THE SOLID-PHASE SYNTHESIS AND SOME PROPERTIES OF TETRAPEPTIDE HYDRAZIDES RELATED TO THE A_{13-16} FRAGMENT OF INSULIN

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For the purpose of the further development of methods for obtaining structural analogs of insulin, we have synthesized the tetrapeptide hydrazides (I-VI) which form structural analogs of the A_{13-16} fragment of insulin at positions 13 (I-IV) and 14 (V, VI) (all amino acids of the L configuration).

13 14 15 16	
. . . -Leu-Tyr-GlN-Leu- . . .	(A_{13-16})
BOC-Gly-Tyr-GlN-Leu-$NHNH_2$	(I)
Bzl	
BOC-Ala-Tyr-GlN-Leu-$NHNH_2$	(II)
Bzl	
BOC-Val-Tyr-GlN-Leu-$NHNH_2$	(III)
Bzl	
BOC-Phe-Tyr-GlN-Leu-$NHNH_2$	(IV)
Bzl	
BOC-Leu-Phe-GlN-Leu-$NHNH_2$	(V)
BOC-Leu-Thr-GlN-Leu-$NHNH_2$	(VI)

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

The C-terminal amino acid was added to the polymeric support in the presence of sodium iodide as catalyst for the reaction [3].

In all the peptide-forming reactions the condensing agent was dicyclohexylcarbodiimide – DCHC. To eliminate the BOC groups we used a 4 N solution of HCl in dioxane [4]. The addition of a glutamine residue was performed by the p-nitrophenyl ester method, and the BOC group in the glutamine residue was removed by the action of a 50% solution of CF_3COOH in CH_2Cl_2 [5]. The peptides obtained were separated from the polymeric support by means of hydrazine hydrate in dimethylformamide (DMFA) [6].

Peptide (V) was synthesized by two methods: by the direct hydrazinolysis of the peptidyl-polymer and also by the preparation of BOC-Leu-Phe-GlN-Leu-OMe (VII) by the transesterification of the peptidyl-polymer with its subsequent hydrazinolysis in solution [7].

The tetrapeptide hydrazides (I) and (V) were then used for the synthesis of the two nonapeptides (VIII and IX) by the method of azide condensation in solution [8] with the hydrobromide of H-Glu-Ala-Tyr-Cys(Bzl)-AsN-OH [3].

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BOC-Gly-Tyr-GLN-Leu-Glu-Ala-Tyr-Cys(Bzl)-As N-OH, (VIII)

BOC-Leu-Phe-GlN-Leu-Glu-Ala-Tyr-Cys(Bzl)-As N-OH. (IX)

EXPERIMENTAL

Methods of Analysis. Ascending chromatography on Filtrak FN4 paper (GDR) was used with the following solvent systems: 1) isoamyl alcohol–pyridine–water (7:7:6), 2) n-butanol–acetic acid–pyridine–water (15:3:12:10), 3) n-butanol–acetic acid–water (4:1:5), 4) isopropanol–25% ammonia–water (14:1:5), and 5) n-butanol saturated with water. Thin-layer chromatography (TLC) was performed by the ascending method on Silufol plates containing silica gel and starch in the same solvent systems as in the case of paper chromatography (PC), and also in systems 6) methanol–chloroform (1:4) and 7) benzene–ethanol (1:4).

The compounds containing free α-amino groups were revealed on the chromatograms and electrophoregrams with ninhydrin. The benzidine reagent (PC) on a 0.05M solution of potassium iodide (TLC) was used to reveal substances containing an imino group. The hydrazides of the peptides were revealed with a mixture of a 5% solution of silver nitrate and 25% aqueous ammonia (9:1) with the subsequent heating of the chromatogram at 105° for 2-3 min. The amounts of the amino acids in hydrolyzates were determined on an automatic amino-acid analyzer.*

Addition of BOC-Leu-OH to the Polymeric Support. The addition of BOC-Leu-OH · H_2O was performed under conditions similar to those described previously [3]. The amount of leucine on the support was 0.33-0.42 mmole/g.

BOC-Gly-Tyr(Bzl)-GlN-Leu-$NHNH_2$ (I). A reaction vessel for solid-phase peptide synthesis was charged with 2 g of BOC-Leu-polymer (0.66 mmole of leucine), and a cycle of reaction similar to that described previously [3] was performed.

After the end of the synthesis, the peptidyl-polymer was washed with glacial acetic acid and with ethanol and was dried in a vacuum desiccator over P_2O_5. Amino-acid analysis: Leu 1.00, Gly 1.00, Glu 1.02, Tyr 0.90.

The peptidyl-polymer obtained was suspended in DMFA, a 100-fold excess of hydrazine hydrate was added, and the mixture was shaken on a shaking machine at room temperature for two days. The polymer support was separated off and was washed with DMFA, and the filtrate and the wash liquids were evaporated in vacuum at 30°C. The residue was treated with water, and the precipitate that formed was separated off, washed with water, and dried in a vacuum desiccator over P_2O_5. The substance was purified by reprecipitation from DMFA with water. The yield of (I) was 200 mg (45% of theoretical), mp 200°C (decomp.), $[\alpha]_D^{25}$ −9° (c 1; DMFA), R_{f1} 0.92, R_{f3} 0.89 (PC); R_{f1} 0.69, R_{f2} 0.78, R_{f3} 0.85, R_{f6} 0.20 (TLC). Amino-acid analysis: Leu 1.00, Gly 0.96, Glu 0.98, Tyr 0.99.

BOC-Ala-Tyr(Bzl)-GlN-Leu-$NHNH_2$ (II). This was synthesized in a similar manner to the preceding compound (I): starting with 1.5 g of BOC-Leu-polymer (0.59 mmole of leucine), the BOC-Ala-Tyr(Bzl)-GlN-Leu-polymer was obtained. Amino-acid analysis: Leu 1.00, Ala 1.00, Glu 0.98 Tyr 0.68. After the treatment of the peptidyl-polymer with hydrazine hydrate in DMFA as before, 120 mg of (II) (30% of theoretical) was isolated with mp 202°C (decomp.), $[\alpha]_D^{25}$ −13°, (c 0.5; DMFA), R_{f1} 0.92, R_{f4} 0.89 (PC); R_{f1} 0.59, R_{f2} 0.79, R_{f3} 0.83, R_{f6} 0.20, R_{f7} 0.64 (TLC). Amino-acid analysis: Leu 1.00, Ala 1.00, Glu 0.96, Tyr 0.74.

BOC-Val-Tyr(Bzl)-GlN-Leu-$NHNH_2$ (III). From 2 g of BOC-Leu-polymer (0.66 mmole of leucine) was obtained BOC-Val-Tyr(Bzl)-GlN-Leu-polymer. Amino-acid analysis: Leu 1.00, Val 0.92, Glu 1.00, Tyr 0.80. After treatment with hydrazine hydrate under the same conditions, 250 mg (52% of theoretical) of (III) was obtained with mp 218°C (decomp.), $[\alpha]_D^{25}$ −13° (c 1; DMFA), R_1 0.92, R_{f4} 0.89 (PC); R_{f1} 0.65, R_{f2} 0.78, R_{f3} 0.84, R_{f6} 0.20 (TLC). Amino-acid analysis: Leu 1.00, Val 0.96, Glu 1.00, Tyr 0.96.

*The amino-acid analyses were performed by workers of the Division of Chromatography of the Interfaculty Laboratory of Bioorganic Chemistry of Moscow State University.

BOC-Phe-Tyr(Bzl)-GlN-Leu-$NHNH_2$ (IV). From 2 g of the BOC-Leu-polymer was obtained the BOC-Phe-Tyr(Bzl)-GlN-Leu-polymer (amino-acid analysis: Leu 1.00, Phe 0.96, Glu 1.05, Tyr 0.98), and then by the action of hydrazine in DMFA, 300 mg of (IV) (59% of theoretical), mp 207°C (decomp.), $[\alpha]_D^{25}$ −20° (c 1; DMFA), R_{f1} 0.92, R_{f3} 0.89 (PC); R_{f1} 0.69, R_{f2} 0.79, R_{f3} 0.83, R_{f6} 0.20 (TLC). Amino-acid analysis: Leu 1.00, Phe 1.07, Glu 1.03, Tyr 0.90.

BOC-Leu-Phe-GlN-Leu-$NHNH_2$ (V). From 3 g of the BOC-Leu-polymer (1.08 mmole of leucine) was obtained the BOC-Leu-Phe-GlN-Leu-polymer (amino-acid analysis: Leu 2.00, Phe 0.65, Glu 1.09) and then 230 mg of (V) (34% of theoretical), mp 224-225°C (decomp.), $[\alpha]_D^{25}$ −22° (c 1; DMFA), R_{f1} 0.90, R_{f2} 0.92, R_{f4} 0.87 (PC); R_{f1} 0.74, R_{f2} 0.74, R_{f3} 0.40, R_{f4} 0.74 (TLC). Amino-acid analysis: Leu 2.00, Phe 0.96, Glu 0.96.

The peptidyl-polymer obtained from 2 g of the BOC-Leu-polymer (0.72 mmole of leucine) was suspended in methanol, a 50-fold excess of triethylamine was added, and the mixture was shaken on the shaking machine at room temperature for 20 h. The polymer support was separated off and washed with methanol, and the filtrate and the wash liquids were evaporated in vacuum. The residue was recrystallized from aqueous methanol, giving 320 mg of (VII) (70% of theoretical), mp 199°C, $[\alpha]_D^{25}$ −35° C 1; DMFA), R_{f1} 0.90, R_{f2} 0.92, R_{f4} 0.85 (PC); R_{f1} 0.86, R_{f2} 0.85, R_{f3} 0.82, R_{f4} 0.67 (TLC). Amino-acid analysis: Leu 2.00, Phe 0.75, Glu 1.07. With stirring, 270 mg (0.37 mmole) of (VII) in 3 ml of DMFA was treated with 0.2 ml (3.7 mmole) of hydrazine hydrate over 48 h at room temperature. Then water was added to the reaction mixture and the precipitate formed was separated off, washed with water, and dried in a vacuum desiccator over P_2O_5. This gave 190 mg of (V) (87% of theoretical), mp 225°C (decomp.), $[\alpha]_D^{25}$ −22° (c 1; DMFA), R_{f1} 0.90, R_{f2} 0.92, R_{f4} 0.85 (PC); R_{f1} 0.74, R_{f2} 0.78, R_{f3} 0.40, R_{f4} 0.74, R_{f5} 0.75 (TLC). Amino-acid analysis: Leu 2.00, Phe 0.85, Glu 1.00.

BOC-Leu-Thr-GlN-Leu-$NHNH_2$ (VI). From 2 g of the BOC-Leu-polymer (0.72 mmole of leucine) was obtained the BOC-Leu-Thr-GlN-Leu-polymer (amino-acid analysis: Leu 2.00, Glu 1.11, Thr 0.21) and, after the action of hydrazine hydrate in DMFA, 130 mg of (VI) (31% of theoretical); mp 211-212°C (decomp.), $[\alpha]_D^{25}$ −21° (c 1; DMFA), R_{f1} 0.90, R_{f2} 0.92, R_{f4} 0.87 (PC); R_{f1} 0.70, R_{f2} 0.73, R_{f3} 0.40, R_{f4} 0.79 (TLC). Amino-acid analysis: Leu 2.00, Glu 0.98, Thr 0.32.

BOC-Gly-Tyr(Bzl)-GlN-Leu-Glu-Ala-Tyr-Cys(Bzl)-AsN-OH (VIII). A solution of 75.2 mg (0.11 mmole) of (I) in 4 ml of absolute DMFA was cooled to −20°C and, with stirring, 0.06 ml (0.39 mmole) of 5.7 N solution of HCl in dioxane and 0.015 ml (0.125 mmole) of tert-butyl nitrite were added. The resulting solution was stirred at −20 to −30°C for 30 min and was then cooled to −40 to −50°C; absolute triethylamine to pH 7 and a solution of 100 mg (0.13 mmole) of the hydrobromide of H-Glu-Ala-Try-Cys(Bzl)-AsN-OH and 0.05 ml (0.39 mmole) of triethylamine in 5 ml of aqueous DMFA (1:1) were added. The condensation reaction was performed with stirring at −10 to −15°C for 4 h and then at +4°C for four days. The reaction mixture was poured into water and the precipitate formed was separated off, washed with water, 5% acetic acid, and water again and was dried in a vacuum desiccator over P_2O_5. The yield of (VIII) was 60 mg (41% of theoretical). The substance was purified by reprecipitation from DMFA − methanol (3:1) with water, $[\alpha]_D^{25}$ −41° (c 0.3; DMFA), R_{f1} 0.50, R_{f2} 0.68, R_{f3} 0.44 (TLC). Amino-acid analysis: Gly 1.00 Ala 0.85, Leu 1.12, Asp 0.94, Glu 1.92, Tyr 2.03, Cys(Bzl) 0.60.

BOC-Leu-Phe-GlN-Leu-Glu-Ala-Tyr-Cys(Bzl)-AsN-OH (IX). As in the preceding case, from 0.11 mmole of (V) was obtained 97 mg of (IX) (70% of theoretical). The substance was reprecipitated from a mixture of DMFA and methanol with water. $[\alpha]_D^{25}$ −31° (c 0.5; DMFA), R_{f1} 0.68, R_{f2} 0.70, R_{f3} 0.86, R_{f4} 0.55, R_{f5} 0.89 (TLC). Amino-acid analysis: Ala 1.00, Leu 2.00, Phe 0.90, Asp 1.03, Glu 2.06, Tyr 0.97, Cys(Bzl) 0.84.

SUMMARY

The following tetrapeptide-hydrazides related to the A_{13-16} fragment of insulin have been synthesized by the solid-phase method: BOC-Gly-Tyr(Bzl)-GlN-Leu-$NHNH_2$, BOC-Ala-Tyr (Bzl)-GlN-Leu-$NHNH_2$, BOC-Val-Tyr(Bzl)-GlN-Leu-$NHNH_2$, BOC-Phe-Tyr(Bzl)-GlN-Leu-$NHNH_2$, BOC-Leu-Phe-GlN-Leu-$NHNH_2$ and BOC-Phe-Tyr(Bzl)-GlN-Leu-$NHNH_2$. BOC-Leu-Phe-GlN-Leu-Ome has also been obtained as an intermediate. By block condensation using the azide method in solution the following analogs of the $A_{13\,21}$ fragment of insulin have been synthesized: BOC-Gly-Tyr(Bzl-GlN-Leu-Glu-Ala-Tyr-Cys(Bzl)AsN-OH and BOC-Leu-Phe-GlN-Leu-Glu-Ala-Tyr-Cys(Bzl)-AsN-OH.

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