[Chem. Pharm. Bull. 21(5) 972—980 (1973)]

UDC 547.466.1.057 : 615.31.011.5.015.11

Synthesis of Antirenin Active Peptides. III.[1-3] C-Terminal Carbinol Analogues of Peptides related to the Partial Structure of Angiotensinogen as Renin Inhibitor

KEISUKE SHIGEZANE and TOMISHIGE MIZOGUCHI

Organic Chemistry Research Laboratory, Tanabe Seiyaku Co., Ltd.[4]

(Received September 8, 1972)

Modifications were attempted on the renin inhibitors, leucylleucylvalyltyrosine ethyl ester or leucylleucylvalylphenylalanine ethyl ester which were reported in the preceding paper.

Borohydride reduction of the above peptide esters, prepared by the solid phase method using bromoacetyl polystyrene, afforded the respective C-terminal carbinol derivatives, which were moderately soluble in water and the renin inhibitory activity was found to be retained.

Elongation of the aforementioned tetrapeptide esters or carbinols by a hydrophilic amino acid residue or introduction of the sodiumsulfomethyl group was rather fruitless with regards to solubility as well as to renin inhibitory activity.

Octapeptide-carbinol derivative, prolylphenylalanylhistidylleucylleucylvalyltyrosylserinol was synthesized, whose dihydrochloride was moderately soluble in water and exhibited the highest renin inhibitory activity among the compounds tested throughout this series of work.

In the previous papers of this series[2,5] we described syntheses of oligopeptide derivatives having amino acid sequences identical or analogous to the structure around the Leu[10]–Leu[11] bond of angiotensinogen. This zymogen is known to undergo cleavage at this bond by the hydrolytic enzyme, renin, releasing angiotensin I which is the precursor of the pressor active hormone, angiotensin II. Several of these synthetic peptide derivatives such as the ester or amide derivatives of leucylleucylvalyltyrosine and leucylleucylvalyl phenylalanine were found to be competivive inhibitors of renin.[6]

However, these compounds have poor solubility in the biological test system (saline-phosphate buffer, pH 6.4), probably owing to the hydrophobic amino acid residues such as leucine, valine and phenylalanine residues in their molecules.

The present paper describes further synthetic studies to find more soluble compounds possessing the renin inhibitory activity and to obtain additional knowledge on the structure-activity relationship. The modified solid phase peptide synthesis employing bromoacetyl-polystyrene[7] was used for preparation of the intermediates of the test compounds.

First, syntheses of the two renin inhibitors, leucylleucylvalyltyrosine and leucylleucylvalylphenylalanine ethyl esters (V and VI)[2] were performed by the modified solid phase method.

1) Presented at the 9th Symposium on Peptide Chemistry, Shizuoka, Nov. 24, 1971.
2) Part II: K. Shigezane, M. Muraki, T. Morikawa and T. Mizoguchi, *Yakugaku Zasshi*, **91**, 987 (1971).
3) The following abbrebiations have been adopted throughout this paper: DCHA: dicyclohexylamine; Boc: *tert*-butoxycarbonyl; Cbz: benzyloxycarbonyl; DCC: dicyclohexylcarbodiimide; Bzl: benzyl.
4) Location: *2-2-50, Kawagishi, Toda-shi, Saitama.*
5) A. Ide, K. Shigezane, S. Shigezane, T. Mizoguchi and S. Saito, *Yakugaku Zasshi*, **90**, 850 (1970).
6) *a*) T. Kokubu, E. Ueda, S. Fujimoto, K. Hiwada, A. Kato, H. Akutsu, Y. Yamamura, S. Saito and T. Mizoguchi, *Nature*, **217**, 456 (1968); *b*) T. Kokubu, Presented at 18th General Assembly of the Japan Medical Congress, Tokyo, April, 1971; *c*) Details of the biological study will be reported elsewhere by T. Kokubu, School of Medicine, Osaka University.
7) *a*) T. Mizoguchi, K. Shigezane and N. Takamura, *Chem. Pharm. Bull.* (Tokyo), **18**, 1465 (1970); *b*) *Idem, ibid.*, **17**, 411 (1969); *c*) F. Weygand, "Peptides 1968," ed. by E. Bricas, North Holland Publishing Co., Amsterdam, 1968, p. 183.

Boc-leucylleucylvalyl-O-Bzl-tyrosyloxyacetyl copolystyrene-divinylbenzene (I) was prepared from the Boc-O-Bzl-tyrosyloxyacetyl resin through three cycles of the deblocking, neutralization and coupling steps. In each coupling step, 2.5—3-fold excess of Boc amino acid was used. The peptide resin I was then subjected to cleavage by mild hydrolysis in dioxane containing 0.5N aqueous sodium hydroxide or by treatment with an excess of sodium thiophenoxide in dimethylformamide containing pyridine, affording Boc-leucylleucylvalyl-O-Bzl-tyrosine (III) in 24 or 21% yield (calculated from the starting Boc amino acid resin). The product was purified as the DCHA salt. As already described in a previous paper,[7a] the total yield of the product was somewhat lower in this method than those obtained in the Merrifield's method.[8] Leucylleucylvalyltyrosine ethyl ester (V) hydrochloride, which was obtained from III by esterification and subsequent removal of the protecting groups, was found to be identical with a specimen[2] prepared by the conventional method. Similarily, leucylleucylvalylphenylalanine ethyl ester (VI) hydrochloride was synthesized, which was identified with a specimen prepared before.[2]

However, as both V and VI hydrochlorides were poorly soluble in water, it was desired to produce more soluble derivatives of these compounds possessing renin inhibitory activity. An attempt was first made on reduction of C-terminal ester groups of V and VI to obtain the corresponding peptide-carbinol analogues, XII and XIII.

Reduction of optically active amino acid esters to the corresponding amino alcohol derivatives by sodium borohydride in aqueous ethanol has been reported.[9] This reaction or an analogous method was later used for reduction of some peptide ester derivatives to yield the corresponding peptide-carbinols.[10,11] Reduction of V and VI with borohydride was successfully carried out in aqueous ethanol at room temperature affording leucylleucylvalyltyrosinol (XII) and leucylleucylvalylphenylalaninol (XIII) respectively in good yields, which were easily isolated and purified as their hydrochlorides. As shown in Table, solubilities of these compounds thus obtained were considerably improved as compared with those of the corresponding ester derivatives, V and VI, and the biological activities were also found to be retained, though they were slightly lower than those of V and VI. The pH values of the saturated solutions of XII-and XIII-hydrochlorides were 5.2 and 5.4 respectively. Advantageously, these values are closer to pH 6.4, optimum pH for renin action, than those of V and VI (4.8—5.0 and 4.9).

Next, introduction of a relatively hydrophilic amino acid residue to leucylleucylvalyltyrosyl sequence was investigated. The Boc-leucylleucylvalyl-O-Bzl-tyrosyl-O-Bzl-seryloxyacetyl resin was synthesized by the modified solid phase method, and cleaved by thiophenoxide to give IX in an overall yield of 20%. IX thus obtained was then subjected to esterification followed by deblocking of the Boc and Bzl groups to give leucylleucylvalyltyrosylserine methyl ester (XI)as the hydrochloride. On the other hand, glycylleucylleucylvalyltyrosinol (XVI) was obtained by the coupling of Cbz-glycine with XII and subsequent removal of the Cbz group. As can be seen in Table, the data on these compounds and on glycylleucylleucylvalyltyrosine ethyl ester and glycylleucylleucylvalylphenylalanine ethyl ester which were prepared previously,[2] indicate that such elongation of the tetrapeptides is not so beneficial for the solubility as well as for renin inhibitory activity.

Introduction of the sodiumsulfomethyl group to the N-termini of the tetrapeptide derivatives, V and XII, was also attempted. When V was heated with sodium hydroxymethane sulfonate dissolved in aqueous ethanol, N-sodiumsulfomethyl-leucylleucylvalyltyrosine ethyl

8) R.B. Merrifield, *Biochemistry*, **3**, 1385 (1964).
9) H. Seki, K. Koga and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **15**, 1948 (1967); H. Seki, K. Koga, H. Matsuo, S. Ohki, I. Matsuo and S. Yamada, *ibid.*, **13**, 955 (1965).
10) O. Yonemitsu, H. Hamada and Y. Kanaoka, *Tetrahedron Letters*, **1968**, 3575; *idem*, *Chem. Pharm. Bull.* (Tokyo), **17**, 2075 (1969).
11) K. Kawamura, S. Kondo, K. Maeda and H. Umezawa, *Chem. Pharm. Bull.* (Tokyo), **17**, 1902 (1969).

ester (VII) was easily obtained in a good yield. Similarly, the corresponding C-terminal carbinol analogue (XIV) was prepared from XII. However, introduction of this group to V or XII was found to be hardly effective in improving solubility and it even caused a considerable fall in the renin inhibitory activity.

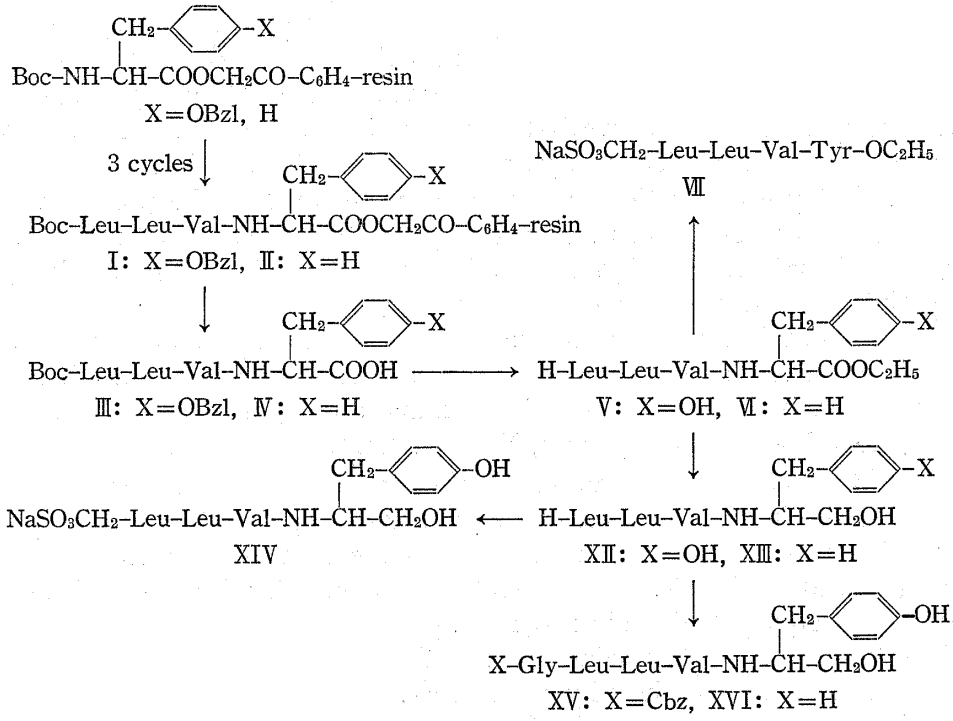

Chart 1

In order to obtain more active compounds, a synthetic study was made on the octapeptide carbinol derivative (XXVI), whose amino acid sequence is identical with the partial structure, prolylphenylalanylhistidylleucylleucylvalyltyrosylserine, of angiotensinogen. The octapeptide of this sequence is considered to be the minimal size of peptide derivatives which act as renin substrates.[12] To the Boc-leucyloxyacetyl resin were coupled successively Boc-im-Bzl-histidine, Boc-phenylalanine and Cbz-proline. N-Methylmorpholine, instead of triethylamine, in chloroform was used in the neutralization steps. Boc-im-Bzl-histidine was coupled in DMF as solvent. The coupling reaction with Boc-im-Bzl-histidine was carried out for five hours, while the reaction time for the other two protected amino acids was three hours. Hydrazinolysis of the resulting protected peptide resin in DMF-methanol afforded XIX, which was identical with a specimen prepared alternatively by the conventional method. Boc-leucylvalyl-O-Bzl-tyrosyl-O-Bzl-serine XX, prepared by the solid phase method followed by thiophenoxide cleavage, was converted to the methyl ester, XXI, which was proved to be identical with the product prepared via an alternative route. Removal of the Boc group from XXI afforded leucylvalyl-O-Bzl-tyrosyl-O-Bzl-serine methyl ester (XXII) as the hydrochloride, which was subjected to the borohydride reduction as described above for Vand VI, providing leucylvalyl-O-Bzl-tyrosyl-O-Bzl-serinol (XXIII) in a good yield.

When XIX was coupled with XXIII by the azide method using 1.1 molar equivalents of iso-amylnitrite, the yield of the product was poor. Therefore, a model reaction was carried out by coupling XIX with leucine methyl ester under the same conditions, but the yield of the product (XXIV) was again low and a considerable amount of the unchanged hydrazide (XIX) was recovered from the reaction mixture. Such unexpected low reactivity of XIX

12) L.T. Skeggs, K.E. Lentz, J.R. Kahn and H. Hochstrasser, *J. Exp. Med.*, **128**, 13 (1968).

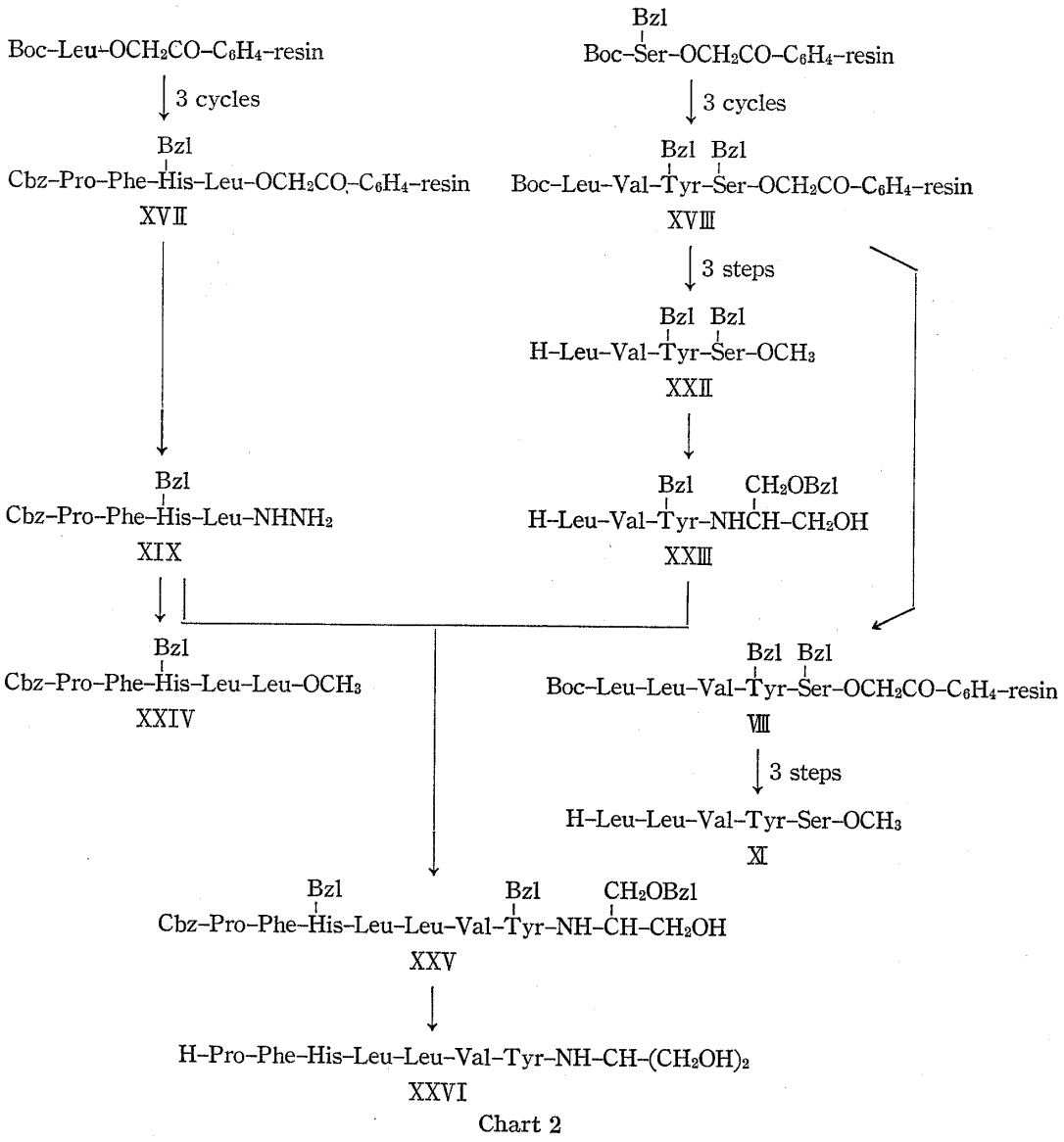

Chart 2

TABLE I

Peptide derivatives	Solubility (water, % w/v)	Inhibitory percent (%)[6b,a] 1.0 (mg)
H-Leu-Leu-Val-Tyr-OC_2H_5 (V)·HCl[2]	<0.5	44.5
H-Leu-Leu-Val-Phe-OC_2H_5 (VI)·HCl[2]	1.0	66.7
H-Leu-Leu-Val-Tyrol[b] (XII)·HCl	4.7	34.4
H-Leu-Leu-Val-Pheol[c] (XIII)·HCl	3.1	52.6
H-Leu-Leu-Val-Tyr-Ser-OCH_3 (XI)·HCl	1.0	—[d]
H-Gly-Leu-Leu-Val-Tyrol[b] (XVI)·HCl	2.1	14.6
H-Gly-Leu-Leu-Val-Tyr-OC_2H_5·HCl[2]	<0.5	+[e]
H-Gly-Leu-Leu-Val-Phe-OC_2H_5·HBr[2]	<0.5	+[e]
$NaSO_3CH_2$-Leu-Leu-Val-Tyr-OC_2H_5 (VII)	0.6	26.9
$NaSO_3CH_2$-Leu-Leu-Val-Tyrol[b] (XIV)	1.4	0.0
H-Pro-Phe-His-Leu-Leu-Val-Tyr-Serol[f] (XXVI)·2HCl	1.7	81.1
H-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser-OCH_3·2HCl[2]	g)	—[d]

a) inhibitory percent $=100-\frac{\text{ng AT formed in Exp.}}{\text{ng AT formed in cont.}}\times 100\%$ *b*) tyrosinol *c*) phenylalaninol *d*) Could not be tested for the biological activity because of poor solubility in saline *e*) The test solution was slightly gelatinous. *f*) serinol, *g*) insoluble in water

toward the nitrite is presumably attributable to a steric environment of the molecule around the hydrazide group. After several attempts were made, use of a large excess of iso-amylnitrite and hydrogen chloride in THF was found to convert XIX completely to its azide derivative. Under these conditions, the coupling reaction of XIX and XXIII took place smoothly affording the protected octapeptide-carbinol derivative (XXV) in a fairly good yield. Cleavage of all the protecting groups from XXV was effected by treatment with metalic sodium in liquid ammonia in the usual manner to furnish the final product, prolylphenylalanylhistidylleucylleucylvalyltyrosylserinol (XXVI). The dihydrochloride of XXVI thus obtained was found to be soluble moderately in water as compared with the corresponding methyl ester derivative[2] which was almost insoluble in water, and exhibited the highest renin inhibitory activity among all the synthetic peptide derivatives tested throughout this series of work.

Experimental[13]

Boc-leucylleucylvalyl-O-Bzl-tyrosyloxyacetyl Resin (I)——Boc-O-Bzl-tyrosyloxyacetyl copolystyrene-divinylbenzene[7a] (2% DVB, 100 mesh, O-Bzl-Tyr: 10.08 mmoles, 14.0 g) was stirred in 140 ml of 2.3N dry HCl in AcOEt for 40 min, filtered and washed with AcOEt and $CHCl_3$ (three times each). The resulting resin was then treated with $CHCl_3$ (140 ml) containing Et_3N (14 ml) for 10 min, filtered and washed with $CHCl_3$ and CH_2Cl_2 (three times each) to give the O-Bzl-tyrosyloxyacetyl resin. This was mixed with Boc-valine, liberated from its DCHA salt (12.1 g, 30.3 mmoles), in CH_2Cl_2 and after stirring for 10 min, DCC (6.25 g, 30.3 mmoles) in CH_2Cl_2 was added and the volume of the mixture was made to 140 ml with CH_2Cl_2. The whole mixture was stirred for 17 hr at room temperature, filtered and the resin was washed successively with CH_2Cl_2, AcOH and AcOEt to give the Boc-valyl-O-Bzl-tyrosyloxyacetyl resin. In the same manner, Boc-leucine·H_2O (7.55 g, 30.3 mmoles, 3 hr) was then coupled twice and the finished peptide resin was washed throughly with AcOEt and MeOH and dried *in vacuo* to give I (13.32 g). Peptide content: 0.39 mmole/g (modified Volhard method[14]).

Boc-leucylleucylvalyl-O-Bzl-tyrosine (III)——a) To a suspension of I (3.0 g, 2.26 mmoles) in dioxane (27 ml) and water (7 ml) was added 1N NaOH (7 ml) and the mixture was stirred for 23 hr at room temperature, filtered and washed with dioxane–water (2:1, v/v). The combined filtrate and washings were adjusted to pH 5 by addition of 1N HCl and extracted repeatedly with AcOEt. The AcOEt extract was washed with satd. NaCl, dried and evaporated *in vacuo* to leave III as a white solid (0.38 g, 24.0% yield from the Boc-O-Bzl-tyrosyloxyacetyl resin), mp 185—187° (decomp.). DCHA salt: mp 151—155° (MeOH–ether), $[\alpha]_D^{24}$ −21.7° (c=0.5, MeOH). *Anal.* Calcd. for $C_{38}H_{56}O_7N_4 \cdot C_{12}H_{23}N$: C, 68.32; H, 9.05; N, 7.96. Found: C, 68.24; H, 9.13; N, 7.81.

b) To a suspension of I (3.0 g) in pyridine–DMF (4:1, v/v, 30 ml) was added sodium thiophenoxide (1.49 g, 11.3 mmoles) and the mixture was stirred for 24 hr at room temperature in a stream of N_2 and then filtered. The filtrate was evaporated *in vacuo* and the residue was taken up in 20 ml of water and treated with $CuCl_2 \cdot 2H_2O$ (0.96 g) in water (2 ml). The mixture was acidified with 10% HCl and extracted with AcOEt. The organic layer was re-extracted with dil. $KHCO_3$, and the extract was acidified with 10% HCl and again extracted with AcOEt. Removal of the solvent gave III as a pale yellow solid (0.34 g, 21.2% from the Boc-O-Bzl-tyrosyloxyacetyl resin), mp 185—187° (decomp.). This was identified with the product obtained by the procedure a) by comparison of their infrared (IR) spectra and thin-layer chromatography (solvent system: MeOH–$CHCl_3$ (1:4, v/v), CCl_4–AcOEt–MeOH (5:5:2, v/v)).

Leucylleucylvalyltyrosine Ethyl Ester (V) Hydrochloride——A solution of III (0.16 g, 0.23 mmole) in EtOH (15 ml) was treated with thionyl chloride (0.11 g, 0.84 mmole) at −25° and then kept at room temperature for 20 hr. The mixture was evaporated *in vacuo*, and the residue thus obtained was dissolved in EtOH (10 ml) and treated with 2.3N dry HCl in AcOEt (25 ml). After standing for 40 min at room temperature, the mixture was evaporated *in vacuo* to give a white powder, which was washed with ether and dried (0.15 g). A 0.14 g portion of this product was dissolved in EtOH (20 ml) containing 2.3N dry HCl in AcOEt (0.5 ml) and hydrogenated over Pd-C (10%, 30 mg) at 50 psi. for 2 hr. The filtered solution was evaporated to leave crude V hydrochloride (0.11 g, 85.0%), which was recrystallized from EtOH–ether to colorless granules, mp 242—244° (decomp.), $[\alpha]_D^{24}$ −5.4° (c=1.4, AcOH). This product was identified with an authentic specimen prepared by the method previously reported[2] (mp 245—245.5° (decomp.), $[\alpha]_D^{25}$ −5.4° (c=2.3, AcOH).

13) All the melting points were uncorrected. Configuration of the optically active amino acids described in this part is L-form unless otherwise noted. Analytical samples were dried over solid KOH at 50—60° overnight under highly reduced pressure.

14) J.M. Stewart and J.D. Young, "Solid Phase Peptide Synthesis," W.H. Freeman and Co., San Francisco, 1969, pp. 6, 55—56.

Boc-leucylleucylvalylphenylalanyloxyacetyl Resin (II)——Boc-phenylalanyloxyacetyl copolystyrene-divinylbenzene[7a] (2% DVB, 100 mesh, Phe: 10.2 mmoles, 17.0 g) was treated with 2.3N dry HCl in AcOEt (170 ml) then with Et_3N 17 ml in $CHCl_3$ (170 ml) to give the phenylalanyloxyacetyl resin, to this were coupled successively Boc-valine (liberated from 12.2 g (30.6 mmoles) of its DCHA salt; 18.5 hr), Boc-leucine·H_2O (7.63 g, 30.6 mmoles; 3 hr) and again Boc-leucine·H_2O (7.63 g, 30.6 mmoles; 3 hr), DCC (6.31 g, 30.6 mmoles) in CH_2Cl_2 being used as condensing agent. The peptide resin (II) thus obtained was washed throughly with AcOEt and MeOH and dried *in vacuo* at room temperature. Yield, 16.66 g. Peptide content: 0.48 mmole/g (modified Volhard method[14]).

Boc-leucylleucylvalylphenylalanine (IV)——To a suspension of the foregoing peptide resin II (8.33 g, 5.1 mmoles) in DMF (83 ml) was added sodium thiophenoxide (3.37 g, 25.5 mmoles) and the mixture was stirred at room temperature for 24 hr under a stream of N_2, filtered and washed with DMF. The combined filtrate and washing were evaporated *in vacuo* and the residue was dissolved in water (40 ml). This solution was treated with air to oxidize an excess of thiophenol to diphenyl disulfide. The precipitated solid was then filtered, dried and washed throughly with ether. The white powder (0.45 g) thus obtained was dissolved in 70% MeOH (22 ml), acidified with 1N HCl (0.8 ml) and concentrated *in vacuo*. The precipitate was filtered, washed with water and dried to give crude IV (0.35 g, mp 200—204° (decomp.)). From the mother liquor, a second crop (0.28 g) was obtained (total yield: 19.0% from the Boc-phenylalanyloxyacetyl resin). DCHA salt: 167—168°, $[\alpha]_D^{26.5}$ −44.3° (c=1.4, MeOH). *Anal.* Calcd. for $C_{31}H_{50}O_7N_4 \cdot C_{12}H_{23}N$: C, 66.90; H, 9.56; N, 9.10. Found: C, 66.68; H, 9.32; N, 8.85.

Leucylleucylvalylphenylalanine Ethyl Ester (VI) Hydrochloride——The foregoing peptide (IV, 0.29 g, 0.49 mmole) was esterified with the EtOH–$SOCl_2$ method as for V described above and then the product was dissolved in EtOH (10 ml) and treated with 2.3N dry HCl in AcOEt (15 ml) for 1 hr at room temperature. After evaporation of the solvent, the residue thus obtained was washed throughly with ether and dried to give VI hydrochloride as a powder (0.23 g, 92.0%), mp 253—255° (decomp.); the melting point was unchanged after recrystallization from EtOH–AcOEt, $[\alpha]_D^{26}$ −18.0° (c=1.6, AcOH). The purified product was identical with an authentic specimen prepared previously[2] (mp 255—256° (decomp.), $[\alpha]_D^{23}$ −18.3° (c=1.2, AcOH)).

Leucylleucylvalyltyrosinol (XII) Hydrochloride——To a cold solution of $NaBH_4$ (6.81 g, 0.18 mole) in 50% EtOH (200 ml) was added V hydrochloride (10.3 g, 18.0 mmoles) in 50% EtOH (260 ml) with cooling in an ice water and the mixture was stirred at room temperature for 22 hr. The resulting solution was acidified (pH 2) with 10% HCl (80 ml), neutralized by adding solid K_2CO_3, concentrated *in vacuo*, then again made alkaline by addition of solid K_2CO_3 and extracted repeatedly with *n*-BuOH. The combined extracts were washed with satd. NaCl and dried and the solvent was removed *in vacuo*. The remaining oily product was dissolved in EtOH and treated with 4.9N HCl in EtOH (5 ml), and the mixture was evaporated to dryness *in vacuo* to leave a white solid, which was washed with ether. Recrystallization from EtOH–ether afforded XII hydrochloride (7.58 g, 79.7%) as white granules, mp 242° (decomp.), $[\alpha]_D^{23}$ −49.7° (c=1.1, MeOH). *Anal.* Calcd. for $C_{26}H_{45}O_5N_4Cl \cdot 1/2C_2H_5OH$: C, 58.64; H, 8.77; N, 10.15; Cl, 6.42. Found: C, 58.20; H, 8.44; N, 10.27; Cl, 6.48.

Leucylleucylvalylphenylalaninol (XIII) Hydrochloride——VI hydrochloride (4.44 g, 8.0 mmoles) in 75% EtOH (250 ml) was reduced by $NaBH_4$ (3.03 g, 80.0 mmoles) in 75% EtOH (250 ml) for 5 hr and the product was isolated as its hydrochloride in the same manner as for XII described above. Yield 3.58 g (87.4%), mp 241—242° (EtOH–ether), $[\alpha]_D^{22.5}$ −35.5° (c=0.5, AcOH). *Anal.* Calcd. for $C_{26}H_{45}O_4N_4Cl$: C, 60.87; H, 8.84; N, 10.92; Cl, 6.91. Found: C, 60.46; H, 8.78; N, 10.76; Cl, 6.76.

Boc-leucylleucylvalyl-O-Bzl-tyrosyl-O-Bzl-seryloxyacetyl Resin (VIII)——Starting from Boc-O-Bzl-seryloxyacetyl copolystyrene-divinylbenzene[7a] (2% DVB, 100 mesh, O-Bzl-Ser: 10.0 mmoles, 10.5 g), the peptide chain was built up by successive couplings of Boc-O-Bzl-tyrosine (11.2 g, 30.0 mmoles), Boc-valine (liberated from 12.0 g, (30.0 mmoles) of DCHA salt), Boc-leucine·H_2O (7.5 g, 30.0 mmoles) and again Boc-leucine·H_2O (7.5 g, 30.0 mmoles). All the deprotection and coupling steps were carried out in the same manner as for I or II described above. The product was then washed with AcOEt and MeOH and dried. Yield: 11.50 g. Peptide content: 0.47 mmole/g (modified Volhard method[14]).

Boc-leucylleucylvalyl-O-Bzl-tyrosyl-O-Bzl-serine (IX)——The foregoing peptide resin VIII (3.2 g) was cleaved by treatment with sodium thiophenoxide (0.79 g, 6.0 mmoles) in DMF (32 ml) and worked up in the same manner as for IV described above affording 0.48 g of IX (20.0% from the Boc-O-Bzl-seryl-oxyacetyl resin), mp 265—267° (decomp.). DCHA salt: mp 184—186°, $[\alpha]_D^{28}$ −29.0° (c=1.7, MeOH). *Anal.* Calcd. for $C_{49}H_{71}O_{11}N_5 \cdot C_{12}H_{23}N$: C, 67.31; H, 8.71; N, 7.72. Found: C, 67.50; H, 8.46; N, 7.72.

Boc-leucylleucylvalyl-O-Bzl-tyrosyl-O-Bzl-serine Methyl Ester (X)——A solution of IX (0.43 g, 0.49 mmole) in MeOH (20 ml) was mixed with a large excess of CH_2N_2 in ether and kept at room temperature for 3 hr. After addition of a few drops of AcOH, the mixture was evaporated to leave a white powder (0.33 g, 77.0%, mp 196—198° (decomp.)), which was recrystallized from MeOH to give white granules, mp 205—206° (decomp.), $[\alpha]_D^{29}$ −46.5° (c=1.4, MeOH). *Anal.* Calcd. for $C_{49}H_{69}O_{10}N_5$: C, 66.20; H, 7.81; N, 7.87. Found: C, 66.13; H, 7.85; N, 7.87.

Leucylleucylvalyltyrosylserine Methyl Ester (XI) Hydrochloride——A solution of X (0.28 g, 0.31 mmole) in a mixture of AcOH (20 ml) and 3N HCl in MeOH (30 ml) was kept at room temperature for 20 min and

then hydrogenated over Pd-C (10%, 0.1 g) for 5 hr. The filtered solution was evaporated *in vacuo* and the residue was washed with ether to give 0.17 g (85.5%) of XI hydrochloride as a white powder, mp 235—237° (decomp.). Precipitation from EtOH–AcOEt afforded 0.13 g of amorphous powder, mp 260° (decomp.), $[\alpha]_D^{28}$ −33.6° (c=0.5, MeOH). *Anal.* Calcd. for $C_{30}H_{50}O_8N_5Cl$: C, 55.95; H, 7.83; N, 10.87; Cl, 5.51. Found: C, 55.72; H, 7.68; N, 10.05; Cl, 5.73.

Cbz-glycylleucylleucylvalyltyrosinol (XV)——To a solution of Cbz-glycine (0.74 g, 3.53 mmoles) in anhyd. THF (20 ml) was added Et_3N (0.36 g, 3.53 mmoles) in THF (5 ml) followed by adding dropwise iso-butyl-chloroformate (0.49 g, 3.53 mmoles) in THF (5 ml) at −15——20°. After stirring for 5 min, a solution of XII hydrochloride (1.87 g, 3.53 mmoles) in DMF–THF (1: 1, v/v, 40 ml) containing 0.36 g of Et_3N was added to and the whole was stirred at −15° for 1 hr and then for 2 hr allowing the temperature to rise to room temperature. The solvent was removed *in vacuo* and the residue, after addition of water was extracted with AcOEt. The AcOEt extract was washed successively with 10% HCl, satd. NaCl, satd. $NaHCO_3$ and satd. NaCl and then dried. Removal of the solvent gave 1.95 g of amorphous powder, an 1.93 g portion of which was purified by column chromatography (Silicic acid 120 g, elution: $CHCl_3$–MeOH (8: 1, v/v)) to give XV (1.59 g, 69.5%), mp 178—181°.

Glycylleucylleucylvalyltyrosinol (XVI) Hydrochloride——The foregoing pentapeptide derivative (XV, 0.75 g, 1.10 mmoles) in 75% MeOH (40 ml) containing AcOH (10 ml) was hydrogenated over Pd-C (10%, 0.1 g) for 4.5 hr at 50 psi. The filtered solution was evaporated and the remaining solid was dissolved in aq. MeOH and treated with 1N HCl (1 ml) and again evaporated to dryness in *vacuo*. The white powder thus obtained was dissolved in EtOH and precipitated by addition of AcOEt to give XVI hydrochloride (0.50 g, 78.1%), mp 220—223° (decomp.), $[\alpha]_D^{23}$ −47.0° (c=1.5, H_2O). Amino acid Ratio[15]: Leu, 2.00: Gly, 1.03: Val, 1.05. *Anal.* Calcd. for $C_{28}H_{48}O_6N_5Cl$: C, 57.37; H, 8.25; N, 11.95: Cl, 6.05. Found: C, 57.09; H, 8.08; N, 11.73; Cl, 6.48.

N-Sodiumsulfomethyl-leucylleucylvalyltyrosine Ethyl Ester (VII)——To a solution V hydrochloride (1.71 g, 3.0 mmoles) in 33% aq. EtOH (30 ml) was added a solution of K_2CO_3 (0.25 g) in water (10 ml) and the mixture was evaporated to dryness *in vacuo*. The residue was washed with water and dried to give the free base, V (1.33 g, mp 198—200°). A 0.64 g-portion (1.2 mmoles) of the base in EtOH was mixed with sodium hydroxymethane sulfonate monohydrate (0.24 g, 1.56 mmoles) in water (10 ml) and the whole mixture was refluxed for 4.5 hr and then evaporated to dryness *in vacuo*. The residue was extracted with 30 ml of hot EtOH and filtered and the filtrate was again evaporated to leave a white powder. This was washed with hot AcOEt and ether and precipitated from EtOH–AcOEt to give VII (0.58 g, 73.8%) as an amorphous powder, mp 186—188° (decomp.), $[\alpha]_D^{15}$ −44.0° (c=0.5, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 1180—1230 (broad), 1050 (SO_2). *Anal.* Calcd. for $C_{29}H_{46}O_9N_4SNa$: C, 53.58; H, 7.13; N, 8.62; S, 4.93. Found: C, 53.36; H, 7.05; N, 8.24; S, 5.12.

N-Sodiumsulfomethyl-leucylleucylvalyltyrosinol (XIV)——XII hydrochloride (0.87 g, 1.64 mmoles) in aq. MeOH (34 ml) was converted to its free base by addition of K_2CO_3 (0.28 g) in water (10 ml). A 0.70 g (1.42 mmoles)-portion of the free base in EtOH (10 ml) was mixed with sodium hydroxymethane sulfonate monohydrate (0.28 g, 1.85 mmoles) in water (10 ml) and the whole was refluxed for 5 hr and then worked up as for VII described above. The product thus obtained (0.79 g) was purified by column chromatogtaphy (Silicic acid, 40 g, elution: $CHCl_3$: MeOH (6: 1, v/v) and precipitated from EtOH–AcOEt to afford XIV as an amorphous powder (0.56 g, 64.8%), mp 154—157° (decomp.). $[\alpha]_D^{17}$ −69.2° (c=0.3, MeOH). *Anal.* Calcd. for $C_{27}H_{45}O_8N_4SNa \cdot 1/2\ H_2O$: C, 52.50; H, 7.51; N, 9.07; S, 5.19. Found: C, 52.62; H, 7.45; N, 9.42; S, 4.84.

Cbz-prolylphenylalanyl-im-Bzl-histidylleucyloxyacetyl Resin (XVII)——Starting from Boc-leucyloxyacetyl copolystyrene-divinylbenzene[7a] (2% DVB, 100 mesh, Leu: 25.0 g, 17.5 mmoles), the peptide was built up by coupling Boc-im-Bzl-histidine (18.3 g, 53.0 mmoles), Boc-phenylalanine (11.7 g, 43.8 mmoles), and then Cbz-proline (13.2 g, 53.0 mmoles) in the same manner as for I described above except that N-methylmorpholine instead of Et_3N was used for neutralization of HCl after deblocking of the Boc group. In the case of Boc-im-Bzl-histidine, coupling was carried out for 5 hr using DMF as solvent. Yield, 26.6 g. Peptide content: 0.59 mmole/g as determined by the modified Volhard method[14] after cleavage by 25% HBr/AcOH.

Cbz-prolylphenylalanyl-im-Bzl-histidylleucine Hydrazide (XIX)——a) To a suspension of the aforementioned peptide resin XVII (18.0 g, 10.6 mmoles) in DMF–MeOH (4: 1, v/v, 180 ml) was added 100% hydrazine hydrate (9.0 g, 180 mmoles) and the mixture was stirred for 20 hr at room temperature. The resin was filtered off and washed with DMF and MeOH and the combined filtrate and washings were evaporated *in vacuo*. The residue was dissolved in water and the solution was saturated with NaCl and extracted repeatedly with AcOEt. The dried AcOEt extract was evaporated *in vacuo* to leave a syrup, which solidified by trituration with ether (2.95 g, 33.4% from the Boc-leucyloxyacetyl resin). Precipitation from EtOH–ether gave XIX as an amorphous powder, mp 160—164°, $[\alpha]_D^{24}$ −64.2° (c=1.1, MeOH). *Anal.* Calcd. for $C_{41}H_{50}O_6N_8 \cdot H_2O$:

15) Determined gas chromatographically. C.W. Gehrke, D. Roach, R.W. Zumwalt, D.L. Stalling and L.L. Wall," Quantitative Gas-Liquid Chromatography of Amino Acids in Protein and Biological Substances, "Analytical Bio-Chemistry Labs., Inc. 1968. Determinations of histidine, tyrosinol and serinol were omitted in this method.

C, 64.05; H, 6.82; N, 14.57. Found: C, 64.00; H, 6.61; N, 14.27.

b) To a solution of Boc-im-Bzl-histidine (3.18 g, 9.23 mmoles) in DMF (55 ml) was added dropwise at −15° DCC (2.10 g, 10.2 mmoles) in DMF (8 ml), followed by addition of leucine methyl ester hydrochloride (1.68 g, 9.23 mmoles) in DMF (8 ml) containing Et_3N (0.93 g, 9.23 mmoles) over the period of 10 min, and the whole mixture was stirred for 20 hr at 5—13°. After evaporation of the solvent *in vacuo*, AcOEt (100 ml) was added to the residue and the insoluble dicyclohexylurea was filtered off. The filtrate was washed successively with 0.5M citric acid, satd. NaCl, satd. $NaHCO_3$ and satd. NaCl and dried. Removal of the solvent by evaporation afforded crude Boc-im-Bzl-histidylleucine methyl ester as a pale yellow powder (3.67 g, 84.2 %). A 3.60 g-portion of the product was then subjected to removal of the Boc group by treatment with TFA (15 ml) in CH_2Cl_2 (15 ml) for 45 min at room temperature. The reaction mixture was evaporated to dryness *in vacuo* and after addition of dil. K_2CO_3 extracted with AcOEt. The extract was washed with water dried and evaporated to afford crude im-Bzl-histidylleucine methyl ester as a light brown oil. This was used for the next step without further purification. An analytical sample was obtained by formation of the dioxalate. Dioxalate: mp 110—112°, $[\alpha]_D^{14}$ −35.0° (c=2.1, MeOH). *Anal.* Calcd. for $C_{20}H_{28}O_3N_4 \cdot C_4H_4O_8$: C, 52.13; H, 5.83; N, 10.13. Found: C, 52.19; H, 5.91; N, 10.57. To a solution of Cbz-prolylphenylalanine hydrazide[12] (2.82 g, 6.85 mmoles) in DMF (20 ml) was added 3.57N dry HCl in THF (7.7 ml) followed by adding dropwise isoamylnitrite (0.88 g, 7.55 mmoles) in DMF (5 ml), at −20——30°. After stirring for 30 min, Et_3N (2.77 g) and then the above mentioned crude im-Bzl-histidylleucine methyl ester (3.2 g) in DMF (15 ml) were added dropwise at −50° and the whole was stirred for 65 hr, allowing the temperature to rise up to 3°. The resulting mixture was evaporated *in vacuo* and the residue obtained was treated with water and extracted with AcOEt. The AcOEt extract was washed successively with 0.5N citric acid, satd. NaCl, 0.5N $NaHCO_3$ and satd. NaCl. The dried extract was evaporated to leave crude Cbz-prolylphenylalanyl-im-Bzl-histidylleucine methyl ester as a brown oil (5.40 g). A 5.34 g-portion of the product was then subjected to hydrazinolysis in EtOH (55 ml) containing 100% hydrazine hydrate (6.81 g) for 23 hr, the mixture was evaporated *in vacuo* and the residue was treated with water. The white precipitate thus obtained was collected, washed with water and dried to give XIX (3.96 g, 77.0% from Cbz-prolylphenylalanine hydrazide), mp 157—160°. Recrystallization from EtOH–ether afforded a pure sample, mp 162—166°, $[\alpha]_D^{25}$ −64.7° (c=1.6, MeOH). This was identified with a sample prepared by the method a) by the mixed melting point test and comparison of their IR spectra and thin-layer chromatographic behavior (solvent systems: MeOH–$CHCl_3$ (1: 4, v/v); CCl_4–AcOEt–MeOH (5: 5: 2, v/v)).

Boc-leucylvalyl-O-Bzl-tyrosyl-O-Bzl-seryloxyacetyl Resin (XVIII)——Boc-O-Bzl-seryloxyacetyl copolystyrene-divinylbenzene[7a] (2% DVB, 100 mesh, O-Bzl-Ser: 20.0 g, 11.2 mmoles), was treated with 2.2N dry HCl in AcOEt, and then with Et_3N in $CHCl_3$ in the usual way to give the O-Bzl-seryloxyacetyl resin. To this amino acid resin were coupled successively Boc-O-Bzl-tyrosine (10.4 g, 28.0 mmoles), Boc-valine (liberated from 11.2 g (28.0 mmoles) of DCHA salt) and Boc-leucine·H_2O (7.0 g, 28.0 mmoles) in the same manner as for I described above. Yield, 21.4 g. Peptide content: 0.52 mmole/g (modified Volhard method[14]).

Boc-leucylvalyl-O-Bzl-tyrosyl-O-Bzl-serine (XX)——To a stirred suspension of the foregoing peptide resin XVIII (6.49 g, 3.37 mmoles) in DMF (50 ml) was added sodium thiophenoxide (2.23 g, 16.9 mmoles) in DMF (15 ml) and the mixture was stirred for 20 hr at room temperature in an atmosphere of N_2. The resin was then filtered and washed with DMF and MeOH. The combined filtrate and washings were evaporated *in vacuo* to leave a syrup, which was mixed with water. After introduction of air for 2.5 hr to this mixture, the precipitate was collected by filtration, dried and washed throughly with ether to give 1.47 g of powder. This was dissolved in 30 ml of 70% methanol and the solution was treated with 1N HCl (2.4 ml) and then concentrated *in vacuo*. The precipitated product was filtered, washed with water and dried to give crude XX (1.31 g, 51.0%, mp 173—174°), which was purified as DCHA salt. Amorphous powder, mp 138—140° (MeOH–AcOEt–ether), $[\alpha]_D^{28}$ −15.1° (c=1.1, MeOH). *Anal.* Calcd. for $C_{42}H_{56}O_9N_4 \cdot C_{12}H_{23}N \cdot CH_3OH$: C, 67.74; H, 8.58; N, 7.18. Found: C, 67.88; H, 8.31; N, 7.06.

Boc-leucylvalyl-O-Bzl-tyrosyl-O-Bzl-serine Methyl Ester (XXI)——a) A solution of XX (2.49 g, 3.27 mmoles) in MeOH (30 ml) was treated with a large excess of CH_2N_2 in ether. Work-up in the same manner as for X described gave XXI (2.01 g, 79.2%), mp 165—167°, mp 168° after recrystallization from AcOEt–ether, $[\alpha]_D^{26}$ −25.4° (c=1.9, MeOH). *Anal.* Calcd. for $C_{43}H_{58}O_9N_4$: C, 66.55; H, 7.54; N, 7.22. Found: C, 66.45; H, 7.56; N, 7.09.

b) To a dried solution of Boc-leucine·H_2O (3.15 g, 12.7 mmoles) in CH_2Cl_2 (50 ml) was added dropwise a solution of DCC (2.62 g, 12.7 mmoles) in CH_2Cl_2 (10 ml) with stirring and cooling at −15° and stirring was continued for 15 min. To the solution was then added valyl-O-Bzl-tyrosyl-O-Bzl-serine methyl ester hydrochloride[2] (6.89 g, 11.5 mmoles) in $CHCl_3$ (200 ml) containing Et_3N (1.16 g, 11.5 mmoles) over a period of 60 min and the whole mixture was stirred for 40 hr at −18——13°. The reaction mixture was evaporated *in vacuo*, the remaining solid was treated with AcOEt and the insoluble material was filtered off. XXI was precipitated from the filtrate by addition of a large volume of ether. Yield, 7.42 g (83.0%), mp 165—168°, $[\alpha]_D^{20.5}$ −23.7° (c=2.5, MeOH). Amino acid ratio[15]: Leu, 1.00: Val, 1.01: Tyr, 0.94: Ser, 1.01. This product was identified with a sample prepared by the method a) by mixed melting test, comparison of their IR spectra and thin-layer chromatography.

Leucylvalyl-O-Bzl-tyrosyl-O-Bzl-serine Methyl Ester (XXII) Hydrochloride——The foregoing ester (XXI, 1.90 g, 2.45 mmoles) was treated with 4N dry HCl in MeOH (50 ml) for 40 min at room temperature and evaporated to dryness *in vacuo*. The residue thus obtained was washed with ether and dried to give XXII hydrochloride (1.66 g, 95.5%), mp 222—224° (decomp.). Recrystallization from MeOH–acetone–ether afforded colorless needles, mp 219° (decomp.). $[\alpha]_D^{24}$ +3.2° (c=1.9, MeOH). The analytical sample was dried *in vacuo* at 70° (over solid KOH). *Anal.* Calcd. for $C_{38}H_{51}O_7N_4Cl \cdot 1/2CH_3OH$: C, 63.36; H, 7.33; N, 7.69; Cl, 4.87. Found: C, 63.25; H, 7.30; N, 8.06; Cl, 5.02.

Leucylvalyl-O-Bzl-tyrosyl-O-Bzl-serinol (XXIII) Hydrochloride——A solution of XXII hydrochloride (4.99 g, 7.0 mmoles) in 70% EtOH (190 ml) was gradually mixed with $NaBH_4$ (2.66 g, 70 mmoles) in 50% EtOH (100 ml) and after dilution with 350 ml of EtOH, the whole mixture was stirred at room temperature for 24 hr. The filtered solution was acidified with 10% HCl (70 ml) and after 20 min, neutralized by addeing solid K_2CO_3 and concentrated *in vacuo*. The remaining solution was then made to pH 11 with solid K_2CO_3 and extracted repeatedly with AcOEt and *n*-BuOH. The combined extracts were washed with satd. NaCl, dried and evaporated *in vacuo* to leave white powder which was washed with water. The product thus obtained was dissolved in MeOH (300 ml) containing 20 ml of water and treated with 1N HCl (12 ml) and the mixture was evaporated to dryness *in vacuo* and dried over solid KOH to afford amorphous XXIII hydrochloride (4.14 g, 86.4%), mp 221—223°, and mp 227° after recrystallization from EtOH. $[\alpha]_D^{24}$ −7.1° (c=1.5, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 1050 (C–O). The analytical sample was dried *in vacuo* at 70° over KOH. *Anal.* Calcd. for $C_{37}H_{51}O_6N_4Cl \cdot 1/2C_2H_5OH$: C, 64.55; H, 7.67; N, 7.89; Cl, 5.01. Found: C, 64.21; H, 7.47; N, 8.11; Cl, 5.43.

Cbz-prolylphenylalanyl-im-Bzl-histidylleucylleucine Methyl Ester (XXIV)——A stirred solution of XIX (1.06 g, 1.4 mmoles) in DMF (15 ml) was acidified with 3.57N dry HCl in THF (1.6 ml)/DMF (3 ml) and then treated with isoamylnitrile (0.18 g, 1.54 mmoles) in DMF (5 ml) with cooling at −30°. The mixture was stirred for 30 min at the same temperature, cooled to −45° and treated with Et_3N (0.56 g, 5.6 mmoles) to adjust pH approximately 8. To the resulting solution was added dropwise a solution of leucine methyl ester hydrochloride (0.25 g, 1.4 mmoles) in DMF (15 ml) containing Et_3N (0.14 g, 1.4 mmoles) with stirring. Stirring was continued for 66 hr at −30——3°. The mixture was evaporated *in vacuo* and the residue was treated with water and extracted repeatedly with AcOEt. The combined extracts were washed successively with 0.5M citric acid, satd. NaCl, satd. $NaHCO_3$ and satd. NaCl and dried. Removal of the solvent gave a solid (0.39 g), which was washed with ether to afford XXIV (0.20 g, 16.5%) as a white powder mp 120—127°, and mp 124—127° after recrystallization from AcOEt–ether, $[\alpha]_D^{14}$ −120.5° (c=1.66, MeOH). *Anal.* Calcd. for $C_{48}H_{61}O_8N_7$: C, 66.73; H, 7.11; N, 11.33. Found: C, 67.14; H, 6.74; N, 11.76. The 0.5M citric acid washing was treated with solid K_2CO_3 (pH 10) and extracted with AcOEt. The AcOEt extract was washed with satd. NaCl, dried and evaporated to leave a syrup, which solidified on trituration with ether to recover starting XIX as a white powder (0.69 g, 64.8%), mp 163—166°.

Cbz-prolylphenylalanyl-im-Bzl-histidylleucylleucylvalyl-O-Bzl-tyrosyl-O-Bzl-serinol (XXV)——To a cooled (−15°) solution of the tetrapeptide hydrazide (XIX, 1.06 g, 1.4 mmoles) in DMF (15 ml) was added 3.41N dry HCl in THF (2.5 ml) followed by adding isoamylnitrite (0.33 g, 2.8 mmoles) in DMF (5 ml) at *ca.* −20°. After being stirred for 40 min, the mixture was neutralized by addition of Et_3N (0.85 g) at −42—−45° and then mixed with the amine (XXIII) hydrochloride (0.96 g, 1.4 mmoles) in DMF (20 ml) containing Et_3N (0.15 g) and the whole was stirred for 40 hr at −20°. The mixture was evaporated *in vacuo* and the residue was treated with water (50 ml) to give a white precipitate, which was collected by filtration, washed successively with water, 0.5M citric acid, water, satd. $NaHCO_3$ and water. The product was reprecipitated from DMF–water to afford 1.28 g (67.0%) of XXV as a white powder, mp 213—215° (decomp.). $[\alpha]_D^{26}$ −34.2° (c=1.2, AcOH). IR ν_{max}^{Nujol} cm^{-1}: 1020 (C–O). *Anal.* Calcd. for $C_{78}H_{96}O_{12}N_{10} \cdot H_2O$: C, 67.71; H, 7.14; N, 10.12. Found: C, 67.73; H, 6.72; N, 9.98.

Prolylphenylalanylhistidylleucylleucylvalyltyrosylserinol (XXVI) Dihydrochloride——To a solution of the above peptide derivative (XXV, 0.75 g, 0.55 mmoles) in liquid NH_3 (450 ml) was added Na (0.15 g, 6.6 m atoms) in small pieces during 90 min. Liquid ammonia was then evaporated, and the residue was then treated with a mixture of water and AcOEt and the precipitate was collected. The aqueous layer was acidified with AcOH, and kept in a refrigerator overnight and the material thus precipitatee was collected by filtration. The combined solid products were treated with 1N HCl in AcOH (50 ml)–water (15 ml)–MeOH (15 ml). After evaporation and washing with ether, 0.41 g (71.7%) of crude XXVI dihydrochloride was obtained as a white powder, mp 215—217° (decomp.). Reprecipitation from DMF–water–AcOEt afforded 0.26 g of pure sample, mp 213—214° (decomp.). $[\alpha]_D^{24}$ −41.4° (c=0.5, DMF). *Anal.* Calcd. for $C_{49}H_{72}O_{10}N_{10} \cdot 2HCl \cdot 4H_2O$: C, 53.21; H, 7.29; N, 12.66; Cl, 6.41. Found: C, 53.03; H, 6.82; N, 12.62; Cl, 6.61. Amino acid ratio[15]: Leu, 2.00: Pro, 1.00: Phe, 1.05: Val, 1.03: Tyr, 1.07.

Acknowledgement The authors are indebted to Professor Y. Yamamura and Dr. T. Kokubu of Osaka University for the biological tests of the compounds prepared in this work. They are also grateful to Professor S. Yamada of Tokyo University and Mr. M. Yamazaki, Manager of this laboratory for their advices and encouragement. Thanks are also due to Dr. K. Kotera and the members of the Analytical Center of this company for analytical, IR and gaschromatographic data.