at each of three doses; the suspending vehicle was administered to a control group of three animals. One hour after treatment, 9 mL of blood was withdrawn by cardiac puncture from each of the conscious animals. Blood samples from each drug-treated group and the control group were pooled and platelet-rich plasma (PRP) was prepared by centrifugation at 200g for 10 min. Platelet concentrations were matched among drug-treated and control PRP pools by diluting when necessary with platelet-poor plasma (prepared by centrifugation at 1400g for 10 min). Forty-five minutes after blood withdrawal, the minimal amount of bovine collagen (Worthington Biochemical, Freehold, N.J., employed as a suspension of 50 mg in 150 mL of physiological saline) required to produce a consistent aggregation of PRP from control animals was added to PRP from drug-treated animals. Aggregation was observed in a chronolog aggregometer at 37 °C and recorded as a curve that describes the rate of increase of light transmittance through PRP as aggregation proceeded. The percentage of inhibition was calculated from the formula $100 \times (\theta$ control PRP $- \theta$ drug PRP$)/\theta$ control PRP where θ stands for the rate of aggregation (curve slope).

Acknowledgment. We thank Mr. K. B. Streeter and his staff for elemental analyses, Mr. W. R. McGaughran and Dr. B. H. Arison for NMR spectra, Mr. R. E. Rhodes for mass spectra, and Miss M. Galavage for expert technical assistance with the in vitro assay.

References and Notes

(1) (a) Merck Institute for Therapeutic Research, Rahway, N.J. 07065; (b) Merck Institute for Therapeutic Research, West Point, Pa. 19486.
(2) J. B. Bicking, C. M. Robb, R. L. Smith, E. J. Cragoe, Jr., F. A. Kuehl, Jr., and L. R. Mandel, *J. Med. Chem.*, **20**, 35 (1977).
(3) (a) J. H. Jones, W. J. Holtz, J. B. Bicking, E. J. Cragoe, Jr., L. R. Mandel, and F. A. Kuehl, Jr., *J. Med. Chem.*, **20**, 44 (1977); (b) R. L. Smith, J. B. Bicking, N. P. Gould, T.-J. Lee, C. M. Robb, F. A. Kuehl, Jr., L. R. Mandel, and E. J. Cragoe, Jr., *ibid.*, **20**, 540 (1977).
(4) J. H. Jones, W. J. Holtz, J. B. Bicking, E. J. Cragoe, Jr., L. R. Mandel, and F. A. Kuehl, Jr., *J. Med. Chem.*, **20**, 1299 (1977).
(5) (a) D. Binder, J. Bowler, E. D. Brown, N. S. Crossley, J. Hutton, M. Senior, L. Slater, P. Wilkinson, and N. C. A. Wright, *Prostaglandins*, **6**, 87 (1974); (b) M. Dukes, W. Russell, and A. L. Walpole, *Nature (London)*, **250**, 330 (1974).
(6) For a recent review see P. W. Howie in "Prostaglandins: Physiological, Pharmacological and Pathological Aspects", S. M. M. Karim, Ed., University Park Press, Baltimore, Md., 1976, p 277.
(7) T. K. Schaaf, *Annu. Rep. Med. Chem.*, 11, 80 (1976).
(8) H. B. Henbest, E. R. H. Jones, and I. M. S. Walls, *J. Chem. Soc.*, 3646 (1950).
(9) J. F. Bagli and T. Bogri, German Offen. 2231244 (1973).
(10) N. A. Nelson, German Offen. 2423155 (1974).
(11) M. Hayashi, S. Kari, and H. Endo, German Offen. 2400451 (1975).
(12) H. Lindlar and R. Dubuis, "Organic Syntheses", Collect. Vol. V, Wiley, New York, N.Y., 1973, p 880.

Synthesis and Biological Activity of Highly Active α-Aza Analogues of Luliberin

Anand S. Dutta,* Barrington J. A. Furr, Michael B. Giles, and Barbara Valcaccia

Imperial Chemical Industries Limited, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, SK10 4TG, England.
Received May 1, 1978

Analogues of luliberin containing an α-azaamino acid in position 6, 9, or 10 (I–XIV) have been synthesized by the solution method of peptide synthesis. Two nonaza analogues, [D-Phe^6]- and [D-Ser(Bu^t)^6,des-Gly-NH$_2$^{10},Pro-ethylamide^9]luliberin, were also synthesized for comparison. The ovulation-inducing activity of the compounds was evaluated in androgen-sterilized constant-estrus rats. A combination of D-amino acid replacement in position 6 with an azaglycine residue at position 10 resulted in highly active compounds which were superior to the corresponding nonaza analogues. The most active compounds, [D-Phe^6,Azgly^{10}]-, [D-Tyr(Me)^6,Azgly^{10}]-, and [D-Ser(Bu^t)^6,-Azgly^{10}]luliberin, were about 100 times as potent as luliberin. *N*-Methylleucine substitution in position 7 in these compounds resulted in decreased activity; [D-Phe^6,MeLeu^7,Azgly^{10}]- and [D-Tyr(Me)^6,MeLeu^7,Azgly^{10}]luliberin were only 50 times as active as luliberin. The presence of either an azaproline residue in position 9, an azaphenylalanine or azaglycine residue in positions 6 and 10, or a *tert*-butyl ether protecting group on the hydroxyl group of the tyrosine residue in position 5 resulted in compounds with significantly reduced biological activity.

Following the discovery and synthesis of luliberin (luteinizing hormone-releasing hormone, <Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH$_2$) it was suggested that longer acting and more potent analogues could be useful for the treatment of infertility states in man and for the improvement of fertility in farm animals;[1,2] it was also concluded that an antagonist of luliberin might offer a new method of birth control.[3,4] Recently, additional information has indicated that luliberin agonists may also be of value as antifertility[5,6] and antitumor[7,8] agents. Thus, agonist and antagonist analogues of luliberin appear to have fairly wide therapeutic potential.

Structure–activity relationship studies on luliberin analogues have indicated that highly active analogues of luliberin can be obtained by replacing the glycine residue in position 6 with D-amino acids and the glycine amide residue in position 10 with various alkylamines.[9,10]

We reported previously the effects of replacing an amino acid residue in a biologically active molecule by an α-azaamino acid.[11] The presence of an α-aza residue in a peptide was expected to be beneficial for two reasons. Firstly, the change in the overall conformation of a molecule might lead to higher affinity for the receptor site. Secondly, the resulting compound might be more stable to enzymic degradation and, therefore, might have a longer duration of action. In addition, the absorption and transport properties of the analogues might also be altered favorably. In the case of luliberin, analogues marginally less active than the parent peptide were prepared by replacing the glycine residue in position 6 or 10 by an azaglycine residue.[11,12] When an azaglycine or azalanine change in position 6 was combined with an ethylamide substitution in position 10, the resulting compounds were two to four times as active as luliberin.[13] We have now

0022-2623/78/1821-1018$01.00/0

Table I. Structures of the Luliberin Analogues

<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (luliberin)

<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-NHN(CH_3)$CONH_2$ (I)

<Glu-His-Trp-Ser-Tyr-NHN($CH_2C_6H_5$)CO-Leu-Arg-Pro-$NHNHCONH_2$ (II)

<Glu-His-Trp-Ser-Tyr-D-Phe-Leu-Arg-N(CH$_2$CH$_2$CH$_2$CH$_2$)NCONHC_2H_5 (III)

<Glu-His-Trp-Ser-Tyr-Sar-Leu-Arg-Pro-$NHNHCONH_2$ (IV)

<Glu-His-Trp-Ser-Tyr-Sar-MeLeu-Arg-Pro-$NHNHCONH_2$ (V)

<Glu-His-Trp-Ser-Tyr-D-Phe-Leu-Arg-Pro-$NHNHCONH_2$ (VI)

<Glu-His-Trp-Ser-Tyr-D-Phe-MeLeu-Arg-Pro-$NHNHCONH_2$ (VII)

<Glu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-$NHNHCONH_2$ (VIII)

<Glu-His-Trp-Ser-Tyr-D-Tyr(Me)-Leu-Arg-Pro-$NHNHCONH_2$ (IX)

<Glu-His-Trp-Ser-Tyr-D-Tyr(Me)-MeLeu-Arg-Pro-$NHNHCONH_2$ (X)

<Glu-His-Trp-Ser-Tyr-D-Ser(Bu^t)-Leu-Arg-Pro-$NHNHCONH_2$ (XI)

<Glu-His-Trp-Ser-Tyr-D-Ser-Leu-Arg-Pro-$NHNHCONH_2$ (XII)

<Glu-His-Trp-Ser-Tyr(Bu^t)-D-Phe-MeLeu-Arg-Pro-$NHNHCONH_2$ (XIII)

<Glu-His-Trp-Ser-Tyr(Bu^t)-NHN($CH_2C_6H_5$)CO-Leu-Arg-Pro-$NHNHCONH_2$ (XIV)

<Glu-His-Trp-Ser-Tyr-D-Phe-Leu-Arg-Pro-Gly-NH_2 (XV)

<Glu-His-Trp-Ser-Tyr-D-Ser(Bu^t)-Leu-Arg-Pro-NHC_2H_5 (XVI)

studied substitution of other α-azaamino acids (azaphenylalanine, azaproline, and azalanine) in positions 6, 9, and 10 and have also investigated the effects of azaglycine replacement in position 10 along with D-amino acid replacement in position 6. Two known compounds, [D-Phe^6]luliberin[14,15] (XV) and [D-Ser(Bu^t)^6,des-Gly-NH_2^{10},Pro-ethylamide^9]luliberin[16] (XVI), have also been prepared for comparison in our biological test systems. The structures of the analogues prepared are shown in Table I.

Synthesis. The general procedure used for the synthesis of analogues I, IV–VI, VIII–XII, XV, and XVI is summarized in Chart I. The synthesis was achieved by coupling three peptide fragments by the azide method. The C-terminal tripeptide derivatives, N^{α}-*tert*-butyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolylglycine amide, N^{α}-*tert*-butyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolylazaglycine amide, and N^{α}-*tert*-butyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolylazalanine amide, were prepared by a stepwise coupling procedure using *N*,*N*′-dicyclohexylcarbodiimide–hydroxybenzotriazole as the coupling agent at each step. Since these tripeptide derivatives were highly water soluble, the purification could only be achieved by a countercurrent distribution procedure using ethyl acetate–water and 1-butanol–water, followed by ion-exchange chromatography to remove unreacted N^{α}-*tert*-butyloxycarbonyl-N^{ω}-nitro-L-arginine.

The middle tripeptide derivatives, *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl-A-B methyl esters (A = Sar, D-Phe, D-Trp, D-Ser, *O*-Bu^t-D-Ser, *O*-Me-D-Tyr; B = Leu, MeLeu), were prepared in a stepwise manner. Coupling *N*-benzyloxycarbonylsarcosine, -D-phenylalanine, -D-tryptophan, -*O*-*tert*-butyl-D-serine, or -*O*-methyl-D-tyrosine with leucine or *N*-methylleucine methyl ester by the dicyclohexylcarbodiimide–hydroxybenzotriazole procedure gave the protected dipeptide derivatives. Most of these

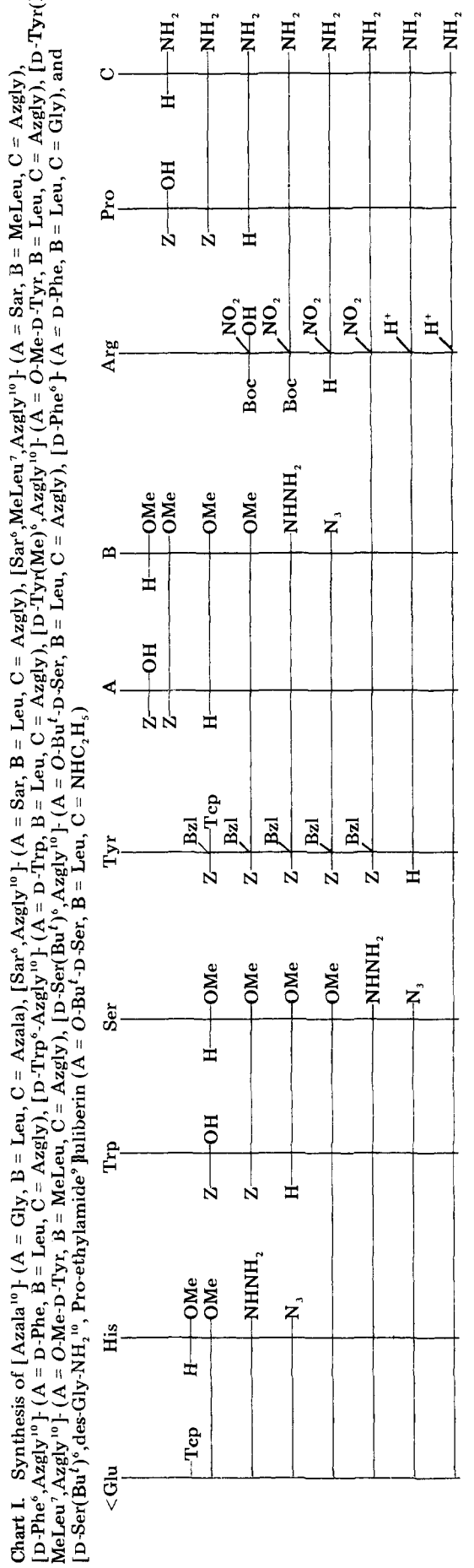

Chart I. Synthesis of [Azala^{10}]- (A = Gly, B = Leu, C = Azala), [Sar^6,Azgly^{10}]- (A = Sar, B = Leu, C = Azgly), [Sar^6,MeLeu^7,Azgly^{10}]- (A = Sar, B = MeLeu, C = Azgly), [D-Phe^6,Azgly^{10}]- (A = D-Phe, B = Leu, C = Azgly), [D-Trp^6-Azgly^{10}]- (A = D-Trp, B = Leu, C = Azgly), [D-Tyr(Me)^6,Azgly^{10}]- (A = *O*-Me-D-Tyr, B = Leu, C = Azgly), [D-Tyr(Me)^6, MeLeu^7,Azgly^{10}]- (A = *O*-Me-D-Tyr, B = MeLeu, C = Azgly), [D-Ser(Bu^t)^6,Azgly^{10}]- (A = *O*-Bu^t-D-Ser, B = Leu, C = Azgly), [D-Phe^6]- (A = D-Phe, B = Leu, C = Gly), and [D-Ser(Bu^t)^6,des-Gly-NH_2^{10}, Pro-ethylamide^9]luliberin (A = *O*-Bu^t-D-Ser, B = Leu, C = NHC_2H_5)

Table II. Physical and Chemical Characteristics of Luliberin (L) Analogues

compd	purificn[a]	yield, % (in final step)	R_f(A)	paper electrophoresis, R_f rel to luliberin		amino acid analysis (16-h acid digest)							
				pH 2.1	pH 6.5	Glu	His	Ser	Tyr	Phe	Leu	Arg	Pro	Gly
[Azala^{10}]-L	A, B	28.0	0.30	1.02	1.01	1.02	0.97	0.84	1.01		1.0	1.01	0.99	
[Azphe^6,Azgly^{10}]-L	A, B	92.4	0.31	1.0	0.92	0.96	0.97	0.82	1.0		1.0	0.97	0.94	
[D-Phe^6,des-Gly-NH$_2^{10}$,Azpro^9-ethylamide]-L	A, C, D	32.2	0.32	0.89	0.98	0.96	0.95	0.80	0.96		1.0	G[c]		
[Sar^6,Azgly^{10}]-L	A, B, C	35.5	0.20	0.95	1.0	F[b]	0.98	0.85	1.01		1.0	1.0	0.94	
[Sar^6,MeLeu^7,Azgly^{10}]-L	A, C	45.4	0.35	0.96	1.04	F[b]	0.96	0.85	1.0			0.99	0.96	
[D-Phe^6,Azgly^{10}]-L	A, B	40.0	0.27	1.0	0.71	0.96	1.02	0.79	1.01	1.04	1.0	0.98	0.92	
[D-Phe^6,MeLeu^7,Azgly^{10}]-L	A, C	46.2	0.35	0.84	0.87	0.98	1.01	0.82	0.98	1.01		0.99	0.96	
[D-Trp^6,Azgly^{10}]-L	A, B, E	15.5	0.37	0.53	0.37	0.97	0.99	0.80	1.02		1.0	0.90	0.87	
[D-Tyr(Me)^6,Azgly^{10}]-L	A, B	15.2	0.25	0.92	0.87	1.0	0.96	0.88	1.87		1.0	0.98	0.97	
[D-Tyr(Me)^6,MeLeu^7,Azgly^{10}]-L	A, C, D	26.4	0.34	0.87	0.90	1.0	0.95	0.87	1.92			1.0	1.01	
[D-Ser(Bu^t)^6,Azgly^{10}]-L	A, C	31.2	0.27	0.94	0.96	0.98	0.96	1.76	1.02		1.0	1.02	0.96	
[D-Ser^6,Azgly^{10}]-L	A, B	95.3	0.25	0.94	0.96	0.96	0.97	1.78	1.01		1.0	1.03	0.97	
[Tyr(Bu^t)^5,D-Phe^6,MeLeu^7,Azgly^{10}]-L	A, C, D	46.0	0.32	0.84	0.87	0.98	0.99	0.86	1.01	1.01		1.01	0.96	
[Tyr(Bu^t)^5,Azphe^6,Azgly^{10}]-L	A, C, D	60.9	0.29	0.90	0.81	0.98	0.98	0.87	1.02		1.0	0.98	0.97	
[D-Phe^6]-L	A, B, D	34.2	0.24	1.01	0.93	1.0	0.96	0.88	1.0	1.01	1.0	1.02	0.98	1.0
[D-Ser(Bu^t)^6,des-Gly-NH$_2^{10}$,Pro^9-ethylamide]-L	A, C, E	22.4	0.36	0.51	0.54	1.03	1.01	1.70	1.01		1.0	G[c]		

[a] A = Sephadex LH-20 column chromatography in dimethylformamide; B = partition chromatography on Sephadex G-25 using 1-butanol–acetic acid–water (4:1:5) solvent system; C = partition chromatography on Sephadex G-25 using 1-butanol–acetic acid–water–pyridine (5:1:5:1) solvent system; D = Sephadex G-25 chromatography in 0.4 M acetic acid; E = column chromatography on CMC-50 using 0.001, 0.005, 0.01, and 0.025 M ammonium acetate. [b] F = glutamic acid and sarcosine eluted together on the amino acid analyzer. [c] G = arginine and ethylamine peaks overlapped.

were obtained as oils after silica gel column chromatography. Debenzyloxycarbonylation, followed by coupling with *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosine, gave the protected tripeptide derivatives. *N*-Methylleucine containing tripeptide methyl esters were also obtained as oils and needed purification by silica gel column chromatography. Hydrazine hydrate treatment then converted the esters to hydrazides.

The N-terminal tetrapeptide derivative, L-pyroglutamyl-L-histidyl-L-tryptophyl-L-serine methyl ester, was prepared by coupling L-pyroglutamyl-L-histidine azide with L-tryptophyl-L-serine methyl ester. L-Pyroglutamyl-L-histidine hydrazide was only sparingly soluble in dimethylformamide, and, therefore, the azide coupling was carried out in a mixture of dimethyl sulfoxide and dimethylformamide.

The coupling of the C-terminal tripeptide derivatives, N^{ω}-nitro-L-arginyl-L-prolylglycine amide, N^{ω}-nitro-L-arginyl-L-prolylazaglycine amide, and N^{ω}-nitro-L-arginyl-L-prolylazalanine amide (prepared by treating the N^{α}-*tert*-butyloxycarbonyl derivatives with HCl in ethyl acetate), with the desired *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl-A-B azide gave the hexapeptide derivatives. Hydrogenolysis over 5% Pd/C in the presence of 2 equiv of hydrogen chloride removed all protecting groups. The resulting dihydrochlorides were converted to monohydrochlorides and reacted with L-pyroglutamyl-L-histidyl-L-tryptophyl-L-serine azide. The guanidino group of arginine was protected by protonation during the coupling procedure. The decapeptide amides were purified by column chromatography on Sephadex LH-20 in dimethylformamide and by partition chromatography on Sephadex G-25 using either 1-butanol–acetic acid–water (4:1:5) or 1-butanol–acetic acid–water–pyridine (5:1:5:1) solvent systems.

Synthesis of [Tyr(Bu^t)^5,D-Phe^6,MeLeu^7,Azgly^{10}]luliberin (XIII) was achieved in a manner similar to the one described in Chart I, except that *N*-benzyloxycarbonyl-*O*-*tert*-butyl-L-tyrosine was used in place of *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosine. The cleavage of the *tert*-butyl ether group from compound XIII by trifluoroacetic acid treatment gave [D-Phe^6,MeLeu^7,Azgly^{10}]luliberin (VII).

The procedure used for the synthesis of [Tyr(Bu^t)^5,-Azphe^6,Azgly^{10}]- (XIV), [Azphe^6,Azgly^{10}]- (II), and [D-Phe^6,des-Gly-NH$_2^{10}$,Azpro-ethylamide^9]luliberin (III) differed from Chart I only in the synthesis of two peptide fragments. One of these, *N*-benzyloxycarbonyl-*O*-*tert*-butyl-L-tyrosylazaphenylalanyl-L-leucine hydrazide, used in the synthesis of compounds II and XIV, was prepared in the following manner. N^1-*tert*-Butyloxycarbonyl-N^2-benzyl hydrazide was reacted with *N*-carbonyl-L-leucine methyl ester to give *N*-*tert*-butyloxycarbonylazaphenylalanyl-L-leucine methyl ester. Removal of the *tert*-butyloxycarbonyl protecting group, followed by a coupling with *N*-benzyloxycarbonyl-*O*-*tert*-butyl-L-tyrosine by the DCCI–HOBt procedure yielded the tripeptide methyl ester which was then converted to the hydrazide by hydrazine hydrate treatment. The other peptide fragment, N^{α}-*tert*-butyloxycarbonyl-N^{ω}-nitro-L-arginylazaproline ethyl amide, used in the synthesis of [D-Phe^6,des-Gly-NH$_2^{10}$,-Azpro-ethylamide^9]luliberin, was prepared in a stepwise manner. *N*-Benzyloxycarbonylpyrazolidine reacted smoothly with ethyl isocyanate to give *N*-benzyloxycarbonylazaproline ethylamide. Debenzyloxycarbonylation, followed by a coupling with N^{α}-*tert*-butyloxycarbonyl-N^{ω}-nitro-L-arginine by the DCCI–HOBT method, yielded the C-terminal dipeptide derivative.

Table III. Ovulation-Inducing Activity of the Luliberin (L) Analogues in Androgen-Sterilized Constant-Estrus Rats

compd	dose, μg per rat	response (no. ovulating/ no. treated)
luliberin	0.5	4/4
	0.25	3/4
	0.10	0/4
[Azala^{10}]-L (I)	1.0	3/3
	0.5	1/3
	0.25	0/3
[Azphe^{6},Azgly^{10}]-L (II)	10.0	3/3
	5.0	2/3
[D-Phe^{6},des-Gly-NH$_2^{10}$,-Azpro-ethylamide^{9}]-L (III)	1.0	3/3
	0.5	2/3
	0.125	0/3
[Sar^{6},Azgly^{10}]-L (IV)	1.0	2/3
[Sar^{6},MeLeu^{7},Azgly^{10}]-L (V)	1.0	2/3
[D-Phe^{6},Azgly^{10}]-L (VI)	0.005	3/3
	0.0025	0/3
[D-Phe^{6},MeLeu^{7},Azgly^{10}]-L (VII)	0.01	3/3
	0.005	0/3
[D-Trp^{6},Azgly^{10}]-L (VIII)	0.062	3/3
	0.031	0/3
[D-Tyr(Me)^{6},Azgly^{10}]-L (IX)	0.005	6/6
	0.0025	0/3
[D-Tyr(Me)^{6},MeLeu^{7},-Azgly^{10}]-L (X)	0.01	3/3
	0.005	0/3
[D-Ser(Bu^t)^{6},Azgly^{10}]-L (XI)	0.0062	3/3
	0.0031	2/6
[D-Ser^{6},Azgly^{10}]-L (XII)	0.05	3/3
	0.01	2/3
	0.001	0/3
[Tyr(Bu^t)^{5},D-Phe^{6},MeLeu^{7},-Azgly^{10}]-L (XIII)	0.1	3/3
	0.05	1/3
[Tyr(Bu^t)^{5},Azphe^{6},-Azgly^{10}]-L (XIV)	100	3/3
	25	1/3
	0.5	0/3
[D-Phe^{6}]-L (XV)	0.01	6/6
	0.005	0/3
[D-Ser(Bu^t)^{6},des-Gly-NH$_2^{10}$,-Pro-ethylamide^{9}]-L (XVI)	0.1	3/3
	0.01	1/3

The physical and chemical characteristics of all the analogues are summarized in Table II.

Biological Activity. The ability of the peptides to induce ovulation was assessed in androgen-sterilized constant-estrus rats.[12] The compounds dissolved in 0.25 mL of saline were injected into the tail vein. The results obtained are given in Table III.

Azaglycine substitution in position 10 and D-amino acid substitution in position 6 gave highly active compounds (VI, VIII, IX, XI, XIII), the potency of which ranged from approximately 5 to 100 times that of luliberin. Azaglycine replacement in position 10 is more favorable for potency than either no substitution or substitution of the glycine amide with ethylamide (compare VI with XV and XI with XVI). In contrast to the increased potency seen when D-amino acids are substituted in position 6, either azaphenylalanine (II) or sarcosine (IV) replacement in this position significantly reduces biological activity. N-Methylation of the leucine residue in position 7 causes only a marginal loss in potency (compare IV with V, VI with VII, and IX with X).

Discussion

The increased potency of the highly active luliberin analogues, synthesized by D-amino acid replacement in position 6 and various alkylamine substitutions in position 10, is said to be due to either a change in the overall conformation of the molecule leading to higher affinity for the receptor site[9,17] or an increase in the stability of the analogues to enzymic degradation.[18,19] Peptide linkages at positions 6 and 10 have, in fact, been shown to be highly susceptible to enzymic degradation.[18,19] When modified in positions 6, 7, and 10, the resulting analogues were less susceptible to degradation by enzymes present in rat brain extract.[20]

We previously investigated the substitution of azaglycine or azalanine in positions 6 or 10 in the hope of increasing the biological potency and duration of action, but analogues marginally less active than luliberin were obtained.[12] Since the aza linkage at position 6 was expected to convey greater stability to enzymic attack, the failure to obtain compounds with significantly increased potency may have been due to a concomitant unfavorable conformational change in the molecule. This speculation is consistent with the finding that azaphenylalanine substitution in position 6 (II) significantly reduces biological activity. When compared with [D-Phe^{6}]luliberin (XV) the reduction in potency of the azaphenylalanine analogue (II) is even more pronounced because compound XV is about 50 times as active as luliberin. The most likely explanation of these findings, therefore, is that because of its planar nature, the α-aza residue at position 6 alters the overall conformation of the molecule in such a manner as to reduce affinity for the receptor; the magnitude of this change increases with increasing bulk of the side chain of the azaamino acid residue.

Since the aza linkages are expected to be stable to enzymic degradation, yet aza^{6} analogues of luliberin have low potency, it can be argued that increased biological activity of the analogues with D-amino acids in position 6 is primarily due to a favorable change in conformation and that stability to enzymic attack is much less important. This view is further strengthened by the relatively poor biological potency of compounds with a sarcosine residue in position 6 with or without an *N*-methylleucine residue in position 7 (IV, V). N-Methylation of the glycine and leucine residue should increase metabolic stability but the compounds are only about half as potent as luliberin.

In contrast to the effect of an aza residue at position 6, replacement of glycine by azaglycine at position 10 combined with substitution of a D-amino acid at position 6 has a most favorable effect on the biological potency. Three of these compounds (VI, IX, and XI) are about 100 times more potent than luliberin. Moreover, [D-Phe^{6},-Azgly^{10}]luliberin (VI) is about twice as active as [D-Phe^{6}]luliberin (XV), and [D-Ser(Bu^t)^{6},Azgly^{10}]luliberin (XI) is about three times as active as [D-Ser(Bu^t)^{6}, des-Gly-NH$_2^{10}$,Pro-ethylamide^{9}]luliberin (XVI). The aza analogue XI is also more active than the ethylamide analogue XVI in inhibiting the increase in ovarian and uterine weights induced by human chorionic gonadotrophin.[19] Furthermore, compound XI is a potent antitumor agent in the rat DMBA mammary tumor model.[21]

[D-Trp^{6},Azgly^{10}]luliberin (VIII) was about eight times more active than luliberin. When compared to [D-Phe^{6},Azgly^{10}]luliberin (VI) this level of activity is surprisingly low. In the corresponding nonaza series [D-Trp^{6}]luliberin has been claimed to be more active than [D-Phe^{6}]luliberin.[15,20,22]

Azalanine substitution in position 10 (I) results in 50% reduction in potency, but in comparison to [L-Ala^{10}]luliberin (20 times less active than luliberin^{10}) the aza analogue is much more active. It is possible that the L-alanine side-chain methyl group interferes with the receptor interaction, whereas in azalanine the methyl group is present in a different spatial position and is unable to interfere so severely with the receptor binding.

Azaproline substitution in position 9 (III) decreases the

Table IV. Dipeptide Derivatives Used in the Synthesis of Luliberin Analogues

compd	yield, %	mp, °C	purifcn	emp formula	analyses	thin-layer chromatography, R_f D	E	H	P	Q
Z-Sar-Leu-OMe	90.2	oil	chloroform[a]	$C_{18}H_{26}N_2O_5$	C, H, N	0.62	0.45	0.49	0.60	0.64
Z-Sar-MeLeu-OMe	69.4	oil	cyclohexane–ether (3:1, 2:1, 1:1) and ether[a]	$C_{19}H_{28}N_2O_5$	C, H, N	0.78	0.58	0.63	0.65	0.69
Z-D-Phe-MeLeu-OMe	64.1	oil	chloroform[a]	$C_{25}H_{32}N_2O_5$	C, H, N	0.76	0.67	0.60	0.68	0.75
Z-D-Tyr(Me)-Leu-OMe	95.2	foam	ethyl acetate–cyclohexane[b]	$C_{25}H_{32}N_2O_6$	C, H, N	0.83	0.69		0.72	0.76
Z-D-Tyr(Me)-MeLeu-OMe	55.4	oil	chloroform[a]	$C_{26}H_{34}N_2O_6$	C, H, N	0.83	0.78	0.79	0.80	0.79
Z-D-Ser(Bu^t)-Leu-OMe	90.4	107–108	methanol–water[b]	$C_{22}H_{34}N_2O_6$	C, H, N	0.80	0.68	0.72	0.72	0.74

[a] The peptide was loaded on a silica gel column and the column was eluted by the solvent mentioned. [b] Crystallized from the indicated solvent.

Table V. Physical and Chemical Properties of the Protected Tripeptide Derivatives

compd	yield, %	mp, °C	purifcn	emp formula	analyses	thin-layer chromatography, R_f A	B	C	D	E	H	P	Q
Z-Tyr(Bzl)-Sar-Leu-OMe	79.4	foam	chloroform[a]	$C_{33}H_{41}N_3O_7$	C, H, N	0.74	0.80	0.84	0.74	0.52	0.69	0.62	0.69
Z-Tyr(Bzl)-Sar-Leu-NHNH$_2$	96.6	92–93		$C_{33}H_{41}N_5O_6$	C, H, N	0.74	0.77	0.84	0.64	0.40	0.63	0.11	0.46
Z-Tyr(Bzl)-Sar-MeLeu-OMe	54.5	oil	ether[a]	$C_{34}H_{40}N_3O_6$	C, H, N	0.73	0.78	0.72	0.79	0.58	0.67	0.63	0.73
Z-Tyr(Bzl)-Sar-MeLeu-NHNH$_2$	83.1	foam		$C_{34}H_{43}N_5O_6 \cdot 0.5H_2O$	C, H, N	0.61	0.67	0.76	0.64	0.44	0.48	0.27	0.54
Z-Tyr(Bu^t)-D-Phe-MeLeu-OMe	58.8	foam	chloroform,[a] system P	$C_{38}H_{49}N_3O_7$	C, H, N							0.71	0.73
Z-Tyr(Bu^t)-D-Phe-MeLeu-NHNH$_2$	80.1	135–136	methanol–ether[b]	$C_{37}H_{49}N_5O_6$	C, H, N				0.75	0.69	0.79		0.73
Z-Tyr(Bzl)-D-Tyr(Me)-Leu-OMe	81.2	190–191	ethyl acetate–petr ether[b]	$C_{41}H_{47}N_3O_8$	C, H, N				0.85	0.73			0.78
Z-Tyr(Bzl)-D-Tyr(Me)-Leu-NHNH$_2$	84.2	212–213	dimethylformamide–water[b]	$C_{40}H_{47}N_5O_7 \cdot 0.5H_2O$	C, H, N					0.49	0.69		0.70
Z-Tyr(Bzl)-D-Tyr(Me)-MeLeu-OMe	62.0	foam	ether[a]	$C_{42}H_{49}N_3O_8$	C, H, N								
Z-Tyr(Bzl)-D-Tyr(Me)-MeLeu-NHNH$_2$	91.1	129–131	methanol–water[b]	$C_{41}H_{49}N_5O_7$	C, H, N				0.75	0.67	0.87	0.70	0.76
									0.79	0.60	0.73		0.77
Z-Tyr(Bzl)-D-Ser(Bu^t)-Leu-OMe	79.4	135–137	ethyl acetate–petr ether[b]	$C_{38}H_{49}N_3O_8$	C, H, N				0.8		0.77		0.81
Z-Tyr(Bzl)-D-Ser(Bu^t)-Leu-NHNH$_2$	56.2	135–136	methanol–water[b]	$C_{37}H_{49}N_5O_7$	C, H, N				0.66		0.64		0.64
Z-Tyr(Bu^t)-Azphe-Leu-OMe	66.4	153–154	2-propanol–water[b]	$C_{36}H_{46}N_4O_7$	C, H, N	0.70	0.73	0.74	0.88	0.70	0.76	0.65	0.76
Z-Tyr(Bu^t)-Azphe-Leu-NHNH$_2$	62.2	175–176	chloroform,[a] system P	$C_{35}H_{46}N_6O_6$	C, H, N				0.67	0.56	0.58	0.30	0.36

[a] Silica gel column chromatography using the solvents mentioned. [b] Crystallized from the indicated solvent.

Table VI. Physical and Chemical Properties of the Penta- or Hexapeptide Derivatives

compd	yield, %	mp, °C	purifcn	emp formula	analyses	thin-layer chromatography, R_f A	B	C	D	H	K
Z-Tyr(Bzl)-Sar-Leu-Arg(NO$_2$)-Pro-Azgly-NH$_2$	31.0	142–143	chloroform,[a] system P	$C_{45}H_{60}N_{12}O_{11} \cdot H_2O$	C, H, N	0.59	0.73	0.53	0.62	0.25	0.45
Z-Tyr(Bzl)-Sar-MeLeu-Arg(NO$_2$)-Pro-Azgly-NH$_2$	34.2	159–160	chloroform,[a] system P	$C_{46}H_{62}N_{12}O_{11}$	C, H, N	0.55	0.69	0.52	0.62	0.20	0.95
Z-Tyr(Bu^t)-D-Phe-MeLeu-Arg(NO$_2$)-Pro-Azgly-NH$_2$	58.5	147–148	ethyl acetate–ether[b]	$C_{49}H_{68}N_{12}O_{11}$	C, H, N				0.72	0.53	0.98
Z-Tyr(Bzl)-D-Tyr(Me)-Leu-Arg(NO$_2$)-Pro-Azgly-NH$_2$	70.9	132 dec	chloroform,[a] system P, Q	$C_{52}H_{66}N_{12}O_{12}$	C, H, N	0.64	0.72	0.55	0.66	0.52	
Z-Tyr(Bzl)-D-Tyr(Me)-MeLeu-Arg(NO$_2$)-Pro-Azgly-NH$_2$	23.83	152–154	chloroform,[a] system P	$C_{53}H_{68}N_{12}O_{12}$	C, H, N	0.67	0.68	0.58	0.59	0.50	0.94
Z-Tyr(Bzl)-D-Ser(Bu^t)-Leu-Arg(NO$_2$)-Pro-Azgly-NH$_2$	38.5	143–145	chloroform,[a] system P	$C_{49}H_{68}N_{12}O_{12} \cdot H_2O$	C, H, N	0.64	0.71	0.55	0.65	0.48	0.93
Z-Tyr(Bu^t)-Azphe-Leu-Arg(NO$_2$)-Pro-Azgly-NH$_2$	27.4	146–148	chloroform[a]	$C_{47}H_{65}N_{13}O_{11}$	C, H, N				0.65	0.37	0.94
Z-Tyr(Bzl)-D-Phe-Leu-Arg(NO$_2$)-Pro-Gly-NH$_2$	55.7	155–157	chloroform,[a] system P, Q	$C_{52}H_{65}N_{11}O_{11}$	C, H, N	0.70	0.75	0.64	0.66	0.59	
Z-Tyr(Bzl)-D-Ser(Bu^t)-Leu-Arg(NO$_2$)-Pro-NHC$_2$H$_5$	82.2	138–140	methanol–ether[b]	$C_{50}H_{70}N_{10}O_{11}$	C, H, N				0.67	0.69	0.42
Z-Tyr(Bzl)-D-Phe-Leu-Arg(NO$_2$)-Azpro-NHC$_2$H$_5$	30.4	121–122	chloroform[a]	$C_{51}H_{65}N_{11}O_{12} \cdot H_2O$	C, H, N	0.82	0.77	0.8	0.73	0.71	0.98

[a] Crude product was purified by silica gel column chromatography using the solvent mentioned. [b] Crystallized from the indicated solvent.

biological activity significantly. When compared with [D-Phe^6]luliberin (XIII), [D-Phe^6,des-Gly-NH$_2^{10}$,Azproethylamide^9]luliberin (III) is about 100 times less active. A proline residue in position 9 therefore appears to be extremely important for high biological activity.

Although a *tert*-butyl protecting group on D-serine in position 6 is favorable for activity (compare XI with XII), when present on the tyrosine residue in position 5 it is decidedly disadvantageous (compare VII with XIII and II with XIV).

It can be concluded from the above results that α-azaamino acid replacement alone in positions 6, 7, 9, and 10 does not have any positive advantage, but when an azaglycine change in position 10 is combined with a D-amino acid replacement in position 6 the resulting aza analogues are highly potent and more active than the corresponding nonaza derivatives.

Experimental Section

Ascending, thin-layer chromatograms were run on Keiselgel G with butan-1-ol–acetic acid–water (4:1:5 v/v) [R_f(A)], butan-1-ol–acetic acid–water–pyridine (15:3:12:10) [R_f(B)], butan-2-ol–3% ammonium hydroxide (3:1) [R_f(C)], acetonitrile–water (3:1) [R_f(D)], acetone–chloroform (1:1) [R_f(E)], ethanol–chloroform (4:1) [R_f(F)], cyclohexane–ethyl acetate–methanol (1:1:1) [R_f(H)], chloroform–methanol–water (11:8:2) [R_f(K)], chloroform–methanol (95:5) [R_f(P)], or chloroform–methanol (9:1) [R_f(Q)]. Spots were revealed with ninhydrin, fluorescamine, or sodium hypochlorite–potassium iodide reagent. Paper electrophoresis was carried out on Whatman chromatography paper (3 mm) using a mixture of 8% acetic acid and 2% formic acid (pH 2.1) and 0.3% acetic acid and 0.9% pyridine (pH 6.5) in deionized water. For amino acid analysis, the peptide in 6 N HCl containing 1% phenol was sealed under vacuum in a glass tube and heated at 110 °C for 16 h.

The synthesis of the following intermediates has been reported earlier:[13,23] N^{α}-*tert*-butyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolylazaglycine amide, N^{α}-*tert*-butyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-proline ethylamide, N^{α}-*tert*-butyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolylazalanine amide, *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl-D-phenylalanyl-L-leucine hydrazide, *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl-D-tryptophyl-L-leucine hydrazide, *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl-D-phenylalanyl-L-leucyl-N^{ω}-nitro-L-arginyl-L-prolylazaglycine amide, *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl-D-tryptophyl-L-leucyl-N^{ω}-nitro-L-arginyl-L-prolylazaglycine amide, and *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosylglycyl-L-leucyl-N^{ω}-nitro-L-arginyl-L-prolylazalanine amide.

N-Benzyloxycarbonylazaproline Ethylamide. Ethyl isocyanate (3.1 mL, 39.3 mmol) was added to a solution of *N*-benzyloxycarbonylpyrazolidine hydrochloride (9.54 g, 39.3 mmol) and triethylamine (5.5 mL, 39.3 mmol) in chloroform (100 mL), and the solution was left overnight at room temperature. Chloroform was removed in vacuo and the residue was taken up in ethyl acetate, washed with water, 20% citric acid solution, and water, and dried (Na_2SO_4). Evaporation of ethyl acetate left an oil which was purified by silica gel column chromatography using chloroform as eluting solvent: yield 8.7 g (80.9%); TLC R_f(D) 0.71, R_f(E) 0.61, R_f(H) 0.66, R_f(P) 0.66, R_f(Q) 0.70. Anal. ($C_{14}H_{19}N_3O_3$) C, H, N.

N^{α}-*tert*-Butyloxycarbonyl-N^{ω}-nitro-L-arginylazaproline Ethylamide. Dicyclohexylcarbodiimide (7.48 g, 36.2 mmol) was added to a cooled (0 °C) and stirred solution of N^{α}-*tert*-butyloxycarbonyl-N^{ω}-nitro-L-arginine (10.0 g, 31.39 mmol), azaproline ethylamide hydrochloride (6.2 g, 34.5 mmol, prepared by the catalytic reduction of the preceeding compound over 5% Pd/C in presence of 1 equiv of hydrogen chloride), triethylamine (4.8 mL, 34.5 mmol), and hydroxybenzotriazole (9.33 g, 69.0 mmol) in dimethylformamide (100 mL), and the stirring was continued overnight at 4 °C. Dicyclohexylurea was filtered off and the filtrate was evaporated to dryness. The oily residue was applied to silica gel column and the column was eluted with 5% v/v methanol in chloroform. The product obtained from the column was contaminated with N^{α}-*tert*-butyloxycarbonyl-N^{ω}-nitro-L-arginine. It was dissolved in a mixture of methanol–water (6:4, 200 mL), and the solution was passed through a column of AG1 X-2 and evaporated to dryness. The residue was dissolved in *tert*-butyl alcohol and freeze-dried to give a fluffy solid: yield 8.3 g (60.3%); TLC R_f(D) 0.61, R_f(F) 0.61, R_f(H) 0.51, R_f(Q) 0.31. Anal. ($C_{16}H_{32}N_8O_6$) C, H, N.

N-Benzyloxycarbonyl-O-*tert*-butyl-D-seryl-L-leucine Methyl Ester. A solution of *N*-benzyloxycarbonyl-*O*-*tert*-butyl-D-serine (2.83 g, 9.6 mmol), leucine methyl ester hydrochloride (1.92 g, 10.6 mmol), 1-hydroxybenzotriazole (2.6 g, 19.2 mmol) and triethylamine (1.6 mL, 11 mmol) in dimethylformamide (30 mL) was cooled to 0 °C, and dicyclohexylcarbodiimide (2.29 g, 11.1 mmol) was added to it. The reaction mixture was stirred overnight at 4 °C and filtered to remove the solid residue, and the filtrate was evaporated to dryness. The residue, in ethyl acetate, was washed with water, 20% citric acid solution, saturated sodium hydrogen carbonate solution, and water and dried (Na_2SO_4). Ethyl acetate was removed in vacuo and the residue was crystallized from aqueous methanol to yield the dipeptide derivative: 3.5 g (90.4%); mp 107–108 °C. Anal. ($C_{15}H_{21}NO_5$) C, H, N.

Several other dipeptide derivatives used in the synthesis of luliberin analogues were prepared using this procedure and are listed in Table IV.

N-Benzyloxycarbonyl-O-benzyl-L-tyrosyl-O-*tert*-butyl-D-seryl-L-leucine Methyl Ester. A solution of *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosine 2,4,5-trichlorophenyl ester (19.17 g, 32.7 mmol) and *O*-*tert*-butyl-D-seryl-L-leucine methyl ester (9.6 g, 32.7 mmol, prepared by the catalytic reduction of the benzyloxycarbonyl derivative) in dimethylformamide (100 mL) was left at room temperature for 48 h. Dimethylformamide was evaporated off in vacuo, and the residue, in ethyl acetate, was washed with water, 20% citric acid solution, saturated sodium hydrogen carbonate solution, and water and dried (Na_2SO_4). The tripeptide ester obtained after evaporating off the ethyl acetate was collected, washed with ether, and dried: yield 17.6 g (79.4%); mp 135–137 °C.

All the other tripeptide derivatives prepared by this procedure are listed in Table V.

N-Benzyloxycarbonyl-O-benzyl-L-tyrosyl-O-*tert*-butyl-D-seryl-L-leucine Hydrazide. Hydrazine hydrate (12.9 mmol) was added to a solution of the preceeding methyl ester (4.32 g, 6.4 mmol) in dimethylformamide (25 mL) and methanol (50 mL), and the reaction mixture was left overnight at room temperature. Methanol was removed in vacuo and the product was precipitated with water, collected, washed with water, and dried: yield 2.4 g (56.2%); mp 134–136 °C. Anal. ($C_{37}H_{49}N_5O_7$) C, H, N.

The tripeptide hydrazides prepared by this procedure are listed in Table V.

General Procedure for the Preparation of N-Benzyloxycarbonyl-O-benzyl- (or *tert*-butyl-) L-tyrosyl-A-B-N^{ω}-nitro-L-arginyl-L-prolyl-C (A = Gly, Sar, D-Phe, D-Trp, D-Tyr(Me), D-Ser(Bu^t), Azphe; B = Leu, MeLeu; C = Azgly-NH$_2$, -NHC$_2$H$_5$). *N*-Benzyloxycarbonyl-*O*-benzyl- (or *tert*-butyl-) L-tyrosyl-A-B hydrazide (5.0 mmol) was dissolved in dimethylformamide (10 mL) and the stirred solution was cooled to 0 °C. Hydrogen chloride (5.92 M) in dioxane (20 mmol) was added, followed by *tert*-butyl nitrite (5.25 mmol). After 2 min, a precooled solution of N^{ω}-nitro-L-arginyl-L-prolyl-C hydrochloride, prepared by treating the *tert*-butyloxycarbonyl derivative with HCl in ethyl acetate for 1 h (5 mmol), and triethylamine (25 mmol) in dimethylformamide (10 mL) was added and the stirring was continued overnight at 4 °C. The reaction mixture was diluted with ethyl acetate (250 mL) and washed with water, 20% citric acid solution, sodium hydrogen carbonate solution, and water. Ethyl acetate was removed in vacuo and the product was purified by crystallization or silica gel column chromatography (Table VI).

L-Pyroglutamyl-L-histidyl-L-tryptophyl-L-serine Methyl Ester. To a cooled (0 °C) and stirred suspension of L-pyroglutamyl-L-histidine hydrazide (2.8 g, 10 mmol) in dimethylformamide (10 mL) and dimethyl sulfoxide (10 mL), 5.7 N hydrogen chloride in dioxane (6.9 mL, 40 mmol) was added. A clear solution was obtained after 5 min of vigorous stirring. The solution was cooled to −20 °C, *tert*-butyl nitrite (1.31 mL, 11 mmol) was added, and the stirring was continued for 25 min. The tem-

perature was lowered to −30 °C and the solution was neutralized by adding triethylamine (5.77 mL, 40 mmol). A precooled (−20 °C) solution of L-tryptophyl-L-serine methyl ester (3.35 g, 11 mmol, prepared by hydrogenating the *N*-benzyloxycarbonyl derivative over 5% Pd/C) and triethylamine in dimethylformamide was added, and the reaction mixture was stirred at −10 °C for 30 min and at 4 °C for 24 h. Triethylamine hydrochloride was removed by filtration and the filtrate was evaporated to dryness. The crude peptide was purified by silica gel column chromatography using 10 and 20% methanol in chloroform and a mixture of chloroform–methanol–water (11:8:2) as eluting solvents: yield 3.85 g (70%); mp 142–145 °C dec; TLC R_f(A) 0.39, R_f(B) 0.72, R_f(C) 0.45, R_f(D) 0.48, R_f(K) 0.61. Anal. ($C_{26}H_{31}N_7O_7$) C, H, N.

L-Pyroglutamyl-L-histidyl-L-tryptophyl-L-serine Hydrazide. The preceding methyl ester (2.98 g, 5.4 mmol) was dissolved in dimethylformamide (70 mL) and was treated with hydrazine hydrate (100 mmol) for 4 h. Dimethylformamide was removed in vacuo, and the residue was triturated with ethanol, collected, washed with ethanol and ether, and dried: yield 2.62 g (88.2%); mp 186–189 °C; TLC R_f(A) 0.18, R_f(B) 0.55, R_f(C) 0.39, R_f(D) 0.27, R_f(K) 0.58. Anal. ($C_{25}H_{31}N_9O_6$) C, H, N.

General Procedure for Coupling L-Pyroglutamyl-L-histidyl-L-tryptophyl-L-serine Azide with L-Tyrosyl-A-B-L-arginyl-L-prolyl-C (A = Gly, Sar, D-Phe, D-Trp, D-Tyr(Me), D-Ser(Bu^t), Azphe; B = Leu, MeLeu; C = Azgly-NH$_2$, Azala-NH$_2$, −NHC$_2$H$_5$). To a cooled (0 °C) and stirred suspension of L-pyroglutamyl-L-histidyl-L-tryptophyl-L-serine hydrazide (0.275 mmol) in dimethylformamide (1.0 mL) was added 5.92 M hydrogen chloride in dioxane (1.1 mmol). The clear solution obtained after several minutes of stirring was further cooled to −20 °C and to it was added *tert*-butyl nitrite (0.29 mmol). Stirring was continued for 20 min at −20 °C and the solution was neutralized by the addition of triethylamine (1.1 mmol). A precooled −20 °C mixture of L-tyrosyl-A-B-L-arginyl-L-prolyl-C dihydrochloride (0.25 mmol), obtained by the hydrogenolysis of *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl-A-B-(*N*$^\omega$-nitro)-L-arginyl-C in 80% v/v aqueous methanol for 40 h over 5% w/w palladium on charcoal in the presence of 2 equiv of hydrogen chloride, and triethylamine (0.25 mmol) in dimethylformamide (1.0 mL) were added. The mixture was stirred for 24 h at 4 °C. The reaction mixture was applied directly to a Sephadex LH-20 column using dimethylformamide as eluent (purification procedure A). Further purification was achieved by the procedures mentioned in Table II which were performed in the same order as listed in the table.

References and Notes

(1) A. V. Schally, A. J. Kastin, and D. H. Coy, *Int. J. Fertil.*, 21, 1 (1976).
(2) G. M. Besser and C. H. Mortimer, *J. Clin. Pathol.*, **27**, 127 (1974).
(3) A. V. Schally, A. Arimura, A. J. Kastin, D. H. Coy, E. J. Coy, J. Vilchez-Martinez, T. W. Redding, R. Geiger, W. König, and H. Wissman, *Pure Appl. Chem.*, **37**, 315 (1974).
(4) R. Guillemin, *Fertil. Steril., Proc. World Congr., 7th, 1971*, 6 (1973).
(5) C. W. Beattie and A. Corbin, *Biol. Reprod.*, **16**, 333 (1977).
(6) R. R. Humphrey, B. L. Windsor, J. R. Reel, R. A. Reel, and R. A. Edgren, *Biol. Reprod.*, 16, 614 (1977).
(7) E. R. DeSombre, E. S. Johnson, and W. F. White, *Cancer Res.*, **36**, 3830 (1976).
(8) A. Danguy, N. Legros, J. A. Heuson-Stiennon, J. L. Pasteels, and J. C. Heuson, *Eur. J. Cancer*, **13**, 1089 (1977).
(9) M. W. Monahan, M. S. Amoss, H. A. Anderson, and W. Vale, *Biochemistry*, **12**, 4616 (1973).
(10) M. Fujino, S. Shinagawa, M. Obayashi, S. Kobayashi, T. Fukuda, I. Yamazabi, R. Nakayama, W. F. White, and R. H. Rippel, *J. Med. Chem.*, 16, 1144 (1973).
(11) A. S. Dutta and J. S. Morley, *Pept., Proc. Eur. Pept. Symp., 14th, 1976*, 517 (1976).
(12) A. S. Dutta, B. J. A. Furr, M. B. Giles, and J. S. Morley, *Clin. Endocrinol. (Oxford)*, **5** (Suppl.), 291 (1976).
(13) A. S. Dutta, B. J. A. Furr, and M. B. Giles, *J. Chem. Soc., Perkin Trans. 1*, in press.
(14) D. H. Coy, F. Labrie, M. Savary, E. J. Coy, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, **67**, 576 (1975).
(15) J. Rivier, N. Ling, M. Monahan, C. Rivier, M. Brown, and W. Vale, "Proceedings of the 4th American Peptide Symposium", Ann Arbor Science Publishers, Ann Arbor, Mich., 1975, p 863.
(16) W. König, J. Sandow, and R. Geiger in ref 15, p 883.
(17) R. H. Rippel, E. S. Johnson, W. F. White, M. Fujino, T. Fukuda, and S. Kobayashi, *Proc. Soc. Exp. Biol. Med.*, **148**, 1193 (1975).
(18) N. Marks and F. Stern, *Biochem. Biophys. Res. Commun.*, **61**, 1458 (1974).
(19) Y. Koch, T. Baram, P. Chobsieng, and M. Friedkin, *Biochem. Biophys. Res. Commun.*, 61, 95 (1974).
(20) W. Vale, C. Rivier, M. Brown, J. Leppaluoto, N. Ling, M. Monahan, and J. Rivier, *Clin. Endocrinol. (Oxford)*, **5** (Suppl.), 261S (1976).
(21) A. S. Dutta, B. J. A. Furr, M. B. Giles, B. Valcaccia, and A. L. Walpole, *Biochem. Biophys. Res. Commun.*, **81**, 382 (1978).
(22) D. H. Coy, J. A. Vilchez-Martinez, E. J. Coy, and A. V. Schally, *J. Med. Chem.*, **19**, 423 (1976).
(23) A. S. Dutta, B. J. A. Furr, and M. B. Giles, *J. Chem. Soc., Perkin Trans. 1*, in press.