[Chem. Pharm. Bull. 36(9)3271—3280(1988)]

Studies on Peptides. CLIX.[1,2] Preparation of a Protected 33-Residue Peptide for the Synthesis of Human Cholecystokinin (hCCK-33)

NOBUTAKA FUJII, SHIROH FUTAKI, SUSUMU FUNAKOSHI, KENICHI AKAJI, HIROSHI MORIMOTO, OSAMU IKEMURA, and HARUAKI YAJIMA*

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

(Received February 9, 1988)

A protected 33-residue peptide corresponding to the entire amino acid sequence of human cholecystokinin (hCCK-33) was synthesized by successive azide condensations of 7 peptide fragments of established purity. β-Cycloheptyl aspartate [Asp(OChp)] was employed to suppress base-catalyzed succinimide formation.

Keywords——human cholecystokinin synthesis; mesitylenesulfonylarginine; β-cycloheptyl aspartate; mesitylenesulfonyltryptophan; methionine sulfoxide; Asp-side reaction; base-catalyzed succinimide formation

In two consecutive papers, we wish to report the synthesis of a 33-residue peptide corresponding to the entire amino acid sequence of human cholecystokinin (hCCK-33), the gene structure of which was elucidated by Takahashi *et al.*[3] in 1985. From the synthetic viewpoint, CCK offers considerable difficulty mainly for two reasons; one is the instability of Tyr(SO_3H) (position 27) to acids, including TFA treatment which is required for N^{α}-deprotection prior to chain elongation, and the other is the fact that sulfation with pyridine–SO_3 complex takes place predominantly at the alcoholic OH group of Ser, rather than at the phenolic OH group of Tyr. Thus, it is clear that the total synthesis of hCCK-33 possessing 4 Ser residues can not be accomplished by the same method employed for the previous synthesis of porcine CCK-8 (positions 26—33), or even CCK-12 (positions 22—33).[4]

We have synthesized hCCK-33 by a method different from that employed for the synthesis of porcine CCK-33[5] by Kurano *et al.*[6] In the final step of their synthesis, the phenoxyacetyl groups, used for OH-protection of 4 Ser residues, were removed, after sulfation

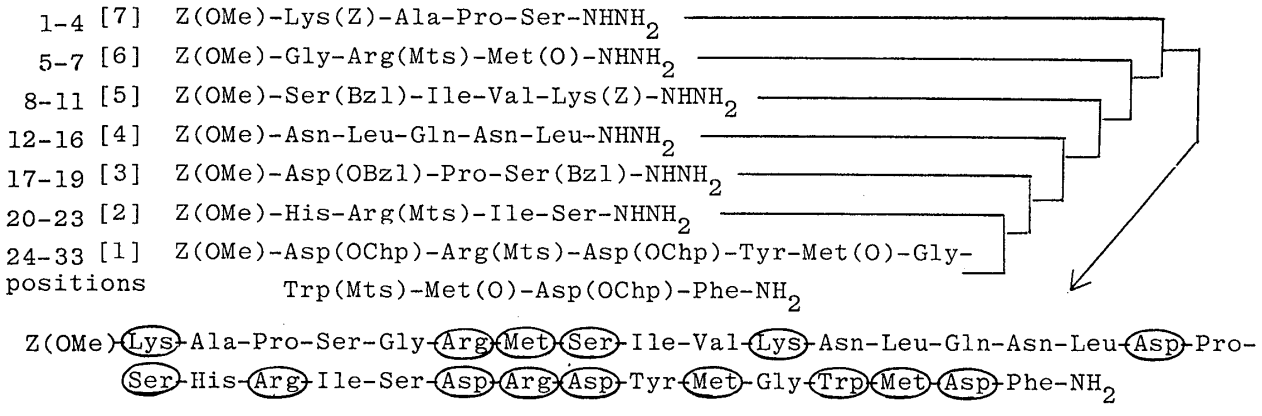


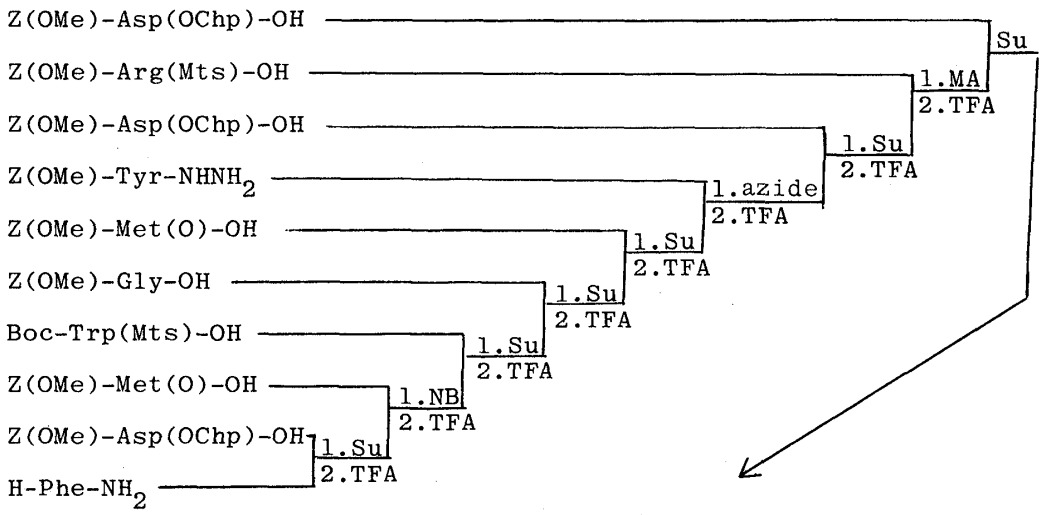
Fig. 1. Synthetic Scheme for Protected hCCK-33 (Free 27-Tyr)

○, protected amino acids: Lys(Z), Arg(Mts), Met(O), Ser(Bzl), Asp(OChp), Asp(OBzl) (position 17), Trp(Mts).

of Tyr, by treatment with 0.1 M NaOH. Even with these precautions, the yield in the final sulfation step was reported to be *ca.* 5%. In our synthesis, a free form of hCCK-33 was first prepared, then 27-Tyr was sulfated with pyridine–SO_3 complex, after reversible masking of other functions with hard base-labile protecting groups, *i.e.*, the amino functions with the Fmoc group[7] and four Ser–OH functions with the *tert*-butyldiphenylsilyl ($tBuPh_2Si$) group.[8]

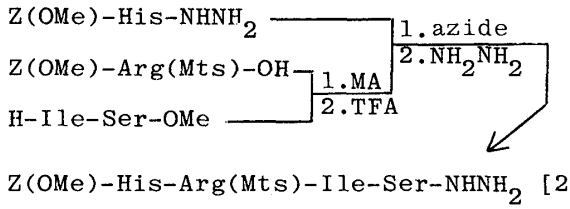
We prepared two forms of protected hCCK-33 by using unprotected Tyr and protected Tyr, Tyr(Cl_2–Bzl).[9] The latter synthesis, *i.e.*, synthesis of protected Tyr(Cl_2–Bzl)–hCCK-33, was conducted to prevent over-acylation at the Tyr residue, and to make purification of each intermediate easier, as will be described in the subsequent paper. In this paper, we wish to report the initial synthesis of protected hCCK-33 (free 27-Tyr), which was accomplished by successive azide condensation[10] of 7 peptide fragments as shown in Fig. 1.

The C-terminal fragment, Z(OMe)–Asp(OChp)–Arg(Mts)–Asp(OChp)–Tyr–Met(O)–Gly–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH_2 [1], was prepared in a stepwise manner starting from H–Phe–NH_2 (Fig. 2). Besides Arg(Mts),[11] Asp(OChp)[12] and Trp(Mts)[13] were employed to suppress side reactions, *i.e.*, the former, base-catalyzed succinimide formation[14] and the latter, indole-alkylation during N^{α}-deprotection.[15] Thus, these three amino acid derivatives introduced by us were successfully employed in the present synthesis. Met was protected as its sulfoxide.[16] The Su ester procedure[17] was employed as a main tool to condense constituent amino acid residues, except for three residues. For condensations of two residues, Met(O) and Arg(Mts) (positions 31 and 25), the NB ester[18] and the MA method[19] respectively gave better yields than the Su method for some reason. The azide procedure was employed for condensation of the unprotected Tyr residue. The purity of fragment [1] was ascertained by thin layer chromatography (TLC), elemental analysis and amino acid analysis after 6 N HCl hydrolysis, as was done with other fragments.



Z(OMe)-Asp(OChp)-Arg(Mts)-Asp(OChp)-Tyr-Met(O)-Gly-Trp(Mts)-Met(O)-Asp(OChp)-Phe-NH_2 [1]

Fig. 2. Synthetic Scheme for the C-Terminal Decapeptide Amide [1] (Positions 24—33)



Z(OMe)-His-Arg(Mts)-Ile-Ser-$NHNH_2$ [2]

Fig. 3. Synthetic Scheme for the Protected Tetrapeptide Hydrazide [2] (Positions 20—23)

Fragment [2], Z(OMe)–His–Arg(Mts)–Ile–Ser–$NHNH_2$, was prepared in a stepwise manner also starting from a TFA-treated sample of Z(OMe)–Ile–Ser–OMe,[20] onto which two residues, Arg(Mts) and His, were condensed successively by the MA and the azide methods, respectively. The resulting protected tetrapeptide ester was converted to [2] by the usual hydrazine treatment (Fig. 3).

Fragment [3], Z(OMe)–Asp(OBzl)–Pro–Ser(Bzl)–$NHNH_2$, was prepared with the aid of the substituted hydrazine, Troc–$NHNH_2$.[21] H–Ser(Bzl)–NHNH–Troc[22] was condensed with Z(OMe)–Pro–OH by DCC[23] in the presence of HOBt,[24] then Z(OMe)–Asp(OBzl)–OH by the MA method. From the resulting tripeptide derivative, the Troc group was removed by treatment with Zn–AcOH[25] to give [3] (Fig. 4). Ser(Bzl) was employed for preparations of fragments [3] and [5] in order to increase the solubilities of these fragments.

Fragment [4], Z(OMe)–Asn–Leu–Gln–Asn–Leu–$NHNH_2$, was prepared in a stepwise manner starting with a TFA-treated sample of Z(OMe)–Asn–Leu–OMe.[26] The Np method[27] was employed as a tool to elongate the peptide chain. The resulting protected pentapeptide ester was converted to [4] by the usual hydrazine treatment (Fig. 5).

Fragment [5], Z(OMe)–Ser(Bzl)–Ile–Val–Lys(Z)–$NHNH_2$, was prepared as shown in

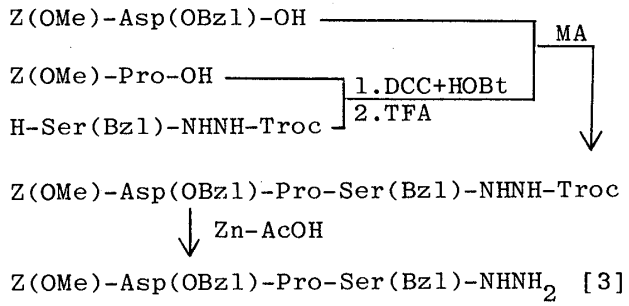


Fig. 4. Synthetic Scheme for the Protected Tripeptide Hydrazide [3] (Positions 17—19)

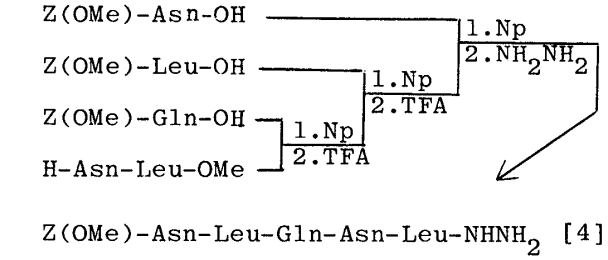


Fig. 5. Synthetic Scheme for the Protected Pentapeptide Hydrazide [4] (Positions 12—16)

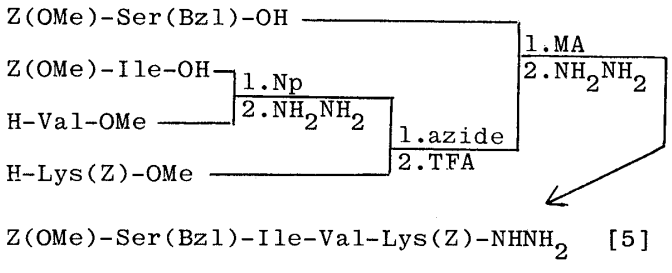


Fig. 6. Synthetic Scheme for the Protected Tetrapeptide Hydrazide [5] (Positions 8—11)

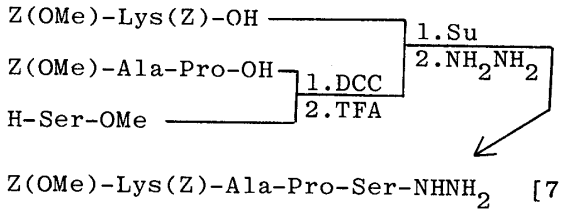


Fig. 7. Synthetic Scheme for the Protected Tetrapeptide Hydrazide [7] (Positions 1—4)

Fig. 6. A dipeptide unit with bulky side chains, Z(OMe)–Ile–Val–$NHNH_2$, was first prepared by the Np method, followed by the usual hydrazine treatment. This was then condensed with H–Lys(Z)–OMe *via* the azide to give Z(OMe)–Ile–Val–Lys(Z)–OMe. This tripeptide, after TFA treatment, was condensed with Z(OMe)–Ser(Bzl)–OH *via* the MA and the resulting tetrapeptide ester was converted to [5] as usual.

Fragment [6], Z(OMe)–Gly–Arg(Mts)–Met(O)–$NHNH_2$, was easily prepared by the Su condensation of Z(OMe)–Gly–OH with a TFA-treated sample of Z(OMe)–Arg(Mts)–Met(O)–OMe,[28] followed by the usual hydrazine treatment.

The N-terminal fragment [7], Z(OMe)–Lys(Z)–Ala–Pro–Ser–$NHNH_2$, was prepared by using an available dipeptide unit, Z(OMe)–Ala–Pro–OH.[29] This dipeptide was condensed with H–Ser–OMe by using DCC and the resulting protected tripeptide ester, after TFA treatment, was condensed with Z(OMe)–Lys(Z)–OH *via* the Su ester to give the protected tetrapeptide ester, which was converted to [7] as usual (Fig. 7).

Seven peptide fragments thus obtained were assembled successively by the azide procedure to minimize racemization. Each reaction was continued until the solution became negative to ninhydrin. The amount of the acyl component was increased from 1.5 to 5 eq as chain elongation progressed. In particular, for condensation of fragment [4], a toral of 8 (5+3) eq of the acyl component was employed. This hydrazide was less soluble in DMF. Thus, a mixture of three solvents, DMF–DMSO–HMPA (1 : 1 : 1), had to be employed in a large excess. Consequently, the solution was too diluted to allow completion of the reaction. The products were purified either by precipitation from DMF with MeOH or by gel-filtration on Sephadex LH-60 using DMF as an eluant. Throughout the synthesis, Phe, the C-terminal residue, was used as a diagnostic amino acid in acid hydrolysis in order to ascertain the homogeneity of each product. Recovery of Phe was compared with those of newly added amino acids after each condensation. Thus, satisfactory incorporation of each fragment was ascertained (Table I).

In the above fragment condensation reactions, we had to use the acyl component in a large excess to bring the reaction to completion. Consequently, the possibility can not be

TABLE I. Amino Acid Ratios in 6 N HCl Hydrolysates of Protected hCCK-33 (Free 27-Tyr) and Its Intermediates

	24—33	20—33	17—33	12—33	8—33	5—33	1—33	
Asp	3.05	3.10	3.82	6.01	6.27	6.12	6.01	(6)
Ser		1.00	1.86	1.90	2.82	2.75	3.45	(4)
Glu				1.10	1.08	1.08	1.03	(1)
Pro			1.00	0.82	1.00	1.00	2.01	(2)
Gly	1.12	1.14	1.18	1.05	1.06	1.98	1.95	(2)
Ala							0.99	(1)
Val					0.53	0.72	0.70	(1)
Met	1.88	1.75	1.74	1.58	1.80	2.68	2.50	(3)
Ile		1.03	0.88	0.94	1.42	1.59	1.60	(2)
Leu				2.07	2.07	2.04	2.00	(2)
Tyr	0.89	1.07	1.01	1.02	1.00	0.99	0.96	(1)
Phe	1.00	1.00	1.00	1.00	1.00	1.00	1.00	(1)
Lys					0.92	0.97	1.90	(2)
His		1.01	0.86	0.92	0.88	0.97	0.81	(1)
Trp[a]	1.21	0.93	0.89	0.85	0.94	1.11	0.88	(1)
Arg	0.98	2.10	1.86	2.00	1.98	2.96	2.86	(3)
Recov. (%)	82	78	84	85	82	73	86	

a) Determined by 4 N MSA hydrolysis.

excluded that over-acylation at the Tyr residue took place to a certain degree. Indeed, we had to purify four products, resulted from azide condensations of fragments from [4] to [7], by gel-filtration on Sephadex LH-60. The yields we obtained were 48% to at best 69%. Considering the difficulty of the sulfation step for the synthesis of hCCK-33, we decided to prepare a certain quantity of protected hCCK-33 by an alternative route using protected Tyr, Tyr(Cl_2–Bzl), as will be described in the subsequent paper.

Experimental

General experimental procedures described herein are essentially the same as described in Part CLVII[30] of this series. Prior to condensation, the N^{α}-Z(OMe) group was removed by TFA treatment in the presence of anisole. The active ester reaction was performed at room temperature, the azide reaction at 4 °C and the MA reaction in an ice-bath. Unless otherwise stated, products were purified by one of the following procedures.

A. Extraction Procedure: After evaporation of the solvent, the product was dissolved in AcOEt or some other organic solvent(s). The organic phase was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and concentrated. The residue was recrystallized or precipitated from appropriate solvent(s).

B. Washing Procedure: After evaporation of the solvent, the residue was treated with 5% citric acid and ether, then the resulting powder was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O, and recrystallized or precipitated from appropriate solvents.

C. Gel-Filtration Procedure: The product, partially purified by procedure B, was dissolved in a small amount of DMF and a solution was applied to a column of Sephadex LH-60, which was eluted with DMF. Fractions corresponding to the front main peak [monitored by ultraviolet (UV) absorption measurement at 280 nm] were combined and the solvent was removed by evaporation *in vacuo*. The residue was precipitated from DMF with AcOEt.

TLC was conducted on silica gel (Kieselgel G, Merck) and *Rf* values refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH–H_2O (8 : 3 : 1), Rf_2 $CHCl_3$–MeOH (10 : 0.5).

Z(OMe)–Asp(OChp)–Phe–NH_2 [1]-(1)——A mixture of Z(OMe)–Asp(OChp)–OSu [prepared from 25.00 g (44.0 mmol) of the DCHA salt], H–Phe–NH_2 [prepared from 14.44 g (44.0 mmol) of the Z(OMe)–derivative] and NMM (4.84 ml, 1 eq) in THF–DMF (60 ml–50 ml) was stirred overnight. The product was purified by procedure A (solvent $CHCl_3$), followed by recrystallization from $CHCl_3$ and ether; yield 16.33 g (69%), Rf_1 0.76, Rf_2 0.25. Physical constants and analytical data are listed in Table II, together with those of other protected peptides.

TABLE II. Physical Constants and Analytical Data for Fragment [1] and Its Intermediates

	Fragment [1] and intermediates	mp (°C)	$[\alpha]_D^{20}$ (°) (DMF)	Formula	Analysis (%) Calcd (Found)		
					C	H	N
(1)	Z(OMe)-Dipeptide (32—33)	163—165	−20.0	$C_{29}H_{37}N_3O_7$	64.54 (64.50	6.91 7.04	7.79 7.78)
(2)	Z(OMe)-Tripeptide (31—33)	197—199	+6.1[a]	$C_{34}H_{46}N_4O_9S$	59.46 (59.21	6.75 6.70	8.16 8.07)
(3)	Boc-Tetrapeptide (30—33)	152—154	−32.2[a]	$C_{50}H_{66}N_6O_{11}S_2$	60.58 (60.72	6.71 6.72	8.48 8.29)
(4)	Z(OMe)-Pentapeptide (29—33)	172—175	−25.0	$C_{56}H_{69}N_7O_{13}S_2 \cdot H_2O$	59.50 (59.77	6.33 6.42	8.68 8.75)
(5)	Z(OMe)-Hexapeptide (28—33)	175—178	+3.1	$C_{61}H_{78}N_8O_{15}S_3 \cdot H_2O$	57.35 (57.52	6.31 6.26	8.77 8.36)
(6)	Z(OMe)-Heptapeptide (27—33)	194—197	−13.3	$C_{70}H_{87}N_9O_{17}S_3 \cdot H_2O$	58.35 (58.53	6.16 6.05	8.75 8.75)
(7)	Z(OMe)-Octapeptide (26—33)	202—204	−22.6	$C_{81}H_{104}N_{10}O_{20}S_3 \cdot 2H_2O$	58.25 (58.32	6.52 6.32	8.39 8.74)
(8)	Z(OMe)-Nonapeptide (25—33)	189—191	−17.0	$C_{96}H_{126}N_{14}O_{23}S_4 \cdot 2H_2O$	57.41 (57.25	6.52 6.56	9.76 10.03)
[1]	Z(OMe)-Decapeptide (24—33)	203—205	−23.6	$C_{107}H_{143}N_{15}O_{26}S_4 \cdot 2H_2O$	57.90 (57.98	6.68 6.79	9.47 9.46)

a) In MeOH.

Z(OMe)–Met(O)–Asp(OChp)–Phe–NH_2 [1]-(2)——A TFA-treated sample of the above dipeptide amide (16.33 g, 30.3 mmol) was dissolved in DMF (300 ml) containing TEA (4.22 ml, 1 eq), then Z(OMe)–Met(O)–ONB [prepared from 10.97 g (33.3 mmol) of Z(OMe)–Met(O)–OH] in DMF–THF (10 ml–35 ml) and NMM (3.33 ml, 30.3 mmol) were added and the mixture was stirred overnight. The product was purified by procedure B, followed by precipitation from DMSO with ether; yield 13.66 g (66%), Rf_1 0.60.

Boc–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH_2 [1]-(3)——A TFA-treated sample of the above tripeptide amide (5.50 g, 8.01 mmol) was dissolved in DMF (50 ml) containing TEA (1.11 ml, 1 eq), then Boc–Trp(Mts)–OSu [prepared from 6.42 g (9.61 mmol) of the DCHA salt] in THF (30 ml) and NMM (0.88 ml, 1 eq) were added and the mixture was stirred overnight. The product was purified by procedure B, followed by precipitation from DMF with ether; yield 5.20 g (66%), Rf_1 0.71.

Z(OMe)–Gly–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH_2 [1]-(4)——A TFA-treated sample of the above tetrapeptide amide (5.30 g, 5.35 mmol) was dissolved in DMF (50 ml) containing TEA (0.75 ml, 1 eq), then Z(OMe)–Gly–OSu (2.16 g, 6.42 mmol) and NMM (0.59 ml, 5.35 mmol) were added and the mixture was stirred for 48 h. The product was purified by procedure B, followed by precipitation from DMF with ether; yield 5.42 g (91%), Rf_1 0.54.

Z(OMe)–Met(O)–Gly–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH_2 [1]-(5)——A TFA-treated sample of the above pentapeptide amide (5.42 g, 4.87 mmol) was dissolved in DMF (30 ml) containing TEA (0.68 ml, 1 eq), then Z(OMe)–Met(O)–OSu [prepared from 1.92 g (5.84 mmol) of Z(OMe)–Met(O)–OH] in THF–DMF (30 ml–2 ml) and NMM (0.34 ml, 4.87 mmol) were added and the mixture was stirred overnight. The product was purified by procedure B, followed by precipitation from DMSO with AcOEt; yield 4.57 g (75%), Rf_1 0.62.

Z(OMe)–Tyr–Met(O)–Gly–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH_2 [1]-(6)——The azide [prepared from 1.71 g (4.76 mmol) of Z(OMe)–Tyr–$NHNH_2$] in DMF (5 ml) and TEA (0.66 ml, 4.76 mmol) were added to an ice-chilled solution of a TFA-treated sample of the above hexapeptide amide (5.00 g, 3.97 mmol) in DMF (20 ml) containing TEA (0.55 ml, 3.97 mmol) and the mixture was stirred for 24 h. The product was purified by procedure B, followed by precipitation from DMF with AcOEt; yield 4.58 g (81%), Rf_1 0.58.

Z(OMe)–Asp(OChp)–Tyr–Met(O)–Gly–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH_2 [1]-(7)——A TFA-treated sample of the above heptapeptide amide (4.50 g, 3.16 mmol) was dissolved in DMF (40 ml) containing TEA (0.44 ml, 1 eq), then Z(OMe)–Asp(OChp)–OSu [prepared from 2.72 g (4.74 mmol) of the DCHA salt] in THF (10 ml) and NMM (0.35 ml, 3.16 mmol) were added and the mixture was stirred overnight. The product was purified by procedure B, followed by precipitation from DMF with MeOH; yield 3.53 g (68%), Rf_1 0.72.

Z(OMe)–Arg(Mts)–Asp(OChp)–Tyr–Met(O)–Gly–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH_2 [1]-(8)——A mixed anhydride [prepared from 2.60 g (1.8 eq) of Z(OMe)–Arg(Mts)–OH·CHA] in THF (20 ml) was added to an ice-chilled solution of a TFA-treated sample of the above octapeptide amide (3.42 g, 2.09 mmol) in DMF (30 ml) containing TEA (0.29 ml, 1 eq) and the mixture was stirred for 6 h. The product was purified by procedure B, followed by precipitation from DMF with AcOEt; yield 3.34 g (81%), Rf_1 0.65.

Z(OMe)–Asp(OChp)–Arg(Mts)–Asp(OChp)–Tyr–Met(O)–Gly–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH_2 [1] (Positions 24—33)——A TFA-treated sample of the above nonapeptide amide (3.30 g, 1.67 mmol) was dissolved in DMF (8 ml) containing TEA (0.23 ml, 1 eq), then Z(OMe)–Asp(OChp)–OSu [prepared from 1.44 g (2.51 mmol) of the DCHA salt] in THF (20 ml) and NMM (0.18 ml, 1.67 mmol) were added and the mixture was stirred overnight. The product was purified by procedure B, followed by precipitation from DMF with AcOEt; yield 3.11 g (85%), Rf_1 0.70. Amino acid ratios in a 6 N HCl hydrolysate are listed in Table I.

Z(OMe)–Arg(Mts)–Ile–Ser–OMe——A mixed anhydride [prepared from 20.43 g (33.0 mmol) of Z(OMe)–Arg(Mts)–OH·CHA] in THF (60 ml) was added to an ice-chilled solution of a TFA-treated sample of Z(OMe)–Ile–Ser–OMe (11.89 g, 30.0 mmol) in DMF (100 ml) containing TEA (4.16 ml 1 eq) and the mixture was stirred for 6 h. The product was purified by procedure A, followed by recrystallization from MeOH and ether; yield 20.20 g (92%), mp 99—101 °C, $[\alpha]_D^{15}$ −1.0° (c=1.0, MeOH), Rf_1 0.75. *Anal.* Calcd for $C_{34}H_{50}N_6O_{10}S \cdot H_2O$: C, 54.23; H, 6.96; N, 11.16. Found: C, 54.55; H, 6.62; N, 10.84.

Z(OMe)–His–Arg(Mts)–Ile–Ser–$NHNH_2$ [2] (20—23)——The azide [prepared from 13.61 g (40.8 mmol) of the Z(OMe)–His–$NHNH_2$] in DMF (100 ml) and TEA (4.55 ml, 32.7 mmol) were added to an ice-chilled solution of a TFA-treated sample of Z(OMe)–Arg(Mts)–Ile–Ser–OMe (20.00 g, 27.2 mmol) in DMF (50 ml) containing TEA (3.79 ml, 27.2 mmol) and the mixture was stirred overnight. The product was purified by procedure A, then dissolved in MeOH (50 ml) and 80% hydrazine hydrate (6.81 ml, 5 eq) was added. The solid formed on standing overnight was precipitated from DMF with MeOH; yield 14.34 g (60%), mp 168—170 °C, $[\alpha]_D^{17}$ −1.2° (c=0.8, DMF), Rf_1 0.36. Amino acid ratios in a 6 N HCl hydrolysate: His 0.99, Arg 0.95, Ile 1.00, Ser 0.96 (recovery of Ile, 81%). *Anal.* Calcd for $C_{39}H_{57}N_{11}O_{10}S \cdot 1/2H_2O$: C, 53.16; H, 6.64; N, 17.49. Found: C, 53.17; H, 6.68; N, 17.63.

Z(OMe)–Pro–Ser(Bzl)–NHNH–Troc——A mixture of Z(OMe)–Pro–OH [prepared from 4.39 g (9.54 mmol) of the DCHA salt], H–Ser(Bzl)–NHNH–Troc [prepared from 5.24 g (9.54 mmol) of the Z(OMe)–derivative], DCC (2.36 g, 11.5 mmol) and HOBt (1.29 g, 9.54 mmol) in DMF (50 ml) was stirred for 18 h, then filtered. The filtrate was concentrated and the product was purified by procedure A, followed by recrystallization from AcOEt and ether; yield 3.70 g (60%), mp 85—87 °C, $[\alpha]_D^{20}$ −2.9° (c=1.0, DMF). Rf_2 0.42. *Anal.* Calcd for $C_{27}H_{31}Cl_3N_4O_8$: C, 50.20; H, 4.84; N, 8.67. Found: C, 50.35; H, 4.92; N, 8.82.

Z(OMe)–Asp(OBzl)–Pro–Ser(Bzl)–NHNH$_2$ [3] (17—19)——A mixed anhydride [prepared from 3.96 g (10.2 mmol) of Z(OMe)–Asp(OBzl)–OH] in THF (30 ml) was added to an ice-chilled solution of a TFA-treated sample of the above dipeptide derivative (5.50 g, 8.51 mmol) in DMF (30 ml) containing TEA (1.18 ml, 1 eq) and the mixture was stirred for 6 h. The product was purified by procedure A. The oily product (3.66 g, Rf_2 0.48) was dissolved in AcOH (30 ml) and treated with Zn powder (5.59 g, 20 eq) at 25 °C for 4 h. The solution was filtered, the filtrate was concentrated and the residue was dissolved in AcOEt and the extract, after being washed with 3% EDTA, was dried over Na_2SO_4 and concentrated. Trituration of the residue with ether afforded a powder, which was recrytallized from MeOH and ether; yield 2.47 g (85%), mp 92—95 °C, $[\alpha]_D^{21}$ −31.1° (c=0.5, DMF), Rf_2 0.40. Amino acid ratios in a 6 N HCl hydrolysate: Asp 1.04, Pro 1.00, Ser 1.04 (recovery of Pro, 77%). *Anal.* Calcd for $C_{35}H_{41}N_5O_9 \cdot 1/2H_2O$: C, 61.39; H, 6.18; N, 10.23. Found: C, 61.69; H, 6.19; N, 10.21.

Z(OMe)–Gln–Asn–Leu–OMe——A mixture of Z(OMe)–Gln–ONp (2.71 g, 6.28 mmol), TEA (1.59 ml, 11.4 mmol) and a TFA-treated sample of Z(OMe)–Asn–Leu–OMe (2.42 g, 5.71 mmol) in DMF (30 ml) was stirred for 48 h. The product was purified by procedure B, followed by precipitation from DMF with MeOH; yield 2.20 g (70%), mp 259—261 °C, $[\alpha]_D^{21}$ −10.9° (c=0.5, DMSO), Rf_1 0.50. *Anal.* Calcd for $C_{25}H_{37}N_5O_9$: C, 54.43; H, 6.76; N, 12.70. Found: C, 54.63; H, 6.72; N, 12.64.

Z(OMe)–Leu–Gln–Asn–Leu–OMe——A mixture of Z(OMe)–Leu–ONp (1.90 g, 4.57 mmol), TEA (1.06 ml, 7.62 mmol) and a TFA-treated sample of the above tripeptide (2.10 g, 3.81 mmol) in DMF (20 ml) was stirred overnight. The product was purified by procedure B, followed by precipitation from DMF with MeOH; yield 2.26 g (89%), mp 246—248 °C, $[\alpha]_D^{21}$ +20.0° (c=0.5, DMSO), Rf_1 0.66. *Anal.* Calcd for $C_{31}H_{48}N_6O_{10}$: C, 56.01; H, 7.28; N, 12.64. Found: C, 55.93; H, 7.37; N, 12.41.

Z(OMe)–Asn–Leu–Gln–Asn–Leu–OMe——A mixture of Z(OMe)–Asn–ONp (1.40 g, 3.34 mmol), TEA (0.78 ml, 5.58 mmol) and a TFA-treated sample of the above tetrapeptide (2.17 g, 2.79 mmol) in DMF (60 ml) was stirred for 18 h. The product was purified by procedure B, followed by precipitation from DMSO with MeOH; yield 2.03 g (80%), mp 261—263 °C, $[\alpha]_D^{21}$ −14.0° (c=0.5, DMSO), Rf_1 0.34. *Anal.* Calcd for $C_{35}H_{54}N_8O_{12}$: C, 53.97; H, 6.99; N, 14.39. Found: C, 53.72; H, 7.02; N, 14.12.

Z(OMe)–Asn–Leu–Gln–Asn–Leu–NHNH$_2$ [4] (12—16)——The above protected pentapeptide ester(6.72 g, 8.63 mmol) in DMSO–MeOH (80 ml–10 ml) was treated with 80% hydrazine hydrate (5.07 ml, 10 eq) for 48 h. The product was precipitated from DMSO with MeOH; yield 4.33 g (64%), mp 250—253 °C, $[\alpha]_D^{21}$ −13.3° (c=0.5, DMSO), Rf_1 0.18. Amino acid ratios in a 6 N HCl hydrolysate: Asp 2.01, Glu 1.01, Leu 2.00 (recovery of Leu, 82%). *Anal.* Calcd for $C_{34}H_{54}N_{10}O_{11} \cdot H_2O$: C, 51.30; H, 7.09; N, 17.60. Found: C, 51.53; H, 7.06; N, 17.38.

Z(OMe)–Ile–Val–OMe——A mixture of Z(OMe)–Ile–ONp (7.05 g, 16.9 mmol), H–Val–OMe·HCl salt (2.84 g, 16.9 mmol) and TEA (4.72 ml, 33.9 mmol) in DMF (70 ml) was stirred overnight. The product was purified by procedure B, followed by precipitation from DMF with ether; yield 5.11 g (74%), mp 116—118 °C, $[\alpha]_D^{21}$ +2.0° (c=0.5, DMF), Rf_2 0.81. *Anal.* Calcd for $C_{21}H_{32}N_2O_6$: C, 61.74; H, 7.90; N, 6.86. Found: C, 61.84; H, 8.07; N, 7.07.

Z(OMe)–Ile–Val–NHNH$_2$——The above dipeptide ester (5.00 g, 12.2 mmol) in MeOH (100 ml) was treated with 80% hydrazine hydrate (6.13 ml, 10 eq) for 48 h. The solid formed on standing was precipitated from DMF with MeOH; yield 2.77 g (55%), mp 252—254 °C, $[\alpha]_D^{30}$ +0.9° (c=1.0, DMF), Rf_1 0.74, Rf_2 0.15. *Anal.* Calcd for $C_{20}H_{32}N_4O_5$: C, 58.80; H, 7.90; N, 13.72. Found: C, 58.90; H, 7.91; N, 13.57.

Z(OMe)–Ile–Val–Lys(Z)–OMe——The azide [prepared from 2.70 g (6.61 mmol) of Z(OMe)–Ile–Val–NHNH$_2$] in DMF (30 ml) and TEA (1.01 ml, 1 eq) were added to an ice-chilled solution of H–Lys(Z)–OMe [prepared from 2.19 g (6.61 mmol) of the HCl salt] in DMF (20 ml) and the mixture was stirred overnight. The product was purified by procedure B, followed by precipitation from DMF with AcOEt; yield 3.60 g (81%), mp 202—204 °C, $[\alpha]_D^{30}$ +1.5° (c=1.0, DMF), Rf_2 0.65. *Anal.* Calcd for $C_{35}H_{50}N_4O_9$: C, 62.67; H, 7.51; N, 8.35. Found: C, 62.44; H, 7.63; N, 8.29.

Z(OMe)–Ser(Bzl)–Ile–Val–Lys(Z)–OMe——A mixed anhydride [prepared from 2.87 g (6.26 mmol) of Z(OMe)–Ser(Bzl)–OH CHA salt] in THF (30 ml) was added to an ice-chilled solution of a TFA-treated sample of the above tripeptide ester (3.50 g, 5.22 mmol) in DMF (10 ml) containing TEA (0.73 ml, 5.22 mmol) and the mixture was stirred for 6 h. The product was purified by procedure B, followed by precipitation from DMF with MeOH; yield 3.25 g (69%), mp 179—181 °C, $[\alpha]_D^{21}$ −1.1° (c=1.0, DMF), Rf_2 0.90. *Anal.* calcd for $C_{45}H_{61}N_5O_{11} \cdot 1/2H_2O$: C, 63.06; H, 7.29; N, 8.17. Found: C, 63.18; H, 7.13; N, 7.81.

Z(OMe)–Ser(Bzl)–Ile–Val–Lys(Z)–NHNH$_2$ [5] (8—11)——The above protected tetrapeptide ester (3.15 g, 3.71 mmol) in DMF (100 ml) was treated with 80% hydrazine hydrate (0.93 ml, 5 eq) overnight. The solid formed during the treatment was precipitated from DMF with MeOH; yield 2.66 g (84%), mp 126—128 °C, $[\alpha]_D^{21}$ +12.6° (c=1.0, DMF), Rf_1 0.77, Rf_2 0.13. Amino acid ratios in a 6 N HCl hydrolysate (96 h): Ser 0.87, Ile 0.94, Val 0.92, Lys 1.00 (recovery of Lys, 79%). *Anal.* Calcd for $C_{44}H_{61}N_7O_{10}$: C, 62.32; H, 7.25; N, 11.56. Found: C, 62.44; H, 7.27; N, 11.29.

Z(OMe)–Gly–Arg(Mts)–Met(O)–NHNH$_2$ [6] (5—7)——A mixture of Z(OMe)–Gly–OSu (2.07 g, 6.16 mmol), TEA (1.43 ml, 2 eq) and a TFA-treated sample of Z(OMe)–Arg(Mts)–Met(O)–OMe (3.50 g, 5.13 mmol) in DMF (30 ml) was stirred for 48 h. The product was purified by procedure A, followed by recrystallization from MeOH and ether. The product was dissolved in MeOH (40 ml), then treated with 80% hydrazine hydrate (1.29 ml, 5 eq) for 24 h. The solvent was evaporated off and the residue was triturated with *n*-BuOH and ether; yield 1.91 g (51%), mp 93—96 °C, $[\alpha]_D^{21}$ −5.5° (c=0.5, DMF), Rf_1 0.59. Amino acid ratios in a 6 N HCl hydrolysate: Gly 1.05, Arg 1.00,

Met+Met(O) 0.87 (recovery of Arg, 81%). *Anal.* Calcd for $C_{31}H_{46}N_8O_9S_2 \cdot 1/2H_2O$: C, 49.78; H, 6.33; N, 14.98. Found: C, 50.05; H, 6.56; N, 14.70.

Z(OMe)–Ala–Pro–Ser–OMe——DCC (4.09 g, 19.8 mmol) was added to a mixture of Z(OMe)–Ala–Pro–OH (6.31 g, 18.0 mmol) and H–Ser–OMe [prepared from 3.36 g (21.6 mmol) of the HCl salt] in DMF (30 ml) and the solution was stirred overnight. The solution was filtered and the filtrate was concentrated. The product was purified by procedure A (solvent *n*-BuOH), followed by recrystallization from MeOH and ether; yield 6.69 g (82%), mp 109—112 °C, $[\alpha]_D^{23}$ −14.5° (c=0.9, MeOH), Rf_1 0.84. *Anal.* Calcd for $C_{21}H_{31}N_3O_9$: C, 55.86; H, 6.47; N, 9.31. Found: C, 56.11; H, 6.71; N, 9.33.

Z(OMe)–Lys(Z)–Ala–Pro–Ser–OMe——A mixture of Z(OMe)–Lys(Z)–OSu (2.35 g, 4.34 mmol), TEA (1.08 ml, 7.76 mmol) and a TFA-treated sample of the above protected tripeptide ester (1.75 g, 3.88 mmol) in DMF (50 ml) was stirred overnight. The product was purified by procedure A, followed by recrystallization from MeOH and AcOEt; yield 1.91 g (69%), mp 153—156 °C, $[\alpha]_D^{23}$ −33.5° (c=1.0, DMF), Rf_1 0.79. *Anal.* Calcd for $C_{35}H_{47}N_5O_{11}$: C, 58.89; H, 6.64; N, 9.81. Found: C, 58.94; H, 6.80; N, 9.80.

Z(OMe)–Lys(Z)–Ala–Pro–Ser–NHNH$_2$ [7] (1—4)——The above protected tetrapeptide ester (2.65 g, 3.71 mmol) in MeOH (30 ml) was treated with 80% hydrazine hydrate (2.32 ml, 10 eq) overnight. The solid formed during the treatment was precipitated from DMF with MeOH; yield 2.43 g (92%), mp 154—159 °C, $[\alpha]_D^{20}$ −36.6° (c=1.0, DMF), Rf_1 0.73. Amino acid ratios in a 6 N HCl hydrolysate: Lys 0.98, Ala 1.06, Pro 1.00, Ser 0.95 (recovery of Pro, 84%). *Anal.* Calcd for $C_{34}H_{47}N_7O_{10} \cdot H_2O$: C, 55.80; H, 6.75; N, 13.40. Found: C, 55.94; H, 6.59; N, 13.25.

Z(OMe)–His–Arg(Mts)–Ile–Ser–Asp(OChp)–Arg(Mts)–Asp(OChp)–Tyr–Met(O)–Gly–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH$_2$ (Positions 20—33)——The azide, prepared from fragment [2] (1.31 g, 1.5 eq), in DMF (6 ml) and NMM (0.13 ml, 1.2 eq) were added to an ice-chilled solution of a TFA-treated sample of fragment [1] (2.18 g, 1.00 mmol) in DMF (6 ml) containing TEA (0.14 ml, 1 eq) and the mixture was stirred for 48 h. H_2O was added and the resulting powder was precipitated from DMF with MeOH; yield 2.45 g (86%), Rf_1 0.66. Physical constants and analytical data are listed in Table III and amino acid ratios after 6 N HCl hydrolysis are listed in Table I, respectively.

Z(OMe)–Asp(OBzl)–Pro–Ser(Bzl)–His–Arg(Mts)–Ile–Ser–Asp(OChp)–Arg(Mts)–Asp(OChp)–Tyr–Met(O)–Gly–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH$_2$ (Positions 17—33)——The azide, prepared from fragment [3] (0.60 g, 1.5 eq), in DMF (3 ml) and NMM (90 μl, 1.2 eq) were added to an ice-chilled solution of a TFA-treated sample of the above 14-residue peptide amide (1.95 g, 0.68 mmol) in DMF (10 ml) containing TEA (0.10 ml, 1 eq) and the mixture was stirred overnight. H_2O was added and the resulting powder was precipitated from DMF with MeOH; yield 1.90 g (84%), Rf_1 0.60.

Z(OMe)–Asn–Leu–Gln–Asn–Leu–Asp(OBzl)–Pro–Ser(Bzl)–His–Arg(Mts)–Ile–Ser–Asp(OChp)–Arg(Mts)–Asp(OChp)–Tyr–Met(O)–Gly–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH$_2$ (Positions 12—33)——The azide, prepared from fragment [4] (1.32 g, 5 eq), in DMF–DMSO–HMPA (1 : 1 : 1, 15 ml) and TEA (47 μl, 1 eq) were added to an ice-chilled solution of a TFA-treated sample of the above 17-residue peptide amide (975 mg, 0.34 mmol) in DMF (5 ml) containing TEA (47 μl, 1 eq) and the mixture was stirred for 24 h. Further azide and TEA (3 eq each) were added and the reaction was continued for an additional 18 h. The solvent was removed by evaporation and the residue was treated with H_2O to give a powder (2.78 g). The product was further purified by procedure C, followed by precipitation from DMF with AcOEt; yield 642 mg (48%), Rf_1 0.57.

TABLE III. Physical Constants and Analytical Data for Protected hCCK-33 and Its Protected Intermediates

Protected peptides (positions)	mp (°C)	$[\alpha]_D^{28}$ (°)	Formula	Analysis (%) Calcd (Found)		
				C	H	N
14-Residue (20—33)	227—230	−15.0 (DMF)	$C_{137}H_{188}N_{24}O_{33}S_5 \cdot 3H_2O$	56.48 (56.47	6.71 6.75	11.54 11.33)
17-Residue (17—33)	230—233	−15.1 (DMF)	$C_{163}H_{217}N_{27}O_{39}S_5 \cdot 4H_2O$	57.39 (57.33	6.65 6.56	11.09 10.95)
22-Residue (12—33)	225—228	−18.0 (DMSO)	$C_{188}H_{259}N_{35}O_{47}S_5 \cdot 7H_2O$	55.78 (55.78	6.80 6.84	12.11 12.40)
26-Residue (8—33)	250—252	−6.7 (DMSO)	$C_{223}H_{308}N_{40}O_{54}S_5 \cdot 8H_2O$	56.77 (56.66	6.92 6.74	11.92 11.97)
29-Residue (5—33)	251—253	−2.0 (DMF)	$C_{245}H_{342}N_{46}O_{60}S_7 \cdot 11H_2O$	55.37 (55.65	6.90 6.63	12.13 11.86)
33-Residue (1—33)	257—259	−33.3 (DMF)	$C_{270}H_{377}N_{51}O_{67}S_7 \cdot 5H_2O$	56.65 (56.58	6.82 6.72	12.48 12.61)

Z(OMe)–Ser(Bzl)–Ile–Val–Lys(Z)–Asn–Leu–Gln–Asn–Leu–Asp(OBzl)–Pro–Ser(Bzl)–His–Arg(Mts)–Ile–Ser–Asp(OChp)–Arg(Mts)–Asp(OChp)–Tyr–Met(O)–Gly–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH_2 (Positions 8—33)——The azide, prepared from fragment [5] (690 mg, 5 eq), in DMF (6 ml) and TEA (27 μl, 1.2 eq) were added to an ice-chilled solution of a TFA-treated sample of the above 22-residue peptide amide (638 mg, 0.16 mmol) in DMF (4 ml) containing TEA (23 μl, 1 eq) and the mixture was stirred for 48 h. Ether and H_2O were added and the resulting powder was purified by procedure C, followed by precipitation from DMF with AcOEt; yield 459 mg (62%), Rf_1 0.59.

Z(OMe)–Gly–Arg(Mts)–Met(O)–Ser(Bzl)–Ile–Val–Lys(Z)–Asn–Leu–Gln–Asn–Leu–Asp(OBzl)–Pro–Ser(Bzl)–His–Arg(Mts)–Ile–Ser–Asp(OChp)–Arg(Mts)–Asp(OChp)–Tyr–Met(O)–Gly–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH_2 (Positions 5—33)——The azide, prepared from fragment [6] (371 mg, 5 eq), in DMF (2 ml) and TEA (17 μl, 1.2 eq) were added to an ice-chilled solution of a TFA-treated sample of the above 26-residue peptide amide (455 mg, 0.10 mmol) in DMF (1 ml) containing TEA (14 μl, 1 eq) and the mixture was stirred for 48 h. H_2O was added and the resulting powder was purified by procedure C, followed by precipitation from DMF with AcOEt; yield 293 mg (57%), Rf_1 0.62.

Z(OMe)–Lys(Z)–Ala–Pro–Ser–Gly–Arg(Mts)–Met(O)–Ser(Bzl)–Ile–Val–Lys(Z)–Asn–Leu–Gln–Asn–Leu–Asp(OBzl)–Pro–Ser(Bzl)–His–Arg(Mts)–Ile–Ser–Asp(OChp)–Arg(Mts)–Asp(OChp)–Tyr–Met(O)–Gly–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH_2 [Positions 1—33, Protected hCCK-33 (Free 27-Tyr)]——The azide, prepared from fragment [7] (63 mg, 5 eq), in DMF (2 ml) and TEA (5 μl, 1.2 eq) were added to an ice-chilled solution of a TFA-treated sample of the above 29-residue peptide amide (90 mg, 18 μmol) in DMF (3 ml) containing TEA (3 ml, 1 eq) and the mixture was stirred for 48 h. H_2O was added and the resulting powder was purified by procedure C, followed by precipitation from DMF with AcOEt; yield 70 mg (69%), Rf_1 0.67.

References and Notes

1) Part CLVIII: N. Fujii, A. Otaka, S. Funakoshi, T. Watanabe, H. Arai, K. Bessho, and H. Yajima, *J. Protein Chem.*, **7**, 151 (1988).
2) The following abbreviations are used: Z = benzyloxycarbonyl, Z(OMe) = *p*-methoxybenzyloxycarbonyl, Mts = mesitylenesulfonyl, Bzl = benzyl, Chp = cycloheptyl, Troc = 2,2,2-trichloroethyloxycarbonyl, Boc = *tert*-butyloxycarbonyl, Fmoc = 9-fluorenylmethyloxycarbonyl, Cl_2-Bzl = 2,6-dichlorobenzyl, (O) = sulfoxide, Su = *N*-hydroxysuccinimidyl, Np = *p*-nitrophenyl, DCC = dicyclohexylcarbodiimide, MA = mixed anhydride, NB = *N*-hydroxy-5-norbornene-2,3-dicarboximidyl, HOBt = *N*-hydroxybenzotriazole, TFA = trifluoroacetic acid, TEA = triethylamine, NMM = *N*-methylmorpholine, HMPA = hexamethylphosphorus triamide, DMF = *N*,*N*-dimethylformamide, DMSO = dimethyl sulfoxide, MSA = methanesulfonic acid, THF = tetrahydrofuran, CHA = cyclohexylamine, DCHA = dicyclohexylamine, EDTA = ethylenediaminetetraacetic acid.
3) Y. Takahashi, K. Kato, Y. Hayashizaki, T. Wakabayashi, E. Ohtsuka, S. Matsuki, M. Ikehara, and K. Matsubara, *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 1931 (1985).
4) M. A. Ondetti, B. Rubin, S. L. Engel, J. Plusces, and J. T. Sheehan, *Am. J. Digestive Diseases*, **15**, 149 (1970); M. A. Ondetti, J. Plusces, E. F. Sabo, J. T. Sheehan, and N. Williams, *J. Am. Chem. Soc.*, **92**, 195 (1970).
5) J. E. Jorpes, V. Mutt, and K. Toczko, *Acta Chem. Scand.*, **18**, 2408 (1964); V. Mutt and J. E. Jorpes, *Eur. J. Biochem.*, **6**, 156 (1968); *idem, Biochem. J.*, **125**, 57 (1971).
6) Y. Kurano, T. Kimura, and S. Sakakibara, *J. Chem. Soc., Chem. Commun.*, **1987**, 323.
7) L. A. Carpino and G. Y. Han, *J. Am. Chem. Soc.*, **92**, 5748 (1970).
8) S. Hanessian and P. Lavallee, *Can. J. Chem.*, **53**, 2975 (1975).
9) B. W. Erickson and R. B. Merrifield, *J. Am. Chem. Soc.*, **95**, 3750 (1973).
10) J. Honzl and J. Rudinger, *Coll. Czech. Chem. Commun.*, **26**, 2333 (1961).
11) H. Yajima, M. Takeyama, J Kanaki, and K. Mitani, *J. Chem. Soc., Chem. Commun.*, **1978**, 482.
12) N. Fujii, M. Nomizu, S. Futaki, A. Otaka, S. Funakoshi, K. Akaji, K. Watanabe, and H. Yajima, *Chem. Pharm. Bull.*, **34**, 864 (1986).
13) N. Fujii, S. Futaki, K. Yasumura, and H. Yajima, *Chem. Pharm. Bull.*, **32**, 2660 (1984).
14) J. Martinez and M. Bodanszky, *Int. J. Peptide Protein Res.*, **12**, 277 (1978) and references cited therein.
15) Y. Omori, Y. Matsuda, S. Aimoto, Y. Shimonishi, and M. Yamamoto, *Chem. Lett.*, **1976**, 805; E. Wunsch, E. Jaeger, L. Kisfaludy, and M. Low, *Angew. Chem.*, **89**, 330 (1977); Y. Masui, N. Chino, and S. Sakakibara, *Bull. Chem. Soc. Jpn*, **53**, 464 (1980); H. Ogawa, T. Sasaki, H. Irie, and H. Yajima, *Chem. Pharm. Bull.*, **26**, 3144 (1978) and references cited therein.
16) B. Iselin, *Helv. Chim. Acta*, **44**, 61 (1961); N. Fujii, S. Kuno, A. Otaka, S. Funakoshi, K. Takagi, and H. Yajima, *Chem. Pharm. Bull.*, **33**, 4587 (1985).
17) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Am. Chem. Soc.*, **85**, 3039 (1963).
18) M. Fujino, S. Kobayashi, M. Obayashi, T. Fukuda, S. Shinagawa, and O. Nishimura, *Chem. Pharm. Bull.*, **22**, 1857 (1974).
19) J. R. Vaughan, Jr., *J. Am. Chem. Soc.*, **73**, 3547 (1951); T. Wieland, W. Kern, and R. Sehring, *Ann. Chem.*, **569**, 117 (1950)' R. A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951).

20) M. Kubota, H. Ogawa, and H. Yajima, *Chem. Pharm. Bull.*, **24**, 2435 (1976).
21) H. Yajima and Y. Kiso, *Chem. Pharm. Bull.*, **19**, 420 (1971).
22) K. Akaji, N. Fujii, H. Yajima, K. Hayashi, K. Mizuta, M. Aono, and M. Moriga, *Chem. Pharm. Bull.*, **33**, 184 (1985).
23) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).
24) W. Konig and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
25) R. B. Woodward, K. Heusler, J. Gosteli, P. Naegeli, W. Oppolzer, R. Ramage, S. Ranganathan, and H. Vorbruggen, *J. Am. Chem. Soc.*, **88**, 852 (1966).
26) Y. Mori, K. Koyama, Y. Kiso, and H. Yajima, *Chem. Pharm. Bull.*, **24**, 2788 (1976).
27) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).
28) S. Kuno, W. Li, N. Fujii, H. Adachi, K. Bessho, T. Segawa, Y. Nakata, A. Inoue, and H. Yajima, *Chem. Pharm. Bull.*, **34**, 4811 (1986).
29) H. Yajima, J. Iwai, H. Watanabe, K. Koyama, M. Nakamura, K. Miyata, and A. Tanaka, *Chem. Pharm. Bull.*, **25**, 2048 (1977).
30) M. Nomizu, K. Akaji, J. Fukata, H. Imura, A. Inoue, Y. Nakata, T. Segawa, N. Fujii, and H. Yajima, *Chem. Pharm. Bull.*, **36**, 122 (1988).