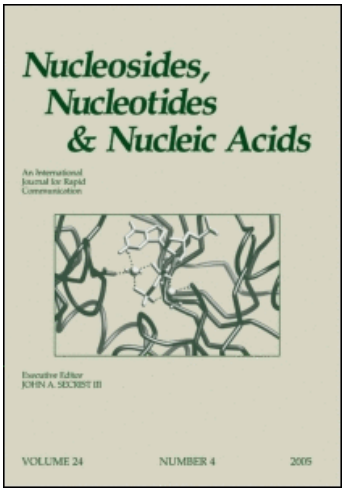

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Synthesis of Chimerical Oligonucleotides Containing Internucleosidic Phosphodiester and *S*-Pivaloyl Mercaptoethyl Phosphotriester Linkages

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SYNTHESIS OF CHIMERICAL OLIGONUCLEOTIDES CONTAINING INTERNUCLEOSIDIC PHOSPHODIESTER AND *S*-PIVALOYL MERCAPTOETHYL PHOSPHOTRIESTER LINKAGES

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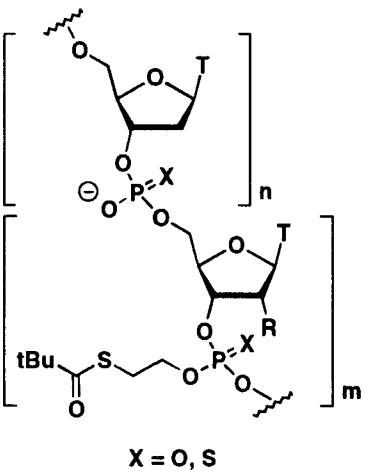
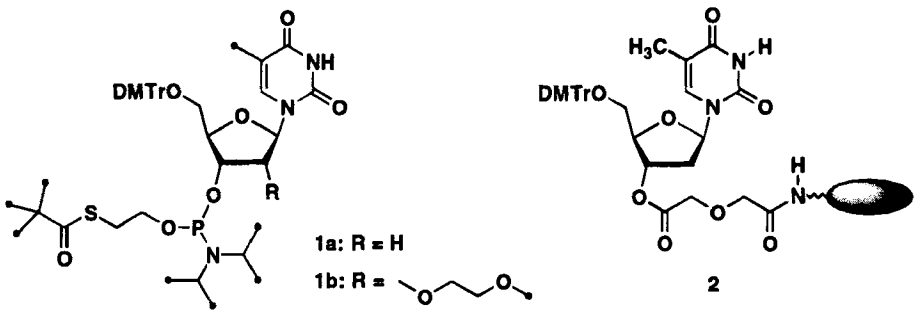
ABSTRACT: Novel oligonucleotide analogs that bear phosphodiester and bioreversible *S*-pivaloyl 2-mercaptoethyl (SPME) phosphate triester internucleosidic linkages and their thioate analogs are described. Their synthesis involves new methodology for the deprotection of base-labile oligonucleotides.

Antisense oligonucleotides are a novel class of potential 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 efficiently permeate biological membranes. Thus both their oral bioavailability and cellular uptake are usually low. Recently, *S*-acyl-2-mercaptoethyl phosphate protection has been introduced to give rise to bioreversible oligonucleotides that may potentially serve as prodrug forms of antisense oligonucleotides.[1]

The major complication in preparation of these oligonucleotides consists in finding chemoselective deprotection procedures. While the base labile (thio)ester function of SPME group is kept intact, both phosphate and nucleic base moieties have to be deprotected which usually requires treatment with a base. So far, oligonucleotides that are comprised of phosphodiester and SPME phosphothionotriester units have been synthesized by postsynthetic alkylation of phosphodiester / phosphorothioate mixed backbone oligonucleotides,[2] whereas no selective removal of cyanoethyl protection in the presence of SPME group have been reported.



X = O, S



Recently we reported synthesis of a phosphoramidite building block **1a** and its ability to efficiently elongate oligonucleotide chain.[3] Now the use of **1a,b** in the oligonucleotide synthesis followed by a new, extremely mild deprotection procedure allows one to prepare chimerical oligonucleotides that are comprised of phosphodiester and SPME phosphotriester internucleosidic units and their thioate analogs.

Chimerical oligonucleotides were assembled on diglycolyl solid support **2**[4] using phosphoramidites **1a,b** to create SPME phosphotriester moiety. A three-step deprotection procedure was elaborated. First, support-bound oligonucleotides were decyanoethylated with 1M piperidine in MeCN. After extensive wash with dioxane, the standard detritylation subroutine was carried out. Finally, oligonucleotide material was released from the support with 0.01M K_2CO_3 in MeOH. The obtained mixtures consisted of chimerical oligonucleotides along with products of methanolysis of SPME group (*ca.* 2% of methanolysis per group). The chimerical oligonucleotides bearing 1 to 8 phosphotriester functions were obtained in 40 to 70% isolated yields depending on the length and the number of SPME groups.

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