SYNTHESIS OF A PROTECTED UNDECAPEPTIDE REPRESENTING THE (6-16) FRAGMENT OF THE N-TERMINAL SEGMENT OF THE HISTONE OF THE F2aI FRACTION OF CALF THYMUS

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The synthesis of models [1] and of the (9-14), (4-9), and (8-14) fragments [2] of the N-terminal segment of the F2aI histone fraction of calf thymus has been reported previously. Continuing this work, we have synthesized a heptapeptide forming the (10-16) fragment and an undecapeptide forming the (6-16) fragment of the N-terminal segment of the nonspiralized section of the glycine-arginine-rich F2aI histone of calf thymus [3].

The methyl esters of benzyloxycarbonylleucylglycyl-N^{ε}-tosyllysylglycylglycylalanyl-N^{ε}-tosyllysine and of benzyloxycarbonylglycylglycyl-N^{ε}-tosyllysylglycylleucylglycyl-N^{ε}-tosyllysylglycylglycylalanyl-N^{ε}-tosyllysine were synthesized by the azide method and the mixed-anhydride method. The synthesis was performed by combining the methods of the successive addition of individual amino acids and of peptide blocks to the N- end. To protect the ε-amino group of lysine we used the tosyl group (Ts− = $CH_3C_6H_4SO_2$−). The α-amino groups of the amino acids and peptides were protected by the benzyloxycarbonyl group (Z− = $C_6H_5CH_2OCO$−); and the carboxy groups of the amino acids and peptides in the form of their methyl esters. Amino acids of the L series were used for the synthesis.

Below we give the scheme of the synthesis of the heptapeptide (VI) and the undecapeptide (IX). The heptapeptide (VI) was synthesized by condensing the pentapeptide (IV) with the hydrobromide of the methyl ester of alanyl-N^{ε}-tosyllysine by the mixed-anhydride method using isobutyl chloroformate. The pentapeptide (II) was obtained as described previously [2] and was saponified in dioxane with 1 N caustic potash solution.

The undecapeptide (IX) was synthesized by the mixed- anhydride method from the saponified tetrapeptide (VII) and the hydrobrmide of the methyl ester of the heptapeptide (VIII). The tetrapeptide (V) was obtained by condensing benzyloxycarbonylglycylglycine with the hydrobromide of the methyl ester of N^{ε}-tosyllysylglycine, which was obtained as described previously [2]. (See scheme on following page.)

EXPERIMENTAL

The purity and individuality of the peptides obtained were checked by thin-layer chromatography in a fixed layer of silica gel in systems 1) benzene–ethanol (2:0.4) and 2) butan-1-ol–acetic acid–water (10:1:3). The hydrochlorides of the methyl esters of the amino acids were obtained by Brenner's method [4]. The methyl esters were saponified in dioxane with 1 N caustic potash solution in 10% excess for 2 h.

In the synthesis of the peptides, the benzyloxy group was removed by the action of a 40% solution of hydrogen bromide in glacial acetic acid. The hydrobromides were repeatedly reprecipitated from ethanol with absolute ether.

The results of the elementary analysis of all the compounds obtained corresponded to the calculated figures.

The methyl ester of benzyloxycarbonylalanyl-N^{ε}-tosyllysine (III) was obtained by the mixed-anhydride method in tetrahydrofuran, using 0.36 ml of isobutyl chloroformate, 0.45 g of benzyloxycarbonylalanine, and

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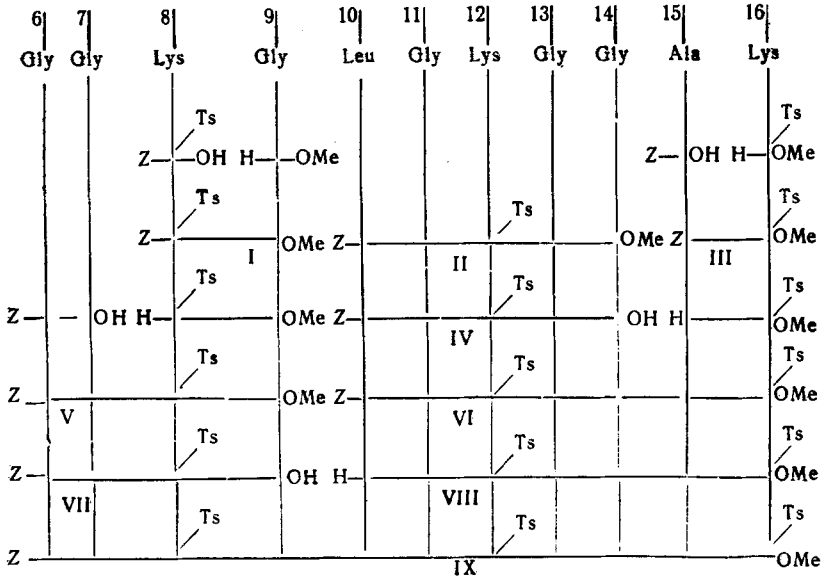
Scheme of the synthesis of the hepta- and undecapeptides



0.71 g of the hydrochloride of the methyl ester of N^{ε}-tosyllysine, mixed with 0.27 ml of triethylamine with cooling to −15°C. After a day, the tetrahydrofuran was distilled off in vacuum and the residue was dissolved in chloroform and washed in the usual way: with water, 1 N hydrochloric acid, water, 5% sodium bicarbonate, and water again. The washed chloroform solution was dried with magnesium sulfate. The solvent was distilled off in vacuum and the oily residue was dried in vacuum over caustic potash. Yield 0.70 g (71.4%), $[\alpha]_D^{20}$ −20.4° (c 1.0; chloroform), R_f 0.66 (1).

Benzyloxycarbonylleucylglycyl-N^{ε}-tosyllysylglycylglycine (IV). A solution of 0.15 g of the pentapeptide (II) in 1 ml of dioxane was treated with 0.25 ml of a 1 N solution of caustic potash and the mixture was left at room temperature for 2 h. After this the dioxane was distilled off in vacuum, the residue was dissolved in water, and the unsaponified peptide was extracted with chloroform. Then the aqueous layer was acidified with a 6 N solution of hydrochloric acid, and the saponified product was extracted with chloroform. The chloroform extracts were dried with sodium sulfate, and the solvent was evaporated off to dryness. The residue was washed several times with ether, giving 0.11 g (80.0%) of a white pulverulent product with mp 130-133°C, $[\alpha]_D^{20}$ −10.5° (c 1.0; chloroform), R_f 0.66 (2).

Methyl Ester of Benzyloxycarbonylglycylglycyl-N^{ε}-tosyllysylglycine (V). This was synthesized from 0.27 g of benzyloxycarbonylglycylglycine and 0.45 g of the hydrobromide of the methyl ester of N^{ε}-tosyllysylglycine mixed with 0.14 ml of triethylamine, using 0.14 ml of triethylamine and 0.20 ml of isobutyl chloroformate in chloroform at −15°C. After a day, the chloroform solution was washed in the usual way, dried with sodium sulfate, and evaporated to dryness in vacuum. On the addition of ether the product crystallized. Yield 0.48 g (80.0%), mp 105-110°C, $[\alpha]_D^{20}$ −7.0° (c 1.0; chloroform), R_f 0.36 (1).

Benzyloxycarbonylglycylglycyl-N^{ε}-tosyllysylglycine (VII). The tetrapeptide (V) (0.25 g) in solution in 2 ml of a mixture of dioxane and methanol (1 : 1) was saponified under the action of 0.41 ml of a 1 N solution of caustic soda for 2 h. The product was worked up as described for compound (IV). After the residue had been treated with ether, 0.13 g (54.2%) of a white crystalline product was obtained with mp 110-113°C, $[\alpha]_D^{20}$ −7.2° (c 1.0; chloroform), R_f 0.66 (1).

Methyl Ester of Benzyloxycarbonylleucylglycyl-N^{ε}-tosyllysylglycylglycylalanyl-N^{ε}-tosyllysine (VI). This was obtained by the mixed-anhydride method from 0.32 g of (IV) and 0.20 g of the hydrobromide of the methyl ester of alanyl-N^{ε}-tosyllysine previously treated with 0.06 ml of triethylamine using 0.06 ml of triethylamine and 0.08 ml of isobutyl chloroformate in tetrahydrofuran. Then the product was worked up as in the case of compound (III). Substance (VI) was crystallized from a mixture of methanol and ether. Yield 0.31 g (64.5%), mp 107-110°C, $[\alpha]_D^{20}$ −16.5° (c 1.0; chloroform), R_f 0.32 (1).

Hydrobromide of the Methyl Ester of Leucylglycyl-N^{ε}-tosyllysylglycylglycylalanyl-N^{ε}-tosyllysine (VIII). A solution of 0.20 g of (VI) in 0.16 ml of glacial acetic acid was treated with 0.16 ml of 40% HBr/CH_3COOH. After 40 min, the hydrobromide was precipitated with absolute ether and was washed several times with ether. For purification, the product was reprecipitated with ether from methanol. The hydrobromide was dried in vacuum over caustic potash. Yield 0.18 g (94.7%), mp 50-55°C (hygroscopic), R_f 0.58 (2).

Methyl Ester of Benzyloxycarbonylglycylglycyl-N^{ε}-tosyllysylglycylleucylglycyl-N^{ε}-tosyllysylglycylglycylalanyl-N^{ε}-tosyllysine (IX). This was obtained in the same way as (III) from 0.10 g of (VII), 0.18 g of (VIII), 0.024 ml of triethylamine, and 0.024 ml of isobutyl chloroformate. The product was crystallized from a mixture of methanol and ether and was dried over caustic potash in vacuum. Yield 0.15 g (60.0%), mp 151-152°C, $[\alpha]_D^{20}$ −30.6° (c 0.8; chloroform), R_f 0.40 (1).

SUMMARY

The synthesis has been performed of a protected heptapeptide – the methyl ester of benzyloxycarbonylleucylglycyl-N^{ε}-tosyllysylglycylglycylalanyl-N^{ε}-tosyllysine (V) – and of a protected undecapeptide – the methyl ester of benzyloxycarbonylglycylglycyl-N^{ε}-tosyllysylglycylleucylglycyl-N^{ε}-tosyllysylglycylglycylalanyl-N^{ε}-tosyllysine (IX) – which represent the (10-16) and (6-16) fragments of the N-terminal section of the histone of the F2aI fraction of calf thymus.

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