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One-pot synthesis of organophosphate monoesters from alcohols

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ABSTRACT

A one-pot procedure for the phosphorylation of alcohols provides the corresponding phosphate monoesters in improved yields. The protocol features the use of tetrabutylammonium hydrogen phosphate and trichloroacetonitrile, followed by purification of the crude product by flash chromatography on silica gel. The final step, cation exchange chromatography, affords the organophosphates as ammonium salts that are usually required for biochemical applications. The mechanism appears to be phosphate rather than alcohol activation by trichloroacetonitrile.

The condensation of a phosphate residue with hydroxyl groups present in biogenic alcohols like carbohydrates, proteins, and other biomolecules to form the corresponding phosphate ester is called phosphorylation. This transformation can significantly change the physical, chemical, and biological properties of the substrate due to change in its solubility, electric charge, conformational properties, and protein recognition, among others. Phosphorylation of biological systems is usually carried out by ATP and is catalyzed by kinases, while phosphatases catalyze the removal of phosphate groups in biological systems. These two families of enzymes are key players in cell signaling and in the regulation of signal transduction cascades involved in cell metabolism. Disruption of their proper functioning has been related to several diseases such as diabetes, cancer, and others.[1]

An enormous variety of biomolecules is encountered in the form of phosphate monoesters such as nucleotides, proteins, and also a great number of natural products arising from the secondary metabolism of, for example, fungi such as (−)-calyculin A (**1**), cytostatin (**2**), and fostriecin (**3**) (Fig. 1).[2]

Phosphate monoesters are important in medicinal chemistry, for example, as water soluble prodrugs and for biological studies.[3] Several approaches to their preparation have been reported but some of them are plagued by the competitive formation of di- and tri-substituted esters or by the formation of the corresponding pyrophosphates; or they require the utilization of a great excess of either the alcohol or the phosphorylation reagent.[4,5] In order to avoid such side products, the use of organic phosphate triesters containing two cleavable ester residues has been established as a valuable method for the preparation of phosphate monoesters. Evans,[6] Johns,[7] and Miyashita[8] have reported methodologies wherein phosphorous trichloride, diethylchlorophosphite, and phosphoryl chloride, respectively, are used to obtain the aforementioned phosphate esters. Some of these reagents are not suitable at all for acid sensitive alcohols, for example, those leading to higher prenylphosphates.

A more direct approach to phosphate monoesters is the phosphorylation of an alcohol substrate with an activated form of phosphoric acid under mild conditions. The pioneering work by Cramer in 1959 set the stage for direct phosphorylation of alcohols.[9]

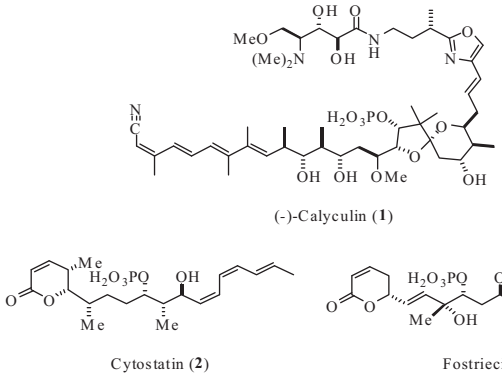

Figure 1. Examples of phosphate monoester natural products.

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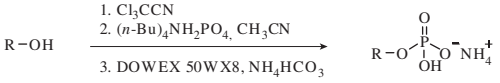
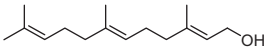
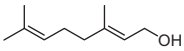
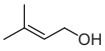
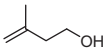
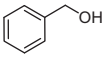
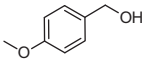
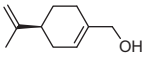
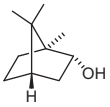


Scheme 1. General phosphorylation procedure.

Table 1
Alcohols phosphorylated according to Scheme 1, and yields of purified organic monophosphates

Entry	Starting alcohol	Isolated yield of phosphate (%)
1		86
2		75
3		66
4		50
5		43
6		59
7		70
8		76
9		57
10		67
11		52

However, unsatisfactory yields were achieved with this original procedure. A few years later, Danilov published a modification of the Cramer procedure with good yields, but with a long and tedious work-up of the crude reaction mixture.[10] It is also known from the work published by Keller in 1993 that isoprenoid diphosphates can be purified by standard flash silica chromatography.[11] More recently, Ishihara[12] and Pascal[5] have shown improvements with direct phosphorylation.

Based on these preceding developments of the phosphorylation reaction, we describe herein a straightforward method for the preparation of phosphate monoesters directly from alcohols. The commercially available tetrabutylammonium dihydrogenphosphate is used as the phosphate donor in combination with trichloroacetonitrile[9d] as a mild esterification agent (Scheme 1).

The reaction was monitored by TLC and a mixture of isopropanol/NH_4OH/H_2O 7:2:1 was used as the eluent. After consumption of the alcohol, the product was purified by flash silica column chromatography using the above solvent mixture and it was finally converted into the corresponding ammonium salt by percolation through a DOWEX 50WX8 ion-exchange column with a 0.025 M NH_4HCO_3 buffer solution, a process which also helps to remove dissolved silica gel.[13]

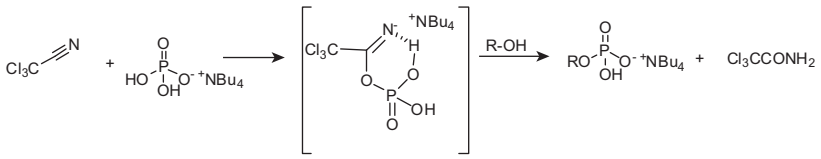
The ammonium phosphate monoesters were characterized by ESI-MS and ^{1}H-, ^{13}C-, and ^{31}P NMR analyses. In the ^{1}H NMR spectra of the allylic and benzylic monoesters the coupling constant ^3J (^{1}H–^{31}P) is evident (4.5–6.0 Hz) while in the ^{13}C NMR spectra the ^{13}C–^{31}P coupling is generally observed for the carbinolic carbon (^2J = 4.0–7.5 Hz) and for the carbon α to the carbinolic center (^3J = 3.0–9.0 Hz). In the ^{31}P NMR spectra the ^{31}P signal appears at δ 1.69–4.95 ppm with the aliphatic phosphate monoesters appearing upfield and the allylic ones downfield in the above region (H_3PO_4 was used as an internal standard). When allyl alcohol was employed, we were able to detect minuscule formation of the corresponding pyrophosphate monoester which displays lower field signals in the ^{1}H- and ^{13}C NMR spectra when compared to the corresponding phosphate monoester. In the ^{31}P NMR spectrum, the phosphate monoester signal appears at δ 4.5 ppm while the signals for the corresponding pyrophosphate are found at δ 5,63 and 9,51 ppm ($^2J_{P-P}$ = 21 Hz) The described method was applied to a series of alcohols with moderate to good results (Table 1). The list of phosphate monoesters comprises those derived from primary and secondary aliphatic alcohols, including benzylic and allylic ones. However, the phosphorylation of *tert*-butyl alcohol and phenol could not be carried out under these conditions.

All yields were calculated based on the amount of starting alcohol and have proved to be superior to those reported in the literature by the previously described methods. Furthermore, the formation of byproducts such as pyrophosphates was absent with all alcohols employed, with the exception of the very reactive allyl alcohol (Table 1, entry 8) showing some minor double activation.

A proposed mechanism for this reaction is shown in Scheme 2, where trichloroacetonitrile reacts with tetrabutylammonium dihydrogenphosphate to give the corresponding reactive phosphorylated trichloroacetimidate, a mixed anhydride-like activated phosphate, followed by nucleophilic attack by the alcohol to yield the desired phosphorylated alcohol and trichloroacetamide.

The proposed mechanism is supported by ^{13}C NMR experiments carried out under the experimental conditions described above: no reaction was observed upon either mixing of 4-methoxybenzyl trichloroacetimidate with tetrabutylammonium dihydrogenphosphate, or by pre-mixing of trichloroacetonitrile with geraniol. However, when geraniol, trichloroacetonitrile, and tetrabutylammonium dihydrogenphosphate have been successively mixed, the disappearance of the ^{13}C signals of trichloroacetonitrile and the appearance of the signals corresponding to the phosphorylated alcohol were observed. The proposed mechanism is supported by a previously reported synthesis of deuterated dimethylallyl diphosphate by treatment of a solution of (*R*)-[1-^{2}H]3-methyl-2-butenol in trichloroacetonitrile with bis-triethylammonium phosphate in acetonitrile to afford the corresponding diphosphate with retention of configuration.[14]

The method described herein is a straightforward approach to phosphate monoesters in a one-pot reaction procedure and should be applicable to a wide range of primary and secondary alcohols.



Scheme 2. Proposed mechanism for the formation of phosphate monoester using trichloroacetonitrile and dihydrogenphosphate via a reactive 'mixed anhydride'.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.01.059.

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13. *Typical experimental procedure:* To a solution of the alcohol (1 mmol) in acetonitrile (1 mL), trichloroacetonitrile (2.4 mmol) is added, followed by dropwise addition of tetrabutylammonium dihydrogenphosphate (2.0 mmol) in acetonitrile (5 mL). The reaction mixture is stirred at room temperature for two hours, or until all the starting material is consumed. The solvent is removed in vacuo and the crude material is pre-purified by flash chromatography on silica gel (isopropanol/conc. aq NH_4OH/H_2O 7:2:1). The column fractions which show the presence of phosphate on TLC (e.g., with molybdate reagent) are combined and concentrated under reduced pressure. A Dowex 50WX8 ion-exchange column of 8 cm tall and 1 cm diameter is loaded and equilibrated with a mixture of conc. NH_4OH/H_2O 3:1 and afterward flushed with a buffer solution of 0.025 M NH_4HCO_3 until the pH reaches 8.0. The silica column residue is percolated through the DOWEX column with the same NH_4OH buffer, collected, and dried either by lyophilization or by evaporation under reduced pressure until excess ammonium hydrogen carbonate is evaporated. The residual ammonium organophosphates are isolated as white solids.
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