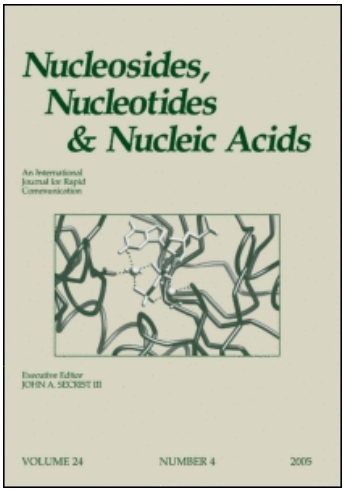


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# Nucleoside Phosphonates. Development of Synthetic Methods and Reagents

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# NUCLEOSIDE PHOSPHONATES. DEVELOPMENT OF SYNTHETIC METHODS AND REAGENTS#

Annika Kers[a], Inger Kers[a], Adam Kraszewski[b], Michał Sobkowski[b], Tomas Szabó[a], Mats Thelin[a¶], Rula Zain[a], and Jacek Stawiński[a]*

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*Abstract:*
*In this paper a short account of our recent research concerning development of new synthetic methods and new reagents for the preparation of DNA and RNA fragments and their analogues is given.*

## INTRODUCTION

The last decade has witnessed a tremendous development in the field of oligonucleotide analogues which is attracting considerable interest due to the emergence of a new kind of therapeutics - antisense[1] and antigene[2] agents. Although a profusion of oligonucleotide analogues, sometimes with ingeniously designed structural variations[2], have been synthesized[3], those which carry a strong resemblance to natural nucleic acids, *eg.*, oligonucleoside phosphorothioate or C-phosphonates, still are in focus of medical research[4-6].

The biological importance and practical significance of phosphorus-containing natural products and their analogues have been the major driving forces for research in

# Dedicated to Professor Yoshihisa Mizuno on the occasion of his 75th birthday.
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various areas of synthetic organic phosphorus chemistry. Though the majority of phosphate esters and their analogues are accessible *via* the well established phosphite[7] and phosphotriester approaches[8], in recent years H-phosphonate methodology emerged as viable alternative[9,10]. It was Todd *et al.*[11,12] who first demonstrated feasibility of the formation of H-phosphonate diesters in a condensation reaction of suitably protected H-phosphonate monoesters with a hydroxylic component, but it is only recently that the synthetic potential of this class of compounds has begun to be appreciated[13] and explored[14-18].

In contradistinction to phosphate analogues, which can be conveniently prepared *via* H-phosphonate intermediates[9], synthesis of C-phosphonate derivatives usually involves lengthy and less efficient routes[19]. A primary rationale of using C-phosphonates as analogues of natural phosphates[19] lies in the fact these compounds, due to the presence of the P-C bond, are usually resistant to enzymatic hydrolysis under conditions used for cleavage of phosphate esters. Despite this favourable property, C-phosphonate derivatives of natural products are less frequently used in biological studies than other phosphate analogues, mainly due to difficulties in their preparation.

In this paper we would like to give a short account of our research on the development of new synthetic methods and new reagents for the preparation of phosphate analogues based on H-phosphonate chemistry, and of our studies directed towards efficient preparation of C-phosphonate derivatives.



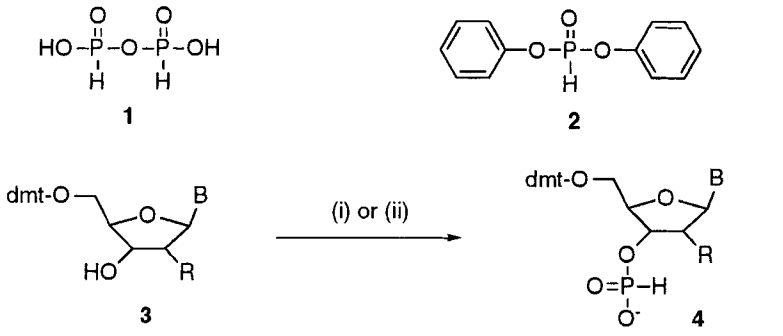
## 1. METHODS FOR THE PREPARATION OF H-PHOSPHONATE AND H-PHOSPHONOTHIOATE ESTERS.

The growing synthetic interest in H-phosphonate chemistry in recent years is probably due to the fact that this chemistry combines advantages of the most important methodologies for the preparation of phosphorus-containing natural products (phosphodiester, phosphotriester and phosphite approaches). The tautomeric equilibrium of mono- or diesters of phosphonic acid, which is practically completely shifted toward the H-phosphonate form, is most favourable from a synthetic point of view. The major advantages of methods based on H-phosphonate intermediates are: (i) starting materials are stable, easy to handle and resistant to air oxidation; (ii) no need for protection of the phosphorus centre which facilitates synchronization of other protecting groups used in a synthesis; (iii) various phosphate analogues can be obtained from one precursor by changing oxidation conditions; (iv) the synthetic procedures are usually time- and cost-effective by comparison with other approaches.

1.1 Preparation of nucleoside H-phosphonate monoesters.

Advances in synthesis of phosphate analogues *via* H-phosphonate intermediates caused high demand for reliable and economical methods for the preparation of nucleoside H-phosphonate monoesters. These have lately been reviewed[9,10].

Among the recently developed methods in our Laboratories are those which make use of the commercial available phosphonylating reagents, namely phosphonic acid and diphenyl H-phosphonate.




(i) **1** in pyridine
(ii) **2** in pyridine, followed by hydrolysis

R = H or t-butyldimethylsilyloxy group ( OTBDMS)

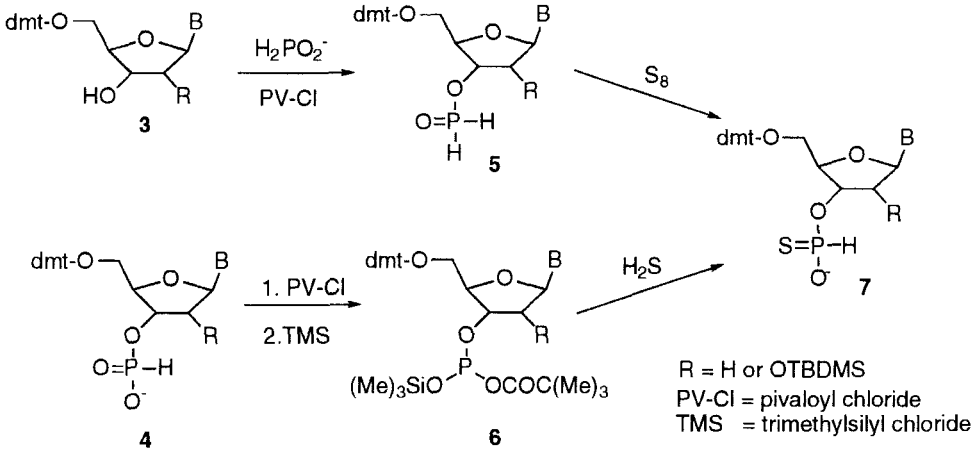
During our studies on the activation pathway of phosphonic acid[20] with condensing agents we found that it was possible to convert phosphonic acid almost exclusively into H-pyrophosphonate **1**, which in contradistinction to other reactive species derived from phosphonic acid, could act as a mild, monofunctional phosphonylating agent. The reaction of **1** with suitably protected deoxyribonucleosides (**3**, R=H) proceeds smoothly affording in ca 90% yield the corresponding 3'-H-phosphonate monoesters **4**[21]. Since 2',5'-protected ribonucleosides react very slowly with **1** (especially those having in the 2'-position a bulky TBDMS group), the reagent probably can be used for a selective phosphonylation of less sterically hindered hydroxyl functions.

We have also developed another phosphonylation procedure based on a commercial available reagent. It consists of transesterification of diphenyl H-phosphonate **2** in pyridine with 5'-protected deoxyribo- or 2',5'-protected ribonucleosides **3**[22] which produces nucleoside phenyl H-phosphonates as intermediates. These are very susceptible to hydrolysis under mild basic conditions and readily afford the nucleoside 3'-H-phosphonates **4** in 80-90% yield.

Taking into account experimental simplicity, mild reaction conditions, and high yields of the H-phosphonate monoesters, both methods can be considered as general procedures for the preparation of this class of compounds.

1.2. Preparation of nucleoside H-phosphonothioate monoesters.

Nucleoside H-phosphonothioates **7** are interesting from a synthetic point of view since they provide an entry to some phosphate analogues inaccessible *via* H-phosphonates **4**. Simple alkyl H-phosphonothioates can be obtained by alkaline hydrolysis of the corresponding diesters[23], however, natural product derivatives having H-phosphonothioate function are most conveniently prepared by one of the methods developed in our Laboratories[24,25].



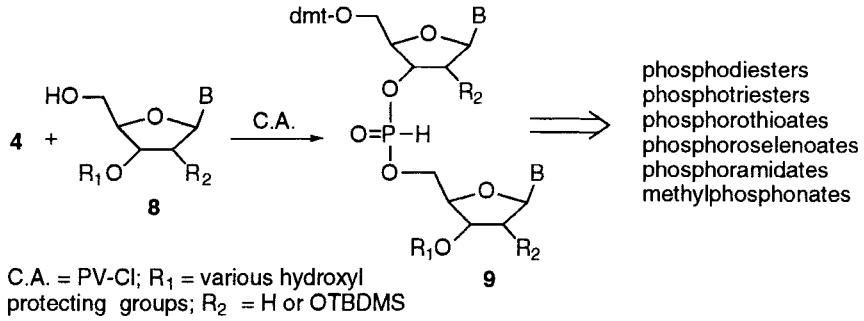

Suitably protected nucleosides **3** can be transformed to the H-phosphonothioates **7** *via* phosphinate intermediates **5**. The procedure involves condensation of nucleosides **3** with triethylammonium phosphinate in the presence of a condensing agent, followed by oxidation of the nucleoside phosphinate **5** with elemental sulfur. Deoxyribo- and ribonucleoside 3'-H-phosphonothioates **7** can be obtained in this way in 80-90% yield[24]. The method is experimentally simple, fast and reliable. The reagents are stable and commercially available.

One can also envisage an approach consisting of the conversion of the H-phosphonate function, which is already present in a molecule, into an H-phosphonothioate. This proved to be non-trivial transformation since during attempted activation of H-phosphonates **4** with a condensing agent, followed by treatment with hydrogen sulfide, the corresponding H-phosphonodithioate derivatives usually are formed as major products[25,26]. To overcome this problem we have developed a

procedure for generation of the silyl acyl phosphites **6**[25] from H-phosphonate monoesters. This intermediate affords the silylated nucleoside H-phosphonothioates upon treatment with hydrogen sulfide, which after aqueous work-up produce the desired products **7** in 80-90% yield[27]. The procedure can be further simplified by using 1,1,1,3,3,3-hexamethyldisilthiane as a silylating agent and source of hydrogen sulfide[27].

1.3. Preparation of nucleoside H-phosphonate diesters.

Formation of H-phosphonate diesters from the corresponding monoesters in condensation reactions probably is the most common application of the H-phosphonate methodology. Diesters of phosphonic acid are important intermediates for the preparation of variety of phosphorus-containing natural products and their analogues, *e.g.*, oligonucleotides[10], nucleopeptides[28,29], phosphorylated carbohydrates[30,31], phospholipids[32,33].




Easy preparation of the starting materials, lack of phosphate protecting group, versatility, and experimental simplicity provide the underlying allure of the H-phosphonate methodology. Due to the very fast condensation, side reactions usually associated with the use of condensing agents (*e.g.*, acylation, sulfonation) can practically be neglected, at least during "solution synthesis".

The principle of the method is shown in the scheme above. It consists of acid chloride (carboxylic, phosphoric or arylsulfonic) promoted condensation of an H-phosphonate monoester with a suitably protected hydroxylic component **8**, followed by oxidation (usually *in situ*) of the formed H-phosphonate diester **9** to produce various phosphodiesters or their analogues. The most important features of the synthetic scheme concerning oligonucleotide synthesis have been discussed in detail elsewhere[10].

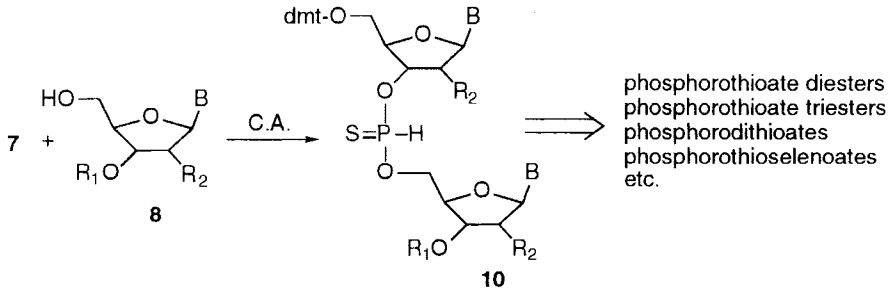
The H-phosphonate approach is gaining interest in oligonucleotide analogue synthesis. It is worth mentioning, that in contradistinction to other synthetic methods for oligonucleotides, the H-phosphonate methodology seems to be even more suited for

RNA than for DNA synthesis[10] due to differences in the kinetics of activation of deoxyribo- and ribonucleoside 3'-H-phosphonate monoesters. Basic studies in oligonucleotide synthesis *via* the H-phosphonate approach provide a firm ground for further development of the method. For example, replacement of pyridine by the less basic quinoline has recently been advocated[34] to have a beneficial effect on the synthesis on a solid support. Also the use of di(pentafluorophenyl) carbonate, instead of pivaloyl chloride, as a condensing agent was claimed[35] to noticeably improve quality of the synthesised oligonucleotides. Studies on synthesis of oligoribonucleotides showed a significant influence of the nature of 2'-protecting groups on yields of the isolated products[36]. The best results were obtained with 2'-TBDMS or 2-chlorobenzoyl groups, while the acetal-type of protecting groups were noticeably inferior[36].

1.4. Preparation of nucleoside H-phosphonothioate diesters.

Nucleoside H-phosphonothioate diesters **10** are useful synthetic intermediates for analogues bearing sulfur and an additional heteroatom (*e.g.*, O, S, Se) in the non-bridging positions of a phosphodiester function. These compounds (**10**) are accessible[37-39] *via* the condensation of the appropriate H-phosphonomonothioate **7** with a hydroxylic component in the presence of a condensing agent. As a starting material one can also use H-phosphonodithioate monoesters[40], however, the coupling is usually slow and rather inefficient[37].

In spite of apparent similarities to H-phosphonate diesters, the P-H bond in the thio derivatives **10** is more reactive and markedly susceptible to some side reactions. For these reasons, the condensations of H-phosphonothioates with hydroxylic components have to be performed under different reaction conditions, preferably using chlorophosphates as condensing agents[38].




C.A. = chlorophosphates; $R_1$= various hydroxyl protecting groups; $R_2$ = H or OTBDMS

The synthetic development of H-phosphonothioate diesters is far less advanced than that of H-phosphonates. From our preliminary studies, the following findings

emerged[37,38]. The rate of condensation of H-phosphonothioate monoesters with nucleosides **8** is comparable to that of H-phosphonate esters. The products of the condensation, the H-phosphonothioates **10**, may undergo subsequent activation with pivaloyl chloride (PV-Cl), which can result in the formation of phosphite triesters and loss of sulfur. PV-Cl also reacts readily with **10** to give the P-acylated products. Replacement of PV-Cl with chlorophosphates (*e.g.*, diphenyl phosphorochloridate or 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane **25**) as condensing agents, eliminates these side reactions. The H-phosphonothioates **10** were also found to be more easy to oxidize with elemental sulfur or with selenium, which is in agreement with the observed rather high reactivity of their P-H bonds[37].

## 2. Reagents for the Conversion of Nucleoside H-Phosphonates and H-Phosphonothioates into Phosphate Derivatives and their Analogues

Though H-phosphonate mono- and H-phosphonate diesters contain phosphorus at the same oxidation state as phosphite triesters (oxidation state +3), there are fundamental differences between these two classes of phosphorus acid derivatives. While phosphite triesters have a lone pair of electrons on the phosphorus atom which dominates the chemistry of these compounds, mono- and diester of phosphorus acid lack this structural element due to the preponderance of the phosphonate form. In consequence, many reactions of these compounds, including the most important one, the oxidation, show different kinetics and involve different catalysis, depending on the degree of esterification. Although a plethora of oxidizing reagents for P(III) compounds, stimulated by the development of the phosphoramidite approach to oligonucleotide synthesis[7,41], has been proposed, their application to H-phosphonate derivatives often requires either modification of the reagents or/and the reaction conditions[42,43].

For these reasons, a significant part of our research has been devoted to the development of new reagents and new reaction conditions which would meet the special requirements of H-phosphonate esters.

### 2.1. Sulfurizing, selenizing, and oxidizing agents based on 3*H*-benzothiol derivatives.

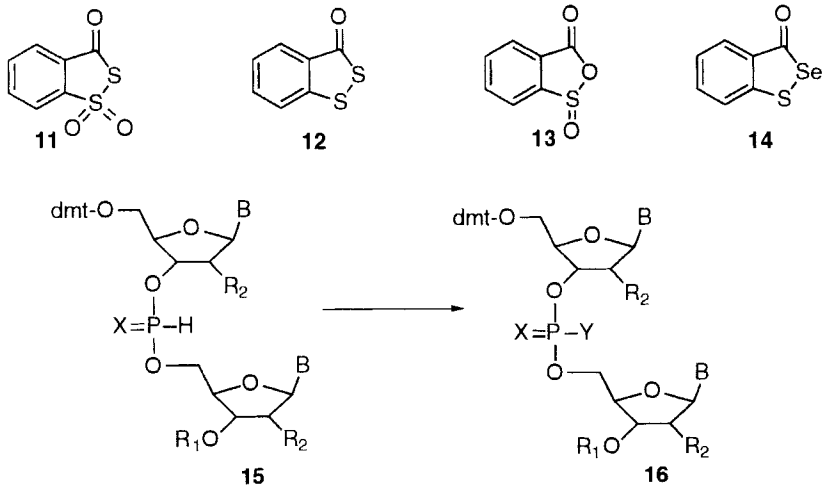
Oligonucleoside phosphorothioates[44] and phosphorodithioates[45] are two important classes of oligonucleotide analogues. Favourable chemical and biological properties, *e.g.*, resistance to nucleases, formation of stable duplexes with RNA and DNA, may constitute a basis for further chemotherapeutic application of these compounds[46]. During preparation of phosphorothioates and phosphorodithioates *via* the phosphoramidite[7] or *via* the H-phosphonate approach[10], the important synthetic step is sulfurization. Though elemental sulfur is often used for this purpose, there are several disadvantages (*e.g.*, low solubility), associated with its use in machine-assisted

syntheses. Recently, several sulfurizing reagents with good solubility in organic solvents have been proposed for oligonucleotide analogues synthesis[7].

In conjunction with our research in H-phosphonate chemistry, we investigated properties of 3*H*-1,2-benzodithiol-3-one 1,1-dioxide[47] **11** as a sulfur-transferring reagent for H-phosphonate and H-phosphonothioate diesters[42]. In contradistinction to clean and fast sulfurization of phosphite triesters with this reagent, we have observed the formation of significant amounts of O-oxidized products during its reaction with H-phosphonate (**15a**) and H-phosphonothioate (**15b**) diesters. Detailed studies revealed, that this phenomenon is due to generation of O-oxidizing agents (*inter alia*, **13**) during the course of sulfurization[43]. Since phosphite triesters and H-phosphonate derivatives **15** show different kinetic of sulfurization, a competing oxidation is observed for the H-phosphonate but not for the phosphite derivatives.

We have proposed two remedies to this problem. Since oxidizing species generated during sulfurization with **11** are susceptible to hydrolysis, we have developed aqueous reaction conditions that practically eliminate formation of O-oxidized products[43]. As an alternative approach, we have developed 3*H*-1,2-benzodithiol-3-one **12** as sulfurizing reagent for H-phosphonate and H-phosphonothioate diesters[43]. This reagent, which is in fact a starting material in the preparation of **11**, does not have oxygen atoms attached to the sulfur, and thus cannot generate O-oxidizing species during the course of sulfurization. In this respect, it can be used for sulfurization of both phosphite and H-phosphonate derivatives. The sulfurization of H-phosphonates **15a** by using the reagent **11** or **12** was found to proceed with retention of configuration at the phosphorus centre[42,43].





The nucleoside phosphorothioate diesters can be obtained either by sulfurization of the corresponding H-phosphonate esters (*e.g.*, **15a**) or by oxidation of H-phosphonothioates (*e.g.*, **15b**). Although various oxidizing agents have been investigated for the purpose of oligonucleotide synthesis *via* the H-phosphonate approach[48], the most commonly used procedure involves oxidation with iodine under aqueous reaction conditions[48,49].

During our studies on the oxidation of H-phosphonate derivatives we have developed a new reagent, 3*H*-2,1-benzoxathiol-3-one 1-oxide **13**, which can efficiently perform oxidation of **15a** or **15b** under anhydrous conditions[50]. The oxidizing agent **13**, which we have previously found to be responsible for competitive oxidation during sulfurization of H-phosphonates with **11**, can conveniently be prepared from the later reagent *via* the disproportionation reaction. Oxidation of diastereomerically pure nucleoside H-phosphorothioate diesters **15b** with **13** was found to be stereospecific and occurred with retention of configuration[50].



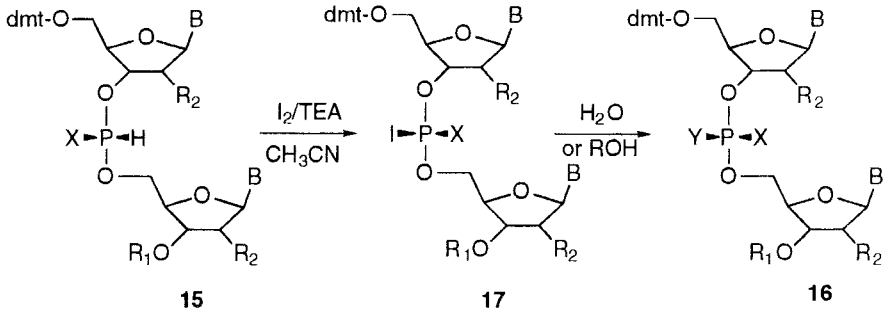
The close resemblance of selenium to sulfur constitutes a strong rationale for the potential therapeutic importance of selenium-containing natural product analogues[51]. Phosphoroselenoate derivatives are usually accessible *via* selenization of phosphite triesters[52] or H-phosphonate diesters[32]. As a source of electrophilic selenium in such reactions elemental selenium or potassium selenocyanate is commonly used. Unfortunately, low solubility in organic solvents, low reactivity, especially toward H-phosphonate derivatives, and often heterogeneous reaction conditions, are the major disadvantages of these reagents.

To overcome these problems, we have developed a new reagent, 3*H*-1,2-benzothiaselenol-3-one **14**[53,54], with good solubility in common organic solvents and with enhanced rate of selenium transfer compared to elemental selenium or potassium selenocyanate. The benzothiaselenol **14** efficiently converts H-phosphonates **15a** or H-phosphonothioates **15b** to the corresponding phosphoroselenoates **16d** or phosphorothioselenoates **16e**. The selenium transfer was found to be stereospecific and occurred with retention of configuration at the phosphorus centre[54]. The reaction conditions are compatible with solid phase synthesis of oligonucleotide. The reagent also proved to be effective in the conversion of phosphite triesters to the corresponding phosphoroselenoates[54].

2.2. Reaction conditions for stereospecific oxidation under aqueous conditions and for stereospecific oxidative coupling.

Oxidation of H-phosphonate and H-phosphonothioate diesters **15** in a stereospecific manner can provide a convenient entry to stereochemically pure

phosphotriesters, oxygen labelled phosphodiesters, or other chiral phosphate analogues. Unfortunately, studies on the oxidation of H-phosphonate diesters **15a** with iodine/[$^{18}$O]$H_2O$ in pyridine showed that this reaction is stereoselective[55] rather than stereospecific (d.e. 30-50%). Also, oxidation of H-phosphonothioates **15b** with iodine in pyridine/water (98:2, v/v), results in extensive epimerization at the phosphorus centre during the course of the reaction[56]. Since the substrates **15b** and the products **16b** are configurationally stable under the reaction conditions, we assumed that lack of stereospecificity in the oxidation is probably due to a pyridine catalyzed epimerization of the phosphorothioiodidate **17**.




$R_1$ = various hydroxyl protecting groups; $R_2$ = H

**15a**, X = O **17a**, X = O **16b**, X=S, Y=O$^-$; **16f**, X=S, Y=OEt
**15b**, X = S **17b**, X = S **16g**, X=O, Y=OEt

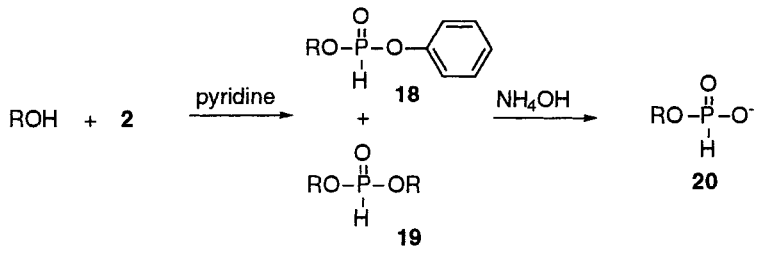
Indeed, when the reaction was carried out with iodine in aqueous acetonitrile (2:98, v/v) in the presence of triethylamine, the oxidation occurred with virtually complete stereospecificity and inversion of the configuration[56]. Also oxidative coupling of **15a** or **15b** with ethanol in acetonitrile in the presence of triethylamine occurred with complete stereospecificity affording pure diastereomeric products **16g** or **16f**, respectively. The analogous reactions in pyridine proved to be stereoselective, yielding one diastereomer in preponderance irrespective of the configuration of the starting material[56].

Since oxidation using **13** occurs with retention while iodine in acetonitrile in the presence of triethylamine gives inversion of configuration, it is possible to obtain both diastereomers of **16b** from one diastereomer of H-phosphonothioate **15b**, depending on the oxidation procedure chosen.

2.3. Alkyl H-phosphonate monoesters

Simple alkyl H-phosphonate monoesters **20**, which are usually crystalline, stable solids, seem to be most interesting as potential phosphonylating reagents. From this point of view, those bearing alkyl groups with electron-withdrawing substituents at the β-carbon can be particularly useful, *e.g.*, 2-cyanoethyl, 2,2,2-trichloroethyl, 2-(p-

nitrophenyl)ethyl. Compounds **20** can be used for transferring of H-phosphonate group to natural product derivatives by their condensation with an appropriate hydroxylic component, followed by the removal of the alkyl group from an asymmetric H-phosphonate diester intermediate. Alternatively, one can take advantage of an easy oxidation of H-phosphonate diesters, and then transform the asymmetric H-phosphonate intermediate to synthetically useful phosphate diesters, monoesters, or their analogues.




R=e.g., 2-cyanoethyl, 2,2,2-trichloroethyl, 2-(*p*-nitrophenyl)ethyl, allyl, benzyl



A major obstacle for wider synthetic applications of alkyl H-phosphonates **20** is the apparent lack of a simple and convenient method for their preparation. To overcome this difficulty, we have developed[57] a general procedure for the synthesis of H-phosphonate monoesters **20**. It consists of a "one-pot" reaction of the commercial available diphenyl H-phosphonate **2** with an appropriate alcohol, followed by ammonolysis of the *in situ* formed dialkyl (**19**) and alkyl phenyl (**18**) H-phosphonates. The H-phosphonates **20** with a variety of simple alkyl groups can be prepared by this methods in good yield[57]. The products are usually of purity > 98% and can be used in phosphonylation reactions without additional purification.

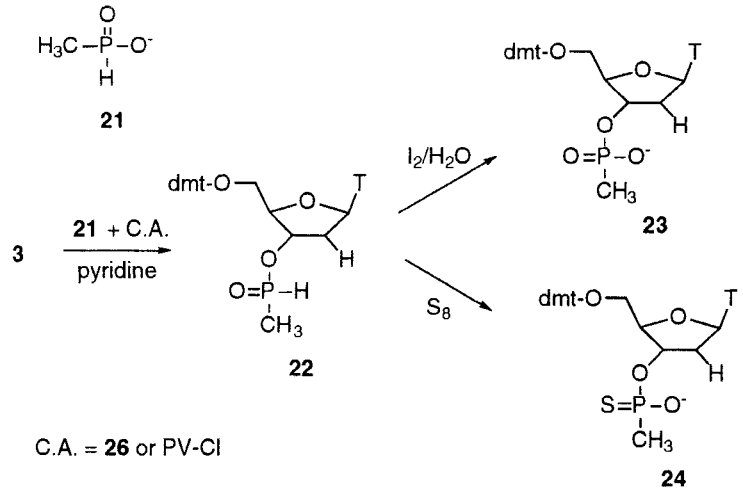
## 3. METHODS FOR THE PREPARATION OF C-PHOSPHONATES.

Replacement of one oxygen atom in the internucleotidic phosphodiester bond by carbon leads to C-phosphonate analogues of oligonucleotides. Though all compounds of this class bear one C-P bond and are collectively referred to as C-phosphonates, in fact they form two very distinctive groups. The replacement of a non-bridging oxygen by carbon introduces a new chiral centre to an oligonucleotide and simultaneously eliminates the negative charge while the substitution of a bridging oxygen by carbon, preserves most of the chemical and stereochemical features of the phosphodiester linkage (charged, achiral functionality). To make a distinction between non-ionic, chiral, C-phosphonate analogues and those ionic, achiral ones, we will refer to the former as alkylphosphonates and the latter ones, as methylenephosphonates. Synthetic methods, chemical, and

biological properties of C-phosphonate analogues of natural products have been reviewed on several occasions[19,58].

3.1. Preparation of nucleoside methylphosphonates and their analogues *via* phosphinate intermediate.

In recent years non-ionic oligonucleotide analogues having chiral methylphosphonate or methylphosphonothioate internucleotidic linkages attracted considerable attention as potential antisense drugs[58]. As a part of our research in this field, we have developed a new method for the preparation of nucleoside methylphosphonate **23** and nucleoside methylphosphonothioates **24** according to the scheme below[59].

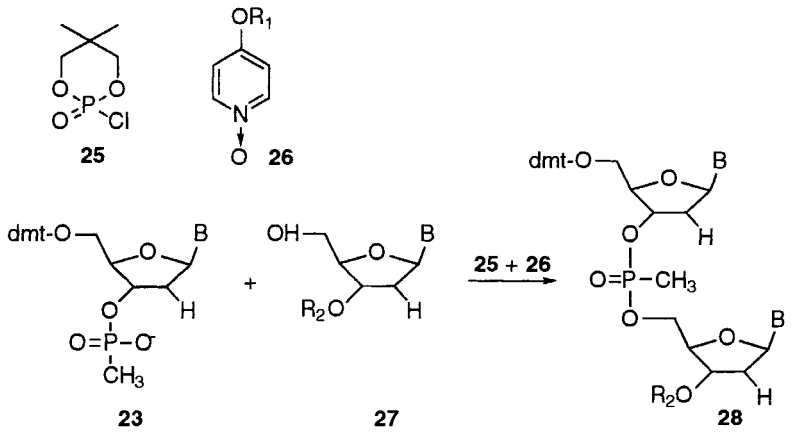



The key synthetic intermediate in this approach is the nucleoside methylphosphinate **22**, accessible *via* condensation of a hydroxylic component **3** with the ammonium salt of methylphosphinic acid **21**. Methylphosphinate **22** is not isolated but converted *in situ* to the nucleoside methylphosphonate **23** *via* oxidation with iodine, or to the nucleoside methylphosphonothioate **24** by sulfurization. Though the oxidation reactions of **22** are rather sluggish, the products **23** and **24** are obtained in rather high yields (~90%)[59] after silica gel column chromatography.

3.2. Preparation of methylphosphonate diesters using a new coupling reagent system.

Methylphosphonate diesters are most often prepared *via* phosphonite [P(III)] intermediates[60] or *via* P(V) derivatives having an activated methylphosphonate function[61,62]. Procedures employing coupling agents, though more convenient than those

involving activated phosphonates, have not found wider applications[63,64]. This is probably due to the inherently lower reactivity of methylphosphonate monoesters compared to that of alkyl aryl phosphodiesters, which usually causes the condensations to be slow and, in consequence, competing side reactions of a condensing agent with a hydroxylic component are usually very much pronounced.




$R_1$ = Et or Me; $R_2$ = various hydroxyl protecting groups

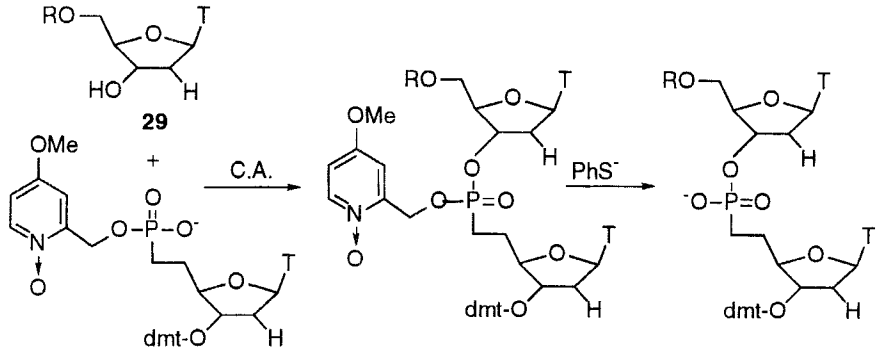
Investigating this problem in more detail we have found that side reactions which are responsible for low yield of condensations of methylphosphonates **23** with nucleosides vary significantly, depending on the combination of condensing agent and nucleophilic catalyst used. Usually, a mild condensing agent in conjunction with a powerful nucleophilic catalyst gave better coupling yields than a reactive coupling agent and mild catalyst. On this basis we designed the coupling reagent system consisting of 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane **25** and a 4-alkoxypyridine-1-oxide (**26**, $R_1$ = Et or Me) which secured relatively fast coupling (~30 min) without detectable formation of side products[65,66]. The procedure was used for the preparation of various dinucleoside methylphosphonates for physicochemical studies[67,68].

The chlorophosphate **25**, which can be prepared[69] in a crystalline state in high yield, has previously been used as a condensing agent[70] in H-phosphonate diester synthesis. This compound is very unreactive towards alcohols[69] which may explain why the reagent system shows a favourable ratio of the rate of condensation and the rate of (undesired) phosphorylation of nucleosidic component during the coupling reactions.

3.3. Preparation of 5'-methylenephosphonates using an intramolecular catalytic phosphonate protecting group.

The problem of chirality at the phosphorus centre in nucleoside alkylphosphonate diesters can be circumvented by placing the P-C bond in the bridging position of the

phosphonate group. This will produce ionic, achiral 3'- or 5'-methylenephosphonate analogues of oligonucleotides (*e.g.*, **32**). Our preliminary structural studies at the dimer level[71] indicate that replacement of the C5'-oxygen by carbon does not introduce notable conformational changes to the ribose rings. It causes, however, significant changes in the distribution of rotamers around the C4'-C5' bond[71], probably due to the absence of the *gauche* effect[72]. Although this kind of analogues have been known for a rather long time[73], they have not received much attention[74,75].




C.A. = 2,4,6-triisopropylbenzenesulfonyl chloride (TPS-Cl) or **25**

Considering the high resemblance of oligonucleoside methylenephosphonates to natural oligonucleotides on the one hand, and the presence of non-hydrolyzable, nuclease resistant P-C bond on the other, we have recently embarked on the development of this type of analogues as potential antisense agents[76-78].

In contradistinction to nucleoside methylphosphonates, in the 5'-methylenephosphonates the P-C bond is an integral part of the nucleoside system and thus two hydroxyl groups at the phosphorus centre are available for esterification. This allowed us to design a synthetic approach which overcomes the problems connected with the low reactivity of C-phosphonates[76,78]. The approach is similar to that previously proposed in phosphotriester chemistry[79,80] and is based on the use of a phosphonate protecting group, 4-methoxy-1-oxido-2-picolyl, enabling intramolecular nucleophilic catalysis. The efficacy of the approach was evaluated in the solid phase synthesis of oligo(thymidin-5'-yl) methylphosphonates. Using the phosphonate component with the catalytic group **30** and TPS-Cl or the chlorophosphate **25** as a condensing agent, several thymidine oligonucleotide analogues **32** with a chain length of up to 20 nucleotidic units have been synthesised[77]. Studies are in progress to optimize and to extend the methodology to the preparation of heterosequences.

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