Syntheses of Peptides Related to the N-Terminal Structure of Corticotropin. IV. The Synthesis of the Amino Acid Sequences, Lys-Pro-Val-Gly and Lys-Pro-Val-Gly-Lys

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In the course of our synthetic studies of corticotropin peptides, the tetra- and pentapeptide derivatives, which have amino acid sequences corresponding to positions 11 to 14 and 11 to 15 respectively of the corticotropin molecule, have been obtained. The ε-amino function of the lysine residues, which were incorporated in these peptides, was protected by the introduction of the *t*-butyloxycarbonyl group after the procedure described by Schwyzer and Rittel.[1] In the coupling steps throughout the synthesis, the *N*, *N'*-dicyclohexylcarbodiimide (DCCI) method[2] was used in combination with the carbobenzoxy-protecting group for α-amino functions. Both of these peptides were built up in a step-by-step procedure from their carboxyl terminals, and at the same time alternative routes of preparing the tripeptide intermediates were also tried. The details of these syntheses will be described in this paper. Synthetic procedures were summarized in Figs. 1 and 2.

The Synthesis of the Lys-Pro-Val-Gly-Lys Sequence.—N^{ε}-*t*-Butyloxycarbonyl-L-lysine[1] was converted as usual into N^{α}-carbobenzoxy-N^{ε}-*t*-butyloxycarbonyl-L-lysine, which was isolated in the form of the crystalline dicyclohexylamine salt (I). The free acid from compound I was then treated with diazomethane to prepare the diacyl-L-lysine methyl ester (II), which was obtained in a crystalline state (m. p. 62~63°C). The catalytic hydrogenation of compound II in methanol containing acetic acid gave crystalline N^{ε}-*t*-butyloxycarbonyl-L-lysine methyl ester acetate (III). The free ester derived from compound III was coupled with carbobenzoxyglycine to obtain carbobenzoxyglycyl-N^{ε}-*t*-butyloxycarbonyl-L-lysine methyl ester (IV). Compound IV was hydrogenated, and the resultant dipeptide ester was condensed with carbobenzoxy-L-valine, thus affording carbobenzoxy-L-valylglycyl-N^{ε}-*t*-butyloxycarbonyl-L-lysine methyl ester (VII). The prolonged hydrogenation of compound IV yielded a large amount of a diketopiperazine like side product, even in the presence of excess acetic acid. The coupling of carbobenzoxy-L-proline with the hydrogenation product of compound VII gave a tetrapeptide derivative, carbobenzoxy-L-prolyl-L-valylglycyl-N^{ε}-*t*-butyloxycarbonyl-L-lysine methyl ester (VIII). The crystalline tetrapeptide ester (free base) (IX) was yielded by the catalytic hydrogenation of compound VIII. The hydrogenation was accelerated considerably by the addition of acetic acid; otherwise it proceeded very slowly. Compound VIII was then united with the free acid from compound I to obtain the crystalline

1) R. Schwyzer and W. Rittel, *Helv. Chim. Acta*, **44**, 159 (1961).
2) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

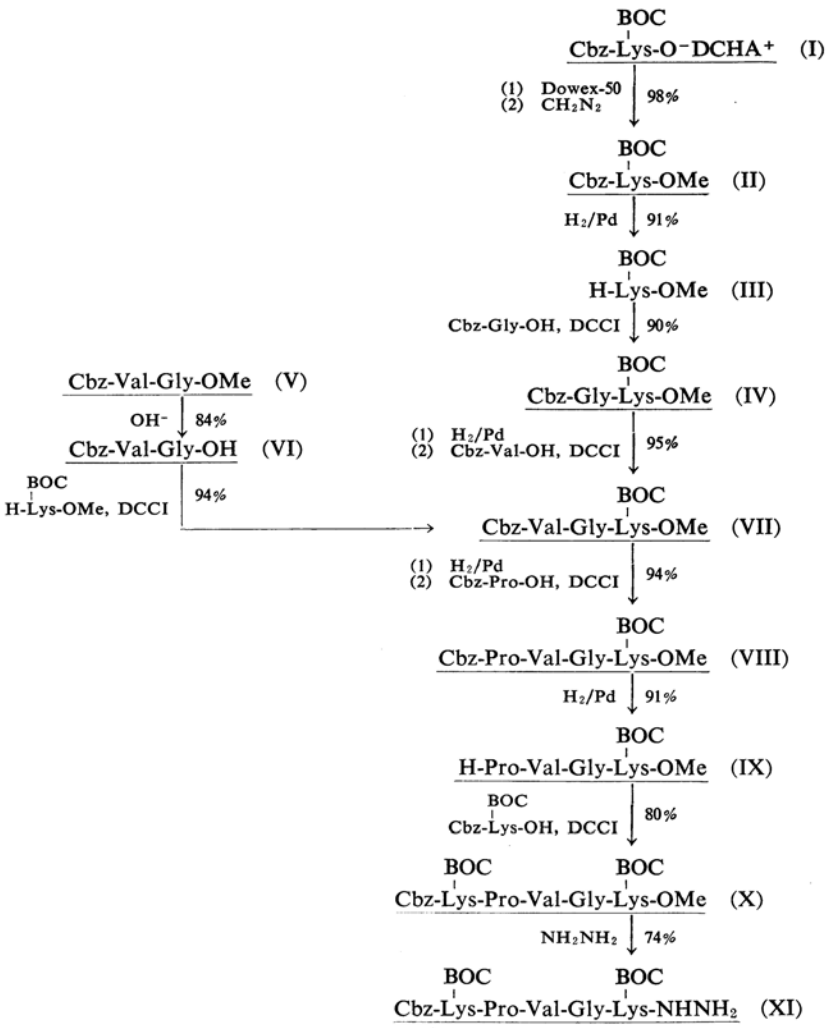
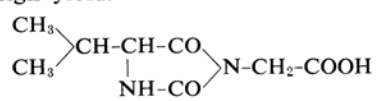


Fig. 1. Procedures for synthesis of the Lys-Pro-Val-Gly-Lys sequence. Cbz, carbobenzoxy; BOC, *t*-butyloxycarbonyl; Me, methyl; DCCI, *N*,*N*'-dicyclohexylcarbodiimide; DCHA, dicyclohexylamine.
Underline indicates that the compound was obtained in a crystalline form.

pentapeptide derivative, N^{α}-carbobenzoxy-N^{ε}-*t*-butyloxycarbonyl-L-lysyl-L-prolyl-L-valylglycyl-N^{ε}-*t*-butyloxycarbonyl-L-lysine methyl ester (X), which could be converted into the corresponding crystalline hydrazide (XI).

The tripeptide intermediate, compound VII, was prepared also by the condensation of the free ester of compound III with carbobenzoxy-L-valylglycine (VI), which was derived from the preceding methyl ester (V) by saponification. Maclaren[3] has described how the saponification of carbobenzoxy-L-leucylglycine ethyl ester is accompanied by a side reaction, producing a cyclic compound with a hydantoin structure, when excess alkali is used. We have observed that the crystalline product, which was obtained by the saponification of compound V, had a little higher nitrogen content and a little lower carbon content than the theoretical values calculated for compound VI. This fact may suggest that the crude sample of compound VI was contaminated, even when an exactly equivalent amount of alkali was used on the saponification, by a small amount of such a side product as is shown below. The pure sample could, however, be isolated after recrystallization from ethanol-water in a moderately high yield.



The Synthesis of the Lys-Pro-Val-Gly Sequence. — Carbobenzoxy-L-valylglycine methyl

3) J. A. Maclaren, *Australian J. Chem.*, **11**, 360 (1958).

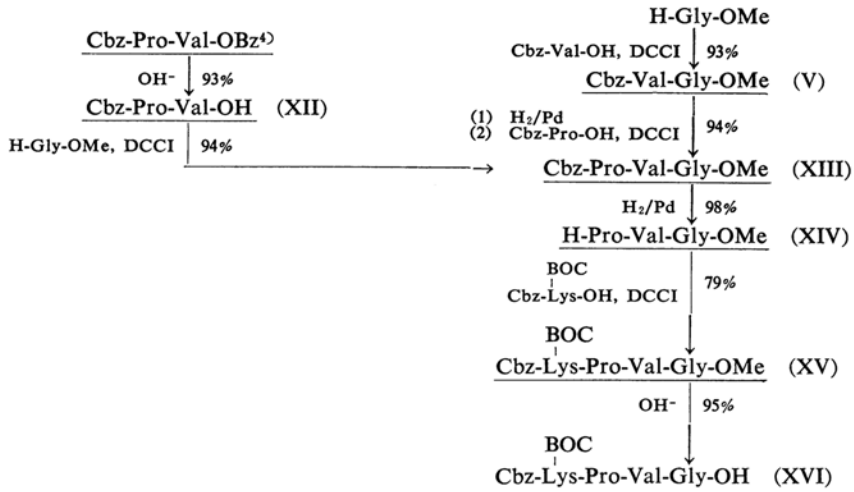


Fig. 2. Procedures for synthesis of the Lys-Pro-Val-Gly sequence. Cbz, carbobenzoxy; BOC, *t*-butyloxycarbonyl; Me, methyl; Bz, benzyl; DCCI, *N*,*N'*-dicyclohexycarbodiimide. Underline indicates that the compound was obtained in a crystalline form.

ester (V) was hydrogenated, and the resultant dipeptide ester was coupled with carbobenzoxy-L-proline to obtain a tripeptide intermediate, carbobenzoxy-L-prolyl-L-valylglycine methyl ester (XIII). This compound was also prepared through another procedure, consisting of the condensation of glycine methyl ester with carbobenzoxy-L-prolyl-L-valine (XII), which was derived from the crystalline benzyl ester[4] in a 90% yield by saponification. The corresponding methyl ester was, however, saponified to obtain compound XII in only a 50% yield.[5,6] This fact may indicate that the steric effect of the isopropyl side group in the valine methyl ester is more remarkable than that in the case of the benzyl ester.

The catalytic hydrogenation of compound XIII gave a crystalline tripeptide ester (XIV); this was next coupled with the free acid from compound I affording the crystalline tetrapeptide, N^{α}-carbobenzoxy-N^{ε}-*t*-butyloxycarbonyl-L-lysyl-L-prolyl-L-valylglycine methyl ester (XV), from which the corresponding acid XVI was derived by saponification.

Experimental

All melting points are uncorrected. The catalytic hydrogenation was carried out in a closed vessel connected with another small one, filled with soda lime, in which the evolved carbon dioxide was to be absorbed.

N^{α}-Carbobenzoxy-N^{ε}-*t*-butyloxycarbonyl-L-lysine Dicyclohexylamine Salt (I).—An 8.62 g.-sample (0.035 mol.) of N^{ε}-*t*-butyloxycarbonyl-L-lysine[1] (m. p. 226~227°C (decomp.), $[\alpha]_D^{25}$ +6.5±2° (*c* 1.085, 2 N ammonia)) and 6.0 g. of sodium bicarbonate were dissolved in 70 ml. of N sodium hydroxide and 30 ml. of water. The mixture was covered with 20 ml. of ether, and to this was added drop by drop 6.58 g. (0.0385 mol.) of benzyl chloroformate while it was being vigorously stirred at 0°C. The reaction mixture was stirred at 0°C for 30 min. and then at room temperature for 2 hr. After the ether phase had been removed, the aqueous solution was washed with ether and acidified to pH 2 at 0°C with ice-cold 4 N hydrochloric acid. The ensuing sirupy precipitates were taken up into cold ethyl acetate, and the organic solution was washed twice with water, dried over anhydrous sodium sulfate, and evaporated to dryness in vacuo. The resultant foamy residue was dissolved in ether, and the solution was mixed with an ethereal solution of 7.0 ml. (0.035 mol.) dicyclohexylamine (DCHA) and kept in a refrigerator overnight. The separated crystals were then collected, washed with ether, and dried; yield, 18.59 g. (94.8%); m. p. 148~149°C (sintering over 135°C). Recrystallization from methanol-ether gave 14.95 g. of colorless needles (recovery 80.5%); m. p. 153~155°C, $[\alpha]_D^{26}$ +5.5± 0.5° (*c* 4.564, methanol).

Found: C, 66.28; H, 9.43; N, 7.39. Calcd. for $C_{19}H_{28}O_6N_2 \cdot C_{12}H_{23}N$: C, 66.28; H, 9.15; N, 7.48%.

N^{α}-Carbobenzoxy-N^{ε}-*t*-butyloxycarbonyl-L-lysine Methyl Ester (II).—A suspension of 11.23 g. (0.02 mol.) of compound I in 160 ml. of 60% ethanol was shaken at room temperature for 30 min. in the presence of 20 ml. (wet volume) of Dowex-50W ×8 (H^+ form). After the resin had been removed by filtration, the filtrate was evaporated in vacuo at 40°C. The resultant oil was dissolved in ether and dried over anhydrous sodium sulfate. To this solution was added an ethereal solution of diazomethane at 0°C until the yellow color persisted; the mixture was then kept at 0°C for 10 min., after which time a few drops of acetic acid were

4) K. Inouye and H. Otsuka, This Bulletin, **34**, 4 (1961).
5) R. L. M. Synge, *Biochem. J.*, **42**, 99 (1948).
6) J. Meienhofer, *Chimia*, **16**, 385 (1962).

added to decompose the excess diazomethane. The solution was next washed successively with water, 5% sodium bicarbonate and water, dried over anhydrous sodium sulfate, and evaporated in vacuo, affording a dense oil which crystallized from ethyl acetate - petroleum ether; yield, 7.70 g. (97.7%); m. p. 60~61.5°C. Recrystallization from the same solvent system gave a sample for analyses; m. p. 62~63°C, $[\alpha]_D^{26}$ $-9.3\pm1°$ (*c* 2.045, acetone). Lit. oil, $[\alpha]_D^{26}$ $-10.6\pm0.5°$ (*c* 1.90, acetone);[1] m. p. 57°C, $[\alpha]_D$ not given.[7]

Found: C, 61.22; H, 7.59; N, 7.10. Calcd. for $C_{20}H_{30}O_6N_2$: C, 60.90; H, 7.67; N, 7.10%.

N^{ε}-*t*-Butyloxycarbonyl-L-lysine Methyl Ester Acetate (III).—Compound II (1.972 g., 5.0 mmol.), which was dissolved in 20 ml. of methanol and 0.5 ml. of acetic acid, was hydrogenated over a palladium black catalyst for 2 hr. After the catalyst had been filtered off, the solvent was removed in vacuo. The sirupy residue was dissolved in ether, and the solution was kept in a refrigerator overnight to afford the ester acetate in the form of needles; yield, 1.465 g. (91.4%); m. p. 78~79°C, $[\alpha]_D^{26}$ $+17.0\pm1°$ (*c* 2.134, methanol).[8]

Found: C, 52.46; H, 8.86; N, 8.77. Calcd. for $C_{12}H_{24}O_4N_2 \cdot CH_3COOH$: C, 52.48; H, 8.81; N, 8.74%.

Carbobenzoxyglycyl-N^{ε}-*t*-butyloxycarbonyl-L-lysine Methyl Ester (IV).—To a solution of 0.802 g. (2.5 mmol.) of compound III in 1 ml. of water were added 15 ml. of methylene chloride and 5 ml. of ice-cold 50% (w/v) potassium carbonate, and the mixture was shaken vigorously at 0°C. The aqueous phase was extracted again with cold methylene chloride. The organic extracts were pooled, dried over anhydrous sodium sulfate, and evaporated in vacuo at a both temperature of 25°C. The resultant oily residue was dissolved in methylene chloride together with 0.523 g. (2.5 mmol.) of carbobenzoxyglycine, and to this was added a methylene chloride solution of 0.517 g. (2.5 mmol.) of *N,N'*-dicyclohexylcarbodiimide (DCCI) at 0°C. The reaction mixture (total volume ca. 20 ml.) was then kept in a refrigerator overnight. After the separated *N,N'*-dicyclohexylurea had been removed by filtration (0.545 g., 97.2%), the filtrate was evaporated in vacuo. The sirupy residue was dissolved in ethyl acetate, and this solution was washed successively with ice-cold N hydrochloric acid, water, 5% sodium bicarbonate and water, dried over anhydrous sodium sulfate, and evaporated in vacuo to give a sirup, which crystallized during storage in a refrigerator; yield, 1.013 g. (90.0%); m. p. 71~73°C. These crystals were recrystallized from ethyl acetate-petroleum ether, affording lustrous thin needles in a recovery of 92.5%, m. p. 74~75°C, $[\alpha]_D^{24.5}$ $-8.04\pm0.5°$ (*c* 3.136, methanol).

Found: C, 58.58; H, 7.44; N, 9.60. Calcd. for $C_{22}H_{33}O_7N_3$: C, 58.52; H, 7.37; N, 9.31%.

Carbobenzoxy-L-valylglycine Methyl Ester (V).—Carbobenzoxy-L-valine (10.05 g., 0.04 mol.) and 3.56 g. (0.04 mol.) of freshly-distilled glycine methyl ester were coupled in the presence of 8.26 g. (0.04 mol.) of DCCI in a methylene chloride solution, almost as in case of compound IV. After the removal of the urea, the crystalline product was isolated from the filtrate during concentration; yield, 11.99 g. (93.2%); m. p. 159~161°C. Recrystallization from 40 ml. of chloroform and 160 ml. of ether gave 11.45 g. of needles (recovery 95.7%); m. p. 161~161.5°C, $[\alpha]_D^{23}$ $-28.6\pm1°$ (*c* 2.205, methanol).

Found: C, 59.45; H, 6.83; N, 8.70. Calcd. for $C_{16}H_{22}O_5N_2$: C, 59.61; H, 6.88; N, 8.69%.

Carbobenzoxy-L-valylglycine (VI).—To a suspension of 3.224 g. (0.01 mol.) of compound V in 25 ml. of methanol was added 10.0 ml. of N sodium hydroxide; the mixture was then shaken at room temperature for 60 min. The resultant solution was diluted by the addition of 20 ml. of water and then acidified with N hydrochloric acid at 0°C. The precipitates were taken into ethyl acetate, and the organic solution was evaporated in vacuo affording a crystalline residue, which was redissolved in 40 ml. of ethyl acetate and extracted three times with 5% sodium bicarbonate. The aqueous extracts were combined and acidified again with 4 N hydrochloric acid at 0°C. The resultant precipitates were extracted with ethyl acetate and crystallized from ethyl acetate - ether; yield, 3.04 g. (98.5%); m. p. (130~132°C) 147~148°C, $[\alpha]_D^{24}$ $-23.5\pm1°$ (*c* 2.032, methanol).

Found: C, 56.47; H, 6.36; N, 9.66. Calcd. for $C_{15}H_{20}O_5N_2$: C, 58.43; H, 6.54; N, 9.09%.

A 2.37 g.-sample of the crude product obtained above was recrystallized from ethanol-water (1 : 7) to give 2.03 g. of needles with a m. p. of 141~142°C. Recrystallization from the same solvent system gave a sample for analyses; m. p. 141~142°C, $[\alpha]_D^{25}$ $-24.9\pm1°$ (*c* 2.474, methanol); lit.[9] m. p. 146°C, $[\alpha]_D^{22}$ $-24.2\pm0.2°$ (*c* 5, methanol).

Found: C, 58.14; H, 6.52; N, 9.03. Calcd. for $C_{15}H_{20}O_5N_2$: C, 58.43; H, 6.54; N, 9.09%.

Carbobenzoxy-L-valylglycyl-N^{ε}-*t*-butyloxycarbonyl-L-lysine Methyl Ester (VII).—*A) By Val plus Gly-Lys.*—A 3.05 g.-sample (6.75 mmol.) of compound IV dissolved in 30 ml. of methanol and 1.5 ml. of acetic acid was hydrogenated over palladium for 2.25 hr. After the catalyst had been removed, the filtrate was evaporated in vacuo at 30°C. The ensuing oily residue was dissolved in 25 ml. of water, and the solution was washed twice with ethyl acetate and concentrated to about 10 ml. at 35~40°C in vacuo. To this concentrate was introduced 40 ml. of methylene chloride; the mixture was then shaken with 20 ml. of ice-cold 50% (w/v) potassium carbonate in an ice-bath. After the aqueous phase had been extracted twice more with cold methylene chloride, the organic extracts were combined, dried over anhydrous sodium sulfate, and evaporated in vacuo to give the dipeptide ester as a sirup in a quantitative yield.

The dipeptide ester obtained above and 1.70 g. (6.75 mmol.) of carbobenzoxy-L-valine were coupled, using 1.395 g. (6.75 mmol.) of DCCI in a methylene chloride solution in the same manner as has been

7) K. Sturm, R. Geiger and W. Siedel, *Chem. Ber.*, **96**, 609 (1963).

8) The value of $[\alpha]_D^{25}$ $+19.0\pm1.0°$ (*c* 1.09, methanol) was given for the corresponding hydrochloride.[1]

9) W. Grassmann and E. Wünsch, *Chem. Ber.*, **91**, 449 (1958).

described above. After the separated urea had been filtered off, the solvent was removed in vacuo to give a crystalline solid mass, which was redissolved in 10 ml. of hot ethyl acetate; then, after the addition of 20 ml. of ether, the solution was kept in a refrigerator overnight. Crystalline precipitates were collected, washed with ether and dried; yield, 3.51 g. (94.5%); m. p. 128.5~129°C. Recrystallization from acetonitrile gave colorless needles with a m. p. of 130~131°C, $[\alpha]_D^{24.5}$ −12.2±1° (*c* 2.483, methanol).

Found: C, 59.22; H, 7.83; N, 10.17. Calcd. for $C_{27}H_{42}O_8N_4$: C, 58.89; H, 7.69; N, 10.18%.

B) By Val-Gly plus Lys.—Compound VI (0.772 g., 2.5 mmol.) and N^{ε}-*t*-butyloxycarbonyl-L-lysine methyl ester (prepared from 0.802 g. (2.5 mmol.) of compound III as described in case of compound IV) were coupled in the presence of 0.517 g. (2.5 mmol.) of DCCI in a methylene chloride solution. The reaction mixture was treated as described in A) to obtain the tripeptide; yield, 1.29 g. (93.7%), m. p. 130~131°C. These crystals were recrystallized from ethyl acetate-ether in a recovery of 94.8%, m. p. 131~132°C, $[\alpha]_D^{25.5}$ −12.1±0.5° (*c* 3.151, methanol).

Found: C, 59.16; H, 7.87; N, 10.19. Calcd. for $C_{27}H_{42}O_8N_4$: C, 58.89; H, 7.69; N, 10.18%.

Carbobenzoxy-L-prolyl-L-valylglycyl-N^{ε}-*t*-butyloxycarbonyl-L-lysine Methyl Ester (VIII).—Compound VII (6.61 g., 0.012 mol.) was dissolved in 60 ml. of methanol and hydrogenated over palladium black for 2.5 hr. After the catalyst had been removed, the filtrate was evaporated in vacuo at a bath temperature of 30°C to afford the tripeptide ester as a colorless foam. The tripeptide ester obtained above and 2.99 g. (0.012 mol.) of carbobenzoxy-L-proline were coupled by the use of 2.48 g. (0.012 mol.) of DCCI in methylene chloride in the same manner as above. After the urea had been removed (2.57 g., 95.5%), the evaporation of the solution gave a foamy residue, which crystallized from ethyl acetate-ether; yield, 7.31 g. (94.1%), m. p. 142~143°C. Recrystallization from ethyl acetate gave a sample for analyses, m. p. 143~144°C, $[\alpha]_D^{23}$ −50.6±1° (*c* 2.558, methanol).

Found: C, 59.47; H, 7.79; N, 10.64. Calcd. for $C_{32}H_{49}O_9N_5$: C, 59.33; H, 7.62; N, 10.81%.

L-Prolyl-L-valylglycyl-N^{ε}-*t*-butyloxycarbonyl-L-lysine Methyl Ester (IX).—Compound VIII (0.972 g.), which was dissolved in 15 ml. of methanol containing 0.5 ml. of acetic acid, was hydrogenated in the presence of a palladium black catalyst for 2 hr. The catalyst was removed by filtration, and the filtrate was evaporated in vacuo to give a colorless and clear oil. The oily residue was dissolved in 5 ml. of water, and the aqueous solution was, after being washed with ethyl acetate, mixed with 10 ml. of methylene chloride. The mixture was then shaken vigorously with 5 ml. of 50% (w/v) potassium carbonate at 0°C and the aqueous phase was extracted again with methylene chloride. The organic extracts were combined, dried over anhydrous sodium sulfate, and evaporated in vacuo to give a crystalline solid mass, which was washed with ether and dried; yield, 0.700 g. (90.9%), m. p. 98~102°C. Recrystallization from methanol-ether gave needles with a m. p. of 104~106°C, $[\alpha]_D^{24.5}$ −34.4±1.5° (*c* 1.762, methanol).

Found: C, 56.13; H, 8.49; N, 13.71. Calcd. for $C_{24}H_{43}O_7N_5$: C, 56.12; H, 8.44; N, 13.64%.

N^{α}-Carbobenzoxy-N^{ε}-*t*-butyloxycarbonyl-L-lysyl-L-prolyl-L-valylglycyl-N^{ε}-*t*-butyloxycarbonyl-L-lysine Methyl Ester (X).—N^{α}-Carbobenzoxy-N^{ε}-*t*-butyloxycarbonyl-L-lysine (prepared from 0.528 g. (0.94 mmol.) of the DCHA salt (I) as described in the preparation of compound II) and 0.483 g. (0.94 mmol.) of the crystalline tetrapeptide ester (IX) were condensed by the use of 0.194 g. (0.94 mmol.) of DCCI in methylene chloride in the same manner as has been described above. After the urea (0.190 g., 90.4%) had been removed, the methylene chloride solution was washed successively with ice-cold N hydrochloric acid, water, 5% sodium bicarbonate and finally with water, dried over anhydrous sodium sulfate, and concentrated in vacuo. Gelatinous precipitates separated upon the addition of ether; yield, 0.728 g. (88.5%), m. p. 98~103°C. The amorphous solid crystallized in needles with a 90.0% recovery during the slow evaporation of the acetone solution in a cold room; m. p. 105~107°C, $[\alpha]_D^{25}$ −55.2±1° (*c* 2.126, methanol).

Found: C, 58.92; H, 7.98; N, 11.14. Calcd. for $C_{43}H_{69}O_{12}N_7$: C, 58.95; H, 7.94; N, 11.19%.

N^{α}-Carbobenzoxy-N^{ε}-*t*-butyloxycarbonyl-L-lysyl-L-prolyl-L-valylglycyl-N^{ε}-*t*-butyloxycarbonyl-L-lysine Hydrazide (XI).—A mixture of 0.876 g. (1.0 mmol.) of compound X and 0.5 ml. of hydrazine hydrate in 10 ml. of methanol was heated under reflux at a bath temperature of 90~95°C. The crystalline hydrazide started to separate in about one hour. After 2 hr. the bath was removed; the reaction mixture was allowed to stand at room temperature overnight and then refrigerated. The crystals were filtered off, washed with ice-cold methanol-ether (1 : 2) and with ether, and dried to give 0.615 g.; m. p. 194~195°C. The concentration of the mother liquor afforded an additional quantity of crystals; 0.084 g., m. p. 190~191.5°C. The total yield amounted to 0.699 g. (79.9%). The recrystallization of these crystals from methanol gave fine needles with a m. p. of 194~195°C in a recovery of 92.5%; $[\alpha]_D^{24.5}$ −31.3±1° (*c* 1.949, dimethylformamide).

Found: C, 57.58; H, 7.87; N, 14.28. Calcd. for $C_{42}H_{69}O_{11}N_9$: C, 57.58; H, 7.94; N, 14.39%.

Carbobenzoxy-L-prolyl-L-valine (XII).—To a solution of 8.77 g. (0.02 mol.) of carbobenzoxy-L-prolyl-L-valine benzyl ester (m. p. 91~92°C), which had been prepared as described previously,[4] in 50 ml. of methanol, 20 ml. of 2 N sodium hydroxide was added, and the mixture was shaken at room temperature for 60 min. The resultant solution was neutralized at 0°C with 40 ml. of ice-cold N hydrochloric acid, concentrated to about 50 ml. at 35°C in vacuo, and extracted three times with ethyl acetate. The organic extracts were combined and then shaken three times with M sodium bicarbonate. The pooled aqueous solution was, after being washed twice with ethyl acetate, acidified with 4 N hydrochloric acid. The oily precipitate was then taken into ethyl acetate. The ethyl acetate extracts were combined, washed twice with water, dried over anhydrous sodium sulfate, and evaporated at 35°C in

vacuo to afford a crystalline mass, which was dissolved in hot ethyl acetate and precipitated by the addition of petroleum ether; yield, 6.48 g. (93.0%), m. p. 136~137°C. A sample for analyses was obtained by recrystallization from ethyl acetate - ether (2 : 5) in the form of needles; m. p. 136.5~137.5°C, $[\alpha]_D^{22.5}$ −56.1±1° (*c* 2.140, methanol.). Lit. m. p. 134~135°C, $[\alpha]_D^{18}$ −58° (*c* 2.8, methanol);[5] m. p. 136°C, $[\alpha]_D^{25}$ −57.8° (*c* 1.2, methanol).[6]

Found: C, 62.34; H, 7.15; N, 7.95. Calcd. for $C_{18}H_{24}O_5N_2$: C, 62.05; H, 6.94; N, 8.04%.

Carbobenzoxy-L-prolyl-L-valylglycine Methyl Ester (XIII). — *A) By Pro plus Val-Gly.* — Carbobenzoxy-L-valylglycine methyl ester (V) (3.22 g., 0.01 mol.), which was suspended in 30 ml. of methanol containing 2 ml. of 20% hydrogen chloride in methanol, was hydrogenated in the presence of palladium black for 2.5 hr. After the catalyst had been removed, the filtrate was evaporated in vacuo at 30°C. The resultant foamy residue was dissolved in 10 ml. of water, and the solution was washed twice with ethyl acetate. To this aqueous solution was added 20 ml. of methylene chloride and solid sodium chloride to the point of saturation. The mixture was then shaken with 10 ml. of ice-cold 50% (w/v) potassium carbonate at 0°C. The aqueous phase was then extracted twice more with cold methylene chloride. The organic extracts were combined, dried over anhydrous sodium sulfate, and evaporated at 25°C in vacuo to give the dipeptide ester as an oil. The obtained dipeptide ester and 2.49 g. (0.01 mol.) of carbobenzoxy-L-proline were coupled using 2.06 g. (0.01 mol.) of DCCI in a methylene chloride solution in the same manner as has been described above. After the removal of the separated urea, the filtrate was washed successively with N hydrochloric acid, water, 5% sodium bicarbonate and water, dried over anhydrous sodium sulfate, and evaporated in vacuo to give a foamy residue which crystallized from acetone-ether; yield, 3.92 g. (93.6%), m. p. 131~132°C (sintering over 127°C). The recrystallization from the same solvent system gave a sample for analyses; m. p. 131~132°C, $[\alpha]_D^{23.5}$ −91.6±1° (*c* 2.130, methanol).

Found: C, 60.13; H, 6.89; N, 10.13. Calcd. for $C_{21}H_{29}O_6N_3$: C, 60.13; H, 6.97; N, 10.02%.

B) By Pro-Val plus Gly.—Compound XII (6.10 g., 0.0175 mol.) and 1.56 g. (0.0175 mol.) of freshly-distilled glycine methyl ester were coupled in the presence of 3.62 g. (0.0175 mol.) of DCCI in methylene chloride as above. The reaction mixture was then treated in the manner described in A) to obtain the tripeptide; yield, 6.85 g. (93.5%); m. p. 132~133°C. Recrystallization from acetone-ether did not alter the m. p.; $[\alpha]_D^{25}$ −91.0±1° (*c* 2.158, methanol). Lit. m. p. 111~112°C, $[\alpha]_D^{25}$ −90.4° (*c* 1.6, methanol);[10] amorph., m. p. 125~127°C, $[\alpha]_D^{24}$ −89.7° (*c* 1.05, methanol).[11]

Found: C, 60.23; H, 7.03; N, 10.36. Calcd. for $C_{21}H_{29}O_6N_3$: C, 60.13; H, 6.97; N, 10.02%.

L-Prolyl-L-valylglycine Methyl Ester (XIV). — Compound XIII (5.25 g., 0.0125 mol.) was dissolved in 80 ml. of methanol and then hydrogenated over a palladium black catalyst for 1.5 hr. After the catalyst had been removed, the solvent was evaporated at 30°C in vacuo. The crystalline solid residue was recrystallized from methanol - ether - petroleum ether to give fine needles; yield, 3.475 g. (97.6%), m. p. 150°C, $[\alpha]_D^{25}$ −76.8±1° (*c* 1.987, methanol); lit.[10] m. p. 140~142°C, $[\alpha]_D$ not given.

Found: C, 54.86; H, 8.17; N, 14.69. Calcd. for $C_{13}H_{23}O_4N_3$: C, 54.72; H, 8.12; N, 14.73%.

N^{α}-Carbobenzoxy-N^{ε}-*t*-butyloxycarbonyl-L-lysyl-L-prolyl-L-valylglycine Methyl Ester (XV).—Compound XIV (2.85 g., 0.01 mol.) and N^{α}-carbobenzoxy-N^{ε}-*t*-butyloxycarbonyl-L-lysine (derived from 5.62 g. (0.01 mol.) of the DCHA salt (I)) were united by the use of 2.06 g. (0.01 mol.) of DCCI in a methylene chloride solution in the same manner as has been described above. The urea was then filtered off (1.99 g., 89.0%), and the filtrate was evaporated in vacuo to afford a foamy residue. The residue was dissolved in ethyl acetate, and the solution was washed successively with ice-cold N hydrochloric acid, water, 5% sodium bicarbonate and water, and dried over anhydrous sodium sulfate. Gelatinous precipitates (5.70 g., 88.1%), which separated during the concentration of the ethyl acetate solution, crystallized from acetone in needles; yield, 5.13 g. (79.2%); m. p. 125~127°C. Recrystallization from acetone afforded thin lustrous needles as a sample for analyses; m. p. 128~129°C, $[\alpha]_D^{24.5}$ −80.4±1° (*c* 2.035, methanol).

Found: C, 59.65; H, 7.70; N, 10.70. Calcd. for $C_{32}H_{49}O_9N_5$: C, 59.33; H, 7.62; N, 10.81%.

N^{α}-Carbobenzoxy-N^{ε}-*t*-butyloxycarbonyl-L-lysyl-L-prolyl-L-valylglycine (XVI). — To a solution of 3.24 g. (0.005 mol.) of compound XV in 12 ml. of methanol, 5.0 ml. of 2 N sodium hydroxide was added at 0°C; the mixture was then shaken at room temperature for 60 min., after which time the solution was cooled in an ice-bath, diluted with 25 ml. of water, and then neutralized with 10.0 ml. of N hydrochloric acid. The resultant sirupy precipitates were taken into ethyl acetate and the organic solution was evaporated in vacuo to remove the methanol. The residue, which was dissolved again in ethyl acetate, was washed with cold water containing a few drops of N hydrochloric acid and with saturated sodium chloride, dried over anhydrous sodium sulfate, and concentrated in vacuo. The addition of twice as much ether gave gelatinous precipitates slowly. These precipitates were washed with ether and dried; yield, 2.87 g. (95.3%), m. p. 107~117°C, $[\alpha]_D^{24.5}$ −79.6±1° (*c* 2.593, methanol); lit.[7] m. p. 116~118°C, $[\alpha]_D^{20}$ −81.4±2° (*c* 1, methanol).

Found: C, 58.34; H, 7.55; N, 11.02. Calcd. for $C_{31}H_{47}O_9N_5$: C, 58.75; H, 7.48; N, 11.05%.

Summary

The amino acid sequence Lys-Pro-Val-Gly-Lys, which corresponds to positions 11 to 15 of the corticotropin molecule, has been synthesized in the form of a crystalline derivative,

10) C. H. Li, J. Meienhofer, E. Schnabel, D. Chung, T-b. Lo and J. Ramachandran, *J. Am. Chem. Soc.*, **83**, 4449 (1961).

11) K. Hofmann, E. Stutz, G. Spühler, H. Yajima and E. T. Schwartz, ibid., **82**, 3727 (1961).

N^{α}-carbobenzoxy-N^{ε}-*t*-butyloxycarbonyl-L-lysyl-L-prolyl-L-valyl-glycyl-N^{ε}-*t*-butyloxycarbonyl-L-lysine methyl ester, from which the crystalline hydrazide has then been obtained. The synthesis of the Lys-Pro-Val-Gly sequence corresponding to positions 11 to 14 of corticotropin has also been described.

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