Supporting Information

Alternative reactivity of leucine 5-hydroxylase using an olefin-containing substrate to construct substituted piperidine ring

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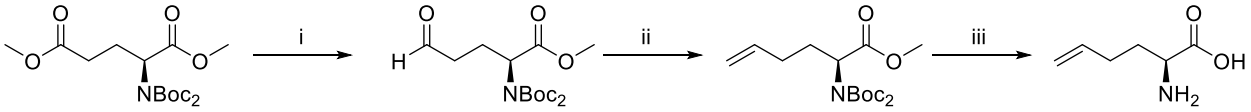
Contents:

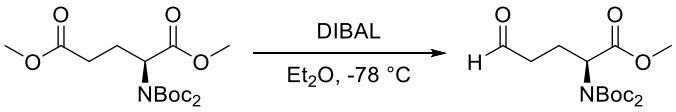
General Experimental Procedures

The chemical shift values are reported in δ values (parts per million, ppm) relative to the standard chemical shift for the proton residue (^{1}H) peak and carbon-13 (^{13}C) peak in the deuterated solvent, $CDCl_3$, D_2O, CD_3OD or DMSO-d_6.[1] The coupling constant (*J*) values are expressed in hertz (Hz). Thin-layer chromatography (TLC) was performed on silica gel plates. Compounds on TLC were visualized by illumination under UV light (254 nm), dipped into $KMnO_4$ solution followed by charring on a hot plate. Solvent systems are expressed as a percentage with respect to total volume (volumetric ratio). 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

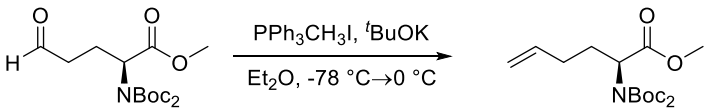


Conditions: (i) DIBAL, Et_2O, -78 °C, 89 % yield; (ii) PPh_3CH_3I, ^{t}BuOK, -78 °C→0 °C, 64 % yield; (iii) (a) LiBr, MeCN, 60 °C; (b) TFA, DCM, room temperature; (c) LiOH, Dioxane/H2O (2:1); 70 % yield (3 steps).



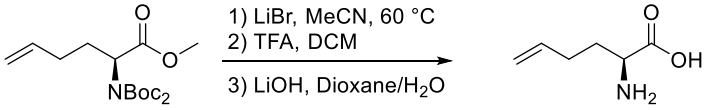
Methyl 2*S*-[bis(*tert*-Butoxycarbonyl)amino]-5-oxopentanoate (4)

According to the literature reported procedure,[2] the protected substrate (1.00 g, 2.67 mmol) was dissolved in anhydrous diethyl ether (Et_2O) (~ 0.2 M) and cooled to -78 °C. DIBAL (1.0 M in hexane, 1.5 equiv., 4.0 mL) was added drop-by-drop to the reaction at -78 °C. After addition, the reaction was kept at the same temperature. Consumption of the substrate was monitored using TLC. The reaction was quenched by adding water (~ 1.5 mL) to the reaction mixture. Following addition of saturated Rochelle's salt solution (~ 15mL), the mixture was allowed to stir vigorously at room temperature until the organic and water layers were separated. The aqueous layer was then extracted using ethyl acetate (~ 15 mL, three times). The combined organic layers were washed with brine and concentrated under reduced pressure. Product **4** was obtained through silica gel column chromatography (6:1, hexanes/ethyl acetate) with 89 % yield.



Methyl 2*S*-[bis(*tert*-Butoxycarbonyl)amino]-hex-5-enoate (5)

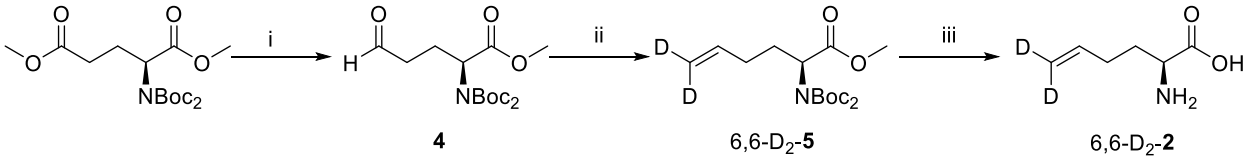
To a solution of PPh_3CH_3I (1.44 g, 3.57 mmol, 1.5 equiv.) dissolved in diethyl ether (~ 0.1 M), ^{t}BuOK (0.40 g, 3.57 mmol, 1.5 equiv.) was added in one portion at room temperature. After ~ 2 h, the reaction mixture was cooled to -78 °C. The aldehyde **4** (0.82 g, 2.38 mmol) dissolved in diethyl ether (~ 0.2 M) was added to the reaction mixture dropwise. After addition, the reaction was warmed to room temperature slowly. The reaction mixture was filtered to remove the solid generated during the reaction. The filtrate was washed with saturated NH_4Cl aqueous solution, brine and concentrated under reduced pressure. The corresponding product **5** (0.52 g, 1.52 mmol) was retrieved via silica gel column chromatography (20:1, hexanes/ethyl acetate) in 64 % yield. Characterization data matches those reported in the literature.[3]



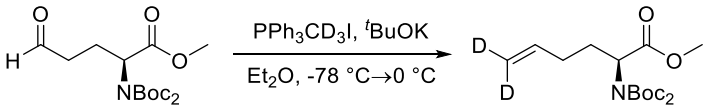
2*S*-amino-hex-5-enoic acid (2)

To a solution of **5** (0.52 g, 1.52 mmol) dissolved in acetonitrile (MeCN) (~ 0.2 M) was added LiBr (0.53 g, 6.08 mmol, 4.0 equiv.). The reaction was then heated up to 60 °C for ~ 14 h. After cooling down to the room temperature, the reaction was diluted with ethyl acetate (~ 5 times volume of MeCN) and washed with brine (~ 1 time volume of MeCN for three times), water (~ 1 time volume of MeCN for two times) and then brine (~ 1 time volume of MeCN). The organic layer was dried using $MgSO_4$ and concentrated under reduced pressure. The crude product was then dissolved in dichloromethane (DCM) (~ 0.2 M). TFA (1.2 mL, 15.2 mmol, 10.0 equiv.) was added dropwise to the reaction at room temperature. After consumption of the starting material which was visualized by TLC, the reaction mixture was concentrated under reduced pressure and subjected to silica gel chromatography (10:1, DCM/MeOH) to give the product at TFA salt form. The crude product was dissolved in a solution mixture of water and 1,4-dioxane (1:2, ~ 0.2 M). Lithium hydroxide (55 mg, 2.28 mmol, 1.5 equiv.) was added to the reaction in one portion. Once the reaction was completed, the mixture was concentrated under reduced pressure to yield the final product **1** (0.14 g, 1.06 mmol) as lithium salt in ~70 % overall yield. ^{1}H NMR (500 MHz, CD_3OD) δ 5.80-5.88 (m, 1H), 5.03 (d, J = 17.0 Hz, 1H), 4.93 (d, J = 10.0 Hz, 1H), 3.22-3.25 (m, 1H), 2.10-2.19 (m, 2H), 1.77-1.84 (m, 1H), 1.58-1.65 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 183.1, 139.6, 115.2, 57.1, 36.1, 31.4.

Preparation of 6,6-$^2H_2$-2 (6,6-D_2-2)

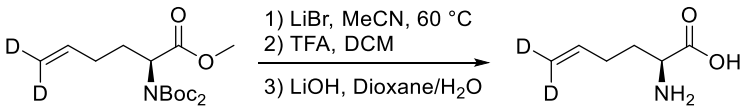


Conditions: (i) DIBAL, Et_2O, -78 °C, 79 % yield; (ii) PPh_3CD_3I, ^{t}BuOK, -78 °C→room temperature, 44 % yield; (iii) (a) LiBr, MeCN, 60 °C; (b) TFA, DCM, room temperature; (c) LiOH, Dioxane/H_2O (2:1); 68 % yield (3 steps).



6,6-$^2H_2$-methyl 2*S*-[bis(*tert*-Butoxycarbonyl)amino]-hex-5-enoate (6,6-D_2-5)

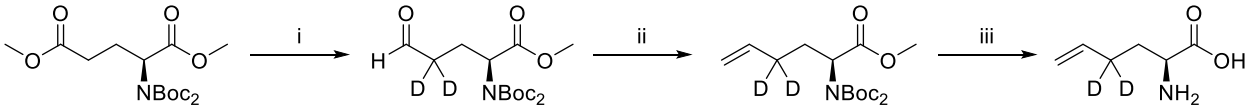
To a solution of PPh_3CD_3I (1.37 g, 3.36 mmol, 0.8 equiv.) dissolved in diethyl ether (~ 0.1 M), ^{t}BuOK (0.38 g, 3.36 mmol, 0.8 equiv.) was added in one portion at room temperature. After ~ 2 h, the reaction mixture was cooled to -78 °C. The aldehyde **4** (1.45 g, 4.20 mmol) dissolved in diethyl ether (~ 0.2 M) was added to the reaction mixture dropwise. After addition, the reaction was warmed to room temperature slowly. Following the workup procedure described for **5**, 6,6-D_2-**5** (0.51 g, 1.48 mmol) was obtained in 44 % yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.73-5.79 (m, 1H), 4.83 (dd, *J* = 9.3, 5.0 Hz), 3.67 (s, 3H), 2.16-2.23 (m, 1H), 2.06-2.10 (m, 2H), 1.90-1.97 (m, 1H), 1.46 (s, 18H), (4.92-5.03, m, residual peaks originate from non-deuterated C6-H. Based on the integration value (0.57 H), deuterium incorporation ratio is ~75 %).



6,6-$^2H_2$-2*S*-aminohex-5-enoic acid (6,6-D_2-2)

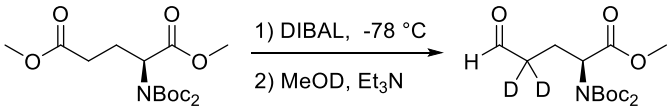
Following the procedure for **2** preparation, 6,6-D_2-**5** (0.51 g, 1.48 mmol) was converted to 6,6-D_2-**2** (0.14 g, 1.01 mmol) as lithium salt in 68 % yield and ~70 % deuterium incorporation. ^{1}H NMR (500 MHz, CD_3OD): δ 5.79-5.88 (m, 1H), 3.24 (dd, *J* = 6.8, 6.3 Hz, 1H), 2.08-2.19 (m, 2H), 1.77-1.84 (m, 1H), 1.58-1.65 (m, 1H), (4.91-5.04, m, residual peaks originate from non-deuterated C6-H. Based on the integration value (0.57 H), deuterium incorporation ratio is ~75 %); ^{13}C NMR (125 MHz, CD_3OD): δ 183.2, 139.3, 114.6-115.1 (m), 57.1, 36.0, 31.2.

Preparation of 4,4-$^2H_2$-2 (4,4-D_2-2)



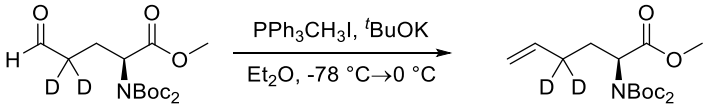
4,4-D_2-**4** 4,4-D_2-**5** 4,4-D_2-**2**

Conditions: (i) (a) DIBAL, Et_2O, -78 °C; (b) Et_3N, MeOD, 35 °C; 77 % yield (2 steps); (ii) PPh_3CH_3I, ^tBuOK, -78 °C→room temperature, 41 % yield; (iii) (a) LiBr, MeCN, 60 °C; (b) TFA, DCM, room temperature; (c) LiOH, Dioxane/H_2O (2:1); 73 % yield (3 steps).



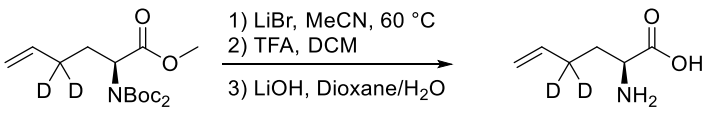
4,4-$^2H_2$-methyl-2*S*-[bis(*tert*-Butoxycarbonyl)amino]-5-oxopentanoate (4,4-D_2-4)

Following the procedures described above, compound **4** was prepared. Compound **4** (0.85 g, 2.46 mmol, 1.0 equiv.) was dissolved in ~ 10 mL of MeOD. Triethylamine (Et_3N) (0.04 mL, 0.25 mmol, 0.1 equiv.) was added to the reaction and the mixture was allowed to stir at 35 °C. Deuterium incorporation ratio was monitored by ^{1}H-NMR. Once 90 % incorporation was achieved (~12 h), the solution was concentrated under reduced pressure to yield crude 4,4-D_2-**4**, which was used directly in the next step without further purification.



4,4-$^2H_2$-methyl 2*S*-[bis(*tert*-Butoxycarbonyl)amino]-hex-5-enoate (4,4-D_2-5)

Following the procedure for6,6-D_2-**5** preparation, 4,4-D_2-**4** (0.71 g, 2.05 mmol) yields 4,4-D_2-**5** (0.29 g, 0.84 mmol) in 51 % yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.78 (dd, *J* = 17.5, 10.0 Hz, 1H), 5.02 (dd, *J* = 17.5 and 2.0 Hz, 1H), 4.97 (dd, *J* = 10.0, 2.0 Hz, 1H), 4.85 (dd, *J* = 9.0, 5.0 Hz, 1H), 3.69 (s, 3H), 2.20 (dd, *J* = 14.0, 5.0 Hz, 1H), 1.94 (dd, *J* = 14.0, 9.0 Hz, 1H), 1.48 (s, 18H), (2.06-2.12, m, residue C4-H peaks originate from the non-deuterated compound. Based on the integration value (0.29), deuterium incorporation is ~ 85%).

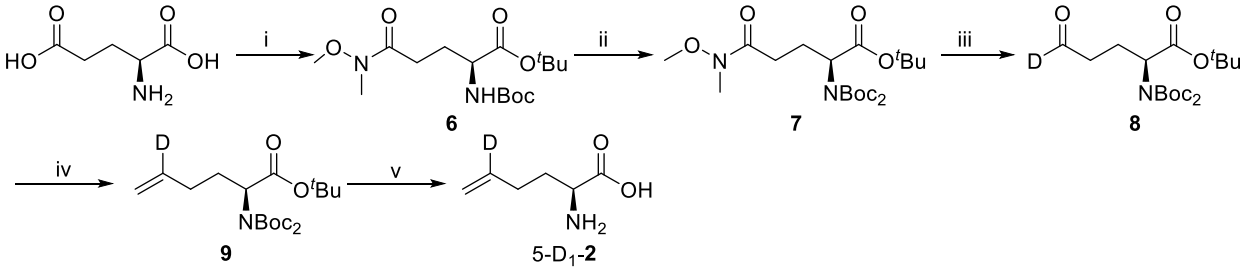


4,4-$^2H_2$-2*S*-aminohex-5-enoic acid (4,4-D_2-2)

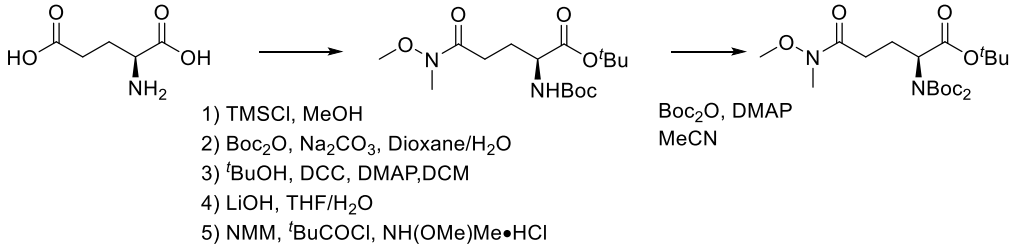
Following the procedure for **2** preparation, using 4,4-D_2-**5** (0.29 g, 0.84 mmol) as the substrate yields 4,4-D_2-**2** (0.08 g, 0.61 mmol) as lithium salt in 73 % yield. ^{1}H NMR (500 MHz, CD_3OD):

δ 5.83 (dd, *J* = 17.0, 10.5 Hz, 1H), 5.03 (dd, *J* = 17.0, 2.0 Hz), 4.93 (dd, *J* = 10.5, 2.0 Hz, 1H), 3.23 (dd, *J* = 7.5, 5.5 Hz, 1H), 1.78 (dd, *J* = 13.5, 5.5 Hz, 1H), 1.59 (dd, *J* = 13.5, 7.5 Hz, 1H), (2.08-2.16, m, residue C4-H peaks originate from the non-deuterated compound. Based on the integration value (0.29), deuterium incorporation is ~ 85%); ^{13}C NMR (125 MHz, CD_3OD): δ 183.0, 139.6, 115.2, 57.2, 36.0, 30.4-31.4 (m).

Preparation of 5-$^2H_1$-2 (5-D_1-2)

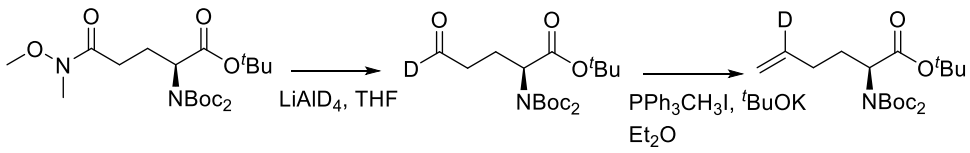


Conditions: (i) (a) TMSCl, MeOH; (b) Boc_2O, Na_2CO_3, Dioxane/H_2O (2:1), 0 °C→room temperature, (c) ^{t}BuOH, DCC, DMAP, DCM; (d) LiOH, THF/H_2O (2;1); (e) NMM, ^{t}BuCOCl, NH(OMe)Me•HCl, DCM; 45 % yield (5 steps); (ii) Boc_2O, DMAP, MeCN, 70 % yield; (iii) $LiAlD_4$, THF, 0 °C→-15 °C, 48 % yield; (iv) PPh_3CH_3I, ^{t}BuOK, -78 °C→room temperature, 72 % yield; (iii) (a) LiBr, MeCN, 60 °C; (b) TFA, room temperature; 65 % yield (2 steps).



^{t}Butyl 2*S*-[bis(*tert*-butoxycarbonyl)amino]-5-(methoxy(methyl)amino)-5-oxopentanoate (7)

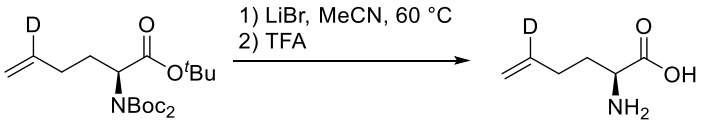
Following the literature procedure,[4-5] compound **7** (1.89 g, 4.23 mmol) was prepared using L-glutamic acid (2.00 g, 13.59 mmol) as the substrate in overall ~ 31 % yield.



5-^{2}H-*tert*-butyl 2*S*-[bis(*tert*-Butoxycarbonyl)amino]-hex-5-enoate (9)

To a tetrahydronfuran (THF, ~ 0.1 M) solution of **7** (1.01 g, 2.26 mmol, 1.0 equiv.), $LiAlD_4$ (57 mg, 1.36 mmol, 0.6 equiv.) was added in one-portion at 0 °C. The reaction was maintained at 0 °C and the progress was monitored by TLC. Once the over reduced alcohol was observed (~ 30 min), the mixture was immediately cooled to -15 °C and quenched by the addition of saturated $KHSO_4$

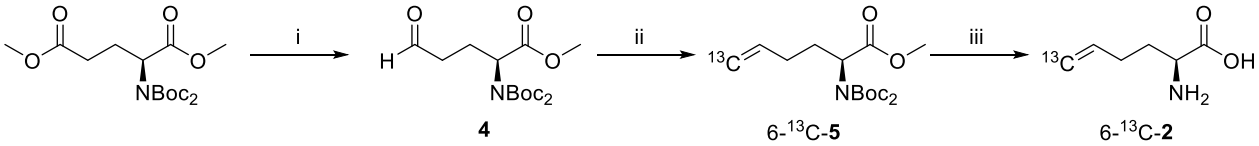
aqueous solution (~ 20 mL) and Et_2O (~ 50 mL). The resulting mixture was stirred vigorously until two phases were separated (~ 30 mins). The aqueous phase was extracted with Et_2O (~ 20 mL, 3 times). The combined organic layers were washed with brine, dried with $MgSO_4$ and concentrated under reduced pressure before purification via silica gel column chromatography (6:1, hexanes/ethyl acetate) to yield **8** in 48 % yield. Prepared following the similar procedures as synthesizing **5**, using **8** (0.42 g, 1.08 mmol) to react with PPh_3CH_3I (0.66 g, 1.62 mmol) yields **9** (0.30 g, 0.78 mmol) in 72 % yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.02 (s, 1H), 4.96 (s, 1H), 4.72 (dd, J = 5.0, 4.8 Hz, 1H), 2.07-2.19 (m, 3H), 1.90-1.97 (m, 1H), 1.49 (s, 18H), 1.43 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.0, 152.6, 137.4 (t, $J_{C\text{-}D}$ = 93.0 Hz), 115.3, 82.8, 81.2, 58.4, 30.5, 28.8, 28.1, 28.1.



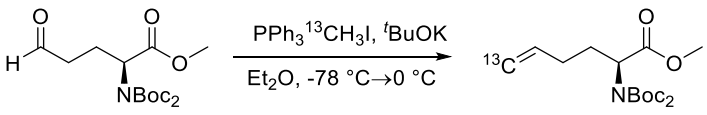
5-^{2}H-2*S*-amino-hex-5-enoic acid (5-D$_1$-2)

To a solution of **7** (0.30 g, 0.78 mmol, 1.0 equiv.) dissolved in MeCN (~ 10 mL) was added LiBr (0.27 g, 3.12 mmol, 4.0 equiv.). The reaction was heated up to 60 °C. After overnight stirring, the reaction was cool down to room temperature and diluted with ethyl acetate (~ 50 mL). It was then washed using brine (~ 10 mL, 3 times), water (~ 10 mL, 2 times) and brine (~ 10 mL). The organic layer was dried using $MgSO_4$ and the resulting solution was concentrated to yield the crude product. TFA (~ 2 mL) was added to the crude product slowly at the room temperature. The reaction was monitored by TLC. Once upon completion, the reaction mixture was concentrated under reduced pressure to yield 5-D$_1$-**2** as TFA salt in 65 % yield. ^{1}H NMR (500 MHz, D_2O): δ 5.13 (s, 1H), 5.08 (s, 1H), 4.02 (dd, J = 7.0, 6.0 Hz, 1H), 2.16-2.25 (m, 2H), 2.04-2.11 (m, 1H), 1.94-2.02 (m, 1H); ^{13}C NMR (125 MHz, D_2O): δ 172.4, 136.1 (t, $J_{C\text{-}D}$ = 94.0 Hz), 116.1, 52.5, 29.0, 28.2.

Preparation of 6-^{13}C-2

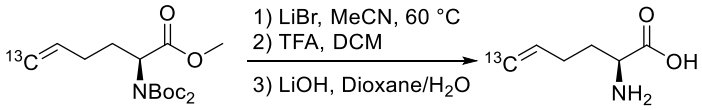


Conditions: (i) DIBAL, Et_2O, -78 °C, 86 % yield; (ii) $PPh_3{}^{13}CH_3I$, ^{t}BuOK, -78 °C→room temperature, 75 % yield; (iii) (a) LiBr, MeCN, 60 °C; (b) TFA, DCM, room temperature; (c) LiOH, Dioxane/H2O (2:1); 70 % yield (3 steps).



6-^{13}C-methyl-2*S*-[bis(*tert*-Butoxycarbonyl)amino]-hex-5-enoate (6-^{13}C-5)

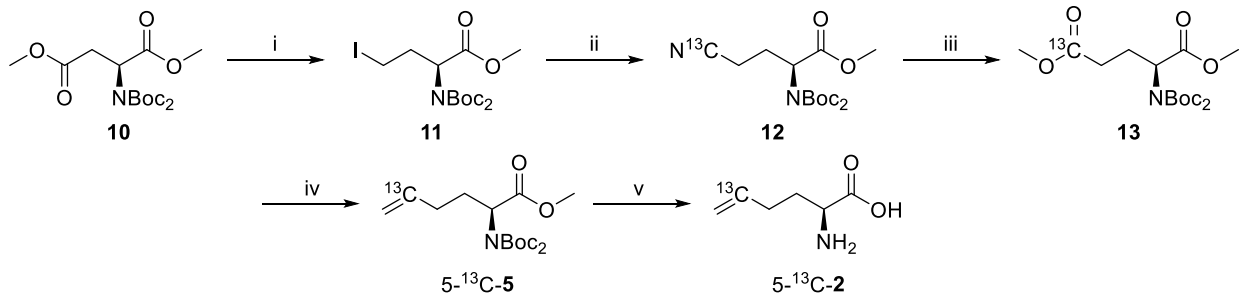
Following the procedure for **5** preparation, reacting **4** (0.79 g, 2.29 mmol) with $PPh_3{}^{13}CH_3I$ (1.39 g, 3.43 mmol) affords 6-^{13}C-**5** (0.59 g, 1.71 mmol) in 75 % yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.75-5.83 (m, 1H), 4.81-5.21 (dm, $J_{C\text{-}H}$ = 154.8 Hz, 1H), 4.86 (dd, J = 5.0, 4.8 Hz, 1H), 3.70 (s, 3H), 2.18-2.25 (m, 1H), 2.08-2.14 (m, 2H), 1.92-2.00 (m, 1H), 1.48 (s, 18H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.5, 152.2, 137.5 (d, $J_{C\text{-}C}$= 68.8 Hz), 115.6, 83.2, 57.7, 52.3, 30.4, 29.4 (d, $J_{C\text{-}C}$ = 3.6 Hz), 28.1.



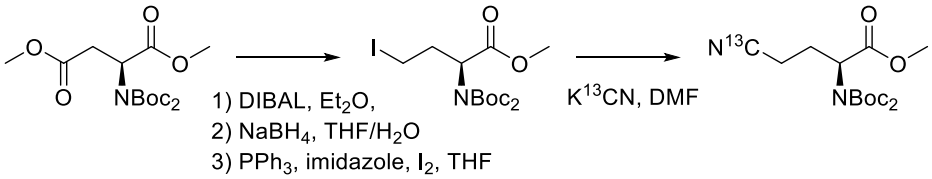
6-^{13}C-2*S*-aminohex-5-enoic acid (6-^{13}C-2)

Following the procedure for **2** preparation, 6-^{13}C-**5** (0.59 g, 1.71 mmol) is converted to 6-^{13}C-**2** (0.16 g, 1.20 mmol) in 70 % yield. ^{1}H NMR (500 MHz, D_2O): δ 5.84-5.92 (m, 1H), 4.82-5.24 (dm, $J_{C\text{-}H}$ = 155.8 Hz, 2H), 3.22 (t, J = 5.5 Hz, 1H), 2.04-2.09 (m, 2H), 1.66-1.73 (m, 1H), 1.56-1.63 (m, 1H); ^{13}C NMR (125 MHz, D_2O): 183.6, 138.8 (d, $J_{C\text{-}C}$ = 68.0 Hz), 114.8, 55.5, 33.9 (d, $J_{C\text{-}C}$ = 3.6 Hz), 29.4.

Preparation of 5-^{13}C-2

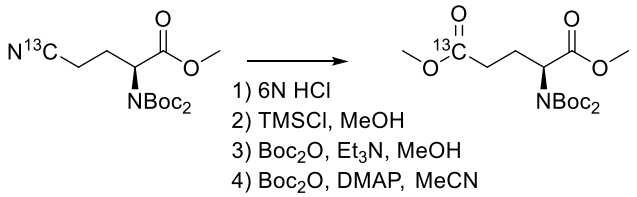


Conditions: (i) (a) DIBAL, Et_2O, -78 °C; (b) $NaBH_4$, THF/H_2O (4:1), 0 °C; (c) PPh_3, imidazole, I_2, THF, room temperature; 80 % yield (3 steps); (ii) K^{13}CN, DMF, room temperature; 87 % yield; (iii) (a) 6N HCl, reflux; (b) TMSCl, MeOH, 0 °C→room temperature; (c) Boc_2O, Et_3N, MeOH; (d) Boc_2O, DMAP, MeCN, room temperature; 42 % yield (4 steps); (iv) (a) DIBAL, Et_2O, -78 °C; (b) PPh_3CH_3I, ^{t}BuOK, -78 °C→room temperature; 56 % yield (2 steps); (v) (a) LiBr, MeCN, 60 °C; (b) TFA, DCM, room temperature; (c) LiOH, Dioxane/H_2O (2:1); 67 % yield (3 steps).



5-^{13}C- methyl 2*S*-[bis(*tert*-butoxycarbonyl)amino]-4-cyanobutanoate (12)

Prepared as literature reported,[2] using **10** (3.00 g, 8.31 mmol) as the substrate yields **11** (2.96 g, 6.68 mmol) in 80 % yield. Compound **11** (2.96 g, 6.68 mmol, 1.0 equiv.) was dissolved in DMF (~ 40 mL). $K^{13}CN$ (0.66 g, 10.02 mmol, 1.5 equiv.) was added in one portion and the reaction was stirred at room temperature overnight. The reaction mixture was diluted by H_2O (~ 40 mL) and extracted with ethyl acetate (~ 40 mL, 5 times). The combined organic phase was washed with saturated $NaHCO_3$ aqueous solution (~ 40 mL), brine (~ 40 mL, 2 times), H_2O (~ 40 mL, 2 times) and brine (~ 40 mL) before concentrated under reduced pressure. Compound **12** was obtained (2.00 g, 5.82 mmol) in 87 % yield using silica gel column chromatography (8:1, hexanes/ethyl acetate). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.92 (dd, J = 8.5Hz, 5.5 Hz, 1H), 3.72 (s, 3H), 2.44-2.55 (m, 3H), 2.16-2.24 (m, 1H), 1.49 (s, 18H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.2, 151.9, 119.0, 84.0, 57.0, 52.6, 28.0, 26.5, 14.6 (d, $J_{C\text{-}C}$ = 56.5 Hz).

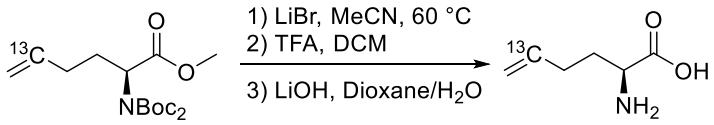


5-^{13}C-dimethyl N,N-bis(*tert*-butoxycarbonyl)-L-glutamate (13)

According to literature reported procedure,[6] **12** (2.00 g, 5.82 mmol, 1.0 equiv.) was mixed with 6N HCl aqueous solution (94 mL, 96.0 equiv.). The reaction was heated and kept refluxing for overnight. Then the solution was concentrated under reduced pressure. The crude product was suspended in ~ 2 mL of 2 % HCl and the suspension was adjusted to pH = 5 using pyridine. Following by addition of ~ 40 mL of ethanol, the mixture was stored at -20 °C overnight. The precipitate was filtered and dried to yield crude 5-^{13}C-L-glutamic acid, which was directly applied to the protecting steps as reported in literature[2] to yield **11** (0.93 g, 2.47 mmol) in 42 % yield (overall 4 steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.92 (dd, J = 9.5, 5.0 Hz, 1H), 3.71 (s, 3H), 3.66 (d, $J_{C\text{-}H}$ = 4.0 Hz, 3H), 2.34-2.52 (m, 3H), 2.14-2.22 (m, 1H), 1.49 (s, 18H).

5-^{13}C-methyl 2(*S*)-[bis(*tert*-Butoxycarbonyl)amino]-hex-5-enoate (5-^{13}C-**5)**

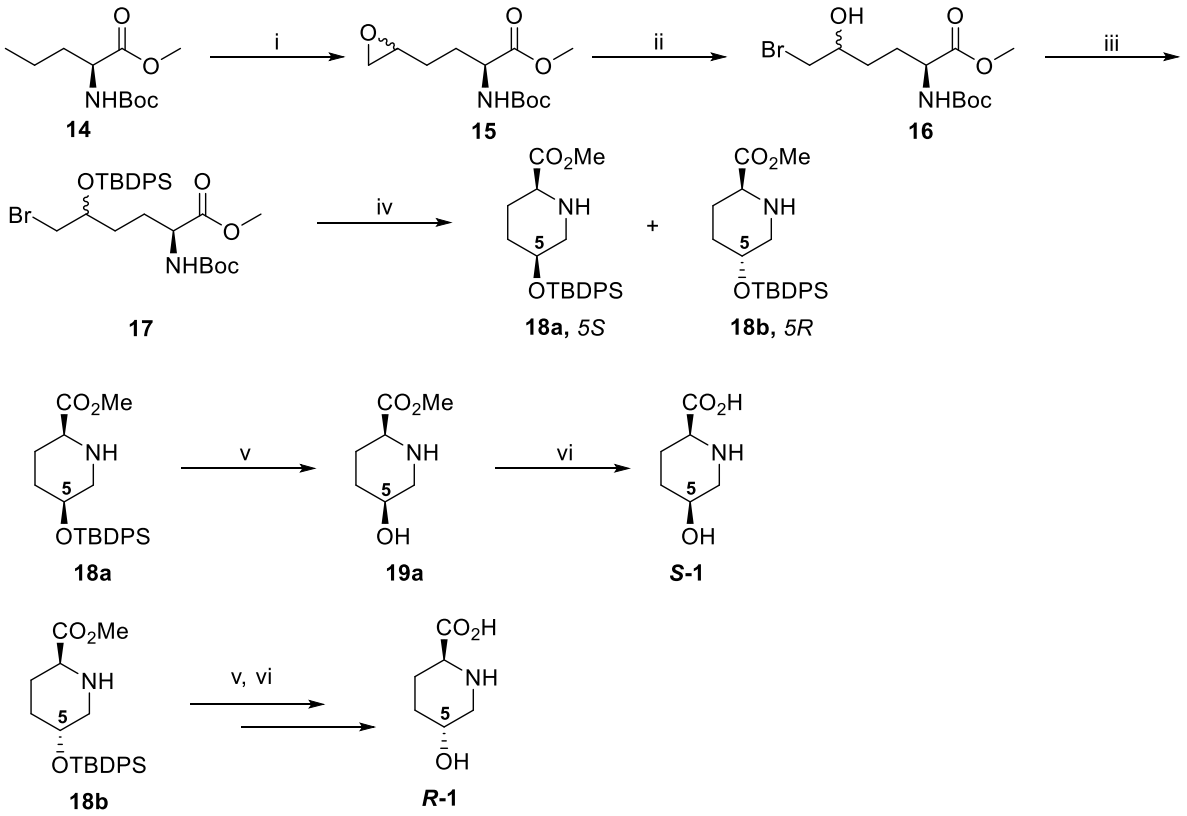
Following the procedure described above, using **13** (0.93 g, 2.47 mmol) as the substrate yields 5-^{13}C-**5** (0.48 g, 1.37 mmol) in 56 % yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.59-5.97 (dm, $J_{C\text{-}H}$ = 151.5 Hz), 5.00-5.05 (m, 1H), 4.95-4.98 (m, 1H), 4.85 (dd, J = 11.5, 5.0 Hz, 1H), 3.69 (s, 3H), 2.17-2.25 (m, 1H), 2.07-2.13 (m, 2H), 1.91-1.99 (m, 1H), 1.47 (s, 18H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.1, 152.2, 137.5, 115.5 (d, $J_{C\text{-}C}$ = 69.0 Hz), 83.2, 57.7, 52.2, 30.4 (d, $J_{C\text{-}C}$ = 41.6 Hz), 29.4 (d, $J_{C\text{-}C}$ = 1.9 Hz), 28.1.



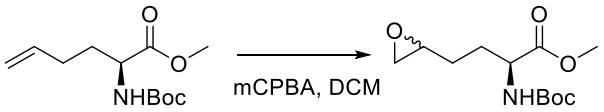
5-^{13}C-2*S*-amino-hex-5-enoic acid (5-^{13}C-2)

Following the procedure for **2** preparation, 5-^{13}C-**5** (0.48 g, 1.39 mmol) is converted to 5-^{13}C-**2** (0.13 g, 0.93 mmol) as lithium salt in 67 % yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.72-6.10 (dm, $J_{C\text{-}H}$ = 152.5 Hz, 1H), 5.08-5.13 (m, 1H), 5.00-5.03 (m, 1H), 3.25 (dd, J = 6.8, 5.8 Hz, 1H), 2.07-2.12 (m, 2H), 1.68-1.76 (m, 1H), 1.59-1.67 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 183.6, 138.9, 114.8 (d, $J_{C\text{-}C}$ = 68.0 Hz), 55.5, 33.9, 29.4 (d, $J_{C\text{-}C}$ = 41.4 Hz).

Preparation of 1

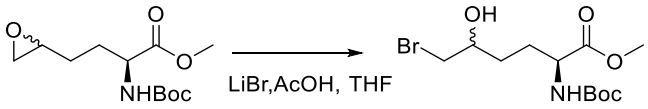


Conditions: (i) mCPBA, DCM; 88 % yield; (ii) LiBr, AcOH, THF, 0 °C→room temperature; 83 % yield; (iii) TBDPSCl, imidazole, DCM; 79 % yield; (iv) (a) TFA, DCM; (b) Na2CO3, MeCN; **16a**, 43 % yield; **16b**, 38 % yield; (v) (a) CbzCl, Na2CO3, THF, (b) TBAF, THF, (c) Pd/C, H2, MeOH; 76 % yield (3 steps); (vi) LiOH, THF/ H2O; 96 % yield. During the reparation of *R*-1,the yeilds for step v and vi are 68% and 92%, respectively.



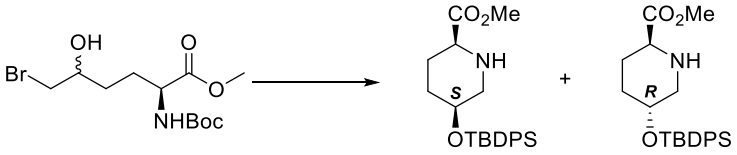
Methyl 2*S*-2-((*tert*-butoxycarbonyl)amino)-4-(oxiran-2-yl)butanoate (15)

14 (1.39 g, 5.72 mmol, 1.0 equiv.) was dissolved in anhydrous DCM (~ 30 mL) followed by the one-portion addition of 75 % mCPBA (3.95 g, 17.15 mmol, 3.0 equiv.). The resulting mixture was allowed to stir at room temperature. The reaction was quenched by adding saturated $Na_2S_2O_3$ aqueous solution. The organic layer was washed with saturated $NaHCO_3$ aqueous solution (~ 30 mL), brine (~ 30 mL) prior to concentration. The crude product was purified via silica gel column chromatography (4:1, hexanes/ethyl acetate) to yield **15** (1.30 g, 5.02 mmol) as a mixture containing both isomers in 88 % yield. ^{1}H NMR spectra match with the reported value.[7]



Methyl 2*S*-6-bromo-2-((*tert*-butoxycarbonyl)amino)-5-hydroxyhexanoate (16)

Following the reported procedures,[7] **15** (1.30 g, 5.02 mmol, 1.0 equiv.) was dissolved in THF (~25 mL) and cooled to 0 °C. It is then followed by addition of acetic aicd (AcOH) (0.89 mL, 15.56 mmol, 3.1 equiv.) and LiBr (0.70 g, 2.76 mmol, 1.6 equiv.) successively at 0 °C. The solution was warmed to room temperature and kept stirring overnight. Once TLC analysis showed complete consumption of the substrate, the reaction mixture was concentrated. It was then dissolved in ethyl acetate (~ 25 mL), washed with saturated $NaHCO_3$ aqueous solution (~ 25 mL), brine (~ 25 mL). The organic layer was concentrated and then purified via silica gel column chromatography (3:1, hexanes/ethyl acetate) to yield **16** (1.56 g, 5.02 mmol) as a mixture containing both isomers in 83 % yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.13-5.22 (m, 1H), 4.31-4.39 (m, 1H), 3.78-3.83 (m, 1H), 3.74 (s, 3H), 3.46-3.50 (m, 1H), 3.34-3.39 (m, 1H), 2.51-2.87 (m, 1H), 1.52-2.05 (m, 4H), 1.43 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.1 (173.2), 155.8 (155.6), 80.3 (80.2), 70.4 (70.9), 52.5 (53.2), 40.0 (40.0), 30.8 (30.7), 29.7 (29.1), 28.4.

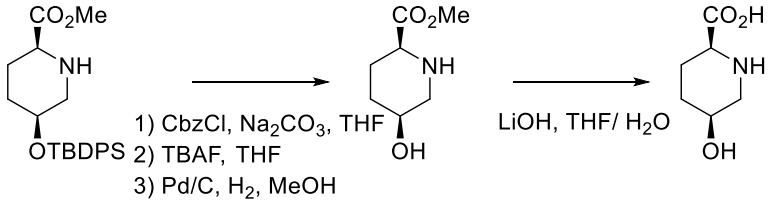


Methyl 2*S*-5-((tert-butyldiphenylsilyl)oxy)piperidine-2-carboxylate (18a and 18b)

To a solution of **16** (0.95 g, 2.55 mmol, 1.0 equiv., in 20 mL of DCM) was added imidazole (0.26 g, 3.82 mmol, 1.5 equiv.) and TBDPSCl (0.86 mL, 3.31 mmol, 1.3 equiv.) sequentially. The reaction mixture was then allowed to stir at room temperature overnight. The reaction mixture was then filtered. The filtrate containing product was concentrated and purified via silica gel column

chromatography (10:1, hexanes/ethyl acetate) to yield **17** (1.16 g, 2.01 mmol) as a mixture of both isomers in 79 % yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.64-7.68 (m, 4H), 7.43-7.46 (m, 2H), 7.38-7.41 (m, 4H), 4.89-4.96 (m, 1H), 4.21-4.26 (m, 1H), 3.83-3.88 (m, 1H), 3.70 (s, 3H), 3.24 (d, *J* = 5.0 Hz, 2H), 1.77-1.88 (m, 1H), 1.57-1.73 (m, 3H), 1.44 (s, 9H), 1.07 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 173.1, 155.4, 135.9, 133.7, 133.4, 130.1, 130.1, 127.9, 127.9, 80.0, 71.6 (71.6), 53.3 (53.2), 52.4, 36.6, 30.5, 28.4, 27.6 (27.5), 27.1, 19.5.

To a solution of **17** (1.16 g, 2.01 mmol, 1.0 equiv., in 15mL of DCM) was added TFA (1.20 mL, 16.08 mmol, 8.0 equiv.) slowly at room temperature. After full consumption of the starting material, the mixture was concentrated under reduced pressure. The crude deprotected product was dissolved in acetonitrile (~ 20 mL). It was followed by addition of Na_2CO_3 (0.53 g, 5.03 mmol, 2.5 equiv.) and the mixture was allowed to stir at room temperature overnight. The reaction was diluted with ethyl acetate (~ 100 mL), washed with brine (~ 20 mL, 3 times), water (~ 20 mL, 2 times) and brine (~ 20 mL). The organic layer was concentrated under reduced pressure and the product was purified via silica gel column chromatography (3:1, hexanes/ethyl acetate) to yield **18b** (0.30 g, 0.76 mmol, 38 % yield). By increasing the eluent polarity to hexanes/ethyl acetate = 1/1, **18a** was obtained (0.34 g, 0.86 mmol) in 43 % yield. **18a**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.64-7.66 (m, 4H), 7.36-7.44 (m, 6H), 3.76-3.79 (m, 4H), 3.42 (dd, *J* = 9.8, 3.3 Hz), 2.86 (ddd, *J* = 13.3, 4.3, 1.3 Hz), 2.69 (dd, *J* = 13.3, 2.3 Hz), 2.25 (broad s, 1H), 1.97-2.04 (m, 1H), 1.66-1.74 (m, 2H), 1.55-1.62 (m, 1H), 1.08 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.0, 135.8, 135.8, 134.2, 134.1, 129.9, 129.9, 127.8, 127.8, 66.1, 57.7, 52.1, 51.2, 31.2, 27.2, 24.8, 19.4. **18b**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.64-7.66 (m, 4H), 7.35-7.44 (m, 6H), 3.60-3.67 (m, 4H), 3.27 (dd, *J* = 11.3, 2.8 Hz), 3.10 (ddd, *J* = 11.6, 4.4, 1.6 Hz), 2.54 (dd, *J* = 11.8, 9.8 Hz), 1.92-2.01 (m, 3H), 1.97-2.04 (m, 1H), 1.45-1.53 (m, 1H), 1.30-1.38 (m, 1H), 1.05 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 173.5, 135.8, 135.8, 134.3, 134.2, 129.8, 129.8, 127.7, 127.7, 68.9, 58.0, 52.8, 52.1, 33.8, 28.5, 27.1, 19.3.



2*S*,5*S*-5-Hydroxypiperidine-2-carboxylic acid (*S*-1)

Following procedure involves derivatization of amine with carboxybenzyl (Cbz) group, which is essential for the purification of the alcohol intermediate obtained through TBDPS deprotection. The product was subjected to hydrogenation to cleave the Cbz group and followed by saponification to afford final product (*S*-**1**). The same reaction sequence was also used to obtain *R*-**1**.

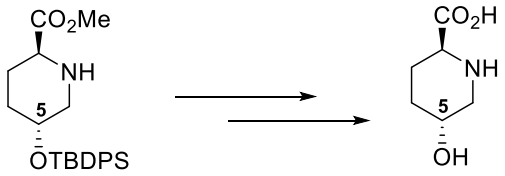
To a solution containing **18a** (0.34 g, 0.86 mmol, 1.0 equiv., in 10 mL THF) was added Na_2CO_3 (0.27 g, 2.57 mmol, 3.0 equiv.) and followed by dropwise addition of CbzCl (0.24 mL, 1.71 mmol,

2.0 equiv.) at room temperature. After stirring overnight, the mixture was concentrated under reduced pressure and the Cbz protected product was obtained by silica gel column chromatography (10:1, hexanes/ethyl acetate) in 99 % yield (0.45 g, 0.85 mmol). ^{1}H NMR (500 MHz, $CDCl_3$, 3:2 rotamers): δ 7.62-7.66 (m, 4H), 7.27-7.45 (m, 11H), 5.03-5.15 (m, 2H), 4.81 (d, *J* = 5.0 Hz, 0.6H), 4.67 (d, *J* = 5.0 Hz, 0.4H), 4.23 (dd, J = 13.0, 5.0 Hz, 0.4H), 4.06 (dd, J = 13.0, 5.0 Hz, 0.6H), 3.75 (s, 1.8H), 3.70 (s, 1.2H), 3.53-3.63 (m, 1H), 2.88 (dd, J = 12.5, 10.5 Hz, 0.6H), 2.83 (dd, J = 12.5, 10.5 Hz, 0.4H), 2.16-2.21 (m, 0.6H), 2.10-2.15 (m, 0.4H), 1.78-1.84 (m, 0.6 H), 1.71-1.76 (m, 0.4H), 1.27-1.56 (m, 2H), 1.05 (s, 3.6H), 1.04 (s, 5.4H).

The Cbz protected product (0.45 g, 0.85 mmol, 1.0 equiv.) was dissolved in THF (~ 10 mL). Tetrabutylammonium fluoride solution (TBAF) (1.3 mL, 1.0 M in THF, 1.5 equiv.) was added dropwise to the reaction. The progress was monitored by TLC. Once completion, the reaction was quenched with H_2O (~ 10 mL), and the mixture was extracted with DCM (~ 10 mL, 3 times). The combined organic layers were washed with brine (~ 10 mL), concentrated and purified via silica gel column chromatography (1:1, hexanes/ethyl acetate) to yield the corresponding alcohol (0.19 g, 0.65 mmol) in 76 % yield. ^{1}H NMR (500 MHz, $CDCl_3$, 3:2 rotamers): δ 7.30-7.37 (m, 5H), 5.10-5.19 (m, 2H), 4.91 (d, *J* = 5.0 Hz, 0.6H), 4.79 (d, *J* = 5.0 Hz, 0.4H), 4.29 (dd, *J* = 12.0, 3.5 Hz, 0.4 H), 4.20 (dd, *J* = 12.0 Hz, 3.5 Hz, 0.6H), 3.75 (s, 1.8H), 3.70 (s, 1.2H), 3.60-3.68 (m, 1H), 2.84 (t, *J* = 11.5 Hz, 0.6 H), 2.74 (t, *J* = 11.5 Hz, 0.4H), 2.30 (t, *J* = 13.5 Hz, 0.6H), 1.97-2.00 (m, 1H), 1.69-1.82 (m, 2H), 1.21-1.29 (m, 1H).

Followed by addition of Pd/C (10% Pd loading, catalytic amount) to a solution (5 mL of MeOH) containing the alcohol (0.19 g, 0.65 mmol), the reaction was subjected to hydrogenation under atmospheric pressure using balloon. Subsequently, the mixture was filtered through celite. The filtrate was concentrated under vacuum to the give **19a** (0.10 g, 0.65 mmol) in 99 % yield. ^{1}H NMR (500 MHz, CD_3OD): δ 3.71-3.75 (m, 4H), 3.52 (dd, *J* = 9.0, 3.0 Hz, 1H), 2.85-2.92 (m, 2H), 1.92-1.99 (m, 1H), 1.77-1.83 (m, 2H), 1.68-1.74 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD): 174.4, 64.8, 57.8, 52.6, 50.9, 31.2, 25.0.

Compound **19a** (0.10 g, 0.65 mmol, 1.0 equiv.) was dissolved in a mixture of water and THF (1:2, ~ 6 mL). Lithium hydroxide (19 mg, 0.78 mmol, 1.2 equiv.) was added to the reaction in one portion. The reaction was monitored by TLC. The mixture was concentrated to yield ***S*-1** (0.62 mmol) in 96 % yield as a lithium salt. ^{1}H NMR (500 MHz, D_2O): δ 3.75-3.78 (m, 1H), 3.16 (dd, *J* = 8.3, 3.8 Hz, 1H), 2.77-2.82 (m, 2H), 1.63-1.82 (m, 4H); ^{13}C NMR (125 MHz, D_2O): δ 181.3, 64.5, 58.6, 48.7, 29.7, 24.4.



2*S*,5*R*-5-Hydroxypiperidine-2-carboxylic acid (*R*-1)

Compound ***R*-1** was obtained in a similar manner using **18b** as the substrate. The corresponding yield and NMR characterization are listed below. **19b** (81 mg, 0.51 mmol) was obtained in 67 % yield. ^{1}H NMR (500 MHz, CD_3OD): δ 3.75 (s, 3H), 3.56-3.62 (m, 1H), 3.34 (dd, *J* = 2.3 Hz, 11.3 Hz, 1H), 3.18 (ddd, *J* = 1.1 Hz, 4.0 Hz, 11.6 Hz, 2H), 2.43 (dd, *J* = 10.0 Hz, 11.3 Hz, 1H), 2.05-2.14 (m, 2H), 1.50-1.58 (m, 1H), 1.38-1.46 (m, 1H); ^{13}C NMR (126 MHz, CD_3OD): 174.2, 67.3, 58.7, 52.8, 52.5, 33.8, 28.8. ***R*-1** (0.47 mmol) was obtained in 92 % yield. ^{1}H NMR (500 MHz, D_2O): δ 3.57-3.61 (m, 1H), 3.11 (ddd, *J* = 12.1, 4.6, 1.5 Hz, 1H), 2.99 (dd, *J* = 11.0, 3.0 Hz, 1H), 2.33 (dd, *J* = 11.8, 10.8 Hz, 1H), 2.05-2.10 (m, 1H), 2.01-2.04 (m, 1H), 1.39-1.44 (m, 1H), 1.33-1.38 (m, 1H); ^{13}C NMR (126 MHz, D_2O): δ 180.9, 67.0, 59.7, 50.6, 32.5, 28.8.

Based on the coupling analysis and the 2D NMR correlation experiments (COSY and NOESY), the stereochemistry at C5 center is at *R*-configuration for ***R*-1**. The COSY and NOESY spectra, and summary is shown in Figure S22 and S23.

Over-expression and purification of LdoA

Over-expression and purification of LdoA was carried out following the reported procedure.[8]

Preparation of NO treated LdoA, EPR and HYSCORE experiment

Preparation of NO treated LdoA EPR samples

Diethylamine (DEA) NONOate (Cayman Chemical) was used as the NO source, which releases 1.5 moles of NO gas from per mole of parent compound with a half-life of 16 mins at 22-25 ºC at pH7.4. To prepare NO treated LdoA solutions, 0.75 mM apo LdoA was first incubated anaerobically with 0.9 equiv. Fe(II) and 10 equiv. 2OG to form the LdoA•Fe(II)•2OG tertiary complex, or with the further addition of 30 equiv. substrates (4-D_2-**2**, 5-D_1-**2**, 6-D_2-**2**) to form the LdoA•Fe(II)•2OG•substrate quaternary complex. DEA NONOate powder was then anaerobically dissolved in buffer containing 100mM Tris-HCl (pH 7.5) to make 100 mM NONOate stock solution. Then an appropriate amount of stock solution, which will generate ~ 5 mM (final concentration) NO gas, was added into the LdoA tertiary complex and the LdoA quaternary complex. The solutions were further incubated anaerobically for 30 min at room temperature before transferred into EPR tubes and frozen in liquid nitrogen. The final sample concentrations are listed in the figure caption of Figure S25.

X-band CW EPR experiment

X-band EPR spectra were measured on a Bruker Elexsys E-500 spectrometer equipped with an Oxford ESP-910 cryostat. The instrument conditions for the measurements were microwave frequency, 9.64 GHz, microwave power, 0.2 mW; modulation frequency, 100 kHz; modulation amplitude, 1 mT. The spectral simulations were performed by using the SpinCount software.[9]

HYSCORE experiment

Q-band (33.83 GHz) pulsed EPR spectra were recorded using a custom-built ELEXSYS E580 spectrometer equipped with SuperQ-FT bridge, SuperQ-FT solid state 10 W amplifier, and EN5107D2 resonator. All spectra were measured at T=3.7 K maintained inside ER 4118CF helium flow cryostat by ER 4112HV-1017 temperature controller (all from BrukerBiospin, Billerica, MA). Durations of 90° and 180° microwave pulses were 8 and 14 ns, respectively.

Hyperfine sublevel correlation spectroscopy (HYSCORE) data were acquired using a four-pulse sequence with 16-step phase cycling scheme.[10] Due to fast electron spin relaxation rates, the interval between the first and the second pulses was set as short as 140 ns. For shorter time separations, the ring-down effects started to be significant and increased the noise of the detected signals. Delays between the second and the third pulses in the first dimension and between the third and the fourth pulses in the second dimension were incremented by 8 ns steps from their initial values of 24 ns and 32 ns, respectively. Pulse delays were defined as time delays between the starting points of the pulses. The difference between the initial delays was set to 6 ns to improve symmetry of the measured HYSCORE spectra with respect to the two dimensions by considering the difference in the pulse durations. Time domain HYSCORE spectra were recorded as [500×500] matrix. A third power polynomial baseline correction was applied, followed by a Hamming window apodization. Time domain data were zero filled to yield [2048×2048] matrix, Fourier transformed, and plotted using a "contour" function of Matlab R2012a (MathWorks, Natick, MA).

Simulations of 2H-HYSCORE spectra were carried out using EasySpin-5.2.20[11] based on treating the observed lower Kramer's doublet $S = \frac{3}{2}$, $m_s = +\frac{1}{2} \leftrightarrow -\frac{1}{2}$ transitions as an effective $S = \frac{1}{2}$ system. In such HYSCORE spectra the electron-nuclear hyperfine interaction, A, is detected as an effective hyperfine interaction $A_{\text{eff}} \approx 2A$ while the quadrupole parameters remain unchanged.[12] No orientational selectivity has been assumed in the simulations. As an approximation, electron-nuclear interactions were considered as "point-dipolar", *i.e.* having the isotropic component of the interaction equal to zero as well as being axially symmetric. This should be a reasonable approximation, since the distances between the iron center and the deuterons were large enough to consider the electron spin density transfer as well as delocalization of electron both be insignificant.

The obtained values of the hyperfine parameters for the substrate **2** labelled at C4, C5 and C6 positions with ^{2}H are listed in the Table S1. It should be noted here, however, that the quadrupole interaction in the experimental spectra is largely masked by line broadening effect caused by the short T_1 relaxation of the electron spins and, thus, cannot be considered as accurate in any sense.

LC-MS and NMR analysis of the LdoA catalyzed reactions

LC-MS experiments

High performance liquid chromatography with detection by mass spectrometry (LC-MS) was carried out 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 package were used for data collection and analysis. Reaction mixtures were separated on an Agilent Poroshell 120 hilic column (4.7 x 50 mm, 2.7 μm particle size) with a gradient elution using solvent A (water with 0.1 % (v/v) of formic acid) and solvent B (acetonitrile) with flow rate of 0.4 mL/min. It started with 5 % solvent A, and a gradient from 5 to 59 % of solvent A was applied from 2-14 mins. It was followed by 59% of solvent A from 14-16 mins. The column was allowed to re-equilibrate for 7 mins prior to next analysis. Detection was performed under electrospray ionization in positive mode (ESI^+). The drying gas temperature was 350 °C with a nebulizer pressure of 35 psi and flow rate of 12 L/min. The capillary voltage is set to 3000 V.

An anaerobic solution of LdoA•Fe(II)•substrate (L-leucine, **2**, 4-D_2-**2** or 5-D_1-**2**)•2OG prepared in the glove box was exposed to air with the final concentrations of 0.10 mM Fe(II), 0.12 mM of protein, 0.5 mM of substrate, and 1.0 mM of 2OG in a total volume of 200 μL at 4 °C. In the assay mixtures, L-glycine with a final concentration of 0.25 mM was used as the standard. After 1 h incubation, acetonitrile (400 μL) was added to the reaction. The reaction mixtures were centrifuged at 14,000 rpm for 40 mins to precipitate the protein. The clean solutions were subjected to LC-MS analysis. The LC-MS chromatograms are shown in Figure 1.

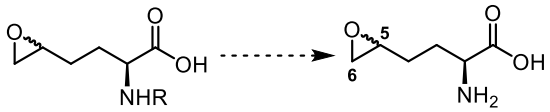
For the time-dependent measurement (Figure 2), the assays were carried analogously. After 1 h incubation, the protein was removed via filtration (2K-filter Poll®, 12,000rpm for 5 min). The filtrate was then instantly subjected to LC-MS. After 14 h, the filtrate left in the vial was then again submitted to LC-MS to obtain the 14h time point result.

^{13}C-NMR experiments

Reaction mixtures containing LdoA, Fe(II), ascorbate, 2OG and substrate (5-^{13}C-**2** or 6-^{13}C-**2**) with the final concentration of 0.44 mM LdoA, 0.4 mM Fe(II), 2.0 mM ascorbate, 4.0 mM 2OG and 1.4 mM substrate with final volume of 700 μL in 50 mM TRIS (pH 7.64) were prepared. The reaction mixtures were left on a plate rotator with a speed of 70 rpm for 12 hours at 4 ˚C. Prior to NMR, 30 μL of d_6-DMSO was added to the reaction and followed by centrifugation at 14,000 rpm for 5 minutes. The supernatant was then transferred to the NMR tube. The ^{13}C NMR spectra were recorded using Bruker NEO 700 MHz with 16 scans. The NMR spectra of LdoA catalyzed reaction are shown in Figure 2 and S24.

Label position	$A_{\perp}$ [MHz]	$A_{\|\|}$ [MHz]	e^2qQ/h [MHz]	η
C4	-0.3	0.6	0.02	0.2
C5	-0.65	1.3	0.04	0.2
C6	-0.45	0.9	0.02	0.2

Table S1. Electron-nuclear and nuclear quadrupole parameters of the simulations shown in the Figure 1.



3

Entry	Conditions	Results
1 (R=Boc)	TFA, DCM	
2 (R=Cbz)	Pd/C, H_2, H_2O	Complete or partial decomposition of the epoxide moiety
3 (R=Cbz)	Pd/C, H_2, H_2O, TFA	
4 (R=Cbz)	Pd/C, H_2, THF, AcOH	
5 (R=Cbz)	Pd/C, H_2, KPi Buffer	

Table S2. Conditions tried to prepare **3**. Under either acidic (TFA) or neutral condition (KPi aqueous buffer), based on the ^{13}C-NMR of the crude reaction products, the epoxide moiety is decomposed during the all conditions listed above.

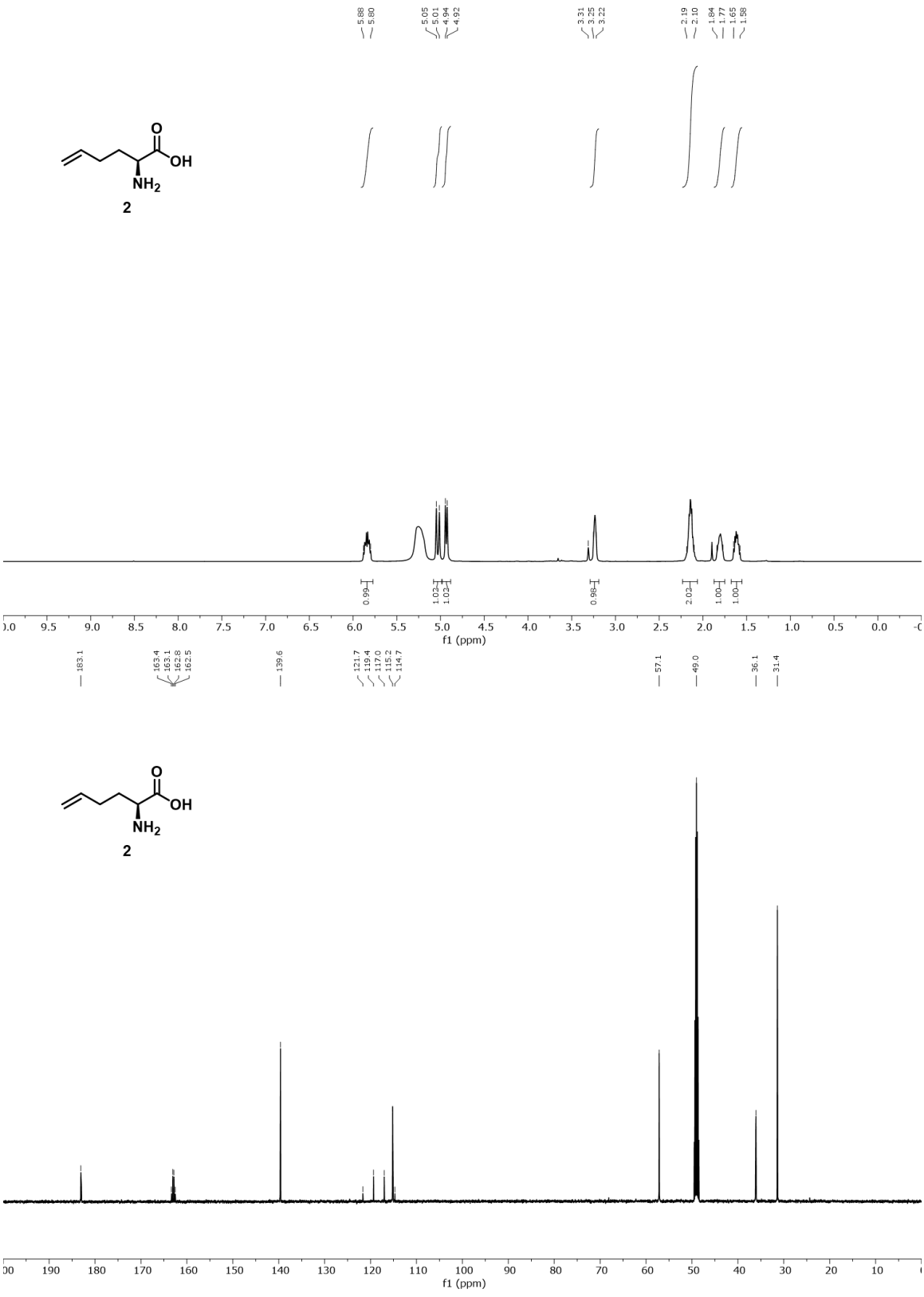


Figure S1. ^{1}H and ^{13}C NMR spectra of compound **2**.

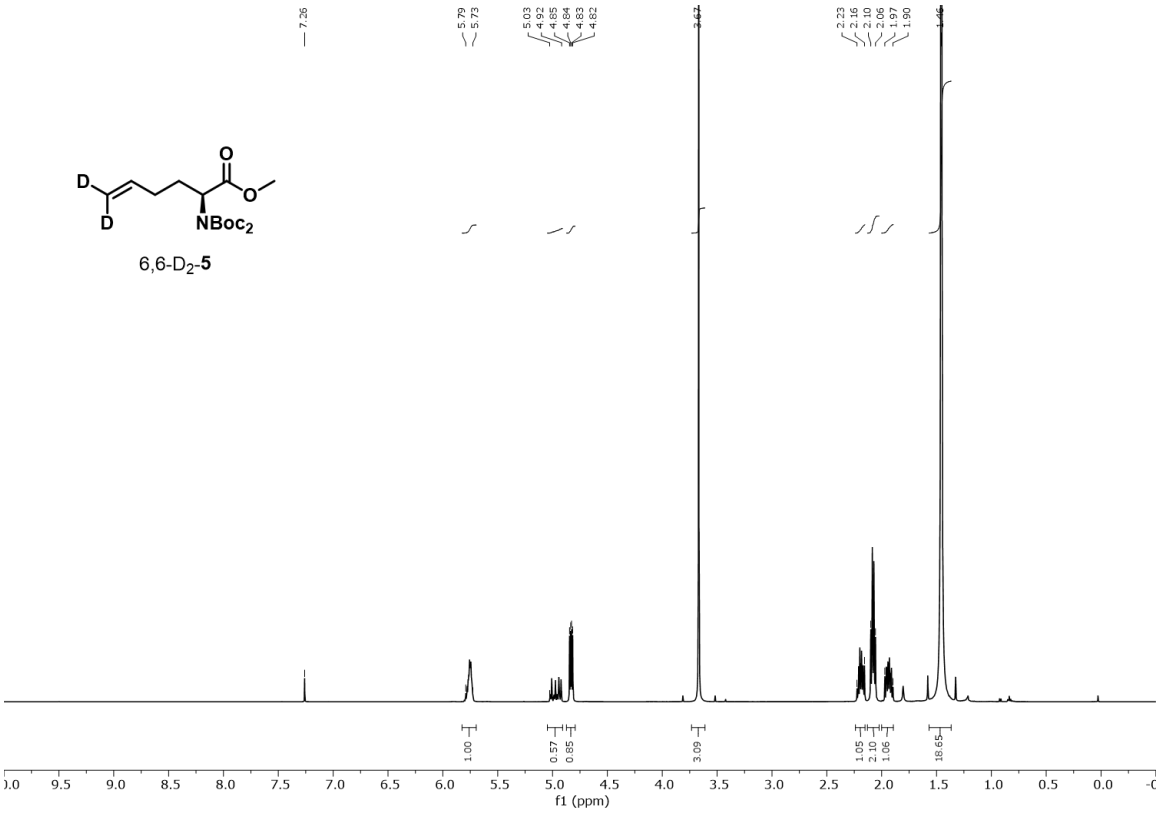


Figure S2. ^{1}H NMR spectra of compound 6,6-D_2-**5**.

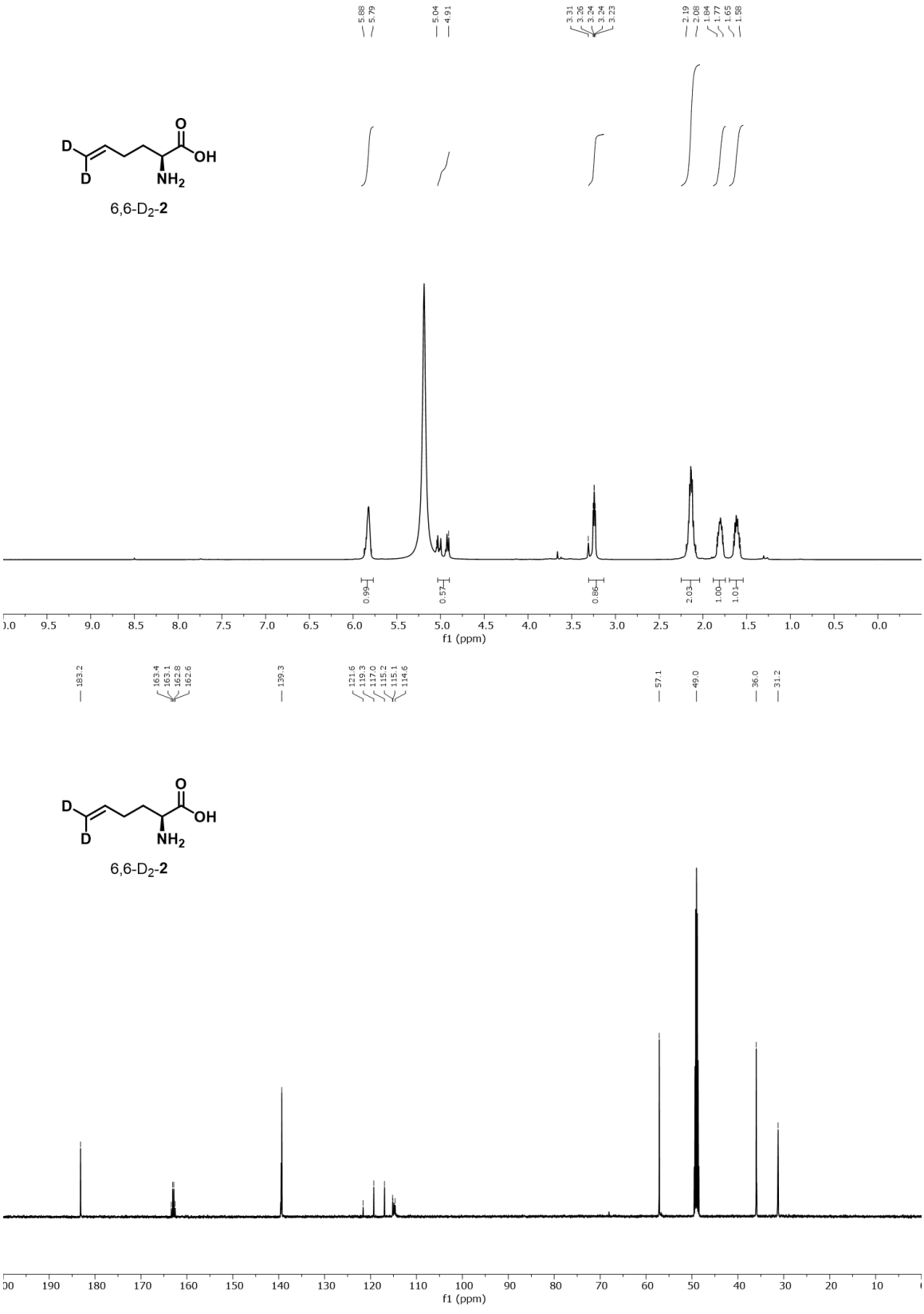


Figure S3. ^{1}H and ^{13}C NMR spectra of compound 6,6-D_2-**2**.

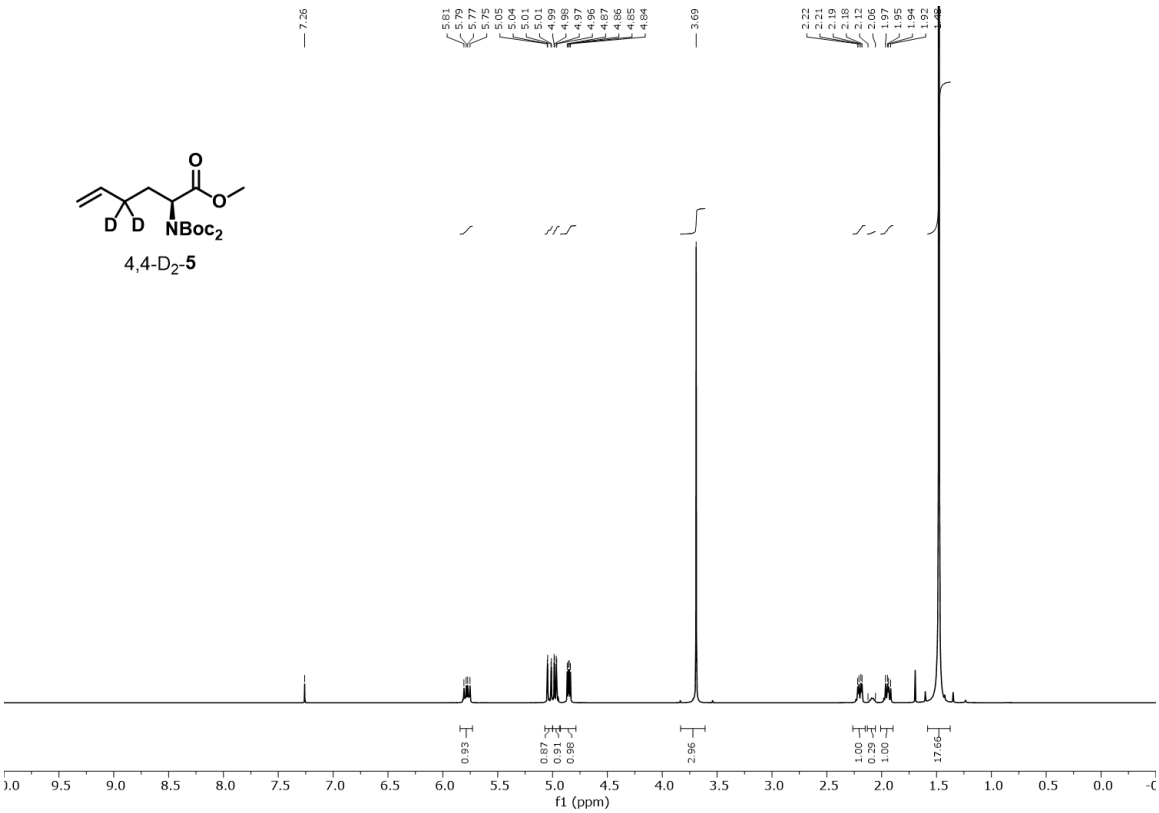


Figure S4. ^{1}H NMR spectra of compound 4,4-D_2-**5**.

Figure S5. ^{1}H and ^{13}C NMR spectra of compound 4,4-D_2-**2**.

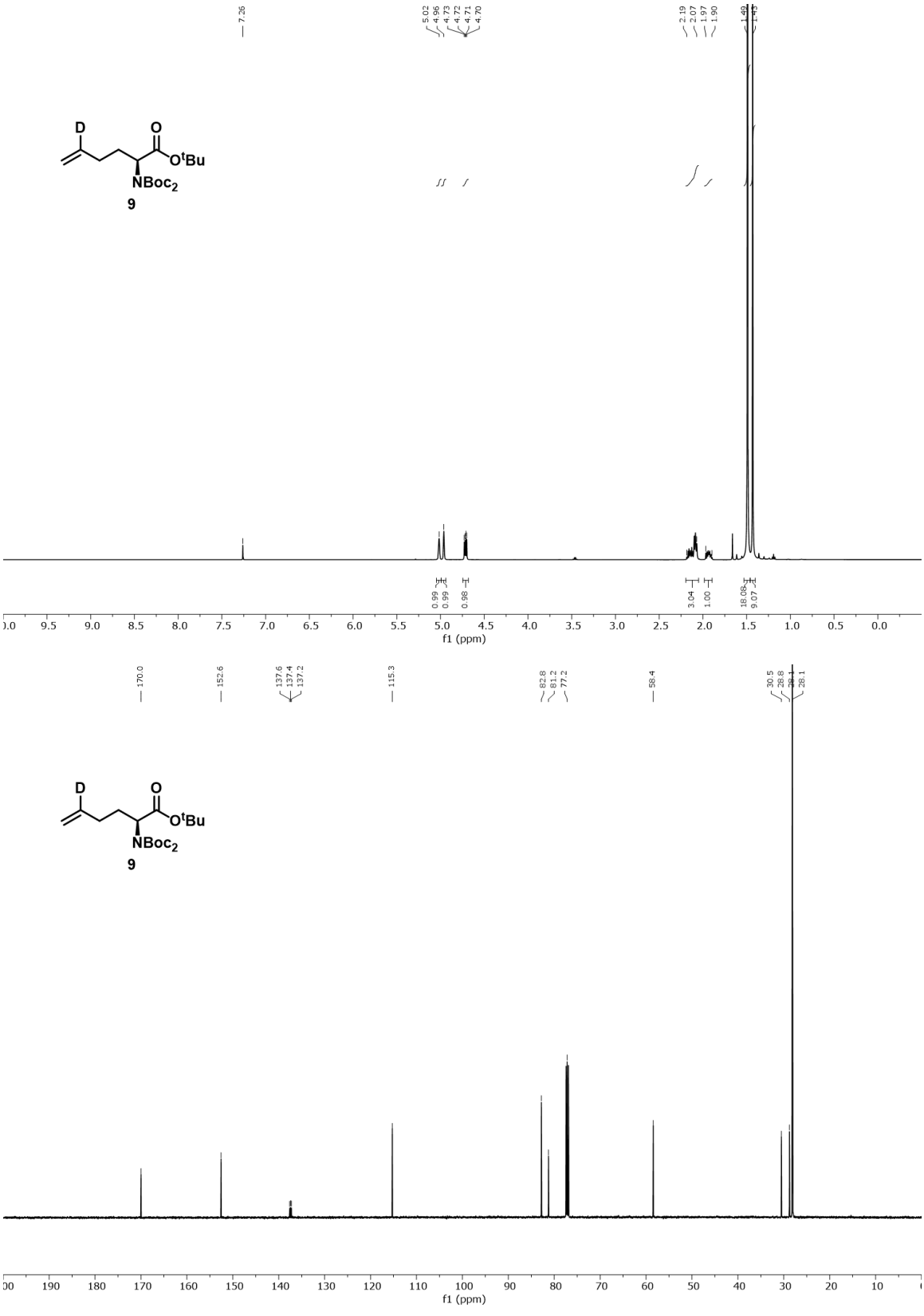


Figure S6. ^{1}H and ^{13}C NMR spectra of compound **9**.

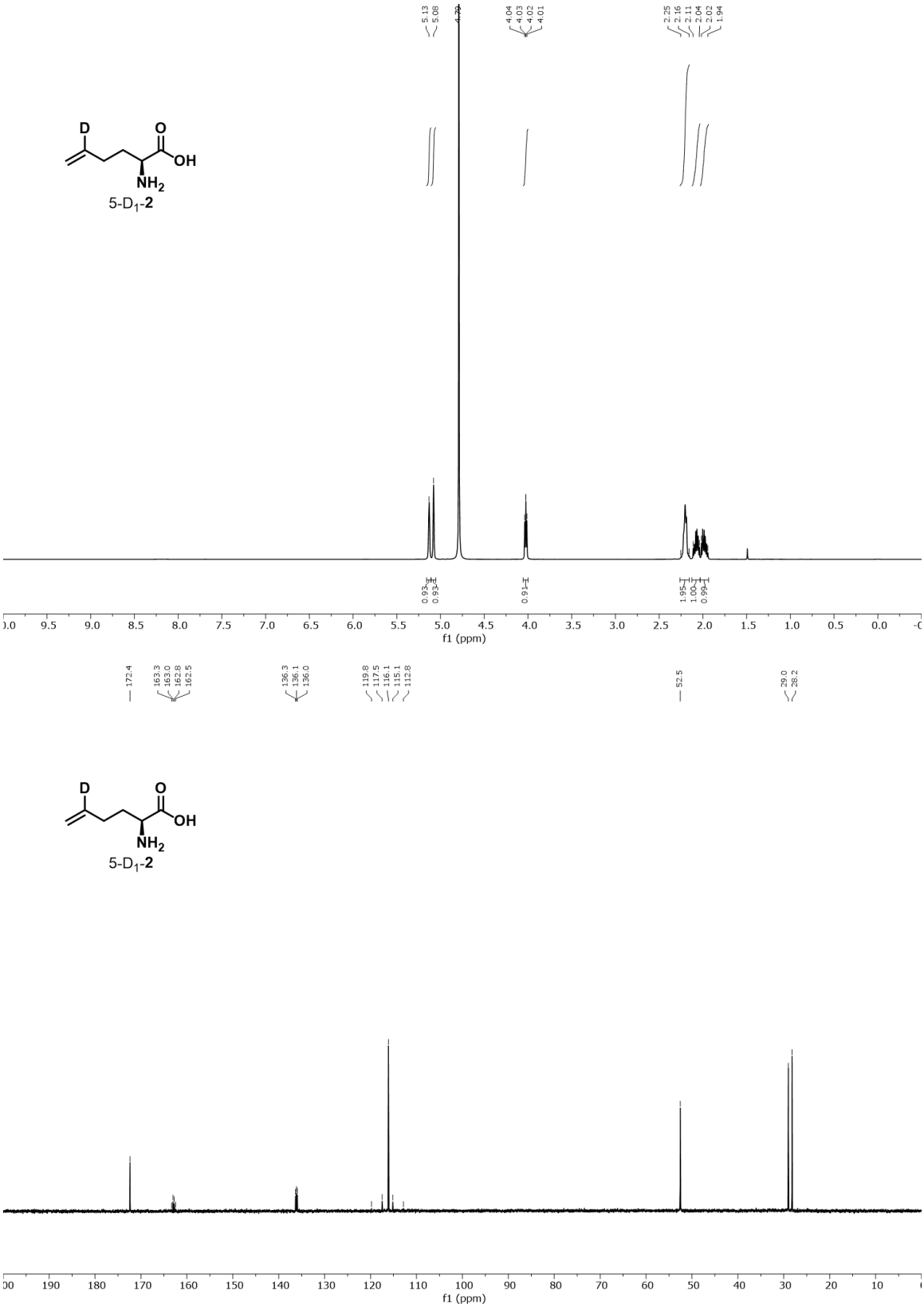


Figure S7. ^{1}H and ^{13}C NMR spectra of compound 5-D_1-**2**.

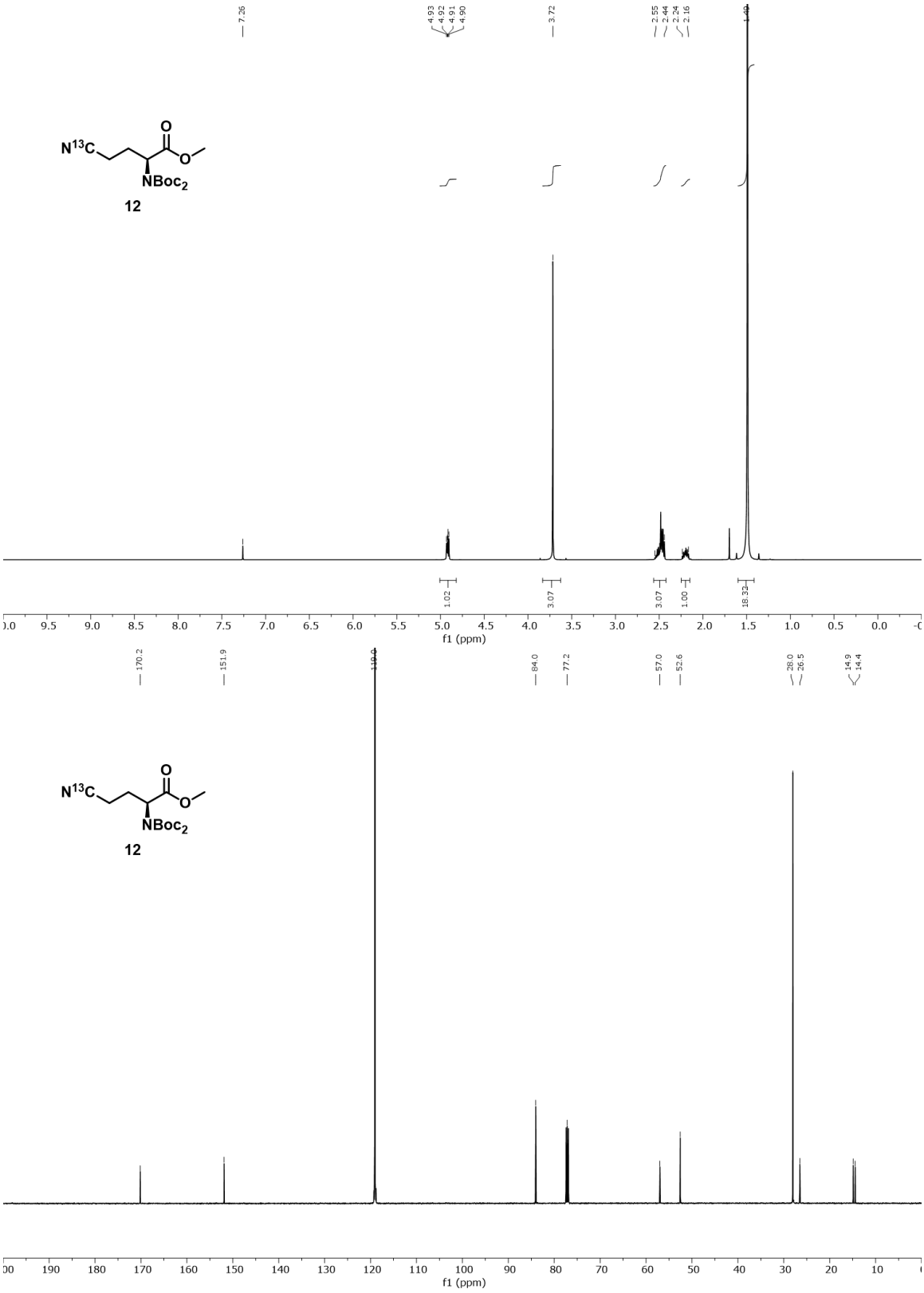
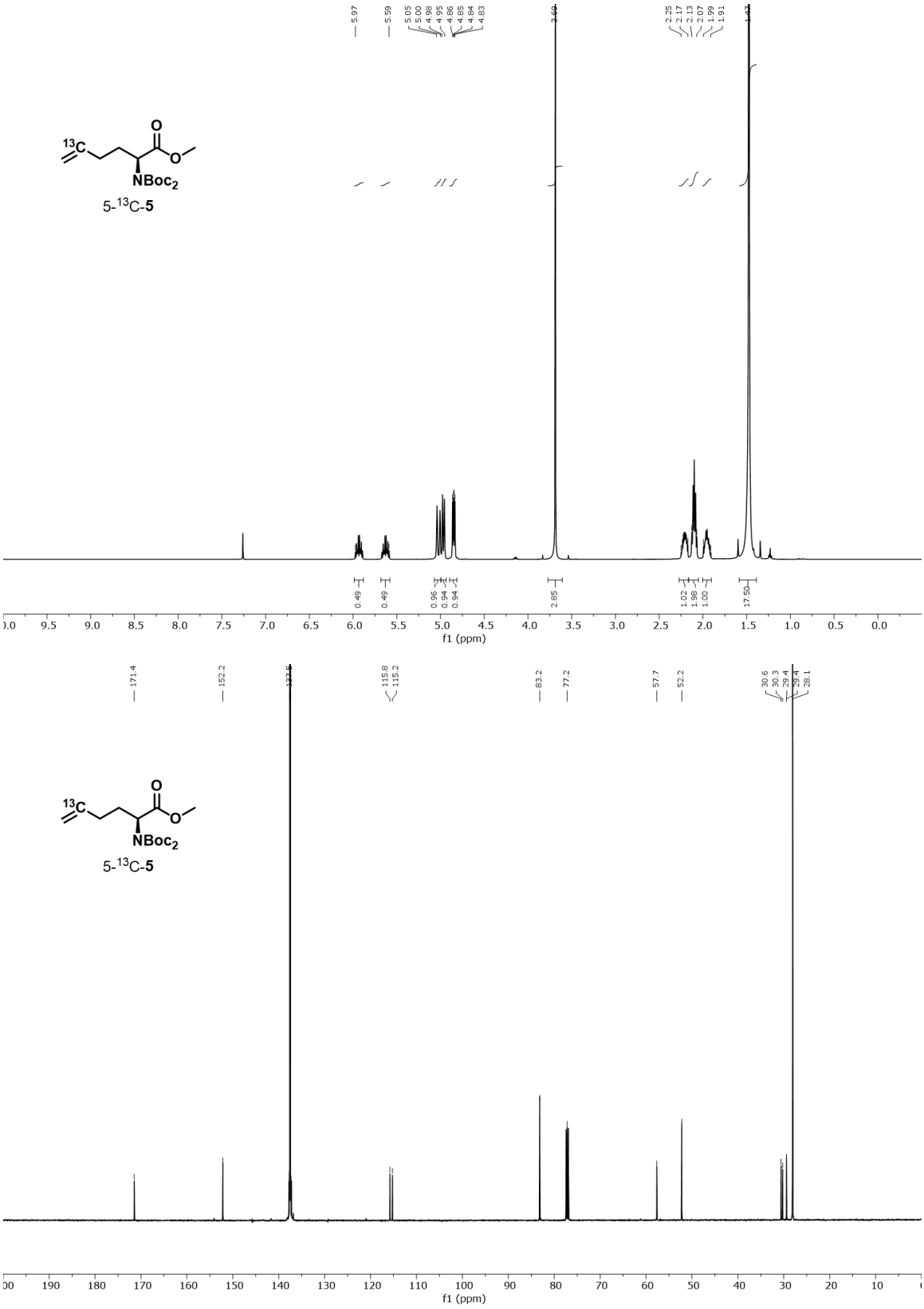


Figure S8. ^{1}H and ^{13}C NMR spectra of compound **12**.



5-^{13}C-**5**

Figure S9. ^{1}H and ^{13}C NMR spectra of compound 5-^{13}C-**5**.

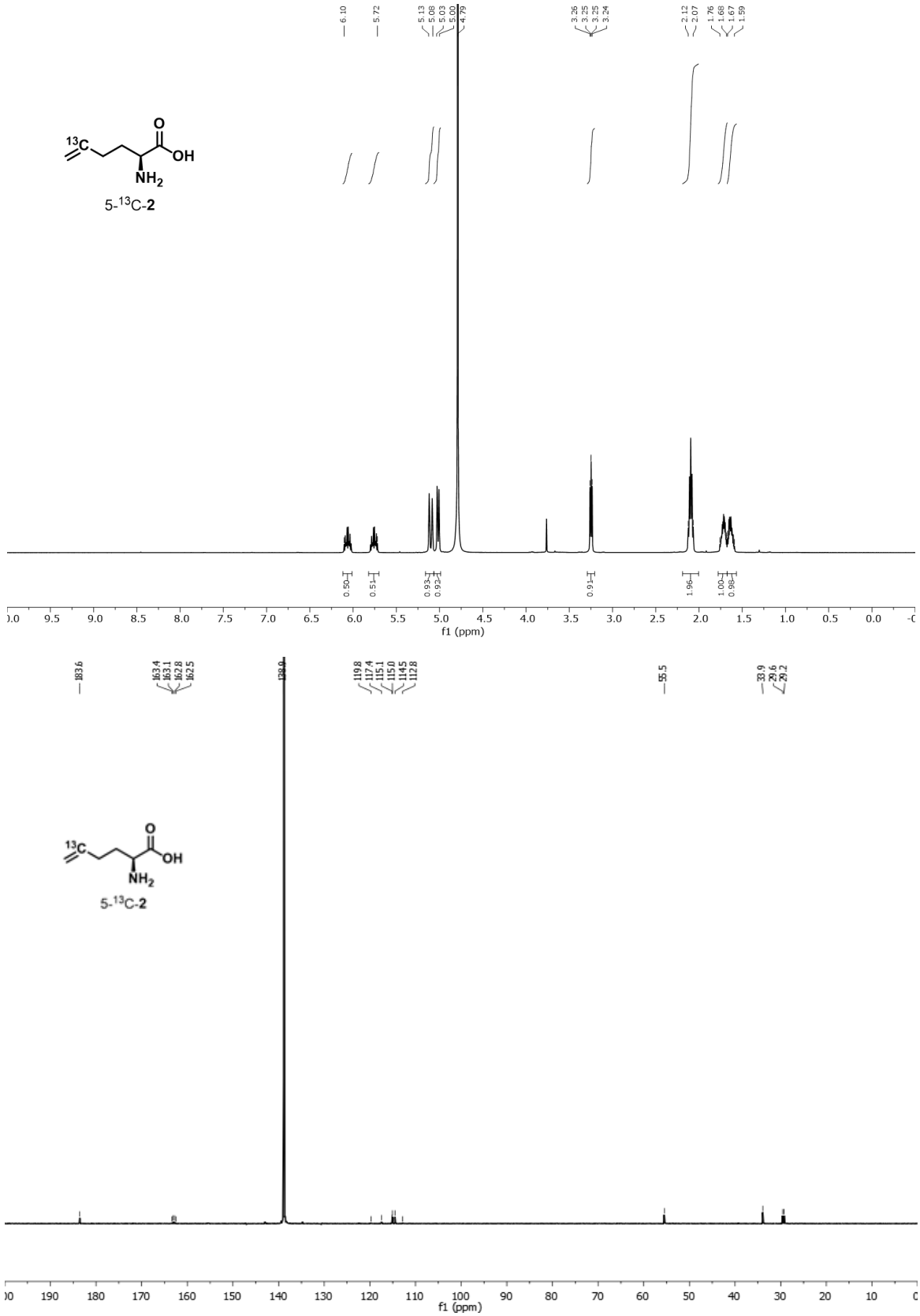
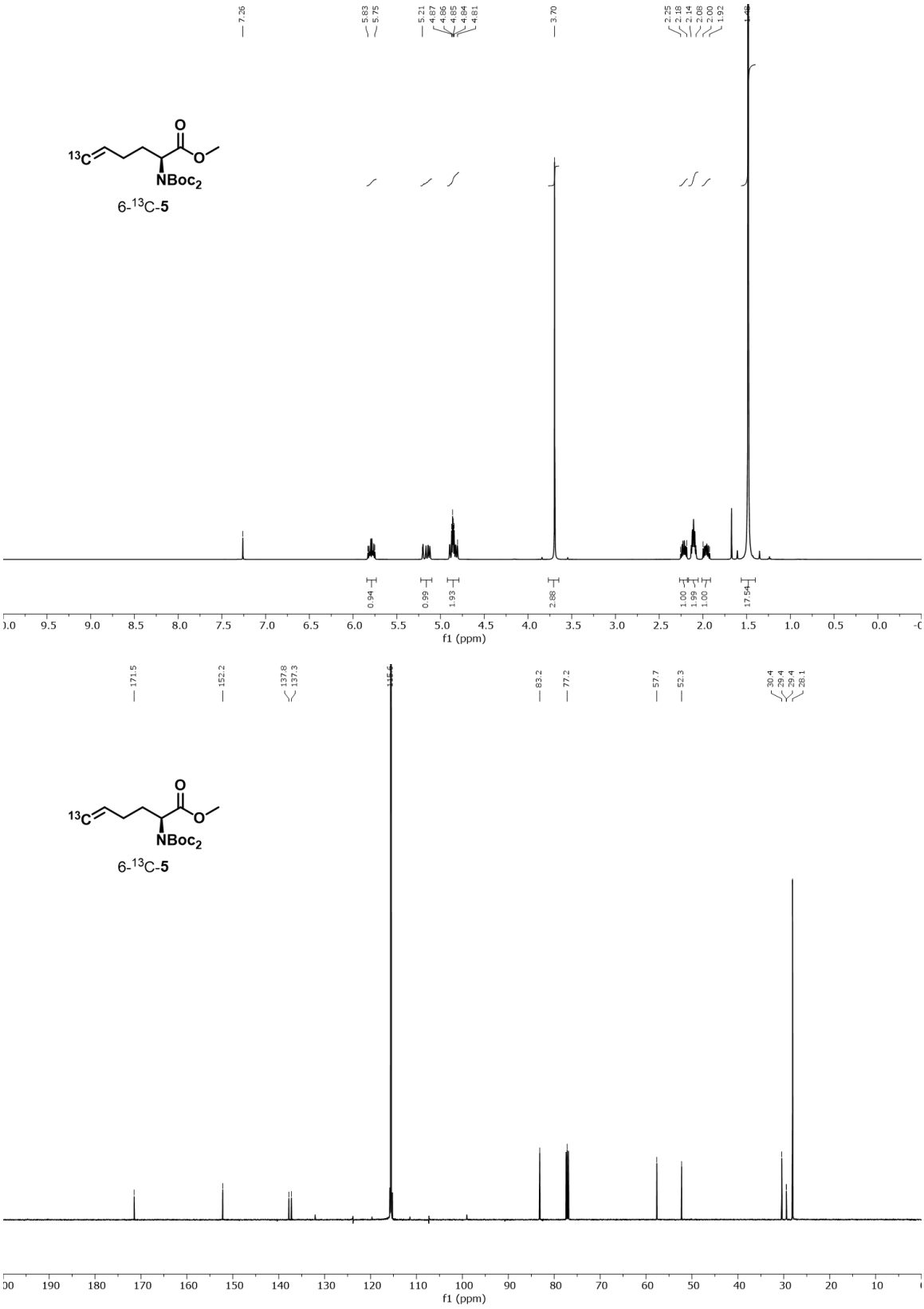


Figure S10. ^{1}H and ^{13}C NMR spectra of compound 5-^{13}C-**2**.



^{13}C

$NBoc_2$

6-^{13}C-**5**

Figure S11. ^{1}H and ^{13}C NMR spectra of compound 6-^{13}C-**5**.

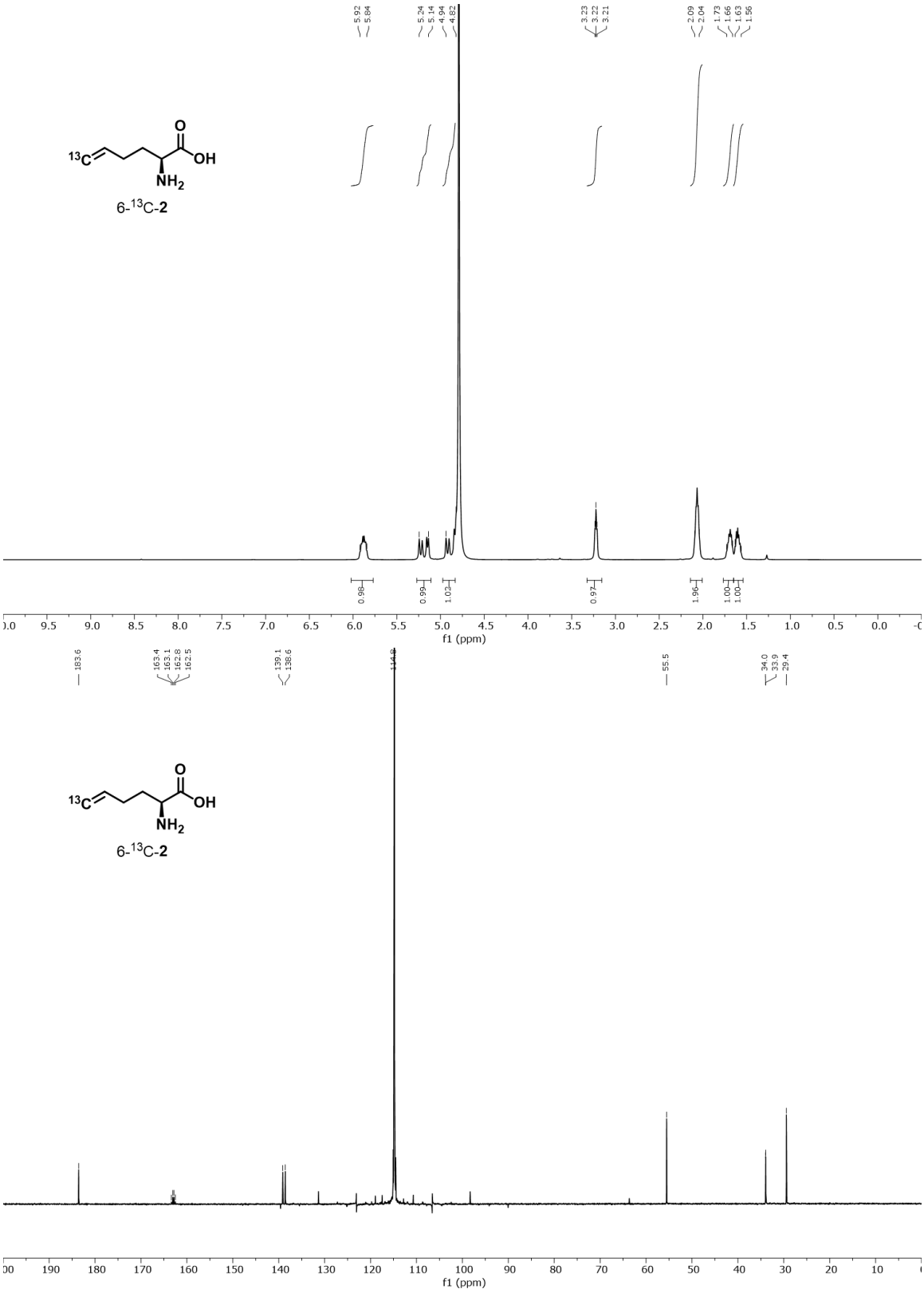


Figure S12. ^{1}H and ^{13}C NMR spectra of compound 6-^{13}C-**2**.

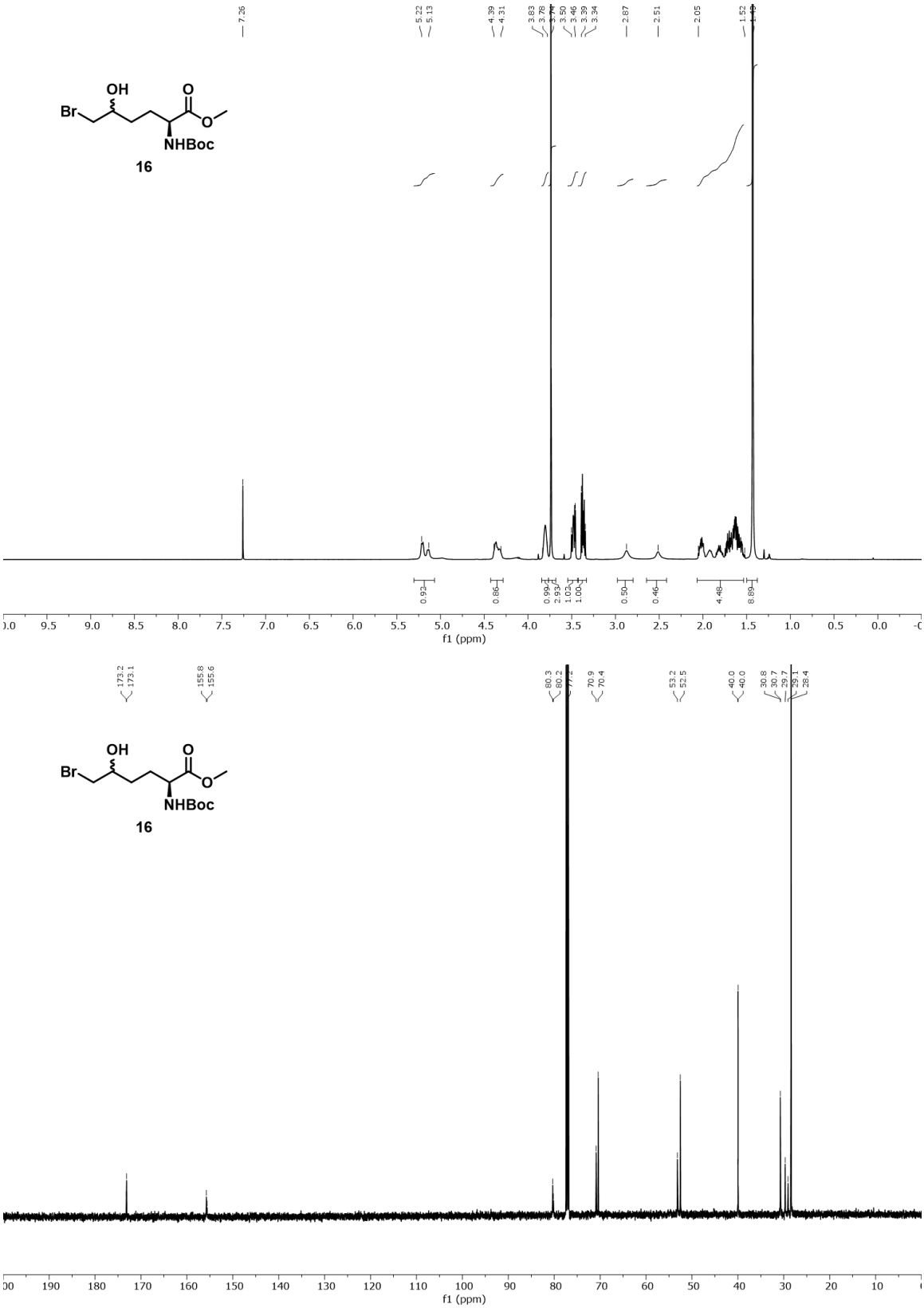


Figure S13. ^{1}H and ^{13}C NMR spectra of compound **16**.

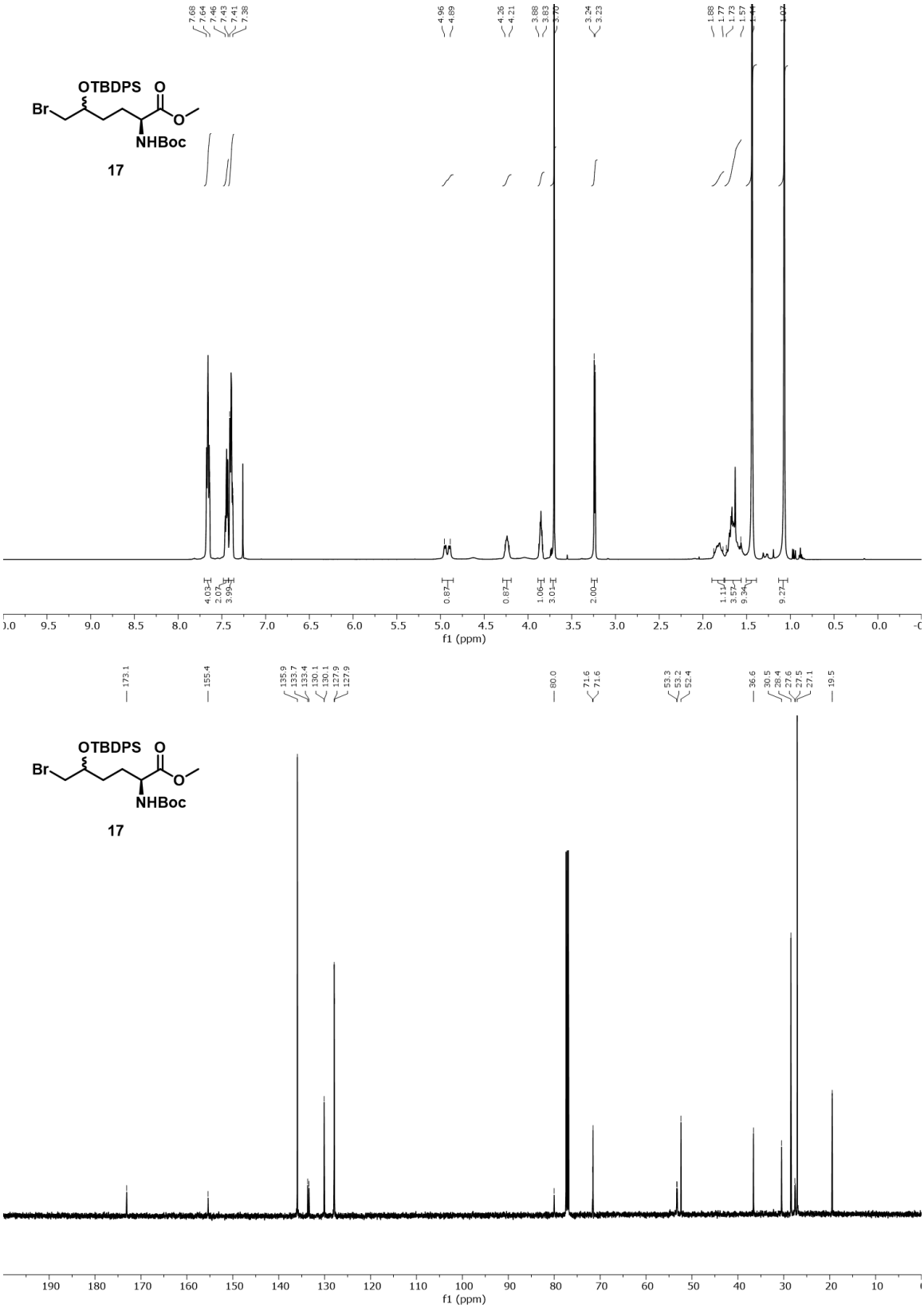


Figure S14. ^{1}H and ^{13}C NMR spectra of compound **17**.

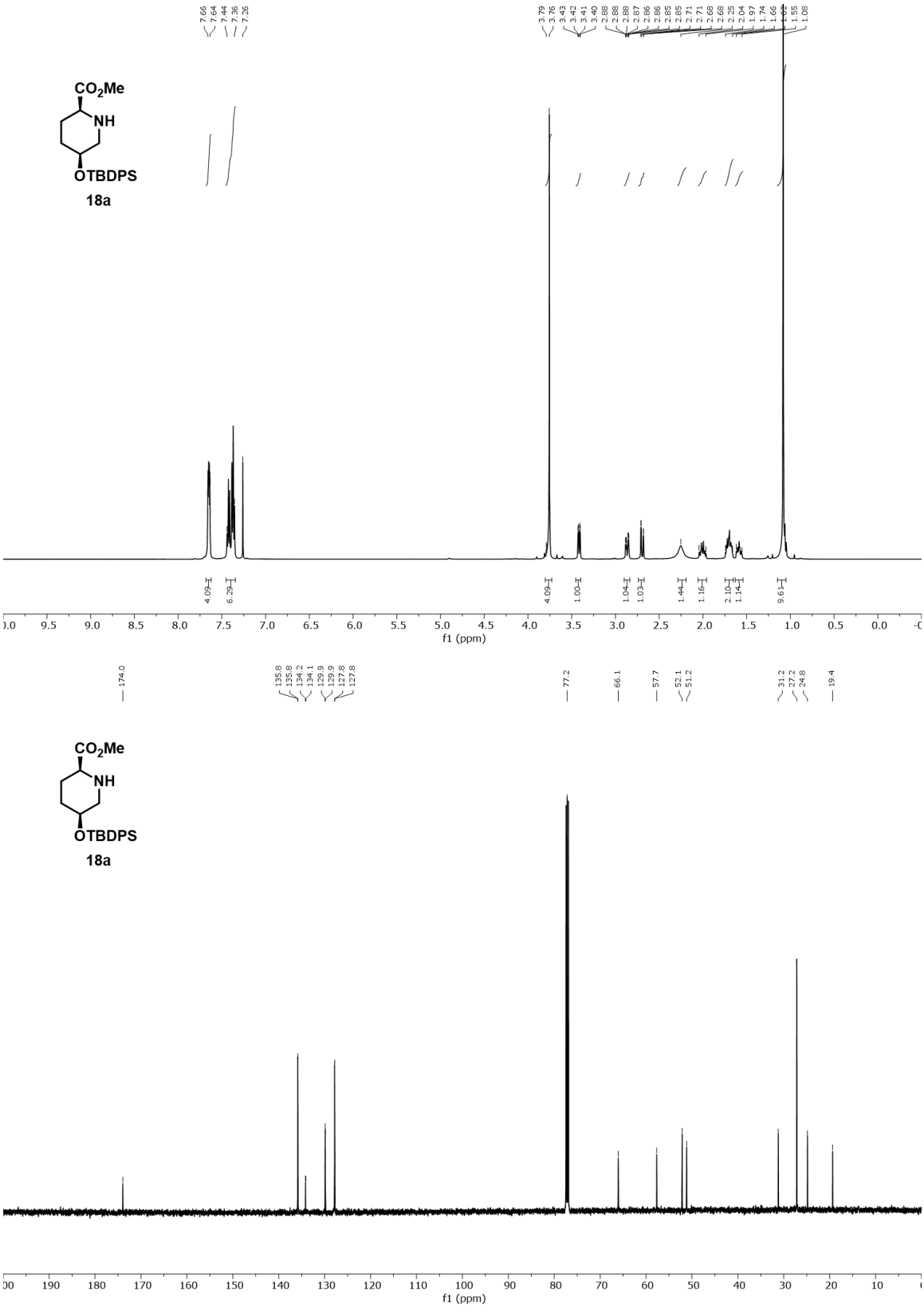


Figure S15. ^{1}H and ^{13}C NMR spectra of compound **18a**.

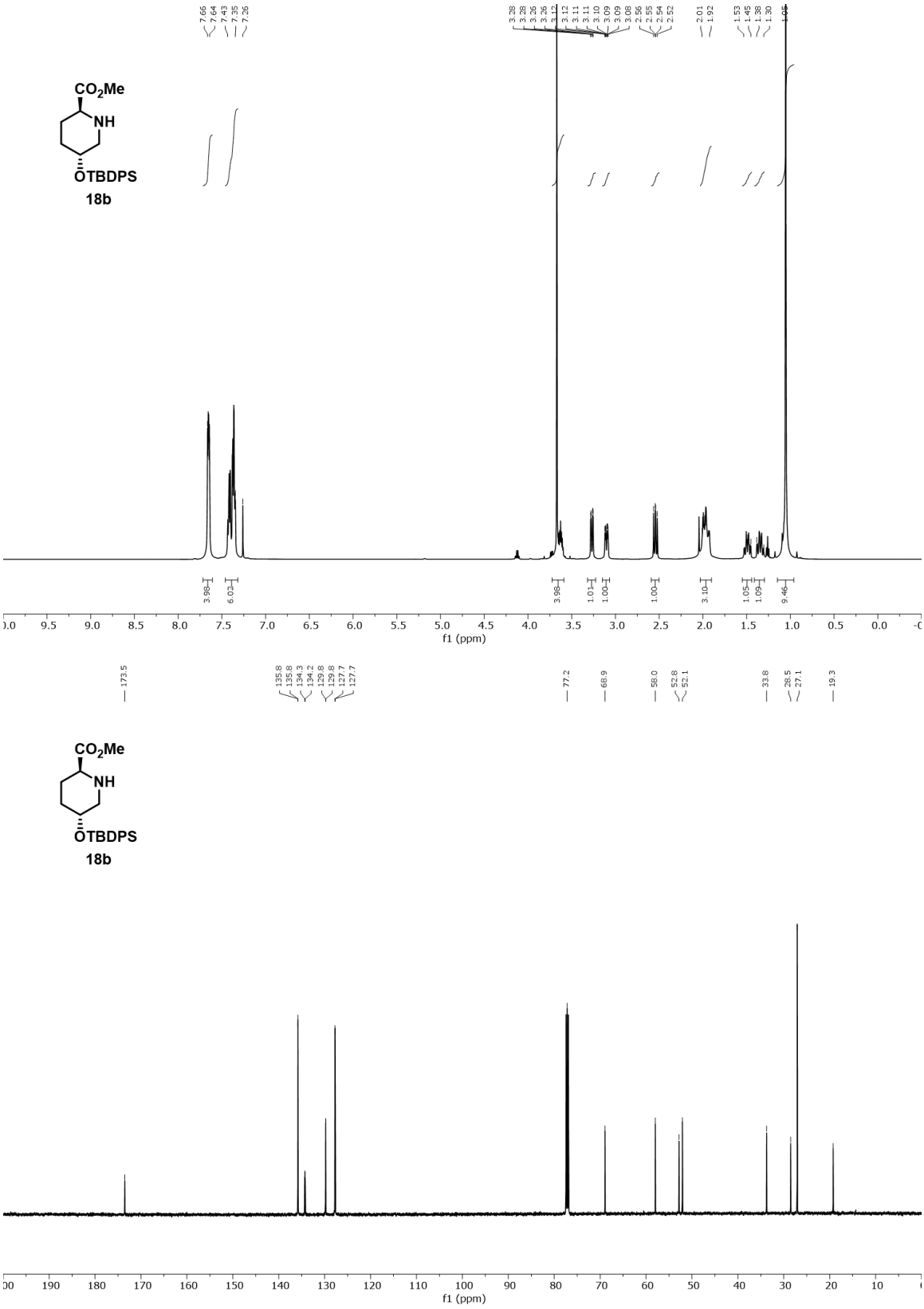


Figure S16. ^{1}H and ^{13}C NMR spectra of compound **18b**.

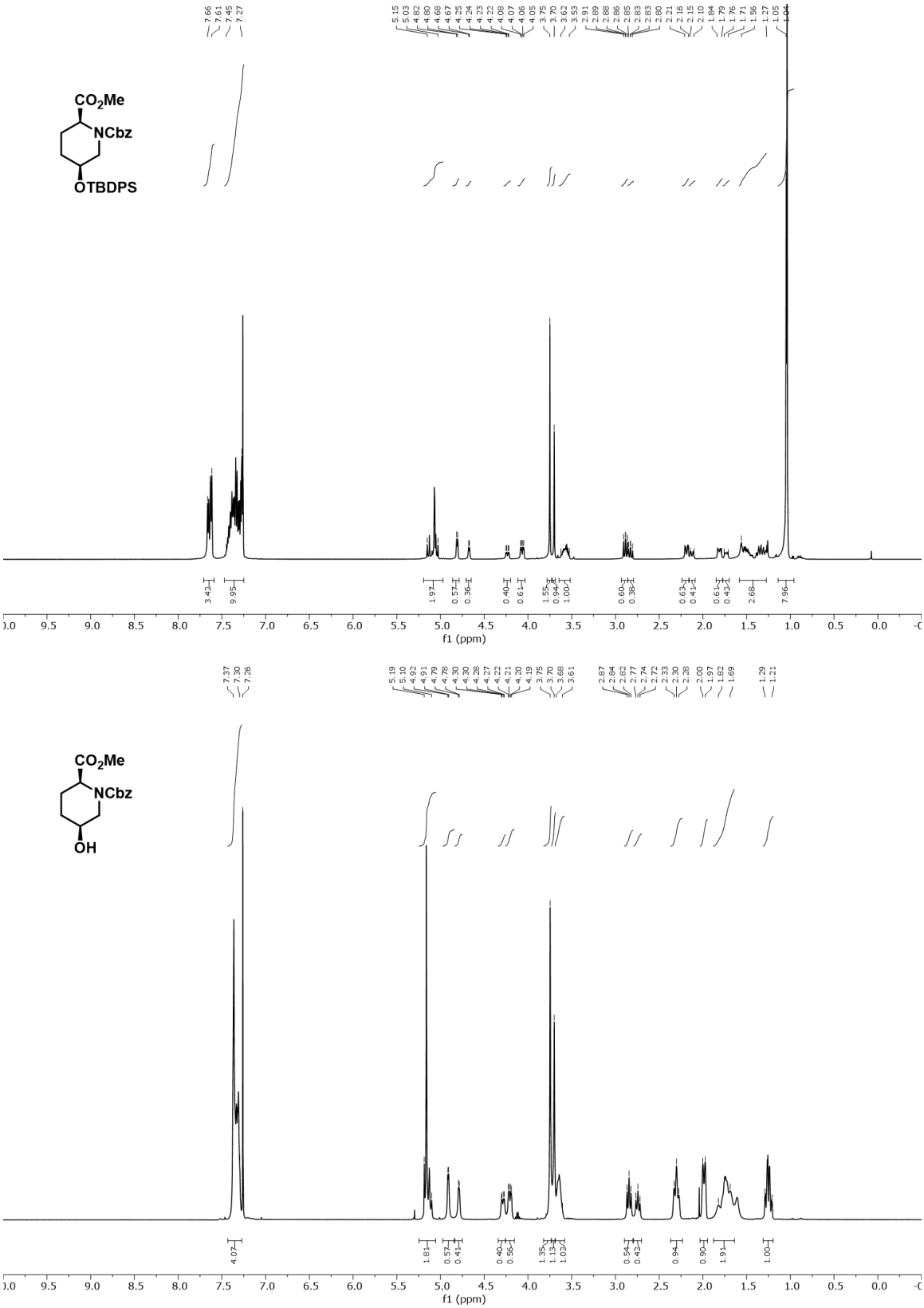


Figure S17. ^{1}H NMR spectra of N-carboxybenzyl protected **19a**.

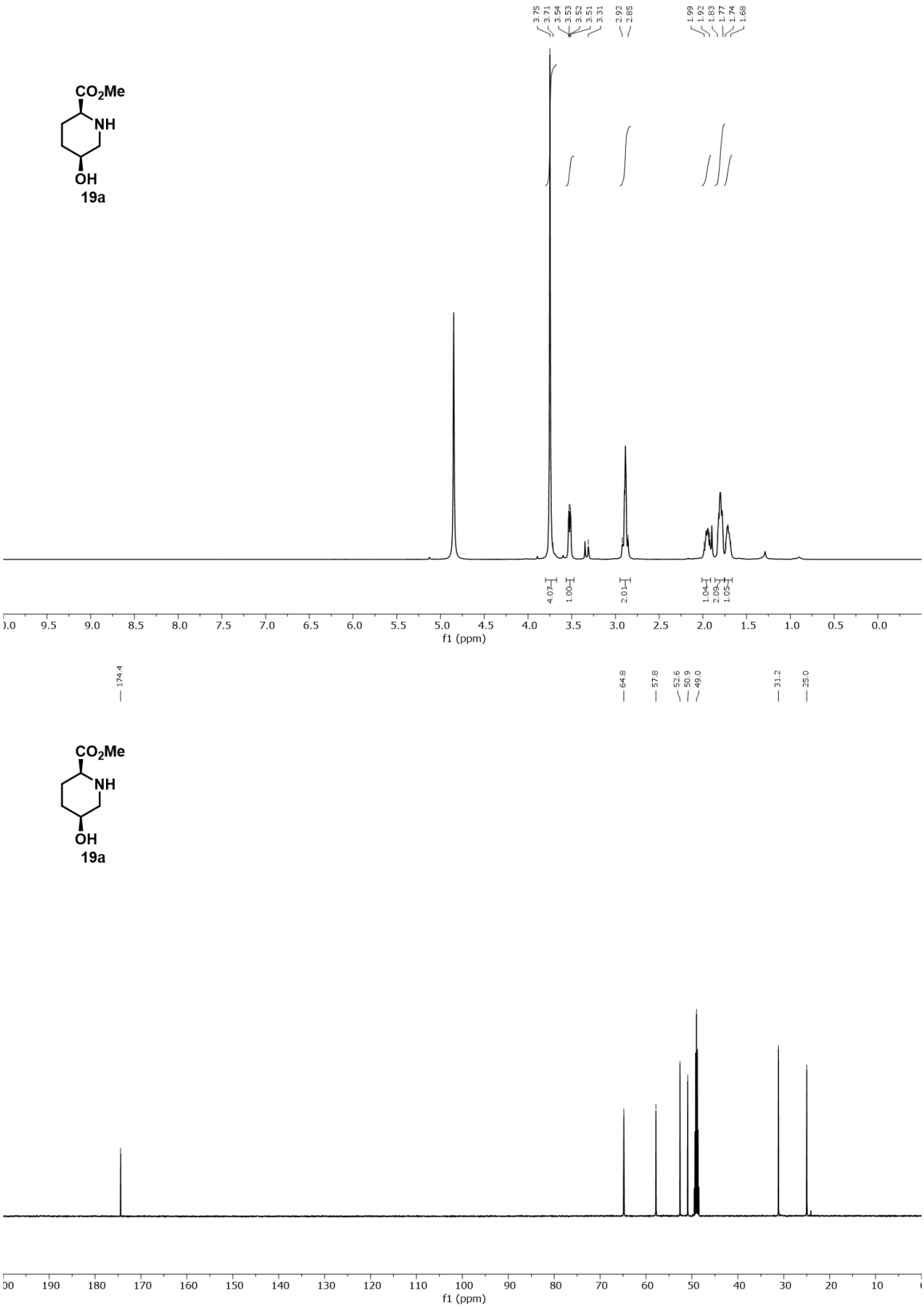


Figure S18. ^{1}H and ^{13}C NMR spectra of compound **19a**.

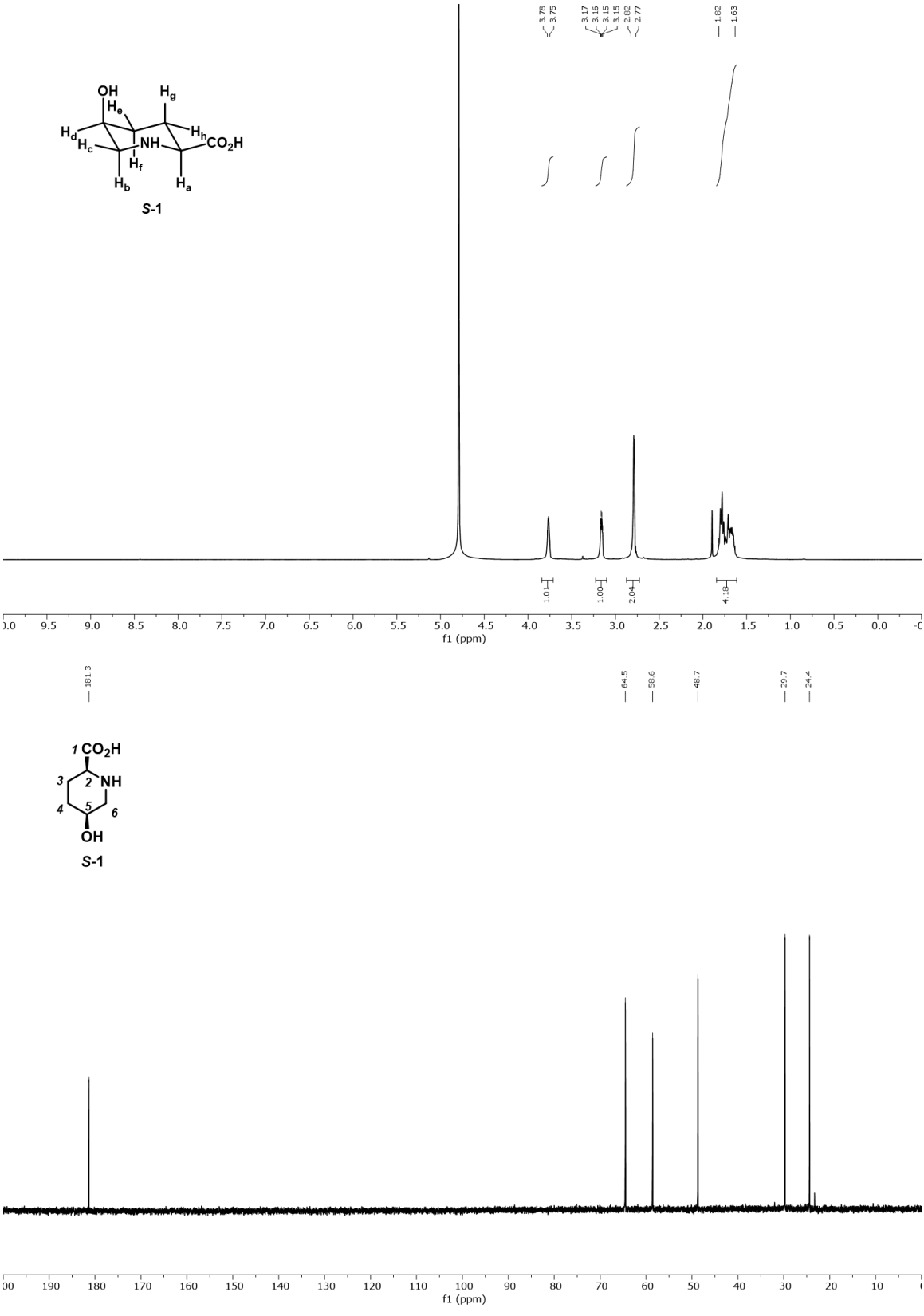


Figure S19. ^{1}H and ^{13}C NMR spectra of compound *S*-**1**.

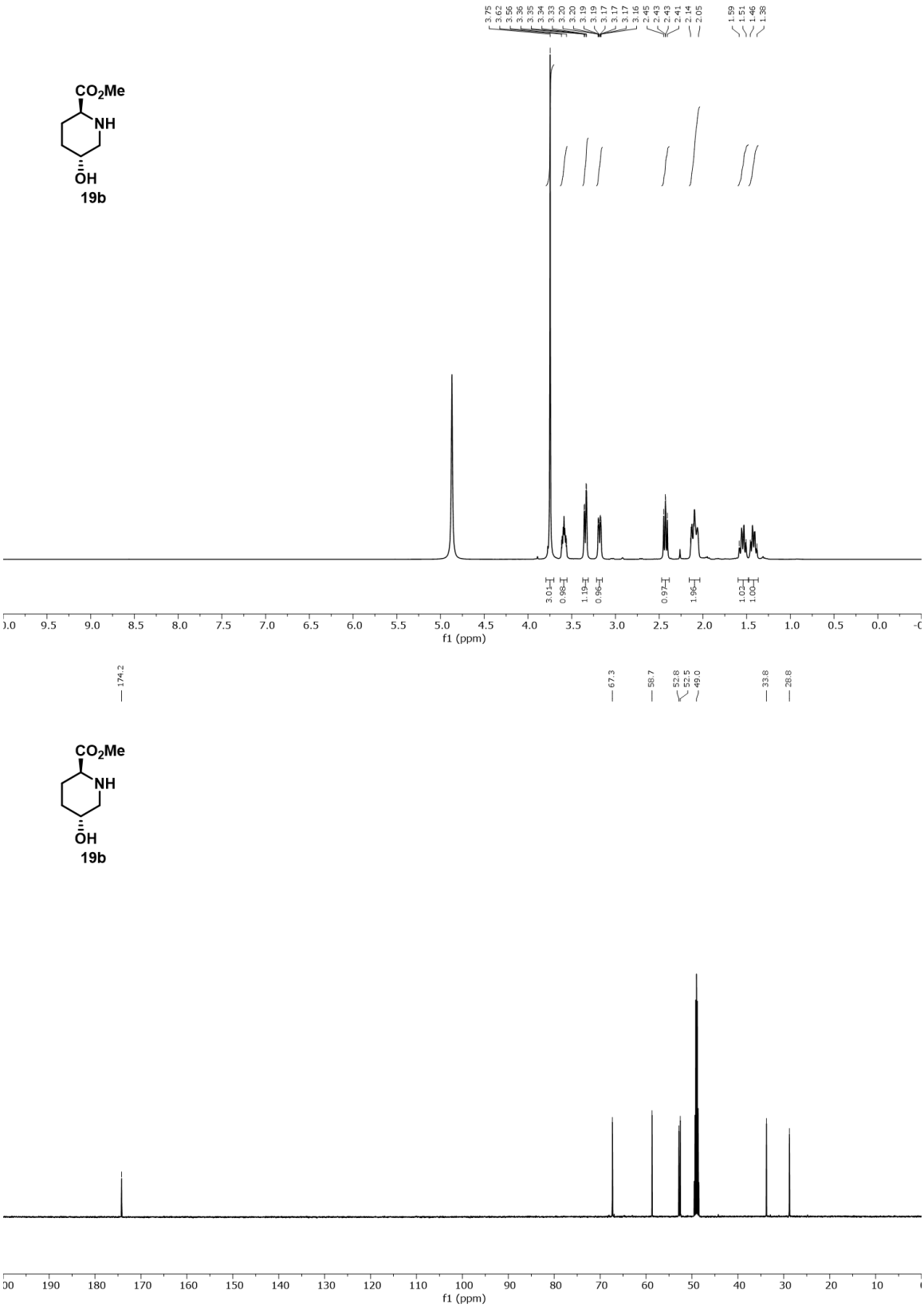


Figure S20. ^{1}H and ^{13}C NMR spectra of compound **19b**.

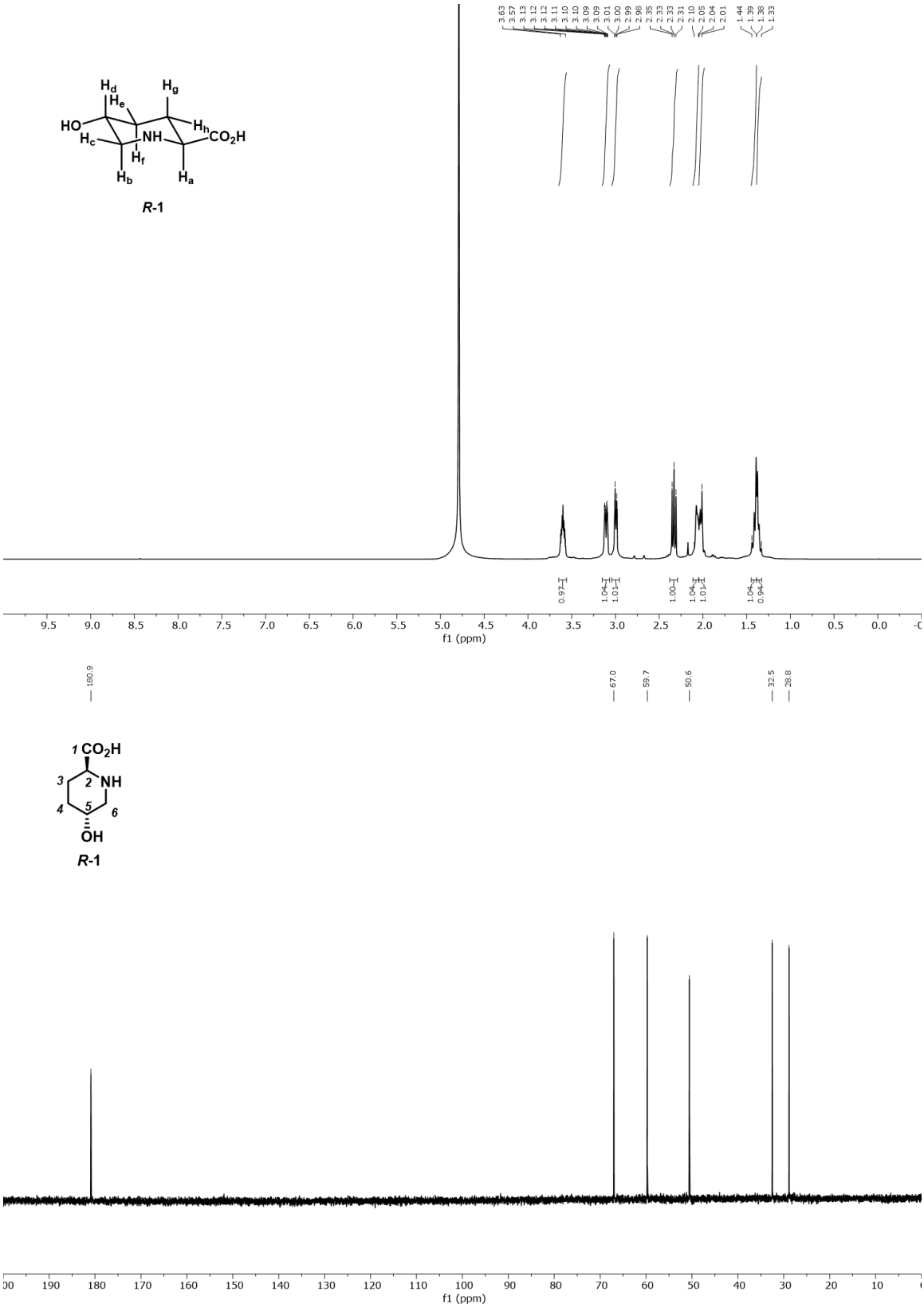


Figure S21. ^{1}H and ^{13}C NMR spectra of compound *R*-**1**.

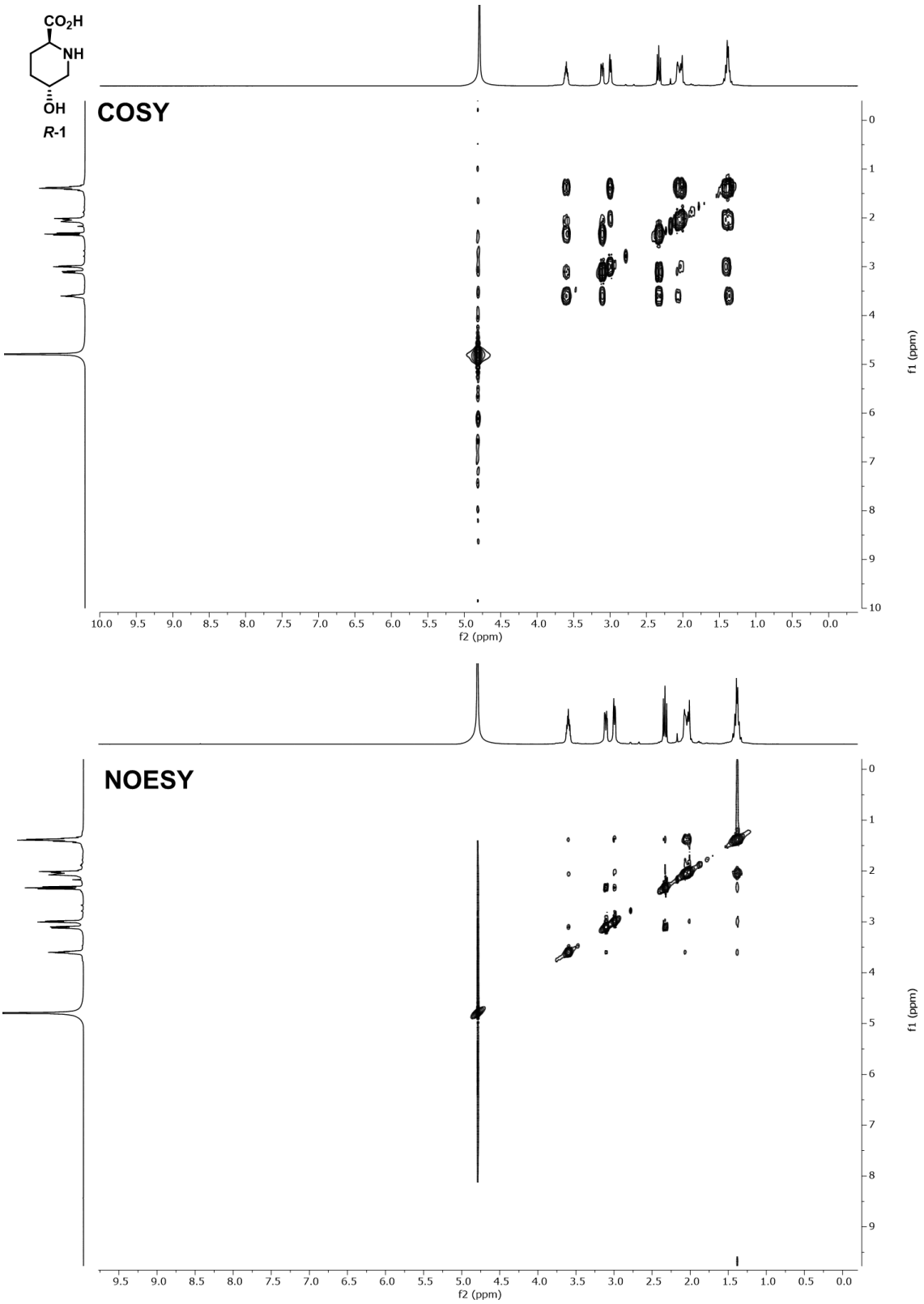


Figure S22. ^{1}H-^{1}H COSY and NOESY NMR spectra of *R*-**1**.

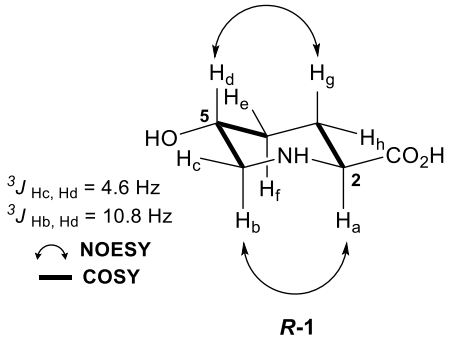


Figure S23. Key correlations deduced from coupling analysis, ^{1}H-^{1}H NOESY and COSY experiments. Based on these results, and preinstalled chiral center at C2 (originated from the substrate, L-glutamic acid), the C5 center most likely has the *R*-configuration.

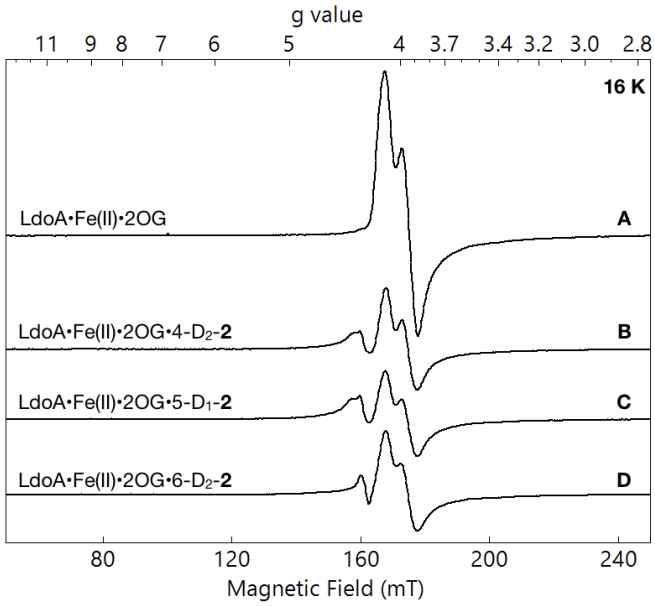


Figure S24. 16 K X-band CW EPR spectra of NO treated LdoA•Fe(II)•2OG complex (A), LdoA•Fe(II)•2OG•4-D_2-**2** complex (B), LdoA•Fe(II)•2OG•5-D_1-**2** complex (C), and LdoA•Fe(II)•2OG•6-D_2-**2** complex (D). The instrument conditions are listed in the X-band CW EPR section of the SI. The sample concentration: [LdoA] = 0.75 mM, [Fe(II)] = 0.68 mM, [2OG] = 7.5 mM, [4-D_2-**2**] = [1-D_1-**2**] = [6-D_2-**2**] = 22 mM, [NO] ~ 5 mM.

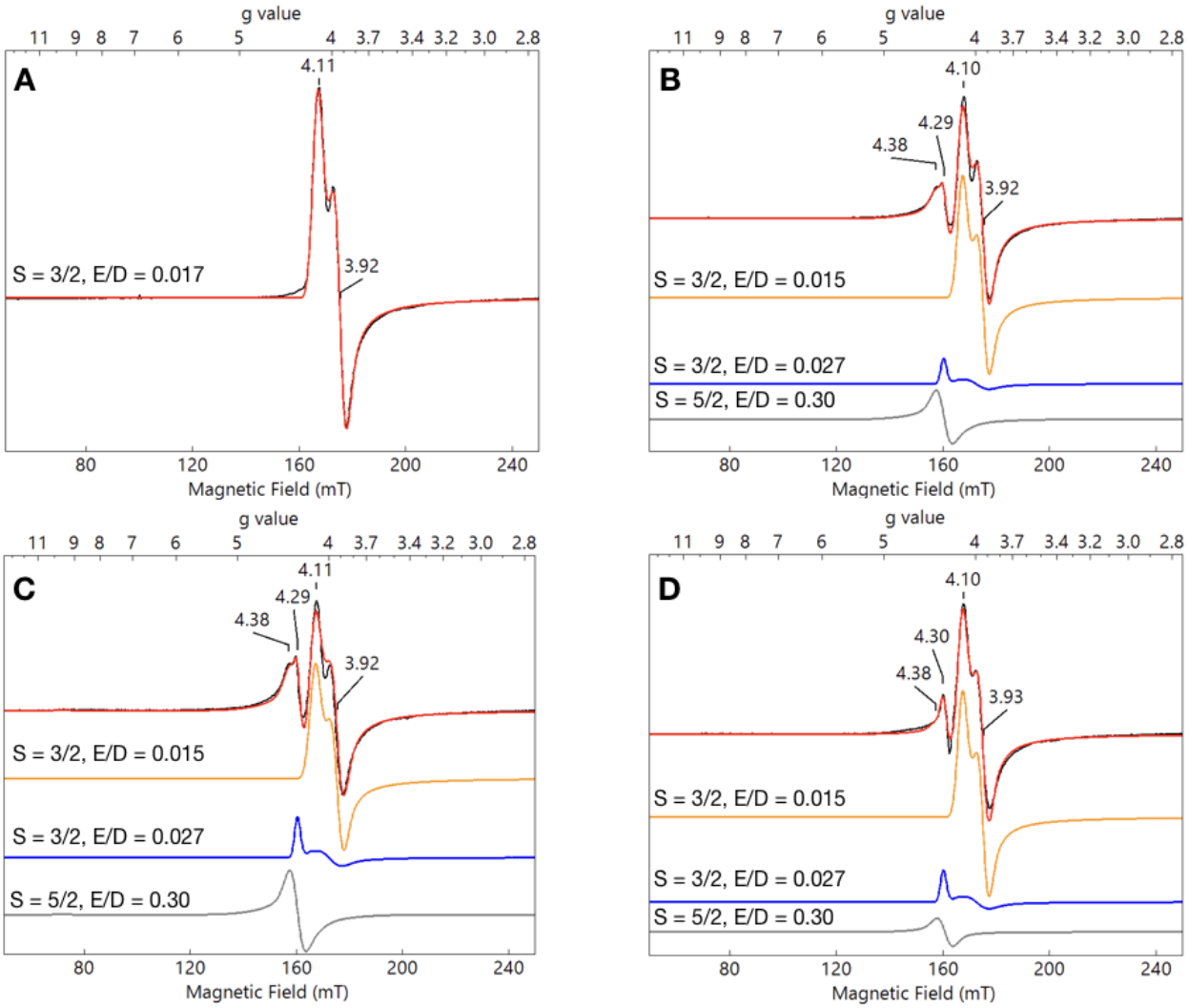


Figure S25. 16 K X-band CW EPR spectra (black) and simulations (red) of different NO treated LdoA samples. (A) The EPR spectrum of the simulation of the LdoA•Fe(II)•2OG complex; only one *S* = 3/2 $\{FeNO\}^7$ species was observed in this sample with E/D = 0.017, $g_x = g_y = 2.02$, $g_z = 2$. For the LdoA quaternary complexes containing 4-D_2-**2** (B), 5-D_1-**2** (C), and 6-D_2-**2** (D), three EPR active species were observed. The main species is an S = 3/2 $\{FeNO\}^7$ with E/D = 0.015, $g_x = g_y = 2.02$, $g_z = 2$ (orange). This species is very similar to the $\{FeNO\}^7$ observed in (A), but having slightly different E/D value. Another S = 3/2 $\{FeNO\}^7$ with E/D = 0.027, $g_x = g_y = 2.08$, $g_z = 2$ (blue) was observed. A third minor species is an S = 5/2 Fe(III) species with E/D = 0.3, which could be developed due to O_2 contaminant in the solution.

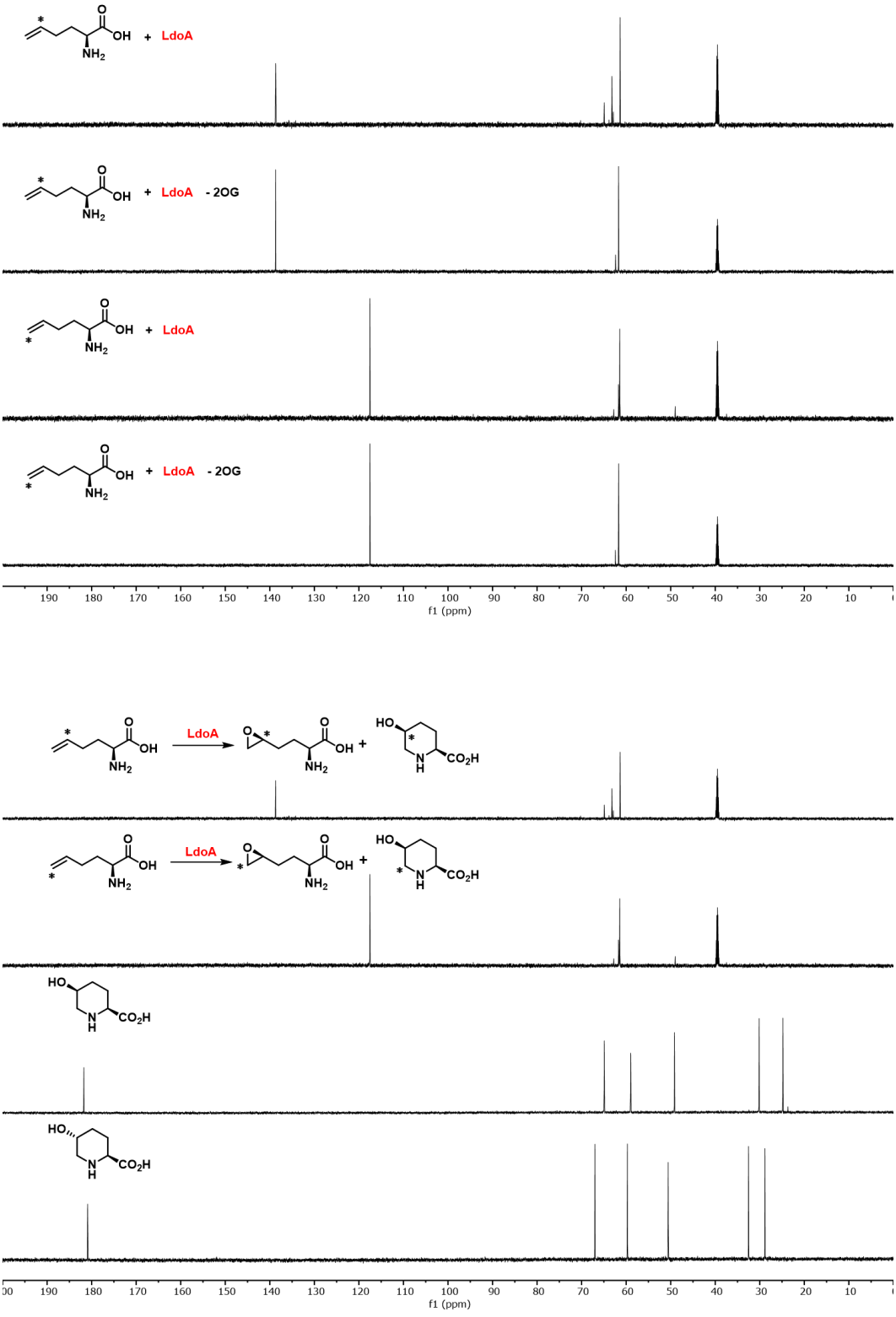


Figure S26. ^{13}C-NMR analysis of LdoA catalyzed oxidation using 5- or 6-^{13}C-**2** as the substrate. The ^{13}C-enriched carbon is labelled with asterisk.

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