[Chem. Pharm. Bull. 30(5)1697—1705(1982)]

Studies on Peptides. CVIII.[1,2] Synthesis of the Protected Eicosapeptide Corresponding to Positions 19 to 38 of Human Parathyroid Hormone

Susumu Funakoshi and Haruaki Yajima*

Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto, 606, Japan

(Received October 29, 1981)

As a starting material for the synthesis of human parathyroid hormone (1—38), a C-terminal-protected eicosapeptide ester (positions 19—38) was synthesized by successive condensation of four peptide fragments; (positions 29—38), (25—28), (22—24) and (19—21).

Keywords——human parathyroid hormone (1—38); β,β,β-trichloroethyloxycarbonylhydrazine; N^{α}-deprotection of Trp-containing peptide; N^{G}-mesitylene-2-sulfonylarginine; methionine sulfoxide; trifluoromethanesulfonic acid-thioanisole system as a deprotecting procedure

Following the structural elucidation of bovine[3,4] and porcine species[5] parathyroid hormone (PTH), Keutmann *et al.*[6] disclosed in 1978 the complete amino acid sequence of the human hormone (hPTH) with the aid of a precise microsequencing technique[7] and reconciled the discrepancy which existed between the sequences proposed by Brewer *et al.*[8] and by Niall *et al.*,[9] at residues 22, 28 and 30 (Fig. 1). The result favored Niall's formulation.

H-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-
Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-
Val-His-Asn-Phe-Val-Ala-Leu-Gly-Ala-Pro-Leu-Ala-Pro-Arg-Asp-
Ala-Gly-Ser-Gln-Arg-Pro-Arg-Lys-Lys-Glu-Asp-Asn-Val-Leu-Val-
Glu-Ser-His-Glu-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-
Asp-Val-Leu-Thr-Lys-Ala-Lys-Ser-Gln-OH

(15, 30, 45, 60, 75, 84)

Fig. 1. Structure of Human Parathyroid Hormone (Keutmann *et al.* 1978)

Based on the earlier observation made by Keutmann *et al.*[10] in 1972 that a fragment of bovine PTH designated as H-(bPTH 1—29)-OH generated by dilute HCl cleavage at the Asp residue (position 30) retained still significant biological potency by both *in vivo* and *vitro* assay methods, solid phase synthesis of H-(bPTH 1—34)-OH[11] was soon undertaken for confirmation of this observation. Solid phase syntheses of H-(hPTH 1—34)-OH or amide corresponding to Niall's sequence[12a,b] and Brewer's sequence[12b] were subsequently reported. In the solid phase method, however, removal of contaminating amino components on the resin, due to incomplete condensation, is practically impossible. Later, Tregear *et al.*[13] reexamined their synthetic H-(bPTH 1—34)-OH by Edman degradation and found the presence of at least 30% of contaminating error peptides which were undetected by other analytical procedures. As far as conventional synthesis of an active fragment of hPTH is concerned, synthesis of H-(hPTH 1—34)-OH (Brewer's 1972 formula) by Andreatta *et al.*[14] has been the only example. Quite recently, Takai *et al.*[15] reported the synthesis of H-(hPTH 1—34)-OH (Niall's formula) in a conventional manner, for which HF was employed as a deprotecting reagent.[16]

Since the complete amino acid sequence of hPTH is firmly established, we decided to synthesize the octatriacontapeptide, H–(hPTH 1—38)–OH, a longer peptide than the active fragment in order to examine the difference in biological activity, if any, and to pave the way for total synthesis of hPTH in future. We wish to report this synthesis in two consecutive papers. In the first paper, synthesis of the protected eicosapeptide corresponding to positions 19—38 of hPTH is presented (Fig. 2).

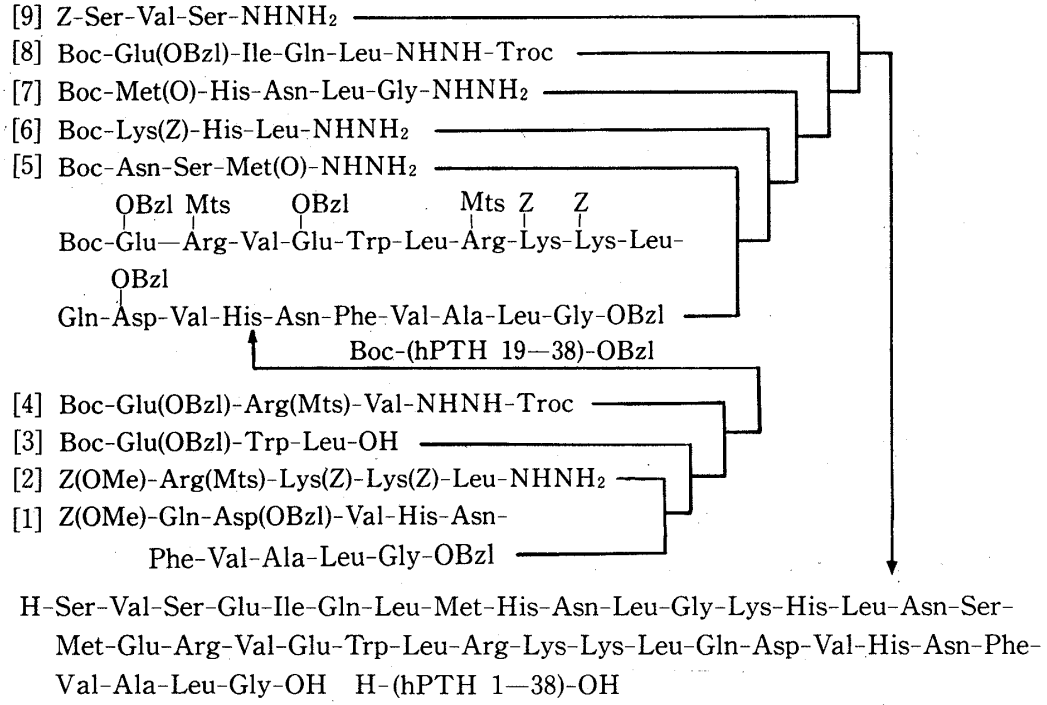

Fig. 2. Synthetic Route to Human Parathyroid Hormone (1—38)

In the present synthesis, we used a new deprotecting procedure, the trifluoromethanesulfonic acid (TFMSA)–thioanisole system,[17,18] in the final step of the synthesis. Thus, amino acid derivatives bearing protecting groups removable by the combination of the above two reagents were employed; *i.e.*, Lys(Z), Glu(OBzl) and Arg(Mts). Arg(Mts)[19] is a new derivative introduced by us in 1979 and its protecting group has been proved to be cleaved smoothly by either HF or methanesulfonic acid. This group was now found to be cleaved also by the above reagents in an ice-bath within 60 min. Each Met residue was protected as the corresponding sulfoxide[20] by oxidation with sodium metaperborate[21] in order to prevent partial oxidation during the synthesis.

In order to construct the octatriacontapeptide backbone, we selected nine peptide fragments (Fig. 2). The azide procedure[22] was applied as a main tool to condense these fragments successively, since much less risk of racemization is involved in this procedure, compared to other amide-forming reactions. However, the following precautions were taken. First, for preparation of the two fragments containing Glu(OBzl), [4] and [8], we employed a substituted hydrazine, Troc–$NHNH_2$,[23] the protecting group of which is known to be removed by Zn[24] without affecting side chain protecting groups such as Z and Bzl. Thus, these fragments were prepared without exposing the corresponding methyl or ethyl esters to hydrazine. Next, the azide procedure is not ideal for condensation of Trp-containing peptides. Thus, fragment [3], Boc–Glu(OBzl)–Trp–Leu–OH, was selected as one unit and this was condensed by DCC–HOBT,[25] instead of the azide procedure, as will be described later. From a synthetic viewpoint, the Trp residue presents some difficulty with regard to the suppression of side reactions involving its functional group during the N^{α}-deprotection by TFA.[26] The Boc group, instead of Z(OMe), was employed as the N^{α}-protecting group

of fragment [3] and subsequent fragments, since our model experiments showed that much less side reaction at the Trp residue is involved during the N^{α}-deprotection by TFA compared to the TFA deprotection of Z(OMe).[27] In addition, anisole containing 2% EDT was applied as a cation scavenger to minimize the side reaction at the Trp residue during the TFA treatment.[28]

Based on the strategies described above, we undertook the synthesis of the octatriacontapeptide (hPTH 1—38). The synthesis of the half molecule, the protected eicosapeptide derived by condensation of four fragments, from [1] to [4], is reported herein.

The synthetic scheme for the C-terminal-protected decapeptide ester, Z(OMe)-(hPTH 29—38)-OBzl [1], is illustrated in Fig. 3. The peptide bonds were constructed successively by the NP active ester procedure,[29] except for the introduction of two dipeptide units, Z(OMe)-Phe-Val-$NHNH_2$ and Z(OMe)-Val-His-$NHNH_2$. These units were introduced by Honzl and Rudinger's azide procedure.[22]

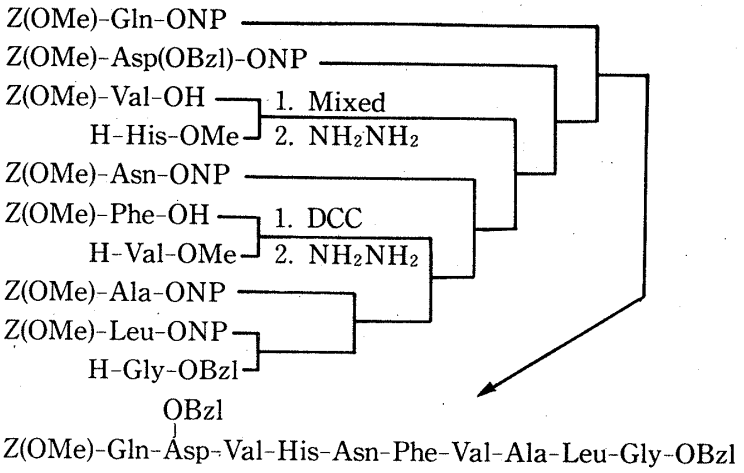

Fig. 3. Synthetic Scheme for the Protected Decapeptide Ester, Z(OMe)-(hPTH 29—38)-OBzl [1]

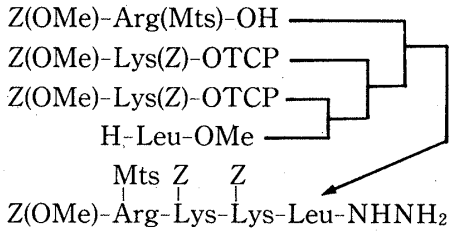

Fig. 4. Synthetic Scheme for the Protected Tetrapeptide Hydrazide, Z(OMe)-(hPTH 25—28)-$NHNH_2$ [2]

The solubility of protected intermediates in DMF decreased remarkably with chain elongation, especially at the pentapeptide stage. Consequently, a mixture of DMSO-DMF had to be employed for subsequent condensation reactions. When the Asn residue (position 33) was incorporated, the protected product stayed at the origin on the thin layer Chromatography (TLC) plate in all solvents so far examined. This situation was not improved until fragment [6] containing the Lys (Z) and His residues was introduced into the chain, as will be described in the subsequent paper.

The poor solubility of protected intermediates resulted in great difficulty in assessment of the homogeneity of each product, but this, in turn, was advantageous for purification of the desired peptides. Because of the great difference in solubilities between the protected products and relatively small acyl components, the latter used in excess during the coupling reaction could be removed easily by repeated precipitation from DMF with methanol or ethyl acetate. Ala was selected as a diagnostic amino acid in acid hydrolysates of protected intermediates, since this amino acid occurs only once at the C-terminal portion of our synthetic target. By comparison of the recovery of Ala with those of newly incorporated amino acids, the satisfactory incorporation of each fragment, together with the homogeneity of each product, could be ascertained.

The fragment, Z(OMe)-(hPTH 25—28)-$NHNH_2$ [2], was synthesized according to the scheme in Fig. 4. The TCP ester procedure[30] was employed for the introduction of two Z(OMe)-Lys(Z)-OH residues to H-Leu-OMe successively and the mixed anhydride procedure[31] was used to introduce Z(OMe)-Arg(Mts)-OH. The resulting tetrapeptide ester was smoothly converted to the corresponding hydrazide [2] in the usual manner.

The fragment [3], Boc–(hPTH 22—24)–OH, was prepared as shown in Fig. 5. Attempts to crystallize Boc–Trp–Leu–OH, prepared by the NP method, were unsuccessful and it was therefore characterized as its DCHA salt. For the reason stated above, the Boc-protecting group, instead of Z(OMe), was used. It was removed from Boc–Trp–Leu–OH by TFA in the presence of anisole containing 2% EDT. The N^{α}-deprotected peptide, although it was found to contain two minor impurities on TLC, was used in the subsequent condensation with Boc–Glu(OBzl)–OH by the Su method,[32] and a homogeneous product [3] was obtained as the corresponding DCHA salt, after recrystallization three times from methanol and ether.

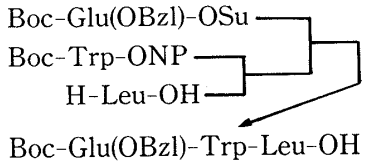

Fig. 5. Synthetic Scheme for the Protected Tripeptide, Boc–(hPTH 22—24)–OH [3]

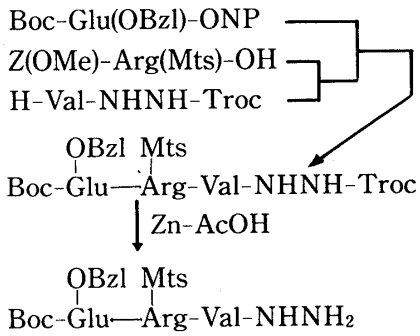

Fig. 6. Synthetic Scheme for the Protected Tripeptide Hydrazide, Boc–(hPTH 19—21)–$NHNH_2$ [4]

The fragment [4], Boc–(hPTH 19—21)–$NHNH_2$, was prepared as shown in Fig. 6, starting with the known substituted hydrazide derivative, Z(OMe)–Val–NHNH–Troc.[33] First, Z(OMe)–Arg(Mts)–Val–NHNH–Troc was prepared by the mixed anhydride procedure. This, after purification by column chromatography on silica gel, was condensed with Boc–Glu-(OBzl)–OH by the NP method. The resulting tripeptide derivative was treated with Zn to remove the Troc group, and the last trace of contaminant zinc acetate was removed by treatment with EDTA to give the tripeptide hydrazide [4] in analytically pure form.

As shown in Fig. 2, four fragments thus prepared were condensed successively by Honzl and Rudinger's azide procedure, except for the fragment [3] as described above. This Trp–containing fragment [3] was condensed by means of DCC in the presence of HOBT in order to suppress possible racemization. For this purpose, the TFA–treated sample of Z(OMe)–(hPTH 25—38)–OBzl was treated with 5% Na_2CO_3 and the resulting free amino component was subjected to condensation with fragment [3] by DCC–HOBT at 4°C. This Et_3N-free procedure seems to be ideal for suppression of racemization at the Leu residue as well as the acyl urea formation of fragment [3], a side reaction of DCC.

A mixture of DMSO and DMF was employed as a solvent in each condensation of these fragments. Even with this solvent system, the above condensation of fragment [3] gave a pasty mixture as the reaction progressed, so that the DC-urea formed during the reaction could not be removed by simple filtration of the reaction mixture. The product was purified by precipitation from hot DMSO with methanol, while DC-urea was removed by filtration.

Gel-filtration on Sephadex LH-20 was employed for purification of the protected tetradecapeptide ester, Z(OMe)–(hPTH 25—38)–OBzl and gel-filtration on Sephadex LH-60 for the protected eicosapeptide ester, Boc–(hPTH 19—38)–OBzl, with DMSO as the eluent in both cases. Especially for the latter peptide, this procedure was so effective that a contaminant, presumably a Trp-modified derivative, was cleanly separated from the main peak.

As noted above, we were unable to record any *Rf* values of the fragment condensation products, because of the unavailability of suitable solvent systems for TLC. Therefore, the homogeneity of each condensation product was ascertained by careful comparison of the recovery of Ala with those of newly incorporated amino acids in acid hydrolysates (Table I).

Table I. Amino Acid Ratios in a 6N HCl Hydrolysate of Boc-(hPTH 19—38)-OBzl and Intermediates

	29—38	25—38	22—38[a)]	19—38
Asp	1.95(2)	2.03(2)	1.97(2)	1.94(2)
Glu	1.05(1)	1.11(1)	1.98(2)	2.97(3)
Gly	0.99(1)	0.99(1)	1.02(1)	1.00(1)
Ala	1.00(1)	1.00(1)	1.00(1)	1.00(1)
Val	1.98(2)	1.82(2)	1.67(2)	2.79(3)
Leu	1.00(1)	1.92(2)	2.84(3)	2.96(3)
Phe	0.99(1)	0.95(1)	0.93(1)	1.00(1)
Lys		2.00(2)	2.00(2)	1.92(2)
His	0.98(1)	0.90(1)	0.78(1)	1.02(1)
Arg		1.04(1)	1.02(1)	1.89(2)
Trp			0.74(1)	ND
Rec.	90%	84%	79%	90%

a) 4N MSA hydrolysate.

By the combination of gel-filtration and acid hydrolysis, we were thus able to synthesize the protected eicosapeptide ester with a high degree of homogeneity. Further chain elongation of this intermediate to the octatriacontapeptide will be described in the following paper.

Experimental

General experimental procedures used were those described in Part LXXXVIII.[34] An azide prepared according to Honzl and Rudinger with isoamylnitrite and a mixed anhydride was prepared according to Vaughan and Osato with isobutylchloroformate. Thin layer chromatography was performed on silica gel (Kieselgel G, Merck). *Rf* values refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH·H_2O (8:3:1), Rf_2 $CHCl_3$–MeOH (10:0.5), Rf_3 $CHCl_3$–MeOH–AcOH (9:1:0.5).

Z(OMe)-Leu-Gly-OBzl——A mixture of Z(OMe)–Leu–ONP (54.57 g, 0.13 mol), H–Gly–OBzl·tosylate (42.10 g, 0.13 mol) and Et_3N (36.68 ml, 0.26 mol) in DMF (200 ml) was stirred at room temperature for 18 h. The solvent was evaporated off and the oily residue was extracted with AcOEt. The extract was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and concentrated. Trituration of the residue with ether afforded a powder, which was recrystallized from MeOH and ether; yield 42.29 g (76%), mp 94—95°C, $[\alpha]_D^{20}$ −7.8° (c=0.8, DMF), Rf_2 0.53. *Anal.* Calcd for $C_{24}H_{30}N_2O_6$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.40; H, 6.67; N, 6.51.

Z(OMe)-Ala-Leu-Gly-OBzl——Z(OMe)–Leu–Gly–OBzl (5.02 g, 11.4 mmol) was treated with TFA–anisole (9.8 ml–2.5 ml) in an ice-bath for 60 min, then TFA was removed by evaporation. The residue was washed with *n*-hexane, dried over KOH pellets *in vacuo* for 3 h and then dissolved in DMF (20 ml) together with Et_3N (3.2 ml, 22.8 mmol) and Z(OMe)–Ala–ONP (4.27 g, 11.4 mmol). After being stirred for 18 h, the solution was concentrated and the residue was treated with ether and 5% citric acid. The resulting powder was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O and precipitated from DMF with MeOH; yield 4.75 g (81%), mp 148—150°C, $[\alpha]_D^{20}$ −13.9° (c=0.6, DMF), Rf_2 0.38. *Anal.* Calcd for $C_{27}H_{35}N_3O_7$: C, 63.14; H, 6.87; N, 8.18. Found: C, 62.87; H, 7.13; N, 7.95.

Z(OMe)-Phe-Val-OMe——This compound was prepared by the usual DCC condensation of Z(OMe)–Phe–OH (49.40 g, 0.15 mol) with H–Val–OMe (prepared from 25.15 g, 0.15 mmol of the hydrochloride) in DMF–THF (250 ml–250 ml) and purified by the extraction procedure with AcOEt as described above, followed by recrystallization from MeOH and ether; yield 47.98 g (72%), mp 132—133°C, $[\alpha]_D^{20}$ −8.7° (c=1.0, DMF), Rf_2 0.69. *Anal.* Calcd for $C_{24}H_{30}N_2O_6$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.00; H, 6.98; N, 6.44.

Z(OMe)-Phe-Val-NHNH$_2$——Z(OMe)–Phe–Val–OMe (44.25 g, 0.1 mol) in MeOH (400 ml) was treated with 80% hydrazine hydrate (25 ml, 5 eq) at room temperature overnight. The resulting mass was collected by filtration, washed with EtOH and precipitated from DMF with EtOH; yield 36.59 g (83%), mp 206—208°C, $[\alpha]_D^{20}$ −8.0° (c=0.8, DMF), Rf_1 0.52, Rf_3 0.60. *Anal.* Calcd for $C_{23}H_{30}N_{45}O_5$: C, 62.42; H, 6.83; N, 12.66. Found: C, 62.16; H, 6.78; N, 12.57.

Z(OMe)-Phe-Val-Ala-Leu-Gly-OBzl——Z(OMe)–Ala–Leu–Gly–OBzl (3.02 g, 5.9 mmol) was treated with TFA–anisole (7.6 ml–1.9 ml) as usual and TFA was removed by evaporation. The oily residue was washed with *n*-hexane, dried over KOH pellets *in vacuo* for 3 h and then dissolved in DMF (50 ml) containing Et_3N (0.82 ml, 5.9 mmol). The azide (prepared from 2.87 g, 6.5 mmol of Z(OMe)–Phe–Val–$NHNH_2$) in DMF (8 ml) and Et_3N (1.09 ml, 7.8 mmol) were added to the above ice-chilled solution and the mixture was stirred

at 4°C for 48 h. The solution was concentrated and the residue was treated with ether and 5% citric acid. The resulting powder was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O and precipitated from DMF with MeOH; yield 3.97 g (88%), mp 249—250°C, $[\alpha]_D^{20}$ −20.1° (c=0.9, DMF), Rf_2 0.26, Rf_3 0.82. Amino acid ratios in a 6 N HCl hydrolysate: Val 0.97, Phe 1.02, Gly 1.00, Ala 1.00, Leu 1.03 (recovery of Ala 85%). *Anal.* Calcd for $C_{41}H_{53}N_5O_9$: C, 64.80; H, 7.03; N, 9.22. Found: C, 64.66; H, 7.06; N, 9.38.

Z(OMe)-Asn-Phe-Val-Ala-Leu-Gly-OBzl——Z(OMe)–Phe–Val–Ala–Leu–Gly–OBzl (3.52 g, 4.63 mmol) was treated with TFA–anisole (6 ml–1.5 ml) as usual, then dry ether was added. The resulting powder was collected by filtration, washed with ether, dried over KOH pellets *in vacuo* for 3 h and then dissolved in DMSO–DMF (1: 1, 20 ml) together with Et_3N (1.3 ml, 9.3 mmol), HOBT (100 mg, 4.63 mmol) and Z(OMe)–Asn–ONP (1.93 g, 4.63 mmol). While being stirred at room temperature for 48 h, the solution became pasty. The solution was concentrated and the residue was treated with ether and 5% citric acid. The resulting powder was washed as described above and precipitated from DMSO with MeOH; yield 3.03 g (75%), mp 260—262°C, $[\alpha]_D^{20}$ −22.1° (c=1.1, DMSO). Amino acid ratios in a 6 N HCl hydrolysate: Asp 0.99, Gly 0.98, Ala 1.00, Val 0.96, Leu 1.01, Phe 0.95 (recovery of Ala 89%). *Anal.* Calcd for $C_{45}H_{59}N_7O_{11}$: C, 61.84; H, 6.80; N, 11.22. Found: C, 61.82; H, 6.83; N, 11.41.

Z(OMe)-Val-His-Asn-Phe-Val-Ala-Leu-Gly-OBzl——The above protected hexapeptide ester (2.73 g, 3.1 mmol) was treated with TFA–anisole (6.8 ml–1.7 ml) as usual, then dry ether was added and the resulting powder, isolated as described above, was dissolved in DMSO–DMF (1: 1, 20 ml) containing Et_3N (0.44 ml, 3.1 mmol). The azide (prepared from 1.50 g, 3.7 mmol of Z(OMe)–Val–His–$NHNH_2$[34]) in DMF (5 ml) and Et_3N (0.63 ml, 4.5 mmol) were added to the above ice-chilled solution and the mixture was stirred at 4°C for 48 h, while the product began to precipitate. The solution was concentrated and the residue was treated with AcOEt and 5% citric acid. The resulting powder was washed as described above and precipitated from DMSO with MeOH; yield 2.81 g (81%), mp 242—244°C, $[\alpha]_D^{20}$ −21.8° (c=1.0, DMSO). Amino acid ratios in a 6 N HCl hydrolysate: Val 1.93, His 1.03, Asp 1.01, Gly 0.98, Ala 1.00, Leu 1.00, Phe 0.98 (recovery of Ala 87%). *Anal.* Calcd for $C_{56}H_{75}N_{11}O_{13}\cdot H_2O$: C, 59.61; H, 6.88; N, 13.66. Found: C, 59.55; H, 6.96; N, 13.59.

Z(OMe)-Asp(OBzl)-Val-His-Asn-Phe-Val-Ala-Leu-Gly-OBzl——The above protected octapeptide ester (2.81 g, 2.53 mmol) was treated with TFA–anisole (6.6 ml–1.6 ml) and the N^{α}-deprotected peptide, isolated as described above, was dissolved in DMSO–DMF (1: 1, 20 ml) together with Et_3N (1.14 ml, 8.1 mmol) and Z(OMe)–Asp(OBzl)–ONP (1.54 g, 3.04 mmol). While being stirred at room temperature for 48 h, the solution became pasty. The solution was concentrated and the product was purified by washing as described above followed by precipitation from DMSO with MeOH; yield 2.55 g (77%), mp dec. 260°C $[\alpha]_D^{20}$ −16.4° (c=1.0, DMSO). Amino acid ratios in a 6 N HCl hydrolysate: Asp 2.05, Gly 0.98, Ala 1.00, Val 2.01, Leu 1.01, Phe 0.99, His 0.87 (recovery of Ala 84%). *Anal.* Calcd for $C_{67}H_{86}N_{12}O_{16}\cdot 3H_2O$: C, 58.76; H, 6.77; N, 12.27. Found: C, 58.84; H, 6.68; N, 11.87.

Z(OMe)-Gln-Asp(OBzl)-Val-His-Asn-Phe-Val-Ala-Leu-Gly-OBzl, Z(OMe)-(hPTH 29—38)-OBzl [1]——The above protected nonapeptide ester (2.55 g, 1.94 mmol) was treated with TFA–anisole (5.0 ml–1.3 ml) and the N^{α}-deprotected peptide, isolated as described above, was dissolved in DMSO–DMF (1: 1, 12 ml) together with Et_3N (0.92 ml, 6.6 mmol) and Z(OMe)–Gln–ONP (1.17 g, 2.72 mmol). While being stirred at room temperature for 48 h, the solution became pasty. The solution was concentrated and the product was purified by washing as described above, followed by precipitation from DMSO with MeOH; yield 2.76 g (99%), mp 248—249°C, $[\alpha]_D^{20}$ −24.6° (c=1.1, DMSO). Amino acid ratios in a 6 N HCl hydrolysate: Glu 1. 05, Asp 1.98, Gly 0.99, Ala 1.00, Val 1.98, Leu 1.00, Phe 0.99, His 0.89. (recovery of Ala 90%). *Anal.* Cal cd for $C_{72}H_{94}N_{14}O_{18}\cdot 1/2H_2O$: C, 59.53; H, 6.59; N, 13.50. Found: C, 59.58; H, 6.71; N, 13.20.

Z(OMe)-Lys(Z)-Leu-OMe——Z(OMe)–Lys(Z)–OTCP (10.0 g, 16 mmol) in THF (50 ml) and Et_3N (2.24 ml, 16 mmol) were added to a stirred solution of H–Leu–OMe (prepared from 2.91 g, 16 mmol of the hydrochloride) in DMF (50 ml). After 20 h, the solution was concentrated and the oily residue was purified by the usual extraction procedure with AcOEt. After concentration of the solution, the oily residue crystallized with ether. The product was recrystallized from MeOH and ether; yield 7.33 g (80%), mp 107—108°C, $[\alpha]_D^{20}$ −21.2° (c=0.9, MeOH), Rf_1 0.73, Rf_3 0.63. *Anal.* Calcd for $C_{30}H_{41}N_3O_8$: C, 63.03; H, 7.23; N, 7.35. Found: C, 63.18; H, 7.23; N, 7.26.

Z(OMe)-Lys(Z)-Lys(Z)-Leu-OMe——Z(OMe)–Lys(Z)–Leu–OMe (5.72 g, 10 mmol) was treated with TFA–anisole (8.7 ml–2.2 ml) as usual, then dry n-hexane was added. The resulting oily precipitate was washed with n-hexane, dried over KOH pellet *in vacuo* for 3 h and dissolved in DMF (20 ml) together with Et_3N (2.8 ml, 20 mmol) and Z(OMe)–Lys(Z)–OTCP (6.24 g, 10 mmol). After being stirred for 20 h, the solution was concentrated and the residue was treated with ether and 5% citric acid. The resulting powder was purified by washing as described above, followed by recrystallization from MeOH and ether; yield 6.71 g (81%), mp 153—154°C, $[\alpha]_D^{20}$ −15.8° (c=1.1, DMF) Rf_2 0.41. *Anal.* Calcd for $C_{44}H_{59}N_5O_{11}$: 63.37; H, 7.13; N, 8.40. Found: C, 63.54; H, 7.21; N, 8.38.

Z(OMe)-Arg(Mts)-Lys(Z)-Lys(Z)-Leu-OMe——Z(OMe)–Lys(Z)–Lys(Z)–Leu–OMe (6.0 g, 7.2 mmol) was treated with TFA–anisole (9.3 ml–2.3 ml) as usual, then dry n-hexane was added. Treatment of the oily residue with ether afforded a powder, which was dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (20 ml) containing Et_3N (1.0 ml, 7.2 mmol). A mixed anhydride (prepared from 4.50 g, 8.6 mmol of

Z(OMe)–Arg(Mts)–OH) in THF (20 ml) was added to the above ice-chilled solution and the mixture, after being stirred in an ice-bath for 4 h, was concentrated. The residue was treated with 5% citric acid and ether to afford a powder, which was purified by washing as described above, followed by precipitation from DMF with AcOEt; yield 5.78 g (69%), mp 151—152°C, $[\alpha]_D^{20}$ −8.9°(c=0.7, DMF), Rf_1 0.76, Rf_3 0.75. *Anal.* Calcd for $C_{59}H_{81}N_9O_{14}S$: C, 60.44; H, 6.96; N, 10.75. Found: C, 60.48; H, 6.97; N, 10.65.

Z(OMe)-Arg(Mts)-Lys(Z)-Lys(Z)-Leu-NHNH$_2$, Z(OMe)-(hPTH 25—28)-NHNH$_2$ [2]——The above protected tetrapeptide ester (5.78 g, 4.9 mmol) in MeOH (50 ml) was treated with 80% hydrazine hydrate (1.23 ml, 5 eq) overnight. The resulting mass was washed with EtOH and recrystallized from MeOH and EtOH; yield 5.25 g (91%), mp 178—180°C, $[\alpha]_D^{20}$ −10.0° (c=0.7, DMF), Rf_1 0.48. Amino acid ratios in a 6 N HCl hydrolysate: Leu 1.00, Lys 1.94, Arg 1.01 (recovery of Leu 87%). *Anal.* Calcd for $C_{58}H_{81}N_{11}O_{13}S$: C, 59.42; H, 6.96; N, 13.14. Found: C, 59.29; H, 7.17; N, 12.87.

Boc-Trp-Leu-OH·DCHA Salt——Boc–Trp–ONP (2.13 g, 5 mmol) in DMF (6 ml) was added to a solution of H–Leu–OH (0.79 g, 6 mmol) and Et_3N (1.39 ml, 10 mmol) in DMF–H_2O (3 ml–2 ml). After being stirred overnight, the solution was concentrated and the residue was dissolved in 5% $NaHCO_3$. The aqueous phase was washed with AcOEt and acidified with 5% citric acid, and the resulting oily residue was extracted with AcOEt. The extract was washed with 5% citric acid and H_2O–NaCl, dried over Na_2SO_4 and concentrated. The oily residue was dissolved in ether and converted to the corresponding DCHA salt in the usual manner; yield 2.03 g (97%), mp 192—195°C, $[\alpha]_D^{20}$ −22.9°, (c=0.6, MeOH), Rf_1 0.67 Rf_3 0.69. *Anal.* Calcd for $C_{21}H_{31}N_3O_5 \cdot C_{12}H_{23}N$: C, 68.19; H, 9.09; N, 9.36. Found: C, 68.07; H, 9.14; N, 9.27.

Boc-Glu(OBzl)-Trp-Leu-OH·DCHA Salt, Boc-(hPTH 22—24)-OH [3]——Boc–Trp–Leu–OH (7.14 g, 17.1 mmol) was treated with TFA (22.3 ml) in the presence of anisole (5.57 ml) containing 2% EDT in an ice-bath for 60 min, then dry *n*-hexane was added. The resulting oily precipitate (Rf_1 0.12 and two minor spots) was washed with *n*-hexane, dried over KOH pellets *in vacuo* for 3 h and then dissolved in DMF (50 ml) together with Et_3N (4.79 ml, 34.2 mmol) and Boc–Glu(OBzl)–OSu (7.43 g, 17.1 mmol). After being stirred at 4°C for 16 h, the solution was concentrated and the residue was dissolved in 5% NH_4OH. The aqueous phase was washed with ether and acidified with citric acid, and the resulitng oily precipitate was extracted with AcOEt. The extract was washed with H_2O–NaCl, dried over Na_2SO_4 and then concentrated. The oily residue was dissolved in a small amount of MeOH and DCHA (2.8 ml) was added. Removal of the solvent by evaporation and trituration of the residue with ether afforded a solid, which was recrystallized three times from MeOH and ether to afford a homogeneous DCHA salt; yield 7.84 g (57%), mp 149—151°C, $[\alpha]_D^{20}$ −23.1° (c=1.3, MeOH), Rf_1 0.53, Rf_3 0.71. Amino acid ratios in a 4 N MSA hydrolysate: Trp 0.76, Glu 1.12, Leu 1.00 (recovery of Leu 79%). *Anal.* Calcd for $C_{34}H_{44}N_4O_8 \cdot C_{12}H_{23}N$: C, 67.53; H, 8.26; N, 8.56. Found: C, 67.23; H, 8.28; N, 8.69.

Z(OMe)-Arg(Mts)-Val-NHNH-Troc——A mixed anhydride (prepared from 25.20 g, 48.4 mmol of Z(OMe)–Arg(Mts)–OH) in THF (100 ml) was added to an ice-chilled solution of H–Val–NHNH–Troc (prepared from 22.79 g, 48.4 mmol of Z(OMe)-derivative by the usual TFA treatment) in DMF (100 ml) and the mixture was stirred in an ice-bath for 5 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 5% citric acid, 1% NH_4OH and H_2O–NaCl, dried over Na_2SO_4 and then concentrated. The oily residue was purified by column chromatography on silica gel (8.0×20 cm) with $CHCl_3$–MeOH (20: 0.5 v/v). Fractions containing the substance with Rf_3 0.46 were combined, the solvent was removed by evaporation and the residue was dissolved in AcOEt. The organic phase was washed with H_2O, dried over Na_2SO_4 and concentrated. Trituration of the residue with *n*-hexane afforded a powder, which was recrystallized from AcOEt and *n*-hexane; yield 29.25 g (75%), mp 114—116°C, $[\alpha]_D^{20}$ −24.6° (c=1.1, MeOH), Rf_1 0.62, Rf_3 0.46. *Anal.* Calcd for $C_{32}H_{44}Cl_3N_7O_9S$: C, 47.50; H, 5.48; N, 12.12. Found: C, 47.24; H, 5.43; N, 11.88.

Boc-Glu(OBzl)-Arg(Mts)-Val-NHNH-Troc—— Z(OMe)–Arg(Mts)–Val–NHNH–Troc (7.30 g, 9 mmol) was treated with TFA–anisole (15.6 ml–3.9 ml) as usual, then dry ether was added. The resulting powder was dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (50 ml) together with Et_3N (25.2 ml, 18 mmol) and Boc–Glu(OBzl)–ONP (4.13 g, 9 mmol). After being stirred for 16 h, the solution was concentrated and the residue was treated with 5% citric acid and ether. The resulting powder was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O and recrystallized from MeOH and ether; yield 6.29 g (73%), mp 159—160°C, $[\alpha]_D^{20}$ −31.9° (c=1.1, MeOH), Rf_1 0.68, Rf_3 0.74. *Anal.* Calcd for $C_{40}H_{57}Cl_3N_8O_{11}S$: C, 49.82; H, 5.96; N, 11.62. Found: C, 49.85; H, 5.97; N, 11.60.

Boc-Glu(OBzl)-Arg(Mts)-Val-NHNH$_2$, Boc-(hPTH 19—22)-NHNH$_2$ [4]——Boc–Glu(OBzl)–Arg(Mts)–Val–NHNH–Troc (6.29 g, 6.5 mmol) in AcOH (15 ml) was treated with Zn powder (4.25 g, 10 eq) at room temperature for 8 h. The solution was filtered, the filtrate was concentrated and the residue was treated with 5% $NaHCO_3$. The resulting powder was washed with a saturated solution of EDTA and H_2O and then recrystallized from MeOH and EtOH; yield 2.64 g (51%), mp 186—187°C, $[\alpha]_D^{20}$ −5.6° (c=0.7, DMF), Rf_1 0.58, Rf_3 0.55. Amino acid ratios in a 6 N HCl hydrolysate: Glu 1.20, Leu 1.00, Arg 1.07 (recovery of Leu 87%). *Anal.* Calcd for $C_{37}H_{56}N_8O_9S \cdot 1/2H_2O$: C, 55.69; H, 7.20; N, 14.04. Found: C, 55.73; H, 7.18; N, 14.17.

Z(OMe)-Arg(Mts)-Lys(Z)-Lys(Z)-Leu-Gln-Asp(OBzl)-Val-His-Asn-Phe-Val-Ala-Leu-Gly-OBzl, Z(OMe)-(hPTH 25—38)-OBzl——Z(OMe)–(hPTH 29—38)–OBzl [1] (2.76 g, 1.92 mmol) was treated with TFA–

anisole (6.6 ml–1.7 ml) in an ice-bath for 60 min, then dry ether was added. The resulting powder was collected by filtration, washed with ether, dried over KOH pellets *in vacuo* for 3 h and then dissolved in DMSO–DMF (1: 1, 10 ml) containing Et_3N (0.54 ml, 3.84 mmol). The azide (prepared from 2.69 g, 2.3 mmol of Z(OMe)–Arg(Mts)–Lys(Z)–Lys(Z)–Leu–$NHNH_2$) in DMF (10 ml) and Et_3N (0.31 ml, 2.3 mmol) were added to the above ice-chilled solution and the mixture was stirred at 4°C for 48 h, during which time the product began to precipitate from the solution. The solution was concentrated and the residue was treated with AcOEt and 5% citric acid. The resulting powder was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O and precipitated twice from DMSO with MeOH; yield 3.50 g (75%). The purity of the product was examined by gel-filtration on Sephadex LH-20. A sample (62 mg) in DMSO (3 ml) was applied to a column of LH-20 (2×141 cm), and eluted with the same solvent at a flow rate of 15 ml/h. When the UV absorption of eluted fractions (5 ml each) was measured at 260 nm, a single peak was detected; recovery 49 mg (80%). mp 260°C (dec.), $[\alpha]_D^{20}$ −15.9° (c=0.6, DMSO), *Anal.* Calcd for $C_{121}H_{163}N_{23}O_{28}S\cdot 1.5H_2O$: C, 59.39; H, 6.84; N, 13.17. Found: C, 59.29; H, 6.83; N, 13.23.

Boc-Glu(OBzl)-Trp-Leu-Arg(Mts)-Lys(Z)-Lys(Z)-Leu-Gln-Asp(OBzl)-Val-His-Asn-Phe-Val-Ala-Leu-Gly-OBzl, Boc-(hPTH 22—38)-OBzl——Z(OMe)–(hPTH 25—38)–OBzl (3.50 g, 1.45 mmol) was treated with TFA–anisole (6.3 ml, 1.6 ml) as described above and *n*-hexane was added. The resulting oily precipitate was triturated with 5% $NaHCO_3$ and ether to afford a powder, which was dried over KOH pellets *in vacuo* for 3 h and then dissolved in DMSO–DMF (1: 1, 20 ml), together with HOBT (0.54 g, 3.5 mmol) and Boc–Glu(OBzl)–Trp–Leu–OH [3] (prepared from 2.33 g, 2.9 mmol of the DCHA salt as usual). Under cooling with ice, DCC (0.72 g, 3.5 mmol) was added and the mixture was stirred at 4°C for 72 h, during which time the solution became pasty. The solvent was evaporated off and the residue was treated with 5% citric acid and ether. The resulting powder was washed as described above and contaminating DC-urea was removed in the purification step by precipitation from DMSO with MeOH; yield 2.85 g (68%), mp 260°C (dec.), $[\alpha]_D^{20}$ −19.8° (c=0.8, DMSO). *Anal.* Calcd for $C_{146}H_{197}N_{27}O_{32}S\cdot 7H_2O$: C, 58.43; H, 6.83; N, 12.61. Found: C, 58.44; H, 7.09; N, 12.58.

Boc-Glu(OBzl)-Arg(Mts)-Val-Glu(OBzl)-Trp-Leu-Arg(Mts)-Lys(Z)-Lys(Z)-Leu-Gln-Asp(OBzl)-Val-His-Asn-Phe-Val-Ala-Leu-Gly-OBzl, Boc-(hPTH 19—38)-OBzl——Boc-(hPTH 22—38)–OBzl (2.81 g, 0.98 mmol) was treated with TFA (8.5 ml)–anisole containing 2% EDT (2.1 ml) as usual and the N^a-deprotected peptide was precipitated with ether as a powder, then dissolved in DMSO–DMF (1: 1, 20 ml) containing Et_3N (0.27 ml, 1.96 mmol). The azide (prepared from 1.54 g, 1.96 mmol of Boc–Glu(OBzl)–Arg(Mts)–Val–$NHNH_2$) in DMF (3 ml) and Et_3N (0.27 ml, 1.76 mmol) were added to the above ice-chilled solution and the mixture, after being stirred at 4°C for 48 h, was concentrated. The residue was treated with 5% citric acid and AcOEt and the resulting powder was purified by washing as described above followed by precipitation from DMSO with MeOH; yield 3.01 g (87%). In order to assess the homogeneity, a sample (61 mg) in DMSO (2 ml) was applied to a column of Sephadex LH-60 (2×141 cm), and eluted with the same solvent. UV absorption of individual fractions (5 ml each) was measured at 280 nm. A main peak (tube Nos. 40—50) with a small front shoulder (30—37) was detected. The solvent of the main fractions was evaporated off and the residue was treated with MeOH to afford a powder; yield 45 mg (74%). The rest of the product was similarly purified. mp 165—168°C, $[\alpha]_D^{20}$ +25.3° (c=0.4, DMF) *Anal* Calcd for $C_{178}H_{241}N_{33}O_{39}S_2\cdot 11H_2O$: C, 57.32; H, 7.11; N, 12.40. Found: C, 56.98; H, 6.65; N, 12.92.

References and Notes

1) Studies on peptides CVII: K. Akaji, N. Fujii, H. Yajima, and D. Pearson, *Chem. Pharm. Bull.*, **30**, 349 (1982).
2) Amino acids, peptides and their derivatives mentioned in this paper are of the L-configuration. Abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochem.*, **5**, 2486 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972); Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Boc=*tert*-butoxycarbonyl, Bzl=benzyl, Mts=mesitylene-2-sulfonyl, Troc=β,β,β-trichloroethyloxycarbonyl, NP=*p*-nitrophenyl, DCC=dicyclohexylcarbodiimide, HOBT=1-hydroxybenzotriazole, TFA=trifluoroacetic acid, DMF=dimethylformamide, Su=*N*-hydroxysuccinimide, TCP=trichlorophenyl, DMSO=dimethyl sulfoxide, EDT=ethanedithiol, DCHA=dicyclohexylamine, THF=tetrahydrofuran, MSA=methanesulfonic acid, EDTA=ethylenediaminetetraacetic acid disodium salt.
3) H.B. Brewer, Jr. and R. Ronan, *Proc. Natl. Acad. Sci. U.S.A.*, **67**, 1862 (1970).
4) H.D. Niall, H.T. Keutmann, R. Sauer, M. L. Hogan, B. Dawson, G.D. Aurbach, and J.T. Potts, Jr., *Z. Physiol. Chem.*, **351**, 1586 (1970).
5) R.T. Sauer, H.D. Niall, M.L. Hogan, H.T. Keutmann, J.L.H. O'Riordan, and J.T. Potts, Jr., *Biochem.*, **13**, 1994 (1974).
6) H.T. Keutmann, M.M. Sauer, G.N. Hendy, J.L.H. O'Riordan, and J.T. Potts, Jr., *Biochem.*, **17**, 5723 (1978).
7) J.W. Jacobs, B. Kemper, H.D. Niall, J.F. Habener, and J.T. Potts, Jr., *Nature*, **249**, 155 (1974).

8) H.B. Brewer, Jr., T. Fairwell, R. Ronan, G.W. Sizemore, and C.D. Arnaud, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 3585 (1972); H.B. Brewer, Jr., T. Fairwell, W. Rittel, T. Littledicke, and C.D. Arnaud, *Amer. J. Med.*, **56**, 759 (1974).
9) H.D. Niall, R.T. Sauer, J.W. Jacobs, H.T. Keutmann, G.V. Segre, J.L.H. O'Riordan, G.D. Aurbach, and J.T. Potts, Jr., *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 384 (1974); H.T. Keutmann, H.D. Niall, J.L.H. O'Riordan, and J.T. Potts, Jr., *Biochem.*, **14**, 1842 (1975).
10) H.T. Keutmann, B.F. Dawson, G.D. Aurbach, and J.T. Potts, Jr., *Biochem.*, **11**, 1973 (1972).
11) J.T. Potts, Jr., G.W. Tregear, H.T. Keutmann, H.D. Niall, R. Sauer, L.J. Deftos, B.F. Dawson, M.L. Hogan, and G.D. Aurbach, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 63 (1971).
12) *a*) G.W. Tregear, J. van Rietschoten, E. Greene, H.D. Niall, H.T. Keutmann, J.A. Parsons, J.L.H. O'Riordan, and J.T. Potts, Jr., *Z. Physiol. Chem.*, **355**, 415 (1974); *b*) J.D. Monet, C.A. Bader, E. Herbingny, and J.L. Funck-Brentano, *FEBS Lett.*, **96**, 76 (1978).
13) G.W. Tregear, J. van Rietschoten, R. Sauer, H.D. Niall, H.T. Keutmann, and J.T. Potts, Jr., *Biochem.*, **16**, 2817 (1977).
14) R.H. Andreatta, A. Hartmann, A. Johl, B. Kamber, R. Maler, B. Riniker, W. Rittel, and P. Sieber, *Helv. Chim. Acta*, **56**, 470 (1973).
15) M. Takai, Y. Kurano, H. Kimura, and S. Sakakibara, "Peptide Chemistry," ed. H. Yonehara, Protein Res. Found., Osaka, 1979, p. 187.
16) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Jpn.*, **40**,21 64(1967).
17) H. Yajima, N. Fujii, H. Ogawa, and H. Kawatani, *J. Chem. Soc., Chem. Commun.*, **1974**, 107; H. Irie, N. Fujii, H. Ogawa, H. Yajima, M. Fujino, and S. Shinagawa, *ibid.*, **1976**, 922; N. Fujii, S. Funakoshi, T. Sasaki, and H. Yajima, *Chem. Pharm. Bull.*, **25**, 3096 (1977).
18) Y. Kiso, S. Nakamura, K. Ito, K. Ukawa, K. Kitagawa, T. Akita, and H. Moritoki, *J. Chem. Soc., Chem. Commun.*, **1979**, 971; Y. Kiso, K. Ito, S. Nakamura, K. Kitagawa, T. Akita, and H. Moritoki, *Chem. Pharm. Bull.*, **27**, 1472 (1979).
19) H. Yajima, M. Takeyama, J. Kanaki, and K. Mitani, *J. Chem. Soc., Chem. Commun.*, **1978**, 482; H. Yajima, M. Takeyama, J. Kanaki, O. Nishimura, and M. Fujino, *Chem. Pharm. Bull.*, **26**, 3572 (1978).
20) B. Iselin, *Helv. Chim. Acta*, **44**, 61 (1961).
21) N. Fujii, T. Sasaki, S. Funakoshi, H. Irie, and H. Yajima, *Chem. Pharm. Bull.*, **26**, 650 (1978).
22) J. Honzl and J. Rudinger, *Collect. Czech. Chem. Comm.*, **26**, 2333 (1961).
23) H. Yajima and Y. Kiso, *Chem. Pharm. Bull.*, **19**, 420 (1971).
24) R.B. Woodward, K. Heusler, J. Gosteli, P. Naegeli, W. Oppolzer, R. Ramage, S. Ranganathan, and H. Vorbrüggen, *J. Am. Chem. Soc.*, **88**, 852 (1966).
25) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
26) Yu. B. Alakhov, A.A. Kiryushkin, V.M. Lipkin, and G.W.A. Milne, *J. Chem. Soc., Chem. Commun.*, **1970**, 406; E. Wünsch, E. Jaeger, L. Kisfaludy, and M. Löw, *Angew. Chem.*, **89**, 330 (1977); E. Jaeger, P. Thamm, S. Knof, E. Wünsch, M. Löw, and L. Kisfaludy, *Z. Physiol. Chem.*, **359**, 1617 (1978); E. Jaeger, P. Thamm, S. Knof, and E. Wünsch, *ibid.*, **359**, 1629 (1978); M. Löw, L. Kisfaludy, E. Jaeger, P. Thamm, S. Knof, and E. Wünsch, *ibid.*, **359**, 1637 (1978); M. Löw, L. Kisfaludy, and P. Sohar, *ibid.*, **359**, 1643 (1978); Y. Mori, Y. Matsuda, S. Aimoto, Y. Shimonishi, and M. Yamamoto, *Chemistry Lett.*, **1976**, 805; K. Hashizume and Y. Shimonishi, in "Peptide Chemistry," ed. by H. Yonehara, Protein Res. Found., Osaka, 1979, p. 77; Y. Masui, N. Chino, and S. Sakakibara, *Bull. Chem. Soc. Japan*, **53**, 464 (1980).
27) H. Ogawa, T. Sasaki, H. Irie, and H. Yajima, *Chem. Pharm. Bull.*, **26**, 3144 (1978).
28) J.J. Sharp, A.B. Robinson, and M.D. Kamen, *J. Am. Chem. Soc.*, **95**, 6097 (1973); H. Yajima, H. Ogawa, M. Kubota, T. Tobe, M. Fujimura, K. Henmi, K. Torizuka, H. Adachi, H. Imura, and T. Taminato, *J. Amer. Chem. Soc.*, **97**, 5593 (1975).
29) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).
30) J. Pless and R.A. Boissonnas, *Helv. Chim. Acta*, **46**, 1609 (1959).
31) Th. Wieland and H. Bernhard, *Ann. Chem.*, **572**, 190 (1951); R.A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951); J.R. Vaughan, Jr. and R.L. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1952).
32) G.W. Anderson, J.E. Zimmerman, and F. Callahan, *J. Am. Chem. Soc.*, **85**, 3039 (1963); *ibid.*, **86**, 1839 (1964).
33) H. Ogawa, M. Kubota, H. Yajima, T. Tobe, M. Fujimura, K. Henmi, K. Torizuka, H. Adachi, H. Imura, and T. Taminato, *Chem. Pharm. Bull.*, **24**, 2447 (1976).
34) N. Fujii and H. Yajima, *J.C.S. Perkin I*, **1981**, 789.