ATRIOPEPTINS. II. SYNTHESIS OF N-TERMINAL FRAGMENTS

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Peptides, corresponding to the N-terminal sequence in atriopeptins, were synthesized by classical methods of peptide chemistry in solution. The obtained peptides were characterized by various physicochemical methods. The scheme and methods of the synthesis are discussed.

In our preceding communication we have described the synthesis of the C-terminal fragments of the atrial natriuretic factors (ANF), corresponding to the sequence (17–27) and (17–28)[1] in α-r-ANF. The present paper concerns the synthesis of several protected N-terminal fragments of the sequence 1–16 (*XX*) for preparation of α-r-ANF, sequence 5–16 (*XII*) for preparation of AP II and AP III, and also sequence 7–16 (*XI*) for synthesis of des-Ser[5]-des-Ser[6]-AP-II (for abbreviations see ref.[1]).

$$\overset{7}{\text{X-Cys(Acm)}}\text{-}\overset{8}{\text{Phe}}\text{-}\overset{9}{\text{Gly}}\text{-}\overset{10}{\text{Gly}}\text{-}\overset{11}{\text{Arg}}\text{-}\overset{12}{\text{Ile}}\text{-}\overset{13}{\text{Asp(OBu}^t\text{)}}\text{-}\overset{14}{\text{Arg}}\text{-}\overset{15}{\text{Ile}}\text{-}\overset{16}{\text{Gly-OH}}$$

XI, X = Boc

$$\text{X} = \overset{5}{\text{Boc-Ser(Bu}^t\text{)}}\text{-}\overset{6}{\text{Ser(Bu}^t\text{)}}$$

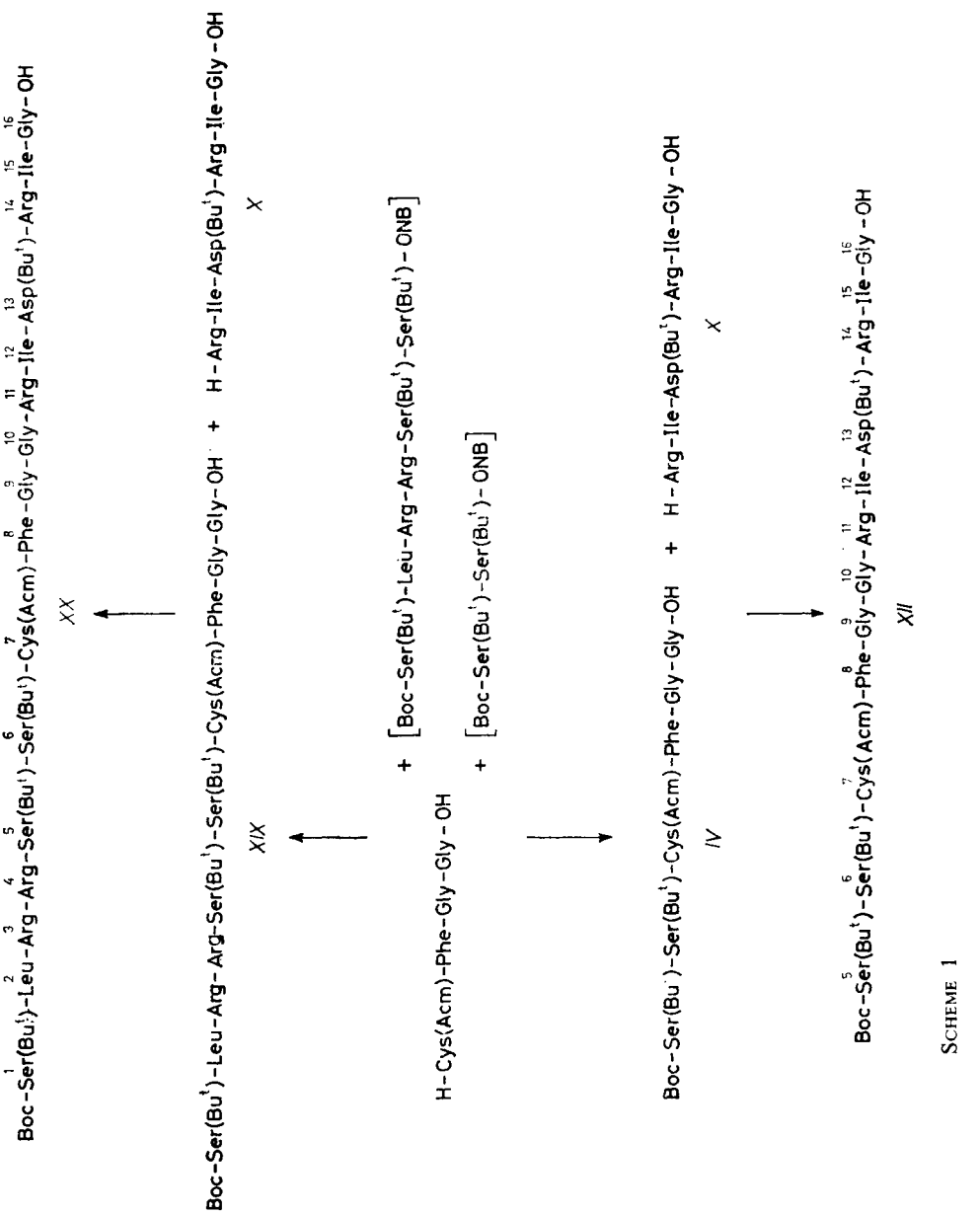
XII,

$$\text{X} = \overset{1}{\text{Boc-Ser(Bu}^t\text{)}}\text{-}\overset{2}{\text{Leu}}\text{-}\overset{3}{\text{Arg}}\text{-}\overset{4}{\text{Arg}}\text{-}\overset{5}{\text{Ser(Bu}^t\text{)}}\text{-}\overset{6}{\text{Ser(Bu}^t\text{)}}$$

XX,

The mentioned compounds were obtained in the following way. The peptide sequence was divided into the fragments (1–6), (7–10) and (11–16) (the two latter being common to all the above-mentioned compounds). Further condensation of the fragments, beginning from the N-terminus, led to compounds *XII* and *XX* (Scheme 1). Compound *XI* was prepared analogously: The protected tetrapeptide Boc--Cys(Acm)-Phe-Gly-Gly-OH (*III*), after activation with DCC-HONB, was condensed with H-Arg-Ile-Asp(OBu[t])-Arg-Ile-Gly-OH (*X*), affording the 7–16 nonapeptide *XI*.

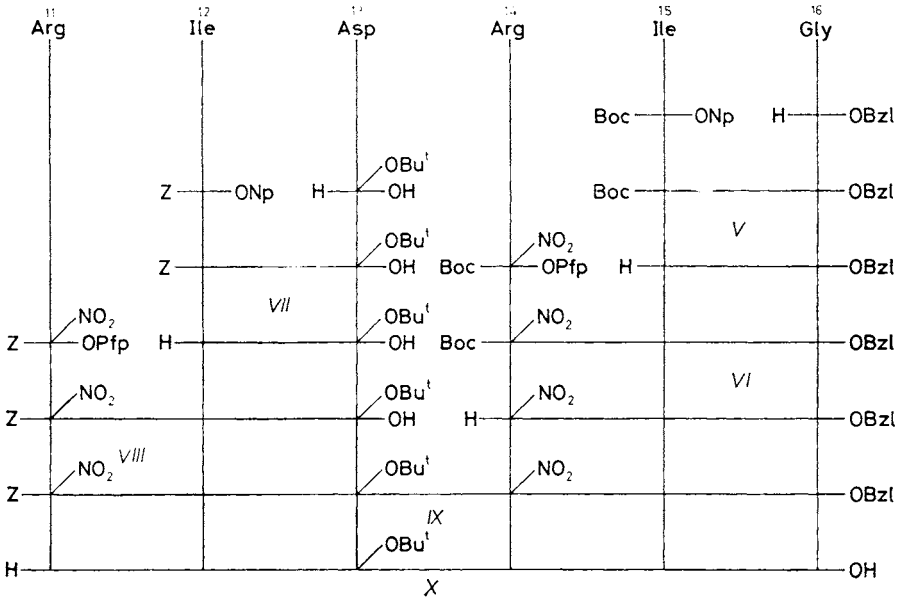
Cysteine was used as its N-α-Boc-S-Acm derivative, the arginine guanidine group was protected using the nitro group. α-Amino groups were blocked with the Z or Boc groups, for the serine and aspartic acid side functions the tert-butyl type protection was employed.



SCHEME 1

Originally, we planned to prepare the 11–16 hexapeptide fragment *X* by stepwise construction of the peptide chain starting from the glycine moiety. However, during the synthesis it appeared that the protected pentapeptide Z-Ile-Asp(OBu^t)-Arg-Ile--Gly-OH is practically insoluble, making thus a selective removal of the Z-group impossible. Therefore, the synthesis was performed by condensation of two tripeptide fragments using "complex F" as the condensing agent (Scheme 2).

After condensation of Boc-isoleucine *p*-nitrophenyl ester with glycine benzyl ester and removal of the tert-butyloxycarbonyl group with trifluoroacetic acid, the mixture was treated with an anion-exchanging resin (Dowex in OH^- form) at −40°C in aqueous isopropyl alcohol. Reaction of the obtained dipeptide H-Ile-Gly--OBzl with N-α-Boc-N-ω-NO_2-arginine pentafluorophenyl ester and removal of the protecting group afforded H-Arg(NO_2)-Ile-Gly-OBzl. In a parallel reaction sequence we prepared the tripeptide Z-Arg(NO_2)-Ile-Asp(OBu^t)-OH (*VIII*) by stepwise addition of the amino acid moieties (Scheme 2).



SCHEME 2

Condensation of the two tripeptides led to the hexapeptide *IX* which was deprotected in acetic acid. This acid was the solvent of choice because in other solvents the compound *IX* was sparingly soluble and its hydrogenation was accompanied by formation of side-products (as shown by TLC). When the crude hydrogenolysis product *X*, obtained by precipitation and air-drying, was subjected to further condensation, a side-product was obtained that was shown by NMR spectra to be acetyl

derivative of *X*.Therefore, we tried to treat the obtained hexapeptide with an anion-exchanging resin to remove the acetate ions. This work-up resulted in a fast (in about 2 h) and specific (TLC) conversion of the peptide into a product of smaller R_F. Isolation of this product and its HPLC analysis showed that it was a 7 : 3 mixture of two compounds. Further ^{1}H NMR investigation led to the conclusion that in this case we encountered the formation of a mixture of α- and β-peptide bonds in the aspartic acid moiety with simultaneous removal of the tert-butyl group, which is an often observed side reaction. According to the literature[2-5], such reactions are characteristic of methyl and benzyl esters of aspartic acid whereas to a substantially smaller extent they have been observed with tert-butyl esters. Such a smooth reaction as observed in our case may be connected with the structure of the peptide studied and particularly with the presence of two arginine moieties in the immediate vicinity of the aspartic acid residue. In connection with the above-mentioned facts, compound *X* was subjected to a prolonged (about 48 h) drying in vacuo over sodium hydroxide before it was used in the further reaction.

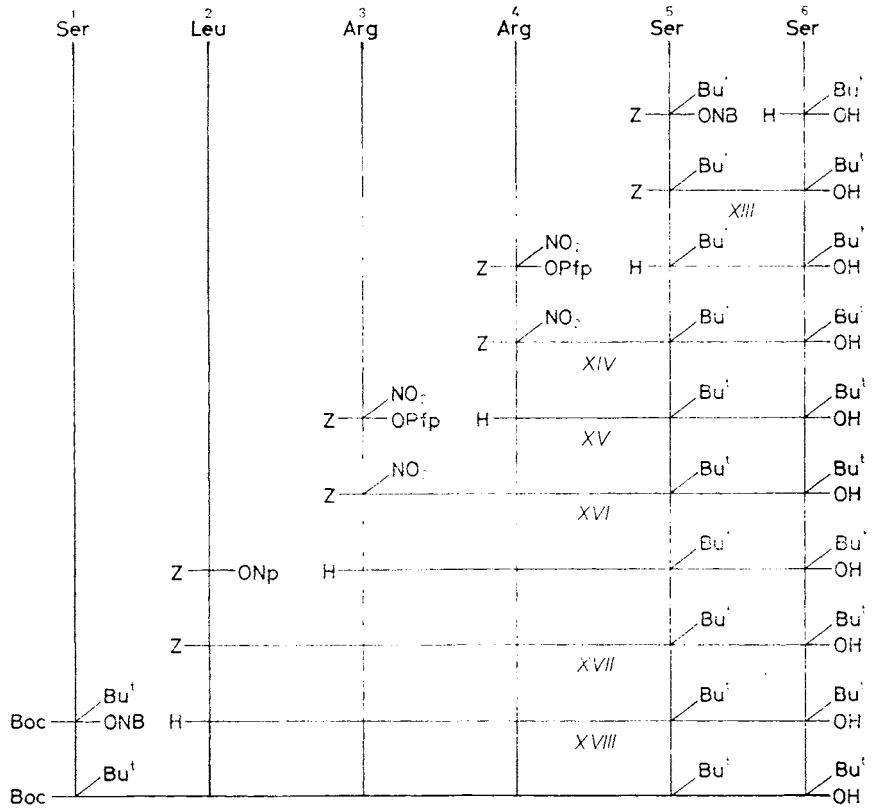
Synthesis of the 7–10 tetrapeptide started from glycylglycine by stepwise construction of the peptide chain. The phenylalanine and cysteine residues were introduced in the form of the respective *p*-nitrophenyl and N-hydroxynorbornene esters. Removal of the Boc protecting group from the prepared tetrapeptide was effected by trifluoroacetic or 85% formic acid, the thus-obtained salt was treated with an anion-exchanger in acetate form and after isolation and prolonged drying in vacuo over sodium hydroxide it was used in the next reaction step.

The dipeptide fragment 5–6 (*I*) was prepared by condensation of tert-butyloxycarbonyl-O-tert-butylserine N-hydroxynorbornyl ester with O-tert-butylserine whose carbonyl group was protected by salt formation with an organic base. The protected dipeptide was obtained as its crystalline salt with dicyclohexylamine.

Synthesis of the N-terminal hexapeptide of the sequence 1–6 (*XVIII*) started from the C-terminal Ser[6] and proceeded by stepwise addition of the amino acid residues. The carboxyl group was protected by salt formation. After introduction of the first arginine residue (Scheme 3) the reaction products were chromatographed on silica gel. During deprotection of the arginine moieties in compounds *XIV* and *XVI* we encountered particular difficulties. Satisfactory results were obtained only when the reaction was performed in acetic acid whereas use of other solvents led to mixture of products.

The fragments were condensed according to Scheme 1. It should be noted that the synthesis of fragments 1–10 (*XIX*), 5–10 (*IV*) and 11–16 (*IX*) used presynthesized active esters of peptides containing serine and aspartate moieties at the C-terminus, which could have been accompanied by racemization. As shown by special ^{1}H NMR investigation and by GLC-analysis of hydrolysates on a chiral phase[6], the synthesis proceeded without appreciable racemization. These results have been confirmed by enzymatic methods[7]. The studied fragment was acid-hydrolyzed and the dry residue

was dissolved in a TRIS-HCl buffer, pH 7, with simultaneous addition of L-amino acid oxidase and catalase. The mixture was then incubated at room temperature for 120 hours and at appropriate time intervals aliquots were taken and the amino acid composition was determined by HPLC[8]. This resulted in gradual decrease in the amino acid content which after 120 hours amounted to less than 0·6% of the starting content in the hydrolysate. In a control experiment with D-Ser and D-Asp under analogous conditions no change in the content of these two acids was observed. These experiments have proven that no racemization took place.



SCHEME 3

Fragments 1 – 10 (*XIX*), 5 – 10 (*IV*) and 7 – 10 (*XI*) were activated with DCC and HONB and the reactions were monitored by TLC. Their subsequent reaction with the hexapeptide *X* afforded the protected N-terminal peptide fragments 1 – 16 (*XX*), 5 – 16 (*XII*) and 7 – 16 (*XI*) (Scheme 1).

Structure of the obtained compounds was confirmed by ^{1}H NMR spectroscopy and amino acid analysis; moreover, their homogeneity was proven by TLC (the presence of only one spot in three different systems).

EXPERIMENTAL

For materials and methods see the preceding contribution (p. 777). In addition, the following chromatographic systems were used: ethyl acetate–pyridine–acetic acid–water (120 : 20 : 6 : 11, J); benzene–acetone (3 : 1, K) and ethyl acetate–hexane (1 : 1, L).

Boc-Ser(Bu^t)-Ser(Bu^t)-OH.DCHA (*I*)

A solution of H-Ser(Bu^t)-OH (1·35 g; 8·3 mmol) in 1M-NaOH (8·3 ml) was added to a solution of Boc-Ser(Bu^t)-ONB (3·51 g; 8·3 mmol) in DMF (50 ml). After stirring at 20°C for 12 h and evaporation, the residue was adjusted to pH 2 with 2% sulfuric acid and the separated oil was extracted with ether. The ethereal extract was washed with water to neutrality, dried, filtered and the drying agent was washed with ether. To the combined filtrates dicyclohexylamine (1·66 ml; 8·3 mmol) was added and the salt was crystallized from hexane; yield 4·66 g (97%) of the product *I*, m.p. 120–127°C; $[\alpha]_D^{15}$ +25·5° (*c* 1; DMF); R_F 0·87 (B), 0·78 (C), 0·88 (D).

Z-Phe-Gly-Gly-OH (*II*)

A suspension of Z-Gly-Gly-OH (5·3 g; 20 mmol) in water (100 ml) was mixed with 1M-NaOH (20 ml) and the mixture was hydrogenated over a Pd/C catalyst for 5 h. The catalyst was filtered off, the filtrate was concentrated to about 30 ml and mixed with DMF (100 ml) and Z-Phe-ONp (8·4 g; 20 mmol). After stirring at 20°C for 15 h, the solvent was evaporated, the residue was dissolved in water (120 ml) and twice extracted with ether. The aqueous layer was acidified with 2% sulfuric acid to pH 2, extracted with ethyl acetate, washed with water to neutrality and dried. Evaporation of the solvent and crystallization from ethyl acetate–hexane afforded 7·0 g (84%) of the title product *II*, m.p. 122–125°C; $[\alpha]_D^{15}$ −11·8° (*c* 1; DMF); R_F 0·65 (A), 0·67 (B), 0·62 (D).

Boc-Cys(Acm)-Phe-Gly-Gly-OH (*III*)

Compound *II* (5·00 g; 11·9 mmol) in methanol (100 ml) was hydrogenated over a Pd/C catalyst in the course of 5 h. A 40% solution of Triton B in methanol (5·94 ml; 11·9 mmol) was added, the catalyst was filtered off and the solvent was evaporated. The residue was dissolved in DMF (60 ml) and Boc-Cys(Acm)-ONp (4·91 g; 11·9 mmol) was added. After keeping the reaction mixture at 20°C for 15 h the solvent was evaporated, the residue was dissolved in water (100 ml), washed twice with ether, acidified to pH 2 with 2% sulfuric acid and extracted with ethyl acetate. The organic phase was washed with water to neutrality, dried and taken down. Crystallization from ethyl acetate–hexane gave 4·60 (71%) of compound *III*, m.p. 178–181°C; $[\alpha]_D^{15}$ +11·9° (*c* 1; DMF); R_F 0·58 (A), 0·54 (D), 0·54 (B). ^{1}H NMR, δ: Acm – 8·56, 1 H (NH), 4·23, 4·11, 2 H (CH_2), 1·83, 3 H (CH_3); Cys – 6·91, 1 H (NH), 4·15, 1 H (α-CH), 2·82, 2·55, 2 H (β-CH_2); Phe – 7·91, 1 H (NH), 4·50, 1 H (α-CH), 3·04, 2·82, 2 H (β-CH_2); Gly – 8·34, 1 H (NH), 3·76, 3·20, 2 H (α-CH_2); Gly – 7·99, 1 H (NH), 3·75, 2 H (α-CH_2), 1·37, 9 H (Boc).

Boc-Ser(Bu^t)-Ser(Bu^t)-Cys(Acm)-Phe-Gly-Gly-OH (*IV*)

A) A suspension of *I* (0·55 g; 0·94 mmol) in ethyl acetate was treated with 2% sulfuric acid. The organic layer was washed with water to neutrality, dried concentrated to about 15 ml. A solution of HONB (0·18 g; 1 mmol) in dioxane (10 ml) was added and, after cooling to −15°C, DCC (0·21 g; 1 mmol) was added under stirring. The reaction mixture was kept at +5°C for 5 h and decomposed with glacial acetic acid (3 drops). After 30 min, the separated N,N'-dicyclohexylurea was filtered off and the filtrate was taken down. The residue was dissolved in ethyl acetate (50 ml), washed with water to neutrality, dried and the solvent was evaporated. The residue was dissolved in DMF (8 ml).

B) Compound *III* (0·57 g; 1·05 mmol) was treated with 85% formic acid (30 ml) for 4 h. After evaporation, the residue was triturated with ether, filtered, washed with ether and dissolved in water (20 ml). The aqueous layer was treated with an ion-exchanging resin (acetate form), filtered and the resin was washed with water. The combined filtrates were taken down and the dry residue was precipitated with ether from methanol. The precipitate was collected on filter, washed with ether and dried over sodium hydroxide in a vacuum desiccator; yield 0·45 g of H-Cys(Acm)-Phe-Gly-Gly-OH. To a solution of this compound (0·24 g; 0·5 mmol) in DMF (8 ml) was added 1M-NaOH (0·5 ml) and the obtained solution was added to the solution prepared as described under *A*). After 2 h, the reaction mixture was evaporated, the residue was dissolved in water and extracted three times with ether. The aqueous layer was acidified to pH 2 with 2% sulfuric acid, extracted with ethyl acetate, washed with water to neutrality and dried. Evaporation and crystallization from ethyl acetate afforded 0·38 g (88%) of compound *IV*, m.p. 143–145°C; $[\alpha]_D^{20}$ −29·4° (*c* 1; DMF); R_F 0·82 (A), 0·62 (D), 0·59 (B). Amino acid analysis: Ser 1·30; Gly 1·91; Phe 1·00.

Boc-Ile-Gly-OBzl (*V*)

Triethylamine (2·76 ml; 20 mmol) and Boc-Ile-ONp (7·01 g; 19·9 mmol) were added to a stirred solution of glycine benzyl ester tosylate (3·28 g; 20 mmol) in DMF (100 ml). The reaction mixture was kept at 20°C for 48 h and the solvent was evaporated. The residue was dissolved in ethyl acetate (150 ml), washed successively with 10% aqueous ammonia, water, 2% sulfuric acid and water, and dried. Evaporation of the solvent and crystallization from ethyl acetate–hexane afforded 7·05 g (92%) of compound *V*, m.p. 93–94°C; $[\alpha]_D^{20}$ −12·7° (*c* 1; DMF); R_F 0·75 (G), 0·69 (K), 0·48 (L).

Boc-Arg(NO_2)-Ile-Gly-OBzl (*VI*)

A solution of compound *V* (3·78 g; 10 mmol) in trifluoroacetic acid (50 ml) was kept at 20°C for 1 h. The acid was evaporated and the residue was dissolved in a 1 : 1 mixture of isopropyl alcohol and water. The solution was cooled to −40°C and adjusted to pH 8 with an ion-exchanging resin (OH^- form). The mixture was filtered, the filtrate was taken down, the residue was codistilled with isopropyl alcohol and dissolved in DMF (50 ml). To the obtained solution was added Boc-Arg(NO_2)-OPfp (4·88 g; 10 mmol) and the mixture was kept at 20°C for 2 h. After evaporation, the residue was dissolved in ethyl acetate (100 ml) and washed successively with 2% sulfuric acid, water, 5% solution of sodium hydrogen carbonate and water. After drying, the solvent was evaporated and the residue crystallized from ether to afford 5·11 g (88%) of compound *VI*, m.p. 104–107°C; $[\alpha]_D^{20}$ −8·9 (*c* 1; DMF); R_F 0·71 (H), 0·64 (G), 0·56 (I).

Z-Ile-Asp(OBu^t)-OH (*VII*)

A solution of Z-Ile-ONp (6·6 g; 18 mmol) in DMF (50 ml) was added under stirring to a solution of H-Asp(OBu^t)-OH (3·4 g; 18 mmol) in 1M-NaOH (18 ml). After stirring at 20°C for 15 h, the mixture was taken down, the residue was dissolved in water and extracted twice with ether. The aqueous layer was acidified to pH 2 with 2% sulfuric acid, extracted with ethyl acetate, the organic layer was washed with water to neutrality and dried. Evaporation and crystallization from ethyl acetate–hexane afforded 6·9 g (88%) of compound *VII*, m.p. 135–140°C; $[\alpha]_D^{20}$ −3·6 (*c* 1; DMF); R_F 0·88 (D), 0·77 (C), 0·73 (H).

Z-Arg(NO_2)-Ile-Asp(OBu^t)-OH (*VIII*)

A solution of compound *VII* (4·36 g; 10 mmol) in methanol (50 ml) was mixed with 40% methanolic solution of Triton B (5 ml; 10 mmol) and hydrogenated over a Pd/C catalyst in the course of 5 h. The catalyst was filtered off, the solvent was evaporated and the residue was mixed with DMF (50 ml) and Z-Arg(NO_2)-OPfp (5·04 g; 10 mmol). After stirring at 20°C for 1 h, the reaction mixture was taken down, the residue was dissolved in ethyl acetate and the solution was washed with 2% sulfuric acid, with water to neutrality, and dried. Evaporation of the solvent and crystallization from ethyl acetate–hexane afforded 5·00 g (80%) of compound *VIII*, m.p. 107–110°C; $[\alpha]_D^{20}$ −6·8° (*c* 1; DMF); R_F 0·87 (B), 0·83 (D), 0·79 (C).

Z-Arg(NO_2)-Ile-Asp(OBu^t)-Arg(NO_2)-Ile-Gly-OBzl (*IX*)

Compound *VI* (1·25 g; 2·25 mmol) was treated with trifluoroacetic acid (40 ml) for 1 h. The acid was evaporated, the residue was mixed with 1M-HCl in ether (5 ml) and the precipitate was filtered, washed with ether and dried in vacuo over sodium hydroxide in a desiccator. The obtained HCl.H-Arg(NO_2)-Ile-Gly-OBzl (0·52 g; 2 mmol) was dissolved in DMF (10 ml). N-Methylmorpholine (0·22 ml; 2 mmol) and compound *VIII* (1·26 g; 2 mmol) were added to this solution. The reaction mixture was cooled to 0°C, complex F (2·29 g; 3 mmol) was added and the mixture was set aside at 20°C for 12 h. The precipitated N,N′-dicyclohexylurea was filtered off, the solvent was evaporated and the residue was mixed with ethyl acetate (100 ml). The precipitate was collected on filter, washed with ethyl acetate and dried in vacuo in a desiccator. Crystallization from ethyl acetate furnished 1·64 g (74%) of compound *IX*, m.p. 204–208°C; $[\alpha]_D^{20}$ −13·0° (*c* 1; DMF); R_F 0·68 (G), 0·45 (H), 0·54 (I).

H-Arg-Ile-Asp(OBu^t)-Arg-Ile-Gly-OH (*X*)

Compound *IX* (1·54 g; 1·4 mmol) was hydrogenated in glacial acetic acid (20 ml) over a Pd/C catalyst for 3 h. The catalyst was filtered off, the solvent was evaporated and the remaining oil was codistilled with isopropyl alcohol. The residue was precipitated with ether from isopropyl alcohol, the precipitate was filtered, washed on the filter with ether and dried in vacuo over sodium hydroxide in an desiccator to give 1·1 g (95%) of compound *X* as amorphous powder, $[\alpha]_D^{20}$ −14·6° (*c* 1; DMF); R_F 0·37 (A), 0·24 (B), 0·42 (C). Amino acid analysis: Asp 1·01; Gly 1·03; Ile 1·91; Arg 2·05. ^{1}H NMR, δ: Arg — 3·88, 1 H (CH), 1·66, 2 H (α-CH_2); Ile — 8·45, 1 H (NH), 4·26, 1 H (α-CH), 1·69, 2 H (β-CH_2); Asp — 8·43, 1 H (NH), 4·60, 1 H (α-CH), 2·65, 2·44 (β-CH_2); Arg — 7·87, 1 H (NH), 4·32, 1 H (α-CH), 1·49, 1·65, 2 H (β-CH_2); Ile — 7·88, 1 H (NH), 4·18, 1 H (α-CH), 1·72, 2 H (β-CH_2); Gly — 8·21, 1 H (NH), 3·77, 3·66, 2 H (α-CH_2), 1·35, 9 H (Bu^t).

Boc-Cys(Acm)-Phe-Gly-Gly-Arg-Ile-Asp(OBu^t)-Arg-Ile-Gly-OH (*XI*)

A mixture of a solution of compound *III* (0·97 g; 1·75 mmol) in DMF (15 ml) and HONB (0·41 g; 2·27 mmol) was cooled to −30°C and DCC (0·41 g; 1·92 mmol) was added under stirring. The stirring was continued at −10°C for 1 h, at +4°C for 2 h and at 20°C for 10 h. The precipitated N,N′-dicyclohexylurea was removed by filtration, the solvent was evaporated and the remaining oil was dissolved in ethyl acetate (30 ml) and precipitated with hexane. After filtration, the solid was reprecipitated with ether from isopropyl alcohol and dried in vacuo in a desiccator to yield 0·90 g (72%) of Boc-Cys(Acm)-Phe-Gly-Gly-ONB. A part of this product (0·72 g; 1 mmol) was dissolved in DMF (10 ml) and compound *X* (0·79 g; 1 mmol) was added. After stirring at 20°C for 5 h, the reaction mixture was mixed with ether (100 ml). The precipitate was filtered, washed with ether, extracted with hot ethyl acetate, filtered and dried in vacuo in a desiccator; yield 1·22 g (92%) of compound *XI* as amorphous powder, $[\alpha]_D^{20}$ −17·2° (*c* 1; DMF); R_F 0·79 (A), 0·25 (B), 0·62 (C). Amino acid analysis: Asp 1·01; Gly 3·00; Ile 1·75; Phe 1·07; Arg 2·05.

Boc-Ser(Bu^t)-Ser(Bu^t)-Cys(Acm)-Phe-Gly-Gly-Arg-Ile-Asp(OBu^t)-Arg-Ile-Gly-OH (*XII*)

HONB (0·08 g; 0·43 mmol) was added to a solution of compound *IV* (0·28 g; 0·33 mmol) in DMF (5 ml). The mixture was cooled to −30°C and DCC (0·08 g; 0·36 mmol) was added with stirring. Stirring was continued at −10°C for 1 h, at +4°C for 2 h and at 20°C for 10 h. The separated N,N′-dicyclohexylurea was filtered off, the solvent was evaporated and the residue was mixed with ether (60 ml). The precipitate was collected on filter, washed with ether, dried and dissolved in DMF (5 ml). Compound *X* (0·26 g; 0·33 mmol) was added to this solution and the reaction mixture was stirred at 20°C for 4 h. After addition of ether (70 ml), the precipitate was filtered, washed with ether and dried in vacuo in a desiccator to give 0·42 g (77%) of compound *XII* as an amorphous powder, $[\alpha]_D^{20}$ −6·4° (*c* 1; DMF); R_F 0·79 (A), 0·34 (B), 0·69 (C). Amino acid analysis: Asp 0·93; Ser 1·92; Gly 3·00; Ile 1·86; Phe 1·04; Arg 2·02.

Z-Ser(Bu^t)-Ser(Bu^t)-OH.DCHA (*XIII*)

Methanolic 40% solution of Triton B (5 ml) was added to a solution of H-Ser(Bu^t)-OH (1·61 g; 10 mmol) in methanol (15 ml). After evaporation of the solvent, the residue was dissolved in DMF (50 ml) and Z-Ser(Bu^t)-ONB (4·56 g; 10 mmol) was added. After standing at 20°C for 15 h, the solvent was evaporated, the residue was dissolved in ethyl acetate (100 ml) and washed with 2% sulfuric acid and then with water to neutrality. The ethyl acetate was driven off, the remaining oil was dissolved in ether (30 ml) and dicyclohexylamine (2 ml; 10 mmol) was added. After 15 h the crystals were collected, washed with ether and dried; yield 5·2 g (81%) of compound *XIII*, m. p. 127–132°C; $[\alpha]_D^{20}$ +28·3° (*c* 1; DMF); R_F 0·71 (G).

Z-Arg(NO$_2$)-Ser(Bu^t)-Ser(Bu^t)-OH (*XIV*)

A suspension of compound *XIII* (6·35 g; 10 mmol) in ethyl acetate (300 ml) was treated with 2% sulfuric acid and then washed with water to neutrality. The solvent was evaporated, the residue was dissolved in ethanol (150 ml) and hydrogenated over a Pd/C catalyst. The catalyst was filtered off, the filtrate was taken down, the residue was codistilled with isopropyl alcohol, dissolved in DMF (50 ml), and Z-Arg(NO$_2$)-OPfp (5·18 g; 10 mmol) was added. After 4 h the mixture was taken down, the residue was dissolved in ethyl acetate (50 ml) and precipitated with diisopropyl ether. The precipitate was filtered, washed with diisopropyl ether and dried to afford 6·11 g (91%) of compound *XIV*, m.p. 103–104°C; $[\alpha]_D^{20}$ +16·0° (*c* 1; DMF); R_F 0·82 (A), 0·42 (G).

H-Arg-Ser(Bu^t)-Ser(Bu^t)-OH (*XV*)

Compound *XIV* (6·0 g; 8·9 mmol) was hydrogenated in 90% acetic acid (70 ml) on Pd/C. The catalyst was filtered off and the filtrate was evaporated. The residue was dissolved in water (50 ml) and treated with ion-exchanging resin (OH^- form) up to pH 10. The resin was filtered off, the filtrate was evaporated and the residue was evaporated with isopropyl alcohol. The residue was dissolved in ethanol and treated with ethyl acetate. The precipitate was filtered, washed with ethyl acetate and dried to afford 3·6 g (84%) of compound *XV* as amorphous powder. $[\alpha]_D^{20}$ +30·4° (*c* 1, DMF), R_F 0·5 (A), 0·27 (B), 0·44 (C).

Z-Arg(NO_2)-Arg-Ser(Bu^t)-Ser(Bu^t)-OH (*XVI*)

Z-Arg(NO_2)-OPfp (2·59 g; 5 mmol) was added to a solution of compound *XV* (2·38 g; 5 mmol) in DMF (30 ml). After standing at 20°C for 4 h, the solvent was removed, the residue was dissolved in chloroform (35 ml) and applied onto a column (2·5 × 40 cm) of silica gel. The column was then eluted with chloroform (3 volumes) and then with a linear gradient of methanol in chloroform (0—100%). The product-containing fractions were combined and the solvent was evaporated. The residue was reprecipitated with ethyl acetate from methanol and dried. Yield 3·50 g (86%) of compound *XVI*, m.p. 157—162°C; $[\alpha]_D^{20}$ +30·0° (*c* 1; DMF); R_F 0·65 (A), 0·44 (B).

Z-Leu-Arg-Arg-Ser(Bu^t)-Ser(Bu^t)-OH (*XVII*)

Compound *XVI* (3·5 g; 4·2 mmol) was hydrogenated in 80% acetic acid (90 ml) for 5 h. The catalyst was filtered off, the solvent was evaporated, the residue was dissolved in water (100 ml) and the solution was adjusted to pH 10 with an ion-exchanging resin (OH^- form). The resin was filtered off, the filtrate was taken down and the residue was codistilled with isopropyl alcohol. The remaining oil was dissolved in DMF (50 ml) and Z-Leu-ONp (1·64 g; 4·2 mmol) was added. After standing at 20°C for 15 h and evaporation, the residue was dissolved in chloroform (40 ml) and applied onto a column (2·5 × 40 cm) of silica gel. The column was washed successively with chloroform (300 ml), chloroform–methanol (1 : 1; 800 ml) and the product was eluted with 1% acetic acid in methanol. The product-containing fractions were combined, the solvent was evaporated and the residue was dissolved in methanol (25 ml) and precipitated with ethyl acetate. The separated product was filtered, washed with ethyl acetate and dried. Yield 2·50 g (70%) of compound *XVII*, m.p. 162—165°C; $[\alpha]_D^{20}$ +2·0° (*c* 0·6; DMF); R_F 0·52 (A), 0·41 (B), 0·71 (C).

Boc-Ser(Bu^t)-Arg-Arg-Ser(Bu^t)-Ser(Bu^t)-OH (*XVIII*)

Compound *XVII* (1·76 g; 2 mmol) was hydrogenated in 80% acetic acid (40 ml) in the presence of a Pd/C catalyst for 5 h. After removal of the catalyst, the solvent was evaporated, the residue was dissolved in water (50 ml) and the solution was adjusted to pH 10 with an ion-exchanging resin (OH^- form). After filtration and evaporation of the solvent, the residue was coevaporated with isopropyl alcohol. The remaining oil was dissolved in DMF (20 ml) and Boc-Ser(Bu^t)-ONB (0·87 g; 2 mmol) was added. The reaction mixture was kept at 20°C for 15 h, stripped of the solvent, the residue was dissolved in a chloroform–methanol mixture (9 : 1) and applied onto a column (2.5 × 40 cm) of silica gel. The column was washed successively with chloroform–methanol (1 : 1; 300 ml) and with 1% solution of acetic acid in methanol. The product fractions were combined, taken down and the residue was precipitated with ethyl acetate from methanol. The precipitate was filtered, washed with ethyl acetate and dried to give 1·62 g (79%) of compound *XVIII*, m.p. 169—173°C; $[\alpha]_D^{20}$ +2·8° (*c* 1; DMF); R_F 0·48 (A), 0·36 (B), 0·64 (C). Amino acid analysis: Ser 2·87; Leu 0·98; Arg 1·92. ^{1}H NMR, δ: Ser — 6·76, 1 H (NH), 4·01, 1 H (α-CH),

3·43, 2 H (β-CH_2); Leu — 7·80, 1 H (NH), 4·38, 1 H (α-CH), 1·42, 2 H (β-CH_2); Arg — 8·25, 1 H (NH), 1·58, 1·87, 2 H (β-CH_2), 4·25, 1 H (α-CH); Arg — 7·95, 1 H (NH), 4·38, 1 H (α-CH), 1·54, 1·72, 2 H (β-CH_2); Ser — 8·47, 1 H (NH), 4·20, 1 H (α-CH), 3·56, 3·73, 2 H (β-CH_2); Ser — 7·37, 1 H (NH), 3·72, 1 H (α-CH), 3·59, 3·52, 2 H (β-CH_2); 1·06, 1·09, 1·12, 3 × 9 H (Bu^t), 1·38, 9 H (Boc).

Boc-Ser(Bu^t)-Leu-Arg-Arg-Ser(Bu^t)-Ser(Bu^t)-Cys(Acm)-Phe-Gly-Gly-OH (*XIX*)

A) To a solution of compound *XVIII* (0·52 g; 0·5 mmol) in DMF (5 ml) was added HONB (0·18 g; 1 mmol) and pyridine hydrobromide (0·16 g; 1 mmol). The mixture was cooled to −30°C and a solution of DCC (0·13 g; 0·61 mmol) in DMF (1 ml) was added. The reaction mixture was stirred at −30°C for 1 h, at +4°C for 15 h and at 20°C for 20 h. After addition of ether (100 ml), the precipitate was filtered, washed on the filter with ether, dried and dissolved in DMF (5 ml).

B) Compound *III* (0·27 g; 0·5 mmol) was treated in the same manner as described for compound *IV* (procedure *B*). The obtained solution was added to the solution obtained above under *A*).

After stirring of the reaction mixture at 20°C for 15 h, 1M-HCl (0·5 ml) and ethyl acetate (60 ml) were added, the precipitate was filtered and reprecipitated from DMF with ethyl acetate. The obtained product was purified on a column of Silasorb RP_{18} (1·6 × 25 cm), elution with a gradient of 0·1% aqueous trifluoroacetic acid in methanol (0—100%). The product-containing fractions were combined and the solvents were evaporated. Reprecipitation with ethyl acetate from methanol afforded 0·44 g (54%) of compound *XIX* as an amorphous powder, $[\alpha]_D^{20}$ −20·7° (*c* 1; DMF); R_F 0·31 (B), 0·61 (C), 0·51 (A). Amino acid analysis: Ser 2·56; Gly 2·04; Arg 1·98; Phe 0·93; Leu 0·97.

Boc-Ser(Bu^t)-Leu-Arg-Arg-Ser(Bu^t)-Ser(Bu^t)-Cys(Acm)-Phe-Gly-Gly-Arg-Ile-Asp(OBu^t)--Arg-Ile-Gly-OH (*XX*)

HONB (0·05 g; 0·28 mmol) was added to a solution of compound *XIX* (0·20 g; 0·14 mmol) in DMF (5 ml), the mixture was cooled to −30°C and DCC (0·03 g; 0·14 mmol) was added. The reaction mixture was stirred at −30°C for 1 h and at 20°C for 36 h. Ether (60 ml) was added, the precipitate was filtered, washed with ether, dried and dissolved in DMF (5 ml). Compound *X* (0·12 g; 0·14 mmol) was added, the mixture was stirred at 20°C for 15 h, diluted with ethyl acetate (50 ml), the precipitate was filtered, washed with ethyl acetate and dried in vacuo (desiccator). Yield 0·29 g (94%) of compound *XX* as an amorphous powder, $[\alpha]_D^{20}$ −21·3° (*c* 1; DMF); R_F 0·39 (A). Amino acid analysis: Asp 0·98; Ser 2·63; Gly 3·02; Ile 1·97; Leu 0·95; Phe 0·92; Arg 3·90.

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