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SYNTHESIS OF CHIMERIC OLIGONUCLEOTIDES CONTAINING INTERNUCLEOSIDIC PHOSPHODIESTER AND *S*–PIVALOYLTHIOETHYL PHOSPHOTRIESTER RESIDUES

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ABSTRACT

Novel oligonucleotide analogs that bear phosphodiester and bioreversible *S*–pivaloyl 2–mercaptoethyl (SPME) phosphate triester internucleosidic linkages are described. Their synthesis employs a novel methodology of oligonucleotide deprotection under mild, non-aqueous conditions.

Antisense oligonucleotides are a novel class of genomic therapeutics. It is well known however that oligonucleotide phosphorothioates are of limited stability in blood and tissues. Besides, being negatively charged molecules, they lack the ability to permeate biological membranes in an efficient manner. Thus, both their oral bioavailability and cellular uptake need further improvement. In order to address this issue, a bioreversible *S*–acyl 2–mercaptoethyl (1) phosphate protecting group has been introduced into synthetic oligonucleotides. The bioreversible oligonucleotides may potentially serve as prodrug forms of antisense oligonucleotides. A search for their reliable and straightforward synthesis is therefore of great interest.

The preparation of oligonucleotide prodrugs using the *S*–acyl 2–mercaptoethyl strategy presents a complex synthetic problem. So far, no conventional deprotection method has been reported in the literature. By an orthogonal protection

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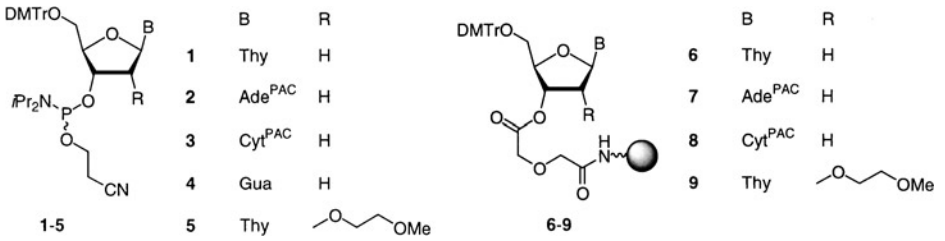

Figure 1. Structures of 2-cyanoethyl phosphoramidites and solid supports.

strategy, preparation of short *S*–pivaloyl 2–mercaptoethyl (SPME) protected oligonucleotides has recently been achieved using photolabile nucleic base protecting groups and solid support (2). However, fully modified SPME oligonucleotides are poorly soluble in water (3) and therefore can hardly be considered as drug candidates.

We reported previously synthesis of a phosphoramidite building block 8 (4) (Fig. 2) and oligothymidylates bearing both SPME and phosphodiester linkages (5). In this communication, we report a novel strategy of oligonucleotide deprotection under mild, non-aqueous conditions. The strategy allows one to deprotect nucleic bases and the backbone while SPME phosphotriester groups are kept intact.

Previously we observed that the SPME group was almost indefinitely stable towards 1 M piperidine in MeCN and of a limited stability towards 0.01 M K_2CO_3 in MeOH (23). With this information in hand, we synthesized a number of model oligonucleotides using phosphoramidites **1**–**3** and solid supports **6**–**8** (Fig. 1) and studied their deprotection. We found that phenoxyacetyl (PAC) protection of deoxycytosine and deoxyadenosine residues was removed under conditions compatible with the SPME group. In contrast, removal of the PAC protection from

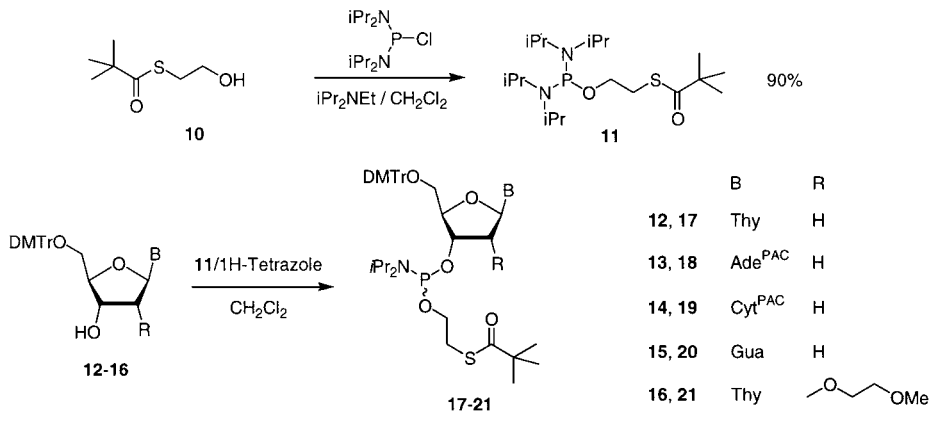

Figure 2. Synthesis of phosphoramidite building blocks **17**–**21**.

deoxyguanosine residue required an unacceptably long treatment with the solution of piperidine (48 h).

This data prompted us to use *N*–unprotected 2–cyanoethyl deoxyguanosine phosphoramidite **4** synthesized as reported earlier (6). In accordance with the previous observations, the use of **4** within the standard protocol of the DNA synthesis demonstrated no deterioration of coupling yields or the purity of synthetic ligonucleotides (6,7).

Having set the deprotection conditions, we synthesized SPME protected nucleoside phosphoramidites **17–21** and evaluated their use in preparation of chimeric oligonucleotides. First, **10** was converted to a bisamidite **11**, which was isolated on a silica gel column as a colorless oil in 90% yield and more than 98% purity (^{31}P NMR). Nucleosides **12–16** were treated with **11** in the presence of **1*H***–tetrazole to give **17–21** (Fig. 2). These were isolated by column chromatography in 75–95% yield and characterized. The phosphoramidites **17–21** were stable compounds whose shelf life exceeded 2 years. Previously we reported that the half-life of disappearance of **17** in 80% aqueous MeCN was 58 h, which indicated its high hydrolytic stability (4).

Oligonucleotides **22–27** (Table) were assembled using phosphoramidite building blocks **1–5** and **17–21** and solid supports **6–9**. For deprotection, a three-step procedure was developed. Solid support-bound oligonucleotides were treated first with 1 M piperidine in MeCN for 8 h and then extensively washed with dioxane. On the second step, the synthetic columns were replaced on the instrument, and the standard detritylation subroutine was carried out. Finally, oligonucleotide material was released from the support with 0.01 M K_2CO_3 in MeOH for 80 min, and the solution was neutralized with glacial AcOH. The obtained mixtures consisted of chimeric oligonucleotides **22–24** along with products of methanolysis of the SPME group. With oligothymidylates, the extent of methanolysis was minimal (*ca*. 2% per SPME group). In other cases it depended on the base composition of an oligonucleotide and varied between 2 and 10%.

Table. Chimeric Oligonucleotides **22–27** Containing SPME Phosphotriester Groups[a]

Compound	Sequence (5′ → 3′)	Backbone
22	***TTT*** T_{13} ***TTT*** T	P=S
23	***T**T*T*** T_{13} ***T**T*T**** T^{*} [b]	P=S
24	CCCCCAA ***T*** T_{10}	P=S
25	***CC*** CCCACCACTTCCCCT ***CT*** C	P=S
26	***CCC*** CCACCACTTCCCC ***TCT*** C	P=S
27	***AG*** CTTCTTTGCACATGT ***AA*** A	P=S

a. Nucleotide residues bearing SPME group at the 3′-phosphate are italicized;

b. T^{*} stands for 2′–*O*–(2–methoxyethyl)–5–methyluridine residue.

In conclusion, the present methodology of mild, non-aqueous oligonucleotide deprotection allows straightforward preparation of potential prodrug oligonucleotides bearing a desired number of the SPME phosphotriester moieties and, potentially, other types of base-labile oligonucleotides.

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