

*Review*

**Nucleoside phosphate analogues of biological interest,  
and their synthesis *via* aryl nucleoside *H*-phosphonates  
as intermediates<sup>★</sup>**

Jacek Ciełak<sup>1</sup>, Michał Sobkowski<sup>1</sup>, Jadwiga Jankowska<sup>1</sup>, Małgorzata Wenska<sup>1</sup>,  
Marzena Szymczak<sup>1</sup>, Barbara Imiończyk<sup>1</sup>, Izabela Zagórska<sup>1</sup>, David Shugar<sup>2</sup>,  
Jacek Stawiński<sup>1,3½</sup> and Adam Kraszewski<sup>1½</sup>

<sup>1</sup>*Institute of Bioorganic Chemistry, Polish Academy of Sciences, Z. Noskowskiego 12/14,  
61-704 Poznań, Poland;* <sup>2</sup>*Institute of Biochemistry and Biophysics, Polish Academy of Sciences,  
Warszawa, Poland;* <sup>3</sup>*Department of Organic Chemistry, Arrhenius Laboratory, Stockholm  
University, S-106 91 Stockholm, Sweden*

Received: 14 February, 2001; revised: 22 May, 2001; accepted: 31 May, 2001

**Key words:** aryl nucleoside *H*-phosphonates, nucleophilic catalysis, *H*-phosphonamides,  
*H*-phosphono(di)thioates, nucleoside 2',3'-cyclic phosphate analogues

**This review presents a brief account of the chemistry and mechanistic aspects of aryl *H*-phosphonates, and selected applications of this class of compounds as intermediates in the synthesis of a wide range of biologically important analogues of nucleoside phosphates, and oligonucleotides, in which the phosphate moieties are replaced by other structurally related groups. The aryl nucleoside *H*-phosphonates, compounds of controlled reactivity, have proven to be more versatile and superior to various mixed anhydrides as synthetic intermediates, particularly for preparation of nucleotide analogues bearing P–N or P–S bonds in various configurational arrangements at the phosphate moiety.**

---

★ Presented at the International Conference on "Molecular Architecture of Evolution, Primary and Secondary Determinants" Poznań, Poland, October 29–31, 2000.

● Financial support from the State Committee for Scientific Research (KBN, Poland) grant 3 T09A 118 14, and from the Swedish Council for Natural Sciences, is gratefully acknowledged.

½ Corresponding authors: Adam Kraszewski, tel: (48 61) 852 8503; fax: (48 61) 852 0532;  
e-mail: <akad@ibch.poznan.pl>; Jacek Stawiński, tel.: (46 8) 162 485; fax: (46 8) 154 908;  
e-mail: <js@organ.su.se>

**Abbreviation:** HMDST, hexamethyldisilathiane.

Intracellular phosphorylation of a multiple of cellular constituents is a key process in the life cycle of a cell, catalysed by variety of enzymes such as nucleoside and nucleotide kinases, protein kinases, etc. Protein phosphorylation, in particular, is now widely recognized as the most important pathway for regulation of protein functions in eukaryotic cells, involved in switching cellular activities from one stage to an other and in this way, regulating gene expression, cellular proliferation and cell differentiation. It is the major mechanism whereby cells respond to extracellular signals, such as hormones and growth factors, in this manner controlling all events in various stages of the cell cycle, as well as the response of a cell to environmental and nutritional stresses.

Intracellular phosphorylation is also regulated *via* dephosphorylation by numerous phosphatases, some of them highly specific. These exquisitely coordinated activities are a striking illustration of the dominant and versatile role of the phosphate esters and anhydrides in living systems, elegantly underlined almost 15 years ago by Frank H. Westheimer [1] in response to the query: "Why were phosphates, and almost no other groups, selected by evolution for biochemical transformations?"

The present report describes the fundamental chemical properties of aryl *H*-phosphonate diesters, and selected applications of this class of compounds to the synthesis of nucleotide analogues in which the phosphate moieties are replaced by other structurally related groups which may be employed to elucidate the mechanisms of action of natural nucleotides, or oligonucleotides, in biochemical systems.

The *H*-phosphonate methodology, due to its efficiency, reliability and experimental simplicity, has emerged in the last decade as a versatile and powerful approach to the synthesis of biologically active phosphate analogues [2, 3]. As part of our studies in this field, we

have recently developed aryl *H*-phosphonates as a new type of active *H*-phosphonate derivatives [4–9]. Their advantages as synthetic intermediates stem from the fact that these compounds possess, in principle, only one electrophilic center located on the phosphorus atom, and in contradistinction to other reactive *H*-phosphonate species (e.g. mixed *H*-phosphono-acyl anhydrides), their reactivity can be modulated by changing electronic or/and steric properties of substituents on the aromatic ring of an aryl moiety. These features significantly broaden synthetic applications of *H*-phosphonate methodology by enabling the syntheses of otherwise difficultly accessible compounds, e.g. nucleoside *H*-phosphonamidates [8].

## SYNTHESIS AND REACTIVITY OF ARYL *H*-PHOSPHONATES

### Synthesis of aryl nucleoside *H*-phosphonates

To secure efficiency and reproducibility of methods making use of aryl *H*-phosphonates as synthetic intermediates, the chemistry of this class of compounds has been investigated, particularly in the context of reactivity of the P–H bond, which may affect the formation and stability of these compounds under various experimental conditions.

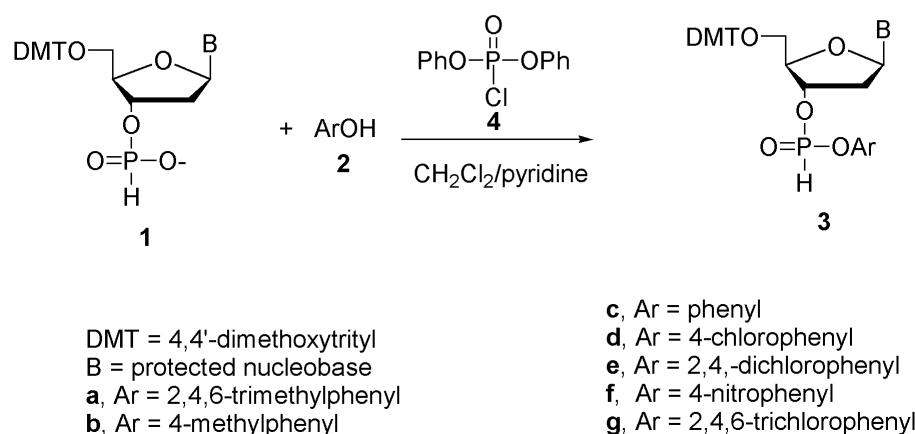
Nucleoside aryl *H*-phosphonate can be prepared either by phosphorylation of a suitable protected nucleoside with an appropriate phosphorylating reagent bearing an aryl moiety [10], or by reacting a nucleoside *H*-phosphonate with an appropriate phenol in the presence of a condensing agent [8, 11]. The latter approach (Scheme 1) alleviates problems connected with preparation of separate phosphorylating reagents for each kind of aryl *H*-phosphonate derivative and, in light of the easily accessible *H*-phosphonate monoesters, appears to be also the most con-

venient and versatile route to these compounds.

We have found that the most important factors influencing formation of nucleoside aryl *H*-phosphonates from nucleoside *H*-phosphonates **1** and the corresponding phenols **2** are: (i) acidity ( $pK_a$ ) of the phenols, (ii) the nature of the coupling agent used for condensation, and (iii) the basicity of the reaction medium. In pyridine, in the presence of different condensing agents, these affect the extent of various side-reactions (e.g. subsequent reac-

### Base and nucleophile catalysis in transesterification of aryl nucleoside *H*-phosphonates

A possible involvement of base or/and nucleophile catalysis in condensation of nucleoside *H*-phosphonate monoesters has been postulated on many occasions [3, 12], but with no clear-cut evidence. Efimov *et al.* [13, 14] found that nucleoside pivaloyl-phosphonate mixed anhydrides reacted with nucleosides about 10 times faster in the pres-



Scheme 1

tions of the produced *H*-phosphonate **3** with condensing agents, disproportionation of **3** and ultimately determine efficiency of generation of aryl *H*-phosphonates **3** [11]. However, these problems can be alleviated when synthesis of aryl *H*-phosphonates **3** is carried out in methylene chloride containing a limited amount of pyridine (3–12 molar equiv.) in the presence of diphenyl phosphorochloridate **4** (1.1 equiv.) as a condensing agent. Under these conditions, coupling of nucleoside *H*-phosphonate **1** with all investigated phenols **2a–g** is clean, relatively fast (about 20 min) [11] and the produced aryl *H*-phosphonates **3a–g** do not undergo any detectable changes within several hours ( $^{31}\text{P}$  NMR spectroscopy). Thus, this procedure can be considered as a general protocol for the formation of aryl *H*-phosphonates **3a–g** from the corresponding *H*-phosphonate monoesters **1**.

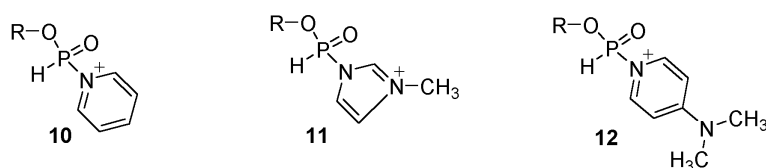
ence of pyridine compared to 4-*N,N*-dimethylaniline, although both bases have similar  $pK_a$  values (5.2 and 5.1, respectively). While these differences are most likely due to nucleophilic catalysis involving pyridine, interpretation of the results was complicated by the fact of a rapid conversion of the mixed carboxylic-phosphonic anhydrides to tervalent bispivaloyl phosphites. In this respect, nucleoside aryl *H*-phosphonates appear to be a more convenient model system for investigation of nucleophilic catalysis, as these compounds (especially those bearing weakly acidic aryl moieties) have less pronounced tendency than mixed anhydrides for conversion into tervalent derivatives.

Thus, for our studies on nucleophilic catalysis in *H*-phosphonate derivatives we selected 4-chlorophenyl nucleoside *H*-phosphonate **3d** (Scheme 1), which is stable in neat pyridine

for at least several hours [11] and at the same time has suitable reactivity towards alcohols [15], enabling monitoring progress of the reactions by  $^{31}\text{P}$  NMR spectroscopy. The set of basic catalysts chosen consisted of amines of different basicity ( $\text{p}K_{\text{a}}$  5.2–10.5) [16] (Table 1) in which pyridine (**9a**), *N*-methylimidazole (**9c**), and 4-*N,N*-dimethylaminopyridine (**9e**) were potential nucleophilic catalysts, while hexamethylenetetramine (**9b**), *N*-ethylmorpholine (**9d**) and triethylamine (**9f**) were intended to act primarily as bases. Experiments were carried out in methylene chloride in which substrate **3d** (1 molar equiv., 0.1 mmol/mL) was allowed to react with ethanol (1.5 molar equiv.) in the presence of an excess of a catalyst (amine **9**, 10 molar equiv.). In all instances the product of transesterification, ethyl nucleoside *H*-phos-

between the  $\text{p}K_{\text{a}}$  values of amines and the rate of transesterification of **3d** was not linear, pointing to a significant contribution of nucleophilic catalysis. Indeed, the catalytic effect of *N*-methylimidazole (**9c**) was much higher than that of the stronger base *N*-ethylmorpholine (**9d**) and the less basic hexamethylenetetramine (**9b**). Thus, it seems likely that, with *N*-methylimidazole and other heteroaromatic amines used in these studies (pyridine **9a** and 4-*N,N*-dimethylaminopyridine **9e**), catalysis occurs not only *via* generation of an alkoxy anion from an alcohol [base catalysis;  $\text{ROH} + \text{B} \rightleftharpoons \text{RO}^- + \text{BH}^+$ ], but also *via* formation of reactive intermediates of type **10**, **11** and **12** (nucleophile catalysis) (Scheme 2).

On the basis of these preliminary data, we can tentatively conclude that, in transesteri-



R = nucleoside moiety

Scheme 2

phonate **6a**, was the only one observed by  $^{31}\text{P}$  NMR spectroscopy.

Results of these experiments are summarised in Table 1. Pyridine, the least basic of the amines investigated, was the least effective as a catalyst (completion within 120 min) while strong bases, e.g. triethylamine (**9f**) and 4-*N,N*-dimethylaminopyridine (**9e**) catalysed the transesterification most efficiently (completion in < 3 min). However, the correlation

of nucleoside aryl *H*-phosphonates, efficiency of nucleophilic catalysis exceeds that of base catalysis. Since strongly basic conditions are detrimental to aryl *H*-phosphonates, nucleophilic catalysis opens a route for transesterification of aryl *H*-phosphonates **3** under mild, basic conditions, without loss of efficiency.

In the next series of experiments we investigated internucleotide bond formation in the

Table 1. Formation of ethyl nucleoside *H*-phosphonate **6a** in transesterification of aryl *H*-phosphonate **3d** with ethanol (**5a**) catalysed by various bases **9**

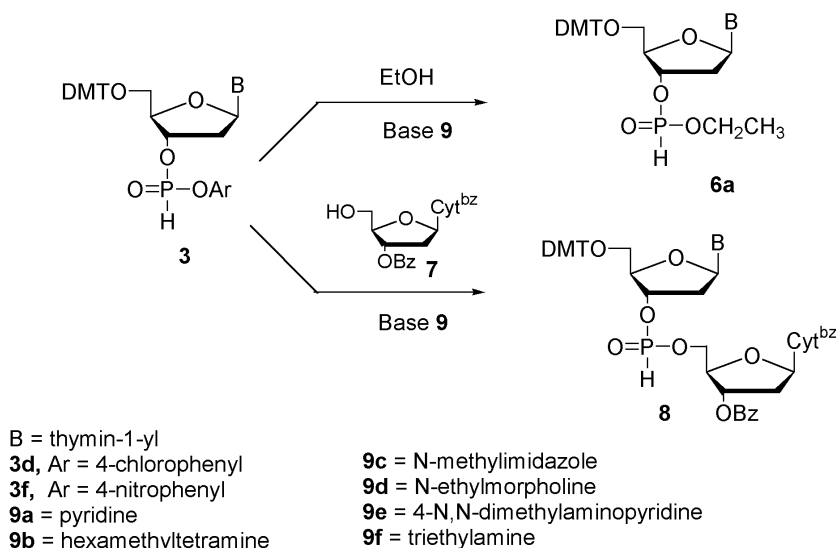
Base	<b>9a</b>	<b>9b</b>	<b>9c</b>	<b>9d</b>	<b>9e</b>	<b>9f</b>
$\text{p}K_{\text{a}}$	5.19	6.30	6.95	7.67	9.70	10.78
Time* (min)	120	50	8	25	< 3	< 3

\*Time for complete disappearance of **3d**

presence of amines **9a–f**, under the same conditions as above, except that ethanol was replaced by *N*<sup>4</sup>,3'-*O*-dibenzoylcytidine **7** as a hydroxylic component (Scheme 3).

For all catalysts used, transesterification of 4-chlorophenyl *H*-phosphonate **3d** with

min). The latter base, being a powerful nucleophilic catalyst, drove the reaction to completion in a time comparable to that observed for most basic amines, triethylamine **9f** and 4-*N,N*-dimethylaminopyridine **9e** (Table 2).



Scheme 3

nucleoside **7** was slower than with ethanol by factor of 10 or more (Table 2). The second, even more important, difference was that for the most basic catalysts **9e** and **9f**, formation of significant amount of side products (9% and 40% respectively, <sup>31</sup>P NMR) was observed. These were identified as nucleoside *H*-phosphonate **1** and bis-aryl nucleoside phosphite ( $\delta_P$  127.54 ppm, d, <sup>3</sup>J<sub>PH</sub> = 9.3 Hz) [11] and were formed due to competing disproportionation [11, 17] of aryl *H*-phosphonate **3d**. Comparing these two catalysts, 4-*N,N*-dimethylaminopyridine (**9e**) and triethylamine (**9f**) (Table 2), one can notice higher yield (91% vs 60%, respectively) and a shorter time (10 min vs 50 min, respectively) for the formation of dinucleoside *H*-phosphonate **8** in the reaction catalysed by nucleophilic base **9e**.

For weaker bases, **9a–9d**, no disproportionation products resulting from **3d** were observed, but transesterification proceeded much slower (700 min for **9a** and **9b**, 660 min for **9d**), except for *N*-methylimidazole **9c** (60

As a final part of these investigations, we studied the transesterification of 4-nitrophenyl *H*-phosphonate **3f**, which is at least 30 times [18] more reactive than 4-chlorophenyl *H*-phosphonate **3d**. Results of these experiments (Table 2) showed that enhanced reactivity of aryl *H*-phosphonate **3f** in the transesterification reaction was counterproductive with strongly basic catalysts **9d–9f** [formation of disproportionation products: nucleoside *H*-phosphonate **1** and bis-4-nitrophenyl nucleoside phosphite ( $\delta_P$  125.78 ppm, d, <sup>3</sup>J<sub>HP</sub> = 9.3 Hz)], but improved the efficiency of the less basic catalysts **9a–9c**.

Although all investigated transesterifications of 4-nitrophenyl *H*-phosphonate **3f** occurred rapidly (< 3 min), only amines with p*K*<sub>a</sub> < 7 (pyridine **9a**, hexamethylenetetramine **9b** and *N*-methylimidazole **9c**) did not promote competing disproportionation of **3f**.

Summing up, efficacy of transesterification of aryl nucleoside *H*-phosphonate diesters depends on (i) basicity and nucleophilicity of the

**Table 2.** Internucleotide bond for ma tion in the pres ence of bases 9a–f

Base	Substrate <b>3d</b>			Substrate <b>3f</b>		
	Time* (min)	<b>8</b> (%)	X (%)**	Time* (min)	<b>8</b> (%)	X (%)**
<b>9a</b>	700	100	0	< 3	100	0
<b>9b</b>	700	100	0	< 3	100	0
<b>9c</b>	60	100	0	< 3	100	0
<b>9d</b>	660	100	0	< 3	92	8
<b>9e</b>	10	91	9	< 3	62	38
<b>9f</b>	50	60	4 0	< 3	20	80

\*Time for complete disappearance of substrate **3d** or **3f**. \*\*X = products of disproportionation of **3d** or **3f**

catalyst used, and (ii) acidity of the P–H bond in aryl *H*-phosphonates of type **3**. High basicity of a catalyst and increased acidity of the P–H bond stimulate disproportionation of aryl *H*-phosphonate diesters **3**. Thus, to perform transesterification of aryl *H*-phosphonates with maximum efficiency, it is important to synchronise the basicity of the catalyst with the acidity of the aryl *H*-phosphonate used.

#### SELECTED SYNTHETIC METHODS FOR NUCLEOTIDE ANALOGUES BASED ON ARYL *H*-PHOSPHONATE INTERMEDIATES

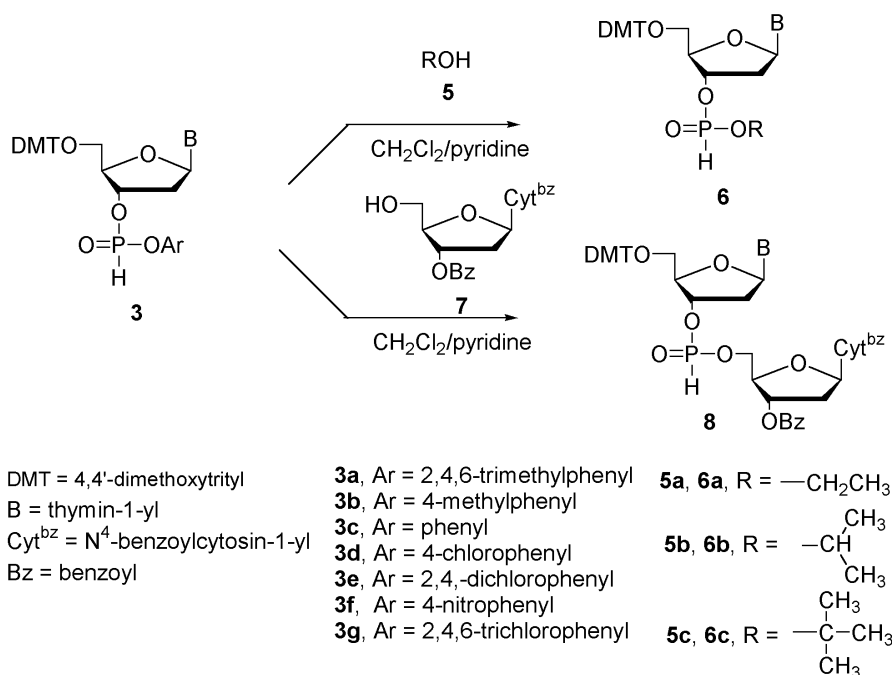
##### Transesterification of aryl nucleoside *H*-phosphonates. Formation of an internucleotide bond

Transesterification of aryl nucleoside *H*-phosphonates **3** was studied as a function of the alcohol used and the  $pK_a$  value of the aryl moiety. All reactions were carried out in methylene chloride/pyridine 9 : 1 (v/v) using aryl nucleoside *H*-phosphonates **3** (1 molar equiv.) and an excess (3 molar equiv.) of primary [e.g. ethanol (**5a**)], secondary [e.g. isopropanol (**5b**)], or tertiary [e.g. *t*-butanol (**5c**)] alcohols (Scheme 4).

Progress of the reaction was monitored with  $^{31}\text{P}$  NMR spectroscopy. In all instances, the corresponding nucleoside alkyl *H*-phospho-

nates **6** were formed as the sole nucleotidic products. For 4-nitrophenyl *H*-phosphonate **3f**, the reaction with ethanol (**5a**) went to completion in less than 3 min, with isopropanol (**5b**) in about 5 min, and with *tert*-butanol (**5c**) in about 8 min ( $^{31}\text{P}$  NMR spectroscopy). These differences apparently reflected changes in steric hindrance of the alkyl substituents and indicated also that transesterification of aryl *H*-phosphonates **3** proceeds most likely *via* an  $S_N2(\text{P})$  mechanism [18].

We also assessed the reactivity of aryl *H*-phosphonate diesters as a function of the aryl moiety present, by reacting **3a–g** with *N*<sup>4</sup>,3'-*O*-dibenzoyldeoxycytidine **7** (Scheme 4). The most reactive among the investigated aryl *H*-phosphonate derivatives were those bearing *p*-nitrophenyl (**3f**) and 2,4,6-trichlorophenyl (**3g**) groups, which produced dinucleoside *H*-phosphonate **8** in less than 3 min. The relative order of reactivity of **3a** : **3b** : **3c** : **3d** : **3e** : **3f** : **3g** was found to be 1 : 4 : 10 : 40 : 350 : 1100 : 1100, and provides estimates of the extent of possible modulation of the reactivity of compounds of type **3**. The data also indicate that reactivities of 4-nitrophenyl- and 2,4,6-trichlorophenyl derivatives **3f** and **3g**, respectively, are close to those of mixed carboxylic-phosphonic anhydrides [12], so that transesterification of an aryl *H*-phosphonate can be considered as a viable alternative for formation of an internucleoside *H*-phosphonate bond [6].



Scheme 4

### Nucleoside *H*-phosphonamides

Searching for a simple and versatile method for the synthesis of nucleoside alkyl-*H*-phosphonamides, we investigated the direct coupling of nucleoside *H*-phosphonates with appropriate amines in the presence of pivaloyl chloride or various chlorophosphates [8]. Unfortunately, such condensations invariably resulted in complex mixtures of products, in which the desired nucleoside *H*-phosphonamides were often minor components. Preactivation of nucleoside *H*-phosphonates with pivaloyl chloride or chlorophosphates, followed by addition of amines, notably diminished these side reactions, but did not eliminate them.

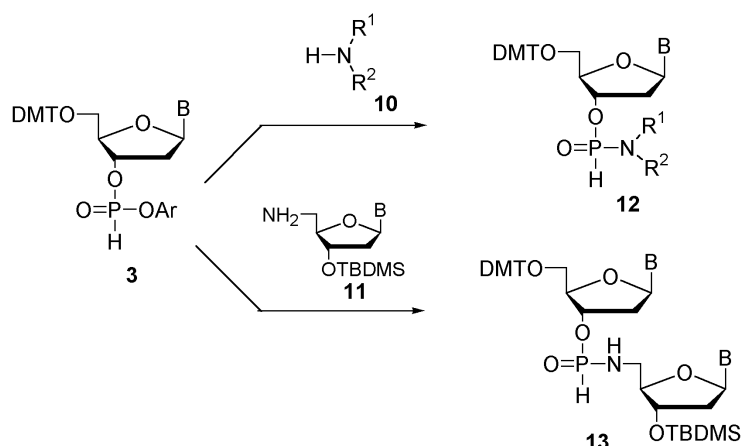
In more detailed studies, we have found that the most important factors affecting the formation of nucleoside *H*-phosphonamides of type **12** in reactions promoted by a condensing agent were (i) reactivity of amines towards coupling reagents, (ii) chemoselectivity of amines towards the reactive species generated during the activation process, and (iii) steric hindrance in the amines.

Problems connected with formation of *H*-phosphonamides from *H*-phosphonate

monoesters and amines **10a–e** in the presence of condensing agents were circumvented by using aryl *H*-phosphonates of type **3**. The ease of preparation, and high susceptibility to nucleophilic substitution at the phosphorus centre, made aryl *H*-phosphonates excellent substrates for synthesis of nucleoside *H*-phosphonamides **12a–e** carrying primary and unhindered secondary amine moieties, including dinucleoside *H*-phosphonamide **13** [19] (Scheme 5). Due to mildness of the reaction conditions, the synthetic protocol developed can be considered as a general method for the preparation of natural product analogues with the P–N bond in a bridging position of the phosphoramidate linkage.

### Nucleoside *H*-phosphonothio- and *H*-phosphonodithioates

We have for some time introduced and investigated various aspects of *H*-phosphonothioates [20–24] as a new class of synthetic intermediates that can supplement and expand applications based on *H*-phosphonate derivatives. Nucleoside *H*-phosphonothioate monoesters can be prepared either from suitably



DMT = 4,4'-dimethoxytrityl  
 B = thymine-1-yl  
 TBDMS = *tert*-butyldimethylsilyl  
 Ar = 2,4,6-trichlorophenyl

a, R<sup>1</sup> = H, R<sup>2</sup> = -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>

b, R<sup>1</sup> = H, R<sup>2</sup> =  $\begin{array}{c} \text{CH}_3 \\ | \\ -\text{HC} \\ | \\ \text{CH}_3 \end{array}$

c, R<sup>1</sup> = H, R<sup>2</sup> =  $\begin{array}{c} \text{CH}_3 \\ | \\ -\text{C}-\text{CH}_3 \\ | \\ \text{CH}_3 \end{array}$

d, R<sup>1</sup> = -CH<sub>3</sub>, R<sup>2</sup> = -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>

e, R<sup>1</sup> = R<sup>2</sup> = -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>

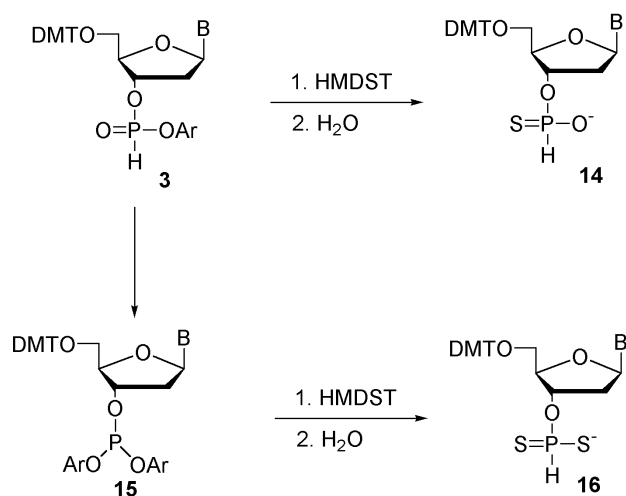
Scheme 5

protected nucleosides using various thio-phosphorylation protocols [21, 24–26] or by non-oxidative thiation of nucleoside *H*-phosphonates [22]. This latter approach seems to be particularly attractive in light of the growing availability of *H*-phosphonate monoesters [4, 27] and has the added advantage that other synthetically useful intermediate, e.g. *H*-phosphonodithioate monoesters [28, 29], can also be prepared from the same substrate [30].

The only protocol hitherto available for transformation of *H*-phosphonate monoesters into the corresponding *H*-phosphonothioate derivatives [22] was based on a P(III) intermediate (a nucleoside pivaloyl silyl phosphite [23]) the reactivity of which was difficult to modulate. To circumvent this, we investigated aryl nucleoside *H*-phosphonates (reactivity of which can be modulated by the electronic properties of the aryl group) as new synthetic intermediates for the preparation of nucleoside *H*-phosphonothioate monoesters

of type **14** [25] and nucleoside *H*-phosphonodithioates of type **16** (Scheme 6).

Thus, by reacting nucleoside aryl *H*-phosphonates **3** with hexamethyldisilathiane (HMDST) various nucleoside *H*-phosphonothioate monoesters **14** were obtained in high yields (over 90%) [31]. For the preparation of nucleoside *H*-phosphonodithioates **16**, two alternative procedures were developed. These consisted of the reaction of *H*-phosphonate monoesters **1** with diphenylchlorophosphate in the presence of hydrogen sulfide (not shown) or involved thiation of the nucleoside diaryl phosphites **15** produced *in situ* with HMDST (Scheme 6). Both transformations [31] were fast, efficient, could be carried out as one-pot reactions, and thus can be recommended as versatile and convenient methods for the preparation of nucleoside *H*-phosphonothioate **14** and nucleoside *H*-phosphonodithioate **16** monoesters from readily accessible nucleoside *H*-phosphonates.



B = N<sup>6</sup>-benzoyladenine-9-yl, N<sup>4</sup>-benzoylcytosine-1-yl,  
 N<sup>2</sup>-isobutrylguanine-9-yl, thymine-1-yl  
 HMDST = hexamethyldisilathian  
 Ar = 2,4,6-trichlorophenyl  
 DMT = 4,4'-dimethoxytrityl

Scheme 6

### Nucleoside phosphorothio- and phosphorodithioates

We have also studied the hitherto poorly defined role of the phosphate group in the binding, by thymidylate synthase, of its substrate (dUMP), its product (dTMP) and classical inhibitor (5-fluoro-dUMP) with the aid of the corresponding 5'-thiophosphates, 5'-dithiophosphates and 5'-*H*-phosphonates [32]. Amongst others, the results provided independent evidence that the enzyme active center exhibits a marked preference for the dianionic phosphate moiety for optimal binding of the nucleotide. In particular the 5'-thiophosphate analogue of 5-fluoro-dUMP was found to be a more effective inhibitor than the parent 5-fluoro-dUMP, undoubtedly associated with the more acidic  $pK_a$  of the former [33].

It was long ago reported that ATP $\gamma$ S is a good competitive inhibitor of the ATP-dependent phosphorylation of proteins. It subsequently turned out that this is due to ATP $\gamma$ S being itself a donor for protein kinases, leading to formation of thiophosphorylated protein residues. These are relatively resistant to phosphatases, thus en-

abling isolation and identification of phosphorylation sites even in crude extracts containing phosphatases [34]. More recently it has been shown that the lability of phosphohistidine residues in a protein can be overcome by the use of ATP $\gamma$ S as a donor, resulting in markedly increased stability of thiophosphohistidine residues [35]. Somewhat surprisingly, ATP $\gamma$ S is a poor donor, if at all, for nucleoside kinases, but is a moderate to good inhibitor, showing that it does not bind to the enzymes [34]. It would be of interest to determine whether ATP $\gamma$ S is a donor for nucleotide kinases.

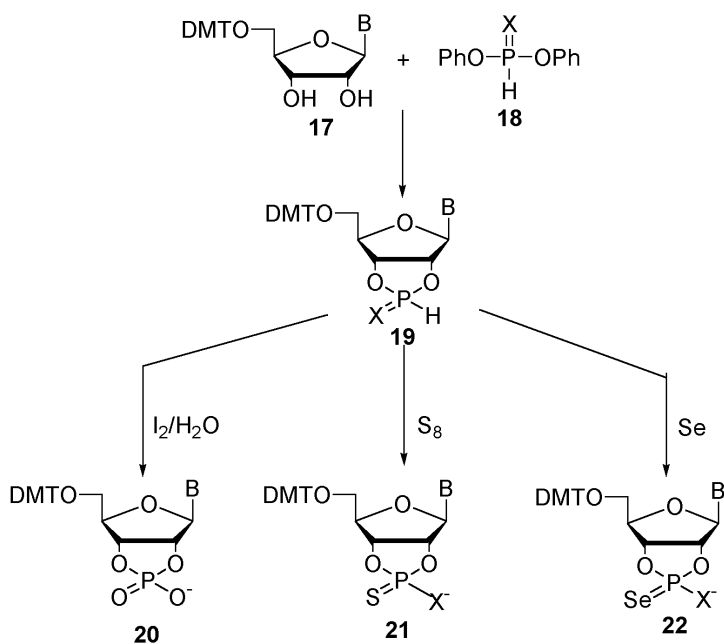
### Nucleoside 2',3'-*O,O*-cyclic phosphates, phosphorothioates, phosphorodithioates, and phosphoroselenoates

Nucleoside 2',3'-cyclic phosphates have for a long time been the focus of chemical research [36]. Although the biological significance of 2',3'-cyclic phosphates *per se* is far from clear, the importance of ribonuclease- [37] and ribozyme-catalysed [38] reactions that involve cyclic phosphates as intermediates, make this class of phosphorus compounds an indispensable

able research tool in mechanistic bioorganic phosphorus chemistry and in molecular biology.

Particularly interesting are the nucleoside 2',3'-*O,O*-phosphorothioates, which have received attention in conjunction with studies on stereochemical aspects of ribonuclease-catalysed reactions [39]. These compounds, however, are usually difficult to prepare and their synthesis involves reactions of 5'-*O,N*-protected ribonucleosides with

nates [43] (**19a**) or 2',3'-*O,O*-cyclic *H*-phosphonothioates (unpublished results) (**19b**) (Scheme 7). These compounds were not stable enough to permit their isolation but appeared to be excellent intermediates in the synthesis of nucleoside 2',3'-cyclic phosphates and their analogues. For example, oxidation of **19a** with I<sub>2</sub>/H<sub>2</sub>O produced nucleoside 2',3'-cyclic phosphates of type **20** (isolated in yields exceeding 90%), while its sulfuration with elemental sulfur afforded nearly quantitatively



DMT = 4,4'-dimethoxytrityl

**18a, 19a, 21a, 22a** X = O

**18b, 19b, 21b, 22b** X = S

B = adenin-9-yl, cytosin-1-yl, guanin-9-yl, thymin-1-yl

Scheme 7

thiophosphoryl chloride [40] or cyclisation of nucleoside 2'(3')-phosphorothioate derivatives [41]. Yields of these reactions (in most instances determined only by UV-spectroscopy) are invariably low (6–10%) [40, 41] and, with the most efficient recent method involving P(III) derivatives [42], do not exceed 40%.

During our studies on phosphorylation of 2',3'-unprotected ribonucleosides **17** we have found that diphenyl *H*-phosphonate (**18a**) or diphenyl *H*-phosphonothioate (**18b**) readily and quantitatively produced the corresponding nucleoside 2',3'-*O,O*-cyclic *H*-phospho-

the respective nucleoside 2',3'-*O,O*-cyclic phosphorothioates **21a** as a mixture of two diastereomers [43].

Analogously, treatment of cyclic *H*-phosphonothioates **19b** with S<sub>8</sub> produced the corresponding nucleoside 2',3'-*O,O*-cyclic *H*-phosphorodithioates **21b** in high yields (50–60% after chromatography). Cyclic *H*-phosphonate **19a** and *H*-phosphonothioate **19b** also underwent readily oxidation with elemental selenium to produce the corresponding nucleoside 2',3'-*O,O*-phosphoroselenoates **22a** and phosphoroselenothioates **22b**, respectively. Thus, the above method, employ-

ing cyclic *H*-phosphonates and cyclic *H*-phosphonothioates represents a new, efficient and general entry to nucleoside 2',3'-*O,O*-cyclic phosphates, phosphorothioates, phosphorodithioates, phosphoroselenoates, and cyclic phosphoroselenothioates. Further chemical and biochemical studies on these new 2',3'-*O,O*-cyclic phosphate analogues are in progress in our laboratories.

In conclusion, aryl *H*-phosphonates, compounds of controlled reactivity, have emerged as convenient intermediates for the preparation of various biologically important

phosphate esters and their analogues, e.g. nucleoside phosphoramidates with the P–N bond in a bridging position, nucleoside *H*-phosphonothio- or nucleoside *H*-phosphodithioates, nucleoside 2',3'-*O,O*-cyclic phosphates bearing single or multiple modifications at the phosphorus centre. As synthetic intermediates, this class of compounds seems to be superior to carboxylic- or phosphoric-phosphonic mixed anhydrides, particularly when high chemoselectivity of substitution at the phosphorus centre is required.

## REFERENCES

1. Westheimer, F.H. (1987) Why nature chose phosphate. *Science* 235, 1173-1178. [MEDLINE](#)
2. Kers, A., Kers, I., Kraszewski, A., Sobkowski, M., Szabo, T., Thelin, M., Zain, R. & Stawinski, J. (1996) Nucleoside phosphonates. Development of synthetic methods and reagents. *Nucleosides & Nucleotides* 15, 361-378.
3. Stawinski, J. (1992) Some aspects of *H*-phosphonate chemistry, in *Handbook of Organophosphorus Chemistry* (Engel, R., ed.) pp. 377-434, Marcel Dekker, Inc., New York.
4. Jankowska, J., Sobkowski, M., Stawinski, J. & Kraszewski, A. (1994) Studies on aryl *H*-phosphonates. I. An efficient method for the preparation of deoxyribo- and ribonucleoside 3'-*H*-phosphonate monoesters by transesterification of diphenyl *H*-phosphonate. *Tetrahedron Lett.* 35, 3355-3358. [MEDLINE](#)
5. Sobkowski, M., Stawinski, J., Sobkowska, A. & Kraszewski, A. (1994) Studies on reactions of nucleoside *H*-phosphonates with bifunctional reagents. Part 2. Stability of nucleoside *H*-phosphonate diesters in the presence of amino alcohols. *J. Chem. Soc. Perkin Trans.* 1, 1803-1808.
6. Cieslak, J., Sobkowski, M., Kraszewski, A. & Stawinski, J. (1996) Aryl *H*-phosphonates. Part IV. A new method for internucleotide bond formation based on transesterification of aryl nucleoside *H*-phosphonate diesters. *Tetrahedron Lett.* 37, 4561-4564. [MEDLINE](#)
7. Kers, A., Stawinski, J., Dembkowski, L. & Kraszewski, A. (1997) Aryl *H*-phosphonates. 7. Studies on the formation of phosphorus-carbon bond in the reaction of trityl and benzyl halides with dialkyl and diphenyl *H*-phosphonates. *Tetrahedron* 53, 12691-12698. [MEDLINE](#)
8. Sobkowska, A., Sobkowski, M., Cieslak, J., Kraszewski, A., Kers, I. & Stawinski, J. (1997) Aryl *H*-phosphonates. 6. Synthetic studies on the preparation of nucleoside *N*-alkyl-*H*-phosphonamides. *J. Org. Chem.* 62, 4791-4794. [MEDLINE](#)
9. Sobkowski, M., Kraszewski, A. & Stawinski, J. (1998) The reactions of *H*-phosphonates with bifunctional reagents. Part V. Functionalization of support-bound oligonucleotides and synthesis of non-radioactive hybridization probes. *Nucleosides & Nucleotides* 17, 253-267.
10. Ozola, V., Reese, C.B. & Song, Q. (1996) Use of ammonium aryl *H*-phosphonates in the preparation of nucleoside *H*-phosphonate building blocks. *Tetrahedron Lett.* 37, 8621-8624. [MEDLINE](#)
11. Cieslak, J., Szymczak, M., Wenska, M., Stawinski, J. & Kraszewski, A. (1999) Aryl *H*-phosphonates. Part 11. Synthetic and <sup>31</sup>P NMR studies on the formation of aryl nucleoside *H*-phosphonates. *J. Chem. Soc. Perkin Trans.* 1, 3327-3331.
12. Garegg, P.J., Regberg, T., Stawinski, J. & Stromberg, R. (1987) Nucleosides *H*-phosphonates. V. The mechanism of hydrogenophosphonate diester formation using acyl chlorides as coupling agents in oligonucleotide synthesis by the hydrogenophosphonate approach. *Nucleosides & Nucleotides* 6, 655-662.
13. Efimov, V.A., Dubey, I.Y. & Chakhmakhcheva, O.G. (1990) NMR Study and improvement of *H*-phosphonate oligonucleotide synthesis. *Nucleosides & Nucleotides* 9, 473-477.
14. Dubey, I.Y., Efimov, V.A., Lyapina, T.V. & Fedoryak, D.M. (1992) Nucleophilic catalysis in *H*-phosphonate internucleotide condensation reaction. *Bioorg. Khim.* 18, 911-919. [MEDLINE](#)
15. Cieslak, J., Jankowska, J., Kers, A., Kers, I., Sobkowska, A., Sobkowski, M., Stawinski, J. & Kraszewski, A. (1996) Synthetic applications of aryl *H*-phosphonates in nucleotide chemistry. *Coll. Czech. Chem. Commun.* 61, 242-245.
16. Perrin, D.D. (1965) *Dissociation constants of organic bases in aqueous solution*. Butterworth, London.
17. Kers, A., Kers, I., Stawinski, J., Sobkowski, M. & Kraszewski, A. (1996) Studies on aryl *H*-phosphonates. 3. Mechanistic investigations related to the disproportionation of diphenyl *H*-phosphonate under anhydrous basic conditions. *Tetrahedron* 52, 9931-9944. [MEDLINE](#)
18. Cieslak, J., Jankowska, J., Sobkowski, M., Kers, A., Kers, I., Stawinski, J. & Kraszewski, A. (1999) The reactions of aryl nucleoside *H*-phosphonates with O-, N-, and S-nucleophiles. *Collection Symposium Series* 2, 63-68.
19. Kers, I., Stawinski, J. & Kraszewski, A. (1999) Aryl *H*-phosphonates. 10. Synthesis of nucleoside phosphoramidate and nucleoside phosphoramidothioate analogues via *H*-phosphonamide intermediates. *Tetrahedron* 55, 11579-11588. [MEDLINE](#)
20. Stawinski, J., Thelin, M. & Zain, R. (1989) Nucleoside *H*-phosphonates. X. Studies on nucleoside hydrogenphosphonothioate diester synthesis. *Tetrahedron Lett.* 30, 2157-2160. [MEDLINE](#)
21. Stawinski, J., Thelin, M., Westman, E. & Zain, R. (1990) Nucleoside *H*-phosphonates. 12. Synthesis of nucleoside 3'-(hydrogen-phosphonothioates)monoesters via phosphinate intermediates. *J. Org. Chem.* 55, 3503-3606. [MEDLINE](#)
22. Zain, R., Stromberg, R. & Stawinski, J. (1995) Nucleoside *H*-phosphonates. 15. Preparation of nucleoside *H*-phosphonothioate monoesters from the corresponding nucleoside *H*-phosphonates. *J. Org. Chem.* 60, 8241-8244. [MEDLINE](#)
23. Zain, R. & Stawinski, J. (1996) Nucleoside *H*-phosphonates. Part 16. <sup>31</sup>P NMR studies on the transformation of nucleoside *H*-phosphonates monoesters into a monofunctional trivalent intermediate, nucleoside acyl silyl phosphite. *J. Chem. Soc. Perkin Trans.* 2, 795-799.
24. Jankowska, J., Cieslak, J., Kraszewski, A. & Stawinski, J. (1997) 9-Fluorenylmethyl *H*-phosphonothioate, a versatile reagent for the preparation of *H*-phosphonothioate, phosphorothioate, and phosphorodithioate monoesters. *Tetrahedron Lett.* 38, 2007-2010. [MEDLINE](#)
25. Kers, I., Kers, A., Stawinski, J. & Kraszewski, A. (1999) Aryl *H*-phosphonates. 8. Simple and efficient method for the preparation of nucleoside *H*-phosphonothioate monoesters. *Tetrahedron Lett.* 40, 3945-3948. [MEDLINE](#)
26. Seeberger, P.H., Jorgensen, P.N., Bankaitis-Davis, D.M., Beaton, G. & Caruthers, M.H. (1996) 5'-Dithiophosphoryl deoxynucleotides: Synthesis and biological studies. *J. Am. Chem. Soc.* 118, 9562-9566.
27. Stawinski, J. & Thelin, M. (1990) Nucleoside *H*-phosphonates. XI. A convenient method for the preparation of nucleoside *H*-phosphonates. *Nucleosides & Nucleotides* 9, 129-135.
28. Porritt, G.M. & Reese, C.B. (1990) Use of the 2,4-dinitrobenzyl protecting group in the synthesis of phosphorodithioate analogues of oligodeoxyribonucleotides. *Tetrahedron Lett.* 31, 1319-1322. [MEDLINE](#)
29. Seeberger, P.H., Yau, E. & Caruthers, M.H. (1995) 2'-Deoxynucleoside dithiophosphates: Synthesis and biological studies. *J. Am. Chem. Soc.* 117, 1472-1478.
30. Stawinski, J., Szabo, T., Thelin, M., Westman, E. & Zain, R. (1990) Studies on the preparation of nucleoside *H*-phosphonothioates. *Coll. Czech. Chem. Commun.* 55, 141-144.
31. Cieslak, J., Jankowska, J., Stawinski, J. & Kraszewski, A. (2000) Aryl *H*-phosphonates. 12. Synthetic and <sup>31</sup>P NMR studies on the preparation of nucleoside *H*-phosphonothioate and nucleoside *H*-phosphonodithioates monoesters. *J. Org. Chem.* 65, 7049-7054. [MEDLINE](#)
32. Jankowska, J., Sobkowska, A., Cieslak, J., Sobkowski, M., Kraszewski, A., Stawinski, J. & Shugar, D. (1998) Nucleoside *H*-phosphonates. 18. Synthesis of unprotected nucleoside 5'-*H*-phosphonates and nucleoside 5'-*H*-phosphonothioates and their conversion into the 5'-phosphorothioate and 5'-phosphorodithioate monoesters. *J. Org. Chem.* 63, 8150-8156. [MEDLINE](#)
33. Golos, B., Dzik, J. M., Rode, W., Jankowska, J., Kraszewski, A., Stawinski, J. & Shugar, D. (1997) Interaction of thymidylate synthase with the 5'-thiophosphates and 5'-*H*-phosphonates of 2'-deoxyuridine, thymidine and 5-fluoro-2'-deoxyuridine; in *Chemistry and Biology of Pteridines and Folates 1997. Proc. Eleventh International Symposium on Pteridines and Folates, Berchtesgaden, Germany, June 15-20, 1997* (Pfleiderer, W., Rokos, H., eds.) pp. 423-426, Blackwell Wissenschafts-Verlag, Berlin-Viena, Oxford, Edinburgh, Boston, London, Melbourne, Paris, Tokyo.
34. Shugar, D. (1999) Viral and host-cell protein kinases: Enticing antiviral targets, and relevance of nucleoside, and viral thymidine, kinases. *Pharmacol. Ther.* 82, 315-335. [MEDLINE](#)
35. Lasker, M., Bul, C.D., Besant, P.G., Sugawara, K., Thai, P., Medzihradszky, G. & Turk, C.W. (1999) Protein histidine phosphorylation: Increased stability of thiophosphohistidine. *Protein Sci.* 8, 2177-2185. [MEDLINE](#)
36. Khorana, H.G., Tener, G.M., Wright, R.S. & Moffatt, J.G. (1957) Cyclic phosphates. III. Some general observations on the formation properties of five-, six- and seven-membered cyclic phosphate esters. *J. Am. Chem. Soc.* 79, 430-436.
37. Cowan, J.A. (1998) Metal activation of enzymes in nucleic acid biochemistry. *Chem. Rev.* 98, 1067-1087. [MEDLINE](#)
38. Bratty, J., Chartrand, P., Ferbeyre, G. & Cedergren, R. (1993) The hammerhead RNA domain, a model ribozyme. *Biochim. Biophys. Acta* 1216, 345-359. [MEDLINE](#)
39. Saenger, W. & Eckstein, F. (1970) Stereochemistry of a substrate for pancreatic ribonuclease. Crystal and molecular structure of the triethylammonium salt of uridine 2',3'-*O,O*-cyclophosphorothioate. *J. Am. Chem. Soc.* 92, 4712-4718.
40. Gerlt, J.A. & Wan, W.H.Y. (1979) Stereochemistry of the hydrolysis of the *endo* isomer of uridine 2',3'-cyclic phosphorothioate catalyzed by the nonspecific phosphohydrolase from *Enterobacter aerogenes*. *Biochemistry* 18, 4630-4638. [MEDLINE](#)
41. Holy, A. & Kois, P. (1980) Synthesis of 2'(3')-phosphorothioates of 5'-*O*-carboxymethylinosine and related compounds. *Coll. Czech. Chem. Commun.* 45, 2817-2829.
42. Ludwig, J. & Eckstein, F. (1989) Rapid and efficient synthesis of nucleoside 5'-*O*-(1-thiotriphosphates), 5'-triphosphates and 2',3'-cyclophosphorothioates using 2-chloro-4*H*-1,3,2-benzodioxaphosphiryn-4-on. *J. Org. Chem.* 54, 631-635. [MEDLINE](#)
43. Jankowska, J., Wenska, M., Popenda, M., Stawinski, J. & Kraszewski, A. (2000) A new, efficient entry to nucleoside 2',3'-*O,O*-cyclophosphorothioates. *Tetrahedron Lett.* 41, 2227-2229. [MEDLINE](#)