The Stereochemical Course of Iodine–Water Oxidation of Dinucleoside Phosphite Triesters

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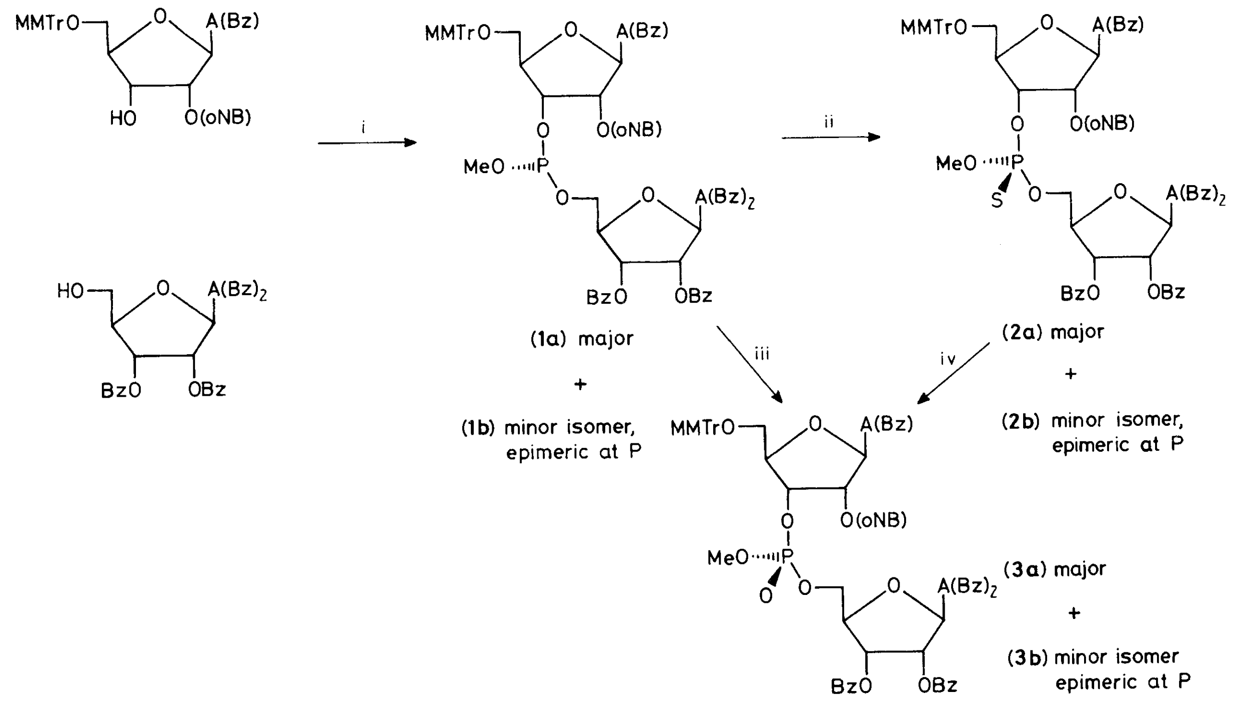
The oxidation of trialkyl phosphites, especially dinucleoside phosphite triesters, by iodine–water and by *m*-chloroperbenzoic acid has been shown to occur stereospecifically with retention of configuration.

A crucial step in the phosphite route[1] for oligonucleotide synthesis is oxidation of the phosphite intermediate to give the phosphate triester. Iodine–water has apparently been adopted as the reagent of choice in the many recent variants of the phosphite coupling method.[2] Despite the importance of this reaction in oligonucleotide synthesis, it appears to have been little studied since its introduction by Letsinger *et al.*[1] We report here the stereochemical course of this reaction.

2′,3′,*N*,*N*′-Tetrabenzoyladenosine[3] and *N*-benzoyl-5′-monomethoxytrityl-2′-*o*-nitrobenzyladenosine[4] were coupled using methoxydichlorophosphite[5] (1 equivalent of each) essentially as described by Benkovic *et al.*[6] to give the diastereoisomeric phosphite triesters (**1a**) and (**1b**) in an approximate ratio of 2 : 1 [$\delta(^{31}\mathrm{P})$ (tetrahydrofuran, THF) 140.63(s); 140.37 (s) p.p.m.; 2 : 1 respectively]. Oxidation of the phosphite triesters (**1a**) and (**1b**) with elemental sulphur gave the diastereoisomeric thiophosphate triesters (**2a**) and (**2b**) in the same ratio [$\delta(^{31}\mathrm{P})$ ($\mathrm{CDCl_3}$) 69.98(s); 69.38 (s) p.p.m.; 2 : 1 respectively]. The absolute configurations of these thiophosphate triesters have previously been assigned;[6] the major diastereoisomer has the R_P configuration (**2a**). Since oxidation of phosphites with elemental sulphur has been shown to occur with retention of configuration[7] the major diasteroisomer of the phosphite triester must have the S_P configuration (**1a**).

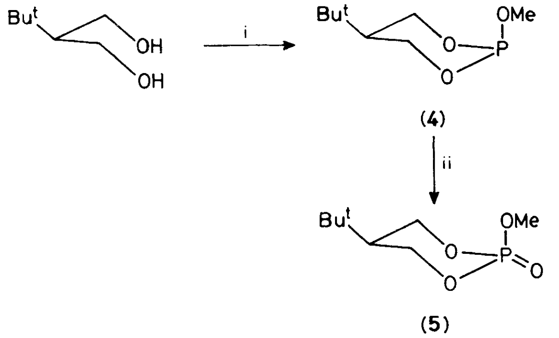
The same mixture of diastereoisomeric phosphites (**1a**) and (**1b**) was oxidised using iodine in water, as described by Letsinger *et al.*[1] to give the corresponding diastereoisomers of



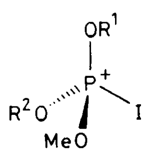
Scheme 1. *Reagents:* i, MeOPCl$_2$; ii, S$_8$, Et$_3$N; iii, I$_2$–H$_2$O or *m*-chloroperbenzoic acid; iv, *m*-chloroperbenzoic acid. *Substituent groups:* A = adenosine, Bz = benzoyl, oNB = *o*-nitrobenzyl, MMTrO = monomethoxytrityl.

the phosphate triesters (**3a**) and (**3b**) in the same ratio as judged by ^{31}P n.m.r. spectroscopy [δ(^{31}P) (CDCl$_3$) +0.60(s); −0.40 (s) p.p.m.; 2:1 respectively]. The correspondence between the isomeric ratios of the phosphites (**1a**) and (**1b**) and their oxidation products (**3a**) and (**3b**), as well as the thiophosphate triesters (**2a**) and (**2b**) implies that the iodine–water oxidation proceeds stereospecifically under these conditions.

To determine whether the reaction occurs with inversion or retention of configuration requires the assignment of the absolute configuration of the phosphate triesters (**3a**) and (**3b**). Recently the absolute configurations of a number of dinucleoside phosphate triesters have been assigned; however there appears to be no secure relationship between ^{31}P shift and absolute configuration at phosphorus.[8] Thiophosphate triesters can be stereospecifically converted into the corresponding phosphate triesters with retention of configuration using *m*-chloroperbenzoic acid.[9] The major diastereoisomer obtained by peracid oxidation of the mixture of diastereoisomers of (**2**) has the downfield ^{31}P resonance and because of the known stereospecificity of this reaction this must have the S_P configuration (**3a**). Since this is also the major diastereoisomer produced on iodine–water oxidation of the phosphite triesters (**1a**) and (**1b**) this reaction must proceed with overall *retention* of configuration. Furthermore, the identical diastereoisomeric ratio is obtained when the phosphites (**1a**) and (**1b**) are oxidised directly with *m*-chloroperbenzoic acid, an alternative method recently introduced for oxidation of phosphite triesters.[10]



Scheme 2. *Reagents:* i, (MeO)$_3$P; ii, I$_2$–H$_2$O or *m*-chloroperbenzoic acid.



(**6**)

As a model reaction, to confirm the above result, *cis*-5-t-butyl-2-methoxy-1,3,2-dioxaphosphorinane[11] (**4**) was prepared and oxidised essentially quantitatively by iodine–water (Scheme 2). Recrystallisation from hexane gave the oxo compound (**5**), m.p. 88—90 °C (lit.[11] m.p. 90—91 °C for the *cis* isomer). ^{1}H and ^{31}P N.m.r. spectroscopy also indicated that this was the *cis* phosphate triester [δ(^{1}H) (CDCl$_3$) 0.95 (9H, s), 2.09 (1H, m), 3.80 (3H, d, $^3J_{PH}$ 11 Hz), 4.20 (2 H, m), and 4.40 (2H, m).; δ(^{31}P) (CDCl$_3$) −5.85 (s) p.p.m. (*cf.* authentic mixture† *trans* −4.84; *cis* −5.85)]. The ^{31}P n.m.r. spectrum unambiguously shows the stereochemistry of the product to be *cis* since it has been shown that in 2-methoxy-2-oxo-1,3,2-dioxaphosphorinane systems the isomer with the axial OMe group has a ^{31}P resonance upfield of the corresponding equatorial isomer.[12] The iodine–water oxidation therefore

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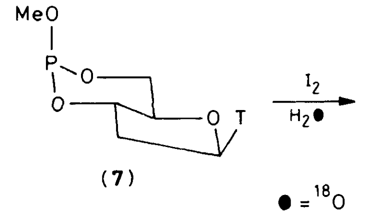
† Prepared by methylation of 5-t-butyl-2-hydroxy-2-oxo-1,3,2-dioxaphosphorinane with methyl iodide. When the product (**5**) was added to the authentic mixture, the upfield ^{31}P resonance was enhanced.



proceeds with retention of configuration. An identical product was obtained when the phosphite triester (**4**) was oxidised by *m*-chloroperbenzoic acid.

The observation of overall retention of configuration in the iodine–water oxidation was not expected if the oxidation proceeds *via* a two-step mechanism involving the phosphonium salt (**6**). Formation of (**6**) by nucleophilic attack of the phosphite on iodine would be expected to occur with retention of configuration. However, the hydrolysis of this intermediate might reasonably be expected to occur with predominant inversion of configuration, which would lead to overall inversion.

Structure (**6**) is also considered to be the intermediate in the Arbusov type reaction. For simple trialkyl phosphites the second step, nucleophilic attack at carbon by iodide to give iodophosphate diesters is rapid.[13] In the presence of water, (**6**) (R^1 and R^2 = nucleoside) must undergo hydrolysis faster than dealkylation, even when one of the ester groups is methyl, since little or no phosphite diester is produced in contrast to simple phosphites. That the iodine–water oxidation may proceed through an entirely different mechanism more consistent with the observation of overall retention of configuration remains a significant possibility.

The use of oxygen isotopically enriched water during the oxidation step offers the opportunity of stereospecifically labelling phosphate esters.[14] Thymidine S_{P}-3′,5′-cyclic phosphite methyl ester (**7**) can be obtained as the thermodynamic product by thermally equilibrating a mixture of diastereoisomers that are epimeric at phosphorus.[15] Oxidation with iodine in the presence of [^{18}O]-water (^{18}O *ca.* 60 atom %, ^{16}O *ca.* 40 atom%) gave thymidine S_{P}-3′,5′-cyclic (^{18}O)phosphate methyl ester (**8**) stereospecifically. Confirmation that the ^{18}O is exclusively in the equatorial position was obtained by ^{31}P n.m.r. spectroscopy. The resonance at δ -3.4 corresponding to the axial 3′,5′-cTMP methyl ester was split into a doublet owing to the incorporation of ^{18}O, the magnitude of the shift, 6.8 Hz, being indicative of ^{18}O in a double bond.[16]

During the course of this work Seela *et al.*[17] reported the use of iodine–water oxidation to introduce isotopes into phosphates; however they did not determine the stereochemistry of this reaction. The observation that this reaction is apparently stereospecific will extend its potential applications in the synthesis of isotopically chiral phosphate esters.

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