Supporting Information

The Dicyclopropylmethyl (Dcpm) Peptide Backbone Protectant†

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Table of Contents

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General

HPLC data were obtained on an automated Waters 2695 Alliance system consisting of a Waters 996 Photodiode Array Detector, associated pumps and an automatic sample injection system. The system was controlled with the Impower Chromatography Manager software system. HPLC/MS data were recorded on an automated Thermo-Finnigan LCQ Advantage system consisting of a Surveyor Photodiode Array Detector, a Surveyor Autosampler and a Surveyor/MS pump. The system was controlled with the X-Calibur Chromatography Manager software system. Microanalyses were carried out by the University of Massachusetts Microanalysis Laboratory under the direction of Dr. Gregory Dabkowski and by Schwarzkopf Laboratories and Galbraith Laboratories. Fmoc protected amino acids and free amino acids were obtained from Novabiochem Inc. and used without purification. All reactions were carried out at room temperature unless otherwise stated. Column chromatography was performed using Silica Gel 60 obtained from Merck Co. (EM-9385, Mesh 230-400 ASTM). TLC was performed using aluminum-backed Merck Silica Gel 60 F254 plates using suitable solvent systems with spots being visualized by a Mineralight UV Lamp Model 51402. Melting points were obtained in open capillary tubes using a Mel-Temp melting point apparatus and are uncorrected. Distillations at reduced pressure were conducted with the use of an electrically heated mineral oil bath. ^{1}H NMR spectra were recorded at 200 MHz on a Bruker AC-200, and 400 MHz on a Bruker AVC-400 spectrometer at ambient temperature. Tetramethylsilane was used as an internal reference for all ^{1}H NMR spectra and all chemical shifts are reported as ppm relative to TMS. Automated peptide syntheses were carried out using Fmoc/piperidine chemistry on an Applied Biosystems 433A peptide synthesizer fitted with a Perkin-Elmer S200 UV monitor and controlled by Synthassist 2.0 software. Infrared spectra were recorded as films between NaCl plates or in the form of KBr pellets using a Perkin-Elmer 1600 FTIR spectrophotometer. Ultraviolet spectra were recorded on a Lambda 2 Perkin-Elmer spectrometer.

Experimental

Synthesis of Dicyclopropylmethanimine Hydrochloride

The method described in a Japanese patent[14] was improved as noted here. To a solution of $TiCl_4$ (55 mL, 94.5 g, 0.50 mol) in a mixture of 1 L of anhydrous benzene and 0.5 L of anhydrous DCM, dicyclopropyl ketone (50.6 mL, 49.5 g, 0.45 mol) was added dropwise while stirring at –10 °C. The solution became yellow and a yellow precipitate began to separate. After all of the ketone had been added, a stream of anhydrous ammonia gas was passed through the reaction mixture at such a rate that the temperature did not rise above 0 °C. The exothermic reaction was completed in about one hour, but ammonia was bubbled in at a low rate overnight (10 bubbles/minute). The reaction mixture was filtered and the filtrate was allowed to warm up to 10 °C. Anhydrous hydrogen chloride gas from a cylinder was bubbled into the filtrate until it was saturated (approx. 1 hour or until no additional white precipitate was formed). The white precipitate, which consisted of dicyclopropylmethanimine hydrochloride and ammonium chloride, began to separate after about 1 minute. Benzene was concentrated *in vacuo* and the crystalline residue was suspended in 500 mL of anhydrous chloroform. The white solid which remained

undissolved was separated by filtration and discarded. The filtrate was concentrated *in vacuo* and 100 mL of anhydrous ether was added. White crystals of dicyclopropylmethanimine hydrochloride were filtered and washed with ether. The crude dicyclopropylmethanimine hydrochloride was recrystallized from chloroform/ether to give 51 g (78%) of the imine salt as a white solid, mp 135-137 °C (lit.[14] mp 125-127 °C); ^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.34-1.67 (m, 8), 1.91-1.99 (m, 2), 11.5-12 (brs, 2); IR (KBr): 1662 cm^{-1} (C=N). The yield exceeded that of the patent description by 28%. In later preparations toluene was substituted for benzene and yields of 40-45% were obtained.

General Procedure for the Synthesis of Dicyclopropylmethylimino (Dcpi) Amino Acid Derivatives

Dicyclopropylmethanimine hydrochloride (6.54 g, 45 mmol) was added to a stirred solution of 30 mmol of amino acid ester in 50 mL of anhydrous DCM (an equivalent amount of triethylamine should be added if the amino acid ester salt is used). The reaction mixture was stirred overnight under nitrogen at room temperature. The solvent was removed *in vacuo* and 50 mL of diethylether was added. After filtration the white solid was washed with ether and the filtrate was concentrated *in vacuo*. The oily residue was distilled at 0.5-1 mm Hg to yield the dicyclopropylmethylimino acid ester as described below.

(a) Dcpi-Ala-OMe
Yield 74.2%, bp 86-87º C (0.5 mm Hg); ^{1}H NMR ($CDCl_3$): δ 0.55-0.95 (m, 8), 1.35 (d, 3), 1.76 (m,2), 3.69 (s, 3), 4.46 (q, 1); IR (NaCl): 1743, 1635 $cm.^{-1}$. Anal. Calcd for $C_{11}H_{17}NO_2$: C, 67.66; H, 8.78; N, 7.17. Found: C 67.57; H, 8.86 N, 7.13.

(b) Dcpi-Val-OMe
Vacuum distillation gave 5.2 g (79%) of the ester as a colorless oil, bp 95-110 °C/1 mm Hg; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 0.55-1.20 (m, 15), 1.76 (m, 1), 2.22 (m, 1), 3.68 (s, 3), 4.09 (d, 1): IR (NaCl): 1746 cm^{-1} (C=O), 1635 cm^{-1} (C=N). Anal. Calcd. for $C_{13}H_{21}NO_2$ (223.32): C, 69.92; H, 9.48; N, 6.27. Found: C, 69.70; H, 9.70, N, 6.38.

(c) Dcpi-Ala-OBn
Vacuum distillation gave 6.4 g (83%) of the ester as a colorless oil, bp 145-148 °C/1 Hg; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.57 (m, 2), 0.8 (m, 4), 0.96 (m, 2), 1.12 (m, 1), 1.39 (d, 3), 1.73 (m, 1), 4.53 (q, 1), 5.14 (s, 2), 7.34 (m, 5); ^{13}C-NMR (CDCl , 75 MHz): *δ* 6.16, 6.20, 7.29, 7.85, 10.67, 12.51, 13.04, 19.15, 57.16, 66.23, 126.69,127.84, 127.97, 128.09, 128.50, 136.25, 173.63, 174.51. IR (NaCl): 1740 cm^{-1} (C=O), 1632 cm^{-1} (C=N). HRMS: $[M + H]^+$ calcd for $C_{17}H_{22}NO_2$: 272.1651; obsd: 272.1606

(d) Dcpi-Ile-OBn
Vacuum distillation gave 7.5 g (79%) of the ester as a pale yellow oil, bp 150-155 °C/1 mm Hg; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.53-0.59 (m, 1), 0.75-0.91 (m, 12), 1.01-1.25 (m, 3), 1.45-1.63 (m, 1) 1.65-1.82 (m, 1), 1.95-2.1 (m, 1), 4.17 (d,

1) 5.15 (q, 2), 7.33 (m, 5); IR (NaCl): 1737 cm^{-1} (C=O), 1635 cm^{-1} (C=N). The crude product was used without further purification for the synthesis of the Dcpm amino acid ester.

(e) Dcpi-Leu-OBn

Vacuum distillation gave 7.9 g (84%) of the ester as a pale yellow oil, bp 158-163 °C/1 mm Hg; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.54-0.60 (m, 1), 0.75-1.2 (m, 12), 1.41-1.92 (m, 6), 4.47 (t, 1) 5.12 (d, 2), 7.33 (m, 5); IR (NaCl): 1734 cm^{-1} (C=O), 1632 cm^{-1} (C=N). The crude product was used without further purification for the synthesis of the Dcpm amino acid ester.

(f) Dcpi-Phe-OBn

Vacuum distillation gave 7.0 g (68%) of the ester as a colorless oil, bp 175-180 °C 1 mm Hg; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 0.51-0.70 (m, 6), 0.82-0.91 (m, 2), 1.07-1.15 (m, 1), 1.45-1.52 (m, 1) 3.15 (dq, 2), 4.66 (m, 1), 5.12 (s, 2) 7.17-7.33 (m, 10); IR (NaCl): 1739 cm^{-1} (C=O), 1631 cm^{-1} (C=N). The crude product was used without further purification for the synthesis of the Dcpm amino acid ester.

General Procedures for Synthesis of *N*-Dicyclopropylmethyl (Dcpm) Amino Acid Esters

1) Reduction via Sodium Triacetoxyborohydride

The dicyclopropylmethylimino acid ester (55.28 mmol) was dissolved in 225 mL of DCM and 3.21 ml of HOAc. Sodium triacetoxyborohydride (17.5 g, 82.92 mmol) was added portionwise over 5 min. The solution became turbid and remained turbid until the end of the reaction. The reaction mixture was stirred at RT for 2 h, washed with saturated $NaHCO_3$ solution (3 x 200 ml), water (3 x 50 ml) and saturated NaCl solution (3 x 50 ml). The DCM layer was dried over $MgSO_4$, and the solvent removed *in vacuo* to give an oil. In the specific case of Dcpm-Ala-OBn ^{1}H NMR analysis of the crude oil did not show contamination by any benzyl alcohol in the case of Dcpm-Ala-OB. Vacuum distillation of the crude oil gave the pure ester.

2) Reduction via Sodium Cyanoborohydride/1% HOAc/MeOH

The dicyclopropylmethylimino acid ester (33.16 mmol) was dissolved in 90 mL of methanol and 0.9 ml of HOAc and treated with $NaBH_3CN$ (2.7g , 43.2 mmol) added in one portion. The reaction mixture which became colorless was stirred at room temperature for 15 – 20 min, cooled in an ice bath, neutralized with saturated $NaHCO_3$ and the resulting solution extracted with EtOAc (3 x 120 mL). The extracts were dried over $MgSO_4$, and the solvent removed *in vacuo* to give an oil which was purified by vacuum distillation. In the specific case of Dcpm-Ala-OBn ^{1}H NMR analysis of the crude ester showed contamination by about 5% of benzyl alcohol whereas after distillation only about 0.5% benzyl alcohol remained.

3) Reduction via Sodium Cyanoborohydride/10% HOAc/MeOH

The dicyclopropylmethylimino acid ester (30 mmol) was dissolved in 60 mL of 10% AcOH/MeOH and $NaBH_3CN$ (40 mmol) was added in one portion. The solution became colorless and the reaction mixture was stirred at room temperature for 30-60

minutes. The reaction mixture was cooled in an ice bath, neutralized with 5N NaOH and the resulting solution extracted with ether (3 x 50 mL). The ether extracts were dried over $MgSO_4$, and the solvent removed *in vacuo* to give an oil which was purified by distillation. Most runs described below were carried out under these conditions but it was later found that this method in the case of Dcpi benzyl esters led to the formation of benzyl alcohol which remained to some degree in the material even after distillation. The presence of benzyl alcohol could be avoided by the use of less acetic acid and shorter reaction periods (method 2) or better by switching to the triacetoxyborohydride procedure (method 1).

(a) Dcpm-Ala-OBn
Vacuum distillation gave 6.4 g (78%) of the ester as a colorless oil, bp 145-155 °C/2 mm Hg; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.07 (m, 3), 0.13 (m, 1), 0.39 (m, 2), 0.49 (m, 2), 0.75 (m, 1), 0.88 (m, 1), 0.93 (q, 1), 1.31 (d, 3), 1.97 (s, 1), 3.82 (q, 1), 5.12 (q, 2) 7.34 (m, 5); ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 0.45, 2.26, 3.45, 4.13, 15.39, 17.46, 19.99, 53.98, 66.04, 66.37, 128.44, 128.48, 128.67, 135.99, 176.35. IR (NaCl): 1735 cm^{-1} (C= O); MS (E.S.I.) m/z $(M+H)^+$ calcd. 274.0; obsd. 274.0. Anal. Calcd. for $C_{17}H_{23}NO_2$ (273.37) C, 74.69; H, 8.48; N, 5.12. Found: C, 74.62; H, 8.67, N, 5.08.

(b)Dcpm-D-Ala-OBn
Vacuum distillation gave 5.5 g (68%) of the ester as a colorless oil, bp 144-150 °C/1mmm Hg; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.02-0.21 (m, 4), 0.35-0.54 (m, 4), 0.72-0.81 (m, 1), 0.82-0.98 (m, 2) 1.32 (d, 3), 3.86 (q, 1), 5.12 (q, 2), 7.36 (m, 5); IR (NaCl): 1735 cm^{-1} (C=O); MS (E.S.I.) m/z $(M+H)^+$ calcd. 274.0; obsd. 274.0. Anal. Calcd. for $C_{17}H_{23}NO_2$ (273.37) C, 74.69; H, 8.48; N, 5.12. Found: C, 74.46; H, 8.69; N, 4.99.

(c)Dcpm-Gly-OBn
Vacuum distillation gave 6.3 g (81%) of the ester as a colorless oil, bp 135-145 °C/1 mm Hg; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.1-0.25 (m, 4), 0.5 (m, 4), 0.81-0.89 (m, 2), 1.11 (t, 1), 3.65 (d, 2), 5.16 (q, 2), 7.36 (m, 5); IR (NaCl): 1738 cm^{-1} (C=O); MS (E.S.I.) m/z $(M+H)^+$ calcd. 260.0; obsd. 260.0. Anal. Calcd. For $C_{16}H_{21}NO_2$ (259.36) C, 74.09; H, 8.16; N, 5.40. Found: C, 74.09; H, 8.32; N, 5.35.

(d)Dcpm-Ile-OBn
Vacuum distillation gave 6.9 g (74%) of the ester as a yellow oil, bp 170-178 °C/1 mm Hg; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.05-0.15 (m, 4), 0.32-0.55 (m, 4), 0.75-0.91 (m, 9), 1.15-1.2 (m, 1), 1.5-1.75 (m, 2), 3.55 (d, 1), 5.10 (q, 2), 7.24 (s, 5); IR (NaCl): 1732 cm^{-1} (C=O); MS (E.S.I.) m/z $(M+H)^+$ calcd. 316.0; obsd. 316.1. Anal. Calcd. for $C_{20}H_{29}NO_2$ (315.45): C, 76.15; H, 9.27; N, 4.44. Found: C, 76.04; H, 9.53; N, 4.73.

(e)Dcpm-Val-OBn
Vacuum distillation gave 7.3 g (81%) of the ester as a yellow oil, bp 175-185 °C/1 mm Hg; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 0.02-0.21 (m, 4), 0.35-0.61 (m, 4), 0.70-1.10 (m, 9), 1.92 (m, 1), 3.46 (d, 1), 5.11 (q, 2), 7.34 (s, 5); IR (NaCl): 1734 cm^{-1}

(C=O); MS (E.S.I.) m/z $(M+Na)^+$ calcd. 324.0; obsd. 324.0. Anal. Calcd. for $C_{19}H_{27}NO_2$ (301.42): C, 75.71; H, 9.03; N, 4.65. Found: C, 75.94; H, 9.09; N, 4.63.

(f) Dcpm-D-Val-OBn

Vacuum distillation gave 6.5 g (73%) of the ester as a yellow oil, bp 165-180 °C/1 mm Hg; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 0.02-0.21 (m, 4), 0.35-0.61 (m, 4), 0.70-1.10 (m, 9), 1.92 (m, 1), 3.46 (d, 1) 5.11 (q, 2), 7.34 (s, 5); IR (NaCl); 1732 cm^{-1} (C=O); MS (E.S.I.) m/z $(M+H)^+$ calcd. 302.0; obsd. 302.0. Anal. Calcd. for $C_{19}H_{27}NO_2$ (301.42): C, 75.70; H, 9.02; N, 4.65. Found: C, 75.95; H, 9.13; N, 4.37

(g) Dcpm-Ala-OMe: Yield 33.2%, bp 65-68° C (1 mm Hg); ^{1}H NMR ($CDCl_3$): 0.16-0.97 (m, 11), 1.30 (d, 3), 1.96 (s, 1), 3.68 (s, 3), 3.60-3.96 (q, 1): IR (NaCl) 1738 $cm.^{-1}$ (C=O). Anal. Calcd for $C_{11}H_{19}NO_2$ (197.27): C, 66.97; H, 9.70; N, 7.10. Found: C, 66.90; H, 9.79; N, 7.24.

(h) Dcpm-Val-OMe

Vacuum distillation gave 5.6 g (84%) of the ester as a colorless oil, bp 85-95 °C/1 mm Hg; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 0.05-0.21 (m, 4), 0.35-0.61 (m, 4), 0.75-1.10 (m, 9), 1.8-1.95 (m, 1), 3.42 (d, 1), 3.67 (s, 3); IR (NaCl); 1733 cm^{-1} (C=O); MS (E.S.I.) m/z $(M+H)^+$ calcd. 226.0; obsd. 226.0. Anal. Calcd. for $C_{13}H_{23}NO_2$ (225.33): C, 69.29; H, 10.28; N, 6.22. Found: C, 69.06; H, 10.38; N, 6.23.

(i) Dcpm-D-Val-OMe

Vacuum distillation gave 5.2 g (78%) of the ester as a colorless oil, bp 85-95 °C/1 mm Hg; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 0.05-0.21 (m, 4), 0.35-0.61 (m, 4), 0.75-1.10 (m, 9), 1.8-1.95 (m, 1), 3.42 (d, 1), 3.67 (s, 3); IR (NaCl); 1733 cm^{-1} (C=O); MS (E.S.I.) m/z $(M+H)^+$ calcd. 226.0; obsd. 226.1. Anal. Calcd. for $C_{13}H_{23}NO_2$ (225.33) C, 69.29; H, 10.28; N, 6.22. Found: C, 69.16; H, 10.38; N, 6.13.

General Procedure for Synthesis of *N*-Dicyclopropylmethyl (Dcpm) Amino Acids from the Corresponding Benzyl Esters

The *N*-dicyclopropylmethyl amino acid benzyl ester (10 mmol) was dissolved in 100 mL of anhydrous MeOH and the reaction mixture was cooled to 5 °C and 0.25 g of Pd/C (5% by weight) was carefully added. The mixture was hydrogenated for 3 hours in a Parr hydrogenator, filtered and the solvent was removed *in vacuo* to yield a white solid that was recrystallized from methanol/ether. The optimum time for hydrogenolysis depended on the exact system under study and the nature of the Pd/C catalyst used.

(a) Dcpm-Ala-OH

Recrystallization gave 1.6 g (92%) of the acid as a white solid, mp 258-260 °C; ^{1}H-NMR (400 MHz, CD_3OD): δ 0.45 (m, 3), 0.56 (sextet, 1), 0.70 (m, 4), 0.85 (m, 2), 1.52 (d, 2), 2.01 (t, 1), 3.98 (q, 1); ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 2.52, 3.91, 4.74, 5.29, 12.66, 14.58, 17.11, 56.45, 68.30, 174.63 ; IR (KBr): 3600-2200 cm^{-1} (OH), 1600, 1568 cm^{-1} (C = O); MS (E.S.I.) m/z $(M+H)^+$ calcd. 184.0;

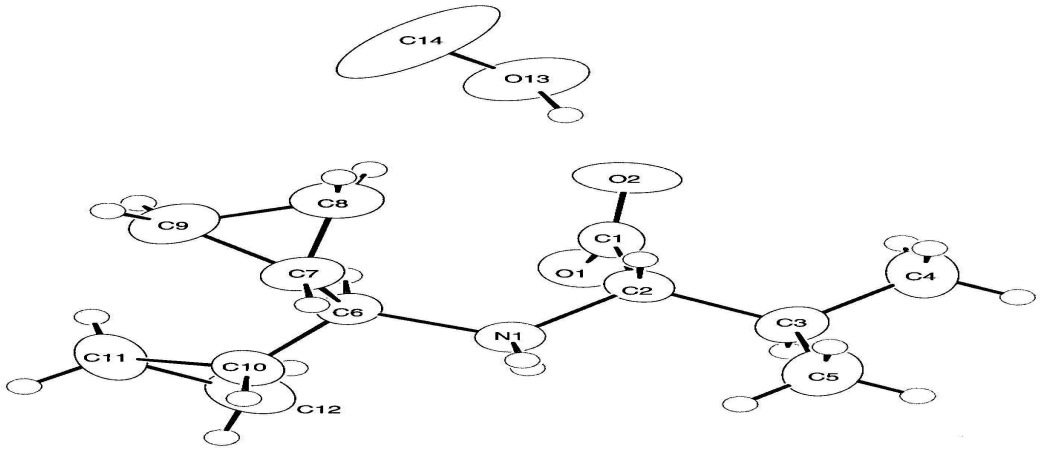
obsd. 184.0; $(M+Na)^+$ calcd. 206.0; obsd. 206.2. Anal. Calcd. for $C_{10}H_{17}NO_2$ (183.25): C, 65.54; H, 9.35; N, 7.64. Found: C, 65.21; H, 9.77; N, 7.41.

(b) Dcpm-Gly-OH

Recrystallization gave 1.4 g (82%) of the acid as a white solid, mp 228-230 °C; ^{1}H-NMR (400 MHz, CD_3OD): δ 0.41-0.49 (m, 2), 0.50-0.59 (m, 2), 0.67-0.76 (m, 4), 1.04-1.13 (m, 2), 2.07 (t, 1), 3.66 (s, 2); IR (KBr): 3600-2200 cm^{-1} (OH), 1623, 1584 cm^{-1} (C = O); MS (E.S.I.) m/z $(M+H)^+$ calcd. 170.0; obsd. 170.0; $(M+Na)^+$ calcd. 192.0; obsd.192.2. Anal. Calcd. for $C_9H_{15}NO_2$ (169.22): C, 63.88; H, 8.93; N, 8.28. Found: C, 64.05; H, 8.94; N, 8.22.

(c) Dcpm-Val-OH

Recrystallization gave 1.9 g (90%) of the acid as a white solid, mp 120-122 °C. Elemental analysis, X-ray crystallography (see crystal structure diagram below) and ^{1}H NMR analysis all showed that the crystalline material separated as a 1:1 adduct with methanol. ^{1}H-NMR (200 MHz, CD_3OD): δ 0.31-0.80 (m, 8), 1.05-1.21 (m, 8), 1.84 (t, 1) 2.21-2.35 (m, 1), 3.36 (d, 1); IR (NaCl): 3700-2200 cm^{-1} (OH), 1600 cm^{-1}(C = O), MS (E.S.I.) m/z $(M+H)^+$ calcd. 212.0; obsd. 212.0; $(M+Na)^+$ calcd. 234.0; obsd. 234.06. Anal. Calcd. for $C_{12}H_{21}NO_2 + CH_3OH$ (243.34): C, 64.16; H, 10.36; N, 5.76. Found: C, 64.32; H, 10.65; N, 5.84.



Crystal structure of the methanol solvate of Dcpm-Val-OH

General Procedure for the Hydrolysis of N-Dicyclopropylmethyl Amino Acid Methyl Esters. Isolation as Hydrochloride Salts of N-Dicyclopropylmethyl Amino Acids

A solution of 1.23 mmol of a Dcpm amino acid methyl ester in 13.5 mL of 1 N NaOH in methanol is stirred for 24-48 h at room temperature. The reaction mixture is acidified with excess dry HCl in MeOH and the mixture concentrated in vacuo to dryness. The residue is extracted with dry ethanol (50 mL). After removing the ethanol *in vacuo* the resulting solid is redissolved in dry ethanol (50 mL) and filtered to remove traces of NaCl. Solvent is removed *in vacuo* and the resulting residue is recrystallized from an appropriate solvent to give the hydrochloride salt. The following compounds were made in this way.

(a) Dcpm-Gly-OH·HCl·H_2O

Recrystallized from EtOH/Me_2CO/ether as white crystals, mp 110-112º C; ^{1}H-NMR (DMSO-d_6) δ 0.47-0.62 (m, 8), 1.17 (m, 2), 1.90-2.19 (m, 1), 3.91 (s, 2); IR (KBr): 1736, 1650 cm.$^{-1}$. Anal. Calcd for $C_9H_{18}ClNO_3$ (223.69): C, 48.32; H, 8.10; N, 6.26. Found: C, 48.46; H, 8.05; N, 6.26.

(b) Dcpm-Ala-OH·HCl

Recrystallized from EtOAc/ether, mp 150-152º C; ^{1}H NMR (acetone-d_6) δ 0.56-0.74 (m, 8), 1.78 (d, 3), 3.66 (br s, 3) 4.58 (q, 1): IR (KBr): 1738 cm.$^{-1}$ Anal. Calcd for $C_{10}H_{18}ClNO_2$ (219.70): C, 54.66; H, 8.25; N, 6.37; Cl 16.13. Found: C, 54.82; H, 8.33; N, 6.44; Cl 16.02.

General Procedure for Synthesis of Fmoc (Dcpm)- and Bsmoc (Dcpm)-Amino Acids

To a stirred solution of a Dcpm amino acid (10 mmol) in 20 mL of dry DCM kept under a nitrogen atmosphere, chlorotrimethylsilane (3.04 mL, 24 mmol) was added. The reaction mixture was stirred under reflux for 3 h and then cooled to 0 °C during the addition first of DIEA (4.17 mL, 24 mmol) and then of Fmoc-Cl or Bsmoc-Cl (3.096 g, 12 mmol). The reaction mixture was stirred at room temperature overnight. DCM was removed with a rotary evaporator and the residue triturated with 10% sodium carbonate and 100 mL of diethyl ether. The ether layer was extracted by means of 10% sodium carbonate (3 x 10 mL). The aqueous layer was collected, acidified by 5% citric acid (Congo Red) and extracted with ethyl acetate (3 x 50 mL). The extract was dried over magnesium sulfate and the solvent removed *in vacuo* to give the crude protected acid as a white solid which was purified by recrystallization.

(a) Fmoc-(Dcpm)Gly-OH

Recrystallization from methanol/diethylether gave 3.0 g (79%) of the acid as a white solid, mp 115-116 °C; ^{1}H-NMR (400 MHz, $CDCl_3$)*: δ -0.3 (m, 1), -0.05 (m, 2), 0.2 (m, 1), 0.31-0.90 (m, 6), 2.25 and 3.25 (2t, 1), 4.0 (d, 2), 4.35 (d, 1), 4.55 (d, 1), 4.60 (d, 1), 7.31-7.43 (m, 4), 7.52-7.57 (m, 2), 7.75 (d, 2); IR (KBr): 1731, 1698, 1669 cm^{-1} (C=O); MS (E.S.I.) m/z $(M+H)^+$ calcd.392.0; obsd. 392.2; $(M+Na)^+$ calcd. 414.0 obsd. 414.0. Anal. Calcd. for $C_{24}H_{25}NO_4$(391.46): C, 73.64; H, 6.44; N, 3.58. Found: C, 73.43; H, 6.34; N, 3.54.

(b) Fmoc-(Dcpm)Ala-OH

Recrystallization from methanol/diethylether gave 3.2 g (81%) of the acid as a white solid, mp 148-150 °C; ^{1}H-NMR (400 MHz, $CDCl_3$)*: δ -0.35 (m, 1), -0.1 (m, 1), 0.17 (m, 2), 0.3 (m, 1), 0.41 (m, 1), 0.49 (m, 1), 0.63 (m, 10), 0.73 (m, 1), 1.31 and 1.51 (2d, 3), 2.05 and 3.06 (2 t, 1), 3.9 and 4.0 (2q, 1), 4.55 (m, 2), 7.29 (t, 2), 7.37 (t, 2), 7.58 (t, 1), 7.71 (d, 2); ^{13}C-NMR (CDCl3, 75 MHz): δ 4.82, 5.51, 5.70, 13.27, 13.42, 13.60, 13.65, 15.77, 16.75, 47.26, 47.56, 51.67, 52.84,

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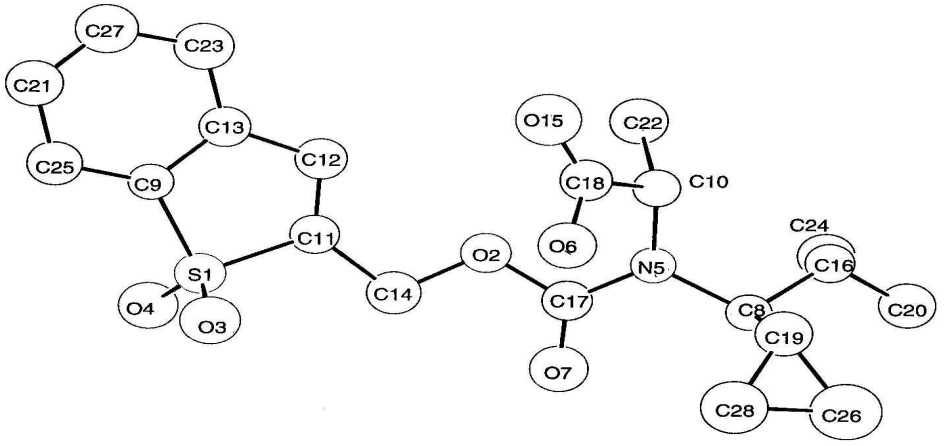
* The effects of restricted rotation were clearly visible in the proton NMR spectra.

53.52, 65.13, 66.38, 66.51, 67.07, 119.83, 124.49, 124.91, 124.98,127.03, 127.10, 12719, 127.55, 127.67, 141.38, 143.96, 144.00, 155.14, 156.03, 176.90, 177.75; IR (KBr): 1745, 1714, 1694 cm^{-1} (C=O); MS (E.S.I.) m/z $(M+H)^+$ calcd. 406.0; obsd. 406.4; $(M+Na)^+$ calcd. 428.0; obsd., 428.1; $(2M+Na)^+$ calcd. 833.0; obsd. 833.1. Anal. Calcd. for $C_{25}H_{27}NO_4$ (405.49): C, 74.05; H, 6.71; N, 3.45. Found: C, 74.10; H, 6.65; N, 3.47.

(d) Bsmoc-(Dcpm)Ala-OH

Recrystallization from methanol/diethylether gave 3.5 g (86%) of the acid as a white solid, mp 140-142 °C; ^1H NMR (400 MHz, $CDCl_3$)*: δ 0.37-1.25 (m, 10), 1.66 (d, 3), 2.95 and 3.10 (2 t, 1), 4.15 (m, 1), 5.08 (s, 2), 7.1 (m, 1), 7.37 (m, 1), 7.52 (m, 2), 7.68 (m, 1); IR (KBr): 3500-2500 (OH), 1740, 1698 cm^{-1} (C = O); MS (E.S.I.) m/z $(M+H)^+$ calcd., 406.0; obsd. 406.3; $(M+Na)^+$ calcd. 428.0; obsd. 428.2; $(2M+Na)^+$ calcd. 833.0; obsd. 832.9. Anal. Calcd. for $C_{20}H_{23}NO_6S$ (405.47): C, 59.24; H, 5.72; N, 3.45. Found: C, 59.04; H, 5.61; N, 3.37.



Crystal structure of Bsmoc-(Dcpm)Ala-OH as determined by x-ray crystallography

(e) Bsmoc-D-(Dcpm)Ala-OH

Recrystallization from methanol/diethylether gave 2.93 g (72%) of the acid as a white solid, mp 132-133 °C; ^1H NMR (400 MHz, $CDCl_3$)*: δ 0.37-1.3 (m, 10), δ 0.68-0.71 (m, 2), 0.88-1.03 (m, 2), 1.66 (t, 3), 3.0 and 3.15 (2 t, 1), 4.20 (m, 1), 5.09 (s, 2), 7.14 (d, 1), 7.37 (d, 1), 7.53 (m, 2), 7.68 (d, 1); IR (KBr): 1740 cm^{-1}, 1700 cm^{-1} (C=O); MS (E.S.I.) m/z $(M+Na)^+$ calcd. 428.0; obsd. 428.0. Anal. Calcd. for $C_{20}H_{23}NO_6S$ (405.47): C, 59.24; H, 5.72; N, 3.45.Found: C, 59.04; H, 5.61; N, 3.37.

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* The effects of restricted rotation were clearly visible in the proton NMR spectra.

Automated Solid Phase Synthesis of H-$(Ala)_{10}$–Arg-OH on a MultiSynTech Syro II Synthesizer using Fmoc/Bsmoc Amino Acids

Assembly of peptides was performed using standard solid phase synthesis procedures using a MultiSynTech Syro II synthesizer (MultiSynTech GmbH, Witten, Germany). Fmoc-Arg(Pbf)-TCP-resin (loading: 0.42 mmol/g; PepChem, Tübingen, Germany) was used for all syntheses. Stepwise synthesis of peptides was performed using 100 mg of resin for each peptide and couplings were accomplished with 4 eq. of Fmoc- or Bsmoc-amino acid N-TBTU (4 eq.)/ DIEA (8 eq) in DMF (coupling concentration: 0.25M, double coupling: 2x20min). Fmoc/Bsmoc-deprotection was achieved by means of 25% piperidine/DMF (2.5 ml; 2x7 min). Peptide-resin cleavage was performed with a solution consisting of 2% triisopropylsilane, 5% water, and 5% phenol in TFA for 2 h.
After precipitation in tert-butyl methyl ether the resulting peptides were analyzed on a LC-MS instrument (MSD Agilent) using a Vydac Protein & Peptide C18 (Cat# 2181P5215) column with a linear gradient 1-70% B in 45 min (A: water, 0.1% TFA; B: ACN, 0.1% TFA) See Table 1 and Fig. 1.

Table 1: MS Values found for major peaks detected via LC-MS.

Sequence	Mcalcd Fmoc/Bsmoc	Mfound $[M+H]^+$/ Fmoc	Mfound $[M+H]^+$/ Bsmoc
AAAAAAR	600.68	601.2	601.2
AAAAAAAR	671.76	672.2	672.2
AAAAAAAAR	742.84	743.2	743.3
AAAAAAAAAR	813.92	814.3	814.3
AAAAAAAAAAR	885.00	885.3	885.3
Fmoc or Bsmoc-AAAAAAR	822.91 or 822.89	823.2	Not Detectable
Fmoc or Bsmoc-AAAAAAAR	893.99 or 893.97	894.2	Not Detectable
Fmoc or Bsmoc-AAAAAAAAR	965.07 or 965.05	965.3	Not Detectable
Fmoc or Bsmoc-AAAAAAAAAR	1036.15 or 1036.13	1036.3	Not Detectable
Fmoc or Bsmoc-AAAAAAAAAAR	1107.23 or 1107.21	1107.3	Not Detectable

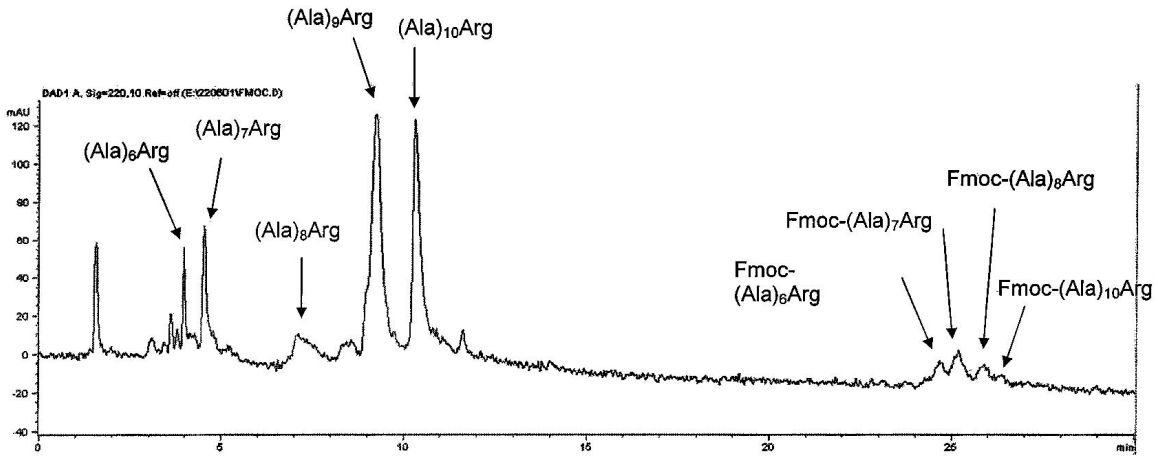


Fig. 1 HPLC trace for the automated assembly of H-$(Ala)_{10}$-Arg-OH using a MultiSynTech Syro II synthesizer via standard Fmoc chemistry. The identities given were established by ESI-MS analysis.

Automated Solid Phase Synthesis of H-$(Ala)_{10}$-Arg-OH using Fmoc/Bsmoc-Amino Acids and Fmoc/Bsmoc-(Dcpm)-Ala-OH at Position 5 from the C-Terminal Arginine

Assembly of peptides was performed using standard solid phase synthesis procedures using a MultiSynTech Syro II synthesizer (MultiSynTech GmbH, Witten, Germany). Fmoc-Arg (Pbf)-TCP resin (loading: 0.42 mmol/g: PepChem, Tübingen, Germany) was used for all syntheses. Stepwise synthesis of peptides was performed using 100 mg of resin for each peptide and couplings were accomplished with 4 eq. of Fmoc- or Bsmoc-amino acid/N-TBTU (4 eq.)/DIEA (8 eq) in DMF (coupling concentration: 0.25M, double coupling: 2x20min). Fmoc/Bsmoc-(Dcpm)-Ala (4eq) was incorporated via activation with N-HATU (4 eq)/ DIEA 8 eq (coupling concentration: 0.25M, double coupling (2x1h). Due to the expected high steric hindrance of the Dcpm-Ala the following Ala was also coupled by means of N-HATU as activation reagent using the same conditions as for the Dcpm-analogs. Fmoc/Bsmoc-deprotection was achieved by means of 25% piperidine/ DMF (2.5ml; 2x7min.) Peptide-resin cleavage was performed with a solution consisting of 2% triisopropysilane, 5% water, and 5% phenol in TFA for 2h. After precipitation in tert-butyl methyl ether the resulting peptides were analyzed on a LC-MS instrument (MSD, Agilent) using a Vydac Protein & Peptide C18 (Cat#2181P5215) column with a linear gradient 1-70% B in 45 min (A: water, 0.1% TFA; B: ACN, 0.1% TFA). See Table 2 and Figures. 1-3.

Table 2: MS values found for major peaks detected via LC-MS.

Sequence	Mcalcd	Mfound $[M+H]^+$/Fmoc	Mfound $[M+H]^+$/Bsmoc
(Dcpm)AAAAAR	623.75	624.5	624.5
AAAAAAAAR	742.84	Not detectable	743.5
AAAAAAAAAAR	885.00	Not detectable	885.6

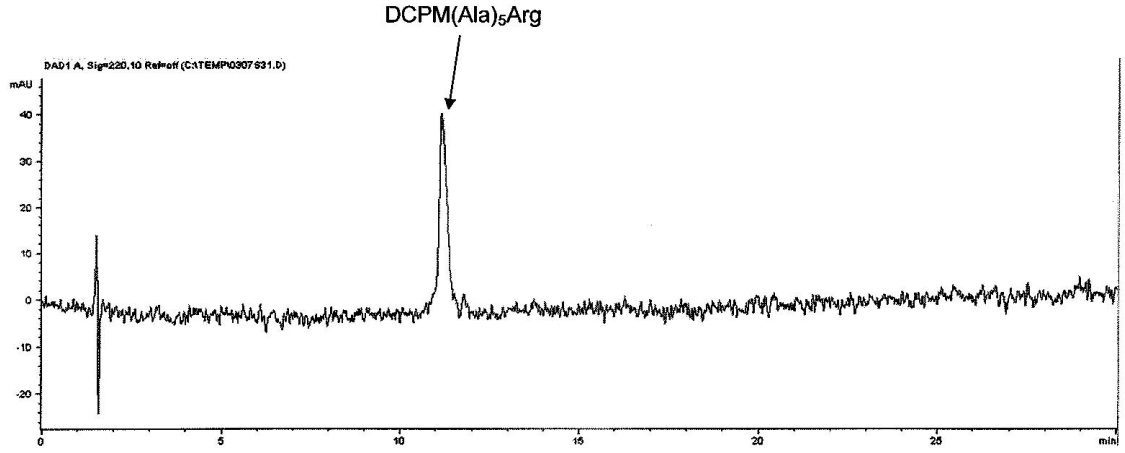


Fig. 2 HPLC trace for the automated assembly of H-$(Ala)_{10}$-Arg-OH using a MultiSynTech Syro II synthesizer via standard Fmoc chemistry with introduction of the 5th Ala unit from the C-terminal arginine via Fmoc-(Dcpm)Ala-OH. The identities given were established by ESI-MS analysis.

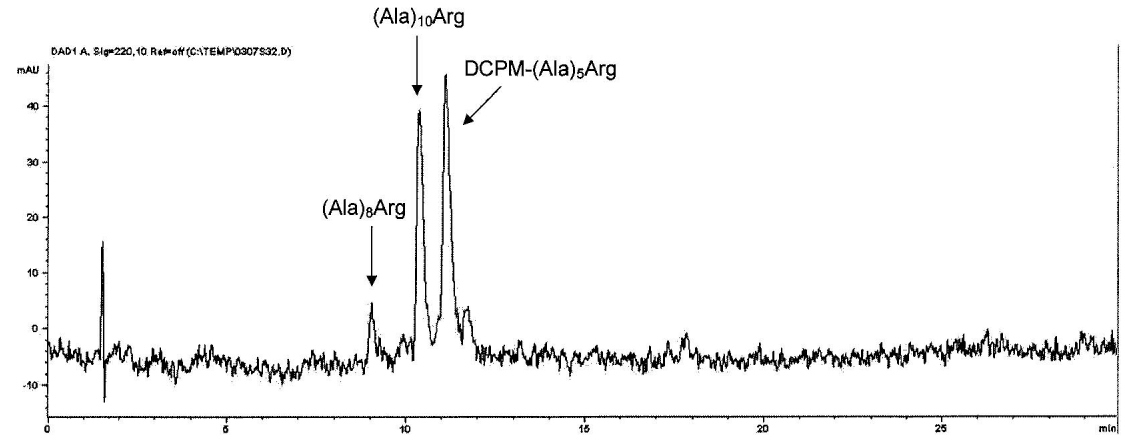


Fig. 3 HPLC trace for the automated assembly of H-$(Ala)_{10}$-Arg-OH using a MultiSynTech Syro II synthesizer via standard Bsmoc chemistry with introduction of the 5th Ala unit from the C-terminal arginine via Bsmoc-(Dcpm)Ala-OH. The identities given were established by ESI-MS analysis.

General Method for the Solid Phase Synthesis of H-$(Ala)_{10}$-Arg-OH on an ABI 433A Synthesizer

A sample of 2-chlorotrityl chloride resin (1g, 1.2 mmol/g, 100-200 mesh, 1% DVB) was transferred to a 10-mL syringe fitted with a polystyrene filter. Separately, Fmoc Arg(Pbf)-OH (0.96 mmol, 0.8 eq) was dissolved in DCM (125µL) and added to the resin (10µL of DMF was added to dissolve the Fmoc-Arg(Pbf)-OH). DIEA (0.371 g, 2.88 mmol, 2.4 eq) were added and the resulting mixture allowed to stand for 1 h in order to quench the remaining unloaded trityl resin. The resin was washed with DMF (3x4mL), DCm (3x4mL), diethyl ether (3x4mL), and dried *in vacuo*. Determination of the loading by UV analysis showed for Fmoc-Arg(Pbf)-Trityl resin that the loading was 0.43

mmol/g. The syntheses were carried out on a standard 20-µmol scale by means of an ABI 433A peptide synthesizer under the following conditions:
Chemistry: Fmoc/piperidine/NMP
Resin: Fmoc-Arg-(Pbf)-Trityl resin (20µmol, 0.43 mmol/g, 0.0465 g),
Amino Acids: 10 cartridges containing Fmoc-Ala-OH (5-fold excess, 100µmol, 0.0311 g)along with 125µK of DMF.
Coupling Reagent: N-HATU (o.38M, 14.4 g/100mL solution in DMF) Base: DIEA (1.60 M, 20.68 g/100mL solution in NMP)
Coupling time: 30 minutes
Preactivation time: 9 seconds
Deblocking time: 2 minutes
Final Peptide Deblocking from the Resin: as described under the MultiSynTech-mediated synthesis. After TFA was removed *in vacuo* at room temperature and twice adding dry DCM followed by removal in the same way, the peptide was precipitated by means of diethyl
ether, dried *in vacuo* and processed by freeze drying from water.
Purity Analysis: HPLC and HPCL/MS analysis was carried out using a Vydac C18 Column (#2181P5215) using the following gradient system:
A: 0.1% TFA/H_2O
B: 1-70% of 0.1% TFA/CH_3CN
With a flow rate of 0.5mL/min over 45 min.

(a) Standard Automated Stepwise SPPS of H-$(Ala)_{10}$-Arg-OH

After freeze drying 8.3 mg (47%) of the crude peptide was obtained. The desired peptide amounted to 34.2%. See Table 3. For the HPLC trace see Fig. 4.

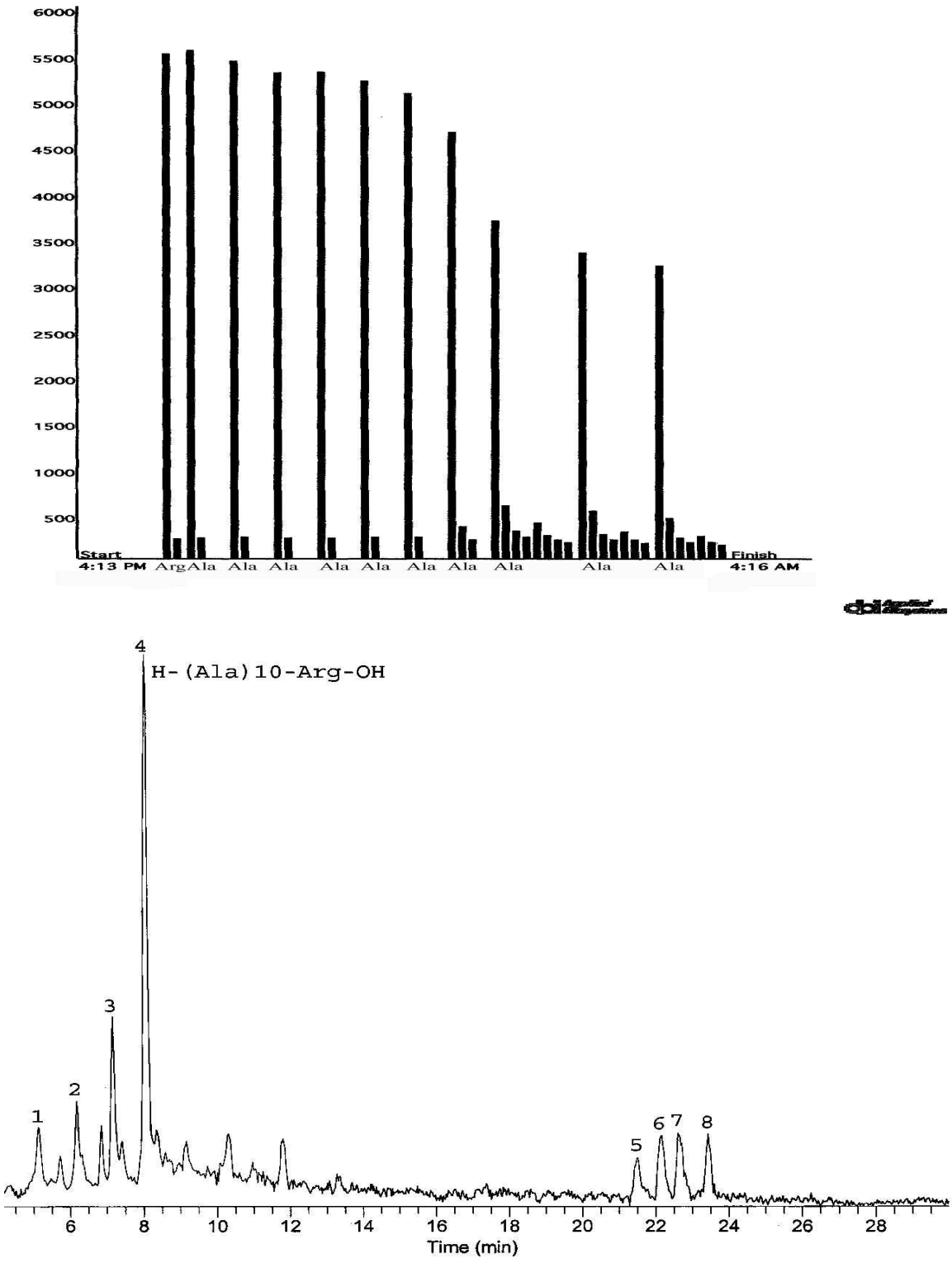


Fig. 4 HPLC trace and UV monitoring trace for the assembly of H-$(Ala)_{10}$-Arg-OH on an ABI 433A synthesizer via standard Fmoc/HBTU chemistry.

Table 3. Identity of Various Segments Formed during the Assembly of Deca-alanine onto a Chlorotrityl Resin by Standard Fmoc Chemistry

Peak #	R_t	m/z	%	Peptide
1	5.12	672	5.51	H-$(Ala)_7$-Arg-OH
2	6.17	743	7.46	H-$(Ala)_8$-Arg-OH
3	7.15	814	8.95	H-$(Ala)_9$-Arg-OH
4	8.02	885	34.21	H-$(Ala)_{10}$-Arg-OH
5	21.51	894	5.55	Fmoc-$(Ala)_7$-Arg-OH
6	22.17	965	7.06	Fmoc-$(Ala)_8$-Arg-OH
7	22.61	1036	7.00	Fmoc-$(Ala)_9$-Arg-OH
8	23.43	1107	4.89	Fmoc-$(Ala)_{10}$-Arg-OH

(b) Automated SPPS of H-$(Ala)_{10}$Arg-OH using Fmoc-(Dcpm)-Ala-OH to Introduce the Fifth Alanine Unit

The synthesis was carried out as noted above except that Fmoc-(Dcpm)-Ala-OH was used to introduce the alanine unit in position 5 from the C-terminal arginine position. After freeze drying 6.5 mg (37%) of the crude peptide was obtained. The desired peptide amounted to 4.79%. See Table 4. For the HPLC trace see Fig. 5.

Table 4: Components Present in the Crude Peptide Based on HPLC/MS Analysis

Peak #	R_t	m/z	%	Peptide
0	4.77	601	0.77	H-$(Ala)_6$-Arg-OH
1	5.02	672	0.91	H-$(Ala)_7$-Arg-OH
2	6.27	743	3.42	H-$(Ala)_8$-Arg-OH
3	7.25	814	1.75	H-$(Ala)_9$-Arg-OH
4	7.95	885	4.79	H-$(Ala)_{10}$-Arg-OH
5	8.84	624	77	Dcpm-$(Ala)_5$Arg-OH

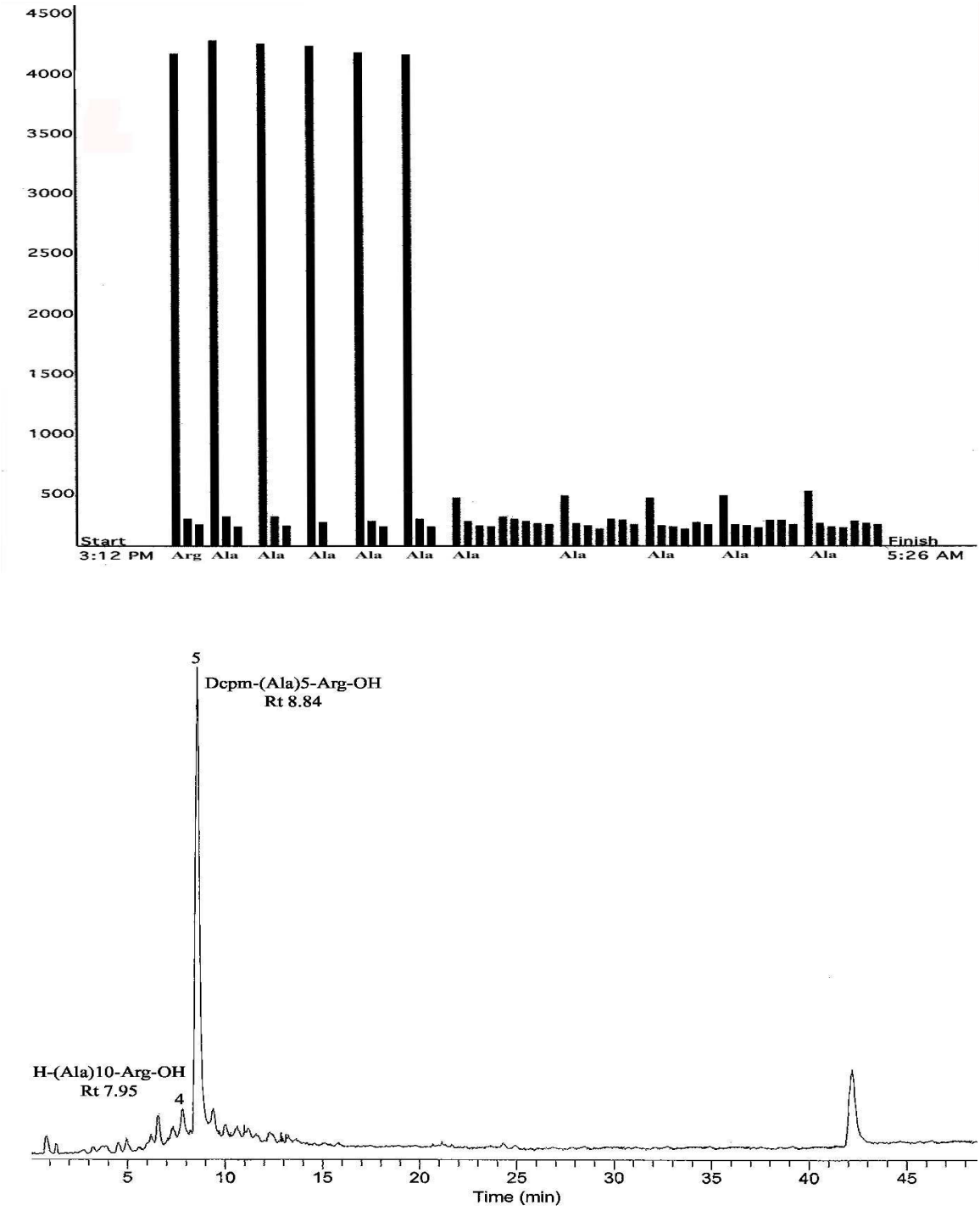


Fig. 5 HPLC trace and UV monitoring trace for the assembly of H-$(Ala)_{10}$-Arg-OH as indicated under Fig. 4 except that Fmoc-(Dcpm)-Ala-OH was used to introduce the 5^{th} alanine unit relative to the C-terminal arginine unit.

General Procedure for Manual Solid Phase Peptide Synthesis

The synthesis was carried out using a plastic syringe attached to a water aspirator as a reaction vessel.

The 20-µmol scale synthesis was carried out as follows:

(1) A sample of preloaded Fmoc-Arg (Pbf)-Trityl resin (0.43 mmol/g, 0.0465 g) was transferred to a 5-mL syringe fitted with a polystyrene filter. The resin was washed with DMF (3x3 mL), DCM (3x3 mL), and DMF (3x3 mL).
(2) The Fmoc-resin was deblocked with 2 mL of 20% piperidine/DMF solution for 9 minutes and washed with DMF (3x3 mL), DCM (3x3 mL) and DMF (3x3 mL)
(3) A mixture containing a 5-fold excess of Fmoc-AA-OH (0.1 mmol, 0.017 mL), a 5-fold excess of N-HATU (0.1 mmol, 0.038 g) and a 5-fold excess of DIEA (0.1 mmol, 0.017 mL) was stirred for 9 seconds and added directly to the resin with another 5-fold excess of DIEA (0.1 mmol, 0.017 mL). After 30 minutes the resin was washed with DMF (3x3 mL), DCM (3x3 mL), and DMF (3x3 mL). For multiple coupling, step 3 was repeated.
(4) Steps 2 and 3 were repeated until the assembly was completed.
(5) The final Fmoc-deblocking was carried out by treating the resin with 2 mL of 20% piperidine/DMF solution for 9 minutes followed by washing with DMF (3x3 mL), DCM (3x3 mL) and DMF (3x3 mL). Finally the resin was washed with diethylether and dried *in vacuo.*
(6) The peptide was deblocked from the resin with 10% TFA/DCM for 1 hour followed by washing with DCM. DCM and TFA were removed *in vacuo* and the oily residue was precipitated by adding cold diethyl ether. The white solid was separated by means of a centrifuge and further processed by freeze drying.

(a) Standard Stepwise Manual Solid Phase Synthesis of H-(Ala)$_{10}$-Arg-OH

After freeze drying 8.6 mg (49%) of the crude peptide was obtained. The desired peptide amounted to 32.21%. See Table 5 and Fig. 6. Note that 15.45% of the desired peptide methyl ester was obtained in this run presumably because of methanol released from the resin during the final TFA deblocking step. During the loading process methanol had been used to quench any unreacted chlorotrityl residues.

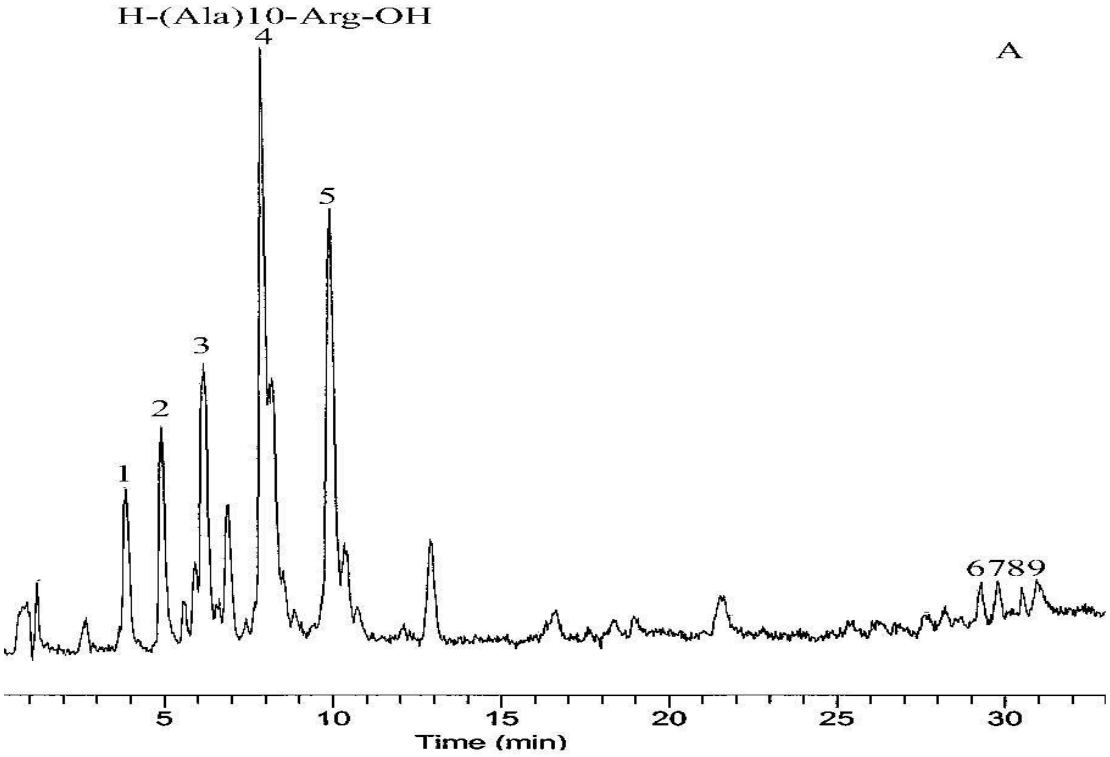


Fig. 6 HPLC trace for the manual assembly of H-$(Ala)_{10}$-Arg-OH according to standard Fmoc/HBTU chemistry.

Table 5: Components Present in the Crude Peptide Based on HPLC/MS Analysis

Peak #	R_t	m/z	%	Peptide
0	3.97	601	9.87	H-$(Ala)_6$-Arg-OH
1	5.02	672	10.61	H-$(Ala)_7$-Arg-OH
2	6.27	743	11.42	H-$(Ala)_8$-Arg-OH
3	6.95	814	4.75	H-$(Ala)_9$-Arg-OH
4	8.02	885	32.21	H-$(Ala)_{10}$-Arg-OH
5	9.98	899	15.45	H-$(Ala)_{10}$-Arg-OMe
6	29.45	894	2.59	Fmoc-$(Ala)_7$-Arg-OH
7	29.87	965	1.53	Fmoc-$(Ala)_8$-Arg-OH
8	30.72	1036	1.06	Fmoc-$(Ala)_9$-Arg-OH
9	31.13	1107	1.89	Fmoc-$(Ala)_{10}$-Arg-OH

(b) Stepwise manual synthesis of H-$(Ala)_{10}$-OH was carried out as noted above except that Fmoc-(Dcpm)-Ala-OH was used to introduce the alanine unit in position 5 from the C-terminal arginine position and Bsmoc-Ala-OH/N-HATU/DIEA/2x2 h coupling was used to introduce the sixth alanine unit. Remaining alanines were introduced in the standard manner via Fmoc-Ala-OH. After freeze drying 12.7 mg (72%) of the crude peptide was obtained. The desired peptide amounted to 40.4%. See Table 6. For the HPLC trace see Fig. 7.

Table 6: Components Present in the Crude Peptide Based on HPLC/MS Analysis

Peak #	R_t	m/z	%	Peptide
1	5.24	672	2.31	H-$(Ala)_7$-Arg-OH
2	6.22	743	2.56	H-$(Ala)_8$-Arg-OH
3	7.15	814	3.38	H-$(Ala)_9$-Arg-OH
4	7.92	885	40.36	H-$(Ala)_{10}$-Arg-OH
5	8.54	624	42.96	Dcpm-$(Ala)_5$-Arg-OH
6	21.51	894	0.45	Fmoc-$(Ala)_7$-Arg-OH
7	22.17	965	0.76	Fmoc-$(Ala)_8$-Arg-OH
8	22.61	1036	0.72	Fmoc-$(Ala)_9$-Arg-OH
9	23.43	1107	0.89	Fmoc-$(Ala)_{10}$-Arg-OH

(a) Stepwise manual synthesis of H-$(Ala)_{10}$-Arg-OH was carried out as noted above except that Fmoc-(Dcpm)-Ala-OH was used to introduce the alanine unit in position 5 from the C-terminal arginine position and Bsmoc-Ala-OH/HOAt/DIC/2x24 h coupling was used to introduce the sixth alanine unit. Remaining alanines were introduced in the standard manner via Fmoc-Ala-OH. After freeze drying 12.1 mg (69%) of the crude peptide was obtained. The desired peptide amounted to 83.4%. See Table 7. For the HPLC trace see Fig. 8.

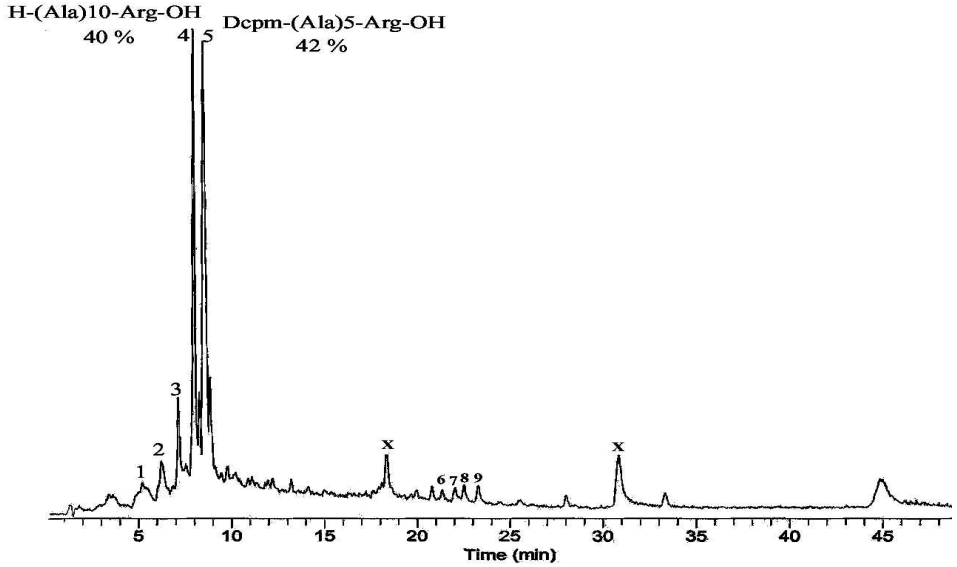


Fig. 7 HPLC trace for the manual assembly of H-$(Ala)_{10}$-Arg-OH according to the method described under Fig. 6 except that the 5th alanine unit relative to the C-terminal arginine was introduced via Fmoc-(Dcpm)Ala-OH and the 6th alanine unit via Bsmoc-Ala-OH (N-HATU/2 x 2 h).

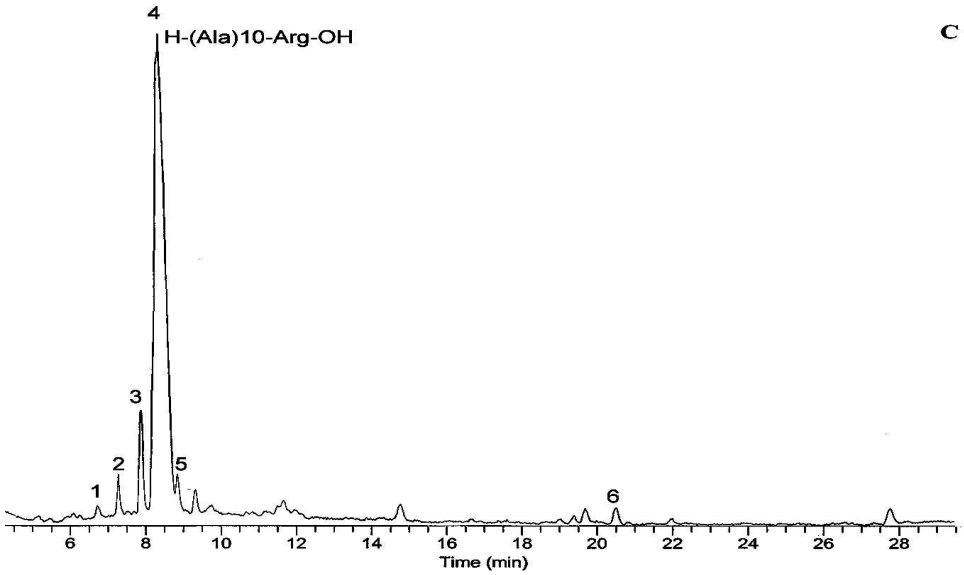


Fig. 8 HPLC trace for the manual assembly of H-$(Ala)_{10}$-Arg-OH according to the method described under Fig. 6 except that the 5th alanine relative to the C-terminal arginine unit was introduced via Fmoc-(Dcpm)Ala-OH and the 6th alanine unit via Bsmoc-Ala-OH (DIC/HOAt/2 x 24 h).

Table 7: Components Present in the Crude Peptide Based on HPLC/MS Analysis

Peak #	R_t	m/z	%	Peptide
1	6.76	672	1.36	H-$(Ala)_7$-Arg-OH
2	7.35	743	1.85	H-$(Ala)_8$-Arg-OH
3	7.86	814	6.55	H-$(Ala)_9$-Arg-OH
4	8.42	885	83.39	H-$(Ala)_{10}$-Arg-OH
5	8.89	624	1.23	Dcpm-$(Ala)_5$-Arg-OH
6	20.51	965	1.85	Fmoc-$(Ala)_8$-Arg-OH

Automated Solid Phase Syntheses of ADGSLDDYNHLV-amide 9

The syntheses were carried out on an ABI 433 A instrument with 300 mg of Tenta Gel-S-RAM resin, loading 0.25 mmol/g, single couplings of 15 min, with molar equivalents of each amino acid, deblocking with 20% piperidine in DMF for 8 min. Peptides were removed from the resin with 95% TFA with titration until the color disappeared with TIPS. After 60 min. rotary evaporation and re-evaporation with heptane followed by precipitation with diethyl ether gave the crude peptides which were examined by HPLC and MS analysis:

(a) Standard synthesis with N-HBTU as coupling reagent. None of the desired product was obtained, only the amino succinimide derivative m/z calc. M+1 1299.3; found 1299.45. See Fig 9a.

(b) Synthesis via Fmoc (Dcpm)-Gly-OH in place of Fmoc-Gly-OH with N-HATU as coupling reagent. The desired product was obtained in 91% purity [m/z calcd. M+1 1317.39; found 1317.41] along with 8% of the des-Ala-Asp deletion peptide [m/z M+1 calcd. 1131.22; found 1131.36], See Fig. 9b.

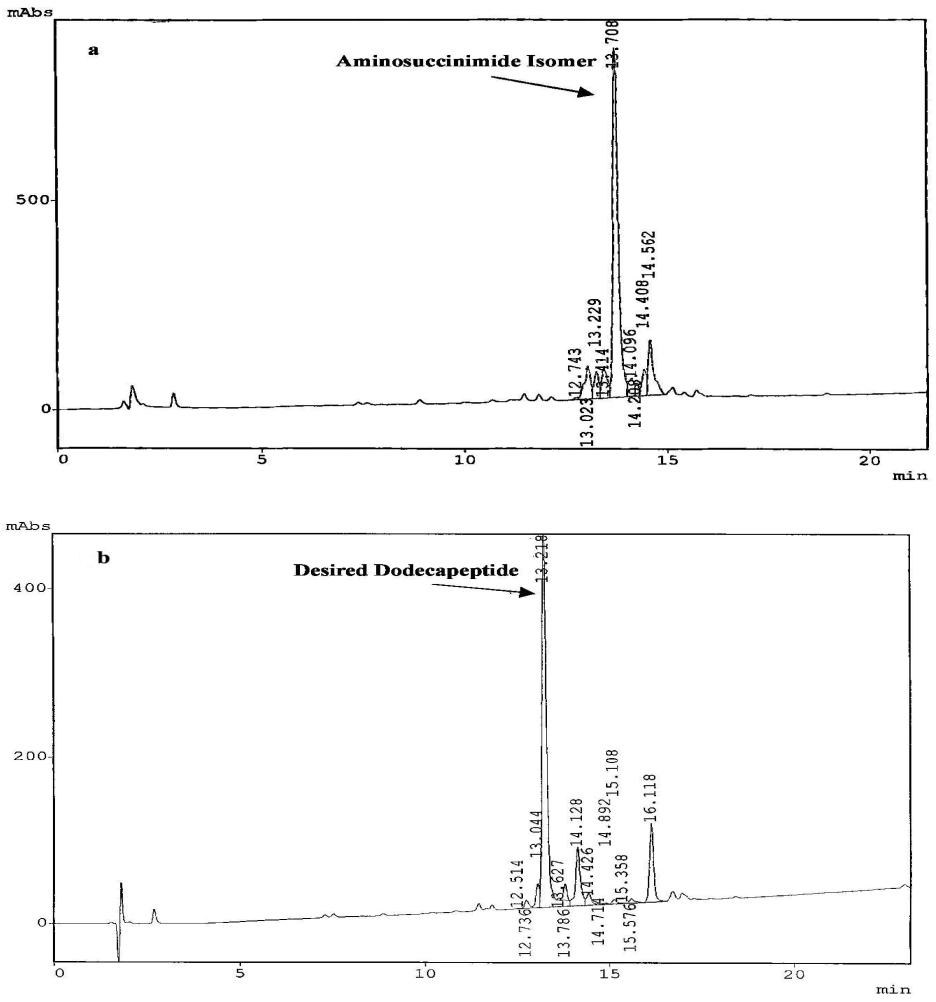


Fig. 9a, b HPLC traces for the synthesis of the Asp-Gly containing sequence **9** on an ABI 433A synthesizer
14a via standard N-HBTU chemistry
14b via standard N-HBTU chemistry except that Fmoc-(Dcpm)Gly-OH was used to introduce the glycine unit and N-HATU was used to couple Fmoc-Asp(O-*t*-Bu)-OH to the Dcpm-Gly unit.

Automated Solid Phase Synthesis of the Prion Peptide (106-126) 10

The synthesis was carried out on an ABI 433A instrument using 250 mg of Gly TCP resin (Pepchem, Tübingen, Germany), loading 0.07mmol/g, single couplings using N-HATU for 15 min. each with 10 molar equivalents of each protected amino acid (Fmoc or Bsmoc) up to position 113 and thereafter double couplings up to the end of the synthesis. Deblocking time 5 min. with 20% piperidine. At positions 114 and 119 Fmoc (Dcpm)-Gly-OH was used and at positions 115 and 120 Bsmoc-Ala-OH was used. Removal from the resin was carried out with 92% TFA/5% H_2O/ 3% TIPS for 75 min.

The crude yield was 41%. LC/MS analysis showed that the desired peptide (crude) was obtained in a purity of 89%; after purification 98.7% [m/z calcd. M+ 1 1913.3; found 1913.7] the only major by-product was the sulfoxide obtained from one of the methionine units [m/z calcd. M+1 1929.3; found 1929.8] along with a by-product derived from the sulfoxide [M-48; m/z calcd.1865.3; found 1865.8] See Figs. 10a and 10b

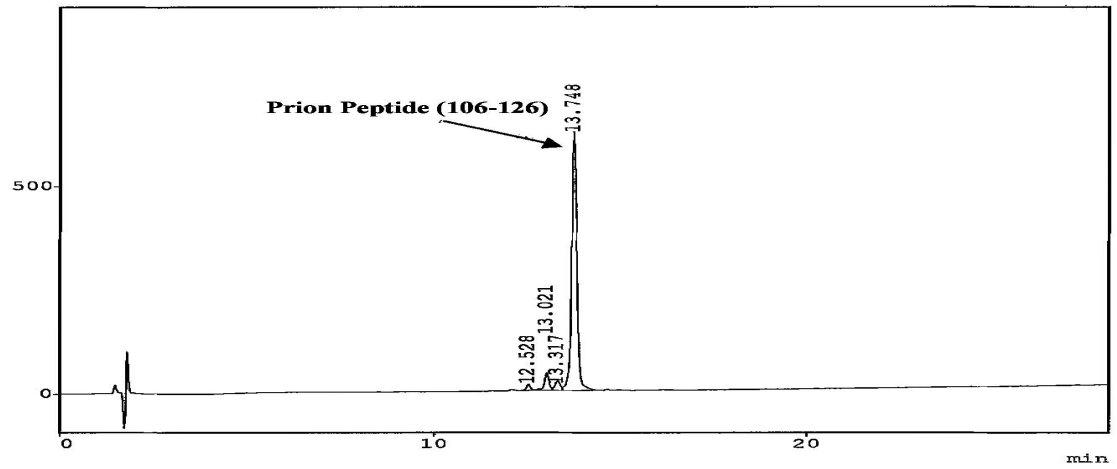


Fig. 10a HPLC trace for the synthesis of the prion peptide (106-126) **10** on an ABI 433A peptide synthesizer using Fmoc-(Dcpm)Gly-OH for the introduction of Gly^{114} and Gly^{119} and Bsmoc-Ala-OH for the introduction of Ala^{113} and Ala^{118}.

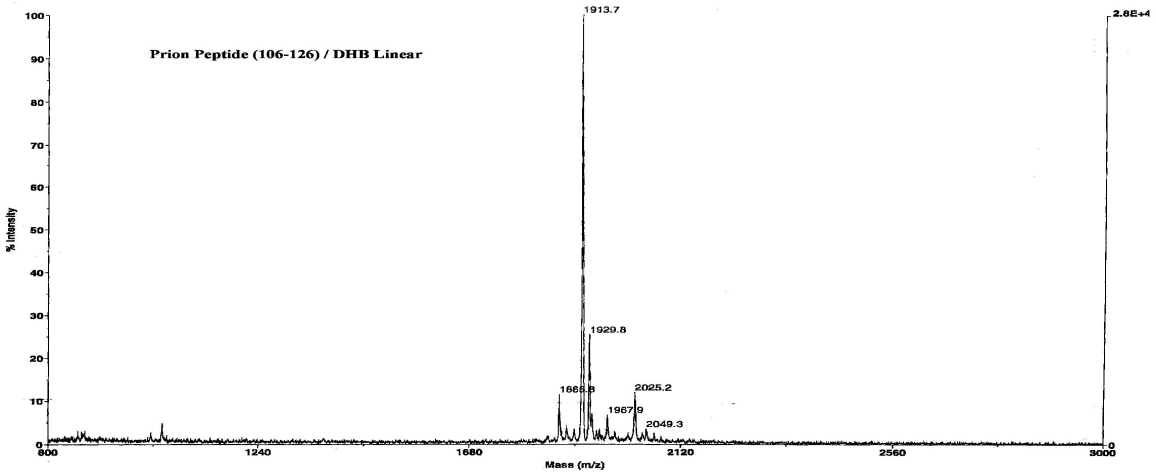


Fig. 10b MALDI trace for prion peptide (106-126). The peak at 1929.8 is assigned to the corresponding methionine sulfoxide.

Determination that no Significant Loss of Configuration Occurs During the Synthesis of Dcpm-Ala-OBn from Dicyclopropylimine Hydrochloride

The method involved the normal coupling (N-HATU/DIEA) of Fmoc-(Dcpm)Ala-OH (derived form Dcpm-Ala-OBn) to a praline unit attached to a Rink amide resin followed by deblocking of the Dcpm residue and removal form the resin by means of TFA to give Fmoc-Ala-Pro-NH_2 and examination of the crude material by HPLC analysis which showed contamination by 0.95 % of the DL- diastereomer. See Scheme 1 and Figs. 11a-11c

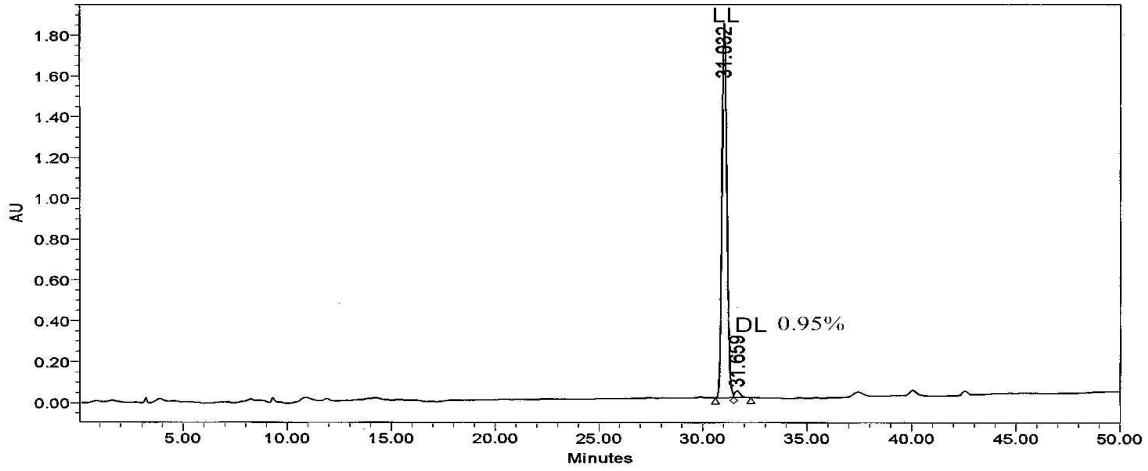


Fig. 11a HPLC trace of crude Fmoc-Ala-Pro-NH_2 derived form Fmoc-(Dcpm)Ala-OH and a proline-substituted Rink amide resin according to Scheme 1

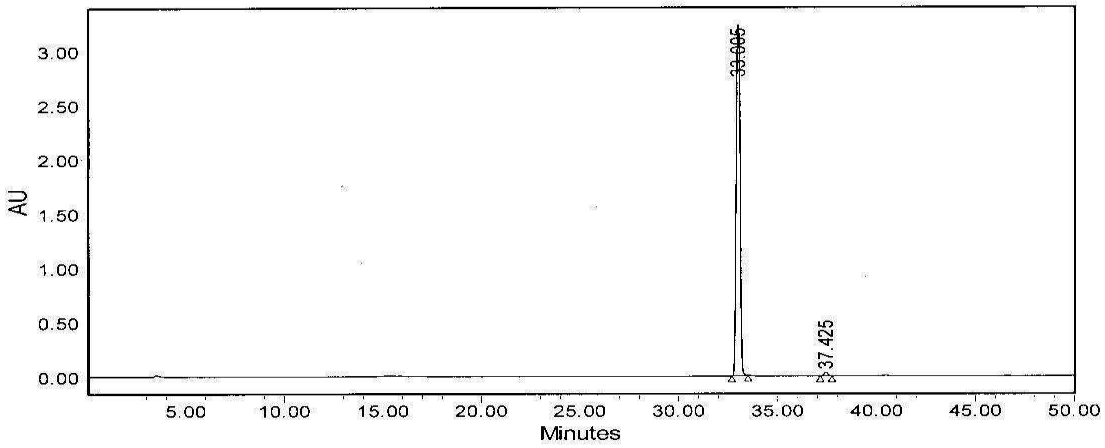


Fig. 11b HPLC trace of analytically pure authentic sample of Fmoc-Ala-Pro-NH_2

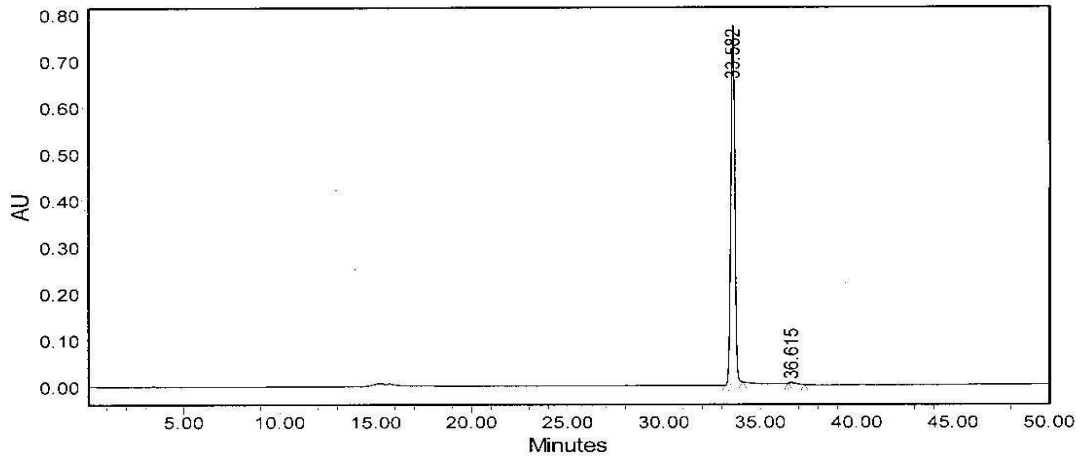


Fig. 11c HPLC trace of analytically pure authentic sample of Fmoc-D-Ala-Pro-NH_2

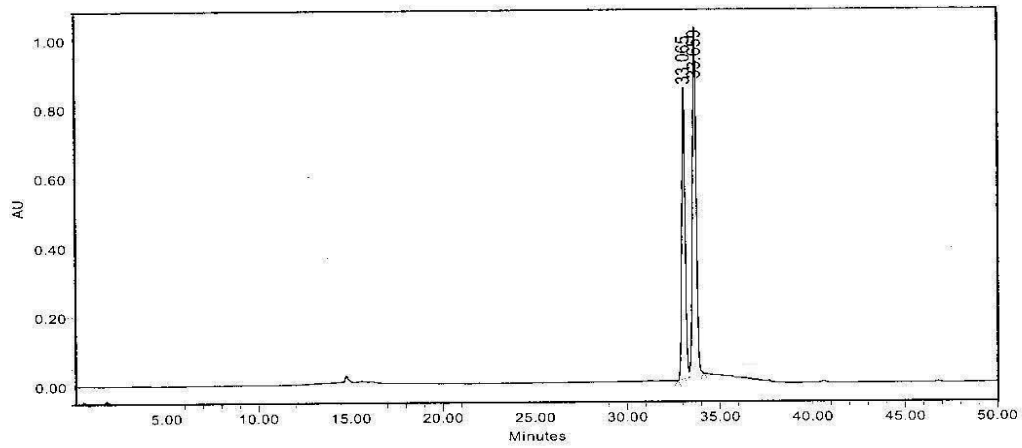
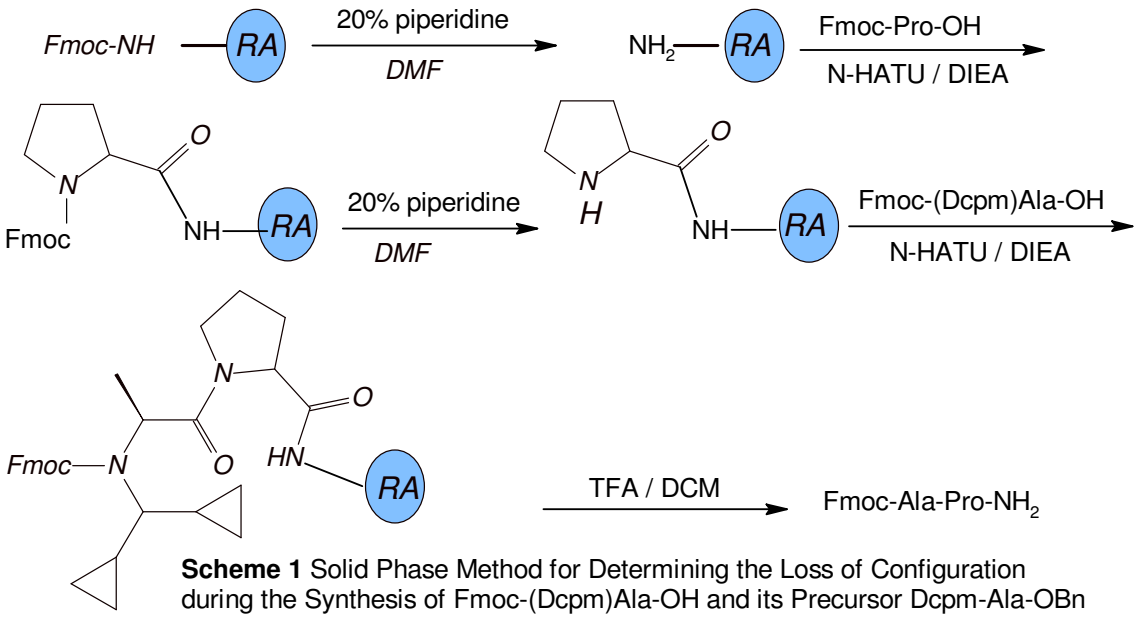


Fig. 11d HPLC trace of a mixture of analytically pure authentic samples of Fmoc-Ala-Pro-NH_2 and Fmoc-D-Ala-Pro-NH_2



Scheme 1 Solid Phase Method for Determining the Loss of Configuration during the Synthesis of Fmoc-(Dcpm)Ala-OH and its Precursor Dcpm-Ala-OBn

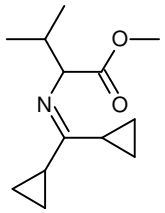
Synthesis of Authentic Fmoc-Ala-Pro-NH_2

To a mixture of Fmoc-Ala-OH (0.622 g, 2 mmol) and N-HATU (0.76 g, 2 mmol) in 20 mL of dry DCM cooled to 0 °C in an ice bath was added TMP (0.52 mL, 4 mmol) and the mixture stirred for 1 min followed by the addition of H-Pro-NH_2 (0.228 g, 2 mmol). The reaction mixture was stirred for 1 h at 0 °C and overnight at room temperature. DCM (30 mL) was added to the mixture which was washed with three 15-mL portions each of 1N HCl, saturated $NaHCO_3$ and saturated NaCl solution, dried over $MgSO_4$ and evaporated at reduced pressure to give the crude dipeptide which was recrystallized from hot EtOAc to give 350 mg (42.9%) of the dipeptide in two crops, m.p 181-183 °C; ^{1}H-NMR (DMSO, 400 MHz)*: δ 1.04-1.2 (several d, 3); 1.7-2.1 (several m, 4); 3.5 (t, 1.6,); 4.0-4.4 (several m, 5); 6.9-7.9 (several m, 11); ^{13}C-NMR (DMSO, 75 MHz): δ 16.84, 24.45, 29.18, 46.67, 47.96, 59.51, 65.54, 120.10, 125.31, 127.06, 127.63, 140.73, 143.84, 155.69, 170.80, 173.60; Anal. Calcd. for $C_{23}H_{25}N_3O_4$ (407.46) C, 67.80; H, 6.18; N, 10.31. Found: C, 67.62; H, 6.13, N, 10.10.

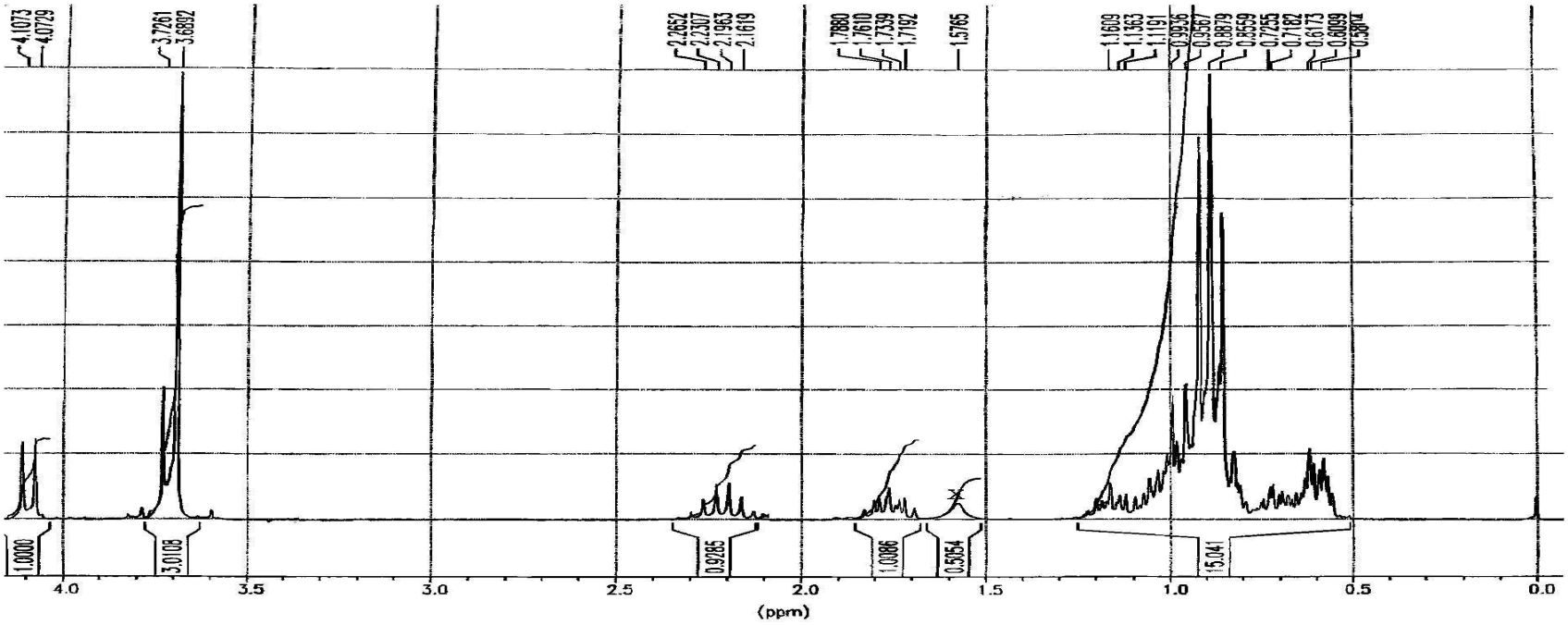
Synthesis of Authentic Fmoc-D-Ala-Pro-NH_2

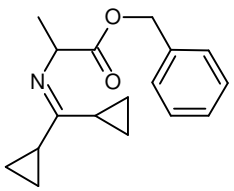
The DL diastereomer was also prepared by the method described above. Recrystalliztion from DCM/hexane gave 450 mg of the crude material which after a second recrystallization gave 370 mg (45.4%) of the pure dipeptide. m.p. 130-135 °C dec; ^{1}H-NMR ($CDCl_3$, 400 MHz)*: δ 1.05-1.15 (three d, 3); 1.7-2.2 (several m, 4); 3.6-4.7 (several m, 7); 6.9-7.9 (several m, 10); ^{13}C-NMR (DMSO + $CDCl_3$, 75 MHz): δ 16.39, 23.81, 28.47, 46.34, 46.45, 48,09, 59.79, 65.91, 129.25, 124.59, 126.47, 127.06, 140.48, 155.75, 171.30, 173.54; HRMS: $(M)^+$ calcd for $C_{23}H_{25}N_3O_4$. 407.1845: obsd. 407.1837

* The effects of restricted rotation were clearly visible in the proton NMR spectra of both isomers.



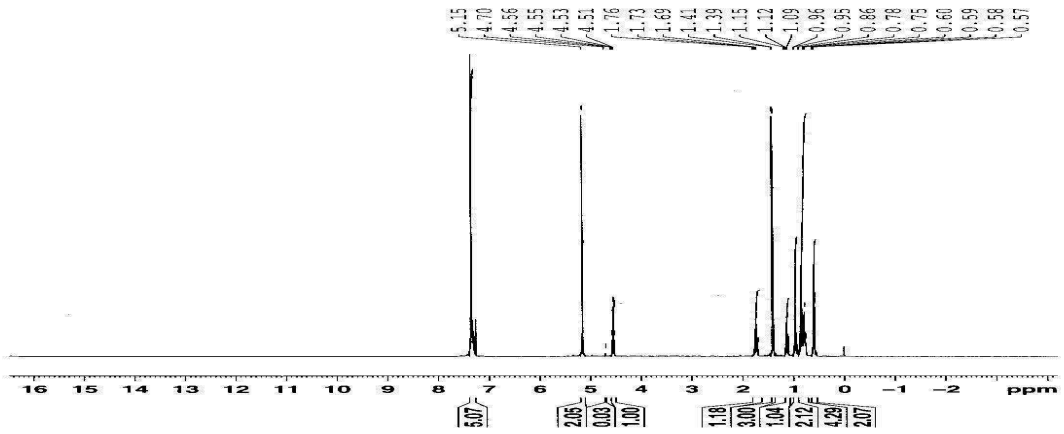
^{1}H-NMR (200 MHz, $CDCl_3$)



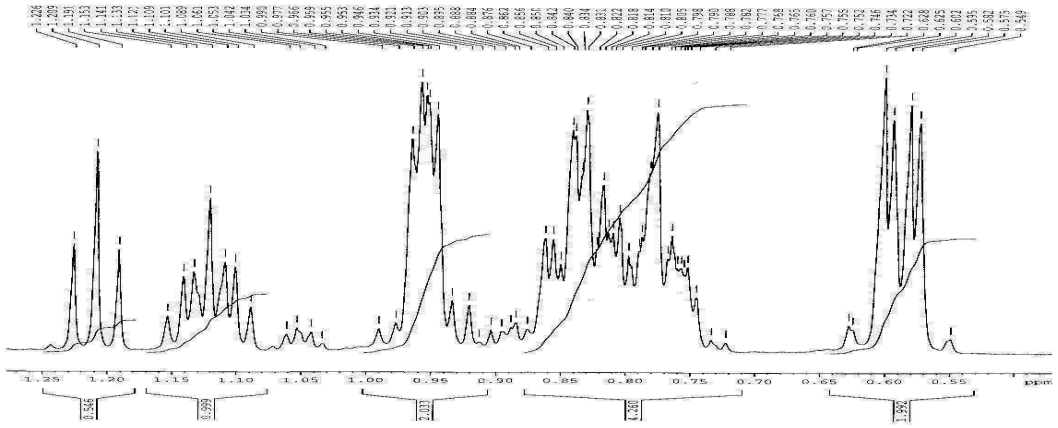


(A) ^{1}H-NMR (400 MHz, $CDCl_3$)
With Selected Regions
Expanded for Clarity (B and C)

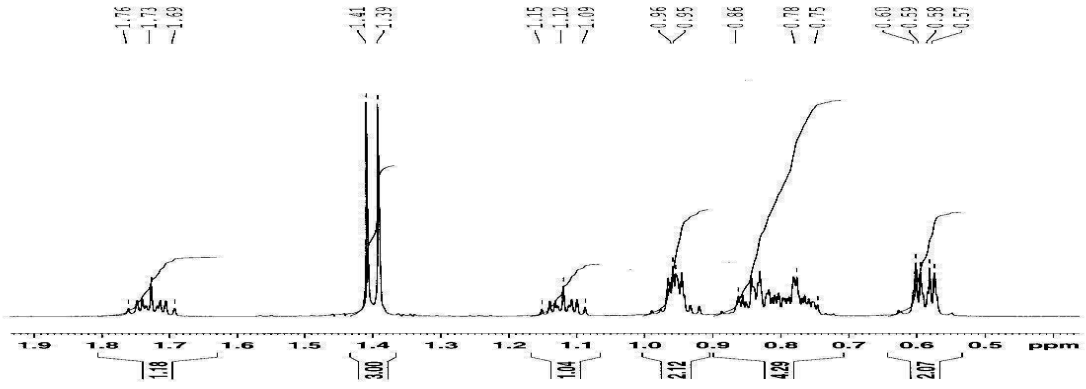
A

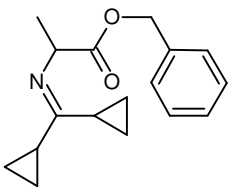


B



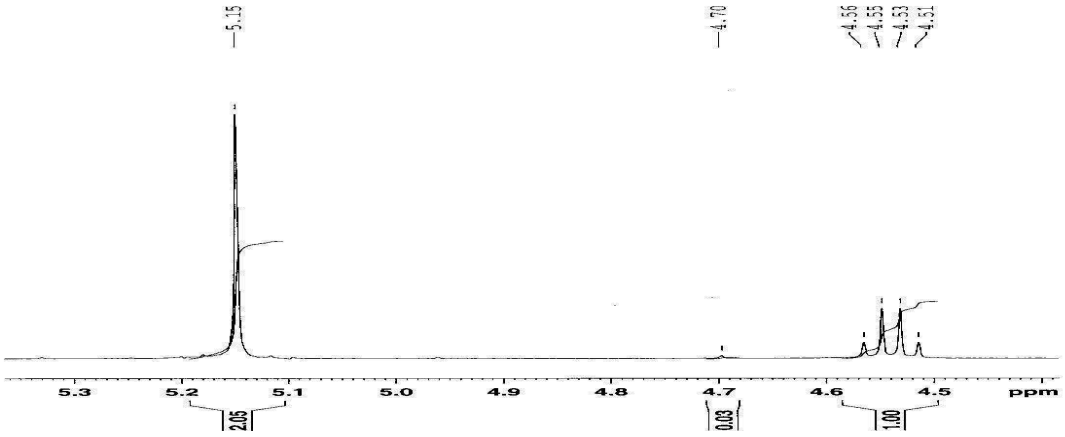
C



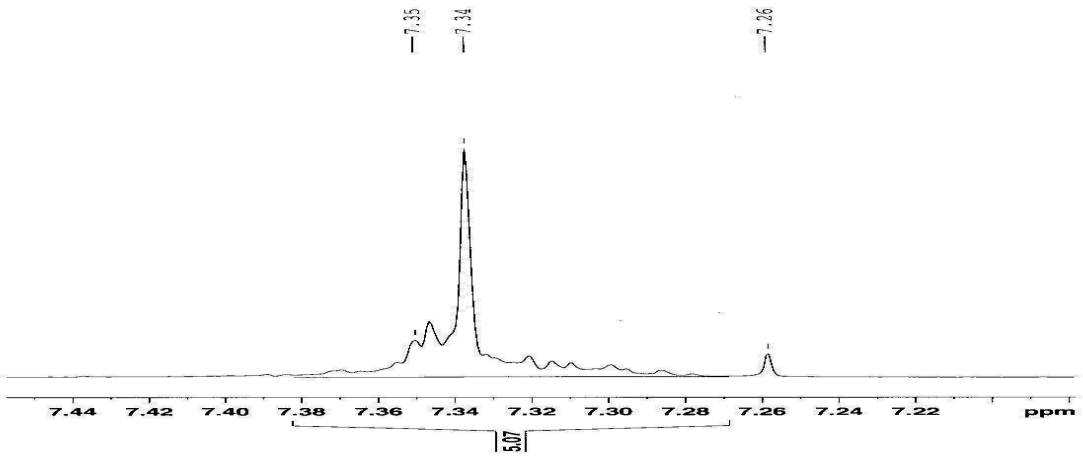


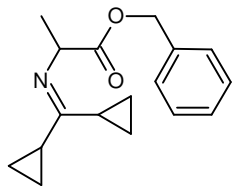
^{1}H-NMR (400 MHz, $CDCl_3$)
Selected Regions Expanded
for Clarity (D and E)

D

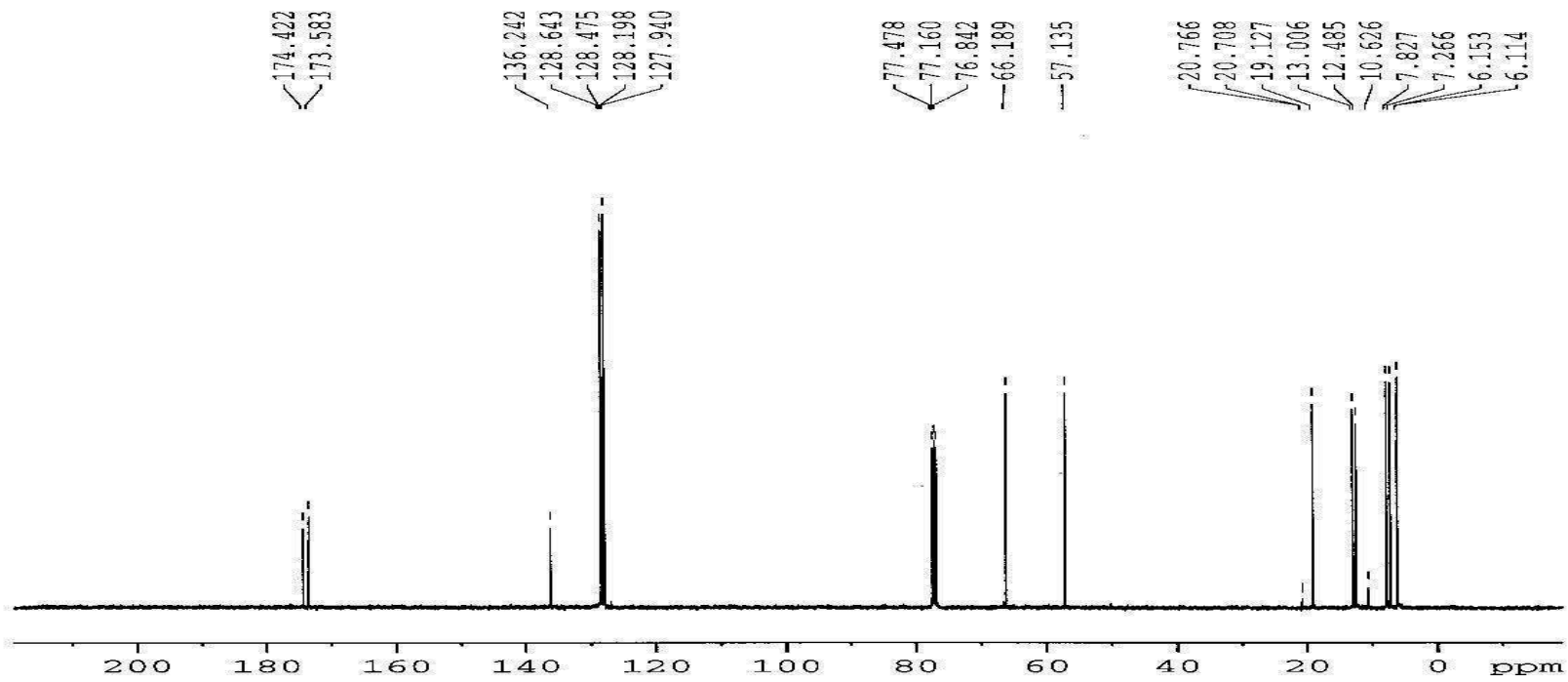


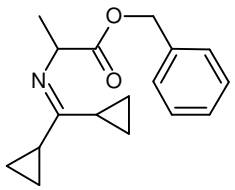
E



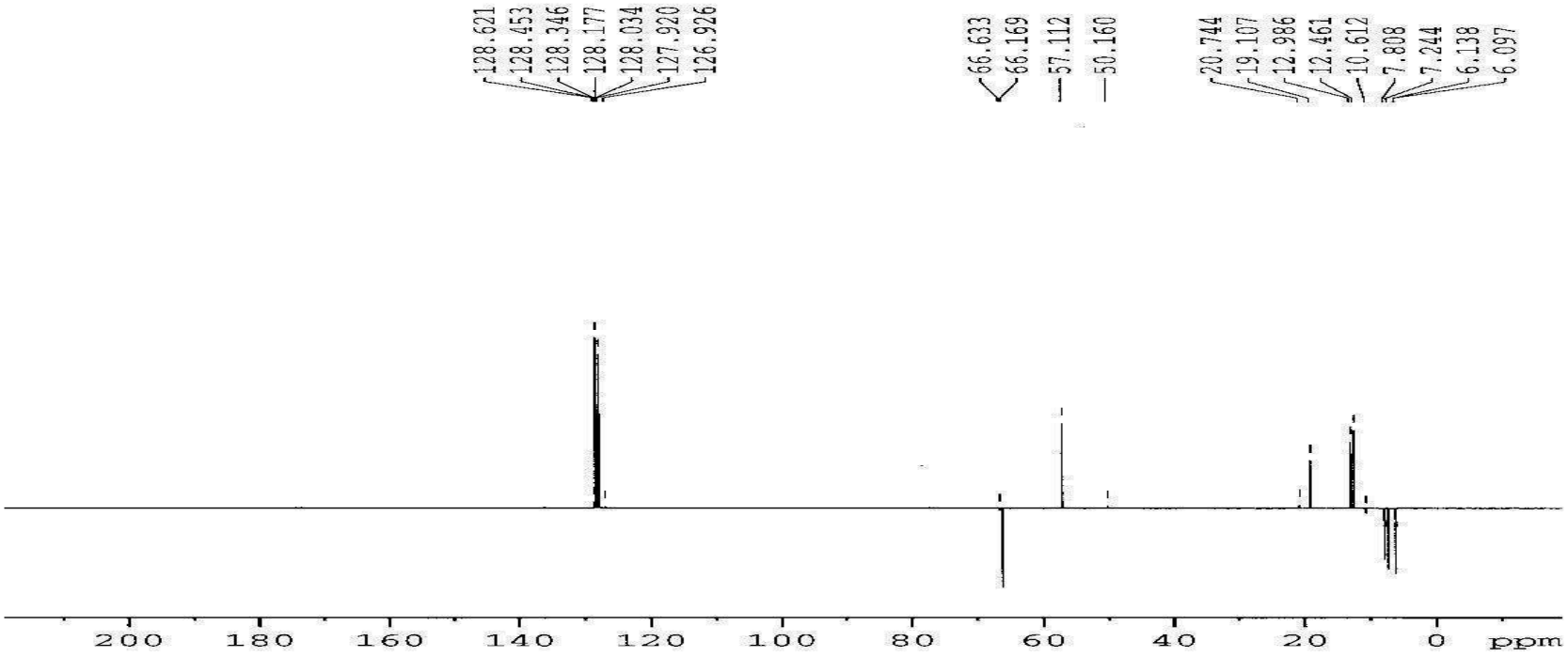


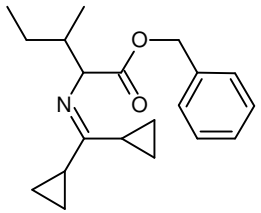
^{13}C-NMR (75 MHz, $CDCl_3$)



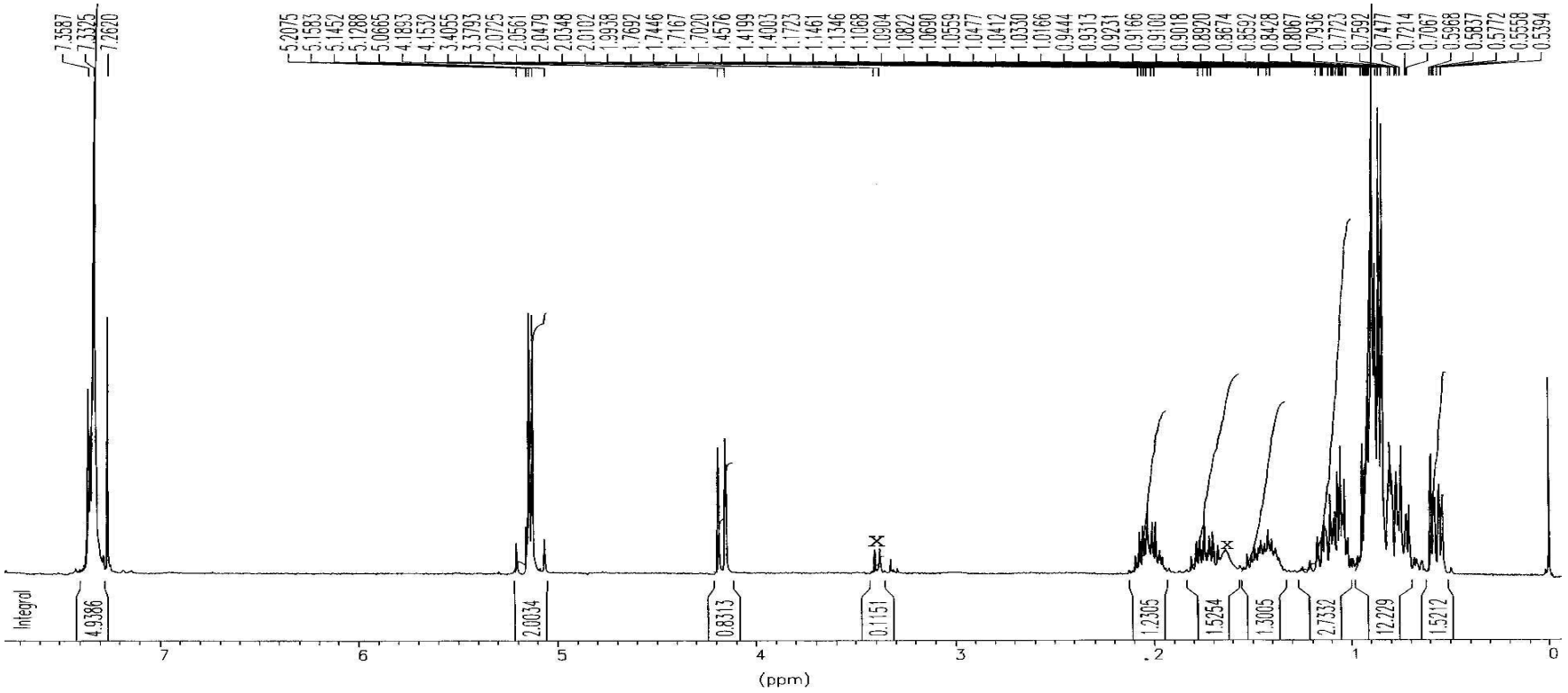


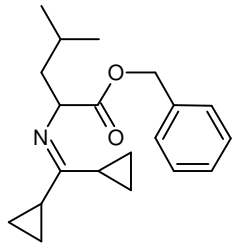
135 dept-NMR (75 MHz, $CDCl_3$)



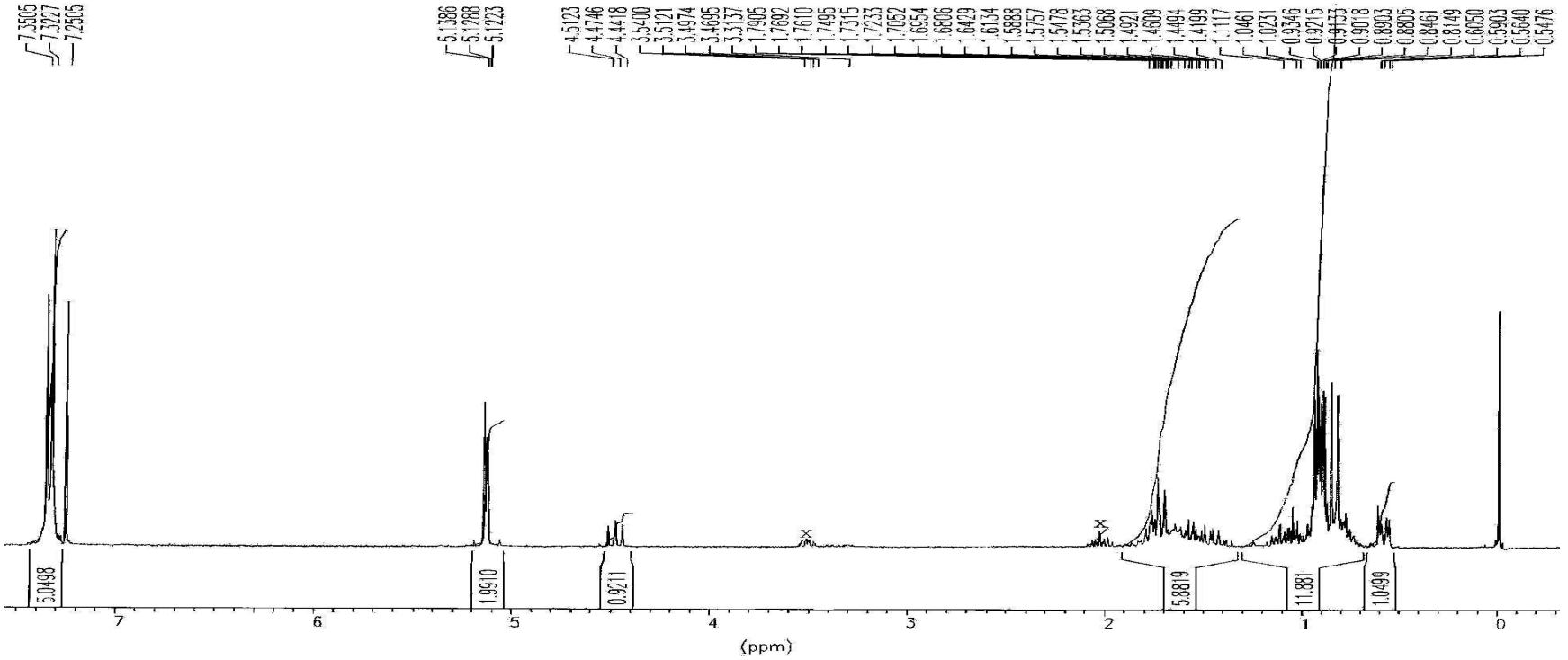


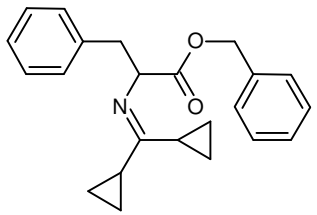
^{1}H-NMR (400 MHz, $CDCl_3$)



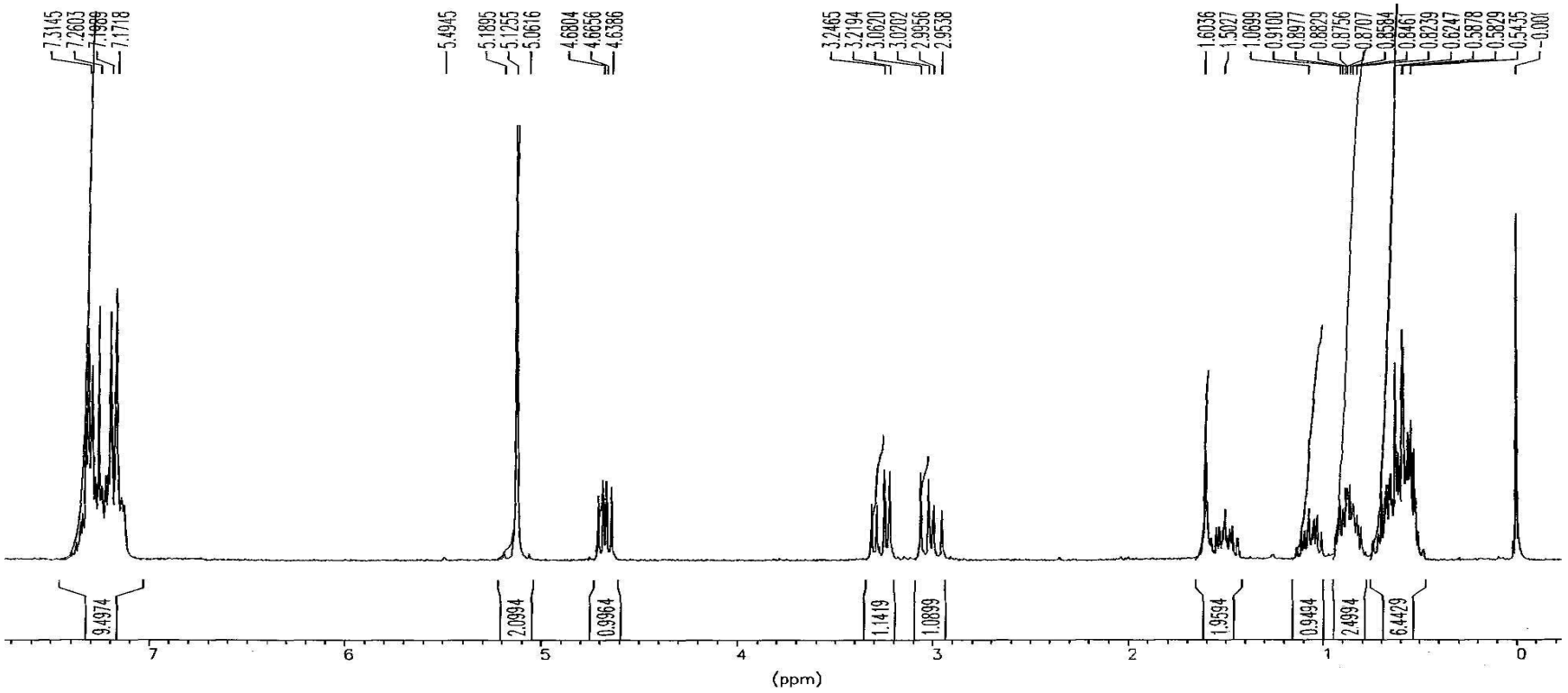


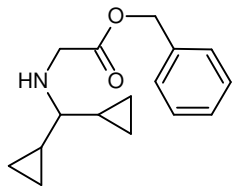
^{1}H-NMR (400 MHz, $CDCl_3$)



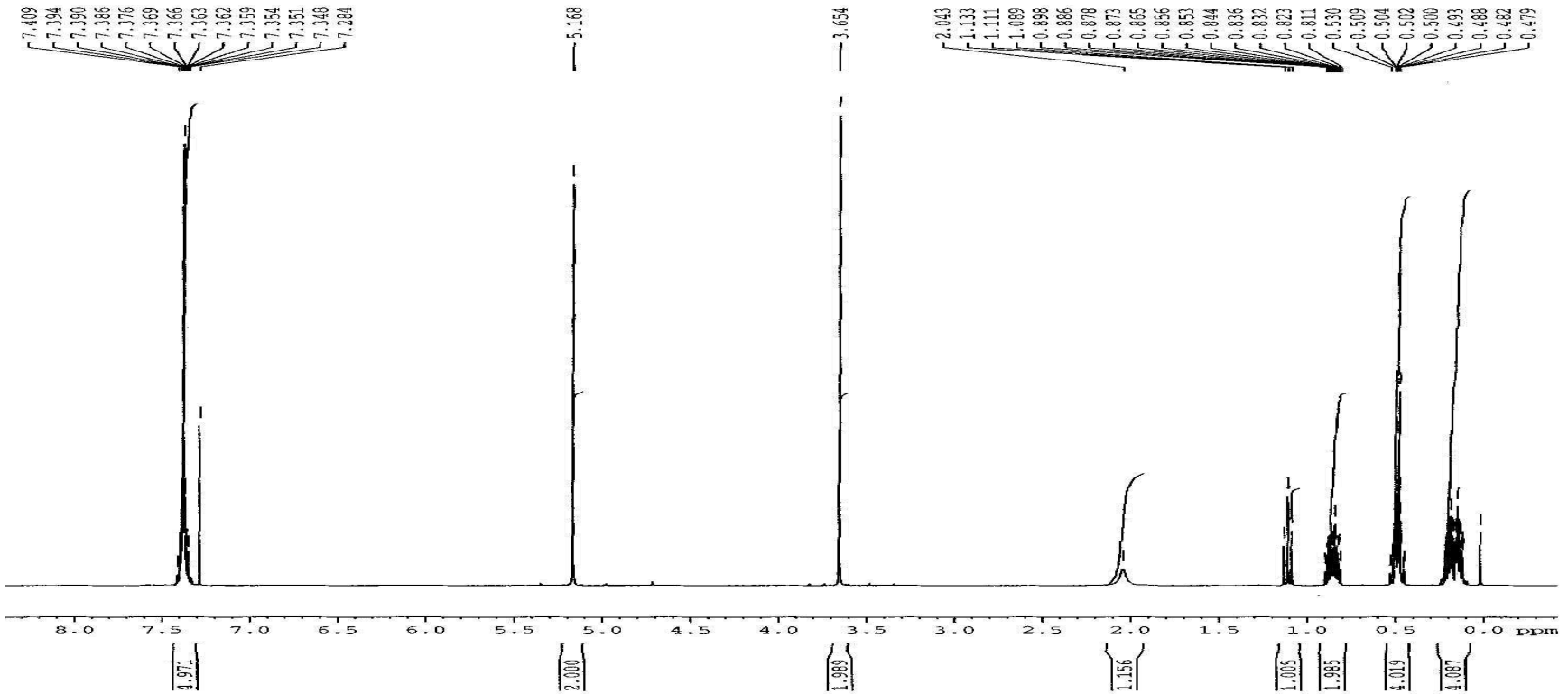


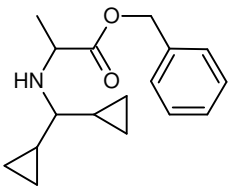
^{1}H-NMR (200 MHz, $CDCl_3$)





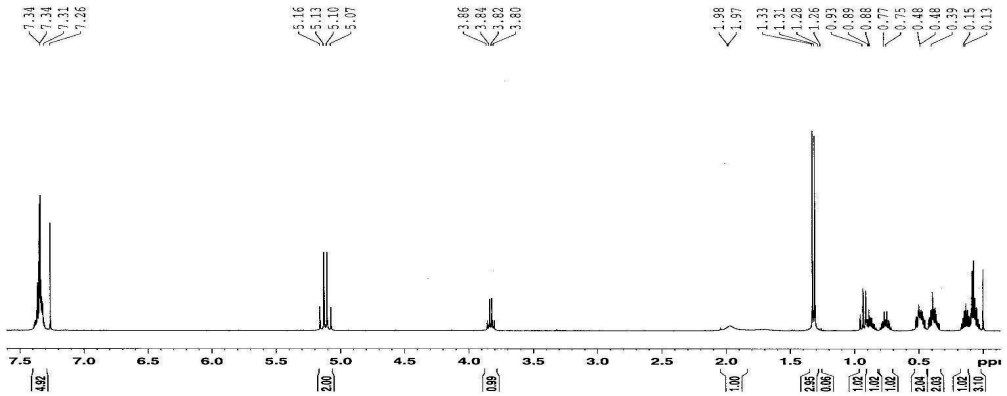
^{1}H-NMR (400 MHz, $CDCl_3$)



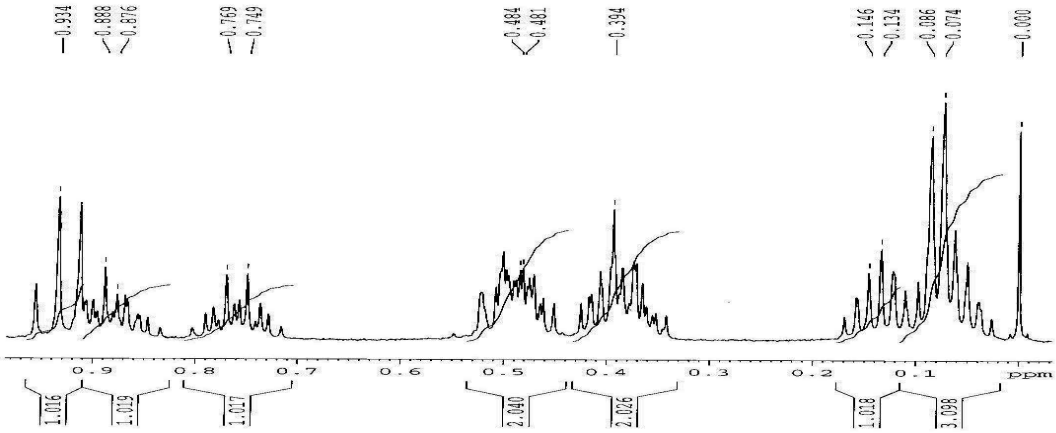


(A) ^{1}H-NMR (400 MHz, $CDCl_3$)
With Selected Regions
Expanded for Clarity (B and C)

A



B



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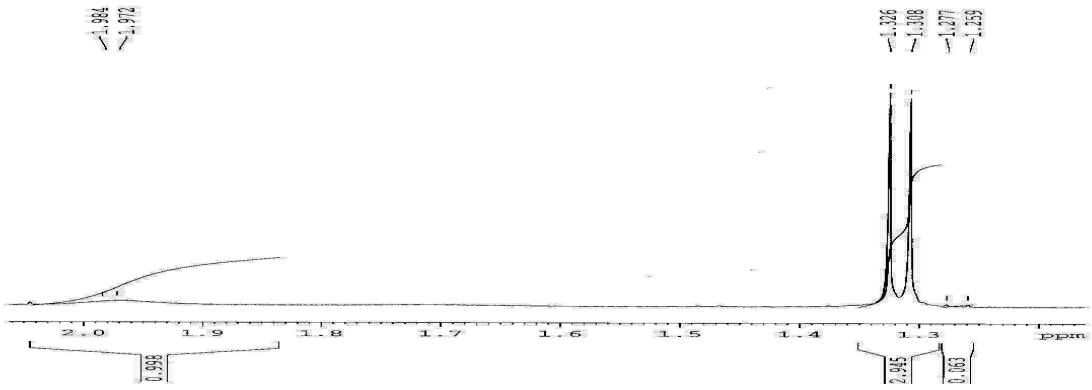
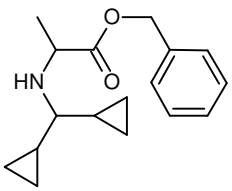
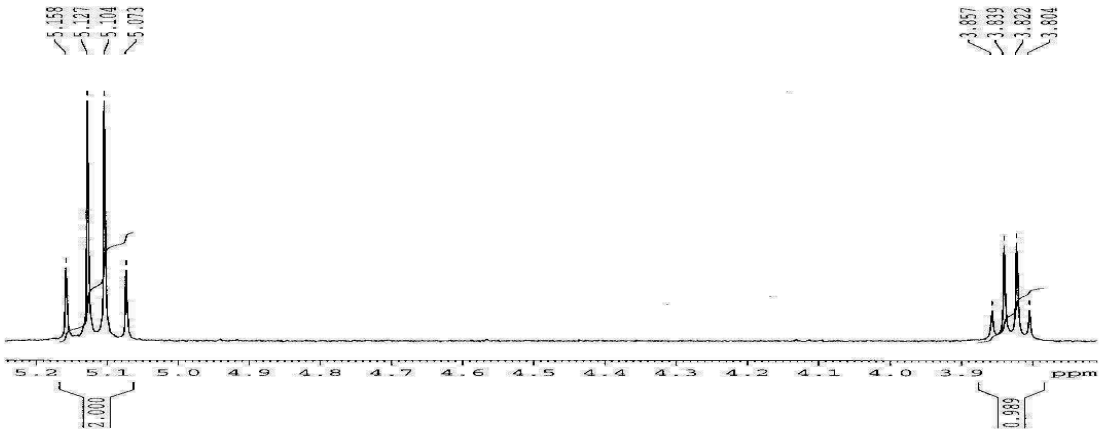




^{1}H-NMR (400 MHz, $CDCl_3$)
Selected Regions Expanded
for Clarity (D and E)

D



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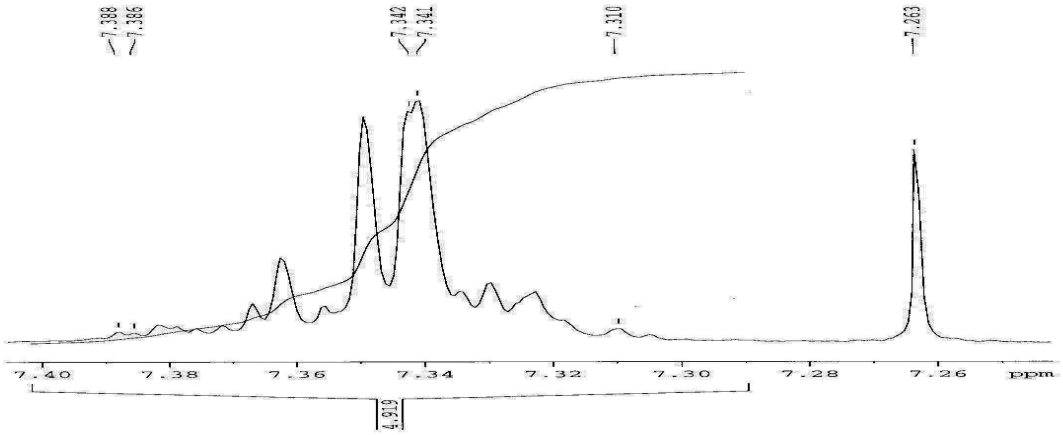
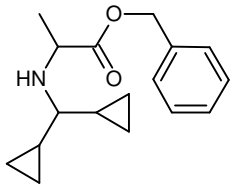
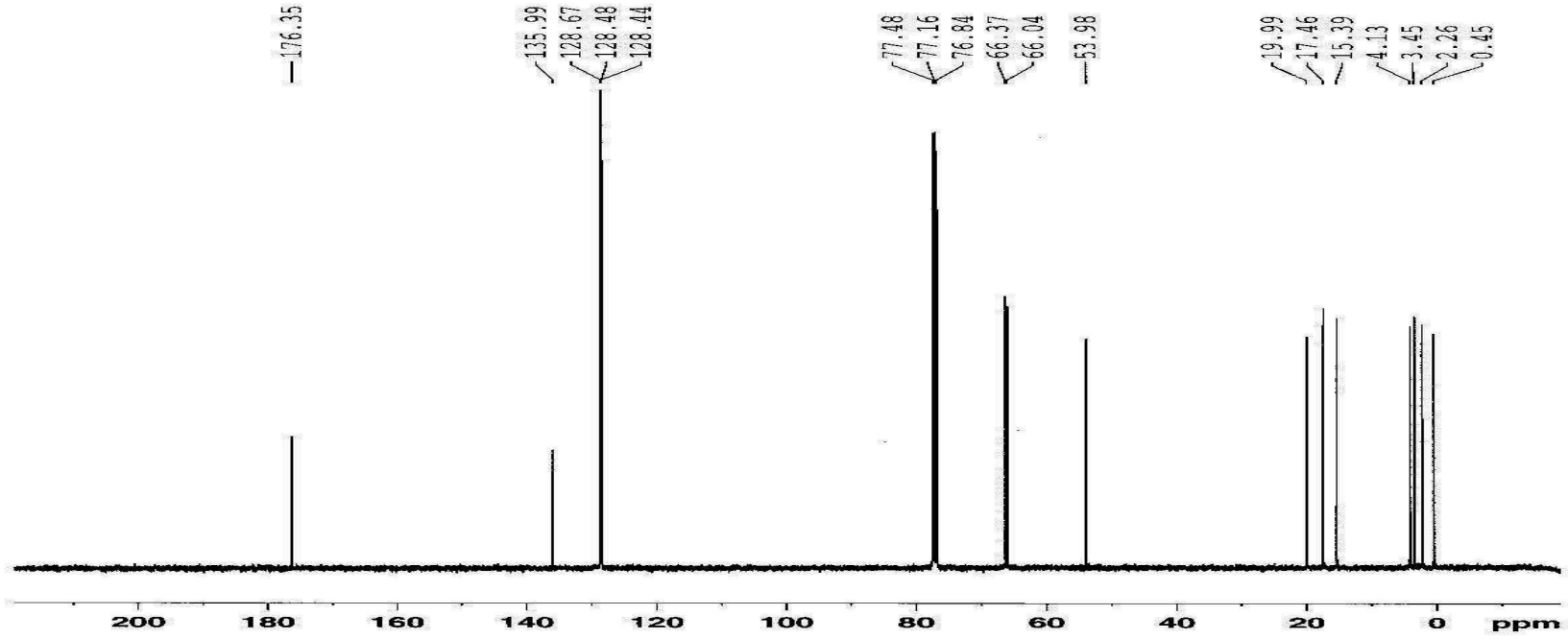
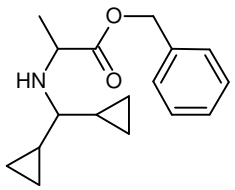


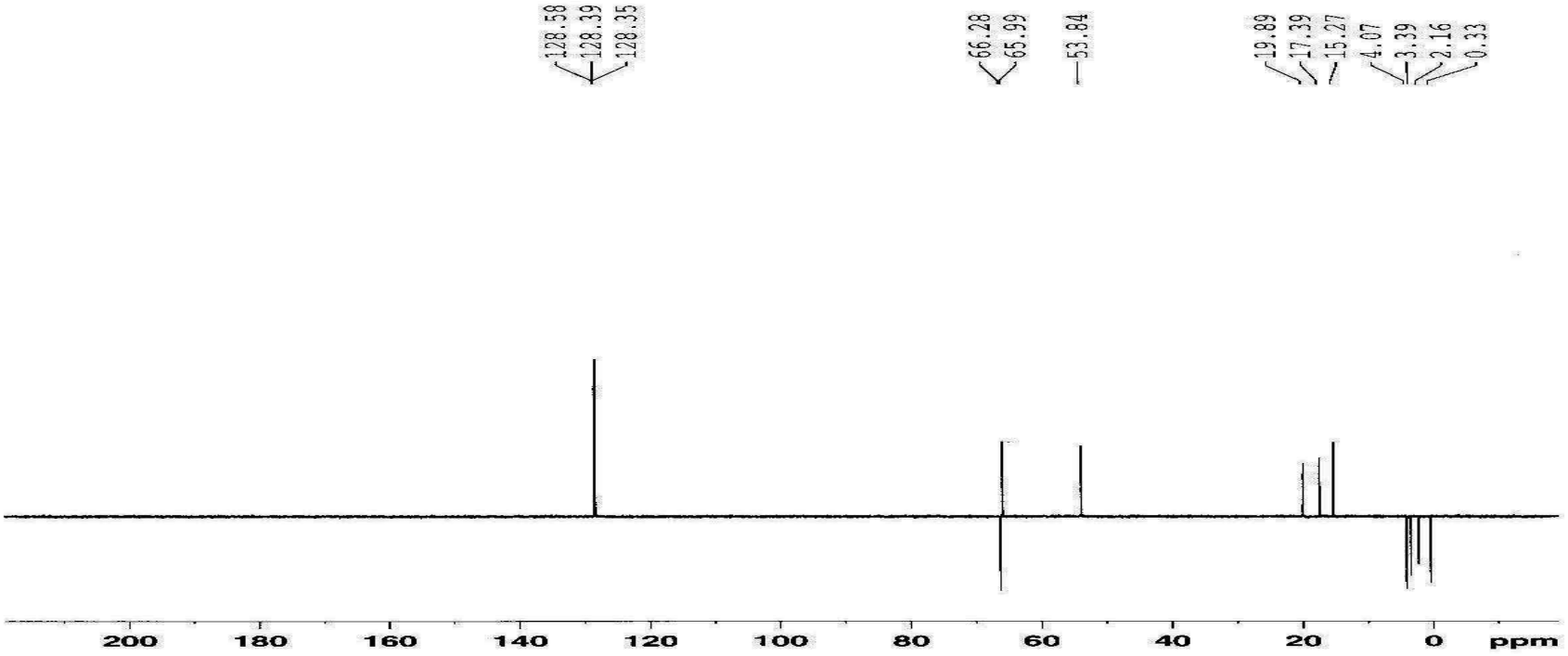


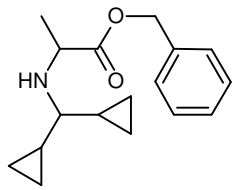
^{13}C-NMR (75 MHz, $CDCl_3$)



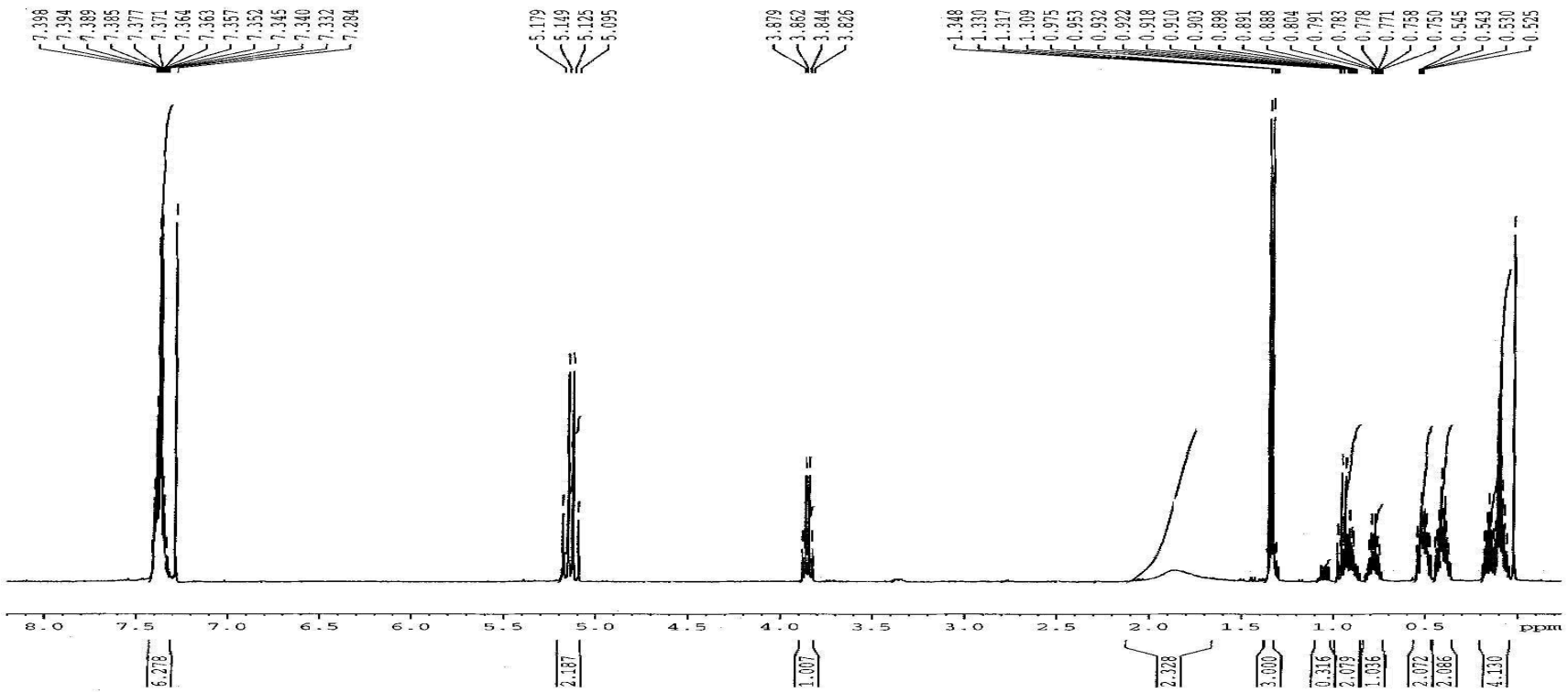


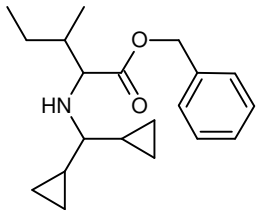
135 dept-NMR (75 MHz, $CDCl_3$)



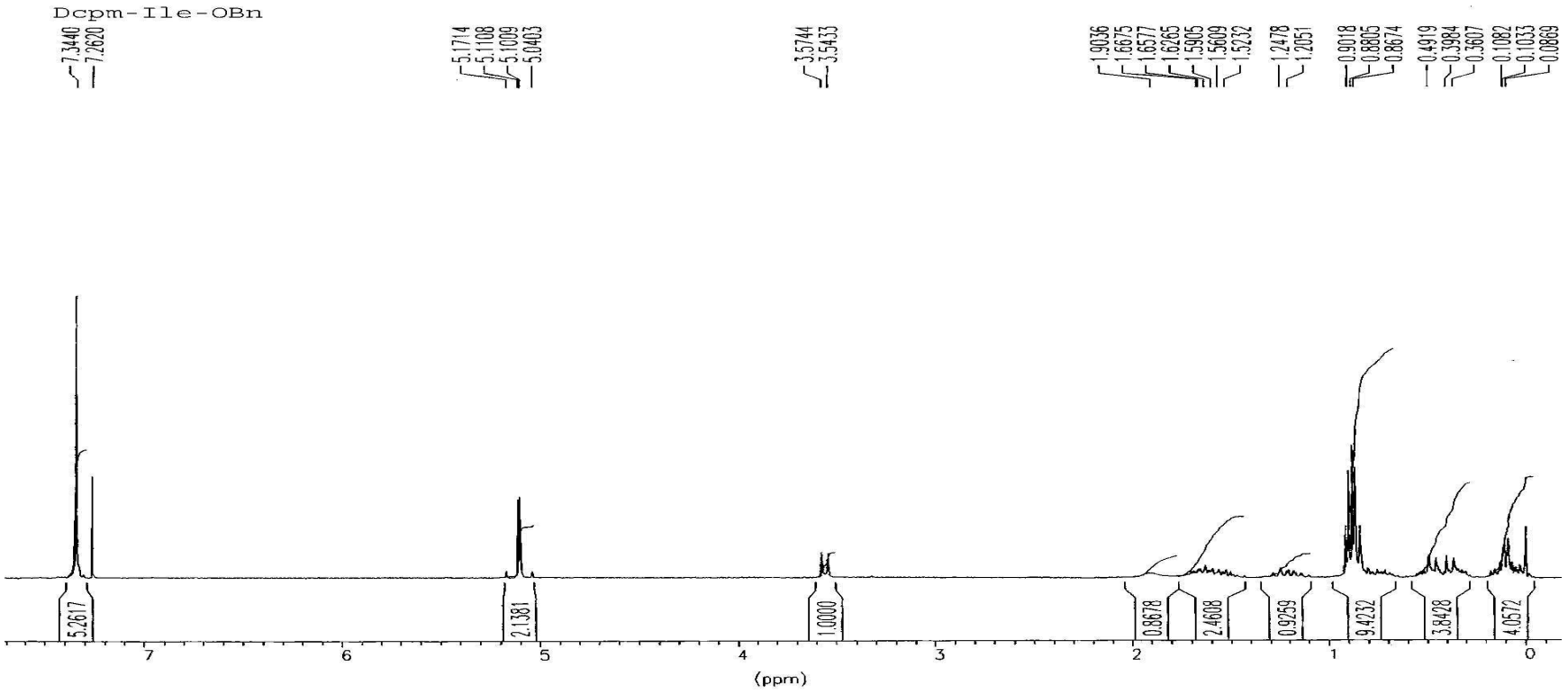


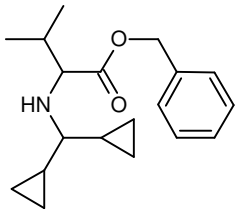
^{1}H-NMR (400 MHz, $CDCl_3$) of Dcpm-D-Ala-OBn



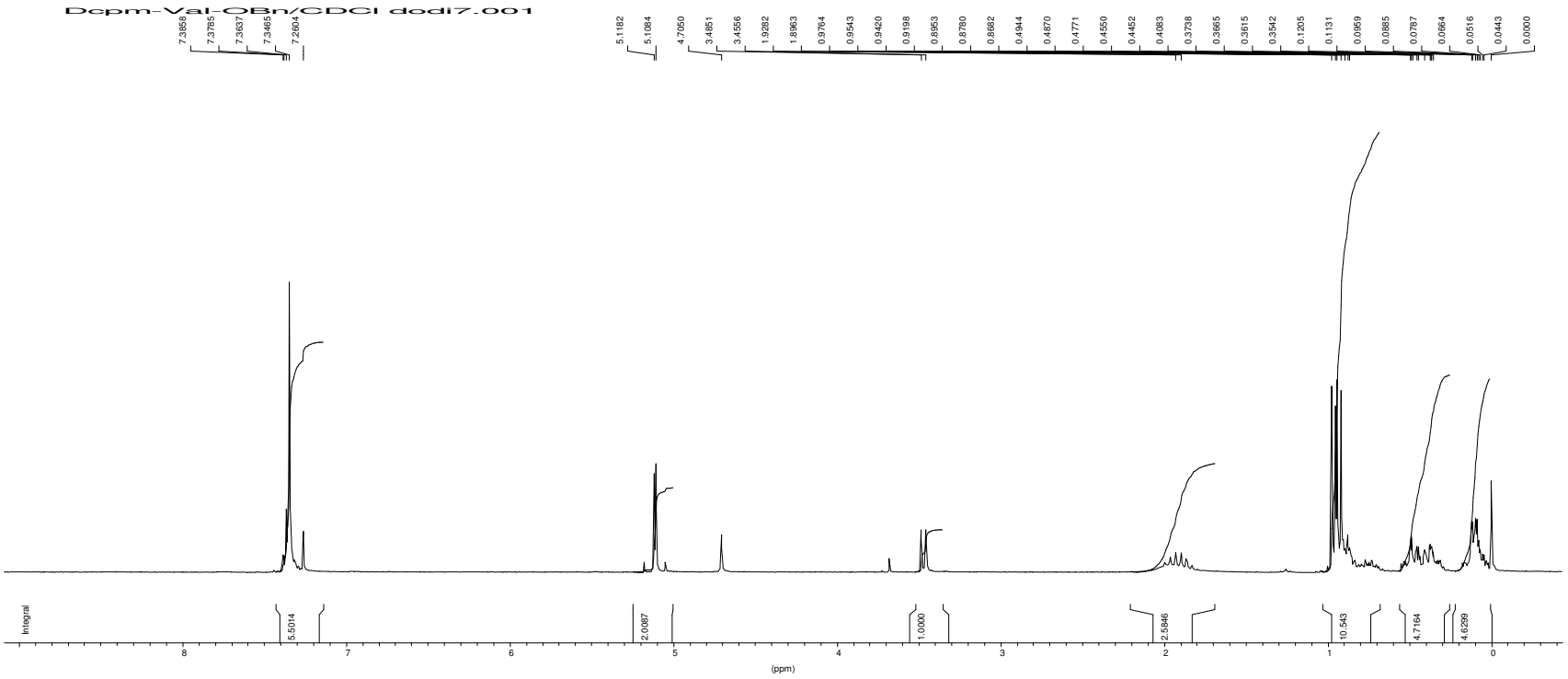


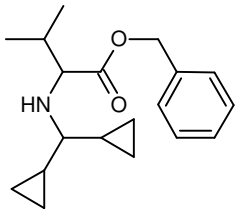
^{1}H-NMR (400 MHz, $CDCl_3$)



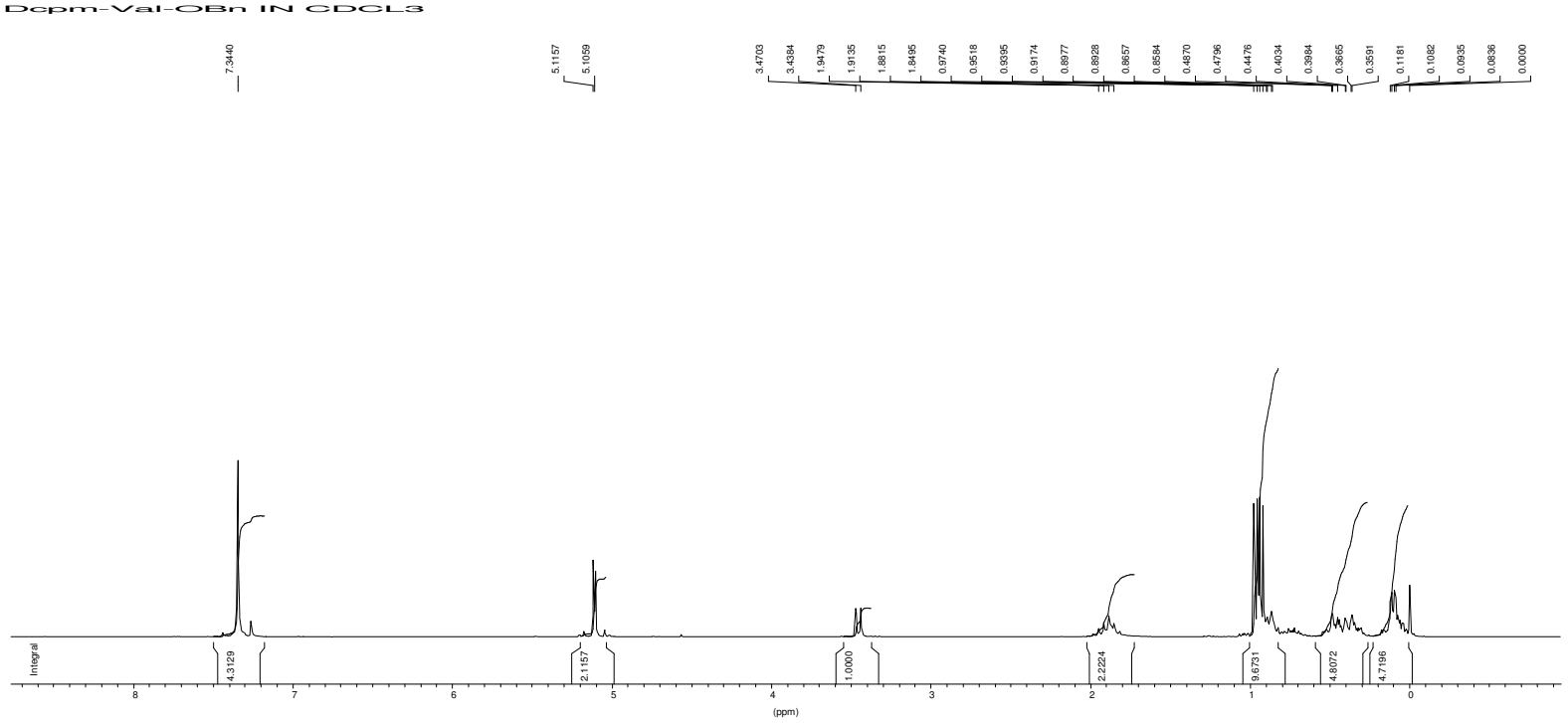


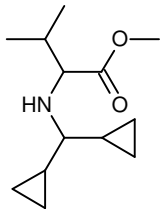
^{1}H-NMR (200 MHz, $CDCl_3$)



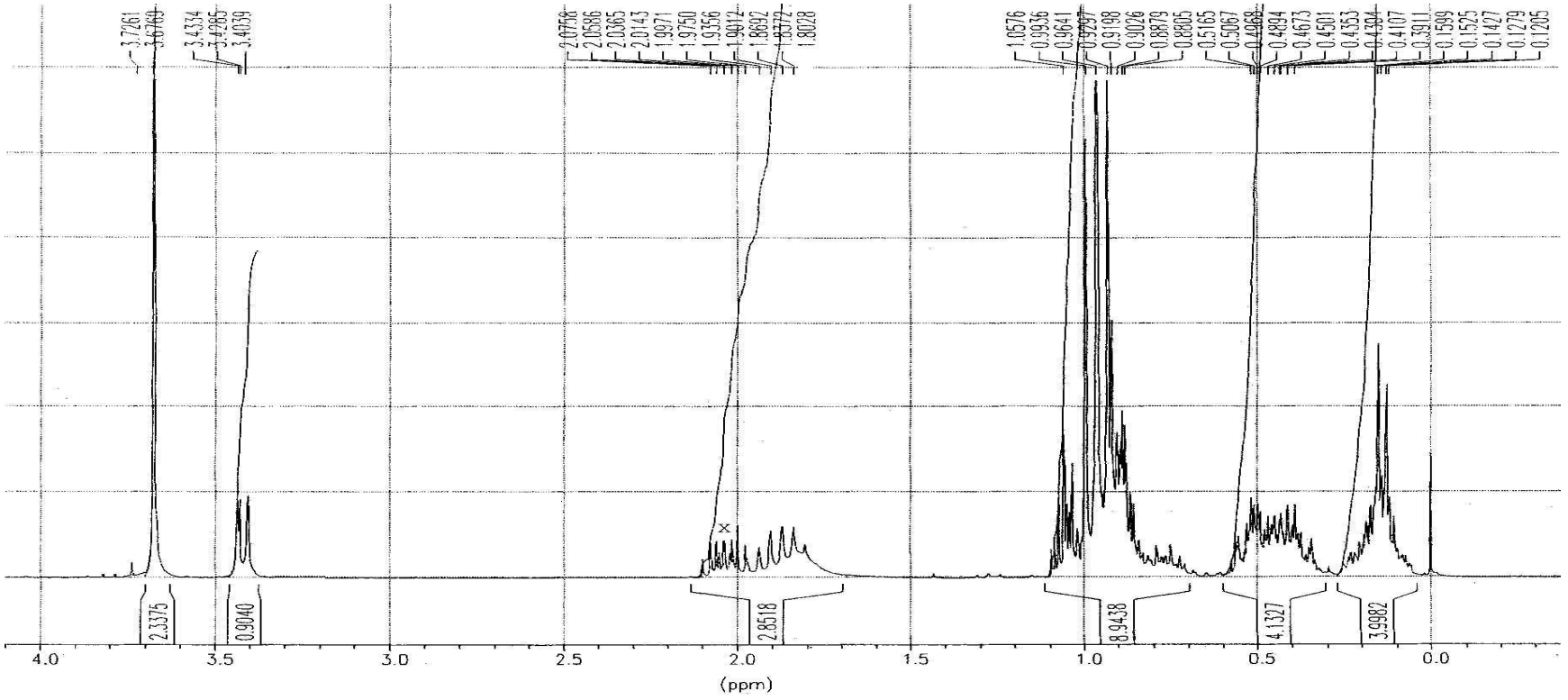


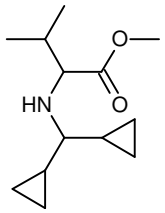
^{1}H-NMR (400 MHz, $CDCl_3$) of Dcpm-D-Val-OBn



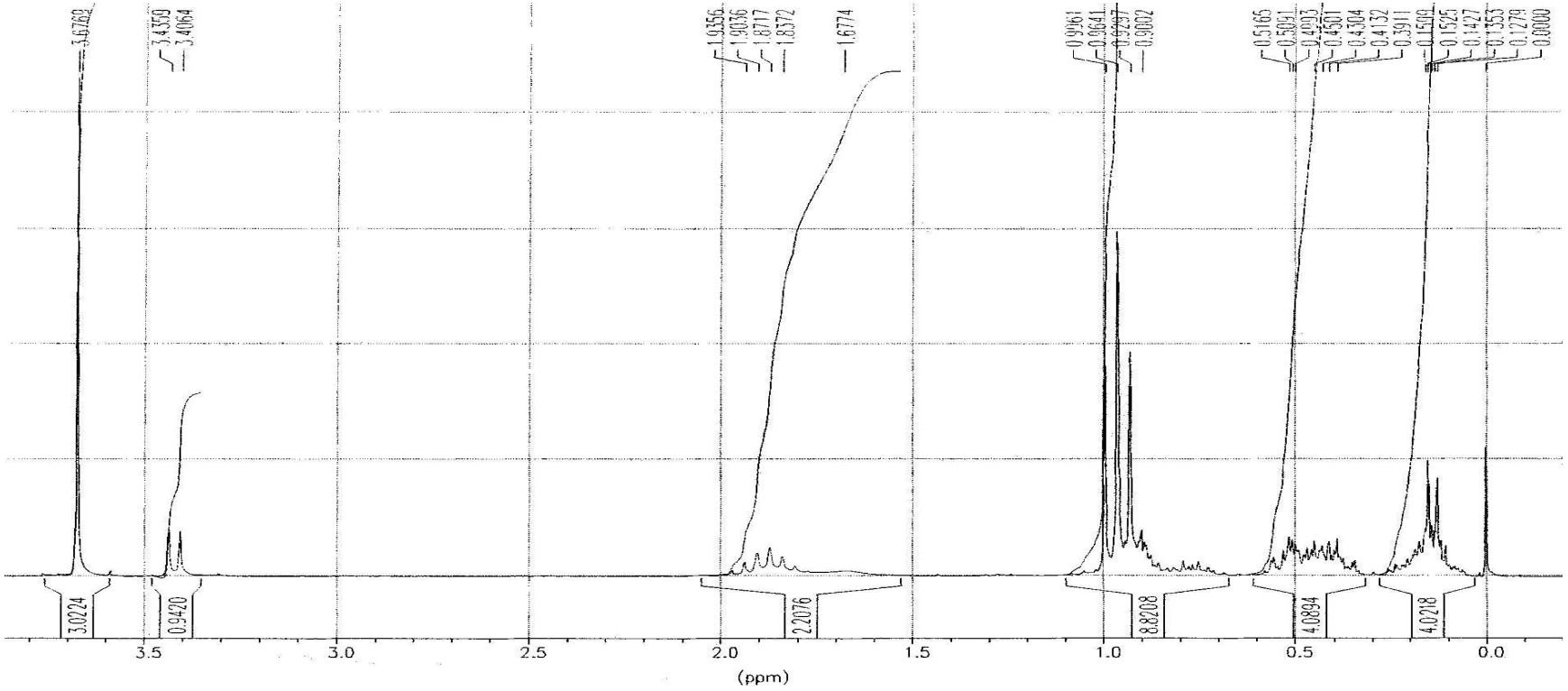


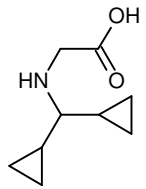
^{1}H-NMR (200 MHz, $CDCl_3$)



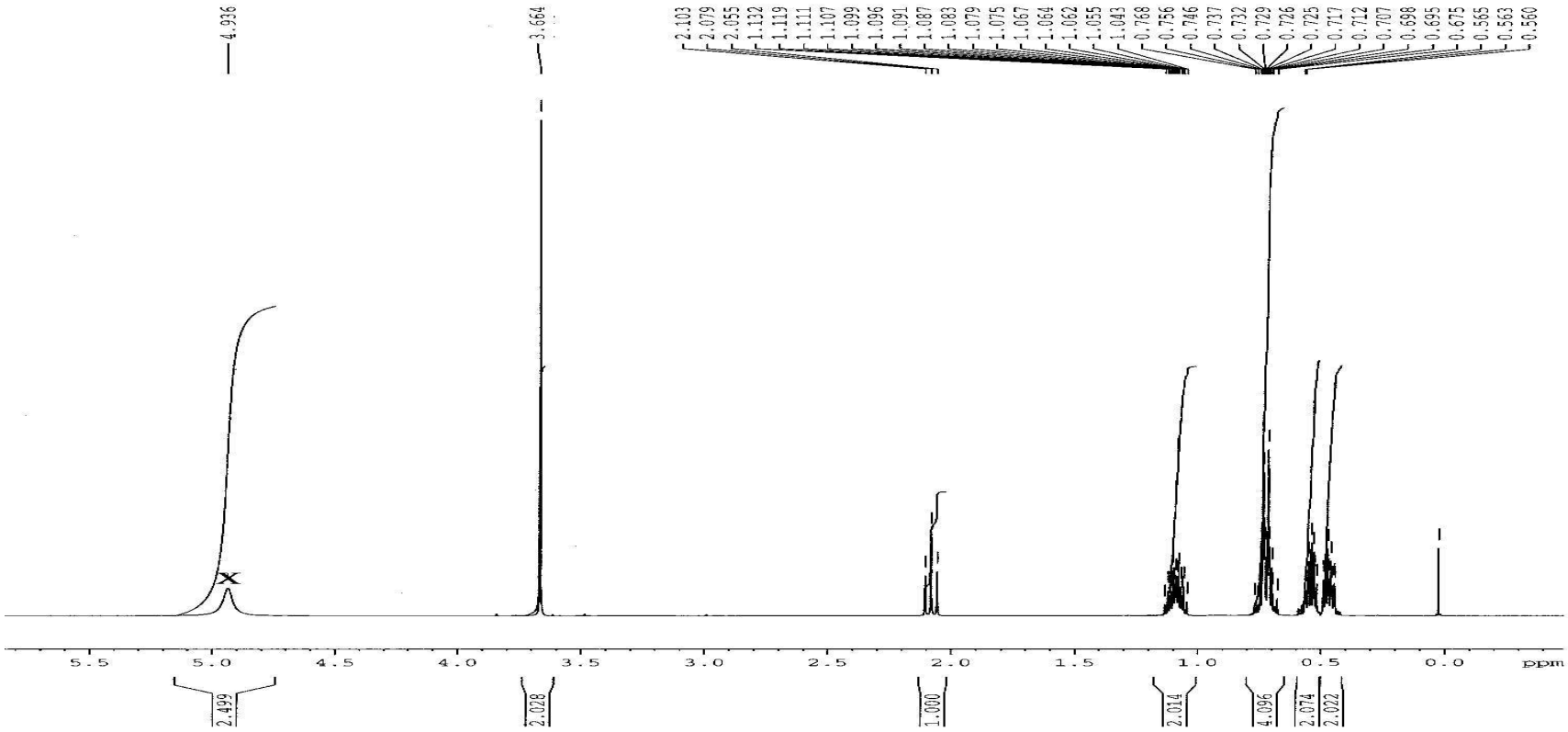


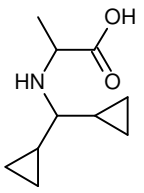
^{1}H-NMR (200 MHz, $CDCl_3$) of Dcpm-D-Val-OMe





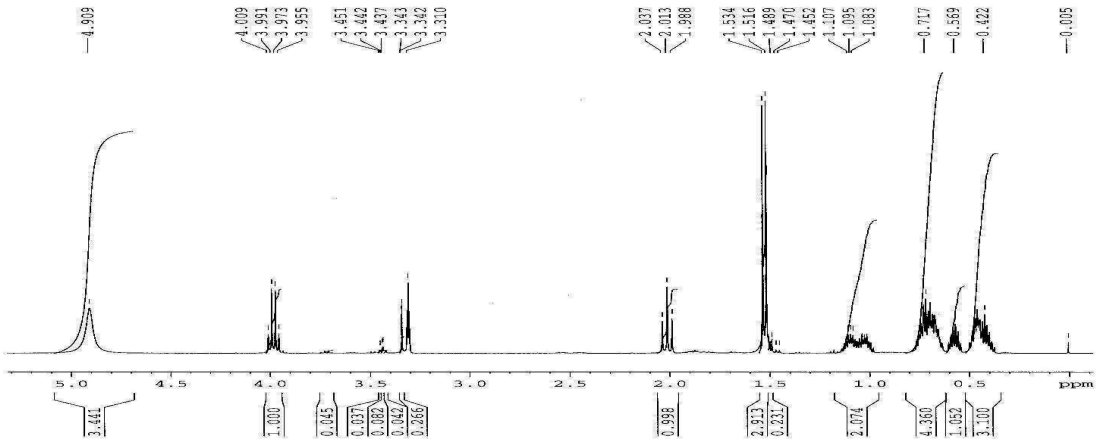
^{1}H-NMR (400 MHz, CD_3OD)



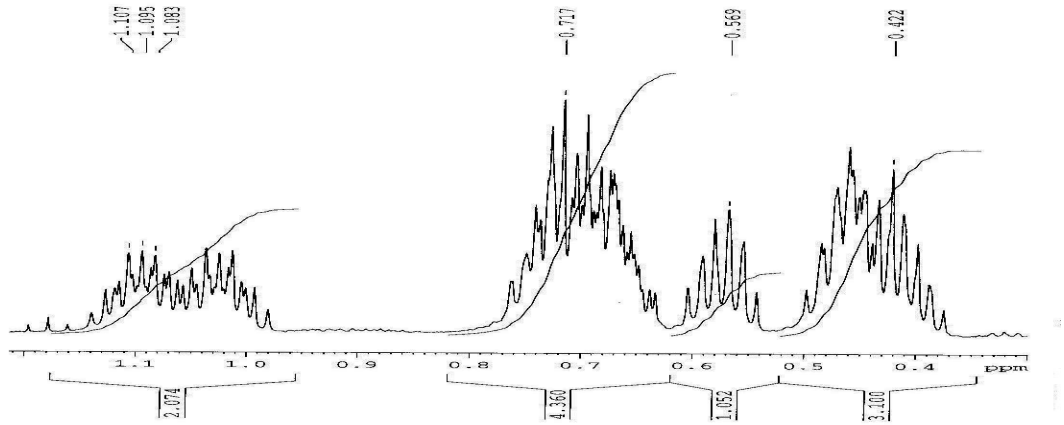


(A) ^{1}H-NMR (400 MHz, $CDCl_3$)
With Selected Regions
Expanded for Clarity (B and C)

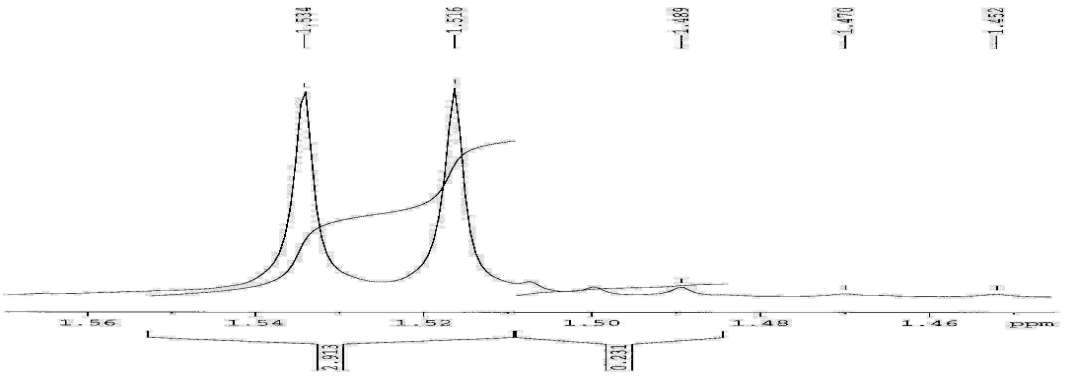
A

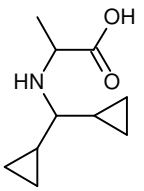


B



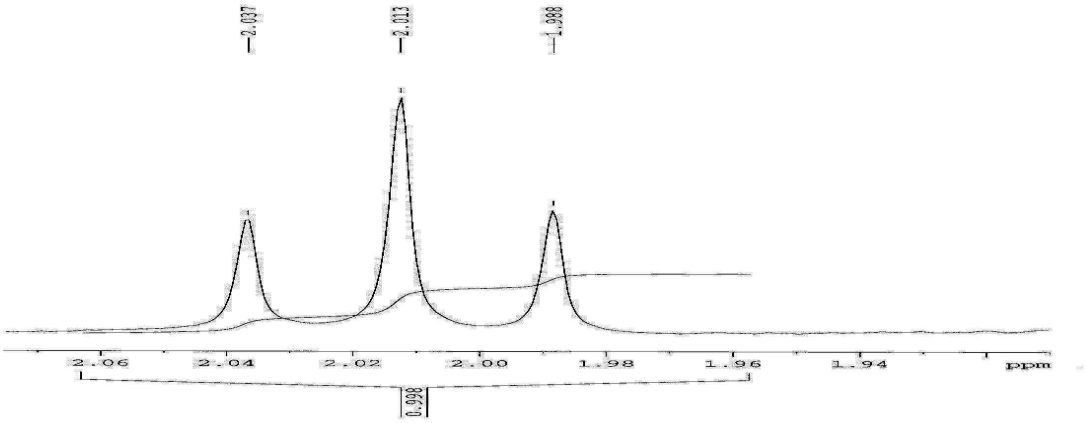
C



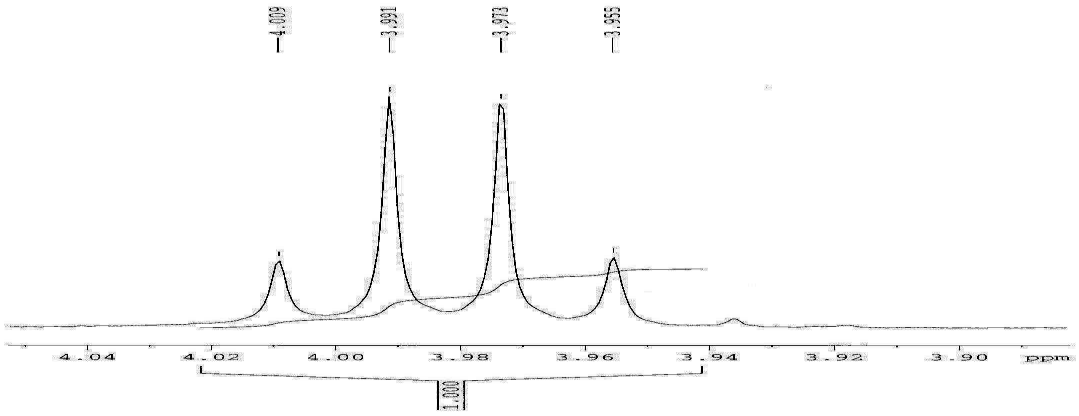


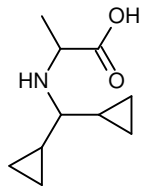
^{1}H-NMR (400 MHz, $CDCl_3$)
Selected Regions Expanded
for Clarity (D and E)

D

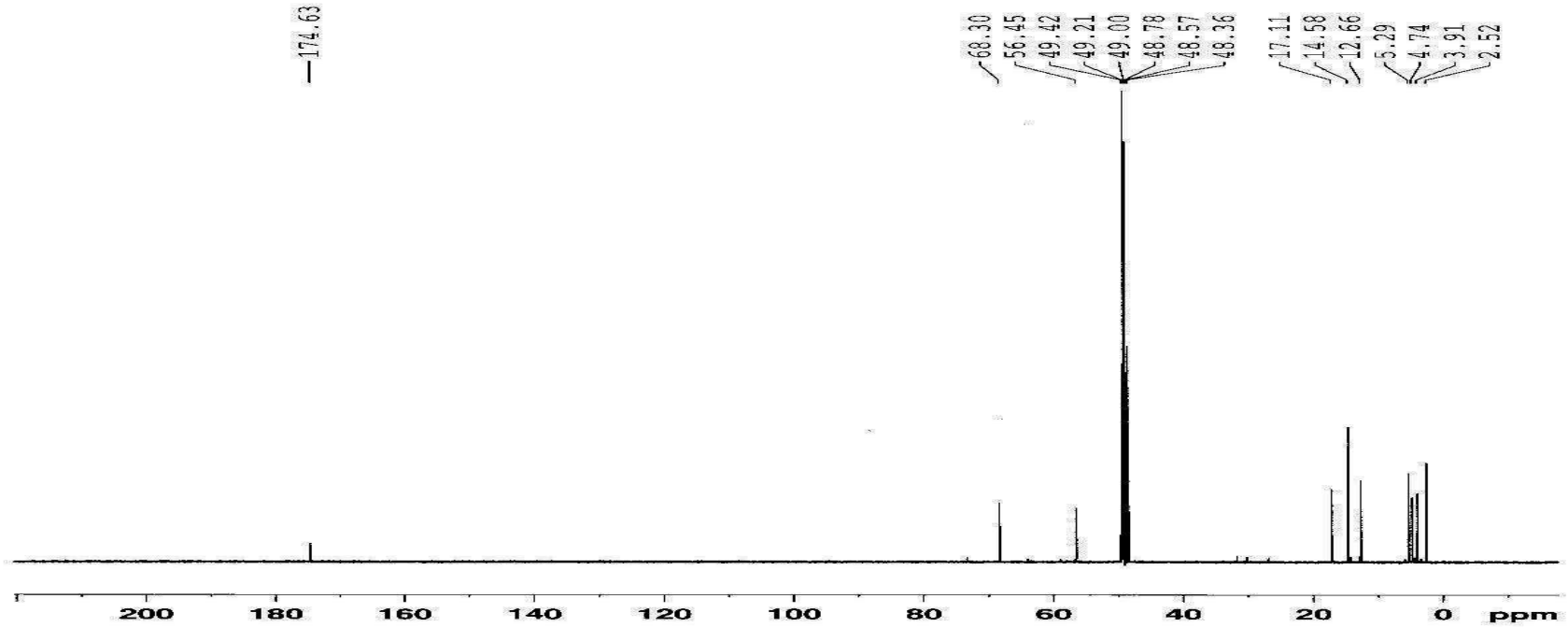


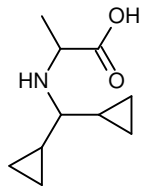
E



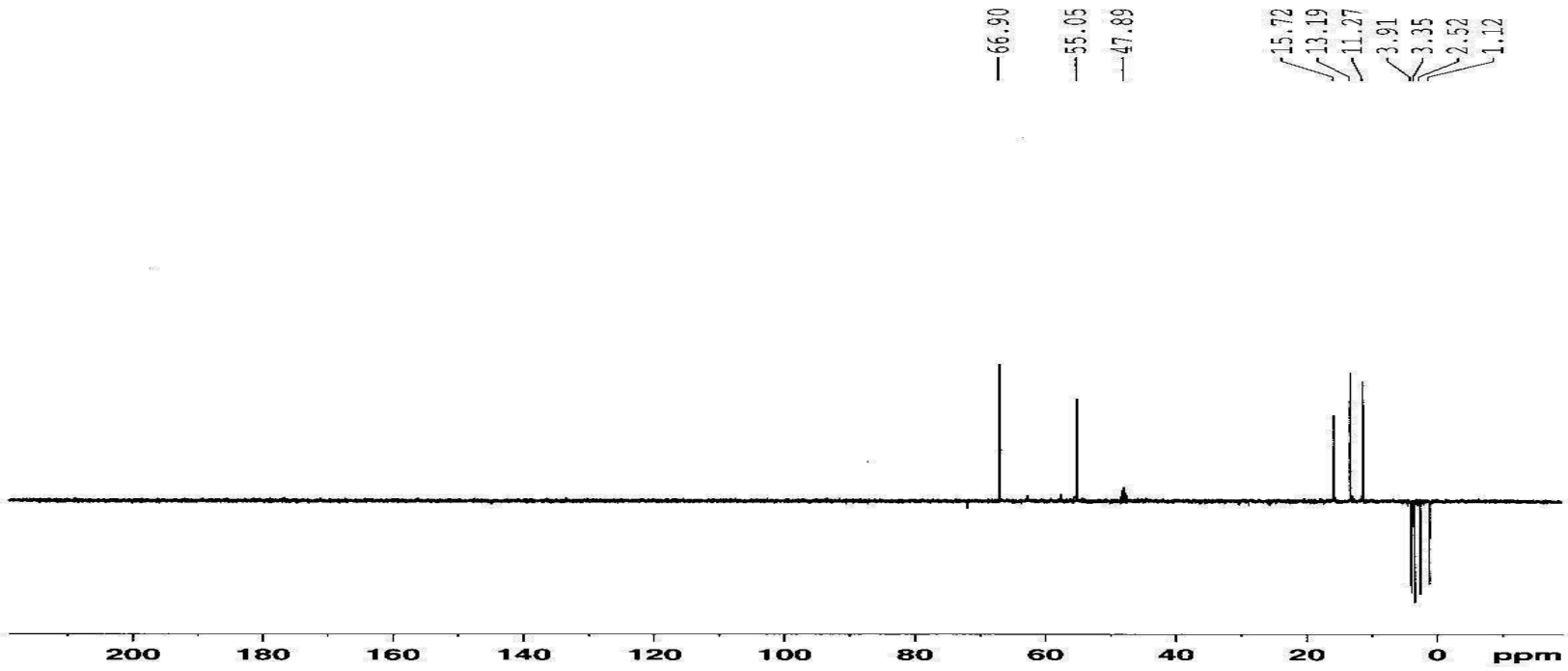


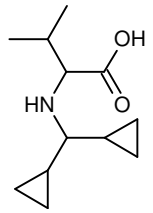
^{13}C-NMR (75 MHz, $CDCl_3$)



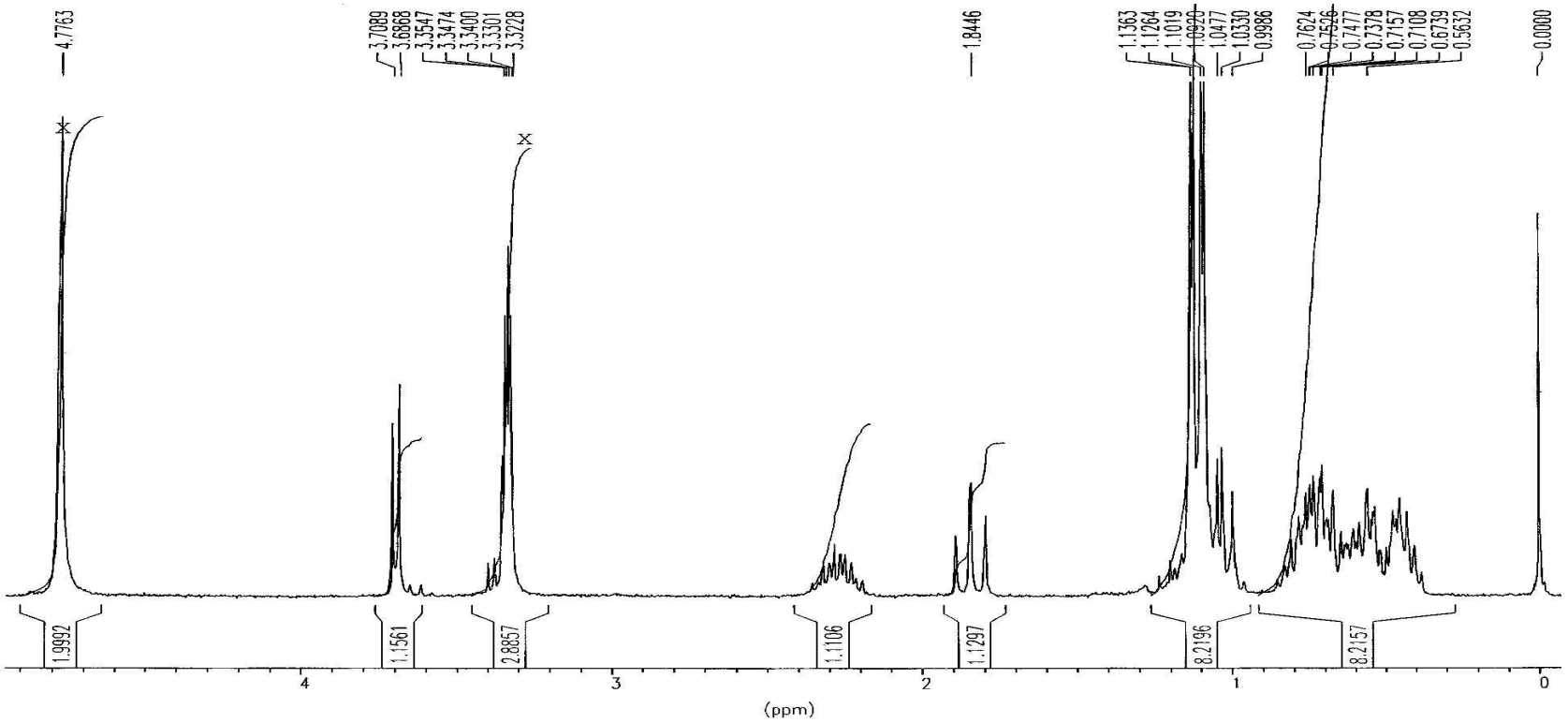


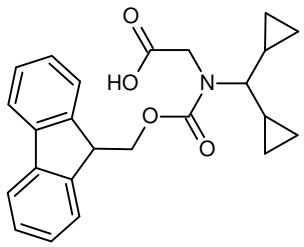
135 dept-NMR (75 MHz, $CDCl_3$)



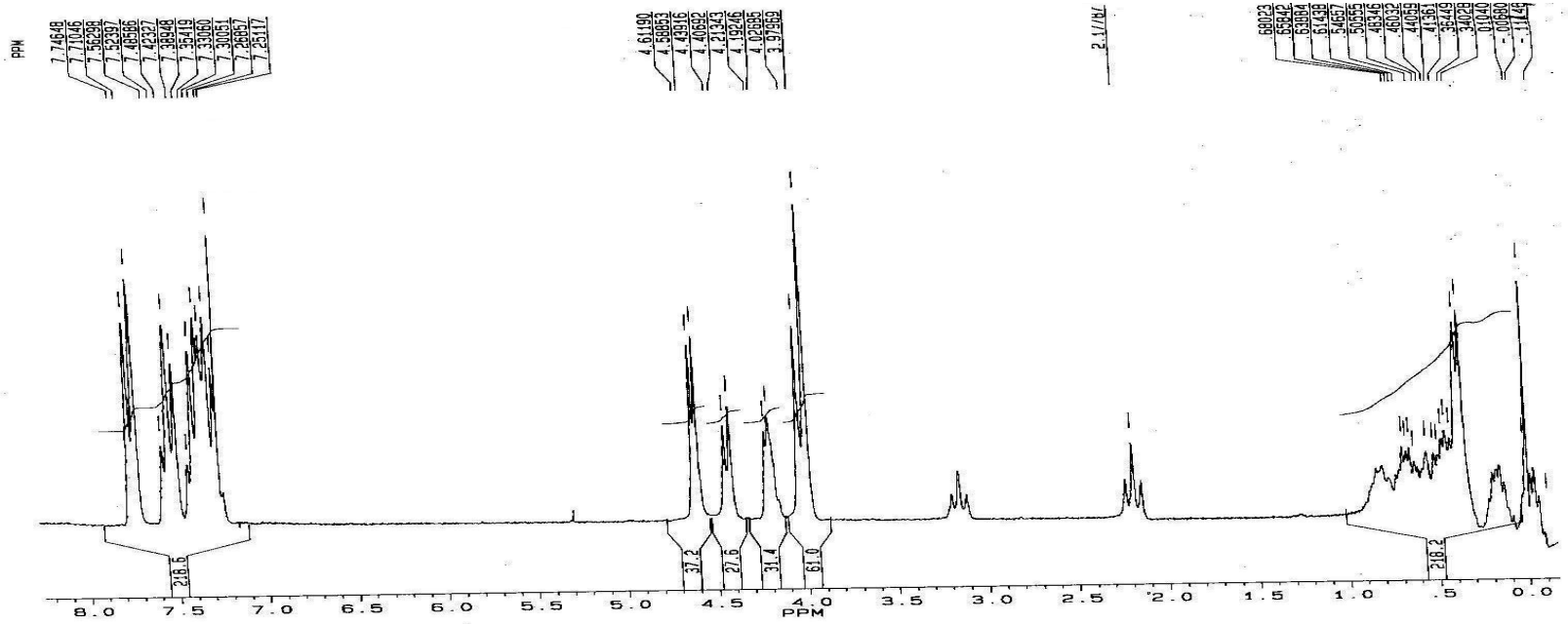


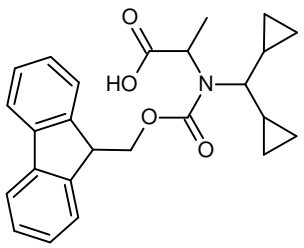
^{1}H-NMR (200 MHz, CD_3OD)





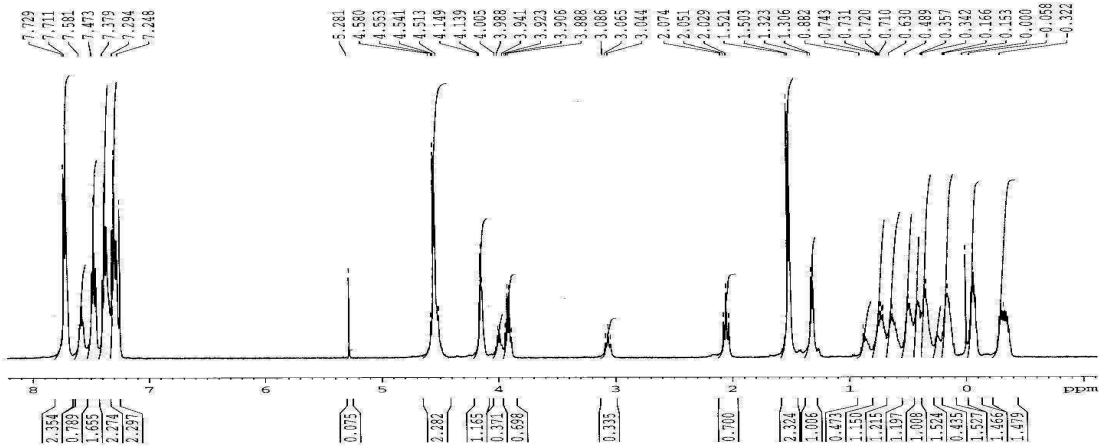
^{1}H-NMR (400 MHz, $CDCl_3$)



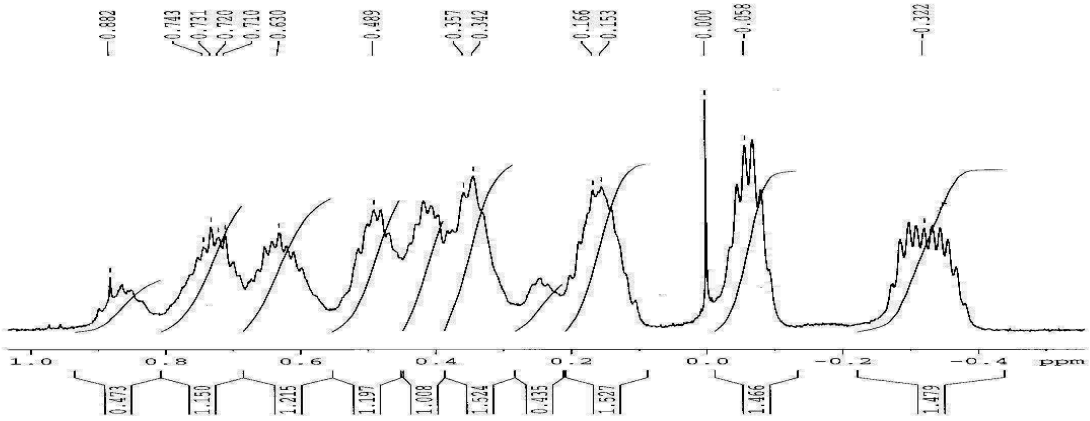


(A) ^{1}H-NMR (400 MHz, $CDCl_3$)
With Selected Regions
Expanded for Clarity (B and C)

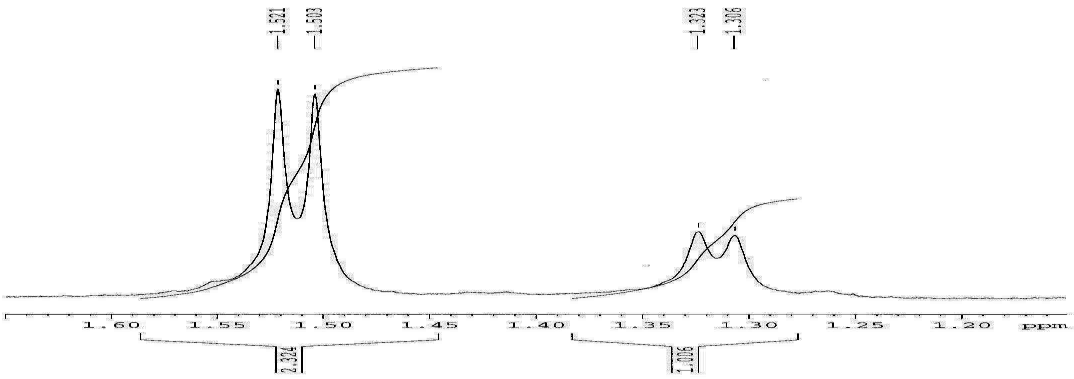
A

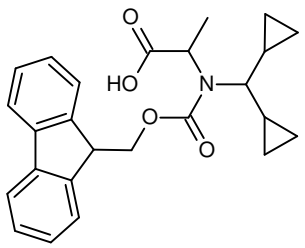


B



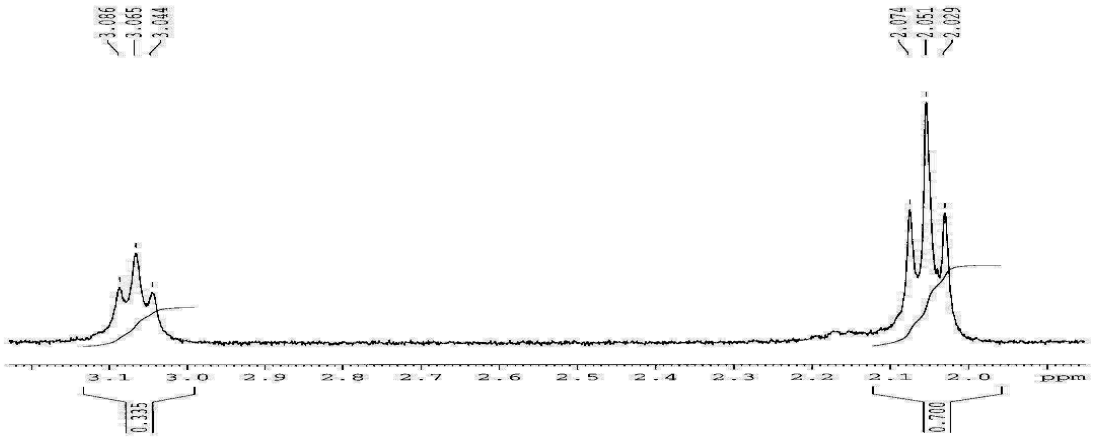
C



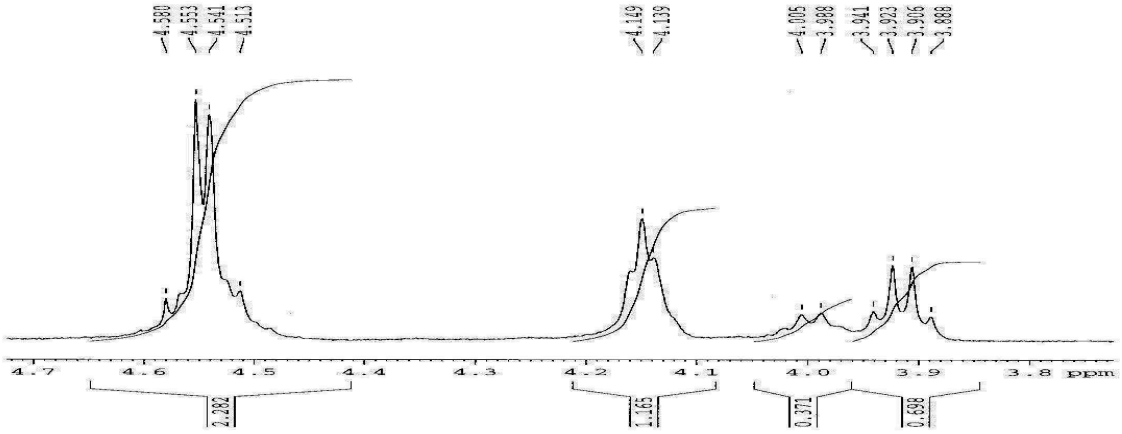


^{1}H-NMR (400 MHz, $CDCl_3$)
Selected Regions Expanded
for Clarity (D-F)

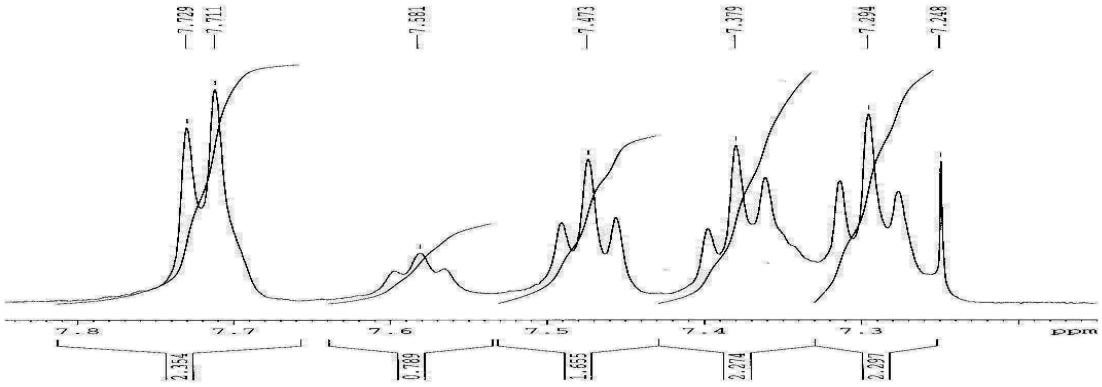
D

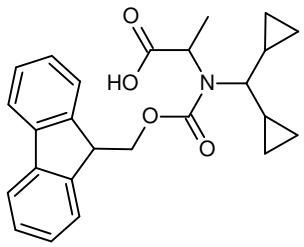


E

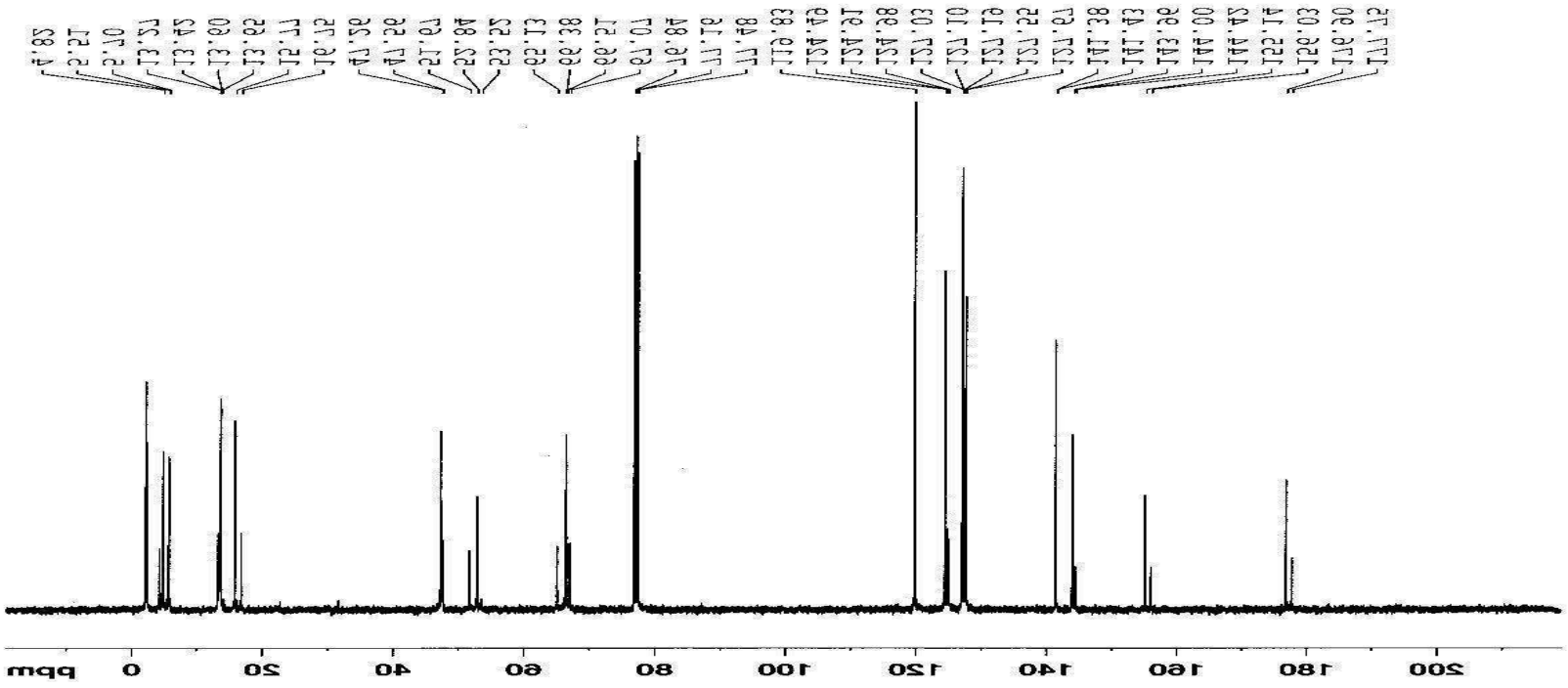


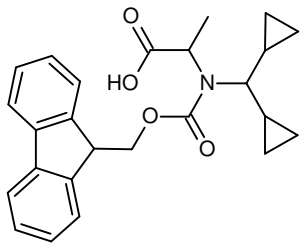
F



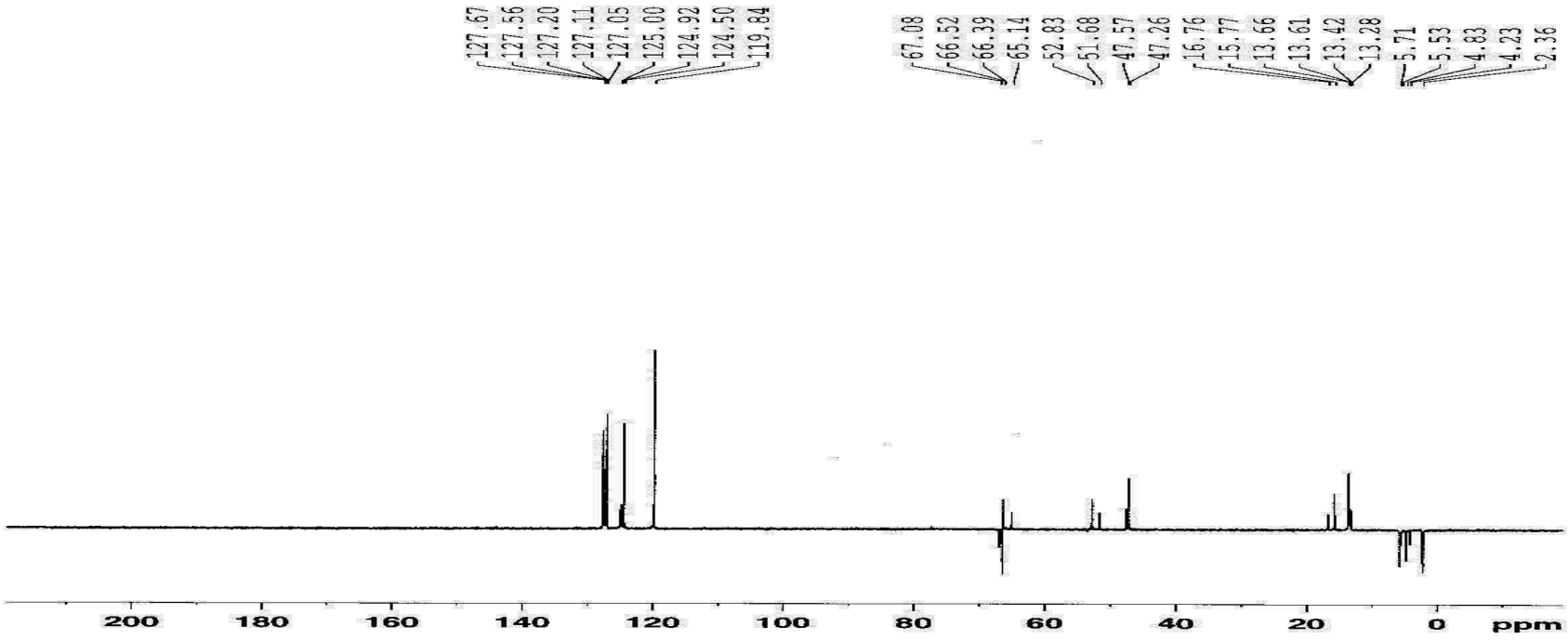


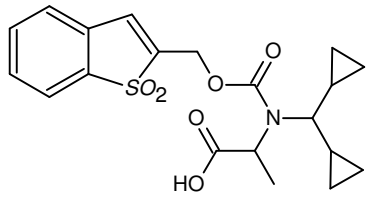
^{13}C-NMR (75 MHz, $CDCl_3$)



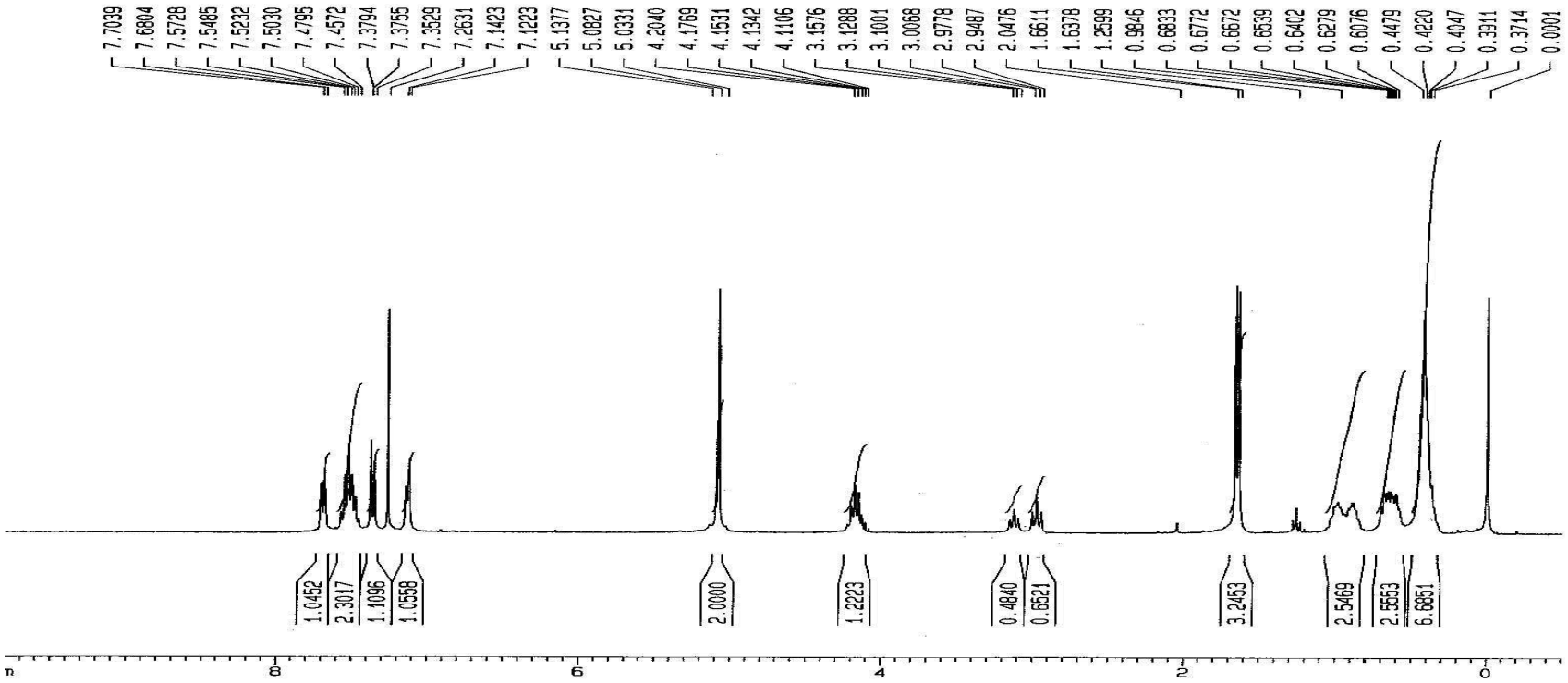


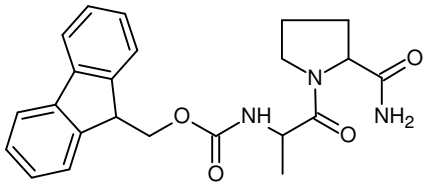
135 dept-NMR (75 MHz, $CDCl_3$)





^{1}H-NMR (200 MHz, $CDCl_3$)

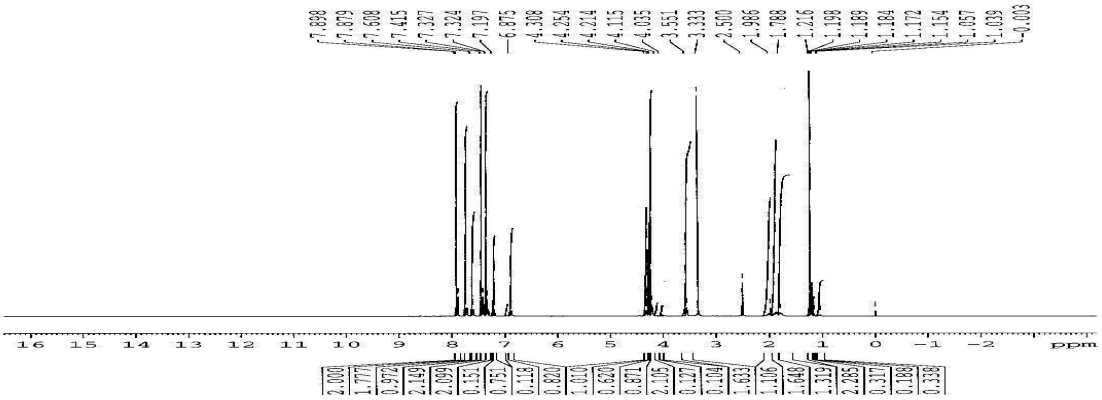




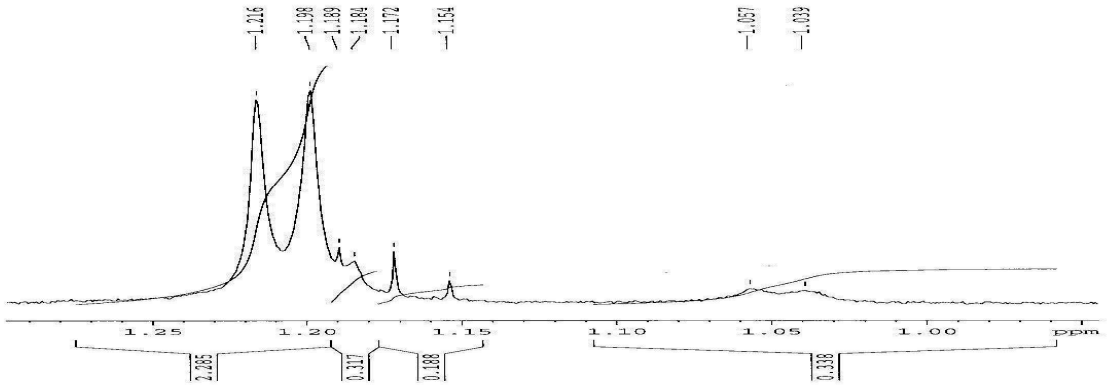
(A) ^{1}H-NMR (400 MHz, $CDCl_3$)
With Selected Regions
Expanded for Clarity (B and C)

^{1}H-NMR (400 MHz, DMSO) for Fmoc-Ala-Pro-NH_2

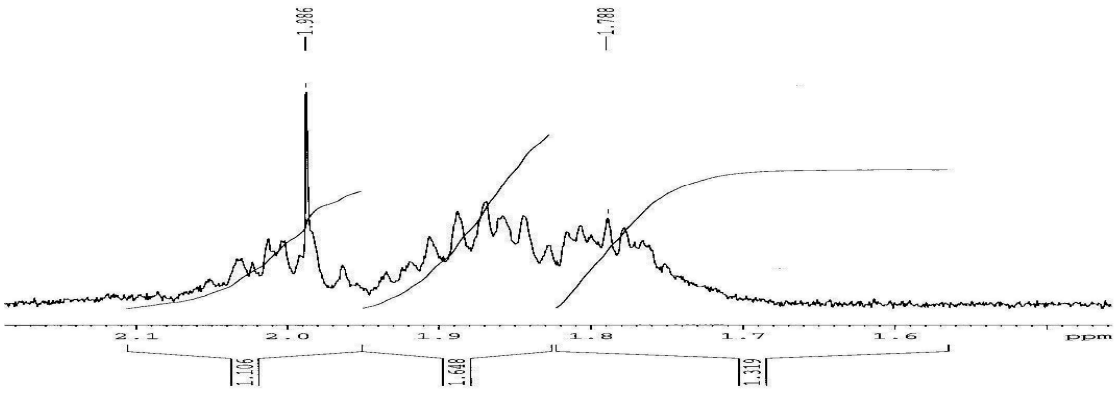
A

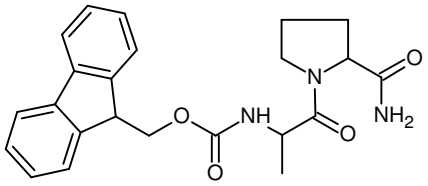


B



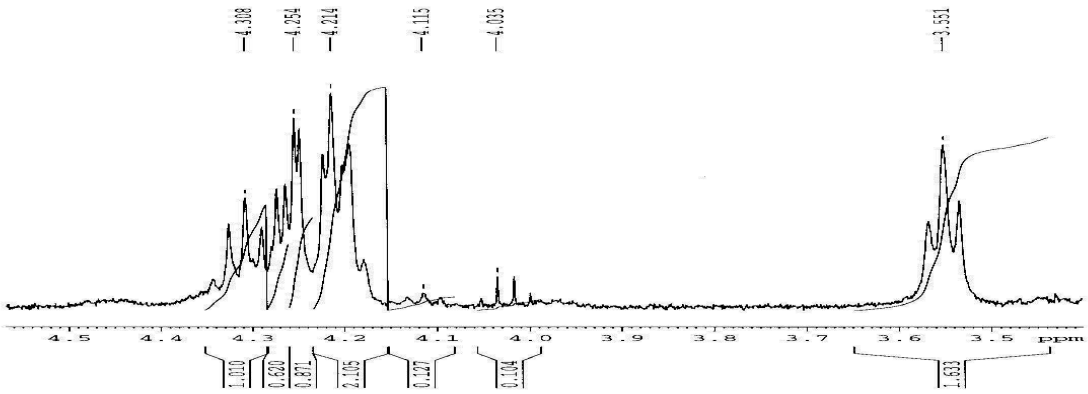
C



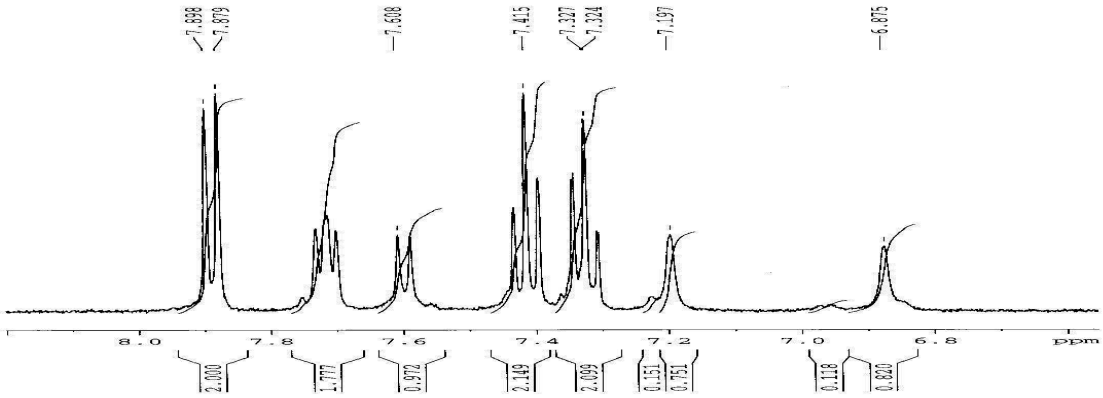


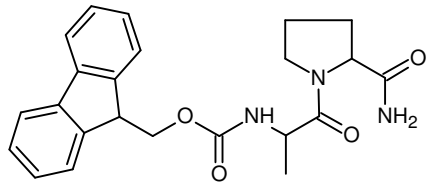
^{1}H-NMR (400 MHz, $CDCl_3$)
Selected Regions Expanded
for Clarity (D and E)

D

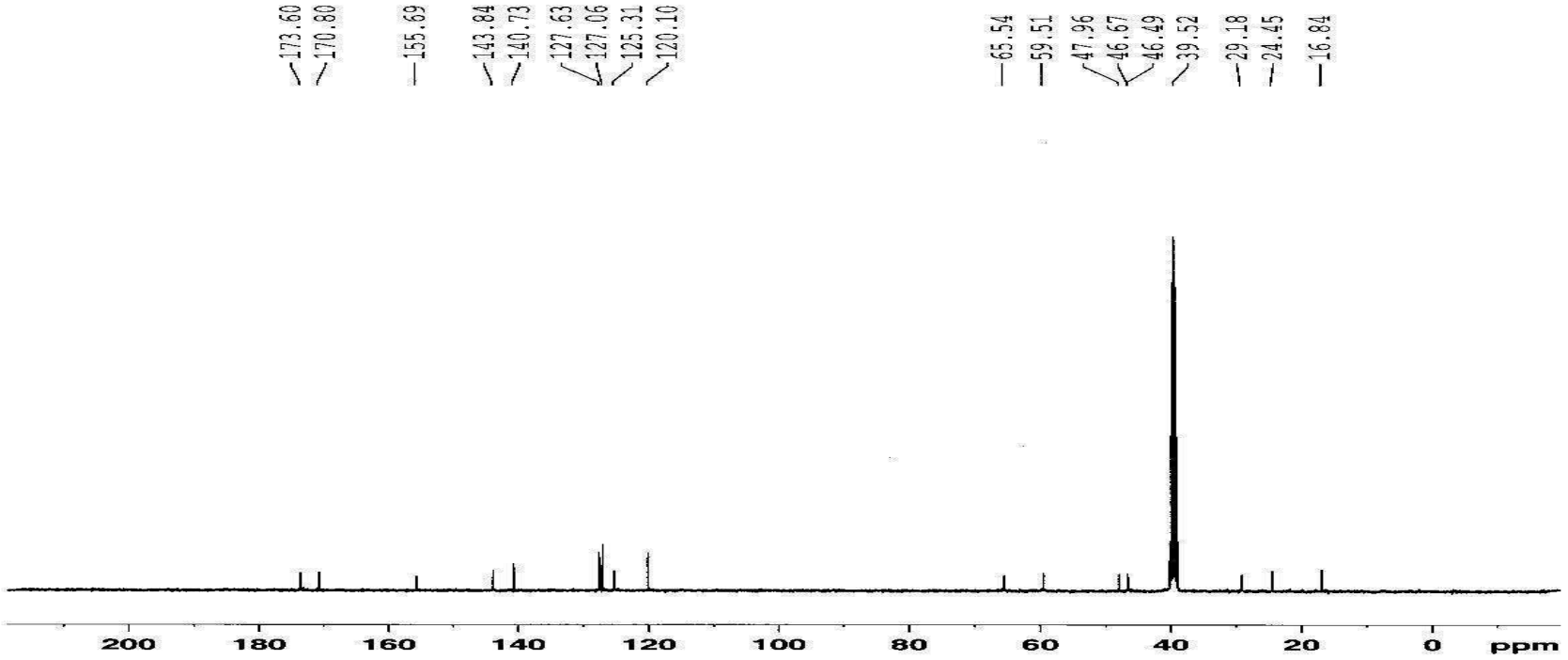


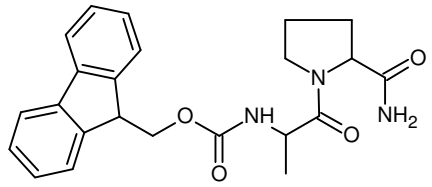
E



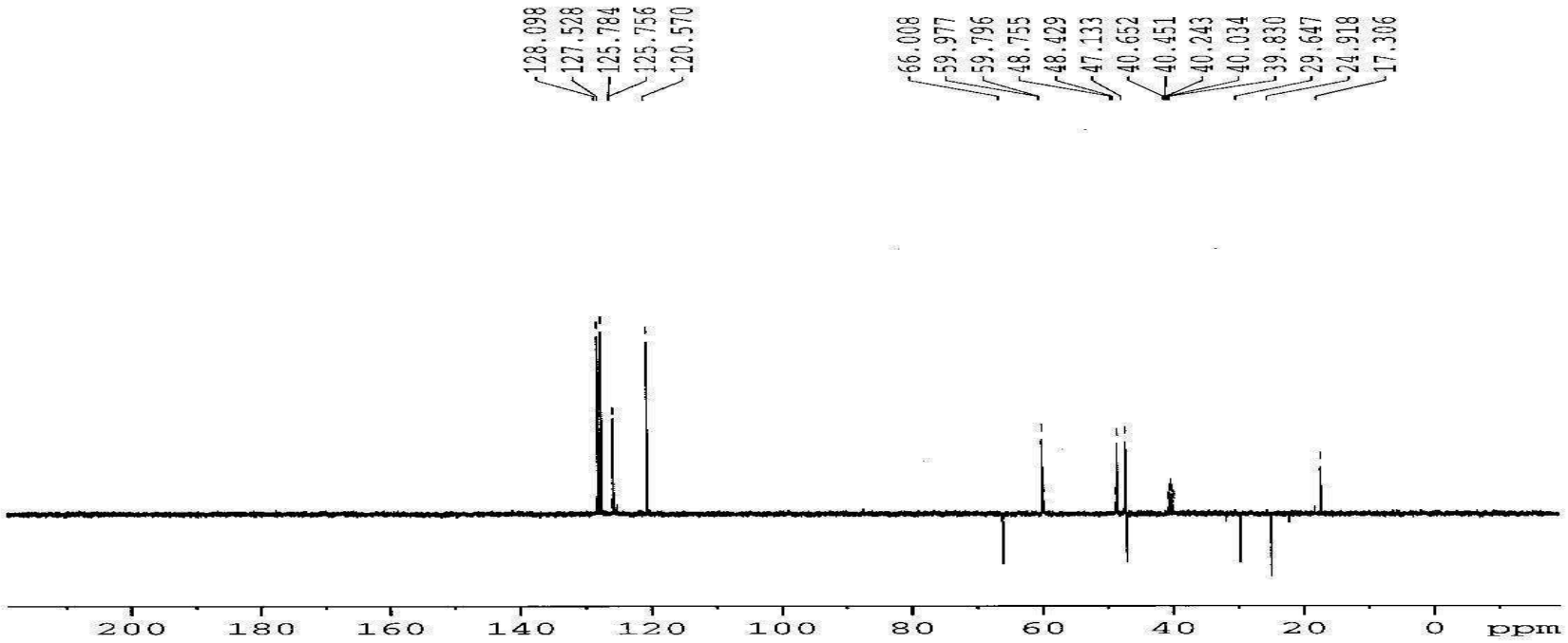


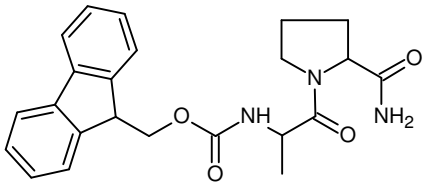
^{13}C-NMR (75 MHz, DMSO) for Fmoc-Ala-Pro-NH_2





135 dept-NMR (75 MHz, DMSO) for Fmoc-Ala-Pro-NH_2

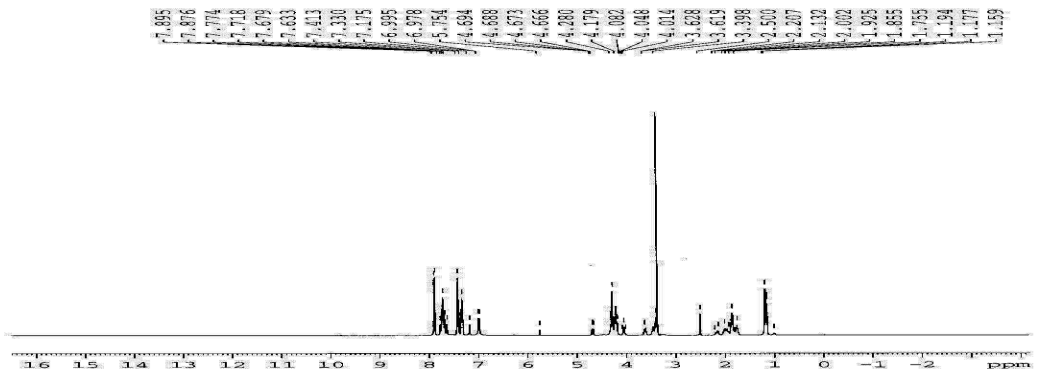




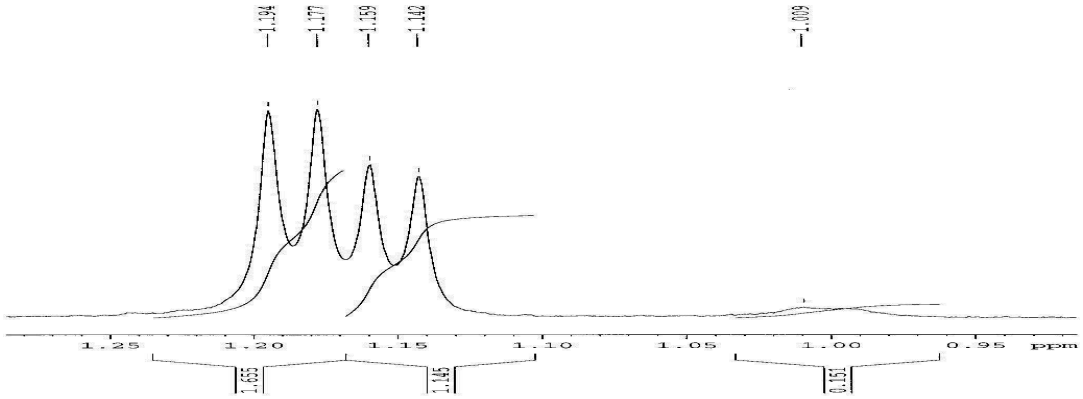
(A) ^{1}H-NMR (400 MHz, $CDCl_3$)
With Selected Regions
Expanded for Clarity (B and C)

^{1}H-NMR (400 MHz, DMSO) for Fmoc-D-Ala-Pro-NH_2

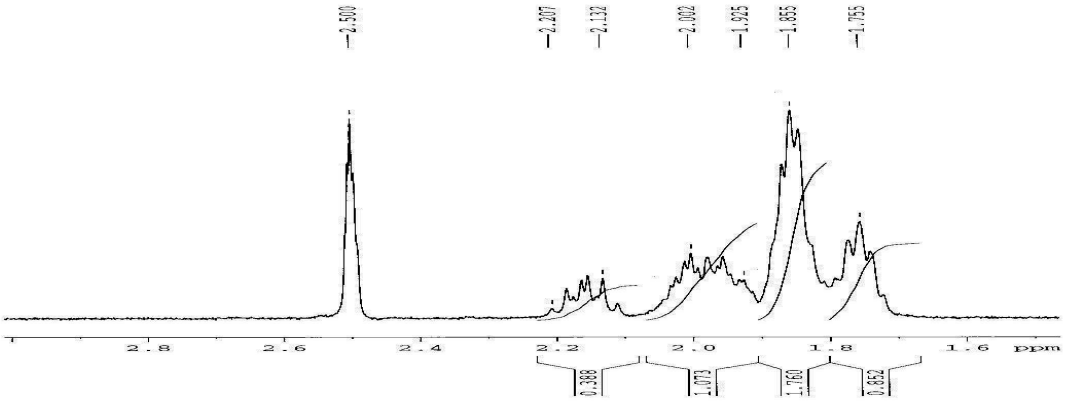
A

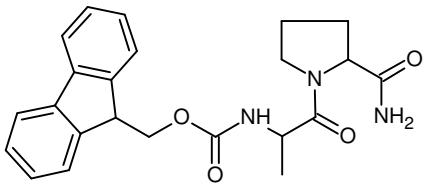


B



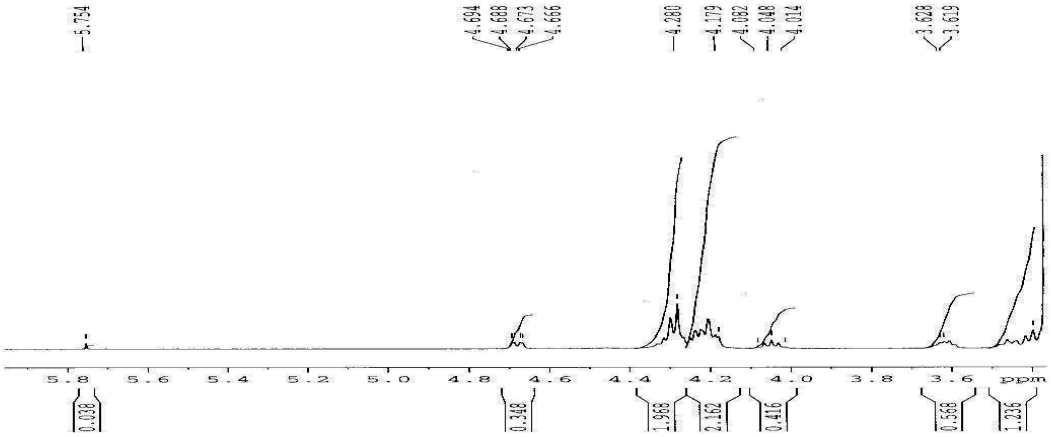
C



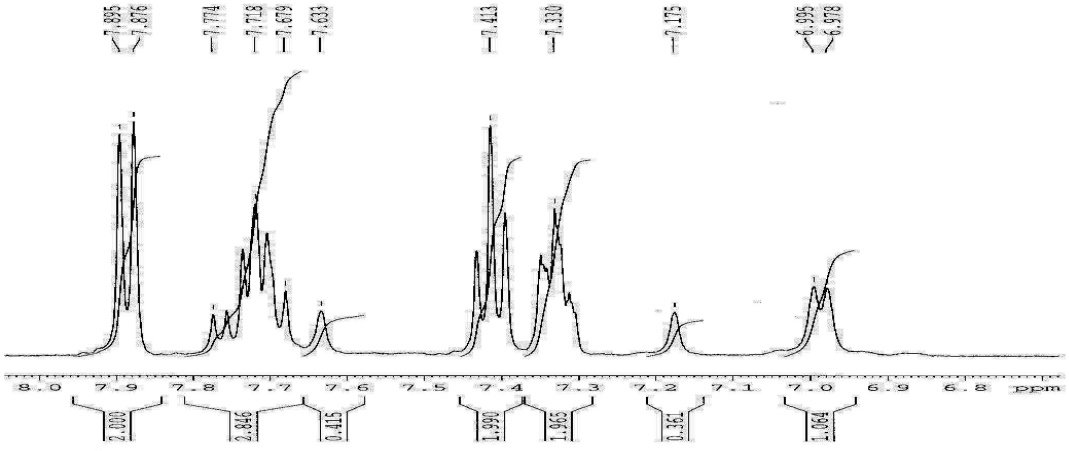


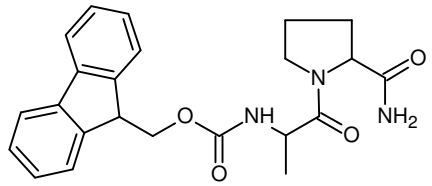
^{1}H-NMR (400 MHz, $CDCl_3$)
Selected Regions Expanded
for Clarity (D and E)

D

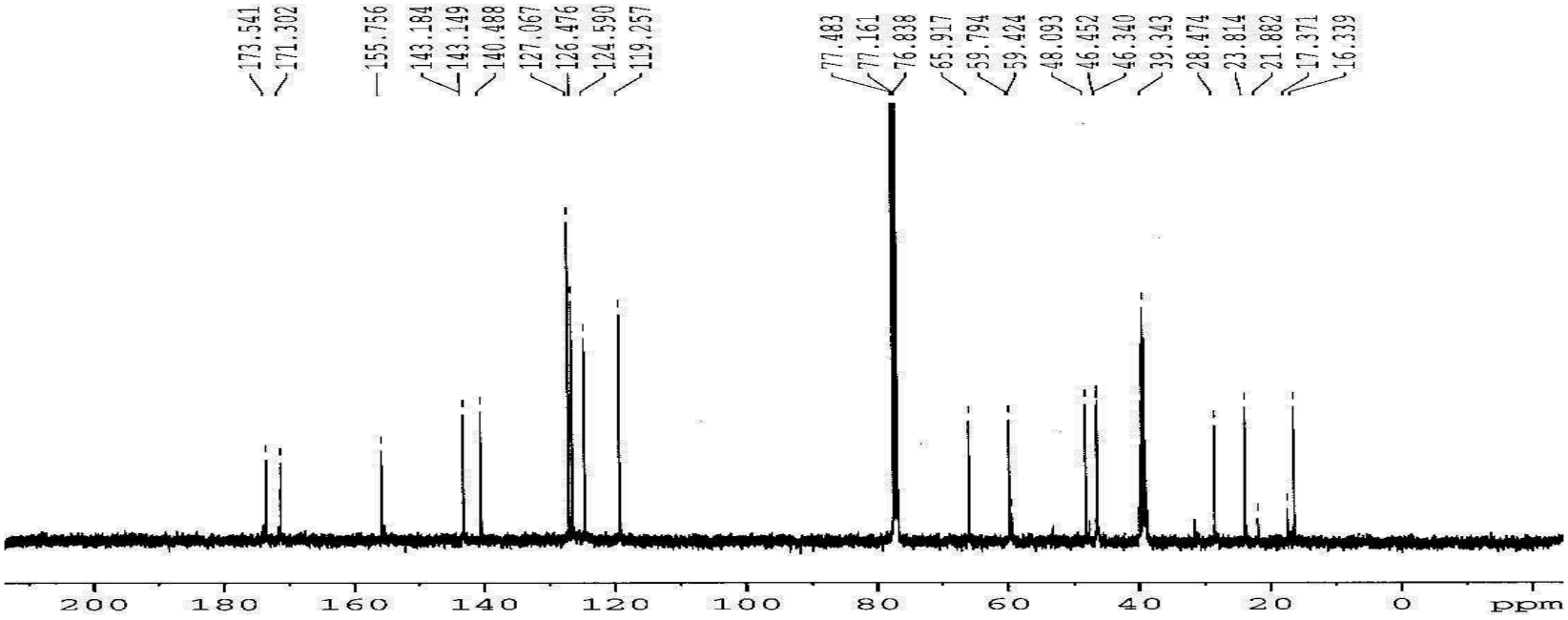


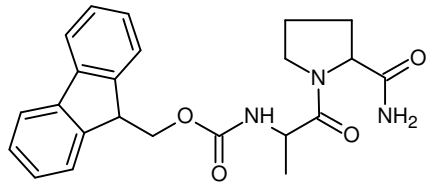
E





^{13}C-NMR (75 MHz, DMSO + $CDCl_3$) for Fmoc-D-Ala-Pro-NH_2





135 dept-NMR (75 MHz, DMSO + $CDCl_3$) for Fmoc-D-Ala-Pro-NH_2