Notes

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**Synthesis and Biological Activity of LH-RH Analogs
substituted by Alkyltryptophans at Position 3¹⁾**

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Seven analogs of luteinizing hormone-releasing hormone (LH-RH), in which the Trp residue at position 3 was replaced by various methyltryptophans and 1-ethyltryptophan, were synthesized and their LH-releasing activities were evaluated. All of these analogs showed reduced hormonal activity compared with synthetic LH-RH.

Keywords—peptide; LH-RH analogs modifying the Trp residue; LH-releasing activity; structure-activity relationship; LH-RH methyltryptophan analogs; LH-RH 1-ethyltryptophan analog

Structure-activity studies on luteinizing hormone-releasing hormone (LH-RH) and its analogs, in which the Trp residue in position 3 was replaced by various aliphatic or aromatic amino acids,³⁾ suggest that the aromaticity of the Trp residue plays an important role in generating the full hormonal activity. Replacement of the Trp residue with Phe resulted in a marked decrease in activity (0.43%).^{3a)} However, we found that analogs substituted by the naphthalene ring, which has a fused ring structure possessing two benzene rings, retained high activity.⁴⁾ In particular, the potency of Nal(1)³-LH-RH was about twice that of the original LH-RH. Consequently, it is probable that the size as well as the aromaticity of the naphthalene ring in the Nal(1) residue is important, and that the naphthalene ring can fit well into the receptor site.

In order to investigate the relationship between the size of the aromatic ring and the biological activity and in the hope of obtaining more potent analogs by substitution of the Trp residue, we synthesized seven analogs substituted by various methyltryptophans and 1-ethyltryptophan.

The homologs used herein for substitution were prepared as follows. Trp(1-Me) and Trp(1-Et) were derived from Trp according to the published method.⁵⁾ The other amino acids, Trp(2-Me), Trp(4-Me), Trp(5-Me), Trp(6-Me) and Trp(7-Me), were obtained by enzymatic optical resolution of acetylated methyl-DL-tryptophans according to the procedure described previously.⁴⁾ L-Isomer were purified by column partition chromatography on Sephadex G-25,^{4,6)} followed by recrystallization from water or 60% aqueous EtOH.

- 1) Amino acids, peptides and their derivatives mentioned in this paper are of the L-configuration. Abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature in May 1971: *J. Biol. Chem.*, **247**, 977 (1972). Abbreviations of the less-common amino acids are as follows: Trp(1-Me), 1-methyl-L-tryptophan; Trp(2-Me), 2-methyl-L-tryptophan; Trp(4-Me), 4-methyl-L-tryptophan; Trp(5-Me), 5-methyl-L-tryptophan; Trp(6-Me), 6-methyl-L-tryptophan; Trp(7-Me), 7-methyl-L-tryptophan; Trp(1-Et), 1-ethyl-L-tryptophan; Nal(1), 3-(1-naphthyl)-L-alanine.
- 2) Location: 2-58, 1-chome, Hiromachi, Shinagawa-ku, Tokyo, 140, Japan.
- 3) a) 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); b) D.H. Coy, E.J. Coy, and A.V. Schally, *J. Med. Chem.*, **16**, 1140 (1973).
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The analogs containing these amino acids were synthesized by the conventional classical methods depicted in chart 1. For the syntheses of N-terminal tripeptide esters, $\langle\text{Glu-His-AA-OMe}\rangle$, $\langle\text{Glu-His-N}_2\text{H}_3\rangle$ was coupled with H-AA-OMe by Rudinger's azide method⁸⁾ as described previously.⁴⁾ The treatment of $\langle\text{Glu-His-AA-OMe}\rangle$ with excess hydrazine hydrate gave the corresponding tripeptide hydrazide, $\langle\text{Glu-His-AA-N}_2\text{H}_3\rangle$.

Chart 1

Condensation of the heptapeptide amide, H-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂,⁴⁾ with $\langle\text{Glu-His-AA-N}_3\rangle$ afforded the decapeptide amides, $\langle\text{Glu-His-AA-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH}_2\rangle$. The crude peptides obtained were purified by column partition chromatography on Sephadex G-25⁶⁾ using a solvent system consisting of *n*-BuOH-AcOH-H₂O (4:1:5). These purified analogs were characterized by amino acid analyses and from their specific optical rotations. The compounds each exhibited a single spot on a thin-layer chromatogram using three different solvent systems (Table I).

TABLE I. Physical Properties and Amino Acid Analyses of LH-RH
Analogues modified at Position 3

Compounds	$[\alpha]_D^{20}$ (<i>c</i> = 0.3, 0.1 N AcOH)	<i>R</i> _f ^{a)}			Amino acid analyses ^{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									
Trp(1-Me) ³⁻ LH-RH	-61.8	0.36	0.58	0.55	0.99	0.98	0.87	0.76	0.97	1.95	1.00	1.02	1.05
Trp(2-Me) ³⁻	-64.6	0.37	0.58	0.50	1.09	1.09	1.09	0.81	0.94	1.95	1.00	0.98	0.96
Trp(4-Me) ³⁻	-48.0	0.35	0.59	0.53	0.99	0.94	0.75	0.81	0.96	1.95	1.00	0.99	0.98
Trp(5-Me) ³⁻	-48.2	0.36	0.61	0.53	1.06	1.09	1.04	0.80	0.98	1.91	1.00	0.99	0.99
Trp(6-Me) ³⁻	-51.3	0.36	0.58	0.51	1.10	1.11	1.01	0.86	0.97	1.93	1.00	1.00	0.96
Trp(7-Me) ³⁻	-38.5	0.36	0.58	0.51	1.12	1.14	1.09	0.84	0.98	1.90	1.00	0.99	0.96
Trp(1-Et) ³⁻	-61.2	0.39	0.61	0.57	0.95	1.18	0.93	0.84	1.00	2.03	1.00	0.98	1.00

a) and b) See Experimental section.

c) AA indicates the particular amino acid replacing Trp. The elution volume for AA was as follows: Trp(1-Me) 1.04, Trp(2-Me) 1.07, Trp(4-Me) 1.12, Trp(5-Me) 1.08, Trp(6-Me) 1.12, Trp(7-Me) 1.07, Trp(1-Et) 1.17 (taking the elution volume for Lys as 1.00).

These LH-RH analogs were tested for LH-releasing activity and compared with synthetic LH-RH at two dose levels. The evaluation of LH-releasing activity was performed *in vivo*

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TABLE II. LH-releasing Activities of LH-RH Analogs modified at Position 3 as compared with Synthetic LH-RH

Compounds	% LH-releasing activity <i>in vivo</i> with 95% confidence limits
Synthetic LH-RH	Assumed 100%
Trp(1-Me) ³ -LH-RH	18.3% (8.4—39.8)
Trp(2-Me) ³ -LH-RH	10.9 (7.1—13.7)
Trp(4-Me) ³ -LH-RH	5.7 (2.0—13.7)
Trp(5-Me) ³ -LH-RH	3.6 (1.2—10.7)
Trp(6-Me) ³ -LH-RH	2.2 (0.7—6.6)
Trp(7-Me) ³ -LH-RH	9.0 (0.2—50.1)
Trp(1-Et) ³ -LH-RH	0.33 (0.22—0.66)

by stimulation of LH release in ovariectomized rats pretreated with estrogen and progesterone,⁹⁾ followed by radioimmunoassay for rat LH according to Niswender, *et al.*¹⁰⁾

The results are summarized in Table II. Contrary to expectation, all the analogs showed much lower activity than the original LH-RH. The potency of Trp(1-Me)³-LH-RH was highest among the ring methylated analogs, but replacement of the methyl group with an ethyl group at the 1-position in the indole ring resulted in a marked diminution in activity.

Experimental

Melting points are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. Thin-layer chromatography was performed on Merck Silica gel 60F₂₅₄. *R_f* values refer to the following solvent systems: *R_f^I* *n*-BuOH-AcOH-H₂O (60:15:25, v/v/v), *R_f^{II}* *n*-BuOH-AcOH-H₂O-pyridine (30:6:24:20), *R_f^{III}* CHCl₃-MeOH-32% aq. AcOH (60:45:20), *R_f^{IV}* CHCl₃-MeOH-32% aq. AcOH (6:3:1). DL-Trp(4-Me), DL-Trp(5-Me), DL-Trp(6-Me) and DL-Trp(7-Me) were purchased from Sigma Chemical Company. Amino acid analyses of peptides were carried out using samples that had been hydrolyzed with constant-boiling HCl containing 4% thioglycolic acid for 24 hr in evacuated, sealed tubes at 110°, and were made with a Hitachi KLA-2 amino acid analyzer.

N^α-Acetyl-methyl-DL-tryptophans—The methyl-DL-tryptophans were acetylated with acetic anhydride (Ac₂O) by the following procedure. Ac₂O (1.0 g, 9.5 mmol) and 1 *N* NaOH (9.5 ml) were added alternately in five portions to a solution of methyl-DL-tryptophan (2.0 g, 9.2 mmol) in 0.5 *N* NaOH (18.4 ml) over 30 min at 0°. The mixture was stirred at 0° for 2 hr, and then at 5° overnight. The reaction mixture was acidified with conc. HCl to pH 3 and the resulting precipitate was collected by filtration. Recrystallization from 30% aq. EtOH gave N^α-acetyl-methyl-DL-tryptophan.

N^α-Acetyl-2-methyl-DL-tryptophan (1)¹¹⁾—Colorless fine needles. Yield 1.35 g (56%). mp 204—206°. *R_f^{IV}* 0.71. *Anal.* Calcd. for C₁₄H₁₆N₂O₃: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.11; H, 6.15; N, 10.31.

N^α-Acetyl-4-methyl-DL-tryptophan (2)—Colorless fine needles. Yield 0.6 g (25%). mp 170—175°. *R_f^{IV}* 0.61. *Anal.* Calcd. for C₁₄H₁₆N₂O₃: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.36; H, 6.11; N, 10.63.

N^α-Acetyl-5-methyl-DL-tryptophan (3)—Colorless fine needles. Yield 1.35 g (56%). mp 160—161°. *R_f^{IV}* 0.66. *Anal.* Calcd. for C₁₄H₁₆N₂O₃: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.21; H, 6.14; N, 10.62.

N^α-Acetyl-6-methyl-DL-tryptophan (4)—Colorless minute leaflets. Yield 1.6 g (67%). mp 192—194°. *R_f^{IV}* 0.68. *Anal.* Calcd. for C₁₄H₁₆N₂O₃·1/4H₂O: C, 63.50; H, 6.19; N, 10.58. Found: C, 63.68; H, 6.09; N, 10.52.

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11) DL-Trp(2-Me) was prepared according to the published method (H.N. Rydon, *J. Chem. Soc.*, **1948**, 705).

N^α-Acetyl-7-methyl-DL-tryptophan (5)—Colorless fine needles. Yield 1.5 g (63%). mp 221—223° (dec.). R_f^{IV} 0.64. Anal. Calcd. for $C_{14}H_{16}N_2O_3$: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.68; H, 6.30; N, 10.86.

Methyl-L-tryptophans—The N^α-acetyl-methyl-DL-tryptophans were resolved with acylase (from *Aspergillus* genus, Tokyo Kasei Co.) according to the methods described in our previous paper.⁴⁾ N^α-Acetyl-methyl-DL-tryptophan (**1**, **2**, **3**, **4** or **5**; 1.3 g, 5 mmol) was suspended in water (50 ml) and dissolved by the addition of conc. NH_4OH . The pH was adjusted to 7.5. Acylase (0.1 g) was added and the reaction mixture was kept at 39° to 40° for 24 hr. Another portion of the enzyme (0.03 g) was added and the incubation was continued for 12 hr. The insoluble material was filtered off and the filtrate was concentrated to dryness *in vacuo*. The residue was dissolved in the upper phase (10 ml) of *n*-BuOH-AcOH-H₂O (16:1:20) and subjected to partition chromatography on Sephadex G-25 (3.5 × 50 cm), collecting 10 ml fractions. The earlier fractions (No. 16—25) contained undigested material and the later fractions (No. 37—105) yielded methyl-L-tryptophan.

2-Methyl-L-tryptophan (6)—Obtained from fractions (No. 55—90). Recrystallization from 60% aq. EtOH gave small leaflets: yield 0.1 g (16%). mp 224—226° (dec.). $[\alpha]_D^{20}$ -9.8° ($c=0.26$, H₂O). R_f^{IV} 0.36. Anal. Calcd. for $C_{12}H_{14}N_2O_2 \cdot H_2O$: C, 56.68; H, 7.13; N, 11.02. Found: C, 56.20; H, 7.34; N, 11.14.

4-Methyl-L-tryptophan (7)—Obtained from fractions (No. 37—72). Recrystallization from water gave small leaflets: yield 0.14 g (56%). mp 229—235° (dec.). $[\alpha]_D^{20}$ 3.3° ($c=1.0$, 0.6 N HCl). R_f^{IV} 0.35. Anal. Calcd. for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.83. Found: C, 66.40; H, 6.49; N, 12.35.

5-Methyl-L-tryptophan (8)—Obtained from fractions (No. 47—89). Recrystallization from water gave leaflets: yield 0.5 g (90%). mp 249—259° (dec.). $[\alpha]_D^{20}$ 10.1° ($c=1.19$, 1 N HCl). R_f^{IV} 0.35. Anal. Calcd. for $C_{12}H_{14}N_2O_2 \cdot 1/4H_2O$: C, 64.70; H, 6.56; N, 12.58. Found: C, 64.95; H, 6.39; N, 12.31.

6-Methyl-L-tryptophan (9)—Obtained from fractions (No. 54—105). Recrystallization from water gave small leaflets: yield 0.49 g (90%). mp 238—248° (dec.). $[\alpha]_D^{20}$ 5.7° ($c=1.2$, 1 N HCl). R_f^{IV} 0.36. Anal. Calcd. for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.83. Found: C, 66.15; H, 6.41; N, 12.71.

7-Methyl-L-tryptophan (10)—Obtained from fractions (No. 56—95). Recrystallization from water gave small leaflets: yield 0.26 g (44%). mp 237—241° (dec.). $[\alpha]_D^{20}$ 2.5° ($c=1.18$, 1 N HCl). R_f^{IV} 0.36. Anal. Calcd. for $C_{12}H_{14}N_2O_2 \cdot H_2O$: C, 61.00; H, 6.83; N, 11.86. Found: C, 60.56; H, 6.24; N, 11.61.

<Glu-His-Trp(1-Me)-OMe (11)—<Glu-His-N₂H₃ (0.45 g, 1.6 mmol) was suspended in dimethylformamide (DMF) (12 ml) and 3.4 N HCl-dioxane (1.6 ml, 5.44 mmol) was added at -60°. After the addition of isoamyl nitrite (0.26 ml, 1.76 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 reduced to -60° again and the mixture was neutralized with N-methylmorpholine (NMM; 0.64 g, 6.4 mmol). A solution of H-Trp(1-Me)-OMe·HCl¹³⁾ (0.43 g, 1.6 mmol) and NMM (0.16 g, 1.6 mmol) in DMF (12 ml) was added to this solution. The reaction mixture was stirred for 17 hr at 5° and concentrated to dryness *in vacuo*. The residue was dissolved in *n*-BuOH saturated with water (30 ml) and the solution was washed with water (twice). The organic layer was concentrated to a small volume (about 2 ml) and CH₂Cl₂ (10 ml) was added. The resulting colorless precipitate was collected by filtration: yield 0.63 g (75%). mp 177—180°. $[\alpha]_D^{20}$ 2.3° ($c=1.15$, DMF). R_f^{IV} 0.52. Anal. Calcd. for $C_{24}H_{28}N_6O_5 \cdot 5/2H_2O$: C, 54.85; H, 6.33; N, 15.99. Found: C, 55.00; H, 6.62; N, 15.81.

<Glu-His-Trp(1-Et)-OMe (12)—Prepared from <Glu-His-N₂H₃ (0.45 g, 1.6 mmol) and H-Trp(1-Et)-OMe·HCl¹³⁾ (0.46 g, 1.6 mmol) as described for the preparation of **11**: yield 0.53 g (61%). mp 129—134°. $[\alpha]_D^{20}$ -5.0° ($c=1.22$, DMF). R_f^{IV} 0.53. Anal. Calcd. for $C_{25}H_{30}N_6O_5 \cdot H_2O$: C, 54.74; H, 6.61; N, 15.32. Found: C, 54.93; H, 6.35; N, 14.92.

<Glu-His-Trp(2-Me)-OMe (13)—Prepared from <Glu-His-N₂H₃ (0.17 g, 0.6 mmol) and H-Trp(2-Me)-OMe·HCl¹²⁾ (0.13 g, 0.5 mmol) as described for the preparation of **11**: yield 0.14 g (84%). R_f^{IV} 0.44. The product was used in the next step without further purification.

<Glu-His-Trp(4-Me)-OMe (14)—Prepared from <Glu-His-N₂H₃ (0.14 g, 0.5 mmol) and H-Trp(4-Me)-OMe·HCl¹²⁾ (0.13 g, 0.5 mmol) as described for the preparation of **11**: yield 0.21 g (78%). mp 217—219° (dec.). $[\alpha]_D^{21}$ 3.3° ($c=0.3$, DMF). R_f^{IV} 0.43. Anal. Calcd. for $C_{24}H_{28}N_6O_5 \cdot 4H_2O$: C, 52.17; H, 6.57; N, 15.21. Found: C, 52.12; H, 6.27; N, 15.25.

<Glu-His-Trp(5-Me)-OMe (15)—Prepared from <Glu-His-N₂H₃ (0.22 g, 0.8 mmol) and H-Trp(5-Me)-OMe·HCl¹²⁾ (0.21 g, 0.8 mmol) as described for the preparation of **11**: yield 0.26 g (62%). mp 225—227° (dec.). $[\alpha]_D^{21}$ 2.3° ($c=0.3$, DMF). R_f^{IV} 0.43. Anal. Calcd. for $C_{24}H_{28}N_6O_5 \cdot 5/2H_2O$: C, 54.85; H, 6.33; N, 15.99. Found: C, 54.93; H, 6.07; N, 15.71.

<Glu-His-Trp(6-Me)-OMe (16)—Prepared from <Glu-His-N₂H₃ (0.22 g, 0.8 mmol) and H-Trp(6-Me)-OMe·HCl¹²⁾ (0.21 g, 0.8 mmol) as described for the preparation of **11**: yield 0.27 g (64%). mp 214—216° (dec.). $[\alpha]_D^{21}$ 4.3° ($c=0.35$, DMF). R_f^{IV} 0.44. Anal. Calcd. for $C_{24}H_{28}N_6O_5 \cdot 5/2H_2O$: C, 54.85; H, 6.33; N, 15.99. Found: C, 54.54; H, 6.72; N, 15.99.

12) Compounds **6**, **7**, **8**, **9** and **10** were esterified in the usual manner using thionyl chloride and MeOH. The product in each case was used in the next step without further purification.

<Glu-His-Trp(7-Me)-OMe (17)——Prepared from <Glu-His-N₂H₃ (0.22 g, 0.8 mmol) and H-Trp-(7-Me)-OMe·HCl¹²⁾ (0.21 g, 0.8 mmol) as described for the preparation of 11: yield 0.28 g (62%). mp 222° (dec.). $[\alpha]_D^{21}$ 4.6° ($c=0.24$, DMF). Rf^{IV} 0.44. Anal. Calcd. for C₂₄H₂₈N₆O₅·5H₂O: C, 50.52; H, 6.71; N, 14.72. Found: C, 50.94; H, 6.80; N, 14.35.

<Glu-His-Trp(1-Me)-N₂H₃ (18)——Hydrazine hydrate (0.5 g, 10 mmol) was added to a solution of 11 (0.48 g, 0.9 mmol) in DMF (10 ml) and the mixture was stirred at room temperature for 24 hr. The colorless precipitate was collected by filtration and washed with DMF and AcOEt: yield 0.28 g (65%). mp 248—250° (dec.). $[\alpha]_D^{16}$ -16.2° ($c=1.1$, DMF). Rf^{IV} 0.28. Anal. Calcd. for C₂₃H₂₈N₈O₄: C, 57.49; H, 5.87; N, 23.32. Found: C, 57.40; H, 5.98; N, 23.31.

<Glu-His-Trp(1-Et)-N₂H₃ (19)——Hydrazine hydrate (0.4 g, 8 mmol) was added to a solution of 12 (0.4 g, 0.73 mmol) in DMF (8 ml) and the mixture was stirred at room temperature for 24 hr. The clear solution was concentrated *in vacuo* to give a solid residue. The residue was triturated with a mixture of AcOEt (6 ml) and EtOH (2 ml) and collected: yield 0.22 g (55%). mp 216—217° (dec.). $[\alpha]_D^{16}$ -15.9° ($c=0.87$, DMF). Rf^{IV} 0.32. Anal. Calcd. for C₂₄H₃₀N₈O₄: C, 58.29; H, 6.11; N, 22.67. Found: C, 58.41; H, 6.02; N, 22.53.

<Glu-His-Trp(2-Me)-N₂H₃ (20)——This compound was prepared from 13 (0.14 g, 0.3 mmol) and hydrazine hydrate (0.15 g, 3 mmol) as described for the preparation of 19: yield 88 mg (63%). mp 163—165°. $[\alpha]_D^{21}$ -1.7° ($c=0.2$, DMF). Rf^{IV} 0.19. Anal. Calcd. for C₂₃H₂₈N₈O₄: C, 57.49; H, 5.87; N, 23.32. Found: C, 57.63; H, 5.62; N, 23.19.

<Glu-His-Trp(4-Me)-N₂H₃ (21), <Glu-His-Trp(5-Me)-N₂H₃ (22), <Glu-His-Trp(6-Me)-N₂H₃ (23) and <Glu-His-Trp(7-Me)-N₂H₃ (24)——These compounds were prepared from 14, 15, 16 or 17 (0.2 g, about 0.4 mmol) and hydrazine hydrate (0.2 g, 4 mmol) as described for the preparation of 19: 21, yield 0.1 g (50%). mp 159—162°. $[\alpha]_D^{21}$ -7.7° ($c=0.3$, DMF). Rf^{IV} 0.19. Anal. Calcd. for C₂₃H₂₈N₈O₄: C, 57.49; H, 5.87; N, 23.32. Found: C, 57.78; H, 6.09; N, 23.40. 22, yield 0.13 g (65%). mp 161—169°. $[\alpha]_D^{21}$ -5.6° ($c=0.3$, AcOH). Rf^{IV} 0.20. Anal. Calcd. for C₂₃H₂₈N₈O₄: C, 57.49; H, 5.87; N, 23.32. Found: C, 57.35; H, 6.03; N, 23.25. 23, yield 0.13 g (65%). mp 153—162°. $[\alpha]_D^{21}$ -4.7° ($c=0.7$, DMF). Rf^{IV} 0.19. Anal. Calcd. for C₂₃H₂₈N₈O₄: C, 57.49; H, 5.87; N, 23.32. Found: C, 57.28; H, 5.94; N, 23.42. 24, yield 0.12 g (60%). mp 163—167°. $[\alpha]_D^{21}$ -6.0° ($c=0.4$, AcOH). Rf^{IV} 0.19. Anal. Calcd. for C₂₃H₂₈N₈O₄: C, 57.49; H, 5.87; N, 23.32. Found: C, 57.32; H, 5.69; N, 23.20.

Trp(1-Me)³-, Trp(1-Et)³-, Trp(2-Me)³-, Trp(4-Me)³-, Trp(5-Me)³-, Trp(6-Me)³- and Trp(7-Me)³-LH-RH (25, 26, 27, 28, 29, 30 and 31)——<Glu-His-AA-N₂H₃ (18—24; 0.1 mmol) was dissolved in DMF (5 ml), and 3.4 N HCl-dioxane (0.1 ml, 0.34 mmol) was added at -60°. After the addition of isoamyl nitrite (0.02 ml, 0.11 mmol), the reaction mixture was raised to -20° and the mixture was stirred at the same temperature for 30 min. The temperature was then reduced to -60° again and NMM (40 mg, 0.4 mmol) was added. A solution of H-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂·2HF⁴⁾ (78 mg, 0.1 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 removed under reduced pressure to give a syrupy residue. The residue was triturated with CH₂Cl₂ (6 ml) and the resulting colorless precipitate was collected by filtration. Yield 100—110 mg. The crude decapeptides (100 mg) obtained were dissolved in the upper phase (1.5 ml) of *n*-BuOH-AcOH-H₂O (4:1:5) and the solution was subjected to partition chromatography on Sephadex G-25 (2.5×90 cm). The eluate was collected in 8 ml portions and the absorbancy at 280 nm was measured after the addition of MeOH (1 ml) to eliminate turbidity. Appropriate fractions (25, No. 61—78; 26, No. 51—60; 27, No. 76—87; 28, No. 61—71; 29, No. 63—80; 30, No. 56—69; 31, No. 64—82) were combined and concentrated to dryness *in vacuo*. The residue was dissolved in 0.1 M AcOH (10 ml), then the solution was treated with a small amount of active charcoal and lyophilized to give a fluffy powder: yield 25, 47.4 mg; 26, 27.6 mg; 27, 24.3 mg; 28, 24.8 mg; 29, 41.8 mg; 30, 39.3 mg; 31, 44.2 mg.

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