SYNTHESIS OF C-EXTENDED ANALOGS OF SERUM THYMIC FACTOR

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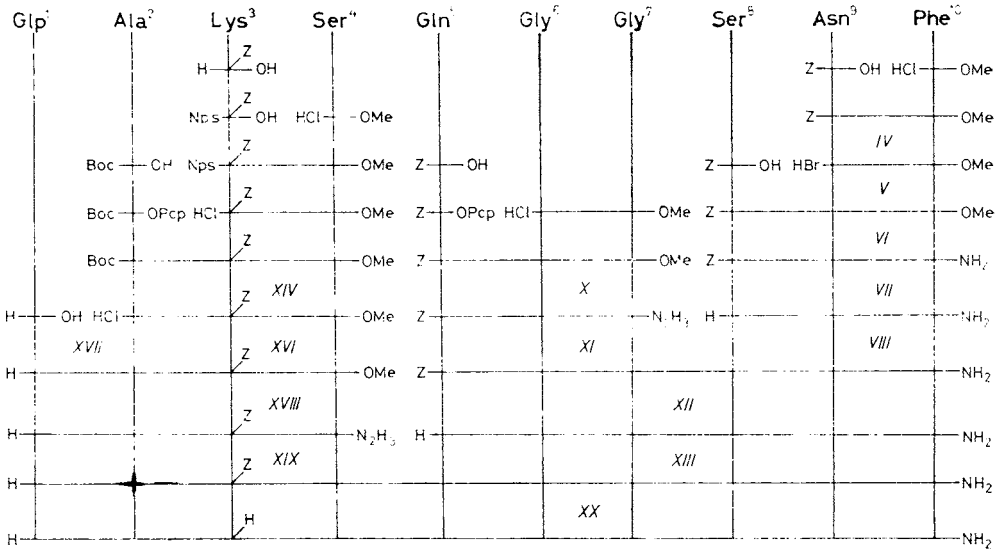
New analogs of the serum thymic factor, (Phe-NH_2^{10})-FTS, (Gln^1, Phe-NH_2^{10})-FTS, and (des Glp^1, Phe-NH_2^{10})-FTS were synthesized. Their biological activities were evaluated in the recovery assay of T lymphocytes. The activity of (Phe-NH_2^{10})-FTS is comparable to the activity of FTS. Nonapeptide (des Glp^1, Phe-NH_2^{10})-FTS shows the highest activity so far observed. Analog (Gln^1, Phe-NH_2^{10})-FTS* is an inhibitor.

Peptides possessing an immunomodulatory effect represent an important group of biologically active compounds[1]. A key position in this group hold thymus hormones[2]. We have focused our interest on the serum thymic factor (FTS) (ref.[3]) and its analogs[4]. The aim of this study was to design and to synthesize analogs with a more pronounced selectivity of their immunomodulatory effects. The FTS analogs which have been reported so far are modified either by substitution or elimination either in the N-terminal part[5] or in the middle part of their polypeptide chains[6]; analogs extended at their C-terminus have been missing as yet. At the same time it is known that biologically active peptides with a free carboxyl group (as in FTS) can form a part of a larger macromolecule from which the active peptide is generated by enzymatic hydrolysis, as, *e.g.* insulin from proinsulin[7] or vespakinin M which represents a bradykinin[8] analog extended at the C-terminus. At present the opiate peptides of the enkephalin[9] type can be regarded as belonging also to this group. The design of our analogs was based on the postulate that the C-extended FTS chain may affect the immunomodulatory activity; another aim of our work was to incorporate an amide group into the C-terminal amino acid of FTS since it has been known that many peptides require a C-terminal amide group for full manifestation of their biological activity[10,11]. The extension of the peptide chain of FTS was limited to the extension by phenylalanine only. To verify the importance of pyroglutamic acid for position 1 also of the C-extended hormone we prepared two more analogs, (des Glp^1, Phe-NH_2^{10})-FTS, and (Gln^1, Phe-NH_2^{10})-FTS.

* Unless stated otherwise all amino acids with the exception of glycine are of L-configuration. The nomenclature and symbols for the compounds comply with the suggestions of the IUPAC-IUB Commission on Biochemical Nomenclature[16,17]. Glp stands for pyroglutamic acid.

(Phe-NH_2^{10})-FTS (*I*) was prepared by azide condensation[12] according to Scheme 1. Nonapeptide (des Glp[1], Phe-NH_2^{10})-FTS (*III*) was prepared by condensation using the carbodiimide method in the presence of 1-hydroxybenztriazole[13] and tripeptide *XV* and hexapeptide *XIII* according to Scheme 2. (Gln[1], Phe-NH_2^{10})-FTS (*II*) was prepared by an analogous procedure from benzyloxycarbonylglutamine and product *XXII* according to the same scheme.

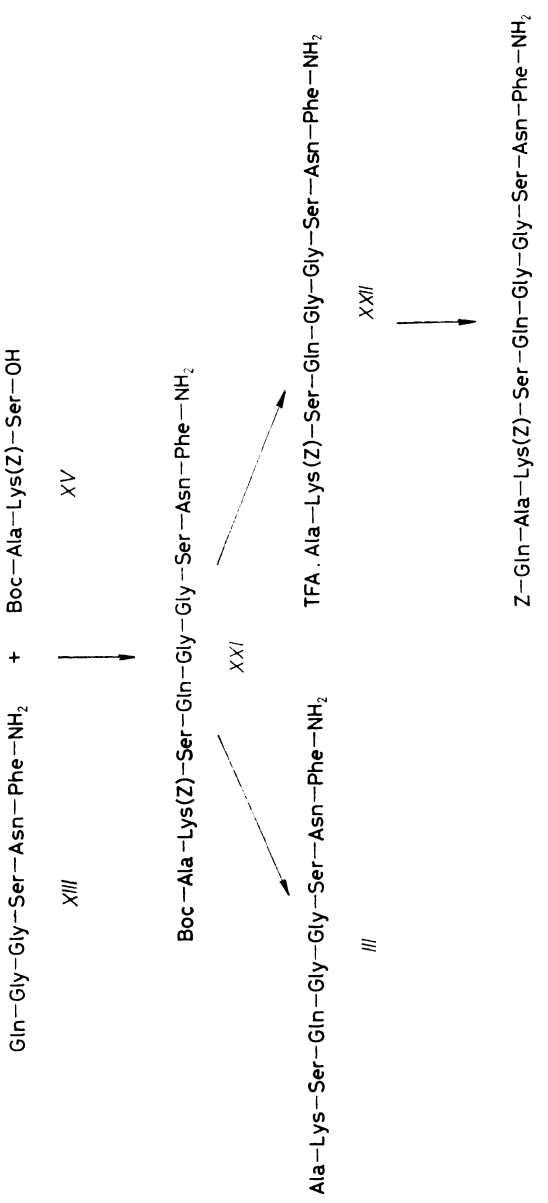


SCHEME 1* *I*

The biological activities were tested in the recovery assay of T lymphocytes[14]. The results are given in Table I. The activities of FTS (ref.[4]) and (Phe-NH_2^{10})-FTS are mutually comparable. If, however, the pyroglutamic acid of FTS is eliminated its activity decreases[4] whereas the same elimination contributes in the case of the C-extended analog to the highest activity obtained. If we compare the replacement of pyroglutamic acid by glutamine from the same viewpoint we find inhibitory effects both in the case of (Gln[1])-FTS (ref.[4]) and of (Gln[1], Phe-NH_2^{10})-FTS.

The results show that an essential change in the quantity of the biological effect is observed with C-extended analogs which possess both active and inhibitory effect. This change is most likely caused not only by the character of the amino acid residue but also by the amide group. If we accept the general assumption that FTS is a part of a larger peptide[15] then the C-extended analogs may suggest a certain resemblance with the native prohormone.

* For compound Z-Gln-OPcp number *IX* is missing.


Gln–Gly–Gly–Ser–Asn–Phe–NH2
XIII
+
Boc–Ala–Lys(Z)–Ser–OH
XV
Boc–Ala–Lys(Z)–Ser–Gln–Gly–Gly–Ser–Asn–Phe–NH2
XXI
Ala–Lys–Ser–Gln–Gly–Gly–Ser–Asn–Phe–NH2
III
TFA . Ala–Lys(Z)–Ser–Gln–Gly–Gly–Ser–Asn–Phe–NH2
XXII
Z–Gln–Ala–Lys(Z)–Ser–Gln–Gly–Gly–Ser–Asn–Phe–NH2
XXIII
Gln–Ala–Lys–Ser–Gln–Gly–Gly–Ser–Asn–Phe–NH2
II

SCHEME 2

EXPERIMENTAL

The melting temperatures were determined in a Kofler block and are not corrected. The optical rotations were measured in a Perkin–Elmer Model 141 polarimeter. The samples for analysis were dried *in vacuo* at 130 Pa over phosphorus pentoxide at room temperature (compounds with melting temperatures below 115°C) or at 105°C (compounds with melting temperatures above 115°C). Chromatography was performed in a thin layer of silica gel (Kiesel-gel G, Merck) in the systems butanol–acetic acid–water (4 : 1 : 1) (S_1) and butanol–acetic acid–pyridine–water (15 : 3 : 10 : 6) (S_2). Electrophoresis was carried out in a thin layer of the same silica gel at a potential gradient of 38 V cm^{-1} (10 min) in the buffer pyridine–acetic acid, pH 5 (E_1). Free amino acids and peptides were detected by ninhydrin, the protected compounds by chlorination and 2-tollidine staining. Standard procedure A represents dissolving of the reaction mixture, washing with 1% solution of citric acid, water, 5% sodium bicarbonate, water, drying over anhydrous sodium sulfate and evaporation. Standard procedure B represents dissolving of the reaction mixture, washing with 1 mol l^{-1} hydrochloric acid, water, 5% sodium bicarbonate, water, drying over anhydrous sodium sulfate and evaporation. Standard procedure C represents trituration of the dry residue of the reaction mixture with 1 mol l^{-1} hydrochloric acid, water, 5% sodium bicarbonate, water and drying in a desiccator over phosphorus pentoxide. The per cent given are weight per cent. The solutions were evaporated *in vacuo* using a rotary vacuum evaporator. Amino acid methyl ester hydrochlorides and glycyl-glycine methyl ester hydrochloride were prepared by esterification according to Brenner[18], N^{α}-benzyloxycarbonyl amino acids were prepared according to Bergman and Zervas[19]. The synthesis of Boc-Ala-Lys(Z)-Ser-OMe (*XIV*) and its hydrolysis to Boc-Ala-Lys(Z)-Ser-OH (*XV*) were described earlier[4].

Benzyloxycarbonylasparaginyl-phenylalanine Methyl Ester (*IV*)

Pivaloyl chloride (6·3 ml) was added to a solution of benzyloxycarbonylasparagine(13·3 g; 50 mmol), N-ethylpiperidine (7·0 ml), and pyridine (5·0 ml) in dichloromethane (100 ml) cooled down to −20°C. The mixture was treated after 8 min of stirring at 0°C with a solution of phenylalanine methyl ester in dichloromethane (100 ml), released from the corresponding hydrochloride (10·7 g; 50 mmol) by the addition of N-ethylpiperidine (7·0 ml) and added during 2—3 min.

TABLE I
Results of E-rosette test

Compound	Difference in number of rosettes newly formed, %
(Phe-NH_2^{10})-FTS (*I*)	7·54
(des Glp^1, Phe-NH_2^{10})-FTS (*III*)	16·09
(Gln^1, Phe-NH_2^{10})-FTS (*II*)	−1·52
FTS	9·20
(des Glp^1)-FTS	3·18
(Gln^1)-FTS	−1·64

The mixture was stirred 2 h at room temperature, the compound which had separated was filtered off and treated according to standard procedure C. The product was crystallized from methanol. The yield was 14·5 g (68%) of *IV*, melting temperature 196—197°C. The sample for analysis was crystallized from dimethylformamide by the addition of 2-propanol, the melting temperature did not change; $[\alpha]_D^{20}$ −2·0° (*c* 0·2; dimethylformamide), R_F 0·71 (S_1), 0·75 (S_2). For $C_{22}H_{25}N_3O_6$ (427·5) calculated: 61·82% C, 5·90% H, 9·83% N; found: 61·68% C, 5·96% H, 9·65% N.

Asparaginyl-phenylalanine Methyl Ester Hydrobromide (*V*)

A 38 wt. % solution (10·0 ml) of hydrogen bromide in acetic acid was added to a solution of *IV* (4·3 g; 10 mmol) in glacial acetic acid (7·0 ml). The mixture was set aside for 1 h at room temperature with occasional stirring. The product was precipitated by the addition of ether. After this period it was filtered off, washed and dried 5 h in a desiccator over sodium hydroxide and phosphorus pentoxide. The yield was 3·6 g (97%) of *V* which was electrophoretically and chromatographically homogeneous: R_F 0·37 (S_1), 0·70 (S_2).

Benzyloxycarbonylseryl-asparaginyl-phenylalanine Methyl Ester (*VI*)

Pivaloyl chloride (4·4 ml) was added to a solution of benzyloxycarbonylserine (8·4 g; 35 mmol), N-ethylpiperidine (4·9 ml), and pyridine (3·5 ml) in dichloromethane (100 ml), cooled down to −20°C. A solution of asparaginyl-phenylalanine methyl ester in dichloromethane (100 ml), released from the corresponding hydrobromide *V* (13·1 g; 35 mmol) by the addition of N-ethylpiperidine (4·9 ml), was added during 2—3 min after the mixture had been stirred for 8 min at 0°C. Stirring was then continued for 2 h at room temperature, the mixture was then set aside for 12 h also at room temperature and evaporated afterwards. The dry residue was dissolved in ethyl acetate, treated according to standard procedure B and crystallized from methanol.The yield was 9·1 g (51%) of *VI*, melting temperature 180—183°C. The sample for analysis was crystallized by the same procedure, melting temperature 184—186°C, $[\alpha]_D^{20}$ −13·8° (*c* 0·2; methanol), R_F 0·71 (S_1), 0·82 (S_2). For $C_{25}H_{30}N_{14}O_8$ (514·5) calculated: 58·36% C, 5·88% H, 10·89% N; found: 58·36% C, 5·83% H, 11·26% N.

Benzyloxycarbonylseryl-asparaginyl-phenylalanine Amide (*VII*)

Liquid ammonia (c. 33 g) was added to a solution of *VI* (5·2 g; 10 mmol) in 98 vol. % methanol (130 ml) cooled down to −5°C. The mixture was set aside for 70 h at room temperature. The product was filtered off after this period and washed and dried to constant weight in a desiccator over sodium hydroxide. The yield was 3·9 g (78%) of *VII*, melting temperature 218—221°C. The sample for analysis was crystallized from the mixture dimethylformamide–methanol (1 : 1) by the addition of ether, melting temperature 222—224°C; $[\alpha]_D^{20}$ −15·90° (*c* 0·2; dimethylformamide) R_F 0·64 (S_1), 0·71 (S_2). For $C_{24}H_{29}N_5O_7$ (499·5) calculated: 57·70% C, 5·86% H, 14·02% N; found: 57·03% C, 5·96% H, 13·90% N.

Seryl-asparaginyl-phenylalanine Amide (*VIII*)

Pd-black (c. 50 mg) was added to a solution of *VII* (5·0 g; 10 mmol) and acetic acid (0·1 ml) in 70 vol. % methanol (400 ml). Hydrogenolysis was carried out at normal pressure, 3 h at room temperature. After Pd-black had been filtered off the mixture was evaporated and the residue was crystalized from 95 vol. % methanol by the addition of ether. The yield was 3·6 g (96%) of *VIII*, melting temperature 169—172°C. The sample for analysis was crystallized by the same

procedure, the melting temperature was unaltered; $[\alpha]_D^{20}$ −19·3° (*c* 0·2; methanol), R_F 0·24 (S_1), 0·57 (S_2). For $C_{16}H_{23}N_5O_5.1/2\ H_2O$ (374·4) calculated: 51·32% C, 6·47% H, 18·70% N; found: 51·67% C, 6·35% H, 18·49% N.

Benzyloxycarbonylglutaminyl-glycyl-glycine Methyl Ester (*X*)

N-Ethylpiperidine (1·4 ml) was added to a suspension of glycyl-glycine methyl ester hydrochloride (1·9 g; 10 mmol) in the mixture dimethylformamide–dichloromethane (1 : 2) (30 ml). Benzyloxycarbonylglutamine pentachlorophenyl ester (*IX*) (5·3 g; 100 mmol) was added after this period. The latter was prepared according to Kovacs and co-workers[20] in a yield of 59%, melting temperature 194—197°C (ref.[21]: m.p. 183—187°C). The mixture was stirred 4 h at room temperature. It was then allowed to stand 8 h at the same temperature, the product was filtered off and crystallized from methanol by the addtion of dichloromethane. The yield was 2·5 g (61%) of *X*, melting temperature 132—134°C (ref.[22]: m.p. 130—136°C), $[\alpha]_D^{20}$ −8·5° (*c* 0·2; 50 vol. % acetic acid), R_F 0·57 (S_1), 0·78 (S_2).

Benzyloxycarbonylglutaminyl-glycyl-glycyl-seryl-asparaginyl-phenylalanine Amide (*XII*)

A 3·6 mol l^{-1} solution of sodium nitrite (2·8 ml) was added to a solution of *XI* (4·1 g; 10 mmol), which had been prepared according to Tsukamoto and co-workers[22] in a yield of 74%; melting temperature 167—170°C (ref.[22]: m.p. 164—167°C), and concentrated hydrochloric acid (4·0 ml) in dimethylformamide (50 ml), cooled down to −20°C. The solution was stirred 10 min at −20°C and its pH was adjusted to pH 6·9 by N-ethylpiperidine afterwards. The solution of *VIII* (3·7 g; 10 mmol) in dimethylformamide (50 ml) was then added and the pH of the mixture was again adjusted to pH 6·9 by N-ethylpiperidine. The mixture was degassed (2 min), set aside for 12 h at 0°C and evaporated. The residue was treated according to standard procedure C. The product was crystallized from butanol by the addition of ethyl acetate. The yield was 5·6 g (74%) of *XII*, melting temperature 225—227°C. The sample for analysis was crystallized by the same procedure, melting temperature 226—229°C; $[\alpha]_D^{20}$ −12·1° (*c* 0·2; dimethylformamide), R_F 0·30 (S_1), 0·58 (S_2). For $C_{33}H_{43}N_9O_{11}.H_2O$ (759·8) calculated: 52·16% C, 5·98% H, 16·59% N; found: 51·85% C, 5·79% H, 16·33% N. Amino acid analysis: Glu 1·00, Gly 2·02, Ser 0·76, Asp 0·94, Phe 0·92.

Glutaminyl-glycyl-glycyl-seryl-asparaginyl-phenylalanine Amide (*XIII*)

Pd-black (c. 25 mg) was added to a solution of *XII* (2·2 g; 3·0 mmol) and acetic acid (0·1 ml) in the mixture dimethylformamide–methanol (1 : 2) (100 ml). Hydrogenolysis was carried out under normal pressure 6 h at room temperature. After Pd-black had been filtered off and the mixture evaporated the product was crystallized from 95 vol. % methanol by the addition of ether. The yield was 1·7 g (86%) of *XIII*, melting temperature 202—204°C. The sample for analysis was crystallized by the same procedure, melting temperature 209—212°C; $[\alpha]_D^{20}$ −21·9° (*c* 0·1; 50 vol. % methanol), R_F 0·05 (S_1), 0·42 (S_2). For $C_{25}H_{37}N_9O_9.\ CH_3COOH.H_2O$ (685·7) calculated: 47·29% C, 6·33% H, 18·39% N; found: 46·91% C, 6·04% H, 18·33% N. Amino acid analysis; Glu 0·97, Gly 2·00, Ser 0·89, Asp 1·02, Phe 1·00.

Alanyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-serine Methyl Ester Hydrochloride (*XVI*)

A 1 mol l^{-1} solution of hydrogen chloride in acetic acid (12·0 ml) was added to a solution of *XIV* (3·4 g; 6·0 mmol) in glacial acetic acid (12·0 ml). The mixture was stirred 1 h at room temperature. The product was then precipitated by ether, filtered off and dried 5 h in a desiccator over sodium hydroxide and phosphorus pentoxide. The yield was 2·7 g (93%) of *XVI*. The product was electrophoretically and chromatographically homogeneous: R_F 0·35 (S_1), 0·62 (S_2).

Pyroglutamyl-alanyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-serine Methyl Ester (*XVIII*)

A solution of pyroglutamic acid (*XVII*) (0·9 g; 7·0 mmol), which had been prepared according to Beecham[23] in a yield of 60%; melting temperature 157—160°C (ref.[23]: m.p. 159—160·5°C), 1-hydroxybenztriazole (0·95 g), and N,N'-dicyclohexylcarbodiimide (1·6 g) in dimethylformamide (50 ml) were stirred 10 min at —10°C. Next was added a precooled solution (—10°C) of alanyl-N$^{\varepsilon}$--benzyloxycarbonyllysyl-serine methyl ester in dimethylformamide (50 ml) which had been released from the corresponding hydrochloride *XVI* (3·4 g; 7·0 mmol) by the addition of N-ethylpiperidine (1·0 ml). The mixture was stirred 2 h at 0°C and then set aside for 8 h at room temperature. After N,N'-dicyclohexylurea which had separated was filtered off, the solution was evaporated. The residue was treated according to standard procedure A. The product was crystallized from 2-propanol. The yield was 2·4 g (59%) of *XVIII*, melting temperature 120—122°C. The sample for analysis was crystallized by the same procedure, melting temperature 135—137°C; $[\alpha]_D^{20}$ —24·4° (*c* 0·1; dimethylformamide), R_F 0·53 (S_1), 0·83 (S_2). For $C_{26}H_{37}N_5O_9.H_2O$ (581·7) calculated: 53·68% C, 6·77% H, 12·04% N; found: 53·96% C, 6·47% H, 11·84% N.

Pyroglutamyl-alanyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-serine Hydrazide (*XIX*)

Hydrazine hydrate (100%; 3·0 ml) was added to a solution of *XVIII* (2·9 g; 5·0 mmol) in the mixture dimethylformamide–methanol (1 : 1) (40 ml). After 8 h of standing at room temperature the product which had separated was filtered off, washed and dried to constant weight. The yield was 2·3 g (79%) of *XIX*, melting temperature 212—214°C. The sample for analysis was crystallized from 80 vol. % methanol, melting temperature 217—218°C; $[\alpha]_D^{20}$ —48·9°C (*c* 0·2; 80 vol. % methanol), R_F 0·54 (S_1), 0·73 (S_2). For $C_{25}H_{37}N_7O_8.H_2O$ (581·6) calculated: 51·62% C, 6·77% H, 16·86% N; found: 52·01% C, 6·55% H, 16·54% N.

Pyroglutamyl-alanyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-seryl-glutaminyl--glycyl-glycyl-seryl-asparaginyl-phenylalanine Amide (*XX*)

A 3·6 mol l^{-1} solution of sodium nitrite (0·5 ml) was added to a solution of *XIX* (0·95 g; 1·63 mmol) and concentrated hydrochloric acid (0·6 ml) in dimethylformamide (20 ml) which had been cooled down to —20°C. After 10 min of stirring of the solution at —20°C the pH was adjusted to 6·9 by the addition of N-ethylpiperidine. The mixture was degassed (2 min), set aside for 12 h at 0°C and evaporated. The residue was triturated with a saturated solution of sodium chloride (2·0 ml), the product which had separated after 1 h of standing at 0°C was filtered off and crystallized from butanol (saturated with water at 20°C) by the addition of ethyl acetate and recrystallized from 80 vol. % methanol by the addition of ethyl acetate. The yield was 0·58 g (33%) of *XX*, melting temperature 248—252°C. The sample for analysis was crystallized from 80 vol. % methanol by the addition of ethyl acetate, melting temperature 259—261°C; $[\alpha]_D^{20}$ —48·2° (*c* 0·1; 50 vol. % acetic acid), R_F 0·08 (S_1), 0·42 (S_2). For $C_{50}H_{70}N_{14}O_{17}.2\,H_2O$ (1 175·3) calculated: 51·10% C, 6·35% H, 16·68% N; found: 51·13% C, 6·26% H, 16·20% N. Amino acid analysis: Glu 1·90, Ala 1·18, Lys 1·06, Ser 1·79, Gly 1·95, Asp 1·05, Phe 1·00.

Pyroglutamyl-alanyl-lysyl-seryl-glutaminyl-glycyl-glycyl--seryl-asparaginyl-phenylalanine Amide (*I*)

Pd-black (c. 20 mg) was added to a solution of *XX* (300 mg; 0·26 mmol) in 10 vol. % acetic acid (100 ml). Hydrogenolysis was carried out under normal pressure 3 h at room temperature. After Pd-black had been filtered off the solution was evaporated, the residue was dissolved in 0·2 mol . . l^{-1} acetic acid (5·0 ml), desalted by gel filtration on a 1·8 × 90 cm column of Sephadex G-15

equilibrated with 0·2 mol l^{-1} acetic acid and eluted by the same solvent. The separation was monitored by thin-layer electrophoresis, homogeneous fractions were pooled, concentrated and lyophilized. The yield was 200 mg (64%) of *I*, R_F 0·02 (S_1), 0·25 (S_2), $[\alpha]_D^{20}$ −67·9° (*c* 0·1; 0·1 mol . l^{-1} acetic acid). For $C_{42}H_{64}N_{17}O_{15}.2\,CH_3COOH.4\,H_2O$ (1 197·3) calculated: 46·47% C, 6·74% H, 17·24% N; found: 46·75% C, 6·25% H, 16·82% N. Amino acid analysis: Glu 1·94, Ala 1·18, Lys 1·10, Ser 1·78, Gly 2·03, Asp 1·04, Phe 1·00.

Tert-butyloxycarbonylalanyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-seryl-glutaminyl-glycyl-glycyl-seryl-asparaginyl-phenylalanine Amide (*XXI*)

A solution of *XV* (0·8 g; 1·49 mmol), 1-hydroxybenztriazole (0·2 g), and N,N′-dicyclohexylcarbodiimide (0·3 g) in dimethylformamide (30 ml) was stirred 10 min at −10°C. A precooled (−10°C) solution of *XIII* (1·0 g, 1·51 mmol) in dimethylformamide (50 ml) was added after this period. The mixture was stirred 2 h at 0°C and set aside for 8 h at room temperature. N,N′-Dicyclohexylurea which had separated was filtered off, the solution was evaporated and the residue triturated with a saturated solution of sodium chloride (3·0 ml). The product was filtered off after 1 h of standing at 0°C, was washed and crystallized from butanol (saturated with water at 20°C) by the addition of ethyl acetate. The yield was 0·4 g (23%) of *XXI*, melting temperature 225—227°C. The sample for analysis was crystallized by the same procedure, the melting temperature was unaltered; $[\alpha]_D^{20}$ −39·9° (*c* 0·1; 50 vol. % acetic acid), R_F 0·25 (S_1), 0·76 (S_2). For $C_{50}H_{73}N_{13}O_{17}.H_2O$ (1 146·2) calculated: 52·39% C, 6·59% H, 15·88% N; found: 51·99% C, 6·55% H, 15·59% N. Amino acid analysis: Ala 1·03, Lys 0·98, Ser 1·77, Glu 0·94, Gly 2·03, Asp 1·00, Phe 1·03.

Alanyl-lysyl-seryl-glutaminyl-glycyl-glycyl-seryl-asparaginyl-phenylalanine Amide (*III*)

Pd-black (c. 20 mg) was added to a solution of *XXI* (0·4 g, 0·35 mmol) in 10 vol. % acetic acid (100 ml). Hydrogenolysis was carried out under normal pressure 2 h at room temperature. After Pd-black had been filtered off the solution was evaporated, the residue dried in a desiccator over sodium hydroxide and phosphorus pentoxide for 5 h and was then dissolved in trifluoroacetic acid (1·8 ml). The solution was stirred 1 h at room temperature; the bistrifluoroacetate, which was precipitated by the addition of ether, was then filtered off, washed, dried 2 h in a desiccator over sodium hydroxide and phosphorus pentoxide and dissolved in 50 vol. % methanol. The product was then converted by Zerolite FF (in CH_3COO^--form) first batchwise (c. 5 ml of resin) and then on a column (1·8 × 25 cm) into crude *III*. The pooled methanolic effluents were evaporated, the residue was dissolved in 0·2 mol l^{-1} acetic acid and treated further as described for compound *I*. The yield was 0·2 g (57%) of product *III*; $[\alpha]_D^{20}$ −40·4° (*c* 0·1; 0·1 mol . l^{-1} acetic acid), R_F 0·02 (S_1), 0·45 (S_2). For $C_{37}H_{59}N_{13}O_{13}.2\,CH_3COOH$ (1 014·1) calculated: 48·56% C, 6·66% H, 17·96% N; found: 49·07% C, 6·42% H, 17·51% N. Amino acid analysis: Ala 1·09, Lys 1·03, Ser 1·65, Glu 1·00, Gly 1·97, Asp 1·02, Phe 0·98.

Alanyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-seryl-glutaminyl-glycyl-glycyl-seryl-asparaginyl-phenylalanine Amide Trifluoroacetate (*XXII*)

A solution of *XXI* (1·0 g, 0·87 mmol) and anisole (0·3 ml) in trifluoroacetic acid (2·7 ml) was stirred 30 min at room temperature. The product was precipitated by the addition of ether, filtered off, washed and dried 2 h in a desiccator over sodium hydroxide and phosphorus pentoxide. The yield was 0·9 g (89%) of *XXII*. The compound was electrophoretically and chromatographically homogeneous: R_F 0·21 (S_1), 0·65 (S_2).

Benzyloxycarbonylglutaminyl-alanyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-seryl--glutaminyl-glycyl-glycyl-seryl-asparaginyl-phenylalanine Amide (*XXIII*)

A solution of benzyloxycarbonylglutamine (250 mg, 0·89 mmol), 1-hydroxybenztriazole (120 mg), and N,N′-dicyclohexylcarbodiimide (200 mg) in dimethylformamide (30 ml) was stirred 10 min at −10°C. A precooled (−10°C) solution was then added of alanyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-seryl-glutaminyl-glycyl-glycyl-seryl-asparaginyl-phenylalanine amide in dimethylformamide (30 ml) released from the corresponding trifluoroacetate *XXII* (1·0 g; 0·89 mmol) by the addition of N-ethylpiperidine (0·15 ml). The mixture was stirred 2 h at 0°C and set aside for 8 h at room temperature. N,N′-Dicyclohexylurea which has separated after concentration of the solution was filtered off and the solution was evaporated. The residue was triturated with water (3·0 ml). The product was filtered off after 1 h of standing at room temperature and was crystallized from acetic acid by the addition of ethyl acetate. The yield was 200 mg (18%) of *XXIII*, melting temperature 251—254°C. The sample for analysis was crystallized by the same procedure, the melting temperature did not change; $[\alpha]_D^{20}$ −38·8° (*c* 0·1; 25 vol. % acetic acid), R_F 0·02 (S_1), 0·03 (S_2). For $C_{58}H_{79}N_{15}O_{19}.2\,H_2O$ (1 326·4) calculated: 52·88% C, 6·27% H, 15·95% N; found: 52·88% C, 6·32% H, 15·32% N. Amino acid analysis: Glu 1·84, Ala 0·95, Lys 1·11, Ser 1·73, Gly 2·04, Asp 1·00, Phe 1·09.

Glutaminyl-alanyl-lysyl-seryl-glutaminyl-glycyl-glycyl-seryl--asparaginyl-phenylalanine Amide (*II*)

Pd-black (c. 30 mg) was added to a solution of *XXIII* (100 mg, 0·08 mmol) in 10 vol. % acetic acid (100 ml). Hydrogenolysis was carried out under normal pressure 3 h at room temperature (the pH of the mixture was adjusted to 2 by 1 mol l^{-1} hydrochloric acid (0·4 ml)). After Pd-black had been filtered off the solution was evaporated, the residue was dissolved in water (5·0 ml) and the solution was again evaporated (twice repeated). The residue was dissolved in 0·2 mol l^{-1} acetic acid and then treated as described for compound *I*. The yield was 70 mg (75%) of *II*; $[\alpha]_D^{20}$ −47·8° (*c* 0·1; 0·1 mol l^{-1} acetic acid), R_F 0·00 (S_1), 0·25 (S_2). For $C_{42}H_{67}N_{15}O_{15}.2$ HCl. $.CH_3COOH.3\,H_2O$ (1 209·3) calculated: 43·70% C, 6·43% H, 17·38% N; found: 43·73% C, 6·20% H, 16·88% N. Amino acid analysis: Glu 1·92, Ala 1·00, Lys 0·99, Ser 1·77, Gly 2·04, Asp 1·01, Phe 0·97.

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