Polypeptides. Part 15.[1,2] Synthesis and Biological Activity of α-Aza-analogues of Luliberin modified in Positions 6 and 10

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Analogues of luliberin (luteinising hormone-releasing hormone) and 2-dehistidyl-luliberin containing α-aza-glycine (–NHNHCO–) or α-aza-alanine (–NHNMeCO–) residues in either position 6 or 10 have been synthesised by classical procedures of peptide synthesis. The agonist and antagonist activities of these compounds were evaluated in androgen-sterilised constant-oestrus rats: [10-Azgly]-, [6-Azgly]-, and [6-Azala]-luliberin were marginally less active than the parent peptide. When the aza-change in position 6 was combined with an ethylamide substitution in position 10 the resulting compounds, [6-Azgly-10-de-Gly-NH_2-9-Pro-ethylamide]- and [6-Azala-10-de-Gly-NH_2-9-Pro-ethylamide]-luliberin, were twice as active as luliberin. [6-D-Ala-10-Azgly]-luliberin was also twice as active as luliberin. [2-De-His-10-Azgly]-luliberin inhibited completely ovulation induced by luliberin (0.5 μg/rat) at a dose of 250 μg/rat, but 2-de-His-analogues of all the other compounds were devoid of antagonist activity in this test system.

ELUCIDATION of the structure of luliberin (luteinising hormone-releasing hormone, ⌐Glu–His–Trp–Ser–Tyr–Gly–Leu–Arg–Pro–Gly–NH_2) [3] has resulted in an intensive search for longer-acting and more potent agonists and antagonists. Agonists could be useful either in treating patients with the 'isolated gonadotrophin deficiency' syndrome,[4,5] or as antitumour agents,[6,7] and the antagonists could lead to the development of a new method of birth control.[8,9] Structure–activity-relationship studies [10] on analogues so far synthesised have indicated that highly active analogues can be obtained by replacing the *C*-terminal glycine amide by various alkylamines, *e.g.* ethylamine [11,12] and 2,2,2-trifluoroethylamine,[13] and also by substituting the glycine residue in position 6 by D-amino-acids, *e.g.* D-alanine [14] and D-leucine.[15,16] When modified in position 2 or 3 these highly active analogues of luliberin give analogues with antagonist activity.[17,18] Rippell *et al.*[16] have ascribed the high potency of [6-D-Leu-10-de-Gly-NH_2-9-Pro-ethylamide]-luliberin to a more favourable conformational fit to the receptor site, whereas Marks and Stern [19] and Koch *et al.*[20] have suggested that the enhanced potency of some of these analogues may be due to increased stability to peptidases present in brain and pituitary tissue.

We have synthesised α-aza-analogues (2)—(7) of luliberin (1) in which the glycine amide residue in position

⌐Glu-X-Trp-Ser-Tyr-Y-Leu-Arg-Pro-Z

(1) X = His, Y = Gly, Z = Gly–NH_2
(2) X = His, Y = Gly, Z = Azgly-NH_2
(3) X = His, Y = Azgly, Z = Gly-NH_2
(4) X = His, Y = Azala, Z = Gly-NH_2
(5) X = His, Y = Azgly, Z = NH–C_2H_5
(6) X = His, Y = Azala, Z = NH–C_2H_5
(7) X = His, Y = D-Ala, Z = Azgly–NH_2
(8) X = nothing, Y = Gly, Z = Azgly–NH_2
(9) X = nothing, Y = Azgly, Z = Gly–NH_2
(10) X = nothing, Y = Azala, Z = Gly–NH_2
(11) X = nothing, Y = Azgly, Z = NH–C_2H_5
(12) X = nothing, Y = Azala, Z = NH–C_2H_5
(13) X = nothing, Y = D-Ala, Z = Azgly–NH_2

10 has been replaced by either azaglycine amide or ethylamide and the glycine residue in position 6 has been replaced by either an azaglycine, aza-alanine, or D-alanine residue. 2-Dehistidyl-analogues (8)—(13) of compounds (2)—(7) were prepared for evaluation as antagonists. It was hoped that the presence of an α-aza-amino acid residue in the molecule might extend the duration of action by increasing the stability of the peptides towards specific peptidases responsible for the metabolic degradation of luliberin. Such an increase in the biological half-life by α-aza-replacement has been reported by Niedrich *et al.* for [5-α-aza-asparagine]-eledoisin-(4-11)-octapeptide.[21] Our own work on model tripeptides has also indicated increased stability of the α-aza-linkages to various enzymes.[1] It is also possible that the presence of an α-aza-amino-acid may change either the local conformation at the α-aza-residue, or the overall conformation of the molecule in such a manner that the analogue may have improved affinity for the receptor and consequently may also have higher biological activity. In addition, the absorption, distribution, and transport characteristics of the azapeptides may be different from the corresponding non-aza-peptides.

RESULTS AND DISCUSSION

The synthesis of [10-azaglycine]-luliberin (2) is described in Figure 1. Semicarbazide was coupled with *N*-benzyloxycarbonyl-L-proline by the *NN*-dicyclohexylcarbodi-imide–1-hydroxybenzotriazole (DCCI–HOBt) procedure of König and Geiger,[22] and the product was hydrogenolysed over 5% Pd–C to give L-prolylazaglycine amide. Coupling (DCCI–HOBt) this azapeptide derivative with *N*-benzyloxycarbonyl-L-leucyl-L-nitroarginine, prepared by reacting *N*-benzyloxycarbonyl-L-leucine and L-nitroarginine by the mixed-anhydride procedure, followed by hydrogenolysis of the product (14), gave L-leucyl-L-arginyl-L-prolylazaglycine amide (15). This *C*-terminal tetrapeptide was coupled with the *N*-terminal hexapeptide, L-pyroglutamyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosylglycine (22), by the DCCI–HOBt method. The hexapeptide was prepared by coupling L-tyrosylglycine t-butyl ester (16) with *N*-benzyloxycarbonyl-L-tryptophyl-L-serine azide (17), followed by hydrogenolysis of the tetrapeptide derivative (18) and coupling with L-pyroglutamyl-L-histidine

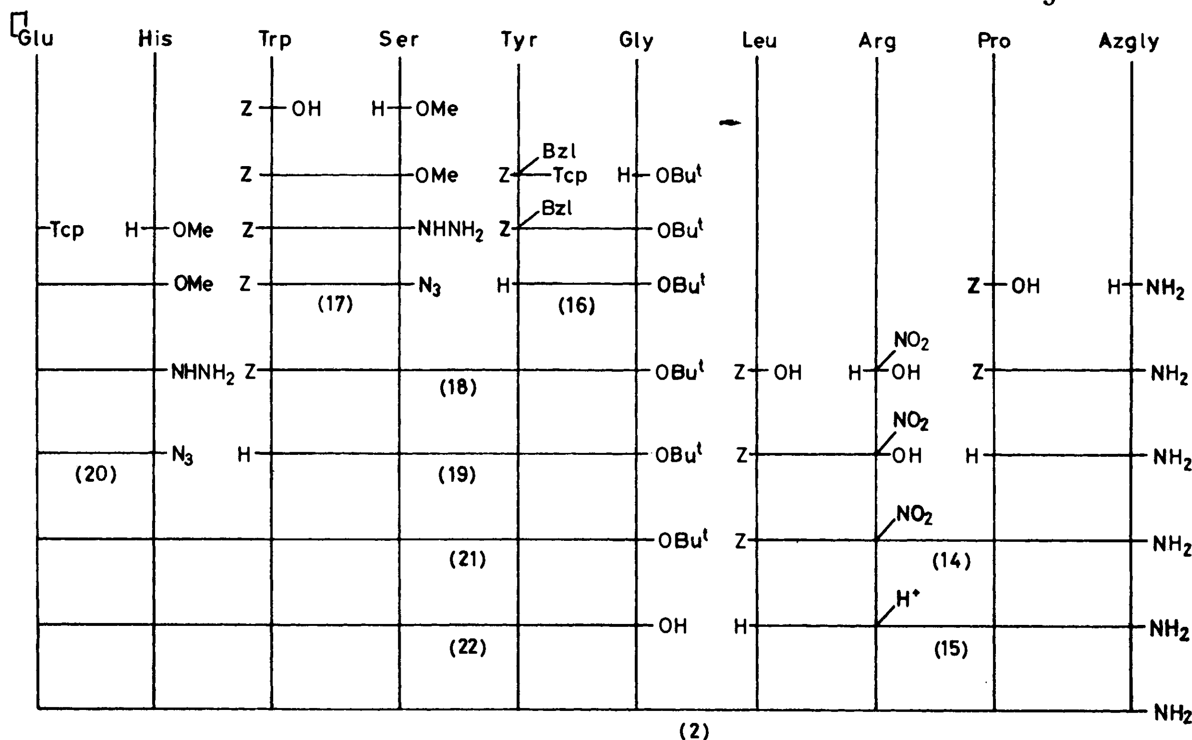


FIGURE 1 Synthesis of [10-azaglycine]-luliberin. Z = Benzyloxycarbonyl; Boc = t-butoxycarbonyl; Bzl = benzyl; Bu^t = t-butyl; Tcp = 2,4,5-trichlorophenyl ester; Azgly = NH–NH–CO–

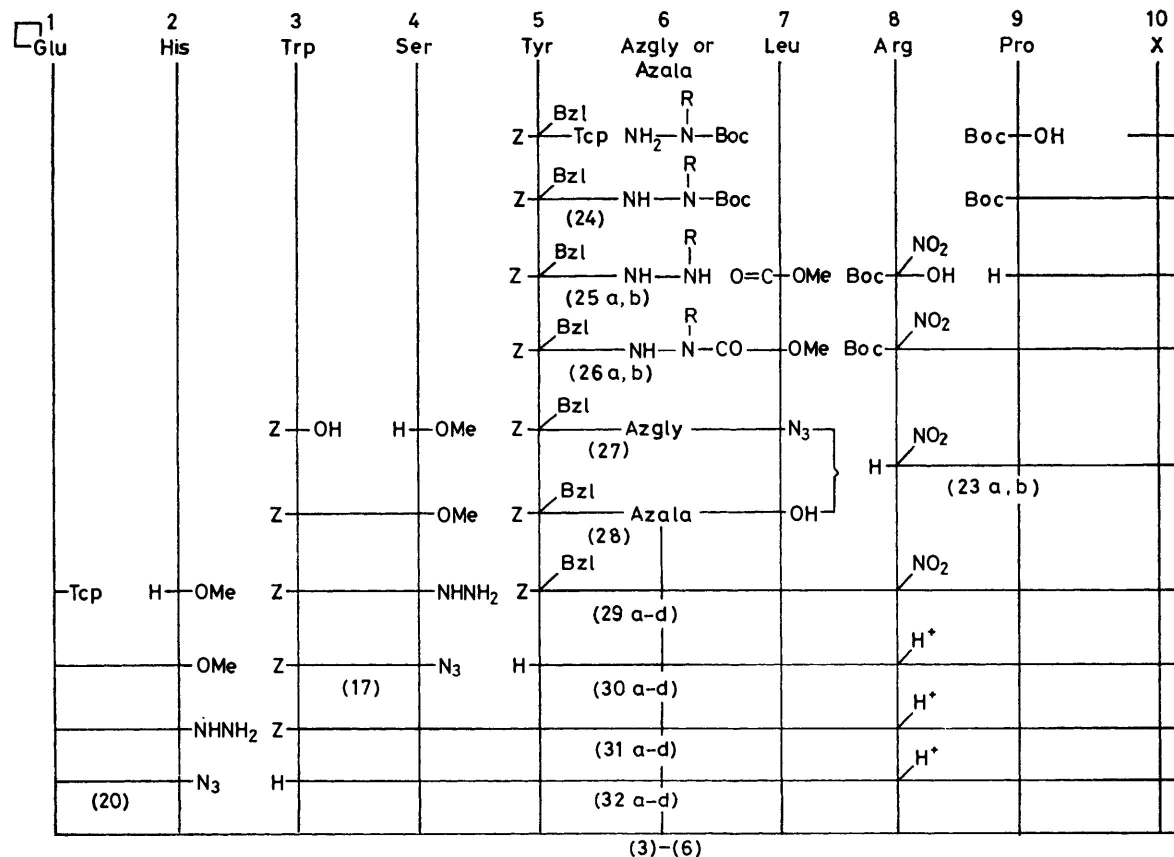
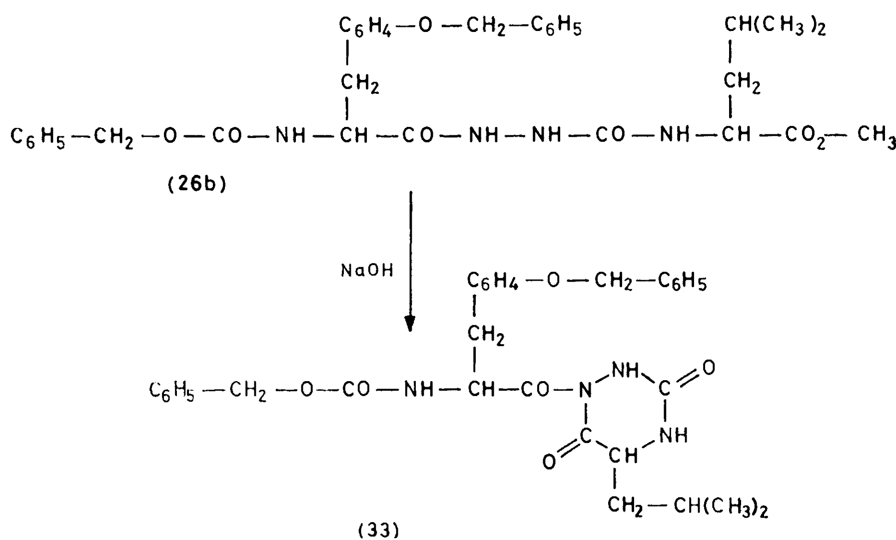


FIGURE 2 Synthesis of [6-Azgly]-luliberin (R = H, X = Gly-NH_2); [6-Azala]-luliberin (R = CH_3, X = Gly-NH_2); [6-Azgly-10-de-Gly-NH_2-9-Pro-ethylamide]-luliberin (R = H, X = NHC_2H_5); and [6-Azala-10-de-Gly-NH_2-9-Pro-ethyl-amide]-luliberin(R = CH_3, X = NHC_2H_5). Abbreviations as in Figure 1

azide [23] (20) by the procedure of Honzl and Rudinger.[24] The decapeptide (2) was purified by gel filtration (Sephadex G-25) and by partition chromatography on Sephadex G-25 using the solvent system, n-butanol–water–acetic acid–pyridine (5 : 5 : 1 : 1).

The synthetic route to [6-Azgly]-, [6-Azala]-, [6-Azgly, 10-de-Gly-NH_2-9-Pro-ethylamide]-, and [6-Azala-10-de-Gly-NH_2-9-Pro-ethylamide]-luliberin is shown in Figure 2. The *C*-terminal tripeptides, N^{ω}-nitro-L-arginyl-L-prolylglycine amide (23a, X = glycine amide) and N^{ω}-nitro-L-arginyl-L-proline ethylamide (23b, X = NHC_2H_5) were prepared by a stepwise coupling procedure starting from glycine amide and ethylamine respectively. Cleavage of the *N*-t-butoxycarbonyl group at each stage was achieved by treatment with HCl in ethyl acetate. The azatripeptides, *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl-aza-alanyl- (26a, R = CH_3) and -azaglycyl-L-leucine methyl ester (26b, R = H) were prepared by reacting either *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosine methyl hydrazide (25a, R = CH_3) or hydrazide (25b, R = H) with *N*-carbonyl-L-leucine methyl ester. Saponification of the aza-alanine containing tripeptide methyl ester (26a, R = CH_3) with sodium hydroxide gave the free acid (28) but even with 1.1 equivalents of aqueous sodium hydroxide, the azaglycine containing tripeptide methyl ester (26b, R = H) gave 1-(*N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl)-5-isobutyl-1,2,4,5-tetrahydro-1,2,4-triazine-3,6-dione (33) in almost quantitative yield in <30 min (characterised by elemental analysis and n.m.r.). However, treatment with hydrazine hydrate afforded the hydrazide which was then converted to the azide (27). Both the *C*-terminal tripeptides (23a,b) were coupled individually with the azatripeptide derivatives (27) and (28) to give protected hexapeptides (29a, *N*-benzyl-oxycarbonyl-*O*-benzyl-L-tyrosylaza-alanyl-L-leucyl-N^{ω}-nitro-L-arginyl-L-prolylglycine amide; 29b, *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosylazaglycyl-L-leucyl-N^{ω}-nitro-L-arginyl-L-prolylglycine amide; 29c, *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosylazaglycyl-L-leucyl-N^{ω}-nitro-L-arginyl-L-proline ethylamide; 29d, *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosylaza-alanyl-L-leucyl-N^{ω}-nitro-L-arginyl-L-proline ethylamide). The protecting groups were cleaved by catalytic hydrogenation and the free peptides (30a—d) were then coupled with *N*-benzyloxycarbonyl-L-tryptophyl-L-serine azide (17) to give the hepta- or octa-peptide derivatives (31a, *N*-benzyloxycarbonyl-L-tryptophyl-L-seryl-L-tyrosylazaalanyl-L-leucyl-L-prolylglycine amide; 31b, *N*-benzyloxycarbonyl-L-tryptophyl-L-seryl-L-tyrosylazaglycyl-L-leucyl-L-arginyl-L-prolylglycine amide; 31c, *N*-benzyloxycarbonyl-L-tryptophyl-L-seryl-L-tyrosylazaglycyl-L-leucyl-L-arginyl-L-proline ethylamide; 31d, *N*-benzyloxycarbonyl-L-tryptophyl-L-seryl-L-tyrosylaza-alanyl-L-leucyl-L-arginyl-L-proline ethylamide). Debenzyloxycarbonylation by catalytic reduction followed by coupling with L-pyroglutamyl-L-histidine azide (20) afforded the desired luliberin analogues (3)—(6). The purification of these compounds was achieved by gel filtration on Sephadex LH-20 in dimethylformamide and by partition chromatography on Sephadex G-25 using the solvent system n-butanol–acetic acid–water (4 : 1 : 5).



The synthetic route to [6-D-Ala-10-Azgly]-luliberin (7) is described in Figure 3. Coupling of *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl-D-alanine azide (36) with L-leucyl-N^{ω}-nitro-L-arginyl-L-prolyl-azaglycine amide (15) gave the fully protected hexapeptide derivative (37). The protecting groups were cleaved from the tyrosine and arginine residues by hydrogenolysis and after conversion to the monohydrochloride the peptide was reacted with *N*-benzyloxycarbonyl-L-tryptophyl-L-serine azide (17). Catalytic reduction of the octapeptide derivative (39) produced (40) which on reaction with L-pyroglutamyl-L-histidine azide gave the decapeptide. Column chromatography on Sephadex LH-20 in dimethylformamide followed by the partition chromatography on Sephadex G-25 using n-butanol–acetic acid–water (4 : 1 : 5) gave [6-D-Ala-10-Azgly]-luliberin (7).

The dehistidyl analogues (8)—(13) were prepared

likewise except that L-pyroglutamic acid 2,4,5-trichlorophenyl ester [25] was used instead of L-pyroglutamyl-L-histidine azide in the final coupling reaction.

The physical and chemical characteristics of the analogues are summarised in Table 1.

TABLE 1

Physical and chemical characteristics of lúliberin analogues

Compound	Method (general procedure)	Purification *	Yield %	R_{FA}	Paper electrophoresis R_F relative to luliberin pH 2.1	pH 6.5	Amino-acid analysis (16-h acid digest) Glu	His	Ser	Tyr	Gly	Ala	Leu	Arg	Pro
[10-Azgly]-luliberin	A	*a, d*	33.5	0.32	0.97	0.97	1.08	1.04	0.92	1.07	1.0		0.98	0.98	0.98
[6-Azgly]-luliberin	A	*a, c*	73.6	0.21	0.97	0.97	1.02	1.07	0.94	1.02	1.0		1.04	1.02	1.08
[6-Azala]-luliberin	A	*a, c*	46.2	0.31	0.99	1.0	1.01	0.96	0.91	1.03	1.0		1.02	0.95	0.97
[6-Azgly-10-de-Gly-NH_2-9-Pro-ethylamide]-luliberin	A	*a, c*	25.4	0.28	0.98	1.03	1.02	1.06	0.85	1.01			0.97	1.10	0.95
[6-Azala-10-de-Gly-NH_2-9-Pro-ethylamide]-luliberin	A	*a, c*	42.0	0.30	0.97	1.0	1.02	0.98	0.89	1.0			1.01	0.97	0.95
[6-D-Ala-10-Azgly]-luliberin	A	*a, c*	32.0	0.30	0.97	1.0	1.0	1.01	0.92	1.0		1.01	0.98	0.98	0.96
[2-de-His-10-Azgly]-luliberin	B	*b, d*	69.1	0.32	0.64	0.76	1.01		0.88	1.02	1.0		1.0	1.02	1.01
[2-de-His-6-Azgly]-luliberin	B	*b, d*	37.1	0.34	0.65	0.75	0.95		0.86	1.0	1.0		0.95	1.06	1.0
[2-de-His-6-Azala]-luliberin	B	*b, d*	63.4	0.34	0.67	0.77	1.03		0.97	1.02	1.0		0.98	0.96	0.95
[2-de-His-6-Azgly-10-de-Gly-NH_2-9-Pro-ethylamide]-luliberin	B	*b, d*	36.5	0.40	0.66	0.79	1.04		0.90	1.0			0.97	0.95	0.97
[2-de-His-6-D-Azala-10-de-Gly-NH_2-9-Pro-ethylamide]-luliberin	B	*b, d*	32.4	0.38	0.70	0.82	1.05		0.90	1.0			0.96	1.02	0.98
[2-De-His-6-Ala-10-Azgly]-luliberin	B	*b, d*	38.7	0.33	0.70	0.78	1.02		0.95	1.01		0.98	0.98	1.01	0.98

* *a* = Sephadex LH-20 column chromatography in DMF; *b* = Sephadex G-25 column chromatography in 0.4M acetic acid; *c* = partition chromatography on Sephadex G-25 using n-butanol–acetic acid–water (4 : 1 : 5) solvent system; *d* = partition chromatography on Sephadex G-25 using n-butanol–acetic acid–water–pyridine (5 : 1 : 5 :1) solvent system.

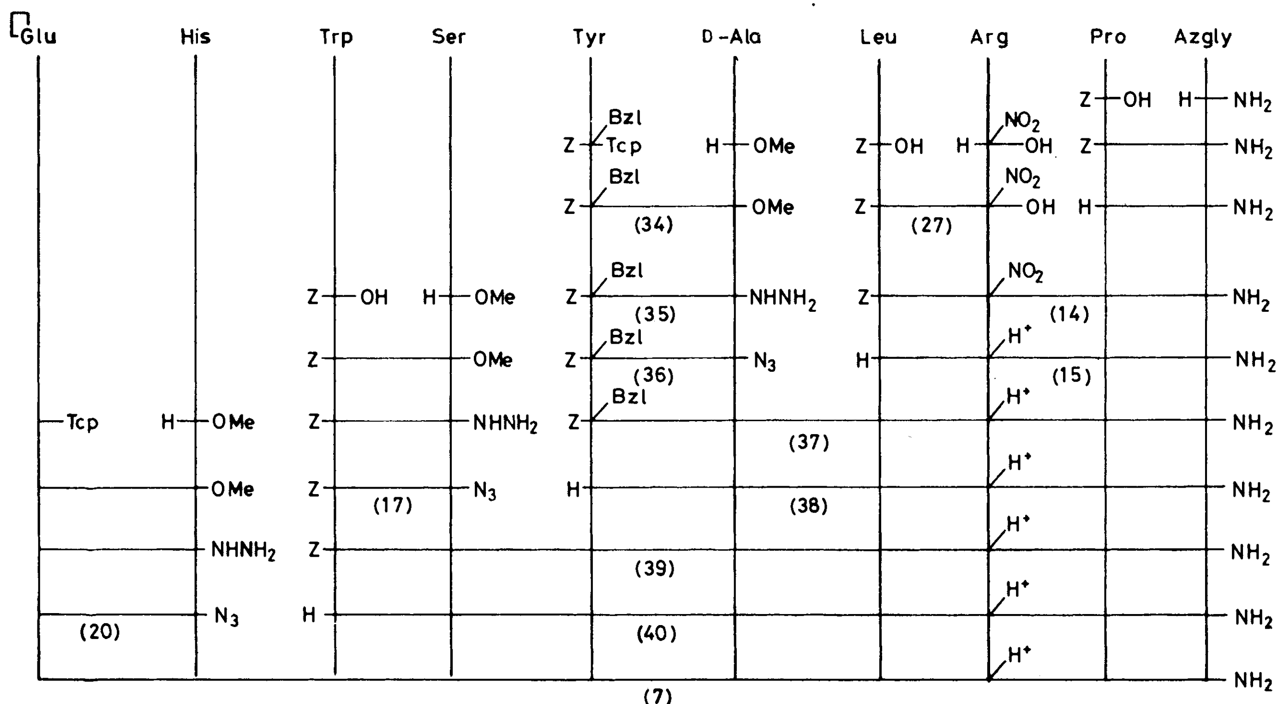


FIGURE 3 Synthesis of [6-D-Ala-10-Azgly]-luliberin. Abbreviations as in Figure 1

The biological activity of the α-aza-analogues was measured in androgen-sterilised constant-oestrus (AFO) rats (Table 2).[2] [10-Azgly]-, [6-Azgly]-, and [6-Azala]-luliberin were marginally less active than luliberin in inducing ovulation; three other analogues, [6-Azgly-10-de-Gly-NH_2-9-Pro-ethylamide]-, [6-Azala-10-de-Gly-NH_2-9-Pro-ethylamide]-, and [6-D-Ala-10-Azgly]-luliberin were about twice as active as luliberin. The de-histidyl analogues (8)—(11) and (13), designed as antagonists, showed no agonist activity in this test even at very high dose levels (500—2 000 μg/rat).

One of the 2-de-His-analogues, [2-de-His-6-Azala-10-de-Gly-NH_2-9-Pro-ethylamide]-luliberin (12) showed a little agonist activity and induced ovulation in 2 out of 3 rats at a dose of 2 mg/rat.

The antagonist activity of the analogues (8)—(13) was measured by injecting the compounds simultaneously with luliberin in the AFO rat model. With the exception of [2-de-His-10-Azgly]-luliberin (8), all were inactive as antagonists. Compound (8) inhibited ovulation induced by luliberin (0.5 μg/rat) completely at a dose of 250 μg/rat and partially at a dose of 62.5—125 μg/rat. When compared with the corresponding non-aza-compound, [2-de-His]-luliberin, reported to have very weak inhibitory activity in an *in vitro* test system,[18] this level of activity *in vivo* is very high.

A strict comparison of the biological activity of the

α-aza-analogues with published data on the corresponding non-aza-compounds is not possible because of the differences in the test systems employed to evaluate these compounds. In general, it appears that azaglycine replacement in position 10 (2) or azaglycine or aza-alanine replacement in position 6 [(3) and (4)] does not produce a significant change in biological activity, but when azaglycine or aza-alanine substitution in position 6 is combined with the replacement of the glycine amide residue in position 10 by ethylamide [(5) and (6)], the biological activity is increased. [6-Azgly-10-de-Gly-NH_2-9-Pro-ethylamide]- and [6-Azala-10-de-Gly-NH_2-9-Pro-ethylamide]-luliberin were about twice as active as luliberin, and they may, therefore, have comparable potency to the corresponding non-aza-analogue, [10-de-Gly-NH_2-9-Pro-ethylamide]-luliberin which was reported to be 1.5 times as active as luliberin.[11,12] It therefore appears that azaglycine or aza-alanine substitution alone in position 6 or 10 does not produce any significant change in the overall conformation of the molecule, but a combination of aza-replacement in position 6 and ethylamide in position 10 results in better interaction with the receptor as in the case of the analogue without any modification in position 6.

L-Alanine substitution in position 6 leads to a significant reduction in the biological activity (25 times less active than luliberin) but D-alanine replacement gives a molecule which is 3—5 times as active as luliberin.[14] Since [6-Azala]-luliberin (4) was as active as luliberin, it would seem that due to the planar structure of the aza-residue the side-chain methyl group does not interfere with the overall conformation but at the same time it does not help to generate a compound with better affinity.

It is difficult to speculate on the high antagonist activity of the [2-de-His-10-Azgly]-luliberin at this time. Since [2-de-His]-luliberin has been shown to be a very weak antagonist and has also been suggested to have some agonist activity, it is possible that in the de-histidyl series azaglycine substitution in position 10 provides compounds with increased affinity towards the receptor site.

TABLE 2

Activity of α-aza-analogues of luliberin in androgen-sterilised, constant-oestrus rats

Compound	Dose (μg/rat i.v.)	Response (no. ovulating/ no. treated)
Luliberin (1)	0.50	4/4
	0.25	3/4
	0.10	0/4
[10-Azgly]-luliberin (2)	0.50	6/6
	0.25	2/6
	0.10	2/6
[6-Azgly]-luliberin (3)	0.50	4/6
	0.25	1/6
	0.10	0/6
[6-Azala]-luliberin (4)	2.0	3/3
	0.50	4/6
	0.25	1/6
[6-Azgly-10-de-Gly-NH_2-9-Pro-ethylamide]-luliberin (5)	0.125	3/3
	0.0625	0/3
[6-Azala-10-de-Gly-NH_2-9-Pro-ethylamide]-luliberin (6)	0.25	3/3
	0.125	2/6
	0.0625	0/3
[6-D-Ala-10-Azgly]-luliberin (7)	0.25	3/3
	0.125	0/6
[2-de-His-10-Azgly]-luliberin (8)	2 000	1/6
	500	0/6
[2-de-His-6-Azgly]-luliberin (9)	1 000	0/3
[2-de-His-6-Azala]-luliberin (10)	1 000	0/3
[2-de-His-6-Azgly-10-de-Gly-NH_2-9-Pro-ethylamide]-luliberin (11)	2 000	0/3
	500	0/3
[2-de-His-6-Azala-10-de-Gly-NH_2-9-Pro-ethylamide]-luliberin (12)	2 000	2/3
	500	0/3
[2-de-His-6-D-Ala-10-Azgly]-luliberin (13)	2 000	0/3
	500	0/3

EXPERIMENTAL

Details of solvent systems, spray reagents used for thin-layer chromatography, and optical rotations, *etc.*, are described in part 13.[26]

N-*Benzyloxycarbonyl*-L-*prolylazaglycine Amide*.—To a stirred and cooled (0 °C) suspension of *N*-benzyloxycarbonyl-L-proline (24.9 g, 100 mmol), semicarbazide hydrochloride (11.2 g, 100 mmol), and triethylamine (14.5 ml, 100 mmol) in dimethylformamide (DMF) (200 ml), dicylohexylcarbodi-imide (DCCI) (20.6 g, 100 mmol) was added and stirring was continued for 16 h at 4 °C. Dicyclohexylurea was removed by filtration and the filtrate was evaporated to a small volume. Water (200 ml) was added and the solution was extracted with ethyl acetate (3 × 50 ml). The product precipitated out of the aqueous solution in about an hour. Recrystallisation from aqueous methanol gave the *dipeptide amide* (16.5 g, 53.9%), m.p. 189—190 °C, $[\alpha]_D^{24}$ −43.6° (*c* 1.4 in DMF), R_{FD} 0.54, R_{FF} 0.52, R_{FH} 0.38, and R_{FK} 0.78 (Found: C, 55.2; H, 6.0; N, 18.6. $C_{14}H_{18}N_4O_4$ requires C, 54.9; H, 5.9; N, 18.3%).

N-*Benzyloxycarbonyl*-L-*leucyl*-(N$^{\omega}$-*nitro*)-L-*arginine*.—Ethyl chloroformate (2.83 ml, 29.5 mmol) was added to a solution of *N*-benzyloxycarbonyl-L-leucine (8.24 g, 31 mmol) and triethylamine (4.55 ml, 32.5 mmol) in THF (100 ml)

at −10 to −15 °C. The reaction mixture was stirred for 3 min at this temperature and was then poured into a vigorously stirred solution of N^{ω}-nitro-L-arginine (5.79 g, 31 mmol) in 2N sodium hydroxide (15.5 ml, 31 mmol) and DMF (50 ml) at −10 °C. Stirring was continued at −10 °C for 30 min and then at room temperature for 1 h. The solvents were removed *in vacuo* and the residue was distributed between ethyl acetate (50 ml) and water (50 ml). The aqueous phase was separated and extracted with two further portions of ethyl acetate. The combined organic phases were washed once more with water (25 ml) and then discarded. The combined aqueous phases were acidified with saturated citric acid solution and extracted with ethyl acetate (3 × 100 ml). The ethyl acetate extracts were combined, washed with water, dried (Na_2SO_4), and evaporated to dryness. Recrystallisation of the residue from ethyl acetate–light petroleum gave the *dipeptide* (8.98 g, 62%), m.p. 150—165 °C (decomp) (Found: C, 51.4; H, 6.5; N, 18.2. $C_{20}H_{30}N_6O_7$ requires C, 51.5; H, 6.6; N, 18.0%).

N-*Benzyloxycarbonyl*-L-*leucyl*-(N^{ω}-*nitro*)-L-*arginyl*-L-*prolylazaglycine Amide*.—A solution of *N*-benzyloxycarbonyl-L-leucyl-(N^{ω}-nitro)-L-arginine (9.2 g, 20 mmol), L-prolylazaglycine amide hydrochloride (4.2 g, 20 mmol, prepared by the catalytic reduction of the benzyloxycarbonyl derivative in DMF in the presence of 2 equiv. of hydrochloric acid), hydroxybenzotriazole (5.4 g, 40 mmol), and triethylamine (3 ml, 20 mmol) in DMF (200 ml) was cooled to 0 °C and DCCI (8.2 g, 40 mmol) was added. The reaction mixture was stirred overnight at room temperature, dicyclohexylurea was removed by filtration and the filtrate was evaporated to dryness. Recrystallisation of the residue from methanol–ether gave the *tetrapeptide derivative* (12.2 g, 98.3%), m.p. 88—90 °C, $[\alpha]_D^{24}$ −30.2° (*c* 1.6 in DMF), R_{FD} 0.57, R_{FF} 0.40, R_{FH} 0.26, and R_{FK} 0.63 (Found: C, 50.2; H, 6.2; N, 22.7. $C_{26}H_{40}N_{10}O_8$ requires C, 50.3; H, 6.4; N, 22.5%).

N-*Benzyloxycarbonyl*-O-*benzyl*-L-*tyrosylglycine t-Butyl Ester*.—Glycine t-butyl ester (8.1 g, 62.4 mmol) was reacted overnight at room temperature with *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosine 2,4,5-trichlorophenyl ester (36.5 g, 62.4 mmol) in DMF (150 ml). The solvent was removed *in vacuo* and the residue was crystallised from ethyl acetate–light petroleum to give the *dipeptide derivative* (16.16 g, 49.4%), m.p. 122—123 °C, R_{FD} 0.75, R_{FF} 0.74, R_{FG} 0.50, R_{FH} 0.80, and R_{FQ} 0.76 (Found: C, 69.5; H, 6.5; N, 5.5. $C_{30}H_{34}N_2O_6$ requires C, 69.3; H, 6.5; N, 5.4%).

N-*Benzyloxycarbonyl*-L-*tryptophyl*-L-*serine Methyl Ester*.—To a vigorously stirred and cooled (−20 °C) solution of *N*-benzyloxycarbonyl-L-tryptophan (33.84 g, 100 mmol) and *N*-methylmorpholine (11.0 ml, 100 mmol) in THF (200 ml), was added ethyl chloroformate (9.0 ml, 95 mmol). After 2 min a pre-cooled (−20 °C) solution of L-serine methyl ester hydrochloride (17.10 g, 110 mmol) and *N*-methylmorpholine (12.1 ml, 110 mmol) in DMF (150 ml) was added and the stirring was continued at −20 °C for 30 min, and then at room temperature for 3 h. *N*-Methylmorpholine hydrochloride was removed by filtration and the filtrate was evaporated to dryness. The remaining oil was dissolved in ethyl acetate and the solution was washed with 20% citric acid solution, water, saturated sodium hydrogencarbonate solution, and water, dried (Na_2SO_4) and then solvent evaporated off to leave an oil. Two crystallisations from ethyl acetate–light petroleum gave the *dipeptide derivative* (30.53 g, 69.5%), m.p. 140.5—141 °C; $[\alpha]_D^{24}$ −22.13° (*c* 1.4 in DMF) (Found: C, 62.6; H, 5.7; N, 9.4. $C_{23}H_{25}N_3O_6$ requires C, 62.8; H, 5.6; N, 9.5%).

N-*Benzyloxycarbonyl*-L-*tryptophyl*-L-*serine Hydrazide*.—The preceding ester (30.53 g. 69.5 mmol) was dissolved in methanol (1 l) and a solution of hydrazine hydrate (62%, 15 ml) was added to it. After 16 h the *hydrazide* was filtered off, washed with methanol and ether, and crystallised from hot ethanol (23.18 g, 75.8%), m.p. 178—179 °C, $[\alpha]_D^{24}$ −25.27° (*c* 1.0 in DMF), R_{FD} 0.65, R_{FE} 0.20, R_{FF} 0.43, and R_{FH} 0.50 (Found: C, 59.9; H, 5.7; N, 16.0. $C_{22}H_{25}N_5O_5$ requires C, 60.1; H, 5.3; N, 15.9%).

N-*Benzyloxycarbonyl*-L-*tryptophyl*-L-*seryl*-L-*tyrosylglycine t-Butyl Ester*.—*N*-Benzyloxycarbonyl-L-tryptophyl-L-serine hydrazide (0.659 g, 1.5 mmol) in DMF (3 ml) was converted to the corresponding azide by treatment with 5.7N HCl in dioxan (1.05 ml, 6.0 mmol) and t-butyl nitrite (162 mg, 1.57 mmol) at −20 °C for 30 min A pre-cooled solution of L-tyrosylglycine t-butyl ester (417 mg, 1.5 mmol) (prepared by hydrogenating the benzyloxycarbonyl dipeptide over 5% palladium–charcoal for 6 h) and triethylamine (607 mg, 6.0 mmol) in DMF (20 ml) was mixed with the azide and the reaction mixture was stirred at 4 °C for four days. DMF was removed *in vacuo* and the residue was partitioned between ethyl acetate and water. The ethyl acetate layer was washed with citric acid solution, saturated sodium hydrogencarbonate solution, and water, dried, and evaporated to dryness *in vacuo*. The yellow solid was recrystallised from ethyl acetate–cyclohexane (790 mg, 75%), $[\alpha]_D^{25}$ −16.4° (*c* 1.0 in methanol), R_{FD} 0.75, R_{FE} 0.36, R_{FF} 0.72, R_{FH} 0.72, R_{FP} 0.26, and R_{FQ} 0.52 (Found: C, 63.3; H, 6.2; N, 10.2. $C_{37}H_{43}N_5O_9$ requires C, 63.3; H, 6.1; N, 9.9%).

L-*Pyroglutamyl*-L-*histidyl*-L-*tryptophyl*-L-*seryl*-L-*tyrosylglycine t-Butyl Ester*.—L-Pyroglutamyl-L-histidine hydrazide (1.74 g, 6.24 mmol) was converted to the azide as described earlier. A solution of L-tryptophyl-L-seryl-L-tyrosylglycine t-butyl ester (3.20 g, 5.64 mmol; prepared by hydrogenating the benzyloxycarbonyl derivative on 5% palladium–charcoal for 8 h) and triethylamine (3.5 ml, 25.0 mmol) in DMF (20 ml) was added and the reaction mixture was stirred overnight at 4 °C. Triethylamine hydrochloride was removed by filtration and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in a mixture of chloroform–methanol–water (11 : 8 : 2, 10 ml), loaded on a silica column (300 g) and the column was eluted with the same solvent mixture to give the *hexapeptide derivative* (3.95 g, 84%), R_{FD} 0.67, and R_{FF} 0.30 (Found: C, 58.8; H, 6.3; N, 15.1. $C_{40}H_{49}N_9O_{10}$ requires C, 58.8; H, 6.0; N, 15.4%).

L-*Pyroglutamyl*-L-*histidyl*-L-*tryptophyl*-L-*seryl*-L-*tyrosylglycyl*-L-*leucyl*-L-*arginyl*-L-*prolylazaglycine Amide* [10-*Azaglycine*]-*luliberin*.—To a cooled (0 °C) and stirred solution of L-pyroglutamyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosylglycine hydrochloride (79.6 mg, 0.10 mmol, prepared by treating the t-butyl ester with trifluoroacetic acid and converting it to the hydrochloride) was added L-leucyl-L-arginyl-L-prolylazaglycine amide dihydrochloride (51.4 mg, 0.10 mmol), 1-hydroxybenzotriazole (27.0 mg, 0.20 mmol) in DMF (1 ml), and *N*-methylmorpholine (1 mmol ml^{-1} solution, 0.20 ml, 0.20 mmol), followed by DCCI (22.7 mg, 0.11 mmol). After stirring for 2 days at room temperature more DCCI (4 mg) was added and the stirring was continued for three days. DMF was evaporated off and the residue, in 1N acetic acid (2 ml), was applied to a Sephadex G-25 column and the column was eluted with the same

solvent. The fractions containing the decapeptide were combined and evaporated to dryness. Final purification by partition column chromatography on Sephadex G-25 using the solvent system n-butanol–water–acetic acid–pyridine (5 : 5 : 1 : 1), gave [10-azaglycine]-luliberin (48 mg, 33.5%), $[\alpha]_D^{24}$ 55.8° (*c* 1.02 in water).

N-*t-Butoxycarbonyl*-L-*prolyglycine Amide*.—DCCI (25.6 g, 124 mmol) was added to a stirred solution of *N*-t-butoxycarbonyl-L-proline (26.7 g, 124 mmol), glycine amide (9.25 g, 124 mmol), and 1-hydroxybenzotriazole (23.9 g, 177 mmol) in DMF (150 ml) at 0 °C. Stirring was continued for 1 h at 0 °C and then overnight at room temperature. Dicyclohexylurea was removed by filtration, the filtrate was evaporated to dryness, and the residue was partitioned between ethyl acetate and water. The aqueous phase was extracted with n-butanol saturated with water (7 × 200 ml) and the n-butanol portions were then extracted in a counter-current manner first with a saturated solution of sodium hydrogencarbonate (20 × 100 ml) and then with water saturated with n-butanol (5 × 50 ml). The n-butanol layers were combined and evaporated to dryness *in vacuo* and the residue was filtered with ether to give the *dipeptide amide* (17.25 g, 40%), m.p. 136—137 °C, $[\alpha]_D^{25}$ −39.61° (*c* 1.05 in methanol), R_{FA} 0.78, R_{FB} 0.70, R_{FC} 0.60, R_{FD} 0.54, R_{FE} 0.16, R_{FF} 0.48, R_{FH} 0.44, and R_{FK} 0.90 (Found: C, 52.1; H, 8.1; N, 15.1; $C_{12}H_{21}N_3O_4$ requires C, 52.2; H, 7.9; N, 15.2%).

N$^{\alpha}$-*t-Butoxycarbonyl*-N$^{\omega}$-*nitro*-L-*arginyl*-L-*prolylglycine Amide*.—A solution of the preceding dipeptide (8.14 g, 30 mmol) in glacial acetic acid (60 ml) was treated with 5N hydrogen chloride in ethyl acetate (18 ml, 90 mmol) for 1 h. Anhydrous ether (1 500 ml) was added and the hydrochloride was collected and reprecipitated from methanol–ether. The hydrochloride (4.6 g, 22.4 mmol) was dissolved in DMF (50 ml) along with N^{α}-t-butoxycarbonyl-N^{ω}-nitro-L-arginine (7.14 g, 22.4 mmol), 1-hydroxybenzotriazole (4.5 g, 33.6 mmol), and triethylamine (3.2 ml, 22.4 mmol) at 0 °C and DCCI (5.06 g, 24.6 mmol) was added. The reaction mixture was then stirred overnight at 4 °C. Dicyclohexylurea was filtered off and the filtrate was evaporated *in vacuo* to leave a solid, which was dissolved in 5% aqueous acetic acid (200 ml) and the solution extracted with ethyl acetate (3 × 50 ml). The aqueous phase was further extracted with n-butanol saturated with 5% aqueous acetic acid (20 × 100 ml). The n-butanol extracts were then extracted with 5% aqueous acetic acid saturated with n-butanol (10 × 50 ml) in a counter-current manner. The combined n-butanol extracts were evaporated to dryness *in vacuo* and the residue was filtered off with ether and dried (7.28 g). The tripeptide derivative thus obtained was contaminated with a small amount of N^{ω}-t-butoxycarbonyl-*N*-nitro-L-arginine. It was divided into two equal parts and each half was chromatographed on a silica gel column (500 g). Each column was eluted with chloroform (350 ml), 5% methanol–chloroform (2 000 ml), and 10% methanol–chloroform (6 800 ml). The fractions containing the desired tripeptide derivative were combined and evaporated to yield the *tripeptide derivative* as a white powder (6.39 g, 60.4%), m.p. 115 °C (decomp.), $[\alpha]_D^{24}$ −27.96° (*c* 1.0 in DMF), R_{FA} 0.45, R_{FB} 0.81, R_{FC} 0.48, R_{FD} 0.57, R_{FF} 0.42, R_{FH} 0.23, and R_{FK} 0.86 (Found: C, 45.5; H, 6.8; N, 23.7. $C_{18}H_{32}N_7O_8$ requires C, 45.7; H, 6.8; N, 23.7%).

1-(N-*Benzyloxycarbonyl*-O-*benzyl*-L-*tyrosyl*)-2-*t-butoxycarbonyl*-2-*methyl Hydrazide*.—A solution of 1-t-butoxycarbonyl-1-methyl hydrazide [26] (3.21 g, 22.0 mmol) and *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosine 2,4,5-trichlorophenyl ester (11.71 g, 20.0 mmol) in DMF (20 ml) was kept at room temperature overnight. It was diluted with ethyl acetate (250 ml), washed with 20% aqueous citric acid and water, dried (Na_2SO_4), and the solvent evaporated off *in vacuo*. Recrystallisation of the residue from ether–cyclohexane gave the *hydrazide* (6.99 g, 65.6%), m.p. 102—104 °C, $[\alpha]_D^{24}$ −15.5° (*c* 1.0 in methanol), R_{FD} 0.76, R_{FE} 0.68, R_{FF} 0.76, and R_{FH} 0.74 (Found: C, 66.9; H, 6.5; N, 7.6. $C_{30}H_{35}N_3O_6$ requires C, 66.9; H, 6.6; N, 7.8%).

N-*Benzyloxycarbonyl*-O-*benzyl*-L-*tyrosylaza-alanyl*-L-*leucine Methyl Ester*.—The preceding hydrazide (5.87 g, 11.0 mmol) in ethyl acetate (50 ml) was treated with 4.5N hydrogen chloride in ethyl acetate (20 ml) for 2 h, and the hydrochloride (3.46 g, 67.5%) was isolated as described before. The hydrochloride (3.28 g, 7 mmol) in dry THF (100 ml) was treated with triethylamine (1.11 ml, 7.7 mmol) and *N*-carbonyl-L-leucine methyl ester (1.19 g, 7 mmol) and the reaction mixture was set aside overnight at room temperature. The solvent was removed *in vacuo* and the residue in ethyl acetate was washed with water and dried (Na_2SO_4). Evaporation of solvent followed by recrystallisation of the residue from ethyl acetate–light petroleum yielded the *tripeptide derivative* (3.93 g, 93.1%), m.p. 145—146 °C, $[\alpha]_D^{25}$ +8.7° (*c* 1.2 in methanol), R_{FA} 0.88, R_{FB} 0.88, R_{FC} 0.83, R_{FD} 0.80, R_{FE} 0.59, R_{FF} 0.78, R_{FH} 0.73 (Found: C, 65.4; H, 6.6; N, 9.3. $C_{33}H_{40}N_4O_7$ requires C, 65.6; H, 6.6; N, 9.2%).

N-*Benzyloxycarbonyl*-O-*benzyl*-L-*tyrosylaza-alanyl*-L-*leucine*.—1N Sodium hydroxide (12 ml, 12 mmol) was added to a stirred solution of the preceding methyl ester (2.41 g, 4 mmol) in methanol (36 ml) at room temperature and the stirring was continued for 3 h. Methanol was removed *in vacuo* and an aqueous solution (40 ml) of the residue was acidified with citric acid (pH 3) and extracted with ethyl acetate. After washing the ethyl acetate extract with water and drying (Na_2SO_4), the solvent was evaporated off and the residue, in a mixture of DMF–water (3 : 2, 200 ml) was applied to a column of AG 1-X2 resin (100 ml). The column was washed with the above solvent (50 ml) and the tripeptide was eluted with 0.2M acetic acid in DMF–water (3 : 2). The tripeptide-containing fractions were combined, evaporated to dryness *in vacuo*, and the residue triturated with ether and collected by filtration (1.22 g, 51.7%), m.p. 195 °C (decomp.), $[\alpha]_D^{24}$ −25.4° (*c* 1.0 in DMF) (Found: C, 65.2; H, 6.3; N, 9.4. $C_{32}H_{38}N_4O_7$ requires C, 65.0; H, 6.4; N, 9.4%).

N-*Benzyloxycarbonyl*-O-*Benzyl*-L-*tyrosylaza-alanyl*-L-*leucyl*-(N$^{\omega}$-*nitro*)-L-*arginyl*-L-*prolylglycine Amide*.—A solution of *N*-t-butoxycarbonyl-(N^{ω}-nitro)-L-arginyl-L-prolylglycine amide (1.41 g, 3.0 mmol) in glacial acetic acid (10 ml) was treated with 5N hydrogen chloride in ethyl acetate (3 ml, 15.0 mmol) for 1 h at room temperature. Acetic acid was removed *in vacuo* and the hydrochloride was obtained as described before (1.20 g, 98.5%).

DCCI (364 mg, 1.76 mmol) was added to a stirred solution of the above hydrochloride (720 mg, 1.76 mmol), triethylamine (0.25 ml, 1.76 mmol), *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosylaza-alanyl-L-leucine (946 mg, 1.60 mmol), and 1-hydroxybenzotriazole (433 mg, 3.20 mmol) in DMF (15 ml) at 0 °C and the stirring was continued for three days at 0—4 °C. Dicyclohexylurea was filtered off and the filtrate was evaporated to dryness. The residue, in ethyl acetate (200 ml) was washed with water, saturated sodium

hydrogencarbonate solution, and saturated sodium chloride solution, and dried (Na_2SO_4). The solution was concentrated to a small volume (10 ml) and the product was precipitated with ether to give the *hexapeptide* (1.45 g, 96%), m.p. 137—140 °C, $[\alpha]_D^{24}$ −27.3° (*c* 1.0 in DMF), R_{FA} 0.61, R_{FB} 0.71, R_{FC} 0.66, R_{FD} 0.65, R_{FF} 0.48, and R_{FH} 0.42 (Found: C, 55.8; H, 6.5; N, 16.9. $C_{45}H_{60}N_{12}O_{11}\cdot 1.5\ H_2O$ requires 55.6; H, 6.5; N, 17.3%). The following amino-acid ratios were found after acid hydrolysis (6N HCl, 16 h): Gly 1.0, Pro 0.97, Leu 0.97, Tyr 0.94, Arg + Orn 0.93.

N-*Benzyloxycarbonyl*-L-*tryptophyl*-L-*seryl*-L-*tyrosylaza-alanyl*-L-*leucyl*-L-*arginyl*-L-*prolylglycine Amide Hydrochloride*.—The preceding hexapeptide (1.70 g, 1.80 mmol) was hydrogenated in 80% acetic acid (100 ml) over 5% palladium–carbon (0.37 g) for 16 h. After removing the solvent *in vacuo* the residue was taken up in water (5 ml), treated with 1N hydrochloric acid (4 ml) and freeze-dried (yield 1.35 g, 100%).

t-Butyl nitrite (1 mmol ml^{-1} solution in DMF, 2.08 ml, 2.08 mmol) was added to a vigorously stirred solution of *N*-benzyloxycarbonyl-L-tryptophyl-L-serine hydrazide (87 mg, 1.98 mmol) in DMF (6 ml) and 5.7N hydrogen chloride in dioxan (1.39 ml, 7.92 mmol) at −20 °C and the stirring was continued for 20 min. Triethylamine (1.15 ml, 7.92 mmol) was added followed by a pre-cooled (−20 °C) solution of the above hexapeptide dihydrochloride (1.35 g, 1.8 mmol) and triethylamine (0.26 ml, 1.8 mmol) in dimethylformamide (6 ml). The pH of the reaction was adjusted to 7.5 and the stirring was continued for 72 h at 0—4 °C. The solvent was evaporated off *in vacuo* and the residue was partitioned between ethyl acetate and water. The aqueous phase was separated and the organic phase was washed with water (2 × 50 ml). The combined aqueous phases were evaporated to dryness and the residue, dissolved in n-butanol saturated with water (30 ml), was extracted with water saturated with n-butanol (6 × 20 ml) and each of these aqueous extracts were back-extracted with n-butanol saturated with water. All the n-butanol phases were combined, evaporated to dryness and the octapeptide was further purified by column chromatography on silica gel (500 g). The column was eluted with chloroform–methanol–water (11 : 8 : 2) and evaporation of the product-containing fractions gave the pure *octapeptide* (904 mg, 46.4%), m.p. 159—160 °C, $[\alpha]_D^{24}$ −28.16° (*c* 1.0 in methanol), R_{FA} 0.44, R_{FB} 0.66, R_{FC} 0.25, R_{FD} 0.18, and R_{FK} 0.90. (Found: C, 55.6; H, 6.4; N, 17.8. $C_{52}H_{70}N_{14}O_{12}\cdot HCl$ requires C, 55.7; H, 6.2; N, 17.5%). Amino-acid analysis of the acid hydrolysate gave the following amino-acid ratios: Gly 1.0, Pro 1.01, Ser 0.95, Leu 0.97, Tyr 1.04, Arg 0.99.

1-(N-*Benzyloxycarbonyl*-O-*benzyl*-L-*tyrosyl*)-2-*t-butoxycarbonyl Hydrazide*.—This was prepared by the procedure already described above for 1-(*N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl)-2-t-butoxycarbonyl-2-methyl hydrazide except that t-butoxycarbonyl hydrazide was used in place of 1-t-butoxycarbonyl-1-methyl hydrazide. Recrystallisation from ether–light petroleum yielded the *hydrazide* as a white powder (67%), m.p. 126—127 °C, $[\alpha]_D^{25}$ −13.2° (*c* 1.0 in methanol, R_{FD} 0.82, R_{FE} 0.65, R_{FF} 0.63, and R_{FH} 0.70 (Found: C, 67.3; H, 6.2; N, 7.9. $C_{29}H_{33}N_3O_6$ requires C, 67.4; H, 6.4; N, 8.1%).

N-*Benzyloxycarbonyl*-O-*benzyl*-L-*tyrosylazaglycyl*-L-*leucine Methyl Ester*.—The procedure used for the preparation of this compound was similar to the one described for the corresponding aza-alanyl tripeptide except that 1-(*N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl)-2-t-butoxycarbonyl hydrazide was used in place of the 1-(*N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl)-2-t-butoxycarbonyl-2-methyl hydrazide. Recrystallisation from ethyl acetate–light petroleum gave the *azatripeptide* derivative (77.7%), m.p. 156—157 °C, $[\alpha]_D^{24}$ −10.3° (*c* 1.0 in methanol), R_{FD} 0.81, R_{FE} 0.45, R_{FP} 0.26, and R_{FQ} 0.47 (Found: C, 64.9; H, 6.4; N, 9.5. $C_{32}H_{38}N_4O_7$ requires C, 65.0; H, 6.5; N, 9.5%).

N-*Benzyloxycarbonyl*-O-*benzyl*-L-*tyrosylazaglycyl*-L-*leucine Hydrazide*.—Hydrazine hydrate (5 ml, 100 mmol) was added to a solution of the preceding methyl ester (2.95 g, 5 mmol) in methanol (50 ml). After 2 h at room temperature the *hydrazide* was precipitated with water and recrystallised from methanol–ether (yield 2.74 g, 92.8%), m.p. 169—170 °C, $[\alpha]_D^{24}$ −9.05° (*c* 1 in dimethylformamide), R_{FA} 0.76, R_{FB} 0.75, R_{FC} 0.73, R_{FD} 0.63, R_{FF} 0.60, and R_{FH} 0.55 (Found: C, 62.8; H, 6.4; N, 14.0. $C_{31}H_{38}N_6O_6$ requires C, 63.0; H, 6.4; N, 14.2%).

N-*Benzyloxycarbonyl*-O-*benzyl*-L-*tyrosylazaglycyl*-L-*leucyl*-(N$^{\omega}$-*nitro*)-L-*arginyl*-L-*prolylglycine Amide*.—A solution of t-butyl nitrite (1 mmol ml^{-1}, 0.76 mmol) was added to a stirred and cooled (−20 °C) reaction mixture containing *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosylazaglycyl-L-leucine hydrazide (455 mg, 0.72 mmol), a 4.14N solution of hydrogen chloride in dioxan (0.70 ml, 2.90 mmol) and DMF (5 ml). After 5 min a pre-cooled (−20 °C) solution of N^{ω}-nitro-L-arginyl-L-prolyglycine amide (245 mg, 0.60 mmol) and triethylamine (0.5 ml, 3.5 mmol) in DMF was added and the reaction mixture was stirred for 48 h at 0—4 °C. DMF was removed by evaporation and the residue, in ethyl acetate (500 ml), was washed with water, 20% citric acid solution, saturated sodium hydrogencarbonate solution, and water. Evaporation of ethyl acetate *in vacuo* left a white solid which was purified by column chromatography on silica gel (50 g). The column was eluted with chloroform (100 ml), 5% methanol–chloroform (100 ml), and 10% methanol–chloroform (1 200 ml). Evaporation of fractions containing *hexapeptide derivative* gave 235 mg (41.5%), m.p. 147—149 °C, $[\alpha]_D^{24}$ −29.6° (*c* 1.1 in methanol), R_{FA} 0.80, R_{FB} 0.93, R_{FC} 0.63, and R_{FD} 0.69 (Found: C, 54.4; H, 6.3; N, 17.2. $C_{44}H_{58}N_{12}O_{11}\cdot 2H_2O$ requires C, 54.6; H, 6.5; N, 17.4%).

N-*Benzyloxycarbonyl*-L-*tryptophyl*-L-*seryl*-L-*tyrosylaza-glycyl*-L-*leucyl*-L-*arginyl*-L-*prolylglycine Amide Hydrochloride*.— *N*-Benzyloxycarbonyl-L-tryptophyl-L-serine hydrazide (242 mg, 0.55 mmol) was converted into the azide, coupled with L-tyrosylazaglycyl-L-leucyl-L-arginyl-L-prolylglycine amide dihydrochloride (367 mg, 0.50 mmol) (prepared by hydrogenating the protected hexapeptide derivative over 5% palladium–charcoal in the presence of 2 equiv. of hydrochloric acid), and purified by counter-current distribution, as described before for the corresponding aza-alanyl octapeptide (yield 215 mg, 40.2%), m.p. 165—167 °C, $[\alpha]_D^{25}$ −41.3° (*c* 1.1 in DMF), R_{FA} 0.49, R_{FB} 0.72, R_{FC} 0.32, R_{FD} 0.10, and R_{FK} 0.90 (Found: C, 55.2; H, 6.3; N, 17.6. $C_{51}H_{68}N_{14}O_{12}\cdot HCl$ requires C, 55.4; H, 6.1; N, 17.9%).

N-*Benzyloxycarbonyl*-L-*proline Ethylamide*.— *N*-Benzyloxycarbonyl-L-proline (19.94 g, 80 mmol) and *N*-methylmorpholine (8.8 ml, 80 mmol) were dissolved in dry THF (200 ml) and the solution was cooled to −20 °C. Ethyl chloroformate (7.15 ml, 76 mmol) was added dropwise and after stirring for 2 min a pre-cooled (−20 °C) 70% aqueous solution of ethylamine (20 ml, 300 mmol) was added and stirring was continued for 18 h at 4 °C. The solvent was

removed *in vacuo* and the residue was partitioned between ethyl acetate and 20% aqueous citric acid solution. The ethyl acetate layer was separated, washed with saturated sodium hydrogencarbonate solution and water, dried (Na_2SO_4), and evaporated to dryness *in vacuo*. The residue was crystallised from ethyl acetate–light petroleum (yield 12.97 g, 58.7%), m.p. 107—108 °C, $[\alpha]_D^{25.5}$ −43.88° (*c* 1.0 in methanol), R_{FD} 0.69 R_{FE} 0.53, R_{FF} 0.67, R_{FH} 0.62, R_{FP} 0.57, and R_{FQ} 0.66 (Found: C, 65.1; H, 7.3; N, 10.1. $C_{15}H_{20}N_2O_3$ requires C, 65.2; H, 7.3; N, 10.1%).

N^{α}-*t-Butoxycarbonyl*-N^{ω}-*nitro*-L-*arginyl*-L-*proline Ethylamide*.—This was prepared by the same procedure as described earlier for N^{α}-t-butoxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolylglycine amide, except that L-proline ethylamide was used in place of L-prolyglycine amide (yield 89%), m.p. 109—111 °C (decomp.), $[\alpha]_D^{25}$ −39.0° (*c* 1.0 in methanol), R_{FA} 0.62, R_{FB} 0.74, R_{FC} 0.59, R_{FD} 0.70, R_{FE} 0.20, R_{FF} 0.60, R_{FH} 0.61, R_{FK} 0.85, R_{FQ} 0.13 (Found: C, 48.7; H, 7.4; N, 22.5; $C_{18}H_{33}N_7O_6$ requires C, 48.7; H, 7.5; N, 22.3%).

N-*Benzyloxycarbonyl*-O-*benzyl*-L-*tyrosylazaglycyl*-L-*leucyl*-(N^{ω}-*nitro*)-L-*arginyl*-L-*proline Ethylamide*.—This was prepared by coupling *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosylazaglycyl-L-leucine azide, as described before with N^{ω}-nitro-L-arginyl-L-proline ethylamide. Purification was achieved by silica gel column chromatography, using chloroform and 3% methanol–chloroform as eluting solvent (yield 38.5%), R_{FA} 0.71, R_{FB} 0.72, and R_{FC} 0.84 (Found: C, 58.4; H, 6.7; N, 16.8. $C_{44}H_{59}N_{11}O_{10}$ requires C, 58.6; H, 6.6; N, 17.1%).

N-*Benzyloxycarbonyl*-L-*tryptophyl*-L-*seryl*-L-*tyrosylazaglycyl*-L-*leucyl*-L-*arginyl*-L-*proline Ethylamide*.—*N*-Benzyloxycarbonyl-L-tryptophyl-L-serine hydrazide was converted into the azide as described before and coupled with L-tyrosylazaglycyl-L-leucyl-L-arginyl-L-proline ethylamide hydrochloride (yield 52.9%), $[\alpha]_D^{25}$ −16.84° (*c* 1.5 in methanol), R_{FA} 0.61, R_{FC} 0.36, R_{FD} 0.67, and R_{FK} 0.90.

N-*Benzyloxycarbonyl*-O-*benzyl*-L-*tyrosylazalanyl*-L-L-*leucyl*-(N^{ω}-*nitro*)-L-*arginyl*-L-*proline Ethylamide*.—The preparation of this was similar to that of the corresponding azaglycyl compound, yield 42.8%, $[\alpha]_D^{25}$ −25.9° (*c* 1.0 in methanol), R_{FA} 0.72, R_{FB} 0.76, and R_{FC} 0.85 (Found: C, 58.1; H, 6.7; N, 17.7. $C_{45}H_{61}N_{12}O_{10}$ requires C, 58.1; H, 6.6; N, 18.0%).

N-*Benzyloxycarbonyl*-L-*tryptophyl*-L-*seryl*-L-*tyrosylazaalanyl*-L-*leucyl*-L-*arginyl*-L-*proline Ethylamide Hydrochloride*.— *N*-benzyloxycarbonyl-L-tryptophyl-L-serine hydrazide (264 mg, 0.6 mmol) was converted into the azide (as described before) and coupled with L-tyrosylaza-alanyl-L-leucyl-L-arginyl-L-proline ethylamide hydrochloride (277 mg, 0.4 mmol). The crude peptide was purified by silica gel column chromatography using chloroform, systems P and Q as eluting solvents. Further purification by Sephadex LH-20 column chromatography in DMF gave the octapeptide derivative yield 414 mg, 42.5%), $[\alpha]_D^{24.5}$ −41.4° (*c* 1.3 in methanol), R_{FA} 0.80, R_{FC} 0.47, R_{FD} 0.65, and R_{FK} 0.95. Amino-acid ratios after 16 h acid digest; Arg 1.06, Ser 0.87, Pro 0.98, Ala 0.96, Leu 1.0, and Tyr 1.03.

N-*Benzyloxycarbonyl*-O-*benzyl*-L-*tyrosyl*-D-*alanine Methyl Ester*.— *N*-Benzyloxycarbonyl-*O*-benzyl-L-tyrosine 2,4,5-trichlorophenyl ester (6.48 g, 11.0 mmol) and D-alanine methyl ester hydrochloride (1.39 g, 10 mmol) were dissolved in DMF (50 ml) and triethylamine (1.4 ml, 10.0 mmol) was added to the solution which was then stirred overnight at room temperature. The reaction mixture was worked-up in the usual manner, and the residue was crystallised from hot ethyl acetate to yield 3.78 g (77.2%) of the protected dipeptide methyl ester, m.p. 163 °C, $[\alpha]_D^{24.8}$ −12.84° (*c* 1.1 in DMF) (Found: C, 68.5; H, 6.2; N, 5.6. $C_{28}H_{30}N_2O_6$ requires C, 68.5; H, 6.1; N, 5.7%).

N-*Benzyloxycarbonyl*-O-*benzyl*-L-*tyrosyl*-D-*alanine Hydrazide*.—The preceding methyl ester (3.43 g, 7.0 mmol) was dissolved in warm methanol (400 ml) and the solution was treated with 62% w/v hydrazine hydrate (10 ml, 120 mmol) and the mixture was set aside at 25 °C overnight. The hydrazide was filtered off, washed with methanol and ether, and crystallised twice from boiling methanol (yield 3.06 g, 89.2%), m.p. 217 °C, $[\alpha]_D^{24}$ −20.44° (*c* 1.1 in DMF), R_{FA} 0.73, R_{FB} 0.75, R_{FC} 0.67, R_{FD} 0.70, R_{FE} 0.50, R_{FF} 0.54, R_{FH} 0.67, R_{FK} 0.85, and R_{FQ} 0.25 (Found: C, 66.3; H, 6.2; N, 11.3. $C_{27}H_{30}N_4O_5$ requires C, 66.4; H, 6.1; N, 11.4%).

N-*Benzyloxycarbonyl*-O-*benzyl*-L-*tyrosyl*-D-*alanyl*-L-*leucyl*-L-*arginyl*-L-*prolylazaglycine Amide*.—To a cooled (−20 °C) and stirred solution of *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl-D-alanine hydrazide (1.18 g, 2.4 mmol) in DMF (10 ml) a 6.02M solution of hydrogen chloride in dioxan (1.6 ml, 9.6 mmol) was added followed by t-butyl nitrite (0.29 ml, 2.52 mmol). After 15 min a pre-cooled (−20 °C) solution of L-leucyl-L-arginyl-L-prolylazaglycine amide dihydrochloride (1.03 g, 2.0 mmol) and triethylamine (1.62 ml, 11.6 mmol) in DMF (15 ml) was added, and the stirring was continued at 4 °C for 24 h. Triethylamine hydrochloride was removed by filtration and the filtrate was evaporated to dryness *in vacuo*. The residue was loaded on a silica gel column and the column was eluted with 5% v/v methanol in chloroform, 10% v/v methanol in chloroform, and chloroform–methanol–water (11 : 8 : 2 v/v). The product-containing fractions were combined and evaporated to dryness and the peptide was chromatographed again on a silica gel column using acetonitrile–water (3 : 1 v/v) as eluting solvent (yield 890 mg, 46.4%), $[\alpha]_D^{25}$ −45.7° (*c* 1.1 in methanol), R_{FA} 0.54, R_{FB} 0.69, and R_{FC} 0.41. Amino-acid ratios; Tyr 0.99, Ala 0.96, Leu 1.0, Arg 1.08, and Pro 0.97.

N-*Benzyloxycarbonyl*-L-*tryptophyl*-L-*seryl*-L-*tyrosyl*-D-*alanyl*-L-*leucyl*-L-*arginyl*-L-*prolylazaglycine Amide*.—The preparation of this was similar to that of *N*-benzyloxycarbonyl-L-tryptophyl-L-seryl-L-tyrosylaza-alanyl-L-leucyl-L-arginyl-L-proline ethylamide (yield 36.5%), $[\alpha]_D^{24.5}$ −24.2° (*c* 0.8 in methanol), R_{FA} 0.57, R_{FC} 0.40, and R_{FD} 0.61. Amino-acid-ratios after a 16-h acid digest; Tyr 1.08, Ser 0.87, Leu 1.0, Arg 1.02, and Pro 0.98.

General Procedure A; Coupling of L-*pyroglutamyl*-L-*histidine Hydrazide to the Hepta- or Octa-peptide Derivatives*.—To a cooled (0 °C) and stirred suspension of L-pyroglutamyl-L-histidine hydrazide (0.2 mmol) in DMF (0.9 ml) and dimethyl sulphoxide (0.7 ml) was added 5.7N hydrogen chloride in dioxan (0.8 mmol). A clear solution was obtained after vigorously stirring for 5 min. The solution was cooled to −20 °C, t-butyl nitrite (0.22 mmol) was added, and the stirring was continued for 25 min. The temperature was then lowered to −30 °C and the solution was neutralised by adding triethylamine (0.8 mmol). A pre-cooled (−20 °C) mixture of L-tryptophyl-L-seryl-L-tryrosyl-A-L-leucyl-L-arginyl-L-prolyl-B-dihydrochloride [0.1 mmol, obtained by the hydrogenolysis of the *N*-benzyloxycarbonyl derivative in aqueous methanol (80% v/v) containing hydrogen chloride (2 equiv.) over 5% w/w palladium–charcoal for 16 h] and triethylamine (0.1 mmol) in DMF (1 ml) was added and the reaction mixture was

stirred for 24 h at 4 °C. DMF was evaporated off *in vacuo* and the residue was chromatographed on Sephadex LH-20 using DMF as eluant. The peptide hydrochloride was further purified by partition chromatography on Sephadex G-25 using the solvent system n-butanol–acetic acid–water–pyridine (5 : 1 : 5 : 1 v/v), or n-butanol–acetic acid–water (4 : 1 : 5).

General Procedure B; Coupling of L-*pyroglutamic Acid* 2,4,5-*Trichlorophenyl Ester to the Hepta- or Octa-peptide Derivatives*.—L-Pyroglutamic acid 2,4,5-trichlorophenyl ester (68 mg, 0.22 mmol) was added to a solution of L-tryptophyl-L-seryl-L-tyrosyl-A-L-leucyl-L-arginyl-L-prolyl-B-dihydrochloride (0.2 mmol) and triethylamine (0.029 ml, 0.2 mmol) in DMF (5 ml), and the reaction mixture was stirred overnight at room temperature. The solvent was removed *in vacuo*, and the residue was partitioned between ethyl acetate and water. The ethyl acetate layer was further extracted with water, and the combined aqueous phases were evaporated to dryness. The crude peptide was purified by gel filtration on Sephadex G-25 in 0.4M acetic acid and by partition chromatography on Sephadex G-25 using the solvent system n-butanol–acetic acid–water–pyridine (5 : 1 : 5 : 1).

The L-tryptophyl-L-seryl-L-tyrosyl-A-L-leucyl-L-arginyl-L-prolyl-B-dihydrochloride was obtained by hydrogenating the *N*-benzyloxycarbonyl derivative over 5% Pd–C in DMF containing 2 equiv. hydrogen chloride.

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