A New and Selective Oxidising Reagent for Oligonucleotide Synthesis†

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A mixture of carbon tetrachloride, *N*-methylmorpholine and water in acetonitrile serves as an excellent reagent for oxidation of nucleoside phosphite to nucleoside phosphates and has been successfully applied to the solid phase synthesis of d(CGTAAAATGG).

Oligonucleotide-based therapeutics are an emerging field in drug discovery.[1,2] The antisense strategy is proving to be extremely promising and hence the production of oligonucleotides has led to the investigation of alternative synthetic strategies aimed at high purity and better yields. The development of the phosphoramidite methodology[3,4] can be regarded as a breakthrough in chemical solid phase oligonucleotide synthesis, due to the high degree of automation of the chain elongation process and the speed of the reactions involved there in. The synthesis is accomplished *via* two key steps namely, condensation of two nucleosides and oxidation of the phosphite intermediate to a phosphate. Extensive investigations have been made dealing mainly with change in the protecting groups of sugar,[5] base, and phosphite moieties,[6,7] and their deprotecting patterns,[8] as well as different materials for the solid matrix and the design of the spacer connecting the starting material covalently to the support.[9] On the other hand only a few effective methods are known for the oxidation of phosphite.[11–16] At present aqueous iodine is most conventionally employed for this purpose;[3–5] however, removal of I_2 from the column requires several washings, and it has also been shown that about 25% cleavage of protecting group occurs in about 2 min.[9]

In continuation of our program to synthesise oligonucleotide derivatives and to get a better understanding of hybridisation,[10] we have observed that the proportion of side products increases with time of oxidation (Fig. 1).

Here we report a new and effective oxidising agent, *i.e.* CCl_4–*N*-methylmorpholine (NMM)–pyridine–H_2O–CH_3CN for the oxidation of dinucleoside phosphite to the corresponding phosphate in oligonucleotide synthesis (Scheme 1). A plausible mechanism is shown in Scheme 2.

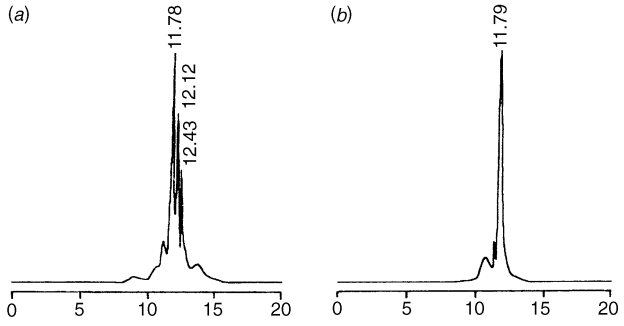
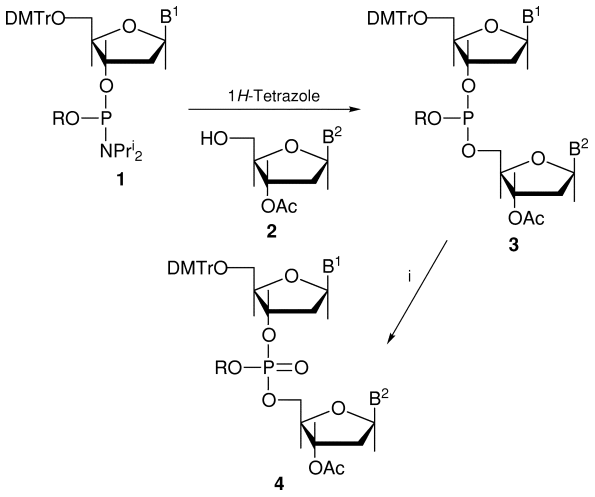


Fig. 1 HPLC of d(CGTAAAATGG) (RP-C-18 column with Et_3N–HOAc (pH = 7) and CH_3CN gradient of 1% min^{-1} starting from 0%; flow rate 1 ml min^{-1}); (*a*) after performing oxidation for 2 min by aqueous I_2; (*b*) after performing oxidation for 20 s by aqueous I_2

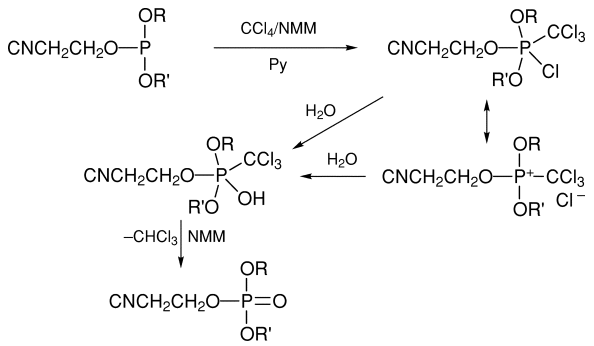


R = $-CH_3$ or $-CH_2CH_2CN$
B_1 and B_2 are nucleoside bases (T, A^{bz}, C^{bz}, G^{ib})

Scheme 1 i, CCl_4–NMM–Py/H_2O–CH_3CN (v/v = 2.5/1.0/6.0/1.0/1.0)

1*H*-Tetrazole-promoted condensation of nucleoside phosphoramidite **1** (0.1 mmol) with nucleoside **2** (0.1 mmol) at room temp. for 10 min, in CH_3CN (3 ml) gave dinucleoside phosphite **3** in 87% yield, after chromatographic purification. Subsequent oxidation of **3** with 6 ml of the reagent, CCl_4–NMM–pyridine–H_2O–CH_3CN (v/v 2.5/1.0/6.0/1.0/1.0) at room temperature in 5 min afforded dinucleoside phosphate **4** in 90% yield. The reaction was studied using ^{31}P NMR spectroscopy, indicating that **3** (δ 142) was oxidised quickly to give **4** (δ 1.05). The UV, ^1H NMR and HPLC analysis indicated that the heterocyclic moiety and all protecting groups (DMTr, $-COR$, $-OCH_3$, $-OCH_2CH_2CN$) remain unaffected under the oxidation conditions.

The reagent has been successfully applied to the solid phase synthesis of oligonucleotides. Here we demonstrate its utility by synthesis of decanucleoside nonaphosphate d(CGTAAAATGG).



Scheme 2 Where R and R′ are nucleosides

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†This is a **Short Paper** as defined in the Instructions for Authors, Section 5.0 [see *J. Chem. Research (S)*, 1998, Issue 1]; there is therefore no corresponding material in *J. Chem. Research (M)*.

Table 1

	Operation	Reagent	*t*/min
1	Washing	CH_3CN	0.5
2	Detritylation	3% CCl_3COOH–CH_2Cl_2	1.8
3	Coupling	0.1 M Phosphoramidite–CH_3CN + 0.5 M Tetrazole–CH_3CN	1.1
4	Washing	CH_3CN	0.2
5	Capping	Ac_2O–2,6-lutidine–THF (1:1:2) + 6.5% DMAP–THF	0.4
6	Oxidation	CCl_4–NMM–pyridine–H_2O–CH_3CN (v/v 2.5/1.0/6.0/0.2/9.3)[a]	0.3
7	Washing	CH_3CN	0.5

[a]The optimum ratio required for oxidation by solid phase oligonucleotide synthesis. The use of THF in place of CH_3CN decreases the rate of reaction. DMAP = 2-dimethylamino-pyridine.

The controlled pore glass (CPG) bound deoxyguanosine was elongated to d(CGTAAAATGG) by repeating the reaction cycle as shown in Table 1. Each coupling sequence was affected in the average yield of 95% as determined by the colourimetric method of released DMTr function. The final oligonucleotide was liberated from the solid support and deprotected by the usual method. The ^{31}P NMR spectrum of the crude product shows no signal due to trivalent phosphorus, indicating the quantitative oxidation. Reverse phase HPLC analysis of the product is shown in Fig. 2(*a*). Enzymatic hydrolysis of purified d(CGTAAAATGG) shows no wrong linkage or base modification.

To prove the inertness of the reagent towards different protecting groups as compared to aqueous I_2 oxidation, d(CGTAAAATGG) has been synthesised in 2 min by oxidation. The reverse phase HPLC analysis of the crude product is shown in Fig. 2(*b*).

Other oxidising reagents such as *tert*-butyl hydroperoxide and bistrimethy(silyl)peroxide[12,17] have been reported for solid phase automated synthesis of oligonucleotides. The described reagent is cheaper and is also stable as compared to these reagents.

Thus the method can be used to obtain oligonucleotides in good yield and high purity by solid phase synthesis, by using CCl_4–NMM–pyridine–H_2O–CH_3CN mixture as an oxidising reagent.

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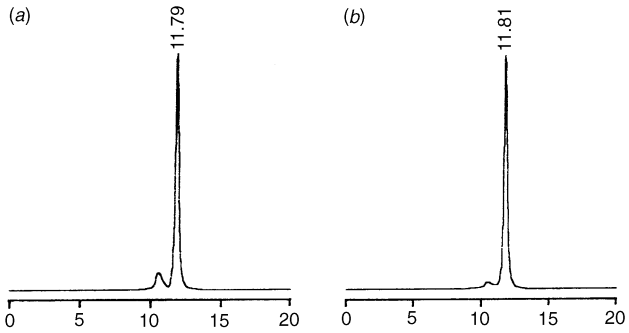

Fig. 2 HPLC (RP-C-18 column with Et_3N–HOAc (pH = 7) and CH_3CN gradient of 1% min^{-1} starting from 0%; flow rate 1 ml min^{-1}): (*a*) after performing oxidation for 2 min by CCl_4–H_2O system; (*b*) after performing oxidation for 20 s by CCl_4–H_2O system

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