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A NOVEL METHOD FOR PHOSPHORYLATION OF NUCLEOSIDES TO 5′-NUCLEOTIDES

Masaharu Yoshikawa, Tetsuya Kato and Tadao Takenishi
Central Research Laboratories, Ajinomoto Company Inc.,
Suzuki-cho, Kawasaki, Japan

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Phosphoryl chloride has not been widely used in the synthesis of 5′-nucleotides because of the very low yields of desired products and the lack of specificity of the reaction.

In a previous paper(1), however, it has been reported that treatment of 2′,3′-O-isopropylidene nucleosides with an excess of phosphoryl chloride in the absence of pyridine reduces the amount of by-products and improves greately the yields of the corresponding 5′-phosphorodichloridates which are readily hydrolyzed by simple treatment with water to give 5′-nucleotides in good yields.

This communication reports the results of an approach to a selective phosphorylation of nucleosides to 5′-nucleotides in a one-step procedure. The syntheses of nucleotides with previously known phosphorochloridates involve several complicated processes. Direct use of phosphoryl chloride in phosphorylation may be more attractive.

During the course of an investigation on accelerators for the phosphorylation , it was found that the use of trialkyl phosphate highly facilitated the phosphorylation with phosphoryl chloride. In addition, 2′,3′-O-isopropylidene nucleosides are moderately soluble in anhydrous trialkyl phosphates, such as trimethyl and triethyl phosphates. These facts suggest the potentiality of these esters as useful solvents for the phosphorylation. When a 2′,3′-O-isopropylidene nucleoside was added to a cold mixture of a trialkyl phosphate and phosphoryl chloride with stirring, it was converted smoothly into the corresponding 5′-phosphorodichloridate in nearly quantitative yield. 5′-Nucleotide was obtained by rapid hydrolysis of the chloridate group followed by removal of the isopropylidene group at 70°C. Results are listed in Table 1.

TABLE I

Phosphorylation of 2′,3′-O-Isopropylidene Nucleoside

2′,3′-O-Isopropylidene nucleoside	Solvent	Time (hr)	Yield of 5′-nucleotide (mole %)
inosine	$(MeO)_3PO$	2	92
"	$(EtO)_3PO$	0.5	93
guanosine	$(MeO)_3PO$	1	90
"	$(EtO)_3PO$	4	88
adenosine	$(MeO)_3PO$	1.5	90
"	$(EtO)_3PO$	2	91
uridine	$(MeO)_3PO$	14	98
"	$(EtO)_3PO$	14	95
cytidine	$(MeO)_3PO$	4	98
"	$(EtO)_3PO$	12	94

Condition; nucleoside: 6.5 mmole, $POCl_3$: 13 mmole, trialkyl phosphate: 65-98 mmole, temperature: -5°C

According to previous works(2,3), direct phosphorylation of unprotected nucleosides is ineffective for preparation of 5′-nucleotides. A reinvestigation by Barker and Foll(4) confirmed that the reaction conducted in aqueous baryta gave only 2′- and 3′-phosphates whereas the reaction in anhydrous pyridine gave mixture of three possible phosphates.

When unprotected nucleosides were treated directly with phosphoryl chloride in trimethyl or triethyl phosphate, the nucleosides were easily phosphorylated in good yields. The products were mainly 5′-phosphates along with 2′ (or 3′),5′-diphosphates. Formation of the diesters could be greatly reduced by the use of the phosphorylating agent previously treated with a small amount of water in trialkyl phosphate. Similar effect of this treatment was also observed in the phosphorylation of 5′-O-acetylinosine giving no phosphorylated product, whereas, without the treatment with water, as much as 22% yield of 2′ (or 3′)-phosphate was obtained.

Xanthosine 5′-phosphate, which was not readily accessible by conventional phosphorylating method, was also obtained in excellent yield by direct phosphorylation in trimethyl phosphate. These results are summarized in Table 2.

TABLE II

Direct Phosphorylation of Unprotected Nucleoside

Nucleoside	Solvent	Water (mmole)	Time (hr)	Yield of 5′-nucleotide (mole %)
inosine	$(EtO)_3PO$	0	2	68
"	"	2.0	2	91
guanosine	$(MeO)_3PO$	0	6	85
"	"	2.0	6	90
adenosine	$(EtO)_3PO$	0	6	84
xanthosine	$(MeO)_3PO$	0	9	80
uridine	$(MeO)_3PO$	0	12	89
cytidine	$(EtO)_3PO$	0	1	88

Condition; nucleoside: 2 mmole, $POCl_3$: 4 mmole (6 mmole in the case of inosine and guanosine), trialkyl phosphate: 5 ml, temperature: 0°C.

Analysis of nucleotides——Aqueous solutions obtained by hydrolysis of the phosphorylated mixtures were applied to paper chromatography by ascending technique on Toyo Roshi No. 51 paper (40x40 cm) with solvent A (20:20:3 v/v n-propyl alcohol - concentrated ammonium hydroxide - water). Nucleotides on paper chromatograms were detected by irradiation with ultraviolet rays and identified by comparison with the Rf values of authentic samples. The Rf values of nucleoside 5′-phosphates and 2′ (or 3′),5′-diphosphates are summarized in Table 3. Two-dimensional chromatograms of the hydrolyzed solution developed with solvent A then with solvent B (2:79:19 v/v i-propyl alcohol - saturated ammonium sulfate - water) proved the amounts of nucleoside 2′(or 3′)-phosphates formed to be trace or naught. The yields were estimated by the optical density of the solutions which were obtained by extracting the scissored spots.

TABLE III

The Rf Values of Nucleotides

Nucleotide	Solv. A	Solv. B
inosine 5′-phosphate	0.30	0.67
inosine 2′(3′)-phosphate	0.34	0.57
inosine 2′(3′),5′-diphosphate	0.16	0.76
guanosine 5′-phosphate	0.21	0.55
guanosine 2′(3′)-phosphate	0.23	0.43
guanosine 2′(3′),5′-diphosphate	0.09	0.47
adenosine 5′-phosphate	0.30	0.32
adenosine 2′(3′),5′-diphosphate	0.15	0.37
xanthosine 5′-phosphate	0.23	0.40
xanthosine 2′(3′),5′-diphosphate	0.15	0.48
uridine 5′-phosphate	0.31	0.69
uridine 2′(3′),5′-diphosphate	0.20	0.77
cytidine 5′-phosphate	0.32	0.73
cytidine 2′(3′),5′-diphosphate	0.23	0.81

(1) M. Yoshikawa and T. Kato, Bull. Chem. Soc. Japan (in press).

(2) J. M. Gulland and G. I. Hobday, J. Chem. Soc., 746 (1940).

(3) G. R. Barker and J. M. Gulland, ibid, 231 (1942).

(4) G. R. Barker and G. E. Foll, ibid, 3798 (1957).