SYNTHESIS OF THE HEPTAPEPTIDE OF THE CYCLOPEPTIDE MOIETY OF POLYMIXIN B

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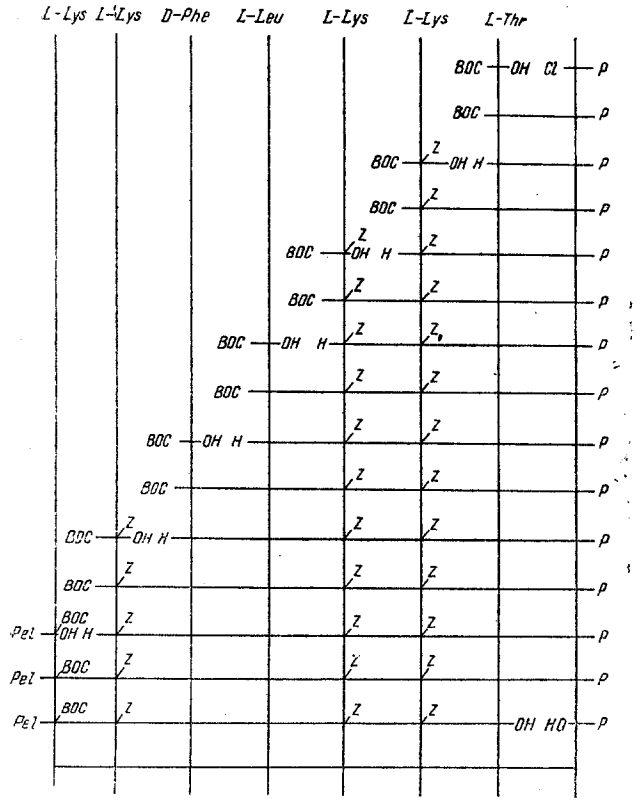
Polymixin B is the most studied representative of the polymixin group. This antibiotic was separated into two components, B_1 and B_2, by countercurrent distribution in 1954 [1]. The structures shown were synthesized in Vogler's laboratory [2].

Each of polymixin B antibiotics is a decapeptide in which seven amino acids form a ring (as with the majority of the other polymixins [3]) to which, at the α-NH_2 group of diaminobutyric acid, is attached a tripeptide in which the α-amino group of the terminal diaminobutyric acid is acylated with (+)-6-methyloctanoic (B_1) or isooctanoic (B_2) acid.

This paper gives the results of the synthesis of a heptapeptide of a protected lysine analog of the cylopeptide moiety of polymixin B in which the lysine forming a peptide bond at the ε-amino group is acylated at the α-amino group by pelargonic acid. The heptapeptide N^{ε}-tert-butoxycarbonyl-N^{α}-pelargonyl-L-lysyl-N^{ε}-benzyloxycarbonyl-L-lysyl-D-phenylalanyl-L-leucyl-N^{ε}-benzyloxycarbonyl-L-lysyl-N^{ε}-benzyloxycarbonyl-L-lysyl-L-threonine was obtained by the solid-phase method [4] in 63% yield according to the scheme shown below.



To form the peptide bond, dicyclohexylcarbodiimide (DCC) was used as the condensing agent. The protective groups used for the amino acids were tert-butoxycarbonyl (BOC) for amino groups participating in the formation of a peptide bond, and benzyloxycarbonyl (Z) for the ε-NH_2 groups of lysine; the hydroxyl group of threonine was not protected. In each cycle, we used a twofold excess of the BOC-amino acid with respect to the L-threonine bound to the polymer.

EXPERIMENTAL

Addition of BOC-L-threonine to the polymer. To 5 g of chloromethylated polymer (a copolymer of styrene with 2% divinylbenzene) containing 9% chlorine was added 2.96 g (0.013 mole) of BOC-L-threonine [5] and 1.82 ml (0.013 mole) of absolute triethylamine, and the mixture was boiled in absolute ethanol for 48 hr. The polymer with the attached amino acid was transferred into a reaction vessel in which all the operations connected with the growth of the peptide chain were carried out. To eliminate the BOC protective group from the threonine (see the scheme), 30 ml of a 10% solution of dry HCl in dioxane was added. After 20 min, the product was filtered off and washed with absolute dioxane, ethanol, methylene chloride, ether, and petroleum ether (5× 30 ml). The hydrochloride was neutralized with a 10% solution of triethylamine in dioxane for 3 min, after which the polymer was washed with dioxane and methanol. The filtrate, after the evaporation of the solvent in vacuo, yielded 0.96 g (0.007 mole) of triethylamine hydrochloride. Consequently, 1.54 g (0.007 mole) or 53.8% of BOC-L-threonine had added to 5 g of resin.

N^{α}-BOC-N^{ε}-Z-L-Lysyl-L-threonyl-polymer **(formation of the peptide bond).** With cooling to −20° C, 5.33 g (0.014 mole) of N^{α}-BOC-N^{ε}-Z-L-lysine [6] in 20 ml of absolute tetrahydrofuran (THF) and 2.88 g (0.014 mole) of DCC in 10 ml of THF were mixed and rapidly added to the L-threonyl-polymer. After stirring until the polymer began to swell, the reaction mixture was left overnight at ~20° C. Then the dipeptide-polymer was filtered off and washed with butan-1-ol heated to 50° C (to remove the dicyclohexylurea). The BOC protective group was eliminated under the conditions described above for the BOC-L-threonyl-polymer.

The polymer-heptapeptide N^{ε}-BOC-N^{α}-pel-L-lysyl-N^{ε}-Z-L-lysyl-D-phenyl-alanyl-L-leucyl-N^{ε}-Z-L-lysyl-N^{ε}-Z-L-lysyl-L-threonyl-polymer. Five cycles with the addition of five amino acid residues in the sequence shown in the scheme were carried out similarly. The white polymer-heptapeptide obtained was dried in vacuo.

The heptapeptide N^{ε}-BOC-N^{α}-pel-L-lysyl-N^{ε}-Z-L-lysyl-D-phenylalanyl-L-leucyl-N^{ε}-Z-L-lysyl-N^{ε}-Z-L-lysyl-L-threonine. The polymer-heptapeptide was treated twice with 6 ml of 2 N NaOH in 120 ml of methanol for 30 min. The filtrate was acidified with citric acid to a weakly acid reaction. The precipitate of sodium citrate was filtered off and the solution was evaporated to dryness in vacuo. The residue was washed with water, ether, and petroleum ether and was dried in vacuo over phosphorus pentoxide. A 6.68 g quantity of the protected heptapeptide was obtained in the form of a white powder, the yield being 63% calculated on the first amino acid, L-threonine, added to the resin. Mp 152–157° C, $[\alpha]_D^{20}$ −43.6° (c 0.5, CH_3OH). The substance was chromatographically homogeneous in the butan-1-ol—acetic acid—water (4 : 1 : 5) and butan-1-ol—acetic acid—pyridine—water (15 : 3 : 10 : 6) systems. Found, %: C 62.53, 62.68; H 7.78, 7.88; N 9.82, 9.64. Calculated for $C_{81}H_{117}N_{11}O_{18} \cdot H_2O$, %: C 62.67; H 7.67; N 9.92.

CONCLUSIONS

The synthesis of the heptapeptide N^{ε}-BOC-N^{α}-pelargonyl-L-lysyl-N^{ε}-Z-L-lysyl-D-phenylalanyl-L-leucyl-N^{ε}-Z-L-lysyl-N^{ε}-Z-L-lysyl-L-threonine, a linear analog of the cyclopeptide part of the antibiotic polymixin B, has been effected (yield 63%). The possibility of using this method for the synthesis of peptides on a polymer support without protecting the hydroxyl group of threonine has been shown.

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