Nucleoside H-phosphonates. Part 16.[1] ^{31}P NMR studies on the transformation of nucleoside H-phosphonate monoesters into a monofunctional tervalent intermediate, nucleoside acyl silyl phosphite

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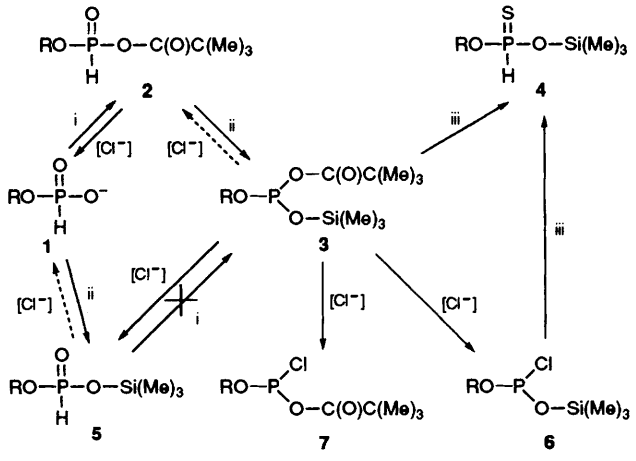
Transformation of nucleoside 3'-H-phosphonate monoester 1 into the corresponding nucleoside pivaloyl† trimethylsilyl phosphite 3 by silylation of the *in situ* formed phosphonic–acyl mixed anhydride 2 with trimethylsilyl chloride (TMSCl), has been investigated using ^{31}P NMR spectroscopy. The conversion was found to be a complex process and its efficiency, due to the involvement of several reversible reactions, depended strongly on the ratio of the reagents used. The tervalent intermediate 3 was found to be unstable under the reaction conditions and underwent at least three parallel reactions, *i.e.* to the nucleoside trimethylsilyl chlorophosphite 6, the nucleoside pivaloyl chlorophosphite 7 and to the monosilylated nucleoside H-phosphonate 5. On the basis of the ^{31}P NMR data the most important factors affecting the stability of 3 were delineated and new reaction conditions for the efficient formation of the monofunctional phosphite derivative 3 from H-phosphonate monoesters 1 were developed.

Introduction

Chemical synthesis of phosphorus-containing natural products *via* H-phosphonate intermediates[2,3] has in recent years begun to emerge as an alternative methodology to the well established phosphite[4,5] and phosphotriester[6,7] approaches. The extended array of synthetic methods available for the preparation of H-phosphonate monoesters[2,8] makes these compounds a convenient starting material for synthesis of oligonucleotides,[9–11] phospholipids,[12,13] phosphorylated carbohydrates[14,15] and a variety of their derived analogues.[2,3] Some phosphate analogues that are rather difficult to obtain from H-phosphonate monoesters, *e.g.* phosphorodithioates and phosphorothioselenoates, can be conveniently prepared *via* H-phosphonothioate intermediates.[16,17]

As a part of our studies in H-phosphonate chemistry we have investigated the possibility of conversion of nucleoside H-phosphonate monoesters into the corresponding H-phosphonothioates.[1,18,19] Taking into account the easy accessibility of H-phosphonate monoesters,[2,8] such a transformation would provide a convenient preparation of the monothio analogues of H-phosphonates.[16] All attempts to achieve this *via* H-phosphonate intermediates, including a reaction of the mixed anhydride of type **2** with hydrogen sulfide, failed to produce the monothio derivatives.[18,19] Instead, H-phosphonodithioates were always formed as the major products. To overcome this problem we have designed a new type of tervalent species, nucleoside silyl acyl phosphite **3**,[19] accessible from H-phosphonate monoesters **1** *via* silylation of the mixed anhydride **2** formed *in situ* (Scheme 1). With the added hydrogen sulfide, the intermediate **3** indeed afforded the desired H-phosphonothioate **4**. In this particular instance[1] it was even possible to simplify the transformation further by using hexamethyldisilathiane, a reagent which simultaneously can act as a silylating agent and a source of an attacking nucleophile (the silylated thiol).

The tervalent intermediate **3** having siloxy and acyloxy groups bound to phosphorus can be considered as a monofunctional phosphitylating agent. Upon attack with a nucleophile only the acyloxy group should depart while the silyl group will remain bound to the phosphorus centre's oxygen or, in principle, may also migrate to another part of the product regenerating the phosphonyl function. The possibility of efficient formation of this type of an intermediate from H-phosphonate monoesters **1** should be helpful in extending further the synthetic application of H-phosphonate derivatives, owing to the different reactivity of tri- *vs.* tetra-coordinated phosphorus species towards nucleophiles.



Scheme 1 **1a–6a**, R = 5'-*O*-(4',4-dimethoxytrityl)-thymidin-3'-yl; **1b–6b**, R = ethyl; (i) pivaloyl chloride in quinoline–acetonitrile (1 : 4, v/v); (ii) trimethylsilyl chloride or bis(trimethylsilyl)acetamide in quinoline–acetonitrile (1 : 4, v/v); (iii) hydrogen sulfide in dioxane

We have noticed, however, that generation of the tervalent intermediate **3** *via* a stepwise addition of pivaloyl chloride and trimethylsilyl chloride to H-phosphonate monoesters **1** is not reproducible, as the amount of **3** in the reaction mixtures varied. Considering the potential synthetic significance of this kind of intermediate, we undertook detailed ^{31}P NMR studies[20] concerning transformation of H-phosphonate monoesters into the tervalent species **3**. The aim was to delineate factors affecting the stability of this intermediate under the reaction conditions used and to develop an efficient and reproducible protocol for generation of nucleoside acyl silyl phosphites of type **3**.

† The IUPAC preferred name for pivaloyl is 2,2-dimethylpropanoyl.

Table 1 ^{31}P NMR data of the substrates **1** and some intermediates[a]

Compound	δ	$^{1}J_{PH}$/Hz	$^{3}J_{PH}$/Hz
1a	1.32	600.7, d	9.6, d
1b	2.86	611.1, d	8.3, t
2a	2.04	748.5, d	9.6, d
	1.95	748.5, d	9.6, d
2b	2.05	731.7, d	9.2, t
3a	121.37	—	9.7, d
	121.19	—	11.3, d
3b	121.96	—	7.3, t
4a	57.10	658.2, d	11.1, d
	56.55	660.0, d	12.8, d
4b	56.79	645.3, d	10.7, t
5a	−2.91	703.4, d	9.1, d
	−3.03	703.4, d	11.6, d
5b	−2.74	689.7, d	9.2, t
6a	153.63	—	12.3, d
6b	154.40	—	8.3, t
7a	152.01	—	12.3, d
7b	153.51	—	7.4, t

[a] Spectra in quinoline–acetonitrile (1:4, v/v).

Results and discussion

The first step in the transformation of H-phosphonate monoesters **1** into the acyl silyl phosphites **3** is the formation of a mixed anhydride of type **2**. In neat pyridine the activation process of **1** promoted by pivaloyl chloride (PV-Cl) does not stop at the stage of the monoacylated species **2**, but proceeds further to the corresponding tervalent diacyl phosphite.[21] Since the rates of both reactions are comparable, a mixture of the mono- and the di-activated species is usually formed, even when a limited amount of acyl chloride is used. However, in the absence of a base[21] or in less basic solvents, *e.g.* in acetonitrile–pyridine[21] or acetonitrile–quinoline[22] mixtures, the second activation step is significantly slower than the reaction of H-phosphonate monoester **1** with PV-Cl, and the mixed anhydride **2** can usually be generated in >95% yield. Since the rate of silylation of **2** should not be significantly affected under such reaction conditions, we chose quinoline–acetonitrile (1:4, v/v) as a standard reaction medium for our ^{31}P NMR experiments.

Reaction of the mixed anhydrides 2 with TMSCl

To check the efficiency of the *in situ* generation of acyl silyl phosphite **3**, the nucleoside H-phosphonate **1a** was treated with PV-Cl (1.5 equiv.) and the reaction was followed by ^{31}P NMR spectroscopy. The activation process was rapid and clean and produced exclusively the mixed anhydride **2a** (two singlets at *ca.* 2 ppm; see also Table 1). Addition of trimethylsilyl chloride (TMSCl) (5 equiv.) to this reaction mixture resulted in an immediate disappearance of the signals corresponding to **2a** and formation of two singlets characteristic of one spin system of P^{III} phosphorus diastereoisomers of the acyl silyl phosphite **3a** at *ca.* 121 ppm. Together with these major resonances (*ca.* 50% of the total nucleotidic material), two singlets at *ca.* 154 ppm (*ca.* 20%) and *ca.* 152 ppm (*ca.* 5%) and two of equal intensities at *ca.* −3 ppm were always observed in the ^{31}P NMR spectra. The resonances related to **3a** and to the two intermediates at *ca.* 150 ppm gradually decreased with time and after a few hours, those at *ca.* −3 ppm became the major signals (*ca.* 80%).

An analogous transformation of ethyl H-phosphonate **1b** followed the same reaction pathway as for **1a**, but the product distribution was different (^{31}P NMR). In this instance, silylation of the *in situ* formed phosphonic–acyl anhydride **2b** (δ_P *ca.* 2 ppm, singlet) with TMSCl (5 equiv.) afforded an intermediate at *ca.* 154 ppm as a major product (*ca.* 45%). The corresponding acyl phosphite **3b** (δ_P *ca.* 121 ppm, singlet) and a compound resonating at *ca.* −3 ppm (singlet), accounted for *ca.* 30 and *ca.* 25%, respectively, of the total phosphorus-containing material. Occasionally, a small singlet (< 5%) at *ca.* 153 ppm was also observed. Similarly to the reaction involving **1a**, a conversion to a product resonating at high field gradually occurred (over a few hours). The larger excess of the silylating agent (10 equiv.) used for the reaction affected neither the intensities of the initially formed intermediates nor the rates of their further transformations.

Identification of intermediates formed in the reactions of mixed anhydrides 2 with TMSCl

The ^{31}P NMR resonances at *ca.* 121 ppm observed during the transformation of **1a** and **1b** were assigned to the postulated tervalent species **3a** and **3b** on the basis of the expected chemical shifts and multiplicity of signals in P–H coupled and {^{1}H}-decoupled spectra. These intermediates have also been produced in different ways[1] (also *vide infra*) and were found to react in a predicted manner with the added hydrogen sulfide or ethanol affording the silylated H-phosphonothioates **4**[1] or the corresponding H-phosphonate diesters, respectively.

Signals in the region of *ca.* 150 ppm and those at *ca.* −3 ppm had similar, but not identical, chemical shifts when produced in the reactions of **2a** and **2b** with TMSCl. It was thus likely that in both reactions the same types of intermediates (with the same arrangement of atoms around the phosphorus centre) were formed.

For the purpose of identification of these intermediates we probed their reactivity by adding hydrogen sulfide at various stages of the reactions. In all instances, signals at *ca.* 150 ppm (which comprised singlets at *ca.* 154 and *ca.* 152 ppm) and those from **3** immediately disappeared, as H-phosphonothioates **4** formed as major products, while resonances at high field (*ca.* −3 ppm) remained unchanged. Small signals at *ca.* 85 ppm (0–5%), whose intensities roughly correlated with those at *ca.* 152 ppm, were related to the corresponding H-phosphonodithioate monoesters. When instead of hydrogen sulfide an excess of ethanol was added, the resonances at *ca.* 150 and at *ca.* 121 ppm were replaced by new ones at *ca.* 8 ppm (major products) and at *ca.* 140 ppm (0–5%). On the basis of the ^{31}P NMR data and by comparison with the authentic samples, the former ones were assigned to the corresponding H-phosphonate diesters (nucleoside ethyl or diethyl H-phosphonates) and the latter ones, to phosphite triester derivatives (nucleoside diethyl and triethyl phosphites, respectively). In these instances the intensities of signals at *ca.* 140 ppm correlated also with those of intermediates resonating at *ca.* 152 ppm.

From these experiments it became apparent that intermediates resonating at *ca.* 154 ppm reacted with hydrogen sulfide and with ethanol in a similar manner as the intermediates **3**, *i.e.* they probably contained only one group susceptible to substitution at the phosphorus centre. In contradistinction to this, the minor intermediates (those resonating at *ca.* 152 ppm) most likely contained two good leaving groups at the phosphorus centre, since they produced di-substituted products (*i.e.* H-phosphonodithioates or phosphite triesters with two ethyl groups). These findings together with the ^{31}P NMR data (in particular the chemical shift values) prompted us to tentatively assign the signals at *ca.* 154 ppm to the corresponding silyl chlorophosphites **6a** and **6b**, and those at *ca.* 152 ppm to the acyl chlorophosphites **7a** and **7b** (see Table 1). These assignments were substantiated by *in situ* generation of the postulated species **6b** and **7b** in the exchange reactions between the appropriate tervalent species [ethyl phosphorodichloridite, ethyl bis(trimethylsilyl) phosphite and ethyl dipivaloyl phosphite; see Experimental section].

The intermediates (δ_P *ca.* −3 ppm) which were unreactive toward hydrogen sulfide (or ethanol) were identified as monosilylated H-phosphonates **5** on the basis of their ^{31}P NMR data and the chemical reactivity. In agreement with the assigned structures, two singlets of the phosphorus diastereo-

isomers were observed in the ^{31}P NMR spectra of **5a** and only one singlet in the instance of **5b**. The values of $^{1}J_{PH}$ coupling constants *ca.* 700 Hz (see Table 1) indicated an H-phosphonate diester-type species. Addition of water to the reaction mixtures containing mainly **5a** or **5b** resulted in regeneration of the corresponding starting H-phosphonate monoester **1**. Finally, silylation of the starting material **1a** and **1b** with TMSCl (see Experimental section) afforded species identical to the observed intermediates at *ca.* 3 ppm (^{31}P NMR data).

Identification of possible sources of the side-products

In principle, only silyl H-phosphonates **5** and acyl chlorophosphites **7** should be considered as side-products, since in reactions with nucleophiles they afford different products compared with those obtained from **3**. Silyl chlorophosphites **6**, on the other hand, react with nucleophiles in a similar manner to the intermediates **3**, and thus their formation provide simply an alternative reaction pathway to a product, *e.g.* formation of H-phosphonothioate **4** (Scheme 1). However, inasmuch as generation of very reactive species of type **6** may contribute to the overall instability of the corresponding reaction mixtures (*vide supra*), we regarded this process as undesirable and wanted to suppress it.

Formation of the two intermediates of type **6** and **7** upon silylation of the mixed anhydrides **2** with TMSCl could have its origin in the presence of chloride anions in the reaction mixtures. One can thus hypothesize that compounds **6** may result from the replacement of the pivaloyloxy group in **3** by chloride, while the acyl chlorophosphite **7**, from the replacement of the siloxy group.‡ The predominant formation of **6** is consistent with the higher leaving ability of the pivaloyloxy *vs.* siloxy group. In a separate experiment we demonstrated (*vide infra*) that **3** indeed can be partially converted to the chlorophosphite **6** upon addition of chloride anion.

However, the reaction pathway *via* **3** is probably not the only way in which silyl chlorophosphites **6** can be generated under the reaction conditions. ^{31}P NMR experiments invariably indicated that the concentration of **6** reached its maximum before the first spectrum could be recorded (*ca.* 3–5 min) and its amount in the reaction mixtures then slowly decreased. Addition of pyridinium hydrochloride (Py·HCl), PV-Cl or TMSCl (or combinations of these reagents) to the reaction mixture containing **6b** (*ca.* 45%), **3b** (*ca.* 30%) and **5b** (*ca.* 25%) (*vide supra*) did not significantly increase the amount of **6b**. This may suggest that a rapid initial burst of formation of **6** may be due to participation of another reaction pathway. One may speculate that coordination of the phosphonyl oxygen in the mixed anhydride **2** by the silicon atom of the silylating reagent can make the phosphorus centre more susceptible to nucleophilic substitution and may result in a rapid displacement of the pivaloyloxy group by chlorine. Thus, **3** and **6** may, in principle, be formed from one intermediate, *i.e.* the mixed anhydride **2**, as a result of two competing reactions.

Formation of the silylated H-phosphonate derivatives **5** during the course of reaction of the mixed anhydrides **2** with TMSCl was troublesome for at least two reasons. First, **5** could not be converted to products of type **4** (or any other product accessible *via* **3**) and thus, even though it remained inert throughout the reaction, it contributed to a lower yield of a desired product formed from the intermediate **3**. Secondly, the amount of **5** (15–30% at the initial stages of the reaction) tended to increase upon standing and after a few hours the silylated H-phosphonate **5** was always the major product.

Silyl acyl phosphites **3** are prone to hydrolysis and thus spurious water may generate the silylated H-phosphonates **5** from these intermediates. This process, however, cannot be the only source of **5**, since the silylated derivatives were formed in comparable amounts even in the presence of a large excess of TMSCl (10 equiv.) in the reaction mixtures. To explain this phenomenon we assumed that a plausible mechanism for the formation of **5** could be deacylation of the acyl silyl phosphite **3** by chlorides. These halide anions can probably also attack the silicon atom in the intermediate **3** regenerating the mixed anhydrides **2** and TMSCl. However, since **2** rapidly reacts with silylating agents, the equilibrium is probably to the right. Accumulation of the silylated H-phosphonates **5**, on the other hand, indicated that these species do not react appreciably under the reaction conditions either with PV-Cl (generated during the deacylation process) or with the silylating agent, or that the appropriate equilibria are attained very slowly.

Attempted synthesis of 3 *via* silyl H-phosphonates

In principle, one can envisage formation of the acyl silyl phosphite **3** *via* acylation of the silylated H-phosphonate **5** instead of silylation of the mixed anhydride **2**. To check this possibility, the H-phosphonate **1a** in quinoline–acetonitrile (1:4, v/v) was treated with TMSCl (5 equiv.) and the reaction was followed by ^{31}P NMR spectroscopy. Only the presence of the monosilylated derivative **5a** could be detected in the reaction mixture (two singlets at *ca.* −3 ppm). A conversion to the corresponding disilyl phosphite (expected chemical shift *ca.* 117 ppm) did not occur to any noticeable extent even after prolonged reaction time (1 h) or upon addition of more TMSCl (total 10 equiv.). The silylated H-phosphonate **5a** reacted very slowly also with the added PV-Cl (3 equiv.) to form the acyl silyl phosphite **3** (< 10% after 20 min). With more PV-Cl, a gradual formation of the corresponding nucleoside dipivaloyl phosphite (δ_P *ca.* 122.2 ppm) was observed.

These findings supported our assumption that if there was any process, *e.g.* deacylation of **3**, which under the reaction conditions would generate the silylated H-phosphonates **5**, it should lead to accumulation of these species in the reaction mixtures. It also seems apparent that a facile conversion of the mixed anhydrides **2** to the tervalent species **3**, is primarily due to the activating effect exerted by electron-withdrawing pivaloyloxy group on the phosphorus centre.

^{31}P NMR experiments related to the putative equilibria 1 ⇌ 2 and 1 ⇌ 5

In order to suppress formation of the side-products **5** occurring upon silylation of the mixed anhydride **2**, we varied the amount of PV-Cl used for the activation of **1**. We noticed that with the increasing excess of the condensing reagent (1.5–5 equiv),§ the amount of **5** significantly decreased (from *ca.* 30 to less than 5%) upon the subsequent silylation of **2** with TMSCl (5 equiv.). We interpreted this as an indication of the existence of a possible equilibrium between the starting H-phosphonate monoester and the corresponding mixed anhydride (**1** ⇌ **2**). The equilibrium is probably more to the right when an excess of PV-Cl is used for the activation of **1**, and thus less **5** is formed during the subsequent silylation. This process together with the above postulated deacylation of acyl silyl phosphite **3** by the chloride anion may be responsible for the observed formation of **5**. Their relative contribution probably will vary depending on the reaction conditions. One should consider that the mixed anhydride **2** may also be engaged in an equilibrium with **3** (Scheme 1), thus affording two species, **1** and **3**, from which the silylated H-phosphonate **5** can be formed.

To probe this rather complex equilibria system we carried out model studies using ethyl H-phosphonate **1b**. First, activation

‡ Since the amount of **7** in the reaction mixtures was always low and varied (depending *inter alia* on the excess of PV-Cl used), it is possible that the origin of **7** is different from that suggested above.

§ For nucleoside H-phosphonate **1a**, a noticeable increase in diacyl phosphite formation was observed under these reaction conditions.

of **1b** with PV-Cl in the presence of excess of chloride anions (Py·HCl, 15 equiv.) was studied. In agreement with the postulated equilibrium **1** ⇌ **2**, in this instance more PV-Cl (3–5 equiv.) was necessary to achieve a complete conversion of **1b** into the mixed anhydride **2b** than in the absence of Py·HCl. Addition of an excess of TMSCl (10 equiv.) to such reaction mixtures had a similar effect as in the analogous reactions without Py·HCl (*vide supra*), but product distribution was different. The silyl H-phosphonate **5b** and the chlorophosphite **6b** were formed in practically equal amounts, while the acyl silyl phosphite **3b** was present in less than 5%.

The transformation of **1b** to the phosphite **3b** was always more complex (monitored by ^{31}P NMR spectroscopy), when only a small excess of TMSCl was used in the silylation step. Thus, addition of TMSCl (2.5 equiv.) to the mixed anhydride **2b** [generated from **1b** with PV-Cl (3 equiv.)] resulted in the reaction mixture (first ^{31}P NMR spectrum, *ca.* 5 min) containing **6b** (< 3%), **3b** (*ca.* 25%) and **5b** (*ca.* 35%). Besides these, the unreacted mixed anhydride **2b** (*ca.* 25%) and dipivaloyl ethyl phosphite (*ca.* 10%, δ_P *ca.* 122.9 ppm) were also present. With time gradual changes occurred which resulted in accumulation in the reaction mixture of the silylated H-phosphonate **5b** and the diacyl phosphite¶ (56 and 23%, respectively, after 40 min). Using less PV-Cl (1.5 equiv.) for the activation of **1b**, silylation with TMSCl (2.5 equiv.) produced less dipivaloyl ethyl phosphite and **2b** (5 and *ca.* 10%, respectively). However, another 2 equiv. of PV-Cl added to this reaction increased the amounts of these two species (*ca.* 12 and 20%, respectively).

The above results indicate that compounds **1**, **2**, **3** and **5** may be involved in a complex equilibria system, presented in a simplified manner in Scheme 1. Owing to these, the transformation **1** ⟶ **3** is rather sensitive to the amount of PV-Cl and TMSCl used for the reaction and proceeds most efficiently when the ratio of these reagents is *ca.* 3:5. The equilibria are apparently reached slowly in quinoline–acetonitrile (1:4, v/v), but may become fast in the presence of pyridine.[23] In agreement with this, addition of pyridine (total 10%) to the reaction mixture containing **5b** and PV-Cl (5 equiv.) in quinoline–acetonitrile resulted in an immediate formation of dipivaloyl ethyl phosphite as the major product (by ^{31}P NMR spectroscopy).

Silylation of the mixed anhydrides 2 with bis(trimethylsilyl)-acetamide

In the light of the experiments discussed above it becomes apparent that the overall complexity of the reaction system can be traced back to the presence of chloride anions in the reaction mixtures. Some of the possible reaction pathways in which these halide anions can be involved are depicted in Scheme 1. One can also envisage participation of TMSCl and/or PV-Cl in some transformations leading to the formation of side-products.

To simplify the reaction system we considered the possibility of using in the silylation step a reagent with leaving groups other than chloride at the silicon centre. These should eliminate (or at least suppress) most of the observed side-reactions and contribute to more efficient and more reproducible generation of the acyl silyl phosphites **3** from the corresponding mixed anhydrides **2**. For this purpose we chose a commercially available reagent bis(trimethylsilyl)acetamide[24] (BSA), which is known to promote salt free silylation of hydroxylic functions.

To check the efficacy of this approach, we reacted the mixed anhydride **2a** or **2b**, generated *in situ* from the appropriate H-phosphonate monoester **1** and PV-Cl (1.5 equiv.) with BSA (5 equiv.). In both instances, an immediate and clean formation of the corresponding acyl silyl phosphites **3a** or **3b**, was observed using ^{31}P NMR spectroscopy. No signals which could be assigned to the previously observed side-products of type **5**, **6** or **7**, were detected. What was even more important, the acyl silyl phosphites **3** produced were stable under the reaction conditions and did not show any noticeable tendency to decompose (*ca.* 1 h). With 2 equiv. of BSA a clean conversion to **3** was also achieved.

BSA proved to be slightly more reactive than TMSCl as judged from the more pronounced formation of the disilylated species in the reactions with H-phosphonates **1** (*ca.* 10% after 15 min). Also, in contradistinction to TMSCl (*vide supra*), this reagent produced almost exclusively the silylated H-phosphonate **5b** (**3b** and **6b**, < 5%) when added to the reaction mixture containing the mixed anhydride **2b** and an excess of chloride anions (15 equiv. of Py·HCl). This can be explained by assuming that in the presence of a large excess of chlorides, the siloxy anion can be generated from BSA and it may act as a nucleophile towards the phosphorus centre in the mixed anhydride **2b**. It is also possible that the acyl silyl phosphite **3b** is formed as a primary product of the reaction, but it rapidly collapses to **5a** in the presence of chloride anions.

The latter option, which we have already postulated as a possible pathway leading to **5**, could now be checked. To this end, the phosphite **3b**, generated from the mixed anhydride **2b** and BSA (3 equiv.), was treated with incremental amounts of Py·HCl. Addition of the first three equivalents did not have any noticeable effect on **3b**, but a larger excess of Py·HCl triggered the expected transformation. Thus, 10 min after addition of 10 equiv. of Py·HCl to **3b**, the reaction mixture consisted of **6b** (*ca.* 13%), **3b** (*ca.* 27%), **5b** (*ca.* 25%) and the starting material **1b** (30%). The lack of the reaction with only a few equivalents of Py·HCl is not clear. We can speculate, however, that owing to the scavenging of chloride anions by BSA *via*, *e.g.* the formation of TMSCl, a larger excess of the salt is needed to initiate the reaction. This is consistent with the observation that under normal reaction conditions no chlorophosphites **6** or the silylated side-products **5** are formed during generation of acyl silyl phosphites **3** with BSA, even though the reaction mixtures are not free from chlorides.

Conclusions

The ^{31}P NMR experiments permitted us a rather detailed description of the transformation process leading from the H-phosphonate monoesters **1** to the acyl silyl phosphites **3**. We found that silylation of the phosphonic acyl mixed-anhydrides **2** with TMSCl to produce the desired intermediates **3** is accompanied by formation of silyl chlorophosphites **6**, acyl chlorophosphites **7** and the silylated H-phosphonates **5**. To explain the observed product distribution in this transformation, we postulated *inter alia* that **1**, **2**, **3** and **5** are involved in a complex equilibria system. Some of these equilibria, *e.g.* **1** ⇌ **2**, **1** ⇌ **5**, are pertinent to the use of TMSCl and PV-Cl as silylating and activating agents, respectively, in synthetic applications of H-phosphonate derivatives.

Since the formation of the side-products **5**, **6** and **7** was traced back to the presence of chloride anions (or chlorine-containing reagents) in the reaction mixtures, we used bis(trimethylsilyl)-acetamide in the silylation step instead of TMSCl. This resulted in the clean generation of the acyl silyl phosphites **3** and these were found to be stable under the reaction conditions.

Experimental

Materials and methods

Pyridine, quinoline, acetonitrile and triethylamine (TEA) were refluxed with CaH_2 and then distilled and stored over molecular sieves or CaH_2 (TEA). Pivaloyl chloride (Aldrich), trimethylsilyl chloride (Fluka), bis(trimethylsilyl)acetamide

¶ The amount of dipivaloyl ethyl phosphite was significantly higher than the reaction of **2b** with pivaloyl chloride could account for. Thus, it seems possible that under the reaction conditions this compound may be formed *via* another route, *e.g.* from **3b** and PV-Cl.

(Aldrich), diethyl H-phosphonate (Aldrich), ethyl phosphorodichloridite (Lancaster) and triethyl phosphite (Aldrich) were commercial grade.

5′-*O*-(4,4′-Dimethoxytrityl)thymidine 3′-H-phosphonate (triethylammonium salt)[8] and ethyl H-phosphonate (ammonium salt)[25] were prepared according to the published procedures. Ethyl H-phosphonate (ammonium salt) was converted into the triethylammonium salt by addition of triethylamine and repeated evaporation of added pyridine. The reference compounds used for the identification of some reaction products or intermediates were obtained as follows: **4a** and **4b**, by silylation of the corresponding H-phosphonothioate monoesters with TMSCl in acetonitrile in the presence of a limited amount of quinoline; **5a** and **5b**,[1] by silylation of the parent H-phosphonate monoesters **1a** and **1b**, respectively, with TMSCl in pyridine; 5′-*O*-(4,4′-dimethoxytrityl)thymidine ethyl H-phosphonate diester,[21] by coupling of the corresponding nucleoside H-phosphonate with ethanol in the presence of PV-Cl; 5′-*O*-(4,4′-dimethoxytrityl)thymidine diethyl phosphite,[26] by reaction of the corresponding nucleoside dipivaloyl phosphite with ethanol. The intermediate **6b** was generated *in situ* in the ligand exchange reaction between ethyl phosphorodichloridite and bis(trimethylsilyl) ethyl phosphite in pyridine and **7b** in an analogous reaction between ethyl phosphorodichloridite and dipivaloyl ethyl phosphite. The stock solution of 1 mol dm^{-3} H_2S was prepared by passing hydrogen sulfide through dioxane until saturation.

The ^{31}P NMR experiments were performed on a JEOL GSX-270 FT spectrometer in 10 mm tubes using 0.04 mmol of phosphorus-containing compounds **1** in 2 cm^3 of a solvent (quinoline–acetonitrile, 1 : 4, v/v, or as indicated in the text). 2% H_3PO_4 in D_2O was used as an external standard (coaxial inner tube). Amounts of pivaloyl chloride, silylating agents and other additives were as indicated in the text. The values of the chemical shifts for the intermediates produced *in situ*, in some experiments varied (± 1 ppm) depending on the reaction conditions.

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References

1 Part 15, R. Zain, R. Strömberg and J. Stawinski, *J. Org. Chem.*, 1995, **60**, 8241.
2 J. Stawinski, in *Handbook of Organophosphorus Chemistry*, ed. R. Engel, Marcel Dekker, New York, 1992, p. 377.
3 J. Stawinski and R. Strömberg, *Trends Org. Chem.*, 1993, **4**, 31.
4 M. H. Caruthers, *Science*, 1985, **230**, 281.
5 J. W. Engels and E. Uhlmann, *Angew. Chem., Int. Ed. Engl.*, 1989, **28**, 716.
6 C. B. Reese, *Tetrahedron*, 1978, **34**, 3143.
7 S. A. Narang, *Tetrahedron*, 1983, **39**, 3.
8 J. Jankowska, M. Sobkowski, J. Stawinski and A. Kraszewski, *Tetrahedron Lett.*, 1994, **35**, 3353.
9 P. J. Garegg, I. Lindh, T. Regberg, J. Stawinski, R. Strömberg and C. Henrichson, *Tetrahedron Lett.*, 1986, **27**, 4051.
10 P. J. Garegg, I. Lindh, T. Regberg, J. Stawinski, R. Strömberg and C. Henrichson, *Tetrahedron Lett.*, 1986, **27**, 4055.
11 B. C. Froehler, P. G. Ng and M. D. Matteucci, *Nucleic Acids Res.*, 1986, **14**, 5399.
12 I. Lindh and J. Stawinski, *Nucleic Acids Sym. Ser.*, 1987, **18**, 189.
13 I. Lindh and J. Stawinski, *J. Org. Chem.*, 1989, **54**, 1338.
14 P. Westerduin, G. H. Veeneman, G. A. van der Marel and J. H. van Boom, *Tetrahedron Lett.*, 1986, **27**, 6271.
15 A. V. Nikolaev, I. A. Ivanova and V. N. Shibaev, *Carbohydr. Res.*, 1993, **242**, 91.
16 J. Stawinski, M. Thelin, E. Westman and R. Zain, *J. Org. Chem.*, 1990, **55**, 3503.
17 J. Stawinski, M. Thelin and R. Zain, *Tetrahedron Lett.*, 1989, **30**, 2157.
18 J. Stawinski, T. Szabó, M. Thelin, E. Westman and R. Zain, *Collect. Czech. Chem. Commun.*, 1990, **55**, 141.
19 J. Stawinski, R. Strömberg and R. Zain, *Nucleosides, Nucleotides*, 1991, **10**, 515.
20 J. Stawinski and R. Zain, *Nucleosides, Nucleotides*, 1995, **14**, 711.
21 P. J. Garegg, T. Regberg, J. Stawinski and R. Strömberg, *Nucleosides, Nucleotides*, 1987, **6**, 655.
22 V. A. Efimov, I. Y. Dubey and O. G. Chakhmakhcheva, *Nucleosides, Nucleotides*, 1990, **9**, 473.
23 A. V. Nikolaev, I. A. Ivanova, V. N. Shibaev and A. V. Ignatenko, *Bioorg. Khim.*, 1991, **17**, 1550.
24 A. M. El-Khawaga and H. M. R. Hoffmann, *J. Prakt. Chem.-Chem. Ztg.*, 1995, **337**, 332.
25 P. R. Hammond, *J. Chem. Soc.*, 1962, 2521.
26 P. J. Garegg, I. Lindh and J. Stawinski, in *Biophosphates and Their Analogues — Synthesis, Structure, Metabolism and Activity*, eds. K. S. Bruzik and W. J. Stec, Elsevier, Amsterdam, 1987, p. 89.

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