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## LETTERS TO THE EDITOR

# Sulfonium Derivatives of Thioxanthenone, a New Class of Photodetritylating Agents for Microarray Oligonucleotide Synthesis

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**Abstract**—The usability of a new class of photo acids, namely, sulfonium hexaphosphates based on thioxanthenone, for the removal of the dimethoxytrityl protective group in the process of oligonucleotide synthesis has been studied in order to search for new detritylating agents for microarray oligodeoxyribonucleotide synthesis. 2,4-Diethyl-9-oxo-10-(4-heptyloxyphenyl)-9*H*-thioxanthenium hexafluorophosphate has been successfully used for the solid-phase synthesis of $(dT)_{10}$.

*Key words: photogenerators of acids, oligodeoxyribonucleotides, thioxanthenone, trityl cation*

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The development of the method of DNA microarray oligonucleotide synthesis has opened up vast opportunities for the biochemical identification of biological molecules and mass using synthetic oligonucleotides in gene engineering. Microarray oligonucleotide synthesis developed by Affimetrix has been known for a rather long time [1]; however, it was impossible to use these oligonucleotides for gene engineering purposes because of their heterogeneity and low availability due to the insufficient yield at the stage of synthesis.

The developers of modern methods of microarray synthesis succeeded in the improvement of the quality of oligodeoxynucleotides synthesized [2] by the use of the approaches earlier applied in photolithography and new protective groups.

In our opinion, the approach using photoactivated detritylation [3] is the most promising scheme for oligonucleotide microarray synthesis, as all other steps of oligonucleotide chain elongation are traditional and have been sufficiently exhausted. At the same time, using triarylsulfonium hexafluoroantimonates as acid photogenerators [4] cannot be considered as optimum because of the potential possibility of the apurinization of the oligonucleotide chain synthesized.

In order to optimize the scheme for oligonucleotide microarray synthesis, we have studied the possibility of using a new class of photo acids, namely, sulfonium hexafluorophosphates on the basis of thioxanthenone derivatives, for the removal of the trimethoxytrityl protective group during the process of oligonucleotide synthesis.

We have synthesized and studied eight representatives of this class of compounds in model experiments on the deprotection of 5'-*O*-(4,4'-dimethoxytrityl)thymidine. One of these derivatives, 2,4-diethyl-9-oxo-10-(4-heptyloxyphenyl)-9*H*-thioxanthenium hexafluorophosphate (Fig. 1), was further used for the model synthesis of decathymidylate $(dT)_{10}$ in an automated DNA synthesizer.

The synthesis of $(dT)_{10}$ was carried out using a modified ASM-800 (Biosett, Russia) synthesizer. The synthesizer was modified for the use of a semiautomated protocol for the 0.5-mmol scale photosynthesis of oligonucleotides on porous glass in the flow of reagents. A solution of 2,4-diethyl-9-oxo-10-(4-heptyloxyphenyl)-9*H*-thioxanthenium hexafluorophosphate (1%) [5] was loaded into the synthesizer for the removal of the protection of the 5'-trityl group instead of trifluoroacetic acid. The oligonucleotide synthesis was performed in quartz columns containing porous glass covalently bound to the first nucleotide unit. The synthesizer was supplied with a mercury-vapor lamp (BKL 120) and an optical block with a shutter, a holder for filters, and lenses for separating and focusing the emission of the mercury-vapor lamp at 365 nm on the reaction column. Detritylation was performed three times, the exposure time for each photo acid portion being

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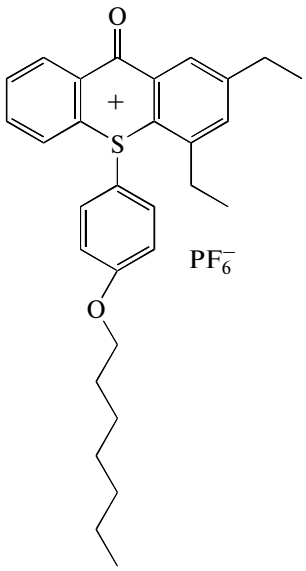


**Fig. 1.** .The structure of 2,4-diethyl-9-oxo-10-(4-heptyloxyphenyl)-9*H*-thioxanthenium hexafluorophosphate used in oligonucleotide synthesis.

1 min. The remaining steps of the oligonucleotide chain elongation when using the phosphoramidite approach, including blocking the unreacted 5'-hydroxyl groups with acetic anhydride, remained invariable. After the synthesis was over, the carrier was treated with concentrated aqueous ammonia for 2 h and the reaction mixture was analyzed with HPLC and PAGE (Fig. 2). The yields for the elon-

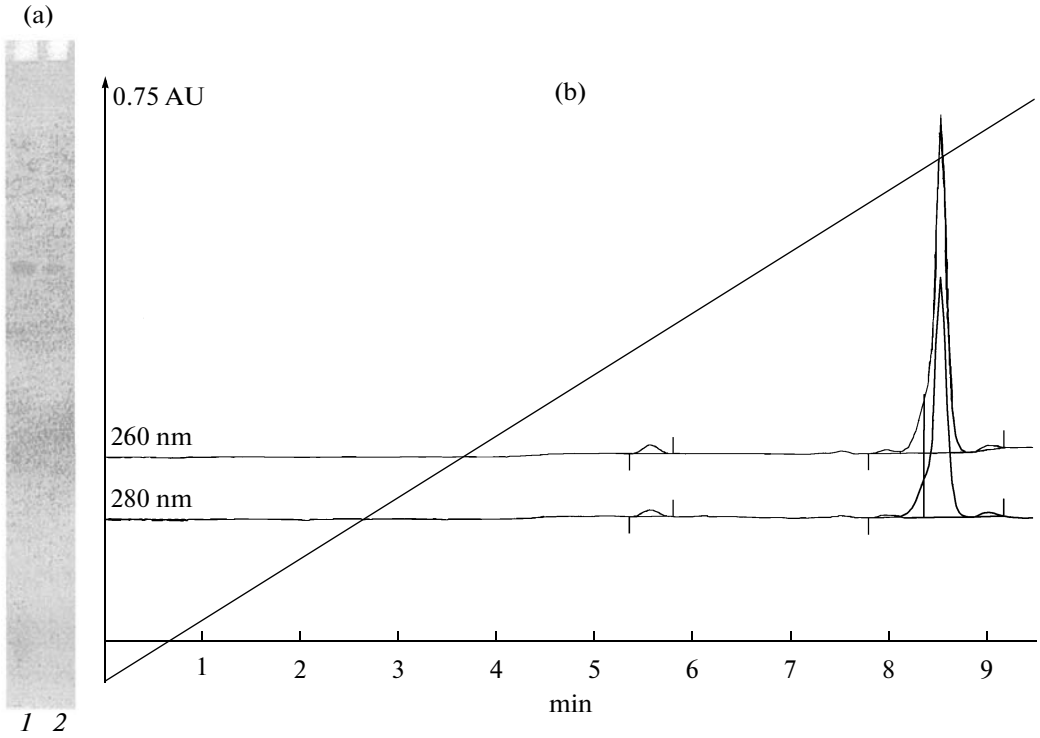


**Fig. 2.** The control of $(dT)_{10}$ synthesis. (a) Electrophoregram in 15% PAG (denaturing conditions) of (*1*), the reaction mixture upon synthesis, (*2*) $(dT)_{10}$ reference; (b) reverse-phase HPLC of the reaction mixture: Millikhrom A-02, ProntoSil-120-5-C18 AQ column with the particle size of 5 μm, 2–20% linear gradient of acetonitrile in 0.05 M triethylammonium acetate (pH 7.5) for 10 min, flow rate is 150 μl/min.

gation step by both methods using either photogenerated or added acid were identical and came to 98%.

Thus, 2,4-diethyl-9-oxo-10-(4-heptyloxyphenyl)-9*H*-thioxanthenium hexafluorophosphate can be successfully used for the generation of hexafluorophosphoric acid during oligonucleotide synthesis, opening up perspectives for its application in microarray nucleotide synthesis.

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