[Chem. Phaem. Bull. 24(12)3149—3157(1976)]

UDC 547.466.1.04 : 615.357.011.4

Analogues of Luteinizing Hormone-Releasing Hormone with Modification in Position 3[1)]

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(Received May 1, 1976)

Six analogues of LH-RH, in which the tryptophan residue in position 3 was replaced by nonprotein amino acids, were synthesized and evaluated for their LH-RH activity. The analogues substituted by amino acids having the fused aromatic ring structure in the side-chain retained relatively high biological activity. In particular, the potency of [3-(1-naphthyl)-L-alanine]^3-LH-RH was 187.1% of that of synthetic LH-RH. The results demonstrate that the fused aromatic ring structure of the side-chain in position 3 is a favorable factor, and that the indolyl NH group in the Trp residue is not essential.

Numerous analogues of luteinizing hormone-releasing hormone (LH-RH) reported from various laboratories have provided valuable information on the structure-activity relationship of this hormone. In our previous papers, we noted that the N-terminal pyroglutamyl residue is not essential for the activity,[3,4)] and that both the size and basicity of the guanidino group as well as the length of the methylene chain in the Arg^8 residue are important factors for generating the full hormonal potency.[5)]

On the other hand, the aromaticity of the Trp residue in position 3 has been suggested to play an important role. Analogues in which Trp was deleted or replaced with an aliphatic amino acid residue such as Leu, possesses virtually no LH-releasing activity.[6)] However, peptides in which replacement was made by aromatic amino acid residues such as Phe,[6)] Tyr,[7)] *p*-NH_2–Phe,[8)] *p*-NO_2–Phe[8)] and pentamethylphenylalanine[8)] at position 3 retained a significant activity although it was considerably decreased.

In this work, we synthesized six analogues in an attempt to obtain more detailed knowledge on the position 3 of LH-RH and to obtain more potent analogues by the substitution at this position.

Special amino acids used herein for the substitution were Nal(1), Nal(2), 7-Azatrp, Bal, Bal(O_2) and Bip. DL-Bip was synthesized by the reaction of 4-chloromethylbiphenyl with diethyl acetamidomalonate, followed by acid hydrolysis and decarboxylation. The other

1) Amino acids, peptides and their derivatives mentioned in this paper are of the L-configuration. Abbreviations used are those recommended by IUPAC-IUB Commission of Biochemical Nomenclature in May 1971: *J. Biol. Chem.*, **247**, 977 (1972). Abbreviations of the less-common amino acids are as follows: Nal(1), 3-(1-naphthyl)-L-alanine; Nal(2), 3-(2-naphthyl)-L-alanine; 7-Azatrp, L-7-azatryptophan; Bal, 3-(3-benzo[*b*]thienyl)-L-alanine; Bal(O_2), 3-(3-benzo[*b*]thiophene-1-dioxide)-L-alanine; Bip, 3-(4-biphenyl)-L-alanine. The other abbreviations are as follows: Ac, acetyl; Z, carbobenzoxy; Bu^t, *tert*-butyl; Tos, tosyl.

2) Location: *1–2–58, Hiromachi, Shinagawa-ku, Tokyo, 140, Japan.*

3) Y. Okada, K. Kitamura, Y. Baba, A. Arimura, and A.V. Schally, *Biochem. Biophys. Res. Commun.*, **53**, 1180 (1973).

4) Y. Okada, H. Horikoshi, and Y. Baba, *Chem. Pharm. Bull.* (Tokyo), **22**, 721 (1974).

5) Y. Yabe, K. Kitamura, C. Miura, and Y. Baba, *Chem. Pharm. Bull.* (Tokyo), **22**, 2557 (1974).

6) N. Yanaihara, T. Hashimoto, C. Yanaihara, K. Tsuji, Y. Kenmochi, F. Ashizawa, T. Kaneko, H. Oka, S. Saito, A. Arimura, and A.V. Schally, *Biochem. Biophys. Res. Commun.*, **52**, 64 (1973).

7) D.H. Coy, E.J. Coy, and A.V. Schally, *J. Med. Chem.*, **16**, 1140 (1973).

8) D.H. Coy, E.J. Coy, Y. Hirotsu, J.A. Vilchez-Martinez, A.V. Schally, J.W. van Nispen, and G.I. Tesser, *Biochemistry*, **13**, 3550 (1974).

DL-amino acids, DL-Nal(1),[9] DL-Nal(2),[10] DL-7-Azatrp[11] and DL-Bal,[12] were synthesized following the already described methods. The optical resolution was performed by the action of acylase (*Aspergillus genus*) on the acetylated DL-amino acids. DL-Amino acids obtained were acetylated with acetic anhydride to give N^{α}-Ac-DL-amino acids in a good yield. N^{α}-Ac-DL-Bal(O_2) was obtained by the oxidation of N^{α}-Ac-DL-Bal with 30% hydrogen peroxide in acetic acid. Necessary L-isomers of Nal(1), Nal(2), Bal, Bal(O_2) and Bip were crystallized out during the incubation and purified by recrystallization. 7-Azatrp was purified by the column partition chromatography on Sephadex G-25[13] in a good yield.

The analogues including these amino acids were synthesized by the conventional classical methods as depicted in Chart 1 and 2. The α-amino groups, the hydroxyl groups of Ser and Tyr, and the guanidino group of Arg were protected by Z, Bu^t and Tos groups, respectively.

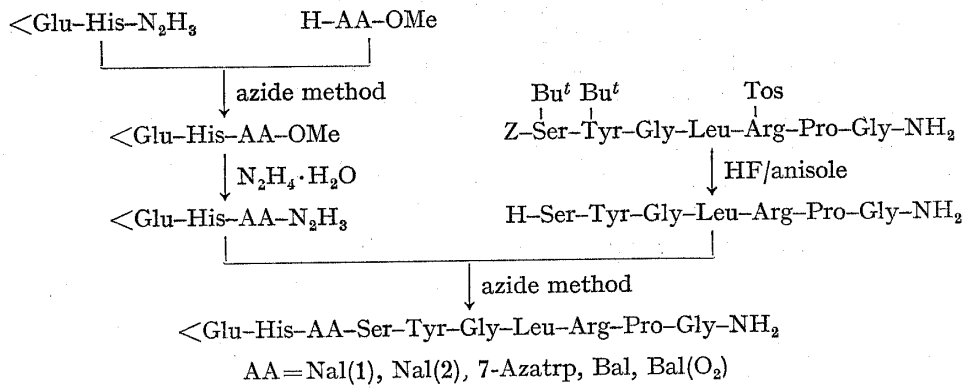


Chart 1

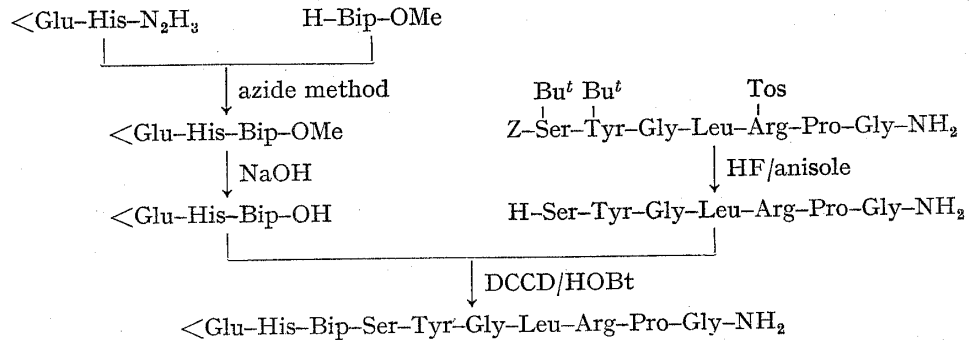


Chart 2

For the syntheses of N-terminal tripeptide esters, <Glu–His–AA–OMe [AA=Nal(1), Nal(2), 7-Azatrp, Bal, Bal(O_2) and Bip], <Glu–His–N_2H_3[14] was coupled with H–AA–OMe by Rudinger's azide method.[15] <Glu–His–N_2H_3, an important intermediate for the synthesis of two hypothalamic hormones, TRH (thyrotropin releasing hormone) and LH–RH, was reported very difficult to dissolve in organic solvents. However, the direct treatment of this dipeptide hydrazide suspension in N,N-dimethylformamide (DMF) with isoamyl nitrite was found to give a clear solution of the corresponding azide. The tripeptide esters obtained above were treated with excess hydrazine hydrate to give <Glu–His–AA–N_2H_3 except for

9) H. Erlenmeyer and W. Grubenmann, *Helv. Chim. Acta*, **30**, 297 (1947).
10) K. Dittmer, W. Herz, and S.J. Cristol, *J. Biol. Chem.*, **173**, 323 (1948).
11) M.M. Robison and B.L. Robison, *J. Am. Chem. Soc.*, **77**, 457 (1955).
12) S. Avakian, J. Moss, and G.J. Martin, *J. Am. Chem. Soc.*, **70**, 3075 (1948).
13) D. Yamashiro, *Nature*, **201**, 76 (1964).
14) D. Gillessen, A.M. Felix, W. Lergier, and R.O. Studer, *Helv. Chim. Acta.*, **53**, 63 (1970).
15) J. Honzl and J. Rudinger, *Coll. Czech. Chem. Commun.*, **26**, 2333 (1961).

the case of <Glu–His–Bip–OMe. These tripeptide derivatives were characterized by the elemental analysis and ultraviolet (UV) spectra.

The protected C-terminal heptapeptide amide, Z–Ser(Bu^t)–Tyr(Bu^t)–Gly–Leu–Arg(Tos)–Pro–Gly–NH_2,[3] was deprotected by the treatment with anhydrous hydrogen fluoride (HF)[16] in the presence of anisole and coupled with <Glu–His–AA–N_2H_3 by Rudinger's azide method[15] to give the decapeptide amide, <Glu–His–AA–Ser–Tyr–Gly–Leu–Arg–Pro–Gly–NH_2. For Bip^3–LH–RH, the deprotected heptapeptide amide was coupled with <Glu–His–Bip–OH by dicyclohexylcarbodiimide (DCCD) in the presence of 1-hydroxybenzotriazole (HOBt),[17] because of the difficulty in preparing <Glu–His–Bip–N_2H_3 from the corresponding methyl ester. The reaction of <Glu–His–Bip–OMe with 30 equivalents of hydrazine hydrate in DMF for a week at room temperature afforded only a slight amount of hydrazide (see Experimental). <Glu–His–Bip–OH was prepared by alkali hydrolysis of <Glu–His–Bip–OMe.

The crude peptides obtained were purified by the column partition chromatography on Sephadex G-25,[13] using the solvent systems consisting of *n*-BuOH–AcOH–CCl_4–H_2O (16:4:1:20) for Nal(1)^3–, Nal(2)^3– and Bip^3–LH–RH, *n*-BuOH–AcOH–CCl_4–H_2O (40:10:1:50) for Bal$(O_2)^3$–LH–RH, *n*-BuOH–AcOH–H_2O (4:1:5) for Bal^3–LH–RH, and *n*-BuOH–0.1M ammonium acetate (1:1) for 7-Azatrp^3–LH–RH. These analogues each exhibited a single spot on thin-layer chromatography by the three different solvent systems, and their acid hydrolysate showed the correct amino acid ratios by an automatic amino acid analyzer as depicted in Table I.

Table I. Chemical and Physical Properties of LH–RH Analogues with Modification in Position 3

Compounds	$[\alpha]_D^{20}$ (c=0.3, 0.1N AcOH)	*Rf*[a]			Amino acid analysis[b]								
		I	II	III	Glu	His	AA[c]	Ser	Tyr	Gly	Leu	Arg	Pro
Synthetic LH–RH	−52.9	0.39	0.58	0.49									
Nal(1)^3–LH–RH	−71.4	0.40	0.62	0.59	1.00	0.98	1.12[d]	0.83	1.00	1.99	1.00	0.95	0.98
Nal(2)^3–LH–RH	−57.2	0.40	0.63	0.58	0.99	0.96	1.11[d]	0.88	1.00	1.97	1.00	0.92	1.00
7-Azatrp^3–LH–RH	−58.1	0.10	0.50	0.38	0.94	0.95	0.93[d]	0.77	1.01	2.02	1.00	0.97	0.99
Bal^3–LH–RH	−62.8	0.38	0.59	0.53	0.93	0.95	0.95[e]	0.87	0.96	1.95	1.00	1.01	0.98
Bal$(O_2)^3$–LH–RH	−39.8	0.34	0.50	0.50	0.95	0.96	0.95[d]	0.83	0.99	1.97	1.00	0.98	0.99
Bip^3–LH–RH	−57.7	0.42	0.65	0.63	0.96	0.97	0.94[d]	0.97	1.00	1.98	1.00	0.95	1.00

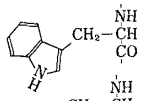
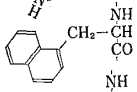
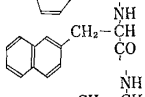
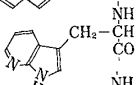
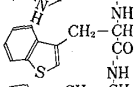
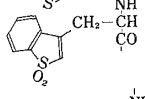
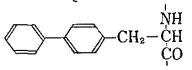
a) *Rf* I, II and III values refer to the solvent systems *n*-BuOH–AcOH–H_2O (60:15:25), *n*-BuOH–AcOH–H_2O–pyridine (30:6:24:20) and $CHCl_3$–MeOH–32% aq. AcOH (60:45:20), respectively.
b) acid hydrolysate (6N HCl, 110°, 24 hr, in the presence of thioglycolic acid).
c) AA indicates individual amino acids substituted for Trp^3.
d) The elution volume for AA was as follows; Nal(1) (1.13), Nal(2) (1.34), 7-Azatrp (0.65), Bal(O_2) (0.48), Bip (2.93) (assuming that the elution volume for His is 1.00). CHROMO-BEADS (type C-2, Technicon, 0.9×15 cm) column eluted with 0.35N citrate buffer pH 5.28 at 50°.
e) Bal was identified by Technicon's amino acid analyzer assembly using a nine chamber gradient buffer systems. The elution volume based on His was 1.209. CHROMO-BEADS (type C-2, Technicon, 0.6×66 cm) column at 70°. Buffer system (Na-citrate buffer) was as follows: (Chamber I) 0.2N, pH 2.75 (33 ml)+MeOH (2 ml), (II) 0.2N, pH 2.75(11 ml)+0.2N, pH 2.875 (24 ml), (III) 0.2N, pH 2.875 (35 ml), (IV and V) 0.2N, pH 3.80 (35 ml), (VI–IX) 1.2N, pH 6.10 (35 ml).

All of these analogues were tested for LH–RH activity and compared with synthetic LH–RH at two dose levels. The evaluations of LH–RH activity was performed *in vivo* by stimulation of release of LH in ovariectomized rats pretreated with estrogen and progesterone,[18] followed by radioimmunoassay for rat LH according to Niswender, *et al.*[19]

16) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Japan*, **40**, 2164 (1967).
17) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
18) V.D. Ramirez and S.M. McCann, *Endocrinology*, **73**, 197 (1963).
19) G.D. Niswender, A.R. Midgley, Jr., S.E. Monroe, and E. Reichart, Jr., *Proc. Soc. Exptl. Biol. Med.*, **128**, 807 (1968).

The results are summarized in Table II. Bip^3-LH-RH in which the indole ring of the Trp residue was displaced by biphenyl showed no LH-RH activity at the dose of 250 ng per animal, although Phe^3-LH-RH retained the potency albeit very low.[6] On the other hand, Bal^3-LH-RH, in which the indolyl NH group of the Trp residue was converted to a sulfur atom, exhibited 73.6% of the activity of synthetic LH-RH. Moreover, replacement of the indole ring in the Trp residue by naphthalene ring resulted in high activity. In particular, Nal(1)^3-LH-RH showed 187.1% of the activity of synthetic LH-RH. The present results demonstrate that the fused ring structure of two aromatic nuclei at the side-chain of position 3 is favorable to elicit LH-releasing activity, while the indolyl NH function makes little contribution.

Table II. LH-RH Activity of LH-RH Analogues with Modification in Position 3 as compared with Synthetic LH-RH

Compounds	Structure in position 3	% LH-RH activity *in vivo* with 95% confidence limits
Synthetic LH-RH		Assumed 100%
Nal(1)^3-LH-RH		187.1% (148.2—236.0)
Nal(2)^3-LH-RH[a]		37.1 (26.3—52.5)
7-Azatrp^3-LH-RH		4.6 (3.6—6.9)
Bal^3-LH-RH		73.6 (48.6—111.4)
Bal(O_2)^3-LH-RH		0 (250 ng/animal)
Bip^3-LH-RH		0 (250 ng/animal)

a) This analogue was reported by K.U. Prasad *et al.*[20] during preparation of this paper.

In comparison with Bal^3-LH-RH, its oxidized analogue, Bal(O_2)^3-LH-RH, showed no activity; and in 7-Azatrp^3-LH-RH, in which another nitrogen atom was introduced into the indole nucleus, a marked decrease in the potency was observed. Incorporation of bulky or polar component to the fused ring system may cause such a considerable reduction of the hormonal activity.

Experimental

Melting points are uncorrected. Optical rotations were measured with a Perkin-Elmer polarimeter 141. Thin-layer chromatography (TLC) was performed on Merck Silica gel $60F_{254}$. *Rf* values refer to the following solvent systems: Rf^{I} *n*-BuOH–AcOH–H_2O (60: 15: 25, v/v/v), Rf^{II} *n*-BuOH–AcOH–H_2O–pyridine (30: 6: 24: 20), Rf^{III} $CHCl_3$–MeOH–32% aq. AcOH (60: 45: 20), Rf^{IV} $CHCl_3$–MeOH–32% aq. AcOH (6: 3: 1) and Rf^{V} $CHCl_3$–EtOH–AcOH (20: 5: 1). Amino acid analyses of peptides were carried out on samples that had been hydrolyzed with constant boiling HCl for 24 hr in evacuated, sealed tubes at 110°, and were recorded on a Hitachi amino acid analyzer KLA-2 and Technicon's amino acid analysis assembly.

N-Acetyl-3-(1-naphthyl)-DL-alanine (1)——3-(1-Naphthyl)-DL-alanine hydrochloride[9] (4.16 g, 16.5 mmol) was dissolved in 1N NaOH (33 ml), and acetic anhydride (Ac_2O; 1.84 g, 18 mmol) and 1N NaOH (18 ml) were added in five portions over 30 min with vigorous stirring and ice-cooling. At the end of the reaction, 2 hr later, the reaction mixture was acidified with conc. HCl to pH 3. Very soon a white mass separated out. After

20) K.U. Prasad, R.W. Roeske, F.L. Weitl, J.A. Vilchez-Martinez, and A.V. Schally, *J. Med. Chem.*, **19**, 492 (1976).

several hours of standing at 0°, colorless crystals were collected by filtration and washed with ice-water. Recrystallization from 60% aq. EtOH gave colorless needles: Yield 3.94 g (93%). mp 186—188°. Rf^{IV} 0.63. *Anal.* Calcd. for $C_{15}H_{15}O_3N$: C, 70.03; H, 5.88; N, 5.44. Found: C, 70.01; H, 5.72; N, 5.29.

N-Acetyl-3-(2-naphthyl)-DL-alanine (2)——The title compound was prepared from 3-(2-naphthyl)-DL-alanine hydrochloride[10] (3.77 g, 15 mmol) and Ac_2O (1.84 g, 18 mmol) in essentially the same way as described for the preparation of **1**. Recrystallization from 60% aq. EtOH gave colorless needles: Yield 3.5 g (91%). mp 171—172°. Rf^{IV} 0.59. *Anal.* Calcd. for $C_{15}H_{15}O_3N \cdot 1/4H_2O$: C, 68.82; H, 5.97; N, 5.35. Found: C, 68.58; H, 5.75; N, 5.18.

N^{α}-Acetyl-DL-7-azatryptophan (3)——Ac_2O (1.12 g, 11 mmol) and 1N NaOH (11 ml) were added alternately in five portions to a solution of DL-7-azatryptophan·$1/3H_2O$[11] (2.11 g, 10 mmol) in 1N NaOH (10 ml) over 30 min at 0° with vigorous stirring. The mixture was stirred at 0° for 1 hr, and then at 5° overnight. The reaction mixture was acidified with conc. HCl to pH 3 and the resulting colorless crystals were collected by filtration. Recrystallized from MeOH: Yield 2.2 g (89%). mp 240—243°. Rf^{III} 0.77. *Anal.* Calcd. for $C_{12}H_{13}O_3N_3$: C, 58.29; H, 5.30; N, 17.00. Found: C, 57.75; H, 5.22; N, 16.84.

N-Acetyl-3-(3-benzo[*b*]thienyl)-DL-alanine (4)——This compound was prepared from 3-(3-benzo[*b*]thienyl)-DL-alanine[12] (2.21 g, 10 mmol) and Ac_2O (1.12 g, 11 mmol) in essentially the same way as described for the preparation of **3**. Recrystallization from 50% aq. EtOH gave colorless needles: Yield 2.2 g (85%). mp 154—156°. Rf^{IV} 0.49. *Anal.* Calcd. for $C_{13}H_{13}O_3NS$: C, 59.30; H, 4.98; N, 5.32; S, 12.18. Found: C, 59.51; H, 4.83; N, 5.27; S, 12.34.

N-Acetyl-3-(3-benzo[*b*]thiophene-1-dioxide)-DL-alanine (5)——**4** (2.3 g, 8.8 mmol) was dissolved in AcOH (7.1 ml) with warming. After cooling, 30% aq. solution of hydrogen peroxide (5.3 ml, 52.8 mmol) was added and the mixture was heated under reflux for 17 min. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in EtOH (5 ml), and ether (30 ml) was added. The resulting colorless precipitate was collected by filtration: Yield 0.65 g (25%). mp 159—163°. Rf^{IV} 0.37. *Anal.* Calcd. for $C_{13}H_{13}O_5NS$: C, 52.87; H, 4.44; N, 4.74; S, 10.86. Found: C, 52.41; H, 4.47; N, 4.62; S, 11.23.

Ethyl α-Acetamido-α-carbethoxy-β-(4-biphenyl)propionate (6)——To a solution of sodium (0.6 g) in absolute EtOH (50 ml), was added diethyl acetamidomalonate (5.5 g, 25 mmol) followed by 4-chloromethylbiphenyl (5.0 g, 25 mmol). The mixture was stirred for 7 hr and allowed to stand for 40 hr at room temperature. The solvent was evaporated *in vacuo* and to the residue water (50 ml) was added. The colorless precipitate was collected by filtration and washed with water and petroleum ether. Recrystallization from benzene–petroleum ether gave colorless fine needles: Yield 8.96 g (93%). mp 138—140°. *Anal.* Calcd. for $C_{22}H_{25}O_5N$: C, 68.91; H, 6.57; N, 3.65. Found: C, 69.08; H, 6.32; N, 3.78.

α-Acetamido-α-carboxy-β-(4-biphenyl)propionic Acid (7)——**6** (8.96 g, 23.4 mmol) was added to a solution of NaOH (5.6 g, 140 mmol) in water (28 ml) and EtOH (56 ml). The mixture was heated under reflux for 3 hr. The insoluble material was filtered off and the filtrate was concentrated *in vacuo*. The residue was dissolved in water (50 ml) and the solution was acidified with conc. HCl. The oily precipitate was extracted with ether and the organic layer was washed with water. After drying over Na_2SO_4, the solvent was evaporated *in vacuo*. On addition of benzene the residue crystallized: Yield 4.85 g (63%). mp 190—192°. *Anal.* Calcd. for $C_{18}H_{17}O_5N$: C, 66.05; H, 5.24; N, 4.28. Found: C, 66.21; H, 5.23; N, 4.45.

3-(4-Biphenyl)-DL-alanine Hydrochloride (8)——A mixture of **7** (4.75 g, 14.5 mmol) and conc. HCl (36 ml) was refluxed for 3 hr, cooled to room temperature, and concentrated *in vacuo*. The residue was dissolved in MeOH (10 ml), and ether was added. The resulting colorless precipitate was collected by filtration: Yield 3.0 g (75%). mp 235—236° (dec.). *Anal.* Calcd. for $C_{15}H_{15}O_2N \cdot HCl$: C, 64.83; H, 5.81; N, 5.04; Cl, 12.76. Found: C, 65.02; H, 5.90; N, 4.95; Cl, 12.83.

N-Acetyl-3-(4-biphenyl)-DL-alanine (9)——**8** (2.8 g, 10 mmol) was dissolved in 1N NaOH (20 ml) with warming and the solution was chilled in ice-water. Ac_2O (1.2 g, 12 mmol) and 1N NaOH (12 ml) were added alternately in five portions over 20 min with vigorous stirring. Water (70 ml) was added to dissolve the precipitate which appeared soon and the stirring was continued for 3 hr at room temperature. The reaction mixture was acidified with conc. HCl to pH 4 and the resulting colorless precipitate was collected by filtration. Recrystallized from EtOH: Yield 1.8 g (64%). mp 208—210.5°. Rf^{IV} 0.81. *Anal.* Calcd. for $C_{17}H_{17}O_3N \cdot 1/4H_2O$: C, 70.94; H, 6.13; N, 4.87. Found: C, 71.06; H, 6.13; N, 4.78.

3-(1-Naphthyl)-L-alanine (10)——**1** (4.7 g, 18.3 mmol) was suspended in water (120 ml) and dissolved by the addition of conc. NH_4OH. The pH was adjusted to 7.5. Acylase (from *Aspergillus genus*, Tokyo kasei; 0.4 g) was added and the reaction mixture was kept at 39° to 40° for 24 hr. Another portion of the enzyme (0.1 g) was added and the incubation was continued for 18 hr. The separating precipitate was collected by filtration and washed with ice-water and MeOH. Recrystallization from 50% aq. EtOH gave colorless fine leaflets: Yield 1.44 g (72%). mp 230—231°. $[\alpha]_D^{20}$ −15.0° (c=0.97, 0.3N HCl). UV $\lambda_{max}^{0.1N\ NaOH}$ nm (ε): 273 (6600), 282.5 (7800), 293.5 (5300, shoulder). Rf^{IV} 0.49. *Anal.* Calcd. for $C_{13}H_{13}O_2N$: C, 72.58; H, 6.09; N, 6.51. Found: C, 72.95; H, 5.92; N, 6.45.

3-(2-Naphthyl)-L-alanine (11)——This compound was obtained by the resolution of **2** (3.6 g, 14 mmol) using acylase as described above. Recrystallization from 50% aq. EtOH gave colorless fine needles: Yield 1.07 g (70%). mp 216—217°. $[\alpha]_D^{22}$ −11.0 (c=0.87, 0.3N HCl). UV $\lambda_{max}^{0.1N\ NaOH}$ nm (ε): 269 (4200), 276 (4300),

286 (3000, shoulder). Rf^{IV} 0.50. *Anal.* Calcd. for $C_{13}H_{13}O_2N$: C, 72.58; H, 6.09; N, 6.51. Found: C, 72.43; H, 5.94; N, 6.45.

L-7-Azatryptophan (12)——3 (2.0 g, 8.1 mmol) was suspended in water (50 ml) and dissolved by the addition of conc. NH_4OH. The pH was adjusted to 7.5. Acylase (0.2 g) was added and the reaction mixture was kept at 40° for 16 hr. Acylase (0.05 g) was added again and the incubation was continued for 24 hr. The insoluble material was filtered off and the filtrate was concentrated *in vacuo*. The residue was dissolved in the upper phase (5 ml) of *n*-BuOH–AcOH–H_2O (4: 1/4: 5) and submitted to partition chromatography on Sephadex G-25[13] (3.5×60 cm) with collection of 10.6 ml fractions. The earlier fractions (No. 38—98) contained undigested material (0.8 g) and the later fractions (No. 147—211) gave **12** (0.9 g). Recrystallized from water: Yield 0.8 g (96%). mp 259—260° (dec.). $[\alpha]_D^{20}$ 28.1° (c=1.0, 1N HCl). UV $\lambda_{max}^{0.1N\ NaOH}$ nm (ε): 290.5 (7400). Rf^{III} 0.50. *Anal.* Calcd. for $C_{10}H_{11}O_2N_3$: C, 58.53; H, 5.40; N, 20.48. Found: C, 58.19; H, 5.31; N, 20.05.

3-(3-Benzo[*b*]thienyl)-L-alanine (13)——**4** (1.2 g, 4.6 mmol) was suspended in water (50 ml) and dissolved by the addition of conc. NH_4OH. The pH was adjusted to 7.5. Acylase (0.2 g) was added and the reaction mixture was kept at 40° for 15 hr. Acylase (0.08 g) was added again and the incubation was continued for 7 hr. The colorless precipitate was collected by filtration and washed with water and MeOH (this portion weighed 0.3 g). The filtrate was concentrated *in vacuo*. The residue and the precipitate described above were combined and dissolved in the upper phase (3 ml) of *n*-BuOH–AcOH–H_2O (4: 1/4: 5) and submitted to partition chromatography on Sephadex G-25[13] (3.5×60 cm) with collection of 9.4 ml fractions. Fractions No. 34—67 gave **13**. Recrystallized from 50% aq. EtOH. Leaflets: Yield 0.47 g (92%). mp 240—242°. $[\alpha]_D^{20}$ −9.5° (c=1.06, 1N HCl). UV $\lambda_{max}^{0.1N\ NaOH}$ nm (ε): 261 (4200), 289.5 (2800), 298.5 (2900). Rf^{V} 0.11. *Anal.* Calcd. for $C_{11}H_{11}O_2NS$: C, 59.71; H, 5.01; N, 6.33; S, 14.49. Found: C, 59.66; H, 4.89; N, 6.25; S, 14.61.

3-(3-Benzo[*b*]thiophene-1-dioxide)-L-alanine Hydrochloride (14)——**5** (2.4 g, 8.1 mmol) was suspended in water (50 ml) and dissolved by the addition of conc. NH_4OH. The pH was adjusted to 7.5. Acylase (0.2 g) was added and the reaction mixture was kept at 39° for 8 hr. The colorless precipitate was collected by filtration and washed with water and MeOH successively. This material was dissolved in 1N HCl (25 ml) and the insoluble material was filtered off. The filtrate was concentrated *in vacuo* and the residual solid was recrystallized from MeOH–ether: Yield 0.48 g (40%). mp 190—192° (dec.). $[\alpha]_D^{20}$ 20.2° (c=1.39, 0.3N HCl). UV $\lambda_{max}^{0.1N\ NaOH}$ nm (ε): 259.5 (6400), 304 (1600). UV $\lambda_{max}^{0.1N\ HCl}$ nm (ε): 307 (2000). Rf^{IV} 0.25. *Anal.* Calcd. for $C_{11}H_{11}O_4NS \cdot HCl \cdot 1/4H_2O$: C, 44.90; H, 4.28; N, 4.76; S, 10.90; Cl, 12.05. Found: C, 45.15; H, 4.06; N, 4.73; S, 10.60; Cl, 12.08.

3-(4-Biphenyl)-L-alanine Hydrochloride (15)——**9** (1.6 g, 5.6 mmol) was suspended in water (30 ml) and dissolved by the addition of 1N NaOH (5.6 ml). The pH was adjusted to 7.5 with 1N NaOH and the mixture was filled up to 50 ml with water. Acylase (0.6 g) was added and the reaction mixture was kept at 38° for 15 hr. The colorless precipitate was collected by filtration and washed with water. The enzyme (0.1 g) was added again to the filtrate and the incubation was continued for 16 hr. The precipitate was collected and washed with water. The combined powder (0.4 g) was dissolved in 1N HCl (15 ml) and the insoluble material was filtered off. The filtrate was concentrated *in vacuo* and the residual solid was recrystallized from MeOH–ether: Yield 0.35 g (49%). mp 235—236° (dec.). $[\alpha]_D^{20}$ 3.7° (c=1.08, 6N HCl–MeOH 1: 19). UV $\lambda_{max}^{0.1N\ NaOH}$ nm (ε): 253.5 (15200). Rf^{IV} 0.62. *Anal.* Calcd. for $C_{15}H_{15}O_2N \cdot HCl$: C, 64.83; H, 5.81; N, 5.04; Cl, 12.76. Found: C, 64.34; H, 5.77; N, 4.98; Cl, 12.74.

H-Nal(1)-OMe·HCl (16)——Thionyl chloride ($SOCl_2$; 1.1 ml) was added to MeOH (50 ml) at −20° with stirring. To this solution, **10** (1.08 g, 5 mmol) was added at the same temperature. The temperature was raised to room temperature and the mixture was allowed to stand for 5 days. The solvent was evaporated *in vacuo* and the residual solid was recrystallized from MeOH–ether to give colorless fine needles: Yield 1.2 g (90%). mp 178—179°. $[\alpha]_D^{22}$ 16.5° (c=1.02, MeOH). Rf^{IV} 0.71. *Anal.* Calcd. for $C_{14}H_{15}O_2N \cdot HCl$: C, 63.28; H, 6.07; N, 5.27; Cl, 13.34. Found: C, 62.73; H, 5.78; N, 5.07; Cl, 13.41.

H-Nal(2)-OMe·HCl (17)——A title compound was prepared from **11** (1.08 g, 5 mmol), $SOCl_2$ (1.1 ml) and MeOH (50 ml) in a similar manner to that described above. Recrystallized from MeOH–ether. Colorless fine needles: Yield 1.2 g (90%). mp 175—176°. $[\alpha]_D^{22}$ 2.4° (c=1.17, MeOH). Rf^{IV} 0.72. *Anal.* Calcd. for $C_{14}H_{15}O_2N \cdot HCl$: C, 63.28; H, 6.07; N, 5.27; Cl, 13.34. Found: C, 62.99; H, 6.17; N, 5.38; Cl, 13.48.

H-7-Azatrp-OMe·2HCl (18)——Prepared from **12** (204 mg, 1 mmol), $SOCl_2$ (0.22 ml) and MeOH (10 ml) in a similar manner to that described above. Recrystallized from MeOH–ether: Yield 240 mg (94%). mp 195—197°. $[\alpha]_D^{22}$ 20.5° (c=1.03, MeOH). Rf^{III} 0.65. *Anal.* Calcd. for $C_{11}H_{13}O_2N_3 \cdot 2HCl \cdot 1/4H_2O$: C, 44.54; H, 5.26; N, 14.16; Cl, 23.90. Found: C, 44.89; H, 5.40; N, 13.92; Cl, 23.58.

H-Bal-OMe·HCl (19)——Prepared from **13** (152 mg, 0.7 mmol), $SOCl_2$ (0.15 ml) and MeOH (10 ml) in a similar manner to that described above. Rf^{IV} 0.54. The product was used in the next step without further purification.

H-Bal(O_2)-OMe·HCl (20)——Prepared from **14** (253 mg, 0.86 mmol), $SOCl_2$ (0.22 ml) and MeOH (10 ml). Recrystallized from MeOH–ether: Yield 232 mg (87%). Rf^{IV} 0.65. The product was used without further purification.

H-Bip-OMe·HCl (21)——Prepared from **15** (253 mg, 1 mmol), $SOCl_2$ (0.46 ml) and MeOH (20 ml). The mixture was heated under reflux for 11 hr and concentrated *in vacuo*. The residual solid was recrystallized from MeOH–ether to give colorless fine needles: Yield 272 mg (89%). mp 201—203°. $[\alpha]_D^{22}$ 9.5° (c=1.01,

MeOH). Rf^{V} 0.43. *Anal.* Calcd. for $C_{16}H_{17}O_2N \cdot HCl$: C, 65.86; H, 6.22; N, 4.80; Cl, 12.15. Found: C, 65.57; H, 6.31; N, 4.87; Cl, 12.34.

<Glu-His-Nal(1)-OMe (22)——<Glu-His-N_2H_3[14] (0.9 g, 3.2 mmol) was suspended in DMF (24 ml) and 3.4N HCl-dioxane (3.2 ml, 10.9 mmol) was added at −60°. After the addition of isoamyl nitrite (0.41 g, 3.5 mmol), the temperature was raised to −20°. The mixture was stirred for 30 min at the same temperature to give a clear solution. The reaction temperature was dropped to −60° again and the mixture was neutralized with N-methylmorpholine (NMM; 1.28 g, 127 mmol). A solution of **16** (0.86 g, 3.2 mmol) and NMM (0.3 g) in DMF (2 ml) was added to this solution. The reaction mixture was stirred for 18 hr at 5°. The colorless precipitate was collected by filtration and washed successively with DMF, CH_2Cl_2 and MeOH: Yield 1.2 g (78%). mp 257—260° (dec.). $[\alpha]_D^{20}$ −45.0° (c=0.8, AcOH). UV λ_{max}^{AcOH} nm (ε): 272.3 (5800), 282.5 (7100), 293 (4900). Rf^{IV} 0.51. *Anal.* Calcd. for $C_{25}H_{27}O_5N_5 \cdot 1/4H_2O$: C, 62.29; H, 5.75; N, 14.52. Found: C, 62.02; H, 5.80; N, 14.40.

<Glu-His-Nal(2)-OMe (23)——Prepared from <Glu-His-N_2H_3 (0.9 g, 3.2 mmol) and **17** (0.86 g, 3.2 mmol) as described for the preparation of **22**: Yield 1.24 g (81%). mp 253—257° (dec.). $[\alpha]_D^{25}$ −5.4° (c=1.12, AcOH). UV λ_{max}^{AcOH} nm (ε): 268.5 (4200), 276 (4400), 285.5 (3000). Rf^{IV} 0.49. *Anal.* Calcd. for $C_{25}H_{27}O_5N_5$: C, 62.88; H, 5.70; N, 14.67. Found: C, 62.47; H, 5.70; N, 14.94.

<Glu-His-7-Azatrp-OMe (24)——A solution of **18** (208 mg, 0.7 mmol) and NMM (142 mg, 1.4 mmol) in DMF (5 ml) was added to a solution of <Glu-His-N_3 [prepared from <Glu-His-N_2H_3 (224 mg, 0.8 mmol), 3.4N HCl-dioxane (0.8 ml, 2.72 mmol), isoamyl nitrite (0.13 ml, 0.88 mmol), NMM (320 mg, 3.16 mmol) and DMF (6 ml)] at −60° with stirring. The stirring was continued at 5° for 21 hr. The solvent was evaporated *in vacuo* and the residue was triturated with CH_2Cl_2. The resulting colorless precipitate was collected and recrystallized from EtOH to give a gelatinous product. The product was washed with ether and dried: Yield 250 mg (77%). mp 205—207° (dec.). $[\alpha]_D^{20}$ 11.1° (c=1.03, DMF). UV λ_{max}^{AcOH} nm (ε): 293.3 (7400). Rf^{III} 0.56. *Anal.* Calcd. for $C_{22}H_{25}O_5N_7 \cdot H_2O$: C, 54.45; H, 5.61; N, 20.20. Found: C, 54.40; H, 5.73; N, 19.77.

<Glu-His-Bal-OMe (25)——A solution of **19** (190 mg, 0.7 mmol) and NMM (71 mg, 0.7 mmol) in DMF (5 ml) was added to a solution of <Glu-His-N_3 [Prepared from <Glu-His-N_2H_3 (224 mg, 0.8 mmol)] in DMF (6 ml) at −60°. The mixture was stirred at 5° for 43 hr. The solvent was evaporated *in vacuo* and the residue was dissolved in *n*-BuOH saturated with water (10 ml). This solution was washed with water (twice) and concentrated *in vacuo*. The residue was triturated with AcOEt (30 ml) and the resulting colorless precipitate was collected: Yield 271 mg (80%). mp 209—213° (dec.). $[\alpha]_D^{20}$ −23.0° (c=1.11, AcOH). UV λ_{max}^{AcOH} nm (ε): 260.5 (6700), 289.7 (3500), 299 (4000). Rf^{IV} 0.42. *Anal.* Calcd. for $C_{23}H_{25}O_5N_5S \cdot 3H_2O$: C, 51.39; H, 5.81; N, 13.02; S, 5.96. Found: C, 51.06; H, 5.38; N, 12.79; S, 5.72.

<Glu-His-Bal(O_2)-OMe (26)——A solution of **20** (232 mg, 0.8 mmol) and NMM (80 mg, 0.8 mmol) in DMF (5 ml) was added to a solution of <Glu-His-N_3 [prepared from <Glu-His-N_2H_3 (224 mg, 0.8 mmol)] in DMF (6 ml) at −60°. The reaction mixture was stirred for 20 hr at 5° and concentrated *in vacuo*. The residue was triturated with *n*-BuOH saturated with water. The resulting colorless precipitate was collected and washed successively with CH_2Cl_2, MeOH and ether. The filtrate was concentrated *in vacuo* and to this residue *n*-BuOH saturated with water (2 ml) was added to obtain the second precipitate. Combined precipitate was washed with CH_2Cl_2 and MeOH: Yield 307 mg (77%). mp 205—208°. $[\alpha]_D^{20}$ −39.4° (c=1.08. AcOH). UV λ_{max}^{AcOH} nm (ε): 302 (2300). Rf^{IV} 0.42. *Anal.* Calcd. for $C_{23}H_{25}O_7N_5S \cdot 3H_2O$: C, 48.50; H, 5.49; N, 12.29; S, 5.62. Found: C, 48.19; H, 5.64; N, 11.92; S, 5.33.

<Glu-His-Bip-OMe (27)——A solution of **21** (204 mg, 0.7 mmol) and NMM (71 mg, 0.7 mmol) in DMF (5 ml) was added to a solution of <Glu-His-N_3 [prepared from <Glu-His-N_2H_3 (224 mg, 0.8 mmol)] in DMF (6 ml) at −60°. The reaction mixture was stirred for 22 hr at 5°. The colorless precipitate was collected and washed successively with DMF, CH_2Cl_2 and ether: Yield 204 mg (58%). mp 278—281° (dec.). $[\alpha]_D^{20}$ −8.2° (c=1.04, AcOH). UV λ_{max}^{AcOH} nm (ε): 254 (15700). Rf^{IV} 0.54. *Anal.* Calcd. for $C_{27}H_{29}O_5N_5$: C, 64.40; H, 5.80; N, 13.91. Found: C, 64.26; H, 5.50; N, 14.17.

<Glu-His-Nal(1)-N_2H_3 (28)——Hydrazine hydrate (1.25 g, 25 mmol) was added to a suspension of **22** (1.2 g, 2.5 mmol) in DMF (60 ml). The mixture was stirred for 3 hr and allowed to stand overnight at room temperature. Additional stirring for 3.5 hr afforded a clear solution and stirring was continued for 6 hr. After the further addition of hydrazine hydrate (0.65 g, 13 mmol), the mixture was allowed to stand overnight. The solvent and the excess hydrazine hydrate were evaporated *in vacuo* to give a colorless solid. The residue was triturated with water (20 ml), and the obtained powder was washed with water and MeOH: Yield 0.7 g (58%). mp 230—232° (dec.). $[\alpha]_D^{22}$ −25.6° (c=0.98, DMF). UV λ_{max}^{AcOH} nm (ε): 272.7 (6100), 282.7 (7400), 293.5 (5100). Rf^{IV} 0.37. *Anal.* Calcd. for $C_{24}H_{27}O_4N_7$: C, 60.37; H, 5.70; N, 20.53. Found: C, 60.50; H, 5.84; N, 20.13.

<Glu-His-Nal(2)-N_2H_3 (29)——Hydrazine hydrate (2.3 g, 46.5 mmol) was added to a suspension of **23** (1.2 g, 2.5 mmol) in DMF (60 ml). The reaction mixture was stirred at room temperature for 4 days and concentrated *in vacuo* to give a gelatinous solid. The residue was triturated with water and the resulting colorless precipitate was collected by filtration: Yield 0.77 g (65%). mp 229—230° (dec.). $[\alpha]_D^{22}$ −18.8° (c=0.96, DMF). UV λ_{max}^{AcOH} nm (ε): 268 (4400), 276 (4500), 285.5 (3000). Rf^{IV} 0.38. *Anal.* Calcd. for $C_{24}H_{27}O_4N_7$: C, 60.37; H, 5.70; N, 20.53. Found: C, 60.16; H, 5.41; N, 20.73.

<Glu-His-7-Azatrp-N_2H_3 (30)——Hydrazine hydrate (250 mg, 5 mmol) was added to a solution of **24** (235 mg, 0.5 mmol) in DMF (2 ml). The mixture was stirred for 24 hr at room temperature and concentrated

in vacuo. The residue was triturated with EtOH (3 ml) and the resulting colorless precipitate was collected: Yield 169 mg (72%). mp 195—196°. $[\alpha]_D^{21}$ −14.7° (c=1.0, DMF). UV λ_{max}^{AcOH} nm (ε): 293 (8300). Rf^{IV} 0.13. *Anal.* Calcd. for $C_{21}H_{25}O_4N_9$: C, 53.95; H, 5.39; N, 26.79. Found: C, 54.13; H, 5.24; N, 27.08.

<Glu-His-Bal-N_2H_3 (31)——Hydrazine hydrate (240 mg, 4.8 mmol) was added to a solution of **25** (240 mg, 0.48 mmol) in DMF (4 ml). A small amount of insoluble material appearing on addition of hydrazine hydrate gradually redissolved during the stirring. The reaction mixture was stirred for 24 hr at room temperature and concentrated *in vacuo*. The residue was triturated with EtOH and the resulting precipitate was collected: Yield 153 mg (66%). mp 224° (dec.). $[\alpha]_D^{21}$ −32.4° (c=1.04, DMF). UV λ_{max}^{AcOH} nm (ε): 259 (5200), 290 (2800), 299 (3000). Rf^{IV} 0.23. *Anal.* Calcd. for $C_{22}H_{25}O_4N_7S$: C, 54.65; H, 5.21; N, 20.28; S, 6.63. Found C, 54.83; H, 5.34; N, 20.41; S, 6.40.

<Glu-His-Bal(O_2)-N_2H_3 (32)——Hydrazine hydrate (200 mg, 4 mmol) was added to a solution of **26** (248 mg 0.4 mmol) in DMF (4 ml). The mixture was stirred for 24 hr at room temperature and concentrated *in in vacuo*. The residue was triturated with EtOH and the resulting precipitate was collected: Yield 148 mg (72%). mp 190—193° (dec.). $[\alpha]_D^{21}$ −1.9° (c=0.78, DMF). UV λ_{max}^{AcOH} nm (ε): 262 (8600). Rf^{IV} 0.20. *Anal.* Calcd. for $C_{22}H_{25}O_6N_7S$: C, 51.25; H, 4.89; N, 19.02; S, 6.22. Found: C, 50.98; H, 5.01; N, 19.23; S, 6.01.

Reaction of <Glu-His-Bip-OMe with Hydrazine Hydrate——Hydrazine hydrate (500 mg, 5 mmol) was added to a suspension of **27** (300 mg, 0.5 mmol) in DMF (15 ml). The mixture was stirred for 2 days at room temperature. Further portions of hydrazine hydrate (1.0 g, 10 mmol) and DMF (15 ml) were then added. The reaction mixture was heated at 80° for 30 min and stirred for 5 days at room temperature. The insoluble starting material (127 mg) was filtered off and the filtrate was concentrated *in vacuo*. The residual solid was washed with EtOH and the colorless precipitate was collected. The product (116 mg) contained two components, <Glu-His-Bip-OMe and <Glu-His-Bip-N_2H_3.

<Glu-His-Bip-OH (33)——1N NaOH (0.4 ml, 0.4 mmol) was added to a suspension of **27** (101 mg, 0.2 mmol) in DMF (5 ml). The mixture was stirred for 1.5 hr at room temperature. The resulting clear solution was neutralized with 1N HCl and concentrated *in vacuo*. The residue was redissolved in a small amount of DMF (0.5 ml) and insoluble material was filtered off. The filtrate was concentrated *in vacuo* and the residue was triturated with AcOEt. The resulting colorless precipitate was collected by filtration: Yield 102 mg. The product exhibited a single spot on TLC (Rf^{IV} 0.33) and was used in the next step without further purification.

H-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2·2HF (34)——Z-Ser (Bu^t)-Tyr (Bu^t)-Gly-Leu-Arg(Tos)-Pro-Gly-NH_2[3] (2.0 g) was treated with anhydrous HF (30 ml) in the presence of anisole (5 ml) in an ice-bath for 30 min. The excess of HF was evaporated *in vacuo* at 0° and the residue was dissolved in water (50 ml). The solution was washed three times with AcOEt and the aqueous layer was lyophilized to give a fluffy powder: Yield 1.1 g. (92%). The deprotected heptapeptide amide was used in the next step without further purification.

Syntheses and Purification of Decapeptide Amides

General Procedure——<Glu-His-AA-N_2H_3 [0.2 mmol; AA=Nal(1) (**28**), Nal(2) (**29**), 7-Azatrp (**30**), Bal (**31**), Bal(O_2) (**32**)] was dissolved in DMF (6 ml), and 3.4N HCl-dioxane (0.2 ml, 0.68 mmol) was added at −60°. After the addition of isoamyl nitrite (0.04 ml, 0.22 mmol), the reaction temperature was raised to −20°. After the mixture was stirred at the same temperature for 30 min, the temperature was dropped to −60° again and NMM (80 mg, 0.79 mmol) was added. To this solution, a solution of H-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2·2HF (**34**, 155 mg, 0.2 mmol) and NMM (20 mg, 0.2 mmol) in DMF (0.5 ml) was added and the reaction mixture was stirred for 24 hr at 5°. The solvent was evaporated *in vacuo* to give a syrupy residue. The residue was triturated with CH_2Cl_2 (10 ml) and the resulting colorless precipitate was collected by filtration; Yield 200—230 mg. The crude decapeptides (100 mg) obtained were dissolved in the upper phase (1.5 ml) of one of the following solvent systems: A, *n*-BuOH-AcOH-CCl_4-H_2O (16: 4: 1: 20); B, *n*-BuOH-AcOH-CCl_4-H_2O (40: 10: 1: 50); C, *n*-BuOH-AcOH-H_2O (4: 1: 5); D, *n*-BuOH-0.1M ammonium acetate (1: 1). The solution was submitted to partition chromatography on Sephadex G-25 (2.5×90 cm) with collection of 8.6—9.6 ml fractions. The column operation was performed according to the method as described in our previous paper.[5]

Nal(1)[3]-LH-RH——Purified by the solvent system A. The eluate was collected in 8.6 ml portions and absorbancy at 280 nm was measured after the addition of MeOH (1 ml) to eliminate turbidity. The necessary fractions (No. 94—119) were combined and concentrated to dryness *in vacuo*. After the residue was dissolved in 0.1M AcOH (10 ml), the solution was treated with a small amount of active charcoal and lyophilized to give a fluffy powder: Yield 53.5 mg.

Nal(2)[3]-LH-RH——Purified by the solvent system A. In this case, the column size used was 2×88 cm. The eluate was collected in 9.4 ml portions and absorbancy at 275 nm was measured. The necessary fractions (No. 64—73) were combined and concentrated *in vacuo*. After the residue was dissolved in 0.1M AcOH (10 ml), the solution was treated with a small amount of active charcoal and lyophilized to give a fluffy powder: Yield 46.4 mg.

7-Azatrp[3]-LH-RH——Purified by the solvent system D. The eluate was collected in 9.6 ml portions and absorbancy at 285 nm was measured. The necessary fractions (No. 50—60) were combined and concentrated *in vacuo*. After the residue was dissolved in 0.1M AcOH (10 ml), the solution was treated with a small amount of active charcoal and lyophilized to give a fluffy powder: Yield 25.7 mg.

Bal^3-LH-RH——Purified by the solvent system C. The eluate was collected in 8.6 ml portions and absorbancy at 265 nm was measured. The neccessary fractions (No. 77—78) were combined and concentrated *in vacuo*. After the residue was dissolved in 0.1M AcOH (10 ml), the solution was treated with a small amount of active charcoal and lyophilized to give a fluffy powder: Yield 32.0 mg.

Bal(O_2)^3-LH-RH——Purified by the solvent system B. The eluate was collected in 9.2 ml portions and absorbancy at 260 nm was measured. The necessary fractions (No. 92—99) were combined and concentrated *in vacuo*. After the residue was dissolved in 0.1M AcOH (5 ml), the solution was treated with a small amount of active charcoal and lyophilized to give a fluffy powder: 12.0 mg.

Bip^3-LH-RH——DCCD (25 mg, 0.12 mmol) was added to a solution of <Glu-His-Bip-OH (33; 49 mg, 0.1 mmol), 34 (77.5 mg, 0.1 mmol), HOBt (25 mg, 0.12 mmol) and NMM (10 mg, 0.1 mmol) in DMF (5 ml) at 0°. The mixture was stirred for 2 hr at 0° and for 22 hr at room temperature. The precipitate (DC urea) was filtered off and the filtrate was concentrated *in vacuo* to give a syrupy residue. The residue was triturated with CH_2Cl_2 (10 ml) and the resulting colorless precipitate was collected by filtration: Yield 127 mg. The crude product (100 mg) was purified by the column partition chromatography on Sephadex G-25 (2.5×90 cm) in the solvent system A. The eluate was collected in 8.8 ml portions and absorbancy at 260 nm was measured. The necessary fractions (No. 61—80) were combined and concentrated *in vacuo*. After the residue was dissolved in 0.1M AcOH (10 ml), the solution was treated with a small amount of active charcoal and lyophilized to give a fluffy powder: Yield 50.3 mg.

Acknowledgement We are indebted to the National Institute of Arthritis, Metabolism and Digestive Diseases for the supply of rat LH immunoassay kit. Thanks are also due to Dr. G. Niswender for his generous supply of rabbit anti-sheep LH serum and Miss H. Miyagawa for her skillful technical assistances.