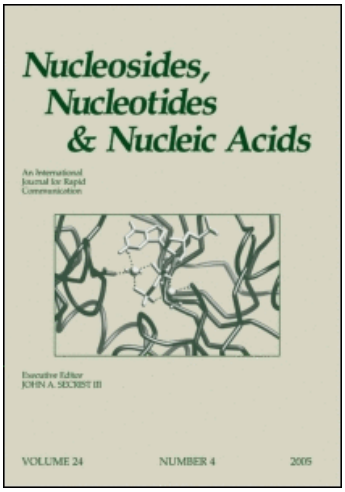

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A NEW PROCEDURE FOR THE PHOSPHORYLATION OF NUCLEOSIDES: APPLICATION TO THE DISCOVERY OF INHIBITORS OF HIV INTEGRASE

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A NEW PROCEDURE FOR THE PHOSPHORYLATION OF NUCLEOSIDES: APPLICATION TO THE DISCOVERY OF INHIBITORS OF HIV INTEGRASE

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ABSTRACT

A new phosphorylating agent for nucleosides, 2-O-(4,4′-dimethoxytrityl) ethylsulfonylethan-2′-yl-phosphate (**1**), has been developed by us. In the many examples studied by us, phosphorylation yields were found to be very high (about 90%). The procedure appears to be remarkably general and can be utilized for the phosphorylation of many biomolecules. Successful application of this phosphorylation method has contributed to the discovery of inhibitors of HIV integrase in our laboratory.

INTRODUCTION

Phosphorylation reactions are ubiquitous in nature. Non-natural phosphorylated nucleosides are of interest for biological studies involving various nucleotide metabolizing enzymes and as potential therapeutic agents (1–4). Chemical methodologies for solution-phase phosphorylations are of critical importance in the preparation of these compounds. Several requirements need to be fulfilled for efficient phosphorylation of nucleosides. The hydroxyl functions of the phosphate group being introduced need to be protected (4). These phosphate protecting groups must possess stability with respect to reaction conditions of subsequent synthetic steps but must also have the property of selective lability at the termination of synthesis.

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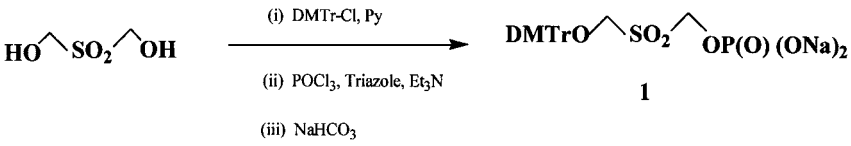
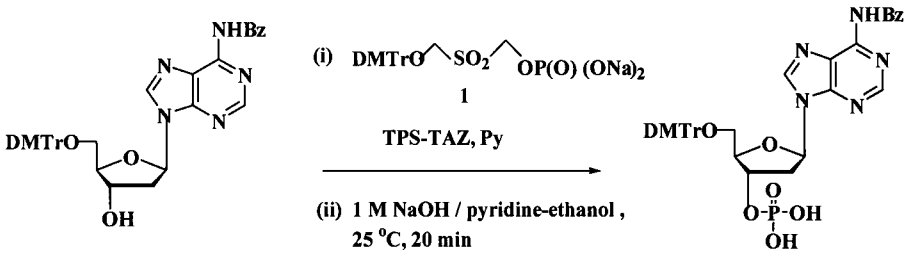


Scheme 1.

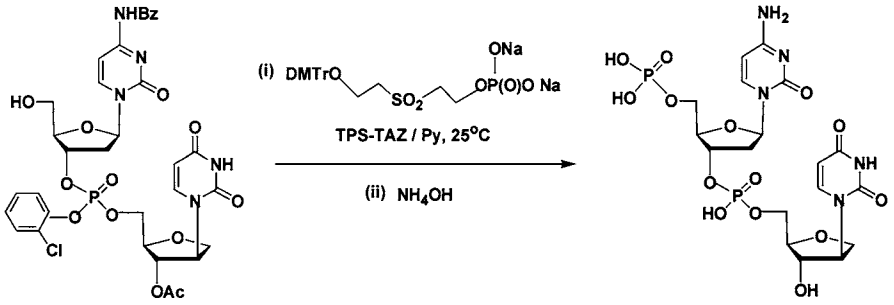
Current methods of chemical phosphorylation have disadvantages that range from lengthy procedures, instability of intermediates, difficulty of purification, problems associated with removal of phosphate protecting groups, issues of monitoring of reaction, and, in many cases low yields in the phosphorylation and deprotection steps (5–18).

We describe herein a new method for phosphorylation of nucleosides and nucleotides which utilizes more effective phosphodiester instead of phosphotriester chemistry and surmounts the problems associated with stability, lability, purification and yields in phosphorylation reactions discussed above. The new phosphorylating reagent is 2′-O-(4,4′-dimethoxytrityl)ethylsulfonylethan-2-yl-phosphate (**1**). It can be synthesized easily from sulfonyldiethanol by selective monotritylation followed by phosphorylation with $POCl_3$/1,2,4-triazole/triethylamine and work up with $NaHCO_3$ (Scheme 1) (19). When **1** is activated by coupling reagents such as TPS-TAZ or TPS-NT, it phosphorylates primary and secondary alcohol groups in nucleosides with remarkable efficiency (of the order of 90%). Deprotection of the phosphodiester intermediate formed after the phosphorylation step is carried out in only one step, but that step constitutes the normal work-up of the reaction. The precursor nucleoside does require protection prior to the phosphorylation step. An example is the 3′-phosphorylation of protected deoxyadenosine which proceeds in 90% overall yield under the conditions shown in Scheme 2.

We have examined many examples of 3′- as well as 5′-phosphorylation of nucleosides to establish the generality of this procedure (20). The results are completely reproducible. The phosphorylation methodology can be utilized in the synthesis of more complex and sensitive molecules, including those that are potent inhibitors of HIV integrase. An example of the utilization of this phosphorylation



Scheme 2.



Scheme 3.

method in the synthesis of one HIV integrase inhibitor is illustrated in Scheme 3. The yield of phosphorylated dinucleotide (HPLC purified) was 79%.

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19. Data for **1**: ^{1}H NMR ($CDCl_3$): δ 7.45–7.25 (m, 9H, Arom), 6.35–6.30 (m, 4H, Arom), 4.20–4.10 (m, 2H, $DMTrOCH_2$), 3.65–3.45 (m, 2H, CH_2OP), 3.20–3.15 (m, 2H, $DMTrOCH_2C\underline{H}_2$), 3.10–3.05 (m, 2H, $C\underline{H}_2CH_2OP$). ^{31}P ($CDCl_3$): δ − 4.90 (br,s).
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