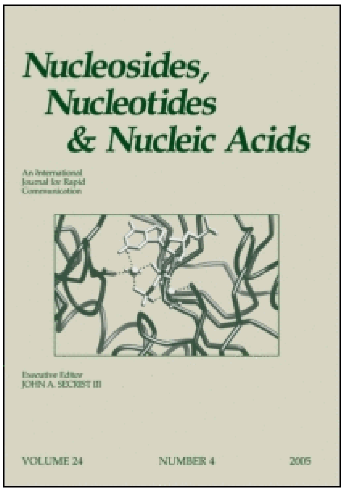

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Synthesis of the 3'-Derivatives of Phosphorothioate Oligonucleotide Analogues

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SYNTHESIS OF THE 3'-DERIVATIVES OF PHOSPHOROTHIOATE OLIGONUCLEOTIDE ANALOGUES.

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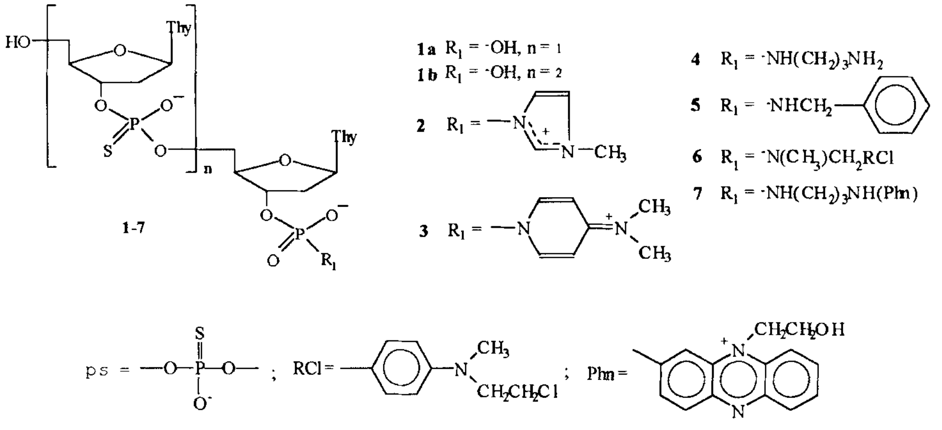
Abstract. 3'-Derivatives of phosphorothioate (PS) oligonucleotide analogues have been synthesized by a selective activation of a 3'-terminal phosphate group of the deprotected PS oligonucleotides using a mixture of triphenylphosphine and 2,2'- dipyridyldisulfide.

Phosphorothioate (PS) oligonucleotide analogues are extensively studied as effective therapeutic agents in the antisense approach[1-6] In order to enhance an antisense effect, reactive, complementary complex stabilizing and other groups have to be attached to PS oligonucleotides. The well-known methods of preparation of the PS oligonucleotide derivatives on polymer supports [4-6] do not allow to introduce residues bearing active chemical groups into PS olgonucleotides. In these cases, the reactive residues may be deactivated during the deprotection of the support-bond blocked PS oligonucleotides.

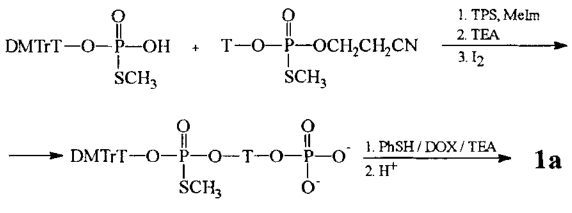
The present paper describes a simple and effective method of the synthesis of the various 3'- PS oligonucleotide derivatives (**1-7**). A selective activation of the 3'-terminal phosphate group of the deblocked PS oligonucleotides with triphenylphosphine (Ph_3P) and 2,2'-dipyridyldisulfide ($(PyS)_2$) mixture in conditions where the internucleotide phosphorothioate groups are not damaged, is essential to this approach. The method was carried out by using di- and trinucleotide phosphorothioates TpsTp (**1a**) and TpsTpsTp (**1b**), bearing a terminal 3'-phosphate group. All obtained derivatives of PS oligonucleotides were detected by ^{31}P-NMR-spectroscopy.

The starting phosphorotioates **1a** and **1b** containing a 3'-terminal phosphate group, were prepared via a phosphotriester approach with using a mixture of N-methylimidazole and 2,4,6-triisopropylbenzenesulfonyl chloride as a condensing reagent in pyridine with deblocking of the protected groups according to Scheme 1. The yields of phosphorothioates **1a** and **1b**, isolated by the ion exchange chromatography, were 60 and 40%, respectively.



The triphenylphosphine and 2,2'-dipyridyldisulfide mixture was used to activate the terminal phosphate group of di- (**1a**) and tri- (**1b**) nucleotide phosphorothioates in the presence of 4-N,N-dimethylaminopyridine (DMAP) or N-methylimidazole (MeIm) as nucleophilic catalysts[7] (Scheme 2). The reaction time was 5-10 minutes. The yields of the 3'-zwitter-ionic derivatives **2a,b** and **3a,b**, obtained and isolated by precipitation with ethyl ether, were about 90%.

The prepared zwitter-ionic DMAP (**2**) or MeIm (**3**) derivatives were used as intermediates for the synthesis of the 3'-phosphoramidate derivatives of **1a** and **1b**, bearing an alkylamino group (**4**), the benzylamine (**5**) or alkylating N-methyl-4-(N-2-chloroethyl-N-methylamino)benzylamine (**6**) residues (Scheme 2). The complete disappearance of the ^{31}P-NMR resonance signals of DMAP (in area -7 ppm) or MeIm (-11 ppm) derivatives, and the appearance of signals in the area of 6-8 ppm corresponding to phosphoramidate derivatives **4-6**, were detected within 5-10 min after addition of



DMTr = dimethoxytrityl
TPS = 2,4,6-triisopropylbenzenesulphonyl chloride
MeIm = N-methylimidazole
TEA = triethylamine
PhSH = thiophenol
DOX = dioxane

Scheme 1

$$\mathbf{1} + Ph_3P + (PyS)_2 + Nu \longrightarrow \mathbf{2} \text{ or } \mathbf{3} \xrightarrow{HR_1} \mathbf{4,5} \text{ or } \mathbf{6}$$

Nu = N-methylimidazole or 4-N,N-dimethylaminopyridine
Ph_3P = Triphenylphosphine
$(PyS)_2$ = 2,2'-dipyridyldisylfide

Scheme 2

10 equivalents of the corresponding amine compounds to the reaction mixtures containing DMAP or MeIm derivatives (0.01 M solution in DMF). The yields of the products, obtained and isolated by precipitation with ethyl ether, were 85-95%.

The N-(2-hydroxyethyl)phenazinium (Phn) derivative **7b** of the PS oligonucleotide was prepared by reaction of the PS olgonucleotide derivative **4,** bearing primary amino group, with Phn^+Cl^- as published in 8. ^{31}P-NMR-signals of Phn derivative **7b** in H_2O were registered in area of 55,3 and 55,4 ppm for ps- and 8,7 ppm for terminal phosphoroamidate groups. The yield of **7b** isolated by the ion exchange chromatography, was 85%.

The method proposed allows to introduce different chemical groups, including high reactive (alkylating) ones, at the 3'-end of PS oligonucleotides without the damage of the internucleotide ps-groups of the PS oligonucleotides and formation of any by-products.

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