POLYPEPTIDES OF REGULAR STRUCTURE WITH THE AMINO-ACID SEQUENCES $(-Ala-Orn-Ala-)_n$, $(-Ala-Orn-Gly-)_n$, AND $(-Ala-Orn-Glu-)_n$

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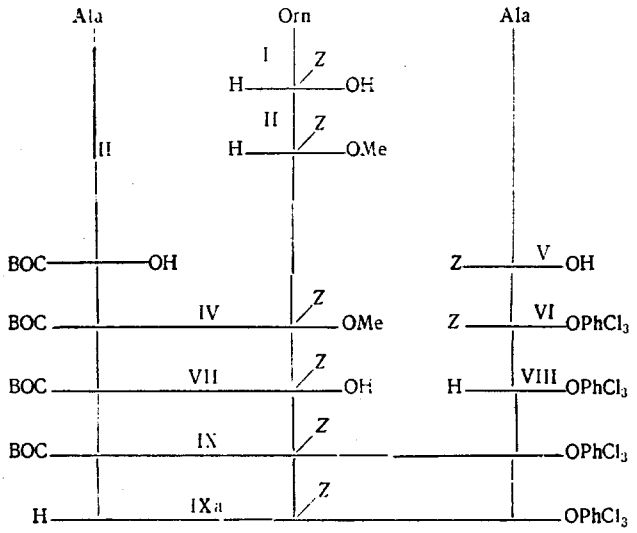
The present work is a continuation of investigations on the synthesis of polypeptides of regular structure in order to study questions of the modeling of protein structures of basic nature – histones and protamines [1-4].

The present paper describes methods for the syntheses of regular polypeptides with compositions $(-Ala-Orn-Ala-)_n$, $(-Ala-Orn-Gly-)_n$, and $(-Ala-Orn-Glu-)_n$, which were obtained by the polymerization of activated esters of tripeptides. We performed the synthesis of the latter from the C end. The δ-amino group of ornithine was blocked by methoxycarbonyl protection through the copper complex [5], the hydrochloride of the methyl ester of N^{δ}-benzyloxycarbonylornithine (III) being obtained by Brenner's method [6]. The γ-carboxy group of glutamic acid was blocked with a benzyl ester group. For the α-amino group of alanine we selected tert-butyloxycarbonyl protection, which is readily removed in the presence of a benzyloxycarbonyl group with trifluoroacetic acid or a 0.1 N solution of hydrogen chloride in benzene.

The initial dipeptide in the synthesis of the monomers was the methyl ester of tert-butyloxycarbonylalanyl-N^{δ}-benzyloxycarbonylornithine (IV), obtained from tert-butoxycarbonylalanine and the hydrochloride of the methyl ester of N^{δ}-benzyloxycarbonylornithine (III) previously treated with an equivalent amount of triethylamine by the method of mixed anhydrides with isobutyl chloroformate.

Scheme 1



Of the large number of activated esters [7] that can be used in the formation of a peptide bond we selected the 2,4,5-trichlorophenyl ester [8]. The 2,4,5-trichlorophenyl ester of tert-butoxycarbonylalanyl-

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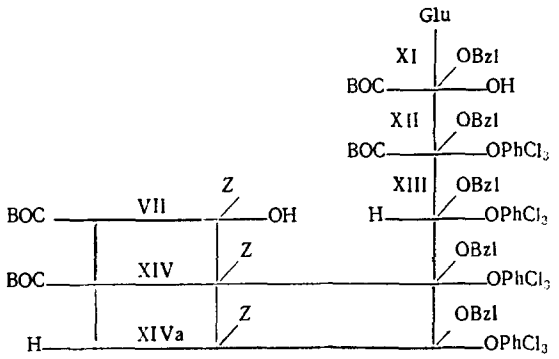
N^{δ}-benzyloxycarbonylornithylalanine (IX), the synthesis of which is shown in Scheme 1, was obtained from tert-butoxycarbonylalanyl-N^{δ}-benzyloxycarbonylornithine (VII) and the 2,4,5-trichlorophenyl ester of alanine (VIII), previously treated with an equivalent amount of triethylamine, as in the synthesis of compound (IV).

The 2,4,5-trichlorophenyl ester of tert-butoxycarbonylalanyl-N^{δ}-benzyloxycarbonylornithylglycine (X) was obtained by Scheme 1, with glycine replacing the alanine at the C-end; the 2,4,5-trichlorophenyl ester of tert-butoxycarbonylalanyl-N^{δ}-benzyloxycarbonylornithyl-γ-benzylglutamic acid (XIV) was obtained as shown in Scheme 2 from tert-butoxycarbonylalanyl-N^{δ}-benzyloxycarbonylornithine (VII) and the trifluoroacetate of the 2,4,5-trichlorophenyl ester of γ-benzylglutamic acid by the mixed-anhydride method.

Scheme 2



The N^{α}-tert-butoxycarbonyl protection was removed from the activated ester of γ-benzylglutamic acid by the action of trifluoroacetic acid at room temperature for 30 min. The activated esters of alanine and glycine were decarboxylated with a 38% solution of hydrogen bromide in glacial acetic acid for 25 and 10 min, respectively. The tert-butoxycarbonyl protection was removed from the tripeptide obtained (IX, X, or XIV) with trifluoroacetic acid at room temperature for 80 min.

The polycondensation of compounds (IXa, Xa, and XIVa) was performed in dimethylformamide in the presence of two equivalents of triethylamine for 6 days. This gave polymers with the following sequences of amino acids: $[-Ala-(N^{\delta}-Z)\ Orn-Ala-]_n$ (XV), $[-Ala-(N^{\delta}-Z)\ Orn-(\gamma-OBzl)\ Glu-]_n$ (XVI), and $[-Ala-(N^{\delta}-Z)Orn-Gly]_n$ (XVII). When the products of the polycondensation reaction were treated with methanol, fractions of polymers soluble and insoluble in methanol were separated. The molecular weights of the methanol-soluble polypeptides, determined by Van Slyke's method were: for compound (XV) 4000, for (XVI) 3400, and for (XVII) 3200; the molecular weights calculated from the IR spectra [9] were : for (XV) 4100, (XVI) 3700, and (XVII) 3000.

The benzyloxycarbonyl protections were removed from the polymers (XV) and (XVII) by the action of dry hydrogen bromide in trifluoroacetic acid at room temperature for 45 min [10] and the benzyloxycarbonyl group and benzyl ester group were removed from the poly-$[-Ala-(N^{\delta}-Z)Orn-(\gamma-OBzl)Glu-]$ simultaneously by the action of an excess of hydrogen bromide in glacial acetic acid at room temperature for 60 min.

EXPERIMENTAL

The removal of the protective groups from the polymers was monitored on a Hitachi EPS-3T spectrophotometer in the UV region (210-360 nm) from the three maxima at 252, 258, and 264 nm characteristic of the benzyloxycarbonyl and benzyl groups for the polymers (XV, XVI, and XVII) (Fig. 1).

For the synthesis we used amino acids of the L series. The individuality of the compounds obtained was checked by thin-layer chromatography on a fixed layer of silica gel in the systems: 1) benzene–ethanol (2 : 0.4) and 2) butan-1-ol–acetic acid–water (100 : 10 : 30).

For all the compounds the results of elementary analysis corresponded to the calculated C, H, and N contents. In the preparation of the protected amino acids and peptides, the reaction mixtures were washed with water, 10% citric acid solution, 5% sodium bicarbonate solution, and water again, and were dried over sodium sulfate.

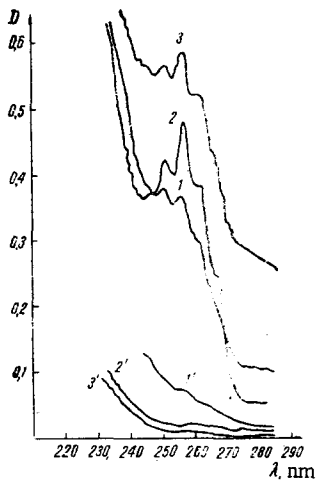


Fig. 1. UV spectra in methanol of the polypeptides [-Ala-(N^{δ}-Z)-Orn-Ala-]$_n$ (1), [-Ala-Orn-Ala-]$_n$ (1'), [-Ala-(N^{δ}-Z)Orn-Gly-]$_n$ (2), [-Ala-Orn-Gly-]$_n$ (2'), [Ala-(N^{δ}-Z)-Orn-(γ-OBzl)-Glu-]$_n$ (3), and [-Ala-Orn-Glu-]$_n$ (3').

Hydrochloride of the Methyl Ester of N^{δ}-Benzyloxycarbonyl-ornithine (III). A mixture of 1 g of N^{δ}-benzyloxycarbonylornithine and 5 ml of dry redistilled methanol was cooled with ice and salt to −10°C and then, with stirring, 0.3 ml of thionyl chloride was added, whereupon the N^{δ}-benzyloxycarbonylornithine gradually dissolved. The reaction mixture was left at room temperature for two days. Then the solvent was distilled off in vacuum and the residue was twice reprecipitated from chloroform with ether. Yield 1.15 g (95.7%), mp 138-139°C, R_f 0.63 (1).

Methyl Ester of tert-Butoxycarbonylalanyl-N^{δ}-benzyloxycarbonyl-ornithine (IV). With stirring, 0.56 ml of triethylamine was added to a solution of 0.75 g of tert-butoxycarbonylalanine (II) in 15 ml of tetrahydrofuran, and then the solution was cooled to −12 to −15°C and with constant stirring 0.53 ml of isobutyl chlorofomate was added. After 25 min, a suspension containing 1.26 g of the hydrochloride of the methyl ester of N^{δ}-benzyloxycarbonylornithine (III), 0.56 ml of triethylamine, and 15 ml of absolute tetrahydrofuran (−15°C) was added. The mixture was stirred for 12 h at room temperature and then the tetrahydrofuran was evaporated off in vacuum and the residue was dissolved in chloroform, washed, and dried. Washing was continued with ether, and then the product was dried in vacuum over caustic potash. Yield 1.78 g (98.8%), R_f 0.54 (2), $[\alpha]_D^{20}$ −5° (c 1.74; chloroform).

tert-Butoxycarbonylalanyl-N^{δ}-benzyloxycarbonylornithine (VII). At room temperature, 4.1 ml of a 1 N solution of caustic soda was added to a solution of 1.7 g of the hydrochloride of the methyl ester of tert-butoxycarbonylalanyl-N^{δ}-benzyloxycarbonylornithine (IV) in 4.1 ml of acetone. After 3 h, part of the acetone was evaporated off and the residual mixture was diluted with water. To remove the unsaponified ester, the reaction mixture was extracted with ethyl acetate (2 × 15 ml). The aqueous layer was acidified with citric acid to pH 2.0 and the saponified product (VII) was again extracted with ethyl acetate (4 × 20 ml). The extract was washed with water, dried over $MgSO_4$, and evaporated. The yield of oil with R_f 0.3 (2) was 1.5 g (91.4%).

2,4,5-Trichlorophenyl Ester of Benzyloxycarbonylalanine (VI). A solution of 1 g of benzyloxycarbonylalanine in 5 ml of absolute chloroform was cooled to −10°C, and 0.62 ml of triethylamine and 0.59 ml of isobutyl chloroformate were added. After 30 minutes' stirring, 0.885 g of 2,4,5-trichlorophenol was also added to the reaction mixture, which was then stirred at 4-5°C for 0.5 h and at room temperature for 4 h. The chloroform solution was diluted 2- to 2.5-fold, washed, dried, and evaporated. The residue was crystallized from a small amount of ethanol. Yield 1.34 g (74%), mp 96°C, R_f 0.75 (2).

Hydrobromide of the 2,4,5-Trichlorophenyl Ester of Alanine (VIII). At room temperature, 2 ml of a 38% solution of hydrogen bromide in glacial acetic acid was added to a solution of 1 g of the 2,4,5-trichlorophenyl ester of benzyloxycarbonylalanine in 2 ml of glacial acetic acid. After 30 min, the hydrobromide (VIII) was precipitated with absolute ether. The precipitate was washed with several portions of ether and was then reprecipitated from methanol with ether. Yield 0.7 g (81.4%), mp 195-196°C, R_f 0.15 (2).

The 2,4,5-trichlorophenyl ester of benzyloxycarbonylglycine was obtained similarly to (VI) from 5 g of benzyloxycarbonylglycine and 4.73 g of 2,4,5-trichlorophenol. Yield 8.0 g (89%), mp 103°C, R_f 0.81 (2).

Hydrobromide of the 2,4,5-Trichlorophenyl Ester of Glycine. With gentle heating, 2.3 g of 2,4,5-trichlorophenyl ester of benzyloxycarbonylglycine was dissolved in 4.8 ml of glacial acetic acid. Then 4.8 ml of 38% hydrogen bromide in glacial acetic acid was added and the mixture was shaken for 10 min. The resulting product was precipitated, washed free from hydrogen bromide with absolute ether, reprecipitated from methanol with ether, and dried. Yield 1.3 g (68%), mp 207°C, R_f 0.2 (2).

The 2,4,5-trichlorophenyl ester of N^{α}-tert-butoxycarbonyl-γ-benzylglutamic acid (XII) was obtained similarly to (VI) from 1.4 g of N^{α}-tert-butoxycarbonyl-γ-benzylglutamic acid and 0.82 g of 2,4,5-trichlorophenol. Yield 2.1 g (98%), mp 104-105°C, R_f 0.84 (1).

2,4,5-Trichlorophenyl Ester of the N^{α}-Trifluoroacetate of γ-Benzylglutamic Acid (XIII). A solution of 1.05 g of (XII) in 0.8 ml of absolute trifluoroacetic acid was kept at room temperature for 30 min.

Then the solvent was driven off in vacuum and the residue was treated with benzene (2× 10 ml) and dried. Yield 0.71 g (75.6%), R_f 0.60 (1).

2,4,5-Trichlorophenyl Ester of tert-Butoxycarbonylalanyl-N^{δ}-benzyloxycarbonylornithylalanine (IX). To a solution of 1.2 g of tert-butoxycarbonylalanyl-N^{δ}-benzyloxycarbonylornithine (VII) and 0.38 ml of triethylamine in 20 ml of absolute tetrahydrofuran at −15°C was added 0.36 ml of isobutyl chloroformate. After 20 min, a suspension containing 0.75 g of the hydrobromide of the 2,4,5-trichlorophenyl ester of alanine, 0.38 ml of triethylamine, and 15 ml of tetrahydrofuran, cooled to −12 to −15°C, was added to the reaction mixture. The residue after the evaporation of the tetrahydrofuran was dissolved in ethyl acetate, the solution was washed and evaporated to dryness, and the residue was recrystallized from a small amount of ethanol. Yield 1.6 g (95%), mp 148-150°C, R_f 0.73 (2), $[\alpha]_D^{19}$ −28° (c 1; chloroform).

The 2,4,5-trichlorophenyl ester of tert-butoxycarbonylalanyl-N^{δ}-benzyloxycarbonylornithylglycine (X) was obtained in the same way as substance (IX) from 1 g of tert-butoxycarbonylalanyl-N^{δ}-benzyloxycarbonylornithine and 0.76 g of the hydrobromide of the 2,4,5-trichlorophenyl ester of glycine. Yield 1.24 g (80%), mp 157-159°C, R_f 0.71 (2), $[\alpha]_D^{19}$ −29° (c 1; chloroform).

The 2,4,5-trichlorophenyl ester of tert-butoxycarbonylalanyl-N^{δ}-benzyloxycarbonylornithyl-γ-benzylglutamic acid (XI) was obtained similarly to substance (IX) from 1.5 g of tert-butoxycarbonylalanyl-N^{δ}-benzyloxycarbonylornithine and 1.81 g of the 2,4,5-trichlorophenyl ester of the N^{α}-trifluoroacetate of γ-benzylglutamic acid. Yield 2.69 g (94%), mp 129-130°C, R_f 0.84 (2) $[\alpha]_D^{19}$ −27.9° (c 0.82; chloroform).

2,4,5-Trichlorophenyl Ester of the Trifluoroacetate of Alanyl-N^{δ}-benzyloxycarbonylornithylalanine (IXa). A solution of 1 g of (IX) in 0.2 ml of trifluoroacetic acid was kept at room temperature for 80 min, and then the solvent was eliminated by evaporation in vacuum. The residue was washed with ether and was reprecipitated with ether from methanol. Yield 0.82 g (80%), R_f 0.41 (1). The trifluoroacetates of (Xa) and (XIVa) were obtained in the same way as that of (IXa).

Polycondensation. A solution of 0.494 g of (IXa) in 0.367 ml of absolute dimethylformamide was treated with 0.20 g of triethylamine and the mixture was left at room temperature for 6 days. Then 5 ml of absolute methanol was added and the precipitate that deposited was separated off and washed with ether. Yield 56%.

The polycondensation of (Xa) and (IXa) was performed in the following way. The yields of the polycondensation stage amounted to 42% and 51%, respectively. The benzyloxycarbonyl group was removed from the poly[-Ala-(N^{δ}-Z)Orn-Ala-] and from the poly[Ala-(N^{δ}-Z)Orn-Gly-] by the passage of dry hydrogen bromide into a trifluoroacetic acid solution for 45 min.

The benzyloxycarbonyl and benzyl ester groups were removed from the poly[-Ala-(N^{δ}-Z)Orn-(γ-OBzl-)Glu-] by the action of an excess of hydrogen bromide in glacial acetic acid at room temperature for 60 min.

CONCLUSION

The synthesis of three regular polypeptides with the compositions $(-Ala-Orn-Ala-)_n$, $(-Ala-Orn-Glu-)_n$, and $(-Ala-Orn-Gly-)_n$ and with molecular weights of 2600, 1900, and 2200, respectively, has been effected.

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