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SIMPLE SYNTHESIS OF 5-VINYL- AND 5-ETHYNYL-2'-DEOXYURIDINE-5'-TRIPHOSPHATES

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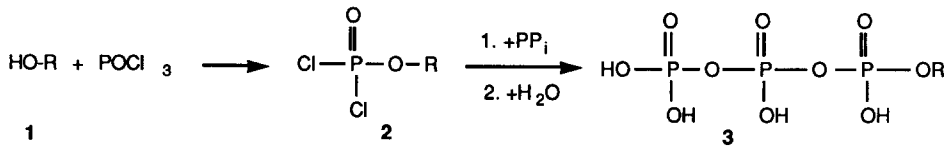
ABSTRACT

Acid-sensitive nucleoside 5'-O-triphosphates can be obtained in good yield directly from the unprotected nucleosides in the presence of a proton sponge.

In the last decade, numerous pyrimidine nucleoside analogues have proven to be remarkably potent and selective in their activity against herpes simplex virus infections (for recent reviews see ref. 1). These nucleoside analogues all share the common feature that their biological function requires their intracellular conversion to the corresponding 5'-nucleotides. Investigations of the mechanism by which these nucleoside analogues interfere with the cellular metabolism therefore require the chemical synthesis of phosphorylated derivatives.

For sometime we have been interested in the synthesis of 5-substituted 2'-deoxyuridine-5'-triphosphate analogues and have studied from the stereochemical point of view their incorporation into a synthetic DNA in the Klenow DNA polymerase-catalysed reaction[2]. We describe here a general method for the phosphorylation of the acid-sensitive 5-vinyl- and 5-ethynyl-2'-deoxyuridines.

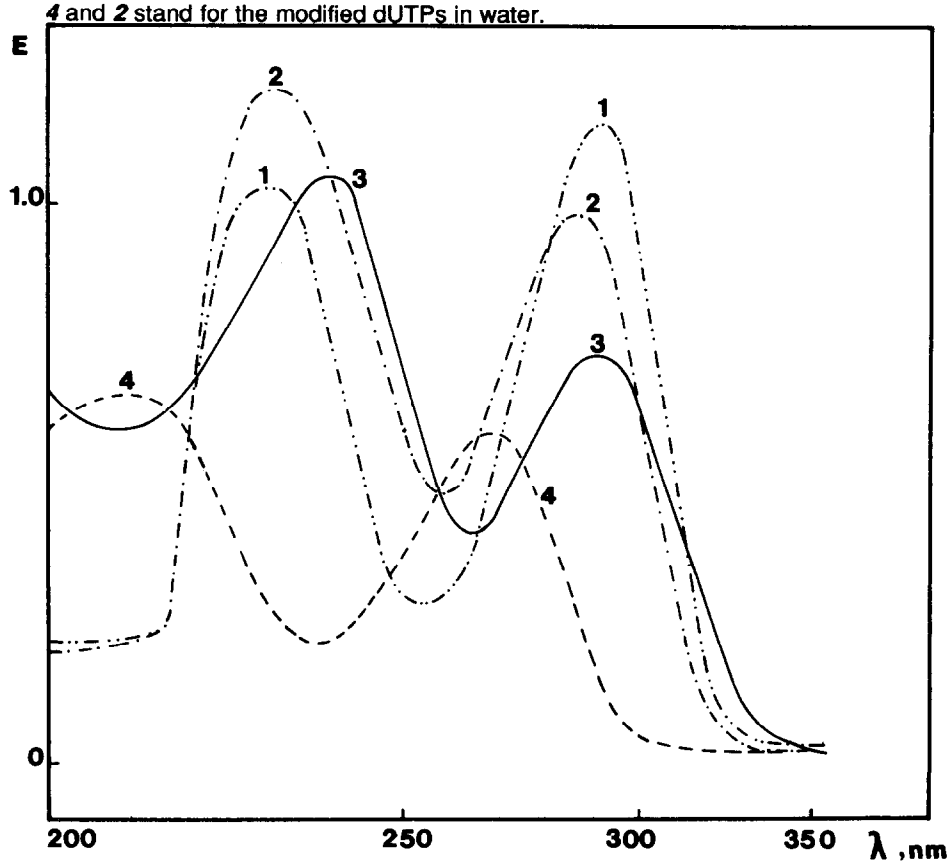
Owing to the great importance of phosphorylated biological molecules there are several reviews dealing with methods of their phosphorylation[3]. Among the reagents described, phosphoryl chloride in a trialkyl phosphate solvent is used extensively for the phosphorylation of unprotected nucleosides (**1**), when the major product after a hydrolytic step is the 5'-phosphate[4]. It is also known that the intermediate phosphorodichloridate (**2**) can easily be transformed into nucleoside 5'-triphosphates (**3**) *in situ,* when **2** is not hydrolysed but is allowed to react directly with pyrophosphate in the same reaction vessel[5]:



Although, this "one-pot-two-steps" procedure has proved to be simple and mild enough to synthesize a series of (E)-5-(1-alkenyl)- and 5-(1-alkynyl)-dUTPs[2], in the case of the title compounds it has failed.We have observed that the triphosphates have different UV absorbtion profiles than the parent 5-vinyl and 5-ethynyl nucleosides (Fig. 1). The reduced λ_{max} values indicate that the structural changes affecting the chromophoric pyrimidine ring[6] are due to the phosphorylation reaction.

This observed hypsochromic shift can be explained by the addition of $POCl_3$ generated hydrogen chloride to the unsaturated side-chain in conjugation with the heterocyclic aromatic π-

Figure 1. The observed UV profiles. ***3*** and ***1*** indicate 5-vinyl and 5-ethynyl dUs in methanol; ***4*** and ***2*** stand for the modified dUTPs in water.



system. This hypothesis is supported by other studies showing the very reactive nature of the 5-vinyl group of 5-vinyl-2'-deoxyuridine in acidic conditions[7]. On the other hand, 5-(1-chlorovinyl)-2'-deoxyuridine can be obtained in good yield by the addition of HCl to 5-ethynyl-2'-deoxyuridine[8].

In order to eliminate the influence of hydrogen chloride we have attempted to carry out the phosphorylation of the title compounds in the presence of a base. Although the applications of $POCl_3$ in basic solution have been reported[5a,9], none of them has proven sufficient to neutralize the liberated HCl. This can be attributed to the high reactivity of the unsaturated side-chain. After further fruitless experience with other bases (e.g. TEA; 2,4,6-collidine; N,N-diisopropylethylamine [Hünig's base]; 1,5-diazabicyclo[4.3.0]non-5-ene [DBN]; 1,8-diazabicyclo[5.4.0]-undec-7-ene [DBU]) we decided to carry out the phosphorylation reaction in the presence of 1,8-bis(dimethylamino)naphthalene (Proton Sponge®, Aldrich), which due to steric effects[10], is known as a very strong base with weak nucleophilic character.

A typical synthesis is described here. A known amount of unsaturated nucleoside (0.3 mmole) was stirred in dry trimethylphosphate (0.75 ml) with powdered proton sponge (0.45 mmole) at 0° C. $POCl_3$ (30 µl, 1.1 eq.) was added and the mixture was stirred at 0-4° C. After observing a maximal formation (85-90%) of the intermediate nucleoside phosphorodichloridate **2** (2 hrs; without base the phosphorylation of pyrimidine nucleosides usually takes more than 12 hours), a mixture of 0.5 M bis-tri-n-butylammonium pyrophosphate in anhydrous DMF (3ml, 5 eq.) and n-Bu_3N (0.3 ml) was quickly added to the reaction mixture under vigorous stirring at 0° C. After 1 min. 0.2 M aqueous $Et_3N{\cdot}H_2CO_3$, pH 7.5 (30 ml) was poured into the solution. After evaporation, the residue was separated by column chromatography on DEAE Cellulose (Whatman, DE 32) using a linear gradient of $Et_3N{\cdot}H_2CO_3$ between 0 and 400 mM (800-800 ml) at 4° C. After purification the 5'-triphosphate was converted into its tetrasodium salt.

On this scale, isolated yields were 62% for 5-vinyl- and 60% for 5-ethynyl-dUTP of theoretical from the corresponding nucleoside. The structure of the modified nucleoside 5'-triphosphate was confirmed by NMR data. The purity of the 5'-nucleotides, which was at least 95%, which was checked by TLC scanning (Shimadzu TLC scanner, CS 920). R_f values on silica plate (Kieselgel 60 F_{254}, Merck) in n-PrOH:cc.NH_4OH:H_2O (11:7:2) are as follows: 5-vinyl-dUTP 0.16 and 5-ethynyl-dUTP 0.13.

The method described above for the preparation of the 5'-triphosphates is suitable for the synthesis of highly acid-sensitive nucleosides. Other advantages of this procedure are the simplicity and shortness, since the chemical manipulations involved are kept to a minimum and only one column is required to obtain the pure end-product. Furthermore, an additional important benefit is that the proton sponge significantly accelerates the reaction.

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