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Review

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

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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.

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Abbreviation: HMDST, hexamethyldisilathiane.

Intracellular phosphorylation of a multiple of cel lu lar con stit u ents is a key pro cess 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 im por tant path way for reg u la tion of pro tein func tions in eukaryotic cells, involved in switching cellular activities from one stage to an other and in this way, reg u lating gene ex pres sion, cel lu lar pro lif er a tion and cell dif fer en ti a tion. It is the ma jor mech a nism whereby cells re spond to extracellular sig nals, such as hormones and growth fac tors, in this manner controlling all events in various stages of the cell cy cle, as well as the re sponse of a cell to environmental and nutritional stresses.

Intracellular phosphorylation is also reg u lated *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 liv ing sys tems, el e gantly un der lined al most 15 years ago by Frank H. Westheimer [1] in re sponse to the query: "Why were phosphates, and al most no other groups, se lected by evolution for biochemical transformations?"

The pres ent re port de scribes the fun da mental chem i cal prop er ties of aryl *H*-phosphonate diesters, and selected applications of this class of com pounds to the syn the sis of nu cle o tide analogues in which the phosphate moieties are re placed by other struc tur ally re lated groups which may be employed to elucidate the mechanisms of action of natural nucleotides, or oligonucleotides, in bio chem i cal sys tems.

The *H*-phosphonate methodology, due to its efficiency, reliability and experimental simplicity, has emerged in the last decade as a ver sa tile and pow er ful ap proach to the syn the sis of biologically active phosphate analogues [2, 3]. As part of our studies in this field, we have re cently de vel oped aryl *H*-phosphonates as a new type of ac tive *H*-phosphonate de riv a tives [4–9]. Their advantages as synthetic intermediates stem from the fact that these compounds possess, in principle, only one electrophilic cen ter lo cated on the phos pho rus 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 prop er ties of sub stitu ents on the aromatic ring of an aryl moiety. These features sig nif i cantly broaden syn thetic ap pli ca tions of *H*-phosphonate methodology by enabling the syntheses of otherwise difficultly ac ces si ble com pounds, e.g. nucleoside *H*-phosphonamidates [8].

SYNTHESIS AND REACTIVITY OF ARYL *H*-PHOSPHONATES

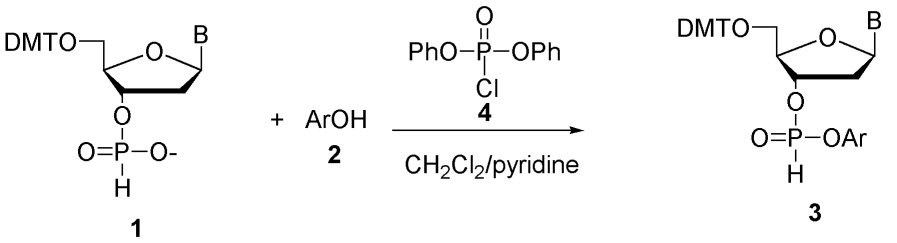
Syn the sis of aryl nucleoside *H*-phosphonates

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, par tic u larly in the con text of re ac tiv ity of the P–H bond, which may af fect the for ma tion and stability of these compounds under various experimental conditions.

Nucleoside aryl *H*-phosphonate can be prepared ei ther by phosphonylation of a suit able protected nucleoside with an appropriate phosphonylating re agent bear ing an aryl moiety [10], or by reacting a nucleoside *H*-phosphonate with an ap pro pri ate phe nol in the presence of a condensing agent [8, 11]. The latter approach (Scheme 1) alleviates prob lems con nected with prep a ra tion of sep a rate phosphonylating reagents for each kind of aryl *H*-phosphonate de riv a tive 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 reactions 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}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**.



DMT = 4,4'-dimethoxytrityl
B = protected nucleobase
a, Ar = 2,4,6-trimethylphenyl
b, Ar = 4-methylphenyl
c, Ar = phenyl
d, Ar = 4-chlorophenyl
e, Ar = 2,4,-dichlorophenyl
f, Ar = 4-nitrophenyl
g, Ar = 2,4,6-trichlorophenyl

Scheme 1

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

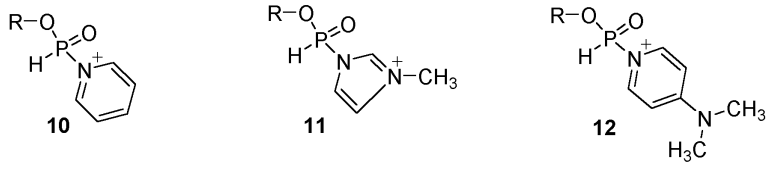
A possible involvement of base or/and nucleophile catalysis in condensation of nucleoside *H*-phosphonate monoesters has been postulated on many occasions [3, 12], but with no a clear-cut evidence. Efimov *et al*. [13, 14] found that nucleoside pivaloyl-phosphonate mixed anhydrides reacted with nucleosides about 10 times faster in the presence of pyridine compared to 4-*N,N*-dimethylaniline, although both bases have similar pK_a values (5.2 and 5.1, respectively). While these difference 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 sev eral hours [11] and at the same time has suitable reactivity towards alcohols [15], enabling monitoring progress of the reac tions by ^{31}P NMR spec tros copy. The set of basic catalysts chosen consisted of amines of different basicity (pK_a 5.2–10.5) [16] (Table 1) in which pyridine (**9a**), *N*-methyl imidazole (**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 eth a nol (1.5 mo lar equiv.) in the pres ence of an ex cess of a cat a lyst (amine **9**, 10 molar equiv.). In all instances the product of transesterification, ethyl nucleoside *H*-phosphonate **6a**, was the only one ob served by ^{31}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 cat a lyst (com ple tion 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 be tween the pK_a val ues of amines and the rate of transesterification of **3d** was not linear, pointing to a significant contribution of nucleophilic ca tal y sis. In deed, the cat a lytic 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**), ca tal y sis oc curs not only *via* gen eration of an alkoxy anion from an alcohol [base catalysis; ROH + B ⇔ RO^- + BH^+], but also *via* for ma tion of re ac tive in ter me di ates of type **10**, **11** and **12** (nucleophile catalysis) (Scheme 2).

On the ba sis of the ses pre lim i nary data, we can tentatively conclude that, in trans esteri-



R = nucleoside moiety

Scheme 2

fication of nucleoside aryl *H*-phosphonates, efficiency of nucleophilic ca tal y sis ex ceeds that of base catalysis. Since strongly basic con di tions are det ri men tal 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 se ries of ex per i ments we in ves tigated internucleotide bond formation in the

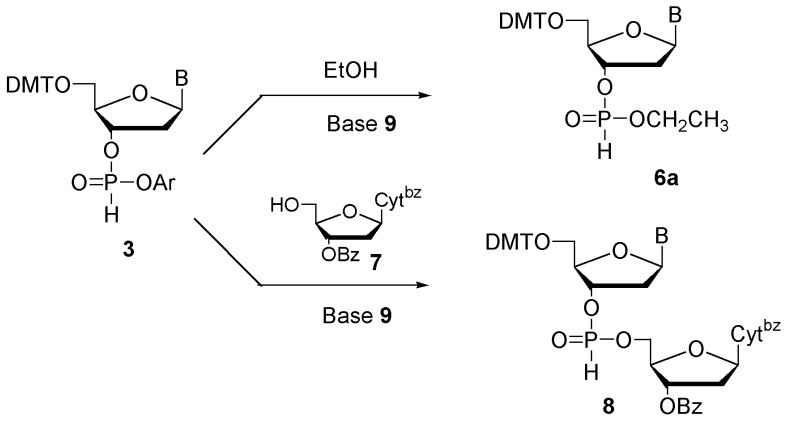
Ta ble 1. For ma tion of ethyl nucleoside *H*-phosphonate 6a **in transesterification of aryl *H*-phosphonate** 3d **with eth a nol (**5a**) catalysed by var i ous bases** 9

Base	**9a**	**9b**	**9c**	**9d**	**9e**	**9f**
pK_a	5.19	6.30	6.95	7.67	9.70	10.78
Time* (min)	120	50	8	25	< 3	< 3

*Time for com plete dis ap pear ance of **3d**

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

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



B = thymin-1-yl
3d, Ar = 4-chlorophenyl
3f, Ar = 4-nitrophenyl
9a = pyridine
9b = hexamethyltetramine
9c = N-methylimidazole
9d = N-ethylmorpholine
9e = 4-N,N-dimethylaminopyridine
9f = triethylamine

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, ^{31}P NMR) was observed. These were identified as nucleoside *H*-phosphonate **1** and bis-aryl nucleoside phosphite (δ_P 127.54 ppm, d, $^3J_{PH}$ = 9.3Hz) [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 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).

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 (δ_P 125.78 ppm, d, $^3J_{HP}$ = 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 pK_a < 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 formation in the presence of bases 9a–f

Base	Substrate **3d**			Substrate **3f**		
	Time* (min)	**8** (%)	X (%)**	Time* (min)	**8** (%)	X (%)**
9a	700	100	0	< 3	100	0
9b	700	100	0	< 3	100	0
9c	60	100	0	< 3	100	0
9d	660	100	0	< 3	92	8
9e	10	91	9	< 3	62	38
9f	50	60	40	< 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.

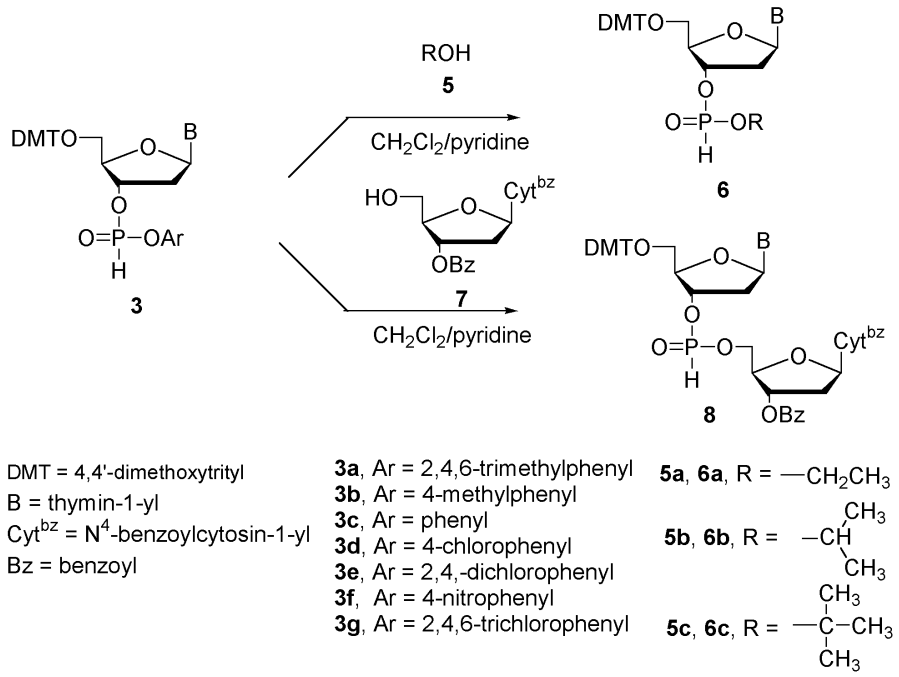
SELECTED SYNTHETIC METHODS FOR NUCLEOTIDE ANALOGUES BASED ON ARYL *H*-PHOSPHONATE INTERMEDIATES

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}P NMR spectroscopy. In all instances, the corresponding nucleoside alkyl *H*-phosphonates **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}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(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^4,3′-*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].



DMT = 4,4'-dimethoxytrityl
B = thymin-1-yl
Cyt^{bz} = N^4-benzoylcytosin-1-yl
Bz = benzoyl

3a, Ar = 2,4,6-trimethylphenyl
3b, Ar = 4-methylphenyl
3c, Ar = phenyl
3d, Ar = 4-chlorophenyl
3e, Ar = 2,4,-dichlorophenyl
3f, Ar = 4-nitrophenyl
3g, Ar = 2,4,6-trichlorophenyl

5a, **6a**, R = $-CH_2CH_3$
5b, **6b**, R = $-CH(CH_3)_2$
5c, **6c**, R = $-C(CH_3)_3$

Scheme 4

Nucleoside *H*-phosphonamidates

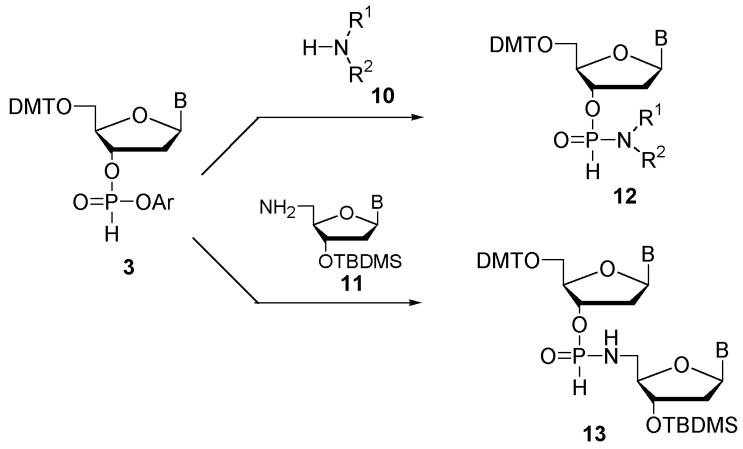
Searching for a simple and versatile method for the synthesis of nucleoside alkyl-*H*-phosphonamidates, we investigated the direct coupling of nucleoside *H*-phosphonates with appropriate amines in the presence of pivaloyl chloride or various chlorophosphates [8]. Unfortunately, such condensations invariably resulted in complex mixtures of products, in which the desired nucleoside *H*-phosphonoamidates 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*-phosphonamidates of type **12** in reactions promoted by a condensing agents 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*-phosphonamidates 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*-phosphonamidates **12a–e** carrying primary and unhindered secondary amine moieties, including dinucleoside *H*-phosphonamidate **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 = thymin-1-yl
TBDMS = *tert*-butyldimethylsilyl
Ar = 2,4,6-trichlorophenyl

a, R^1 = H, R^2 = $-CH_2CH_2CH_2CH_3$

b, R^1 = H, R^2 = $-HC(CH_3)_2$

c, R^1 = H, R^2 = $-C(CH_3)_3$

d, R^1 = $-CH_3$, R^2 = $-CH_2CH_2CH_2CH_3$

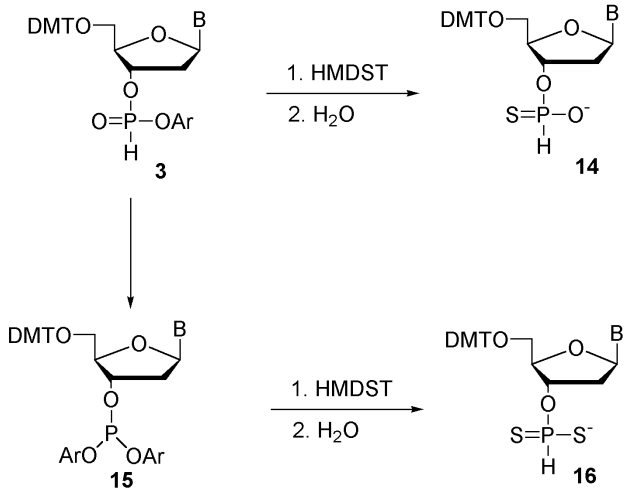
e, R^1 = R^2 = $-CH_2CH_2CH_2CH_3$

Scheme 5

protected nucleosides using various thiophosphonylation 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^6-benzoyladenin-9-yl, N^4-benzoylcytosin-1-yl,
N^2-isobutyrylgunanin-9-yl, thymin-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].

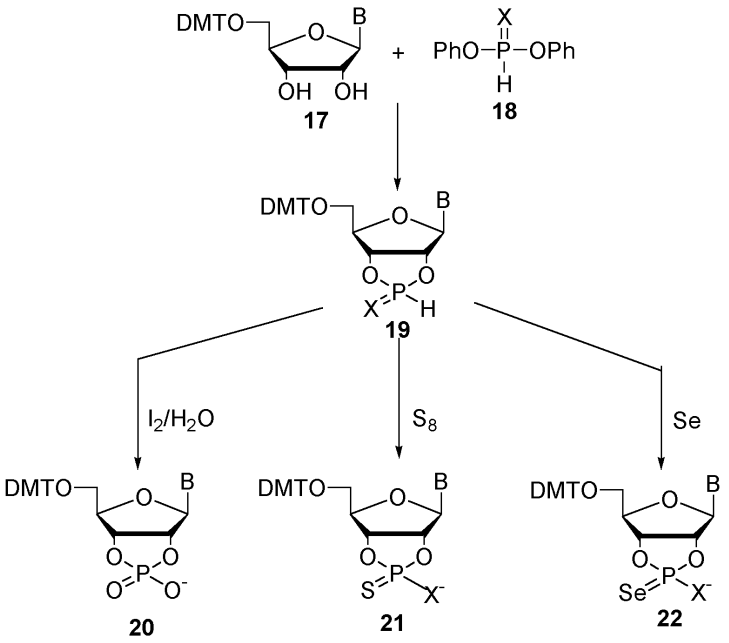
It was long ago reported that ATPγS is a good competitive inhibitor of the ATP-dependent phosphorylation of proteins. It subsequently turned out that this is due to ATPγS being itself a donor for protein kinases, leading to formation of thiophosphorylated protein residues. These are relatively resistant to phosphatases, thus enabling 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 ATPγS as a donor, resulting in markedly increased stability of thiophosphohistidine residues [35]. Somewhat surprisingly, ATPγS is a poor donor, if at all, for nucleoside kinases, but is a moderate to good inhibitor, showing that it does bind to the enzymes [34]. It would be of interest to determine whether ATPγS is a donor for nucleotidekinases.

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 indispens

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



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 invariable 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 phosphonylation 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*-phosphonates [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_2/H_2O produced nucleoside 2′,3′-cyclic phosphates of type **20** (isolated in yields exceeding 90%), while its sulfurisation with elemental sulfur afforded nearly quantitatively the respective nucleoside 2′,3′-*O,O*-cyclophosphorothioates **21a** as a mixture of two diastereomers [43].

Analogously, treatment of cyclic *H*-phosphonothioates **19b** with S_8 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*-phosphonodithioates, 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.

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