(14) F. E. Norrington, R. M. Hyde, S. G. Williams, and R. Wootton, preceding paper in this issue.
(15) J. G. Topliss and R. J. Costello, *J. Med. Chem.*, 15, 1066 (1972).
(16) R. M. Hyde, F. E. Norrington, S. G. Williams, and R. Wootton, manuscript in preparation.
(17) Y. C. Martin and W. J. Dunn, *J. Med. Chem.*, 16, 578 (1973).
(18) The computer selections given in this paper were all made using interactive computer programs written in the Focal language (PS/8 Focal) on a PDP8-e computer with 12k of core store and DECtape backup store. Copies of these programs are available on request from the authors.

Synthesis and Biological Activity of Luteinizing Hormone-Releasing Hormone and Related Peptides

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Syntheses of the decapeptide luteinizing hormone-releasing hormone, <Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 are described. The basic properties of arginine can provide a simple repetitive isolation procedure for arginine-containing peptides. The biological activities of the decapeptide, of a range of fragments and modified fragments, and of two analogs with alteration in the serine at position 4 were measured by in vitro incubation with sheep pituitary slices, measuring the liberated LH by bioassay. None of the compounds of shortened sequence were active, with the exception of <Glu-His-Trp which showed 1% of the activity of the decapeptide in one of four experiments. Neither [Ser(Bu^t)^4]-LH-RH nor [Leu^4]-LH-RH showed significant activity indicating (despite the known activity of [Ala^4]-LH-RH) the importance of this part of the structure for full biological activity.

Following the discovery of the decapeptide structure of luteinizing hormone-releasing hormone (LH-RH) of both porcine[1] and ovine[2] origin, a number of syntheses have been described[3-14] and the structure–activity relationships of the molecule are emerging from the study of synthetic analogs.[15-33] Replacement of single amino acid residues often leads to a dramatic reduction in biological activity, particularly with residues 1, **3**, and **9** or in replacing glycine at position 6 with L-amino acids. Analogs with a D-amino acid such as D-alanine in position 6 show,[32] in contrast, a remarkably high level of biological activity. Lower but significant activity has resulted by replacing histidine with phenylalanine[19] in position 2, by replacing tyrosine with phenylalanine[23] in position 5, by replacing leucine with isoleucine and other amino-acids[19] in position 7, and by replacing arginine in position 8 with lysine,[19] ornithine,[19] or glutamine.[21] Replacement of serine in position 4 by alanine,[19,22,27] threonine,[19,28] or glutamine[19] gave significantly active analogs, and replacement of the C-terminal glycineamide residue by ethylamido and other groups[19,20,24,28,29] gave analogs with high activity. Smaller peptides or fragments of the decapeptide have generally been inactive[18,33] although the tripeptide amide <Glu-His-Trp-NH_2 was reported as having significant activity,[25] a claim subsequently retracted.[30] There are also conflicting reports about the activity of the corresponding acid.[18,31]

The present work describes our syntheses of LH-RH and of [Ser(Bu^t)^4]- and [Leu^4]-LH-RH and the activity of these compounds and of a range of smaller fragments of LH-RH in releasing luteinizing hormone from ovine pituitary tissue in vitro.

Synthesis. Luteinizing hormone-releasing hormone was synthesized as shown in Charts I–III, using either unprotected serine or *tert*-butyl ether protection for the hydroxy group. In Chart I, the protected heptapeptide **11** corresponding to sequence 4–10 was synthesised by a stepwise active ester approach starting from glycineamide hydrochloride and protecting the arginine side chain with a nitro group. Serine and tyrosine were left unprotected. Benzyloxycarbonyl (Z) groups were used for α-amino protection and were removed by HBr in AcOH. At the heptapeptide stage, hydrogenation removed the nitro and Z groups and tryptophan was introduced using Z-Trp-ONp. Hydrogenation and coupling with <Glu-His-N_3 gave LH-RH. A scheme similar in part to this was adopted by Yanaihara et al.

The dipeptide **1** has been reported as having different melting points, which seem to be best explained by there being two crystalline forms melting at ca. 120° [34] and at ca. 145°,[12] respectively. In our work we obtained initially the form with mp 120°; this was difficult to recrystallize and tended to form a gel. Subsequently the compound crystallized in the higher melting form. The deprotected dipeptide salt **2** analyzed as the dihydrobromide, as did other hydrobromides in this series, possibly by formation of a weak salt with the C-terminal amide group. Countercurrent distribution was used to purify several protected intermediates of Chart I and was carried out either with relatively few transfers using separating funnels (tripeptide **3**, for example) or with more transfers using an automatic (steady state) machine (peptides **11** and **13**). The LH-RH (**15a**) was purified by ion-exchange chromatography on CM-Sephadex C-25 using pyridine–AcOH buffers, followed by partition chromatography on Sephadex LH20. The chromatographically pure decapeptide had the expected amino acid and elemental analyses and optical rotation.

A second approach to the synthesis of LH-RH is shown in Charts II and III. For several stages, use was made of the basic properties of arginine peptides to provide a simple separation of protected peptides from neutral coproducts of the coupling reaction.[35] The approach was based on the similar use of 4-picolyl esters[36-38] and of the basic properties of the histidine side chain[39] when this is present in the peptide. The coupling reaction is carried out with excess acylating agent until no amino component is detected and the product is separated from neutral and acidic coproducts by absorption into an acidic phase. It was found suf-

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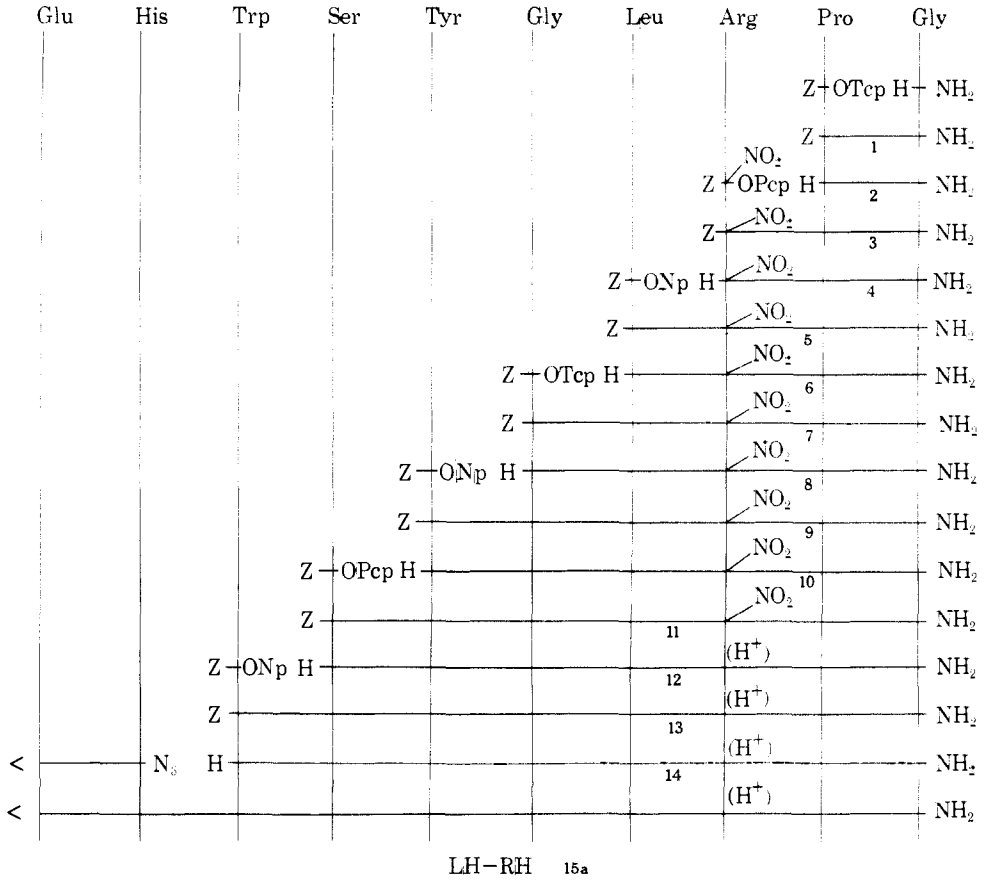
Chart I[41,a]



[a] OPcp = pentachlorophenyl; OTcp = 2,4,5-trichlorophenyl.

Chart II

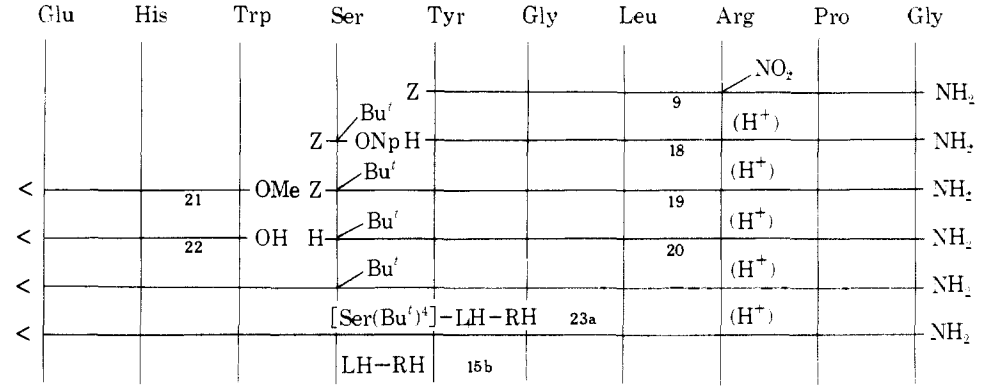
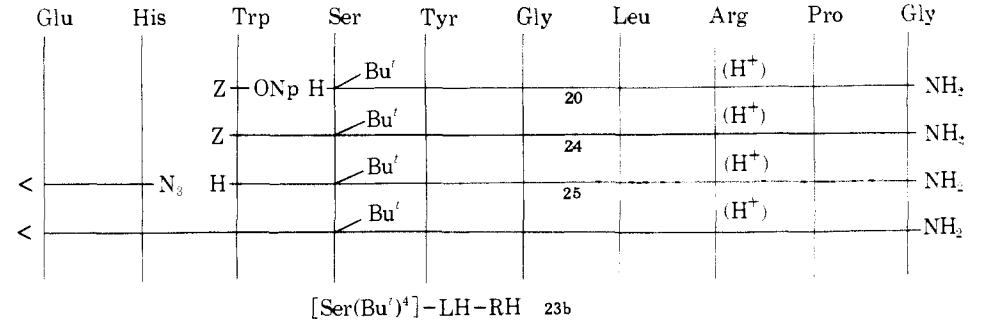
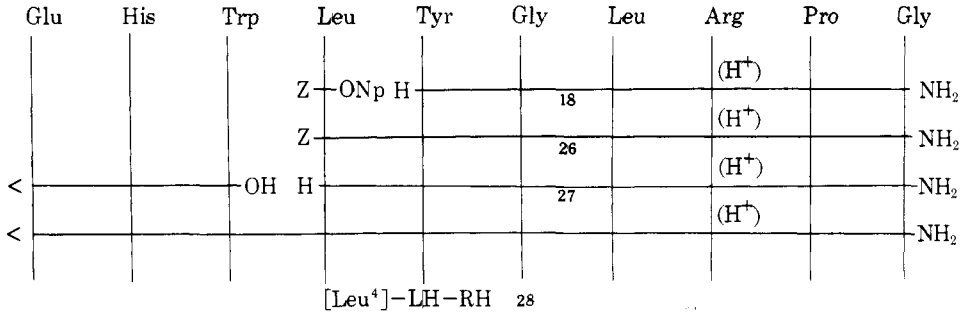


Chart III



ficient to filter the coupling reaction mixture in DMF–H_2O (3:1) through CM-Sephadex resin; the coproducts were washed out with this solvent and the arginine peptides were washed off the resin with pyridine–AcOH–DMF–H_2O mixtures. The crude products frequently had satisfactory chromatographic and analytical properties and could be

Chart IV



used immediately for the next deprotection and coupling, thus providing a simple repetitive procedure. In some cases, coupling with unprotected arginine gives by-products which are not separated in this way, but use can be made in these cases of ion-exchange chromatography which is well established[40] for the chromatographic purification of arginine peptides. In order to avoid acetylation during coupling reactions, the acetates were converted to pivalates by filtering an aqueous solution through DEAE-Sephadex (pivalate form) and evaporating. The approach was tried by coupling Z-Tyr-ONp to Gly-Leu-Arg-Pro-Gly-NH_2 and then to prepare peptides **19**, **24**, and **23b** (Charts II and III) and **26** (Chart IV).

Coupling of the tripeptide <Glu-His-Trp-OH to **20** with *N*,*N*′-dicyclohexylcarbodiimide (DCCI) and *N*-hydroxysuccinimide (HOSu) gave [Ser(Bu^t)^4]-LH-RH in 26% yield. The alternative 2 + 8 route (Chart III) gave the same compound in 76% yield. Removal of the *tert*-butyl group with trifluoroacetic acid–H_2O gave LH-RH with only trace impurities on TLC, and a final purification by CM-Sephadex chromatography gave LH-RH with similar properties to the product from Chart I.

Chart IV shows the synthesis of [Leu^4]-LH-RH which was carried out by a route analogous to the synthesis of **23** (Chart II). The final product was purified by preparative TLC and CM-Sephadex chromatography.

Biological Activity. The LH-releasing activity of synthetic LH-RH and related peptides was assessed by incubation of ovine pituitary tissue and biological assay of the incubation medium for LH as described previously.[42-44] The results are expressed in terms of NIH-LH-S17 (National Institutes of Health, Bethesda, Md.). Responses to synthetic peptides were examined at four dose levels. In the case of synthetic LH-RH the doses were 0.01, 0.10, 1.00, and 10.00 ng per incubation flask. All other peptides were tested at doses of 0.10, 1.00, 10.00, and 100.00 ng per flask. The incubation volume in each flask was 2.5 ml in all cases. The data are given in Tables I–V.

Table I. Typical[a] Experiment Showing LH-Releasing Activity of Synthetic LH-RH in Vitro

Dose/incubation flask	LH release[b] (μg of NIH-LH-S17 equiv/mg of pituitary tissue)	Significance[c]
10.00 ng	3.28 (2.39–4.83)	s.
Control	1.41 (1.03–1.92)	
1.00 ng	2.74 (2.01–3.97)	s.
Control	1.39 (1.02–1.89)	
0.10 ng	2.20 (1.63–3.10)	s.
Control	1.28 (0.92–1.74)	
0.01 ng	1.94 (1.43–2.67)	s.
Control	1.37 (1.00–1.85)	

[a] In other experiments the 0.01-ng dose gave no significant response. [b] Figures in parentheses are fiducial limits of error at p = 0.95. λ = 0.13. [c] n.s. = not significant, s. = significant.

Table II. Typical Experiment on LH-Releasing Activity of Trp-Ser(Bu^t)-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (**25**)[b]

Dose/incubation flask	LH release[a] (μg of NIH-LH-S17 equiv/mg of pituitary tissue)	Significance[c]
100.00 ng	0.59 (0.31–0.94)	n.s.
Control	0.44 (0.20–0.72)	
10.00 ng	0.61 (0.32–0.97)	n.s.
Control	0.37 (0.16–0.62)	
1.00 ng	0.33 (0.13–0.57)	n.s.
Control	0.25 (0.10–0.45)	
0.10 ng	0.26 (0.10–0.46)	n.s.
Control	0.26 (0.09–0.47)	

[a] Figures in parentheses are fiducial limits of error at p = 0.95. λ = 0.17. [b] Similar negative results were obtained with peptides **16**, **18**, and **20**. [c] n.s. = not significant, s. = significant.

Table III. Two Experiments on LH-Releasing Activity of <Glu-His-Trp-OH (**22**)

	Dose/incubation flask	LH release[a] (μg of NIH-LH-S17 equiv/mg of pituitary tissue)	Significance[b]
Expt 1[c]	100.00 ng	0.96 (0.41–1.88)	n.s.
	Control	0.97 (0.31–1.93)	
	10.00 ng	0.71 (0.21–1.39)	n.s.
	Control	1.25 (0.57–2.55)	
	1.00 ng	0.70 (0.33–1.37)	n.s.
	Control	1.20 (0.46–2.35)	
	0.10 ng	1.67 (0.79–3.52)	n.s.
	Control	0.86 (0.27–1.68)	
	λ = 0.28		
Expt 2	100.00 ng	2.34 (1.64–3.28)	s.
	Control	1.28 (0.78–1.87)	
	10.00 ng	2.04 (1.38–2.90)	s.
	Control	1.04 (0.63–1.50)	
	1.00 ng	1.08 (0.67–1.55)	n.s.
	Control	0.96 (0.55–1.42)	
	0.10 ng	1.03 (0.59–1.55)	n.s.
	Control	0.85 (0.48–1.27)	
	λ = 0.13		

[a] Figures in parentheses are fiducial limits of error at p = 0.95. [b] n.s. = not significant, s. = significant. [c] Three further experiments gave similar results to expt 1.

Table IV. Two Experiments on LH-Releasing Activity of [Ser(Bu^t)^4]-LH-RH (23)

	Dose/incubation flask	LH release[a] (μg of NIH-LH-S17 equiv/mg of pituitary tissue)	Significance[b]
Expt 1[c]	100.00 ng	0.78 (0.45–1.19)	n.s.
	Control	0.78 (0.47–1.20)	
	10.00 ng	1.44 (1.06–1.99)	n.s.
	Control	1.40 (1.03–1.90)	
	1.00 ng	0.82 (0.50–1.25)	n.s.
	Control	0.91 (0.54–1.37)	
	0.10 ng	0.66 (0.37–1.00)	n.s.
	Control	0.61 (0.35–0.94)	
		$\lambda = 0.18$	
Expt 2	100.00 ng	1.25 (0.79–1.80)	s.
	Control	0.66 (0.33–1.03)	
	10.00 ng	0.82 (0.47–1.22)	n.s.
	Control	0.77 (0.44–1.14)	
	1.00 ng	0.81 (0.45–1.20)	n.s.
	Control	0.59 (0.27–0.95)	
	0.10 ng	0.60 (0.30–0.93)	n.s.
	Control	0.83 (0.42–1.30)	
		$\lambda = 0.15$	

[a]Figures in parentheses are fiducial limits of error at $p = 0.95$. [b]n.s. = not significant, s. = significant. [c]Two further experiments gave similar results to expt 1.

Table V. Typical Experiment on LH-Releasing Activity of [Leu^4]-LH-RH (28)

Dose/incubation flask	LH release[a] (μg of NIH-LH-S17 equiv/mg of pituitary tissue)	Significance[b]
100.00 ng	1.16 (0.88–1.51)	n.s.
Control	1.37 (1.04–1.76)	
10.00 ng	1.17 (0.86–1.53)	n.s.
Control	1.05 (0.75–1.40)	
1.00 ng	1.10 (0.80–1.44)	n.s.
Control	1.37 (1.00–1.82)	
0.10 ng	2.22 (1.42–3.53)	n.s
Control	1.48 (0.98–2.25)	
	$\lambda = 0.11$	

[a]Figures in parentheses are fiducial limits of error at $p = 0.95$. [b]n.s. = not significant, s. = significant.

The results of Table I show typical activity of LH-RH in the in vitro system. In the experiment shown all doses down to 0.01 ng/incubation flask (0.004 ng/ml) gave significant responses, although the minimum effective dose in other similar experiments was 0.10 ng/flask. Table II shows a typical result from a series of fragments or modified fragments, all of which were inactive at up to 100 ng/flask. This general lack of activity in fragments containing deletions in the N-terminal part of the LH-RH molecule confirms other work.[13,17,33] The tripeptide acid <Glu-His-Trp-OH (22) showed significant activity at two dose levels (100 and 10 ng/flask) in one out of four experiments (Table III). This is an intriguing result in view of other conflicting reports of activity or lack of activity in this tripeptide[18,31] and the corresponding amide.[25,30] The analog containing a Ser(Bu^t) group in place of Ser failed to show activity except at the highest dose level in one of four experiments (Table IV). This lack of activity indicates structural importance for the residue in position 4, but this is not associated with the hydroxyl group since [Ala^4]-LH-RH is reported to be active.[19,22,27] The lack of activity found in [Leu^4]-LH-RH (Table V) may indicate that steric constraints in position 4 are an important factor. The replacement of Ser by Ser(Bu^t) in position 6 of bradykinin also abolishes activity[45] although the glycine analog is fully active.

Experimental Section

Thin-layer chromatograms were run on silica gel GF_{254} (Merck). R_f values refer to the following systems: MeOH–$CHCl_3$ mixtures, R_f^{A1} 1:19, R_f^{A2} 1:9, R_f^{A3} 3:17, R_f^{A4} 1:4; *n*-BuOH–AcOH–H_2O mixtures, R_f^{B1} 10:1:3, R_f^{B2} 4:1:1, R_f^{B3} 3:1:1; R_f^C *n*-BuOH–AcOH–pyridine–H_2O (15:3:10:6); R_f^D cyclohexane–AcOEt–MeOH (1:1:1); $CHCl_3$–MeOH–AcOH mixtures, R_f^{E1} 10:2:1, R_f^{E2} 20:2:1; R_f^F *n*-BuOH–AcOH–H_2O–AcOEt (1:1:1:1); R_f^G *i*-PrOH–pyridine–AcOH–H_2O (10:5:4:4); $CHCl_3$–MeOH–AcOH–H_2O mixtures, R_f^{H1} 30:20:4:6, R_f^{H2} 30:20:2:3, R_f^{H3} 60:18:2:3, R_f^{H4} 45:30:4:6. Spots were detected by use of ninhydrin, chlorine and starch iodide, and uv illumination. Optical rotations were measured with a Perkin-Elmer 141 automatic polarimeter (1-dm cell). A Locarte high-voltage apparatus was used for electrophoresis (HVE) at 100–120 V cm^{-1} (Whatman 3MM paper). Melting points were determined with a Kofler hot-stage apparatus. Organic solutions were dried over sodium sulfate. Samples for amino acid analysis were hydrolyzed at 110° for 16–18 hr in constant boiling HCl with the addition of phenol. Analyses were carried out on a Jeol JLC 6AH machine. Countercurrent distribution (>20 transfers) was carried out using a Q and Q 100 tube steady-state apparatus.

Synthesis of LH-RH (15a) (Chart I). Z-Pro-Gly-NH_2 (1). Z-Pro-OTcp (18 g, 0.04 *M*) and Et_3N (4.9 ml) were added to a stirred mixture of Gly-NH_2, HCl (3.9 g, 0.035 *M*), and DMF (40 ml). The mixture was stirred for 2 hr, diluted with AcOEt (300 ml) and EtOH (50 ml), washed (H_2O, saturated $NaHCO_3$, H_2O; each saturated with NaCl), dried, and evaporated. The residue with Et_2O gave white crystalline **1**: 5.7 g (53%); mp 118–120° (lit.[32] mp 120°); R_f^{A2} 0.42, R_f^{E1} 0.86; attempted recrystallization gave gel-like material. A subsequent preparation starting from Z-Pro-ONp gave 58% of compound **1** as white needles: mp 144–145° (from MeOH–Et_2O) (lit.[12] mp 145–146°); R_f^{E2} 0.51.

Z-Arg(NO_2)-Pro-Gly-NH_2 (3). A solution of HBr in AcOH (45% w/v, 45 ml) was added to a stirred solution of compound **1** (6.0 g, 5 m*M*) in AcOH (15 ml). After 1.5 hr ether (450 ml) was added and the crystalline **Pro-Gly-NH_2 · 2HBr (2)** washed with ether: 5.9 g (88%); $[\alpha]^{22}D$ −14.4° (*c* 2, H_2O); R_f^C 0.27, R_f^{H1} 0.46. Anal. ($C_7H_{13}N_3O_2 \cdot 2HBr \cdot 0.5H_2O$) C, H, N, Br. A mixture of compound **2** (5.9 g, 17 m*M*), Et_3N (7 ml), and Z-Arg(NO_2)-OPcp (17.3 g, 29 m*M*) in DMF (35 ml) was stirred for 20 hr. The mixture was filtered, evaporated, triturated with Et_2O, and partitioned between the upper (1 vol) and lower (3 vol) layers of a mixture of AcOEt–AcOH–H_2O (3:1:5) for 20 transfers. Fractions containing product (TLC) were combined, evaporated, and repeatedly triturated with Et_2O giving tripeptide **3** as an amorphous powder: 7.2 g (82%); $[\alpha]^{25}D$ −22.7°; $[\alpha]^{25}_{546}$ −27.1° (*c* 1, DMF) [lit.[12] $[\alpha]^{23}D$ −25.4° (*c* 1, DMF)]; R_f^{B1} 0.36, R_f^{B3} 0.46, R_f^C 0.60, R_f^{E1} 0.30, R_f^F 0.63. Anal. ($C_{21}H_{30}N_8O_7$) C, H, N.

Z-Leu-Arg(NO_2)-Pro-Gly-NH_2 (5). Removal of the Z group from compound **3** (6.7 g, 13 m*M*) as described for compound **2** gave white solid **Arg(NO_2)-Pro-Gly-NH_2 · 2HBr (4)**: $[\alpha]^{25}D$ −11.9° (*c* 1, DMF); R_f^C 0.26, R_f^G 0.49, R_f^{H1} 0.34. Anal. ($C_{13}H_{24}N_8O_5 \cdot 2HBr$) Br: calcd, 30.5; found, 29.9. A mixture of this dihydrobromide, Et_3N (5 ml), and Z-Leu-ONp (5.9 g, 15 m*M*) in DMF (30 ml) was stirred for 1 hr and kept at 0° for 16 hr. The mixture was filtered, evaporated, dissolved in $CHCl_3$ (800 ml) and EtOH (150 ml), washed (2 *N* HCl, H_2O, saturated $NaHCO_3$, H_2O; each saturated with NaCl), dried, and evaporated. Repeated trituration with Et_2O gave white solid tetrapeptide **5**: 5.6 g (68%); $[\alpha]^{26}D$ −41.1° (*c* 1, DMF) [lit.[13] $[\alpha]^{28}D$ −49.0° (*c* 1, MeOH)]; R_f^{B1} 0.42, R_f^{B3} 0.52, R_f^C 0.64, R_f^{E1} 0.39, R_f^F 0.68. Anal. ($C_{27}H_{41}N_9O_8$) C, H, N.

Z-Gly-Leu-Arg(NO_2)-Pro-Gly-NH_2 (7). Removal of the Z group from compound **5** (25 g, 0.04 *M*) as described for compound **2** gave white solid **Leu-Arg(NO_2)-Pro-Gly-NH_2·1.8HBr (6)**: $[\alpha]^{20}D$ −16.2° (*c* 1, DMF); R_f^C 0.50. Anal. ($C_{19}H_{35}N_9O_6$·1.8HBr) C, H, N, Br. This hydrobromide **6** was dissolved in DMF (100 ml), and Et_3N (12 ml) and Z-Gly-OTcp (18.7 g, 0.048 *M*) were added. The mixture was stirred for 2 hr and worked up as for compound **5** giving white solid **7**: 24.4 g (89%); $[\alpha]^{20}D$ −34.7° (*c* 1, DMF) [lit.[13]

$[\alpha]^{26}D$ −51.0° (c 1, MeOH)]; R_f^{E1} 0.55, R_f^{H3} 0.61. Anal. ($C_{29}H_{44}N_{10}O_9$) C, H, N.

Z-Tyr-Gly-Leu-Arg(NO_2)-Pro-Gly-NH_2 (9). Removal of the Z group from compound **7** (22.6 g, 33 m*M*) as described for compound **2** gave white solid **Gly-Leu-Arg(NO_2)-Pro-Gly-NH_2·2HBr (8):** R_f^C 0.51, R_f^G 0.76, R_f^{H1} 0.58. Anal. ($C_{21}H_{38}N_{10}O_7$·2HBr) C, N; H: calcd, 5.7; found, 5.2; Br: calcd, 22.7; found 23.6. A mixture of hydrobromide **8** (95% of the total prepared above), Et_3N (18 ml), and Z-Tyr-ONp (16.0 g, 37 m*M*) in DMF (100 ml) was stirred for 18 hr. The reaction mixture was worked up as for compound **5** giving hexapeptide **9** after precipitation with EtOH–Et_2O: 24.1 g (89%); $[\alpha]^{20}D$ −32.6° (c 1, EtOH); R_f^{B2} 0.45 with trace impurities. Anal. ($C_{38}H_{53}N_{12}O_{11}$) H; C: calcd, 53.45; found, 52.8; N: calcd, 19.7; found, 20.2.

Z-Ser-Tyr-Gly-Leu-Arg(NO_2)-Pro-Gly-NH_2 (11). Removal of the Z group from compound **9** (22 g, 26 m*M*) as described for compound **2** with the addition of phenol (5% w/v) gave the hydrobromide **10:** R_f^{H3} 0.32 with impurities. This hydrobromide was dissolved with stirring in DMF (100 ml), with the addition of excess Et_3N and Z-Ser-OPcp (13 g, 48 m*M*) and kept at 4° for 2 days. Work-up of the reaction mixture as for compound **5** gave a product showing one major spot with impurities. The crude product was purified by countercurrent distribution between the upper (18 ml) and lower (25 ml) layers of a mixture of AcOEt–EtOH–H_2O (10:2:5), 670 transfers, K = 2.3. Fractions containing product (TLC) were combined, evaporated, and precipitated with EtOH–Et_2O giving heptapeptide **11:** 11 g (45%); $[\alpha]^{20}D$ −44.0° (c 1, 10% AcOH); R_f^{B3} 0.55, R_f^C 0.69, R_f^G 0.90, R_f^{H3} 0.38. Anal. ($C_{41}H_{58}N_{13}O_{13}\cdot H_2O$) C, H, N.

Z-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2·AcOH (13). Compound **11** (7 g, 7.3 m*M*) was hydrogenated in 50% AcOH (100 ml) and 10 *N* HCl (1.4 ml) over 10% Pd/C (0.4 g) for 24 hr (room temperature, 1 atm). The mixture was filtered (Celite) and evaporated, EtOH (100 ml) was added and evaporated (twice), and the residue precipitated from EtOH with Et_2O to give **Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2·2HCl (12)** as a white powder: 6.7 g; R_f^C 0.50; Tyr 0.97, Gly 2.05, Leu 0.98, Pro 1.04, Arg 0.96, Ser present. To a solution of compound **12** (5.8 g) and Et_3N (2.1 ml) in DMF (35 ml) was added Z-Trp-ONp (5.5 g, 12 m*M*). The mixture was kept at −15° for 66 hr, filtered, and evaporated. The crude product was purified by countercurrent distribution between upper (18 ml) and lower (25 ml) layers of a mixture of AcOEt–pyridine–AcOH–H_2O (20:1:1:8), 677 transfers, K = 0.1. Evaporation of the fraction containing product (TLC) and trituration with Et_2O gave white powdery octapeptide acetate **13:** 3.0 g (40% from compound **11**); $[\alpha]^{20}D$ −44.0°; $[\alpha]^{20}_{546}$ −50.3° (c 1, 10% AcOH); R_f^{B3} 0.50, R_f^C 0.65, R_f^G 0.81, R_f^{H1} 0.87; Tyr 0.98, Leu 0.97, Gly 1.93, Pro 1.05, Arg 1.07, Ser, Trp present. Anal. ($C_{52}H_{69}N_{13}O_{12}\cdot CH_3CO_2H\cdot 3H_2O$) C, H, N.

<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (LH-RH, 15a). Octapeptide **13** (2 g, 1.7 m*M*) was hydrogenated in 60% AcOH (24 ml) over 10% Pd/C (0.3 g) for 2 hr (room temperature, 1 atm). The mixture was filtered (Celite) and evaporated to give octapeptide acetate **14** as a gum: R_f^C 0.25, R_f^{H2} 0.24. A sample was dissolved in 10% AcOH, filtered, and lyophilized. Anal. ($C_{44}H_{63}N_{13}O_{10}\cdot 3CH_3CO_2H\cdot 7H_2O$) C, N; H: calcd, 7.2; found, 6.5. To a solution of the remaining compound **14** in DMF (12 ml) and Et_3N (slight excess) was added a solution of <Glu-His-N_3 prepared from the hydrazide (1.04 g, 3.7 m*M*) in DMF (21 ml) as described.[46] The mixture was stirred at −20° (1 hr), 0° (5.5 hr), kept at −15° overnight, and filtered, and the crude product precipitated by adding Et_2O. Cl^- ions were removed by filtering a solution in 1% AcOH (50 ml) through DEAE-Sephadex A25 resin (50 m*M*) and evaporating. The crude acetate was chromatographed (a) on a column (97 × 2.5 cm) of CM-Sephadex C-25 (pyridinium form) equilibrated with 1% AcOH and 1% pyridine (v/v) and eluted with a gradient to 5% AcOH and 5% pyridine (v/v) and then (b) by partition chromatography on a Sephadex LH 20 column (100 × 2.5 cm) with $CHCl_3$–AcOH–H_2O–EtOH (10:1:9:15). Fractions containing product were combined, evaporated, and triturated with Et_2O, and an aqueous solution was lyophilized, giving LH-RH-acetate (**15a**): 1.14 g (52%); $[\alpha]^{20}_{578}$ −49.8° (c 1, 10% AcOH); R_f^{B3} 0.1, R_f^C 0.43, R_f^G 0.76, R_f^{H1} 0.50; Tyr 1.00, Leu 0.97, Gly 1.99, Pro 1.03, Glu 1.00, His 1.00, Ser 0.84, Arg 1.01. Anal. ($C_{55}H_{75}N_{17}O_{13}\cdot 2CH_3CO_2H\cdot H_2O$) C, H, N.

Gly-Leu-Arg-Pro-Gly-NH_2-pivalate (16). Hydrogenation of compound **7** (1.2 g, 1.7 m*M*) in 70% AcOH (15 ml) over 10% Pd/C (0.1 g; room temperature, 1 atm) for 19 hr, followed by chromatography on a column (20 × 1.5 cm) of CM-Sephadex C-25 resin [eluting with HOAc–pyridine–H_2O from (v/v) 1:1:98 (pH 4.7) to 5:5:98 (pH 4.7)] gave pentapeptide acetate: one spot on TLC. A solution of the acetate in H_2O (20 ml) was filtered through DEAE-Sephadex A25 (pivalate form, 10 m*M*), and the filtrate was evaporated and triturated repeatedly with Et_2O giving pentapeptide pivalate (**16**): 0.89 g (77%); $[\alpha]^{25}D$ −37.5°: $[\alpha]^{25}_{546}$ −44.6° (c 1, DMF); R_f^{B3} 0.03, R_f^C 0.26, R_f^F 0.16; Arg 0.99, Pro 0.99, Gly 2.02, Leu 1.02. Anal. ($C_{21}H_{39}N_9O_5\cdot 1.25(CH_3)_3CCO_2H\cdot H_2O$) C, H, N.

Simplified Isolation of Arginine Peptides Z-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2-acetate (17). A solution of pentapeptide **16** (0.53 g, 0.8 m*M*) and Z-Tyr-ONp (0.39 g, 0.9 m*M*) in DMF (4 ml) was kept overnight; no compound **16** could then be detected (TLC). The solution was evaporated, dissolved in DMF–H_2O (3:1 v/v; 10 ml), and filtered through CM-Sephadex C-25 resin [H^+ form; ca. 5 m*M;* equilibrated with DMF–H_2O (3:1)] in a 20-ml separating funnel plugged with cotton wool. The resin was washed (DMF–H_2O 3:1) and the filtrate and washings (containing HONp and Z-Tyr-ONp but not product, TLC) were rejected. Product was washed off the resin with pyridine–AcOH–DMF–H_2O (1:1:6:2); evaporation and trituration with ether gave hexapeptide acetate **17:** 0.69 g (97%); $[\alpha]^{25}D$ −39.7°; $[\alpha]^{25}_{546}$ −47.3°; R_f^{B3} 0.35, R_f^C 0.57, R_f^F 0.63; Tyr 1.00, Gly 2.02, Leu 1.02, Arg 0.97, Pro 1.00. Anal. ($C_{38}H_{54}N_{10}O_9\cdot CH_3CO_2H\cdot H_2O$) C, H, N.

Synthesis of LH-RH (15b) (Chart II). Tyr-Gly-Leu-Arg-Pro-Gly-NH_2-pivalate (18). A solution of compound **9** (3.5 g, 4 m*M*) in 70% AcOH (60 ml) was hydrogenated over 10% Pd/C (0.6 g; room temperature, 1 atm) for 24 hr. The crude product was chromatographed on a CM-Sephadex C-25 column (85 × 2.5 cm) as described for compound **16** and on trituration with Et_2O gave **18** acetate (2.4 g): $[\alpha]^{25}D$ −40.5° (c 2, DMF); R_f^{B3} 0.30, R_f^C 0.45, R_f^G 0.70, R_f^{H1} 0.75. Treatment of the acetate (1.02 g) with DEAE-Sephadex (pivalate form) as for compound **16** gave hexapeptide pivalate (**18**): 0.91 g (63%); $[\alpha]^{25}D$ −45.0° (c 1, DMF); R_F^{B3} 0.30, R_f^C 0.45, R_f^G 0.70, R_f^F 0.47, R_f^{H1} 0.75. Anal. ($C_{30}H_{48}N_{10}O_7\cdot 1.5(CH_3)_3CCO_2H\cdot H_2O$) H, N; C: calcd, 54.1; found, 54.6.

Z-Ser(Bu^t)-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2-acetate (19). A solution of compound **18** (1.03 g, 1.2 m*M*), Et_3N (0.05 ml), and Z-Ser(Bu^t)-ONp (0.78 g, 1.9 m*M*) in DMF (7 ml) was kept at 0° for 16 hr. The mixture was worked up as for compound **17** [washing the product off the resin with pyridine–AcOH–DMF–H_2O (1:1:4:4)] giving heptapeptide **19:** 1.14 g (92%); $[\alpha]^{25}D$ −35.1°; $[\alpha]^{25}_{546}$ −42.3° (c 1, DMF); R_f^{B3} 0.45, R_f^C 0.60, R_f^F 0.67, R_f^G 0.87, R_f^{H1} 0.75 with only trace impurities. Anal. ($C_{45}H_{67}N_{11}O_{11}\cdot CH_3CO_2H$) C, H, N. Trace impurities were removed by chromatography on a column of CM-Sephadex C-25 as described for compound **16** (overall yield 65%).

<Glu-His-Trp-OMe (21). A solution of <Glu-His-N_3 in DMF (2.5 ml) prepared[46] from the hydrazide (0.18 g, 0.65 m*M*) was stirred at −40° and H-Trp-OMe·HCl (0.33 g, 1.3 m*M*) and excess Et_3N (0.2 ml) were added. The mixture was partitioned between upper (2 ml) and lower (2 ml) layers of pyridine–0.1% AcOH–$CHCl_3$–EtOH (3:5:4:4) for 19 transfers and fractions containing product were combined, evaporated, and dissolved in EtOH (10 ml). The solution, on standing, deposited crystalline **21:** yield (after recrystallization from EtOH–Et_2O) 81 mg (27%); mp 237–240° dec; $[\alpha]^{20}D$ −52.0°; $[\alpha]^{20}_{546}$ −72.0° (c 1, 10% AcOH); R_f^C 0.57, R_f^G 0.74, R_f^{H1} 0.69, R_f^{H3} 0.24. Anal. ($C_{23}H_{26}N_6O_5$) C, H, N. A preparation via Z-His-Trp-OMe gave a similar product.

<Glu-His-Trp-OH (22). A mixture of compound **21** (45 mg, 0.1 m*M*) and 2 *M* NaOH (2 ml) was stirred for 30 min, AcOH (1 ml) and H_2O (10 ml) were added, and the solution was filtered through SP-Sephadex C-25 resin (12 m*M*) and washed off with pyridine–H_2O (1:19) (no Cl^- ions detected). Evaporation and washing the resulting solid with EtOH gave **22:** 45 mg (94%); $[\alpha]^{20}_{578}$ −19.0°; $[\alpha]^{20}_{546}$ −21.6° (c 0.5, 50% EtOH); R_f^C 0.38, R_f^G 0.53, R_f^{H1} 0.48. Anal. ($C_{22}H_{24}N_6O_5\cdot 2.5H_2O$) C, H; N: calcd, 16.9; found, 16.45.

Ser(Bu^t)-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2-pivalate (20). Hydrogenation of compound **19** (0.87 g, 0.87 m*M*) in 60% AcOH (15 ml) over 10% Pd/C (75 mg; room temperature, 4 hr) and conversion to the pivalate as described for compound **16** gave heptapeptide pivalate **20** as a white power: 0.63 g (67%); $[\alpha]^{25}D$ −34.1° (c 1.3, DMF); R_f^C 0.53, R_f^F 0.60 (streaking), R_f^G 0.84, R_f^{H1} 0.72; Arg 0.97, Ser 0.99 (corrected), Pro 0.98, Gly 2.05, Leu 1.01, Tyr 0.98. Anal. ($C_{37}H_{61}N_{11}O_9\cdot (CH_3)_3CCO_2H\cdot H_2O$) C, H, N.

<Glu-His-Trp-Ser(Bu^t)-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2·AcOH ([Ser(Bu^t)^4]-LH-RH, 23a). A solution of compound **20** (0.60 g, 0.65 m*M*), compound **22** (0.35 g, 0.70 m*M*), and triethylamine (10 mg) in DMF (3 ml) was cooled to −15°. DCCI (0.32 g, 1.55 m*M*) and HONSu (0.18 g, 1.55 m*M*) were added and the mixture was kept at −15° for 5 days. The mixture was filtered, evaporated, and triturated with Et_2O and the crude product chromato-

graphed on a CM-Sephadex C-25 column (31 × 1.5 cm) as described for compound **16** giving white, powdery decapeptide **23a**: 0.23 g (26%); $[\alpha]^{25}D$ −29.4° (*c* 0.8, DMF); R_f^C 0.34, R_f^{H1} 0.80, R_f^{H2} 0.40. Anal. ($C_{59}H_{83}N_{17}O_{13} \cdot 2CH_3CO_2H$) C, H, N.

LH-RH (15b). A solution of decapeptide **23a** (0.20 g, 0.15 m*M*) in TFA–H_2O (85:15, 6.5 ml) was kept at −10° under N_2 for 30 min and at room temperature for 3 hr. Addition of Et_2O (90 ml) precipitated a white solid which was washed with Et_2O, dissolved in H_2O, filtered through DEAE-Sephadex A-25 (AcO^- form, 5 m*M*), evaporated, and triturated with Et_2O to give crude **15**: 0.17 g (84%); R_f^C 0.25, R_f^G 0.78 with only trace impurities. Chromatography on a column (35 × 2 cm) of CM-Sephadex C-25 resin as for compound **16** gave white powdery LH-RH acetate **15b** (after trituration with Et_2O): 0.14 g (70%); $[\alpha]^{20}D$ −53.6°; $[\alpha]^{20}_{546}$ −60.0° (*c* 0.5, H_2O); R_f^C, R_f^G, R_f^{H1} identical with **15a**; Arg 0.99, Glu 1.04, Gly 1.94, His 0.99, Leu 1.00, Pro 1.04, Ser 0.83, Tyr 1.01, NH_3 1.33; HVE, R_{His} 0.60 (pH 5.3), Pauly and Sakaguchi reagents. Anal. ($C_{55}H_{75}N_{17}O_{13} \cdot 2.5CH_3CO_2H$) C, H, N.

Synthesis of [Ser(Bu^t)^4]-LH-RH (23b) (Chart III). Z-Trp-Ser(Bu^t)-Tyr-Gly-Leu-Arg-Pro-Gly-NH$_2$-acetate (24). A solution of compound **20** (0.67 g, 0.7 m*M*) and Z-Trp-ONp (0.40 g, 0.9 m*M*) in DMF (3 ml) was kept at 0° overnight. Work-up as described for compound **19** gave **24**: 0.87 g (97%); TLC, one major spot with only trace impurities. Chromatography as described for compound **16** gave (after trituration with Et_2O) octapeptide **24**: 0.62 g (69%); $[\alpha]^{25}D$ −33.0°; $[\alpha]^{25}_{546}$ −39.5° (*c* 0.8, DMF); R_f^{B3} 0.43, R_f^C 0.68, R_f^F 0.73, R_f^{H3} 0.50, R_f^{H2} 0.60. Anal. ($C_{56}H_{77}N_{13}O_{12} \cdot CH_3CO_2H \cdot H_2O$) C, H, N.

Trp-Ser(Bu^t)-Tyr-Gly-Leu-Arg-Pro-Gly-NH$_2$-acetate (25). Octapeptide **24** (0.60 g, 0.5 m*M*) was hydrogenated in 60% AcOH (15 ml) over 10% Pd/C (0.1 g; room temperature, 1 atm) for 4 hr, and the mixture was filtered, evaporated, and triturated with Et_2O giving acetate **25**: 0.56 g (98%); R_f^{B3} 0.26, R_f^C 0.59, R_f^{H2} 0.39; HVE, 1 spot (Sakaguchi), pH 6.5. Anal. ($C_{48}H_{71}N_{13}O_{10} \cdot 2CH_3CO_2H \cdot 2H_2O$) C, H, N.

[Ser(Bu^t)^4]-LH-RH (23b). To a stirred solution of compound **25** (0.51 g, 0.44 m*M*) with a trace of Et_3N in DMF (2.5 ml) at −25° was added a solution of <Glu-His-N_3 prepared[32] from the hydrazide (0.15 g, 0.55 m*M*) in DMF (2 ml). The mixture was kept at −15° for several days and worked up as for compound **19**, washing with pyridine–DMF–H_2O (1:5:5) to remove all coproducts. Trituration with Et_2O gave decapeptide **23b** as an off-white solid: 0.46 g (76%); R_f^C, R_f^{H2} identical with **23a**; HVE, 1 spot (Pauly, Sakaguchi), pH 6.5.

[Leu^4]-LH-RH (28) (Chart IV). Z-Leu-Tyr-Gly-Leu-Arg-Pro-Gly-NH$_2$-acetate (26). A solution of compound **18** (0.06 g, 0.07 m*M*), Z-Leu-ONp (0.06 g, 0.15 m*M*), and $(CH_3)_3CCO_2H$ (0.1 g, 1 m*M*) in DMF (2.5 ml) was kept at 0° overnight. Work-up as for compound **19** gave heptapeptide acetate **26** (after trituration with Et_2O): 0.06 g (76%); R_f^C 0.65, R_f^F 0.57, R_f^{H1} 0.81; Arg 1.02, Leu 1.98, Tyr 0.99, Gly 2.01, Pro present. Anal. ($C_{44}H_{65}N_{11}O_{16} \cdot 2CH_3CO_2H \cdot H_2O$) C, H, N.

Leu-Tyr-Gly-Leu-Arg-Pro-Gly-NH$_2$ · 2HCl (27). A solution of compound **26** (60 mg, 0.06 m*M*) in MeOH (3.5 ml) and 0.1 *M* HCl (1.8 ml) was hydrogenated over 10% Pd/C (10 mg) for 1.5 hr. The mixture was filtered (Celite), evaporated, dissolved in H_2O (4 ml), and lyophilized to give **27**: 52 mg (89%); R_f^{B3} 0.40, R_f^C 0.58, R_f^{H1} 0.65; Tyr 0.99, Gly 2.01, Pro 0.99, Arg present. Anal. ($C_{36}H_{59}N_{11}O_8 \cdot 2HCl \cdot 10H_2O$) H, N; C: calcd, 42.5; found, 43.0.

<Glu-His-Trp-Leu-Tyr-Gly-Leu-Arg-Pro-Gly-NH$_2$ · AcOH ([Leu^4]-LH-RH, 28). To a solution of heptapeptide **27** (39 mg, 0.05 m*M*), tripeptide **22** (42 mg, 0.09 m*M*), and Et_3N (10 μl) in DMF at −15° (0.2 ml) was added HONSu (10.5 mg, 0.09 m*M*) and DCCI (18.8 mg, 0.09 m*M*). The mixture was kept at 0–5° overnight, diluted with buffer (pyridine–AcOH–H_2O 1:1:98), and chromatographed as for compound **16** on CM-Sephadex C-25 (50 × 1.3 cm column). The product (36 mg) with trace impurities on TLC was purified by preparative TLC (solvent H1) and the product (29 mg) rechromatographed on CM-Sephadex giving, after lyophilization, decapeptide acetate **28**: 20 mg (41%); $[\alpha]^{20}D$ −41.2°; $[\alpha]^{20}_{546}$ −48.8° (*c* 0.7, 10% AcOH); R_f^{B3} 0.38, R_f^C 0.51, R_f^{H4} 0.45; His 0.96, Glu 1.04, Pro 1.04, Gly 2.00, Leu 1.96, Tyr 1.00.

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References and Notes

(1) H. Matsuo, Y. Baba, R. M. G. Nair, A. Arimura, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, **43**, 1334 (1971).
(2) R. Burgus, M. Butcher, N. Ling, M. Monahan, J. Rivier, R. Fellows, M. Amoss, R. Blackwell, W. Vale, and R. Guillemin, *C. R. Acad. Sci., Paris, Ser. D*, **273**, 1611 (1971).
(3) H. Matsuo, A. Arimura, R. M. G. Nair, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, **45**, 822 (1971).
(4) P. Rivaille, A. Robinson, M. Kamen, and G. Milhaud, *Helv. Chim. Acta*, **54**, 2772 (1971).
(5) R. Geiger, W. König, H. Wissman, K. Geisen, and F. Enzmann, *Biochem. Biophys. Res. Commun.*, **45**, 767 (1971).
(6) M. W. Monahan and J. Rivier, *Biochem. Biophys. Res. Commun.*, **48**, 1100 (1972).
(7) H. Sievertsson, J.-K. Chang, A. von Klaudy, C. Bogentoft, B. Currie, K. Folkers, and C. Bowers, *J. Med. Chem.*, **15**, 222 (1972).
(8) J.-K. Chang, H. Sievertsson, B. L. Currie, C. Bogentoft, K. Folkers, and C. Y. Bowers, *J. Med. Chem.*, **15**, 623 (1972).
(9) W. Voelter and K. Zech, *Chimia*, **26**, 313 (1972).
(10) H. C. Beyerman, H. Hindriks, J. Hirt, E. W. B. de Leer, and A van der Wiele, *Recl. Trav. Chim. Pays-Bas*, **91**, 1239 (1972).
(11) M. Fujino, T. Fukuda, S. Kobayashi, and M. Obayashi, *Chem. Pharm. Bull.*, **21**, 87 (1973).
(12) G. R. Flouret, W. H. Arnold, J. W. Cole, R. L. Morgan, W. F. White, M. T. Hedlund, and R. H. Rippel, *J. Med. Chem.*, **16**, 369 (1973).
(13) N. Yanaihara, C. Yanaihara, M. Sakagami, K. Tsuji, T. Hashimoto, T. Kaneko, H. Oka, A. V. Schally, A. Arimura, and T. W. Redding, *J. Med. Chem.*, **16**, 373 (1973).
(14) W. H. Arnold, W. White, and G. Flouret, *J. Med. Chem.*, **16**, 1054 (1973).
(15) M. W. Monahan, J. Rivier, W. Vale, R. Guillemin, and R. Burgus, *Biochem. Biophys. Res. Commun.*, **47**, 551 (1972).
(16) J.-K. Chang, R. H. Williams, A. J. Humphries, N.-G. Johansson, K. Folkers, and C. Y. Bowers, *Biochem. Biophys. Res. Commun.*, **47**, 727 (1972).
(17) J.-K. Chang, A. J. Humphries, R. H. Williams, H. Sievertsson, K. Folkers, and C. Y. Bowers, *Biochem. Biophys. Res. Commun.*, **47**, 1256 (1972).
(18) A. V. Schally, A. Arimura, W. H. Carter, T. W. Redding, R. Geiger, W. König, H. Wissman, G. Jaeger, J. Sandow, N. Yanaihara, C. Yanaihara, T. Hashimoto, and M. Sakagami, *Biochem. Biophys. Res. Commun.*, **48**, 366 (1972).
(19) 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).
(20) 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).
(21) N. Yanaihara, C. Yanaihara, T. Hashimoto, Y. Kenmochi, T. Kaneko, H. Oka, S. Saito, A. V. Schally, and A. Arimura, *Biochem. Biophys. Res. Commun.*, **49**, 1280 (1972).
(22) R. Geiger, H. Wissmann, W. König, J. Sandow, A. V. Schally, T. W. Redding, L. Debeljuk, and A. Arimura, *Biochem. Biophys. Res. Commun.*, **49**, 1467 (1972).
(23) D. H. Coy, E. J. Coy, and A. V. Schally, *J. Med. Chem.*, **16**, 83 (1973).
(24) 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).
(25) J. Rivier, M. Monahan, W. Vale, G. Grant, M. Amoss, R. Blackwell, R. Guillemin, and R. Burgus, *Chimia*, **26**, 300 (1972).
(26) N. Yanaihara, K. Tsuji, C. Yanaihara, T. Hashimoto, T. Kaneko, H. Oka, A. Arimura, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, **51**, 165 (1973).
(27) 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).
(28) D. H. Coy, E. J. Coy, and A. V. Schally, *J. Med. Chem.*, **16**, 1140 (1973).
(29) 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).
(30) J. Rivier, W. Vale, R. Burgus, N. Ling, M. Amoss, R. Blackwell, and R. Guillemin, *J. Med. Chem.*, **16**, 545 (1973).
(31) R. S. Mecklenburg, K. Noda, Y. Miyachi, E. Gross, and M. B. Lipsett, *Endocrinology*, **93**, 993 (1973).
(32) M. W. Monahan, M. S. Amoss, H. A. Anderson, and W. Vale, *Biochemistry*, **12**, 4616 (1973).

(33) J. Rivier, M. Amoss, C. Rivier, and W. Vale, *J. Med. Chem.*, 17, 230 (1974).
(34) P.-A. Jaquenoud and R. A. Boissonnas, *Helv. Chim. Acta*, **45**, 1462 (1962).
(35) D. J. Schafer and A. D. Black, *Tetrahedron Lett.*, 4071 (1973).
(36) R. Camble, R. Garner, and G. T. Young, *Nature (London)*, **217**, 247 (1968); R. Camble, R. Garner, and G. T. Young, *J. Chem. Soc C*, 1911 (1969).
(37) D. J. Schafer, G. T. Young, D. F. Elliot, and R. Wade, *J. Chem. Soc. C*, 46 (1971).
(38) D. J. Schafer, *J. Chem. Soc., Perkin Trans. 1*, 1452 (1972).
(39) D. J. Schafer and L. Carlsson, *J. Chem. Soc., Chem. Commun.*, 276 (1972).
(40) N. Izumiya, K. Noda, and C. B. Anfinsen, *Arch. Biochem. Biophys.*, **144**, 237 (1971).
(41) Abbreviations follow the recommendations of the IUPAC–IUB Commission on Biological Nomenclature [*Biochem. J.*, **104**, 17 (1967); **126**, 773 (1972)].
(42) B. M. Hartley, D. B. Crighton, and G. E. Lamming, *J. Endocrinol.*, **58**, 363 (1973).
(43) D. B. Crighton, B. M. Hartley, and G. E. Lamming, *J. Endocrinol.*, **58**, 377 (1973).
(44) D. B. Crighton, *J. Endocrinol.*, **58**, 387 (1973).
(45) D. J. Schafer, Doctor of Philosophy Thesis, Oxford, 1970.
(46) D. Gillessen, A. M. Felix, W. Lergier, and R. O. Studer, *Helv. Chim. Acta*, **53**, 63 (1970).

Notes

Effect of 9-Hydroxylation on Benzomorphan Antagonist Activity

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In a benzomorphan bearing an antagonist side chain, introduction on the methano bridge of a hydroxyl oriented away from nitrogen has little effect on antagonist activity whereas a hydroxyl oriented toward nitrogen enhances this activity. Hydroxylation tends to decrease analgesic activity.

In a series of pioneering papers,[1-4] May and coworkers demonstrated the effects on analgesic activity of introducing an OH group on the methano bridge of benzomorphans.[5]

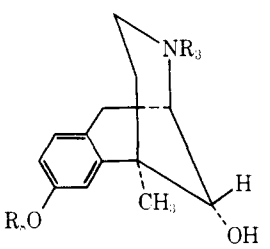
In more recent years morphine derivatives having antagonist side chains of varying degrees of potency have been oxygenated in the analogous position to give naloxone, naltrexone, nalmexone, and nalbuphine, and this oxygenation has been extended to the morphinan series to give oxilorphan and butorphanol.

The present note provides some data on the effect of this hydroxy group on antagonist activity in the benzomorphan series. Workers at Bristol-Myers have described some 9-hydroxybenzomorphans[6] but apparently have not prepared the compounds listed in Table I.

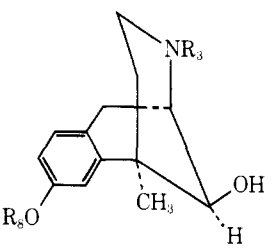
Chemistry. May's procedures were used in the synthesis of **1a**, **2a**, and **3a**.

Treatment of **1a** with CNBr proceeded uneventfully as noted by Kugita and May.[3] However, treatment of **2a** or **3a** with CNBr gave considerable by-product which proved to be **4**.

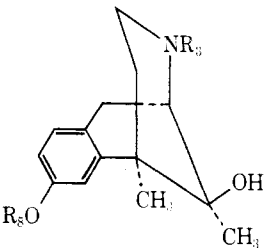
Elemental analysis, the strong band in the ir at 1750 cm^{-1}, and the lack of a CN band were the most conclusive evidence for structure **4a**, but uv, NMR, and MS were confirmatory. The structure of **4b** was inferred by analogy and by the ir spectrum.



1



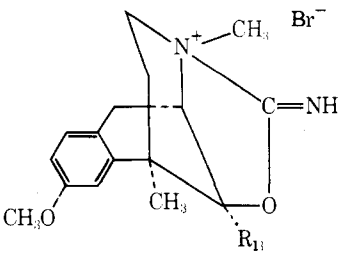
2



3

a, $R_3 = R_8 = CH_3$
b, $R_3 = CN$; $R_8 = CH_3$
c, $R_3 = H$; $R_8 = CH_3$
d, $R_3 = R_8 = H$

Recently Vaughan, Hill, and Mitchard[7] published a method using mass spectrometry for distinguishing the configuration of the hydroxy group at C_{11} when only one isomer is available. The determination depended upon the relative peak heights of the mass ion, $M^+ - C_{11}CO$, and the



4a, $R_{11} = H$
b, $R_{11} = CH_3$