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Studies on Peptides. CLXIV.[1,2] Solution-Phase Synthesis of a 36-Residue Peptide Amide Corresponding to the Entire Amino Acid Sequence of Chicken Antral Peptide

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A 36-residue peptide amide corresponding to the entire amino acid sequence of chicken antral peptide was synthesized by assembling seven peptide fragments *via* the azide, followed by thioanisole-mediated deprotection with trimethylsilyl bromide and trimethylsilyl trifluoromethanesulfonate in trifluoroacetic acid. The synthetic peptide stimulated gastric secretion, but not pancreatic secretion.

Keywords——chicken antral peptide synthesis; β-cycloheptylaspartate; N^{im}benzyloxymethylhistidine; N^{G}-mesitylenesulfonylarginine; thioanisole-mediated deprotection; hard acid deprotection; trimethylsilyl trifluoromethanesulfonate deprotection; trimethylsilyl bromide; Chou–Fasman prediction

In 1986, Dimaline *et al.*[3] elucidated the structure of a novel 36-residue peptide isolated from chicken antrum. This peptide shares the common C-terminal tetrapeptide amide unit, Trp–Met–Asp–Phe–NH_2, with mammalian gastrin[4] and cholecystokinin (CCK)[5] and was reported to be a potent stimulant of avian gastric acid secretion, but not pancreatic secretion. In order to examine its biological effects on the mammalian digestive tract, we undertook the synthesis of this avian gastrin and CCK family-peptide in two ways, by the solution-phase method and by the solid-phase method. In the latter case, our newly introduced amide precursor reagent[6] was employed.

First, we wish to report the solution-phase synthesis of a 36-residue peptide corresponding to the entire amino acid sequence of chicken antral peptide (cAP). Seven peptide fragments were selected as the building blocks to construct its entire peptide backbone as shown in Fig. 1 and amino acid derivatives bearing protecting groups removable by treatment with 1 M TMSOTf–thioanisole/TFA[7] were employed, *i.e.*, Glu(OBzl), Lys(Z), Arg(Mts),[8] Tyr(Cl_2–Bzl),[9] Trp(Mts),[10] and His(Bom).[11] In addition, Asp(OChp)[12] was employed to suppress the base-catalyzed side reaction, *i.e.*, succinimide formation.[13] The Met residue was protected as its sulfoxide[14] to prevent partial air-oxidation, which would otherwise take place during manipulation, and S-alkylation, which would occur in each TFA-N^{α}-deprotection step. The synthetic peptide obtained by the present solution-phase method served as the standard sample for monitoring the subsequent solid-phase synthesis, which often gives a product contaminated with many partially deleted peptides.

The C-terminal fragment [1], Boc–Tyr(Cl_2–Bzl)–Pro–Asp(OChp)–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH_2, was prepared in a stepwise manner starting with Boc–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH_2, an intermediate of our previous synthesis of human CCK-33,[15] as shown in Fig. 2. Onto this tetrapeptide unit, Z(OMe)–Asp(OChp)–OH was introduced by the Su active ester procedure,[16] Z(OMe)–Pro–OH by the mixed anhydride (MA)[17] pro-

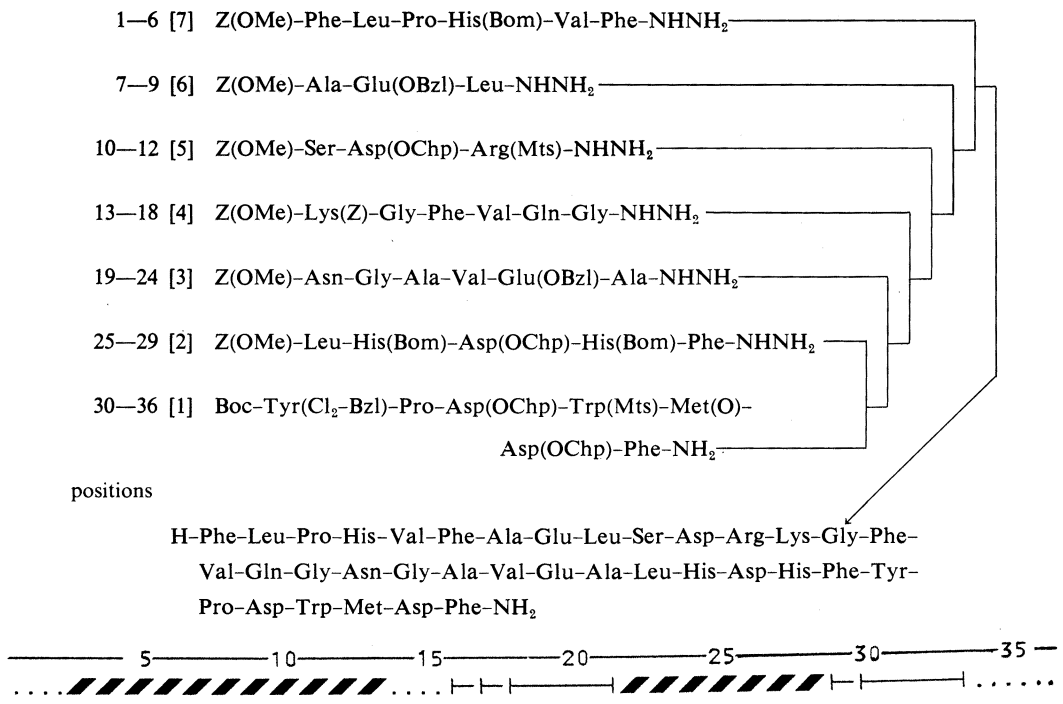

Fig. 1. Amino Acid Sequence of Chicken Antral Peptide and Chou-Fasman Prediction of Its Conformation

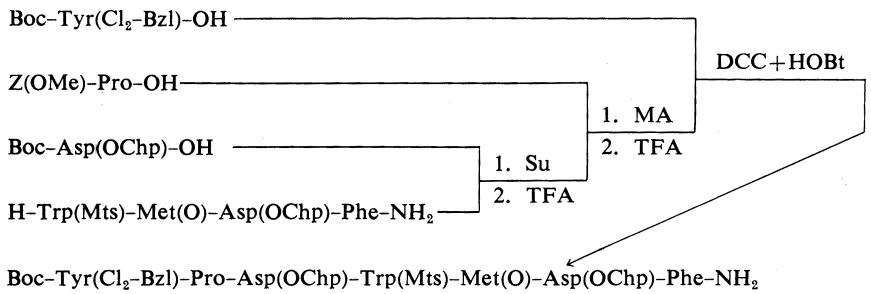

Fig. 2. Synthetic Scheme for the Protected Heptapeptide Amide [1] (Positions 30—36)

cedure, and Boc–Tyr–(Cl_2–Bzl)–OH by the DCC plus HOBt procedure,[18] successively. The purity of the protected heptapeptide amide thus obtained was ascertained by thin layer chromatography (TLC) and amino acid analysis, after 4 M methanesulfonic acid (MSA)[19] or 6 N HCl hydrolysis, as was done with other fragments.

Fragment [2], Z(OMe)–Leu–His(Bom)–Asp(OChp)–His(Bom)–Phe–NH_2, was prepared with the aid of a substituted hydrazine, Troc–NH–NH_2,[20] as shown in Fig. 3. First, Boc–Asp(OChp)–His(Bom)–Phe–NHNH–Troc was prepared in a stepwise manner. A TFA-treated sample of Z(OMe)–Phe–NHNH–Troc[21] was condensed with Boc–His(Bom)–$NHNH_2$ by the azide procedure,[22] then with Boc–Asp(OChp)–OH *via* the Su ester. Next, Z(OMe)–Leu–His(Bom)–$NHNH_2$ was prepared by the Np condensation[23] of Z(OMe)–Leu–OH with a TFA-treated sample of Boc–His(Bom)–OMe, followed by the usual hydrazine treatment. Finally, two fragments thus obtained were condensed by the azide procedure, then

the Troc group was removed from the resulting pentapeptide derivative by Zn–AcOH treatment[24] to give [2].

Fragment [3], Z(OMe)–Asn–Gly–Ala–Val–Glu(OBzl)–Ala–$NHNH_2$, was prepared in a stepwise manner starting with a TFA-treated sample of Z(OMe)–Ala–NHNH–Troc.[25] The respective amino acid residues were successively introduced by the active ester procedure, as

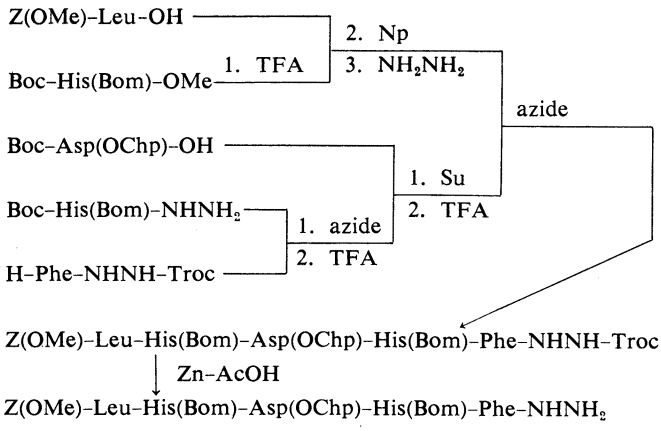

Fig. 3. Synthetic Scheme for the Protected Pentapeptide Hydrazide [2] (Positions 25—29)

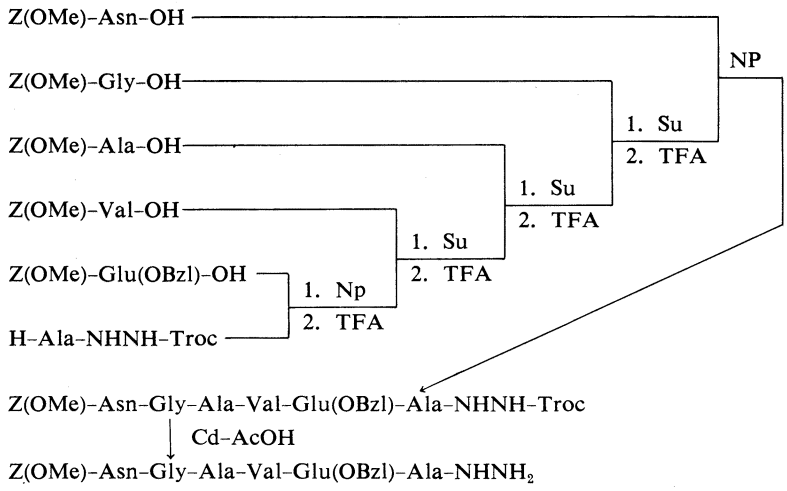

Fig. 4. Synthetic Scheme for the Protected Hexapeptide Hydrazide [3] (Positions 19—24)

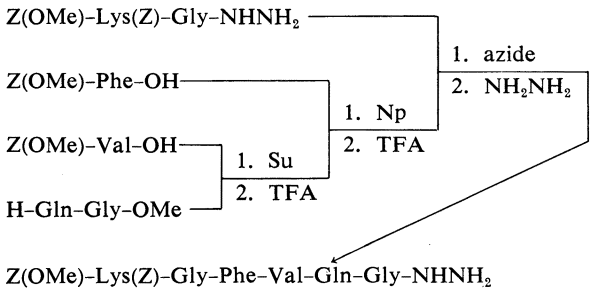

Fig. 5. Synthetic Scheme for the Protected Hexapeptide Hydrazide [4] (Positions 13—18)

shown in Fig. 4, then the Troc group was cleaved from the resulting hexapeptide derivatives by treatment with Cd powder[26] in a mixture of AcOH and DMF. In this step, Cd-treatment gave a more homogeneous product than Zn-treatment.

Fragment [4], Z(OMe)–Lys(Z)–Gly–Phe–Val–Gln–Gly–$NHNH_2$, was prepared by the azide condensation of two components, Z(OMe)–Lys(Z)–Gly–$NHNH_2$[27] and Z(OMe)–Phe–Val–Gln–Gly–OMe, followed by the usual hydrazine treatment, as shown in Fig. 5. A known dipeptide, Z(OMe)–Gln–Gly–OMe,[28] served as a starting material to prepared the latter component. This dipeptide, after being treated with TFA, was allowed to react with Z(OMe)–Val–OSu, then with Z(OMe)–Phe–ONp.

Initially, we selected Z(OMe)–Phe–Val–Gln–Gly–$NHNH_2$ as one of the fragments. However, treatment of the corresponding tetrapeptide methyl ester with hydrazine hydrate gave a product with poor solubility in DMF, and even in DMSO. Thus, we decided to add the Lys(Z)–Gly unit onto this tetrapeptide unit to improve the solubility. Indeed, in this way, we were able to obtain fragment [4], which could be purified by precipitation from DMF with MeOH as usual.

Fragment [5], Z(OMe)–Ser–Asp(OChp)–Arg(Mts)–$NHNH_2$ was prepared in a stepwise manner starting with Z(OMe)–Arg(Mts)–NHNH–Troc,[29] as shown in Fig. 6. The two residues were introduced by the Su active ester procedure and the azide procedure, respectively. From the resulting tripeptide derivative, the Troc group was cleaved off by Zn–AcOH treatment to give [5], which could be purified by precipitation from AcOEt with ether.

Fragment [6], Z(OMe)–Ala–Glu(OBzl)–Leu–$NHNH_2$, was prepared in a stepwise man-

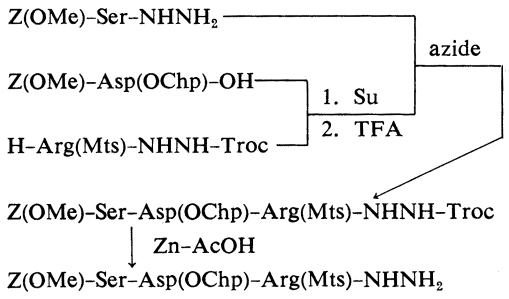

Fig. 6. Synthetic Scheme for the Protected Tripeptide Hydrazide [5] (Positions 10—12)

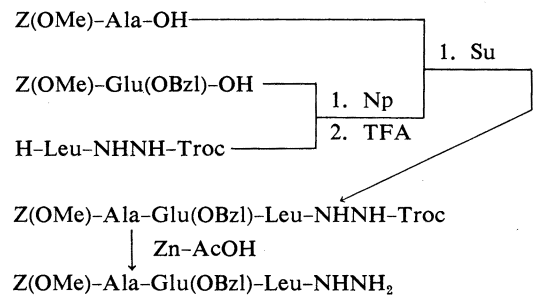

Fig. 7. Synthetic Scheme for the Protected Tripeptide Hydrazide [6] (Positions 7—9)

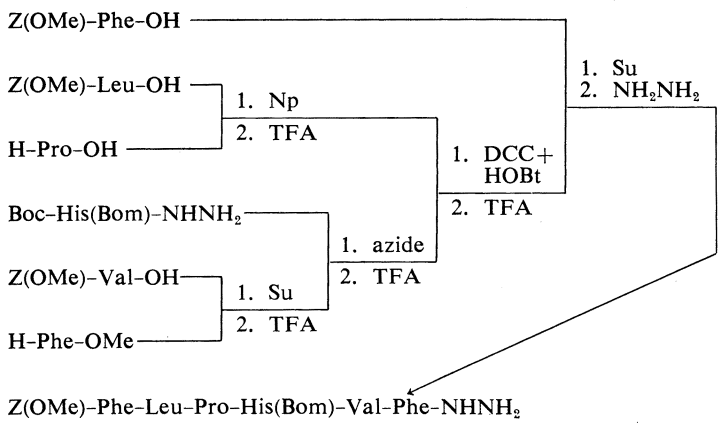

Fig. 8. Synthetic Scheme for the Protected Hexapeptide Hydrazide [7] (Positions 1—6)

ner by the active ester procedure, starting with a TFA-treated sample of Z(OMe)–Leu–NHNH–Troc.[30] The Np and the Su esters were employed to introduce the Glu(OBzl) and the Ala residues, respectively, as shown in Fig. 7. From the resulting tripeptide derivative, the Troc group was cleaved by Zn-treatment to give [6], soluble in MeOH and even in AcOEt.

The N-terminal hexapeptide fragment [7], Z(OMe)–Phe–Leu–Pro–His(Bom)–Val–Phe–$NHNH_2$, was prepared according to the scheme shown in Fig. 8. First, Boc–His(Bom)–Val–Phe–OMe was prepared in a stepwise manner starting with H–Phe–OMe, onto which the amino acid residues were successively condensed by the Su and the azide procedures, respectively. Next, Z(OMe)–Leu–Pro–OH was prepared by the Np method, but an attempt to crystallize this compound was unsuccessful. The above tripeptide ester, after TFA-treatment, was condensed with Z(OMe)–Leu–Pro–OH by DCC in the presence of HOBt.[18] Z(OMe)–Leu–Pro–His(Bom)–Val–Phe–OMe was obtained in a satisfactory yield and this, after TFA-treatment, was allowed to react with Z(OMe)–Phe–OSu to give the protected pentapeptide ester, which was converted to [7] by the usual hydrazine treatment.

Seven peptide fragments thus obtained were then assembled successively according to the route illustrated in Fig. 1. The azide procedure was employed extensively to minimize racemization. As a solvent, DMF or DMF–DMSO (1 : 1) was employed. The fragment condensations from [1] to [5] proceeded as usual, when each acyl component was employed in a slight excess (1.5 to 2 eq). However, for condensation of fragment [6], the acyl component was used in larger excess (5 eq) and the reaction was continued for 72 h in order to bring the reaction to completion. We encountered considerable difficulty in incorporating fragment [7] into the peptide chain, even by the use of a large excess of the acyl component (5 plus 5 eq). Even under these conditions, the acyl component was incorporated into the peptide chain to the extent of only 50 to 60%. Previously, we observed similar phenomena in the syntheses of human and ovine corticotropin releasing factors,[31] the circular dichroism (CD) spectra of which indicated predominantly α-helical character.[32] The Chou–Fasman technique[33] predicted such α-helical conformation at the N-terminal portion of cAP (Fig. 1, positions 3 to 13). Thus, the possibility can not be excluded that such a conformational feature may be present in the protected form of cAP and this may account more or less for the difficulty in the coupling reaction mentioned above.

An attempt to purify protected cAP by gel-filtration on Sephadex LH-60 was unsuccessful, since the desired compound emerged from the column with the unreacted amino component, as a single peak, when DMF–DMSO was used as an eluant. It is interesting to note that elution with DMF gave two peaks with similar amino acid compositions (both a mixture of protected cAP and the amino component) (Fig. 9). These results suggested that protected cAP and even the amino component have a tendency to aggregate in DMF, but less in DMSO. Thus, we decided to subject crude protected cAP to the deprotection reaction

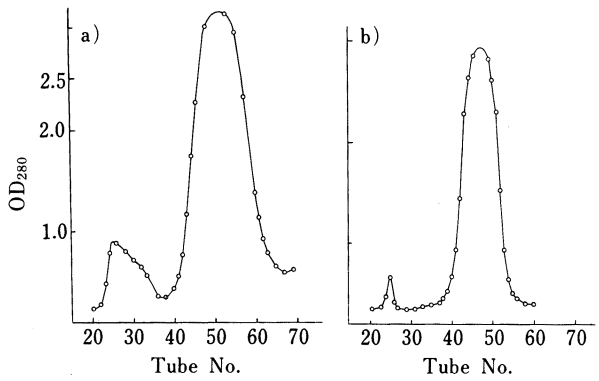

Fig. 9. Gel-Filtration of Protected Antral Peptide

a) Eluted with DMF. b) Eluted with DMF–DMSO (1 : 1).

TABLE I. Amino Acid Ratios in 6 N HCl Hydrolysates of Synthetic Chicken Antral Peptide and Its Protected Intermediates

Positions	Protected peptides							Syn. cAP	Theory
	30—36	25—36	19—36	13—36	10—36	7—36	1—36		
Asp	1.98	2.64	3.68	3.68	4.74	4.97	5.65	4.98	(5)
Ser					0.91	1.06	1.12	1.01	(1)
Glu			1.02	2.02	2.20	3.33	3.66	3.24	(3)
Pro	0.98	0.97	0.91	0.95	0.91	1.00	1.79	2.24	(2)
Gly			1.05	3.02	3.30	3.34	3.80	3.25	(3)
Ala			2.12	2.11	2.20	3.24	3.68	3.14	(3)
Val			1.04	1.94	2.13	2.14	3.09	2.83	(3)
Met	0.95	0.88	0.91	0.93	0.92	0.90	0.98	0.98	(1)
Leu		1.00	1.00	1.00	1.00	2.00	3.00	3.00	(3)
Tyr	0.92	0.91	0.95	0.96	0.96	1.05	1.16	0.98	(1)
Phe	1.00	1.88	1.93	2.75	2.82	3.07	4.63	5.08	(5)
Lys					1.10	1.09	1.23	1.10	(1)
His		1.93	1.90	1.90	1.85	2.05	3.03	3.24	(3)
Trp[a)]	—	—	—	—	—	—	—	—	(1)
Arg					1.02	1.09	1.23	1.12	(1)
Recov. (%)	92	99	99	98	98	99	98	89	

a) Not determined.

without further purification.

Except for this protected cAP, other protected intermediates were purified by flash silica gel chromatography[34] or by gel-filtration on Sephadex LH-20 or LH-60. Throughout this synthesis, Leu was used as a diagnostic amino acid in acid hydrolysis. By comparison of the recovery of Leu with those of newly incorporated amino acis, satisfactory incorporation of each fragment, except fragment [7], was ascertained (Table I). Elemental analysis of relatively large peptides offers little information about their homogeneity. Therefore, this was not done.

In the final step, crude protected cAP was treated with 1 M TMSBr–thioanisole/TFA,[35] then with 1 M TMSOTf–thioanisole/TFA. The former reagent has an ability to reduce Met(O) to Met and to remove most of the protecting groups, except for the Chp group. The latter treatment was performed to ensure complete deprotection, including the Chp group mentioned above. The deprotected peptide was treated with 1 N NH_4F containing 6 M guanidine HCl at pH 8.0 to reverse any possible N→O shift[36] and to ensure complete hydrolysis of the trimethylsilyl moieties and then incubated with 2-mercaptoethanol at pH 6.0 for 12 h to ensure complete reduction of Met(O). The treated peptide was purified by gel-filtration on Sephadex G-25 using 1 N AcOH as an eluant. The product was purified to homogeneity by high-performance liquid chromatography (HPLC) on a Cosmosil 5-C18 column using gradient elution with acetonitrile in 0.1% TFA. As predicted, two large peaks were detected. After acid hydrolysis, the front peak was found to be the triacontapeptide missing the N-terminal hexapeptide unit of cAP and the product obtained from the latter eluate was found to possess the amino acid ratios predicted by theory. The desired product thus isolated exhibited a sharp single spot on TLC and a single band on disk isoelectrofocusing (Pharmalyte pH 3—10). Its purity was further ascertained by enzymatic digestion and its molecular weight was ascertained by fast atom bombardment mass spectrometry (FAB-MS).

The effects of this synthetic avian peptide on mammalian gastrointestinal functions were examined in rats and the results were compared with those in the case of synthetic CCK-8 (sulfated). First, the effects on pancreatic secretion were tested by using the Love–Tachibana

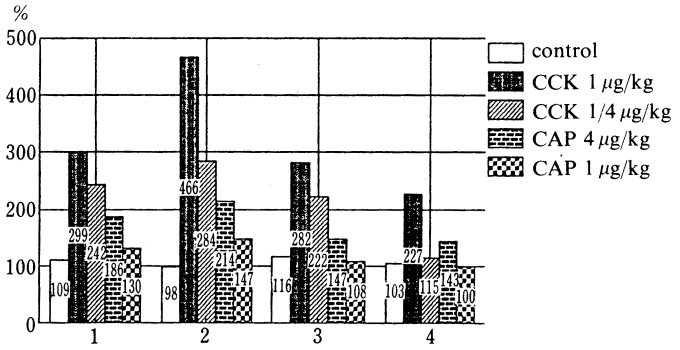

Fig. 10. Effects of Synthetic Chicken Antral Peptide on Gastric Pepsin Secretion

rat preparation.[37] Synthetic cAP (0.25, 1 and 4 μg/kg) and CCK-8 (1 μg/kg) were given as a bolus injection, then secreted pancreatic juice was collected every 30 min and the volume was measured. No significant increase or decrease of the pancreatic flow was observed at any dose of synthetic cAP administered, while synthetic CCK-8 increased the pancreatic flow by 222%.

Next, the effects on gastric acid and pepsin secretion were tested in the Ghosh-Lai rat preparation.[38] Synthetic cAP (1 and 4 μg/kg) and CCK-8 (0.25 and 1 μg/kg) were given as a bolus injection, then secreted gastric juice was collected every 15 min, and the amounts of gastric acid and pepsin were determined by titration with alkali and by the method of Anson and Mirsky,[39] respectively. Gastric acid secretion was increased by 57 and 157%, respectively, by the administrations of synthetic cAP mentioned above. CCK-8 at doses of 0.25 and 1 μg/kg also increased acid secretion by 87 and 146%, respectively. Thus, the effects of cAP and CCK-8 on gastric acid stimulation were judged to be nearly equivalent on a molar basis. The increases of gastric pepsin secretion at doses of 1 and 4 μg/kg of synthetic cAP were 47 and 114%, respectively, while CCK-8 at doses of 0.25 and 1 μg/kg increased pepsin secretion by 184 and 366%, respectively (Fig. 10). Thus, in this assay system, synthetic cAP is less active than CCK-8.

This new type of antral peptide has never been isolated from mammalian sources. The antisera raised by this synthetic peptide may serve to identify such a peptide possibly in mammalian intestinal tissues. As described above, we encountered some difficulty in the condensation of peptide fragments. Thus, we decided to perform an alternative synthesis of cAP by the stepwise solid-phase method, which may overcome such dfficulty in the chain elongation reaction. This synthesis will be reported in the subsequent paper.

Experimental

General experimental procedures employed in this investigation are essentially the same as described for the sauvagine synthesis.[40]

N^{α}-Deprotection——The N^{α}-protecting groups, Z(OMe) or Boc, were cleaved by TFA (*ca.* 10 ml per 1 g of a peptide) in the presence of anisole (2 eq or more) at ice-bath temperature for 60 min. After evaporation of the TFA *in vacuo* at 30 °C or below, the residue was treated with dry ether. If a powder was obtained, it was collected by filtration, dried over KOH pellets *in vacuo* for 3 h and then used for the condensation reaction. If an oily precipitate was obtained, it was washed with *n*-hexane, dried over KOH pellets *in vacuo* for 3 h and used for the condensation reaction.

Condensation Reactions——Every reaction was continued until the solution became negative to the ninhydrin test. The active ester and DCC condensation reactions were performed at room temperature (17—25 °C). The azide was prepared with isoamyl nitrite and usually the reaction was conducted at 4 °C. An MA was prepared with isobutyl chloroformate and the reaction was performed in an ice-bath.

Purification——Unless otherwise stated, products were purified by one of the following procedures.

Procedure A: For purification of protected peptides soluble in AcOEt, the extract was washed with 5% citric

acid, 5% $NaHCO_3$ and H_2O, dried over Na_2SO_4 and concentrated. The residue was recrystallized from appropriate solvents.

Procedure B: For purification of protected peptides less soluble in AcOEt; the crude product was triturated with ether and 5% citric acid. The resulting powder was washed with 5% citric acid, 5% $NaHCO_3$, and H_2O and recrystallized or precipitated from appropriate solvents.

Silica Gel Chromatography: For purification of protected peptides which could not be purified by the above methods, the product was dissolved in a small amount of $CHCl_3$–MeOH (10 : 0.5) and the solution was applied to a column of silica (Kieselgel 60-H, Merck), which was eluted with the same solvent under N_2 pressure. The eluates (10 ml each) were collected and examined by TLC. The eluates containing the desired product were combined and the solvent was removed by evaporation. The residue was triturated with appropriate solvents.

Gel-Filtration: For purification of protected peptides less soluble in $CHCl_3$–MeOH (10 : 0.5), the crude product was dissolved in a small amount of DMF and the solution was applied to a column of Sephadex LH-60, which was eluted with the same solvent. Individual fractions (10 ml each) were collected and examined by ultraviolet (UV) absorption measurement at 275 nm. The desired fractions were combined, the solvent was removed by evaporation and the residue was precipitated from appropriate solvents.

TLC was performed on silica (Kieselgel G, Merck). *Rf* values refer to the following solvent systems (v/v): Rf_1 $CHCl_3$–MeOH (10 : 0.5), Rf_2 $CHCl_3$–MeOH–H_2O (8 : 3 : 1), Rf_3 *n*-BuOH–AcOH–pyridine–H_2O (4 : 1 : 1 : 2), Rf_4 *n*-BuOH–AcOH–AcOEt–H_2O (1 : 1 : 1 : 1).

HPLC was conducted with a Waters compact model 204. FAB-mass spectra were obtained on a ZAB SE instrument (VG Analytical Co., England). Leucine-aminopeptidase (LAP) and sulfated CCK-8 were purchased from Sigma (lot No. L-6007) and Peptide Institute Inc. (Osaka, Japan), respectively.

Boc–Asp(OChp)–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH_2——Boc–Asp(OChp)–OSu (2.56 g, 1.2 eq) and NMM (0.66 ml, 1.2 eq) were added to a solution of a TFA-treated sample of Z(OMe)–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH_2 (5.00 g, 5.0 mmol) in DMF (40 ml) containing TEA (0.78 ml, 1.1 eq), then the mixture was stirred overnight. The product was purified by procedure B, followed by recrystallization from MeOH and AcOEt; yield 4.80 g (80%), mp 160—162 °C, $[\alpha]_D^{15}$ −23.4° (c = 0.6, DMF), Rf_1 0.41. *Anal.* Calcd for $C_{61}H_{83}N_7O_{14}S_2$: C, 60.93; H, 6.96; N, 8.15. Found: C, 60.63; H, 7.03; N, 7.97.

Z(OMe)–Pro–Asp(OChp)–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH_2——A mixed anhydride [prepared from 1.40 g (1.2 eq) of Z(OMe)–Pro–OH] in THF (30 ml) was added to an ice-chilled solution of a TFA-treated sample of the above pentapeptide amide (5.05 g, 4.19 mmol) in DMF–THF (1 : 1, 20 ml) containing TEA (0.58 ml, 1 eq) and the mixture was stirred for 5 h. The product was purified by procedure A, followed by recrystallization from MeOH and ether; yield 4.77 g (84%), mp 156.5—158 °C, $[\alpha]_D^{15}$ −39.8° (c = 0.5, DMF); Rf_1 0.19, Rf_2 0.76. *Anal.* Calcd for $C_{70}H_{90}N_8O_{16}S_2 \cdot H_2O$: C, 60.85; H, 6.71; N, 8.11. Found: C, 60.76; H, 6.62; N, 8.17.

Boc–Tyr(Cl_2–Bzl)–Pro–Asp(OChp)–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH_2 [1]——Boc–Tyr(Cl_2–Bzl)–OBt [prepared from Boc–Tyr(Cl_2–Bzl)–OH (1.13 g, 1.2 eq), HOBt (0.39 g, 1.2 eq) and DCC (0.53 g, 1.2 eq)] in THF (20 ml) was added to an ice-chilled solution of a TFA-treated sample of the above hexapeptide amide (2.91 g, 2.13 mmol) in DMF (30 ml) containing TEA (0.36 ml, 1.2 eq) and the mixture was stirred overnight. The product was purified by procedure A, followed by flash column chromatography on silica (4 × 25 cm) using $CHCl_3$–MeOH (10 : 0.5) as an eluant. The product was finally recrystallized from AcOEt and ether; yield 2.45 g (71%), mp 119—121 °C, $[\alpha]_D^{15}$ −33.8° (c = 0.9, DMF), Rf_1 0.32. Amino acid ratios in a 6 N HCl hydrolysate: Asp 1.98 (2), Pro 0.98 (1), Met 0.95 (1), Tyr 0.92 (1), Phe 1.00 (1, recovery 92%). *Anal.* Calcd for $C_{82}H_{103}Cl_2N_9O_{17}S_2 \cdot H_2O$: C, 60.06; H, 6.33; N, 7.69. Found: C, 59.84; H, 6.44; N, 7.60.

Boc–His(Bom)–Phe–NHNH–Troc——The azide [prepared from 6.94 g (1.0 eq) of Boc–His(Bom)–$NHNH_2$] in DMF (10 ml) and TEA (2.73 ml, 1.1 eq) were added to an ice-chilled solution of a TFA-treated sample of Z(OMe)–Phe–NHNH–Troc (8.40 g, 16.2 mmol) in DMF (20 ml) containing TEA (2.26 ml, 1 eq) and the mixture was stirred overnight. The product was purified by procedure A, followed by recrystallization from AcOEt and ether; yield 7.68 g (67%), mp 144—145 °C, $[\alpha]_D^{15}$ −11.3° (c = 0.3, DMF), Rf_2 0.74. *Anal.* Calcd for $C_{31}H_{37}Cl_3N_6O_7$: C, 52.29; H, 5.24; N, 11.80. Found: C, 52.35; H, 5.20; N, 11.70.

Boc–Asp(OChp)–His(Bom)–Phe–NHNH–Troc——A mixture of Boc–Asp(OChp)–OSu (2.64 g, 6.2 mmol), TEA (1.72 ml, 2.2 eq) and a TFA-treated sample of the above dipeptide derivative (4.00 g, 5.60 mmol) in DMF (40 ml) was stirred overnight. The product was purified by procedure A, followed by recrystallization from AcOEt and *n*-hexane; yield 5.07 g (98%), mp 88.5—90 °C, $[\alpha]_D^{15}$ −28.6° (c = 0.5, DMF), Rf_1 0.28. *Anal.* Calcd for $C_{42}H_{54}Cl_3N_7O_{10}$: C, 54.64; H, 5.90; N, 10.62. Found: C, 54.38; H, 5.85; N, 10.63.

Z(OMe)–Leu–His(Bom)–$NHNH_2$——A mixture of Z(OMe)–Leu–ONp (10.76 g, 1.1 eq), TEA (7.20 ml, 2.2 eq) and a TFA-treated sample of Boc–His(Bom)–OMe (10.00 g, 23.5 mmol) in DMF (40 ml) was stirred overnight and the product was purified by procedure A. The dipeptide ester thus obtained was dissolved in MeOH (100 ml) and treated with 80% hydrazine hydrate (5.9 ml, 5 eq) for 24 h. The solvent was evaporated off, then the residue was treated with H_2O and the resulting powder was precipitated from DMF with MeOH; yield 8.17 g (62%), mp 127—129 °C $[\alpha]_D^{15}$ −18.5° (c = 0.8, DMF), Rf_2 0.79. *Anal.* Calcd for $C_{29}H_{38}N_6O_6$: C, 61.47; H, 6.76; N, 14.83. Found: C, 61.46; H, 6.73; N, 14.93.

Z(OMe)–Leu–His(Bom)–Asp(OChp)–His(Bom)–Phe–NHNH–Troc——The azide [prepared from 1.72 g, 1.1 eq) of Z(OMe)–Leu–His(Bom)–$NHNH_2$] in DMF (10 ml) and NMM (0.37 ml, 1.1 eq) were added to an ice-chilled solution of a TFA-treated sample of Boc–Asp(OChp)–His(Bom)–Phe–NHNH–Troc (2.55 g, 2.76 mmol) in DMF (10 ml) containing TEA (0.38 ml, 1 eq) and the mixture was stirred overnight. Additional azide [prepared from 0.2 eq of the dipeptide hydrazide] in DMF (3 ml) and TEA (77 μl, 0.2 eq) were added and stirring was continued for 24 h, then the product was purified by procedure A, followed by recrystallization from MeOH and ether; yield 2.51 g (67%), mp 156—157 °C, $[\alpha]_D^{15}$ −31.8° (c=0.5, DMF), Rf_2 0.53. *Anal.* Calcd for $C_{66}H_{80}Cl_3N_{11}O_{14}$: C, 58.38; H, 5.94; N, 11.35. Found: C, 58.28; H, 5.92; N, 11.38.

Z(OMe)–Leu–His(Bom)–Asp(OChp)–His(Bom)–Phe–$NHNH_2$ [2]——The above pentapeptide derivative (2.51 g, 1.85 mmol) in AcOH (12 ml) was treated with Zn powder (1.2 g, 10 eq) at room temperature for 2 h. The solution was filtered, the filtrate was concentrated *in vacuo* and the residue was treated with 3% EDTA. The resulting powder was washed with H_2O and precipitated from DMF with MeOH; yield 1.29 g (59%), mp 171—173 °C, $[\alpha]_D^{15}$ −33.7° (c=0.6, DMF), Rf_2 0.64. Amino acid ratios in a 6 N HCl hydrolysate: Asp 1.02 (1), Leu 1.03 (1), His 1.87 (2), Phe 1.00 (1, recovery 85%). *Anal.* Calcd for $C_{63}H_{79}N_{11}O_{12}\cdot H_2O$: C, 63.03; H, 6.80; N, 12.84. Found: C, 63.00; H, 6.61; N, 12.84.

Z(OMe)–Glu(OBzl)–Ala–NHNH–Troc——A mixture of Z(OMe)–Glu(OBzl)–ONp (21.26 g, 1.2 eq), TEA (11.20 ml, 2.4 eq) and a TFA-treated sample of Z(OMe)–Ala–NHNH–Troc (14.96 g, 33.8 mmol) in DMF (150 ml) was stirred overnight and the product was isolated by procedure A, followed by recrystallization from AcOEt and ether; yield 20.00 g (94%), mp 145.5—147 °C, $[\alpha]_D^{20}$ −40.6° (c=0.9, DMF), Rf_2 0.79. *Anal.* Calcd for $C_{27}H_{31}Cl_3N_4O_9$: C, 48.99; H, 4.72; N, 8.47. Found: C, 48.92; H, 4.66; N, 8.54.

Z(OMe)–Val–Glu(OBzl)–Ala–NHNH–Troc——A mixture of Z(OMe)–Val–OSu (27.70 g, 1.2 eq), NMM (16.0 ml, 2.4 eq) and a TFA-treated sample of Z(OMe)–Glu(OBzl)–Ala–NHNH–Troc (40.40 g, 61.0 mmol) in DMF (200 ml) was stirred overnight and the product was isolated by procedure B, followed by recrystallization from AcOEt and ether; yield 35.88 g (77%), mp 145—147 °C, $[\alpha]_D^{20}$ −56.0° (c=0.8, MeOH), Rf_1 0.39. *Anal.* Calcd for $C_{32}H_{40}Cl_3N_5O_{10}$: C, 50.50; H, 5.30; N, 9.20. Found: C, 50.43; H, 5.21; N, 9.27.

Z(OMe)–Ala–Val–Glu(OBzl)–Ala–NHNH–Troc——A mixture of Z(OMe)–Ala–OSu (7.94 g, 1.2 eq), TEA (6.4 ml, 2.4 eq), and a TFA-treated sample of the above tripeptide derivative (14.40 g, 18.9 mmol) in DMF (150 ml) was stirred overnight. The product was purified by procedure B, followed by recrystallization from MeOH; yield 10.39 g (66%), mp 186—188 °C, $[\alpha]_D^{20}$ −23.4° (c=0.9, DMF), Rf_1 0.28. *Anal.* Calcd for $C_{35}H_{45}Cl_3N_6O_{11}$: C, 50.52; H, 5.45; N, 10.10. Found: C, 50.80; H, 5.52; N, 10.31.

Z(OMe)–Gly–Ala–Val–Glu(OBzl)–Ala–NHNH–Troc——A mixture of Z(OMe)–Gly–OSu (5.38 g, 1.3 eq), TEA (4.10 ml, 2.4 eq) and a TFA-treated sample of the above tetrapeptide derivative (10.23 g, 12.3 mmol) in DMF (250 ml) was stirred overnight. The product was purified by procedure B, followed by precipitation from DMF with MeOH; yield 8.83 g (81%), mp 204—206 °C, $[\alpha]_D^{20}$ −28.6° (c=1.4, DMF), Rf_1 0.25. *Anal.* Calcd for $C_{37}H_{48}Cl_3N_7O_{12}$: C, 49.98; H, 5.44; N, 11.03. Found: C, 50.27; H, 5.66; N, 11.12.

Z(OMe)–Asn–Gly–Ala–Val–Glu(OBzl)–Ala–NHNH–Troc——A mixture of Z(OMe)–Asn–ONp (1.84 g, 1.2 eq), TEA (1.22 ml, 2.4 eq), and a TFA-treated sample of the above pentapeptide derivative (3.27 g, 3.68 mmol) in DMF (150 ml) was stirred overnight. The product was purified by procedure B, followed by precipitation from DMF with MeOH; yield 3.50 g (95%), mp 215—217 °C, $[\alpha]_D^{20}$ −29.1° (c=1.1, DMSO), Rf_1 0.44. *Anal.* Calcd for $C_{41}H_{54}Cl_3N_9O_{14}$: C, 49.08; H, 5.42; N, 12.56. Found: C, 49.36; H, 5.54; N, 12.82.

Z(OMe)–Asn–Gly–Ala–Val–Glu(OBzl)–Ala–$NHNH_2$ [3]——The above hexapeptide derivative (7.00 g, 6.98 mmol) in DMF–AcOH (200 ml–35 ml) was treated with Cd powder (7.9 g, 10 eq) at room temperature overnight. The solution was filtered, the filtrate was concentrated, and the residue was treated with 2% EDTA. The resulting powder was washed with H_2O and precipitated from DMSO with MeOH; yield 5.29 g (92%), mp 233—234 °C, $[\alpha]_D^{20}$ −25.5° (c=0.7, DMSO), Rf_1 0.47. Amino acid ratios in a 6 N HCl hydrolysate: Asp 0.98 (1), Glu 0.95 (1), Gly 0.98 (1), Val 0.95 (1), Ala 2.00 (2, recovery 98%). *Anal.* Calcd for $C_{38}H_{53}N_9O_{12}\cdot H_2O$: C, 53.96; H, 6.55; N, 14.90. Found: C, 54.09; H, 6.38; N, 14.62.

Z(OMe)–Val–Gln–Gly–OMe——A mixture of Z(OMe)–Val–OSu (11.40 g, 1.2 eq), TEA (8.4 ml, 2.4 eq) and a TFA-treated sample of Z(OMe)–Gln–Gly–OMe (9.52 g, 25.0 mmol) in DMF (100 ml) was stirred overnight. The product was purified by procedure B, followed by precipitation from DMF–DMSO (1 : 1) with MeOH; yield 9.25 g (77%), mp 221—223 °C, $[\alpha]_D^{20}$ −1.4° (c=0.7, DMSO), Rf_2 0.72. *Anal.* Calcd for $C_{22}H_{32}N_4O_8$: C, 54.99; H, 6.71; N, 11.66. Found: C, 55.01; H, 6.75; N, 11.82.

Z(OMe)–Phe–Val–Gln–Gly–OMe——A mixture of Z(OMe)–Phe–ONp (10.30 g, 1.2 eq), TEA (6.4 ml, 2.4 eq), and a TFA-treated sample of the above tripeptide (9.20 g, 19.1 mmol) in DMF–DMSO (1 : 1, 200 ml) was stirred for 60 h. The product was precipitated from DMSO with MeOH; yield 11,45 g (96%), mp 260—262 °C, $[\alpha]_D^{20}$ −2.5° (c= 1.6, DMSO), Rf_2 0.71. *Anal.* Calcd for $C_{31}H_{41}N_5O_9$: C, 59.32; H, 6.58; N, 11.16. Found: C, 59.23; H, 6.56; N, 11.16.

Z(OMe)–Lys(Z)–Gly–Phe–Val–Gln–Gly–OMe——The azide [prepared from 4.76 g (1.2 eq) of Z(OMe)–Lys(Z)–Gly–$NHNH_2$] in DMF (30 ml) and TEA (2.35 ml, 2.2 eq) were added to an ice-chilled solution of a TFA-treated sample of the above tetrapeptide ester (4.83 g, 7.69 mmol) in DMF–DMSO (20 ml–40 ml) and the mixture was stirred overnight. H_2O was added and the resulting powder was washed with H_2O and precipitated from DMF with MeOH;

yield 6.80 g (93%), mp 235—237 °C, $[\alpha]_D^{20}$ −5.6° (c = 1.1, DMSO), Rf_2 0.83. *Anal.* Calcd for $C_{47}H_{62}N_8O_{13}$: C, 59.61; H, 6.60; N, 11.83. Found: C, 59.49; H, 6.53; N, 11.88.

Z(OMe)–Lys(Z)–Gly–Phe–Val–Gln–Gly–NHNH$_2$ [4]——The above hexapeptide ester (2.00 g, 2.11 mmol) in DMF (90 ml) was treated with 80% hydrazine hydrate (2.54 ml, 20 eq) at room temperature for 48 h. MeOH was added and the resulting solid was precipitated from DMF with MeOH; yield 1.83 g (92%), mp 221—224 °C, $[\alpha]_D^{20}$ −2.7° (c = 1.5, DMSO), Rf_2 0.59. Amino acid ratios in a 6 N HCl (72 h) hydrolysate: Glu 1.00 (1), Gly 2.00 (2), Phe 0.97 (1), Lys 0.97 (1), Val 1.00 (1, recovery 98%). *Anal.* Calcd for $C_{46}H_{62}N_{10}O_{12}\cdot H_2O$: C, 57.25; H, 6.68; N, 14.51. Found: C, 57.39; H, 6.65; N, 14.68.

Z(OMe)–Asp(OChp)–Arg(Mts)–NHNH–Troc——A mixture of Z(OMe)–Asp(OChp)–OSu (4.80 g, 1.1 eq), TEA (2.96 ml, 2.4 eq) and a TFA-treated sample of Z(OMe)–Arg(Mts)–NHNH–Troc (6.30 g, 8.87 mmol) in DMF (50 ml) was stirred overnight. The product was purified by procedure A, followed by recrystallization from ether and n-hexane. The product was further purified by column chromatography on silica using $CHCl_3$–MeOH (10 : 0.5) as an eluant; yield 5.80 g (71%), mp 93—95 °C, $[\alpha]_D^{20}$ −16.6° (c = 1.2, MeOH), Rf_1 0.70. *Anal.* Calcd for $C_{38}H_{52}Cl_3N_7O_{11}S$: C, 49.54; H, 5.69; N, 10.64. Found: C, 49.70; H, 5.81; N, 10.62.

Z(OMe)–Ser–Asp(OChp)–Arg(Mts)–NHNH–Troc——The azide [prepared from 1.87 g (1.2 eq) of Z(OMe)–Ser–NHNH$_2$] in DMF (25 ml) and NMM (0.73 ml, 1.2 eq) were added to an ice-chilled solution of a TFA-treated sample of the above dipeptide derivative (5.07 g, 5.50 mmol) in DMF (20 ml) containing TEA (0.85 ml, 1.1 mmol) and the mixture was stirred for 48 h. The product was purified by procedure A, followed by column chromatography on silica using $CHCl_3$–MeOH (10 : 0.5) as an eluant; yield 4.71 g (85%), mp 101—103 °C, $[\alpha]_D^{20}$ −19.8° (c = 0.9, MeOH), Rf_1 0.46. *Anal.* Calcd for $C_{41}H_{57}Cl_3N_8O_{13}S$: C, 48.84; H, 5.70; N, 11.11. Found: C, 49.03; H, 5.87; N, 10.82.

Z(OMe)–Ser–Asp(OChp)–Arg(Mts)–NHNH$_2$ [5]——The above protected tripeptide derivative (1.00 g, 0.99 mmol) in DMF–AcOH (9 ml–1 ml) was treated with Zn powder (2.80 g, 43 eq) for 3.5 h. The solution was filtered, the filtrate was concentrated and the residue was treated with 2% EDTA. The resulting powder was washed with H_2O and recrystallized from AcOEt and ether; yield 0.56 g (68%), mp 97—99 °C, $[\alpha]_D^{20}$ −12.4° (c = 0.9, MeOH), Rf_1 0.33. Amino acid ratios in a 6 N HCl hydrolysate: Ser 0.93 (1), Arg 0.97 (1), Asp 1.00 (1, recovery 99%). *Anal.* Calcd for $C_{38}H_{56}N_8O_{11}S$: C, 54.79; H, 6.78; N, 13.45. Found: C, 54.64; H, 6.89; N, 13.17.

Z(OMe)–Glu(OBzl)–Leu–NHNH–Troc——A mixture of Z(OMe)–Glu(OBzl)–ONp (7.70 g, 1.1 eq), TEA (4.48 ml, 2.4 eq) and a TFA-treated sample of Z(OMe)–Leu–NHNH–Troc (6.50 g, 13.4 mmol) in DMF (50 ml) was stirred overnight. The product was purified by procedure A, followed by recrystallization from ether and n-hexane; yield 7.7 g (82%), mp 129—130 °C, $[\alpha]_D^{20}$ −43.8° (c = 1.0, MeOH), Rf_1 0.40. *Anal.* Calcd for $C_{30}H_{37}Cl_3N_4O_9$: C, 51.18; H, 5.30; N, 7.96. Found: C, 51.37; H, 5.28; N, 7.91.

Z(OMe)–Ala–Glu(OBzl)–Leu–NHNH$_2$ [6]——A mixture of Z(OMe)–Ala–OSu (3.03 g, 1.2 eq), NMM (1.90 ml, 2.4 eq) and a TFA-treated sample of the above dipeptide derivative (5.07 g, 7.20 mmol) in DMF (25 ml) was stirred overnight. The product was purified by procedure A, followed by column chromatography on silica using $CHCl_3$–MeOH (10 : 0.5) as an eluant; yield 2.74 g (49%), Rf_1 0.42. The above tripeptide derivative (2.60 g, 3.35 mmol) in MeOH–AcOH (20 ml–5 ml) was treated with Zn powder (7.0 g, 32 eq) at 4 °C overnight. The solution was filtered, the filtrate was concentrated and the residue was treated with 3% EDTA. The resulting powder was washed with H_2O and purified by column chromatography on silica using a gradient of MeOH (2% to 5%) in $CHCl_3$. The solvent of the main eluate was removed by evaporation and the residue was triturated with n-hexane to give a powder; yield 1.36 g (68%), mp 149—151 °C, $[\alpha]_D^{20}$ −41.9° (c = 0.9, MeOH), Rf_1 0.39. Amino acid ratios in a 6 N HCl hydrolysate: Glu 1.15 (1), Leu 0.97 (1), Ala 1.00 (1, recovery 98%). *Anal.* Calcd for $C_{30}H_{41}N_5O_8$: C, 60.09; H, 6.89; N, 11.68. Found: C, 60.29; H, 6.99; N, 11.39.

Z(OMe)–Val–Phe–OMe——A mixture of Z(OMe)–Val–OSu (5.26 g, 1 eq), HOBt (2.13 g, 1 eq), TEA (1.93 ml, 1 eq) and H–Phe–OMe [prepared from 3.0 g (1 eq) of the HCl salt] in DMF (20 ml) was stirred overnight and the product was purified by procedure B, followed by precipitation from DMF with ether; yield 4.10 g (67%), mp 159—162 °C, $[\alpha]_D^{15}$ −4.9° (c = 0.8, DMF), Rf_1 0.96. *Anal.* Calcd for $C_{24}H_{30}N_2O_6$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.30; H, 6.91; N, 6.33.

Boc–His(Bom)–Val–Phe–OMe——The azide [prepared from 3.87 g (1.1 eq) of Boc–His(Bom)–NHNH$_2$] in DMF (30 ml) and TEA (1.53 ml, 1.2 eq) were added to an ice-chilled solution of a TFA-treated sample of the above dipeptide ester (4.00 g, 9.0 mmol) in DMF (10 ml) containing TEA (1.26 ml, 1 eq) and the mixture was stirred overnight. The product was purified by procedure B, followed by recrystallization from MeOH and ether; yield 5.02 g (88%), mp 150—152 °C, $[\alpha]_D^{15}$ −12.0° (c = 0.7, DMF), Rf_2 0.84. *Anal.* Calcd for $C_{34}H_{45}N_5O_7$: C, 64.23; H, 7.14; N, 11.02. Found: C, 64.44; H, 7.24; N, 11.31.

Z(OMe)–Leu–Pro–OH——Z(OMe)–Leu–ONp (3.0 g, 7.2 mmol) was added to a solution of H–Pro–OH (1.66 g, 2 eq) in H_2O (5 ml) containing TEA (6.02 ml, 6 eq) and the mixture was stirred overnight. The solvent was removed by evaporation and the residue was dissolved in 5% NH_4OH. The aqueous phase was washed with ether, then acidified with citric acid and the resulting precipitate was dissolved in AcOEt. The organic phase was washed with H_2O–NaCl, dried over Na_2SO_4 and concentrated to give an oily product.

Z(OMe)–Leu–Pro–His(Bom)–Val–Phe–OMe——A TFA-treated sample of Boc–His(Bom)–Val–Phe–OMe (3.00 g, 4.70 mmol) was dissolved in AcOEt, then the organic phase was washed with 5% $NaHCO_3$ and H_2O–NaCl,

dried over Na_2SO_4 and concentrated. The residue was dissolved in THF (10 ml) and mixed with a solution of Z(OMe)–Leu–Pro–OH (2.20 g, 1.2 eq) in THF (20 ml). DCC (1.07 g, 1.1 eq) and HOBt (0.72 g, 1 eq) were added and the mixture was stirred overnight. The product was purified by procedure A, followed by recrystallization from AcOEt and ether; yield 2.65 g (62%), mp 102—104 °C, $[\alpha]_D^{20}$ −36.1° (c=0.8, DMF), Rf_1 0.29, Rf_2 0.90. Amino acid ratios in a 6 N HCl hydrolysate: Pro 0.93 (1), Val 0.96 (1), Leu 1.08 (1), His 1.01 (1), Phe 1.00 (1, recovery 89%). *Anal.* Calcd for $C_{49}H_{63}N_7O_{10}\cdot 3H_2O$: C, 61.04; H, 6.80; N, 10.17. Found: C, 61.06; H, 6.78; N, 10.19.

Z(OMe)–Phe–Leu–Pro–His(Bom)–Val–Phe–OMe——A solution of Z(OMe)–Phe–OSu (1.46 g, 1.2 eq) and NMM (0.35 ml, 1.1 eq) in DMF (5 ml) was added to a solution of a TFA-treted sample of the above pentapeptide ester (2.60 g, 2.86 mmol) in DMF (5 ml) containing TEA (0.40 ml, 1 eq) and the mixture was stirred overnight. The product was purified by procedure A, followed by recrystallization from AcOEt and ether; yield 2.71 g (90%), mp 112—114 °C, $[\alpha]_D^{20}$ −70.4° (c=0.8, MeOH), Rf_1 0.25, Rf_2 0.73. *Anal.* Calcd for $C_{58}H_{72}N_8O_{11}\cdot 3H_2O$: C, 62.68; H, 7.08; N, 10.08. Found: C, 62.49; H, 6.79; N, 10.13.

Z(OMe)–Phe–Leu–Pro–His(Bom)–Val–Phe–NHNH$_2$ [7]——The above hexapeptide ester (2.60 g, 2.46 mmol) in MeOH (20 ml) was treated with 80% hydrazine hydrate (0.60 ml, 5 eq) overnight. The solid formed during the above treatment was precipitated from DMF with MeOH; yield 1.25 g (50%), mp 164—166 °C, $[\alpha]_D^{15}$ −47.5° (c=0.6, DMF), Rf_2 0.70. Amino acid ratios in a 6 N HCl hydrolysate: Pro 0.95 (1), Val 0.98 (1), Leu 1.11 (1), His 1.04 (1), Phe 2.00 (2, recovery 80%). *Anal.* Calcd for $C_{57}H_{72}N_{10}O_{10}$: C, 64.75; H, 6.86; N, 13.25. Found: C, 64.45; H, 6.77; N, 13.17.

Z(OMe)–Leu–His(Bom)–Asp(OChp)–His(Bom)–Phe–Tyr(Cl$_2$Bzl)–Pro–Asp(OChp)–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH$_2$ (Positions 25—36)——The azide, prepared from 1.09 g (1.5 eq) of fragment [2], in DMF (1.5 ml) and TEA (0.14 ml, 1.5 eq) were added to an ice-chilled solution of a TFA-treated sample of fragment [1] (1.00 g, 0.62 mmol) in DMF (4 ml) containing TEA (86 μl, 1 eq) and the mixture was stirred overnight. The product was purified by procedure B, followed by silica gel chromatography, using $CHCl_3$–MeOH (10 : 0.7). After evaporation of the solvent, the residue was triturated with ether; yield 1.31 g (80%), mp 117—119 °C, $[\alpha]_D^{20}$ −42.7° (c=0.6, DMF), Rf_2 0.82. Amino acid ratios in 6 N HCl are listed in Table I, together with other protected intermediates.

Z(OMe)–Asn–Gly–Ala–Val–Glu(OBzl)–Ala–Leu–His(Bom)–Asp(OChp)–His(Bom)–Phe–Tyr(Cl$_2$–Bzl)–Pro–Asp(OChp)–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH$_2$ (Positions 19—36)——The azide, prepared from 725 mg (1.8 eq) of fragment [3], in DMF (3 ml) and TEA (122 μl, 1.8 eq) were added to an ice-chilled solution of a TFA-treated sample of the above protected dodecapeptide amide (1.30 g, 0.487 mmol) in DMF (4 ml) containing TEA (68 μl, 1 eq) and the mixture was stirred for 2 d. The product was purified by procedure B, followed by gel-filtration on Sephadex LH-20. Trituration of the product with MeOH afforded a powder; yield 1.04 g (65%), mp 228—232 °C, $[\alpha]_D^{20}$ −31.3° (c=0.6, DMF), Rf_2 0.63.

Z(OMe)–Lys(Z)–Gly–Phe–Val–Gln–Gly–Asn–Gly–Ala–Val–Glu(OBzl)–Ala–Leu–His(Bom)–Asp(OChp)–His(Bom)–Phe–Tyr(Cl$_2$–Bzl)–Pro–Asp(OChp)–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH$_2$ (Positions 13—36)——The azide, prepared from fragment [4] (549 mg, 2 eq), and TEA (87 μl, 2 eq) were added to an ice-chilled solution of a TFA-treated sample of the above octadecapeptide amide (1.03 g, 0.314 mmol) in DMF (5 ml) containing TEA (70 μl, 1.5 eq) and the mixture was stirred overnight. The product was purfied by procedure B, followed by gel-filtration on Sephadex LH-60. The purified product was triturated with MeOH; yield 1.06 g (83%), mp 149—151 °C, $[\alpha]_D^{20}$ −26.8° (c=0.2, DMSO), Rf_2 0.60.

Z(OMe)–Ser–Asp(OChp)–Arg(Mts)–Lys(Z)–Gly–Phe–Val–Gln–Gly–Asn–Gly–Ala–Val–Glu(OBzl)–Ala–Leu–His(Bom)–Asp(OChp)–His(Bom)–Phe–Tyr(Cl$_2$–Bzl)–Pro–Asp(OChp)–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH$_2$ (Positions 10—36)——The azide, prepared from fragment [5] (606 mg, 3.0 eq), in DMF (1 ml) and TEA (101 μl, 3 eq) were added to an ice-chilled solution of a TFA-treated sample of the above protected tetracosapeptide amide (985 mg, 0.243 mmol) in DMF–DMSO (1 : 1, 50 ml) containing TEA (34 μl, 1 eq) and the mixture was stirred for 2 d. The product was purified by procedure B, followed by gel-filtration on Sephadex LH-60. The purified product was triturated with MeOH; yield 890 mg (78%), mp 252—254 °C, $[\alpha]_D^{20}$ −23.5° (c=0.5, DMSO), Rf_2 0.65.

Z(OMe)–Ala–Glu(OBzl)–Leu–Ser–Asp(OChp)–Arg(Mts)–Lys(Z)–Gly–Phe–Val–Gln–Gly–Asn–Gly–Ala–Val–Glu(OBzl)–Ala–Leu–His(Bom)–Asp(OChp)–His(Bom)–Phe–Tyr(Cl$_2$–Bzl)–Pro–Asp(OChp)–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH$_2$ (Positions 7—36)——The axide, prepared from fragment [6] (338 mg, 3.0 eq), in DMF (1 ml) and TEA (78 μl, 3 eq) were added to an ice-chilled solution of a TFA-treated sample of the above heptacosapeptide amide (880 mg, 0.188 mmol) in DMF–DMSO (1 : 1, 12 ml) containing TEA (26 μl, 1 eq) and the mixture was stirred overnight. Additional azide (3 eq) in DMF (1.5 ml) and TEA (3 eq) were added and the stirring was continued for an additional 24 h. H_2O was added and the resulting solid was purified by gel-filtration on Sephadex LH-60. The purified product was triturated with MeOH; yield 0.55 g (57%), mp 233—235 °C, $[\alpha]_D^{20}$ −46.0° (c=0.6, DMSO), Rf_2 0.76.

Z(OMe)–Pne–Leu–Pro–His(Bom)–Val–Phe–Ala–Glu(OBzl)–Leu–Ser–Asp(OChp)–Arg(Mts)–Lys(Z)–Gly–Phe–Val–Gln–Gly–Asn–Gly–Ala–Val–Glu(OBzl)–Ala–Leu–His(Bom)–Asp(OChp)–His(Bom)–Phe–Tyr(Cl$_2$–Bzl)–Pro–Asp(OChp)–Trp(Mts)–Met(O)–Asp(OChp)–Phe–NH$_2$, Protected Antral Peptide——The azide, prepared from fragment [7] (156 mg, 5.0 eq), in DMF (2 ml) and TEA (20 μl, 5 eq) were added to an ice-chilled solution of a TFA-treated sample of the above triacontapeptide amide (150 mg, 0.029 mmol) in DMF–DMSO (1 : 1, 4 ml) containing TEA (5 μl, 1.1 eq) and the mixture was stirred for 48 h. Additional azide (5 eq) in DMF–DMSO (1 : 1, 2 ml) and TEA (5 eq) were added and the stirring was continued for another 72 h. The ninhydrin test was still positive. The solvent was removed

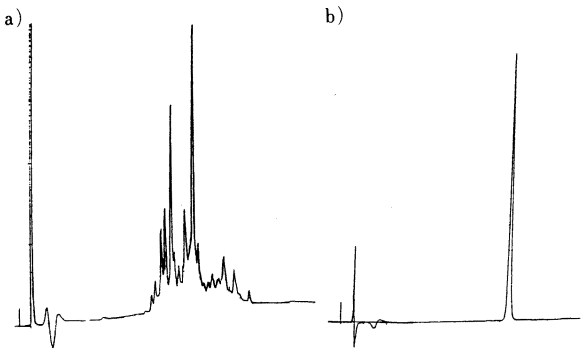

Fig. 11. HPLC of Synthetic cAP (OD at 220 nm)
a) Gel-filtered sample. b) HPLC-purified sample.

by evaporation and the residue was subjected to gel-filtration on Sephadex LH-60 (3.2 × 133 cm), which was eluted with DMF–DMSO (1 : 1). The product emerged from the column as nearly a single component and exhibited a single spot on TLC. However, its acid hydrolysis indicated that the acyl component was incorporated in only *ca.* 60%. Amino acid ratios in a 6 N HCl hydrolysate of this partially purified product are listed in Table I.

In this purification step, when the column was eluted with DMF, we observed another peak in front of the main peak (Fig. 9). Amino acid ratios in a 6 N HCl hydrolysate were similar to those of the main peak.

Synthetic Chicken Antral Peptide——The crude sample of protected antral peptide (76 mg, 12.76 μmol) was treated with 1 M TMSBr–thioanisole/TFA (5 ml) in an ice-bath for 120 min in the presence of *m*-cresol (244 μl, 183 eq) and EDT (100 μl, 88 eq), then dry ether was added. The resulting powder was treated with 1 M TMSOTf–thioanisole/TFA (5 ml) in an ice-bath for 120 min in the presence of *m*-cresol (244 μl, 183 eq) and EDT (100 μl, 88 eq), and dry ether was added. The resulting powder was dissolved in MeOH (3 ml), then 1 M NH_4F (200 μl) was added. The pH of the solution was adjusted to 8.0 with 0.2 M Tris–HCl buffer pH 8.0 containing 6 M guanidine hydrochloride and after 20 min to 6.0 with 1 N AcOH. The solution was incubated with 2-mercaptoethanol (200 μl, 224 eq) at room temperature for 5 h, and then applied to a column of Sephadex G-25 (2.2 × 130 cm), which was eluted with 1 N AcOH. The fractions (8.0 ml each) corresponding to the front main peak (tube Nos. 26—36, monitored by UV absorption measurement at 280 nm) were collected and the solvent was removed by lyophilization to give a powder; 50.8 mg. The crude deprotected peptide was purified by HPLC on a Cosmosil 5C18 column (10 × 250 mm), which was eluted with a gradient of MeCN (27—42%, 45 min) in 0.1% TFA at a flow rate of 2 ml/min (Fig. 11a). Two relatively large peaks were detected. The eluates corresponding to these peaks were collected and the solvent was removed from each by lyophilization; peak-1 1.0 mg, peak-2 2.7 mg (5.0%). Amino acid ratios of peak-1 matched with those of the amino component (position 7—36). Amino acid ratios in a 6 N HCl hydrolysate of peak-2 are listed in Table I. The product peak-2 was characterized as follows: single spot on TLC, Rf_3 0.40, Rf_4 0.55, $[\alpha]_D^{25}$ −46.5° (c=0.1, 0.5 N AcOH), FAB-MS m/z: 4194.1 $(M+H)^+$ (Calcd 4193.7), retention time on an analytical Cosmosil 5C18 column (4.6 × 150 mm): 22 min (Fig. 11b) on gradient elution with MeCN (20—45%, 30 min) in 0.1% TFA at a flow rate of 1.5 ml/min. Mobility on polyacrylamide gel containing Pharmalyte, pH 3.0—10.0, was 1.3 cm from the origin toward the cathodic end of the gel (0.5 × 6.3 cm), after running for 4 h at 200 V. Amino acid ratios in a LAP digest: Asp 3.86 (4), Gln 1.20 (1), Ser + Asn N.D., Glu 2.42 (2), Pro 1.99 (2), Gly 3.48 (3), Ala 3.46 (3), Val 3.34 (3), Met 0.89 (1), Leu 3.45 (3), Tyr 1.17 (1), Phe 5.00 (5), Lys 1.10 (1), His 3.05 (3), Trp 0.94 (1), Arg 0.89 (1), recovery of Phe 82%.

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References and Notes

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2) Amino acids used in this investigation are of the L-configuration. The following abbreviations are used: Z = benzyloxycarbonyl, Z(OMe) = *p*-methoxybenzyloxycarbonyl, Boc = *tert*-butyloxycarbonyl, Bzl = benzyl, Cl_2Bzl = 2,6-dichlorobenzyl, Mts = mesitylenesulfonyl, Troc = 2,2,2-trichloroethyloxycarbonyl, Chp = cycloheptyl, Bom = benzyloxymethyl, Np = *p*-nitrophenyl, Su = *N*-hydroxysuccinimidyl, DCC = dicyclohexylcarbodiimide, HOBt = *N*-hydroxybenzotriazole, TFA = trifluoroacetic acid, TMSBr = trimethylsilyl bromide, TMSOTf = trimethylsilyl trifluoromethanesulfonate; DMF = dimethylformamide, DMSO = dimethyl sulfoxide, THF = tetrahydrofuran, EDT = ethanedithiol; EDTA = ethylenediaminetetraacetic acid disodium salts.
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