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Synthesis of the Untriacontapeptide corresponding to the Entire Amino Acid Sequence of Human β-Endorphin[1]

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In a conventional manner, the untriacontapeptide corresponding to the entire amino acid sequence of human β-endorphin was synthesized using amino acid derivatives, Lys(Z) Glu(OBzl), bearing protecting groups removable by methanesulfonic acid. Potency ratios of the synthetic peptide to morphin (taken as 1) was 6.5, when assayed the analgesic effect on tail pinch method in mice.

Keywords——conventional synthesis of human β-endorphin; deprotection with methanesulfonic acid; 2,2,2-trichloroethyloxycarbonhylhydrazine; sodium metaperiodate oxidation of Met to Met(O); [5-Met(O)]-human β-endorphin; N→O shift at Ser and Thr; reduction of Met(O) to Met by mercaptoethanol; analgesic effect on tail pinch method in mice

In the preceding paper,[3] synthesis of the protected octadecapeptide, Z(OMe)-Leu-Val-Thr-Leu-Phe-Lys(Z)-Asn-Ala-Ile-Ile-Lys(Z)-Asn-Ala-Tyr-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OBzl, corresponding to positions 14 to 31 of human β-endorphin[4] was described. We wish to report in this paper, the synthesis of untriacontapeptide which corresponds to the entire amino acid sequence of this endogeneous opiate peptide. The above protected octadecapeptide, selected as the starting material in this synthesis, was prepared by the successive azide condensation[5] of six peptide fragments and the additional three fragments shown in Fig. 1, (VII, VIII and IX), were newly synthesized.

Considering the racemization-free condensation of a proline-terminal peptide fragment, the protected tetrapeptide, Z(OMe)-Ser-Gln-Thr-Pro-OH (VII) was first synthesized as illustrated in Fig. 2. The TFA labile Z(OMe) group[6] was employed for the α-amino protection. The Rudinger's azide procedure[5] was employed for condensation of the threonine and serine residues possessing the hydroxyl function and the *p*-nitrophenyl ester procedure[7] for the glutamine residue. Z(OMe)-Gln-Thr-Pro-OH and (VII) are the compounds partially soluble in water and therefore *n*-butanol, instead of ethyl acetate, was used as the solvent for extraction during purification of these two derivatives.

Synthetic scheme of the protected tetrapeptide hydrazide, Z(OMe)-Thr-Ser-Glu(OBzl)-Lys(Z)-$NHNH_2$ (VIII), is shown in Fig. 3. In order to incorporate the Glu(OBzl) residue

1) Amino acids, peptides and their derivatives mentioned in this communication are of the L-configuration. Abbreviations used are those recommended by IUPAC-IUB Commission on Biochemical Nomenclature: *Biochem.*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972). Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Bzl=benzyl, Troc=2,2,2-trichloroethyloxycarbonyl, NP=*p*-nitrophenyl, DCC=dicyclohexylcarbodiimide, TFA=trifluoroacetic acid, DMF=dimethylformamide, DMSO=dimethylsulfoxide, HMPA=hexamethylphosphoramide, HOBT=1-hydroxybenzotriazole, MSA=methanesulfonic acid.

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3) M. Kubota, T. Hirayama, O. Nagase, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **26**, 2132 (1978).

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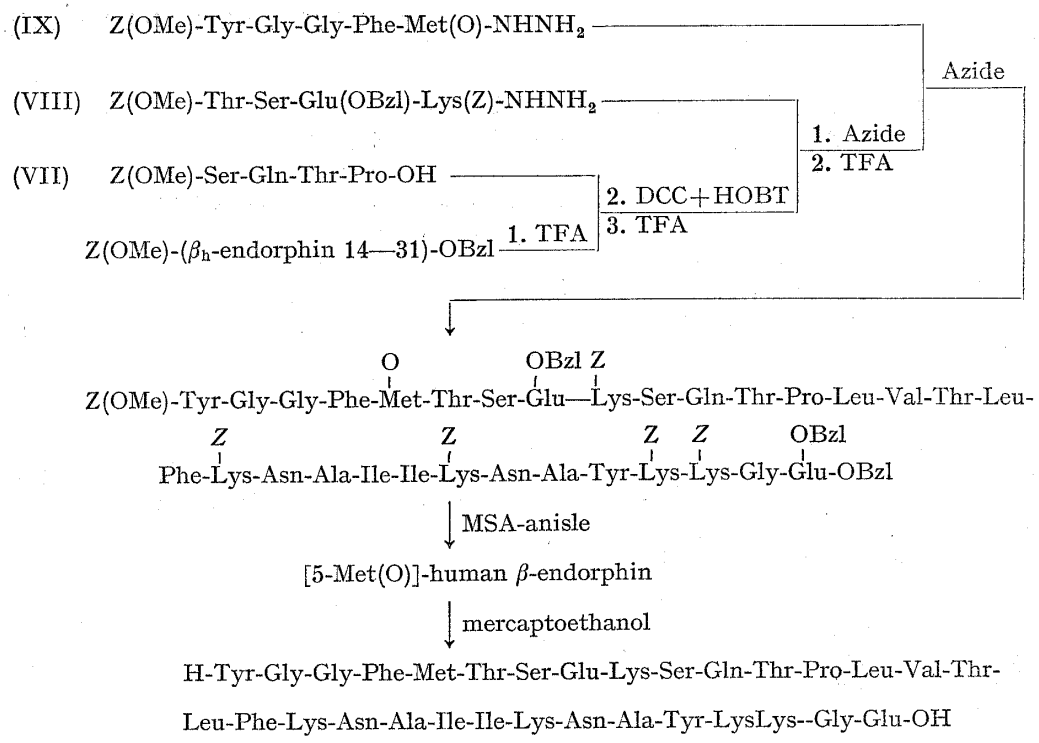

Fig. 1. Synthetic Route to Human β-Endorphin

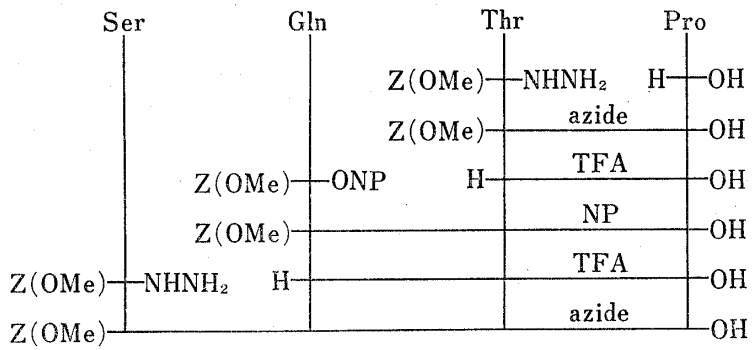

Fig. 2. Synthetic Scheme of the Protected Tetrapeptide, Z(OMe)-(βh-endorphin 10—13)-OH

to the hydrazide (VIII), Troc-$NHNH_2$[8] was first condensed with Z(OMe)-Lys(Z)-OH by DCC[9] and the resulting Z(OMe)-Lys(Z)-NHNH-Troc,[10] after the usual TFA treatment, was further condensed with Z(OMe)-Glu(OBzl)-OH by the *p*-nitrophenyl ester procedure to give Z(OMe)-Glu(OBzl)-Lys(Z)-NHNH-Troc. Subsequently, the azide procedure was employed to introduce the dipeptide unit, Thr-Ser, to the above hydrazide derivative. The necessary hydrazide, Z(OMe)-Thr-Ser-$NHNH_2$, was derived from the known dipeptide ester, Z(OMe)-Thr-Ser-OMe,[10] by the usual hydrazine treatment. Removal of the Troc group from the resulting tetrapeptide derivative, Z(OMe)-Thr-Ser-Glu(OBzl)-Lys(Z)-NHNH-Troc, was performed by treatment with zinc dust in a mixture of DMF and acetic acid and the last trace of metal contamination was removed from the product (VIII) by ethylenediamine tetraacetate (EDTA).

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10) H. Ogawa, M. Sugiura, H. Yajima, H. Sakurai, and K. Tsuda, *Chem. Pharm. Bull.* (Tokyo), **26**, 1549 (1978).

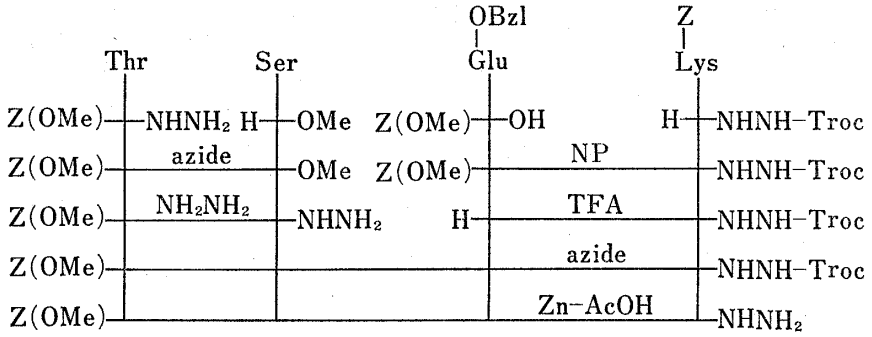

Fig. 3. Synthetic Scheme of the Protected Tetrapeptide Hydrazide, Z(OMe)-(β_h-endorphin 6—9)-$NHNH_2$

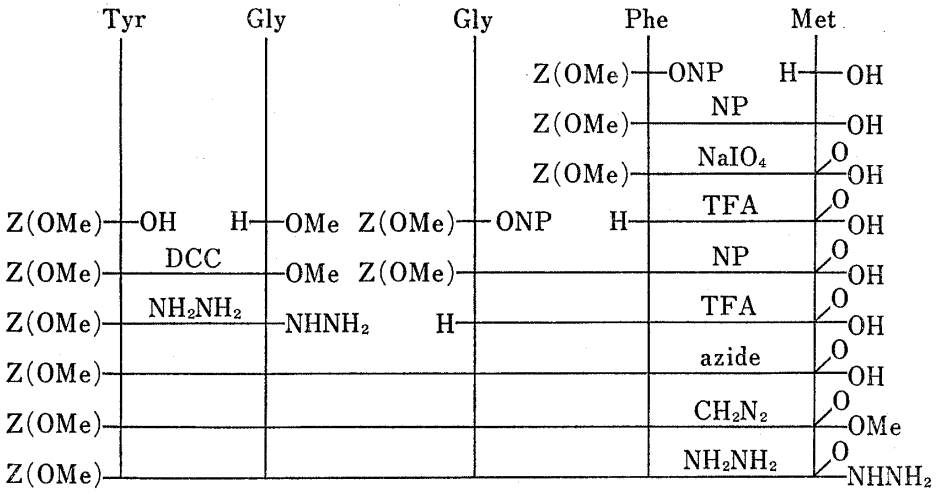

Fig. 4. Synthetic Scheme of the Protected Pentapeptide Hydrazide, Z(OMe)-(β_h-endorphin 1—5)-$NHNH_2$

The N-terminal pentapeptide unit, Tyr-Gly-Gly-Phe-Met, is a peptide termed as enkephalin[11] and its synthesis was reported by several authers.[12] We decided to prepare this unit as the hydrazide, Z(OMe)-Tyr-Gly-Gly-Phe-Met(O)-$NHNH_2$ (IX) as shown in Fig. 4. First, the known dipeptide, Z(OMe)-Phe-Met-OH,[13] was oxidized to the corresponding sulfoxide, Z(OMe)-Phe-Met(O)-OH by sodium metaperiodate. This oxidant was proved to give mixture of diastereoisomers at the sulfur atom of methionine.[14] Thus the methionine residue was protected, since otherwise the alkylation may take place, when protecting groups was cleaved by the MSA-anisole system[15] at the final step of the synthesis. The Z(OMe) group was removed from Z(OMe)-Phe-Met(O)-OH as usual and the resulting dipeptide was allowed to condense with Z(OMe)-Gly-ONP to give Z(OMe)-Gly-Phe-Met(O)-OH, which after the similar α-deprotection, was condensed with Z(OMe)-Tyr-Gly-$NHNH_2$ by the Rudinger's

11) J. Hughes, T.W. Smith, H.W. Kosterlitz, L.A. Fothergill, B.A. Morgan, and H.R. Morris, *Nature*, (London), **258**, 577 (1975).
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14) N. Fujii, T. Sasaki, S. Funakoshi, H. Irie, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **26**, 650 (1978).
15) H. Irie, N. Fujii, H. Ogawa, H. Yajima, M. Fujino, and S. Shinagawa, *J.C.S. Chem. Comm.*, **1976**, 922; *idem*, *Chem. Pharm. Bull.* (Tokyo), **25**, 2929 (1977).

azide procedure. The protected pentapeptide, Z(OMe)-Tyr-Gly-Gly-Phe-Met(O)-OH thus obtained, was converted to the hydrazide (IX) through the corresponding methyl ester as usual.

Three peptide fragments, (VII, VIII and IX), thus obtained, were then assembled according to the scheme illustrated in Fig. 1. Condensation between the proline-terminal tetrapeptide (VII) and the α-deprotected octadecapeptide was performed by means of DCC in the presence of HOBT.[16] As a solvent, HMPA had to be used, because of the poor solubility of the amino component. The azide condensation of (VIII) was perfomed in a mixture of HMPA and DMSO, but the final condensation of (IX) could be perfomed in DMF, since some improvement of the solubility of the amino component was noted, despite of the chain elongation. The protected intermediates and the protected untriacontapeptide ester, Z(OMe)-Tyr-Gly-Gly-Phe-Met(O)-Thr-Ser-Glu(OBzl)-Lys(Z)-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-(Z)-Asn-Ala-Ile-Ile-Lys(Z)-Asn-Ala-Tyr-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OBzl, were purified by batchwise washing with 5% citric acid and water followed by precipitation from DMSO or DMF with methanol and their purities were assessed by three criteria; thin layer chromatography, elemental analysis and hydrolysis with 3 N *p*-toluenesulfonic acid.[17] As mentioned previously,[3] recovery of isoleucine in a 3 N *p*-toluenesulfonic acid hydrolysate was low and the satisfactory recovery could be obtained, when peptides were hydrolyzed with 6 N hydrochloric acid for 72 hours. However, the former hydrolysis gave the satisfactory recovery of tyrosine, compared to the low recovery of tyrosine in the latter.[18] Under both conditions, Met(O) was partially converted to methionine, as demonstrated with Z(OMe)-Met(O)-OH under identical conditions.

For deprotection, the protected untriacontapeptide ester obtained above was then exposed to MSA in the presence of a cation scavenger, anisole, in an ice-bath for 30 minutes and an additional 60 minutes at room temperature. The deprotected product, [5-Met(O)-β_h-endorphin), was precipitated with ether as a powder, converted to the corresponding acetate by Dowex 1×2 (acetate form) and then treated with 2 N ammonia for 30 minutes. The latter treatment was applied by the reason, that it will cause a reversible N→O shift at the serine and threonine residues, if this shift occurred. This possible side reaction and the reversible shift are known in the treatment of serine and threonine rich peptides by hydrogen fluoride.[19] The product was purified by column chromatography on Sephadex G-25 with 0.5 N acetic acid as eluent and subsequently incubated with mercaptoethanol to reduce the Met(O) residue to the parent amino acid residue with monitoring the progress of reduction by thin layer chromatography. The synthetic untriacontapeptide was then purified by column chromatography on CM-cellulose using gradient elution with 0.5 M ammonium acetate buffer at pH 6.5 and finally desalted by Sephadex G-25. Homogeneity of the synthetic human β-endorphin was assessed by thin layer chromatography in three different solvent systems, acid hydrolysis and digestion with aminopeptidase (AP-M).[20]

Biological assay was conducted by Professor H. Takagi of Faculty of Pharmaceutical Sciences, Kyoto University. Potency ratio of our synthetic peptide to morphin (taken as 1) was 6.5, when assayed, by intracisternal injection, the analgesic effect on tail pinch method in mice.

Experimental

General experimental methods employed are essentially the same as described in the preceding paper. Thin-layer chromatography was performed on silica gel (Kieselgel G Merck), Rf_1 $CHCl_3$–MeOH–H_2O (8:3:1)

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19) S. Sakakibara, in "Chemistry and Biochemistry of Amino Acids, Peptides and Proteins," Vol. 1, ed. B. Weinstein, Marcel Dekker, New York, 1971, p. 51.
20) G. Pfleiderer and G.P. Celliers, *Biochem. Z.*, **339**, 186 (1963).

and on cellulose F (Merck), Rf_2 *n*-BuOH–AcOH–H_2O (4:1:5), Rf_3 *n*-BuOH–AcOH–pyridine–H_2O (30:6:20:24), Rf_4 *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2).

Z(OMe)-Thr-Pro-OH——The azide (prepared from 11.89 g of Z(OMe)–Thr–$NHNH_2$ with 23.2 ml of 3.79 N HCl–DMF, 5.91 ml of isoamylnitrite and 18.4 ml of Et_3N) in DMF (60 ml) was added to an ice-chilled solution of H–Pro–OH (9.21 g) and Et_3N (22.3 ml) in H_2O (90 ml). After stirring at 4° for 48 hr, the solvent was evaporated and the residue was dissolved in 3% NH_4OH. The aqueous phase was washed with AcOEt and then acidified with citric acid. The resulting precipitate was extracted with AcOEt. The extract was washed with 5% citric acid and H_2O–NaCl, dried over Na_2SO_4 and evaporated. The resulting mass was recrystallized from AcOEt and ether; yield 9.36 g (62%). mp 144—146°, $[\alpha]_D^{24}$ −65.6° (c=0.3, MeOH), Rf_1 0.40. *Anal.* Calcd. for $C_{18}H_{24}N_2O_7$: C, 56.83; H, 6.36; N, 7.36. Found: C, 57.01; H, 6.30; N, 7.23.

Z(OMe)-Gln-Thr-Pro-OH——Z(OMe)–Thr–Pro–OH (5.71 g) was treated with TFA (11 ml)–anisole (6 ml) in an ice-bath for 60 min and dry ether was added. The resulting powder was dissolved in DMF (10 ml). To this solution, Et_3N (2.8 ml) and Z(OMe)–Gln–ONP (6.47 g) were added. The mixture was stirred at room temperature for 24 hr, the solvent was evaporated and the residue was dissolved in H_2O. The aqueous phase, after washing with AcOEt, was acidified with citric acid. The resulting precipitate was extracted with *n*-butanol. The extract was washed with H_2O and then evaporated. The residue was triturated with ether and recrystallized from MeOH and ether; yield 4.60 g (60%), mp 106—110°, $[\alpha]_D^{24}$ −62.8° (c=0.4, MeOH), Rf_1 0.24. *Anal.* Calcd. for $C_{23}H_{32}N_4O_9$: C, 54.32; H, 6.34; N, 11.02. Found: C, 54.21; H, 6.35; N, 10.71.

Z(OMe)-Ser-Gln-Thr-Pro-OH (VII)——Z(OMe)–Gln–Thr–Pro–OH (3.33 g) was treated with TFA (5 ml)-anisole (3 ml) as usual and dry ether was added. The resulting powder was dissolved in DMF (9 ml) containing Et_3N (0.98 ml). To this ice-chilled solution, the azide (prepared from 2.27 g of Z(OMe)–Ser–$NHNH_2$ with 4.64 ml of 3.79 N HCl–DMF, 1.18 ml of isoamylnitrite and 3.68 ml of Et_3N) in DMF (12 ml) was added and the mixture was stirred at 4° for 48 hr. The solvent was evaporated and the residue was dissolved in 5% $NaHCO_3$. The aqueous phase was washed with AcOEt and then acidified with citric acid. The resulting precipitate was extracted with *n*-butanol. The extract was washed with H_2O and then dried over Na_2SO_4 and then evaporated. The residue was triturated with ether and then recrystallized from MeOH and ether; yield 1.85 g (47%), mp 118—123°, $[\alpha]_D^{24}$ −67.3° (c=0.4, DMF), Rf_1 0.17. *Anal.* Calcd. for $C_{26}H_{37}N_5O_{11}\cdot 1/2\text{-}H_2O$: C, 51.64; H, 6.34; N, 11.58. Found: C, 51.65; H, 6.17; N, 11.47.

Z(OMe)-Glu(OBzl)-Lys(Z)-NHNH-Troc——Z(OMe)–Lys(Z)–NHNH–Troc[10] (9.51 g) was treated with TFA (11 ml)-anisole (6.5 ml) as usual and *n*-hexane was added. The resulting oil dried over KOH pellets *in vacuo* for 4 hr, was dissolved in DMF (15 ml). To this solution Et_3N (2.1 ml) and Z(OMe)–Glu(OBzl)–ONP (7.84 g) were added. After stirring for 48 hr, the solvent was evaporated and the residue was dissolved in AcOEt. The extract was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and then evaporated. The residue was triturated with ether and recrystallized from AcOEt and ether; yield 8.88 g (69%), mp 100—102°, $[\alpha]_D^{24}$ −24.8° (c=0.3, MeOH), Rf_1 0.79. *Anal.* Calcd. for $C_{38}H_{44}N_5O_{11}Cl_3$: C, 53.49; H, 5.20; N, 8.21. Found: C, 53.89; H, 5.18; N, 8.21.

Z(OMe)-Thr-Ser-$NHNH_2$——To a solution of Z(OMe)–Thr–Ser–OMe[10] (7.69 g) in MeOH (200 ml), 80% hydrazine hydrate (10 ml) was added. After standing overnight, the resulting mass was collected by filtration, washed with MeOH and then precipitated from DMF with MeOH; yield 5.22 g (68%), mp 214—216°, $[\alpha]_D^{24}$ +13.0° (c=0.3, DMSO), Rf_1 0.40. *Anal.* Calcd. for $C_{16}H_{24}N_4O_7$: C, 50.00; H, 6.29; N, 14.58. Found: C, 50.30; H, 6.27; N, 14.57.

Z(OMe)-Thr-Ser-Glu(OBzl)-Lys(Z)-NHNH-Troc——Z(OMe)–Glu(OBzl)–Lys(Z)–NHNH–Troc (8.53 g) was treated with TFA (8 ml)–anisole (4 ml) as usual and dry *n*-hexane was added. The resulting oil isolated as stated above was dissolved in DMF (20 ml) containing Et_3N (1.4 ml). To this ice-chilled solution, the azide (prepared from 3.84 g of Z(OMe)–Thr–Ser–$NHNH_2$ with 1.48 ml of 3.79 N HCl–DMF, 1.5 ml of isoamylnitrite and 4.6 ml of Et_3N) in DMF (30 ml) was added. The mixture was stirred at 4° for 48 hr, the solvent was evaporated and the residue was treated with ether and H_2O. The resulting powder was washed batchwisely with 5% citric acid and H_2O and then precipitated from DMF with ether; yield 7.00 g (67%), mp 140—144°, $[\alpha]_D^{24}$ −7.0° (c=0.4, DMF), Rf_1 0.64. *Anal.* Calcd. for $C_{45}H_{56}N_7O_{15}Cl_3$: C, 51.90; H, 5.42; N, 9.42. Found: C, 51.80; H, 5.36; N, 9.35.

Z(OMe)-Thr-Ser-Glu(OBzl)-Lys(Z)-$NHNH_2$——To a solution of Z(OMe)–Thr–Ser–Glu(OBzl)–Lys(Z)–NHNH–Troc (5.21 g) in DMF (20 ml)–AcOH (20 ml), zinc dust (20 g) was added. After stirring at 50° for 2 hr, the solution was filtered, the filtrate was condensed *in vacuo* and the residue was dissolved in DMF (50 ml) and 17% EDTA (100 ml) was added. The pH of the solution was adjusted at 8 with $NaHCO_3$. After standing overnight, the resulting mass was collected by filtration, washed with H_2O and then precipitated from DMF with MeOH; yield 3.29 g (76%), mp 183—187°, $[\alpha]_D^{24}$ −1.3° (c=0.4, DMSO), Rf_1 0.58. *Anal.* Calcd. for $C_{42}H_{55}N_7O_{13}$: C, 58.25; H, 6.40; N, 11.32. Found: C, 58.10; H, 6.37; N, 11.17.

Z(OMe)-Phe-Met(O)-OH——To a solution of Z(OMe)–Phe–Met–OH[13] (6.91 g) in MeOH (100 ml), a solution of $NaIO_4$ (3.85 g) in H_2O (100 ml) was added. After stirring at room temperature for 3 hr, the solvent was evaporated and the residue was treated with MeOH. The insoluble mass was removed by filtration and the filtrate was condensed. The residue was washed with a small amount of H_2O and then recrystallized

from MeOH and ether; yield 6.28 g (88%), mp 152—156°, $[\alpha]_D^{24}$ −1.3° (c=0.4, MeOH), Rf_1 0.21. *Anal.* Calcd. for $C_{23}H_{28}N_2O_7S \cdot 1/2H_2O$: C, 56.89; H, 6.02; N, 5.77. Found: C, 56.97; H, 5.81; N, 5.68.

Z(OMe)-Gly-Phe-Met(O)-OH——Z(OMe)–Phe–Met(O)–OH (6.18 g) was treated with TFA (15 ml)-anisole (5.6 ml) as usual and dry ether was added. The resulting powder was then dissolved in DMF (30 ml)–H_2O (10 ml). To this solution, Et_3N (3.6 ml) and Z(OMe)–Gly–ONP (4.68 g) in DMF (30 ml) were added. After stirring at room temperature for 24 hr, the solvent was evaporated and the residue was treated with 5% citric acid. The resulting powder was washed with H_2O and then precipitated from DMF with AcOEt; yield 4.58 g (66%), mp 165—169°, $[\alpha]_D^{24}$ −2.8° (c=0.4, MeOH), Rf_1 0.35. *Anal.* Calcd. for $C_{25}H_{31}N_3O_8S \cdot 1.5H_2O$: C, 55.34; H, 5.94; N, 7.75. Found: C, 55.04; H, 5.75; N, 8.13.

Z(OMe)-Tyr-Gly-OMe——DCC (16.51 g) was added to a stirred solution of Z(OMe)–Tyr–OH (27.0 g) and H–Gly–OMe (prepared from 10.04 g of the hydrochloride with 11.2 ml of Et_3N) in THF (150 ml). After 24 hr, the solution was filtered, the filtrate was condensed and the residue was dissolved in AcOEt. The solution was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O–NaCl and then condensed. The resulting mass was recrystallized from DMF and ether; yield 24.12 g (72%), mp 164—166°, $[\alpha]_D^{24}$ −16.7° (c=0.3, MeOH), Rf_1 0.70. *Anal.* Calcd. for $C_{21}H_{24}N_2O_7$: C, 60.57; H, 5.81; N, 6.73. Found: C, 60.24; H, 5.85; N, 6.66.

Z(OMe)-Tyr-Gly-NHNH$_2$——To a solution of Z(OMe)–Tyr–Gly–OMe (3.56 g) in MeOH (100 ml), 80% hydrazine hydrate (8.5 ml) was added. The gelatinous mass formed on standing overnight, was collected by filtration and precipitated from DMF with MeOH; yield 3.05 g (86%), mp 218—222°, $[\alpha]_D^{24}$ −26.8° (c=0.3, DMF), Rf_1 0.50. *Anal.* Calcd. for $C_{20}H_{24}N_4O_6$: C, 57.68; H, 5.81; N, 13.46. Found: C, 57.50; H, 5.81; N, 13.30.

Z(OMe)-Tyr-Gly-Gly-Phe-Met(O)-OH——Z(OMe)–Gly–Phe–Met(O)–OH (4.44 g) was treated with TFA (13 ml)-anisole (4 ml) as usual and dry ether was added. The resulting powder was then dissolved in DMF (15 ml) containing Et_3N (2.3 ml). To this ice-chilled solution, the azide (prepared from 3.0 g of Z(OMe)–Tyr–Gly–NHNH$_2$ with 4.22 ml of 3.79 N HCl–DMF, 1.07 ml of isoamylnitrite and 3.3 ml of Et_3N) in DMF (15 ml) was added. After stirring at 4° for 48 hr, the mixture was condensed and the residue was treated with 5% citric acid and ether. The resulting powder was washed with H_2O and then precipitated from DMF with AcOEt; yield 3.01 g (40%), mp 111—115°, $[\alpha]_D^{24}$ −10.5° (c=0.4, MeOH), Rf_1 0.16. Amino acid ratios in 3 N Tos–OH hydrolysis: Tyr 0.93; Gly 2.05, Phe 1.00, Met+Met(O) 0.88 (average recovery 92%). *Anal.* Calcd. for $C_{36}H_{43}N_5O_{11}S$: C, 56.01; H, 5.88; N, 9.07. Found: C, 55.74; H, 5.78; N, 8.87.

Z(OMe)-Tyr-Gly-Gly-Phe-Met(O)-OMe——An etheral solution of diazomethane was added to an ice-chilled solution of Z(OMe)–Tyr–Gly–Gly–Phe–Met(O)–OH (0.24 g) in MeOH (5 ml) and the yellow color was persisted for 15 min. After addition of a few drops of AcOH, the solvent was evaporated. The residue was triturated with ether and recrystallized from MeOH and ether; yield 0.11 g (44%); mp 198—203°, $[\alpha]_D^{24}$ −17.1° (c=0.3, MeOH), Rf_1 0.71. *Anal.* Calcd. for $C_{37}H_{45}N_5O_{11}S \cdot 1.5H_2O$: C, 55.91; H, 6.09; N, 8.81. Found: C, 56.04; H, 5.80; N, 8.85.

Z(OMe)-Tyr-Gly-Gly-Phe-Met(O)-NHNH$_2$——To a solution of Z(OMe)–Tyr–Gly–Gly–Phe–Met(O)–OMe (0.96 g) in DMF (10 ml), 80% hydrazine hydrate (1.3 ml) was added. After standing overnight, the solution was condensed and the residue was treated with MeOH. The resulting mass was precipitated from DMF with MeOH; yield 0.45 g (47%), mp 185—190°, $[\alpha]_D^{24}$ −30.6° (c=0.4, DMF), Rf_1 0.38. Amino acid ratios in 3 N Tos–OH hydrolysate: Tyr 0.89, Gly 2.02, Phe 1.00, Met+Met(O) 0.88 (average recovery 92%). *Anal.* Calcd. for $C_{36}H_{45}N_7O_{10}S \cdot H_2O$: C, 55.02; H, 6.03; N, 12.48. Found: C, 55.13; H, 5.88; N, 12.86.

Z(OMe)-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys(Z)-Asn-Ala-Ile-Ile-Lys(Z)-Asn-Ala-Tyr-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OBzl, Z(OMe)-(β_h-endorphin 10—31)-OBzl——The protected octadecapeptide ester, Z(OMe)-(β_h-endorphin 14—31)-OBzl, (2.93 g) was treated with TFA (4 ml)–anisole (2.2 ml) as usual and the deprotected peptide was precipitated with ether and 5% $NaHCO_3$. The resulting powder was dissolved in HMPA (20 ml), to which Z(OMe)–Ser–Gln–Thr–Pro–OH (1.11 g), HOBT (0.25 g) and DCC (0.38 g) were successively added. The mixture was stirred at room temperature for 72 hr and then filtered. The filtrate was condensed and the residue was treated with H_2O. The resulting powder was washed batchwisely with 5% citric acid, 5% Na_2CO_3 and H_2O and then precipitated from DMSO with MeOH; yield 1.99 g (59%), mp 309—313°, $[\alpha]_D^{24}$ −20.0° (c=0.3, DMSO), Rf_1 0.54. Amino acid ratios in 3 N Tos–OH hydrolysate: Asp 2.28, Thr 2.18, Ser 0.96, Glu 1.80, Pro 1.04, Gly 1.00, Ala 2.09, Val 1.03, Ile 0.96, Leu 2.14; Tyr 1.03, Phe 1.18, Lys 4.03 (average recovery 86%). *Anal.* Calcd. for $C_{168}H_{231}N_{29}O_{43} \cdot 4H_2O$: C, 59.05; H, 7.05; N, 11.89. Found: C, 59.17; H, 6.84; N, 11.77.

Z(OMe)-Thr-Ser-Glu(OBzl)-Lys(Z)-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys(Z)-Asn-Ala-Ile-Ile-Lys(Z)-Asn-Ala-Tyr-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OBzl, Z(OMe)-(β_h-endorphin 6—31)-OBzl——The above protected docosapeptide ester (1.88 g) was treated with TFA (3 ml)–anisole (1.8 ml) as usual and dry ether was added. The resulting powder was then dissolved in HMPA-DMSO (5 ml–4 ml). To this solution Et_3N (0.08 ml) and the azide (prepared from 0.73 g of Z(OMe)–Thr–Ser–Glu(OBzl)–Lys(Z)–NHNH$_2$ with 0.49 ml of 3.79 N HCl–DMF, 0.13 ml of isoamylnitrite and 0.39 ml of Et_3N) in DMF (5 ml) were added under cooling with ice-NaCl. After stirring at 4° for 48 hr, the solution was condensed and the residue was treated with H_2O. The resulting powder was washed batchwisely as stated above and then precipitated from DMF with MeOH; yield 0.60 g (28%), mp 289—293°, $[\alpha]_D^{24}$ −23.3° (c=0.2, DMSO), Rf_1 0.52. Amino acid ratios

in 3 N Tos–OH hydrolysate; Lys 5.16; Asp 2.37, Thr 3.10, Ser 1.94, Glu 2.76, Pro 1.14, Gly 0.90, Ala 2.14, Val 0.95, Ile 0.93, Leu 2.06, Tyr 1.00, Phe 1.21 (average recovery 94%). *Anal.* Calcd. for $C_{185}H_{258}N_{34}O_{50}\cdot 5H_2O$: C, 57.74; H, 7.02; N, 12.38. Found: C, 58.19; H, 6.76; N, 11.70.

Z(OMe)-Tyr-Gly-Gly-Phe-Met(O)-Thr-Ser-Glu(OBzl)-Lys(Z)-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys(Z)-Asn-Ala-Ile-Ile-Lys(Z)-Asn-Ala-Tyr-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OBzl, Z(OMe)-(βh-endorphin 1—31)-OBzl——The above protected hexacosapeptide ester (0.52 g) was treated with TFA (0.75 ml)–anisole (0.8 ml) as usual and dry ether was added. The resulting powder was then dissolved in DMF (3 ml) containing Et_3N (0.04 ml). To this ice-chilled solution, the azide (prepared from 0.20 g of Z(OMe)–Tyr–Gly–Gly–Phe–Met(O)–$NHNH_2$ with 0.15 ml of 3.79 N HCl–DMF, 0.04 ml of isoamylnitrite and 0.12 ml of Et_3N) in DMF (3 ml) was added. After stirring at 4° for 72 hr, the solution was condensed and the residue was treated with H_2O. The resulting powder was washed batchwisely as stated above and then precipitated from DMF with MeOH; yield 0.53 g (88%), mp 284—288°, $[\alpha]_D^{24}$ −22.8° (c=0.4, DMSO). Rf_1 0.46. Amino acid ratios in 3 N Tos–OH hydrolysate: Asp 2.07, Thr 2.88, Ser 1.74, Glu 2.53, Pro 0.98, Gly 3.06, Ala 2.07, Val 0.96, Ile 0.77, Leu 2.00, Tyr 1.90, Phe 2.14, Lys 5.36 (average recovery 70%). *Anal.* Calcd. for $C_{212}H_{291}N_{39}O_{57}S\cdot 5H_2O$: C, 57.61; H, 6.86; N, 12.36. Found: C, 58.20; H, 6.58; N, 11.74.

H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu-OH, (Human β-Endorphin)——The above protected untriacontapeptide ester (303 mg) was treated with MSA (3.3 ml) in the presence of anisole (0.6 ml) in an ice-bath for 30 min and an additional 60 min at room temperature and dry ether was added. The resulting powder was collected by filtration and dissolved in H_2O (25 ml). The solution was treated with Dowex 1×2 (acetate from, approximately 5 g) for 30 min, then filtered and the filtrate was lyophilized. The resulting powder was dissolved in 2 N NH_4OH (10 ml) and the solution, after stirring in an ice-bath for 30 min, was lyophilized. The resulting powder was dissolved in 0.5 N AcOH (4 ml) and the solution was applied to a column of Sephadex G-25 (3.5×145 cm), which was eluted with the same solvent. Individual fractions (4.2 ml each) were collected and absorbancy at 275 mμ was determined (Fig. 5a). Fractions corresponding to the main peak (tube No. 137—160) were collected and the solvent was removed by lyophilization to give a white powder; yield 107 mg (44%). [5-Met(O)]-human endorphin thus obtained was dissolved in H_2O (2 ml) and the solution was incubated with mercaptoethanol (0.1 ml) under the nitrogen atmosphere at 50° for 48 hr, while Rf_2 0.38 was fully converted to Rf_2 0.42 on TLC. The solution, after addition of H_2O (4 ml), was applied to a column of CM-cellulose (1.8×8 cm), which was eluted with pH 6.5, 0.5 M NH_4OAc (1000 ml) through a mixing flask containing H_2O (1000 ml). Individual fractions (10 ml each) were collected and absorbancy at 275 mμ was determined. Fractions corresponding to the single peak (Tube No. 46—58, Fig. 5b) were collected and the solvent was removed by lyophilization. For desalting, the product was dissolved in 0.5 N AcOH and the solution was applied to a column of Sephadex G-25 (1.8×145 cm), which was eluted with the same solvent. Individual farctions (4.2 ml each) were collected and the desired fractions (Tube No. 30—49) were collected in essentially the same manner as described above. Lyophilization gave a white fluffy powder; yield 53 mg

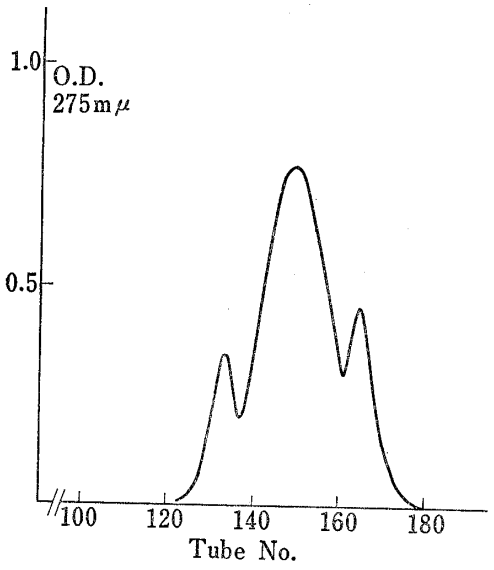

Fig. 5-a. Purification of [5-Met(O)]-Human β-Endorphin

Sample	303 mg.
Column	3.5×145 cm.
Solvent	0.5 N AcOH.
Fraction	4.2 ml.

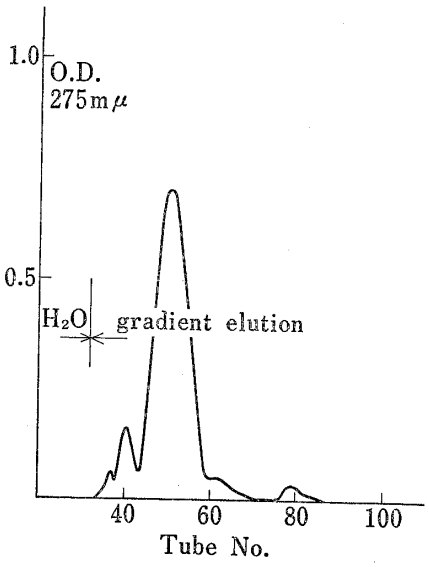

Fig. 5-b. Purification of Synthetic Human β-Endorphin

Column	1.8×8 cm.
Buffer	0.5 M NH_4OAc.
Mixing flask H_2O	1000 ml.
Fraction	10 ml.

(22%), $[\alpha]_D^{24}$ −76.6° (c=0.3, 0.5 N AcOH). Single spot positive to ninhydrin, Pauly and methionine tests; Rf_2 0.42, Rf_3 0.61, Rf_4 0.52. Amino acid ratios in 3 N Tos–OH hydrolysate: Lys 4.97, Asp 2.55, Thr 3.46, Ser 2.00, Glu 3.38, Pro 1.10, Gly 3.34, Ala 2.48, Val 0.98, Met 0.91, Ile 0.37, Leu 2.00, Tyr 2.00 Phe 2.12 (average recovery 87%). Amino acid ratios in a AP–M digest (number in parentheses indicate the theory); Lys 4.88 (5), Thr+Gln 3.33 (3+1, Calcd. as Thr), Ser+Asn 3.11 (2+2, Calcd. as Ser), Glu 2.26 (2), Pro 1.06 (1), Gly 2.86 (3), Ala 2.06 (2), Val 0.99 (1), Met 0.81 (1), Ile 2.13 (2), Leu 2.00 (2), Tyr 1.86 (2), Phe 1.93 (2) (average recovery 83%).

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