Synthesis of Phenolic Group Containing Analogues of Porcine Secretin and Their Immunological Properties

Noboru Yanaihara,* Mari Kubota, Masanori Sakagami, Haruko Sato, Tohru Mochizuki, Naoki Sakura, Tadashi Hashimoto, Chizuko Yanaihara,

Laboratory of Bioorganic Chemistry, Shizuoka College of Pharmacy, Shizuoka, Japan

Ken Yamaguchi, Fujio Zeze, and Kaoru Abe

Endocrine Division, National Cancer Center Research Institute, Tokyo, Japan. *Received August 17, 1976*

Syntheses by the conventional method are described of N^{α}-tyrosylsecretin, [Tyr^1]secretin, and N^{α}-β-(4-hydroxyphenyl)propionylsecretin. Secretin and [Tyr^6]secretin were also prepared by the synthetic route identical with those employed for construction of the above analogues. Purification of secretin and the analogues was conducted by ion-exchange column chromatography on CM-Sephadex and gel filtration. Immunological reactivities of these analogues were examined in the radioimmunoassay system for secretin using two different antisera raised against synthetic secretin in rabbits. The tracers used in this study were [^{125}I]-[Tyr^1]secretin and [^{125}I]-[Tyr^6]secretin. The dose–response curves of N^{α}-tyrosylsecretin, [Tyr^1]secretin, and N^{α}-β-(4-hydroxyphenyl)propionylsecretion were essentially superimposable upon those of natural and synthetic preparations of porcine secretin in the systems used, while [Tyr^6]secretin showed discrepancy in the curve. In addition, biological activities of the synthetic polypeptides were compared with that of natural porcine secretin in terms of exocrine pancreatic secretory response in anesthetized dogs.

The difficulty in preparing a satisfactorily labeled antigen hampered the development of a radioimmunoassay for secretin that lacks the Tyr residue in the molecules.[1a] Artificial introduction of a Tyr residue to the molecule may provide a way to overcome this problem, and [Tyr^6]secretin has been prepared[1b] by the solid-phase synthesis for this purpose. Chemical modification of the secretin molecule, however, may or may not have effect on its immunological property that causes another problem in the radioimmunoassay. Recently, Straus et al.[2] described the disadvantage of the use of [^{125}I]-[Tyr^6]secretin as a tracer in the immunoassay for secretin, because of its diminished immunological reactivity.

In order to find more suitable tracers for secretin radioimmunoassay, we have synthesized novel, phenolic group containing secretin analogues. The present communication describes the syntheses by the conventional method and immunological properties of porcine secretin, N^{α}-tyrosylsecretin, [Tyr^1]secretin, N^{α}-β-(4-hydroxyphenyl)propionylsecretin, and [Tyr^6]secretin. The effect of the synthetic peptides on pancreatic juice secretion in dogs is also described. Studies on the synthesis of porcine secretin by different approaches have been reported.[3-7]

Synthesis. Constructions of porcine secretin and its analogues, N^{α}-tyrosylsecretin, [Tyr^1]secretin, and [Tyr^6]secretin, were achieved by acylation of secretin$_{7-27}$ (**40**) with azides of protected peptide hydrazides followed by hydrogenolysis. The protected peptide hydrazides used for acylation are Z-His-Ser-Asp-Gly-Thr-Phe-NHNH-Boc (**23**), Z-Tyr-His-Ser-Asp-Gly-Thr-Phe-NHNH-Boc (**25**), di-Z-Tyr-Ser-Asp-Gly-Thr-Phe-NHNH-Boc (**28**), and Z-His-Ser-Asp-Gly-Thr-Tyr-NHNH$_2$ (**34**). Synthesis of N^{α}-β-(4-hydroxyphenyl)propionylsecretin was accomplished by the coupling of **40** with N^{α}-β-(4-hydroxyphenyl)propionyl-His-Ser-Asp-Gly-Thr-Phe-NHNH-Boc (**27**). The stepwise procedure[8] using the benzyloxycarbonyl (Z) group for α-amino protection was selected for the preparation of these protected peptide hydrazides with a minimum of side-chain protection.[9] Z-Thr-Phe-NHNH-Boc or Z-Thr-Tyr-OMe were used as starting materials. Hydrogenolysis was employed exclusively for removal of amino-protecting groups. The Ser and His residues were introduced via Z-Ser-N$_3$ or Z-His-N$_3$ and other amino acid residues were coupled by the active ester method using the Z-amino acid *N*-hydroxysuccinimido esters[10] or the mixed anhydride procedure.[11] Introduction of the β-(4-hydroxyphenyl)propionyl function was performed by the active ester method. The benzyl (Bz) group was used to protect the β-carboxyl group of the Asp residue.

Secretin$_{7-27}$ (**40**), which was used as an amino component at the final coupling stage in preparation of secretin and the analogues, was constructed exclusively by the Honzl–Rudinger azide procedure[12] starting from the C-terminal decapeptide amide, H-Arg(H$^+$)-Leu-Gln-Arg-(H$^+$)-Leu-Leu-Gln-Gly-Leu-Val-NH$_2$ (positions 18–27) (**35**). The decapeptide amide **35** had been prepared by coupling of Z-Arg(NO$_2$)-Leu-Gln-N$_3$ with H-Arg(NO$_2$)-Leu-Leu-Gln-Gly-Leu-Val-NH$_2$ (**36**) followed by hydrogenolysis. In the manner as shown in Scheme I, the peptide chain was elongated by the fragment condensation method using, as acylating agents, azides derived from Z-Arg(NO$_2$)-Asp-Ser-Ala-NHNH-Boc (positions 14–17) (**11**), Z-Ser-Arg-Leu-NHNH$_2$ (positions 11–13) (**14**), and Z-Thr-Ser-Glu-Leu-NHNH$_2$ (positions 7–10) (**17**), consecutively. These protected peptide hydrazides were constructed mainly by the stepwise procedure. The Bz ester protected the β- or α-carboxyl of Asp and Glu and the NO$_2$ group was used to protect the guanidino group of Arg. Hydrogenolysis resulted in simultaneous removal of the Z and Bz or NO$_2$ functions. Synthesis of porcine secretin by azide fragment condensation using different fragments had been adopted by Ondetti et al.[4]

The fragment condensation reactions were monitored by TLC of the reaction mixture using chlorine–tolidine reagent as well as by the ninhydrin color reaction of the reaction mixture on filter paper. In order to minimize the amino component unreacted in the fragment condensation, the acylating component was used in excess. Countercurrent distribution was used to purify some of the protected intermediates. Purification of secretin$_{11-27}$ (**39**) and secretin$_{7-27}$ (**40**) was conducted by gel filtration on Bio-Gel P-6 with aqueous AcOH as eluent.

After acylation of **40** with an azide of the respective protected peptide fragment, the reaction mixture was distributed between 1-BuOH and 2% AcOH. The crude protected heptacosa- or octacosapeptide amides, obtained by evaporation of the organic layers, were hydrogenated and the final product was purified by gel filtration on Bio-Gel P-10 or Sephadex G-25 using 3 M AcOH as eluent and then ion-exchange chromatography on CM-Sephadex with NH$_4$HCO$_3$ buffer. Finally, the products were desalted

Scheme I

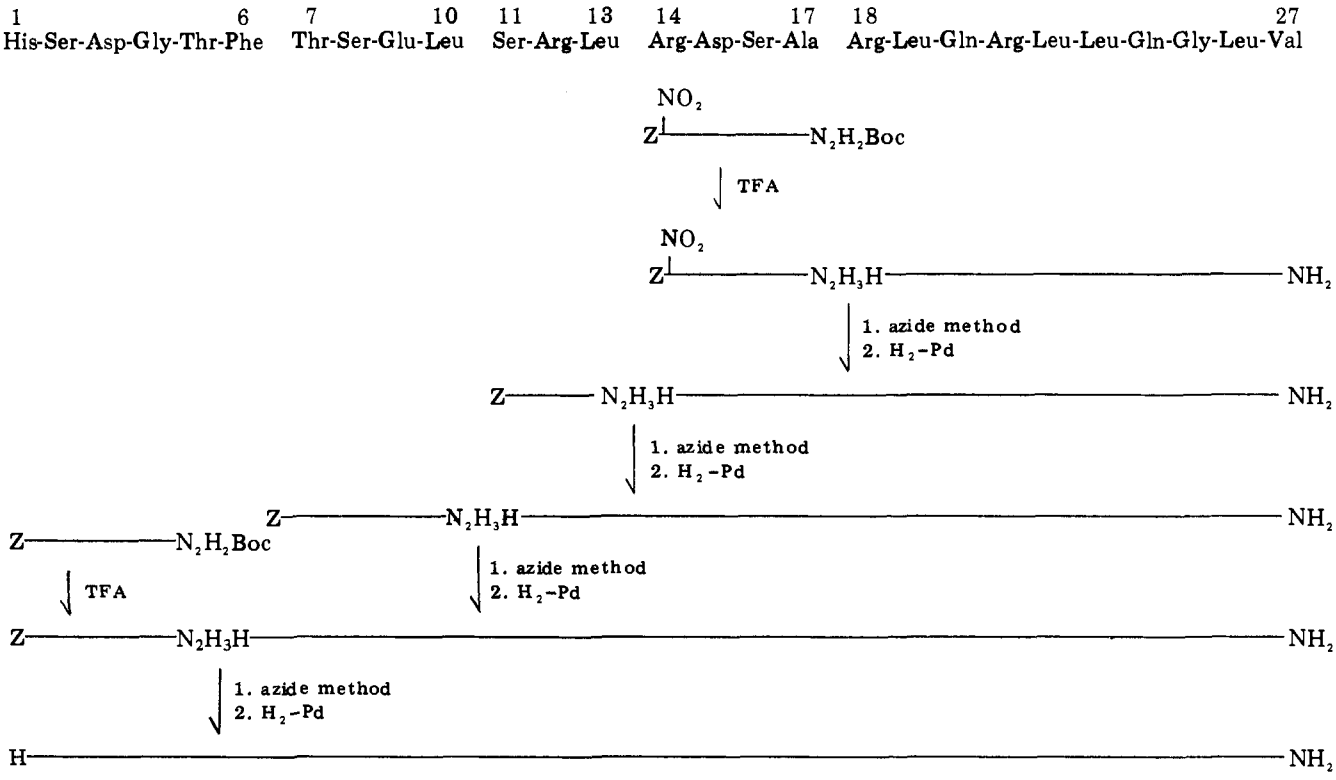

Table I. Radioimmunoassay Systems for Secretin

System no.	Rabbit antisynthetic secretin antiserum (final dilution)	Tracer
System 1	NCC-R-1 (1:64 000)	[^{125}I]-[Tyr^1]secretin
System 2	NCC-R-1 (1:20 000)	[^{125}I]-[Tyr^6]secretin
System 3	R-802 (1:54 000)	[^{125}I]-[Tyr^1]secretin
System 4	R-802 (1:54 000)	[^{125}I]-[Tyr^6]secretin

by gel filtration on Bio-Gel P-6. Each of the products behaved as a homogeneous component on TLC when detected by Sakaguchi, Pauly, chlorine–tolidine, and ninhydrin reagents. Their acid hydrolysates and AP-M digests contained the constituent amino acids in theoretical ratios, respectively.

Immunological Reactivity. Immunological reactivities of the synthetic secretin analogues and fragments prepared in the present study were determined by the double-antibody radioimmunoassay,[14] in a manner similar to that described previously,[15] using antisera against synthetic porcine secretin, NCC-R-1 and R-802, raised in rabbits and [^{125}I]-[Tyr^1]secretin and [^{125}I]-[Tyr^6]secretin as tracers. Four radioimmunoassay systems employed are shown in Table I.

Effect on Pancreatic Juice Secretion in Dogs. The amounts of pancreatic juice secreted in anesthetized dogs[13] after intravenous injection of the synthetic peptides were compared with that observed by administration of pure natural porcine secretin (Karolinska Institute, Stockholm). The responses to peptides were examined in four-point assays.

Results and Discussion

As a part of our continuing studies on gastrointestinal hormones by the chemical synthetic approach, we synthesized porcine secretin analogues modified at the N-terminal portion of the molecule which may facilitate preparation of satisfactory tracers for secretin immunoassay. The synthetic approach employed in this study proved to be effective for syntheses of homogeneous preparations of secretin and its analogues.

Table II. Relative Biological Potency of Synthetic Secretin and Its Analogues[a]

Compound	Rel potency, % (n = 5)
Natural porcine secretin (Karolinska)	Accepted as 100%
Synthetic porcine secretin	107.5 ± 1.5
N^{α}-Tyrosylsecretin	25.6 ± 3.1
[Tyr^1]secretin	4.3 ± 3.1
N^{α}-β-(4-Hydroxyphenyl)-propionylsecretin	4.1 ± 0.2
[Tyr^6]secretin	1.3 ± 0.8

[a] Biological activities of the compounds were examined with respect to the effect on pancreatic juice secretion in dogs.

Synthetic secretin exhibited a potency of approximately 4300 cu/mg which is identical with that of pure natural secretin. Relative potencies of synthetic secretin and the analogues are summarized in Table II. N^{α}-Tyrosylsecretin was found to possess significant activity, approximately 1000 cu/mg, while N^{α}-β-(4-hydroxyphenyl)propionylsecretin showed a markedly lower potency, indicating that removal of the α-amino function from N^{α}-tyrosylsecretin results in a considerable decrease in the activity on pancreatic juice secretin. [Tyr^1]secretin showed a potency of 150–200 cu/mg. The low but definite activity of [Tyr^1]secretin suggests that the aromatic property of the His residue in the N terminus may in part contribute to the physiological activity of secretin. Bodanszky et al.[16] described very little activity of secretin$_{2-27}$, which lacks the His residue. [Tyr^6]secretin was found to possess extremely low activity, which was about 50 cu/mg. The low activity of this analogue on pancreatic juice secretion has been reported.[17]

Figures 1a,b and 2a,b show the displacement curves of synthetic secretin-related peptides in immunoassay systems 1, 2, 3, and 4, respectively. In any of the systems

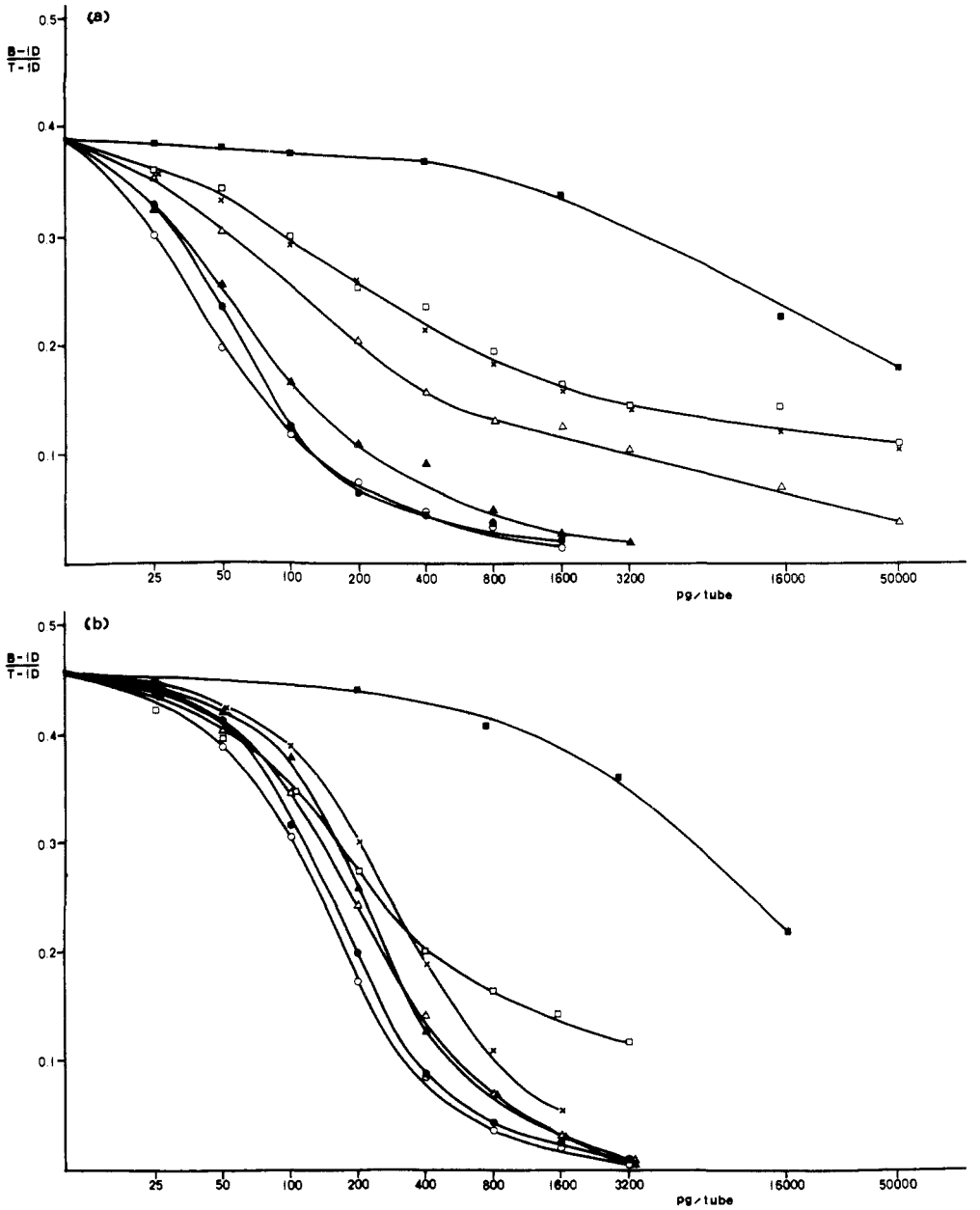

Figure 1. Double-antibody radioimmunoassays for synthetic peptides related to porcine secretin (a) using rabbit antisynthetic secretin antiserum NCC-R-1 (1:64 000 final dilution) and [^{125}I]-[Tyr^1]secretin (system 1) and (b) using antiserum NCC-R-1 (1:20 000 final dilution) and [^{125}I]-[Tyr^6]secretin (system 2): (○) synthetic secretin; (●) N^{α}-tyrosylsecretin, [Tyr^1]secretin, and N^{α}-β-(4-hydroxyphenyl)-propionylsecretin; (△) [Tyr^6]secretin; (▲) secretin$_{4-27}$ and secretin$_{5-27}$; (×) secretin$_{7-27}$; (□) secretin$_{11-27}$ and secretin$_{14-27}$; and (■) secretin$_{18-27}$.

used, natural and synthetic secretin preparations, N^{α}-tyrosylsecretin, [Tyr^1]secretin, and N^{α}-β-(4-hydroxyphenyl)propionylsecretin competed with the tracers for sites in the antisecretin antisera used. The cross reactivities of these peptides were essentially equivalent to each other and parallel displacement curves were obtained with them. On the other hand, in system 1 (Figure 1a), the cross reactivities of [Tyr^6]secretin and secretin$_{7-27}$ (**40**) were considerably less than those of other phenolic group containing analogues. The distinct decrease in immunological reactivities of both [Tyr^6]secretin and **40** seems to indicate that the Phe residue in position 6 contributes, conformationally or/and sequentially, to the interaction between secretin and its particular antiserum (NCC-R-1). Bodanszky et al.[16] described a possibility of unique conformation in this region of the secretin molecule. In addition, in system 2 (Figure 1b), higher concentration of the antiserum (final dilution 1:20 000) was required for antibody binding of the tracer comparable to that obtained in system 1. Diminished immunoreactivity of [Tyr^6]secretin was also observed in systems 3 and 4, although not so markedly as in system 1. Peptide **40** showed significant reactivity in these systems. Shorter fragments such as secretin$_{11-27}$ (**39**) and secretin$_{14-27}$ (**38**) showed cross reactions of various degrees in the systems using both antisera. Secretin$_{18-27}$ (**35**) exhibited a slight but still significant reaction for both antisera. However, the dose–response curve of this peptide was not parallel to that of secretin. These results indicate that the major antigenic determinant(s) for the two antisera presently used is mainly located in the 14–27 sequence of secretin. Boden and Chey[18] reported that the cross reactivity of secretin$_{5-27}$ was equal to that of secretin in the system using [^{125}I]-secretin as tracer, while secretin$_{14-27}$ (**38**) was approximately 50% as reactive as secretin.

In the present investigation, we observe more or less diminished cross reactivity of [Tyr^6]secretin and secretin$_{7-27}$ (**40**) in four different assay systems used. On the

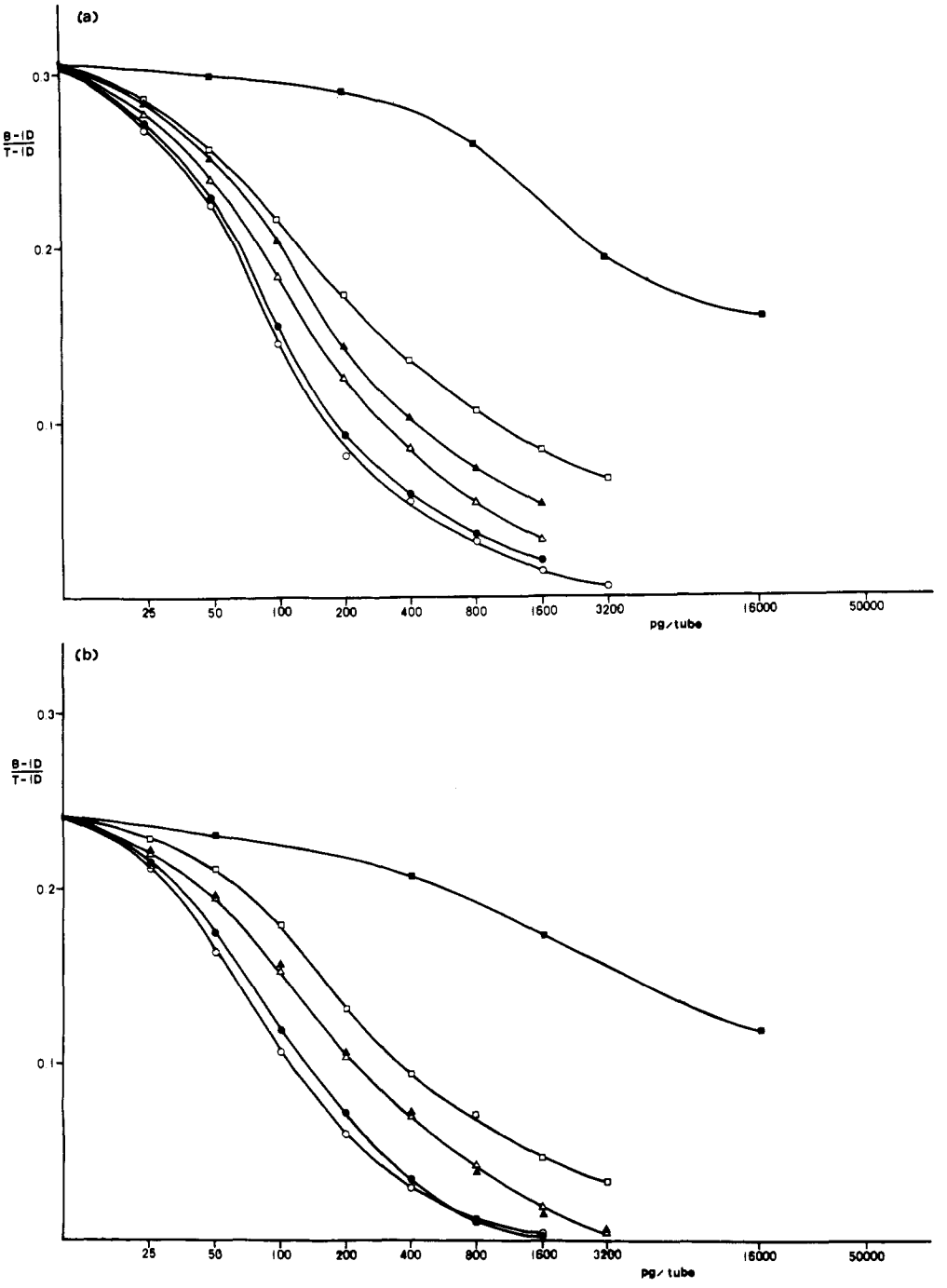

Figure 2. Double-antibody radioimmunoassays for synthetic peptides related to porcine secretin (a) using rabbit antisynthetic secretin antiserum R-802 (1:54 000 final dilution) and [^{125}I]-[Tyr^1]secretin (system 3) and (b) using antiserum R-802 (1:54 000 final dilution) and [^{125}I]-[Tyr^6]secretin (system 4): (○) synthetic secretin, N^{α}-tyrosylsecretin, [Tyr^1]secretin, and N^{α}-β-(4-hydroxyphenyl)propionylsecretin; (●) [Tyr^6]secretin; (△) secretin$_{4-27}$ and secretin$_{5-27}$; (▲) secretin$_{7-27}$; (□) secretin$_{11-27}$ and secretin$_{14-27}$; and (■) secretin$_{18-27}$.

other hand, N^{α}-tyrosylsecretin, [Tyr^1]secretin, or N^{α}-β-(4-hydroxyphenyl)propionylsecretin exhibited identical cross reactivity with that of secretin. The results suggest that N^{α}-tyrosylsecretin, [Tyr^1]secretin, or N^{α}-β-(4-hydroxyphenyl)propionylsecretin as well as secretin itself could be at least more safely used for labeling in the development of radioimmunoassay for secretin of higher sensitivity.

Experimental Section

Melting points were determined on a Mitamura Riken capillary melting point apparatus and are uncorrected. Microanalyses were performed by the Analytical Center of Shizuoka College of Pharmacy. All analytical samples gave combustion values for C, H, and N within 0.4% of the theoretical values. R_f^{I} and R_f^{II} values refer to the solvent systems 1-BuOH-AcOH-H_2O (4:1:5) (upper layer) and 1-BuOH–pyridine–AcOH–H_2O (30:20:6:24), respectively. Optical rotations were measured on a Yanaco automatic polarimeter OR-50. Amino acid analyses were performed with a Hitachi Model KLA-3B amino acid analyzer. Acid hydrolysis of samples for amino acid analysis was conducted with 6 N HCl at 110 °C for 24 h in evacuated sealed tubes. When tyrosine-containing peptides were hydrolyzed, phenol was added to the hydrolysis tubes. Aminopeptidase M (AP-M) digestion of peptides was carried out according to the method described by Hofmann et al.[19] All solvents were of reagent grade and were distilled before use. Evaporations were carried out in vacuo at 40–45 °C in rotary evaporators. CM-Sephadex C-25 (Na form) was washed twice with 10% AcOH and then with H_2O before use. On column chromatography or gel filtration, the eluted fractions were examined by ninhydrin and Sakaguchi reagents on filter paper and

by chlorine–tolidine reagent on TLC.

Z-Leu-Gln-NHNH-Boc (1). Z-Leu-OSu (5.44 g, 15 mmol) was added to a stirred solution of H-Gln-NHNH-Boc (3.90 g, 15 mmol) and Et_3N (2.10 mL, 15 mmol) in THF (30 mL). After 24 h at room temperature, the solvent was removed and the residue dissolved in EtOAc. The solution was washed successively with 1 N citric acid, saturated $NaHCO_3$, and saturated NaCl, dried, and evaporated. The residue was solidified by addition of Et_2O and precipitated from MeOH with Et_2O: yield 5.01 g (66%); mp 152–154 °C; $[\alpha]^{24}D$ −48.0° (*c* 0.48, MeOH); R_f^I 0.82, R_f^{II} 0.82. Anal. ($C_{24}H_{37}N_5O_7$) C, H, N.

Z-Leu-Gln-Gly-Leu-Val-NH_2 (4). A solution of Z-Gly-Leu-Val-NH_2[19] (2.10 g, 5 mmol) in 25% HBr in AcOH (15 mL) was allowed to stand at room temperature for 1 h. Anhydrous Et_2O was added and the resulting H-Gly-Leu-Val-NH_2·HBr (2) was collected by filtration, washed with Et_2O, and dried over KOH: R_f^I 0.56. Compound 1 (3.81 g, 7.5 mmol) was dissolved in chilled TFA (10 mL) and the solution kept at room temperature for 30 min. Z-Leu-Gln-$NHNH_2$·TFA (3) was precipitated by addition of ice-cold Et_2O, washed with Et_2O, and dried over KOH: R_f^I 0.65. The hydrazide 3 was dissolved in DMF (25 mL) and cooled to −15 °C and 6 N HCl in dioxane (3.80 mL, 22.5 mmol) and *i*-AmONO (1.01 mL, 7.5 mmol) were added. The mixture was stirred at −10 °C for 5 min and neutralized with Et_3N, when an ice-cold solution of 2 and Et_3N (0.70 mL, 5.0 mmol) in DMF (20 mL) was added. The mixture was stirred at −10 °C for 2 h and at 4 °C for a further 24 h. The bulk of the solvent was evaporated. Addition of H_2O gave a solid which was collected, washed with MeOH, and dried: yield 2.95 g (89%); mp 270–272 °C (lit.[19] mp 270–272 °C); $[\alpha]^{23}D$ −41.5° (*c* 1.01, AcOH) [lit.[19] $[\alpha]^{20}D$ −35° (*c* 2, AcOH)]; R_f^I 0.82, R_f^{II} 0.88. Anal. ($C_{32}H_{51}N_7O_8$) C, H, N.

Z-Leu-Leu-Gln-Gly-Leu-Val-NH_2 (5). Compound 4 (1.99 g, 3 mmol) was decarbobenzoxylated in the manner as described for 2: R_f^I 0.30. Z-Leu-OSu (1.63 g, 4.5 mmol) was added to a solution of the above pentapeptide amide hydrobromide and Et_3N (0.42 mL, 3 mmol) in DMF (40 mL). The mixture was stirred at room temperature for 20 h. The solvent was evaporated and saturated $NaHCO_3$ added to the residue. The precipitate was collected, washed with H_2O, and dried. The powder was washed with MeOH: yield 1.88 g (81%); mp 263–265 °C (lit.[19] mp 264–267 °C); $[\alpha]^{23}D$ −45.0° (*c* 1.02, AcOH) [lit.[19] $[\alpha]^{28}D$ −47° (*c* 2, AcOH)]; R_f^I 0.75, R_f^{II} 0.93. Anal. ($C_{38}H_{62}N_8O_9$) C, H, N.

Z-Arg(NO_2)-Leu-Leu-Gln-Gly-Leu-Val-NH_2 (6). Compound 5 (4.26 g, 5.5 mmol) was decarbobenzoxylated in the manner as described for 2: R_f^I 0.55. A solution of Z-Arg(NO_2)-OH (2.72 g, 7.7 mmol) and *N*-methylmorpholine (0.79 mL, 7.7 mmol) in DMF (20 mL) was cooled to −15 °C and isobutyl chloroformate (1.02 mL, 7.7 mmol) added. After 1 min, the mixed anhydride was combined with an ice-cooled solution of the above hexapeptide amide hydrobromide and Et_3N (0.77 mL, 5.5 mmol) in DMF (20 mL). The mixture was kept at 0 °C for 5 min and at 15 °C for additional 30 min. Compound 6 was obtained in the manner as described for 5: yield 4.57 g (85%); mp 244–246 °C (lit.[19] mp 245–247 °C); $[\alpha]^{23}D$ −42.3° (*c* 1.02, AcOH) [lit.[19] $[\alpha]^{25}D$ −40° (*c* 2, AcOH)]; R_f^I 0.62, R_f^{II} 0.86. Anal. ($C_{44}H_{73}N_{13}O_{12}·H_2O$) C, H, N.

Z-Arg(NO_2)-Leu-Gln-NHNH-Boc (7). Compound 1 (3.05 g, 6 mmol) was hydrogenated for 24 h over Pd black (wet weight 500 mg) in MeOH (30 mL): R_f^I 0.39. The hydrogenated material was coupled with a mixed anhydride which was prepared from Z-Arg(NO_2)-OH (2.33 g, 6.6 mmol), *N*-methylmorpholine (0.70 mL, 6.6 mmol), and isobutyl chloroformate (0.90 mL, 6.6 mmol) in the same manner as described for 6. The desired product was isolated in the manner as described for 1: yield 3.03 g (70%); mp 126–130 °C; $[\alpha]^{23}D$ −26.7° (*c* 1.01, AcOH); R_f^I 0.73, R_f^{II} 0.75. Anal. ($C_{30}H_{48}N_{10}O_{10}·H_2O$) C, H, N.

Z-Ser-Ala-OH (8). Z-Ser-$NHNH_2$ (7.60 g, 30 mmol) was converted to the corresponding azide according to the method as described for 4 using 6 N HCl in dioxane (15 mL, 90 mmol) and *i*-AmONO (4.43 mL, 33 mmol). The azide was coupled with H-Ala-OH (2.67 g, 30 mmol) in a mixture of Et_3N (4.2 mL, 30 mmol), H_2O (50 mL), and DMF (10 mL) in the same manner as described. The solvents were evaporated and the residue was dissolved in H_2O (100 mL) containing Et_3N (5 mL). The solution was washed with four portions of EtOAc. The aqueous layer was acidified to congo red with 3 N citric acid and the resulting oily material was extracted with EtOAc, which was washed with saturated NaCl, dried, and evaporated. The crystalline product was collected, washed with Et_2O, and recrystallized from MeOH with EtOAc: yield 4.88 g (52%); mp 154–156 °C; $[\alpha]^{24}D$ −15.9° (*c* 1.00, MeOH); R_f^I 0.75, R_f^{II} 0.68. Anal. ($C_{14}H_{18}N_2O_6$) C, H, N.

Z-Ser-Ala-NHNH-Boc (9). A solution of 8 (4.66 g, 15 mmol) and *t*-Boc-$NHNH_2$ (1.98 g, 15 mmol) in THF (60 mL) was cooled to −10 °C and DCCI (3.40 g, 16.5 mmol) was added. The mixture was stirred for 18 h at 4 °C and filtered. The filtrate was evaporated and the residue dissolved in EtOAc. Isolation of the product was performed in the manner as described for 1: yield 5.36 g (82%); mp 133–136 °C; $[\alpha]^{24}D$ −43.5° (*c* 1.04, MeOH); R_f^I 0.85, R_f^{II} 0.87. Anal. ($C_{19}H_{28}N_4O_7·0.5H_2O$) C, H, N.

Z-Asp(OBz)-Ser-Ala-NHNH-Boc (10). Compound 9 (5.20 g, 12 mmol) was hydrogenated for 24 h over Pd black (wet weight 500 mg) in MeOH (40 mL) in the usual manner to give H-Ser-Ala-NHNH-Boc: R_f^I 0.39; amino acid ratios in AP-M digest $Ser_{1.05}Ala_{0.95}$ (recovery 93%). The dipeptide hydrazide was coupled with a mixed anhydride prepared from Z-Asp(OBz)-OH (6.43 g, 18 mmol), *N*-methylmorpholine (1.84 mL, 18 mmol), and isobutyl chloroformate (2.38 mL, 18 mmol) in the manner as described for 6. The product was isolated in the manner as described for 1 and purified by reprecipitation from EtOAc with petroleum ether: yield 6.20 g (82%); mp 95–98 °C; $[\alpha]^{24}D$ −13.2° (*c* 0.99, DMF); R_f^I 0.85, R_f^{II} 0.85. Anal. ($C_{30}H_{39}N_5O_{10}$) C, H, N.

Z-Arg(NO_2)-Asp-Ser-Ala-NHNH-Boc (11). Compound 10 (3.15 g, 5 mmol) was hydrogenated for 48 h over Pd black (wet weight 500 mg) in MeOH (50 mL) in the usual manner to give H-Asp-Ser-Ala-NHNH-Boc: R_f^I 0.35; amino acid ratios in AP-M digest, $Asp_{1.07}Ser_{0.98}Ala_{0.95}$ (recovery 95%). The tripeptide hydrazide was coupled by the mixed anhydride method with Z-Arg(NO_2)-OH (2.21 g, 6.3 mmol) using *N*-methylmorpholine (0.64 mL, 6.3 mmol) and isobutyl chloroformate (0.83 mL, 6.3 mmol) in the same manner as described for 6. The solvent was evaporated and the residue dissolved in H_2O (50 mL). The solution was washed with three portions of EtOAc and extracted with five portions of 1-BuOH. The extracts were washed with five portions of 2% AcOH. Evaporation of 1-BuOH layers gave an oily residue which was solidified by addition of Et_2O. Reprecipitation of the solid from MeOH with Et_2O gave 11: yield 3.50 g (95%); mp 174–177 °C; $[\alpha]^{24}D$ −14.6° (*c* 1.03, DMF); R_f^I 0.70, R_f^{II} 0.72. Anal. ($C_{29}H_{44}N_{10}O_{13}$) C, H, N.

Z-Arg(NO_2)-Leu-OMe (12). A mixed anhydride prepared from Z-Arg(NO_2)-OH (5.30 g, 15 mmol), *N*-methylmorphiline (1.53 mL, 15 mmol), and isobutyl chloroformate (1.98 mL, 15 mmol) was coupled with H-Leu-OMe·HCl (2.72 g, 15 mmol) in the manner as described for 6. After evaporation of the solvent, 1 N citric acid was added and the precipitate collected, washed with H_2O, and dried. The product was reprecipitated from MeOH with EtOAc and washed with EtOAc: yield 5.63 g (78%); mp 158–160 °C; $[\alpha]^{24}D$ −20.2° (*c* 0.62, MeOH); R_f^I 0.79, R_f^{II} 0.84. Anal. ($C_{21}H_{32}N_6O_7$) C, H, N.

Z-Ser-Arg-Leu-OMe (13). Compound 12 (1.92 g, 4 mmol) was hydrogenated in the presence of 6 N HCl (1.33 mL, 8 mmol) for 48 h over Pd black (wet weight 300 mg) in a mixture of MeOH (50 mL) and H_2O (20 mL) to give H-Arg-Leu-OMe·2HCl: R_f^I 0.18. Z-Ser-$NHNH_2$ (2.22 g, 8.8 mmol) was coupled via the corresponding azide with the hydrogenated material in the manner as described for 4 using *i*-AmONO (1.30 mL, 9.7 mmol) and 6 N HCl in dioxane (7.34 mL, 44 mmol). The bulk of the solvent was evaporated and the residue dissolved in 1 M AcOH. The solution was washed with three portions of EtOAc. The aqueous layer was made alkaline with Et_3N and extracted with five portions of 1-BuOH, which were washed with five portions of H_2O. The 1-BuOH layers were evaporated and the residue was dissolved in EtOAc. Addition of Et_2O gave 13: yield 1.43 g (64%); mp 73–80 °C; $[\alpha]^{24}D$ −30.4° (*c* 0.49, MeOH); R_f^I 0.53, R_f^{II} 0.73. Anal. ($C_{24}H_{38}N_6O_7·2H_2O$) C, H, N.

Z-Ser-Arg-Leu-$NHNH_2$ (14). Hydrazine hydrate (90%) (1.40 mL, 25 mmol) was added to a solution of 12 (1.40 g, 2.5 mmol) in MeOH (25 mL). The mixture was kept at room temperature for 24 h and the solvent evaporated. The residue was extracted with eight portions of 1-BuOH, which were washed with two portions of H_2O. The organic layers were evaporated and the residue was solidified from MeOH with EtOAc: yield 1.14 g (80%); mp 137–140 °C; $[\alpha]^{24}D$ −21.8° (*c* 0.53, MeOH); R_f^I 0.45, R_f^{II} 0.69.

Anal. ($C_{23}H_{38}N_8O_6 \cdot 2.5H_2O$) C, H, N.

Boc-Glu(OBz)-Leu-OMe (15). A mixed anhydride prepared from Boc-Glu(OBz)-OH (5.06 g, 15 mmol), *N*-methylmorpholine (1.53 mL, 15 mmol), and isobutyl chloroformate (1.98 mL, 15 mmol) was coupled with H-Leu-OMe·HCl (2.72 g, 15 mmol) in DMF (20 mL) containing Et_3N (2.10 mL, 15 mmol) as described for **6**. The mixture was worked up in the manner as described for 1 and the product was solidified by addition of petroleum ether: yield 5.70 g (82%); mp 67–69 °C; $[\alpha]^{26}D$ −29.2° (*c* 1.03, MeOH); R_f^I 0.81, R_f^{II} 0.89. Anal. ($C_{24}H_{36}N_2O_7$) C, H, N.

Z-Thr-Ser-Glu-Leu-OMe (16). Compound **15** (2.56 g, 5.5 mmol) was hydrogenated for 48 h over Pd black (wet weight 500 mg) in MeOH (50 mL). The resulting Boc-Glu-Leu-OMe was dissolved in TFA (4 mL) and the solution was allowed to stand at room temperature for 30 min. The bulk of TFA was removed in vacuo and petroleum ether added. The supernatant was discarded and the resulting H-Glu-Leu-OMe was dried over KOH: R_f^I 0.25. Z-Thr-Ser-$NHNH_2$[20] (1.95 g, 5.5 mmol) was coupled by the azide method with the above deblocked material in the manner as described for **4**. After 15 h of the reaction, the solvent was removed and the residue dissolved in H_2O (60 mL) containing Et_3N (1.30 mL). The solution was washed with two portions of EtOAc, acidified with 1 N HCl, and combined with EtOAc (50 mL). The mixture was shaken, and the resulting precipitate was collected and washed with H_2O: yield 2.72 g (83%); mp 121–122 °C; $[\alpha]^{24}D$ −12.6° (*c* 1.03, DMF); R_f^I 0.75, R_f^{II} 0.83. Anal. ($C_{27}H_{40}N_4O_{11}$) C, H, N.

Z-Thr-Ser-Glu-Leu-$NHNH_2$ (17). A solution of **16** (1.25 g, 2.1 mmol) and hydrazine hydrate (90%) (1.20 mL, 20 mmol) in MeOH (10 mL) was allowed to stand at room temperature for 24 h. The solvent was evaporated and the residue extracted with five portions of 1-BuOH, which were washed with three portions of 2% AcOH. The 1-BuOH layers were combined and evaporated and the residue was solidified with EtOAc: yield 1.12 g (88%); mp 184–185 °C; $[\alpha]^{24}D$ −51.8° (*c* 1.10, 50% AcOH); R_f^I 0.43, R_f^{II} 0.75. Anal. ($C_{26}H_{40}N_6O_{10} \cdot 0.5H_2O$) C, H, N.

Z-Thr-Phe-NHNH-Boc (18). A mixed anhydride prepared from Z-Thr-OH (6.13 g, 24.2 mmol), *N*-methylmorpholine (2.50 mL, 24.2 mmol), and isobutyl chloroformate (3.20 mL, 24.2 mmol) was coupled to H-Phe-NHNH-Boc derived from Z-Phe-NHNH-Boc (6.76 g, 24.2 mmol) in the manner as described for **6**. The product was isolated in the manner as described for 1 and solidified from Et_2O with petroleum ether: yield 12.1 g (99%); mp 79 °C; $[\alpha]^{26}D$ −26.7° (*c* 1.01, MeOH); R_f^I 0.83, R_f^{II} 0.81. Anal. ($C_{26}H_{34}N_4O_7$) C, H, N.

Z-Gly-Thr-Phe-NHNH-Boc (19). Compound **18** (11.99 g, 23.3 mmol) was hydrogenated in the usual manner to give H-Thr-Phe-NHNH-Boc, R_f^I 0.48, which was coupled with a mixed anhydride prepared from Z-Gly-OH (6.07 g, 29.0 mmol), *N*-methylmorpholine (2.96 mL, 29.0 mmol), and isobutyl chloroformate (3.80 mL, 29.0 mmol) in the manner as described for **6**. The product was isolated in the same manner as described for 1 and solidified from Et_2O: yield 12.5 g (94%); mp 138–139 °C; $[\alpha]^{26}D$ −27.4° (*c* 0.98, MeOH); R_f^I 0.81, R_f^{II} 0.87. Anal. ($C_{28}H_{37}N_5O_8$) C, H, N.

Z-Asp(OBz)-Gly-Thr-Phe-NHNH-Boc (20). Compound **19** (10.18 g, 17.8 mmol) was hydrogenated in the usual manner to give H-Gly-Thr-Phe-NHNH-Boc, R_f^I 0.39, which was coupled with a mixed anhydride prepared from Z-Asp(OBz)-OH (9.29 g, 26.0 mmol), *N*-methylmorpholine (2.70 mL, 26.0 mmol), and isobutyl chloroformate (3.40 mL, 26.0 mmol) in the manner as described for **6**. After evaporation of the solvent, 1 N citric acid (80 mL) was added. The mixture was extracted with three portions of EtOAc and the extracts were washed with two portions of 1 N citric acid and five portions of H_2O. Evaporation of the solvent gave an oil which was solidified from EtOAc with Et_2O: yield 11.4 g (82%); mp 164–166 °C dec; $[\alpha]^{26}D$ −12.7° (*c* 1.02, DMF); R_f^I 0.89, R_f^{II} 0.89. Anal. ($C_{39}H_{48}N_6O_{11}$) C, H, N.

Z-Ser-Asp-Gly-Thr-Phe-NHNH-Boc (21). Compound **20** (10.02 g, 12.9 mmol) was hydrogenated over Pd black (wet weight 800 mg) in MeOH (150 mL) containing 10% AcOH (30 mL) in the usual manner to give H-Asp-Gly-Thr-Phe-NHNH-Boc (**22**), R_f^I 0.46. The material was coupled by the azide method with Z-Ser-$NHNH_2$ (4.91 g, 19.4 mmol) in the manner as described for **4**. The product was isolated in the manner as described for **20**. Evaporation of the solvent gave a solid which was washed with Et_2O: yield 10.1 g (99%); mp 147–149 °C dec; $[\alpha]^{26}D$ −11.6° (*c* 1.03, DMF); R_f^I 0.74, R_f^{II} 0.77. Anal. ($C_{35}H_{47}N_7O_{13} \cdot H_2O$) C, H, N.

Z-His-Ser-Asp-Gly-Thr-Phe-NHNH-Boc (23). Compound **21** (4.30 g, 5.4 mmol) was hydrogenated over Pd black (wet weight 500 mg) in MeOH (70 mL) containing 30% AcOH (40 mL) in the usual manner to give H-Ser-Asp-Gly-Thr-Phe-NHNH-Boc (**24**), R_f^I 0.37. Compound **24** was coupled by the azide method with Z-His-$NHNH_2$ (2.53 g, 8.3 mmol) in the same manner as described for **4**. After evaporation of the solvent, the residue was dissolved in 1-BuOH. The solution was washed with eight portions of 3% AcOH, which were extracted with two portions of 1-BuOH. The 1-BuOH layers were evaporated and the product was solidified by addition of Et_2O and washed with EtOH: 3.11 g (61%); mp 167–169 °C dec; $[\alpha]^{26}D$ −16.3° (*c* 1.04, DMF); R_f^I 0.44, R_f^{II} 0.61. Anal. ($C_{41}H_{54}N_{10}O_{14} \cdot 2H_2O$) C, H, N.

Z-Tyr-His-Ser-Asp-Gly-Thr-Phe-NHNH-Boc (25). Compound **23** (1.10 g, 1.2 mmol) was hydrogenated in the usual manner to give H-His-Ser-Asp-Gly-Thr-Phe-NHNH-Boc (**26**), R_f^I 0.22. DCCI (0.35 g, 1.7 mmol) was added to an ice-cold solution of Z-Tyr-OH (0.54 g, 1.7 mmol) and *N*-hydroxysuccinimide (0.20 g, 1.7 mmol) in THF (15 mL). The mixture was stirred at 4 °C for 20 h and filtered. The filtrate was evaporated and the residue dried in vacuo and dissolved in DMF (20 mL). The solution was added to a solution of **26** in DMF (20 mL) containing Et_3N (0.32 mL, 2.3 mmol), and the mixture was stirred at room temperature for 20 h. Isolation of the product was performed in the manner as described for **23**, and the product was solidified by addition of EtOAc and triturated with EtOH: yield 0.86 g (65%); mp 188–190 °C dec; $[\alpha]^{24}D$ −20.5° (*c* 1.20, DMF); R_f^I 0.51, R_f^{II} 0.67. Anal. ($C_{50}H_{63}N_{11}O_{16} \cdot CH_3CO_2H$) C, H, N.

N^{α}-β-(4-Hydroxyphenyl)propionyl-His-Ser-Asp-Gly-Thr-Phe-NHNH-Boc (27). Compound **26** which was derived from **23** (1.10 g, 1.2 mmol) as described was coupled with *N*-hydroxysuccinimido β-(4-hydroxyphenyl)propionate (0.45 g, 1.7 mmol) in DMF (20 mL) in the usual way. The product was isolated in the manner as described for **23** and precipitated from EtOH with EtOAc: yield 0.92 g (81%); mp 163–165 °C dec; $[\alpha]^{24}D$ −13.4° (*c* 0.52, DMF); R_f^I 0.43, R_f^{II} 0.70. Anal. ($C_{42}H_{56}N_{10}O_{14} \cdot CH_3CO_2H$) C, H, N.

Di-Z-Tyr-Ser-Asp-Gly-Thr-Phe-NHNH-Boc (28). Compound **24** which was derived from **21** (0.96 g, 1.2 mmol) was coupled with di-Z-Tyr-OSu (1.23 g, 2.3 mmol) in the usual way. The product was isolated in the manner as described for **23** and purified by washing with MeOH: yield 1.08 g (83%); mp 163–165 °C; $[\alpha]^{26}D$ −31.9° (*c* 0.56, 50% AcOH); R_f^I 0.81, R_f^{II} 0.85. Anal. ($C_{52}H_{62}N_8O_{17} \cdot H_2O$) C, H, N.

Z-Thr-Tyr-OMe (29). A mixed anhydride prepared from Z-Thr-OH (3.80 g, 15 mmol), *N*-methylmorpholine (1.53 mL, 15 mmol), and isobutyl chloroformate (1.98 mL, 15 mmol) in THF (25 mL) was coupled with an ice-cold solution of H-Tyr-OMe·HCl (3.48 g, 15 mmol) in THF (40 mL) containing Et_3N (2.10 mL, 15 mmol) in the same manner as described for **6**. The product was isolated in the manner as described for 1: yield 5.85 g (91%); mp 59–62 °C; $[\alpha]^{26}D$ −4.6° (*c* 0.76, MeOH); R_f^I 0.93, R_f^{II} 0.88. Anal. ($C_{22}H_{26}N_2O_7$) C, H, N.

Z-Gly-Thr-Tyr-OMe (30). Compound **29** (5.60 g, 13 mmol) was hydrogenated over Pd black (wet weight 500 mg) in the presence of 1 N HCl (13 mL) in MeOH (50 mL) to give H-Thr-Tyr-OMe·HCl, R_f^I 0.51. The material was coupled with a mixed anhydride prepared from Z-Gly-OH (2.72 g, 13 mmol), *N*-methylmorpholine (1.33 mL, 13 mmol), and isobutyl chloroformate (1.72 mL, 13 mmol) in the manner as described for **6**. The solvents were evaporated and the residue was solidified by addition of EtOAc (20 mL) and 1 N citric acid (40 mL). The solid was collected, washed with H_2O, and dried: yield 4.05 g (64%); mp 174–177 °C; $[\alpha]^{26}D$ −6.3° (*c* 0.47, MeOH); R_f^I 0.79, R_f^{II} 0.92. Anal. ($C_{24}H_{29}N_3O_8$) C, H, N.

Z-Asp(OBz)-Gly-Thr-Tyr-OMe (31). Compound **30** (2.78 g, 5.7 mmol) was hydrogenated in the usual manner to give H-Gly-Thr-Tyr-OMe, R_f^I 0.28. The product was coupled with a mixed anhydride prepared from Z-Asp(OBz)-OH (2.47 g, 6.9 mmol), *N*-methylmorpholine (0.70 mL, 6.9 mmol), and isobutyl chloroformate (0.91 mL, 6.9 mmol) in the manner as described for **6**. The product was isolated in the same manner as described for 1: 3.53 g (89%); mp 167–169 °C; $[\alpha]^{26}D$ −7.4° (*c* 0.54, MeOH);

R_f^I 0.90, R_f^{II} 0.91. Anal. ($C_{35}H_{40}N_4O_{11}$) C, H, N.

Z-Ser-Asp-Gly-Thr-Tyr-OMe (32). Compound **31** (2.08 g, 3.0 mmol) was hydrogenated in the usual manner to give H-Asp-Gly-Thr-Tyr-OMe, R_f^I 0.28. The material was coupled by the azide method with Z-Ser-$NHNH_2$ (1.14 g, 4.5 mmol) in the manner as described for **4**. The solvents were evaporated and the residue was dissolved in EtOAc, which was washed with three portions of 1 N citric acid and three portions of H_2O. The organic layers were evaporated and the product was solidified from MeOH with Et_2O: yield 1.46 g (67%); mp 162–166 °C dec; $[\alpha]^{26}D$ −14.2° (*c* 0.49, MeOH); R_f^I 0.67, R_f^{II} 0.77. Anal. ($C_{31}H_{39}N_5O_{13}\cdot 0.5H_2O$) C, H, N.

Z-His-Ser-Asp-Gly-Thr-Tyr-OMe (33). Compound **32** (1.43 g, 2.0 mmol) was hydrogenated in the usual manner to give H-Ser-Asp-Gly-Thr-Tyr-OMe, R_f^I 0.34. The hydrogenated material was coupled by the azide method with Z-His-$NHNH_2$ (0.94 g, 3.1 mmol) in the manner as described for **4**. Isolation of the product was performed in the manner as described for **23** and the product purified by precipitation from MeOH with EtOAc and washing with EtOH: yield 1.31 g (78%); mp 143–145 °C; $[\alpha]^{26}D$−19.8° (*c* 0.53, 50% AcOH); R_f^I 0.39, R_f^{II} 0.61. Anal. ($C_{37}H_{46}N_8O_{14}\cdot H_2O$) C, H, N.

Z-His-Ser-Asp-Gly-Thr-Tyr-$NHNH_2$ (34). A solution of **33** (1.28 g, 1.5 mmol) and hydrazine hydrate (90%) (0.85 mL, 15 mmol) in DMF (20 mL) was allowed to stand at room temperature for 24 h and the solvent was evaporated. The residue was solidified from MeOH with Et_2O. The crude material was distributed between eight portions of 1-BuOH and 2% AcOH in the same manner as described for **17**. The organic layers were evaporated and the residue was solidified from MeOH with EtOAc: yield 0.98 g (73%); mp 190–192 °C; $[\alpha]^{26}D$−26.6° (*c* 0.51, 50% AcOH); R_f^I 0.35, R_f^{II} 0.64. Anal. ($C_{36}H_{46}N_{10}O_{13}\cdot CH_3CO_2H$) C, H, N.

H-Arg(H$^+$)-Leu-Gln-Arg(H$^+$)-Leu-Leu-Gln-Gly-Leu-Val-NH_2 (35). Compound **6** (4.30 g, 4.4 mmol) was decarbobenzoxylated in AcOH (20 mL) with 25% HBr in AcOH (20 mL) and the reaction mixture was worked up as described for **2** to afford H-Arg(NO_2)-Leu-Leu-Gln-Gly-Leu-Val-NH_2·HBr (**36**), R_f^I 0.71. Compound **7** (6.40 g, 8.8 mmol) was deprotected partially with TFA (10 mL) as described for **3**, R_f^I 0.95. The ensuing Z-tripeptide hydrazide was coupled by the azide method with **36** in the same manner as described for **4**. After evaporating the solvents, 1 N citric acid was added to give a solid, which was reprecipitated from MeOH with EtOAc to yield Z-Arg(NO_2)-Leu-Gln-Arg(NO_2)-Leu-Leu-Gln-Gly-Leu-Val-NH_2 (**37**): yield 4.67 g (74%); mp 250–259 °C (lit.[19] mp 255–264 °C); $[\alpha]^{23}D$ −40.3° (*c* 1.04, AcOH) [lit.[19] $[\alpha]^{25}D$ −36° (*c* 2, AcOH)]; R_f^I 0.47, R_f^{II} 0.78. Anal. ($C_{61}H_{103}N_{21}O_{18}\cdot H_2O$) C, H, N. Compound **37** (992 mg, 0.69 mmol) was hydrogenated over Pd black (wet weight 200 mg) in 50% AcOH (30 mL) for 48 h and the reaction mixture was worked up in the usual manner. The hydrogenated material was dissolved in 1 M AcOH (5 mL) and the solution was subjected to gel filtration on a column (4.5 × 42 cm) of Sephadex G-10, which was eluted with 1 M AcOH. The fractions containing the desired product (R_f^I 0.23) were collected and the solvent was evaporated. The residue was lyophilized: yield 761 mg (80%); $[\alpha]^{26}D$ −55.8° (*c* 0.95, 50% AcOH); R_f^I 0.23, R_f^{II} 0.69. Amino acid ratios in acid hydrolysate: $Glu_{2.06}Gly_{0.97}Val_{1.10}Leu_{3.97}Arg_{2.90}(NH_3)_{2.44}$ (recovery 90%). Anal. ($C_{53}H_{99}N_{19}O_{12}\cdot 2CH_3CO_2H\cdot 4H_2O$) C, H, N.

H-Arg(H$^+$)-Asp-Ser-Ala-Arg(H$^+$)-Leu-Gln-Arg(H$^+$)-Leu-Leu-Gln-Gly-Leu-Val-NH_2 (38). A solution of **11** (1.38 g, 1.9 mmol) was partially deblocked with TFA (10 mL) as described for **3**. The ensuing Z-Arg(NO_2)-Asp-Ser-Ala-$NHNH_2$·TFA (R_f^I 0.45) was coupled with **35** (860 mg, 0.62 mmol) by the azide method in the presence of 6 N HCl (0.95 mL, 5.7 mmol) and *i*-AmONO (0.25 mL, 1.9 mmol) as described for **4**. The solvent was evaporated and the residue distributed between 1-BuOH and 2% AcOH in the manner as described for **11**. Evaporation of the 1-BuOH layers gave an oily residue which was solidified by addition of Et_2O. The crude product thus obtained was hydrogenated in the usual manner. The hydrogenated material (major spot R_f^I 0.15) was dissolved in 1 M AcOH (3 mL) and the solution was subjected to gel filtration on a column (3.7 × 120 cm) of Bio-Gel P-4, which was eluted with 1 M AcOH. Fractions containing the desired material (R_f^I 0.15) were pooled and the solvent was evaporated. The residue was lyophilized: yield 573 mg (57%); $[\alpha]^{26}D$ −47.2° (*c* 1.04, 50% AcOH); R_f^I 0.15, R_f^{II} 0.52. Amino acid ratios in acid hydrolysate: $Asp_{1.09}Ser_{0.96}Glu_{2.06}Gly_{0.95}Ala_{1.04}Val_{0.95}Leu_{3.95}Arg_{3.00}(NH_3)_{2.80}$ (recovery 93%).

H-Ser-Arg(H$^+$)-Leu-Arg(H$^+$)-Asp-Ser-Ala-Arg(H$^+$)-Leu-Gln-Arg(H$^+$)-Leu-Leu-Gln-Gly-Leu-Val-NH_2 (39). Compound **14** (426 mg, 0.75 mmol) was converted to the corresponding azide with 6 N HCl in dioxane (1.25 mL, 7.5 mmol) and *i*-AmONO (0.11 mL, 0.75 mmol) in DMF (8 mL), which was coupled with **38** (262 mg, 0.15 mmol) in the manner as described for **38**. The resulting crude material was hydrogenated over Pd black (wet weight 100 mg) in a mixture of 50% AcOH (15 mL), MeOH (10 mL), and 1-BuOH (10 mL) for 24 h. The hydrogenated product (major spot R_f^I 0.17) was purified by gel filtration on a column (3.5 × 170 cm) of Bio-Gel P-6 using 3 M AcOH as eluent in the manner as described for **38**: yield 156 mg (53%); $[\alpha]^{23}D$ −37.2° (*c* 0.51, 50% AcOH); R_f^I 0.17, R_f^{II} 0.50. Amino acid ratios in acid hydrolysate: $Asp_{1.00}Ser_{1.73}Glu_{2.05}Gly_{1.05}Ala_{1.02}Val_{1.03}Leu_{5.04}Arg_{3.81}(NH_3)_{2.63}$ (recovery 95%).

H-Thr-Ser-Glu-Leu-Ser-Arg(H$^+$)-Leu-Arg(H$^+$)-Asp-Ser-Ala-Arg(H$^+$)-Leu-Gln-Arg(H$^+$)-Leu-Leu-Gln-Gly-Leu-Val-NH_2 (40). Compound **17** (485 mg, 0.80 mmol) was converted to the corresponding azide with 6 N HCl in dioxane (0.80 mL, 4.8 mmol) and 10% *i*-AmONO in DMF (1.18 mL, 0.88 mmol), which was coupled with **39** (334 mg, 0.16 mmol) in DMF (10 mL) in the same manner as described for **38**. The resulting crude material was hydrogenated over Pd black (wet weight 100 mg) in 50% AcOH for 24 h in the usual way and the hydrogenated product (major spot R_f^I 0.16) was purified by gel filtration on a column (3.5 × 170 cm) of Bio-Gel P-6 using 3 M AcOH as eluent in the manner as described for **38**: yield 190 mg (49%); $[\alpha]^{23}D$ −34.7° (*c* 0.52, 50% AcOH); R_f^I 0.11, R_f^{II} 0.50. Amino acid ratios in acid hydrolysate: $Asp_{0.99}Thr_{1.05}Ser_{3.07}Glu_{3.08}Gly_{0.96}Ala_{1.06}Val_{0.94}Leu_{5.96}Arg_{3.89}(NH_3)_{2.52}$ (recovery 91%).

H-His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg(H$^+$)-Leu-Arg(H$^+$)-Asp-Ser-Ala-Arg(H$^+$)-Leu-Gln-Arg(H$^+$)-Leu-Leu-Gln-Gly-Leu-Val-NH_2 (Porcine Secretin) (41). Compound **23** (331 mg, 0.35 mmol) was partially deprotected with TFA (2 mL) as described for **3**. The deblocked material was converted to the corresponding azide with 6 N HCl in dioxane (0.58 mL, 3.5 mmol) and 10% *i*-AmONO in DMF (0.51 mL, 0.38 mmol) and the azide was coupled with **40** (135 mg, 0.05 mmol) in DMF (10 mL) in the manner as described for **4**. The resulting crude material was hydrogenated over Pd black (wet weight 100 mg) in 50% AcOH and the product was subjected to gel filtration on a column (3.0 × 180 cm) of Sephadex G-25 using 3 M AcOH as eluent. Fractions (10 g each) containing the desired product (major spot R_f^I 0.19) (fraction no. 50–61) were evaporated and the residue was lyophilized from H_2O. The fluffy powder was dissolved in H_2O (100 mL) and the solution was applied to a column (2.5 × 6 cm) of CM-Sephadex C-25, which was eluted successively with H_2O (200 mL) and NH_4HCO_3 buffer (pH 7.8): 0.02 M (200 mL), 0.04 M (200 mL), 0.06 M (200 mL), 0.08 M (200 mL), and 0.1 M (200 mL). The eluate was collected in 10-g weight per tube. The 0.04–0.06 M NH_4HCO_3 eluate (tube no. 45–73) was evaporated and the residue was lyophilized from H_2O. The product was dissolved in 3 M AcOH (2 mL) and the solution was filtered on a column (3.0 × 137 cm) of Bio-Gel P-10 using 3 M AcOH as eluent. Fractions (10 g each) containing the desired product (tube no. 50–60) were evaporated and the residue was lyophilized from H_2O: 72 mg (47%); $[\alpha]^{21}D$ −59.2° (*c* 0.15, 50% AcOH); R_f^I 0.19, R_f^{II} 0.58. Amino acid ratios in acid hydrolysate: $Asp_{2.07}Thr_{1.99}Ser_{3.65}Glu_{3.06}Gly_{1.97}Ala_{0.98}Val_{1.02}Leu_{5.94}Phe_{1.05}His_{0.90}Arg_{4.02}(NH_3)_{3.85}$ (recovery 90%). Amino acid ratios in AP-M digest: $Asp_{1.86}Glu_{0.87}Gly_{2.00}Ala_{1.01}Val_{1.23}Leu_{6.58}Phe_{0.85}His_{0.86}Arg_{3.80}$ (Thr, Ser, and Gln were not determined) (recovery on the basis of Gly 84%).

H-Tyr-His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg(H$^+$)-Leu-Arg(H$^+$)-Asp-Ser-Ala-Arg(H$^+$)-Leu-Gln-Arg(H$^+$)-Leu-Leu-Gln-Gly-Leu-Val-NH_2 (N^{α}-Tyrosylsecretin) (42). Compound **25** (227 mg, 0.20 mmol) was partially deblocked with TFA (1 mL) as described for **3**. The partially deblocked heptapeptide hydrazide was converted to the corresponding azide using 6 N HCl in dioxane (0.33 mL, 2.0 mmol) and 10% *i*-AmONO in DMF (0.28 mL, 0.21 mmol), and the azide was coupled to **40** (68 mg, 0.025 mmol) in DMF (10 mL). The crude material thus obtained was hydrogenated over Pd black

and the product was subjected to gel filtration on a column (3.0 × 137 cm) of Bio-Gel P-10 using 3 M AcOH as eluent. Fractions (10 g each) containing the desired product (major spot R_f^I 0.18) (tube no. 52–58) were pooled and the solvent was evaporated. Further purification was performed by ion-exchange chromatography on a column (2.5 × 6 cm) of CM-Sephadex C-25, which was eluted successively with H_2O and NH_4HCO_3 buffer in the manner as described for purification of **41**. The 0.02–0.04 M eluate (tube no. 35–60) containing the desired material was pooled and evaporated. The residue was desalted by gel filtration on Sephadex G-25 and lyophilized: 45 mg (56%); $[\alpha]^{22}D$ −62.2° (*c* 0.26, 1 M AcOH); R_f^I 0.18, R_f^{II} 0.52. Amino acid ratios in acid hydrolysate: $Asp_{2.10}Thr_{1.90}Ser_{3.68}Glu_{3.04}Gly_{1.98}Ala_{1.10}Val_{1.09}Leu_{5.88}Tyr_{0.85}Phe_{0.98}His_{0.84}Arg_{3.97}(NH_3)_{3.87}$ (recovery 89%).

H-Tyr-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg(H⁺)-Leu-Arg(H⁺)-Asp-Ser-Ala-Arg(H⁺)-Leu-Gln-Arg(H⁺)-Leu-Leu-Gln-Gly-Leu-Val-NH₂ ([Tyr¹]secretin) (43). Compound **28** (109 mg, 0.10 mmol) was partially deblocked with TFA (1 mL) as described for **3**. The product was converted to the corresponding azide in the presence of 6 N HCl in dioxane (0.17 mL, 1.00 mmol) and 10% *i*-AmONO in DMF (0.13 mL, 0.10 mmol), which was coupled with **40** (41 mg, 0.015 mmol) in DMF (8 mL). The mixture was worked up as described and the resulting crude material was hydrogenated over Pd black in the usual manner. The product was subjected to gel filtration on a column (3.0 × 137 cm) of Bio-Gel P-10, which was eluted with 3 M AcOH. Fractions (10 g each) containing the desired product (major spot R_f^I 0.20) (tube no. 48–55) were pooled and evaporated. Further purification was performed by ion-exchange chromatography on a column (2.5 × 5 cm) of CM-Sephadex C-25, which was eluted successively with H_2O and NH_4HCO_3 buffer in the manner as described for **41**. The 0.04–0.06 M NH_4HCO_3 eluate (tube no. 46–72) was pooled and evaporated. The product was finally desalted by gel filtration on Sephadex G-25 and lyophilized: 28 mg (61%); $[\alpha]^{24}D$ −44.5° (*c* 0.46, 1 M AcOH); R_f^I 0.20, R_f^{II} 0.56. Amino acid ratios in acid hydrolysate: $Asp_{1.93}Thr_{1.79}Ser_{3.70}Glu_{2.94}Gly_{1.92}Ala_{1.06}Val_{1.03}Leu_{6.00}Tyr_{0.83}Phe_{0.99}Arg_{4.08}(NH_3)_{3.48}$ (recovery 91%).

4-Hydroxyphenyl-CH₂CH₂CO-His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg(H⁺)-Leu-Arg(H⁺)-Asp-Ser-Ala-Arg(H⁺)-Leu-Gln-Arg(H⁺)-Leu-Leu-Gln-Gly-Leu-Val-NH₂ [N^{α}-β-(4-Hydroxyphenyl)propionylsecretin] (44). Compound **27** (158 mg, 0.16 mmol) was partially deblocked with TFA (1 mL) as described, and the product (R_f^I 0.40) was converted to the corresponding azide with 6 N HCl in dioxane (0.27 mL, 1.6 mmol) and 10% *i*-AmONO in DMF (0.24 mL, 0.18 mmol). The azide was coupled with **40** (54 mg, 0.020 mmol) in DMF (8 mL). The crude material obtained was purified by ion-exchange chromatography on a column (2.5 × 5 cm) of CM-Sephadex C-25, which was eluted with H_2O and NH_4HCO_3 buffer as described for **41**. The 0.04–0.06 M NH_4HCO_3 eluate (tube no. 45–75) was pooled and evaporated. The residue was dissolved in 3 M AcOH (3 mL) and the solution was submitted to gel filtration on Bio-Gel P-10 (3.0 × 137 cm) using 3 M AcOH as eluent. Fractions containing the desired material were collected and lyophilized: 32 mg (50%); $[\alpha]^{24}D$ −53.5° (*c* 0.52, 1 M AcOH); R_f^I 0.20, R_f^{II} 0.58. Amino acid ratios in acid hydrolysate: $Asp_{2.01}Thr_{1.82}Ser_{3.75}Glu_{3.01}Gly_{2.03}Ala_{1.02}Val_{5.97}Phe_{1.03}His_{0.88}Arg_{4.01}(NH_3)_{3.68}$ (recovery 88%).

H-His-Ser-Asp-Gly-Thr-Tyr-Thr-Ser-Glu-Leu-Ser-Arg(H⁺)-Leu-Arg(H⁺)-Asp-Ser-Ala-Arg(H⁺)-Leu-Gln-Arg(H⁺)-Leu-Leu-Gln-Gly-Leu-Val-NH₂ ([Tyr⁶]secretin) (45). Compound **34** (89 mg, 0.10 mmol) was converted to the corresponding azide with 6 N HCl in dioxane (0.17 mL, 1.00 mmol) and 10% *i*-AmONO in DMF (0.13 mL, 0.10 mmol). The azide was coupled with **40** (41 mg, 0.015 mmol) in DMF (8 mL) and the resulting crude material was subjected, successively, to gel filtration on Sephadex G-25, ion-exchange chromatography on CM-Sephadex C-25, and gel filtration on Bio-Gel P-10 as described for purification of **41**: 25 mg (54%); $[\alpha]^{24}D$ −56.4° (*c* 0.56, 1 M AcOH); R_f^I 0.18, R_f^{II} 0.56. Amino acid ratios in acid hydrolysate: $Asp_{2.05}Thr_{1.80}Ser_{3.80}Glu_{3.02}Gly_{2.05}Ala_{0.98}Val_{0.95}Leu_{6.05}Tyr_{0.88}His_{0.91}Arg_{3.96}(NH_3)_{3.39}$ (recovery 92%).

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References and Notes

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