than twice the inhibitory dose the analogues were always inactive. This holds true also for MIF itself.[25]

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Synthesis and Biological Activity of Peptide Antagonists of Luliberin (Luteinizing Hormone-Releasing Hormone)

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A series of des-His[2] octa- and nonapeptide analogues of luliberin (luteinizing hormone-releasing hormone) with modifications in the 1 and 6 positions, and in some instances the 10 position, has been prepared. Some of these analogues are potent inhibitors of luliberin in vitro and in vivo. The use of ultraviolet absorption measurements for evaluating peptides containing tyrosine and tryptophan is described. An efficient synthesis of *O*-methyl-D-tyrosine is reported.

The first analogue reported to be an antagonist of luliberin [luteinizing hormone-releasing hormone (LH-RH)] was [des-His[2]]-LH-RH, described by Vale et al.[4] Since that time, a large number of antagonists to luliberin have been synthesized and tested. The most potent analogues have been modified at the 2, 3, 6, and 10 positions of LH-RH. A review by Schally et al.[5] summarizes the literature in this field. Recent reports from Folkers' group[6-8] and Schally's group[9] describe additional analogues based on this approach.

Luliberin agonist analogues in which the 1 position has been modified have been reported.[10-12] However, only a few reports have appeared in which antagonists of luliberin have been obtained by modification of the 1 position. Bowers et al.[13] reported that replacement of <Glu[1] with the nitrogen mustard chlorambucil (Chl) in [Chl[1],Leu[2],-

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Leu^3,D-Ala^6]-LH-RH resulted in an antagonist analogue. Coy et al.[14] reported that [D-<Glu^1,des-His^2,D-Trp^3,D-Ala^6,des-Gly^{10}]-LH-RH ethylamide had little antagonist activity. Recently, Rivier and Vale[15] reported that [D-<Glu^1,D-Phe^2,D-Trp^{3,6}]-LH-RH was a potent antagonist of luliberin in vitro and an inhibitor of ovulation in the rat.

We now report the synthesis and biological activity of a series of des-His^2 octa- and nonapeptide analogues of luliberin in which modifications have been made in the 1 and 6 positions and in some instances the 10 position.

Chemistry. Solution-phase synthetic procedures were used for the preparation of the peptides. This method allowed the preparation of large quantities of individual fragments which could be isolated and easily purified. These fragments could then be utilized as common intermediates in preparing a large variety of peptides with modifications at specific positions, e.g., the 1 and 6 positions. The synthetic procedures involved the following recognized procedures: (1) protection by amine acylation using acyl chloride (aryloxycarbonyl chloride), acyl azide (*tert*-butoxycarbonyl azide), or aryloxycarbonyl azide; (2) deprotection involving reductive removal of aryloxycarbonyl; (3) deprotection involving removal of *tert*-butoxycarbonyl by hydrogen chloride in tetrahydrofuran or by trifluoroacetic acid in dichloromethane; (4) coupling by acyl azide; (5) coupling by use of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT); (6) coupling by use of diphenylphosphoryl azide; (7) coupling by mixed anhydride formation using ethyl chloroformate; (8) coupling by use of active esters (pentachlorophenyl or *p*-nitrophenyl); (9) ester hydrazinolysis; (10) ester aminolysis; and (11) exchange of hydrochloride for acetate.

Specific examples illustrating these reactions are described under the Experimental Section.

The final step in the synthesis of the octa- and nonapeptides listed in Table I involved azide coupling reactions. In most cases, azides were generated in situ from hydrazide intermediates of N-terminus protected tetra-, penta-, or hexapeptides. The majority of the peptides were obtained by coupling protected N-terminus hexapeptide azides with monoprotonated C-terminus amides, i.e., Arg-Pro-NHC_2H_5·HCl to obtain compounds **1**, **7**, **8**, **10**, **17–19**, and **23–29** or Arg-Pro-Gly-NH_2·HCl to obtain compounds **30**, **32**, **34–36**, **40–44**, **46**, **50–61**, **63**, **64**, **66**, and **68**. Protected N-terminus pentapeptide azides were coupled with Leu-Arg-Pro-NHC_2H_5·HCl to obtain compounds **3–5** and **9** or Leu-Arg-Pro-Gly-NH_2·HCl to obtain compound **45**. Protected N-terminus tetrapeptide azides were coupled with monoprotonated (HCl) NH_2-CH(R or Ar)-CO-Leu-Arg-Pro-NHC_2H_5 to obtain compounds **2** [$N(CH_3)_2$ for NHC_2H_5], **6**, **11–16**, and **20–22** or NH_2-CH(R or Ar)-CO-Leu-Arg-Pro-Gly-NH_2·HCl to obtain compounds **37–39** and **47–49**. Compound **31** was obtained by coupling the hexapeptide azide prepared from Z-His-Trp-Ser-Tyr-Gly-Leu-$NHNH_2$ with Arg-Pro-OCH_3·HCl. The resulting octapeptide methyl ester was hydrolyzed with dilute base to give compound **31**. Compound **67** was obtained by coupling Z-His-N_3 with Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2·HCl and compound **65** was the hydrogenolysis product of compound **67**. Compounds **33** and **62** were obtained by coupling the azides prepared in situ from the cyclohexylurea of Trp-$NHNH_2$ or <Glu-Trp-$NHNH_2$, respectively, with Ser-Trp-Gly-Leu-Arg-Pro-Gly-NH_2·HCl. Compound **69** was obtained by coupling <Glu-D-Phe-Trp-Ser-Tyr-N_3 with D-Phe-Leu-Arg-Pro-Gly-NH_2·HCl. Protection of the arginine guanidino group by monoprotonation during peptide synthesis was first described by Anderson.[16]

Table II contains the microanalytical data (elements for which data were obtained), rotation and an ultraviolet comparison of tyrosine and tryptophan content of the synthetic peptides, the procedure for which is described in the following section. Amino acid analyses were obtained only in special cases, since the fragment-coupling approach allowed thin-layer chromatography, infrared and ultraviolet spectroscopy, and, in some cases, nuclear magnetic resonance spectroscopy to establish the success of the coupling.

Ultraviolet Absorption Analysis. An assay for tyrosine and tryptophan content helped to establish the identity and purity of the synthetic peptides, using a modified version of an early spectrophotometric method.[17] The procedure is described under the Experimental Section. Ratios of % found/% theory are listed in Table II; a value of 1.00 is ideal.

Biological Assays. Inhibition of Luteinizing Hormone (LH) Release in Vitro. Anterior pituitaries were obtained from diestrus Charles River CD rats (200–250 g), and pooled glands were dissociated with collagenase and hyaluronidase followed by Viokase® as described by Vale et al.,[18] Baker et al.,[19] and Reel et al.[20] Monodispersed cells were suspended (1.67×10^5 cells/mL) in Dulbecco's modified Eagle's medium (DME) supplemented with 10% horse serum and 2.5% fetal calf serum, and 1.0-mL aliquots of cells were dispensed into individual wells of Falcon multiwell plates (no. 3008). After 4 days in culture, the growth medium was removed and the cell monolayers were washed twice with serum-free DME. The 4-day cultures then were employed to test LH-RH analogues for their ability to inhibit LH-RH-stimulated LH release. One milliliter of serum-free DME containing either (1) no addition (control), (2) LH-RH ($2–5 \times 10^{-10}$ M), or (3) LH-RH ($2–5 \times 10^{-10}$ M) in combination with an analogue (at one or more concentrations) was added to duplicate cultures, and incubation was conducted at 37 °C for 4 h. The medium was removed and frozen for subsequent radioimmunoassay of LH as described previously by Dermody et al.[21] The data in Table I were expressed as a percent inhibition of LH-RH-stimulated LH release when a single concentration of analogue was tested against a single concentration of LH-RH. In those instances when multiple concentrations of an analogue were tested against a single concentration of LH-RH, the results were expressed as the ED_{50} molar ratio, i.e., the ratio of molar concentrations of analogue to LH-RH that yielded a 50% inhibition of LH-RH-stimulated LH release.

Blockade of Ovulation in the Cycling Rat. The procedure for testing analogues for their ability to block ovulation in the cycling rat was essentially the same as that employed by Corbin and Beattie.[22] On the afternoon of proestrus, Charles River CD rats received subcutaneous injections of analogues in 0.1 mL of propylene glycol or of vehicle alone (controls) at 1200, 1230, 1300, 1350, 1400, and 1450 h. The rats were sacrificed the next morning (estrus). The number of animals ovulating was determined and the number of ova in the oviducts was counted under a dissecting microscope. The data in Table I were expressed as the percent of the animals in which ovulation was completely blocked (percent ovulation blockade) or as the unit dose that completely inhibited ovulation in 50% of the treated rats (ED_{50}). Where ovulation blockade was not achieved, the result is indicated as greater than the largest dose level employed.

Results and Discussion

Table I lists the compounds that were prepared along

with the results of the in vitro and in vivo biological assays. Some of the compounds were known previously and were prepared for purposes of comparison. The 1 position of LH-RH was modified with various protected amino acids. Some of the amino acids were protected at the N terminus only, while others were protected at the N terminus and in the side chain. The N terminus was protected to simulate the uncharged <Glu[1] moiety in LH-RH. The rationale for the introduction of side-chain protected amino acids at the 1 position of LH-RH was based on our earlier discovery that certain protected LH-RH analogues containing benzylated tyrosine and/or serine showed potent in vitro activity.[23] The data in Table I show that incorporation of Z-Ser(Bzl) or Z-Gln in the 1 position combined with an aromatic D-amino acid or D-proline in the 6 position in the octapeptide series gave compounds that were potent inhibitors of LH-RH. In all cases, the C terminus was proline ethylamide with the exception of compound **2**, which was proline dimethylamide. The most potent inhibitors were compounds **1**, **3**, **5**, **6**, **11**, **17**, and **18**. Similar modifications of the 1 and 6 positions were employed in the nonapeptide series. Here, the requirements for activity seem to be more specific. Boc-Ser(Bzl) in position 1 gave more active antagonists than other 1-position substitutions. D-Trp in position 6 gave rise to greater potency than other aromatic D-amino acids in this position. Compounds **38** and **39** (the acetate salt of **38**) were potent inhibitors of LH-RH. The substitution of prolylglycinamide in compounds **35**, **47**, **49**, and **59** with proline ethylamide (compounds **1**, **20**, **22**, and **26**) seemed to have little or no effect on the antagonist activity. Compounds **1**, **3**, **5**, **6**, **11**, **17**, **18**, **38**, and **39** were compared to compound **69**, reported by Beattie et al.[24] to have antiovulatory activity. Compounds **5**, **38**, and **39** were more potent than **69**, compounds **3** and **18** were equipotent to **69**, while compounds **1**, **6**, **11**, and **17** were less potent than **69**. It can be seen that incorporation of uncharged bulky amino acid residues at the 1 position in combination with an aromatic D-amino acid or D-proline in the 6 position in [des-His[2]]-LH-RH results in antagonists of LH-RH.

A number of these peptides showed a very low solubility (less than 1 mg/mL) in water. However, several others were highly water soluble as the acetic acid salt but were less soluble as the hydrochloride. The finding that solubility was diminished in physiological saline was in keeping with this observation. The most striking examples were compounds **38** and **39**. While completely water soluble at 3 mg/mL in the acetate form, compound **39** was soluble to only 0.016 mg/mL in 0.9% sodium chloride. Compound **38**, the hydrochloride salt, was soluble in water at 1 mg/mL.

Collectively, these newly synthesized des-His[2] octa- and nonapeptide analogues with modifications in the 1, 6, and/or 10 positions present still another series of potential LH-RH antagonists.

Experimental Section

Melting points (mp) were determined in a Thomas-Hoover capillary melting point apparatus or on a Fisher-Johns melting point apparatus and are uncorrected. Infrared data were recorded on a Beckman IR-9 or IR-7 prism grating instrument or on a Digilab FTS-14 interferometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter in a 1-dm tube. Nuclear magnetic resonance measurements (NMR) were made on a Bruker WH-90 pulsed Fourier transform instrument. Amino acid analyses were determined on peptide hydrolysates by vapor-phase chromatography as trimethylsilyl derivatives on 3% OV-17 on Gas Chrom Q, temperature programmed. Homogeneity of the products was determined by ascending thin-layer chromatography (TLC) on silica gel coated glass plates using principally the solvent systems (1) $CHCl_3$–MeOH–H_2O (60:45:10) and (2) $CHCl_3$–MeOH–32% HOAc in H_2O (60:45:20). The TLC of the synthetic peptides described in Table I were homogeneous single spots when visualized with UV and/or I_2 vapors.

The amino acids, benzyloxycarbonyl-protected amino acids and *tert*-butoxycarbonyl-protected amino acids were commercial materials obtained from Ajinomoto Co., Inc., Tokyo, Japan; Aldrich Chemical Co., Inc., Milwaukee, Wis.; Bachem, Inc., Torrence, Calif.; Nutritional Biochemicals Corp., Cleveland, Ohio; or Vega-Fox Biochemicals, Tucson, Ariz.

threo-D-Phenylserine (*threo*-D-Pse)[25] and D- and DL-pentamethylphenylalanine[26] were obtained according to preparations in the literature. *O*-Methyl-DL-tyrosine was prepared by the hydantoin procedure for amino acids and resolved by enzymatic hydrolysis (Takadiastase) of the *N*-acetyl derivative. Since this procedure is an efficient and novel route to the previously known *O*-methyl-D-tyrosine,[27] it is described here.

Ultraviolet Absorption Assay. A Cary Model 11 spectrophotometer was used. Spectra of standards, intermediates, and products were obtained first in methanol at a scan rate of 12 nm/min, followed by a rerun after the addition of 1 drop of 12 N KOH solution to each cuvette. Tyrosine was measured by its change in absorbance with ionization at 300 and 244 nm and tryptophan by its intense absorption at 281.5 nm. The absorption readings were evaluated as follows. Tyrosine: (1) At 300 nm, $\Delta E'$ of the sample is obtained from the increase in absorbance from neutral to alkaline (ΔA) divided by the percent (weight/volume) concentration. $\Delta E'$ at this wavelength for the 4-cresyl moiety (M_r 107) is 203. $(\Delta E' \times 100)/205$ = % 4-cresyl moiety. (2) Again, at 244 nm $\Delta E'$ of the sample is obtained from the absorbance change (ΔA) divided by the concentration. $\Delta E'$ for the 4-cresyl moiety is 1090. Then, $(\Delta E' \times 100)/1090$ = % 4-cresyl moiety. The mean of these two percent values divided by the required percent for a weight of 107 in the sample (mol % of 4-cresyl moiety) gives the ratio shown in Table II for each sample. Tryptophan: (1) At 281.5 nm, the A in neutral solution is corrected for underlying tyrosyl absorption by subtracting 0.70 of the tyrosine ΔA at 300 nm. The E' of the indolyl moiety (M_r 130) at this wavelength is 450. Then, $(E' \times 100)/450$ = % indolyl moiety. (2) At 281.5 nm, the A in alkaline solution is corrected for tyrosyl absorption by subtracting 0.122 of the tyrosine ΔA at 244 nm and determining E'. Then, $(E' \times 100)/450$ = % indolyl moiety. The mean of these percent values divided by the required value for a weight of 130 in the sample (mol % of indolyl moiety) gives the ratio for tryptophan.

Ser-Tyr-OMe·HCl (I). Z-Ser-Tyr-OMe,[28] 8.328 g (0.02 mol), dissolved in 70 mL of MeOH containing 6.67 mL (0.02 mol) of 3 N HCl in THF and 500 mg of 20% Pd/C was stirred under H_2 at 1-in. water pressure for 3 h or until TLC indicated completion. The mixture was filtered and evaporated under reduced pressure to a glass (I), which was suitable for use without further purification.

Z-Gln-Trp-OMe (II). To a solution of 17.9 g (0.064 mol) of Z-Gln and 17.8 g (0.07 mol) of Trp-OMe·HCl in 100 mL of DMF at 5 °C was added cold solutions of 15.1 mL (0.07 mol) of diphenylphosphoryl azide in 50 mL of DMF and 19.6 mL (0.014 mol) of Et_3N in 50 mL of DMF. The reaction mixture was stirred for 2.5 h with ice-bath cooling and then overnight at room temperature. Then the reaction mixture was evaporated in vacuo to a syrup, which was dissolved in EtOAc and washed successively with 1 N HCl, saturated $NaHCO_3$, and H_2O. The H_2O wash caused a solid to precipitate. The solid was isolated by filtration and washed with EtOAc and Et_2O to give 23.5 g of product (II), mp 190–191 °C, and recrystallized from MeOH: mp 194–195 °C; $[\alpha]^{25}_D$ +12.4° (*c* 1.0, DMF); UV (MeOH) λ_{max} 289 nm (E' 108.5), 281 (126), 274 (118). Anal. ($C_{25}H_{28}N_4O_6$) C, H, N.

Z-Gln-Trp-NHNH$_2$ (III). To a hot solution of 23.5 g (0.049 mol) of II in 1 L of MeOH and 50 mL of DMF was added 12.5 mL (0.25 mol) of 99% hydrazine hydrate. After 2 days, the reaction mixture was warmed and filtered. The solid hydrazide III was washed with MeOH and Et_2O to yield 20.8 g of product III: mp 252–253 °C; −7.4° (*c* 2.0, DMF). Anal. ($C_{24}H_{28}N_6O_5$) C, H, N.

Z-Gln-Trp-Ser-Tyr-OMe (IV). To a solution of 20.2 g (0.042 mol) of III in 300 mL of DMF and 63.5 mL (0.126 mol) of 1.98 N HCl in THF at −10 °C was added 6.2 mL (0.0465 mol) of isoamyl nitrite. The reaction mixture was stirred at −15 °C for 1 h. After cooling to −30 °C, 24.7 mL (0.176 mol) of Et_3N and

Table I. Biological Activities of LH-RH Analogues

no.	compd	in vitro activity: molar ratio of analogue/LH-RH	in vitro activity: % inhibn of LH-RH-induced LH release	in vitro activity: ED_{50} molar ratio[a]	in vivo activity: unit dose, mg × 6	in vivo activity: % ovulation blockade	in vivo activity: ED_{50}, mg × 6
1	Z-[Ser(Bzl)^1,des-His^2,D-Phe^6,des-Gly^{10}]-LH-RH ethylamide·HCl	10	93	1.3 (5)	5	80	2.5
2	Z-[Ser(Bzl)^1,des-His^2,D-Phe^6,des-Gly^{10}]-LH-RH dimethylamide·HOAc	20	100	5.9 (1)	1	40	>3.0[b]
3	Z-[Ser(Bzl)^1,des-His^2,D-Tyr(Me)^6,des-Gly^{10}]-LH-RH ethylamide·HOAc	20	100		1	100	0.1
4	Z-[Ser(Bzl)^1,des-His^2,Phe(Me$_5$)^6,des-Gly^{10}]-LH-RH ethylamide·HOAc	200	74		1	0	>1.0[b]
5	Z-[Ser(Bzl)^1,des-His^2,D-Phe(Me$_5$)^6,des-Gly^{10}]-LH-RH ethylamide·HOAc	1	90	0.4 (2)	0.3	100	0.055
6	Z-[Ser(Bzl)^1,des-His^2,DL-Phe(Me$_5$)^6,des-Gly^{10}]-LH RH ethylamide·HOAc	20	100	0.2 (3)	1	80	0.325
7	Boc-[Ser(Bzl)^1,des-His^2,D-Phe^6,des-Gly^{10}]-LH-RH ethylamide·HCl	57	91	19 (1)	1	0	>10.0[b]
8	Boc-[Ser(Bzl)^1,des-His^2,des-Gly^{10}]-LH-RH ethylamide·HCl	2 800	40				
9	MeSO$_2$-[Ser(Bzl)^1,des-His^2,D-Tyr(Me)^6,des-Gly^{10}]-LH-RH ethylamide·HOAc	200	75		1	0	>1.0[b]
10	Z-[Gln^1,des-His^2,D-Ala^6,des-Gly^{10}]-LH-RH ethylamide·HCl	1 000	100		1	20	>3.0[b]
11	Z-[Gln^1,des-His^2,D-Pro^6,des-Gly^{10}]-LH-RH ethylamide·HOAc	200	74		3	100	1.0
12	Z-[Gln^1,des-His^2,D-Ser^6,des-Gly^{10}]-LH-RH ethylamide·HOAc	200	50		1	0	>1.0[b]
13	Z-[Gln^1,des-His^2,D-Asn^6,des-Gly^{10}]-LH-RH ethylamide·HOAc	2 000	100	300 (1)	1	20	>1.0[b]
14	Z-[Gln^1,des-His^2,D-Gln^6,des-Gly^{10}] LH-RH ethylamide·HOAc	28	77		1	20	>1.0[b]
15	Z-[Gln^1,des-His^2,D-Met^6,des-Gly^{10}]-LH-RH ethylamide·HCl	200	85		3	60	3.0
16	Z-[Gln^1,des-His^2,D-Arg^6,des-Gly^{10}]-LH-RH ethylamide·HCl	2 800	86	20 (1)	1	0	>1.0[b]
17	Z-[Gln^1,des-His^2,D-Pse^6,des-Gly^{10}]-LH-RH ethylamide·HCl[c]	28	82	12.7 (1)	1	100	0.4
18	Z-[Gln^1,des-His^2,D-Pse^6,des-Gly^{10}]-LH-RH ethylamide·HOAc[c]	5	77	1.7 (1)	0.3	100	0.1
19	Z-[Gln^1,des-His^2,D-Leu^6,des-Gly^{10}]-LH-RH ethylamide·HCl	28	60	22.3 (1)	1	20	>1.0[b]
20	Z-[Gln^1,des-His^2,D-Trp^6,des-Gly^{10}]-LH-RH ethylamide·HCl	100	100	3.2 (5)	1	20	>1.0[b]
21	Z-[Gln^1,des-His^2,D-Phe^6,des-Gly^{10}]-LH-RH ethylamide·HCl	40	70	12.8 (5)	10	0	>10.0[b]
22	Z-[Gln^1,des-His^2,D-Phe^6,des-Gly^{10}]-LH-RH ethylamide·HOAc	100	70	7.7 (11)	5	0	>5.0[b]
23	Z-[Gln(NH-Bzl)^1,des-His^2,des-Gly^{10}]LH-RH ethylamide·HCl	100	88	350 (2)	1	20	>1.0[b]
24	Z-[Gln(NH-Bzl)^1,des-His^2,D-Phe^6,des-Gly^{10}]-LH-RH ethylamide·HCl	10	62	2.7 (6)			
25	Z-[Gln(NH-Bzl)^1,des-His^2,D-Phe^6,des-Gly^{10}]-LH-RH ethylamide·HOAc	20	71	1.3 (1)	1	0	>1.0[b]
26	Z-[Ile^1,des-His^2,D-Trp^6,des-Gly^{10}]-LH-RH ethylamide·HCl	20	100	1.5 (2)	5	0	>5.0[b]
27	Z-[Leu^1,des-His^2,D-Phe^6,des-Gly^{10}]-LH-RH ethylamide·HCl	20	100	3.0 (1)	1	0	>1.0[b]
28	Z-[Leu^1,des-His^2,des-Gly^{10}]-LH-RH ethylamide·HCl	1 000	81		1	20	>1.0[b]
29	Z-[D-Leu^1,des-His^2,des-Gly^{10}]-LH-RH ethylamide·HCl	2 800	51				
30	[des<Glu^1,des-His^2]-LH-RH·HCl[d,e]	10 000	15				
31	Z[des<Glu^1,Pro-OH^9,des-Gly^{10}]-LH-RH·HCl	4 000	10				
32	PhAc-[des<Glu^1,des-His^2]-LH-RH·HCl[f]	1 000	51				
33	CHAc-[des<Glu^1,des-His^2]-LH-RH·HCl[g]	1 000	48				
34	Z-[Ser(Bzl)^1,des-His^2]-LH-RH·HCl	2 800	100	100 (1)	3	20	>3.0[b]
35	Z-[Ser(Bzl)^1,des-His^2,D-Phe^6]-LH-RH·HCl	10	99	1.5 (2)	3	40	>3.0[b]
36	Z-(*p*-MeO)[Ser(Bzl)^1,des-His^2,D-Phe^6]-LH-RH·HCl	10	100	1.3 (1)	1	0	>1.0[b]
37	Boc-[Ser(Bzl)^1,des-His^2,D-Trp^6,Gly-OCH$_3^{10}$]-LH-RH·HCl	20	99	1.7 (3)	1	40	>1.0[b]
38	Boc-[Ser(Bzl)^1,des-His^2,D-Trp^6]-LH-RH·HCl	1.7	64	1.3 (4)	0.1	100	0.055
39	Boc-[Ser(Bzl)^1,des-His^2,D-Trp^6]-LH-RH·HOAc	20	71	2.0 (4)	0.1	100	0.055
40	Boc-[Ser(Bzl)^1,des-His^2,D-Ser^6]-LH-RH·HCl	200	68		1	0	>1.0[b]
41	Z-[Tyr(Bzl)^1,des-His^2]-LH-RH·HCl	2 800	74		1	20	>1.0[b]
42	Z-[Tyr(Bzl)^1,des-His^2,D-Leu^6]-LH-RH·HCl	20	57	7.0 (1)	1	0	>1.0[b]
43	Z-[Tyr^1,des-His^2]-LH-RH·HCl	1 000	16				
44	Z-[D-Phe^1,des-His^2]-LH-RH·HCl	2 800	76		1	0	>1.0[b]
45	Z-[D-Phe^1,des-His^2,D-Tyr^6]-LH-RH·HCl	200	83		1	0	>1.0[b]
46	Z-[Gln^1,des-His^2]-LH-RH·HCl	4 000	33				
47	Z-[Gln^1,des-His^2,D-Trp^6]-LH-RH·HCl	200	87	8.0 (2)	1	0	>1.0[b]
48	Z-[Gln^1,des-His^2,D-Phe^6]-LH-RH·HCl	200	83				

49	Z-[Gln^1,des-His^2,D-Phe^6]-LH-RH·HOAc	200	93	22 (1)	1	20	>3.0[b]
50	Z-[Gln^1,des-His^2,Gly-NH-Et^{10}]-LH-RH·HCl	1 000	40				
51	Boc-[Gln^1,des-His^2]-LH-RH·HCl	1 000	15				
52	HCO-[Gln^1,des-His^2]-LH-RH·HCl	1 000	0				
53	Z-[*i*-Gln^1,des-His^2]-LH-RH·HCl	1 000	59				
54	Z-[Gln(NH-Bzl)^1,des-His^2]-LH-RH·HCl	100	96	360 (1)	1	40	>1.0[b]
55	Z-[Asn^1,des-His^2]-LH-RH·HCl	1 000	35				
56	Z-[Leu^1,des-His^2]-LH-RH·HCl	1 000	95	750 (1)	1	40	>3.0[b]
57	Z-[Leu^1,des-His^2,D-Asn^6]-LH-RH·HCl	100	42	134 (1)	1	0	>1.0[b]
58	Z-[Ile^1,des-His^2]-LH-RH·HCl	1 000	70		1	20	>1.0[b]
59	Z-[Ile^1,des-His^2,D-Trp^6]-LH-RH·HOAc	20	95	1.8 (1)	1	20	>1.0[b]
60	Z-[Val^1,des-His^2]-LH-RH·HCl	1 000	48				
61	HCO-[Pro^1,des-His^2]-LH-RH·HCl	10 000	53				
62	[des-His^2]-LH-RH·HOAc[d,h]	10 000	60				
63	[<Glu(Bzl)^1,des-His^2]-LH-RH·HCl	10 000	59				
64	[<Glu(Tos)^1,des-His^2]-LH-RH·HCl	1 000	26				
65	[des<Glu^1]-LH-RH·HCl[e]	10 000	42				
66	Z-[des<Glu^1,Gly-OH^{10}]-LH-RH·HCl	4 000	17				
67	Z-[des<Glu^1]-LH-RH·HCl[i]	10 000	58				
68	Z-[des<Glu^1,des-His^2]-LH-RH·HCl[i,j]	10 000	68		1	20	>1.0[b]
69	[D-Phe^2,D-Phe^6]-LH-RH·HCl[k]	500	98	76 (2)	0.3	100	0.1

[a] Number of determinations are in parentheses. [b] Highest dose used that was ineffective in blocking ovulation. [c] *threo*-D-Phenylserine, D-Pse. [d] J. Rivier, M. Amoss, C. Rivier, and W. Vale, *J. Med. Chem.*, **17**, 230 (1974). [e] T. Kaneko, S. Saito, H. Oka, T. Oda, and N. Yanaihara, *Metabolism*, **22**, 77 (1973). [f] Phenylacetyl, PhAc. [g] Cyclohexylaminocarbonyl, CHAc. [h] M. W. Monahan, J. Rivier, W. Vale, R. Guillemin, and R. Burgus, *Biochem. Biophys. Res. Commun.*, **47**, 551 (1972). [i] N. Yanaihara, C. Yanaihara, M. Sakagami, K. Tsuji, T. Hashimoto, T. Kaneko, H. Oka, A. V. Schally, A. Arimura, and T. W. Redding, *J. Med. Chem.*, **16**, 373 (1973). [j] Reference 30[b] (reported as Tos-OH salt). [k] Reference 24.

13.6 g (0.042 mol) of I in 20 mL of DMF were added. The reaction mixture was stirred for 1 h while the temperature came to 0 °C and then was refrigerated at 0 °C overnight. The reaction mixture was then filtered and the filtrate concentrated in vacuo. The residue was triturated with MeOH and the solid filtered. Recrystallization from MeOH yielded 17.0 g of IV: mp 248–249 °C; $[\alpha]^{25}_D$ −2.9° (*c* 1.0, DMF); UV (MeOH) λ_{max} 289.5 nm (*E*′ 75.6), 280 (100). Anal. ($C_{37}H_{42}N_6O_{10}$) C, H, N.

Z-Gln-Trp-Ser-Tyr-NHNH$_2$ (V). IV, 58.3 g (0.08 mol), was dissolved in 500 mL of DMF with heating. Hydrazine hydrate (99%, 20 mL, 0.4 mol) was added to the DMF filtrate. After 4 days, the solid was collected by filtration and washed with hot MeOH to give 36.4 g of the hydrazide product V, mp 251–253 °C. The filtrate was evaporated in vacuo and the residue triturated with hot MeOH to obtain an additional 15.9 g of V: $[\alpha]^{25}_D$ −16.1° (*c* 1.0, DMF); UV (MeOH) λ_{max} 289.5 nm (*E*′ 75), 280 (98.6). Anal. ($C_{36}H_{42}N_8O_9$) C, H, N.

Z-Leu-Arg-Pro-NHEt·HCl (VI). A solution of 6.23 g (0.0186 mol) of Arg-Pro-NHEt-HCl[29] and 7.2 g (0.0186 mol) of Z-Leu-*p*-nitrophenyl ester in 30 mL of DMF was allowed to stand for 4 days, warmed to 50 °C for 0.5 h, and evaporated at reduced pressure and 40 °C to yield an oil. The product was purified by chromatography on a Brinkmann silica gel Q-60 column developed with a $CHCl_3$–MeOH solvent mixture containing increasing percentages (to 20%) of MeOH. Fraction selection was made on the basis of analytical TLC, and product VI was obtained by precipitating from MeOH solution with Et_2O. The solid was dried at 40 °C in vacuo: yield 6.74 g; $[\alpha]^{25}_D$ −68.4° (*c* 1, MeOH); UV (MeOH) λ_{max} 257 nm (*E*′ 3.5). Anal. ($C_{27}H_{43}N_7O_5 \cdot 1.125HCl \cdot 0.5CH_3OH$) C, H, N, Cl.

Z-D-Phe-Leu-Arg-Pro-NHEt·HCl (VII). A solution of 1.8 g (0.003 mol) of VI in 100 mL of MeOH was treated with 400 mg of 20% Pd/C, and the mixture was stirred under a H_2 atmosphere for 4 h. Disappearance of the starting material was determined by TLC of samples of the solution. The catalyst was removed by filtration and the filtrate was evaporated to dryness. The product was mixed with 900 mg (0.003 mol) of Z-D-Phe and 400 mg (0.003 mol) of HOBt in 40 mL of DMF, cooled, and then treated with 700 mg (0.0034 mol) of DCC. The reaction was stirred at 23 °C for 3 days and filtered, and the filtrate was evaporated at 50 °C under reduced pressure. The residue was obtained solid by repeated precipitation from MeOH by Et_2O and by EtOAc and Et_2O: yield 1.95 g; liquifies at 130–135 °C; $[\alpha]^{23}_D$ −69° (*c* 1.02, MeOH); UV (MeOH) λ_{max} 258 nm (*E*′ 5.8). Anal. ($C_{36}H_{52}N_8O_6 \cdot 1.125HCl \cdot 0.5CH_3OH$) C, H, N, Cl.

Z-Gln-Trp-Ser-Tyr-D-Phe-Leu-Arg-Pro-NHEt·HCl (21; Table I). A solution of 4.15 g (0.0057 mol) of VII in 100 mL of MeOH was stirred with 500 mg of 20% Pd/C under a H_2 atmosphere at room temperature for 3 h. The disappearance of the starting material was followed by TLC. The catalyst was removed by filtration and the filtrate evaporated at 40 °C under reduced pressure. The residue was dissolved in 30 mL of DMF and used in the azide coupling as follows. A suspension of 4.16 g (0.0057 mol) of V in 90 mL of DMF was dissolved by the addition of 16.4 mL of 2.08 N HCl (0.0342 mol, 6 equiv) in THF at 10 °C with stirring. The solution was cooled to −25 °C and treated with 0.88 mL (0.0057 mol) of isopentyl nitrite for 3 h at −10 to −20 °C and then with 4.8 mL (0.0342 mol) of Et_3N at −40 °C. The azide solution thus obtained was treated with the DMF solution of D-Phe-Leu-Arg-Pro-NHEt·HCl using 10 mL of DMF to rinse the flask. The reaction was then stirred at −10 to 5 °C for several hours and for 2 days at room temperature. The mixture was filtered and the filtrate evaporated at 50 °C under reduced pressure. Compound **21** (Table I) was obtained as a solid by precipitation from MeOH with EtOAc and Et_2O and then by recrystallization from MeOH.

Z-Pro-Gly-NH$_2$ (VIII).[30a,b] Z-Pro, 74.3 g (0.297 mol), dissolved in 600 mL of CH_2Cl_2 was cooled to −10 °C and treated with 41.2 mL (0.297 mol) of Et_3N and then with 32.4 g (0.297 mol) of ethyl chloroformate with stirring. After 20 min at −10 °C, a solution of 42 g (0.3 mol) of glycine ethyl ester hydrochloride and 41.2 g (0.297 mol) of Et_3N in 250 mL of CH_2Cl_2 was added at −10 °C. The reaction was stirred at −10 °C for 1 h, at −5 °C for 1 h, at 5 °C for 1 h, and at 25 °C for 20 h. Water (100 mL) was added, and the CH_2Cl_2 layer was separated; washed twice with 5% $NaHCO_3$ solution (100 mL each), with H_2O (100 mL), twice with

Table II. Chemical and Physical Properties of LH-RH Analogues

no.	formula	microanal	$[\alpha]^{23}_D$, deg (*c*, solvent)	UV assay found/theory, %	
				Tyr	Trp
1	$C_{69}H_{87}N_{13}O_{13}\cdot 1.5HCl\cdot 2H_2O$	C, H, N, Cl	−26 (1.085, DMF), −60 (*c* 1.0, MeOH)	1.06	1.02
2	$C_{69}H_{87}N_{13}O_{13}\cdot C_2H_4O_2\cdot H_2O$	C, H, N	−34.2 (1.02, DMF)	1.02	1.02
3	$C_{70}H_{89}N_{13}O_{14}\cdot C_2H_4O_2\cdot 2H_2O$	C, H, N	−29.4 (1.03, DMF)	0.97	1.00
4	$C_{74}H_{97}N_{13}O_{13}\cdot C_2H_4O_2\cdot 2H_2O$	C, H, N	−32 (1.0, DMF)	1.01	1.02
5	$C_{74}H_{97}N_{13}O_{13}\cdot C_2H_4O_2\cdot H_2O$	C, H, N	−35.2 (1.0, DMF)	1.02	1.00
6	$C_{74}H_{97}N_{13}O_{13}\cdot C_2H_4O_2\cdot 2H_2O$	C, H, N	−37.2 (1.0, MeOH)	1.05	1.04
7	$C_{68}H_{89}N_{13}O_{13}\cdot 1.5HCl\cdot 3H_2O$	C, H, N, Cl	−58 (1.02, MeOH)	1.07	1.05
8	$C_{59}H_{83}N_{13}O_{13}\cdot 1.33HCl\cdot 2H_2O$	C, H, N, Cl	−43.2 (1.0, MeOH)	1.03	1.01
9	$C_{63}H_{85}N_{13}O_{14}S\cdot C_2H_4O_2\cdot 2H_2O$	C, H, N	−29.8 (1.05, DMF)	1.02	0.99
10	$C_{58}H_{80}N_{14}O_{13}\cdot 2C_2H_4O_2\cdot 5H_2O$	C; H,[a] N[b]	−51 (0.99, MeOH)	1.05	1.07
11	$C_{60}H_{82}N_{14}O_{13}\cdot 3.5C_2H_4O_2\cdot 2.5H_2O$	C, H, N	−43.5 (1.025, MeOH)	1.01	1.01
12	$C_{58}H_{80}N_{14}O_{14}\cdot 3C_2H_4O_2\cdot 4H_2O$	C, H, N	−48 (1.0, MeOH)	0.96	0.95
13	$C_{59}H_{81}N_{15}O_{14}\cdot 2C_2H_4O_2\cdot 3H_2O$	C, H, N	−32.8 (1.025, DMF)	1.00	1.02
14	$C_{60}H_{83}N_{15}O_{14}\cdot C_2H_4O_2\cdot 3H_2O$	C, H, N	−36.6 (1.021, DMF)	0.91	0.86
15	$C_{60}H_{84}N_{14}O_{13}S\cdot HCl\cdot 2H_2O$	C, H, N		1.00	1.01
16	$C_{61}H_{87}N_{17}O_{13}\cdot 3.5HCl\cdot 5H_2O$	C, H, N, Cl	−40.0 (1.0, MeOH)	0.98	0.98
17	$C_{64}H_{84}N_{14}O_{14}\cdot 1.7HCl\cdot 3H_2O$	C, N, Cl; H[c]	−17.2 (1.035, DMF)	1.01	0.99
18	$C_{64}H_{84}N_{14}O_{14}\cdot C_2H_4O_2\cdot 4H_2O$	C, H, N	−64.5 (1.05, MeOH)	0.97	0.96
19	$C_{61}H_{86}N_{14}O_{13}\cdot 2HCl\cdot 3H_2O$	C, H, N, Cl	−31.4 (1.029, DMF)	1.07	1.07
20	$C_{66}H_{85}N_{15}O_{13}\cdot 1.25HCl\cdot 2CH_3OH$	C, H, N, Cl	−18.2 (1.02, DMF)	1.12	1.03
21	$C_{64}H_{84}N_{14}O_{13}\cdot 1.25HCl\cdot 2CH_3OH$	C, H, N, Cl	−27.4 (1.03, DMF), −71 (1.004, MeOH)	1.04	1.06
22	$C_{64}H_{84}N_{14}O_{13}\cdot C_2H_4O_2\cdot 3H_2O$	C, H, N	−27 (1.0, DMF), −65.5 (1.03, MeOH)	1.01	1.01
23	$C_{64}H_{84}N_{14}O_{13}\cdot HCl\cdot 3H_2O$	C, H, N, Cl	−48 (0.94, MeOH)	0.99	1.00
24	$C_{71}H_{90}N_{14}O_{13}\cdot HCl\cdot 2.5H_2O$	C, H, Cl; N[d]	−22.8 (1.02, MeOH)	1.03	1.01
25	$C_{71}H_{90}N_{14}O_{13}\cdot C_2H_4O_2\cdot 3H_2O$	C, H, N	−30 (1.015, DMF)	0.92	1.00
26	$C_{67}H_{88}N_{14}O_{12}\cdot 1.5HCl\cdot 3H_2O$	C, H, N, Cl	−19.4 (1.01, DMF)	1.06	1.02
27	$C_{65}H_{87}N_{13}O_{12}\cdot 1.25HCl\cdot 3H_2O$	C, H, N, Cl	−70.5 (1.01, DMF)	1.02	1.06
28	$C_{58}H_{81}N_{13}O_{12}\cdot HCl\cdot 2H_2O$	C, H, N, Cl	−55 (1.03, MeOH)	1.03	1.00
29	$C_{58}H_{81}N_{13}O_{12}\cdot HCl\cdot 4H_2O$	C, H, N, Cl	−24.6 (1.01, DMF)	1.03	1.02
30	$C_{44}H_{63}N_{13}O_{10}\cdot 2.5HCl\cdot 4H_2O$	C, H, N, Cl	−31.3 (1.04, MeOH)	1.13	0.98
31	$C_{56}H_{72}N_{14}O_{13}\cdot 2.3HCl\cdot 4H_2O$	C, H, N, Cl	−36.0 (1.04, DMF)	1.05	1.02
32	$C_{52}H_{69}N_{13}O_{11}\cdot 1.7HCl\cdot 3H_2O$	C, H, N, Cl	−34 (1.0, MeOH)	0.98	0.98
33	$C_{51}H_{74}N_{14}O_{11}\cdot 1.6HCl\cdot 5.5H_2O$	C, H, N, Cl	−29 4 (1.0, MeOH)	1.04	0.96
34	$C_{62}H_{80}N_{14}O_{14}\cdot 2HCl\cdot 3H_2O$	C, H, N, Cl	−30.7 (1.025, MeOH)	1.07	1.01
35	$C_{69}H_{86}N_{14}O_{14}\cdot 1.5HCl\cdot 4H_2O$	C, H, N, Cl	−23.4 (1.0, DMF)	1.03	1.00
36	$C_{70}H_{88}N_{14}O_{15}\cdot 1.5HCl\cdot 3.5H_2O$	C, H, N, Cl	−23.4 (1.05, DMF)	1.06	0.99
37	$C_{69}H_{90}N_{14}O_{15}\cdot 1.5HCl\cdot 4H_2O$	C, H, N, Cl	−42.2 (1.01, MeOH)	1.01	0.96
38	$C_{68}H_{89}N_{15}O_{14}\cdot 1.5HCl\cdot 4H_2O$	C, H, N, Cl	−41.8 (1.0, MeOH)	1.05	0.96
39	$C_{68}H_{89}N_{15}O_{14}\cdot C_2H_4O_2\cdot 3H_2O$	C, H, N	−50 (1.0, MeOH)	1.04	1.02
40	$C_{60}H_{85}N_{14}O_{15}\cdot 1.5HCl\cdot 4H_2O$	C, N, Cl; H[e]	−27.2 (1.038, DMF)	1.08	1.06
41	$C_{68}H_{84}N_{14}O_{14}\cdot 1.9HCl\cdot 2.5H_2O$	C, H, N, Cl	−31.7 (1.0, DMF)	1.01	0.94
42	$C_{72}H_{92}N_{14}O_{14}\cdot 1.25HCl\cdot 4H_2O$	C, H, N, Cl	−33.2 (1.04, DMF)	1.07	1.02
43	$C_{61}H_{78}N_{14}O_{14}\cdot 1.75HCl\cdot 2H_2O$	C, H, N, Cl	−35 (1.0, MeOH)	1.02	1.01
44	$C_{61}H_{78}N_{14}O_{13}\cdot 1.8HCl\cdot 3H_2O$	C, H, N, Cl	−22.2 (1.04, MeOH)	1.07	1.00
45	$C_{68}H_{84}N_{14}O_{14}\cdot 2HCl\cdot 3H_2O$	C, H, N, Cl	−29 (1.035, MeOH), −17.5 (1.0, DMF)	0 98	0.99
46	$C_{57}H_{77}N_{15}O_{14}\cdot 3HCl\cdot 4H_2O$	C, H, N, Cl	−41 (1.03, MeOH)	1.03	0.92
47	$C_{66}H_{84}N_{16}O_{14}\cdot 1.5HCl\cdot 3H_2O$	C, N, Cl; H[f]	−59 (1.0, MeOH)	0.98	0.95
48	$C_{64}H_{83}N_{15}O_{14}\cdot 1.25HCl\cdot 2H_2O$	C, H, N, Cl	−60.5 (1.0, MeOH)	1.03	1.02
49	$C_{64}H_{83}N_{15}O_{14}\cdot C_2H_4O_2\cdot 2.5H_2O$	C, H, N	−63 (1.007, MeOH)	1.01	1.02
50	$C_{59}H_{81}N_{15}O_{14}\cdot 1.6HCl\cdot 3H_2O$	C, H, N, Cl	−39 (1.035, MeOH)	1.01	0.99
51	$C_{54}H_{79}N_{15}O_{14}\cdot 2HCl\cdot 3H_2O$	C, H, N, Cl	−34 (1.027, MeOH)	1.01	1.00
52	$C_{50}H_{71}N_{15}O_{14}\cdot 1.5HCl\cdot 2H_2O$	C, H, N, Cl	−41 (1.01, MeOH)	1.03	0.99
53	$C_{57}H_{77}N_{15}O_{14}\cdot 1.7HCl\cdot 3H_2O$	C, H, N, Cl	−32.6 (0.977, MeOH)	1.00	0.95
54	$C_{64}H_{83}N_{15}O_{14}\cdot 1.5HCl\cdot 3H_2O$	C, H, N, Cl	−35.9 (0.907, MeOH)	1.02	0.92
55	$C_{56}H_{75}N_{15}O_{14}\cdot 2HCl\cdot 3H_2O$	C, H, N, Cl	−38 (1.0, MeOH)	1 01	0.99
56	$C_{58}H_{80}N_{14}O_{13}\cdot 1.5HCl\cdot 3H_2O$	C, H, N, Cl	−42 (1.02, MeOH)	1.06	1.06
57	$C_{60}H_{83}N_{15}O_{14}\cdot 2HCl\cdot 5H_2O$	C, H, N, Cl	−36.4 (1.0, DMF)	1.04	1.06
58	$C_{58}H_{80}N_{14}O_{13}\cdot 1.5HCl\cdot 2.5H_2O$	C, H, N, Cl	−43 (1.0, MeOH)	1.02	1.03
59	$C_{67}H_{87}N_{15}O_{13}\cdot C_2H_4O_2\cdot 5H_2O$	C, H, N	−20.4 (1.0, DMF)	0.94	0.86
60	$C_{57}H_{78}N_{14}O_{13}\cdot 1.5HCl\cdot 3H_2O$	C, H, N, Cl	−42.8 (1.0, MeOH)	1.00	1.00
61	$C_{50}H_{70}N_{14}O_{12}\cdot 2.2HCl\cdot 4H_2O$	C, H, N, Cl	−46.2 (1.01, MeOH)	1.05	1.02
62	$C_{49}H_{68}N_{14}O_{12}\cdot C_2H_4O_2\cdot 6H_2O$	C, H, N	−40.2 (1.03, MeOH)	1.00	1.01
63	$C_{56}H_{74}N_{14}O_{12}\cdot 2.25HCl\cdot 5H_2O$	C, H, N, Cl	−23.8 (0.975, MeOH)	1.06	0.97
64	$C_{56}H_{74}N_{14}O_{14}S\cdot 1.8HCl\cdot 4H_2O$	C, H, N, Cl	−36.5 (1.0, MeOH)	0.96	1.02
65	$C_{50}H_{70}N_{16}O_{11}\cdot 2.7HCl\cdot 5H_2O$	C, H, N, Cl	−28.5 (1.0, MeOH)	0.93	0.87
66	$C_{58}H_{75}N_{15}O_{14}\cdot 2.9HCl\cdot 4H_2O$	C, H, N, Cl	−41.5 (1.04, MeOH)	1.03	0.96
67	$C_{58}H_{76}N_{16}O_{13}\cdot 3.1HCl\cdot 7H_2O$	C, H, N, Cl	−24.2 (1.03, MeOH)	1.03	0.95
68	$C_{52}H_{69}N_{13}O_{12}\cdot 1.5HCl\cdot 3.5H_2O$	C, H, N, Cl	−34.4 (1.02, MeOH)	1.03	1.00
69	$C_{65}H_{83}N_{15}O_{13}\cdot HCl\cdot 3.5H_2O$	C, H, N, Cl	−27 (1.025, DMF)	1.00	1.00

[a] H: calcd, 7.10; found, 6.31. [b] N: calcd, 14.09; found, 13.59. [c] H: calcd, 6.65; found, 6.07. [d] N: calcd, 13.72; found, 13.23. [e] H: calcd, 6.95; found, 6.41. [f] H: calcd, 6.43; found, 6.87.

dilute HCl (30 mL each), and with H_2O (100 mL); and dried over $MgSO_4$. The solution was filtered and evaporated at 50 °C under reduced pressure to an oil. The oil was dissolved in 100 mL of MeOH, added at 0–10 °C to 500 mL of MeOH saturated with NH_3 at 10 °C and kept at 0 °C for 20 h; at 25 °C for 6 h; and again at 0 °C for 20 h. The solution was evaporated under reduced pressure to a small volume where crystallization was started. While the solid was still wet with MeOH, 100 mL of THF was added and the mixture warmed gently to partially dissolve the solid. The mixture was cooled in an ice bath and after 4 h was filtered and the solid air-dried to give 59 g of VIII: mp 140–142 °C; $[\alpha]^{23}_D$ −32.8° (c 2.0, DMF). Anal. ($C_{15}H_{19}N_3O_4$) C, H, N. A further amount, 15.2 g, mp 139–143 °C, was obtained by concentration of the mother liquors.

Z-Arg-Pro-Gly-NH_2·HCl (IX). A solution of 11.9 g of VIII (0.039 mol) in 120 mL of MeOH was stirred vigorously under 1 atm of H_2 with 1.0 g of 10% Pd/C until TLC showed that removal of the benzyloxycarbonyl group was complete (about 1 h). The catalyst was filtered and the solvent evaporated under reduced pressure to a foam, which was dried for 2 h in vacuo and used immediately.

A solution of Z-Arg (11.3 g, 0.037 mol) dissolved in 20 mL of DMF was cooled to 5 °C and 16.20 mL (0.037 mol) of 2.27 N HCl in THF added. Then, 5.43 g (0.04 mol) of HOBt was added and, finally, 8.29 g (0.037 mol) of DCC. An ice-cold solution of Pro-Gly-NH_2 (above) in 20 mL of DMF was next added, together with 15 mL of DMF washings. The reaction mixture was chilled with an ice bath and stirred for 4 h; then the ice was allowed to melt spontaneously while stirring was continued overnight and then for 24 h longer at room temperature. Solids were filtered and washed with two 30-mL portions of DMF. The combined filtrate and washings containing the product were evaporated, and the residue was suspended in 100 mL of H_2O and stirred in an ice bath for 1 h. Again, solid byproducts were filtered and washed with 50 mL of H_2O, and the combined aqueous filtrate and washing were extracted four times with EtOAc. The aqueous phase containing product IX was lyophilized. The resultant glass was dissolved in 80 mL of MeOH and triturated with EtOAc. The product crystallized after 4 h at room temperature (seeding is beneficial) and was then stored in the refrigerator overnight before filtration: yield 11.9 g; mp 145–146 °C; $[\alpha]^{25}_D$ −34.4° (c 1.0 MeOH). Anal. ($C_{21}H_{31}N_7O_5$·HCl) C, H, N, Cl.

Z-Leu-Arg-Pro-Gly-NH_2·HCl (X). A solution of IX (21.2 g, 0.0425 mol) in 200 mL of MeOH was stirred under H_2 with 1.0 g of 20% Pd/C for 4 h and allowed to stand overnight. The solution was filtered and the filtrate evaporated at 40 °C under reduced pressure to a white foam. This material was combined with 17 g (0.044 mol) of Z-Leu-*p*-nitrophenyl ester and 85 mL of DMF. The reaction was allowed to stand at 25 °C for 48 h, warmed to 50 °C for 10 min, and let stand for 6 h at 25 °C. The solution was poured slowly into 800 mL of stirred dry Et_2O. The sticky solid was separated by decanting and dissolved in 50 mL of MeOH with warming, and the solution was poured into 500 mL of stirred Et_2O. The solid was seprated and dried at 40 °C under reduced pressure. It was dissolved in 50 mL of MeOH and 450 mL of $CHCl_3$, chromatographed over a column of 170 g of silica gel (prepared in 10% MeOH in $CHCl_3$), and eluted with 10–20% MeOH in $CHCl_3$. The desired fractions, as shown by TLC, were combined and evaporated to dryness at 40 °C under reduced pressure, the residue was dissolved in 50 mL of MeOH and 25 mL of EtOAc, and the solution was dropped slowly into 350 mL of stirred dry Et_2O. The solid was separated and dried at 40 °C under reduced pressure to give X: yield 11.6 g; $[\alpha]^{23}_D$ −51.6° (c 1.0, MeOH); UV (MeOH) λ_{max} 257 nm (E' 3.24). Anal. ($C_{27}H_{42}N_8O_6$·HCl·2H_2O) C, H, N, Cl.

Z-D-Phe-Leu-Arg-Pro-Gly-NH_2·HCl (XI). A solution of 6.43 g (0.01 mol) of X in 200 mL of MeOH was stirred under H_2 with 250 mg of 20% Pd/C for 3 h, the catalyst was removed by filtration, and the filtrate was evaporated to dryness at 45 °C under reduced pressure. The dried residue, the hydrochloride of Leu-Arg-Pro-Gly-NH_2,[30a,31] was treated with 4.3 g (0.01 mol) of Z-D-Phe-4-nitrophenyl ester[32] and 30 mL of DMF. The mixture was stirred to solution and allowed to stand at 25 °C for 48 h. The solution was filtered and evaporated to dryness at 40 °C under reduced pressure. The residue was dissolved in MeOH (40 mL) and EtOAc (30 mL) and dropped into 300 mL of stirred dry Et_2O. The granular solid which precipitated was separated by decanting and the precipitation repeated. The solid was separated and dried at 45 °C under reduced pressure to give 6.5 g of XI: $[\alpha]^{23}_D$ −54.6° (c 1.0, MeOH); UV (MeOH) λ_{max} 257 nm (E' 5.8). Anal. ($C_{36}H_{51}N_9O_7$·HCl·1.5H_2O) C, H, N, Cl.

Z-Gln-Trp-Ser-Tyr-D-Phe-Leu-Arg-Pro-Gly-NH_2·HCl (48; Table I). A solution of 3.9 g (0.005 mol) of XI in 100 mL of MeOH was stirred under H_2 with 500 mg of 20% Pd/C at slightly above atmospheric pressure for 4 h. The solution was filtered and the filtrate evaporated to dryness at 40 °C under reduced pressure to a white foam, which was used as such in the following coupling reaction. A solution of 3.68 g (0.005 mol) of V in 90 mL of DMF was cooled to 0 °C and treated with 8.8 mL of 3.4 N HCl in THF (0.03 mol) with stirring. At 10 °C, the hydrazide was completely dissolved in 0.5 h, the solution was cooled to −20 °C, and 0.84 mL (0.0055 mol) of isopentyl nitrite was added. The reaction was stirred at −40 to 15 °C for 3 h and 4.2 mL (0.03 mol) of Et_3N was added, followed by a solution of the above D-Phe-Leu-Arg-Pro-Gly-NH_2·HCl in 30 mL of DMF. The reaction was stirred at −20 °C for 2 h, allowed to warm to 20 °C in 1 h, and stirred for 20 h. The solution was filtered and the filtrate evaporated at 45 °C under reduced pressure. The residue was dissolved in 50 mL of MeOH and the solution added dropwise to 100 mL of EtOAc while stirring. The tan solid which separated was filtered and dissolved in 30 mL of warm MeOH, and 20 mL of EtOAc and 50 mL of $CHCl_3$ were added slowly to the warm solution. The solution was cooled in ice for 1 h, and the solid was filtered, washed with EtOAc, and dried at 40 °C under reduced pressure; 6.3 g was collected. A part of this product (600 mg) was stirred with 10 mL of warm MeOH, and the solution was cooled and filtered. The solid which separated was washed with 5 mL of MeOH. The filtrate (15 mL) was diluted with 15 mL of $CHCl_3$ and chromatographed over a column (1.2 × 12 cm) of 6 g of silica gel (prepared in $CHCl_3$), eluting the column with increasing concentrations of MeOH (5–20%) in $CHCl_3$. The fractions containing the desired product, as shown by TLC, were combined and evaporated to dryness at 40 °C under reduced pressure, and the residue was dissolved in 5 mL of MeOH and allowed to stand and slowly evaporate. The solid which separated was filtered, washed with ether, and dried at 50 °C under reduced pressure to give 120 mg of **48**.

The remainder of the product, 5.7 g, was stirred in 25 mL of MeOH at 50 °C and allowed to cool and stand at 20 °C, and the solid was filtered and washed with a little cold MeOH. This process was repeated with 30 mL of MeOH, and the solid was separated by filtration, washed with cold MeOH and dry Et_2O, and dried at 35 °C under reduced pressure; 3.7 g was collected. The solid was dissolved in 300 mL of hot MeOH with stirring, the solution was filtered, the filtrate was put over a column (2.4 × 25 cm) of 50 g of Dowex 1 × 2 resin (acetate form), and the product was eluted with MeOH. Fractions containing the desired product as shown by TLC were combined and filtered. The filtrate was concentrated to 75 mL by a stream of warm, filtered air and the solution allowed to stand overnight. Solvent was pipetted from the gel which precipitated, and the gel was washed with MeOH (5 mL) twice, pipetting off the solvent. The gel was stirred with dry Et_2O (150 mL) for 1 h and filtered, and the solid was washed with Et_2O and dried at 50 °C under reduced pressure to give 3.24 g of **49**.

Z-Trp-Ser-Tyr-OCH_3 (XII). To an ice-cold solution of 5.76 g (0.018 mol) of I in 60 mL of DMF were added 2.56 mL (0.018 mol) of Et_3N and then, in succession, 6.1 g (0.018 mol) of Z-Trp, 2.68 g (0.0199 mol) of HOBT, and 4.09 g (0.0198 mol) of DCC. The reaction mixture was stirred for 2 h with ice-bath cooling. Stirring was continued overnight while the ice melted spontaneously; then the reaction mixture was stirred at room temperature for 24 h longer and finally filtered. The filtrate and DMF wash were evaporated to an oil, which was dissolved in 200 mL of EtOAc and washed with 1 N HCl, 5% $NaHCO_3$, and H_2O (all washes were saturated with NaCl). The EtOAc was dried over $MgSO_4$, evaporated to ca. 100 mL, and chilled in the refrigerator overnight, and then the gelatinous precipitate was isolated by filtration. The solid was suspended in Et_2O and stirred for 2 h, then filtered, and dried: yield 9.7 g; mp 167–170 °C; $[\alpha]^{23}_D$ −5.8° (c 1, DMF); UV (MeOH) λ_{max} 289.5 nm (E' 89.6), 280 (118). Anal. ($C_{32}H_{34}N_4O_8$) C, H, N.

Z-Trp-Ser-Tyr-$NHNH_2$ (XIII). To a solution of 4.5 g (0.0075 mol) of XII in 50 mL of MeOH was added 4.5 mL (0.09 mol) of 99% hydrazine hydrate. The reaction mixture was allowed to stand at room temperature for 48 h, during which time the hydrazide precipitated. The product XIII was filtered, washed with MeOH, suspended in Et_2O, stirred for 2 h, and finally filtered and dried: yield 4.18 g; mp 226–229 °C; $[\alpha]^{23}_D$ −15.6° (*c* 1, DMF); UV (MeOH) λ_{max} 289.5 nm (*E'* 92.6) 280 (122). Anal. ($C_{31}H_{34}N_6O_7 \cdot 0.5H_2O$) C, H, N.

Z-D-Phe-Leu-OMe (XIV). To a solution of 6.14 g (0.021 mol) of Z-D-Phe and 4.09 g (0.021 mol) of Leu-OMe[33] in 75 mL of DMF at 5 °C was added 2.77 mL (0.021 mol) of Et_3N, 3.65 g (0.023 mol) of HOBt, and finally 4.6 g (0.022 mol) of DCC. The reaction mixture was stirred for 2 h in an ice bath and then stirred and allowed to come to room temperature overnight. After stirring for an additional 24 h, the solid was filtered and the filtrate containing the product was evaporated in vacuo. The resultant residue was dissolved in EtOAc and washed in succession with 0.5 N HCl, 5% $NaHCO_3$, and H_2O saturated with NaCl. The EtOAc solution was dried over $MgSO_4$ and evaporated. The product XIV was crystallized by dissolving in 50 mL of EtOAc and adding low-boiling petroleum ether to turbidity: yield 7.5 g; mp 124–125 °C; $[\alpha]^{25}_D$ −20.3° (*c* 1.0, MeOH). Anal. ($C_{24}H_{30}N_2O_5$) C, H, N.

Z-Trp-Ser-Tyr-D-Phe-Leu-$OCH_3 \cdot CH_3OH$ (XV). A solution of 6.5 g (0.015 mol) of XIV in 85 mL of MeOH and 15.3 mL of 0.995 N methanolic HCl (0.015 mol) was stirred vigorously with 450 mg of 10% Pd/C under H_2 at atmospheric pressure until TLC indicated cleavage of the protecting group was complete (ca. 15–30 min). The catalyst was removed, and the filtrate was evaporated to a glass, dried for 2 h in vacuo, and used immediately. XIII (8.5 g, 0.014 mol) was dissolved in 150 mL of DMF in a three-necked 500-mL flask equipped with a stirrer and thermometer and chilled to −20 °C (internal temperature) with an acetone–dry ice bath. Then, 34.4 mL (0.088 mol) of freshly prepared 2.56 N HCl/THF was added, followed by 2.68 mL (0.018 mol) of isopentyl nitrite. The reaction mixture was stirred for 30 min at −20 °C, chilled to −25 °C, and 13.73 mL (0.098 mol) of cold Et_3N was added, followed by 4.9 g (0.015 mol) of D-Phe-Leu-OCH_3 (above). Stirring was continued at −20 °C for 30 min, then −20 to −10 °C during 15 min, and finally at 0 °C for 3 h. The reaction mixture was placed in the refrigerator overnight and then was filtered, and the filtrate was evaporated in vacuo. The residue was dissolved in 500 mL of EtOAc and washed successively with 50-mL portions of 0.5 N HCl, 5% $NaHCO_3$, and H_2O (all washes were saturated with NaCl). The EtOAc solution was dried over $MgSO_4$ and evaporated. The product XV crystallized from 125 mL of MeOH on standing in the refrigerator overnight. The filtered solid was then suspended in 200 mL of Et_2O, stirred for 2 h, filtered, and dried: yield 6.25 g; mp 221–223 °C; $[\alpha]^{23}_D$ −21° (*c* 1.0, DMF); UV (MeOH) λ_{max} 289.5 nm (*E'* 66.5), 280 (86.5). Anal. ($C_{47}H_{54}N_6O_{10} \cdot CH_3OH$) C, H, N.

Z-Ser(Bzl) (XVI). Five grams (0.0169 mol) of Boc-Ser(Bzl) (Bachem, Inc.) was dissolved in 20 mL of TFA at 5 °C. After 15 min, the solvent was evaporated with the aid of an aspirator pump with repeated additions of benzene to remove excess acid. The crystalline Ser(Bzl)·F_3OAc salt was dried overnight in vacuo and then dissolved in 17.0 mL of ice-cold 2.0 N NaOH (0.034 mol). The solution was stirred magnetically while 3.1 g (0.025 mol) of benzyloxycarbonyl chloride was added in three portions, with concurrent additions of 14-mL portions of ice-cold 2 N NaOH. After the reaction mixture had stirred at 5 °C for 45 min following the final addition, it was extracted twice with Et_2O and then acidified (to Congo red) with 4 N HCl. The product was extracted into EtOAc (three extractions). The combined extracts were dried over $MgSO_4$ and evaporated. The crystalline residue was purified by recrystallization from EtOAc–hexane: yield 4.75 g; mp 98–99 °C; $[\alpha]^{23}_D$ +13.4° (*c* 2.0, MeOH). Anal. ($C_{18}H_{19}NO_5$) C, H, N.

Z-Ser(Bzl)-Trp-Ser-Tyr-D-Phe-Leu-OCH_3 (XVII). XV (7 g, 0.0078 mol) was dissolved in 200 mL of MeOH with warming, and then the solution was cooled to room temperature and 900 mg of 20% Pd/C was added. The reaction was stirred vigorously under H_2 at atmospheric pressure until TLC indicated complete removal of the benzyloxycarbonyl group. The catalyst was filtered and the filtrate was evaporated to a glass, Trp-Ser-Tyr-D-Phe-Leu-OCH_3, which was dried for 2 h in vacuo. This product (5.68 g, 0.0078 mol) was dissolved in 120 mL of DMF, cooled to 5 °C in an ice bath, and stirred while the following were added in sequence: Z-Ser(Bzl) (XVI), 2.57 g (0.0078 mol); HOBt, 1.16 g (0.0086 mol); and DCC, 1.77 g (0.0086 mol). Stirring was continued for 2 h with ice-bath chilling. The reaction mixture was then stirred overnight while allowing the ice to melt spontaneously. After stirring 48 h longer at room temperature, the filtrate was evaporated in vacuo and the residual oil dissolved in 600 mL of wet EtOAc. The EtOAc solution was washed successively with 50 mL of 0.5 N HCl, 5% $NaHCO_3$, and H_2O (all solvents were saturated with NaCl). The product began to crystallize when the EtOAc solution had been evaporated to ca. 150 mL. After storing overnight in the refrigerator, the solid (XVII) was filtered; 8.2 g was collected: mp 242–244 °C; $[\alpha]^{25}_D$ −14.5° (*c* 1.0, DMF); UV (MeOH) λ_{max} 289.5 nm (*E'* 51.8), 280 (67.8). Anal. ($C_{57}H_{65}N_7O_{12} \cdot 2H_2O$) C, H, N.

Z-Ser(Bzl)-Trp-Ser-Tyr-D-Phe-Leu-$NHNH_2$ (XVIII). To 8.2 g (0.077 mol) of XVII dissolved in 50 mL of DMF was added 8.0 mL (0.16 mol) of 99% hydrazine hydrate. After the mixture was left standing overnight at room temperature, a few milligrams of extraneous solid was filtered, and the filtrate was diluted with 350 mL of *i*-PrOH, allowed to stand for 24 h at room temperature, while the hydrazide product separated, and then transferred to the refrigerator for 24 h. The filtered product was suspended in Et_2O and stirred for 3 h before finally filtering and drying: yield 6.0 g; mp 242–244 °C; $[\alpha]^{23}_D$ −31.2° (*c* 0.9, DMF); UV (MeOH) λ_{max} 290 nm (*E'* 52.5), 280 (68.7). Anal. ($C_{56}H_{65}N_9O_{11} \cdot 2H_2O$) C, H, N.

Z-Ser(Bzl)-Trp-Ser-D-Phe-Leu-Arg-Pro-Gly-$NH_2 \cdot HCl$ (35; Table I). A solution containing 1.2 g (0.0024 mol) of Z-Arg-Pro-Gly-$NH_2 \cdot HCl$ (IX) dissolved in 60 mL of MeOH was stirred vigorously with 350 mg of 10% Pd/C under H_2 at 1 atm until TLC indicated that the benzyloxycarbonyl group had been cleaved. Brinkmann silica gel plates developed with $CHCl_3$–MeOH–H_2O (60:45:10) were used. The catalyst was removed by filtration through a thin layer of Hy-Flo-Supercel on sintered glass, and the filtrate was evaporated to a glass, which was dried overnight in vacuo and used immediately.

A solution of 1.6 g (0.015 mol) of XVIII in 80 mL of DMF was chilled to −20 °C using an acetone–dry ice bath and 3.42 mL (0.009 mol) of 2.63 N HCl in THF was added, followed by 0.30 mL (0.002 mol) of isopentyl nitrite (90%). The reaction mixture was stirred for 30 min at −20 °C (internal temperature), then was chilled to −25 °C, and 1.25 mL (0.009 mol) of Et_3N was added, followed by 600 mg of Arg-Pro-Gly-$NH_2 \cdot HCl$ (above). The reaction mixture was stirred for 30 min at −20 °C, then for 30 min at −20 to −10 °C, and finally at 0 °C for 3 h. The reaction mixture was kept at 5 °C overnight and then was filtered through a sintered glass funnel, and the filtrate was evaporated to an oil, which was triturated with 150 mL of THF for 3 h in the refrigerator. The supernatant was decanted, and the residue was dissolved in 20 mL of MeOH and added dropwise to 250 mL of EtOAc with magnetic stirring. The precipitate was filtered after 48 h at 5 °C and dried in vacuo; 1.81 g was collected. The product (**35**) was purified by chromatography on a Brinkmann silica gel G-60 column, 70–230 mesh, which was developed with a 60:45 mixture of $CHCl_3$–MeOH. Samples of eluate fractions were spotted on Brinkmann silica gel plates, TLC, which were developed with $CHCl_3$–MeOH–H_2O (60:45:10). Fractions showing only one spot were combined and evaporated, and the residue was dissolved in H_2O and lyophilized; 1.29 g of fluffy white product (**35**) was obtained.

***O*-Methyl-D-tyrosine Hydrochloride (XIX).** A suspension of 136 g (1.0 mol) of 4-methoxybenzaldehyde, 110 g (1.1 mol) of hydantoin, and 20 g of β-alanine in 400 mL of HOAc was heated under reflux for 7 h. Solution was achieved after short reflux, but a solid appeared after a few hours. The mixture was stirred at room temperature overnight, diluted with 400 mL of H_2O, and filtered, and the solid was washed with MeOH and with H_2O. The solid was then slurried in 1.5 L of boiling EtOH and filtered hot. The solid was dried in a vacuum oven to give 171.5 g of 5-*p*-methoxybenzylidenehydantoin (XX) as a bright yellow solid, mp 250–253 °C. Anal. ($C_{11}H_{10}N_2O_3$) C, H, N.

Compound XX (171.5 g, 0.785 mol) in 680 mL of H_2O with 63 g of 50% NaOH solution (31.5 g, 0.785 mol) and 6 g of 20% Pd/C with 51.5 psi of H_2 was shaken for about 1.5 h until the H_2 pressure

no longer diminished. The reaction was vented and the mixture filtered to separate the catalyst. The filtrate was cooled and taken to pH 2 with concentrated HCl. The solid was separated by filtration, washed with H_2O, and dried at 50 °C in a vacuum oven; 148 g of 5-*p*-methoxybenzylhydantoin (XXI) was obtained, mp 177–179 °C.

Compound XXI (148 g, 0.67 mol) was dissolved in 1 L of H_2O containing 188 g (4.7 mol) of NaOH and heated at reflux in a stainless-steel container for 25 h. The solution was cooled and made acid to pH 1 with concentrated HCl. The solid was separated by filtration, washed with H_2O, and dried at 60 °C in a vacuum oven to give 114 g of *O*-methyl-DL-tyrosine (XXII), mp 255 °C dec.

Compound XXII (114 g, 0.58 mol) was dissolved in 500 mL of a solution of 46 g (1.15 mol) of NaOH in H_2O with cooling. Acetic anhydride (80 mL, 0.85 mol) and 50% NaOH to maintain pH at 9–11 were added with continued cooling. The reaction was stirred for 20 min at pH 11 and then acidified to pH 1 with continued cooling. The precipitated solid was collected by filtration, washed with a small amount of H_2O, and dried at 60 °C in a vacuum oven to give 129.9 g of *N*-acetyl-*O*-methyl-DL-tyrosine (XXIII), mp 144–146 °C. Anal. ($C_{12}H_{15}NO_4$) C, H, N.

Compound XXIII (129.9 g, 0.55 mol) was dissolved in 400 mL of H_2O by addition of NH_4OH to pH 7.5. The solution was treated with 5.46 mL of 0.1 M $CoCl_2$, 27.3 mL of 1% formalin, and a filtered solution of 12 g of takadiastase in 50 mL of H_2O. The pH was adjusted to 7.5, and the solution was diluted to 650-mL volume and incubated at 41–42 °C for 5 days. The mixture was filtered and the solid washed with *i*-PrOH, giving 38.1 g of *O*-methyltyrosine: mp 264–265 °C dec; $[\alpha]^{25}_D$ −6.6° (*c* 2, 1 N HCl). The filtrate was concentrated to one-half volume and treated with decolorizing carbon, and the filtrate was cooled. The precipitated solid was collected to yield an additional 5.4 g, mp 255–256 °C. The filtrate was made acid to pH 1, filtered, washed with H_2O, and dried to yield 51 g of *N*-acetyl-*O*-methyl-D-tyrosine (XXIV): mp 146–148 °C; $[\alpha]^{25}_D$ −51.7° (*c* 5, EtOH). Anal. ($C_{12}H_{15}NO_4$) C, H, N.

Compound XXIV (24 g, 0.1 mol) was heated in 100 mL of H_2O and 100 mL of concentrated HCl for 7 h on a steam bath and let cool to room temperature overnight. The flask was swirled gently and the product crystallized. It was separated by filtration, washed with a small amount of *i*-PrOH and then with Et_2O, and dried at 50 °C to give 16.1 g of *O*-methyl-D-tyrosine hydrochloride (XIX): $[\alpha]^{23}_D$ +7.4° (*c* 2.02, 1 N HCl).

O-Methyltyrosine Methyl Ester Hydrochloride (XXV). HCl was bubbled into a suspension of *O*-methyltyrosine (43.5 g, 0.22 mol) in 300 mL of MeOH for 2 h. The resulting solution was stirred overnight and then evaporated to dryness. The residue was recrystallized from EtOH, with charcoal treatment, to yield 23.5 g of product (XXV): mp 194–196 °C; $[\alpha]^{23}_D$ +68° (*c* 1.04, pyridine). Anal. ($C_{11}H_{15}NO_3$·HCl) C, H, N. Additional crops yielded 17.0 g.

O-Methyl-D-tyrosine Methyl Ester Hydrochloride (XXVI). A solution of 10.0 g (0.043 mol) of *O*-methyl-D-tyrosine hydrochloride (XIX) in 100 mL of MeOH was cooled in an ice bath and treated slowly with 3.4 mL (0.047 mol) of $SOCl_2$. The reaction was stirred for 1 h with cooling and then overnight at room temperature. The solution was evaporated to dryness and the residue crystallized from MeOH–Et_2O to give 5.2 g of XXVI: mp 189–190 °C; $[\alpha]^{23}_D$ −68° (*c* 1.04, pyridine). Anal. ($C_{11}H_{15}NO_3$·1.1HCl) C, H, N. Additional crops yielded 2.5 g.

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