isomer (6.15 ppm, $J_{1,2}$ = 6.2 Hz) was 4 times as large as that from the trans isomer (5.90 ppm, $J_{1,2}$ = 1.7 Hz).

Reaction of Porfiromycin with CH_3CO_2H. A solution of porfiromycin (30 mg) in 2 mL of CH_3CO_2H was stirred for 3 h. The solvent was removed under reduced pressure and the purple residue was divided into two parts: a 5-mg part to be used for UV analysis and the main part. The main part was purified by preparative TLC with ethyl acetate/acetone (8:2) as solvent. Two main spots and a trace of a third spot with intermediate R_f were observed. The spot with highest R_f gave 8.6 mg of purple solid, which had a ^1H NMR spectrum identical with that of the mixture of *cis*- and *trans*-diacetates **9** described above. The R_f of this spot also was identical with that of the diacetates. From the spot with lowest R_f was obtained 6.5 mg of a new purple compound, which was shown to be *cis*-2-(acetylmethylamino)-7-amino-1-hydroxymitosene (**11**) by its conversion into the *trans*-diacetate on acetylation with acetic anhydride and by the following eivdence: IR (KBr) 3240–3440, 1725, 1700, 1600 cm^{-1}; NMR (Me_2SO-d_6) δ 1.76 (s, 3 H), 2.05 (s, 3 H), 2.7–2.9 (d, 3 H), 3.3–3.5 (m, 1 H), 4.10–4.60 (m, 2 H), 5.03 (s, 2 H), 4.9–5.4 (m, 1 H), 5.5 (d, 1 H), 6.5 (s, 2 H), 6.6 (s, 2 H) ppm. Anal. ($C_{17}H_{20}N_4O_6 \cdot 1.5CH_3OH$) H; C: calcd, 52.36; found, 52.80. N: calcd, 13.19; found, 12.49.

The 5-mg portion of crude product was dissolved in methanol and a 0.1-mL aliquot was placed on a silica gel plate (5 × 20 cm). After the place dried it was developed with ethyl acetate/acetone (8:2). The purple spots were scraped off the plate and extracted with 25 mL of CH_3OH, each, and the UV absorption spectra of these extracts were measured. They showed a ratio of diacetates **9**/**11** of 59:41 at both 255 and 305 nm.

Reaction of Mitomycin F with CH_3CO_2H. This reaction was carried out in exactly the same way as described for porfiromycin, except that it was done on a one-third scale. Two strong and one very faint orange spots were seen on preparative TLC. The spot with highest R_f was identical in R_f with the *cis*- and *trans*-diacetate mixture **10** obtained from mitomycin F. The spot with lowest R_f gave on treatment with 0.5 N NH_4OH in methanol a product identical in R_f and infrared spectrum with compound **11** obtained from porfiromycin. Therefore, the spot with lowest R_f must have contained *cis*-2-(acetylmethylamino)-1-hydroxy-7-methoxymitosane (**12**).

Chromatography of the small portion of the crude product, followed by UV absorption analysis, showed a ratio of 28.5:71.5 for the diacetates **10**/**12**.

Reaction of Mitomycin C with DCl. A solution of mitomycin C (20 mg) in 3.2 mL of D_2O (99.8%, Aldrich) containing 0.05 N DCl was stirred under N_2 for 40 min. The resulting purple solution was cooled in an ice bath and treated with aqueous sodium acetate until the pH was 9.5. Three extractions with 50-mL portions of ethyl acetate were made and the combined extracts were dried (Na_2SO_4) and concentrated. The residual solid was treated with 3 mL of pyridine and 1 mL of acetic anhydride. After 16 h the mixture was worked up as described above and the crude product was purified by TLC on silica gel with CH_3OH–$CHCl_3$ (2:8) as solvent. Considerable material remained at the origin. The main purple band was scraped off the plate and extracted with methanol. Concentration of this extract gave about 3 mg of purple solid. A ^1H NMR spectrum of this solid (Me_2SO-d_6) showed it to be the diacetate **6**, with the C-1 protons occurring in a ratio of about 9:1 cis (6.1 ppm, $J_{1,2}$ = 6.2 Hz) to trans (5.9 ppm, $J_{1,2}$ = 1.7 Hz). The ratio of these combined peak integrals to those of the three methyl groups showed that about 55% of the theoretical amount of H-1 was still present.

Acknowledgment. This investigation was supported by Grant CA 21430, awarded by the National Cancer Institute, HHS. We thank Dr. Terrence Doyle of Bristol-Meyers and Dr. Julius Rebek and Dr. Charles Kowal of the University of Pittsburgh for helpful comments on the manuscript.

Registry No. **1**, 50-07-7; **1** (**1a**-acetyl), 1102-95-0; **2**, 801-52-5; **3**, 4055-39-4; **4**, 18209-14-8; **6** (isomer 1), 96412-15-6; **6** (isomer 2), 96479-82-2; **7** (isomer 1), 96412-16-7; **7** (isomer 2), 96479-83-3; **8** (X = CH_3O, Y = CH_3), 15973-07-6; **9** (isomer 1), 96412-17-8; **9** (isomer 2), 96479-44-6; **10** (isomer 1), 96479-45-7; **10** (isomer 2), 96479-46-8; **11**, 96412-18-9; **12**, 96412-19-0.

Improved Antagonists of Luteinizing Hormone-Releasing Hormone Modified in Position 7[1]

Simon J. Hocart,* Mary V. Nekola, and David H. Coy

Department of Medicine, Tulane University School of Medicine, New Orleans, Louisiana 70112. *Received November 2, 1984*

The structure–activity relationship of position 7 in the antagonist [N-Ac-D-Nal[1],D-pClPhe[2],D-Trp[3],D-Arg[6],D-Ala[10]]-LH-RH has been investigated by the incorporation of a series of amino acids at this position. The analogues were prepared by solid-phase peptide synthesis. All purifications were performed in two stages: gel permeation followed by preparative reversed-phase high-performance liquid chromatography. The analogues were assayed in the standard rat antiovulatory assay using a 40% propylene glycol–saline vehicle. The results demonstrated that position 7 requires a hydrophobic aromatic amino acid for greatest antiovulatory activity. The compound [N-Ac-D-Nal[1],D-pClPhe[2],D-Trp[3],D-Arg[6],Phe[7],D-Ala[10]]-LH-RH caused 65% blockade of ovulation at the 500-ng dose and is approximately twice as active as the parent analogue in this assay system. The enhanced activity may indicate the stabilization of the active conformation via intramolecular hydrophobic or π–π interactions.

One aim for the synthesis of antagonists of luteinizing hormone-releasing hormone (LH-RH), a decapeptide with the sequence Glp-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, is the control of conception by the effective blockade of ovulation. Toward this goal, over 1000 analogues have been synthesized internationally in the last several years in the search for ever more potent antagonists. Currently, the most active analogues are 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],D-Ala[10]]-LH-RH.[2] The incorporation of the bulky, hydrophobic residue D-Nal[1] in position 1 resulted in a pronounced increase in antiovulatory activity over the previous halogenated phenylalanines, when combined with D-Arg in position 6.[3] However, past experience has dem-

(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-Nal, 3-(2-naphthyl)-D-alanine; D-pClPhe, 4-chlorophenyl-D-alanine; D-Abu, D-2-aminobutanoic acid; D-3-Pal, 3-pyridyl-D-alanine; Glp, pyroglutamic acid; NMePhe, *N*-methylphenylalanine; pFPhe, 4-fluorophenylalanine; pMePhe, 4-methylphenylalanine; F_5Phe, 2,3,4,5,6-pentafluorophenylalanine.

(2) Horvath, A.; Coy, D. H.; Nekola, M. V.; Coy, E. J.; Schally, A. V.; Teplan, I. *Peptides* **1982**, *3*, 969.

0022-2623/85/1828-0967$01.50/0

Table I. Antiovulatory Activity of Several Position-7 LH-RH Analogues

	analogue	antiovulatory[a] activity
I	[N-Ac-D-Nal[1],D-pClPhe[2],D-Trp[3],D-Arg[6],(Leu[7]),D-Ala[10]]-LH-RH	100% @ 3 (9) 77% @ 1 (13)
II	Lys[7]	0% @ 7.5 (13)
III	Thr[7]	20% @ 3 (10)
IV	Tyr[7]	100% @ 3 (7) 89% @ 1 (9) 31% @ 0.5 (13)
V	Phe[7]	64% @ 0.5 (11)
VI	NMePhe[7]	11% @ 3 (9) 13% @ 1 (8)
VII	pFPhe[7]	75% @ 3 (8) 0% @ 1 (10)
IIX	F_5Phe[7]	100% @ 3 (11) 20% @ 1 (5)
IX	Nal[7]	100% @ 3 (10) 0% @ 0.5 (10)
X	pMePhe[7]	22% @ 1 (9)
XI	pClPhe[7]	100% @ 3 (12) 9% @ 1 (11)
XII	Trp[7]	85% @ 3 (8) 10% @ 0.5 (10)

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

Table II. Antiovulatory Activity of Several LH-RH Analogues

	analogue		antiovulatory[a] activity
I	[N-Ac-D-Nal[1],D-pClPhe[2],D-Trp[3],D-Arg[6],(Leu[7]),D-Ala[10]]-LH-RH		77% @ 1 (13)
XIIV	D-Nal[2]		0% @ 1 (11)
IV	[N-Ac-D-Nal[1],D-pClPhe[2],D-Trp[3],D-Arg[6],Tyr[7],D-Ala[10]]-LH-RH		89% @ 1 (9)
XIV	D-Nal[1,2]	Tyr[7]	11% @ 1 (9)
V	[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)
XV	D-pClPhe[1]	Phe[7]	20% @ 1 (10)

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

onstrated 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 the agonist series of analogues, replacement of Leu in position 7 is followed by a marked reduction in LH-releasing activity,[4] despite the enhanced resistance to enzymatic attack the modification may confer in the standard pituitary extract degradation assay.[4,5] In this paper, we describe a new series of analogues with modifications in position 7 that were made to reexamine the structure–activity relationship at this position of the antagonist with respect to the hydrophobic amino terminus.

Results and Discussion

The active conformation of LH-RH is currently thought to consist of a loose cyclic structure in which the amino and carboxyl termini are in close proximity and held by hydrogen bonding.[6-8] This notion is reinforced by the fact that minor modifications to the size of the termini can cause a large reduction in antiovulatory activity (AOA). For example, the replacement of acetyl by propionyl in [N-Ac-D-pClPhe[1,2],D-Trp[3],D-Arg[6],D-Ala[10]]-LH-RH caused a drastic loss of activity at the 3-μg dose.[9] Similarly, the replacement of D-Ala by D-Abu in [N-Ac-D-pClPhe[1,2],D-3-Pal[3],D-Arg[6],D-Ala[10]]-LH-RH, caused a greater than 10-fold loss in activity.[10] In the purported cyclic structure, residue 7, Leu, resides within the polar, hydrophilic half of the analogue. We therefore investigated the effect on the antiovulatory activity of several diverse substitutions at this position.

The replacement of Leu by Lys caused a catastrophic reduction in activity in excess of 2 orders of magnitude (I, 100% AOA at 3 μg compared with II, 0% at 7.5 μg, respectively; see Table I). Substitution by Thr also caused a loss of activity (III, 20% at 3 μg). However, replacement by Tyr gave an essentially equipotent analogue (IV, 89% AOA at 1 μg compared with 77% at 1 μg for the Leu[7] analogue). The incorporation of Phe produced an unexpectedly potent analogue, approximately twice as active as the Tyr[7] modification (V, 64% AOA at 0.5 μg compared with IV, 31% at 0.5 μg).

To study the effect of electron-donating and -withdrawing groups in the aromatic ring, several substituted phenylalanines were incorporated. Both pFPhe and F_5Phe were highly effective at the 3-μg dose (VII and IIX, 75% AOA and 100% at 3 μg, respectively), despite vastly different electron-withdrawing properties and hydrophobicity of the side chains. This activity was also retained in the pClPhe and in the electron-donating pMePhe analogues (XI, 9% AOA and X, 22% at 1 μg, respectively). However, none of these replacements produced analogues of higher

(3) Coy, D. H.; Horvath, A.; Nekola, M. V.; Coy, E. J.; Erchegyi, J.; Shally, A. V. *Endocrinology* **1982**, *110*, 1445.
(4) Hazum, E.; Fridkin, M.; Baram, T.; Koch, Y. *FEBS Lett.* **1981**, 123, 300.
(5) Koch, Y.; Baram, T.; Hazum, E.; Fridkin, M. *Biochem. Biophys. Res. Commun.* **1977**, *74*, 2.
(6) Coy, D. H.; Seprodi, J.; Vilchez-Martinez, J. A.; Pedroza, E.; Gardner, J.; Schally, A. V. "Central Nervous System Effects of Hypothalamic Hormones and Other Peptides"; Collu, R., Barbeau, A., Ducharme, J. R., Rochefort, J.-G., Eds.; Raven Press: New York, 1979; pp 317–323.
(7) Momany, F. A. *J. Am. Chem. Soc.* **1976**, *98*, 2990.
(8) Momany, F. A. *J. Am. Chem. Soc.* **1976**, *98*, 2996.
(9) Coy, D. H.; Horvath, A., unpublished results.
(10) Folkers, K.; Bowyers, C. Y.; Kubiak, T.; Stepinski, J. *Biochem. Biophys. Res. Commun.* **1983**, *3*, 3, 1089.

Table III. Antiovulatory Activity of Several Cyclic LH-RH Analogues

	analogue	antiovulatory[a] activity
XVI	[N-Ac-D-Nal[1],D-pClPhe[2],D-Trp[3],Cys[4],D-Arg[6],Cys[7],D-Ala[10]]-LH-RH	0% @ 50 (8)
XVII	Cys[5] Cys[7]	0% @ 50 (8)
XIIX	D-Cys[3] Cys[7]	0% @ 50 (10)
XIX	D-Cys[2] Cys[7]	0% @ 50 (8)

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

potency than that of the Phe[7] peptide. Since any electronic effect appeared to be of marginal importance for the enhanced activity of the Phe analogue, the effect of steric bulk was investigated. Replacement by Trp and NMePhe gave less active analogues (XII, 10% AOA at 0.5 μg and VI, 13% at 1 μg, respectively). This was also true of the L-Nal[7] analogue (IX, 0% AOA at 0.5 μg).

The antiovulatory results obtained with the position 7 analogues indicate a preference for hydrophobic residues. (Recently, this was also found to be the case in a related series of antagonists.[11]) However, beyond a certain point, the degree of hydrophobicity appears to have little effect on antagonist potency. It is likely that the position-7 side chain occupies the same water-shielded region of the molecule as the rest of the aromatic side chains. To examine the effect of increased hydrophobicity in the amino terminal region, several peptides containing modified amino termini were synthesized. The results are given in Table II. In the Tyr[7] antagonist, replacement of D-pClPhe[2] by D-Nal[2] caused a reduction in the antiovulatory activity, but this reduction was less than that seen in the corresponding Leu[7] analogues (IV, 89% AOA and XIV, 11% vs. I, 77% and XIIV, 0% at 1 μg, respectively). Additionally, the replacement of D-Nal[1] by D-pClPhe[1] in the Phe[7] analogue also caused a loss of activity (V, 64% AOA at 0.5 μg and XV, 20% AOA at 1 μg, respectively).

The enhanced activity of this antagonist sequence produced by the substitution of Phe at position 7 and this modification's greater tolerance of a large aromatic amino acid in position 2 suggested the possibility of intramolecular hydrophobic or π–π bonding between the Phe side chain and an aromatic side chain in the amino terminus, thereby stabilizing the purported cyclic conformation of the molecule. To test this hypothesis, a series of cyclic analogues, constrained by cystine bridges, was synthesized. The results are given in Table III.

None of the peptides exhibited any antiovulatory activity at a dose of 50 μg. The lack of success of this approach was not wholly unexpected since many previous attempts to obtain antagonists through conformational restriction had proven somewhat unsuccessful.[12-14] The aromatic amino acids that were responsible for the enhanced activity seen in the linear analogues, possibly stabilized an active conformation of the antagonist through π–π or hydrophobic bonding. However, their replacement with cystine bridges in the cyclic analogues caused a complete loss of activity. Thus, the original side chains of the residues involved may also be necessary for receptor recognition.

Materials and Methods

Materials. Benzhydrylamine hydrochloride resin (ca. 0.5 mequiv g^{-1}) and *tert*-butoxycarbonyl (Boc) protected amino acids were purchased from Bachem Inc. The reactive side chains of amino acids were masked as follows: Arg, N^{g}tosyl; Ser and Thr, *O*-benzyl. 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 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, 2% cross-linked polystyrene resin[15] on a 0.5-mmol scale utilizing either a Beckman Model 990B automatic peptide synthesizer or a Vega Model 50 synthesiser, using a modified solid-phase procedure.[16] All protected amino acids were coupled with use of *N,N'*-diisopropylcarbodiimide (Aldrich) until completion, as judged by the Kaiser ninhydrin test.[17] After coupling, Boc deprotection was effected by using either 33% trifluoroacetic acid in dichloromethane or 20% boron trifluoride-diethyl etherate in glacial acetic acid,[18] an equally efficacious, but much less expensive reagent. The decapeptides were cleaved from the resin support, with simultaneous side-chain deprotection, by acidolysis with anhydrous hydrogen fluoride containing anisole and dithiothreitol as scavengers.

Purification. The crude peptides were initially subjected 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 use of a Chromat-a-Trol Model II (Eldex) 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 using 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 cm^3 min^{-1}. Column eluent was monitored at 215 nm. The retention time and purity of each peptide was 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–

(11) Folkers, F. A.; Bowers, C. Y.; Shieh, H-M.; Yin-Zeng, L.; Shao-Bo, X.; Tang, P. F. L.; Li-Yu, C. *Biochem. Biophys. Res. Commun.* **1984**, *123*, 1221.
(12) Smith, C. W.; Skala, G.; Wilks, J. W. *Int. J. Pept. Protein Res.* **1983**, *21*, 127.
(13) Roeske, R. W.; Anantharamaiah, G. M.; Momany, F. A.; Bowers, C. W. "Peptides, Structure and Function, Proceedings of the Seventh American Peptide Symposium"; Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Co.: Rockford, IL, 1983; p 333.
(14) Rivier, J.; Rivier, C.; Perrin, M.; Porter, J.; Vale, W. "Peptides 1980—Proceedings of the Sixth European Peptide Symposium"; Brunfeldt, K., Ed.; Scriptor: Copenhagen, 1981; p 566.
(15) Pietta, P. G.; Marshall, G. R. *J. Chem. Soc. D* **1970**, 650.
(16) Coy, D. H.; Coy, E. J.; Schally, A. V.; Vilchez-Martinez, J. A.; Delseljuk, L.; Carter, W. A.; Arimura, A. *Biochemistry* **1974**, *13*, 323.
(17) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.
(18) Schnabel, E.; Klostermeyer, H.; Berndt, H. *Justus Leibigs Ann. Chem.* **1971**, *90*, 749.

Table IV. Peptide Chromatographic and Purity Data

peptide	HPLC		TLC				
	t_R, min	purity, %	R_f 1	R_f 2	R_f 3	R_f 4	R_f 5
I	22.8	97.3	0.25	0.75	0.56	0.20	0.53
II	22.4	98.3	0.07	0.63	0.59	0.18	0.28
III	23.7	95.8	0.27	0.66	0.56	0.28	0.49
IV	22.4	99.4	0.21	0.76	0.71	0.35	0.57
V	25.2	99.5	0.23	0.80	0.69	0.38	0.56
VI	23.6	99.1	0.32	0.85	0.62	0.27	0.55
VII	23.5	98.5	0.33	0.83	0.60	0.28	0.57
IIX	26.2	99.9	0.40	0.80	0.70	0.53	0.61
IX	26.3	97.5	0.34	0.83	0.61	0.30	0.59
X	25.3	99.2	0.24	0.78	0.60	0.26	0.59
XI	25.4	98.6	0.32	0.83	0.66	0.43	0.53
XII	25.6	98.5	0.24	0.85	0.61	0.37	0.55
XIIV	25.7	94.8	0.23	0.78	0.55	0.37	0.51
XIV	24.4	98.8	0.34	0.89	0.59	0.33	0.39
XV	24.4	97.4	0.24	0.78	0.59	0.28	0.56
XVI	24.7	98.7	0.28	0.68	0.51	0.21	0.47
XVII	23.7	98.6	0.23	0.65	0.49	0.21	0.41
XIIX	22.7	98.9	0.21	0.72	0.53	0.17	0.46
XIX	20.5	94.6	0.27	0.68	0.50	0.18	0.45

Table V. Amino Acid Analyses

peptide	Ser	Pro	Ala	Cys	Tyr	Phe	pClPhe	Trp	Nal	Arg	X	
I	0.97	0.90	0.98		0.98		1.01	0.76	0.99	1.99	1.02	Leu[2]
II	1.07	1.00	1.10		0.85		0.98	0.85	0.98	1.07	0.95	Lys
III	0.88	1.00	0.99		0.94		0.93	0.91	0.97	0.95	0.92	Thr
IX	1.03	1.02	1.03		1.84		0.95	1.04	1.02	1.82		
V	0.98	1.08	1.05		0.95	0.95	0.98	0.85	0.95	2.03		
VI	0.96	0.97	1.00		1.01		1.01	1.00	+[a]	1.89	+	NMePhe
VII	0.94	0.93	1.00		0.99		1.02	1.00	+	1.90	0.90	pFPhe
IIX	0.94	0.96	1.00		1.00		1.01	1.06	+	1.95	0.98	F_5Phe
IX	0.89	0.95	1.00		0.97		1.01	1.00	+	1.84		
X	0.94	1.11	1.00		1.05		0.89	0.77	1.01	1.74	0.89	pMePhe
XI	0.92	1.00	0.98		0.96		1.87	0.90	0.93	2.08		
XII	0.91	0.99	1.00		0.95		0.98	0.80	0.95	0.98		
XIIV	1.00	1.00	1.03		0.82			0.80	1.82	1.89	0.94	Leu
XIV	0.93	0.83	1.00		0.92			0.83	+	1.93		
XV	0.96	0.93	1.00		0.94	0.96	2.12	0.93		1.89		
XVI		1.21	1.00	0.83	0.92		1.02	0.89	1.33	1.86		
XVII	0.88	1.11	1.00	0.87			1.10	0.99	1.40	1.92		
XIIX	0.97	1.03	1.01	0.91	1.10		0.95		0.98	2.04		
XIX	0.99	1.10	1.03	0.90	1.03			0.84	1.09	2.03		

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

iodide reagents:[19] 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 hydrolyzing the peptides in vacuo (110 °C, 20 h) in 4 M methanesulfonic acid containing 0.1% tryptamine.[20] 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[21] using a 40% propylene glycol/saline vehicle. The results are expressed as the percentage of (n) rats that did not ovulate at a dose of x micrograms of analogue.

Acknowledgment. We gratefully acknowledge the technical assistance of E. D. Yauger for liquid chromatography and amino acid analyses and K. Brown and L. V. Mackey for conducting the antiovulatory bioassays. This work was supported by NIH Contract NO1-HD-4-2832 to D.H.C.

Registry No. I, 86044-77-1; II, 96394-79-5; III, 96394-80-8; IV, 96394-81-9; V, 96394-82-0; VI, 96394-83-1; VII, 96394-84-2; VIII, 96394-85-3; IX, 96394-86-4; X, 96394-87-5; XI, 96411-88-0; XII, 93128-25-7; XIII, 96394-88-6; XIV, 96394-89-7; XV, 96394-90-0; XVI, 96394-91-1; XVII, 96394-92-2; XVIII, 96394-93-3; XIX, 96394-94-4; LH-RH, 9034-40-6.

(19) Stewart, J. M.; Young, J. D. "Solid Phase Peptide Synthesis", 2nd ed.; Pierce Chemical Co.: Rockford, IL, 1984; p 120.
(20) Simpson, R. J.; Neuberger, M. R.; Liu, T. Y. *J. Biol. Chem.* **1976**, *251*, 1936.
(21) Vilchez-Martinez, J. A.; Coy, D. H.; Coy, E. J.; Arimura A.; Schally, A. V. *Endocrine Res. Commun.* **1976**, *3*, 231.