extracts were washed with H_2O (2 × 5 mL), dried over Na_2SO_4, and concentrated to afford a yellow solid residue, still containing pyrrolidone, which was removed under high vacuum. The product was chromatographed on 10 g of SiO_2 (first with CH_2Cl_2 to remove an impurity and then with CH_2Cl_2–MeOH, 9:1) to afford a yellow material, which after trituration with ether gave 410 mg (44%) of thiocornigerine (**4**) as a canary-yellow solid: mp 140 °C; $[\alpha]^{20}_D$ −155° (*c* 0.65, $CHCl_3$); NMR ($CDCl_3$) δ 1.85–2.45 (m, 4 H, 2 H_5 and 2 H_6), 2.06 (s, 3 H, NHAc), 2.50 (s, 3 H, SCH_3), 3.81 (s, 3 H, OCH_3), 4.70 (m, 1 H, H_7), 6.02 (s, 4 H, OCH_2O), 6.48 (s, 1 H, H_4), 7.18–7.46 (m, 3 H, H_8, H_{11}, H_{12}); CIMS, *m/e* 388 (M^+ + 1). Anal. ($C_{20}H_{21}NO_5S$) C, H, N, S.

1-Demethyl-1-acetylthiocolchicine (**8**) (according to a modified procedure of Bladé-Font[9]). To a cold (0–5 °C) solution of 5 g (12.05 mmol) of thiocolchicine (**1**) in 50 mL of CH_2Cl_2 and 15 mL of acetyl chloride was added 5 mL of $SnCl_4$, and the mixture stirred at room temperature. After 24 h a substantial amount of thiocolchicine was still present in the reaction mixture, and addition of $SnCl_4$ and prolonged reaction time had no influence on the ratio of starting material and product. The reaction mixture was cooled and cold water was added slowly. After extraction with CH_2Cl_2 (3×), the CH_2Cl_2 layer was washed with saturated $NaHCO_3$ solution (2×), dried, and evaporated to give 5.2 g of a yellow solid, which was directly hydrolyzed to the mixture of thiocolchicine (**1**) and phenol **7** by stirring for 30 min at room temperature in a mixture of 20 mL of MeOH and 50 mL of 1 N NaOH. The MeOH was evaporated and the residue extracted extensively with Et_2O to get all the thiocolchicine (**1**) out. The water phase was acidified (pH 4) with 2 N HCl, and the yellow precipitate filtered, washed with H_2O, and dried overnight to give 1.32 g (66% based on recovered **1**) of **7**. Acetylation with 6 mL of acetic anhydride and 12 mL of pyridine gave 1.28 g (88%) of **8** as yellow crystals, mp 218–219 °C (from ethyl acetate, lit.[9] mp 210 °C then 220 °C).

1-Demethyl-1-acetyl-8,10,11,12-tetrahydro-10-demethoxycolchicine (**9**) **and 1-Demethyl-1-acetylcolchicide** (**10**). A mixture of 1 g (2.26 mmol) of **8** in 100 mL of acetone was treated with Raney nickel catalyst as described earlier (see preparation of **2**). After 1 h, starting material could not be detected on TLC, but two new spots corresponding to compounds **9** and **10** were observed. Flash chromatography ($CHCl_3$–MeOH, 100:7) gave 460 mg of **9** as the faster moving compound and 280 mg of **10**.

9: light yellow foam; UV (EtOH) λ_{max} nm (log ε) 256 (4.04); CIMS, *m/e* 402 (M^+ + 1).

10: yellow crystals from MeOH–ether, mp 163–164 °C; $[\alpha]^{20}_D$ −235° (*c* 0.26, MeOH);[16] UV (EtOH) λ_{max} nm (log ε) 232 (4.18), 328 (3.78); IR (Nujol) 1765, 1670, 1605, 1590 cm^{-1}; NMR (CD_3OD) δ 1.87 and 2.19 (m, 2 H, H_6), 1.98 (s, 3 H, NHAc), 2.18 (s, 3 H, OAc), 2.46 and 2.70 (m, 2 H, H_5), 3.81 and 3.93 (s, 6 H, OCH_3), 4.44 (m, 1 H, H_7), 6.90 (s, 1 H, H_4), 7.05 (m, 2 H, H_{10}, H_{12}), 7.18 (d, *J* = 2.5 Hz, 1 H, H_8), 7.40 (dd, J_1 = 11.5, J_2 = 9.5 Hz, 1 H, H_{11}), 8.61 (d, *J* = 7 Hz, 1 H, NH); CIMS, *m/e* 398 (M^+ + 1). Anal. ($C_{22}H_{23}NO_6 \cdot 0.75H_2O$) C, H, N.

Refluxing **9** in toluene with Pd/C catalyst (see preparation of **3**) gave a compound in 72% yield, which was in all respects identical with **10**.

1-Demethylcolchicide (**11**). A mixture of 80 mg (0.2 mmol) of **10**, 240 mg of anhydrous K_2CO_3, and 5 mL of MeOH was stirred at room temperature under argon for 2 h. After filtration, 50 mL of $CHCl_3$ was added, together with 2 N HCl, until the pH reached 5. The H_2O phase was extracted once with $CHCl_3$, and the combined organic layer was washed with brine, dried over $MgSO_4$, and evaporated to give 70 mg of a yellow solid. Crystallization from EtOH–ether gave 60 mg (84%) of **11** as slightly yellow crystals: mp 213–214 °C; $[\alpha]^{20}_D$ −261° (*c* 0.3, MeOH); UV (EtOH) λ_{max} nm (log ε) 234 (4.47), 329 (4.01); IR (Nujol) 3350, 1665, 1610, 1590 cm^{-1}; NMR (CD_3OD) δ 1.87 and 2.17 (m, 2 H, H_6), 1.98 (s, 3 H, NHAc), 2.37 and 2.59 (m, 2 H, H_5), 3.82 and 3.89 (s, 6 H, OCH_3), 4.47 (m, 1 H, H_7), 6.48 (m, 1 H, H_4), 7.06 (m, 2 H, H_{10}, H_{12}), 7.21 (d, *J* = 3 Hz, 1 H, H_8), 7.43 (dd, J_1 = 12, J_2 = 9 Hz, 1 H, H_{11}); CIMS, *m/e* 356 (M^+ + 1). Anal. ($C_{20}H_{21}NO_5 \cdot 1.25H_2O$) C, H, N.

2-Demethylcolchicide (**21**). A mixture of 100 mg (0.27 mmol) of colchicide (**3**) in 2 mL of concentrated H_2SO_4 was stirred at 55–60 °C (oil bath temperature). After 4 h the reaction mixture was poured on ice and the pH adjusted to 5 with 10% NaOH. Extraction with $CHCl_3$–MeOH (4:1) (3×), drying over $MgSO_4$, and evaporation gave a yellow solid, which was purified by flash chromatography ($CHCl_3$–MeOH, 100:8) to give 80 mg (83%) of a yellow amorphous solid: $[\alpha]^{20}_D$ −268° (*c* 0.11, $CHCl_3$); UV (EtOH) λ_{max} nm (log ε) 234 (4.44), 330 (3.99); IR ($CHCl_3$) 3580, 3490, 3320, 1690, 1645, 1575 cm^{-1}; NMR ($CDCl_3$) δ 1.79 and 2.19 (m, 2 H, H_6), 1.99 (s, 3 H, NHAc), 2.49 (m, 2 H, H_5), 3.67 and 3.93 (s, 6 H, OCH_3), 4.59 (m, 1 H, H_7), 6.51 (s, 1 H, H_4), 7.04–7.27 (m, 4 H, H_8, H_{10}, H_{11}, H_{12}); CIMS, *m/e* 356 (M^+ + 1). Anal. ($C_{20}H_{21}NO_5 \cdot 0.5CH_3OH$) C, H, N.

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Structure–Activity Studies of Antagonists of Luteinizing Hormone-Releasing Hormone with Emphasis on the Amino-Terminal Region[1]

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The structure–activity relationship of the hydrophobic amino terminal region of the antagonist [N-Ac-D-Nal^1,D-p$ClPhe^2$,D-Trp^3,D-Arg^6,Phe^7,D-Ala^{10}]-LH-RH has been investigated by the incorporation of a variety of amino acids with emphasis on positions 1, 2, and 3. The analogues were prepared by routine solid-phase peptide synthesis. All purifications were performed in two stages: gel permeation chromatography followed by preparative, reversed-phase, high-performance chromatography. The analogues were assayed in a standard rat antiovulatory assay using a 40% propane-1,2-diol–saline vehicle. A simplified antagonist was developed that allowed the removal of the custom-synthesized D-pClPhe and the labile D-Trp while retaining antiovulatory potency. The compound [N-Ac-D-Nal^1,D-$Phe^{2,3}$,D-Arg^6,Phe^7,D-Ala^{10}]-LH-RH caused a 56% blockade of ovulation at the 500-ng dose and is approximately equipotent with the parent analogue in this system.

Since the discovery of the postulated luteinizing hormone-releasing hormone (LH-RH), a decapeptide with the sequence Glp-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, extensive structure–activity studies have produced both

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Table I. Antiovulatory Activity of N-Terminal-Modified LH-RH Analogues

	analogue				antiovulatory activity[a]
I	[N-Ac-D-Nal[1],	D-pClPhe[2],	D-Trp[3],	D-Arg[6],Phe[7],D-Ala[10]]-LH-RH	64% @ 0.5 (11)
II			D-Tyr[3]		86% @ 0.5 (14)
III			D-pClPhe[3]		63% @ 3 (8)
					0% @ 1 (8)
IV			D-Phe[3]		56% @ 1 (9)
					10% @ 0.5 (10)
V		D-Phe[2]	D-Phe[3]		100% @ 1 (9)
					56% @ 0.5 (9)
VI		D-Tyr[2]	D-Phe[3]		14% @ 3 (7)
					0% @ 1 (16)
VII		D-Tyr[2]	D-Tyr[3]		0% @ 1 (8)
VIII		D-Phe[2]	D-Tyr[3]		0% @ 1 (9)
IX	D-pClPhe[1]	D-Phe[2]	D-Phe[3]		63% @ 3 (8)
X	D-Phe[1]	D-Phe[2]	D-Phe[3]		0% @ 3 (8)
XI	D-Trp[1]	D-Phe[2]	D-Phe[3]		0% @ 3 (10)
XII	D-pClPhe[1]	D-pClPhe[2]	D-Trp[3]		100% @ 3 (10)
					70% @ 1 (10)
XIII	D-Nal[1]	D-Nal[2]	D-Tyr[3]		100% @ 7.5 (9)
					38% @ 3 (8)
					9% @ 1 (11)
XIV	D-Nal[1]	D-Nal[2]	D-His[3]		0% @ 1 (10)
XV	D-Nal[1]	D-Nal[2]	D-Phe[3]		100% @ 3 (10)
XVI	D-Nal[1]	D-Nal[2]	D-F_5Phe[3]		0% @ 7.5 (9)
XVII	D-Nal[1]	D-Nal[2]	D-Nal[3]		15% @ 3 (9)

[a] Expressed as the percentage of (n) rats blocked at a dose of x μg.

agonists and antagonists of the native hormone. Antagonists are of interest since, theoretically, they can control fertility either by the suppression of spermatogenesis or by the effective blockade of ovulation and thus are potential contraceptive agents. In the search for ever more potent antagonists, many analogues have been synthesized internationally during the last decade. Presently, the most active analogues are linear peptides, characterized by distinct hydrophobic and hydrophilic regions, and are typified by the antagonist [N-Ac-D-Nal[1],D-pClPhe[2],D-Trp[3],D-Arg[6],Phe[7],D-Ala[10]]-LH-RH.[2] The incorporation of the hydrophobic residue D-Nal in position 1 resulted in a marked increase in potency in a standard antiovulatory assay, when combined with D-Arg in position 6 as compared with the previous series of antagonists. Recently, this potency was further increased by the incorporation of an aromatic residue in position 7, most notably Phe.[3,4] However, past studies have illustrated the necessity to reexamine the structure–activity relationship of new substitutions with regard to the other proven sensitive positions of the new highly active analogue. In this paper we describe a series of analogues that were synthesized in order to investigate the structure–activity relationship of the hydrophobic amino terminus of the antagonist with respect to the new substitution of Phe in position 7.

Results and Discussion

Initially, the substitution of D-Trp at position 3 of LH-RH was studied since Trp is known to be a source of side reactions and hence gives rise to lowered yields of peptide compared to analogues without Trp. The replacement of D-Trp by D-Tyr caused a minor increase in antiovulatory activity (AOA) (I, 64% AOA and II, 86% AOA at 0.5 μg; see Table I). Substitution of the very hydrophobic residue D-pClPhe[3] for D-Trp[3] was accompanied by a dramatic loss in activity (III, 63% AOA at 3 μg) whereas replacement by the moderately hydrophobic D-Phe[3] caused only a slight loss of activity (IV, 10% at 0.5 μg). Since position 3 was intolerant of a large increase in the hydrophobic nature of the residue, the effect of reduced hydrophobicity in positions 1 and 2 was studied. Replacement of D-pClPhe by D-Phe in position 2 together with D-Phe in position 3 produced an analogue that was as active as the original compound (V, 56% AOA and I, 65% AOA at 0.5 μg, respectively) but which contained two relatively inexpensive D-Phe residues in place of the custom-synthesized D-pClPhe and the labile D-Trp. Substitution of the hydrophilic residue D-Tyr in position 2 markedly reduced the activity of the analogue (VI, 14% AOA at 3 μg) as did the incorporation of D-Tyr in positions 2 and 3 (VII, 0% at 1 μg). Additionally, the exchange of D-Phe[3] for D-Tyr[3] in the D-Phe[2,3] analogue was also associated with a reduction in activity (respectively VIII, 0% AOA at 1 and V, 56% at 0.5 μg). A further reduction in the hydrophobic nature of the N-terminus of analogue V by the replacement of D-Nal in position 1 by D-pClPhe caused a large loss of activity (IX, 63% AOA at 3 μg) and this trend was continued with the substitution of D-Phe (X, 63% AOA at 3 μg) and D-Trp (XI, 0% AOA at 3 μg). Additionally, the exchange of D-Nal[1] for D-pClPhe[1] in analogue I was accompanied by a halving of activity (XII, 70% at 1 μg vs. I, 64% at 0.5 μg).[3]

Since the D-Tyr[3] substitution had produced a potent analogue and the previous series had shown a marked preference for the bulky hydrophobic residue D-Nal in position 1, a series of analogues were made with the D-Nal residue also in position 2. The placement of D-Nal in 2 caused a large reduction in activity compared with the

(1) Abbreviations used in this paper for amino acids, protecting groups, and peptides follow the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature and Symbols as described in *Eur. J. Biochem.* **1972**, *27*, 201 and *J. Biol. Chem.* **1975**, *250*, 3215: D-Bta, 3-(benzothien-3-yl)-D-alanine: Cha, cyclohexyl-L-alanine; D-Et_2hArg, N^g,N'^g-diethyl-homo-D-arginine: F_5Phe, 2,3,4,5,6-pentafluorophenyl-L-alanine; Glp, pyroglutamic acid: D-Nal, 3-(2-naphthyl)-D-alanine; D-pClPhe, 4-chlorophenyl-D-alanine.

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Table II. Antiovulatory Activity of Several LH-RH Analogues Modified in Position 6

	analogue	antiovulatory activity[a]
XII	[N-Ac-D-pClPhe^1,D-pClPhe^2,D-Trp^3,D-Arg^6,Phe^7,D-Ala^{10}]-LH-RH	100% @ 3 (10) 70% @ 1 (10)
XVIII	D-Nal^6	0% @ 7.5 (8)
XIX	D-Phe^6	8% @ 7.5 (8)
I	[N-Ac-D-Nal^1, D-pClPhe^2,D-Trp^3,D-Arg^6,Phe^7,D-Ala^{10}]-LH-RH	64% @ 0.5 (11)
XX	D-Lys^6	70% @ 1 (10)
XXI	D-Et$_2$hArg^6	63% @ 1 (8)

[a] Expressed as the percentage of (n) rats blocked at a dose of x μg.

Table III. Antiovulatory Activity of Several LH-RH Analogues Modified in Position 7

	analogue	antiovulatory activity[a]
V	[N-Ac-D-Nal^1, D-Phe^2, D-Phe^3,D-Arg^6,Phe^7,D-Ala^{10}]-LH-RH	56% @ 0.5 (9)
XXII	Cha^7	80% @ 0.5 (10)
XXIII	[N-Ac-D-pClPhe^1,D-pClPhe^2,D-Trp^3,D-Arg^6,D-Ala^{10}]-LH-RH	67% @ 3 (9) 10% @ 1.5 (10)
XXIV	D-Bta^3	100% @ 1 (8) 33% @ 0.5 (10)
XXV	[N-Ac-D-Nal^1, D-pClPhe^2,D-Trp^3,D-Arg^6,D-Ala^{10}]-LH-RH	77% @ 1 (13)
XXVI	D-Bta^3	100% @ 1 (4) 60% @ 0.5 (5)

[a] Expressed as the percentage of (n) rats blocked at a dose of x μg.

D-pClPhe compound (XIII, 9% AOA at 1 μg compared with II, 86% at 0.5 μg). Replacement of D-Tyr^3 with D-His^3 did not increase activity (XIV, 0% at 1 μg) nor did replacement with D-Phe^3 (XV, 11% at 1 μg),D-F$_5$Phe^3 (XVI, 0% at 7.5), or D-Nal^3 (XVII, 15% at 3 μg), with the D-F$_5$Phe^3 substitution being particularly unfavorable. Comparison of analogues XV and IV, and XIII and II illustrates, again, that although position 2 requires a hydrophobic residue, the additional size of the D-Nal residue may sterically inhibit the analogue from adopting a suitable conformation for antiovulatory activity.

In a previous series of antagonists, potent antiovulatory activity had been obtained by inserting D aromatic residues in positions 2, 3, and 6.[5] Several analogues were synthesized to explore the effect of both other basic D-amino acids and D aromatic amino acids in position 6 relative to the substitution of Phe in 7. The substitution of D-Nal^6 for D-Arg^6 in the D-pClPhe^{1,2} antagonist (XII) was accompanied by a complete loss of activity even at the high dosage level of 7.5 μg (XVIII, 0% AOA at 7.5 μg compared with XII, 70% at 1 μg; see Table II). Substitution with D-Phe^6 also gave an inactive compound (XIX, 8% at 7.5 μg). The great reduction in activity of these compounds may in part be due to their marked insolubility in most aqueous buffers whereas the active D-Arg^6 analogue was freely soluble. The effect of other basic residues was investigated with the D-Nal^1 analogue (I). Substitution with D-Lys^6 produced an analogue approximately half as active (XX, 70% AOA at 1 μg compared with I, 64% at 0.5 μg). A similar reduction in activity was also caused by the substitution of D-Et$_2$hArg^6 (XXI, 56% at 1 μg).

The insertion of D-Phe^2 and D-Phe^3 had a marginal effect on the AOA while considerably simplifying the structure and synthesis of the antagonist. The replacement of Phe^7 by Cha^7 restored the activity of the analogue (V, 56% at 0.5 μg and XXII, 80% at 0.5 μg; see Table III), perhaps the result of a subtle change in side-chain hydrophobicity or flexibility.

Recently, the substitution of benzothienylalanine for Trp in position 3 has been reported in a closely related analogue resulting in increased antiovulatory potency.[6] This analogue (XXIV) was indeed more active than the original D-Trp^3 analogue XXIII (100% AOA at 1 μg vs. 10% at 1.5 μg, respectively). However, this potentiation is apparently sequence dependent since the substitution of D-Nal^1 for D-pClPhe^1 was accompanied by a much less dramatic change in activity (XXV, 77% at 1 μg and XXVI, 100% at 1 μg; 60% at 0.5 μg), perhaps a result of steric crowding or the increased hydrophobicity of D-Nal. Moreover, these analogues were subject to major side reactions during synthesis and were particularly difficult to obtain in good yields.

In this paper we describe the results of an ongoing investigation of potential luteinizing hormone-releasing hormone antagonists with emphasis on the N-terminal region. Potent analogues were prepared with a simplified structure that permitted the removal of expensive, custom-synthesized D-pClPhe and the labile D-Trp from a previously described family of analogues.[3] Recently, LH-RH antagonists have been shown to release histamine and produce transient edema of the face and extremeties when injected into rats at 50–100 times the antiovulatory dose.[7] In a standard in vitro rat peritoneal cell histamine release assay,[8] analogue V was found to have an ED_{50} of 0.11 ± 0.04 μg mL^{-1} (LH-RH has an ED_{50} of 328 ± 62 and the superagonist [D-Trp^6]-LH-RH as an ED_{50} of 46 ± 7 in the same assay system). This value is typical of many current LH-RH antagonists,[8] and further work is now in progress to study the structure–activity relationship of these D-Phe^{2,3} analogues to decrease their histamine release by modifying the basic side chains while retaining the advantage of sequence simplification and facile preparation.

Materials and Methods

Materials. Benzhydrylamine hydrochloride resin (ca. 0.4 mequiv g^{-1}) was obtained from Vega Biotechnologies Inc. Most *tert*-butoxycarbonyl (Boc) protected amino acids were purchased

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Table IV. Peptide Chromatographic and Purity Data

peptide	HPLC		TLC				
	t_R, min	purity, %	R_f1	R_f2	R_f3	R_f4	R_f5
I	22.8	99.5	0.23	0.80	0.69	0.38	0.56
II	22.2	99.9	0.31	0.75	0.59	0.30	0.57
III	26.2	98.3	0.61	0.25	0.61	0.25	0.63
IV	25.0	99.9	0.23	0.74	0.58	0.26	0.54
V	23.7	97.7	0.35	0.79	0.73	0.17	0.44
VI	21.9	99.7	0.38	0.69	0.56	0.17	0.56
VII	20.2	99.6	0.23	0.67	0.55	0.19	0.56
VIII	22.1	99.3	0.25	0.69	0.57	0.18	0.54
IX	23.0	97.0	0.32	0.71	0.67	0.22	0.53
X	22.0	92.3	0.70	0.61	0.51	0.22	0.49
XI	22.2	98.2	0.38	0.67	0.55	0.23	0.52
XII	24.4	99.2	0.49	0.79	0.59	0.29	0.57
XIII	24.0	99.9	0.25	0.85	0.61	0.34	0.54
XIV	21.1	98.4	0.19	0.66	0.51	0.16	0.35
XV	25.7	99.0	0.35	0.78	0.59	0.32	0.56
XVI	27.6	99.1	0.41	0.84	0.49	0.22	0.56
XVII	27.6	99.1	0.33	0.78	0.62	0.35	0.58
XVIII	28.5	99.3	0.59	0.95	0.73	0.54	0.69
XIX	29.6	98.9	0.67	0.95	0.74	0.57	0.72
XX	25.1	99.7	0.30	0.51	0.57	0.26	0.51
XXI	26.4	99.6	0.84	0.63	0.59	0.24	0.51
XXII	25.0	99.9	0.43	0.70	0.60	0.29	0.53
XXIII	24.0	99.8	0.29	0.75	0.63	0.44	0.55
XXIV	24.9	99.9	0.51	0.76	0.58	0.31	0.58
XXV	24.5	99.9	0.29	0.80	0.65	0.35	0.53
XXVI	26.0	94.2	0.51	0.76	0.59	0.32	0.58

Table V. Amino Acid Analyses

peptide	Ser	Pro	Ala	Leu	Tyr	Phe	pClPhe	Trp	Nal	Arg	X
I	0.98	1.08	1.05	–	0.95	0.95	0.98	0.85	0.95	2.03	
II	0.93	0.98	1.00	–	1.99	0.98	0.99	–	0.94	2.05	
III	0.94	1.00	1.03	–	0.96	1.02	2.00	–	0.95	2.02	
IV	0.95	1.03	1.03	–	0.96	2.01	1.22	–	1.10	2.01	
V	0.93	1.13	1.03	–	1.03	3.18	–	–	0.94	2.10	
VI	1.03	1.14	1.08	–	2.08	2.12	–	–	0.94	2.05	
VII	0.92	1.05	1.03	–	3.06	1.22	–	–	0.91	1.97	
VIII	1.02	1.08	1.06	–	2.03	2.13	–	–	0.89	2.08	
IX	0.96	0.94	1.01	–	0.98	3.09	0.94	–	–	1.96	
X	1.11	1.09	1.10	–	1.05	3.78	–	–	–	2.14	
XI	0.82	1.09	1.12	–	1.01	3.28	–	–	–	2.18	
XII	0.96	0.93	1.00	–	0.94	0.96	2.12	0.93	1.11	1.89	
XIII	0.91	1.02	0.97	–	1.94	0.97	–	–	2.28	1.91	
XIV	0.98	+[a]	1.00	–	1.03	1.14	–	–	1.98	1.94	1.05 His
XV	0.93	1.00	1.00	–	1.00	1.86	–	–	1.65	2.08	
XVI	0.95	0.94	1.00	–	0.96	0.94	–	–	1.58	1.75	1.11 F_5Phe
XVII	0.92	0.97	1.00	–	1.01	1.00	–	–	2.34	2.08	
XVIII	1.02	1.05	1.06	–	0.97	0.94	1.07	0.82	1.10	0.96	
XIX	1.00	1.06	1.00	–	1.00	2.01	2.08	0.87	–	0.96	
XX	0.94	0.92	1.09	–	0.97	0.93	1.06	0.68	1.42	1.00	1.00 Lys
XXI	0.92	0.99	1.01	–	0.95	1.00	0.97	0.78	1.08	0.93	1.11 Et_2hArg
XXII	1.00	1.03	1.04	–	0.97	1.98	–	–	1.09	2.12	0.94 Cha
XXIII	0.80	0.97	1.00	1.03	1.00	–	2.00	–	0.66	1.81	
XXIV	0.90	0.93	1.00	0.95	0.94	–	1.96	–	–	1.81	0.94 Bta
XXV	0.97	0.90	0.98	1.02	0.98	–	+[a]	+	0.76	1.99	
XXVI	0.97	0.94	1.00	0.99	1.00	–	0.64	–	0.73	2.15	0.78 Bta

[a] (+) present, but not quantified.

from Bachem Inc. The reactive side chains of amino acids were masked as follows: Arg, N^g-tosyl; Ser, *O*-benzyl; His, N^{im}-tosyl. Tyr and Trp were incorporated without side-chain protection. Boc-3-(2-naphthyl)-D-alanine and Boc-4-chlorophenyl-D-alanine were provided by the Southwest Foundation for Research and Education, San Antonio, TX, through the courtesy of Dr. Marvin Karten, Center for Population Research, National Institutes of Health, Bethesda, MD. All reagents and solvents were ACS grade or better and used without further purification, except *N,N*-dimethylformamide, which was stored over 4A molecular sieves before use.

Peptide Synthesis. Peptides were synthesized on benzhydrylamine functionalized, 1% cross-linked polystyrene resin[9] on a 0.5-mmol scale utilizing either a Beckman Model 990B automatic peptide synthesizer or a Vega Model 50 synthesizer, using a modified solid-phase procedure.[10] All protected amino acids were coupled with *N,N'*-diisopropylcarbodiimide[11] (Aldrich) until completion, as judged by the Kaiser ninhydrin test.[12] After coupling, Boc deprotection was effected with 20% boron trifluoride etherate in glacial acetic acid.[13] The decapeptides were cleaved

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from the resin support, with simultaneous side-chain deprotection, by acidolysis with anhydrous hydrogen fluoride containing anisole (~15% v/v) and dithiothreitol (~0.3% w/v) as scavengers.

Purification. The crude peptides were subjected initially to gel permeation chromatography on Sephadex G25 (2.5 × 100 cm) with 50% acetic acid eluent. Final purification was effected by preparative, reversed-phase, high-performance liquid chromatography on C_{18} bonded silica gel (LRP-1, Whatman, 2.5 × 45 cm) eluted with a linear acetonitrile gradient with a constant concentration of trifluoroacetic acid (0.1% v/v). The linear gradient was generated with a Chromat-A-Trol Model II (Eldex Laboratories Inc) gradient maker. The separations were monitored at 280 nm and by thin-layer chromatography (TLC) on silica gel plates (Merck F60). The purity of the final peptides was assessed by HPLC and TLC in five solvent systems, and the results are given in Table IV. Reversed-phase HPLCs were recorded with a 5-μm Vydac phenyl support (4.6 × 250 mm, 5 μm, 30-nm pore size, Liquid Separations Group). Buffer A, 0.1 M triethylammonium phosphate, pH 2; buffer B, 20% buffer A in acetonitrile. A linear gradient of 10% B to 70% B over 30 min was employed for all the analyses at a flow rate of 1.5 mL min^{-1}. Column eluent was monitored at 215 nm. The retention time and purity of each peptide were assessed by an LKB 2220 recording integrator. Each peptide produced only one spot in each of the following solvent systems when visualized by both Ehrlich and chlorine/starch–iodide reagents:[14] 1, ethyl acetate–pyridine–acetic acid–water, 10:5:1:3; 2, ethyl acetate–pyridine–acetic acid–water, 5:5:1:3; 3, butan-1-ol–acetic acid–water–ethyl acetate, 1:1:1:1; 4, butan-1-ol–acetic acid–water, 4:1:1; and 5, propan-2-ol–1 M acetic acid, 2:1. Amino acid analyses were performed with an LKB 4150 analyzer, equipped with an LKB 2220 recording integrator, after the peptides were hydrolyzed in vacuo (110 °C, 20 h) in 4 M methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole[15] (Pierce). The results of the amino acid analyses are given in Table V.

Biological Assays. The antiovulatory activity of each analogue was determined in Sprague–Dawley rats in a standard assay[16] using a 40% propane-1,2-diol–0.9% saline vehicle, in which the peptides were freely soluble. The compounds were injected subcutaneously at noon on the day of proestrus and the oviducts examined for the presence of ova on the following day. The results are expressed as the percentage of (n) rats that did not ovulate at a dose of x μg of analogue.

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Effect of Reductive Alkylation of D-Lysine in Position 6 on the Histamine-Releasing Activity of Luteinizing Hormone-Releasing Hormone Antagonists[1]

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The reductive alkylation of the D-Lys side chain in position 6 of the LH-RH antagonist [*N*-Ac-D-Nal[1],D-Phe[2,3],D-Arg[6],Phe[7],D-Ala[10]]LH-RH was investigated in an attempt to reduce the histamine-releasing activity inherent to most potent antagonists while retaining high antiovulatory activity. The protected parent analogue was prepared by conventional solid-phase peptide synthesis. After selective removal of the Lys Fmoc side-chain protection, the resin-bound peptide was readily and conveniently alkylated at the ε amino group with various aldehydes and ketones in the presence of $NaCNBH_3$. The analogues were then cleaved from the resin with simultaneous deprotection by anhydrous hydrogen fluoride and purified to homogeneity in two stages: gel permeation followed by preparative reversed-phase liquid chromatography. The analogues were assayed in standard rat antiovulatory and in vitro histamine-release assays. Simple alkyl groups such as ethyl, isopropyl, neopentyl, and cyclohexyl caused little reduction in histamine-releasing activity while exhibiting antiovulatory activity similar to that of the parent peptide. The presence of benzyl and substituted benzyl groups resulted in substantial losses of both histamine-releasing and antiovulatory activities. Thus, results showed that alterations in the hydrophobicity and size of the position-6 side chain have little effect on histamine-releasing activity or antiovulatory activity as long as a high degree of basicity is retained.

Since the elucidation of the structure of the luteinizing hormone-releasing hormone (LH-RH) Glp-His-Trp-Ser-Tyr-Gly-Arg-Leu-Pro-Gly-NH_2 by Matsuo et al.[2] in 1971, many hundreds of analogues have been synthesized in the search for ever more potent agonists and antagonists. The antagonists are of particular interest for the control of fertility by the blockade of ovulation and also in the control of hormone-dependent tumors. Recently, however, the antagonists, typified by the compound [*N*-Ac-D-Nal[1],D-*p*ClPhe[2],D-Trp[3],D-Arg[6],Phe[7],D-Ala[10]]LH-RH[3] have been shown to cause transient edema of the face and extremities when injected subcutaneously into rats at 50–100 times the effective antiovulatory dose.[4] Additionally, many compounds are mast cell secretagogues, release histamine, and

(1) Abbreviations used in this paper for amino acids, protecting groups, and peptides follow the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature and Symbols as described in the following: *Eur. J. Biochem.* **1972**, *27*, 201; *J. Biol. Chem.* **1975**, *250*, 3215. Glp, pyroglutamic acid; D-Nal, 3-(2-naphthyl)-D-alanine; D-pClPhe, 4-chlorophenyl-D-alanine.

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