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A Convenient Synthesis of Nucleoside 3'-H-Phosphonate Monoesters Using Triphosgene

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Abstract: A practical method for the preparation of deoxyribonucleoside and ribonucleoside 3'-H-phosphonate monoesters via triphosgene-mediated coupling of nucleosides to phosphonic acid is described.

Hydrogenphosphonates(H-phosphonates) have been used as key intermediates in the multistep synthesis of various natural products and synthetic medicinals like oligonucleotides,[1,2] nucleopeptides,[3] sugar phosphates[4] and phospholipids.[5] These synthons are easy to handle and can be conveniently oxidized at phosphorous in high yields using simple experimental procedures.[6]

Nucleoside H-phosphonates are used in solid phase as well as solution phase synthesis of nucleic acids.[7] The advantages of the H-phosphonate method include: i) nucleoside H-phosphonates are relatively stable, ii) the internucleotide H-phosphonate bonds are stable to the conditions of synthesis such as exposure to weak acids and basic pyridine solutions, iii) internucleotide linkages are oxidized at the end of synthesis, rather than after each coupling. Although a number of syntheses have been developed with the goal of providing nucleoside H-phosphonate monoesters, they vary in terms of reaction conditions, availability of the phosphonylating reagent, efficiency of the coupling and isolation of the final product. Phosphorous trichloride/imidazole[8] and phosphorous trichloride/1,2,4-triazole[2] systems have been used to make deoxyribo- and ribonucleoside 3'-H-phosphonates. A major disadvantage of this method is that phosphorous trichloride is highly toxic. Van Boom et al. employed bis(N,N-diisopropylamino)chlorophosphine and salicylchlorophosphite as phosphitylating reagents.[9] A limitation in the use of the former reagent is that it requires carefully-controlled acidic hydrolysis as the final reaction to form H-phosphonate. Although salicylchlorophosphite showed good coupling efficiency, the isolation of some nucleoside H-phosphonates from hydrolysis products of the reagent was cumbersome. Transesterification of reactive H-phosphonate diesters with nucleosides has been used to prepare nucleoside H-phosphonates. Diphenyl H-phosphonate,[10] Bis(1,1,1,3,3,3-hexafluoro-2-propyl)-H-phosphonate[11] and bis(2,2,2-trifluoroethyl)-H-phosphonate[12] are some of the reagents of choice in this protocol. Recently Stawinski and co-workers have developed a convenient method to synthesize nucleoside H-phosphonates from phosphonic acid. Either pivaloyl chloride or 5,5-dimethyl-2-oxo-2-chloro-1,3,2-dioxaphosphorinane was used as coupling agent.[13] In most of the methods mentioned above pyridine is used as the solvent. Pyridine, however, is not suitable to use in large scale processes because of its toxicity and odor.

As a part of our ongoing program directed towards the process development of oligonucleotide phosphorothioates we needed a convenient method to prepare various nucleoside H-phosphonates. We report herein a practical procedure to make these synthons by using triphosgene[bis(trichloromethyl) carbonate] to couple phosphonic acid to nucleosides. Triphosgene is a commercially available, stable, crystalline solid which has proved to be a versatile substitute for phosgene gas. It is safe and can be exactly weighed out in milligram amounts to perform desired chemical transformations. It has been used in the reactions such as carbonylation, chloroformylation, chlorination, oxidation of alcohols and dehydration.[14]

Treatment of 5'-O-dimethoxytritylthymidine(**1a**) with phosphonic acid(**2**)(10 eq.), triphosgene(**3**)(3.33 eq.) and triethylamine in acetonitrile slowly gave the expected product **4a**(scheme 1). TLC analysis of the reaction mixture after 12 hr at room temperature showed both **4a** and starting nucleoside. However, after addition of another portion of **3**(1.67 eq) the reaction was found to be complete within 2 hr at room temperature(TLC). A large excess of **2** is required to speed up the reaction and suppress the undesired side reactions.[13] The ^{31}P NMR examination of the reaction mixture indicated the formation of pyrophosphonate(**5**) as a reactive intermediate(δ = -9.0 ppm, $^{1}J_{PH}$ = 628.7 Hz). Stawinski et al. have studied the mechanism of phosphonic acid activation by acid chlorides in detail and have proposed the intermediacy of **5**.[15] Workup of the reaction mixture with dichloromethane and triethylammonium bicarbonate(TEAB) buffer followed by silica gel chromatography[16] gave the pure H-phosphonate **4a**. The aqueous washes removed excess of phosphonic acid and decomposition products of triphosgene completely. The reaction also works well with other nucleosides(table 1).

The following procedure is representative: To a stirring solution of **1a**(0.500g, 0.91 mmol) and **2**(0.753 g, 9.18 mmol) in 16 ml acetonitrile/triethylamine(1:1) was added **3**(0.907 g, 3.06 mmol) in small lots. External cooling is required to maintain the room temperature. After stirring the reaction at room temperature for two hr additional amount of triethylamine(5 ml) and **3**(0.455 g, 1.53 mmol) were added sequentially. The resulting mixture was stirred for two hr at room temperature. The reaction then was quenched with 10 ml 1M TEAB buffer and was stirred for 15 min. The volatiles were evaporated off under reduced pressure and the residue was partitioned between methylene chloride(25 ml) and 0.5M TEAB buffer(25 ml). The organic layer was washed with 0.5M TEAB buffer. The combined aqueous phase was extracted with methylene chloride, and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Silica gel chromatography of the residue gave the product **4a** as a colorless solid (0.481 g, 73.9%).

In summary, we have developed a simple and one-pot procedure to make nucleoside 3'-H-phosphonate monoesters by utilizing triphosgene, an easily handled reagent. The reaction is operationally convenient, offers good yields and the isolation of the product is easy. We believe that this protocol will find use in the other areas like lipids, peptides and carbohydrates.

Scheme 1

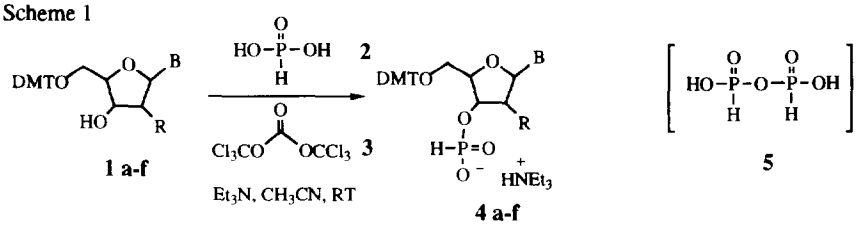
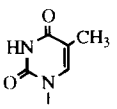
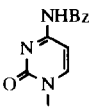
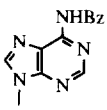
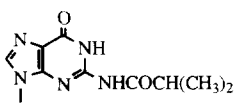
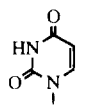
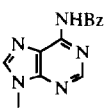


Table 1: Data of the products 4 a-f.

Entry	B	% Yield[a, b, c]	^{31}P NMR($CDCl_3$)[e] δ(ppm), $^{1}J_{PH}$(Hz)
	DNA, R = H		
a		73.9	2.98, 618.8
b		57.8[d]	3.10, 620.3
c		76.5	3.02, 617.6
d		33.8[c,d]	2.46, 620.3
	RNA, R = OMe		
e		74.8	3.57, 628.7
f		60.2	3.51, 626.4

a: All yields correspond to isolated pure compounds. b: Reaction conditions were not optimized. c: Trace amounts of dinucleside H-phosohonate was detected(^{31}P NMR) in crude products in some cases. 5-8% was detected in crude 4d. d: The reactions were conducted at 0 °C(4h) as many products were formed at RT. ~30% of 1b and ~50% of 1d was recovered. e: Analytical data(TLC, ^{1}H and ^{31}P NMR) was found to be identical with that obtained from commercial samples.

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16. 2.5% and 5.0% of methanol in methylene chloride(containing 1% of triethyl amine) was used as eluent.

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