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SYNTHESIS OF A BIOLOGICALLY ACTIVE AMIDE OF THE C-TERMINAL TETRAPEPTIDE FRAGMENT OF GASTRIN ON A RESIN

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In a study of various fragments of gastrin, Tracy and Gregory [1, 2] found that only the amide of the C-terminal tetrapeptide

$$H - L - Try - L - Met - L - Asp - L - Phe NH_2$$

was necessary for the practically complete development of the physiological action caused by the natural hormone on the gastric and pancreatic cells and also on the gastrointestinal musculature.

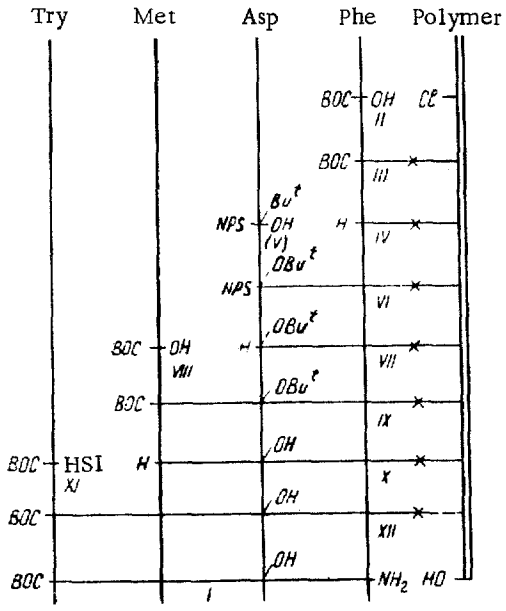
Subsequent investigations have shown that N-acylated derivatives of this tetrapeptide and, in particular, the derivative I retain this activity completely [3]. These results have served as a basis for the synthesis of a N-acylated amide of the C-terminal tetrapeptide (I) using the solid-phase method of peptide synthesis.

$$BOC - L - Try - L - Met - L - Asp - L - Phe NH_2. \quad (I).$$

We have carried out the solid-phase synthesis of this peptide under conditions differing somewhat from those described by Merrifield [4].

The polymer on which the peptide synthesis was carried out was obtained by the emulsion copolymerization of styrene with divinylbenzene. To form a styrene/divinylbenzene polymer with a porous structure, carbon tetrachloride was added to the medium [5]. The swellability of the polymer obtained corresponds to that of a polymer containing 0.5-0.8% of divinylbenzene. The use of such a polymer in the solid-phase synthesis of peptides enables the yield of peptides per gram of resin to be increased.

The maximum concentration of reactants in the condensation stage was achieved from a solution in such an amount of solvent as was necessary only for the complete swelling of the resin without the presence of a liquid phase, and therefore the synthesis of the peptide was carried out in a reaction vessel [4] without stirring or shaking.



Scheme of the synthesis (conventional abbreviations for the names of the amino acids and respective groups; x represents a bond of the benzyl ester type).

We have previously shown the possibility of using the o-nitrophenylsulfenyl (NPS) protective group in place of BOC protection in the solid-phase method of peptide synthesis [6, 7]. In the present work we used the properties of the NPS group for being split off under mild acidolysis conditions, which enabled the NPS protective group to be removed selectively by the action of two equivalents of hydrogen chloride in glacial acetic acid at the stage of the production of (VII) (see scheme). The splitting off of the β-tert-butyl (Bu^t) ester of L-aspartic acid [in the production of (X)] was effected by the action of 98% CH_3COOH on (IX), the BOC protective grouping from the N-terminal L-methionine being removed simultaneously. The subsequent addition of the BOC-L-Try residue was performed successfully by means of its N-hydroxysuccinimide ether in the presence of triethylamine.

The ammolysis of the ester linkage between the peptide and the polymer was effected by the action of a saturated solution of ammonia in absolute methanol at 0° C for a day, as a result of which the peptide I was obtained with a yield of 40%.

Experimental

For analysis the substances were dried at 40° C in vacuum for 4–6 hr. Their homogeneity was determined by thin-layer chromatography on alumina and hydrated silica with the addition of 30% of gypsum. The following systems were used in chromatography: 1) pyridine–isoamyl alcohol–water (10 : 10 : 7), and 2) 1-butanol–acetic acid–water (4 : 1 : 5).

Preparation of the polymer. To a mixture of 25 ml of styrene, 2 ml of 30% divinylbenzene, and 6 ml of CCl_4 was first added 2.5 g of finely ground copolymer containing 20% of divinylbenzene and then 1 g of benzoyl peroxide. The resulting mixture was transferred to a flask containing a 1% aqueous solution of poly(vinyl alcohol) and the emulsion formed was stirred at 70° C for 24 hr.

Chloromethylation. Twenty grams of polymer was placed in a flask containing 300 ml of methylene chloride, 10 ml of chloromethyl ether, and 1.5 ml of stannic chloride. The mixture was kept at 40° C for 2 hr. After filtration, the polymer was washed with methylene chloride, ethanol, water, and ethanol again. The chlorine content of the polymer was 5.7%; its swellability in toluene was 400%. The polymer was stored in CCl_4.

Addition of BOC-L-Phe (II) to the polymer. A mixture of 5 g of the chloromethylated polymer, 1.3 g of BOC-L-Phe [9] and 0.6 ml of Et_3N in 20 ml of absolute ethanol was boiled for 12 hr, and the solid matter was filtered off and washed with ethanol, dioxane, and ethanol. The polymer bearing the BOC-L-Phe (III) was placed in a reaction vessel [4] in which all the operations connected with the further growth of the peptide chain were carried out.

To split off the BOC protective group and to obtain (IV), 25 ml of a 7% solution of dry HCl in glacial acetic acid was added to the (III). After 30 min the mixture was filtered, and the residue was washed with absolute dioxane and ethanol. The amount of L-Phe attached to the resin was determined from the amount of triethylamine hydrochloride formed (0.0015 mole).

Preparation of (VII). The synthesis of the NPS-derivative of the β-tert-butyl ester of L-aspartic acid (V) was carried out by treating the β-tert-butyl L-aspartate [10] with o-nitrophenylsulfenyl chloride under the usual conditions [11]. Yield 72%. Compound (V) was characterized in the form of a dicyclohexylammonium salt. Mp 148–150° C; $[\alpha]_D^{20}$ -12° (c 2; chloroform).

Found, %: C 59.23; H 7.92; N 7.83; S 5.66. Calculated for $C_{26}H_{11}O_6N_3S$, %: C 59.65; H 7.84; N 8.03; S 6.11.

Preparation of (VI). A solution of compound (V) in 10 ml of methylene chloride cooled to 5° C was mixed with a solution of 0.63 g of dicyclohexylcarbodiimide in 10 ml of methylene chloride and the mixture was rapidly added to the (IV) present in the reaction vessel. The gel formed was kept at room temperature for 2 hr, after which it was filtered off and washed with dioxane at 50° C, and then with chloroform and ethanol until the dicyclohexylurea had been eliminated completely.

The splitting off of NPS protective group and the preparation of (VII) were performed by treating (VI) with 20 ml of a 0.6% solution of dry HCl in glacial acetic acid at 0° C for 15 min. After filtration, the resulting hydrobromide was treated with 30 ml of a 2% solution of Et_3N in DMF and was washed first with DMF and then with ethanol.

Addition of BOC-L-methionine (VIII) and preparation of (IX). 0.75 g of BOC-L-methionine [12] (VIII) was condensed with (VII) in the same way as in the preparation of (VI).

Preparation of (X). 30 ml of 98% trifluoroacetic acid was added to (IX) and the mixture was kept at 20° C for 30 min and was then filtered, and the residue was washed with dioxane and ethanol. The trifluoroacetate salt that had formed was neutralized with 30 ml of a 2% solution of triethylamine in DMF, filtered off, and washed with DMF and ethanol.

Addition of BOC-L-tryptophan. .0.15 ml of Et_3N was added to a solution of 2.4 g of the N-hydroxysuccinimide ester of BOC-L-tryptophan [8] (XI) in 20 ml of DMF cooled to -15° C. The resulting mixture was rapidly poured onto the (X) in the reaction vessel and the mixture was kept at 0° C for 12 hr and at room temperature for 12 hr. After the (XII) had been filtered off, it was repeatedly washed with DMF, water, and ethanol, and carefully dried in vacuum at 40° C.

Preparation of the tetrapeptide (I). The (XII) was added to 30 ml of a methanolic solution of ammonia saturated at 0° C and cooled to 0° C and the mixture was kept at 0° C for 24 hr. The methanolic solution was removed by filtration and the resin was washed with DMF and methanol. The combined filtrates were evaporated in vacuum at 30° C to dryness. The residue (0.83 g) was washed with chloroform, treated with a 3% solution of citric acid, filtered, washed with water, and dried in vacuum. The protected tetrapeptide (I) so obtained was dissolved in ethanol and precipitated with ethyl acetate, and the precipitate was filtered off, dissolved in 90% ethanol, and treated with activated carbon. On evaporation in vacuum, the peptide (I) crystallized. After recrystallization from ethanol, 0.43 g (40.5%) of the

amide of tert-butoxycarbonyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine (I) was obtained. Mp 207–209° C; $[\alpha]_D^{20}$ -34° (c 1; DMF); compare [3].

Summary

The solid-phase synthesis of the biologically active fragment of gastrin BOC-L-Try-L-Met-L-Asp-L-PheNH_2 has been carried out on a resin obtained by the copolymerization of styrene with divinyl benzene. The swellability of the resin used corresponded to a content of 0.5–0.8% of divinylbenzene in it.

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