

SYNTHESIS OF ADENOSINE-5'-DIPHOSPHATE USING
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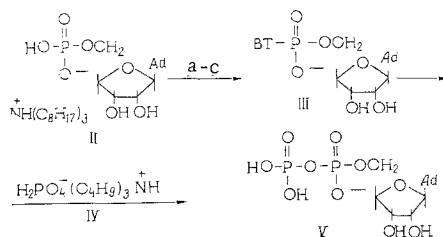
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It has already been found [1-3] that N,N'-carbonyldiimidazole and N,N'-thionyl-diimidazole can be successfully used for the preparation of nucleoside polyphosphates. Information on the diazolides based on benzotriazole is not available in the literature, and it is not improbable that these reagents can be used for the synthesis of nucleoside polyphosphates and their analogs with definite advantages.

In the present work, we report a new method of synthesis of adenosine 5'-diphosphate using N,N'-acyl-dibenzotriazoles BT-X-BT (Ia-c) (BT = benzotriazolyl; a) X = CO, b) X = CS, c) X = SO) for the activation of the phosphate group of the nucleotide.

For a comparative evaluation of the reactivity of Ia-c, we studied the rate of decomposition of these compounds by the action of water in a tetrahydrofuran solution. The kinetic studies were carried out by a spectrophotometric method under the conditions of a 30,000-fold excess of water. The observed kinetic curves are well described by a first-order equation. As expected, the reactivity of the thionyl compounds in the reaction is considerably higher than that of the thiocarbonyl and carbonyl compounds, as in the case of N,N'-acyldiimidazoles [4]. The observed rate constant and time of hydrolysis of half the amount of Ia-c was calculated by the method described in [5]. The results are shown in Table 1.

We prepared adenosine-5'-diphosphate according to the following scheme:



Compounds Ia-c were condensed with the tri-n-octylammonium salt of adenosine-5'-monophosphate (II) in a solution of absolute DMFA at room temperature or with heating. The rate of formation of the activated derivative, the benzotriazolide of adenosine-5'-monophosphate (III), was directly dependent on the reactivity of the initial diazolides (Ia-c) (Table 2).

The formation of (III) was controlled by TLC in an isopropanol-ammonia-water (7:1:2) system.

The purity of III was determined by chromatography and paper electrophoresis in systems 1-3 (see below).

The absorption maxima in the UV light of the initial nucleotide II and the nucleotide obtained (III) are situated at the same wavelength, which indicates that the amino group of the purine base (II) was not acylated.

The electrophoretic mobility of III agrees well with the number of charges in this compound (Table 3). Moreover, the positive reaction for cis-glycol grouping [6] serves as an additional confirmation of the structure of III.

Adenosine-5'-diphosphate (V) was obtained by condensation of III with the tri-n-butylammonium salt of phosphoric acid (IV). The reaction was carried out at room temperature in absolute DMFA for 48 h. The

TABLE 1. Rates of Hydrolysis and Half-Conversion Periods of N,N'-Acyldibenzotriazoles (Ia-c)

Compound	Hydrolysis (pH 7.2, 25°C)	
	$K \cdot 10^2, \text{ min}^{-1}$	$\tau_{1/2}, \text{ min}$
Ic	7.1	9.76
Ib	0.5	138.50
Ia	1.9	35.47

TABLE 2. Synthesis of N,N'-Acyldibenzotriazoles Ia-c

Initial compounds*		Time of reaction, h	Reaction temperature, °C	Yield, %
HN ⁺ (C ₈ H ₁₇) ₃ — salt [†]	diazolides			
II	Ia	6	20	95
II	Ia	1	20	70
II	Ib	5	60	96
II	Ic	2	20	98

* In all the experiments, the ratio of the nucleotide to diazolid (in moles) is 1:2.

† Absolute DMFA was used as solvent.

TABLE 3. Chromatographic (R_f) and Electrophoretic (E_f) Characteristics of V and Intermediates in Its Synthesis

Compound	R _f in system			E _f ^{PA} at pH	
	1	2	3	3.8	7.5
III	0.35	0.52	0.33	0.8	0.62
II	0.15	0.21	0.31	1.0	1.0
V	0.11	0.08	0.25	1.96	—
VI	0.13	—	—	1.16	—
VII	0.80	0.57	0.70	—	—

Note. E_f^{PA} is the electrophoretic mobility of the reaction product in relation to the electrophoretic mobility of AMP.

course of the reaction was controlled by TLC. According to the chromatographic data, the reaction product consisted of the following compounds: V; III, II; P¹, P²-diadenosine-5'-pyrophosphate (VI) and benzotriazole (VII).

The structure and the purity of V were confirmed by chromatography, electrophoresis and comparison of the results obtained with the literature data [1, 7] (see Table 3). The yield of V was 40-45% with respect to the amount of III used in the reaction.

EXPERIMENTAL

The IR spectra were run on the Spectromom-2000 apparatus in mineral oil. The UV spectra were run on the Specord spectrophotometer in dry tetrahydrofuran, and the kinetic studies were carried out on the SF-26 and Varian-Chart spectrophotometers [the latter from LKB (Sweden)].

The electrophoretic characteristics were obtained on the REF-1 apparatus; the voltage gradient was 24 V/cm, time 1½ h, using 0.1 M acetate buffer at pH 3.8 and 0.05 M ammonium acetate at pH 7.5. The chromatog-

raphy was carried out on FN-11 paper in systems 1) isopropanol-ammonia-water, 7:1:2; 2) butanol-water-acetic acid, 5:3:2; 3) n-propanol-water-acetic acid, 20:12:3. The TLC was carried out on the Silufol plates UV-254 (CSSR) in system 1.

N,N'-Acyldibenzotriazoles (Ia-c). These compounds were synthesized by the method described in [8]. The kinetics of hydrolysis of Ia-c were studied spectrophotometrically under the conditions of a 30,000-fold excess of water. The kinetic curves observed were described well by a first-order equation; the observed rate constants of hydrolysis and the half-conversion periods of Ia-c are listed in Table 1.

Adenosine-5'-monophosphate benzotriazolide (III). A solution of 0.212 g of tri-n-octylammonium salt of II in 2 ml of absolute DMFA is added at room temperature to a solution of 0.2 g of Ia in 2 ml of absolute DMFA, and the mixture is stirred for 6 h.

Compound III is isolated by preparative TLC in a crystalline state in system 1, R_f 0.45. UV spectrum: λ_{\max} 262 nm, λ_{\min} 230 nm.

Adenosine-5'-diphosphate (V). A solution of 0.75 mM of the mono-tributylammonium salt of phosphoric acid in 7.5 ml of absolute DMFA is added to a solution of 0.15 mmole of III in 2.5 ml of absolute DMFA (preliminarily treated with 1.2 mmole of methanol to remove excess of Ia-c).

The reaction mixture is stirred at room temperature for 48 h. Compound V is isolated by preparative paper chromatography in system 1. The yield of V was 40-45% with respect to compound III used in the reaction. The chromatographic and electrophoretic data are listed in Table 3.

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