Chem. Pharm. Bull. 22(8)1879—1888(1974)

UDC 547.466.1.057

Studies on Peptides. XLVI.[1,2] Synthesis of Human Type Corticotropin-like Intermediate Lobe Peptide (CLIP)

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(Received February 21, 1974)

The docosapeptide, H–Arg–Pro–Val–Lys–Val–Tyr–Pro–Asn–Gly–Ala–Glu–Asp–Glu–Ser–Ala–Glu–Ala–Phe–Pro–Leu–Glu–Phe–OH, corresponding to positions 18 to 39 of human ACTH (human type corticotropin-like intermediate lobe peptide, CLIP) was synthesized by the conventional method and by the fragment condensation procedure on a polymer support.

When Scott, *et al.*[4] isolated corticotropin-like intermediate lobe peptide (CLIP) from porcine pituitaries, they also separated a corresponding peptide from a human bronchial carcinoid tumour associated with the ectopic ACTH syndrome. The amino acid composition of this peptide is identical with that of the C-terminal portion of human ACTH (positions 18 to 39). Leading from the newly revised amino acid sequence of human ACTH,[5] we wish to report the synthesis of the docosapeptide corresponding to positions 18 to 39 of this hormone, which should be defined as human type CLIP.

Synthetic route and choice of the protecting groups employed for the synthesis of human type CLIP are similar to those of bovine type CLIP.[1] Some alternate experiments were added. In addition to such a conventional way of synthesis of CLIP, the fragment condensation procedure on a polymer support was examined for this synthesis. Our present methods are different from those taken during the total syntheses of human ACTH by Sieber, *et al.*[6]

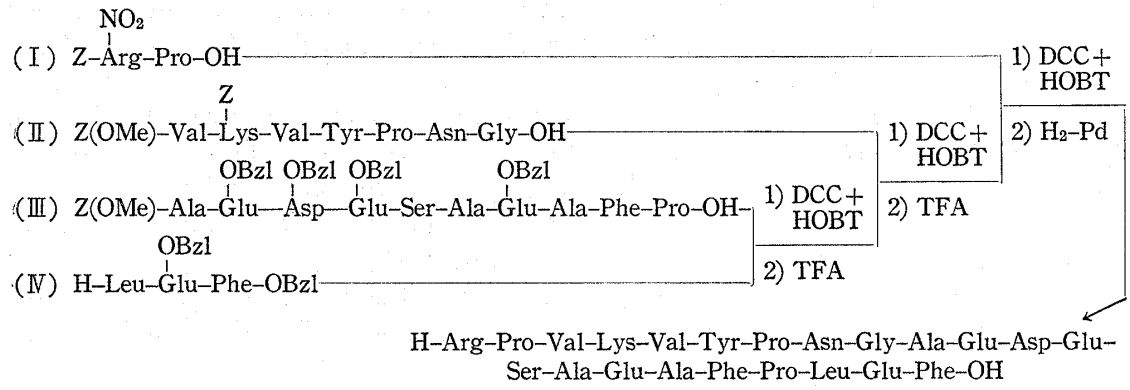

Fig. 1. Synthetic Route to Human Type CLIP

1) Part XLV: H. Kawatani and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **22**, 1872 (1974).
2) Peptide and their derivatives, except Gly, are of the L-configuration. Abbreviations used are those recommended by IUPAC–IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972). Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Bzl=benzyl, ONP=*p*-nitrophenyl ester, OPCP=pentachlorophenyl ester, OSU=*N*-hydroxysuccinimide ester.
3) Location: *Sakyo-ku, Kyoto.*
4) A.P. Scott, J.G. Ratcliffe, L.H. Rees, J. Landon, H.P.J. Bennett, P.J. Lowry and C. McMartin, *Nature New Biol.*, **244**, 65 (1973).
5) B. Riniker, P. Sieber and W. Rittel, *Nature New Biol.*, **235**, 114 (1972).
6) P. Sieber, W. Rittel and B. Riniker, *Helv. Chim. Acta*, **55**, 1243 (1972).

Kisfaludy, *et al.*,[7] Nishimura and Fujino,[8] and Yamashiro and C.H. Li.[9] As shown in Fig. 1, four fragments bearing the protecting groups all removable by catalytic hydrogenation or hydrogen fluoride served to construct the entire sequence of this peptide, *i.e.*, Z–Arg(NO_2)–Pro–OH[10] (I), Z(OMe)–Val–Lys(Z)–Val–Tyr–Pro–Asn–Gly–OH (II), Z(OMe)–Ala–Glu(OBzl)–Asp(OBzl)–Glu(OBzl)–Ser–Ala–Glu(OBzl)–Ala–Phe–Pro–OH (III) and Z(OMe)–Leu–Glu(OBzl)–Phe–OBzl (IV).[1] Among those, two fragments, (I) and (IV), are those previously synthesized.[1] In the synthesis of bovine type CLIP, Z(OMe)–Val–Lys(Z)–Val–Tyr–Pro–OH was taken as one unit, but in the present synthesis, the protected heptapeptide (II) was adopted as one fragment.

Synthetic route to the protected heptapeptide (II) is illustrated in Fig. 2. This sequence contains Asn–Gly bond which is sensitive to alkaline conditions as mentioned by Gráf, *et al.*[11] Therefore, during the course of this synthesis, the base saponification step was avoided. Z(OMe)–Asn–Gly–OBzl was first prepared by the *p*-nitrophenyl ester method,[12] This, after treat-

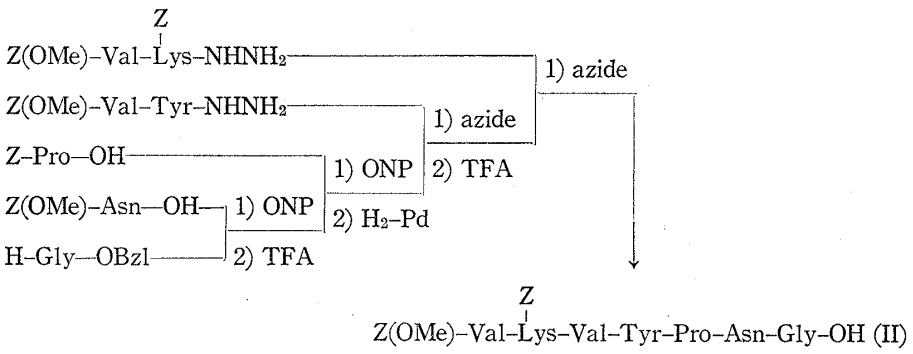

Fig. 2. Synthetic Route to the Protected Heptapeptide (II), Z(OMe)–(ACTH 20–26)–OH

ment with trifluoroacetic acid (TFA),[13] was condensed with Z–Pro–OH again by the *p*-nitrophenyl ester method to give the protected tripeptide ester, Z–Pro–Asn–Gly–OBzl. At this stage, both of the protecting groups attached at the carboxyl and the α-amino groups were removed by catalytic hydrogenation. The resulting free tripeptide, H–Pro–Asn–Gly–OH, was then condensed with Z–Val–Tyr–$NHNH_2$[14] by the modified azide procedure according to Honzl and Rudinger.[15] The protecting group of the resulting pentapeptide, Z–Val–Tyr–Pro–Asn–Gly–OH, was removed by hydrogenation to give H–Val–Tyr–Pro–Asn–Gly–OH. This was then coupled with Z(OMe)–Val–Lys(Z)–$NHNH_2$[14] *via* the modified azide procedure to afford (II). Elemental analysis and careful ammonia determination in an acid hydrolysate supported the empirical formula of this Asn-containing heptapeptide.

Synthetic route to the protected decapeptide (III) is illustrated in Fig. 3. Z–Ala–Phe–$NHNH_2$[16] was coupled with the triethylammonium salt of H–Pro–OH *via* the azide procedure

7) L. Kisfaludy, M. Law, T. Szirtes, I. Schon, M. Sarkozi, S. Bajusz, A. Turan, A. Juhasz, R. Bake, L. Gráf and K. Medzihradszky, "Chemistry and Biology of Peptides," Ed. by J. Meienhofer, Ann Arbor Protein Science, Michigan, U.S., 1972, p. 299.
8) O. Nishimura and M. Fujimo, "The 10th Symposium on Peptide Chemistry," Ed. By J. Noguchi, Res. Foundation, Osaka, 1972, p. 154.
9) D. Yamashiro and C.H. Li, *J. Am. Chem. Soc.*, **95**, 1310 (1973).
10) H. Yajima and K. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **21**, 682 (1973).
11) L. Gráf, S. Bajusz, A. Patthy, E. Barat and G. Cseh, *Acta, Biochim. Biophys. Acad. Sci. Hung.*, **6**, 415 (1971).
12) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).
13) F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 1 (1962).
14) H. Yajima and H. Kawatani, *Chem. Pharm. Bull.* (Tokyo), **19**, 1905 (1971).
15) J. Honzl and J. Rudinger, *Collection Czech. Chem. Commun.*, **26**, 2333 (1961).
16) E. Schröder, *Ann. Chem.*, **679**, 207 (1964).

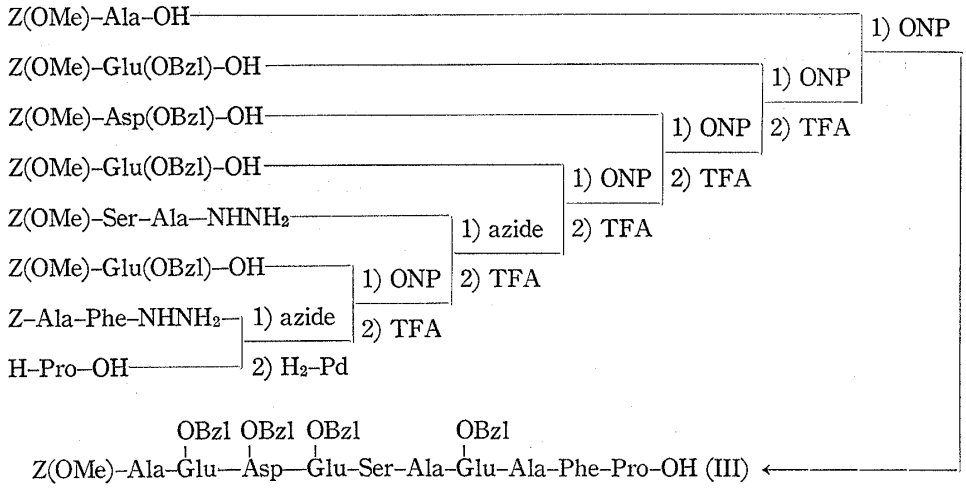

Fig. 3. Synthetic Route to the Protected Decapeptide (III), Z(OMe)-(h-ACTH 27-36)-OH

to afford Z-Ala-Phe-Pro-OH, which was subsequently hydrogenated to give the free tripeptide, H-Ala-Phe-Pro-OH. An alternate synthesis of this tripeptide was reported by Bajusz and Lázár.[17] To this tripeptide, Z(OMe)-Glu(OBzl)-ONP was allowed to react to give Z(OMe)-Glu(OBzl)-Ala-Phe-Pro-OH, which after treatment with TFA, was condensed with Z(OMe)-Ser-Ala-$NHNH_2$[1] by the azide procedure. Along this synthetic route, the protected heptapeptide, Z(OMe)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-OH, which differs from the subunit of bovine CLIP, was synthesized in satisfactory yield. Next combination of the TFA treatment for deprotection of the Z(OMe) group and the *p*-nitrophenyl ester method was applied to elongate this hexapeptide chain to the decapeptide stage (III). Extraction of this protected decapeptide (III), as well as all of protected intermediates, with ethyl acetate was possible. Therefore these peptides were purified by the extraction procedure.

Four peptide fragments obtained as outlined above were then assembled according to the route illustrated in Fig. 1. As previously demonstrated in the synthesis of bovine type CLIP, the dicyclohexylcarbodiimide (DCC) plus N-hydroxybenzotriazole (HOBT) procedure, developed by König and Geiger,[18] was applied for the condensation of these fragments, and the TFA procedure was served to remove the Z(OMe) protecting group from intermediates.

Synthesis of the protected tridecapeptide, Z(OMe)-Ala-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-OBzl, abbreviated as Z(OMe)-(h-ACTH 27—39)-OBzl, was performed by two alternate routes. First, condensation of (III) with H-Leu-Glu(OBzl)-Phe-OBzl derived from (IV) was carried out by DCC in the presence of HOBT. The product, Z(OMe)-(h-ACTH 27—39)-OBzl, was purified by column chromatography on silica using the solvent, chloroform-methanol-water (40: 15: 5). This solvent system was quite effective for the purification of this protected tridecapeptide ester as well as the latter protected intermediates.

Alternatively, we have synthesized Z(OMe)-(h-ACTH 27—39)-OBzl according to the route illustrated in Fig. 4. The protected nonapeptide ester, Z(OMe)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-OBzl, was prepared, like the previous synthesis of bovine CLIP,[1] by condensation of Z(OMe)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-OH and H-Leu-Glu(OBzl)-Phe-OBzl with DCC in the presence of HOBT. Elongation of the nonapeptide chain up to the tridecapeptide was performed stepwisely by the *p*-nitrophenyl ester procedure. In this route, purification of the desired protected tridecapeptide was achieved by batchwise washing with acid and base followed by recrystallization without an aid of column chromato-

17) S. Bajusz and T. Lázár, *Acta Chim. Acad. Sci. Hung.*, **48**, 111 (1966).
18) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).

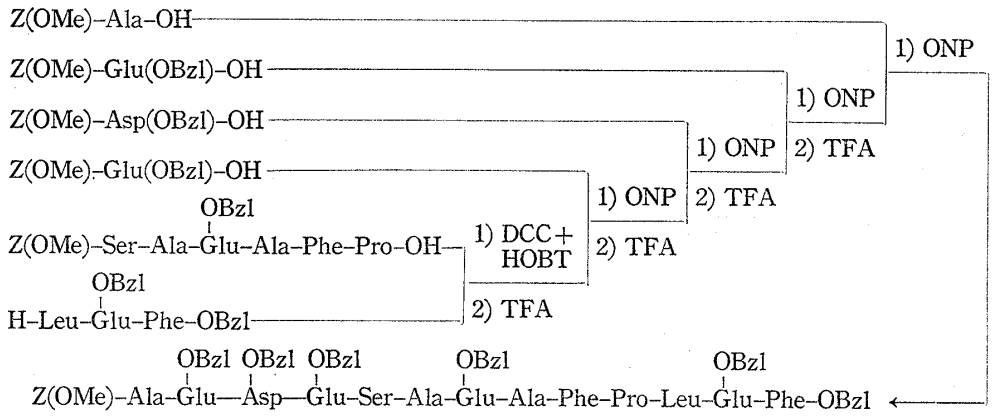

Fig. 4. Alternate Synthetic Route to the Protected Tridecapeptide Ester, Z(OMe)-(h-ACTH 27-39)-OBzl

graphy. The protected tridecapeptide ester was then treated with TFA. The resulting trifluoroacetate was once converted to the corresponding hydrochloride and subsequently to the free base by neutralization with triethylamine. The deprotected tridecapeptide ester was coupled with Z(OMe)-(ACTH 20—26)-OH (II) by the DCC plus HOBT procedure to give the protected eicosapeptide ester, Z(OMe)-Val-Lys(Z)-Val-Tyr-Pro-Asn-Gly-Ala-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-OBzl, abbreviated as Z(OMe)-(h-ACTH 20—39)-OBzl, which was purified by column chromatography on silica.

The final coupling reaction was performed in essentially the same manner as described previously.[1] The above protected eicosapeptide ester, Z(OMe)-(h-ACTH 20—39)-OBzl, after treatment with TFA, was condensed with Z-Arg(NO_2)-Pro-OPCP[10] to give the fully protected docosapeptide ester, Z-Arg(NO_2)-Pro-Val-Lys(Z)-Val-Tyr-Pro-Asn-Gly-Ala-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-OBzl, which was purified also by column chromatography on silica. Removal of the protecting groups from this protected human type CLIP; *i.e.*, the Z, NO_2 and benzyl ester groups, was performed by hydrogen fluoride according to Sakakibara, *et al.*,[19] rather by catalytic hydrogenation, since the latter reductive cleavage of the NO_2 group was found to be a time consuming process.[1] The deblocked product, after treatment with a small amount of Amberlite IR-4B, was purified by column chromatography on CM-cellulose and the desired compound was eluted by means of the gradient elution with pyridine acetate buffer. As stated previously,[1] the absorbancy due to the Tyr residue was the guide for this chromatographic purification. Homogeneity of the synthetic human type CLIP was assessed by thin-layer chromatography and elemental and amino acid analyses.

As an alternate route, synthesis of human type CLIP was performed by the fragment condensation procedure on a polymer support. Fragments, I, II, and III, prepared by the above conventional method were directly available for this purpose, except for the C-terminal tripeptide unit, Z(OMe)-Leu-Glu(OBzl)-Phe-OH. This protected tripeptide was prepared in a stepwise manner by the active ester procedure.

Along the scheme illustrated in Fig. 5, esterification of this protected tripeptide onto the bromomethylated copolymer of styrene and 2% divinylbenzene[20] instead of the chloromethylated resin,[21] was first carried out. Condensation of succeeding peptide fragments, III, II and I, by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) were performed

19) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada and H. Sugihara, *Bull. Chem. Soc. Japan*, **40**, 2164 (1967).
20) H. Yajima, H. Kawatani and H. Watanabe, *Chem. Pharm. Bull.* (Tokyo), **18**, 1333 (1970).
21) R.B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963).

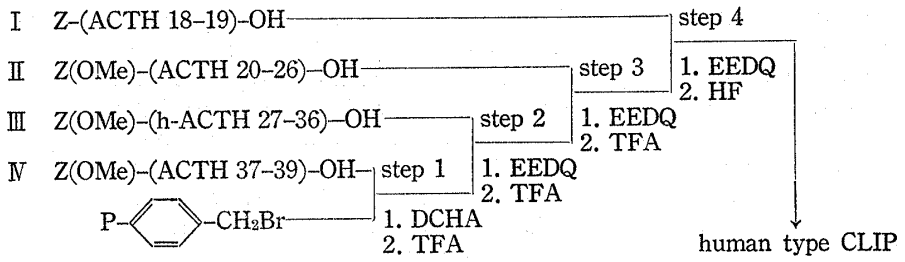

Fig. 5. Synthetic Chart of Human Type CLIP by Fragment Condensation Procedure on a Polymer Support

in essentially the same manner as described previously.[22] In this approach, tendency was noticed that quantitative coupling was difficult to achieve, when a relatively large peptide, such as the protected decapeptide (III), was used as a carboxyl component and a sterically hindered amino acid residue, such as the Leu residue in the tripeptideresin, served as an amino component. The coupling yield of III and H–Leu–Glu(OBzl)–Phe–resin was 58% when 2 equimoles of III were employed. Two succeeding coupling reaction proceeded in nearly 90% yield, as judged by the newly incorporated amino acid content. In each step, unreacted amino components on the resin were masked by acetylation prior to the next coupling reaction. Cleavage of the growing peptide from the resin and removal of all protecting groups were achieved in one step by hydrogen fluoride.[19] Purification of the product was carried out by column chromatography on CM-cellulose as stated earlier by applying the gradient elution with pyridine acetate buffer. Rechromatography was necessary to isolate a peptide which gave rational constituent amino acid ratios in an acid hydrolysate. As the result of a series of incompleted reactions on the polymer, purification of the final peptide was, as expected, more laborious compared to that of the above mentioned conventional synthesis, though the purification step of intermediates was simplified in this approach.

Syntheses of sheep and pig type CLIP's are in progress and biological evaluation of synthetic CLIP's (bovine[1] and human) are under investigations.

Experimental

General experimental methods employed are essentially the same as described in the Part XXII[23] of this series. Thin-layer chromatography was performed on silica gel (Kieselgel G, Merck). *Rf* values refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH–H_2O (40:15:5). Rf_2 $CHCl_3$–MeOH–AcOH (9:1:0.5), Rf_3 *n*-butanol–pyridine–AcOH–H_2O (4:1:1:2).

Z(OMe)-Asn-Gly-OBzl——Z(OMe)–Asn–ONP (28.3 g) was added to a solution of H–Gly–OBzl (prepared from 15.0 g of the hydrobromide with 20.2 ml of Et_3N) in dimethylformamide (DMF) (150 ml) and the mixture was stirred at room temperature for 48 hr. After evaporation of the solvent, ether and H_2O were added to the residue and the resulting gelatinous mass was washed batchwisely with 5% citric acid, 5% Na_2CO_3 and H_2O and recrystallized twice from tetrahydrofuran (THF) and AcOEt; yield 18.1 g (67%). mp 164—167°. $[\alpha]_D^{26}$ −3.1° (c=0.8, DMF). Rf_1 0.60. *Anal.* Calcd. for $C_{22}H_{25}O_7N_3$: C, 59.58; H, 5.68; N, 9.48. Found: C, 59.74; H, 5.61; N, 9.48.

Z-Pro-Asn-Gly-OBzl——In the presence of anisole (20 ml), Z(OMe)–Asn–Gly–OBzl (39.0 g) was treated with trifluoroacetic acid (TFA) (40 ml) at room temperature for 45 min. Petroleum ether was added and the resulting oily precipitate was treated with dry ether to give fine powder, which after drying over KOH pellets *in vacuo*, was dissolved in DMF (250 ml). To this solution, Et_3N (24.8 ml) and Z–Pro–ONP (35.0 g) were added and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated and the resulting gelatinous mass was washed batchwisely with ether, 5% citric acid, 5% Na_2CO_3 and H_2O and recrystallized from dioxane and MeOH; yield 35.0 g (74%). mp 157—161°. $[\alpha]_D^{22}$ −43.6° (c=0.9, DMF). Rf_1 0.45. *Anal.* Calcd. for $C_{27}H_{32}O_8N_4$: C, 59.99; H, 5.97; N, 10.37. Found: C, 60.52; H, 5.89; N, 11.03.

H-Pro-Asn-Gly-OH——Z–Pro–Asn–Gly–OBzl (34.6 g) in a mixture of dioxane and MeOH (3:2 v/v, 300 ml) was hydrogenated over a Pd catalyst in the usual manner. The product was recrystallized from

22) H. Yajima and H. Kawatani, *Chem. Pharm. Bull.* (Tokyo), **19**, 1905 (1971).
23) H. Yajima, Y. Okada, H. Kawatani and N. Mizokami, *Chem. Pharm. Bull.* (Tokyo), **17**, 1229 (1969).

H_2O and EtOH; yield 18.2 g (99%), mp 238° dec., $[\alpha]_D^{26}$ −61.3° (c=0.9, 30% AcOH). Rf_3 0.66. *Anal.* Calcd. for $C_{11}H_{18}O_5N_4$: C, 46.15; H, 6.34; N, 19.57. Found: C, 45.90; H, 6.41; N, 19.36.

Z-Val-Tyr-Pro-Asn-Gly-OH——In the usual manner, Z-Val-Tyr-$NHNH_2$ (12.8 g) in 1.83N HCl-DMF (33 ml) was converted to the corresponding azide with isoamyl nitrite (4.0 ml) and the solution, after neutralization with Et_3N (8.3 ml), was combined with a solution of H-Pro-Asn-Gly-OH (8.6 g) and Et_3N (8.3 ml) in H_2O (50 ml). The mixture was stirred at 4° for 48 hr and then the solvent was evaporated. The residue was dissolved in 5% NH_4OH, which after washing with AcOEt, was acidified with citric acid and the resulting precipitate was extracted with AcOEt. The crystalline solid formed on standing in a refrigerator was recrystallized from 80% MeOH; yield 17.4 g (85%). mp 169—173°. $[\alpha]_D^{22}$ −37.8° (c=1.1, DMF). Rf_1 0.18, Rf_3 0.70. *Anal.* Calcd. for $C_{33}H_{42}O_{10}N_6 \cdot H_2O$: C, 56.56; H, 6.33; N, 11.99. Found: C, 56.30; H, 6.29; N, 11.82.

H-Val-Tyr-Pro-Asn-Gly-OH——In the usual manner, Z-Val-Tyr-Pro-Asn-Gly-OH (17.0 g) in a mixture of MeOH (160 ml) and AcOH (20 ml) was hydrogenated over a Pd catalyst. During the hydrogenolysis, H_2O (50 ml) was added. The clear solution, after removing the catalyst, was condensed. H_2O was added and evaporation was repeated. Tritulation of the residue with EtOH afforded the solid, which was recrystallized from H_2O and EtOH; yield 12.5 g (92%). mp 194—197°. $[\alpha]_D^{27}$ −50.9° (c=0.8, 10% AcOH). Rf_3 0.38. *Anal.* Calcd. for $C_{25}H_{36}O_8N_6 \cdot 1.5H_2O$: C, 52.16; H, 6.83; N, 14.60. Found: C, 52.39; H, 7.00; N, 14.22.

Z(OMe)-Val-Lys(Z)-Val-Tyr-Pro-Asn-Gly-OH (II), Z(OMe)-(ACTH 20-26)-OH——In the usual manner, Z(OMe)-Val-Lys(Z)-$NHNH_2$ (8.4 g) in 1N HCl-DMF (30 ml) was converted to the corresponding azide with isoamyl nitrite (2.0 ml) and the solution, after neutralization with Et_3N (2.8 ml) was added to a solution of H-Val-Tyr-Pro-Asn-Gly-OH (5.5 g) and Et_3N (2.8 ml) in 60% DMF (50 ml). The mixture was stirred at 4° for 48 hr and the solvent, after addition of a few drop of AcOH, was evaporated. The gelatinous mass formed by addition of AcOEt, was washed with 5% citric acid and H_2O and recrystallized twice from MeOH; yield 5.9 g (55%). mp 158—162°, $[\alpha]_D^{26}$ −32.6° (c=1.1, DMF). Rf_3 0.80. Amino acid ratios in an acid hydrolysate, $Val_{2.23}Lys_{0.95}Tyr_{0.33}Pro_{1.06}Asp_{1.12}Gly_{1.00}(NH_4)_{1.04}$ (average recovery 96%).[24] *Anal.* Calcd. for $C_{53}H_{71}O_{15}N_9 \cdot H_2O$: C, 58.28; H, 6.74; N, 11.54. Found: C, 58.07; H, 6.77; N, 11.49.

Z-Ala-Phe-OMe——The compound was obtained by the DCC procedure; yield 64%. mp 99—101°, $[\alpha]_D^{26}$ +1.1° (c=0.9, DMF). (lit.[15] mp 99—100°. $[\alpha]_D$ −9.3° in EtOH). Rf_1 0.93. *Anal.* Calcd. for $C_{21}H_{24}O_5N_2$: C, 65.61; H, 6.29; N, 7.29. Found: C, 65.35; H, 6.36; N, 7.29.

Z-Ala-Phe-$NHNH_2$——Z-Ala-Phe-OMe (24.3 g) in MeOH (140 ml) was converted to the corresponding hydrazide in the usual manner; yield 24.0 g (98%). mp 206—209° (lit.[15] mp 198—199°). *Anal.* Calcd. for $C_{20}H_{24}O_4N_4$: C, 62.48; H, 6.29; N, 14.58. Found: C, 62.34; H, 6.31; N, 14.41.

Z-Ala-Phe-Pro-OH——(a) Z-Ala-Phe-$NHNH_2$ (24.0 g) was dissolved in DMF (100 ml) with an aid of 4N HCl-DMF (42.5 ml). To this chilled solution (−5°), isoamyl nitrite (11.3 ml) was added. The hydrazine test became negative within 5 min, when the solution was neutralized with Et_3N (23.4 ml). This solution was then combined to a solution of H-Pro-OH (19.6 g) in H_2O (80 ml) containing Et_3N (35.2 ml). The mixture was stirred at 4° for 48 hr and then evaporated. The residue was dissolved in 3% NH_4OH, which after washing with AcOEt, was acidified with citric acid. The resulting precipitate was extracted with AcOEt, which was washed with H_2O-NaCl, dried over Na_2SO_4 and then evaporated. Treatment of the residue with petroleum ether afforded the solid, which was recrystallized from AcOEt and petroleum ether; yield 26.9 g (68%). mp 91—93°. $[\alpha]_D^{27}$ −30.3° (c=1.0, DMF). Rf_1 0.62. *Anal.* Calcd. for $C_{25}H_{29}O_6N_3$: C, 64.22; H, 6.25; N, 8.99. Found: C, 63.73; H, 6.31; N, 9.01.

(b) Z-Ala-ONP (28.0 g) in tetrahydrofuran (140 ml) was combined with a solution of H-Phe-Pro-OH[1] (21.0 g) in H_2O (140 ml) containing Et_3N (24 ml) and the mixture was stirred at room temperature for 48 hr. After evaporation of the solvent, the residue was dissolved in H_2O, which after washing with ether, was acidified with citric acid and the resulting precipitate was extracted with AcOEt. The organic phase was washed with H_2O, dried over Na_2SO_4 and then evaporated. The residue was treated as stated above; yield 24.0 g (64%). mp 91—93°.

H-Ala-Phe-Pro-OH——Z-Ala-Phe-Pro-OH (31.1 g) in MeOH (250 ml) was hydrogenated over a Pd catalyst in the usual manner. It was recrystallized from H_2O and EtOH; yield 21.9 g (99%). mp 153—155°. $[\alpha]_D^{26}$ −33.2° (c=1.0, 30% AcOH). (lit.[17] mp 159—161°, $[\alpha]_D$ −51° in H_2O). Rf_1 0.15, Rf_3 0.64. *Anal.* Calcd. for $C_{17}H_{23}O_4N_3 \cdot 1.5H_2O$: C, 56.65; H, 7.27; N, 11.66. Found: C, 57.12; H, 7.55; N, 11.68.

Z(OMe)-Glu(OBzl)-Ala-Phe-Pro-OH——Z(OMe)-Glu(OBzl)-ONP (23.0 g) in a mixture of tetrahydrofuran (120 ml) and pyridine (60 ml) was added to a solution of H-Ala-Phe-Pro-OH (12.0 g) in H_2O (230 ml) containing Et_3N (10.9 ml) and the mixture was stirred at room temperature for 48 hr. After evaporation of the solvent, the residue was dissolved in H_2O, which was washed with AcOEt and then acidified with citric acid. The resulting precipitate was extracted with AcOEt, which was washed with H_2O-NaCl, dried over Na_2SO_4 and then evaporated. The semisolid residue was applied to a column of silica (3×20 cm), which was eluted with $CHCl_3$. The product was recrystallized from AcOEt and petroleum ether; yield 22.6 g

24) Low recovery of Tyr in the acid hydrolysis was mentioned by B. Iselin, *Helv. Chim. Acta*, **45**, 1510 (1962).

(79%). mp 78—82°. $[\alpha]_D^{26}$ −21.5° (c=1.0, DMF). Rf_1 0.62. *Anal.* Calcd. for $C_{38}H_{44}O_{10}N_4 \cdot H_2O$: C, 62.11; H, 6.31; N, 7.63. Found: C, 62.40; H, 6.50; N, 7.57.

Z(OMe)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-OH——Isoamylnitrite (4.0 ml) was added to a chilled solution (−5°) of Z(OMe)–Ser–Ala–$NHNH_2$[1] (10.5 g) in 1N HCl–DMF (60 ml). When the hydrazine test became negative within 5 min, the solution was neutralized with Et_3N (12.4 ml). This solution was combined with a solution of H–Glu(OBzl)–Ala–Phe–Pro–OH (prepared by treatment of 18.6 g of Z(OMe)–Glu(OBzl)–Ala–Phe–Pro–OH with 18 ml of TFA in the presence of 9 ml of anisole for 45 min followed by precipitation with ether and neutralization with 7.2 ml of Et_3N) in DMF (180 ml) and the mixture was stirred at 4° for 48 hr. The solvent was dissolved in 3% NH_4OH, which after washing with AcOEt, was acidified with citric acid. The resulting precipitate turned to solid, which was washed with 5% citric acid and H_2O and then recrystallized from MeOH and AcOEt; yield 8.9 g (39%). mp 156—159°. $[\alpha]_D^{27}$ −21.0° (c=0.9, DMF). Rf_1 0.55. *Anal.* Calcd. for $C_{44}H_{54}O_{13}N_6$: C, 60.40; H, 6.22; N, 9.61. Found: C, 60.10; H, 6.24; N, 9.51.

Z(OMe)-Glu(OBzl)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-OH——In the presence of anisole (4 ml), Z(OMe)–Ser–Ala–Glu(OBzl)–Ala–Phe–Pro–OH (8.7 g) was treated with TFA (17 ml) at room temperature for 40 min and the product was precipitated by addition of petroleum ether. The oily precipitate turned to solid powder by treatment with ether. The TFA salt thus obtained was dissolved in DMF (80 ml). Et_3N (4.1 ml) and Z(OMe)–Glu(OBzl)–ONP (6.8 g) were added and the mixture was stirred at room temperature for 48 hr, when the solvent was evaporated. The residue was dissolved in AcOEt, which was washed with 5% citric acid and H_2O–NaCl, dried over Na_2SO_4 and then evaporated to give gelatinous mass. It was recrystallized three times from AcOEt and ether; yield 9.3 g (85%). mp 135—139°. $[\alpha]_D^{29}$ −24.7° (c=1.0, DMF). Rf_1 0.55. *Anal.* Calcd. for $C_{56}H_{67}O_{16}N_7$: C, 61.47; H, 6.17; N, 8.96. Found: C, 61.46; H, 6.00; N, 8.78.

Z(OMe)-Asp(OBzl)-Glu(OBzl)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-OH——The above protected heptapeptide (9.0 g) was similarly treated with TFA (9 ml) in the presence of anisole (4 ml). The TFA salt precipitated by addition of ether, was dried over KOH pellets *in vacuo* and then dissolved in DMF (90 ml). Et_3N (4.1 ml) and Z(OMe)–Asp(OBzl)–ONP (6.5 g) were added and the mixture was stirred at room temperature for 48 hr. After evaporation of the solvent, the residue was extracted with AcOEt, which was washed with 5% citric acid and H_2O, dried over Na_2SO_4 and then evaporated. The solid obtained after addition of ether, was precipitated from AcOEt with ether and recrystallized from MeOH; yield 8.8 g (83%). mp 158—162°. $[\alpha]_D^{29}$ −23.1° (c=1.0, DMF). Rf_1 0.47. *Anal.* Calcd. for $C_{67}H_{78}O_{19}N_8 \cdot 4H_2O$: C, 58.67; H, 6.32; N, 8.17. Found: C, 58.85; H, 6.11; N, 8.12.

Z(OMe)-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-OH——The above protected octapeptide (8.5 g) was similarly treated with TFA (8.5 ml) in the presence of anisole (4 ml). The fine powder obtained by addition of dry ether, was dried over KOH pellets *in vacuo* and then dissolved in DMF (60 ml). Et_3N (2.7 ml) and Z(OMe)–Glu(OBzl)–ONP (4.7 g) was added and the mixture was stirred at room temperature for 48 hr. A few drop of AcOH was added and the solvent was evaporated. The residue was dissolved in AcOEt, which was washed with 5% citric acid and then evaporated. Addition of ether afforded the solid which was recrystallized twice from MeOH and ether; yield 8.7 g (88%), mp 152—156°. $[\alpha]_D^{27}$ −19.0° (c=0.4, DMF). Rf_1 0.64. *Anal.* Calcd. for $C_{79}H_{91}O_{22}N_9 \cdot 3H_2O$: C, 60.33; H, 6.22; N, 8.02. Found: C, 59.92; H, 6.18; N, 8.56.

Z(OMe)-Ala-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-OH (III), Z(OMe)-(h-ACTH 27-36)-OH——The above protected nonapeptide (8.5 g) was treated with TFA (9 ml) in the presence of anisole (2.5 ml) as stated above. The TFA salt obtained as fine powder by addition of dry ether, was dissolved in DMF (50 ml). After addition of Et_3N (2.5 ml) and Z(OMe)–Ala–ONP (2.9 g), the mixture was stirred at room temperature for 48 hr. The solvent was evaporated and the residue was extracted with AcOEt, which was washed with 5% citric acid and then evaporated. The resulting solid formed by addition of ether was recrystallized twice from AcOEt and ether; yield 8.2 g (92%), mp 176—178°. $[\alpha]_D^{26}$ −20.3° (c=0.8, DMF). Rf_1 0.46. Amino acid ratios in an acid hydrolysate: $Ala_{2.85}Glu_{3.21}Asp_{0.97}Ser_{0.82}Phe_{1.00}Pro_{1.01}$ (average recovery 91%). *Anal.* Calcd. for $C_{82}H_{96}O_{23}N_{10} \cdot 4H_2O$: C, 59.26; H, 6.30; N, 8.42. Found: C, 59.03; H, 5.81; N, 9.19.

Z(OMe)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-OBzl——Z(OMe)–Leu–Glu(OBzl)–Phe–OBzl (12.4 g) was treated with TFA (12 ml) in the presence of anisole (4 ml) for 45 min. AcOEt was added and the solution was washed with ice-chilled 5% Na_2CO_3, dried over Na_2SO_4 and then filtered. The filtrate was combined with a solution of Z(OMe)–Ser–Ala–Glu(OBzl)–Ala–Phe–Pro–OH (13.1 g) in DMF (70 ml). DCC (3.7 g) and HOBT (4.1 g) were added and the mixture was stirred at room temperature for 48 hr. After filtration, the solvent was evaporated and the residue was extracted with AcOEt, which was washed with 5% citric acid, 5% Na_2CO_3 and H_2O and then evaporated. Addition of ether to the residue gave fine powder, which was washed batchwisely with 5% citric acid and 5% Na_2CO_3 and recrystallized from AcOEt and ether; yield 21.1 g (98%), mp 114—115°. $[\alpha]_D^{20}$ −26.1° (c=0.9, DMF). Rf_2 0.69. Amino acid ratios in an acid hydrolysate $Ser_{0.91}Ala_{2.00}Glu_{2.27}Phe_{2.02}Pro_{1.05}Leu_{0.95}$ (average recovery 99%). *Anal.* Calcd. for $C_{78}H_{93}O_{18}N_9 \cdot 1.5H_2O$: C, 63.66; H, 6.58; N, 8.57. Found: C, 63.65; H, 6.58; N, 9.19.

Z(OMe)-Glu(OBzl)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-OBzl——In the presence of anisole (5 ml), the above protected nonapeptide ester (20.0 g) was treated with TFA (20 ml) at room tem-

perature for 60 min. Dry ether was added to form fine powder, which after drying over KOH pellets *in vacuo* overnight, was dissolved in DMF (180 ml). Et_3N (3.8 ml) and Z(OMe)–Glu(OBzl)–ONP (9.5 g) were added and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated and the residue was extracted with AcOEt, which was washed successively with 5% citric acid, 5% Na_2CO_3 and H_2O–NaCl and then evaporated. Fine powder formed by addition of ether was washed batchwisely with 5% citric acid and 5% Na_2CO_3 and then recrystallized from AcOEt and ether. Batchwise washing and recrystallization were repeated; yield 21.2 g (92%), mp 123—127°. $[\alpha]_D^{25}$ −26.1° (c=0.9, DMF). Rf_1 0.96. *Anal.* Calcd. for $C_{90}H_{106}O_{21}N_{10}\cdot 3H_2O$: C, 62.92; H, 6.57; N, 8.15. Found: C, 62.58; H, 6.50; N, 8.05.

Z(OMe)-Asp(OBzl)-Glu(OBzl)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-OBzl——The Z(OMe) group was cleaved from the above protected decapeptide ester (21.0 g) by TFA (21 ml) in the presence of anisole (7 ml) as stated above. The TFA salt thus obtained was dissolved in DMF (180 ml). To this solution, Et_3N (3.6 ml) and Z(OMe)–Glu(OBzl)–ONP (8.6 g) were added and the mixture was stirred at room temperature for 48 hr. The product was purified in essentially the same manner as stated above; yield 21.6 g (92%). mp 109—114°. $[\alpha]_D^{25}$ −26.5° (c=1.0, DMF). Rf_1 0.89. *Anal.* Calcd. for $C_{101}H_{117}O_{24}N_{11}\cdot 4H_2O$: C, 62.49; H, 6.49; N, 8.24. Found: C, 62.41; H, 6.29; N, 8.14.

Z(OMe)-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-OBzl——The above protected undecapeptide ester (21.4 g) was treated with TFA (25 ml) in the presence of anisole (7 ml) as stated above. The TFA salt thus obtained was dissolved in DMF (150 ml). Et_3N (3.2 ml) and Z(OMe)–Glu(OBzl)–ONP (7.8 g) were added and the mixture was stirred at room temperature for 48 hr. The product was isolated in essentially the same manner as stated above; yield 22.3 g (93%) mp 103—105°. $[\alpha]_D^{25}$ −25.9° (c=1.0, DMF). Rf_1 0.89. *Anal.* Calcd. for $C_{113}H_{130}O_{27}N_{12}\cdot 4H_2O$: C, 62.82; H, 6.44; N, 7.78. Found: C, 62.74; H, 6.14; N, 8.19.

Z(OMe)-Ala-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-OBzl, Z(OMe)-(h-ACTH 27-39)-OBzl——(a) In the presence of anisole (7 ml), the above protected dodecapeptide ester (22.2 g) was treated with TFA (25 ml) for 45 min and the product was precipitated with dry ether, dried over KOH pellets *in vacuo* and then dissolved in DMF (180 ml). Et_3N (3.3 ml) and Z(OMe)–Ala–ONP (5.8 g) were combined with this solution, which was stirred at room temperature for 48 hr. The product was isolated in essentially the same manner as stated above; yield 19.9 g (78%). mp 127—131°. $[\alpha]_D^{25}$ −26.8° (c=1.0, DMF). Rf_1 0.85. Amino acid ratios in an acid hydrolysate $Ala_{2.85}Glu_{4.21}Asp_{1.00}Ser_{0.81}Phe_{1.96}Pro_{0.84}Leu_{1.00}$ (average recovery 91%). *Anal.* Calcd. for $C_{116}H_{135}O_{28}N_{13}\cdot 4H_2O$: C, 62.43; H, 6.45; N, 8.16. Found: C, 62.03; H, 6.10; N, 8.71.

(b) Z(OMe)–Leu–Glu(OBzl)–Phe–OBzl (3.8 g) was treated with TFA (3.7 ml) in the presence of anisole (1 ml) at room temperature for 45 min. Addition of ether or petroleum ether did not give any precipitate as stated previously. In this experiment, the TFA was removed *in vacuo* and after addition of 1N HCl-dioxane (7.5 ml), the solvent was removed by lyophilization. The residue was dissolved in DMF (35 ml) and Et_3N (0.68 ml) was added. To this solution, Z(OMe)–Ala–Glu(OBzl)–Asp(OBzl)–Glu(OBzl)–Ser–Ala–Glu(OBzl)–Ala–Phe–Pro–OH (4.0 g), HOBT (0.68 g) and DCC (1.03 g) were combined and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated and the residue was extracted with AcOEt, which was washed with 5% citric acid, 5% Na_2CO_3 and H_2O, dried over Na_2SO_4 and then evaporated. The resulting solid was washed batchwisely with 5% citric acid and 5% Na_2CO_3 and recrystallized from AcOEt and ether. For further purification, the product was applied to a column of silica (3×15 cm), which was eluted with $CHCl_3$–MeOH–H_2O (40: 15: 5) and recrystallized from AcOEt and ether; yield 4.47 g (80%), mp 126—131°. $[\alpha]_D^{25}$ −27.9° (c=1.1, DMF). Rf_1 0.85. Amino acid ratios in an acid hydrolysate: $Ala_{2.80}Glu_{4.28}Asp_{1.00}Ser_{0.81}Phe_{2.22}Pro_{0.99}Leu_{1.03}$ (average recovery 89%).

Z(OMe)-Val-Lys(Z)-Val-Tyr-Pro-Asn-Gly-Ala-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-OBzl——In the usual manner, the above tridecapeptide ester (8.6 g) was treated with TFA (10 ml) in the presence of anisole (4 ml) at room temperature for 45 min. The solid precipitate formed by addition of dry ether, was dissolved in 1N HCl-dioxane and again precipitated by dry ether. The solid hydrochloride was dissolved in dioxane containing Et_3N (0.55 ml) and dry ether was added. The fine powder thereby obtained was then dissolved in DMF (60 ml). To this solution, Z(OMe)–Val–Lys(Z)–Val–Tyr–Pro–Asn–Gly–OH (4.29 g), HOBT (1.62 g) and DCC (1.65 g) were combined and the mixture was stirred at room temperature for 48 hr. After evaporation of the solvent, the solid precipitate formed by addition of ether, was washed with 5% citric acid and H_2O and applied to a column of silica (3×25 cm), which was eluted with $CHCl_3$–MeOH–H_2O (40: 15: 5). The eluates were examined by thin-layer chromatography. The solvent of the fractions containing the substance of Rf_1 0.58, was evaporated and the residue was treated with ether to afford fine powder, which after washing with H_2O, was recrystallized from DMF and ether; yield 9.23 g (74%). mp 134—138°. $[\alpha]_D^{28}$ −27.9° (c=1.1, DMF). Rf_1 0.58, Rf_2 0.77. Amino acid ratios in an acid hydrolysate: $Val_{2.33}Lys_{0.95}Tyr_{0.45}Pro_{2.46}Asp_{1.90}Gly_{1.00}Ala_{2.97}Glu_{4.23}Ser_{0.76}Phe_{1.72}Leu_{0.87}$ (average recovery 97%). *Anal.* Calcd. for $C_{160}H_{196}O_{39}N_{22}\cdot 3H_2O$: C, 61.88; H, 6.56; N, 9.92. Found: C, 61.69; H, 6.89; N, 10.33.

Z-Arg(NO$_2$)-Pro-Val-Lys(Z)-Val-Tyr-Pro-Asn-Gly-Ala-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser-Ala-Glu-(OBzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-OBzl, Z(h-ACTH 18-39)-OBzl——Z(OMe)–(h-ACTH 20–39)–OBzl (0.61 g) was treated with TFA (1.6 ml) in the presence of anisole (0.3 ml) for 60 min. After addition of dry

ether, the resulting powder was collected by filtration, dried over KOH pellets *in vacuo* and then dissolved in DMF (5 ml). To this solution, Et_3N (0.06 ml) and Z–Arg(NO_2)–Pro–OPCP[10] (0.21 g) were added and the mixture was stirred at room temperature for 72 hr. The solvent was evaporated and the powder formed by addition of ether, was washed batchwisely with 5% citric acid and H_2O and then applied to a column of silica (2×30 cm), which was eluted with $CHCl_3$–MeOH–H_2O (8:3:1). Eluates which contained the desired product (Rf_1 0.70) were combined and the solvent was evaporated. The residue was washed with H_2O and then precipitated from DMF with AcOEt; yield 0.40 g (61%). mp 151—153°. $[\alpha]_D^{22}$ −28.3° (c=0.3, DMF). Amino acid ratios in an acid hydrolysate: $Arg_{0.70}Pro_{3.29}Val_{1.95}Lys_{1.37}Tyr_{0.32}Asp_{2.00}Gly_{0.78}Ala_{3.32}Glu_{4.21}Ser_{0.79}Phe_{1.85}Leu_{0.95}$ (average recovery 86%). *Anal.* Calcd. for $C_{170}H_{212}O_{42}N_{28}\cdot 4H_2O$: C, 60.20; H, 6.54; N, 11.56. Found: C, 59.78; H, 6.36; N, 12.02.

H-Arg-Pro-Val-Lys-Val-Tyr-Pro-Asn-Gly-Ala-Glu-Asp-Glu-Ser-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe-OH (human type CLIP)——Z–(h-ACTH 18–39)–OBzl (0.33 g) was treated with HF (approximately 10 ml) in the presence of anisole (1 ml) at 0° for 60 min. The excess of HF was evaporated *in vacuo* and the residue was dissolved in H_2O (20 ml), which was treated with Amberlite IR-4B (acetate form, approximately 5 g) for 15 min. The solution was filtered and the filtrate, after washing with ether, was condensed *in vacuo*. The residue was lyophilized and then applied to a column of CM-cellulose (2×20 cm), which was eluted with H_2O (300 ml) and then pH 6.9, 0.05M pyridine acetate buffer (550 ml) through a mixing flask containing H_2O (50 ml). Absorbancy at 275 mμ in individual fraction (10 ml) was examined. Chromatographic pattern indicated the presence of a main symmetrical peak and two other minor peaks (4 mg and 6 mg each). Fractions of this main peaks were combined and the solvent was removed by evaporation. The residue was lyophilized to give fluffy white powder; yield 89 mg (36%). $[\alpha]_D^{22}$ −92.8° (c=0.1, H_2O). Rf_3 0.50. Amino acid ratios in an acid hydrolysate: $Arg_{1.10}Pro_{3.25}Val_{2.00}Lys_{0.95}Tyr_{0.78}Asp_{2.01}Gly_{0.90}Ala_{3.34}Glu_{4.16}Ser_{0.84}Phe_{2.08}Leu_{1.00}$ (average recovery 78%). *Anal.* Calcd. for $C_{112}H_{165}O_{36}N_{27}\cdot 3CH_3COOH\cdot 6H_2O$: C, 51.45; H, 6.92; N, 13.74. Found: C, 51.33; H, 6.46; N, 13.45.

Synthesis of Human Type CLIP by Fragment Condensation Procedure on a Polymer Support

Z(OMe)-Glu(OBzl)-Phe-OH——Z(OMe)–Glu(OBzl)–ONP (40.2 g) was added to a solution of H–Phe–OH (11.6 g) and Et_3N (19.6 ml) in a mixture of H_2O (150 ml) and THF (300 ml). The solution was stirred at room temperature for 24 hr. The solvent was evaporated and the residue was acidified with 10% citric acid. The resulting precipitate was extracted with AcOEt, which after washing with NaCl–H_2O, was dried over Na_2SO_4 and then evaporated. The residue was tritulated and the resulting solid was recrystallized from AcOEt; yield 33.0 g (86%). mp 143—144°. $[\alpha]_D^{22}$ +2.5° (c=0.6, DMF). Rf_1 0.60. *Anal.* Calcd. for $C_{30}H_{32}O_8N_2$: C, 65.68; H, 5.88; N, 5.11. Found: C, 65.74; H, 5.95; N, 5.20.

Z(OMe)-Leu-Glu(OBzl)-Phe-OH——Z(OMe)–Glu(OBzl)–Phe–OH (21.9 g) was treated with TFA (25 ml) in the presence of anisole (8 ml) at room temperature for 60 min. Dry ether was added and the resulting precipitate (19.9 g), after drying over KOH pellets *in vacuo*, was dissolved in 50% aqueous THF (200 ml) and Et_3N (11.2 ml) was added. This solution was combined with a solution of Z(OMe)–Leu–OSU (23.5 g) in THF (200 ml) and the mixture was stirred at room temperature for 24 hr. The solvent was evaporated and the residue, after acidification with 10% citric acid, was extracted with AcOEt, which was washed with H_2O–NaCl, dried over Na_2SO_4 and then evaporated. The residue was tritulated with ether and the resulting solid was recrystallized from AcOEt and ether; yield 23.2 g (88%). mp 124—126°. $[\alpha]_D^{22}$ −2.6° (c=1.0, DMF). Rf_1 0.47. Amino acid ratios in an acid hydrolysate: $Leu_{1.00}Glu_{1.13}Phe_{1.16}$ (average recovery 91%). *Anal.* Calcd. for $C_{36}H_{43}O_9N_3$: C, 65.34; H, 6.55; N, 6.35. Found: C, 65.63; H, 6.47; N, 6.34.

Z(OMe)-Leu-Glu(OBzl)-Phe-Resin——To a suspension of the bromomethylated resin[20] (5.0 g, bromo content 1.1 mM/g) in DMF (20 ml), Z(OMe)–Leu–Glu(OBzl)–Phe–OH (1.19 g) and dicyclohexyalmine (DCHA, 0.74 ml) were added and the mixture was shaked at room temperature for 72 hr. The resin was collected by filtration and washed successively with DMF, H_2O and EtOH (20 ml×3 each). A part of the resin was submitted to acid hydrolysis: Phe content 0.11 mM/g (coupling yield 31%).

Z-Arg(NO_2)-Pro-Val-Lys(Z)-Val-Tyr-Pro-Asn-Gly-Ala-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser-Ala-Glu-(OBzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-Resin——The above protected tripeptide resin (1.0 g) was treated with 50% TFA in CH_2Cl_2 (8 ml) for 45 min, collected by filtration and washed with CH_2Cl_2 and DMF (10 ml× 3 each). To a suspension of this resin in DMF (8 ml), Z(OMe)–Ala–Glu(OBzl)–Asp(OBzl)–Glu(OBzl)–Ser–Ala–Glu(OBzl)–Ala–Phe–Pro–OH (III) (440 mg, 2 equimoles) and EEDQ (164 mg, 6 equimoles) were combined and the mixture was shaked at room temperature for 48 hr. AcOH (0.06 ml) was then added and the suspension was shaken overnight. The resin was collected by filtration and washed with DMF (10 ml× 3). After similar deprotection and subsequent condensation of Z(OMe)–Val–Lys(Z)–Val–Tyr–Pro–Asn–Gly–OH (II) (354 mg, 3 equimoles) and Z–Arg(NO_2)–Pro–OH (149 mg, 3 equimoles) were repeated stepwisely. Calculation of molar ratios of amino acids newly incorporated in each reaction step revealed the coupling yield: 58% (Asp/Leu) in step 2, 87% (Val/Ala) in step 3 and 92% (increase of the Pro content) in step 4. The peptide resin 1.15 g (yield 42% in weight basis) was obtained.

Isolation of the Synthetic Human Type CLIP——The protected docosapeptide resin obtained above (1.15 g) was treated with HF (approximately 10 ml) in the presence of anisole (1 ml) for 60 min. The excess HF was removed by evaporation *in vacuo* and the residue was dissolved in H_2O, which was treated with Amberlite IR-4B (acetate form, approximately 4 g) for 15 min. The solution was filtered, the filtrate

was condensed *in vacuo* and the residue was lyophilized. This lyophilized sample was applied to a column of CM-cellulose (1.6×17 cm), which was eluted with H_2O (100 ml) and 0.05 M pyridine acetate buffer (300 ml) through a mixing flask containing H_2O (100 ml). Absorbancy at 275 mμ was determined in each fraction (10 ml). Amino acid ratios in the acid hydrolysate of the product obtained from the main peak (42 mg) were $Arg_{1.23}Pro_{1.83}Val_{2.23}Lys_{1.11}Tyr_{1.03}Asp_{1.55}Gly_{1.11}Ala_{1.22}Glu_{2.44}Ser_{0.30}Phe_{1.39}Leu_{1.00}$. This sample was submitted to rechromatography on CM-cellulose (1.6×9 cm); yield 14 mg (9.3% starting from the peptide resin), Rf_3 0.50, single spot positive to ninhydrin, Sakaguchi and Pauly tests. $[\alpha]_D^{22}$ −96.4° (c=0.1, H_2O). Amino acid ratios in an acid hydrolysate: $Arg_{0.80}Pro_{3.11}Val_{1.77}Lys_{1.02}Tyr_{0.82}Asp_{0.86}Gly_{1.19}Ala_{2.77}Glu_{4.06}Ser_{1.00}$ $Phe_{1.84}Leu_{1.00}$ (average recovery 90%).