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Stereospecific Oxidation and Oxidative Coupling of H-Phosphonate and H-Phosphonothioate Diesters

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Abstract: Oxidation of dinucleoside H-phosphonothioate diesters with the aid of iodine in aqueous acetonitrile and triethylamine, and oxidative coupling of H-phosphonate and H-phosphonothioate diesters with ethanol under similar conditions in anhydrous acetonitrile were found to be stereospecific reactions.

Efficient oxidation is an important requirement for successful synthesis of phosphodiesters or their analogues in procedures where H-phosphonate and H-phosphonothioate diesters are used as intermediates. H-Phosphonate diesters can be oxidized efficiently[1] under mild conditions by treatment with iodine in aqueous pyridine. It was recently reported[2] that oxidation of deoxydinucleoside H-phosphonate diesters with iodine/[O-18] H_2O in pyridine showed some stereoselectivity. The Sp diastereomers were always formed in excess (d.e. 30-50%) regardless of the configuration of the starting H-phosphonate diester.

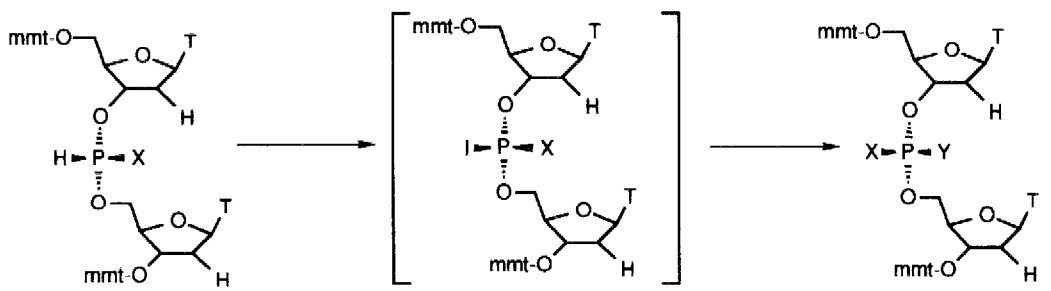
Our interest in stereospecific transformations of H-phosphonate[3] and H-phosphonothioate[3] diesters prompted us to start a more detailed study on the stereochemistry of oxidation with iodine. Oxidation of these compounds in a stereospecific manner would provide new possibilities to design an efficient synthesis of stereochemically pure phosphotriesters, oxygen labelled phosphodiesters, phosphorothioate diesters, and other chiral phosphate analogues. In this communication we describe our present results on the stereochemistry of oxidation[4] and oxidative coupling of H-phosphonate and H-phosphonothioate diesters.

As model compounds for these studies we chose a suitably protected dinucleoside H-phosphonate (**1**, X=O) and dinucleoside H-phosphonothioate (**1**, X=S). Both dimers were prepared according to standard methods[5] and the diastereomeric pairs (**1a** and **1b**, or **1c** and **1d**) were separated using column chromatography on silica gel with toluene:ethyl acetate (1:3, v/v for **1a**/**1b** and 1:1, v/v for **1c**/**1d**) as eluent.

Preliminary experiments on the oxidation of H-phosphonothioates[6] **1c** and **1d** with iodine under standard aqueous conditions[1] (2 equiv. of iodine in pyridine/water 98:2, v/v) showed that oxidation was fast (less than 1 min) and clean[7]. Unfortunately, extensive epimerization was observed during the course of the reaction. A most likely pathway, for the reaction of H-phosphonothioate diesters with iodine, would involve intermediate formation of a

phosphorothioiodidate **2** (X=S)[8]. Since substrates (**1c** and **1d**) and products (**3c** and **3d**) are configurationally stable under the reaction conditions, we thought that the lack of stereospecificity during the oxidation could be due to a pyridine and/or iodide mediated epimerization at the phosphorus centre in the phosphorothioiodidate **2**. To evaluate if the presence of pyridine is a major cause of epimerization in **2**, we carried out the oxidation in aqueous acetonitrile (2:98, v/v) containing triethylamine (2-4 equiv.) as a base. Under these new reaction conditions the conversion of H-phosphonothioate **1c** or **1d** into the corresponding phosphorothioates **3** (X=S, Y=O$^-$) was found to be much slower (several hours), but contrary to the reaction in aqueous pyridine, the oxidation occurred with virtually complete stereospecificity[9]. Thus, the H-phosphonothioate **1c** (resonating at higher field in ^{31}P NMR, relative to the other diastereomer) was converted into the phosphorothioate **3c** (also resonating at higher field in ^{31}P NMR), while the other diastereomer (**1d**) was oxidized to the phosphorothioate **3d**. The absolute configurations of compounds **3c** and **3d** were found to be Sp and Rp, respectively, by comparison to the corresponding phosphorothioates[10] of known configurations.

Scheme 1



1 **2** **3**

1 a, X=O, (Rp) ("fast") (δ_p=8.43 ppm)
1 b, Sp diastereomer of **1 a** ("slow") (δ_p=9.37 ppm)
1 c, X=S, (Rp) ("fast") (δ_p=71.89 ppm)
1 d, Sp diastereomer of **1 c** ("slow") (δ_p=72.71 ppm)

3 a, X=O, Y=OEt, (Rp) (δ_p= -1.66 ppm)
3 b, Sp diastereomer of **3 a** (δ_p= -1.52 ppm)
3 c, X=S, Y=O$^-$, (Sp) (δ_p=57.07 ppm)
3 d, Rp diastereomer of **3 c** (δ_p=57.15 ppm)
3 e, X=S, Y=OEt, (Sp) (δ_p=67.58 ppm)
3 f, Rp diastereomer of **3 e** (δ_p=67.91 ppm)

Detailed studies on the stereochemical course of the oxidation of H-phosphonothioate diesters with iodine/water/base have not been reported. Assuming, however, the reaction pathway in Scheme 1, it seems most likely that the reaction would proceed with overall inversion of configuration when triethylamine is used as the base. This would then mean that the absolute configuration of the starting H-phosphonothioate **1d** should be Sp and, by the same token, **1c** should have the Rp configuration.[11]

These findings encouraged us to investigate the possibility of stereospecific oxidative coupling of H-phosphonothioate and H-phosphonate diesters with alcohols. The significance of such reactions cannot be overestimated especially in chemical synthesis of oligonucleotide analogues that are chiral at the phosphorus centre. To this end model reactions involving condensation of separate diastereomers of H-phosphonate or H-phosphonothioate diesters with ethanol, aided by iodine and TEA in acetonitrile, have been carried out. In all instances stereospecific formation of the corresponding phosphate or phosphorothioate triester was indeed observed[9]. Thus, the H-phosphonate diesters **1a** and **1b** were converted into the phosphotriesters **3a** and **3b**, and the H-phosphonothioates **1c** and **1d**, into the phosphorothioate triesters **3e** and **3f** (Fig. 1), respectively. Analogous reactions in which acetonitrile and TEA were replaced by pyridine, proved to be stereoselective rather than stereospecific. Under these conditions both H-phosphonate and H-phosphonothioate diesters afforded one diastereomer (phosphotriester **3b** or phosphorothioate triester **3f**) in preponderance (d.e.~35-40%), irrespective of the configuration of the starting material.

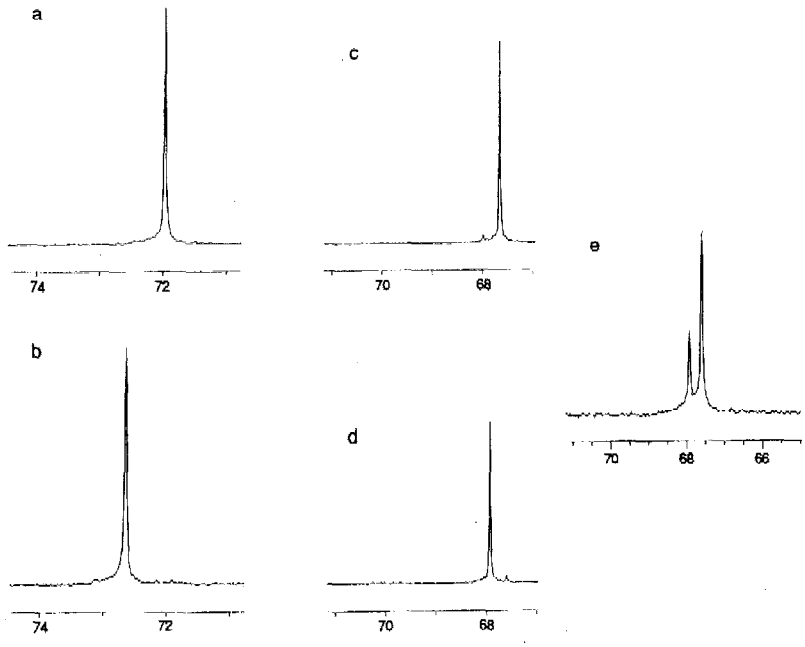


Figure 1. ^{31}P NMR spectra of the oxidative coupling of H-phosphonothioate diesters **1** (X=S) with ethanol in the presence of iodine/TEA in MeCN.
a) "fast" diastereomer **1c** (Rp); b) "slow" diastereomer **1d** (Sp); c) product of the oxidative coupling of **1c** with ethanol, (**3e**); d) product of the oxidative coupling of **1d** with ethanol, (**3f**); e) 2:1 mixture of **3e** and **3f**.

It thus seems that oxidation with iodine/water as well as oxidative coupling of H-phosphonate and H-phosphonothioate diesters with alcohols can be carried out with virtually

complete stereospecificity (since oxidative coupling of H-phosphonate diesters with alcohols is a stereospecific reaction, it would be unlikely to find a different behaviour for their oxidation with iodine/[O-18] H_2O). It also seems reasonable to suggest a mechanism in which these reactions occur with overall inversion of configuration. The first part of such a mechanism would be base catalyzed formation of phosphoroiodidate (**2**, X=O) or phosphorothioiodidate (**2**, X=S), with retention of configuration. The oxygen nucleophile (water or ethanol) would then attack **2** and cause inversion of configuration. An important requirement for these reactions is that powerful nucleophilic catalysts such as pyridine (as it seems here iodide is not) should be absent or else the stereospecificity will be lost due to epimerisation of the intermediate **2**.

Acknowledgements

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5. Compounds **1a-d** were prepared by condensation of 5´-O-monomethoxytritylthymidine 3´-H-phosphonate or 3´-H-phosphonothioate with 3´-O-monomethoxytritylthymidine in pyridine, in the presence of pivaloyl chloride (**1a** and **1b**) or 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphorinane 2-oxide (**1c** and **1d**) (see also P.J. Garegg, T. Regberg, J. Stawiński, R. Strömberg, *Chemica Scr.*, **25**, 280 (1985) and Ref. 6).
6. Stawiński, J.; Thelin, M.; Zain, R. *Tetrahedron Lett.*, **30**, 2157 (1989).
7. As we reported previously (Ref. 4) excess of iodine may cause desulfurization of the product.
8. During oxidation of H-phosphonothioates **1c** and **1d** a signal at ~4 ppm was observed in the ^{31}P NMR spectra, which we tentatively assigned to the phosphorothioiodidate **2** (X=S).
9. We noticed that in all reactions the products were contaminated (ca 3%) by the other diastereomers (TLC, ^{31}P NMR). This can be due to incomplete diastereomeric purity of the substrates, or the reaction may not be completely stereospecific. Since the detection level by ^{31}P NMR critically depends on the half-width of a signal and phosphates usually give sharper signals than H-phosphonate esters, we consider the first possibility as the most likely one. More experiments to elucidate this problem are in progress.
10. The phosphorothioates **3c** and **3d** were obtained independently by sulfurization of **1a** and **1b**, respectively, with elemental sulfur (most likely, the sulfurization occurs with retention of configuration at the phosphorus centre) and their absolute configurations were established by enzymatic methods (for stereochemical analysis using SVPD, see P.M.J. Burgers, F. Eckstein, *Biochem.*, 1979, **18**, 592-596 and F.R. Bryant, S.J. Benkovic, *Biochem.*, 1979, **18**, 2825-2828).
11. This assignment was further substantiated by conversion (m-chloroperoxybenzoic acid[12]) of **1c** and **1d** with retention of configuration into the H-phosphonates **1a** and **1b**, respectively (J. Stawiński, R. Strömberg, R. Zain, unpublished results), followed by their sulfurization[10].
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