pyridine—acetic acid (1:1) at 20°C for 24 h. The polymer was washed by method (a) and after three evaporations with aqueous and then with absolute pyridine the nucleotide material was precipitated with ether. The precipitate was dissolved in concentrated ammonia solution, kept for a day at 20°C and for 3 h at 50°C, and chromatographed on paper.


## SUMMARY

A simple method is proposed for obtaining two kinds of polymeric supports of the polystyrene type for the solid-phase synthesis of oligonucleotides containing 5'-terminal phosphate groups. For both kinds of supports the optimum conditions of adding the first nucleotide and of splitting out the nucleotide material from the polymer have been worked out and the synthesis of a number of di- and trinucleotides of the deoxy series has been performed.


## LITERATURE CITED


1. M. J. Gait and R. C. Sheppard, Nucl. Acid Res., 1135 (1977).
2. H. Koster, A. Pollak, and F. Gramer, Ann. Chem., 946 (1974).
3. Yu. V. Tumanov, M. G. Shlyapnikov, V. K. Potapov, and Z. A. Shabarova, Bioorg. Khim., 1, 1291 (1975).
4. R. L. Letsinger and T. Shimidzu, Bull. Chem. Soc. Jpn., 44, 1673 (1971).
5. K. F. Yip and K. C. Tsou, J. Am. Chem. Soc., 93, 3272 (1971).
6. T. M. Chapman and D. G. Kleid, J. Chem. Soc. Chem. Commun., 5, 193 (1973).
7. H. Koster and K. Heyns, Tetrahedron Lett., 16, 1531 (1972).
8. S. I. Turkin, V. K. Potapov, Z. A. Shabarova, V. F. Zarytova, and D. G. Knorre, Bioorg. Khim., 1, 1430 (1975).
9. E. Ohtsuka, S. Morioka, and M. Ikehara, J. Am. Chem. Soc., 94, 3229 (1972).
10. M. M. Kabachnik, N. G. Timofeeva, M. V. Budanov, V. K. Potapov, and Z. A. Shabarova, Zh. Obshch. Khim., 43, 379 (1973).
11. R. K. Ralph, W. J. Connors, H. Shaller, and H. G. Khorana, J. Am. Chem. Soc., 85, 1983 (1963).
12. H. Shaller and H. G. Khorana, J. Am. Chem. Soc., 85, 3821 (1963).


# SYNTHESIS OF A FRAGMENT OF HISTONE H2B WITH THE AMINO ACID SEQUENCE 30-39 AND ITS $Lys^{31,33}$ ANALOG


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UDC 547.962:541.61


Recently, great attention has been devoted to the study of the structure and function of histones, which affect the template activity of DNA. It is assumed that DNA is capable of transmitting genetic information only if it is not bound to histones.

On the phosphorylation of the histone fraction H2B, of the fairly large number of serine residues only $Ser^{14}$ and $Ser^{36}$ are phosphorylated by phosphokinase. This shows a high specificity of the elements of the primary structure of the histones in relation to such processes as phosphorylation, acetylation, etc. But the process of phosphorylation of histones is directly connected with the stability of the DNP complexes and, consequently, with the transmission of genetic information. Upon what factors does such a high specificity of the enzyme depend? Is it explained by the immediate environment of the serine residue or does it depend on the secondary structure of the molecule as a whole?

In order to answer these questions, we have synthesized a fragment of the histone H2B with the sequence Lys-Arg-Ser-Arg-Lys-Glu-Ser-Tyr-Ser-Val and its $Lys^{31,33}$ analog. This fragment is interesting because, in addition to $Ser^{36}$, it contains two serine residues which do not undergo phosphorylation by phosphokinase in the natural material.

Fragment 30-39 was synthesized by condensing two pentapeptides by the carbodiimide method with the addition of N-hydroxysuccinimide. The synthesis of its $Lys^{31,33}$ analog was also


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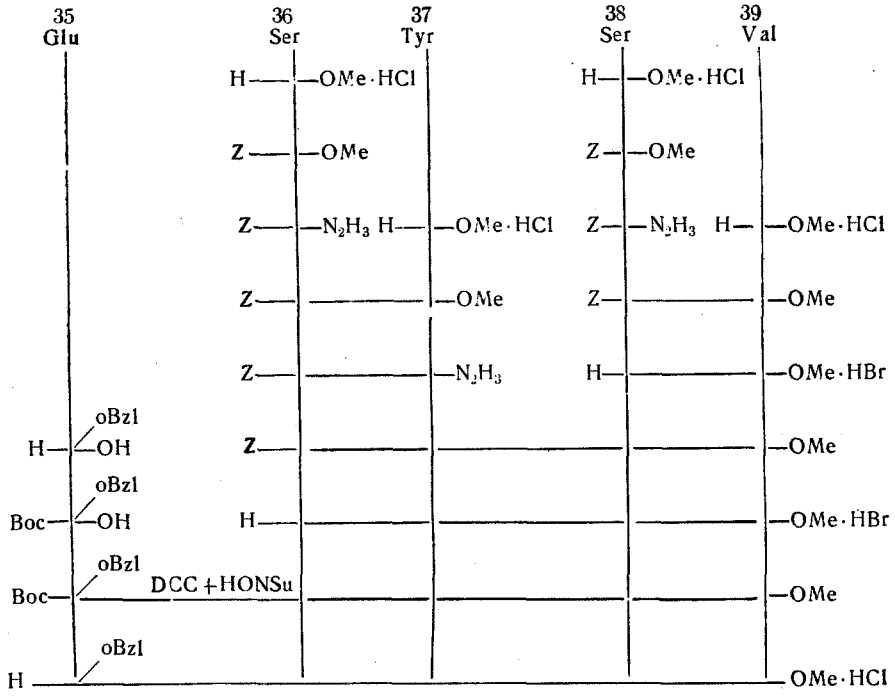



Scheme 1

carried out not by condensing two pentapeptides, but by a modified azide method [1] using the addition of N-hydroxysuccinimide [2, 3], (Scheme 3), since N-hydroxysuccinimide esters formed via azides lead to the products of block condensation more effectively than the azides themselves. The N-hydroxysuccinimide esters obtained as intermediates can be removed by washing with hot ethanol.

As the protective groups we used the benzyloxycarbonyl (Z) and tert-butoxycarbonyl (Boc) groups. The protective groups were eliminated by the action of hydrogen halides in various absolute solvents and by anhydrous HF in anisole at the support stage of the synthesis. The final products were purified by chromatography and rechromatography on Sephadex G-15. The yields and constants of the compounds obtained are given in Table 1.

## EXPERIMENTAL

All the amino acids were of the L form. The individuality of the compounds obtained was checked by TLC on 75 × 25 mm plates with a fixed layer [300 mesh, silica gel—gypsum—water (1:0.25:3.1)] in systems: 2) sec-butanol—3% $NH_4OH$ (100:44); 3) chloroform—methanol (8:1); 4) ethanol—benzene (2:0.3); and 5) benzene—ethanol (2:0.3), and also by electrophoresis (600 V and 7 mA) in 30% $CH_3COOH$ and 0.2 M $CH_3COOH$ buffers.

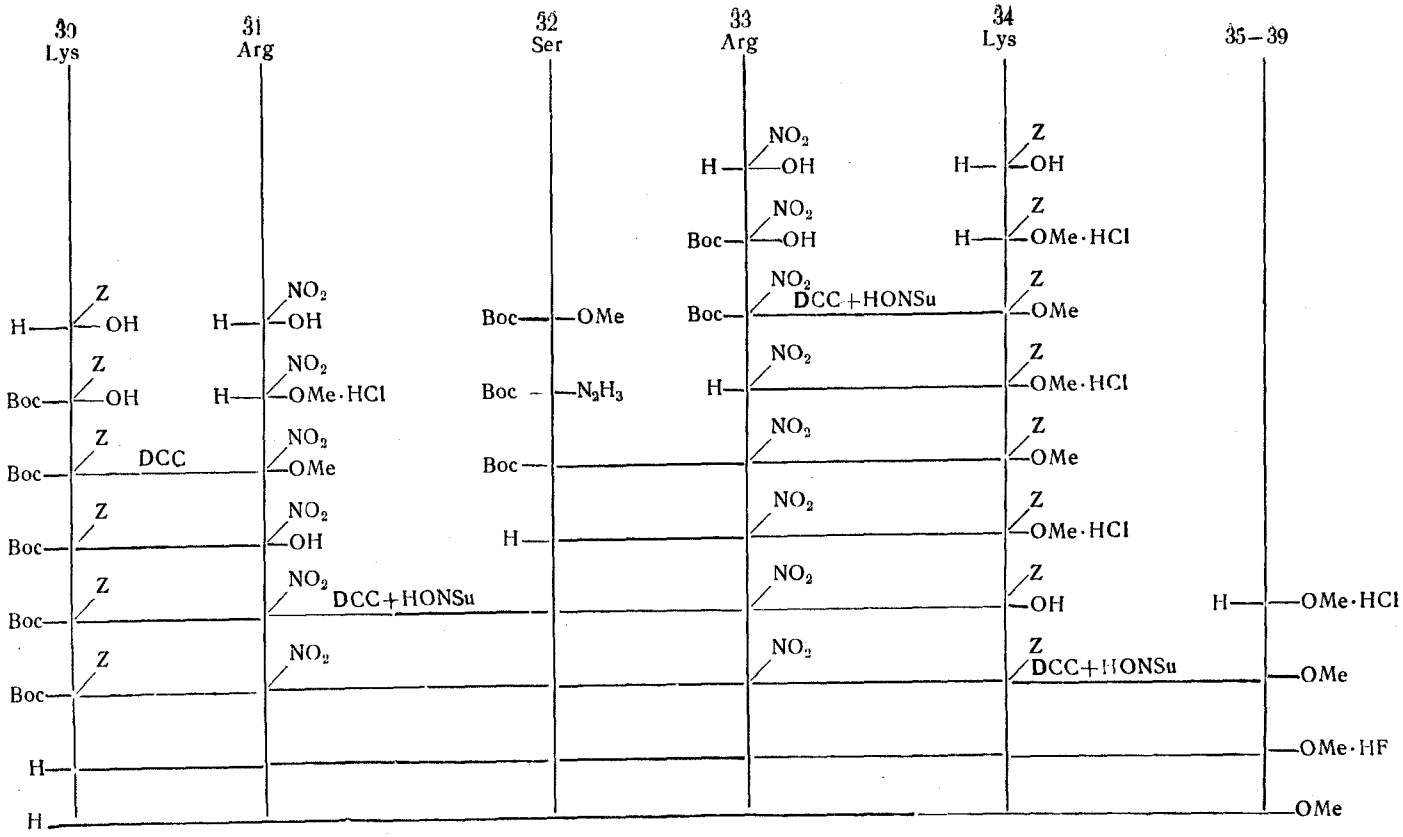
The protected peptides were purified on a column (100 × 1.5 cm) of Sephadex LH-20, V = 6 ml/h.

The angles of rotation were determined on an AI-EPL automatic polarimeter. The protected amino acids, and also their methyl and benzyl ethers and hydrazides, were obtained by standard methods [4].

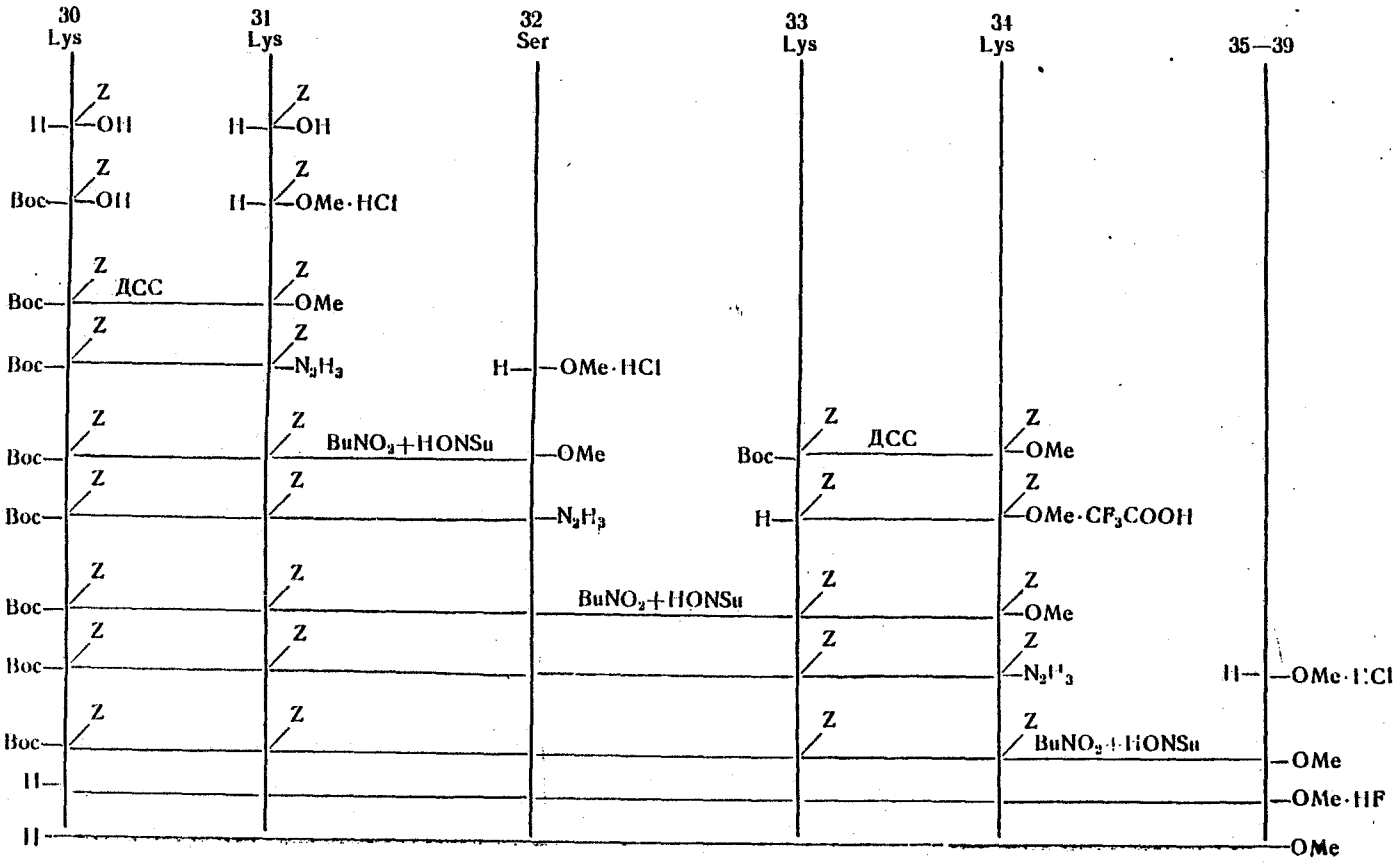
After each stage of condensation in the production of the protected peptides, the reaction mixture was washed with 10% citric acid, $NaHCO_3$ solution, and with water, and was dried over $Na_2SO_4$, and the solvent was evaporated off.

Preparation of the Hydrides of the Protected Amino Acids and Peptides. A solution of 1 mmole of a protected methyl ester of an amino acid or peptide in absolute $C_2H_5OH$ was treated with 10 mmole of hydrazine hydrate. The mixture was kept at room temperature for 24 h, and the precipitate that had deposited was filtered off, washed with water, $C_2H_5OH$, and ether, dried, and recrystallized from hot ethanol or dioxane.

Preparation of Compounds (I), (II), and (IV). The hydrazide of a protected amino acid or peptide (2 mmole) was dissolved at −10°C in the minimum amount of ice-cooled 1 N HCl, and the calculated amount of 2 M $NaNO_2$ was added. After 5 min, the azide that had deposited was


30 Lys
31 Arg
32 Ser
33 Arg
34 Lys
35–39
H
Z
OH
Boc
NO2
OMe·HCl
DCC
DCC+HONSu
OMe
N2H3
OMe·HF

Scheme 2


30 Lys
31 Lys
32 Ser
33 Lys
34 Lys
35—39
H—
Z
OH
Boc—
OMe·HCl
ДСС
OMe
N2H3
BuNO2+HONSu
OMe·CF3COOH
OMe·HF

Scheme 3

TABLE 1

| Compound | Yield, % | mp, °C | $[\alpha]_D^{15}$, deg (DMFA) | $R_f$ systems |
|---|---|---|---|---|
| I. Z-Ser-Tyr-OMe | 70 | | | 0,56 (2)<br>0,66 (3) |
| II. Z-Ser-Val-OMe | 98 | 60 Ether—hexane | +7,1 (*c* 1,4) | 0,56 (2)<br>0,58 (5) |
| III. Z-Ser-Tyr-$N_2H_3$ | 74 | 214—215 Dioxane | | 0,3 (3) |
| IV. Z-Ser-Tyr-Ser-Val-OMe | 98 | 187—189 $C_2H_5OH$ | +12,5 (*c* 0,96) | 0,83 (2)<br>0,37 (5) |
| V. H-Ser-Tyr-Ser-Val-OMe·HBr | 90 | 140—141<br>$C_2H_5OH$—ether | | 0,66 (2)<br>0,2 (3) |
| VI. Boc-Glu(OBzl)-Ser-Tyr-Ser-Val-OMe | 69 | 196—198<br>$C_2H_5OH$ | +3 (*c* 1,46) | 0,92 (2)<br>0,66 (3) |
| VII. H-Glu(OBzl)-Ser-Tyr-Ser-Val-OMe·HCl | 88 | 168—170<br>$C_2H_5OH$—ethyl acetate | | 0,58 (2)<br>0,79 (4) |
| VIII. Boc-Lys(Z)-Arg($NO_2$)-OMe | 80 | | −5 (*c* 1,2) | 0,95 (2) |
| IX. Boc-Lys(Z)-Arg($NO_2$)-OH | 90 | | −13,72<br>(*c* 0,9; CHOH) | 0,7 (2) |
| X. Boc-Arg($NO_2$)-Lys(Z)-OMe | 85 | | −5,0 (*c* 1,2) | 0,94 (2)<br>0,91 (3) |
| XI. H-Arg($NO_2$)-Lys(Z)-OMe·HCl | 94,5 | 162—163<br>$C_2H_5OH$ | | 0,63 (2)<br>0,33 (3) |
| XII. Boc-Ser-Arg($NO_2$)-Lys(Z)-OMe | 89 | 105—106<br>$C_2H_5OH$—ether | −6 (*c* 1,05) | 0,81 (2)<br>0,61 (3) |
| XIII. Boc-Lys(Z)-Arg($NO_2$)-Ser-Arg($NO_2$)-Lys(Z)-OMe | 80 | 154—156<br>$C_2H_5OH$ | −9,0 (*c* 1,0) | 0,8 (2)<br>0,32 (3) |
| XIV. Boc-Lys(Z)-Arg($NO_2$)-Ser-Arg($NO_2$)-Lys(Z)-OH | 80 | 113—114<br>$C_2H_5OH$—ether | | 0,7 (2)<br>0,12 (3) |
| XV. Boc-Lys(Z)-Lys(Z)-$N_2H_3$ | 97,5 | 147—148<br>$C_2H_5OH$ | −20,9 (*c* 1,34; 50% $CH_3COOH$) | 0,36 (5) |
| XVI. Boc-Lys(Z)-Lys(Z)-Ser-OMe | 40 | 157—158<br>$C_2H_5OH$—ether | −10,0 (*c* 1,05) | 0,91 (2)<br>0,53 (5) |
| XVII. Boc-Lys(Z)-Lys(Z)-Ser-$N_2H_3$ | 83 | 163 $C_2H_5OH$ | | 0,18 (5) |
| XVIII. Boc-Lys(Z)-Lys(Z)-Ser-Lys(Z)-Lys(Z)-OMe | 85,8 | 159—160<br>$C_2H_5OH$—ether | −12,3 (*c* 1,3) | 0,98 (3)<br>0,5 (5) |
| XIX. Boc-Lys(Z)-Lys(Z)-Ser-Lys(Z)-Lys(Z)-Glu(OBzl)-Ser-Tyr-Ser-Val-OMe | 60 | 214—216<br>$C_2H_5OH$ | −35,4 (*c* 1,0) | 0,53 (2) |
| XX. Boc-Lys(Z)-Lys-(Z)-Ser-Lys(Z)-Lys(Z)-$N_2H_3$ | 75 | 166—169 | | |
| XXI. Boc-Lys(Z)-Arg($NO_2$)-Ser-Arg($NO_2$)-Lys(Z)-Glu(OBzl)-Ser-Tyr-Ser-Val-OMe | 58 | 207 $C_2H_5OH$ | −30,0 (*c* 1,0) | 0,81 (2)<br>0,79 (3) |

transferred into ethyl acetate, and the solution was washed with 1 M $K_2CO_3$ and with saturated NaCl solution and was dried over $Na_2SO_4$. The solution was filtered into a flask containing 2 mmole of the second component dissolved in DMFA in the presence of 2 mmole of methylmorpholine. All the operations were carried out in the cold. Then the reaction mixture was left at 0°C for 68 h and was worked up by the usual method. Recrystallization was carried out from the solvents shown in Table 1.

Preparation of Compounds (XII), (XVI), (XVIII), (XX), and (XXI). A solution of 2 moles of compound (XV), (XVII), or (XIX) in 2 ml of DMFA was cooled to −30°C and was acidified with 10 N HCl in dioxane to pH 2.5. Then a 30% solution of butyl nitrite (4 mmole) [5] in DMFA cooled to −30°C was added. The mixture was stirred at 30°C for 45 min, and then 3 mmole of N-hydroxysuccinimide was added, the temperature was lowered to −40°C, and the pH was brought to 6 with TEA. The solution was kept at 0°C for 15 h and was then again cooled to −40°C and 2 mmole of the second component in DMFA was added. The pH of the solution was brought to 7.5 with TEA. Then the reaction mixture was kept at 0°C for 48 h and at room temperature for

24 h, after which it was poured cold into 50 ml of 10% citric acid. The precipitate that deposited was filtered off, washed with water, and recrystallized from $C_2H_5OH$. The compound (XX) was purified on a column of silica gel in the chloroform–methanol (8:1) system.

Preparation of Compounds (VI), (VIII), (X), (XIII), and (XXI). An N-protected amino acid or compound (IX) or (XIV) (1 mmole) was dissolved in absolute DMFA at −10°C and the solution was stirred and was treated with 1 mmole of DCC and 1 mmole of N-hyroxysuccinimide. The reaction mixture was stirred at −20°C for 5 h, at 0°C for 1-4 days, and at room temperature 1-2 days. Then the filtrate was evaporated. Compounds (VI), (VIII), (X), and (XIII) were worked up in the usual way in ethyl acetate. Compound (XXI) was recrystallized from hot $C_2H_5OH$ and was purified on a column of Sephadex LH-20.

Elimination of the Protective Groups. The benzyloxycarbonyl group was removed from compounds (II) and (IV) by the action of a current of dry HBr on a solution of the protected peptide ester in absolute $CF_3COOH$ for 20 min. Then the solvent was distilled off with $C_6H_6$, and the residue was treated with ether. Recrystallization was carried out from the solvents shown in Table 1.

The Boc group was removed from compounds (VI), (X), and (XII) by the action of 4 N HCl in methanol for 20-25 min. After the solvent had been distilled off, the peptide was precipitated with ether and was recrystallized (see Table 1).

All the protective groups were removed from compounds (XX) and (XXI) by anhydrous HF in anisole. The HF was eliminated on Amberlite IRA-401($OH^-$). Then the solution was freeze-dried and the compounds were purified on a column of Sephadex G-15.

## SUMMARY

An oligopeptide with the amino acid sequence 30-39 of histone H2B (Lys-Arg-Ser-Arg-Lys-Glu-Ser-Tyr-Ser-Val) and its $Lys^{31,33}$ analog have been synthesized.

## LITERATURE CITED

1. J. Honzl and J. Rudinger, Collect. Czech. Chem. Commun., 26, 2333 (1961).
2. V. F. Krivtsov, L. A. Kolomeitseva, T. V. Andrusenko, N. A. Kocharova, S. K. Girin, L. V. Ivanovskaya, E. N. Voluiskaya, S. P. Krasnoshchekova, and Yu. P. Shvachkin, Abstracts of Lectures at the Third All-Union Symposium on Peptide and Protein Chemistry [in Russian], Kiev (1974), p. 74.
3. GFR Patent No. 1,918,549 (1969); Chem. Abstr., 72, 32,215 (1970).
4. E. Schroeder and K. Lübke, The Peptides, Academic Press, New York (1965).
5. W. Noyes, Organic Syntheses [Russian translation], Moscow (1949), p. 131.