9. Houben-Weyl, Methoden der Organischen Chemie, Vol. 4, Georg Thieme, Leipzig (1924), p. 818.
10. R. Boyce, W. S. Murphy, and K. P. Klein, J. Chem. Soc. Perkin Trans., 1, 1292 (1972).
11. P. Girard, J. L. Namy, and H. B. Kagan, J. Am. Chem. Soc., 102, 2693 (1980).
12. J. E. McMurry, M. G. Silvestri, M. P. Fleming, T. Hoz, and M. W. Grayston, J. Org. Chem., 43, 3249 (1978).
13. S. W. Ela and D. J. Cram, J. Am. Chem. Soc., 88, 5777 (1966).
14. H. Gilman and J. W. Morton, Organic Reactions, Vol. 8, Wiley, New York, Chapman and Hall, London (1954), p. 286.
15. J. Nelles and R. Streicher, Z. Chem., 3, 188 (1963).
16. V. A. Rabinovich and Z. Ya. Khavin, Short Handbook of Chemistry [in Russian], 2nd ed., Khimiya, Leningrad (1978).

AMINOACYL DERIVATIVES OF NUCLEOSIDES, NUCLEOTIDES, AND POLYNUCLEOTIDES

17.* POSSIBLE USE OF N,N'-THIOCARBONYLDIIMIDAZOLE FOR SYNTHESIS OF 2'(3')-O-AMINOACYLNUCLEOTIDES

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The method of synthesis of 2'(3')-O-aminoacylnucleotides using N,N'-carbonyldiimidazole for the activation of the carboxylic group of the amino acids has been described in several papers [1-3]. The disadvantage of this method is the high sensitivity of N,N'-carbonyldiimidazole to moisture [4], so that the preparation of imidazolides of amino acids must be carried out in absolute solvents. The selective aminoacylation of the nucleotides also proceeds in an aqueous–organic medium with a water content of > 50% [5]. As the result, the amino acid imidazolides not only react with nucleotides but are also subjected to hydrolysis, and therefore 4 to 10 moles of the amino acid imidazolide per mole of nucleotide are introduced into the reaction [4]. There is only one paper in the literature on the possible use of diethyl phosphate imidazolide as an activating agent for the synthesis of 2'(3')-O-aminoacyl nucleotides in an aqueous medium [6].

Our attention was drawn to N,N'-thiocarbonyldiimidazole, which is used for the activation of the carboxyl group of amino acids in the peptide synthesis [7], as a possible condensing agent. Its half-decomposition period in an aqueous–organic medium is several hours [8].

In the present work we report on the possible use, in principle, of N,N'-thiocarbonyldiimidazole for the synthesis of 2'(3')-O-aminoacylnucleotides in an aqueous medium. The synthesis was carried out by starting from an imidazolide of N-tert-butyloxycarbonylalanine BOC-Ala-Im (III), obtained from BOC-Ala-OH (I) and N,N,-thiocarbonyldiimidazole (II) and nucleoside 5'-phosphates (IV) (scheme 1). The main studies on the selection of the condensation conditions were carried for adenosine 5'-phosphate (pA) and imidazolide (III). Compound (I) was condensed with (II) and (III) with (IV) in water at ~20°C. The initial nucleotides were introduced into the reaction in the form of a disodium or diammonium salt. Determination of the yields of 2'(3')-O-BOC-alanylnucleotides depending on the time of reaction showed that the highest yields are obtained after 2.5 h and are 15-20% (Table 1). Imidazolide (III) was taken in excess with respect to nucleotide (IV). The above yields were obtained at a ratio (III) : (IV) = 10:1 (moles). Further increase in this ratio does not influence the yield of 2'(3')-O-BOC-alanylnucleotides (V).

The isolation, purification, and verification of the structure of 2'(3')-O-BOC-alanyl-pA (V), 2'(3')-O-alanyl-pA (VI) were carried out by chromatography, electrophoresis, and UV spectroscopy (Table 2).

The absorption maxima in the UV spectra of the initial compounds (IV) and esters (V) and (VI) are located at the same wavelength, while the electrophoretic mobility of (VI) at pH 2.5 corresponds to the number of charges on it, which indicates the absence of acylation of the heterocyclic bases.

* Article 16, see [1].

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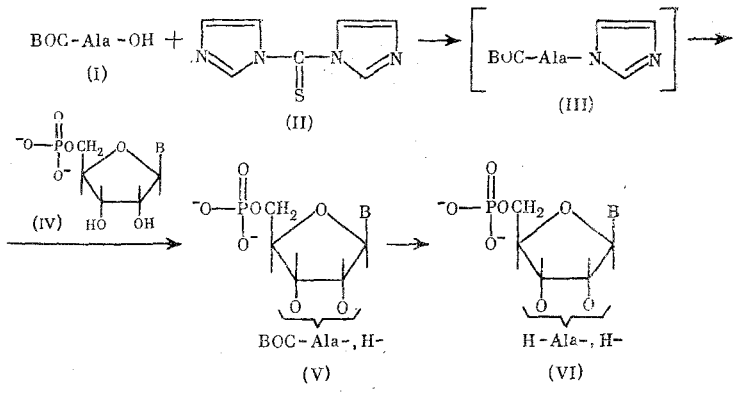
TABLE 1. Synthesis of 2'(3')-O-BOC-Aminoacylnucleotides (V) in Aqueous Medium Starting from BOC-Alanine (I) and Nucleotide (IV)

Initial compounds	Yield of BOC-alanyl-nucleotides (V), %		Initial compounds	Yield of BOC-alanyl-nucleotides (V), %	
nucleotide (IV)	per nucleotide introduced into reaction	per reacted nucleotide	nucleotide (IV)	per nucleotide introduced into reaction	per reacted nucleotide
pU	30	85	pA	26	84
pG	27	80	pC	15	72

TABLE 2. Values of R_f during Chromatography and E_f during Paper Electrophoresis

Compound	Paper chromatography, R_f in systems			Paper electrophoresis in 6% AcOH, 800 V, 1 h	
				mobility with reference to	
	1	2	3	Gly	His
pA-(BOC-Ala)	0,52	0,48	0,33	–	–
pA-(H-Ala)	0,33	0,54	0,87	0,85	0,59
pG-(BOC-Ala)	0,61	0,35	0,59	–	–
pG-(H-Ala)	0,36	0,44	–	0,73	0,45
pU-(BOC-Ala)	0,34	0,40	0,45	–	–
pU-(H-Ala)	0,29	0,38	0,54	0,75	0,40
pC-(BOC-Ala)	0,57	0,39	0,75	–	–
pC-(H-Ala)	0,47	0,44	–	0,79	0,42

Scheme 1



B = residues of adenine (A), guanine (G), uracil (U), and cytosine (C)

An alternative synthesis from pA and imidazolide (III) by a known method [3] serves as a final confirmation of the structure of (V). In all cases, only (V) and initial (IV) were detected among the reaction products.

EXPERIMENTAL

For preparative and analytical purposes, descending chromatography was used on "Leningradskaya" paper brand B in the systems: n-butanol–water–CH_3COOH = 5:3:3 (1); saturated solution of $(NH_4)_2SO_4$–isopropanol–water = 79:2:17 (2), and $(CH_3)_2CHCOOH$–CH_3COOH–water = 100:50:1 (3). The R_f values are listed in Table 2. The electrophoresis was carried out on "Leningradskaya" paper brand M in 6% AcOH at pH 2.5, voltage 800 V, current density 22 V/cm, in the course of 1-2 h. The E_f values, given with reference to glycine and histidine, are listed in Table 2.

2'(3')-O-tert-Butyloxycarbonyl-L-alanaylnucleotides. N,N'-Thiocarbonyldiimidazole, 34.3 mg (0.187 mmole, mp 105-106°C), was added to a solution of 35.4 mg of BOC-L-alanine (0.187 mmole, mp 72-73°C) in 0.1 ml of distilled water, and the mixture was stirred for 30 min at 20-22°C. The imidazolide formed was added to a solution of 0.019 mmole of nucleotide (IV) in 0.4 ml of water, and the mixture was stirred for 2.5 h at 20-22°C.

Compounds (IV) and (VI) were isolated and identified according to [2]. The experimental results are listed in Table 1.

CONCLUSIONS

It was shown that it is possible to use N,N'-thiocarbonyldiimidazole for the synthesis of 2'(3')-O-aminoacyl derivatives of nucleotides in an aqueous medium.

LITERATURE CITED

1. L. A. Aleksandrova, T. M. Kutuzova, A. A. Kraevskii, M. K. Kukhanova, and B. P. Gottikh, Izv. Akad. Nauk SSSR, Ser. Khim., 647 (1977).
2. B. P. Gottikh, A. A. Kraevskii, and P. P. Purygin, Izv. Akad. Nauk SSSR, Ser. Khim., 2529 (1971).
3. B. P. Gottich, A. A. Krayevsky, N. B. Tarussova, P. P. Purygin, and T. L. Tsilevish, Tetrahedron, 26, 4419 (1970).
4. A. A. Kraevskii, P. P. Purygin, L. N. Rudzite, Z. S. Belova, and B. P. Gottikh, Izv. Akad. Nauk SSSR, Ser. Khim., 378 (1968).
5. B. P. Gottikh, A. A. Kraevskii, P. P. Purygin, T. L. Tsilevich, Z. N. Belova, and L. N. Rudzite, Izv. Akad. Nauk SSSR, Ser. Khim., 2571 (1967).
6. A. A. Kraevskii, E. V. Degtyarev, V. P. Gottikh, and L. N. Nikolenko, Izv. Akad. Nauk SSSR, Ser. Khim., 1730 (1971).
7. E. Corey and A. Winter, J. Am. Chem. Soc., 89, 2677 (1963).
8. H. A. Staab, Angew. Chem., 74, 402 (1962).