[Chem. Pharm. Bull. 23(9)2033—2038(1975)]

UDC 547.466.1.057 : 615.357.011.5

Synthesis of Some Di- and Tri-Substituted Analogs of Luteinizing Hormone Releasing Hormone (LH-RH)[1]

Shigeru Kobayashi, Susumu Shinagawa, Mikihiko Obayashi, Tsunehiko Fukuda and Masahiko Fujino

Medicinal Research Laboratories, Central Research Division, Takeda Chemical Industries, Ltd.[2]

(Received January 22, 1975)

Eight new di- and tri-substituted luteinizing hormone releasing hormone (LH-RH) analogs, *i.e.*, des-Gly^{10}-[Thr^{4}]-LH-RH-ethylamide, des-Gly^{10}-[Phe^{5}]-LH-RH-ethylamide, des-Gly^{10}-[Phe^{5}, Ile^{7}]-LH-RH-ethylamide, des-Gly^{10}-[Nle^{7}]-LH-RH-ethylamide, des-Gly^{10}-[Met^{7}]-LH-RH-ethylamide, des-Gly^{10}-[Orn^{8}]-LH-RH-ethylamide, des-Gly^{10}-[Lys^{8}]-LH-RH-ethylamide and des-Gly^{10}-[Gly^{9}]-LH-RH-ethylamide, were synthesized by the solution method, and tested *in vitro* for the ability to induce secretion of luteinizing hormone. On the basis of structure-activity relations of these analogs, it is concluded that the effect of several structural changes in the LH-RH-molecule on its hormonal activity is "additive".

Since the elucidation of the structure of luteinizing hormone releasing hormone (LH-RH) as a decapeptide amide, <Glu–His–Trp–Ser–Tyr–Gly–Leu–Arg–Pro–Gly–NH_2 [I],[3] several syntheses of LH-RH analogs have been reported and structure-activity relations have been discussed for this clinically important peptide.[4] We recently reported that replacement of the C-terminal Gly–NH_2 of the hormone by various alkylamines resulted in retention of hormonal activity[5] and one of these alkylamine-substituted analogs, des-Gly^{10}–LH-RH-ethylamide [II], was found to be more than 5 times as potent as the synthetic LH-RH standard in the ovulation-inducing assay.[6] We also found that the analog has considerably more prolonged action in proestrous rat than LH-RH itself.[7]

This paper describes the synthesis and biological evaluation of eight di- and tri-substituted analogs with structures based on the highly potent analog II to obtain more detailed information regarding structure-activity relations of the LH-RH molecule. The newly synthesized analogs are des-Gly^{10}-[Thr^{4}]-LH-RH-ethylamide [III], des-Gly^{10}-[Phe^{5}]-LH-RH-ethylamide [IV], des-Gly^{10}-[Phe^{5}, Ile^{7}]-LH-RH-ethylamide [V], des-Gly^{10}-[Nle^{7}]-LH-RH-ethylamide [VI], des-Gly^{10}-[Met^{7}]-LH-RH-ethylamide [VII], des-Gly^{10}-[Orn^{8}]-LH-RH-ethylamide [VIII], des-Gly^{10}-[Lys^{8}]-LH-RH-ethylamide [IX] and des-Gly^{10}-[Gly^{9}]-LH-RH-ethylamide [X].

---

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 of Biochemistry Nomenclature in July 1965 and July 1966; *Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967). Other abbreviations used are IBOC=*l*-isobornyloxycarbonyl, NH-Et=ethylamide, NO_2=nitro, HONB=N-hydroxy-5-norbornene-2,3-dicarboximide, DCC=N,N′-dicyclohexylcarbodiimide, ODNP=2,4-dinitrophenyl ester.

2) Location: *Juso-Honmachi, Yodogawa-ku, Osaka, 532, Japan.*

3) H. Matsuo, Y. Baba, R.M.G. Nair, A. Arimura and A.V. Schally, *Biochem. Biophys. Res. Commun.*, **43**, 1334 (1971).

4) A recent review: W.F. White, "Annual Report in Medicinal Chemistry," Vol. 8, ed. R.V. Heinzelman, Academic Press, Inc., New York, N.Y., 1973, p. 204.

5) M. Fujino, S. Kobayashi, M. Obayashi, S. Shinagawa, T. Fukuda, C. Kitada, R. Nakayama, I. Yamazaki, W.F. White and R.H. Rippel, *Biochem. Biophys. Res. Commun.*, **49**, 863 (1972).

6) M. Fujino, S. Shinagawa, I. Yamazaki, S. Kobayashi, M. Obayashi, T. Fukuda, R. Nakayama, W.F. White and R.H. Rippel, *Arch. Biochem. Biophys.*, **154**, 488 (1973).

7) R.H. Rippel, E.S. Johnson, W.F. White, M. Fujino, I. Yamazaki and R. Nakayama, *Endocrinology*, **93**, 1449 (1973).

The essential strategy for synthesizing the peptide analogs shown in Fig. 1 has been described in detail previously.[8]

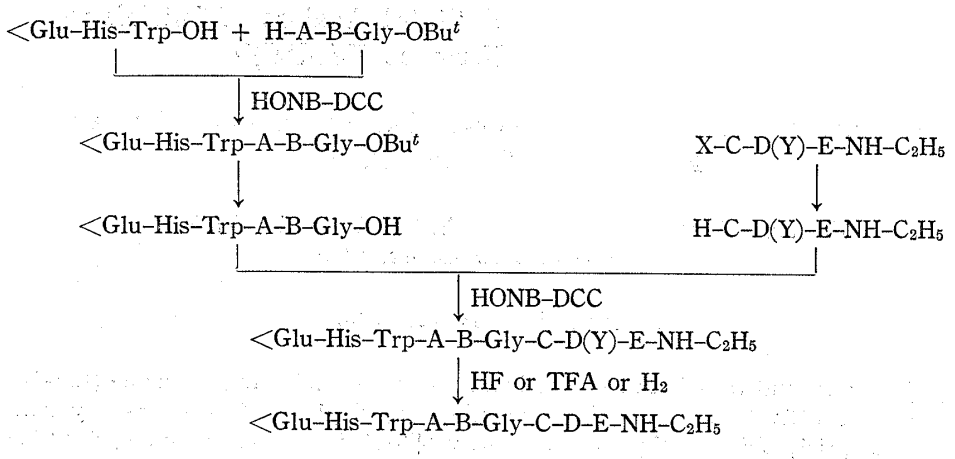

Fig. 1. Syntheses of LH-RH Analogs

The amino acid residues (A—E) are those of compound II unless otherwise stated (<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-NH-C_2H_5). X=Z or Boc; D(Y)=Arg(NO_2) or Orn(Z) or Lys(IBOC).
III, A=Thr; IV, B=Phe; V, B=Phe, C=Ile; VI, C=Nle; VII, C=Met; VIII, D=Orn; IX, D=Lys; X, E=Gly

Both intermediates, C-terminal tripeptides and N-terminal hexapeptides, were prepared by the conventional solution method using HONB activated ester.[8] The physicochemical properties of the intermediates are listed in Table I.

Table I. Physicochemical Properties of Intermediates

Compound	mp (°C)	$[\alpha]_D$ (temp., conc., solvent)	TLC[a] Rf^1	Formula	Analysis (%) Calcd. (Found) C	H	N
<Glu-His-Trp-Thr-Tyr-Gly-OBu^t (XIV)	—	−28.6° (23, 0.5, MeOH)	0.64[b]	$C_{41}H_{51}O_{10}N_9 \cdot 2H_2O$	57.17 (57.21)	6.43 (6.05)	14.02 (14.11)
<Glu-His-Trp-Ser-Phe-Gly-OBu- (XV)	—	−20.6° (23, 1.0, DMF)	0.23[c]	$C_{40}H_{49}O_9N_9 \cdot 2H_2O$	58.41 (58.59)	6.21 (6.52)	18.41 (18.38)
Z-Leu-Arg(NO_2)-Pro-NH-C_2H_5 (XXV)	144—146	−58.0° (24, 1.0, MeOH)	0.40	$C_{27}H_{42}O_7N_8 \cdot H_2O$	53.28 (53.30)	7.28 (6.99)	18.41 (18.07)
Z-Ile-Arg(NO_2)-Pro-NH-C_2H_5 (XVI)	103—105	−58.1° (23, 1.0, MeOH)	0.48	$C_{27}H_{42}O_7N_8 \cdot 1/2H_2O$	54.08 (53.80)	7.23 (7.15)	18.68 (18.84)
Z-Nle-Arg(NO_2)-Pro-NH-C_2H_5 (XVII)	109—110	−50.4° (22, 0.5, MeOH)	0.41	$C_{27}H_{42}O_7N_8 \cdot 1/2H_2O$	54.08 (53.79)	7.23 (7.09)	18.68 (18.34)
Boc-Met-Arg($NO)_2$-Pro-NH-C_2H_5 (XVIII)	101—104	−64.4° (22, 1.0, MeOH)	0.50	$C_{23}H_{42}O_7N_8S \cdot 1/2H_2O$	47.37 (47.88)	7.42 (7.23)	19.20 (18.94)
Boc-Leu-Orn(Z)-Pro-NH-C_2H_5 (XIX)	83— 87	−55.6° (23, 1.0, MeOH)	0.59	$C_{31}H_{49}O_7N_5 \cdot 1/2H_2O$	60.96 (61.11)	8.25 (8.20)	11.47 (11.10)
Z-Leu-Lys(IBOC)-Pro-NH-C_2H_5 (XX)	73— 75 (decomp.)	−56.9° (22, 1.0 EtOH)	0.80	$C_{38}H_{59}O_7N_5 \cdot 1/2H_2O$	64.56 (64.36)	8.55 (8.67)	9.91 (9.36)
Z-Leu-Arg(NO_2)-Gly-NH-C_2H_5 (XXIII)	105—109	−17.1° (23, 1.0, MeOH)	0.37	$C_{24}H_{38}O_7N_8 \cdot 1/2H_2O$	51.51 (51.61)	7.02 (7.05)	20.26 (20.55)

a) See Experimental. b) Rf^4 c) Rf^2

Coupling of N-terminal hexapeptides and C-terminal tripeptides was achieved by the HONB/DCC method,[8] and the resulting protected nonapeptides were purified by column chromatography on Amberlite XAD-2 in a manner similar to that described for our synthesis

8) M. Fujino, S. Kobayashi, M. Obayashi, T. Fukuda, S. Shinagawa and O. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **22** 1857 (1974).

of the other LH-RH analogs.[9] The purified protected peptides were deblocked by treatment with hydrogen fluoride[10] or trifluoroacetic acid,[11] or by catalytic hydrogenation using palladium black as catalyst. The resulting crude peptides thus obtained were purified by column chromatography on carboxymethylcellulose and Amberlite XAD-2, and the products were finally desalted on a column of Sephadex LH-20. All the peptide analogs obtained were chromatographically pure and gave the correct amino acid ratios. The physicochemical properties of the analogs synthesized are listed in Table II.

Table II. Physicochemical Properties of LH-RH Analogs

Analog III:	<Glu–His–Trp–Thr–Tyr–Gly–Leu–Arg–Pro–NH–C_2H_5 $[\alpha]_D^{23}$ −53.0°[a]; Rf^3 0.55; Rf^4 0.32 amino acid analysis: His 1.02; Arg 1.02; Trp 0.87; Thr 0.90; Glu 1.04; Pro 1.00; Gly 1.00; Leu 1.02; Tyr 0.90; ethylamine 1.01 (95%)[b]
Analog IV:	<Glu–His–Trp–Ser–Phe–Gly–Leu–Arg–Pro–NH–C_2H_5 $[\alpha]_D^{23}$ −58.3°[a]; Rf^3 0.66; Rf^4 0.37 amino acid analysis: His 1.03; Arg 1.03; Trp 0.92; Ser 0.89; Glu 0.92; Pro 0.96; Gly 0.92; Leu 1.00; Phe 0.96; ethylamine 0.96 (79.2%)[b]
Analog V:	Glu–His–Trp–Ser–Phe–Gly–Ile–Arg–Pro–NH–C_2H_5 $[\alpha]_D^{23}$ −59.4°[a]; Rf^3 0.63; Rf^4 0.41 amino acid analysis: His 1.10; Arg 1.10; Trp 0.87; Ser 0.81; Glu 1.03; Pro 100.; Gly 1.00; Ile 1.06; Phe 0.97; ethylamine 0.95 (82.0%)[b]
Analog VI:	<Glu–His–Trp–Ser–Tyr–Gly–Nle–Arg–Pro–NH–C_2H_5 $[\alpha]_D^{22}$ −52.2°[a]; Rf^3 0.54; Rf^4 0.44 amino acid analysis: His 1.00; Arg 1.02; Trp 1.00; Ser 0.91; Glu 1.00; Pro 1.05; Gly 0.98; Tyr 1.00; Nle 1.02; ethylamine 1.01 (82.5%)[b]
Analog VII:	<Glu–His–Trp–Ser–Tyr–Gly–Met–Arg–Pro–NH–C_2H_5 $[\alpha]_D^{23}$ −43.2°[a]; Rf^3 0.50; Rf^4 0.44 amino acid analysis: His 0.96; Arg 0.96; Trp 0.84; Ser 0.84; Glu 0.96; Pro 1.12; Gly 1.00; Met 0.92; Tyr 0.96; ethylamine 1.04 (95.0%)[b]
Analog VIII:	<Glu–His–Trp–Ser–Tyr–Gly–Leu–Orn–Pro–NH–C_2H_5 $[\alpha]_D^{23}$ −51.4°[a]; Rf^3 0.60; Rf^4 0.33 amino acid analysis: His 1.00; Trp 0.84; Orn 0.88; Ser 0.96; Glu 0.96; Pro 1.00; Gly 1.00; Leu 0.96; Tyr 0.84; ethylamine 1.15 (85.0%)[b]
Analog IX:	<Glu–His–Trp–Ser–Tyr–Gly–Leu–Lys–Pro–NH–C_2H_5 $[\alpha]_D^{21}$ −55.2°[a]; Rf^3 0.49; Rf^4 0.35 amino acid analysis: Lys 1.00; His 0.96; Trp 0.88; Ser 0.88; Glu 0.96; Pro 0.96; Gly 1.00; Leu 0.92; Tyr 0.96; ethylamine 1.12 (92%)[b]
Analog X:	<Glu–His–Trp–Ser–Tyr–Gly–Leu–Arg–Gly–NH–C_2H_5 $[\alpha]_D^{22}$ −31.8°[a]; Rf^3 0.51; Rf^4 0.40 amino acid analysis: His 0.80; Arg 0.96; Trp 0.92; Ser 0.90; Glu 1.00; Gly 2.08; Leu 1.04; Tyr 1.06; ethylamine 0.95 (85.5%)[b]

a) *c* 0.5, 5% AcOH b) peptide content

To elucidate the biological properties of these analogs, the *in vitro* LH-releasing activities were measured by a modification of the method of Mittler and Meites[12] using hemisected anterior pituitaries from rats. The methodology employed in these assays has been previously described in detail by White, *et al.*[13]

As can be seen in Table III, analogs IV, VI and VII are more potent than LH-RH itself, and moreover, analog V in which three amino acid residues at positions 5, 7 and 10 are replaced

9) M. Fujino, S. Shinagawa, M. Obayashi, S. Kobayashi, T. Fukuda, I. Yamazaki, R. Nakayama, W.F. White and R.H. Rippel, *J. Med. Chem.*, **16**, 1144 (1973).
10) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada and H. Sugihara, *Bull. Chem. Soc. Japan*, **40**, 2164 (1967).
11) M. Fujino, S. Shinagawa, O. Nishimura and T. Fukuda, *Chem. Pharm. Bull.* (Tokyo), **20**, 1017 (1972).
12) J.C. Mittler and J. Meites, *Proc. Soc. Exp. Biol. Med.*, **117**, 309 (1964).
13) W.F. White, M.T. Hedlund, R.H. Rippel, W. Arnold and G.R. Flouret, *Endocrinology*, **78**, 96 (1973).

by the other analogous residues, still released significant LH. From the results in Table III and those of the corresponding monosubstituted analogs reported previously from this laboratory,[14] we concluded that the effect of several structural changes in the LH-RH molecule on its hormonal activity is generally "additive."

This information is very important for synthesizing a more potent agonist or effective antagonist, and very intense agonists, des-Gly^{10}-[D-Ala^{6}]-LH-RH-ethylamide[15] and des-Gly^{10}-[D-Leu^{6}]-LH-RH-ethylamide,[16] have been prepared along this line.

Table III. Relative Activity of LH-RH Analogs[a]

Analog	LH release (*in vitro*) %	Analog	LH release (*in vitro*) %
LH-RH[b]	100	VII	280—330
Analog III	38— 42	VIII	35— 42
IV	190	IX	20
V	100—190	X	5
VI	220—230		

a) These assays were performed by Drs. White and Rippel of Abbott Laboratories.
b) standard

Experimental

Melting points were taken in open capillaries and are uncorrected. Rotations were determined with a Perkin-Elmer Model 141 polarimeter. Amino acid analyses were performed on a Hitachi KLA-3B amino acid analyzer. Acid hydrolyses were carried out according to the method of Matsubara and Sasaki.[17] Thin-layer chromatography (TLC) was run on Silica gel G (Merck) in the following solvent systems: Rf^1 $CHCl_3$–MeOH–AcOH (18: 2: 1); Rf^2 EtOAc–pyridine–AcOH–H_2O (60: 20: 6: 11); Rf^3 *n*–BuOH–pyridine–AcOH–H_2O (15: 10: 3: 12); Rf^4 *n*–BuOH–EtOAc–AcOH–H_2O (1: 1: 1: 1). Evaporations were carried out in rotatory evaporators under reduced pressure at a temperature of 40—45°. Catalytic hydrogenations were performed at room temperature with palladium black as catalyst.

Z-Thr-Tyr-Gly-OBu^t (XI)——Z-Thr-OH (2.53 g, 10 mmoles), H-Tyr-Gly-OBu^t[8] (2.94 g, 10 mmoles), HONB (1.97 g, 11 mmoles), and DCC (2.27 g, 11 mmoles) were combined in a mixture of dioxane–EtOAc (20 ml, 3: 1 v/v) at 0°. The solution was stirred at 0° for 3 hr and at room temperature for 16 hr. After filtration and evaporation, the residue was extracted with EtOAc (100 ml). The extract was washed with 0.5N HCl, and 5% $NaHCO_3$, then dried (Na_2SO_4). After evaporation, the residue was purified by reprecipitation from EtOAc–Pet. benzine: 3.74 g (70.6%); mp 119—120°; $[\alpha]_D^{23}$ −26.9° (*c* 1.0, MeOH); Rf^1 0.20. *Anal.* Calcd. for $C_{27}H_{35}O_8N_3$: C, 61.23; H, 6.66; N, 7.94. Found: C, 61.01; H, 6.63; N, 7.57.

Z-Phe-Gly-OBu^t (XII)——Z-Phe-OSu (3.96 g, 10 mmoles) and H-Gly-OBu^t (1.57 g, 12 mmoles) in EtOAc were stirred at room temperature for 12 hr. The reaction mixture was washed with 5% $NaHCO_3$, 1N HCl, and water, then dried ($MgSO_4$). Evaporation of the solvent yielded an oily residue which crystallized upon addition of EtOAc–pet. ether: 3.50 g (85.5%); mp 78—79°; $[\alpha]_D^{23}$ −16.7° (*c* 1.0, EtOH); Rf^1 0.72. *Anal.* Calcd. for $C_{23}H_{28}O_5N_2$: C, 66.97; H, 6.87; N, 6.79. Found: C, 67.15; H, 6.93; N, 6.79.

Z-Ser-Phe-Gly-OBu^t (XIII)——Compound XII (2.00 g, 4.85 mmoles) was hydrogenated in MeOH (50 ml) to give H-Phe-Gly-OBu^t, which was allowed to react with Z-Ser-ODNP (1.62 g, 4 mmoles) in CH_3CN (10 ml) at room temperature for 5 hr. After the usual work-up, the residue was triturated with ether to give crystals which were recrystallized from EtOAc–ether–pet. ether: 1.41 g (70.8%); mp 105—106°; $[\alpha]_D^{23}$ −26.6° (*c* 0.7, EtOH); Rf^1 0.66. *Anal.* Calcd. for $C_{26}H_{33}O_7N_3 \cdot 1/2H_2O$: C, 61.43; H, 6.74; N, 8.27. Found: C, 61.85; H, 6.69; N, 8.21.

<Glu-His-Trp-Thr-Tyr-Gly-OBu^t (XIV)——Compound XI (1.06 g, 2 mmoles) was hydrogenated in MeOH (50 ml). The resulting H-Thr-Tyr-Gly-OBu^t was dissolved in N,N′-dimethylformamide (DMF) (5 ml)

14) M. Fujino, S. Kobayashi, M. Obayashi, T. Fukuda, S. Shinagawa, I. Yamazaki, R. Nakayama, W.F. White and R.H. Rippel, *Biochem. Biophys. Res. Commun.*, **49**, 698 (1972).
15) M. Fujino, I. Yamazaki, S. Kobayashi, T. Fukuda, S. Shinagawa, R. Nakayama, W.F. White and R.H. Rippel, *Biochem. Biophys. Res. Commun.*, **57**, 1248 (1974).
16) M. Fujino, T. Fukuda, S. Shinagawa, S. Kobayashi, I. Yamazaki, R. Nakayama, J.H. Seely, W.F. White and R.H. Rippel, *Biochem. Biophys. Res. Commun.*, **60**, 406 (1974).
17) H. Matsubara and K. Sasaki, *Biochem. Biophys. Res. Commun.*, **35**, 176 (1969).

together with <Glu-His-Trp-OH[8] (813 mg, 1.8 mmoles), HONB (358 mg, 2 mmoles), and DCC (412 mg, 2 mmoles) at −2°. The mixture was stirred at 0° for 2 hr and at room temperature for 16 hr, then filtered. The filtrate was evaporated to give the residue which solidified upon trituration with ether. The solid was purified by reprecipitation from DMF–EtOAc: 1.05 g (63.3%); $[\alpha]_D^{23}$ −28.6° (*c* 0.5, MeOH); Rf^4 0.64. *Anal.* Calcd. for $C_{41}H_{51}O_{10}N_9 \cdot 2H_2O$: C, 57.17; N, 6.43; N, 14.02. Found: C, 57.21; N, 6.05; N, 14.11.

<Glu-His-Trp-Ser-Phe-Gly-OBu^t (XV) was synthesized in a similar manner to that described for XIV from <Glu-His-Trp-OH and Z-Ser-Phe-Gly-OBu^t (XIII): yield, 67.4%.

Z-Ile-Arg(NO_2)-Pro-NH-C_2H_5 (XVI)——Z-Ile-OH (795 mg, 3 mmoles), HONB (590 mg, 3.3 mmoles), and DCC (680 mg, 3.3 mmoles) were mixed in EtOAc (10 ml) at 0° and the mixture was stirred at room temperature for 2 hr then filtered. The filtrate was stirred into a solution of H-Arg(NO_2)-Pro-NH-C_2H_5 hydrobromide[8] (3 mmoles) and triethylamine (TEA), (0.84 ml, 0.6 mmoles) in DMF (10 ml), and the mixture was stirred at room temperature for 8 hr. After evaporation of the solvent, the residue was dissolved in $CHCl_3$ (50 ml) and the extract was washed with 0.1N HCl, 5% $NaHCO_3$, and water, then dried ($MgSO_4$). The solvent was evaporated, and the residue was extracted with EtOAc (20 ml). The insolubles were filtered and the was evaporated to give the residue which was solidified upon trituration with ether. The solid was purified by reprecipitation from EtOH–ether: 1.12 g (62.1%); mp 103—105°; $[\alpha]_D^{23}$ −58.1° (*c* 1.0, MeOH); Rf^1 0.48. *Anal.* Calcd. for $C_{27}H_{42}O_7N_8 \cdot 1/2H_2O$: C, 54.08; H, 7.23; N, 18.68. Found: C, 53.80; H, 7.15; N, 18.84. Z-Nle-Arg(NO_2)-Pro-NH-C_2H_5 (XVII), and Boc-Met-Arg(NO_2)-Pro-NH-C_2H_5 (XVIII) were synthesized by using Z-Nle-OH and Boc-Met-OH, respectively, in a similar manner to that described for XVI.

Boc-Leu-Orn(Z)-Pro-NH-C_2H_5 (XIX)——Z-Pro-NH-C_2H_5 (0.99 g, 3.6 mmoles) was hydrogenated in MeOH (50 ml). The resulting H-Pro-NH-C_2H_5 was dissolved in tetrahydrofuran (THF), (10 ml) together with IBOC-Orn-(Z)-OH (oil, 1.60 g, 3.6 mmoles), HONB (716 mg, 4 mmoles), and DCC (824 mg, 4 mmoles) at 0°. The mixture was stirred at 0° for 2 hr and at room temperature for 5 hr, and the solvent was evaporated. The residue was dissolved in EtOAc (100 ml) and the solution was washed in the usual manner, then dried (Na_2SO_4). Evaporation of the solvent gave IBOC-Orn(Z)-Pro-NH-C_2H_5 as an oily residue: 1.60 g (77.5%); Rf^1 0.58. This compound (1.60 g, 2.8 mmoles) was then treated with TFA (10 ml) at room temperature for 30 min. The solvent was evaporated and the residue was dried over NaOH. The free dipeptide trifluoroacetate and Boc-Leu-ONB (oil, 1.11 g, 2.7 mmoles) were mixed in dioxane (10 ml) and TEA (0.38 ml) was added. After 10 hr at room temperature, the solvent was evaporated and the residue was dissolved in EtOAc (100 ml). The solution was washed with 5% $NaHCO_3$, and 0.1N HCl, then dried (Na_2SO_4). The solvent was evaporated to give the residue which solidified upon trituration with pet. ether: 1.40 g (86.0%); mp 83—87°; $[\alpha]_D^{23}$ −55.6° (*c* 1.0, MeOH); Rf^1 0.59. *Anal.* Calcd. for $C_{31}H_{49}O_7N_5 \cdot 1/2H_2O$: C, 60.96; H, 8.25; N, 11.47. Found: C, 61.11; H, 8.20; N, 11.10.

Z-Leu-Lys(IBOC)-Pro-NH-C_2H_5 (XX)——Z-Pro-NH-C_2H_5 (0.99 g, 3.6 mmoles) was hydrogenated in MeOH (50 ml) to give H-Pro-NH-C_2H_5, which was dissolved in dioxane (10 ml) together with Z-Lys(IBOC)-OH[18] (1.66 g, 3.6 mmoles), HONB (650 mg, 3.6 mmoles), and DCC (750 mg, 3.6 mmoles) at 0°. The mixture was stirred at room temperature for 8 hr and filtered, then the solvent was evaporated. The residue was dissolved in EtOAc (100 ml) and the solution was washed in the usual manner, then dried (Na_2SO_4). Evaporation of the solvent gave Z-Lys(IBOC)-Pro-NH-C_2H_5 as an oily residue: 1.50 g (70.5%); Rf^1 0.67. This compound (1.50 g, 2.5 mmoles) was hydrogenated in MeOH (50 ml) to give H-Lys(IBOC)-Pro-NH-C_2H_5, which was allowed to react with Z-Leu-OSu (965 mg, 2.6 mmoles) in dioxane (20 ml) at room temperature for 16 hr. After evaporation, the residue was dissolved in EtOAc (100 ml), and the solution was washed in the usual manner, then dried (Na_2SO_4). Evaporation of the solvent gave the crude product which was purified by reprecipitation from EtOAc–pet. benzine: 1.30 g (74.5%); mp 73—75° (decomp.); $[\alpha]_D^{22}$ −56.9° (*c* 1.0, EtOH); Rf^1 0.80. *Anal.* Calcd. for $C_{38}H_{59}O_7N_5 \cdot 1/2H_2O$: C, 64.56; H, 8.55; N, 9.91. Found: C, 64.36; H, 8.67; N, 9.36.

Z-Gly-NH-C_2H_5 (XXI)——Z-Gly-OH (4.18 g, 20 mmoles), ethylamine hydrochloride (1.95 g, 24 mmoles), TEA (3.36 ml, 24 mmoles), and DCC (4.47 g, 22 mmoles) were mixed in THF (20 ml) at 0° and the mixture was stirred at 10° for 5 hr. After filtration and evaporation, the residue was dissolved in EtOAc (100 ml). The usual work-up gave the product which was recrystallized from EtOAc–pet. ether: 4.21 g, (89.1%); mp 99—100°; Rf^1 0.69. *Anal.* Calcd. for $C_{12}H_{16}O_3N_2$: C, 61.00; H, 6.83; N, 11.86. Found: C, 59.83; H, 6.59; N, 11.62.

IBOC-Arg(NO_2)-Gly-NH-C_2H_5 (XXII)——Compound XXI (1.18 g, 5 mmoles) was hydrogenated in MeOH (20 ml) to give H–Gly–NH–C_2H_5, which was dissolved in THF (50 ml) together with IBOC–Arg(NO_2)–OH[19] (1.98 g, 5.0 mmoles), HONB (0.99 g, 5.5 mmoles), and DCC (1.13 g, 5.5 mmoles) at 0°. The solution was stirred at 0° for 2 hr and at room temperature for 3 hr, then filtered. After evaporation, the residue was dissolved in *n*–BuOH (100 ml). The usual work-up gave the product which was reprecipitated from EtOH–pet. ether: 2.03 g (83.5%); mp 115—117°; $[\alpha]_D^{27}$ −19.6° (*c* 0.5, MeOH); Rf^1 0.12. *Anal.* Calcd. for $C_{21}H_{37}O_6N_7$: C, 52.16; H, 7.71; N, 20.27. Found: C, 52.07; H, 7.87; N, 19.62.

Z-Leu-Arg(NO_2)-Gly-NH-C_2H_5 (XXIII)——Compound XXII (1.21 g, 2.5 mmoles) was treated with TFA (10 ml) at room temperature for 30 min and the solution was diluted with dry ether. The precipitate

18) mp 104—105°; $[\alpha]_D^{32}$ −6.9° (*c* 0.1, EtOH).
19) mp 135—136°; $[\alpha]_D^{24}$ −28.7° (*c* 1.0, EtOH).

was collected, washed with dry ether, and dried over NaOH. The free peptide trifluoroacetate was dissolved in DMF (10 ml) together with Z–Leu–OSu (907 mg, 2.5 mmoles), and TEA (0.35 ml, 2.5 mmoles) was added. After being stirred at room temperature for 8 hr, the mixture was concentrated to a small volume. To this, *n*–BuOH (100 ml) was added, and the solution was washed with water. After evaporation, the residue was triturated with ether and reprecipitated from EtOH–ether: 1.10 g (80.4%); mp 105—109°; $[\alpha]_D^{23}$ −17.1° (*c* 1.0, MeOH); Rf^1 0.37. *Anal.* Calcd. for $C_{24}H_{38}O_7N_8 \cdot 1/2H_2O$: C, 51.51; H, 7.02; N, 20.26. Found: C, 51.61; H, 7.05; N, 20.55.

Synthesis of the LH–RH Analogs. <Glu–His–Trp–Thr–Tyr–Gly–Leu–Arg–Pro–NH–C_2H_5 (III)——Compound XIV (250 mg, 0.3 mmoles) was treated with TFA (5 ml) in the presence of 2-mercaptoethanol (0.1 ml) and 6N HCl (0.05 ml) at room temperature for 40 min. The mixture was diluted with ether (50 ml) to give the precipitate which was collected and dried. The resulting N-terminal hexapeptide hydrochloride was dissolved in DMF (5 ml) together with H–Leu–Arg(NO_2)–Pro–NH–C_2H_5[8] (213 mg, 0.36 mmoles), HONB (81 mg, 0.45 mmoles), and DCC (93 mg, 0.45 mmoles) at 0°. The mixture was stirred at 0° for 2 hr and at room temperature for 16 hr, then filtered. The filtrate was diluted with EtOAc (50 ml) and the precipitate was collected: 450 mg. The product (400 mg) was dissolved in 10% EtOH and applied on a column (2 × 16 cm) of Amberlite XAD-2 which was developed with a linear EtOH gradient (10—80%, 200 ml each). The fractions containing the product showing a single spot on TLC were combined, evaporated to a small volume, and lyophilized to a constant weight: 162 mg.

This material (130 mg) was treated with anhydrous HF (*ca.* 5 ml) in the presence of anisole (0.03 ml) and 2-mercaptoethanol (0.03 ml) at 0° for 1 hr. Volatile components were removed and the residue was dried over NaOH and dissolved in water (10 ml). After filtration, the filtrate was passed through a column (1 × 4 cm) of Amberlite IRA-410 (acetate form). The eluate and washings were combined and lyophilized to give the crude product as acetate. The acetate was dissolved in water and applied on a column (1 × 26 cm) of CM-cellulose which was developed with a linear NH_4OAc gradient (0.005—0.2M, 200 ml each). The product III was eluted in fractions 200—230 ml. The fractions were combined and lyophilized to give a white fluffy material which was dried over P_2O_5 at 50° for 5 hr under reduced pressure: 104 mg (40%); $[\alpha]_D^{23}$ −53.0° (*c* 0.5, 5% AcOH); Rf^3 0.55; Rf^4 0.32. Amino acid analysis: His 1.02; Arg 1.02; Trp 0.87; Thr 0.90; Glu 1.04; Pro 1.00; Gly 1.00; Leu 1.02; Tyr 0.90; ethylamine 1.01 (peptide content 95%).

Other LH–RH analogs were prepared from the N-terminal hexapeptides and the corresponding tripeptide ethylamide by exactly the same procedure as described for the synthesis of analog III. Deblocking of the IBOC group of the protected nonapeptide was carried out by treatment with TFA, and that of the Z group by catalytic hydrogenation. Pysicochemical properties of LH–RH analogs are shown in Table II.

Acknowledgements The authors express their gratitude to Drs. E. Ohmura and K. Morita of this division, and Dr. W.J. Close of Abbott Laboratories, North Chicago, for their encouragement throughout this work. We also wish to thank Drs. W.F. White and R.H. Rippel of Abbott Laboratories, for their performing the *in vitro* biological assays.