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New Methods for the Synthesis of 3'-*S*-Phosphorothiolate Internucleoside Linkages

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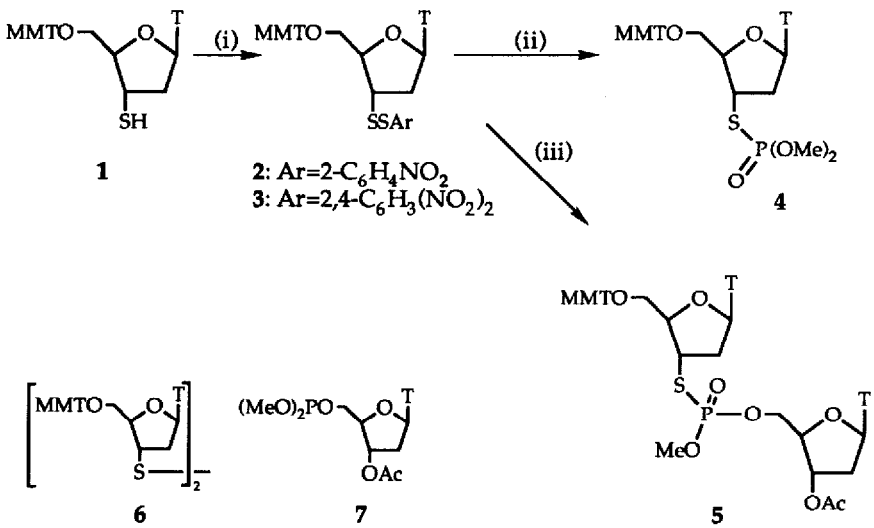
Abstract: *Efficient procedures are described for the synthesis of dinucleoside phosphorothiolates using either a Michaelis-Arbusov-type reaction or a phosphotriester approach.*

The utility of backbone-modified polynucleotides in molecular biology[1,2] and as potential therapeutic drugs[2-4] makes such compounds targets of considerable interest. DNA analogues in which sulphur is substituted for oxygen in the phosphodiester linkages are minimally perturbed from the natural structure[5,6] and in the case of 3'-*S*-phosphorothiolates[7] avoid problems associated with an asymmetric phosphorus centre. We have previously described[8-11] the incorporation of a single such modification into several di- and oligo-nucleotides and some of their biochemical properties. Although we are continuing to develop the chemistry for solid-supported synthesis of phosphorothiolate DNA,[12] the quantities available from such preparations are not sufficient for some of the biophysical studies (e.g. NMR and X-ray crystallography) required to explain emerging biochemical data. Furthermore, the phosphorothioamidite intermediates used in solid-phase synthesis have been found by us and other workers[13,14] to undergo acid catalysed ligand exchange reactions which, in solution, lead to poor yields and involve laborious purification of the product. Here we report the solution synthesis of dinucleosides containing 3'-*S*-phosphorothiolate linkages using either a Michaelis-Arbusov-type reaction or a modification of the phosphotriester approach both of which are high yielding and clean.

Whilst Michaelis-Arbusov-type reactions have attracted relatively little attention for the synthesis of modified internucleotide linkages,[15-17] 3'-*S*-phosphorothiolate analogues are particularly attractive targets for their application; it is established that both thioimides[18] and disulphides[19,20] react with phosphite triesters to give phosphorothiolates under mild, non-acidic conditions. Thus, 5'-*O*-monomethoxytrityl-3'-thiothymidine (**1**) was prepared according to the published protocol[9] and treated with 2,4-dinitrophenylsulphenyl chloride (1.5 equiv.) with triethylamine (1.5 equiv.) in THF at 0°C for 90 minutes to give 5'-*O*-monomethoxytrityl-3'-*S*-(2-nitrophenyldithio)thymidine (**2**, 72%)[21] and the symmetrical dinucleoside disulphide (**6**, about 20%, **Scheme 1**) after silica gel column chromatography. This method was also employed to prepare the 2,4-dinitrophenyl-disulphide derivative (**3**, 72%). In model reactions, excess trimethyl phosphite reacted smoothly with either **2**

or **3** in THF at room temperature to give the nucleoside phosphorothiolate (**4**) in greater than 80% yield. The fully protected dinucleoside phosphorothiolate (**5**) was prepared in an analogous fashion by reaction of the

Scheme 1



(i) 2,4-dinitrophenylsulphenyl chloride or 2-nitrophenylsulphenyl chloride (1.5 equiv.), triethylamine (1.5 equiv.), THF; (ii) trimethyl phosphite (10 equiv.), THF; (iii) 3'-*O*-acetylthymidine-5'-dimethylphosphite (2 equiv.), toluene.

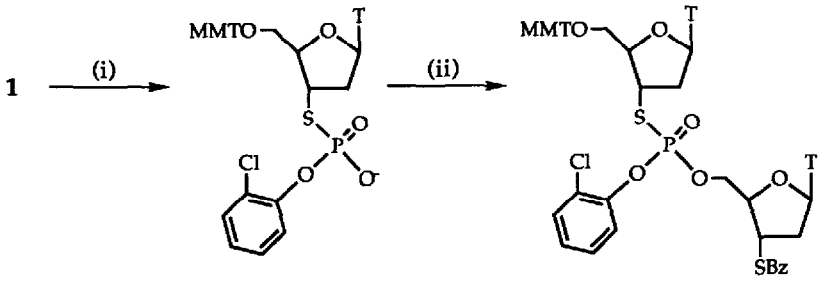
dinitrophenyldisulphide (**3**) and 3'-*O*-acetylthymidine-5'-dimethylphosphite[22] (**7**, 2 equiv.) in toluene at room temperature. Optimum yields (89%) were achieved after 16 hours using a 2:1 ratio of phosphite to disulphide. Similar reaction conditions with the nitrophenyldisulphide (**2**) were less efficient and several side reactions were observed including nucleophilic attack at the aryl sulphur by the phosphite (**7**), sulphurisation of **7** and demethylation of **5**. The second step of this general Arbusov reaction involves S_N2 displacement at methyl by thiophenolate and work is currently in progress using phosphorus ligands which give this step an S_N1 mechanism.[15]

To establish the compatibility of the methyl protecting group with the phosphorothiolate linkage, demethylation of **5** was studied by NMR; treatment with thiophenol:triethylamine:dioxan (1:2:4) for 1 hour at room temperature gave quantitative deprotection with no cleavage of the internucleoside linkage and the phosphate deprotected dinucleoside was isolated in 82% yield after chromatography.

More recently we have investigated the potential of phosphotriester chemistry for the preparation of 3'-*S*-phosphorothiolate linkages (**Scheme 2**). The thionucleoside (**1**) was phosphorylated using 2-chlorophenyl-phosphorodi-1,2,4-triazolide (2 equiv.) in THF at room temperature for 9 hours. After chromatography and precipitation from heptane containing 1% triethylamine the phosphorothiolate diester (**8**) was isolated as its triethylammonium salt in 69% yield. The reaction is considerably slower than the phosphorylation of the corresponding 3'-hydroxy nucleoside, but despite the extended reaction period no side-reactions leading to modification of the thymine base were observed. Coupling of **8** with 3'-*S*-benzoyl-3'-thiothymidine[23] using 1-

(2-mesitylenesulphonyl)-3-nitro-1,2,4-triazole (MSNT; 2.5 equiv.) in pyridine gave about 50% reaction (by ^{31}P NMR) after 23 hours at room temperature. Addition of a further aliquot of MSNT (1 equiv.) gave complete reaction within 1 hour and the dinucleoside phosphorothiolate (**9**) was obtained in 76% yield.

Scheme 2



8 **9**

(i) 2-chlorophenylphosphorodi-1,2,4-triazolide (2 equiv.), THF; (ii) 3'-*S*-benzoyl-3'-thiothymidine (1.5 equiv.), MSNT (3.5 equiv.), pyridine.

Oximate mediated removal of aryl phosphorus protecting groups proceeds *via* nucleophilic attack at phosphorus with potential displacement of nucleoside thiolate from **9**. This deprotection step was therefore investigated by ^{31}P NMR and TLC using 0.3 *M* N^1,N^1,N^3,N^3-tetramethylguanidinium 2-nitrobenzaldoximate under either aqueous[24] or anhydrous[25] conditions. In both cases deprotection was complete within 1 minute, and under anhydrous conditions was accompanied by only 1% cleavage of the phosphorus-sulphur bond. Under aqueous conditions, scission of this bond occurred to a greater extent (4-10%) which we attribute to the presence of hydroxide ions in the reaction mixture (pH 10).

In conclusion, both of the methods described here have significant advantages (higher yields and cleaner products) over those previously published and should be readily applicable to the large scale synthesis of short oligonucleotides containing multiple phosphorothiolate linkages.

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