Interstrand Dipole–Dipole Interactions Can Stabilize the Collagen Triple Helix

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EXPERIMENTAL PROCEDURES

General—Commercial chemicals were of reagent grade or better, and were used without further purification. Anhydrous CH_2Cl_2 and DMF were obtained from a CYCLE-TAINER® solvent delivery system (J. T. Baker, Phillipsburg, NJ). In all reactions involving anhydrous solvents, glassware was either oven- or flame-dried. $NaHCO_3$ and brine (NaCl) refer to saturated aqueous solutions. Flash chromatography was performed with columns of silica gel 60, 230–400 mesh (Silicycle, Québec City, Canada). The term "concentrated under reduced pressure" refers to the removal of solvents and other volatile materials using a rotary evaporator at water aspirator pressure (<20 torr) while maintaining the water-bath temperature below 40 °C. Residual solvent was removed from samples at high vacuum (<0.1 torr). The term "high vacuum" refers to vacuum achieved by a mechanical belt-drive oil pump. NMR spectra were acquired with a Bruker DMX-400 Avance spectrometer (^{1}H, 400 MHz; ^{13}C, 100.6 MHz) at the National Magnetic Resonance Facility at Madison. NMR spectra were obtained at ambient temperatures on samples dissolved in $CDCl_3$, unless otherwise noted. Mass spectrometry was performed with a Micromass LCT (electrospray ionization, ESI) in the Mass Spectrometry Facility in the Department of Chemistry.

Representative Procedure for the Synthesis of Boc-Yaa-OBn—A solution of Cs_2CO_3 (5.4 mmol) in water (31 ml) at 0 °C was added to a solution of Boc-Yaa-OH (10.7 mmol) in MeOH (41 ml) at 0 °C. The mixture was concentrated under reduced pressured, and dried briefly under high vacuum. Anhydrous DMF (63 ml) was added to the residue under Ar(g). BnBr (10.7 mmol) was added, and the resulting solution was stirred for 12 h. The white precipitate was removed by vacuum filtration and the filtrate was concentrated under high vacuum. The residue was dissolved in EtOAc, washed with water (3 × 100 ml), dried over anhydrous $MgSO_4$(s), and concentrated under reduced pressure to yield Boc-Yaa-OBn as a white solid. Both Boc-Hyp-OBn (**3**) and Boc-Flp-OBn (**4**) were described previously (1).

Representative Procedure for Synthesis of Boc-XaaYaa-OBn—The appropriate Boc-Yaa-OBn amino acid (5.4 mmol) was dissolved in 4 N HCl in dioxane (50 ml) under Ar(g) and stirred for 2 h. The resulting solution was concentrated under reduced pressure, and dried briefly under high vacuum. The residue was dissolved in anhydrous CH_2Cl_2 (100 ml) under Ar(g), and the appropriate Boc-Xaa-OH amino acid (5.4 mmol) was added. The resulting solution was cooled to 0 °C. DIEA (21.6 mmol) and PyBroP (5.4 mmol) were added, and the solution was stirred for 12 h. The reaction mixture was washed with 100 ml of aqueous citric acid (10% w/v), $NaHCO_3$(aq), and brine. The organic layer was dried over anhydrous $MgSO_4$(s), and concentrated under reduced pressure. The crude residue was purified by flash chromatography over silica gel to afford Boc-XaaYaa-OBn as a white solid.

*N-tert-Butyloxycarbonyl-(2S,4R)-4-fluoroprolyl–(2S,4R)-4-hydroxylproline Benzyl Ester (**5**)*—Boc-FlpHyp-OBn (**5**) was obtained in 73% yield. ^{1}H NMR δ: 1.41 and 1.42 (s, 9H), 1.87–2.14 (m, 1H), 2.18–2.51 (m, 2.8H), 2.66–2.71 (m, 0.2H), 3.54–3.96 (m, 4H), 4.24–4.34 (m, 0.8H), 4.45 (bs, 0.8H), 4.52–4.72 (m, 1.4H), 4.79 (t, J = 8.6, 1H), 5.01–5.33 (m, 3H), 7.27–7.39 (m, 5H); ^{13}C NMR δ: 28.5, 36.7, 36.8, 36.9, 37.0, 37.3, 38.1, 53.2, 53.4, 53.7, 54.0, 54.4, 55.3, 55.6, 56.4, 57.7, 58.1, 67.0, 67.2, 70.4, 70.5, 80.6, 81.2, 90.8, 91.6, 92.6, 93.4, 128.2, 128.4, 128.5, 128.6, 128.7, 135.8, 153.8, 154.7, 171.2, 171.8, 172.2; ESI–EMM (m/z): $[M + Na]^+$ calcd for $C_{22}H_{29}FN_2O_6Na$ 459.1902; found 459.1917.

*N-tert-Butyloxycarbonyl-(2S,4R)-4-hydroxyprolyl–(2S,4R)-4-fluoroproline Benzyl Ester (**6**)*—Boc-HypFlp-OBn (**6**) was obtained in 92% yield. ^{1}H NMR δ: 1.39 and 1.45 (s, 9H), 1.90–2.23 (m, 4H), 2.49–2.69 (m, 1H), 3.39–3.82 (m, 3H), 3.95–4.29 (m, 1H), 4.39–4.50 (m, 1H), 4.52–4.77 (m, 2H), 5.02–5.10 (m, 1H), 5.19–5.30 (m, 1.5H), 5.31–5.40 (m, 0.5H), 7.28–7.40 (m, 5H); ^{13}C NMR δ: 28.4, 28.5, 35.5, 35.7, 37.7, 38.4, 52.9, 53.2, 53.4, 55.0, 55.1, 58.0, 67.2, 67.3, 69.8, 70.4, 80.2, 80.5, 90.8, 91.1, 92.6, 92.9, 128.4, 128.5, 128.6, 128.7, 135.5, 135.6, 154.1, 154.7, 171.5, 171.7, 171.8; ESI–EMM (m/z): $[M + Na]^+$ calcd for $C_{22}H_{29}FN_2O_6Na$ 459.1902; found 459.1899.

*N-tert-Butyloxycarbonyl-(2S,4R)-4-fluoroprolyl–(2S,4R)-4-fluoroproline Benzyl Ester (**7**)*—Boc-FlpFlp-OBn (**7**) was obtained in 66% yield. ^{1}H NMR δ: 1.40 and 1.45 (s, 9H), 1.90–2.19 (m, 2H), 2.35–2.69 (m, 2H), 3.53–3.74 (m, 1.5H), 3.75–3.96 (m, 1.5H), 3.99–4.12 (m, 0.6H), 4.31 (dd, J = 11.7 and 21.4, 0.4H), 4.57 and 4.65 (t, J = 7.8, 1H), 4.67–4.78 (m, 1H), 5.01–5.13 (m, 1.5H), 5.19–5.30 (m, 2H), 5.33–5.41 (m, 0.5H), 7.29–7.41 (m, 5H); ^{13}C NMR δ: 28.4, 28.5, 35.5, 35,7, 36.1, 36.3, 36.9, 37.2, 53.0, 53.2, 53.4, 53.5, 53.7, 56.1, 56.2, 58.0, 67.2, 67.3, 80.4, 80.8, 90.7, 91.1, 91.6, 92.5, 92.8, 93.3, 128.4, 128.5, 128.6, 128.7, 135.4, 135.5, 153.6, 154.3, 171.2, 171.3, 171.6; ESI–EMM (m/z): $[M + Na]^+$ calcd for $C_{22}H_{28}F_2N_2O_5Na$ 461.1859; found 461.1852.

*N-tert-Butyloxycarbonyl-(2S)-prolyl–(2S,4R)-4-fluoroproline Benzyl Ester (**8**)*—Boc-ProFlp-OBn (**8**) was obtained in 98% yield. ^{1}H NMR δ: 1.39 and 1.45 (s, 9H), 1.40–1.52 (m, 0.7H), 1.73–2.19 (m, 5.3H), 2.49–2.67 (m, 1H), 3.32–3.84 (m, 3H), 4.38 (dd, J = 3.9 and 8.4, 0.6H), 4.49 (dd, J = 2.8 and 8.1, 0.4H), 4.66–4.79 (m, 1H), 5.02–5.11 (m, 1H), 5.18–5.40 (m, 2H), 7.28–7.40 (m, 5H); ^{13}C NMR δ: 23.6, 24.2, 28.4, 28.6, 29.1, 30.0, 35.4, 35.5, 35.6, 35.7, 46.7, 46.9, 52.8, 53.1, 53.3, 57.9, 58.0, 67.2, 67.3, 79.7, 80.0, 90.8, 91.2, 92.6, 93.0, 128.4, 128.5, 128.6, 128.7, 135.5, 135.6, 153.9, 154.7, 171.5, 171.6, 171.8, 171.9; ESI–EMM (m/z): $[M + Na]^+$ calcd for $C_{22}H_{29}FN_2O_5Na$ 443.1953; found 443.1950.

Representative Procedure for Synthesis of Fmoc-GlyXaaYaa-OBn—The appropriate Boc-XaaYaa-OBn amino acid (5.2 mmol) was dissolved in 4 N HCl in dioxane (60 ml) under Ar(g) and stirred for 2 h. The resulting solution was concentrated under reduced pressure and dried briefly under high vacuum. The residue was dissolved in anhydrous DMF (100 ml) under Ar(g). DIEA (26 mmol) was added, followed by Fmoc-Gly-OPfp (16.8 mmol). The solution was stirred for 12 h and then concentrated by rotary evaporation under high vacuum. The crude residue was purified by flash chromatography over silica gel to afford Fmoc-GlyXaaYaa-OBn as a white solid.

*N-9-Fluorenylmethoxycarbonyl-glycyl-(2S,4R)-4-fluoroprolyl–(2S,4R)-4-hydroxylproline Benzyl Ester (**9**)*—Fmoc-GlyFlpHyp-OBn (**9**) containing a slight impurity, which was removed after the subsequent step, was obtained in 75% yield. ESI–EMM (*m*/*z*): $[M + Na]^+$ calcd for $C_{34}H_{34}FN_3O_7Na$ 638.2273; found 638.2272.

*N-9-Fluorenylmethoxycarbonyl-glycyl-(2S,4R)-4-hydroxyprolyl–(2S,4R)-4-fluoroproline Benzyl Ester (**10**)*—Fmoc-GlyHypFlp-OBn (**10**) containing a slight impurity, which was removed after the subsequent step, was obtained in 72% yield. ESI–EMM (*m*/*z*): $[M + Na]^+$ calcd for $C_{34}H_{34}FN_3O_7Na$ 638.2273; found 638.2251.

*N-9-Fluorenylmethoxycarbonyl-glycyl-(2S,4R)-4-fluoroprolyl–(2S,4R)-4-fluoroproline Benzyl Ester (**11**)*—Fmoc-GlyFlpFlp-OBn (**11**) containing a slight impurity, which was removed after the subsequent step, was obtained in 82% yield. ESI–EMM (*m*/*z*): $[M + Na]^+$ $C_{34}H_{33}F_2N_3O_6Na$ 640.2230; found 640.2240.

*N-9-Fluorenylmethoxycarbonyl-glycyl-(2S)-prolyl–(2S,4R)-4-fluoroproline Benzyl Ester (**12**)*—Fmoc-GlyProFlp-OBn (**12**) containing a slight impurity that was removed after the subsequent step was obtained in 75% yield. ESI–EMM (*m*/*z*): $[M + Na]^+$ $C_{34}H_{34}FN_3O_6Na$ 622.2354; found 622.2334.

Representative Procedure for Synthesis of Fmoc-GlyXaaYaa-OH—MeOH (150 ml) was added carefully to a mixture of the appropriate Fmoc-GlyXaaYaa-OBn amino acid (3.5 mmol) and Pd/C (10% w/w, 0.4 g) under Ar(g). The resulting black suspension was stirred under H_2(g) for ~2 h. Careful monitoring by TLC was necessary to prevent hydrogenolysis of the Fmoc group. The suspension was filtered through a pad of Celite and concentrated under reduced pressure. The crude product was purified by flash chromatography over silica gel.

*N-9-Fluorenylmethoxycarbonyl-glycyl-(2S,4R)-4-fluoroprolyl–(2S,4R)-4-hydroxylproline (**13**)*—Fmoc-GlyFlpHyp-OH (**13**) was obtained in 79% yield. ^{1}H NMR (DMSO-d_6) δ: 1.82–2.35 (m, 3H), 2.47–2.85 (m, ~1H-peaks under DMSO), 3.24–3.81 (m, 4.5H), 3.82–4.08 (m, 2.25H), 4.08–4.48 (m, 5.25H), 4.67 and 5.04 (t, J = 8.0, 1H), 5.19–5.55 (m, 1H), 7.26–7.55 (m, 5H), 7.56–7.78 (m, 2H), 7.83–7.94 (m, 2H), 12.53 (bs, 0.7H); ^{13}C (DMSO-d_6) NMR δ: 34.5, 34.7, 36.8, 37.1, 41.9, 42.6, 52.3, 52.5, 54.2, 54.3, 55.4, 56.1, 57.7, 57.8, 65.7, 68.9, 69.1, 90.0, 91.7, 92.0, 93.8, 120.1, 125.3, 127.1, 127.5, 127.7, 140.7, 156.5, 160.3, 167.2, 167.8, 169.6, 169.6, 173.0, 173.1 ESI–EMM (*m*/*z*): $[M - H]^-$ calcd for $C_{27}H_{27}FN_3O_7$ 524.1838; found 524.1844.

*N-9-Fluorenylmethoxycarbonyl-glycyl-(2S,4R)-4-hydroxyprolyl–(2S,4R)-4-fluoroproline (**14**)*—Fmoc-GlyHypFlp-OH (**14**) was obtained in 73% yield. ^{1}H NMR (DMSO-d_6) δ: 1.81–2.34 (m, 2.8H), 2.43–2.62 (m, ~0.3H-peaks under DMSO), 3.25–3.97 (m, 7H), 4.07–4.34 (m, 5H), 4.34–4.46 (m, 1H), 4.64 and 5.03 (t, J = 7.7, 1H), 5.30–5.52 (m, 1H), 7.28–7.50 (m, 5H), 7.60–7.75 (m, 2H), 7.85–7.92 (m, 2H), 12.60 (bs, 0.7H); ^{13}C NMR (DMSO-d_6) δ: 35.1, 35.2, 36.4, 42.6, 46.6, 56.1, 56.5, 57.4, 65.7, 66.8, 69.0, 91.8, 93.6, 120.1, 125.3, 127.1, 127.6, 140.7, 143.9, 156.5, 167.2, 167.8, 170.3, 175.1; ESI–EMM (*m*/*z*): $[M + Na]^+$ calcd for $C_{27}H_{28}FN_3O_7Na$ 548.1804; found 548.1827.

*N-9-Fluorenylmethoxycarbonyl-glycyl-(2S,4R)-4-fluoroprolyl–(2S,4R)-4-fluoroproline (**15**)*—Fmoc-GlyFlpFlp-OH (**15**) was obtained in 82% yield. ^{1}H NMR DMSO-d_6 (MeOH-d_4) δ: 2.01–2.29 (m, 2H), 2.52–2.73 (m, 2H), 3.53–4.03 (m, 4H), 4.03–4.15 (m, 1H), 4.17–4.38 (m, 4H), 4.40–4.56 (m, 1H), 5.24–5.48 (m, 2H), 7.24–7.46 (m, 4H), 7.54–7.73 (m, 2H), 7.73–7.84 (m, 2H); ^{13}C NMR (DMSO-d_6) δ: 34.6, 34.9, 35.1, 35.3, 41.8, 42.6, 46.6, 52.2, 52.5, 52.9, 53.2, 55.3, 56.1, 57.4, 91.8, 92.0, 93.5, 93.8, 120.1, 125.3, 127.1, 127.6, 140.7, 143.9, 156.5, 167.3, 169.7, 169.9, 172.4; ESI–EMM (*m*/*z*): $[M - H]^-$ calcd for $C_{27}H_{27}F_2N_3O_6Na$ 550.1761; found 550.1749.

*N-9-Fluorenylmethoxycarbonyl-glycyl-(2S)-prolyl–(2S,4R)-4-fluoroproline (**16**)*—Fmoc-GlyProFlp-OH (**16**) was obtained in 85% yield. ^{1}H NMR (DMSO-d_6) δ: 1.62–2.35 (m, 5H), 2.45–2.70 (m, 1H; peak under DMSO), 3.21–3.53 (m, 2H), 3.62–3.80 (m, 2.4H), 3.83–3.98 (m, 1.6H), 4.06–4.42 (m, 5H), 4.63 and 4.96 (dd, J = 3.2, 8.6, 1H), 5.31–5.53 (m, 1H), 7.27–7.48 (m, 5H), 7.57–7.76 (m, 2H), 7.85–7.93 (m, 2H), 12.61 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ: 21.6, 242.2, 27.7, 29.9, 34.9, 35.2, 42.1, 45.6, 46.6, 52.8, 53.1, 57.1, 57.3, 57.5, 65.7, 91.9, 93.6, 120.1, 125.3, 127.1, 127.6, 140.7, 143.9, 156.5, 166.8, 167.2, 169.9, 170.3, 172.6; ESI–EMM (*m*/*z*): $[M - H]^-$ calcd for $C_{27}H_{27}FN_3O_6$ 508.1889; found 508.1892.

*Synthesis of N-9-Fluorenylmethoxycarbonyl-(2S,4R)-4-tert-butoxyprolyl–(2S,4S)-4-methylprolylglycyl benzyl ester (**18**)*—Boc-MepGly-OBn (**17**) (0.69 g, 1.8 mmol), prepared as described previously (2), was dissolved in 4 N HCl in dioxane (60 ml) under Ar(g) and stirred for 3 h. The resulting solution was concentrated under reduced pressure, and dried briefly under high vacuum. The residue was dissolved in anhydrous CH_2Cl_2 (40 ml) under Ar(g), and Fmoc-Hyp(*t*Bu)-OH (1.47 g, 3.6 mmol) was added. The resulting solution was cooled to 0 °C. DIEA (0.93 g, 7.2 mmol) and PyBroP (1.68 g, 3.6 mmol) were added, and the solution was stirred for 12 h. The reaction mixture

was washed with 100 ml of aqueous citric acid (10% w/v), $NaHCO_3$(aq), and brine. The organic layer was dried over anhydrous $MgSO_4$(s), and concentrated under reduced pressure. The crude residue was purified by flash chromatography over silica gel (gradient: 60% v/v EtOAc in hexanes to 90% v/v EtOAc in hexanes) to afford Fmoc-Hyp(*t*Bu)MepGly-OBn **(18)** (0.46 g, 39%) as a white solid containing a slight impurity, which was removed after the subsequent step. ESI–EMM (*m*/*z*): $[M + H]^+$ calcd for $C_{39}H_{46}N_3O_7$ 668.3331; found 668.3333.

*Synthesis of N-9-Fluorenylmethoxycarbonyl-(2S,4R)-4-tert-butoxyprolyl–(2S,4S)-4-methylprolylglycine (**19**)*—MeOH (40 ml) was added carefully to a mixture of Fmoc-Hyp(*t*Bu)MepGly-OBn (**18**) (0.43 g, 0.7 mmol) and Pd/C (10% w/w, 0.07 g) under Ar(g). The resulting black suspension was stirred under H_2(g) for ~2 h. Careful monitoring by TLC was necessary to prevent hydrogenolysis of the Fmoc group. The suspension was filtered through a pad of Celite and concentrated under reduced pressure. The crude product was purified by flash chromatography over silica gel (CH_2Cl_2 to elute byproducts, then 5% v/v MeOH in CH_2Cl_2 containing 0.1% v/v formic acid) to afford Fmoc-Hyp(*t*Bu)MepGly-OH (**19**) (0.38 g. 0.7 mmol, quant.) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 1.01 (t, J = 4.2, 3H), 1.13 (s, 9H), 1.27–1.41 (m, 1H), 1.99–2.37 (m, 4H), 2.89–3.02 (m, 1H), 3.10–3.21 (m, 1H), 3.46–3.55 (m, 1H), 3.62–3.86 (m, 3H), 3.92–4.01 (m, 1H), 4.11–4.35 (m, 5H), 4.51–4.60 (m, 1H), 7.28–7.46 (m, 4H), 7.51–7.68 (m, 2H), 7.85–7.94 (m, 2H), 8.06–8.19 (m, 1H); ^{13}C NMR (DMSO-d_6) δ: 16.7, 16.8, 28.0, 33.3, 33.4, 36.8, 36.9, 37.1, 37.4, 40.5, 46.6, 46.8, 53.5, 53.7, 53.8, 53.9, 56.6, 56.7, 59.9, 60.0, 66.5, 68.1, 69.2, 73.5, 120.1, 120.2, 125.0, 125.0, 127.1, 127.7, 140.7, 140.7, 140.8, 143.8, 143.9, 153.8, 153.9, 169.5, 169.6, 171.2, 171.7; ESI–EMM (*m*/*z*): $[M - H]^-$ calcd for $C_{32}H_{38}N_3O_7$ 576.2715; found 576.2719.

Synthesis and Characterization of Longer Peptides—The peptides $(GlyFlpHyp)_{10}GlyGlyTyr$, $(GlyHypFlp)_{10}GlyGlyTyr$, $(GlyProHyp)_{10}GlyGlyTyr$, $(GlyProFlp)_{10}GlyGlyTyr$, and $(GlyHypHyp)_{10}GlyGlyTyr$ were prepared using a procedure similar to that described in the main text for the synthesis of 21-mers, but employing a pre-loaded Fmoc-Tyr Wang resin to enable addition of the C-terminal GlyGlyTyr amino acid triplet. Conformational analyses of these peptides were performed using the protocol described in the main text, and the results are shown in Figure S1 and listed in Table S1. These peptides are not a main focus of this paper because both $(GlyHypFlp)_{10}GlyGlyTyr$ and $(GlyProFlp)_{10}GlyGlyTyr$ formed triple helices too stable for biophysical characterization (*e.g.*, values of T_m could not be determined due to the absence of a high-temperature baseline) and because peptides of this length could not be synthesized for other sequences reported in the main text. Nevertheless, data with longer peptides recapitulate data reported in the main paper. That is, $(GlyHypHyp)_{10}GlyGlyTyr$ and $(GlyFlpHyp)_{10}GlyGlyTyr$ both form triple helices more stable than does $(GlyProHyp)_{10}GlyGlyTyr$, and both $(GlyHypFlp)_{10}GlyGlyTyr$ and $(GlyProFlp)_{10}GlyGlyTyr$ form highly stable triple helices with similar values of T_m.

REFERENCES

1. Hodges, J. A., and Raines, R. T. (2005) *J. Am. Chem. Soc.* **127**, 15923–15932
2. Shoulders, M. D., Hodges, J. A., and Raines, R. T. (2006) *J. Am. Chem. Soc.* **128**, 8112–8113

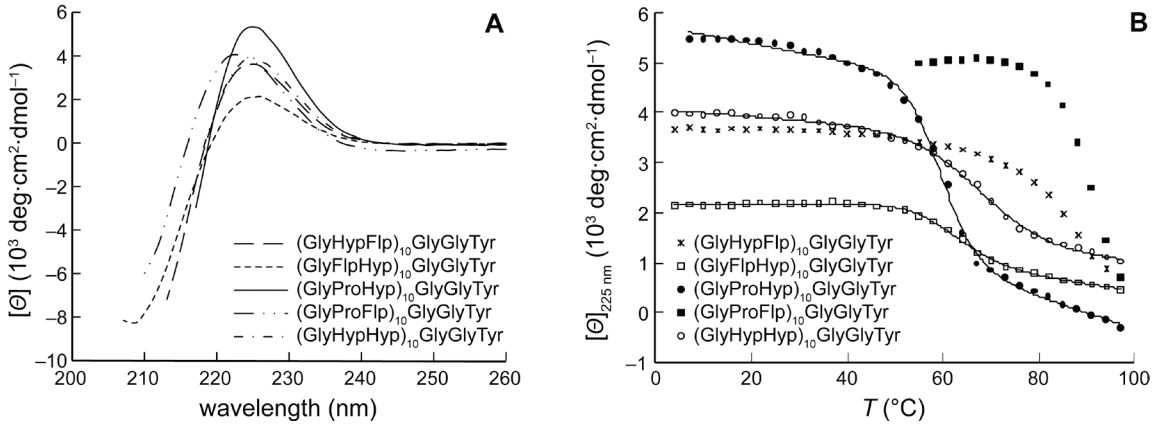
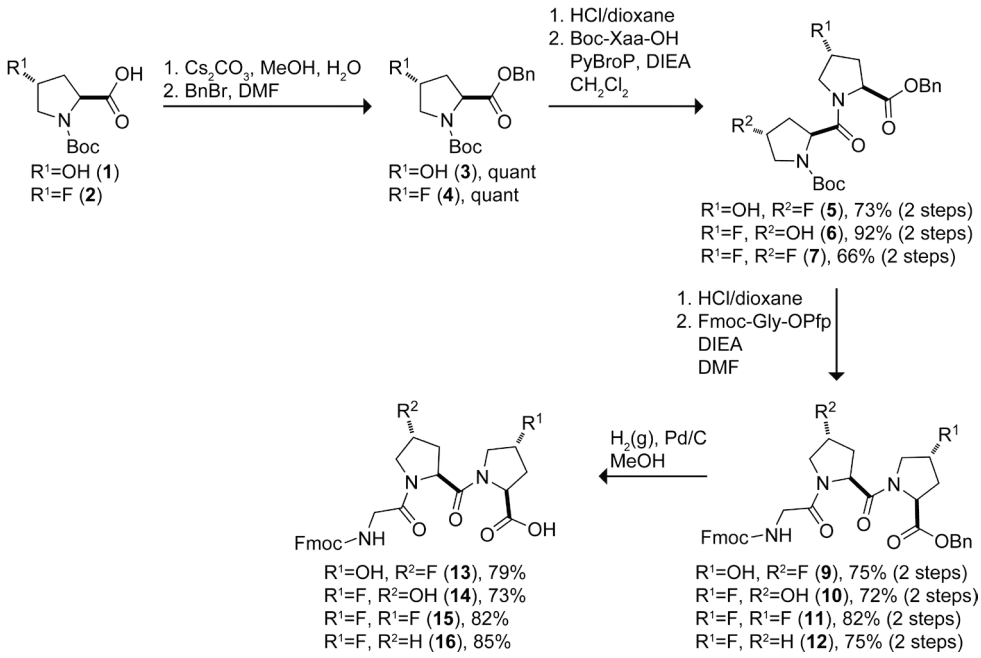


Figure S1. Conformational analysis of longer peptides. (A) Circular dichroism spectra of peptide solutions (~0.2 mM in 50 mM sodium phosphate buffer, pH 7.0. (B) Effect of temperature on the molar ellipticity at 225 nm. Data were recorded at 3-°C intervals after a 5-min equilibration.

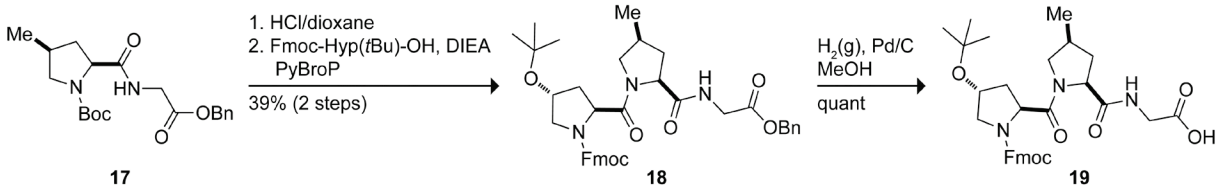
Table S1. Values of T_m for the Unfolding of Longer Collagen Triple Helices

peptide	T_m [a]
$(GlyProFlp)_{10}GlyGlyTyr$	≥80[b]
$(GlyProFlp)_{10}GlyGlyTyr$	>80[b]
$(GlyHypHyp)_{10}GlyGlyTyr$	68
$(GlyFlpHyp)_{10}GlyGlyTyr$	62
$(GlyProHyp)_{10}GlyGlyTyr$	58

[a]Values (±1 °C) were determined by CD spectroscopy. [b]A value of T_m could not be determined due to the high triple-helix stability.

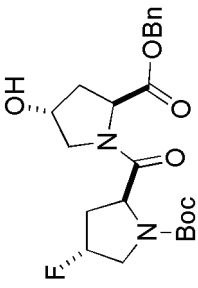


SCHEME S1. Synthesis of Fmoc-GlyHypFlp-OH (**13**), Fmoc-GlyFlpHyp-OH (**14**), Fmoc-GlyFlpFlp-OH (**15**), and Fmoc-GlyProFlp-OH (**16**)

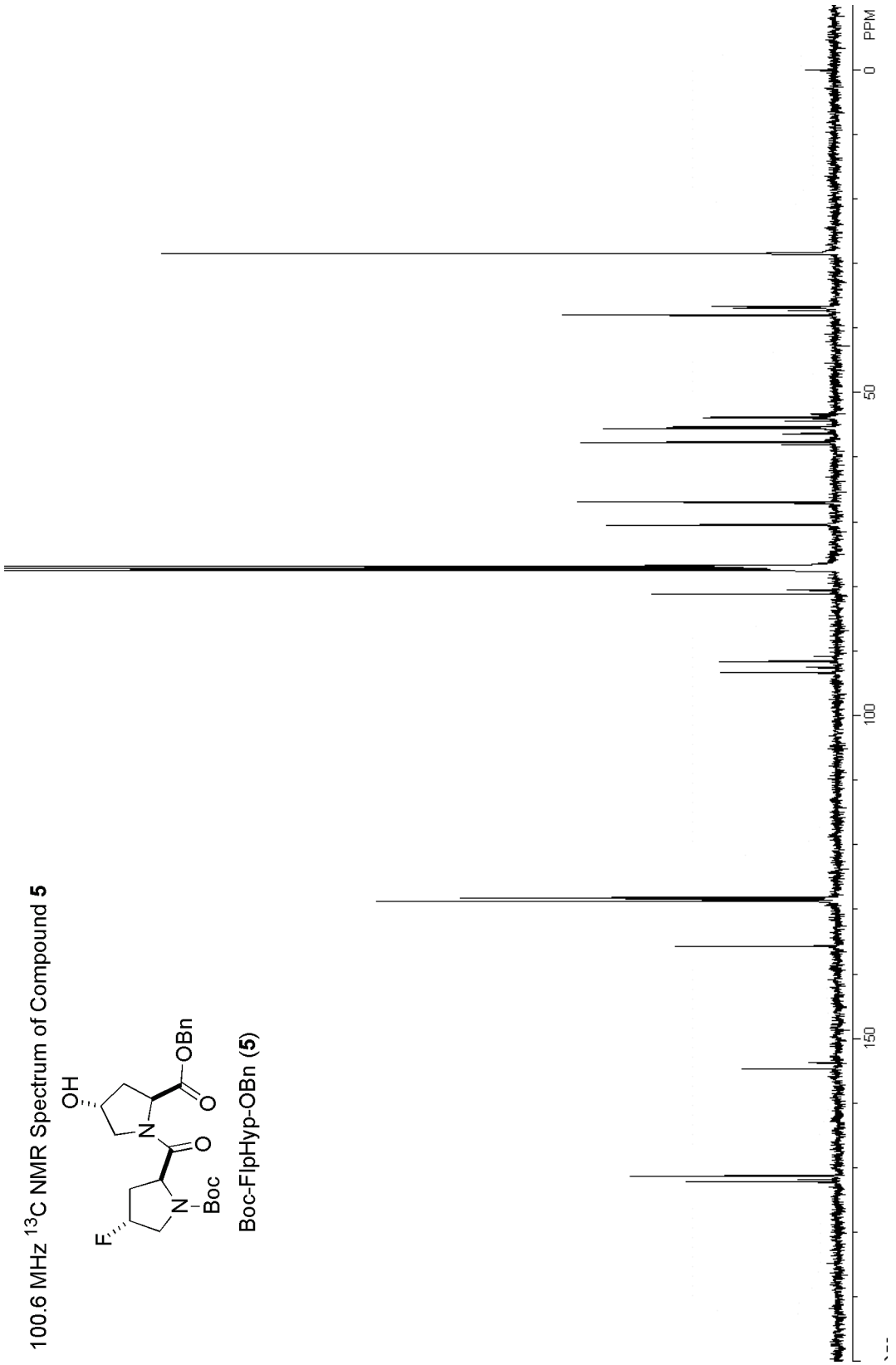


Scheme S2. Synthesis of Fmoc-Hyp(*t*Bu)MepGly-OH (**19**).

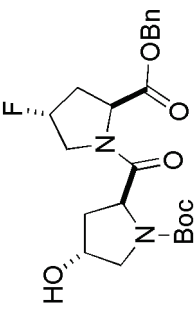
400 MHz ^{1}H NMR Spectrum of Compound **5**



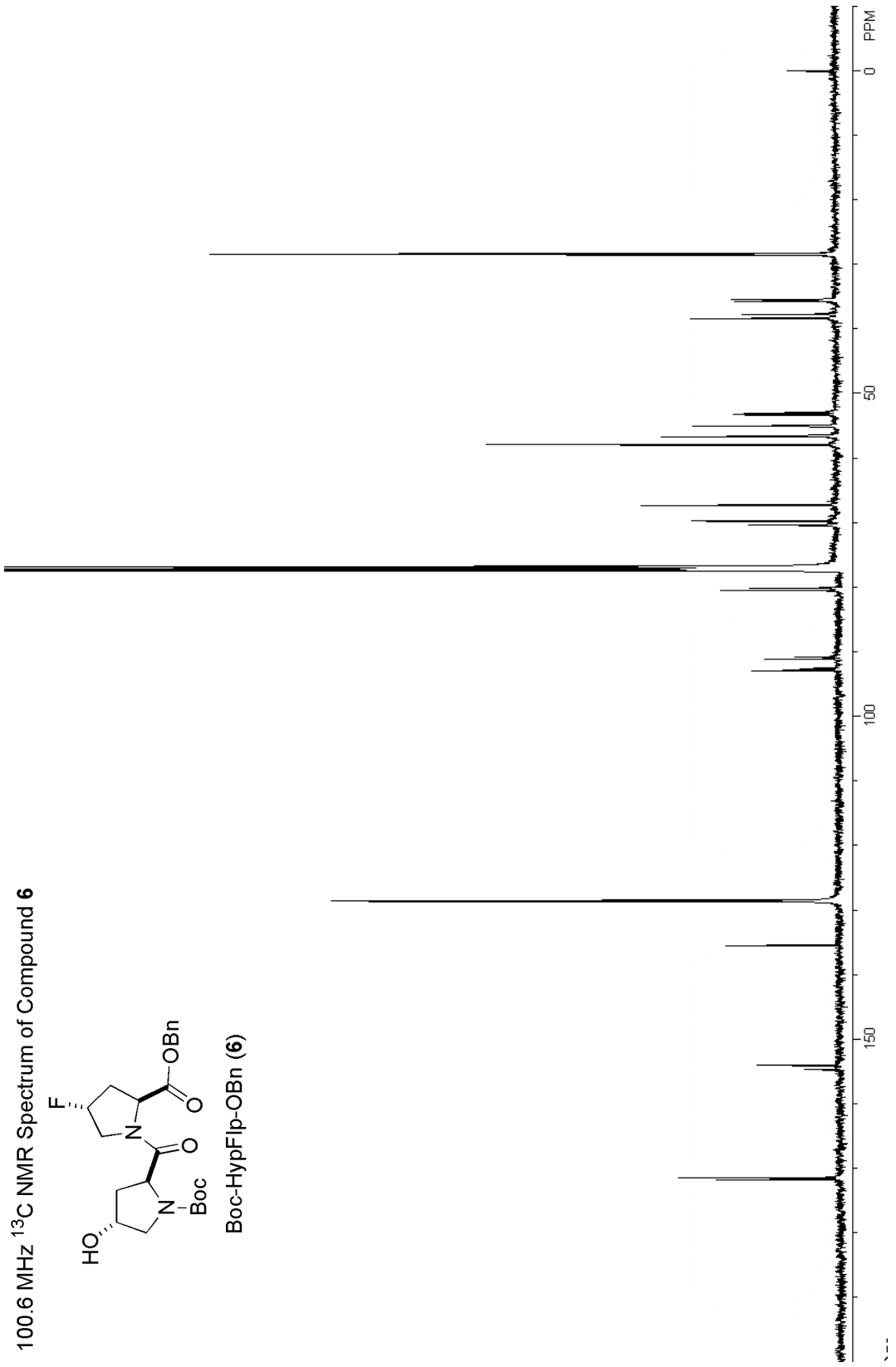
Boc-FlpHyp-OBn (**5**)


100.6 MHz ^{13}C NMR Spectrum of Compound **5**
Boc-FlpHyp-OBn (**5**)
OH
OBn
O
N
O
N
Boc
F
PPM
0
50
100
150

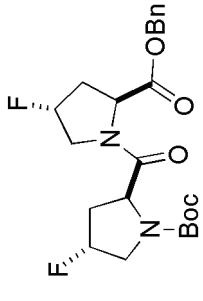
400 MHz ^{1}H NMR Spectrum of Compound **6**



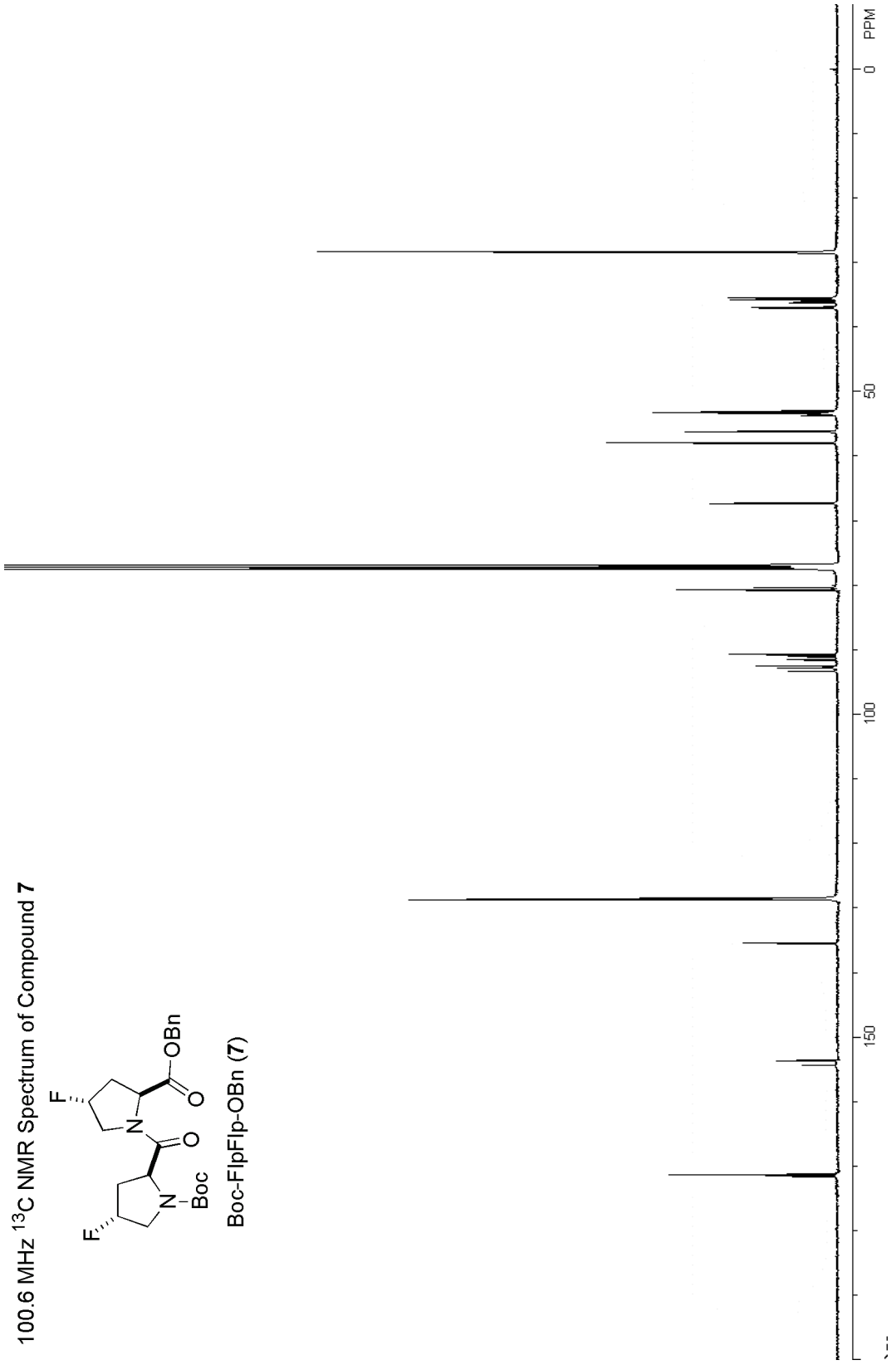
Boc-HypFlp-OBn (**6**)


100.6 MHz ^{13}C NMR Spectrum of Compound **6**
Boc-HypFlp-OBn (**6**)
F
OBn
O
O
N
N
Boc
HO
PPM
0
50
100
150

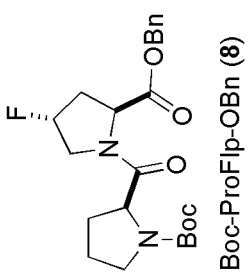
400 MHz ^{1}H NMR Spectrum of Compound **7**



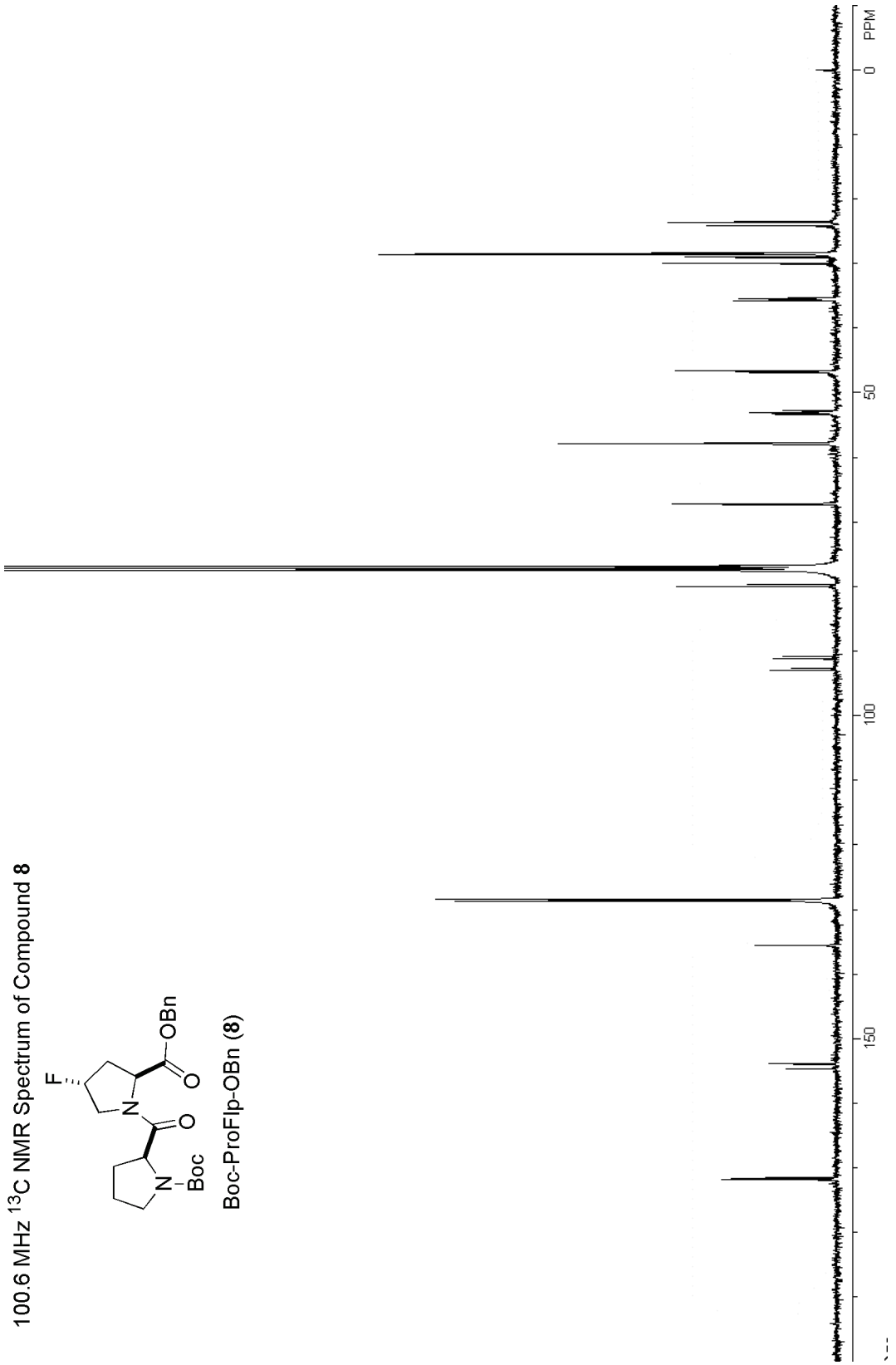
Boc-FlpFlp-OBn (**7**)

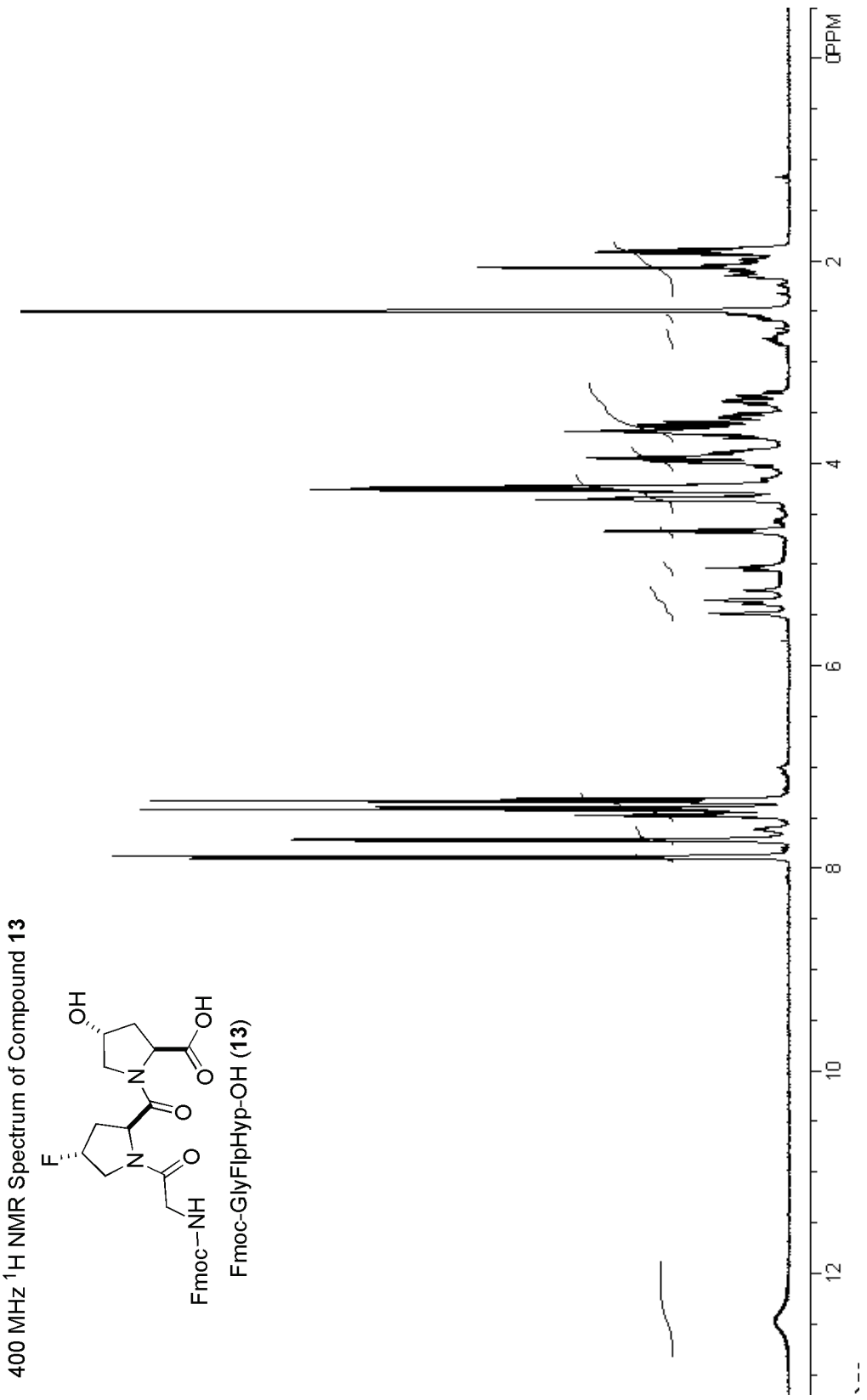

100.6 MHz ^{13}C NMR Spectrum of Compound **7**
Boc-FlpFlp-OBn (**7**)
F
OBn
O
N
O
N
Boc
F
PPM
0
50
100
150

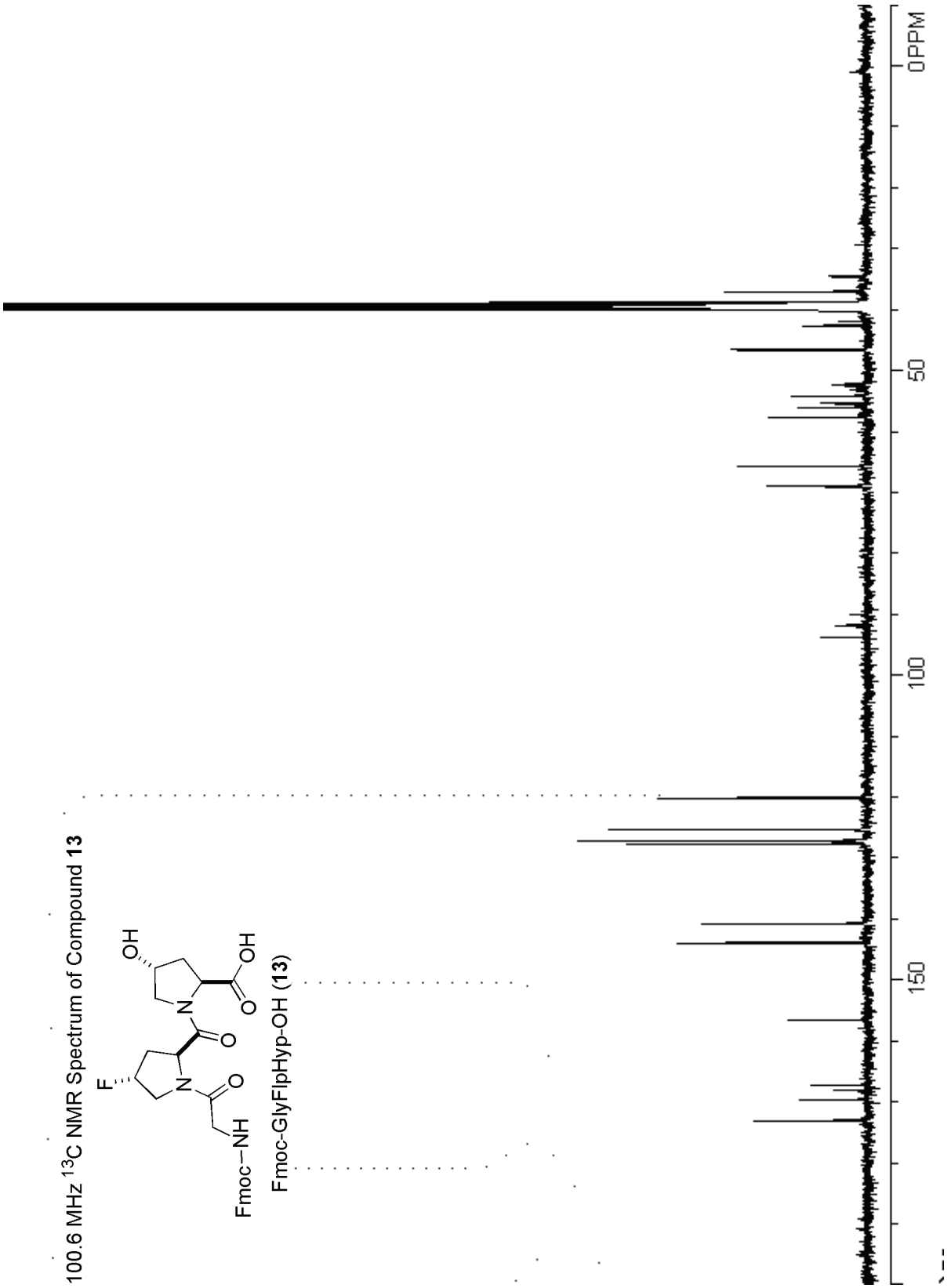
400 MHz ^{1}H NMR Spectrum of Compound **8**

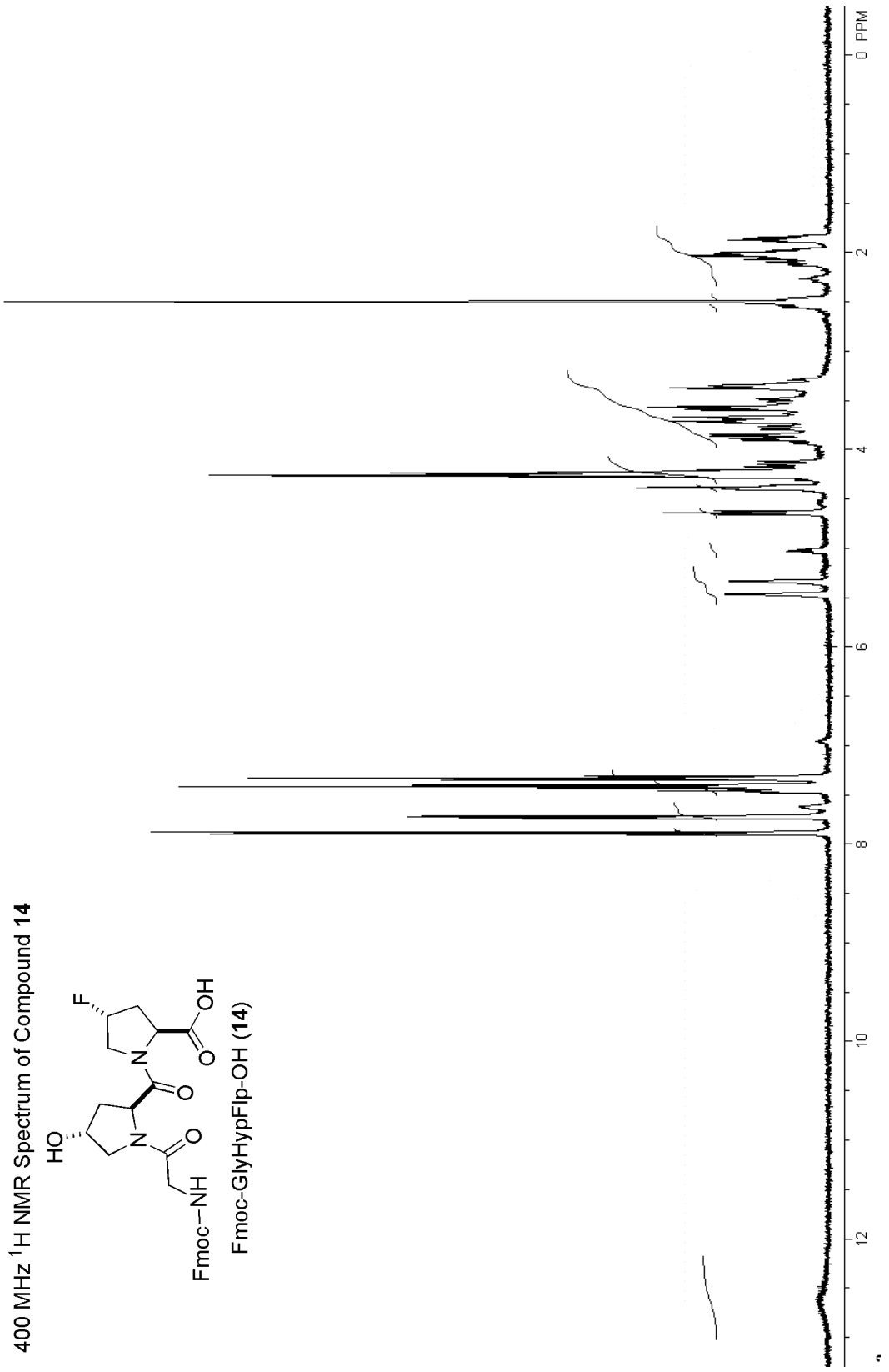


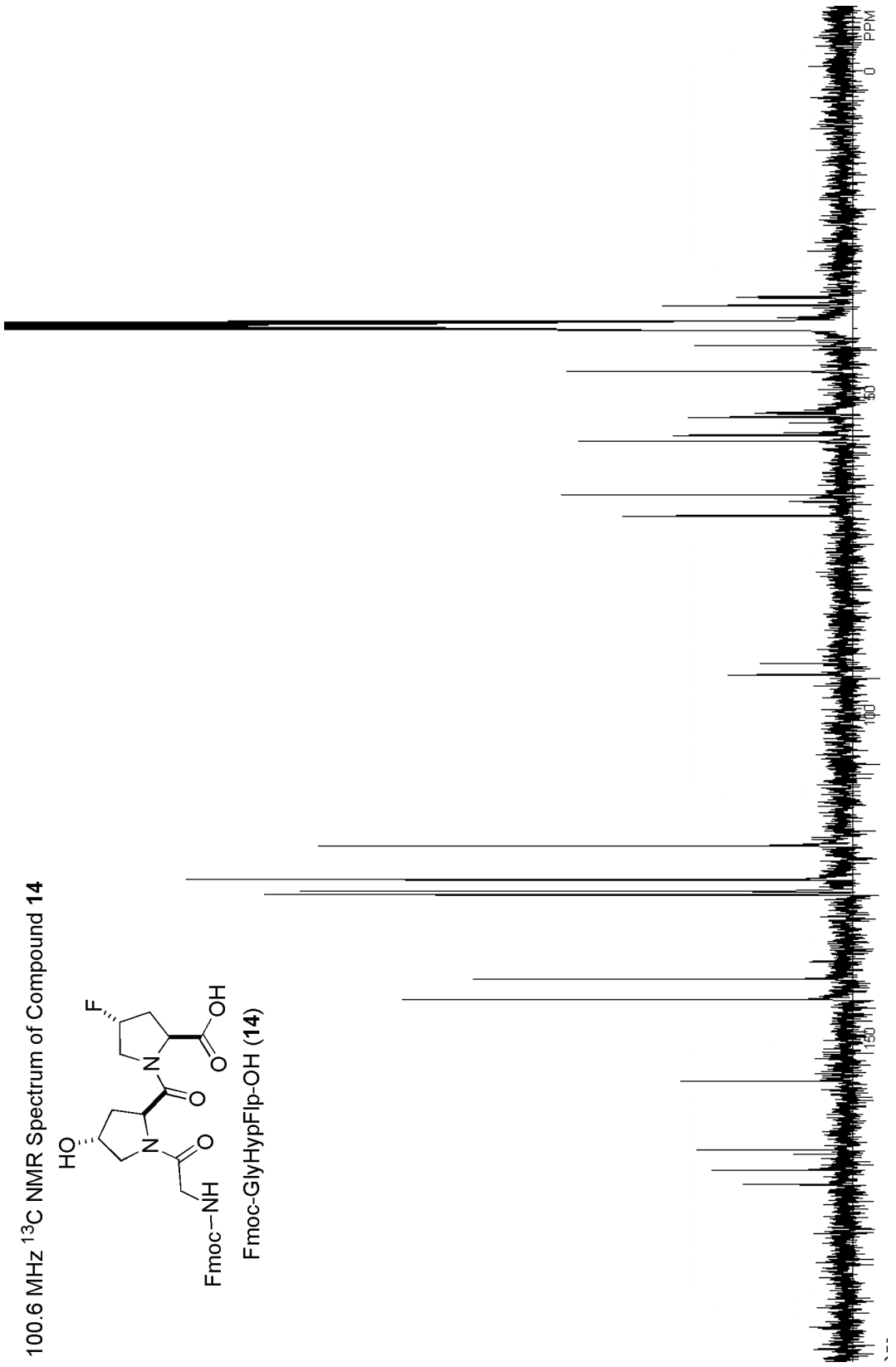
Boc-ProFlp-OBn (**8**)

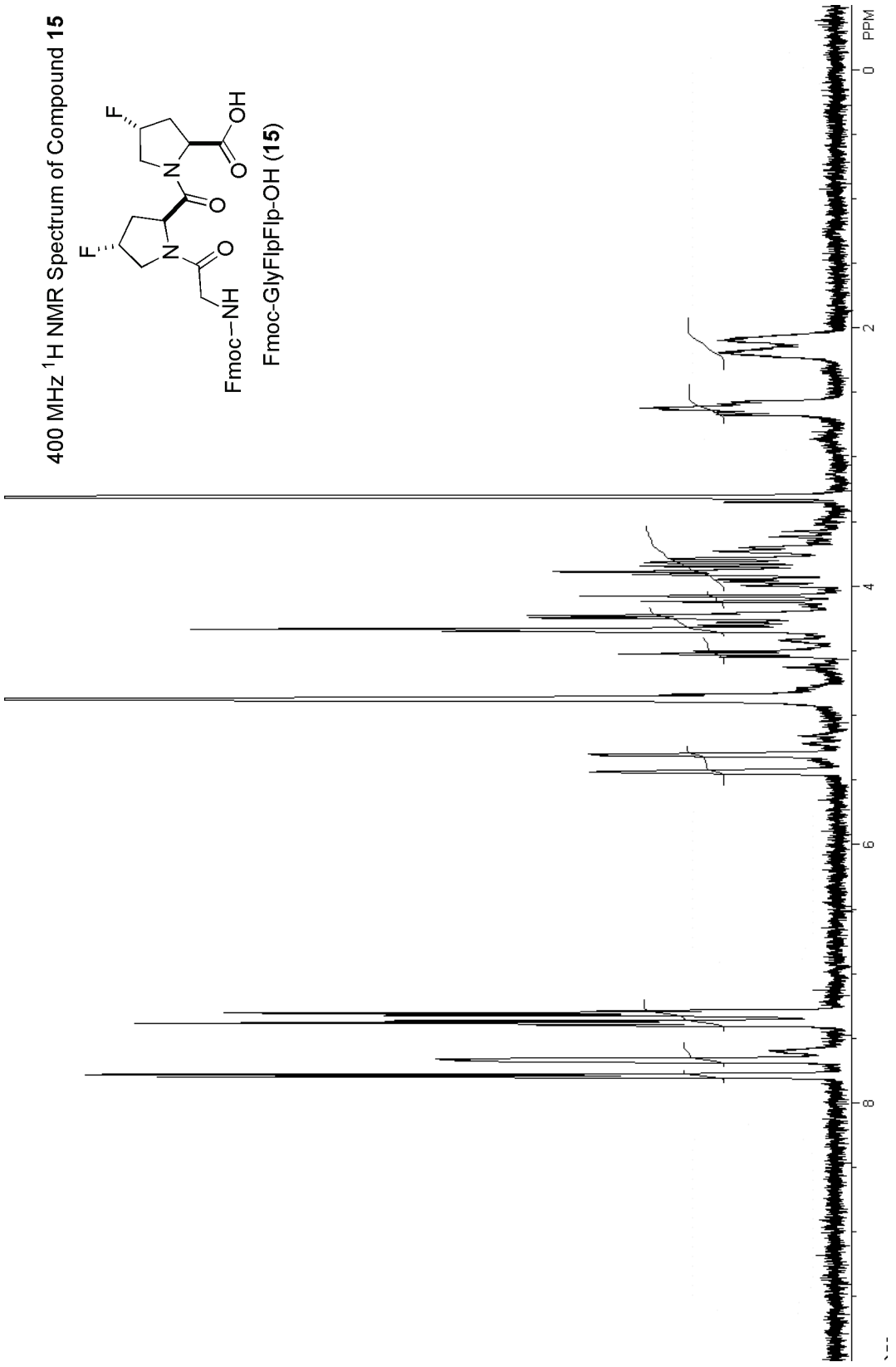

100.6 MHz ^{13}C NMR Spectrum of Compound **8**
Boc-ProFlp-OBn (**8**)
F
OBn
O
O
N
N
Boc
PPM
0
50
100
150

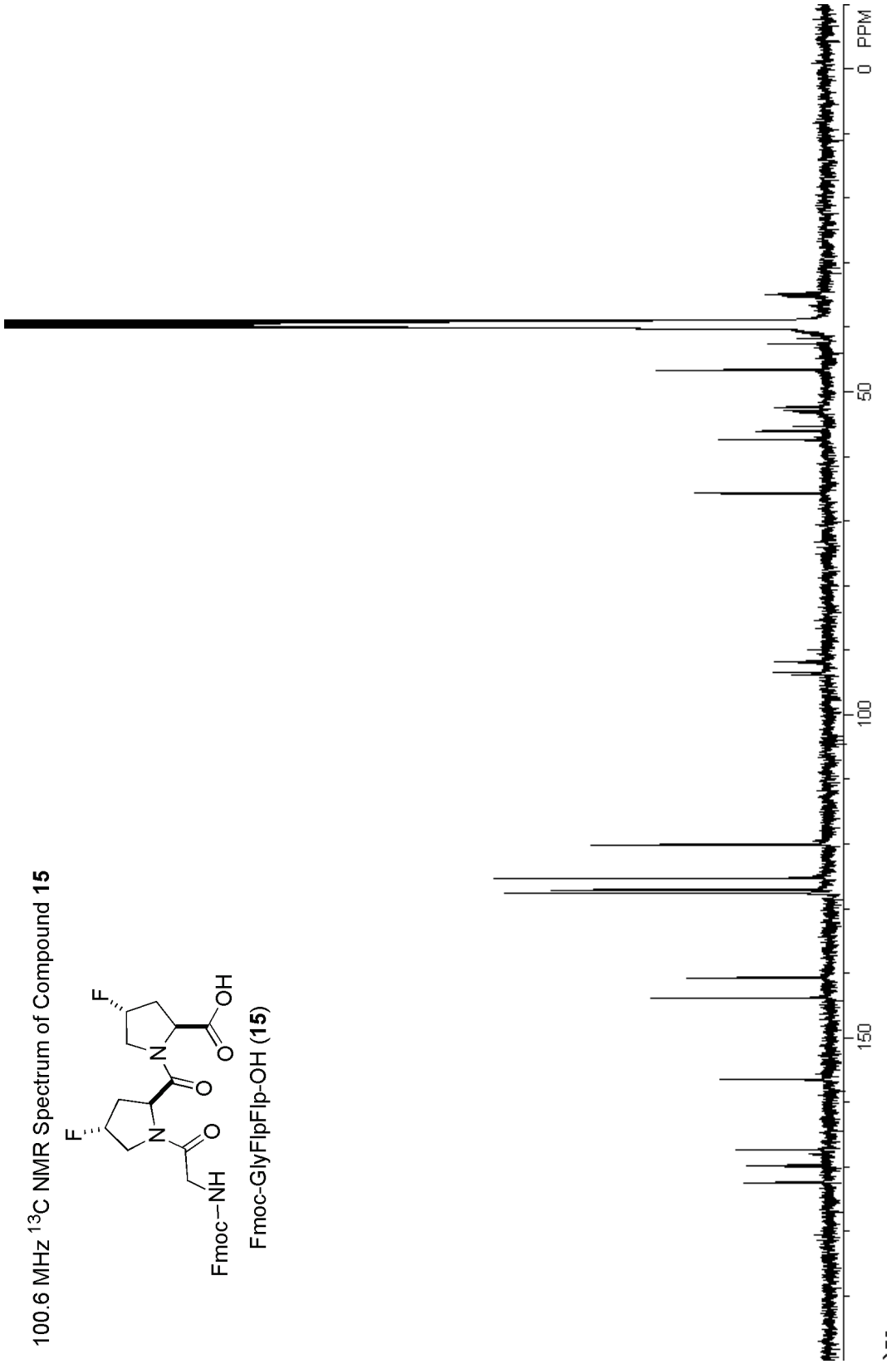

400 MHz ^{1}H NMR Spectrum of Compound **13**
Fmoc-GlyFlpHyp-OH (**13**)
Fmoc—NH
F
OH
OH
O
O
O
N
N
PPM
2
4
6
8
10
12


100.6 MHz ^{13}C NMR Spectrum of Compound **13**
Fmoc-GlyFlpHyp-OH (**13**)
Fmoc—NH
F
OH
OH
O
O
O
N
N
150
100
50
0PPM

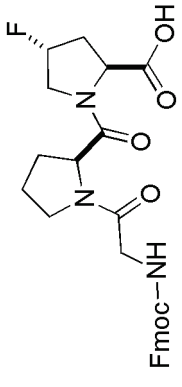

400 MHz ^{1}H NMR Spectrum of Compound **14**
Fmoc-GlyHypFlp-OH (**14**)
Fmoc—NH
HO
F
OH
O
N
0 PPM
2
4
6
8
10
12


100.6 MHz ^{13}C NMR Spectrum of Compound **14**
Fmoc-GlyHypFlp-OH (**14**)
HO
F
OH
O
N
O
N
O
Fmoc—NH
PPM
0
50
100
150


400 MHz ^{1}H NMR Spectrum of Compound **15**
Fmoc-GlyFlpFlp-OH (**15**)
Fmoc—NH
F
N
O
OH
PPM
0
2
4
6
8

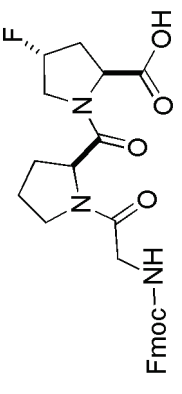

100.6 MHz ^{13}C NMR Spectrum of Compound **15**
Fmoc-GlyFlpFlp-OH (**15**)
Fmoc—NH
F
F
N
N
O
O
O
OH
0 PPM
50
100
150

400 MHz ^{1}H NMR Spectrum of Compound **16**

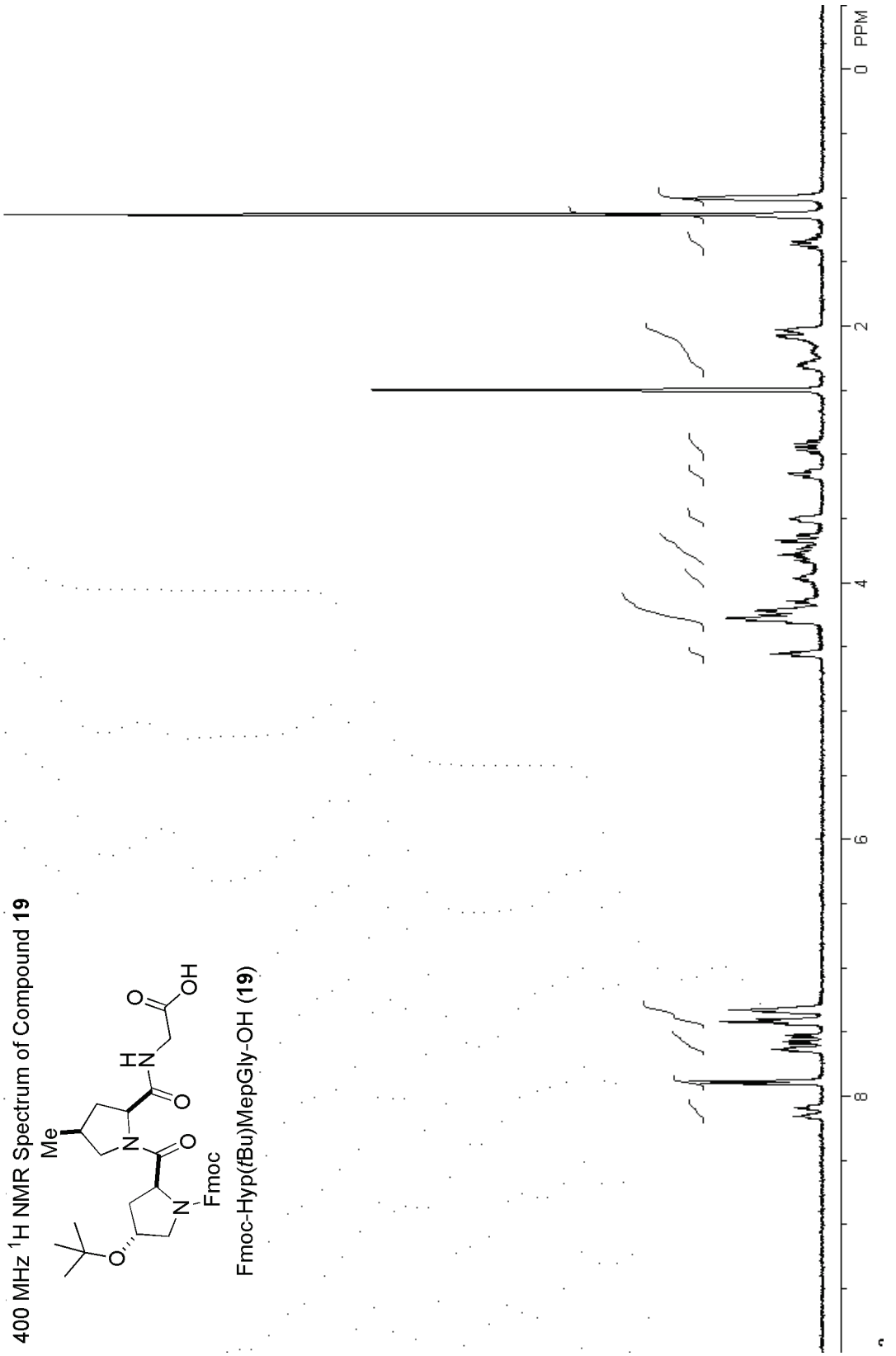


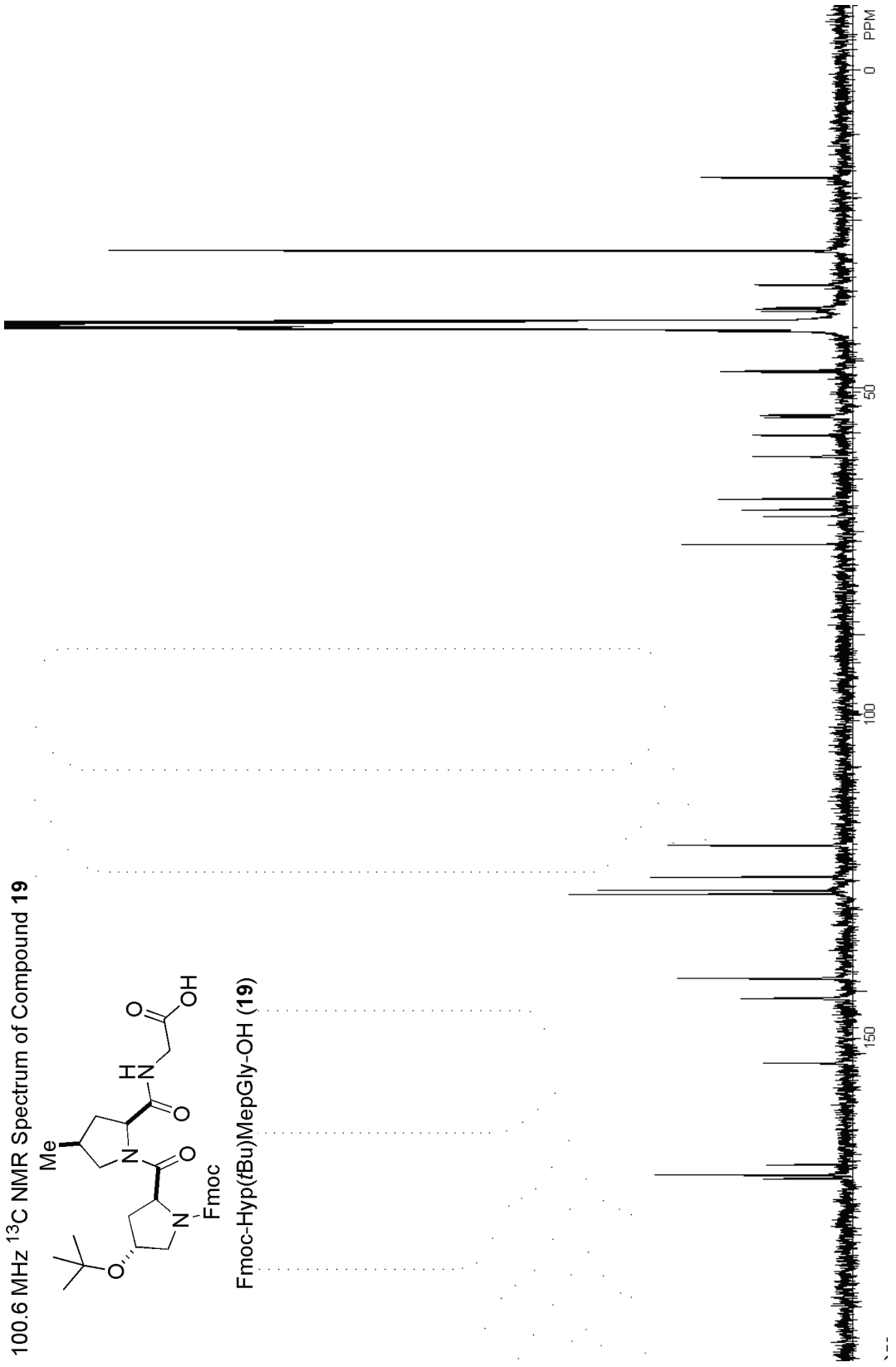
Fmoc-GlyProFlp-OH (**16**)

100.6 MHz ^{13}C NMR Spectrum of Compound **16**



Fmoc-GlyProFlp-OH (**16**)


400 MHz ^{1}H NMR Spectrum of Compound **19**
Fmoc-Hyp(*t*Bu)MepGly-OH (**19**)
Me
Fmoc
OH
O
N
H
0 PPM
2
4
6
8


100.6 MHz ^{13}C NMR Spectrum of Compound **19**
Fmoc-Hyp(*t*Bu)MepGly-OH (**19**)
Me
Fmoc
PPM
0
50
100
150