SYNTHESIS OF A PROTECTED HEXADECAPEPTIDE CORRESPONDING TO SEQUENCE 1-16 OF THE N-TERMINAL PART OF THE HISTONE OF FRACTION F 2aI OF CALF THYMUS

N. I. Koryakina and V. K. Burichenko

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We have previously reported the synthesis of peptides corresponding to the fragments of the N-terminal segment of the histone of fraction F 2aI of calf thymus [1, 2] where tosyl groups were used to protect the side chains of the lysine residues. We synthesized simultaneously the same peptides using the benzyloxycarbonyl group to protect the side chains of the lysine and arginine residues [3], but at the stage of the saponification of the methyl ester of O,N-diacetylseryl-glycyl-N^G-benzyloxycarbonylarginine, corresponding to sequence 1-3, the yields proved to be low. In order to circumvent the stage of saponifying the tripeptide, in the present investigation to obtain O,N-diacetylserylglycyl-N^G-nitroarginine we used the activated ester method. The pentachlorophenyl ester of the dipeptide O,N-diacetylserylglycine (IX) reacted with N^G-nitroarginine in which the carboxy group was free. This gave the tripeptide O,N-diacetylserylglycyl-N^G-nitroarginine with a free carboxy group (X) (Scheme).

The synthesis was performed by the successive addition of amino acids to tripeptyl fragments selected in such a way that, so far as possible, the C-terminal residue was glycine. In this case, the fragments were added by the mixed-anhydride method using isobutyl chloroformate. In this way we obtained the hexapeptide of sequence of 11-16 by the (3+3) scheme, the decapeptide of sequence 7-16 (VI) by the (3 + 7) scheme, and the tridecapeptide of the 4-16 sequence (VII) by the (3 + 10) scheme. If the C-terminal residue was an optically active amino acid, we used the carbodiimide method with the addition of N-hydroxysuccinimide since, according to the literature [4], no racemization is observed with this method in the majority of cases. By the carbodiimide method we obtained the methyl ester of tert-butoxycarbonylalanyl-N^ε-benzyloxycarbonyllysine (I), the methyl ester of tert-butoxycarbonylglycyl-N^ε-benzyloxycarbonyllysylglycine (III) by the (2 + 1) scheme, and the heptapeptide of the sequence 10-16 (V) by the addition of tert-butoxycarbonylleucine to the trifluoroacetate of the hexapeptide (IVa). We also used the carbodiimide method at the stage of obtaining the protected hexadecapeptide (XI) corresponding to the sequence 1-16 by the (3 + 13) scheme, since it was impossible to obtain a hydrazide at an arginine residue (see Scheme).

The ε-amino group of lysine was protected by the benzyloxycarbonyl group (Z), and the α-amino groups of the amino acids and peptides by the tert-butoxycarbonyl group (Boc). The synthesis was performed with N^G-nitroarginine and the methyl esters of the amino acids and peptides.

EXPERIMENTAL

The synthesis was performed with optically active amino acids of the L series. The methyl esters of the peptides synthesized, in suitable solvents (ethanol, dioxane, or mixtures of them), were washed with 1 N caustic soda for 30-40 min. The tert-butoxycarbonyl groups were removed from the dipeptide (I) and the tripeptide (II) by the action of gaseous hydrogen chloride on solutions of the peptides in dioxane. The higher-molecular-weight peptides were deblocked by the action of trifluoroacetic acid. As a rule, the trifluoroacetates were crystalline. The pentachlorophenyl ester of benzyloxycarbonylglycine was obtained as describe by Kovacs et al. [5]. In the preparation of the protected peptides, their solutions in organic solvents were washed successively with 10% citric acid solution, water, 5% sodium bicarbonate solution,

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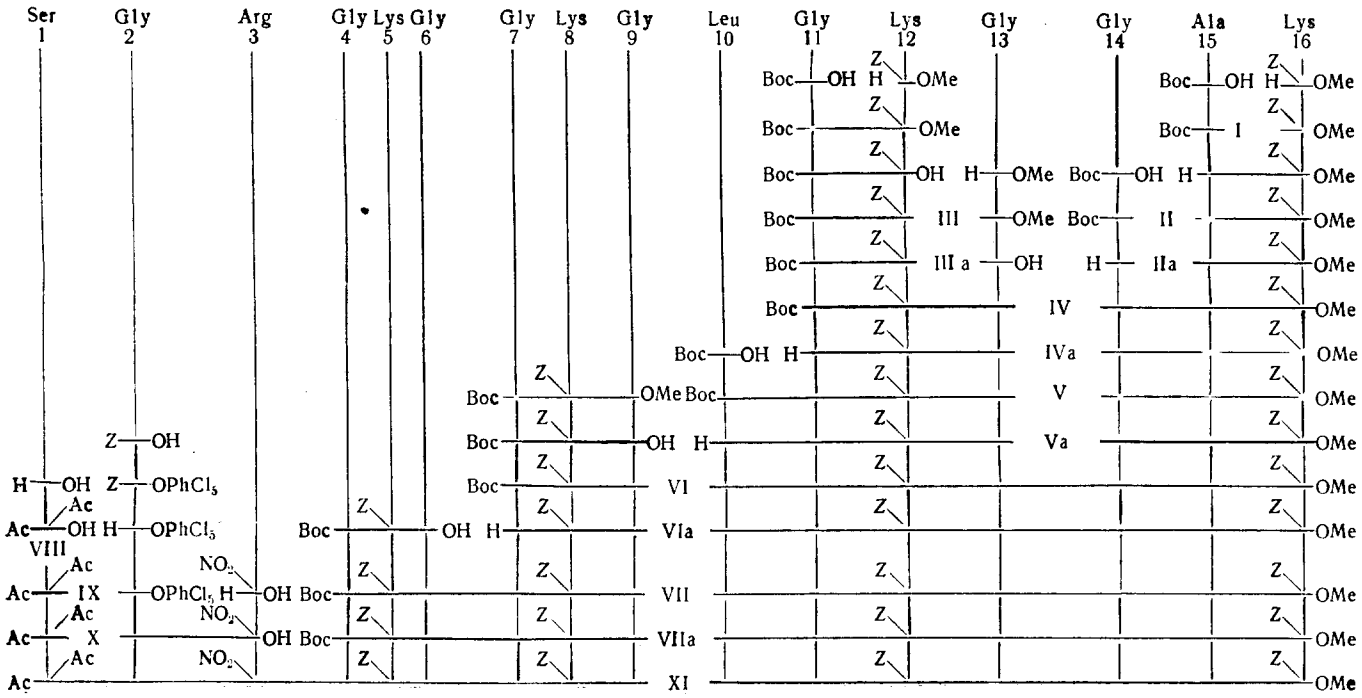
and again with water and were then dried with anhydrous sodium sulfate. The purity of the peptides obtained was checked by chromatography in a layer of silica gel fixed with gypsum as on standard plates of the "Silufol UV_{254}" brand marketed by the "Kavalier" enterprise (Czechoslovakia) in the following systems: 1) benzene–ethanol (2 :0.4); 2) benzene–ethanol (2 :0.5); 3) benzene–ethanol–water (2 :1 :0.1); 4) water–formic acid–tert-butyl alcohol (1.5 :1.5:7); 5) butan-1-ol–acetic acid–water (10 :1 :3); 6) chloroform–ethanol–20% ammonia (1 :1 :1); 7) chloroform–methanol–acetic acid (90 :8 :2). The analyses of all the compounds corresponded to the calculated figures.

SCHEME OF THE SYNTHESIS OF THE HEXAPEPTIDE (XI)



Methyl Ester of tert-Butoxycarbonylalanyl-N^{ε}-benzyloxycarbonyllysine (I). A solution of 1.14 g of tert-butoxycarbonylalanine in 30 ml of tetrahydrofuran was cooled to −15°C, and 1.25 g of N-hydroxysuccinimide and 1.25 g of dicyclohexylcarbodiimide were added to the solution. After 15 min the hydrochloride of the methyl ester of N^{ε}-benyloxycarbonyllysine was added in the dry form. The mixture was stirred with cooling for 5 h and was then left in the refrigerator for a day. The dicyclohexylurea that had deposited was filtered off and washed with tetrahydrofuran. The filtrate was evaporated, the residue was dissolved in chloroform, the solution was washed and dried, and the solvent was driven off in vacuum to dryness. The resulting oil, amounting to 2.4 g (88.8%), was chromatographically homogeneous, R_f 0.62 (system 1), $[\alpha]_D^{25}$ −20.0° (c 1.0; chloroform).

Methyl Ester of tert-Butoxycarbonylglycylalanyl-N^{ε}-benzyloxycarbonyllysine (II). A solution of 0.22 g of tert-butoxycarbonylglycine in 10 ml of chloroform was cooled to −20°C, and to this solution were added 0.17 ml of triethylamine and 0.23 ml of isobutyl chloroformate. After 15 min, a cooled solution of 0.60 g of the hydrochloride of the methyl ester of alananyl-N^{ε}-benyloxycarbonyllysine containing 0.17 ml of triethylamine was added to the reaction mixture and it was stirred at −15°C for 2 h and at room temperature for a day. Then it was washed and dried, and the solvent was distilled off in vacuum. After treatment with ether and drying in vacuum, 0.57 g (87.7%) of a chromatographically homogeneous oily product was obtained with R_f 0.59 (system 1), $[\alpha]_D^{25}$ −18.5° (c 1.0; chloroform).

Methyl Ester of tert-Butoxycarbonylglycyl-N^{ε}-benyloxycarbonyllysylglycine (III). Azide Method. A solution of 0.7 g of the hydrazide of tert-butoxycarbonylglycyl-N^{ε}-benzyloxycarbonyllysine in water–acetic acid (8 :6) was mixed with 30 ml of chloroform, the mixture was cooled to 0°C, and 1 ml of a cooled 6 N solution of hydrochloric acid and, immediately, a cold solution of 0.40 g of sodium nitrite in water were added. After 5 minutes' stirring, the organic layer was separated off, washed with cold 5% sodium bicarbonate solution and with water, and dried with cooling, and the filtered solution was mixed with a cold solution of 0.20 g of the hydrochloride of the methyl ester of glycine containing 0.22 ml of trieth-

ylamine. The mixture was kept in the refrigerator for a day, and at room temperature for a day, and was then washed and dried, and the solvent was distilled off in vacuum. This gave a chromatographically pure colorless oily product in an amount of 0.57 g (73.0%), R_f 0.64 (system 1); $[\alpha]_D^{25}-18.0°$ (c 0.78; chloroform).

Carbodiimide Method. To a solution of 4.12 g of tert-butoxycarbonylglycyl-N^{ε}-benzyloxycarbonylglycine in 30 ml of tetrahydrofuran was added 1.8 g of N-hydroxysuccinimide, and the mixture was cooled to −5°C and 2.20 g of dicyclohexylcarbodiimide was added. After 15 min, 1.18 g of the dry hydrochloride of the methyl ester of glycine and 1.3 ml of triethylamine were added. The mixture was kept in the refrigerator for a day and the dicyclohexylurea that had separated out was filtered off and washed with ethyl acetate. The filtrate was evaporated to dryness, the residue was dissolved in ethyl acetate, the solution was washed and dried, and the solvent was distilled off in vacuum. This gave 4.67 g (99.0) of a crude oily product which, after repeated treatment with ether, began to crystallize. The product was left under ether in the refrigerator for crystallization. After filtration and drying, 3.95 g (84.0%) of a crystalline product with mp 86-90°C was obtained; R_f 0.63 (system 1); $[\alpha]_D^{25}-18.0°$ (c 0.80; chloroform).

tert-Butoxycarbonylglycyl-N^{ε}-benzyloxycarbonyllysylglycine (IIIa). To a solution of 0.86 g of the protected tripeptide (III) in a mixture of ethanol and dioxane (2 :5) was added 1.9 ml of a 1 N solution of caustic soda. After 30 min, the contents of the flask were evaporated to half-volume, and the residue was diluted with water and was washed free from unsaponified tripeptide with chloroform. The aqueous phase was acidified with dry citric acid to pH 3 and extracted with chloroform. The chloroform extract was dried and the solvent was distilled off in vacuum. The residue was treated with several portions of ether and dried in vacuum. The product was converted into a dry white foam, and this was ground into a powder. The yield was 0.60 g (72.3%), mp 60-63°C; R_f 0.53 (system 1); $[\alpha]_D^{25}-6.06°$ (c 1.32; chloroform).

Methyl Ester of tert-Butoxycarbonylglycyl-N^{ε}-benzyloxycarbonyllysylglycylglycylalanyl-N^{ε}-benzyloxycarbonyllysine (IV). A solution of 0.49 g of the saponified tripeptide (IIIa) in 20 ml of chloroform was cooled to −20°C and 0.14 ml of triethylamine and 0.2 ml of isobutylchloroformate were added. After 15 min, a cold solution of 0.52 g of the hydrochloride of the methyl ester of glycylalanyl-N^{ε}-benzyloxycarbonyllysine containing 0.14 ml of triethylamine was added. After stirring for 2 h with cooling, the mixture was left at room temperature for a day. Then it was washed in the usual way and dried, and the solvent was distilled off to dryness. The residue was treated with ether, whereupon the product crystallized; it was washed with ether and dried in vacuum, giving 0.63 g (71.0%) of the protected hexapeptide (IV), mp 110°C; R_f 0.33 (system 2), $[\alpha]_D^{25}-26.7°$ (c 1.0; chloroform).

Methyl Ester of tert-Butoxycarbonylleucylglycyl-N^{ε}-benzyloxy-carbonyllysylglycylglycylalanyl-N^{ε}-benzyloxycarbonyllysine (V). This compound was obtained by the carbodiimide method in tetrahydrofuran in a similar manner to (III) from 0.12 g of tert-butoxycarbonylleucine, 0.43 g of the trifluoroacetate of the hexapeptide (IVa) and 0.08 ml of triethylamine, using 0.11 g of N-hydroxysuccinimide and 0.12 g of dicyclohexylcarbodiimide. Yield 0.41 g (85.4%), mp 103°C; R_f 0.44 (system 2); $[\alpha]_D^{25}-25.4°$ (c 1.0; chloroform).

The Methyl Ester of tert-Butoxycarbonylglycyl-N^{ε}-benzyloxycarbonyllysylglycylleucylglycyl-N^{ε}-benzyloxycarbonyllysylglycylglycylalanyl-N^{ε}-benzyloxycarbonyllysine (VI). Compound (VI) was obtained in a similar manner to (IV) from 0.08 g of the saponified tripeptide (IIIa) with 0.03 ml of triethylamine and 0.17 g of the trifluoroacetate of the methyl ester of the heptapeptide (Va), mixed with 0.03 ml of triethylamine and 0.17 g of the trifluoroacetate of the methyl ester of the heptapeptide (Va), mixed with 0.03 ml of triethylamine and 0.04 ml of isobutyl chloroformate. Yield 0.17 g (75.0%), mp 140°C, R_f 0.42 (system 2), 0.40 (system 3), $[\alpha]_D^{25}-55.7°$ (c 1.0; chloroform).

Methyl Ester of tert-Butoxycarbonylglycyl-N^{ε}-benzyloxycarbonyllysylglycylglycyl-N^{ε}-benzyloxycarbonyllysylglycylleucylglycyl-N^{ε}-benzyloxycarbonyllysylglycylglycylalanyl-N^{ε}-benzyloxycarbonyllysine (VII). A solution of 0.10 g of the saponified tripeptide (IIIa) in 20 ml of chloroform was cooled to −20°C, and 0.04 ml of triethylamine and 0.05 ml of isobutyl chloroformate were added. After 20 min at −20°C, 0.28 g of the trifluoroacetate of the methyl ester of decapeptide (VIa) and 0.04 ml of triethylamine were added. After 3 hours' stirring at −20°C, the temperature was gradually raised to that of the room, and stirring was continued for another 48 h. Then the reaction mixture was washed in the usual way and dried, and the solvent was distilled off in vacuum. The residue was treated several times with ether, whereupon the product crystallized. The yield after drying was 0.34 g (97.1%); mp 145°C (decomp.) R_f 0.15 (system 2), 0.83 (system 4), $[\alpha]_D^{25}-36.1°$ (c 1.0; chloroform).

O,N-Diacetylserine (VIII). Serine was acetylated by a method proposed for tyrosine [6]. A solution of 0.8 g of serine in 24 ml of 2 N caustic soda was cooled to between 0 and −2°C and, with stirring

and cooling, 30 ml of acetic anhydride and 24 ml of 2 N caustic soda were added dropwise over 5 h, and then the mixture was acidified with 6 N hydrochloric acid to pH 2. The water was distilled off in vacuum to dryness. The O,N-diacetylserine was extracted from the residue with methanol. The methanolic solution was dried with sodium sulfate and the solvent was distilled off. The resulting oil was treated several times with ether and acetone and dried in vacuum. This gave 0.125 g (86.8%) of an oily product with R_f 0.77 (system 6); $[\alpha]_D^{25} - 5.2°$ (c 0.91; dimethylformamide). The product did not give a coloration with ninhydrin.

The pentachlorophenyl ether of O,N-diacetylserylglycine (IX) was obtained by the mixed-anhydride method in dimethylformamide at −20°C from 0.29 g of O,N-diacetylserine, 0.20 ml of triethylamine, and a cold solution of 0.62 g of the hydrobromide of the pentachlorophenyl ester of glycine mixed with 0.20 ml of triethylamine with the aid of 0.26 ml of isobutyl chloroformate, in a similar manner to (II). After the reaction, the dimethylformamide was distilled off, the residue was dissolved in ethyl acetate, the solution was washed and dried, and the solvent was distilled off to dryness. The residue was treated with ether. This gave 0.40 g (52.6%) of an amorphous product with R_f 0.71 (system 8), $[\alpha]_D^{25} - 6.6°$ (c 1.1; chloroform).

O,N-Diacetylserylglycyl-N^G-nitroarginine (X). Compound (X) was obtained by the activated-ester method. A solution of 0.40 g of the pentachlorophenyl ester of O,N-diacetylserylglycine (IX) in 20 ml of methylene chloride was treated with 0.02 g (catalytic amount) of 2-hydroxypyridine. A suspension of 0.17 g of N^G-nitroarginine in 20 ml of methylene chloride, to which 0.16 ml of dicyclohexylamine was added, was prepared separately. The suspension so obtained was added in three portions to the first flask with continuous stirring. Stirring was continued at room temperature for 24 h. Then the reaction mixture was treated repeatedly with 1 N hydrochloric acid until the unchanged N^G-nitroarginine had been completely eliminated, and it was dried with sodium sulfate, and the solvent was distilled off to dryness. The residue was extracted with ether. The ethereal extract was dried with sodium sulfate and evaporated to dryness. This gave 0.30 g (88.2%) of chromatographically pure oily product with R_f 0.84 (system 1), $[\alpha]_D^{25} - 19.4$ (c 0.8; chloroform).

Methyl Ester of O,N-Diacetylserylglycyl-N^G-nitroarginylglycyl-N-benzyloxycarbonyllysylglycylglycyl-N^ε-benzyloxycarbonylysylglycylleucylglycyl-N^ε-benzyloxycarbonyllysylglycylglycylalanyl-N^ε-benzyloxycarbonylglycine (XI). A solution of 0.07 g of O,N-diacetylserylglycyl-N^G-nitroarginine (X) in tetrahydrofuran was cooled to −18°C, and 0.04 g of N-hydroxysuccinimide and 0.04 g of dicyclohexylcarbodiimide were added. After 20 min, 0.20 g of the trifluoroacetate of the methyl ester of the tridecapeptide (VIIa) and 0.015 ml of triethylamine were added to the reaction mixture. It was stirred with cooling for 5 h and was left in the refrigerator for two days. The solvent was evaporated to dryness, the residue was dissolved in ether (it did not dissolve in chloroform and ethyl acetate), and the solution was washed in the usual way; during this process a white precipitate of urea deposited in the separatory funnel, which was filtered off. The ethereal solution was dried, the solvent was distilled off to small volume, and the product was crystallized from ether. Yield 0.16 g (70.0%) mp 99-100°C (decomp.), R_f 0.90 (system 4), $[\alpha]_D^{25} - 29.0°$ (c 0.64; dimethylformamide).

SUMMARY

A protected hexadecapeptide corresponding to the sequence 1-16 of the N-terminal part of the histone of fraction F 2aI of calf thymus (XI) has been synthesized.

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