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Human Chorionic Gonadotropin. V.[1,2] Synthesis of a Triacontapeptide corresponding to the C-Terminal Sequence 116—145 of the β-Subunit of Human Chorionic Gonadotropin (hCG)

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The triacontapeptide corresponding to sequence 116—145 of the β-subunit of human chorionic gonadotropin (hCG) according to the amino acid sequence elucidated by Morgan *et al.* was synthesized by successive azide condensation of 5 peptide fragments, followed by TFA treatment and catalytic hydrogenation. This peptide was conjugated with bovine serum albumin (BSA). A 1% binding of ^{125}I-hCG was observed with the antiserum produced against the conjugate obtained above at a 1: 500 dilution.

Keywords——human chorionic gonadotropin; β-subunit; C-terminal triacontapeptide; chemical synthesis; antiserum

Following to the preceding paper,[1] we describe here the synthesis of a triacontapeptide corresponding to the sequence 116—145 of the β-subunit of human chorionic gonadotropin (hCG),[4-6] and report on its immunological properties. The synthetic route to the triacontapeptide is illustrated in Fig. 1. Starting with the C-terminal protected hexadecapeptide (I) prepared previously,[1] peptide fragments, Z–Ser(Bzl)–Leu–Pro–NHNHBoc (II), Z–Ala–Pro–Pro–Pro–NHNHBoc (IV), Z–Ser–Ser–Lys(Boc)–$NHNH_2$ (VI) and Z–Gln–Asp(OBzl)–Ser–Ser–NHNHBoc (VIII) were coupled successively by the azide procedure.[7]

The protected tripeptide, Z–Ser(Bzl)–Leu–Pro–NHNHBoc (II), was synthesized by the coupling of H–Leu–Pro–NHNHBoc[1] with Z–Ser(Bzl)–OH[8] using the DCC and 1-hydroxybenzotriazole (HOBt) method.[9] The protected tripeptide hydrazide (VI) was prepared as shown in Fig. 2. Z–Ser–$NHNH_2$[10] was coupled with H–Lys(Boc)–OH[11] by the azide procedure to afford Z–Ser–Lys(Boc)–OH (**1**), which was hydrogenated to furnish H–Ser–Lys(Boc)–OH (**2**). The peptide (**2**) was coupled with Z–Ser–$NHNH_2$ to give Z–Ser–Ser–Lys(Boc)–OH (**3**). **3** was converted to the corresponding methyl ester (**4**) by treatment with diazomethane. **4** was further converted to the hydrazide (VI).

The N-terminal protected tetrapeptide (VIII) was synthesized as illustrated in Fig. 3. Z–Ser–$NHNH_2$ and H–Ser–NHNHBoc[12] were coupled by the azide method to afford Z–Ser–

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[2] Amino acid, peptides and their derivatives mentioned in this paper are of the L-configuration except in the case of glycine.

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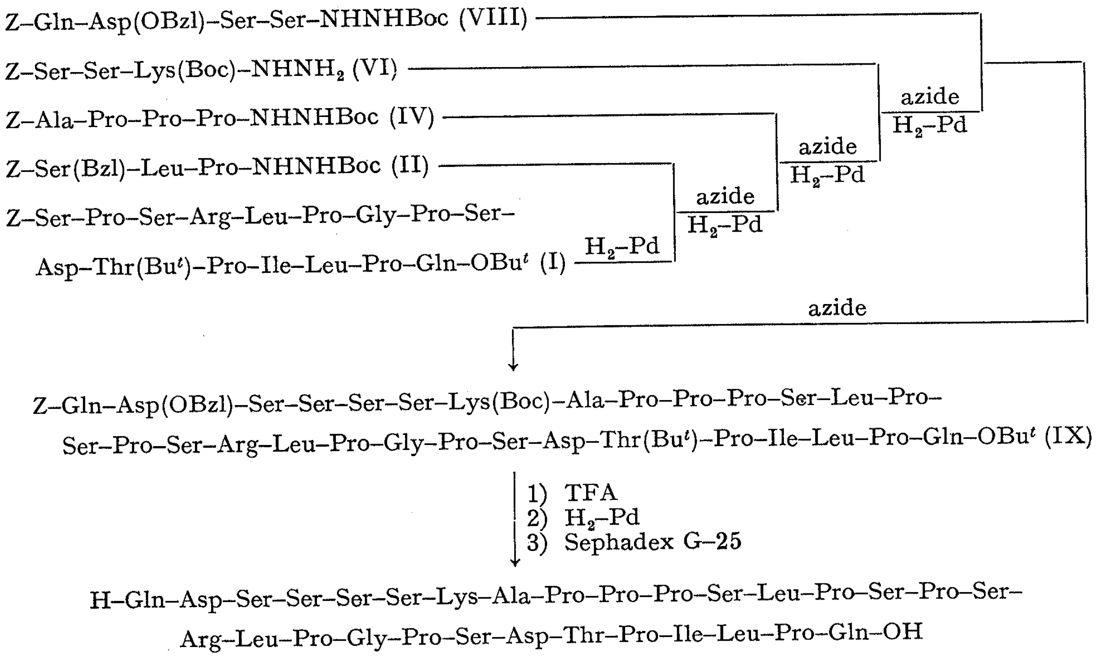

Fig. 1. Synthetic Scheme for the Triacontapeptide corresponding to Sequence 116—145 of the β-Subunit of Human Chorionic Gonadotropin

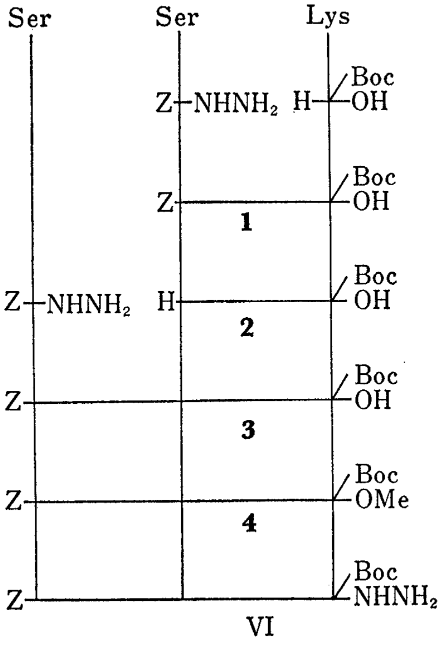

Fig. 2. Synthetic Scheme for the Tripeptide Hydrazide (VI)

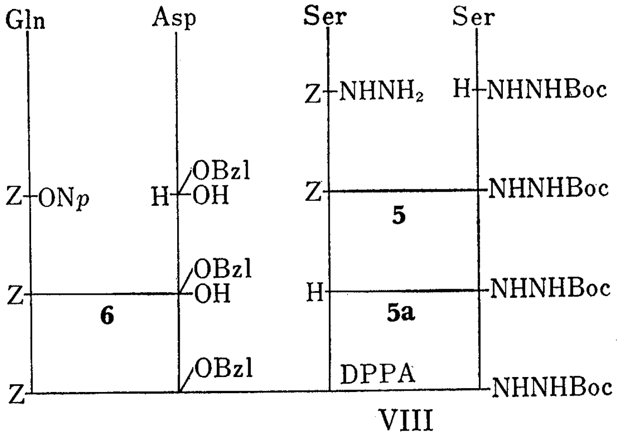

Fig. 3. Synthetic Scheme for the Protected Tetrapeptide (VIII)

Ser–NHNHBoc (**5**), which was converted to the corresponding amine (**5a**) by catalytic hydrogenation. Another peptide (**6**) was prepared by the coupling of Z–Gln–ONp[13] and H–Asp(OBzl)–OH[14] to give Z–Gln–Asp(OBzl)–OH (**6**). The peptides **5a** and **6** were coupled by the diphenylphosphorazidate (DPPA) method[15] to give Z–Gln–Asp(OBzl)–Ser–Ser–NHNHBoc (VIII).

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The protected hexadecapeptide, Z–Ser–Pro–Ser–Arg–Leu–Pro–Gly–Pro–Ser–Asp–Thr(Bu^t)–Pro–Ile–Leu–Pro–Gln–OBu^t (I),[1] was converted to the corresponding amine (Ia) by catalytic hydrogenation. Ia was subjected to coupling with the azide derived from 3 equivalents of II by treatment with HCl, followed by isopentylnitrite, to afford the protected nonadecapeptide, Z–Ser(Bzl)–Leu–Pro–Ser–Pro–Ser–Arg–Leu–Pro–Gly–Pro–Ser–Asp–Thr(Bu^t)–Pro–Ile–Leu–Pro–Gln–OBu^t (III). III was purified by gel-filtration on Sephadex LH-20 and further by silica gel column chromatography. The Z group and benzyl group on Ser residue were removed by catalytic hydrogenation over a palladium catalyst, monitoring the progress of the reaction by thin–layer chromatography (TLC). The nonadecapeptide amine (IIIa) thus obtained was coupled with Z–Ala–Pro–Pro–Pro–NHNHBoc (IV)[16] by the azide procedure to afford the tricosapeptide, Z–Ala–Pro–Pro–Pro–Ser–Leu–Pro–Ser–Pro–Ser–Arg–Leu–Pro–Gly–Pro–Ser–Asp–Thr(Bu^t)–Pro–Ile–Leu–Pro–Gln–OBu^t (V) in a thin–layer chromatographically pure form after purification by gel-filtration on Sephadex LH-20. V was converted to the corresponding amine by catalytic hydrogenation and coupled with VI by the azide procedure to afford Z–Ser–Ser–Lys(Boc)–Ala–Pro–Pro–Pro–Ser–Leu–Pro–Ser–Pro–Ser–Arg–Leu–Pro–Gly–Pro–Ser–Asp–Thr(Bu^t)–Pro–Ile–Leu–Pro–Gln–OBu^t (VII). In this coupling reaction, 3 equivalents of the azide were employed and the product was purified by gel-filtraion on Sephadex LH-20 using EtOH, followed by silica gel column chromatography using CHCl$_3$, MeOH and H$_2$O (8: 3: 1). The hexacosapeptide amine (VIIa) derived from VII by catalytic hydrogenation was finally coupled with 4 equivalents of VIII by the azide method to afford the protected triacontapeptide, Z–Gln–Asp(OBzl)–Ser–Ser–Ser–Ser–Lys(Boc)–Ala–Pro–Pro–Pro–Ser–Leu–Pro–Ser–Pro–Ser–Arg–Leu–Pro–Gly–Pro–Ser–Asp–Thr(Bu^t)–Pro–Ile–Leu–Pro–Gln–OBu^t (IX). This peptide was isolated by gel-filtration on Sephadex LH-20 using DMF, and further purified by silica gel column chromatography with *n*-butanol, AcOH and H$_2$O (4: 1: 5, upper layer). The homogeneity of the peptide was assessed by TLC, amino acid analysis of an acid hydrolysate and elemental analysis. In order to remove the protecting groups, the peptide (IX) was treated with trifluoroacetic acid (TFA) for 3 hr and then hydrogenated over a palladium catalyst. The deblocked peptide was purified by gel-filtration on Sephadex G-25, equilibrated and eluted with 5% AcOH; the eluted material was detected by the method of Lowry *et al.*[17] A single symmetrical peak was detected in terms of the absorbancy at 750 nm. The white fluffy powder contained from this peak exhibited a sharp single spot positive to ninhydrin and Sakaguchi reagent on TLC. Amino acid analysis of an acid hydrolysate gave molar ratios in good agreement with the theoretically expected values. Although this peptide contained 9 Pro residues,[18] aminopeptidase (AP–M)[19] digested the peptide reasonably well, and amino acid analysis of the digest gave molar ratios in good agreement with the expected values, except for Ala, Pro and Ser residues, for which the results were slightly less than the expected values. However, the presence of Gln instead of Glu was confirmed by this amino acid analysis of the digest.

The triacontapeptide was converted to the corresponding hydrochloride and conjugated with bovine serum albumin (BSA) by the DCC method in the usual manner.[20] An antiserum was produced in New Zealand White rabbits by multiple intradermal injection of the conjugate in Freund's Complete adjuvant. A 1% binding of the labeled hCG (^{125}I-hCG) was observed with the antiserum obtained above at a 1: 500 dilution . In order to obtain antisera specific to hCG for practical diagnostic use, further elongation of the peptide chain and production of

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antisera against these synthetic peptide antigens are in progress, and the results will be reported in the future.

Experimental

Thin-layer chromatography was performed on silica gel (Kieselgel G, Merck). *Rf* values refer to the following solvent systems: Rf^1 = *n*-butanol, AcOH and H_2O (4: 1: 5), Rf^2 = *n*-butanol, pyridine, AcOH and H_2O (4: 1: 1: 2), Rf^3 = $CHCl_3$, MeOH and AcOH (90: 8: 2), Rf^4 = $CHCl_3$, MeOH and H_2O (8: 3: 1), Rf^5 = *n*-butanol, AcOEt, AcOH and H_2O (1: 1: 1: 1), Rf^6 = *n*-butanol, pyridine, AcOH and H_2O (30: 20: 6: 24), Rf^7 = *n*-butanol, pyridine, AcOH and H_2O (2: 1: 1: 2).

Z-Ser(Bzl)-Leu-Pro-NHNHBoc (II)——DCC (1.3 g) and 1-hydroxybenzotriazole (0.9 g) were added to a cold solution of H–Leu–Pro–NHNHBoc (prepared from 2.5 g of Z–Leu–Pro–NHNHBoc by catalytic hydrogenation) and Z–Ser(Bzl)–OH (1.7 g) in DMF (20 ml). The reaction mixture was stirred at room temperature overnight. After removal of the urea derivative and the solvent, the residue was extracted with AcOEt. The extract was washed with 5% Na_2CO_3, 10% citric acid and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford an amorphous powder, which was collected by filtration and dried, yield 1.7 g (48.6%), $[\alpha]^{23}_{D}$ −88.1° (c=1.0, MeOH), Rf^3 0.62. *Anal.* Calcd for $C_{34}H_{47}N_5O_8$: C, 62.5; H, 7.25; N, 10.7. Found: C, 62.4; H, 7.28; N, 10.6. Amino acid ratios in an acid hydrolysate: Ser 0.96; Pro 1.04; Leu 1.00 (average recovery 90%).

Z-Ser(Bzl)-Leu-Pro-Ser-Pro-Ser-Arg-Leu-Pro-Gly-Pro-Ser-Asp-Thr(Bu^t)-Pro-Ile-Leu-Pro-Gln-OBu^t (III)——Ia (prepared from 200 mg of I by catalytic hydrogenation and converted to the corresponding hydrochloride) was dissolved in DMF (2 ml), and the pH of the solution was adjusted to 8 with triethylamine. To this cold solution, Z–Ser(Bzl)–Leu–Pro–N_3 prepared as follows was added. II (210 mg) was dissolved in 5.4 N HCl/dioxane (0.34 ml), and after 5 min this solution was diluted with dioxane (0.5 ml) and stored at room temperature for 30 min. This was diluted with DMF (5 ml). Isopentylnitrite (0.04 ml) was then added after cooling this solution to −20°. After 5 min, the pH of the solution was adjusted to 8 with triethylamine (0.26 ml). This solution was poured into the DMF solution of Ia prepared above. The reaction mixture was stirred in a cold room (4°) for 48 hr. After removal of the solvent, the residue was extracted with *n*-butanol. The extract was washed with water and concentrated. Ether was added to the residue, providing a white precipitate. This was dissolved in EtOH (2 ml) and applied to a Sephadex LH-20 column (1 × 180 cm), equilibrated and eluted with EtOH. Individual fractions (3 g each) were collected. The solvent (tube Nos. 18—21) was removed and addition of ether to the residue gave a white precipitate. This was further purified by column chromatography on silica gel (2.3 × 30 cm), equilibrated and eluted with $CHCl_3$, MeOH and H_2O (8: 3: 1). The eluate (3 g each, tube Nos. 17—21) was collected and the solvent was removed by evaporation. Ether was added to the residue to give a precipitate; yield 176 mg (73.6%), mp 154—159°, $[\alpha]^{25}_{D}$ −113.9° (c=0.4, MeOH), Rf^2 0.81, Rf^4 0.52. *Anal.* Calcd for $C_{110}H_{171}N_{23}O_{30} \cdot HCl \cdot 2H_2O$: C, 55.8; H, 7.49; N, 13.6. Found: C, 55.4; H, 7.43; N, 13.6. Amino acid ratios in an acid hydrolysate: Asp 1.03; Thr 1.03; Ser 3.83; Glu 1.02; Pro 6.40; Gly 1.00; Ile 0.94; Leu 3.03; Arg 0.97 (average recovery 89%).

Z-Ala-Pro-Pro-Pro-Ser-Leu-Pro-Ser-Pro-Ser-Arg-Leu-Pro-Gly-Pro-Ser-Asp-Thr(Bu^t)-Pro-Ile-Leu-Pro-Gln-OBu^t (V)——III (150 mg) in EtOH (8 ml) and 5% AcOH (4 ml) was hydrogenated over a palladium catalyst for 2 days. The progress of the reaction was monitored by TLC (debenzyloxycarbonylated and debenzylated nonadecapeptide Rf^4 0.26, debenzyloxycarbonylated nonadecapeptide Rf^4 0.50). After removal of the palladium and the solvent, the residue was treated with 1 N HCl (0.13 ml) and lyophilized to give the nonadecapeptide amine dihydrochloride (IIIa) as a fluffy powder (Rf^4 0.26). IIIa was dissolved in DMF (3 ml) and cooled to 0°. The pH of the solution was adjusted to 8 with triethylamine (0.018 ml). Z–Ala–Pro–Pro–Pro–N_3 (prepared from 121 mg of IV by treatment with TFA followed by isopentylnitrite) in DMF (3 ml) was added to the cold solution of IIIa prepared above. The reaction mixture was stirred in a cold room for 2 days, then the solvent was removed by evaporation. The residue was extracted with *n*-butanol. The extract was washed with water and concentrated. Addition of ether to the residue gave a white precipitate. This was purified by gel-filtration on Sephadex LH-20 using EtOH as a solvent, yield 117 mg (68.6%), mp 174—180°, $[\alpha]^{23}_{D}$ −151.5° (c=0.2, DMF). *Anal.* Calcd for $C_{121}H_{191}N_{27}O_{34} \cdot HCl \cdot 5H_2O$: C, 53.9; H, 7.56; N, 14.0. Found: C, 53.9; H, 7.32; N, 14.2. Amino acid ratios in an acid hydrolysate: Asp 0.98; Thr 0.96; Ser 3.83; Glu 0.96; Pro 9.09; Gly 1.00; Ala 1.02; Ile 0.87; Leu 2.61; Arg 0.90 (average recovery 90%).

Z-Ser-Lys(Boc)-OH (1)——Z–Ser–N_3 (prepared from 2.53 g of Z–Ser–$NHNH_2$ and 1.18 ml of *t*-butylnitrite in the usual manner) in DMF (15 ml) was mixed with a solution of H–Lys(Boc)–OH (1.97 g) in 50% aqueous DMF (20 ml) containing triethylamine (1.1 ml). The reaction mixture was stirred in a cold room for 2 days, then the solvent was removed by evaporation. The residue was dissolved in H_2O and the solution was washed with AcOEt. The water layer was acidified with citric acid and the resulting precipitate was collected by filtration, washed with H_2O and recrystallized from MeOH; yield 2.6 g (70%), mp 126—128°, $[\alpha]^{23}_{D}$ +6.7° (c=1.0, DMF), Rf^1 0.85, Rf^4 0.78. *Anal.* Calcd for $C_{22}H_{33}N_3O_8 \cdot 1/2H_2O$: C, 55.5; H, 7.19; N, 8.8. Found: C, 55.4; H, 7.19; N, 8.9.

H-Ser-Lys(Boc)-OH (2)——**1** (1.2 g) was hydrogenated over a palladium catalyst in 80% MeOH (70 ml). After removal of the palladium and the solvent, the residue was washed with AcOEt and lyophilized from dioxane and water; yield 805 mg (94%), mp 219—221° (dec.), $[\alpha]_D^{24}$ +6.0° (c=1.0, 50% AcOH), Rf^1 0.50, Rf^4 0.15. *Anal.* Calcd for $C_{14}H_{27}N_3O_6$: C, 50.4; H, 8.10; N, 12.6. Found: C, 50.3; H, 8.20; N, 12.8.

Z-Ser-Ser-Lys(Boc)-OH (3)——A solution of Z–Ser–N_3 (prepared from 759 mg of Z–Ser–$NHNH_2$) in DMF (5 ml) was combined with a solution of **2** (500 mg) in 50% aqueous DMF (3 ml) containing triethylamine (0.21 ml). The reaction mixture was stirred in a cold room for 20 hr and the solvent was removed by evaporation. The residue was dissolved in H_2O (10 ml), and the solution was washed with AcOEt. The water layer was acidified with citric acid and the resulting precipitate was extracted with AcOEt. The extract was washed with H_2O and dried over Na_2SO_4. After removal of the solvent, the residue was precipitated from AcOEt and ether; yield 540 mg (77%), mp 72—75°, $[\alpha]_D^{23}$ +6.6° (c=0.9, DMF), Rf^1 0.86, Rf^4 0.36. *Anal.* Calcd for $C_{25}H_{38}N_4O_{10}\cdot 1/2H_2O$: C, 53.3; H, 7.0; N, 9.9. Found: C, 53.2; H, 6.8; N, 9.8.

Z-Ser-Ser-Lys(Boc)-OMe (4)——An ethereal solution of diazomethane (prepared from nitrosomethylurea) was added to a solution of **3** (500 mg) in MeOH (30 ml) until the mixture aquired a yellow color. The mixture was kept at 0° for 10 min, then decolorized with AcOH. The solvent was evaporated off and the residue was dissolved in AcOEt. The extract was washed with H_2O and concentrated after drying over Na_2SO_4. The residue was recrystallized from AcOEt; yield 488 mg (86%), mp 114—116°, $[\alpha]_D^{24}$ −2.7° (c=0.9, DMF), Rf^4 0.67. *Anal.* Calcd for $C_{26}H_{40}N_4O_{10}$: C, 54.9; H, 7.09; N, 9.9. Found: C, 54.6; H, 7.08; N, 9.7.

Z-Ser-Ser-Lys(Boc)-NHNH$_2$ (VI)——Hydrazine hydrate (80%, 1.8 ml) was added to a solution of **4** (480 mg) in MeOH (5 ml). The mixture was stirred for 40 hr at room temperature and the resulting precipitate was collected by filtration. It was recrystallized from EtOH; 400 mg (83%), mp 180—182°, $[\alpha]_D^{23}$ +3.5° (c=0.8, DMF), Rf^4 0.58. *Anal.* Calcd for $C_{25}H_{40}N_6O_9$: C, 52.8; H, 7.09; N, 14.8. Found: C, 52.9; H, 7.11; N, 14.7.

Z-Ser-Ser-Lys(Boc)-Ala-Pro-Pro-Pro-Ser-Leu-Pro-Ser-Pro-Ser-Arg-Leu-Pro-Gly-Pro-Ser-Asp-Thr(Bu^t)-Pro-Ile-Leu-Pro-Gln-OBu*t* (VII)——Z–Ser–Ser–Lys(Boc)–N_3 (prepared from 86.6 mg of VI with isopentylnitrite) was added to a cold solution of Va (prepared from 100 mg of V by catalytic hydrogenation). After 48 hr at 4°, the solvent was removed by evaporation and the residue was extracted with *n*-butanol. The extract was washed with water and concentrated. Addition of ether to the residue gave a white precipitate. The solid material was purified by column chromatography twice on Sephadex LH-20 (1×180 cm) using DMF. Individual fractions (3 g each) were collected. The solvent of the effluent (tube Nos. 17—21) was removed and ether was added to the residue to give a white precipitate; yield 70 mg (60.9%), mp 182—184°, $[\alpha]_D^{23}$ −85.7° (c=0.2, DMF), Rf^2 0.43, Rf^4 0.19. *Anal.* Calcd for $C_{138}H_{221}N_{31}O_{41}\cdot HCl\cdot 5H_2O$: C, 53.5; H, 7.55; N, 14.0. Found: C, 53.5; H, 7.45; N, 14.0. Amino acid ratios in an acid hydrolysate: Asp 1.05; Thr 0.92; Ser 5.00; Glu 0.96; Pro 9.30; Gly 1.00; Ala 1.07; Ile 0.95; Leu 2.85; Lys 0.92; Arg 0.90 (average recovery 85%).

Z-Ser-Ser-NHNHBoc (5)——Z–Ser–N_3 (prepared from 3.63 g of Z–Ser–$NHNH_2$ in the usual manner) was added to a cold solution of H–Ser–NHNHBoc (prepared from 5.1 g of Z–Ser–NHNHBoc by catalytic hydrogenation) in DMF (20 ml). The reaction mixture was stirred for two days in a cold room. After removal of the solvent, the residue was dissolved in AcOEt. The extract was washed with 10% citric acid and water, dried over Na_2SO_4 and concentrated. Ether was added to the residue to give a solid mass, which was collected by filtration and recrystallized from AcOEt and ether; yield 3.2 g (50.3%), mp 128—129°, $[\alpha]_D^{23}$ −22.6° (c=1.0, MeOH), Rf^3 0.27. *Anal.* Calcd for $C_{19}H_{28}N_4O_8$: C, 51.8; H, 6.4; N, 12.7. Found: C, 52.0; H, 6.4, N, 12.7.

Z-Gln-Asp(OBzl)-OH (6)——H–Asp(OBzl)–OH (5.23 g) and Z–Gln–ON*p* (10.03 g) were dissolved in 50% dioxane (200 ml) containing triethylamine (3.5 ml) and the solution was stirred at room temperature overnight. After removal of the solvent, the residue was dissolved in 5% $NaHCO_3$ (100 ml), and the solution was washed with AcOEt. The water layer was acidified with conc. HCl to pH 3 to give a crystalline precipitate, which was collected by filtration, washed with AcOEt and dried; yield 11.24 g (92.7%), mp 183—184°, $[\alpha]_D^{23}$ −2.3° (c=1.0, DMF), Rf^1 0.78, Rf^2 0.80, Rf^3 0.32. *Anal.* Calcd for $C_{24}H_{27}N_3O_8$: C, 59.4; H, 5.61; N, 8.7. Found: C, 59.4; H, 5.51; N, 8.7.

Z-Gln-Asp(OBzl)-Ser-Ser-NHNHBoc (VIII)——A solution of H–Ser–Ser–NHNHBoc (**5a**) (prepared from 5.63 g of **5** by catalytic hydrogenation) and **6** (3.88 g) in DMF (60 ml) was cooled to 0°, then diphenylphosphorazidate (DPPA, 4.23 ml) and triethylamine (1.46 ml) were added to the solution. This reaction mixture was stirred in a cold room overnight. After removal of the solvent, AcOEt and water were added to the residue to give a gelatinous material, which was collected by filtration and recrystallized from MeOH; yield 3.5 g (56.6%), mp 181—191°, $[\alpha]_D^{22}$ −15.2° (c=1.0, DMF), Rf^2 0.76, Rf^3 0.12. *Anal.* Calcd for $C_{35}H_{47}N_7O_{13}\cdot H_2O$: C, 53.1; H, 6.2; N, 12.4. Found: C, 53.2; H, 6.0; N, 12.2.

Z-Gln-Asp(OBzl)-Ser-Ser-Ser-Ser-Lys(Boc)-Ala-Pro-Pro-Pro-Ser-Leu-Pro-Ser-Pro-Ser-Arg-Leu-Pro-Gly-Pro-Ser-Asp-Thr(Bu^t)-Pro-Ile-Leu-Pro-Gln-OBu^t (IX)——A solution of VIII (68 mg) in TFA (0.6 ml) containing anisole (0.04 ml) was stored at room temperature for 30 min. On addition of ether (10 ml), tetrapeptide hydrazide was precipitated. It was collected by centrifugation, washed with ether and dried over KOH pellets *in vacuo*. This hydrazide was dissolved in DMF (1 ml) and cooled to −20°, then 0.54 N HCl in dioxane (0.37 ml) followed by isopentylnitrite (0.012 ml) were added to the solution. After 5 min, the pH of the solution was adjusted to 8 with triethylamine. This azide was added to a cold solution of VIIa (prepared from 50 mg of VII by catalytic hydrogenation and converted to the dihydrochloride, Rf^{2} 0.29, Rf^{4} 0.16) in DMF (1 ml). This reaction mixture was stirred in a cold room for 48 hr and applied to a Sephadex LH-20 column (1 × 180 cm) equilibrated and eluted with DMF. Fractions (3 g each) were collected. The solvent of the eluate (tube Nos. 15—17) was evaporated off and ether was added to give a white precipitate. The peptide (IX) was further purified by column chromatography on silica gel (1.5 × 22 cm), equilibrated and eluted with *n*-butanol, AcOH and H_2O (4: 1: 5, upper phase). The eluate (2 g each, tube Nos. 10—20) was collected and the solvent was removed. Ether was added to the residue to give a white precipitate; yield 45 mg (77.3%), mp 245° (dec.) with sintering at 160°, $[\alpha]_D^{23}$ −77.0° ($c=0.2$, DMF), Rf^{1} 0.17, Rf^{2} 0.59, Rf^{4} 0.10. *Anal.* Calcd for $C_{160}H_{250}N_{36}O_{50} \cdot HCl \cdot 5H_2O$: C, 53.3; H, 7.30; N, 14.0. Found: C, 53.1; H, 7.15; N, 14.3. Amino acid ratios in an acid hydrolysate: Asp 2.10; Thr 1.09; Ser 7.19; Glu 2.25; Pro 8.16; Gly 1.00; Ala 1.21; Ile 0.95; Leu 2.88; Lys 0.95; Arg 0.89 (average recovery 87%).

H-Gln-Asp-Ser-Ser-Ser-Ser-Lys-Ala-Pro-Pro-Pro-Ser-Leu-Pro-Ser-Pro-Ser-Arg-Leu-Pro-Gly-Pro-Ser-Asp-Thr-Pro-Ile-Leu-Pro-Gln-OH——A solution of IX (100 mg) in TFA (0.5 ml) containing anisole (0.05 ml) was stored at room temperature for 3 hr. On addition of ether, partially deblocked triacontapeptide precipitated, and was collected by centrifugation, washed with ether and dried over KOH pellets *in vacuo*. This solid material was dissolved in EtOH (6 ml) and H_2O (4 ml) containing AcOH (0.2 ml) and hydrogenated over a palladium catalyst for 6 hr. After removal of the palladium and the solvent, the residue was lyophilized from water to give a fluffy powder. It was applied to a Sephadex G-25 column (3 × 140 cm) equilibrated and eluted with 5% AcOH. The eluted material was detected by the method of Lowry *et al.* Fractions (3 g each) were collected and the solvent of the effluent (tube Nos. 59—66) was removed. The residue was lyophilized from water to afford purified triacontapeptide, yield 43 mg (51.3%). This was converted to the corresponding hydrochloride. $[\alpha]_D^{25}$ −176.5° ($c=0.2$, H_2O), Rf^{5} 0.10, Rf^{6} 0.33. *Anal.* Calcd for $C_{132}H_{216}N_{36}O_{46} \cdot 3HCl \cdot 20H_2O$: C, 45.2; H, 7.37; N, 14.4. Found: C, 44.9; H, 6.92; N, 13.7. Amino acid ratios in an acid hydrolysate: Asp 2.12; Thr 0.91; Ser 7.36; Glu 2.19; Pro 9.07; Gly 1.00; Ala 1.04; Ile 0.98; Leu 3.02; Lys 1.00; Arg 0.92 (average recovery 87%), amino acid ratios in an AP–M[21] digest: Asp 1.81; Thr+Gln 2.85; Ser 6.93; Glu 0.75; Pro 7.16; Gly 1.00; Ala 0.85; Ile 1.08; Leu 2.85; Lys 0.90; Arg was not determined (average recovery 87%) (Gln emerged at the same position as Thr, and was calculated as Thr).

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21) Pierce Chemical Co., Lot. 08307.33. This enzyme partially digested glutamine to glutamic acid; the data are not corrected.