Factors affecting conformation in proline-containing peptides

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SUPPORTING INFORMATION

Procedures for the preparation of compounds 1, 2, 4, 5 and 6; ^{1}H and ^{13}C NMR spectra for all intermediates and final products and tables of NMR and thermodynamic data.

Experimental Procedures

General

Synthesis of dipeptides: reagents were purchased and purified as described previously.[1]

Purification of dipeptides: this was performed by RP-HPLC using 250 mm long C18 columns (4.6 mm diameter Spherisorb S10 ODS2, 10 mm diameter Econosil, 22 mm diameter Econosil), eluting with flow rates of 0.6, 2.8 and 12 mL min^{-1} respectively. The following gradient of acetonitile in water was employed: 20-30% over 8 min; 30-95% over 2 min; 95% for 5 min; 95-20% over 5 min. The eluent was monitored at 218 nm and 259 nm; the compounds absorbed approximately 10-fold less strongly at 259 nm than they did at 218 nm. Relevant fractions of eluent were concentrated by lyophilization.

[1] (a) Weir, C. A.; Taylor, C. M. *J. Org. Chem.* **1999**, *64*, 1554-1558; (b) Taylor, C. M.; Barker, W. D.; Weir, C. A.; Park, J. H. *J. Org. Chem.* **2002**, *67*, 4466-4474.

NMR studies. Samples for NMR experiments were prepared by dissolving 2-3 mg of compound in D_2O (0.5 mL). The pH value, without correction for isotope effects, was 7.56. The spectra were recorded on a Bruker AVANCE 400 spectrometer. Assignments have been given only for compounds where these have been determined unequivocally using COSY, HMQC, HMBC and NOESY experiments. In the many cases where a spectrum reflects the presence of two species arising from *trans* and *cis* rotamers about the X-Pro amide bond, the following conventions have been adopted for reporting. For ^{1}H spectra: chemical shift (multiplicity, #protons^{trans}/#protons^{cis}, assignment/s). For ^{13}C spectra: where signals are clearly part of a pair they are listed, X.X (X.X), where the first signal refers to the *trans* species and the second, in parentheses, to the *cis*. ^{1}H spectra were referenced relative to residual HOD at 4.65ppm. ^{13}C spectra were referenced relative to external DSS (2,2-dimethyl-2-silapentane-5-sulfonate, sodium salt). The ratio of *trans*:*cis* rotamers was determined by integration of all well-resolved peaks in the one-dimensional ^{1}H NMR spectra, and averaging of these results. Variable temperature experiments were conducted from 25-80 °C with a 5 °C interval.

Ac-Pro-OMe (**1**) – this compound is commercially available from Bachem and Advanced ChemTech, but was prepared by acetylation of proline methyl ester hydrochloride and purified by flash chromatography, eluting with 0-5% MeOH in CH_2Cl_2. ^{1}H NMR (400 MHz, D_2O, $K_{t/c}$ = 5.4 at 298K) δ 1.65-1.82 (m, 1H^{cis}, Proγ'^{cis}), 1.85-1.95 (m, 3H^{trans}/1H^{cis}, Proβ'^{trans}, Proγ'^{trans}, Proγ^{trans}, Proγ^{cis}), 1.88 (s, 3H^{cis}, Ac^{cis}), 2.00 (s, 3H^{trans}, Ac^{trans}), 2.06-2.30 (m, 1H^{trans}/2H^{cis}, Proβ^{trans}, Proβ'^{cis}, Proβ^{cis}), 3.30-3.46 (m, 2H^{cis}, Proδ'^{cis} Proδ^{cis}), 3.46-3.59 (m, 1H^{trans}, Proδ'^{trans}), 3.53 (dd, J = 14.6, 7.3 Hz, 1H^{trans}, Proδ^{trans}), 3.64 (s, 3H^{trans}, OMe^{trans}), 3.68 (s, 3H^{cis}, OMe^{cis}), 4.32 (dd, J = 8.4, 4.2 Hz, 1H^{trans}, Proα^{trans}), 4.58 (dd, J = 8.7, 2.5 Hz, 1H^{cis}, Proα^{cis}); ^{13}C NMR (D_2O, 100 MHz,) δ 24.0, 27.1 (25.2), 32.0 (33.5), 51.2 (49.4), 55.6 (56.0), 61.8 (63.4), 175.5 (175.9), 177.7 (177.2).

Ac-Pro-NHMe (**2**)

1. *Boc-Pro-NHMe*. Boc_2O (948 mg, 4.34 mmol, 2 equiv.) was added to a solution of *L*-proline (250 mg, 2.17 mmol, 1.0 equiv.) and triethylamine (303 μL 2.17 mmol, 1.0 equiv.) in dry methanol (6 mL). The solution was heated at reflux for 3 h and then concentrated. The mixture was diluted with ethyl

acetate (50 mL) and washed with ice-cold 1 M aq. HCl (8 mL). The aqueous layer was extracted with ethyl acetate (4 x 50 mL). The organic layers were combined and dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in dichloromethane (5 mL) and methylamine hydrochloride (147 mg, 2.17 mmol, 1.0 equiv.), BOP reagent (960 mg, 2.17 mmol, 1.0 equiv.) and triethylamine (760 μL, 5.42 mmol, 2.5 equiv.) were added. The mixture was stirred at room temperature for 16 h. The mixture was evaporated and the residue dissolved in CH_2Cl_2 (100 mL), washed with 1 M aq. HCl (5 mL), sat'd aq. $NaHCO_3$ (10 mL) and brine (10 mL). The last two aqueous solutions were extracted with further dichloromethane (2 x 70 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash column chromatography eluting with ethyl acetate-hexanes (8:1). Relevant fractions were combined to give *Boc-Pro-NHMe* (407 mg, 82 %). R_f = 0.38 (EtOAc); ^{1}H NMR (CD_3OD, 400 MHz) δ 1.45 (s, 9H^{trans}), 1.50 (s, 9H^{cis}), 1.81-2.01(m, 3H^{trans}/3H^{cis}), 2.13-2.32 (m, 1H^{trans}/1H^{cis}), 2.78 (s, 3H^{trans}/3H^{cis}), 3.38-3.49 (m, 1H^{trans}/1H^{cis}), 3.49-3.58 (m, 1H^{trans}/1H^{cis}), 4.14 (dd, J = 7.9, 3.8 Hz, 1H^{trans}), 4.19 (br. d, J = 8.2 Hz, 1H^{cis}); ^{13}C NMR (CD_3OD, 100 MHz) δ 24.7 (25.3), 26.3 (26.3), 28.6 (28.7), 32.4 (31.5), 47.8 (48.3), 62.0 (61.7), 81.3 (79.5), 155.9 (156.4), 176.3 (175.9).

II. *Ac-Pro-NHMe (2).* Boc-Pro-NHMe (57 mg, 0.25 mmol) was dissolved in a 1:1 (v/v) solution of TFA and dichloromethane (2 mL). The solution was stirred at room temperature for 30 min and concentrated. The residue was dissolved in pyridine (1 mL) and acetic anhydride (1 mL). The solution was stirred at room temperature for 19 h and concentrated. The residue was purified by flash column chromatography, eluting with 0-10 % MeOH in ethyl acetate. Relevant fractions were combined to give *Ac-Pro-NHMe* (**2**) (35 mg, 83%). ^{1}H NMR (400 MHz, D_2O, $K_{t/c}$ = 2.6 at 298K) δ 1.64-2.26 (m, 4H^{trans}/4H^{cis}, Proβ'^{trans}, Proβ^{trans}, Proγ'^{trans}, Proγ^{trans}, Proβ'^{cis}, Proβ^{cis}, Proγ'^{cis}, Proγ^{cis}), 1.82 (s, 3H^{cis}, Ac^{cis}), 1.96 (s, 3H^{trans}, Ac^{trans}), 2.57 (s, 3H^{trans}, NHMe^{trans}), 2.62 (s, 3H^{cis}, NHMe^{cis}), 3.25-3.59 (m, 2H^{trans}/2H^{cis}, Proδ'^{trans}, Proδ^{trans}, Proδ'^{cis}, Proδ^{cis},), 4.18 (dd, J = 7.6, 3.9 Hz, 1H^{trans}, Proα^{trans}), 4.33 (dd, J = 8.7, 2.9 Hz, 1H^{cis}, Proα^{cis}); ^{13}C NMR (100 MHz, D_2O) δ 24.2 (23.9), 26.8 (25.2), 28.5 (28.7), 32.7 (34.3), 51.4 (49.8), 63.1 (64.6), 176.1 (176.3), 177.7 (177.6).

Ac-Gly-Pro-NHMe (4).

I. *Boc-Gly-Pro-NHMe***.** BOP reagent (650 mg, 1.47 mmol, 1.0 equiv.) was added to a solution of Boc-Gly-Pro-OH (400 mg, 1.47 mmol, 1.0 equiv.), methylamine hydrochloride (99 mg, 1.47 mmol, 1 .0 equiv.) and triethylamine (1.3 mL, 9.23 mmol, 3.0 equiv.) in acetonitrile (15 mL). The solution was stirred at room temperature for 18 h and then concentrated. The mixture was diluted with ethyl acetate (75 mL), washed with 1 M aq. HCl (40 mL), sat'd aq. $NaHCO_3$.(40 mL), water (20 mL) and brine (40 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash column chromatography, eluting with 0-10 % MeOH in ethyl acetate. After evaporation of the relevant fractions, *Boc-Gly-Pro-NHMe* was obtained (217 mg, 52 %). R_f = 0.37 (EtOAc-MeOH 9:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.45 (s, 9H), 1.74-2.34 (m, 4H), 2.70 (d, *J* = 4.8 Hz, 3H), 3.37 (dd, *J* = 16.2, 8.1 Hz, 1H), 3.47-3.60 (m, 1H), 3.88 (d, *J* = 4.6 Hz, 2H), 4.48 (dd, *J* = 7.7, 2.2 Hz, 1H), 5.50 (d, *J* = 4.8 Hz, 1H), 6.92 (d, *J* = 4.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 24.8, 26.2, 27.9, 28.3, 43.1, 46.4, 60.2, 79.8, 155.9, 168.7, 171.4; HRMS (FAB$^+$, NBA, MeOH) calcd for $(MH)^+$ $C_{13}H_{23}$ N_3O_4: 286.1759; obsd 286.1767.

II. *Ac-Gly-Pro-NHMe* **(4).** Boc-Gly-Pro-NHMe (100 mg, 0.35 mmol) was dissolved in a 1:1 (v/v) solution of TFA and dichloromethane (6 mL). The solution was stirred at room temperature for 30 min and concentrated. The residue was dissolved in pyridine (1 mL) and acetic anhydride (1 mL). The solution was stirred at room temperature for 19 h and concentrated. The residue was purified by flash column chromatography, eluting with 0-20 % MeOH in ethyl acetate. Relevant fractions were combined to give *Ac-Gly-Pro-NHMe* (**4**) (35 mg, 44%). ^{1}H NMR (400 MHz, D_2O, $K_{t/c}$ = 5.5 at 298K) δ 1.67-1.77 (m, $1H^{cis}$, Proγ'^{cis}), 1.77-1.96 (m, $3H^{trans}$/$1H^{cis}$, Proβ'^{trans}, Proγ'^{trans}, Proγ^{trans}, Proγ^{cis}), 1.92 (s, $3H^{cis}$, Ac^{cis}),1.93 (s, $3H^{trans}$, Ac^{trans}), 1.96-2.04 (m, $1H^{cis}$, Proβ'^{cis}), 2.06-2.15 (m, $1H^{trans}$, Proβ^{trans}), 2.17-2.26 (m, $1H^{cis}$, Proβ^{cis}), 2.61 (s, $3H^{trans}$, NHMe^{trans}), 2.66 (s, $3H^{cis}$, NHMe^{cis}), 3.35-3.58 (m, $2H^{trans}$/$2H^{cis}$, Proδ'^{trans}, Proδ^{trans}, Proδ'^{cis}, Proδ^{cis}), 3.61 (d, *J* = 16.9 Hz, $1H^{cis}$, Glyα'^{cis}), 3.89 (d, *J* = 16.9 Hz, $1H^{cis}$, Glyα^{cis}), 3.96 (br. s, $2H^{trans}$, Glyα'^{trans}, Glyα^{trans}), 4.25 (dd, *J* = 9.0, 4.4 Hz, $1H^{trans}$, Proα^{trans}), 4.41 (dd, *J* = 8.8, 2.7 Hz, $1H^{cis}$, Proα^{cis}); ^{13}C NMR (100 MHz, D_2O) d 24.3, 27.0 (24.6), 28.6 (28.8), 32.2 (34.5), 44.5 (44.3), 49.7 (50.3), 63.6 (63.2), 172.5 (172.8), 177.4, 177.5 (177.0, 177.3); HRMS (FAB$^+$, glycerol, MeOH) calcd for $(MH)^+$ $C_{10}H_{18}N_3O_3$: 228.1348; obsd 228.1348.

Ac-Phe-Pro-OMe (**5**). Diphenylphosphoryl azide (150 μL, 160 mg, 0.69 mmol, 1.1 equiv.) was added to a solution of *N*-acetylphenylalanine (143 mg, 0.69 mmol, 1.1 equiv.), proline methyl ester hydrochloride (102 mg, 0.62 mmol, 1.0 equiv.) and triethylamine (220 μL, 160 mg, 1.57 mmol, 2.5 equiv.) in dry acetonitrile (5 mL). The solution was stirred at room temperature under nitrogen for 18 h and then concentrated. The residue was partitioned between ethyl acetate (60 mL) and water (60 mL). The organic layer was washed with brine (60 mL), dried over $MgSO_4$, filtered and concentrated. The product was isolated from the residue by RP-HPLC to give *Ac-Phe-Pro-OMe* (**5**) (15 mg, 28%). R_f 0.33 (95:5 CH_2Cl_2-MeOH); ^{1}H NMR (400 MHz, D_2O, $K_{t/c}$ = 4.20 at 298K) δ 1.46-1.65 (m, 4H^{cis}, Proβ'^{cis}, Proβ^{cis} Proγ'^{cis}, Proγ^{cis}), 1.67-1.86 (m, 3H^{trans}, Proβ'^{trans}, Proγ'^{trans}, Proγ^{trans}), 1.76 (s, 3H^{trans}, Ac^{trans}), 1.78 (s, 3H^{cis}, Ac^{cis}), 2.02-2.16 (m, 1H^{trans}, Proβ^{trans}), 2.78 (dd, J = 13.9, 8.2 Hz, 1H^{trans}, Pheβ'^{trans}), 2.76 (m, 2H^{cis}, Pheβ'^{cis}, Pheβ^{cis}), 2.96 (dd, J = 13.9, 6.5 Hz, 1H^{trans}, Pheβ^{trans}), 3.10-3.27 (m, 1H^{trans}, 1H^{cis}, Proδ'^{trans}, Proδ'^{cis}), 3.35 (q, J = 5.9 Hz, 1H^{cis}, Proδ^{cis}), 3.57 (s, 3H^{cis}, OMe^{cis}), 3.60 (s, 3H^{trans}, OMe^{trans}), 3.52-3.66 (m, 1H^{trans}, Proδ^{trans}), 3.84 (br. d, J = 7.6 Hz, 1H^{cis}, Proα^{cis}), 4.28 (dd, J = 8.2, 4.4 Hz, 1H^{trans}, Proα^{trans}), 4.52 (t, J = 7.8 Hz, 1H^{cis}, Pheα^{cis}), 4.69 (t, J = 6.9 Hz, 1H^{trans}, Pheα^{trans}), 7.06-7.28 (m, 5H^{trans},/5H^{cis}, Ar); ^{13}C NMR (100 MHz, D_2O) δ 24.1 (24.2), 27.2 (24.3), 31.4 (33.1), 39.2 (41.0), 50.4 (49.4), 55.6 (55.5), 55.7 (55.9), 62.4 (62.3), 129.9 (130.2), 131.4, 132.1, (131.6, 132.0), 138.9 (138.9), 174.6 (174.5), 176.5, 177.2 (175.7, 176.7); HRMS (EI$^+$) calcd for $C_{17}H_{22}O_4N_2$ (M^+): 318.15796; obsd 318.15789.

Ac-Phe-Pro-NHMe (**6**).

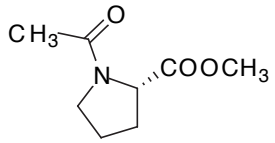
I. *Fmoc-Phe-Pro-NHMe*. *N*-Hydroxysuccinimide (150 mg, 1.29 mmol, 1.0 equiv.), followed by DCC (265 mg, 1.29 mmol, 1.0 equiv.) was added to a solution of Fmoc-Phe-OH (500 mg, 1.29 mmol, 1.0 equiv.) in CH_2Cl_2 (15 mL) at 0 °C under N_2. The solution was stirred at 0 °C for 20 min and then warmed to room temperature and stirred for a further 4 h. The suspension was filtered through a plug of cotton in a Pasteur pipette. The filtrate was concentrated to 4 mL and then refrigerated for 2 h. The suspension was filtered again and the residue evaporated to give a colorless foam which was dissolved in DMF (7.5 mL) and cooled to 0 °C under N_2. *L*-Proline (150 mg, 1.29 mmol, 1.0 equiv.) was added as a

solid in one portion, followed by the dropwise addition of diisopropylethylamine (225 μL, 165 mg, 1.29 mmol, 1.0 equiv.). The solution was gradually warmed to room temperature and left to stir overnight. The mixture was diluted with ethyl acetate (70 mL) and washed with 2M HCl (70 mL). The acidic aqueous layer was back-extracted with ethyl acetate (70 mL). The organic layers were combined, washed with water (140 mL) and brine (140 mL), filtered through $MgSO_4$ and concentrated to give Fmoc-Phe-Pro-OH. This residue was dissolved in CH_2Cl_2 (15 mL) and cooled to 0 °C under N_2. Methylamine hydrochloride (87 mg, 1.29 mmol, 1.0 equiv.) was added, followed by triethylamine (430 μL, 313 mg, 3.09 mmol, 2.4 equiv.) and finally BOP reagent (571 mg, 1.29 mmol, 1.0 equiv). The mixture was stirred at room temperature for 16 h and then diluted with CH_2Cl_2 (100 mL), washed with 2M HCl (100 mL), water (100 mL) and brine (100 mL), filtered through $MgSO_4$ and concentrated. The product was isolated by flash chromatography, eluting with 2% MeOH in EtOAc to give *Fmoc-Phe-Pro-NHMe* (375 mg, 58%). R_f 0.73 (9:1 CH_2Cl_2-MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.68-1.98 (m, 3H), 2.28-2.38 (m, 1H), 2.75 (d, J = 4.7 Hz, 3H), 2.98-3.08 (m, 2H), 3.42-3.66 (m, 2H), 4.20 (t, J = 7.0 Hz, 1H), 4.32 (dd, J = 10.5, 7.1 Hz, 1H), 4.42 (dd, J = 10.5, 7.5 Hz, 1H), 4.54 (dd, J = 7.1, 1.8 Hz, 1H), 4.76 (dd, J = 14.5, 7.6 Hz, 1H), 5.73 (d, J = 8.6 Hz, 1H), 6.44 (br s, 1H), 7.18-7.33 (m, 7H), 7.31 (t, J = 7.5 Hz, 2H), 7.58 (m, 2H), 7.77 (d, J = 7.5 Hz, 2H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.9, 26.2, 27.1, 39.1, 47.0, 47.4, 53.5, 59.9, 67.0, 120.0, 125.0, 125.1, 127.0, 127.2, 127.7, 128.5, 129.3, 135.6, 141.2, 143.7, 155.6, 170.9, 171.4; HRMS (FAB, NBA, MeOH) calcd for $(MH)^+$ $C_{30}H_{32}N_3O_4$: 498.2393; obsd 498.2389.

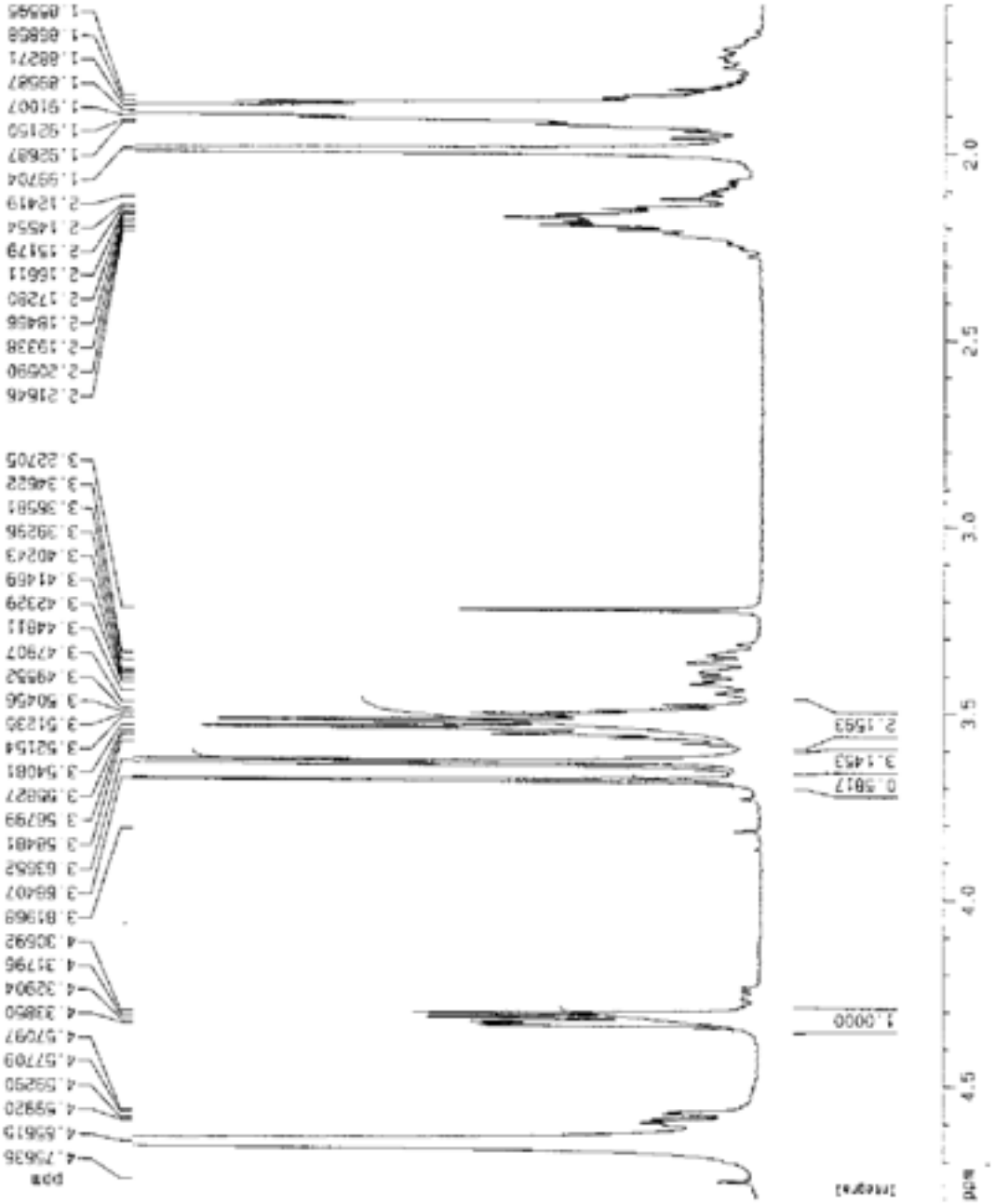
II. *Ac-Phe-Pro-NHMe* **(6).** Diethylamine (2 mL) was added to a solution of Fmoc-Phe-Pro-NHMe (115 mg, 0.230 mmol, 1.0 equiv.) in acetonitrile (15 mL). The solution was stirred at room temperature for 1 h, concentrated and then concentrated twice more from acetonitrile. The residue was suspended in CH_2Cl_2 (10 mL). Triethylamine (153 μL, 111 mg, 2.0 equiv.) was added, followed by acetyl chloride (41 μl, 45 mg, 1.05 equiv.). The solution was stirred at room temperature for 16 h, then evaporated. The residue was filtered through a plug of silica gel washing with ethyl acetate (200 mL), followed by a mixture of 9:1 EtOAc-methanol (300 mL). The latter solution was concentrated to give a colorless foam (170 mg) which was further purified by RP-HPLC to give *Ac-Phe-Pro-NHMe* (**6**) (73 mg; 42%). R_f

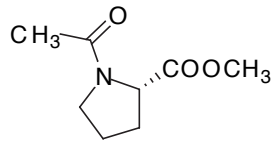
0.69 (95:5 CH_2Cl_2-MeOH); ^{1}H NMR (D_2O, 400 MHz, $K_{t/c}$ = 2.10 at 298K) δ 1.33-1.58 (m, 3H^{cis}, Proβ'^{cis}, Proγ'^{cis}, Proγ^{cis}), 1.63-1.84 (m, 3H^{trans}/1H^{cis}, Proβ'^{trans}, Proγ'^{trans}, Proγ^{trans}, Proβ^{cis}), 1.80 (s, 3H^{trans}, Ac^{trans}), 1.85 (s, 3H^{cis}, Ac^{cis}), 1.99-2.12 (m, 1H^{trans}, Proβ^{trans}), 2.57 (s, 3H^{cis}, NHMe^{cis}), 2.59 (s, 3H^{trans}, NHMe^{trans}), 2.78 (dd, *J* = 14.0, 8.5 Hz, 1H^{trans}, Pheβ'^{trans}), 2.80 (dd, *J* = 12.9, 9.1 Hz, 1H^{cis}, Pheβ'^{cis}), 2.85 (dd, *J* = 12.9, 6.6 Hz, 1H^{cis}, Pheβ^{cis}), 3.00 (dd, *J* = 14.0, 6.1 Hz, 1H^{trans}, Pheβ^{trans}), 3.17 (dt, *J* = 11.7, 8.1 Hz, 1H^{cis}, Proδ'^{cis}), 3.31-3.42 (m, 1H^{cis}, Proδ^{cis}), 3.36 (dd, *J* = 10.0, 6.5 Hz, 1H^{trans}, Proδ'^{trans}), 3.47 (d, *J* = 6.0 Hz, 1H^{cis}, Proα^{cis}), 3.65 (dt, *J* = 10.0, 6.8 Hz, 1H^{trans}, Proδ^{trans}), 4.38 (dd, *J* = 9.1, 6.6 Hz, 1H^{cis}, Proα^{cis}), 4.70 (dd, *J* = 8.5, 6.1 Hz, 1H^{trans}, Pheα^{trans}), 5.20 (dd, *J* = 8.6, 5.1 Hz, 1H^{trans}, Pheα^{trans}), 7.01-7.23 (m, 5H, Ar); ^{13}C NMR (100 MHz, D_2O) δ 24.2 (24.3), 27.3 (24.4), 28.7 (28.8), 32.0 (33.8), 39.2 (41.0), 50.7 (49.8), 55.8 (55.8), 63.7 (63.5), 130.0 (130.3), 131.5, 132.0, (131.7, 132.0), 139.1 (138.3), 174.7 (174.9), 176.3, 177.1, (175.6, 176.2); HRMS (FAB$^+$, NBA, MeOH) calcd for $(MH)^+$ $C_{17}H_{24}O_3N_3$ $(MH)^+$: 318.1818; obsd 318.1811.

^{1}H and ^{13}C NMR spectra

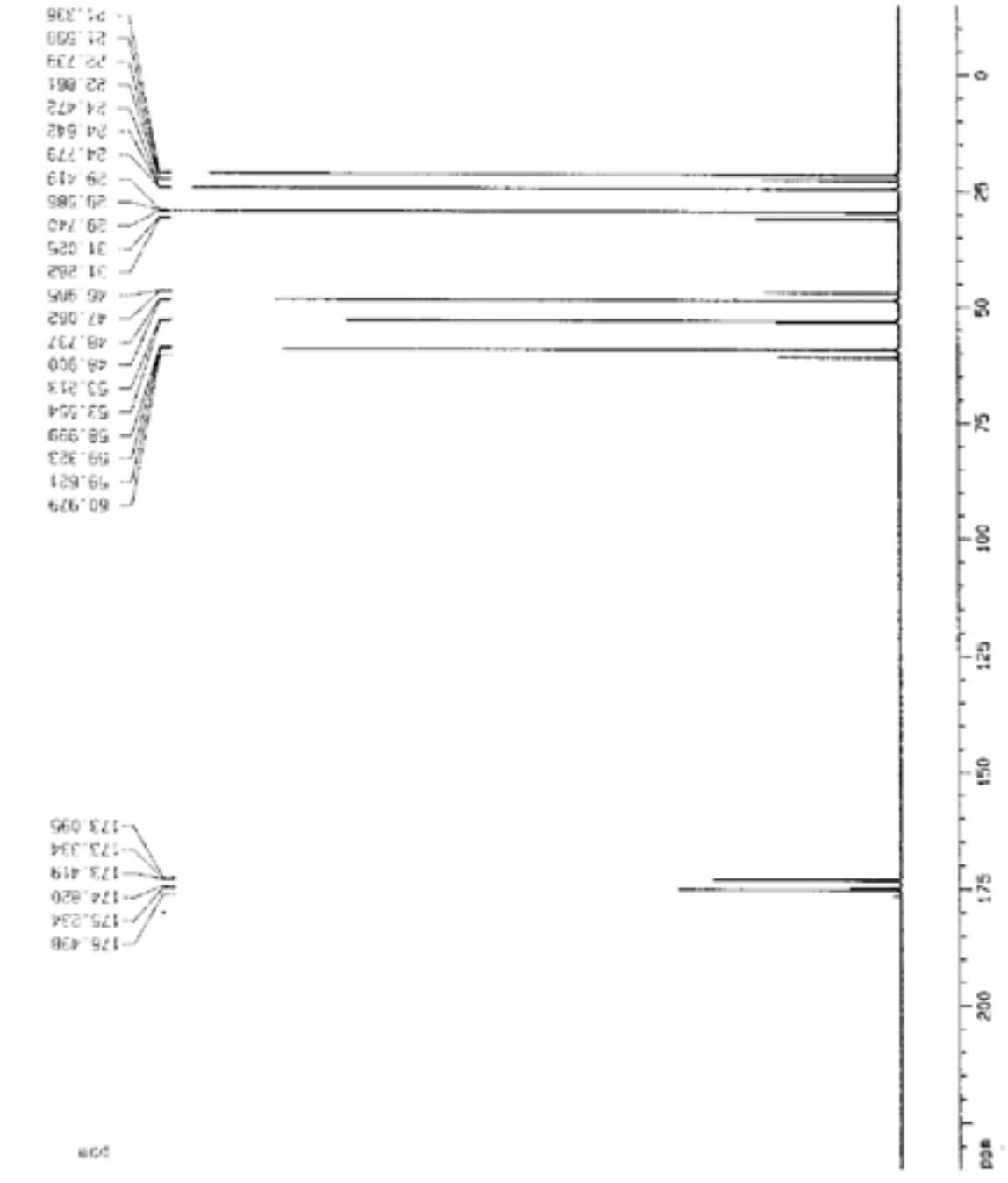


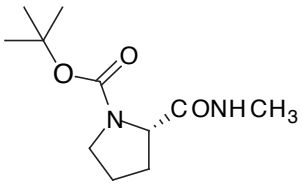
Compound **1**
^{1}H NMR spectrum at 400 MHz in D_2O



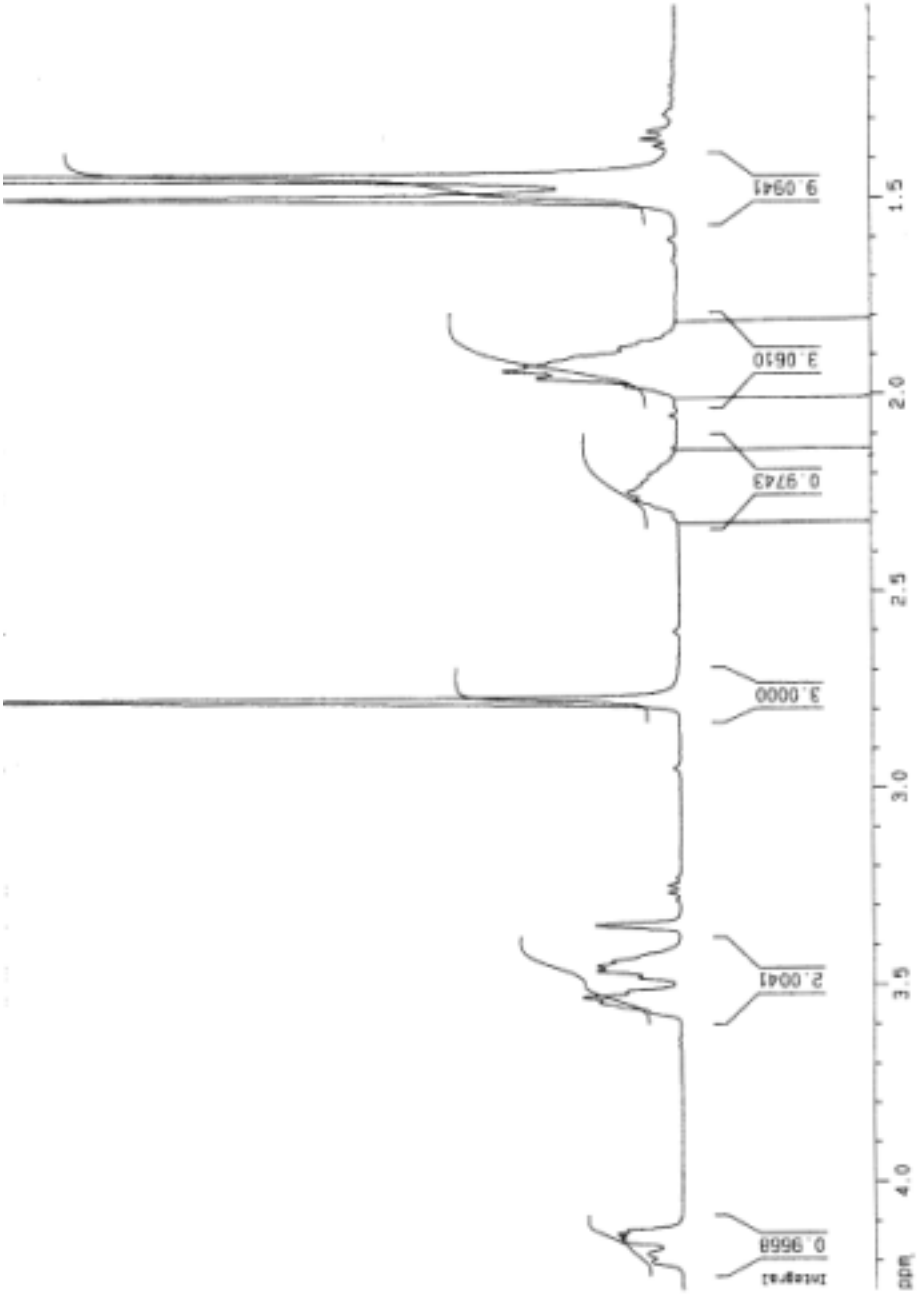


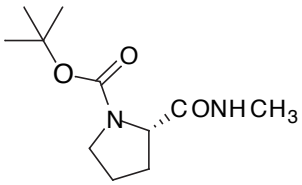
Compound **1**
^{13}C NMR spectrum at 100 MHz in D_2O





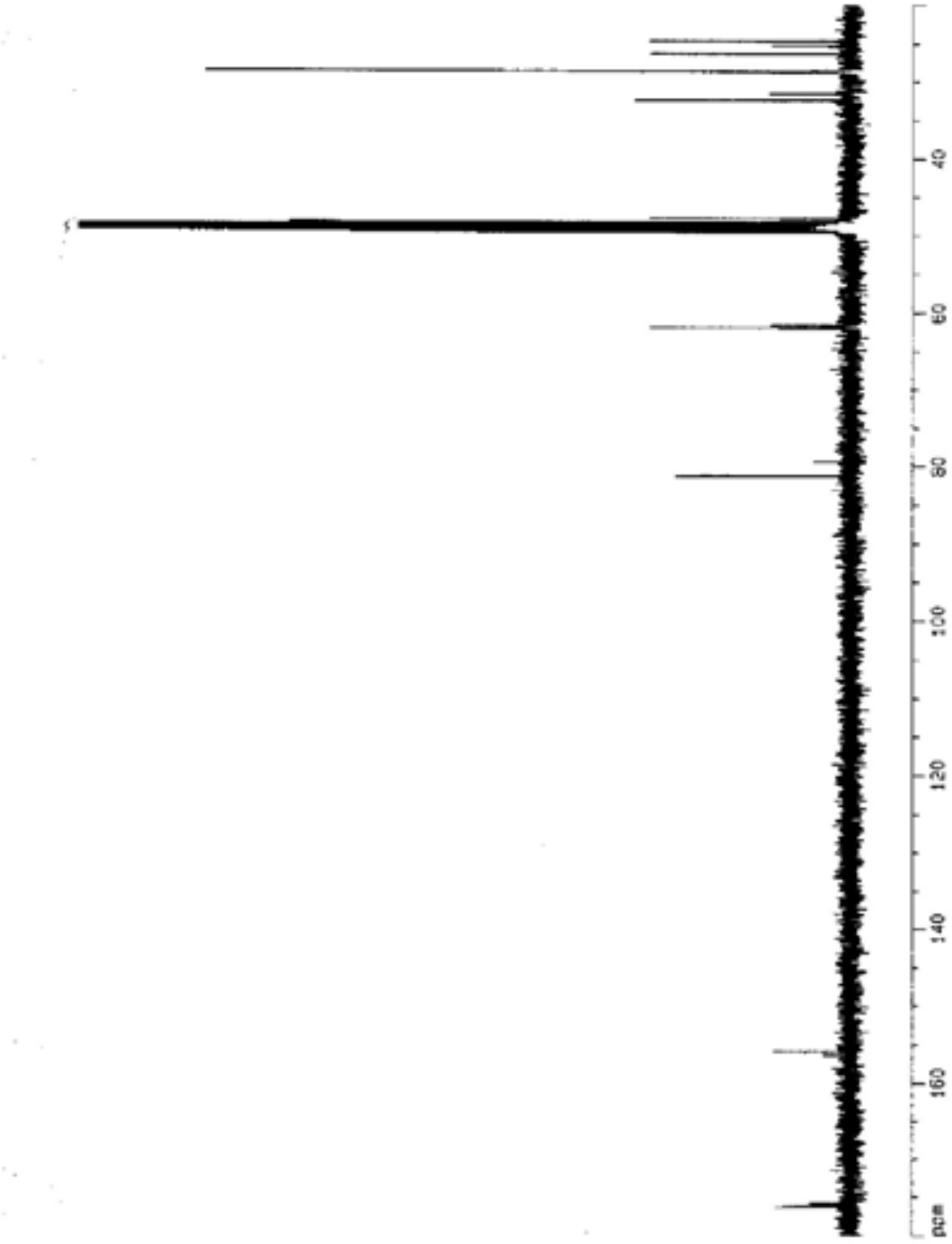
Boc-Pro-NHMe
^{1}H NMR spectrum at 400 MHz in CD_3OD

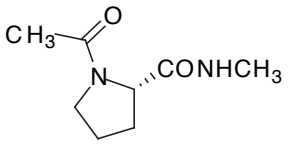




Boc-Pro-NHMe

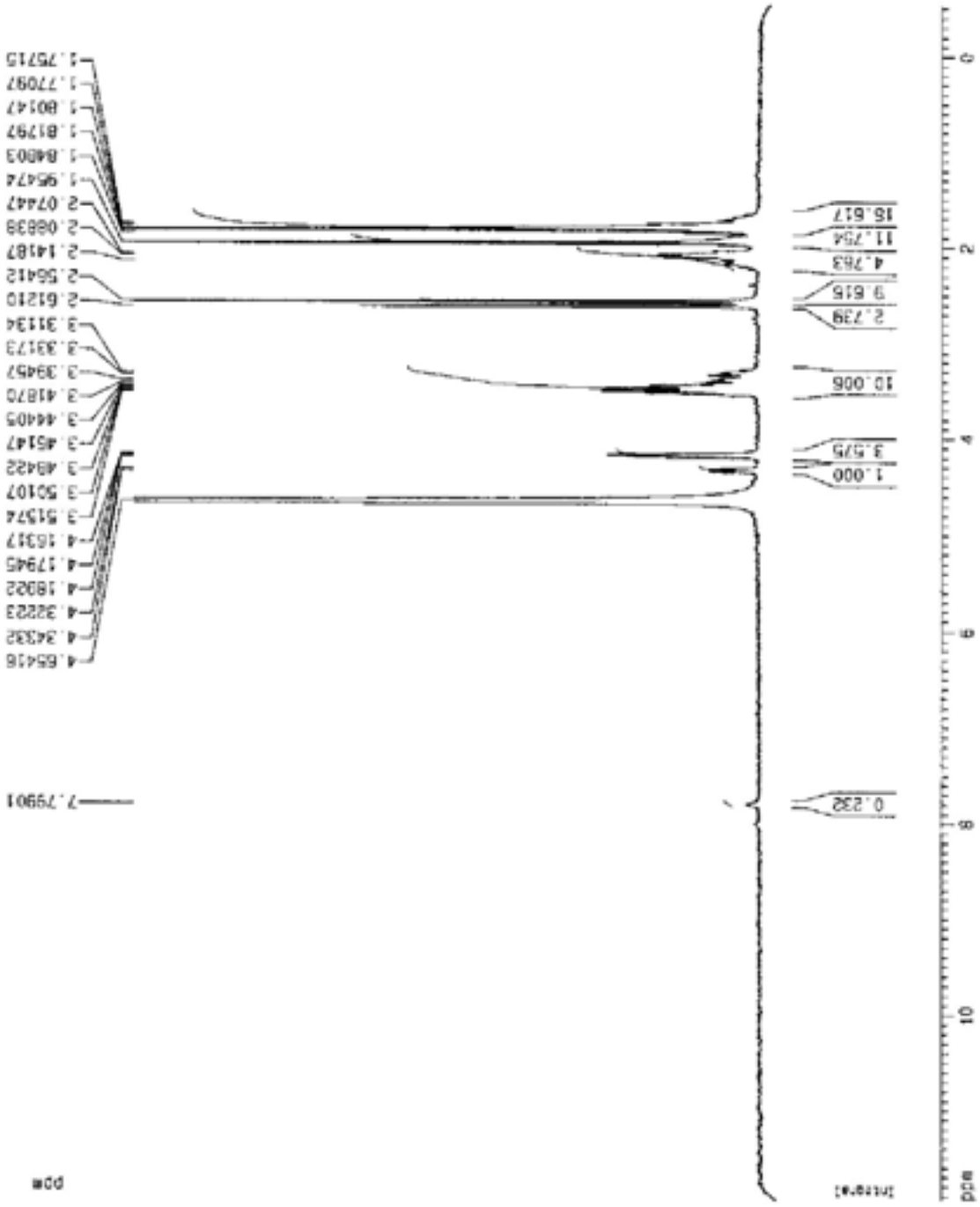
^{13}C NMR spesctrum at 100 MHz in $CDCl_3$

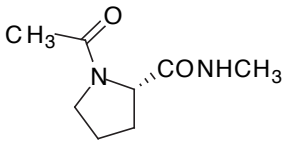




Compound **2**

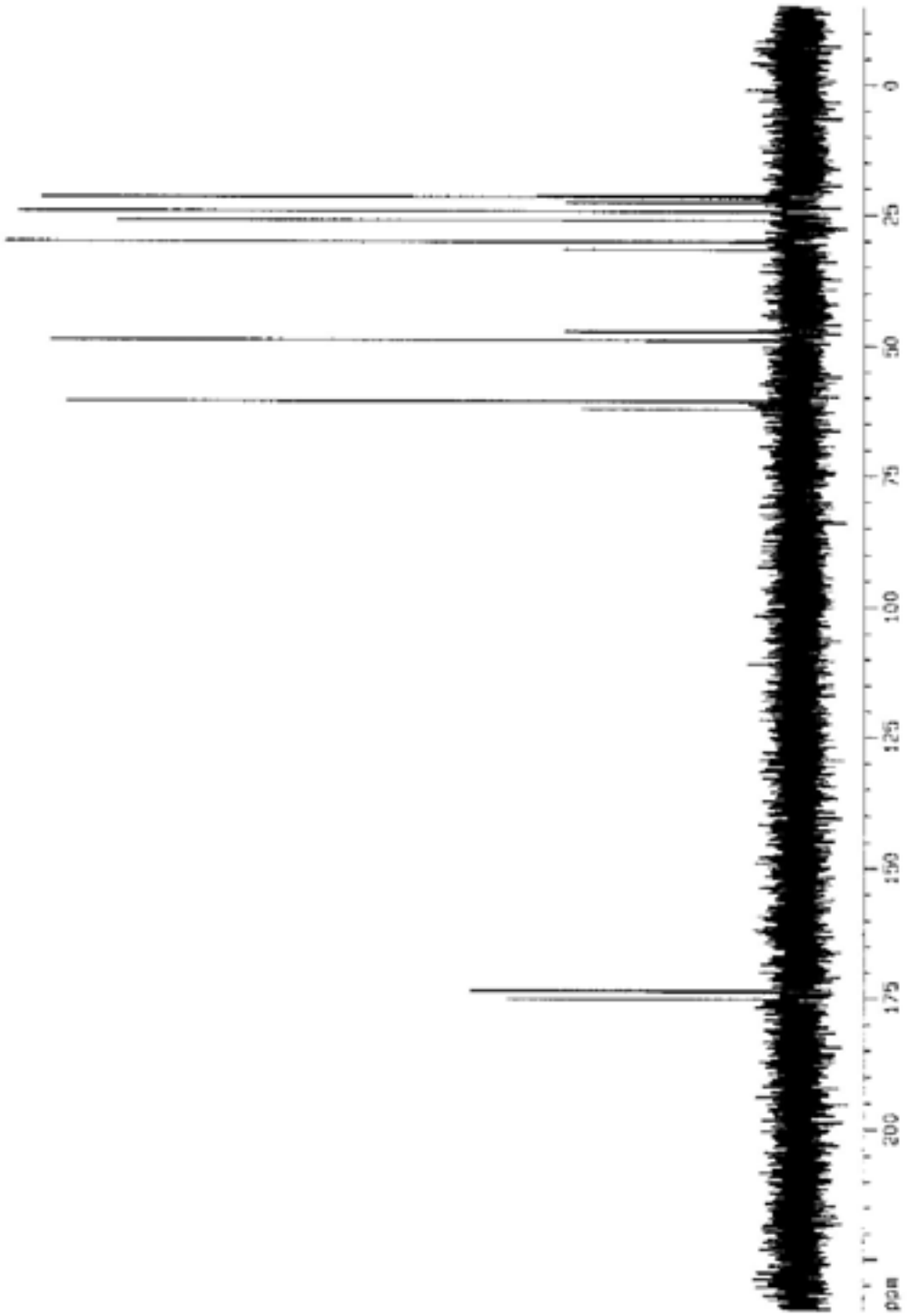
^{1}H NMR spectrum at 400 MHz in D_2O

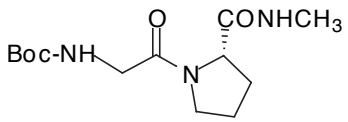




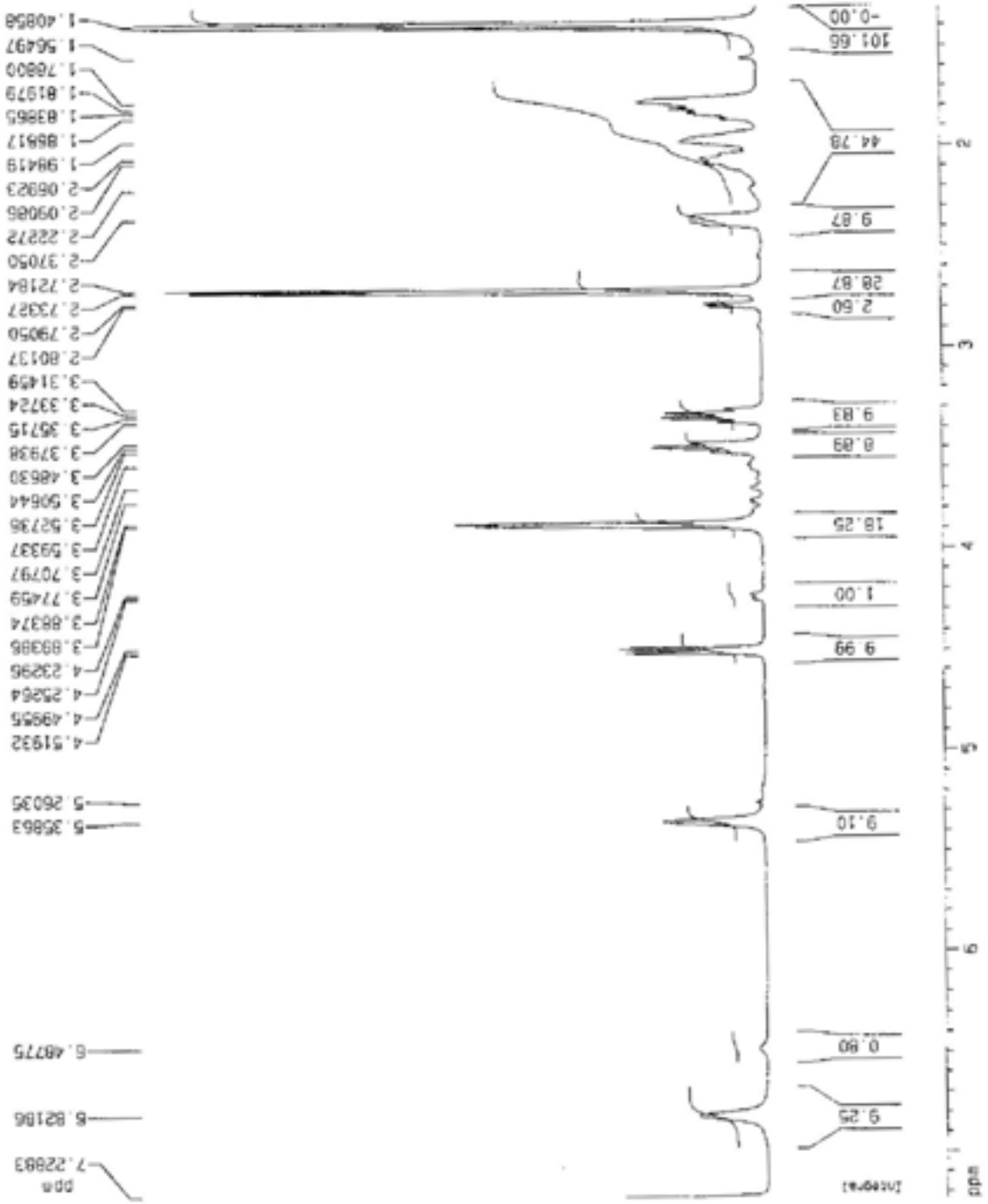
Compound **2**

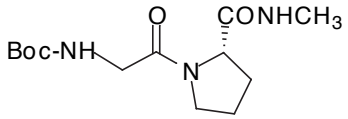
^{13}C NMR spectrum at 100 MHz in D_2O



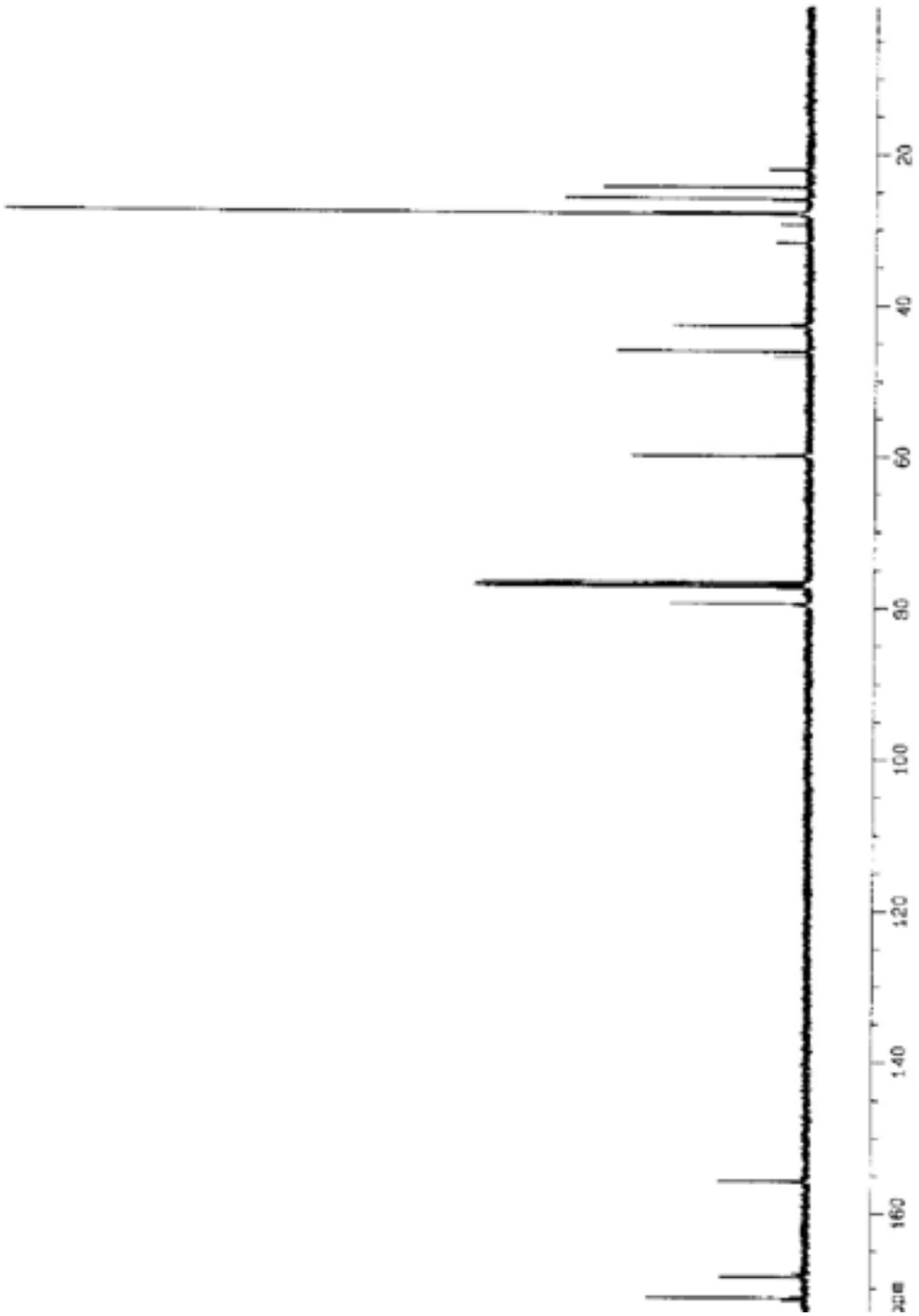


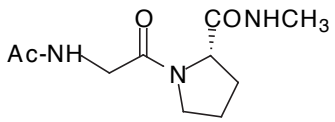
Boc-Gly-Pro-NHMe
^{1}H NMR spectrum at 400 MHz in $CDCl_3$



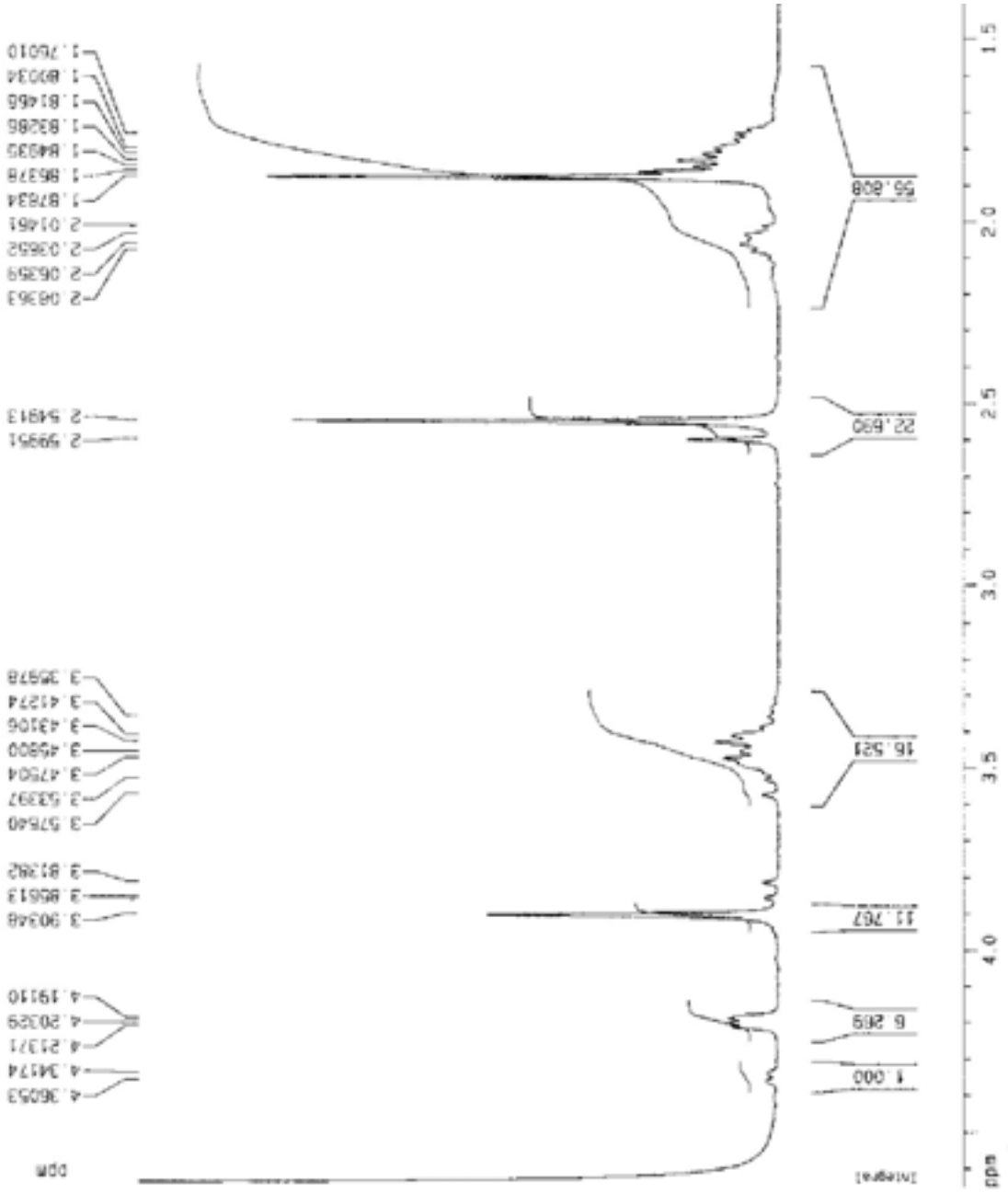


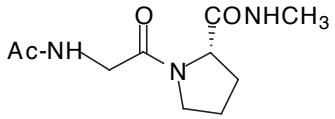
Boc-Gly-Pro-NHMe
^{13}C NMR spectrum at 100 MHz in $CDCl_3$





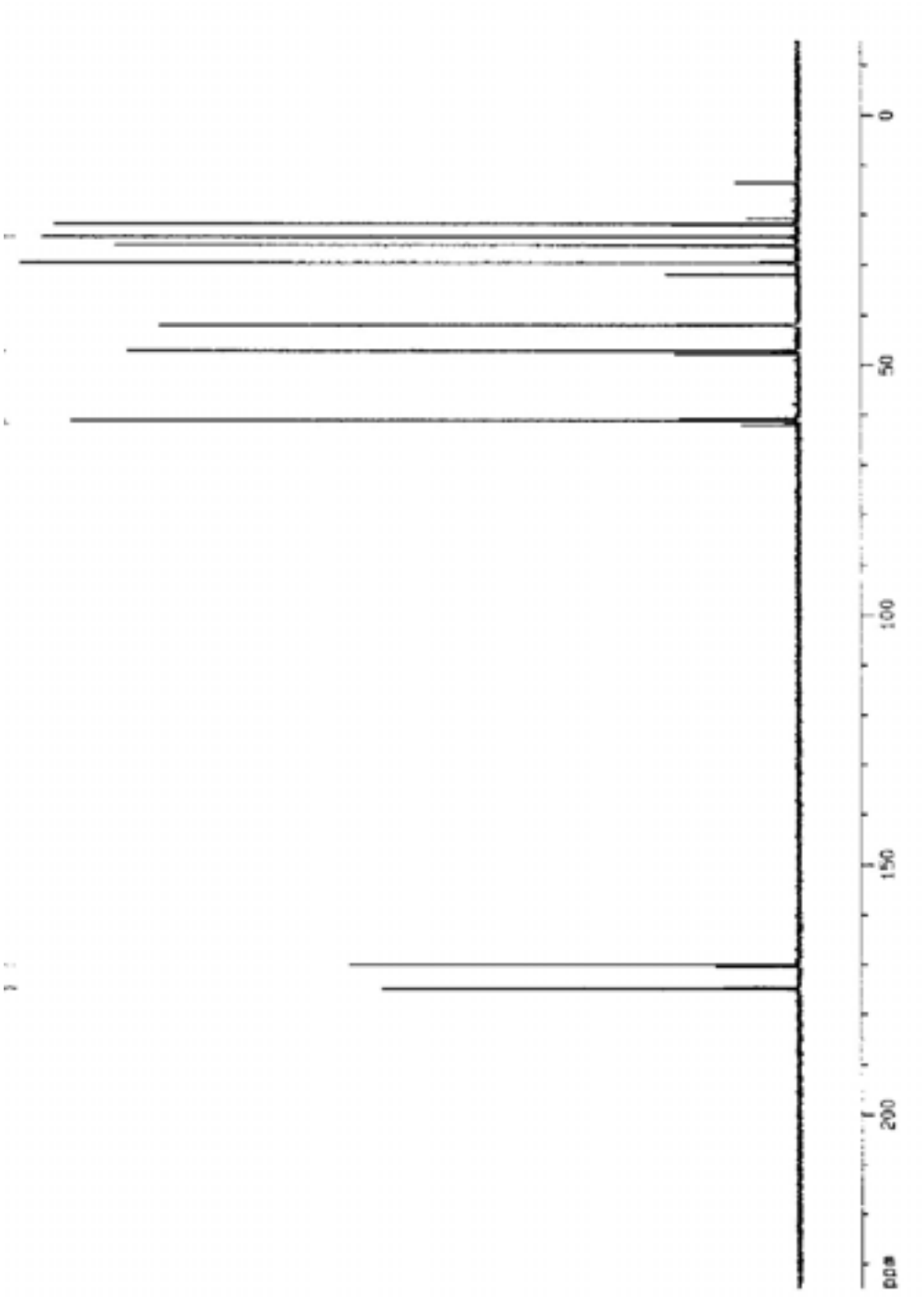
Compound **4**
^{1}H NMR spectrum at 400 MHz in D_2O

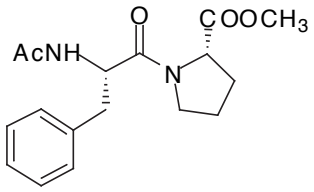




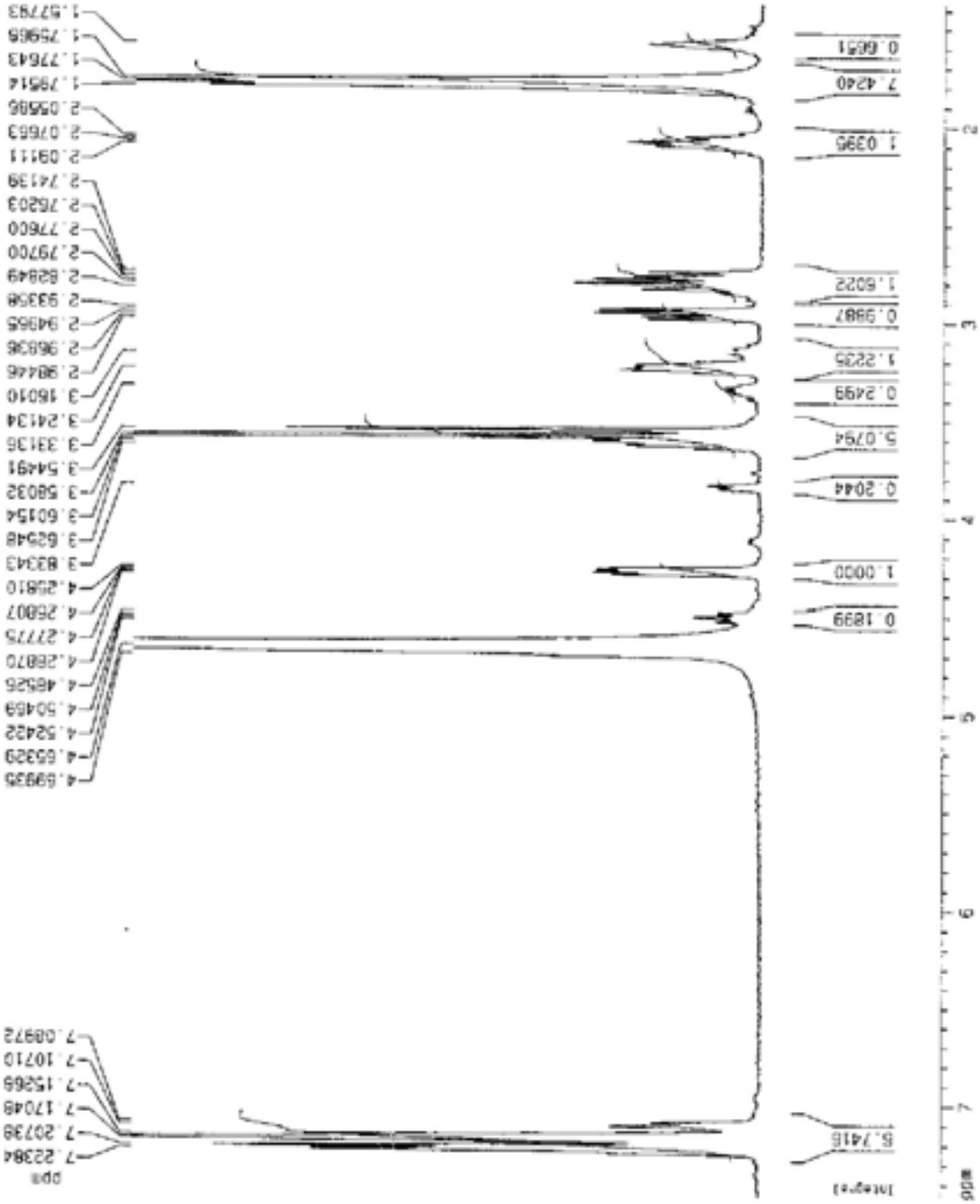
Compound **4**

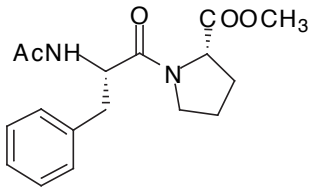
^{13}C NMR spectrum at 100 MHz in D_2O



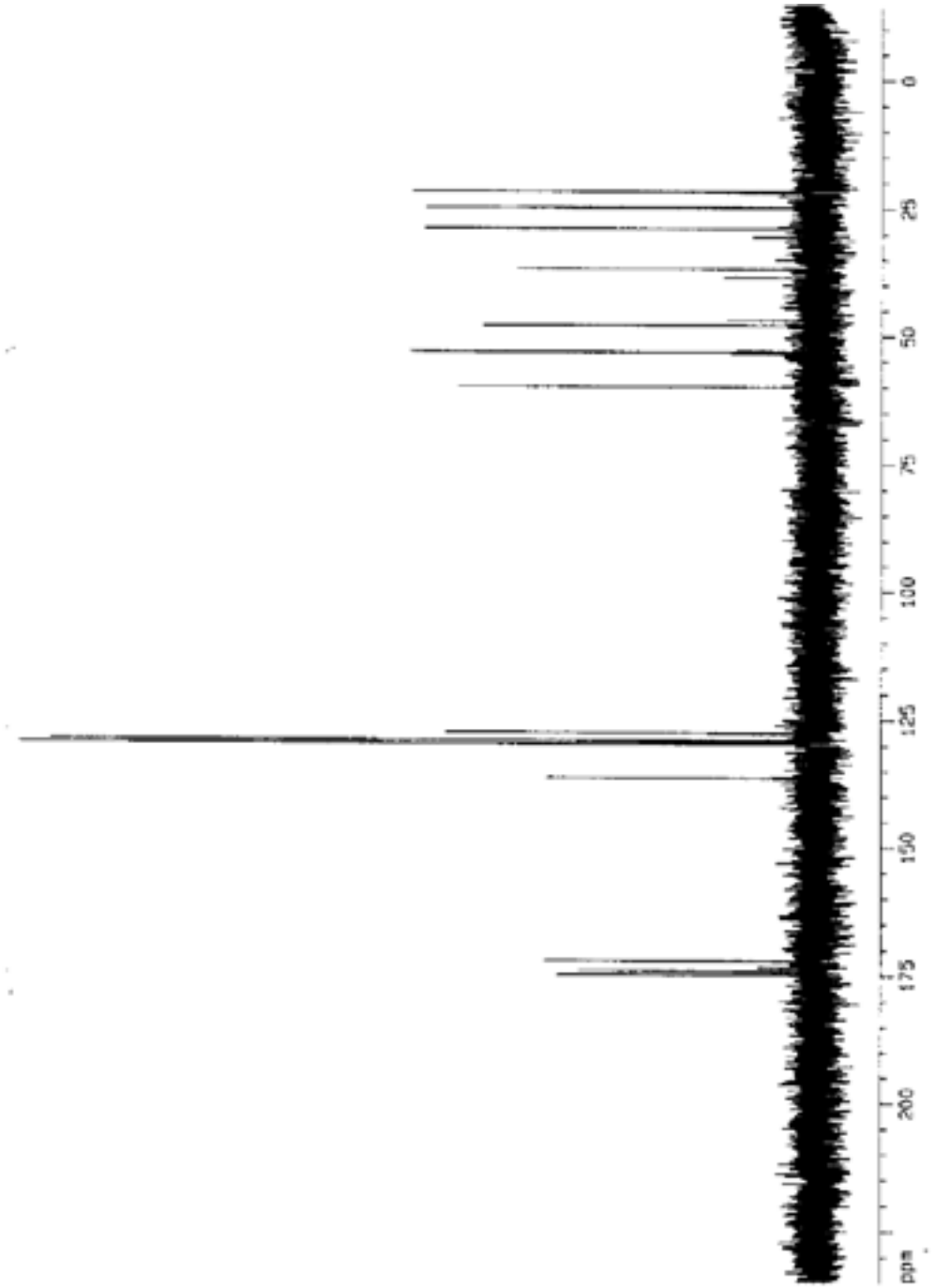


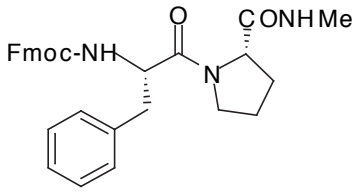
Compound **5**
^{1}H NMR at 400 MHz (D_2O)



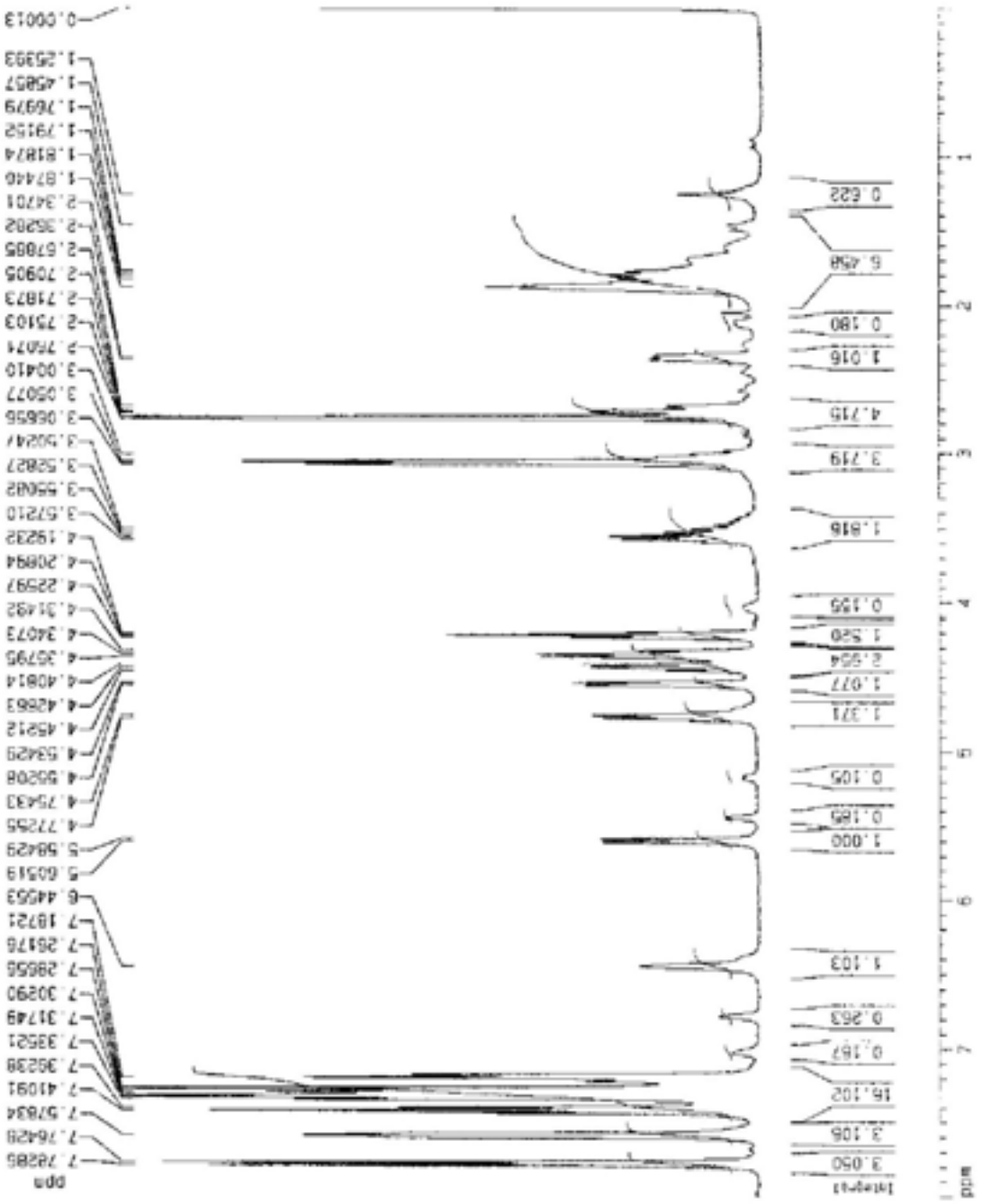


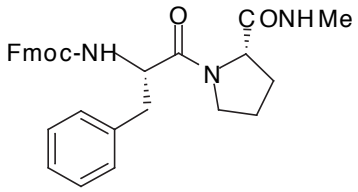
Compound **5**
^{13}C NMR at 100 MHz (D_2O)



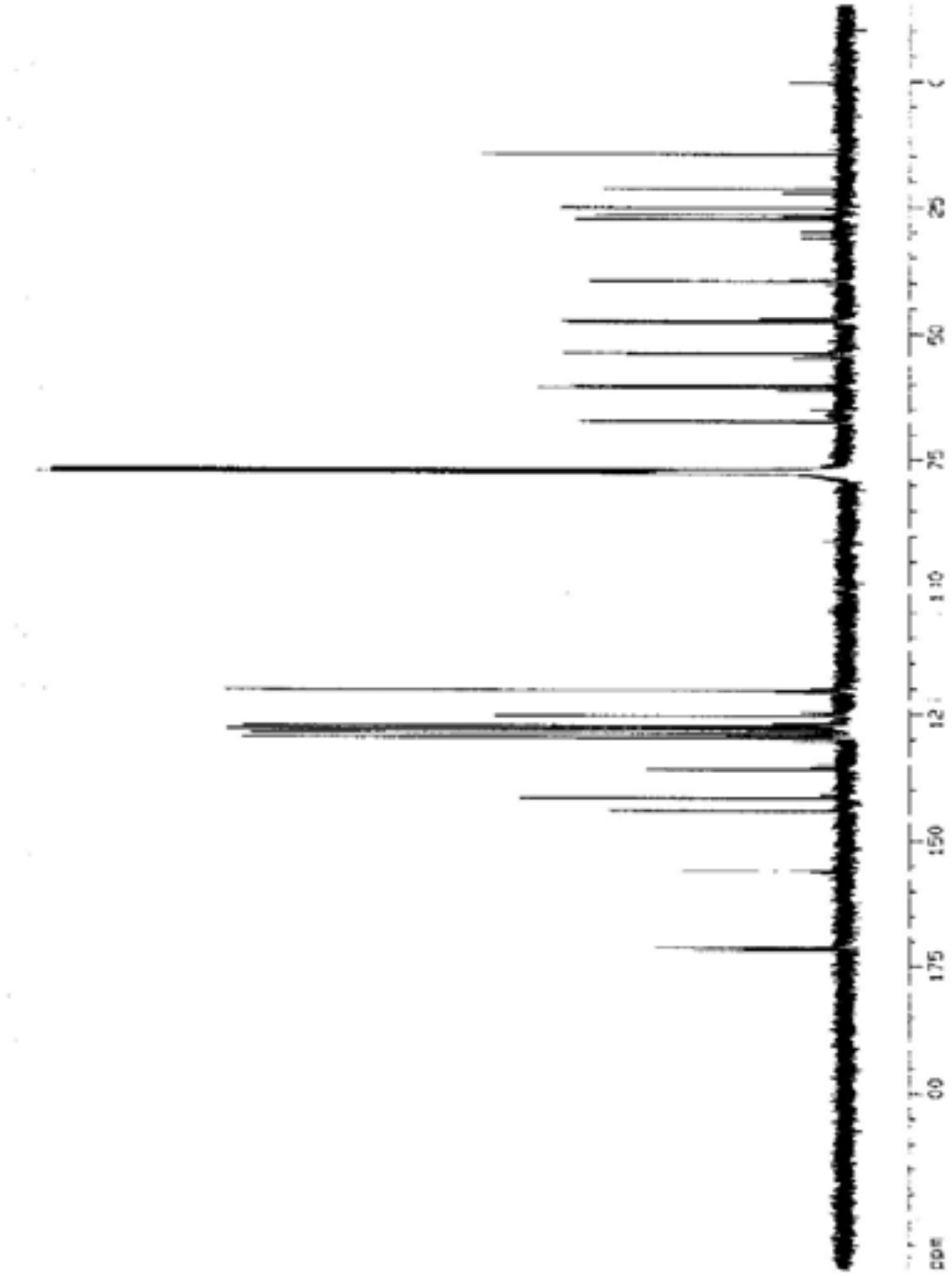


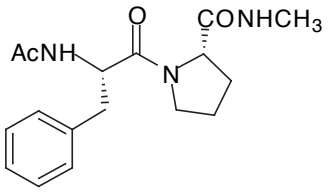
Fmoc-Phe-Pro-NHMe
^{1}H NMR spectrum at 400 MHz in $CDCl_3$



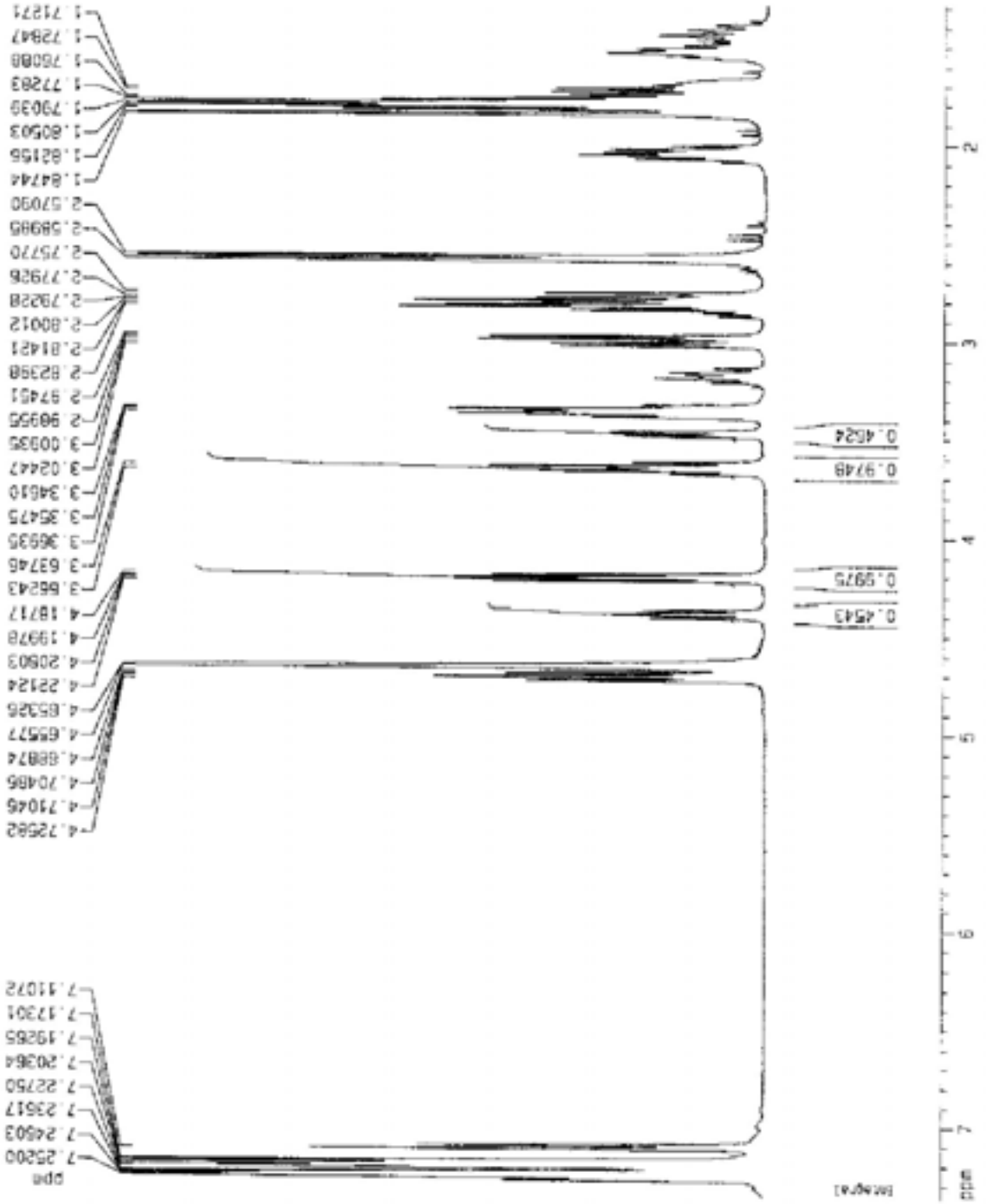


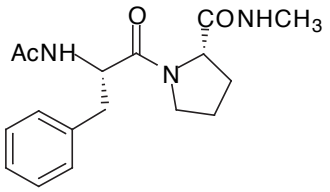
Fmoc-Phe-Pro-NHMe
^{13}C NMR spectrum at 100 MHz in $CDCl_3$



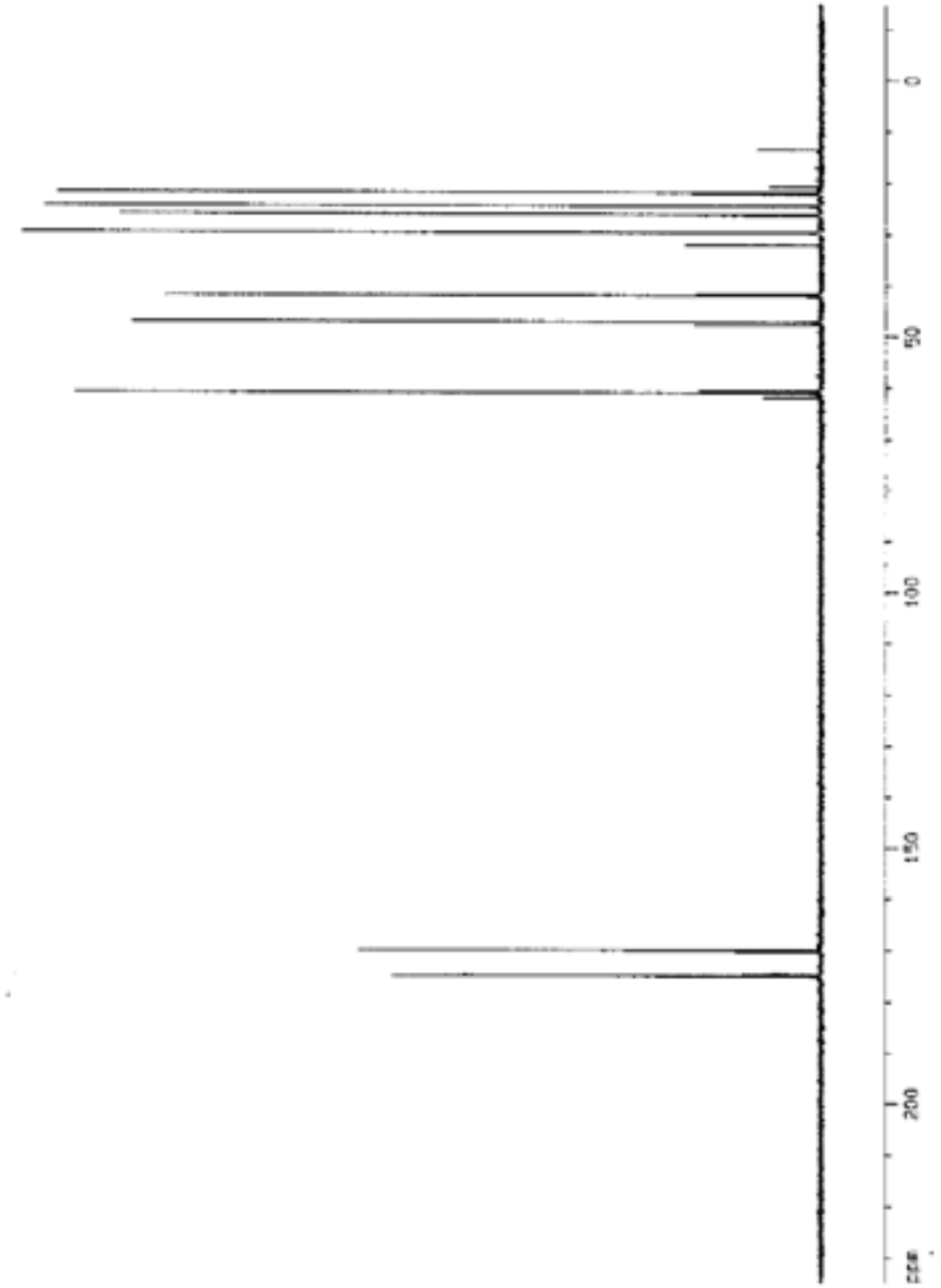


Compound **6**
^{1}H NMR at 400 MHz in D_2O





Compound **6**
^{13}C NMR at 100 MHz in D_2O



*ln*K values for Compounds at Various Temperatures

Temperature (° C)	80	75	70	65	60	55	50	45	40	35	30	25
Temperature (K)	353	348	343	338	333	328	323	318	313	308	303	298
1/T x 10^{-3} (K^{-1})	2.83	2.87	2.92	2.96	3.00	3.05	3.10	3.14	3.19	3.25	3.30	3.36
Ac-Pro-OMe	1.369	1.345	1.386	1.396	1.430	1.440	1.459	1.515	1.530	1.627	1.593	1.686
Ac_5Pro-NHMe	0.755	0.657	0.799	0.760	0.807	0.759	0.820	0.812	0.946	0.938	0.989	0.940
Ac-Gly-Pro-OMe*	1.68	1.72	1.74	-	-	1.81	1.84	1.89	-	-	-	-
Ac-Gly-Pro-NHMe	1.482	1.481	1.517	-	1.536	-	-	1.485	1.665	1.67	1.722	1.712
Ac-Phe-Pro-OMe	1.371	1.388	1.370	1.371	1.415	1.381	1.396	1.407	1.428	1.441	1.430	1.434
Ac-Phe-Pro-NHMe	0.798	0.765	0.788	0.829	0.811	0.806	0.765	0.765	0.788	0.728	0.732	-

*Estimated from: Eberhardt, E. S.; Loh, S. N.; Raines, R. T. *Tetrahedron Lett.* **1993**, *34*, 3055-3056.
(the compound was ^{13}C-labelled in the β and δ positions)

Van't Hoff Plots

Compound	Equation	Slope	Intercept	ΔH kJ/mol	ΔS J/K mol
Ac-Pro-OMe	y = - 0.31831 + 588.59x R^2 = 0.946	588.89	-0.31831	-4.89	-2.6
Ac-Pro-NHMe	y = - 0.67259 + 491.98x R^2 = 0.793	491.98	-0.67259	-4.09	-5.6
Ac-Gly-Pro-NHMe	y = 0.20119 + 450.22x R^2 = 0.790	450.22	0.20119	-3.74	1.7
Ac-Phe-Pro-OMe	y = 0.99215 + 126.90x R^2 = 0.205	126.9	0.99215	-1.06	8.2
Ac-Phe-Pro-NHMe	y = 1.1552 - 123.89x R^2 = 0.400	-123.89	1.1552	1.03	9.6

THERMODYNAMIC PARAMETERS FOR COMPOUNDS 1-7.

Compound	Number	$K_{t/c}$ D_2O, 298 K	ΔG, 298K (kcal mol^{-1} K^{-1})	ΔH (kcal mol^{-1})	ΔS (cal mol^{-1} K^{-1})
Ac-Pro-OMe	**1**	5.40[a]	-0.99	-1.17[b]	-0.62[c]
Ac-Pro-NHMe	**2**	2.56	-0.57	-0.97	-1.34
Ac-Gly-Pro-OMe	**3**	~7.2[d]	-1.20[d]	-1.27[d]	-0.25[d]
Ac-Gly-Pro-NHMe	**4**	5.54	-1.01	-0.89	+0.41
Ac-Phe-Pro-OMe	**5**	4.2	-0.83	-0.25	+1.96
Ac-Phe-Pro-NHMe	**6**	2.1	-0.43	+0.25	+2.29
Ac-Gly-Phe-Pro-Gly-NH_2	**7**	4.8[e]	-0.93[e]	-0.53[e]	-1.4[e]

[a]Lit. $K_{t/c}$ = 4.6 in D_2O at 25 °C [Bretscher, L. E.; Jenkins, C. L.; Taylor, K. M.; DeRider, M. L.; Raines, R. T. *J. Am. Chem. Soc.* **2001**, *123*, 777-778]; [b]Lit. ΔH = -1.04 ± 0.02 kcal mol^{-1} [Eberhardt, E. S.; Panasik, N., Jr.; Raines, R. T. *J. Am. Chem. Soc.* **1996**, *118*, 12261-12266]; [c]Lit ΔS = -0.46 ± 0.05 cal mol^{-1} K^{-1} [Eberhardt, E. S.; Panasik, N., Jr.; Raines, R. T. *J. Am. Chem. Soc.* **1996**, *118*, 12261-12266]; [d]Results taken from: Eberhardt, E. S.; Loh, S. N.; Raines, R. T. *Tetrahedron Lett.* **1993**, *34*, 3055-3056; [e]Results taken from: Wu, W.-J.; Raleigh, D. P. *Biopolymers* **1998**, *45*, 381-394.