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The low-molecular-weight synthetic analogs of histones and protamines can be used to obtain complexes with DNA and to study their thermal stabilities [1]. The synthesis of such oligopeptides as the simplest model compounds is therefore of definite interest.

In this paper we report the synthesis of oligopeptides with the following sequences: $BOC-[(N^{\varepsilon}-Cb)-Lys]_{6}-OCH_{3}$; $BOC-[(N^{\varepsilon}-Cb)-Lys]_{8}-OCH_{3}$; $BOC-[(N^{\varepsilon}-Cb)-Lys]_{6}-Ala-[(N^{\varepsilon}-Cb)-Lys]_{4}-Ala-OCH_{3}$; and $BOC-Ala-[(N^{\varepsilon}-Cb)-Lys]_{6}-Ala-OCH_{3}$. These oligopeptides are intermediates for the preparation of the indicated model compounds.* The synthesis was accomplished by the method of mixed anhydrides and by the carbodiimide method. A BOC group was used to protect the α -amino group of lysine and alanine, while a carbobenzoxy group was used to protect the N^{\varepsilon}-amino group of lysine. The intermediate BOC-peptides were obtained by saponifica-tion of their methyl esters.

EXPERIMENTAL METHOD

Thin-layer chromatography was carried out on a fixed layer of silica gel (250 mesh with 75×25 mm plates). The systems were as follows: water-acetic acid-n.butanol (30:10:100) (A), 3% ammonium hydrox-ide-sec.butanol (44:100) (B), and toluene-dioxane-heptane-acetic acid (5:3:1.5:0.5) (C). All of the amino acids used were L forms.

 $\frac{\text{BOC}-[(N^{\epsilon}-\text{Cb})-\text{Lys}]_2-\text{OCH}_3 \text{ (I)}}{20 \text{ ml of THF and 3.7 ml of TEA at -15°.} After 15 min, a solution of 9.12 g of the hydrochloride of (N^{\epsilon}-\text{Cb})-\text{Lys}-\text{OCH}_3 in 3 ml of THF with 3.7 ml of TEA was added. At the end of the reaction the solvent was evaporated, the residue was dissolved in 100 ml of ethyl acetate, and the solution was treated successively with 0.1 N NaHCO₃ (three 5 ml portions), 10% citric acid (three 5 ml portions), and water. The solution was dried over Na₂SO₄ to give 13.6 g (80%) of (I) with mp 95-97° (ethyl acetate -hexane), <math>[\alpha]_D^{24} - 5.6°$ (C 1.02, alcohol), R_f (A) 0.96, and R_f (B) 0.84.

BOC-[(N^{ε}-Cb)-Lys]₂-COOH (II). A solution of 3 g of (I) in 50 ml of acetone was added to 50 ml of 0.1 N NaOH, and the mixture was held at 20° for 2 h. The unchanged material was extracted with ethyl acetate. The aqueous phase was acidified with citric acid at 0° to pH 3-4 and extracted with four 20 ml portions of ethyl acetate. The ethyl acetate solution was washed with water to neutral pH, dried over Na₂SO₄, and evaporated to give 2.8 g (93%) of oily (II) with $[\alpha]_D^{2^2} - 7.1^\circ$ (C 2.5, alcohol), R_f (A) 0.79, and R_f (B) 0.72.

 $\frac{\text{BOC}-[(N^{\epsilon}-\text{Cb})-\text{Lys}]_{3}-\text{OCH}_{3} \text{ (III)}}{\text{III}} \text{ A total of 0.8 g of DCHCD was added to a solution of 2.33 g of (II)} in 6 ml of THF at -10°, and, after 15 min, 2.274 g of the hydrochloride of (N^{<math>\epsilon$}-Cb)-Lys-OCH₃ in 4 ml of THF and 0.54 ml of TEA was added to the mixture. After 24 h, 0.3 ml of glacial acetic acid was added. The solution was then evaporated, and the residue was dissolved in 70 ml of ethyl acetate. The dicyclohexylurea was filtered, and the solution was evaporated. The product was dissolved in ethyl acetate and worked up as in the preparation of (I) to give 2.9 g (90%) of oily (III) with $[\alpha]_{\text{D}}^{2^{2}}-8.98^{\circ}$ (C 2.7, alcohol), R_{f} (A) 0.98, and R_{f} (B) 0.96.

 $BOC-[(N^{\varepsilon}-Cb)-Lys]_{3}-COOH$ (IV). A total of 20 ml of 0.1 N NaOH was added to 1.3 g of (III) in 20 ml of acetone, and the mixture was held at 20° for 160 min. The subsequent workup and isolation were similar

*BOC is a tert-butoxycarbonyl group, Cb is a carbobenzoxy group, THF is tetrahydrofuran, TEA is triethylamine, DCHCD is dicyclohexylcarbodiimide, and IBCF is isobutyl chloroformate.

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© 1973 Consultants Bureau, a division of Plenum Publishing Corporation, 227 West 17th Street, New York, N. Y. 10011. All rights reserved. This article cannot be reproduced for any purpose whatsoever without permission of the publisher. A copy of this article is available from the publisher for \$15.00. to the methods used to obtain (II). The yield of amorphous (IV), reprecipitated from chloroform with ether, was 1 g (77%); the product had $[\alpha]_D^{22} - 8.6^\circ$ (C 1.28, alcohol) and R_f (B) 0.6.

 $H-[(N^{\varepsilon}-Cb)-Lys]_3-OCH_3$ Hydrochloride (V). A solution of 1.2 g of (III) in 30 ml of 2.93 N HCl in ethyl acetate was held at 20° for 2 h and evaporated. The residue was triturated with absolute ether. Two reprecipitations from ethyl acetate with ether gave 1.05 g (92%) of amorphous V with R_f (A) 0.82.

BOC-[(N^{ε}-Cb)-Lys]₆-OCH₃ (VI). A total of 0.26 g of DCHCD was added to a solution of 1 g of (IV) in 3 ml of CH₂Cl₂ at -5°. After 30 min, 0.935 g of (V) in 3 ml of CH₂Cl₂ and 0.17 ml of TEA was added to this mixture. After 24 h, 0.3 ml of glacial acetic acid was added, and the mixture was worked up as in the case of (III) to give 1.44 g (77.4%) of amorphous (VI) with mp 86-88° (ethyl acetate -hexane), $[\alpha]_D^{23}$ -17.9° (C 1.7, alcohol), R_f (A) 0.88, R_f (B) 0.94, and R_f (C) 0.68.

 $\frac{\text{CF}_3\text{COOH}\cdot\text{H}-[(N^{\epsilon}-\text{Cb})-\text{Lys}]_6-\text{OCH}_3 \text{ (VII).}}{\text{was allowed to stand for 80 min and was then treated with 2 ml of absolute benzene and vacuum evaporated (this operation was repeated twice). The residue was triturated with absolute ether and then dissolved in 1 ml of ethanol and precipitated with absolute ether to give 0.8 g (80%) of powdery (VII) with R_f(A) 0.85.$

 $\frac{\text{BOC}-[(\text{N}^{\text{E}}-\text{Cb})-\text{Lys}]_8-\text{OCH}_3 \text{ (VIII)}. A \text{ total of 0.11 g of DCHCD was added to 0.361 g of (II) in 2 ml of THF at -5°. After 30 min, 0.85 g of (VII) in 4 ml of THF and 0.075 ml of TEA was added. After 24 h, 0.1 ml of acetic acid was added. The subsequent workup and isolation were similar to those used to obtain (III). Two reprecipitations from 1 ml of ethanol with ether gave 0.85 g (78%) of powdery (VIII) with <math>[\alpha]_{\text{D}}^{24}-21^{\circ}$ (C 0.91, alcohol), R_f (A) 0.92, and R_f (B) 0.91.

BOC-Ala-[(N^{ε}-Cb)-Lys]₃-OCH₃ (IX). A total of 0.612 g of DCHCD was added to 1.35 g of BOC-Ala-(N^{ε}-Cb)-Lys-COOH in 5 ml of THF at -10°. After 20 min, 2 g of H-[(N^{ε}-Cb)-Lys]₂-OCH₃ trifluoroacetate in 5 ml of THF and 0.416 ml of TEA was added. The conditions used to carry out the reaction, workup, and isolation were similar to those used to obtain (III). The yield of amorphous (IX) was 1.6 g (77%) (chloroform -ether); the product had $[\alpha]_D^{20}$ -21.9° (C 1.24, alcohol), R_f (A) 0.88, R_f (B) 0.91, and R_f (C) 0.62.

 $\frac{\text{BOC}-[(N^{\varepsilon}-\text{Cb})-\text{Lys}]_{3}-\text{Ala-OCH}_{3}(X)}{\text{ml of THF at -5°. After 20 min, 1.35 g of H-(N^{\varepsilon}-\text{Cb})-\text{Lys}-\text{Ala-OCH}_{3} trifluoroacetate in 4 ml of THF and 0.375 ml of TEA was added to this mixture. The reaction was carried out and worked up in the same way as in the preparation of (III) to give 1.5 g (55%) of (X) with mp 79-80° (ethyl acetate - ether), <math>[\alpha]_{D}^{22} - 16.9^{\circ}$ (C 1.85, alcohol), R_{f} (A) 0.95, R_{f} (B) 0.95, and R_{f} (C) 0.65.

BOC-Ala- $[(N^{\varepsilon}-Cb)-Lys]_{3}$ -COOH (XI). A total of 4 ml of 1 N NaOH was added to a solution of 1 g of (X) in 8 ml of acetone, and the mixture was allowed to stand for 2 h and worked up in the same was as in the preparation of (II) to give 0.9 g (90%) of oily (XI) with R_f (B) 0.7.

 $\frac{\text{CF}_{3}\text{COOH} \cdot \text{H}[(\text{N}^{\text{E}}-\text{Cb})-\text{Lys}]_{3}-\text{Ala}-\text{OCH}_{3} \text{ (XII).} A \text{ solution of 1.5 g of (X) in 2.5 ml of absolute trifluoro-acetic acid was allowed to stand for 30 min, 3 ml of absolute benzene was added, and the mixture was vacuum evaporated three times. Two reprecipitations from alcohol with ether gave 1.3 g (89%) of amorphous (XII) with R_f (A) 0.71.$

BOC-Ala-[(N^{ε}-Cb)-Lys]₆-Ala-OCH₃ (XIII). A total of 0.22 g of DCHCD was added to a solution of 1 g of (II) in 3 ml of THF at -10°. After 20 min, 1 g of (XII) in 3 ml of THF and 0.14 ml of TEA was added to the mixture. The reaction was carried out and worked up in the same way as in the preparation of (III). The product was purified by column chromatography with silica gel using alcohol as the eluent to give 1.2 g (62.8%) of powdery (XIII) with $[\alpha]_D^{20} - 40^\circ$ (C 2.00, alcohol), R_f (A) 0.86, and R_f (B) 0.84.

 $\frac{\text{CF}_{3}\text{COOH} \cdot \text{H-}[(\text{NH}^{\text{E}}-\text{Cb})-\text{Lys}]_{2}-\text{OCH}_{3} \text{ (XIV).} \text{ A solution of 2 g of (I) in 4 ml of trifluoroacetic acid was allowed to stand for 45 min, 4 ml of absolute benzene was added, and the mixture was vacuum evaporated. The residue was dissolved in 2 ml of alcohol and precipitated with ether to give 1.8 g (88%) of (XIV) with <math>[\alpha]_{D}^{2^{2}}-2.24^{\circ}$ (C 2.27, alcohol) and R_f (A) 0.71.

 $\frac{\text{BOC-Ala-[(N^{\epsilon}-\text{Cb})-\text{Lys}]_2-\text{OCH}_3 (XV).}{\text{A total of 1.1 g of DCHCD was added to a solution of 2 g of BOC-Ala in 3 ml of THF at -10°. After 20 min, 3.54 g of (XIV) in 10 ml of THF and 0.736 ml of TEA was added to the mixture. The reaction was worked up as in the case of (III) to give 3 g (86%) of oily (XV) with <math>[\alpha]_D^{24}$ -18.8° (C 2.7, alcohol), R_f (A) 0.96, and R_f (B) 0.95.

 $\frac{BOC-[(N^{\varepsilon}-Cb)-Lys]_2-Ala-OCH_3 (XVI).}{(II) in 11 ml of THF at -10^{\circ}. After 20 min, 0.656 g of H-Ala-OCH_3 hydrochloride in 2 ml of THF and 0.65 ml$

of TEA was added to the mixture. The mixture was worked up as in the case of (III) to give 2 g (60%) of (XVI) with $[\alpha]_D^{23}-6.5^\circ$ (C 2.2, alcohol), R_f (A) 0.96, and R_f (B) 0.92.

BOC-Ala- $[(N^{\epsilon}-Cb)-Lys]_2$ -COOH (XVII). (XV) (2.25 g) was dissolved in 16 ml of acetone, 8 ml of 1 N NaOH was added, and the mixture was allowed to stand for 2 h. The mixture was then worked up as in the case of (II) to give 1.75 g (79%) of amorphous (XVII) (reprecipitated from chloroform-ether with hexane) with $R_f(A)$ 0.7 and $R_f(B)$ 0.69.

 $\frac{\text{CF}_{3}\text{COOH} \cdot \text{H} - (\text{N}^{\epsilon} - \text{Cb}) - \text{Lys} - \text{Ala} - \text{OCH}_{3} \text{ (XVIII)}. \text{ (XVI) (1 g) was dissolved in 0.8 ml of} absolute trifluoroacetic acid, and the solution was allowed to stand for 50 min. The mixture was evaporated twice with absolute benzene and reprecipitated twice from alcohol with ether to give 0.76 g (76%) of powdery (XVIII) with R_f (A) 0.89.$

BOC-Ala-[(N^{ε}-Cb)-Lys]₄-Ala-OCH₃ (XIX). A solution of 0.76 g of (XVIII) in 2 ml of THF, 0.152 ml of TEA, and 0.22 ml of DCHCD was added at -5° to 0.75 g of (XVII) in 3 ml of THF. The mixture was worked up as in the case of (III). The product was reprecipitated from ethyl acetate with ether and then triturated with hexane to give 1 g (72.4%) of (XIX) with $[\alpha]_D^{23}$ -30° (C 2.1, alcohol), R_f (A) 0.89, and R_f (B) 0.88.

CONCLUSIONS

Oligopeptides containing lysine and alanine and having the following sequences were obtained: BOC- $[(N^{\varepsilon}-Cb)-Lys]_{6}-OCH_{3}, BOC-[(N^{\varepsilon}-Cb)-Lys]_{8}-OCH_{3}, BOC-Ala-[(N^{\varepsilon}-Cb)-Lys]_{4}-Ala-OCH_{3}, and BOC-Ala-[(N^{\varepsilon}-Cb)-Lys]_{6}-Ala-OCH_{3}.$

LITERATURE CITED

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