SYNTHESIS OF A SEVEN-MEMBERED ANALOG OF ELEDOISIN USING THE SOLID-PHASE METHOD OF PEPTIDE SYNTHESIS

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Recently, Lübke and Schröder [1] have reported the synthesis of the heptapeptide H-Gly-L-Ala-L-Phe-L-Val-Gly-L-Leu-L MetNH$_2$ (I), which possesses an activity fairly close to that of eledoisin [2].

We have now synthesized the peptide (I) using the solid-phase method of peptide synthesis [3–5] in order to obtain a protected N-terminal pentapeptide, tert-butoxycarbonyl (BOC)-Gly-L-Ala-L-Phe-L-Val-GlyOH (II).

As the solid support we used chloromethylated polystyrene containing 2% of divinylbenzene cross-linkages and 7.1% of chlorine*.

The peptide (II) after separation from the resin, was condensed with L-Leu-L-MetNH$_2$ (III) [6] by the dicyclohexylcarbodiimide method. The purification of the peptide (I), obtained after the elimination of the N-protecting tert-butoxycarbonyl group did not give rise to any complications characteristic for the purification of eledoisin and its fragments synthesized by the classical methods of peptide chemistry.

To confirm the possibility of using an o-nitrophenylsulfenyl (NPS) protective group [8] in the solid-phase method of peptide synthesis, we also obtained the peptide (II) with the partial use of NPS-protected amino acids.

In performing this variant of the synthesis of peptide (II), it was found that NPS-protected amino acids can be used successfully in the building up of central sections of a peptide chain. An attempt to add NPS-glycine to the resin as a C-terminal amino acid was unsuccessful, since when NPS-glycine was boiled with the resin in the presence of Et_3N in alcohol for a long time, considerable oxidation and decomposition of the NPS-protective group was found. So far as concerns the possibility of using an NPS-protective group for the N-terminal amino acid (NPS-Gly), additional complications also arose here in connection with the difficulties of purifying the peptide (II); this did not arise when BOC-glycine was used as the N-terminal amino acid.

Experimental**

Synthesis of peptide (II). A. Using BOC-protected amino acids. A mixture of 5 g of chloromethylated polystyrene resin, 1.75 g (0.01 mole) of BOC-GlyOH [7], and 1 g (0.01 mole) of triethylamine in 20 ml of alcohol was boiled for 48 hr, and the product was then filtered off and washed with alcohol.

The amount of BOC-GlyOH bound to the resin was determined from the amount of chlorine split off (0.002 mole). The resin bearing the BOC-Gly was placed in a reaction vessel. To eliminate the BOC protective group, 25 ml of a 1 N solution of hydrochloric acid in glacial acetic acid was added. After 30 min, the solution was filtered off under vacuum and the resin was washed with acetic acid, dimethylformamide (DMF), and alcohol, each solvent being used three times (30-ml portions). The glycine hydrochloride attached to the resin was treated with 15 ml of a 3% solution of triethylamine in dimethylformamide and was carefully washed with DMF.

Then the successive addition of the BOC derivatives of L-valine, L-phenyl-alanine, L-alanine [7], and glycine was carried out, for which purpose 0.006 mole of the BOC derivative of each amino acid to be added, in 15 ml of dichloroethane, was added to the resin bearing a peptide with a free amino group. After 10 min, a solution of 0.006 mole of dicyclohexylcarbodiimide in 15 ml of dichloroethane was added. The condensation was carried out for 2 hr with shaking (vibrator), after which the solvent was sucked off and the residue was washed with DMF, acetic acid, and hot alcohol until the dicyclohexylurea formed had dissolved completely.

The elimination of the BOC protective group and the production of the free base of the peptide in the intermediate stages of the synthesis and the subsequent washings were carried out under the above conditions.

To detach the peptide (II) from the resin, 3 ml of 2 N caustic soda in 30 ml of alcohol was added to the reaction

* This sample of resin was kindly given to us by E. B. Trostyanskaya of the All-Union Scientific Research Institute for Chemical Reagents and Particularly Pure Chemical Substances.

** All the substances synthesized were characterized by quantitative analyses agreeing with the calculated figures.

vessel and stirring was carried out for 1 hr. The solution was filtered and the resin was carefully washed with alcohol.

The resulting solution was neutralized with 10% alcoholic hydrogen chloride to pH 7.0. After evaporation to dryness in vacuum, the residue was treated with 25 ml of water and carefully triturated; then the peptide (II) was filtered off and washed with water, alcohol, and petroleum ether. Yield 0.8 g (73%); mp 178°–181° C $[\alpha]_D^{21}$ −24.6° (c 1; acetic acid).

B. With the partial use of NPS derivatives of the amino acids. Resin (5 g) bearing 0.002 mole of BOC-glycine was placed in a reaction vessel, and after the elimination of the BOC protective group condensation was carried out successively with 0.006 mole of the NPS-derivatives of L-valine, L-phenylalanine, and L-alanine [)], and then with 0.006 mole of BOC-glycine. The conditions for the creation of the peptide bonds and the elimination of the N-protective groups were analogous to those described above for the synthesis of the peptide (II) using BOC derivatives of the amino acid (see Section A). The yield of BOC-protected pentapeptides (II) was 0.76 g (69%), mp 176°–181° C.

Synthesis of the peptide (I). A solution of 0.55 g (0.001 mole) of the peptide (II) obtained by method A or B and 0.58 g (0.002 mole) of the hydrochloride of the peptide (III) [6] in 30 ml of DMF containing 0.2 g (0.002 mole) of triethylamine, at 0° C, was treated with 0.4 g (0.002 mole) of dicyclohexylcarbodiimide in 10 ml of DMF. The solution was left for 24 hr at 0° C and for 24 hr at room temperature, and then a few drops of acetic acid were added and the solvent was distilled off in vacuum. The dry residue was carefully triturated with hot chloroform, filtered off, and washed with chloroform until the dicyclohexylurea had been eliminated. Yield 0.6 g (72%), mp 252°–255° C, $[\alpha]_D^{21}$ −28.4° (c 1; DMF) (cf. [1]).

The elimination of the BOC protective group from 0.4 g of N-substituted peptide (I) was carried out under the conditions described above. The hydrochloride of the peptide (I) was dissolved in 20 ml of glacial acetic acid and the solution was treated in the cold with activated carbon. After filtration, the substance was isolated by the addition of absolute ether. This gave 0.32 g (60%) of the hydrochloride of the peptide (I), mp 226°–234° C, $[\alpha]_D^{22}$ −42.5° (c 1; acetic acid). On chromatography on paper in the acetic acid–n-butyl alcohol–water (1:4:5) system, it had R_f 0.60.

A test of the biological activity of both samples of the peptide (I) showed that at a concentration of $1 \times 10^{-7} - 1 \times \times 10^{-8}$ g/kg weight they lower blood pressure in the rat.

Summary

1. The solid-phase method of peptide synthesis has been used to obtain the heptapeptide HGly-L-Ala-L-Phe-L-Val-Gly-L-Leu-L-Met NH_2, which is an analog of eledoisin.

2. The possibility has been shown of using o-nitrophenylsulphenyl derivatives of amino acids in the solid-phase method of peptide synthesis.

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