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How To Get the Most Out of Two Phosphorus Chemistries. Studies on H-Phosphonates

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ABSTRACT

The biological importance and practical significance of phosphate esters and their analogues have been the major driving forces for research in various areas of synthetic organic phosphorus chemistry. In this Account, the authors' studies on the development of a comprehensive H-phosphonate methodology and the underlying chemistry for the preparation of biologically important phosphate esters and their analogues are briefly discussed.

I. Introduction

In the past two decades, advances in our knowledge regarding the flow of genetic information, DNA → mRNA → protein, resulted in the emergence of two powerful experimental techniques: antisense[1] and antigene[2] approaches for modulation of gene expression. Both techniques hold the promise of a novel therapy for hard-to-treat diseases (including those associated with human genetic disorders) by making use of synthetic oligonucleotide analogues for blocking access to genetic information in mRNA (antisense approach) or in DNA (antigene approach). Although the idea of an antisense and antigene therapy seems simple, a practical solution to this problem is exceedingly complex. In 1998, a cumulative effort of chemists, biochemists, biologists, and clinical physicians culminated in the development and the approval for marketing of the first drug based on an antisense therapy for the treatment of cytomegalovirus-induced retinitis in AIDS patients, Isis' Vitravene (ISIS 2922).

The key role in antisense/antigene therapies is played by a therapeutic agent, usually a synthetic oligonucleotide that targets templates from which a harmful protein is synthesized rather than the protein itself. Due to high specificity of formation of stable complexes with complementary RNA and DNA sequences, oligonucleotides appear as a new generation of drugs with potentially higher selectivity and with fewer side effects than conventional drugs. Since oligonucleotides with natural 3′−5′ internucleoside phosphodiester linkages undergo rapid degradation in cell, nuclease-resistant analogues were required for the antisense and antigene therapies. Although a profusion of oligonucleotide analogues, sometimes with ingeniously designed structural variations, have been synthesized for this purpose,[3] those bearing modifications at phosphorus centers became the main focus of medical research.[4−6] During recent years, the therapeutic importance of oligonucleotide analogues constituted a strong incentive for bioorganic phosphorus chemistry to develop new, efficient methodologies for the preparation of novel phosphate derivatives and also to set new challenges for synthetic organic chemistry in general, and phosphorus chemistry in particular.

To synthesize a phosphorus-containing compound with a given structural feature, chemists have two kinds of phosphorus chemistries at their disposal, referred to as P(V) and P(III) chemistry. The choice between these two is often not easy, as both of them have their own strong merits.

II. Reactivity of P(V) vs P(III) Phosphorus Compounds

Three classes of phosphorus compounds that are most frequently used in the synthesis of biologically important phosphate esters and their analogues are shown in Scheme 1. These are tetracoordinated P(V) compounds of type A (oxidation state +5), tricoordinated P(III) compounds of type B (oxidation state +3), and tetracoordinated P(III) compounds of type C (oxidation state +3).

Factors that govern the reactivity of P(V) and P(III) compounds are most diverse. P(V) compounds of type A (e.g., phosphate esters) have tetrahedral geometry, and their chemistry is dominated by the presence of a very stable phosphoryl group (P═O), for which formation often is a driving force for reactions. The phosphorus atom in A is a hard, electrophilic center and is subject to reactions with hard nucleophiles. Conversely, P(III) compounds of type B (e.g., phosphite triesters) have the shape of a trigonal pyramid with a lone electron pair located on the phosphorus atom. Due to this, the phosphorus center in these compounds is basic and is a soft nucleophile that may react with various (preferably soft) electrophiles. However, when the phosphorus center in B is protonated,

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Adam Kraszewski was born in 1947 in Kobylec, Poland. He studied chemistry at A. Mickiewicz University, Poznan, and obtained his Ph.D. degree (Prof. M. Wiewiórwski) from the Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw. His postdoctoral stay (1977−1978) in City of Hope, Duarte, USA (with Dr. K. Itakura), involved the first chemical synthesis of the human insulin gene and its expression in *E. coli*. From 1978 until now, he has carried out his work at the Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland, in 1985 becoming associate professor and in 1999 full professor. His main research interests include the chemistry, synthesis, and structure of nucleotides, oligonucleotides, and their analogues.

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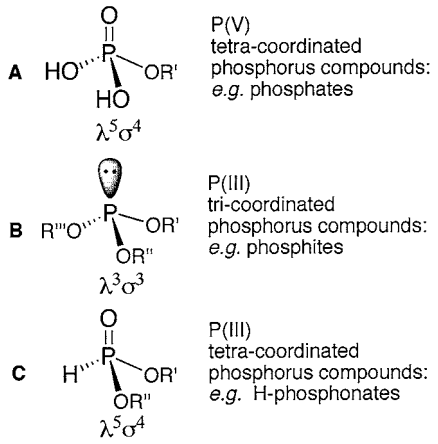
Scheme 1. Different Types of Phosphorus Compounds: Phosphates, Phosphites, and H-Phosphonates[a]



[a] Indices λ and σ stand for the valency and the coordination number of the phosphorus atom, respectively.

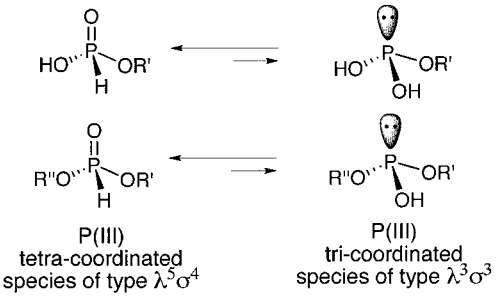
or when it bears electron-withdrawing substituents, such P(III) compounds may also react with nucleophiles. The reactions with electrophiles and nucleophiles are both rapid and make P(III) derivatives an attractive starting material in the synthesis of various phosphorus compounds, although they are often difficult to handle.

The third class of phosphorus compounds shown in Scheme 1, P(III) derivatives of type C, is unique. Due to the presence of a phosphoryl group (P═O) and the tetrahedral structure, compounds C bear strong resemblance to P(V) derivatives of type A, but the oxidation state +3 clearly relates them to P(III) compounds B. However, in contrast to the latter, they are distinct in that they lack a lone electron pair on the phosphorus center.

A feature which is unique for compounds of type C and is usually emphasized in their names (H-phosphonates) is the presence of a P−H bond. This class of phosphorus compounds is less numerous than classes A and B, and its chemistry has been less explored. Since compounds C are structurally related to P(V) derivatives A, one can predict that the phosphorus center in H-phosphonate derivatives should be electrophilic, and the compounds, although harboring the phosphorus atom in the +3 oxidation state but lacking of a lone pair of electrons, will be less prone to oxidation than P(III) derivatives of type B.

The wide range of structural types of phosphorus compounds has led to the increasing use of two descriptors for their classification: the valency term λ and the coordination number σ. According to this notation, compounds of type A are quinquevalent, tetracoordinated phosphorus derivatives ($\lambda^5\sigma^4$), compounds of type B are tervalent, tricoordinated derivatives ($\lambda^3\sigma^3$), and compounds of type C are quinquevalent, tetracoordinated phosphorus compounds($\lambda^5\sigma^4$). One should note that these structural indices for species of type A and C are identical and indicate that, although the compounds belong to different classes, they share common structural features, and, as a consequence, some of their chemical properties can be similar.

Scheme 2. Tautomeric Equilibria in H-Phosphonate Derivatives



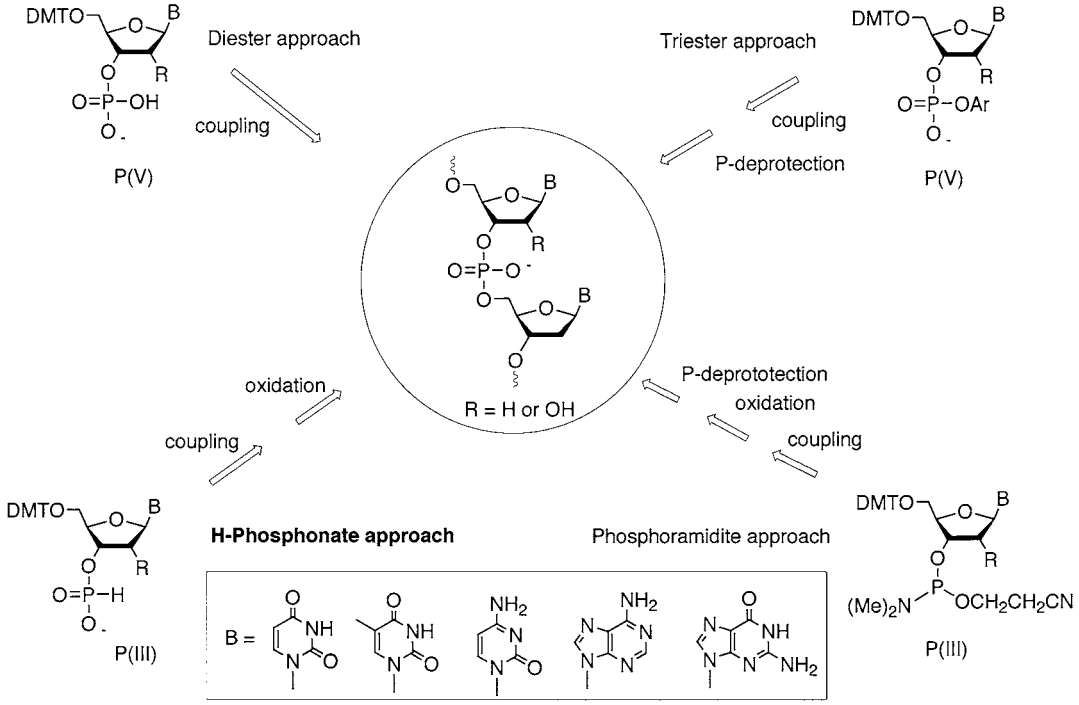
III. Why H-Phosphonates?

Although tervalent P(III) derivatives of type B are frequently used in the synthesis of phosphorus compounds, their high reactivity makes them susceptible to spontaneous oxidation (even by atmospheric oxygen) and hydrolysis upon storage. In marked contrast to this, P(V) compounds of type A are stable during storage and convenient to handle. However, they have disadvantages of typically being less efficient in synthetic transformations, as they react significantly more slowly (even upon activation with condensing agents) than tervalent P(III) derivatives. Thus, from the point of view of a synthetic chemist, it would be ideal to deal with compounds that are stable on the shelf and convenient to manipulate but that become highly reactive (preferably toward both nucleophiles and electrophiles) when placed in a reaction flask. These requirements, which may sound like wishful thinking, are in fact met by class C of the phosphorus compounds shown in Scheme 1, H-phosphonates.

H-Phosphonate derivatives, having a phosphorus atom in the +3 oxidation state, can be easily converted into various P(V) derivatives using different oxidizing reagents (e.g., iodine/water, elemental sulfur, elemental selenium, etc.). However, since they lack an electron pair on the phosphorus center, they are at the same time significantly more resistant to spontaneous oxidation than tervalent P(III) derivatives and are also more stable under acidic conditions.

Similar to P(V) compounds, the phosphorus atom in H-phosphonates is an electrophilic center; however, this nature can be modified so as to provide a nucleophilic phosphorus center. This is due to the fact that H-phosphonate derivatives in solution exist as an equilibrium mixture of two tautomeric forms:[7,8] a tetracoordinate phosphonate form $\lambda^5\sigma^4$ [which resembles P(V) derivatives] and a tricoordinated phosphite form $\lambda^3\sigma^3$ [which is a typical tervalent P(III) derivative] (Scheme 2). Since these equilibria are usually heavily shifted to the left,[9] it is the phosphonate form that determines the overall reactivity of these compounds. The phosphonate−phosphite equilibria, however, can be shifted to the right by fixing a phosphite form with a suitable reagent (e.g., with a silylating agent), and thus causing the nucleophilic nature of the phosphorus center to become predominant. Since the phosphite form also retains the soft electrophilic phosphorus center typical for tervalent P(III) compounds, H-phosphonates can react with both soft nucleophiles

Scheme 3. Methods for Oligonucleotide Synthesis



(utilizing a phosphite form) and hard nucleophiles (via a tetracoordinate H-phosphonate form).

Although H-phosphonate derivatives have been present in chemical laboratories for more than a century, somewhat surprisingly they did not attract much attention from synthetic chemists. After pioneering work by Michaelis and Becker[10] and later by Nylén[8,11] on the structure determination of phosphonic acid and the simple alkyl derivatives, these compounds have only been sporadically used in synthesis. Most notable are the early studies by Todd and co-workers,[12,13] who in the mid-1950s briefly investigated the chemistry of these compounds for possible applications in nucleic acid synthesis and even prepared a 5′,5′-dinucleoside phosphate in this way.

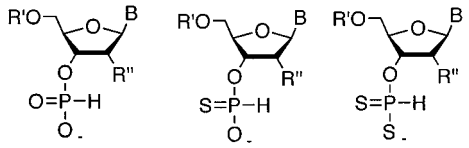
Unfortunately, the synthetic potential of H-phosphonates had not been appreciated at that time and the methodology did not gain popularity as a means of preparing phosphorus compounds. Instead, P(V) chemistry dominated the early phase of chemical synthesis of nucleic acids, and other biologically important phosphate esters (the phosphodiester and phosphotriester approaches to oligonucleotide synthesis, Scheme 3) as tetracoordinate P(V) compounds appeared more easy to work with than P(III) derivatives. In the late 1970s, when this chemistry had apparently reached its limits, methodologies based on P(III) chemistry entered the stage (the phosphoramidite and H-phosphonate approaches, Scheme 3) as more efficient and versatile means for synthesising oligonucleotides and their various analogues that were difficult to prepare using P(V) chemistry.

For our part, we became interested in H-phosphonate chemistry in the middle of the 1980s, when we attempted (in vain) to convert nucleoside H-phosphonate diesters (that are usually formed as side products during the syntheses of oligonucleotides via the phosphoramidite approach) into the desired nucleoside phosphite triester products. Since H-phosphonate diesters turned out to be rather stable compounds that could be purified by silica gel chromatography, we set out to explore this class of compounds as possible intermediates in the synthesis of phosphate esters and their analogues.

The initial allure of nucleoside H-phosphonate diesters as intermediates in the synthesis of oligonucleotides[14] and their analogues was that, being uncharged and rather stable, they resembled phosphate triesters, but with one important difference: there was no phosphate protecting group in these compounds. Due to favorable phosphonate−phosphite equilibria (Scheme 2), the hydrogen atom attached to the phosphorus center in these compounds might be perceived as being the smallest possible protecting group. It is lost during oxidation, and thus the corresponding phosphodiesters (or their analogues) can be produced directly from H-phosphonate intermediates.

However, there is much more to H-phosphonate diesters than merely being "phosphotriester-like" compounds. As the basic studies on H-phosphonate derivatives progressed,[15−18] a more complete picture of the synthetic potential of these compounds unfolded.[19,20] Today, it is apparent that H-phosphonates possess numerous traits that lend themselves to a powerful methodology for the preparation of biologically important phosphate esters and their analogues. Apart from those mentioned above, other synthetically important features of this class of phosphorus compounds are the following: (i) the coupling rate of H-phosphonate monoesters to the corresponding diesters is high and comparable to that of phosphoramidite derivatives; (ii) an array of condensing agents can be used to promote the condensation; (iii)

Scheme 4. H-Phosphonate Monoesters and Their Analogues



R' = a protecting group, e.g. 4,4'-dimethoxytrityl
R" = H or OTBDMS
B = a nucleobase

products of the condensation, H-phosphonate diesters, are usually resistant to further activation by condensing agents, and thus formation of side products is suppressed; (iv) H-phosphonate diesters are configurationally stable and their oxidation to P(V) derivatives as well as their conversion into the corresponding phosphite forms is usually stereospecific; (v) by changing oxidation conditions, various phosphate analogues can be produced from the same intermediates; (vi) H-phosphonate derivatives, due to the presence of the P−H bond, can be used as substrates in palladium(0)-catalyzed phosphorus−carbon bond formation; (vii) multiple modifications at the phosphorus center can be introduced by a good choice of starting materials and oxidation protocols.

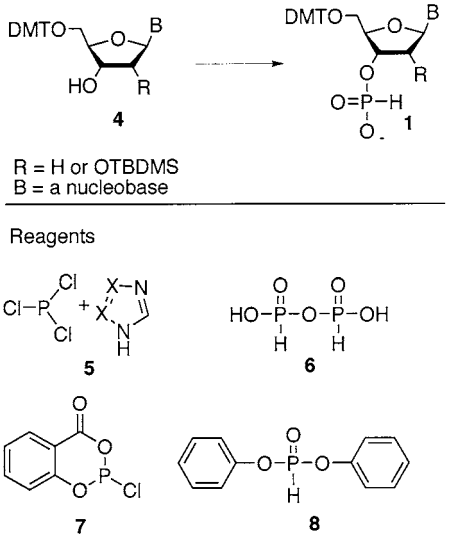
IV. H-Phosphonate Methodology

A. Preparation of H-Phosphonate Monoesters. To explore the synthetic potential of H-phosphonate derivatives, easy access to the starting materials, H-phosphonate monoesters **1** (Scheme 4), was of prime importance. When we started our studies some 15 years ago, a handful of synthetic methods had been available for this purpose,[21] but all of them suffered from harsh reaction conditions, variable yields, or incompatibility with the common protecting groups utilized in natural product chemistry.

Our goal was thus to develop simple, general, and efficient methods for the preparation of H-phosphonate monoesters based preferably on inexpensive, commercial reagents. Scheme 5 shows four reagents which seem to be most efficient for the introduction of an H-phosphonate monoester moiety into various hydroxylic compounds.[22,23] All of them are commercially available and allow the formation of nucleoside H-phosphonates **1** in high yield (80−95%) under mild reaction conditions (room temperature, pyridine/acetonitrile mixture, from a few minutes to a few hours).

Since the phosphonylating agents **5**−**8** differ in their reactivity, the choice between them may be determined by special structural features of a hydroxylic compound. For example, the use of PCl_3/imidazole reagent system **5**[24] or salicylchlorophosphite **7**[25] requires that amino functions in nucleosides **4** are protected, while H-pyrophosphonate **6**[26] and diphenyl H-phosphonate **8**[27] can tolerate the analogous substrates with unprotected amino groups. The reagents also differ in their reactivity toward hydroxyl functions: reagents **5**, **7**, and **8** can transfer an H-phosphonate moiety to a 3′-OH group of 2′-protected

Scheme 5. Synthesis of H-Phosphonate Monoesters



R = H or OTBDMS
B = a nucleobase

Reagents

ribonucleosides, while H-pyrophosphonate **6** is practically unreactive toward sterically hindered hydroxyl functions.

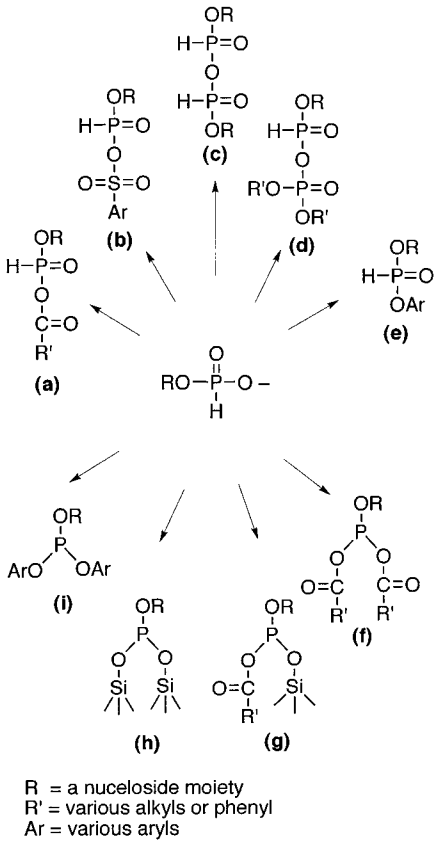
Probably the most versatile among these is diphenyl H-phosphonate **8**, which being cheap, stable, and commercially available can be considered as an all-purpose phosphonylating agent for a variety of types of hydroxylic compounds. It is also worth noting here that this synthetic protocol affords crude H-phosphonate monoesters of purity 90−95%, and thus, for many applications, an aqueous workup of the reaction mixture is sufficient. Other reagents or procedures that can be used for the preparation of H-phosphonate monoesters include, e.g., 2-cyanoethyl phosphordiamidite,[14] 2-cyanoethyl H-phosphonate,[28] aryl H-phosphonates,[29] and others.[22]

Apart from H-phosphonates **1**, there are two other important starting materials used in the synthesis of phosphate analogues: H-phosphonothioate **2** and H-phosphonodithioate **3** monoesters. These can be prepared directly from the corresponding alcohols by separate synthetic methods[30−32] or by using H-phosphonate monoesters **1** as starting materials.[33,34] Compounds **2** and **3** are most useful as precursors for the preparation of sulfur-containing phosphate analogues, such as phosphoromono- and phosphorodithioate diesters or various analogues of phosphate monoesters.[20] A few synthetic applications of these compounds are discussed further in the text.

B. Activation of H-Phosphonate Monoesters. Tri- vs Tetracoordinated Species. To react with nucleophiles or electrophiles, H-phosphonates **1** have first to be converted into reactive derivatives. This can be achieved with the help of various activating reagents. The array of reagents that can efficiently activate H-phosphonate monoesters toward nucleophiles is broader than that for P(V) compounds and also includes acyl halides and halophosphate diesters.[21]

Reactions of H-phosphonate monoesters with activating agents may produce a variety of reactive species that can be divided into two groups: tetracoordinated intermediates [e.g., **a**−**e**] and tricoordinated intermediates [**i**−**f**]

Scheme 6. Examples of Tetra- and Tricoordinated Species Derived from H-Phosphonate Monoesters



(Scheme 6). For the same activating agent, various intermediates can be formed from H-phosphonate monoesters, and these in reactions with nucleophiles or electrophiles may afford different products. For this reason, a detailed knowledge of an activation pathway of H-phosphonate monoesters for each kind of activating agent used is of crucial importance when designing syntheses of phosphorus compounds.[35−38]

Probably best understood is the activation process of H-phosphonate monoesters by acyl chlorides.[35,38] Depending on the reaction conditions, three reactive species can be formed: mixed anhydrides (**a**), H-pyrophosphonates (**c**), or bisacyl phosphite (**f**). These intermediates can be detected by ^{31}P NMR spectroscopy when the activation process is carried out in the absence of a hydroxylic component. Tetracoordinated intermediates **a** and **c** are most reactive and with alcohols afford H-phosphonate diesters. In contrast, tervalent intermediates **f** react with alcohols to give phosphite triesters. By changing the reaction conditions, these two types of intermediates can be formed as single species, and thus it is possible to steer the reaction of H-phosphonate monoesters with alcohols to proceed toward H-phosphonate diesters[39] or phosphite triesters.[40]

In contradistinction to P(V) derivatives, anhydrides **a** and **c** are exceedingly reactive, and in the absence of external nucleophiles, they undergo rapid transformations to less reactive species of type **f**.[35] Thus, to make use of the high reactivity of H-phosphonate derivatives, the activation process has to be carried out in the presence of a hydroxylic component that traps most reactive intermediates. Alcohols usually react so quickly with carboxylic (**a**), sulfonic (**b**), or phosphoric (**d**) mixed anhydrides that side products (due to competing reactions with condensing agents) do not occur to any significant extent.[39] Another important feature of these intermediates is the high degree of chemoselectivity observed in the reaction with alcohols, as only the P(III) phosphorus center in **a**, **b**, or **d** is attacked by hydroxylic compounds.

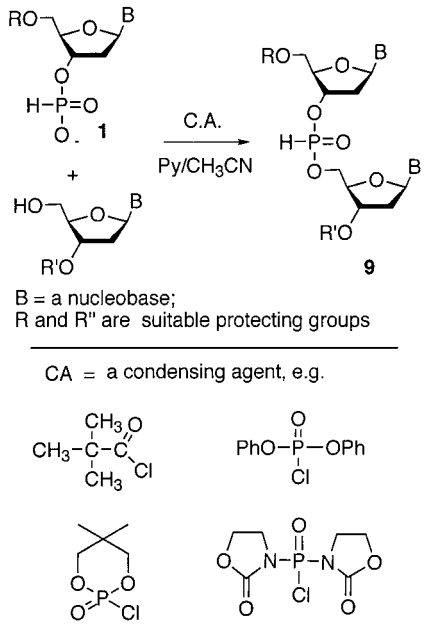
For other nucleophiles, e.g., amines, chemoselectivity is less pronounced and side products are usually formed.[41] To remedy this problem, we explored aryl H-phosphonates (**e**) as a novel type of intermediate.[42,43] Since these species have only one electrophilic center located on the phosphorus atom, the problem of chemoselectivity in the reactions with nucleophiles is alleviated. This type of intermediates can be produced in situ and also has the added advantage that their reactivity can be modulated by substituents on the aromatic ring of the aryl moiety. Some synthetic applications of this class of reactive H-phosphonate esters will be discussed later.

In contrast to the tetracoordinate H-phosphonate derivatives **a**−**e**, tervalent intermediates of types **i**, **h**, **g**, and **f** bear phosphorus atoms of various degrees of nucleophilicity and electrophilicity. As a consequence, the tervalent intermediates may react with both electrophiles and nucleophiles or selectively with only one of these. For example, bis-silyl phosphites (**h**)[44,45] react rapidly with electrophiles (e.g., sulfur), and this feature has been exploited in the syntheses of various analogues of phosphate monoesters. Acyl silyl phosphite (**g**),[46] on the other hand, reacts readily with soft nucleophiles (e.g., HS^-), producing H-phosphonothioate monoesters **2**. The latter cannot be obtained from tetracoordinate intermediates **a**−**e**, as their reactions with the sulfide anion produced H-phosphonodithioates **3** instead. Diaryl phosphite (**i**) is another synthetically useful intermediate.[47] Here, the phosphorus atom has highly pronounced electrophilic character and affords cyclic phosphites in its reactions with diols, from which analogues of five-membered cyclic phosphates can be produced.[47]

It can thus be concluded that in the synthesis of phosphorus compounds using H-phosphonate methodology, it is possible to choose an intermediate that best fits a particular transformation by tuning nucleophilicity, electrophilicity, hardness, and softness of the phosphorus center in the intermediate by changing the coordination number and chemical properties of ligands attached to the phosphorus atom.

C. Synthetic Methods Based on H-Phosphonate Chemistry. *(i) Oxidative Transformations of H-Phosphonate Diesters.* H-Phosphonate diesters **9** (Scheme 7) are probably the most important intermediates in the synthesis of phosphate diesters and their analogues using the H-phosphonate methodology. The coupling reactions are usually carried out in a mixture of pyridine/acetonitrile using acyl chlorides or chlorophosphate derivatives as condensing agents. Various aspects of this and the analo-

Scheme 7



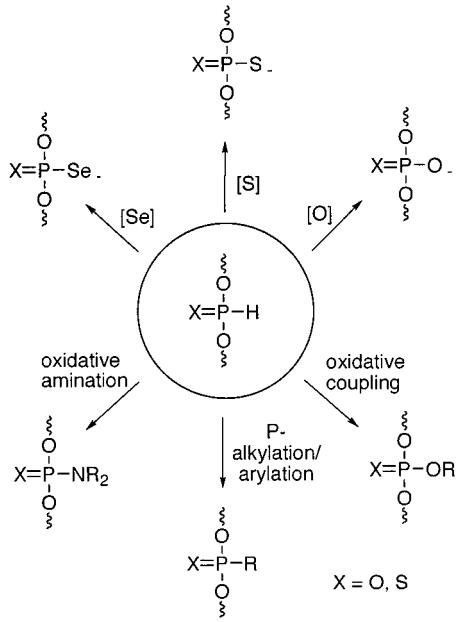
gous reaction that lead to H-phosphonothioate diester formation have been studied in detail.[35,48,49] On this basis, highly efficient synthetic protocols for the preparation of these important intermediates have been developed.[50]

Since the phosphorus center in H-phosphonate and H-phosphonothioate diesters is chiral and the oxidative transformations of these compounds are usually stereospecific (except in the cases where fluoride is used as a nucleophile), phosphodiester analogues with defined stereochemistry at the phosphorus center can be obtained in this way. Oxidation is a reaction step where the H-phosphonate method can offer a great versatility and the opportunity to make oligonucleotides bearing various backbone modifications, as outlined in Scheme 8 and in a recently published paper.[51] These include syntheses of phosphate diesters,[52] phosphoromonothioate and phosphorodithioate diesters,[53] phosphoroselenoate diesters,[54] fluorophosphate and fluorothiophosphate diesters,[55] etc.

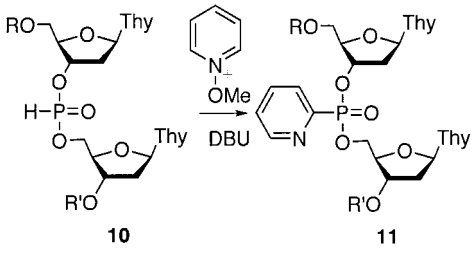
An important class of phosphate analogues that also can be obtained from H-phosphonate diesters are C-phosphonate derivatives. In contrast to phosphate esters, these compounds bear one P−C bond that makes them resistant to enzymatic hydrolysis and, thus, of potential interest as antisense/antigene agents. Recently, we have developed an efficient synthesis of a novel class of nucleotide analogues bearing this functionality, namely, pyridylphosphonates. In Scheme 9, the synthesis of 2-pyridylphosphonate derivatives **11** from the corresponding H-phosphonate diester **10** and *N*-methoxypyridinium salt in the presence of DBU is depicted.[56] The reaction is rapid (few minutes), high yielding (>90%), and completely stereospecific. Separate methods based on H-phosphonate chemistry have been developed for the synthesis of isomeric 3- and 4-pyridylphosphonate derivatives of type **11**.[57,58]

The modifications of the phosphorus center described above were all located at nonbridging positions of the

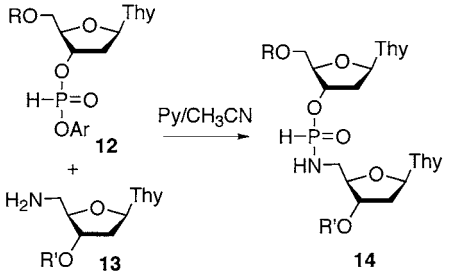
Scheme 8. Some Oxidative Transformations of H-Phosphonate Diesters



Scheme 9. Synthesis of Dinucleoside 2-Pyridylphosphonates



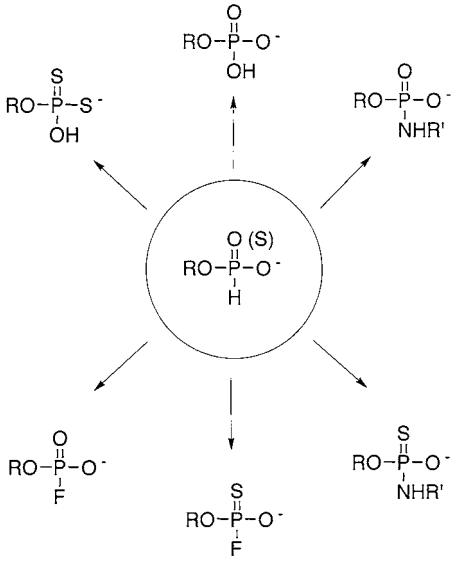
Scheme 10. Synthesis of Dinucleoside H-Phosphonamidates



phosphodiester function. Making changes at bridging positions of phosphodiesters, however, is a more difficult task and called for intermediates different from the H-phosphonate diester type. Inspired by favorable antisense properties of oligonucleoside phosphoramidates with the nitrogen atom in a bridging position of the phosphoramidate internucleotide linkage,[59,60] we designed a new synthesis of such of compounds, involving H-phosphonamidates **14** as key intermediates (Scheme 10).[43]

Attempted condensations of H-phosphonate monoester **1** with aminonucleosides failed to produce H-

Scheme 11. Some Oxidative Transformations of H-Phosphonate and H-Phosphonothioate Monoesters



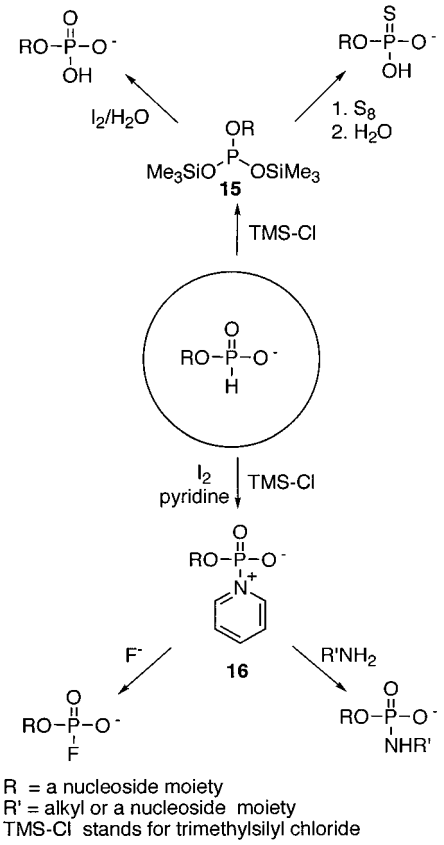
phosphonamidate **14** due to extensive side reactions of **13** with condensing agents. All of these problems, however, were alleviated by using nucleoside aryl H-phosphonate **12** as starting material (Scheme 10). Thus, intermediates **14** could be produced practically quantitatively and then converted either to the corresponding phosphoramidate diesters (oxidation with iodine/water) or to phosphoramidothioate derivatives (oxidation with sulfur).[43]

(ii) Oxidative Transformations of H-Phosphonate Monoesters. Various analogues of phosphate monoesters were found to be potent inhibitors of essential enzymes in living organisms and thus are of interest as potential therapeutic agents.[61] To provide new entries to phosphate analogues bearing P−S, P−Se, P−F, and P−N bonds, we investigated oxidative transformation of H-phosphonate **1** and H-phosphonothioate **2** monoesters (Scheme 11).

Due to the presence of a negative charge at the H-phosphonate functionality, compounds **1** and **2** are significantly less susceptible to oxidation than the corresponding diesters, and they usually require activation to react with electrophiles.[21] There are two types of reactive intermediates that appear to be most useful in the synthesis of phosphate analogues using H-phosphonate monoesters **1** as starting materials: bis-silyl phosphites **15** and pyridinium adducts of type **16** (Scheme 12).

Transformation of H-phosphonate monoesters into tervalent derivatives **15**[44,52] increases nucleophilicity of the phosphorus center and makes them react rapidly with electrophiles, e.g., with elemental sulfur (to produce thiophosphate analogues)[45] or iodine/water (to produce phosphate monoesters)[62] (Scheme 12). The intermediate **16**, on the other hand, is already an oxidized P(V) species, and thus its phosphorus center can be efficiently attacked by nucleophiles, e.g., fluoride ion (to produce fluorophosphate analogues),[63] or by amines (to produce phosphoramidate derivatives).[43] By replacing H-phosphonate **1** by H-phosphonothioate **2** or H-phosphonodithioate **3** monoesters in these reactions, additional phosphate analogues bearing multiple modifications at the phosphorus center can be obtained.[45,63]

Scheme 12. Some Oxidative Transformations of H-Phosphonate Monoesters



V. Conclusions and Outlook

In the past two decades, synthesis of phosphorus-containing natural products via H-phosphonate intermediates emerged as an alternative methodology[21] to well-established synthetic methods based on phosphate [P(V)][64,65] and phosphite [P(III)][66,67] derivatives. This also helped launch H-phosphonate chemistry as a distinctive field of phosphorus chemistry in its own right.

Although the focus of our research has been nucleotide and oligonucleotide chemistry,[19] the H-phosphonate methodology is not, by any means, confined to this class of compounds. Growing applications of H-phosphonates as synthetic intermediates in carbohydrate,[68,69] peptide,[70,71] lipid,[72,73] and general phosphorus chemistry[74,75] support this view. One should stress, however, that synthesis of nucleotides, oligonucleotides, and their analogues provides a rigorous test for a synthetic methodology in terms of the efficiency, versatility, and mildness of the reaction conditions used. Synthetic methods based on H-phosphonate chemistry passed this test with distinction.

It is likely that the demand for oligonucleotide analogues and other bioactive phosphorus compounds as potential therapeutic and diagnostic agents will grow. In

this context, H-phosphonate chemistry might provide the necessary tools for fine-tuning chemical, biological, and pharmacokinetic properties of phosphorus compounds by means of introducing multiple modifications at the phosphorus center and controlling its stereochemistry.

Since H-phosphonate chemistry appears to combine the advantages of two other phosphorus chemistries, that of P(V) phosphoryl compounds and that of tervalent P(III) derivatives, its synthetic potential certainly is worth further exploration.

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