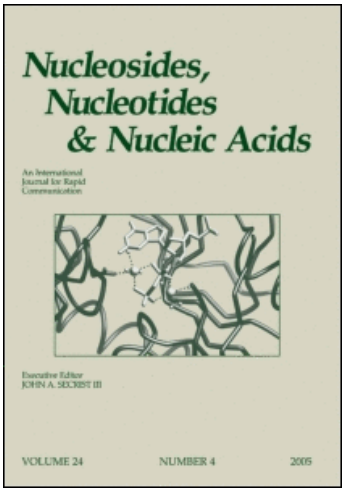

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STUDIES ON THE OXIDATION OF NUCLEOSIDE HYDROGENPHOSPHONATES

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Abstract. Oxidation of nucleoside H-phosphonate diesters has been investigated using dipyridyl disulphide, hexachloroacetone and iodine, under various reaction conditions.

Recently we have reported a most efficient method for the synthesis of nucleoside H-phosphonate diesters based on the reaction of nucleoside 3'-H-phosphonate monoesters with suitably protected nucleosides in the presence of various condensing reagents[1]. To develop it as a method for oligonucleotide synthesis via H-phosphonate intermediates[2], we faced the problem of an efficient conversion of H-phosphonate diesters into the desired phosphorodiesters. This was a matter of prime importance in our approach to oligonucleotide synthesis which involves a formation of oligonucleotides having all internucleotidic bonds in the form of H-phosphonate diesters, which in turn, have to be converted at the end of synthesis into the corresponding oligonucleotides with natural phosphorodiester linkages.

It is known from the literature that phosphite diesters, which exist predominantely in the H-phosphonate form, are much more difficult to oxidize than phosphite triester, and require stronger oxidizing agents[3].

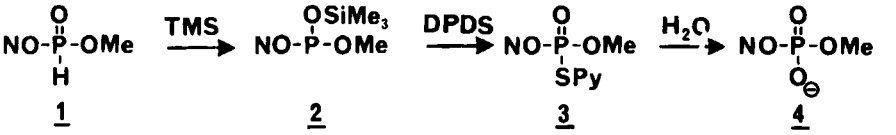
Preliminary experiments showed that three oxidizing agents may be suitable for our purpose: (i) dipyridyl disulphide, introduced by Hata et al.[4] for the oxidation of nucleoside 5'-H-phosphonates, (ii) hexachloroacetone, used by Holy and Smrt[5] for the conversion of nucleoside 3'-H-phosphonates into the corresponding phosphates, and (iii) iodine, routinely used for the oxidation of phosphite triesters during oligo-

nucleotide synthesis[6]. Since none of these reagents have been used for the oxidation of H-phosphonate diesters[7], we undertook studies using different reaction conditions.

Oxidation with dipyridyl disulphide (DPDS)

Hata et al.[4] have found that H-phosphonate monoesters are more susceptible to oxidation when converted into a trivalent form of bis-silyl phosphite in the reaction with trimethylsilyl chloride (TMS).

An oxidation procedure involving presilylation, was found to be most efficient also in the case of H-phosphonate diesters[8]. Addition of TMS to a pyridine solution of 1 produced the silylphosphite 2, which upon addition of DPDS was immediately converted into a phosphorothioate 3.



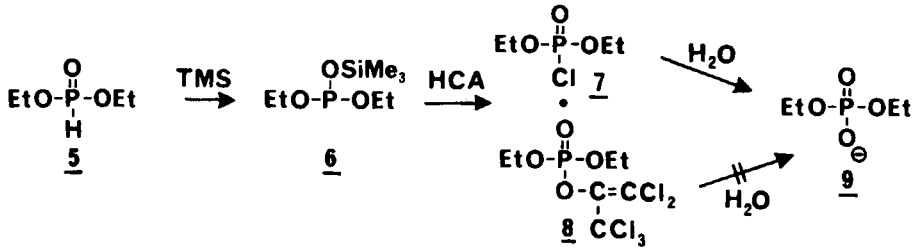
1 2 3 4

Water, however, caused slow hydrolysis of 3 (ca 2 h) into the corresponding diester 4. As a side-reaction, the formation of nucleoside 3'-phosphate was observed (^{31}P NMR analysis).

Oxidation with hexachloroacetone (HCA)

^{31}P NMR studies revealed that oxidation of H-phosphonate diester 1 with HCA in pyridine resulted in formation of nucleoside 3'-methyl-2-(1,1,3,3,3-pentachloro)propen-1 phosphorotriester, which underwent demethylation (within 2 h), affording after hydrolysis nucleoside 3'-phosphate (and its pyrophosphate).

Presilylation of 1 made the subsequent oxidation faster (3 min), but demethylation also seemed to be accelerated. Oxidation of diethyl



5 6

H-phosphonate, which did not undergo dealkylation under reaction conditions, showed (^{31}P NMR) the formation of two intermediates: diethyl chlorophosphate 7 and enol phosphate 8. Unfortunately, this latter

compound was found to be rather difficult to hydrolyse and remained virtually unchanged in aq. pyridine during several hours.

Oxidation with iodine

We have found that oxidation of 1 using iodine in tetrahydrofuran -2,6-lutidine-water (94:5:1), standard condition for the oxidation of nucleoside phosphite triesters), is considerably slower than that for phosphite triesters, and it took ca 30-40 min for the reaction to go to completion. Addition of triethylamine accelerated the reaction, but such conditions can be detrimental for the H-phosphonate diestres bonds. However, when the oxidation with iodine was carried out in pyridine-water (98:2) solution, it went to completion in 4-5 min.

Presilylation of H-phosphonate diester 1 followed by addition of iodine in anhydrous pyridine, accelerated the oxidation. However, we have noticed the formation of side-products, especially when the reaction mixture was kept for some time (5 min or longer) before the addition of water. Fortunatelly, it was found that the presilylation procedure is compatible with aq. oxidizing reagents. Thus, addition of iodine in aq. pyridine into a presilylated compound 1, produced immediately (1-2 min) the desired phosphorodiester 4 as a sole reaction product (^{31}P NMR).

As a final stage of these investigations, we prepared dodeca-thymidyl H-phosphonate diester, $(Tp\text{-}H)_{11}T$, bound to solid support (Controlled Pore Glass 500) and oxidized it under the above conditions. After releasing the oligomer from the support (ammonia, 55°C, 4 h), the reaction mixture was phosphorylated using Polynucleotide kinase and γ-(^{32}P) ATP and analysed electrophoretically (PAGE). Oligomers which were oxidized with 2% I_2 in pyridine-water (98:2) during 5 min and those which were presilylated with TMS and than treated with the above oxidizing solution for 5 min, looked identical on PAGE, showing $(Tp_{11})T$ as the sole reaction product. These findings indicate that oxidation of oligonucleotide H-phosphonate diesters with iodine in aq. pyridine is efficient even without presilylation, however, the presilylation procedure may prove to be superior for the oxidation of longer oligomers having H-phosphonate internucleotidic linkages.

We have compared the above oxidation procedure to that recently reported for the same purpose by Froehler and Matteucci[9] (0.2 M I_2 in tetrahydrofurane-pyridine-water 90:5:5; 5 min). Unfortunately, the latter method did not work in our hands and invariably led to extensive

degradation of oligomers after ammonia treatment. The desired thymidyl dodecamer was detected by PAGE as a minor product or was not present at all. Such a extensive degradation indicates an incomplete oxidation and it is in agreement with our findings concerning the reactivity of iodine in different solvents.

CONCLUSIONS

The mildest, most efficient and convenient oxidation procedure for the transformation of nucleoside H-phosphonate diesters into the corresponding phosphorodiesters, consists of oxidation with iodine in pyridine-water solution.

Other oxidizing reagents investigated during these studies proved to be less suitable for that purpose. Despite the fact that DPDS and HCA oxidize H-phosphonate diesters only slightly slower than iodine, the oxidation pathways involve the formation of rather stable intermediates of type 3 or 9, and thus an additional reaction step would be necessary to obtain the desired phosphorodiesters.

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