Supporting Information

Oxidative damage of proline residues by nitrate radicals ($NO_3^{\bullet}$): A kinetic and product study

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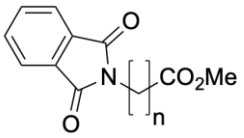
Contents

1. Supplementary Table

Table S1. Absolute second-order rate coefficients k for the reaction of $NO_3^\bullet$ with N-phthaloylated amino acid methyl esters.[a,b]



n = 1: Phth-Gly-OMe
n = 2: Phth-βAla-OMe
n = 3: Phth-Gaba-OMe
n = 4: Phth-Ava-OMe
n = 5: Phth-Ahx-OMe

Substrate	k / $\times 10^6$ M^{-1} s^{-1}
Phth-Gly-OMe	0.6[c]
Phth-βAla-OMe	0.7
Phth-Gaba-OMe	1.4
Phth-Ava-OMe	1.4
Phth-Ahx-OMe	1.6

[a]In acetonitrile, at 298 $\pm$ 1 K. [b]Experimental error $\pm$15%.
[c]From ref. [11] in the manuscript.

Synthetic procedures, spectroscopic details and kinetic plots for these compounds are provided in the sections below.

2. General Experimental Procedures

All amino acid, di- and tripeptide derivatives were prepared as enantiomerically pure compounds. The starting materials for the synthesis were purchased from commercial suppliers (Sigma-Aldrich Australia, Chem-Supply Australia, AK Scientific USA or Chem-Impex USA) and used without purification. Thin layer chromatography (TLC) was performed to monitor the reactions using aluminium plates coated with silica gel 60 F_{254} (Merck) and visualized with UV light at 254 nm or stained with phosphomolybdic acid (PMA), ninhydrin or $KMnO_4$ stain followed by heating. The crude products were purified by recrystallization from hot solvent or silica column chromatography with approximately 30–50 g of dry silica (Davisil Chromatography Silica Gel LC60A, 40–60 micron, 230–400 mesh) per 1 g of the crude product mixture. The eluting solvent consisted of a mixture of either petroleum ether and ethyl acetate or chloroform and methanol. Solvents were removed under reduced pressure and elevated temperature using a rotary evaporator (Büchi). The purity was assessed by analytical reversed-phase HPLC on an Alltech Hypersil BDS-C18 5 µm 150 x 4.6 mm (Gradient: 100% water buffered with 0.1% TFA to 100% acetonitrile buffered with 0.1% TFA over 25 minutes, 4% min^{-1}, flow rate: 1 mL min^{-1}).

^{1}H and ^{13}C spectra were recorded on an Agilent MR 400 MHz NMR spectrometer, an Agilent DD2 500 MHz NMR spectrometer or a Varian Inova 600 MHz NMR spectrometer in deuterated dimethylsulfoxide (DMSO-d_6) at 25.0 °C. Chemical shifts are reported in ppm (δ) using respective residual solvents as the reference (DMSO: δ = 2.50 ppm for ^{1}H NMR, δ = 39.5 ppm for ^{13}C NMR). ^{1}H NMR data are reported as follows: chemical shift (δ), multiplicity, coupling constants (in Hz, if any) and relative integral. High Resolution Mass Spectrometry (HRMS) was performed by ionising the sample using Electrospray Ionization (ESI) into a Thermo Scientific Exactive Plus Orbitrap mass spectrometer. Parent ions are denoted by $[M + H]^+$ or $[M + Na]^+$, and in the case of salts, they are $[M – Cl]^+$ or $[M – C_2O_2F_3]^+$. Melting points were recorded with EZ-Melt Automated Melting Point Apparatus MPA120 from Stanford Research Systems.

Spectroscopic details are provided for all isolated intermediates and substrates used in the laser flash photolysis and product studies. Compounds for which no spectroscopic details are provided, were obtained from suppliers mentioned above. NMR spectra and HPLC chromatograms are provided for all substrates used in the laser flash photolysis and product studies. Compounds containing a tertiary amide moiety usually exist as *trans*- and *cis*-isomeric mixtures around the peptide bond. The appearance of these isomers in the NMR spectra has been well established in the literature in various solvent systems.[1-4] By analogy, all compounds with the tertiary amide moiety in this work also show both isomers in their ^{1}H NMR spectra, except for those where steric hindrance or ring strain disfavours one isomer such as in Ac-(Me)Aib-OMe, Ac-(*t*Bu)Gly-OMe, Me-Glp-OMe and Me(d_3)-Glp-OMe(d_3). Only chemical shifts for the major isomer (usually *s-trans*) are assigned. The ratio of isomers is included on top of the respective ^{1}H NMR spectrum.

GP1. General procedure for the N-acetylation of the amino acids[5]

The amino acid (53.4 mmol) was suspended in 5% aqueous sodium bicarbonate solution (150 mL) and cooled to 0 °C. Acetic anhydride (6.1 mL, 64.5 mmol) was added dropwise over a period of 1 hour. The resulting mixture was stirred at room temperature for 4 hours until starting material was fully consumed as monitored by TLC (9:1 EtOH/1 M AcOH, ninhydrin stain). The reaction mixture was then acidified to pH 2–3 with 6 M hydrochloric acid and cooled overnight. The resulting precipitate was collected by filtration, washed with cold water (2 x 10 mL) and dried to give the N-acetylated amino acid. In the absence of solid, the solvent was removed under reduced pressure and elevated temperature. Methanol (20 mL) was added and the solid was filtered off. The filtrate was concentrated under reduced pressure and elevated temperature to give a colourless oil. Toluene (10 mL) was repeatedly added and removed under reduced pressure and elevated temperature to get rid of residual acetic acid and give the N-acetylated amino acid.

GP2. General procedure for the C-methylation of the amino acids[5]

The amino acid (78.8 mmol) was suspended in methanol (250 mL) and cooled to 0 °C. Thionyl chloride (10 mL, 138 mmol) was added dropwise. The resulting mixture was stirred overnight at room temperature until starting material was fully consumed as monitored using TLC (9:1 EtOH/1 M AcOH, ninhydrin stain). The solvent was removed under reduced pressure and elevated temperature to give the hydrochloride salt of the amino acid methyl ester.

GP3. General procedure for the C-methylation of the N-protected amino acids[6]

The N-protected amino acid (12.0 mmol) was suspended in methanol (11 mL) and cooled to 0 °C. Thionyl chloride (1.0 mL, 13.8 mmol) was added dropwise. The resulting mixture was stirred for 4 hours until starting material was fully consumed as monitored using TLC (EtOAc, UV light or $KMnO_4$ stain). The solvent was removed under reduced pressure and elevated temperature. Water (20 mL) was then added and the product was extracted with dichloromethane (3 x 15 mL). The combined organic layers were dried over anhydrous sodium sulfate and filtered. The solvent was removed under reduced pressure and elevated temperature to give the N- and C-protected amino acid.

GP4. General procedure for the peptide coupling

Method (a):[7] The N-protected amino acid (3.09 mmol) and HOBt·H_2O (0.48 g, 3.12 mmol) were suspended in dichloromethane (10 mL) under inert atmosphere and cooled to 0 °C. Diisopropylethylamine (0.65 mL, 3.73 mmol), followed by HBTU (1.15 g, 3.04 mmol), was then added and the resulting mixture was stirred for 1 hour at room temperature. This solution was then added dropwise to an ice-cold solution of the hydrochloride salt of the amino acid methyl ester (3.28 mmol) and diisopropylethylamine (0.65 mL, 3.73 mmol) in dichloromethane (10 mL). The resulting mixture was stirred at 0 °C for 3 hours and quenched by the addition of 10% aqueous citric acid solution (15 mL). The mixture was filtered and the solid was washed with dichloromethane (30 mL). The filtrate was washed sequentially with 10% aqueous citric acid solution (15 mL), saturated aqueous sodium bicarbonate solution (15 mL) and brine (15 mL). The organic layer was dried over anhydrous sodium sulfate and

filtered. The solvent was removed under reduced pressure and elevated temperature. The crude product was purified by column chromatography to give the dipeptide.

Method (b):[8] The N-protected amino acid (10.1 mmol) was suspended in dichloromethane (20 mL) and cooled to −20 °C. *N*-Methylmorpholine (1.2 mL, 10.9 mmol), followed by isobutylchloroformate (1.5 mL, 11.6 mmol), was added dropwise. After 10 minutes, the hydrochloride salt of the amino acid methyl ester (10.1 mmol) was added, followed by *N*-methylmorpholine (1.2 mL, 10.9 mmol). The resulting mixture was stirred for 1 hour at room temperature and then washed sequentially with 0.2 M hydrochloric acid (2 x 20 mL), 1% aqueous sodium bicarbonate solution (2 x 20 mL) and brine (1 x 10 mL). The organic layer was dried over anhydrous sodium sulfate and filtered. The solvent was removed under reduced pressure and elevated temperature to give a yellow oil. Diethyl ether (20 mL) was added to the residue and the resulting precipitate was collected by filtration and washed with ice-cold diethyl ether (2 x 10 mL) to give the dipeptide.

Method (c):[9] The N-protected amino acid (5.73 mmol) was suspended in dichloromethane (15 mL) and cooled to 0 °C, followed by addition of HOBt·H_2O (1.32 g, 8.59 mmol) and EDC·HCl (1.65 g, 8.59 mmol). After 10 minutes, the hydrochloride salt of the amino acid methyl ester (6.30 mmol) was added, followed by dropwise addition of diisopropylethylamine (3.0 mL, 17.2 mmol). After the resulting mixture was stirred overnight at room temperature, saturated aqueous sodium bicarbonate solution (50 mL) was added. The aqueous layer was then extracted with dichloromethane (3 x 50 mL). The combined organic layers were dried over anhydrous sodium sulfate and filtered. The solvent was removed under reduced pressure and elevated temperature. The crude product was purified by column chromatography or by recrystallisation to give the dipeptide.

Method (d):[5] The N-protected amino acid (10.0 mmol), the hydrochloride salt of amino acid methyl ester (10.1 mmol) and HBTU (3.75 g, 9.9 mmol) were suspended in dimethylformamide (15 mL) and cooled to 0 °C. Triethylamine (4.2 mL, 30.0 mmol) was added dropwise and the resulting mixture was stirred overnight at room temperature. The mixture was then partitioned between 1 M hydrochloric acid (100 mL) and ethyl acetate (100 mL). The aqueous layer was extracted with ethyl acetate (2 x 100 mL), and the combined organic layers were washed sequentially with 5% aqueous sodium bicarbonate solution (100 mL) and brine (100 mL). The organic layer was dried over anhydrous magnesium sulfate and filtered. The solvent was removed under reduced pressure and elevated temperature. The crude product was purified by column chromatography or by recrystallisation to give the dipeptide.

GP5. General procedure for the N- and C-methylation of the amino acids[10]

The amino acid or the N-acetylated amino acid (20.1 mmol) was added to a suspension of sodium hydride (60% dispersion in mineral oil, 2.19 g, 54.8 mmol) in dimethylformamide (60 mL) under inert atmosphere at 0 °C. The resulting mixture was stirred for 1 hour at room temperature and cooled again to 0 °C, followed by dropwise addition of methyl iodide (3.45 mL, 55.4 mmol) or methyl(d_3) iodide (3.46 mL, 55.4 mmol). The resulting mixture was stirred overnight at room temperature. Nitrogen was blown over the mixture for 3 days to remove the solvent and water (75 mL) was then added. The aqueous layer was extracted with

dichloromethane (3 x 100 mL). The combined organic layers were washed sequentially with water (75 mL) and 5% aqueous lithium chloride solution (50 mL). The organic layer was dried over anhydrous magnesium sulfate and filtered. The solvent was removed under reduced pressure and elevated temperature to give a yellow liquid. The crude product was purified by column chromatography to give the N- and C-protected amino acid.

GP6. General procedure for the N-phthaloylation of the amino acids

(a) Synthesis of the phthaloylating agent:[11] Trimethyl phosphite (8 mL, 67.7 mmol) was added dropwise to 90% phthaloyl chloride (10 mL, 62.5 mmol) over 30 mins so that the temperature was kept below 50 °C. The mixture was stirred for a few hours and excess trimethyl phosphite was removed under reduced pressure and elevated temperature. The crude solid was washed with petroleum ether to give 3-chloro-3-(dimethoxyphosphoryl)isobenzofuran-1(3*H*)-one as a white solid (16.1 g, 86%). This acylphosphonate derivative was directly used in the next step to introduce the phthaloyl group in the amino acid.

(b) Synthesis of N-Phth protected amino acids:[12] The acylphosphonate derivative (1.38 g, 5.0 mmol) and the amino acid (5.3 mmol) were dissolved in a 1:1 acetonitrile/water mixture (25 mL). Diisopropylethylamine (3.5 mL, 20.1 mmol) was added dropwise and the mixture was stirred for 30 minutes at room temperature. Acetonitrile was removed under reduced pressure and elevated temperature. The remaining solution was acidified with 1 M hydrochloric acid dropwise. The resulting precipitate was collected by filtration and washed with 1 M cold hydrochloric acid to give the *N*-Phth protected amino acid.

GP7. General procedure for the N-Boc protection of the amino acids[13]

The amino acid (20.0 mmol) was suspended in a 1:1 water/1,4-dioxane mixture (25 mL). Sodium hydroxide (1.60 g, 40.0 mmol) was added at 0 °C and the resulting mixture was stirred for 5 minutes. Boc anhydride (5.46 g, 25.0 mmol) was then added in portions and the reaction mixture was stirred overnight at room temperature. 1,4-Dioxane was removed under reduced pressure and elevated temperature. The remaining solution was washed with diethyl ether (3 x 20 mL) and then slowly acidified with 1 M hydrochloric acid at 0 °C. The product was extracted with ethyl acetate (3 x 40 mL) and the combined organic layers were dried over anhydrous sulfate and filtered. The solvent was removed under reduced pressure and elevated temperature to give the *N*-Boc-protected amino acid.

GP8. General procedure for the N-Boc deprotection of the N-Boc protected peptide methyl esters[14]

The N-Boc protected peptide methyl ester (10.0 mmol, synthesised according to GP4) was dissolved in dichloromethane (8 mL) and cooled to 0 °C. Trifluoroacetic acid (8 mL, 104.5 mmol) was added dropwise. The resulting mixture was stirred for 4 hours at room temperature until starting material was fully consumed as monitored using TLC (EtOAc, ninhydrin stain). The solvent was removed under reduced pressure and elevated temperature to give a yellow oil. Toluene (10 mL) was repeatedly added and removed under reduced pressure and elevated temperature to get rid of residual trifluoroacetic acid and give the trifluoroacetate salt of the peptide methyl ester.

3. Spectroscopic details for the isolated compounds

N-Acetyl-L-proline methyl ester (Ac-Pro-OMe)

(a) N-Acetyl-L-proline:[15] L-Proline (8.07 g, 70.1 mmol) was dissolved in water (8 mL). The solution was heated to 55 °C, followed by dropwise addition of acetic anhydride (10 mL, 105 mmol). The resulting mixture was heated to 70 °C and stirred for 1 hour. Half of the solvent was then removed under reduced pressure. The solution was cooled to room temperature and the resulting solid was collected by filtration. The crude product was recrystallised from hot water and washed with cold water and ethanol (2 x 5 mL) to give the product as white shards (6.99 g, 63%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 12.53 (s, 1H), 4.18 (dd, *J* = 8.8, 3.7 Hz, 1H), 3.57–3.42 (m, 2H), 2.22–1.98 (m, 2H), 1.96 (s, 3H), 1.95–1.85 ppm (m, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 173.5, 168.2, 58.1, 47.2, 29.1, 24.4, 22.1 ppm. HRMS (ESI) *m/z* calcd. for $[C_7H_{12}NO_3]^+$: 158.0812 $[M + H]^+$, found 158.0813, HRMS (ESI) *m/z* calcd. for $[C_{14}H_{23}N_2O_6]^+$: 315.1551 $[2M + H]^+$, found 315.1550.

(b) N-Acetyl-L-proline methyl ester: Prepared according to GP3 using 0.99 g (6.28 mmol) of *N*-acetyl-L-proline. A colourless liquid (0.88 g, 82%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 4.25 (dd, *J* = 8.8, 4.2 Hz, 1H), 3.60 (s, 3H), 3.58–3.46 (m, 2H), 2.22–2.08 (m, 1H), 1.97 (s, 3H), 1.96–1.86 (m, 2H), 1.85–1.78 ppm (m, 1H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 172.6, 168.3, 58.0, 51.7, 47.2, 29.0, 24.4, 22.0 ppm. HRMS (ESI) *m/z* calcd. for $[C_8H_{14}NO_3]^+$: 172.0969 $[M + H]^+$, found 172.0969, HRMS (ESI) *m/z* calcd. for $[C_8H_{13}NO_3Na]^+$: 194.0788 $[M + Na]^+$, found 194.0789.

N-Acetyl-L-prolyl-L-proline methyl ester (Ac-Pro-Pro-OMe)

Prepared according to GP4 method (a), purified by column chromatography (49:1 $CHCl_3$/MeOH, $KMnO_4$ stain). A colourless liquid (0.16 g, 19%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 4.54 (dd, *J* = 8.8, 3.8 Hz, 1H), 4.28 (dd, *J* = 8.7, 4.5 Hz, 1H), 3.69 (t, *J* = 5.0 Hz, 1H), 3.60 (s, 3H), 3.57–3.45 (m, 3H), 2.22–2.06 (m, 2H), 1.97–1.89 (m, 6H), 1.90–1.76 (m, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 172.3, 170.1, 167.8, 58.3, 57.0, 51.8, 47.5, 46.2, 28.4, 28.2, 24.6, 24.1, 22.1 ppm. HRMS (ESI) *m/z* calcd. for $[C_{13}H_{21}N_2O_4]^+$: 269.1496 $[M + H]^+$, found 269.1496, HRMS (ESI) *m/z* calcd. for $[C_{13}H_{20}N_2O_4Na]^+$: 291.1316 $[M + Na]^+$, found 291.1315

N-Acetyl-L-prolyl-L-phenylalanine methyl ester (Ac-Pro-Phe-OMe)

(a) L-Phenylalanine methyl ester hydrochloride salt: Prepared according to GP2. A white solid (16.8 g, 99%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.74 (s, 3H), 7.38–7.28 (m, 2H), 7.32–7.20 (m, 3H), 4.23 (dd, *J* = 7.4, 5.9 Hz, 1H), 3.65 (s, 3H), 3.21 (dd, *J* = 14.0, 5.7 Hz, 1H), 3.10 ppm (dd, *J* = 14.0, 7.5 Hz, 1H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 169.3, 134.7, 129.4, 128.6, 127.2, 53.2, 52.5, 35.8 ppm. HRMS (ESI) *m/z* calcd. for $[C_{10}H_{14}NO_2]^+$: 180.1020 $[M - Cl]^+$, found 180.1010.

(b) N-Acetyl-L-prolyl-L-phenylalanine methyl ester: Prepared according to GP4 method (b). A white solid (2.12 g, 66%); mp 109.4–112.9 °C. ^{1}H NMR (500 MHz, DMSO-d_6) major isomer: δ 8.47 (d, *J* = 8.5 Hz, 1H), 7.23–7.17 (m, 5H), 4.61 (ddd, *J* = 10.9, 8.5, 4.6 Hz, 1H), 4.21 (dd, *J* =

8.9, 2.5 Hz, 1H), 3.65 (s, 3H), 3.37 (ddd, *J* = 11.6, 9.3, 3.0 Hz, 1H), 3.27 (ddd, *J* = 11.3, 8.5, 6.8 Hz, 1H), 3.14 (dd, *J* = 13.8, 4.6 Hz, 1H), 2.93 (dd, *J* = 13.7, 2.2 Hz, 1H), 2.11–2.04 (m, 1H), 1.72–1.63 (m, 3H), 1.61 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 171.84, 171.79, 168.64, 137.46, 129.03, 128.12, 126.41, 60.30, 52.88, 52.00, 46.16, 36.03, 31.49, 23.97, 22.21 ppm. HRMS (ESI) *m/z* calcd. for $[C_{17}H_{23}N_2O_4]^+$: 319.1653 $[M + H]^+$, found 319.1658, HRMS (ESI) *m/z* calcd. for $[C_{17}H_{22}N_2O_4Na]^+$: 341.1472 $[M + Na]^+$, found 341.1468.

_N_-Acetyl-L-phenylalanyl-L-proline methyl ester (Ac-Phe-Pro-OMe)

(a) N-Acetyl-L-phenylalanine: Prepared according to GP1 using 11.2 g (67.9 mmol) of L-phenylalanine. A white solid (12.7 g, 90%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.65 (s, 1H), 8.18 (d, *J* = 8.1 Hz, 1H), 7.31–7.25 (m, 2H), 7.24–7.17 (m, 3H), 4.40 (ddd, *J* = 9.6, 8.1, 4.9 Hz, 1H), 3.03 (dd, *J* = 13.8, 4.9 Hz, 1H), 2.83 (dd, *J* = 13.8, 9.6 Hz, 1H), 1.77 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 173.2, 169.2, 137.7, 129.0, 128.2, 126.4, 53.5, 36.8, 22.3 ppm. HRMS (ESI) *m/z* calcd. for $[C_{11}H_{14}NO_3]^+$: 208.0969 $[M + H]^+$, found 208.0985, HRMS (ESI) *m/z* calcd. for $[C_{22}H_{27}N_2O_6]^+$: 415.1864, $[2M + H]^+$, found 415.1893.

(b) N-Acetyl-L-phenylalanyl-L-proline methyl ester: Prepared according to GP4 method (c) using 2.12 g (10.2 mmol) of *N*-acetyl-L-phenylalanine and 1.87 g (11.3 mmol) of L-proline methyl ester hydrochloride salt, purified by column chromatography (49:1 $CHCl_3$/MeOH, $KMnO_4$ stain). A colourless liquid (0.67 g, 21%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 8.29 (d, *J* = 8.3 Hz, 1H), 7.29–7.26 (m, 4H), 7.23–7.16 (m, 1H), 4.68 (td, *J* = 8.6, 5.3 Hz, 1H), 4.31 (dd, *J* = 8.7, 4.7 Hz, 1H), 3.69 (dt, *J* = 9.8, 6.9 Hz, 1H), 3.61 (s, 3H), 3.44 (dt, *J* = 9.8, 6.6 Hz, 1H), 2.93 (dd, *J* = 13.8, 5.3 Hz, 1H), 2.74 (dd, *J* = 13.8, 9.0 Hz, 1H), 2.18–2.10 (m, 1H), 1.93–1.85 (m, 2H), 1.84–1.77 (m, 1H), 1.75 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 172.2, 170.0, 168.9, 137.6, 129.2, 128.1, 126.4, 58.6, 51.9, 51.8, 46.5, 36.9, 28.6, 24.6, 22.3 ppm. HRMS (ESI) *m/z* calcd. for $[C_{17}H_{23}N_2O_4]^+$: 319.1653 $[M + H]^+$, found 319.1653, HRMS (ESI) *m/z* calcd. for $[C_{17}H_{22}N_2O_4Na]^+$: 341.1472 $[M + Na]^+$, found 341.1471.

_N_-Acetylglycyl-L-proline methyl ester (Ac-Gly-Pro-OMe)

Prepared according to GP4 method (c), purified by column chromatography (97:3 $CHCl_3$/MeOH, $KMnO_4$ stain). A colourless liquid (0.57 g, 43%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 8.00 (t, *J* = 5.5 Hz, 1H), 4.29 (dd, *J* = 7.9, 3.3 Hz, 1H), 3.97 (dd, *J* = 17.3, 4.8 Hz, 1H), 3.82 (dd, *J* = 17.3, 4.8 Hz, 1H), 3.61 (s, 3H), 3.53 (q, *J* = 7.2 Hz, 2H), 1.92 (p, *J* = 7.8 Hz, 2H), 1.85 (s, 3H), 1.87–1.79 ppm (m, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 172.8, 169.8, 167.7, 58.9, 52.2, 46.0, 41.4, 29.0, 24.9, 22.8 ppm. HRMS (ESI) *m/z* calcd. for $[C_{10}H_{17}N_2O_4]^+$: 229.1183 $[M + H]^+$, found 229.1183, HRMS (ESI) *m/z* calcd. for $[C_{10}H_{16}N_2O_4Na]^+$: 251.1003 $[M + Na]^+$, found 251.1001.

_N_-Acetyl-L-leucyl-L-proline methyl ester (Ac-Leu-Pro-OMe)

(a) N-Acetyl-L-leucine: Prepared according to GP1 using 5.65 g (43.1 mmol) of L-leucine. A white solid (6.61 g, 89%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.46 (s, 1H), 8.07 (d, *J* = 7.9 Hz, 1H), 4.19 (ddd, *J* = 9.1, 8.1, 6.0 Hz, 1H), 1.83 (s, 3H), 1.69–1.54 (m, 1H), 1.48 (ddd, *J* = 8.5, 5.5, 3.0 Hz, 2H), 0.89 (d, *J* = 6.5 Hz, 3H), 0.84 ppm (d, *J* = 6.5 Hz, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 174.3, 169.2, 50.2, 40.0, 24.3, 22.8, 22.3, 21.3 ppm. HRMS (ESI) *m/z* calcd. for

$[C_8H_{16}NO_3]^+$: 174.1125 $[M + H]^+$, found 174.1133, HRMS (ESI) *m/z* calcd. for $[C_{16}H_{31}N_2O_6]^+$: 347.2177 $[2M + H]^+$, found 347.2182.

(b) N-Acetyl-L-leucyl-L-proline methyl ester: Prepared according to GP4 method (c), purified by column chromatography (99:1 $CHCl_3$/MeOH, $KMnO_4$ stain). White sticky crystals (0.41 g, 26%); mp 51.9–53.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 8.10 (d, *J* = 8.0 Hz, 1H), 4.55 (q, *J* = 7.8 Hz, 1H), 4.31 (dd, *J* = 8.5, 5.1 Hz, 1H), 3.75 (dt, *J* = 9.7, 6.8 Hz, 1H), 3.60 (s, 3H), 3.51 (dt, *J* = 9.5, 6.6 Hz, 1H), 2.16 (ddd, *J* = 12.5, 8.2, 6.0 Hz, 1H), 1.93 (p, *J* = 6.8 Hz, 2H), 1.81 (s, 3H), 1.83–1.76 (m, 1H), 1.61 (dq, *J* = 13.4, 6.7 Hz, 1H), 1.41 (dd, *J* = 8.3, 6.0 Hz, 2H), 0.90 (d, *J* = 6.8 Hz, 3H), 0.88 ppm (d, *J* = 6.6 Hz, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 172.3, 170.7, 169.0, 58.4, 51.7, 48.4, 46.5, 40.1, 28.5, 24.6, 24.0, 23.0, 22.3, 21.7 ppm. HRMS (ESI) *m/z* calcd. for $[C_{14}H_{25}N_2O_4]^+$: 285.1809 $[M + H]^+$, found 285.1803, HRMS (ESI) *m/z* calcd. for $[C_{14}H_{24}N_2O_4Na]^+$: 307.1629 $[M + Na]^+$, found 307.1619.

N-Acetyl-β-alanyl-L-proline methyl ester (Ac-βAla-Pro-OMe)

(a) N-Acetyl-β-alanine: Prepared according to GP1. A colourless oil (6.30 g, 92%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.98–7.88 (m, 1H), 3.20 (q, *J* = 6.7 Hz, 2H), 2.35 (t, *J* = 6.9 Hz, 2H), 1.77 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 172.9, 169.3, 34.8, 33.9, 22.5 ppm. HRMS (ESI) *m/z* calcd. for $[C_5H_{10}NO_3]^+$: 132.0656 $[M + H]^+$, found 132.0655, HRMS (ESI) *m/z* calcd. for $[C_5H_9NO_3Na]^+$: 154.0475 $[M + Na]^+$, found 154.0473.

(b) N-Acetyl-β-alanyl-L-proline methyl ester: Prepared according to GP4 method (c), purified by column chromatography (24:1 $CHCl_3$/MeOH, $KMnO_4$ stain). A colourless oil (0.17 g, 12%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 7.84 (t, *J* = 5.4 Hz, 1H), 4.28 (dd, *J* = 8.8, 4.1 Hz, 1H), 3.61 (s, 3H), 3.49 (q, *J* = 7.4, 6.9 Hz, 2H), 3.25–3.16 (m, 2H), 2.43 (td, *J* = 7.1, 2.0 Hz, 2H), 2.18–2.12 (m, 1H), 1.90 (p, *J* = 6.8 Hz, 2H), 1.86–1.79 (m, 1H), 1.77 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 172.5, 169.3, 169.2, 58.1, 51.8, 46.5, 34.6, 33.7, 28.8, 24.4, 22.6 ppm. HRMS (ESI) *m/z* calcd. for $[C_{11}H_{19}N_2O_4]^+$: 243.1340 $[M + H]^+$, found 243.1343, HRMS (ESI) *m/z* calcd. for $[C_{11}H_{18}N_2O_4Na]^+$: 265.1159 $[M + Na]^+$, found 265.1162.

N-Acetyl-N-methylglycine methyl ester (Ac-(Me)Gly-OMe)

(a) N-Methylglycine methyl ester hydrochloride salt: Prepared according to GP2 using 1.43 g (16.0 mmol) of *N*-methylglycine. A white solid (1.92 g, 86%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.55 (s, 2H), 3.93 (s, 2H), 3.73 (s, 3H), 2.54 ppm (s, 3H). ^{13}C {^{1}H} NMR (126 MHz, DMSO-d_6): δ 167.1, 52.5, 47.8, 32.5 ppm. HRMS (ESI) *m/z* calcd. for $[C_4H_{10}NO_2]^+$: 104.0707 $[M - Cl]^+$, found 104.0709.

(b) N-Acetyl-N-methylglycine methyl ester:[16] *N*-Methylglycine methyl ester hydrochloride salt (1.03 g, 7.41 mmol) was suspended in dichloromethane and cooled to 0 °C. Triethylamine (2.2 mL, 15.8 mmol), followed by acetyl chloride (0.55 mL, 7.74 mmol), was added dropwise and the resulting mixture was stirred at room temperature for 1 hour. The solvent was then removed under reduced pressure and ethyl acetate (15 mL) was added. The mixture was filtered, and the solvent was removed under reduced pressure to give a yellow oil. The crude product was purified by column chromatography (EtOAc, $KMnO_4$ stain). A colourless oil (0.59

g, 55%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 4.05 (s, 2H), 3.63 (s, 3H), 3.01 (s, 3H), 2.02 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 170.5, 169.9, 51.6, 48.7, 36.8, 21.1 ppm. HRMS (ESI) *m*/*z* calcd. for $[C_6H_{12}NO_3]^+$: 146.0812 $[M + H]^+$, found 146.0812, HRMS (ESI) *m*/*z* calcd. for $[C_6H_{11}NO_3Na]^+$: 168.0632 $[M + Na]^+$, found 168.0631.

_N_-Acetyl-_N_-methylglycyl-_N_-methylglycine methyl ester (Ac-(Me)Gly-(Me)Gly-OMe)

(a) N-Acetyl-N-methylglycine: Prepared according to GP1. White crystals (3.83 g, 58%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.67 (s, 1H), 3.95 (s, 2H), 2.99 (s, 3H), 2.01 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 170.8, 170.4, 48.7, 36.8, 21.2 ppm. HRMS (ESI) *m*/*z* calcd. for $[C_5H_{10}NO_3]^+$: 132.0656 $[M + H]^+$, found 132.0657, HRMS (ESI) *m*/*z* calcd. for $[C_5H_9NO_3Na]^+$: 154.0475 $[M + Na]^+$, found 154.0475.

(b) N-Acetyl-N-methylglycyl-N-methylglycine methyl ester: Prepared according to GP4 method (c), purified by column chromatography (97:3 $CHCl_3$/MeOH, $KMnO_4$ stain). A colourless oil (0.61 g, 49%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 4.18 (s, 2H), 4.06 (s, 2H), 3.62 (s, 3H), 2.98 (s, 3H), 2.92 (s, 3H), 1.99 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 170.6, 170.0, 169.0, 52.2, 49.4, 48.6, 35.6, 34.8, 21.7 ppm. HRMS (ESI) *m*/*z* calcd. for $[C_9H_{17}N_2O_4]^+$: 217.1183 $[M + H]^+$, found 217.1178, HRMS (ESI) *m*/*z* calcd. for $[C_9H_{16}N_2O_4Na]^+$: 239.1003 $[M + Na]^+$, found 239.0996.

_N_-Acetyl-_N_-methylglycyl-L-phenylalanine methyl ester (Ac-(Me)Gly-Phe-OMe)

Prepared according to GP4 method (d), purified by column chromatography (49:1 $CHCl_3$/MeOH, $KMnO_4$ stain). A colourless viscous oil (1.65 g, 56%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 8.31 (d, *J* = 7.8 Hz, 1H), 7.30–7.27 (m, 2H), 7.22–7.19 (m, 3H), 4.49 (td, *J* = 8.5, 5.6 Hz, 1H), 3.88 (s, 2H), 3.60 (s, 3H), 2.93 (dd, *J* = 9.7, 2.6 Hz, 1H), 2.89 (dd, *J* = 9.6, 2.3 Hz, 1H), 2.82 (s, 3H), 1.98 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 171.8, 170.2, 168.4, 137.1, 129.1, 128.3, 126.6, 53.5, 52.6, 52.0, 49.3, 36.6, 36.5, 34.0, 21.4 ppm. HRMS (ESI) *m*/*z* calcd. for $[C_{15}H_{21}N_2O_4]^+$: 293.1496 $[M + H]^+$, found 293.1494, HRMS (ESI) *m*/*z* calcd. for $[C_{15}H_{20}N_2O_4Na]^+$: 315.1316 $[M + Na]^+$, found 315.1312.

_N_-Acetyl-L-phenylalanyl-_N_-methylglycine methyl ester (Ac-Phe-(Me)Gly-OMe)

Prepared according to GP4 method (c), purified by column chromatography (EtOAc, PMA stain). A colourless gel (1.35 g, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 8.27 (d, *J* = 8.6 Hz, 1H), 7.27–7.23 (m, 4H), 7.18–7.16 (m, 1H), 4.94 (td, *J* = 8.7, 5.5 Hz, 1H), 4.13 (d, *J* = 17.2 Hz, 1H), 3.99 (d, *J* = 17.1 Hz, 1H), 3.63 (s, 3H), 3.03 (s, 3H), 2.93 (dd, *J* = 13.7, 5.5 Hz, 1H), 2.74 (dd, *J* = 13.8, 8.9 Hz, 1H), 1.76 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ171.8, 169.5, 168.7, 137.5, 129.2, 128.1, 126.4, 51.7, 49.7, 49.2, 37.2, 36.0, 22.2 ppm. HRMS (ESI) *m*/*z* calcd. for $[C_{15}H_{21}N_2O_4]^+$: 293.1496 $[M + H]^+$, found 293.1496, HRMS (ESI) *m*/*z* calcd. for $[C_{15}H_{20}N_2O_4Na]^+$: 315.1316 $[M + Na]^+$, found 315.1313.

_N_-Acetylglycyl-_N_-methylglycine methyl ester (Ac-Gly-(Me)Gly-OMe)

Prepared according to GP4 method (c), purified by column chromatography (97:3 $CHCl_3$/MeOH, $KMnO_4$ stain). A white solid (0.19 g, 17%); mp 80.8–81.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 7.97 (t, *J* = 5.5 Hz, 1H), 4.09 (s, 2H), 3.97 (d, *J* = 5.5 Hz, 2H), 3.63 (s,

3H), 3.01 (s, 3H), 1.86 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 169.7, 169.4, 169.3, 51.8, 49.2, 40.2, 35.2, 22.4 ppm. HRMS (ESI) *m/z* calcd. for $[C_8H_{15}N_2O_4]^+$: 203.1027 $[M + H]^+$, found 203.1027, HRMS (ESI) *m/z* calcd. for $[C_8H_{14}N_2O_4Na]^+$: 225.0846 $[M + Na]^+$, found 225.0846.

_N_-Acetyl-L-leucyl-_N_-methylglycine methyl ester (Ac-Leu-(Me)Gly-OMe)

Prepared according to GP4 method (c), purified by column chromatography (99:1 $CHCl_3$/MeOH, $KMnO_4$ stain). A colourless oil (1.36 g, 89%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 8.09 (d, *J* = 8.4 Hz, 1H), 4.80 (td, *J* = 8.6, 5.6 Hz, 1H), 4.23 (d, *J* = 17.2 Hz, 1H), 3.92 (d, *J* = 17.2 Hz, 1H), 3.63 (s, 3H), 3.09 (s, 3H), 1.82 (s, 3H), 1.65–1.54 (m, 1H), 1.43–1.37 (m, 2H), 0.89 (d, *J* = 6.5 Hz, 3H), 0.88 ppm (d, *J* = 6.5Hz, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 172.7, 169.7, 169.0, 51.7, 49.2, 46.5, 40.4, 36.0, 24.2, 23.1, 22.3, 21.6 ppm. HRMS (ESI) *m/z* calcd. for $[C_{12}H_{23}N_2O_4]^+$: 259.1653 $[M + H]^+$, found 259.1652, HRMS (ESI) *m/z* calcd. for $[C_{12}H_{22}N_2O_4Na]^+$: 281.1472 $[M + Na]^+$, found 281.1470.

_N_-Acetyl-L-valyl-_N_-methylglycine methyl ester (Ac-Val-(Me)Gly-OMe)

Prepared according to GP4 method (c), purified by column chromatography (49:1 $CHCl_3$/MeOH, $KMnO_4$ stain) and recrystallised from hot acetonitrile and pentane. White crystals (0.66 g, 47%); mp 104.9–108.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 8.05 (d, *J* = 8.8 Hz, 1H), 4.58 (t, *J* = 8.6 Hz, 1H), 4.29 (d, *J* = 17.2 Hz, 1H), 3.89 (d, *J* = 17.2 Hz, 1H), 3.63 (s, 3H), 3.13 (s, 3H), 1.98–1.91 (m, 1H), 1.84 (s, 3H), 0.88 (d, *J* = 7.0 Hz, 3H), 0.86 ppm (d, *J* = 6.9Hz, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 172.7, 169.6, 169.1, 53.2, 51.7, 49.2, 36.4, 30.1, 22.2, 19.0, 18.2 ppm. HRMS (ESI) *m/z* calcd. for $[C_{11}H_{21}N_2O_4]^+$: 245.1496 $[M + H]^+$, found 245.1497, HRMS (ESI) *m/z* calcd. for $[C_{11}H_{20}N_2O_4Na]^+$: 267.1316 $[M + Na]^+$, found 267.1315.

_N_-Acetylglycine methyl ester (Ac-Gly-OMe)

Prepared according to GP3. A colourless oil (0.59 g, 37%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.28 (t, *J* = 6.0 Hz, 1H), 3.80 (d, *J* = 6.0 Hz, 2H), 3.62 (s, 3H), 1.85 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 170.5, 169.7, 51.6, 40.6, 22.2 ppm. HRMS (ESI) *m/z* calcd. for $[C_5H_9NO_3Na]^+$: 154.0475 $[M + Na]^+$, found 154.0477, HRMS (ESI) *m/z* calcd. for $[C_{10}H_{19}N_2O_6]^+$: 263.1238 $[2M + H]^+$, found 263.1241.

_N_-Acetyl-β-alanine methyl ester (Ac-βAla-OMe)

Prepared according to GP3 using 3.62 g (27.6 mmol) of *N*-acetyl-β-alanine, purified by column chromatography (97:3 $CHCl_3$/MeOH, $KMnO_4$ stain). White crystals (1.11 g, 28%); mp 32.4–34.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.06–7.81 (m, 1H), 3.59 (s, 3H), 3.24 (td, *J* = 6.9, 5.6 Hz, 2H), 2.44 (t, *J* = 6.8 Hz, 2H), 1.77 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 171.8, 169.3, 51.4, 34.7, 33.7, 22.5 ppm. HRMS (ESI) *m/z* calcd. for $[C_6H_{12}NO_3]^+$: 146.0812 $[M + H]^+$, found 146.0813, HRMS (ESI) *m/z* calcd. for $[C_6H_{11}NO_3Na]^+$: 168.0632 $[M + Na]^+$, found 168.0632.

N_α,N_ε-Diacetyl-L-lysine methyl ester (Ac-Lys(NHAc)-OMe)

(a) N_α,N_ε-Diacetyl-L-lysine: Prepared according to GP1 using 3.28 g (17.4 mmol) of L-lysine monohydrochloride salt. A colourless liquid (1.00 g, 24%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.93 (d, *J* = 7.8 Hz, 1H), 7.81 (t, *J* = 5.5 Hz, 1H), 4.07 (td, *J* = 8.2, 5.0 Hz, 1H), 2.98 (q, *J* = 6.6 Hz, 3H), 1.83 (s, 3H), 1.77 (s, 3H), 1.64 (ddd, *J* = 12.6, 8.9, 4.9 Hz, 1H), 1.52 (td, *J* = 14.7, 14.1, 8.0 Hz, 1H), 1.39–1.32 (m, 2H), 1.29–1.23 ppm (m, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 174.12, 169.04, 168.97, 52.39, 38.39, 31.23, 28.87, 22.94, 22.63, 22.50 ppm.

(b) N_α,N_ε-Diacetyl-L-lysine methyl ester: Prepared according to GP3 using 1.00 g (4.34 mmol) of N_α,N_ε-Diacetyl-L-lysine. A white solid (0.18 g, 17%); mp 96.3–99.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20 (d, *J* = 7.4 Hz, 1H), 7.79 (t, *J* = 5.3 Hz, 1H), 4.17 (ddd, *J* = 8.8, 7.3, 5.3 Hz, 1H), 3.61 (s, 3H), 2.99 (q, *J* = 6.5 Hz, 2H), 1.84 (s, 3H), 1.77 (s, 3H), 1.71–1.58 (m, 1H), 1.55 (ddt, *J* = 13.8, 8.8, 3.9 Hz, 1H), 1.42–1.30 (m, 2H), 1.33–1.20 ppm (m, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 172.8, 169.4, 168.9, 51.9, 51.7, 38.2, 30.6, 28.7, 22.8, 22.6, 22.2 ppm. HRMS (ESI) *m/z* calcd. for $[C_{11}H_{21}N_2O_4]^+$: 245.1496 $[M + H]^+$, found 245.1496, HRMS (ESI) *m/z* calcd. for $[C_{22}H_{41}N_4O_8]^+$: 489.2919 $[2M + H]^+$, found 489.2920.

***N*-Acetyl-*N*-methyl(d_3)glycine methyl(d_3) ester (Ac-(Me(d_3))Gly-OMe(d_3))**

Prepared according to GP5 using 2.35 g (20.1 mmol) of *N*-acetylglycine and 3.46 mL (55.4 mmol) of methyl(d_3) iodide, purified by column chromatography (49:1 $CHCl_3$/MeOH, $KMnO_4$ stain). A colourless oil (0.73 g, 24%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 4.05 (s, 2H), 2.02 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 170.5, 169.9, 48.6, 21.1 ppm. HRMS (ESI) *m/z* calcd. for $[C_6H_6D_6NO_3]^+$: 152.1188 $[M + H]^+$, found 152.1189, HRMS (ESI) *m/z* calcd. for $[C_6H_5D_6NO_3Na]^+$: 174.1108 $[M + Na]^+$, found 174.1108.

***N*-methyl-L-pyroglutamic acid methyl ester (Me-Glp-OMe)**

Prepared according to GP5 using 2.60 g (20.1 mmol) of L-pyroglutamic acid and 3.45 mL (55.4 mmol) of methyl iodide, purified by column chromatography (49:1 $CHCl_3$/MeOH, $KMnO_4$ stain). A colourless oil (0.58 g, 18%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.25 (dd, *J* = 8.5, 3.2 Hz, 1H), 3.70 (s, 3H), 2.69 (s, 3H), 2.37–2.25 (m, 1H), 2.28–2.17 (m, 2H), 1.99–1.87 ppm (m, 1H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 174.3, 172.4, 60.6, 52.2, 28.8, 28.3, 22.1 ppm. HRMS (ESI) *m/z* calcd. for $[C_7H_{12}NO_3]^+$: 158.0812 $[M + H]^+$, found 158.0813, HRMS (ESI) *m/z* calcd. for $[C_7H_{11}NO_3Na]^+$: 180.0632 $[M + Na]^+$, found 180.0631.

***N*-methyl(d_3)-L-pyroglutamic acid methyl(d_3) ester (Me(d_3)-Glp-OMe(d_3))**

Prepared according to GP5 using 2.60 g (20.1 mmol) of L-pyroglutamic acid and 3.46 mL (55.4 mmol) of methyl(d_3) iodide, purified by column chromatography (49:1 $CHCl_3$/MeOH, $KMnO_4$ stain). A colourless oil (0.19 g, 6%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.35–4.14 (m, 1H), 2.35–2.27 (m, 1H), 2.26–2.17 (m, 2H), 1.97–1.90 ppm (m, 1H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 174.4, 172.5, 60.6, 28.8, 22.1 ppm. HRMS (ESI) *m/z* calcd. for $[C_7H_6D_6NO_3]^+$: 164.1188 $[M + H]^+$, found 164.1189, HRMS (ESI) *m/z* calcd. for $[C_7H_5D_6NO_3Na]^+$: 186.1108 $[M + Na]^+$, found 186.1108.

_N_-Acetyl-_N_-methyl-L-alanine methyl ester (Ac-(Me)Ala-OMe)

Prepared according to GP5 using 2.64 g (20.1 mmol) of *N*-acetyl-L-alanine, purified by column chromatography (49:1 $CHCl_3$/MeOH, $KMnO_4$ stain). A colourless oil (0.63 g, 20%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 4.86 (q, *J* = 7.2 Hz, 1H), 3.61 (s, 3H), 2.90 (s, 3H), 2.01 (s, 3H), 1.28 ppm (d, *J* = 7.2 Hz, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 171.9, 170.0, 52.1, 51.9, 32.6, 21.7, 14.2 ppm. HRMS (ESI) *m/z* calcd. for $[C_7H_{14}NO_3]^+$: 160.0968 $[M + H]^+$, found 160.0968, HRMS (ESI) *m/z* calcd. for $[C_7H_{13}NO_3Na]^+$: 182.0788 $[M + Na]^+$, found 182.0788.

_N_-Acetyl-_N_-methyl-L-valine methyl ester (Ac-(Me)Val-OMe)

Prepared according to GP5 using 3.20 g (20.1 mmol) of *N*-acetyl-L-valine, purified by column chromatography (99:1 $CHCl_3$/MeOH, $KMnO_4$ stain). A colourless oil (0.09 g, 3%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 4.68 (d, *J* = 10.5 Hz, 1H), 3.62 (s, 3H), 2.90 (s, 3H), 2.16–2.09 (m, 1H), 2.03 (s, 3H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.77 ppm (d, *J* = 6.7 Hz, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 171.1, 170.6, 60.8, 51.6, 31.9, 26.9, 21.6, 19.6, 18.7 ppm. HRMS (ESI) *m/z* calcd. for $[C_9H_{18}NO_3]^+$: 188.1282 $[M + H]^+$, found 188.1283, HRMS (ESI) *m/z* calcd. for $[C_9H_{17}NO_3Na]^+$: 210.1101 $[M + Na]^+$, found 210.1100.

_N_-Acetyl-_N_-methyl-2-aminoisobutyric acid methyl ester (Ac-(Me)Aib-OMe)

(a) N-Acetyl-2-aminoisobutyric acid: Prepared according to GP1 (2.97 g, 38%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 12.08 (s, 1H), 8.00 (s, 1H), 1.77 (s, 3H), 1.31 ppm (s, 6H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 175.6, 168.6, 54.6, 25.0, 22.6 ppm. HRMS (ESI) *m/z* calcd. for $[C_6H_{12}NO_3]^+$: 146.0812 $[M + H]^+$, found 146.0812, HRMS (ESI) *m/z* calcd. for $[C_6H_{11}NO_3Na]^+$: 168.0632 $[M + Na]^+$, found 168.0631.

(b) N-Acetyl-N-methyl-2-aminoisobutyric acid methyl ester: Prepared according to GP5 using 2.92 g (20.1 mmol) of *N*-acetyl-2-aminoisobutyric acid, purified by column chromatography (49:1 $CHCl_3$/MeOH, $KMnO_4$ stain). A white solid (0.42 g, 12%); mp 66.1–68.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.51 (s, 3H), 2.91 (s, 3H), 1.97 (s, 3H), 1.29 ppm (s, 6H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 174.2, 169.8, 59.4, 51.5, 30.9, 22.9, 22.7 ppm. HRMS (ESI) *m/z* calcd. for $[C_8H_{16}NO_3]^+$: 174.1125 $[M + H]^+$, found 174.1125, HRMS (ESI) *m/z* calcd. for $[C_8H_{15}NO_3Na]^+$: 196.0945 $[M + Na]^+$, found 196.0944.

_N_-Acetyl-_N_-_t_-butylglycine methyl ester (Ac-(*t*Bu)Gly-OMe)

(a) N-t-Butylglycine methyl ester: To a mixture of *t*-butylamine (23.6 mL, 225 mmol) and toluene (20 mL) at 45 °C was added methyl bromoacetate (4.8 mL, 50 mmol). The mixture was stirred for 1 hour at 55 °C. The temperature was then increased to 75 °C for 1 hour and cooled down to 0 °C. After 1 hour, the solid was removed by filtration and the filtrate was concentrated under reduced pressure to give the desired product as a colourless liquid (5.80 g, 80%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.62 (s, 3H), 3.29 (s, 2H), 2.30 (s, 1H), 1.00 ppm (s, 9H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 173.2, 51.4, 49.8, 44.3, 28.5 ppm. HRMS (ESI) *m/z* calcd. for $[C_7H_{16}NO_2]^+$: 146.1176 $[M + H]^+$, found 146.1177.

(b) N-Acetyl-N-t-butylglycine methyl ester:[16] *N-t*-Butylglycine methyl ester (5.80 g, 39.9 mmol) was suspended in dichloromethane and cooled to 0 °C. Triethylamine (11.7 mL, 83.9 mmol), followed by acetyl chloride (2.99 mL, 42.1 mmol), was added dropwise and the resulting mixture was stirred at room temperature for 1 hour. The solvent was then removed under reduced pressure and ethyl acetate (50 mL) was added. The mixture was filtered, and the solvent was removed under reduced pressure to give a dark orange oil. The crude product was purified by column chromatography (EtOAc, $KMnO_4$ stain). A colourless liquid (0.32 g, 4%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.18 (s, 2H), 3.68 (s, 3H), 1.92 (s, 3H), 1.32 ppm (s, 9H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 171.6, 171.1, 56.6, 52.1, 47.7, 28.2, 24.8 ppm. HRMS (ESI) *m/z* calcd. for $[C_9H_{18}NO_3]^+$: 188.1282 $[M + H]^+$, found 188.1290, HRMS (ESI) *m/z* calcd. for $[C_9H_{17}NO_3Na]^+$: 210.1101 $[M + Na]^+$, found 210.1102.

_N_-Phthaloylglycyl-_N_-methylglycine methyl ester (Phth-Gly-(Me)Gly-OMe)

(a) N-Phthaloylglycine. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.26 (s, 1H), 7.97–7.89 (m, 2H), 7.91–7.84 (m, 2H), 4.32 ppm (s, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 168.9, 167.2, 134.8, 131.4, 123.4, 38.9 ppm.

(b) N-Phthaloylglycyl-N-methylglycine methyl ester: Prepared according to GP4 method (c) using 1.01 g (4.90 mmol) of *N*-phthaloylglycine and 0.74 g (5.31 mmol) of *N*-methylglycine methyl ester hydrochloride salt, purified by column chromatography ($CHCl_3$, UV light). A white solid (0.24 g, 17%); mp 145.7–146.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 7.93–7.91 (m, 2H), 7.89–7.87 (m, 2H), 4.58 (s, 2H), 4.10 (s, 2H), 3.63 (s, 3H), 3.15 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 169.4, 167.5, 166.4, 134.7, 131.6, 123.3, 51.8, 49.2, 39.2, 35.3 ppm. HRMS (ESI) *m/z* calcd. for $[C_{14}H_{15}N_2O_5]^+$: 291.0976 $[M + H]^+$, found 291.0975, HRMS (ESI) *m/z* calcd. for $[C_{14}H_{14}N_2O_5Na]^+$: 313.0795 $[M + Na]^+$, found 313.0793.

_N_-Phthaloyl-β-alanyl-_N_-methylglycine methyl ester (Phth-βAla-(Me)Gly-OMe)

(a) N-Phthaloyl-β-alanine: Prepared according to GP6 using 0.38 g (4.22 mmol) of β-alanine. A white solid (0.79 g, 90%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.36 (s, 1H), 7.88–7.85 (m, 2H), 7.85–7.81 (m, 2H), 3.79 (t, J = 7.4 Hz, 2H), 2.60 ppm (t, J = 7.4 Hz, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 172.1, 167.6, 134.4, 131.6, 123.0, 33.6, 32.4 ppm. HRMS (ESI) *m/z* calcd. for $[C_{11}H_{10}NO_4]^+$: 220.0605 $[M + H]^+$, found 220.0603, HRMS (ESI) *m/z* calcd. for $[C_{11}H_9NO_4Na]^+$: 242.0424 $[M + Na]^+$, found 242.0422.

(b) N-Phthaloyl-β-alanyl-N-methylglycine methyl ester: Prepared according to GP4 method (c) using 0.63 g (2.89 mmol) of *N*-phthaloyl-β-alanine and 0.46 g (3.31 mmol) of *N*-methylglycine methyl ester hydrochloride salt, purified by recrystallisation from hot acetonitrile. White crystals (0.51 g, 57%); mp 140.9–142.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 7.87–7.86 (m, 2H), 7.84–7.82 (m, 2H), 4.07 (s, 2H), 3.81–3.72 (m, 2H), 3.62 (s, 3H), 2.99 (s, 3H), 2.76 ppm (t, J = 7.8 Hz, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 170.6, 169.7, 167.7, 134.4, 131.7, 123.0, 51.7, 48.8, 36.0, 33.7, 30.8 ppm. HRMS (ESI) *m/z* calcd. for $[C_{15}H_{17}N_2O_5]^+$: 305.1132 $[M + H]^+$, found 305.1134, HRMS (ESI) *m/z* calcd. for $[C_{15}H_{16}N_2O_5Na]^+$: 327.0952 $[M + Na]^+$, found 327.0953.

_N_-Phthaloyl-γ-aminobutyryl-_N_-methylglycine methyl ester (Phth-Gaba-(Me)Gly-OMe)

(a) N-Phthaloyl-γ-aminobutyric acid: Prepared according to GP6. A white solid (0.92 g, 79%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05 (s, 1H), 7.86–7.84 (m, 2H), 7.83–7.80 (m, 2H), 3.60 (t, *J* = 6.8 Hz, 2H), 2.27 (t, *J* = 7.2 Hz, 2H), 1.82 ppm (p, *J* = 7.0 Hz, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 173.8, 168.0, 134.3, 131.7, 123.0, 36.9, 31.0, 23.3 ppm. HRMS (ESI) *m*/*z* calcd. for $[C_{12}H_{12}NO_4]^+$: 234.0761 $[M + H]^+$, found 234.0759, HRMS (ESI) *m*/*z* calcd. for $[C_{12}H_{11}NO_4Na]^+$: 256.0581 $[M + Na]^+$, found 256.0578.

(b) N-Phthaloyl-γ-aminobutyryl-N-methylglycine methyl ester: Prepared according to GP4 method (c) using 0.90 g (3.85 mmol) of *N*-phthaloyl-γ-aminobutyric acid and 0.61 g (4.35 mmol) of *N*-methylglycine methyl ester hydrochloride salt, purified by column chromatography (99:1 $CHCl_3$/MeOH, UV light). A white solid (0.33 g, 27%); mp 109.0–111.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 7.86–7.84 (m, 2H), 7.84–7.81 (m, 2H), 4.00 (s, 2H), 3.61 (s, 3H), 3.59–3.55 (m, 2H), 2.97 (s, 3H), 2.41 (t, *J* = 7.2 Hz, 2H), 1.85–1.78 ppm (m, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 172.1, 169.9, 168.0, 134.3, 131.7, 122.9, 51.6, 48.9, 37.2, 36.0, 29.5, 23.4 ppm. HRMS (ESI) *m*/*z* calcd. for $[C_{16}H_{19}N_2O_5]^+$: 319.1289 $[M + H]^+$, found 319.1288, HRMS (ESI) *m*/*z* calcd. for $[C_{16}H_{18}N_2O_5Na]^+$: 341.1108 $[M + Na]^+$, found 341.1106.

_N_-Phthaloyl-δ-aminovaleryl-_N_-methylglycine methyl ester (Phth-Ava-(Me)Gly-OMe)

(a) N-Phthaloyl-δ-aminovaleric acid: Prepared according to GP6. A white solid (0.96 g, 77%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.01 (s, 1H), 7.90–7.77 (m, 4H), 3.57 (t, *J* = 6.8 Hz, 2H), 2.23 (t, *J* = 7.2 Hz, 2H), 1.62–1.56 (m, 2H), 1.52–1.46 ppm (m, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 174.2, 168.0, 134.4, 131.6, 123.0, 37.1, 33.1, 27.4, 21.8 ppm. HRMS (ESI) *m*/*z* calcd. for $[C_{13}H_{14}NO_4]^+$: 248.0918 $[M + H]^+$, found 248.0917, HRMS (ESI) *m*/*z* calcd. for $[C_{13}H_{13}NO_4Na]^+$: 270.0737 $[M + Na]^+$, found 270.0735.

(b) N-Phthaloyl-δ-aminovaleryl-N-methylglycine methyl ester: Prepared according to GP4 method (c) using 0.86 g (3.49 mmol) of *N*-phthaloyl-δ-aminovaleric acid and 0.54 g (3.89 mmol) of *N*-methylglycine methyl ester hydrochloride salt, purified by column chromatography (199:1 $CHCl_3$/MeOH, UV light). A colourless oil (0.27 g, 23%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 7.87–7.82 (m, 4H), 4.04 (s, 2H), 3.61 (s, 3H), 3.58 (t, *J* = 7.0 Hz, 2H), 3.00 (s, 3H), 2.37 (t, *J* = 7.2 Hz, 2H), 1.63–1.57 (m, 2H), 1.50–1.45 ppm (m, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 172.5, 169.9, 167.9, 134.4, 131.6, 123.0, 51.6, 48.9, 37.3, 36.1, 31.5, 27.6, 21.8 ppm. HRMS (ESI) *m*/*z* calcd. for $[C_{17}H_{21}N_2O_5]^+$: 333.1445 $[M + H]^+$, found 333.1444, HRMS (ESI) *m*/*z* calcd. for $[C_{17}H_{20}N_2O_5Na]^+$: 355.1265 $[M + Na]^+$, found 355.1263.

_N_-Phthaloyl-ε-aminocaproyl-_N_-methylglycine methyl ester (Phth-Ahx-(Me)Gly-OMe)

(a) N-Phthaloyl-ε-aminocaproic acid: Prepared according to GP6 using 0.56 g (4.27 mmol) of ε-aminocaproic acid. A white solid (0.94 g, 88%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.97 (s, 1H), 7.87–7.84 (m, 2H), 7.84–7.80 (m, 2H), 3.55 (t, *J* = 7.1 Hz, 2H), 2.18 (t, *J* = 7.3 Hz, 2H), 1.58 (p, *J* = 7.5 Hz, 2H), 1.50 (p, *J* = 7.5 Hz, 2H), 1.30–1.25 ppm (m, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 174.3, 167.9, 134.4, 131.6, 123.0, 37.3, 33.4, 27.7, 25.8, 24.0 ppm. HRMS (ESI)

m/z calcd. for $[C_{14}H_{16}NO_4]^+$: 262.1074 $[M + H]^+$, found 262.1075, HRMS (ESI) *m/z* calcd. for $[C_{14}H_{15}NO_4Na]^+$: 284.0891 $[M + Na]^+$, found 284.0893.

(b) N-Phthaloyl-ε-aminocaproyl-N-methylglycine methyl ester: Prepared according to GP4 method (c) using 1.10 g (4.20 mmol) of *N*-phthaloyl-ε-aminocaproic acid and 0.66 g (4.73 mmol) of *N*-methylglycine methyl ester hydrochloride salt, purified by column chromatography (99:1 $CHCl_3$/MeOH, UV light). White crystals (0.45 g, 31%); mp 56.7–58.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 7.86–7.82 (m, 4H), 4.03 (s, 2H), 3.60 (s, 3H), 3.56 (t, *J* = 6.8 Hz, 2H), 2.99 (s, 3H), 2.31 (t, *J* = 7.3 Hz, 2H), 1.62–1.56 (m, 2H), 1.53–1.48 (m, 2H), 1.31–1.26 ppm (m, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 172.7, 169.9, 168.0, 134.4, 131.6, 123.0, 51.6, 48.9, 37.3, 36.1, 31.9, 27.8, 25.9, 24.1 ppm. HRMS (ESI) *m/z* calcd. for $[C_{18}H_{23}N_2O_5]^+$: 347.1602 $[M + H]^+$, found 347.1603, HRMS (ESI) *m/z* calcd. for $[C_{18}H_{22}N_2O_5Na]^+$: 369.1421 $[M + Na]^+$, found 369.1421.

_N_-Phthaloylglycylglycyl-_N_-methylglycine methyl ester (Phth-Gly-Gly-(Me)Gly-OMe)

(a) N-Boc-glycyl-N-methylglycine methyl ester: Prepared according to GP4 method (c), purified using column chromatography (3:7 Pet. ether/EtOAc, ninhydrin stain). A colourless oil (0.53 g, 36%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 6.78 (t, *J* = 6.0 Hz, 1H), 4.08 (s, 2H), 3.82 (d, *J* = 5.9 Hz, 2H), 3.63 (s, 3H), 2.99 (s, 3H), 1.37 ppm (s, 9H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 169.8, 169.6, 155.8, 77.9, 51.8, 49.1, 41.6, 35.0, 28.2 ppm. HRMS (ESI) *m/z* calcd. for $[C_{11}H_{21}N_2O_5]^+$: 261.1445 $[M + H]^+$, found 261.1443, HRMS (ESI) *m/z* calcd. for $[C_{11}H_{20}N_2O_5Na]^+$: 283.1265 $[M + Na]^+$, found 283.1262.

(b) Glycyl-N-methylglycine methyl ester trifluoroacetate salt: Prepared according to GP8 using 0.53 g (2.02 mmol) of *N*-Boc-glycyl-*N*-methylglycine methyl ester. A colourless oil used in the next step without further purification (quantitative yield). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 8.12 (s, 3H), 4.17 (s, 2H), 3.95 (d, *J* = 4.6 Hz, 2H), 3.65 (s, 3H), 3.01 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 169.7, 167.4, 52.4, 49.5, 39.9, 35.5 ppm. HRMS (ESI) *m/z* calcd. for $[C_6H_{13}N_2O_3]^+$: 161.0921 $[M - C_2O_2F_3]^+$, found 161.0921, HRMS (ESI) *m/z* calcd. for $[C_{12}H_{25}N_4O_6]^+$: 321.1769 $[2M - C_4HO_4F_6]^+$, found 321.1768.

(c) N-Phthaloylglycylglycyl-N-methylglycine methyl ester: Prepared according to GP4 method (c) using 0.44 g (2.16 mmol) of *N*-phthaloylglycine and 0.61 g (2.36 mmol) of glycyl-*N*-methylglycine methyl ester trifluoroacetate salt, purified by column chromatography (49:1 $CHCl_3$/MeOH, UV light). A white solid (0.36 g, 49%); mp 193.9–196.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 8.45 (t, *J* = 5.3 Hz, 1H), 7.92–7.89 (m, 2H), 7.88–7.85 (m, 2H), 4.27 (s, 2H), 4.10 (s, 2H), 4.04 (d, *J* = 5.4 Hz, 2H), 3.64 (s, 3H), 3.00 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 169.7, 168.8, 167.5, 166.2, 134.6, 131.7, 123.2, 51.8, 49.2, 40.5, 39.9, 35.2 ppm. HRMS (ESI) *m/z* calcd. for $[C_{16}H_{18}N_3O_6]^+$: 348.1191 $[M + H]^+$, found 348.1189, HRMS (ESI) *m/z* calcd. for $[C_{16}H_{17}N_3O_6Na]^+$: 370.1010 $[M + Na]^+$, found 370.1003.

_N_-Phthaloylglycyl-β-alanyl-_N_-methylglycine methyl ester (Phth-Gly-βAla-(Me)Gly-OMe)

(a) N-Boc-β-alanine: Prepared according to GP7. A white solid (3.13 g, 82%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.16 (s, 1H), 6.78 (t, *J* = 5.6 Hz, 1H), 3.11 (td, *J* = 7.1, 5.6 Hz, 2H), 2.34 (t, *J* = 7.1 Hz, 2H), 1.36 ppm (s, 9H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 172.9, 155.5, 77.7, 36.2,

34.2, 28.2 ppm. HRMS (ESI) *m/z* calcd. for $[C_8H_{16}NO_4]^+$: 190.1074 $[M + H]^+$, found 190.1073, HRMS (ESI) *m/z* calcd. for $[C_8H_{15}NO_4Na]^+$: 212.0894 $[M + Na]^+$, found 212.0892.

(b) N-Boc-β-*alanyl-N-methylglycine methyl ester:* Prepared according to GP4 method (c), purified using column chromatography (3:7 Pet. ether/EtOAc, ninhydrin stain). A colourless oil (1.39 g, 89%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 6.65 (t, *J* = 5.8 Hz, 1H), 4.06 (s, 2H), 3.63 (s, 3H), 3.13–3.10 (m, 2H), 2.99 (s, 3H), 2.48 (t, *J* = 7.2 Hz, 2H), 1.37 ppm (s, 9H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 171.3, 169.8, 155.4, 77.6, 51.7, 48.8, 36.2, 36.0, 32.7, 28.2 ppm. HRMS (ESI) *m/z* calcd. for $[C_{12}H_{23}N_2O_5]^+$: 275.1602 $[M + H]^+$, found 275.1601, HRMS (ESI) *m/z* calcd. for $[C_{12}H_{22}N_2O_5Na]^+$: 297.1421 $[M + Na]^+$, found 297.1420.

(c) β-*Alanyl-N-methylglycine methyl ester trifluoroacetate salt:* Prepared according to GP8 using 1.39 g (5.05 mmol) of *N*-Boc-β-alanyl-*N*-methylglycine methyl ester. A colourless oil used in the next step without further purification (quantitative yield). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 7.74 (s, 3H), 4.12 (s, 2H), 3.64 (s, 3H), 3.01 (s, 3H), 3.00–2.96 (m, 2H), 2.72 ppm (t, *J* = 6.6 Hz, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 170.5, 169.7, 51.8, 48.8, 35.9, 35.1, 29.8 ppm. HRMS (ESI) *m/z* calcd. for $[C_7H_{15}N_2O_3]^+$: 175.1077 $[M – C_2O_2F_3]^+$, found 175.1078, HRMS (ESI) *m/z* calcd. for $[C_7H_{14}N_2O_3Na]^+$: 197.0897 $[M – C_2HO_2F_3 + Na]^+$, found 197.0897.

(d) N-Phthaloylglycyl-β-*alanyl-N-methylglycine methyl ester:* Prepared according to GP4 method (c) using 0.93 g (4.54 mmol) of *N*-phthaloylglycine and 1.46 g (5.05 mmol) of β-alanyl-*N*-methylglycine methyl ester trifluoroacetate salt, purified by column chromatography (24:1 $CHCl_3$/MeOH, UV light). A white solid (0.57 g, 35%); mp 157.4–159.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 8.27 (t, *J* = 5.9 Hz, 1H), 7.92–7.89 (m, 2H), 7.88–7.87 (m, 2H), 4.18 (s, 2H), 4.08 (s, 2H), 3.64 (s, 3H), 3.27 (q, *J* = 6.0 Hz, 2H), 3.00 (s, 3H), 2.52 ppm (d, *J* = 6.9 Hz, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 171.2, 169.8, 167.5, 166.0, 134.6, 131.7, 123.2, 51.7, 48.8, 40.1, 36.0, 35.0, 32.2 ppm. HRMS (ESI) *m/z* calcd. for $[C_{17}H_{20}N_3O_6]^+$: 362.1347 $[M + H]^+$, found 362.1347, HRMS (ESI) *m/z* calcd. for $[C_{17}H_{19}N_3O_6Na]^+$: 384.1167 $[M + Na]^+$, found 384.1167.

_N_-Phthaloylglycyl-γ-aminobutyryl-_N_-methylglycine methyl ester (Phth-Gly-Gaba-(Me)Gly-OMe)

(a) N-Boc-γ-*aminobutyric acid:* Prepared according to GP7. A white solid (3.78 g, 93%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.01 (s, 1H), 6.80 (t, *J* = 5.4 Hz, 1H), 2.91 (q, *J* = 6.6 Hz, 2H), 2.19 (t, *J* = 7.4 Hz, 2H), 1.58 (p, *J* = 7.3 Hz, 2H), 1.37 ppm (s, 9H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 174.2, 155.6, 77.4, 39.3, 31.0, 28.3, 24.9 ppm. HRMS (ESI) *m/z* calcd. for $[C_9H_{18}NO_4]^+$: 204.1231 $[M + H]^+$, found 204.1231, HRMS (ESI) *m/z* calcd. for $[C_9H_{17}NO_4Na]^+$: 226.1050 $[M + Na]^+$, found 226.1049.

(b) N-Boc-γ-*aminobutyryl-N-methylglycine methyl ester:* Prepared according to GP4 method (c) using 1.09 g (5.36 mmol) of *N*-Boc-γ-aminobutyric acid and 0.85 g (6.07 mmol) of *N*-methylglycine methyl ester hydrochloride salt, purified using column chromatography (3:7 Pet. ether/EtOAc, ninhydrin stain). A colourless oil (1.32 g, 85%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 6.81 (t, *J* = 5.6 Hz, 1H), 4.06 (s, 2H), 3.63 (s, 3H), 3.00 (s, 3H), 2.93 (q, *J* = 7.4, 7.0 Hz, 2H), 2.32 (t, *J* = 7.4 Hz, 2H), 1.62–1.56 (m, 2H), 1.37 ppm (s, 9H). ^{13}C {^{1}H} NMR (101

MHz, DMSO-d_6) major isomer: δ 172.5, 169.9, 155.6, 77.4, 51.7, 48.9, 39.3, 36.1, 29.6, 28.5, 24.9 ppm. HRMS (ESI) *m/z* calcd. for $[C_{13}H_{25}N_2O_5]^+$: 289.1758 $[M + H]^+$, found 289.1758, HRMS (ESI) *m/z* calcd. for $[C_{13}H_{24}N_2O_5Na]^+$: 311.1578 $[M + Na]^+$, found 311.1577.

(c) γ*-Aminobutyryl-N-methylglycine methyl ester trifluoroacetate salt:* Prepared according to GP8 using 1.32 g (4.57 mmol) of *N*-Boc-γ-aminobutyryl-*N*-methylglycine methyl ester. A brown oil used in the next step without further purification (Quantitative yield). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 7.76 (s, 3H), 4.08 (s, 2H), 3.63 (s, 3H), 3.01 (s, 3H), 2.81–2.75 (m, 2H), 2.47 (t, *J* = 7.3 Hz, 2H), 1.76 ppm (p, *J* = 7.4 Hz, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 172.0, 169.9, 51.7, 49.0, 38.5, 36.0, 29.1, 22.5 ppm. HRMS (ESI) *m/z* calcd. for $[C_8H_{17}N_2O_3]^+$: 189.1234 $[M - C_2O_2F_3]^+$, found 189.1233, HRMS (ESI) *m/z* calcd. for $[C_{16}H_{33}N_4O_6]^+$: 377.2395 $[2M - C_4HO_4F_6]^+$, found 377.2392.

*(d) N-Phthaloylglycyl-*γ*-aminobutyryl-N-methylglycine methyl ester:* Prepared according to GP4 method (c) using 0.98 g (4.78 mmol) of *N*-phthaloylglycine and 1.38 g (4.57 mmol) of γ-aminobutyryl-*N*-methylglycine methyl ester trifluoroacetate salt, purified by column chromatography (24:1 $CHCl_3$/MeOH, UV light). A white solid (0.93 g, 54%); mp 141.9–143.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 8.22 (t, *J* = 5.5 Hz, 1H), 7.92–7.89 (m, 2H), 7.88–7.85 (m, 2H), 4.18 (s, 2H), 4.06 (s, 2H), 3.63 (s, 3H), 3.09 (q, *J* = 6.8 Hz, 2H), 3.00 (s, 3H), 2.35 (t, *J* = 7.4 Hz, 2H), 1.63 ppm (p, *J* = 7.4 Hz, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 172.5, 169.9, 167.6, 165.9, 134.5, 131.8, 123.2, 51.7, 48.9, 40.2, 38.3, 36.1, 29.5, 24.4 ppm. HRMS (ESI) *m/z* calcd. for $[C_{18}H_{22}N_3O_6]^+$: 376.1504 $[M + H]^+$, found 376.1507, HRMS (ESI) *m/z* calcd. for $[C_{18}H_{21}N_3O_6Na]^+$: 398.1323 $[M + Na]^+$, found 398.1324.

_N_-Phthaloylglycyl-δ-aminovaleryl-_N_-methylglycine methyl ester (Phth-Gly-Ava-(Me)Gly-OMe)

*(a) N-Boc-*δ*-aminovaleric acid:* Prepared according to GP7. A white solid (3.68 g, 84%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.97 (s, 1H), 6.77 (t, *J* = 5.8 Hz, 1H), 2.89 (q, *J* = 6.5 Hz, 2H), 2.19 (t, *J* = 7.2 Hz, 2H), 1.49–1.42 (m, 2H), 1.37 (s, 9H), 1.36–1.32 ppm (m, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 174.4, 155.6, 77.3, 39.5, 33.3, 29.0, 28.3, 21.8 ppm. HRMS (ESI) *m/z* calcd. for $[C_{10}H_{20}NO_4]^+$: 218.1387 $[M + H]^+$, found 218.0917, HRMS (ESI) *m/z* calcd. for $[C_{10}H_{19}NO_4Na]^+$: 240.1207 $[M + Na]^+$, found 240.1205.

*(b) N-Boc-*δ*-aminovaleryl-N-methylglycine methyl ester:* Prepared according to GP4 method (c), purified using column chromatography (3:7 Pet. ether/EtOAc, ninhydrin stain). A colourless oil (1.11 g, 64%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 6.77 (t, *J* = 6.1 Hz, 1H), 4.05 (s, 2H), 3.62 (s, 3H), 3.00 (s, 3H), 2.90 (q, *J* = 6.3, 2H), 2.32 (t, *J* = 7.1 Hz, 2H), 1.48–1.43 (m, 2H), 1.43–1.40 (m, 2H), 1.37 ppm (s, 9H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 172.8, 169.9, 155.6, 77.3, 51.6, 48.9, 39.6, 36.1, 31.8, 29.1, 28.3, 21.8 ppm. HRMS (ESI) *m/z* calcd. for $[C_{14}H_{27}N_2O_5]^+$: 303.1915 $[M + H]^+$, found 303.1913, HRMS (ESI) *m/z* calcd. for $[C_{14}H_{26}N_2O_5Na]^+$: 325.1734 $[M + Na]^+$, found 325.1731.

(c) δ*-Aminovaleryl-N-methylglycine methyl ester trifluoroacetate salt:* Prepared according to GP8 using 1.11 g (3.66 mmol) of *N*-Boc-δ-aminovaleryl-*N*-methylglycine methyl ester. A colourless oil used in the next step without further purification (Quantitative yield). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 7.77 (s, 3H), 4.07 (s, 2H), 3.63 (s, 3H), 3.01 (s, 3H), 2.79–

2.74 (m, 2H), 2.38 (t, *J* = 6.5 Hz, 2H), 1.56–1.54 (m, 2H), 1.52–1.49 ppm (m, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 172.6, 170.0, 51.7, 49.0, 38.7, 36.1, 31.5, 26.7, 21.2 ppm. HRMS (ESI) *m/z* calcd. for $[C_9H_{19}N_2O_3]^+$: 203.1391 [M – $C_2O_2F_3$]$^+$, found 203.1391.

(d) N-Phthaloylglycyl-δ-aminovaleryl-N-methylglycine methyl ester: Prepared according to GP4 method (c) using 0.80 g (3.90 mmol) of *N*-phthaloylglycine and 1.32 g (4.17 mmol) of δ-aminovaleryl-*N*-methylglycine methyl ester trifluoroacetate salt, purified by column chromatography (24:1 $CHCl_3$/MeOH, UV light). A white solid (0.12 g, 8%); mp 129.1–131.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 8.21 (t, *J* = 5.8 Hz, 1H), 7.92–7.89 (m, 2H), 7.88–7.85 (m, 2H), 4.17 (s, 2H), 4.05 (s, 2H), 3.63 (s, 3H), 3.07 (q, *J* = 6.2 Hz, 2H), 3.01 (s, 3H), 2.34 (t, *J* = 7.0 Hz, 2H), 1.51–1.46 (m, 2H), 1.43–1.39 ppm (m, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 172.7, 169.9, 167.6, 165.8, 134.5, 131.8, 123.2, 51.7, 48.9, 40.2, 38.5, 36.1, 31.7, 28.6, 21.8 ppm. HRMS (ESI) *m/z* calcd. for $[C_{19}H_{24}N_3O_6]^+$: 390.1660 [M + H]$^+$, found 390.1660, HRMS (ESI) *m/z* calcd. for $[C_{19}H_{23}N_3O_6Na]^+$: 412.1480 [M + Na]$^+$, found 412.1479.

_N_-Phthaloylglycyl-ε-aminocaproyl-_N_-methylglycine methyl ester (Phth-Gly-Ahx-(Me)Gly-OMe)

(a) N-Boc-ε-aminocaproic acid: Prepared according to GP7. A white solid (4.34 g, 90%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.96 (s, 1H), 6.75 (t, *J* = 5.7 Hz, 1H), 2.88 (q, *J* = 6.6 Hz, 2H), 2.18 (t, *J* = 7.4 Hz, 2H), 1.47 (p, *J* = 7.5 Hz, 2H), 1.37 (s, 9H), 1.35–1.31 (m, 2H), 1.22 ppm (p, *J* = 7.9, 7.4 Hz, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 174.4, 155.6, 77.3, 39.7, 33.6, 29.2, 28.3, 25.9, 24.2 ppm. HRMS (ESI) *m/z* calcd. for $[C_{11}H_{22}NO_4]^+$: 232.1544 [M + H]$^+$, found 232.1540, HRMS (ESI) *m/z* calcd. for $[C_{11}H_{21}NO_4Na]^+$: 254.1363 [M + Na]$^+$, found 254.1359.

(b) N-Boc-ε-aminocaproyl-N-methylglycine methyl ester: Prepared according to GP4 method (c) using 1.29 g (5.58 mmol) of *N*-Boc-ε-aminocaproic acid and 0.88 g (6.33 mmol) of *N*-methylglycine methyl ester hydrochloride salt, purified using column chromatography (3:7 Pet. ether/EtOAc, ninhydrin stain). A colourless oil (1.49 g, 85%). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 6.74 (t, *J* = 5.2 Hz, 1H), 4.05 (s, 2H), 3.63 (s, 3H), 3.01 (s, 3H), 2.89 (q, *J* = 6.6, 2H), 2.31 (t, *J* = 7.4 Hz, 2H), 1.54–1.46 (m, 2H), 1.46–1.41 (m, 2H), 1.37 (s, 9H), 1.29–1.22 ppm (m, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 172.8, 169.9, 155.6, 77.3, 51.6, 48.9, 39.7, 36.1, 32.1, 29.4, 28.3, 25.9, 24.2 ppm. HRMS (ESI) *m/z* calcd. for $[C_{15}H_{29}N_2O_5]^+$: 317.2071 [M + H]$^+$, found 317.2064, HRMS (ESI) *m/z* calcd. for $[C_{15}H_{28}N_2O_5Na]^+$: 339.1891 [M + Na]$^+$, found 339.1882.

(c) ε-Aminocaproyl-N-methylglycine methyl ester trifluoroacetate salt: Prepared according to GP8 using 1.49 g (4.72 mmol) of *N*-Boc-ε-aminocaproyl-*N*-methylglycine methyl ester. A yellow oil used in the next step without further purification (Quantitative yield). ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 7.74 (s, 3H), 4.06 (s, 2H), 3.63 (s, 3H), 3.01 (s, 3H), 2.78–2.74 (m, 2H), 2.33 (t, *J* = 7.2 Hz, 2H), 1.55–1.51 (m, 2H), 1.48–1.44 (m, 2H), 1.34–1.29 ppm (m, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 172.7, 170.0, 51.7, 48.9, 38.7, 36.1, 31.8, 26.8, 25.4, 23.9 ppm. HRMS (ESI) *m/z* calcd. for $[C_{10}H_{21}N_2O_3]^+$: 217.1547 [M − $C_2O_2F_3$]$^+$, found 217.1542, HRMS (ESI) *m/z* calcd. for $[C_{20}H_{41}N_4O_6]^+$: 433.3021 [2M – $C_4HO_4F_6$]$^+$, found 433.3011.

(d) N-Phthaloylglycyl-ε-aminocaproyl-N-methylglycine methyl ester: Prepared according to GP4 method (c) using 1.01 g (4.94 mmol) of *N*-phthaloylglycine and 1.56 g (4.72 mmol) of ε-aminocaproyl-*N*-methylglycine methyl ester trifluoroacetate salt, recrystallised from hot acetonitrile. A white solid (1.34 g, 70%); mp 149.0–151.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) major isomer: δ 8.18 (t, *J* = 5.1 Hz, 1H), 7.92–7.89 (m, 2H), 7.88–7.85 (m, 2H), 4.17 (s, 2H), 4.06 (s, 2H), 3.63 (s, 3H), 3.07–3.04 (m, 2H), 3.01 (s, 3H), 2.32 (t, *J* = 7.4 Hz, 2H), 1.51–1.46 (m, 2H), 1.43–1.35 (m, 2H), 1.32–1.22 ppm (m, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) major isomer: δ 172.7, 169.9, 167.5, 165.7, 134.5, 131.8, 123.2, 51.6, 48.9, 40.1, 38.6, 36.1, 32.1, 28.8, 25.9, 24.1 ppm. HRMS (ESI) *m/z* calcd. for $[C_{20}H_{26}N_3O_6]^+$: 404.1817 $[M + H]^+$, found 404.1814, HRMS (ESI) *m/z* calcd. for $[C_{20}H_{25}N_3O_6Na]^+$: 426.1636 $[M + Na]^+$, found 426.1632.

N-Phthaloylglycylglycine methyl ester (Phth-Gly-Gly-OMe)

(a) Glycine methyl ester hydrochloride salt: Prepared according to GP2. A white solid (9.64 g, 97%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.58 (s, 3H), 3.76 (s, 2H), 3.72 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 168.0, 52.5, 39.5 ppm.

(b) N-Phthaloylglycylglycine methyl ester: Prepared according to GP4 method (d), recrystallised from hot ethyl acetate. A white solid (1.91 g, 69%); mp 203.2–204.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.71 (t, *J* = 5.9 Hz, 1H), 7.95–7.86 (m, 2H), 7.92–7.83 (m, 2H), 4.26 (s, 2H), 3.88 (d, *J* = 5.8 Hz, 2H), 3.63 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 170.0, 167.4, 166.7, 134.6, 131.7, 123.2, 51.8, 40.6, 39.9 ppm. HRMS (ESI) *m/z* calcd. for $[C_{13}H_{13}N_2O_5]^+$: 277.0819 $[M + H]^+$, found 277.0819, HRMS (ESI) *m/z* calcd. for $[C_{13}H_{12}N_2O_5Na]^+$: 299.0639 $[M + Na]^+$, found 299.0637.

N-Phthaloylglycyl-β-alanine methyl ester (Phth-Gly-βAla-OMe)

(a) β*-Alanine methyl ester hydrochloride salt:* Prepared according to GP2 using 2.89 g (32.5 mmol) of β-alanine. A white solid (4.50 g, 99%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.23 (s, 3H), 3.63 (s, 3H), 2.99 (h, *J* = 6.4 Hz, 2H), 2.72 ppm (t, *J* = 7.1 Hz, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 170.7, 51.8, 34.6, 31.3 ppm. HRMS (ESI) *m/z* calcd. for $[C_4H_{10}NO_2]^+$: 104.0707 $[M – Cl]^+$, found 104.0709.

*(b) N-Phthaloylglycyl-*β*-alanine methyl ester:* Prepared according to GP4 method (c), recrystallised from hot acetonitrile. White needles (0.62 g, 38%); mp 179.9–182.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.34 (t, *J* = 5.6 Hz, 1H), 7.93–7.88 (m, 2H), 7.88–7.85 (m, 2H), 4.16 (s, 2H), 3.60 (s, 3H), 3.29 (q, *J* = 6.6 Hz, 2H), 2.47 ppm (t, *J* = 6.8 Hz, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 171.7, 167.5, 166.1, 134.6, 131.8, 123.2, 51.5, 40.1, 34.9, 33.5 ppm. HRMS (ESI) *m/z* calcd. for $[C_{14}H_{15}N_2O_5]^+$: 291.0976 $[M + H]^+$, found 291.0976, HRMS (ESI) *m/z* calcd. for $[C_{14}H_{14}N_2O_5Na]^+$: 313.0795 $[M + Na]^+$, found 313.0796.

N-Phthaloylglycyl-γ-aminobutyric acid methyl ester (Phth-Gly-Gaba-OMe)

(a) γ*-Aminobutyric acid methyl ester hydrochloride salt:* Prepared according to GP2 using 3.31 g (32.1 mmol) of γ-aminobutyric acid. A white solid (4.84 g, 98%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.16 (s, 3H), 3.59 (s, 3H), 2.78 (h, *J* = 5.7 Hz, 2H), 2.44 (t, *J* = 7.5 Hz, 2H), 1.81 ppm (p, *J* = 7.5 Hz, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 172.7, 51.4, 38.0, 30.2, 22.4 ppm. HRMS (ESI) *m/z* calcd. for $[C_5H_{12}NO_2]^+$: 118.0863 $[M – Cl]^+$, found 118.0865.

(b) N-Phthaloylglycyl-γ-aminobutyric acid methyl ester: Prepared according to GP4 method (c), recrystallised from hot acetonitrile. White needles (0.74 g, 43%); mp 161.0–163.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.23 (t, *J* = 5.7 Hz, 1H), 7.93–7.89 (m, 2H), 7.88–7.85 (m, 2H), 4.17 (s, 2H), 3.58 (s, 3H), 3.08 (q, *J* = 6.5 Hz, 2H), 2.31 (t, *J* = 7.4 Hz, 2H), 1.65 ppm (p, *J* = 7.2 Hz, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 173.1, 167.6, 166.0, 134.5, 131.8, 123.2, 51.3, 40.2, 38.0, 30.6, 24.4 ppm. HRMS (ESI) *m/z* calcd. for $[C_{15}H_{17}N_2O_5]^+$: 305.1132 $[M + H]^+$, found 305.1129, HRMS (ESI) *m/z* calcd. for $[C_{15}H_{16}N_2O_5Na]^+$: 327.0952 $[M + Na]^+$, found 327.0947.

_N_-Phthaloylglycyl-δ-aminovaleric acid methyl ester (Phth-Gly-Ava-OMe)

(a) δ-Aminovaleric acid methyl ester hydrochloride salt: Prepared according to GP2 using 3.70 g (31.5 mmol) of δ-aminovaleric acid. A white solid (4.34 g, 82%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.98 (s, 3H), 3.59 (s, 3H), 2.80–2.70 (m, 2H), 2.34 (t, *J* = 6.3 Hz, 2H), 1.59–1.54 ppm (m, 4H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 173.1, 51.3, 38.4, 32.7, 26.4, 21.4 ppm. HRMS (ESI) *m/z* calcd. for $[C_6H_{14}NO_2]^+$: 132.1020 $[M – Cl]^+$, found 132.1021.

(b) N-Phthaloylglycyl-δ-aminovaleric acid methyl ester: Prepared according to GP4 method (c), recrystallised from hot acetonitrile. White crystals (0.55 g, 32%); mp 173.9–175.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20 (t, *J* = 5.7 Hz, 1H), 7.92–7.89 (m, 2H), 7.88–7.85 (m, 2H), 4.16 (s, 2H), 3.58 (s, 3H), 3.06 (q, *J* = 6.5 Hz, 2H), 2.31 (t, *J* = 7.3 Hz, 2H), 1.55–1.47 (m, 2H), 1.44–1.37 ppm (m, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 173.2, 167.5, 165.8, 134.5, 131.8, 123.2, 51.2, 40.1, 38.3, 32.9, 28.4, 21.8 ppm. HRMS (ESI) *m/z* calcd. for $[C_{16}H_{19}N_2O_5]^+$: 319.1289 $[M + H]^+$, found 319.1285, HRMS (ESI) *m/z* calcd. for $[C_{16}H_{18}N_2O_5Na]^+$: 341.1108 $[M + Na]^+$, found 341.1105.

_N_-Phthaloylglycyl-ε-aminocaproic acid methyl ester (Phth-Gly-Ahx-OMe)

(a) ε-Aminocaproic acid methyl ester hydrochloride salt: Prepared according to GP2 using 4.22 g (32.2 mmol) of ε-aminocaproic acid. A white solid (5.85 g, 100%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.06 (s, 3H), 3.58 (s, 3H), 2.72 (q, *J* = 6.6 Hz, 2H), 2.30 (t, *J* = 7.4 Hz, 2H), 1.54 (dp, *J* = 12.7, 7.5 Hz, 4H), 1.38–1.23 ppm (m, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 173.2, 51.3, 38.5, 33.1, 26.7, 25.3, 23.9 ppm. HRMS (ESI) *m/z* calcd. for $[C_7H_{16}NO_2]^+$: 146.1176 $[M – Cl]^+$, found 146.1176.

(b) N-Phthaloylglycyl-ε-aminocaproic acid methyl ester: Prepared according to GP4 method (c), recrystallised from hot acetonitrile. A white solid (1.24 g, 67%); mp 169.5–171.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.18 (t, *J* = 5.6 Hz, 1H), 7.92–7.88 (m, 2H), 7.88–7.85 (m, 2H), 4.16 (s, 2H), 3.58 (s, 3H), 3.04 (q, *J* = 6.6 Hz, 2H), 2.28 (t, *J* = 7.4 Hz, 2H), 1.51 (p, *J* = 7.5 Hz, 2H), 1.39 (p, *J* = 7.1 Hz, 2H), 1.24 ppm (p, *J* = 7.3, 6.4 Hz, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 173.3, 167.6, 165.8, 134.5, 131.8, 123.2, 51.2, 40.1, 38.5, 33.2, 28.6, 25.8, 24.1 ppm. HRMS (ESI) *m/z* calcd. for $[C_{17}H_{21}N_2O_5]^+$: 333.1445 $[M + H]^+$, found 333.1439, HRMS (ESI) *m/z* calcd. for $[C_{17}H_{20}N_2O_5Na]^+$: 355.1265 $[M + Na]^+$, found 355.1257.

_N_-Phthaloylglycine methyl ester (Phth-Gly-OMe)

Prepared according to GP3 using 1.24 g (6.06 mmol) of *N*-phthaloylglycine. A white powder (1.21 g, 91%); mp 110.7–114.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.98–7.90 (m, 2H), 7.94–7.86 (m, 2H), 4.45 (s, 2H), 3.70 ppm (s, 3H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 168.0, 167.1,

134.9, 131.3, 123.5, 52.5, 38.7 ppm. HRMS (ESI) *m*/*z* calcd. for $[C_{11}H_{10}NO_4]^+$: 220.0605 [M + H]$^+$, found 220.0603, HRMS (ESI) *m*/*z* calcd. for $[C_{11}H_9NO_4Na]^+$: 242.0424 [M + Na]$^+$, found 242.0422.

_N_-Phthaloyl-β-alanine methyl ester (Phth-βAla-OMe)

Prepared according to GP3 using 0.62 g (2.83 mmol) of *N*-phthaloyl-β-alanine. A white solid (0.56 g, 84%); mp 72.8–74.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.89–7.85 (m, 2H), 7.85–7.82 (m, 2H), 3.82 (t, *J* = 7.2 Hz, 2H), 3.57 (s, 3H), 2.68 ppm (t, *J* = 7.2 Hz, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 171.0, 167.6, 134.3, 131.6, 123.1, 51.6, 33.5, 32.2 ppm. HRMS (ESI) *m*/*z* calcd. for $[C_{12}H_{12}NO_4]^+$: 234.0761 [M + H]$^+$, found 234.0760, HRMS (ESI) *m*/*z* calcd. for $[C_{12}H_{11}NO_4Na]^+$: 256.0580 [M + Na]$^+$, found 256.0579.

_N_-Phthaloyl-γ-aminobutyric acid methyl ester (Phth-Gaba-OMe)

Prepared according to GP3 using 0.71 g (3.02 mmol) of *N*-phthaloyl-γ-aminobutyric acid. A white solid (0.69 g, 93%); mp 88.3–90.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.87–7.85 (m, 2H), 7.84–7.81 (m, 2H), 3.60 (t, *J* = 6.8 Hz, 2H), 3.52 (s, 3H), 2.36 (t, *J* = 7.2 Hz, 2H), 1.85 ppm (p, *J* = 7.0 Hz, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 172.8, 168.0, 134.3, 131.7, 122.9, 51.2, 36.7, 30.6, 23.2 ppm. HRMS (ESI) *m*/*z* calcd. for $[C_{13}H_{14}NO_4]^+$: 248.0918 [M + H]$^+$, found 248.0917, HRMS (ESI) *m*/*z* calcd. for $[C_{13}H_{13}NO_4Na]^+$: 270.0737 [M + Na]$^+$, found 270.0735.

_N_-Phthaloyl-δ-aminovaleric acid methyl ester (Phth-Ava-OMe)

Prepared according to GP3 using 0.95 g (3.84 mmol) of *N*-phthaloyl-δ-aminovaleric acid. White crystals (0.83 g, 83%); mp 38.7–40.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.88–7.81 (m, 4H), 3.59–3.56 (m, 2H), 3.56 (s, 3H), 2.33 (t, *J* = 7.1 Hz, 2H), 1.59 (ddt, *J* = 11.0, 7.1, 3.2 Hz, 2H), 1.52 ppm (tdd, *J* = 7.2, 4.9, 1.5 Hz, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 173.1, 168.0, 134.4, 131.6, 123.0, 51.2, 37.0, 32.7, 27.3, 21.7 ppm. HRMS (ESI) *m*/*z* calcd. for $[C_{14}H_{16}NO_4]^+$: 262.1074 [M + H]$^+$, found 262.1074, HRMS (ESI) *m*/*z* calcd. for $[C_{14}H_{15}NO_4Na]^+$: 284.0894 [M + Na]$^+$, found 284.0892.

_N_-Phthaloyl-ε-aminocaproic acid methyl ester (Phth-Ahx-OMe)

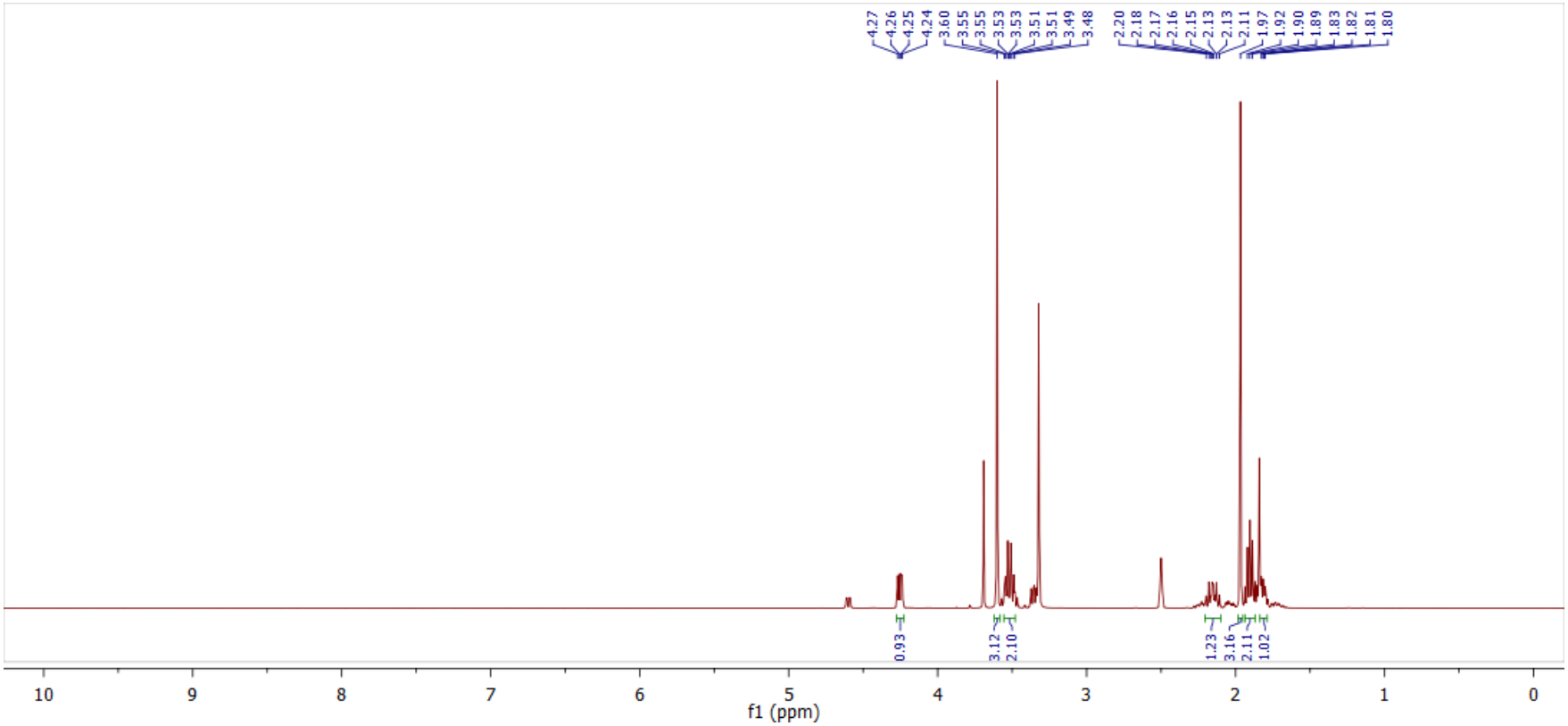
Prepared according to GP3 using 0.89 g (3.40 mmol) of *N*-phthaloyl-ε-aminocaproic acid. A white solid (0.67 g, 72%); mp 47.1–48.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.87–7.85 (m, 2H), 7.84–7.81 (m, 2H), 3.55 (s, 3H), 3.58–3.53 (m, 2H), 2.28 (t, *J* = 7.4 Hz, 2H), 1.62–1.56 (m, 2H), 1.56–1.49 (m, 2H), 1.31–1.22 ppm (m, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 173.2, 167.9, 134.3, 131.6, 123.0, 51.1, 37.2, 33.1, 27.6, 25.6, 24.0 ppm. HRMS (ESI) *m*/*z* calcd. for $[C_{15}H_{18}NO_4]^+$: 276.1231 [M + H]$^+$, found 276.1230, HRMS (ESI) *m*/*z* calcd. for $[C_{15}H_{17}NO_4Na]^+$: 298.1050 [M + Na]$^+$, found 298.1046.

4. Spectra and chromatograms of substrates used in the laser flash photolysis study

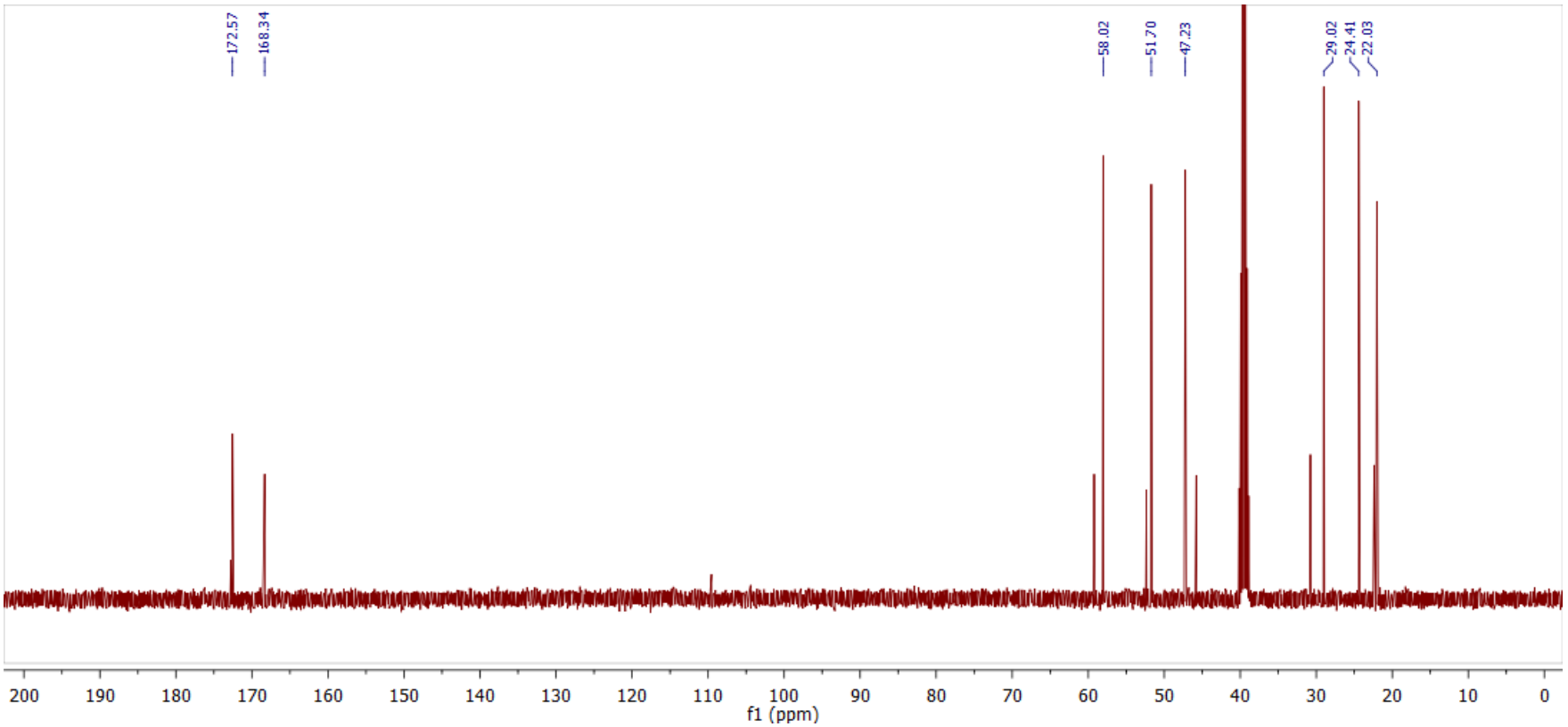
4.1. ^{1}H NMR and ^{13}C NMR spectra

4.1.1. *N*-Acetyl-L-proline methyl ester (Ac-Pro-OMe)

^{1}H NMR spectrum of Ac-Pro-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 3.5 : 1.

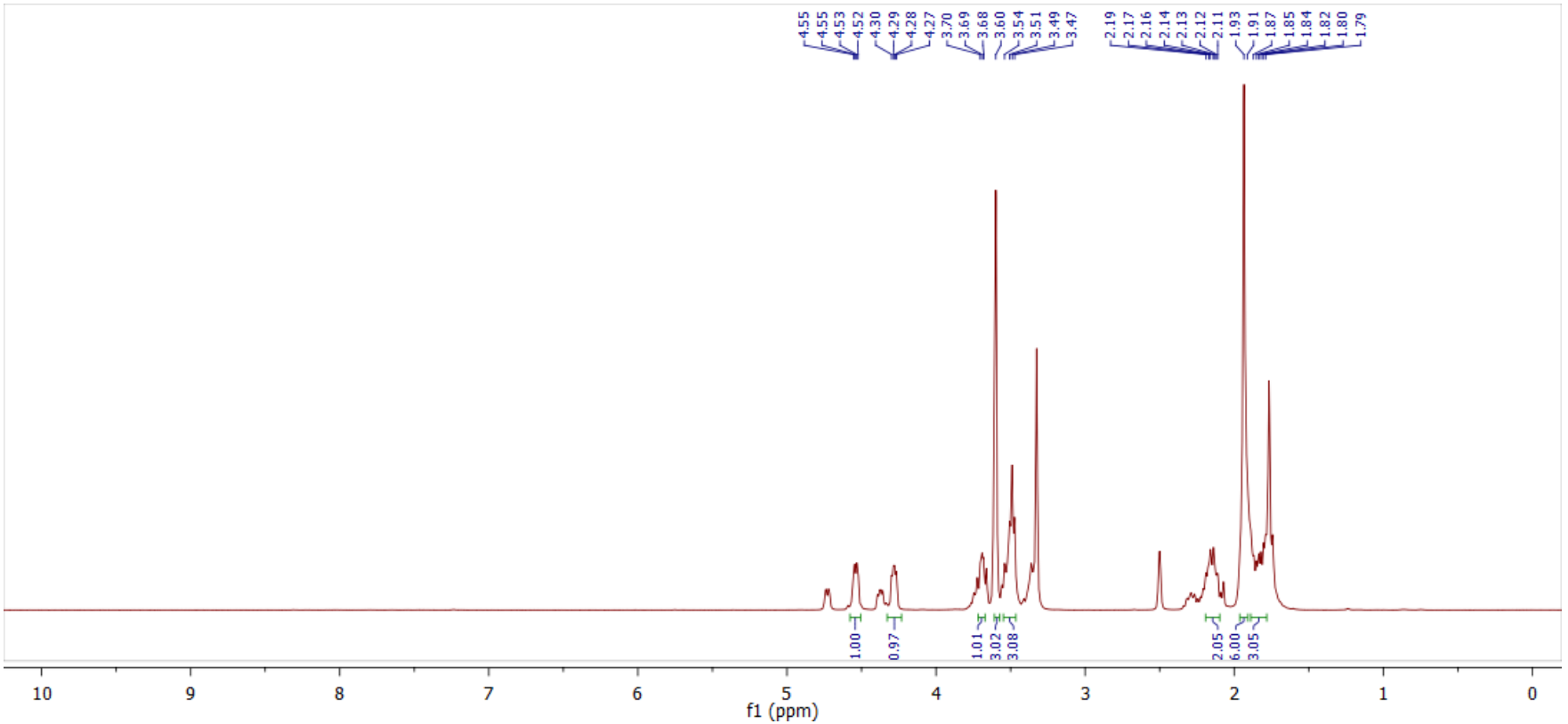


^{13}C NMR spectrum of Ac-Pro-OMe

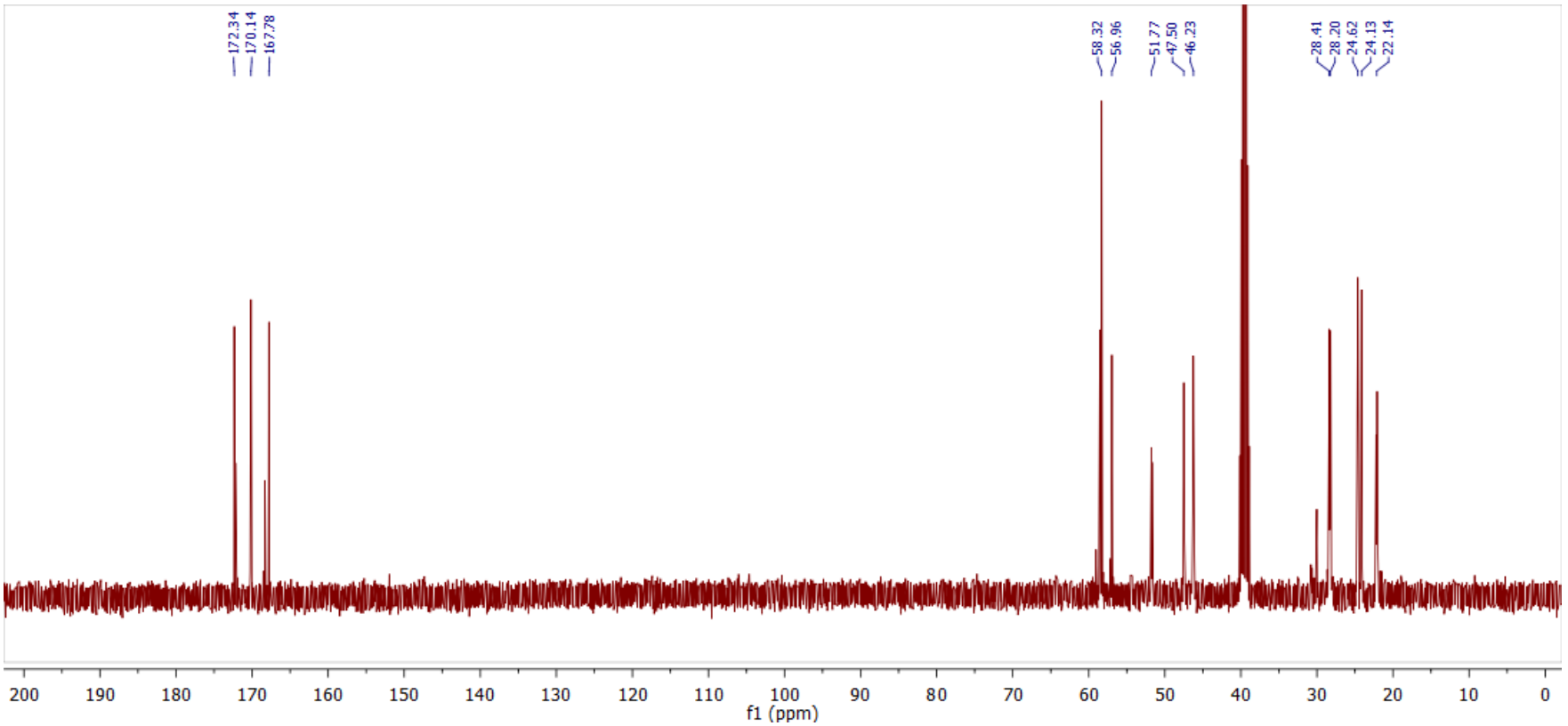


4.1.2. *N*-Acetyl-L-prolyl-L-proline methyl ester (Ac-Pro-Pro-OMe)

^{1}H NMR spectrum of Ac-Pro-Pro-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 1.5 : 1.

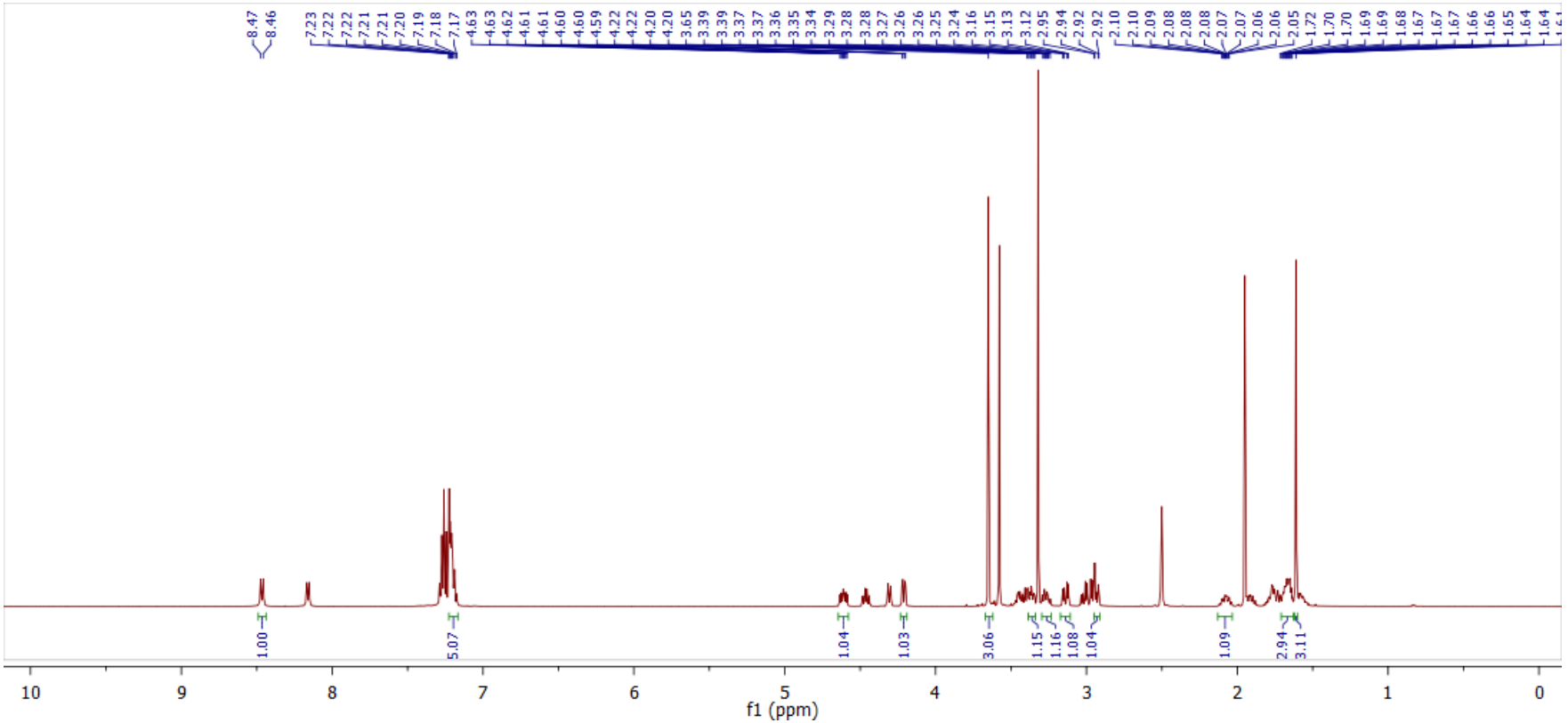


^{13}C NMR spectrum of Ac-Pro-Pro-OMe

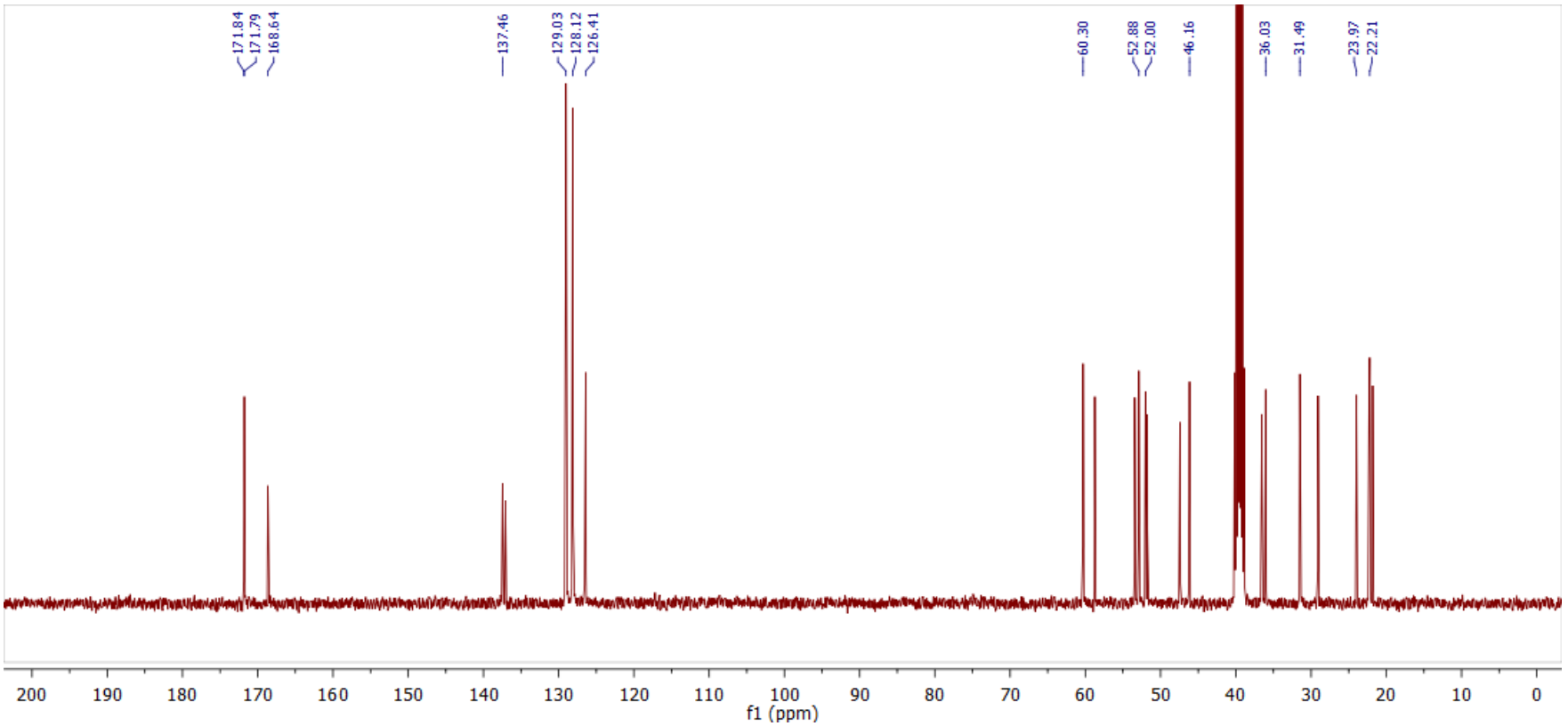


4.1.3. *N*-Acetyl-L-prolyl-L-phenylalanine methyl ester (Ac-Pro-Phe-OMe)

^{1}H NMR spectrum of Ac-Pro-Phe-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 1.25 : 1.

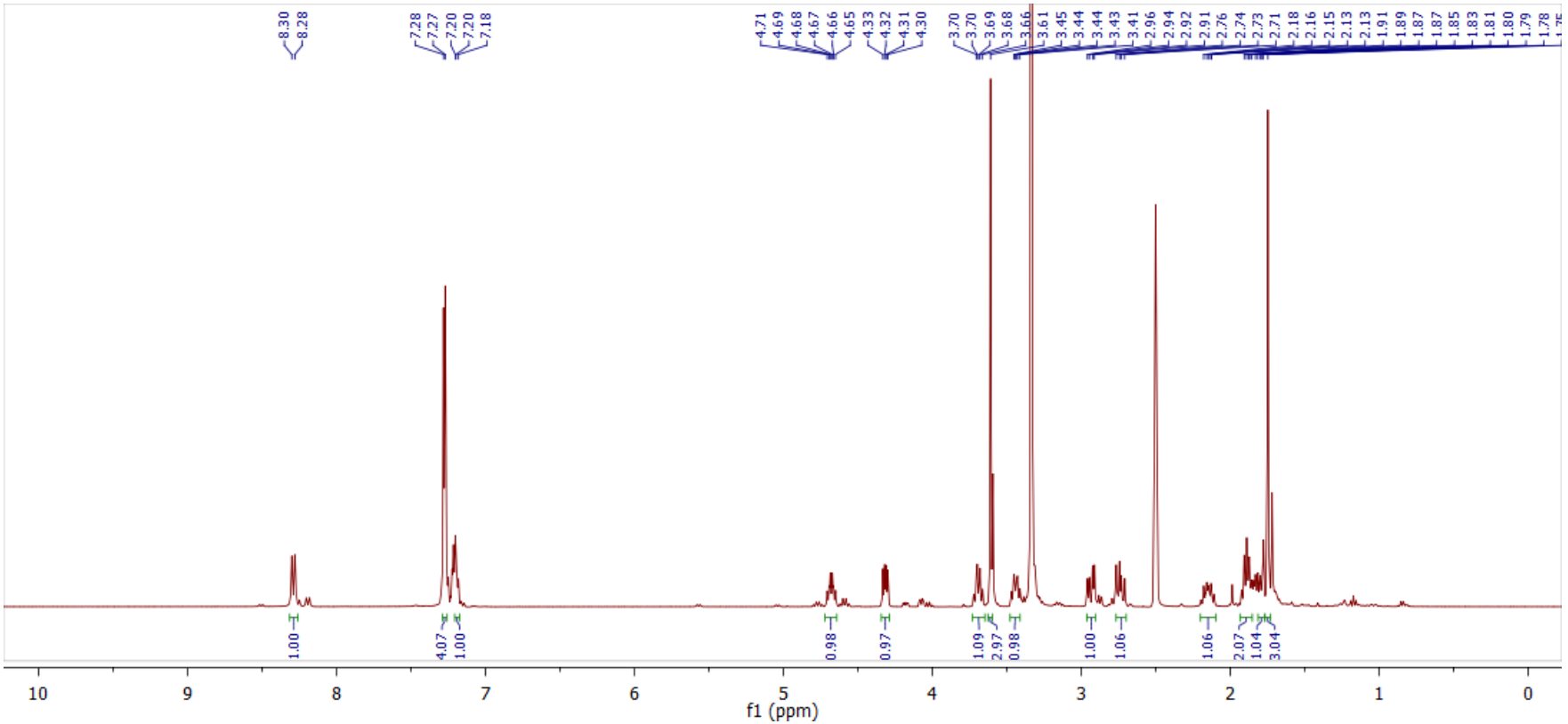


^{13}C NMR spectrum of Ac-Pro-Phe-OMe

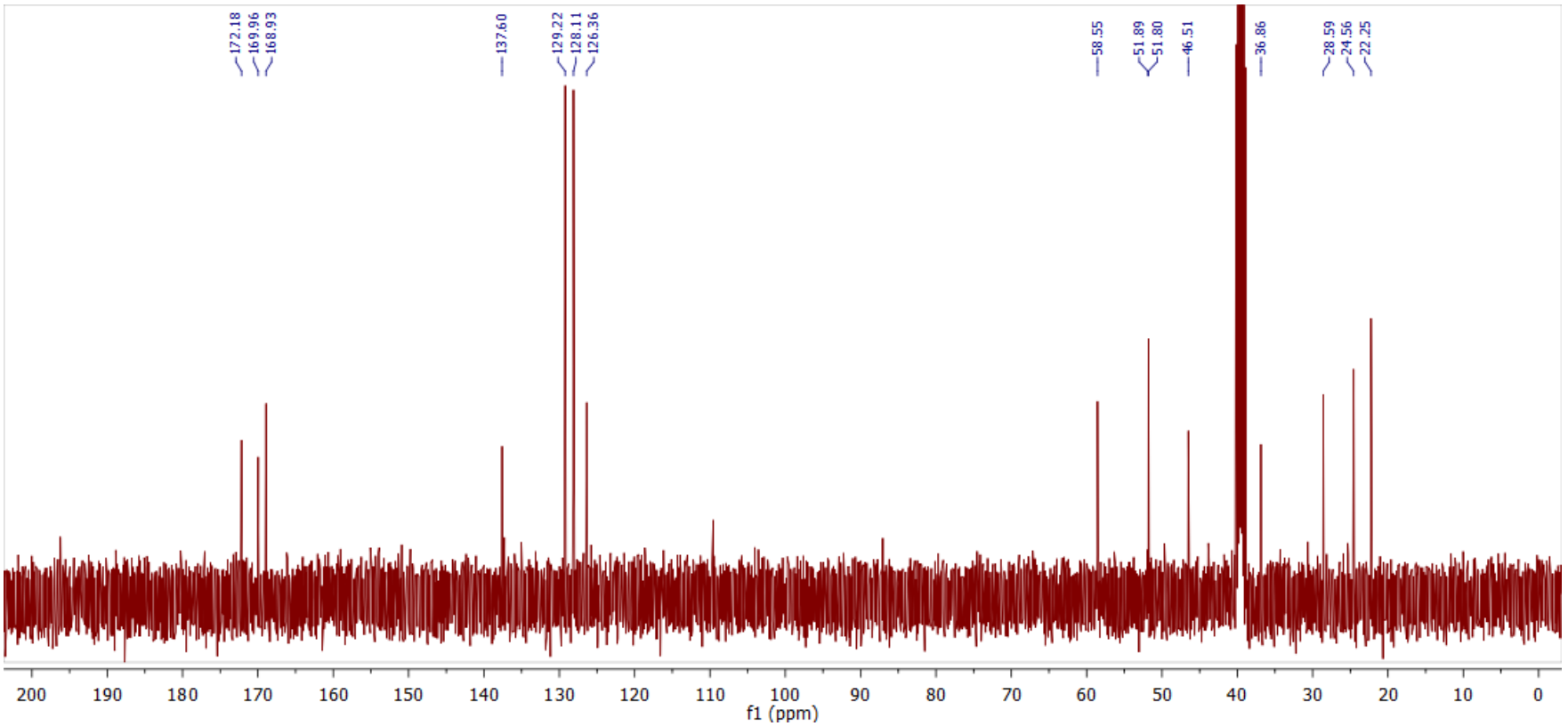


4.1.4. *N*-Acetyl-L-phenylalanyl-L-proline methyl ester (Ac-Phe-Pro-OMe)

^{1}H NMR spectrum of Ac-Phe-Pro-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 6.7 : 1.

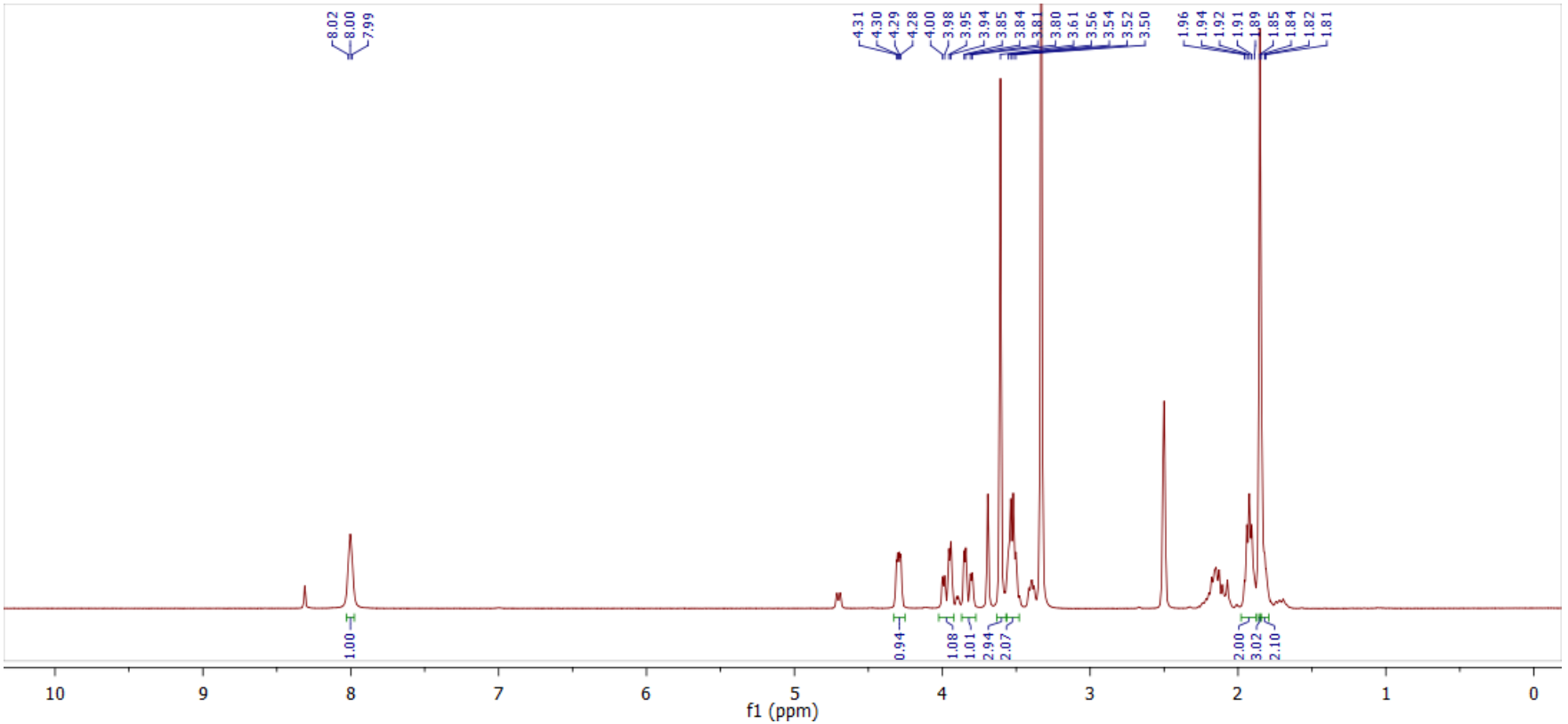


^{13}C NMR spectrum of Ac-Phe-Pro-OMe

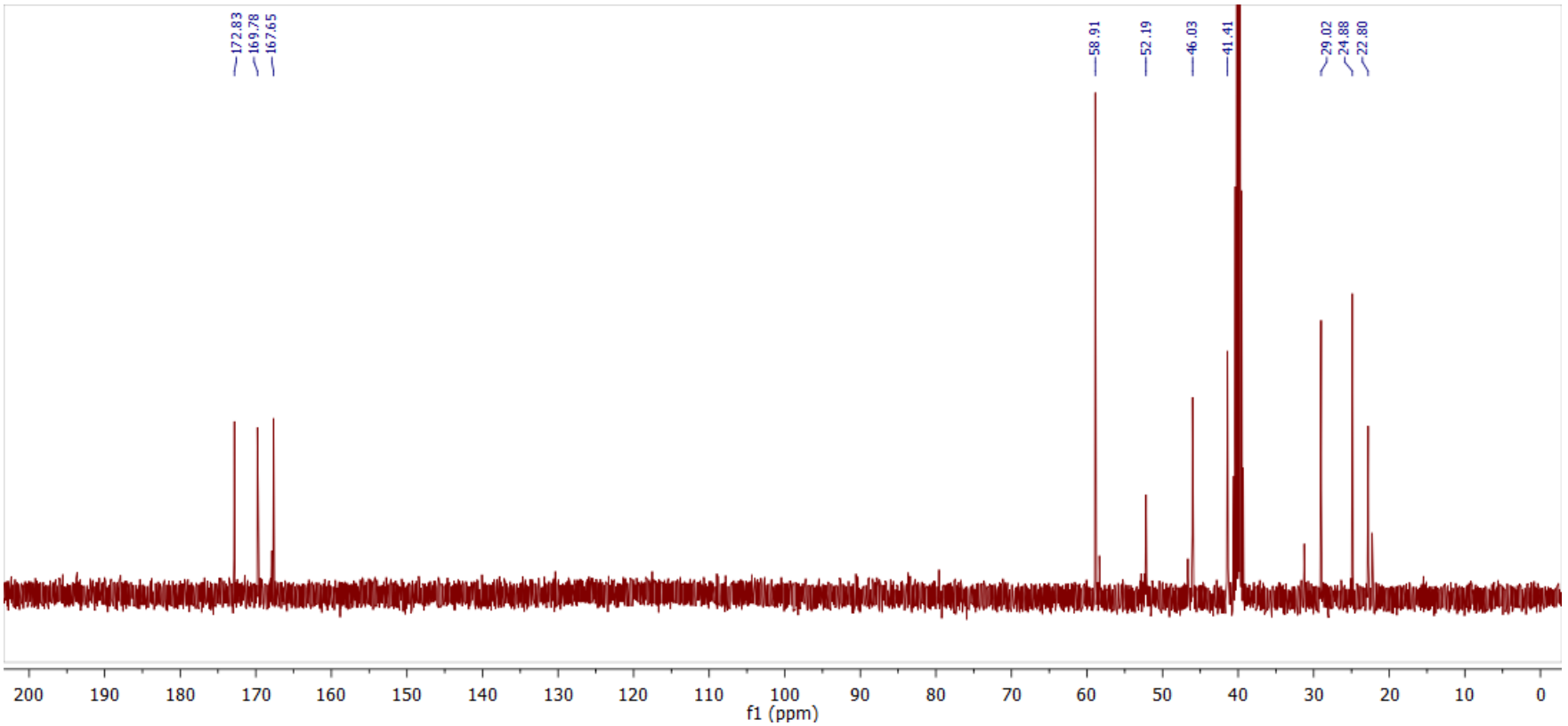


4.1.5. *N*-Acetyl-glycine-L-proline methyl ester (Ac-Gly-Pro-OMe)

^{1}H NMR spectrum of Ac-Gly-Pro-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 5 : 1.

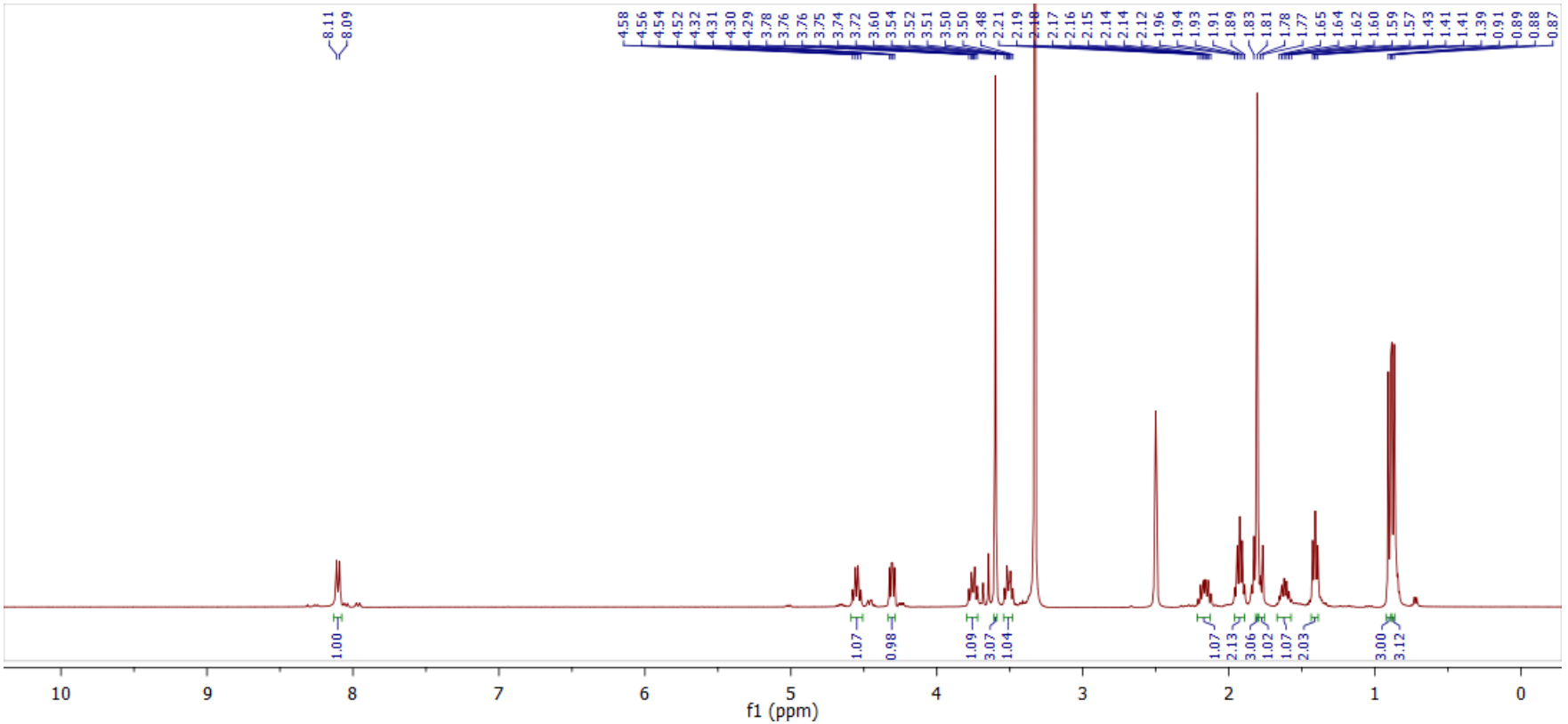


^{13}C NMR spectrum of Ac-Gly-Pro-OMe

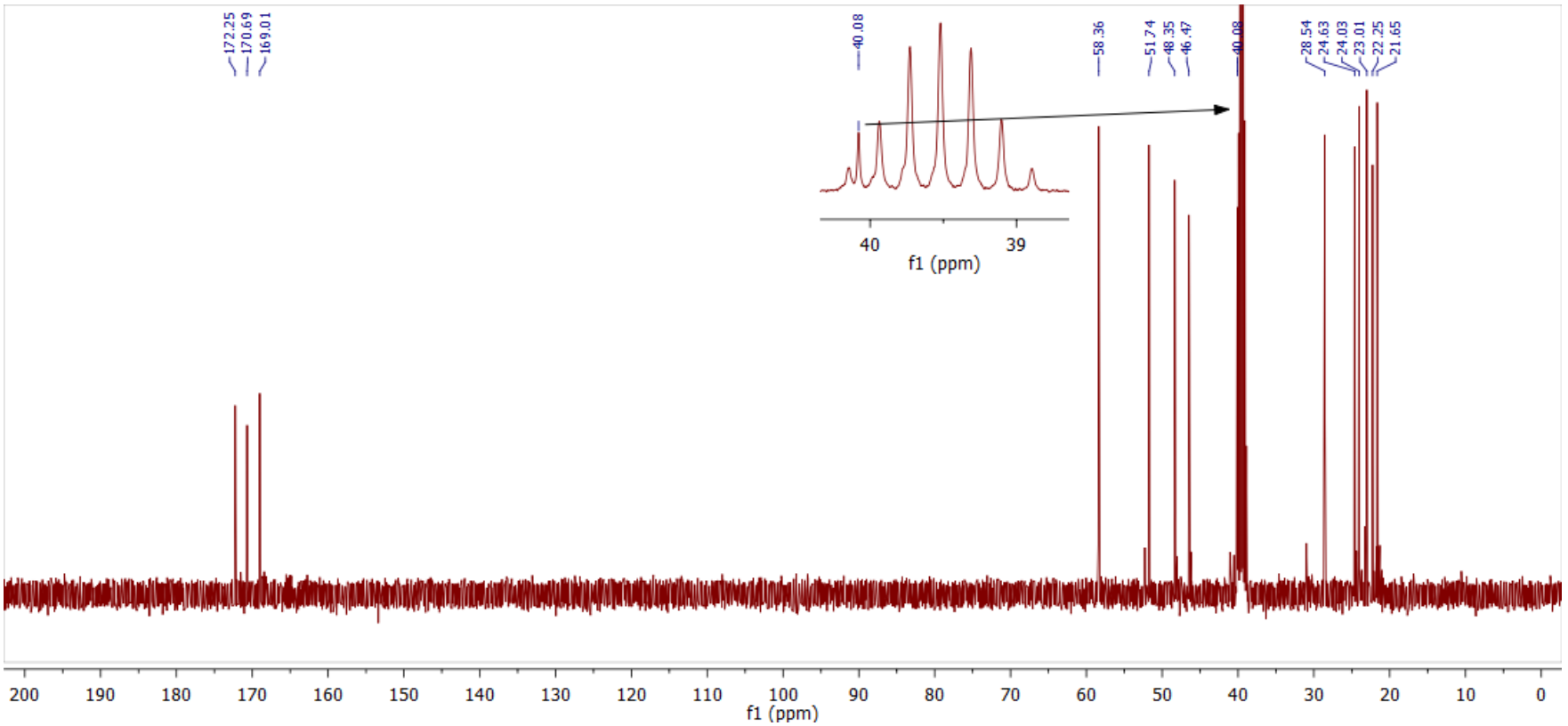


4.1.6. *N*-Acetyl-L-leucyl-L-proline methyl ester (Ac-Leu-Pro-OMe)

^{1}H NMR spectrum of Ac-Leu-Pro-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 10 : 1.

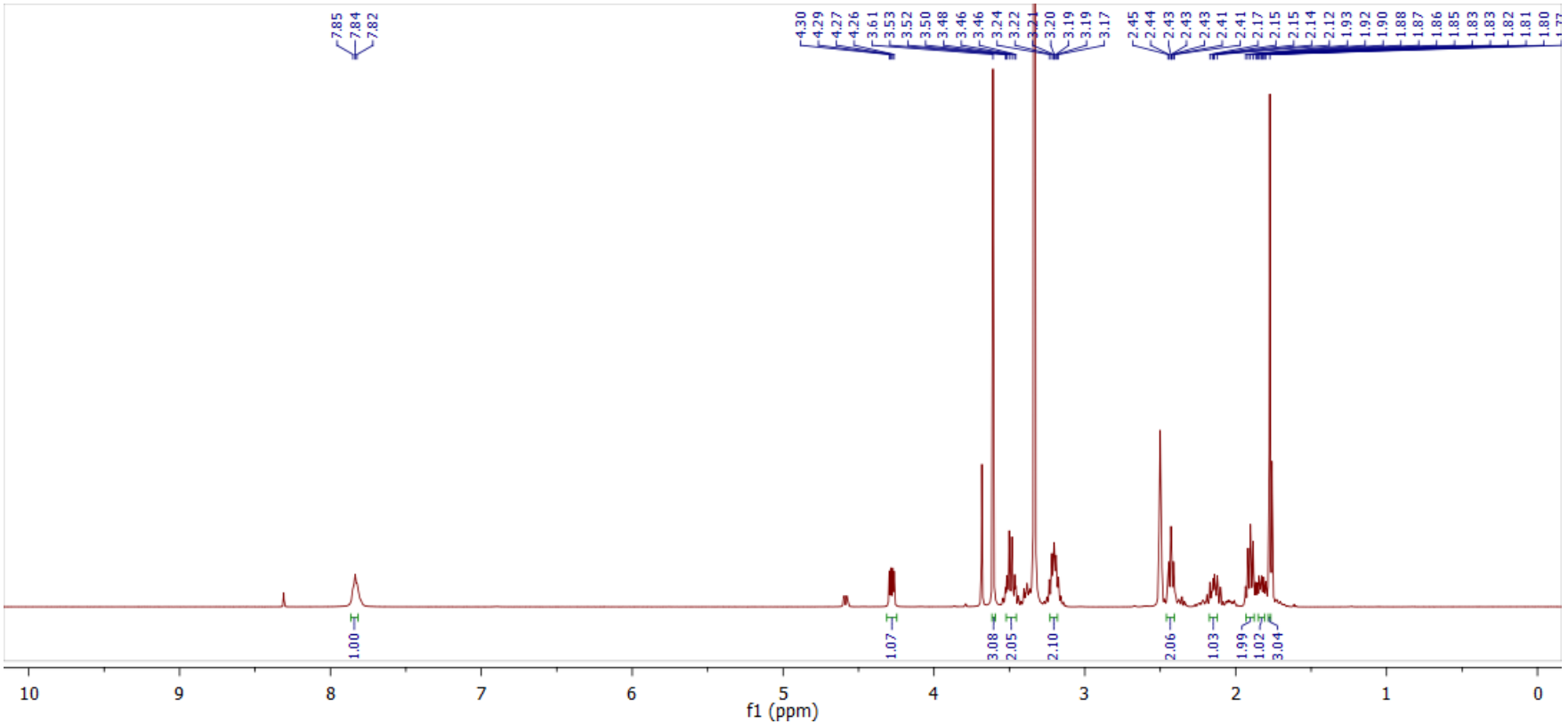


^{13}C NMR spectrum of Ac-Leu-Pro-OMe

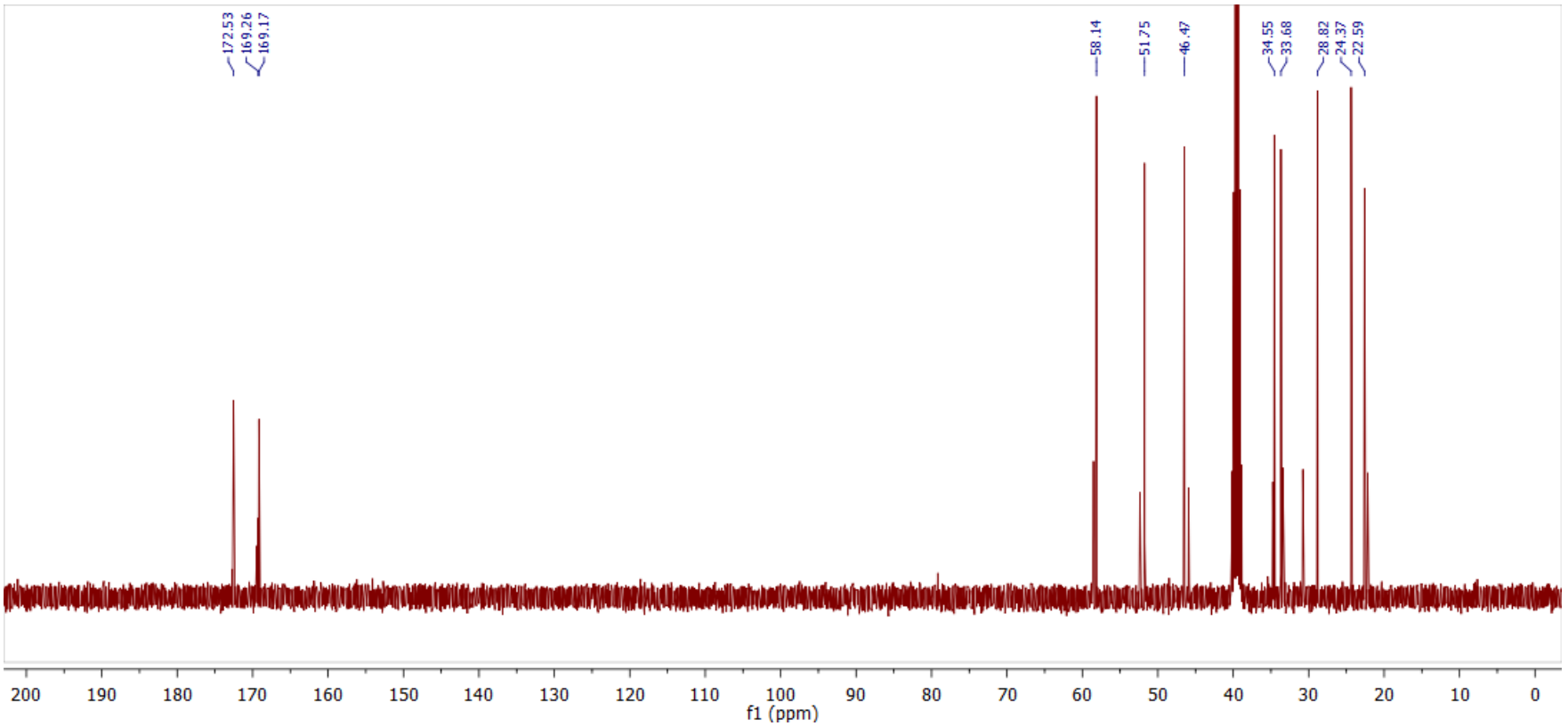


4.1.7. *N*-Acetyl-β-alanyl-L-proline methyl ester (Ac-βAla-Pro-OMe)

^{1}H NMR spectrum of Ac-βAla-Pro-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 4 : 1.

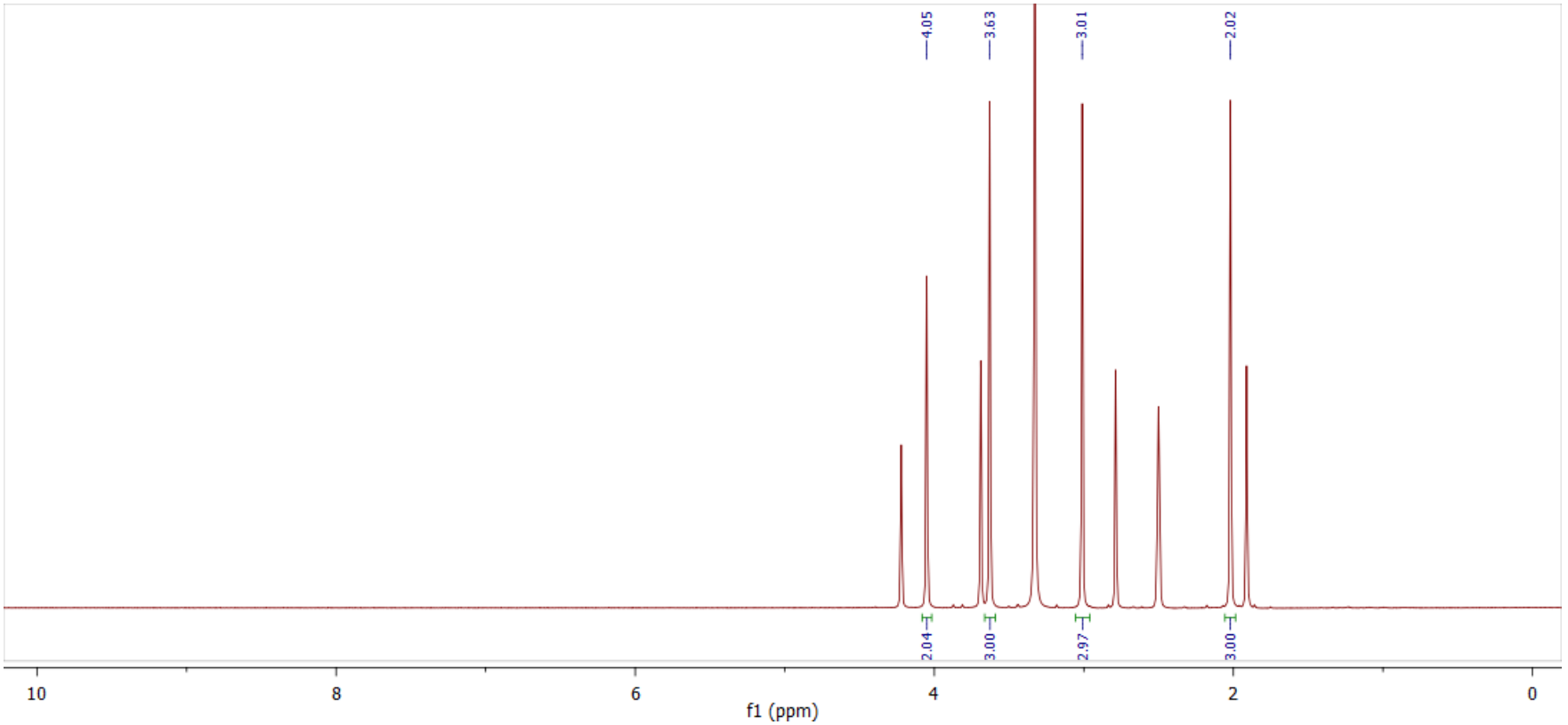


^{13}C NMR spectrum of Ac-βAla-Pro-OMe

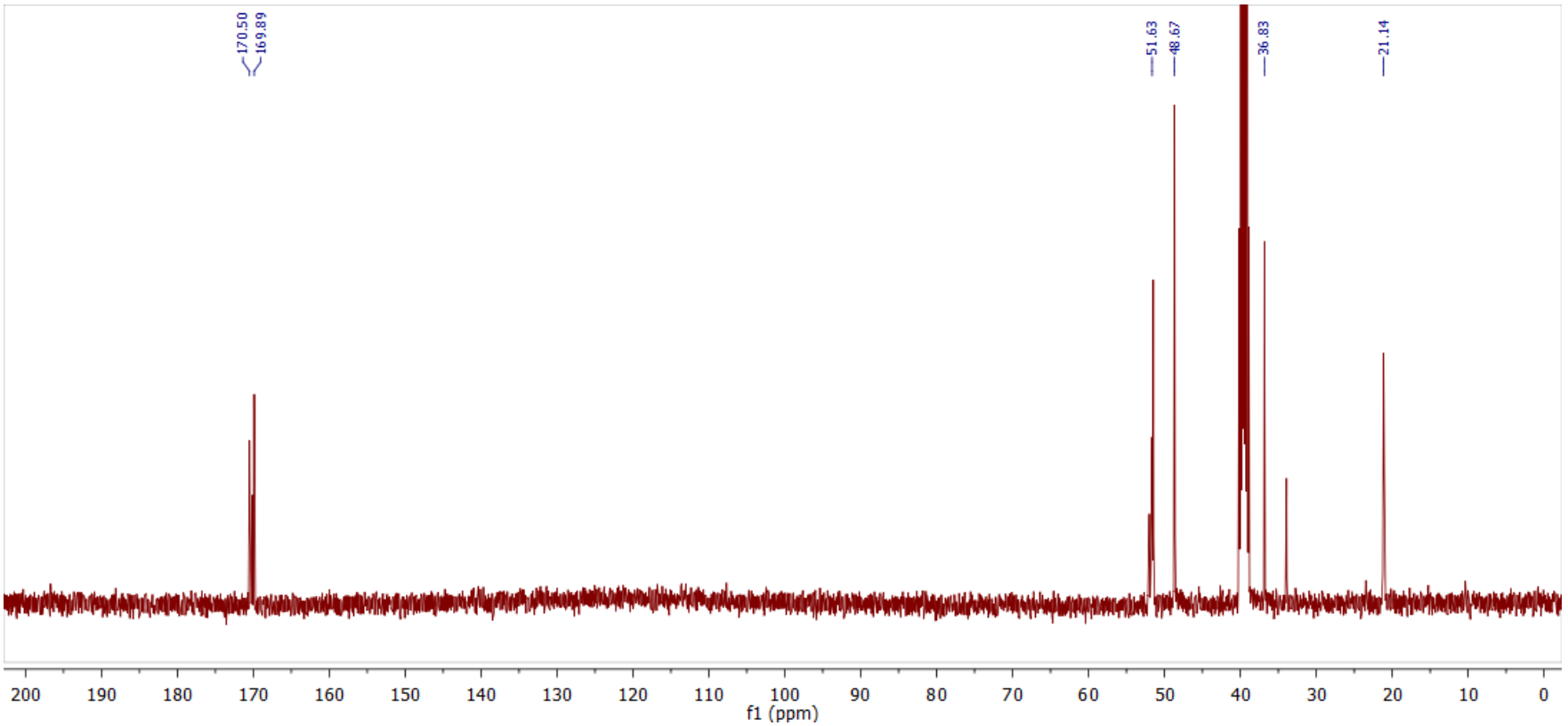


4.1.8. *N*-Acetyl-*N*-methylglycine methyl ester (Ac-(Me)Gly-OMe)

^{1}H NMR spectrum of Ac-(Me)Gly-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 2 : 1.



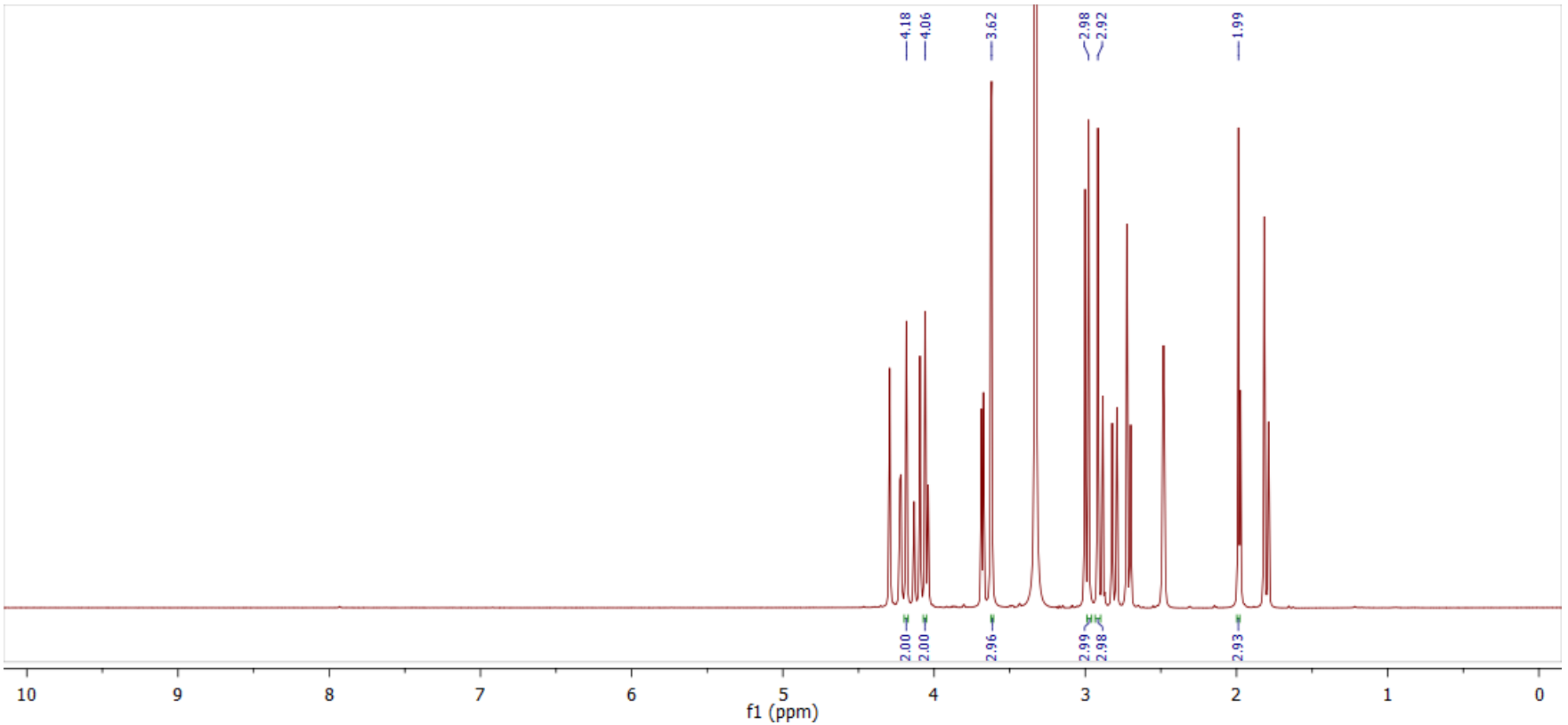
^{13}C NMR spectrum of Ac-(Me)Gly-OMe



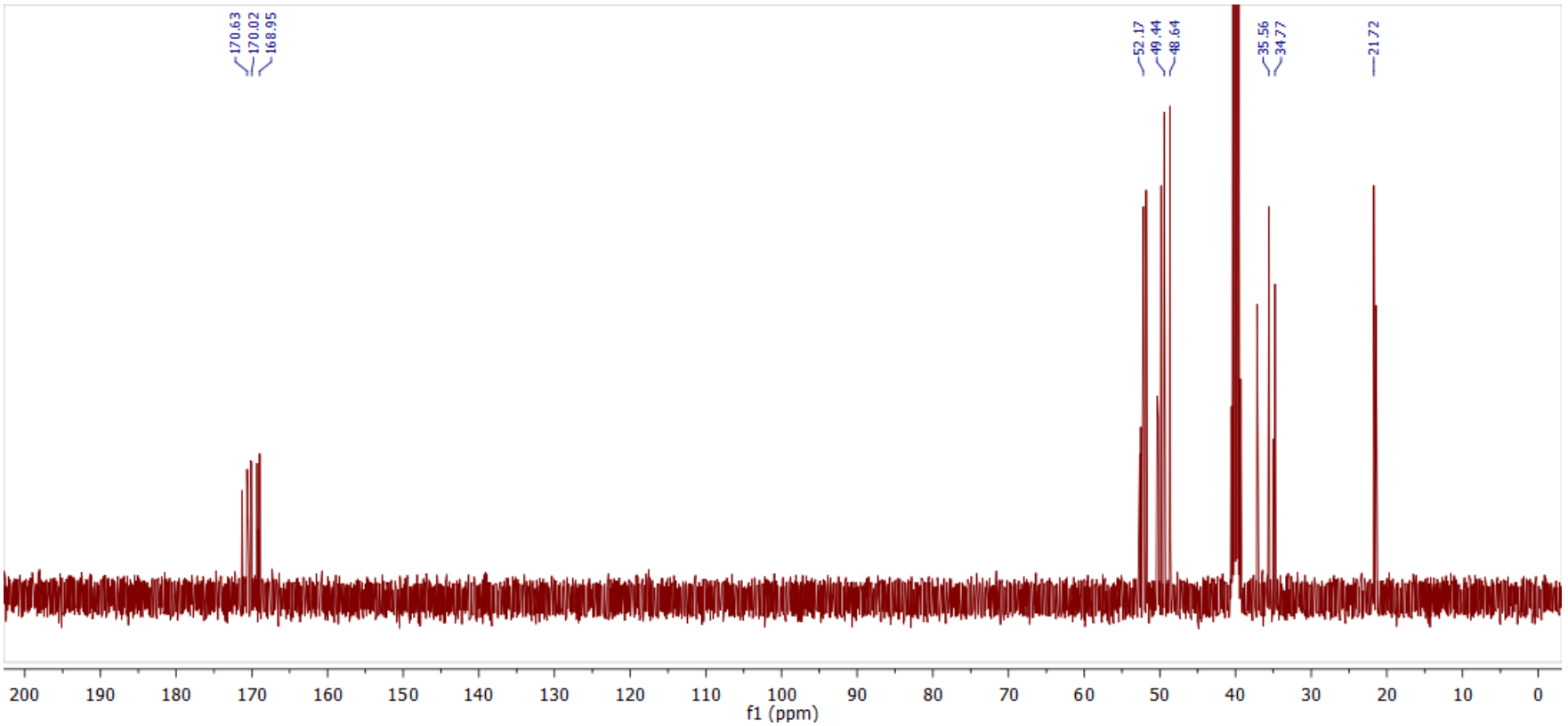
4.1.9. *N*-Acetyl-*N*-methylglycyl-*N*-methylglycine methyl ester (Ac-(Me)Gly-(Me)Gly-OMe)

^{1}H NMR spectrum of Ac-(Me)Gly-(Me)Gly-OMe, only the major isomer was assigned (there are 4 isomers total).

The isomers are observed in a ratio of 2.6 : 2.2 : 1.2 : 1.

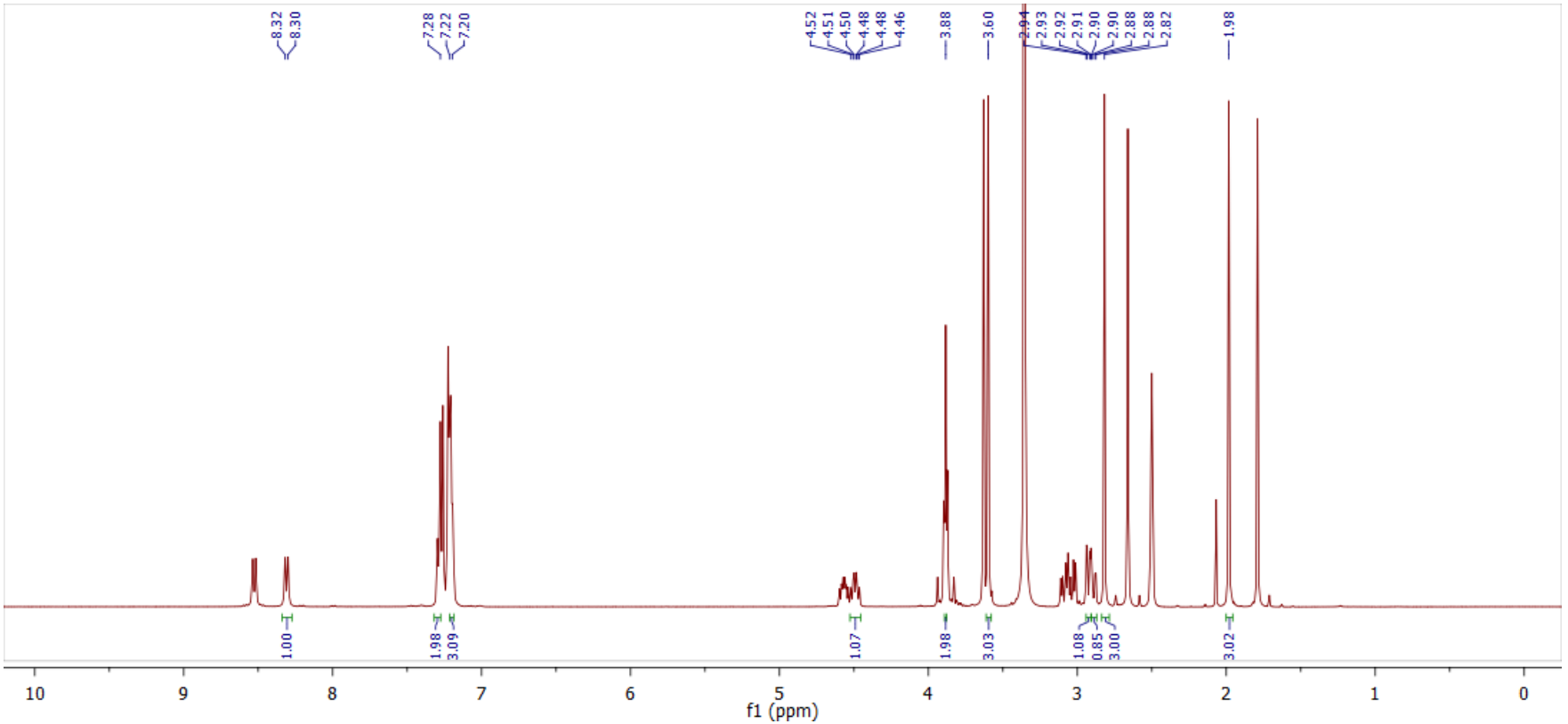


^{13}C NMR spectrum of Ac-(Me)Gly-(Me)Gly-OMe

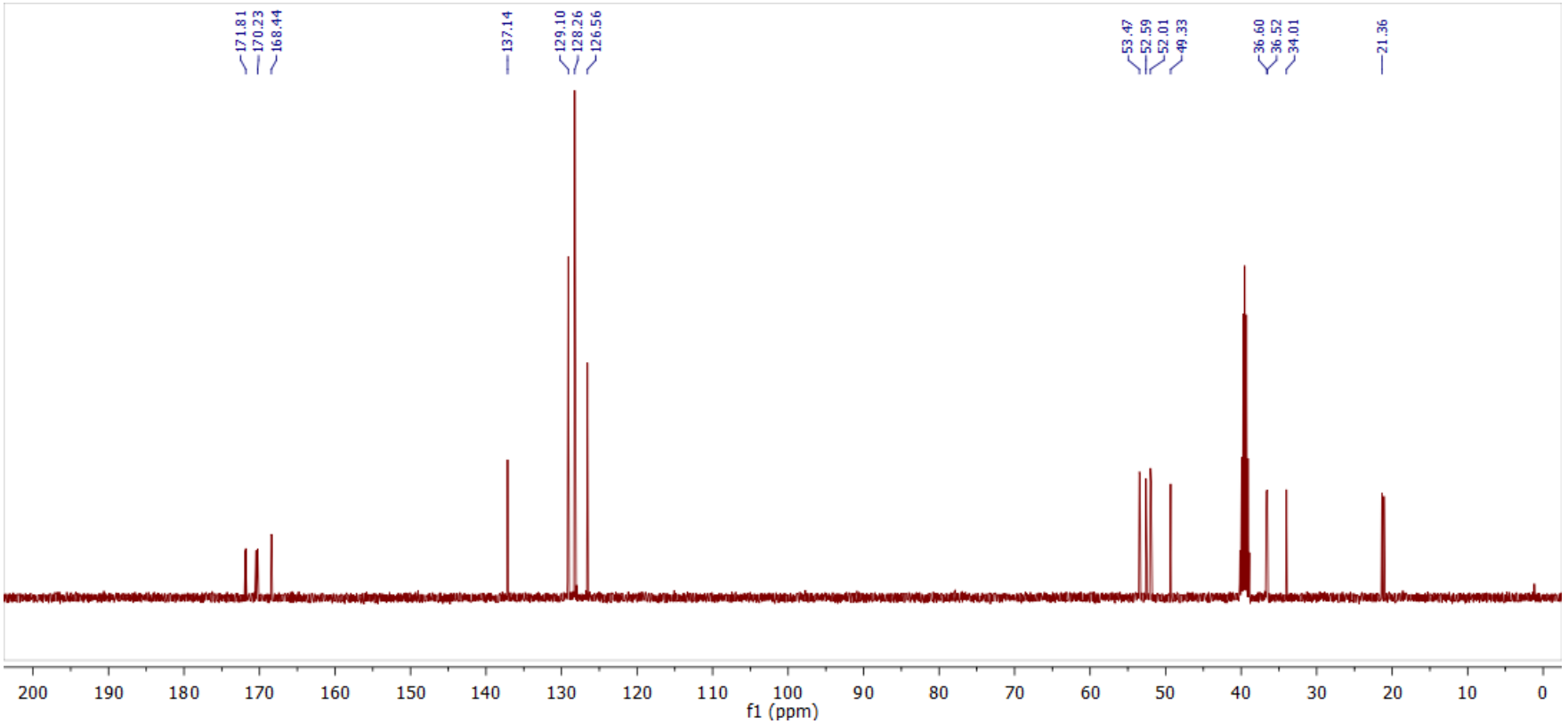


4.1.10. *N*-Acetyl-*N*-methylglycyl-L-phenylalanine methyl ester (Ac-(Me)Gly-Phe-OMe)

^{1}H NMR spectrum of Ac-(Me)Gly-Phe-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 1.03 : 1.

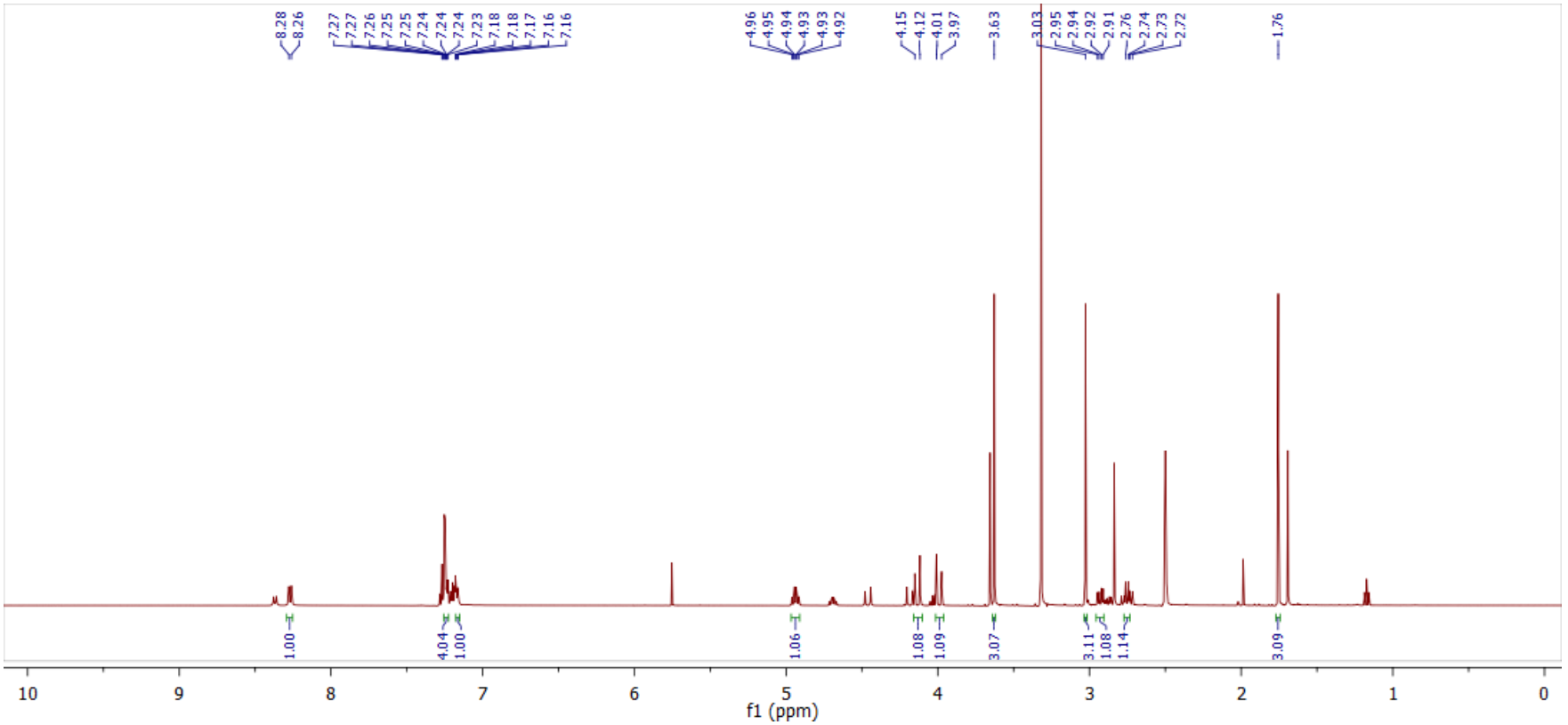


^{13}C NMR spectrum of Ac-(Me)Gly-Phe-OMe

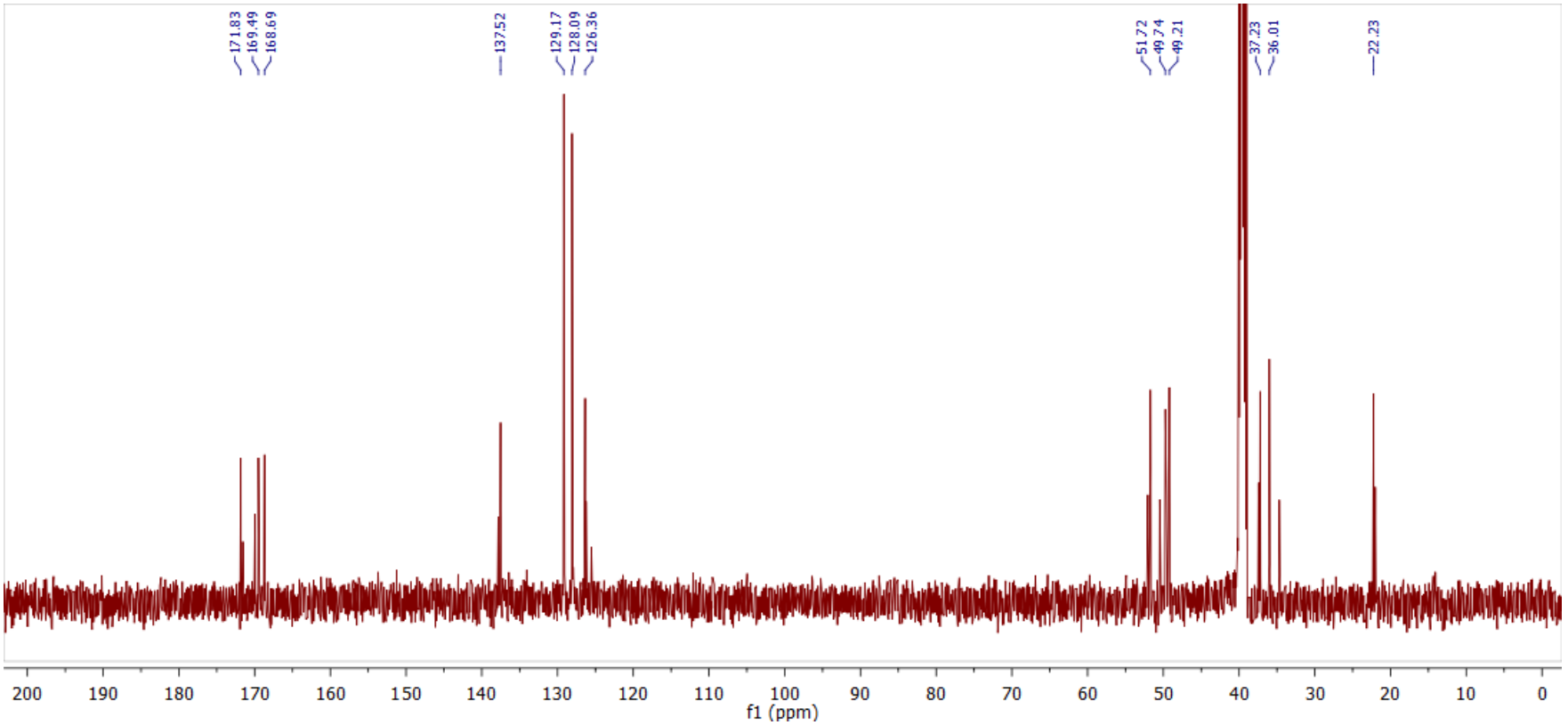


4.1.11. *N*-Acetyl-L-phenylalanyl-*N*-methylglycine methyl ester (Ac-Phe-(Me)Gly-OMe)

^{1}H NMR spectrum of Ac-Phe-(Me)Gly-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 2 : 1. There are traces of ethyl acetate and dichloromethane.

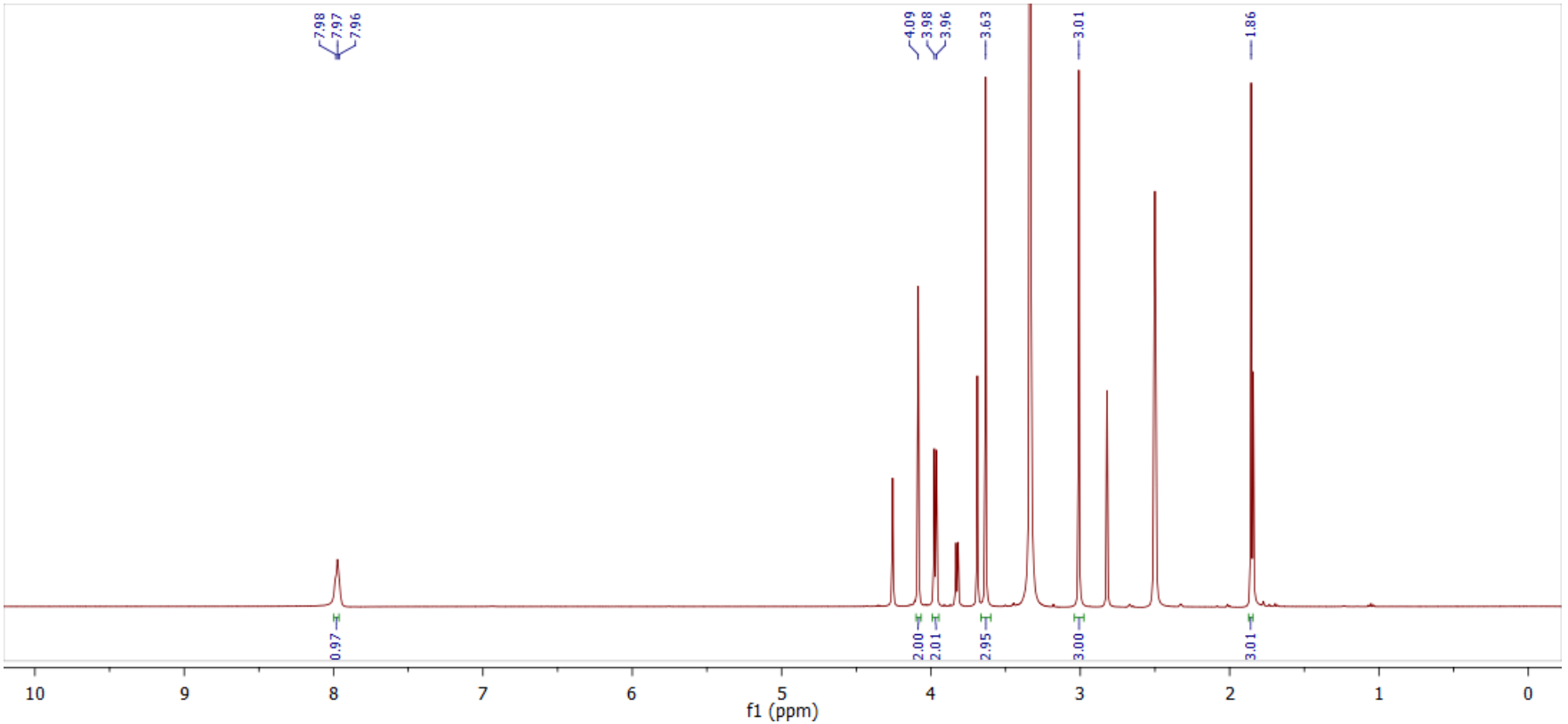


^{13}C NMR spectrum of Ac-Phe-(Me)Gly-OMe

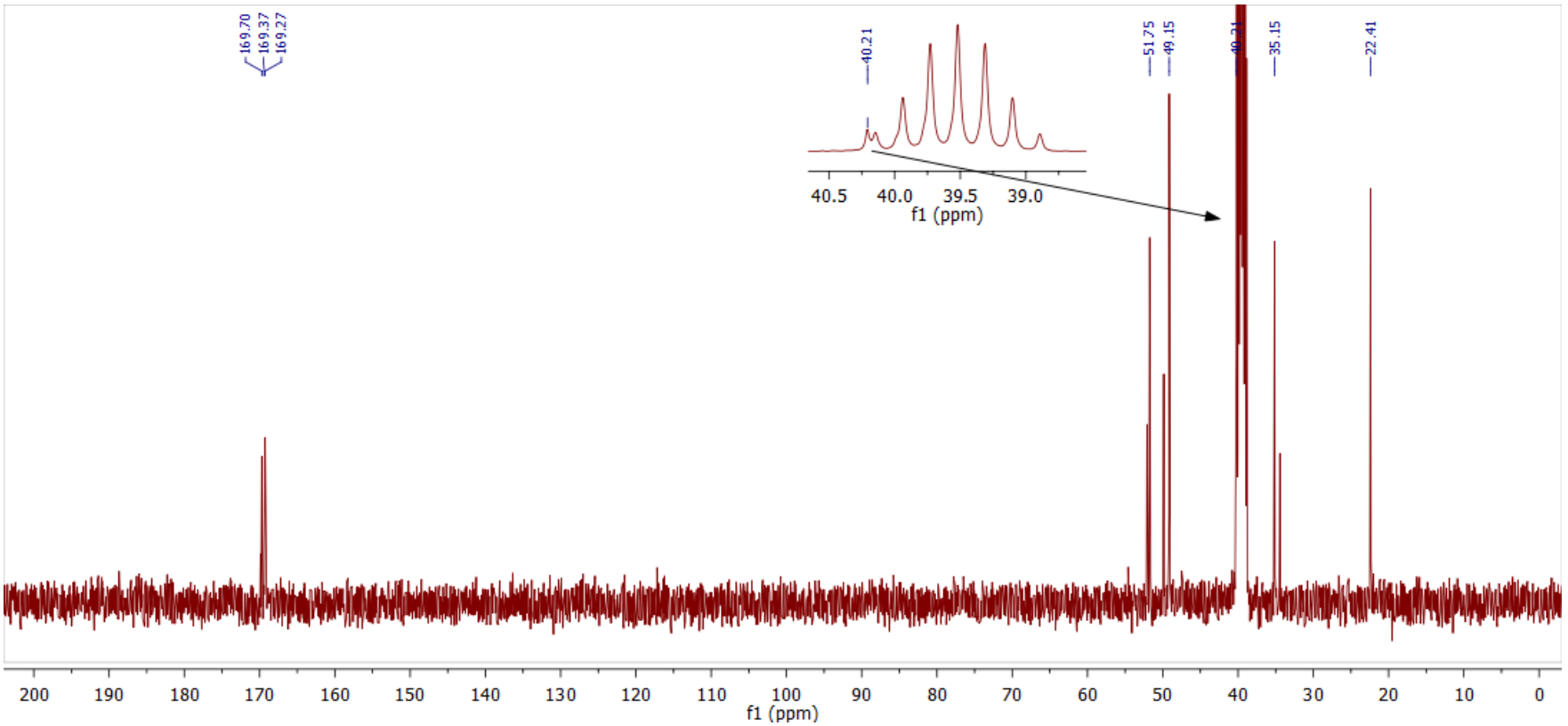


4.1.12. *N*-Acetylglycyl-*N*-methylglycine methyl ester (Ac-Gly-(Me)Gly-OMe)

^{1}H NMR spectrum of Ac-Gly-(Me)Gly-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 2 : 1.

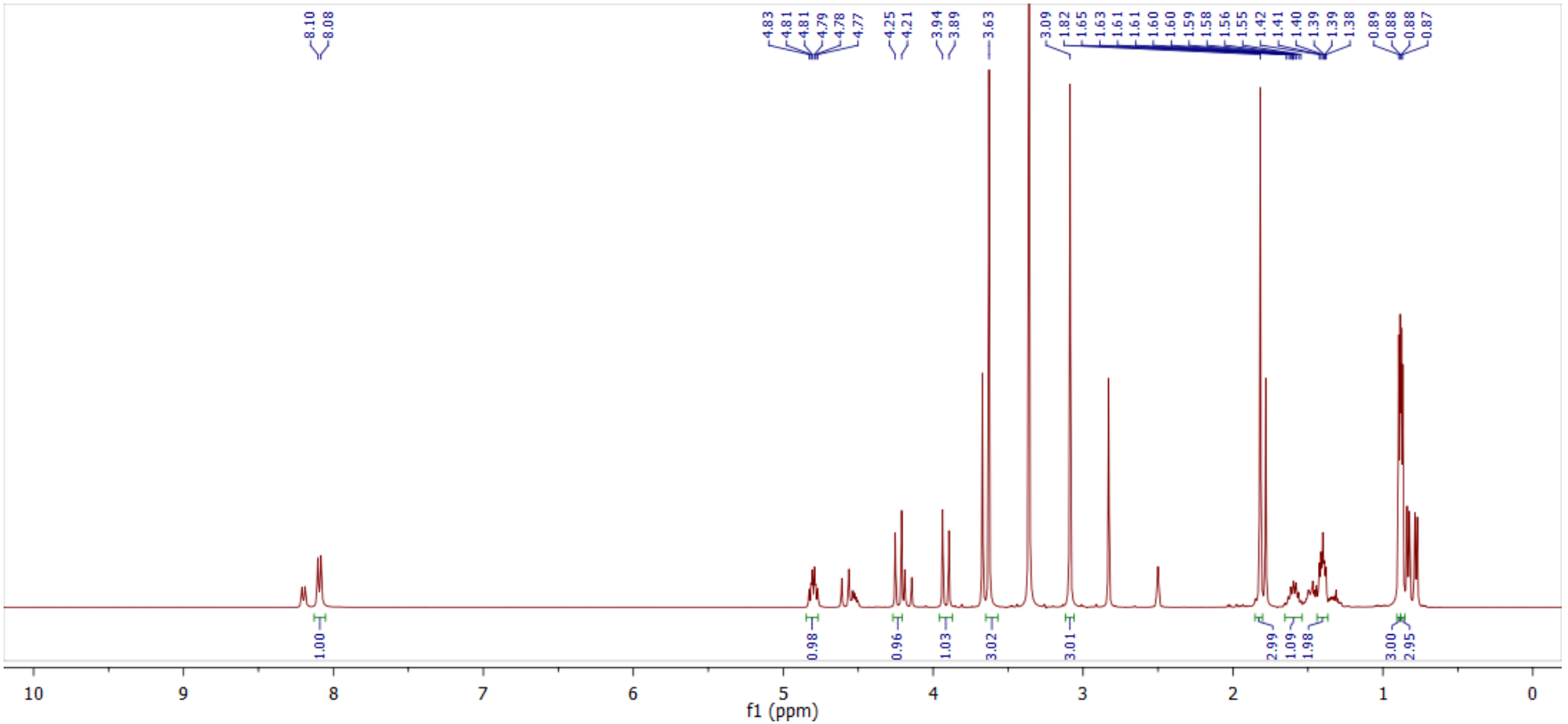


^{13}C NMR spectrum of Ac-Gly-(Me)Gly-OMe

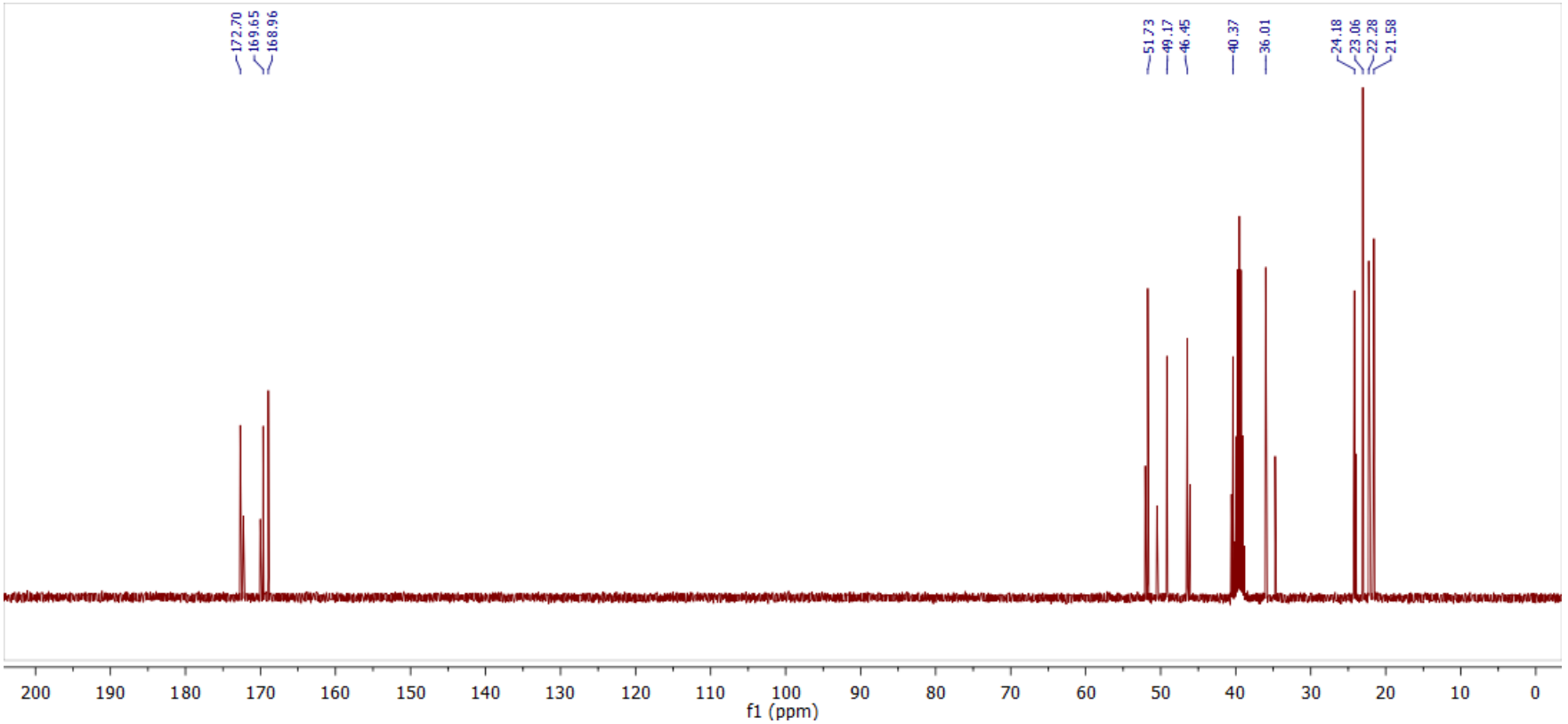


4.1.13. *N*-Acetyl-L-leucyl-*N*-methylglycine methyl ester (Ac-Leu-(Me)Gly-OMe)

^{1}H NMR spectrum of Ac-Leu-(Me)Gly-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 2.5 : 1.

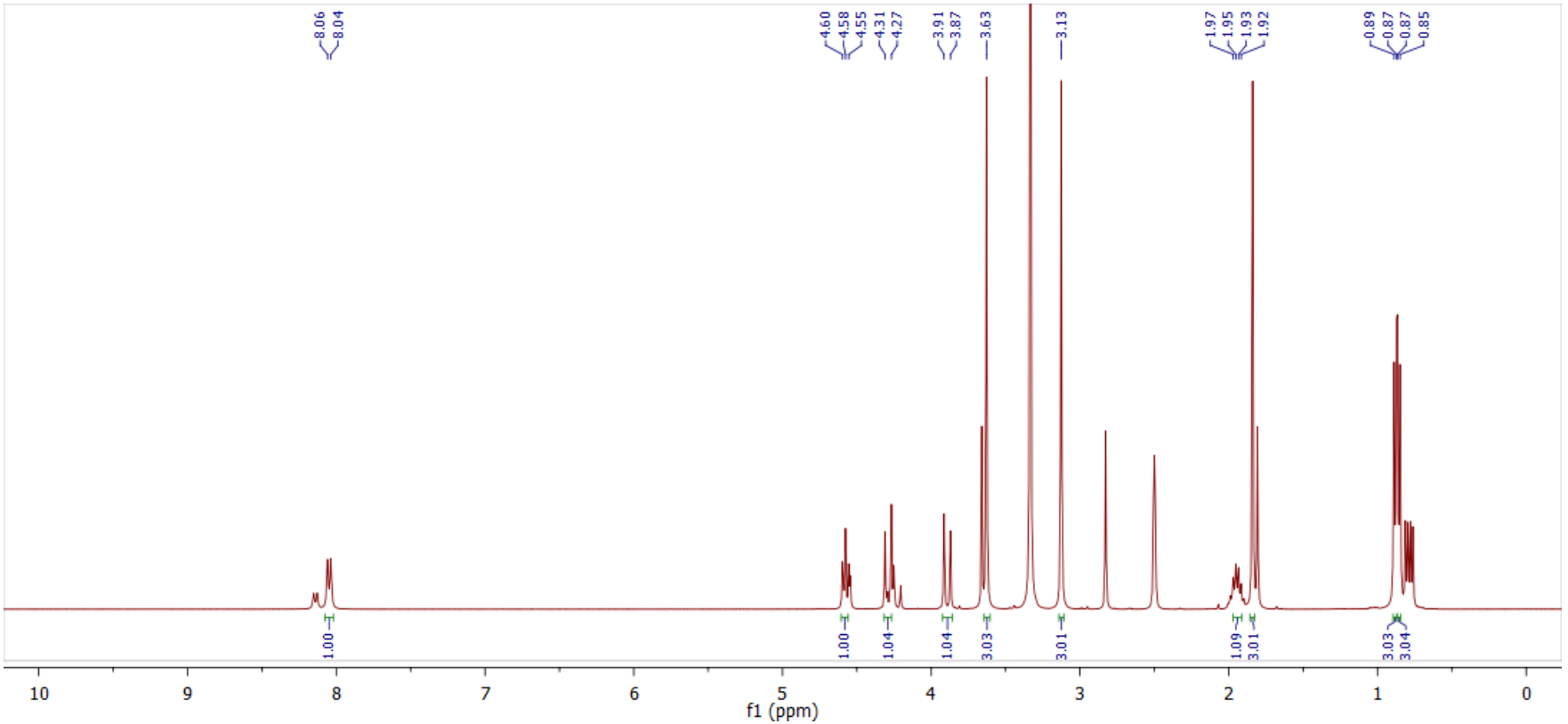


^{13}C NMR spectrum of Ac-Leu-(Me)Gly-OMe

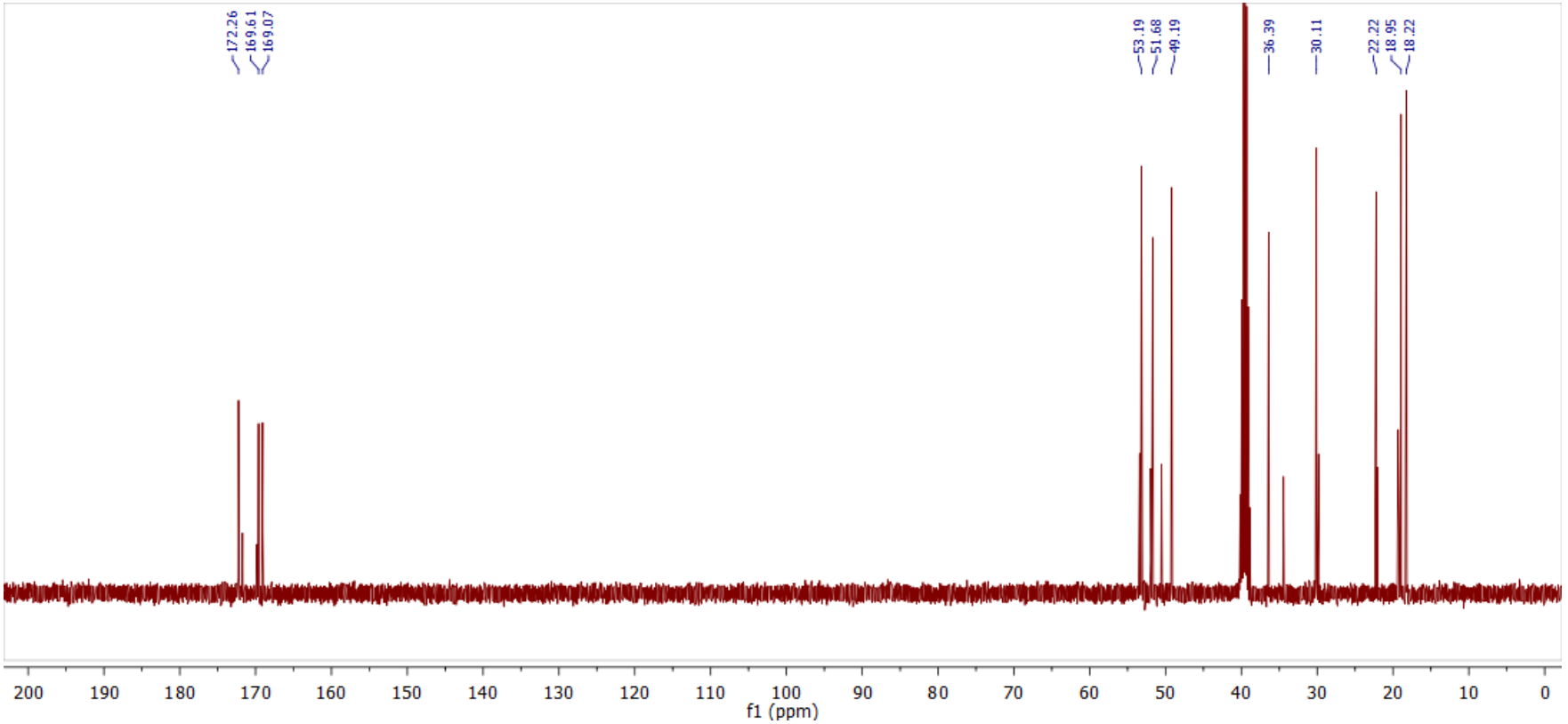


4.1.14. *N*-Acetyl-L-valyl-*N*-methylglycine methyl ester (Ac-Val-(Me)Gly-OMe)

^{1}H NMR spectrum of Ac-Val-(Me)Gly-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 3 : 1.

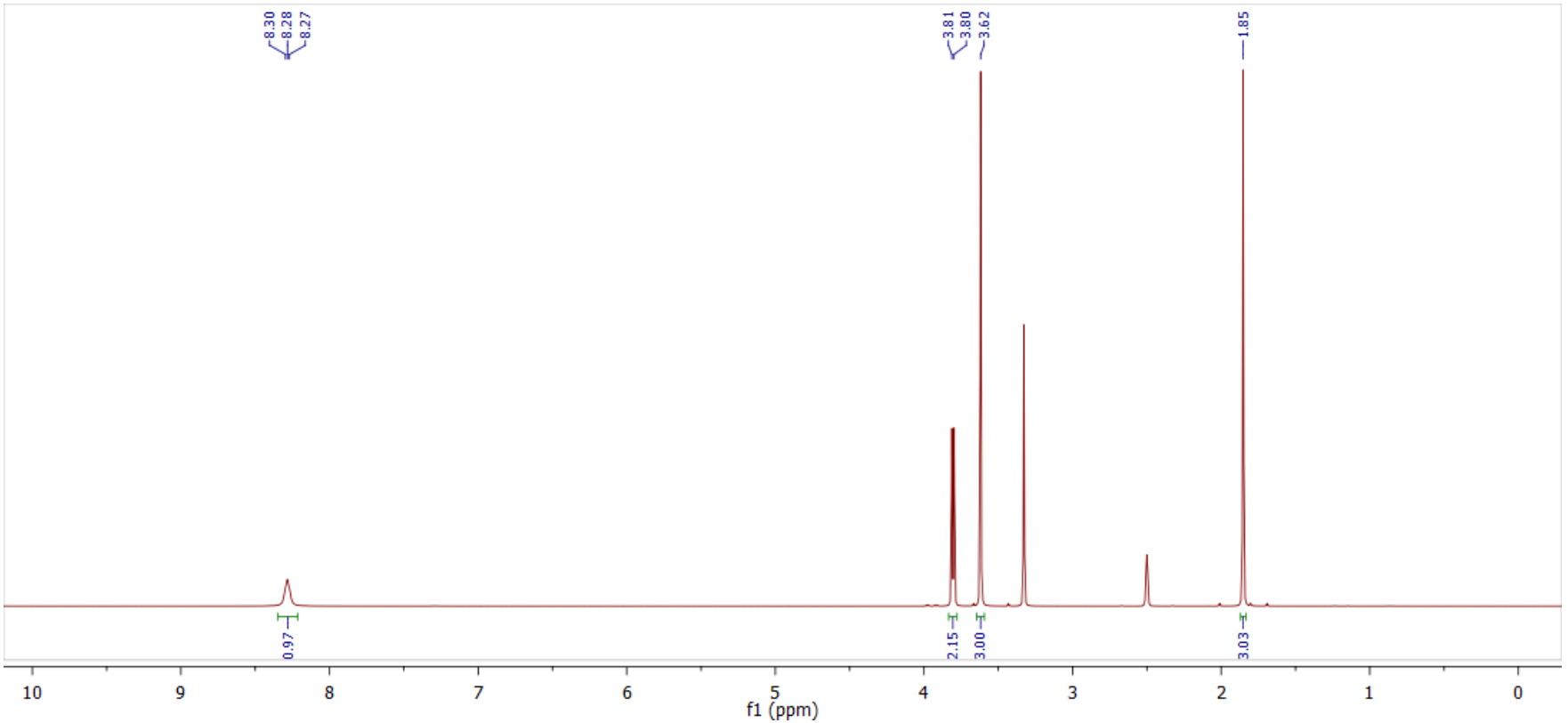


^{13}C NMR spectrum of Ac-Val-(Me)Gly-OMe

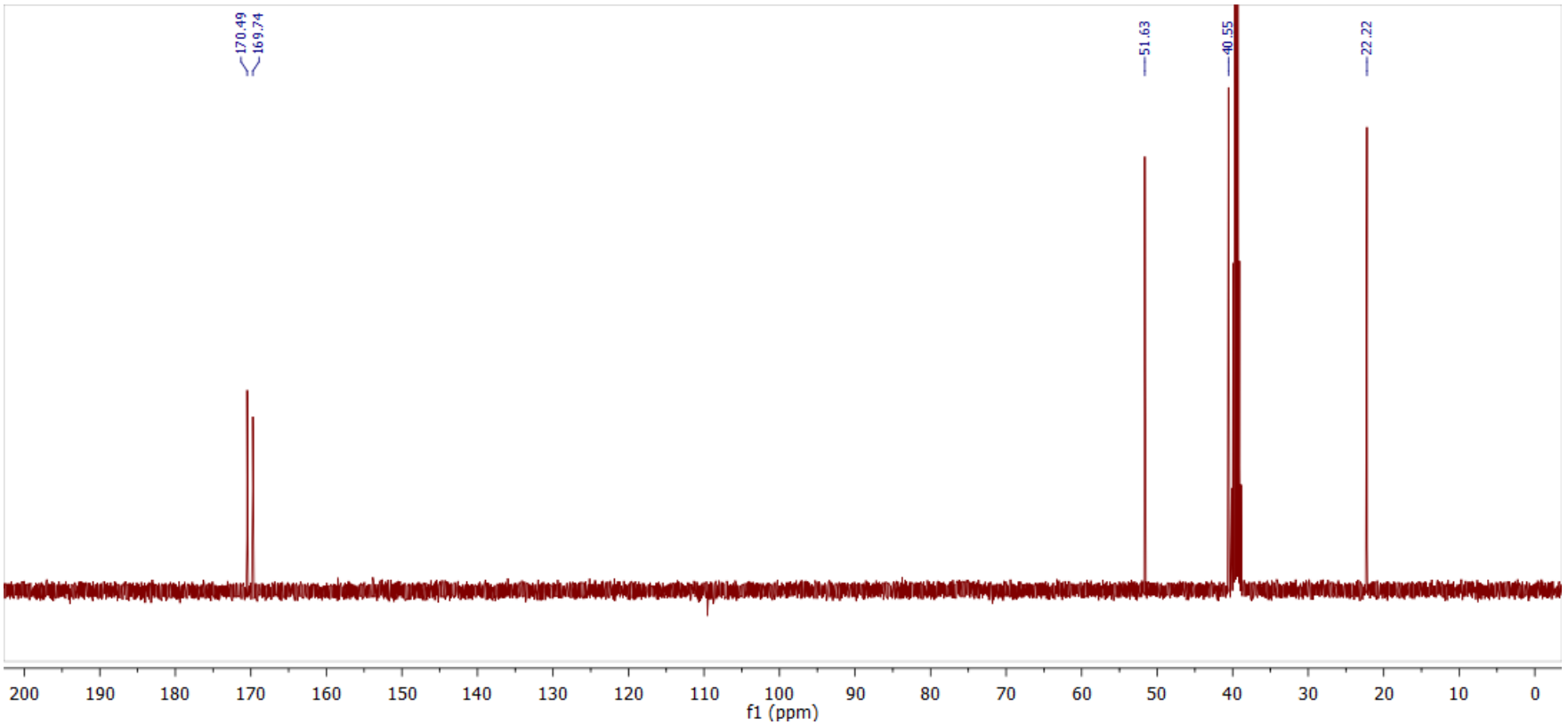


4.1.15. *N*-Acetylglycine methyl ester (Ac-Gly-OMe)

^{1}H NMR spectrum of Ac-Gly-OMe

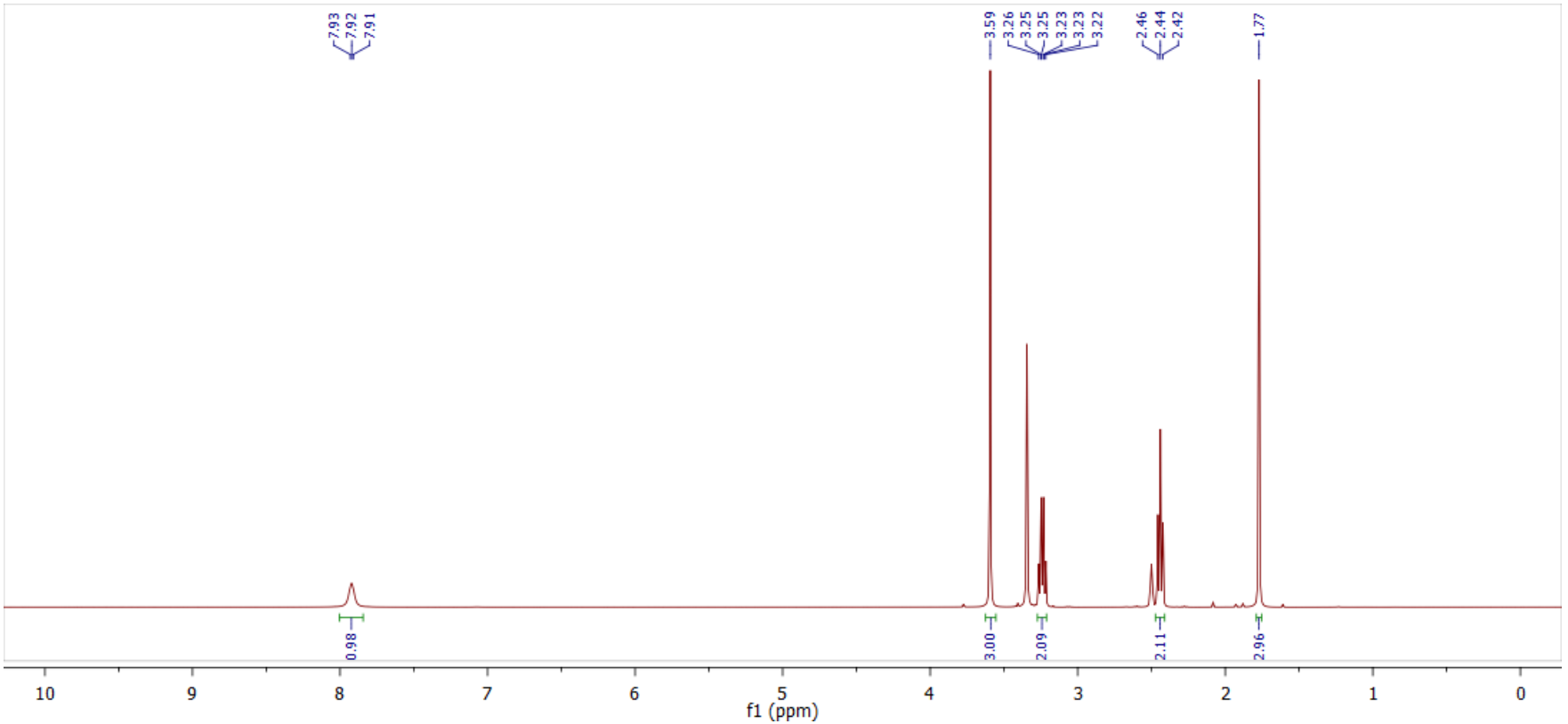


^{13}C NMR spectrum of Ac-Gly-OMe

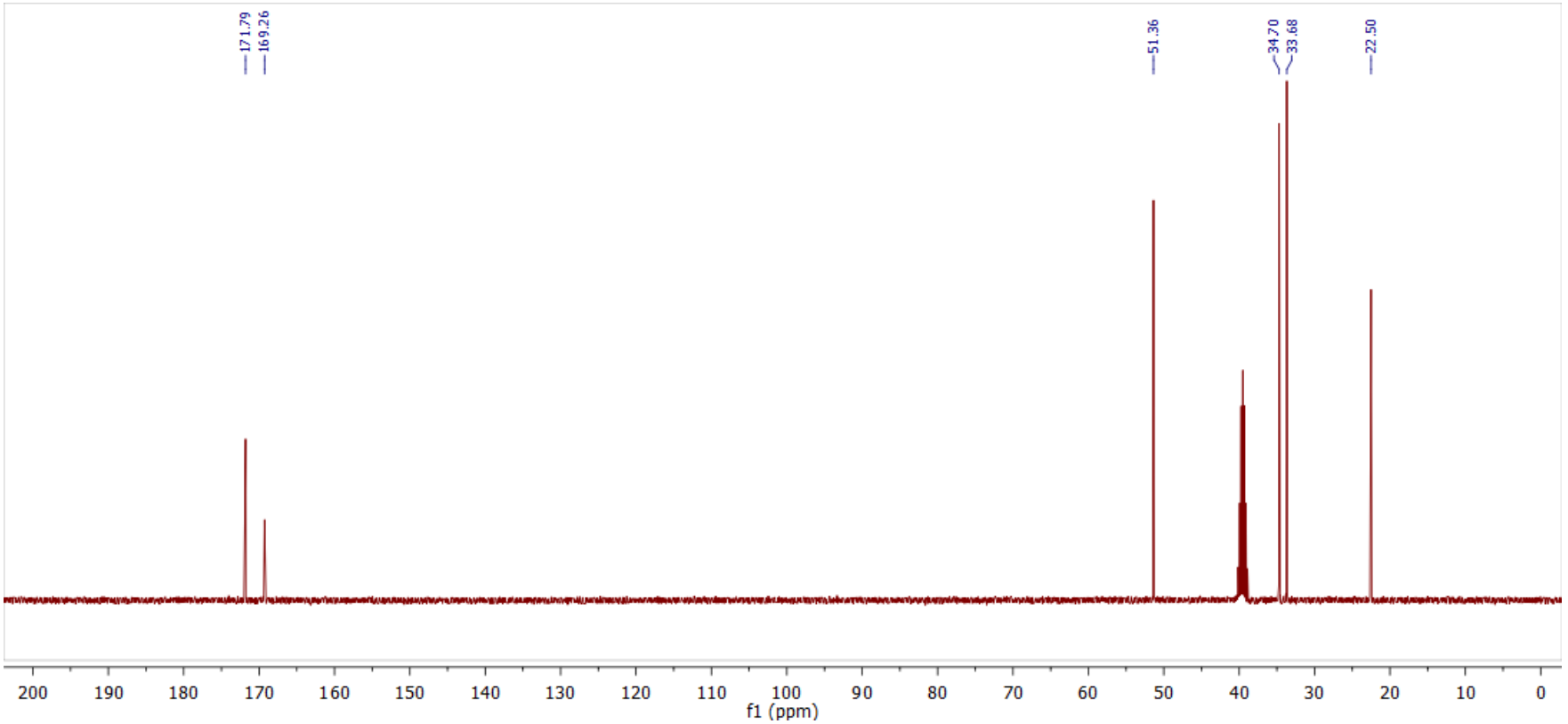


4.1.16. *N*-Acetyl-β-alanine methyl ester (Ac-βAla-OMe)

^{1}H NMR spectrum of Ac-βAla-OMe

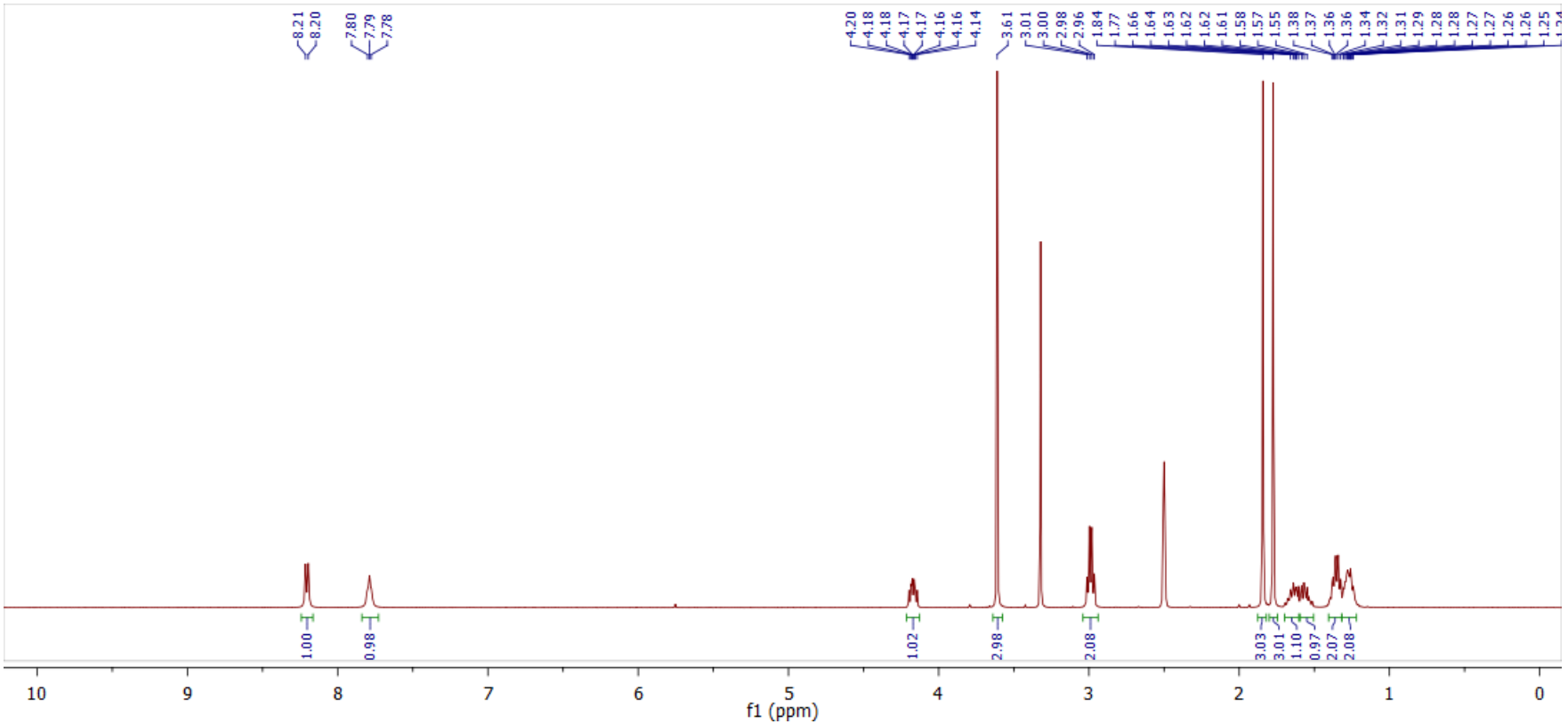


^{13}C NMR spectrum of Ac-βAla-OMe

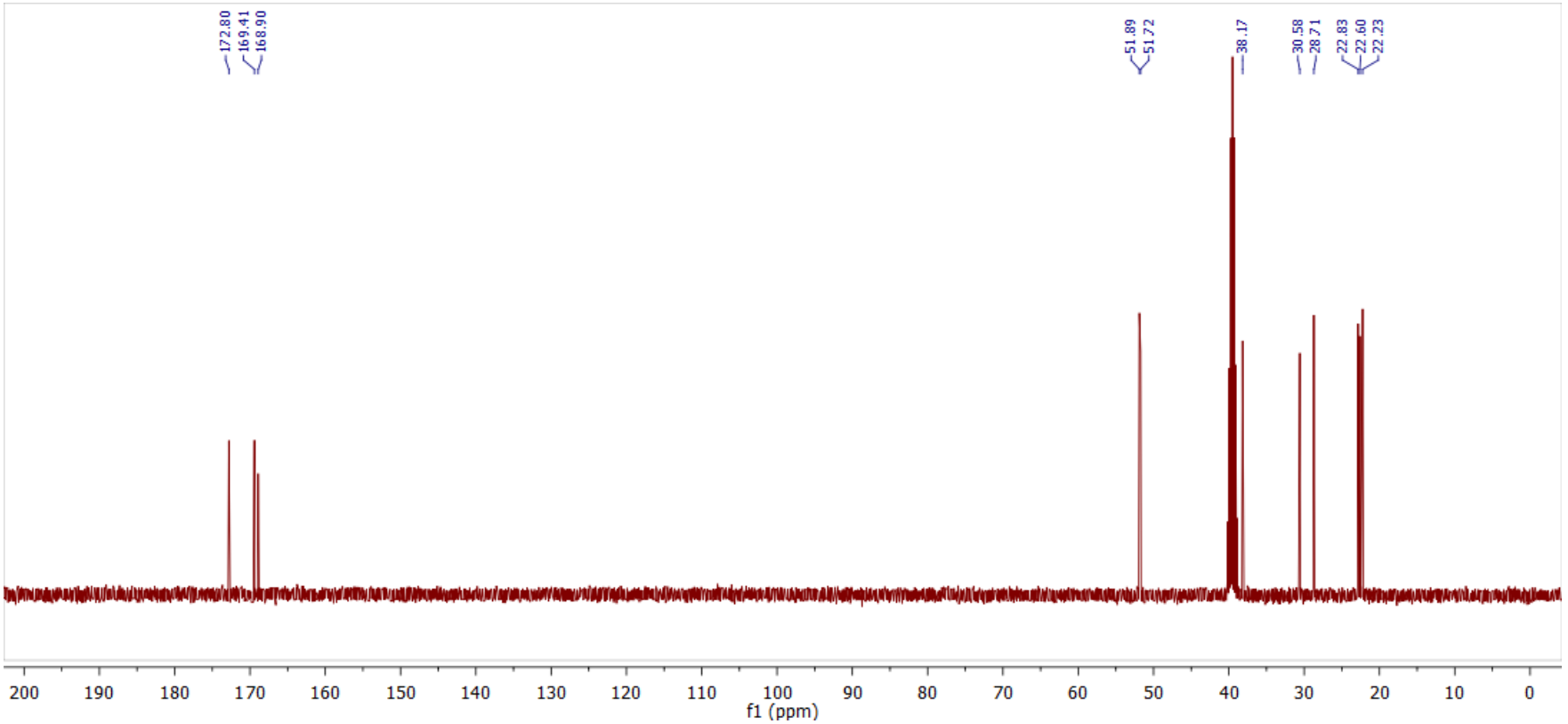


4.1.17. N_{α},N_{ε}-Diacetyl-L-lysine methyl ester (Ac-Lys(NHAc)-OMe)

^{1}H NMR spectrum of Ac-Lys(NHAc)-OMe

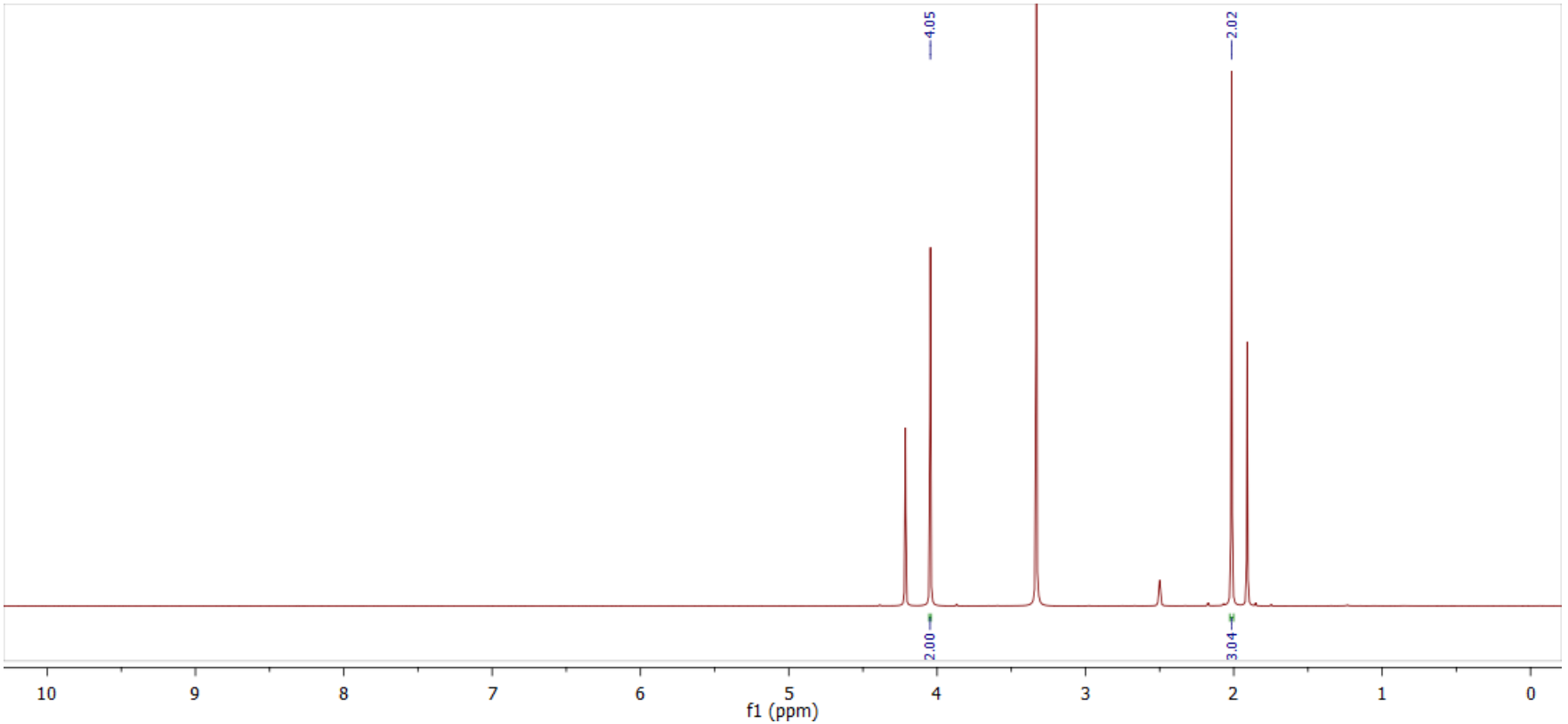


^{13}C NMR spectrum of Ac-Lys(NHAc)-OMe

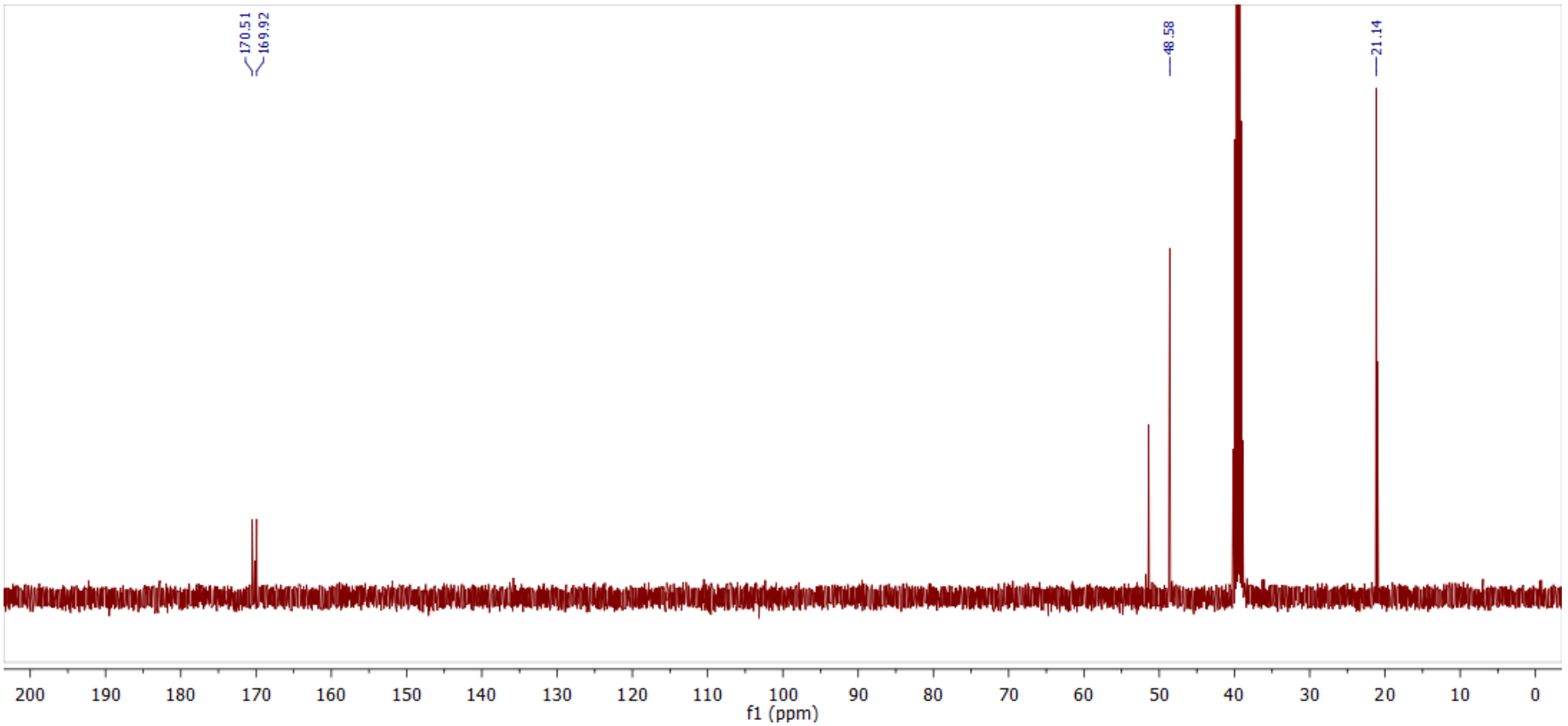


4.1.18. *N*-Acetyl-*N*-methyl(d_3)glycine methyl(d_3) ester (Ac-(Me(d_3))Gly-OMe(d_3))

^{1}H NMR spectrum of Ac-(Me(d_3))Gly-OMe(d_3), only the major isomer was assigned. The isomers are observed in a ratio of 2 : 1.

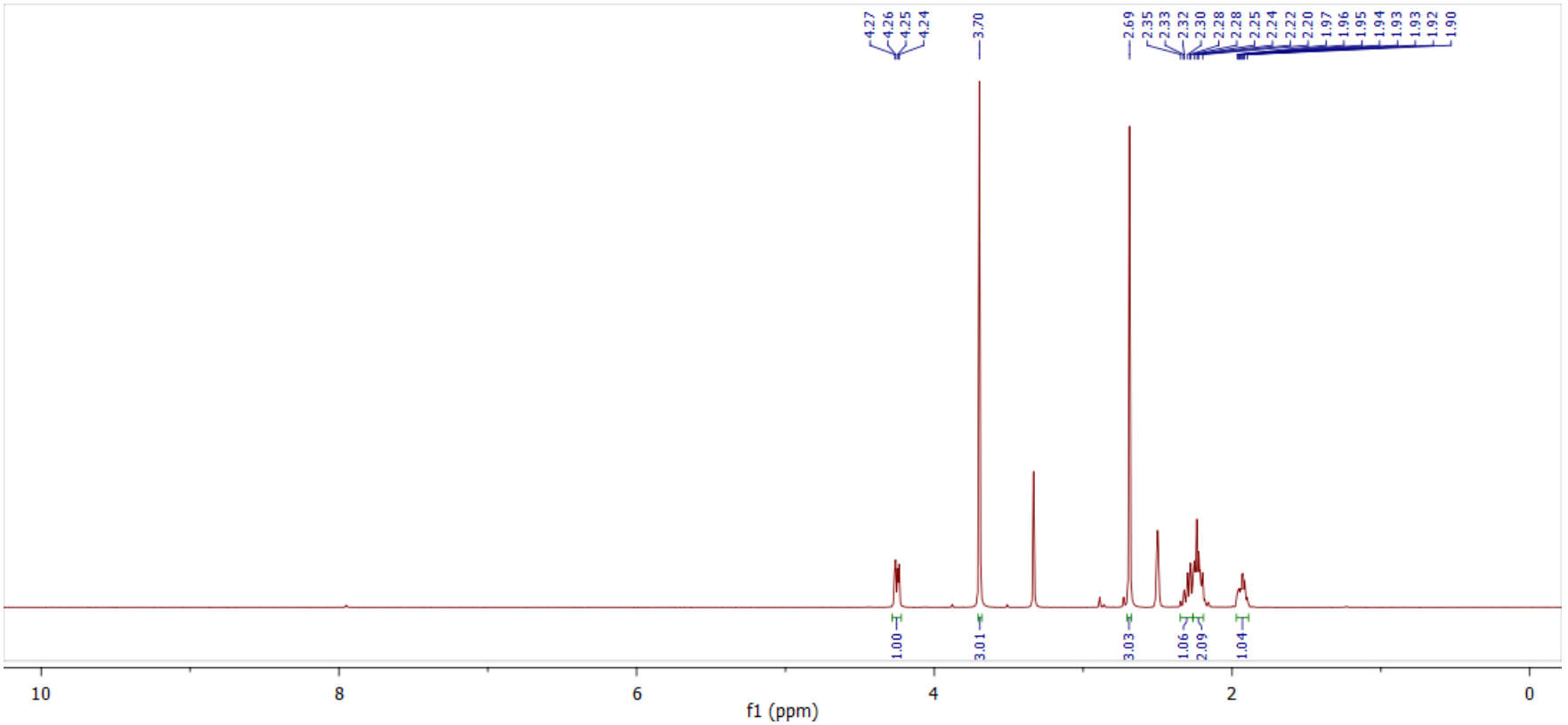


^{13}C NMR spectrum of Ac-(Me(d_3))Gly-OMe(d_3)

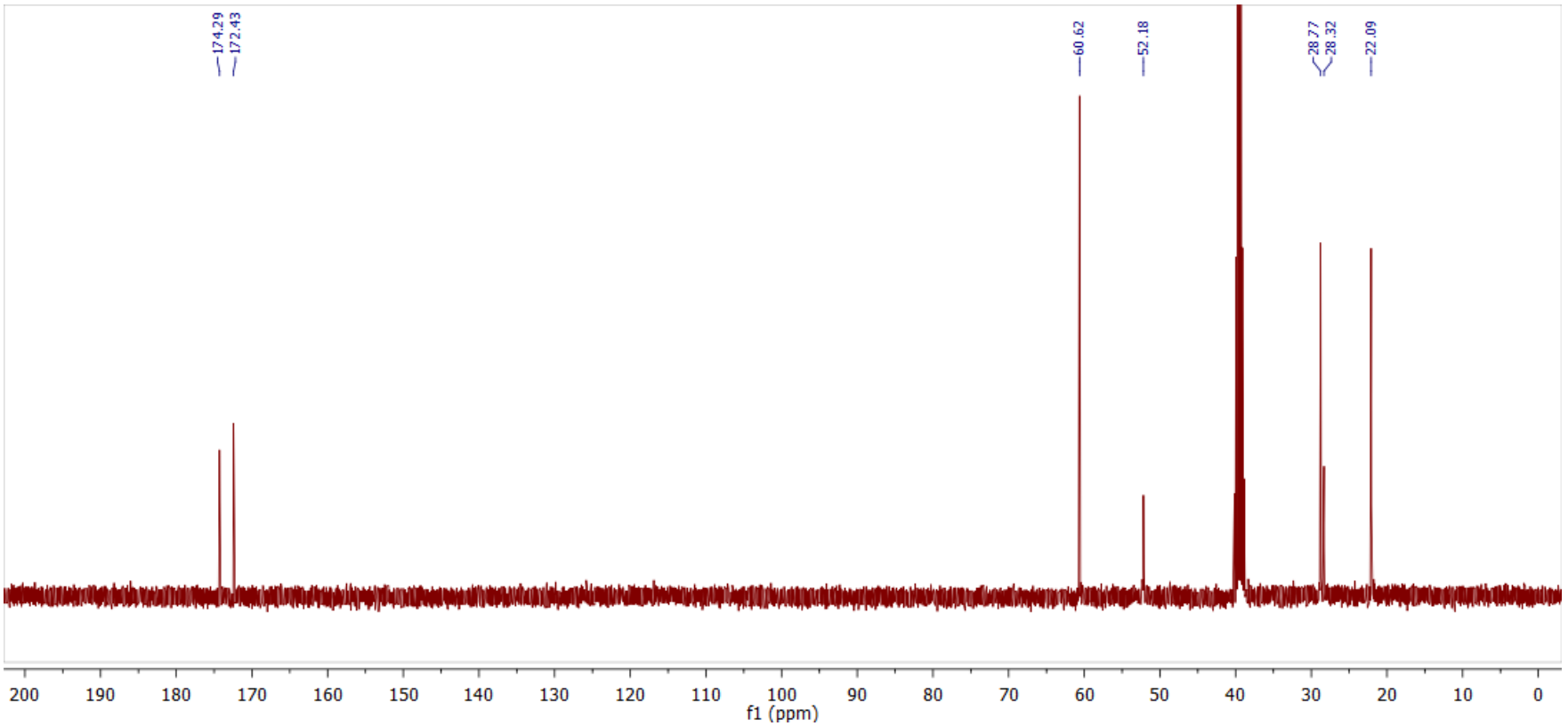


4.1.19. *N*-Methyl-L-pyroglutamic acid methyl ester (Me-Glp-OMe)

^{1}H NMR spectrum of Me-Glp-OMe

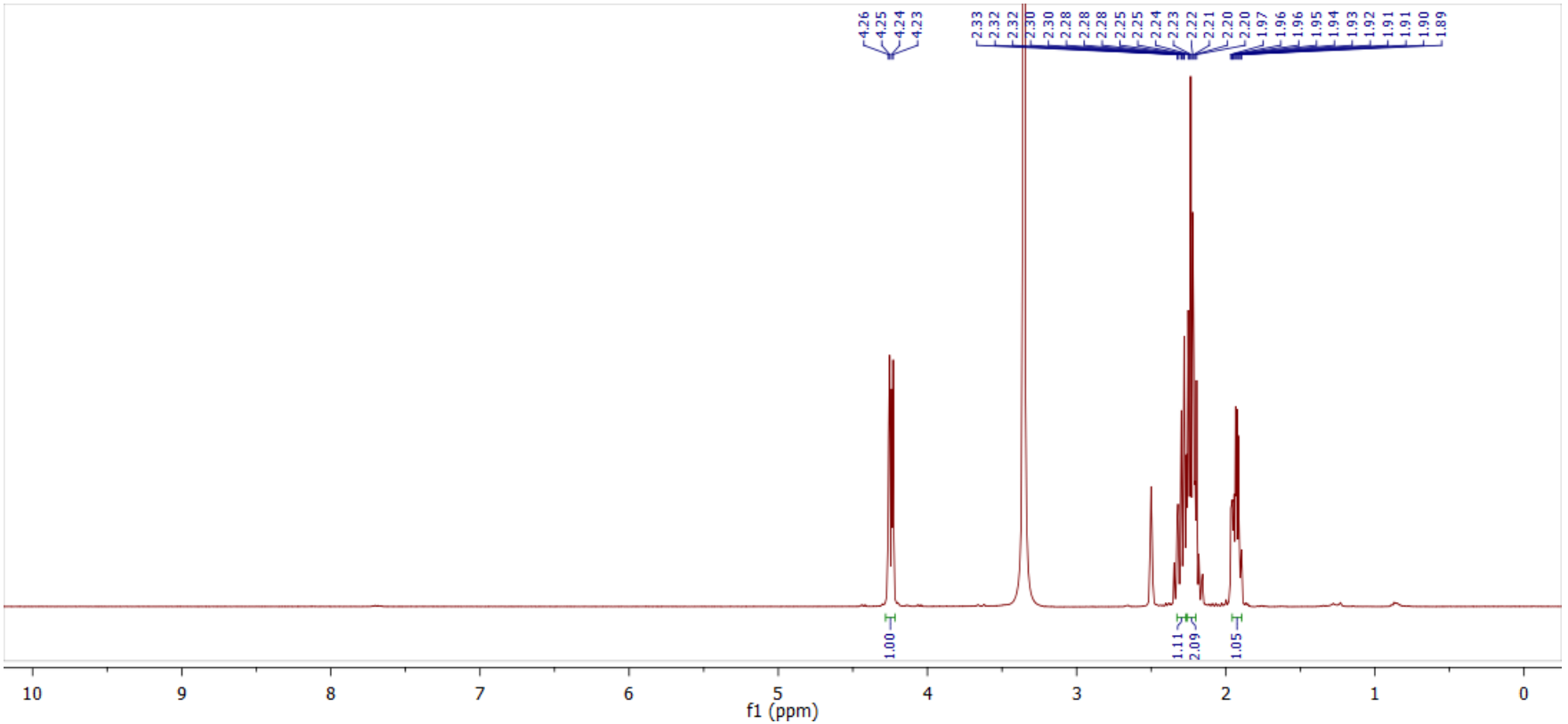


^{13}C NMR spectrum of Me-Glp-OMe

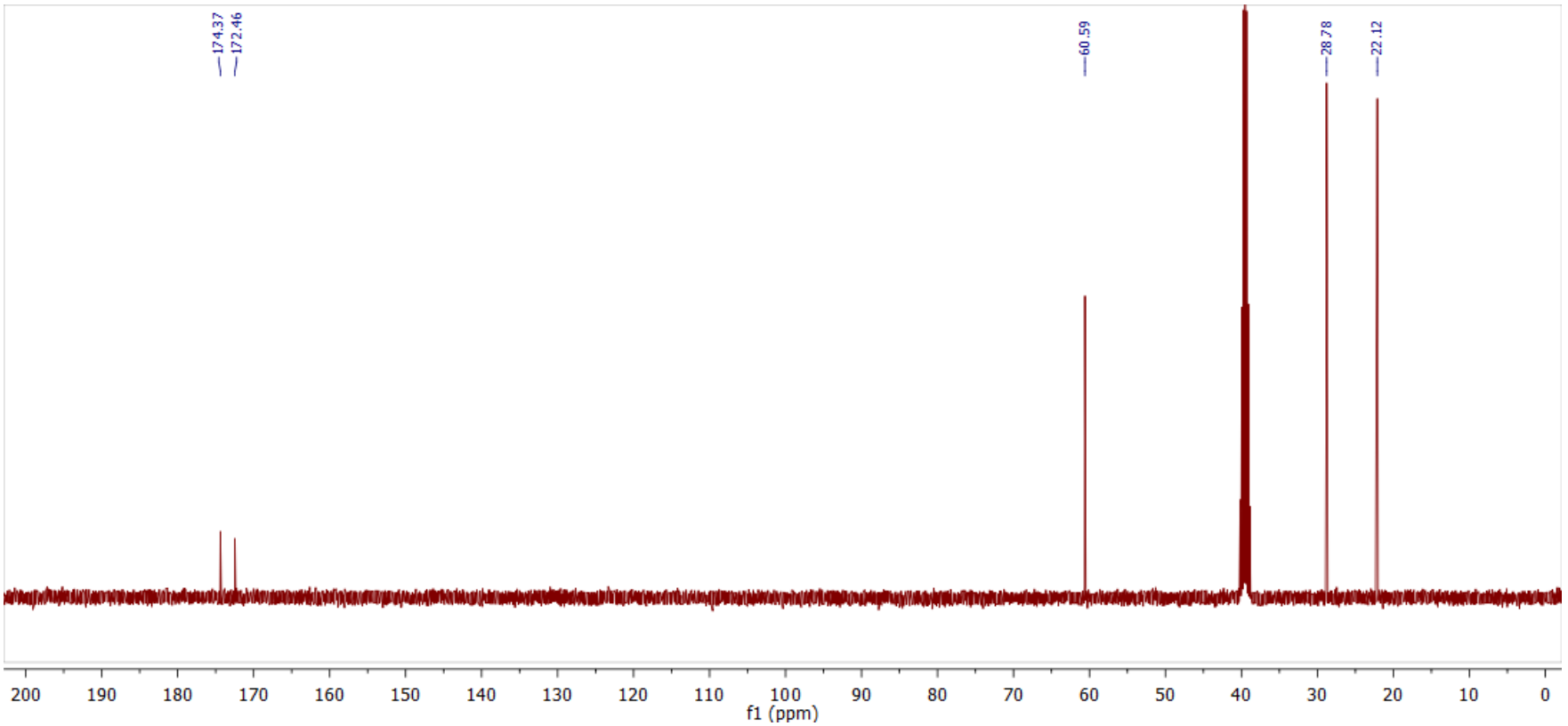


4.1.20. *N*-Methyl(d_3)-L-pyroglutamic acid methyl(d_3) ester (Me(d_3)-Glp-OMe(d_3))

^{1}H NMR spectrum of Me(d_3)-Glp-OMe(d_3)

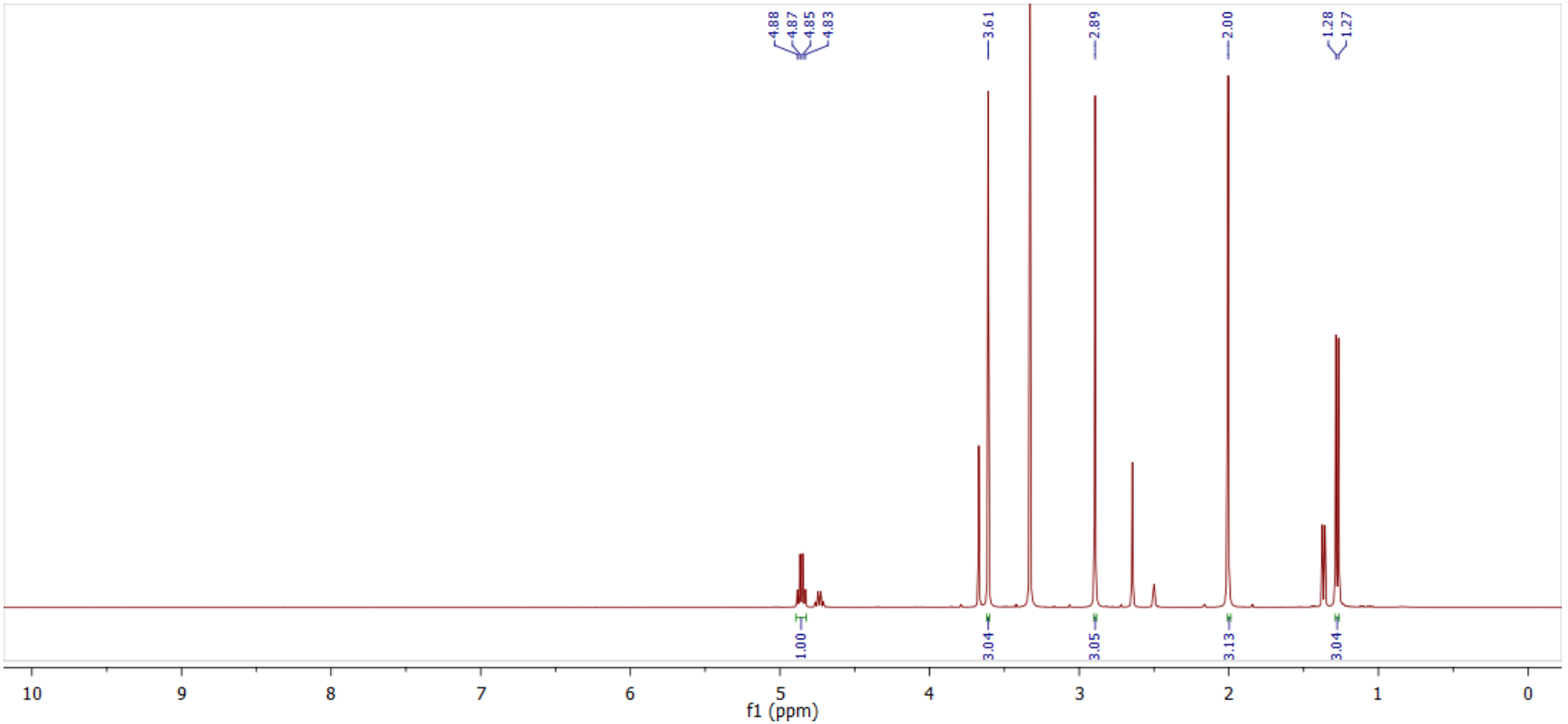


^{13}C NMR spectrum of Me(d_3)-Glp-OMe(d_3)

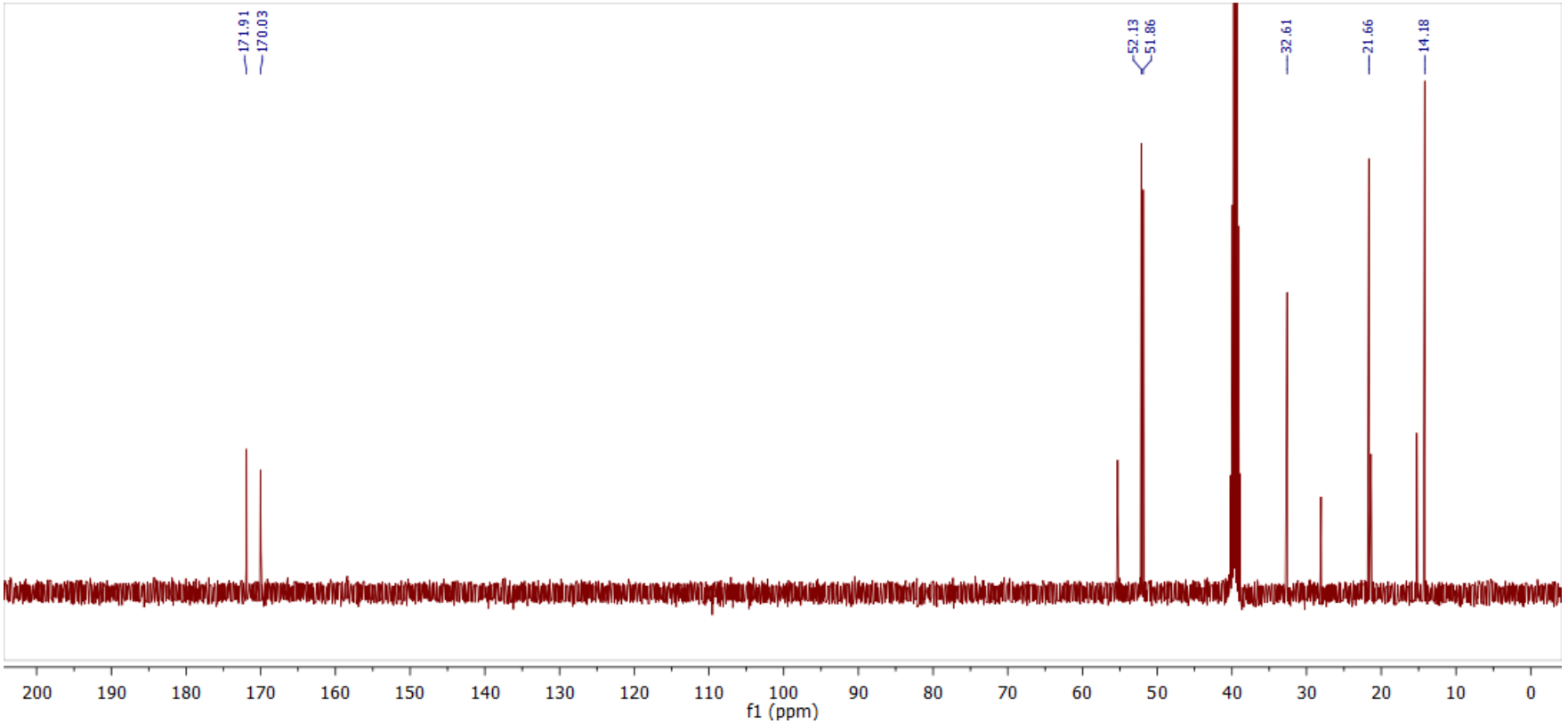


4.1.21. *N*-Acetyl-*N*-methyl-L-alanine methyl ester (Ac-(Me)Ala-OMe)

^{1}H NMR spectrum of Ac-(Me)Ala-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 3 : 1.

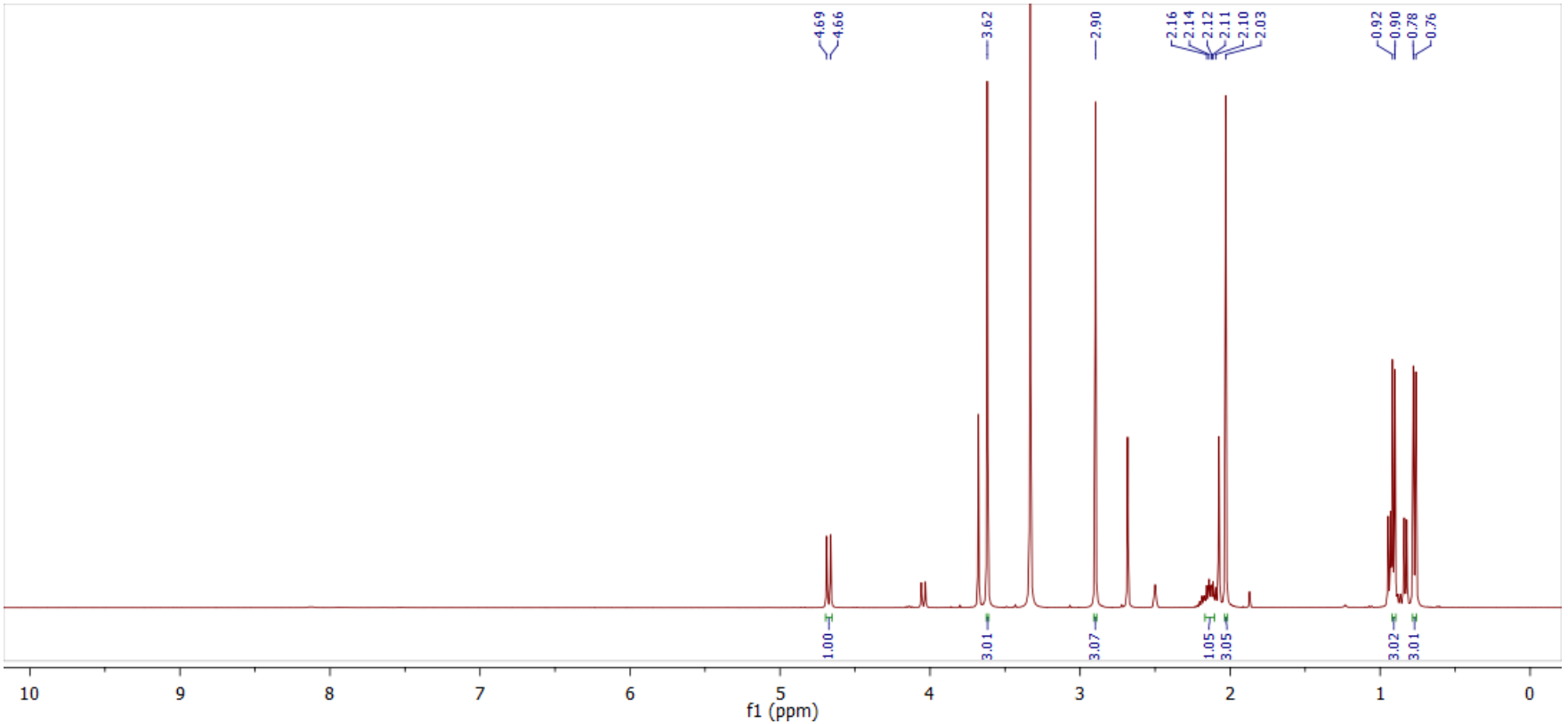


^{13}C NMR spectrum of Ac-(Me)Ala-OMe

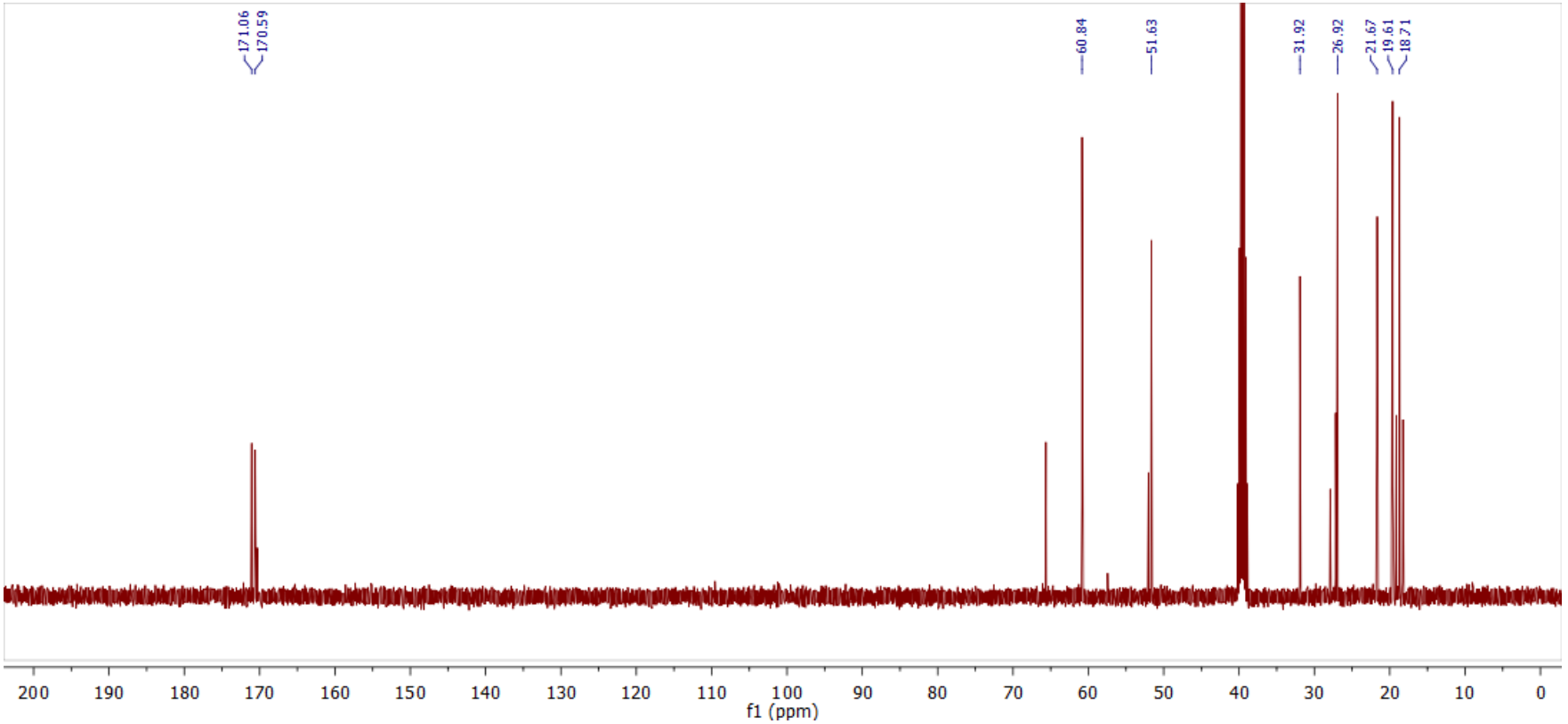


4.1.22. *N*-Acetyl-*N*-methyl-L-valine methyl ester (Ac-(Me)Val-OMe)

^{1}H NMR spectrum of Ac-(Me)Val-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 2.5 : 1.

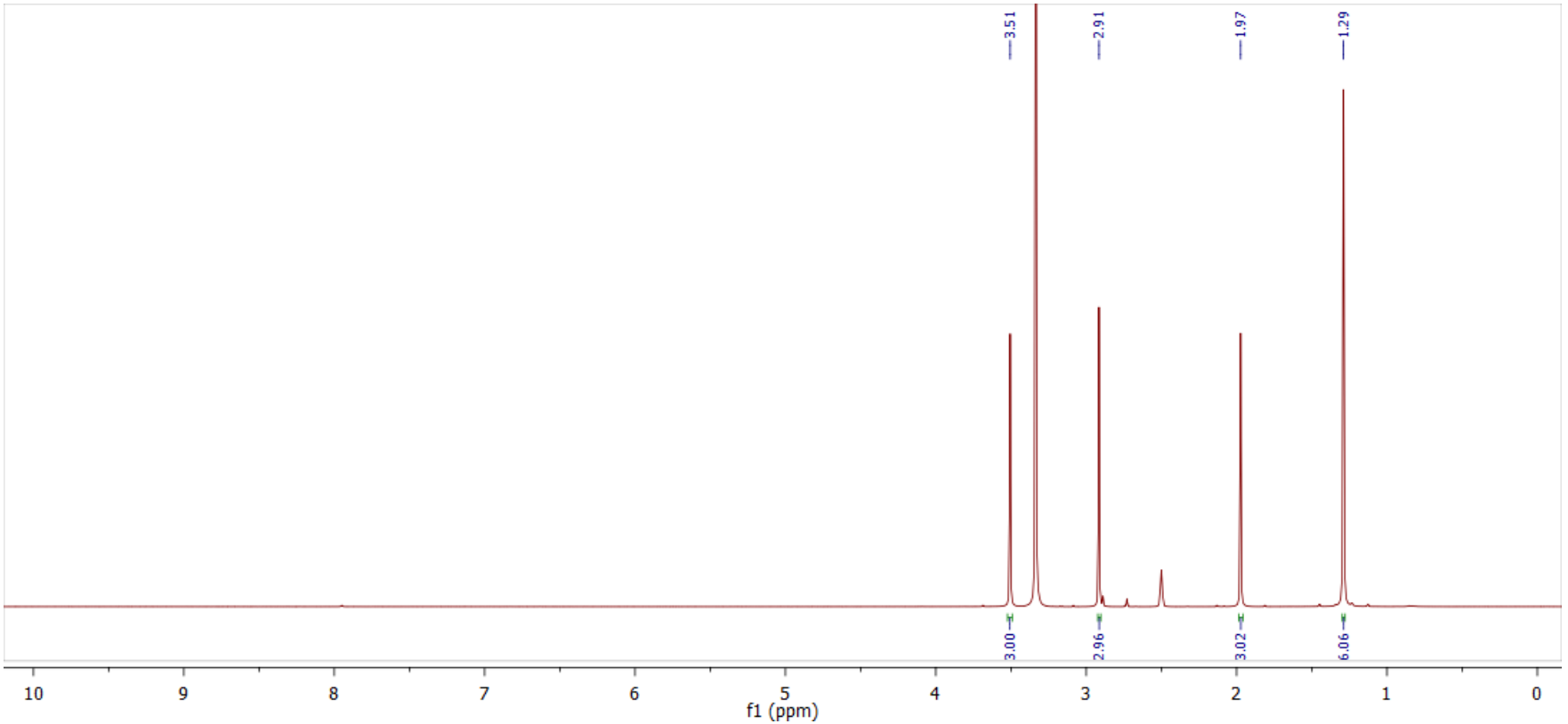


^{13}C NMR spectrum of Ac-(Me)Val-OMe

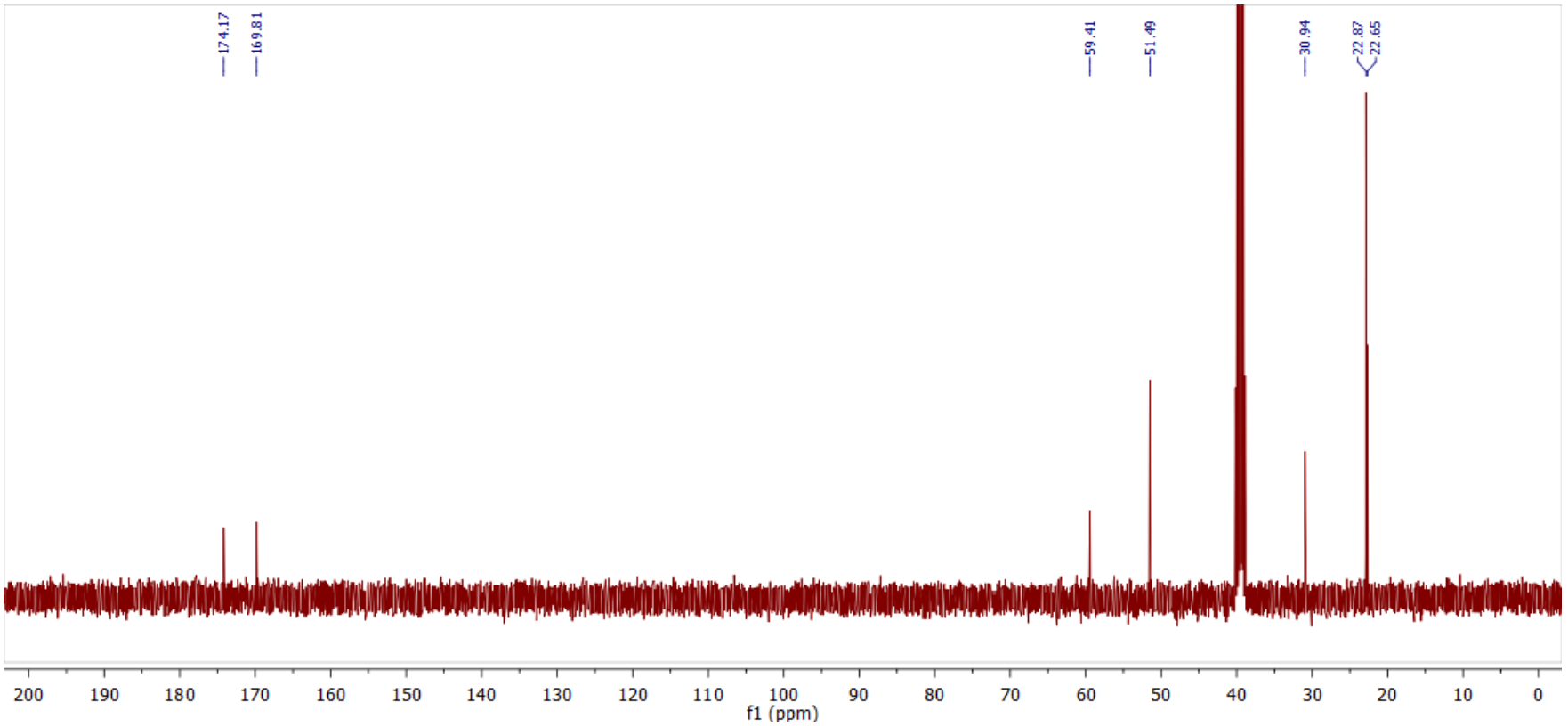


4.1.23. *N*-Acetyl-*N*-methyl-2-aminoisobutyric acid methyl ester (Ac-(Me)Aib-OMe)

^{1}H NMR spectrum of Ac-(Me)Aib-OMe

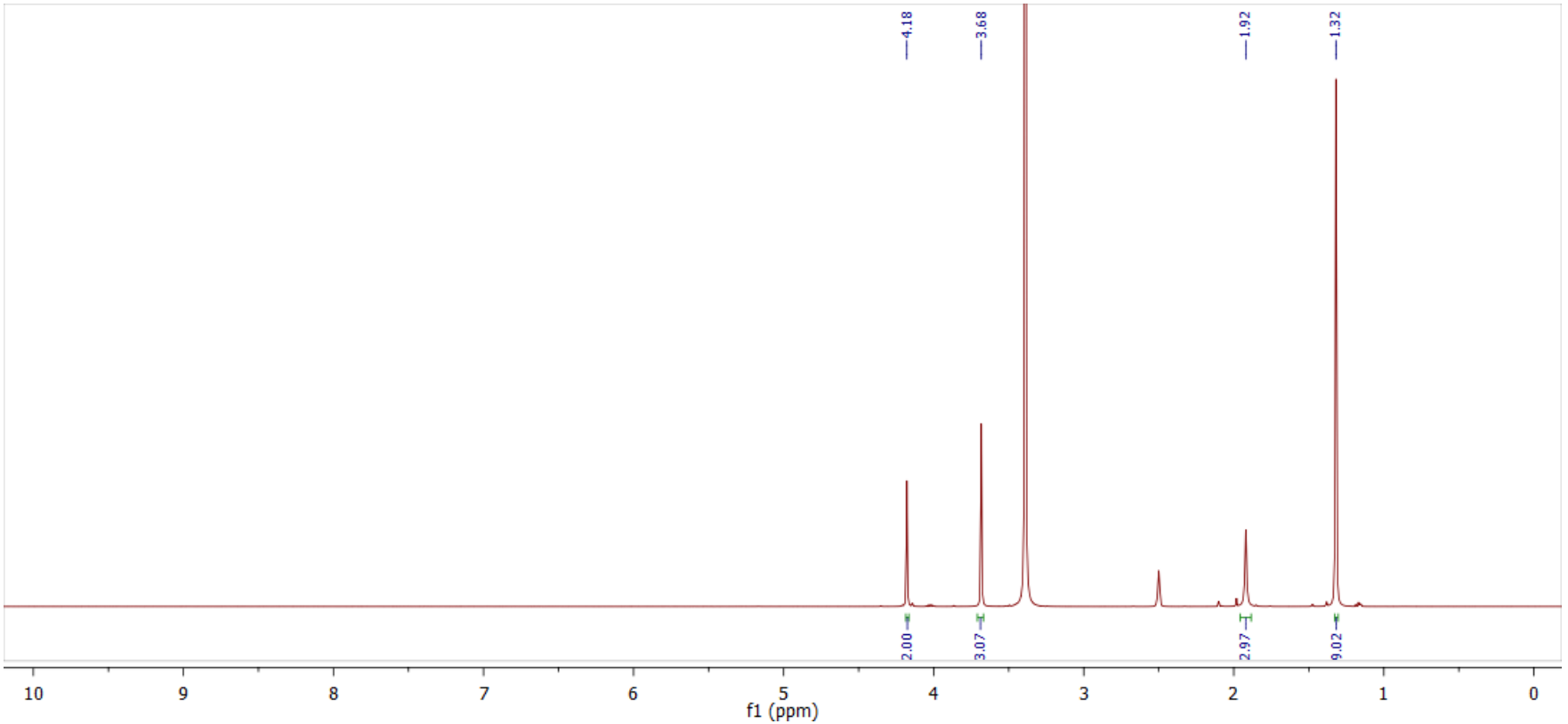


^{13}C NMR spectrum of Ac-(Me)Aib-OMe

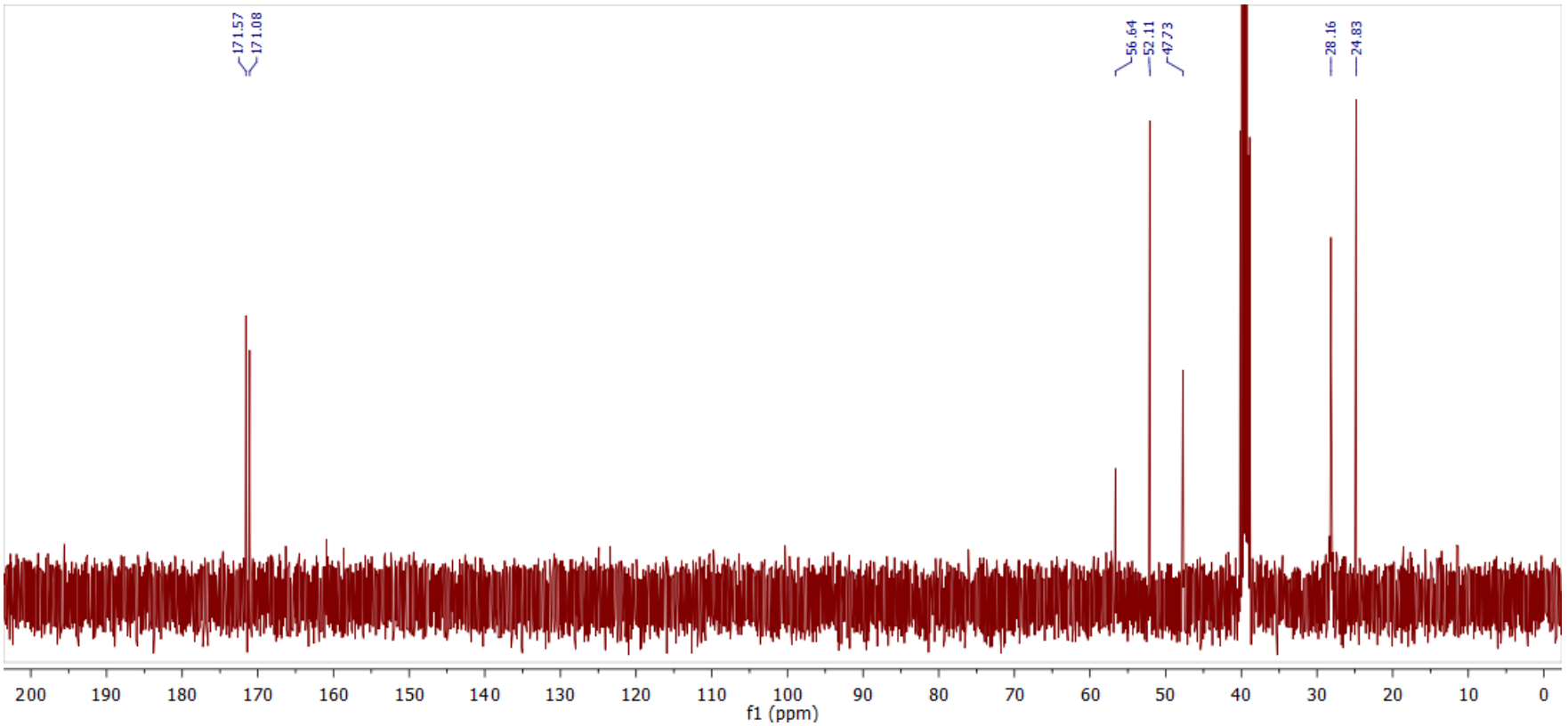


4.1.24. *N*-Acetyl-*N*-*t*-butylglycine methyl ester (Ac-(*t*Bu)Gly-OMe)

^{1}H NMR spectrum of Ac-(*t*Bu)Gly-OMe

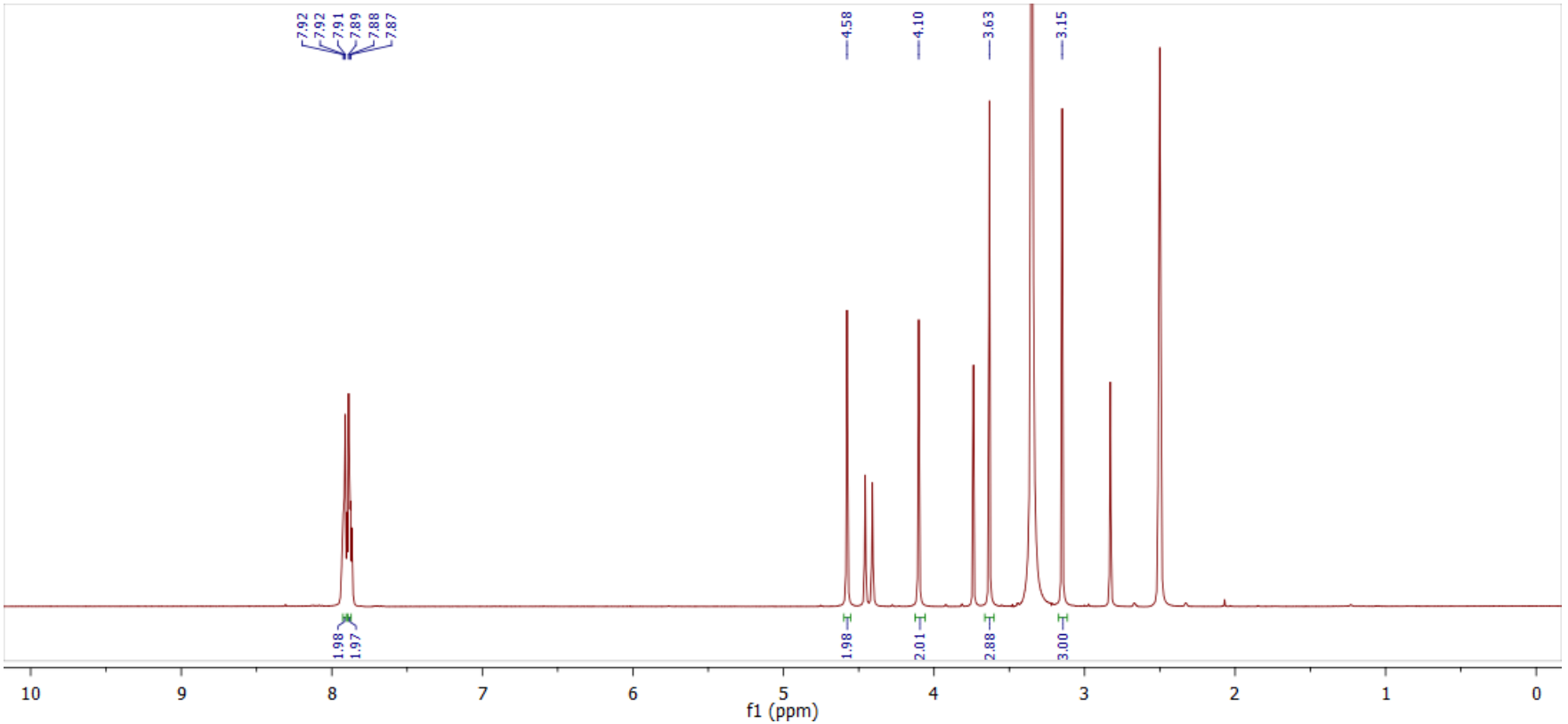


^{13}C NMR spectrum of Ac-(*t*Bu)Gly-OMe

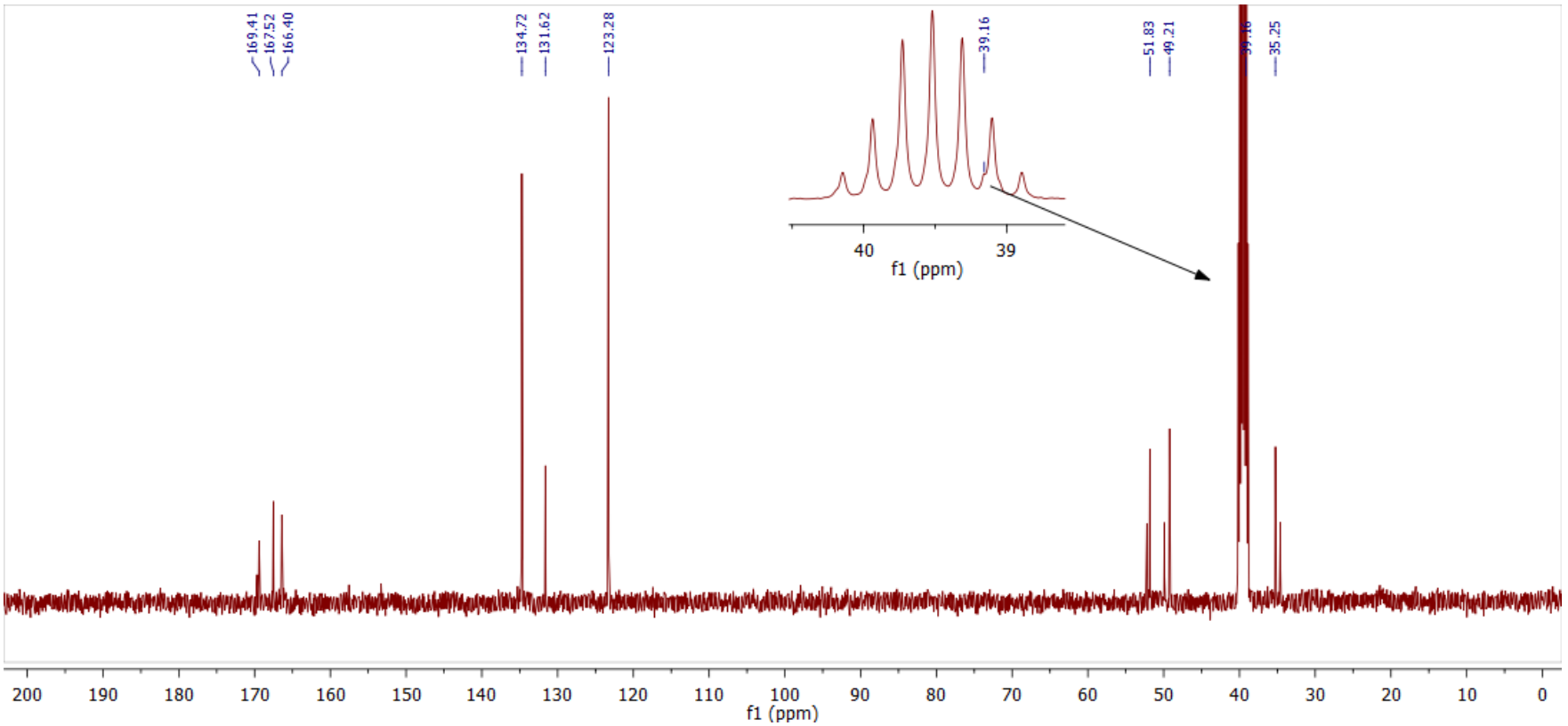


4.1.25. *N*-Phthaloylglycyl-*N*-methylglycine methyl ester (Phth-Gly-(Me)Gly-OMe)

^{1}H NMR spectrum of Phth-Gly-(Me)Gly-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 2.2 : 1.

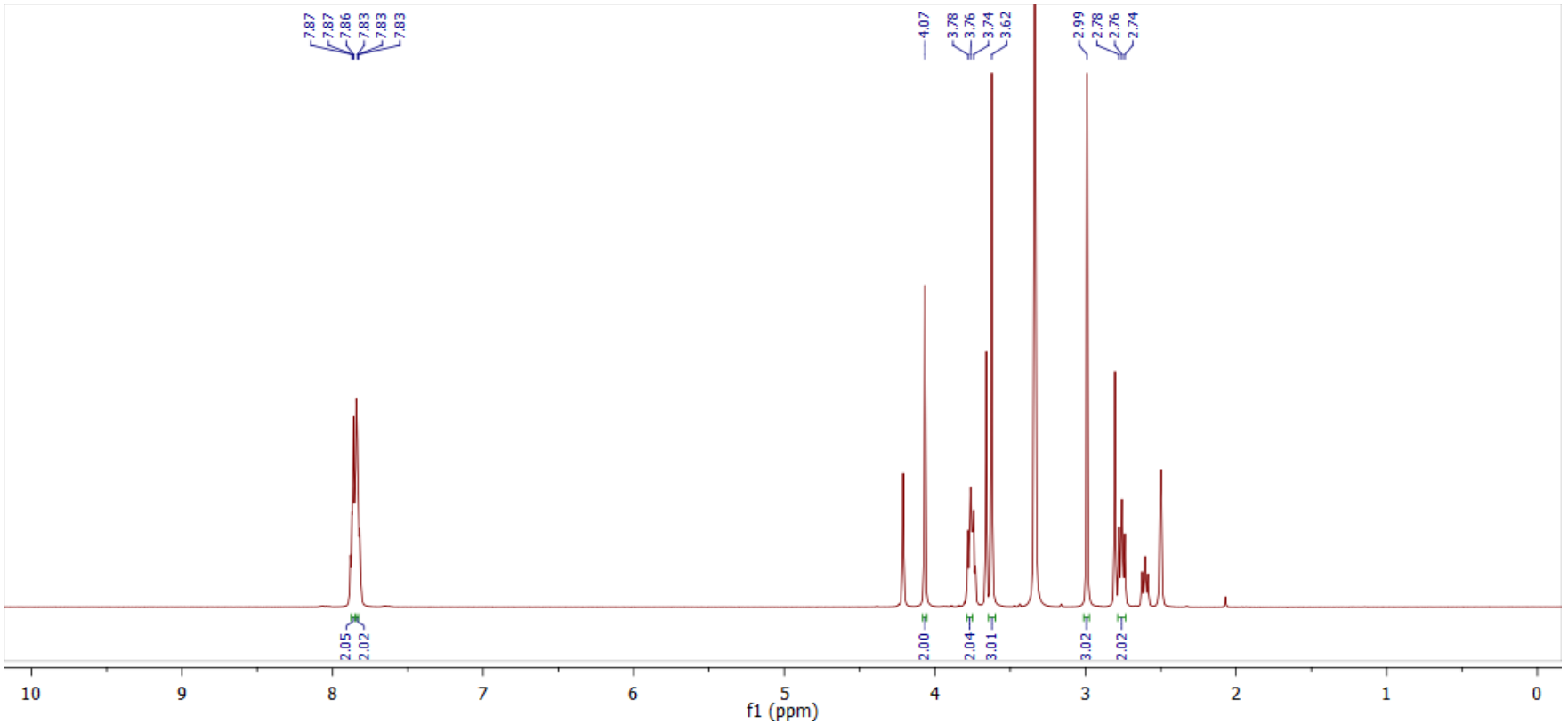


^{13}C NMR spectrum of Phth-Gly-(Me)Gly-OMe

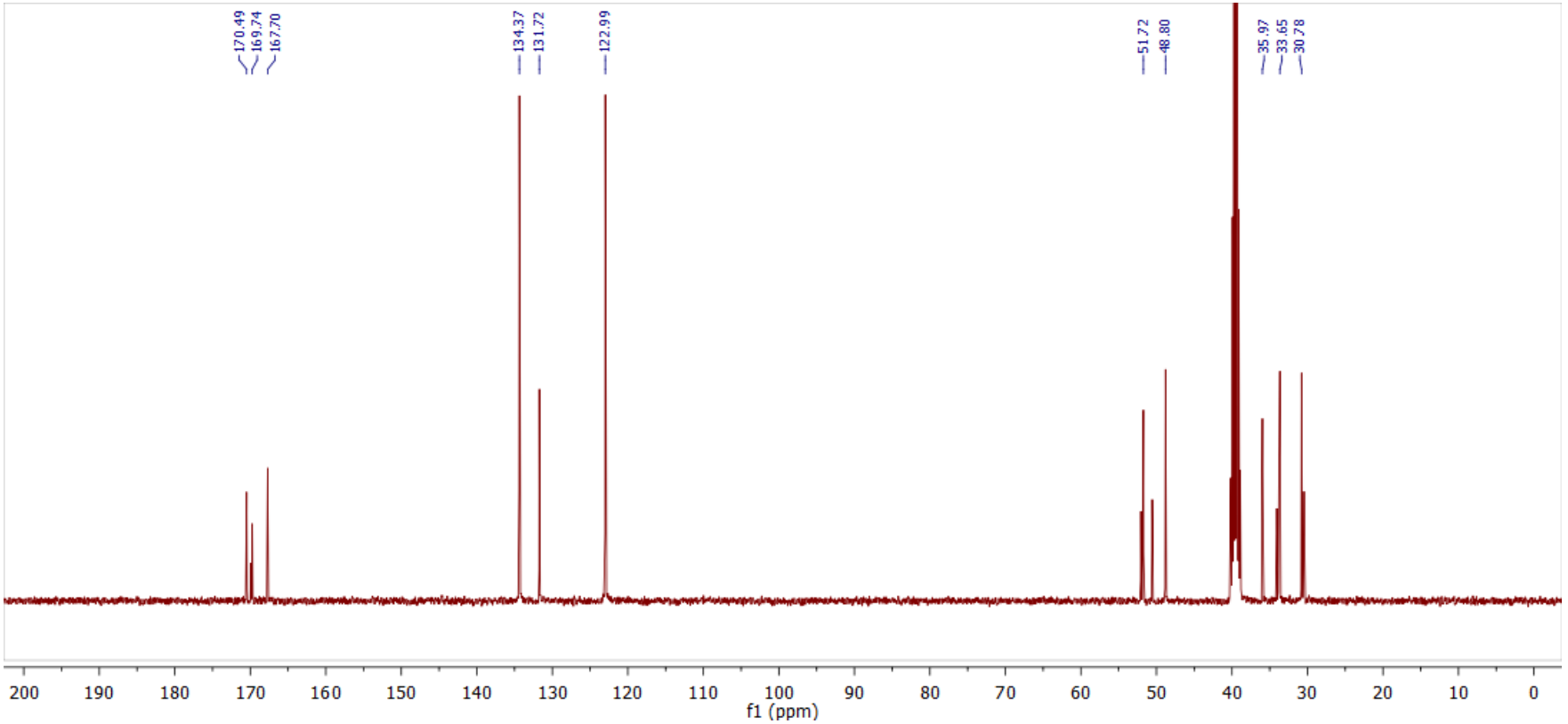


4.1.26. *N*-Phthaloyl-β-alanyl-*N*-methylglycine methyl ester (Phth-βAla-(Me)Gly-OMe)

^{1}H NMR spectrum of Phth-βAla-(Me)Gly-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 2.2 : 1.

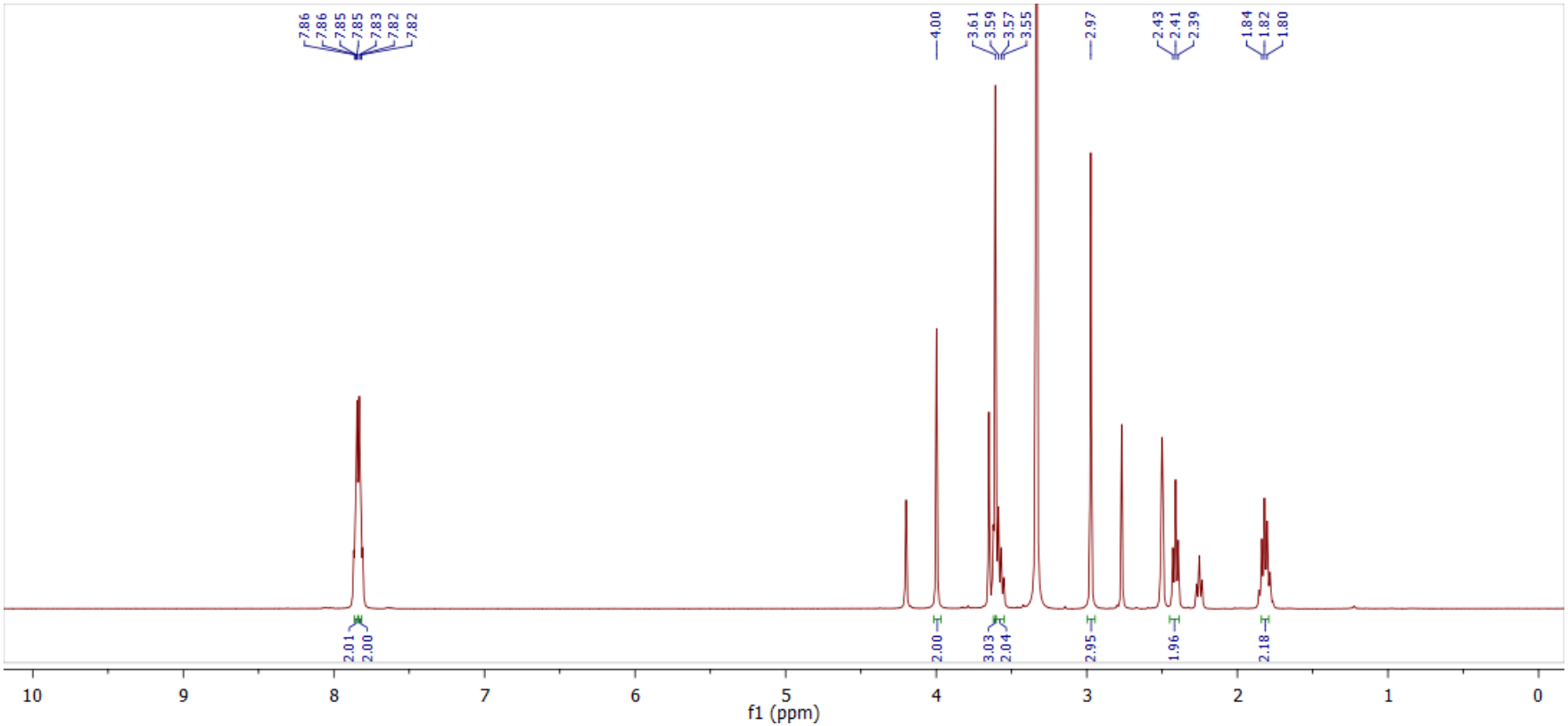


^{13}C NMR spectrum of Phth-βAla-(Me)Gly-OMe

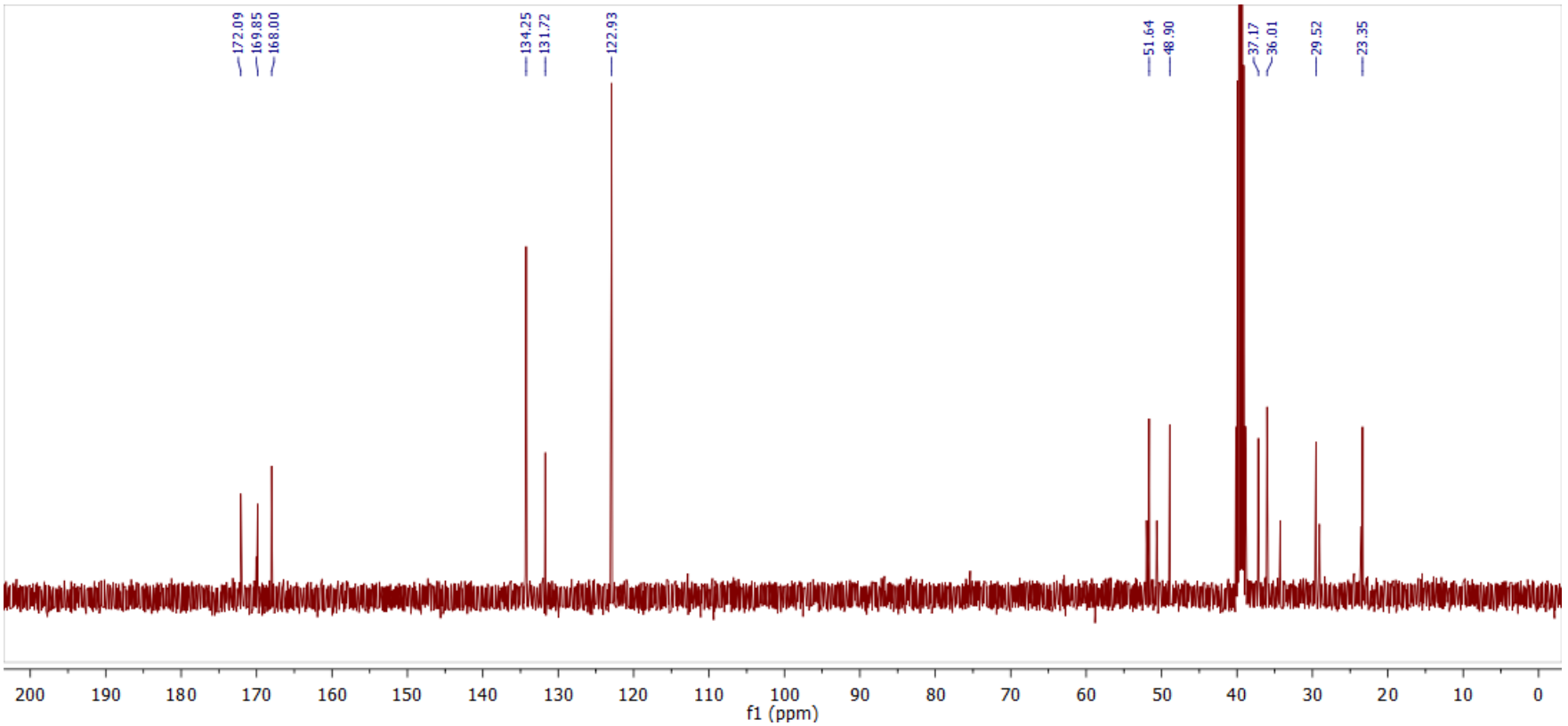


4.1.27. *N*-Phthaloyl-γ-aminobutyryl-*N*-methylglycine methyl ester (Phth-Gaba-(Me)Gly-OMe)

^{1}H NMR spectrum of Phth-Gaba-(Me)Gly-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 2.4 : 1.

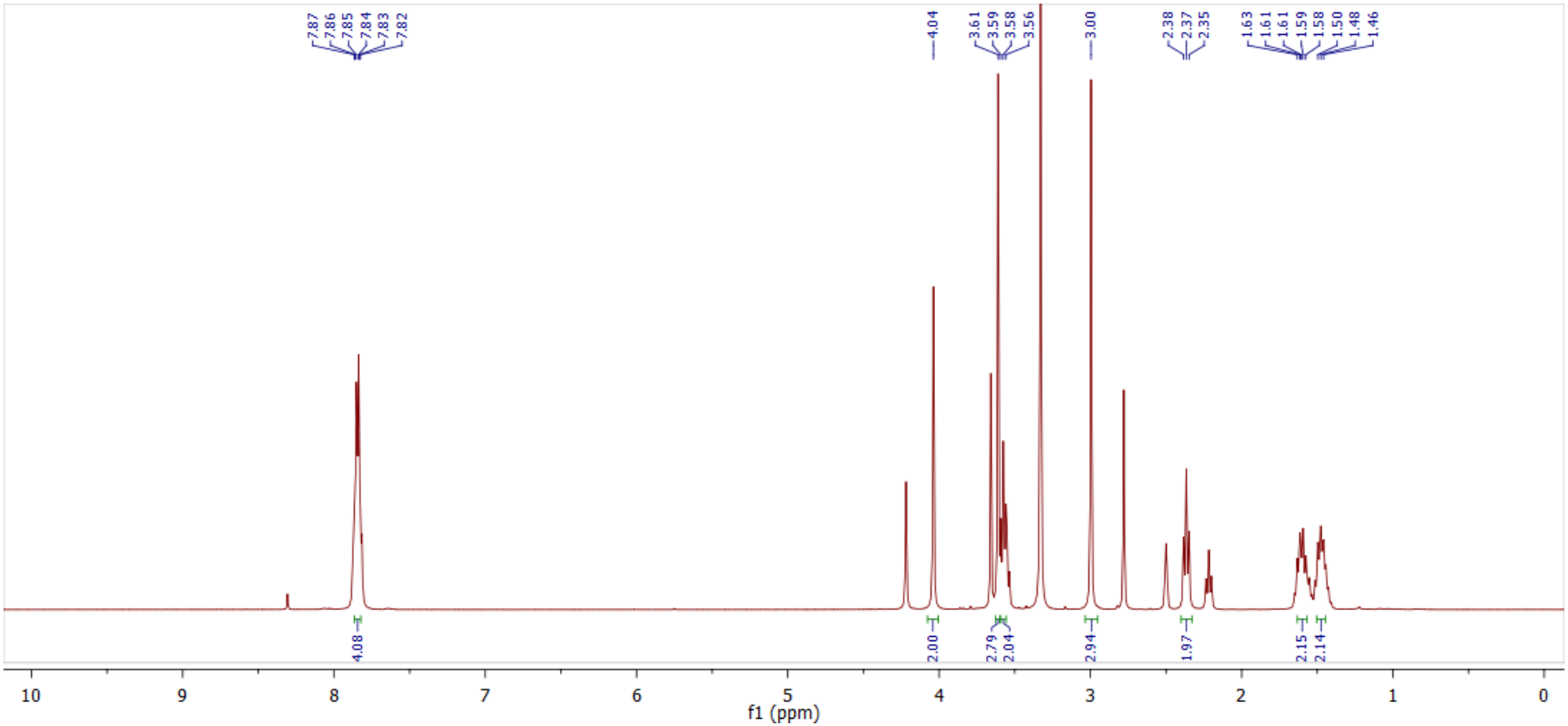


^{13}C NMR spectrum of Phth-Gaba-(Me)Gly-OMe

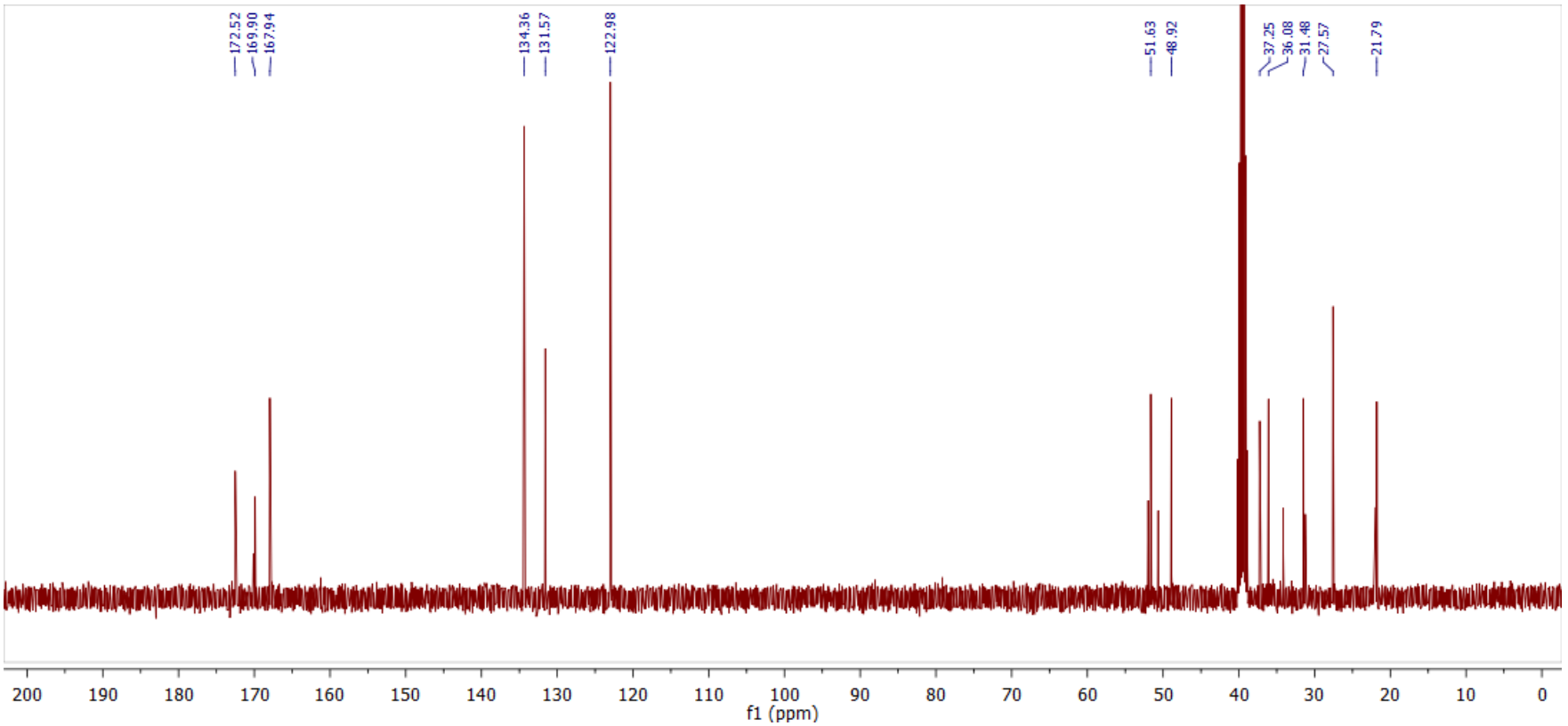


4.1.28. *N*-Phthaloyl-δ-aminovaleryl-*N*-methylglycine methyl ester (Phth-Ava-(Me)Gly-OMe)

^{1}H NMR spectrum of Phth-Ava-(Me)Gly-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 2.2 : 1.

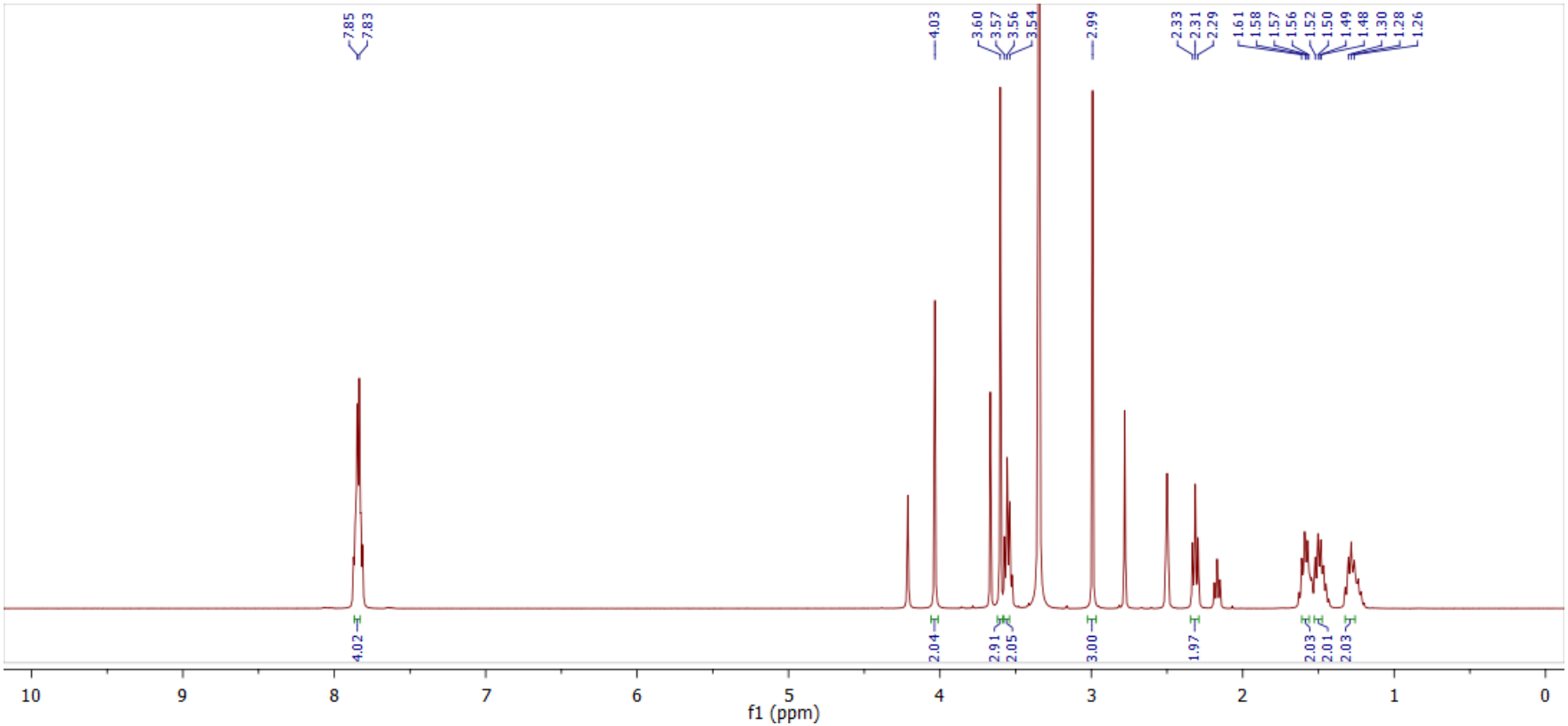


^{13}C NMR spectrum of Phth-Ava-(Me)Gly-OMe

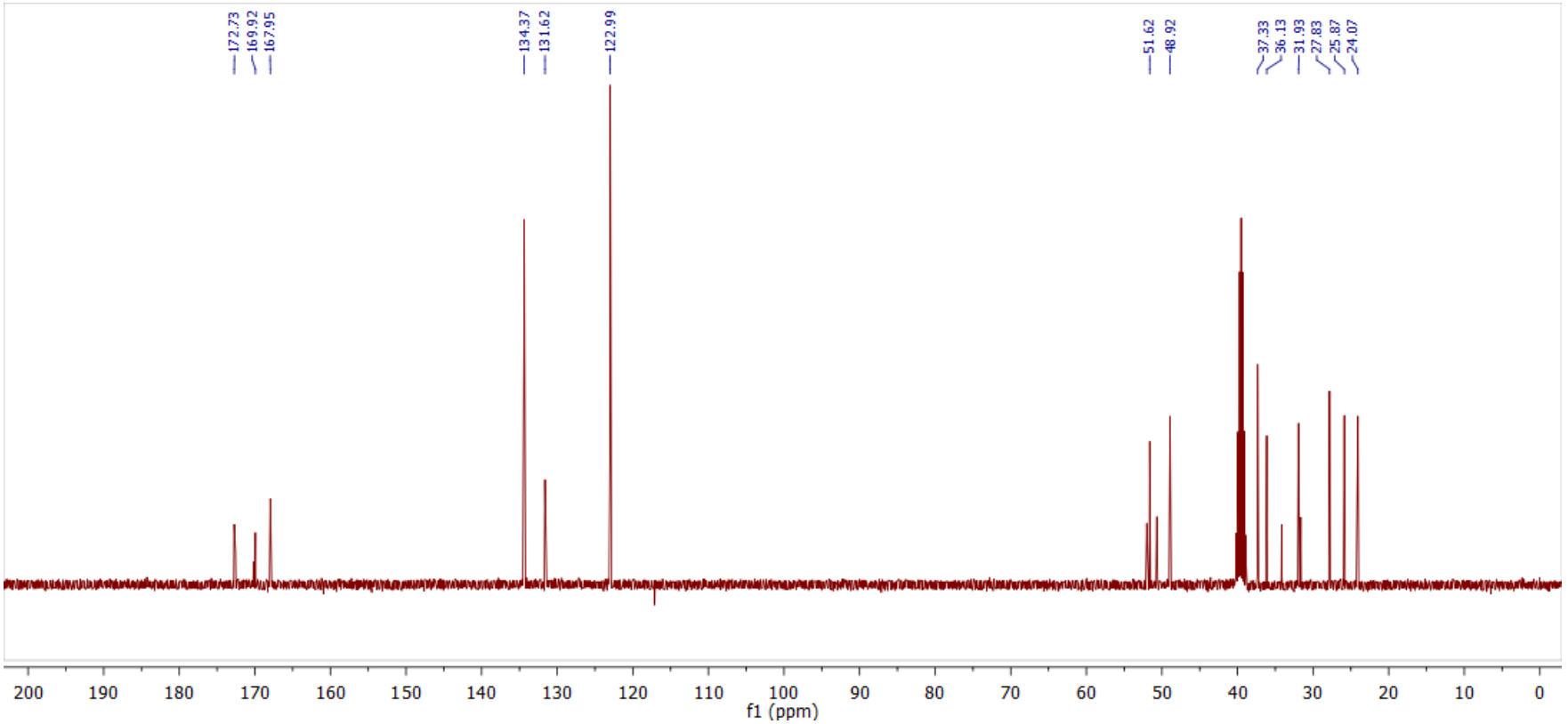


4.1.29. *N*-Phthaloyl-ε-aminocaproyl-*N*-methylglycine methyl ester (Phth-Ahx-(Me)Gly-OMe)

^{1}H NMR spectrum of Phth-Ahx-(Me)Gly-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 2.4 : 1.

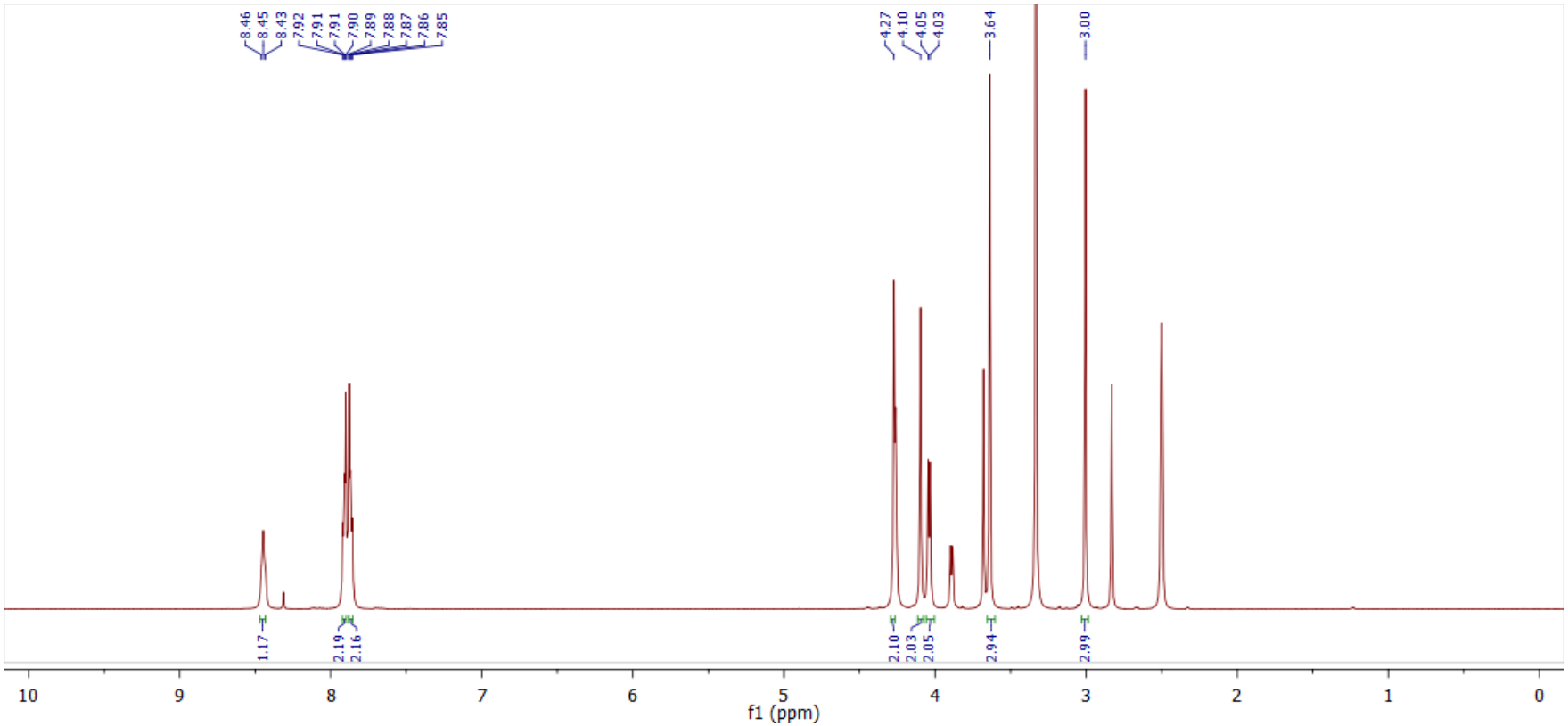


^{13}C NMR spectrum of Phth-Ahx-(Me)Gly-OMe

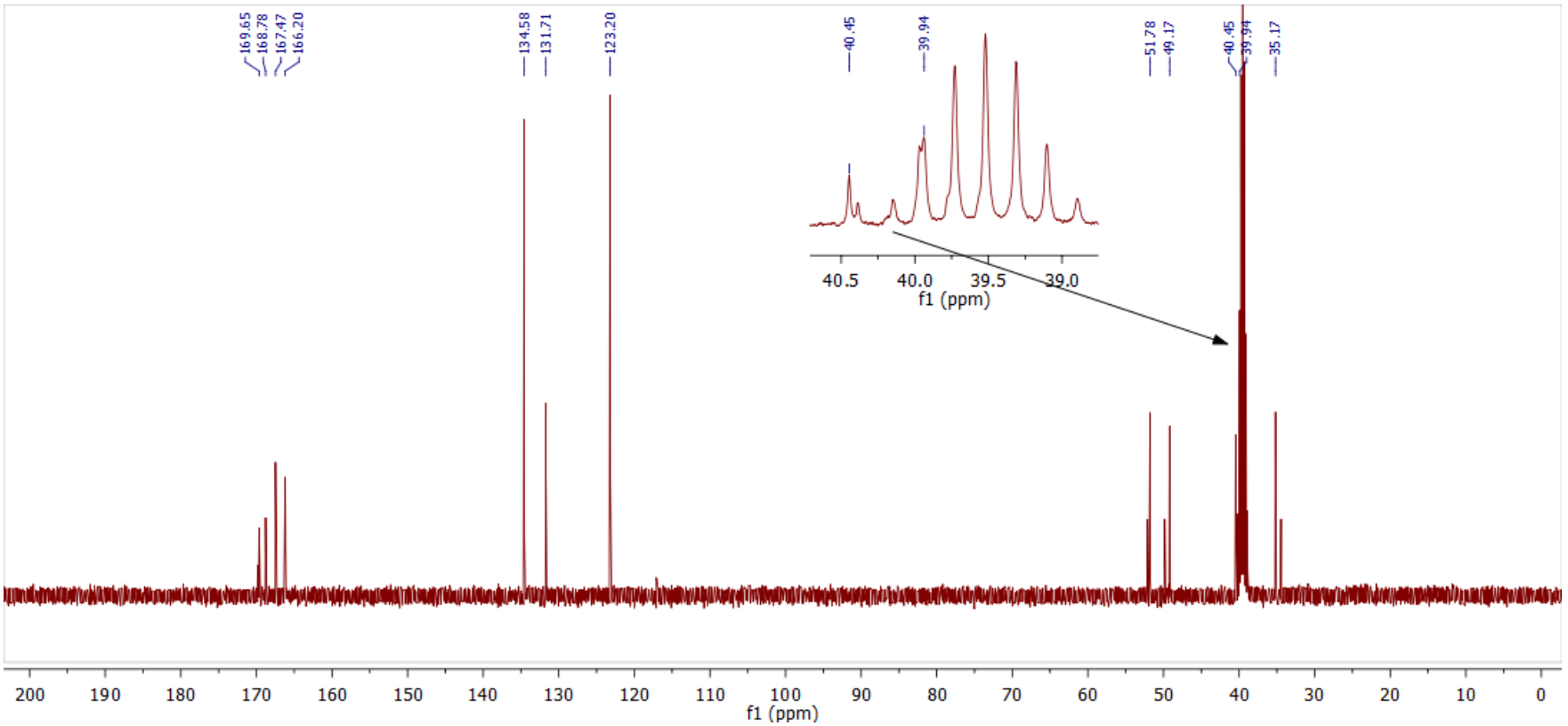


4.1.30. *N*-Phthaloylglycylglycyl-*N*-methylglycine methyl ester (Phth-Gly-Gly-(Me)Gly-OMe)

^{1}H NMR spectrum of Phth-Gly-Gly-(Me)Gly-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 2 : 1.

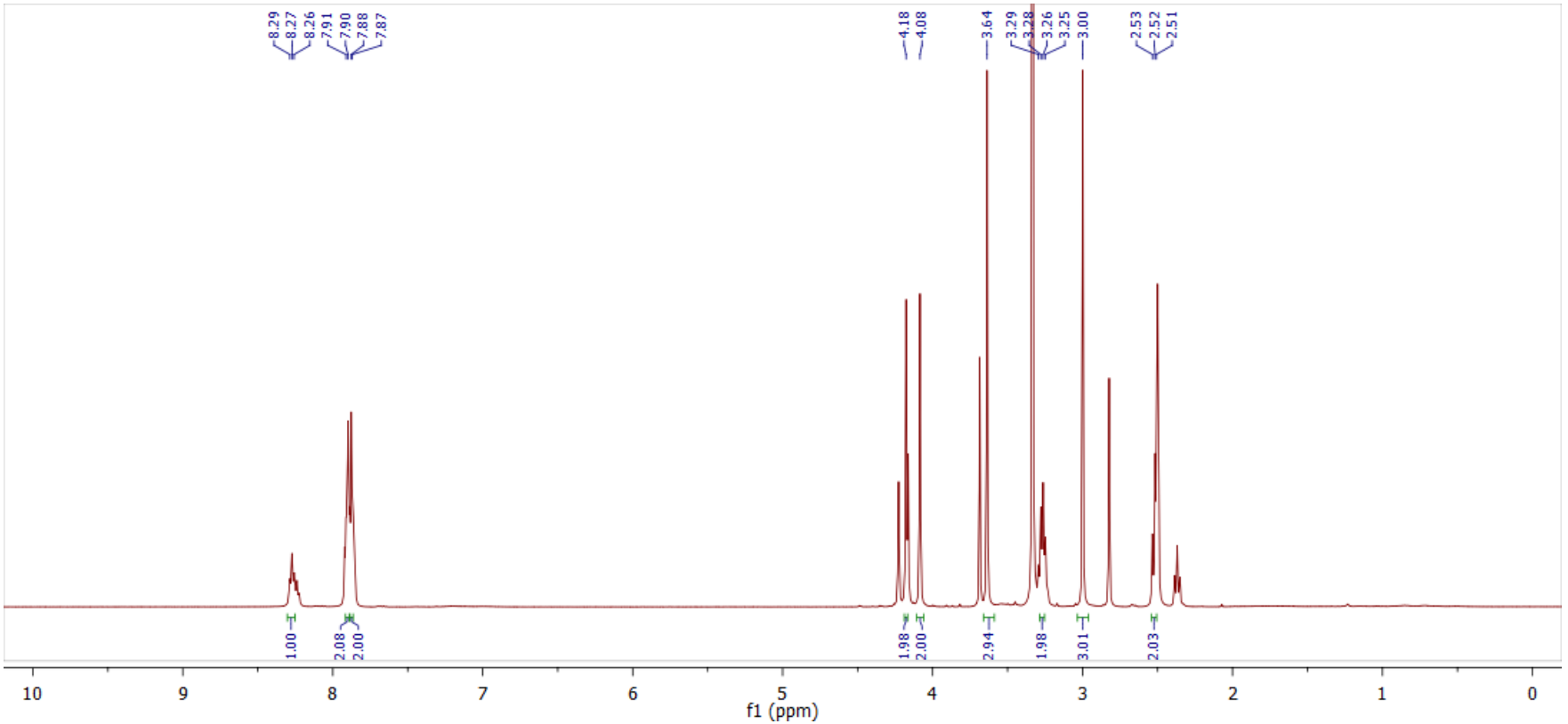


^{13}C NMR spectrum of Phth-Gly-Gly-(Me)Gly-OMe

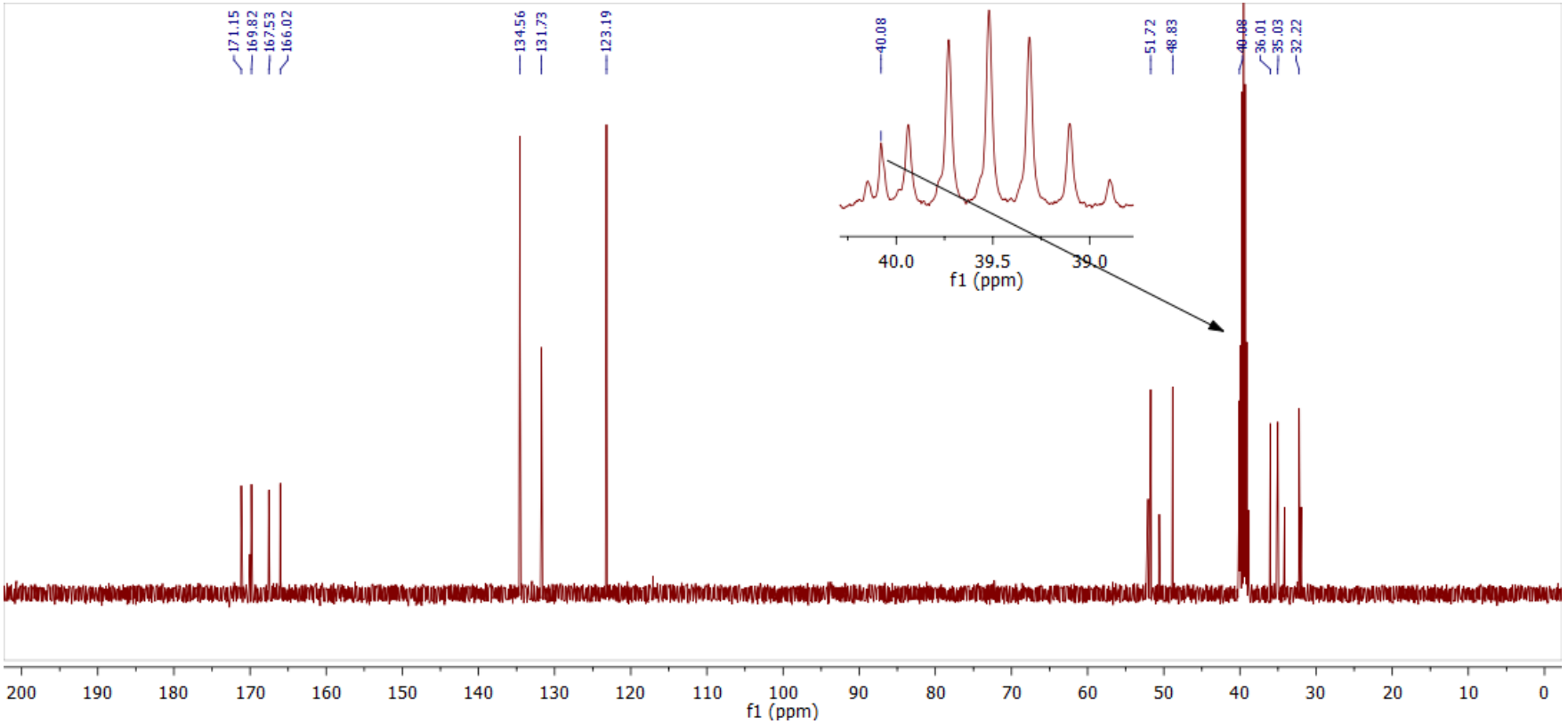


4.1.31. *N*-Phthaloylglycyl-β-alanyl-*N*-methylglycine methyl ester (Phth-Gly-βAla-(Me)Gly-OMe)

^{1}H NMR spectrum of Phth-Gly-βAla-(Me)Gly-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 2 : 1.

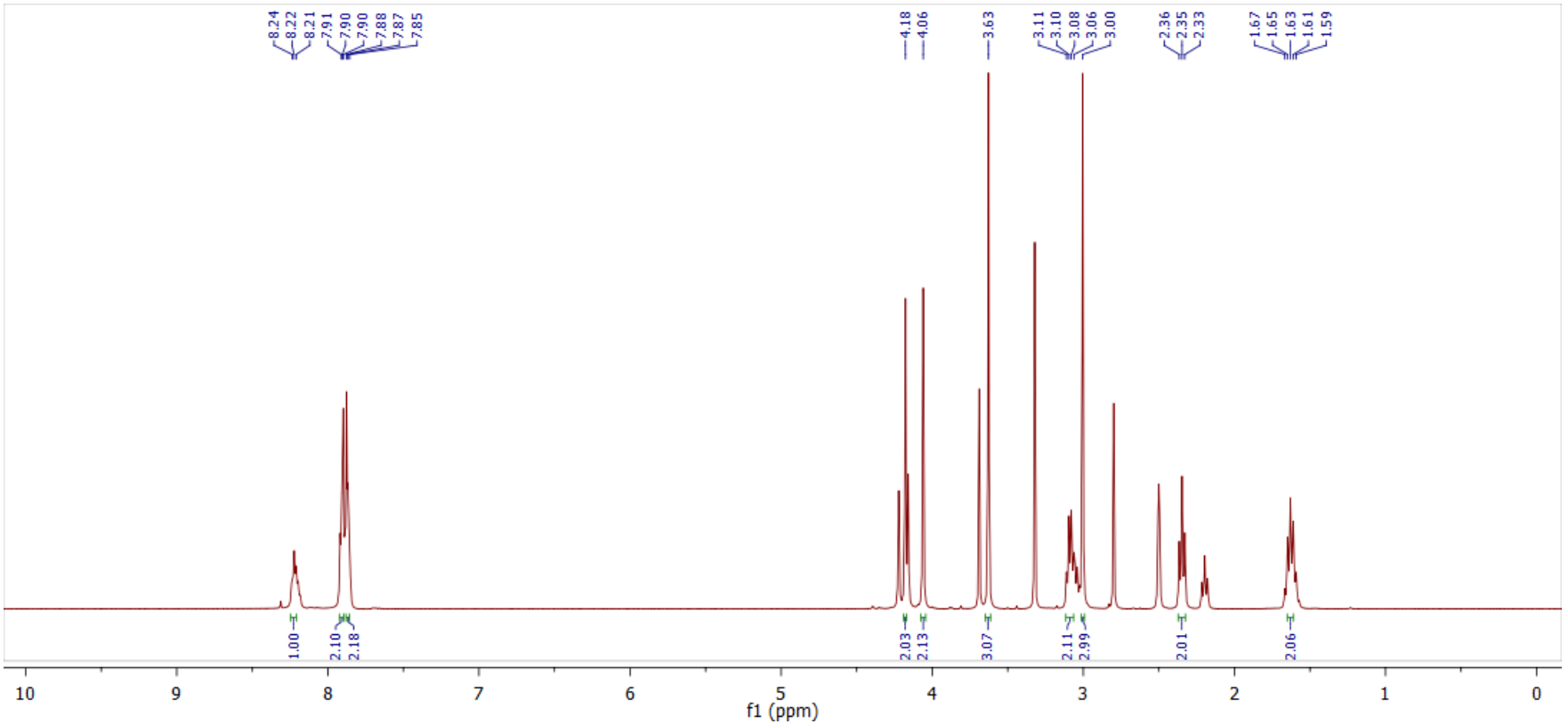


^{13}C NMR spectrum of Phth-Gly-βAla-(Me)Gly-OMe

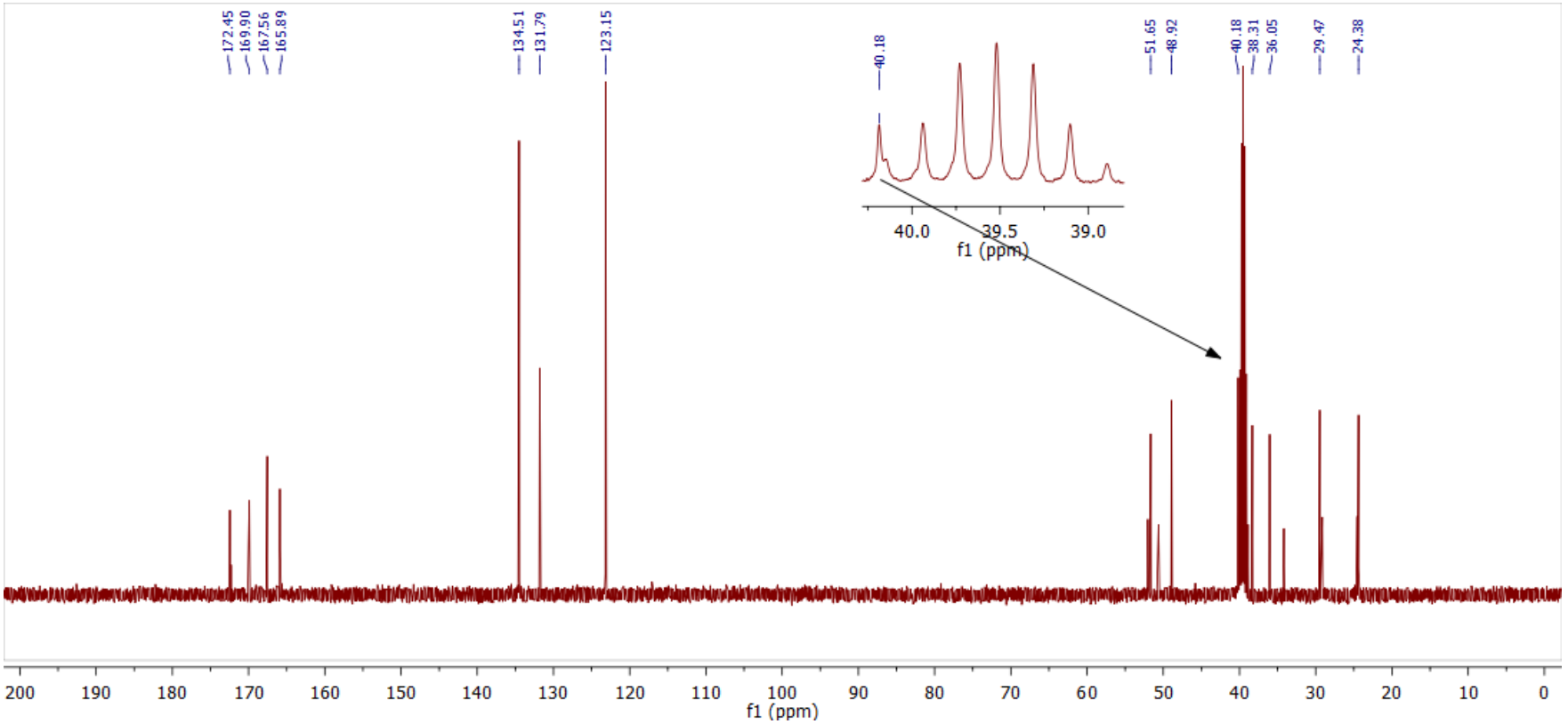


4.1.32. *N*-Phthaloylglycyl-γ-aminobutyryl-*N*-methylglycine methyl ester (Phth-Gly-Gaba-(Me)Gly-OMe)

^{1}H NMR spectrum of Phth-Gly-Gaba-(Me)Gly-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 2.5 : 1.

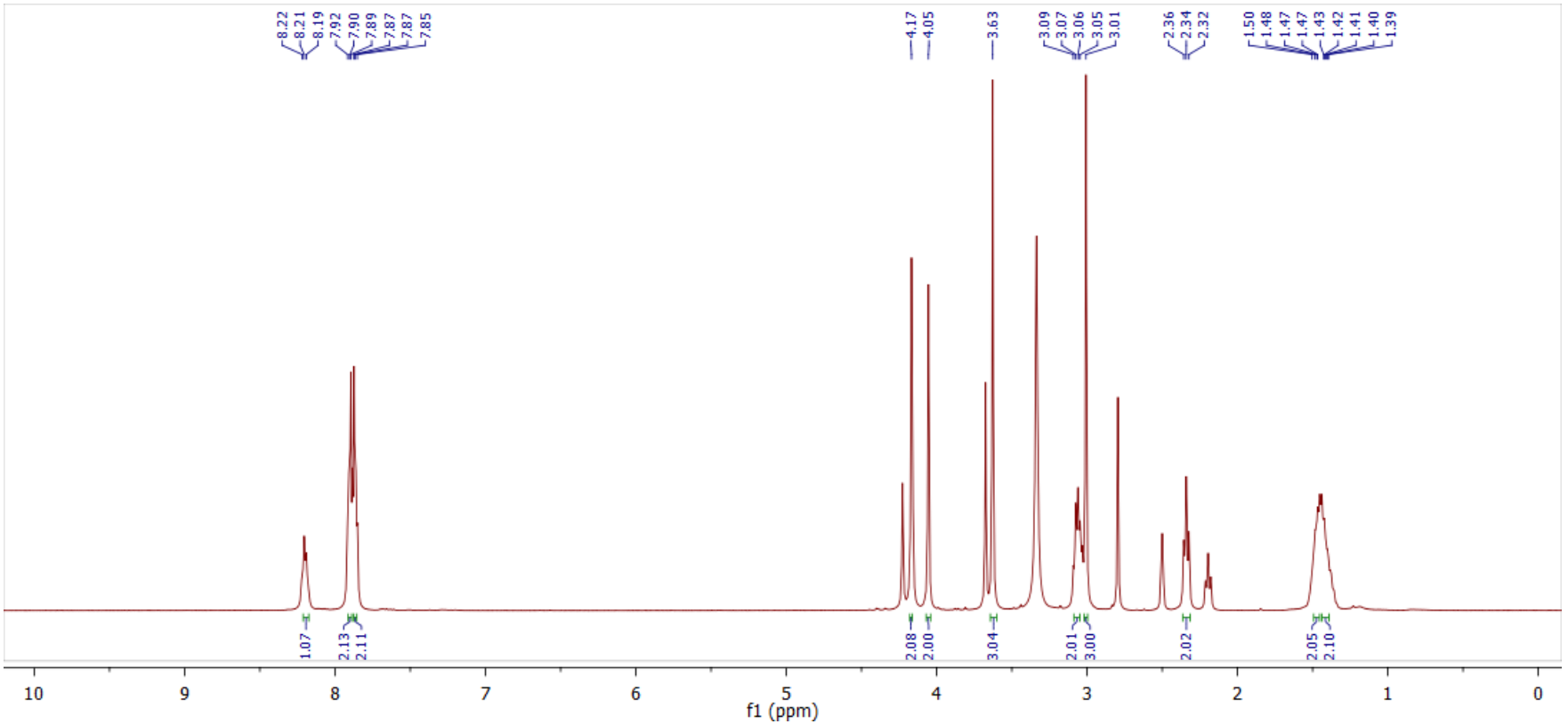


^{13}C NMR spectrum of Phth-Gly-Gaba-(Me)Gly-OMe

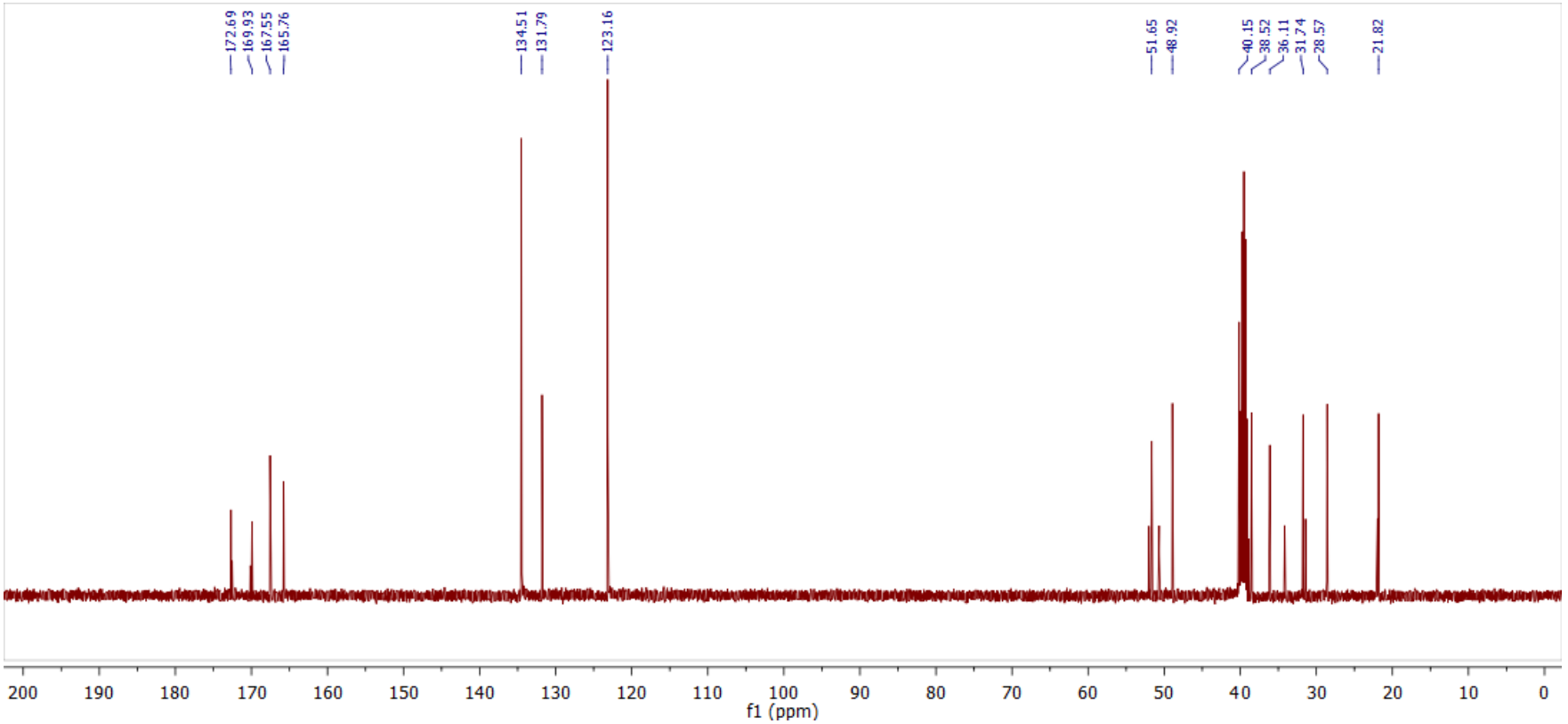


4.1.33. *N*-Phthaloylglycyl-δ-aminovaleryl-*N*-methylglycine methyl ester (Phth-Gly-Ava-(Me)Gly-OMe)

^{1}H NMR spectrum of Phth-Gly-Ava-(Me)Gly-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 2.2 : 1.

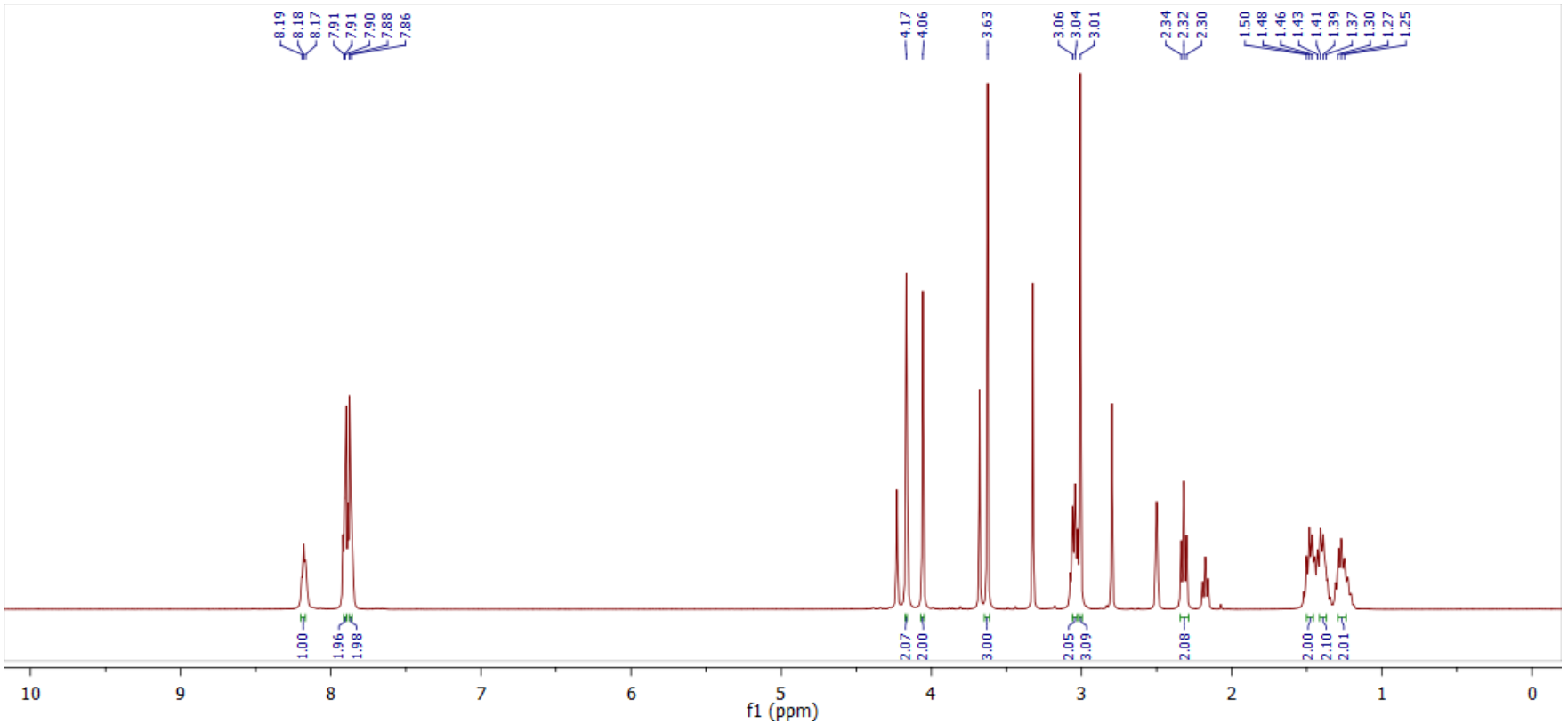


^{13}C NMR spectrum of Phth-Gly-Ava-(Me)Gly-OMe

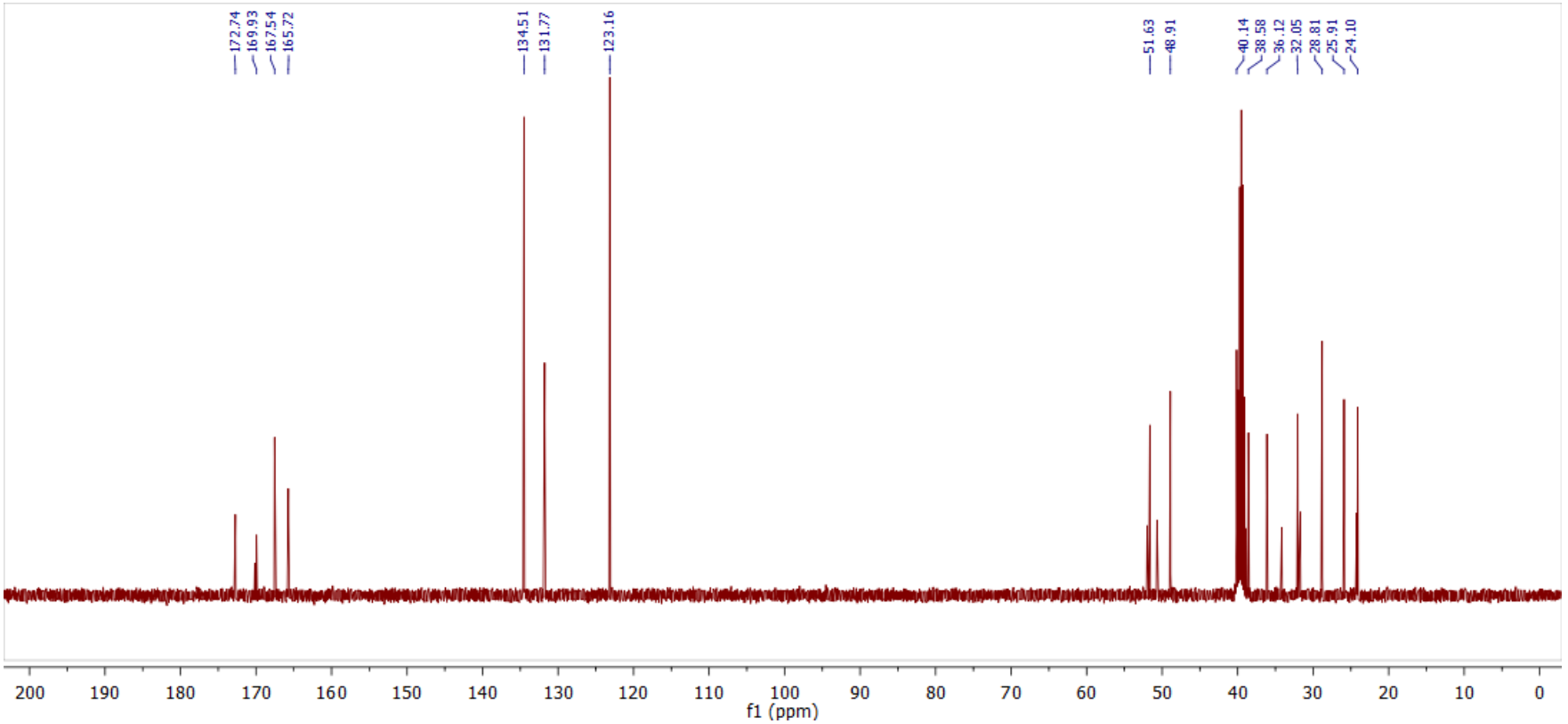


4.1.34. *N*-Phthaloylglycyl-ε-aminocaproyl-*N*-methylglycine methyl ester (Phth-Gly-Ahx-(Me)Gly-OMe)

^{1}H NMR spectrum of Phth-Gly-Ahx-(Me)Gly-OMe, only the major isomer was assigned. The isomers are observed in a ratio of 2.4 : 1.

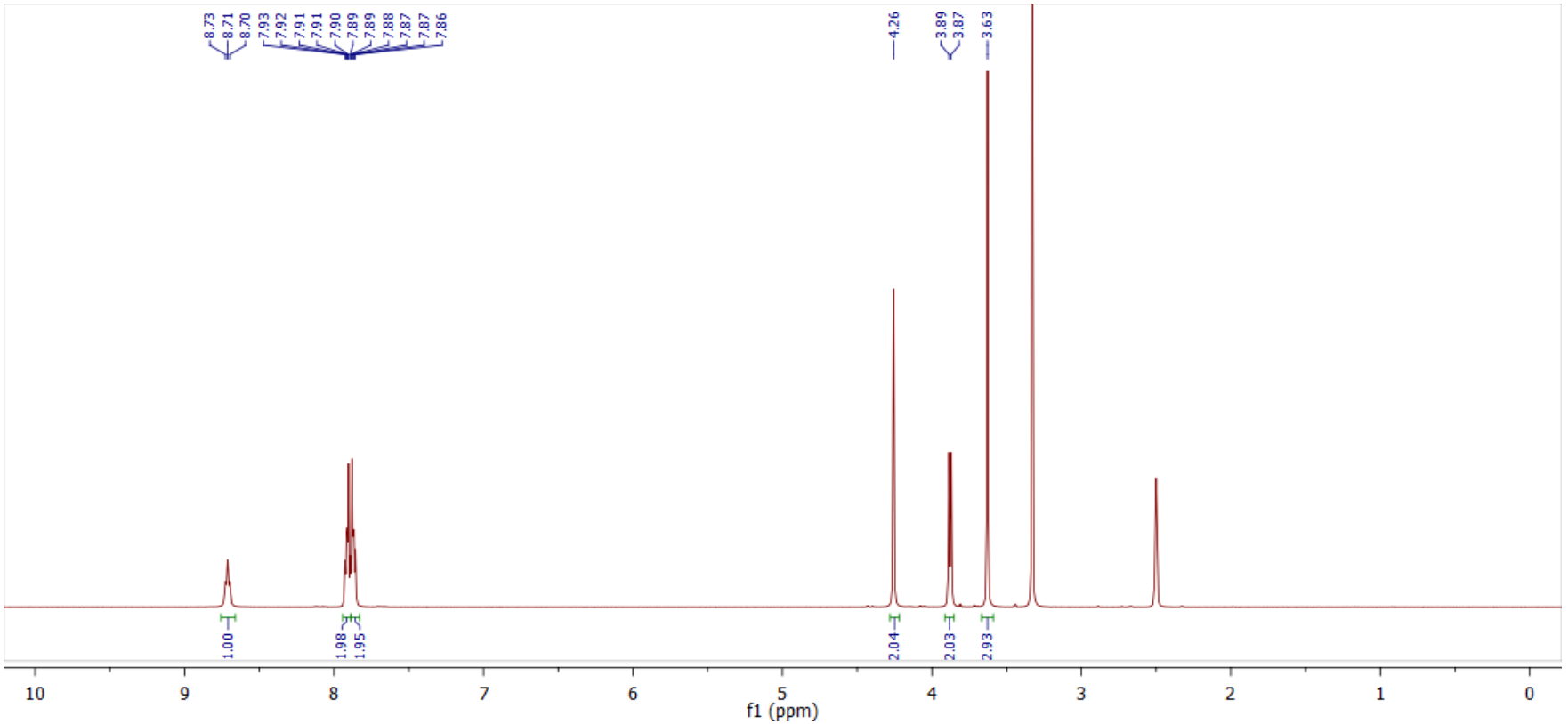


^{13}C NMR spectrum of Phth-Gly-Ahx-(Me)Gly-OMe

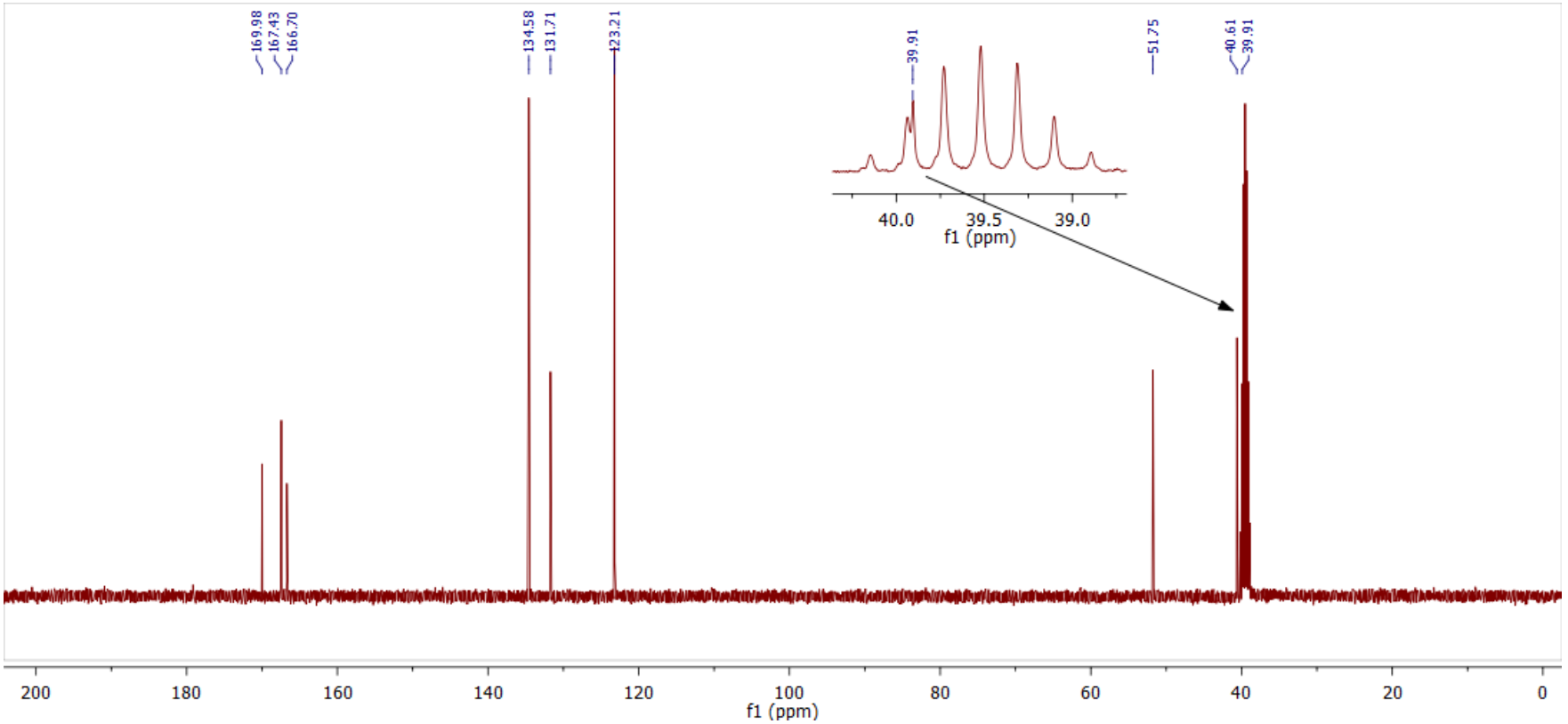


4.1.35. *N*-Phthaloylglycylglycine methyl ester (Phth-Gly-Gly-OMe)

^{1}H NMR spectrum of Phth-Gly-Gly-OMe

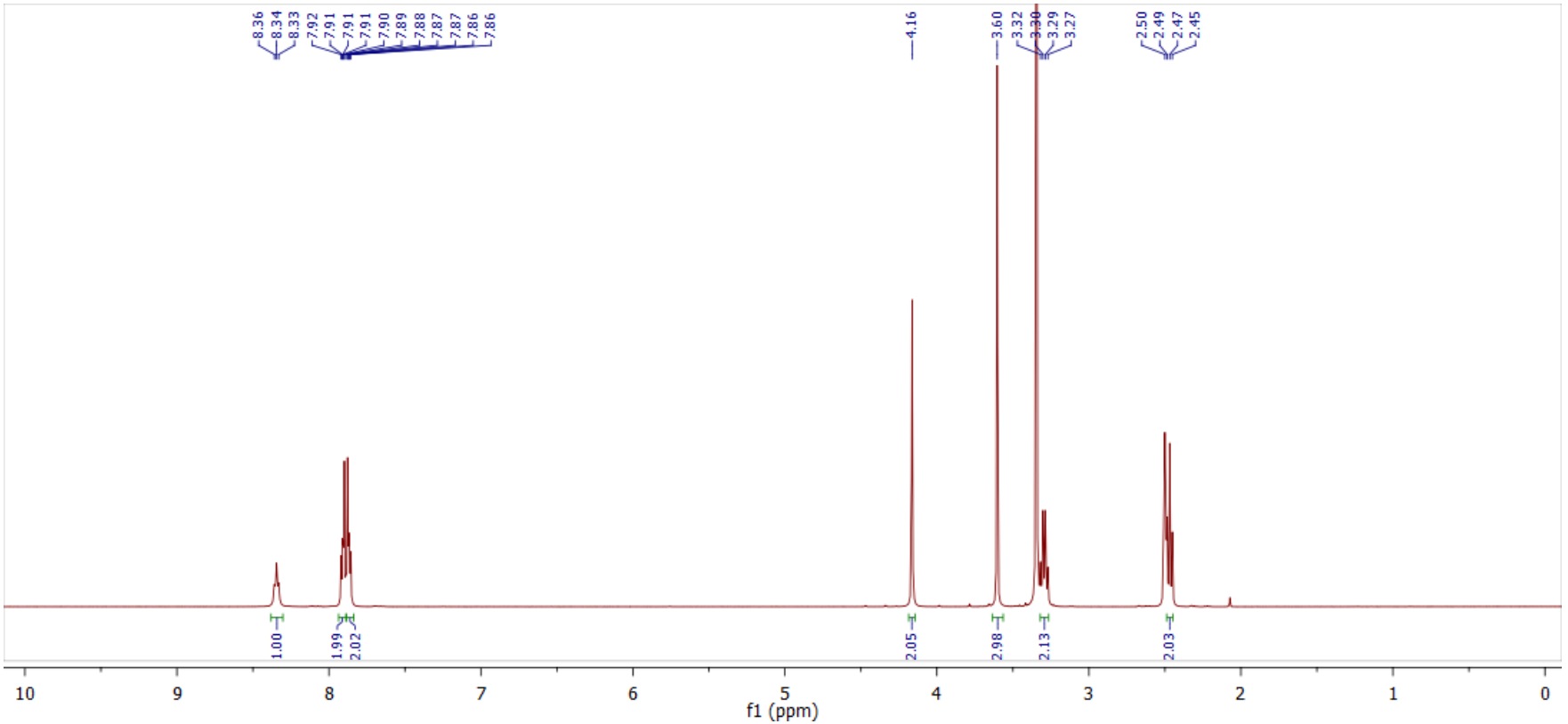


^{13}C NMR spectrum of Phth-Gly-Gly-OMe

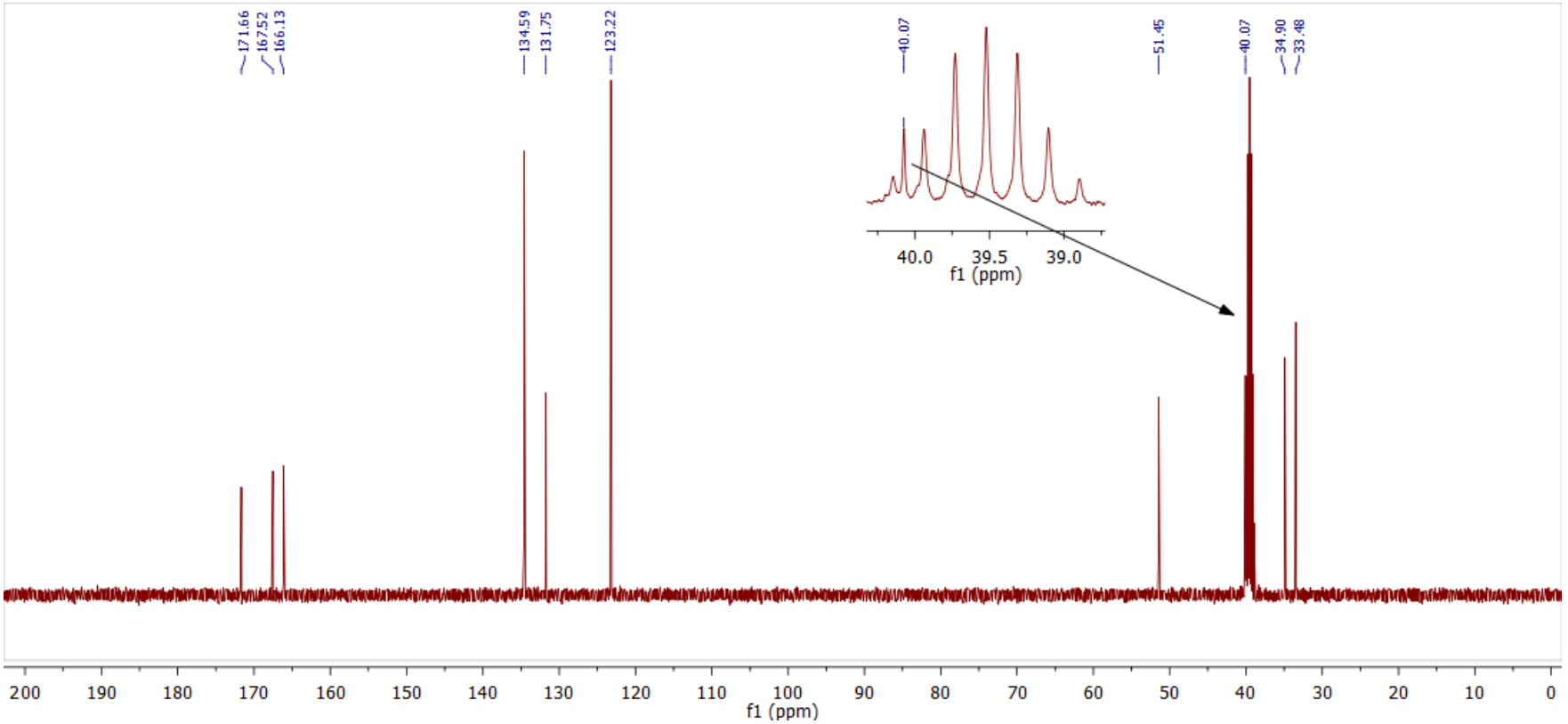


4.1.36. *N*-Phthaloylglycl-β-alanine methyl ester (Phth-Gly-βAla-OMe)

^{1}H NMR spectrum of Phth-Gly-βAla-OMe

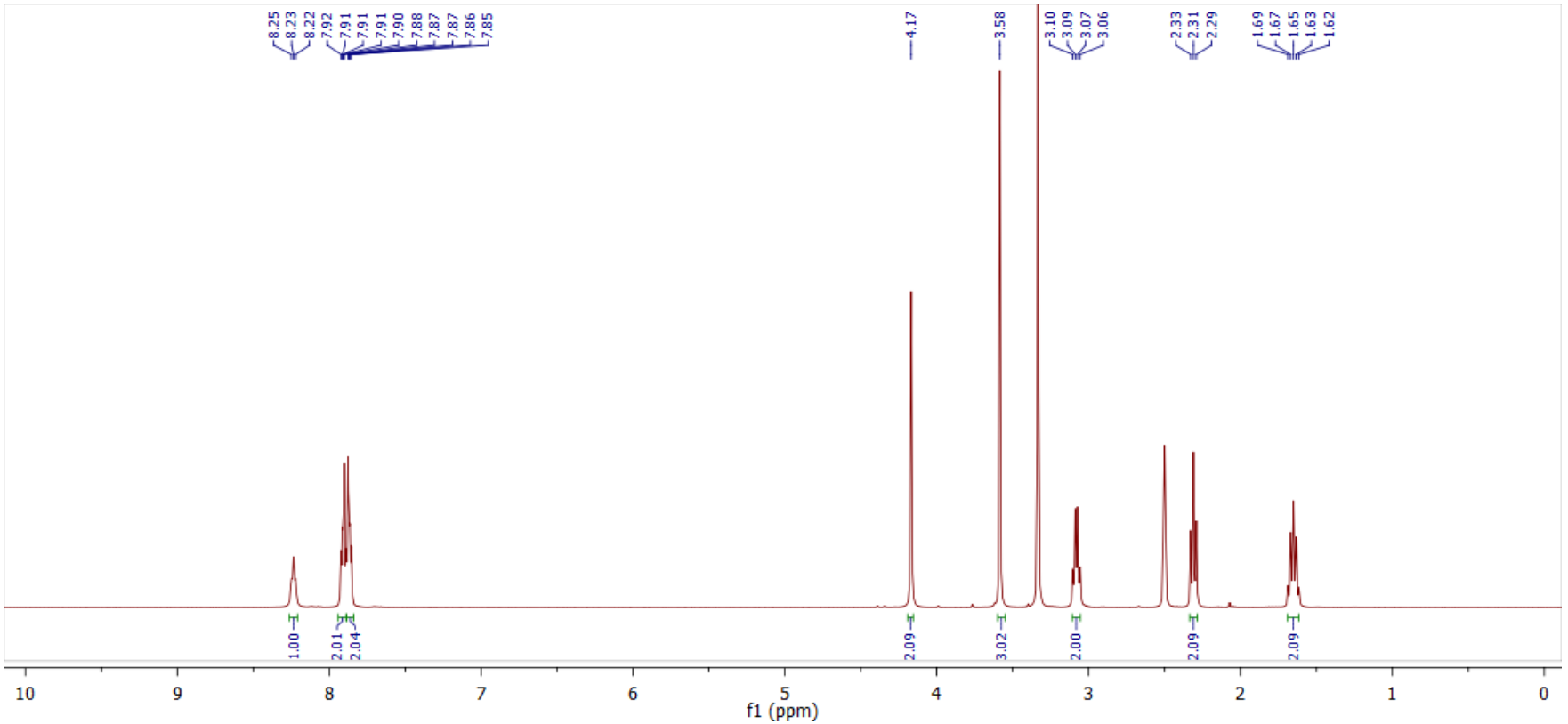


^{13}C NMR spectrum of Phth-Gly-βAla-OMe

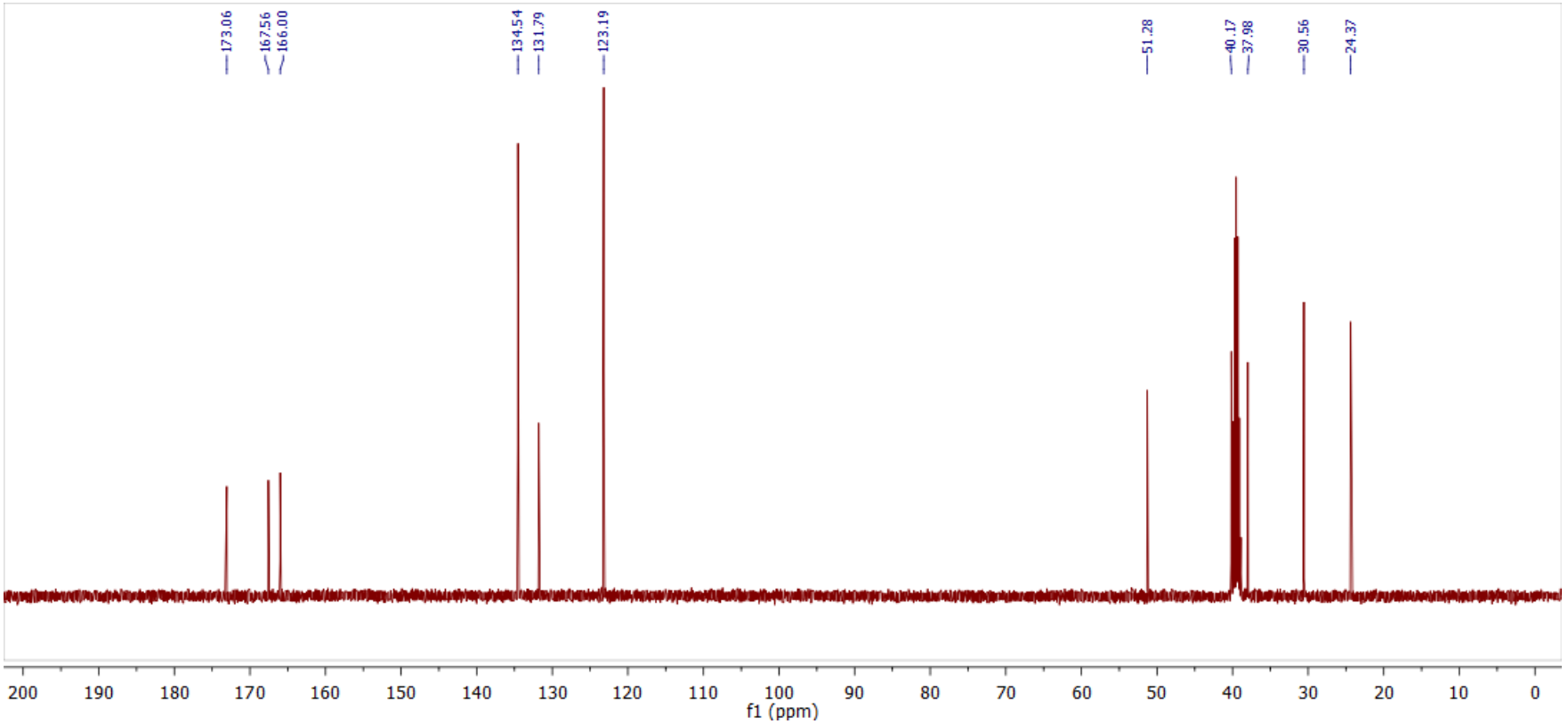


4.1.37. *N*-Phthaloylglycl-γ-aminobutyric acid methyl ester (Phth-Gly-Gaba-OMe)

^{1}H NMR spectrum of Phth-Gly-Gaba-OMe

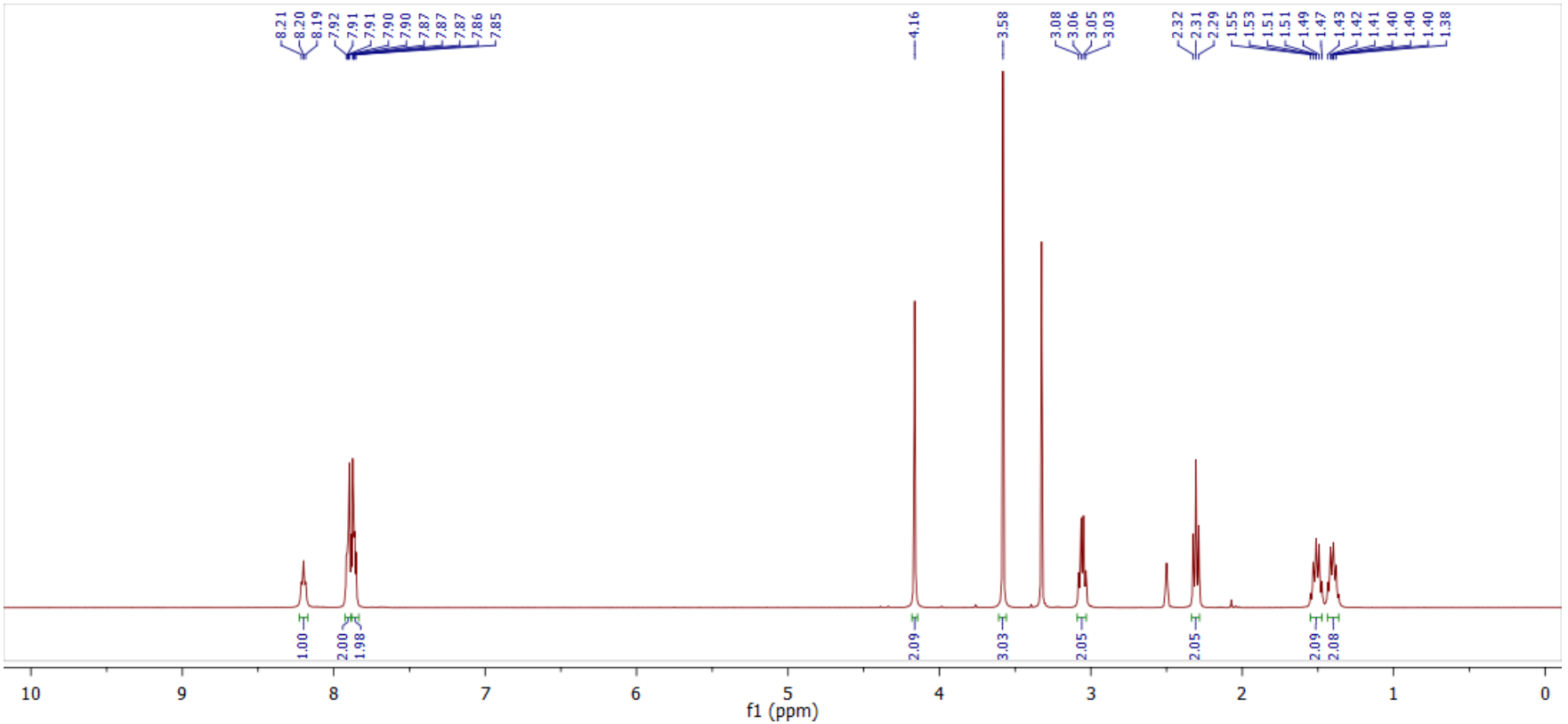


^{13}C NMR spectrum of Phth-Gly-Gaba-OMe

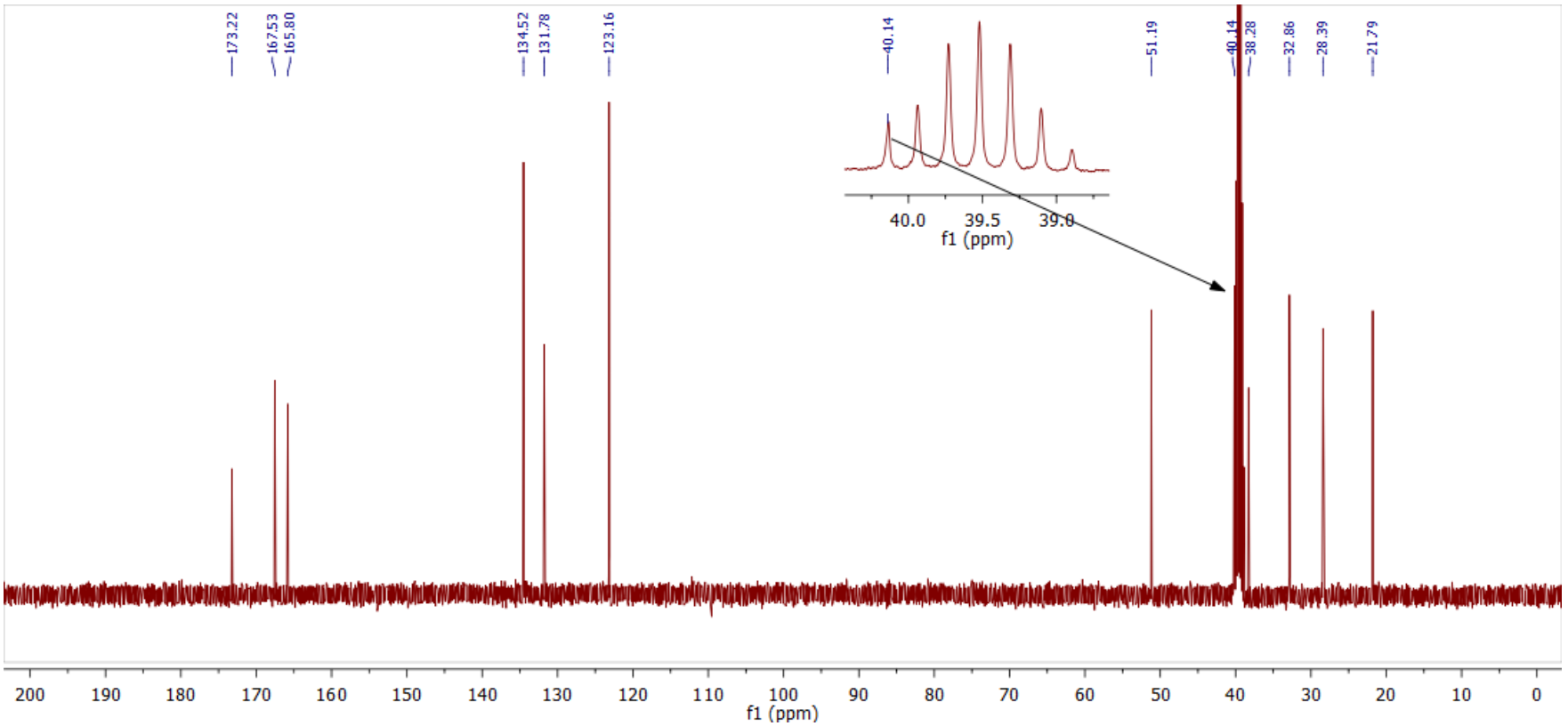


4.1.38. *N*-Phthaloylglycl-δ-aminovaleric acid methyl ester (Phth-Gly-Ava-OMe)

^{1}H NMR spectrum of Phth-Gly-Ava-OMe

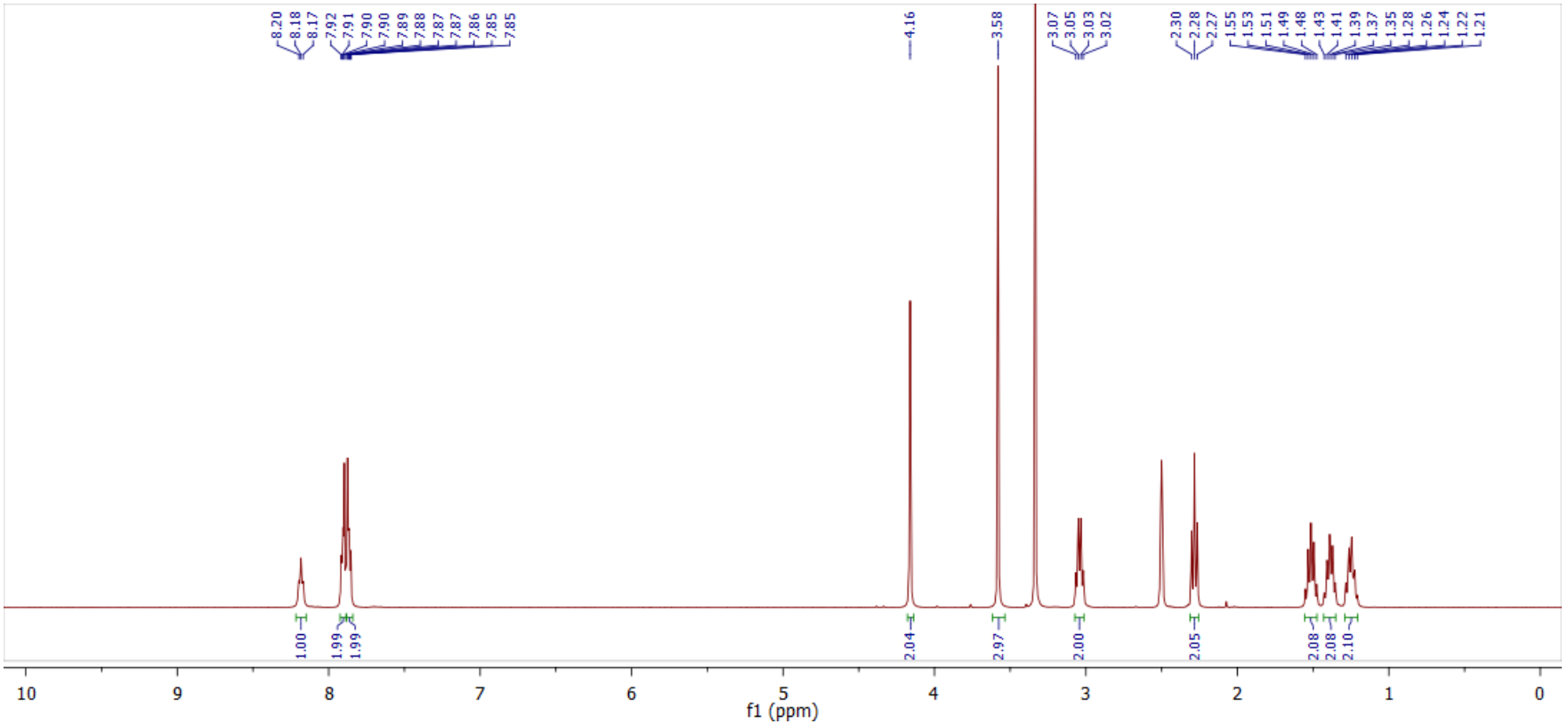


^{13}C NMR spectrum of Phth-Gly-Gaba-OMe

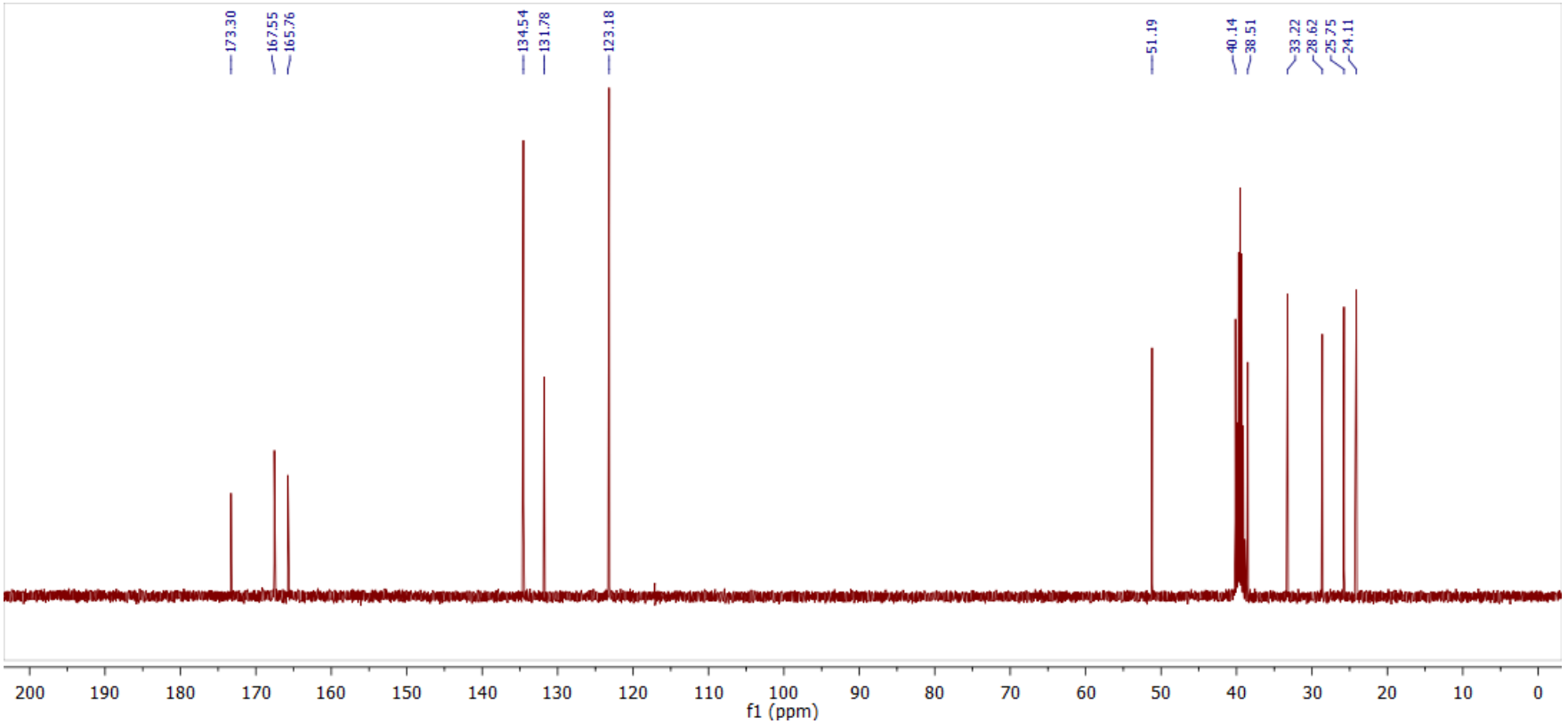


4.1.39. *N*-Phthaloylglycl-ε-aminocaproic acid methyl ester (Phth-Gly-Ahx-OMe)

^{1}H NMR spectrum of Phth-Gly-Ahx-OMe

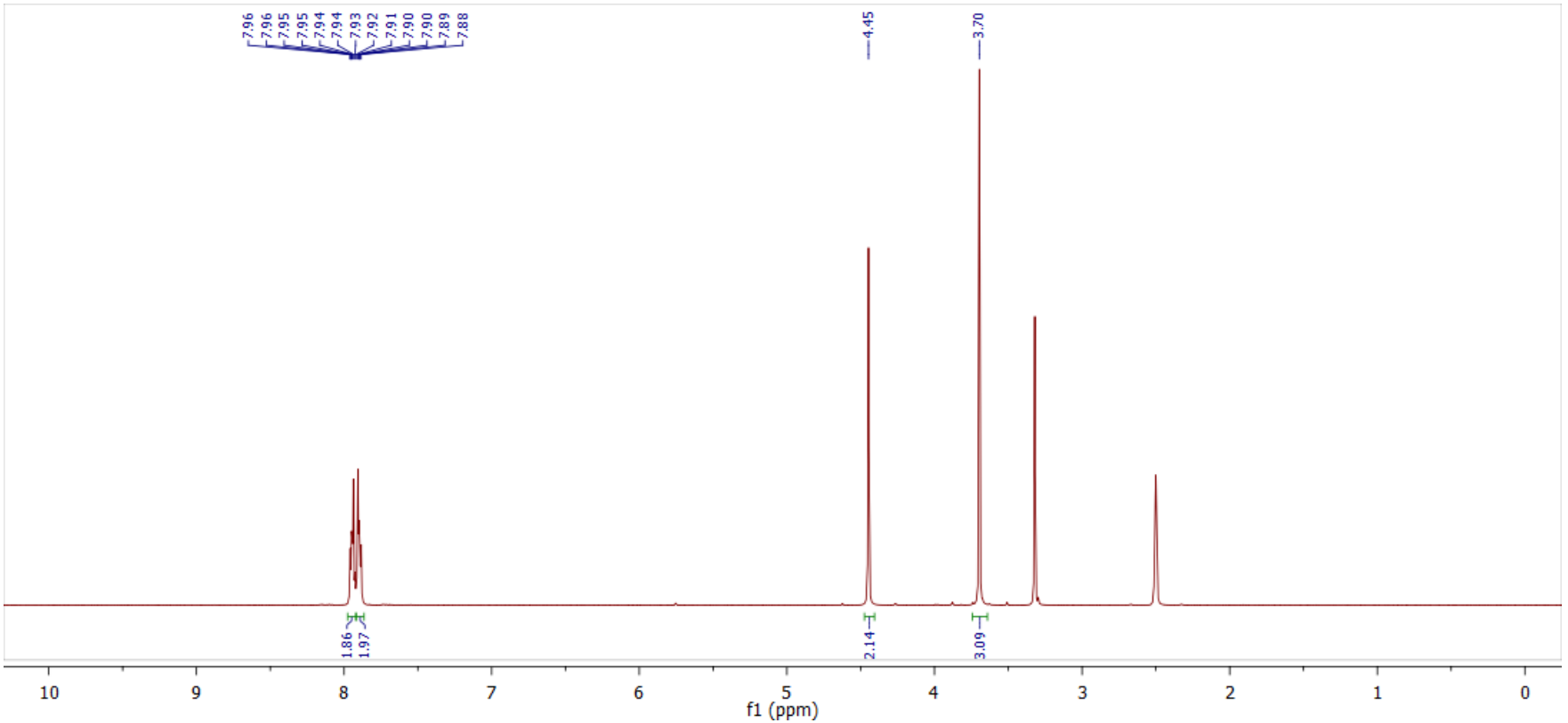


^{13}C NMR spectrum of Phth-Gly-Ahx-OMe

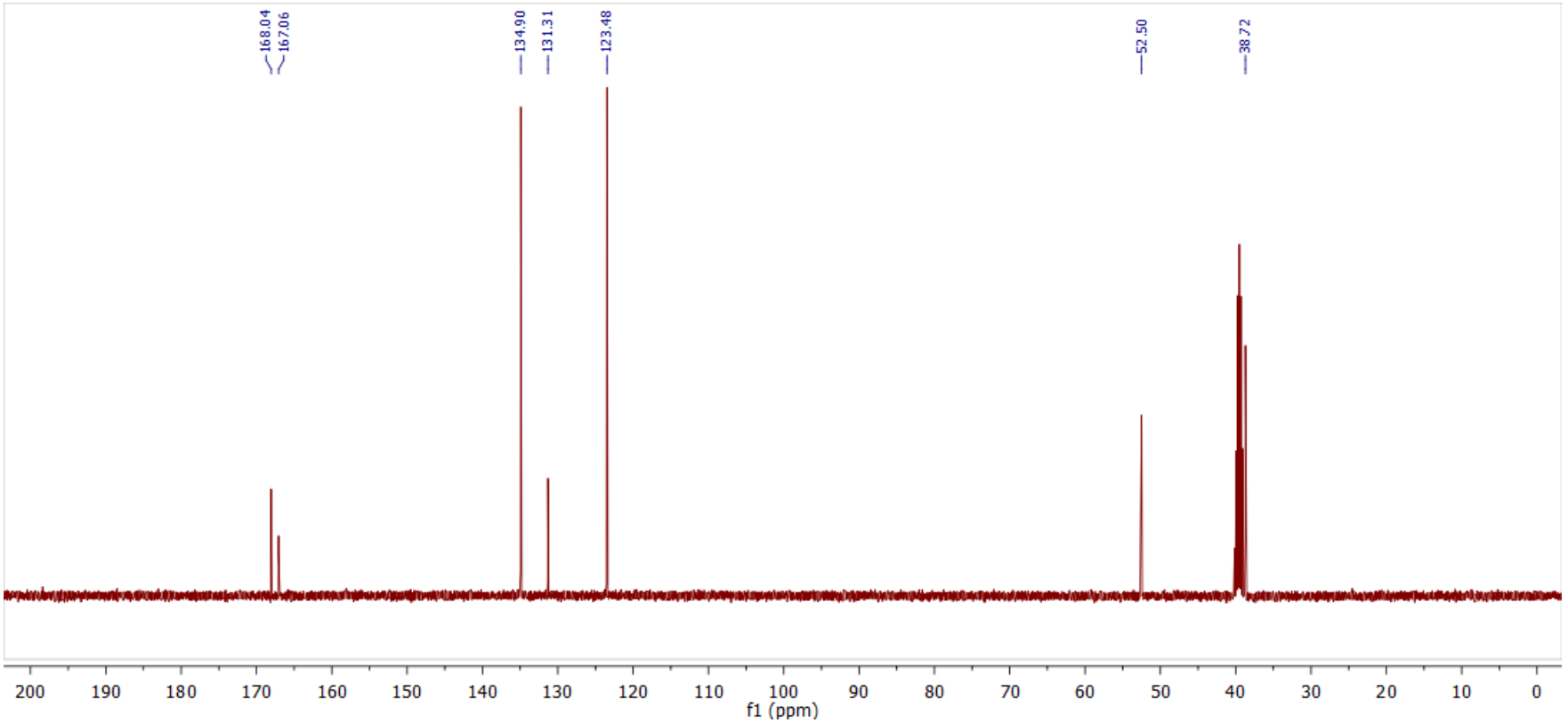


4.1.40. *N*-Phthaloylglycine methyl ester (Phth-Gly-OMe)

^{1}H NMR spectrum of Phth-Gly-OMe

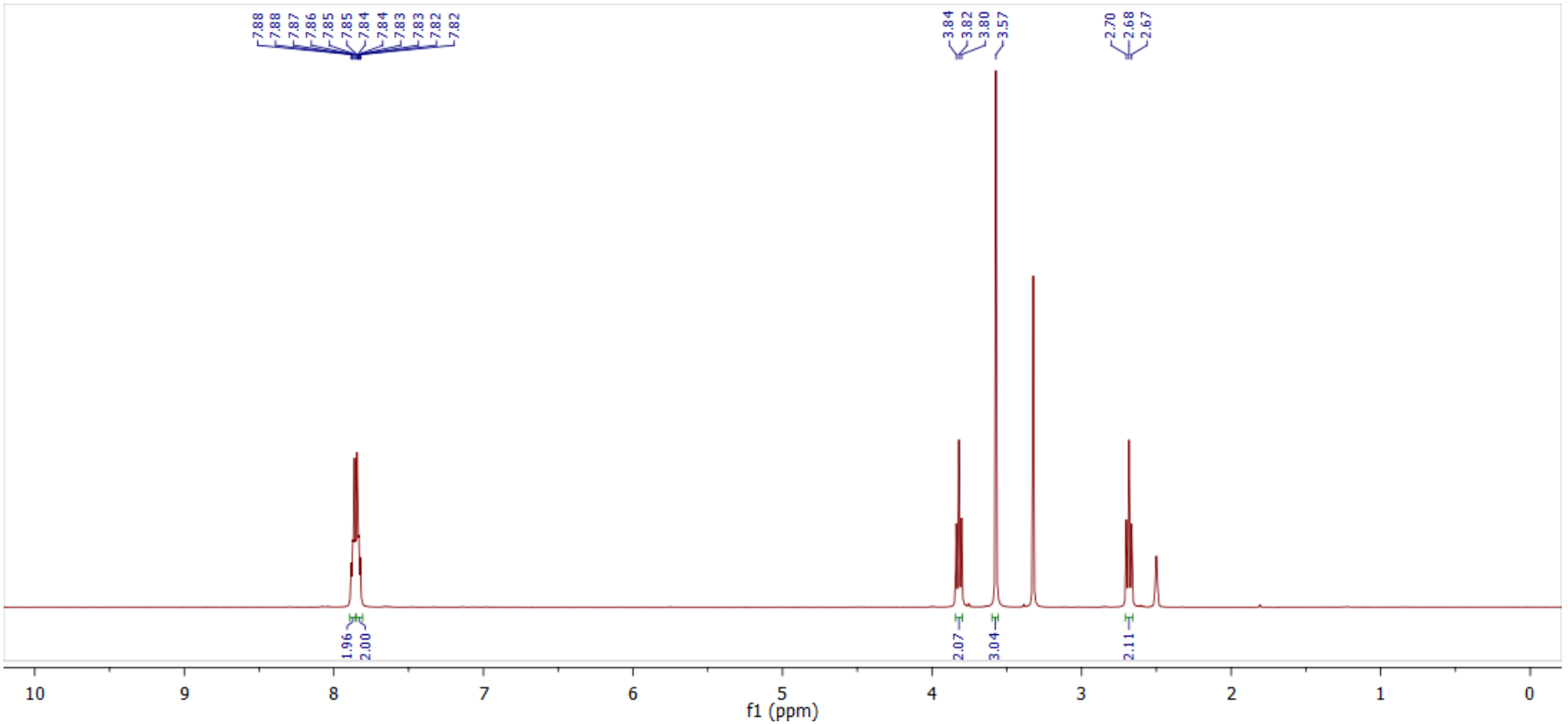


^{13}C NMR spectrum of Phth-Gly-OMe

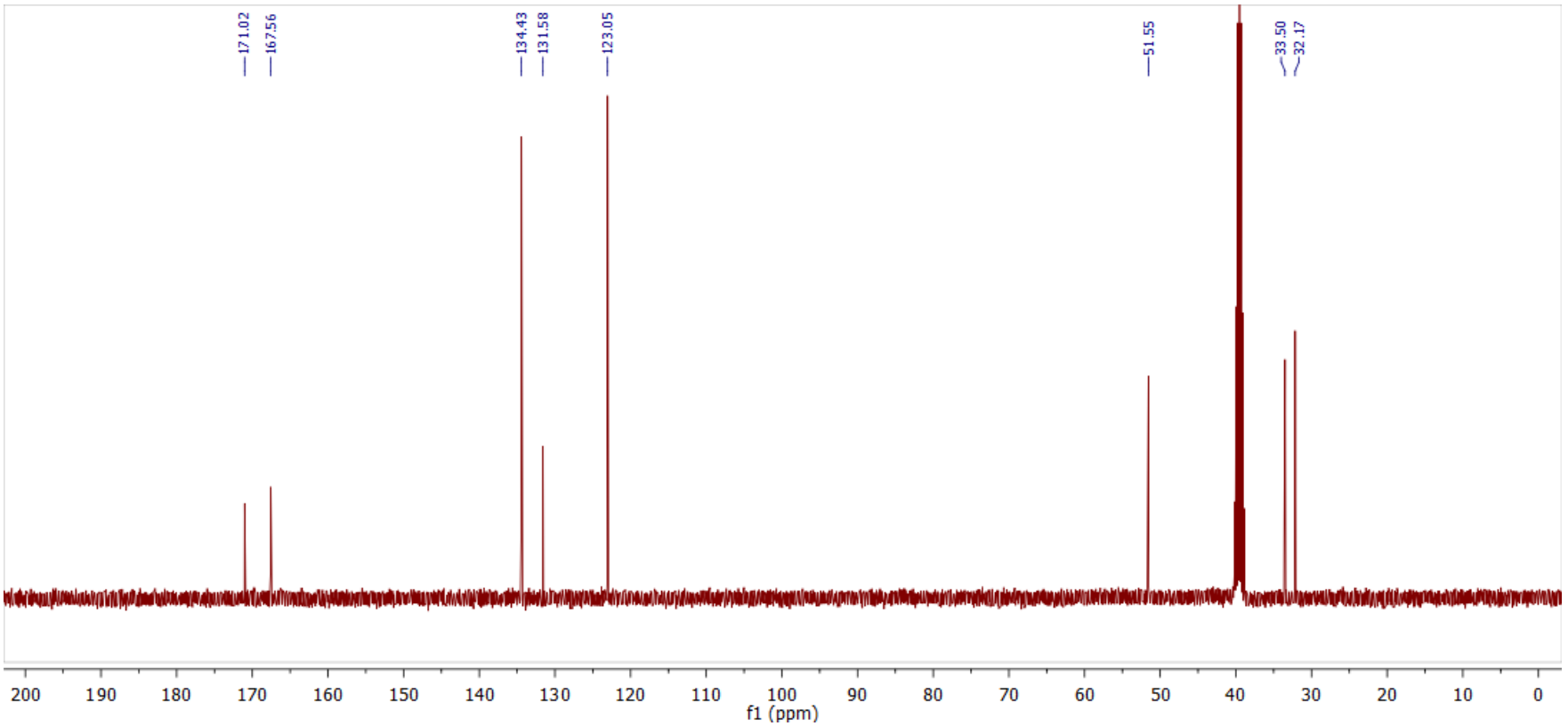


4.1.41. *N*-Phthaloyl-β-alanine methyl ester (Phth-βAla-OMe)

^{1}H NMR spectrum of Phth-βAla-OMe

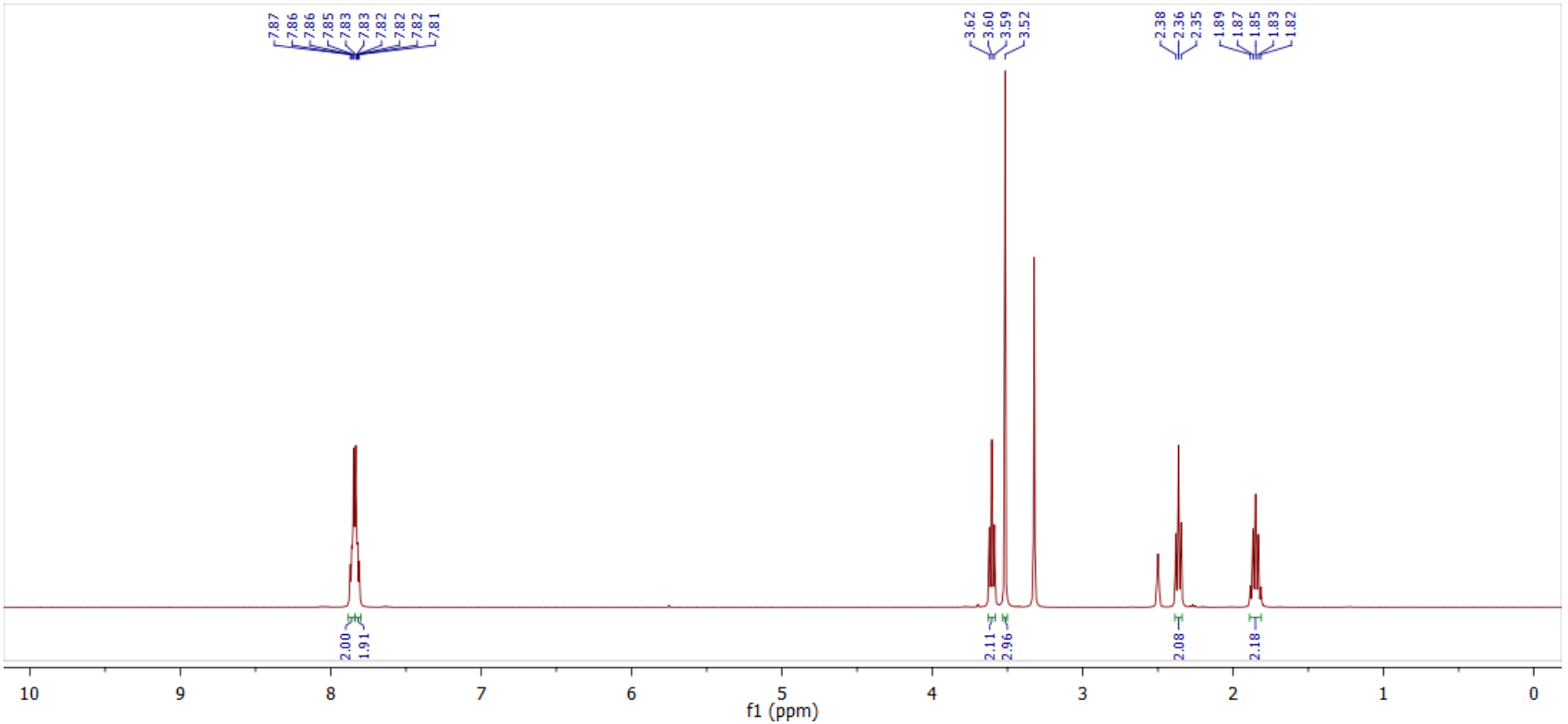


^{13}C NMR spectrum of Phth-βAla-OMe

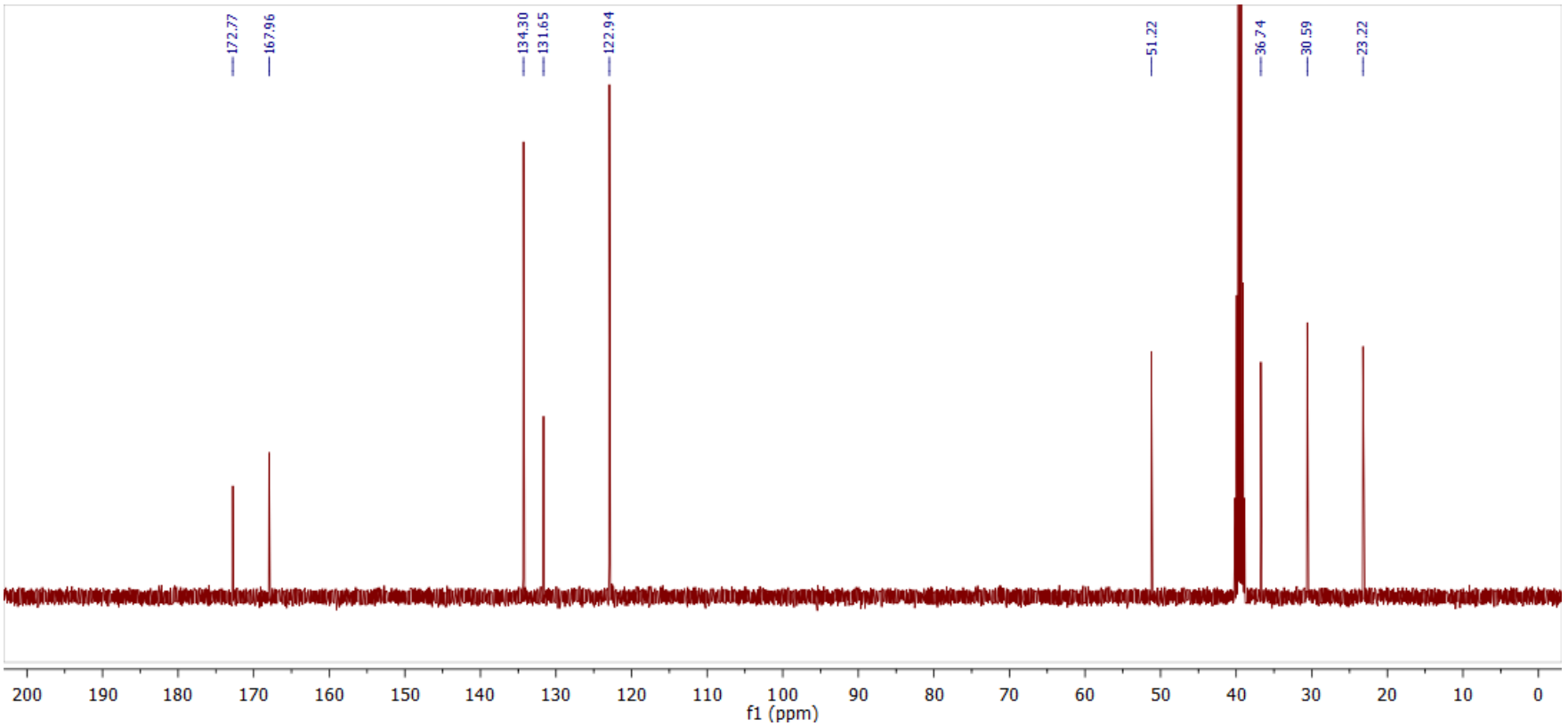


4.1.42. *N*-Phthaloyl-γ-aminobutyric acid methyl ester (Phth-Gaba-OMe)

^{1}H NMR spectrum of Phth-Gaba-OMe

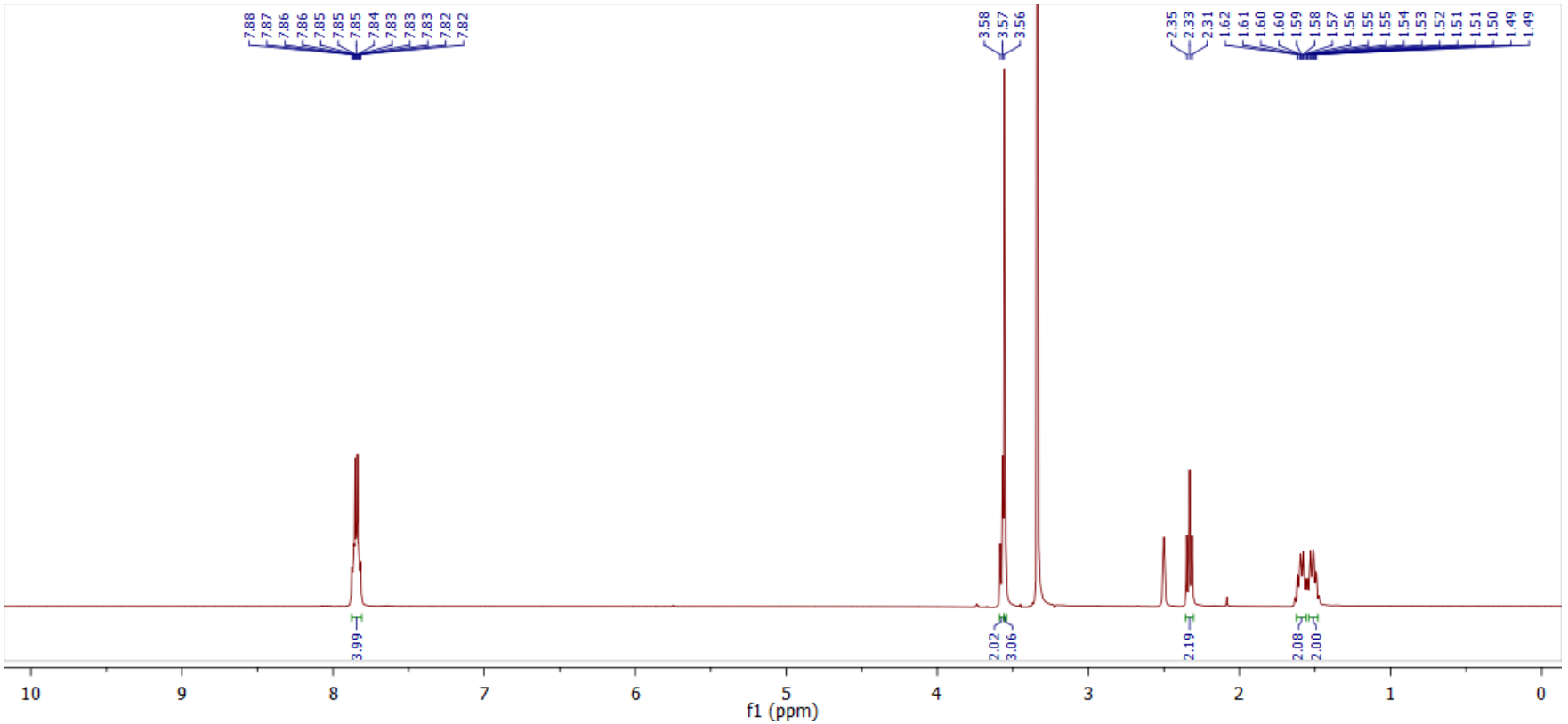


^{13}C NMR spectrum of Phth-Gaba-OMe

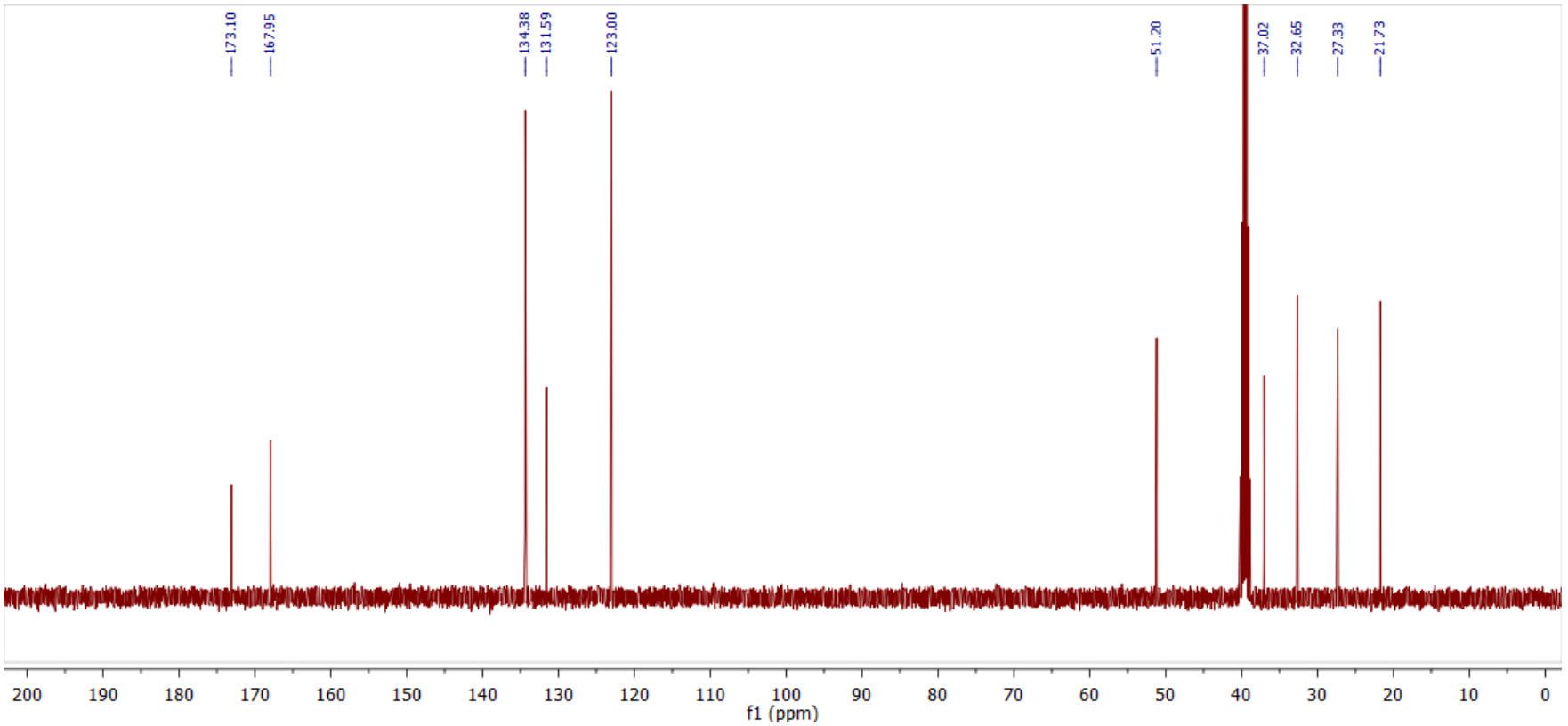


4.1.43. *N*-Phthaloyl-δ-aminovaleric acid methyl ester (Phth-Ava-OMe)

^{1}H NMR spectrum of Phth-Ava-OMe

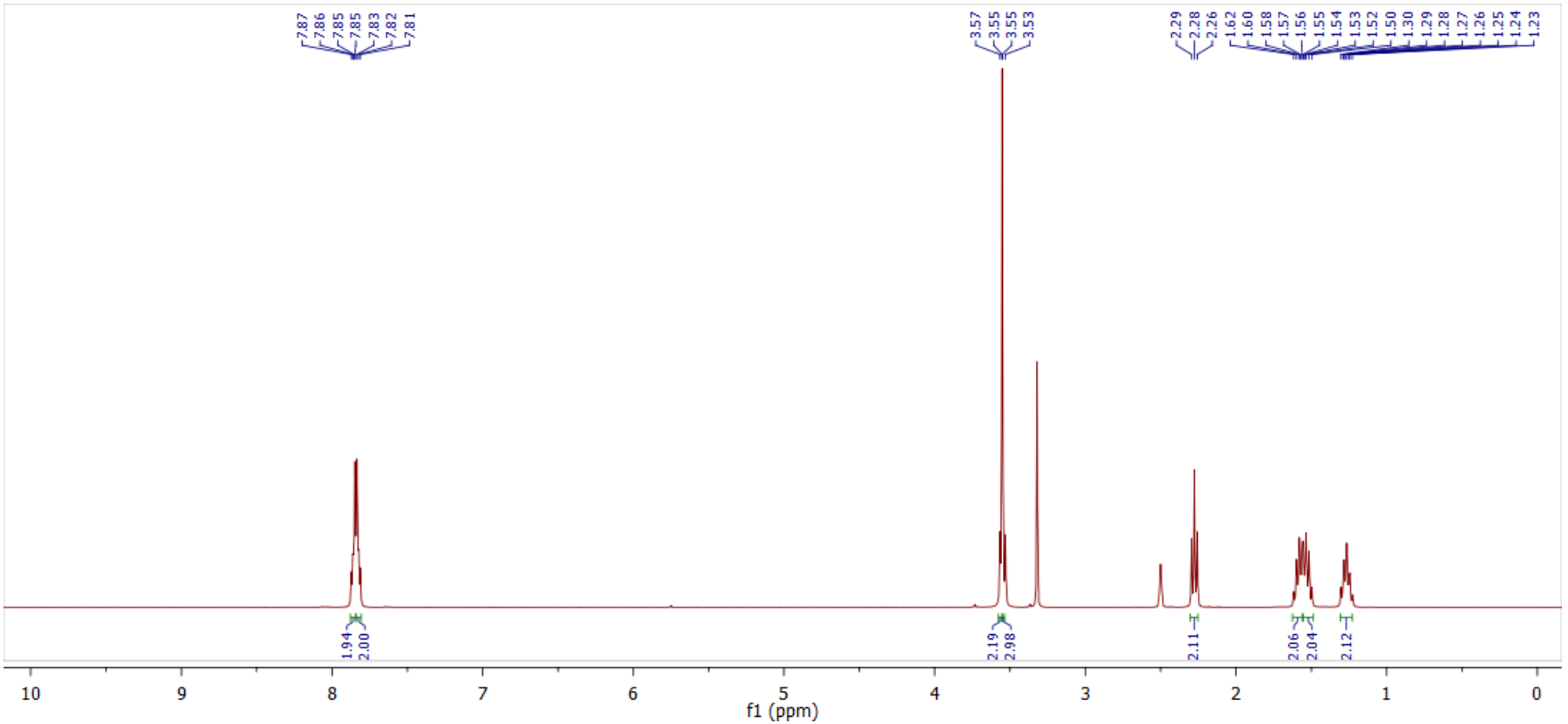


^{13}C NMR spectrum of Phth-Ava-OMe

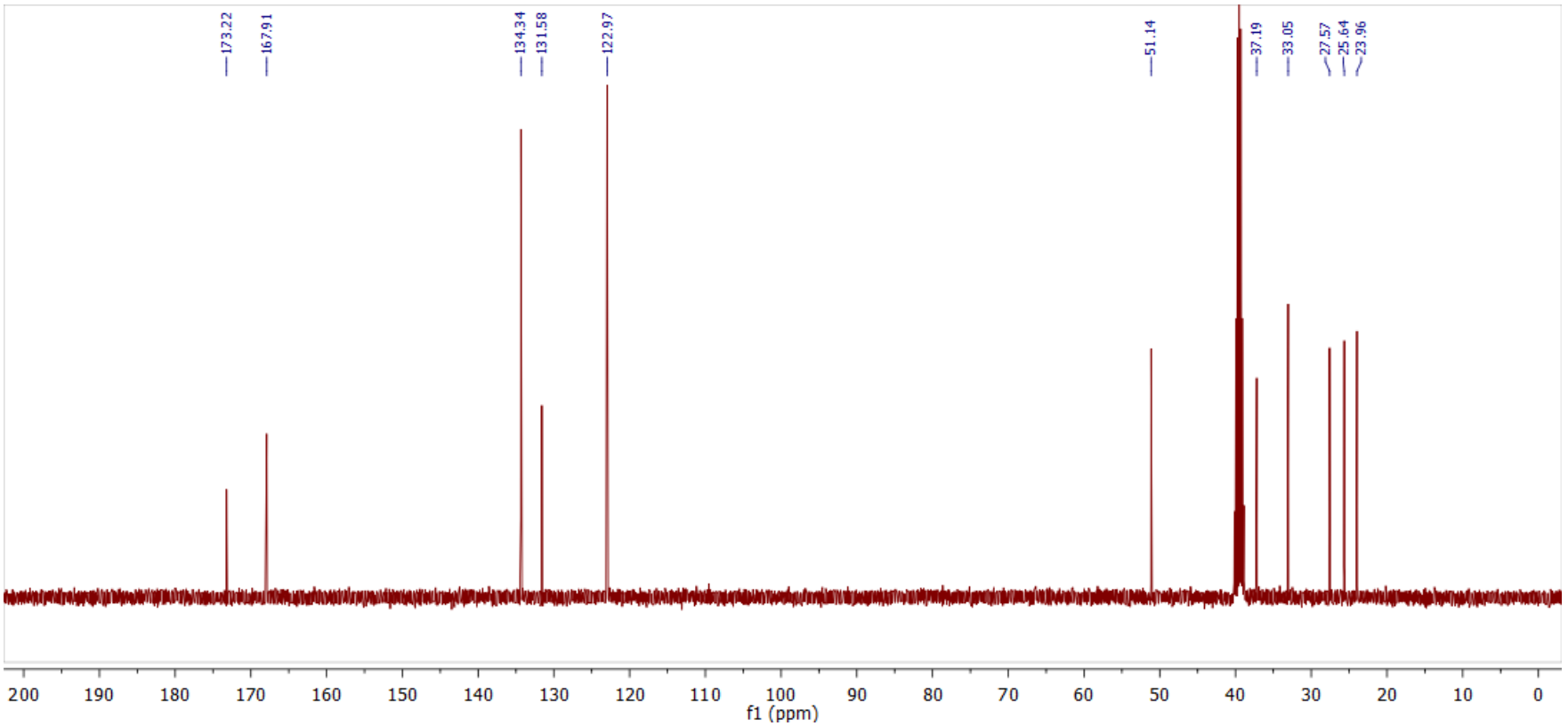


4.1.44. *N*-Phthaloyl-ε-aminocaproic acid methyl ester (Phth-Ahx-OMe)

^{1}H NMR spectrum of Phth-Ahx-OMe

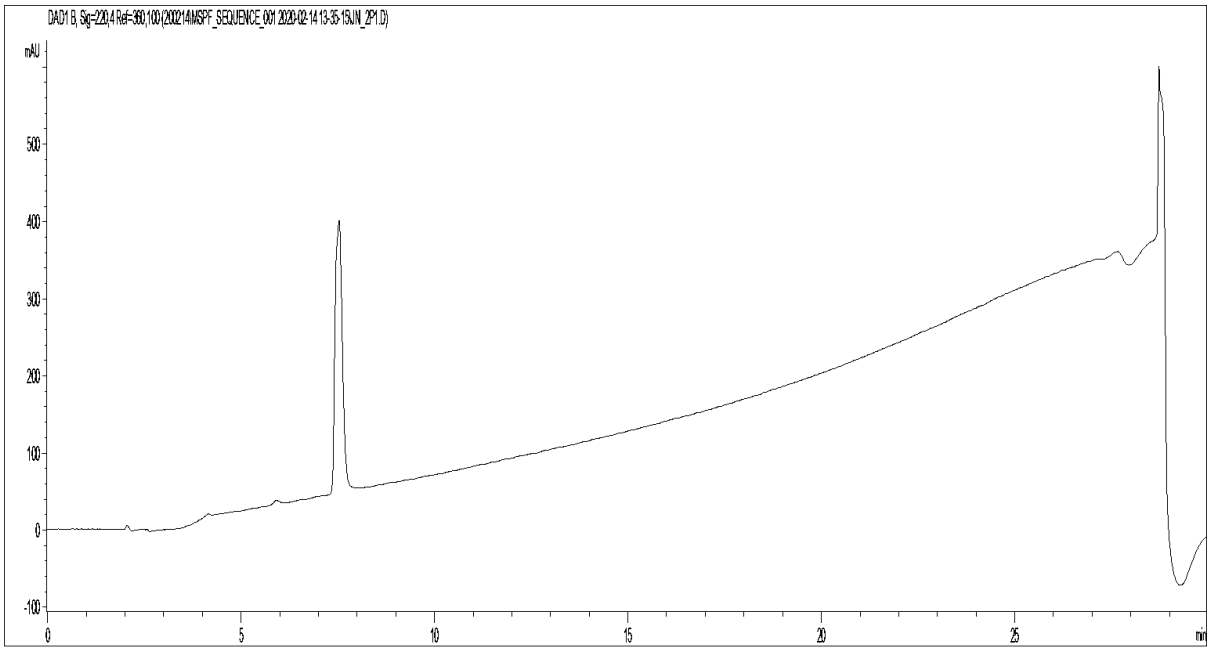


^{13}C NMR spectrum of Phth-Ahx-OMe

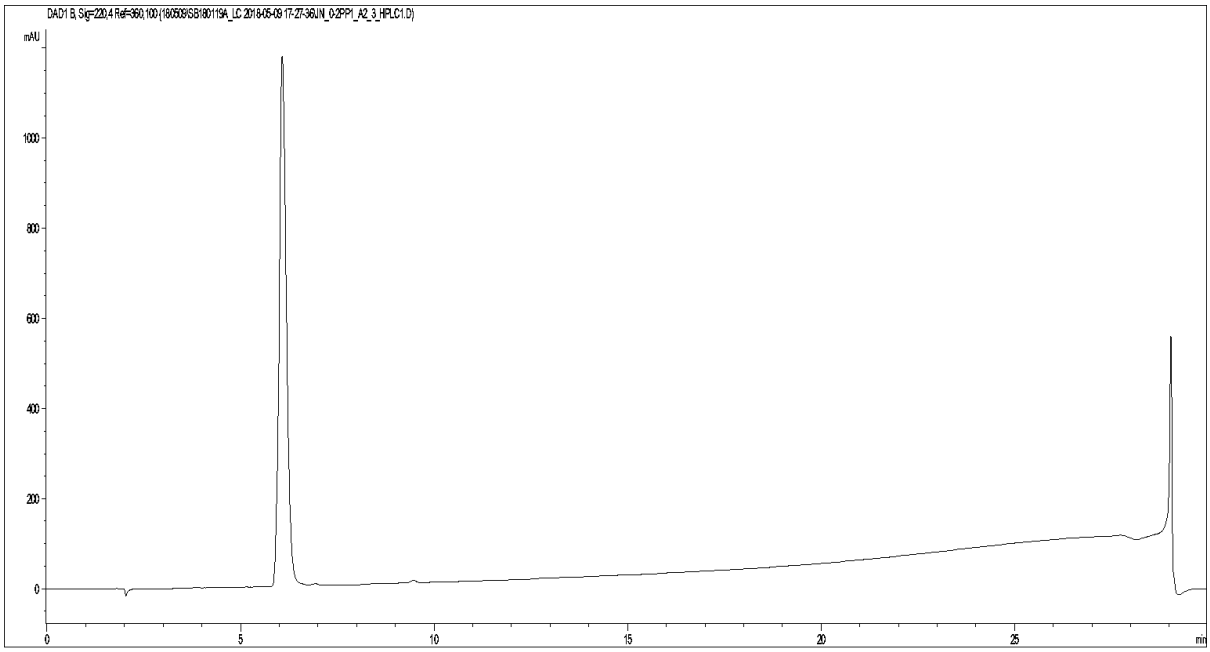


4.2. HPLC chromatograms

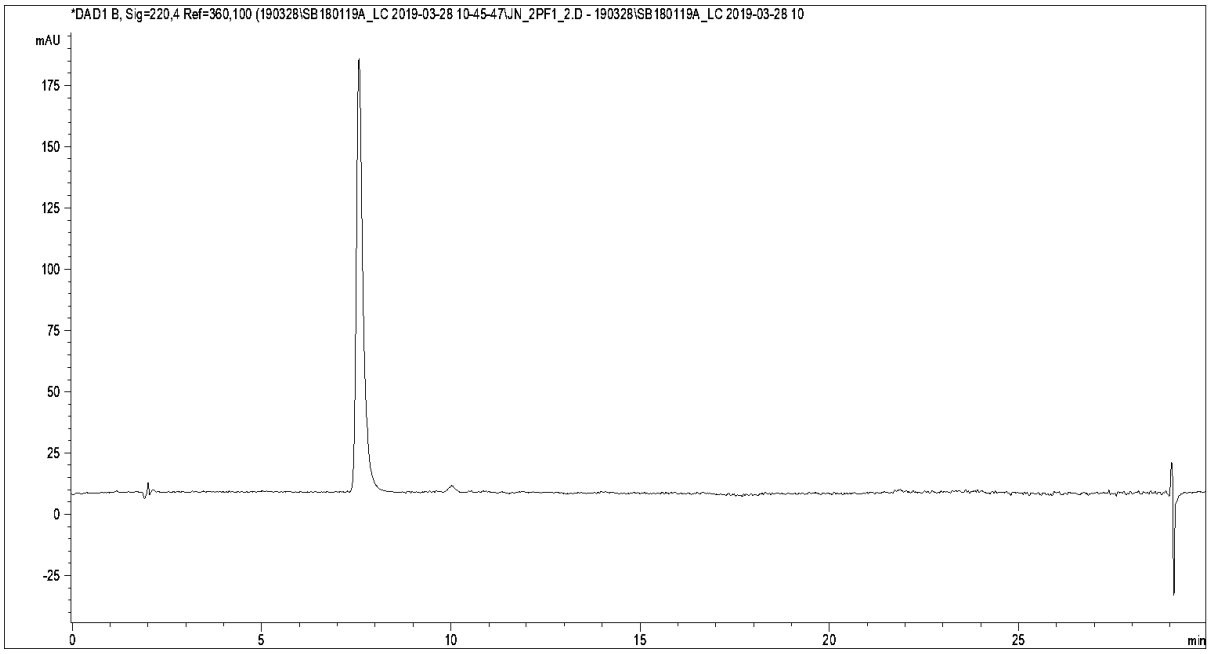
4.2.1. *N*-Acetyl-L-proline methyl ester (Ac-Pro-OMe)



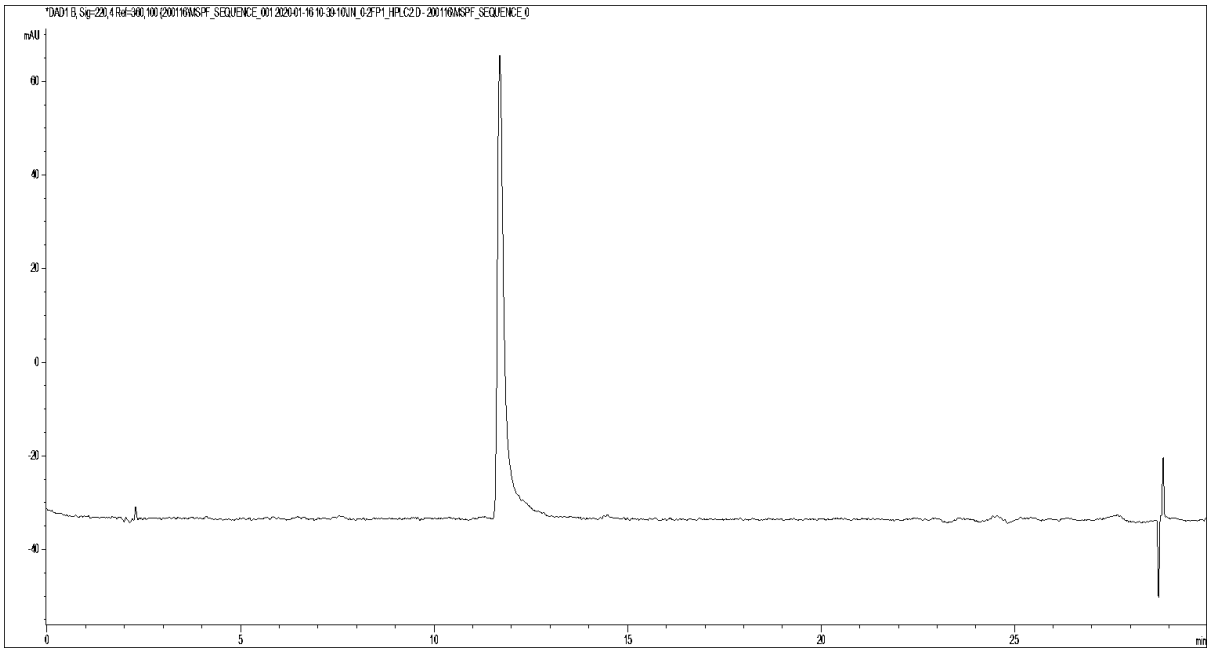
4.2.2. *N*-Acetyl-L-prolyl-L-proline methyl ester (Ac-Pro-Pro-OMe)



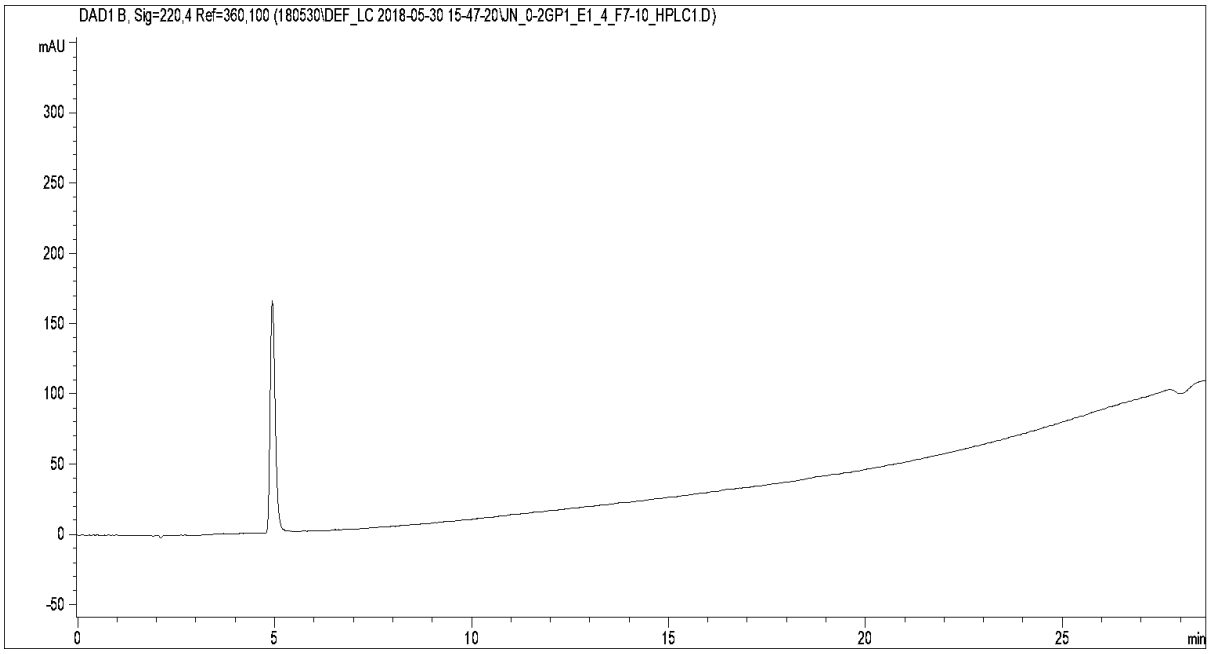
4.2.3. *N*-Acetyl-L-prolyl-L-phenylalanine methyl ester (Ac-Pro-Phe-OMe)



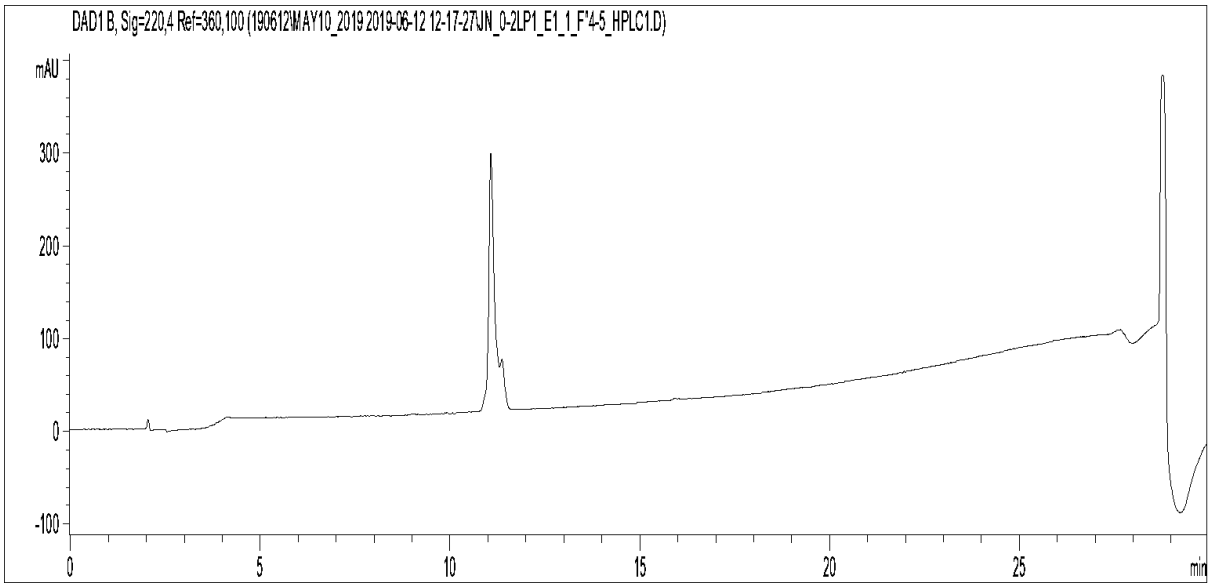
4.2.4. *N*-Acetyl-L-phenylalanyl-L-proline methyl ester (Ac-Phe-Pro-OMe)



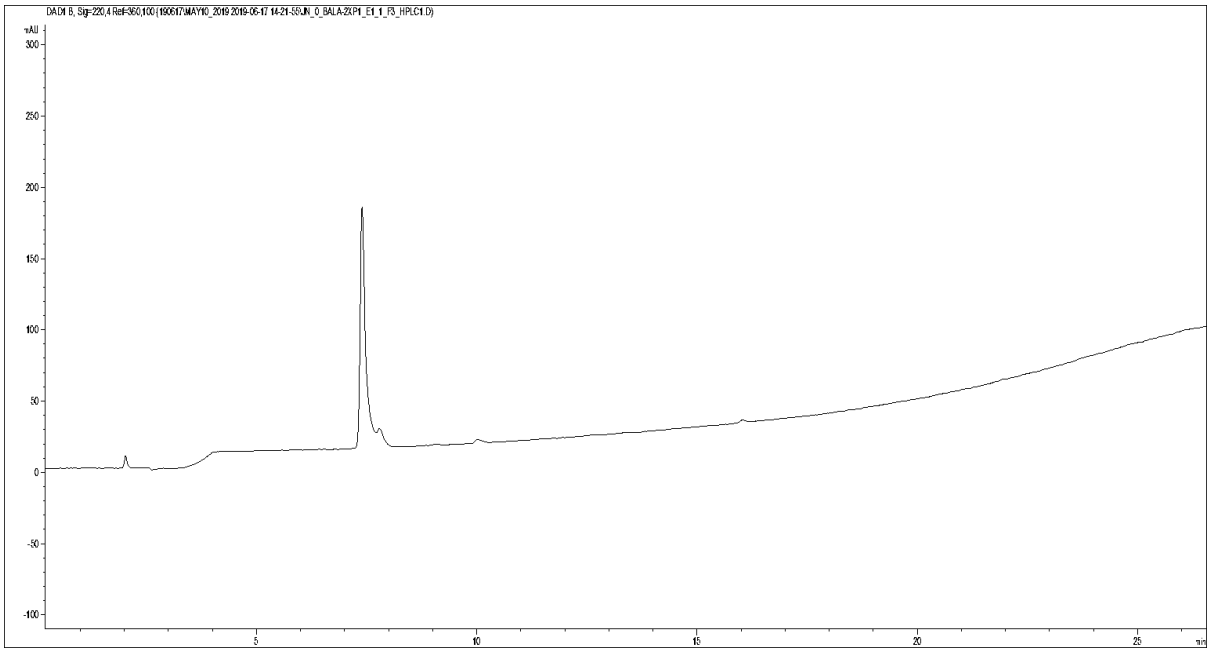
4.2.5. *N*-Acetyl-glycine-L-proline methyl ester (Ac-Gly-Pro-OMe)



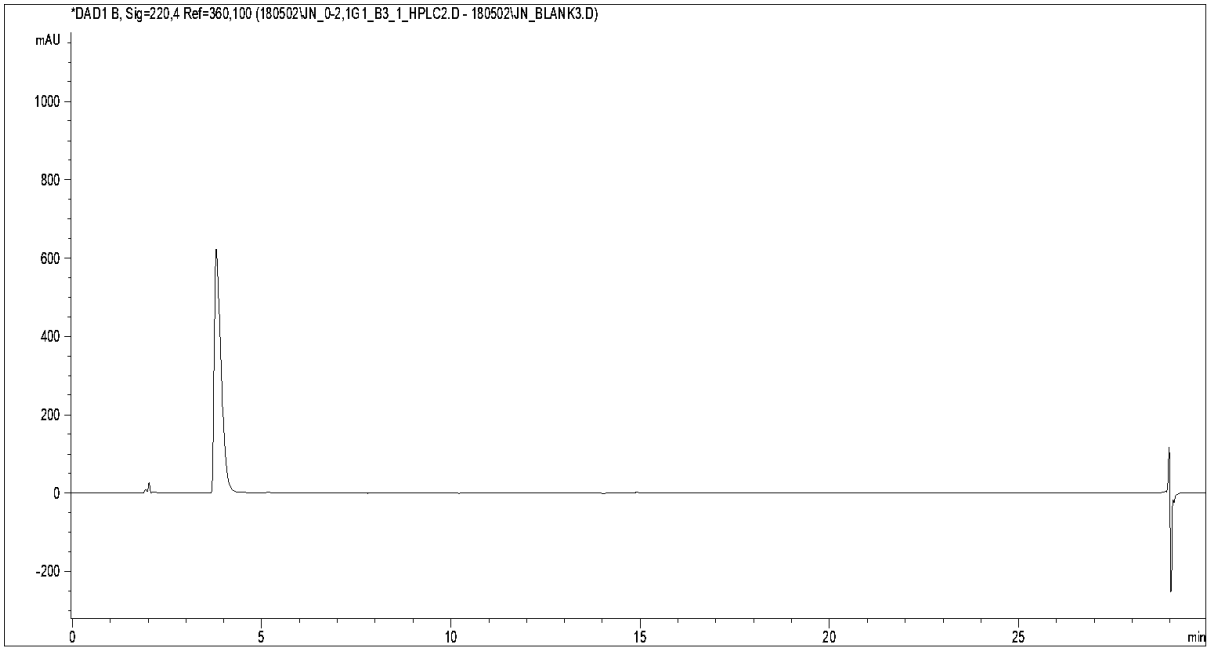
4.2.6. *N*-Acetyl-L-leucyl-L-proline methyl ester (Ac-Leu-Pro-OMe)



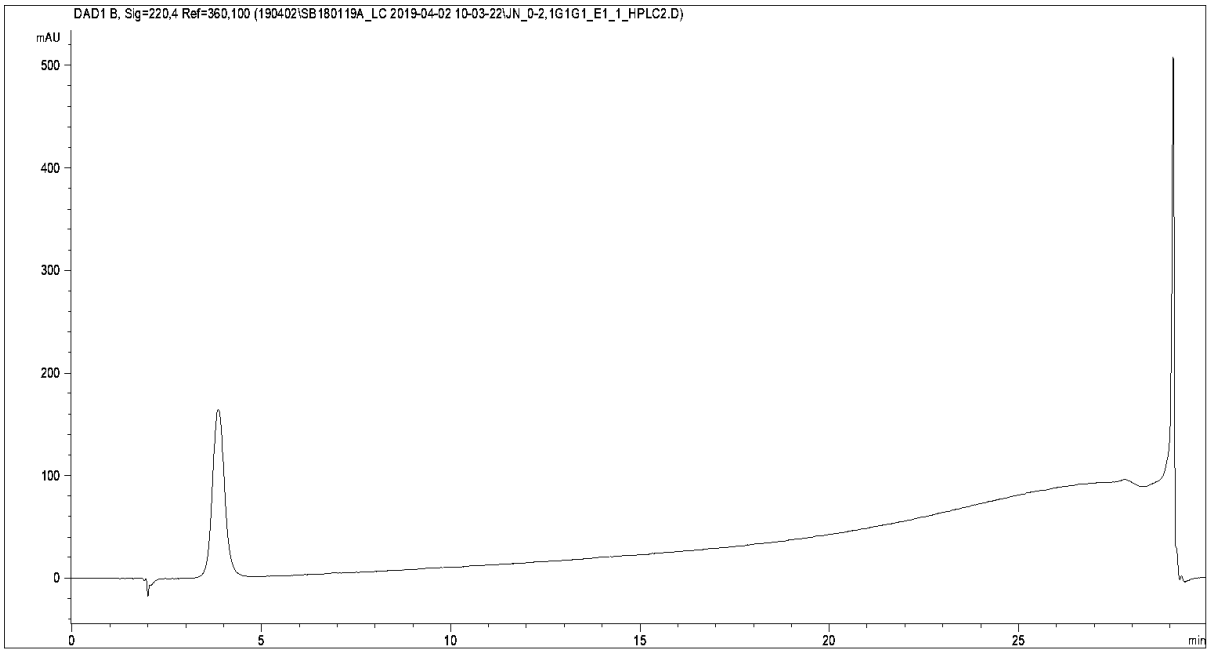
4.2.7. *N*-Acetyl-β-alanyl-L-proline methyl ester (Ac-βAla-Pro-OMe)



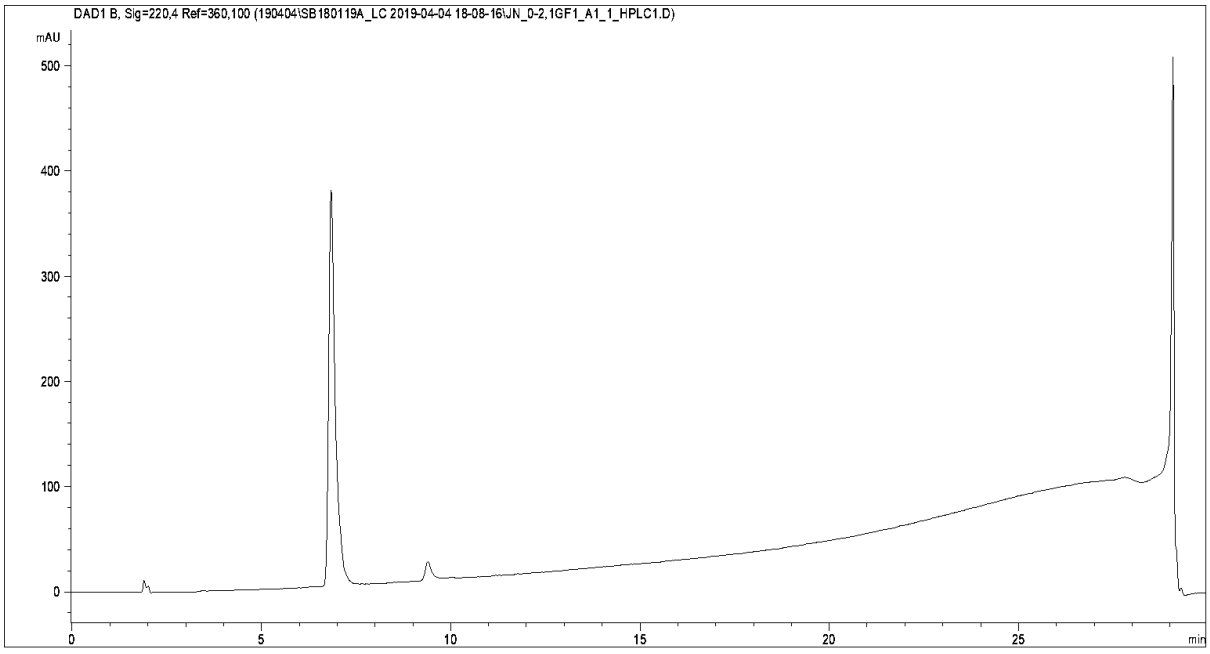
4.2.8. *N*-Acetyl-*N*-methylglycine methyl ester (Ac-(Me)Gly-OMe)



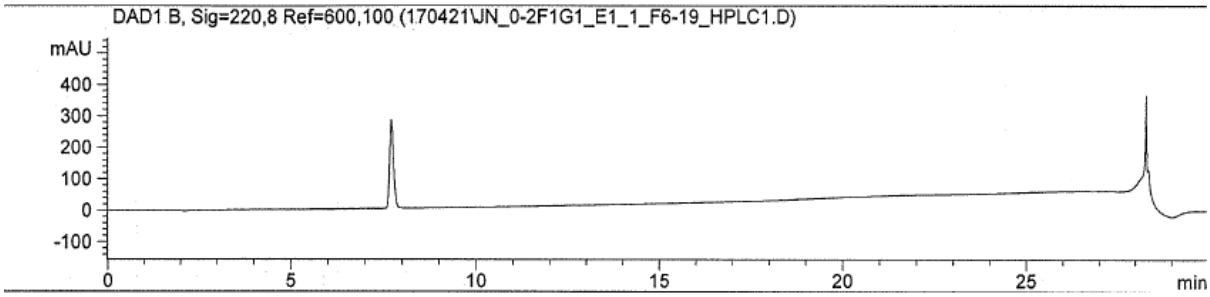
4.2.9. *N*-Acetyl-*N*-methylglycyl-*N*-methylglycine methyl ester (Ac-(Me)Gly-(Me)Gly-OMe)



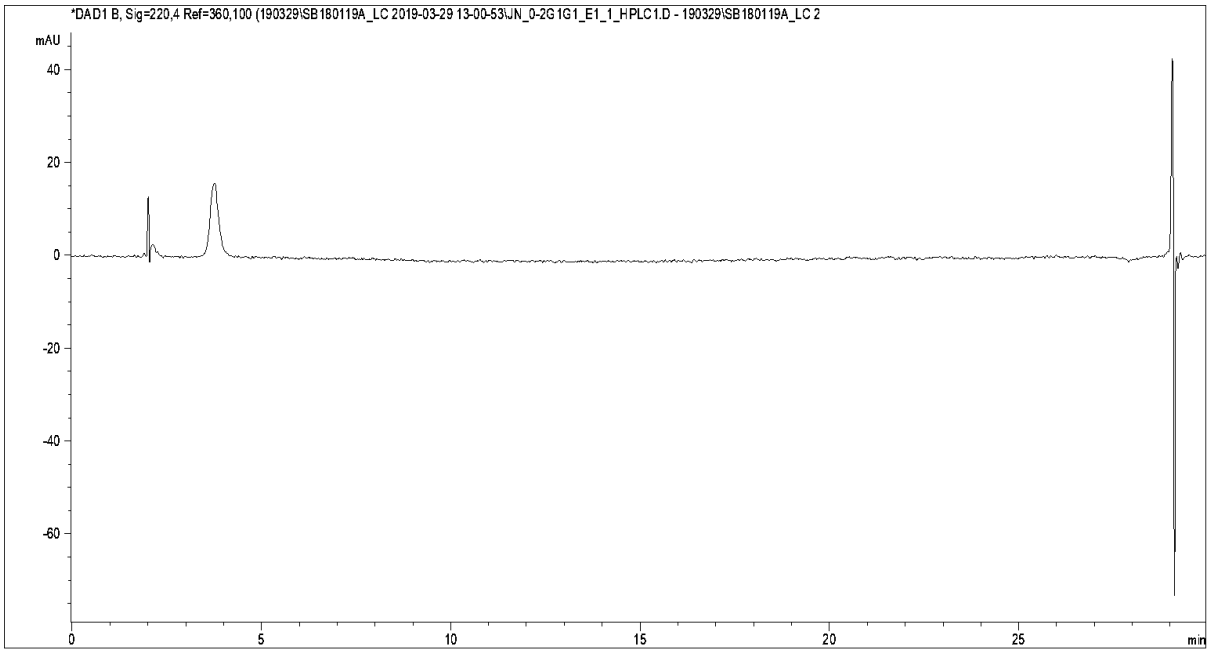
4.2.10. *N*-Acetyl-*N*-methylglycyl-L-phenylalanine methyl ester (Ac-(Me)Gly-Phe-OMe)



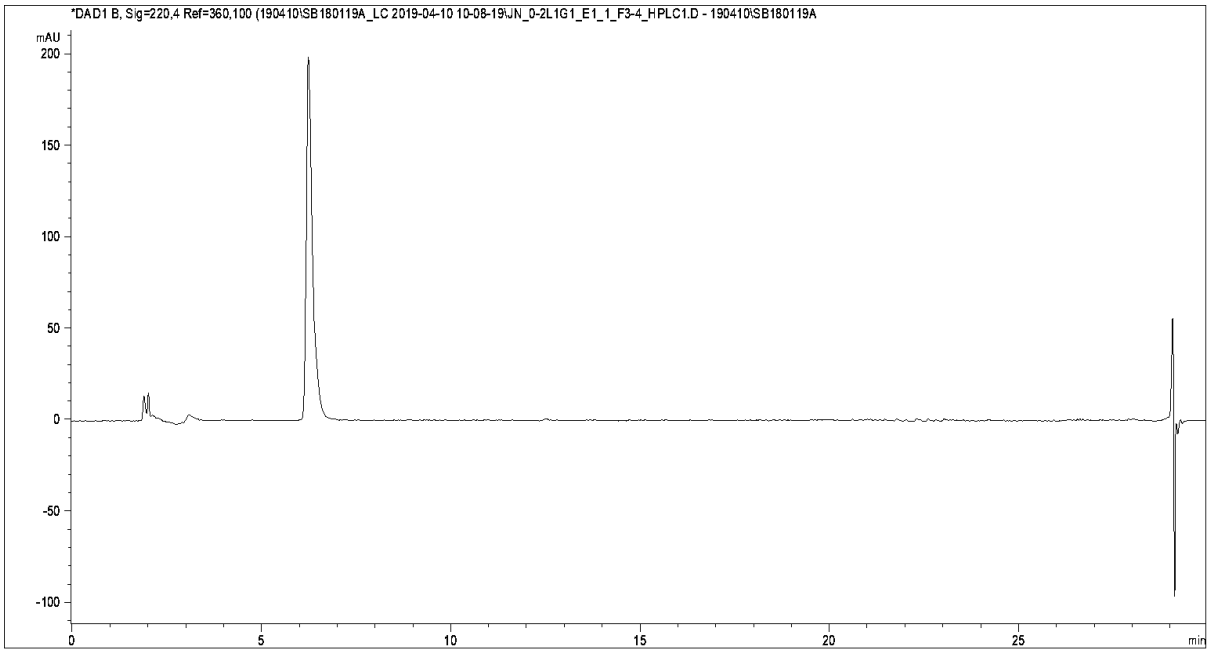
4.2.11. *N*-Acetyl-L-phenylalanyl-*N*-methylglycine methyl ester (Ac-Phe-(Me)Gly-OMe)



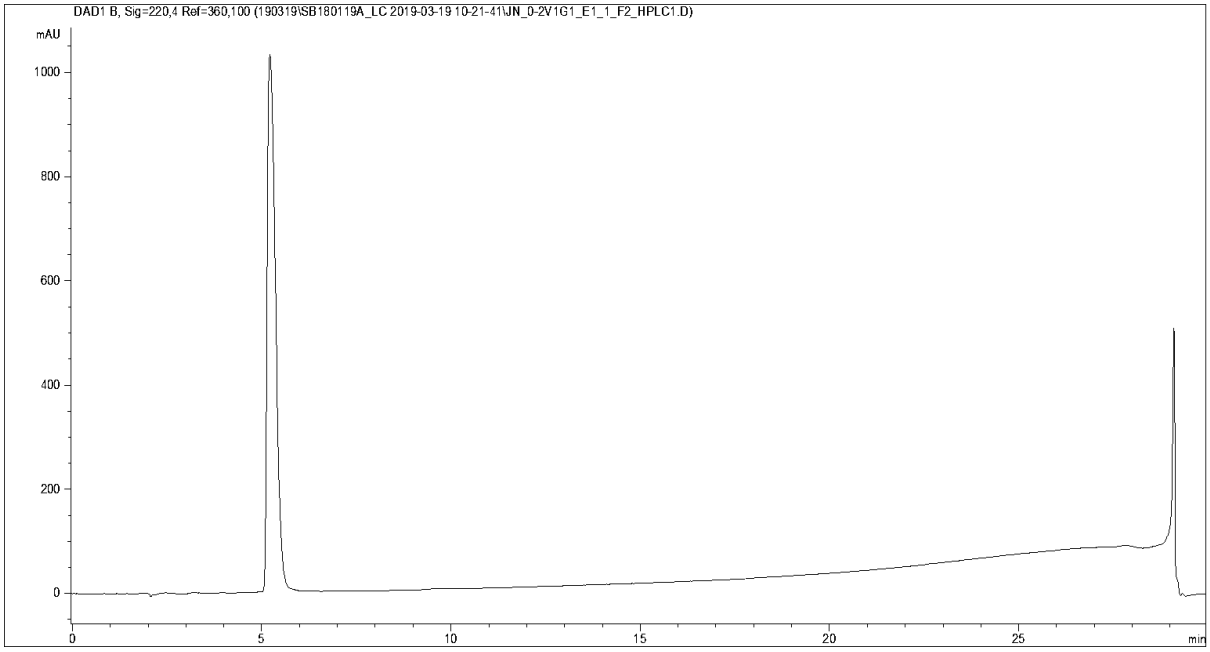
4.2.12. *N*-Acetylglycyl-*N*-methylglycine methyl ester (Ac-Gly-(Me)Gly-OMe)



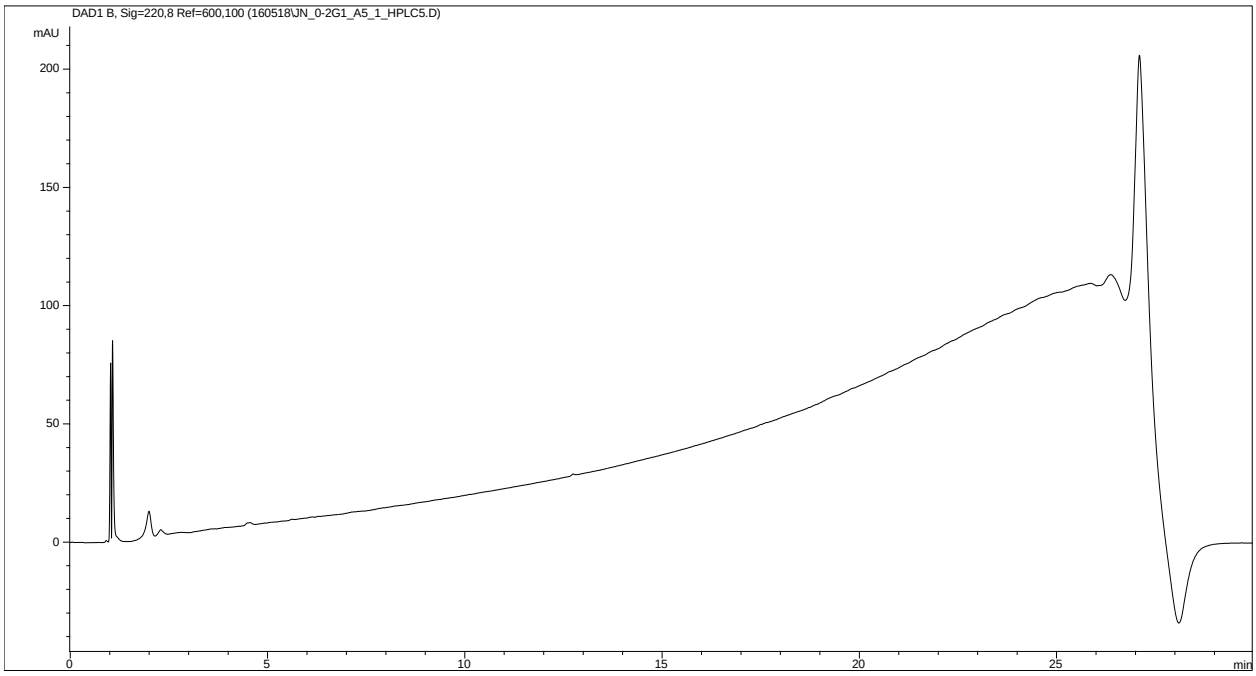
4.2.13. *N*-Acetyl-L-leucyl-*N*-methylglycine methyl ester (Ac-Leu-(Me)Gly-OMe)



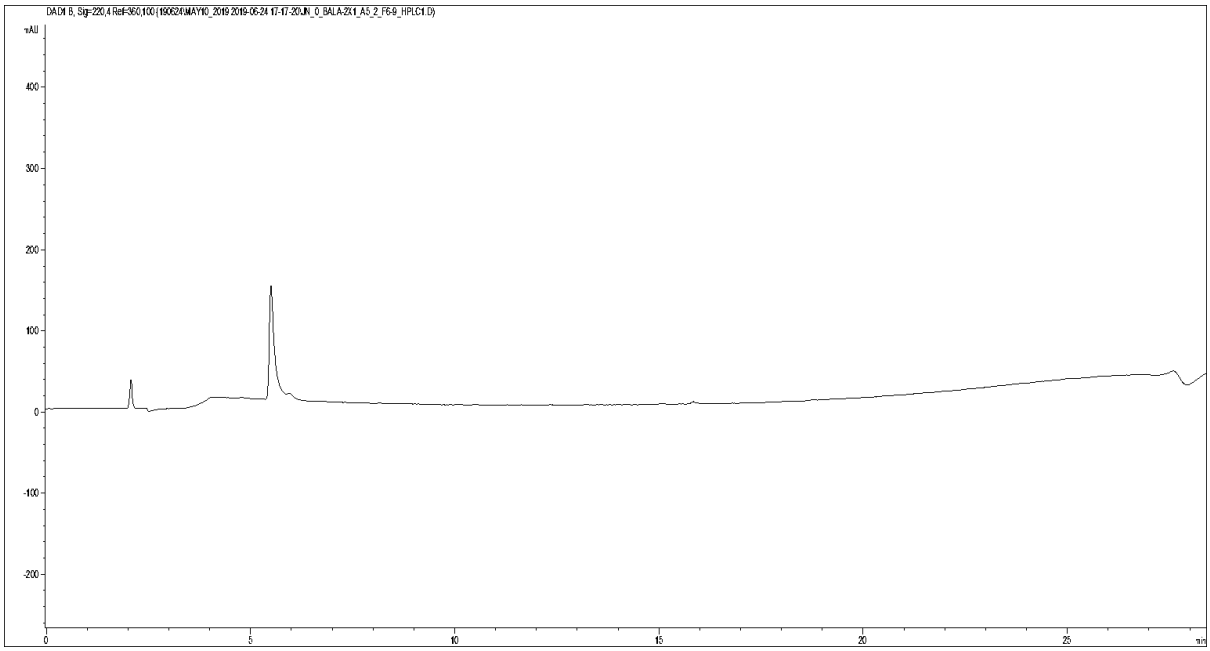
4.2.14. *N*-Acetyl-L-valyl-*N*-methylglycine methyl ester (Ac-Val-(Me)Gly-OMe)



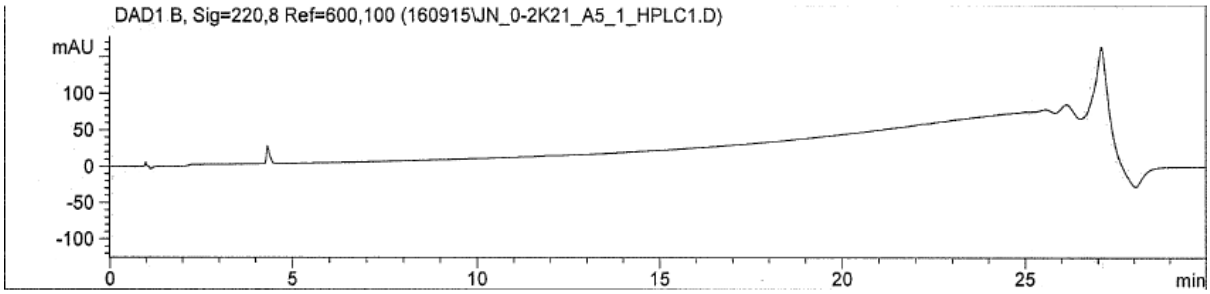
4.2.15. *N*-Acetylglycine methyl ester (Ac-Gly-OMe)



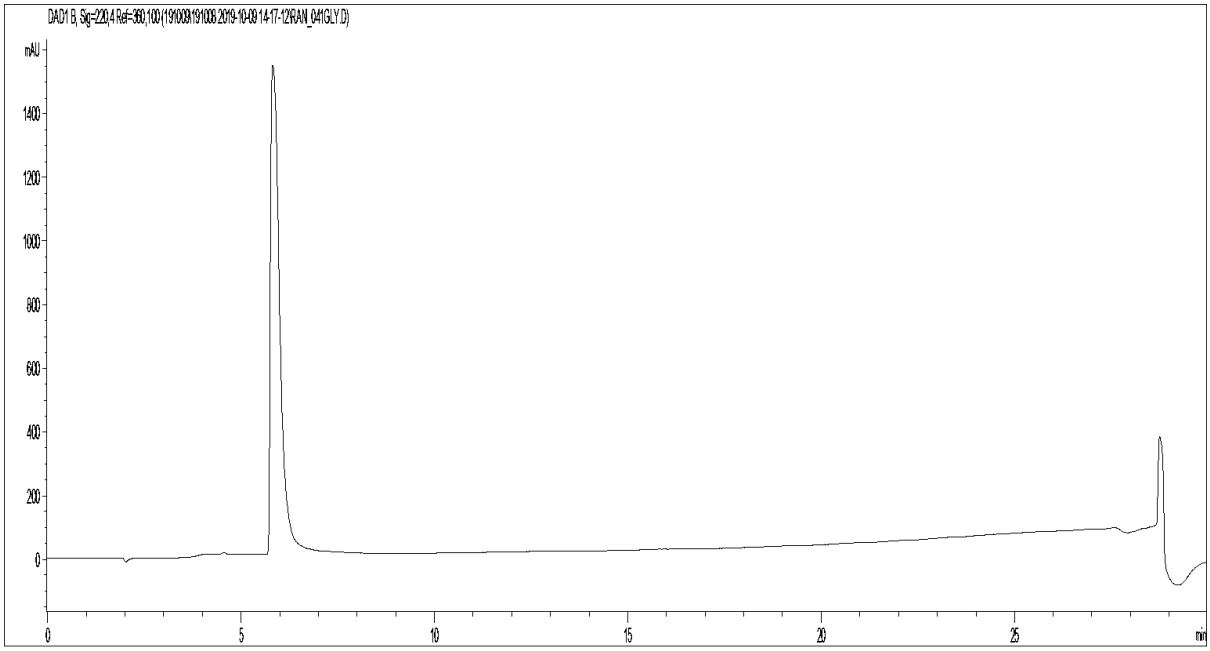
4.2.16. *N*-Acetyl-β-alanine methyl ester (Ac-βAla-OMe)



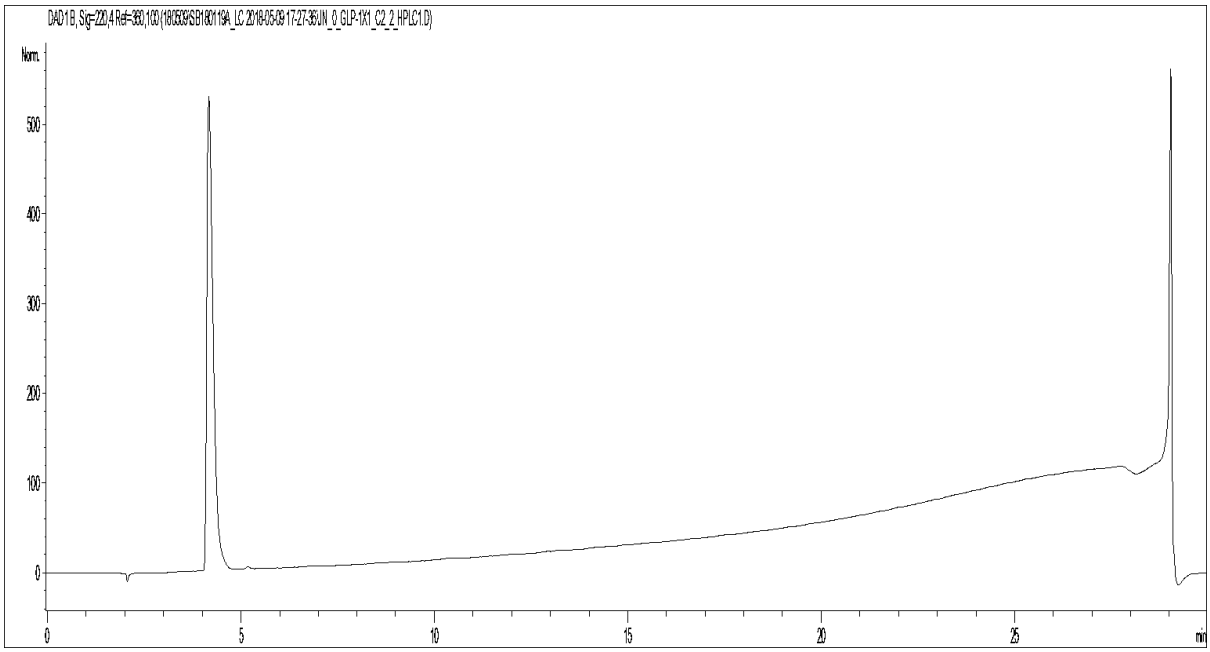
4.2.17. N_α,N_ε-Diacetyl-L-lysine methyl ester (Ac-Lys(NHAc)-OMe)



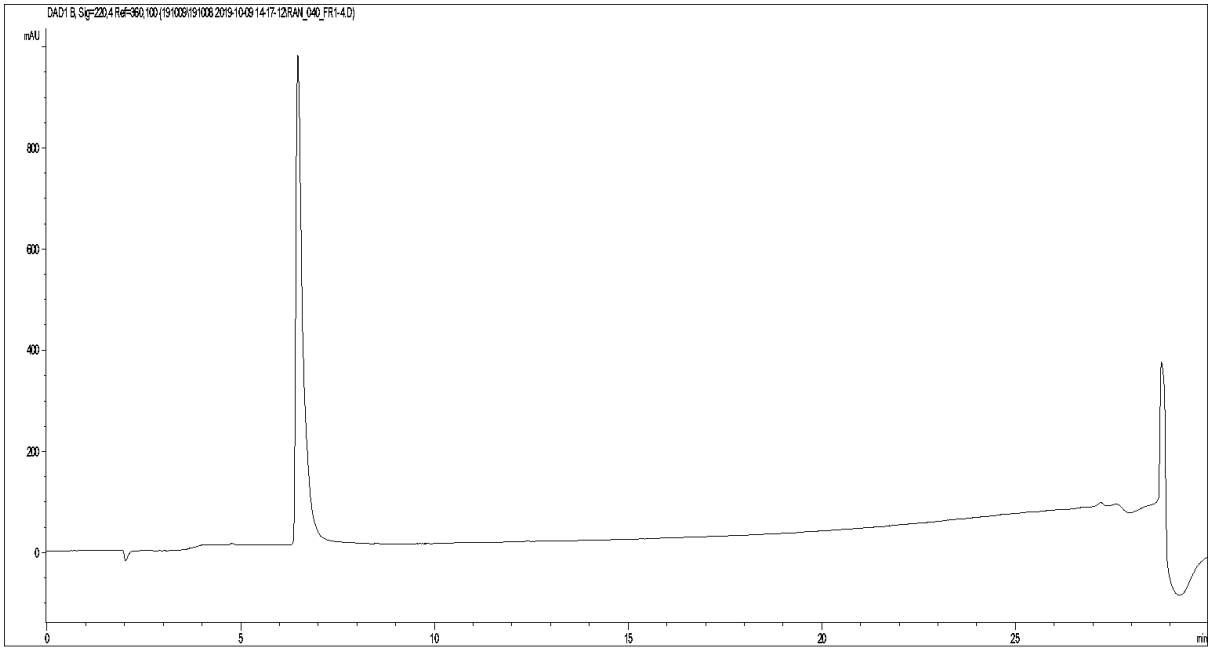
4.2.18. *N*-Acetyl-*N*-methyl(d_3)glycine methyl(d_3) ester (Ac-(Me(d_3))Gly-OMe(d_3))



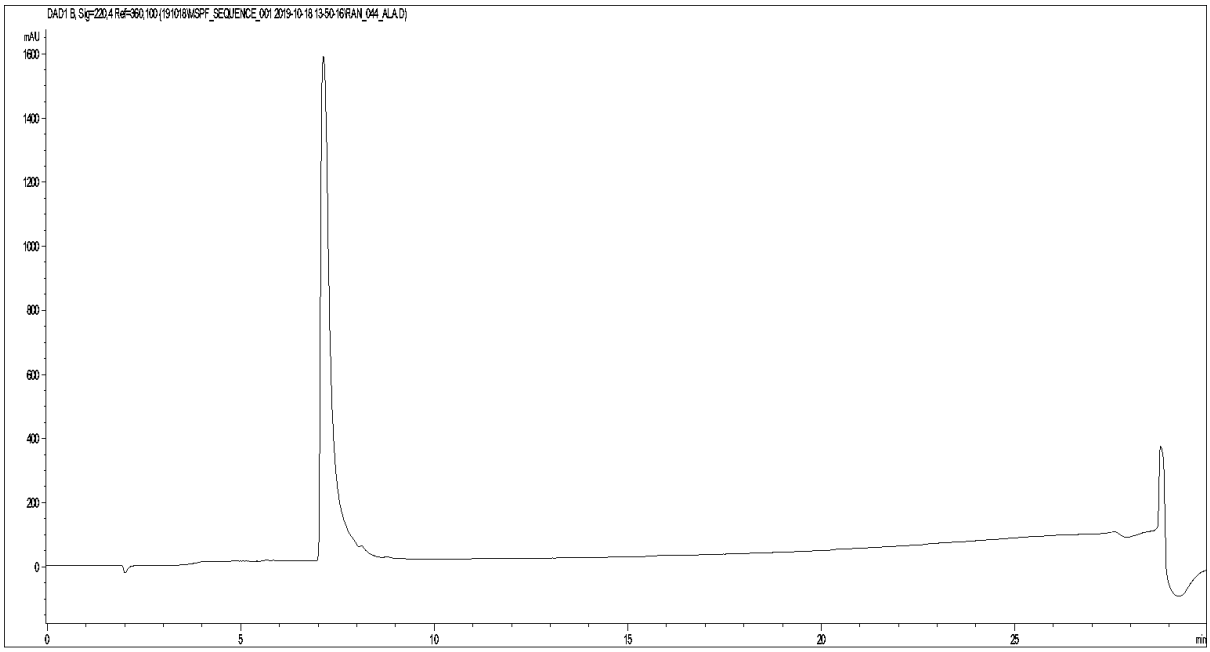
4.2.19. *N*-Methyl-L-pyroglutamic acid methyl ester (Me-Glp-OMe)



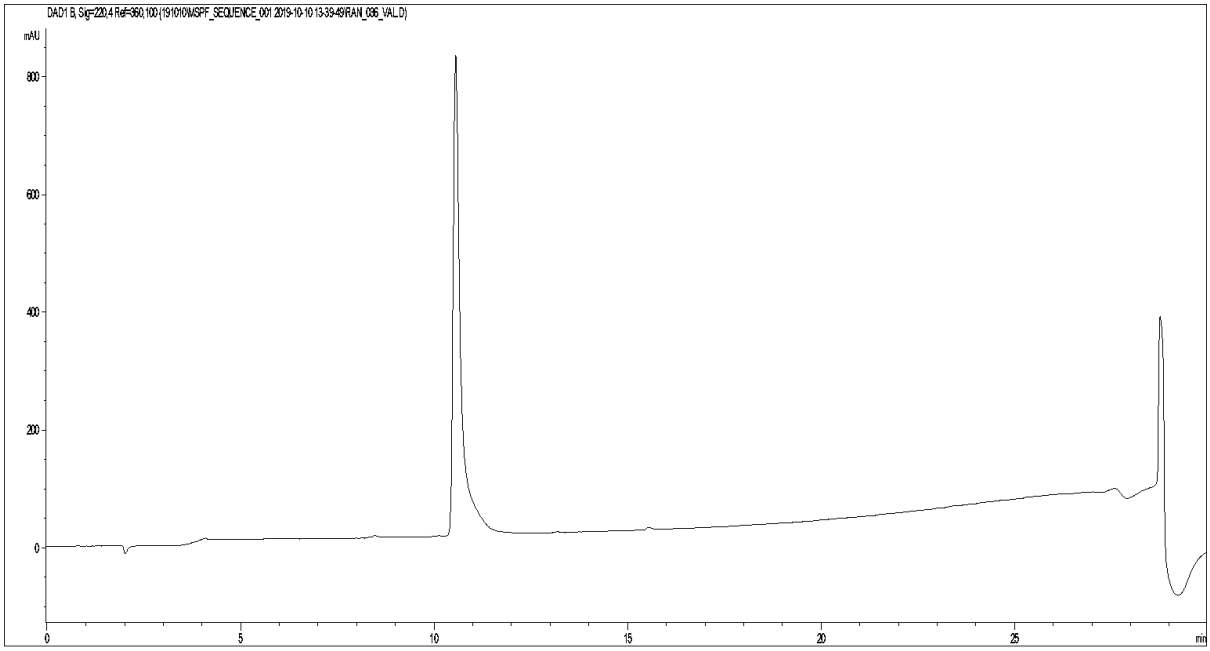
4.2.20. *N*-Methyl(d_3)-L-pyroglutamic acid methyl(d_3) ester (Me(d_3)-Glp-OMe(d_3))



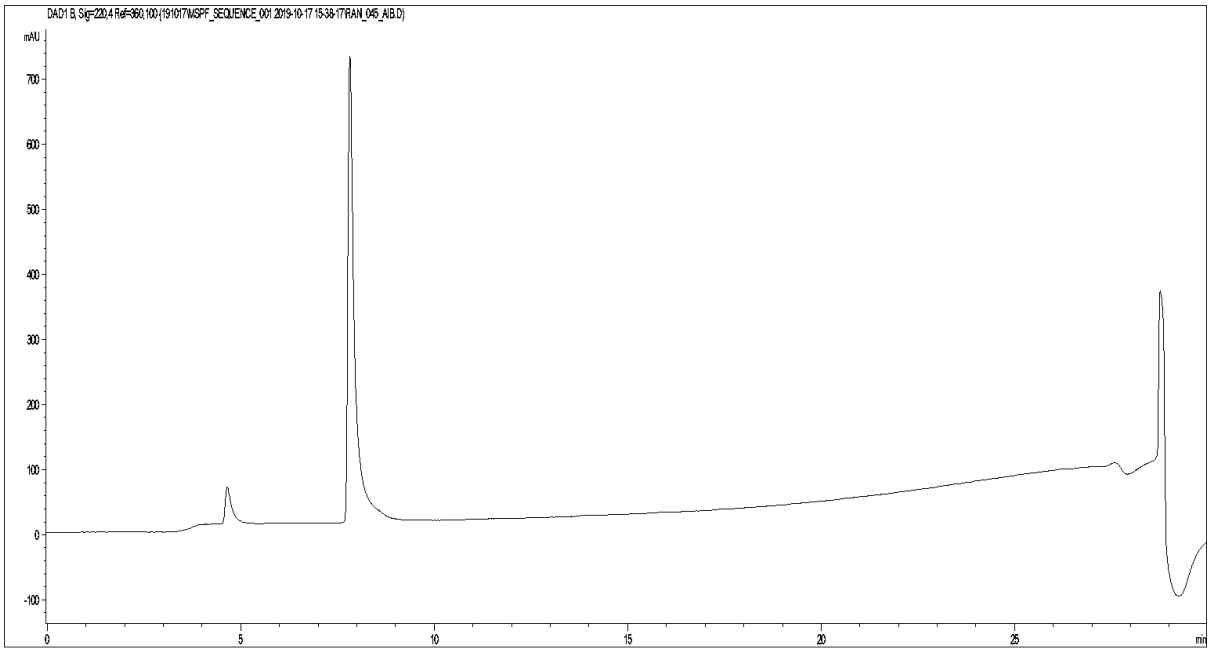
4.2.21. *N*-Acetyl-*N*-methyl-L-alanine methyl ester (Ac-(Me)Ala-OMe)



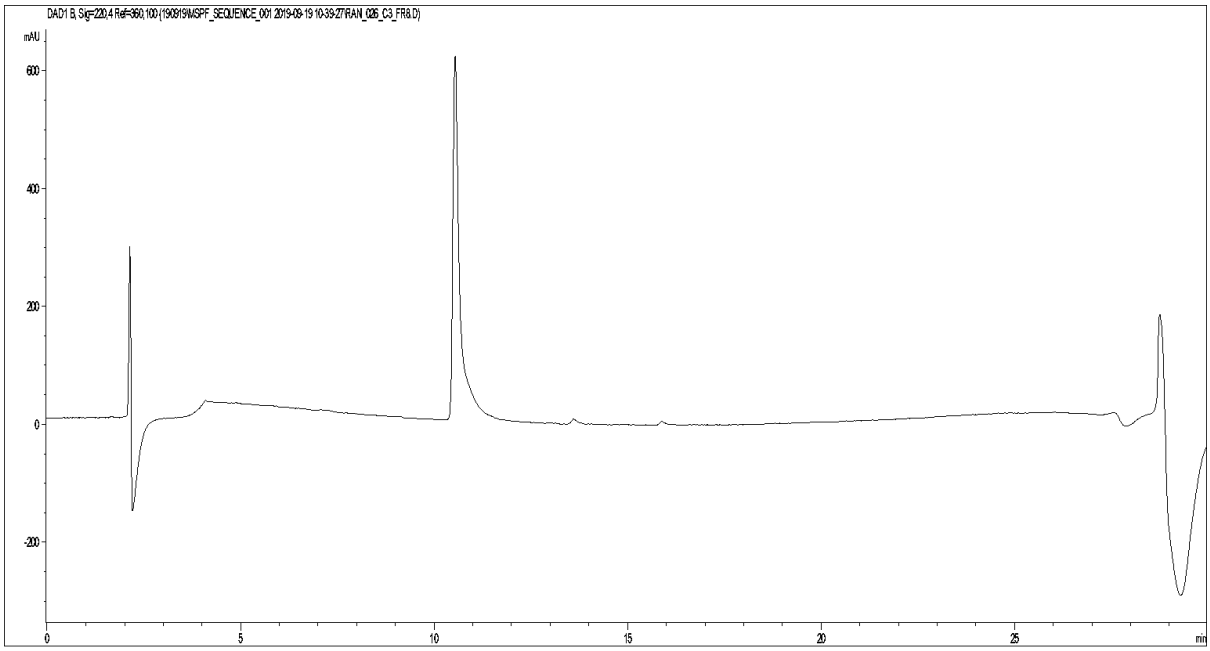
4.2.22. *N*-Acetyl-*N*-methyl-L-valine methyl ester (Ac-(Me)Val-OMe)



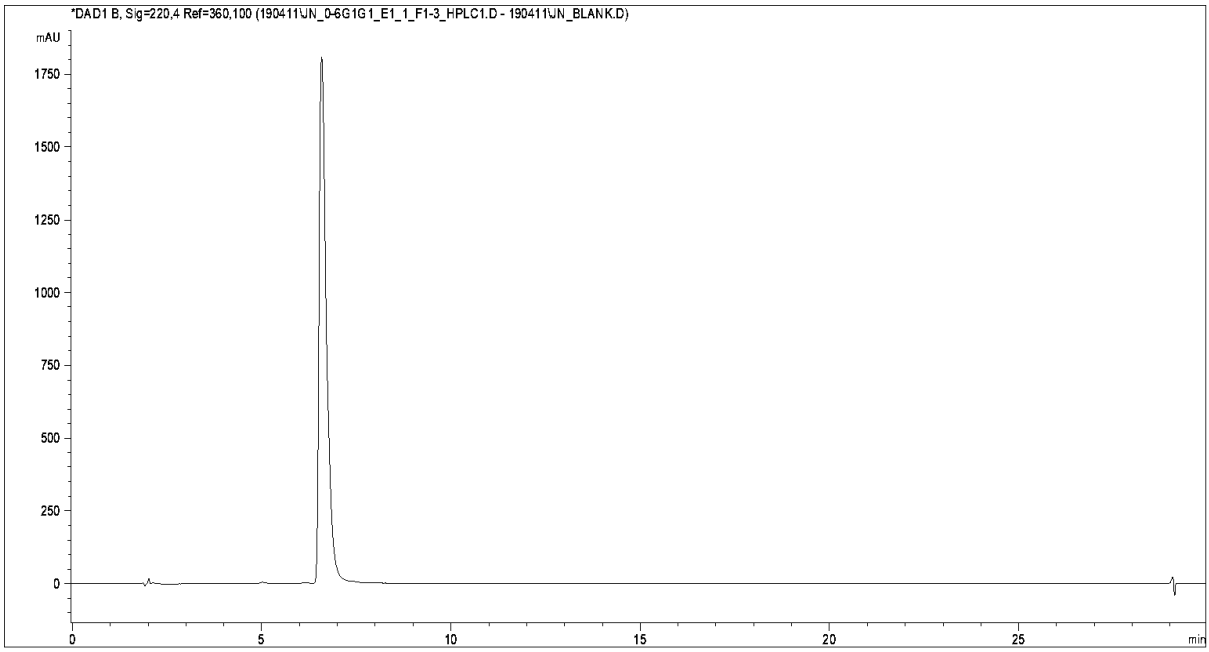
4.2.23. *N*-Acetyl-*N*-methyl-2-aminoisobutyric acid methyl ester (Ac-(Me)Aib-OMe)



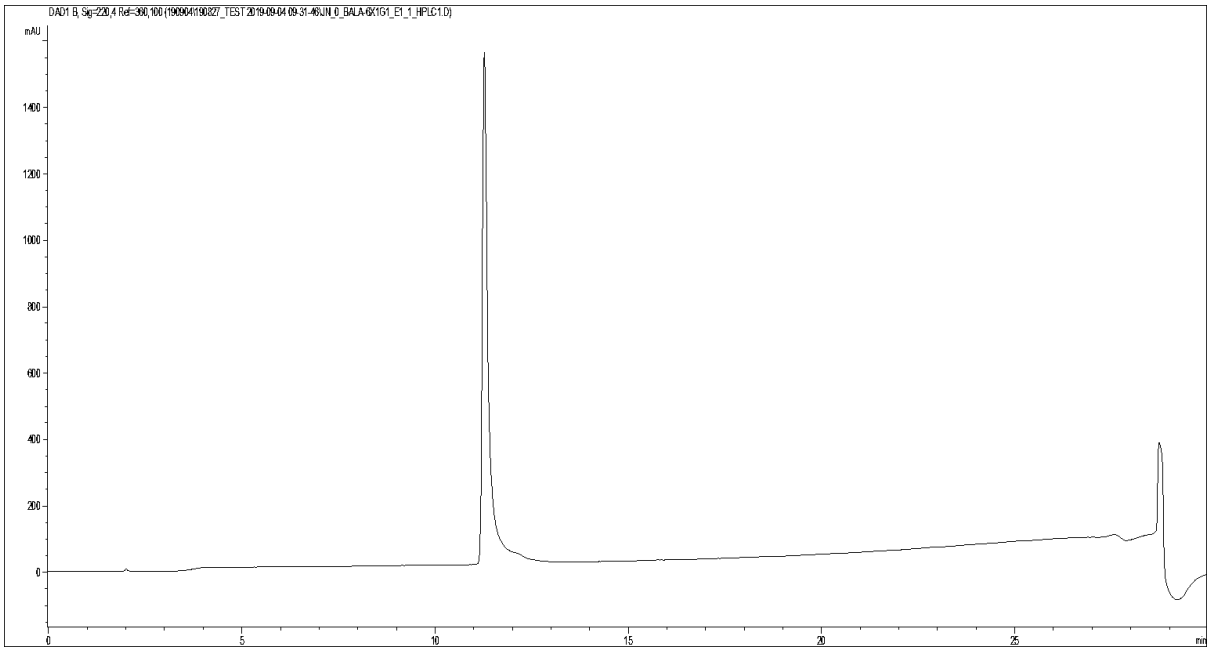
4.2.24. *N*-Acetyl-*N*-*t*-butylglycine methyl ester (Ac-(*t*Bu)Gly-OMe)



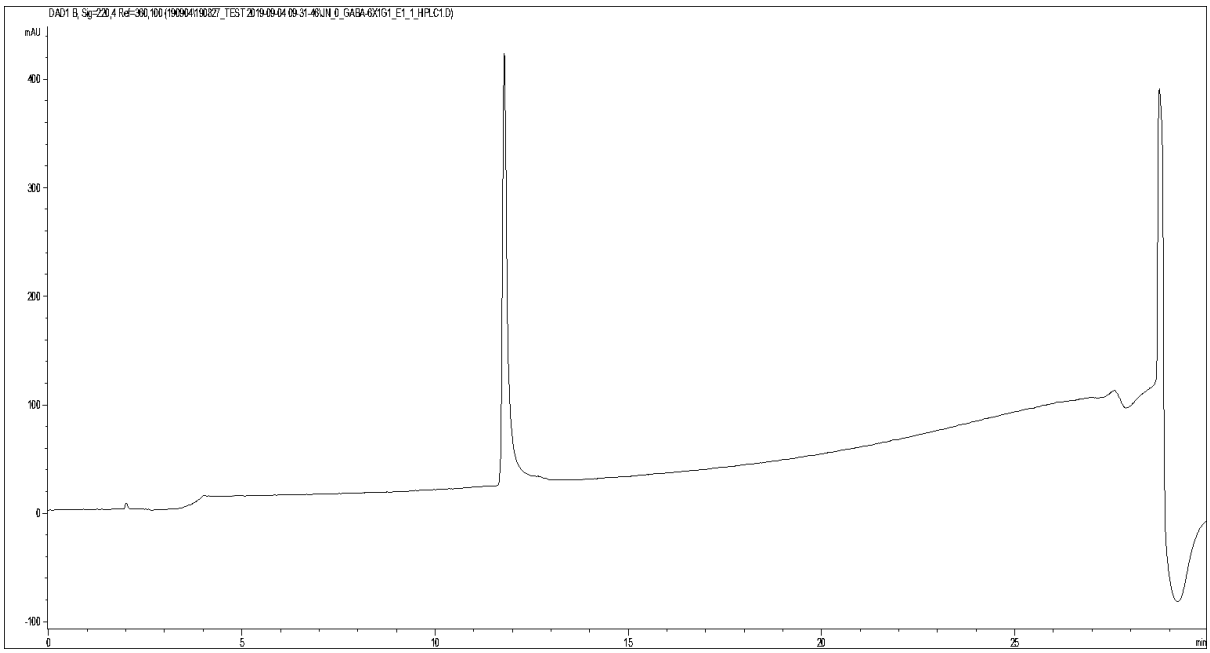
4.2.25. *N*-Phthaloylglycyl-*N*-methylglycine methyl ester (Phth-Gly-(Me)Gly-OMe)



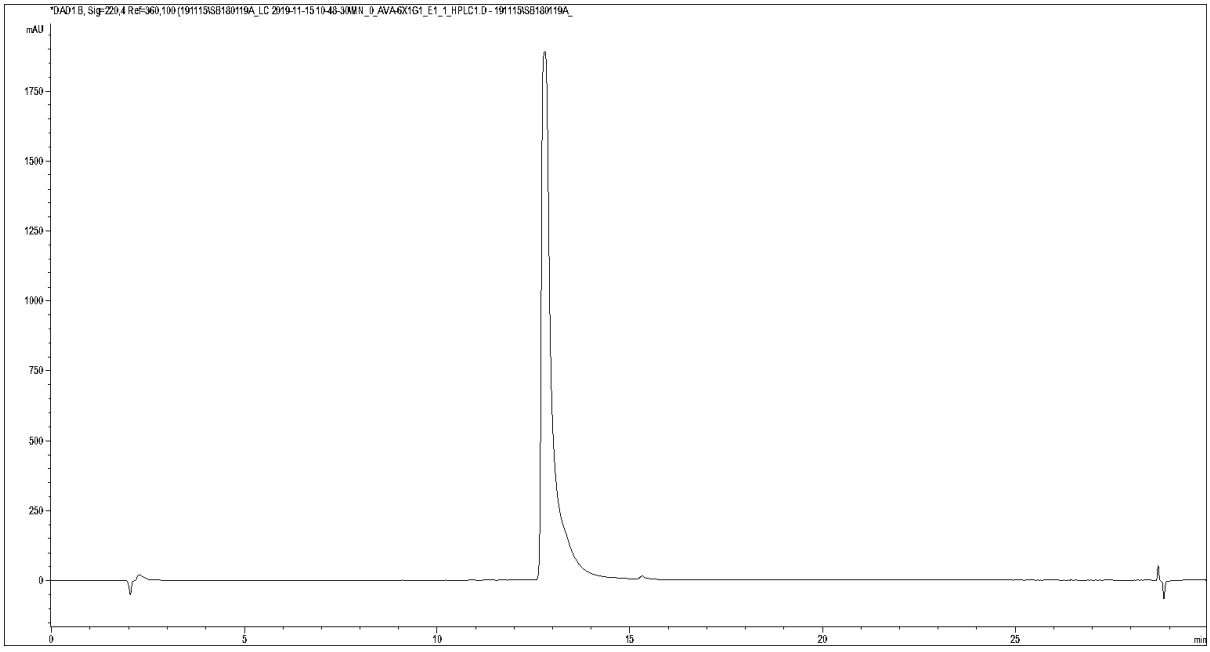
4.2.26. *N*-Phthaloyl-β-alanyl-*N*-methylglycine methyl ester (Phth-βAla-(Me)Gly-OMe)



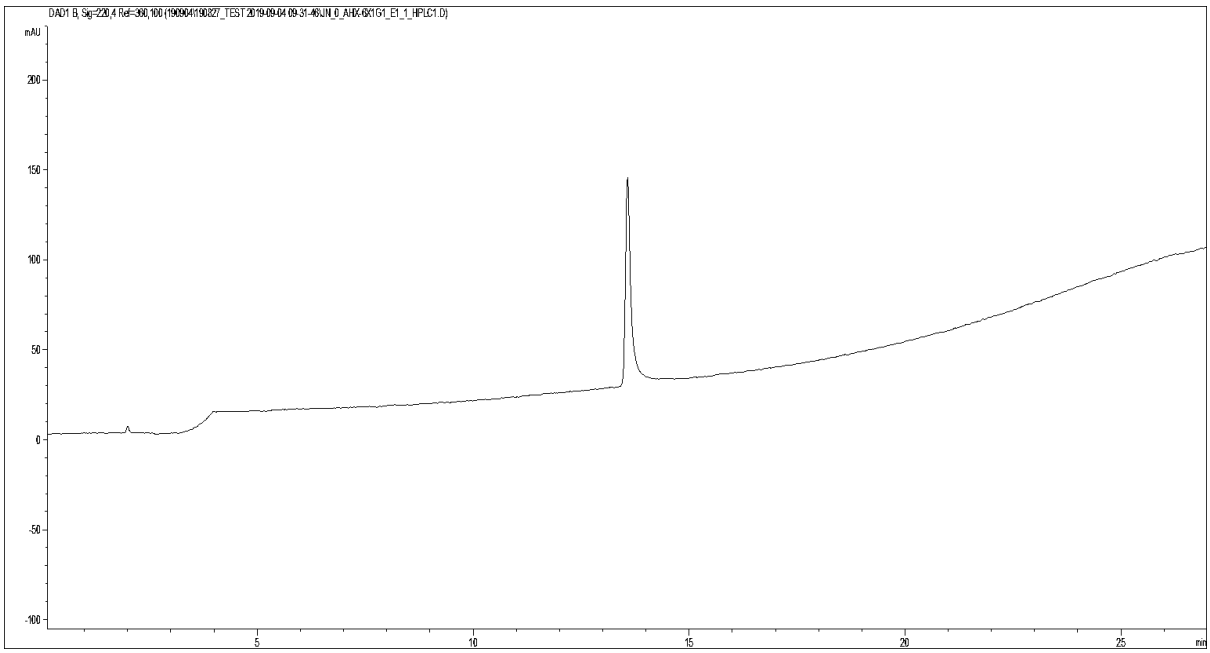
4.2.27. *N*-Phthaloyl-γ-aminobutyryl-*N*-methylglycine methyl ester (Phth-Gaba-(Me)Gly-OMe)



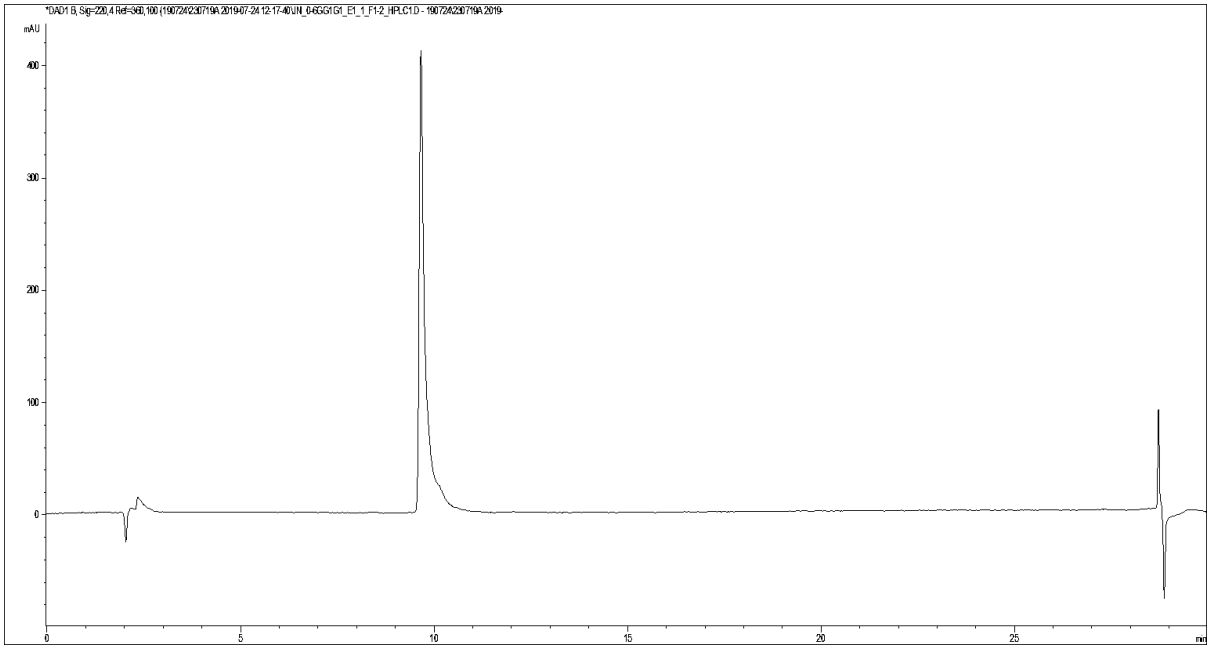
4.2.28. *N*-Phthaloyl-δ-aminovaleryl-*N*-methylglycine methyl ester (Phth-Ava-(Me)Gly-OMe)



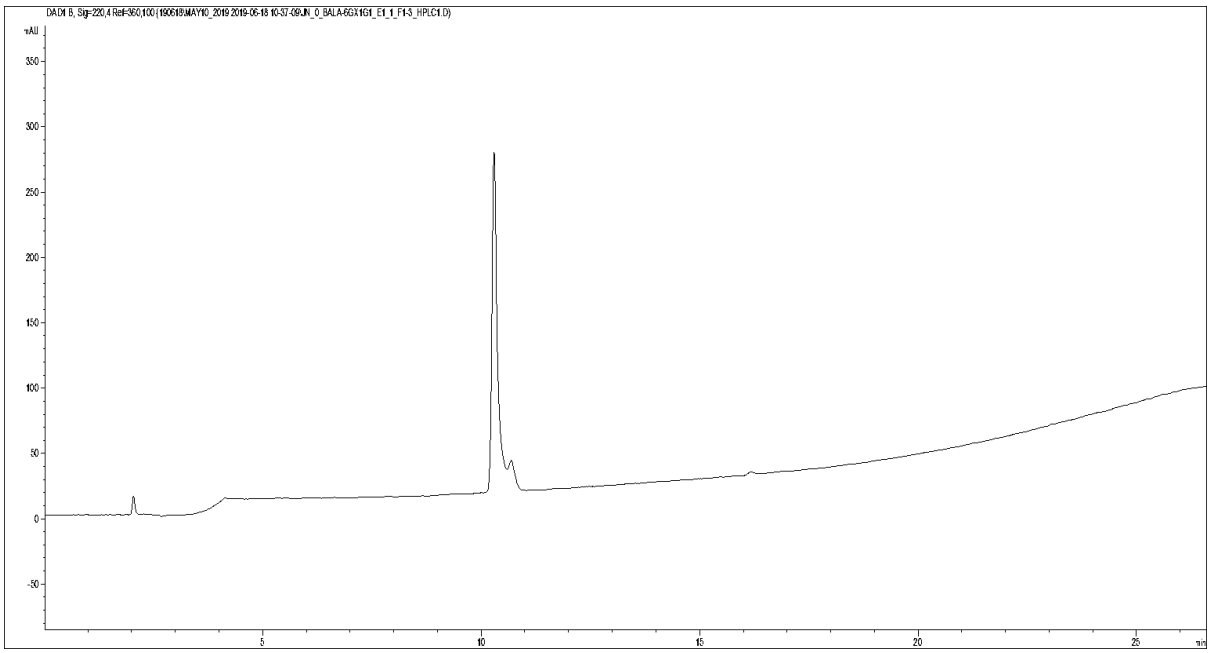
4.2.29. *N*-Phthaloyl-ε-aminocaproyl-*N*-methylglycine methyl ester (Phth-Ahx-(Me)Gly-OMe)



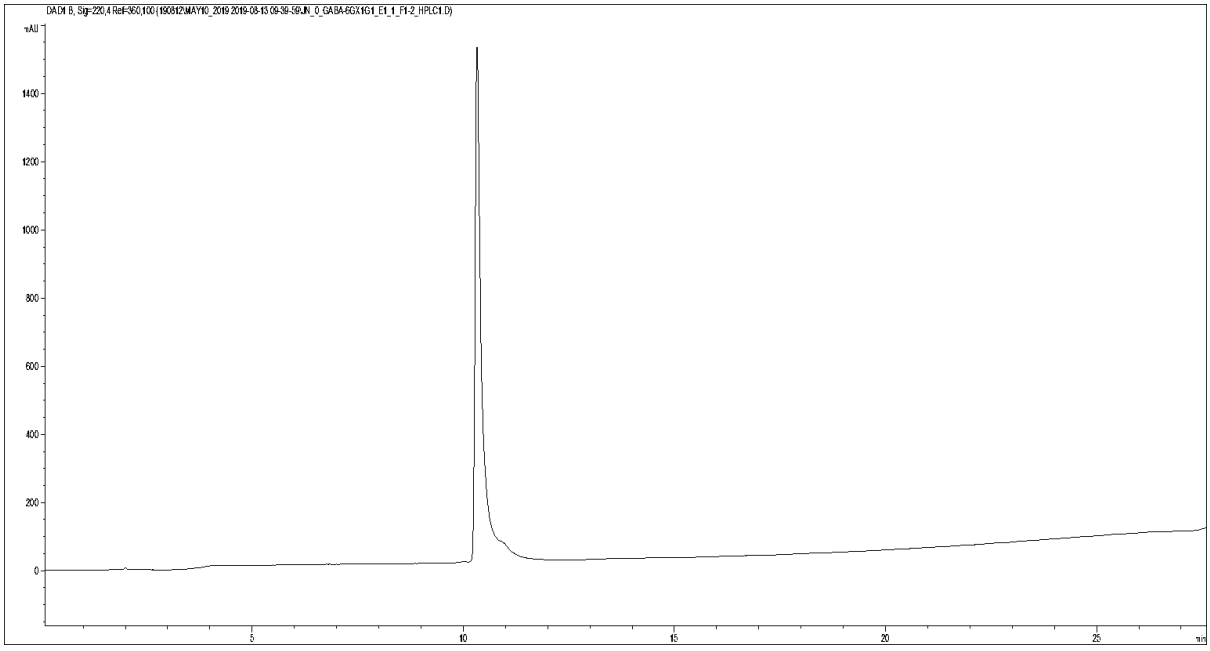
4.2.30. *N*-Phthaloylglycylglycyl-*N*-methylglycine methyl ester (Phth-Gly-Gly-(Me)Gly-OMe)



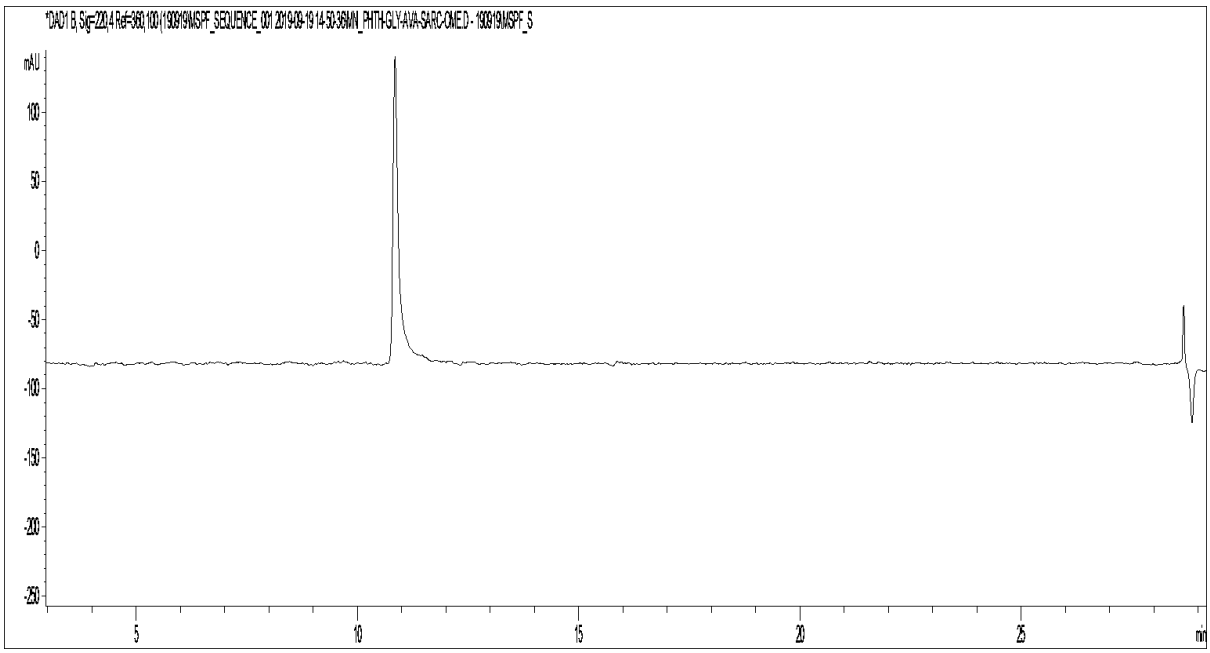
4.2.31. *N*-Phthaloylglycyl-β-alanyl-*N*-methylglycine methyl ester (Phth-Gly-βAla-(Me)Gly-OMe)



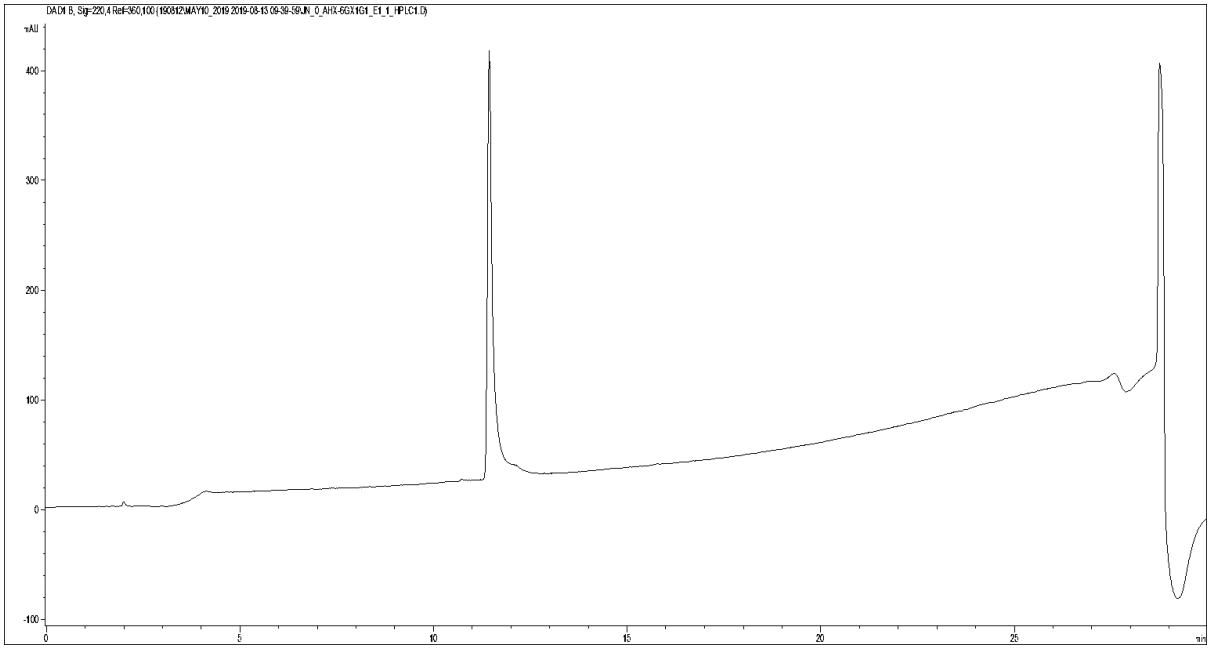
4.2.32. *N*-Phthaloylglycyl-γ-aminobutyryl-*N*-methylglycine methyl ester (Phth-Gly-Gaba-(Me)Gly-OMe)



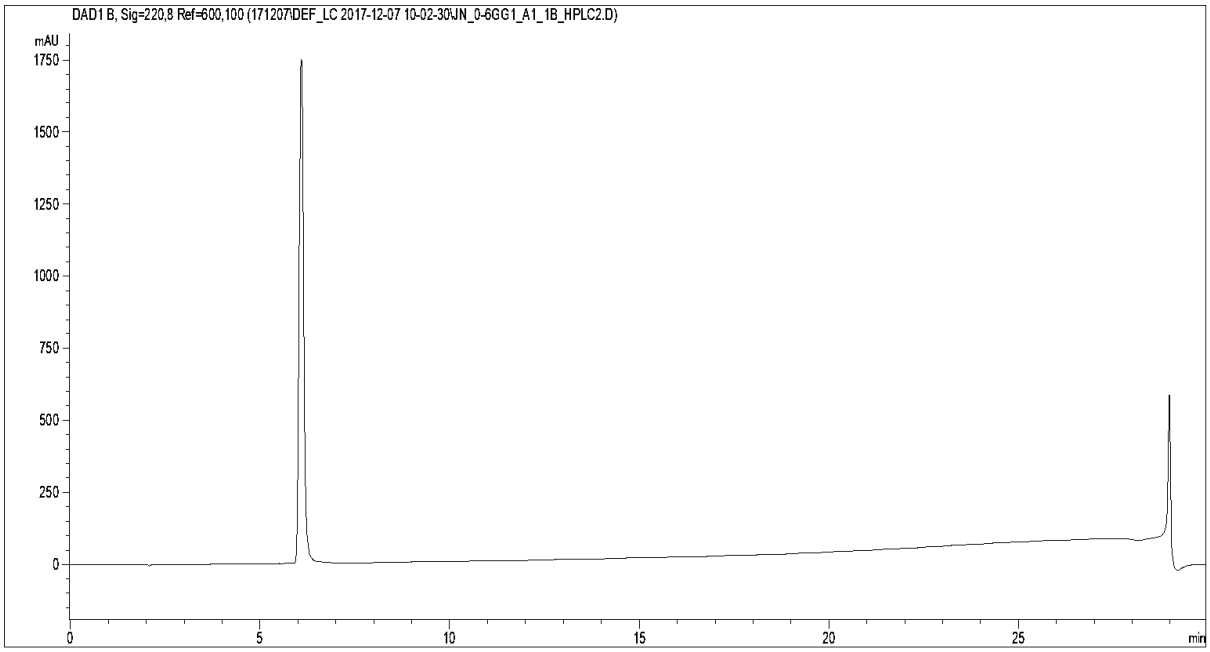
4.2.33. *N*-Phthaloylglycyl-δ-aminovaleryl-*N*-methylglycine methyl ester (Phth-Gly-Ava-(Me)Gly-OMe)



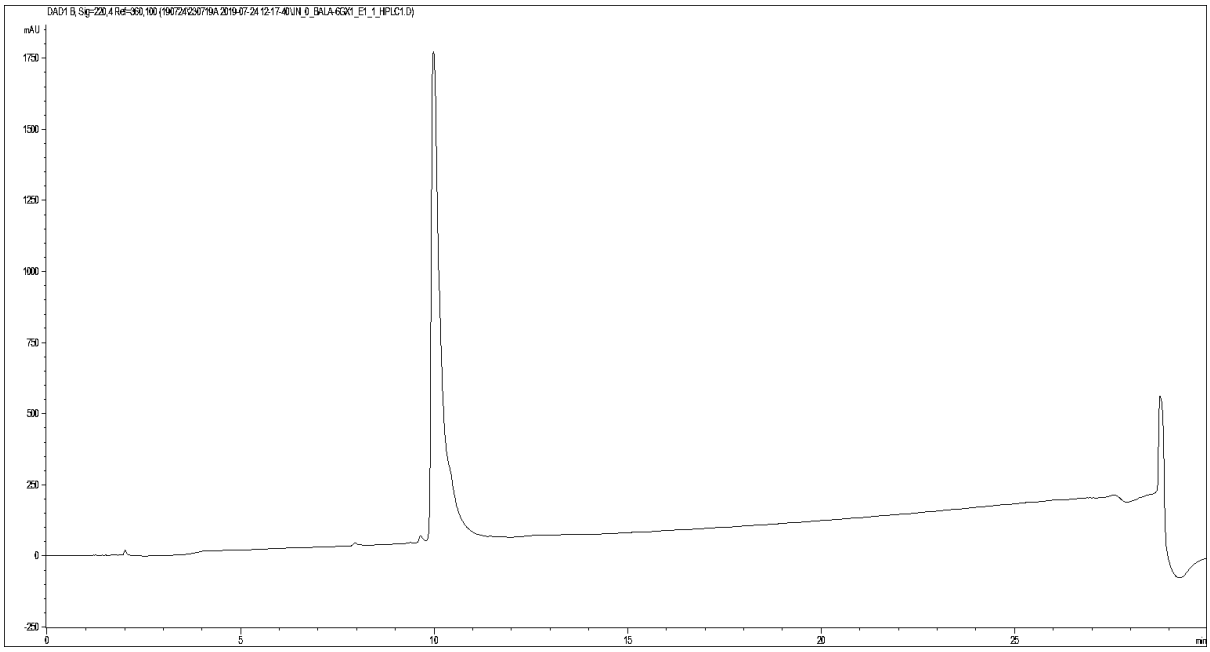
4.2.34. *N*-Phthaloylglycyl-ε-aminocaproyl-*N*-methylglycine methyl ester (Phth-Gly-Ahx-(Me)Gly-OMe)



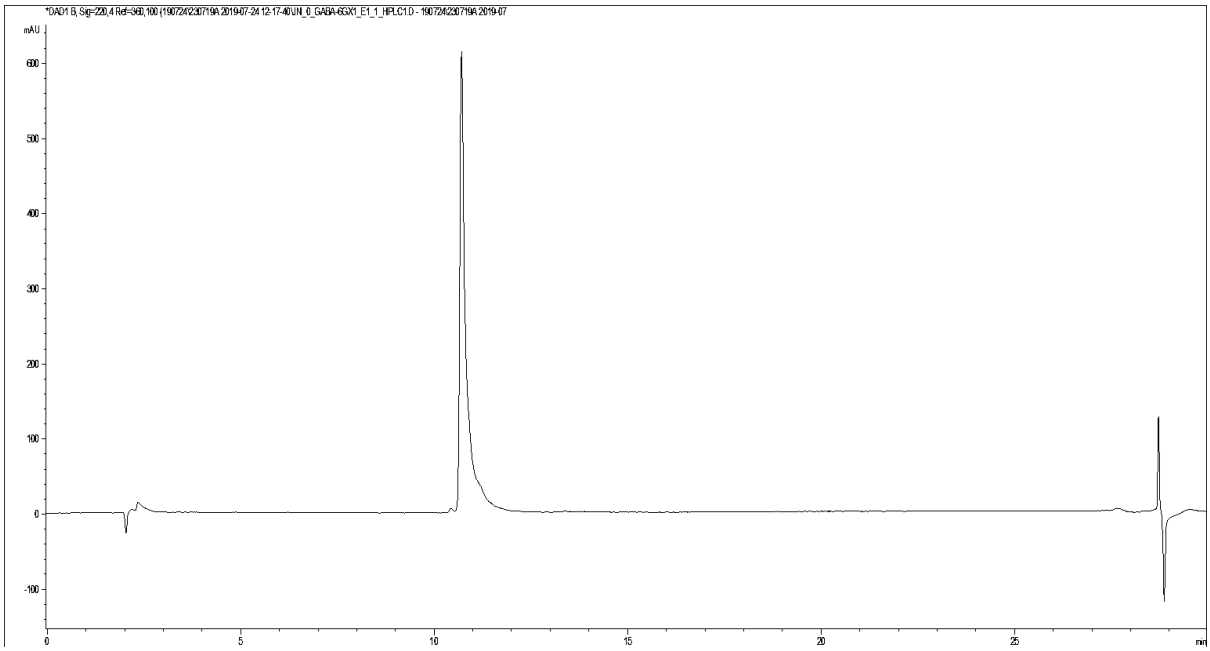
4.2.35. *N*-Phthaloylglycylglycine methyl ester (Phth-Gly-Gly-OMe)



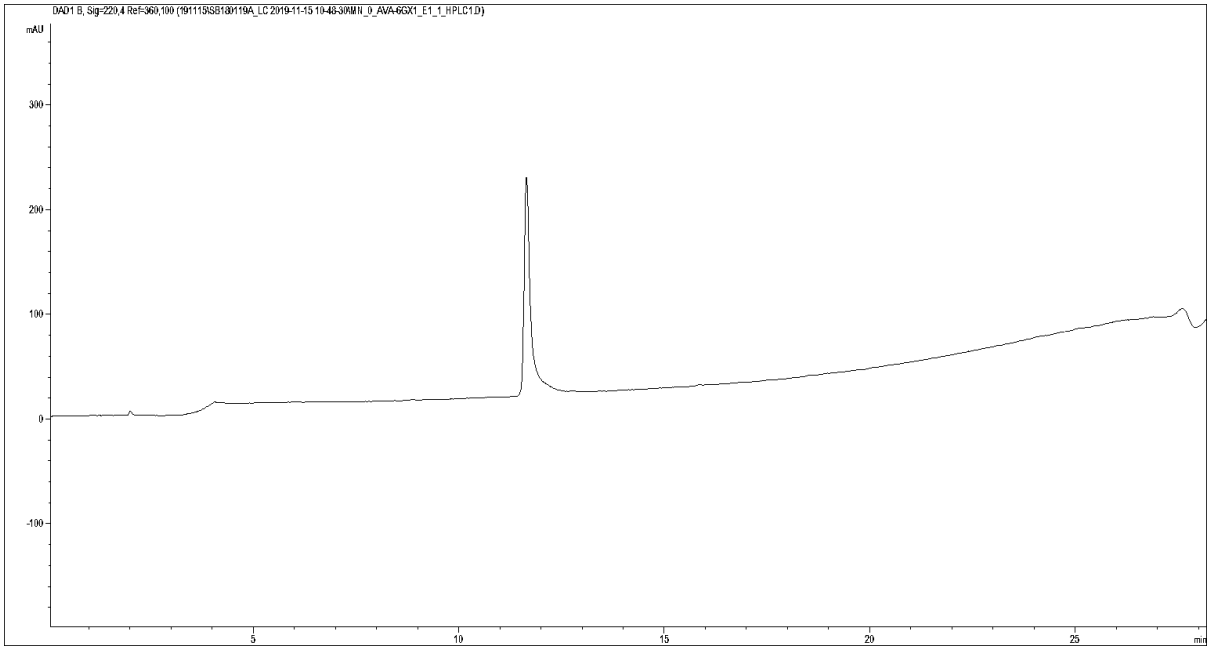
4.2.36. *N*-Phthaloylglycl-β-alanine methyl ester (Phth-Gly-βAla-OMe)



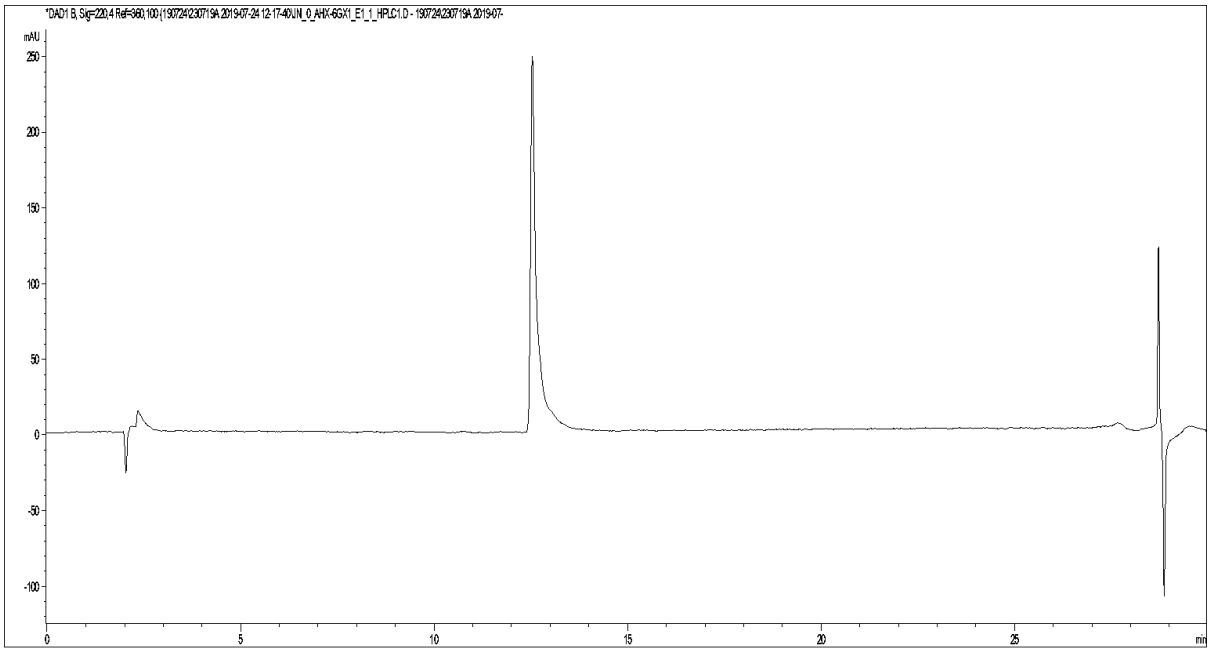
4.2.37. *N*-Phthaloylglycl-γ-aminobutyric acid methyl ester (Phth-Gly-Gaba-OMe)



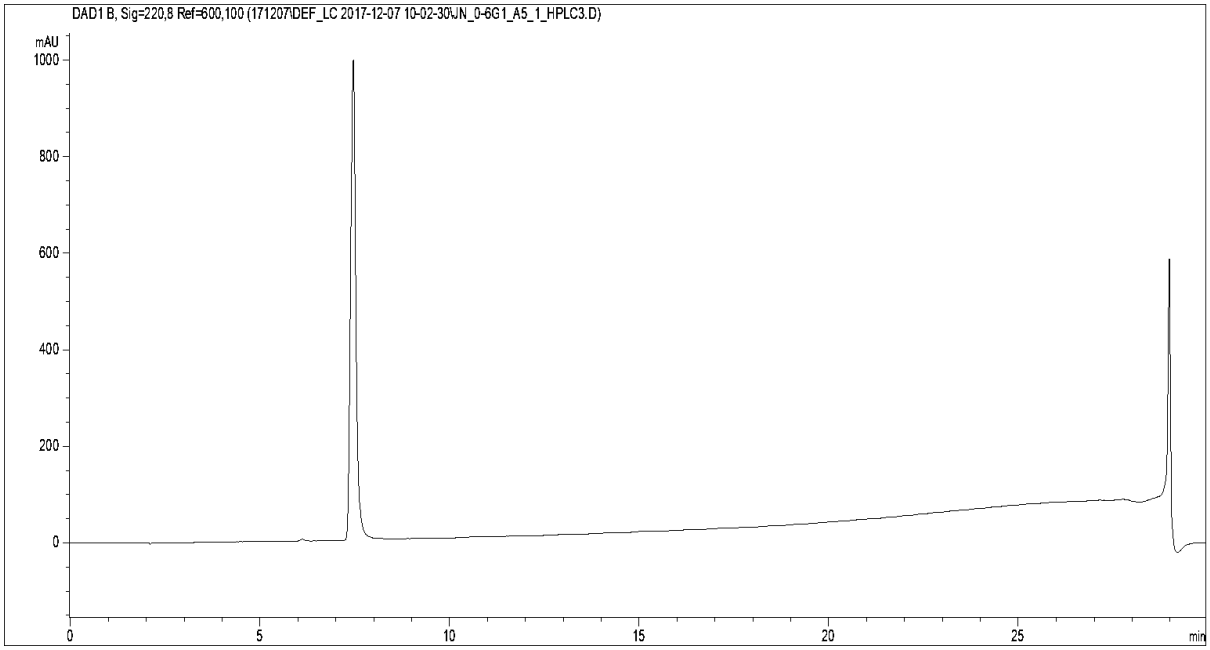
4.2.38. *N*-Phthaloylglycl-δ-aminovaleric acid methyl ester (Phth-Gly-Ava-OMe)



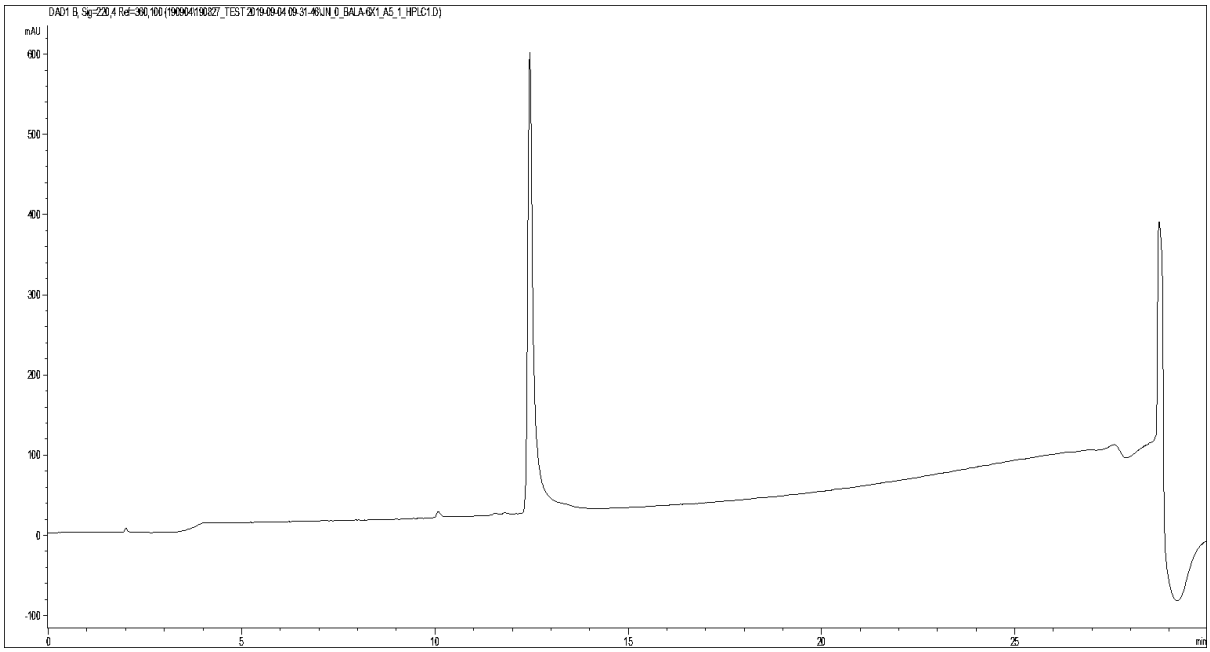
4.2.39. *N*-Phthaloylglycl-ε-aminocaproic acid methyl ester (Phth-Gly-Ahx-OMe)



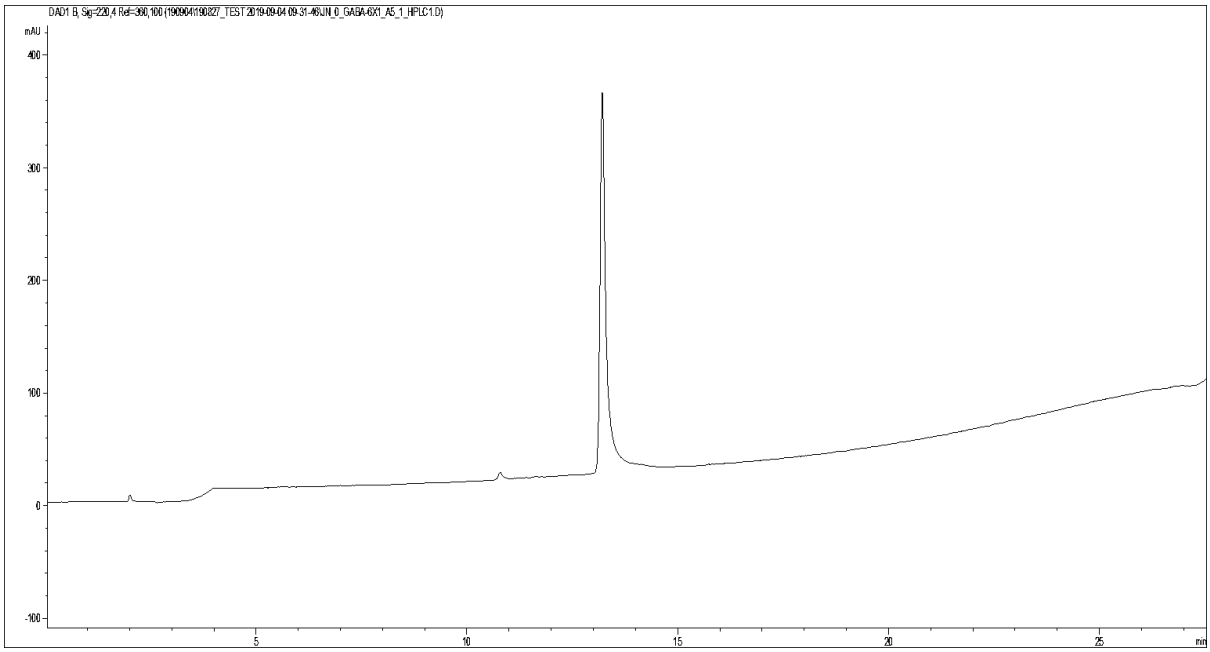
4.2.40. *N*-Phthaloylglycine methyl ester (Phth-Gly-OMe)



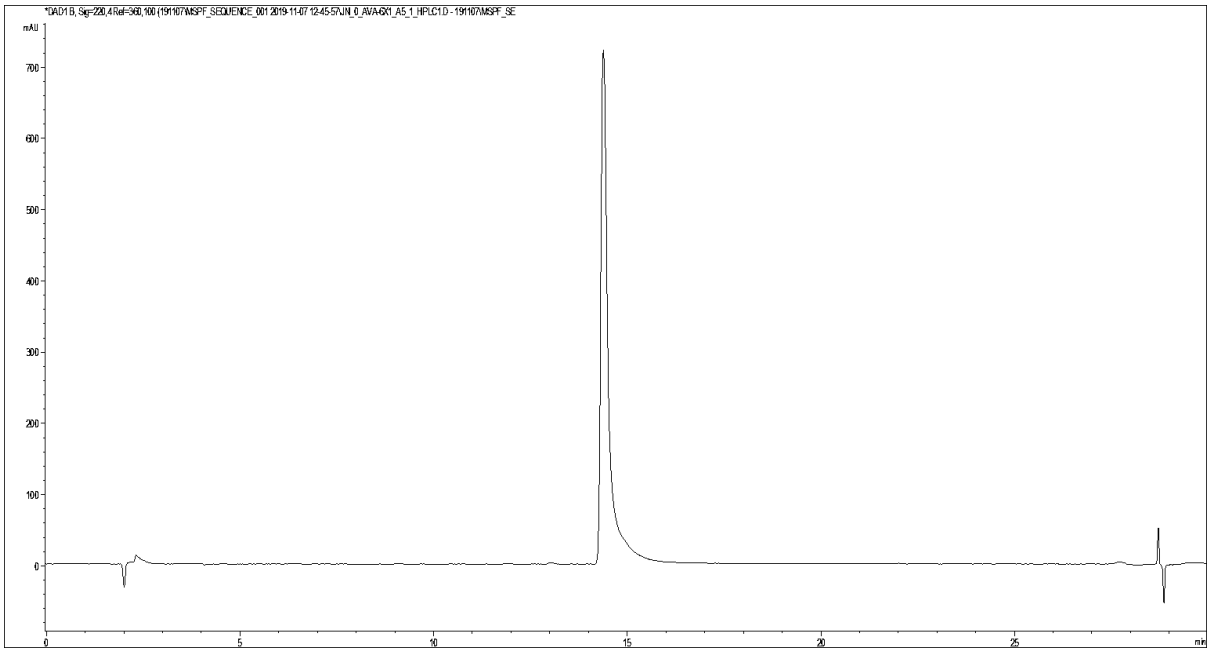
4.2.41. *N*-Phthaloyl-β-alanine methyl ester (Phth-βAla-OMe)



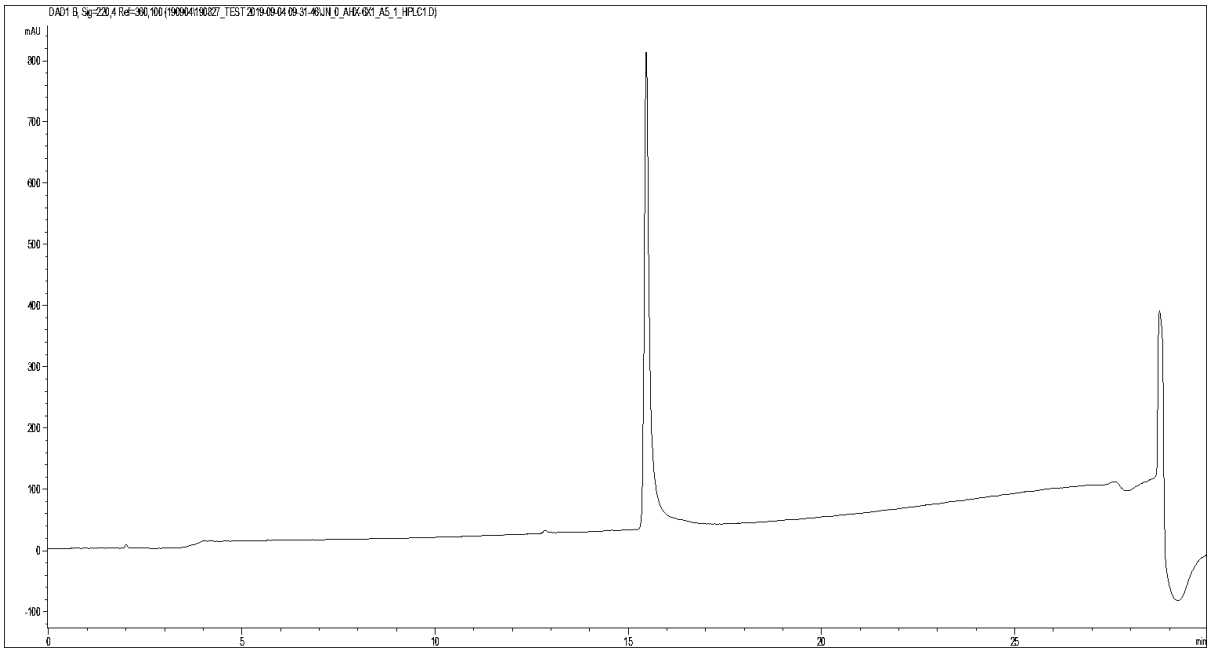
4.2.42. *N*-Phthaloyl-γ-aminobutyric acid methyl ester (Phth-Gaba-OMe)



4.2.43. *N*-Phthaloyl-δ-aminovaleric acid methyl ester (Phth-Ava-OMe)



4.2.44. *N*-Phthaloyl-ε-aminocaproic acid methyl ester (Phth-Ahx-OMe)



5. Laser flash photolysis studies

Time-dependent decay plots of $NO_3^\bullet$ signals at 630 nm for its reaction with each compound are fitted according to the exponential decay fitting available on Igor Pro 6.03 (WaveMetrics, Inc.; Lake Oswego, OR, USA). The fitting used for the calculations follows the equation: $y = y_o + Ae^{-invTau*x}$; where y is the optical density, OD (proportional to [$NO_3^\bullet$]) and x is the time (unit in ns). The value of k_{obs} corresponds to the value of $invTau$.

Second-order rate coefficients, k, for the reactions of $NO_3^\bullet$ with various substrates were obtained from the slopes of k_{obs} vs [substrate] plots. These plots are shown in Figure S1–8 below. The intercept is due to the reaction of $NO_3^\bullet$ with the solvent (see ref [17] in the manuscript).

Error determination: each result is reported as the average of three different runs. For example, if three different runs produced this set of data: $(x_1 \pm \sigma_1)$, $(x_2 \pm \sigma_2)$ and $(x_3 \pm \sigma_3)$, then the result would be taken as $\frac{x_1 + x_2 + x_3}{3}$ and σ is the overall standard deviation obtained following the statistical equation below:

$\sigma = \sqrt{\frac{{\sigma_1}^2 + {\sigma_2}^2 + {\sigma_3}^2}{3} + \frac{2}{9}({x_1}^2 + {x_2}^2 + {x_3}^2 - x_1 \cdot x_2 - x_2 \cdot x_3 - x_3 \cdot x_1)}$, where

x_1, x_2, x_3 are the result from each respective run and $\sigma_1, \sigma_2, \sigma_3$ are the standard deviation of each result. Overall errors are given as 2σ statistical uncertainties. Typical errors measured in these experiments are around 5–15%.

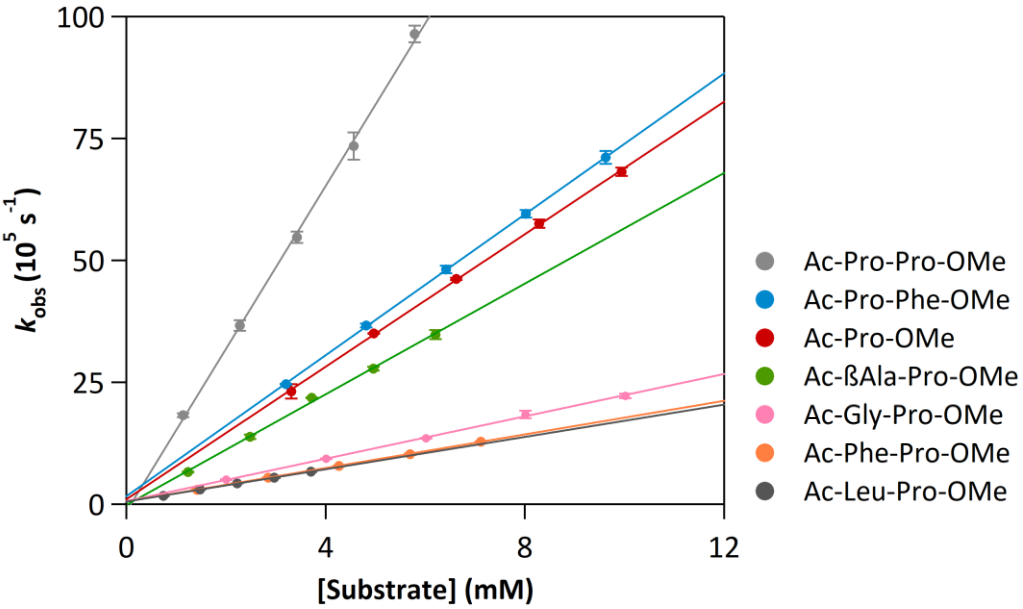


Figure S1. Plot of pseudo-first-order rate coefficient (k_{obs}) versus [substrate] containing proline residue (see Table 1). Error bars shown are 2σ statistical uncertainties. From the linear regression analysis: Ac-Pro-Pro-OMe: intercept = −1.5 x 10^5 s^{-1}, k = 16.7 x 10^8 M^{-1} s^{-1}, R^2 = 0.9988; Ac-Pro-Phe-OMe: intercept = 1.5 x 10^5 s^{-1}, k = 7.2 x 10^8 M^{-1} s^{-1}, R^2 = 0.9999; Ac-Pro-OMe: intercept = 9.9 x 10^4 s^{-1}, k = 6.8 x 10^8 M^{-1} s^{-1}, R^2 = 0.9996; Ac-βAla-Pro-OMe: intercept = −1.5 x 10^4 s^{-1}, k = 5.7 x 10^8 M^{-1} s^{-1}, R^2 = 0.9980; Ac-Gly-Pro-OMe: intercept = 6.5 x 10^4 s^{-1}, k = 2.2 x 10^8 M^{-1} s^{-1}, R^2 = 0.9903; Ac-Phe-Pro-OMe: intercept = 5.2 x 10^4 s^{-1}, k = 1.7 x 10^8 M^{-1} s^{-1}, R^2 = 0.9999; Ac-Leu-Pro-OMe: intercept = 5.5 x 10^4 s^{-1}, k = 1.7 x 10^8 M^{-1} s^{-1}, R^2 = 0.9998.

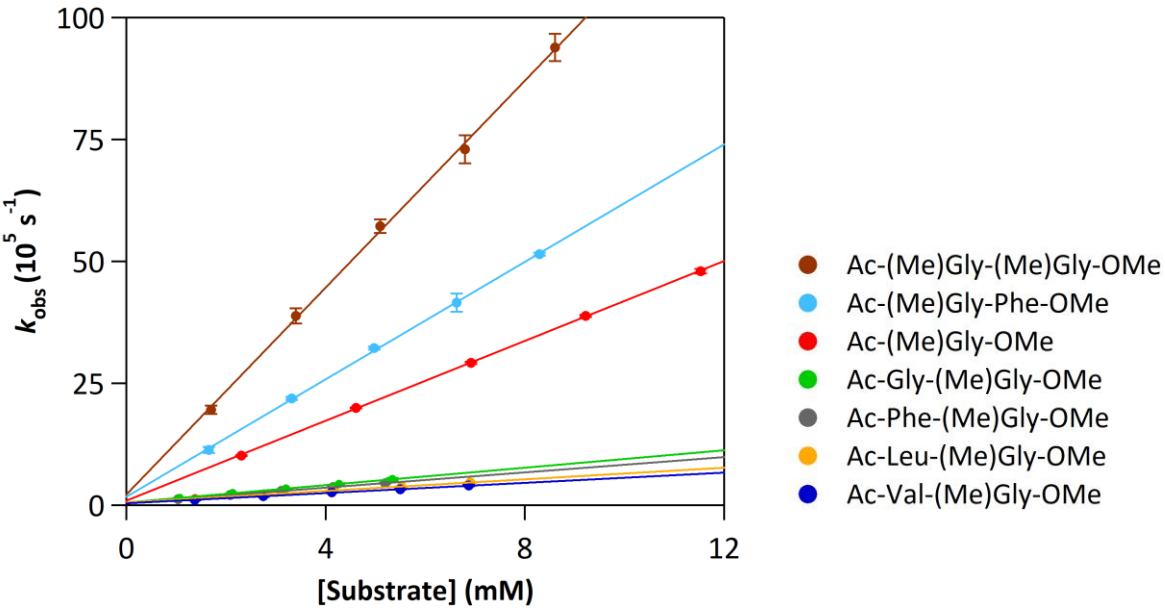


Figure S2. Plot of pseudo-first-order rate coefficient (k_{obs}) versus [substrate] containing *N*-methylglycine residue (see Table 1). Error bars shown are 2σ statistical uncertainties. From the linear regression analysis: Ac-(Me)Gly-(Me)Gly-OMe: intercept = 2.1 x 10^5 s^{-1}, k = 10.6 x 10^8 M^{-1} s^{-1}, R^2 = 0.9989; Ac-(Me)Gly-Phe-OMe: intercept = 1.7 x 10^5 s^{-1}, k = 6.0 x 10^8 M^{-1} s^{-1}, R^2 = 0.9995; Ac-(Me)Gly-OMe: intercept = 9.3 x 10^4 s^{-1}, k = 4.1 x 10^8 M^{-1} s^{-1}, R^2 = 0.9999; Ac-Gly-(Me)Gly-OMe: intercept = 5.6 x 10^4 s^{-1}, k = 0.9 x 10^8 M^{-1} s^{-1}, R^2 = 0.9996; Ac-Phe-(Me)Gly-OMe: intercept = 4.8 x 10^4 s^{-1}, k = 0.8 x 10^8 M^{-1} s^{-1}, R^2 = 0.9996; Ac-Leu-(Me)Gly-OMe: intercept = 5.3 x 10^4 s^{-1}, k = 0.6 x 10^8 M^{-1} s^{-1}, R^2 = 0.9976; Ac-Val-(Me)Gly-OMe: intercept = 4.1 x 10^4 s^{-1}, k = 0.5 x 10^8 M^{-1} s^{-1}, R^2 = 0.9995.

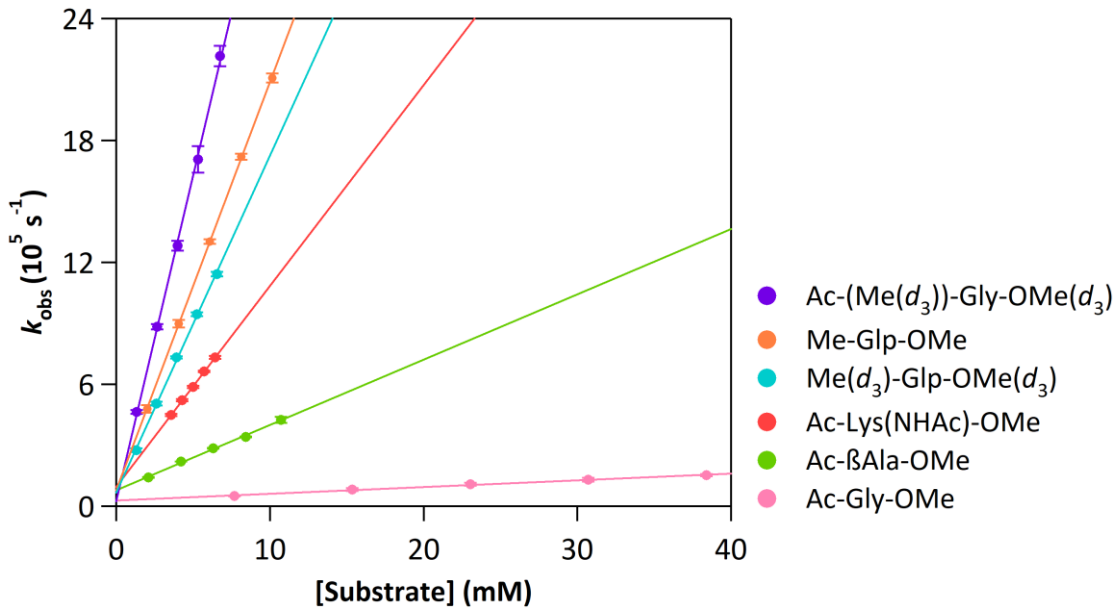


Figure S3. Plot of pseudo-first-order rate coefficient (k_{obs}) versus [amino acid] with various N- and C-terminal protecting groups (see Table 2). Error bars shown are 2σ statistical uncertainties. From the linear regression analysis: Ac-(Me(d_3))Gly-OMe(d_3): intercept = 2.5 x 10^4 s^{-1}, k = 3.2 x 10^8 M^{-1} s^{-1}, R^2 = 0.9990; Me-Glp-OMe: intercept = 7.8 x 10^4 s^{-1}, k = 2.0 x 10^8 M^{-1} s^{-1}, R^2 = 0.9998; Me(d_3)-Glp-OMe(d_3): intercept = 6.7 x 10^4 s^{-1}, k = 1.7 x 10^8 M^{-1} s^{-1}, R^2 = 0.9990; Ac-Lys(NHAc)-OMe: intercept = 9.6 x 10^4 s^{-1}, k = 1.0 x 10^8 M^{-1} s^{-1}, R^2 = 0.9997; Ac-βAla-OMe: intercept = 7.9 x 10^4 s^{-1}, k = 0.3 x 10^8 M^{-1} s^{-1}, R^2 = 0.9970; Ac-Gly-OMe: intercept = 3.0 x 10^4 s^{-1}, k = 0.03 x 10^8 M^{-1} s^{-1}, R^2 = 0.9892.

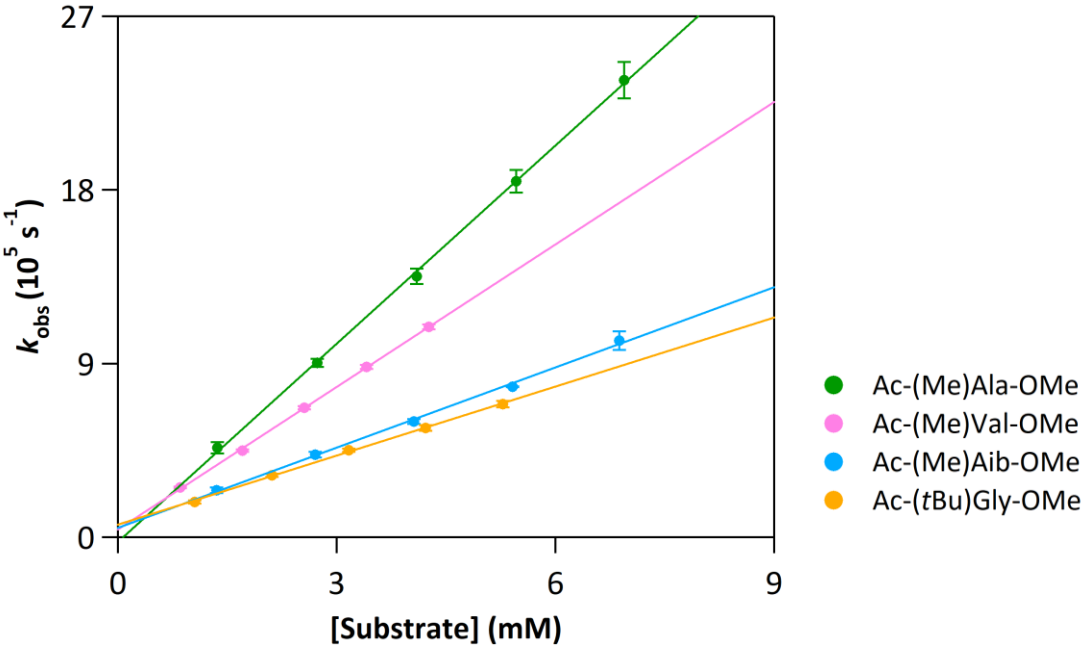


Figure S4. Plot of pseudo-first-order rate coefficient (k_{obs}) versus [amino acid] with N-terminal tertiary amide (see Table 2). Error bars shown are 2σ statistical uncertainties. From the linear regression analysis: Ac-(Me)Ala-OMe: intercept = −2.5 x 10^4 s^{-1}, k = 3.4 x 10^8 M^{-1} s^{-1}, R^2 = 0.9994; Ac-(Me)Val-OMe: intercept = 4.1 x 10^4 s^{-1}, k = 2.5 x 10^8 M^{-1} s^{-1}, R^2 = 0.9996; Ac-(Me)Aib-OMe: intercept = 4.8 x 10^4 s^{-1}, k = 1.4 x 10^8 M^{-1} s^{-1}, R^2 = 0.9978; Ac-(*t*Bu)-Gly-OMe: intercept = 6.5 x 10^4 s^{-1}, k = 1.2 x 10^8 M^{-1} s^{-1}, R^2 = 0.9987.

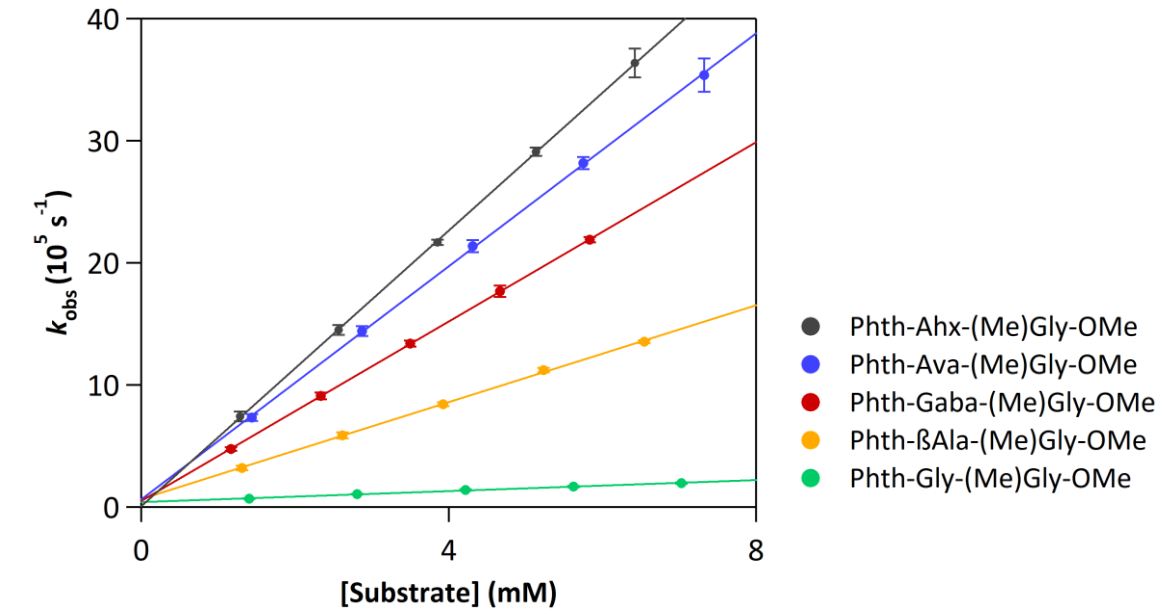


Figure S5. Plot of pseudo-first-order rate coefficient (k_{obs}) versus [dipeptide] possessing *N*-methylglycine residue (see Table 3). Error bars shown are 2σ statistical uncertainties. From the linear regression analysis: Phth-Ahx-(Me)Gly-OMe: intercept = 7.4 x 10^3 s^{-1}, k = 5.6 x 10^8 M^{-1} s^{-1}, R^2 = 0.9999; Phth-Ava-(Me)Gly-OMe: intercept = 6.3 x 10^4 s^{-1}, k = 4.8 x 10^8 M^{-1} s^{-1}, R^2 = 0.9998; Phth-Gaba-(Me)Gly-OMe: intercept = 5.0 x 10^4 s^{-1}, k = 3.7 x 10^8 M^{-1} s^{-1}, R^2 = 0.9999; Phth-βAla-(Me)Gly-OMe: intercept = 6.5 x 10^4 s^{-1}, k = 2.0 x 10^8 M^{-1} s^{-1}, R^2 = 0.9994; Phth-Gly-(Me)Gly-OMe: intercept = 4.1 x 10^4 s^{-1}, k = 0.2 x 10^8 M^{-1} s^{-1}, R^2 = 0.9971.

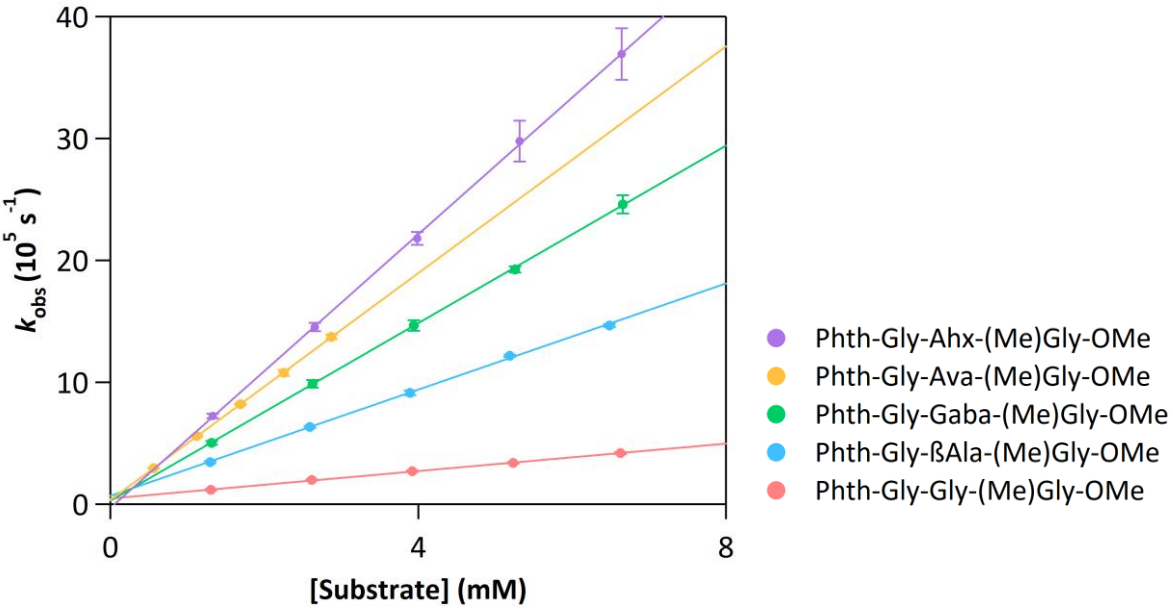


Figure S6. Plot of pseudo-first-order rate coefficient (k_{obs}) versus [tripeptide] possessing *N*-methylglycine residue (see Table 3). Error bars shown are 2σ statistical uncertainties. From the linear regression analysis: Phth-Gly-Ahx-(Me)Gly-OMe: intercept = −3.1 x 10^4 s^{-1}, k = 5.6 x 10^8 M^{-1} s^{-1}, R^2 = 0.9997; Phth-Gly-Ava-(Me)Gly-OMe: intercept = 3.5 x 10^4 s^{-1}, k = 4.6 x 10^8 M^{-1} s^{-1}, R^2 = 0.9999; Phth-Gly-Gaba-(Me)Gly-OMe: intercept = 2.7 x 10^4 s^{-1}, k = 3.6 x 10^8 M^{-1} s^{-1}, R^2 = 0.9998; Phth-Gly-βAla-(Me)Gly-OMe: intercept = 6.8 x 10^4 s^{-1}, k = 2.2 x 10^8 M^{-1} s^{-1}, R^2 = 0.9992; Phth-Gly-Gly-(Me)Gly-OMe: intercept = 4.7 x 10^4 s^{-1}, k = 0.6 x 10^8 M^{-1} s^{-1}, R^2 = 0.9990.

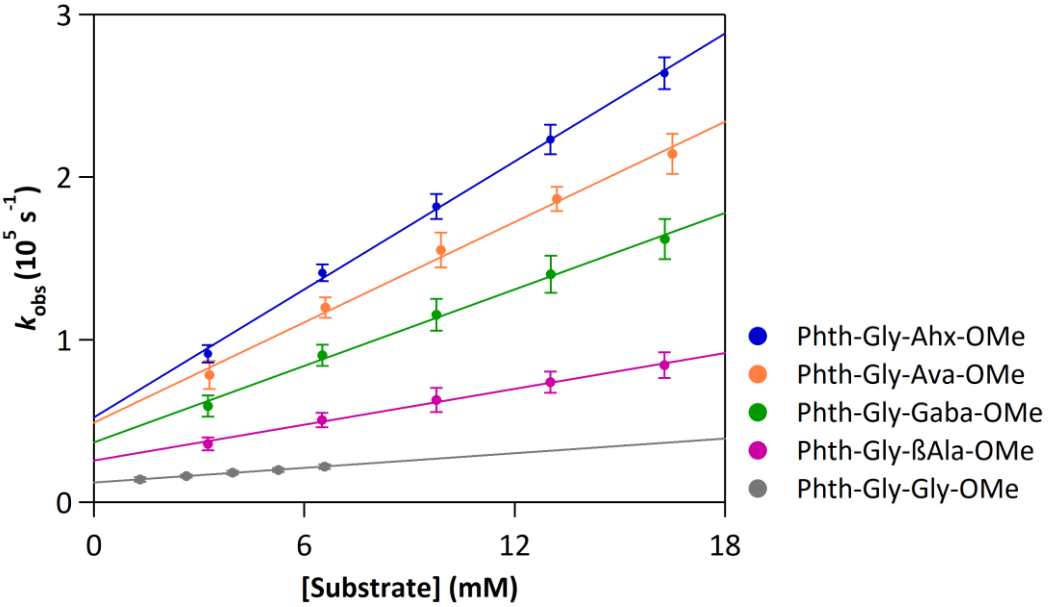


Figure S7. Plot of pseudo-first-order rate coefficient (k_{obs}) versus [dipeptide] (see Table 4). Error bars shown are 2σ statistical uncertainties. From the linear regression analysis: Phth-Gly-Ahx-OMe: intercept = 5.2 x 10^4 s^{-1}, k = 13.1 x 10^6 M^{-1} s^{-1}, R^2 = 0.9983; Phth-Gly-Ava-OMe: intercept = 4.9 x 10^4 s^{-1}, k = 10.3 x 10^6 M^{-1} s^{-1}, R^2 = 0.9937; Phth-Gly-Gaba-OMe: intercept = 3.7 x 10^4 s^{-1}, k = 7.8 x 10^6 M^{-1} s^{-1}, R^2 = 0.9959; Phth-Gly-βAla-OMe: intercept = 2.6 x 10^4 s^{-1}, k = 3.7 x 10^6 M^{-1} s^{-1}, R^2 = 0.9949; Phth-Gly-Gly-OMe: intercept = 1.2 x 10^4 s^{-1}, k = 1.5 x 10^6 M^{-1} s^{-1}, R^2 = 0.9993.

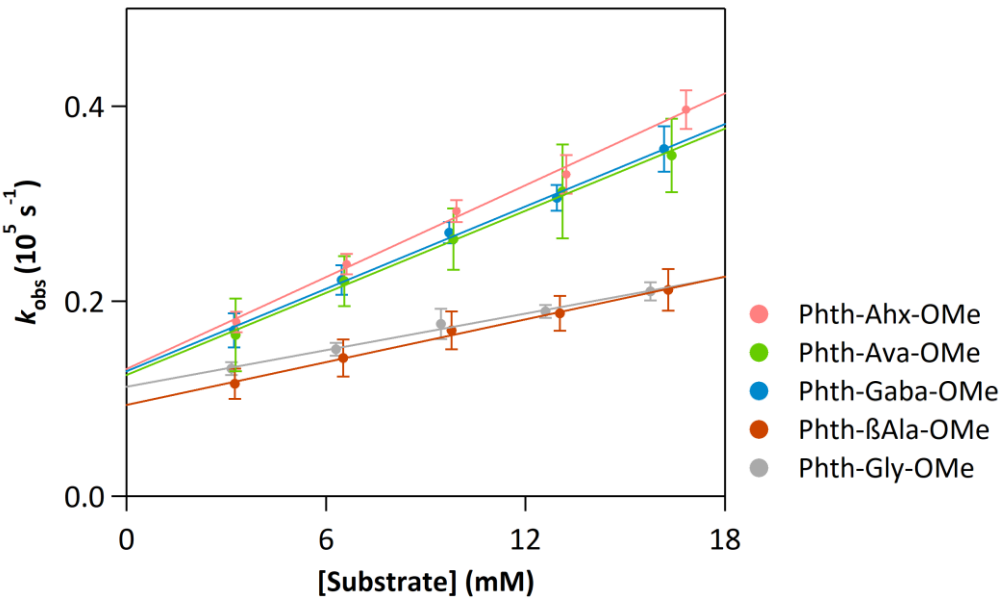


Figure S8. Plot of pseudo-first-order rate coefficient (k_{obs}) versus [amino acid] (see Table S1). Error bars shown are 2σ statistical uncertainties. From the linear regression analysis: Phth-Ahx-OMe: intercept = 1.3 x 10^4 s^{-1}, k = 1.6 x 10^6 M^{-1} s^{-1}, R^2 = 0.9952; Phth-Ava-OMe: intercept = 1.2 x 10^4 s^{-1}, k = 1.4 x 10^6 M^{-1} s^{-1}, R^2 = 0.9959; Phth-Gaba-OMe: intercept = 1.3 x 10^4 s^{-1}, k = 1.4 x 10^6 M^{-1} s^{-1}, R^2 = 0.9967; Phth-βAla-OMe: intercept = 0.9 x 10^4 s^{-1}, k = 0.7 x 10^6 M^{-1} s^{-1}, R^2 = 0.9943; Phth-Gly-OMe: intercept = 1.1 x 10^4 s^{-1}, k = 0.6 x 10^6 M^{-1} s^{-1}, R^2 = 0.9919.

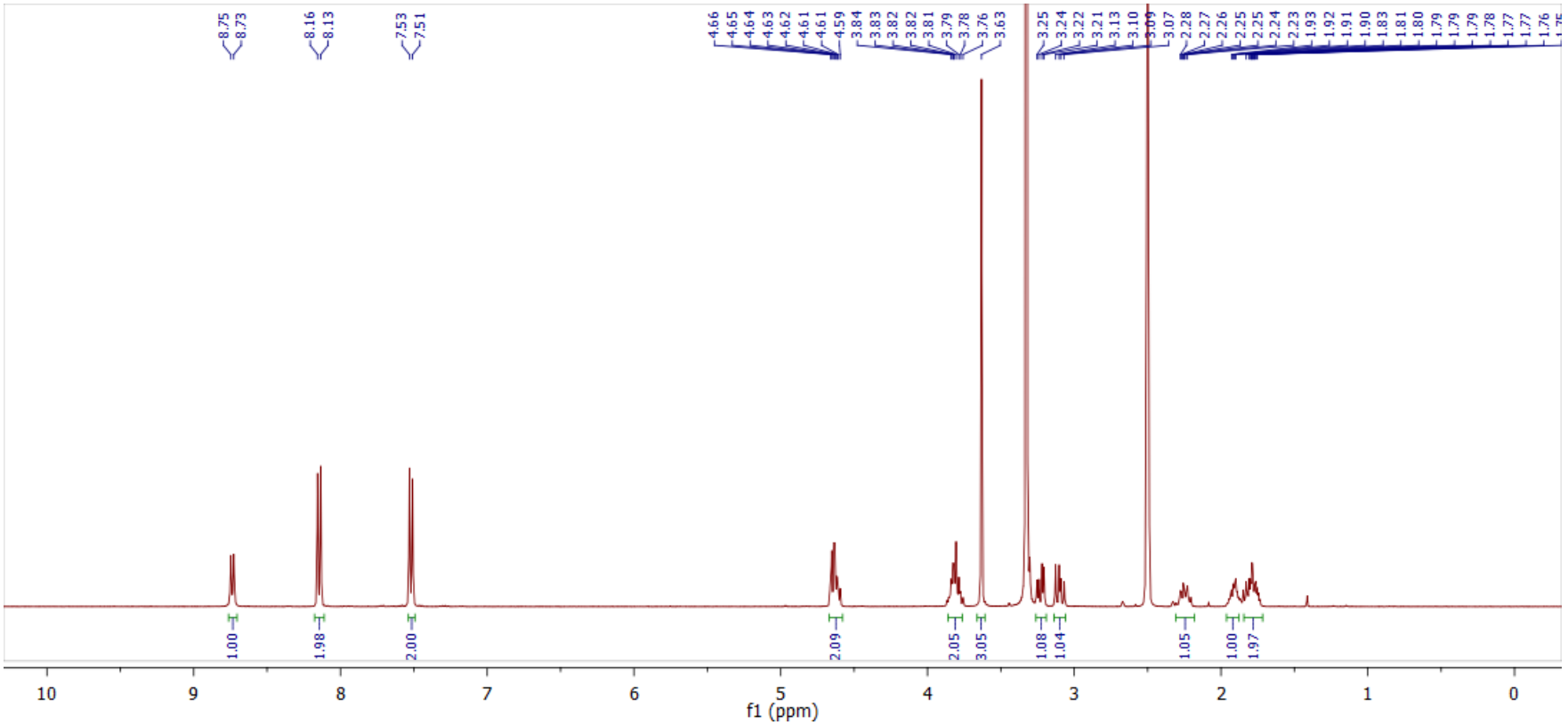
6. Product Studies – reactions with $NO_2^{\bullet}/O_3$ mixtures

Product studies were carried out using the method that was previously developed in our group.[5] The liquid $NO_2^{\bullet}$ (0.25 mL, condensed at −20 °C) was injected into a solution of the peptide (0.5 mmol) in acetonitrile (50 mL) at 10 °C, with a stream of ozonised oxygen passing through the solution. The reaction mixture was stirred for 20 minutes and saturated aqueous sodium bicarbonate solution (25 mL) was added. Acetonitrile was then removed under reduced pressure and elevated temperature. The aqueous layer was extracted with ethyl acetate (3 x 50 mL) and the combined organic layers were dried over magnesium sulfate and filtered. The solvent was removed under reduced pressure and elevated temperature to give a yellow oil. The crude product was purified by column chromatography. The product was analysed by ^{1}H NMR, ^{13}C NMR, HPLC and HRMS, when possible.

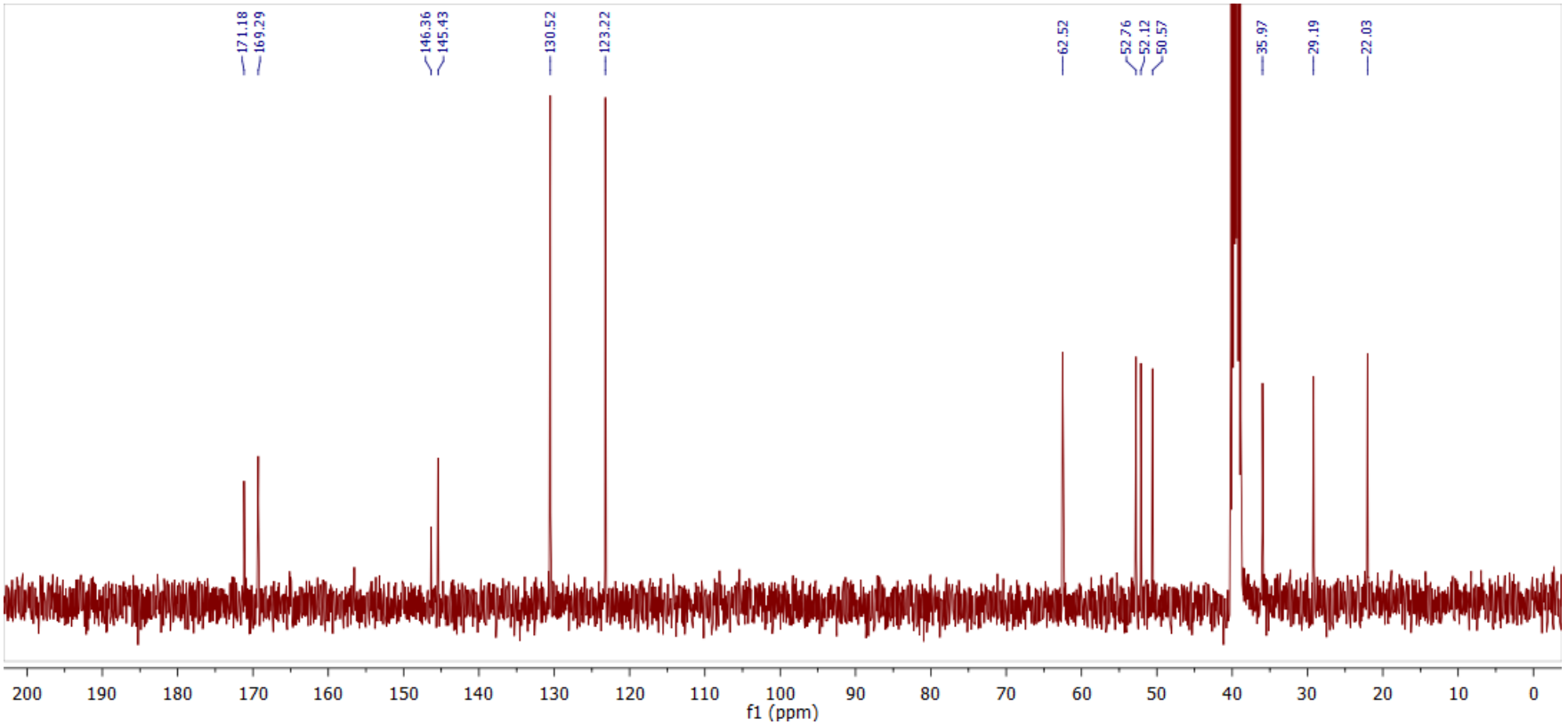
_N_-Acetyl-L-prolyl-L-phenylalanine methyl ester (Ac-Pro-Phe-OMe)

The reaction with $NO_2^{\bullet}/O_3$ mixtures led to formation of some products that were purified by column chromatography (2:3 Pet. ether/EtOAc, UV light). *N*-nitro-L-prolyl-L-*p*-nitrophenylalanine methyl ester (NO_2-Pro-Phe(NO_2)-OMe) was isolated as the major product. White needles; mp 172.1–172.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.74 (d, *J* = 8.0 Hz, 1H), 8.15 (d, *J* = 8.7 Hz, 2H), 7.52 (d, *J* = 8.6 Hz, 2H), 4.67–4.59 (m, 2H), 3.84–3.57 (m, 2H), 3.63 (s, 3H), 3.23 (dd, *J* = 13.9, 5.3 Hz, 1H), 3.10 (dd, *J* = 13.9, 9.6 Hz, 1H), 2.29–2.20 (m, 1H), 1.96–1.88 (m, 1H), 1.84–1.74 ppm (m, 2H). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6): δ 171.2, 169.3, 146.4, 145.4, 130.5, 123.2, 62.5, 52.8, 52.1, 50.6, 36.0, 29.2, 22.0 ppm. HRMS (ESI) *m*/*z* calcd. for $[C_{15}H_{19}N_4O_7]^+$: 367.1249 $[M + H]^+$, found 367.1249, HRMS (ESI) *m*/*z* calcd. for $[C_{15}H_{18}N_4O_7Na]^+$: 389.1068 $[M + Na]^+$, found 389.1064.

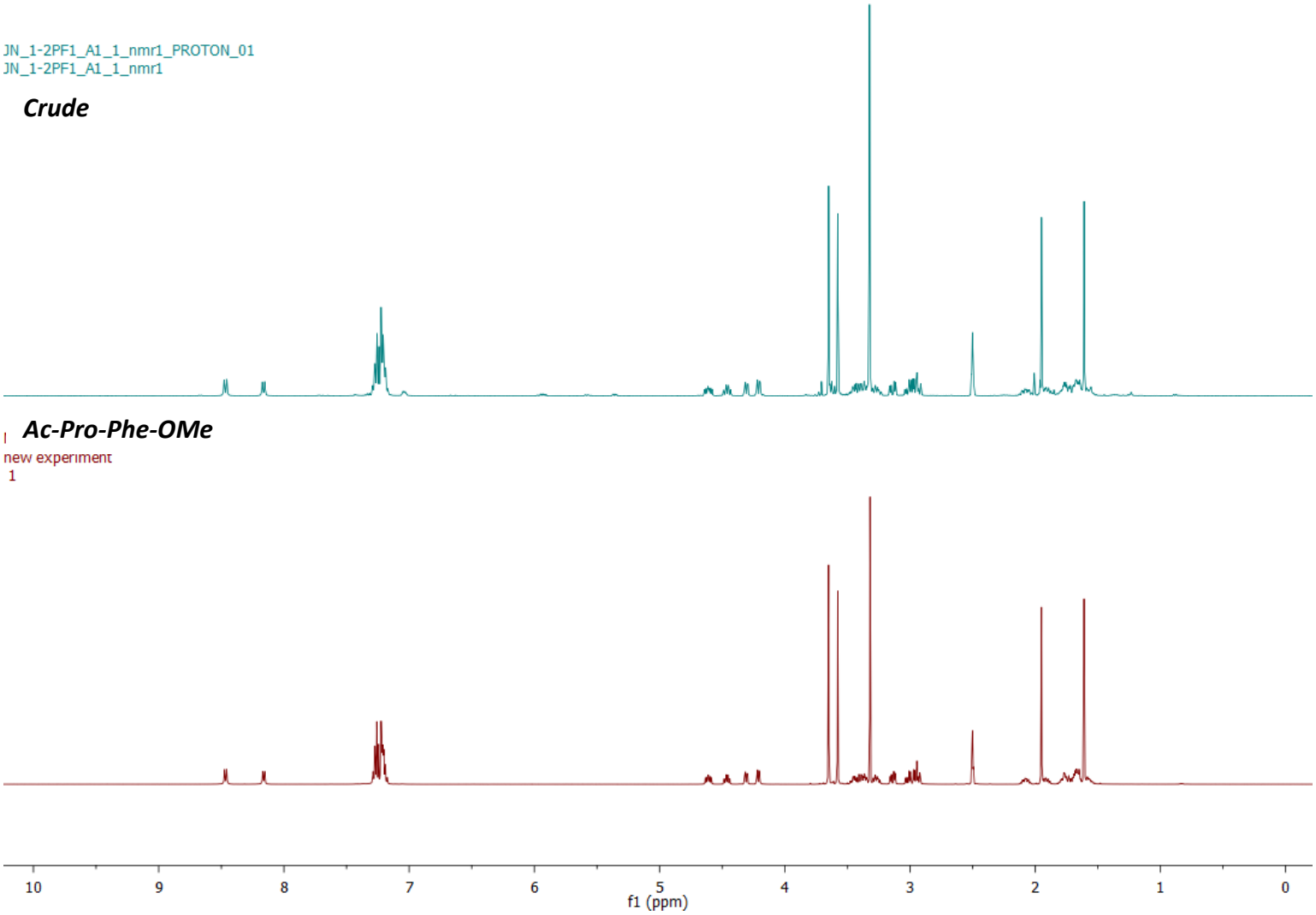
^{1}H NMR spectrum of *N*-nitro-L-prolyl-L-*p*-nitrophenylalanine methyl ester (NO_2-Pro-Phe(NO_2)-OMe)



^{13}C NMR spectrum of *N*-nitro-L-prolyl-L-*p*-nitrophenylalanine methyl ester (NO_2-Pro-Phe(NO_2)-OMe)

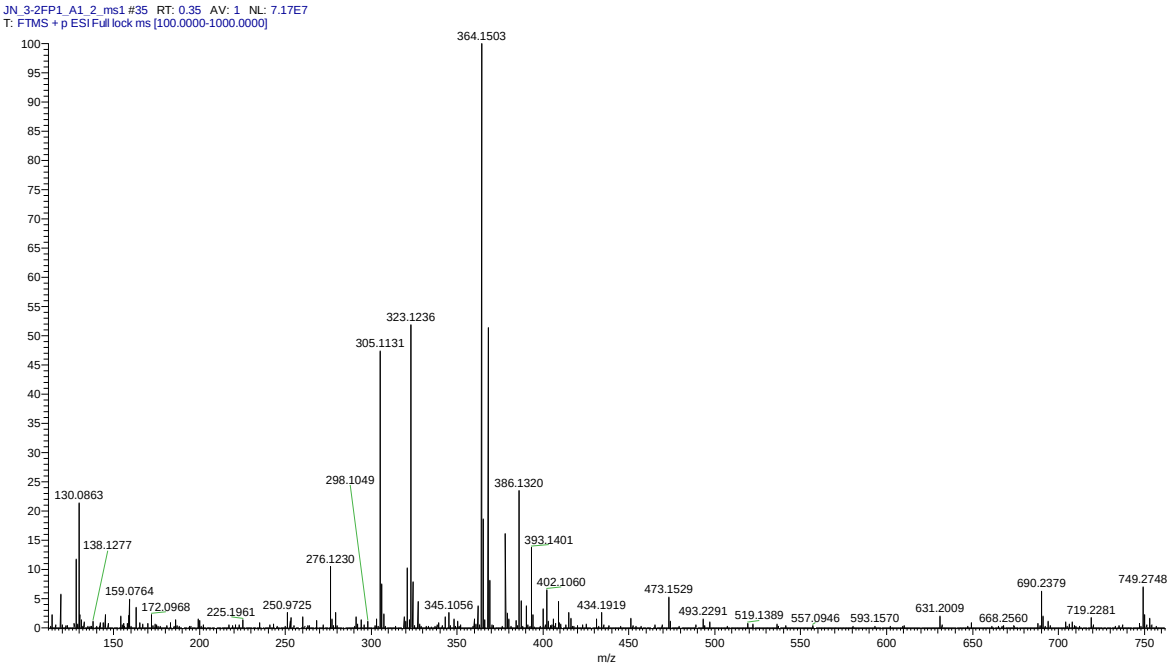


Control experiments were performed in the absence of O_3 (no stream of ozonised oxygen). No significant reaction took place as shown in the ^1H NMR spectrum of the crude reaction mixture below, compared to the ^1H NMR spectrum of the starting material (Ac-Pro-Phe-OMe).

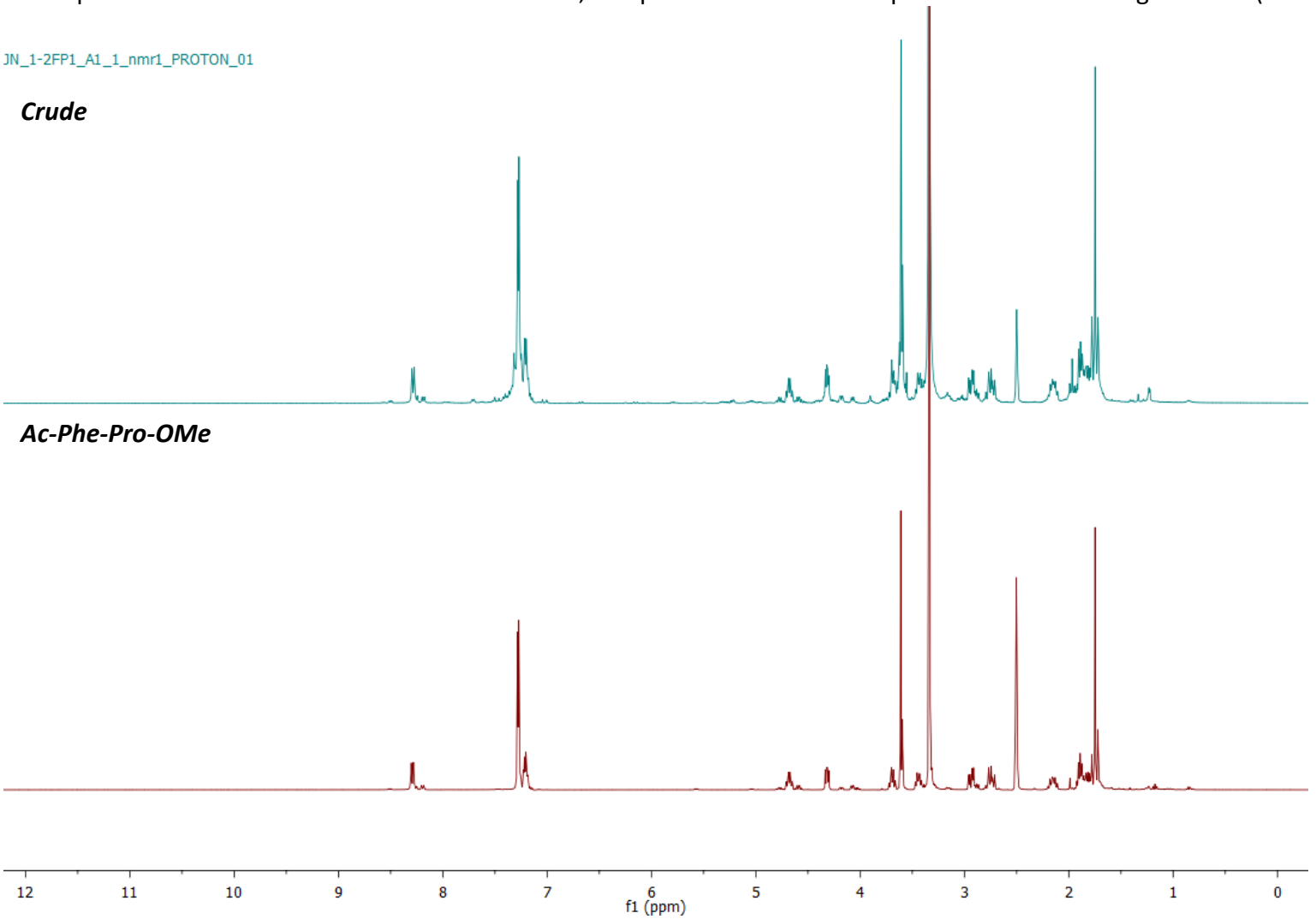


_N_-Acetyl-L-phenylalanyl-L-proline methyl ester (Ac-Phe-Pro-OMe)

The reaction with $NO_2^{\bullet}/O_3$ mixtures led to formation of several products. Purification was attempted by column chromatography but not successful. The major product was tentatively assigned by ESI-HRMS as *N*-acetyl-L-nitrophenylalanyl-L-proline methyl ester (Ac-Phe(NO_2)-Pro-OMe). HRMS (ESI) *m/z* calcd. for $[C_{17}H_{22}N_3O_6]^+$: 364.1504 $[M + H]^+$, found 364.1503, HRMS (ESI) *m/z* calcd. for $[C_{17}H_{21}N_3O_6Na]^+$: 386.1323 $[M + Na]^+$, found 386.1320. The mass spectrum of the crude reaction mixture is shown below.

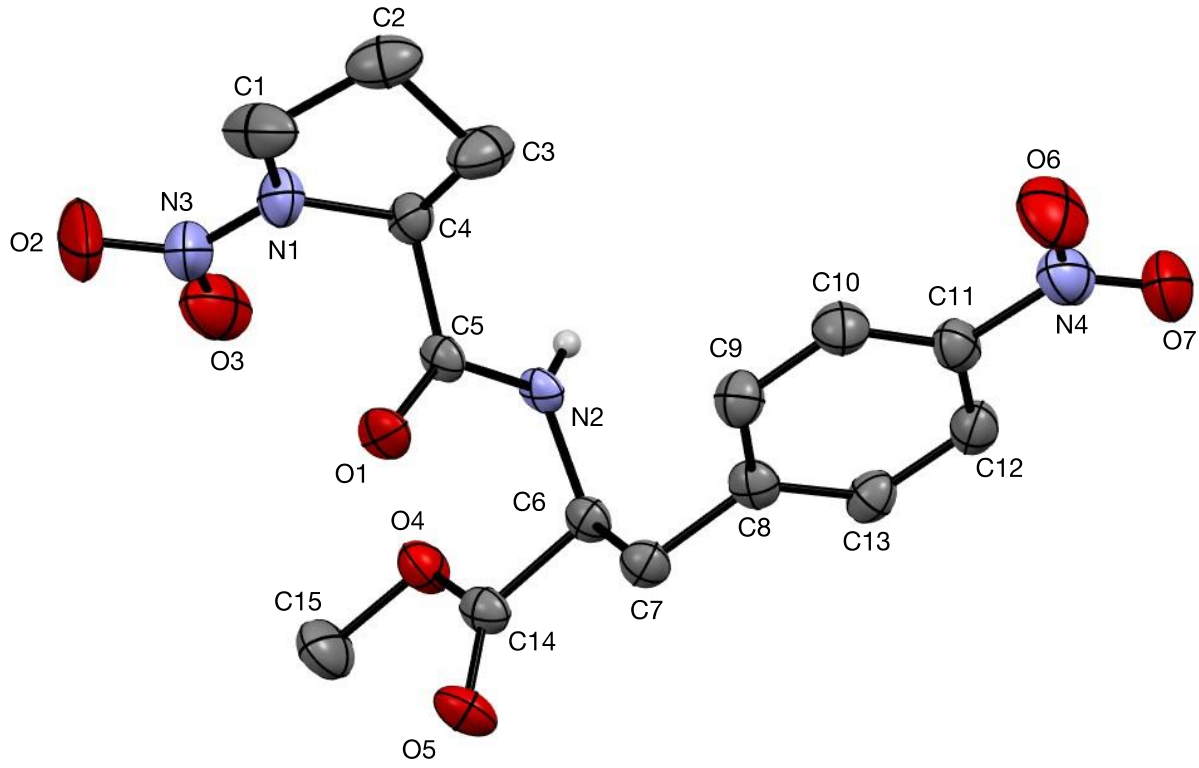


Control experiments were performed in the absence of O_3 (no stream of ozonised oxygen). No significant reaction took place as shown in the ^1H NMR spectrum of the crude reaction mixture below, compared to the ^1H NMR spectrum of the starting material (Ac-Phe-Pro-OMe).



7. Crystallographic Data

Intensity data for NO_2-Pro-Phe(NO_2)-OMe were collected with an Oxford Diffraction SuperNova CCD diffractometer using Cu-Kα microsource radiation (graphite crystal monochromator λ = 1.54814). The temperature during the data collection was maintained at 130.0(1). The structure was solved by direct methods and difference Fourier synthesis.[17] Thermal ellipsoid plots were generated using the program Mercury[18] integrated within the WINGX[19] suite of programs.



Crystal data for NO_2-Pro-Phe(NO_2)-OMe: $C_{15}H_{18}N_4O_7$ M = 366.33, T = 130.0(1) K, λ = 1.54184 Å, Monoclinic, space group $P2_1$ a = 4.7625(2), b = 13.0451(8), c = 13.8262(10) Å, β = 95.126(5)°, V = 855.55(9) Å^3, Z = 2, D_c = 1.422 Mg/m^3, μ(Cu-Kα) = 0.977 mm^{-1}, F(000) = 384. Crystal dimensions 0.508 x 0.062 x 0.027 mm^3. θ_{max} = 74.73°, 4959 reflections measured, 2723 independent reflections (R_{int} = 0.0400) the final R was 0.0447 [I > 2σ(I), 2335 data] and $wR(F^2)$ was 0.1183 (all data) GOOF = 1.070. CCDC deposit code 1991811.

Crystal data and structure refinement for NO_2-Pro-Phe(NO_2)-OMe

Identification code	shelx	
Empirical formula	C15 H18 N4 O7	
Formula weight	366.33	
Temperature	130.0(1) K	
Wavelength	1.54184 Å	
Crystal system	Monoclinic	
Space group	P 21	
Unit cell dimensions	a = 4.7625(2) Å	α= 90°.
	b = 13.0451(8) Å	β= 95.126(5)°.
	c = 13.8262(10) Å	γ = 90°.
Volume	855.55(9) $Å^3$	
Z	2	
Density (calculated)	1.422 Mg/m^3	
Absorption coefficient	0.977 mm^{-1}	
F(000)	384	
Crystal size	0.508 x 0.062 x 0.027 mm^3	
Theta range for data collection	4.669 to 74.735°.	
Index ranges	-5<=h<=5, -13<=k<=16, -16<=l<=16	
Reflections collected	4959	
Independent reflections	2723 [R(int) = 0.0400]	
Completeness to theta = 67.684°	99.6 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	1.00000 and 0.83605	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2723 / 2 / 239	
Goodness-of-fit on F^2	1.070	
Final R indices [I>2sigma(I)]	R1 = 0.0447, wR2 = 0.1079	
R indices (all data)	R1 = 0.0555, wR2 = 0.1183	
Absolute structure parameter	0.3(3)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.180 and -0.240 $e.Å^{-3}$	

8. Gaussian Archive Entries for Computational Data (Scheme 3)

The calculations were performed using the Gaussian software package (ref. [28] in the manuscript).

Compound 6

1\1\GINC-SPARTAN-RC049\FOpt\RM062X\6-31+G(d)\C8H13N2O5(1+)\UWILLE\06-N
ov-2018\0\\#p M062X/6-31+G* scf=(qc,direct) nosymm scrf=(cpcm,solvent=
acetonitrile) opt=(calcfc,maxcycle=500) freq=noraman\\geom&freq\\1,1\C
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,-1.0471058275\H,-2.4750949588,0.707263738,-1.7581546838\\Version=AM64
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57943,-0.9886162,-1.1945351\Quadrupole=4.8224429,1.3859331,-6.208376,5
.947319,4.8443295,-1.1415022\PG=C01 [X(C8H13N2O5)]\\@

Sum of electronic and thermal Free Energies= -797.495087 Hartrees

TS Compound 6 → Product Complex 7

1\1\GINC-SPARTAN-RC169\FTS\RM062X\6-31+G(d)\C8H13N2O5(1+)\UWILLE\12-No
v-2018\0\\#p M062X/6-31+G* scf=(qc,direct) nosymm scrf=(cpcm,solvent=a
cetonitrile) opt=(ts,noeigentest,calcfc,z-matrix,maxcycle=500) freq=no
raman\\geom&freq\\1,1\C,-0.1130745229,0.8641143309,1.4745202672\H,-0.3
994173031,1.3937003517,2.3836745809\C,0.1695153691,1.8844759738,0.3757
081941\C,-2.7513592825,1.2631485215,0.2233570227\O,-0.5540938784,2.054
7031792,-0.5868475928\O,1.2819927413,2.542057201,0.6084325503\C,1.6468
089433,3.5485262824,-0.3591582627\H,0.8648054891,4.3071075846,-0.40930
99472\H,2.5784436054,3.971344421,0.0074447959\H,1.7857167175,3.0813379
401,-1.3347158222\O,-2.9366232479,2.0313317309,1.0420749563\C,1.033082
6348,-0.1352790927,1.6505406532\H,0.8644601999,-0.7030226296,2.5706364
095\H,1.9999512737,0.3636320641,1.7237435612\C,-0.6217243241,-1.035336
5383,0.0941565821\H,-0.8356345391,-0.7518260675,-0.9361676304\H,-1.106
8371127,-1.9872238385,0.3013460895\C,0.8910311795,-1.0185307799,0.4043
245522\H,1.2702108745,-2.028093643,0.5663444559\H,1.4380558168,-0.5826
12809,-0.4364610785\N,-1.2327131298,0.0140626926,0.9877568354\O,-2.724
9785453,-1.4785312746,1.76617191\O,-1.9389257556,-0.0085863597,3.14713
91799\N,-2.0053913304,-0.5580687674,2.0752672298\C,-3.1424625178,0.602
7343519,-1.0189998338\H,-3.3306700268,-0.4555019437,-0.8196052928\H,-4
.0577013629,1.1022334301,-1.3499470717\H,-2.344000966,0.7298076881,-1.
7512322945\\Version=AM64L-G09RevB.01\HF=-797.6751948\RMSD=0.000e+00\RM

SF=1.451e-05\Dipole=0.056217,0.0681883,-2.2326872\Quadrupole=9.400345,
1.1861413,-10.5864863,-1.1214914,9.3896438,-0.2587184\PG=C01 [X(C8H13N
2O5)]\\@
ν_{imag}: -136.9617 cm^{-1}
Sum of electronic and thermal Free Energies= -797.492546 Hartrees

Product Complex 7

1\1\GINC-SPARTAN-RC194\FOpt\RM062X\6-31+G(d)\C8H13N2O5(1+)\UWILLE\16-N
ov-2018\0\\#p M062X/6-31+G* scf=(qc,direct) nosymm scrf=(cpcm,solvent=
acetonitrile) opt=(calcfc,z-matrix,maxcycle=500) freq=noraman\\geom&fr
eq\\1,1\C,0.6246911876,0.2061043358,1.8226653884\H,0.82537445,0.614312
7718,2.8151231308\C,0.3675947667,1.3512676114,0.8514437704\C,-2.859733
7802,2.0267690661,-0.4040951271\O,-0.4947746538,1.3302434589,-0.007575
7539\O,1.222511131,2.3376287283,1.0285489854\C,1.1140397731,3.45626988
74,0.1275699147\H,0.1366013048,3.9268278387,0.2434510985\H,1.908017678
3,4.1390236545,0.4200807701\H,1.2512416107,3.1159695073,-0.8997475231\
O,-3.0235533011,2.6087477934,0.5360377985\C,1.7401921855,-0.7280436198
,1.3158113841\H,2.1505915448,-1.2690625923,2.172742091\H,2.5493369248,
-0.1738709146,0.8364768718\C,-0.3544025362,-1.9196226235,1.0352082071\
H,-1.1819088858,-1.9992320303,0.3273981634\H,-0.3619961888,-2.79181139
3,1.6946177019\C,1.0050146675,-1.6845697937,0.3654135965\H,1.548780965
2,-2.6184787572,0.2155463341\H,0.8585973462,-1.2117052643,-0.610194092
6\N,-0.5100908481,-0.6990088985,1.8418171316\O,-2.6677642772,-1.011464
2166,2.0615404818\O,-1.7787725353,0.8858808509,2.6667522239\N,-1.70965
99736,-0.2549419474,2.202923645\C,-2.7540394564,1.2979828384,-1.636188
8097\H,-2.5048670311,0.2623819621,-1.3851627209\H,-3.7201598629,1.3695
646707,-2.1459640407\H,-1.949486206,1.7538980752,-2.221524621\\Version
=AM64L-G09RevB.01\HF=-797.6884194\RMSD=0.000e+00\RMSF=4.977e-06\Dipole
=-0.4455557,1.308041,-4.2637697\Quadrupole=5.3913407,6.7122279,-12.103
5687,-13.0002734,20.5435988,-8.8711612\PG=C01 [X(C8H13N2O5)]\\@
Sum of electronic and thermal Free Energies= -797.508899 Hartrees

9. References

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