Stereochemical Evidence for a Phosphorylpyridinium Intermediate in the Iodine-mediated Desulphurisation of a Phosphorothioate Diester

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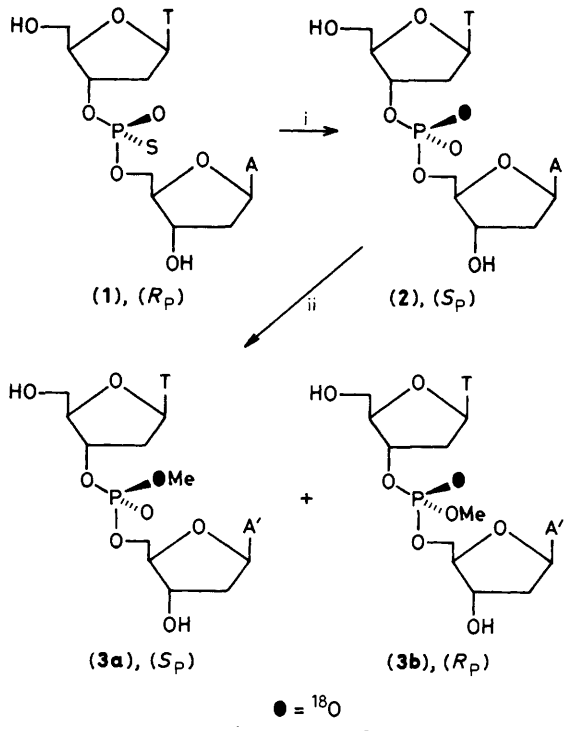
Desulphurisation of (S_P)-5′-*O*-(2′-deoxyadenosyl)-3′-*O*-thymidyl phosphorothioate to 5′-*O*-(2′-deoxyadenosyl)-3′-*O*-thymidyl [^{18}O]phosphate proceeds with epimerisation at phosphorus using iodine in aqueous pyridine but with predominant inversion of configuration in aqueous 2,6-lutidine.

Current interest in investigating the stereochemical course of enzyme-catalysed transformations at phosphorus has led to the syntheses of a number of chiral phosphorothioates[1] and oxygen chiral phosphates.[2] Moreover, considerable interest has centred on the conversion of the former into the latter.[3—10] Thus, methods reported so far exploit the oxidative displacement of sulphur using reagents such as bromine,[3] *N*-bromosuccinimide,[4] cyanogen bromide,[5] and dimethyl sulphoxide[6] in the presence of isotopically labelled water (all proceeding with inversion of configuration at phosphorus), as well as the displacement of *S*-methyl groups using labelled hydroxide[7] and the use of specially synthesised reagents such as ^{18}O-oxiranes[8,9] and ^{18}O-chloral[9,10] (reactions using these latter reagents proceeding with retention of configuration at phosphorus). However, in some cases side reactions can occur with nucleotide bases, especially guanine, during these procedures. This would have serious consequences if the desulphurisation of oligonucleotide phosphorothioates were attempted using some of these reagents.

Recently it has been demonstrated that phosphorothioate-containing oligonucleotides can be cleanly desulphurised under mild conditions using a solution of iodine in pyridine.[11] The resulting oligonucleotides are substrates for restriction endonucleases which have very stringent recognition requirements, attesting to the cleanness of the reaction. The potential usefulness of this reaction as a mild route to ^{17}O- or ^{18}O-containing oligomers prompted us to investigate the stereochemical course of this replacement reaction using a dinucleoside phosphorothioate.

(R_P)-5′-*O*-(2′-deoxyadenosyl)-3′-*O*-thymidyl phosphorothioate, (R_P)-d[Tp(S)A] (**1**),[12] was desulphurised using *N*-bromosuccinimide[4,13] in ^{18}O-labelled water to (S_P)-[^{18}O]d[TpA] (**2**). After addition of an approximately equivalent amount of [^{16}O]d[TpA], methylation of the potassium 18-crown-6 salt with methyl iodide[14] gave a mixture of the diastereoisomeric [^{16}O]- and [^{18}O]-d[TpA′] methyl esters (**3a,b**) (Scheme 1, ^{16}O-esters not shown). Inspection of the ^{31}P n.m.r. spectrum of the resulting mixture (Figure 1a) indicated the presence of two ^{31}P(^{18}O) isotope shifts,[15] one of 1.95 Hz and the other of 4.97 Hz. Since the ^{18}O-isotope shift has been shown to be dependent on bond order[16] we may assign the small isotope shift on the downfield resonance to ester (**3a**) and the larger isotope shift on the higher field resonance to (**3b**) {assignments are shown on the spectrum, other small



Scheme 1. *Reagents:* i, *N*-bromosuccinimide, $H_2{}^{18}O$–dioxane; ii, (a) K^+-Dowex (b) 18-crown-6 (c) MeI-dimethyl sulphoxide (DMSO).

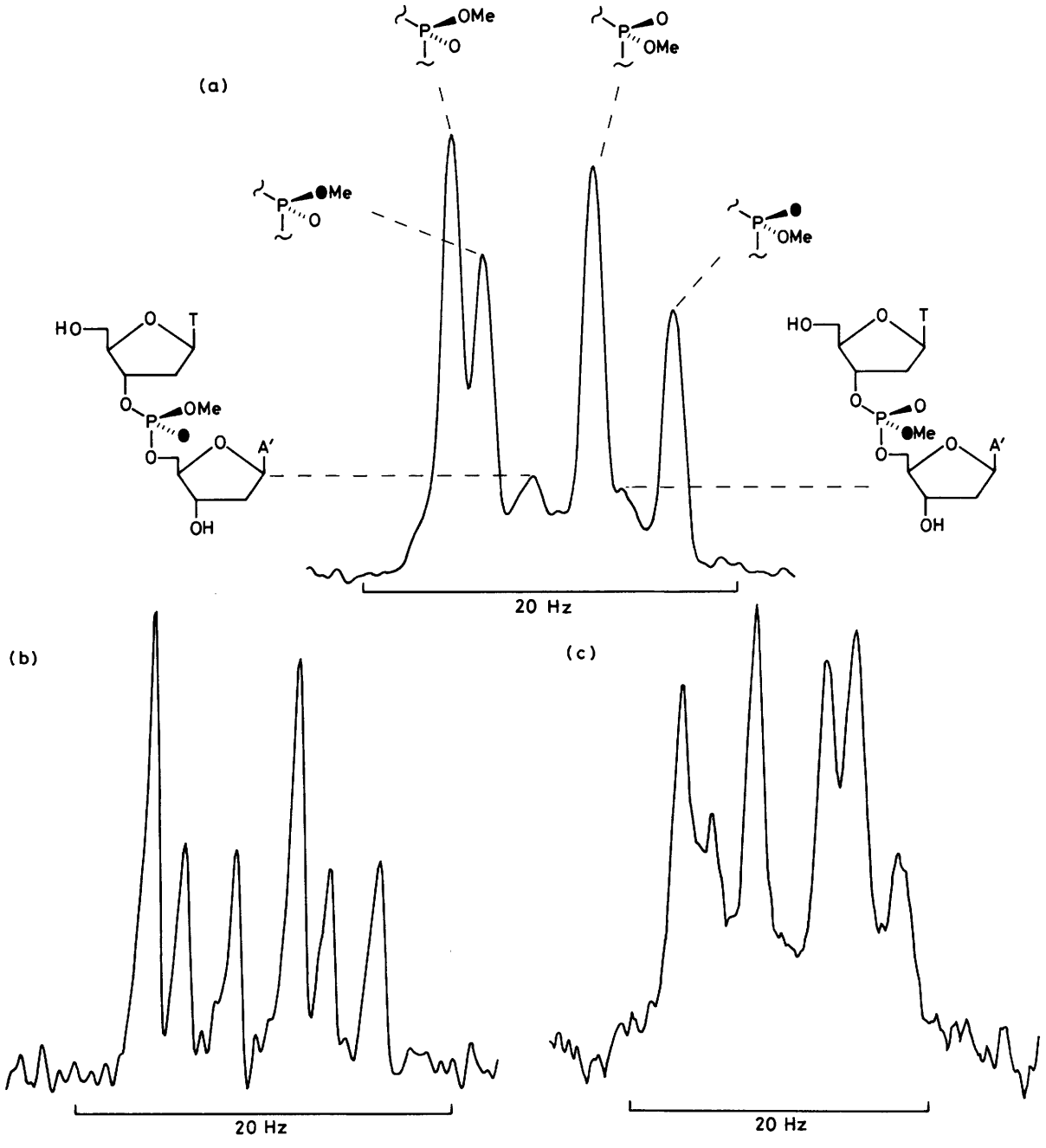
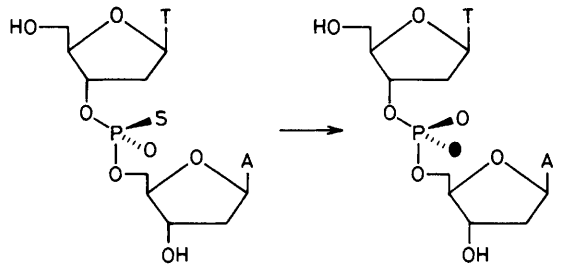


Figure 1. 121.5 MHz ^{31}P n.m.r. spectra of the diastereoisomers of [^{16}O]d[TpA'] methyl ester and [^{18}O]d[TpA'] methyl ester (*ca.* 10—20 mM; δ 0.88 and 0.80 p.p.m. for unlabelled compound) in 2:1 v/v DMSO–[$^2H_6$]DMSO containing 8-hydroxyquinoline. Typical n.m.r. parameters were: sweep width, 1000 Hz; pulse width, 7 μs; acquisition time, 4 s; data collection in 16K. Spectra: (a) methylation of (S_P)-[^{18}O]d[TpA] [contaminated with *ca.* 15% (R_P)-isomer] after mixing with [^{16}O]d[TpA]; (b) methylation of [^{18}O]d[TpA] obtained by desulphurisation of (S_P)-d[Tp(S)A] [contaminated with *ca.* 15% (R_P)-isomer] with I_2–pyridine–$H_2{}^{18}O$, after mixing with [^{16}O]d[TpA]; (c) methylation of [^{18}O]d[TpA] obtained as in (b) but with 2,6-lutidine instead of pyridine, and using *ca.* 66 atom% $H_2{}^{18}O$.

peaks on the spectrum are due to the presence of *ca.* 15% of (S_P)-d[Tp(S)A] in the starting material}. Thus, it is now possible to locate the position of an ^{18}O-isotope in a sample of [^{18}O]d[TpA] of unknown configuration at phosphorus.

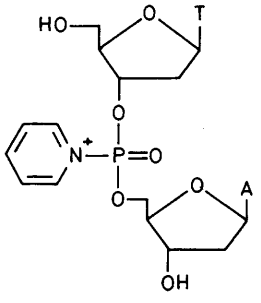
(S_P)-d[Tp(S)A] (**4**) (8 μmol) was desulphurised by iodine in pyridine–^{18}O-labelled water (10 equiv. I_2, 400 μl 3:1 pyridine:$H_2{}^{18}O$) to [^{18}O]d[TpA]. After 1.5 h when the reaction was judged to be complete by n.m.r. spectroscopy the iodine was extracted with diethyl ether. After conversion into the potassium 18-crown-6 salt and methylation the ^{31}P n.m.r. spectrum shown in Figure 1b was obtained. Here it can be seen that for each diastereoisomer both isotope-shifted peaks are equal in height and the reaction has therefore proceeded with epimerisation at phosphorus. When this desulphurisation was repeated using iodine in the presence of 2,6-lutidine–^{18}O-labelled water, however, a considerably slower reaction ensued. The product (**5**) was stereochemically analysed as above and the ^{31}P n.m.r. spectrum shown in Figure 1c was obtained. Taking into account a 15% contamination of the starting material with (R_P)-d[Tp(S)A] it is clear from this



(4), (S_P) (5), (R_P)

● = ^{18}O

Reagents: I_2–$H_2{}^{18}O$–2,6-lutidine.



(6)

spectrum that desulphurisation has proceeded with predominant inversion of configuration at phosphorus with a stereospecificity of *ca.* 75%.

That this reaction should proceed with epimerisation in aqueous pyridine but with considerable stereospecificity in the presence of the less nucleophilic 2,6-lutidine is best explained in terms of the involvement of pyridine as a nucleophilic catalyst in this reaction in the former case, presumably by way of the pyridinium species (**6**). Similar intermediates have been invoked by Mikolajczyk,[17] and by Frey.[18] Moreover, direct observation by ^{31}P n.m.r. spectroscopy of a reactive pyridinium intermediate consequent on the addition of 4-dimethylaminopyridine to diphenylphosphorochloridate has recently been claimed.[19]

Consequently, we may formulate the reaction as follows: initial activation of sulphur by iodine in pyridine occurs {possibly by way of the $[(pyridine)_2I]^-$ I_3-complex[20]} and displacement of sulphur by pyridine and epimerisation at phosphorus by successive displacements with pyridine at this centre take place, before final base-catalysed reaction with water yields the phosphate diester. In 2,6-lutidine the activated intermediate presumably has a lifetime long enough to permit base-catalysed direct displacement of the sulphur by water without extensive participation by the heterocycle, and consequently the displacement reaction proceeds with predominant inversion of configuration, in a similar fashion to related reactions with other activating agents.[3–6]

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