Supplementary Information (SI) for Organic & Biomolecular Chemistry.

SUPPORTING INFORMATION

Synthesis and stability of collagen mimetic peptides featuring δ-heteroatom-substituted prolines

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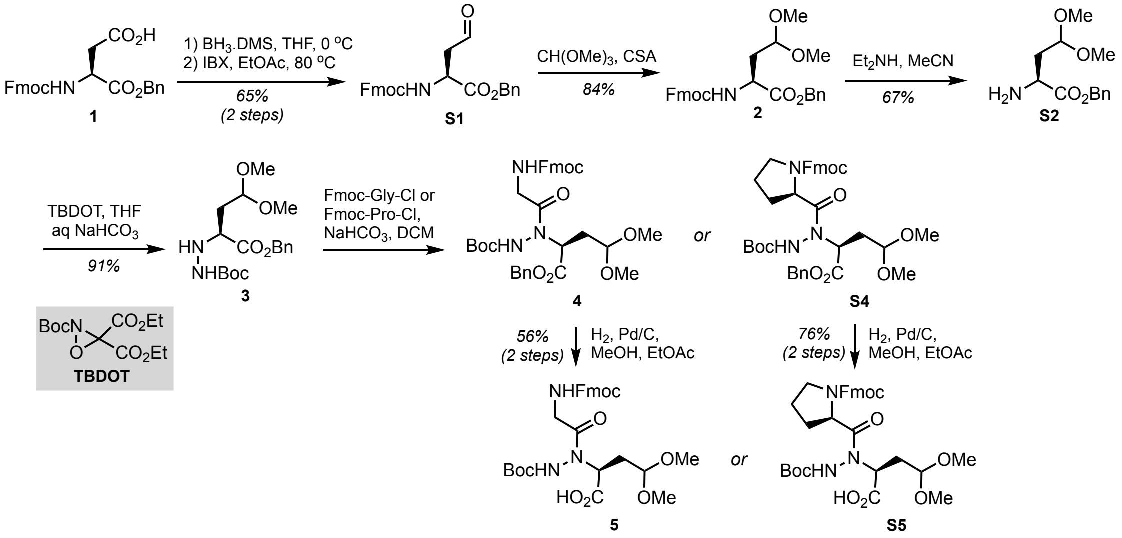
SOLUTION-PHASE BUILDING BLOCK SYNTHESIS

General notes. Unless stated otherwise, reactions were performed in flame-dried glassware under a positive pressure of nitrogen gas using dry solvents. TBDOT was synthesized according to our previously reported procedure,[1] which is a modification to the original protocol from Armstrong and co-workers.[2] Commercial grade reagents and solvents were used without further purification except where noted. Acetonitrile, diethyl ether, and dichloromethane were used following passage through a Pure Process Technologies solvent purification system. Other anhydrous solvents were purchased directly from chemical suppliers and used without further purification. Analytical HPLC chromatograms were acquired with a reverse-phase column (C12, 150 mm × 4.6 mm, 4 µm, 90 Å) using linear gradients of MeCN in H_2O (mobile phases modified with 0.1% formic acid) over 20 minutes and spectra are provided for λ = 220 or 280 nm. HRMS spectra were acquired using a Bruker Impact II ESI-QTOF.



Scheme S1. Synthesis of Fmoc-Gly-(Boc)aAdb-OH (**5**) and Fmoc-Pro-(Boc)aAdb-OH (**S5**).

(*S*)-3-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-4-(benzyloxy)-4-oxobutanoic acid (S1). A solution of commercially available Fmoc-Asp-OBn (5.00 g, 11.2 mmol) in THF was treated with $BH_3 \cdot DMS$ complex (5.32 mL, 56.1 mmol) at 0 °C over 1 h. The reaction was stirred at 0 °C for 2 h and then at rt for 18 h. The reaction was quenched with sat aq NH_4Cl, extracted with EtOAc, and washed with 1 M aq $KHSO_4$ and brine. The organic layer was then dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude residue was dissolved in EtOAc and IBX (9.35 g, 33.3 mmol) was added. After stirring at 80 °C for 3 h, the reaction was filtered and then dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude residue was purified by flash chromatography over silica gel (30% EtOAc/hexanes, then 100% EtOAc) to afford **S1** as a white solid (3.10 g, 65% yield over 2 steps). ^1H NMR (400 MHz, $CDCl_3$) δ 9.70 (s, 1H), 7.77 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.43–7.29 (m, 9H), 5.81 (d, *J* = 8.2 Hz, 1H), 5.20 (s, 2H), 4.72 (ddd, *J* = 8.7, 4.7 Hz, 1H), 4.45–4.34 (m, 2H), 4.21 (dd, *J* = 7.0 Hz 1H), 3.10 (dd, *J* = 18.6, 4.7 Hz, 1H), 3.12 (dd, *J* = 18.6, 4.7 Hz, 1H). $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 199.2, 170.5, 155.9, 143.7, 143.6, 141.3, 141.2, 135.0, 131.7 128.6, 128.5, 128.4, 128.3, 127.9, 127.7, 127.6, 127.0, 125.1, 120.0, 119.9, 49.1, 47.0, 45.7. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{26}H_{24}NO_5$ 430.1649, found 430.1643.

(*S*)-2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-4,4-dimethoxybutanoate (2). Compound **S1** (3.10g, 7.28 mmol) was dissolved in 39.5 mL of trimethoxymethane and camphorsulfonic acid (168 mg, 0.72 mmol) was added to the solution. The reaction was stirred for 2 h, concentrated, and the crude residue was purified by flash chromatography (30% EtOAc/hexanes then 100% EtOAc) to afford **2** as a red oil (2.87 g, 84% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.6 Hz, 2H), 7.63–7.57 (m, 2H), 7.43–7.27 (m, 9H), 5.78 (d, *J* = 8.2 Hz, 1H), 5.19 (m, 2H), 4.53 (ddd, *J* = 7.2, 4.9 Hz, 1H), 4.38 (ddd, *J* = 10.6, 7.2 Hz, 3H), 4.24 (dd, *J* = 7.2 Hz, 1H), 3.31 (s, 3H), 3.28 (s, 3H), 2.19–2.00 (m, 2H). ^{13}C{1H} NMR (126 MHz, $CDCl_3$) δ 172.4, 156.6, 144.6, 144.4, 142.0, 136.0, 129.3, 129.2, 129.1, 129.0, 128.6, 128.4, 127.7, 125.8, 120.7, 103.0, 68.4, 67.9, 67.8, 54.4, 47.8, 35.4. HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{28}H_{29}NNaO_6$ 498.1880, found 498.1902.

Benzyl (*S*)-2-amino-4,4-dimethoxybutanoate (S2). A solution of **2** (2.87 g, 4.88 mmol) in MeCN was treated with diethylamine (8.74 mL, 84.5 mmol). The reaction was stirred for 2 h, concentrated, and the crude residue was purified by flash chromatography (30–100% EtOAc/hexanes then 20% MeOH/EtOAc) to afford **S2** as a red oil (1.02 g, 67% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31–7.25 (m, 5H), 5.10 (d, *J* = 2.6 Hz, 2H), 4.51 (dd, *J* = 6.4, 4.9 Hz, 1H), 3.55 (dd, *J* = 8.2, 4.7 Hz, 1H), 3.24 (s, 3H), 324 (s, 3H), 2.05 (ddd, *J* = 13.9, 6.5, 4.8 Hz, 1H), 1.78 (ddd, *J* = 13.7, 8.2, 4.8 Hz, 1H), 1.61 (s, 2H) ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 175.1, 135.4, 128.2, 128.0, 102.0, 66.3, 53.0, 52.7, 51.0, 37.0. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{13}H_{20}NO_4$ 254.1387, found 254.1386.

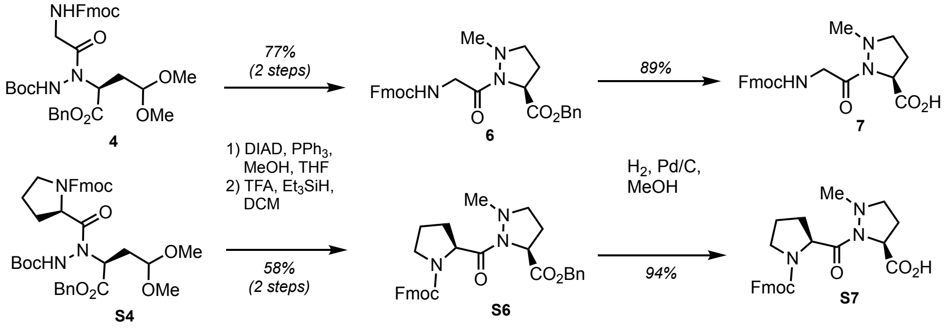
***tert*-Butyl (*S*)-2-(1-(benzyloxy)-4,4-dimethoxy-1-oxobutan-2-yl)hydrazine-1-carboxylate (3).** A solution of **S2** (1.02 g, 4.09 mmol) in a biphasic mixture of THF and sat aq $NaHCO_3$ (1:1) was treated with 2-(*tert*-butyl)-3,3-diethyl 1,2-oxaziridine-2,3,3-tricarboxylate (TBDOT) (1.16 g, 4.03 mmol) and the reaction mixture was allowed to stir at rt for 1 h. The reaction was diluted with EtOAc and the aqueous layer separated. The organic layer was washed with additional water, then dried over anhydrous Na_2SO_4, filtered, concentrated, and the crude residue was purified by flash chromatography (30–100% EtOAc/hexanes) to afford **3** as a yellow oil (980 mg, 91% yield). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 7.30 (s, 5H), 6.57 (s, 1H), 5.11 (m, 2H), 4.58 (dd, *J* = 5.5 Hz, 1H), 4.24 (m, 1H), 3.75 (dd, *J* = 7.2, 5.1 Hz, 1H), 3.32–3.22 (m, 3H), 1.98 (m, 2H), 1.39 (s, 9H), 1.23 (m, 2H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$, mixture of rotamers) δ 173.0, 156.2, 135.5, 128.7, 128.4, 128.3, 102.5, 80.5, 66.9, 60.2, 53.8, 53.6, 33.6, 28.3. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{18}H_{29}N_2O_6$ 369.2020, found 369.2026.

Fmoc-Gly-(Boc)aAdb-OBn (4). Freshly prepared Fmoc-Gly-Cl[3] (1.11 g, 3.53 mmol) was added to a solution of **3** (1.00 g, 2.71 mmol) and 10 equiv of $NaHCO_3$ in dry DCM at a concentration of 0.2 M. The reaction was stirred for 2 h. The solution was diluted with DCM and washed with water and brine. The organic layer was then dried over anhydrous Na_2SO_4, filtered, concentrated, and purified by flash chromatography (30–100% EtOAc/hexanes) to afford **4** as a white powder (1.22 g, 69% yield). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 7.79 (d, *J* = 7.5 Hz, 2H), 7.63 (d, *J* = 7.4 Hz, 2H), 7.44–7.32 (m, 9H), 5.60 (s, 1H), 5.23–5.18 (m, 2H), 4.64–4.63 (m, 1H), 4.40 (d, *J* = 7.2 Hz, 2H), 4.26 (t, *J* = 7.1 Hz, 1H), 3.39 (d, *J* = 16.5 Hz, 6H), 1.51 (s, 9H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$, mixture of rotamers) δ 170.0, 156.3, 144.0, 141.4, 135.3, 128.9, 128.8, 128.7, 128.6, 128.4, 128.3, 127.8,127.2, 125.3, 120.1, 91.3, 84.1, 83.8, 67.8, 67.5, 67.3, 57.7, 56.6, 50.9, 47.2, 42.7, 42.4, 36.7, 28.2, 28.1, 28.1. HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{35}H_{41}N_3NaO_9$ 670.2735, found 670.2731.

Fmoc-Gly-(Boc)aAdb-OH (5). To a round-bottom flask containing **4** (1.22 g, 1.88 mmol) was added 401 mg of 5% Pd/C followed by a small amount of EtOAc. The mixture was diluted with MeOH and the flask was charged with H_2. After 3 h the reaction was filtered through a pad of Celite® and the filtrate was concentrated *in vacuo.* Purification by silica gel flash chromatography (30–100% EtOAc/hexanes, then 20% MeOH in EtOAc) afforded **5** as a white solid (852 mg, 81 % yield). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 7.75 (d, *J* = 7.6 Hz, 2H), 7.59 (d, *J* = 7.5 Hz, 2H), 7.39 (dd, *J* = 7.5 Hz , 2H), 7.30 (dd, *J* = 7.4 Hz, 2H), 5.64 (m, 1H), 4.55–4.19 (m, 5H), 3.90 (m, 1H), 3.38 (s, 6H), 2.32–2.30 (m, 2H), 1.51 (s, 9H). $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$, mixture of rotamers) δ 156.7, 156.6, 143.9, 141.4, 127.8, 127.2,125.3,120.1, 67.4, 57.0, 52.5, 47.1, 42.5, 31.1, 28.3, 28.2, 28.0. HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{28}H_{35}N_3NaO_9$ 580.2266, found 580.2256.

Fmoc-Pro-(Boc)aAdb-OBn (S4). Freshly prepared Fmoc-Pro-Cl[3] (1.26 g, 3.53 mmol) was added to a solution of **3** (1.00 g, 2.71 mmol) and 10 equiv of $NaHCO_3$ in 15 mL of dry DCM. The reaction was stirred for 2 h. The solution was diluted with DCM and washed with water and brine. The organic layer was then dried over anhydrous Na_2SO_4, filtered, concentrated, and purified by flash chromatography (30% EtOAc/hexanes) to afford **S4** as a white powder. (1.50 g, 80% yield). ^1H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 7.78 (d, *J* = 7.5 Hz, 2H), 7.58 (t, *J* = 7.9 Hz, 2H), 7.44–7.28 (m, 9H), 5.98–5.95 (m, 1H), 5.33–5.29 (m, 1H), 5.17 (s, 2H), 4.92–4.89 (m, 1H), 4.38–4.20 (m, 3H), 3.77–3.68 (m, 1H), 3.58–3.54 (m, 1H), 2.62–2.56 (m, 1H), 2.43–2.16(m, 4H), 2.00–1.94 (m, 1H), 1.49 (s, 9H). $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$, mixture of rotamers) δ 177.1, 170.0, 155.2, 154.7, 143.9, 143.7, 141.4, 141.3, 135.3, 128.6, 128.4, 128.2, 127.8, 127.1, 125.2, 120.1, 120.0, 83.9, 83.1, 68.1, 67.2, 58.2, 55.6, 47.2, 47.1, 39.4, 30.5, 28.1, 25.0. HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{38}H_{45}N_3NaO_9$ 710.3048, found 710.3055.

Fmoc-Pro-(Boc)aAdb-OH (S5). To a round-bottom flask containing **S4** (1.50 mg, 2.18 mmol) was added 464 mg of 5% Pd/C followed by a small amount of EtOAc. The mixture was diluted with MeOH and the flask was charged with H_2. After 3 h the reaction was filtered through a pad of Celite® and the filtrate was concentrated *in vacuo.* Purification by silica gel flash chromatography (30–100% EtOAc/hexanes, then 20% MeOH in EtOAc) afforded **S5** as a white solid (1.24 mg, 95% yield). ^1H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 8.29 (s, 0.3H), 7.76 (m, 2H), 7.60–7.56 (m, 1.7H), 7.42–7.29 (m, 4H), 4.98–4.21 (m, 6H), 3.69–3.15 (m, 8H), 2.35–1.93 (m, 6H), 1.54–1.20 (m, 9H). $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$, mixture of rotamers) δ 178.1, 171.4, 155.4, 155.3, 155.0, 144.0, 143.8, 141.5, 141.4, 128.0, 127.9, 127.3, 127.2, 127.1, 127.0, 125.4, 125.3, 125.2, 120.2, 120.1, 120.0, 93.2, 84.2, 84.1, 68.2, 58.8, 58.7, 55.8, 55.6, 47.3, 47.2, 47.1, 47.0, 41.9, 38.5, 34.6, 30.4, 29.5, 28.3, 28.1, 28.0, 27.2, 25.1, 24.7, 14.1, 11.9. HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{31}H_{39}N_3NaO_9$ 620.2579, found 620.2565.



Scheme S2. Synthesis of Fmoc-Gly-(Me)aPro-OH (**7**) and Fmoc-Pro-(Me)aPro-OH (**S7**).

Fmoc-Gly-(Me)aPro-OBn (6). A solution of **4** (1.29 g, 1.99 mmol), triphenylphosphine (1.31 g, 4.98 mmol), and methanol (201 µL, 4.98 mmol) in THF was treated with DIAD (968 µL, 4.98 mmol). The reaction was allowed to stir for 1 h. The reaction was concentrated and purified by flash chromatography over silica gel (25% EtOAc/hexanes) to afford the methylated intermediate, which contained a small amount of diisopropyl hydrazine-1,2-dicarboxylate byproduct. The semi-crude material was used directly in the next step.

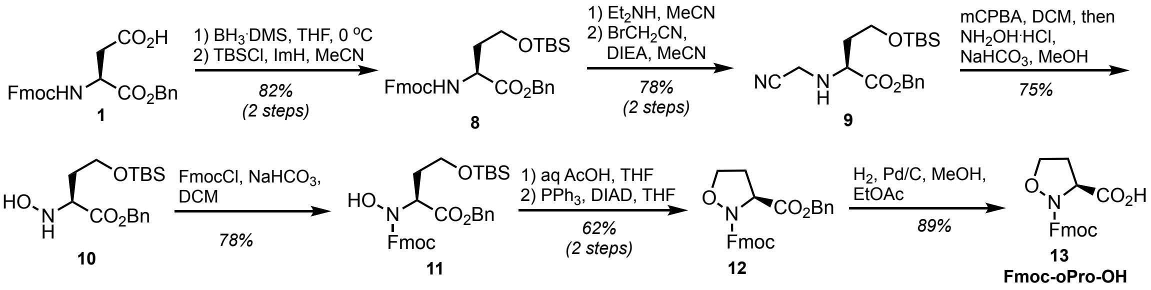
A solution of product above in 10.5 mL of 95:5 TFA/DCM was allowed to stir for 15 min. After 15 min, the reaction was treated dropwise with triethylsilane (784 µL, 4.91 mmol), and this stirred for 1 h. The reaction was concentrated and purified by flash chromatography over silica gel (30% EtOAc/hexanes) to afford **6** as a white solid (752mg, 77% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 7.5 Hz, 2H), 7.54 (d, *J* = 7.6 Hz, 2H), 7.41 – 7.19 (m, 9H), 5.56 (t, *J* = 4.7 Hz, 1H), 5.13 (s, 2H), 4.60 (m, 1H), 4.38 – 3.96 (m, 5H), 2.98 – 2.83 (m, 2H), 2.51 (m, 5H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 156.4, 144.1, 144.0, 141.4, 135.5, 128.8, 128.6, 128.3, 127.8, 127.2, 125.3, 120.1, 67.5, 67.2, 58.5, 55.3, 47.3, 45.0, 43.1, 28.9. HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{29}H_{29}N_3NaO_5$ 522.2000, found 522.2001.

Fmoc-Gly-(Me)aPro-OH (7). To a round-bottom flask containing **6** (752 mg, 1.51 mmol) was added 320 mg of 5% Pd/C followed by a small amount of EtOAc. The mixture was diluted with MeOH and the flask was charged with H_2. After 30 min the reaction was filtered through a pad of Celite® and the filtrate was concentrated *in vacuo.* Purification by silica gel flash chromatography (0–10% MeOH/$CHCl_3$) afforded **7** as a white solid (552 mg, 89% yield). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.60 (d, *J* = 7.6 Hz, 2H), 7.48–7.25 (m, 4H), 5.64 (bs, 1H), 4.67–4.09 (m, 6H), 3.16–2.85 (m, 2H), 2.77–2.36 (m, 5H). $^{13}C\{^1H\}$ NMR (201 MHz, $CDCl_3$) δ 156.7, 144.0, 141.4, 127.9, 127.2, 125.3, 120.1, 67.4, 55.2, 47.2, 45.1, 42.9. HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{22}H_{23}N_3NaO_5$ 432.1530, found 432.1536.

Fmoc-Pro-(Me)aPro-OBn (S6). A solution of **S4** (1.49 g, 2.17 mmol), triphenylphosphine (1.42 g, 5.42 mmol), and methanol (219 µL, 5.42 mmol) in THF was treated with DIAD (1.05 mL, 5.42 mmol). The reaction was allowed to stir for 1 h. The reaction was concentrated and purified by flash chromatography over silica gel (25% EtOAc/hexanes) to afford the methylated intermediate which contained a small amount of diisopropyl hydrazine-1,2-dicarboxylate byproduct. The semi-crude material was used directly in the next step.

A solution of product above in 10.5 mL of 95:5 TFA:DCM was allowed to stir for 15 min. After 15 min, the reaction was treated dropwise with triethylsilane (920 µL, 5.76 mmol), and this stirred for 1 h. The reaction was concentrated and purified by flash chromatography over silica gel (30% EtOAc/hexanes) to afford **S6** as a white solid (680 mg, 58% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 7.79–7.75 (m, 2H), 7.68–7.58 (m, 2H), 7.43–7.28 (m, 9H),5.25–5.19 (m, 4H), 4.46–4.02 (m, 5H), 3.78–3.59 (m, 2H), 3.17–2.56 (m, 4H), 2.30–2.03 (m, 5H) $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$, mixture of rotamers) δ 144.2, 141.5, 128.8, 128.6, 128.5, 127.8, 127.3, 127.2, 125.5, 125.4, 125.3, 120.1, 67.6, 67.3, 47.6, 47.4, 47.1, 28.8. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{32}H_{34}N_3O_5$ 540.2493, found 540.2504.

Fmoc-Pro-(Me)aPro-OH (S7). To a round-bottom flask containing **S6** (680 mg, 1.26 mmol) was added 268 mg of 5% Pd/C and wetted with minimal EtOAc. The mixture was diluted with MeOH and the flask was charged with H_2. After 30 min the reaction was filtered through a pad of Celite® and the filtrate was concentrated *in vacuo.* Purification by silica gel flash chromatography (30% EtOAc/hexanes, then 60% EtOAc/hexanes) to afford **S7** as a white solid (530 mg, 94% yield). ^{1}H NMR (400 MHz, $CDCl_3$-d_6, mixture of rotamers) δ 7.79–7.76 (m, 2H), 7.66–7.56 (m, 2H), 7.44–7.28 (m, 4H), 5.21 (s, 1H), 4.45–4.15 (m, 3H), 3.75–3.61 (m, 3H), 3.21–2.90 (m, 2H), 2.54 (d, *J* = 28.8 Hz, 3H), 2.33–1.97 (m, 5H). $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$, mixture of rotamers) δ 171.6, 155.3, 144.2, 141.7, 128.1, 127.4, 125.6, 125.5, 120.4, 67.9, 63.9, 60.8, 55.4, 51.3, 47.5, 47.2, 46.0, 32.0, 25.2, 23.0, 22.2, 21.9, 21.5, 14.6, 14.5. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{25}H_{28}N_3O_5$ 450.2023, found 450.2028.



Scheme S3. Synthesis of Fmoc-oPro-OH (**13**).

Benzyl *N*-(((9H-fluoren-9-yl)methoxy)carbonyl)-*O*-(tert-butyldimethylsilyl)-L-homoserinate (8). A solution of commercially available Fmoc-Asp-OBn (5.00 g, 11.2 mmol) in THF was treated with BH_3·DMS complex (5.32 mL, 56.1 mmol) at 0 °C over 1 h. The reaction was stirred at 0 °C for 2 h and then at rt for 18 h. The reaction was quenched with sat aq NH_4Cl, extracted with EtOAc, and washed with 1 M aq $KHSO_4$ and brine. The organic layer was then dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude residue was dissolved in MeCN and imidazole (1.53 g, 22.4 mmol) was added followed by TBSCl (6.76 g, 44.8 mmol). After 3 h the reaction mixture was concentrated. The resulting oil was taken up in EtOAc, transferred to a separatory funnel, and washed with water (3×) followed by brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated. Purification by flash chromatography over silica gel (0–20% EtOAc/hexanes) afforded **8** (5.02 g, 82% yield over 2 steps) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.62 (dd, *J* = 7.6, 3.9 Hz, 2H), 7.45 – 7.28 (m, 9H), 6.14 (d, *J* = 7.7 Hz, 1H), δ 5.23 (d, *J* = 12.3 Hz, 1H), 5.17 (d, *J* = 12.3 Hz, 1H), 4.55 (m, 1H), 4.48 – 4.29 (m, 2H), 4.23 (t, *J* = 7.2 Hz, 1H), 3.78 – 3.58 (m, 2H), 2.18 – 1.84 (m, 2H), 0.91 (s, 9H), 0.05 (s, 6H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 172.1, 156.2, 144.2, 143.9, 141.4, 141.4, 135.6, 128.7, 128.4, 128.3, 127.8, 127.7, 127.1, 127.1, 125.3, 125.2, 120.0, 67.1, 60.2, 53.1, 47.3, 33.5, 26.0, 18.3, -5.5. HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{32}H_{39}NNaO_5Si$ 568.2490, found 568.2480.

Benzyl *O*-(tert-butyldimethylsilyl)-*N*-(cyanomethyl)- L-homoserinate (9). To a solution of **8** (5.02 g, 9.20 mmol) in MeCN was added diethylamine (47.6 mL, 460 mmol). After 2 h the reaction mixture was then concentrated. The residue was dissolved in MeCN and DIEA (3.21 mL, 18.4 mmol) was added followed by bromoacetonitrile (760 µL, 10.1 mmol). The mixture was stirred for 18 h at 50 °C. Volatiles were removed under vacuum and the crude material was partitioned between DCM and sat aq $NaHCO_3$. The aqueous layer was extracted with DCM. The organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash chromatography on a silica gel column (20% EtOAc/hexanes) to afford **9** as a colorless oil (2.59 g, 78% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (m, 5H), 5.19 (d, *J* = 12.2 Hz, 1H), 5.17 (d, *J* = 12.2 Hz, 1H), 3.73 (m, 2H), 3.66–3.52 (m, 3H), 2.26 (bs, 1H), 1.98 (m, 1H), 1.79 (m, 1H), 0.88 (s, 9H), 0.04 (s, 6H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 173.7, 135.5, 128.8, 128.6, 128.5, 117.6, 67.1, 60.1, 58.3, 36.1, 35.5, 26.0, 18.3, -5.3. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{19}H_{31}N_2O_3Si$ 363.2098, found 363.2094.

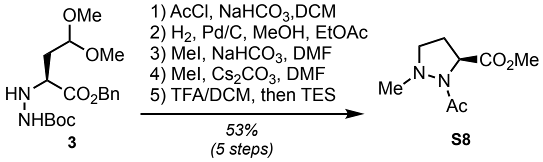
Benzyl *O*-(tert-butyldimethylsilyl)-*N*-hydroxy-L-homoserinate (10). To a solution of **9** (2.59 g, 7.14 mmol) in DCM at 0 °C was added mCPBA (3.52 g, 14.3 mmol) portion-wise over 20 min. The reaction was allowed to warm to rt and stirred for 1 h. A solution of sat aq $Na_2S_2O_3$ and sat aq $NaHCO_3$ was added and the mixture was stirred for 30 min. Water was added and the mixture was extracted with DCM. The combined organic layers were dried over anhydrous Na_2SO_4, and concentrated. The resulting residue was dissolved in MeOH and $NaHCO_3$ (3.00 g, 35.7 mmol) and hydroxylamine hydrochloride (2.48 g, 35.7

mmol) were added. The reaction was fitted with a reflux condenser and heated at 60 °C for 2 h. The reaction mixture was concentrated, and the crude residue was taken up in DCM and sat aq $NaHCO_3$ and extracted with DCM. The organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash chromatography on a silica gel column (30% EtOAc/hexanes) to give **10** as a colorless oil (1.81 g, 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.29 (m, 5H), 6.11 (bs, 1H), 5.23 (d, *J* = 12.3 Hz, 1H), 5.19 (d, *J* = 12.3 Hz, 1H), 3.85 (dd, *J* = 8.0, 5.1 Hz, 1H), 3.69 (m, 2H), 1.96–1.73 (m, 2H), 0.88 (s, 9H), 0.03 (s, 6H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 173.4, 135.7, 128.6, 128.3, 127.0, 66.8, 63.0, 60.1, 32.2, 26.0, 18.3, -5.4. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{17}H_{30}NO_4Si$ 340.1939, found 340.1943.

Benzyl *N*-(((9H-fluoren-9-yl)methoxy)carbonyl)-*O*-(tert-butyldimethylsilyl)-*N*-hydroxy-L-homoserinate (11). A mixture of **10** (1.81 g, 5.34 mmol) and $NaHCO_3$ (2.24 g, 26.7 mmol) in DCM was treated with FmocCl (2.07 g, 8.00 mmol) portion-wise over 10 min. After 1 h, the reaction was quenched with water and transferred to a separatory funnel. The organic layer was washed with water and dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by silica gel flash chromatography (20% EtOAc/hexanes) gave **11** as a pale yellow oil (2.32 g, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81–7.57 (m, 4H), 7.46 – 7.19 (m, 9H), 5.24 (d, *J* = 12.3 Hz, 1H), 5.18 (d, J = 12.3 Hz, 1H), 5.03 (m, 1H), 4.53–4.32 (m, 2H), 4.25 (m, 1H), 3.82 (m, 2H), 2.28 (q, *J* = 6.2 Hz, 2H), 0.93 (s, 9H), 0.10 (s, 6H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 171.0, 157.8, 143.7, 141.3, 135.3, 128.6, 128.4, 128.1, 127.8, 127.2, 125.3, 120.0, 68.6, 67.4, 59.7, 47.0, 31.1, 26.0, 18.3, -5.4. HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{32}H_{39}NNaO_6Si$ 584.2439, found 584.2436.

2-((9H-fluoren-9-yl)methyl) 3-benzyl (*S*)-isoxazolidine-2,3-dicarboxylate (12). To a solution of **11** (2.00 g, 3.56 mmol) in 1:1 H_2O/THF mixture was added acetic acid (7.12 mL, 125 mmol). After 18 h the reaction mixture was carefully diluted with sat aq $NaHCO_3$ and transferred to a separatory funnel. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The resulting oil was dissolved in THF and triphenylphosphine (1.40 g, 5.33 mmol) was added followed by dropwise addition of DIAD (760 μL, 3.91 mmol). After 45 min the reaction was concentrated and purification by silica gel flash chromatography (25% EtOAc/hexanes) gave **12** as a colorless oil (950 mg, 62% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79–7.72 (m, 2H), 7.62 (dd, *J* = 11.8, 7.5 Hz, 2H), 7.44–7.24 (m, 9H), δ 5.23 (d, J = 12.3 Hz, 1H), 5.17 (d, J = 12.3 Hz, 1H)., 4.74 (dd, *J* = 9.3, 5.0 Hz, 1H), 4.53 (dd, *J* = 10.5, 7.1 Hz, 1H), 4.40 (dd, *J* = 10.5, 7.3 Hz, 1H), 4.28–4.14 (m, 2H), 3.84–3.74 (m, 1H), 2.66–2.43 (m, 2H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 170.2, 157.0, 143.6, 143.5, 141.5, 141.4, 135.3, 128.8, 128.6, 128.4, 128.0 127.3, 127.3, 125.5, 125.3, 120.1, 69.0, 68.6, 67.6, 59.8, 47.1, 33.0. HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{26}H_{23}NNaO_5$ 452.1468, found 452.1472.

(*S*)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)isoxazolidine-3-carboxylic acid (13). To a round-bottom flask containing **12** (949 mg, 2.21 mmol) was added 470 mg of 5% Pd/C followed by a small amount of EtOAc. The mixture was diluted with MeOH and the flask was charged with H_2. After 30 min the reaction was filtered through a pad of Celite® and the filtrate was concentrated. Purification by silica gel flash chromatography (0–10% MeOH/$CHCl_3$) afforded **13** as a white solid (705 mg, 94% yield). Spectroscopic data was consistent with those previously reported in every regard.[4,5]

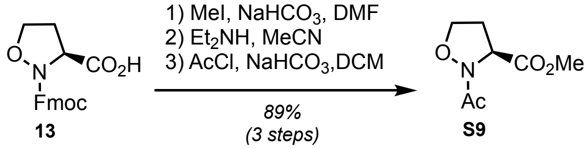


Scheme S4. Synthesis of Ac-(Me)aPro-OMe (**S8**).

Ac-(Me)-aPro-OMe (S8). To a solution of **3** (500 mg, 1.48 mmol) in DCM was added $NaHCO_3$ (1.87 g, 22.2 mmol) followed by dropwise addition of AcCl (1.74 g, 2.22 mmol). After 1 h the reaction mixture was transferred to a separatory funnel and washed with water. The organic layer was collected, dried over Na_2SO_4, filtered, and concentrated. Purification by silica gel flash chromatography (30% EtOAc/hexanes) afforded **Ac-(Boc)aAdb-OBn** as a colorless oil (540 mg, 89% yield).

To a round-bottom flask containing **Ac-(Boc)aAdb-OBn** (540 mg, 1.32 mmol) was added 280 mg of 5% Pd/C followed by a small amount of EtOAc. The mixture was diluted with MeOH and the flask was charged with H_2. After 1 h the reaction was filtered through a pad of Celite® and the filtrate was concentrated. The resulting oil was dissolved in DMF and $NaHCO_3$ (551 mg, 6.56 mmol) was added to the mixture followed by dropwise addition of MeI (164 µL, 2.62 mmol). After 1 h the reaction was filtered and concentrated. Purification by silica gel flash chromatography (30% EtOAc/hexanes) afforded **Ac-(Boc)aAdb-OMe** as a colorless oil (362 mg, 83% yield).

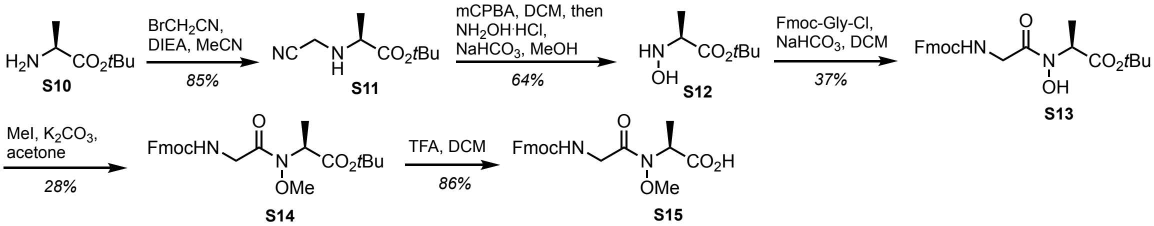
To a solution of **Ac-(Boc)aAdb-OMe** (362 mg, 1.08 mmol) in DMF was added Cs_2CO_3 (1.41 g, 4.33 mmol) followed by MeI (203 µL, 3.25 mmol). After 18 h, the reaction mixture was diluted with EtOAc and transferred to a separatory funnel. The mixture was washed with water followed by brine. The organic layer was collected, dried over Na_2SO_4, filtered, and concentrated. The resulting oil was dissolved in 5:95 DCM/TFA and allowed to stir. After 15 min triethylsilane (421 µL, 2.63 mmol) was added dropwise. After an additional 45 min the reaction mixture was concentrated. Purification by silica gel flash chromatography (60% EtOAc/hexanes) afforded **S8** as a colorless oil (142 mg, 72% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.55 (t, *J* = 8.6 Hz, 1H), 3.70 (s, 3H), 2.95 (m, 2H), 2.56 (s, 3H), 2.49–2.24 (m, 2H), 2.14 (s, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 172.6, 170.8, 57.8, 55.0, 52.5, 44.9, 29.2, 20.9. HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_8H_{14}NaN_2O_3$ 209.0897, found 209.0891.



Scheme S5. Synthesis of Ac-oPro-OMe (**S9**).

Ac-oPro-OMe (S9). To a solution of **13** (150 mg, 442 µmol) in DMF was added $NaHCO_3$ (186 mg, 2.21 mmol) followed by dropwise addition of MeI (55.5 µL, 884 µmol). After 3 h, the reaction was diluted with EtOAc and transferred to a separatory funnel. The mixture was washed with 1 M aq HCl and brine. The organic layer was collected, dried over Na_2SO_4, filtered, and concentrated. (Purification by silica gel flash chromatography (30% EtOAc/hexanes) afforded **Fmoc-oPro-OMe** as a white solid (149 mg, 95% yield).

To a solution of **Fmoc-oPro-OMe** (149 mg, 422 μmol) in MeCN was added diethylamine (2.18 mL, 21.1 mmol) and allowed to stir for 1 h. The reaction was concentrated and the resulting oil was taken up in DCM. To the solution was added $NaHCO_3$ (176 mg, 2.10 mmol) followed by dropwise addition of AcCl (60 μL, 839 μmol). After 20 min the reaction was filtered, concentrated, and purified via silica gel flash chromatography (75% EtOAc/hexanes) to give **S9** (68.1 mg, 94% yield) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 4.80 (dd, J = 9.5, 5.3 Hz, 1H), 4.14 (td, J = 7.7, 4.6 Hz, 1H), 3.80 (td, J = 8.1, 6.9 Hz, 1H), 3.71 (s, 3H), 2.66–2.55 (m, 1H), 2.51–2.36 (m, 1H), 2.10 (s, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 171.0, 170.6, 69.1, 56.2, 52.8, 32.7, 20.2. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_7H_{12}NO_4$ 174.0761, found 174.0760.



Scheme S6. Synthesis of Fmoc-Gly-(Me)hAla-OH (**S15**).

***tert*-Butyl (cyanomethyl)-L-alaninate (S11).** A mixture of **S10** (HCl salt, 5.00 g, 27.5 mmol) and DIEA (14.4 mL, 82.6 mmol) in MeCN was treated with bromoacetonitrile (2.3 mL, 33.05 mmol) dropwise over 10 min at rt. The reaction was stirred for 18 h at 50 ºC prior to the removal of MeCN. The residue was dissolved in DCM and washed with sat. aq. $NaHCO_3$. The organic layer was collected, and the aq. phase was extracted with additional DCM. The combined organic layers were dried over anhydrous Na_2SO_4, then filtered and concentrated. Purification by silica gel flash chromatography (20% EtOAc/hexanes), gave **S11** as a colorless oil (4.32 g, 85% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 3.60 (d, J = 1.3 Hz, 2H), 3.37 (q, J = 7.0 Hz, 1H), 1.97 (s, 1H), 1.47 (s, 10H), 1.29 (d, J = 7.0 Hz, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 173.3, 117.6, 81.9, 55.9, 35.5, 28.0, 18.6. HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_9H_{16}N_2NaO_2$ 207.1104, found 207.1101.

***tert*-Butyl hydroxy-L-alaninate (S12).** To a solution of **S11** (4.32 g, 23.4 mmol) in DCM at 0 °C was added 75% mCPBA (10.8 g, 46.9 mmol) portion-wise over 20 min. The reaction was allowed to warm to rt and stirred for 1 h. A solution of sat aq $Na_2S_2O_3$ and sat aq $NaHCO_3$ was added and the mixture was stirred for 30 min. Water was added and the mixture was extracted with DCM. The combined organic layers were dried over anhydrous Na_2SO_4, and concentrated. The resulting residue was dissolved in MeOH and hydroxylamine hydrochloride (8.15 g, 117 mmol) was added. The reaction was fitted with a reflux condenser and heated at 60 °C for 6 h. The reaction mixture was concentrated, and the crude residue was taken up in DCM and sat aq $NaHCO_3$ and extracted with DCM. The organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated. Purification by flash chromatography on a silica gel column (40% EtOAc/hexanes) gave **S12** as a colorless oil (2.4 g, 64% yield over 2 steps). ^1H NMR (400 MHz, $CDCl_3$) δ 3.55 (q, J = 7.1 Hz, 1H), 1.44 (s, 9H), 1.18 (d, J = 7.1 Hz, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 173.3, 81.5, 60.9, 28.0, 14.6. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_7H_{16}NO_3$, 162.1125 found 162.1121.

Fmoc-Gly-hAla-O*t*Bu (S13). A solution of Fmoc-Gly-Cl[3] (783 mg, 2.48 mmol) in DCM was added to a solution of **S12** (400 mg, 2.48 mmol) and $NaHCO_3$ (4.17 g, 49.6 mmol) in DCM. The reaction was stirred for 2 h at rt prior to the removal of DCM. The residue was diluted with EtOAc and washed with 1 M aq HCl, sat. aq $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4, then filtered and concentrated under reduced pressure. Purification by silica gel flash chromatography (40% EtOAc/hexanes) gave **S13** as a white solid (401 mg, 37% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 7.5 Hz, 2H), 7.63 (d, *J* = 7.4 Hz, 2H), 7.42 (td, *J* = 7.5, 1.2 Hz, 3H), 7.33 (td, *J* = 7.4, 1.2 Hz, 2H), 5.68 (t, *J* = 4.9 Hz, 1H), 5.19 (q, *J* = 7.3 Hz, 1H), 4.41 (d, *J* = 7.2 Hz, 2H), 4.33 – 4.19 (m, 3H), 1.50 (d, *J* = 4.2 Hz, 14H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 171.6, 169.7, 156.6, 143.9, 141.3, 127.7, 127.1, 125.2, 120.0, 83.3, 77.3, 67.3, 54.5, 47.1, 42.5, 28.0, 14.2. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{24}H_{29}N_2O_6$ 441.2020, found 441.2026.

Fmoc-Gly-(Me)hAla-O*t*Bu (S14). A mixture of **S13** (300 mg, 0.68 mmol) and methyl iodide (967 mg, 6.80 mmol) in acetone was treated with K_2CO_3 (94 mg, 0.68 mmol). The reaction was stirred for 5 h at rt prior to the removal of acetone. The residue was diluted with EtOAc and washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, then filtered and concentrated under reduced pressure. Purification by silica gel flash chromatography (20% EtOAc/hexanes) gave **S14** as a white solid (88 mg, 28% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 7.5 Hz, 2H), 7.55 (d, *J* = 7.2 Hz, 2H), 7.33 (td, *J* = 7.5, 1.2 Hz, 2H), 7.25 (td, *J* = 7.5, 1.2 Hz, 2H), 5.53 (t, *J* = 4.8 Hz, 1H), 4.86–4.70 (m, 1H), 4.32 (d, *J* = 7.4 Hz, 2H), 4.24–4.11 (m, 3H), 3.75 (s, 3H), 1.46 (d, *J* = 7.4 Hz, 3H), 1.38 (s, 9H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 169.1, 156.3, 143.9, 143.9, 141.3, 127.7, 127.1, 125.21, 125.19, 120.0, 82.3, 77.3, 67.2, 65.0, 57.7, 47.2, 42.9, 28.0, 14.0. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{25}H_{31}N_2O_6$ 455.2177, found 455.2173.

Fmoc-Gly-(Me)hAla-OH (S15). A solution of **S14** (80 mg, 0.2 mmol) in DCM was treated with TFA (7.0 mL, 0.1 mol). The reaction was stirred for 2 h at rt before removing the solvent. The residue was recrystallized with Et_2O and dried under reduced pressure to give **S15** as a white solid (62 mg, 86% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.65 (s, 1H), 7.76 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.5 Hz, 2H), 7.35 (dt, *J* = 35.8, 7.4 Hz, 5H), 5.84 (t, *J* = 5.0 Hz, 1H), 4.93 (q, *J* = 8.0 Hz, 1H), 4.53 (s, 1H), 4.39 (d, *J* = 7.2 Hz, 1H), 4.31–4.16 (m, 2H), 4.12–3.94 (m, 1H), 3.91–3.57 (m, 3H), 1.58 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 174.1, 173.2, 156.6, 143.88, 143.86, 141.3, 127.7, 127.1, 125.2, 120.0, 77.4, 77.2, 77.0, 76.7, 67.3, 64.9, 56.7, 47.1, 42.7, 13.7. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{21}H_{23}N_2O_6$ 399.1551, found 399.1541.

SOLID-PHASE PEPTIDE SYNTHESIS

Automated solid-phase peptide synthesis was carried out on a CEM Liberty Blue peptide synthesizer or a PurePrep Chorus peptide synthesizer using ProTide Rink amide resin (100-200 mesh, 0.63 mmol/g, 0.1 mmol scale). The following derivatives suitable for Fmoc SPPS were used: Fmoc-Pro-OH, Fmoc-Gly-OH, Ac-Gly-OH, Fmoc-Hyp(*t*Bu)-OH, Fmoc-Tyr(*t*Bu)-OH, Fmoc-Gly-(Boc)aAdb-OH (**5**), Fmoc-Gly-(Me)aPro-OH (**7**), Fmoc-oPro-OH (**13**), Fmoc-Pro-(Boc)aAdb-OH (**S5**), Fmoc-Pro-(Me)aPro-OH (**S7**), Fmoc-Gly-(Me)hAla-OH (**S15**). Fmoc deprotection steps were carried out by treating the resin with a solution of 20% (v/v) piperidine/DMF once at rt (5 min), then at 75 °C (2 min). After Fmoc deprotection the resin was washed with DMF 3×. Coupling of Fmoc-protected building blocks was achieved using 5 equiv HCTU (0.25 M in DMF), 10 equiv NMM (1 M in DMF), and 5 equiv of Fmoc-protected amino acid or dipeptide building block (0.2 M in DMF) at 50 °C (10 min), then at 75 °C (5 min). Deprotection and coupling steps were repeated until peptide synthesis was complete and then a final Fmoc deprotection was performed to unmask the N-terminus. Peptides were N-terminally acetylated using 5% acetic anhydride and 10% pyridine in DCM (15 min). In the case of **(Me)hAla11-CMP**, the N-terminal residue was introduced as Ac-Gly-OH.

After synthesis, the resin was washed with DCM (5 mL × 4) and dried under vacuum. Cleavage from the solid support and global deprotection was effected by incubating the dried resin in 5 mL of TFA:TIPS:H_2O (95:2.5:2.5) for 2.5 h. The resin was filtered, and the filtrate was collected in a 50 mL centrifuge tube. The resin was washed with DCM (10 mL), filtered, and crude peptides were precipitated from the combined filtrate by the addition of cold Et_2O (45 mL). The mixture was centrifuged and the supernatant decanted. The pellet was washed with Et_2O (25 mL × 2) and dried thoroughly under vacuum. All peptides were purified by preparative HPLC with a reverse-phase column (C12 or C18, 250 mm × 21.2 mm, 4 μm, 90 Å) using linear gradients of MeCN in H_2O (mobile phases modified with 0.1% formic acid) over 30 min. For the peptides containing oPro, the resin was removed from the peptide synthesizer after coupling and Fmoc deprotection of the oPro residue. The resin was washed with DMF (5 mL × 3) before treating with 5 equiv of Fmoc-Gly-Cl or Fmoc-Pro-Cl[1], and 10 equiv 2,4,6-collidine (130 μL, 1.0 mmol) in DMF (10 mL) for 30 min. Automated SPPS was resumed following this condensation.

CIRCULAR DICHROISM

CD Wavescans. CMP samples were prepared by dissolving lyophilized powder in 1× PBS, pH 7.4, at a concentration of 150 µM. The single-strand PPII peptides were dissolved in 5 mM Na_3PO_4, 25mM KF (pH 7.0) at a concentration of 150 µM. CD spectra were acquired using a JASCO J-1700 CD spectrometer in a 1 mm path length quartz cell with 2 s digital integration time, 1 nm bandwidth, 0.5 nm datapitch, and a scan speed of 100 nm/min at 20 °C. Mean residue ellipticity at a given wavelength (MRE; deg cm^2 $dmol^{-1}$ $residue^{-1}$) was calculated based on the equation MRE = q / (10 × b × M × n), where q is ellipticity (mdeg), b is the pathlength (cm), M is the peptide concentration (mol L^{-1}), and n is the total number of residues. All ellipticity values are reported as the mean across 3 scans.

Thermal Denaturation. Temperature-dependent CD spectra were acquired using the same parameters described above from 7–90 °C in 1 °C increments at a ramp rate of 2 °C per minute. The melting temperature was determined by plotting the change in ellipticity at 225 nm as a function of temperature and applying the non-linear regression model for two-state protein unfolding described by Shortle and coworkers.[6] Melting temperatures are reported as the mean and standard deviation across 2 separate experiments.

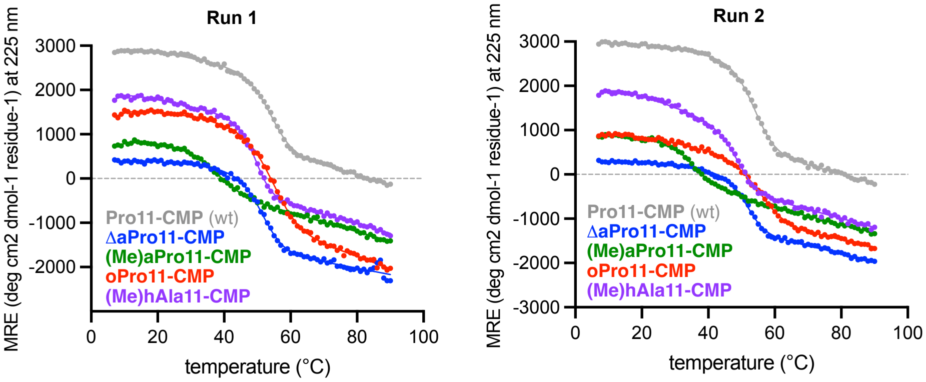


Fig. S1. Thermal denaturation of CMPs at 150 µM in aq PBS (pH 7.4) from two independent experiments. MRE at 225 nm was recorded as a function of temperature by CD.

Refolding Kinetics. CMPs were prepared as described above and heated at 95 °C for 20 min before being added to a pre-cooled cuvette at 7 °C. The molar ellipticity was tracked at 225 nm for 300 minutes, starting after 1 min delay to allow transfer of the sample to the cuvette. The data was fit to a 3rd order kinetic model.[7] The time required to reach a folded population of 50% ($t_{1/2}$) was derived from non-linear regression and values are reported as the mean and standard deviation across 2 separate experiments.

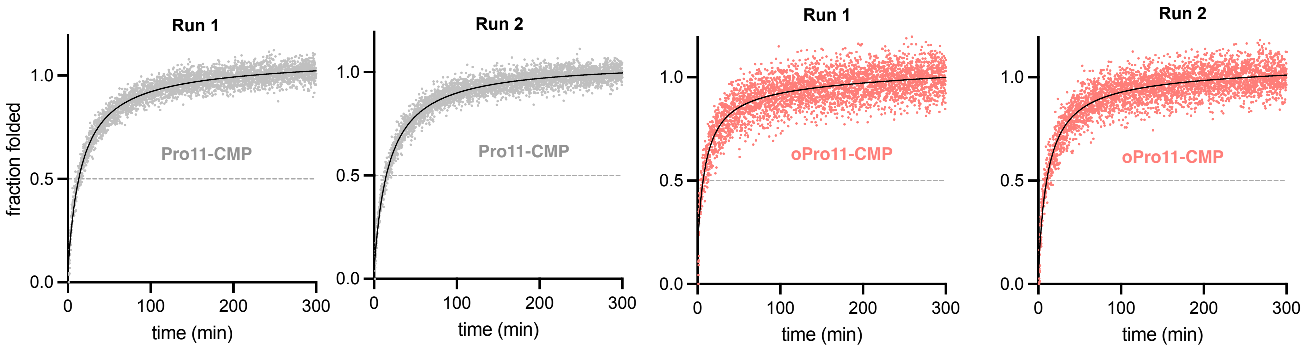
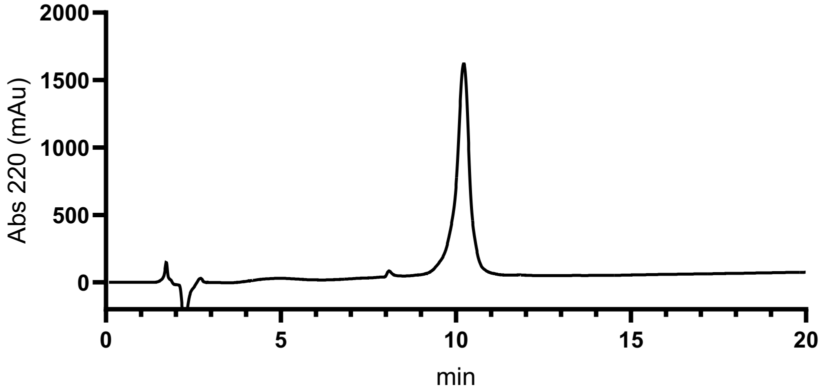


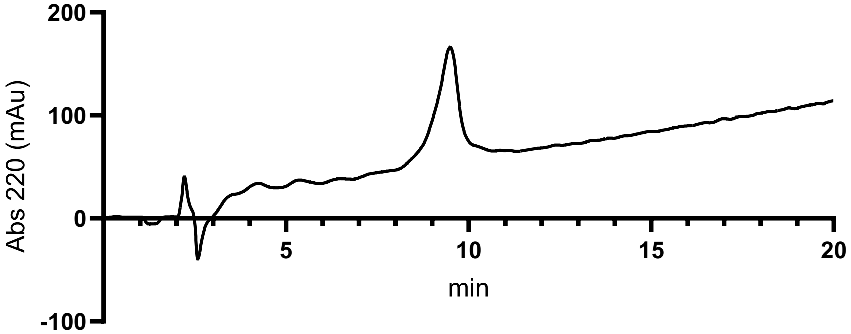
Fig. S2. Refolding kinetics of **Pro11-CMP** and **oPro11-CMP**. Two independent experiments are shown for each peptide with MRE at 225 nm plotted as a function of time upon re-cooling. Third-order non-linear fit (black curve) are indicated on each graph.

ANALYTICAL HPLC CHROMATOGRAMS

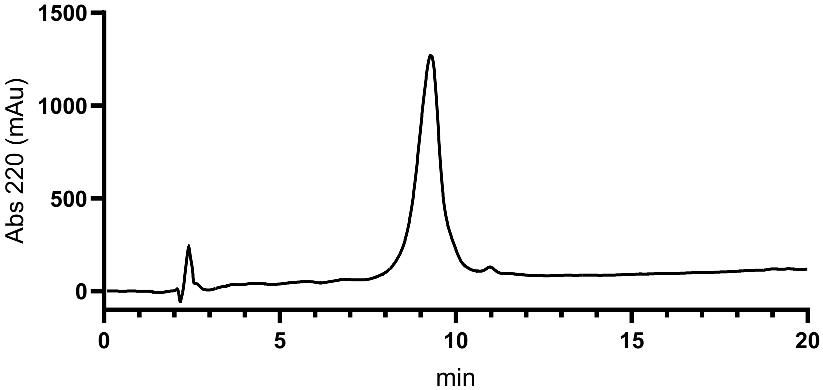
Pro11-CMP. The crude peptide was purified by preparative scale RP-HPLC using a 5–25% MeCN/H_2O gradient (with 0.1% formic acid). t_R = 10.2 min. The pure peptide was obtained in 5% overall yield based on initial resin loading. HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{86}H_{124}N_{22}NaO_{29}$ 1951.8797, found 1951.8793.



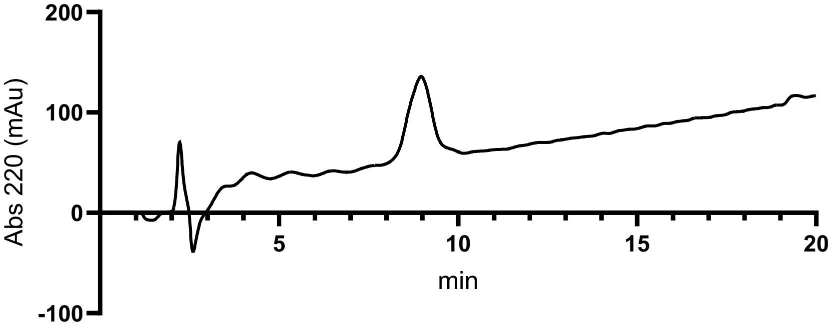
ΔaPro11-CMP: The crude peptide was purified by preparative scale RP-HPLC using a 5–40% MeCN/H_2O gradient (with 0.1% formic acid). t_R = 9.5 min. The pure peptide was obtained in 12% overall yield based on initial resin loading. HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{85}H_{121}N_{23}NaO_{29}$ 1950.8593, found 1950.8600.



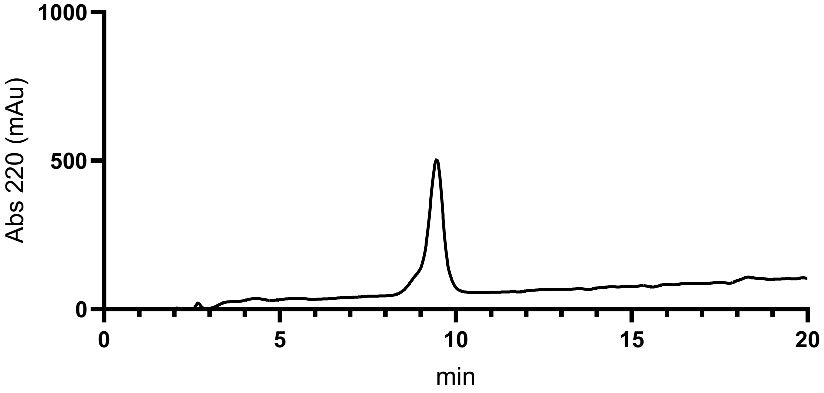
(Me)aPro11-CMP. The crude peptide was purified by preparative scale RP-HPLC using a 5–40% MeCN/H_2O gradient (with 0.1% formic acid). t_R = 9.3 min. The pure peptide was obtained in 16% overall yield based on initial resin loading. HRMS (ESI-TOF) *m/z* $[M + 2Na]^{2+}$ calcd for $C_{86}H_{125}N_{23}Na_2O_{29}$ 994.9399, found 994.9392.



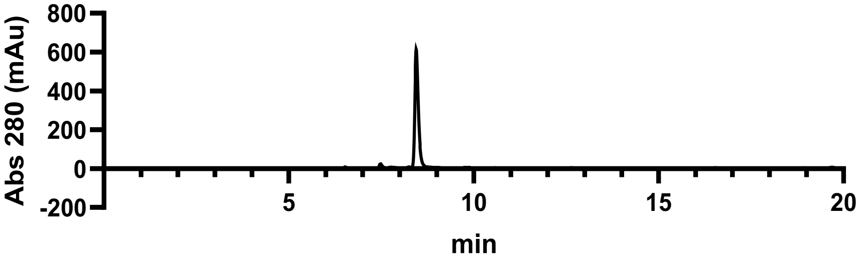
oPro11-CMP. The crude peptide was purified by preparative scale RP-HPLC using a 5–40% MeCN/H_2O gradient (with 0.1% formic acid). t_R = 8.9 min. The pure peptide was obtained in 17% overall yield based on initial resin loading. HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{85}H_{122}N_{22}NaO_{30}$ 1953.8589, found 1953.8600.



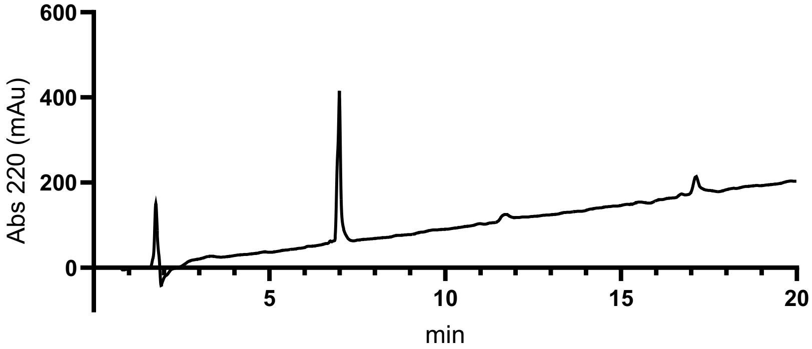
(Me)hAla11-CMP. The crude peptide was purified by preparative scale RP-HPLC using a 5-40% MeCN/H_2O gradient (with 0.1% formic acid). t_R = 9.5 min. The pure peptide was obtained in 14% overall yield based on initial resin loading. HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{85}H_{124}N_{22}NaO_{30}$ 1955.8746, found 1955.8779.



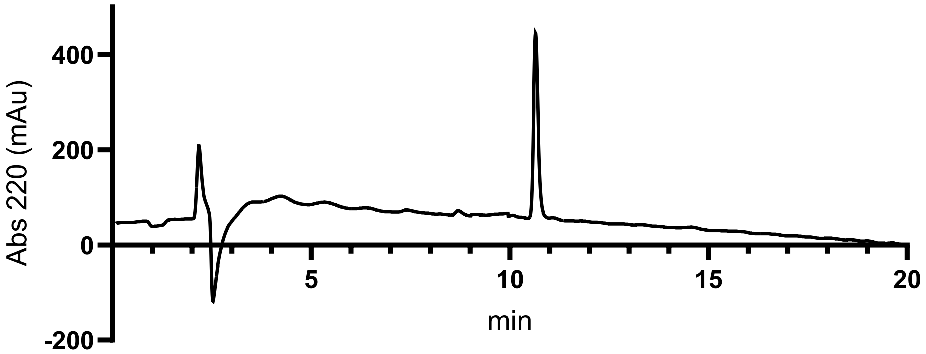
ΔaPro4-PP2. The crude peptide was purified by preparative scale RP-HPLC using a 5-40% MeCN/H_2O gradient (with 0.1% formic acid). t_R = 8.4 min. The pure peptide was obtained in 10% overall yield based on initial resin loading. HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{39}H_{52}N_{10}NaO_{10}$ 843.3760, found 843.3772.

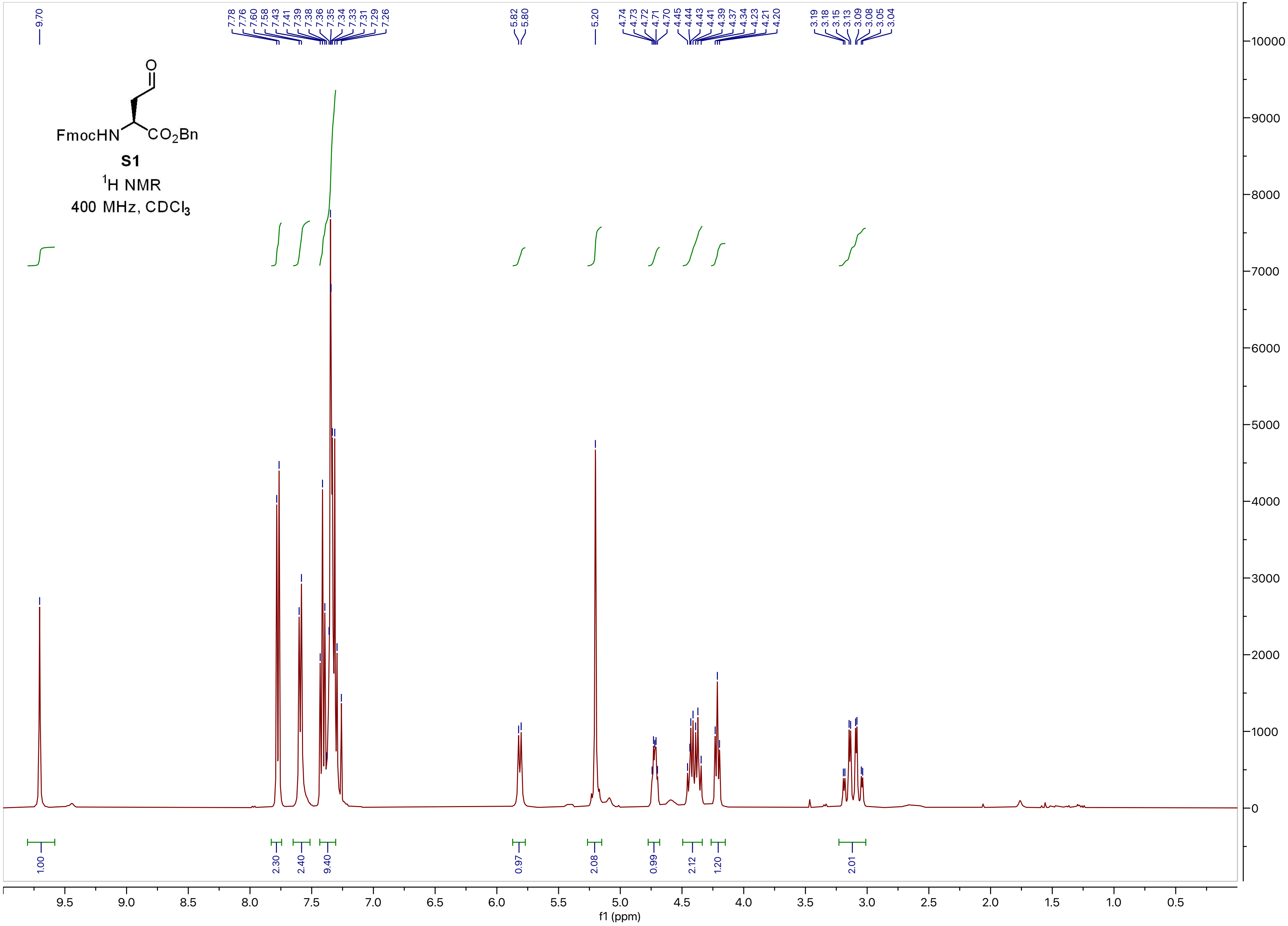


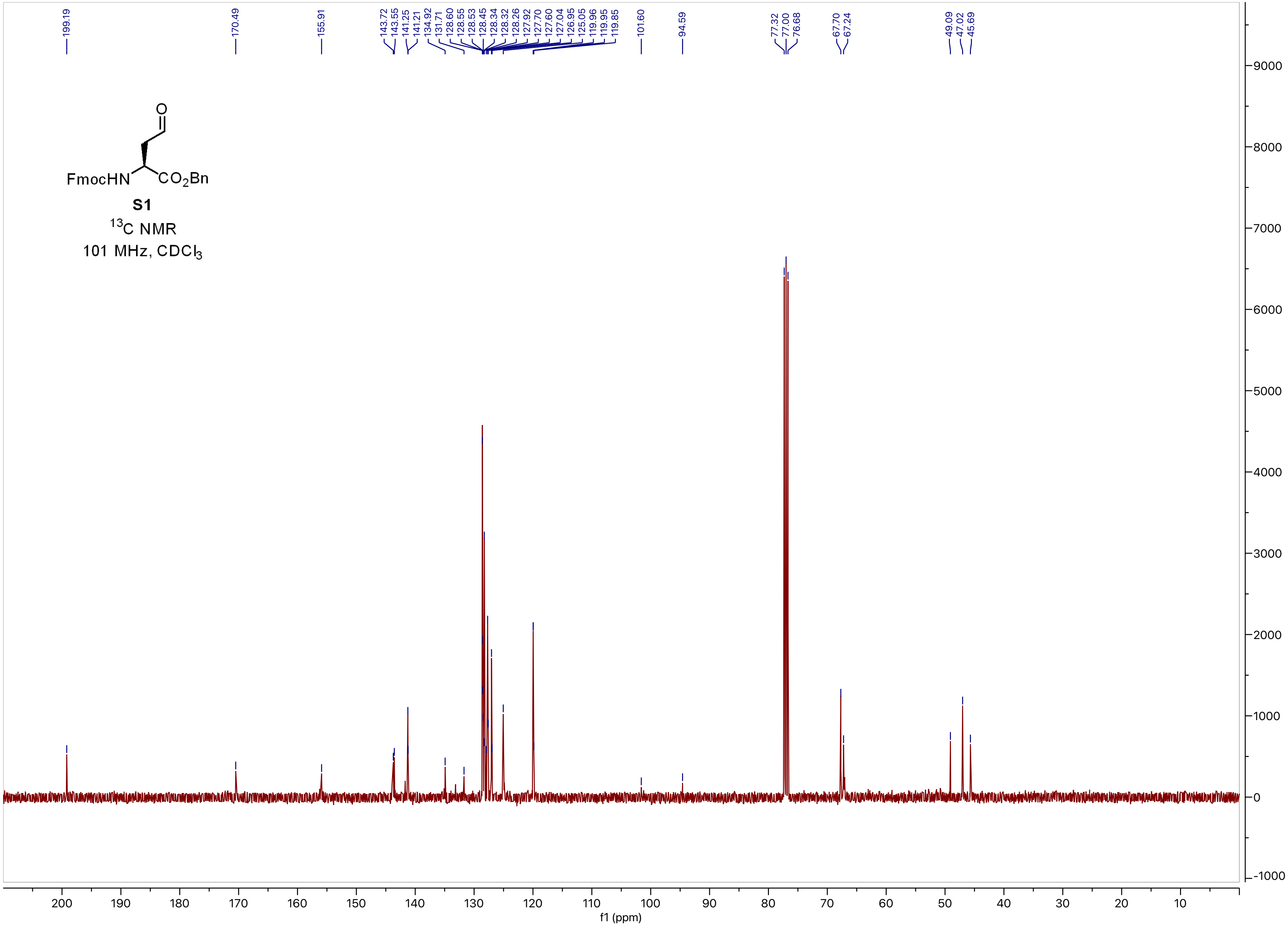
(Me)aPro4-PP2. The crude peptide was purified by preparative scale RP-HPLC using a 5-60% MeCN/H_2O gradient (with 0.1% formic acid). t_R = 7.0 min. The pure peptide was obtained in 8% overall yield based on initial resin loading. HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{40}H_{57}N_{10}O_{10}$ 837.4254, found 837.4258.



oPro4-PP2. The crude peptide was purified by preparative scale RP-HPLC using a 5–60% MeCN/H_2O gradient (with 0.1% formic acid). t_R = 10.1 min. The pure peptide was obtained in 17% overall yield based on initial resin loading. HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{39}H_{53}N_9NaO_{11}$ 846.3757, found 846.3766.



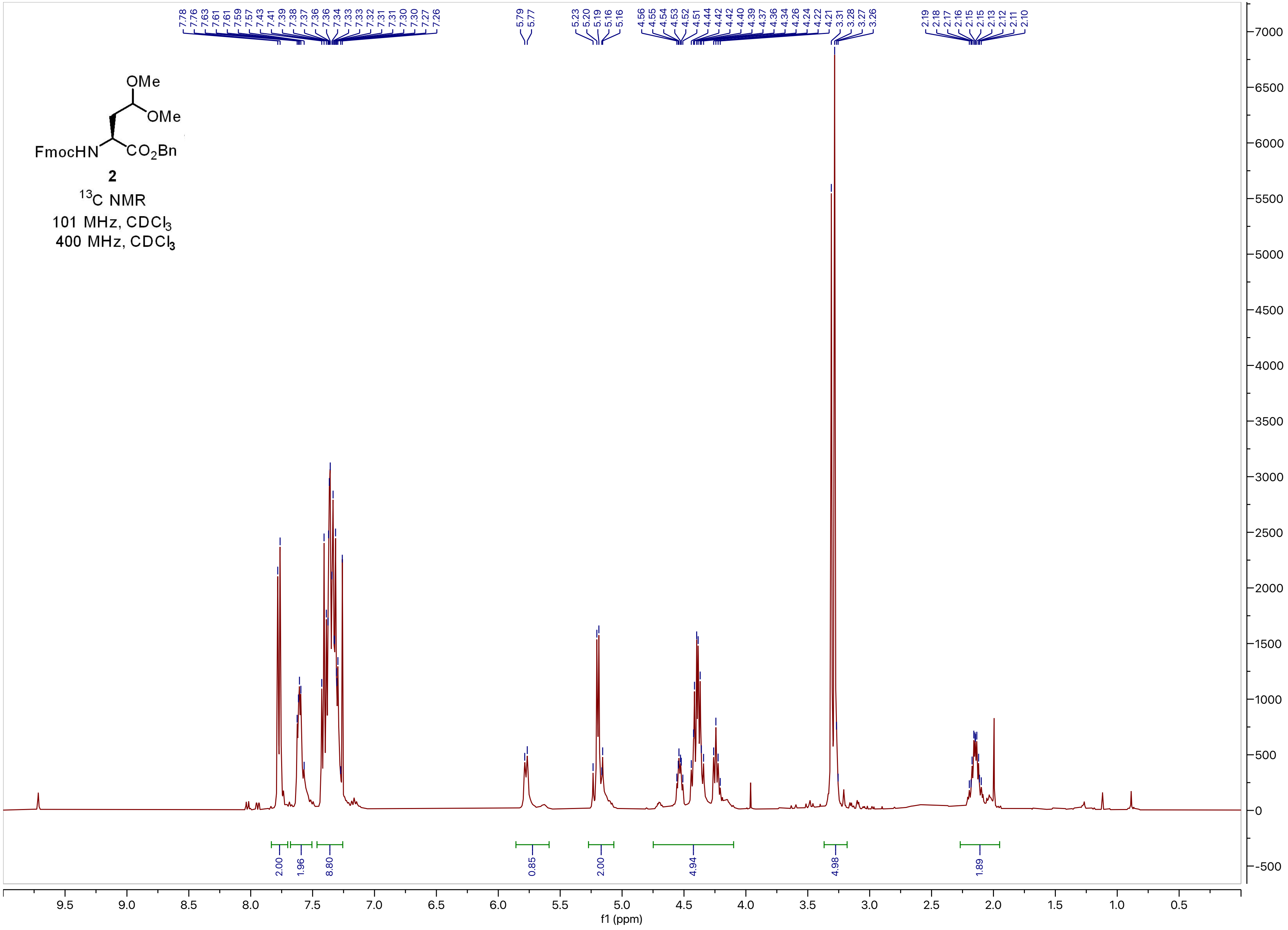

O
FmocHN
CO2Bn
S1
1H NMR
400 MHz, CDCl3
9.70
7.78
7.76
7.60
7.58
7.43
7.41
7.39
7.38
7.36
7.35
7.34
7.33
7.31
7.29
7.26
5.82
5.80
5.20
4.74
4.73
4.72
4.71
4.70
4.45
4.44
4.43
4.41
4.39
4.37
4.34
4.23
4.21
4.20
3.19
3.18
3.15
3.13
3.09
3.08
3.05
3.04
1.00
2.30
2.40
9.40
0.97
2.08
0.99
2.12
1.20
2.01
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)



S1

^{13}C NMR

101 MHz, $CDCl_3$

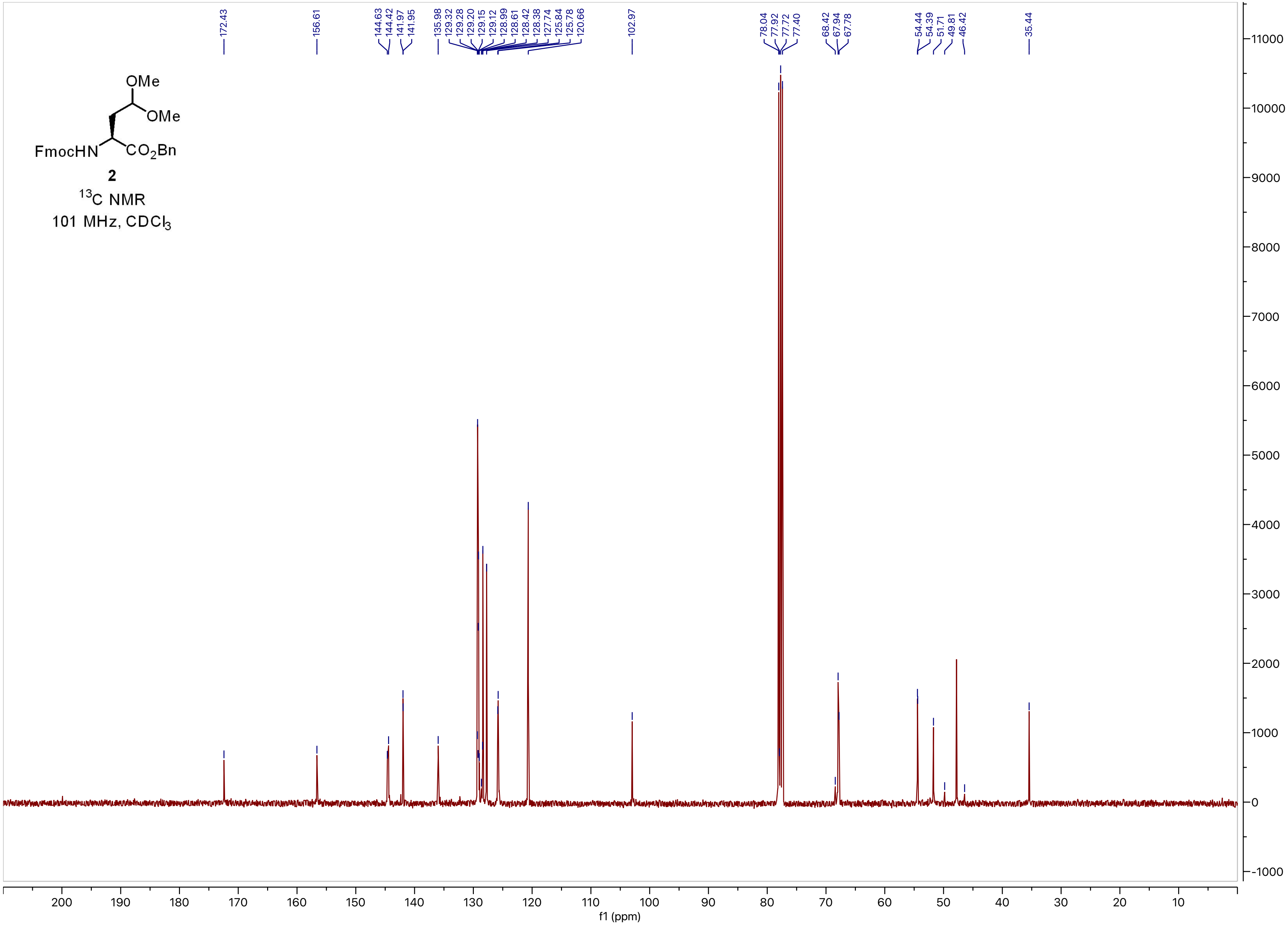


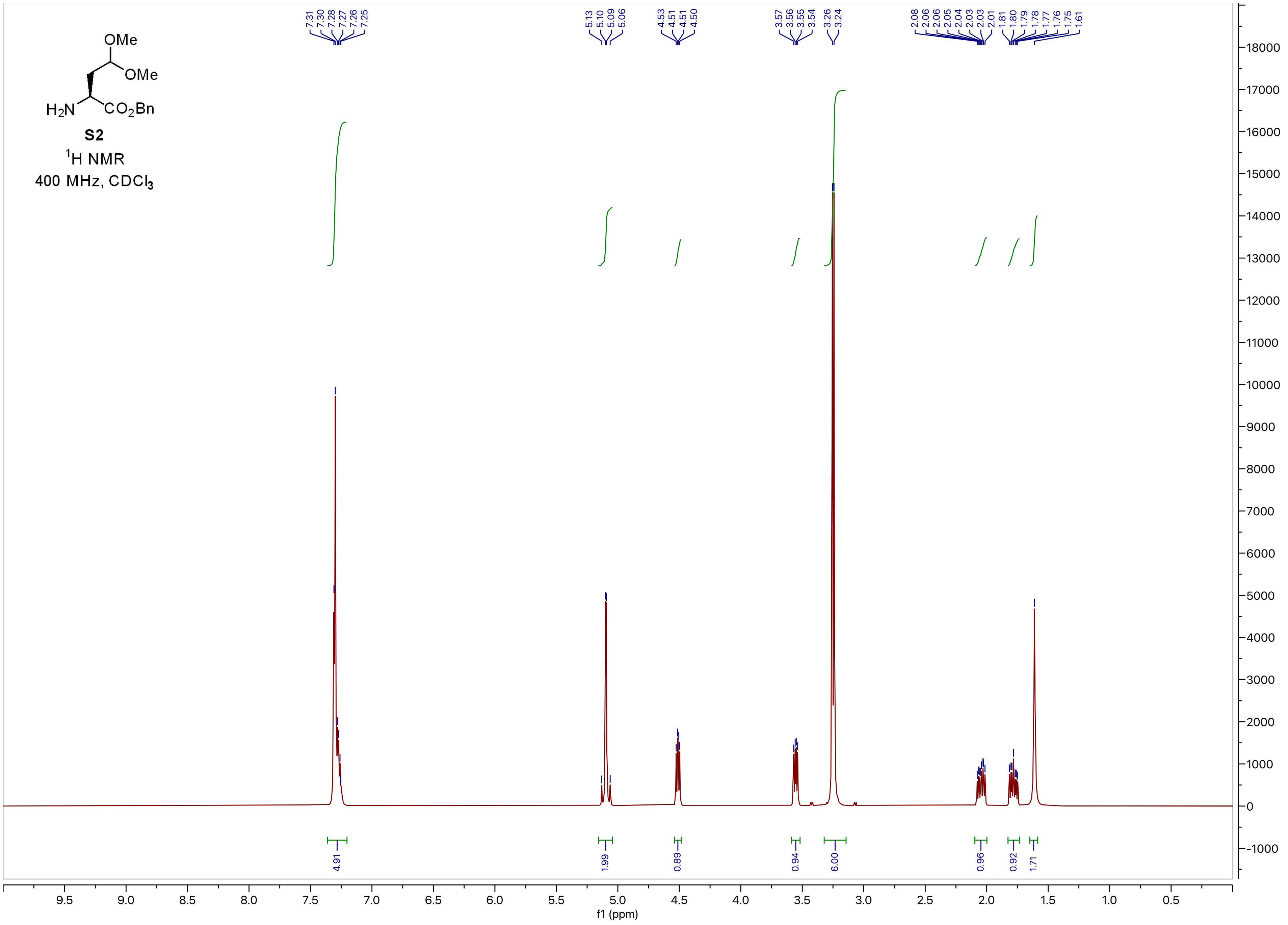
2

^{13}C NMR

101 MHz, $CDCl_3$

400 MHz, $CDCl_3$

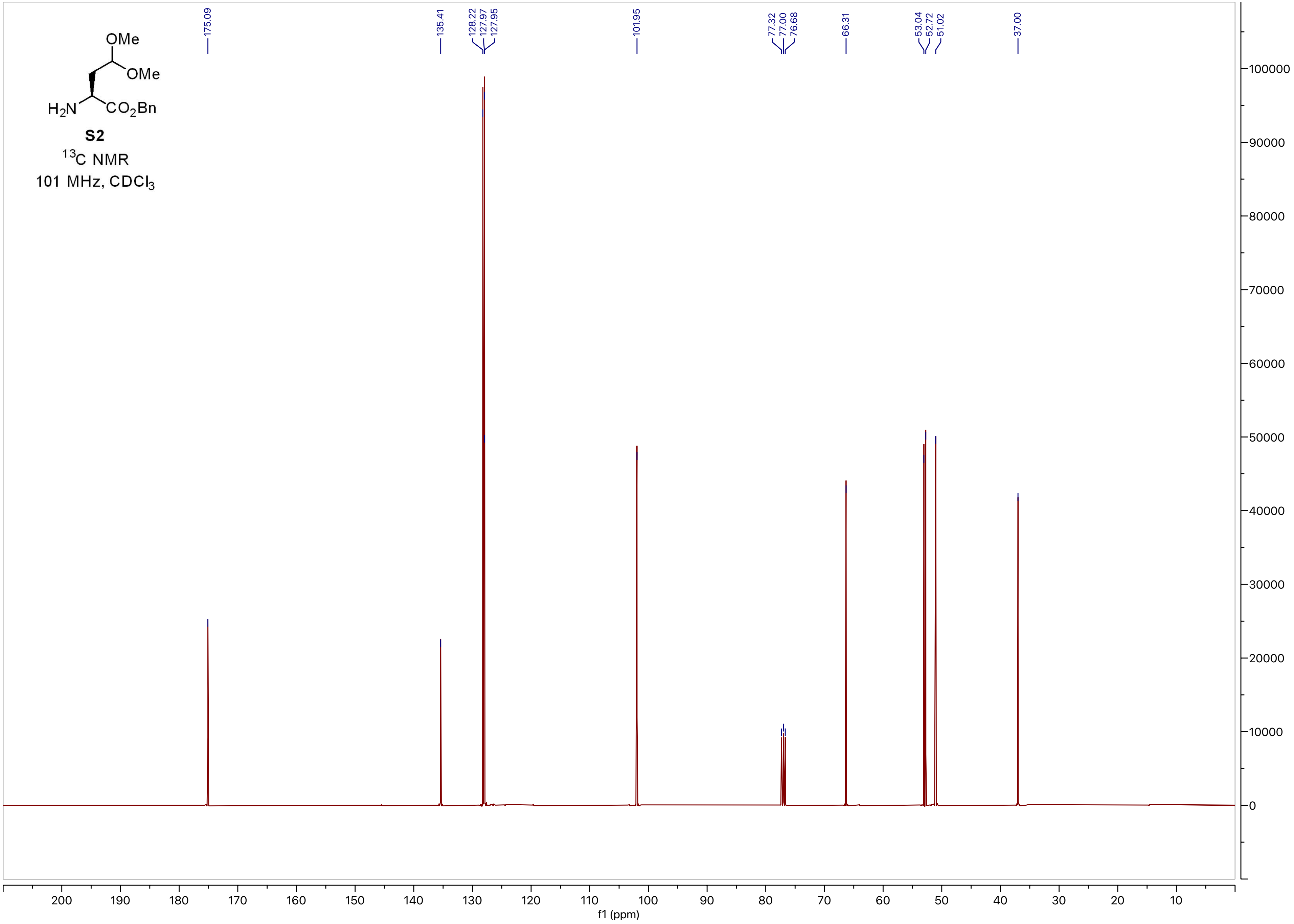

172.43
156.61
144.63
144.42
141.97
141.95
135.98
129.32
129.28
129.20
129.15
129.12
128.99
128.61
128.42
128.38
127.74
125.84
125.78
120.66
102.97
78.04
77.92
77.72
77.40
68.42
67.94
67.78
54.44
54.39
51.71
49.81
46.42
35.44
OMe
OMe
FmocHN
CO2Bn
2
13C NMR
101 MHz, CDCl3
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)



S2

^1H NMR

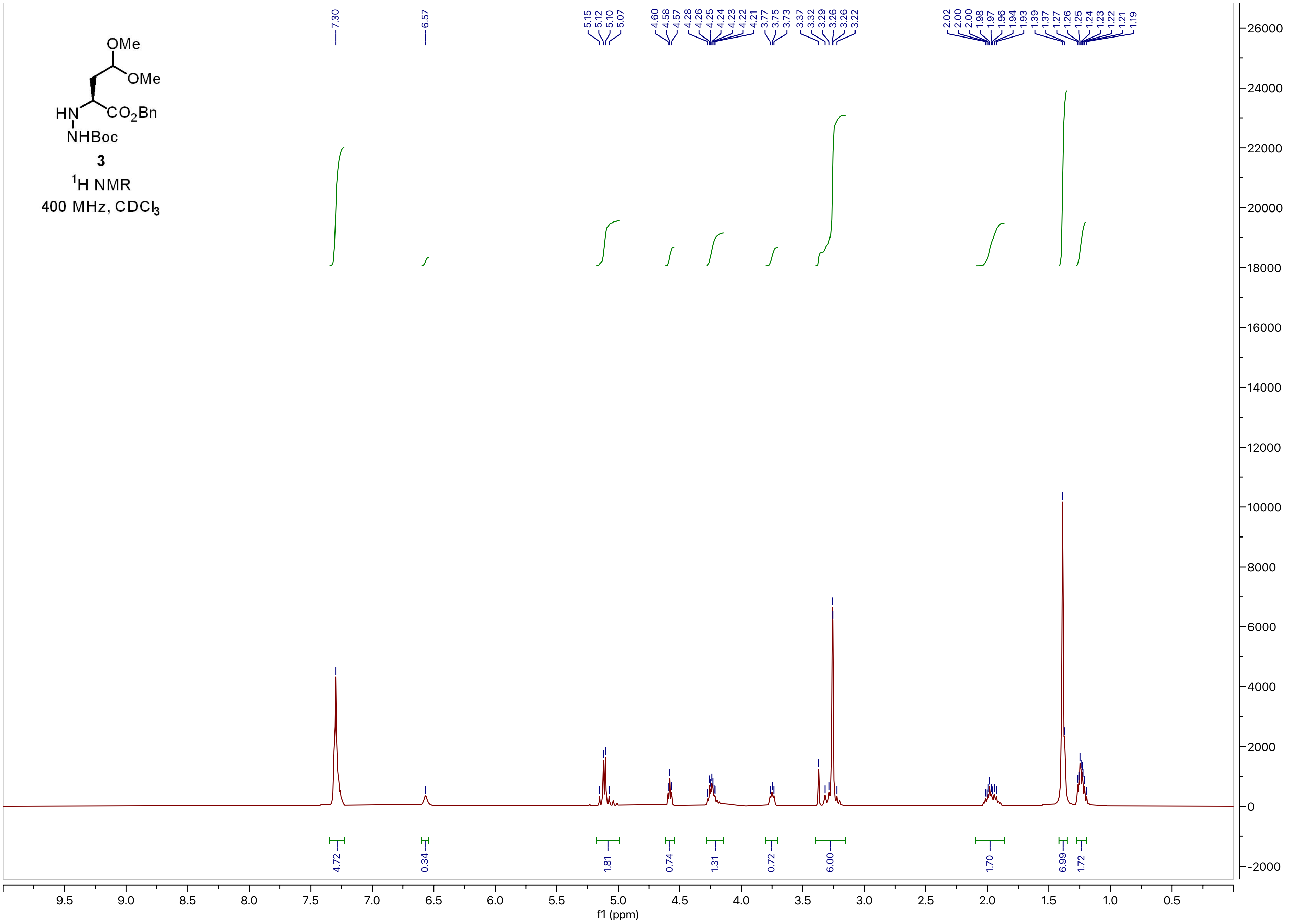
400 MHz, $CDCl_3$



S2

^{13}C NMR

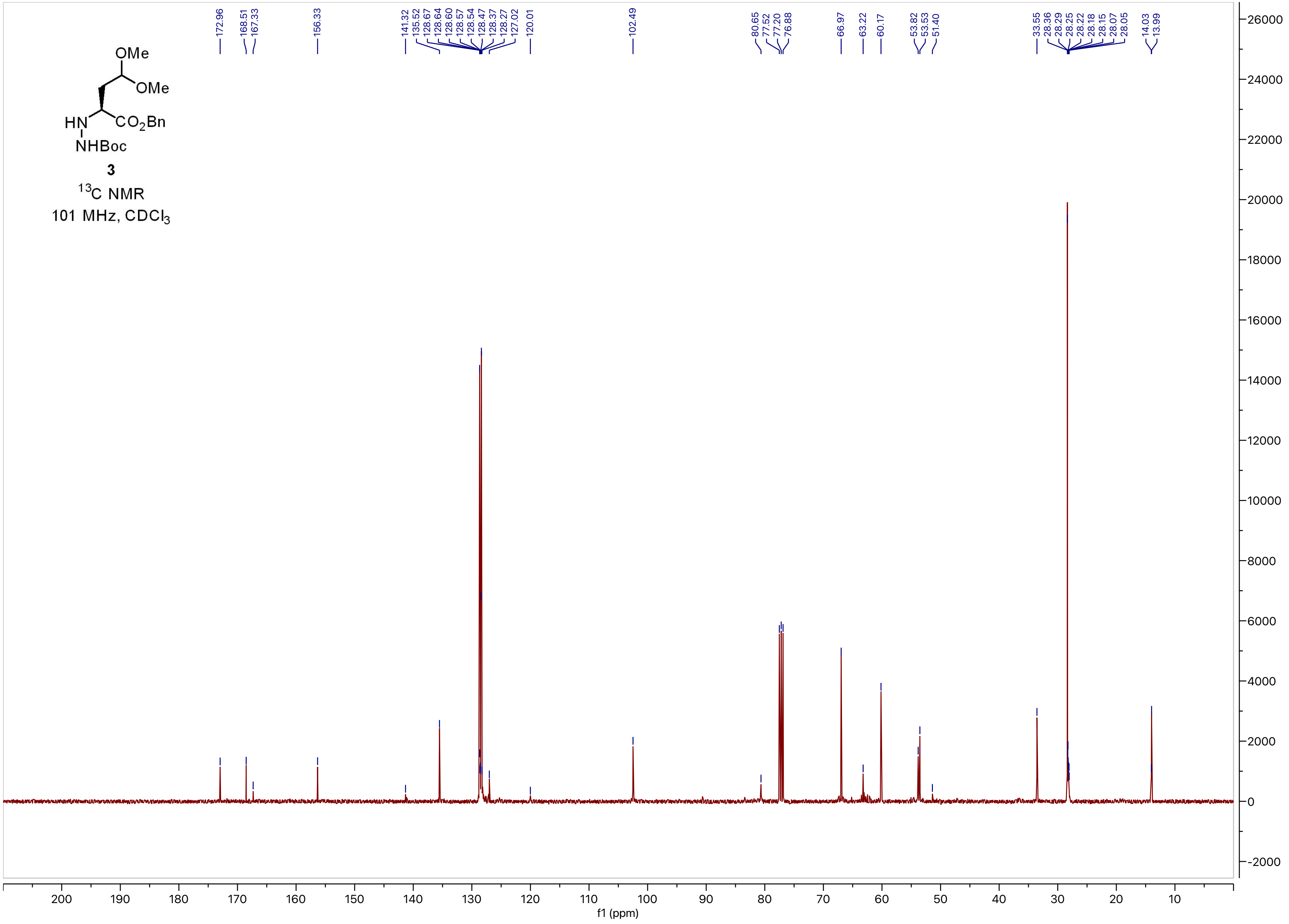
101 MHz, $CDCl_3$



3

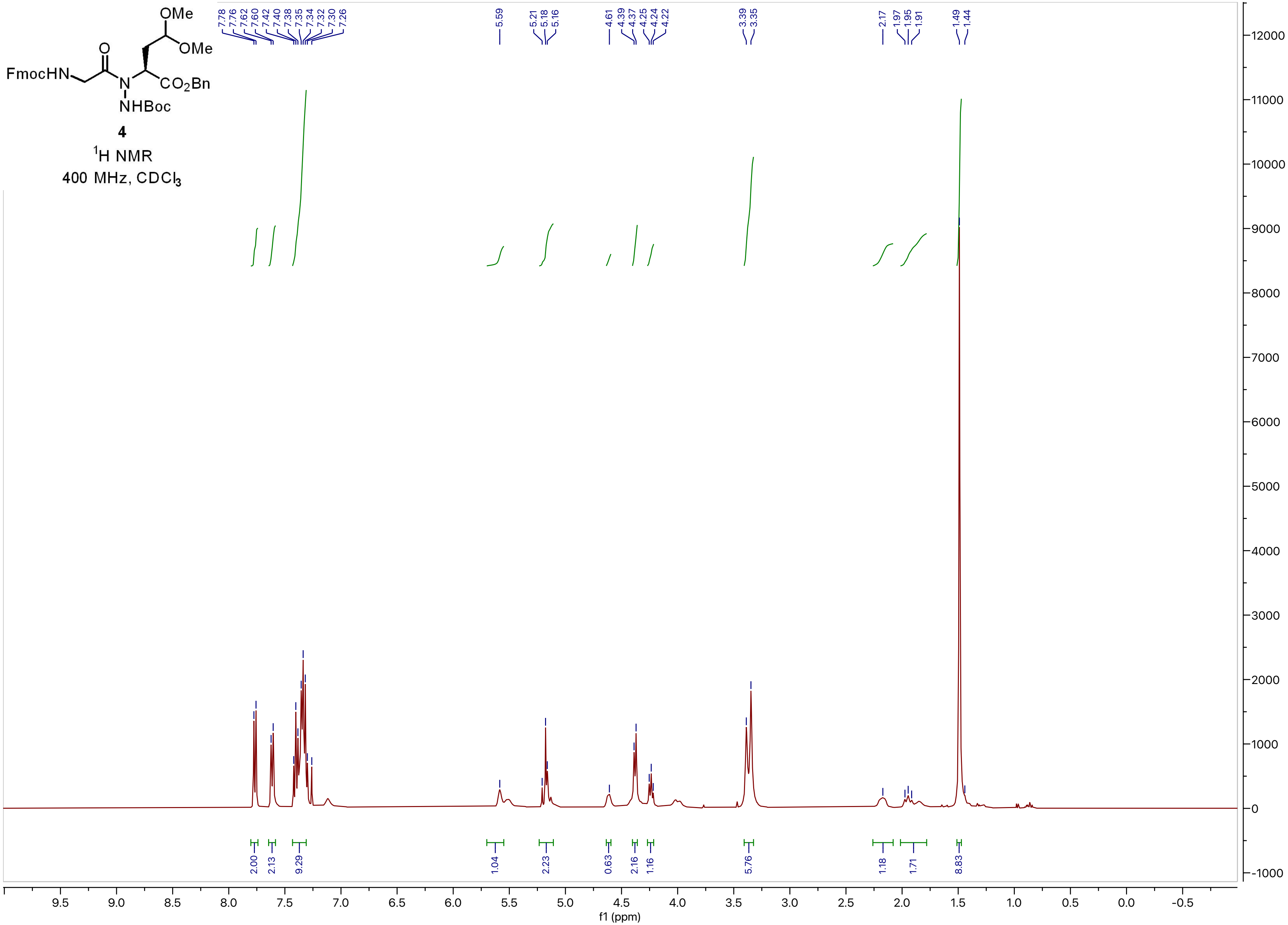
^{1}H NMR

400 MHz, $CDCl_3$


3

^{13}C NMR

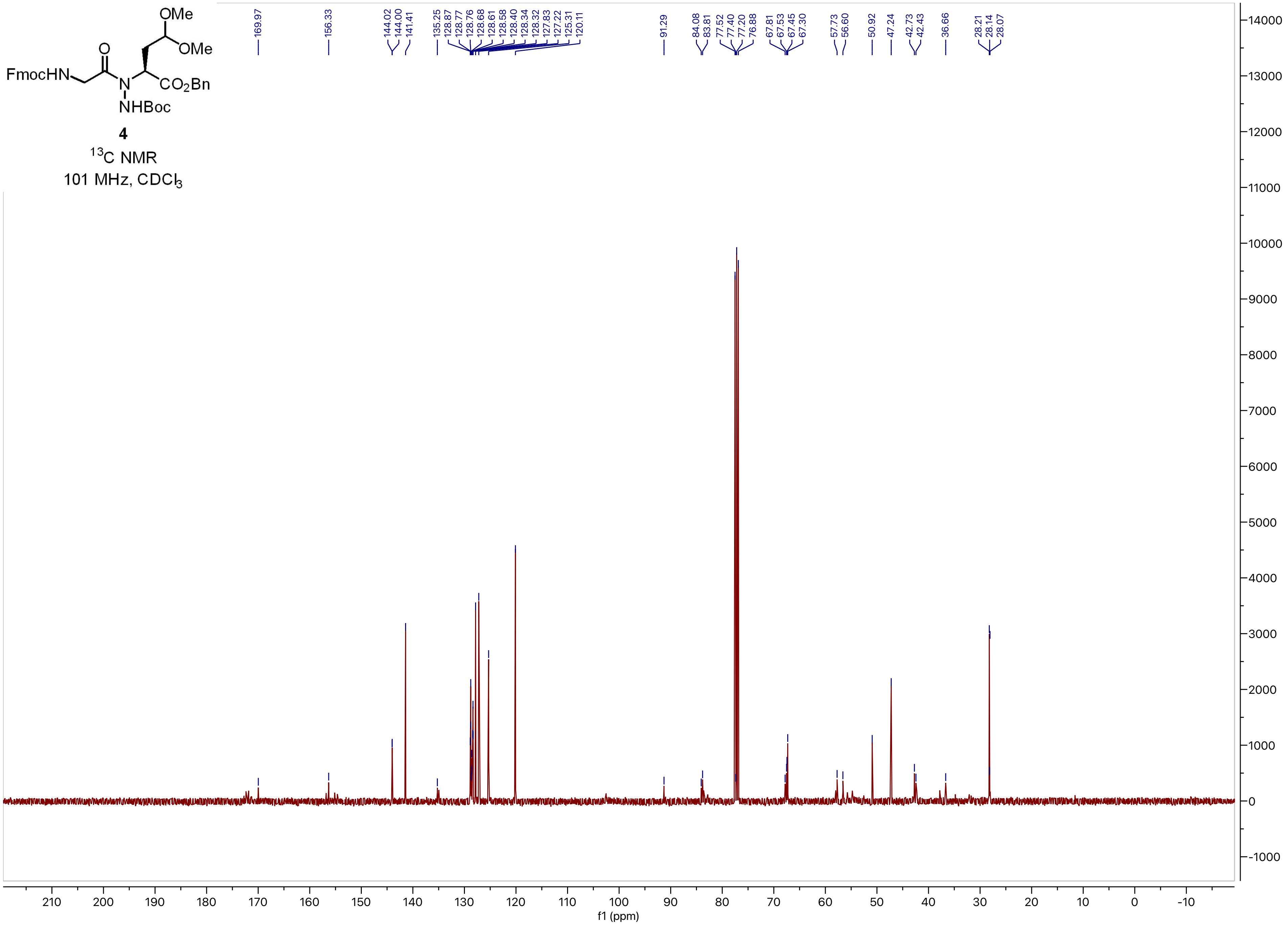
101 MHz, $CDCl_3$



4

^{1}H NMR

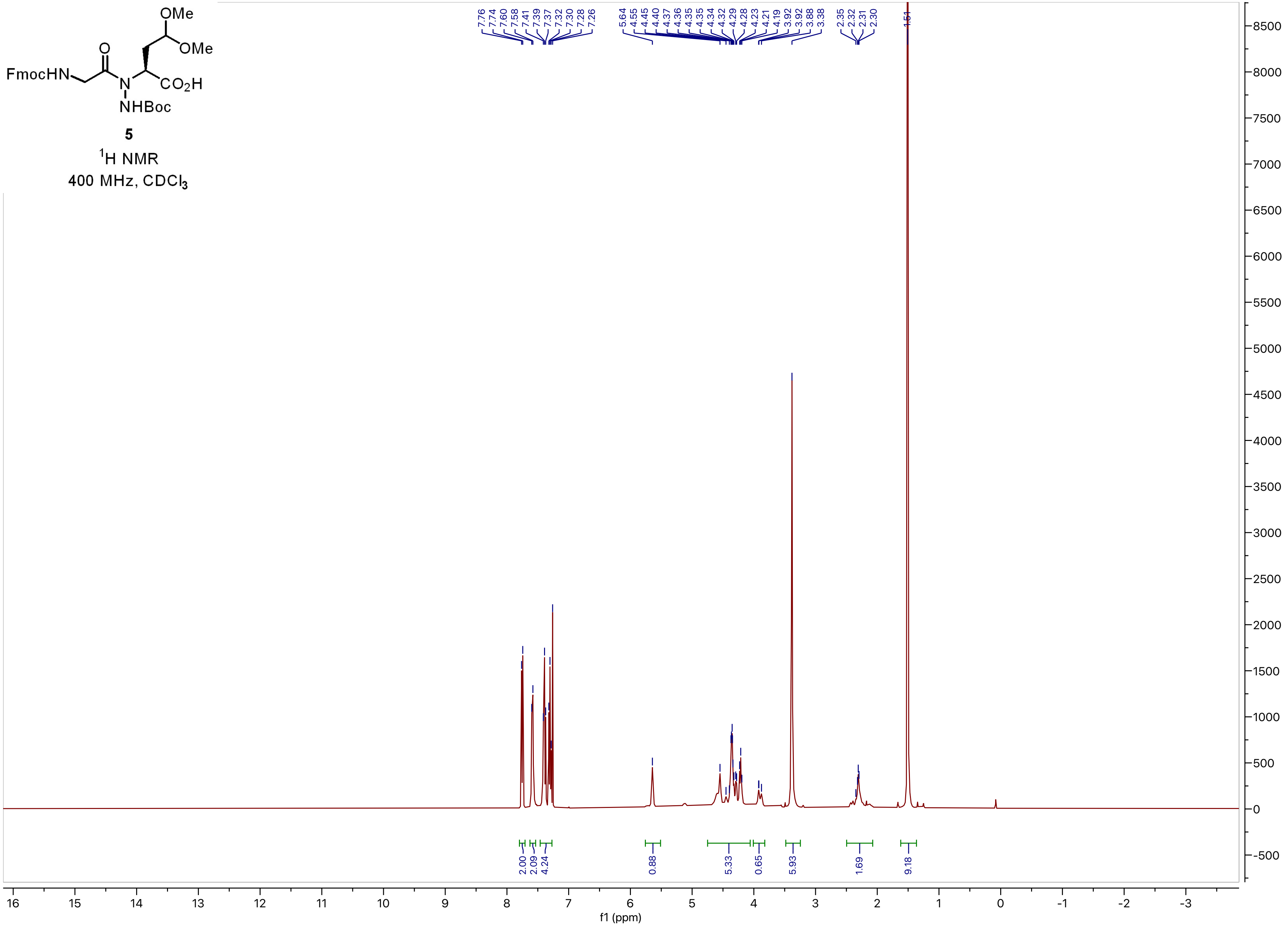
400 MHz, CDCl$_3$



4

^{13}C NMR

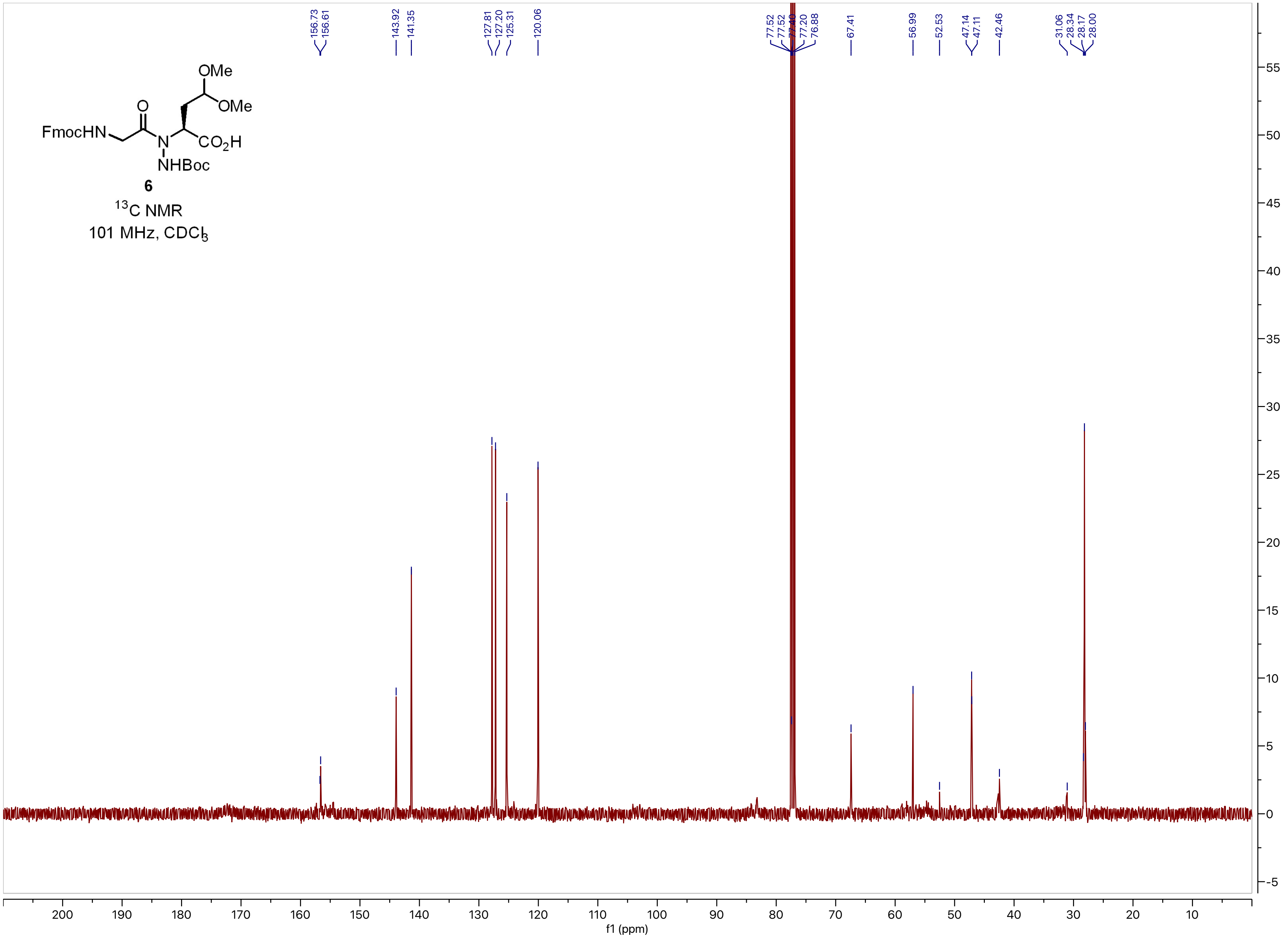
101 MHz, $CDCl_3$



5

^{1}H NMR

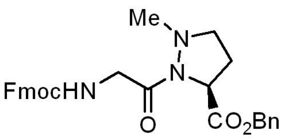
400 MHz, CDCl$_3$



6

^{13}C NMR

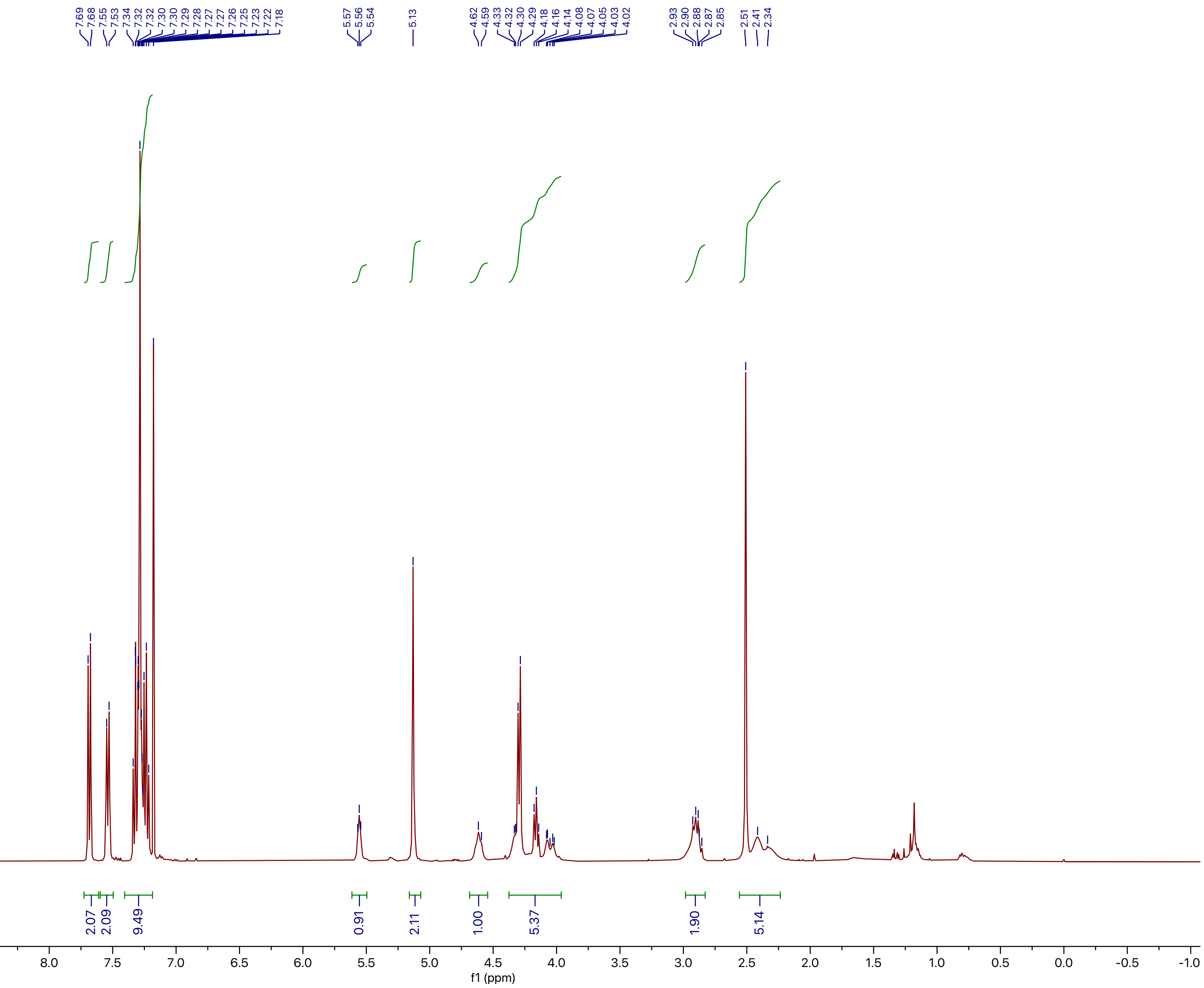
101 MHz, CDCl$_3$

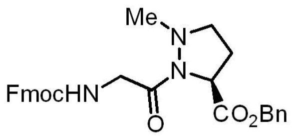


6

^{1}H NMR

400 MHz, CDCl$_3$

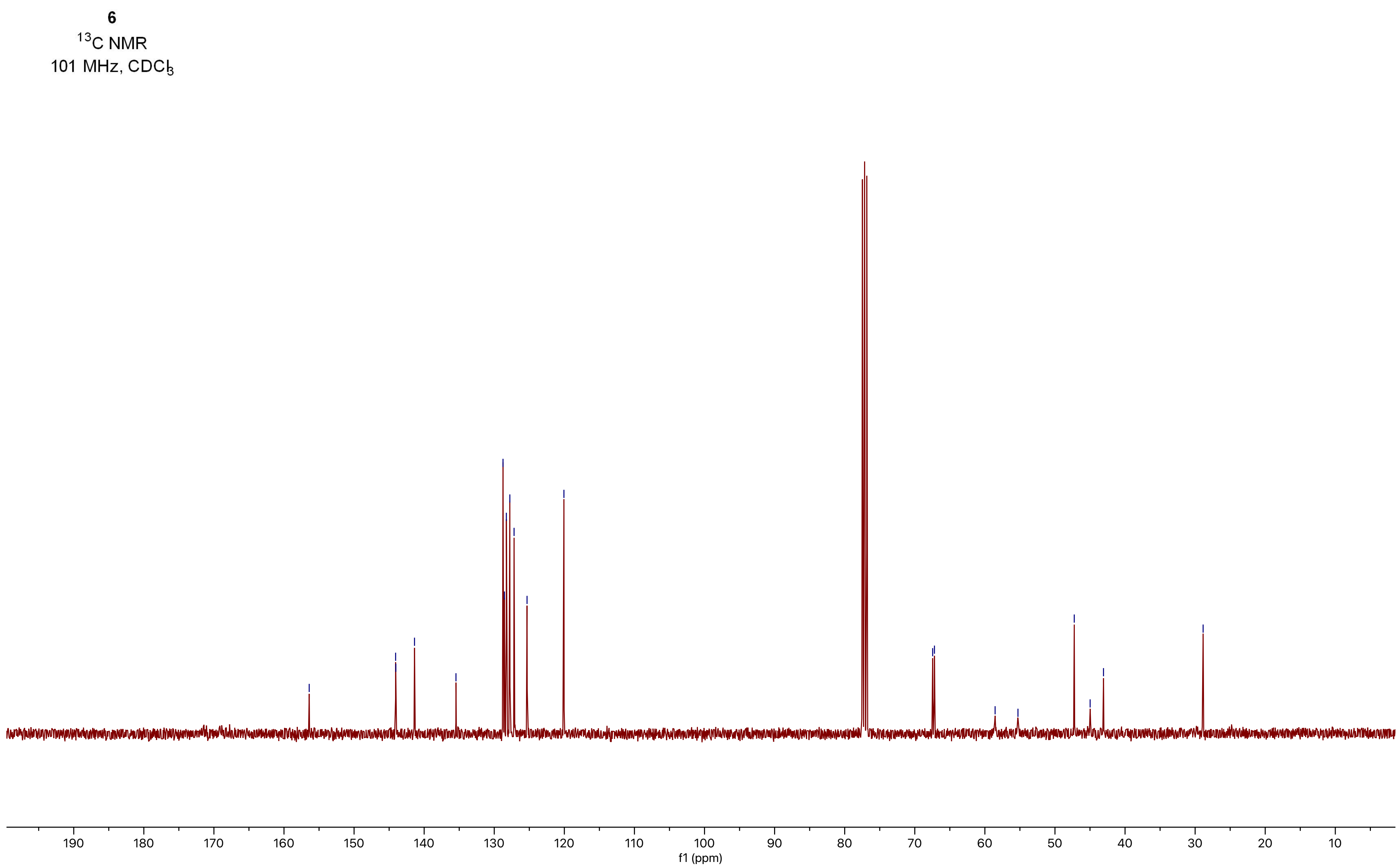


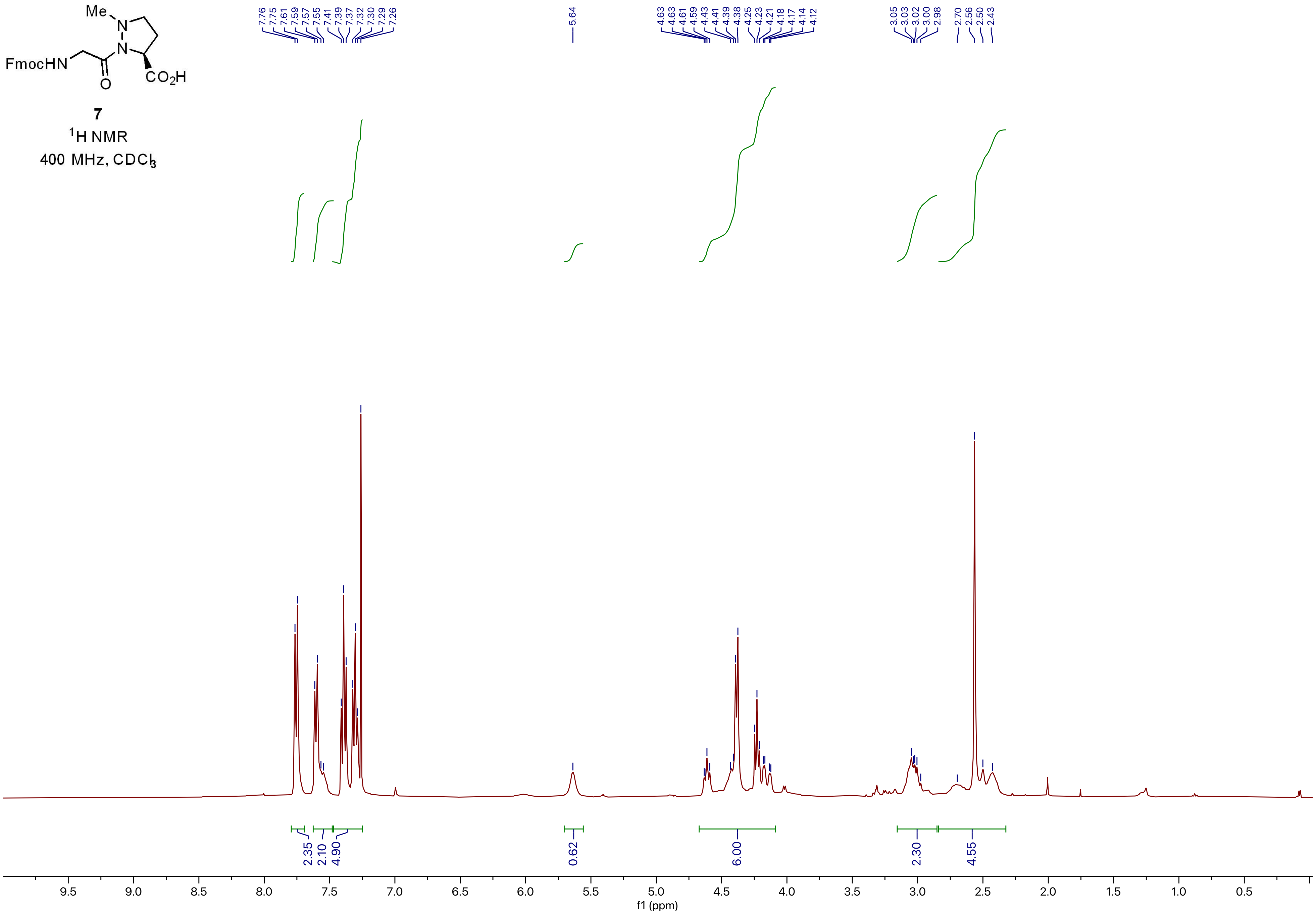


6

^{13}C NMR

101 MHz, CDCl$_3$

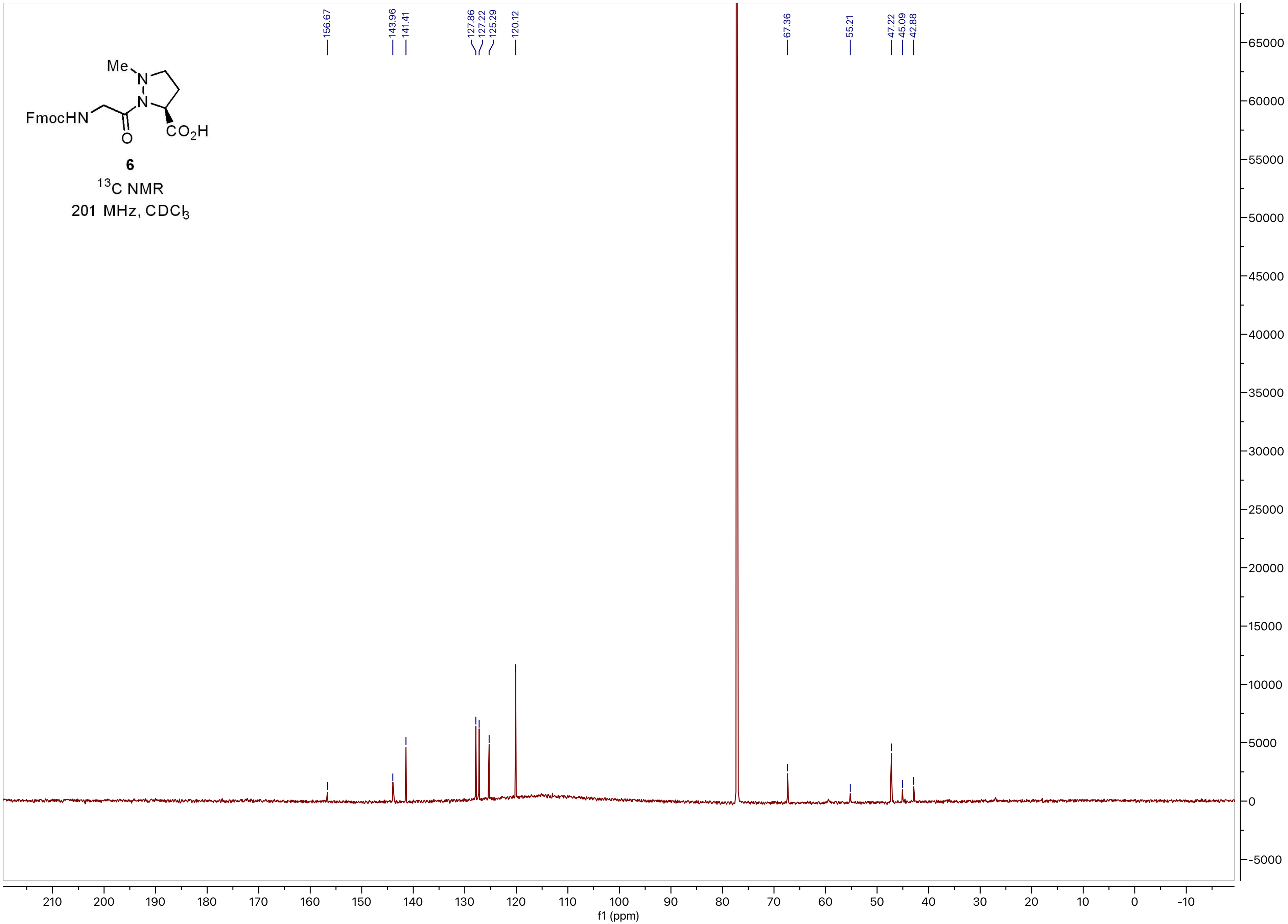




7

^{1}H NMR

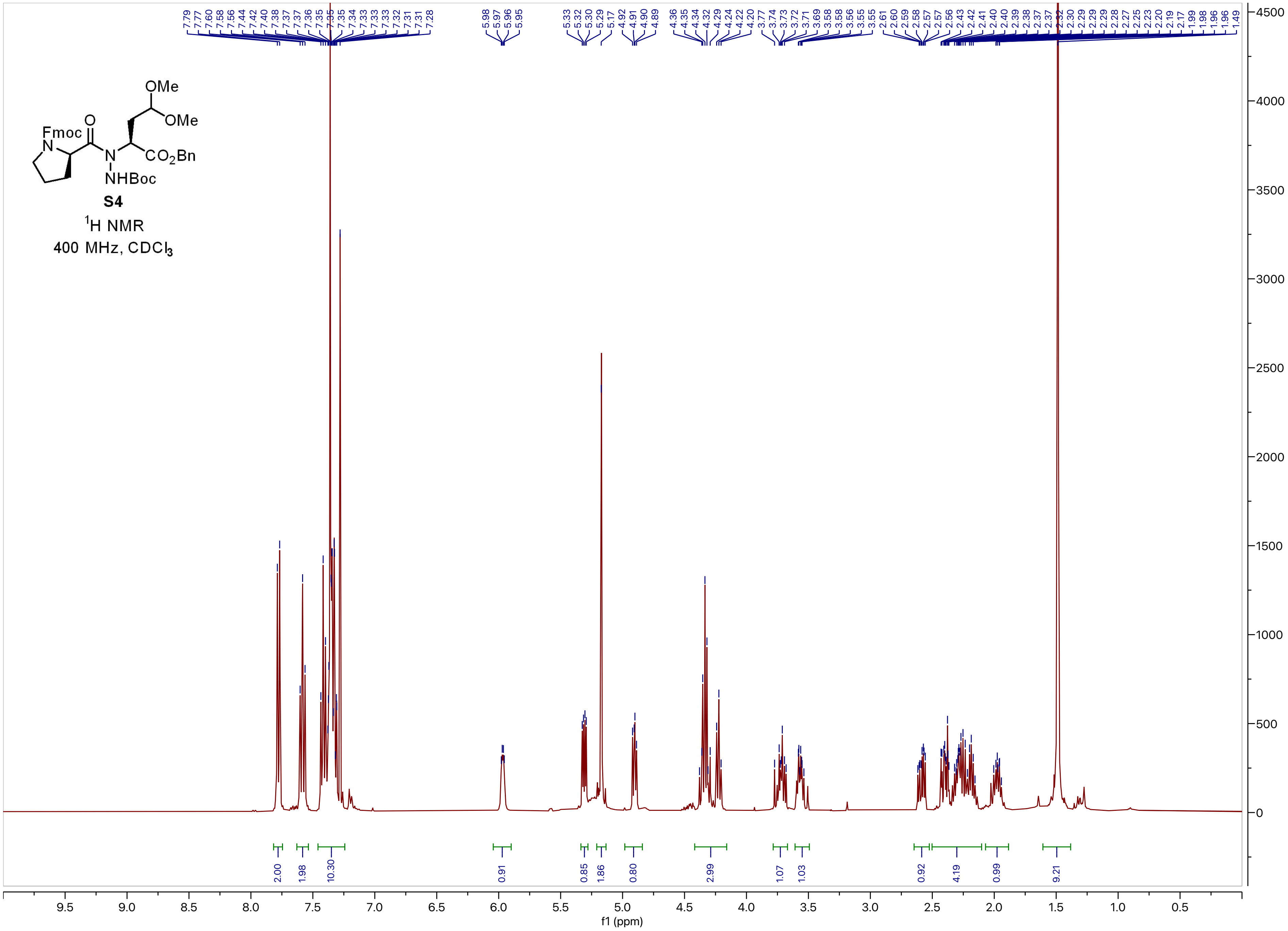
400 MHz, $CDCl_3$



6

^{13}C NMR

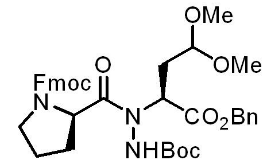
201 MHz, $CDCl_3$



S4

^{1}H NMR

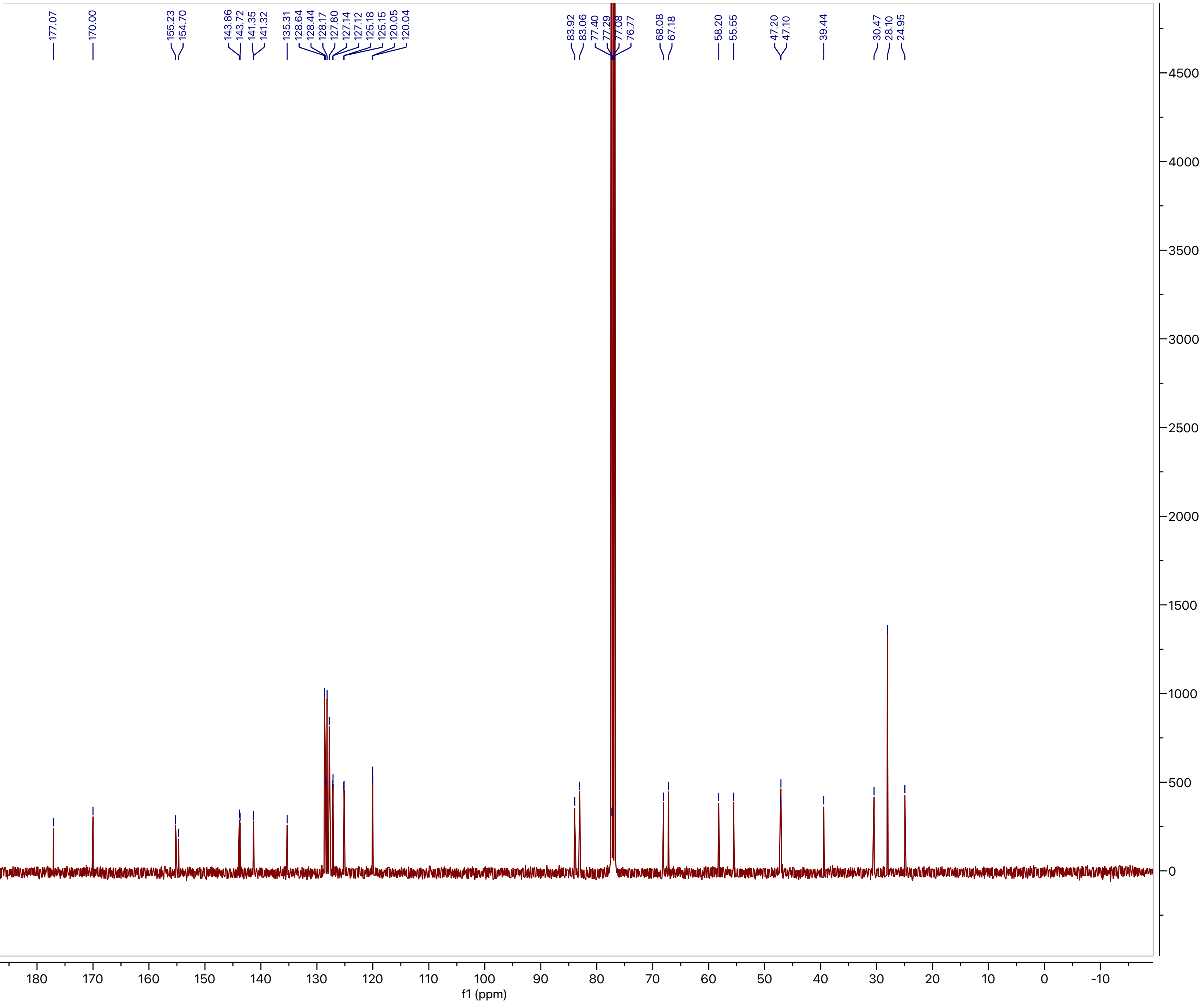
400 MHz, $CDCl_3$

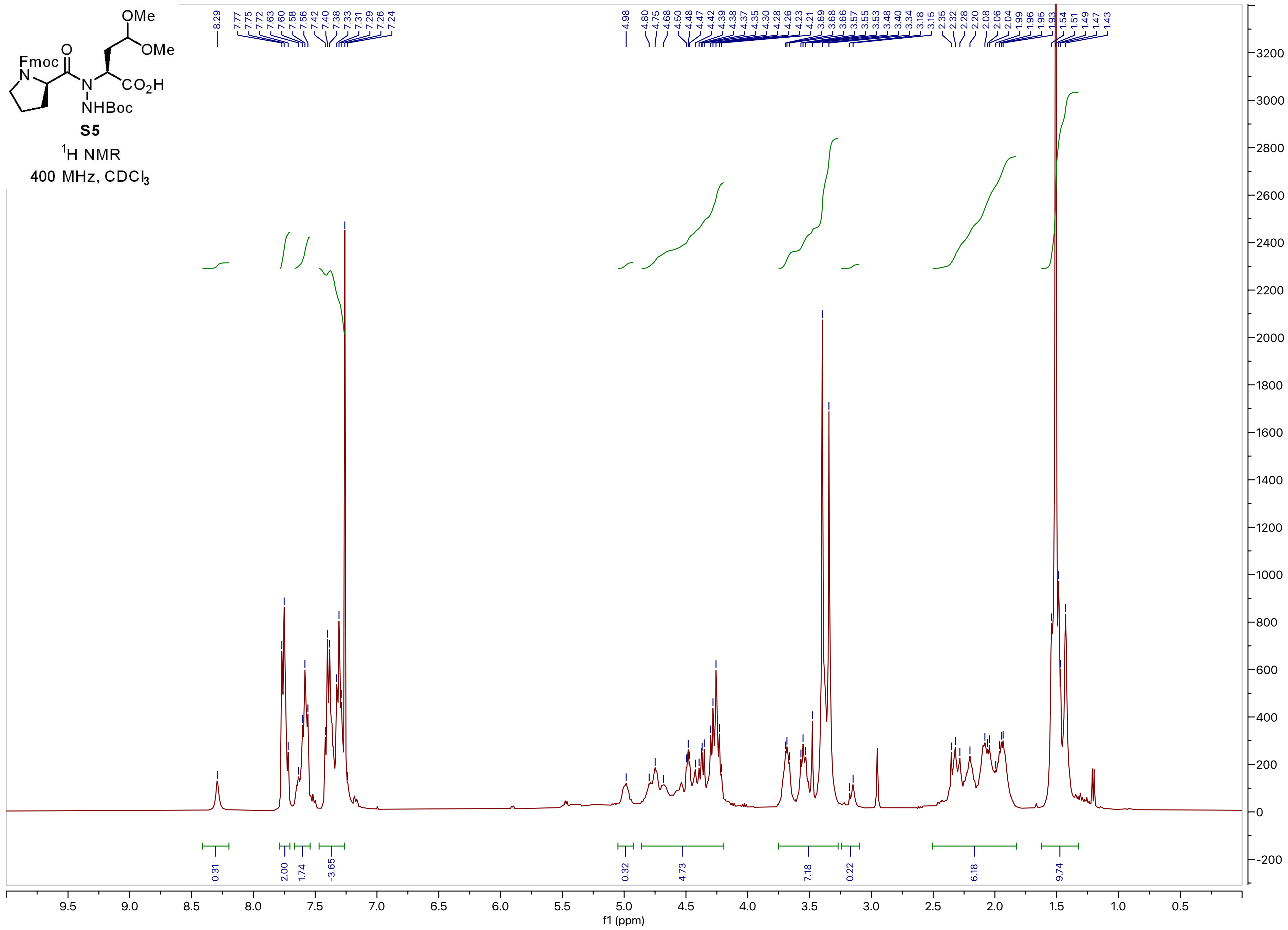


S4

^{13}C NMR

101 MHz, CDCl$_3$

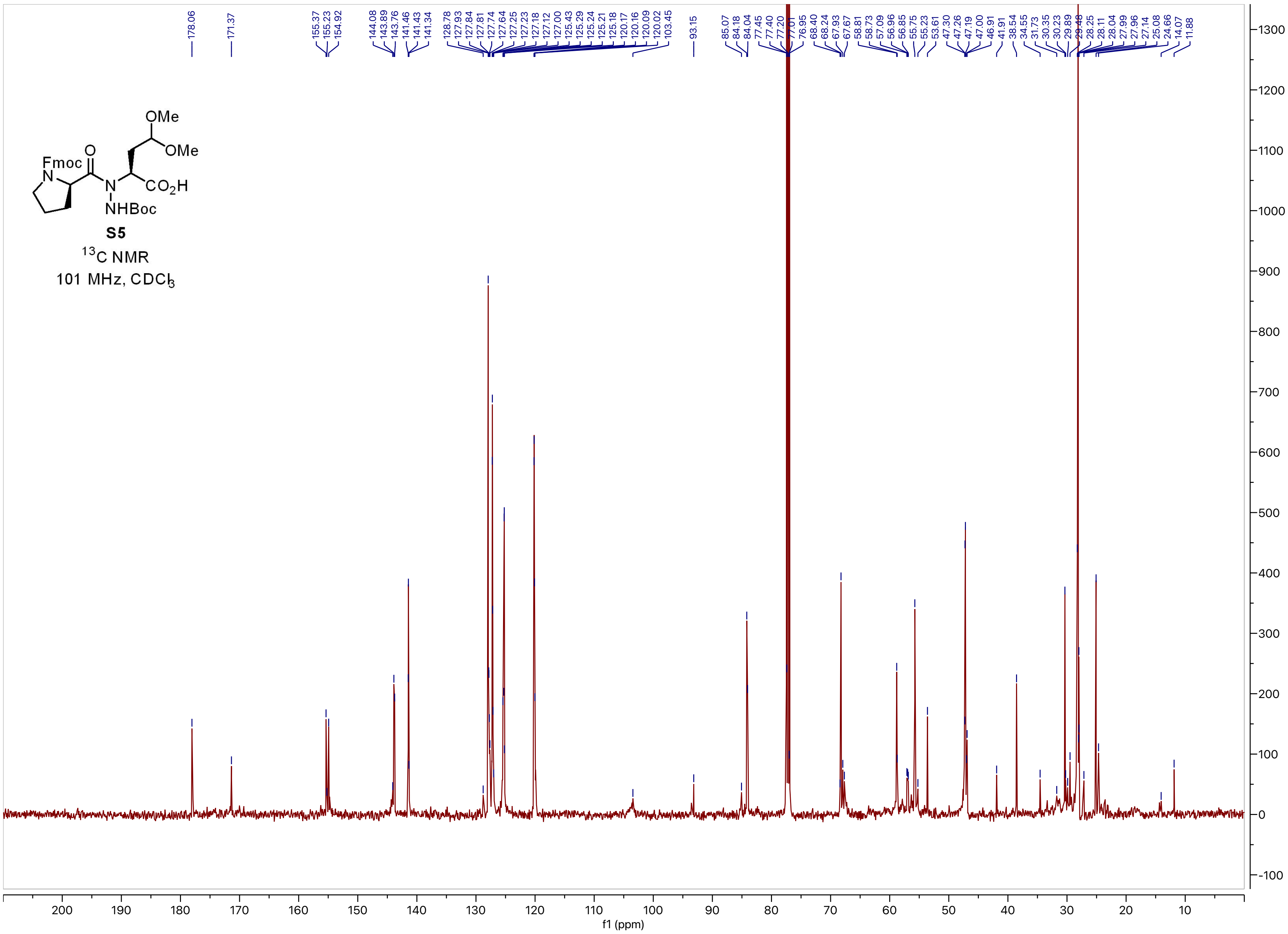




S5

^{1}H NMR

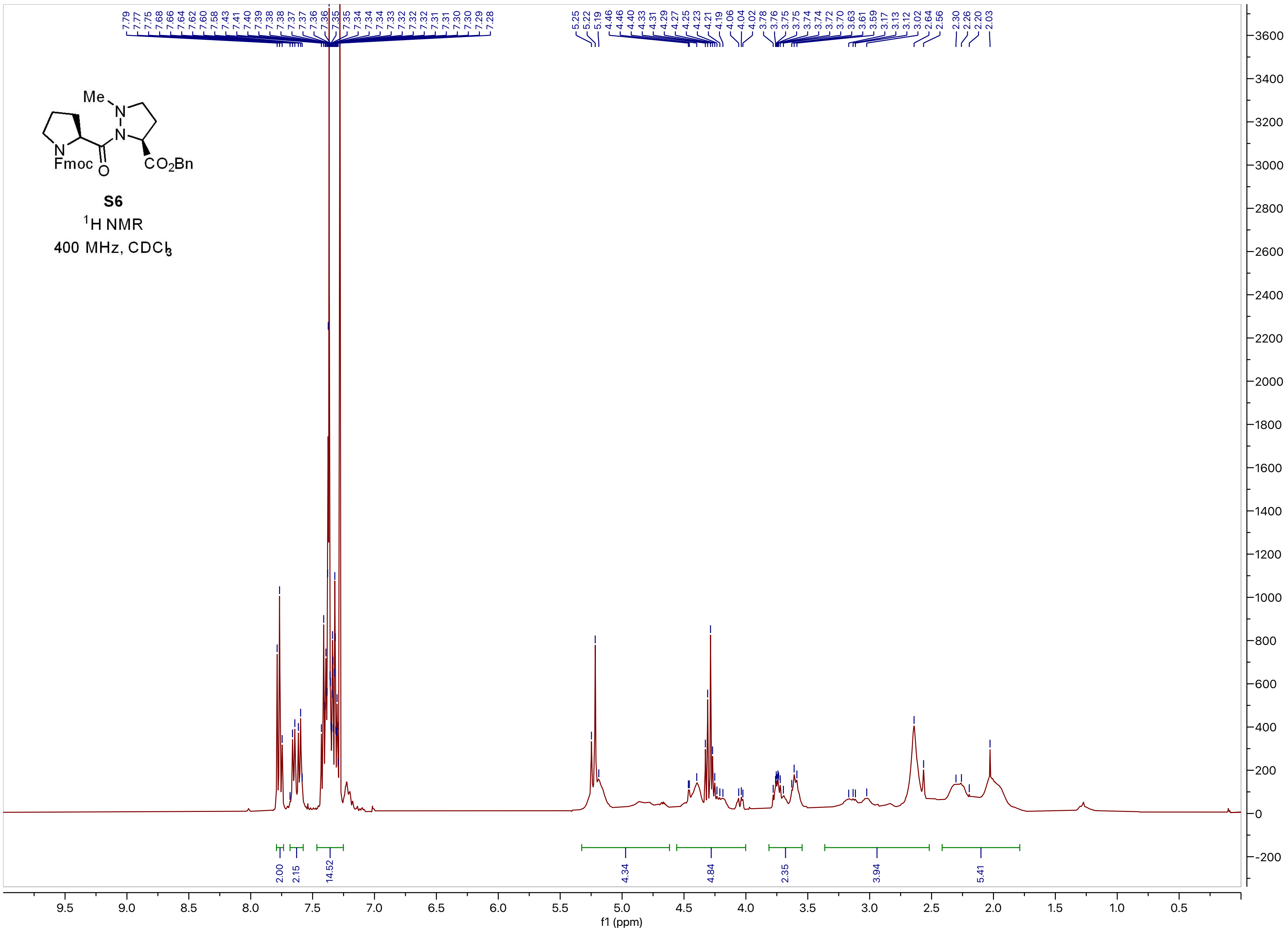
400 MHz, CDCl$_3$



S5

^{13}C NMR

101 MHz, $CDCl_3$


Me
N
N
N
Fmoc
O
CO$_2$Bn
S6
^{1}H NMR
400 MHz, CDCl$_3$
7.79
7.77
7.75
7.68
7.66
7.64
7.62
7.60
7.58
7.43
7.41
7.40
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7.37
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7.29
7.28
5.25
5.22
5.19
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4.31
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4.27
4.25
4.23
4.21
4.19
4.06
4.04
4.02
3.78
3.76
3.75
3.75
3.74
3.74
3.72
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3.02
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2.56
2.30
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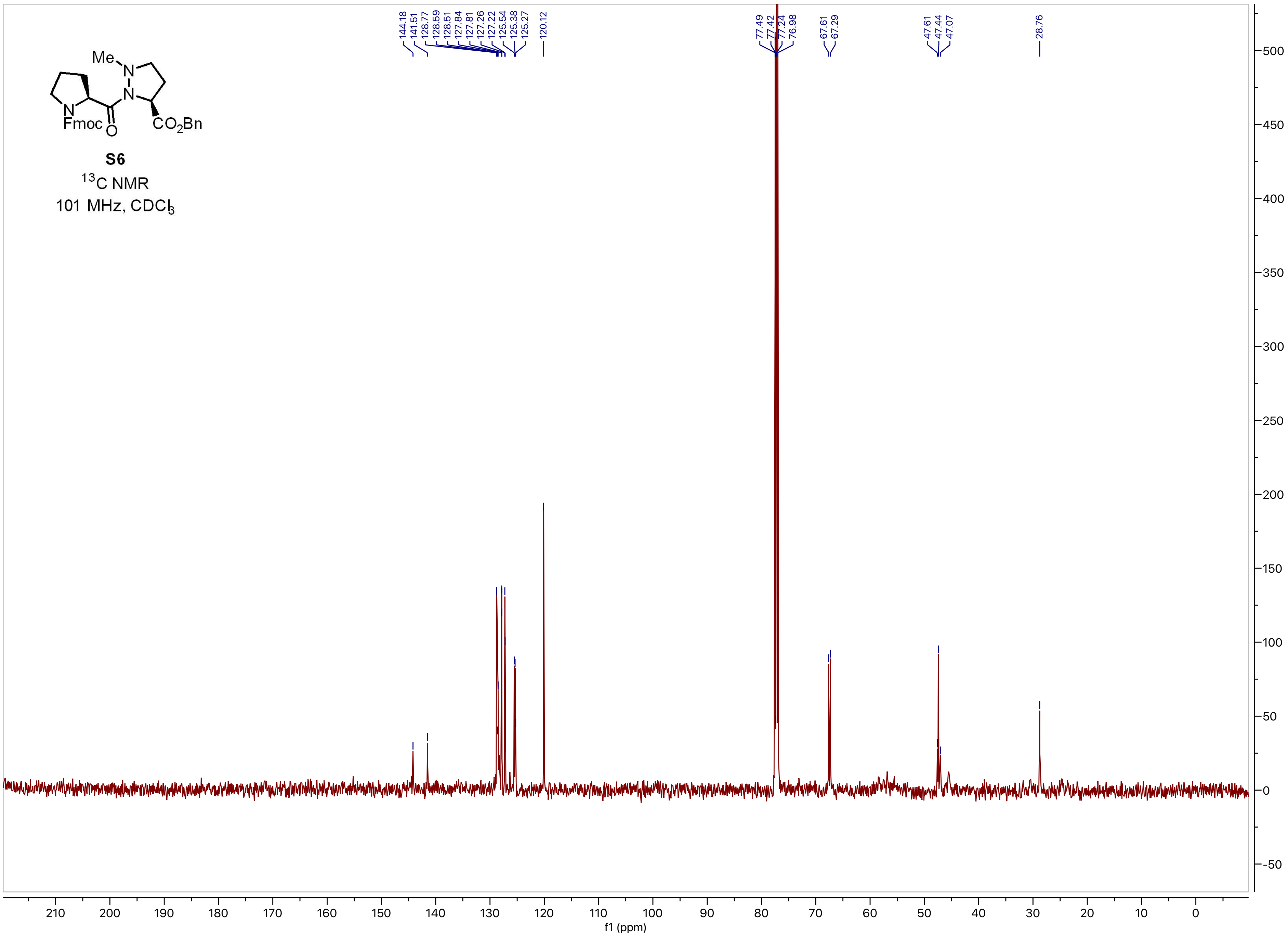
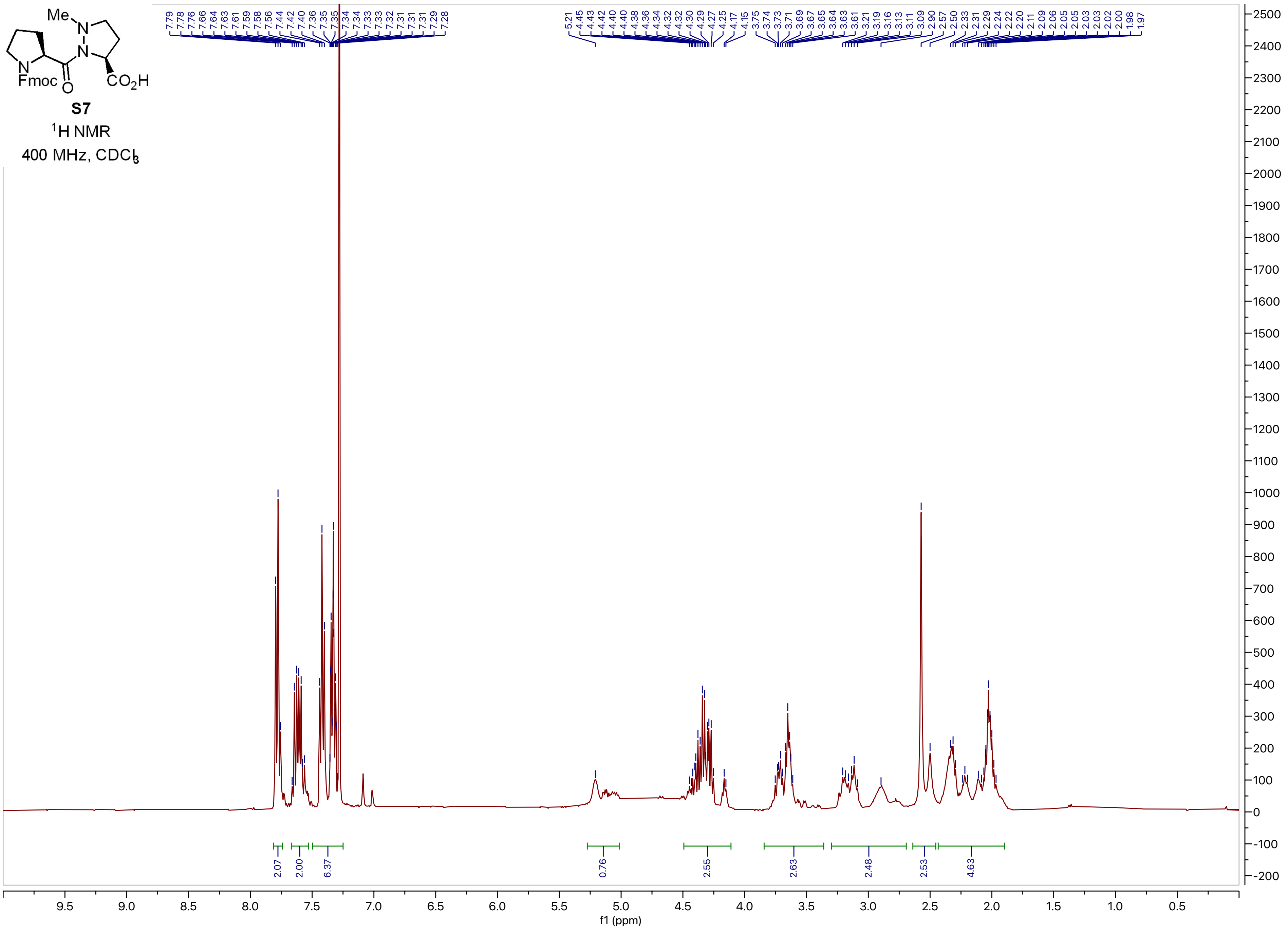
2.20
2.03
2.00
2.15
14.52
4.34
4.84
2.35
3.94
5.41
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)
3600
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200



S6

^{13}C NMR

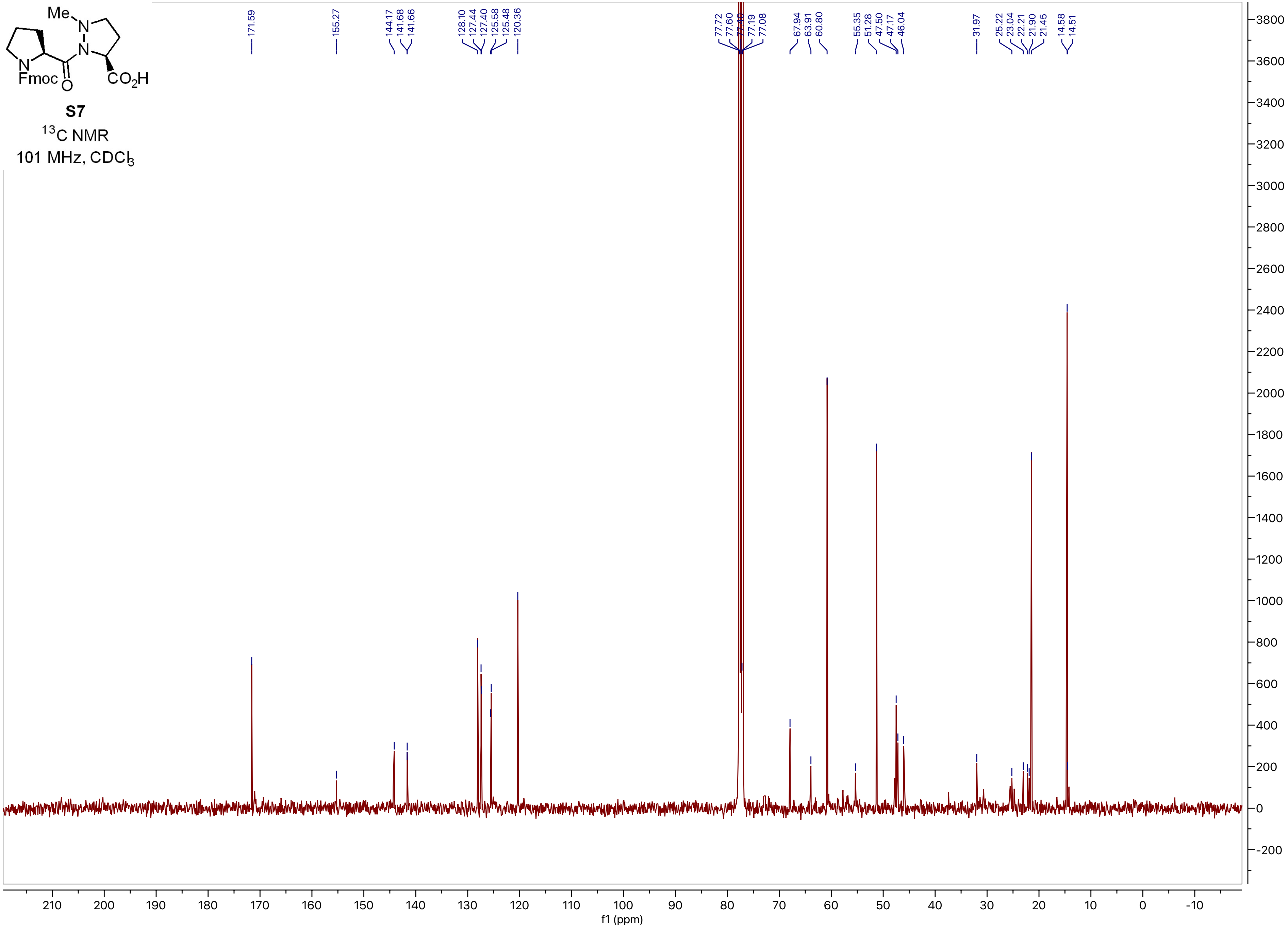
101 MHz, $CDCl_3$



S7

^{1}H NMR

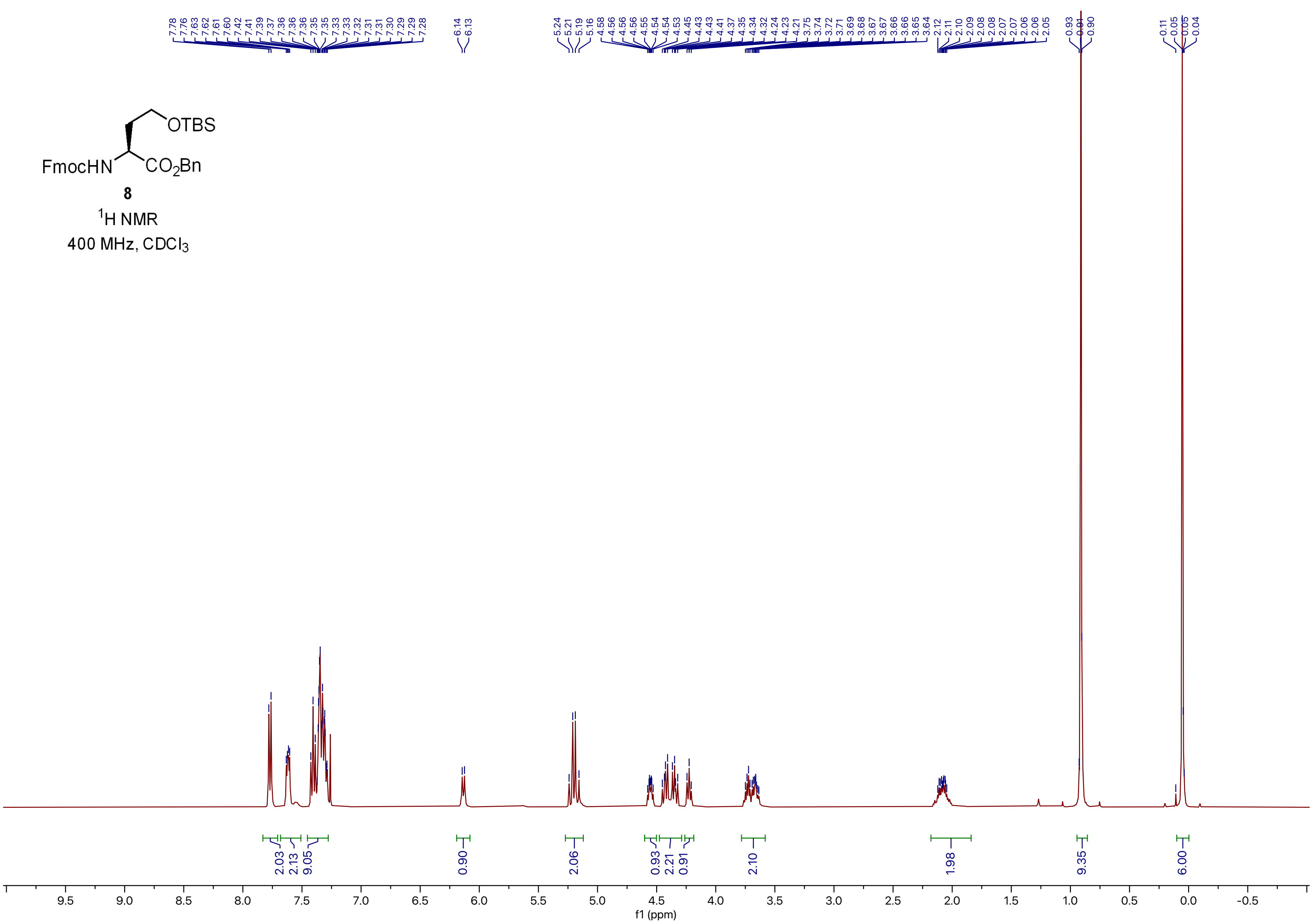
400 MHz, $CDCl_3$



S7

^{13}C NMR

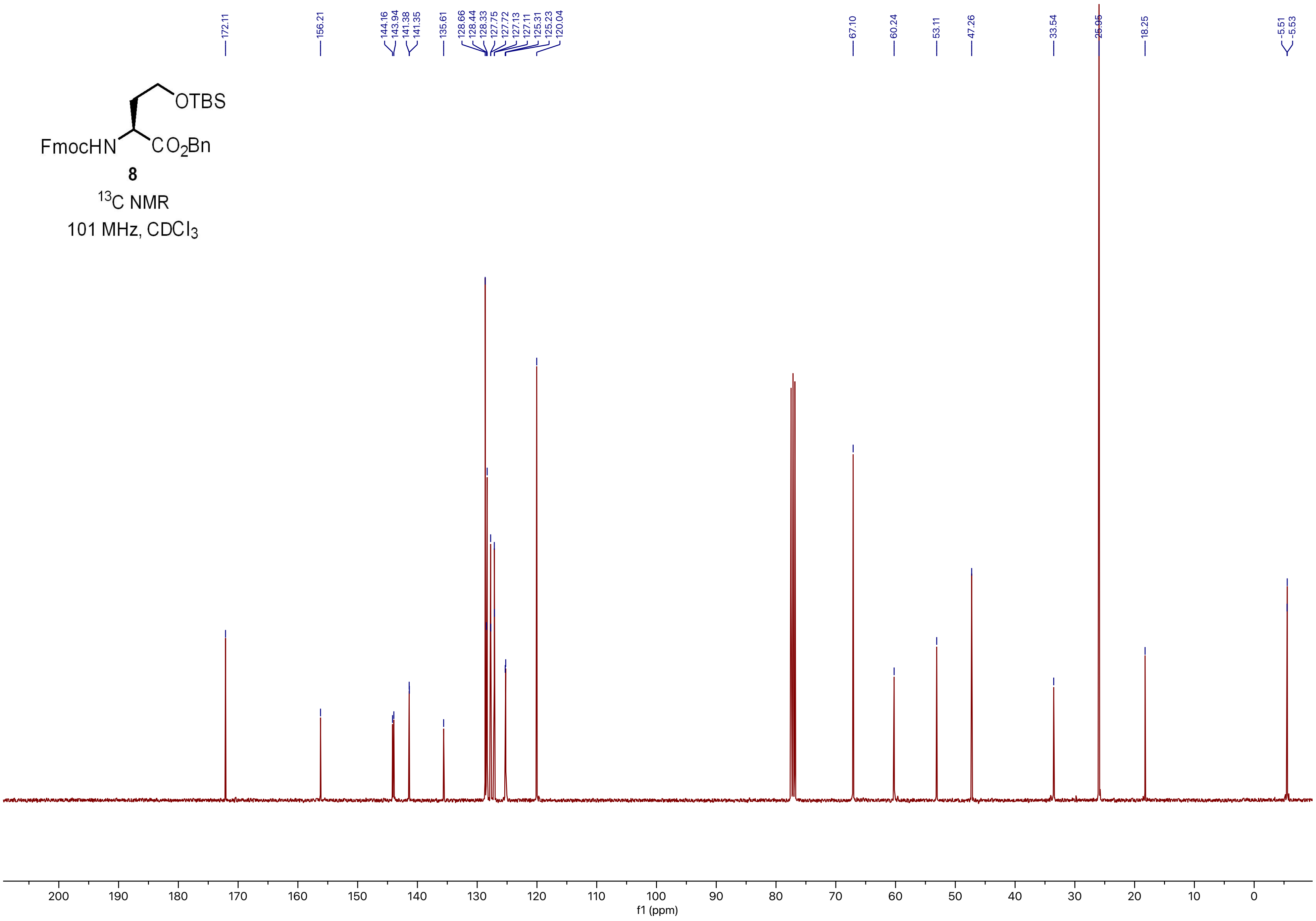
101 MHz, CDCl$_3$



8

^1H NMR

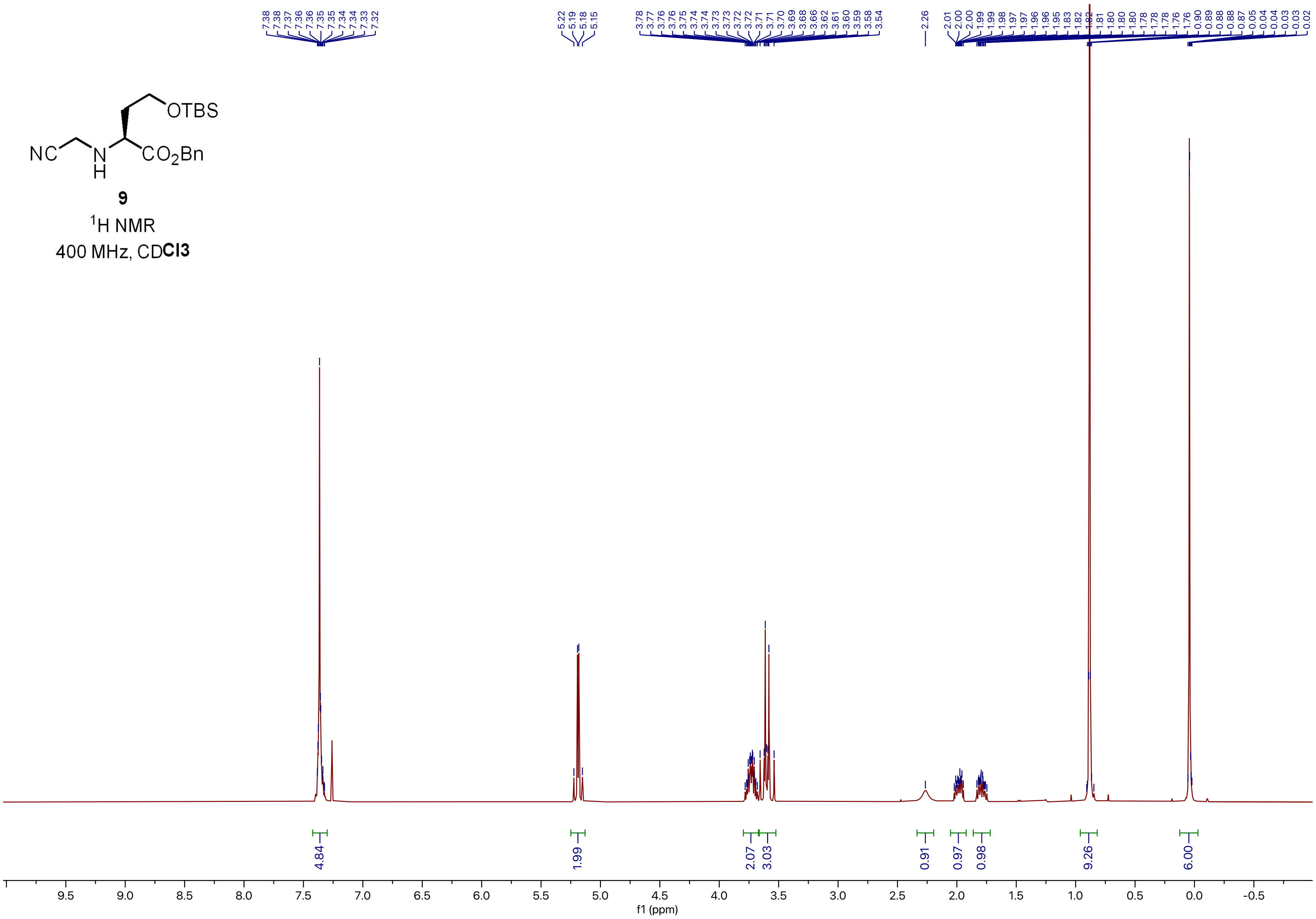
400 MHz, $CDCl_3$



8

^{13}C NMR

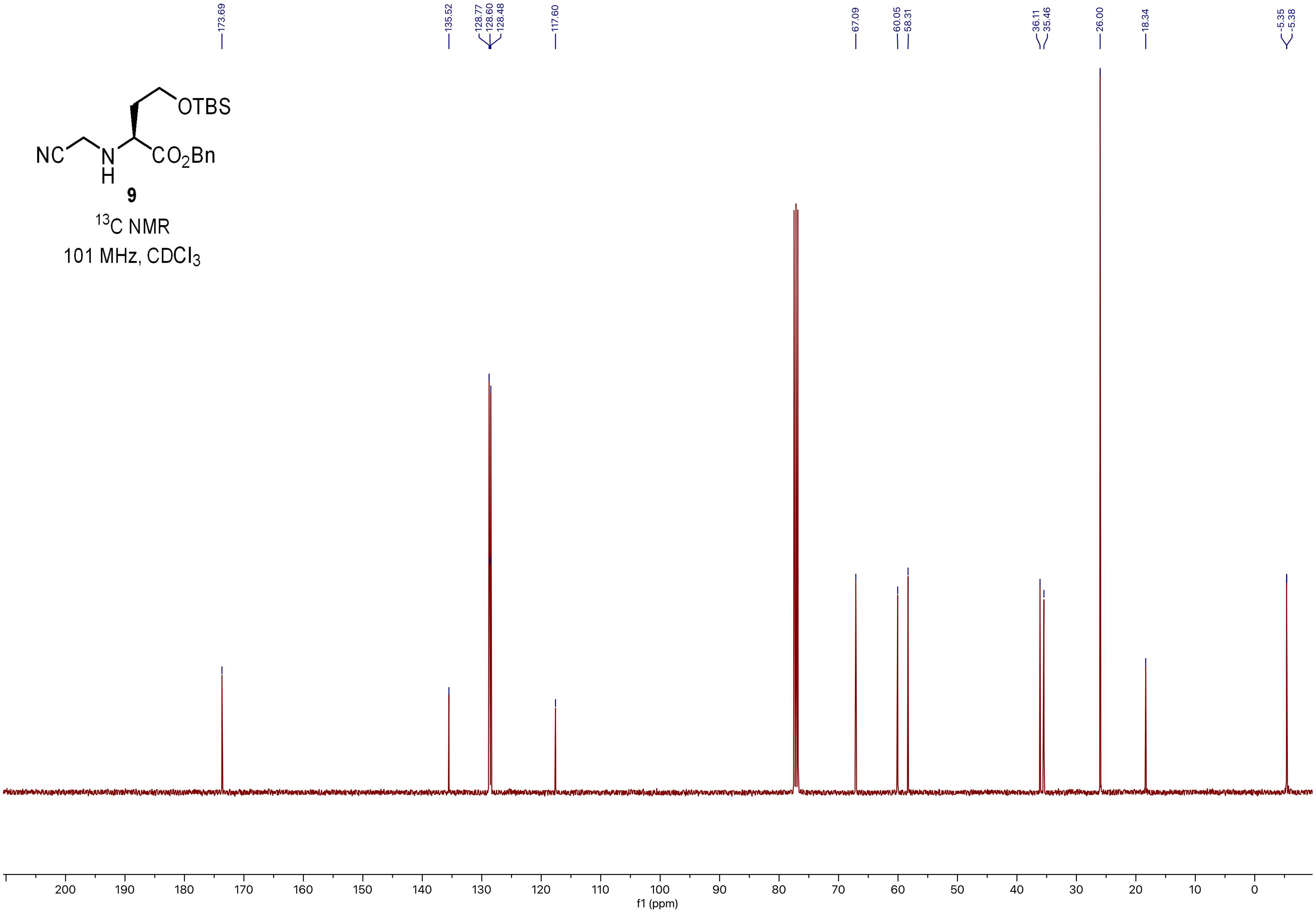
101 MHz, CDCl$_3$



9

^{1}H NMR

400 MHz, CD**Cl3**

173.69
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128.77
128.60
128.48
117.60
67.09
60.05
58.31
36.11
35.46
26.00
18.34
-5.35
-5.38
OTBS
NC
N
H
CO2Bn
9
13C NMR
101 MHz, CDCl3
200
190
180
170
160
150
140
130
120
110
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90
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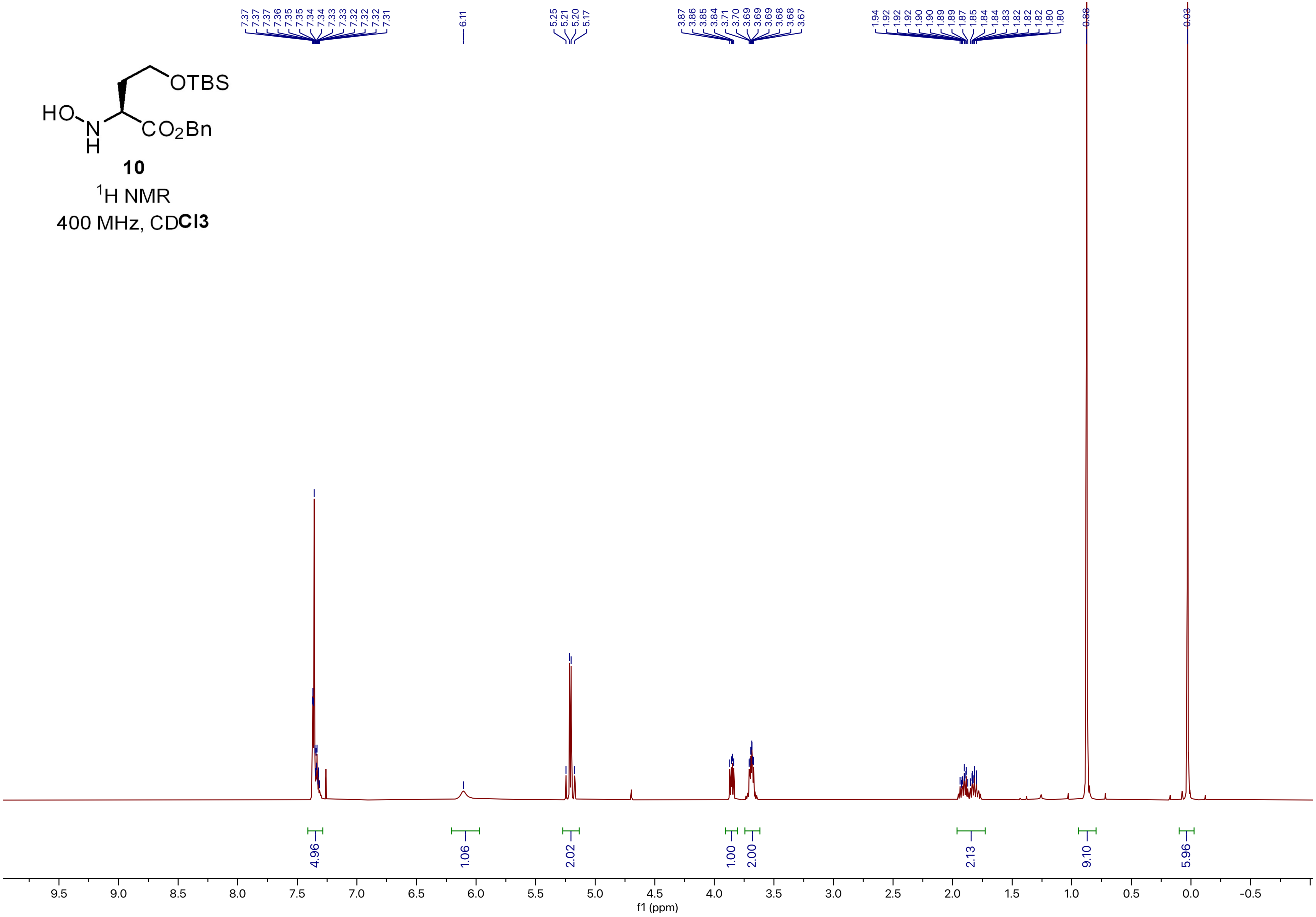
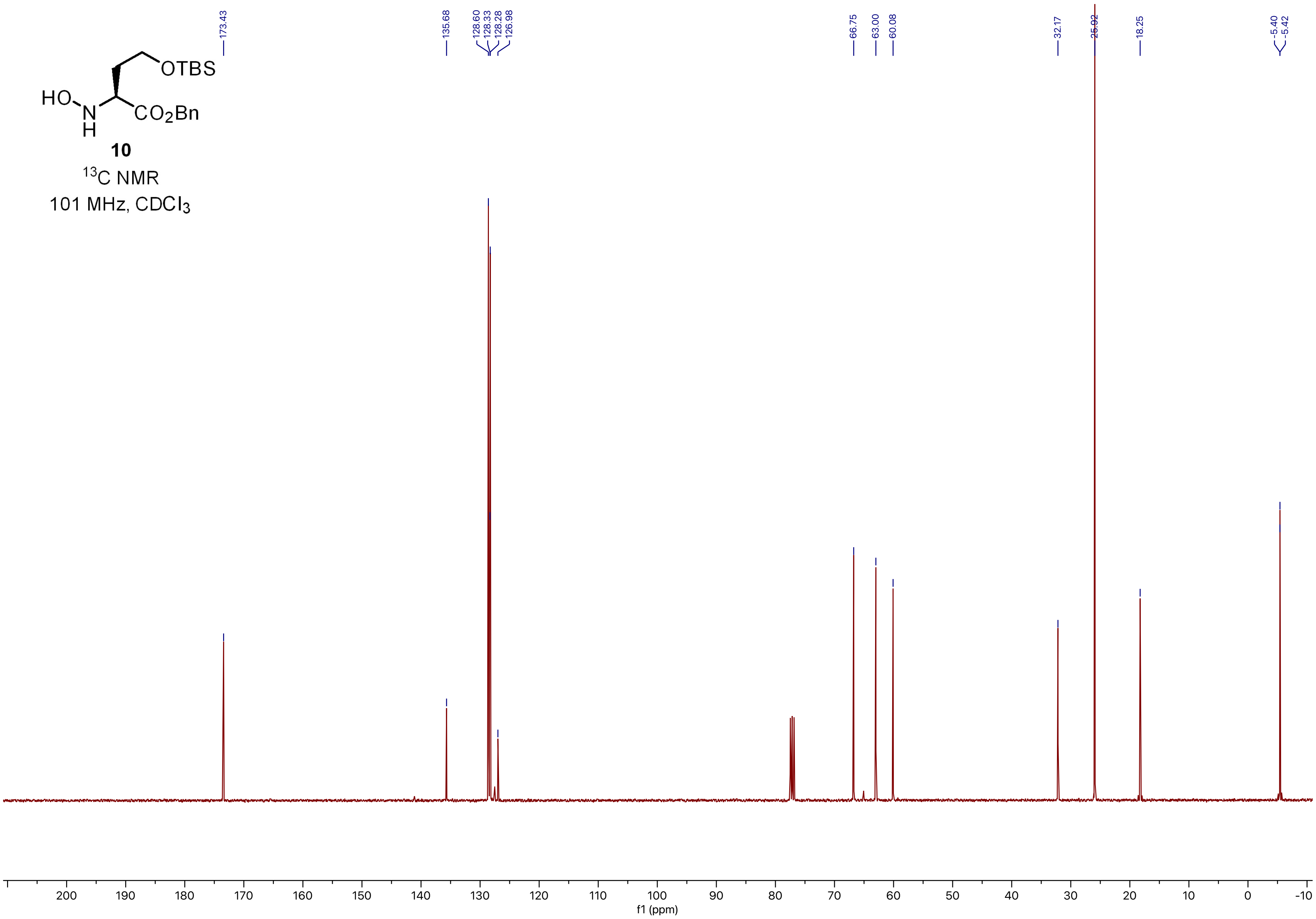
30
20
10
0
f1 (ppm)



10

^{1}H NMR

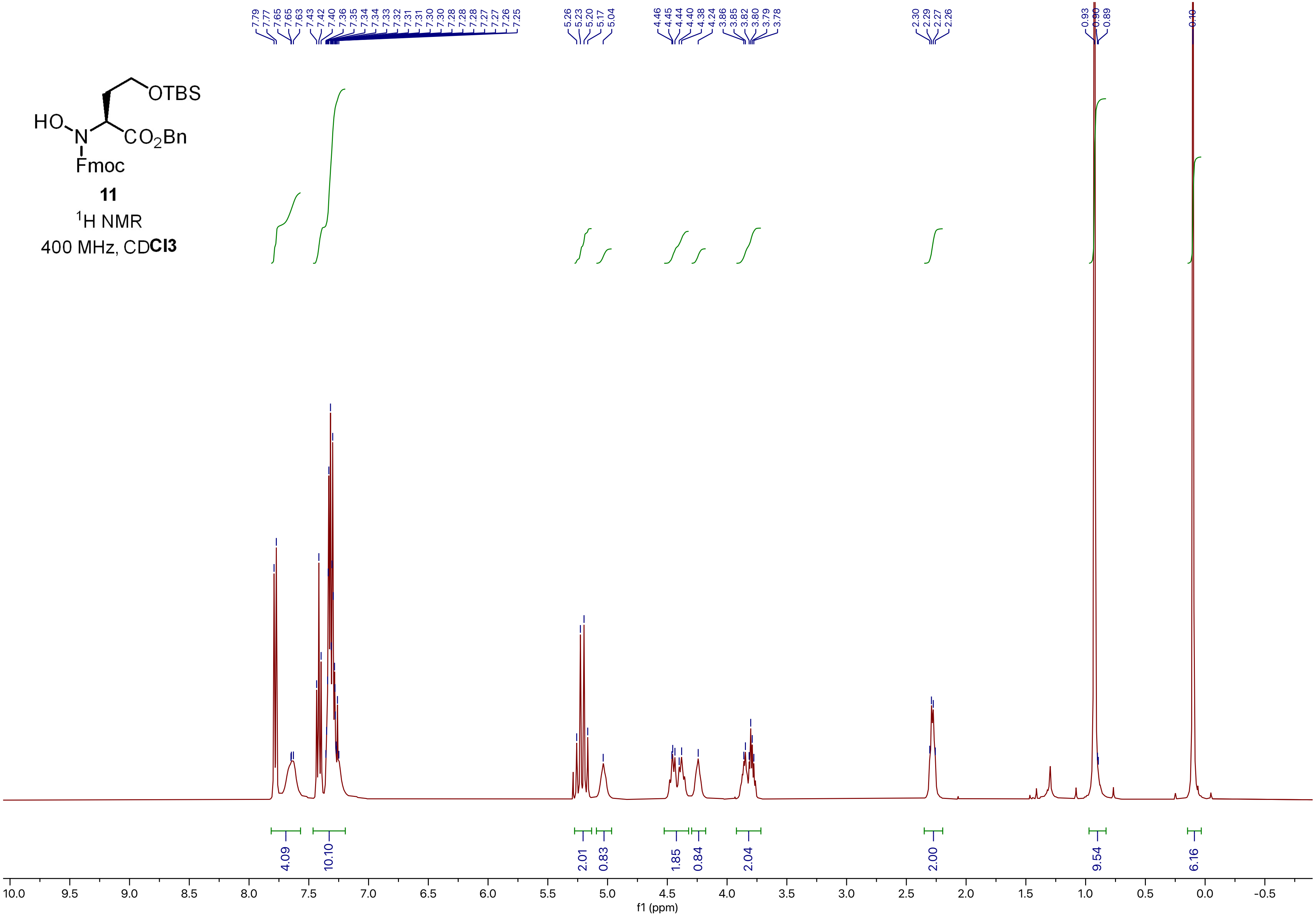
400 MHz, CDCl3



10

^{13}C NMR

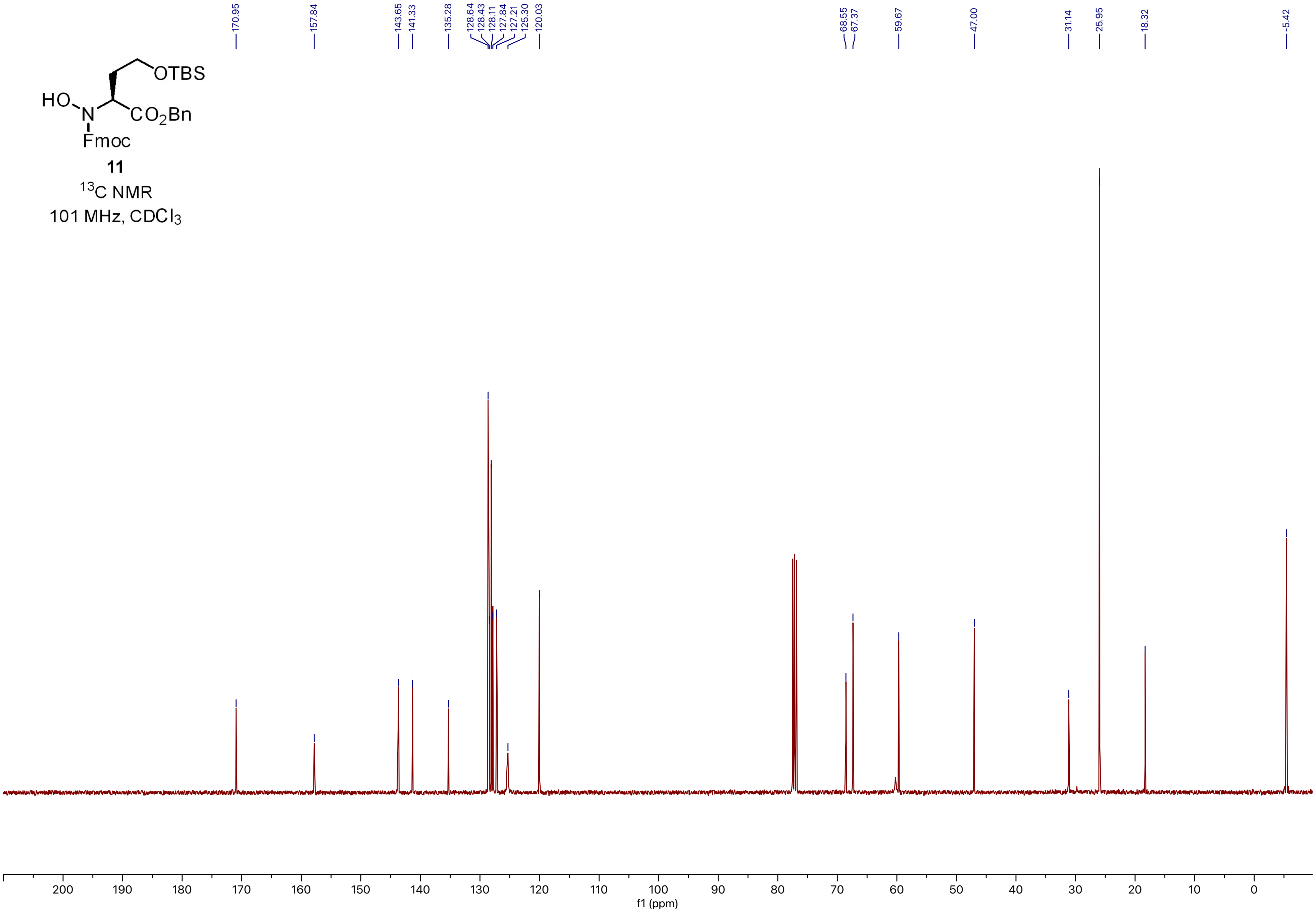
101 MHz, $CDCl_3$



11

^{1}H NMR

400 MHz, CDCl3


170.95
157.84
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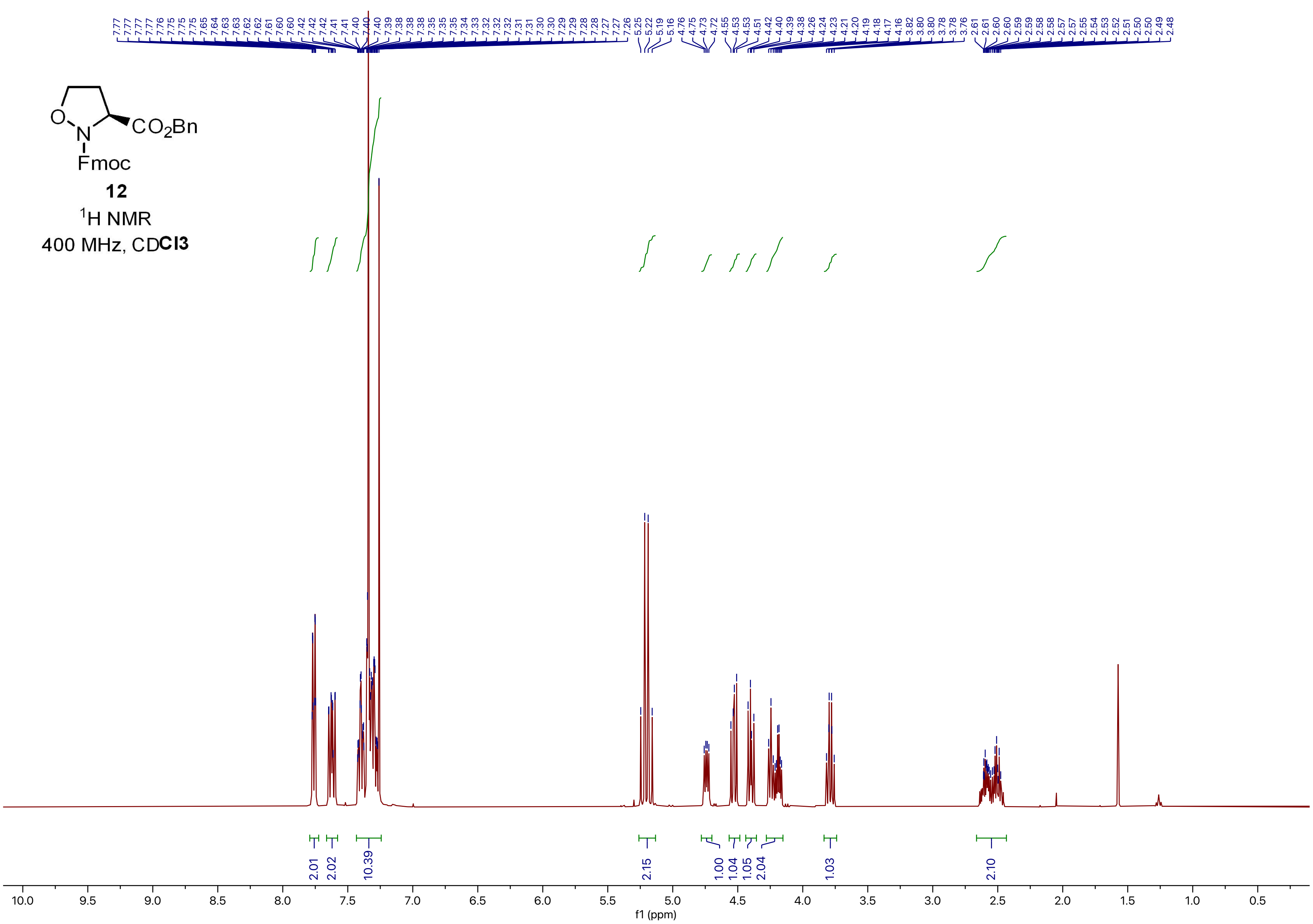
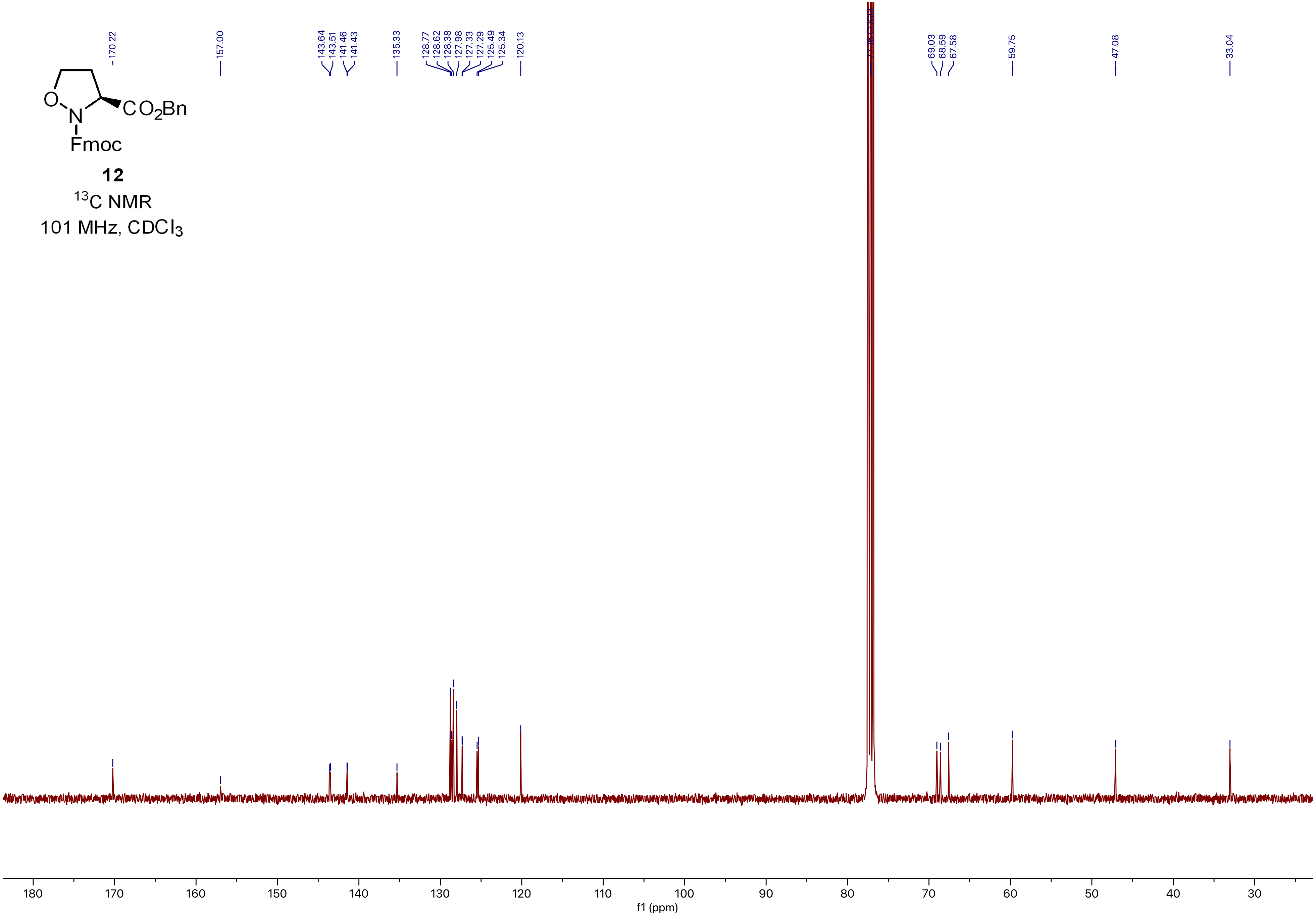
141.33
135.28
128.64
128.43
128.11
127.84
127.21
125.30
120.03
68.55
67.37
59.67
47.00
31.14
25.95
18.32
-5.42
OTBS
HO
N
CO$_2$Bn
Fmoc
11
^{13}C NMR
101 MHz, CDCl$_3$
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

12

^{1}H NMR

400 MHz, $CDCl_3$

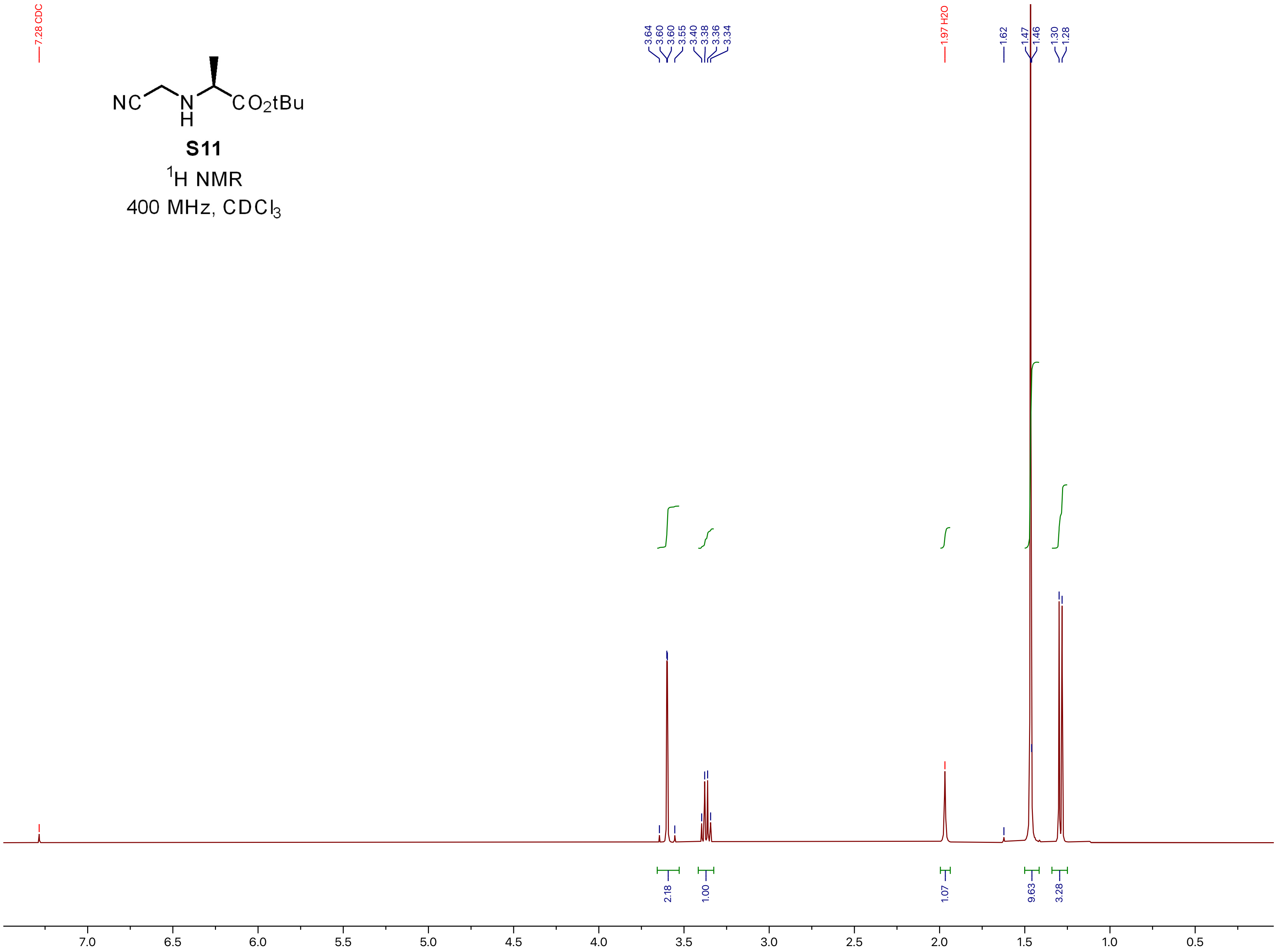




12

^{13}C NMR

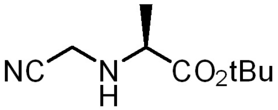
101 MHz, CDCl$_3$



S11

^{1}H NMR

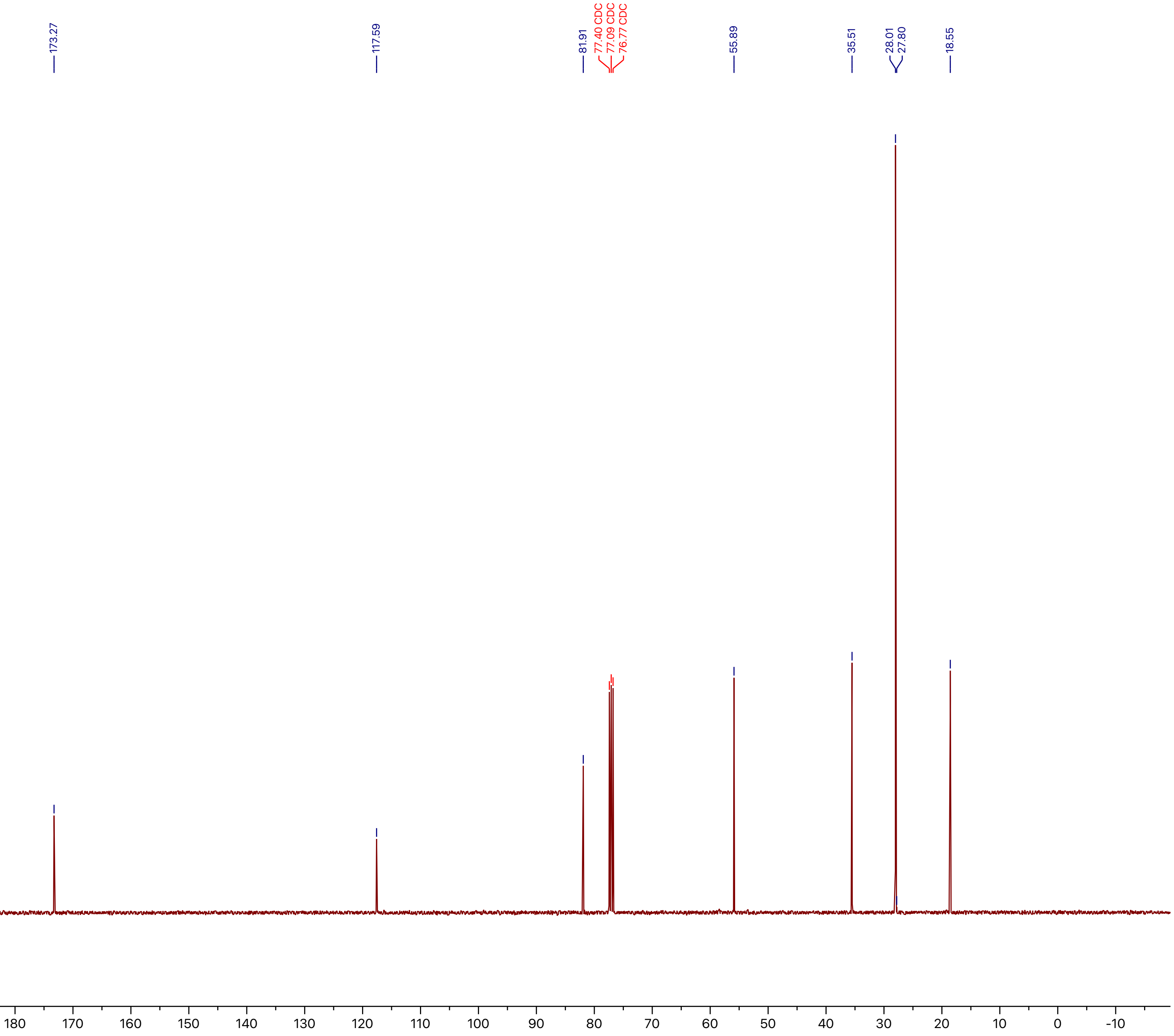
400 MHz, $CDCl_3$

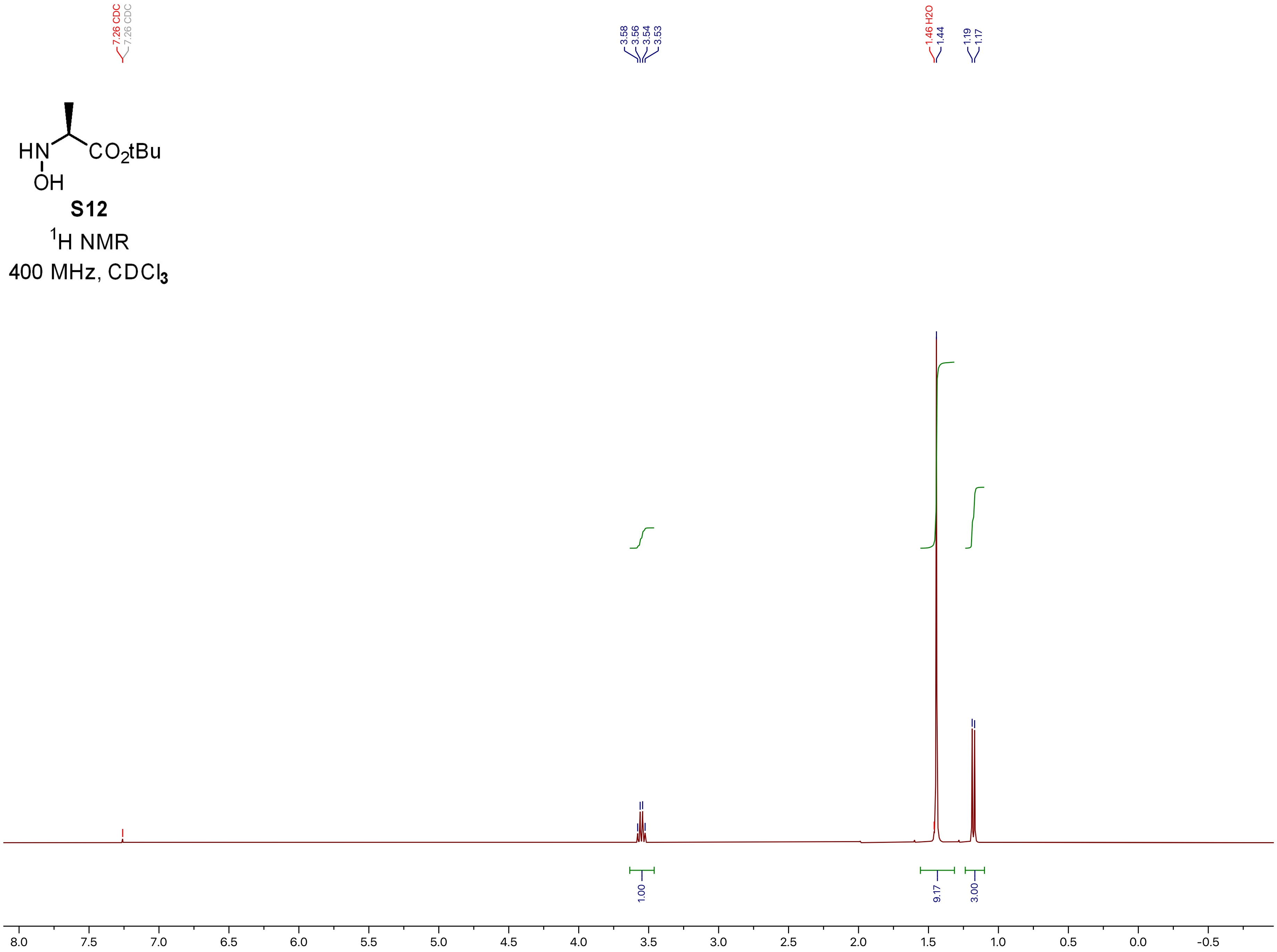


S11

^{13}C NMR

101 MHz, $CDCl_3$

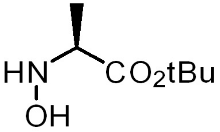




S12

^{1}H NMR

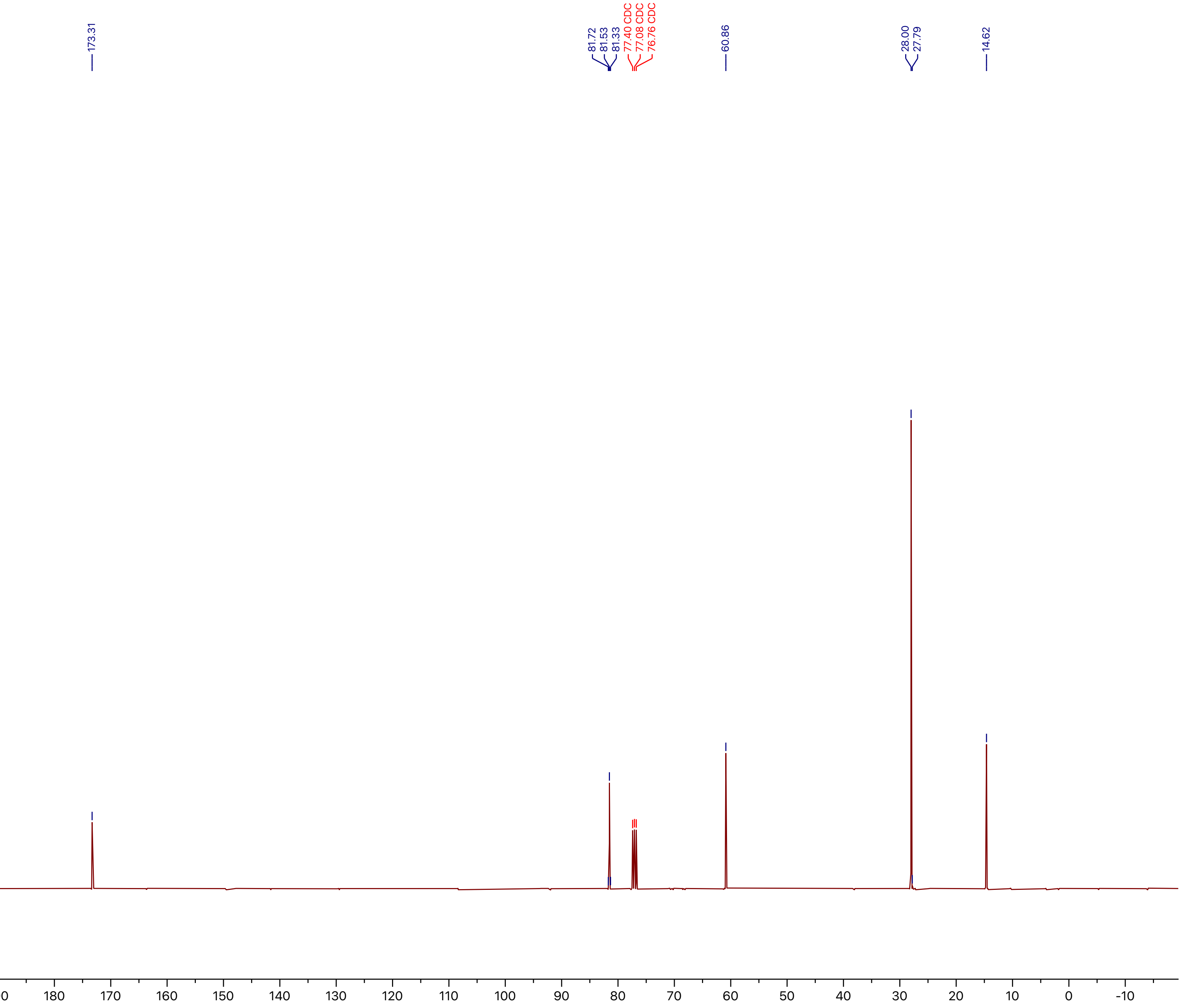
400 MHz, CDCl$_3$

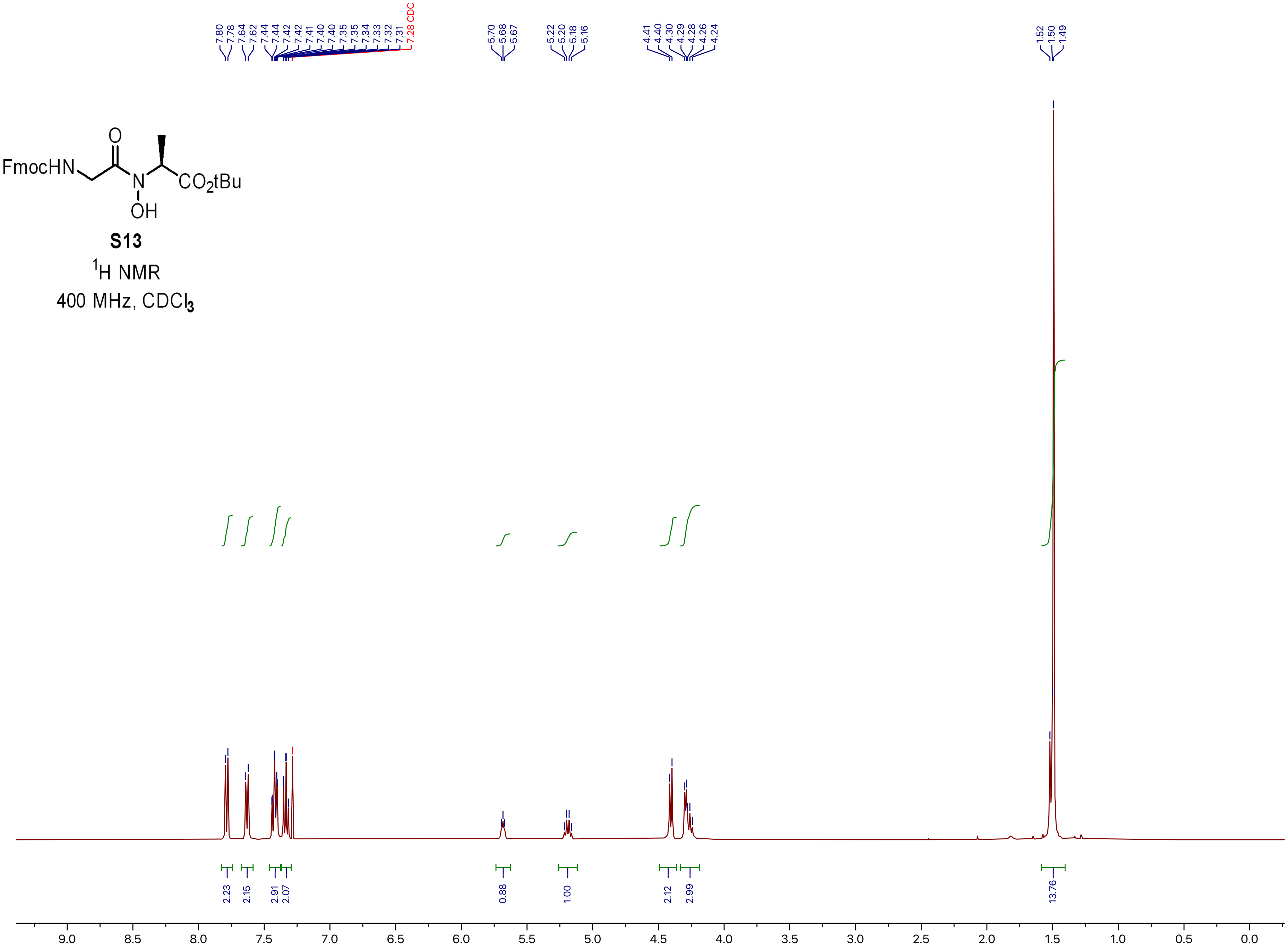


S12

^{13}C NMR

101 MHz, $CDCl_3$

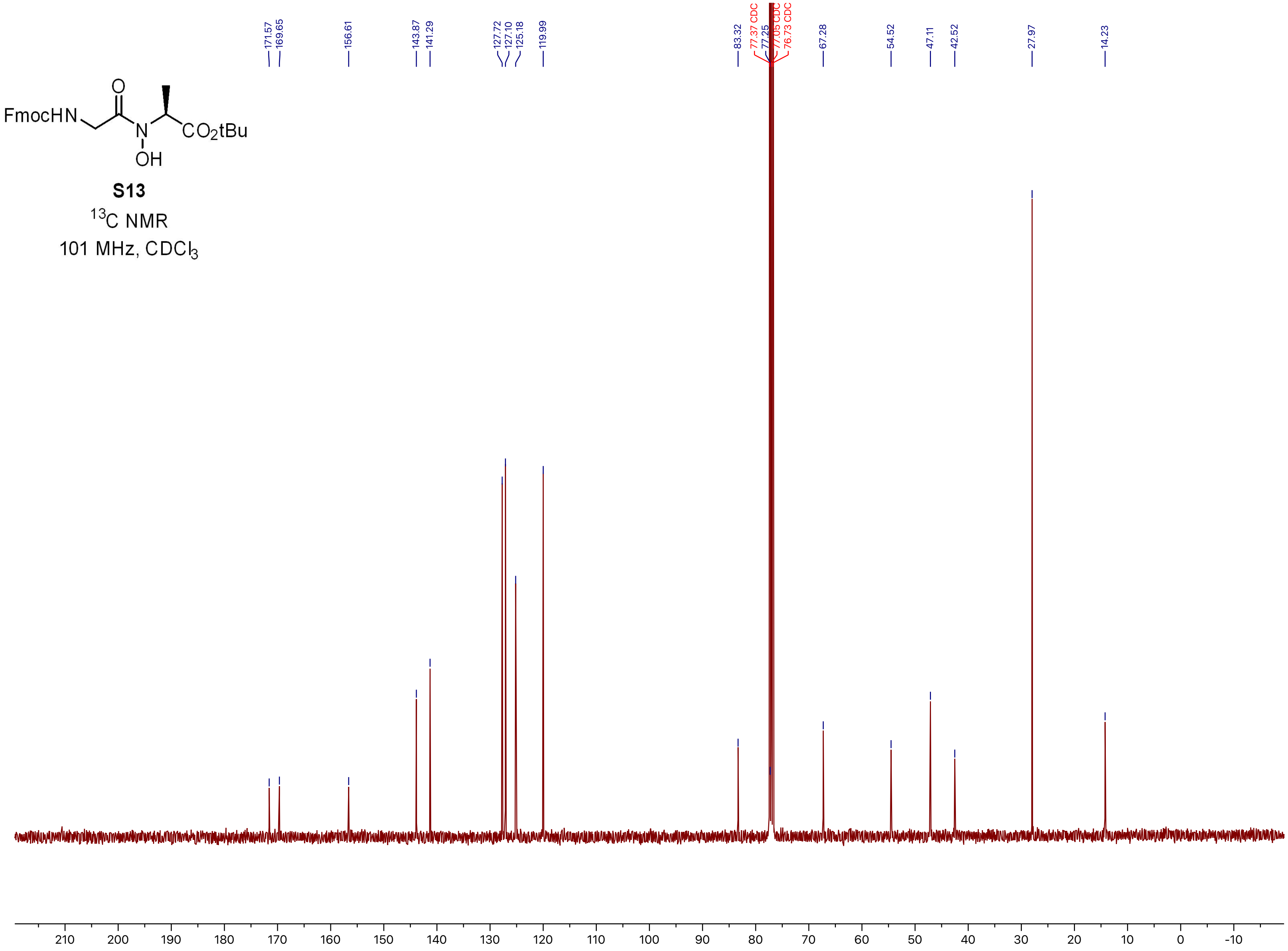




S13

^{1}H NMR

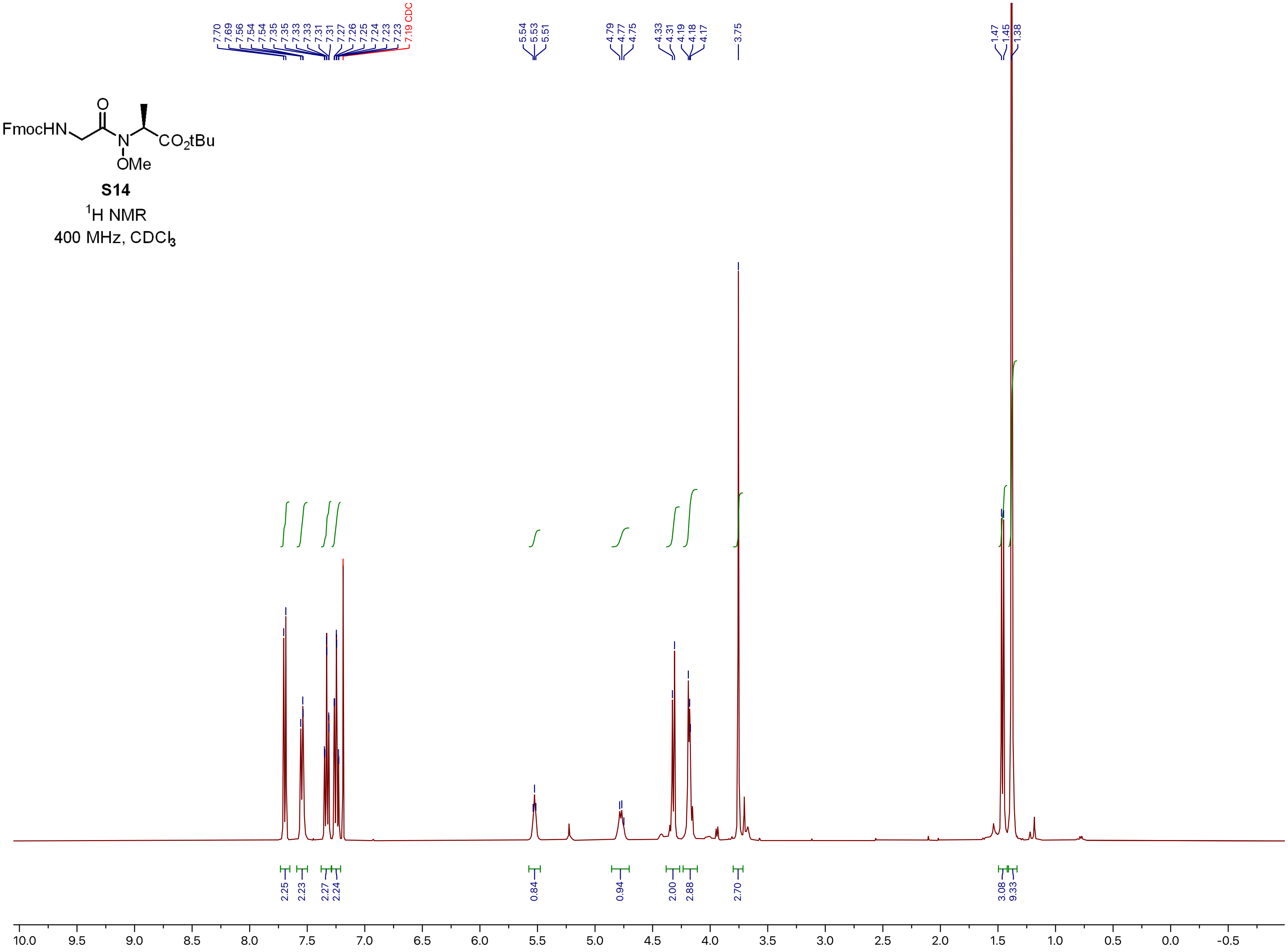
400 MHz, CDCl$_3$



S13

^{13}C NMR

101 MHz, $CDCl_3$



S14

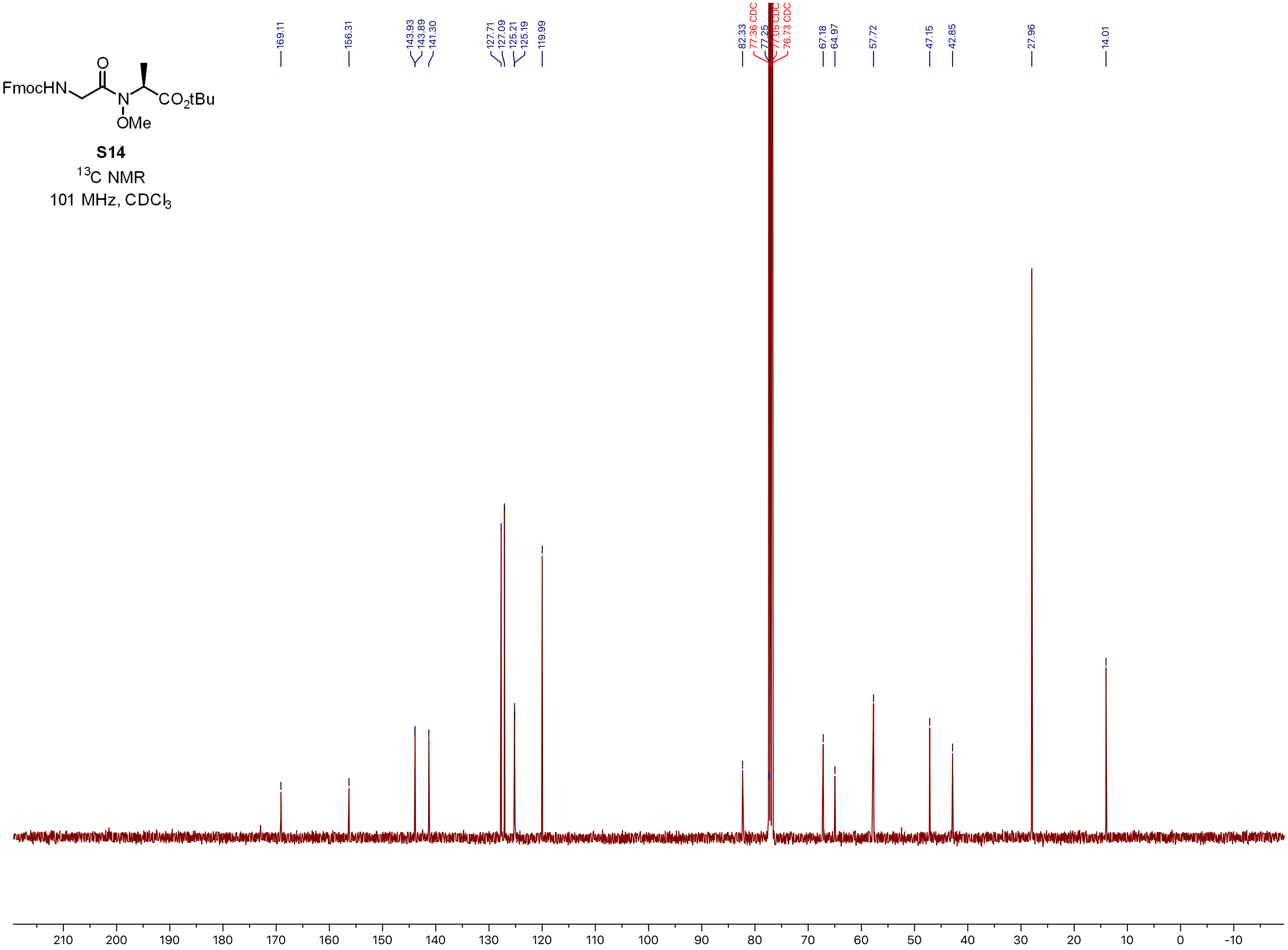
^{1}H NMR

400 MHz, CDCl$_3$

S14

^{13}C NMR

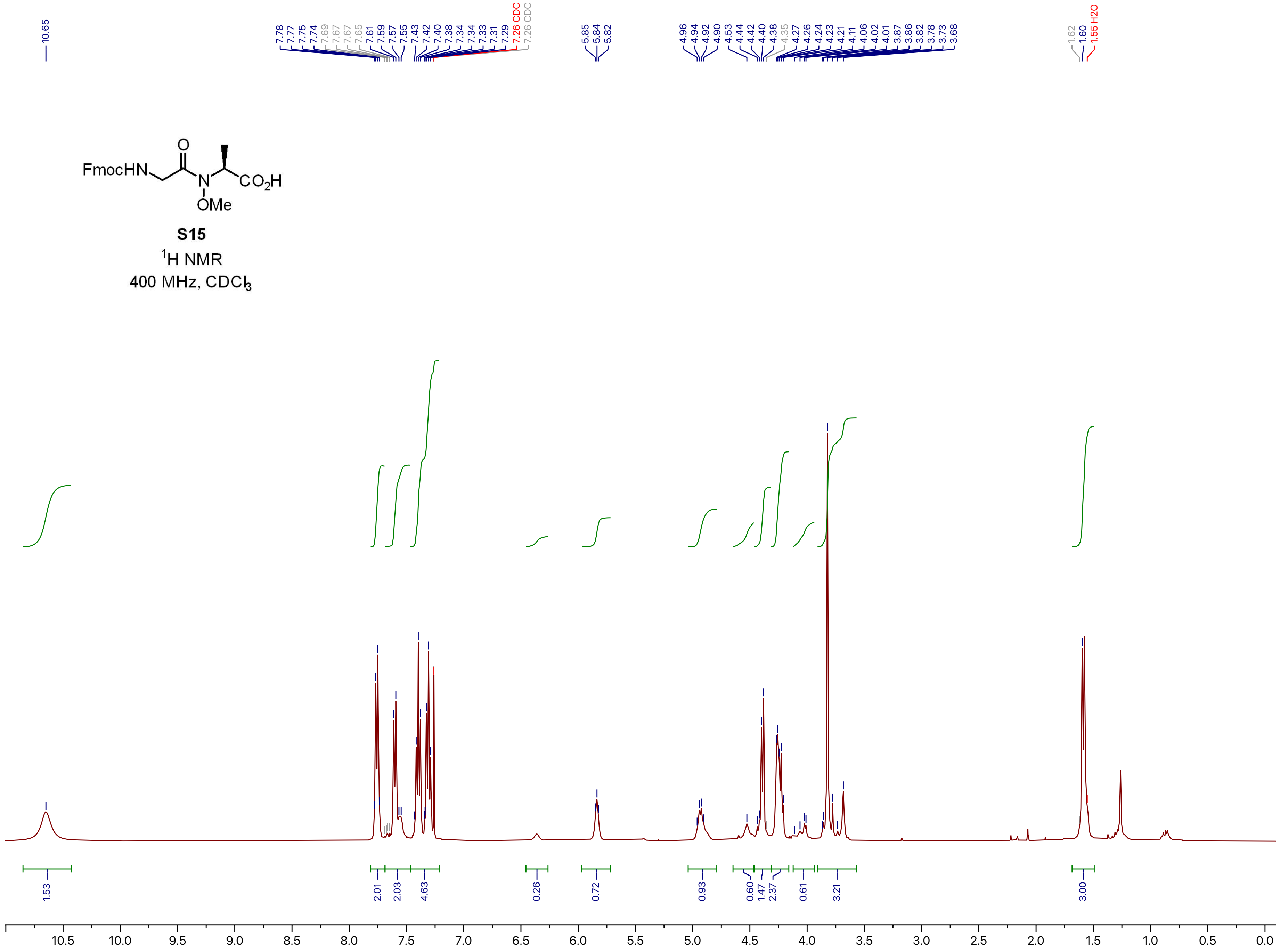
101 MHz, $CDCl_3$

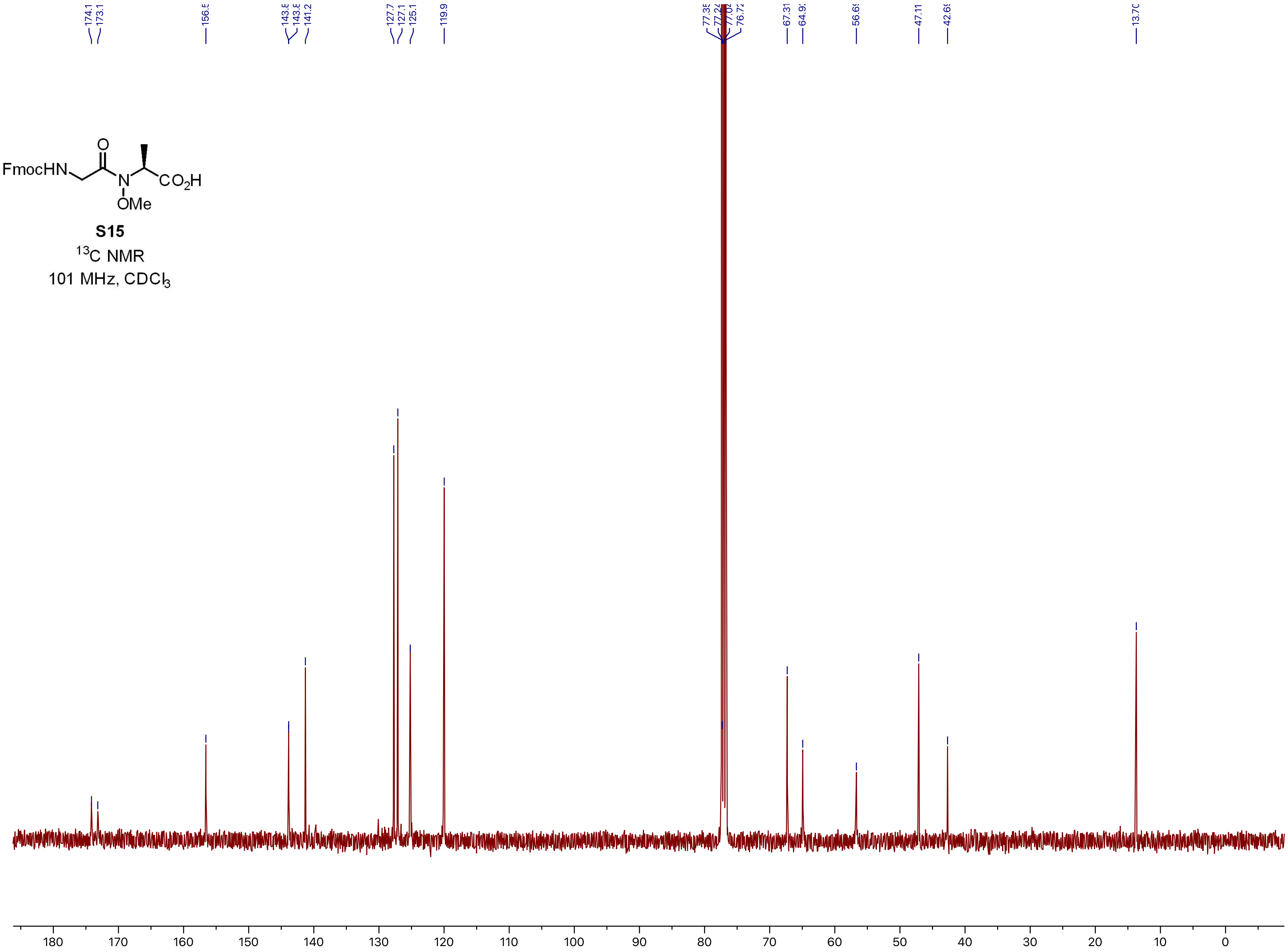


S15

^{1}H NMR

400 MHz, $CDCl_3$

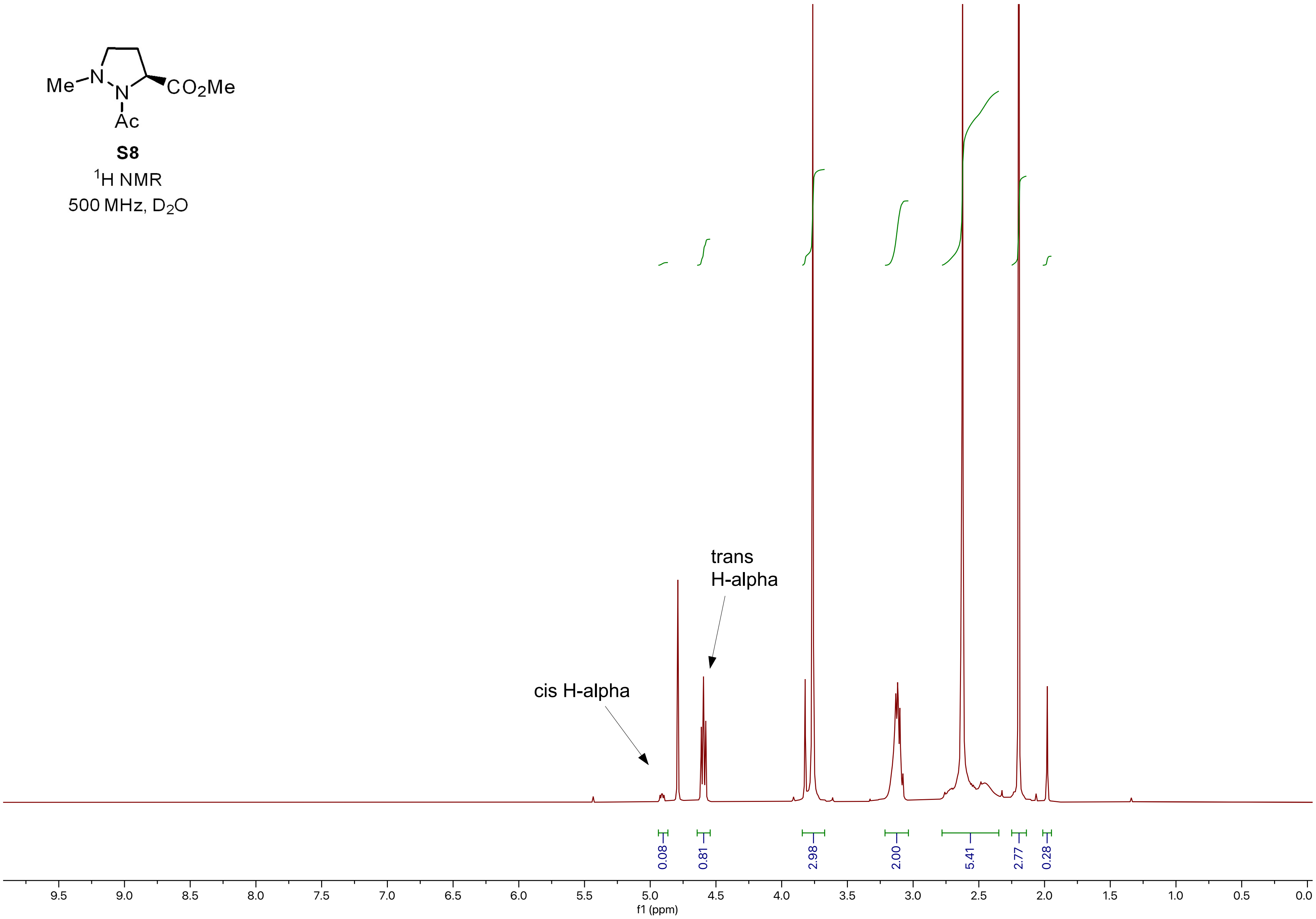




S15

^{13}C NMR

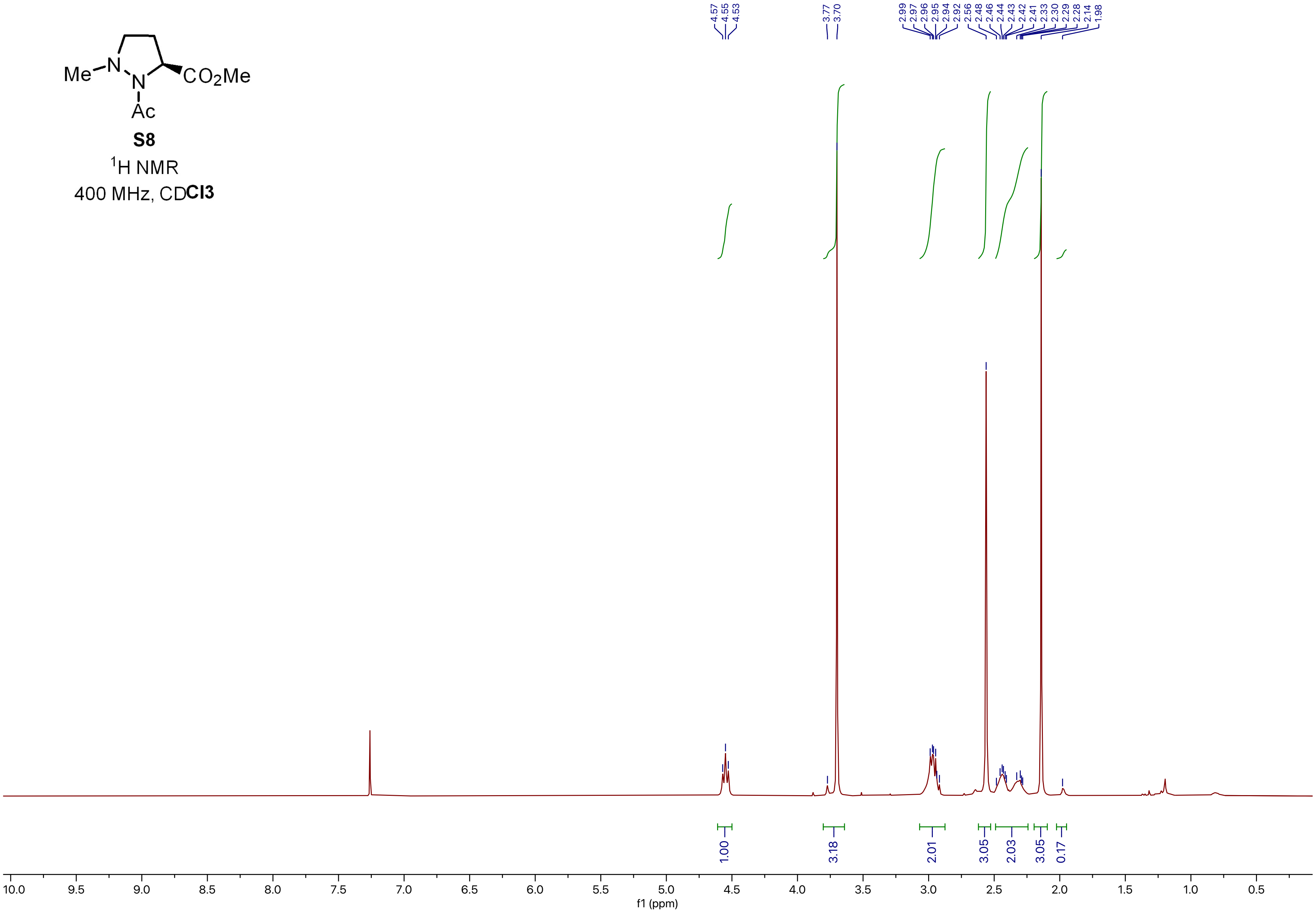
101 MHz, $CDCl_3$



S8

^{1}H NMR

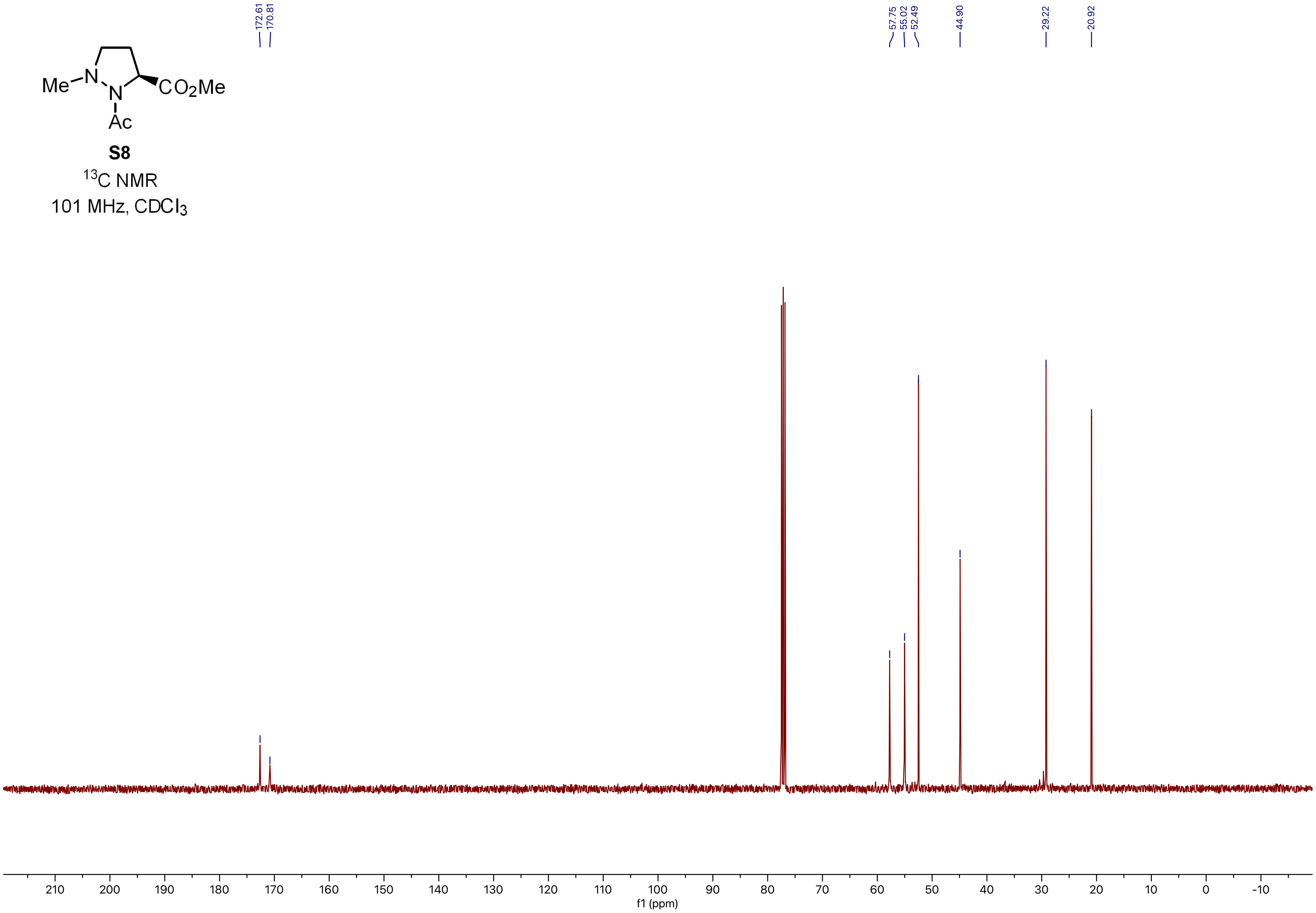
500 MHz, D_2O



S8

^1H NMR

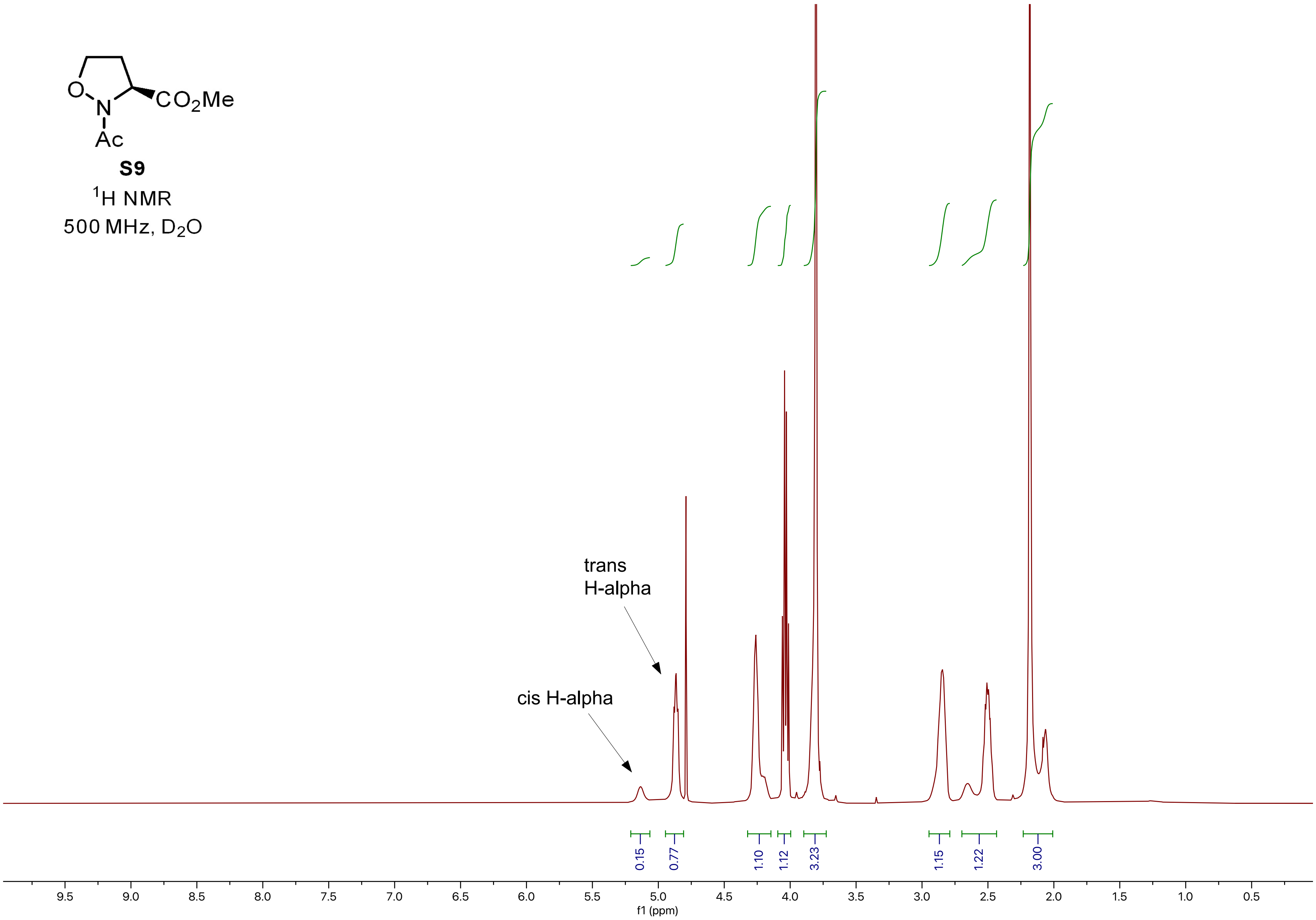
400 MHz, $CDCl_3$



S8

^{13}C NMR

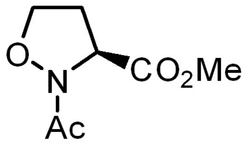
101 MHz, $CDCl_3$



S9

^{1}H NMR

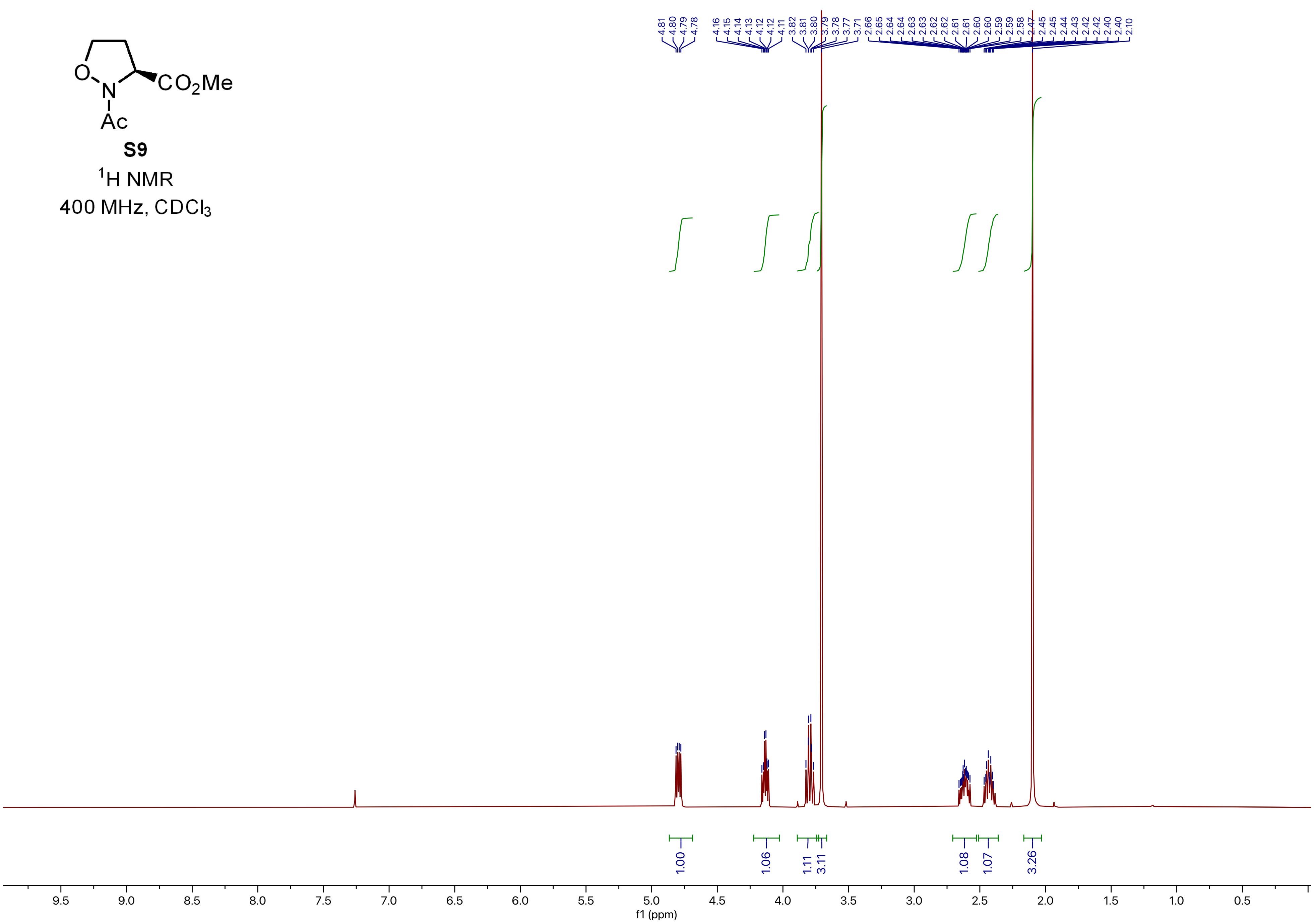
500 MHz, D_2O

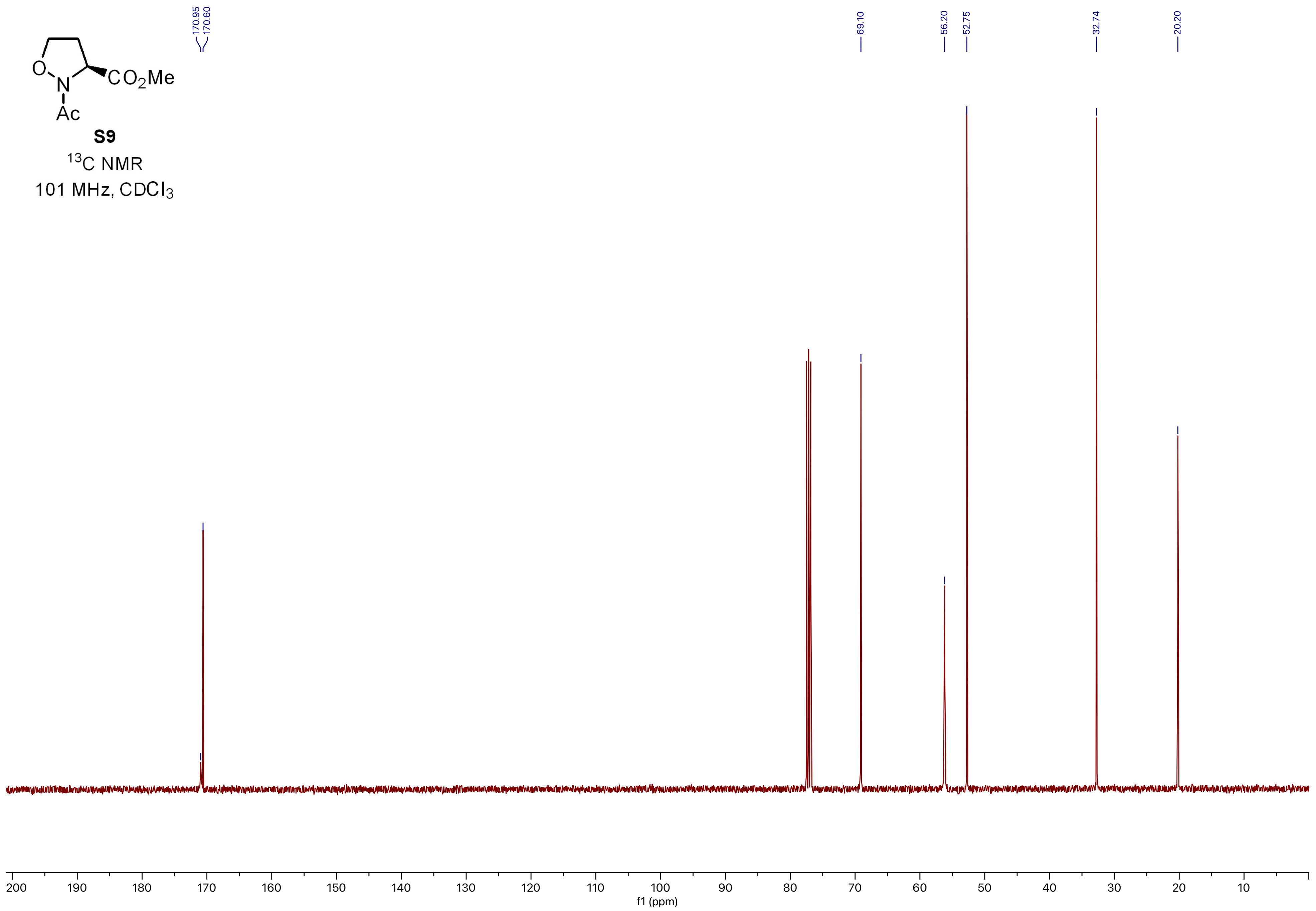


S9

^{1}H NMR

400 MHz, $CDCl_3$





S9

^{13}C NMR

101 MHz, $CDCl_3$

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