Synthesis and Biological Activity of Peptides Related to Eledoisin. I. Hexapeptide Amides Containing α-Hydroxy Acids[1,2]

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(Received April 27, 1972)

A new series of eledoisin-like depsipeptides was synthesized in order to obtain some information about the relationship between the structure of the peptide backbone of eledoisin and the biological activity. Specific amino acids in the biologically-active hexapeptide analog of eledoisin, H–Lys–Phe–Ile–Gly–Leu–Met–NH_2 (**1**), were replaced by the corresponding α-hydroxy acids. The biological activities of these analogs were then compared with that of the standard one (**1**). On the blood pressure in rabbits, H–Lys–Phe–Ile–Glyc–Leu–Met–NH_2 (**4**) and H–Lys–Phlac–Ile–Gly–HyIc–Met–NH_2 (**6**) possessed a substantial activity, though lower than **1**, and H–Lys–Phlac–Ile–Gly–Leu–Met–NH_2 (**2**) and H–Lys–Phe–Ile–Gly–HyIc–Met–NH_2 (**5**) were almost as active as **1**. On the other hand, H–Lys–Phe–HyMeV–Gly–Leu–Met–NH_2 (**3**) exhibited much less activity. The results indicate that the amide bond between the phenylalanine residue and the isoleucine residue is essential for the hypotensive activity.

Eledoisin, Pyroglu–Pro–Ser–Lys–Asp–Ala–Phe–Ile–Gly–Leu–Met–NH_2, was isolated by Erspamer from extracts of salivary glands of *Eledone moshata* and *Aldrovandi*.[4] Its pharmacological action includes the induction of acute arterial hypotension and the stimulation of smooth muscles.[5]

Since the primary structure of eledoisin was elucidated and confirmed by synthesis,[6] a large number of analogs in which amino acids are replaced by other amino acids or in which the peptide chains are shortened have been synthesized.[7] These synthetic studies have given us much information about the sequence and specific amino acid residues required for pharmacological activity. That is, the *C*-terminal hexapeptide analog, H–Lys–Phe–Ile–Gly–Leu–Met–NH_2 (**1**), obtained by the replacement of alanine with lysine possesses a high biological activity.[8] The exchange of the phenylalanine residue and the isoleucine residue almost always leads to the formation of compounds with lower activity. It is, however, relatively harmless to exchange the glycine residue. The exchange of the methionine residue produces inactive compounds almost exclusively.[7]

However, there is no knowledge about the relationship between the structure of the peptide backbone and the specific biological activity of eledoisin. In order to obtain some information about the role of the peptide chain of eledoisin, especially about the necessity of the presence of an amide group for the compound to manifest its biological properties, the present authors synthesized several hexadepsipeptides in which phenylalanine, isoleucine, glycine, and leucine in the compound **1** were replaced with the corresponding α-hydroxy acids and assayed for eledoisin-like activity. The compound **1** was chosen as the model peptide of eledoisin in this study because it had been reported that the *C*-terminal pentapeptide, H–Phe–Ile–Gly–Leu–Met–NH_2, was needed in order to have an appreciable activity of eledoisin[7] and the compound **1** was almost as active as natural eledoisin.

Shemyakin *et al.* attempted to exchange the amide groups by ester groups in the biologically-active peptides, such as glutathione, opthalmic acid, and bradykinin.[9] However, no systematic studies of depsipeptide analogs of eledoisin have ever been performed.

We synthesized such depsipeptides as H–Lys–Phlac–Ile–Gly–Leu–Met–NH_2 (**2**), H–Lys–Phe–HyMeV–Gly–Leu–Met–NH_2 (**3**), H–Lys–Phe–Ile–Glyc–Leu–Met–NH_2 (**4**), H–Lys–Phe–Ile–Gly–HyIc–Met–NH_2 (**5**), and H–Lys–Phlac–Ile–Gly–HyIc–Met–NH_2 (**6**).

Optically active α-hydroxy acids were prepared from the corresponding amino acids by the method reported by Winitz *et al.*[10] The acids were converted into benzyl esters by refluxing them with benzyl alcohol in benzene in the presence of a catalytic amount of sulfuric acid or *p*-toluenesulfonic acid. In the synthesis of the depsipeptides, an ester bond was formed with the aid of benzenesulfonyl chloride in pyridine according to the method of Shemyakin *et al.*[11] All the

1) Presented in part at the 8th Symposium on Peptide Chemistry, Osaka, November, 1970.

2) The abbreviations recommended by the IUPAC-IUB commission on Biochemical Nomenclature (*J. Biol. Chem.*, **241**, 2491 (1966); **242**, 555 (1967)) have been used throught. In addition: Glyc=glycolic acid, HyMeV=α-hydroxy-β-methylvaleric acid, Phlac=β-phenyllactic acid and HyIc=α-hydroxyisocaproic acid. Amino acid and α-hydroxy acid symbols except Gly and Glyc denote the L-configuration.

3) To whom inquiries should be addressed.

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5) V. Erspamer and A. Glaesser, *Brit. J. Pharmacol.*, **20**, 516 (1963). V. Erspamer and G. F. Erspamer, *ibid.*, **19**, 337 (1962).

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9) M. M. Shemyakin, L. A. Shchukina, E. I. Vinogradova, G. A. Ravdel, and Yu. A. Ovchinnikov, *ibid.*, **22**, 353 (1966) and literatures cited therein. G. A. Ravdel, M. P. Filatova, L. A. Shchukina, T. S. Paskina, M. S. Surovikina, S. S. Trapeznikova, and T. P. Egorova, *J. Med. Chem.*, **10**, 242 (1967).

10) M. Winitz, L. Bloch-Frankenthal, N. Izumiya, S. M. Birnbaum, C. G. Baker, and J. P. Greenstein, *J. Amer. Chem. Soc.*, **78**, 2423 (1956).

11) M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, and A. A. Kiryushkin, *Tetrahedron*, **19**, 581 (1963); M. M. Shemyakin, E. I. Vinogradova, M. Yu. Feigina, N. A. Aldanova, Yu. A. Ovchinnikov, and A. A. Kiryushkin, *J. Gen. Chem. USSR.*, **34**, 1796 (1964).

hexadepsipeptide amides obtained as dihydrochloride were found to be homogeneous by the criteria of paper chromatography, paper electrophoresis, thin-layer chromatography, and elemental analysis. The presence of the ester bond was confirmed by IR, which showed an ester carbonyl band near 1750 cm^{-1}.

The syntheis of H–Lys–Phlac–Ile–Gly–Leu–Met–NH_2 (**2**) was carried out according to Scheme 1:

di-BOC–Lys–OH + H–Phlac–OBzl (**7**)
↓ $C_6H_5SO_2Cl$/Pyridine
di-BOC–Lys–Phlac–OBzl (**8**)
↓ H_2/Pd
di-BOC–Lys–Phlac–OH (**9**) + H–Ile–Gly–Leu–Met–NH_2
↓ EtOCOCl/Et_3N
di-BOC–Lys–Phlac–Ile–Gly–Leu–Met–NH_2 (**10**)
↓ HCl in AcOH
H–Lys–Phlac–Ile–Gly–Leu–Met–NH_2·2HCl (**2**)

Scheme 1.

The di-*t*-butyloxycarbonyl(BOC)-didepsipeptide ester (**8**), obtained in a crystalline form, was converted to the acid (**9**) by hydrogenolysis. The di-BOC-hexapeptide amide (**10**), obtained by the mixed anhydride (MA) method, was treated with 2.5N hydrogen chloride in acetic acid to get the hygroscopic amorphous dihydrochloride (**2**).

Subsequently, H–Lys–Phe–HyMeV–Gly–Leu–Met–NH_2 (**3**) was synthesized as is shown in Scheme 2:

BOC–Phe–OH + H–HyMeV–OBzl (**11**)
↓ $C_6H_5SO_2Cl$/Pyridine
BOC–Phe–HyMeV–OBzl
↓ H_2/Pd
BOC–Phe–HyMeV–OH (**12**) + H–Gly–Leu–Met–NH_2
↓ EtOCOCl/Et_3N
BOC–Phe–HyMeV–Gly–Leu–Met–NH_2 (**13**)
↓ i) HCl in Dioxane ii) di-BOC-Lys-ONP (**14**)
di-BOC–Lys–Phe–HyMeV–Gly–Leu–Met–NH_2 (**15**)
↓ HCl in AcOH
H–Lys–Phe–HyMeV–Gly–Leu–Met–NH_2·2HCl (**3**)

Scheme 2.

The oily BOC-didepsipeptide benzyl ester, obtained through column chromatography on silica gel, was hydrogenolyzed with palladium on charcoal to yield the corresponding acid (**12**). **12** was coupled with glycyl-leucyl-methionine amide by the MA method to yield the amorphous BOC-pentapeptide amide (**13**) in a good yield. The protected hexapeptide amide (**15**) was prepared from the di-BOC-lysine *p*-nitrophenyl ester (**14**) and the pentapeptide amide which had been obtained after the removal of the protecting group of **13**.

H–Lys–Phe–Ile–Glyc–Leu–Met–NH_2 (**4**) was synthesized as is shown in Scheme 3:

BOC–Ile–OH + $ClCH_2COOBzl$
↓ Et_3N in AcOEt
BOC–Ile–Glyc–OBzl
↓ H_2/Pd
BOC–Ile–Glyc–OH (**16**) + H–Leu–Met–NH_2
↓ EtOCOCl/Et_3N
BOC–Ile–Glyc–Leu–Met–NH_2 (**17**)
↓ i) HCl in Dioxane ii) BOC-Phe-ONP (**18**)
BOC–Phe–Ile–Glyc–Leu–Met–NH_2 (**19**)
↓ i) HCl in Dioxane ii) di-BOC-Lys-ONP (**14**)
di-BOC–Lys–Phe–Ile–Glyc–Leu–Met–NH_2 (**20**)
↓ HCl in AcOH
H–Lys–Phe–Ile–Glyc–Leu–Met–NH_2·2HCl (**4**)

Scheme 3.

The benzyl ester of BOC-isoleucyl-glycolic acid was prepared by the reaction of BOC-isoleucine with benzyl chloroacetate in the presence of an equimolar amount of triethylamine. The ester was hydrogenolyzed to produce BOC-didepsipeptide acid (**16**). BOC-tetradepsipeptide amide (**17**) was synthesized by the MA method. Di-BOC-hexapeptide amide (**20**) was obtained by the stepwise elongation method.

H–Lys–Phe–Ile–Gly–HyIc–Met–NH_2 (**5**) was synthesized as follows (Scheme 4):

BOC–Gly–OH + H–HyIc–OBzl (**21**)
↓
BOC–Gly–HyIc–OBzl
↓ H_2/Pd
BOC–Gly–HyIc–OH (**22**) + H–Met–NH_2
↓ EtOCOCl/Et_3N
BOC–Gly–HyIc–Met–NH_2 (**23**)
↓ HCl in Dioxane
AOC–Phe–Ile–N_2H_3 (**24**) + H–Gly–HyIc–Met–NH_2·HCl (**25**)
↓ HNO_2
AOC–Phe–Ile–Gly–HyIc–Met–NH_2 (**26**)
↓ i) HCl in Dioxane ii) di-BOC-Lys-ONP (**14**)
di-BOC–Lys–Phe–Ile–Gly–HyIc–Met–NH_2 (**27**)
↓ HCl in AcOH
H–Lys–Phe–Ile–Gly–HyIc–Met–NH_2·2HCl (**5**)

Scheme 4.

The BOC-dipeptide benzyl ester was hydrogenolyzed to yield an acid (**22**), which was then coupled with methionine amide to afford the crystalline BOC-tripeptide amide (**23**). The condensation of the azide derived from AOC-dipeptide hydrazide (**24**) with tripeptide amide (**25**) gave an acylpentapeptide amide (**26**).

H-Lys-Phlac-Ile-Gly-HyIc-Met-NH_2 (**6**) was synthesized according to Scheme 5:

BOC-Ile-OH + H-Gly-HyIc-Met-NH_2 (**25**)
↓ EtOCOCl/Et_3N
BOC-Ile-Gly-HyIc-Met-NH_2 (**28**)
↓ HCl in Dioxane
di-BOC-Lys-Phlac-OH (**9**) + H-Ile-Gly-HyIc-Met-NH_2
↓ EtOCOCl/Et_3N
di-BOC-Lys-Phlac-Ile-Gly-HyIc-Met-NH_2 (**29**)
↓ HCl in AcOH
H-Lys-Phlac-Ile-Gly-HyIc-Met-NH_2·2HCl (**6**)

Scheme 5.

The BOC-tetradepsipeptide amide (**28**) was obtained by acylating tripeptide amide (**25**) by a mixed anhydride of BOC-isoleucine. The di-BOC-hexapeptide amide (**29**) was obtained from **9** and the tetradepsipeptide amide by the MA method. The removal of the BOC group lead to the desired depsipeptide amide dihydrochloride (**6**).

TABLE 1. PHARMACOLOGICAL RESULTS ON THE BLOOD PRESSURE IN RABBITS.

Compound	Relative activity[a)]
H-Lys-Phe-Ile-Gly-Leu-Met-NH_2 (**1**)	100
H-Lys-Phlac-Ile-Gly-Leu-Met-NH_2 (**2**)	90
H-Lys-Phe-HyMeV-Gly-Leu-Met-NH_2 (**3**)	10
H-Lys-Phe-Ile-Glyc-Leu-Met-NH_2 (**4**)	30
H-Lys-Phe-Ile-Gly-HyIc-Met-NH_2(**5**)	120
H-Lys-Phlac-Ile-Gly-HyIc-Met-NH_2(**6**)	30

a) Compared on the basis of the dose which caused fall of 20 mmHg of the blood pressure

A biological comparison of the five depsipeptide analogs to the standard compound (**1**) is given in Table 1. In the study of rabbit blood pressure, **2** and **5** were almost identical with **1**, and **4** and **6** possessed substantial activity, though it was lower than that of the parent hexapeptide, while **3** showed much less activity. These results show that the amide bond between the phenylalanine residue and the isoleucine residue play an important role in manifesting the hypotensive activity. Thus, it was found that, in the case of the replacement of the amide bond in the compound **1** by the ester bond, with no change in the amino acid side chain, the potency of the biological activity was strictly dependent upon the position replaced. To clarify this point, more precise investigations are required; such studies will be reported in the near future.

Experimental

All the melting points are uncorrected.

H-Phlac-OBzl (7). A solution of β-phenyllactic acid[10] (60 g, 360 mmol), *p*-toluenesulfonic acid monohydrate (5.5 g), and benzyl alcohol (180 ml) in benzene (360 ml) was refluxed in a Dean and Stark apparatus[12] for 30 min. The solution was washed with water, 4% sodium bicarbonate, and again water, dried over magnesium sulfate, and fractionated. A fraction boiling at 165—170°C/1 mmHg was collected; yield, 30 g (32.5%); $[\alpha]_D^{25}$ −13.0° (*c* 1, MeOH); $[\alpha]_D^{25}$ −10.9° (*c* 1.5, EtOH).

Di-BOC-Lys-Phlac-OBzl (8). To a solution of di-BOC-Lys-OH (34.6 g, 100 mmol) in a mixture of pyridine (100 ml) and tetrahydrofuran (100 ml), was added with stirring benzenesulfonyl chloride (19.5 g, 110 mmol) at −10°C over a period of 10 min. After 10 min, a solution of compound **7** (25.6 g, 100 mmol) in pyridine (40 ml) was added to the solution. The mixture was then allowed to stand at 0°C for 1 hr and at room temperature for 8 hr, and subsequently poured into water (1000 ml). The oil separated was extracted with ether. The extract was washed repeatedly with 2% hydrochloric acid, 4% sodium bicarbonate, and water, and dried over magnesium sulfate. The solvent was distilled off *in vacuo*, and crude product obtained as crystals was recrystallized from ether-*n*-hexane; yield, 57 g (85%); mp 60—62°C; $[\alpha]_D^{25}$ −28.1° (*c* 1, MeOH). Found: C, 65.73; H, 7.59; N, 4.48%. Calcd for $C_{32}H_{44}O_8N_2$: C, 65.73; H, 7.59; N, 4.79%.

Di-BOC-Lys-Phlac-OH (9). Compound **8** (5.84 g, 10 mmol) was dissolved in methanol (50 ml) and hydrogenolyzed in the presence of 10% palladium on charcoal. The filtrate from the catalyst was evaporated to dryness; yield of oil, 4.9% (100%).

Di-BOC-Lys-Phlac-Ile-Gly-Leu-Met-NH_2 (10). To a solution of **9** (4.5 g, 8 mmol) and triethylamine (1.2 ml) in chloroform (40 ml), was added ethyl chloroformate (880 mg, 8 mmol) at −15—−10°C. After 15 min, there was added a solution of H-Ile-Gly-Leu-Met-NH_2·HCl[13] (4.3 g, 8 mmol) and triethylamine (1.2 ml) in dimethylformamide (100 ml) at −15—−10°C, and this mixture was allowed to stand at room temperature overnight. The mixture was poured into cold water (1000 ml), and the resulting solid was extracted with ethyl acetate. The extract was washed successively with 1% hydrochloric acid, 4% sodium bicarbonate, and water. The solvent was then evaporated *in vacuo*, and the crude product was recrystallized from ethyl acetate-methanol-petroleum ether; yield, 4.6 g (62%); mp 190—192°C; $[\alpha]_D^{20}$ −35.6° (*c* 0.5, MeOH). Found: C, 57.43; H, 7.89; N, 10.53; S, 3.75%. Calcd for $C_{44}H_{73}O_{11}N_7S\cdot H_2O$: C, 57.08; H, 8.10; N, 10.59; S, 3.45%.

H-Lys-Phlac-Ile-Gly-Leu-Met-NH_2·2HCl (2). Compound **10** (200 mg) was dissolved in 2N hydrogen chloride in acetic acid (5 ml), and the solution was allowed to stand at room temperature for 20 min. The product was precipitated by adding dry ether, and the precipitate was collected by filtration, washed well with ether, and dried over sodium hydroxide *in vacuo*. It was dissolved in 60% methanol (4 ml), and an insoluble material was filtered off. The filtrate was con-

12) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John Wiley & Sons, Inc., New York London (1961), p. 940.
13) E. Schröder, R. Schmiechen, and H. Gibian, *Ann. Chem.*, **679**, 195 (1964).

centrated to dryness over phosphorus pentoxide *in vacuo* to obtain the product; yield, 170 mg; mp 160—165°C $[\alpha]_D^{25}$ −48.1° (*c* 0.305, H_2O); R_f, 0.70.[14] Found: C, 50.83; H, 7.92; N, 12.36; S, 4.44; Cl, 8.84%. Calcd for $C_{34}H_{57}O_7N_7S \cdot 2HCl \cdot H_2O$: C, 51.12; H, 7.64; N, 12.28; S, 4.01; Cl, 8.89%. The amino acid ratios in the acid hydrolyzate were Lys 1.00, Ile 1.03, Gly 1.05, Leu 1.00, and Met 0.91.

H-HyMeV-OBzl (11). This was obtained from α-hydroxy-β-methylvaleric acid[10] (52.8 g, 400 mmol), benzyl alcohol (200 ml), and sulfuric acid (2 ml) as described for the preparation of **7**; yield of oil, 62 g (70%); bp 122—125°C /1 mmHg; $[\alpha]_D^{25}$ −12.7° (*c* 1.9, EtOH).

BOC-Phe-HyMeV-OH (12). BOC-Phe-OH (26.5 g, 100 mmol) was condensed with **11** (24.5 g, 110 mmol) by the benzenesulfonyl chloride method to yield an oil (33.8 g, 72%); this oil was then hydrogenolyzed in the presence of 10% palladium on charcoal; yield of oil, 24.6 g (64%).

BOC-Phe-HyMeV-Gly-Leu-Met-NH_2 (13). This compound was obtained from **12** (6.4 g, 16.8 mmol) and H-Gly-Leu-Met-$NH_2 \cdot HCl$[13] (6.9 g, 19 mmol) as described for the preparation of **10**; yield, 7.5 g (66%); mp 165—168°C; $[\alpha]_D^{25}$ −39.5° (*c* 1, MeOH). Found: C, 58.34; H, 7.89; N, 10.41; S, 4.81%. Calcd for $C_{33}H_{53}O_8N_5S$: C, 58.29; H, 7.85; N, 10.30; S, 4.71%.

Di-BOC-Lys-Phe-HyMeV-Gly-Leu-Met-NH_2 (15). Compound **13** (5.4 g, 8 mmol) was dissolved in 3N hydrogen chloride in dioxane (70 ml) at room temperature. After 20 min, the solution was evaporated to dryness in *vacuo*. The pentadepsipeptide amide hydrochloride thus obtained was dissolved in dimethylformamide (40 ml) and then neutralized with triethylamine (1.2 ml). Di-BOC-Lys-ONP (**14**)[15] (4.2 g, 9 mmol) was added to the solution, and the mixture was allowed to stand at room temperature for 20 hr. Water was added to the reaction mixture to precipitate the product, which was then collected by filtration, washed well with 2% hydrochloric acid, N ammonia, and water, and dried. Recrystallization from ethyl acetate-petroleum ether gave 4.4 g (60%) of a product; mp 192—195°C; $[\alpha]_D^{25}$ −37.8° (*c* 0.5, MeOH). Found: C, 58.13; H, 8.03; N, 10.57; S, 3.62%. Calcd for $C_{44}H_{73}N_7O_{11}S$: C, 58.19; H, 8.10; N, 10.79; S, 3.53%.

H-Lys-Phe-HyMeV-Gly-Leu-Met-$NH_2 \cdot 2HCl$ (3). This compound was obtained from **15** (200 mg) as described for the preparation of **2**; yield, 170 mg; mp 60—80°C; $[\alpha]_D^{25}$ −35.2° (*c* 0.25, H_2O); R_f, 0.72.[14] Found: C, 51.05;H, 7.83; N, 12.05; S, 4.25; Cl, 9.05%. Calcd for $C_{34}H_{57}O_7N_7S \cdot 2HCl \cdot H_2O$: C, 51.12; H, 7.64; N, 12.28; S, 4.01; Cl, 8.89%. The amino acid ratios in the acid hydrolyzate were Lys 0.98, Phe 0.96, Gly 1.00, Leu 1.00, and Met 0.76.

BOC-Ile-Glyc-OH (16). A solution of BOC-Ile-OH (15 g, 60 mmol), triethylamine (8 ml), and benzyl chloroacetate[16] (10.5 g, 60 mmol) in ethyl acetate (150 ml) was refluxed for 5 hr. The solution was washed with 2% hydrochloric acid, 4% sodium bicarbonate and water, dried over magnesium sulfate, and concentrated to dryness. The resulting oil (16 g) was dissolved in methanol (80 ml) and hydrogenolyzed in the presence of 5% palladium on charcoal. The solvent was distilled off *in vacuo*, and the crude acid was dissolved in 4% sodium bicarbonate, washed with ether, and acidified with 1% hydrochloric acid. The oil thus separated was extracted into ether. The ether was removed under reduced pressure; yield of oil, 7.7 g (60%).

BOC-Ile-Glyc-Leu-Met-NH_2 (17). This was obtained from **16** (7 g, 24.2 mmol) and H-Leu-Met-$NH_2 \cdot HCl$[13] (8.3 g, 25 mmol) as described for the preparation of **10**. The crude product was recrystallized from ethyl acetate-petroleum ether; yield, 8.7 g (68%); mp 171—177°C; $[\alpha]_D^{25}$ −39.0° (*c* 1, MeOH). Found: C, 54.01; H, 8.13; N, 10.82; S, 6.47%. Calcd for $C_{24}H_{44}N_4O_7S$: C, 54.13; H, 8.27; N, 10.52; S, 6.01%.

BOC-Phe-Glyc-Leu-Met-NH_2 (19). Compound **17** (4 g, 8 mmol) was dissolved in 3N hydrogen chloride in dioxane (60 ml) at room temperature. After 20 min, the solution was evaporated to dryness under reduced pressure. The tetradepsipeptide amide hydrochloride thus obtained was dissolved in dimethylformamide (30 ml), neutralized with triethylamine (1.1 ml), and then subjected to a reaction with BOC-Phe-ONP (**18**)[15] (3.1 g, 8 mmol). After standing overnight at roomt emperature, the solution was diluted with ethyl acetate (350 ml), washed with 2% hydrochloric acid, 4% sodium bicarbonate, and water, and dried over magnesium sulfate. The solvent was distilled off under reduced pressure, and the solid material was recrystallized from ethyl acetate-petroleum ether; yield, 4 g, (75%); mp 156—158°C; $[\alpha]_D^{25}$ −25.3° (*c* 1, MeOH). Found: C, 56.73; H, 7.97; N, 10.06; S, 4.84%. Calcd for $C_{33}H_{53}O_8N_5S \cdot H_2O$: C, 56.81; H, 7.89; N, 10.04; S, 4.59%.

Di-BOC-Lys-Phe-Ile-Glyc-Leu-Met-NH_2 (20). Compound **19** (1.3 g, 2 mmol) was treated with 3N hydrogen chloride in dioxane. The pentadepsipeptide amide hydrochloride thus obtained was dissolved in dimethylformamide (10 ml), neutralized with triethylamine (0.28 ml), and then subjected to a reaction with compound **14** (1 g, 2.1 mmol). The mixture was then treated as described for the preparation of **15**; yield, 1.5 g (80%); mp 146—149°C; $[\alpha]_D^{25}$ −36.0° (*c* 1, MeOH). Found: C, 57.39; H, 8.25; N, 10.29; S, 3.36%. Calcd for $C_{44}H_{93}O_{11}N_7S \cdot H_2O$: C, 57.08; H, 8.10; N, 10.59; S, 3.45%.

H-Lys-Phe-Ile-Glyc-Leu-Met-$NH_2 \cdot 2HCl$ (4). This compound was obtained from **20** (200 mg) as described for the preparation of **2**; yield, 170 mg, mp 65—80°C; $[\alpha]_D^{25}$ −26.7° (*c* 0.5, H_2O); R_f, 0.68.[14] Found: C, 51.05; H, 7.82; N, 12.13; S, 3.53; Cl, 9.13%. Calcd for $C_{34}H_{57}O_7N_7S \cdot 2HCl \cdot H_2O$: C, 51.12; H, 7.64; N, 12.28; S, 4.01; Cl, 8.89%. The amino acid ratios in the acid hydrolyzate were Lys 1.08, Phe 0.98, Ile 1.12, Leu 1.00, and Met 0.85.

H-HyIc-OBzl (21). This compound was obtained from α-hydroxyisocaproic acid[10] (25.4 g, 200 mmol), benzyl alcohol and sulfuric caid as described for the preparation of **7**; yield of oil, 26 g (60%); bp 113—115°C/1 mmHg; $[\alpha]_D^{25}$ −16.0° (*c* 2.1, EtOH).

BOC-Gly-HyIc-OH (22). Benzenesulfonyl chloride (17.7 g, 100 mmol) was added to a solution of BOC-Gly-OH (17.5 g, 100 mmol) in a mixture of tetrahydrofuran (200 ml) and pyridine (100 ml) at −10°C. Then compound **21** (22.2 g, 100 mmol) was added. The product was isolated and purified by column chromatography. The resulting oil (38 g) was hydrogenolyzed in the presence of 5% palladium on charcoal; yield of oil, 17 g (60%).

BOC-Gly-HyIc-Met-NH_2 (23). To a solution of **22** (18.86 g, 65 mmol) and triethylamine (9.2 ml) in chloroform (200 ml), was added ethyl chloroformate (7.6 g, 70 mmol) at −12——10°C. After 15 min, a solution of H-Met-$NH_2 \cdot HCl$[17] (12.8 g, 70 mmol) and triethylamine (9.8 ml) in dimethylformamide (50 ml) was added to the solution. The mixture was stirred at room temperature for 2 hr and then poured into water (500 ml), and the oil thus separated was

14) Paper chromatography was carried out on Toyo Roshi No.50 with *n*-butanol-acetic acid-water (4: 1: 1).

15) E. Sandrin and R. A. Boissonnas, *Helv. Chim. Acta*, **46**, 1637 (1963).

16) K. Seubert, *Chem. Ber.*, **21**, 281 (1888).

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extracted with chloroform. The extract was washed with 1% hydrochloric acid, 4% sodium bicarbonate, and water, and dried over magnesium sulfate. The solvent was distilled off under reduced pressure, and the product was recrystallized from ethyl acetate–petroleum ether; yield, 17 g (73%); mp 148—150°C; $[\alpha]_D^{25}$ −34.5° (*c* 1.25, MeOH). Found: C, 51.71; H, 8.04; N, 9.53; S, 7.66%. Calcd for $C_{18}H_{33}O_6N_3S$: C, 51.54; H, 7.93; N, 10.02; S, 7.63%.

AOC-Phe-Ile-NHNH$_2$ (24). A solution of AOC-Phe-Ile-OEt (8.4 g, 20 mmol) and hydrazine hydrate (10 g, 200 mmol) in methanol (20 ml) was refluxed for 48 hr. After ocoling, the crystal thus precipitated out was filtered, washed well with water, and recrystallized from methanol–water; yield, 6.4 g (78%); 171—172°C; $[\alpha]_D^{20}$ −45.9° (*c* 1, AcOH). Found: C, 62.16; H, 8.43; N, 13.53%. Calcd for $C_{21}H_{34}O_4N_4$: C, 62.04; H, 8.43; N, 13.78%.

AOC-Phe-Ile-Gly-HyIc-Met-NH$_2$ (26). Compound **23** (3 g, 7.1 mmol) was dissolved in 3N hydrogen chloride in dioxane (50 ml) at room temperature. After 30 min, the solvent was distilled off under reduced pressure, and the residue was triturated with ether. The product was dried *in vacuo* over sodium hydroxide; yield, 2.5 g (99%). This material (**25**) was used in the following reaction without further purification. To a solution of **24** (2.85 g, 7 mmol) in a mixture of 3N hydrochloric acid (11.2 ml) and dimethylformamide (280 ml), was added with stirring a chilled solution of sodium nitrite (483 mg, 7 mmol) in water (3 ml) at −18—−15°C. After 20 min, the solution was neutralized with triethylamine and there was added a solution of **25** (2.5 g, 7 mmol) obtained above and triethylamine (0.98 ml) in dimethylformamide (35 ml) at −15—−10°C. After the mixture had been stirred at room temperature for 3 hr, it was treated as described for the preparation of **10**; yield, 3.5 g (73%); mp 191—192°C; $[\alpha]_D^{25}$ −47.0° (*c* 1, MeOH). Found: C, 58.63; H, 8.14; N, 10.13; S, 4.40%. Calcd for $C_{34}H_{55}O_8N_5S$: C, 58.87; H, 7.93; N, 10.10; S, 4.61%.

Di-BOC-Lys-Phe-Ile-Gly-HyIc-Met-NH$_2$ (27). Compound **26** (1 g, 1.4 mmol) was treated with 3N hydrogen chloride in dioxane for 30 min at room temperature. The solvent was distilled off under reduced pressure. The product, which corresponded to H-Phe-Ile-Gly-HyIc-Met-NH_2·HCl, was added to a solution of **14** (700 mg, 1.5 mmol) in dimethylformamide (7 ml), together with triethylamine (0.2 ml); the mixture was then treated as described for the preparation of **15**; yield, 1 g, (77%); mp 199—201°C; $[\alpha]_D^{25}$ −44.4° (*c* 0.5, MeOH). Found: C, 57.48; H, 8.45; N, 10.36; S, 3.35%. Calcd for $C_{44}H_{73}O_{11}N_7S\cdot H_2O$: C, 57.08; H, 8.10; N, 10.59; S, 3.45%.

H-Lys-Phe-Ile-Gly-HyIc-Met-NH$_2$·2HCl (5). This compound was obtained from **27** (200 mg) as described for the preparation of **2**; yield, 180 mg; mp 230—233°C; $[\alpha]_D^{25}$ −28.8° (*c* 0.25, H_2O); R_f, 0.68.[14] Found: C, 50.84; H, 7.93; N, 12.59; S, 3.67; Cl, 9.25%. Calcd for $C_{34}H_{57}O_7N_7S\cdot 2HCl\cdot H_2O$; C, 51.12: H, 7.64; N, 12.28; S, 4.01; Cl, 8.89%. The amino acid ratios in the acid hydrolyzate were Lys 0.97, Phe 1.01, Ile 1.00, Gly 1.04, and Met 0.85.

BOC-Ile-Gly-HyIc-Met-NH$_2$ (28). To a solution of BOC-Ile-OH (1 g, 4 mmol) and triethylamine (0.56 ml, 4 mmol) in chloroform (50 ml), was added ethyl chloroformate (0.44 g, 4 mmol) at −12—−10°C. After 15 min, there was added a solution of H-Gly-HyIc-Met-NH_2·HCl (**25**) obtained from **23** (1.5 g, 3.6 mmol) and triethylamine (0.5 ml) in dimethylformamide (20 ml); the mixture was then stirred at room temperature for 3 hr. Then it was poured into water (100 ml), and the solid thus precipitated was extracted with ethyl acetate. The extract was washed with 1% hydrochloric acid, 4% sodium bicarbonate, and water, and dried over magnesium sulfate. The solvent was distilled off *in vacuo*, and the crude product was recrystallized from ethyl acetate–petroleum ether; yield, 1.25 g (59%); mp 140—142°C; $[\alpha]_D^{25}$ −46.0° (*c* 1, MeOH). Found: C, 53.68; H, 8.28; N, 10.78; S, 6.06%. Calcd for $C_{24}H_{44}O_7N_4S$: C, 54.12; H, 8.33; N, 10.52; S, 6.00%.

Di-BOC-Lys-Phlac-Ile-Gly-HyIc-Met-NH$_2$ (29). Compound **28** (1.7 g, 3.2 mmol) was dissolved in 3N hydrogen chloride in dioxane at room temperature. After 40 min, the solvent was distilled off *in vacuo*, and the residue, which corresponded to H-Ile-Gly-HyIc-Met-NH_2·HCl, was used in the following reaction without further purification. To a solution of compound **9** (2 g, 4 mmol) and triethylamine (0.56 ml) in chloroform (50 ml), was added ethyl chloroformate (440 mg, 4 mmol) at −10—−8°C. Then, a solution of the tetradepsipeptide amide hydrochloride obtained above in dimethylformamide (16 ml) was added and the mixture was treated as described for the preparation of **10**; yield, 1.9 g (52%); mp 148—150°C; $[\alpha]_D^{25}$ −50.0° (*c* 0.5, MeOH). Found: C, 57.26; H, 7.97; N, 9.25; S, 3.76%. Calcd for $C_{44}H_{72}O_{12}N_6S\cdot H_2O$: C, 57.01; H, 7.99; N, 9.07; S, 3.45%.

H-Lys-Phlac-Ile-Gly-HyIc-Met-NH$_2$·2HCl (6). This compound was obtained from **29** (200 mg) as described for the preparation of **2**; yield, 180 mg; mp 155—160°C; $[\alpha]_D^{25}$ −52.3° (*c* 0.285, H_2O): R_f, 0.75.[14] Found: C, 51.17; H, 7.70; N, 10.79; S, 4.23; Cl, 8.76%. Calcd for $C_{34}H_{56}O_8N_6S\cdot 2HCl\cdot H_2O$: C, 51.05; H, 7.56; N, 10.50; S, 4.00; Cl, 8.86%. The amino acid ratios in the acid hydrolyzate were Lys 0.93, Ile 1.00, Gly 1.05, and Met 0.83.

Pharmacological Assay. White male rabbits (2—3 kg) were anesthetized subcutaneously with 1.2 g/kg of urethane. Anesthesia was maintained with additional doses as needed. The arterial blood pressure was measured from a canulated carotid artery and recorded on a Kymograph by means of a mercury manometer. All the peptides were dissolved in physiological saline and injected into the femoral vein. Each dose was tested on five animals and always checked against the reference standard material, **1**. The activity of the peptides was compared on a weight basis. The data were obtained with five animals per group.

The authors wish to thank Professor N. Izumiya of Kyushu University and Director Dr. I. Chibata of Tanabe Seiyaku for their encouragement in the course of this study.