SOLID-PHASE SYNTHESIS OF THE PARTIALLY PROTECTED A_{13-21} FRAGMENT OF INSULIN

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Continuing the development of methods for obtaining structural analogs of insulin, we have synthesized the nonapeptides (I-III), which consist of the partially protected A_{13-21} fragment of insulin [1] and structural analogs of this fragment (II and III) (all amino acids of the L configuration).

13 14 15 16 17 18 19 20 21

...-Leu-Tyr-GlN-Leu-Glu-AsN-Tyr-Cys-AsN-OH (A_{13-21})

HBr·H-Leu-Tyr-GlN-Leu-Glu-AsN-Tyr-Cys-AsN-OH (I)

Bzl

HBr·H-Ala-Tyr-GlN-Leu-Glu-AsN-Tyr-Cys-AsN-OH (II)

Bzl

HBr·H-Leu-Tyr-Ala-Leu-Glu-Ala-Tyr-Cys-AsN-OH. (III)

Bzl

The synthesis was performed by Merrifield's solid-phase method [1, 2]. The polymeric support was a chloromethylated copolymer of styrene and 1% of divinylbenzene containing 8% of chlorine.

The general scheme of the synthesis of peptides (I)-(III) was similar, on the whole, to that given in the preceding paper [3].

EXPERIMENTAL

Ascending thin-layer chromatography (TLC) on cellulose and on Silufol plates in the following solvent systems was used: 1) isoamyl alcohol–pyridine–water (7:7:60, and 2) isopropanol–25% aqueous ammonia–water (14:1:5).

The amounts of amino acids in the hydrolyzates were determined on an automatic amino acid analyzer.*

Addition of BOC–AsN–OH to the Polymeric Support. The addition reaction was performed under conditions analogous to those given previously [3]. The amount of asparagine on the support amounted to 0.35 mmole/g.

Preparation of the Hydrobromide of H–Leu–Tyr–GlN–Leu–Glu–AsN–Tyr–Cys (Bzl)–AsN–OH [1]. A reaction vessel for solid-phase peptide synthesis was charged with 2 g of BOC–AsN–polymer (0.70 mmole of asparagine), 20 ml of dioxane was added, and the suspension was shaken for 10 min. The aminoacylpolymer was washed twice with dioxane, and then the BOC group was eliminated by the action of 20 ml of a 4 N solution of HCl in dioxane for 30 min. After washing with dioxane (3 × 20 ml) and CH_2Cl_2 (3 × 20 ml), the reaction mixture was neutralized with 20 ml of a 10% solution of triethylamine in CH_2Cl_2 over 10 min. Then the polymer was washed with CH_2Cl_2 (3 × 20 ml) and with methanol (2 × 15 ml), and 545

* The amino acid analyses were performed by workers of the division of chromatography of the interfaculty laboratory of bioorganic chemistry of Moscow State University.

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mg (1.75 mmole) of BOC–Cys (Bzl)–OH was added and, after 5 min, 360 mg (1.75 mmole) of dicyclohexylcarbodiimide. Condensation was performed for 4 h with shaking. After the end of the peptide-forming reaction, the polymer support was washed with CH_2Cl_2 (3 × 20 ml), with ethanol (3 × 20 ml), and then again with CH_2Cl_2 (3 × 20 ml).

At the stages of the introduction of asparagine and glutamine residues into the peptide chain, after the elimination of the BOC group the aminoacylpolymer was washed with dimethylformamide (DMFA) (3 × 20 ml) and was then neutralized with a 10% solution of triethylamine in DMFA for 10 min, was washed again with DMFA (3 × 20 ml), and 2.8 mmole of BOC–AsN–ONP or BOC–GlN–ONP was added. Condensation was performed for 16 h with shaking.

By repeating the cycle of reactions and washings that has been described, the BOC–Leu–Tyr(Bzl)–GlN–Leu–Glu(OBzl)–AsN–Tyr(Bzl)–Cys(Bzl)–AsN– polymer was obtained. The substance was washed with glacial acetic acid and with ethanol and was dried in a vacuum desiccator over P_2O_5. Amino acid analysis: Asp 2.04, Glu 2.10, Leu 2.00, Tyr 1.13.

The peptidylpolymer was suspended in a mixture of 15 ml of freshly distilled CF_3COOH and 10 ml of anisole and a slow current of HBr freed from bromine was passed through the suspension for 80 min. The polymer support was separated off and washed with CF_3COOH (3 × 10 ml), and the filtrate and washing liquids were evaporated in vacuum at 30°C. Ether was added to the residue, and the precipitate that deposited was separated off, washed with ether, and dried in vacuum. Yield 480 mg (52% of theoretical).

The product obtained (60 mg) was purified by descending preparative chromatography on Whatman No. 3 paper in system 1. The zone corresponding to the strongest coloration with ninhydrin reagent and absorption in the UV region was eluted from the paper with water or DMFA, and the extract was evaporated in vacuum. This gave 10 mg of chromatographically pure compound (I) with $[\alpha]_D^{20}$ −32° (c 1; DMFA); TLC – R_{f1} 0.73, R_{f2} 0.71 (on cellulose), R_{f1} 0.60, R_{f2} 0.50 (on Silufol plates). Amino acid analysis: Asp 2.10, Glu 2.20, Leu 2.00, Tyr 1.40.

Hydrobromide of H–Ala–Tyr–GlN–Leu–Glu–AsN–Tyr–Cys(Bzl)–AsN–OH (II). In a similar manner (here and below) to the preceding experiment, starting from 2 g of the BOC–AsN–polymer (0.70 mmole of asparagine) we synthesized the BOC–Ala–Tyr(Bzl)–GlN–Leu–Glu (OBzl)–AsN–Tyr (Bzl)–Cys (Bzl) AsN–polymer. Amino acid analysis: Asp 2.10, Glu 2.10, Ala 1.00, Leu 1.00, Tyr 0.96.

Treatment with HBr in CF_3COOH gave 400 mg of (II) (45% of theoretical) which, to achieve chromatographic purity, was purified in the same way as (I); $[\alpha]_D^{20}$ −40° (c 1; DMFA); TLC – R_{f1} 0.65, R_{f2} 0.70 (on cellulose); R_{f1} 0.59, R_{f2} 0.52 (on Silufol plates). Amino acid analysis: Asp 1.92, Glu 1.88: Ala 0.82, Leu 1.00, Tyr 0.96.

Hydrobromide of H–Leu–Tyr–Ala–Leu–Glu–Ala–Tyr–Cys (Bzl)–AsN–OH (III). Starting from 2 g of the BOC–AsN–polymer (0.70 mmole of asparagine) we synthesized the BOC–Leu–Tyr (Bzl)–Ala–Leu–Glu (OBzl)–Ala–Tyr (Bzl)–Cys (Bzl)–AsN–polymer. Amino acid analysis: Asp 0.97, Glu 1.15, Ala 2.07, Leu 2.00, Tyr 0.80. Treatment with HBr in CF_3COOH for 60 min gave 450 mg of crude (III) (52% of theoretical), which was converted by reprecipitation from DMFA with water into the chromatographically pure compound (III) with $[\alpha]_D^{20}$ −23° (c 1; AcOH); TLC R_{f1} 0.57, R_{f2} 0.55 (on cellulose); R_{f1} 0.65, R_{f2} 0.60 (on Silufol plates). Amino acid analysis: Asp 0.98, Glu 1.01, Ala 2.00, Leu 2.00, Tyr 1.06.

SUMMARY

Using the solid-phase method, the synthesis has been effected of a partially protected A_{13-21} fragment of insulin and of analogs of this fragment: the hydrobromides of H–Leu–Tyr–GlN–Leu–Glu–AsN–Tyr–Cys(Bzl)–AsN–OH, H–Ala–Tyr–GlN–Leu–Glu–AsN–Tyr–Cys(Bzl)–AsN–OH and H–Leu–Tyr–Ala–Leu–Glu–Ala–Tyr–Cys(Bzl)–AsN–OH.

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