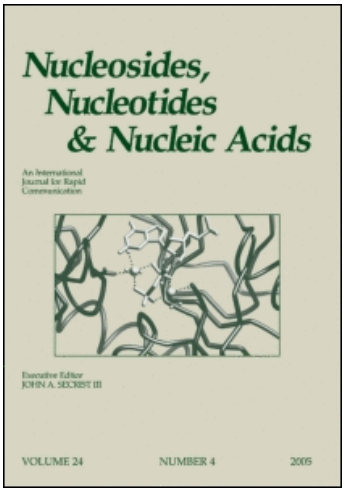

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Synthesis of Oligodeoxynucleoside Phosphoro-Monothioates and Phosphorodithioates by a Phosphotriester Method

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SYNTHESIS OF OLIGODEOXYNUCLEOSIDE PHOSPHORO-MONOTHIOATES AND PHOSPHORODITHIOATES BY A PHOSPHOTRIESTER METHOD

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ABSTRACT. A phosphotriester method for the synthesis of dithymidine phosphoromonothioates and phosphorodithioates with new *S*-protecting groups has been investigated. Four of the *S*-protecting groups possesed catalytic activity, however side reactions occurred during deprotection. The best *S*-protecting group was 4-chloro-2-nitrobenzyl which could be removed with a minimum of side reactions (0.3 %). The coupling reagent PyFNOP (**14**) gave protected dithymidine phosphoromonothioate in 96 % yield after 15 min coupling. Furthermore PyFNOP chemoselectively activates oxygen in nucleoside phosphorodithioate monomers **9** and can be used for the synthesis of oligodeoxynucleoside phosphorodithioates with mixed base sequences.

Oligonucleoside phosphoromonothioates (POS-ODN) and phosphorodithioates (PS_2-ODN) are among the most intensively investigated nuclease-resistant antisense analogues [1-3] and have been shown to have markedly antiviral properties and to inhibit a variety of oncogenes. We have investigated a phosphotriester method (**Figure 2**) for the synthesis of dithymidine phosphoromonothioates and phosphorodithioates and for the solid phase synthesis of oligodeoxynucleoside phosphorodithioates with mixed base sequences.

The method we used to synthesize the nucleoside phosphorothioate monomers **8a-8h** and **9a-9h** with the new *S*-protecting groups is outlined in **Figure 1**. The key step is alkylation of the *O*-cyanoethyl protected thymidine phosphorothioates **4** or **5**, which were prepared from commercially available phosphoramidite **1** as shown.

These monomers were used to synthesise fully protected dithymidine phosphorothioates **10a-10h** and **11a-11h** in order to investigate if the S-protecting groups 1) could be selectively removed to give **12** or **13** without side reactions and 2) gave any rate acceleration in the coupling step (i.e. could function as catalytic protecting groups). Our results are presented in the table in **Figure 2**. The phosphoromonothioates and phosphorodithioates behaved similarly in the deprotection step with a tendency for the *S*-protecting groups to be more labile in the phosphorodithioates. With the exception of the (2-pyridyl)methyl *N*-oxide dimers **10f** and **11f**, the only side products detected by ^{31}P-NMR were the *S*-protected

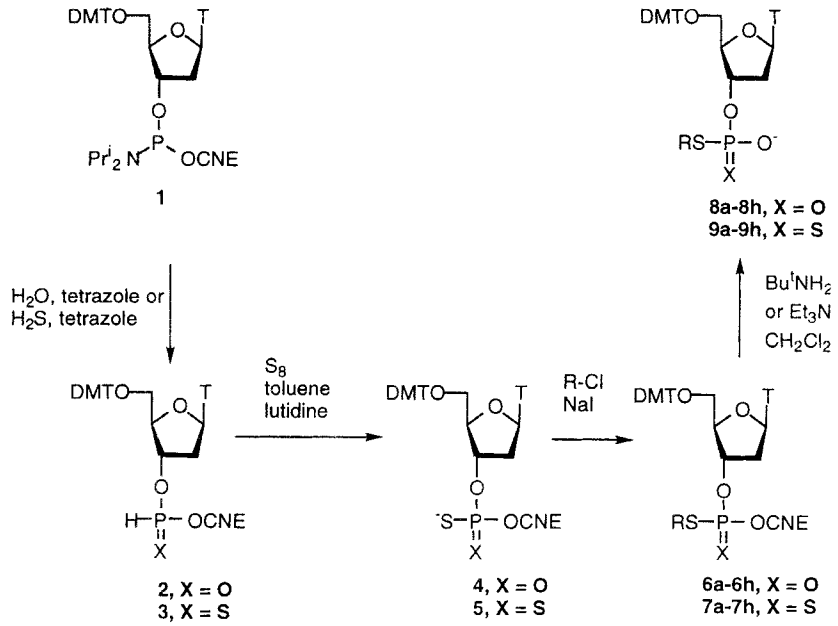
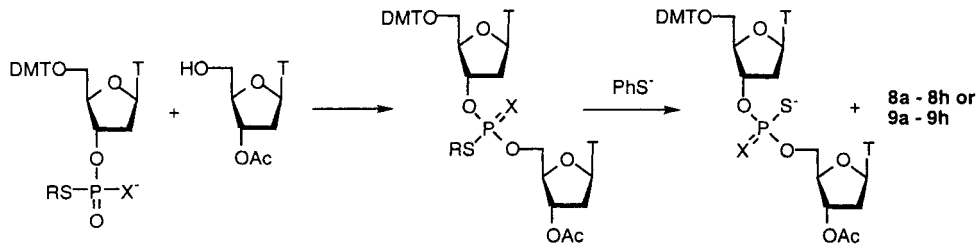


Figure 1. Synthesis of the phosphorothioate monomers **8a-8h** and **9a-9h**. R is shown in **fig. 2**. **4** or **5** was converted to the fully protected thymidine phosphorothioates **6a-6h** or **7a-7h**, respectively, by alkylation, and the cyanoethyl group was easily and selectively removed by treatment with either 10% *tert*-butylamine in dry pyridine or 20% triethylamine in dry methylene chloride to give the ammonium salts **8a-8h** and **9a-9h** in almost quantitative yields.

thymidine phosphorothioate monomers **8a-8h** and **9a-9h** formed by cleavage of the internucleotide bridge by attack of thiophenolate ions at the 5'-position of the dimers **10** and **11**. Deprotection of the (2-pyridyl)methyl *N*-oxide dimers **10f** and **11f** were accompanied by the formation of 1 - 2% phosphorothioate and phosphate impurities, which is clearly unacceptable.

The best *S*-protecting group was 4-chloro-2-nitrobenzyl which could be removed with a minimum of side reactions (0.3 %). Four of the new *S*-protecting groups (**d**, **f-h**) showed catalytic activity with the (*N*-methyl-2-imidazolyl)methyl group being the most efficient. However the amount of side reactions during deprotection was judged unacceptable for the use of these new *S*-protecting groups in the synthesis of oligodeoxynucleoside phosphoromonothioates and phosphorodithioates.

To accelerate the couplings we turned our attention to the coupling reagent 4-nitro-6-trifluoromethylbenzotriazol-1-yl-oxy-tris(pyrrolidino)-phosphonium hexafluorophosphate (PyFNOP) **14** (**Figure 3**) [4-7]. PyFNOP is a very reactive coupling reagent and the phosphoromonothioate dimer **10c** could be synthesised fast (coupling reaction completed in less than 2 min.) and could be isolated in high yield (96 %).



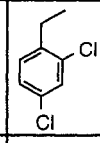
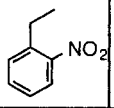
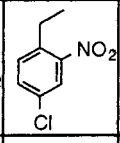
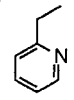
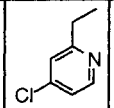
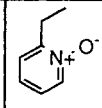
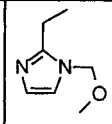
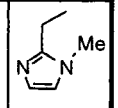
	a	b	c	d	e	f	g	h
R								
% 5'-cleavage in 10 (POS)	1.4	0.8	0.3	6.4	3.0	0.9 [b]	2.0	0.8
% 5'-cleavage in 11 (PS_2)	1.2	nd	0.3	3.0	1.0	0.4 [c]	nd	0.8
coupling time (min), 10 (POS)	8 > 60[a]	8	8	< 2	8	< 2	< 4[a]	< 2[a]

Figure 2. Synthesis, *S*-deprotection and concomitant cleavage of the internucleoside linkage (5'-cleavage) in the phosphorothioate dimers **10a-10h** and **11a-11h**. The amount of 5'-cleavage was determined by ^{31}P-NMR. Coupling reactions to **10** were performed with 3-6 eq. triisopropyl benzenesulfonyl chloride (TIPSCl) and 6-10 eq. *N*-methylimidazole (NMI) in dry pyridine. Coupling reactions to **11** were performed with 3 eq. PyFNOP and 10 eq. NMI in dry acetonitrile. Deprotection was performed by treating the fully protected dimers (0.1 mmol) with pyridine/triethylamine/thiophenol (0.1 ml : 0.1 ml : 0.1 ml) for 3-10 h. nd = not determined.

a) NMI was not added. b) Phosphate (0.9%) was also detected. c) Phosphoromonothioate (1%) and phosphate (1%) were also detected.

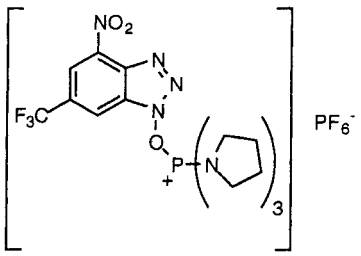


Figure 3. PyFNOP **14**

Furthermore PyFNOP is a chemoselective coupling reagent, which primarily activate the hard oxygen in the phosphorodithioate monomers **9**, thereby allowing the solution phase synthesis of phosphorodithioate dimers **11** without concomitant formation of phosphoromonothioate **10**. However, during solid phase synthesis the chemoselectivity was found to depend on the *S*-protecting group. When a pentamer thymidine phosphorodithioate was synthesised on a solid support using **9h**, **9c** and **9a** respectively the products after deprotection were contaminated with 15%, 1% and 0% phosphoromonothioate respectively (^{31}P NMR, detection limit 0.5 %). From these results we conclude, that although the S-protecting groups of **9c** and **9h** are removed more selectively than the 2.4-dichlorobenzyl group of **9a** from the products with thiophenolate ions, the monomer **9a** is preferred for solid phase synthesis of oligodeoxynucleoside phosphorodithioates when the amount of phosphoromonothioate contamination is to be kept small. In this way we succeded in developing a method for the solid phase synthesis of oligodeoxynucleoside phosphorodithioates with mixed base sequences [5].

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