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A New Route to Nucleoside 5'-triphosphates

(Short Communication)

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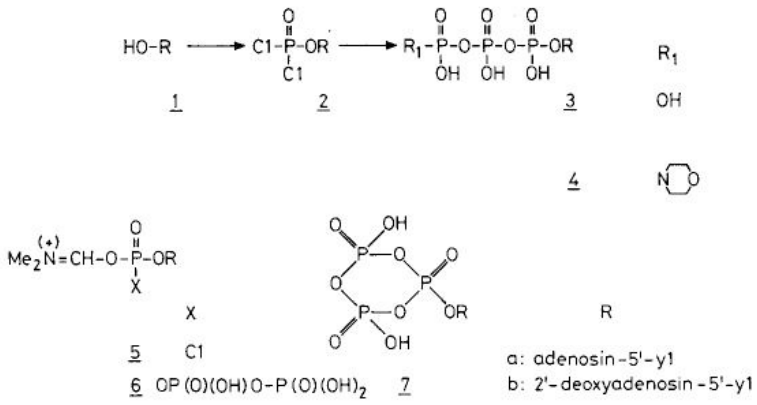
The Yoskihawa reaction, i. e. phosphorylation of unblocked nucleosides *(1)* with $POCl_3$ in trialkyl phosphates gives predominantly nucleoside 5'-phosphorodichloridates *(2)* (Yoshikawa et al. 1969). Subsequent *in situ* neutral (acidic) or alkaline hydrolysis, ammonolysis or alcoholysis of compounds *2* results in the formation of nucleoside *5'*-phosphates (Yoskihawa et al., 1969; Slotin, 1977) or nucleoside *3',5'*-cyciic phosphates (Tazawa et al., 1972) nucleoside *5'*-phosphorodiamidates (Simoncsits, Tomasz, 1975; Bottka, Tomasz, 1979) or nucleoside *5'*-alkylphosphates (Kim, Rosowsky, 1979).

It was found that compounds *2* can be transformed also into nucleoside 5'-triphosphates (*3*) by a short treatment performed *in situ* with an excess of tri-n-butylammonium pyrophosphate in DMF under anhydrous conditions followed by neutral hydrolysis, as shown on the examples of adenosine 5'-triphosphate (*3a*) and 2'-deoxyadenosine 5'-triphosphate (*3b*).

The reaction may proceed *via* the highly reactive imidoyl phosphate (*5* and *6*) and trimetaphosphate (*7*) intermediates. This supposition is based on the following observations. Phosphorodichloridates and DMF immediately form imidoyl phosphates which in turn react with phosphates to give pyrophosphates (Cramer, Winter, 1961). Trimetaphosphates *7* are readily formed from nucleoside 5'-(γ-imidoyl) triphosphate derivatives by intramolecular condensation (Glonek et al., 1974; Knorre et al., 1976; Webb, 1980). On this basis it seems reasonable to suppose that nucleoside 5'-(α-imidoyl) triphosphates (*6*) behave similarly. The γ-morpholidate *4a* was thus formed instead of *3a* when morpholine was added to the reaction mixture instead of aqueous $Et_3N \cdot H_2CO_3$. (Compound *4a* was previously described by Wehrli et al. (1965).

$POCl_3$ (0.26 mmol) was pipetted into a suspension of adenosine (*1a*, 0.20 mmol) in dry $(MeO)_3PO$ (0.5 ml) and the mixture was stirred at 0° for 1.5 h. A mixture of 0.5 M bis-tri-n-butylammonium pyrophosphate in anhydrous DMF (2 ml) and Bu_3N (0.2 ml) was quickly added under vigorous stirring. [The preparation of 0.5 M bis-tri-n-butylammonium pyrophosphate was carried out according to Moffat et al. (1964). By omitting the additional quantity of Bu_3N the yield significantly decreases.] After 1 min 1 M aqueous $Et_3N \cdot H_2CO_3$, pH = 7.5, was poured into the solution. After evaporation the residue was separated on a DEAE



cellulose [HCO_3^- form] column with a linear gradient of aqueous $Et_3N \cdot H_2O_3$, pH = 7.5. Yield: 86% of TLC pure *3a*, $R_f^A = 0.40$, $R_f^B = 0.29$. (Thin-layer chromatography was carried out on cellulose in a nPrOH: cc · NH_4OH : H_2O = = 11/7/2 (A) and on PEI-cellulose in 1.5 M NaCl (B). Adenine: $P_{total} = 1.00 : 2.98$.

3b was prepared in exactly the same manner except that the phosphorylation was performed at −20° for 2 h. Yield: 78%, $R_f^A = 0.38$, $R_f^B = 0.25$; adenine: $P_{total} = 1.00 : 2.96$.

The simplicity, shortness and the possibility of using unlocked nucleoside as starting material (instead of 5'-nucleotide) render the method more advantageous than the earlier described procedures (e.g. anion-exchange, (Michelson, 1964) morpholidate (Moffatt, Khorana, 1961) and imidazolidate method (Hoard, Ott, 1965). The non-selectivity of the Yoshikawa reaction (Bottka, Tomasz, 1979; Dawson et al., 1977) (which depends on the nature of the heterobase) may cause the contamination of deoxyribonucleoside 5'-triphosphates with the isomeric 3'-triphosphates. In the case of *1b* this side product was formed to less than 0.5%. On the other hand ribonucleoside 2'(3')-triphosphates are unstable compounds and readily decompose to ribonucleoside 2',3'-cyclic phosphates (Khorana, 1961).

After the preparation of this manuscript a similar approach was described for the synthesis of several thymidine and 2'-deoxycytidine analogue 5'-triphosphates (Ruth, Cheng, 1981).

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