tense), 1685 (ketone C=O, intense), 1630 cm^{-1} (H-bonded ester C=O). *Anal.* ($C_{18}H_{24}O_5$) C, H.

Estrogen Assay. This assay measures uterine weight increase in mice. Adult female CF No. 1 mice were ovariectomized and allowed to recover for 14 days. The compounds were dissolved in sesame oil and administered in three graded doses by gavage for 3 days. On the fourth day the animals were sacrificed, and uteri were removed and weighed.

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Synthetic Luteinizing Hormone Releasing Factor Analogs. Series of Short-Chain Amide LRF Homologs Converging to the Amino Terminus

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The synthesis by solid phase on a benzhydrylamine resin of a series of analogs of the luteinizing hormone releasing factor, LRF, is described. The amidated derivatives of the natural decapeptide LRF (<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) described are peptides successively shortened by deletion of 1 (<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-NH_2), 2, 3, etc., amino acids from the C terminus. These peptide amides were purified by ion-exchange and partition chromatography and were characterized by amino acid analysis, nuclear magnetic resonance spectrometry, and, when possible, mass spectrometry after derivatization. Their specific rotations are reported. Homogeneity of these peptides was tested by thin-layer chromatography in six different solvent systems. The *in vitro* and *in vivo* LRF and follicle stimulating hormone releasing activities and the *in vivo* thyrotropin stimulating hormone releasing activity of these peptides are compared to that of the synthetic LRF and TRF (<Glu-His-Pro-NH_2).

The primary structure of the hypothalamic luteinizing hormone releasing factor (LRF) of porcine[1,2] and ovine[3,4] origins has been demonstrated to be that of the decapeptide <Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2. This peptide stimulates the secretion of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), by the anterior pituitary of several species,[5] including man.[6] Synthesis of this decapeptide was started[7] following the Merrifield method on a benzhydrylamine resin first described by Pietta and Marshall[8] and used extensively by us for the synthesis of many peptides.[9] Synthesis of that resin was reported by Monahan, *et al.*,[9] and Rivaille, *et al.*;[10] more details on the chemistry of that resin, as well as a convenient way of controlling its final substitution, will be found in the Experimental Section. Cleavage and deprotection of the peptide is achieved in one step by liquid HF yielding, in the case of LRF, a decapeptide amide showing a single spot in six different tlc systems and which has full biological activity after purification.

Once the methodology was well defined, we undertook the synthesis of the LRF analogs **2–8** in an attempt to find the smallest fragment from the N terminus to have biological activity (Table I).

The protected peptide resins were synthesized in a stepwise manner beginning with a benzhydrylamine resin and using dicyclohexylcarbodiimide[11] (DCI) as the sole coupling agent. The couplings were carried out in CH_2Cl_2 and, in some cases, a mixture of DMF-CH_2Cl_2 (1:1). Thorough washes with MeOH (which contracts the resin) and CH_2Cl_2 (which expands it) to eliminate side products and by-products of the reaction were performed after every coupling step. A ninhydrin test[12] after each coupling was seldom found to be positive; when it was positive, a second coupling with the same BOC amino acid was performed; alternatively, acylation with acetic anhydride in CH_2Cl_2 was employed to

Table I. Synthetic LRF and LRF Analogs

<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2-(LRF) (1)
<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-NH_2-des-Gly^{10}-LRF (2)
<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-NH_2-des-(Pro^9-Gly^{10})-LRF (3)
<Glu-His-Trp-Ser-Tyr-Gly-Leu-NH_2-des-(Arg^8-Gly^{10})-LRF (4)
<Glu-His-Trp-Ser-Tyr-Gly-NH_2-des-(Leu^7-Gly^{10})-LRF (5)
<Glu-His-Trp-Ser-Tyr-NH_2-des-(Gly^6-Gly^{10})-LRF (6)
<Glu-His-Trp-Ser-NH_2-des-(Tyr^5-Gly^{10})-LRF (7)
<Glu-His-Trp-NH_2-des-(Ser^4-Gly^{10})-LRF (8)
<Glu-His-NH_2-des-(Trp^3-Gly^{10})-LRF (9)
<Glu-NH_2-des-(His^2-Gly^{10})-LRF (10)

terminate potential failure sequences. When the ninhydrin test was negative, deblocking with TFA was applied,[13] followed by neutralization by Et_3N in DMF. Repeated washes with MeOH and then CH_2Cl_2 yielded the α-amino deprotected peptide (ninhydrin test strongly positive) which was recycled through a new coupling procedure. Following the coupling of the final residue, the washed and dried resin appeared by amino acid analysis after hydrolysis[14] to have the same substitution as the starting resin with acceptable ratios of the different amino acids. Cleavage and concomitant deprotection was effected by HF[15a] in the presence of redistilled anisole. Time and temperature for that reaction was dependent on the presence or the absence of *O*-benzyltyrosine. Elimination of the HF under high vacuum was followed by addition of 0.1 *N* acetic acid. Filtration gave a slightly yellow solution which was subsequently lyophilized. The material was dissolved in water (≃0.3 g) and applied on a cation-exchange cellulose (CMC) column. The peptides which were adsorbed on the column were washed with water and were eluted with ammonium acetate. Monitoring by optical density (280 nm), or Pauly spot test on filter paper, amino acid analysis and nmr permitted us to localize the desired major product and isolate it at an estimated purity of 75% or greater. Column partition chromatography was monitored by tlc in two different systems (basic and acidic) and cuts were made accordingly which yielded, upon lyophilization, the different peptide acetates **1–8**. Peptide **9** was obtained by amidation of the dipeptide methyl ester described by Gillessen, *et al.*[16] <Glu amide **10** was obtained by ammonolysis of its mixed anhydride. Both the dipeptide amide **9**[17] and tripeptide amide **8**[18] have already been reported.

Amino acid analysis along with thin-layer chromatography (all the products show only one spot in six different systems), nmr spectrometry, and mass spectrometry do not exclude a contamination of the peptides **1–4** by a maximum of 5% of very closely related peptides, presumably failure sequences.

To examine our estimate of >95% purity, compound **1** was further purified on a long (2 m) partition chromatography column (system 6) which was monitored by optical density. The outside fractions I and X representing less than 1% of the total amount of material appeared by amino acid analysis to be respectively low in His (only 35%) and rich in Gly (125%). Fractions II and VIII, however, did not show any obvious differences from V verifying that our estimate of 5% impurity is quite conservative.

Comparative sequencing of an equivalent preparation after CMC and one partition chromatography with natural LRF from ovine origin was described by Burgus, *et al.*[4] The results of both analyses are comparable, thus helping to prove the structure of natural LRF as well as providing further evidence for the homogeneity of the synthetic material.

The results illustrate the definite advantages exhibited by the benzhydrylamine resin for the synthesis of peptide amides: (a) it yields the C-terminal amide directly after cleavage and deprotection by HF; (b) the amide linkage of the first amino acid to the resin is carried out like any other coupling with DCI or active ester; (c) the amide linkage is less susceptible to hydrolysis than the ester bond on Merrifield resin during peptide synthesis.

Biological Activity. The activity of the peptides to stimulate LH and FSH release was determined *in vitro* by the method of Vale, *et al.*,[19] and *in vivo* by the procedure of Amoss and Guillemin[20] and Blackwell, *et al.*[21] The potency of each analog relative to that of LRF is reported in Table II. In addition, each analog was tested for possible ability to antagonize LRF as described by Vale, *et al.*[22] Assays for thyrotropin releasing activity were carried out as reported by Vale, *et al.*[23]

All of the peptides that release LH also release FSH. However, in view of the difficulties in quantitating the FSH releasing potencies of LRF or its analogs,[24] the potencies illustrated in Table II are only based on the abilities of the various peptides to stimulate the secretion of LH.

The elimination of the C-terminal amino acid, *i.e.*, Gly^{10}, decreases the biological activity by a factor of 10, demonstrating that, in this series, the decapeptide is required for full biological activity. The remainder of the analogs of this series have considerably reduced specific activities.† The active nonapeptide showed dose-response curves parallel to those of LRF with similar maximum responses, indicating that the differences in specific biological activities result from altered affinities of the analog for the LRF receptor. The amounts of LH released during the simultaneous administration of submaximal doses of LRF and the active analog *in vitro* indicate an additive pharmacological interaction. Furthermore, as the inactive analogs have no influence on the response to LRF, we can conclude that under the conditions of our experiments, none of the peptides acted as competitive antagonists of LRF. The ability of our *in vitro* tests to detect peptides which antagonize LRF was demonstrated by our recent report that des-His^2-LRF and [Gly^2]-LRF are competitive inhibitors of LRF.[22] All the analogs of the series reported had less than 0.1% of the potency of TRF in stimulating the secretion of TSH.

Experimental Section‡

Benzhydrylamine Resin.[7] Bio-Beads S-XI (200–400 mesh) from Bio-Rad Lab. were successively boiled for 1 hr in 1 *N* NaOH and 1 *N* HCl and brought to 80° in DMF with intermediate washes with H_2O and MeOH. Fines were eliminated by decantation.

(a) The Friedel–Crafts reaction (resin, 100 g; C_6H_5COCl, 70 g; $AlCl_3$, 70 g) was carried out in $NO_2C_6H_5$ (1 l.) for 2 hr at 15° with stirring. After successive washes in H_2O, AcOH, MeOH, CH_2Cl_2, and MeOH and drying, the resin had gained 25–30 g indicating 2.0–2.3 mmol of benzoyl residue per gram of resin.

†We want to correct reports[9b,5e] where the tripeptide Glu-His-Trp-NH_2 and the octapeptide <Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-NH_2 were found to have 0.1–0.4 and 0.01% LRF biological activity, respectively. The most probable explanation is that LRF from previous syntheses contaminated the Sephadex used in purifying these analogs. Indeed, at the doses tested, minute amounts of LRF could account for the biological activity. In subsequent syntheses of LRF analogs, we have been aware of this problem and use fresh column support material for each analog. Present results on the biological activity of the tripeptide are in agreement with results recently published.[18]

‡Melting points (Thomas-Hoover capillary melting point apparatus) are uncorrected. Ir data were recorded on Beckman IR-18A spectrophotometer (KBr pellets). Nmr spectra were obtained with a Varian 220-MHz spectrometer. In view of their interest for the assignment of all LRF protons and for the subsequent study of LRF conformation in solution, the nmr data will be reported elsewhere.§ Mass spectra were obtained at 70 eV with a Varian CH-5; data were recorded with the Varian 620 I computer. High-resolution mass spectrometry was used to characterize the final products as such in the case of <Glu-NH_2 and <Glu-His-NH_2 and permethylated[25] in the case of <Glu-His-Trp-NH_2, <Glu-His-Trp-Ser-NH_2, <Glu-His-Trp-Ser-Tyr-NH_2, and <Glu-His-Trp-Ser-Tyr-Gly-NH_2 at low resolution. Amino acid analyses were performed on peptide hydrolysates (6 *N* HCl, 0.5% thioglycolic acid at 110° in evacuated sealed tubes for 20 hr; no corrections were made for decomposition of tryptophan, serine, and tyrosine) using a Beckman/Spinco, Model 119 amino acid analyzer. Resin peptides were hydrolyzed in 6 *N* HCl–propionic acid (1/1 v/v) at 130° for 2 hr.[14] Peak areas were determined by an Infotronics Model CRS-100A electronic integrator. Optical rotations were measured in 1% HOAc (v/v) on a Perkin-Elmer Model 141 polarimeter.

§J. Rivier, unpublished work, The Salk Institute, Aug 1972.

Table II. Specific Biological Activity of LRF Analogs

Analog	% specific activity of LRF		
	In vitro	*In vivo*	FSH[a]
1 LRF	100	100	+
2 des-Gly[10]-LRF	11[b]	10	+
3 des-(Pro[9]-Gly[10])-LRF	<0.01	<0.01	–
4 des-(Arg[8]-Gly[10])-LRF	<0.01	<0.01	–
5 des-(Leu[7]-Gly[10])-LRF	<0.01	<0.01	–
6 des-(Gly[6]-Gly[10])-LRF	<0.01	<0.01	–
7 des-(Tyr[5]-Gly[10])-LRF	<0.01	<0.01	–
8 des-(Ser[4]-Gly[10])-LRF	<0.01	<0.01	–
9 des-(Trp[3]-Gly[10])-LRF	<0.01	<0.01	–
10 des-(His[2]-Gly[10])-LRF	<0.01	<0.01	–

[a]Release of FSH *in vivo* and *in vitro* different from controls: +, significant; –, not significant. [b]Confidence limit 6.2–22.0.

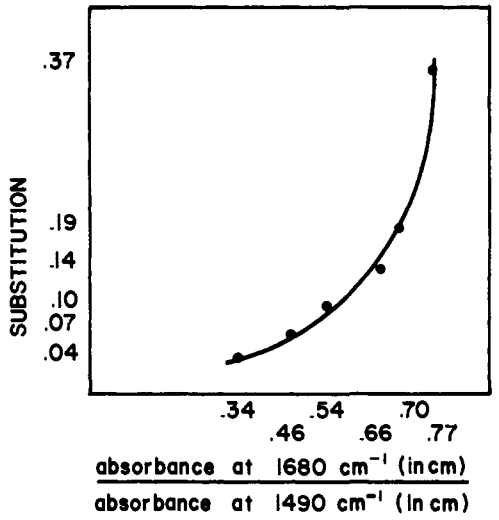

Figure 1. Correlation of the γ C=O absorbance of the formyl group at 1680 cm^{-1} to the final substitution of the benzhydrylamine resin. The γ C=O absorbance at 1680 cm^{-1} of the formyl group (measured in cm from base line) relative to the aromatic skeletal in-plan vibrations at 1490 cm^{-1} (also expressed in cm)[27] grossly indicates the substitution obtained after 6 *N* HCl hydrolysis and complete coupling of an amino acid. The data on the substitution were obtained by coupling proline to the free benzhydrylamine resin and quantitation by amino acid analysis. These data are particularly helpful in correlating substitution with a physical characteristic that can be measured as a function of time during the course of the reaction.

(b) Leuckart reductive amination.[26] Ammonium formate (ten times the weight of the resin to be treated) was used and the temperature kept at 165°. The reaction time depends on the substitution desired (see Figure 1).

(c) Hydrolysis was done by refluxing the washed formylated benzhydrylamine resin in 6 *N* HCl for 8 hr. The resin was then successively washed with H_2O, AcOH, MeOH, CH_2Cl_2, and MeOH and dried.

Deblocking Procedure. The resin was then cycled through a deblocking procedure in the reaction vessel. (a) Two 10-min treatments with TFA–CH_2Cl_2 (1:1, containing 5% 1,2-ethanedithiol) were followed by the regular CH_2Cl_2–MeOH washes. Two 5-min treatments with 12.5% Et_3N in DMF were then applied and followed by thorough washes (MeOH, CH_2Cl_2).

Starting Materials. *tert*-Butyloxycarbonylamino acids# were bought from Bachem and further purified when necessary. All amino acids were of the L configuration.**

#<Glu was introduced as carbobenzoxypyroglutamic acid, Boc-im-tosylhistidine, Boc-*O*-benzylserine, Boc-*O*-benzyltyrosine, Boc-glycine, Boc-leucine, Boc-proline. Boc-NO_2-arginine contained an unidentified amino acid (probably Boc-ornithine) eliminated by repeated extraction in boiling $CHCl_3$. Boc-tryptophan was freed from an unknown contaminant by repeated extractions in boiling CCl_4.

**The starting materials were found to be optically pure. The final products were not analyzed for optical purity in view of the low probability of the occurrence of racemization.[15b]

Coupling Procedure (Generally Applied through All the Syntheses). To 1 g of resin (0.3 mmol of NH_2/g) suspended in 5 ml of CH_2Cl_2 was added *tert*-Boc-L-amino acid (2 mequiv) in solution in CH_2Cl_2 (a mixture of DMF–CH_2Cl_2, 1:1, was added when Boc-NO_2-arginine, Boc-leucine after Boc-NO_2-arginine, and *Z*-<Glu were coupled). DCI (1 mequiv) was added and the second milliequivalent after 1 hr. The resin was washed (MeOH, CH_2Cl_2) 3 hr after the last addition of DCI (total of 4 hr of coupling). When the ninhydrin test[12] was negative, the synthesis was carried on through a deblocking procedure; when the test was positive, acylation was either repeated with the same Boc-amino acid or acetylation (acetic anhydride) was performed until a negative test was obtained.

Cleavage from the Resin. The resin peptide (1 g ≃ 0.3 mequiv/g of resin), thoroughly washed and dried, was treated with redistilled HF in the presence of redistilled anisole (1.5 ml) for 0.5 hr at 0° and 0.5 hr at room temperature when *O*-benzyltyrosine was present and 0.5 hr at 0° in the absence of *O*-benzyltyrosine.[28] HF was completely eliminated under high vacuum (discoloration of the resin from bright red to yellow). The resin was then washed with 0.1 *N* HOAc and filtered. The filtrate after lyophilization was a fluffy white powder. A strong violet coloration due to the decomposition of some tryptophan has been observed from time to time during the elimination of HF; although no quantitative studies have been made, these degradation products do not appear to be of great importance.[29] Approximately 200–400 mg of crude product was obtained.

Ion Exchange Chromatography. Ion exchange chromatography was performed on Microgranular CM-32 carboxymethylcellulose (CMC) from Whatman that was first precycled as indicated through a basic (1 *N* NaOH, room temperature for 0.5 hr) then acidic (1 *N* HCl, room temperature for 0.5 hr) step. CMC columns (11 × 2.5 cm) were packed in 50-ml disposable syringes and equilibrated before use with an ammonium acetate buffer (0.01 *M*, pH ≃4.0). The material (200–400 mg of crude product obtained from the cleavage by HF) was applied in 20 ml of the same buffer. Elution by 0.075 *M* ammonium acetate (pH 7) selectively displaced different components of the mixture. Cuts were made according to the optical density curves obtained at 280 nm or by gross estimation of the content of histidine in the fractions determined by Pauly test[30] (a known and constant volume of each fraction was spotted on a filter paper dried and sprayed with Pauly; variation in intensity of the spots is a good and quick indication of where the peptide fractions are located). Nmr spectroscopy was then decisive in choosing the right fraction which was confirmed by amino acid analysis. Peptides (30–80 mg) of greater than 75% purity were obtained and further purified in a partition system.

Partition Chromatography. Partition chromatography was performed on two columns different in diameter (0.9 × 45 cm and 1.6 × 90 cm; void volumes 13 and 45 ml, respectively) depending on the amount of material to be purified (up to 60 and 120 mg, respectively), packed with Sephadex G-25 F in 0.2 *N* AcOH (degassed). It was saturated with the lower phase of system 6 described below and equilibrated with the upper phase. The sample was applied as a solution in a maximum of 1 ml of upper phase. Fractions of *ca.* 2 ml were collected. The retention time of the different products is given in intervals of void volumes. After examination of the profile of the optical density curves obtained by analysis of each fraction or by spotting of an aliquot of each fraction on a tlc run in the two systems 6 and 4, the final cuts were made, yielding, after evaporation of the solvent mixture and lyophilization from water, the final products to be described as acetate salts. These products were analyzed by thin-layer chromatography.

Tlc Solvent Systems. Ascending tlc was conducted on silica gel supported on 20 cm long plastic sheets (type 6061 with fluorescent indicator from Eastman). Solvent systems for tlc: (1) 1-butanol–pyridine–0.1 *N* acetic acid, 5:3:11 (upper phase); (2) ethyl acetate–pyridine–acetic acid–water, 5:5:1:3; (3) isoamyl alcohol–pyridine–water, 7:7:6; (4) 2-propanol–1 *N* ammonia, 2:1; (5) 2-propanol–1 *N* acetic acid, 2:1; (6) 1-butanol–acetic acid–water, 4:1:5 (upper phase). Uv, I_2 (always positive), ninhydrin spray (always negative), and Pauly reagent[30] were successively used in all cases. Loads were >20 μg of peptide.

All the new peptides appeared homogeneous under these conditions (see Table III of R_f values). Peptide yields are calculated on the basis of millimoles of peptides isolated after final purification relative to the total millimoles of starting Boc-amino acid, *viz.*, as resin amide.

<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (1) LRF. LRF was purified following the general procedure mentioned before; after CMC, it was applied on a partition chromatography column (system 6) and was obtained in between 2.9 and 3.6 vv (void volumes), yield 15–20%.

Table III. R_f Values of Synthetic LRF Analogs

Compd	Tlc system[a] (1) BPA	(2) EPAW	(3) IaPW	(4) IpN	(5) IpA	(6) BAW
1	0.51	0.67	0.49	0.70	0.81	0.31
2	0.52	0.66	0.51	0.72	0.80	0.33
3	0.56	0.67	0.58	0.74	0.83	0.35
4	0.77	0.79	0.75	0.89	0.93	0.42
5	0.70	0.71	0.70	0.83	0.84	0.37
6	0.81	0.80	0.82	0.80	0.85	0.43
7	0.71	0.71	0.70	0.74	0.74	0.34
8	0.77	0.74	0.79	0.69	0.76	0.43
9	0.48	0.58	0.52	0.71	0.60	0.26

[a] See Experimental Section tlc solvent systems.

Its nmr will be published in detail elsewhere[§] as well as its mass spectral[††] characteristics; amino acid analysis, Glu 1.05, His 0.96, Trp 0.46, Ser 0.92, Tyr 1.00, Gly 1.98, Leu 1.00, Arg 1.06, Pro 1.10, NH_3 4.54; $[\alpha]^{22}D$ −50° (*c* 1) [lit.[31] −51° (*c* 1)].

<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-NH_2 (2) des-Gly[10]-LRF. After it was eluted from CMC by the usual procedure, the product (90 mg) was applied on a partition chromatography column (system 6) and was obtained in between 3.0 and 3.6 vv: yield 19%; amino acid analysis, Glu 0.97, His 0.94, Trp 0.71, Ser 0.86, Tyr 0.98, Gly 0.94, Leu 1.00, Arg 0.99, Pro 1.10, NH_3 1.06; $[\alpha]^{22}D$ −53.5° (*c* 1).

<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-NH_2 (3) des-(Pro[9]-Gly[10])-LRF. After it was eluted from CMC by the usual procedure, the product (75 mg) was applied on a partition chromatography column (system 6) (small column) and was obtained in between 3.2 and 4.3 vv: yield 17%; amino acid analysis, Glu 1.01, His 0.89, Trp 0.60, Ser 0.90, Tyr 1.01, Gly 1.00, Leu 1.04, Arg 0.95, NH_3 1.05; $[\alpha]^{22}D$ −28° (*c* 1).

<Glu-His-Trp-Ser-Tyr-Gly-Leu-NH_2 (4) des-(Arg[8]-Gly[10])-LRF. After it was eluted from CMC by the usual procedure, the product (75 mg) was applied on a partition chromatography column (system 6) and was obtained in between 3.1 and 3.8 vv: yield 15%, amino acid analysis, Glu 0.95, His 0.93, Trp 0.72, Ser 0.86, Tyr 0.96, Gly 0.95, Leu 1.00, NH_3 1.03; $[\alpha]^{22}D$ −23.5° (*c* 1).

<Glu-His-Trp-Ser-Tyr-Gly-NH_2 (5) des-(Leu[7]-Gly[10])-LRF. After it was eluted from CMC by the usual procedure, the product (50 mg) was applied on a partition chromatography column (system 6) and was obtained in between 3.0 and 3.8 vv: yield 20%; amino acid analysis, Glu 1.02, His 0.98, Trp 0.69, Ser 0.91, Tyr 1.04, Gly 1.00, NH_3 1.12; mass spectrum of permethylated derivative *m/e* (rel intensity) 95 (420), 98 (1000), 121 (750), 126 (250), 144 (650), 263 (40), 276 (110), 291 (50), 334 (120), 449 (27), 477 (14), 505 (3), 573 (2), 592 (2), 663 (2), 739 (<1), 751 (<1), 779 (<1), 811 (<1), 822 (<1), M^+ 926 not observable; $[\alpha]^{22}D$ −22° (*c* 1).

<Glu-His-Trp-Ser-Tyr-NH_2 (6) des-(Gly[6]-Gly[10])-LRF. After a pass on CMC, the product (50 mg) was applied on a partition chromatography column (system 6) and was obtained in between 2.6 and 3.1 vv: yield 17%; amino acid analysis, Glu 0.96, His 1.00, Trp 0.90, Ser 0.96, Tyr 1.09, NH_3 0.86; mass spectrum of permethylated derivative *m/e* (rel intensity) 95 (430), 98 (1000), 121 (740), 144 (58), 156 (230), 206 (490), 235 (100), 263 (30), 291 (90), 318 (20), 321 (20), 347 (15), 503 (4), 505 (5), 532 (2), 535 (4), 560 (2), 564 (4), 617 (1), 667 (2), M^+ 855 not observable; $[\alpha]^{22}D$ −22.5° (*c* 1).

<Glu-His-Trp-Ser-NH_2 (7) des-(Tyr[5]-Gly[10])-LRF. After a pass on CMC, the product (50 mg) was applied on a partition chromatography column (system 6) and was obtained in between 3.8 and 4.3 vv: yield 20%; amino acid analysis, Glu 1.06, His 1.00, Trp 0.92, Ser 0.98, NH_3 0.77; mass spectrum of permethylated derivative *m/e* (rel intensity) 95 (42), 98 (1000), 130 (100), 144 (700), 156 (270), 187 (90), 263 (40), 291 (70), 311 (40), 321 (10), 343 (40), 476 (5), 477 (4), 505 (4), 535 (3), 560 (10), 588 (<1), M^+ 664 not observable; $[\alpha]^{22}D$ −21° (*c* 0.867).

<Glu-His-Trp-NH_2 (8)[17] des-(Ser[4]-Gly[10])-LRF. It was at least 80% pure (nmr criterium) after cleavage from the resin. It was directly applied on a partition chromatography column (120 mg, system 6) and was obtained in between 3.6 and 4.9 vv: yield 29%; amino acid analysis, Glu 1.00, His 0.91, Trp 1.12, NH_3 0.75; mass spectrum of permethylated derivative *m/e* (rel intensity) 95 (67), 98 (80), 144 (88), 155 (40), 229 (46), 263 (9), 286 (5), 291 (9), 320 (1), 321 (5), 394 (<1), 423 (<1), 451 (2), 447 (<1), 505 (<1), M^+ 549 (2); $[\alpha]^{22}D$ −26.7° (*c* 1) [lit.[18] −11.2° (95% EtOH)].

<Glu-His-NH_2[16] (9). <Glu-His-OMe[15] (500 mg) was dissolved in absolute MeOH (40 ml) saturated at 0° with NH_3. After standing for 78 hr at room temperature, the solution was evaporated to dryness and twice recrystallized from MeOH: yield 75%; mp (MeOH) 216° dec; $[\alpha]^{22}D$ −20° (*c* 1); amino acid analysis, Glu 1.00, His 1.03; mass spectrum *m/e* (rel intensity) 44 (100), 81 (100), 84 (100), 112 (27), 137 (64), 138 (27), 181 (90), 221 (48), 249 (5), M^+ 265 (4). *Anal.* Calcd mass for $C_{11}H_{15}N_5O_2$: 265.117. Found: 265.118.

<Glu-NH_2 (10). <Glu- (2.6 g, 20 mmol) was dissolved in 60 ml of CH_3CN–DMF (5/1) and *N*-methylmorpholine (2.2 ml, 20 mmol) was added. After cooling at −15°, isobutyl chloroformate (2.68 ml, 20 mmol) was added. The mixture was stirred for 4 min and NH_3 was then added until saturation. Yield greater than 90% was obtained: mp (MeOH) 217.5°; $[\alpha]^{22}D$ 0° (*c* 1); amino acid analysis, Glu 1.00, NH_3 1.05; mass spectrum *m/e* (rel intensity) 41 (100), 44 (56), 56 (62), 84 (100), 100 (3), 110 (2), M^+ 128 (26). *Anal.* Calcd mass for $C_5H_8N_2O_2$: 128.058. Found: 128.058.

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Synthesis and Properties of Fluorine-Containing Heterocyclic Compounds. 8. α-(2-Pyridyl)- and α-(2-Piperidyl)-2-(trifluoromethyl)-4-azaphenanthrenemethanols

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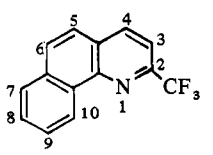
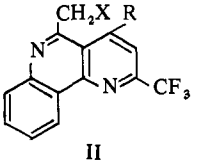
Some α-(2-pyridyl)- and α-(2-piperidyl)-2-(trifluoromethyl)-4-azaphenanthrenemethanols were synthesized as potential antimalarials. These compounds were prepared by a route involving the condensation of aminonaphthalenes or aminoquinolines with ethyl trifluoroacetoacetate. The resulting 2-(trifluoromethyl)azaphenanthren-4-ols were converted to the corresponding 4-chloro and 4-bromo derivatives. The 4-bromo derivatives exchanged rapidly with *n*-butyllithium. The lithio derivatives thus obtained were treated with 2-pyridinecarboxaldehyde or converted to the parent 4-carboxylic acids which were then treated with 2-lithiopyridine. The products of these reactions were reduced to the desired amino alcohols with hydrogen and platinum oxide. α-(2-Piperidyl)-2-(trifluoromethyl)-4-benzo[*h*]quinolinemethanol and its 6-chloro derivative were found to be curative in mice infected with *Plasmodium berghei* and active in chicks infected with *Plasmodium gallinaceum*. These compounds exhibited higher antimalarial activity than the corresponding 2-(trifluoromethyl)-4-quinolinemethanols but were also phototoxic. The α-(2-pyridyl)-4-azaphenanthrenemethanols were inactive.

Quinolinemethanols have long been known as active antimalarial agents.[1] Unfortunately, many of these compounds are also photosensitizers.[2] In a search for useful antimalarial chemotherapeutic agents, we investigated the synthesis of some 2-(trifluoromethyl)-4-azaphenanthrenemethanols. These compounds incorporate both a quinoline nucleus and a phenanthrene nucleus in a single structure. Such nuclei[3,4] have shown antimalarial activity when appropriately substituted with amino or amino alcohol groups. Suitable amino alcohol substituents such as the α-(2-piperidyl)-methanol group were introduced at the 4 position to impart the desired activity. It was hoped that the 2-trifluoromethyl group would prevent metabolic oxidation at the 2 position and would decrease the phototoxic side effects associated with similar structures.[2] The 2-(trifluoromethyl)azaphenanthrenes previously synthesized in our laboratory were chosen as the parent nuclei for these potential antimalarials.[5,6] They included 2-trifluoromethyl derivatives of the following heterocycles: benzo[*h*]quinoline (I), benzo[*h*]-1,6-naphthyridine (II), 1,7-phenanthroline (III), 1,8-phenanthroline (IV), and 1,10-phenanthroline (V).



I



II

17, R = Br; X = H
18, R = Br; X = Br
33, R = CO-(2-pyridyl); X = H

Chemistry. The synthetic routes used to prepare α-(2-pyridyl)- and α-(2-piperidyl)-2-(trifluoromethyl)-4-azaphenanthrenemethanols are outlined in Schemes I and II.

The 2-(trifluoromethyl)azaphenanthren-4-ols† were formed by a Conrad-Limpach-type condensation of the appropriate amine and ethyl trifluoroacetoacetate in polyphosphoric acid. 2-(Trifluoromethyl)benzo[*h*]quinolin-4-ol (**1**), 6-chloro-2-(trifluoromethyl)benzo[*h*]quinolin-4-ol (**9**), 6-cyano-2-(trifluoromethyl)benzo[*h*]quinolin-4-ol (**10**), 7-nitro-2-(trifluoromethyl)benzo[*h*]quinolin-4-ol (**11**), 2-(trifluoromethyl)-1,7-phenanthrolin-4-ol (**12**), 2-(trifluoromethyl)-1,8-phenanthrolin-4-ol (**13**), 2-(trifluoromethyl)-1,10-phenanthrolin-4-ol (**14**), 5-methoxy-2-(trifluoromethyl)-1,10-phenanthrolin-4-ol (**15**), and 5-methyl-2-(trifluoromethyl)benzo[*h*]-1,6-naphthyridin-4-ol (**16**) were prepared from 1-naphthylamine, 4-chloro-1-naphthylamine, 4-amino-1-naphthalenecarbonitrile, 5-nitro-1-naphthylamine, 5-aminoisoquinoline, 8-aminoisoquinoline, 8-amino-6-methoxyquinoline, and 4-aminoquinaldine, respectively. The conversion of the 4-hydroxy compounds into the corresponding 4-bromo derivatives was accomplished with either phosphorus pentabromide, phosphorus oxybromide,[8] phosphorus tribromide, or a mixture of phosphorus oxybromide and

†The 4-hydroxy derivatives of azaphenanthrenes exist in equilibrium with their keto tautomers which are believed to be the predominant form.[7] For convenience, however, we refer to them simply as azaphenanthren-4-ols.