CHAPTER IV

RESULTS AND DISCUSSION

In this research, twelve amide derivatives of valproic acid expected to possess anticonvulsant activity were synthesized. These compounds can be divided into two groups based on chemical structures: the compounds of which chemical structures related to amino acid, and the hydroxy-, alkoxy-, acyloxy-methyl derivatives of valproic acid.

For the first group of compounds, amino acid or amino acid ester was acylated with 2-propylpentanoyl chloride to provide the N-(2-propylpentanoyl) amino acid and N-(2-propylpentanoyl) amino acid ester. The N-(2-propylpentanoyl) amino acid benzylamide derivatives were obtained from the coupling of the corresponding N-(2-propylpentanoyl) amino acids with benzylamine in the presence of N,N′-dicyclohexylcarbodiimide as the coupling reagent.

Compounds in the latter group were prepared from the N-alkylation of valpromide with methoxymethyl chloride for methoxymethyl derivative, or formaldehyde in basic condition for hydroxymethyl derivative. Esterification of N-hydroxymethyl-2-propylpentamide gave the acyloxymethyl derivative.

Table 4 concludes some physical properties and percent yields of the products.

Table 4. Some physical properties and percent yield of the products

Compounds	Description	m.p.(°C)	Yield (%)	CHN analysis
1.CU 763-15-01	White needles	79-80	95	Calcd.:C,64.700:H,9.605:N, 5.805 Found:C,64.711:H,9.501:N, 5.897
2.CU 763-15-02	Colorless liquid	-	68	Calcd.:C,66.878:H,10.104:N, 5.200 Found:C,66.867:H,10.054:N, 5.159
3.CU 763-15-03	Colorless liquid	-	56	Calcd.:C,72.690:H,9.151:N,8.476 Found:C,72.727:H,9.109:N,8.630
4.CU 763-15-15	White needles	57-58	83	Calcd.:C,58.752:H,9.449:N,5.711 Found:C,58.749:H,9.350:N,5.751
5.CU 763-15-04	White needles	107-108	76	Calcd.:C,57.121:H,9.153:N,6.057 Found:C,57.144:H,9.070:N,6.082
6.CU 763-15-05	White needles	99-101	73	Calcd.:C,60.204:H,9.717.N,5.402 Found:C,60.254:H,9.757:N,5.494
7.CU 763-15-06	White solid	145-146	56	Calcd.:C,67.472:H,8.807:N,8.741 Found:C,67.576:H,8.838:N,8.697
8.CU 763-15-07	White needles	79-80	61	Calcd.:C,66.878:H,10.104:N,5.200 Found:C,66.867:H,10.054:N,5.159

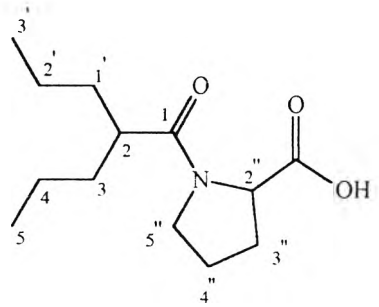
Table 4.(continued) Some physical properties and percent yield of the products

Compounds	Description	m.p. (°C)	Yield (%)	CHN analysis
9.CU 763-15-08	White solid	108-110	56	Calcd.:C,70.556:H,8.708:N,9.679 Found:C,70.583:H,8.835:N,9.690
10.CU 763-15-09	White needles	110-111	80	Calcd.:C,62.428:H,10.983:N,8.092 Found:C,62.446:H,10.933:N,8.042
11.CU 763-15-10	White needles	-	-	-
12.CU 763-15-11	White solid	51-52	41	Calcd.:C,64.130:H,11.303:N,7.480 Found:C,64.149:H,11.290:N,7.485

2-Propylpentanoyl chloride

This compound was prepared from 2-propylpentanoic acid and thionyl chloride following the known general method. The two by-products, sulfur dioxide and hydrogen chloride are gaseous and were removed concurrently with the excess thionyl chloride during distillation. The residual 2-Propylpentanoyl chloride was pure enough to use without further purification.

N-(2-Propylpentanoyl)-L- proline (CU 763-15-01)



This N-acylated amino acid was obtained from the acylation of L-proline with 2-propylpentanoyl chloride. The reaction took place in the presence of 10% aqueous sodium hydroxide solution, which helped to suppress the charge on the amino group and neutralize the mole of acid formed during the reaction.

2-Propylpentanoyl chloride is an excellent acylating agent. The general mechanism is nucleophilic substitution as shown in figure 14 (page 42). The product forms as carboxylate ion, and needs neutralization before extraction with organic solvent.

The chemical structure of this compound was proven by IR, ^{1}H-NMR, ^{13}C-NMR, CH COSY, mass spectrometry, and elemental analysis.

The IR spectrum of this compound (Figure 18) showed a broad band of O-H stretching (3446-2457 cm^{-1}) which overlapped the signal of aliphatic C-H stretching (2959-2870 cm^{-1}). The band at 1749 and 1734 cm^{-1} represented C=O stretching of carboxylic acid and amide, respectively. The N-C=O stretching showed a strong band at 1594 cm^{-1}. Peaks at 1218cm^{-1} and 918 cm^{-1} arose from C-O stretching and out-of-plane O-H bending, respectively.

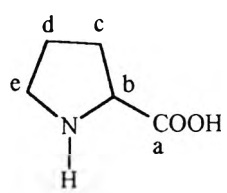
The ^{1}H-NMR spectrum of a solution of this compound in $CDCl_3$ had eleven signals (Figure 19). The triplet, six-proton peak at 0.86 ppm represented methyl

protons of C-5 and C-3′. The multiplet at 1.18-1.34 ppm was assigned to the four protons of C-4 and C-2′. The two-proton multiplets at 1.39 ppm and 1.61 ppm arose from methylene protons of C-3 and C-1′. The signal of C-2 proton was shown as the multiplet at 2.52 ppm.

The methylene protons at C-5″ were non-equivalent and showed signals as one-proton peaks at 3.50 and 3.61 ppm. Both were ddd pattern due to geminal coupling with each other (J=10.2 Hz) and the vicinal coupling with two protons of C-4″ (J=7.2 and 3.4 Hz). The methine proton at C-2″ showed a signal at 4.57 ppm as a one-proton double doublet. The broad one-proton peak at 9.71 ppm represented the carboxylic proton. However, using only ^{1}H-NMR does not enable the clear assignment of the methylene protons at C-3″ and C-4″.

The ^{13}C-NMR spectrum of this compound is shown in figure 22. The peak at 14.15 ppm represented two carbons; C-5 and C-3′. Peaks at 20.60 and 20.88 ppm were for C-4 and C-2′. The peaks at 34.81 and 35.22 ppm were for C-3 and C-1′. The signal at 43.77 ppm represented the methine carbon, C-2.

Comparing to the reported assignment of zwitterionic DL-proline in water (Fresenius, Huber, Pungor, Rechnitz, Simon, and West, 1989, Figure 91), peaks at 59.74, 27.47, 24.72, and 47.81 ppm were assigned to C-2″, C-3″, C-4″, and C-5″, respectively. C-1 and the carboxylic carbon showed peaks at 173.03 and 178.45 ppm.



(a) 175.2 ppm

(b) 62.4 ppm

(c) 30.3 ppm

(d) 25.0 ppm

(e) 47.4 ppm

Figure 91. Assignment of carbon atoms on DL-proline.

C-H shift correlation spectrum, CH COSY (Figure 23) showed correlation between each carbon atom and protons attached to it. The spectrum confirmed the assigned peaks and allowed further identification. The methylene protons at C-4'' showed the signal in the complex at 1.93-2.06 ppm. The two protons at C-3'' were non-equivalent. One signal appeared at 2.36 ppm as a ddd peak resulted from geminal coupling (J=11.1 Hz) and vicinal coupling with two protons at C-4'' and a proton at C-2'' (J=6.6 Hz, 6.6 Hz and 6.0 Hz, respectively). Another signal was overlapped in the complex at 1.93-2.06 ppm.

The EIMS spectrum of this compound is shown in figure 25. A peak at m/e 242 represented (M+1) peak. Proton rearrangement followed by hydrogen radical loss and amide clleavage gave a peak at m/e 126. Elimination of cyclopropane gave a peak at m/e 199. Decarboxylation of this ion resulted in a peak at m/e 155, loss of a hydrogen radical from this ion gave a peak at 154.

An amide cleavage gave a peak at m/e 127, loss of CO followed by loss of cyclopropane yielded peaks at m/e 99, and 57, respectively. Elimination of ethylene, followed by proton rearrangement resulted in a peak at m/e 170. The ion underwent amide cleavage to give a peak at m/e 83. Pattern of fragmentation is shown in figure 92.

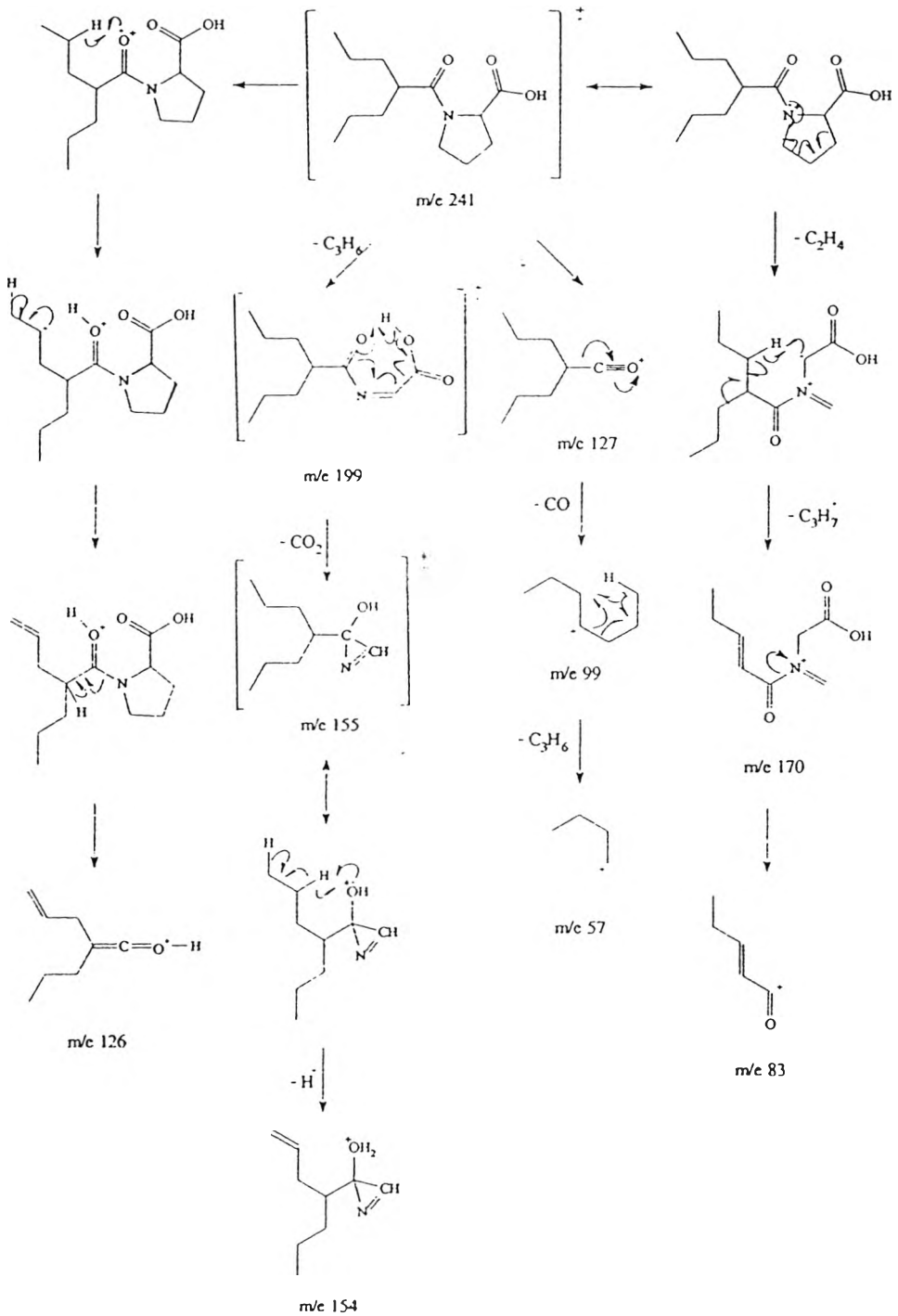


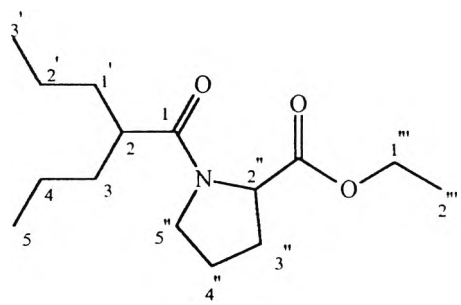
Figure 92. Mass fragmentation of N-(2-propylpentanoyl)-L-proline

L-Proline ethyl ester hydrochloride

L-Proline ethyl ester hydrochloride was prepared from treatment of the amino acid with ethanol containing thionyl chloride.

The IR spectrum of this compound (Figure 26) showed a broad band (3682-2287 cm^{-1}) arose from N-H stretching of the hydrochloride salt overlapped with the signal of aliphatic C-H stretching. The strong peak at 1738 cm^{-1} represented C=O stretching of ester. The C-C(=O)-O stretching was associated with the band at 1240 cm^{-1}.

N-(2-Propylpentanoyl)-L-proline ethyl ester (CU 763-15-02)



This compound was obtained from N-acylation of amino acid ester. Triethylamine (2 equivalent) was added in order to generate the free ester and neutralize the hydrochloric acid eliminated from the acylation. Mechanism of reaction is shown in figure 14 (page 42).

The IR spectrum of this compound (Figure 27) showed a signal of aliphatic C-H stretching at wave number 2957-2872 cm^{-1}. The strong peak at 1743 and 1650 cm^{-1} represented C=O stretching of ester and amide respectively. The band at 1045 and 1031 cm^{-1} were attributed to C-C(=O)-O stretching .

The ^{1}H-NMR spectrum of a solution of this compound in $CDCl_3$ had eleven signals. (Figure 28) The triplet, six-proton peak at 0.84 ppm represented methyl protons of C-5 and C-3′. The nine-proton complex at 1.16-1.40 ppm consisted of four protons at C-4 and C-2′, two methylene protons at C-1 and C-3 and three methyl protons at C-2‴. The signal of the other C-1 and C-3 protons was observed at 1.59 ppm. Signal of the methine proton on C-2 appeared as a multiplet at 2.47 ppm. The methylene protons of ethyl ester were shown as a quartet at 4.11 ppm.

According to the assignment of N-(2-Propylpentanoyl)-L-proline (CU 763-15-01), the methylene protons at C-4″ showed the signal in the complex at 1.84-2.06 ppm. The two protons at C-3″were non-equivalent. One signal appeared at 2.13 ppm as a ddd peak. Another signal was observed in the complex at 1.84-2.06 ppm. The one-proton multiplets at 3.52 and 3.63 ppm represented the non-equivalent methylene ptotons at C-5″. The methine proton at C-2″ showed a signal at 4.43 ppm as a one-proton double doublet.

The ^{13}C-NMR spectrum of this compound is shown in figure 31. Peaks at 14.01 and 14.12 ppm represented two carbons; C-5 and C-3′. Peaks at 20.41 and 20.79 ppm were C-4 and C-2′. The peaks at 34.97 and 35.28 ppm were C-3 and C-1′. The signal at 43.29 ppm represented the methine carbon, C-2.

The peaks at 58.74, 29.11, 24.75, and 46.99 ppm were assigned to C-2″, C-3″, C-4″, and C-5″, respectively. C-1 and 2″-carbonyl carbon showed peaks at 172.37 and 175.21 ppm, respectively. C-1‴ and C-2‴ showed signals at 58.74 and 14.19 ppm, respectively.

The CH COSY spectrum (Figure 32, 33) confirmed the assignment of this compound.

The EIMS spectrum of this compound is shown in figure 34. A peak at m/e 270 represented (M+1) peak. Cleavage of ester bond followed by loss of CO resulted in the peaks at m/e 196. Loss of cyclopropane from this ion resulted in a peak at m/e 154. Elimination of 3-propylbutane radical followed by loss of CO resulted in a peak at m/e 142. McLafferty rearrangement with an elimination of propylene gave a peak at m/e 227.

The amide cleavage followed by loss of CO and cyclopropane gave peaks at m/e 127, 99, and 57, respectively. Loss of ethylene from the pyrrolidine ring followed by McLafferty rearrangement with loss of propyl radical resulted in a peak at m/e 198. Inductive cleavage of amide gave a peak at m/e 83. Pattern of fragmentation is shown in figure 93.

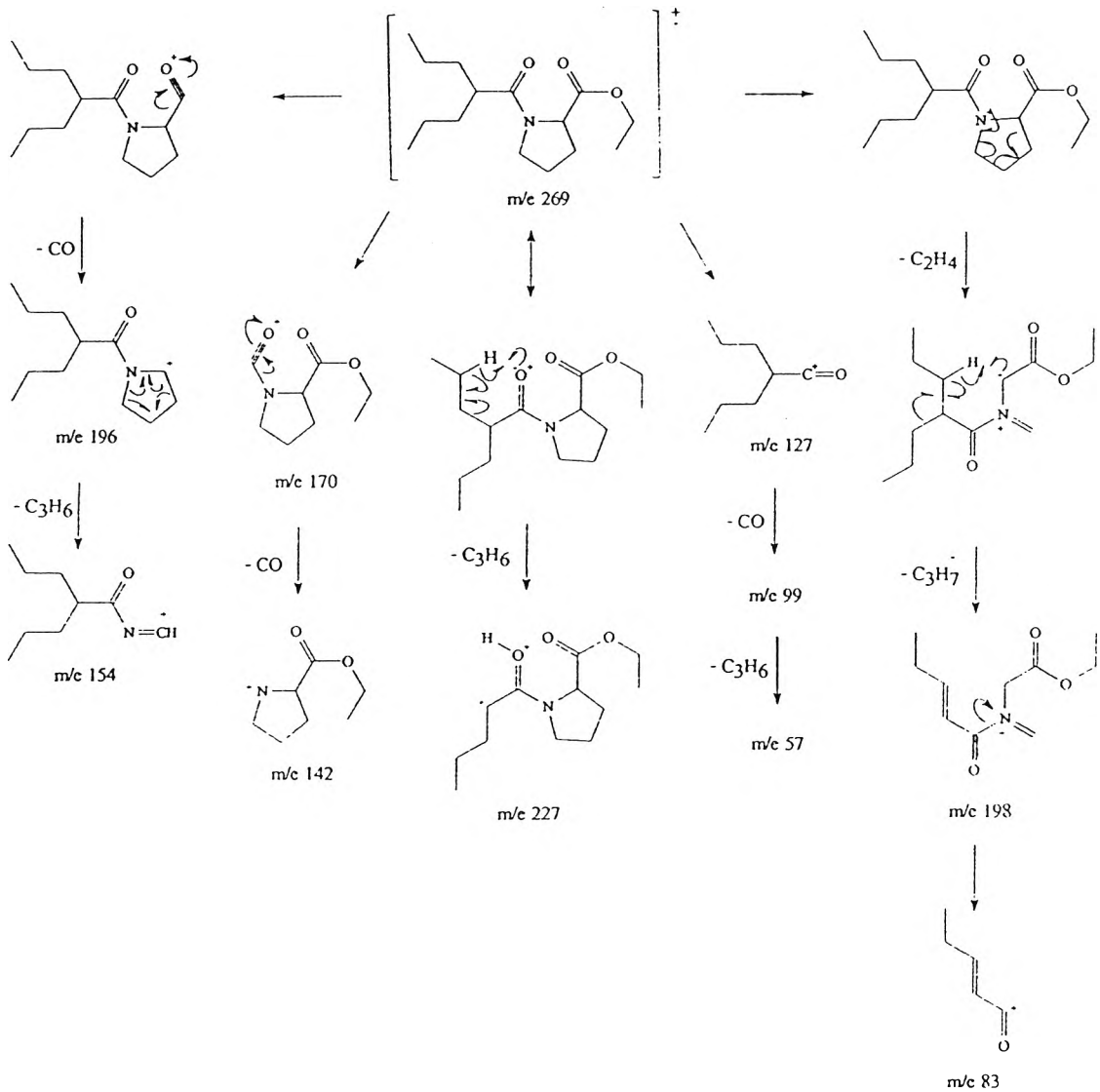
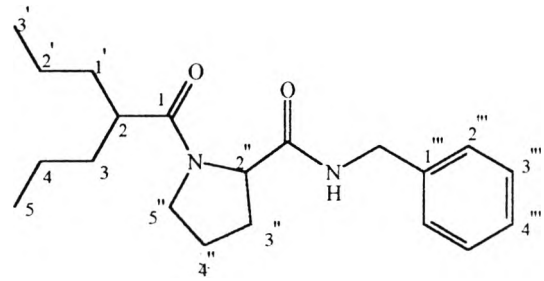


Figure 93. Mass fragmentation of N-(2-propylpentanoyl)-L-proline ethyl ester

N-(2-Propylpentanoyl)-L-proline benzylamide (CU 763-15-03)



This compound was obtained by coupling of N-acylated amino acid with benzylamine in the presence of N,N′-dicyclohexylcarbodiimide. Mechanism of this reaction is shown in figure 13 (page 40).

After a 20-hour period of reaction, the reaction mixture was added 5% acetic acid in order to decompose the unreacted coupling reagent. The N,N′-dicyclohexylurea formed from the reaction is removed by filtration because of poor solubility in organic solvent.

Considering this coupling reaction, the possible by-products are guanidine derivative, O-acyl isourea, N-acyl urea, and dicyclohexylurea. (See figure 94) All are neutral compounds, hence, removal of these by-products by acid-base extraction is impossible. Therefore, the crude product obtained from this reaction was purified by column chromatography method.

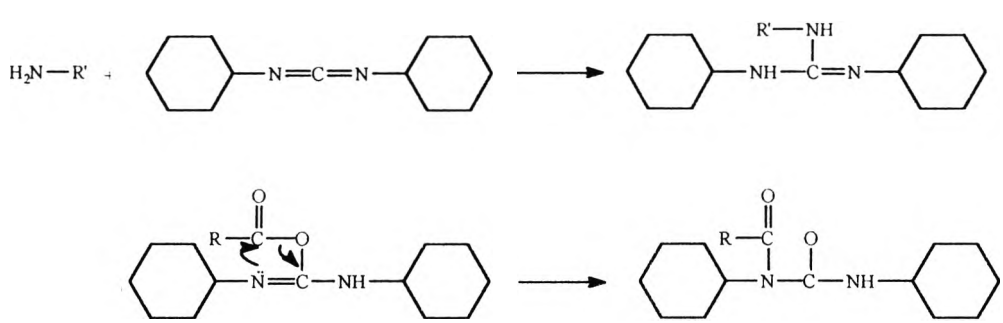


Figure 94. The formation of by-products from the coupling with N,N′-dicyclohexylcarbodiimide.

The IR spectrum of this compound (Figure 35) showed N-H stretching at 3295 cm^{-1}. A signal of aliphatic C-H stretching appeared between 2955-2871 cm^{-1}. The strong band between 1679-1621 cm^{-1} represented C=O stretching of amide. The aromaticity of this compound was shown by aromatic C-H stretching, overtone, and combination bands, and out-of-plane C-H bending of aromatic ring at 3084-3033, 1952-1749, and 736 and 699 cm^{-1}.

The ^{1}H-NMR spectrum of a solution of this compound in $CDCl_3$ had sixteen signals. (Figure 36) The triplet, three-proton peaks at 0.80 and 0.89 ppm represented methyl protons of C-5 and C-3′. The multiplet, two-proton peaks at 1.16 and 1.26 ppm represented methylene protons at C-4 and C-2′. The two-proton multiplets at 1.37 and 1.58 ppm were methylene protons at C-3 and C-1′. The signal of the methine proton on C-2 was incorporated in the multiplet peak at 2.52 ppm.

According to the assignment of N-(2-Propylpentanoyl)-L-proline (CU 763-15-01), the methylene protons at C-4″ showed the signal in the multiplets at 1.98 and 2.13 ppm. The two protons at C-3″were non-equivalent as usual and one proton showed peak at 2.52 ppm as a ddd peak while the other gave the signal at 1.79 ppm. The one-proton multiplets at 3.49 and 3.57 ppm represented the non-equivalent methylene protons at C-5″. Both were ddd due to geminal coupling with each other (J=9.7 Hz) and the vicinal coupling with two protons of C-4″ (J=9.8 and 7.0 Hz). The methine proton at C-2″ showed a signal at 4.70 ppm as a one-proton double doublet. The benzylic protons had a vicinal coupling with NH proton (J=30.4 Hz) and long-range coupling with the aromatic protons (J=15.0 Hz) resulted in a couple of double doublets at 4.39 ppm.

The ^{13}C-NMR spectrum of this compound is shown in figure 39. The peak at 14.23 ppm represented two carbons; C-5 and C-3′. Peaks at 20.66 and 20.91 ppm

were C-4 and C-2′. The peaks at 34.89 and 35.45 ppm were C-3 and C-1′. The signal at 43.51 ppm represented the methine carbon, C-2.

The peaks at 59.50, 26.75, 25.07, and 47.56 ppm were assigned to C-2″, C-3″, C-4″, and C-5″, respectively. The signal of the benzylic carbon appeared at 43.46 ppm. C-1 and 2″-carbonyl carbon showed peaks at 171.18 and 177.13 ppm, respectively.

The CH COSY spectrum (Figure 40) confirmed the assignment of this compound.

The EIMS spectrum of this compound is shown in figure 42. A peak at m/e 330 represented molecular ion peak. Elimination of benzylamine radical followed by loss of CO and cyclopropane resulted in a peak at m/e 154.

Elimination of cyclopropane resulting from cleavage within pyrrolidine ring gave a peak at m/e 288. The amide cleavage followed by loss of CO and cyclopropane gave peaks at m/e 127, 99, and 57, respectively. Elimination of 1-propylbutyl radical, followed by loss of CO and NH resulted in a peak at m/e 91. The amide cleavage with loss of 2-propylpentanoyl radical gave a peak at m/e 203. Pattern of fragmentation is shown in figure 95.

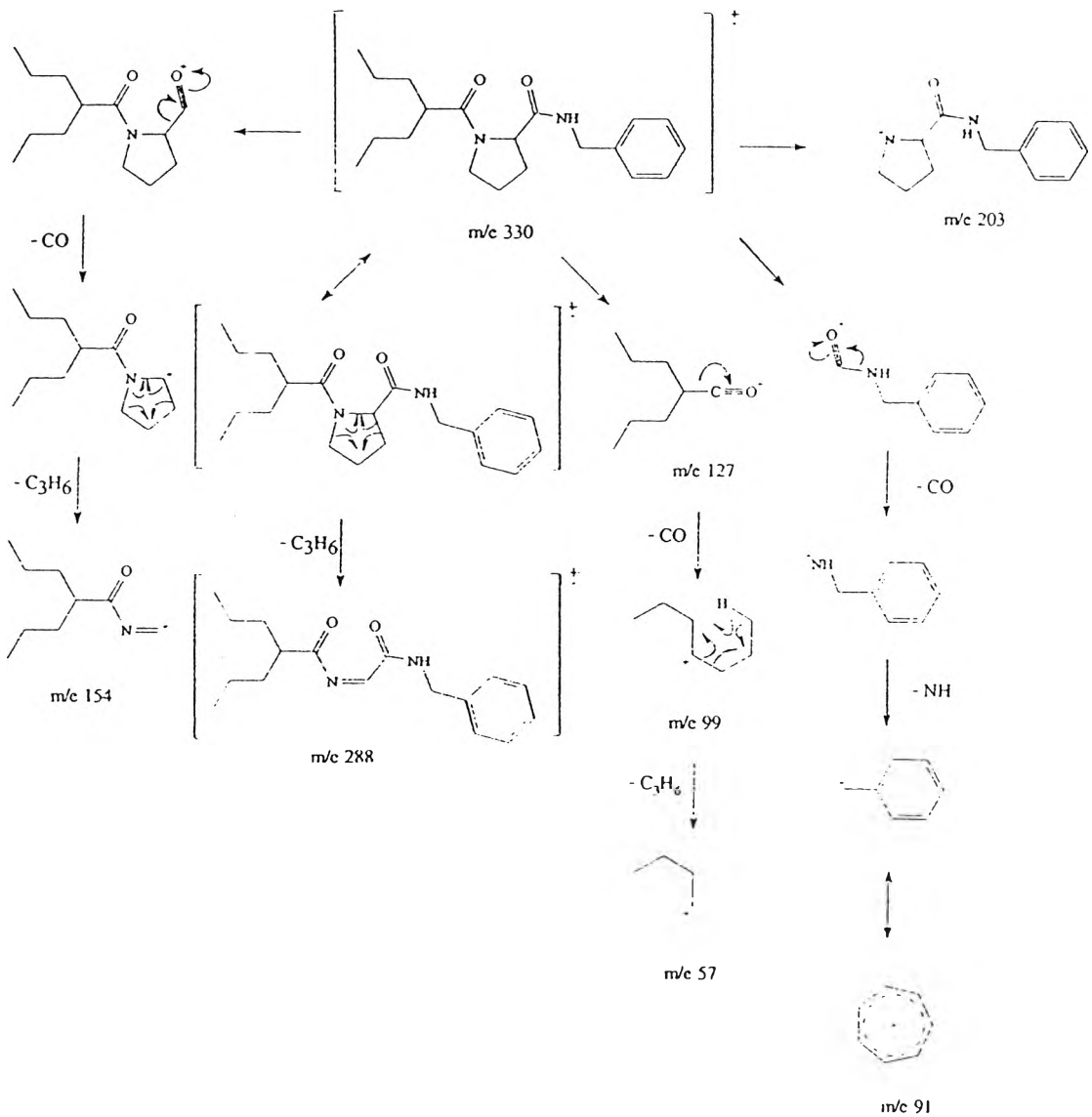


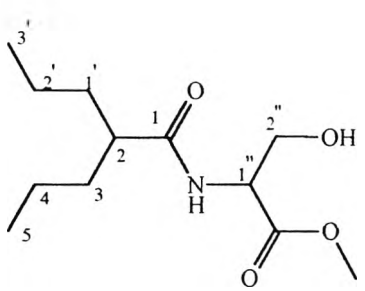
Figure 95. Mass fragmentation of N-(2-propylpentanoyl)-L-proline benzylamide

DL-Serine methyl ester hydrochloride

DL-Serine methyl ester hydrochloride was prepared from treatment of the amino acid with methanol containing thionyl chloride.

The IR spectrum of this compound (Figure 43) showed O-H stretching at 3402 cm^{-1}. A broad band of N-H stretching of the hydrochloride salt appeared between 3612-2241 cm^{-1} overlapped with the aliphatic C-H stretching of the compound and Nujol (2952-2848 cm^{-1}). The strong band at 1738 cm^{-1} represented C=O stretching of ester. The signal at 1583 cm^{-1} was attributed to N-H bending of NH_3^+. The signal of C-C(=O)-O-, and O-C-C stretching were shown at 1251, and 1037 cm^{-1}, respectively.

N-(2-Propylpentanoyl)-DL-serine methyl ester (CU 763-15-15)



As described in the preparation of N-(2-Propylpentanoyl)-L-proline ethyl ester (CU 763-15-02). This compound was also obtained from N-acylation of amino acid ester in the presence of triethylamine.

The IR spectrum of this compound (Figure 44) showed a broad band of N-H overlapped with O-H stretching at 3579-3143 cm^{-1}. The aliphatic C-H stretching band Appeared at 2959-2863 cm^{-1}. The ester characteristic peaks; C=O stretching, C-C(=O)-O-, and O-C-C stretching were shown at 1755, 1217, and 1050 cm^{-1},

respectively. The amide characteristic peaks; C=O stretching and N-H bending were attributed to the bands at 1649 and 1548 cm^{-1}.

The ^{1}H-NMR spectrum of a solution of this compound in $CDCl_3$ had nine signals. (Figure 45) The triplet, six-proton peak at 0.90 ppm represented methyl protons of C-5 and C-3′. The six-proton complex at 1.20-1.47 ppm consisted of four protons at C-4 and C-2′, two protons at C-1′ and C-3 and three methyl protons at C-4″. The signal of two protons at C-3 and C-1′ was observed at 1.60 ppm. Signal of the methine proton at C-2 appeared as a multiplet at 2.17 ppm. The protons of methyl ester were shown as a singlet at 3.80 ppm.

The one-proton broad peak at 2.61 ppm represented the hydroxylic proton. Intramolecular hydrogen bond formed between the proton and the ester carbonyl reduced rate of proton interchange, thus, resulted in the coupling of this proton and adjacent methylene protons (J=5.8 Hz). Furthermore, this hydrogen bond also made the methylene protons at C-2″ non-equivalent. They gave the signals at slightly different chemical shifts. Each proton coupled with hydroxylic proton and the methine proton at C-1″ resulted in two two-proton ddd peaks overlapped at 3.96 ppm. The one-proton doublet at 6.39 ppm represented the amidic proton.

The ^{13}C-NMR spectrum of this compound is shown in figure 47. The peak at 14.08 ppm represented two carbons; C-5 and C-3′. Peaks at 20.67 and 20.76 ppm were C-4 and C-2′. The peak at 35.15 represented to C-3 and C-1′. The signal at 47.43 ppm represented the methine carbon, C-2.

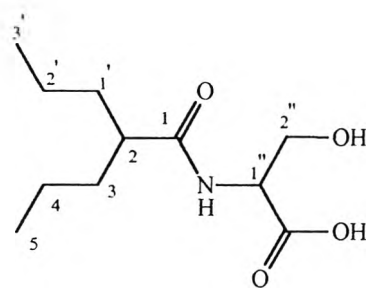
The peaks at 52.74, 54.58, and 63.94 ppm were assigned to the methyl ester carbon, C-1″, and C-2″, respectively. The carbonyl carbon of amide and carboxylic acid showed peaks at 171.01 and 176.71 ppm, respectively.

The EIMS spectrum of this compound is shown in figure 48. A peak at m/e 246 represented (M+1) peak. McLafferty rearrangement with the loss of propylene resulted in a peak at m/e 203. Elimination of ethyl radical from this ion gave a peak at m/e 174, loss of formaldehyde resulted in peaks at m/e 144 and 114.

The amide cleavage resulted in a peak at m/e 127. Fragmentation within the alkyl side chain provided peaks at m/e 99 and 57. Elimination of methoxy radical followed by decarboxylation and loss of formaldehyde gave a peak at m/e 156.

McLafferty rearrangement initiated at the ester group gave a peak at m/e 215. Elimination of formaldehyde from this ion gave a peak at m/e 185. Pattern of fragmentation is shown in figure 96.

N-(2-Propylpentanoyl)-DL-serine (CU 763-15-04)



This compound was obtained from basic hydrolysis of the methyl ester in the presence of 1 N sodium hydroxide solution at room temperature. Mechanism of the reaction is $B_{AC}2$ as described in figure 16 (page 51).

Acid hydrolysis using the mixture of 40 ml acetone, 28 ml water, and 12 ml concentrated hydrochloric acid under reflux for 2 hours gave the desired acid in poor yield.

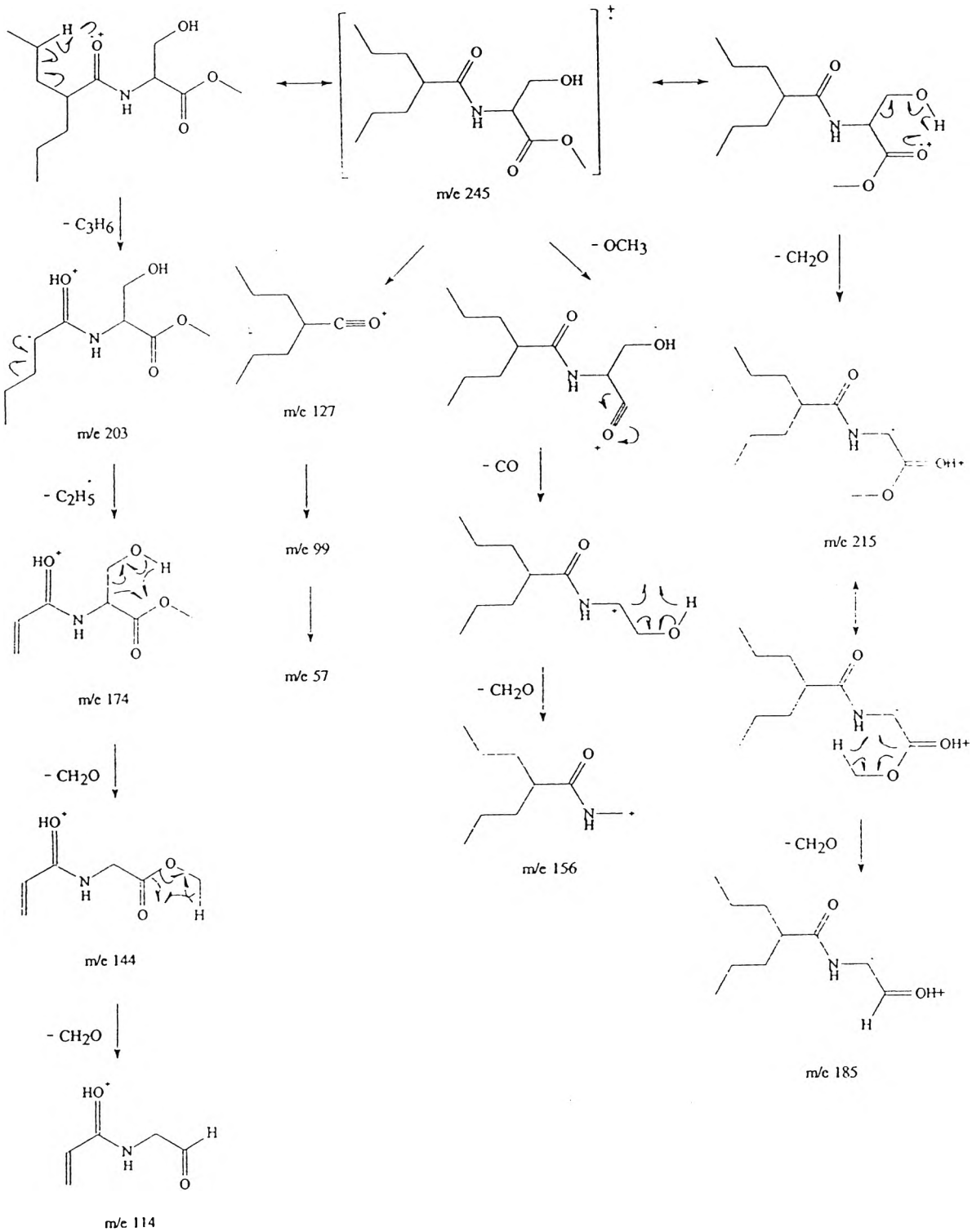


Figure 96. Mass fragmentation of N-(2-propylpentanoyl)-DL-serine methyl ester

The IR spectrum of this compound (Figure 49) showed a broad band of O-H stretching (3586-2206 cm^{-1}) overlapped the signal of N-H stretching (3372 cm^{-1}) and aliphatic C-H stretching (2960-2870 cm^{-1}). The strong bands at 1736 and 1647 cm^{-1} represented C=O stretching of carboxylic acid and amide respectively. The N-H bending was attributed to a strong band at 1540 cm^{-1}. The peaks at 1234, 1087, and 919 cm^{-1} arose from C-O stretching, C-C-O stretching and out-of-plane O-H bending, respectively.

The ^{1}H-NMR spectrum of a solution of this compound in DMSO-d_6 had eight signals (Figure 50). The triplet, six-proton peak at 0.82 ppm represented methyl protons of C-5 and C-3′. The complex, six-proton peak at 1.10-1.32 ppm represented four methylene protons at C-4 and C-2′, two protons at C-3 and C-1′. Two protons at C-3 and C-1′ appeared as a multiplet at 1.41 ppm. The signal of the methine proton at C-2 appeared as a multiplet at 2.27 ppm.

The hydroxylic proton showed a signal as a broad peak at 3.35 ppm. Intramolecular hydrogen bond formed between the proton and the carboxylic group made the adjacent methylene protons of C-2″ non-equivalent. They coupled with the hydroxylic proton (J=20.2 Hz) and the methine proton at C-1″ (J=10.8 Hz) and resulted in the two-proton complex at 3.63 ppm. The signal of the methine proton of C-1″ at 4.26 ppm showed the coupling of this proton with amidic proton (J=7.6 Hz) and methylene protons at C-2″(J=4.9 Hz). The one-proton doublet at 7.90 ppm represented the amidic proton.

The ^{13}C-NMR spectrum of this compound is shown in figure52. The peak at 16.85 ppm represented two carbons; C-5 and C-3′. Peaks at 20.13 and 20.27 ppm were C-4 and C-2′. The peaks at 35.00 and 35.14 ppm were C-3 and C-1′. The signal at 45.01 ppm represented the methine carbon, C-2.

The peaks at 54.60 and 61.70 ppm were assigned to C-1″and C-2″, respectively. The carbonyl carbon of amide and carboxylic acid showed peaks at 172.31 and 175.28 ppm, respectively.

The assignment of carbon atoms was confirmed by DEPT 135 spectrum (See figure 53).

The EIMS spectrum of this compound is shown in figure 54. A peak at m/e 232 represented (M+1) peak. McLafferty rearrangement with loss of propylene yielded a peak at m/e 189. Elimination of ethyl radical resulting from α-cleavage gave a peak at m/e 160.

The (M-17) peak caused by loss of hydroxy radical resulted in a peak at m/e 214. α-Cleavage gave a peak at m/e 142. The amide cleavage and fragmentation within the alkyl side chain resulted in peaks at m/e 127, 99, and 57.

McLafferty rearrangement with the loss of formaldehyde resulted in a peak at m/e 201. Dehydration from the ion gave a peak at m/e 183. The pattern of decomposition is shown in figure 97.

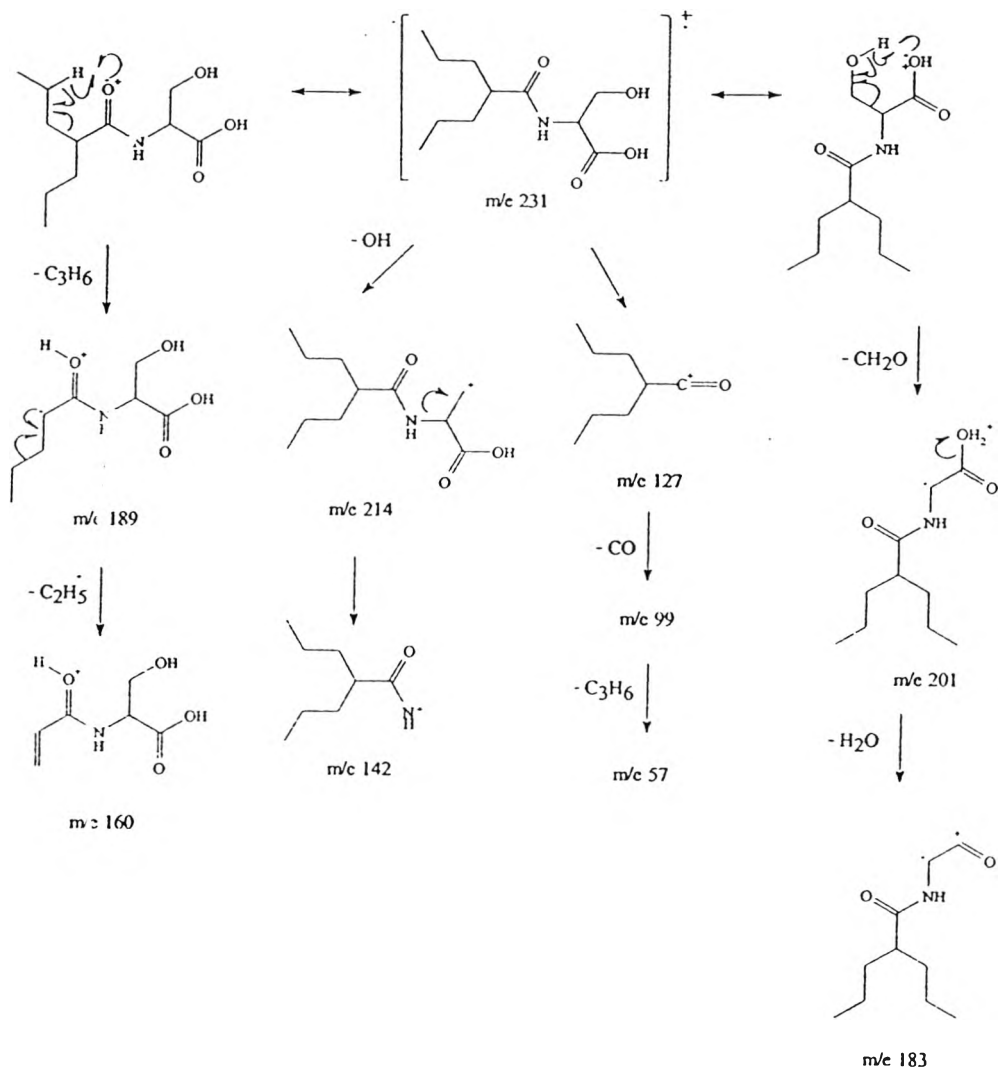


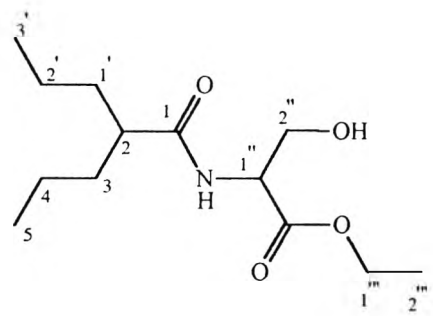
Figure 97. Mass fragmentation of N-(2-propylpentanoyl)-DL-serine

DL-Serine ethyl ester hydrochloride

DL-Serine ethyl ester hydrochloride was prepared from treatment of the amino acid with ethanol containing thionyl chloride.

The IR spectrum of this compound (Figure 55) showed a broad band of N-H stretching of the hydrochloride salt between 3616-2243 cm^{-1} overlapped with O-H stretching (3380 cm^{-1}) and aliphatic C-H stretching of the compound and Nujol (2952-2850 cm^{-1}). The strong band at 1742 cm^{-1} represented C=O stretching of ester. The signal at 1582 cm-1 was attributed to N-H bending of NH_3^+. The signal of C-C(=O)-O-and O-C-C stretching were shown at 1240, and 1027 cm^{-1}, respectively.

N-(2-Propylpentanoyl)-DL-serine ethyl ester (CU 763-15-05)



As described in the preparation of N-(2-Propylpentanoyl)-l -proline ethyl ester (CU 763-15-02) This compound obtained from N-acylation of amino acid ester in the presence of triethylamine.

The IR spectrum of this compound (Figure 56) showed ban ls of N-H and O-H stretching at 3476 and 3282 cm^{-1}.The aliphatic C-H stretching band appeared at 2957-2870 cm^{-1}. The ester characteristic peaks; C=O stretching. C-C(=O)-O-, and O-C-C stretching were shown at 1748, 1287, and 1079 cm^{-1}, respectively. The amide characteristic peaks; C=O stretching and N-H bending were attributed to the bands at 1645 and 1554 cm^{-1}.

The ^{1}H-NMR spectrum of a solution of this compound in $CDCl_3$ had nine signals (Figure 57). The triplet, six-proton peak at 0.90 ppm represented methyl protons of C-5 and C-3′. The nine-proton complex at 1.25-1.47 ppm consisted of four protons at C-4 and C-2′, two protons at C-1′, C-3 and three methyl protons at C-2″. The signal of protons at C-3 and C-1′ was observed at 1.62 ppm. Signal of the methine proton at C-2 appeared as a multiplet at 2.16 ppm. The methylene protons of ethyl ester were shown as a quartet at 4.25 ppm.

The one-proton triplet peak at 2.66 ppm represented the hydroxylic proton which coupled with the adjacent methylene protons (J=5.8 Hz). The methylene protons at C-2″ were non-equivalent. Each proton coupled with hydroxylic proton and the methine proton at C-1″ resulted in two two-proton dd peaks overlapping at 3.96 ppm. The one-proton doublet at 6.40 ppm represented the amidic proton. The signal of the proton at C-1″ appeared at 4.69 ppm as multiplet.

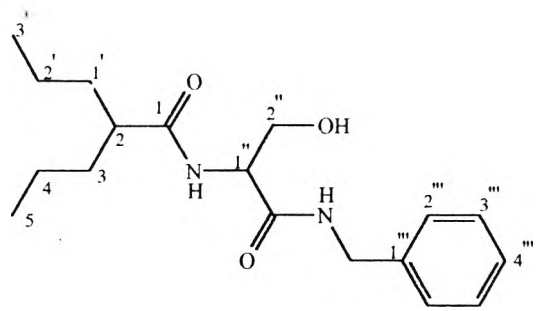
The ^{13}C-NMR spectrum of this compound is shown in figure 59. The peak at 14.0 ppm represented two carbons; C-5 and C-3′. Peak at 20.9 ppm was C-4 and C-2′. The peak at 35.2 represented to C-3 and C-1′. The signal at 47.4 ppm represented the methine carbon, C-2.

The peaks at 54.8, 62.1, and 64.0 ppm were assigned to C-1″, C-2″, and C-1‴ respectively. The carbonyl carbon of amide and carboxylic acid showed peaks at 170.8 and 176.8 ppm, respectively.

The EIMS spectrum of this compound is shown in figure 60. A peak at m/e 260 represented (M+1) peak. Elimination of hydroxyl group resulted in a peak at m/e 242. β-Cleavage gave a peak at m/e 142. Loss of O=C=NH followed by cleavage within alkyl chain gave peaks at m/e 99 and 57.

McLafferty rearrangement initiated at the ester group gave a peak at m/e 229. While cleavage of the amide bond in this ion yielded a peak at m/e 102, loss of ethanol gave a peak at m/e 183. McLafferty rearrangement initiated at the amide carbonyl site generated a peak at m/e 217. Loss of ethyl radical from this ion gave a peak at m/e 188. Pattern of fragmentation is shown in figure 98.

N-(2-Propylpentanoyl)-DL-serine benzylamide (CU 763-15-06)



As described in the preparation of N-(2-Propylpentanoyl)-L-proline benzylamide (CU 763-15-03) This compound was obtained by coupling of N-acylated amino acid with benzylamine in the presence of N,N′-dicyclohexylcarbodiimide.

The IR spectrum of this compound (Figure 61) showed a band generated from N-H stretching overlapped by O-H stretching at 3275 cm^{-1}. Aliphatic C-H stretching band appeared at 2959-2878 cm^{-1}. The amide characteristic peaks; C=O stretching, N-H bending were shown at 1639 and 1553 cm^{-1}, respectively. The aromatic C-H stretching, overtone and combination bands, and out-of-plane C-H bending of aromatic ring appeared at 3092-3025. 1955-1745, and 742-694 cm^{-1}, respectively.

The ^{1}H-NMR spectrum of a solution of this compound in $CDCl_3$ had nine signals (Figure 62). The multiplet, six-proton peak at 0.85 ppm represented methyl protons of C-5 and C-3′. The multiplet, six-proton peak at 1.10-1.44 ppm represented the four methylene protons at C-4 and C-2′, two protons at C-3 and C-1′.

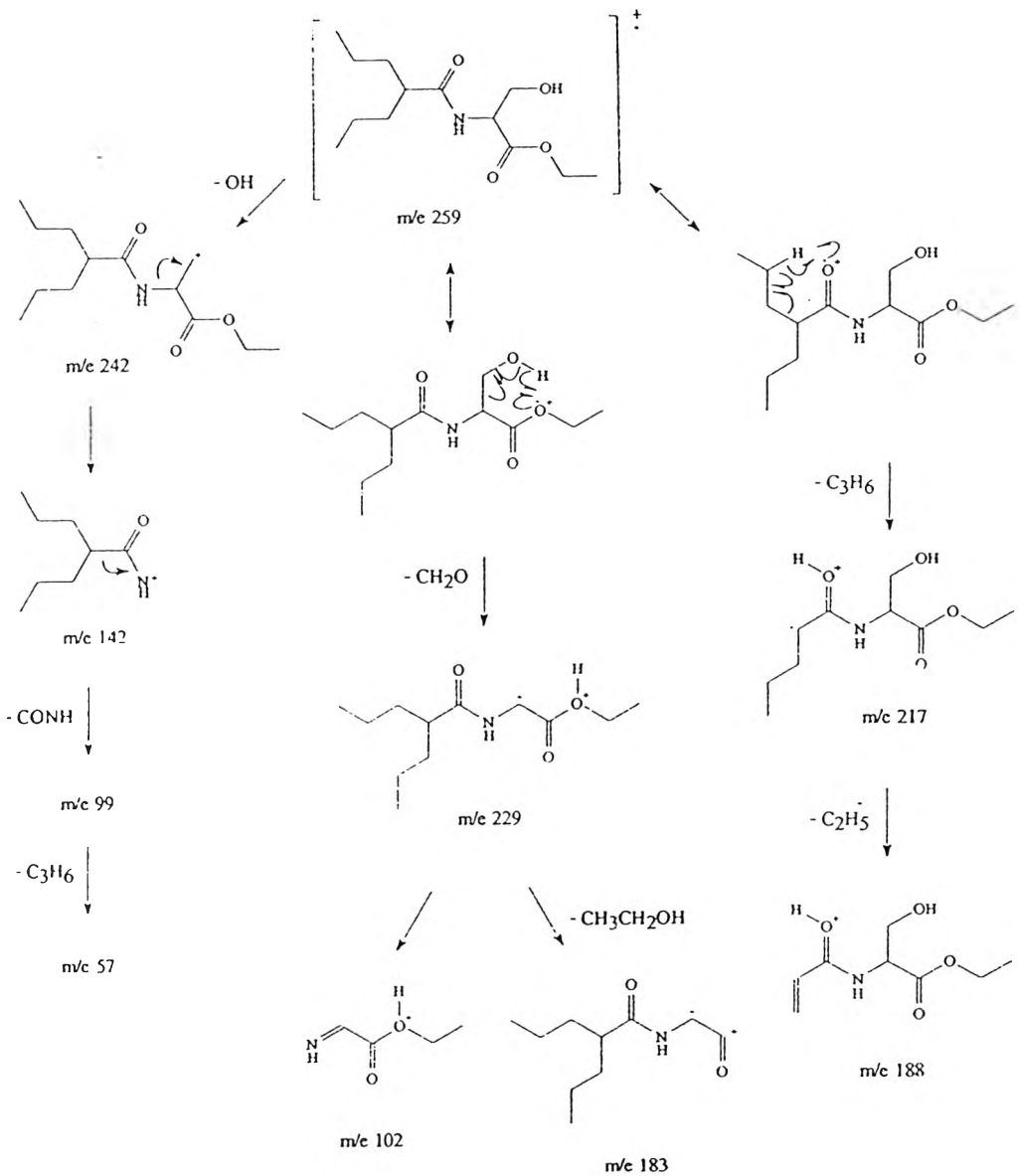


Figure 98. Mass fragmentation of N-(2-propylpentanoyl)-DL-serine ethyl ester

The other two protons at C-3 and C-1′ appeared as the signal at 1.44-1.61 ppm. The signal of the methine proton at C-2 appeared as a multiplet at 2.13 ppm.

Protons of the phenyl ring showed a signal as a multiplet at 7.23-7.31 ppm. The two amidic protons of 2-propylpentamide moiety and of benzamide moiety gave signals at 6.69 and 7.32 ppm, respectively.

The assignment of methine proton at C-1″, methylene proton at C-2″, and the methylene protons of benzylic moiety did not enable with this ^{1}H-NMR technique.

The ^{13}C-NMR spectrum of this compound (Figure 64) showed three signals of the aromatic carbons. The peaks at 127.52,127.55,128.69, and 137.61 ppm represented the C-4‴, C-3‴, C-2‴, and C-1‴ of benzylic moiety, respectively. The carbonyl carbon of 2-propylpentamide, C-1 and the other carbonyl carbon showed peaks at 171.20 and 177.44 ppm, respectively. The signals of aliphatic carbons could not be assigned obviously from ^{13}C-NMR.

In order to assign the peaks of aliphatic carbons, DEPT 135 spectrum (figure 65) was considered. Peaks at 14.04, 20.72, 35.06 and 35.08 ppm, and 47.24 ppm represented C-5 and C-3′, C-4 and C-2′, C-3 and C-1′, and C-2 respectively. The positive peak at 53.40 ppm was the methine carbon, C-1″. The negative peaks at 43.43 and 62.75 ppm represented the methylene carbon of benzylic moiety and the methylene C-2″ respectively. The peak at 137.61 ppm was not observable in this experiment, thus, this confirm the assignment of this carbon as C-1‴, the quaternary carbon.

At this point, all carbon atoms have been assigned. The heteronuclear multiple quantum coherence (HMQC) spectrum (Figure 66) enable the assignment for the

protons directly attached to each carbon atom. Then, the proton signals at 3.63 and 4.16 ppm were assigned to the unequivalent methylene protons on C-2″. The three-proton complex lying between 4.34-4.50 ppm represented the methine proton at C-1″, and the methylene protons of benzylic moiety.

The EIMS spectrum of this compound is shown in figure 67. A peak at m/e 322 represented (M+2) peak. Elimination of 1-propylbutyl radical followed by loss of CO gave a peak at m/e 193. Loss of CO and O=C=NH yielded peaks at m/e 106 and 91 (tropylium ion) respectively.

McLafferty rearrangement initiated at the amide with the loss of propylene resulted in a peak at m/e 278. This ion underwent α-cleavage with loss of ethanol resulting in a peak at m/e 249. Cleavage of benzylamide moiety yielded a peak at m/e 214. Elimination of CO gave a peak at m/e 186.

McLafferty rearrangement initiated at hydroxyl group and dehydration gave a peak at m/e 169. Amide cleavage followed by loss of CO and cleavage within the alkyl side chain resulted in peaks at m/e 127, 99, and 57 respectively. Pattern of fragmentation is shown in figure 99.

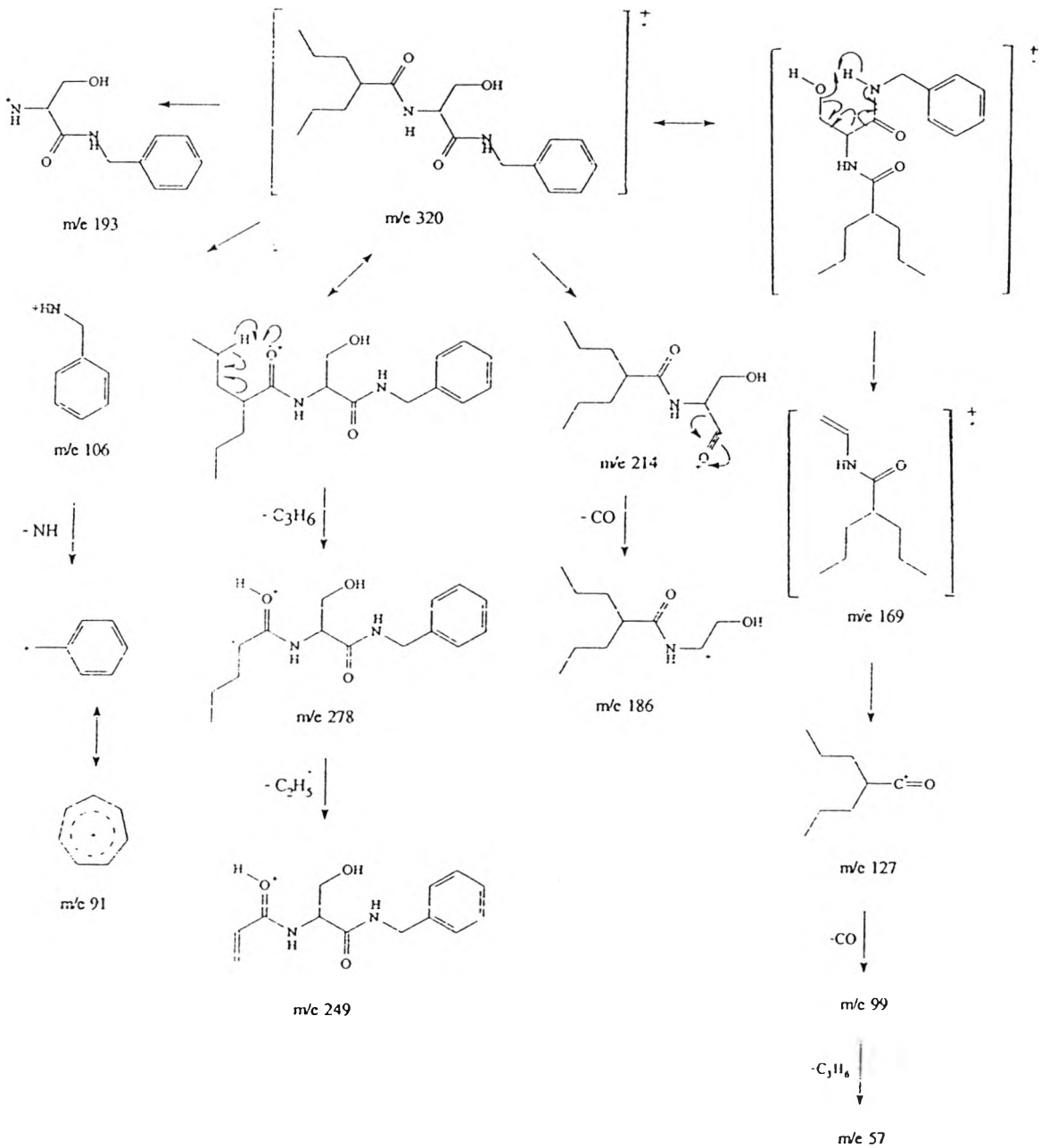


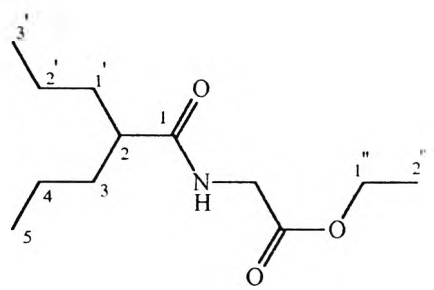
Figure 99. Mass fragmentation of N-(2-propylpentanoyl)-DL-serine benzylamide

Glycine ethyl ester hydrochloride

Glycine ethyl ester hydrochloride was prepared from treatment of the amino acid with ethanol containing thionyl chloride.

The IR spectrum of this compound (Figure 68) showed a broad band of N-H stretching of the hydrochloride salt between 3682-2265 cm^{-1} overlapped with aliphatic C-H stretching of the compound and Nujol (2952-2856 cm^{-1}). The strong band at 1744 cm^{-1} represented C=O stretching of ester. The signal of C-C(=O)-O-, and O-C-C stretching were shown at 1255, and 1048 cm^{-1}, respectively.

N-(2-Propylpentanoyl) glycine ethyl ester (CU 763-15-07)



As described in the preparation of N-(2-Propylpentanoyl)-L-proline ethyl ester (CU 763-15-02). This compound was also obtained from N-acylation of amino acid ester in the presence of triethylamine.

The IR spectrum of this compound (Figure 69) showed bands of N-H at 3296 cm^{-1}. The aliphatic C-H stretching band appeared at 2953-2870 cm^{-1}. The ester characteristic peaks; C=O stretching, C-C(=O)-O-, and O-C-C stretching were shown at 1731, 1243, and 1040 cm^{-1}, respectively. The amide characteristic peaks; C=O stretching and N-H bending were attributed to the bands at 1641 and 1550 cm^{-1}.

The ^{1}H-NMR spectrum of a solution of this compound in $CDCl_3$ had seven signals (Figure 70). The multiplet, six-proton peak at 0.90 ppm represented methyl protons of C-5 and C-3′. The nine-proton complex at 1.20-1.46 ppm consisted of four protons at C-4 and C-2′, two protons at C-1′, C-3 and the methyl protons at C-2″. The signal of protons at C-3 and C-1′ was observed at 1.62 ppm. Signal of the methine proton at C-2 appeared as a multiplet at 2.12 ppm. The methylene protons of C-1″ were shown as a quartet at 4.23 ppm.

Two glycilic protons showed the signal as a doublet at 4.05 ppm. The NH proton appeared as a broad peak at 5.92 ppm.

The ^{13}C-NMR spectrum of this compound is shown in figure 72. The peak at 14.06, 20.73, 35.18, and 47.42 ppm represented C-5 and C-3, C-4 and C-2, C-3 and C-1′, and C-2, respectively.

The peak at 41.19 ppm was assigned to glycilic carbon. Peaks at 14.06 and 61.46 ppm were attributed to C-2″and C-1″, respectively. The carbonyl carbon of amide and ester showed peaks at 170.18 and 176.20 ppm, respectively.

The EIMS spectrum is shown in figure 73. A peak at m/e 230 represented the (M+1) peak. Amide cleavage of the molecular ion led to a peak at m/e 127. Loss of CO followed by cleavage of alkyl chain gave peaks at m/e 99 and 57.

McLafferty rearrangement initiated at amide moiety gave a peak at m/e 187. This ion underwent two pathways of decomposition, loss of ethyl radical and charge migration with α-cleavage yielded peaks at m/e 158 and 85 respectively. Elimination of ethanol from the molecular ion resulted in a peak at m/e 184, loss of n-heptane gave a peak at m/e 83. Pattern of fragmentation of this compound is shown in figure 100.

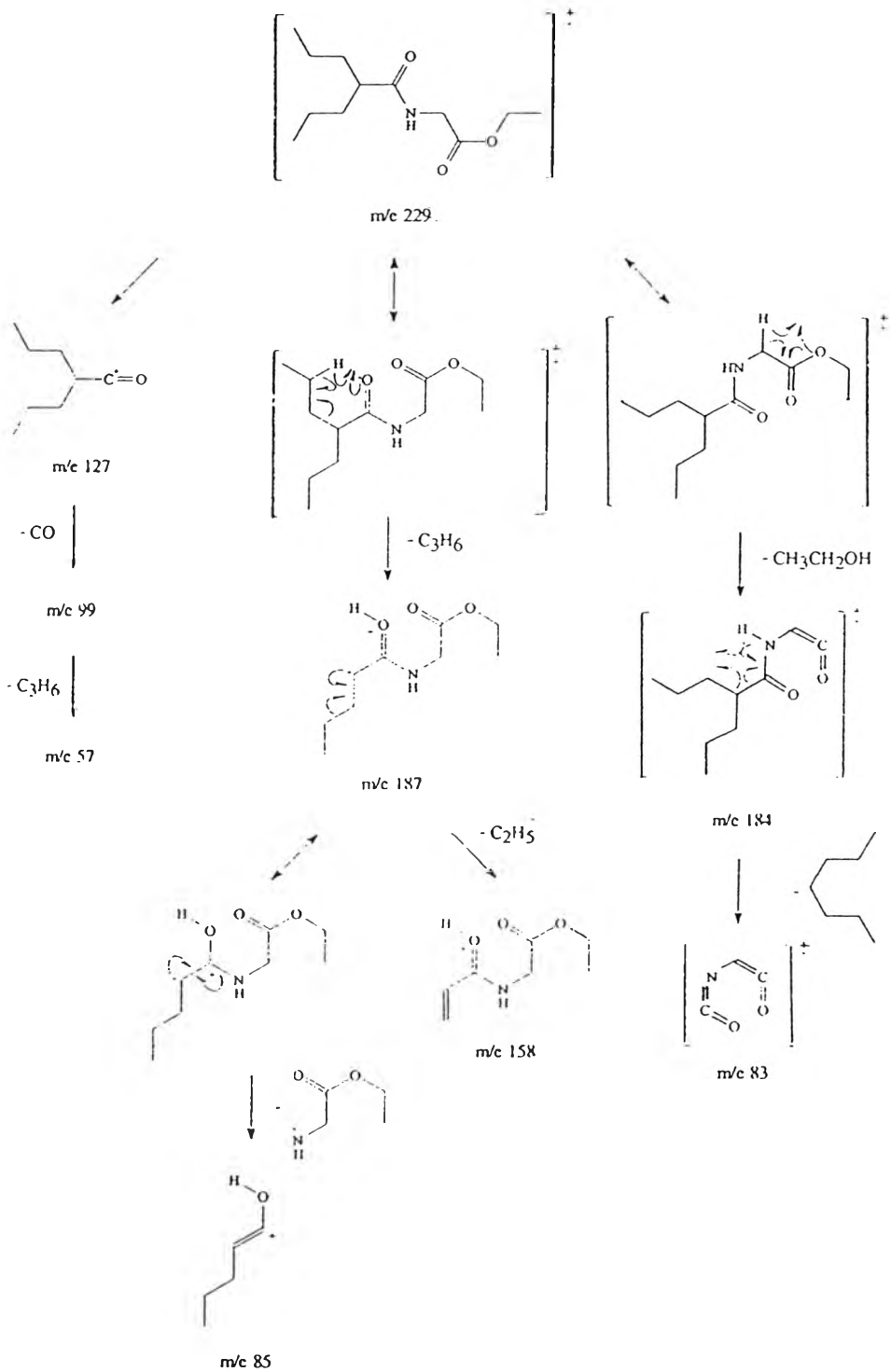
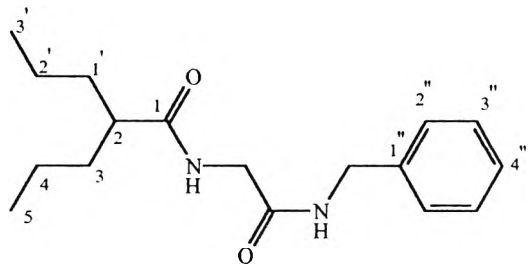


Figure 100. Mass fragmentation of N-(2-propylpentanoyl)-glycine ethyl ester

N-(2-Propylpentanoyl)-glycine benzylamide (CU 763-15-08)



As described in the preparation of N-(2-Propylpentanoyl)-L-proline benzylamide (CU 763-15-03) This compound was obtained by coupling of N-acylated amino acid with benzylamine in the presence of N,N′-dicyclohexylcarbodiimide.

The IR spectrum of this compound (Figure 74) showed a band of N-H stretching at 32793 cm^{-1}. The aliphatic C-H stretching appeared at 2944-2870 cm^{-1}. The amide characteristic peaks; C=O stretching, N-H bending were shown at 1639 and 1558 cm^{-1}, respectively. The interaction between N-H bending and C N stretching were observed at 1256 cm^{-1}. The aromatic C-H stretching, overtone and combination bands, C=C stretching, and out-of-plane C-H bending of aromatic ring appeared at 3089, 1963-1815, 1454 $cm^{-1}$and 744-694 cm^{-1}, respectively.

The ^{1}H-NMR spectrum of a solution of this compound in $CDCl_3$ had nine signals (Figure 75). The triplet, six-proton peak at 0.85 ppm represented methyl protons of C-5 and C-3′. The six-proton complex at 1.15-1.42 ppm consisted of four protons at C-4 and C-2′, two protons at C-1′ and C-3. The signal of protons at C-3 and C-1′ was observed at 1.55 ppm. Signal of the methine proton at C-2 appeared as a multiplet at 2.11 ppm.

The glycilic protons showed the signal as a doublet at 3.96 ppm due to the coupling with NH proton of 2-propylpentamide moiety (J=5.3 Hz). The methylene protons of benzylic moiety coupled with the adjacent NH proton, resulted in a doublet

at 4.43 ppm (J=5.7 Hz). The NH protons of 2-propylpentamide and benzylamide moieties appeared as broad bands at 6.33 and 6.65 ppm, respectively. The signal of aromatic protons appeared as five-proton multiplet at 7.29 ppm.

The ^{13}C-NMR spectrum of this compound is shown in figure 77. The peaks at 14.06, 20.78, 35.13, and 43.64 ppm represented C-5 and C-3′, C-4 and C-2′, C-3 and C-1′, and benzylic carbon, respectively. C-1 and C-2″ appeared at 168.96 and 176.91 ppm, respectively.

The signals of C-2 and glycilic carbon could not be distinguished clearly by the ^{13}C-NMR technique. In the DEPT 135 spectrum (Figure 78), the methine carbon, C-2 appeared as the positive peak at 47.29 ppm while the glycilic carbon appeared as the negative peak at 43.47 ppm.

The EIMS spectrum of this compound is shown in figure 79. A peak at m/e 290 represented molecular ion peak. Elimination of 1-propylbutyl radical followed by loss of CO resulted in a peak at m/e 163.

Cleavage of benzylamide resulted in a peak at m/e 106, which underwent two pathways of decomposition. β-Cleavage gave a peak at m/e 77, loss of NH resulted in a peak at m/e 91. Cleavage of benzamide moiety in the opposite side yielded a peak at m/e 184.

McLafferty rearrangement with loss of propylene yielded an ion with m/e 248. Elimination of ethylene resulted in a peak at m/e 219. Amide cleavage of the molecular ion yielded a peak at m/e 127. Cleavage within alkyl chain gave peakks at m/e 99 and 57. Pattern of fragmentation of this compound is shown in figure 101.

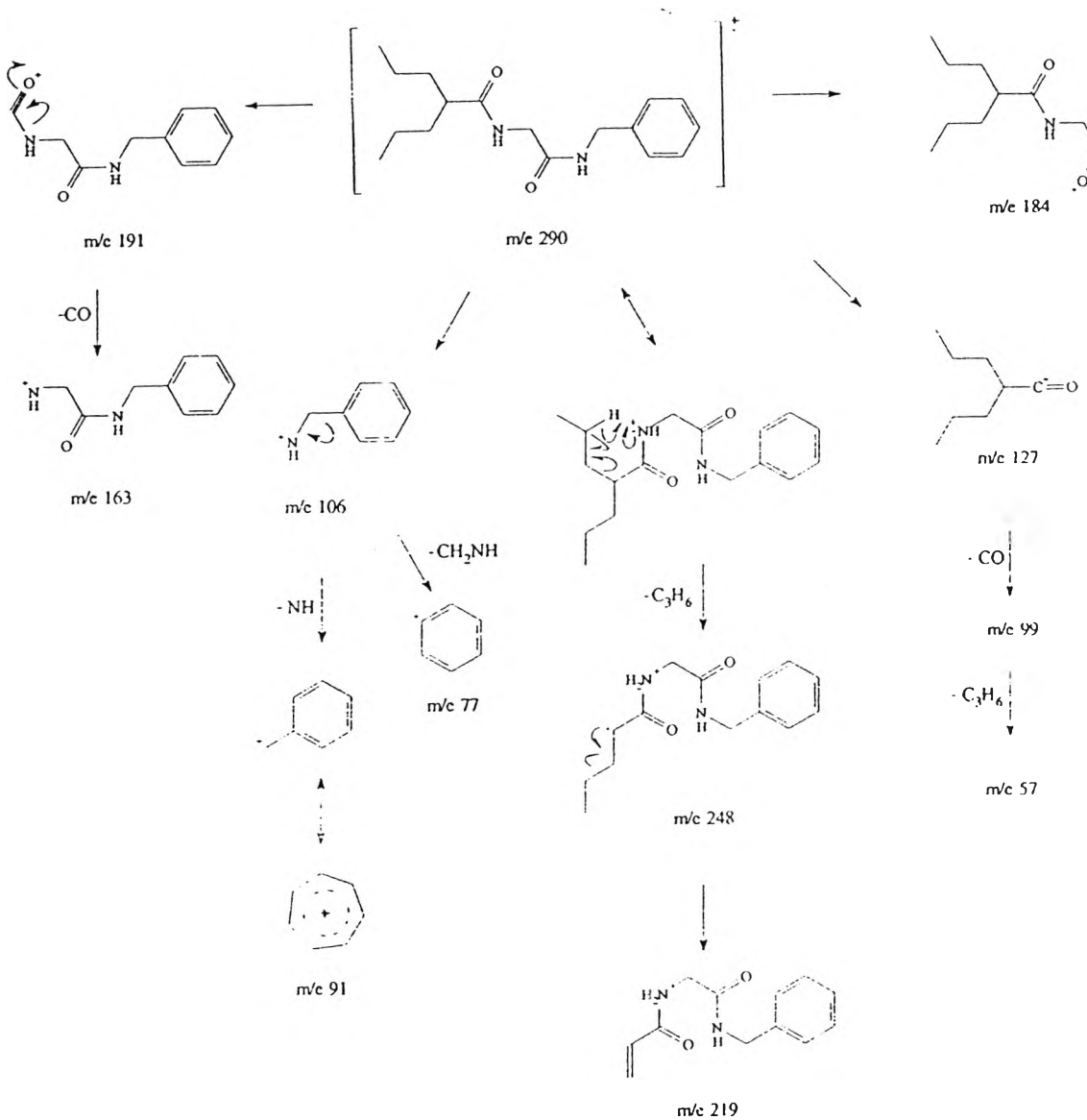


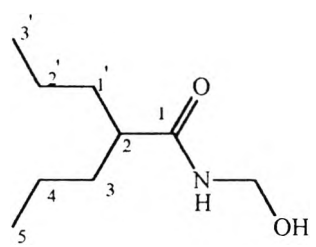
Figure 101. Mass fragmentation of N-(2-propylpentanoyl)-glycine benzylamide

2-Propylpentamide

This compound was synthesized readily from the general method of synthesis of primary amide by treatment of 2-propylpentanoyl chloride with concentrated ammonia solution at low temperature.

The IR spectrum of this compound (Figure 80) showed two N-H stretching bands at 3390 and 3195 cm^{-1}. The aliphatic C-H stretching band appeared at 2957-2870 cm^{-1}. The amide characteristic peaks; C=O stretching and C-N stretching appeared as the bands at 1654 and 1465 cm^{-1}.

N-Hydroxymethyl-2-propylpentamide (CU 763-15-09)



This compound was prepared from the reaction of 2-propylpentamide with 37% formaldehyde solution in the presence of potassium carbonate. Mechanism of reaction undergoes base-catalyzed addition as described in figure 17 (page 52). The reaction proceed smoothly with only one product occurred.

An attempt to synthesize this compound by acid-catalyzed addition was unsuccessful. The reaction gave the alkylidene bisamide derivative. The formation of this bisamide product could be explained as followed; after N-hydroxymethyl-2-propylpentamide was generated, the acidic condition caused protonation at the hydroxyl group which made this species as the target for nucleophilic substitution (S_N1) by another molecule of 2-propylpentamide. The proposed mechanism is suggested as follow. (See figure 102)

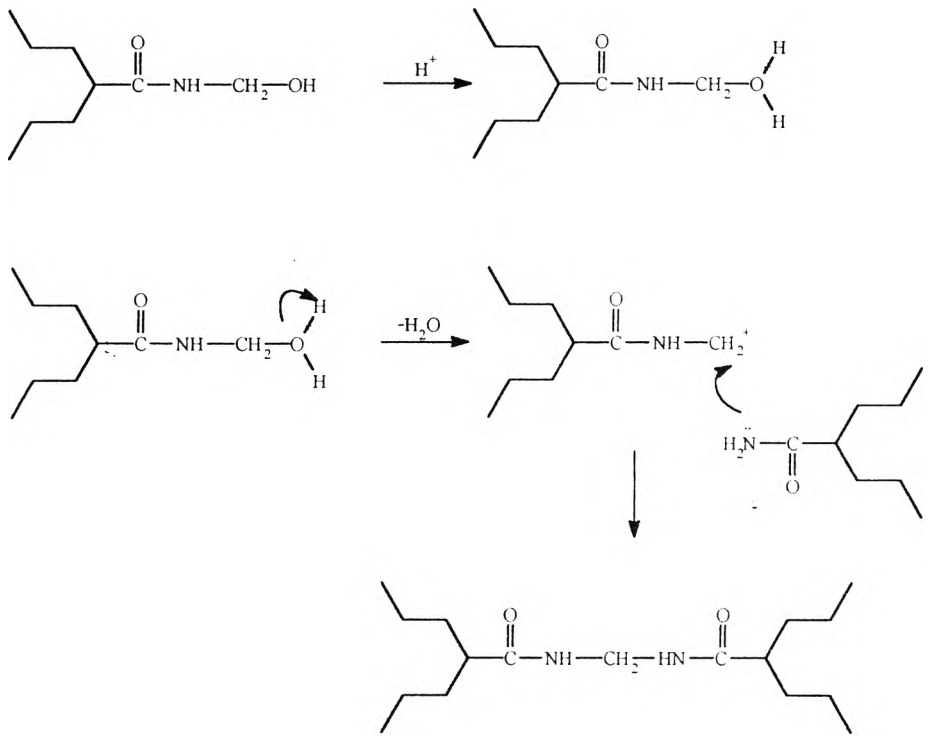


Figure 102. Proposed mechanism of formation of the alkylidene bisamide derivative in acid-catalyzed reaction.

The IR spectrum of this compound (Figure 81) showed a broad band resulting from overlapping between N-H stretching (of which the peak shown at 3302 cm^{-1}) and O-H stretching (of which the peak shown at 3217 cm^{-1}). The aliphatic C-H stretching band appeared at 2959-2870 cm^{-1}. The amide characteristic peaks; C=O stretching and N-H bending were attributed to the bands at 1655 and 1546 cm^{-1}. The C-O stretching was shown at 1049 and 1015 cm^{-1}.

The ^{1}H-NMR spectrum of a solution of this compound in $CDCl_3$ had seven signals. (Figure 82) The triplet, six-proton peak at 0.88 ppm represented methyl protons of C-5 and C-3′. The six-proton multiplet at 1.19-1.45 ppm was assigned to four protons at C-4 and C-2′, two protons at C-1′ and C-3. The signal of protons at C-3 and C-1′ was observed at 1.55 ppm. Signal of the methine proton at C-2 appeared as a multiplet at 2.05 ppm.

The one-proton triplet peak at 3.36 ppm represented the hydroxylic proton. The coupling (J=7.7 Hz) between this proton and aminal protons was observable due to intramolecular hydrogen bond of hydroxylic proton and the amide carbonyl.

The two-proton triplet peak at 4.73 ppm represented methylene protons of hydroxymethyl moiety and the NH proton appeared as a broad peak at 6.39 ppm.

The ^{13}C-NMR spectrum of this compound is shown in figure 83. The peaks at 14.07, 20.72, 35.13, and 47.40 ppm represented C-5 and C-3′, C-4 and C-2′, C-3 and C-1′, and C-2, respectively. The peaks at 64.92 and 177.96 ppm were associated with the aminal methylene and the amide carbonyl C-1, respectively.

The EIMS spectrum of this compound is shown in figure 84. A peak at m/e 174 represented a (M+1) peak. Elimination of hydroxyl radical yielded a peak at m/e 156. Rearrangement with loss of OCNH resulted in a peak at m/e 113. McLafferty rearrangement initiated at the hydroxyl group followed by elimination of ethyl radical yielded a peak at m/e 144.

McLafferty rearrangement initiated at nitrogen atom with loss of propylene followed by McLafferty rearrangement with loss of formaldehyde yielded a peak at m/e 101. β-Cleavage with elimination of $CONH_2$ resulted in a peak at m/e 57.

McLafferty rearrangement at the carbonyl group with elimination of formaldehyde followed by rearrangement with β-cleavage yielded a peak at m/e 101. α-Cleavage with loss of ethyl radical resulted in the peak at m/e 72. Pattern of fragmentation is shown in figure 103.

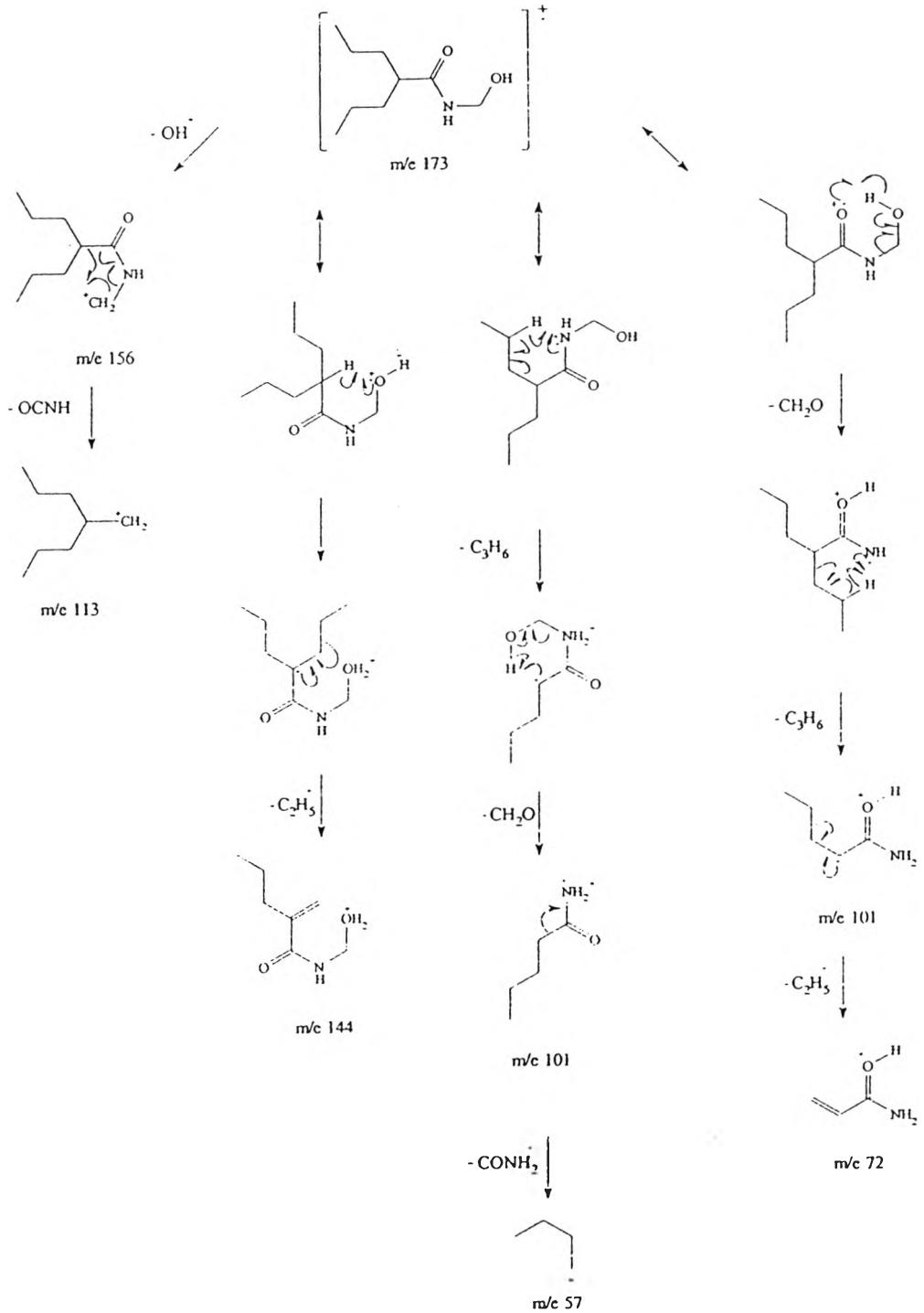
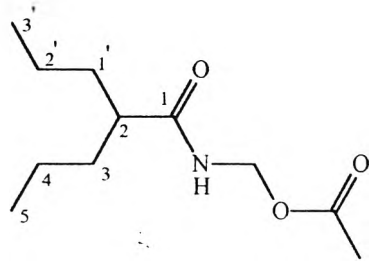


Figure 103. Mass fragmentation of N-hydroxymethyl-2-propylpentamide

N-Acetoxymethyl-2-propylpentamide (CU 763-15-10)



This compound was obtained by esterification of N-hydroxymethyl-2-propylpentamide with acetic anhydride in the presence of pyridine.

This acid-catalyzed esterification also generated the undesired product, alkylidene bisamide. The mechanism of this formation is the same as described for N-hydroxymethyl-2-propylpenytamide.

The IR spectrum of this compound (Figure 85) showed a band of N-H stretching at 3307 cm^{-1}. Aliphatic C-H stretching band appeared at 2958-2870 cm^{-1}. The ester characteristic peaks; C=O stretching, C-C(=O)-O-, and O-C-C stretching were shown at 1744, 1200, and 1017 cm^{-1}, respectively. The amide characteristic peaks; C=O stretching and N-H bending were attributed to the bands at 1673 and 1537 cm^{-1}.

The ^{1}H-NMR spectrum of a solution of this compound in $CDCl_3$ had seven signals (Figure 86). The triplet, six-proton peak at 0.80 ppm represented methyl protons of C-5 and C-3′. The six-proton multiplet at 1.20-1.46 ppm was assigned to four protons at C-4 and C-2′, two protons at C-1′ and C-3. The signal of two protons at C-3 and C-1′ was observed at 1.50 ppm. Signal of the methine proton at C-2 appeared as a multiplet at 2.06 ppm.

The three-proton singlet peak at 1.98 ppm was obviously represented the protons on acetyl group. The methylene protons showed signal as a doublet at 5.18 ppm. The NH proton appeared as a broad peak at 7.02 ppm.

Unfortunately, this compound was found unstable. Upon standing, the ester bond was hydrolyzed and gave back N-hydroxymethyl-2-propylpentamide and acetic acid which were parent compounds. This decomposition was confirmed by ^{1}H-NMR spectrum (Figure 104).

Bungaard (1991) suggested the mechanism of decomposition of N-α-acyloxymethyl derivatives of primary amide, carbamate, and sulfonamide and proposed that these derivatives decompose by an elimination-addition mechanism involving a reactive N-acylimine intermediate. The mechanism of decomposition of this compound is shown as figure 105.

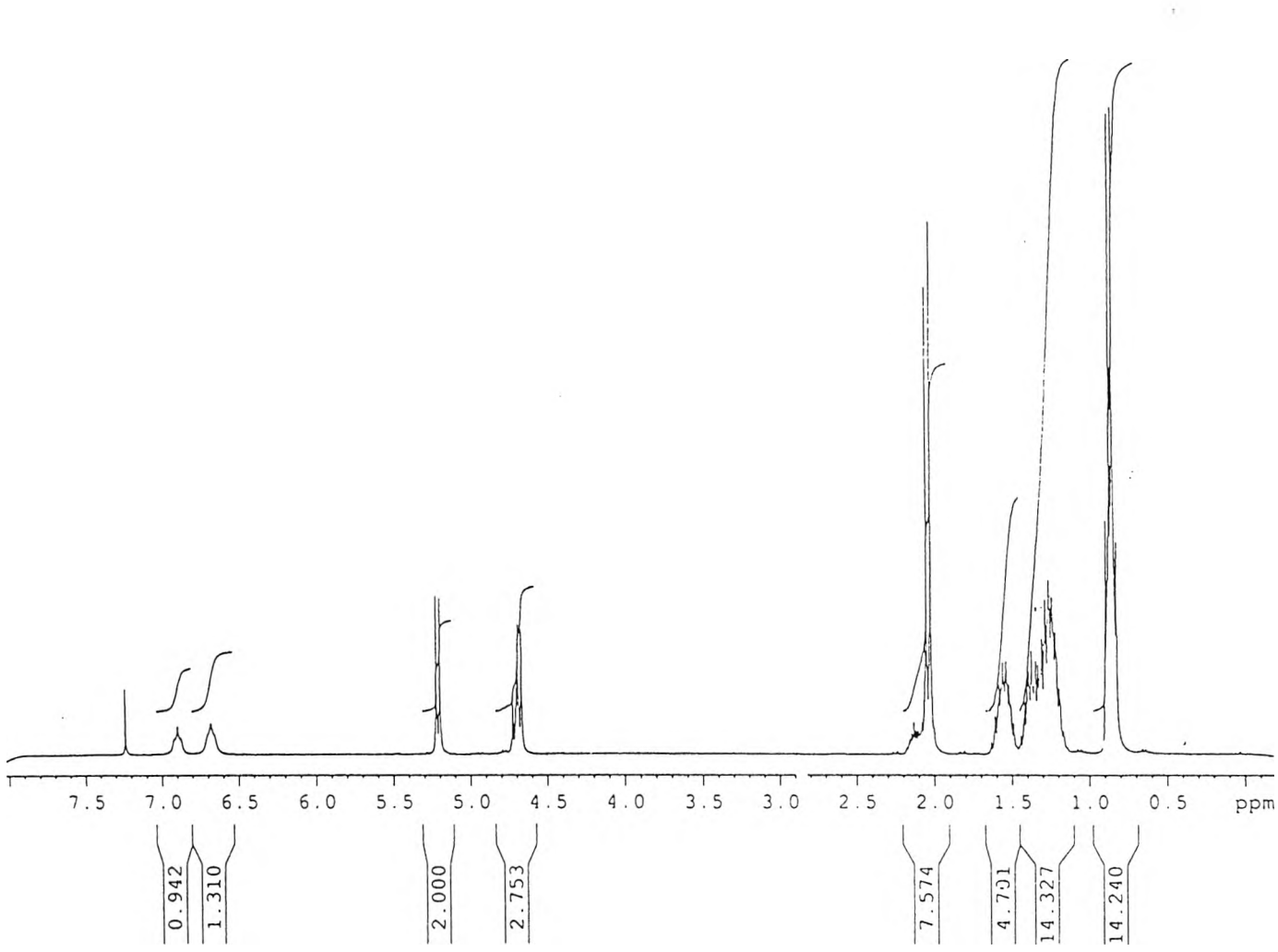


Figure 104. ^{1}H-NMR spectrum of decomposed product of N-acetoxymethyl-2-propylpentamide.

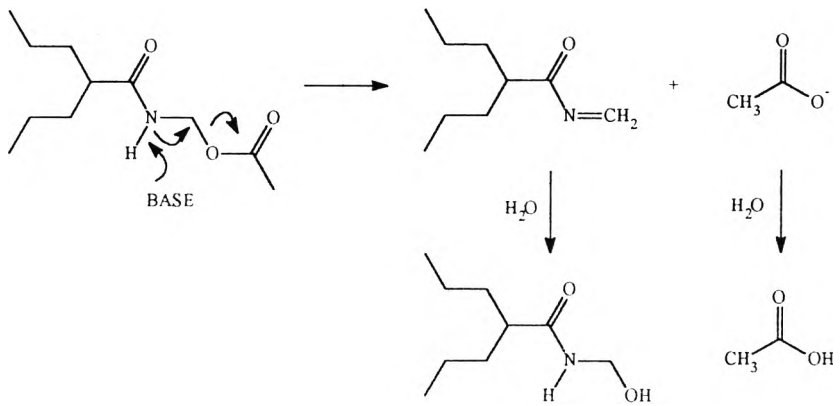
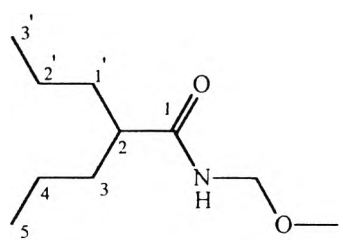


Figure 105. Mechanism of decomposition of N-acetoxymethyl-2-propylpentamide.

N-Methoxymethyl-2-Propylpentamide (CU 763-15-11)



This compound was prepared from the reaction of 2-propylpentamide with methoxymethyl chloride. However, the basicity of this amide was too weak and not enough to directly attack the alkyl halide , hence, its nucleophilicity was increased by reacting of this amide with sodium hydride to generate the anion species. This anion was counterparted by sodium ion and was precipitated from the solution. The mechanism of this generation is shown in figure 106.

The by-product which normally formed from this reaction was N,N-dimethoxymethyl-2-propylpentamide. This by-product was generated by the dialkylation reaction of the starting 2-propylpentamide. In order to decrease the generation of this by-product, the reaction was carried out at low temperature.

The products obtained from this reaction are neutral and the purification of these compounds was achieved by column chromatography.

The IR spectrum of this compound (Figure 87) showed a band of N-H stretching at 3298 cm^{-1}.The aliphatic C-H stretching band appeared at 2959-2870 cm^{-1}. The amide characteristic peaks; C=O stretching and N-H bending were attributed to the bands at 1655 and 1544 cm^{-1}. The peak at 1128 cm^{-1} was C-O-C stretching.

The ^{1}H-NMR spectrum of a solution of this compound in $CDCl_3$ had seven signals. (Figure 88) The triplet, six-proton peak at 0.89 ppm represented methyl protons of C-5 and C-3′. The six-proton multiplet at 1.20-1.46 ppm was assigned to four protons at C-4 and C-2′, two protons at C-1′ and C-3. The signal of two protons at C-3 and C-1′ was observed at 1.60 ppm. Signal of the methine proton at C-2 appeared as a multiplet at 2.08 ppm.

The three-proton singlet peak at 3.34 ppm was obviously assigned to the methoxylic protons. The aminal methylene protons coupled with an amidic proton resulted in a doublet two-proton peak at 4.70 ppm. The NH proton appeared as a broad peak at 6.06 ppm.

The ^{13}C-NMR spectrum of this compound is shown in figure 89. The peaks at 14.07, 20.78, 35.11, and 47.89 ppm represented C-5 and C-3′, C-4 and C-2′, C-3 and C-1′, and C-2, respectively. The peaks at 56.07, 71.25, and 176.83 ppm were associated with the methoxy carbon, aminal carbon and the amide carbonyl C-1, respectively.

The EIMS spectrum of this compound is shown in figure 90. A peak at m/e 188 represented the (M+1) peak. Amide cleavage followed by loss of CO and the cleavagre of alkyl chain yielded peaks at m/e 127, 99, and 57, respectively. Elimination of

methoxyl radical resulted in the peak at m/e 156. Elimination of OCNH from this ion gave a peak at m/e 113.

McLafferty rearrangement initiated at carbonyl group with loss of propene gave a peak at m/e 145.

McLafferty rearrangement initiated at nitrogen atom with loss of propylene resulted in a peak at m/e 145, which underwent inductive cleavage to yield a peak at m/e 84. Loss of methyl radical yielded a peak at m/e 172. Pattern of fragmentation is shown in figure 107.

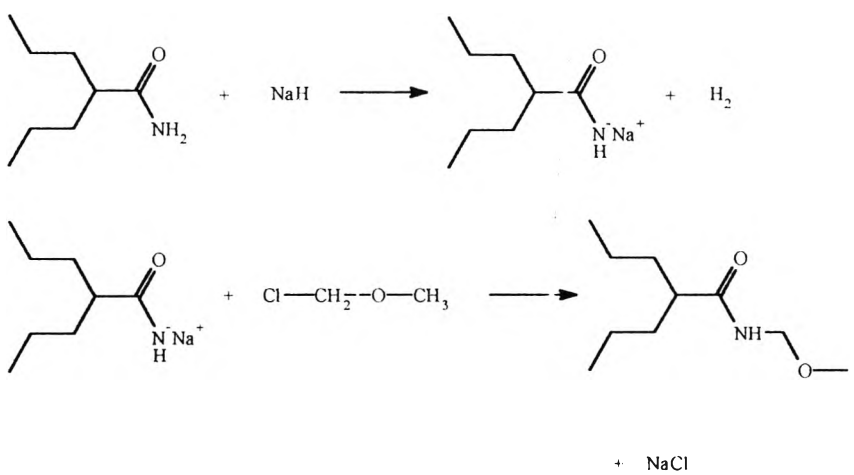


Figure 106. Mechanism of formation of N-Methoxymethyl-2-propylpentamide.

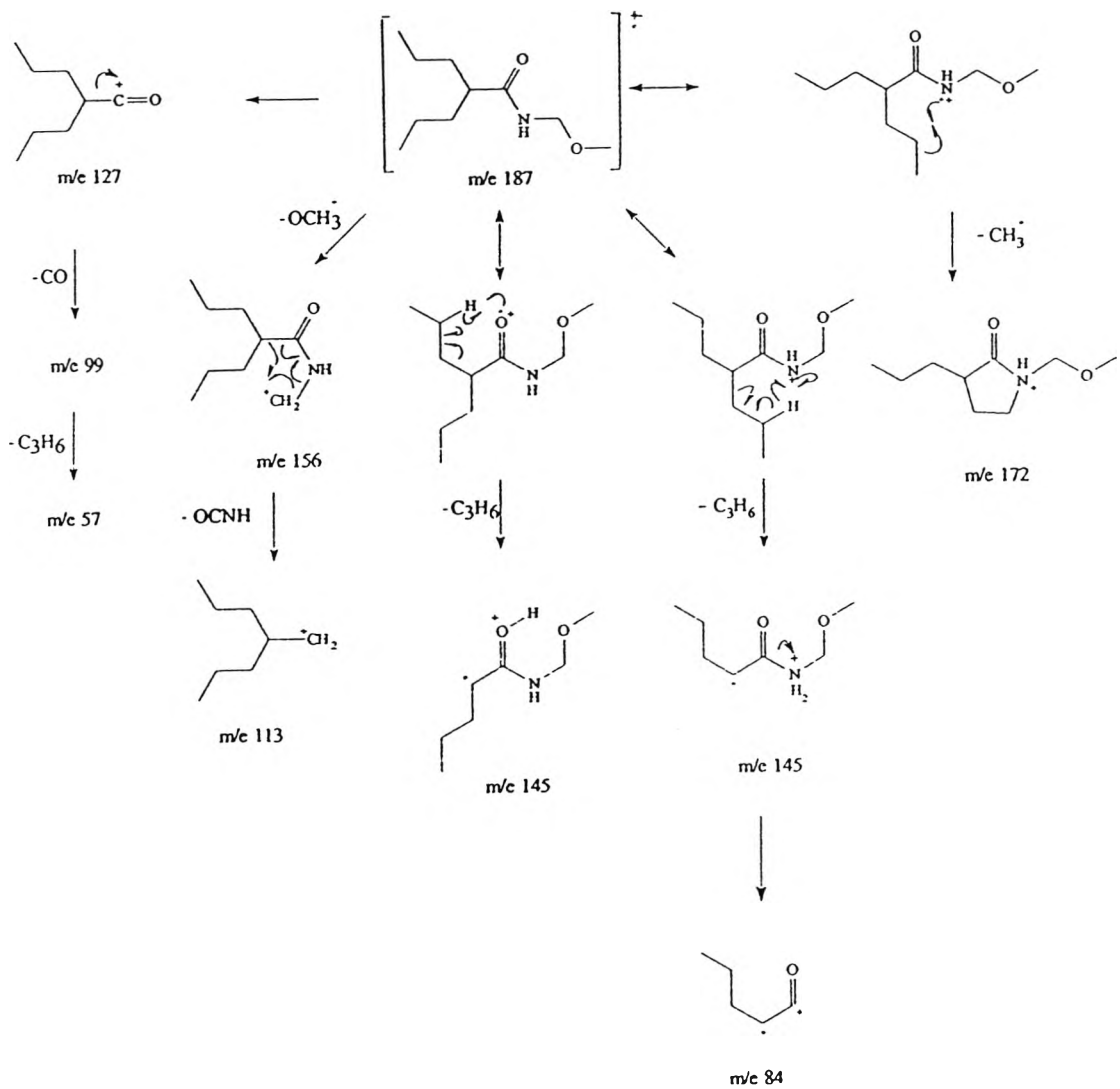


Figure 107. Mass fragmentation of N-methoxymethyl-2-propylpentamide