

p-Nitrophenyl Phosphate as a Phosphorylating Reagent in Nucleotide Synthesis

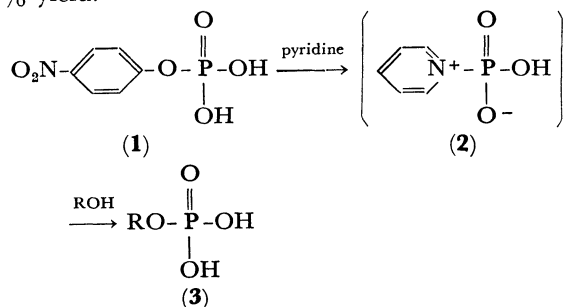
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It is known that tri-substituted esters of phosphoric acid such as triethyl phosphate are transesterified into the corresponding trialkyl phosphate by treatment with alcohol or sodium alkoxide.¹⁾ In the case of mono-substituted phosphates, however, the interchange reaction proceeds very sluggishly and therefore can not be applied to the syntheses of desired monoalkyl phosphates. It was found that pyridine was a remarkably effective solvent for the ester-interchange reaction of *p*-nitrophenyl phosphate (**1**) with alcohols.²⁾ The reaction is of particular interest since it is an example of ester-interchange reaction, probably proceeding through a reactive *N*-phosphopyridinium intermediate (**2**) towards alcohols.

Phosphorylation of nucleosides by the use of **1** was investigated. When 2',3'-*O*-isopropylidene adenosine (0.3 mmole) was allowed to react with **1** (0.6 mmole) in dry pyridine (0.3 ml) at 110°C for two hours, 2',3'-*O*-isopropylidene adenosine 5'-phosphate was isolated by using DEAE cellulose column chromatography. After removal of the protecting group by treating 30% formic acid, adenosine 5'-phosphate was obtained in 85% yield.



Similarly, other nucleotides were obtained in high yields as shown in Table 1.

It is noted that the yields of nucleotides depend on

the concentrations and the molar ratios of **1** to nucleosides. When a large excess of pyridine was employed, nucleoside polyphosphates were formed. 2',3'-*O*-Isopropylidene adenosine (0.05 mmol) was treated with **1** (0.25 mmol) in 1.0 ml of dry pyridine at 110°C for four hours. After removal of the protecting group, adenosine 5'-diphosphate (ADP) and adenosine 5'-triphosphate (ATP) were obtained in 32% and 33% yields, respectively, along with adenosine 5'-phosphate (35%). These polyphosphates were isolated by means of DEAE cellulose column chromatography.

Phosphorylation of unprotected free nucleoside by the present method was attempted. When adenosine (0.1 mmol) was treated with **1** (0.7 mmol) in dry pyridine (3.0 ml) in the presence of tri-*n*-butylamine, adenosine 2',3'-cyclic phosphate (**4**) and adenosine 2'(3'),5'-diphosphate (**5**) were obtained in 43% and 39% yields, respectively. The cyclic phosphate (**4**) was converted with 0.1 *N* hydrochloric acid into adenosine 2'(3')-phosphate in quantitative yield. When **4** (0.1 mmol) was further treated with **1** (0.7 mmol) in dry pyridine (3.0 ml), adenosine 2',3'-cyclic phosphate 5'-phosphate (**6**) was obtained in 57% yield. Data on the chromatographic and spectral properties of these compounds (**4**, **5** and **6**) agree with those of authentic samples.^{3,4)}

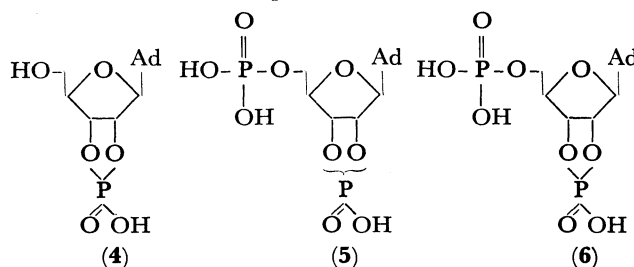


TABLE 1. PREPARATION OF NUCLEOTIDES

Nucleotide ^{a)}	Yield (%)	<i>R_f</i> value ^{c)}	UV Spectra (pH 2)	
			$\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (m μ)	$\lambda_{\text{min}}^{\text{H}_2\text{O}}$ (m μ)
Adenosine 5'-phosphate	85	0.14	257	231
Guanosine 5'-phosphate	80	0.09	256	228
Uridine 5'-phosphate	82	0.16	262	231
Cytidine 5'-phosphate	70	0.16	279	242
4-S-Thiouridine 5'-phosphate	52	0.17	331	285
Thymidine 3'-phosphate ^{b)}	43	0.15	267	235

a) 2',3'-*O*-Isopropylidene nucleoside was used as a starting material except in the case of thymidine derivative.

b) 5'-*O*-Trityl thymidine was used.

c) Paper chromatography was performed by descending technique using Toyo Roshi No. 51 paper. Solvent system used: isopropyl alcohol, concentrated ammonium hydroxide, water (7 : 1 : 2 v/v).

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4) J. G. Moffatt and H. G. Khorana, *ibid.*, 663 (1961).