SYNTHESIS OF REGULAR POLYPEPTIDES WITH THE SEQUENCES -Lys-Lys-Lys-Gly- AND -Orn-Orn-Orn-Gly-

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Regular polypeptides including lysine and ornithine residues can be considered as possible models of histones. They are used in the investigation of the conformational characteristics of polypeptides of this class [1] and as possible inhibitors of biosynthesis [2]. In relation to the latter, ornithine polypeptides differ considerably from polypeptides containing lysine residues. Consequently, it was of interest to determine whether this difference is connected with conformational changes in these polypeptides or is only a function of the length of the side chain of the amino-acid residue. For this purpose we have synthesized two polytetrapeptides with the sequences -Lys-Lys-Lys-Gly- and -Orn-Orn-Orn-Gly-, obtained by the polymerization of the pentachlorophenyl esters of the corresponding peptides. As the protection for the N^{α}-NH_2 terminal groups we used the tert-butoxycarbonyl (BOC) group, and for the N^{ζ} and N^{δ} amino groups the benzyloxycarbonyl (Cbo) group. The mixed-anhydride method was used to form the peptide bond. The completeness of the removal of the Cbo group was checked spectrophotometrically. The molecular weights of the polypeptides were determined by the Van Slyke method.

EXPERIMENTAL

The work was carried out with amino acids of the L form, and thin-layer chromatography was performed in a fixed layer (250 mesh, plates 75 × 25 mm) with the following solvent systems: 1) water–acetic acid–butan-1-ol (30 : 10 : 100); 2) chloroform–methanol (63.7 : 6.33); and 3) 3% ammonia–sec-butanol (100 : 44). BOC denotes the tert-butoxycarbonyl group, Cbo the benzyloxycarbonyl group, $OPhCl_5$ the pentachlorophenyl group, and DMFA dimethylformamide.

Pentachlorophenyl Ester of N^{α}-tert-Butoxycarbonyl-(N^{ζ}-benzyloxycarbonyllysyl)$_3$glycine (I). To a solution of 2.1 g of BOC-(N^{ζ}-Cbo-Lys)$_2$OH in 10 ml of tetrahydrofuran containing 0.31 ml of triethylamine and cooled to −15°C was added 0.3 ml of isobutyl chloroformate and, after 15 min, 0.935 g of HBr · H-Gly-$OPhCl_5$ in 5 ml of tetrahydrofuran containing 0.31 ml of triethylamine. Then the reaction mixture was kept at 0°C for 2 h and at 20°C for 12 h, after which the solution was evaporated in vacuum at a temperature not exceeding 35°C, and the residue was dissolved in 50 ml of ethyl acetate. The ethyl acetate solution was extracted with cooled 10% citric acid (3 × 10 ml), water, a cooled 0.5 N solution of $NaHCO_3$ (3 × 10 ml), and water again and was dried over calcined sodium sulfate and evaporated in vacuum. The crystalline residue was recrystallized from 3 ml of methanol. Yield of (I) 1.1 g (43%), mp 149–150°C, $[\alpha]_D^{25}$ −12.5° (c 0.8; methanol); R_f 0.98 (1) and 0.71 (2).

Methyl Ester of N^{α}-tert-Butoxycarbonyl(N^{δ}-benzyloxycarbonylornithine)$_3$ (II). As in the preparation of (I), 0.9 g of BOC-Orn(N^{δ}-Cbo)-Orn(N^{δ}-Cbo)-OH, 0.2 ml of triethylamine, 0.195 ml of isobutyl chloroformate, 0.46 g of HCl · H-Orn(N^{δ}-Cbo)-OCH_3, and 0.2 ml of triethylamine in 25 ml of tetrahydrofuran yielded 0.86 g (68.5%) of BOC-(N^{δ}-Cbo-Orn)$_3$-OCH_3 with mp 112–114°C, $[\alpha]_D^{25}$ −19.6° (c 1.15; DMFA); R_f 0.98 (1) and 0.8 (93).

N^{α}-tert-Butoxycarbonyl-(N^{δ}-benzyloxycarbonylornithine)$_3$ (III). To a solution of 3 g of (II) in 10 ml of acetone containing 30 ml of methanol was added 40 ml of 1 N NaOH. The reaction mixture was shaken

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for 75 min, and then the pH was brought to 7 and the solvent was partially evaporated off at 30°C. The mixture was acidified to pH 4-5, and the precipitate that deposited was washed with a large amount of water and acetone. The residue could be dissolved only in a large amount of ethanol, from which it was recrystallized in dimethylformamide and dimethyl sulfoxide. The yield of (III) was 2 g (66%), mp 177-179°C, $[\alpha]_D^{25}$ −14.1° (c 1.13; DMFA); R_f 0.7 (3).

Pentachlorophenyl Ester of N^{α}-tert-Butoxycarbonyl-(N^{δ}-benzyloxycarbonylornithyl)$_3$glycine (IV). As for the preparation of (I), 1.7 g of BOC-[Orn(N^{δ}-CbO)]$_3$-OH, 0.28 ml of triethylamine, 0.26 ml of isobutyl chloroformate, 0.8 g of HBr · H-Gly-OPhCl$_5$, and 0.28 ml of triethylamine in 13 ml of dimethylformamide yielded 1.5 g of BOC-[(N^{δ}-Cbo)Orn]$_3$Gly-OPhCl$_5$. The substance is insoluble in the usual organic solvents, and it was filtered off and was washed with water, ethyl acetate, and cooled ethanol. Mp 169-170°C, $[\alpha]_D^{25}$ −24° (c 1.0; DMFA); R_f 0.7 (2); 0.96 (1).

Trifluoroacetate of the Pentachlorophenyl Ester of (N^{ζ}-Benzyloxycarbonyllysyl)$_3$glycine (V). Compound (I) (1.1 g) was dissolved in 3 ml of absolute trifluoroacetic acid. The process of removing the BOC group took 1 h. Then 3 ml of benzene was added and the mixture was evaporated in vacuum without heating. The residue was triturated with ether and reprecipitated from ethanol with ether. The yield of (V) was 0.8 g (73%); mp 131-132°C, $[\alpha]_D^{26}$ −8° (c 16; methanol); R_f 0.81 (1).

Trifluoroacetate of the Pentachlorophenyl Ester of (N^{δ}-Benzyloxycarbonylornithyl)$_3$glycine (VI). A solution of 1.5 g of (IV) in 3 ml of absolute trifluoroacetic acid was left for 70 min, and was then evaporated to dryness, and the residue was triturated with ether. The yield of (VI) was 1 g (66%), mp 180°C (decomp.), $[\alpha]_D^{25}$ −4.7° (c 1.22; DMFA); R_f 0.83 (1), 0.39 (2).

Polycondensation. Polycondensation was performed in absolute dimethylformamide (25% solution) in the presence of one equivalent of triethylamine at 20°C for 7 h. Then the reaction mixture was treated with methanol, and the methanol-insoluble residue was separated off. The yield of product was 60%. The Cbo group was removed by passing HBr through a solution of the protected polypeptide in glacial acetic acid for 60 min, after which absolute ether was added to the reaction mixture until the reaction product had been completely precipitated. The polypeptide was fractionated on Sephadex G-25 in a column 2 cm in diameter and 100 cm long. The rate of elution was 20 ml/h, and the yield of polypeptides after fractionation was 30-40%.

The molecular weights determined by the Van Slyke method were 25,000 for H-(Lys$_3$-Gly)$_n$-OH and 11,000 for H-(Orn$_3$-Gly)$_n$-OH.

CONCLUSIONS

The synthesis of polypeptides of regular structure with the sequences -Lys-Lys-Lys-Gly- and -Orn-Orn-Orn-Gly- with molecular weights of 25,000 and 11,000, respectively, have been performed.

LITERATURE CITED

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