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Studies on Peptides. CIX.[1,2] Synthesis of the Octatriacontapeptide Corresponding to Positions 1 to 38 of Human Parathyroid Hormone

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The octatriacontapeptide corresponding to positions 1 to 38 of human parathyroid hormone (hPTH), a half of the whole molecule, was synthesized by assembling 9 peptide fragments in a conventional manner. Arg(mesitylene-2-sulfonyl), a new arginine derivative bearing an acid-labile protecting group, was employed in combination with a new deprotecting procedure with 1 M trifluoromethanesulfonic acid–thioanisole in TFA. The synthetic peptide exhibited an activity of 1400 IU/mg when assayed by the mouse bone adenyl cyclase activity assay.

Keywords——synthesis of an active fragment of human parathyroid hormone; β,β,β-trichloroethyloxycarbonylhydrazine; N^G-mesitylene-2-sulfonylarginine; reduction of methionine sulfoxide; 1 M trifluoromethanesulfonic acid in TFA as a deprotecting reagent; thioanisole as a cation scavenger; mouse bone adenyl cyclase activity.

As described in the preceding paper,[1] the discrepancy between two sequence proposals[3,4] for the N-terminal active fragment of human parathyroid hormone (hPTH 1—34) was resolved by Keutmann *et al.*[5] in 1978. Thus, we decided to synthesize the N-terminal octatriacontapeptide, a longer peptide than the active fragment which was previously synthesized by other authors.[6-8] As reported in the preceding paper, the protected eicosapeptide ester corresponding to residues 19 to 38 of our target, Boc-(hPTH 19—38)-OBzl, was synthesized by assembling four peptide fragments; Z(OMe)-(29—38)-OBzl [1], Z(OMe)-(25—28)-$NHNH_2$ [2], Boc-(22—24)-OH [3] and Boc-(19—21)-$NHNH_2$ [4].

We wish to report in this paper that we succeeded in synthesizing the octatriacontapeptide by further chain elongation of the above intermediate followed by deprotection of all protecting groups employed by a new deprotecting reagent with TFMSA[9] in the presence of thioanisole.[10,11] As shown in Fig. 1, five hydrazides, [5] to [9], were newly synthesized using amino acid derivatives bearing protecting groups removable by the above reagent, *i.e.*,; Lys(Z) and Glu(OBzl). The Met residue was protected as its sulfoxide[12,13] to prevent partial oxidation during the synthesis.

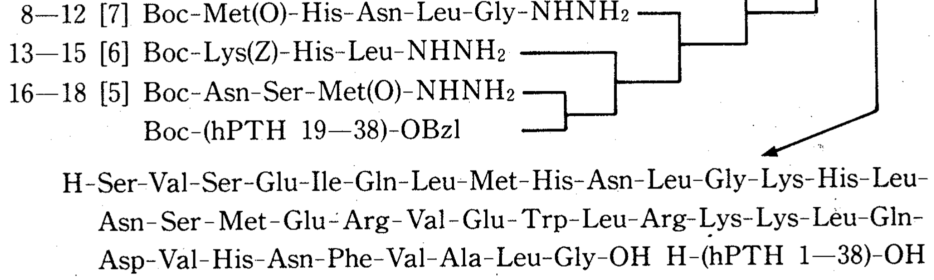

Fig. 1. Synthetic Route to Human Parathyroid Hormone (1—38)

First, the fragment Boc–Asn–Ser–Met(O)–$NHNH_2$ [5] was synthesized according to the scheme illustrated in Fig. 2. Z(OMe)–Ser–Met(O)–OMe, prepared by the azide procedure,[14] was found to be freely soluble in water. The analytically pure product was obtained by extraction with *n*-butanol, followed by column chromatographic purification on silica gel. This product, after TFA treatment, was subjected to condensation with Boc–Asn–OH by the NP active ester procedure.[15] The product, Boc–Asn–Ser–Met(O)–OMe, was still partially soluble in water and therefore was purified solely by recrystallization from organic solvents, without water-washing. Conversion of the tripeptide ester to the corresponding hydrazide [5] proceeded smoothly without particular difficulty.

As shown in Fig. 3, Boc–Lys(Z)–His–Leu–$NHNH_2$ [6] was prepared starting with H–Leu–OMe by successive condensation with Z(OMe)–His–$NHNH_2$ and Boc–Lys(Z)–OSu followed by the usual hydrazine treatment of the resulting ester, Boc–Lys(Z)–His–Leu–OMe. But conversion of the above tripeptide ester to the corresponding hydrazide required somewhat more prolonged treatment with hydrazine than usual, presumably due to the steric hindrance of the Leu residue. Even after 48 h's treatment with hydrazine hydrate at room temperature, a certain amount of the starting material was still detected on thin layer chromatography (TLC). The product, which is soluble in MeOH and EtOH, was purified by repeated precipitation from MeOH with AcOEt.

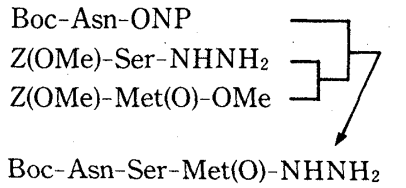

Fig. 2. Synthetic Scheme for the Protected Tripeptide Hydrazide Boc-(hPTH 16—18)-$NHNH_2$ [5]

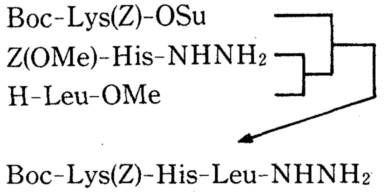

Fig. 3. Synthetic Scheme for the Protected Tripeptide Hydrazide Boc-(hPTH 13—15)-$NHNH_2$ [6]

The synthetic scheme for fragment [7] is illustrated in Fig. 4. This fragment possesses the Gly residue at the C-terminus, so that racemization -free condensation is feasible. However, considering the superiority of the azide reaction for fragment condensation, we decided to introduce this fragment by the azide procedure. The starting dipeptide, Z(OMe)–Leu–Gly–OMe, was prepared by the NP method. This, after TFA treatment, was subjected to the next condensation with Z(OMe)–Asn–OH by the same active ester procedure. Subsequent introduction of a His residue was performed by the usual azide procedure to afford Z(OMe)–His–Asn–Leu–Gly–OMe, which was subjected to further condensation with Boc–Met(O)–OH by the mixed anhydride procedure.[16] For some reason, alternative condensation by the NP method afforded a product containing some impurities. The resulting protected pentapeptide ester, Boc–Met(O)–His–Asn–Leu–Gly–OMe, was smoothly converted to the corresponding hydrazide [7] in the usual manner.

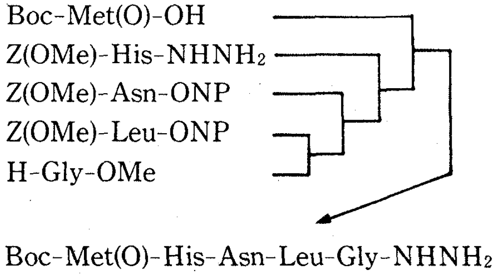

Fig. 4. Synthetic Scheme for the Protected Pentapeptide Hydrazide Boc-(hPTH 8—12)-$NHNH_2$ [7]

The synthetic scheme for fragment [8] is illustrated in Fig. 5. In order to prepare the fragment containing Glu(OBzl), a substituted hydrazine, Troc–$NHNH_2$,[17] was employed, as described for the preparation of fragment [4]. Z(OMe)–Leu–NHNH–Troc, which was prepared with Z (OMe)–Leu–OH and Troc–$NHNH_2$ by the mixed anhydride procedure, was treated with TFA as usual and then condensed with Z(OMe)–Gln–ONP to afford Z(OMe)–

Gln–Leu–NHNH–Troc. Next, the NP method was employed to introduce Z(OMe)–Ile–OH and the Su method[18] for Boc–Glu(OBzl)–OH. Treatment of the resulting tetrapeptide derivative, Boc–Glu(OBzl)–Ile–Gln–Leu–NHNH–Troc, with Zn in AcOH afforded the fragment [8] in a satisfactory yield. The last trace of contaminating Zn acetate was removed by treatment with EDTA. The hydrazide test, elemental analysis and amino acid analysis were consistent with homogeneity of the desired product.

The N-terminal fragment, Z–Ser–Val–Ser–NHNH$_2$ [9], was prepared according to the scheme illustrated in Fig. 6. Z(OMe)–Val–Ser–OMe was prepared by the DCC procedure,[19] though the yield was reduced because of the acylurea formation, a side reaction of DCC.[20] The product, after recrystallization from THF with EtOH, was condensed with Z–Ser–NHNH$_2$. The resulting tripeptide ester, Z–Ser–Val–Ser–OMe, was converted smoothly to the corresponding hydrazide [9] in the usual manner.

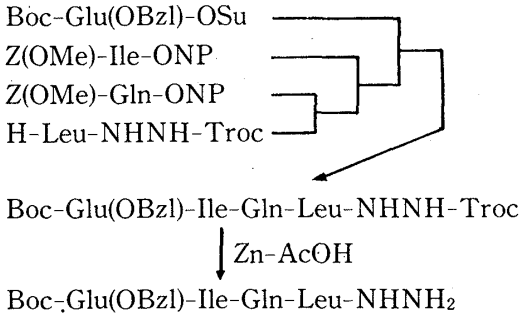

Fig. 5. Synthetic Scheme for the Protected Tetrapeptide hydrazide Boc-(hPTH 4—7)-NHNH$_2$ [8]

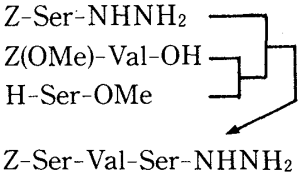

Fig. 6. Synthetic Scheme for the Protected Tripeptide Hydrazide Z-(hPTH 1—3)-NHNH$_2$ [9]

Five fragments thus obtained were then assembled successively onto the eicosapeptide, Boc–(hPTH 19—38)–OBzl, by the azide procedure. As discussed in the preceding paper, the TFA treatment of Trp-containing peptide leads to side reaction at its indole moiety.[21] Therefore, anisole containing 2% EDT[22] was employed to suppress this side reaction. As far as estimated on TLC, the amount of side product was reduced when the Boc group was removed from peptides containing Trp not at the N-terminus, rather than from N$^\alpha$-Trp residue. In the azide condensation of fragment [5] with the TFA-treated sample of Boc–(hPTH 19—38)–OBzl, two equivalents of the acyl component were sufficient to bring the reaction to completion, though the reaction had to be performed in a mixture of DMSO and DMF. At this stage, we were able to follow the progress of the reaction and the homogeneity of the product by TLC, because of increasing solubility of the product in DMF. As reported in the preceding paper,[1] we were not able to record the *Rf* values of the protected eicosapeptide and some other peptides because of the unavailability of suitable solvents for TLC. Our ability to assess the homogeneity of the desired product was thus improved immensely at this step. The product, Boc–(hPTH 16—38)–OBzl, was isolated in a satisfactory yield after purification by column chromatography on Sephadex LH-60, where a small peak, presumably due to the Trp-modified product, was separated. Throughout the synthesis, as described previously, Ala (which occurs once near the C-terminal portion in the molecule) was selected as a diagnostic amino acid in acid hydrolysates of fragment condensation products. By comparison of the recovery of Ala in an acid hydrolysate with those of newly incorporated amino acids, satisfactory incorporation of each fragment could be ascertained, as shown in Table 1.

Chain elongation of Boc–(hPTH 16—38)–OBzl to Boc–(hPTH 13—38)–OBzl was performed in DMF, without the aid of DMSO, using in total 4 eq of fragment [6]. To obtain Boc–(hPTH 8—38)–OBzl, condensation of fragment [7] with the TFA-treated sample of Boc–(hPTH 13—38)–OBzl was next performed using two additions of 2 eq of the acyl component at 48-h intervals, and the crude product was purified by column chromatography on silica

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Table I. Amino Acid Ratios in a 6N HCl Hydrolysate of Z–(hPTH 1—38)–OBzl and Intermediates

	16—38	13—38	8—38	4—38	1—38	
Asp	2.94	2.91	3.87	3.60	4.14	4
Ser	0.78	0.79	0.62	0.72	2.21	3
Glu	3.12	3.11	3.16	5.36	5.47	5
Gly	1.01	1.08	1.94	1.91	2.01	2
Ala	1.00	1.00	1.00	1.00	1.00	1
Val	2.85	2.91	3.08	2.92	3.98	4
Met[a]	0.61	0.82	1.27	1.22	1.54	2
Ile				1.22	0.94	1
Leu	3.02	4.18	4.67	6.00	6.39	6
Phe	0.99	0.96	0.97	0.99	0.93	1
Lys	2.01	3.48	2.84	3.00	2.99	3
His	0.93	1.87	2.16	2.84	2.70	3
Arg	2.03	1.61	1.91	2.02	2.01	2
Trp	ND	ND	ND	ND	ND	1
Rec.	92%	80%	79%	82%	80%	

a) Met+Met(O).

gel with $CHCl_3$–MeOH–H_2O (8 : 3 : 1) as the eluent; some impurity with low *Rf* was removed in this step. Next, condensation of fragment [8] with the TFA-treated sample of Boc–(hPTH 8—38)–OBzl was also performed in DMF with two additions of 4 eq of the acyl component at 20-h intervals. Since the solubility of the product was not sufficient to perform silica gel chromatography as stated above, we tried to remove the unreacted acyl component by repeated precipitation from DMF with AcOEt and we then purified the product by gel-filtration on Sephadex LH-60 using DMF. The product showed essentially a single spot on TLC ,but the recovery of newly incorporated Ile in an acid hydrolysate was somewhat higher than the theoretical value indicating that the product, Boc–(hPTH 4—38)–OBzl, was still contaminated with a certain amount of the unreacted acyl component (*ca.* 22%). We decided to remove the impurity after performing the final condensation.

The final condensation was performed using 4 eq of acyl component [9] and after 30 h, additional azide (4 eq) was added. The product was then purified by gel-filtration on Sephacryl S-200 using DMF containing 5% H_2O.[23] A main peak with shoulders in the front and tail portions was detected (Fig. 7a). An acid hydrolysate of the main peak revealed contamination with the acyl component (*ca.* 10%). This product was then rechromatographed (Fig. 7b). The main product thus obtained gave satisfactory recovery of constituent amino acids in an acid hydrolysate with the ratios predicted by theory. With the aid of this new purification technique, we were thus able to obtain the protected octatriacontapeptide ester, Z-(hPTH 1—38)–OBzl, with a high degree of homogeniety.

Next, deprotection and subsequent purification were carried out according to the following scheme (Fig. 8). The protected octatriacontapeptide ester, Z–(hPTH 1—38)–OBzl, was treated with 1 M TFMSA in TFA[24] in the presence of thioanisole and *m*-cresol in an ice-bath for 60 min, then at 10°C for 60 min. This acid concentration in TFA has sufficient acidity to cleave the Mts group from Arg residue[25] within 60 min at 0°C, as well as all other protecting groups based on benzyl alcohol, without accompanying marked aminosuccinimide formation at the Asp(OBzl) residue.[26] Besides *m*-cresol, thioanisole was used as an additional scavenger, since this sulfur compound has the ability to accelerate the acidolytic cleavage by trapping alkyl cations as *S*-sulfonium compounds.[13] This treatment was repeated twice to ensure complete deprotection. The deprotected peptide, H–[8–Met(O), 18–Met(O)–hPTH 1—38]–OH was precipitated by ether, converted to the corresponding acetate by treatment with Amberlite

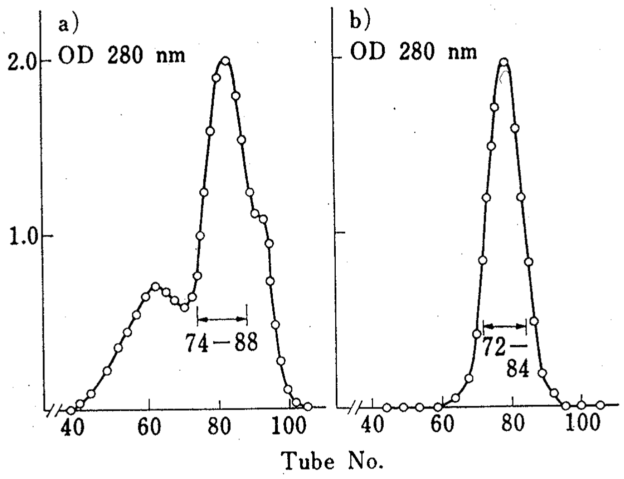

Fig. 7. Purification of Z-(hPTH 1—38)-OBzl by Gel-filtration on Sephacryl S-200

Z-(hPTH 1—38)-OBzl
1. 1 M TFMSA-thioanisole in TFA, *m*-cresol
2. Amberlite CG-4B (acetate)
↓
H-[8-Met(O), 18-Met(O)-hPTH 1—38]-OH
1. reduction with dithiothreitol (60°C 36 h)
2. Sephadex G-25
3. CM-cellulose ($AcONH_4$buffer-8 M urea)
4. reduction with dithiothreitol
5. Sephadex G-25
↓
purified H-(hPTH 1—38)-OH

Fig. 8. Deprotection and Purification Scheme for H-(hPTH 1—38)-OH

CG-4B and then treated with dilute ammonia to reverse a possible N→O shift at the Ser residues.[27]

The treated product was next incubated with dithiothreitol to reduce two Met(O) residues[28] in H_2O at 60°C for 36 h. It seems worthwhile to note that TFMSA–thioanisole in TFA reduced Met(O) to methionine in *ca.* 80% yield within 60 min at 0°C in a model experiment.[29] Thus, reduction of two Met(O) residues was ensured by two successive treatments; first by acid-thioanisole treatment and secondly by dithiothreitol treatment. After removing salts and the reducing reagent by gel-filtration on Sephadex G-25, the product was purified by ion-exchange chromatography on CM-cellulose. According to Tregear *et al.*,[6] the product was eluted with a gradient up to 0.1 M ammonium acetate in 8 M urea in order to prevent coagulation. As shown in Fig. 9, two side peaks were detected in front of the main peak. These two peaks seem to be due to the unreduced mono and di-Met(O) derivatives, since Met recoveries in these acid hydrolysates were lower than in that of the main product. The result indicates that from a practical standpoint, complete reduction of the two Met(O) residues becomes difficult with increasing molecular size, despite the two-stage treatments mentioned above. After elution of the main peak, another side peak was detected. This peak seems to be due to incomplete deprotection of products. This material was less soluble in water. The main product was rechromatographed on CM-cellulose. The product thus obtained was treated with dithiothr-

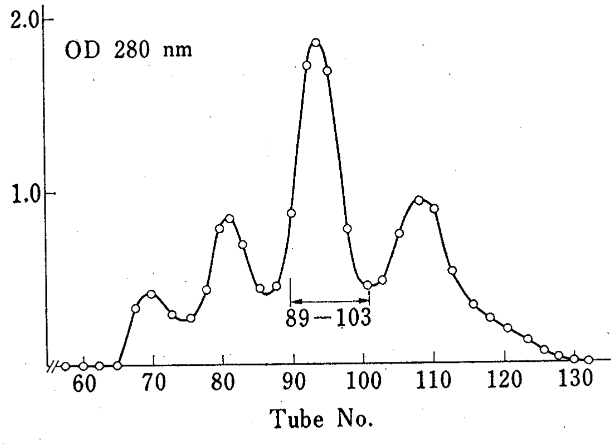

Fig. 9. Purification of Synthetic H-(hPTH 1—38)-OH by Ion-exchange Chromatography on CM-cellulose

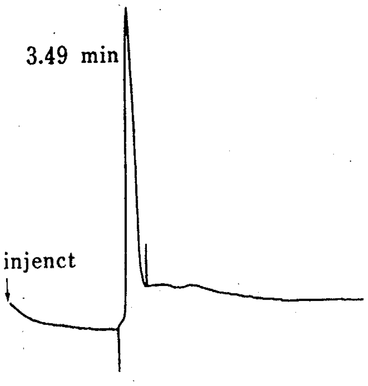

Fig. 10. HPLC of Synthetic H-(hPTH 1—38)-OH

eitol to reduce possible re-oxidized Met residue and then desalted by gel-filtration on Sephadex G-25.

The product thus purified exhibited a single spot on TLC and a single peak in HPLC (Fig. 10). It behaved as a singel component upon disc-electrophoresis and disc-isoelectrofocusing. Its homogeneity was confirmed by amino acid analysis after acid hydrolysis and enzymatic digestion. Especially in the latter case, the presence of Trp, Gln and Asn residues in the synthetic peptide was ascertained. The peptide obtained here is very sensitive to air oxidation. When a solution stored at room temperature for several days was examined by HPLC, two small peaks were detected in front of the main peak. These peaks became prominent when 5% hydrogen peroxide was added to the solution.

Our synthetic peptide exhibited an activity of 1400 IU/mg, when the mouse bone adenyl cyclase activity was assayed according to Luben *et al.*,[30] using calvaria cells obtained from newborn mouse. The recorded activity of natural human PTH is 350 IU/mg. The reported activities of synthetic active fragments, H-(hPTH 1—34)-NH_2[7] and H-(hPTH 1—34)-OH,[8] are 1574 IU/mg and 2457 IU/mg, respectively. As we will report in a future paper, our synthetic H-(hPTH 1—34)-NH_2 exhibited an activity of 3380 IU/mg. In comparison with the reported activities of a series of bovine PTH peptides,[31] *i.e.*, native bPTH (3000 IU/mg), H-(bPTH 1—34)-OH (5400 IU/mg), H-(bPTH 1—26)-OH (0.3% of that of the native bPTH), H-(bPTH 1—27)-OH (2%) and H-(bPTH 1—28)-OH (5%), it appears that as far as the activity on a weight basis is concerned, the activity of PTH molecules, including hPTH, increases sharply with chain elongation from 28 to 34, reaches a maximum at 34 and then declines somewhat.

Experimental

General experimental methods in this investigation were essentially the same as described in Part LXXXVIII[32] of this series. Synthetic hPTH (1—34) was purchased from the Protein Research Foundation (Lot No. 300331). HPLC was performed using a Waters Compact Model with a μBondapak C_{18} (0.25″×1′) column; gradient elution was carried out starting from acetonitrile–0.1 M H_3PO_4 in 0.1% AcOH (30:70) to (50:50), flow rate=1 ml/min. 8 M urea solution for ion-exchange chromatography on CM-cellulose was freshly prepared and passed through an Amberlite MB-3 deionizing resin to remove traces of cyanate or other ions. TLC was performed on silica gel (Kiesel gel G, Merck) and *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), Rf_4 *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2). TLC of the synthetic hPTH (1—38) was performed on DC-Alufolien Cellulose (Merck) and *Rf* values refer to the following solvent systems; Rf_{I} *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2), Rf_{II} *n*-BuOH–AcOH–AcOEt–H_2O (1:1:1:1).

Z(OMe)-Met(O)-OMe——An ethereal solution of diazomethane was added to an ice-chilled solution of Z(OMe)–Met(O)–OH (28.46 g) in MeOH (70 ml); the yellow color persisted for 30 min. After addition of a few drops of AcOH, the solvent was evaporated off. The residue was triturated with ether and recrystallized from AcOEt and ether; yield 27.18 g (92%); mp 81—82°C, $[\alpha]_D^{18}$ −41.8° (*c*=1.0, MeOH), Rf_1 0.72. *Anal.* Calcd for $C_{15}H_{21}NO_6S$: C, 52.46; H, 6.16; N, 4.08. Found: C, 52.16; H, 6.09; N, 4.20.

Z(OMe)-Ser-Met(O)-OMe——Z(OMe)–Met(O)–OMe (9.02 g, 26.3 mmol) was treated with TFA–anisole (22.8—5.7 ml) as usual, then TFA was removed by evaporation. The residue was washed with *n*-hexane, dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (40 ml) containing Et_3N (3.7 ml, 26.3 mmol). The azide [prepared from 8.95 g (31.6 mmol) of Z(OMe)–Ser–$NHNH_2$] in DMF (20 ml) and Et_3N (5.3 ml, 37.9 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 extracted with *n*-BuOH. The extract was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O, dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel (7.2×20 cm), with $CHCl_3$–MeOH (10:0.5, v/v) as an eluent. The fractions containing the substance of Rf_1 0.60 were combined and the solvent was removed by evaporation. The residue was triturated with AcOEt and recrystallized from MeOH and AcOEt; yield 7.22 g, (64%), mp 109—110°C, $[\alpha]_D^{20}$ −36.4° (*c*=1.0, DMF), Rf_1 0.60, Rf_3 0.25. *Anal.* Calcd for $C_{18}H_{26}N_2O_8S$: C, 50.22; H, 6.09; N, 6.51. Found: C, 50.31; H, 6.05; N, 6.43.

Boc-Asn-Ser-Met(O)-OMe——Z(OMe)–Ser–Met(O)–OMe (5.97 g, 13 mmol) was treated with TFA–anisole (11—2.8 ml) as usual, then dry ether was added. The resulting powder, after being dried over KOH pellets *in vacuo* for 3 h, was dissolved in DMF (25 ml) together with Et_3N (3.6 ml, 26 mmol) and Boc–Asn–ONP (4.58 g, 13 mmol). After being stirred at room temperature for 18 h, the solution was concentrated

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and the residue was treated with AcOEt. The resulting powder was washed with AcOEt and recrystallized from MeOH and AcOEt; yield 4.99 g (80%), mp 181—182°C, $[\alpha]_D^{20}$ −49.3° (c=0.8, DMF), Rf_1 0.46, Rf_3 0.06. *Anal.* Calcd for $C_{18}H_{32}N_4O_9S$: C, 44.99; H, 6.71; N, 11.66. Found: C, 45.10; H, 6.83; N, 11.45.

Boc-Asn-Ser-Met(O)-NHNH$_2$ [5]——Boc–Asn–Ser–Met(O)–OMe (5.25 g, 10.9 mmol) in MeOH (30 ml) was treated with 80% hydrazine hydrate (2.7 ml, 5 eq) overnight. The resulting mass was precipitated from DMSO with MeOH; yield 4.73 g (90%), mp 218—219°C, $[\alpha]_D^{20}$ −26.0° (c=1.0, DMSO), Rf_1 0.06, Rf_4 0.49. Amino acid ratios in a 6 N HCl hydrolysate: Asp 1.00, Ser 0.88, Met+Met(O) 0.75 (recovery of Asp, 74%). *Anal.* Calcd for $C_{17}H_{32}N_6O_8S \cdot 1/2H_2O$: C, 41.71; H, 6.79; N, 17.17. Found: C, 41.64; H, 6.59; N, 17.07.

Z(OMe)-His-Leu-OMe——The azide [prepared from 20.0 g (60 mmol) of Z(OMe)–His–NHNH$_2$] in DMF (20 ml) and Et_3N (7.0 ml, 50 mmol) were added to an ice-chilled solution of H–Leu–OMe [prepared from 9.08 g (50 mmol) of the hydrochloride as usual] in DMF (50 ml) and the mixture, after being stirred at 4°C for 20 h, was concentrated. The residue was extracted with AcOEt and the extract was washed with 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 16.32 g (75%), mp 110—111°C, $[\alpha]_D^{20}$ −24.1° (c=1.1, MeOH), Rf_1 0.65, Rf_3 0.17. *Anal.* Calcd for $C_{22}H_{30}N_4O_6$: C, 59.18; H, 6.77; N, 12.55. Found: C, 58.93; H, 6.70; N, 12.32.

Boc-Lys(Z)-His-Leu-OMe——Z(OMe)–His–Leu–OMe (5.11 g, 11.4 mmol) was treated with TFA–anisole (10—2.5 ml) and dry ether was added as described above. The resulting powder was dried over KOH pellets *in vacuo* for 3 h, then dissolved in DMF (20 ml) together with Et_3N (3.2 ml, 22.8 mmol) and Boc–Lys(Z)–OSu (5.44 g, 11.4 mmol). After being stirred for 20 h, the solution was concentrated and the residue was extracted with AcOEt. The extract was washed with 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 5.42 g (74%), mp 144—147°C, $[\alpha]_D^{20}$ −33.6° (c=0.7, MeOH), Rf_1 0.65. *Anal.* Calcd for $C_{32}H_{48}N_6O_8$: C, 59.61; H, 7.50; N, 13.04. Found: C, 59.61; H, 7.45; N, 13.03.

Boc-Lys(Z)-His-Leu-NHNH$_2$ [6]——Boc–Lys(Z)–His–Leu–OMe (2.65 g, 4.1 mmol) in MeOH (20 ml) was treated with 80% hydrazine hydrate (1.0 ml, 5 eq) for 48 h. The solvent was evaporated off and the residue was treated with H_2O to form a powder, which was recrystallized twice from MeOH and AcOEt; yield 2.10 g (79%), mp 167—169°C, $[\alpha]_D^{20}$ −21.4° (c=1.0, DMF), Rf_1 0.48. Amino acid ratios in a 6 N HCl hydrolysate: Leu 1.00, Lys 0.97, His 0.91 (recovery of Leu 100%). *Anal.* Calcd for $C_{31}H_{48}N_8O_7$: C, 57.74; H, 7.50; N, 17.38. Found: C, 57.78; H, 7.52; N, 17.38.

Z(OMe)-Leu-Gly-OMe——Z(OMe)–Leu–ONP (41.64 g, 0.1 mol) was added to a solution of H–Gly–OMe [prepared from 12.56 g (0.1 mol) of the hydrochloride as usual] in DMF (60 ml). After further addition of Et_3N (14 ml, 0.1 mol), the mixture was stirred at room temperature for 18 h. The solvent was evaporated off and the residue was dissolved in AcOEt. The organic phase was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and then concentrated. Trituration of the residue with ether afforded a powder, which was recrystallized from MeOH and ether; yield 28.98 g (79%), mp 78—79°C, $[\alpha]_D^{25}$ +13.1° (c=0.7, DMF), Rf_1 0.71. *Anal.* Calcd for $C_{18}H_{26}N_2O_6$: C, 59.00; H, 7.15; N, 7.65. Found: C, 59.17; H, 7.16; N, 7.59.

Z(OMe)-Asn-Leu-Gly-OMe——Z(OMe)–Leu–Gly–OMe (11.18 g, 30.5 mmol) was treated with TFA–anisole (26.5—6.6 ml) as usual, then TFA was removed by evaporation. The oily residue was washed with *n*-hexane, dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (50 ml) together with Et_3N (8.5 ml, 61 mmol) and Z(OMe)–Asn–ONP (12.73 g, 30.5 mmol). The mixture was stirred overnight and then the solvent was evaporated off. 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 then recrystallized from DMF and AcOEt; yield 11.21 g (77%), mp 196—197°C, $[\alpha]_D^{22}$ −15.4° (c=0.8, DMF), Rf_1 0.56, Rf_3 0.33. *Anal.* Calcd for $C_{22}H_{32}N_4O_8$: C, 54.99; H, 6.71; N, 11.66. Found: C, 55.14; H, 6.86; N, 11.60.

Z(OMe)-His-Asn-Leu-Gly-OMe——Z(OMe)–Asn–Leu–Gly–OMe (14.03 g, 29.2 mmol) was treated with TFA–anisole (38—9.5 ml) as usual, then TFA was removed by evaporation and dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* for 3 h and then dissolved in DMF (70 ml) containing Et_3N (4.1 ml, 29.2 mmol). The azide [prepared from 11.67 g (35 mmol) of Z(OMe)–His–NHNH$_2$] in DMF (20 ml) and Et_3N (4.9 ml, 35 mmol) were added to the above ice-chilled solution and the mixture was stirred at 4°C for 20 h. The solvent was evaporated off and the residue was treated with AcOEt and 5% $NaHCO_3$. The resulting powder was washed with H_2O and precipitated from DMF with AcOEt; yield 15.13 g (84%), mp 226—227°C, $[\alpha]_D^{22}$ −11.1° (c=0.8, DMF), Rf_1 0.37. *Anal.* Calcd for $C_{28}H_{32}N_4O_9$: C, 54.45; H, 6.36; N, 15.88. Found: C, 54.31; N, 6.38; N, 15.73.

Boc-Met(O)-His-Asn-Leu-Gly-OMe——Z(OMe)–His–Asn–Leu–Gly–OMe (5.0 g, 8.1 mmol) was treated with TFA–anisole (10.6—2.6 ml) as usual and the N^{α}-deprotected peptide isolated as stated above was dissolved in DMF (25 ml) containing Et_3N (2.3 ml, 16.2 mmol). The mixed anhydride [prepared from 2.58 g (9.7 mmol) of Boc–Met(O)–OH] in DMF (15 ml) was added to the above ice-chilled solution and the mixture was stirred in an ice-bath for 4 h. The solvent was evaporated off and the residue was treated with EtOH. The resulting powder was washed with EtOH and precipitated from DMF with EtOH; yield 3.19 g (56%), mp 218—219°C, $[\alpha]_D^{22}$ +11.4° (c=0.9, DMF) Rf_1 0.22, *Anal.* Calcd for $C_{29}H_{48}N_8O_{10}S$: C, 49.70; H, 6.90; N, 15.99. Found: C, 49.74; H, 6.80; N, 15.74.

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Boc-Met(O)-His-Asn-Leu-Gly-NHNH$_2$ [7]——Boc–Met(O)–His–Asn–Leu–Gly–OMe (5.21 g, 7.4 mmol) in MeOH–DMF (20—30 ml) was treated with 80% hydrazine hydrate (1.9 ml, 5 eq) overnight. After removal of the solvent by evaporation, the residue was treated with EtOH and the resulting powder was precipitated from DMF with MeOH; yield 4.39 g (84%), mp 197—198°C, $[\alpha]_D^{18}$ −19.7° (c=1.0, DMSO), Rf_1 0.13. Amino acid ratios in a 6 N HCl hydrolysate: Met+Met(O) 0.82, Asp 1.02, Gly 1.00, Leu 1.03, His 0.98 (recovery of Gly 92%). *Anal.* Calcd for $C_{28}H_{48}N_{10}O_9S$: C, 47.98; H, 6.90; N, 19.99. Found: C, 47.72; H, 6.84; N, 19.70.

Z(OMe)-Leu-NHNH-Troc——Troc–NHNH$_2$ (25.72 g, 0.12 mol) was added to a solution of mixed anhydride [prepared from 43.12 g (0.15 mol) of Z(OMe)–Leu–OH] in THF (200 ml). After the mixture had been stirred in an ice-bath for 4 h, the solvent was evaporated off and the residue was dissolved in AcOEt. The organic phase was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and then concentrated to give an oily product; yield 45.96 g (79%), Rf_2 0.64.

Z(OMe)-Gln-Leu-NHNH-Troc——Z(OMe)–Leu–NHNH–Troc (5.0 g, 10.3 mmol) was treated with TFA–anisole (9.0—2.2 ml), then excess TFA was removed by evaporation and the residue, after being washed with *n*-hexane, was dissolved in DMF (30 ml) together with Et_3N (2.9 ml, 20.6 mmol) and Z(OMe)–Gln–ONP (4.44 g, 10.3 mmol). After being stirred overnight, 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 from DMF with AcOEt; yield 5.18 g (82%), mp 188—190°C, $[\alpha]_D^{20}$ −17.1° (c=1.0, DMF), Rf_1 0.65, Rf_3 0.44. *Anal.* Calcd for $C_{23}H_{32}Cl_3N_5O_8$: C, 45.07; H, 5.26; N, 11.43. Found: C, 45.52; H, 5.41; N, 11.52.

Z(OMe)-Ile-Gln-Leu-NHNH-Troc——Z(OMe)–Gln–Leu–NHNH–Troc (5.18 g, 8.5 mmol) was treated with TFA–anisole (11—2.8 ml) as usual and *n*-hexane was added. The oily precipitate was dried over KOH pellets *in vacuo* for 3 h then dissolved in DMF (50 ml) together with Et_3N (2.4 ml, 16.9 mmol) and Z(OMe)–Ile–ONP (3.52 g, 8.5 mmol). After being stirred overnight, the solution was concentrated and the residue was treated with AcOEt and 5% citric acid. The resulting powder was purified as stated above; yield 4.86 g (79%), mp 205—207°C, $[\alpha]_D^{20}$ −15.3° (c=0.9, DMF), Rf_1 0.62, Rf_3 0.32. *Anal.* Calcd for $C_{29}H_{43}Cl_3N_6O_9$: C, 47.97; H, 5.97; N, 11.58. Found: C, 48.16; H, 6.17; N, 11.66.

Boc-Glu(OBzl)-Ile-Gln-Leu-NHNH-Troc——Z(OMe)–Ile–Gln–Leu–NHNH–Troc (15.0 g, 20.7 mmol) was treated with TFA–anisole (35.7—9.0 ml) as usual, then dry ether was added. The resulting powder was dried over KOH pellets *in vacuo* for 3 h then dissolved in DMF (50 ml). Et_3N (5.8 ml, 41.4 mmol) and Boc–Glu(OBzl)–OSu (8.99 g, 20.7 mmol) were added. After being stirred overnight, the mixture was concentrated and the residue was treated with 5% citric acid and AcOEt. The resulting powder was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O, and then precipitated from DMF with MeOH; yield 15.89 g (87%), mp 223—225°C, $[\alpha]_D^{18}$ −26.3° (c=0.6, DMSO), Rf_1 0.59, Rf_3 0.49. *Anal.* Calcd for $C_{37}H_{56}Cl_3N_7O_{11}$: C, 50.43; H, 6.41; N, 11.13. Found: C, 50.33; H, 6.52; N, 11.63.

Boc-Glu(OBzl)-Ile-Gln-Leu-NHNH$_2$ [8]——Boc–Glu(OBzl)–Ile–Gln–Leu–NHNH–Troc (9.0 g, 10.2 mmol) in a mixture of DMF (40 ml) and AcOH (6.8 ml, 10 eq) was treated with Zn dust (6.67 g, 10 eq) at room temperature for 17 h. The solution was filtered, the filtrate was concentrated *in vacuo* at 35°C and the residue was treated with a saturated solution of EDTA, then with $NaHCO_3$ to adjust the pH to neutral. The resulting powder was washed with H_2O and precipitated from DMF with MeOH; yield 5.31 g (74%), mp 260°C (dec.), $[\alpha]_D^{18}$ −22.3° (c=0.6, DMSO), Rf_1 0.48. Amino acid ratios in a 6 N HCl hydrolysate: Glu 2.06, Ile 1.01, Leu 1.00 (recovery of Leu 84%). *Anal.* Calcd for $C_{34}H_{55}N_7O_9$: C, 57.85; H, 7.85; N, 13.89. Found: C, 57.76; H, 8.00; N, 13.64.

Z(OMe)-Val-Ser-OMe——DCC (24.76 g) was added to a mixture of Z(OMe)–Val–OH (28.13 g, 0.1 mol) and H–Ser–OMe [prepared from 15.56 g (0.1 mol) of the hydrochloride as usual] in DMF–THF (80—140 ml). After being stirred at 0°C for 3 h and at room temperature for 15 h, the solution was filtered, the filtrate was concentrated and the residue was treated with EtOH. The resulting powder was recrystallized from THF and EtOH; yield 22.19 g (58%), mp 160—161°C, $[\alpha]_D^{20}$ +9.9° (c=0.9, DMF), Rf_1 0.67, Rf_3 0.64. *Anal.* Calcd for $C_{18}H_{26}N_2O_7$: C, 56.53; H, 6.85; N, 7.33. Found: C, 56.70; H, 6.96; N, 7.36.

Z-Ser-Val-Ser-OMe——Z(OMe)–Val–Ser–OMe (7.15 g, 18.7 mmol) was treated with TFA–anisole (16.2—4.1 ml) as usual, then dry *n*-hexane was added. The oily residue was dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (35 ml) containing Et_3N (2.6 ml, 18.7 mmol). The azide [prepared from 5.68 g (22.4 mmol) of Z–Ser–NHNH$_2$] and Et_3N (3.8 ml, 26.9 mmol) in DMF (10 ml) were added to the above ice-chilled solution and the mixture was stirred at 4°C for 48 h. After neutralization with a few drops of AcOH, the solution was concentrated. The residue was triturated with EtOH and precipitated from DMF with MeOH; yield 4.94 g (60%), mp 211—213°C, $[\alpha]_D^{20}$ +6.4° (c=0.9, DMF). Rf_1 0.66. *Anal.* Calcd for $C_{20}H_{29}N_3O_8$: C, 54.66; H, 6.65; N, 9.56. Found: C, 54.75; H, 6.52; N, 9.45.

Z-Ser-Val-Ser-NHNH$_2$ [9]——Z–Ser–Val–Ser–OMe (3.27 g, 7.4 mmol) in MeOH–DMF (10—20 ml) was treated with 80% hydrazine hydrate (1.9 ml, 5 eq) overnight. The resulting powder was collected by filtration, washed with EtOH and precipitated from DMSO with MeOH; yield 2.65 g (81%), mp 240°C (dec.), $[\alpha]_D^{22}$ −3.4° (c=0.6, DMSO), Rf_1 0.44. Amino acid ratios in a 6 N HCl hydrolysate: Ser 1.86, Val 1.00 (recovery of Val 94%). *Anal.* Calcd for $C_{19}H_{29}N_5O_2$: C, 51.92; H, 6.65; N, 15.94. Found: C, 51.63; H, 6.65; N, 15.98.

Boc-Asn-Ser-Met(O)-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 16—38)-OBzl——Boc-(hPTH 19—38)-OBzl

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(2.95 g, 0.84 mmol) was treated with TFA (9.1 ml) in the presence of anisole (2.3 ml) containing 2% EDT in an ice-bath for 60 min, then dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* for 3 h and then dissolved in DMSO–DMF (1: 1, 10 ml) containing Et_3N (0.23 ml, 1.67 mmol). The azide [prepared from 0.80 g (1.67 mmol) of Boc–Asn–Ser–Met(O)–$NHNH_2$] in DMF (3 ml) was added to the above ice-chilled solution and the mixture was stirred at 4°C for 48 h. After removal of the solvent by evaporation, the residue was treated with 5% citric acid and ether. The resulting powder was washed with H_2O and precipitated from DMSO with AcOEt: yield 3.20 g. For further purification, the product (1.20 g) was dissolved in DMSO (5 ml) and the solution was applied to a column of Sephadex LH-60 (6.0 × 150 cm), which was eluted with the same solvent with a flow rate of 45 ml/h. The UV absorption at 280 nm was determined in each fraction (15 ml). A peak with a small shoulder in front was detected. The fractions corresponding to the main peak (tube Nos. 95—116) were combined and the solvent was removed by evaporation. Treatment of the residue with ether afforded a powder; yield 0.90 g. The rest was similarly purified; total yield 2.40 g (74%), mp 160—161°C, $[\alpha]_D^{20}$ $-9.9°$ ($c=0.8$, DMF), Rf_1 0.45. *Anal.* Calcd for $C_{190}H_{261}N_{37}O_{45}S_3 \cdot 10H_2O$: C, 56.21; H, 6.98; N, 12.77. Found: C, 56.18; H, 6.65; N, 13.27.

Boc-Lys-(Z)-His-Leu-Asn-Ser-Met(O)-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 13—38)-OBzl——Boc–(hPTH 16—38)–OBzl (2.37 g, 0.61 mmol) was treated with TFA–anisole containing 2% EDT (5.3—1.3 ml) and the N^α-deprotected peptide isolated as described above was dissolved in DMF (15 ml) containing Et_3N (0.17 ml, 1.22 mmol). The azide [prepared from 0.79 g (1.22 mmol) of Boc–Lys(Z)–His–Leu–$NHNH_2$] in DMF (2 ml) and Et_3N (0.2 ml, 1.46 mmol) were added to the above ice-chilled solution and the mixture, after being stirred at 4°C for 18 h, was concentrated. Treatment of the residue with 5% $NaHCO_3$ and ether afforded a powder, which was washed with 3% AcOH, 5% $NaHCO_3$ and H_2O and precipitated five times from DMF with AcOEt; yield 2.22 g (83%), mp 149—151°C, $[\alpha]_D^{20}$ $+1.3°$ ($c=0.8$, DMF), Rf_1 0.59. *Anal.* Calcd for $C_{216}H_{297}N_{43}O_{50}S_3 \cdot 6H_2O$: C, 57.65; H, 6.92; N, 13.38. Found: C, 57.35; H, 6.86; N, 13.69.

Boc-Met(O)-His-Asn-Leu-Gly-Lys (Z)-His-Leu-Asn-Ser-Met (O)-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 8—38)-OBzl——Boc–(hPTH 13—38)–OBzl (1.57 g, 0.36 mmol) was treated with TFA–anisole containing 2% EDT (4.7—1.2 ml) and the N^α-deprotected peptide isolated as described above was dissolved in DMF (15 ml) containing Et_3N (0.15 ml, 0.86 mmol). The azide [prepared from 0.50 g, 0.72 mmol of Boc–Met(O)–His–Asn–Leu–Gly–$NHNH_2$] in DMF (5 ml) and Et_3N (0.12 ml, 0.86 mmol) were added and the mixture was stirred at 4°C for 48 h. Additional azide [prepared from 0.50 g (0.72 mmol) of the hydrazide] in DMF (5 ml) and Et_3N (0.12 ml, 0.86 mmol) were added and stirring was continued for an additional 36 h. After neutralization with a few drops of AcOH, the solution was concentrated and the residue was treated with 5% AcOH and ether. The resulting powder was washed with 5% AcOH, 5% $NaHCO_3$ and H_2O and precipitated from DMF with AcOEt. The crude product was dissolved in $CHCl_3$–MeOH–H_2O (8: 3: 1 v/v, 5 ml) and the solution was applied to a column of silica gel (Kieselgel 60 Merck, 2 × 14 cm), which was eluted with the same solvent. Two fractions with different Rf_1 (0.46 and 0.14) were isolated. The fractions with Rf_1 0.46 were combined and the solvent was removed by evaporation. Treatment of the residue with ether afforded a powder; yield 0.87 g (49%), mp 158—160°C, $[\alpha]_D^{18}$ $-20.8°$ ($c=0.2$, DMSO), Rf_1 0.46. *Anal.* Calcd for $C_{239}H_{333}N_{51}O_{57}S_4 \cdot 3H_2O$: C, 57.24; H, 6.81; N, 14.25. Found: C, 57.07; H, 6.96; N, 14.41.

Boc-Glu (OBzl)-Ile-Gln-Leu-Met (O)-His-Asn-Leu-Gly-Lys (Z)-His-Leu-Asn-Ser-Met (O)-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 4—38)-OBzl——Boc–(hPTH 8—38)–OBzl (0.54 g, 0.11 mmol) was treated with TFA–anisole containing 2% EDT (1.9—0.48 ml) and the N^α-deprotected peptide isolated as described above was dissolved in DMF (3 ml) containing Et_3N (61 µl, 0.44 mmol). The azide (prepared from 0.31 g (0.44 mmol) of Boc–Glu(OBzl)–Ile–Gln–Leu–$NHNH_2$] in DMF (1 ml) and Et_3N (74 µl, 0.53 mmol) were added and the mixture was stirred at 4°C for 20 h. Additional azide (prepared from the same amount of the hydrazide) in DMF (1 ml) and Et_3N (74 µl, 0.53 mmol) were added and stirring was continued for an additional 20 h. After neutralization with a few drops of AcOH, the solution was concentrated and the residue was treated with 5% AcOH and ether. The resulting powder was washed with 5% AcOH, 5% $NaHCO_3$ and H_2O and precipitated from DMF with AcOEt. The crude product was dissolved in DMF (10 ml) and the solution was applied to a column of Sephadex LH-60 (3.4 × 140 cm), which was eluted with the same solvent. Individual fractions (8 ml each) were collected and the absorbancy at 280 nm was determined. The fractions corresponding to the main peak (tube Nos. 58—70) were combined, the solvent was evaporated off, and the residue was treated with AcOEt to afford a powder; yield 0.44 g (73%), mp 155—157°C, $[\alpha]_D^{20}$ $+30.4°$ ($c=0.1$, DMF), Rf_1 0.52. *Anal.* Calcd for $C_{268}H_{376}N_{56}O_{64}S_4 \cdot 6H_2O$: C, 57.04; H, 6.93; N, 13.90. Found: C, 56.79; H, 7.19; N, 14.39.

Z-Ser-Val-Ser-Glu(OBzl)-Ile-Gln-Leu-Met(O)-His-Asn-Leu-Gly-Lys(Z)-His-Leu-Asn-Ser-Met(O)-Glu-(O-Bzl)-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, Z-(hPTH 1—38)-OBzl——Boc–(hPTH 4—38)–OBzl (0.42 g, 79.5 µmol) was treatedwith TFA–anisole containing 2% EDT (1.6—0.4 ml) and the N^α-deprotected peptide isolated as stated above was dissolved in DMF (5 ml) containing Et_3N (42.5 µl, 0.3 mmol). The azide [prepared from 0.133 g (0.30 mmol) of Z–Ser–Val–Ser–$NHNH_2$] in DMF (1 ml) and Et_3N (51 µl, 0.36 mmol) were added and the mixture was stirred at 4°C for 18 h. Additional azide (prepared from the same amount of the hydrazide) in DMF (1 ml) and Et_3N (51 μl, 0.36 mmol) were added and stirring was continued for an additional 36 h. After neutralization with a few drops of AcOH, the solution was concentrated and the residue was treated with 5% AcOH and ether. The resulting powder was washed with 5% AcOH, 5% $NaHCO_3$ and H_2O and precipitated from DMF with AcOEt. The crude product was then dissolved in DMF (10 ml) and the solution was applied to a column of Sephacryl S-200 (3 × 140 cm), which was eluted with DMF containing 5% H_2O. Individual fractions (10 ml each) were collected and the absorbancy at 280 nm was determined. The fractions corresponding to the main peak (Fig. 7a, tube Nos. 74—88) were concentrated and rechromatographed on the same column. A symmetrical single peak was detected (Fig. 7b). The fractions (tube Nos. 72—84) were collected and the solvent was evaporated off. Treatment of the residue with AcOEt afforded a powder; yield 0.18 g (40%), mp 159—160°C, $[\alpha]^{18}_{D}$ $-17.9°$ (c=0.2, DMSO). Rf_1 0.48. *Anal.* Calcd for $C_{282}H_{393}N_{59}O_{69}S_4 \cdot 7H_2O$: C, 56.75; H, 6.87; N, 13.85. Found: C, 56.55; H, 6.54; N, 13.72.

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-OH, H-(hPTH 1—38)-OH——The protect-ed octatriacontapeptide ester, Z (hPTH 1—38)-OBzl (150 mg, 25.7 μmol) was treated with 1 M TFMSA–thioanisole in TFA (5.65 ml) in the presence of *m*-cresol (0.59 ml, 5.65 mmol) in an ice-bath for 60 min and at 10°C for 60 min and then *n*-hexane was added. The resulting oily precipitate was washed with *n*-hexane and treated with ether. The resulting powder was collected by filtration and dried over KOH pellets *in vacuo* for 30 min. This treatment was repeated twice to ensure complete deprotection. The deprotected peptide thus obtained was dissolved in H_2O (10 ml), and treated with Amberlite CG-4B type II (acetate form, approximately 2 g) for 30 min. After filtration, the filtrate was adjusted to pH 10 with 5 N NH_4OH and stirred in an ice-bath for 30 min. The pH of the solution was adjusted to 6 with a few drops of AcOH and the solution was lyophilized to give a white fluffy powder.

The crude deprotected product was dissolved in H_2O (4 ml) and incubated with dithiothreitol (793 mg, 200 eq) under N_2 gas at 60°C for 36 h, then applied to a column of Sephadex G-25 (3 × 93 cm) and eluted with 1 N AcOH. Individual fractions (8 ml each) were collected and the absorbancy at 280 nm was determined. The front peak (tube Nos. 48—62) was collected and the solvent was removed by lyophilization; yield 91.4 mg (70%).

Next, the Sephadex-purified sample was dissolved in 0.01 M ammonium acetate buffer in 8 M urea (pH 5.1) and the solution was applied to a column of CM-cellulose (2.4 × 11 cm, Whatman CM-52). The CM-cellulose column was eluted first with the above buffer (40 ml) and then with a linear gradient formed from the starting buffer (200 ml) and 0.1 M ammonium acetate buffer in 8 M urea (200 ml). Individual fractions (4 ml each) were collected and the absorbancy at 280 nm was determined (Fig. 9). The main peak (tube Nos. 89—103) was collected and then rechromatographed on CM-cellulose (2.4 × 10 cm) with a linear gradient from H_2O (250 ml) to 0.2 M ammonium acetate buffer (250 ml). Individual fractions (5 ml each) were collected and the absorbancy at 280 nm was determined. The main peak (tube Nos. 62—85) was collected and then the solvent was removed by lyophilization. The resulting powder was reduced again with dithiothreitol as described above and then desalted by gel-filtration on Sephadex G-25 (1.8 × 140 cm column), which was eluted with 0.1 N AcOH. The desired fractions (tube Nos. 29—39) were combined and the solvent was removed by lyophilization to give a white fluffy powder; yield 25.9 mg (19.8%), $[\alpha]^{18}_{D}$ $-62.3°$ (c=0.2, 0.1 N AcOH), Rf_{I} 0.57, Rf_{II} 0.72 (on DC-Alufoline Cellulose, Merck). The synthetic peptide exhibited a single peak on HPLC at a retention time of 3.49 min and a single band on disc-electrophoresis in 15% polyacrylamide gel (0.5 × 6.0 cm) at pH 2.3 (mobility, 3.9 cm from the origin toward the cathode, after running at 5 mA per tube for 120 min) and on disc-isoelectrofocusing[33] in 8 M urea-acrylamide gel (0.5 × 6.0 cm) at pH 3—10 (Pharmalyte) (mobility, 1.4 cm from the origin toward the acidic end of the gel, after running at 1 mA per tube for 1 h and then at 200 V for 3 h). Amino acid ratios in a 6 N HCl hydrolysate: Asp 3.94, Ser 2.26, Glu 4.62, Gly 2.00, Ala 1.00, Val 4.06, Met 1.86, Ile 1.01, Leu 5.96, Phe 0.85, Trp ND, Lys 2.81, His 2.81, Arg 1.90 (recovery of Ala 91%). Amino acid ratios in LAP digest: Asp 0.87, Asn 2.65, Ser 2.65, Gln 2.10, Glu 3.09, Gly 2.00, Ala 1.00, Val 3.61, Met 1.52, Ile 1.01, Leu 5.94, Phe 0.89, Trp 1.00, Lys 3.35, His 2.75, Arg 1.97 (recovery of Ala 74%). *Anal.* Calcd for $C_{197}H_{319}N_{59}O_{55}S_2 \cdot 9CH_3COOH \cdot 17H_2O$: C, 48.67; H, 7.39; N, 15.58. Found: C, 48.27; H, 7.09; N, 15.87.

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References and Notes

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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**, 2485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972). Z=benzyloxycarbonyl, Z(OMe)=

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p-methoxybenzyloxycarbonyl, Boc = *tert*-butoxycarbonyl, Bzl = benzyl, Mts = mesitylene-2-sulfonyl, Troc = β,β,β-trichloroethyloxycarbonyl, NP = *p*-nitrophenyl, Su = *N*-hydroxysuccinimide, DCC = dicyclohexylcarbodiimide, TFA = trifluoroacetic acid, DMF = dimethylformamide, DMSO = dimethyl sulfoxide, THF = tetrahydrofuran, TFMSA = trifluoromethanesulfonic acid, EDT = ethanedithiol, EDTA = ethylenediaminetetraacetic acid disodium salt.

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