Synthesis of Corticotropin Peptides. XIII. The Synthesis of a Hexacosapeptide and a Heptacosapeptide Corresponding to the First Twenty-six and Twenty-seven Amino Acid Residues of Corticotropin (ACTH)*

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The syntheses are described of a hexacosapeptide H–Ser–Tyr–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys–Pro–Val–Gly–Lys–Lys–Arg–Arg–Pro–Val–Lys–Val–Tyr–Pro–Asn–Gly–OH (I) and a heptacosapeptide H–Ser–Tyr–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys–Pro–Val–Gly–Lys–Lys–Arg–Arg–Pro–Val–Lys–Val–Tyr–Pro–Asn–Gly–Ala–OH (II) corresponding to the first twenty-six and twenty-seven amino acid residues, respectively, of corticotropin (ACTH), in which a new protecting group 1-methylcyclohexyloxycarbonyl (Mhoc) is utilized for the temporary protection of the ε-amino function of lysines. The *in vivo* steroidogenic potencies of the synthetic peptides I and II are compared on a molar basis with those of ACTH(1—18)-NH_2 and synthetic human ACTH (α_h-ACTH) to demonstrate that the relative potencies of I and II *vs.* ACTH(1—18)-NH_2 are 2.3 and 2.3—3.8, respectively, and those *vs.* α_h-ACTH are 1.1 and 1.1—1.8, respectively.

The primary structure of porcine corticotropin (ACTH) and that of human ACTH were first proposed by Shepherd *et al.*[1] in 1956 and by Lee *et al.*[2] in 1961, respectively. The proposed structure of porcine ACTH was synthesized by Schwyzer and Sieber[3] in 1963 and that of human ACTH by Bajusz *et al.* in 1967.[4]

In a previous communication we reported the synthesis of a heptacosapeptide corresponding to the first twenty-seven amino acid residues of human ACTH.[5] In 1971, however, Gráf *et al.*[6] followed by Riniker *et al.*[7] revised the amino acid sequences of porcine and human ACTH's. In addition, the structures of bovine and ovine ACTH's have also been revised recently not only to establish the identity between these two mammalian hormones,[8,9] but also to show that the only structural differences among the human, porcine, bovine and ovine hormones are confined to the two positions 31 and 33. Thus, we have carried out the synthesis of ACTH(1—27)-OH according to the revised amino acid sequence, H–Ser–Tyr–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys–Pro–Val–Gly–Lys–Lys–Arg–Arg–Pro–Val–Lys–Val–Tyr–Pro–Asn–Gly–Ala–OH (II), which differs from the one previously proposed for the human hormone[2] in positions 25 through 27, Asn–Gly–Ala instead of Asp–Ala–Gly. In parallel with peptide II, a hexacosapeptide, H–Ser–Tyr–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys–Pro–Val–Gly–Lys–Lys–Arg–Arg–Pro–Val–Lys–Val–Tyr–Pro–Asn–Gly–OH, ACTH(1—26)-OH (I), has also been synthesized. For their size peptides I and II may be placed halfway between the nonatriacontapeptide of natural ACTH and the octadecapeptide ACTH(1—18)-NH_2 which is the smallest peptide endowed with all the structural features required for the manifestation of steroidogenic activity.[10] Comparison of I or II with these peptides for the hormonal action will, therefore, provide data important for evaluating the role of the C-terminal half of the ACTH molecule. This paper describes the details of the synthesis of

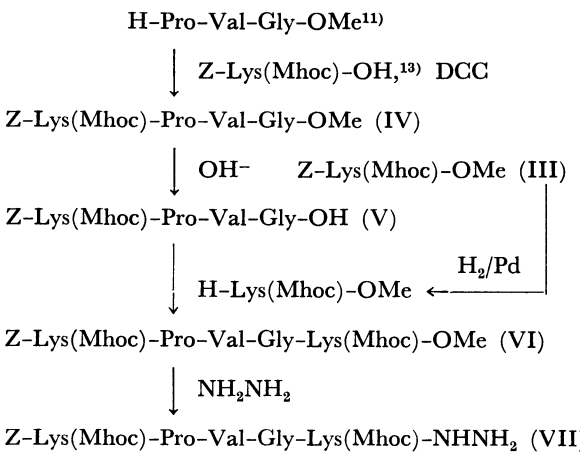

Fig. 1. Synthesis of amino acid sequence 11—15 of ACTH. Mhoc: 1-methylcyclohexyloxycarbonyl.

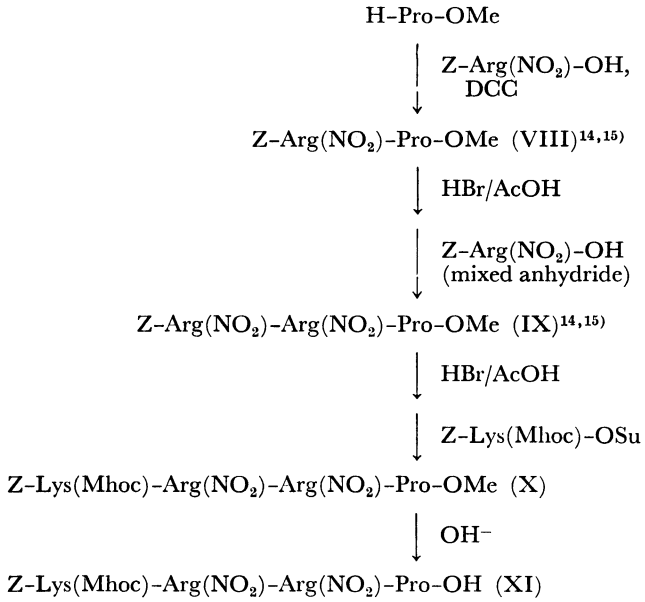

Fig. 2. Synthesis of amino acid sequence 16—19 of ACTH. Mhoc: 1-methylcyclohexyloxycarbonyl.

* All the amino acid residues mentioned in this communication are of the L-configuration. Abbreviations used are those recommended by the IUPAC-IUB Commission of Biochemical Nomenclature [*Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972)] and include Mhoc: 1-methylcyclohexyloxycarbonyl.

peptides I and II.

The pentapeptide derivative (VII) corresponding to positions 11—15 of the ACTH molecule was synthesized according to the procedure illustrated in Fig. 1. This procedure is much the same as that employed for the synthesis of Z-Lys(Boc)-Pro-Val-Gly-Lys-(Boc)-OMe (and $-NHNH_2$)[11,12] except that the ε-amino function of lysines was blocked by the 1-methylcyclohexyloxycarbonyl (Mhoc) groups. The Mhoc group is cleavable by acidolysis in almost the same rate as the Boc group. However, a highly lipophilic nature of the new protecting group would increase the solubility of protected peptides in organic solvents, thus facilitating their chromatographic purification remarkably. A full account of the Mhoc and other new protecting groups will be given elsewhere.[13]

The amino acid sequence 16—19 was synthesized in the form of Z-Lys(Mhoc)-Arg(NO_2)-Arg(NO_2)-Pro-OH (XI) as shown in Figure 2. The two arginines were successively incorporated by the dicyclohexylcarbodiimide (DCC) method and by the mixed anhydride procedure to give IX.[14,15] Removal of the Z group with hydrogen bromide in acetic acid yielded the N^{α}-free tripeptide which was coupled with the *N*-hydroxysuccinimide (HOSu) ester of Z-Lys(Mhoc)-OH to produce X. Saponification of ester X gave the corresponding acid (XI). An alternative synthesis of XI has recently been accomplished by the use of H-Arg(NO_2)-Arg(NO_2)-Pro-OH, derived from Z-Arg(NO_2)-Arg(NO_2)-Pro-OBzl with hydrogen bromide

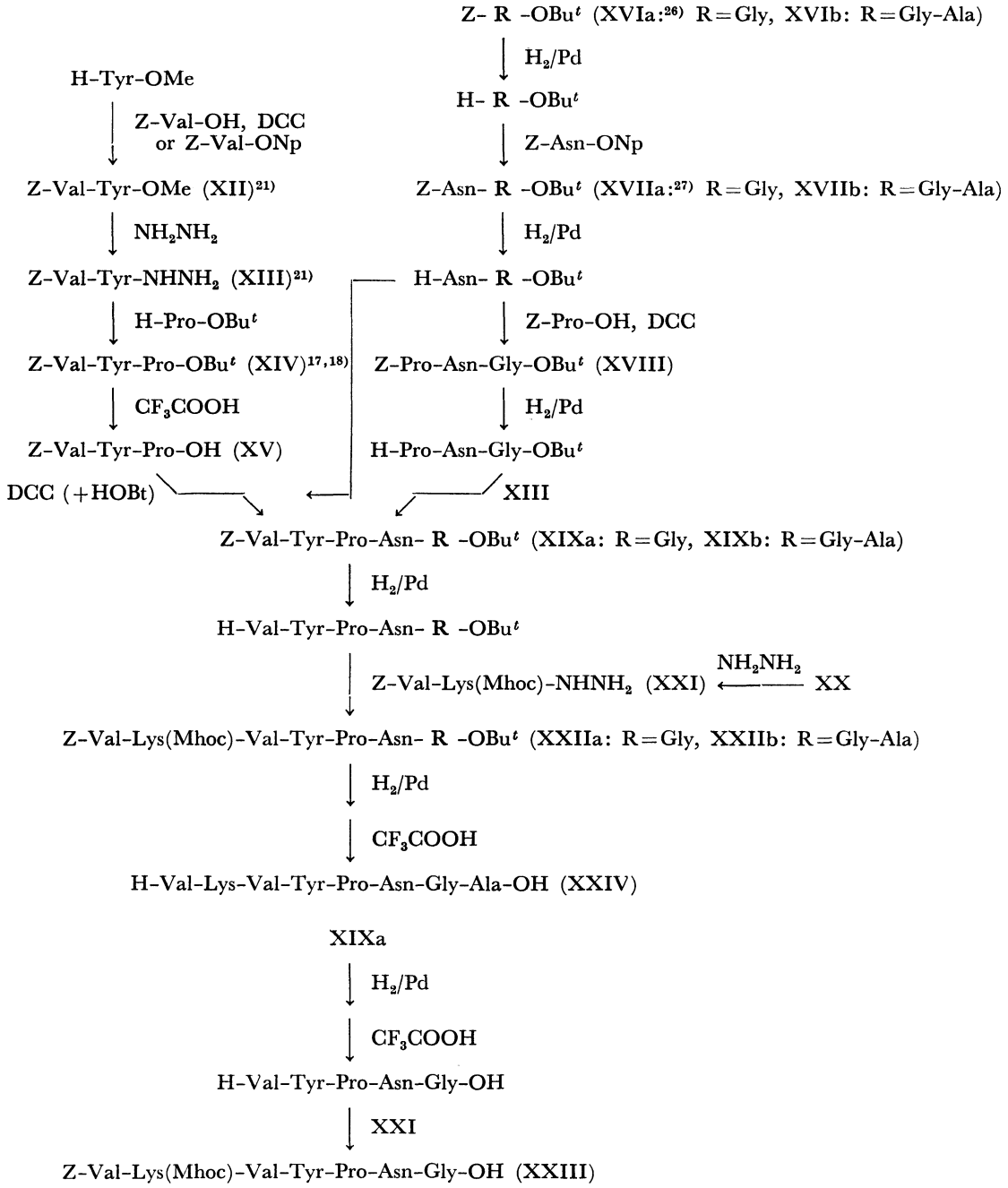

Fig. 3. Snythesis of amino acid sequences 20—26 and 20—27 of ACTH.
Mhoc: 1-methylcyclohexyloxycarbonyl.

in acetic acid, as intermediate.[16] The optical rotation of the product was identical with that of the one derived from the methyl ester. This provides a proof for the optical purity of XI.

The synthetic routes to the amino acid sequences 20—26 and 20—27 are summarized in Fig. 3. The tripeptide derivative XV corresponding to positions 22—24 was derived from the *t*-butyl ester (XIV)[17,18] by the treatment with trifluoroacetic acid. Compound XV could not be obtained from Z–Val–Tyr–Pro–OMe without any danger of racemization, because the methyl ester was remarkably resistant to the action of alkali. In contrast to the methyl ester the corresponding benzyl ester may be used as an intermediate to XV, since Ramachandran and Li have reported that Z–Val–Lys(Tos)–Val–Tyr–Pro–OBzl can be saponified to the corresponding acid in an 84% yield.[19] Coupling of XV with H–Asn–Gly–OBu^t and H–Asn–Gly–Ala–OBu^t by means of the DCC method in the presence of 1-hydroxybenzotriazole (HOBt)[20] gave crystalline compounds XIXa and XIXb, respectively, in moderate yields. Compound XIXa was also obtained by the reaction of Z–Val–Tyr–N_3, derived from the preceding hydrazide (XIII)[21] by the treatment with nitrous acid, with a crystalline tripeptide H–Pro–Asn–Gly–OBu^t (free base) in a comparable yield. Catalytic hydrogenolysis of XIXa and XIXb yielded the N^α-free penta- and hexapeptides, which were then acylated with Z–Val–Lys(Mhoc)–N_3, derived from the hydrazide (XXI) with nitrous acid in the usual manner, to give crystalline derivatives XXIIa and XXIIb, respectively, in excellent yields.

A portion of XXIIb was fully deblocked by catalytic hydrogenolysis and subsequent treatment with trifluoroacetic acid to afford an octapeptide (XXIV) corresponding to ACTH(20—27). The acid hydrolysate of XXIV was found to contain the constituent amino acids (and ammonia derived from Asn) in the ratios predicted by theory. Compound XIXa was deprotected in the same manner as above and the resulting pentapeptide was acylated with Z–Val–Lys(Mhoc)–N_3 to give XXIII in a crystalline form. Compound XXIII may be used as an intermediate for the synthesis of peptides related to the C-terminal half of the ACTH molecule.

The fragment peptides obtained above were assembled as illustrated in Fig. 4, leading to the formation of a hexadecapeptide and a heptadecapeptide corresponding to ACTH(11—26) and ACTH(11—27), respectively. The N^α-free peptides, derived from XXIIa and XXIIb by catalytic hydrogenolysis, were coupled with XI by the DCC method in the presence of HOBt[20] to yield XXVa and XXVb, respectively, both of which were purified on silica gel columns with chloroform–methanol systems as solvent. For removal of the N^α-benzyloxycarbonyl (Z) group and nitro groups, XXVa and XXVb were hydrogenolyzed; the completion of reaction was checked by TLC and by the disappearance of absorption at 275 nm characteristic of the nitro group. The resulting partially protected peptides XXVIa and XXVIb were then allowed to react with the azide, derived from hydrazide VII with nitrous acid in the usual manner, to give a hexadecapeptide (XXVIIIa) and a heptadecapeptide (XXVIIIb) corresponding to ACTH(11—26) and ACTH(11—27), respectively. They were obtained in moderate yields after purification by silica gel column chromatography with chloroform–methanol systems as solvent. The acid hydrolysates of XXVIIIa and XXVIIIb were found to contain the constituent amino acids in the ratios expected by theory.

A portion of XXVIa was treated with trifluoroacetic acid in the presence of anisole and 2-mercaptoethanol as scavengers to liberate a free undecapeptide XXVIIa, that is ACTH(16—26). In the same manner a free dodecapeptide (XXVIIb) corresponding to ACTH(16—27) was derived from XXVIb. Both XXVIIa and XXVIIb, purified on a carboxymethyl cellulose column, behaved as a single component in TLC.

The final coupling process for connecting the N-terminal decapeptide with the C-terminal hexadecapeptide and the subsequent deprotection to lead to

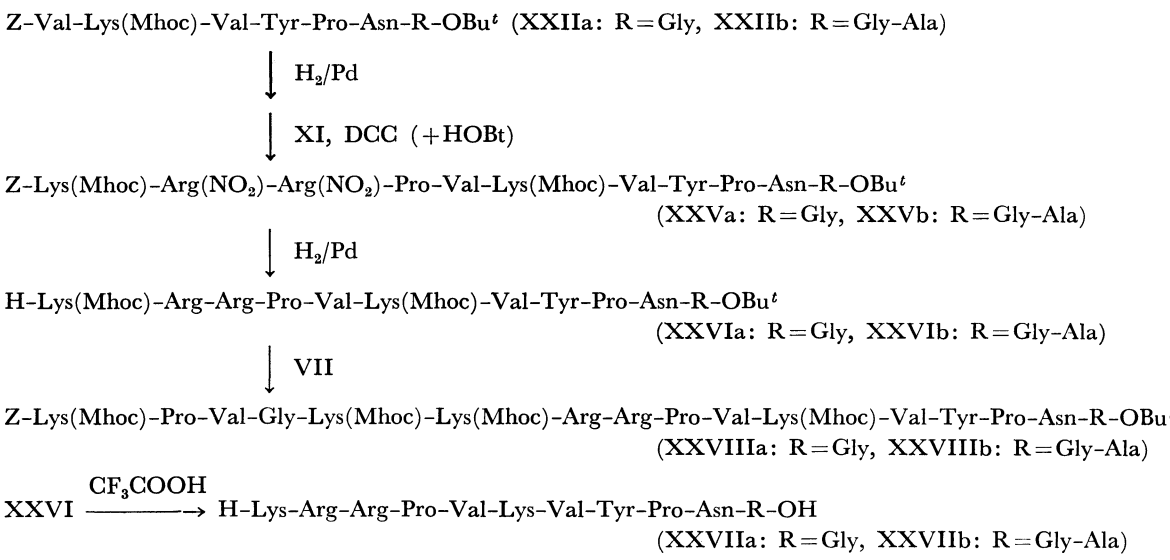

Fig. 4. Synthesis of amino acid sequences 11—26 and 11—27 of ACTH.
Mhoc: 1-methylcyclohexyloxycarbonyl.

the production of a hexacosapeptide, ACTH(1—26)-OH (I), were performed as follows. Boc-Ser-Tyr-Ser-Met-Glu(OBu^t)-His-Phe-Arg(H^+)-Trp-Gly-O$^-$ [22] was converted into the hydrochloride Boc-Ser-Tyr-Ser-Met-Glu(OBu^t)-His-Phe-Arg(HCl)-Trp-Gly-OH and this was esterified with HOSu by the DCC method. The resulting active ester hydrochloride was coupled with the N^{α}-free hexadecapeptide, derived from XXVIIIa by catalytic hydrogenolysis, to give the protected hexacosapeptide, Boc-Ser-Tyr-Ser-Met-Glu(OBu^t)-His-Phe-Arg-Trp-Gly-Lys(Mhoc)-Pro-Val-Gly-Lys(Mhoc)-Lys(Mhoc)-Arg-Arg-Pro-Val-Lys(Mhoc)-Val-Tyr-Pro-Asn-Gly-OBu^t. Finally, this was submitted for deprotection to the trifluoroacetic acid treatment in the presence of anisole and 1,2-ethanedithiol as scavengers to liberate hexacosapeptide I as the trifluoroacetate, which was converted into the acetate by passing through a column of Amberlite CG-400 (acetate form). The heptacosapeptide ACTH(1—27)-OH (II) was obtained in exactly the same manner as described above except that the N^{α}-free heptadecapeptide, derived from XXVIIIb by catalytic hydrogenolysis, was used as the amine component in the coupling reaction. The deblocked peptides were then purified by partition chromatography on a column of Sephadex G-25 with 1-butanol–acetic acid–pyridine–water (12 : 3 : 4 : 6) as solvent[10b] and by carboxymethyl cellulose column chromatography in an ammonium acetate buffer. The overall yields of peptide I and peptide II for the final coupling, deprotection and purification processes were 26% and 27%, respectively. These preparations were found to be homogeneous in TLC, and their acid hydrolysates contained the constituent amino acids in the ratios expected by theory except for tryptophan. The Tyr/Trp ratio was 2.0 for both peptides, as determined spectrophotometrically.[23]

The synthetic peptides thus obtained were assayed for the *in vivo* steroidogenic activity by a method based on the elevated levels of 11-hydroxycorticosteroids in the adrenal venous blood of the hypophysectomized rat in response to intravenous administration of the peptide. The results are shown in Table 1,[24] in which the potencies of ACTH(1—26)-OH (I) and ACTH(1—27)-OH (II) are compared with those of ACTH(1—18)-NH_2[10b] and the synthetic human hormone (α_h-ACTH).[25] The *in vivo* steroidogenic potencies of I and II appear to be comparable, although the heptacosapeptide is rather variable and tends to give potencies somewhat higher than that of the hexacosapeptide. When compared on a molar basis, peptide I and peptide II are more than twice as active as the octadecapeptide and equal to, or even more active than, the intact hormone; the relative potencies of I and II *vs.* ACTH(1—18)-NH_2 are 2.3 and 2.3—3.8, respectively, and those *vs.* α_h-ACTH are 1.1 and 1.1—1.8, respectively, as calculated from Table 1. More of the comparative studies including a detailed examination of the time-action relations are obviously needed to evaluate the effect of chain length on biological activity. Such studies are now in progress.

Table 1. Steroidogenic activity of snythetic peptides[24]

Compound	Molecular weight[a]	*In vivo* steroidogenic activity	
		units/mg[b]	units/μmol
ACTH(1—18)-NH_2[10b]	2249	96.7	217
ACTH(1—26)-OH (I)	3105	160	497
ACTH(1—27)-OH (II)	3176	155—259	492—823
Synthetic α_h-ACTH[25]	4541	98.6	448

a) As anhydrous free base. b) The Third USP Corticotropin Reference Standard was used as reference.

Experimental

Thin layer chromatography was (TLC) performed on "silica gel" plates [Kieselgel GF_{254} or Kieselgel $60F_{254}$ (precoated), Merck] or precoated "cellulose" plates (Cellulose F, Merck), and the following solvent systems were employed: A, benzene-ethyl acetate (7 : 3); B, ethyl acetate-methanol (8 : 2); C, chloroform-methanol (9 : 1); D, chloroform-methanol (85 : 15); E, chloroform-methanol (8 : 2); F, chloroform-methanol-acetic acid (95 : 5 : 3); G, chloroform-methanol-acetic acid (90 : 10 : 3); H, chloroform-methanol-acetic acid (8 : 1 : 1); I, chloroform-methanol-acetic acid (80 : 20 : 3); J, 1-butanol-acetic acid-water (4 : 1 : 2); K, 1-butanol-acetic acid-pyridine-water (15 ; 3 : 10 : 12); L, 1-butanol-acetic acid-pyridine-water (15 : 3 : 10 : 15).

Z-Lys(Mhoc)-OMe (III). Z-Lys(Mhoc)-OH, derived from the dicyclohexylamine (DCHA) salt (12.04 g, 20 mmol)[13] by the treatment with Dowex 50W×8 (H^+ form) in 60% ethanol in the usual manner, was esterified with diazomethane to give III as amorphous solid; wt 8.38 g (96%), $[\alpha]_D^{23}$ −9.6± 0.3° (*c* 2.0, acetone). TLC (silica gel): a single component (ninhydrin, after pretreatment with hydrobromic acid) in system A and system B.

Found: C, 63.60; H, 8.07; N, 6.56%. Calcd for $C_{23}H_{34}N_2O_6$: C, 63.57; H, 7.89; N, 6.54%.

Z-Lys(Mhoc)-Pro-Val-Gly-OMe (IV). Z-Lys-(Mhoc)-OH, derived from the DCHA salt (15.05 g, 25 mmol)[13] in the usual manner, and H-Pro-Val-Gly-OMe (7.13 g, 25 mmol)[11] were coupled with DCC (5.16 g, 25 mmol) in dichloromethane at 4 °C overnight. The reaction mixture was worked up in the usual manner to give the desired tetrapeptide, which was separated as gelatinous precipitates from ether; wt 11.0 g (64%), mp 71—73 °C, $[\alpha]_D^{23}$ −73.1±1.1° (*c* 1.0, methanol). TLC (silica gel): a single component (sulfuric acid) in system C and system F.

Found: C, 60.88; H, 7.70; N, 10.11%. Calcd for $C_{35}H_{53}N_5O_9$: C, 61.12; H, 7.77; N, 10.18%.

Z-Lys(Mhoc)-Pro-Val-Gly-OH (V). Compound IV (1.38 g, 2 mmol) in methanol (5 ml) was saponified with 2 M sodium hydroxide (2 ml) at 0 °C for 60 min to give V as amorphous solid; wt 1.26 g (92%), $[\alpha]_D^{23}$ −71.1±1.1° (*c* 1.0, methanol). TLC (silica gel): a single component (sulfuric acid) in system G.

Found: C, 59.97; H, 7.84; N, 9.92%. Calcd for $C_{34}H_{51}N_5O_9 \cdot 1/2H_2O$: C, 59.81; H, 7.68; N, 10.26%.

Z-Lys(Mhoc)-Pro-Val-Gly-Lys(Mhoc)-OMe (VI). Compound III (5.21 g, 12 mmol) was hydrogenolyzed over palladium in methanol in the presence of acetic acid (1 ml) for 2 h to give the acetic acid salt of H-Lys(Mhoc)-OMe as a sirupy residue. This was then treated with 50% potassium carbonate at 0 °C in the presence of dichloromethane. The resulting free base and V (6.74 g, 10 mmol) were coupled

with dicyclohexylcarbodiimide (DCC, 2.48 g, 12 mmol) in dichloromethane at 4 °C for 20 h to give VI, which was separated as gelatinous precipitates from ether. Reprecipitation from ethyl acetate-ether afforded the pure material; wt 7.51 g (79%), mp 84—85 °C, $[\alpha]_D^{23}$ −51.1±0.9° (*c* 1.0, methanol). TLC (silica gel): a single component (sulfuric acid) in system C.

Found: C, 61.84; H, 8.37; N, 10.02%. Calcd for $C_{49}H_{77}N_7O_{12}$: C, 61.55; H, 8.12; N, 10.25%.

Z-Lys(Mhoc)-Pro-Val-Gly-Lys(Mhoc)-NHNH$_2$ (VII). Compound VI (6.69 g, 7 mmol) was treated with hydrazine hydrate (3.4 ml, 70 mmol) in methanol (30 ml) at room temperature for 45 h, after which the product was separated as gelatinous precipitates by the addition of ether. Reprecipitation from methanol-ether gave VII in pure form; wt 6.50 g (97%), mp 165—166 °C, $[\alpha]_D^{22.5}$ −52.0±0.9° (*c* 1.0, methanol), −28.3±0.4° [*c* 2.0, *N*,*N*-dimethylformamide (DMF)]. TLC (silica gel): a single component (sulfuric acid) in system B and system C.

Found: C 60.19; H, 8.46; N, 13.21%. Calcd for $C_{48}H_{77}N_9O_{11}$: C, 60.29, H, 8.12; N, 13.18%.

Z-Lys(Mhoc)-Arg(NO$_2$)-Arg(NO$_2$)-Pro-OMe (X). Compound IX (2.00 g, 3 mmol) was treated with 25% hydrogen bromide in acetic acid (7 ml) at room temperature for 120 min to give H-Arg(NO$_2$)-Arg(NO$_2$)-Pro-OMe hydrobromide (2.44 g).

Z-Lys(Mhoc)-OH, derived from the DCHA salt (1.80 g, 3 mmol)[13] in the usual manner, and *N*-hydroxysuccinimide (HOSu, 0.35 g, 3 mmol) were coupled with DCC (0.62 g, 3 mmol) in acetonitrile at 0 °C for 3 h. The resulting active ester was allowed to react with the N^{α}-free tripeptide ester obtained above in DMF (15 ml) in the presence of triethylamine (1.7 ml, 12 mmol) at 4 °C for 2 days. The product was dissolved in 10% 1-butanol in ethyl acetate and the solution was washed with ice-cold M hydrochloric acid and M sodium hydrogencarbonate, dried over magnesium sulfate and evaporated *in vacuo*. The resulting sirupy residue was reprecipitated from methanol-ether yielded X in pure form; wt 2.40 g (86%), mp 111—119 °C, $[\alpha]_D^{24.5}$ −45.2±0.9° (*c* 1.0, methanol). TLC (silica gel): a single component (sulfuric acid) in system D.

Found: C, 50.67; H, 6.87; N, 18.81%. Calcd for $C_{40}H_{63}N_{13}O_{13}\cdot H_2O$: C, 50.46; H, 6.88; N, 19.13%.

Z-Lys(Mhoc)-Arg(NO$_2$)-Arg(NO$_2$)-Pro-OH (XI). Compound X (2.15 g, 2.3 mmol) in methanol (7 ml) was saponified with sodium hydroxide (4.6 ml) at room temperature for 2 h followed by neutralization with M hydrochloric acid (4.6 ml) at 0 °C and concentration *in vacuo* to remove methanol. The resulting aqueous mixture was extracted three times with 10% 1-butanol in ethyl acetate. The organic extracts were combined, dried over magnesium sulfate and evaporated *in vacuo*. The crude product thus obtained was purified on a silica gel column (100g, Kieselgel 60, Merck) with chloroform-methanol-acetic acid systems (90 : 10 : 3, 400 ml; 85 : 15 : 3, 500 ml; 80 : 20 : 3, 600 ml) as solvent. The fractions were examined by TLC (silica gel, in system I) and those containing the desired compound as a single component were combined and evaporated *in vacuo* to give a residue which was solidified by treatment with ether; wt 1.72 g (80%), mp 137—142 °C, $[\alpha]_D^{21}$ −36.8±0.8° (*c* 1.0, methanol).

Found: C, 50.16; H, 6.79; N, 19.25%. Calcd for $C_{39}H_{61}N_{13}O_{13}\cdot H_2O$: C, 49.94; H, 6.77; N, 19.41%.

Z-Val-Lys(Mhoc)-OMe (XX). Compound III (2.17 g, 5 mmol) was hydrogenolyzed over palladium in 10% acetic acid in methanol for 2 h. The solvent was removed by evaporation *in vacuo* to give an oily residue which was treated with aqueous potassium carbonate at 0 °C in the presence of dichloromethane. The resulting free base of H-Lys(Mhoc)-OMe and Z-Val-OH (1.26 g, 5 mmol) were coupled with DCC (1.03 g, 5 mmol) in dichloromethane at 4 °C overnight to give XX; wt 2.31 g (87%), mp 65—67 °C, $[\alpha]_D^{25}$ −18.7±0.5° (*c* 1.0, methanol). TLC (silica gel): a single component (sulfuric acid) in system G.

Found: C, 63.23; H, 8.24; N, 8.14%. Calcd for $C_{28}H_{43}N_3O_7$: C, 63.02; H, 8.12; N, 7.87%.

Z-Val-Lys(Mhoc)-NHNH$_2$ (XXI). Compound XX (1.87 g, 3.5 mmol) was treated with hydrazine hydrate (1.7 ml) in ethanol (15 ml) at room temperature overnight. The product was reprecipitated from ethanol-ether to afford XXI; wt 1.46 g (78%), mp 147—149 °C, $[\alpha]_D^{23.5}$ −25.8±0.7° (*c* 1.0, acetic acid).

Found: C, 60.80; H, 8.11; N, 13.04%. Calcd for $C_{27}H_{43}N_5O_6$: C, 60.77; H, 8.12; N, 13.12%.

Z-Pro-Asn-Gly-OBu^t (XVIII). Compound XVIIa (4.93 g, 13 mmol) was hydrogenolyzed over palladium in methanol for 4 h to give H-Asn-Gly-OBu^t (free base) as an oil. This was then coupled with Z-Pro-OH (3.24 g, 13 mmol) by means of DCC (2.68 g, 13 mmol) in dichloromethane at 4 °C overnight. The product was repeatedly recrystallized from acetonitrile; wt 4.84 g (78%), mp 146—147.5 °C, $[\alpha]_D^{23}$ −64.6±1.0° (*c* 1.0, methanol). TLC (silica gel): a single component (sulfuric acid) in system G.

Found: C, 57.52; H, 6.73; N, 11.63%. Calcd for $C_{23}H_{32}N_4O_7$: C, 57.97; H, 6.77; N, 11.76%.

Z-Val-Tyr-Pro-Asn-Gly-OBu^t (XIXa). *a)* Via *Val-Tyr-Pro + Asn-Gly.* Compound XVIIa (0.38 g, 1 mmol) was hydrogenolyzed over palladium in methanol for 4 h to give H-Asn-Gly-OBu^t (free base) as an oil.

Compound XIV (0.57 g, 1 mmol) was treated with trifluoroacetic acid (5 ml) at room temperature for 60 min. The solvent was evaporated *in vacuo* and the residue was lyophilized from acetic acid. The resulting powder was dissolved in ethyl acetate and the solution was extracted with 5% sodium hydrogencarbonate three times. The aqueous solutions combined were acidified with hydrochloric acid followed by extraction with ethyl acetate. The combined extracts were dried over magnesium sulfate and evaporated *in vacuo* to give XV as a sirupy residue. This was then coupled with the above dipeptide ester by means of DCC (0.21 g, 1 mmol) in acetonitrile in the presence of 1-hydroxybenzotriazole (HOBt, 0.14 g, 1 mmol) at 4 °C overnight. The crystalline precipitates which had formed were filtered off and recrystallized from acetonitrile (20 ml) to afford XIXa in pure form; wt 0.50 g (66%), mp 152—155 °C, $[\alpha]_D^{22}$ −33.0±2.0° (*c* 0.4, DMF). TLC (silica gel): a single component (sulfuric acid) in system H.

Found: C, 58.79; H, 6.89; N, 10.92%. Calcd for $C_{37}H_{50}N_6O_{10}\cdot H_2O$: C, 58.72; H, 6.93; N, 11.10%.

b) Via *Val-Tyr + Pro-Asn-Gly.* Compound XVIII 2.38 g, 5 mmol) was hydrogenolyzed over palladium in methanol for 4 h to give H-Pro-Asn-Gly-OBu^t (free base) in a crystalline form. This was then coupled with Z-Val-Tyr-N$_3$ [derived from XIII (2.14 g, 5 mmol) by the treatment with nitrous acid in the usual manner in DMF-ethyl acetate at 4 °C overnight. Evaporation of the solvent *in vacuo* gave a residue which was crystallized from acetonitrile. Recrystallization from the same solvent gave the desired XIXa; wt 2.22 g (59%), mp 154—156 °C, $[\alpha]_D^{23}$ −34.2±1.5° (*c* 0.5, DMF).

Found: C, 58.58; H, 6.84; N, 10.91%.

Z-Gly-Ala-OBu^t (XVIb). Z-Ala-OH (11.2 g, 50 mmol) was treated with 2-methylpropene (isobutene, 50 ml) in dichloromethane in the presence of sulfuric acid (1.5 ml)

at room temperature for 3 days to give Z-Ala-OBu^t as an oily residue (13.7 g). This was hydrogenolyzed over palladium in methanol for 7 h to yield H-Ala-OBu^t (free base) as an oil (6.97 g).

The alanine ester obtained above was coupled with Z-Gly-OH (9.41 g, 45 mmol) with DCC (9.33 g, 45 mmol) in dichloromethane at 4 °C overnight to give a crude preparation of XVIb (15.9 g). A 1.06-g portion was chromatographed on a column of silica gel (20 g, Kieselgel 60, Merck) with benzene-ethyl acetate (3 : 2) as solvent. The fractions were examined by TLC (silica gel, in the same solvent as that for the column) and those containing the desired compound as a single component were combined and evaporated *in vacuo*; wt 0.95 g (94%), $[\alpha]_D^{24.5}$ −31.5±0.8° (*c* 0.9, methanol).

Found: C, 60.77; H, 7.33; N, 8.30%. Calcd for $C_{17}H_{24}N_2O_5$: C, 60.70; H, 7.19; N, 8.33%.

Z-Asn-Gly-Ala-OBu^t (XVIIb). Compound XVIb (6.57 g, 19.5 mmol) was hydrogenolyzed over palladium in methanol for 10 h to give H-Gly-Ala-OBu^t as an oil. This was then coupled with Z-Asn-ONp (7.55 g, 19.5 mmol) in DMF at 4 °C overnight. The product was crystallized from acetonitrile. Recrystallization from the same solvent yielded XVIIb; 6.63 g (74%), mp 159—160 °C, $[\alpha]_D^{23.5}$ −20.4±0.6° (*c* 1.0, methanol). TLC (silica gel): a single component (sulfuric acid) in system E.

Found: C, 56.19; H, 6.78; N, 12.56%. Calcd for $C_{21}H_{30}N_4O_7$: C, 55.99; H, 6.71; N, 12.44%.

Z-Val-Tyr-Pro-Asn-Gly-Ala-OBu^t (XIXb). Compound XVIIb (1.80 g, 4 mmol) was hydrogenolyzed over palladium in methanol for 3 h to give H-Asn-Gly-Ala-OBu^t as an oily residue.

Compound XIV (2.27 g, 4 mmol) was treated with trifluoroacetic acid (20 ml) at room temperature for 60 min to give XV, which was isolated in the manner described above in the synthesis of XIXa. Compound XV was coupled with the above tripeptide ester by means of DCC (0.82 g, 4 mmol) in acetonitrile in the presence of HOBt (0.54 g, 4 mmol) at 4 °C overnight. The desired XIXb was isolated in the same manner as described above for XIXa; wt 1.93 g (58%), mp (70) 140—145 °C, $[\alpha]_D^{23.5}$ −57.2±0.8° (*c* 1.0, methanol). TLC (silica gel): a single component (sulfuric acid) in system H.

Found: C, 57.82; H, 6.98; N, 12.18%. Calcd for $C_{40}H_{55}N_7O_{11}\cdot H_2O$: C, 58.03; H, 6.94; N, 11.84%.

Z-Val-Lys(Mhoc)-Val-Tyr-Pro-Asn-Gly-OBu^t (XXIIa). Compound XIXa (2.22 g, 3 mmol) was hydrogenolyzed over palladium in acetic acid (20 ml) for 4 h to give H-Val-Tyr-Pro-Asn-Gly-OBu^t acetate as amorphous solid (1.93 g).

To a DMF solution of XXI (1.04 g, 1.95 mmol) previously chilled in an ice-bath were added ice-cold M hydrochloric acid (5 ml) and 2 M sodium nitrite (1.07 ml) and the mixture was shaken at 0 °C for 4 min. The resulting azide was extracted with ethyl acetate and the combined extracts were washed twice with M sodium hydrogencarbonate and dried over magnesium sulfate at 0 °C. This was combined with a solution of the above pentapeptide ester (0.87 g, 1.3 mmol) and triethylamine (0.28 ml, 2 mmol) in DMF and the mixture was kept at 4 °C for 2 days. The crude product was crystallized and recrystallized from methanol-ethyl acetate to afford the pure XXIIa; wt 1.21 g (84%), mp 152—155 °C, $[\alpha]_D^{23}$ −66.9±2.0° (*c* 0.5, methanol). TLC (silica gel): a single component (ninhydrin, after preheating at 150 °C) in system H.

Found: C, 60.54; H, 7.72; N, 11.26%. Calcd for $C_{56}H_{83}N_9O_{14}$: C, 60.80; H, 7.56; N, 11.40%.

Z-Val-Lys(Mhoc)-Val-Tyr-Pro-Asn-Gly-OH (XXIII). A 0.81-g portion (1.3 mmol) of H-Val-Tyr-Pro-Asn-Gly-OBu^t acetate obtained above was treated with trifluoroacetic acid at room temperature for 30 min to give the trifluoroacetic acid salt of H-Val-Tyr-Pro-Asn-Gly-OH as amorphous solid (0.88 g).

This pentapeptide was then coupled with Z-Val-Lys-(Mhoc)-N_3 [derived from XXI (0.88 g, 1.65 mmol) in the same manner as described above] in DMF in the presence of triethylamine (0.23 ml, 1.65 mmol) at 4 °C overnight. The crude product was purified on a silica gel column (30 g, Kieselgel 60, Merck) with chloroform-methanol-acetic acid (8 : 2 : 1) as solvent. The fractions were examined by TLC (silica gel, in the same solvent as that for the column) and those containing the desired compound as a single component were combined and evaporated *in vacuo*. The resulting residue was crystallized from methanol-ether; wt 0.90 g (65%), mp 173—177 °C decomp., $[\alpha]_D^{23.5}$ −33.5±1.5° (*c* 0.5, DMF). Amino acid ratios in acid hydrolysate (theoretical values are given in parentheses): Lys 0.97 (1), NH_3 1.22 (1), Asp 1.07 (1), Pro 1.00 (1), Gly 1.07 (1), Val 2.00 (2), Tyr 0.97 (1).

Found: C, 57.94; H, 7.31; N, 11.67%. Calcd for $C_{52}H_{75}N_9O_{14}\cdot 3/2\,H_2O$: C, 57.98; H, 7.30; N, 11.70%.

Z-Val-Lys(Mhoc)-Val-Tyr-Pro-Asn-Gly-Ala-OBu^t (XXIIb). Compound XIXb (1.62 g, 2 mmol) was hydrogenolyzed over palladium in methanol for 3.5 h to give the N^α-free hexapeptide ester as a sirupy residue. This was then coupled with Z-Val-Lys(Mhoc)-N_3 [derived from XXI (1.63 g, 3 mmol) in the usual manner as described above] in DMF at 4 °C overnight. The crude product was crystallized and recrystallized from methanol-ethyl acetate to give the pure XXIIb; wt. 2.02 g (86%), mp 214—217 °C decomp., $[\alpha]_D^{24}$ −25.7±0.5° (*c* 1.0, DMF). TLC (silica gel): a single component (ninhydrin, after preheating at 150 °C) in system H.

Found: C, 59.89; H, 7.50; N, 11.87%. Calcd for $C_{59}H_{88}N_{10}O_{15}$: C, 60.19; H, 7.53; N, 11.90%.

H-Val-Lys-Val-Tyr-Pro-Asn-Gly-Ala-OH Acetate (XXIV). Compound XXIIb (0.15 g) was hydrogenolyzed over palladium in acetic acid for 2.5 h. The resulting N^α-free peptide was then treated with trifluoroacetic acid (2 ml) in the presence of anisole (0.1 ml) and 2-mercaptoethanol (0.1 ml) at room temperature for 30 min. The precipitates which formed upon addition of ether were filtered off, thoroughly washed with ether and dried *in vacuo*. This was dissolved in water (10 ml) and the solution was passed through a column (0.9×10 cm) of Amberlite CG-400 (acetate) with portions of water. The aqueous solutions were combined, concentrated *in vacuo* at a bath temperature of 50 °C and lyophilized to give XXIV; wt 0.10 g (86%), $[\alpha]_D^{26}$ −74.3±2.3° (*c* 0.5, 0.1 M hydrochloric acid). TLC (cellulose): a single component (ninhydrin) in system J. Amino acid ratios in acid hydrolysate: Lys 1.11 (1), NH_3 1.14 (1), Asp 1.02 (1), Pro 0.99 (1), Gly 1.07 (1), Ala 1.05 (1), Val 2.00 (2), Tyr 0.95 (1).

Z-Lys(Mhoc)-Arg(NO$_2$)-Arg(NO$_2$)-Pro-Val-Lys(Mhoc)-Val-Tyr-Pro-Asn-Gly-OBu^t (XXVa). Compound XXIIa (1.11 g, 1 mmol) was hydrogenolyzed over palladium in acetic acid (10 ml) for 3 h to give the acetic acid salt of H-Val-Lys(Mhoc)-Val-Tyr-Pro-Asn-Gly-OBu^t as fluffy powder (1.15 g). A half portion of this material was dissolved in a mixture of 1-butanol (8 ml) and ethyl acetate (16 ml) and the solution was shaken with 50% potassium carbonate (5 ml) at 0 °C. The organic phase was dried over magnesium sulfate followed by evaporation *in vacuo* to give the free base which was crystallized from methanol-ether; wt 0.46 g, $[\alpha]_D^{21}$ −36.5±1.5° (*c* 0.5, DMF).

Compound XI (0.41 g, 0.45 mmol) and the heptapeptide

ester obtained above (0.44 g, 0.45 mmol) were coupled with DCC (0.19 g, 0.9 mmol) in DMF (9 ml) in the presence of HOBt (0.12 g, 0.9 mmol) at 4 °C overnight. The crude product was submitted to chromatography on a silica gel column (100 g, Kieselgel 60, Merck) with chloroform-methanol systems (95 : 5, 500 ml; 90 : 10, 200 ml; 85 : 15, 1200 ml) as solvent. The fractions were examined by TLC (silica gel, in system D) and those containing XXVa as a single component were combined and evaporated *in vacuo* to give a residue which was precipitated from methanol-ether; wt 0.74 g (87%), mp 150—155 °C, $[\alpha]_D^{21.5}$ −78.5±2.3° (*c* 0.5, methanol).

Found: C, 55.62; H, 7.06; N, 16.35%. Calcd for $C_{87}H_{136}N_{22}O_{24}$: C, 55.75; H, 7.31; N, 16.44%.

H–Lys(Mhoc)–Arg–Arg–Pro–Val–Lys(Mhoc)–Val–Tyr–Pro–Asn–Gly–OBu^t Acetate (XXVIa). Compound XXVa (0.94 g) was hydrogenolyzed over palladium in acetic acid (15 ml) for 40 h to give XXVIa; wt 1.09 g $\lambda_{max}^{0.1M\ HCl}$ 275 nm ($E_{1cm}^{1\%}$ 6.8). TLC (cellulose): almost homogeneous in system J.

H–Lys–Arg–Arg–Pro–Val–Lys–Val–Tyr–Pro–Asn–Gly–OH Acetate (XXVIIa). Compound XXVIa (0.33 g) was treated with trifluoroacetic acid (9 ml) in the presence of anisole (0.1 ml) and 2-mercaptoethanol (0.1 ml) at room temperature for 30 min. An aqueous solution of the product was treated with Amberlite CG-400 (acetate) and lyophilized (0.23 g).

A 0.1-g portion of this product was submitted to a column (1.7×21 cm) of carboxymethyl cellulose (CM-52, Whatman) using an ammonium acetate buffer (pH 6, 1500 ml) with a linear concentration gradient of 0—0.6 M. Fractions (7 ml/tube) were collected and their absorption at 275 nm was measured. The fractions corresponding to a major peak (tubes 110—133) were pooled, evaporated *in vacuo* and lyophilized to give XXVIIa as fluffy powder; wt 82 mg, $[\alpha]_D^{23}$ −85.7±3.0° (*c* 0.5, 0.1 M hydrochloric acid). TLC (cellulose): a single component (ninhydrin) in system K. Amino acid ratios in acid hydrolysate: Lys 1.99 (2), NH_3 1.13 (1), Arg 1.92 (2), Asp 1.03 (1), Pro 1.90 (2), Gly 1.00 (1), Val 2.08 (2), Tyr 0.99 (1).

Z–Lys(Mhoc)–Arg(NO_2)–Arg(NO_2)–Pro–Val–Lys(Mhoc)–Val–Tyr–Pro–Asn–Gly–Ala–OBu^t (XXVb). Compound XXIIb (0.47 g, 0.4 mmol) was hydrogenolyzed over palladium in acetic acid for 4 h to give the acetic acid salt of N^{α}-free octapeptide ester. This was dissolved in a mixture of 1-butanol (5 ml) and ethyl acetate (5 ml) and the solution was shaken with 50% potassium carbonate (5 ml) at 0 °C. The organic phase was dried and evaporated *in vacuo* to give the crystalline free base (0.42 g).

Compound XI (0.37 g, 0.4 mmol) and the octapeptide ester obtained above (0.42 g) were coupled with DCC (0.17 g, 0.8 mmol) in DMF (4 ml) in the presence of HOBt (0.11 g, 0.8 mmol) at 4 °C for 2 days. The crude product (1.03 g) was purified on a silica gel column in the same manner as described above for XXVa; wt 0.50 g (64%), mp 150—156 °C, $[\alpha]_D^{26}$ −73.4±2.3° (*c* 0.5, methanol).

Found: C, 54.70; H, 7.31; N, 15.94%. Calcd for $C_{90}H_{141}N_{23}O_{25}\cdot 2H_2O$: C, 54.56; H, 7.38; N, 16.26%.

H–Lys(Mhoc)–Arg–Arg–Pro–Val–Lys(Mhoc)–Val–Tyr–Pro–Asn–Gly–Ala–OBu^t Acetate (XXVIb). Compound XXVb (0.39 g) was hydrogenolyzed over palladium in acetic acid (8 ml) for 21 h. The catalyst was filtered off and the filtrate was lyophilized; wt 0.51 g, $\lambda_{max}^{0.1M\ HCl}$ 275 nm ($E_{1cm}^{1\%}$ 5.0). TLC (cellulose): almost homogeneous in system J.

H–Lys–Arg–Arg–Pro–Val–Lys–Val–Tyr–Pro–Asn–Gly–Ala–OH Acetate (XXVIIb). Compound XXVIb (0.13 g) was deprotected with trifluoroacetic acid (3 ml) in the presence of anisole (0.1 ml) and 2-mercaptoethanol (0.1 ml) at room temperature for 30 min. The product was treated with Amberlite CG-400 (acetate) in the usual manner to give the crude XXVIIb. This was then purified on a carboxymethyl cellulose column in the same manner as described above for XXVIIa; wt. 81 mg, $[\alpha]_D^{23}$ −86.9±2.5° (*c* 0.5, 0.1 M hydrochloric acid). TLC (cellulose): a single component (ninhydrin) in system L. Amino acid ratios in acid hydrolysate: Lys 1.75 (2), NH_3 0.91 (1), Arg 2.04 (2), Asp 1.06 (1), Pro 1.05 (1), Gly 1.05 (1), Ala 1.05 (1), Val 2.00 (2), Tyr 0.88 (1).

Z–Lys(Mhoc)–Pro–Val–Gly–Lys(Mhoc)–Lys(Mhoc)–Arg–Arg–Pro–Val–Lys(Mhoc)–Val–Tyr–Pro–Asn–Gly–OBu^t (XXVIIIa). A solution of VII (0.29 g, 0.3 mmol) in 90% aqueous tetrahydrofuran (5 ml) was chilled in an ice-bath and to this were added ice-cold M hydrochloric acid (0.75 ml) and ice-cold 2 M sodium nitrite (0.17 ml) successively. The mixture was stirred at 0 °C for 5 min, after which triethylamine (0.27 ml, 1.9 mmol) was added. The azide solution thus obtained was combined with a solution of XXVIa (0.28 g, 0.15 mmol) in 95% aqueous DMF (8 ml) and the mixture was stirred at 4 °C overnight. The product (0.51 g) was submitted to chromatography on a silica gel column (50 g, Kieselgel 60, Merck) with chloroform-methanol systems (9 : 1, 300 ml; 8 : 2, 400 ml; 7 : 3, 400 ml) as solvent. The fractions were examined by TLC (silica gel, in system J) and those containing the desired hexadecapeptide as a single component were pooled and evaporated *in vacuo* to give a residue which was precipitated from methanol-ether; wt 0.25 g (64%), mp 170—175 °C decomp., $[\alpha]_D^{23}$ −65.0±2.0° (*c* 0.5, methanol). Amino acid ratios in acid hydrolysate: Lys 3.97 (4), NH_3 1.10 (1), Arg 2.00 (2), Asp 1.12 (1), Pro 3.25 (3), Gly 2.09 (2), Val 3.00 (3), Tyr 1.01 (1).

A sample was reprecipitated from methanol-water and lyophilized from acetic acid for elemental analysis.

Found: C, 54.45; H, 7.45; N, 13.75%. Calcd for $C_{127}H_{205}N_{27}O_{29}\cdot 2CH_3COOH\cdot 10H_2O$: C, 54.74; H, 8.17; N, 13.16%.

Z–Lys(Mhoc)–Pro–Val–Gly–Lys(Mhoc)–Lys(Mhoc)–Arg–Arg–Pro–Val–Lys(Mhoc)–Val–Tyr–Pro–Asn–Gly–Ala–OBu^t (XXVIIIb). Compound XXVIb (0.22 g, 0.1 mmol) and the azide derived from VII (0.19 g, 0.2 mmol) in the manner as described above were coupled in aqueous tetrahydrofuran–DMF in the presence of triethylamine (0.13 ml, 0.9 mmol) at 4 °C overnight. The crude product was purified on a silica gel column in the same manner as described above for XXVIIIa; wt 0.14 g (52%), $[\alpha]_D^{24}$ −64.0±2.0° (*c* 0.5, methanol). TLC (silica gel): almost homogeneous (ninhydrin, after preheating at 150 °C) in system J. Amino acid ratios in acid hydrolysate: Lys 3.72 (4), NH_3 1.17 (1), Arg 1.98 (2), Asp 1.03 (1), Pro 2.98 (3), Gly 2.10 (2), Ala 1.11 (1), Val 3.00 (3), Tyr 0.93 (1).

A sample for elemental analysis was reprecipitated from methanol-water and lyophilized from acetic acid.

Found: C, 55.02; H, 7.99; N, 13.80%. Calcd for $C_{130}H_{210}N_{28}O_{30}\cdot 2CH_3COOH\cdot 8H_2O$: C, 55.32; H, 8.11; N, 13.48%.

H–Ser–Tyr–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys–Pro–Val–Gly–Lys–Lys–Arg–Arg–Pro–Val–Lys–Val–Tyr–Pro–Asn–Gly–OH Acetate, ACTH(1—26)–OH (I). Compound XXVIIIa (0.14 g, 0.05 mmol) was hydrogenolyzed over palladium in methanol containing acetic acid (0.5 ml) for 5 h. Removal of the solvent by evaporation *in vacuo* followed by lyophilization from acetic acid gave the N^{α}-free hexadecapeptide derivative (0.13 g).

To a solution of Boc–Ser–Tyr–Ser–Met–Glu(OBu^t)–His–Phe–Arg–Trp–Gly–OH (0.11 g, 0.075 mmol)[22] in DMF (2 ml) previously chilled in an ice-bath was added M hydro-

chloric acid (0.15 ml) and the solution was introduced into ice-cold ethyl acetate–ether (1 : 1, 50 ml). The resulting precipitates of the decapeptide hydrochloride were dissolved in DMF (3 ml) together with *N*-hydroxysuccinimide (HOSu, 0.035 g, 0.3 mmol) and to this was added DCC (0.062 g, 0.3 mmol) at 0 °C. The mixture was kept at 4 °C overnight, after which it was introduced into ethyl acetate–ether (1 : 1, 50 ml) to give the decapeptide active ester hydrochloride as amorphous precipitates (0.12 g). The active ester thus obtained was added to a solution of the N^{α}-free hexadecapeptide obtained above and triethylamine (0.07 ml) in DMF (3 ml), and the mixture was kept at room temperature for 2 days. The product was isolated as precipitates which formed when the reaction mixture was introduced into ethyl acetate (60 ml). The crude protected hexacosapeptide thus obtained (0.24 g) was mixed with anisole (0.1 ml) and 1,2-ethanedithiol (0.1 ml) and chilled in an ice-bath. To this was added trifluoroacetic acid (5 ml) and the mixture was kept at 0 °C for 60 min. The precipitates which formed upon addition of ether were filtered off, washed with ether and dried *in vacuo*. This was then dissolved in water and the solution was passed through a column (1.1×20 cm) of Amberlite CG-400 (acetate form) with additional portions of water. The aqueous solutions were combined and lyophilized to give the deprotected hexacosapeptide (0.24 g).

The crude peptide obtained above was submitted to partition chromatography on a column (2.0×75 cm) of Sephadex G-25 (medium) with 1-butanol–acetic acid–pyridine–water (12 : 3 : 4 : 6) as solvent and 5-ml fractions were collected. The tubes (no. 25—50) containing the desired peptide as a major component, as examined by TLC (cellulose, in system L) with the ninhydrin and Ehrlich reagents as reagents for locating peptide spots, were combined and evaporated *in vacuo* at a bath temperature of 45 °C and the residue was lyophilized (0.096 g). This was then chromatographed on a column (1.7×20 cm) of carboxymethyl cellulose (CM-52, Whatman) using an ammonium acetate buffer (pH 6.0, 2000 ml) with a linear concentration gradient of 0—0.6 M. Ten-ml fractions were collected and their absorption at 280 nm was measured. The fractions corresponding to a main peak (tubes 134—159) were combined, evaporated and lyophilized (0.051 g). For final purification this was submitted to gel filtration on a column (2.0×75 cm) of Sephadex G-25 (medium) with 0.1 M acetic acid as solvent. Five-ml fractions were collected and those corresponding to a single peak (tubes 20—35), as monitored by absorption at 280 nm, were combined, lyophilized and dried over sodium hydroxide pellets and phosphorus pentoxide *in vacuo* to afford the pure hexacosapeptide (I) as colorless fluffy powder; wt 0.048 g (26%), $[\alpha]_D^{25}$ −87.6±2.5° (*c* 0.5, 0.1 M acetic acid). $\lambda_{max}^{0.1M\ HCl}$ 276.5 nm ($E_{1cm}^{1\%}$ 20.5), $\lambda_{shoulder}^{0.1M\ HCl}$ 288 nm ($E_{1cm}^{1\%}$ 13.7); $\lambda_{max}^{0.1M\ NaOH}$ 283 nm ($E_{1cm}^{1\%}$ 22.9), 289 nm ($E_{1cm}^{1\%}$ 23.5). TLC (cellulose): a single component (ninhydrin and Ehrlich reagents) in system L. Amino acid ratios in acid hydrolysate: Lys 3.80 (4), His 1.00 (1), Arg 3.08 (3), Asp 1.00 (1), Ser 1.80 (2), Glu 1.10 (1), Pro 3.02 (3), Gly 3.05 (3), Val 3.15 (3), Met 1.00 (1), Tyr 2.05 (2), Phe 1.02 (1). The Tyr/Trp ratio in intact I was 1.99 as determined spectrophotometrically.[23]

H–Ser–Tyr–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys–Pro–Val–Gly–Lys–Lys–Arg–Arg–Pro–Val–Lys–Val–Tyr–Pro–Asn–Gly–Ala–OH Acetate, ACTH(1—27)–OH (II). Compound XXVIIIb (0.15 g, 0.05 mmol) was hydrogenolyzed over palladium in methanol containing acetic acid (0.5 ml) for 3 h to give the N^{α}-free heptadecapeptide derivative (0.13 g). This was allowed to react with the decapeptide *N*-hydroxysuccinimide ester [prepared from Boc–Ser–Tyr–Ser–Met–Glu(OBu^t)–His–Phe–Arg–Trp–Gly–OH (0.11 g, 0.075 mmol)[22] in exactly the same manner as described above] in DMF (3 ml) in the presence of triethylamine (0.07 ml) at room temperature for 2 days. The resulting protected peptide (0.24 g) was treated with trifluoroacetic acid (5 ml) at 0 °C for 60 min in the presence of anisole (0.1 ml) and 1,2-ethanedithiol (0.1 ml). The product was then treated with Amberlite CG-400 (acetate form) to give a crude preparation of II (0.23 g).

The crude peptide obtained above was purified by partition chromatography on a column of Sephadex G-25 and by carboxymethyl cellulose column chromatography in the same manner as described above for I. The resulting material (0.054 g) was then submitted to gel filtration on a Sephadex G-25 column with 0.1 M acetic acid as solvent to give the pure heptacosapeptide (II); wt 0.050 g (27%), $[\alpha]_D^{25}$ −86.6± 2.3° (*c* 0.5, 0.1 M acetic acid). $\lambda_{max}^{0.1M\ HCl}$ 276.5 nm ($E_{1cm}^{1\%}$ 21.5), $\lambda_{shoulder}^{0.1M\ HCl}$ 288 nm ($E_{1cm}^{1\%}$ 14.5); $\lambda_{max}^{0.1M\ NaOH}$ 283 nm ($E_{1cm}^{1\%}$ 23.1), 289 nm ($E_{1cm}^{1\%}$ 23.6). TLC (cellulose): a single component (ninhydrin and Ehrlich reagents) in system L. Amino acid ratios in acid hydrolysate: Lys 4.00 (4), His 1.00 (1), Arg 3.10 (3), Asp 1.00 (1), Ser 1.85 (2), Glu 1.08 (1), Pro 3.08 (3), Gly 3.10 (3), Ala 1.08 (1), Val 3.05 (3), Met 0.98 (1), Tyr 1.93 (2), Phe 0.98 (1). The Tyr/Trp ratio in intact II was 1.98 as determined spectrophotometrically.[23]

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