Synthesis and Biological Activity of Peptides Related to Eledoisin. II.[1] Hexapeptide Amides Containing *N*-Methylamino Acids[2,3]

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Eledoisin-like hexapeptides were synthesized in order to obtain longer-lasting hypotensive analogs. A part of the amino acid of the standard peptide, H–Lys–Phe–Ile–Gly–Leu–Met–NH_2 (**1**), was replaced by *N*-methylamino acid. It was found, in these syntheses, that when the *C*-terminal amino acid of a carboxy component was an *N*-methylamino acid, a system of dicyclohexylcarbodiimide plus 1-hydroxybenzotriazole was a useful coupling agent. With regard to the hypotensive effect in rabbits, H–Lys–Phe–MeIle–Gly–Leu–Met–NH_2 (**2**) and H–Lys–Phe–Ile–Gly–MeIle–Met–NH_2 (**6**) show much less activity; H–Lys–MePhe–Ile–Gly–Leu–Met–NH_2 (**3**) and H–Lys–MePhe–Ile–Gly–MeLeu–Met–NH_2 (**7**) show a substantial activity, though weaker than the standard one. On the other hand, H–Lys–Phe–Ile–Gly–MeLeu–Met–NH_2 (**5**) and H–Lys–Phe–Ile–Sar–Leu–Met–NH_2 (**4**) show a higher activity than **1**. These results indicate that, in some cases, the replacement of an amide bond by an *N*-methylamide bond without any change in the side chain of amino acid would have an important influence on the activity. The duration of the action of *N*-methypeptildes synthesized was unexpectedly of the same order of magnitude as that of **1**.

In the preceding paper,[1] we described the synthesis and the biological activity of several hexadepsipeptide analogs of eledoisin, in which the amide bond was replaced with the ester bond in order to avoid cleavage by the proteolytic enzyme of the organism and, consequently, in order to obtain a longer-lasting compound. However, the duration of the depsipeptides synthesized was unexpectedly of the same order of magnitude as that of the standard peptide, H–Lys–Phe–Ile–Gly–Leu–Met–NH_2 (**1**). This shows that the ester bond would be hydrolyzed by the enzyme as readily as the original peptide bond and that, in order to produce a longer-lasting action, the peptide bond must be replaced by some other bond which can not be attacked by the enzyme.

Andreatta and Scheraga recently reported that a peptide involving *N*-methylamino acid was not attacked by the protease.[5] Thus, the His–MeAla bond in [Val[5], MeAla[7]]-angiotensin II is totally resistant to the aminopeptidase. Baldwin also showed that a peptide bond involving methylated arginine could not be hydrolyzed by trypsin.[6]

In the present investigation, from this point of view, the authors synthesized several hexapeptides, H–Lys–Phe–MeIle–Gly–Leu–Met–NH_2 (**2**), H–Lys–MePhe–Ile–Gly–Leu–Met–NH_2 (**3**), H–Lys–Phe–Ile–Sar–Leu–Met–NH_2 (**4**), H–Lys–Phe–Ile–Gly–MeLeu–Met–NH_2 (**5**), H–Lys–Phe–Ile–Gly–MeIle–Met–NH_2 (**6**), and H–Lys–MePhe–Ile–Gly–MeLeu–Met–NH_2 (**7**). In these peptides, a part of the amino acid of the standard peptide **1** was replaced with *N*-methylamino acid, and their biological activity was compared with that of **1**.

In compounds **2**, **3**, **4**, **5**, and **7**, none of the amino-acid side chains are altered, whereas the amino-acid side chain of compound **6** is different from the reference standard in the part of leucine.

Synthesis

Optically active *N*-methylamino acids were prepared by the method reported previously by Quitt *et al.*[7]

H–Lys–Phe–MeIle–Gly–Leu–Met–NH_2 (**2**) was synthesized according to Scheme 1. *t*-Amyloxycarbonyl (AOC)-phenylalanine was condensed with the *N*-methylisoleucine methyl ester (**8**) with dicyclohexylcarbodiimide (DCC) to yield the AOC-phenylalanyl-*N*-methylisoleucine methyl ester. The ester did not crystallize and was converted into AOC-dipeptide acid (**9**) by

AOC–Phe–OH + H–MeIle–OMe (**8**)
↓ DCC
AOC–Phe–MeIle–OMe
↓ NaOH
AOC–Phe–MeIle–OH (**9**) + H–Gly–Leu–Met–NH_2
7% ↓ $EtOCOCl/Et_3N$
AOC–Phe–MeIle–Gly–Leu–Met–NH_2 (**10**)
↓ i) HCl in Dioxane ii) di-BOC-Lys-ONP
di-BOC–Lys–Phe–MeIle–Gly–Leu–Met–NH_2 (**11**)
↓ HCl in AcOH
H–Lys–Phe–MeIle–Gly–Leu–Met–$NH_2 \cdot 2HCl$ (**2**)

Scheme 1.

1) Part I of this series; H. Sugano, K. Higaki, and M. Miyoshi, This Bulletin, **46**, 226 (1973).

2) Presented in part at the 91 st Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, April, 1971.

3) The abbreviations recommended by the IUPAC-IUB commission on Biological Nomenclature (*J. Biol. Chem.*, **241**, 2491 (1966); **242**, 555 (1967)) have been used throughout. Amino acid and *N*-methylamino acid symbols except Gly and Sar denote the L-configuration.

4) To whom inquiries should be addressed.

5) R. H. Andreatta and H. A. Scheraga, *J. Med. Chem.*, **14**, 489 (1971).

6) G. S. Baldwin and P. R. Carnegie, *Science*, **171**, 579 (1971).

7) P. Quitt, J. Hellerbach, and K. Vogler, *Helv. Chim. Acta*, **46**, 327 (1963).

saponification, which did not proceed smoothly because of the bulkiness of the side chain and, in addition, that of the *N*-methyl group of *N*-methylisoleucine. The acid (**9**) was coupled with glycyl-leucyl-methionine amide to produce AOC-pentapeptide amide in a rather poor yield. This low yield may also be due to the bulky *C*-terminal amino acid in the carboxy component.

Di-*t*-butyloxycarbonyl (BOC)-hexapeptide amide (**11**) was prepared from the di-BOC-lysine *p*-nitrophenyl ester and the pentapeptide amide which had been obtained from AOC-pentapeptide amide (**10**) using 2N hydrogen chloride in dioxane. The BOC group was removed from compound **11** using 2N hydrogen chloride in acetic acid.

The route of synthesizing H–Lys–MePhe–Ile–Gly–Leu–Met–NH_2 (**3**) is shown in Scheme 2:

A)

di-BOC–Lys–OH + H–MePhe–OBzl (**12**)

↓ DCC

di-BOC–Lys–MePhe–OBzl

↓ H_2/Pd

di-BOC–Lys–MePhe–OH (**13**) + H–Ile–Gly–Leu–Met–NH_2

20% ↓ $EtOCOCl/Et_3N$

di-BOC–Lys–MePhe–Ile–Gly–Leu–Met–NH_2 (**15**)

B)

di-BOC–Lys–MePhe–OH (**13**) + H–Ile–Gly–Leu–Met–OMe

75% ↓ DCC/HOBt

di-BOC–Lys–MePhe–Ile–Gly–Leu–Met–OMe (**14**)

↓ NH_3 in MeOH

di-BOC–Lys–MePhe–Ile–Gly–Leu–Met–NH_2 (**15**)

↓ HCl in AcOH

H–Lys–MePhe–Ile–Gly–Leu–Met–NH_2·2HCl (**3**)

Scheme 2.

Compound **13**, obtained from the di-BOC-lysyl-*N*-methylphenylalanine benzyl ester by hydrogenolysis, was coupled with *C*-terminal tetrapeptide amide by the mixed anhydride method to afford the protected hexapeptide amide (**15**) in a low yield, accompanied by a considerable amount of ethoxycarbonyl-isoleucyl-glycyl-leucyl-methionine amide.

On the other hand, using DCC together with the 1-hydroxybenzotriazole (HOBt) developed by König and Geiger,[8] the di-BOC-hexapeptide ester (**14**) was obtained in a good yield from compound **13** and the tetrapeptide ester. No acylurea was detected on thin-layer chromatography.[9] Thus, the DCC–HOBt is recommendable for a coupling agent if the *C*-terminal amino acid of a carboxy component is a bulky *N*-methyl-amino acid.

Hexapeptide, H–Lys–Phe–Ile–Sar–Leu–Met–NH_2 (**4**), was synthesized in the following way (Scheme 3):

BOC–Ile–OH + H–Sar–OBzl (**16**)

↓ DCC

BOC–Ile–Sar–OBzl

↓ H_2/Pd

BOC–Ile–Sar–OH (**17**) + H–Leu–Met–OMe

↓ DCC/HOBt

BOC–Ile–Sar–Leu–Met–OMe (**18**)

↓ NH_3 in MeOH

BOC–Ile–Sar–Leu–Met–NH_2 (**19**)

↓ i) HCl in Dioxane ii) BOC–Phe–ONP

BOC–Phe–Ile–Sar–Leu–Met–NH_2 (**20**)

↓ i) HCl in Dioxane ii) di-BOC–Lys–ONP

di-BOC–Lys–Phe–Ile–Sar–Leu–Met–NH_2 (**21**)

↓ HCl in AcOH

H–Lys–Phe–Ile–Sar–Leu–Met–NH_2·2HCl (**4**)

Scheme 3.

BOC-isoleucyl-sarcosine, which was prepared by allowing BOC-isoleucine to react with the sarcosine benzyl ester by the DCC method, followed by hydrogenolysis over palladium on charcoal, was coupled with the leucyl-methionine ester by DCC–HOBt. Amidation followed by two successive stepwise elongations using the *p*-nitrophenyl ester and subsequent deprotection afforded the desired compound (**4**).

H–Lys–Phe–Ile–Gly–MeLeu–Met–NH_2 (**5**) and H–Lys–Phe–Ile–Gly–MeIle–Met–NH_2 (**6**) were synthesized according to Scheme 4:

BOC–Gly–OH + H–X–OMe (**22**) or (**8**)

↓ DCC

BOC–Gly–X–OMe

↓ NaOH

BOC–Gly–X–OH + H–Met–OMe (**23a**) or (**23b**)

↓ DCC/HOBt

BOC–Gly–X–Met–OMe (**24a**) or (**24b**)

↓ NH_3 in MeOH

BOC–Gly–X–Met–NH_2 (**25a**) or (**25b**)

↓ HCl in Dioxane

AOC–Phe–Ile–N_2H_3 + H–Gly–X–Met–NH_2

↓ HONO

8) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).

9) J. C. Sheehan and D. D. H. Young, *J. Amer. Chem. Soc.*, **80**, 1158 (1958); K. Hofmann, N. Yanaihara, S. Lande, and H. Yajima, *ibid.*, **84**, 4470 (1962); H. Schüssler and H. Zahn, *Chem. Ber.*, **95**, 1076 (1962).

AOC–Phe–Ile–Gly–X–Met–NH_2 (**26a**) or (**26b**)

↓ i) HCl in Dioxane ii) di-BOC-Lys-ONP

di-BOC–Lys–Phe–Ile–Gly–X–Met–NH_2 (**27a**) or (**27b**)

↓ HCl in AcOH

H–Lys–Phe–Ile–Gly–X–Met–NH_2·2HCl (**5**) or (**6**)

Scheme 4. X=MeLeu or MeIle

BOC-dipeptide acids (**23a** and **23b**) were obtained as crystals and were coupled with the methionine ester by the use of HObt and DCC. The BOC-tripeptide esters (**24a** and **24b**) thus obtained were amidated and deprotected to give tripeptide amide hydrochlorides. They were converted into AOC-pentapeptide amides (**26a** and **26b**) by the azide method. Deprotection and subsequent reaction with the di-BOC-lysine *p*-nitrophenyl ester afforded di-BOC-hexapeptide amides (**27a** and **27b**), which were then subjected to acidolysis to yield the desired compounds (**5** and **6**).

Further, H–Lys–MePhe–Ile–Gly–MeLeu–Met–NH_2 (**7**) was synthesized as follows:

BOC–Gly–MeLeu–Met–OMe (**23a**)

↓ i) HCl in MeOH ii) BOC–Ile–OH $EtOCOCl/Et_3N$

BOC–Ile–Gly–MeLeu–Met–OMe (**28**)

↓ i) HCl in MeOH ii) di-BOC-Lys-MePhe-OH (**13**) DCC/HObt

di-BOC–Lys–MePhe–Ile–Gly–MeLeu–Met–OMe (**29**)

↓ NH_3 in MeOH

di-BOC–Lys–MePhe–Ile–Gly–MeLeu–Met–NH_2 (**30**)

↓ HCl in AcOH

H–Lys–MePhe–Ile–Gly–MeLeu–Met–NH_2·2HCl (**7**)

Scheme 5.

Compound **23a** was used as the starting material (Scheme 5). Thus, **23a** was deprotected and was condensed with a mixed anhydride of BOC-isoleucine to afford the BOC-tetrapeptide ester (**28**). After the removal of the BOC group, compound **28** was coupled with di-BOC-dipeptide acid (**13**) with the aid of HObt and DCC. Amidation and deprotection gave the desired hexapeptide amide dihydrochloride (**7**).

None of the protected hexapeptide amides reported here, except di-BOC–Lys–Phe–Ile–Gly–MeIle–Met–NH_2 (**27b**), crystallized; they were purified by column chromatography on silica-gel. Compound **27b** was recrystallized from ethyl acetate. The hexapeptide amide dihydrochlorides were all amorphous and were shown to be homogeneous by paper chromatography, paper electrophoresis, and elemental analysis.

Results

The pharmacological results concerning rabbit blood pressure are summarized in Table 1.

The time of 50% recovery as well as that of 100% recovery shows that the duration of action of the *N*-methylpeptides synthesized here is of the same order of magnitude as that of the reference standard, **1**. These results suggest that the *N*-methylpeptide bond would be cleaved by the proteolytic enzyme of the organism as fast as the usual peptide bonds, or that other factors, as yet unknown, would participate in the deactivation of the peptides. As regards this point, a more precise study will be reported in a following paper.

Table 1 also shows that compound **2** and compound **6** have much less activity and that compounds **3** and **7** possess a substantial activity, though weaker than the standard one, **1**. On the other hand, compound **5** and compound **4** show a higher activity than **1**. It is reasonable that **6** exhibits a marked decrease in activity, because it has a different amino-acid side chain in the part of leucine which has been reported to be essential for activity.[10] Though the other compounds, **2**, **3**, **4**, **5**, and **7**, have the same side chains as **1**, they vary in the degree of biological activity. As may be seen in the case of depsipeptide analogs,[1] these results suggest that, in some cases, the replacement of an amide bond by an *N*-methylamide bond would have an important influence on the activity. To clarify this point, a more precise investigation is required; studies are currently in progress.

Experimental

All the melting points are uncorrected. Column chromatography was carried out on silica gel (Merck, 70—325 mech). The amino acid analysis was performed by the method reported by Kono *et al.*,[11] in which the acid hydrolysate was converted into the *t*-butyl ester of trifluoroacetylamino acid and the composition was determined by gas chromatography. The pharmacological assay was carried out as had been described previously.[1]

H–MeIle–OMe·HCl (8). Thionyl chloride (83 g, 0.7 mol) was stirred into methanol (120 ml) at −2—2°C. To this solution, *N*-methyl-L-isoleucine (18 g, 0.124 mol) prepared by the procedure developed by Quitt *et al.*[7] ($[\alpha]_D^{25}$ +29.0° (*c* 1, H_2O) was added; the solution was stirred at room temperature for 1 hr, and then refluxed for 3 hr. After evaporation, this procedure was repeated two times. The oily residue thus obtained was dissolved in water (50 ml), and the solution was basified with sodium bicarbonate and repeatedly extracted with ether. The ethereal solution was washed with a saturated sodium chloride solution and dried over magnesium sulfate. Into the filtered solution, dry hydrogen chloride was bubbled, and the white crystals thus obtained were collected and recrystallized from methanol–ether; yield, 15.7 g (65%); mp 156—157°C; $[\alpha]_D^{25}$ +40.1° (*c* 1, ethanol) [lit,[12] mp 154—155°C; $[\alpha]_D^{25}$ +39° (*c* 1, ethanol)]. (Found: C, 49.47; H, 9.30; N, 7.16; Cl, 17.95%).

AOC–Phe–MeIle–OH (9). Into a solution of *t*-amyloxycarbonyl-L-phenylalanine dicyclohexylammonium salt[13]

10) E. Schröder and K. Lübke, "The Peptides," Academic Press, New York and London (1966), p. 127.

11) Presented by T. Kono, M. Ishii, and K. Kotera at the 19th Annual Meeting of the Analytical Chemistry of Japan, Nagoya, November, 1970.

12) M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, and A. A. Kiryushkin, *Tetrahedron*, **19**, 581 (1963).

13) S. Sakakibara, I. Honda, K. Takada, M. Miyoshi, T. Ohnishi, and K. Okumura, This Bulletin, **42**, 809 (1969).

TABLE 1. PHARMACOLOGICAL RESULTS ON THE BLOOD PRESSURE IN RABBITS

Compound	Dose μg/kg i.v.	Change mean arterial pressure mmHg	Change mean arterial pressure After sec	Average duration sec Recovery 50%	Average duration sec Recovery 100%
H-Lys-Phe-Ile-Gly-Leu-Met-NH_2 (**1**)	0.1	20± 4	17± 1	38± 7	74± 14
	1	45± 3	30± 5	60±15	140± 98
	5	58± 6	30± 5	121±29	323±148
H-Lys-Phe-MeIle-Gly-Leu-Met-NH_2 (**2**)	5	0	0	0	0
	10	4	15	29	10
	100	21± 3	26± 7	33± 6	88± 28
	500	26±24	33± 4	49±21	114± 58
H-Lys-MePhe-Ile-Gly-Leu-Met-NH_2 (**3**)	0.1	0	0	0	0
	0.5	16± 1	15± 2	20± 2	40± 12
	1	22± 3	25± 2	25± 4	63± 27
	5	27± 1	29± 2	32± 3	78± 12
	10	32± 4	36± 5	68±40	242±142
H-Lys-Phe-Ile-Sar-Leu-Met-NH_2 (**4**)	0.1	42± 4	24± 4	38± 5	175± 53
	0.5	50± 3	28± 3	69±11	299± 65
	1	52± 4	43± 8	99±10	372± 91
	5	57± 7	49±11	114±5	331± 53
H-Lys-Phe-Ile-Gly-MeLeu-Met-NH_2 (**5**)	0.1	24± 3	17± 2	17± 1	35± 7
	0.5	35± 2	20± 4	23± 2	95± 21
	1	36± 2	21± 3	32± 3	113± 20
	5	39±32	31± 5	57± 1	116± 22
	10	47± 4	33± 7	103±16	307±69
H-Lys-Phe-Ile-Gly-MeIle-Met-NH_2 (**6**)	1	0	0	0	0
	5	3± 2	5± 3	6± 4	8± 5
	10	5± 4	6± 4	6± 4	7± 5
	50	13	11	13	18
H-Lys-MePhe-Ile-Gly-MeLeu-Met-NH_2 (**7**)	0.1	1± 1	3± 3	2± 2	3± 3
	0.5	13± 2	13± 2	11± 1	46± 22
	1	21± 3	13± 2	14± 1	37± 7
	5	34± 3	19± 3	35±11	111± 28
	10	35± 1	19± 5	50±14	193± 38

(50 g, 0.11 mol) and **8** (20 g, 0.1 mol) in chloroform (300 ml), DCC[14] (22.7 g, 0.11 mol) was stirred at −5°C. After this mixture had been stirred overnight at room temperature, the dicyclohexylurea and dicyclohexylammonium chloride were filtered off. The filtrate was washed successively with 4% sodium bicarbonate, N hydrochloric acid, and water, and then dried over magnesium sulfate. The filtrate was evaporated *in vacuo* to yield an oil (31 g), which contained the acylurea[9] in some degree. This oil (30 g) was dissolved in a mixture of methanol (100 ml) and 4N sodium hydroxide (30 ml), and the solution was stirred for 20 hr at 0—5°C. After the dicyclohexylurea originating from the acylurea had been filtered off, the methanol was distilled off under reduced pressure below 40°C. The aqueous solution was washed with ether, acidified with N hydrochloric acid, and repeatedly extracted with ether. The combined ether extracts were washed with water and dried over magnesium sulfate. Evaporation gave crude crystals, which were recrystallized from ethyl acetate–petroleum ether; yield, 24 g (60%); mp 115—116°; $[\alpha]_D^{25}$ −61.9° (*c* 1, methanol). Found: C, 65.49; H, 8.47; N, 7.10%. Calcd for $C_{22}H_{34}O_5N_2$: C, 65.00; H, 8.43; N, 6.89%.

*AOC-Phe-MeIle-Gly-Leu-Met-NH_2 (**10**).* To a solution of **9** (8 g, 20 mmol) and triethylamine (3.1 ml) in chloroform (100 ml), ethyl chloroformate (2.4 g, 22 mmol) was added over a 15 min period at −12——10°C. After 15 min, a solution of glycyl-leucyl-methionine amide hydrochloride[15] (8 g, 22 mmol) and triethylamine (3.1 ml) in dimethylformamide (100 ml) was added, after which the solution was stirred overnight at room temperature. Then, it was poured into water (600 ml) and extracted with ethyl acetate. The ethyl acetate solution was washed successively with N hydrochloric acid, 4% sodium bicarbonate, and water, and then dried over magnesium sulfate. The filtrate was evaporated *in vacuo*, and the crude product was recrystallized from ethyl acetate–petroleum ether; yield, 1 g (7.05%); mp 204—206°C; $[\alpha]_D^{25}$ +22.1° (*c* 1, methanol). Found: C, 58.16; H, 8.41; N, 11.45; S, 4.65%. Calcd for $C_{35}H_{58}N_6O_7S\cdot H_2O$: C, 58.01; H, 8.28; N, 11.60; S, 4.42%.

*Di-BOC-Lys-Phe-MeIle-Gly-Leu-Met-NH_2 (**11**).* Compound **10** (400 mg, 0.6 mmol) was dissolved in 3N hydrogen chloride in dioxane (10 ml), and the solution was allowed to stand at room temperature for 30 min. The solvent was then distilled off *in vacuo*, and the residue was treated with ether. The pentapeptide amide hydrochloride thus obtained was dissolved in dimethylformamide (3 ml), neutralized with triethylamine (0.1 ml), and then subjected to a reaction with the di-BOC-lysine *p*-nitrophenyl ester[1] (400

14) J. C. Sheehan and G. P. Hess, *J. Amer. Chem. Soc.*, **77**, 1067 (1955).

15) K. Lübke, E. Schröder, R. Schmiechen, and H. Gibian, *Ann. Chem.*, **679**, 195 (1964).

mg, 0.85 mmol). After standing overnight at room tempetature, the solution was diluted with ethyl acetate (200 ml), washed successively with N hydrochloric acid, 4% sodium bicarbonate, and water, and dried over magnesium sulfate. The solvent was distilled off *in vacuo*, and the residue was precipitated as an amorphous powder from ethyl acetate–petroleum ether; yield, 420 mg (75%); mp 196—197°C; $[\alpha]_D^{25}$ −5.0° (*c* 0.5, methanol). Found: C, 57.98; H, 8.39; N, 11.73; S, 3.35%. Calcd for $C_{45}H_{76}O_{10}H_8S \cdot H_2O$: C, 57.50; H, 8.30; N, 11.92; S, 3.40%.

H–Lys–Phe–MeIle–Gly–Leu–Met–NH₂·2HCl (2) Compound **11** (200 mg) was dissolved in 2N hydrogen chloride in acetic acid (5 ml), and the solution was allowed to react at room temperature for 20 min. The product was precipitated by adding ice-cold dry ether (20 ml), and the precipitate was collected by filtration, washed well with ether, and dried over sodium hydroxide *in vacuo*. The product was dissolved in 60% methanol (4 ml), and an insoluble material was filtered off. The filtrate was concentrated to dryness over phosphorus pentoxide *in vacuo* to obtain the final product; yield, 170 mg; mp 159—164°C; $[\alpha]_D^{25}$ +27.3° (*c* 0.52, water); R_f, 0.79;[16] amino acid ratios in the acid hydrolyzate: Lys, 1.09; Phe, 1.01; Gly, 0.925; Leu, 1.00; Met, 0.99.[17] Found: C, 50.65; H, 7.99; N, 13.38; S, 3.48%. Calcd for $C_{35}H_{60}O_6N_8S \cdot 2HCl \cdot 2H_2O$: C, 50.66; H, 7.96; N, 13.51; S, 3.86%.

H–MePhe–OBzl–Tos–OH (12). A solution of *N*-methyl-L-phenylalanine[7] (18 g, 0.1 mol), *p*-toluenesulfonic acid monohydrate (21 g, 0.1 mol), and benzyl alcohol (100 ml) in benzene (100 ml) was refluxed in a Dean and Stark apparatus[18] for 48 hr. Ether (100 ml) was added to the solution, and it was kept at −20°C for 10 hr. The product, precipitated as crystals, was collected and recrystallized from methanol–ether; yield, 36 g (82%); mp 110—113°C; $[\alpha]_D^{25}$ +1.2° (*c* 1, ethanol). Found: C, 65.49; H, 6.09; N, 3.09; S, 7.21%. Calcd for $C_{24}H_{27}O_5NS$: C, 65.29; H, 6.16; N, 3.17; S, 7.25%.

Di-BOC–Lys–MePhe–OH (13). Into a solution of di-BOC-L-lysine dicyclohexylammonium salt[13] (28 g, 50 mmol) and **12** (22 g, 50 mmol) in chloroform (250 ml), DCC was stirred at −5°C. After it had been allowed to stand overnight, the dicyclohexylurea and dicyclohexylammonium chloride were filtered off. The filtrate was washed successively with 4% sodium bicarbonate, N hydrochloric acid, and water, and then dried over magnesium sulfate. The filtrate was evaporated *in vacuo* to yield an oil (25 g). This oil (25 g) was dissolved in methanol (300 ml) and was subjected to hydrogenolysis in the presence of 5% palladium on charcoal. After the filtration and evaporation of the solvent, the crude material was dissolved in 4% sodium bicarbonate, extracted with ether, acidified with N hydrochloric acid, and extracted into ether. The ether was then removed *in vacuo*, leaving a colorless, clear oil; yield, 20 g (80%); $[\alpha]_D^{25}$ −52.9° (*c* 1.55, methanol).

Di-BOC–Lys–MePhe–Ile–Gly–Leu–Met–OMe (14). Into a solution of **13** (5 g, 10 mmol) and HOBt[8] (1.4 g, 10 mmol) in tetrahydrofuran (50 ml), DCC was stirred (2.1 g, 10 mmol) at −5°C. After stirring for 1 hr at −5—5°C and then for 1 hr at room temperature, a solution of isoleucyl-glycyl-leucyl-methionine methyl ester hydrochloride[15] (4.8 g, 10 mmol) and triethylamine (1.4 ml) in tetrahydrofuran (20 ml) was added. After the mixture had then been allowed to stand overnight at room temperature, the dicyclohexylurea was filtered off. The filtrate was evaporated *in vacuo*, and the remaining oil was dissolved in ethyl acetate (200 ml). The ethyl acetate solution was washed successively with 1% hydrochloric acid, N sodium bicarbonate, and water, and dried over magnesium sulfate. The filtrate was evaporated to dryness under reduced pressure, and the crude product obtained was purified by column chromatography, being eluted with a mixture of ethyl acetate–benzene (1: 1); yield, 7.2 g (75%); $[\alpha]_D^{25}$ − 25.7°(*c* 1, methanol). Found: C, 58.29; H, 8.12; N, 10.25; S, 3.20%. Calcd for $C_{46}H_{77}O_{11}N_7S \cdot H_2O$: C, 57.89; H, 8.34; N, 10.27; S, 3.36%.

Di-BOC–Lys–MePhe–Ile–Gly–Leu–Met–NH₂ (15). a) To a solution of **13** (4 g, 8 mmol) and triethylamine (1.2 ml) in chloroform (100 ml), ethyl chloroformate (0.95 g, 8.8 mmol) was added over a 15 min period at −12—−10°C. After 15 min, a solution of isoleucyl-glycyl-leucyl-methionine amide hydrochloride[15] (3.8 g, 8 mmol) and triethylamine (1.2 ml) in dimethylformamide (20 ml) was added, and the solution was stirred overnight. Then, it was poured into water (200 ml) and extracted with chloroform. The chloroform extract was washed successively with 1% hydrochloric acid, 4% sodium bicarbonate, and water, dried over magnesium sulfate, and evaporated to dryness. The product was recrystallized from ethyl acetate–methanol–petroleum ether to yield ethoxycarbonyl-isoleucyl-glycyl-leucyl-methionine amide; yield, 2.3 g (58%); mp 247—249°C. Found: C, 52.10; H, 8.10; N, 13.65%. Calcd for $C_{22}H_{41}N_5O_6S$: C, 52.47; H, 8.21; N, 13.91%. From the mother liquor, the desired compound, **15**, was obtained; 1.5 g (20%); mp 180—182°C; $[\alpha]_D^{25}$ +14.6° (*c* 1, methanol). Found: C, 57.60; H, 8.28; N, 11.58; S, 3.18%. Calcd for $C_{45}H_{76}O_{10}N_8S \cdot H_2O$: C, 57.50; H, 8.30; N, 11.92; S, 3.40%.

b) A solution of **14** (4.7 g, 5 mmol) in methanol (100 ml) was saturated with dry ammonia gas at 0°C, and the solution was allowed to stand for 24 hr at room temperature. The reaction mixture was then concentrated to dryness under reduced pressure, and the residue was crystallized from ethyl acetate–methanol–petroleum ether; yield, 3.8 g (82%); mp 181—183°C; $[\alpha]_D^{25}$ +14.8° (*c* 1, methanol).

H–Lys–MePhe–Ile–Gly–Leu–Met–NH₂·2HCl (3). This was obtained from **15** (200 mg) by the method described for the preparation of **2**; yield, 180 mg; mp 103—105°C; $[\alpha]_D^{25}$ −19.1° (*c* 0.2, water), R_f, 0.77;[16] amino acid ratios in the acid hydrolyzate; Lys, 1.15; MePhe,1. 10; Ile, 1.08; Gly, 1.15; Leu, 1.00; Met, 0.85. Found: C, 51.06; H, 8.01; N, 13.60; S, 4.08%. Calcd for $C_{35}H_{60}O_6N_8S \cdot 2HCl \cdot 2H_2O$: C, 50.66; H, 7.96; N, 13.51; S, 3.86%.

H–Sar–OBzl·HCl (16). A solution of sarcosine (50 g, 0.6 mol), *p*-toluenesulfonic acid monohydrate (125 g, 0.66 mol) and benzyl alcohol (600 ml) in benzene (600 ml) was treated as described for the preparation of **12**. The crude product precipitated as *p*-toluenesulfonate was dissolved into water (100 ml), and the solution was basified with sodium bicarbonate and extracted with ethyl acetate. The extract was washed with a saturated sodium chloride solution and then dried over magnesium sulfate. To the filtered solution, 3N hydrogen chloride in dioxane (200 ml) was added, and the solution was evaporated *in vacuo* to dryness. The remaining crystal was recrystallized from methanol–ether; yield, 93 g (72%); mp 174—176°C. Found: C, 55.20; H, 6.58; N, 6.56; Cl, 16.46%. Calcd for $C_{10}H_{13}NO_2 \cdot HCl$: C, 55.68; H, 6.49; N, 6.49; Cl, 16.47%.

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

17) MeIle was present but not determined. Its presence was proved by paper chromatography of the acid hydrolysate [*n*-butanol–acetic acid–water (4: 1: 1) system] in which MeIle was perfectly separated from the other slower moving amino acids.

18) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John Wiley & Sons, Inc., New York and London (1961), p. 940.

BOC-Ile-Sar-OH (17). This was obtained from BOC-isoleucine[13] and **16** (21 g, 100 mmol) as described for the preparation of **13**; yield of oil, 19.2 g (64%); $[\alpha]_D^{25}$ −31.0° (*c* 1.3, methanol).

BOC-Ile-Sar-Leu-Met-OMe (18). This was obtained from **17** (9.1 g, 30 mmol), HObt (4 g, 30 mmol), DCC (6.8 g, 33 mmol), and leucyl-methionine methyl ester hydrochloride[15] (9.5 g, 30 mmol) as described for the preparation of **14**. The crude product was purified by column chromatography on silica-gel, being eluted with ethyl acetate; yield of oil, 15.5 g (92%); $[\alpha]_D^{25}$ −51.3° (*c* 1.25, methanol).

BOC-Ile-Sar-Leu-Met-NH_2 (19). A solution of **18** (11.2 g, 20 mmol) in methanol (300 ml) was saturated with dry ammonia gas at 0°C, after which the solution was allowed to stand for 40 hr at room temperature. The reaction mixture was concentrated to dryness under reduced pressure, and the residual crystal was recrystallized from ethyl acetate–petroleum ether; yield, 9 g (80%); mp 188—191°C; $[\alpha]_D^{25}$ −55.0° (*c* 1, methanol). Found: C, 55.08; H, 8.44; N, 12.77; S, 6.06%. Calcd for $C_{25}H_{47}O_6N_5S$: C, 55.04; H, 8.62; N, 12.84; S, 5.87%.

BOC-Phe-Ile-Sar-Leu-Met-NH_2 (20). Compound **19** (5.5 g, 10 mmol) was dissolved in 3N hydrogen chloride in dioxane (100 ml), and the solution was allowed to stand at room temperature for 40 min. The solvent was then distilled off *in vacuo*, and the residue was triturated with ether. The tetrapeptide amide hydrochloride thus obtained was dissolved in dimethylformamide (40 ml), neutralized with triethylamine (1.6 ml), and then subjected to a reaction with the BOC-phenylalanine *p*-nitrophenyl ester (5.2 g, 13 mmol). The solution was treated as described for the preparation of **11**, and the crude product was recrystallized from ethyl acetate–methanol–petroleum ether; yield, 4.9 g (90%); mp 162—167°C; $[\alpha]_D^{25}$ −54.1° (*c* 1, methanol). Found: C, 58.47; H, 7.70; N, 11.85; S, 4.35%. Calcd for $C_{34}H_{56}O_7N_6S$: C, 58.95; H, 8.09; N, 12.13; S, 4.62%.

Di-BOC-Lys-Ile-Sar-Leu-Met-NH_2 (21). This was obtained from the di-BOC-lysine *p*-nitrophenyl ester (700 mg, 1.5 mmol) and the pentapeptide amide derived from compound **20** (1 g, 1.4 mmol) as described for the preparation of **11**; yield, 825 mg (63%); mp 154—160°C; $[\alpha]_D^{25}$ −57.3° (*c* 1, methanol). Found: C, 57.46; H, 8.30; N, 11.59; S, 3.26%. Calcd for $C_{45}H_{76}O_{10}N_8S\cdot H_2O$: C, 57.50; H, 8.30; N, 11.92; S, 3.40%.

H-Lys-Phe-Ile-Sar-Leu-Met-$NH_2\cdot 2HCl$ (4). This was obtained from **21** (200 mg) as described for the preparation of **2**; yield, 170 mg; mp 80—90°C; $[\alpha]_D^{25}$ −33.8° (*c* 0.5, water); R_f, 0.73;[16] amino acid ratios in the acid hydrolyzate; Lys, 1.07; Phe, 1.05; Ile, 1.05; Sar, 1.15; Leu, 1.00; Met, 0.94. Found: C, 51.64; H, 7.79; N, 13.62; S, 3.83%. Calcd for $C_{35}H_{60}O_6N_8S\cdot 2HCl\cdot H_2O$: C, 51.78; H, 7.89; N, 13.81; S, 3.94%.

H-MeLeu-OMe·HCl (22). This was obtained from *N*-methyl-L-leucine[7] as described for the preparation of **8**; yield (77%); mp 126.5—127°C; $[\alpha]_D^{25}$ +31.4° (*c* 1, ethanol) (lit,[12] mp 127—128°C; $[\alpha]_D^{25}$ +30° (ethanol)). (Found: C, 49.32; H, 9.21; N, 7.19; Cl, 18.17%).

BOC-Gly-MeLeu-OH (23a). Into a solution of BOC-glycine[13] (17.5 g, 100 mmol) **22** (20 g, 100 mmol), and triethylamine (14 ml) in chloroform (300 ml), DCC was stirred at −5°C. After the mixture had been allowed to stand overnight at room temperature, the dicyclohexylurea was filtered off and the filtrare was treated as described for the preparation of **9**. The crude product was recrystallized from ether–*n*-hexane; yield, 10.6 g (35%); mp 93—95°C; $[\alpha]_D^{25}$ −24.6° (*c* 1, methanol). Found: C, 55.46; H, 8.82; N, 9.18%. Calcd for $C_{14}H_{26}O_5N_2$: C, 55.61; H, 8.67; N, 9.27%.

BOC-Gly-MeIle-OH (23b). This was obtained from BOC-glycine (17.5 g, 100 mmol) and **8** (20 g, 100 mmol) as described above. The crude product was recrystallized from ethyl acetate–petroleum ether; yield, 10.2 g (33.8%); mp 139.5—140°C; $[\alpha]_D^{25}$ −1.1° (*c* 2, methanol). Found: C, 55.76; H, 8.53; N, 9.41%. Calcd for $C_{14}H_{26}O_5N_2$: C, 55.61; H, 8.67; N, 9.27%.

BOC-Gly-MeLeu-Met-OMe (24a). This compound was obtained from **23a** (20 g, 67 mmol), HObt (9.1 g, 67 mmol), DCC (16 g, 80 mmol), methionine methyl ester hydrochloride (14 g, 70 mmol), and triethylamine (9.8 ml, 70 mmol) as described for the preparation of **14**. The crude oil was purified by column chromatography on silica gel being eluted with a mixture of benzene and ethyl acetate (7: 3); yield of oil, 27 g (90%); $[\alpha]_D^{25}$ −9.2° (*c* 1, methanol).

BOC-Gly-MeIle-Met-OMe (24b). This was obtained from **23b** (9 g, 30 mmol), HObt (4 g, 30 mmol), DCC (6.6 g, 33 mmol), methionine methyl ester hydrochloride (6.6 g, 33 mmol), and triethylamine (4.6 ml, 33 mmol) as described above; yield of oil, 11.6 g, (87%); $[\alpha]_D^{25}$ +11.0° (*c* 1, methanol).

BOC-Gly-MeLeu-Met-NH_2 (25a). A solution of **24a** (16.5 g, 37 mmol) in methanol (300 ml) was saturated with dry ammonia gas at 0°C, and the solution was treated as described for the preparation of **19**; yield of oil, 13.2 g (84%); $[\alpha]_D^{25}$ −6.2° (*c* 1, methanol).

BOC-Gly-MeIle-Met-NH_2 (25b). This was obtained from **24b** (4.5 g, 10 mmol) as described for the preparation of **19**; yield of oil, 3.6 g (80%); $[\alpha]_D^{25}$ −17.8° (*c* 1, methanol).

AOC-Phe-Ile-Gly-MeLeu-Met-NH_2 (26a). Into a solution of *t*-amyloxycarbonyl-phenylalanyl-isoleucine hydrazide[1] (4 g, 10 mmol) in a mixture of 2N hydrochloric acid (20 ml, 40 mmol) and dimethylformamide (200 ml), a chilled solution of sodium nitrite (700 mg, 10 mmol) in water (1 ml) was stirred over a 15 min period at −15——13°C. After 20 min, the solution was neutralized with triethylamine; then a solution of triethylamine (1.4 ml) and the glycyl-*N*-methylleucyl-methionine amide hydrochloride obtained from **25a** (4.3 g, 10 mmol) in dimethylformamide (20 ml) was added at −10——5°C. After the reaction mixture had been stirred at room temperature for 3 hr, it was poured into water (1000 ml) and extracted with ethyl acetate. The extract was washed successively with N hydrochloric acid, 4% sodium bicarbonate, and water, dried over magnesium sulfate, and evaporated *in vacuo*. The oily residue (6.6 g) was purified by column chromatography on silica gel (Merck 0.2—0.5 mm) being eluted with a mixture of ethyl acetate and methanol (8: 2); yield of oil, 5.8 g (80%); $[\alpha]_D^{25}$ −23.9° (*c* 1, methanol). Found: C, 58.33; H, 8.14; N, 11.62; S, 4.24%. Calcd for $C_{35}H_{58}O_7N_6S\cdot H_2O$: C, 58.01; H, 8.28; N, 11.60; S, 4.42%.

AOC-Phe-Ile-Gly-MeIle-Met-NH_2 (26b). To a solution of the azide derived from *t*-amyloxycarbonyl-phenylalanyl-isoleucine hydrazide (4 g, 10 mmol) in dimethylformamide (200 ml), a solution of the glycyl-*N*-methylisoleucyl-methionine amide hydrochloride obtained from **25b** (4.3 g, 10 mmol) and triethylamine (1.4 ml) in dimethylformamide (20 ml) was added. The crude product was obtained as described above and was purified by column chromatography on silica-gel, being eluted with a mixture of ethyl acetate and methanol; yield of oil, 5.9 g (82%); $[\alpha]_D^{25}$ −15.8° (*c* 1, methanol). Found: C, 58.38; H, 8.25; N, 11.40; S, 4.23%. Calcd for $C_{35}H_{58}O_7N_6S\cdot H_2O$: C, 58.01; H, 8.28; N, 11.60; S, 4.42%.

Di-BOC-Lys-Phe-Ile-Gly-MeLeu-Met-NH_2 (27a). This was obtained from the di-BOC-lysine *p*-nitrophenyl ester (2.3 g, 5 mmol) and the pentapeptide amide derived from compound **26a** (3 g, 4.4 mmol) as described for the preparation of **11**. The crude product was purified by column chromatography on silica-gel, being eluted first with ethyl

acetate and then with a mixture of ethyl acetate and methanol (8: 2); yield of oil, 3 g (72%); $[\alpha]_D^{25}$ −26.5° (*c* 1, methanol). Found: C, 57.82; H, 8.18; N, 11.73; S, 3.18%. Calcd for $C_{45}H_{76}O_{10}N_8S \cdot H_2O$: C, 57.50; H, 8.30; N, 11.92; S, 3.40%.

Di-BOC-Lys-Phe-Ile-Gly-MeIle-Met-NH$_2$ (27b). This was obtained from the di-BOC-lysine *p*-nitrophenyl ester (1.9 g, 4 mmol) and the pentapeptide amide derived from compound **26b** (2.7 g, 3.8 mmol). The crude product was dissolved in ethyl acetate, and the product was precipitated gradually as crystals; yield, 2.1 g (61%); mp 212—213°C; $[\alpha]_D^{25}$ +8.3° (*c* 1, methanol). Found: C, 57.97; H, 8.18; N, 11.90; S, 3.36%. Calcd for $C_{45}H_{76}O_{10}N_8S \cdot H_2O$: C, 57.50; H, 8.30; N, 11.92; S, 3.40%.

H-Lys-Phe-Ile-Gly-MeLeu-Met-NH$_2$·2HCl (5). This was obtained from **27a** (200 mg) as described for the preparation of **2**; yield, 180 mg; mp 107—112°C; $[\alpha]_D^{25}$ −7.3° (*c* 0.3, water); R_f, 0.70;[16] amino acid ratios in the acid hydrolyzate: Lys, 1.14; Phe, 1.05; Ile, 1.00; Gly, 1.05; MeLeu, 1.02; Met, 0.99. Found: C, 50.42; H, 7.94; N, 13.65; S, 3.48%. Calcd for $C_{35}H_{60}O_6N_8S \cdot 2HCl \cdot 2H_2O$: C, 50.66; H, 7.96; N, 13.51; S, 3.86%.

H-Lys-Phe-Ile-Gly-MeIle-Met-NH$_2$·2HCl (6). This was obtained from **27b** (200 mg) as described for the preparation of **2**; yield, 170 mg, mp 85—100°C; $[\alpha]_D^{25}$ +34.0° (*c* 0.2, water); R_f, 0.70;[16] amino acid ratios in the acid hydrolyzate: Lys, 0.92; Phe, 0.99; Ile, 1.00; Gly, 0.97; Met, 0.98.[17] Found: C, 50.95; H, 8.11; N, 13.46; S, 3.93%. Calcd for $C_{35}H_{60}O_6N_8S \cdot 2HCl \cdot 2H_2O$: C, 50.66; H, 7.96; N, 13.51; S, 3.86%.

BOC-Ile-Gly-MeLeu-Met-OMe (28). Into a solution of BOC-isoleucine (2.3 g, 10 mmol) and triethylamine (1.4 ml) in chloroform (50 ml), ethyl chloroformate (1.1 g, 10 mmol) was stirred over a 15 min period at −11—−10°C. After 15 min, there was added a solution of triethylamine (1.4 ml) and the glycyl-*N*-methylleucyl-methionine methyl ester hydrochloride derived from **23a** (4.5 g, 10 mmol) in chloroform (30 ml), the reaction mixture was then stirred at room temperature overnight. It was washed successively with N hydrochloride acid, 4% sodium bicarbonate, and water, and dried over magnesium sulfate. The filtered solution was evaporated to dryness under reduced pressure to yield the product; yield of oil, 4.9 g (88%); $[\alpha]_D^{25}$ −23.0° (*c* 1, methanol).

Di-BOC-Lys-MePhe-Ile-Gly-MeLeu-Met-OMe (29). This was obtained from **13** (7.6 g, 15 mmol), HOBt (2 g, 15 mmol), DCC (3.5 g, 17 mmol), and the isoleucyl-glycyl-*N*-methylleucyl-methionine methyl ester derived from **28** (8.2 g, 15 mmol) as described for the preparation of **14**. The crude oil was purified by column chromatography on silica-gel, being eluted with ethyl acetate; yield of oil, 10.6 g (77%); $[\alpha]_D^{25}$ −30.8° (*c* 1, methanol). Found: C, 58.01; H, 8.32; N, 10.33; S, 3.51%. Calcd for $C_{47}H_{79}O_{11}N_7S \cdot H_2O$: C, 58.32; H, 8.37; N, 10.13; S, 3.30%.

Di-BOC-Lys-MePhe-Ile-Gly-MeLeu-Met-NH$_2$ (30). A solution of compound **29** (9.7 g, 10 mmol) in methanol was saturated with ammonia gas at 0°C, after which the solution was allowed to stand for 24 hr at room temperature. The reaction mixture was then concentrated to dryness under reduced pressure, and the residue was dissolved in ethyl acetate and chromatographed on silica-gel, being eluted first with ethyl acetate and then with a mixture of ethyl acetate and methanol (8: 2); yield of oil, 7.7 g (81%); $[\alpha]_D^{25}$ −30.2° (*c* 1, methanol). Found: C, 57.56; H, 8.25; N, 11.28; S, 3.27%. Calcd for $C_{46}H_{78}O_{10}N_8S \cdot H_2O$: C, 57.98; H, 8.40; N, 11.76; S, 3.36%.

H-Lys-MePhe-Ile-Gly-MeLeu-Met-NH$_2$·2HCl (7). This was obtained from **30** (200 mg) as described for the preparation of **2**; yield, 180 mg; mp 109—115°C; $[\alpha]_D^{25}$ −22.7° (*c* 0.1, water); R_f, 0.74;[16] amino acid ratios in the acid hydrolyzate: Lys, 0.89; MePhe, 0.93; Ile, 1.00; Gly, 0.95; MeLeu, 1.06; Met, 0.92. Found: C, 51.02; H, 8.09; N, 13.26; S, 3.42%. Calcd for $C_{36}H_{62}O_6N_8S \cdot 2H_2O$: C, 51.24; H, 8.06; N, 13.28; S, 3.79%.

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