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INTRODUCTION OF A NONAQUEOUS OXIDATION PROCEDURE IN THE PHOSPHITE TRIESTER ROUTE FOR OLIGONUCLEOTIDE SYNTHESIS

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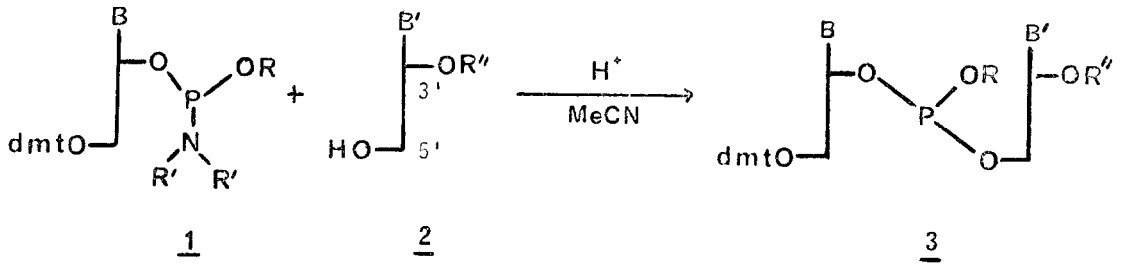
Summary:
Iodobenzene diacetate 5 or tetrabutylammonium periodate 6 oxidize phosphite into phosphate under nonaqueous conditions and can be conveniently used in oligodeoxynucleotide synthesis via the phosphite triester route.

Nucleoside phosphoramidites of general structure 1 are now well recognized as most valuable tools for oligonucleotide synthesis[1]. The phosphorylation of a free 5'-OH nucleoside derivative 2 by a phosphoramidite 1 is usually accomplished in two steps as indicated in the Scheme. Thus, upon activation by an appropriate protonating agent, the phosphoramidite 1 undergoes a condensation with a nucleosidic component 2 to give a dinucleoside phosphite 3 which is subsequently oxidized by aqueous iodine to the phosphate 4. When the deoxynucleoside 2 is covalently attached to a solid support, such as silica gel, unreacted molecules 2 are acylated (capping step) before a new elongation cycle is repeated[2].

Especially for automated synthesis, this sequence would be simplified if oxidation and capping steps could be accomplished simultaneously by using a nonaqueous oxidation system compatible with an acylating agent. The immediate advantage would be a shorter operation cycle which, moreover, could be run under strictly anhydrous conditions thereby rendering the drying step superfluous[3]. Towards this objective we have examined two oxidants: iodobenzene diacetate 5[4] and tetrabutylammonium periodate 6[5].

We have introduced arylphosphoramidites 1a[6] for solution and solid phase syntheses of oligodeoxynucleotides, and we show now that oxidation by these reagents of the condensation product 3a formed by coupling 1a and 2a leads to dinucleoside phosphates 4a in good yield.

Thus, addition of an acetonitrile solution of 1a (B=T)(1.2 equ.) to a solution in the same solvent of 2a (B'=T)(1 equ.) in the presence of N-methylanilinium trifluoroacetate (2.2 equ.) resulted as reported in the immediate disappearance of 2a as indicated by TLC to give phosphite 3a (B=B'=T). Oxidation of the latter to the dinucleoside phosphate 4a (B=B'=T) was accomplished by adding to the reaction mixture either a methylene chloride solution of N-tetrabutylammonium periodate 6 (2 equ.) or a THF/pyridine/acetic anhydride (2/1/1) solution of iodobenzene diacetate 5(2 equ.).



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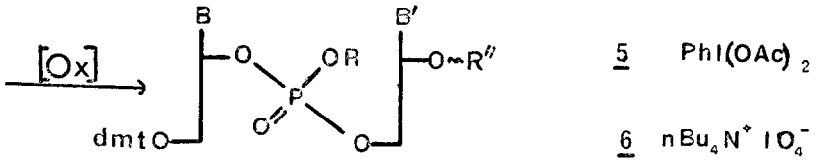


5 $PhI(OAc)_2$

6 $nBu_4N^+IO_4^-$

4

SCHEME:

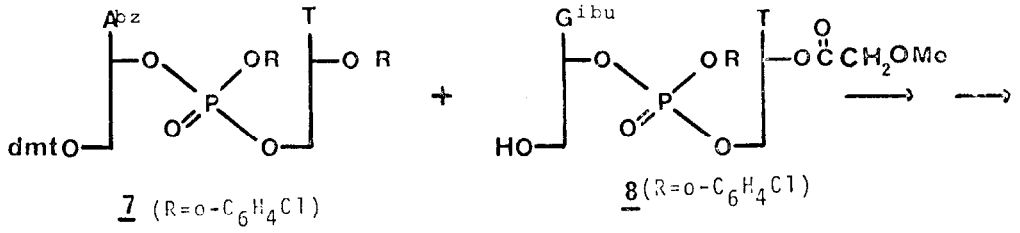
	R	R'	R"
1a	o-C_6H_4Cl	$(CH_2CH_2)_2O$	$COCH_2OMe$
2a	-	-	"
3a	"	"	-
4a	"	"	-

In either case, the reaction was instantaneous and the dinucleoside phosphate 4a (B=B'=T) was obtained in good yield (76%) after purification by silica gel column chromatography[6,7]. However, we prefer to use iodobenzene diacetate 5 since with periodate 6 the recovered material 4a (B=B'=T) could never be obtained as a white foam although its ^1H NMR spectrum was identical with that of the same compound obtained by using oxidant 5.

The same reaction was repeated with phosphoramidites 1a having B=N^4-benzoylcytosine, N^6-benzoyladenine and N^2-isobutyrylguanosine, respectively. In every case the corresponding dinucleoside phosphate 4a (B'=T) was obtained in excellent yield ($\sim$75%) after purification[7]. Accordingly, iodobenzene diacetate 5 in THF/pyridine/acetic anhydride solution is perfectly suitable for oligodeoxynucleotide synthesis chemistry based on the phosphoramidite methodology.

This was further illustrated by the preparation of the pentamer CATGT in solution. Fully protected dinucleoside phosphate 7a was O-deacylated at the 3' position (MeOH: conc. NH_4OH / 100: 1) at room temperature and the resulting compound 7b[7] was transformed into the corresponding phosphoramidite 7c[7] by using our standard procedure[6]. This phosphoramidite 7c

in acetonitrile solution was added to a solution of dinucleoside phosphate 8 and N-methylanilinium trifluoroacetate in the same solvent and the condensation product was oxidized by iodobenzene diacetate 5 to give the fully protected tetramer ATGT (9a)[7] in 60% yield after purification on a short column of silica gel. The dimethoxytrityl group at the 5' end of this tetramer was removed giving 9b which on further reaction with the phosphoramidite derived from deoxycytidine 1a ($B=C^{bz}$)(3 equ.) and oxidation provided the expected fully protected CATGT 10 in 75% yield. Its complete deprotection was accomplished by successive treatments with 2-nitrobenzaldoxime [8],concentrated ammonia and 80% aqueous acetic acid.



7 ($R=o\text{-}C_6H_4Cl$)

8 ($R=o\text{-}C_6H_4Cl$)

7a $R'=COCH_2OMe$

7b $R'=H$

7c $R'=P(OR)N(CH_2CH_2)_2O$

$HO\,5'\,A^{bz}pT\,p\,G^{ibu}pT\,3'\,OCCH_2OMe \xrightarrow{+1a\,(B=C^{bz})}$ **9b**

$dmtO\,C^{bz}pA^{bz}pT\,p\,G^{ibu}pT\,OCCH_2OMe \longrightarrow CATGT$

10

Finally, it was of interest to show that iodobenzene diacetate 5 can also be used to oxidize a phosphite triester in the methyl series. Accordingly , the methyl phosphite 3 (R=Me,B=B'=T)) was prepared by sequential addition of 5'-dimethoxytritylthymidine and 3'-methoxyacetylthymidine 2a (B'=T) to bis-triazolylmethoxyphosphine in THF[9]. The corresponding phosphate 4 (R=Me B=B'=T) was obtained after in situ treatment of the above mixture with a solution of 5 in THF/Pyridine (3/1). The yield was identical to the aqueous iodine oxidation of the same phosphite 3 (R=Me, B=B'=T)

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2 : M.D. MATTEUCI and M.H. CARUTHERS, J.Am.Chem.Soc., (1981), 103, 3185

3 : K.K. OGILVIE and M.J. NEMER (Tetrahedron Lett., (1981), 22, 2531) have proposed m-chloroperbenzoic acid in methylene chloride to convert phosphite to phosphate during oligonucleotide synthesis. However, this method did not find wide application, probably because under the reaction conditions this chemical which contains a significant amount (~15%) of m-chlorobenzoic acid, can also be considered as a detritylation agent.

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5 : E. SANTANIELLO, A. MANZOCCHI and C. FARACHI, Synthesis, (1980), 563.

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7 : The final overall yields (coupling + oxidation) of chromatographically pure products are based on the 5'-OH free component : 2a (B'=T), 8 and 9. Products 4a (having B and B' as indicated), 7b, 7c and 9a exhibited high field ^{1}H n.m.r. spectra consistent with the proposed structures.

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