tone. The filtrates were diluted with H_2O and extracted with EtOAc. The extract was washed with H_2O, dried (Na_2SO_4), and concentrated *in vacuo*. The residue was chromatographed on 25 g of Florisil. The product was eluted with $CHCl_3$–EtOH (19:1): yield, 20 mg.

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Synthesis and Biological Activity of Some Analogs of the Gonadotropin Releasing Hormone

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Twenty-one analogs of gonadotropin releasing hormone, <Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (GnRH), were synthesized by the solid-phase method. The derivatives were <Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-OCH_3 (GnRH methyl ester), GnRH *N*-methyl amide, GnRH free acid, <Glu-His-Trp-Ser-Tyr-Gly-NH_2 (GnRH N-terminal hexapeptide), and also [Ac-Ala^1]-, [Ac-gly^1]-, [D-<Glu^1]-, [Pro^1]-, [Arg^2]-, [Tyr(Me)^3]-, [Ser^5]-, [des-Gly^6]-, [Sar^6]-, [des-Pro^9]-, [des-Gly^{10},Pro-NH_2^{9}]-, [des-Gly^{10},Pro-NHC_2H_5^{9}]-, [Tyr^{11}]-, [Tyr(Me)^{3,5}]-, [des-His^2,des-Pro^9]-, [des-His^2,Sar^6], and [Sar^6·des-Tyr^5]-GnRH. All analogs were purified at both the protected and the deblocked stage. The final products were characterized by chemical and physical methods and assayed *in vitro* for both LH and FSH release using rat pituitaries. Those derivatives which showed less than 0.2% of the activity of GnRH itself were further tested for inhibition of gonadotropin release.

There have now appeared several descriptions of the total synthesis of the gonadotropin releasing hormone (GnRH),[1-6] as well as of GnRH analogs.[7-13] In a collaborative project with Takeda Industries, Ltd., the effects of simple substitution of several of the amino acids in the GnRH molecule with other naturally occurring amino acids have been studied.[12] In a recent report the effect of modification of an amide function on proline in position 9 has been explored. From these efforts have emerged several noteworthy agonists in the [des-Gly^{10}] series,[13] one of which demonstrates up to five times the activity of GnRH. These results are indeed encouraging, but more elusive goals such as finding an antagonist have yet to be met. In the work reported here an attempt has been made to eliminate agonist activity by making a variety of substitutions, deletions, and other structural changes in the hope of finding an antagonist.

The procedures used in this laboratory for the preparation of GnRH[6,14] were based chiefly on the solid-phase method.[15] These same procedures have provided a means of producing GnRH analogs with the following advantages: (a) the rapidity and versatility of synthesizing analogs by the solid-phase method, (b) standardization of methods for isolating the protected GnRH analogs by a general purification method involving silica gel column chromatography, and (c) purification of the analogs by the general method of gel filtration on Sephadex. Examples of the purification methods employed are shown in Figures 1–4. The silica gel column chromatography (first pass) of the methyl ester III shown in Figure 1 clearly demonstrates the removal of impurities encountered in the solid-phase preparation. After a second pass (not shown), the product was obtained as a single spot on tlc (R_f's of Table I). This purification was typical of all analogs prepared and, in fact, more than half required only one purification by silica gel column chromatography. Figures 2 and 4 show the isolation of a single product on Sephadex G-25 for a large preparation of GnRH-free acid (VIII, 769 mg) and a moderate size preparation of [Ac-Gly^1]-GnRH (XIV, 143 mg). After passage through the first Sephadex G-25 column most of the analogs showed a single spot by tlc (R_f's of Table II). Figure 3 demonstrates the use of Sephadex G-15 to improve the quality of VIII (1.0 mg) which had been previously obtained by chromatography on Sephadex G-25.

The use of nmr spectra (Tables III and IV) has been a particularly convenient tool in following structural modification of the GnRH molecule. Some general statements can be made to describe the spectra. <Glu was difficult to observe but a peak centered at 2.4 ppm due to the $-CH_2$ group was visible as a broadening at the base of the Tos-CH_3 singlet. The Tos group was easily determined by the resonance of the aromatic proton at 8.68 ppm. Trp was somewhat difficult to detect but generally showed aromatic proton resonance at 7.6 ppm and also a 7.02-ppm singlet attributable to the hydrogen at position 1. Ser(Bzl) was easily seen by the singlet resonance of the Bzl-CH_2 at 4.47 ppm. In the absence of the blocking group, the presence of Ser could be determined by the integral of the 3.5–4.2-ppm region. Tyr(Bzl) was also easily seen by the Bzl-CH_2 proton at 4.93 ppm and also by the aromatic resonances (½ AA^1BB^1 multiplet) centered at 6.87 ppm. The number of Gly present could be obtained from the integral in the 4.0-ppm region which was due to the CH_2 resonances. Leu was very easily determined by $(CH_3)_2$ resonances (two doublets) at 0.9 ppm. The presence of Arg(Tos) could be detected by the occurrence of a CH_3 singlet at 2.36 ppm and also by aromatic resonance at 7.75 ppm. Pro had no easily detected resonances.

Other physical data include melting points (Table I), yields (Tables I and II), optical rotations (Table I and II), and amino acid analysis (Tables V and VI). The biological

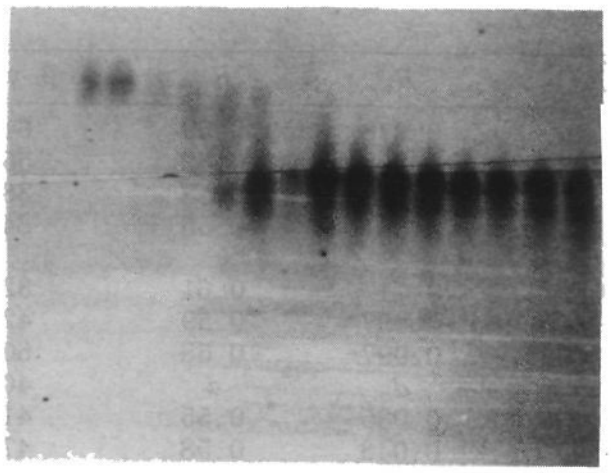


Figure 1. Ascending thin-layer chromatography (silica gel) of *fractions from the first column pass of the crude protected GnRH* methyl ester III, developed with 33% MeOH–$CHCl_3$ and indicated with Ehrlich spray.

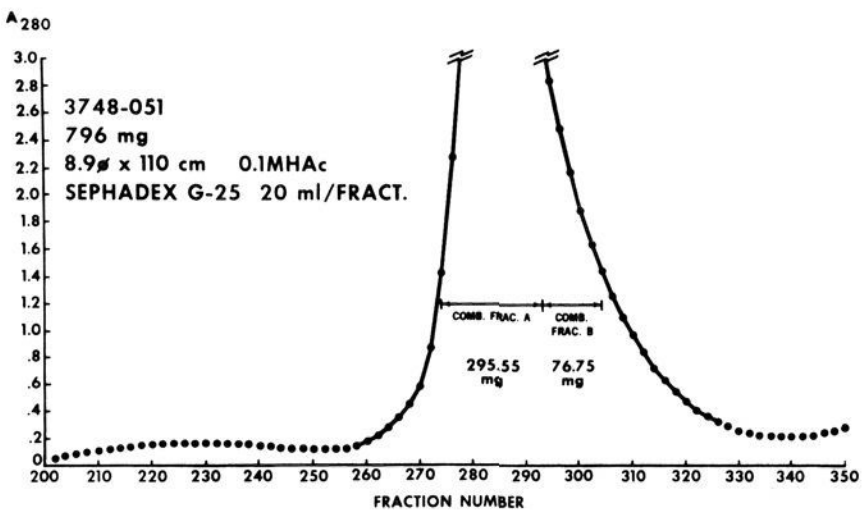


Figure 2. Stage one chromatography of 796 mg of the GnRH free acid VIII on Sephadex G-25 (8.0 × 110 cm), eluting with 0.1 *N* HOAc.

activity of the synthetic peptides was determined by *in vitro* release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from male rat hemipituitaries.[16] The LH and FSH releasing activity of the analogs is presented in Table VII.

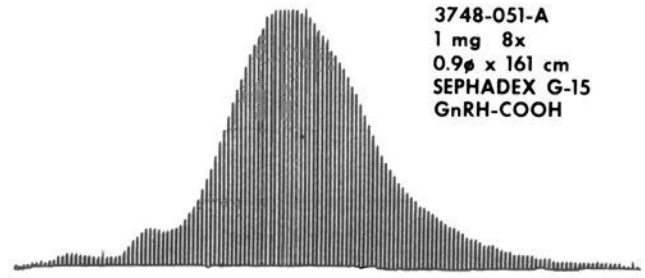


Figure 3. Chromatography of 1.0 mg of the purified (stage one) GnRH free acid VIII on Sephadex G-15 (0.9 × 161 cm), eluting with 0.1 *N* HOAc.

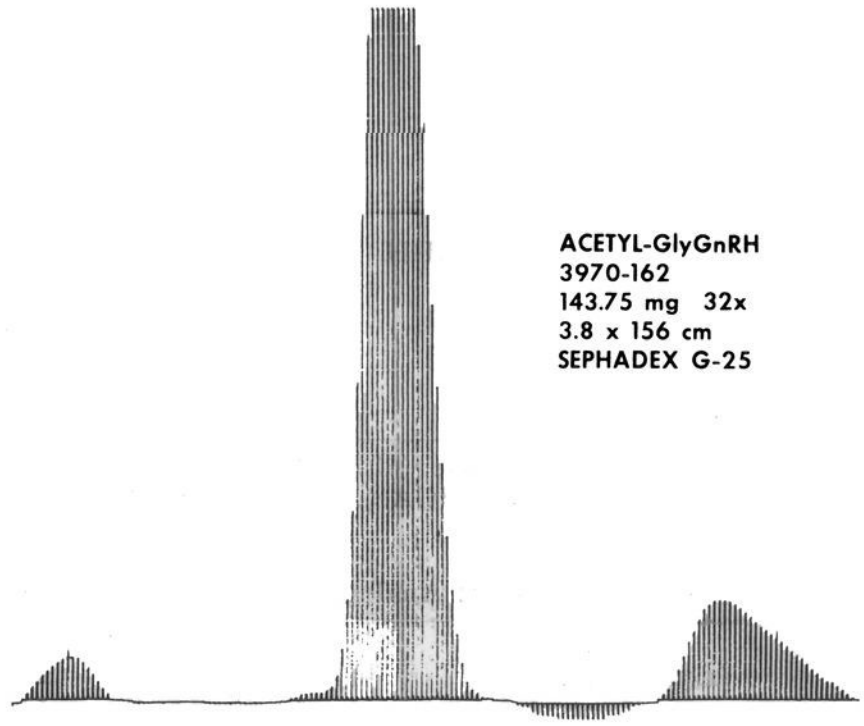


Figure 4. Stage one chromatography of 143.75 mg of the [Ac-Gly[1]]-GnRH on Sephadex G-25 (3.8 × 156 cm), eluting with 0.1 *N* HOAc.

Discussion

All four of the analogs involving substitution for pyroglutamic acid in position 1 show very little bioactivity. These results conform to findings for TRH analogs[17] as well as to previously described results for GnRH.[12] It appears that the N-terminal pyroglutamic acid residue in the releasing hormones is important to bioactivity. As yet,

Table I. Protected GnRH Analogs Ser(Bzl), Tyr(Bzl), and Arg(Tos)—Yields, Melting Points, Optical Rotations, and Tlc Data

Compound	$[\alpha]^{25}D$, deg (*c* 1, HOAc)	R_f[a]	R_f[b]	R_f[c]	Mp, °C (uncor)	% yield[d]
GnRH	−27	0.79	0.17	0.78	163–169	29.2
GnRH–OMe	−26	0.90	0.40	0.82	165–167	32.3
GnRH–$NHCH_3$	−22	0.93	0.55	0.98	165–169 dec	20.7
GnRH–OBzl	−30	0.76	0.23	0.58	157–160	37.3
GnRH N-terminal hexapeptide amide	−9	0.74	0.13	0.64	184–186	16.2
[Ac-Ala[1]]-GnRH	−24	0.88	0.24	0.72	165–175 dec	19.1
[Ac-Gly[1]]-GnRH	*g*	0.84	0.16	0.70	155–165	16.1
[D<Glu[1]]-GnRH	−17	*g*	*g*	*g*	164–167	17.4
[Pro[1]]-GnRH[f]	*g*	*g*	*g*	*g*	*g*	20.8
[Arg[2]]-GnRH[e]	−28	0.84	0.15	0.78	166–169 dec	10.1
[Tyr(Me)[3]]-GnRH	−22	0.84	0.25	0.81	177–178	4.8
[Ser[5]]-GnRH[e]	−25	0.76	0.066	0.35	168–169 dec	3.2
[des-Gly[6]]-GnRH	*g*	0.89	0.092	0.56	160–169 dec	4.4
[Sar[6]]-GnRH	−15	0.79	0.22	0.80	152–155	8.5
[des-Pro[9]]-GnRH	−14	0.83	0.22	0.78	180–182	4.5
[des-Gly[10],Pro-NH_2[9]]-GnRH	−27	0.92	0.54	0.95	157–159	17.8
[des-Gly[10],Pro-NHC_2H_5[9]]-GnRH	−25	0.93	0.38	0.80	159–160	13.4
[Tyr[11]]-GnRH	−20	0.88	0.28	0.83	152–154	27.0
[Tyr(Me)[3,5]]-GnRH	−20	0.82	0.19	0.64	162–164	31.7
[des-His[2],des-Pro[9]]-GnRH	−6	0.98	0.50	0.96	166–169	43.6
[Sar[6],des-His[2]]-GnRH	*g*	0.95	0.50	0.91	143–146	3.7
[Sar[6],des-Tyr[5]]-GnRH	−24	0.75	0.099	0.58	160–169 dec	34.9

[a] $CHCl_3$–MeOH–33% HOAc (60:45:20). [b] MeOH–$CHCl_3$ (2:8). [c] MeOH–$CHCl_3$ (4:6). [d] Based on starting millimoles of amino acid per gram of starting resin. [e] NO_2–Arg protection. [f] Boc decapeptide. [g] Not obtained.

Table II. GnRH Analogs—Yield, Optical Rotations, and Tlc Data

Compound	$[\alpha]^{25}D$, deg (c 1, 1% HOAc)	R_f[a]	R_f[b]	R_f[c]	% yield[h]
GnRH	−49	0.50	0.078	0.59	61.8
GnRH–OMe	−20[f]	0.63	0.093	0.62	55.0
GnRH–$NHCH_3$	−53	0.55	0.11	0.58	38.5
GnRH–OH	−107[e]	0.47	0.53	0.55	64.0
GnRH N-terminal hexapeptide amide	−7[g]	0.53	0.71	0.61	32.3
[Ac-Ala^1]-GnRH	−22	0.53	0.097	0.59	42.2
[Ac-Gly^1]-GnRH	−44	0.49	0.097	0.58	60.5
[D<Glu^1]-GnRH	−51.2	*d*	*d*	*d*	40.2
[Pro^1]-GnRH	−49	0.25	0.035	0.55	41.2
[Arg^2]-GnRH	−47	0.47	0.048	0.58	47.2
[Tyr(Me)^3]-GnRH	−40	0.51	0.063	0.57	57.8
[Ser^5]-GnRH	−57	0.44	0.063	0.59	56.0
[des-Gly^6]-GnRH	−34	0.52	0.071	0.58	48.9
[Sar^6]-GnRH	−44	0.45	0.07	0.54	31.5
[des-Pro^9]-GnRH	−28	0.42	0.088	0.63	36.6
[des-Gly^{10},Pro-NH_2^{9}]-GnRH	−55	0.61	0.081	0.66	37.9
[des-Gly^{10},Pro-NHC_2H_5^{9}]-GnRH	−48	0.61	0.078	0.61	56.3
[Tyr^{11}]-GnRH	−44	0.65	0.98	0.65	40.7
[Tyr(Me)^{3,5}]-GnRH	−52	0.59	0.070	0.63	29.1
[des-His^2,des-Pro^9]-GnRH	−19	0.67	0.11	0.65	37.3
[Sar^6,des-His^2]-GnRH	−18	0.68	0.083	0.64	34.7
[Sar^6,des-Tyr^5]-GnRH	−52	0.43	0.069	0.67	53.8

[a] $CHCl_3$–MeOH–33% HOAc (60:45:20). [b] EtOH–H_2O (7:3). [c] *n*-BuOH–Pyr–HOAc–H_2O (30:20:6:24). [d] Not obtained. [e] *c* 2 (1% HOAc). [f] *c* 0.5 (1% HOAc). [g] *c* 0.1 (1% HOAc). [h] Based on weight of purified, protected peptide exposed to HF.

Table III. Protected GnRH Analogs Ser(Bzl), Tyr(Bzl), and Arg(Tos)—Nmr Data

Compound	H_2–His	H_2–Trp	OBzl–CH_2–Ser	OBzl–CH_2–Tyr	Gly–CH_2	Leu–$(CH_3)_2$	Arg(Tos)	Other
GnRH	8.68	7.02	4.47	4.93	3.98, 3.98	0.90	2.36	
GnRH–OMe	8.66	6.00	4.47	4.92	3.93, 4.02	0.88	2.35	OCH_3, 3.70
GnRH–$NHCH_3$	8.67	7.03	4.46	4.91	3.96, 3.96	*a*	2.34	NCH_3, 2.74
GnRH–OBzl	8.67	7.02	4.46	4.92	3.94, 4.08	0.89	2.34	$PhCH_2O$, 5.15
GnRH N-terminal hexapeptide amide	8.70	7.09	4.42	4.93	3.89			
[Ac-Ala^1]-GnRH	8.63	7.00	4.46	4.93	4.00, 3.95	0.90	2.36	CH_3–Ala, 1.26
[Ac-Gly^1]-GnRH	8.64	7.02	4.46	4.93	4.00, 3.43	0.89	2.36	Gly–CH_2, 3.95
[Arg^2]-GnRH		7.01	4.45	4.94	4.01, 3.95	0.89	*c*	*a*
[Tyr(Me)^3]-GnRH	8.65		4.47	4.92	3.92, 3.97	0.86	2.32	OCH_3, 3.68
[Ser^5]-GnRH	8.67	7.03	4.52[b]		4.02, *a*	0.88	*c*	OCH_2–Ser,[b] 4.56
[Sar^6]-GnRH	7.1	7.1	4.41	4.92	*a*	0.89	2.33	NCH_3, *a*
[des-Pro^9]-GnRH	8.68	6.99	4.45	4.92	4.02, 3.93	0.89	2.35	*a*
[des-Gly^{10},Pro-NH_2^{9}]-GnRH	8.68	7.07	4.46	4.92	3.95	0.90	2.36	OCH_3, 3.68
[des-Gly^{10},Pro-NHC_2H_5^{9}]-GnRH	8.66	7.04	4.43	4.90	3.92	0.86	2.32	CH_3 of NEt, 1.04
[Tyr^{11}]-GnRH	8.68	6.99	4.45	4.91	3.95, 3.95	0.88	2.33	Tyr–OCH_2, 5.02
[Tyr(Me)^{3,5}]-GnRH	8.71		4.51		3.97, 3.97	0.89	2.36	OCH_3, 3.68, 3.73
[des-His^2,des-Pro^9]-GnRH								
[Sar^6,des-His^2]-GnRH		7.06	4.48	4.98	4.00	0.88	2.35	NCH_3, 3.05,[d] 2.92
[Sar^6,des-Tyr^5]-GnRH	8.70	7.14	4.53		4.00	0.90	2.38	NCH_3, 3.18

[a] Not determined. [b] Cannot differentiate. [c] NO_2 derivative. [d] Two peaks due to syn–anti isomers.

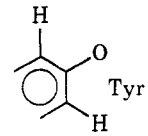
only substitution by cyclic serine[12] and Glu[9] has resulted in analogs with substantial residual bioactivity. Our work further suggests that it is the specific spatial structure of pyroglutamic acid which is important, as opposed to a presumed effect in protecting the GnRH molecule against degradation by N-terminal exopeptidases. All four of our derivatives would be resistant to aminopeptidase-like action. Even though the position 1 analogs have very little activity as agonists of GnRH, they have not shown any appreciable antagonist activity.

The marked loss in bioactivity attendant upon the substitution of arginine for histidine in position 2 appears to be another example of the unique importance of histidine in biologically active peptides resulting from the special properties of the imidazole group.[18]

The substantial reduction in bioactivity resulting from the substitution of serine for tyrosine in position 5 is not surprising since both steric and functional considerations are probably involved. The inherent importance of tyrosine in the bioactivity of GnRH is also shown by the low activity of the *O*-methyltyrosine derivatives. In this regard, GnRH is different from bradykinin[19] and physalaemin[20] where substituents on the tyrosine residue either have no effect or enhance agonistic activity.

Elision of the chain by omission of glycine in position 6 did not result in as great a loss in activity as expected. Apparently other forces are able to sbustitute for spatial relationships conferred by the glycine residue. On the other hand, addition of a methyl group on the peptide nitrogen between tyrosine and glycine by the use of sarcos-

Table IV. GnRH Analogs—Nmr Data

Compound	H_2–His	H_2–Trp		Gly–CH_2	Leu–$(CH_3)_2$	Other
GnRH	8.68	7.03	6.77	4.01, 4.01	0.90	
GnRH–OMe	8.67	7.05	6.78	4.05, 4.05	0.90	OCH_3, 3.72
GnRH–$NHCH_3$	8.66	7.05	6.77	3.96, 4.02	0.91	NHC_3, 2.78
GnRH–OH	8.67	7.03	6.77	4.05, 4.05	0.90	
GnRH N-terminal hexapeptide amide	8.66	7.01	6.75	3.98		
[Ac–Ala^1]–GnRH[e]	7.1	7.0	6.69	*a*	0.91	CH_3–Ala, 1.25
[Ac–Gly^1]–GnRH[e]	7.1	7.1	6.67	*a*	0.89	CH_3CO, 1.94
[Tyr(Me)^3]–GnRH	8.71		6.75	4.01, 4.01	0.90	OCH_3, 3.73
[Ser^6]–GnRH	8.73	7.21		4.05, 4.05	0.93	*a*
[des–Gly^6]–GnRH	8.67	7.02	6.77	4.00	0.91	
[Sar^6]–GnRH	8.69	7.09	6.77	4.00	0.90	NCH_3, 2.83, 3.02[d]
[des–Gly^{10},Pro–$NH_2{}^9$]–GnRH	8.67	7.03	6.76	4.02	0.91	OCH_3, 3.71
[des–Gly^{10},Pro–$NHC_2H_5{}^{10}$]–GnRH	8.66	7.02	6.75	4.01	0.90	$NCCH_3$, 1.08
[Tyr^{11}]–GnRH	8.67	7.03	6.75	4.00, 4.00	0.89	Arom, 6.75
[Tyr(Me)^{3,5}]–GnRH	8.72		6.77	4.00, 4.00	0.91	OCH_3, 3.71, 3.74[b]
[des–His^2,des–Pro^9]–GnRH		7.05	6.75	4.00, 4.00	0.89	
[Sar^6,des–Tyr^5]–GnRH	8.71	7.14		4.00	0.91	NCH_3, 3.18

[a]Not determined. [b]Cannot differentiate. [c]NO_2 derivative. [d]Two peaks due to syn–anti isomers. [e]Solvent is methanol-d_4.

Table V. Protected GnRH Analogs Ser(Bzl), Tyr(Bzl), and Arg(Tos)–Amino Acid Analysis[a]

Compound	Glu	His	Ser	Tyr	Gly	Leu	Arg	Pro	Other	Comments
GnRH	1.03	1.02	0.96	1.00	2.02	1.07	0.99	0.92		
GnRH–OMe	1.04	0.92	0.99	1.00	2.04	1.07	0.95	1.00		
GnRH–$NHCH_3$	1.00	0.83	1.00	1.00	2.08	1.07	0.99	1.05		
GnRH–OBzl	1.08	0.95	1.02	0.88	2.12	1.03	0.95	0.94		
GnRH N-terminal hexapeptide amide	1.50	0.89	0.86	0.85	0.92					
[Ac–Ala^1]–GnRH		1.03	0.97	1.04	1.72	1.11	1.11	0.85	*b*, Ala	
[Ac–Gly^1]–GnRH		0.97	1.00	1.00	3.08	1.05	0.97	1.00		
[D-<Glu^1]–GnRH	*b*									
[Pro^1]–GnRH	*b*									
[Arg^2]–GnRH	1.12		0.85	0.85	1.95	1.05	2.02	1.13		No tryptophan
[Tyr(Me)^3]–GnRH	1.02	0.99	0.99	1.37	2.01	1.02	0.86	1.11		No tryptophan
[Ser^5]–GnRH	0.99	0.97	1.88		2.22	1.10	0.81	1.08		
[des–Gly^6]–GnRH	1.45	0.83	0.85	0.92	0.99	1.00	1.00	0.80		
[Sar^6]–GnRH	1.18	0.92	0.98	0.97	1.04	1.04	0.98	0.98	*b*, Sar	
[des–Pro^9]–GnRH	1.01	1.00	0.88	0.96	1.95	0.97	1.12			
[des–Gly^{10},Pro–$NH_2{}^9$]–GnRH	0.98	0.94	0.99	1.06	0.98	1.06	0.99	1.02		
[des–Gly^{10},Pro–$NHC_2H_5{}^9$]–GnRH	1.04	0.94	1.00	0.97	1.02	1.06	0.77	1.01		
[Tyr^{11}]–GnRH	0.81	0.96	1.03	2.01	2.12	1.05	0.86	1.18		
[Tyr(Me)^{3,5}]–GnRH	1.14	0.80	1.16	0.82	2.21	1.10	1.00	1.16		No tryptophan
[des–His^2,des–Pro^9]–GnRH	0.91		1.01	0.99	2.11	1.05	0.99			
[Sar^6,des–His^2]–GnRH	0.94		0.99	0.97	2.04	1.05	0.94	1.08	*b*, Sar	
[Sar^6,des–Tyr^5]–GnRH	1.77[c]	1.17	0.87		0.98	1.05	1.02	0.92	*b*, Sar	

[a]Tryptophan noted only when completely absent. [b]Not determined. [c]Includes partial integration of sarcosine.

ine in position 6 has apparently resulted in restriction of freedom in a central bond system. This, in turn, could inhibit attainment of the biologically active conformation.

Our bioassay value for the des-Gly^{10} analog is appreciably lower than that reported by Rivier, *et al.*[21] The reasons for this discrepancy are not known. None of the peptides having less than 0.2% the activity of GnRH were active inhibitors of *in vitro* gonadotropin release.

Experimental Section

The procedural details can be considered to also apply to those analogs whose synthesis is not specifically described. In general, the protected peptides were obtained by aminolysis of the methyl esters. Analogs such as the free acid and the *N*-methyl amide were obtained *via* the benzyl ester and direct aminolytic cleavage from the resin, respectively. Individual analogs were subjected to extensive chemical and physical characterization (Tables I–VI) and biological testing (Table VII).

Melting points were performed on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The nmr spectra were obtained on a Varian HA-100 and chemical shifts reported in parts per million relative to internal TMS. Optical rotations were measured on a Hilger and Watts polarimeter with 0.01° of precision. Amino acid analyses were carried out on a Beckman-Spinco 120-B with samples which were hydrolyzed in 6 *N* HCl for 14 hr in sealed, evacuated tubes at 105°. All solutions were made up on a volume/volume basis. Solvent concentrations were carried out under reduced pressure evaporation with bath temperature not exceeding 40°.

Monitoring of Sephadex columns by differential refractometry was performed using a Waters Associates W-4 instrument. Chloromethylated resin was obtained commercially from Schwarz-Mann as 2% cross linked beads, Cl content of 1.7 mequiv/g. Silica gel 60 was obtained from Brinkman Instruments, particle size 0.063–0.200 mm (70–230 mesh ASTM). Acetate anion exchange resin AG1-X2 (200–400 mesh) was purchased from Bio-Rad. For column chromatography, silica gel columns were prepared in 5% MeOH–$CHCl_3$. For thin-layer chromatography, Anal Tech silica gel GF precoated (250 μ) plates were used.

Boc-Gly-resin (I). The method of preparation has previously been described.[6] Amino acid analysis showed 0.435 mmol of Gly/g of resin.

<Glu-His-Trp-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(Tos)-Pro-Gly-resin (II). The protected decapeptide-resin was built up starting with Boc-Gly-resin (56 g, 24.3 mmol) by the method previously described.[6]

<Glu-His-Trp-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(Tos)-Pro-Gly-OMe (III). A portion (20.5 g, 6 mmol) of the decapeptide-resin II was stirred in MeOH (800 ml) and Et_3N (42 ml) for 24 hr

Table VI. GnRH Analogs—Amino Acid Analysis[a]

Compound	Glu	His	Ser	Tyr	Gly	Leu	Arg	Pro	Other	Comments
GnRH	1.03	1.02	0.93	1.00	1.02	1.03	0.96	1.04		
GnRH-OMe	*b*									
GnRH-$NHCH_3$	*b*									
GnRH-OH	1.05	0.94	1.00	0.97	2.09	1.03	0.97	0.97		
GnRH N-terminal hexapeptide amide	1.10	1.04	0.80	1.00	1.06					
[Ac-Ala^1]-GnRH		1.00	0.98	1.02	2.02	1.02	0.99	1.01	0.96	Ala
[Ac-Gly^1]-GnRH		1.01	0.97	1.03	3.01	1.01	1.01	0.96		
[D-<Glu^1]-GnRH	0.84	1.30	0.94	0.93	2.00	0.93	0.91	0.89		
[Pro^1]-GnRH		0.88	0.93	0.95	2.00	0.95	0.99	1.84		
[Arg^2]-GnRH	1.04		0.94	1.02	1.97	1.04	1.96	0.98		No tryptophan
[Tyr(Me)^3]-GnRH	1.10	0.98	0.98	1.54[c]	2.08	1.02	1.00	0.84		No tryptophan
[Ser^5]-GnRH	0.99	1.02	1.88		2.02	1.08	1.04	0.94		
[des-Gly^6]-GnRH	1.06	0.99	0.95	0.96	1.00	0.99	0.99	1.10		
[Sar^6]-GnRH	1.02	0.98	0.96	0.98	1.04	1.00	1.00	1.04	*b*	
[des-Pro^9]-GnRH	1.09	0.98	0.81	1.00	2.10	1.04	1.04			
[des-Gly^{10},Pro-$NH_2{}^9$]-GnRH	1.05	0.98	1.00	0.97	1.00	1.01	0.98	0.99		
[des-Gly^{10},Pro-$NHC_2H_5{}^9$]-GnRH	1.06	1.00	0.95	0.98	1.01	1.01	1.00	1.00		
[Tyr^{11}]-GnRH	1.01	0.99	0.95	1.98	2.03	1.01	1.02	1.01		
[Tyr(Me)^{3,5}]-GnRH	1.05	0.92	0.98	0.98[c]	2.08	1.04	1.03	1.05		No tryptophan
[des-His^2,des-Pro^9]-GnRH	1.03		0.94	0.98	2.06	1.05	0.98			
[Sar^6,des-His^2]-GnRH	1.08		0.96	0.97	1.00	1.01	0.99	0.97	*b*	
[Sar^6,des-Tyr^5]-GnRH	1.02	1.04	0.95		0.99	1.02	1.02	0.98	*b*	

[a] Tryptophan noted only when completely absent. [b] Not determined. [c] Incomplete conversion of *O*-methyltyrosine.

Table VII. GnRH Analogs—Biological Activity

Compound	*In vitro* LH release	*In vitro* FSH release	Inhibition test
GnRH	100	100	
GnRH-OMe	1.4 (RIA)[a]	1.0 (RIA)[a]	
GnRH-$NHCH_3$	1.7 (RIA)	1.9 (SP)	
GnRH-OH	0.5 (P)	0.5 (RIA)	
GnRH N-terminal hexapeptide amide	1.8 (RIA)	1.3 (SP)	
[Ac-Ala^1]-GnRH	~0.25 (RIA)	0.1–0.25 (SP, RIA)	
[Ac-Gly^1]-GnRH	>0.25 (RIA)	0.1–0.5 (SP)	
[D-<Glu^1]-GnRH	~1 (P)	0.6 (SP)	
[Pro^1]-GnRH	0.02 (P)	<0.5 (RIA)	None
[Arg^2]-GnRH	~0.03 (RIA)	0.01 (SP)	None
[Tyr(Me)^3]-GnRH	~2 (RIA)	~2 (SP)	
[Ser^5]-GnRH	~0.5 (P, RIA)	~0.5 (RIA)	
[des-Gly^6]-GnRH	~5 (P, RIA)	5–7 (SP, RIA)	
[Sar^6]-GnRH	~2 (RIA)	~2 (SP, RIA)	
[des-Pro^9]-GnRH	~1 (P, RIA)	0.8 (SP, RIA)	
[des-Gly^{10},Pro-$NH_2{}^9$]-GnRH	<5 (P, RIA)	3 (SP)	
[des-Gly^{10},Pro-$NHC_2H_5{}^9$]-GnRH	120 (P, RIA)	158 (SP)	
[Tyr^{11}]-GnRH	0.08 (RIA)	0.1 (SP, RIA)	None
[Tyr(Me)^{3,5}]-GnRH	0.5 (P, RIA)	0.5 (SP, RIA)	
[des-His^2,des-Pro^9]-GnRH	≪0.1 (RIA)	≪0.1 (SP)	None
[Sar^6,des-His^2]-GnRH	≪0.1 (RIA)	≪0.1 (SP)	None
[Sar^6,des-Tyr^5]-GnRH	≪0.1 (RIA)	≪0.1 (SP)	None

[a] P = Parlow, SP = Steelman–Pohley, and RIA = radioimmunoassay; see ref 16.

at room temperature. The suspension was filtered and the resin subjected to the same treatment for an additional 30 hr. The combined filtrates were concentrated, dissolved in a minimum of HOAc (15 ml), and applied to a 4.5 × 40 cm silica gel column. After preliminary elution with 5% MeOH–$CHCl_3$ (150 ml), the decapeptide methyl ester was eluted with 33% MeOH–$CHCl_3$. Detection of desired fractions was accomplished by tlc using 33% MeOH–$CHCl_3$–Ehrlich spray. The protected decapeptide methyl ester was purified a second time by column chromatography as above. One-third of this material was crystallized from MeOH–EtOH and dried over P_2O_5 in a vacuum desiccator: yield, 0.9888 g (32.3%); mp 165–167°; $[\alpha]^{25}D$ −26° (c 1, HOAc).

<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-OMe (IV). The blocking groups of the protected decapeptide methyl ester III (100 mg) were cleaved by treatment with liquid HF (5 ml) in the presence of anisole (100 mg) for 1 hr at 0°. The HF was swept away with a slow stream of N_2 and the residue precipitated with Et_2O. The precipitate was filtered and then dissolved in 10% HOAc (100 ml). This solution was washed in a separatory funnel with fresh Et_2O and then lyophilized. The resulting white powder was dissolved in 0.1 *N* HOAc (100 ml) and the solution was chromatographed on an anion exchange column containing acetate ion exchange resin AG1-X2 (5 g) using additional 0.1 *N* HOAc. The effluent was lyophilized, dissolved in 0.1 *N* HOAc, and subjected to a two-stage gel filtration purification by chromatography first on Sephadex G-25 and in a subsequent step on Sephadex G-15 columns using 0.1 *N* HOAc as eluent. Detection of fractions on Sephadex was accomplished by differential refractometry. The desired fractions corresponding to the main peak were combined and lyophilized: yield, 43.0 mg (55.0%); $[\alpha]^{25}D$ −20° (c 0.5, 1% HOAc).

<Glu-His-Trp-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(Tos)-Pro-Gly-NH_2 (V). A portion (2 mmol) of the purified methyl ester III was dissolved in a minimum of MeOH (50 ml) and added carefully to freshly distilled liquid NH_3 (350 ml) with stirring. The resulting solution was stirred overnight and concentrated, and the residue was crystallized from MeOH–EtOH to give the protected decapeptide amide V: yield, 0.8861 g (29.2%); mp 163–169°; $[\alpha]^{25}D$ −12° (c 1, HOAc).

<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (VI). The blocking groups of the protected amide V (100 mg) were cleaved with HF. The procedure for deblocking and subsequent purification was identical with that used for the methyl ester IV: yield, 55 mg (61.8%); $[\alpha]^{25}D$ −49° (c 1, 1% HOAc).

<Glu-His-Trp-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(Tos)-Pro-Gly-OBzl (VII). A portion (13.6 g, 4 mmol) of the decapeptide resin II was suspended in benzyl alcohol (540 ml) and Et_3N (28 ml) for 24 hr at room temperature with stirring. The material was

filtered and the resin treated under the same conditions for an additional 30 hr. The filtrates were combined and chromatographed on a silica gel column (6 × 100 cm). Eluting with 5% MeOH–$CHCl_3$ removed much of the benzyl alcohol, but product eventually began appearing in the effluent. At this point, the product was eluted with 33% MeOH–$CHCl_3$ and desired fractions were concentrated as much as possible. The remaining benzyl alcohol was removed completely by two additional chromatography steps on silica gel columns. Elution of the third column with 33% MeOH–$CHCl_3$ gave the protected decapeptide benzyl ester VII which was crystallized from MeOH–EtOH: yield, 2.3903 g (37.3%); mp 157–160°; $[\alpha]^{25}D$ −30° (c 1, HOAc).

<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-OH (VIII). The blocking groups of the decapeptide benzyl ester VII (250 mg) were cleaved by treatment with anhydrous, liquid HF (5 ml) in the presence of anisole (250 mg) at 0° for 1 hr. The liquid HF was removed with a stream of N_2 in approximately 15 min at 25° and the residue was precipitated with Et_2O. The precipitate was dissolved in 10% HOAc (150 ml) and lyophilized. The resulting lyophilizate was dissolved in 0.1 *N* HOAc (150 ml) and passed through AG1-X2 (acetate) exchange resin (10 g) using additional 0.1 *N* HOAc. The crude product was subjected to gel filtration on Sephadex G-25 yielding a major peak (differential refractometry). This material was chromatographed on Sephadex G-15 from which a single peak was isolated. The desired fractions were conservatively selected from the central portion of the peak, combined, and lyophilized: yield, 108 mg (64.0%); $[\alpha]^{25}D$ −107° (c 2, 0.1 *N* HOAc).

<Glu-His-Trp-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(Tos)-Pro-Gly-$NHCH_3$ (IX). A portion (6.83 g, 2 mmol) of the decapeptide-resin II was suspended in MeOH (25 ml) and DMF (25 ml). CH_3NH_2 was bubbled through the liquid until saturation occurred. The suspension was stirred for 12 hr at room temperature. After resaturation with CH_3NH_2 and stirring for 4 additional hours, the material was filtered and the filtrate concentrated. The residue was dissolved in a minimum of HOAc (5 ml) and applied to a silica gel column (4.5 × 40 cm). After preliminary washing with 5% MeOH–$CHCl_3$, the product was eluted with 33% MeOH–$CHCl_3$. The peptide was detected by tlc with 33% MeOH–$CHCl_3$–Ehrlich spray. After chromatography on silica gel for a second time (3.5 × 40 cm) the protected decapeptide methyl amide was crystallized from MeOH–EtOH: yield, 0.6342 g (20.7%); mp 165–169° dec; $[\alpha]^{25}D$ −22° (c 1, HOAc).

<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-$NHCH_3$ (X). A sample of the protected decapeptide methyl amide IX (250 mg) was deprotected with liquid HF. The procedure for deblocking and subsequent purification was similar to that described used for the free acid VIII: yield, 75 mg (38.5%); $[\alpha]^{25}D$ −53° (c 1, HOAc).

His-Trp-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(Tos)-Pro-Gly-resin (XI). The protected nonapeptide-resin was built up starting with Boc-Gly-resin (18.4 g, 8.0 mmol).

Ac-Gly-His-Trp-Ser(Bzl)-Tyr(Bzl)-Tyr(Bzl)-Gly-Leu-Arg-(Tos)-Pro-Gly-resin (XII). A sample of the nonapeptide-resin (3.4 g, 1.0 mmol) was taken through one additional coupling cycle using *N*-acetylglycine (0.468 g, 4.0 mmol) in place of pyroglutamic acid.

Ac-Gly-His-Trp-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(Tos)-Pro-Gly-NH_2 (XIII). The decapeptide-resin XII was suspended in MeOH (240 ml) and Et_3N (13.9 ml) for 24 hr at room temperature. The suspension was filtered and the resin resuspended as described above for an additional 30 hr. The crude product was dissolved in MeOH (50 ml) and added carefully to freshly distilled NH_3 (300 ml). The resulting solution was stirred overnight and concentrated and the residue subjected to the usual silica gel purification. Crystallization of the column purified material from MeOH–EtOH gave the desired product: yield, 0.253 g (16.1%); mp 155–165°.

Ac-Gly-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (XIV). The blocking groups of the protected decapeptide XIII (180 mg) were cleaved by treatment with liquid HF in the presence of anisole (180 mg) for 1 hr at 0°. The procedure for purification and isolation was similar to that for VI: yield, 81.0 mg (60.2%); $[\alpha]^{25}D$ −44° (c 1, HOAc).

<Glu-His-Trp-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(Tos)-Pro-resin (XV). The protected nonapeptide-resin was built up starting with Boc-Pro-resin (4.25 g, 2.0 mmol) prepared by the usual methods.[6]

<Glu-His-Try-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(Tos)-Pro-NHC_2H_5 (XVI). The nonapeptide-resin XV was suspended in MeOH (240 ml) and Et_3N (13.9 ml) and stirred for 24 hr at room temperature. The material was filtered and the resin methanolyzed as above for an additional 30 hr. The suspension was again filtered and the combined filtrates were concentrated. The residue was dissolved in MeOH (50 ml) and added carefully to freshly distilled $C_2H_5NH_2$ (500 ml). The resulting solution was stirred for 12 hr and concentrated. The residue was dissolved in a minimum of HOAc (5 ml) and subjected to silica gel purification. The purified product was crystallized from MeOH–EtOH: yield, 0.3993 g (13.4%); mp 159–160°; $[\alpha]^{25}D$ −25° (c 1, HOAc).

<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-NHC_2H_5 (XVII). A sample of the protected nonapeptide ethyl amide XVI (300 mg) was deprotected by treatment with liquid HF (15 ml) in the presence of anisole (300 mg). The isolation and Sephadex purification was identical with that described for VIII: yield, 88.0 mg (37.7%); $[\alpha]^{25}D$ −48° (c 1, HOAc).

Biological Assay Procedures. *In vitro* bioassay procedures for agonist activity were described previously.[16] Usually the analogs were assayed at two dose levels in a 2 × 2 design following an exploratory one-level assay. Those peptides having less than 0.2% the activity of GnRH were also assayed for antagonist activity. This assay procedure involved the addition of GnRH (2 ng/pit) to the media 10 min after initiation of the incubation in the presence of 2000 ng of antagonist. Media was sampled 6 hr later and assayed for LH and FSH by bioassay and/or radioimmunoassay.[16]

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