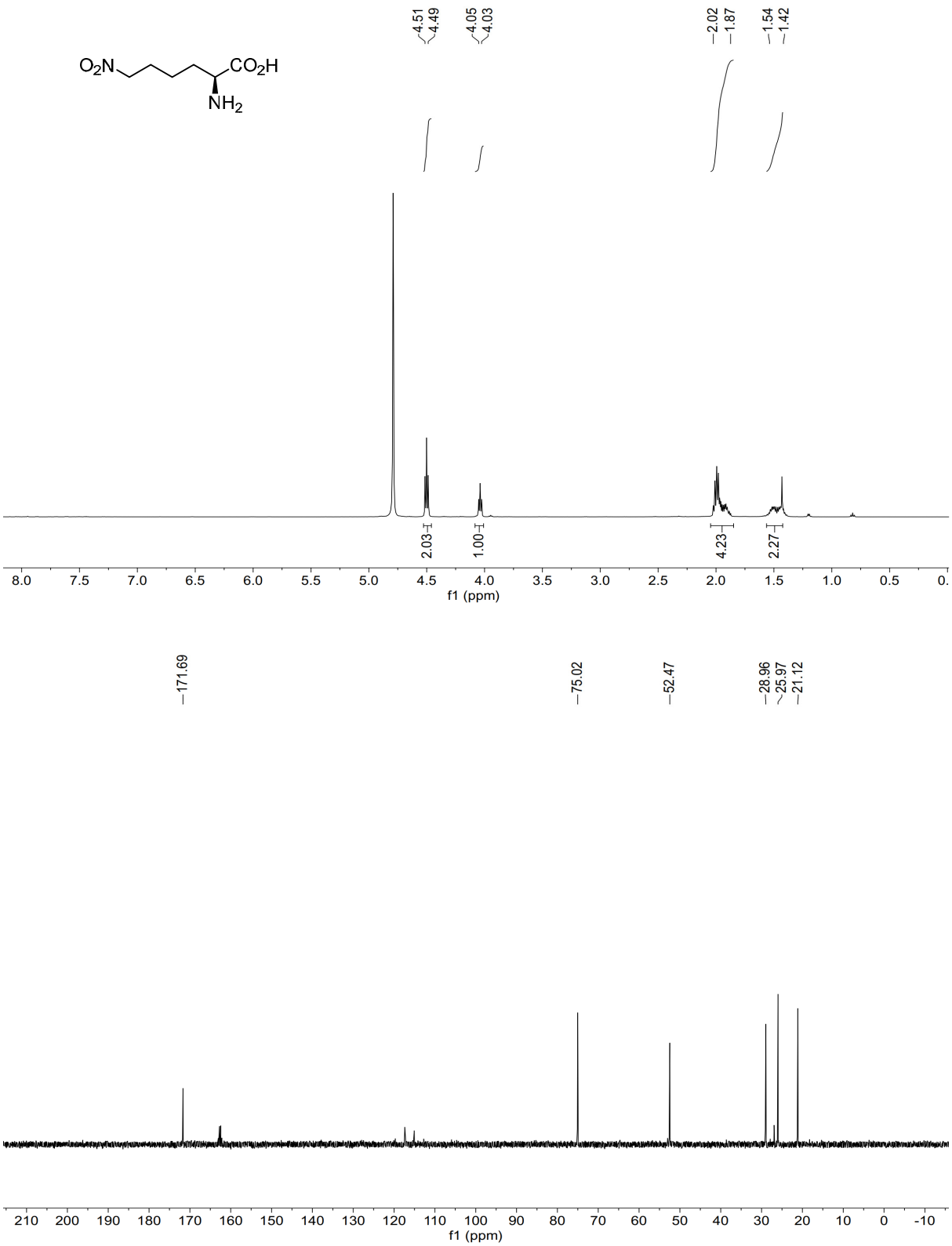


Figure S18. ^{1}H and ^{13}C NMR of (*S*)-2-amino-6-nitrohexanoic acid.

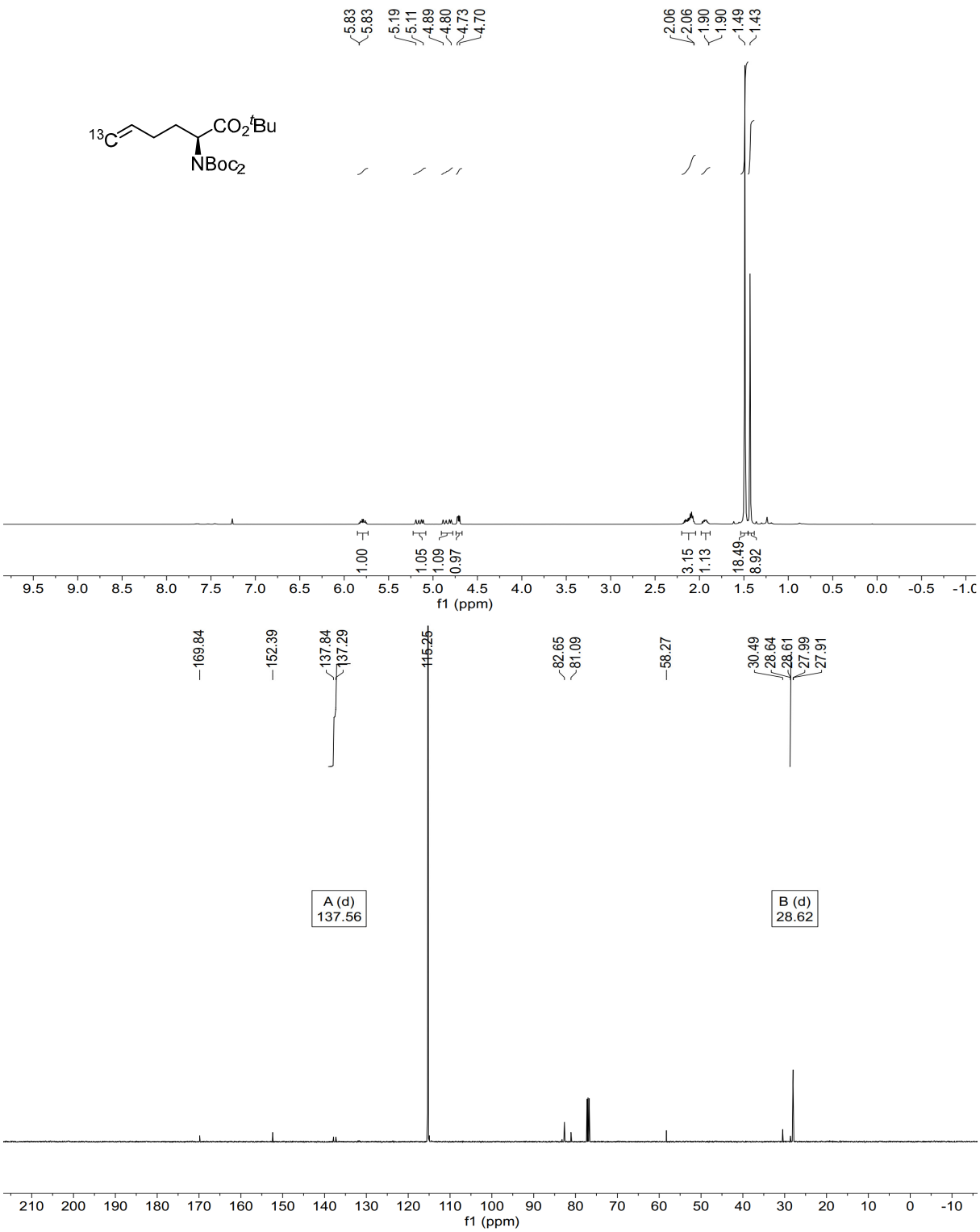


Figure S19. ^{1}H and ^{13}C NMR of [6-^{13}C]*tert*-butyl (*S*)-2-(bis(*tert*-butoxycarbonyl)amino)hex-5-enoate.

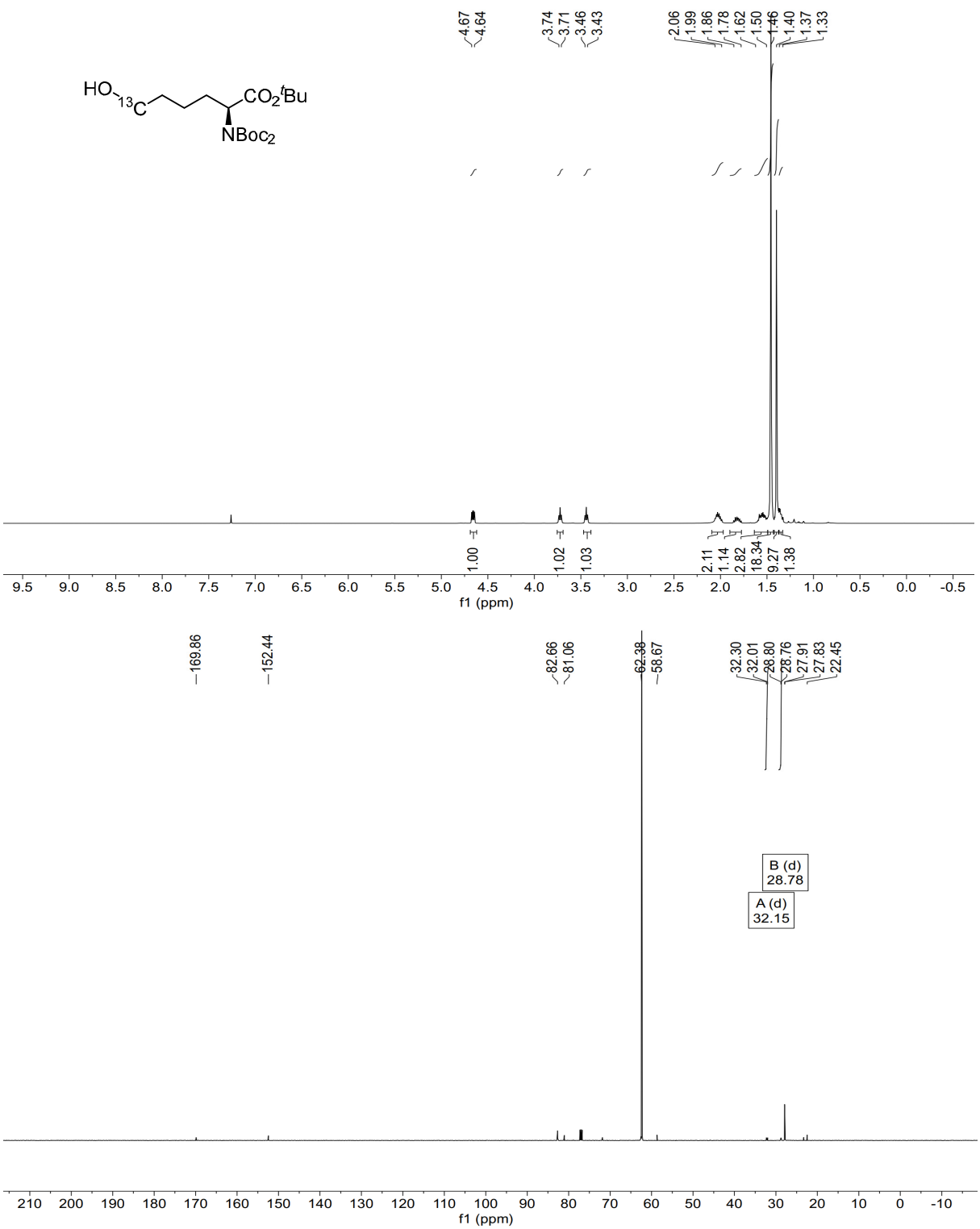


Figure S20. ^{1}H and ^{13}C NMR of [6-^{13}C]*tert*-butyl (*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-6-hydroxyhexanoate.

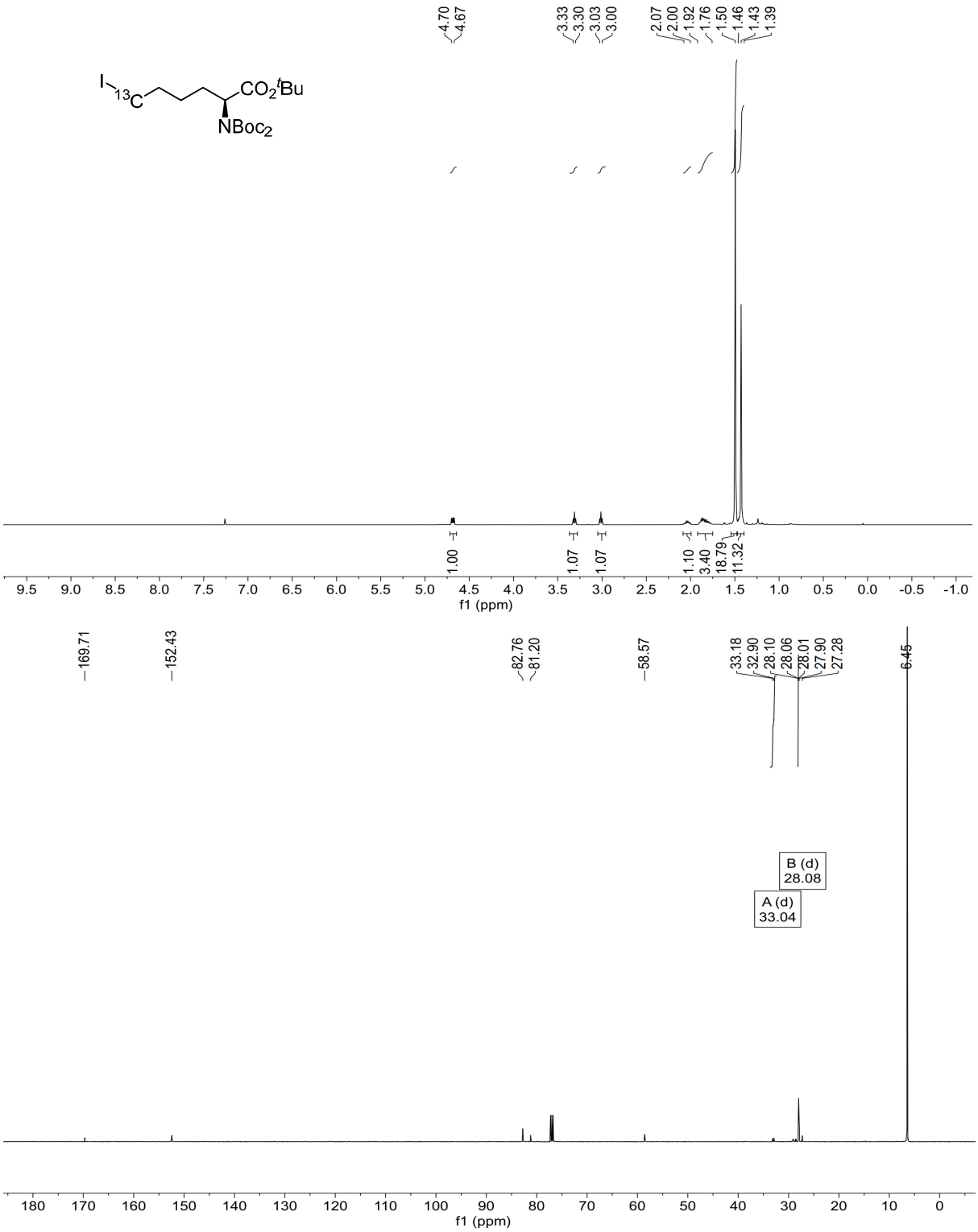


Figure S21. ^{1}H and ^{13}C NMR of [6-^{13}C]*tert*-butyl (*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-6-iodohexanoate.

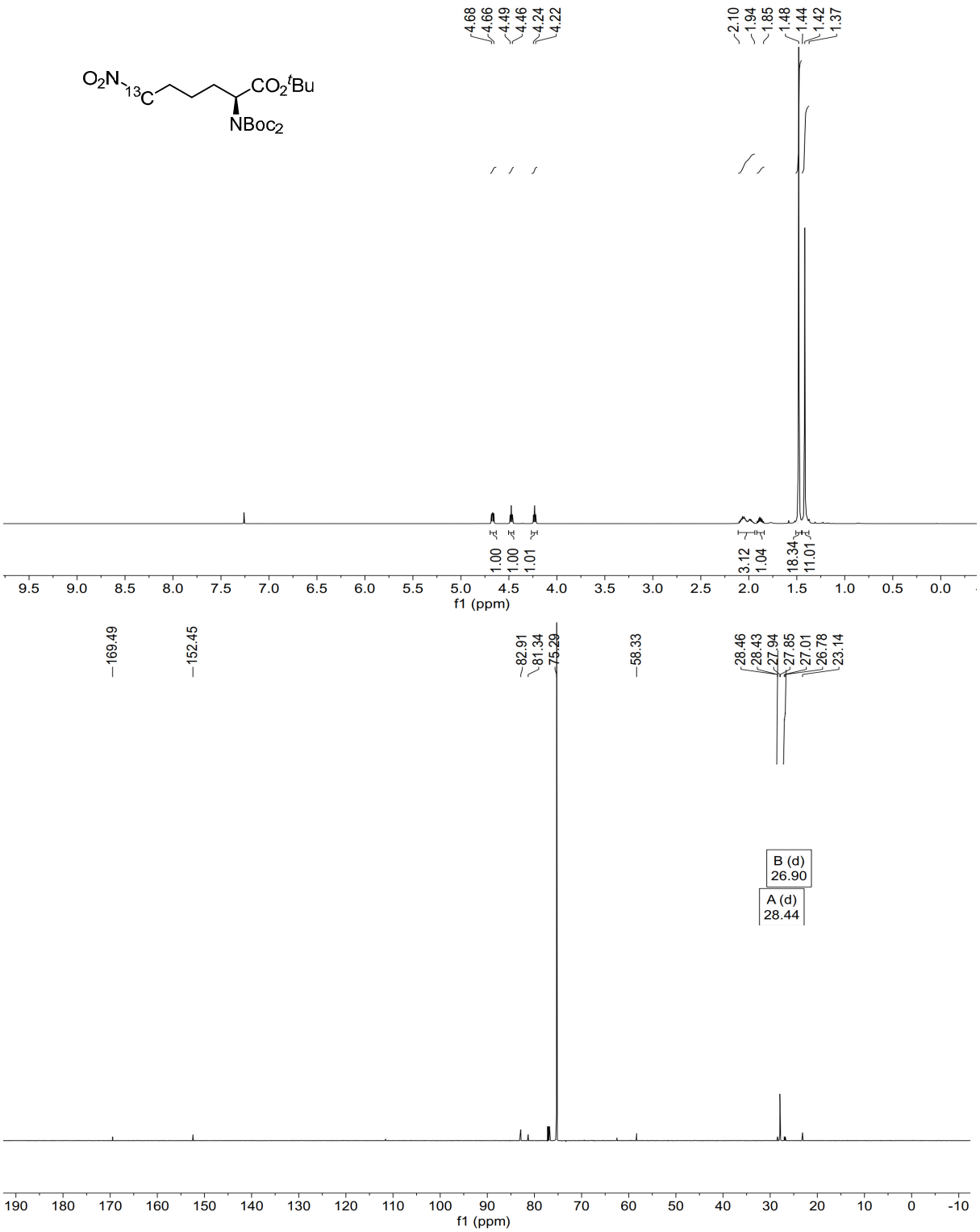


Figure S22. ^{1}H and ^{13}C NMR of **[**6-^{13}C]*tert*-butyl (*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-6-nitrohexanoate.

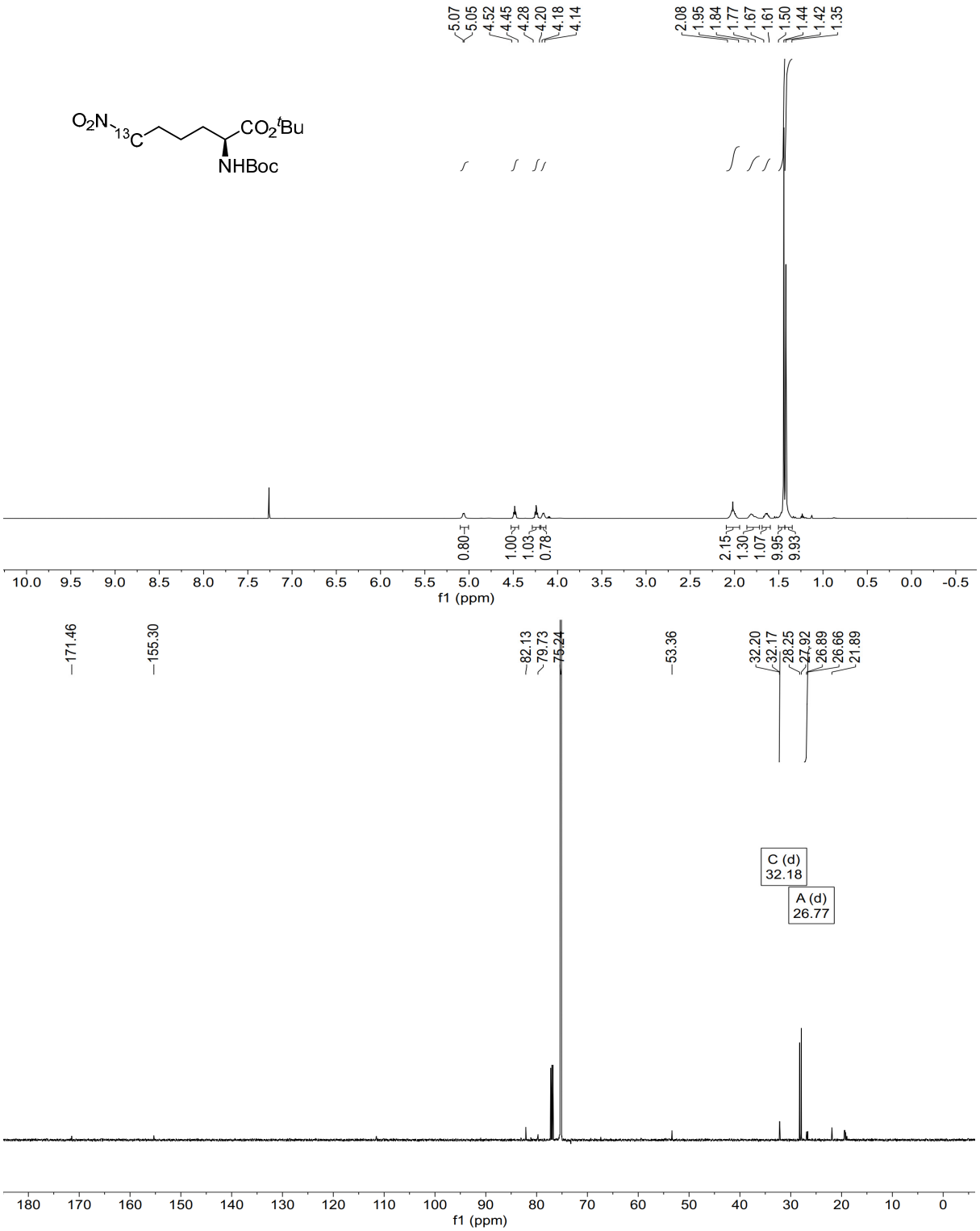


Figure S23. ^{1}H and ^{13}C NMR of **[**6-^{13}C]*tert*-butyl (*S*)-2-((*tert*-butoxycarbonyl)amino)- 6-nitrohexanoate.

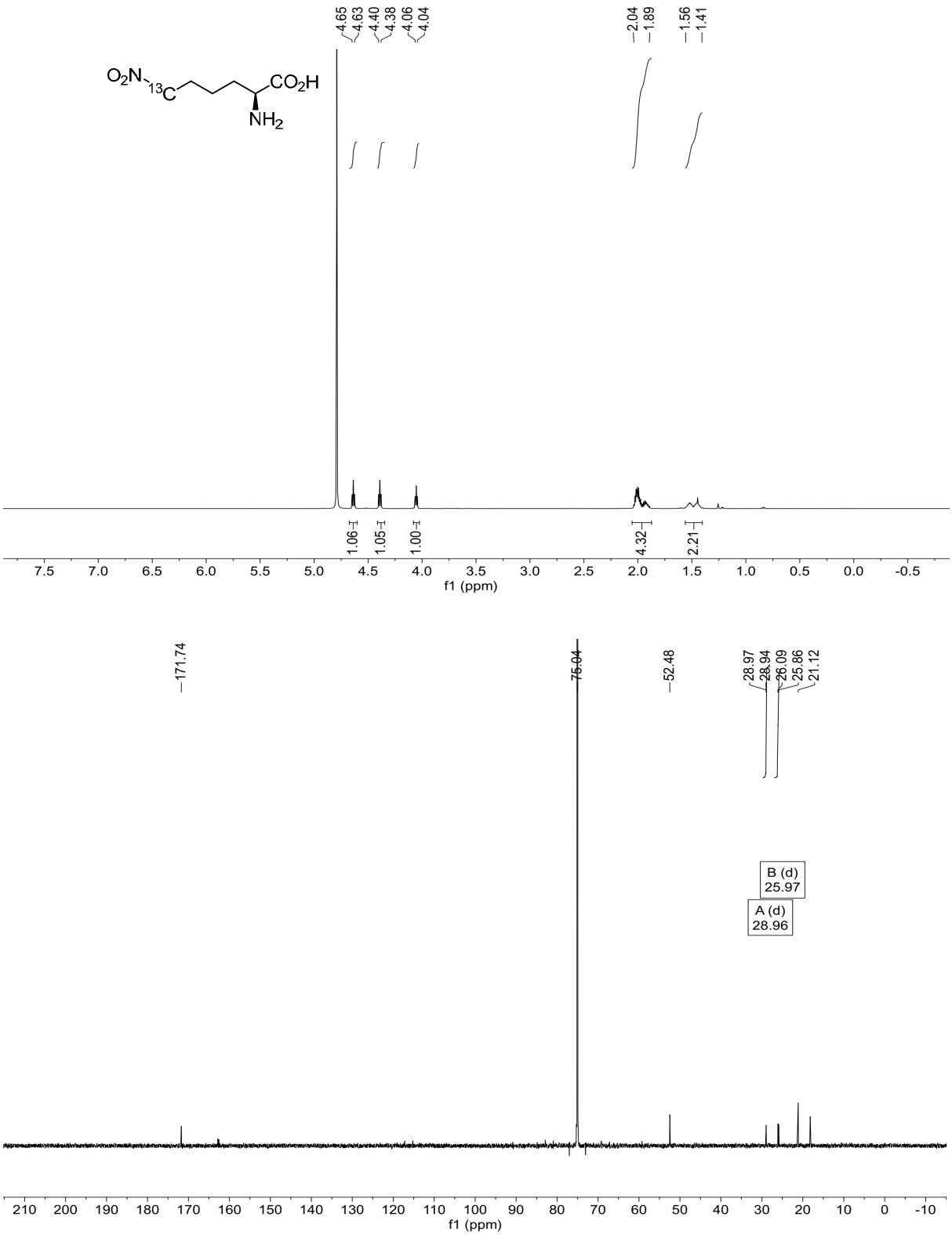


Figure S24. ^{1}H and ^{13}C NMR of [6-^{13}C](*S*)-2-amino-6-nitrohexanoate (**2**).

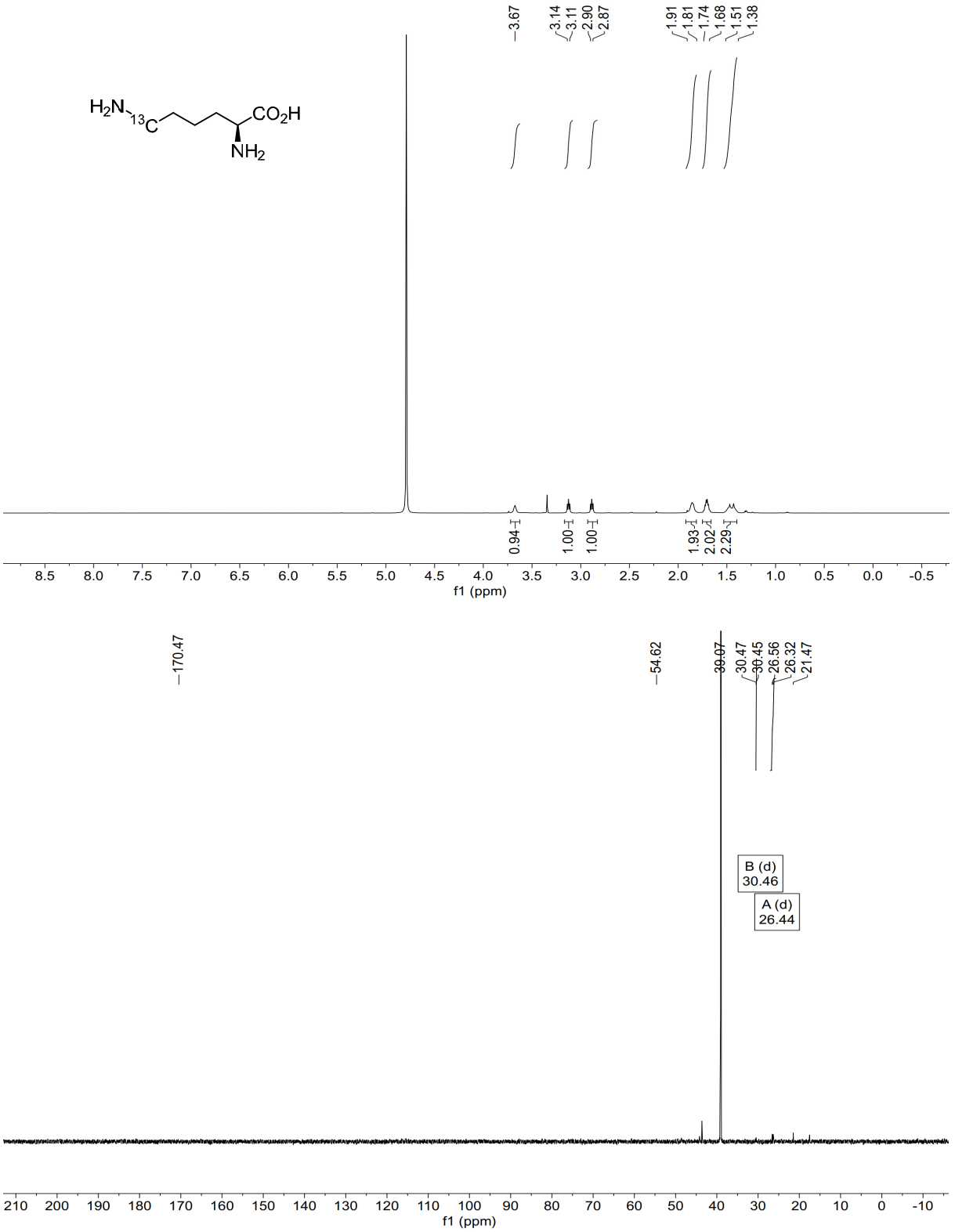


Figure S25. ^{1}H and ^{13}C NMR of [6-^{13}C]L-lysine.

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