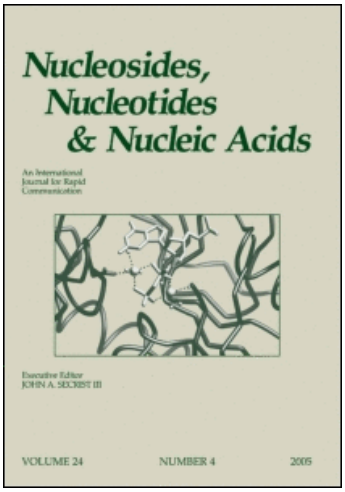

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The Use of Triisopropylsilyl-oxymethyl (TOM) in the Synthesis of Anti-telomerase 2-5A Antisense Compound RBI 011

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ABSTRACT

The 2-5A antisense compound RBI 011 targeting telomerase RNA was synthesized using the triisopropylsilyl-oxymethyl (TOM) group for the 3′-hydroxyl protection of 2′,5′-linked RNA.

INTRODUCTION

A novel class of chimeric oligonucleotides for use as tools in antisense technology was introduced by Torrence, Silverman and coworkers in 1993.[1,2] These molecules are composed of a 5′-phosphorylated 2′,5′-linked oligoadenylate part, which is linked to the antisense domain by means of a short linker such as two butanediol phosphate molecules. The 5′-phosphorylated 2′-5′-linked oligoadenylate known as 2-5A is able to activate the endogenous and ubiquitous enzyme RNase L, while

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the antisense portion of the chimera ensures specific hybridization to the targeted RNA thereby directing the nuclease activity of RNase L to the targeted molecule. The 2-5A antisense strategy combines the ability of 2-5A to activate RNase L with the specificity of antisense.

Recently, telomerase has become a focus of interest among oncologists as a target for treating cancer cells. Telomerase, a holoenzyme with RNA and protein components, elongates telomeric DNA repeats (TTAGGG) n and is important in protecting and replicating DNA.[3] The almost exclusive expression of telomerase in tumor cells, and not in most normal cells, offers an exciting opportunity for cancer therapy by inhibiting its function.

RBI 011 is a 2-5A antisense compound that targets telomerase RNA. It specifically induces apoptosis in a telomerase-positive ovarian carcinoma cell line HEY-1B.[4] Furthermore, treatment of tumors grown in nude mice with the antisense oligonucleotide inhibited tumor growth. Targeting telomerase RNA with 2-5A antisense, therefore, may represent an effective and novel approach for treatment of a broad range of cancers.

During the synthesis of a 2-5A antisense compound the ribose's 3′-hydroxyl functions of the 2-5A part requires protection analogously to the 2′-hydroxyl functions in RNA synthesis. Here, we report for the first time the use of the triisopropylsilyl-oxymethyl (TOM) group for the 3′-hydroxyl protection of 2′,5′-linked RNA. The TOM group was recently introduced to RNA synthesis by Pitsch et al.[5]

SYNTHESIS

RBI 011 was synthesized by means of solid phase synthesis using phosphoramidite methodology and an automated synthesizer.[6,7] Thereby, it is possible to synthesize the different moieties of the 2-5A antisense compound without the need of postconjugation steps.

For incorporating the linker, a 2-(cyanoethyl) -N,N-diisopropyl-4-O-(dimethoxytrityl) butyl phosphoramidite is coupled twice to the growing chain. For the synthesis of the 2-5A part we employed a 3′-O-TOM protected RNA phosphoramidite [5′-O-dimethoxytrityl-N^6-acetyl-3′-O-(triisopropylsilyl-oxymethyl)adenosine 2′-(N,N-diisopropylcyanoethyl)-phosphoramidite (Xeragon)] in combination with the activator ethylthio-tetrazole (ETT).

The final step of the chimera synthesis is the 5′-phosphorylation. The introduction of a 5′-phosphorothioate can be accomplished by using the commercially available phosphorylation reagent bis- cyanoethyl-N,N-diisopropyl-phosphoramidite and a sulfurization reagent (3H-1,2-benzodithiole-3-one-1,1-dioxide, Beaucage reagent) for the oxidation step.

For cleavage from the solid support and base deprotection the CPG is dried and treated with 8 M anhydrous ethanolic methylamine. Thereafter, the TOM group is removed by a 1 M TBAF solution in THF or alternatively with a cocktail made up of TEA 3HF, TEA and NMP.[8] The completely deprotected 2-5A antisense compound is then purified using a phenyl column and a tetrabutylammoniumphosphate (TBAP) buffer or a Dionex PA-100 SAX column using a sodium perchlorate gradient. The final product is obtained after desalting and cation exchange if needed.

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RESULTS AND DISCUSSION

We were able to significantly decrease synthesis and deprotection times by replacing the routinely used TBDMS with the TOM sugar protecting group. We found that 200 sec is sufficient for the coupling 3′-O-TOM protected sugars, while the TBDMS group required 600 sec. We were able to decrease sugar deprotection times from 24 h to 15 min at room temperature using TBAF or from 2 h at 65 C to 15 min at room temperature using TEA 3HF/TEA/NMP.

A 5′-phosphorothioate group on 2-5A compounds is very sensitive to aqueous base treatment. Therefore, it is important to perform base deprotection of such derivatives under anhydrous conditions such as ethanolic methylamine. Since CPG tends to collect moisture from air it is also routinely dried prior to base deprotection.

The addition of water or aqueous buffer to the TEA 3HF solution after sugar deprotection also leads to the complete degradation of a 2-5A analog. Therefore, before the compound can be further worked up all excess HF has to be removed by adding 2-propyl trimethylsilyl ether to the TEA 3HF solution using the method from Song and Jones.[9] The 2-propyl trimethylsilyl ether reacts with any excess of HF to form volatile compounds, which can be lyophilized in the speed-vac.

Before commencing with the HPLC purification it is necessary to add water or aqueous buffer to finalize the TOM deprotection. This necessity was already noticed when the TOM group was introduced into RNA synthesis by Pitsch et al.[5]

The method of Song and Jones[9] removes all excess fluoride in the TEA 3HF solution. Therefore, compounds can now be purified using strong anion exchange (SAX) or other fluoride sensitive columns without the need of an additional desalting step.

5′-Phosphorothioated 2-5A antisense compounds easily dimerize during work-up especially during fluoride treatment with TEA 3HF in an oxidative process. The dimerization of 5′-phosphorothioated oligonucleotides is well documented in the literature.[10] The dimer can easily be reduced back into the monomer with mild reducing agent such as 2-mercaptoethanol.

CONCLUSIONS

The TOM group was found to be an attractive sugar protecting group in 2-5A and 2-5A antisense synthesis. It allows for shorter coupling and deprotecting times. End products were of similar or higher quality. Yields were slightly higher.

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