SYNTHESIS OF DERIVATIVES AND PEPTIDES OF α-AMINO-β-GUANIDINOPROPIONIC ACID AND α-AMINO-γ-GUANIDINOBUTYRIC ACID*

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Reaction of α-benzyloxycarbonylamino-β-aminopropionic acid (L, *IIIa*; D, *IIIb*) and α-benzyloxycarbonylamino-γ-aminobutyric acid (*VII*) with 2-methyl-1-nitroisourea afforded α-benzyloxycarbonylamino-β-nitroguanidinopropionic acid (L, *IVa*; D, *IVb*) and α-benzyloxycarbonylamino-γ-nitroguanidinobutyric acid (*VIII*), resp. The corresponding protected dipeptide amides were obtained by condensation of compounds *IVa*, *IVb*, and *VIII* with glycine amide. Decarbobenzoxylation (HBr/CH_3COOH) of D-α-benzyloxycarbonylamino-β-nitroguanidinopropionyl-glycine amide (*IXa*), acylation of the resulting dipeptide amide hydrobromide with benzyloxycarbonylproline according to the REMA method, and decarbobenzoxylation (HBr/CH_3COOH) of the resulting benzyloxycarbonylprolyl-D-α-amino-β-nitroguanidinopropionyl-glycine amide (*X*) yielded prolyl-D-α-amino-β-nitroguanidinopropionyl-glycine amide hydrobromide. The azide of β-benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine was condensed with prolyl-D-α-amino-β-nitroguanidinopropionyl-glycine amide hydrobromide to afford β-benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-D-α-amino-β-nitroguanidinopropionyl-glycine amide (*XIII*).

From the standpoint of investigations on the relationship between chemical structure and biological activity of peptidic compounds, the existing set of proteinogenic amino acids is not sufficient[1]: it contains incomplete homologous series and a limited number of amino acid derivatives of the particular type (L). In the present paper, the synthesis of some lacking members of this set is reported, namely, of peptides derived from lower homologues of arginine α-amino-β-guanidinopropionic acid (Agp)** and α-amino-γ-guanidinobutyric acid (Agb). This work was made in connection with investigations on the importance of position 8 for biological effects of vasopressins. The synthesis of Agp and Agb peptides cannot be underestimated

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** Symbols and abbreviations in this paper are in accordance with recommendations of the IUPAC-IUB Commision on Biochemical Nomenclature[2-4]. Unless stated otherwise, the optically active amino acids are of the L-series.

since the steric disposition of functional groups in the two lower homologues of arginine makes possible numerous intramolecular interactions. Some problems accompanying the preparation of Agp and Agb peptides have been encountered earlier. An attempt was therefore made to circumvent this difficulty by the indirect* "guanidination procedure"[5,6]. The guanidination procedure[7] has been used in the synthesis of arginine peptides[8-10] including arginine-vasopressin[11]. The results obviously depend on the nature of the reagent and on the complexity of the peptide. A synthesis of some peptide derivatives of α-amino-γ-nitroguanidinobutyric acid by nitroguanidination of the corresponding peptides of α,γ-diaminobutyric acid has been recently reported by Arold and Gersch[12].

The guanidination procedure did not prove quite satisfactory in the synthesis of arginine-vasopressin analogues[5,6]; in these syntheses, partially protected linear vasopressin peptides (with a free ω-amino group in the position 8) were guanylated with the use of O-methylisourea, S-methylisourea, and 1-guanidino-3,5-dimethylpyrazole nitrate. The guanidinations were incomplete (60–80%) and the purification of products was difficult and accompanied by loss of material both in the stage of the linear peptide and after removal of protecting groups and closure of the disulphide ring. The thus-prepared analogues of arginine-vasopressin contained despite the purification about 10% of the nonguanidinated contaminant. The presence of the non-guanidinated product is due not only to the incomplete guanidination reaction but also to a partial loss of the guanidine residue during the last step of the synthesis comprising reduction with sodium in liquid ammonia and purification. A similar loss was observed when a sample was hydrolysed for purposes of the amino acid analysis. Difficulties encountered in the guanidination procedure prompted us to investigate the synthesis of Agp and Agb peptides in detail. In the meantime, the accessibility of Agb peptides from α-amino-γ-nitroguanidinobutyric acid has been demonstrated by van Nispen and Tesser[13]. The more complex problem of the synthesis of Agp peptides remained to be examined. Our attention was particularly focussed to this question.

In the selection of the starting compounds it appeared advisable to use the same type for both groups of peptides. It was also taken into account that cupric complexes[14] could not be used in the preparation of α,β-diaminopropionic acid β-monoacyl derivatives and that the attempted benzyloxycarbonylation of α-tosylamino-β-guanidinopropionic acid or α-tosylamino-γ-guanidinobutyric acid was not successful in our hands. Considering these observations, α-tosylamino-β-aminopropionic acid and α-tosylamino-γ-aminobutyric acid were chosen as the starting material. Both acids are readily accessible from tosylasparagine and tosylglutamine[15] and have been used earlier in the preparation of lysine-vasopressin analogues[1,16,17].

* In contrast to direct syntheses starting from the corresponding α-amino-ω-guanidino acids.

In view of the above mentioned difficult acylation of the guanidino group in α-tosylamino-β-guanidinopropionic acid and α-tosylamino-γ-guanidinobutyric acid it appeared advisable to introduce the guanidino residue in the protected form. In this respect, the nitroguanidino group seemed to be the most advantageous (see ref.[18,19]). Nitroguanidinations are effected readily and in fair yields with the use of 2-methyl-1-nitroisourea[13,20]. The combination of a tosyl residue and a nitroguanidino group is not too suitable in the synthesis of peptides. Owing to the properties of the nitroguanidino group and the character of both starting acids, the benzyloxycarbonyl residue was used to protect the α-amino groups. The tosyl residue in both starting acids was replaced by the benzyloxycarbonyl group according to a slightly modified known procedure[21]. The resulting α-benzyloxycarbonylamino-β-aminopropionic acid (L, *IIIa*; D, *IIIb*) and α-benzyloxycarbonylamino-γ-aminobutyric acid (*VII*) were treated with 2-methyl-1-nitroisourea (the use of 1 equivalent of 1M-NaOH proved to be essential) to afford α-benzyloxycarbonylamino-β-nitroguanidinopropionic acid (L, *IVa*; D, *IVb*) and α-benzyloxycarbonylamino-γ-nitroguanidinobutyric acid (*VIII*), resp., in fair yields. The protected acids *IVa*, *IVb*, and *VIII* were used in the preparation of peptide fragments from the carboxylic end of the vasopressin molecule and the linear protected peptide containing the amino-acid sequence of [Mpr[1], D-Agp(G-NO_2)[8]]vasopressin. Thus, condensation of compounds *IVa*, *IVb*, and *VIII* with glycine amide[22] afforded α-benzyloxycarbonylamino-β-nitroguanidinopropionyl-glycine amide (L, *IXa*; D, *IXb*) and α-benzyloxycarbonylamino-γ-nitroguanidinobutyryl-glycine amide (*XI*) in good yields. Some complications were encountered in the second step of the synthesis, *i.e.*, in the condensation of benzyloxycarbonylproline with α-amino-β-nitroguanidinopropionyl-glycine amide (obtained by decarbobenzoxylation of compound *IXa* with a solution of hydrogen bromide in glacial acetic acid[23]). The dicyclohexylcarbodiimide method in the presence of N-hydroxybenzotriazole afforded a very low yield of the required product. A somewhat better yield was obtained by the method of mixed anhydrides (ethyl chloroformate)[24,25], but the yield was not high enough to make possible a preparative utilisation of the method. As shown by thin-layer chromatography and paper electrophoresis, the reaction mixture (with the use of both the carbodiimide method and the method of mixed anhydrides) contained a substance, probably 2-nitriminoimidazoline-4-carbonyl-glycine amide (or the corresponding degradation product) formed by cyclisation involving the α-amino group of α-amino-β-nitroguanidinopropionyl-glycine amide and the nitroguanidino group. The cyclisation reaction is obviously considerably fast. In order to decrease its extent and improve conditions for the formation of the peptide bond, the method of mixed anhydrides was exclusively adopted as a method with a very fast course and the amount of the mixed anhydride was increased. However, optimum yields were recorded with the use of the REMA method[26,27]. Under such conditions, benzyloxycarbonylprolyl-D-α-amino-β-nitroguanidinopropionyl-glycine amide (*X*) was obtained in a satisfactory yield. The product was not crystalline,

but of a high purity. Benzyloxycarbonylprolyl-α-amino-γ-nitroguanidinobutyryl--glycine amide (*XII*) was prepared analogously from α-benzyloxycarbonylamino--γ-nitroguanidinobutyric acid (*VIII*). The applicability of the thus-obtained nitroguanidino derivatives in the preparation of vasopressin peptides was demonstrated as follows. Compound *X* was decarbobenzoxylated on treatment with a solution of hydrogen bromide in acetic acid and the resulting prolyl-D-α-amino-β-nitroguanidinopropionyl-glycine amide hydrobromide was condensed with β-benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine azide to afford a very good yield of the corresponding protected octapeptide amide derivative *XIII*.

EXPERIMENTAL

Melting points (uncorrected) were taken on a heated microscope stage (Kofler block). The optical rotation was measured on a Perkin-Elmer Type 141 polarimeter. The UV spectra were recorded on a CF 4 spectrophotometer (Optica Milano). The IR spectra were taken on an IR-20 apparatus (Carl Zeiss, Jena). The purity of products was checked by thin-layer chromatography on ready--for-use Silufol (Kavalier Glassworks, Votice, Czechoslovakia) silica gel sheets in the solvent systems A, 1-butanol–acetic acid–water (4 : 1 : 1); B, 1-butanol–acetic acid–water (4 : 1 : 5); C, 1-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 12); D, acetonitrile–water (3 : 1); E, ethanol –chloroform (4 : 1); F, 1-butanol–ethyl acetate–acetic acid–water (1 : 1 : 1 : 1), and G, cyclohexane–ethyl acetate–methanol (1 : 1 : 1), and by electrophoresis on paper Whatman No 3 (5% aqueous acetic acid and pyridine acetate buffer solution, pH 5·7). Analytical samples were dried at 80°C/0·1 Torr for 8 h.

α-Tosylamino-β-tert-butyloxycarbonylaminopropionic Acid (*Ia*)

A suspension of α-tosylamino-β-aminopropionic acid[15] (82 g; 0·32 mol) in water (80 ml) was treated with 4M sodium hydroxide (80 ml) and tert-butyloxycarbonyl azide (115 g; 0·82 mol) in dioxane (160 ml). The whole mixture was heated at 50°C for 8 h, the pH being maintained at the value of 10 by additions of 4M sodium hydroxide. The mixture was then washed with five 100 ml portions of ether and the aqueous layer adjusted with hydrochloric acid to pH 3. The precipitate was extracted with ethyl acetate (one 1 000 ml portion, one 500 ml portion, and two 100 ml portions), the extracts combined, washed with two 100 ml portions of water, dried over anhydrous sodium sulphate, and evaporated. The residue was crystallised from ethyl acetate–light petroleum to afford 109 g (96%) of compound *Ia*, m.p. 129–131°C and $[\alpha]_D^{22}$ −71·2° (*c* 4, 1M-NaOH); reported[21], m.p. 125–126°C to 128–129°C and $[\alpha]_D^{22}$ −71·1° (*c* 4, 1M-NaOH). The product was chromatographically homogeneous in solvent systems D and E.

D-α-Tosylamino-β-tert-butyloxycarbonylaminopropionic Acid (*Ib*)

Analogously to the preparation of compound *Ia*, D-α-tosylamino-β-aminopropionic acid (100 g; 0·39 mol) and tert-butyloxycarbonyl azide (140 ml; 1 mol) yielded 135 g (97%) of the title compound *Ib*, m.p. 130–131°C (after recrystallisation from ethyl acetate–light petroleum) and $[\alpha]_D^{24}$ +71·65° (*c* 4, 1M-NaOH). The product was homogeneous in solvent systems D and E. For $C_{15}H_{22}N_2O_6S$ (358·4) calculated: 50·41% C, 6·19% H, 7·82% N, 8·95% S; found: 50·41% C, 6·32% H, 7·93% N, 9·13% S.

α-Benzyloxycarbonylamino-β-tert-butyloxycarbonylaminopropionic Acid (*IIa*)

The detosylation of compound *Ia* (35·8 g; 0·1 mol) was effected according to a known procedure[21] except for the removal of the blue color (excess sodium) of the reaction mixture, accomplished by the addition of acetic acid. The ammonia was evaporated, the residue dissolved in water (50 ml), and the aqueous solution extracted with three 100 ml portions of ether. Carbobenzoxylation (*cf.* ref.[21]) yielded 22·3 g (66%) of the title compound *IIa*, m.p. 145—146°C (after two recrystallisations from ethyl acetate–light petroleum); reported[21], 145—146°C. Optical rotation: $[\alpha]_D^{22}$ −20·1° (*c* 4, 1M-NaOH) and $[\alpha]_D^{22}$ −9·45° (*c* 1, methanol). The product was homogeneous in solvent systems B, D, and E.

D-α-Benzyloxycarbonylamino-β-tert-butyloxycarbonylaminopropionic Acid (*IIb*)

The preparation was effected analogously to that of compound *IIa*. Yield, 21·7 g (64%) of compound *IIb*, m.p. 145—146°C (after three recrystallisations from ethyl acetate–light petroleum), $[\alpha]_D^{24}$ +11·8° (*c* 1, methanol) and $[\alpha]_D^{24}$ +23·0° (*c* 4, 1M-NaOH). The product was chromatographically homogeneous in solvent systems B, D, and E. The analytical sample was recrystallised from aqueous ethanol. For $C_{16}H_{22}N_2O_6$ (338·4) calculated: 56·80% C, 6·55% H, 8·28% N; found: 56·80% C, 6·55% H, 8·29% N.

α-Benzyloxycarbonylamino-β-aminopropionic Acid (*IIIa*)

A solution of trifluoroacetic acid (20 ml) in dichloromethane (5 ml) was added to the acid *IIa* (5·7 g; 17 mmol), the mixture kept at room temperature for 20 min, and evaporated under diminished pressure. The residue was triturated with ether and the precipitate dissolved in water (50 ml). The aqueous solution was adjusted to pH 7—8 by the addition of concentrated aqueous ammonia to deposit a solid which was collected with suction and dried over phosphorus pentoxide. Recrystallisation from water yielded compound *IIIa*, m.p. 240—241° (decomp.) and $[\alpha]_D^{22}$ −7·4° (*c* 0·4, 1M-NaOH). The product was chromatographically homogeneous in solvent systems A, B, and C. For $C_{11}H_{14}N_2O_4$ (238·2) calculated: 55·46% C, 5·92% H, 11·76% N; found: 55·41% C, 6·05% H, 11·77% N.

D-α-Benzyloxycarbonylamino-β-aminopropionic Acid (*IIIb*)

The preparation was effected analogously to that of compound *IIIa*. Thus, compound *IIb* (11·2 g; 33 mmol) yielded (after recrystallisation of the crude product from water) 7·2 g (91%) of compound *IIIb*, m.p. 240°C (decomp.) and $[\alpha]_D^{22}$ +7·5° (*c* 0·4, 1M-NaOH). The product was chromatographically homogeneous in solvent systems A, B, and C. For $C_{11}H_{14}N_2O_4$ (238·2) calculated: 55·46% C, 5·92% H, 11·76% N; found: 55·48% C, 5·84% H, 11·87% N.

α-Benzyloxycarbonylamino-β-nitroguanidinopropionic Acid (*IVa*)

A stirred suspension of acid *IIIa* (11·2 g; 47 mmol) in water (50 ml) was treated at 0—5°C with such an amount of 1M-NaOH to dissolve the solid. 2-Methyl-1-nitroisourea[20] (6·2 g; 52 mmol) and additional 1M-NaOH (total amount, 1 equivalent) were then portionwise introduced in the solution. The stirring was continued for 60—90 min until the solid material dissolved. The mixture was then washed with two 50 ml portions of ethyl acetate and the aqueous layer adjusted to pH 2 by the addition of hydrochloric acid. The precipitate was extracted with ethyl acetate, the extract dried over anhydrous magnesium sulfate, and evaporated. Recrystallisation of the residue from

ethyl acetate yielded 11·2 g (73%) of compound *IVa*, m.p. 162—163·5° (decomp.), chromatographically homogeneous in solvent systems A and B. The analytical sample was recrystallised from the solvent mixture methanol–ethyl acetate–light petroleum; m.p. 164·5—165·5°C (decomp.); $[\alpha]_D^{22}$ —4·3° (*c* 4, 1M-NaOH); $[\alpha]_D^{22}$ —9·65° (*c* 1, methanol); $[\alpha]_D^{22}$ —34·4° (*c* 1, dimethylformamide). IR spectrum: benzyloxycarbonyl 1691 cm^{-1}, 1535 cm^{-1}, 1546 cm^{-1}, 3450 cm^{-1}, 3350 cm^{-1}; —COOH 1712 cm^{-1}, 2800—3500 cm^{-1}; nitroguanidino group 1635 cm^{-1}, 1503 cm^{-1}. UV spectrum (methanol): λ_{max} 208 nm, λ_{min} 233 nm, λ_{max} 268 nm. For $C_{12}H_{15}N_5O_6$ (325·3) calculated: 44·31% C, 4·65% H, 21·53% N; found: 44·25% C, 4·83% H, 21·56% N.

D-α-Benzyloxycarbonylamino-β-nitroguanidinopropionic Acid (*IVb*)

The preparation was effected analogously to that of compound *IVa*. Thus, compound *IIIb* (10 g) and 2-methyl-1-nitroisourea (5·5 g; 46 mmol) yielded 10 g (73%) of compound *IVb*. The product was recrystallised from the solvent mixture methanol–ether–light petroleum; m.p. 164·5—165·5°C; $[\alpha]_D^{22}$ +3·5° (*c* 4, 1M-NaOH); $[\alpha]_D^{22}$ +8·5° (*c* 0·9, methanol); $[\alpha]_D^{22}$ +34·6° (*c* 0·4, dimethylformamide). The recrystallised product was chromatographically homogeneous in solvent systems A and B. For $C_{12}H_{15}N_5O_6$ (325·3) calculated: 44·31% C, 4·65% H, 21·53% N; found: 44·49% C, 4·77% H, 21·79% N.

α-Tosylamino-γ-tert-butyloxycarbonylaminobutyric Acid (*V*)

The preparation was analogous to that of compound *Ia*. Thus, α-tosylamino-γ-aminobutyric acid[15] (44 g; 0·16 mol) and tert-butyloxycarbonyl azide (65 ml; 0·46 mol) yielded 58 g (96%) of compound *V*, m.p. (after recrystallisation from ethyl acetate–light petroleum) 149·5—150·0°C (decomp.); $[\alpha]_D^{25}$ —50·5° (*c* 1, 1M-NaOH); $[\alpha]_D^{22}$ —8·1° (*c* 0·5, dimethylformamide); $[\alpha]_D$ —15·3° *c* 1, methanol). The product was chromatographically homogeneous in solvent systems D and F. Reported[28], m.p. 149·5—150·0°C (decomp.) and $[\alpha]_D$ —1·2° (*c* 0·5, dimethylformamide).

α-Benzyloxycarbonylamino-γ-tert-butyloxycarbonylaminobutyric Acid (*VI*)

Compound *V* (37·2 g; 0·1 mol) was detosylated (*cf.* ref.[28]) and the resulting α-amino-γ-tert-butyloxycarbonylaminobutyric acid carbobenzoxylated (*cf.* ref.[21]) to afford the title acid *VI* in the form of a chromatographically homogeneous oil (solvent systems B, D, and E). The properties of the dicyclohexylammonium salt were identical with those reported earlier[29].

α-Benzyloxycarbonylamino-γ-aminobutyric Acid (*VII*)

A mixture of compound *VI* (10 g; 0·27 mol), dichloromethane (5 ml), and trifluoroacetic acid (20 ml) was kept at room temperature for 20 min, evaporated under diminished pressure, the residue triturated at 20°C with ether, and the precipitate dissolved in water (50 ml). The aqueous solution was adjusted to pH 7—8 by the addition of concentrated aqueous ammonia, diluted with acetone (5000 ml), and the whole mixture kept at 0°C overnight. The solid was collected with suction and recrystallised from acetic acid–ether. Yield, 5 g (74%) of compound *VII*, m.p. 194·5—195·0°C (decomp.) and $[\alpha]_D^{23}$ —9·25° (*c* 1, water). The product was chromatographically homogeneous in solvent systems A, B, and C. For $C_{12}H_{16}N_2O_4$ (252·3) calculated: 57·13% C, 6·39% H, 11·10% N; found: 57·01% C, 6·35% H, 11·17% N.

α-Benzyloxycarbonylamino-γ-nitroguanidinobutyric Acid (*VIII*)

A stirred solution of acid *VII* (2·5 g; 10 mmol) in water (10 ml) was treated portionwise at 0°C to +5°C over 30 min with 1M-NaOH and 2-methyl-1-nitroisourea (1·4 g; 12 mmol) in such a manner to keep both components in solution and to maintain the pH above 9. The stirring was continued for additional one hour and the mixture processed analogously to the preparation of compound *IVa*. Yield, 3·0 g (83%) of compound *VIII* as an oil homogeneous on chromatography in solvent systems A and B. Dicyclohexylammonium salt, m.p. 140–141°C (after recrystallisation from ethyl acetate) and $[\alpha]_D^{24}$ −7·6° (*c* 0·7, methanol). For $C_{25}H_{40}N_6O_6$ (520·6) calculated: 57·68% C, 7·74% H, 16·14% N; found: 57·43% C, 7·75% H, 15·97% N. UV spectrum (methanol): λ_{min} 237 nm, λ_{max} 271 nm.

α-Benzyloxycarbonylamino-β-nitroguanidinopropionyl-glycine Amide (*IXa*)

A solution of glycine amide hydrobromide (1·55 g; 10 mmol) and N-methylmorpholine (1·12 ml; 10 mmol) in dimethylformamide (10 ml) was added to a solution of compound *IVa* (3·25 g; 10 mmol) and N-hydroxybenzotriazole (1·35 g; 10 mmol) in dimethylformamide (8 ml), the mixture cooled down to 0°C and treated (stirring) with a solution of N,N′-dicyclohexylcarbodiimide (2·3 g; 11 mmol) in dimethylformamide (6 ml). The whole mixture was kept at 0°C for 1 h and at room temperature for 15 h. The precipitate of N,N′-dicyclohexylurea was filtered off and the filtrate evaporated. The residue was successively triturated with three 30 ml portions of saturated aqueous sodium hydrogen carbonate, saturated aqueous sodium chloride (30 ml), three 30 ml portions of 1% aqueous hydrochloric acid, saturated aqueous sodium chloride again, and finally crystallised from aqueous methanol. Compound *IXa* was homogeneous on chromatography in solvent systems A, B, and C; m.p. 130–132°C; $[\alpha]_D^{20}$ −7·3° (*c* 1, ethanol); $[\alpha]_D^{20}$ −17·2° (*c* 1, dimethylformamide). For $C_{14}H_{19}N_7O_6 \cdot H_2O$ (399·4) calculated: 42·10% C, 5·30% H, 24·55% N; found: 42·20% C, 5·29% H, 24·39% N. UV spectrum (ethanol): λ_{min} 238 nm, λ_{max} 268 nm.

D-α-Benzyloxycarbonylamino-β-nitroguanidinopropionyl-glycine Amide (*IXb*)

Compound *IVb* (3·25 g; 10 mmol) and corresponding amounts of other reactants yielded 3 g (75%) of compound *IXb*, m.p. 129–131°C (after three crystallisations from aqueous methanol) and $[\alpha]_D^{21}$ +7·7° (*c* 1, ethanol); $[\alpha]_D^{21}$ +16·9° (*c* 1, dimethylformamide). The product was homogeneous on chromatography in solvent systems A, B, and C. For $C_{14}H_{19}N_7O_6 \cdot H_2O$ (399·4) calculated: 42·10% C, 5·30% H, 24·55% N; found: 42·26% C, 5·25% H, 24·56% N.

Benzyloxycarbonylprolyl-D-α-amino-β-nitroguanidinopropionyl-glycine Amide (*X*)

Hydrogen bromide in glacial acetic acid (25 ml of a 35% solution) was added to a solution of compound *IXb* (3·8 g; 10 mmol) in glacial acetic acid (15 ml), the whole heated at 60°C for 7 min, cooled down, and precipitated with ether (200 ml). The solid hydrobromide was collected with suction and dried over phosphorus pentoxide and potassium hydroxide for one week; yield, 4 g (100%).

A solution of benzyloxycarbonylproline (4·64 g; 20 mmol) in dimethylformamide (10 ml) was treated at −10°C with N-methylmorpholine (2·23 ml; 20 mmol), the mixture stirred at this temperature for 5 min, treated with ethyl chloroformate (1·91 ml; 20 mmol), and the stirring continued for additional 5 min. The resulting suspension was then treated at −30°C with a solution of the above dipeptide amide hydrobromide (4 g; 10 mmol; homogeneous on chromatography in solvent systems A and B) in dimethylformamide (15 ml) followed by N-methylmorpholine

(2·23 ml; 20 mmol) at the same temperature. The mixture was stirred at −15°C for 4 h, adjusted to pH 8 by the addition of 2·5M potassium hydrogen carbonate (7 ml), and stirred at 0°C for 30 min more. On the addition of 30% aqueous sodium chloride, the mixture deposited an oil which was extracted with chloroform (100 ml), the extract washed with two 25 ml portions of 33% aqueous sodium chloride, dried over anhydrous sodium sulfate, and evaporated under diminished pressure. The residue was triturated with ethyl acetate to afford 3·9 g (81%) of the amorphous compound *X*, homogeneous on chromatography in solvent systems A, B, and C. Amino acid analysis[30]: Pro 1·03, Gly 0·98.

α-Benzyloxycarbonylamino-γ-nitroguanidinobutyryl-glycine Amide (*XI*)

A solution of α-benzyloxycarbonylamino-γ-nitroguanidinobutyric acid dicyclohexylammonium salt (*VIII*) (3·0 g) in ethyl acetate (50 ml) was shaken with three 30 ml portions of 5% aqueous sulfuric acid and with water (twice), dried over anhydrous sodium sulfate, and evaporated under diminished pressure. The residual α-benzyloxycarbonylamino-γ-nitroguanidinobutyric acid (1·7 g; 5 mmol) was condensed with glycine amide hydrobromide (0·8 g; 5 mmol) dissolved in 5 ml of dimethylformamide containing 0·6 ml (5 mmol) of N-methylmorpholine, with the use of a solution of N,N′-dicyclohexylcarbodiimide (1·03 g; 5 mmol) and N-hydroxybenzotriazole (0·7 g; 5 mmol) in dimethylformamide (10 ml). The crude product was recrystallised from 90% aqueous ethanol. Yield, 1·43 g (73%) of compound *XI*, m.p. 179–180°C and $[\alpha]_D$ −4·8° (*c* 0·47, dimethylformamide). For $C_{15}H_{21}N_7O_6$ (395·4) calculated: 45·56% C, 5·35% H, 24·80% N; found: 45·79% C, 5·47% H, 24·62% N.

Benzyloxycarbonylprolyl-α-amino-γ-nitroguanidinobutyryl-glycine Amide (*XII*)

The preparation was effected analogously to that of compound *X* from the amide *IXb* (1·0 g; 2·53 mmol), benzyloxycarbonylproline (1·26 g; 5·06 mmol), N-methylmorpholine (1·12 ml), and ethyl chloroformate (0·48 ml; 5·06 mmol). Yield, 0·83 g (67%) of the amorphous benzyloxycarbonylprolyl-α-amino-γ-nitroguanidinobutyryl-glycine amide (*XII*), homogeneous on chromatography in solvent systems A, B, F, and G. Amino acid analysis[30]: Pro 1·09, Gly 0·90.

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-D-α-amino-β-nitroguanidinopropionyl-glycine Amide (*XIII*)

A solution of β-benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine hydrazide[17] (1·43 g; 1·5 mmol) in dimethylformamide (15 ml) was treated with 4M hydrogen chloride in dioxane (0·8 ml). Amyl nitrite (0·175 g; 1·5 mmol) in dimethylformamide (1 ml) was then added at −20°C, the mixture kept at this temperature for 20 min (the reaction was checked by the test with the Griess agent), cooled down to −40°C, neutralised with triethylamine, and treated with a precooled (−10°C) solution of prolyl-D-α-amino-β-nitroguanidinopropionyl-glycine amide hydrobromide (0·753 g; 2·25 mmol; chromatographically homogeneous in solvent systems A, B, and F; prepared analogously to compound *X*, see above) in dimethylformamide (5 ml) containing triethylamine (0·32 ml). The whole mixture was kept at 0°C for 3 days and then 3 h at room temperature. The triethylammonium salt was filtered off and the filtrate evaporated under diminished pressure. The residue was kept with 1% dilute hydrochloric acid (200 ml) for 30 min at 3°C, the precipitate collected with suction, washed with two 50 ml portions of 5% aqueous sodium hydrogen carbonate and one 50 ml portion of water (40°C), and dried over phosphorus pentoxide. The crude product (2 g) was crystallised from dimethylformamide–water to afford 1·8 g (94%) of compound *XIII*, m.p. 200–204°C and $[\alpha]_D^{20}$ −30·2° (*c* 1, dimethylforma-

mide). For $C_{58}H_{73}N_{15}O_{14}S_2$ (1268·45) calculated: 54·92% C, 5·80% H, 16·56% N, 5·06% S; found: 54·77% C, 5·75% H, 16·30% N, 4·99% S. Amino acid analysis: Tyr 0·96, Phe 1·00, Glu 1·03, Asp 0·99, Pro 1·08, Gly 0·95.

REFERENCES

1. Zaoral M., Šorm F.: This Journal *31*, 90 (1966).
2. IUPAC-IUB *Commission on Biochemical Nomenclature. Symbols for Amino-Acid Derivatives and Peptides. Recommendations (1971).* Biochemistry *11*, 1726 (1972).
3. *Rules for Naming Synthetic Modifications of Natural Peptides. Amendments.* Eur. J. Biochem. *45*, 3 (1974).
4. IUPAC *Commission on the Nomenclature of Organic Chemistry and* IUPAC-IUB *Commission on Biochemical Nomenclature. Nomenclature of α-Amino Acids.* Biochemistry *14*, 449 (1975).
5. Zaoral M., Flegel M.: This Journal *37*, 3350 (1972).
6. Zaoral M., Brtník F.: This Journal *40*, 905 (1975).
7. Fruton J. S.: Advan. Protein Chem. *5*, 64 (1949).
8. Katchalski E., Spitnik P.: Nature (London) *164*, 1092 (1949).
9. Zaoral M., Rudinger J.: Proc. Chem. Soc., London *1957*, 176.
10. Barras B. C., Elmore D. T.: J. Chem. Soc. *1957*, 3134.
11. Bodanszky M., Ondetti M. A., Birkhimer C. A., Thomas P. L.: J. Amer. Chem. Soc. *86*, 4452 (1964).
12. Arold H., Gersch D.: J. Prakt. Chem. *317*, 682 (1975).
13. van Nispen J. W., Tesser G. I.: Syn. Commun. *2*, 207 (1972).
14. Albert A.: Biochem. J. *50*, 690 (1952).
15. Rudinger J., Poduška K., Zaoral M.: This Journal *25*, 2022 (1960).
16. Zaoral M., Šorm F.: This Journal *31*, 310 (1966).
17. Zaoral M., Kolc J., Šorm F.: This Journal *35*, 1716 (1970).
18. Schröder E., Lübke K.: *The Peptides*, Vol. I, p. 167. Academic Press, New York and London 1965.
19. *Methoden der Organischen Chemie (Houben-Weyl)* (E. Müller, Ed.), 15/1, *Synthese von Peptiden* I, 507. Thieme, Stuttgart 1974.
20. Heyboer N., Visser G. H., Kerling K. E. T.: Rec. Trav. Chim. Pays-Bas *81*, 69 (1962).
21. Broadbent W., Morley J. S., Stone B. E.: J. Chem. Soc. C *1967*, 2632.
22. König W., Geiger R.: Chem. Ber. *103*, 788 (1970).
23. Ben-Ishai D.: J. Org. Chem. *19*, 62 (1954).
24. Wieland T., Bernhard A.: Justus Liebigs Ann. Chem. *572*, 190 (1951).
25. Boissonnas R. A.: Helv. Chim. Acta *34*, 874 (1951).
26. Tilak M. A.: Tetrahedron Lett. *1970*, 849.
27. Beyerman H. C. in the book: *Chemistry and Biology of Peptides, Proc. 3rd Amer. Symp., Boston 1972*, p. 351. Ann Arbor Scientific Publications, Ann Arbor, Michigan 1972.
28. Poduška K.: This Journal *31*, 2955 (1966).
29. Vogler K., Studer R. O., Lanz P., Lergier W., Boehmi E.: Helv. Chim. Acta *48*, 1161 (1965).
30. Spackman D. H., Stein W. H., Moore S.: Anal. Chem. *30*, 1190 (1958).

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