SYNTHESIS OF N-METHYLAMIDES OF N^{α}-ACETYLATED OLIGOLYSINES

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Histones – the basic protein component of the main structural unit of chromatin, a deoxyribonucleoprotein (DNP) – have been the subject of prolonged and multifaceted investigations. Histones are proteins of a basic nature; the polycationic nature of the histones is responsible for the basic electrostatic form of their interaction with DNA, but the positive charge is distributed nonuniformly along the polypeptide chain: The basic amino acids are grouped in clusters of 3-5 amino acids [1]. For a model study of the interaction of histones with DNA we have synthesized two oligopeptides of L-lysine with the general formula CH_3CO-$(Lys)_n$-$NHCH_3$, n=1 or 2, by the following routes

$n = 1$

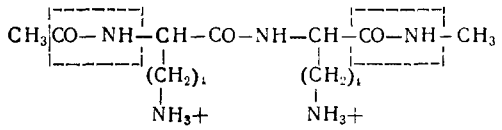
$$HCl \cdot H\text{-}Lys(Cbz)\text{–}OH \xrightarrow[SOCl_2]{MeOH} HCl \cdot H\text{-}Lys(Cbz)\text{-}OMe\ (I) \xrightarrow{AcOSu} Ac\text{-}Lys(Cbz)\text{–}OMe\ (II) \xrightarrow[MeOH]{CH_3NH_2} Ac\text{-}Lys(Cbz)\ NHCH_3\ (III) \xrightarrow{H_2/Pd} X\Gamma\ Ac\text{-}Lys\text{-}NHCH_3\ (IV)$$

$n = 2$

$$HCl \cdot H\text{-}Lys(Cbz)\text{-}OH \xrightarrow{Boc\ N_3} Boc\text{-}Lys(Cbz)\text{-}OH\ (V) \xrightarrow[HOSu]{DCHC} Boc\text{-}Lys(Cbz)\text{-}OSu\ (VI) \xrightarrow[(C_2H_5)_3N]{HCl \cdot H\text{-}Lys(Cbz)\text{-}OMe} Boc\text{-}Lys(Cbz)\text{-}Lys(Cbz)\text{-}OMe\ (VII) \xrightarrow{CH_3NH_2} Boc\text{-}Lys(Cbz)\text{-}Lys(Cbz)\text{-}NHCH_3\ (VIII) \xrightarrow{CF_3COOH} [CF_3COOH \times H_2N\text{-}Lys(Cbz)\text{-}Lys(Cbz)\text{-}NHCH_3]\ (IX) \xrightarrow{AcOSu} Ac\text{-}Lys(Cbz)\text{-}Lys(Cbz)\text{-}NHCH_3\ (X) \xrightarrow{H_2Pd} 2\ HCl \cdot Ac\text{-}(Lys)_2\text{-}NHCH_3\ (XI)$$

The following abbreviations have been used: Ac – acetyl group; OMe – methoxy group; DCHC – N,N-dicyclohexylcarbodiimide; HCl – hydrochloride; Boc – tert-butoxy carbonyl group; Cbz – benzyloxycarbonyl group; HOSu – N-hydroxysuccinimide; –Osu – succinimidyl ester; AcOSu – N-acetoxysuccinimide.

The advantages of these models as compared with the oligopeptides elimination of "end effects" and the creation of additional peptide bonds:



As protective groups for the synthesis we selected groups of the urethane type – the benzyloxycarbonyl and tert-butoxycarbonyl groups – since the combination of these groups ensures selectivity in the removal of the protection and a high optical purity of the reaction products. The peptide bond was formed by the carbodiimide method using N-hydroxysuccinimide esters, which enabled the synthesis to be performed with practically no racemization [3].

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EXPERIMENTAL

Chromatography. The purity of the compounds obtained was determined by paper chromatography (PC) on Whatman MN 261 paper in the butanol–CH_3COOH–H_2O (4:1:5) (1) and methyl ethyl ketone–pyridine–H_2O (65:15:20) (2) systems and by thin-layer chromatography (TLC) in nonfixed silicic acid in the ethyl acetate–benzene (4:1) (3), (1:1) (4), and (1:2) (5) and dioxane–benzene (1:2) (6) systems. The spots of the compounds were revealed in iodine vapor in the case of thin-layer chromatography and with a solution of ninhydrin in the case of paper chromatography; the spots of some completely protected derivatives and of peptides containing a Boc group can be detected on a paper chromatogram by means of ninhydrin, since the Boc group is probably split off during the heating of the chromatogram.

Hydrochloride of the Methyl Ester of N^{ε}-Benzyloxycarbonyl-L-lysine (I). This was obtained by the method of Ionov and Morozova [4], with additional purification of the reaction product. To 32 ml of absolute MeOH, 2 ml of thionyl chloride was added dropwise and 10 g of HCl · N^{ε}-benzyloxycarbonyl-L-lysine in portions. The mixture was stirred at room temperature for 4 h and was then left to stand for 13 h. The solvent was eliminated in vacuum, and the residue was dissolved in ethyl acetate and the solution was washed with 10% sodium carbonate solution and then with water to neutrality. After drying with magnesium sulfate, the ethyl acetate solution was saturated with dry hydrogen chloride; then the ethyl acetate was distilled off in vacuum, the residue was dissolved in a small amount of absolute MeOH, and the chromatographically pure reaction product was precipitated by the addition of absolute ether. 6.7 g (61%), mp 114-116°C, $[\alpha]_D^{21}$ + 16.7° (c 2; methanol), R_f 0.75 [PC (1)]. Literature data: mp 75-77°C [4]; mp 117°C, $[\alpha]_D^{21}+16.7\pm0.5°$ (c 2; methanol) [5].

Methyl Ester of N^{α}-Acetyl-N^{ε}-benzyloxycarbonyl-L-lysine (II). With stirring, a solution of 3.2 g of (I) in 30 ml of absolute chloroform and 1.25 ml of triethylamine was added to 1.5 g of N-acetylsuccinimide in 20 ml of absolute chloroform. The mixture was stirred at room temperature for 2 h and was left for 24 h, and was then washed with 1 N hydrochloric acid, 10% sodium carbonate solution, and water, and dried with magnesium sulfate. The chloroform was driven off in vacuum, and the reaction product was isolated in the form of an oil. Yield quantitative, R_f 0.42 [TLC (3)].

Methylamide of N^{α}-Acetyl-N^{ε}-benzyloxycarbonyl-L-lysine (III). This was obtained from (II) by Zann's method [6]. Yield 95%, mp 176°C, $[\alpha]_D^{20}-8.25°$ (c 2; methanol). Literature data: mp 176°C [6].

Hydrochloride of the Methylamide of N^{ε}-Acetyl-L-lysine (IV). The hydrogenation of the methylamide of N^{α}-acetyl-N^{ε}-benzyloxycarbonyl-L-lysine by Zann's method [6] gave the hydrochloride of the methylamide of N^{α}-acetyl-L-lysine. Yield quantitative; mp 161-163°C, $[\alpha]_D^{20}-18.4°$ (c 2; water), R_f 0.45 [PC (1)], R_f 0.52 [PC (2)]. Literature data: mp 166°C, $[\alpha]_D^{25}-20.1°$ (c 2; water) [6].

N^{α}-tert-Butoxycarbonyl-N^{ε}-benzyloxycarbonyl-L-lysine (V). A solution of 5 g of the hydrochloride of N^{α}-benzyloxycarbonyl-L-lysine in 20 ml of a 1 N solution of caustic soda was treated with 50 ml of dioxane. Then, at 45°C, 5 ml of tert-butoxycarbonyl azide and, with stirring, over 10-12 h, 45 ml of a 1 N solution of caustic soda were added. After 48 h, 50 ml of water was added and the whole volume was washed with ethyl acetate. The organic layer was washed twice with water and, at 0°C, the combined aqueous extract was covered with ethyl acetate; the solution was acidified with cold 2 N hydrochloric acid to pH ~ 3, and the organic layer was separated off, washed with water, and dried with magnesium sulfate. The solvent was driven off in vacuum and the reaction product was isolated in the form of an oil. Yield 5.8 g (86%), R_f 0.85 [TLC (3)], R_f 0.73 [PC (1)]. Literature data: isolated in the form of an oil, no characteristics given [7].

N-Hydroxysuccinimide Ester of N^{α}-Benzyloxycarbonyl-N^{ε}-tert-butoxycarbonyl-L-lysine (VI). To 5.8 g of N^{ε}-benzyloxycarbonyl-N^{α}-tert-butoxycarbonyl-L-lysine in 70 ml of absolute dioxane was added 2.12 g of N-hydroxysuccinimide, and then the reaction mixture was cooled to +10°C and 3.5 g of N,N-dicyclohexylcarboxiimide was added. The resulting mixture was stirred at room temperature for 12 h; the dicyclohexylurea that had deposited was filtered off, the mother solution was diluted with 70 ml of ethanol, 0.39 ml of CH_3COOH was added, and it was left in the refrigerator for 30-60 min, after which dicyclohexylurea was again separated off, and the procedure was repeated with the reaction mixture being left in the refrigerator overnight; the procedure was repeated again until no more dicyclohexylurea separated out, and then the mother solution was evaporated to dryness in vacuum, the residue was dissolved in isopropanol, and the reaction product was isolated by the addition of n-hexane. Yield quantitative, composition $C_{23}H_{31}N_3O_8$, mp 95-100°C, $[\alpha]_D^{20}-20°$ (c 2; ethanol), R_f 0.42 [TLC (5)]. The substance decomposed on standing.

Methyl Ester of N^{ε}-Benzyloxycarbonyl-N^{α}-tert-butoxycarbonyl-L-lysyl-N^{ε}-benzyloxycarbonyl-L-lysine (VII). To a solution of 7.7 g of the N-hydroxysuccinimide ester of N^{α}-benzyloxycarbonyl-N^{ε}-tert-

butoxycarbonyl-L-lysine (VI) in 40 ml of absolute dioxane was added 3.45 g of the hydrochloride of the methyl ester of N^{ε}-benzyloxycarbonyl-L-lysine (I) in 40 ml of methylene chloride, and the mixture was cooled to 0°C, after which 3.93 ml of triethylamine was added; the reaction mixture was left at room temperature for 24 h, and then 600 ml of water was added, and the oil that separated out was extracted with ethyl acetate; the ethyl acetate extract was washed with 10% citric acid, a 10% solution of sodium carbonate, and with water After drying with sodium sulfate and evaporation of the solvent in vacuum, the residual oil was crystallized from petroleum ether. The yield was quantitative; composition $C_{34}H_{48}O_9N_4$, mp 110-111°C (from methanol), $[\alpha]_D^{20} -15.25°$ (c 2; methanol), R_f 0.23 [TLC (5)], R_f 0.95 [PC (1)].

Methylamide of N^{α}-Benzyloxycarbonyl-N^{ε}-tert-butoxycarbonyl-L-lysyl-N^{ε}-benzyloxycarbonyl-L-lysine (VIII). To a solution of 5.5 g of Boc-Lys(Cbz)-Lys(Cbz)-OMe (VII) in 100 ml of absolute ether was added 4 ml of condensed dehydrated methylamine. The reaction mixture was left at room temperature for several days, and then the solvent was driven off in vacuum, and the solid residue was recrystallized from methanol; composition $C_{34}O_{49}N_5O_9$. Yield 5.3 g (95%), mp 150-152°C, $[\alpha]_D^{25} -16.5°$ (c 1.7; methanol), R_f 0.6 [TLC (3)].

Methylamide of N^{α}-Acetyl-N^{ε}-benzyloxycarbonyl-L-lysyl-N^{ε}-benzyloxycarbonyl-L-lysine (X). A solution of 3.3 g of Boc-Lys(Cbz)-L-Lys(Cbz)-$NHCH_3$ (VIII) in 30 ml of freshly distilled trifluoroacetic acid was stirred without the access of moisture for 1 h; after the reaction mixture had been dissolved in ethyl acetate and the solvent had been evaporated off in vacuum three times, the methylamide of the trifluoroacetate of N^{ε}-benzyloxycarbonyl-L-lysyl-N^{ε}-benzyloxycarbonyl-L-lysine (XI) was isolated, and it was used in the synthesis without purification and identification. To 2.3 g of (IX) in 20 ml of dimethylformamide were added 0.66 g of N-acetoxysuccinimide in 20 ml of dimethylformamide and 4 ml of triethylamine to pH 5-6. The reaction mixture was stirred for 24 h (during which time a precipitate deposited), after which 100 ml of water was added; the precipitate was filtered off and washed on the filter with 1 N hydrochloric acid and with water to neutrality. Yield 1.5 g (60%), composition $C_{31}H_{43}N_5O_7$. After reprecipitation from dimethylformamide with water, mp 218-219°C, $[\alpha]_D^{25} -13°$ (c 1; dimethylformamide) R_f 0.45 [TLC (6)].

After the protective groups had been removed from part of the product by treatment with glacial acetic acid saturated with hydrogen bromide, on a paper chromatogram (system 1) a main spot with R_f 0.14 was found, with a small amount of impurity at the start. To obtain a chromatographically pure preparation, 0.5 g of CH_3CO-Lys(Cbz)-Lys(Cbz)-$NHCH_3$ (X) was dissolved in 500 ml of a mixture of dimethylformamide and water (4:1), 50 ml of Dowex 50×8 resin (H^+) equilibrated with the same solvent was added, and the suspension was stirred for 24 h to eliminate the nonacetylated material. The resin was removed by filtration, the mother solution was evaporated in vacuum to small volume, and the reaction product was isolated by the addition of water.

Dihydrochloride of the Methylamide of N^{α}-Acetyl-L-lysyl-L-lysine (XI). Without additional purification, 0.6 g of CH_3CO-Lys(Cbz)-Lys(Cbz)-$NHCH_3$ was dissolved in 100 ml of dimethylformamide and hydrogenated in a current of hydrogen over Pd black at room temperature for 6 h. After elimination of the solvent in vacuum, the residue was dissolved in 6 ml of absolute methanol and the reaction product was isolated by the addition of ether. The substance is hygroscopic and therefore after the decantation of the bulk of the solvent it was dried in a vacuum desiccator over calcium chloride. Yield 0.1 g (25%), composition $C_{15}H_{35}N_5O_3Cl_2$, mp 155-158°C, $[\alpha]_D^{20} -20.12°$ (c 1.62; water) R_f 0.14 [PC (1)], and R_f 0.54; the amount of impurity with R_f 0.54 can be considerably reduced by washing the precipitate with ethyl acetate after the decantation of the bulk of the solvent and before drying.

The ^{14}C derivatives of compounds (II, III, IV, X, and XI) were obtained by the same method. The ^{14}C was introduced at the acetylation stage with the aid of N-([^{14}C]acetoxy)succinimide.

SUMMARY

For model investigatians, two representatives of the N-methylamides of N^{α}-acetylated oligolysines of the general formula CH_3CO-$(Lys)_n$-$NHCH_3$, with n=1 and 2, have been synthesized.

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