

PHOSPHORYLATION OF NUCLEOSIDES
WITH TRICHLOROMETHANEPHOSPHONIC ACID DERIVATIVES

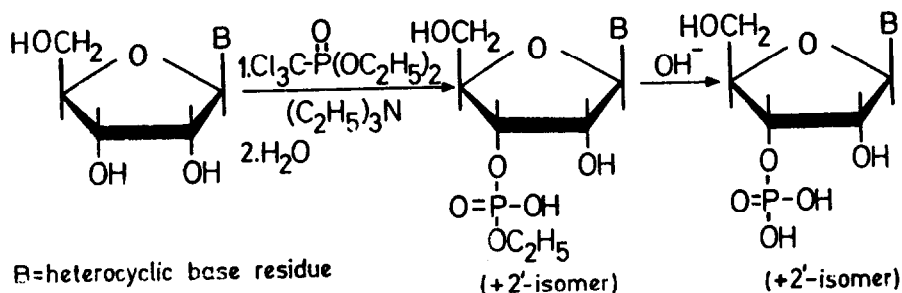
A. Holý

Institute of Organic Chemistry and Biochemistry,
Czechoslovak Academy of Sciences, Prague

(Received in UK 30 November 1971; accepted for publication 9 December 1971)

The reagents used for the phosphorylation of nucleosides are based mostly on the activated derivatives of phosphoric and phosphorous acids which, upon reaction with a free hydroxyl group of the nucleoside, afford phosphoric acid esters of nucleosides. The method described here presents a different principle - cleavage of the carbon-phosphorus linkage of trichloromethanephosphonic acid derivatives, catalyzed by alkali¹.

Unprotected ribonucleosides react with ethyl trichloromethanephosphonate in the presence of triethylamine. The reaction proceeds selectively at the 2',3'-cis-diol group, giving rise to 2'(3')-ribonucleotide ethyl esters. These compounds can be either isolated or transformed directly to 2'(3')-nucleotides on treatment with alkali:



Thus, the ribonucleoside (5 mmol), dimethylformamide (5 ml), ethyl trichloromethanephosphonate (4 ml) and triethylamine (5 ml) were stirred at room temperature overnight, the mixture diluted with water (100 ml) and extracted with ether (25 ml). The aqueous phase was evaporated to dryness and the ethyl ester of ribonucleoside 2'(3')-phosphate isolated by DEAE-cellulose column chromatography. In an alternative procedure, the

reaction mixture was treated directly with 2 N lithium hydroxide (50 ml) 2 h at 50°C, neutralized with a cation exchanger (acid form), concentrated in vacuo and the 2'(3')-nucleotide isolated as lithium salt by ethanol-acetone precipitation, or, by DEAE-cellulose column chromatography. The yields of nucleotides depend upon the character of the heterocyclic base: uridine, 67%, cytidine, 23%, adenosine, 45%, 6-azauridine, 3%, α -uridine, 4%.

The participation of the cis-diol system in the mechanism of the reaction was confirmed by the failure of thymidine or 2',3'-O-isopropylideneuridine to undergo reaction. The cyclic character of the reaction intermediate is emphasized by the reaction with 9-(α -L-lyxofuranosyl)adenine in which the conformation allows the formation of two cyclic intermediates². The reaction mixture (contains 95% of nucleotides) is composed of the 2'-(23%), 3'-(37%) isomers and the 3',5'-cyclic phosphate (35%).

There is essentially no reaction without the catalyst or in the presence of an anhydrous acid. Similarly, no reaction was observed with the free trichloromethane-phosphonic acid and triethylamine. On the other hand, trichloromethane-phosphonic acid dichloride reacts (in the presence of 2 equivs of triethylamine) in dimethylformamide solution with ribonucleosides giving rise to 2'(3')-ribonucleotides as the sole products.

The selective phosphorylation by ethyl trichloromethane-phosphonate represents one of the simplest procedures of this type, comparable only with the direct metaphosphate reaction with ribonucleosides in alkali³.

REFERENCES

1. I.S. Bengelsdorf, J. Am. Chem. Soc. 77, 6611 (1955).
2. A. Holý and F. Šorm, Collection Czechoslov. Chem. Commun. 34, 3523 (1969).
3. R. Saffhill, J. Org. Chem. 35, 2881 (1970).