# Mild and Regiospecific Phosphorylation of Nucleosides

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*Received 13 May 1997*

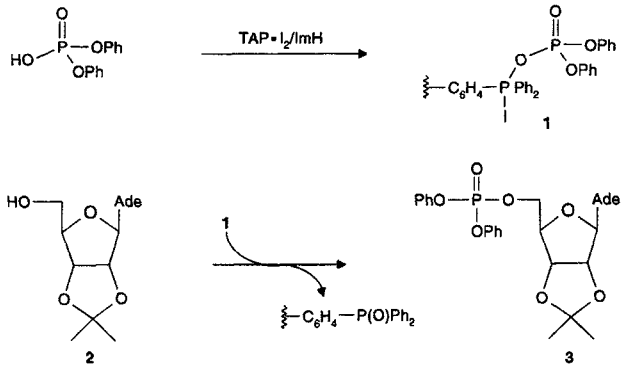
**Abstract**: Nucleosides are reported to be conveniently phosphorylated by diphenylphosphate previously activated with a triarylphosphine-$I_2$ complex. The reaction is characterised by mild conditions, high yields, easy work-up and purification of the final product. The activation of the phosphorylating agent, unlike Mitsunobu reaction, enables to avoid the usual inversion of configuration at C-3'.

Phosphate esters are compounds widely present in many biological systems. Through the past years, chemists have developed a great interest for the synthesis of phosphonic acids along with their derivatives that may be considered as analogs of natural phosphates and show modified chemical and biological properties[1].

Several methods[2-4] are available for the synthesis of phosphonates and the most broadly used is the Mitsunobu reaction[5] –and all of its modifications[6]– that allows to couple phosphonic acids with primary and secondary alcohols under mild and efficient conditions, by an alkoxyphosphonium salt attack onto the activated alcohol. Unfortunately the Mitsunobu reaction, when applied to chiral secondary alcohols, leads to complete inversion of configuration and is also reported[7,8] to fail in the preparation of C-5' phosphorylated nucleosides bearing a purine base, due to the formation of a $N^3$,5'-cyclonucleoside as the main product coming from the intramolecular nucleophilic attack of the purine base itself onto the C-5' carbon.

Hereby, we would like to report a new method for the synthesis of phosphoric acid monoesters *via* direct coupling of alcohols with a suitable phosphorylating agent in the presence of polystyryl diphenylphosphine-$I_2$ (TAP•$I_2$) complex[9], thus under mild and neutral conditions suitable to acid sensitive hydroxyl groups as is the case of ribo- and deoxyribonucleosides.

In fact the TAP•$I_2$ complex, used as a condensing agent, essentially activates the phosphate (Scheme 1) which may undergo a nucleophilic attack by the alcohol. The addition of imidazole (ImH) to the reaction ensures neutral conditions by trapping the protons released during the reaction, even though an active role of imidazole in the esterification process cannot be excluded[10].




**Scheme 1**

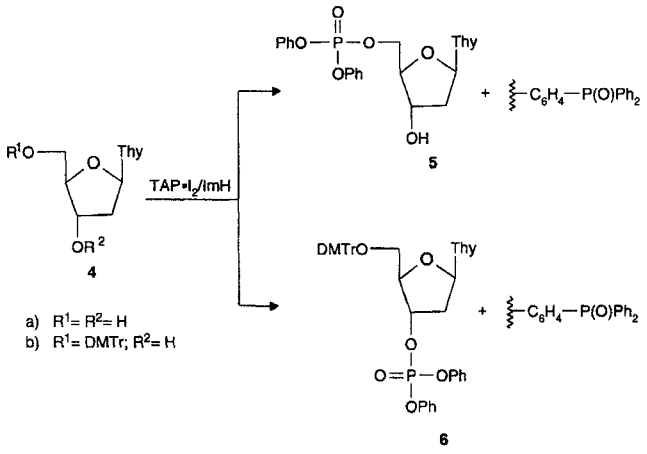
The choice of a polymer bound triarylphosphine, as is polystyryl diphenylphosphine, ensures the phosphine oxide, which actually represents the only by-product of the reaction, to be linked to a polymeric matrix and consequently to be separated by simple filtration. This avoids time consuming and circumstantial purification procedures to obtain the pure phosphorylated compound that is directly crystallised from a suitable solvent.

Such phosphorylation conditions led to successful results by using diphenylphosphate as the phosphorylating agent and 2',3'-*O*-isopropylideneadenosine[11] **2** as a nucleoside model.

In a typical experiment, a magnetically stirred suspension of dry polystyryl diphenylphosphine (1.0 g; ≅ 3 mmol/phosphine) in anhydrous $CH_2Cl_2$ (20 mL) was titrated by 0.1 M iodine solution (30 mL) at room temperature and under nitrogen (or argon) atmosphere. After 15 min, diphenylphosphate (0.4 g; 1.6 mmol) was added to the suspension, stirring being gently continued for an additional 40 min. A solution of imidazole (0.4 g; 6.0 mmol) and pure 2',3'-*O*-isopropylideneadenosine **2** (0.5 g; 1.6 mmol) in the same solvent (10 mL) was then added. After 2 h, the reaction was quenched and the solid polymeric triarylphosphine oxide formed filtered off. Final evaporation of the solvent under reduced pressure led to a semicrystalline solid which, by hexane crystallisation, gave the pure[12] phosphorylated **3** (1.5 mmol; 92% yield). No traces of $N^3$,5'-cycloadenosine were detected in the crude reaction product.

It is noteworthy that this phosphorylation reaction is regiospecific towards the primary hydroxyl group as was shown by treating the fully deprotected thymidine **4a** under the above conditions that led exclusively to 5'-phosphorylated thymidine **5** (92% yield).

The retention of configuration at C-3' was ascertained by treating the dimethoxytrityl derivative **4b** under the above conditions that gave the pure phosphorylated compound **6** (95% yield) without inversion at C-3' (Scheme 2). The survival of both the isopropylidene and trityl functions to the phosphorylation conditions (as is the case of compounds **2** and **4b**) indicates that this reaction can be appropriate for phosphorylating alcohols containing acid sensitive functions.




**Scheme 2**

Feasibility, mildness and high-yielding, beside regiospecificity and configuration retention as well, are all features for a broad application of this procedure in the phosphorylation of nucleosides and other fragile molecules.

**Acknowledgements**

Financial support from National Research Council - Progetto Finalizzato "Chimica Fine" to R.C. is gratefully acknowledged.

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(12) m.p. 48-49 °C; $[\alpha]_D$ = -25.0 (c = 1.0 in $CHCl_3$); $^1H$ NMR (400 MHz; $CDCl_3$): δ 1.29 (*s*, 3H, Me), 1.54 (*s*, 3H, Me), 4.30-4.42 (*m*, 1H, H-4'), 4.41-4.50 (*m*, 2H, H-5'), 4.99 (*dd*, 1H, $J_{3',4'}$ = 2.6 Hz; $J_{3',2'}$ = 6.3 Hz, H-3'), 5.25 (*dd*, 1H, $J_{2',-1'}$ = 2.8 Hz; $J_{2',3'}$ = 6.3 Hz, H-2'), 5.87 (*bs*, 2H, $NH_2$), 6.03 (*d*, 1H, $J_{1',2'}$ = 2.2 Hz; H-1'), 7.02-7.12 (*m*, 6H, *ortho* and *para* phenyl H), 7.15-7.28 (*m*, 4H, *meta* phenyl H), 7.79 (*s*, 1H, H-2), 8.23 (*s*, 1H, H-7).