Nucleoside Phosphonates: Part 7. Studies on the Oxidation of Nucleoside Phosphonate Esters

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Oxidation of phosphonate mono- and di-esters with various oxidizing agents including diaryl disulphides, hexachloroacetone, and iodine, has been investigated under various reaction conditions. The most efficient oxidation procedure consists of treatment of phosphonate esters with iodine in pyridine–water. When the phosphonate esters were presilylated by treatment with trimethylsilyl chloride, the subsequent oxidation with aqueous iodine was faster. ^{31}P N.m.r. spectroscopic studies have enabled us to propose the most likely pathway for the majority of the oxidations.

We have recently reported a new and efficient method for the synthesis of oligonucleotides *via* nucleoside phosphonate intermediates.[2] Since one of the critical steps in that approach involves the oxidation of phosphonate–diester bonds into phosphorodiester linkages, we have been searching for a suitably mild and efficient oxidation procedure applicable.

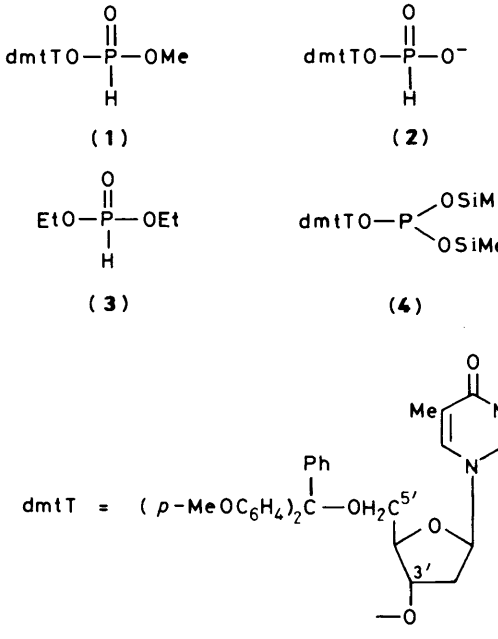
Oxidation of dialkyl or monoalkyl phosphites which exist predominantly in the phosphonate form, usually requires strong oxidizing agents[3] which may cause degradation of these rather fragile compounds. Mild oxidizing reagents such as benzoyl peroxide, perbenzoic acid, or active manganese dioxide do not react with phosphonates, and mercuric acetate or oxide react slowly and a rather troublesome work-up of the reaction mixtures is required.[3]

Preliminary experiments showed that three oxidizing agents might be suitable for our purpose: (*i*) dipyridyl disulphide, introduced by Hata *et al.*[4] for the oxidation of nucleoside 5′-phosphonates, (*ii*) hexachloroacetone, used by Holy *et al.*[5] for the conversion of nucleoside 3′-phosphonates into the corresponding phosphates, and (*iii*) iodine, routinely used for the oxidation of the nucleoside phosphite triesters in oligonucleotide synthesis.[6] Since none of these reagents have been used for the oxidation of phosphonate diesters, we undertook studies using various reaction conditions and to gain some insight into the mechanisms involved, the progress of the reactions was followed by ^{31}P n.m.r. spectroscopy.

The model compounds used for our investigations were 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl methyl phosphonate (**1**) (a phosphonate diester), 5′-*O*-(4,4′-dimethoxytrityl)thymidine-3′-yl phosphonate (**2**) (a phosphonate monoester), and in some experiments also diethyl phosphonate (**3**).

Oxidation with Dipyridyl Disulphide (DPDS).—Typical conditions for the oxidation of nucleoside 5′-phosphonates involve the silylation of the starting material with trimethylsilyl chloride (TMSCl) and subsequent addition of DPDS.[4] The silylation step is necessary to transform the phosphonate esters into the trivalent form of bis-silylphosphites (**4**) which are much more susceptible to oxidation than the tetraco-ordinated species of type (**2**).

^{31}P N.m.r. spectroscopic studies showed that the silylation of compound (**2**) (1.5 p.p.m.) is fast [complete reaction in a few seconds; the new singlet at 118.0 p.p.m. indicated the presence of compound (**4**)]. Addition of DPDS immediately produced the corresponding silylated phosphorothioate diester (**6a**) (8.5 p.p.m.). Addition of water then caused immediate desilylation (singlet at 9.7 p.p.m.) and transformation of the phosphorothioate (**7a**) into the corresponding nucleoside 3′-phosphate (0.2 p.p.m.) in *ca.* 40 min.



The reaction of the phosphonate diester (**1**) followed a similar course. Addition of TMSCl to a pyridine solution of (**1**) produced two singlets at 127.8 and 127.0 p.p.m. [the silyl phosphite (**5**)] in the ^{31}P n.m.r. spectrum, which, on addition of DPDS, immediately disappeared. Two new singlets at 21.6 and 21.2 p.p.m. were then observed [the phosphorothioate (**8a**)] and addition of water caused the slow hydrolysis of (**8a**) (*ca.* 2 h; pH

a, R = 2-Pyridyl; **b**, R = Ph

Table. ^{31}P N.m.r. data of phosphonate esters and intermediates observed during oxidation

Compd.	δ_P/p.p.m.[a]	$^1J_{PH}$ (Hz)[b]	$^3J_{PH}$ (Hz)[b]
(1)	8.3	704	7.6 (2 × q)
(2)	1.5	608	8.8 (2 × d)
(3)	7.3	689	8.9 (2 × q)
(4)	118.0		7.9 (d)
(5)	127.8, 127.0		7.8 (2 × d)
(6a)	8.5		7.6 (d)
(6b)	10.9		7.6 (d)
(7a)	9.7		8.1 (d)
(7b)	12.5		8.1 (d)
(8a)	21.6, 21.2		*c* (m)
(8b)	23.5, 23.0		*c* (m)
(9a)	−7.4, −7.6		7.6 (2 × q)
(9b)	−7.7		7.9 (q)
(11)	−5.7		7.6 (d)
(12a)	3.9, 3.7		9.1 (2 × q)
(12b)	2.8		9.7 (q)

[a] Chemical shifts relative to 2% H_3PO_4 in D_2O (inner tube). Spectra with ^1H heteronuclear decoupling. [b] Spectra without ^1H heteronuclear decoupling.

8.6) producing the corresponding nucleoside phosphoro diester (0.9 p.p.m.). As a side-reaction we also observed demethylation, as revealed by ^{31}P n.m.r. spectroscopy [singlet at 9.7 p.p.m.; the phosphorothioate (**7a**)]. Thus the most likely reaction pathway, based on ^{31}P n.m.r. spectroscopic data, for the oxidation using DPDS is described in Scheme 1, also consistent with the mechanism proposed by Hata *et al.*[4]

(1) ⟶ (5) ⟶ [(**15a** or **b**)]* ⟶ (8)

(2) ⟶ (4) ⟶ [(**14a** or **b**)]* ⟶ (6) ⟶ (7)

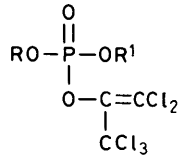
Scheme 1. Oxidation of phosphonate mono- and di-esters by DPDS or DPhDS

When DPDS was replaced by diphenyl disulphide,[7] the same type of intermediates were observed in the ^{31}P n.m.r. spectra. However, the *S*-phenyl nucleoside 3′-phosphorothioate [(**7b**) singlet at 12.5 p.p.m.] was completely stable in pyridine–water solution and no conversion into the phosphate monoester was observed. Also the methyl *S*-phenyl nucleoside phosphorothioate [(**8b**) two singlets at 23.5 and 23.0 p.p.m.] was resistent to hydrolysis, and when allowed to stand in aqueous pyridine (pH 8.6) overnight, only demethylation (by thiophenol) was observed [compound (**7b**) singlet at 12.5 p.p.m. *S*-phenyl nucleoside 3′-phosphorothioate].

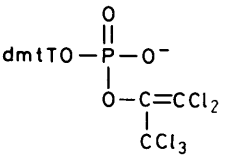
The above experiments indicate that oxidation of compounds (**1**) and (**2**) is much faster than that reported in literature.[4] However, the transformation of the *S*-pyridyl- or *S*-phenyl-nucleoside 3′-phosphorothioate of type (**8**) into the desired nucleoside phosphorodiester, proceeds rather slowly and this may be disadvantageous when such an oxidation procedure is applied to oligomers containing several phosphonate diester bonds.

Oxidation with Hexachloroacetone (HCA).—The original oxidation procedure[5] involves the treatment of nucleoside 3′-phosphonates in acetonitrile with a 30 molar excess of HCA overnight. We have found that replacment of acetonitrile by pyridine speeds up the reaction but, nevertheless, a reaction time of more than 3 h was needed to completely oxidize compound (**1**).

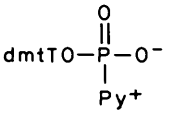
In the oxidation of the phosphonate diester (**1**), the ^{31}P n.m.r. spectrum of the reaction mixture at the beginning consisted of a signal from the starting material (**1**) and two singlets at −7.4 and −7.6 p.p.m. As the reaction proceeded, an additional singlet at −5.7 p.p.m. appeared. When the phosphonate diester had been completely consumed, the ^{31}P n.m.r. spectrum consisted of a singlet at −5.7 p.p.m. and two close singlets at *ca.* −7 p.p.m. all of them of equal intensity. After a further 2 h, the singlets at *ca.* −7 p.p.m. disappeared and only the singlet at −5.7 p.p.m. remained. Since the oxidation of these phosphonates occurs at the expense of the reduction of the carbon–halogen linkage of HCA,[5] it seems likely that the mechanism of the oxidation involves formation of the enol phosphate (**9a**) (two singlets at −7.4 and −7.6 p.p.m.),



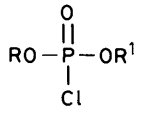
(**9**)



(**10**)



(**11**)



(**12**)

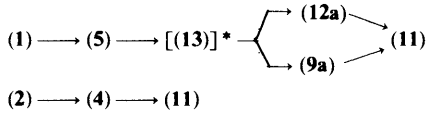
a, R = dmtT, R^1 = Me; **b**, R = R^1 = Et

analogous to the intermediate (**10**) proposed by Holy *et al.*[5] for the oxidation of phosphonate monoesters. However, the signal at −5.7 p.p.m. in the ^{31}P n.m.r. spectra [internal salt (**11**)] unequivocally indicates that demethylation occurs under the reaction conditions. To explain this, it can be assumed that a similar mechanism is operating to that which occurs in methyl nucleoside chlorophosphates of type (**12**), which is known to undergo conversion into the pyridine adduct of metaphosphate (**11**) in the presence of pyridine.[8] In the case of the phosphorotriester (**9a**), demethylation probably results in the formation of intermediate phosphorodiester (**10**), which is instantaneously converted into the pyridine adduct (**11**). An alternative reaction pathway, involving the formation of the chlorophosphate (**12a**) followed by demethylation, seems to be less likely. We failed to produce any detectable amount (^{31}P n.m.r.) of chlorophosphate (**12b**) in the reaction of the phosphorotriester (**9b**) with chloride anions, whereas the chlorophosphates (**12a** and **b**) can be produced in a different way (see below), and were found to be relatively stable [especially (**12b**), which does not undergo dealkylation].

Presilylation of compound (**1**) by TMSCl made the subsequent oxidation much faster. Addition of 3 equiv. of HCA to the silylated phosphonate diester (**1**) resulted in a spectrum (after 3 min) similar to that obtained in the oxidation without presilylation (three resonances at −5.7, −7.4, and −7.6 p.p.m.), but with two additional singlets at 3.9 and 3.7 p.p.m. After a further 3 h, only compound (**11**) (−5.7 p.p.m.) was detected in the reaction mixture. Addition of water produced two compounds which were identified as the nucleoside 3′-phosphate (0.2 p.p.m.) and the corresponding pyrophosphate (−11.9 p.p.m.). When water was added at an earlier stage of the reaction, when all three intermediates were present, the ^{31}P n.m.r. spectrum showed that the two singlets at −7.4 and −7.6 p.p.m. [the phosphorotriester (**9a**)], as expected, remained

unchanged, while the two other intermediates disappeared and new signals at 0.9 p.p.m. (singlet, nucleoside methyl 3′-phosphate), 0.2 p.p.m. (singlet, nucleoside 3′-phosphate), *ca.* −10 p.p.m. (five signals, a characteristic pattern for P^1-methyl-P^1,P^2 dinucleoside pyrophosphates[9]), and −11.9 p.p.m. (singlet, dinucleoside pyrophosphate) were observed. Thus, the chemical reactivity, together with the chemical shift values and the number of signals in the ^{31}P n.m.r. spectrum indicate, that the two singlets at 3.9 and 3.7 p.p.m. come from the two diastereoisomers of the chlorophosphate diester (**12a**).

Since the first step of the oxidation probably consists of an attack by the phosphorus atom on the carbonyl centre of HCA,[10] it is likely that enol phosphate (**9a**) and chlorophosphate (**12a**) are formed from the same intermediate [possible structure (**13**)] *via* two different reaction pathways Scheme 2. Opening of the three-membered ring, followed by elimination of chloride anion (and trimethylsilyl cation) would lead to the enol phosphate (**9a**), while an intramolecular attack of chloride on the phosphorus centre, followed by elimination of pentachloroacetone (and trimethylsilyl cation) would result in the formation of chlorophosphate (**12a**). Alternatively, one can assume the formation of (**12a**) by the attack of nucleophilic phosphorus on the halogen atom in HCA, but such a mechanism is less likely for that type of reaction.[10]



Scheme 2. Oxidation of phosphonate mono- and di-esters by HCA[1]

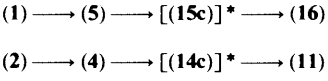
Further support for the hypothesis that reaction of the phosphonate diester with HCA can proceed *via* two parallel reaction pathways [however not necessarily *via* reactive species of type (**13**) as the sole intermediate] was obtained from studies on the oxidation of diethyl phosphonate (**3**) under identical conditions. The ^{31}P n.m.r. spectrum of that reaction mixture showed two singlets (intensities 2:1) at 2.8 p.p.m. [diethylchlorophosphate (**12b**)] and −7.7 p.p.m. (the phosphoro triester (**9b**), and no further change was observed during the next 3 h. Addition of water caused the disappearance of the singlet at 2.8 p.p.m., which was replaced by two resonances at 0.1 (diethyl phosphate) and −13.4 p.p.m. (symmetrical tetraethylpyrophosphate), while the signal at −7.7 p.p.m. remained.

The presilylation procedure also proved to be most efficient for the oxidation of the phosphonate monoester (**2**). Addition of 3 equiv. of HCA to the presilylated compound (**2**) resulted in the formation of the pyridinium adduct (**11**) as a single reactive intermediate observed in the ^{31}P n.m.r. spectrum. Subsequent hydrolysis with water afforded a mixture of nucleoside 3′-phosphate and a symmetrical pyrophosphate, as reported by Holy *et al.*[5] However, under those reaction conditions, we did not observe any formation of the hypothetical intermediate (**10**) (enol phosphoro diester), postulated by Holy.[5]

The above results indicate that HCA can be conveniently used for the oxidation of phosphonate monoesters of type (**2**) when combined with the presilylation procedure. Oxidation of phosphonate diesters by HCA, however, seems to offer little advantage, because it would be necessary to add an additional deprotection step for the conversion of the enol phosphate (**9**) into the desired phosphoro diester.

Oxidation with Iodine.—First we investigated the oxidation of the phosphonate diester (**1**) under conditions commonly employed in the phosphite triester method for oligonucleotide synthesis (I_2 in tetrahydrofuran–2,6-dimethylpyridine–water 94:5:1).[6] The reaction proved to be considerably slower than that for phosphite triesters taking *ca.* 30—40 min to reach completion. However, when pyridine–water (98:2) was used as the solvent, the oxidation was faster and was usually complete after 4—5 min. The phosphonate monoester (**2**) was found to be rather resistant to the oxidation even in pyridine–water and no reaction was observed even after 3 h. However, when compound (**2**) was converted into the silyl ester [compound (**4**)] before the addition of I_2 in anhydrous pyridine, the reaction was practically over within 2 min. The oxidation of the phosphonate diester (**1**) was also faster (*ca.* 1—2 min) when the starting material was silylated before the oxidation.

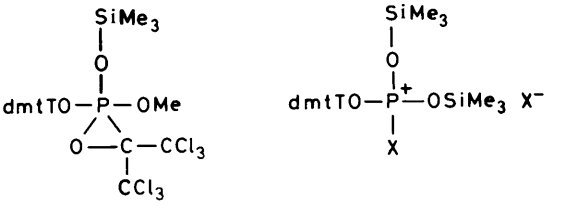
The progress of the reactions was followed by ^{31}P n.m.r. spectroscopy. Addition of 2 equiv. of iodine to the presilylated reaction mixture from the phosphonate monoester (**2**), resulted in an immediate disappearance of the signal due to the silyl phosphite [compound (**4**) 118.0 p.p.m.] and the formation of a singlet at −5.7 [compound (**11**)]. The reaction (Scheme 3) probably proceeds *via* the initial formation of phosphonium salt (**14c**) which undergoes a rapid transformation into the pyridine adduct (**11**), which in turn can be converted into the corresponding phosphate and pyrophosphate by the addition of water.



Scheme 3. Oxidation of phosphonate mono- and di-esters using I_2

For phosphonate diester (**1**), the addition of iodine to the presilylated reaction mixture in pyridine, resulted in the formation of an intermediate which gave two singlets at −12.8 and −12.9 p.p.m. in the ^{31}P n.m.r. spectrum [compound (**15c**), (**16**) or symmetrical pyrophosphate].[15]

Despite the fact that the intermediates of type (**11**), (**15**), and (**16**) are easy to hydrolyse, we would consider it an advantage,



a, X = 2-Pyridylthio; b, X = Phenylthio; c, X = I

if the presilylation procedure could be compatible with the aqueous oxidizing conditions. If oxidation is faster than hydrolysis of the silyl esters [compound (**4**) or (**5**)], we should be able to oxidize compounds (**1**) and (**2**) using iodine in aqueous pyridine. In addition, our previous findings[2] have shown that phosphite diesters produced *in situ* from trivalent species, are very reactive and reaction with an electrophilic reagent is faster than isomerisation into the rather unreactive phosphonate forms. This should ensure rapid oxidation even though silyl

* Postulated intermediates not detected in the ^{31}P n.m.r. spectra are given in square brackets.

esters hydrolyse rapidly upon addition of the aqueous oxidating reagent.

Indeed, we have found that addition of iodine in pyridine–water (98:2) to the presilylated phosphonate monoester (**2**), immediately produced the desired nucleoside 3′-phosphate (0.2 p.p.m.). No formation of the corresponding pyrophosphate was observed. In an analogous reaction, the phosphonate diester (**1**) also produced immediately, as judged from the ^{31}P n.m.r. spectrum, the methyl nucleoside phosphoro diester (0.9 p.p.m.). In addition, the above reaction conditions were the only ones for which we did not observe demethylation of compound (**1**) during the oxidation.

Other Oxidizing Reagents.—We also checked the efficiency of the oxidation of the phosphonate diesters (**1**) and (**3**) using two reagents, recently recommended for oxidation in the automated synthesis of DNA fragments *via* the phosphite triester approach, namely, iodobenzene diacetate[11] and tetrabutylammonium periodate.[11] Both compounds proved to be rather unreactive towards the phosphonate diesters under the reaction conditions stated in the literature for the oxidation of phosphite triesters, and the reaction did not go to completion overnight. The reactions were faster in pyridine, but unidentified intermediates formed during the oxidation were rather difficult to hydrolyse in pyridine–water for several hours. The presilylation procedure, speeded up the oxidation, but reaction was still much slower (*ca.* 30 min) with both reagents, as compared to the oxidation with iodine. In addition, the ^{31}P n.m.r. spectra showed formation of some unidentified side products.

Conclusions.—The mildest, most efficient, and convenient oxidation procedure found for the transformation of nucleoside phosphonate esters into the corresponding phosphates, consists of a silylation of the phosphonate esters and subsequent oxidation with iodine in pyridine–water (98:2). The oxidation procedure, presented in this paper, with iodine (with or without presilylation) has recently been successfully applied by us to the transformation of oligonucleotide phosphonates into oligonucleotides with the desired phosphoro diester bonds during the chemical synthesis of DNA[12] and RNA[13] fragments *via* phosphonate intermediates.

Other oxidizing reagents investigated during these studies proved to be less suitable for our purpose, *i.e.* conversion of phosphonate diesters into the corresponding phosphoro diesters in one step. Despite the fact, that DPDS, DPhDS, and HCA oxidize phosphonate esters only slightly slower than iodine, the oxidation pathways involve formation of rather stable intermediates [*e.g. O,O*-dialkyl *S*-aryl phosphorothioate, phosphoro triester (**9**)], and thus an additional reaction step would be necessary to obtain the desired phosphoro diesters.

Experimental

Materials.—5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl phosphonate (triethylammonium salt (**2**) and 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl methyl phosphonate (**1**) were prepared as described previously.[2] Dipyridyl disulphide, diphenyl disulphide, hexachloroacetone, diethyl phosphonate (**3**), diethyl chlorophosphate (**12b**), trimethylsilyl chloride, and iodine were commercial grade (Aldrich). Pyridine was refluxed and distilled over P_2O_5, then distilled over CaH_2 and stored over 4 Å molecular sieves. Acetonitrile (p.a.) was distilled from CaH_2 and stored over molecular sieves 3 Å.

Methods.—All ^{31}P n.m.r. spectra were recorded on 12 mm tubes with a Varian Associates XL-100 FT spectrometer operating at 40.48 MHz. Chemical shifts are reported relative to 2% H_3PO_4 in D_2O (inner tube). Experiments were usually carried out in 3—4 ml of the appropriate solvent in n.m.r. tubes.

The following compounds were synthesized for comparison with reaction products or intermediates observed during various oxidation reactions. 5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl phosphate was prepared as described in literature[14] and isolated using silica gel short column chromatography. The corresponding symmetrical pyrophosphate and the pyridine adduct metaphosphate (**11**) were prepared from the above compound by reaction with 1 and 3 equiv. of 2,4,6-triisopropylbenzenesulphonyl chloride (TPSCl) respectively. 5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl *S*-phenyl phosphorothioate (**7b**) was obtained from 5′-*O*-(4,4′-dimethoxytrityl)-thymidine and *S*-phenyl phosphorodichloridate (1.5 equiv.) in pyridine, followed by hydrolysis and separation on a silica gel column. 5′-*O*-(4,4′-Dimethoxytrityl)thymidine-3′-yl *S*-phenyl phosphorothioate trimethylsilyl ester (**6b**) was produced from the phosphorothioate (**7b**) and trimethylsilyl chloride (5 equiv.) in pyridine. 5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl *S*-phenyl methylphosphate (**8b**) was prepared by reaction of compound (**1**) and benzenesulphenyl chloride (2 equiv.) in pyridine. Diethyl phosphate was obtained from diethyl chlorophosphate by hydrolysis in pyridine and the enol phosphate (**9b**) was prepared by the Perkow reaction from triethyl phosphite and HCA (1.5 equiv.) in acetonitrile or in pyridine. 5′-*O*-(4′,4′-Dimethoxytrityl)thymidin-3′-yl methyl phosphorochloridate (**12a**) was synthesized by treating 5′-*O*-(4,4′-dimethoxytrityl)thymidine with methyl phosphorodichloridate (1.2 equiv.) in pyridine–acetonitrile (1:1).

Oxidation with Dipyridyl Disulphide.—The nucleoside phosphonate (**1**) or (**2**) (0.2 mmol) was dissolved in pyridine (3 ml) and trimethylsilyl chloride (5 equiv.) was added, followed by dipyridyl disulphide (2 equiv.). After each step, a ^{31}P n.m.r. spectrum was recorded. Hydrolysis was carried out by the addition of water (0.5 ml).

Oxidation with diphenyl disulphide was carried out analogously.

Oxidation with Hexachloroacetone.—Oxidation in acetonitrile was carried out as described in the literature,[5] using the presilylation procedure. The nucleoside phosphonate (**1**) or (**2**) (0.2 mmol) was dissolved in pyridine (3 ml) and trimethylsilyl chloride (5 equiv.) was added, followed by hexachloroacetone (3 equiv.). The progress of the reaction was monitored by ^{31}P n.m.r. spectroscopy.

Oxidation with Iodine.—(*a*) *Procedure without presilylation.* The nucleoside phosphonate (**1**) or (**2**) (0.2 mmol) was dissolved in pyridine (3 ml) after which iodine (2 equiv.) in pyridine (0.5 ml) or in aqueous pyridine was added.

(*b*) *Procedure with presilylation.* The nucleoside phosphonate (**1**) or (**2**) (0.2 mmol) was treated, in pyridine (3 ml), with trimethylsilyl chloride (5 equiv.). Iodine (2 equiv.) in pyridine (0.5 ml) was then added, followed by water (0.5 ml). Alternatively, the oxidizing reagent [iodine (2 equiv.)] in aqueous pyridine (98:2; 0.5 ml) could be added to the presilylated reaction mixture. ^{31}P N.m.r. spectra were recorded after each step.

Acknowledgements

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15 To our knowledge ^{31}P n.m.r. chemical shifts of diester iodophosphates in pyridine have not been reported. In methylene dichloride, diethyl iodophosphate resonates at −41.0 p.p.m. (A. Skowronska *et al.*, *Tetrahedron Lett.*, 1980, **21**, 321), however, addition of pyridine to such a solution results in immediate replacement of the original signal by a new one, at *ca.* −13 p.p.m. (J. Stawinski and R. Strömberg, unpublished results).

Since the nature of this phenomenon is not clear, further studies are necessary to establish a structure of the intermediate formed during the reaction of (**5**) with iodine.

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