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Studies on Peptides. CXXXIII.[1,2] Synthesis and Biological Activity of Galanin, a Novel Porcine Intestinal Polypeptide

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A new porcine gastrointestinal 29-residue peptide, galanin, was synthesized in a satisfactory yield by assembling seven peptide fragments followed by deprotection with 1 M trifluoromethanesulfonic acid–thioanisole in trifluoroacetic acid. N^{in}-Mesitylenesulfonyltryptophan was employed to suppress indole alkylation during N^{α}-deprotection. β-Cycloheptylaspartate was employed to suppress base-catalyzed succinimide formation. The synthetic peptide exhibited a powerful contractile activity on rat ileum, but not on guinea pig ileum and caused sustained hyperglycemia in dogs.

Keywords——galanin solution synthesis; β-cycloheptylaspartate; N^{in}-mesitylenesulfonyltryptophan; Asp side reaction; Trp side reaction; thioanisole-mediated deprotection; trifluoromethanesulfonic acid deprotection; smooth muscle contractile activity; hyperglycemic activity

Galanin is a 29-residue peptide isolated from porcine intestine by Tatemoto *et al.*[3] in 1983. Previously, Tatemoto and Mutt[4] explored a new chemical technique to identify peptides containing a C-terminal amidated structure from tissue extracts. Using this chemical method, galanin was discovered in extracts of porcine intestine as a peptide with C-terminal Ala–NH_2.

We wish to report the first solution-phase synthesis of a nonacosapeptide corresponding to the entire amino acid sequence of galanin. The synthesis was conducted by a strategy based on the use of the thioanisole-mediated TFMSA deprotecting procedure,[5] the superior properties of which were recently reviewed.[6] In order to suppress side reactions at the Trp and the Asp residues, two new derivatives were employed in the present synthesis.

As shown in Fig. 1, in combination with the TFA-labile Z(OMe)[7] or Boc group for N^{α}-protection, amino acid derivatives bearing protecting groups removable by 1 M TFMSA–thioanisole/TFA were employed, *i.e.*, Lys(Z), Ser(Bzl), Arg(Mts),[8] Trp(Mts)[9] and Asp(OChp).[1] Of these, Boc–Trp(Mts)–OH was introduced by us in 1984 in order to minimize indole alkylation during the N^{α}-TFA deprotection[10,11] and the susceptibility of its protecting group to 1 M TFMSA–thioanisole/TFA was preliminarily examined by syntheses of small model peptides.[12] Boc–Asp(OChp)–OH[1] was applied to the present synthesis for the first time. Galanin possesses the Asp–Asn sequence (positions 17-18). This particular sequence is known to be particularly susceptible to rearrangement by base (succinimide formation),[13] like Asp–Gly,[14] when Asp(OBzl) is employed for the synthesis. In order to avoid this sequence-

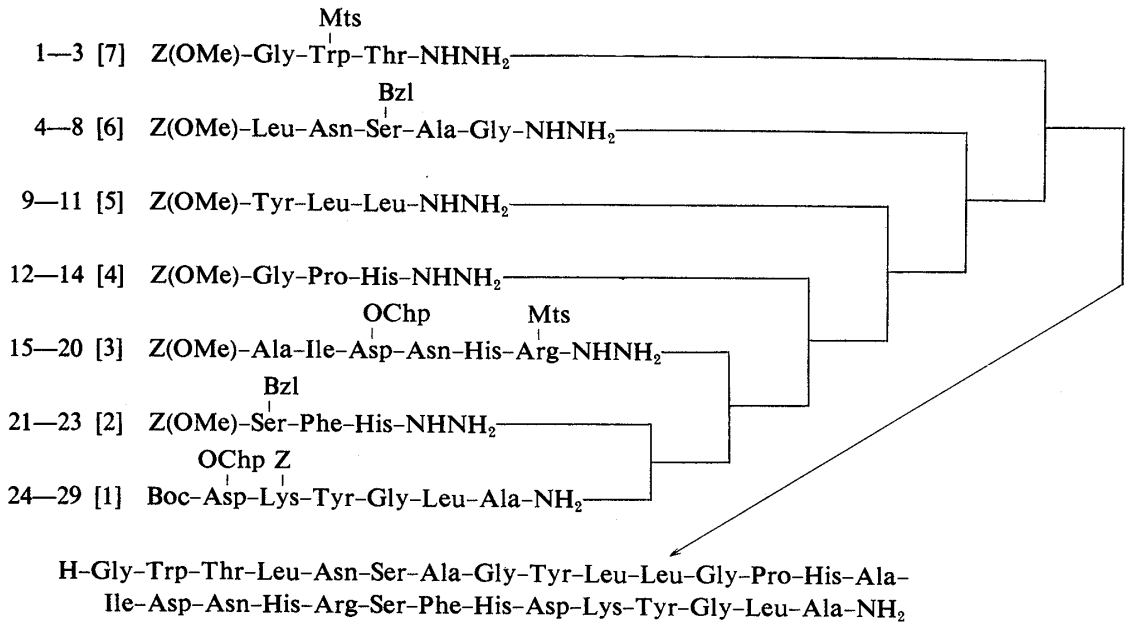

Fig. 1. Synthetic Route to Galanin

dependent and base-catalyzed side reaction, removal of the Bzl group by hydrogenolysis, immediately after introduction of Asp(OBzl), is a method of choice, as demonstrated by the syntheses of VIP (vasoactive intestinal polypeptide)[13,15] possessing this particular sequence. Alternatively, protection of the β-functional group of Asp by sterically hindered secondary alcohols, such as cyclopentyl or cyclohexyl alcohol, was examined by Blake[16] and Tam *et al.*,[17] respectively. We found preliminarily that the β-Chp group fulfills several criteria required for peptide synthesis, *i.e.*, it is stable to TFA, resistant to Et_3N treatment and removable by 1 M TFMSA–thioanisole/TFA.[1] Further, when Boc–Asp(OChp)–Asn–OH was prepared and exposed to Et_3N, it gave significantly less imide than Boc–Asp(OBzl)–Asn–OH. Galanin possesses the Asp–Lys sequence (positions 24-25). From available information, this sequence was expected to be safely synthesized using Asp(OBzl), because of its low base-sensitivity. However, Asp(OChp) was employed in order to make the protecting groups as uniform as possible.

Seven peptide fragments were selected to construct the entire peptide backbone of galanin (Fig. 1). Of these, the hydrazide [3] containing Asp(OChp) was prepared with the aid of Troc–$NHNH_2$,[18] the protecting group of which can be removed by Zn[19] or Cd[20] in AcOH or Zn in methanol in the presence of NH_4Cl,[21] without affecting other functional protecting groups.

The C-terminal hexapeptide, Boc–Asp(OChp)–Lys(Z)–Tyr–Gly–Leu–Ala–NH_2 [1], was synthesized according to the scheme illustrated in Fig. 2. The known dipeptide ester, Z(OMe)–Leu–Ala–OMe,[22] was exposed to NH_3 in MeOH in a sealed tube to obtain the corresponding amide, which after TFA treatment, was condensed with Z(OMe)–Tyr–Gly–$NHNH_2$[23] *via* the azide.[24] The peptide chain of the resulting tetrapeptide amide was elongated by azide condensation with Boc–Asp(OChp)–Lys(Z)–$NHNH_2$, which was prepared by the Su condensation[25] of Boc–Asp(OChp)–OH with H–Lys(Z)–NHNH–Troc, followed by Zn–AcOH treatment. Thus the C-terminal amide fragment [1], recrystallizable from MeOH and ether, was obtained as a relatively soluble amide in organic solvents. The purity of [1] was confirmed by thin layer chromatography (TLC), amino acid analysis after 6 N HCl hydrolysis and elemental analysis, as was also done with other fragments.

Fragment [2], Z(OMe)–Ser(Bzl)–Phe–His–$NHNH_2$, was synthesized by the azide con-

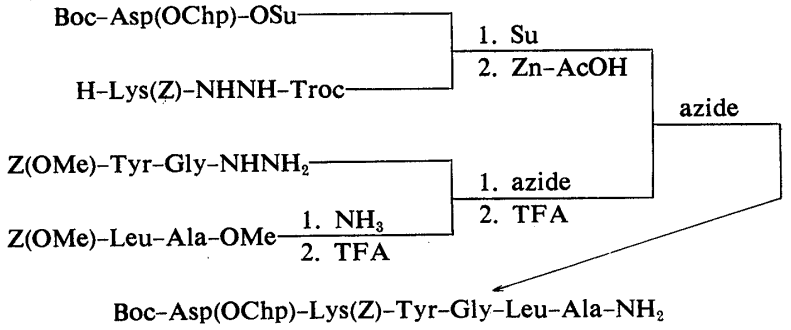

Fig. 2. Synthetic Scheme for the Protected Hexapeptide Amide, Boc-(galanin 24—29)-NH_2 [1]

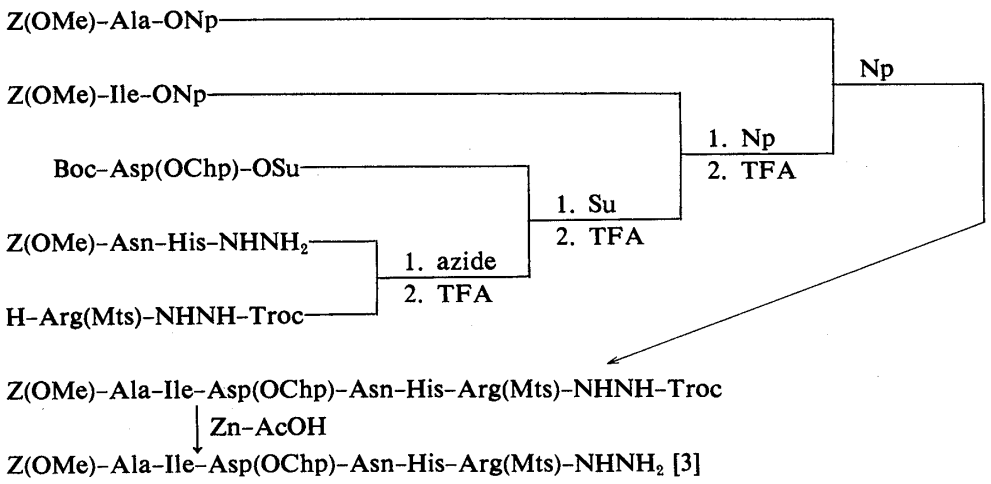

Fig. 3. Synthetic Scheme for the Protected Hexapeptide Hydrazide, Z(OMe)-(galanin 15—20)-$NHNH_2$ [3]

densation of Z(OMe)–Ser(Bzl)–Phe–$NHNH_2$ and H–His–OMe, followed by the usual hydrazine treatment. The former dipeptide derivative was easily prepared by the DCC condensation[26] of Z(OMe)–Ser(Bzl)–OH and H–Phe–OMe, followed by the usual hydrazine treatment.

Fragment [3], Z(OMe)–Ala–Ile–Asp(OChp)–Asn–His–Arg(Mts)–$NHNH_2$, was prepared according to the scheme illustrated in Fig. 3. Z(OMe)–Asn–His–$NHNH_2$, prepared by hydrazinolysis of Z(OMe)–Asn–His–OMe,[12] was condensed with H–Arg(Mts)–NHNH–Troc *via* the azide. Next, the Su ester was employed to introduce Boc–Asp(OChp)–OH onto a TFA-treated sample of the protected tripeptide derivative obtained above. Using NMM as a base, this coupling reaction and the subsequent condensation reactions with Z(OMe)–Ile–OH and Z(OMe)–Ala–OH *via* the corresponding Np esters[27] could be conducted smoothly, without producing any extra spots on TLC due to succinimide formation. The presence of the bulky Chp side chain protecting group did not interfere with the coupling reaction in either case, when Asp(OChp) was used as an acyl component or even an amino component, as judged from the yields of the protected tetrapeptide and protected pentapeptide derivatives. Finally, the Troc group was removed from the protected hexapeptide derivative by Zn–AcOH treatment to give Z(OMe)–(galanin 15—20)–$NHNH_2$ [3]. This protected hexapeptide hydrazide, after deprotection with 1 M TFMSA–thioanisole in TFA, was subjected to aminopeptidase digestion. Amino acid ratios of Ile and Asp were 1 : 0.8, indicating that the β-Chp group had resisted succinimide formation fairly well under the above basic conditions and this acid

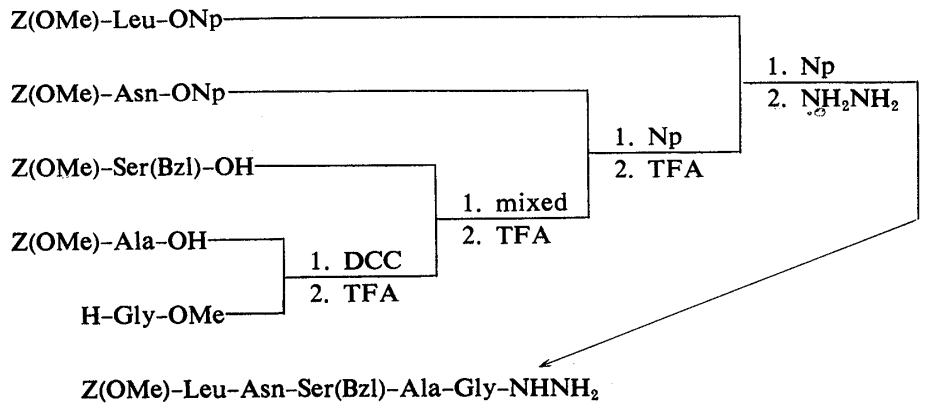

Fig. 4. Synthetic Scheme for the Protected Pentapeptide Hydrazide, Z(OMe)–(galanin 4—8)–$NHNH_2$ [6]

treatment as well.

The next two fragments were easily prepared as follows. Fragment [4], Z(OMe)–Gly–Pro–His–$NHNH_2$, was prepared by the Su condensation of H–His–OMe and Z(OMe)–Gly–Pro–OH followed by the usual hydrazine treatment of the resulting tripeptide ester. The latter dipeptide was prepared by the Np method and characterized as its DCHA salt. Fragment [5], Z(OMe)–Tyr–Leu–Leu–$NHNH_2$, was prepared by the azide condensation of Z(OMe)–Tyr–$NHNH_2$ with a TFA-treated sample of Z(OMe)–Leu–Leu–OMe followed by the usual hydrazine treatment. The latter dipeptide was easily prepared by the mixed anhydride procedure.[28]

The next pentapeptide fragment, Z(OMe)–Leu–Asn–Ser(Bzl)–Ala–Gly–$NHNH_2$ [6], was prepared in a stepwise manner starting with H–Gly–OMe. First, Z(OMe)–Ala–Gly–OMe was prepared by the DCC procedure. The mixed anhydride procedure was employed for introduction of Z(OMe)–Ser(Bzl)–OH and the Np method for Z(OMe)–Asn–OH and Z(OMe)–Leu–OH. The resulting pentapeptide ester was smoothly converted to [6] by the usual hydrazine treatment as shown in Fig. 4.

The N-terminal fragment [7], Z(OMe)–Gly–Trp(Mts)–Thr–$NHNH_2$, was prepared in a stepwise manner also starting with H–Thr–OMe. Boc–Trp(Mts)–OH was introduced by using DCC to give Boc–Trp(Mts)–Thr–OMe, from which the Boc group was removed by TFA treatment in the presence of anisole as usual, without formation of any extra spots on TLC or any pink color in a solution. Next, Z(OMe)–Gly–OH was condensed by the Np procedure and the resulting protected tripeptide ester was smoothly converted to the corresponding hydrazide [7] as stated above.

The necessary fragments thus obtained were assembled successively onto a TFA-treated sample of the C-terminal fragment [1] *via* the azide as shown in Fig. 1. The amount of the acyl component was increased from 1.5 to 3 eq as the chain elongation progressed. Every condensation proceeded smoothly without encountering any solubility problem. The protected galanin and the two protected intermediates that resulted from the azide condensations of relatively large fragments [3] and [6] were purified by gel-filtration on Sephadex LH-60. The rest of the protected intermediates were purified by repeated precipitation from MeOH or DMF with AcOEt. Throughout this synthesis, Lys was used as a diagnostic amino acid. Each intermediate was subjected to acid hydrolysis and the recovery of Lys was compared with those of newly added amino acids in order to ascertain satisfactory incorporation, after each condensation, as shown in Table I.

In the final step, deprotection and subsequent purification were carried out according to the following scheme (Fig. 5). The protected nonacosapeptide amide, Z(OMe)–(galanin 1—

TABLE I. Amino Acid Ratios in 6 N HCl Hydrolysates[a] of Synthetic Galanin and Its Intermediates

	Protected peptides							Synthetic galanin
	24—29 (6)	21—29 (9)	15—29 (15)	12—29 (18)	9—29 (21)	4—29 (26)	1—29 (29)	
Asp	0.99	1.02	3.16	3.06	3.07	3.97	3.95	3.87 (4)
Thr							1.01	0.93 (1)
Ser		0.94	0.87	0.89	0.89	1.81	1.78	1.82 (2)
Pro				0.95	1.00	0.83	0.86	0.97 (1)
Gly	0.94	1.01	0.99	1.88	1.88	2.85	3.90	3.96 (4)
Ala	0.90	1.06	2.13	2.06	2.06	3.19	3.21	3.09 (3)
Ile			1.06	0.99	0.99	0.96	0.97	0.97 (1)
Leu	0.98	1.06	1.00	0.98	3.00	3.81	3.77	4.04 (4)
Tyr	0.84	0.90	0.93	0.93	1.86	1.68	1.66	2.05 (2)
Phe		0.98	0.96	0.96	0.97	0.95	0.95	0.99 (1)
Trp[b]							0.90	0.75 (1)
Lys	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00 (1)
His		0.97	1.98	2.77	2.76	2.77	2.76	2.89 (3)
Arg			1.02	0.98	0.99	0.95	0.99	0.94 (1)
Recov.	70%	85%	84%	83%	75%	90%	89%	74%

a) With phenol. *b*) Determined by 4 N MSA hydrolysate.

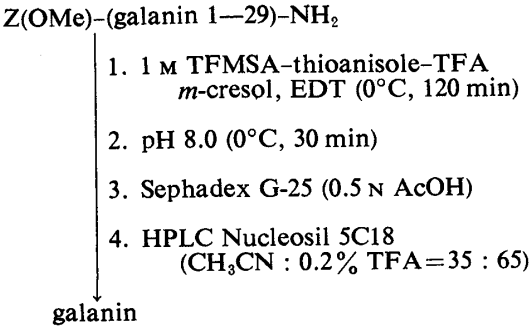

Fig. 5. Deprotection and Purification of Galanin

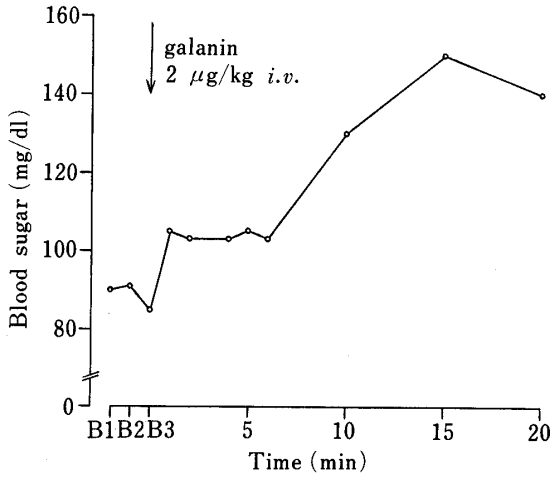

Fig. 6. Hyperglycemic Activity of Synthetic Galanin

29)–NH_2, was treated with 1 M TFMSA–thioanisole (molar ratios 1 : 1) in TFA in the presence of *m*-cresol and EDT in an ice-bath for 120 min. EDT is particularly important to suppress indole modification in this acidolytic deprotection as reported previously.[12] The deprotected peptide was treated with dilute ammonia at pH 8.0 in order to reverse the possible N→O shift at the Ser and Thr residues[29] and then subjected to gel-filtration on Sephadex G-25 using 0.5 N AcOH as an eluant. High performance liquid chromatography (HPLC) examination of the deprotected peptide obtained here showed it to be fairly pure. The product was then purified by preparative HPLC on a column of Nucleosil $5C_{18}$. The main product, obtained after desalting on Sephadex G-25, exhibited a single spot on TLC and a single band on disc isoelectrofocusing (Pharmalyte pH 3—10). The purified product exhibited a single peak on HPLC with a retention time identical with that of natural galanin. Its acid hydrolysate gave

the amino acid ratios predicted by theory. Its purity was further confirmed by enzymatic digestion, in which satisfactory recoveries of Asp and Trp were obtained. From the experimental data obtained here, we conclude that the two derivatives, Trp(Mts) and Asp(OChp), are both useful for practical peptide synthesis.

Galanin was reported to contract smooth muscle preparations and to cause sustained hyperglycemia in dogs. The contractile activity of synthetic galanin on isolated rat ileum was 5 times greater than that of substance P, but galanin (3×10^{-5} M) had no effect on guinea-pig ileum. Thus, galanin exhibited tissue specificity. When administered intravenously to dogs, our synthetic peptide (2 μg/kg) exhibited remarkable hyperglycemic activity, as shown in Fig. 6. Thus our synthetic peptide was found to have the same spectrum of biological activities as natural galanin.

Experimental

General experimental methods employed here are essentially the same as described in Part 88[30] of the present series.

N^{α}-Deprotection——The N^{α}-protecting group, Z(OMe) or Boc, was 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 TFA *in vacuo* at 15—25 °C, 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 then used for the condensation reaction.

Condensation Reactions——The DCC and the active ester condensations were performed at room temperature. Each hydrazide was converted to the corressponding azide by isoamyl nitrite treatment and the azide condensation was performed at 4 °C. A mixed anhydride was prepared using isobutyl chloroformate and allowed to react with an amino component in an ice-bath for 5 h.

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

Procedure A: For purification of a protected peptide soluble in AcOEt, the extract was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and concentrated. The residue was crystallized or precipitated from appropriate solvents.

Procedure B: For purification of a peptide 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 crystallized or precipitated from appropriate solvents. For purification of peptides containing the Asp residue, 5% citric acid and H_2O were used for washing. For purification of peptides containing the His residue, 5% $NaHCO_3$ and H_2O were used for washing.

Procedure C: For purification of a protected peptide partially soluble in water, the crude product was extracted with *n*-BuOH and the extract was washed with H_2O saturated with *n*-BuOH, and concentrated. The residue was crystallized or precipitated from appropriate solvents.

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

Leucine aminopeptidase (LAP, Lot. No. L-6007) was purchased from Sigma. HPLC was conducted with a Waters 204 compact model. Fast atom bombardment (FAB) mass spectra were obtained on a JEOL JMS-HX 100 double-focusing spectrometer, equipped with an FAB ion source, a data processor (JEOL JMA-3100) and a mass data analysis system. Typical experimental conditions were as follows: A xenon atom beam source at 7-keV accelerating potential. Mass assignment was made by using a mixture of CsI and KI as a mass reference. A sample solution containing 15—40 μg of peptide was loaded on a stainless steel plate and mixed with glycerol and α-thioglycerol on the plate.

Model Experiments

Boc–Asp(OBzl)–Asn–OH——This compound was prepared by the Su procedure and purified by recrystallization from MeOH and ether; yield 62%, mp 136—139 °C, $[\alpha]_D^{20}$ +3.8° (c=1.0, MeOH), Rf_3 0.71. *Anal.* Calcd for $C_{20}H_{27}N_3O_8$: C, 54.91; H, 6.22; N, 9.61. Found: C, 55.10; H, 6.26; N, 9.63.

Boc–Asp(OChp)–Asn–OH——This compound was prepared similarly by the Su method and purified by recrystallization from AcOEt and ether; yield 52%, mp 70—73 °C, $[\alpha]_D^{20}$ −4.0° (c=0.5, MeOH), Rf_3 0.83. *Anal.* Calcd for $C_{20}H_{33}N_3O_8 \cdot 1/4H_2O$: C, 53.62; H, 7.48; N, 9.38. Found: C, 53.56; H, 7.50; N, 9.69.

Base Treatment of Boc–Asp(OR)–Asn–OH (R = Bzl, Chp)——A solution of Boc–Asp(OR)–Asn–OH (5 mg each) in DMF (50 μl) containing Et_3N (1 eq) or NMM (1 eq) was periodically examined by TLC. The plate was stained with HCl–ninhydrin, and the succinimide formed was measured with a dual-wavelength TLC scanner. The results are listed in Table II.

Table II. Succinimide Formation from Boc-Asp(OR)-Asn-OH (%) by Base Treatment

R	Triethylamine 10 h	Triethylamine 20 h	Triethylamine 40 h	*N*-Methylmorpholine 10 h	*N*-Methylmorpholine 20 h	*N*-Methylmorpholine 40 h
Bzl	20.8	21.4	28.3	1.4	7.8	11.6
Chp	2.8	4.8	5.1	≒0	≒0	1.1

Z(OMe)-Leu-Ala-NH_2——In a sealed flask, Z(OMe)-Leu-Ala-OMe[22] (5.70 g, 15.00 mmol) in MeOH (60 ml) was treated with NH_3 for 2 d. The product was precipitated from DMF with ether; yield 4.30 g (78%), mp 170—172 °C, $[\alpha]_D^{17}$ +7.1° (c=1.0, DMF), Rf_1 0.70. *Anal.* Calcd for $C_{18}H_{27}N_3O_5$: C, 59.16; H, 7.45; N, 11.50. Found: C, 58.88; H, 7.52; N, 11.28.

Z(OMe)-Tyr-Gly-Leu-Ala-NH_2——A TFA-treated sample of Z(OMe)-Leu-Ala-NH_2 (4.20 g, 11.50 mmol) was dissolved in DMF (40 ml) containing Et_3N (1.75 ml, 12.60 mmol). The azide [prepared from 5.75 g (13.80 mmol) of Z(OMe)-Tyr-Gly-$NHNH_2$[23]] in DMF (60 ml) and Et_3N (1.92 ml, 13.80 mmol) were added to the above ice-chilled solution and the mixture, after being stirred overnight, was concentrated. The product was purified by procedure C, followed by precipitation from MeOH with AcOEt; yield 3.95 g (59%), mp 161—163 °C, $[\alpha]_D^{17}$ −37.9° (c=1.0, DMF), Rf_1 0.66. *Anal.* Calcd for $C_{29}H_{39}N_5O_8 \cdot 1/2H_2O$: C, 58.57; H, 6.78; N, 11.78. Found: C, 58.68; H, 6.78; N, 11.74.

Boc-Asp(OChp)-Lys(Z)-NHNH-Troc——A mixture of H-Lys(Z)-NHNH-Troc [prepared from 4.05 g (6.40 mmol) of the Z(OMe)-derivative[31]], Boc-Asp(OChp)-OSu (3.00 g, 7.00 mmol) prepared as usual and NMM (0.71 ml, 7.00 mmol) in DMF (40 ml) was stirred overnight and concentrated. The product was purified by procedure A, followed by precipitation from AcOEt with *n*-hexane; yield 4.51 g (90%), mp 61—64 °C, $[\alpha]_D^{18}$ −24.4° (c=1.0, MeOH), Rf_1 0.76. *Anal.* Calcd for $C_{33}H_{48}Cl_3N_5O_{10}$: C, 50.74; H, 6.19; N, 8.97. Found: C, 51.00; H, 6.24; N, 8.75.

Boc-Asp(OChp)-Lys(Z)-$NHNH_2$——Boc-Asp(OChp)-Lys(Z)-NHNH-Troc (0.76 g, 0.97 mmol) in AcOH (6 ml) was treated with Zn powder (1.27 g, 20 eq) at room temperature for 6 h, then the mixture was filtered and concentrated. The residue was dissolved in AcOEt and the organic phase, after being washed with 5% EDTA and H_2O, was dried over Na_2SO_4 and concentrated. The residue was treated with ether to afford a powder, which was recrystallized from MeOH and ether; yield 0.58 g (98%), mp 91—93 °C, $[\alpha]_D^{18}$ −13.2° (c=1.0, DMF), Rf_1 0.69. *Anal.* Calcd for $C_{30}H_{47}N_5O_8$: C, 59.48; H, 7.82; N, 11.56. Found: C, 59.68; H, 7.82; N, 11.53.

Boc-Asp(OChp)-Lys(Z)-Tyr-Gly-Leu-Ala-NH_2 [1], Boc-(galanin 24—29)-NH_2——The azide [prepared from 0.80 g (1.32 mmol) of Boc-Asp(OChp)-Lys(Z)-$NHNH_2$] in DMF (2 ml) and Et_3N (0.18 ml, 1.32 mmol) were added to the ice-chilled solution of H-Tyr-Gly-Leu-Ala-NH_2 [obtained from 0.65 g (1.10 mmol) of the Z(OMe)-derivative] in DMF (5 ml), and the mixture, after being stirred for 24 h, was concentrated. The product was purified by procedure C, followed by recrystallization from MeOH and ether; yield 0.74 g (68%), mp 127—130 °C, $[\alpha]_D^{25}$ −38.7° (c=0.2, MeOH), Rf_1 0.67. Amino acid ratios in 6 N HCl hydrolysate: Asp 1.01, Lys 1.02, Tyr 0.86, Gly 0.96, Leu 1.00, Ala 0.91 (recovery of Leu 69%). *Anal.* Calcd for $C_{50}H_{74}N_8O_{13} \cdot 1/2H_2O$: C, 59.80; H, 7.53; N, 11.16. Found: C, 59.60; H, 7.56; N, 11.26.

Z(OMe)-Ser(Bzl)-Phe-OMe——The above dipeptide ester was prepared by DCC condensation and purified by procedure A, followed by recrystallization from MeOH and *n*-hexane; yield 1.35 g (86%), mp 97—99 °C, $[\alpha]_D^{17}$ −1.9° (c=1.0, DMF), Rf_1 0.77. *Anal.* Calcd for $C_{29}H_{32}N_2O_7$: C, 66.91; H, 6.20; N, 5.38. Found: C, 66.83; H, 6.28; N, 5.90.

Z(OMe)-Ser(Bzl)-Phe-$NHNH_2$——Z(OMe)-Ser(Bzl)-Phe-OMe (3.00 g, 5.76 mmol) in MeOH (30 ml) was treated with 80% hydrazine hydrate (3.60 ml, 10 eq) and the solid formed on standing at room temperature overnight was precipitated from DMF with MeOH; yield 2.25 g (75%), mp 208—210 °C, $[\alpha]_D^{29}$ −12.4° (c=1.0, DMF), Rf_1 0.98, Rf_2 0.30. *Anal.* Calcd for $C_{28}H_{32}N_4O_6$: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.82; H, 6.13; N, 10.85.

Z(OMe)-Ser(Bzl)-Phe-His-OMe——The azide [prepared from 2.00 g (3.84 mmol) of Z(OMe)-Ser(Bzl)-Phe-$NHNH_2$] in DMF (10 ml) was allowed to react with H-His-OMe [prepared from 0.95 g (4.61 mmol) of the HCl salt] in DMF (5 ml) containing Et_3N (0.59 ml, 4.18 mmol) for 12 h and the product was purified by procedure C, followed by precipitation from MeOH with ether; yield 2.32 g (92%), mp 163—166 °C, $[\alpha]_D^{29}$ −18.4° (c=1.0, DMF), Rf_1 0.65. *Anal.* Calcd for $C_{35}H_{39}N_5O_8 \cdot 2H_2O$: C, 60.59; H, 6.25; N, 10.10. Found: C, 60.83; H, 5.82; N, 10.12.

Z(OMe)-Ser(Bzl)-Phe-His-$NHNH_2$ [2], Z(OMe)-(galanin 21—23)-$NHNH_2$——The above tripeptide ester (2.22 g, 3.38 mmol) in DMF-MeOH (1 : 2, 20 ml) was treated with 80% hydrazine hydrate (2.20 ml, 10 eq) at room temperature for 12 h and the solvent was removed by evaporation. The product was precipitated from DMF with EtOH; yield 1.54 g (69%), mp 165—167 °C, $[\alpha]_D^{16}$ +7.1° (c=1.0, DMF), Rf_1 0.58. Amino acid ratios in 6 N HCl hydrolysate: Ser 0.98, Phe 1.00, His 1.06 (recovery of Phe 61%). *Anal.* Calcd for $C_{34}H_{39}N_7O_7 \cdot 1/2H_2O$: C, 61.25; H, 6.05; N, 14.71. Found: C, 60.98; H, 6.00; N, 14.89.

Z(OMe)-Asn-His-$NHNH_2$——A mixture of Z(OMe)-Asn-His-OMe[12] (3.00 g, 6.70 mmol) and 80% hydrazine hydrate (4.20 ml, 10 eq) in MeOH-DMF (1 : 1, 30 ml) was allowed to stand overnight and then concentrated. The

product was recrystallized from DMF with MeOH; yield 2.35 g (78%), mp 197—199 °C, $[\alpha]_D^{18}$ +23.6° (c=0.8, DMF), Rf_1 0.23. *Anal.* Calcd for $C_{19}H_{25}N_7O_6$: C, 51.00; H, 5.63; N, 21.91. Found: C, 51.03; H, 5.56; N, 22.07.

Z(OMe)–Asn–His–Arg(Mts)–NHNH–Troc——In the presence of Et_3N (0.56 ml, 4.02 mmol), the azide [prepared from 1.50 g (3.35 mmol) of Ż(OMe)–Asn–His–$NHNH_2$] in DMF (5 ml) was allowed to react with H–Arg(Mts)–NHNH–Troc[32] [obtained from 2.86 g (4.02 mmol) of the Z(OMe)-derivative] in DMF (15 ml) overnight and the product was purified by procedure A, followed by precipitation from MeOH with ether; yield 3.05 g (95%), mp 130—132 °C, $[\alpha]_D^{18}$ +40.4° (c=0.5, MeOH), Rf_1 0.59. *Anal.* Calcd for $C_{37}H_{48}Cl_3N_{11}O_{11}S$: C, 46.23; H, 5.17; N, 15.63. Found: C, 46.23; H, 5.03; N, 16.03.

Boc–Asp(OChp)–Asn–His–Arg(Mts)–NHNH–Troc——A mixture of a TFA-treated sample of the above tripeptide (2.90 g, 3.02 mmol), Boc–Asp(OChp)–OSu (1.54 g, 3.62 mmol) and NMM (0.68 ml, 6.84 mmol) in DMF (30 ml) was stirred overnight and concentrated. The product was purified by procedure A, followed by precipitation from MeOH with ether; yield 3.09 g (92%), mp 136—139 °C, $[\alpha]_D^{18}$ −13.0° (c=1.0, MeOH), Rf_1 0.63. *Anal.* Calcd for $C_{44}H_{65}Cl_3N_{12}O_{13}S \cdot 2H_2O$: C, 46.17; H, 6.07; N, 14.69. Found: C, 46.47; H, 5.77; N, 14.48.

Z(OMe)–Ile–Asp(OChp)–Asn–His–Arg(Mts)–NHNH–Troc——A mixture of a TFA-treated sample of the above tetrapeptide (3.09 g, 2.79 mmol), Z(OMe)–Ile–ONp (1.39 g, 3.35 mmol) and NMM (0.62 ml 6.17 mmol) in DMF (30 ml) was stirred for 24 h and concentrated. The product was purified by procedure B, followed by precipitation from DMF with EtOH; yield 2.75 g (77%), mp 175—178 °C, $[\alpha]_D^{15}$ −10.6° (c=1.0, DMF), Rf_1 0.60. *Anal.* Calcd for $C_{54}H_{76}Cl_3N_{13}O_{15}S$: C, 50.44; H, 5.96; N, 14.16. Found: C, 50.47; H, 6.06; N, 14.16.

Z(OMe)–Ala–Ile–Asp(OChp)–Asn–His–Arg(Mts)–NHNH–Troc——A mixture of a TFA-treated sample of the above pentapeptide (2.70 g, 2.10 mmol), Z(OMe)–Ala–ONp (1.02 g, 2.73 mmol), HOBt (0.14 g, 1.05 mmol) and NMM (0.50 ml, 4.83 mmol) in DMF (30 ml) was stirred overnight and the product was purified by procedure C, followed by two precipitations from DMF with EtOH; yield 2.33 g (82%), mp 169—172 °C, $[\alpha]_D^{15}$ −10.6° (c=1.0, DMF), Rf_1 0.55. *Anal.* Calcd for $C_{57}H_{81}Cl_3N_{14}O_{16}S$: C, 50.46; H, 6.02; N, 14.45. Found: C, 50.22; H, 5.99; N, 14.31.

Z(OMe)–Ala–Ile–Asp(OChp)–Asn–His–Arg(Mts)–$NHNH_2$ [3], Z(OMe)–(galanin 15—20)–$NHNH_2$——The above hexapeptide (2.30 g, 1.69 mmol) in DMF–AcOH (1 : 1, 20 ml) was treated with Zn powder (1.11 g, 10 eq) at room temperature for 8 h, then the mixture was filtered and concentrated. The residue was treated with 5% EDTA to afford a powder, which was washed with 5% EDTA and H_2O and precipitated from DMF with MeOH; yield 1.01 g (50%), mp 190—192 °C, $[\alpha]_D^{25}$ −12.8° (c=0.6, DMF), Rf_1 0.40. Amino acid ratios in 6 N HCl hydrolysate: Ala 1.06, Ile 1.02, Asp 2.03, His 1.00, Arg 1.00 (recovery of Arg 80%). *Anal.* Calcd for $C_{54}H_{80}N_{14}O_{14}S \cdot 2H_2O$: C, 53.27; H, 6.96; N, 16.11. Found: C, 53.01; H, 6.71; N, 15.85.

The protected hexapeptide thus obtained (30 mg) was treated with 1 M TFMSA–thioanisole in TFA (1.3 ml) in the presence of *m*-cresol (28 μl, 10 eq) in an ice-bath for 2 h, then dry ether was added. The resulting powder, after conversion to the corresponding acetate, was digested with leucine aminopeptidase. Amino acid ratios were Ala 1.13, Ile 1.00, Asp 0.80, Asn N.D., His 0.91, Arg 0.93 (recovery of Ile 83%).

Z(OMe)–Gly–Pro–OH · DCHA——A mixture of H–Pro–OH (8.63 g, 75.00 mmol), Z(OMe)–Gly–ONp (18.00 g, 50.00 mmol) and Et_3N (17.50 ml, 125.00 mmol) in DMF–H_2O (1 : 1, 200 ml) was stirred overnight and concentrated. The residue was dissolved in 5% $NaHCO_3$ and washed with ether. The aqeuous solution was acidified with citric acid and the product was extracted with *n*-BuOH. The organic phase was washed with H_2O and concentrated. The residue was converted to the corresponding DCHA salt as usual and recrystallized from MeOH and ether; yield 17.20 g (67%), mp 120—122 °C, $[\alpha]_D^{18}$ −30.1° (c=1.0, DMF), Rf_1 0.20. *Anal.* Calcd for $C_{28}H_{43}N_3O_6$: C, 64.96; H, 8.37; N, 8.12. Found: C, 64.77; H, 8.44; N, 8.18.

Z(OMe)–Gly–Pro–His–$NHNH_2$ [4], Z(OMe)–(galanin 12—14)–$NHNH_2$——A mixture of Z(OMe)–Gly–Pro–OH [prepared from 10.40 g (20.00 mmol) of the DCHA salt], HOSu (2.53 g, 22.00 mmol) and DCC (4.54 g, 22.00 mmol) in THF (100 ml) was stirred for 4 h and filtered. The filtrate was combined with a solution of H–His–OMe [prepared from 6.17 g (30.00 mmol) of the HCl salt] in DMF (100 ml) together with Et_3N (2.80 ml, 20.00 mmol) and the mixture, after being stirred overnight, was concentrated. The residue was extracted with *n*-BuOH. The organic phase was washed with H_2O and concentrated. The residue dissolved in MeOH (50 ml) was treated with 80% hydrazine hydrate (6.30 ml, 5 eq) overnight and the product was purified by precipitation from MeOH with ether; yield 6.85 g (70%), mp 99—102 °C, $[\alpha]_D^{18}$ −58.3° (c=1.0, MeOH), Rf_1 0.18. Amino acid ratios in 6 N HCl hydrolysate: Gly 1.00, Pro 1.18, His 0.93 (recovery of Gly 82%). *Anal.* Calcd for $C_{22}H_{29}N_7O_6 \cdot 1/2H_2O$: C, 53.21; H, 6.09; N, 19.74. Found: C, 53.55; H, 6.29; N, 19.32.

Z(OMe)–Leu–Leu–OMe——This dipeptide ester was prepared by the mixed anhydride procedure and purified by procedure A, followed by recrystallization from AcOEt and isopropyl ether; yield 5.71 g (62%), mp 62—63 °C, $[\alpha]_D^{17}$ −38.6° (c=1.0, MeOH), Rf_1 0.94, Rf_2 0.75. *Anal.* Calcd for $C_{22}H_{34}N_2O_6$: C, 62.54; H, 8.11; N, 6.63. Found: C, 62.47; H, 8.14; N, 6.68.

Z(OMe)–Tyr–Leu–Leu–OMe——The azide [prepared from 5.35 g (14.90 mmol) of Z(OMe)–Tyr–$NHNH_2$] in DMF (50 ml) was allowed to react with H–Leu–Leu–OMe [obtained from 5.70 g (13.50 mmol) of the Z(OMe)-derivative] in DMF (20 ml) in the presence of Et_3N (2.07 ml, 14.90 mmol) overnight and the product was purified by procedure A, followed by recrystallization from AcOEt and isopropyl ether; yield 5.70 g (72%), mp 70—73 °C, $[\alpha]_D^{18}$ −27.1° (c=1.0, MeOH), Rf_1 0.92, Rf_2 0.42. *Anal.* Calcd for $C_{31}H_{43}N_3O_8$: C, 63.57; H, 7.40; N, 7.18. Found: C,

63.80; H, 7.37; N, 6.98.

Z(OMe)–Tyr–Leu–Leu–$NHNH_2$ [5], Z(OMe)–(galanin 9—11)–$NHNH_2$——The above tripeptide ester (5.50 g, 9.39 mmol) dissolved in MeOH–DMF (1 : 1, 50 ml) was treated with 80% hydrazine hydrate (2.82 ml, 5 eq) overnight and the product was purified by precipitation from DMF with EtOH; yield 2.86 g (52%), mp 220—222 °C, $[\alpha]_D^{20}$ +5.0° (c=1.0, DMF), Rf_1 0.69, Rf_2 0.18. Amino acid ratios in 6 N HCl hydrolysate: Tyr 0.87, Leu 2.00 (recovery of Leu 95%). *Anal.* Calcd for $C_{30}H_{43}N_5O_7$: C, 61.52; H, 7.40; N, 11.96. Found: C, 61.62; H, 7.49; N, 11.95.

Z(OMe)–Ala–Gly–OMe——This dipeptide ester was prepared by the DCC condensation and purified by procedure, B, followed by recrystallization from AcOEt and ether; yield 18.50 g (72%), mp 104—105 °C, $[\alpha]_D^{22}$ +5.0° (c=1.0, DMF), Rf_1 0.92, Rf_2 0.37. *Anal.* Calcd for $C_{15}H_{20}N_2O_6$: C, 55.55; H, 6.22; N, 8.64. Found: C, 55.95; H, 6.38; N, 8.72.

Z(OMe)–Ser(Bzl)–Ala–Gly–OMe——A mixed anhydride [prepared from 9.61 g (21.53 mmol) of Z(OMe)–Ser(Bzl)–OH·CHA] in THF (50 ml) was allowed to react with H–Ala–Gly–OMe [prepared from 5.82 g (17.94 mmol) of the Z(OMe)-derivative] in DMF (30 ml) and the product was purified by procedure A, followed by recrystallization from AcOEt and ether; yield 6.98 g (78%), mp 110—113 °C, $[\alpha]_D^{18}$ −8.3° (c=1.0, MeOH), Rf_1 0.81. *Anal.* Calcd for $C_{25}H_{31}N_3O_8$: C, 59.87; H, 6.23; N, 8.38. Found: C, 60.14; H, 6.50; N, 8.39.

Z(OMe)–Asn–Ser(Bzl)–Ala–Gly–OMe——A mixture of H–Ser(Bzl)–Ala–Gly–OMe [obtained from 5.27 g (10.50 mmol) of the Z(OMe)-derivative], Z(OMe)–Asn–ONp (4.82 g, 11.00 mmol) and Et_3N (1.53 ml, 11.00 mmol) in DMF (50 ml) was stirred overnight and concentrated. The product was purified by procedure B, followed by precipitation from DMF with AcOEt; yield 6.29 g (97%), mp 210—213 °C, $[\alpha]_D^{16}$ +6.0° (c=1.0, DMF), Rf_1 0.67. *Anal.* Calcd for $C_{29}H_{37}N_5O_{10}$: C, 56.57; H, 6.06; N, 11.38. Found: C, 56.65; H, 6.00; N, 11.27.

Z(OMe)–Leu–Asn–Ser(Bzl)–Ala–Gly–OMe——A mixture of H–Asn–Ser(Bzl)–Ala–Gly–OMe [obtained from 6.27 g (10.19 mmol) of the Z(OMe)-derivative], Et_3N (1.42 ml, 10.19 mmol) and Z(OMe)–Leu–ONp (6.37 g, 15.29 mmol) in DMF (60 ml) was stirred overnight and concentrated. The product was purified by procedure B, followed by precipitation from DMF with MeOH; yield 5.63 g (76%), mp 231—233 °C, $[\alpha]_D^{15}$ −3.0° (c=1.0, DMF), Rf_1 0.74. *Anal.* Calcd for $C_{35}H_{48}N_6O_{11}$: C, 57.68; H, 6.64; N, 11.53. Found: C, 57.75; H, 6.72; N, 11.48.

Z(OMe)–Leu–Asn–Ser(Bzl)–Ala–Gly–$NHNH_2$ [6], Z(OMe)–(galanin 4—8)–$NHNH_2$——The above protected pentapeptide ester (1.50 g, 2.05 mmol) in DMF (20 ml) was treated with 80% hydrazine hydrate (1.29 ml, 10 eq) for 24 h and the product was purified by precipitation from DMF with MeOH; yield 1.21 g (81%), mp 119—122 °C, $[\alpha]_D^{16}$ −2.9° (c=1.0, DMF), Rf_1 0.62. Amino acid ratios in 6 N HCl hydrolysate: Leu 1.01, Asp 1.01, Ser 0.94. Ala 0.99, Gly 1.00 (recovery of Gly 92%). *Anal.* Calcd for $C_{34}H_{48}N_8O_{10}\cdot 1/2H_2O$: C, 55.35; H, 6.69; N, 15.18. Found: C, 55.36; H, 6.50; N, 15.30.

Boc–Trp(Mts)–Thr–OMe——Boc–Trp(Mts)–OH [prepared from 6.68 g (10.00 mmol) of the DCHA salt] and H–Thr–OMe [prepared from 2.04 g (12.00 mmol) of the HCl salt] in THF–DMF (1 : 1, 40 ml) were condensed with DCC (2.27 g, 11.00 mmol) as usual and the product was purified by procedure B, followed by precipitation from AcOEt with *n*-hexane; yield 4.90 g (81%), mp 88—90 °C, $[\alpha]_D^{15}$ −3.7° (c=0.3, MeOH), Rf_2 0.33. *Anal.* Calcd for $C_{30}H_{39}N_3O_8S$: C, 59.88; H, 6.53; N, 6.98. Found: C, 60.35; H, 6.86; N, 6.70.

Z(OMe)–Gly–Trp(Mts)–Thr–OMe——A mixture of H–Trp(Mts)–Thr–OMe [prepared from 4.75 g (7.89 mmol) of the Boc-derivative], Et_3N (1.10 ml, 7.89 mmol) and Z(OMe)–Gly–ONp (2.90 g, 9.47 mmol) in DMF (20 ml) was stirred for 24 h and concentrated. The product was purified by procedure A, followed by recrystallization from AcOEt and ether; yield 2.45 g (43%), mp 92—94 °C, $[\alpha]_D^{18}$ −10.3° (c=1.0, MeOH), Rf_2 0.37. *Anal.* Calcd for $C_{36}H_{42}N_4O_{10}S$: C, 59.82; H, 5.86; N, 7.75. Found: C, 59.79; H, 5.90; N, 7.61.

Z(OMe)–Gly–Trp(Mts)–Thr–$NHNH_2$ [7], Z(OMe)–(galanin 1—3)–$NHNH_2$——The above protected tripeptide ester (2.43 g, 3.36 mmol) in MeOH (20 ml) was treated with 80% hydrazine hydrate (0.84 ml, 5 eq) overnight and the product was purified by precipitation from DMF with MeOH; yield 2.26 g (93%), mp 186—188 °C, $[\alpha]_D^{29}$ −7.2° (c=1.0, DMF), Rf_1 0.61. Amino acid ratios in 4 N MSA hydrolysate: Gly 1.00, Trp 0.84, Thr 0.91 (recovery of Gly 95%). *Anal.* Calcd for $C_{35}H_{42}N_6O_9S$: C, 58.16; H, 5.86; N, 11.63. Found: C, 58.39; H, 6.01; N, 11.49.

Z(OMe)–Ser(Bzl)–Phe–His–Asp(OChp)–Lys(Z)–Tyr–Gly–Leu–Ala–NH_2, Z(OMe)–(galanin 21—29)–NH_2——A TFA-treated sample of Boc–(galanin 24—29)–NH_2 [1] (700 mg, 0.70 mmol) was dissolved in DMF (3 ml) containing Et_3N (0.10 ml, 0.70 mmol). The azide [prepared from 600 mg (0.91 mmol) of [2]] in DMF (3 ml) and Et_3N (0.14 ml, 1.00 mmol) were added to the above ice-chilled solution and the mixture was stirred for 12 h, then the additional azide (0.25 eq) in DMF (1.5 ml) and Et_3N (0.25 eq) were added. The mixture, after being stirred for an additional 12 h, was concentrated and the product was purified by procedure C, followed by precipitation from MeOH with AcOEt; yield 810 mg (76%), mp 180—183 °C, $[\alpha]_D^{15}$ −25.0° (c=0.8, DMF), Rf_1 0.67. *Anal.* Calcd for $C_{79}H_{101}N_{13}O_{18}\cdot 4H_2O$: C, 59.57; H, 6.90; N, 11.43. Found: C, 59.76; H, 6.39; N, 11.23.

Z(OMe)–Ala–Ile–Asp(OChp)–Asn–His–Arg(Mts)–Ser(Bzl)–Phe–His–Asp(OChp)–Lys(Z)–Tyr–Gly–Leu–Ala–NH_2, Z(OMe)–(galanin 15—29)–NH_2——The azide [prepared from 780 mg (0.66 mmol) of [3]] in DMF (5 ml) and Et_3N (0.10 ml, 0.73 mmol) were added to an ice-chilled solution of H–(galanin 21—29)–NH_2 [obtained from 770 mg (0.51 mmol) of the Z(OMe)-derivative] in DMF (3 ml) and the mixture was stirred for 12 h, then the additional azide (0.2 eq) in DMF (1.5 ml) and Et_3N (0.2 eq) were added. The mixture, after being stirred for an additional 12 h, was concentrated and the residue was treated with H_2O to afford a powder, which was precipitated from DMF with

AcOEt. For further purification, the product was dissolved in DMF (1 ml) and the solution was applied to a column of Sephadex LH-60 (3.1 × 132 cm), which was eluted with the same solvent. The fractions (8 ml each) corresponding to the front peak (tube Nos. 64—79, detected by measuring the ultraviolet (UV) absorption at 280 nm) were combined, the solvent was removed by evaporation and the residue was treated with AcOEt to afford a fine powder; yield 645 mg (51%), mp 220—223 °C, $[\alpha]_D^{15}$ +6.0° (c=0.5, DMF), Rf_1 0.48. *Anal.* Calcd for $C_{124}H_{169}N_{25}O_{29}S \cdot 2H_2O$: C, 58.59; H, 6.86; N, 13.78. Found: C, 58.54; H, 6.87; N, 13.74.

Z(OMe)–Gly–Pro–His–Ala–Ile–Asp(OChp)–Asn–His–Arg(Mts)–Ser(Bzl)–Phe–His–Asp(OChp)–Lys(Z)–Tyr–Gly–Leu–Ala–NH_2, Z(OMe)–(galanin 12—29)–NH_2——The azide [prepared from 156 mg (0.32 mmol) of [4]] in DMF (3 ml) and Et_3N (49 μl, 0.35 mmol) were added to an ice-chilled solution of H–(galanin 15—29)–NH_2 [obtained from 500 mg (0.20 mmol) of the Z(OMe)-derivative] in DMF (2 ml) and the mixture, after being stirred for 12 h, was concentrated. The residue was treated with H_2O to afford a powder, which was precipitated from DMF with AcOEt; yield 506 mg (91%), mp 226—228 °C, $[\alpha]_D^{18}$ −16.1° (c=0.5, DMF), Rf_1 0.48. *Anal.* Calcd for $C_{137}H_{186}N_{30}O_{32}S \cdot 3H_2O$: C, 57.71; H, 6.79; N, 14.74. Found: C, 57.71; H, 6.64; N, 14.93.

Z(OMe)–Tyr–Leu–Leu–Gly–Pro–His–Ala–Ile–Asp(OChp)–Asn–His–Arg(Mts)–Ser(Bzl)–Phe–His–Asp(OChp)–Lys(Z)–Tyr–Gly–Leu–Ala–NH_2, Z(OMe)–(galanin 9—29)–NH_2——The azide [prepared from 205 mg (0.35 mmol) of [5]] in DMF (2 ml) and Et_3N (54 μl, 0.39 mmol) were added to an ice-chilled solution of H–(galanin 12—29)–NH_2 [obtained from 490 mg (0.18 mmol) of the Z(OMe)-derivative] in DMF (3 ml) and the mixture, after being stirred for 12 h, was concentrated. The product was purified as described above; yield 533 mg (96%), mp 228—231 °C, $[\alpha]_D^{18}$ +5.1° (c=0.4, DMF), Rf_1 0.48. *Anal.* Calcd for $C_{158}H_{217}N_{33}O_{36}S \cdot 2H_2O$: C, 58.88; H, 6.91; N, 14.34. Found: C, 58.56; H, 7.02; N, 14.24.

Z(OMe)–Leu–Asn–Ser(Bzl)–Ala–Gly–Tyr–Leu–Leu–Gly–Pro–His–Ala–Ile–Asp(OChp)–Asn–His–Arg(Mts)–Ser(Bzl)–Phe–His–Asp(OChp)–Lys(Z)–Tyr–Gly–Leu–Ala–NH_2, Z(OMe)–(galanin 4—29)–NH_2——The azide [prepared from 298 mg, (0.41 mmol) of [6]] in DMF (3 ml) and Et_3N (63 μl, 0.45 mmol) were added to an ice-chilled solution of H–(galanin 9—29)–NH_2 [obtained from 521 mg (0.16 mmol) of the Z(OMe)-derivative] in DMF (3 ml) and the mixture, after being stirred for 48 h, was poured into H_2O (100 ml) to form a powder, which was purified by gel-filtration on Sephadex LH-60 (3.1 × 132 cm) using DMF as an eluant as described above; yield 385 mg (63%), mp 224—226 °C, $[\alpha]_D^{18}$ +3.3° (c=0.6, DMF), Rf_1 0.57. *Anal.* Calcd for $C_{183}H_{253}N_{39}O_{43}S \cdot 5H_2O$, 57.70; H, 6.96; N, 14.34. Found: C, 57.74; H, 6.90; N, 14.28.

Z(OMe)–Gly–Trp(Mts)–Thr–Leu–Asn–Ser(Bzl)–Ala–Gly–Tyr–Leu–Leu–Gly–Pro–His–Ala–Ile–Asp(OChp)–Asn–His–Arg(Mts)–Ser(Bzl)–Phe–His–Asp(OChp)–Lys(Z)–Tyr–Gly–Leu–Ala–NH_2, Z(OMe)–(galanin 1—29)–NH_2——The azide [prepared from 170 mg (0.24 mmol) of [7]] in DMF (3 ml) and Et_3N (36 μl, 0.26 mmol) were added to an ice-chilled solution of H–(galanin 4—29)–NH_2 [obtained from 350 mg (94 μmol) of the Z(OMe)-derivative] in DMF (2 ml) and the mixture, after being stirred for 48 h, was poured into H_2O (100 ml). The resulting powder was purified by gel-filtration on Sephadex LH-60 (3.1 × 132 cm) as described above; yield 335 mg (84%), mp 223—225 °C, $[\alpha]_D^{25}$ −11.3° (c=1.0, DMF), Rf_1 0.64. *Anal.* Calcd for $C_{209}H_{283}N_{43}O_{49}S_2 \cdot 3H_2O$: C, 58.38; H, 6.77; N, 14.01. Found: C, 58.10; H, 6.95; N, 13.90.

H–Gly–Trp–Thr–Leu–Asn–Ser–Ala–Gly–Tyr–Leu–Leu–Gly–Pro–His–Ala–Ile–Asp–Asn–His–Arg–Ser–Phe–His–Asp–Lys–Tyr–Gly–Leu–Ala–NH_2 (Galanin)——The above protected nonacosapeptide (100 mg, 24 μmol) was treated with 1 M TFMSA–thioanisole in TFA (6 ml) in the presence of *m*-cresol (197 μl, 80 eq) and EDT (49 μl, 20 eq) in an ice-bath for 120 min, then dry ether was added. The resulting powder was collected by centrifugation and dissolved in ice-chilled H_2O (10 ml). The pH of the solution was adjusted to 8.0 with 10% NH_4OH and after 30 min to 4.0 with 10% AcOH, then the solution was lyophilized. The residue was dissolved in 0.5 N AcOH (1 ml) and the solution was applied to a column of Sephadex G-25 (3.3 × 126 cm), which was eluted with the same solvent. The fractions (7 ml each) corresponding to the front main peak (tube Nos. 69—91, determined by measuring the UV absorption at 280 nm) were combined and the solution was lyophilized to give a fluffy powder; yield 66 mg (86%).

The product obtained here was found to be fairly pure by HPLC examination (Fig. 7, ii-a). Subsequent purification was carried out by reversed-phase HPLC. A part of the sample (3 mg each) was applied to a column of Nucleosil $5C_{18}$ (10 × 250 mm), which was eluted with 35% CH_3CN in 0.2% TFA under isocratic conditions at a flow rate of 1.4 ml/min. The eluate corresponding to the main peak (retention time 19.5 min) was collected. The rest of the sample was similarly purified and the solvent of the combined eluates was removed by lyophilization. The resulting powder was dissolved in 0.5 N AcOH (0.5 ml) and subjected to gel-filtration on Sephadex G-25 (1.8 × 70 cm) using 0.5 N AcOH as an eluant. The desired fractions were collected and the solvent was removed by lyophilization to give a white fluffy powder; yield 25.7 mg. Total yield from the protected peptide amide was 33%. $[\alpha]_D^{25}$ −61.2° (c=0.1, 0.5 N AcOH), Rf_3 0.29. FAB mass, 3209.6 $(M+H)^+$, a single peak in HPLC on a Nucleosil $5C_{18}$ column (4 × 150 mm), retention time 4.0 min by isocratic elution with CH_3CN–0.2% TFA (35 : 65) at a flow rate of 0.8 ml/min (Fig. 7, ii-b), a single band in disk isoelectrofocusing on 7.5% polyacrylamide gel (0.5 × 7.3 cm) containing Pharmalyte (pH 3.0—10.0): mobility 6.3 cm (stained with Coomassie Brilliant Blue G-250, Sigma) from the origin toward the cathodic end of the gel after running at 200 V for 4 h (Fig. 7, i). Amino acid ratios in a 6 N HCl hydrolysate are shown in Table I. Amino acid ratios in LAP digest: 2Asp 1.84, 1Thr 0.91, 2Ser 2.01, 1Pro 0.78, 4Gly 3.59, 3Ala 3.02, 1Ile 1.11, 4Leu 3.96, 2Tyr 1.78, 1Phe 1.00, 1Trp 0.73, 1Lys 1.00, 3His 2.70, 1Arg 1.02, 2Asn N.D. (recovery of Lys 90%).

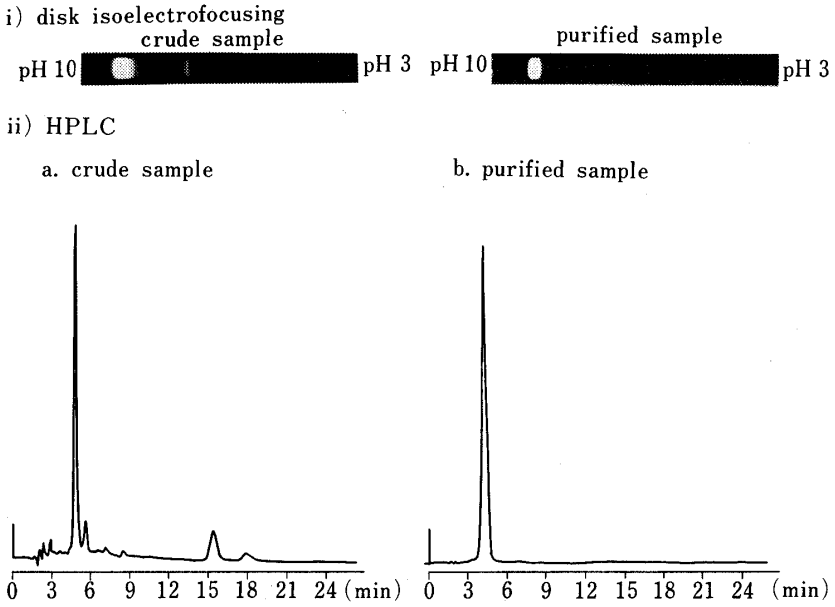

Fig. 7. Disk Isoelectrofocusing and HPLC Pattern of Synthetic Galanin

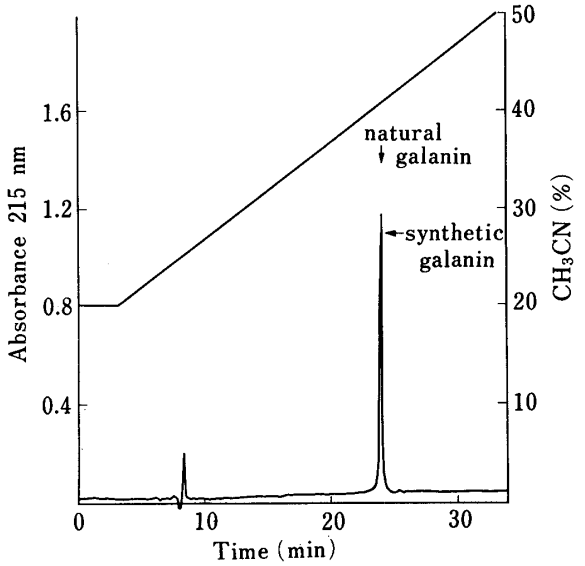

Fig. 8. HPLC Elution Pattern of a Mixture of Synthetic and Natural Galanin

Sample: Mixture of Natural and synthetic galanin, 10 μg each. Column: TSK-ODS-120T 5 μm, 4.6 × 250 mm. Solvent system: A, 0.1% TFA–H_2O; B, 0.1% TFA–CH_3CN; linear gradient 20%—50% in 30 min. Flow rate: 1.0 ml/min. Absorbance: 215 nm, scale OD. 2.0.

The HPLC elution pattern of synthetic and natural galanin was examined. A mixture of the two compounds emerged from the column as a single peak (Fig. 8).

Bioassay——Smooth Muscle Contractile Assay: The effect of synthetic galanin on isolated intestinal tissues was compared on a molar basis. ED_{50} values of synthetic galanin, substance P and acetylcholine for contracting rat ileum were 6×10^{-8} M, 3.2×10^{-7} M and 1×10^{-7} M respectively. Therefore, the relative potencies of synthetic galanin and acetylcholine with respect to substance P were approximately 5 and 3, respectively. Synthetic galanin had no effect on guinea pig ileum at 3×10^{-5} M, while substance P contracted this smooth muscle even at 1×10^{-10} M.

Hyperglycemic Activity Assay: After overnight fasting a mongrel dog was used under pentobarbital anesthesia (30 mg/kg of body weight). Synthetic galanin (2 μg/kg) was given intravenously as a bolus. Peripheral blood samples were collected serially before and after the injection. Blood glucose levels were measured by the reflomat method (*ortho*-toluidine method). The result is shown in Fig. 6.

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2) Amino acids, peptides and their derivatives are of the L-configuration. The following abbreviations are used: Z = benzyloxycarbonyl, Z(OMe) = *p*-methoxybenzyloxycarbonyl, Boc = *tert*-butoxycarbonyl, Bzl = benzyl, Mts = mesitylene-2-sulfonyl, Chp = cycloheptyl, Np = *p*-nitrophenyl, Su = *N*-hydroxysuccinimidyl, DCC = dicyclohexylcarbodiimide, HOBt = *N*-hydroxybenzotriazole, TFA = trifluoroacetic acid, MSA = methanesulfonic acid, TFMSA = trifluoromethanesulfonic acid, EDT = ethanedithiol, DMF = dimethylformamide, THF = tetrahydrofuran, DCHA = dicyclohexylamine, NMM = *N*-methylmorpholine, EDTA = ethylenediaminetetraacetic acid.
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