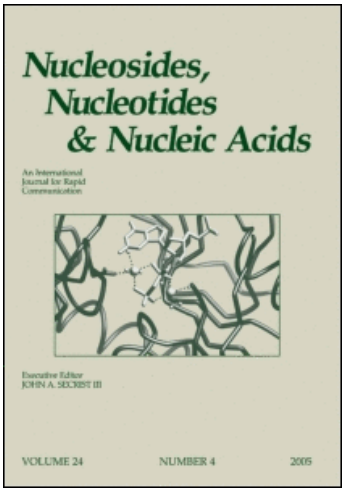

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A Novel and Convenient Method for the Synthesis of Free 5′-Thiol Modified Oligonucleotides

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ABSTRACT

The synthesis of free 5′-thiol-modified oligonucleotides using a 4,4′,4″-trimethoxytrityl (TMTr)-protected linker and standard Poly-Pak^{TM} purification has been described.

Key Words: 5′-Thiol linker; TMTr removal; Oligonucleotide conjugation; Phosphoramidite.

The synthesis of DNA bioconjugates often requires 5′-thiol modified oligonucleotides. The most frequently applied protecting group on thiol-modifier linkers is the trityl (Tr) group. However, removal of this group is usually problematic: heavy metal cations (Ag^{+}, Hg^{2+}) are used[1] for removal and residual ion impurity may hamper the application of the desired DNA in biological systems. In our preliminary experiments it was found that using the trityl deprotection method and a modified

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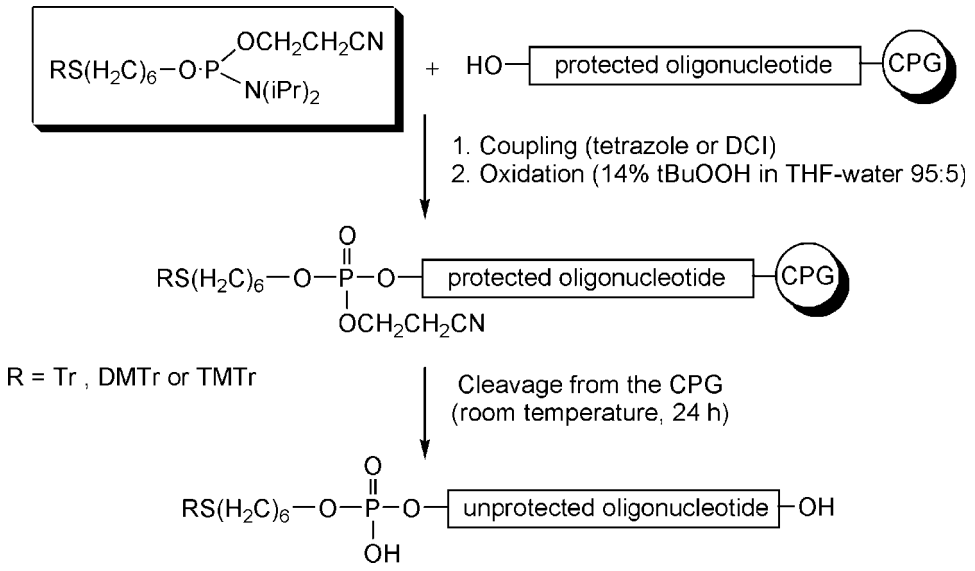
Poly-Pak™ purification procedure[2] there was a significant loss of oligonucleotide and undesired scavenger impurities appeared in the product.

Our aim was to develop a similarly simple purification and thiol deprotecting method for 5′-*S*-protected oligonucleotides as the Poly-Pak™ purification procedure for native oligonucleotides without using scavengers applied in the standard protocol.

RESULTS

6-*S*-Tr-, -DMTr- and -TMTr-protected 6-mercaptohexanol-derived phosphoramidites were prepared from the corresponding protected 6-mercaptohexanols[3] using standard procedures. The oligonucleotides containing a 5′-thiol modifier have been prepared according to Sch. 1.[4]

Three methods have been applied and evaluated for the cleavage and purification: 1. standard Poly-Pak™ column and purification procedure; 2. mixed column (Poly-Pak™ and thiol-sepharose, as an immobilized scavenger) and a slightly modified Poly-Pak™ purification; 3. extraction in the presence of triethylsilane scavenger. The following conclusions could be drawn: 1. Purification with extraction: based on HPLC study, the aqueous phase did not contain oligonucleotides. 2. Purification on mixed columns gave similar results as the standard Poly-Pak™ method with somewhat lower yields, DTT (used for activating the sepharose-linked thiol scavenger) impurities were present in the product. 3. Standard Poly-Pak™ purification affords the best results, only use of TMTr group results in high yield of deprotection (80–90%), it does not require use of heavy metal ions and scavengers, in the case of TMTr protecting group product of deprotection can directly be used for conjugation



Scheme 1.

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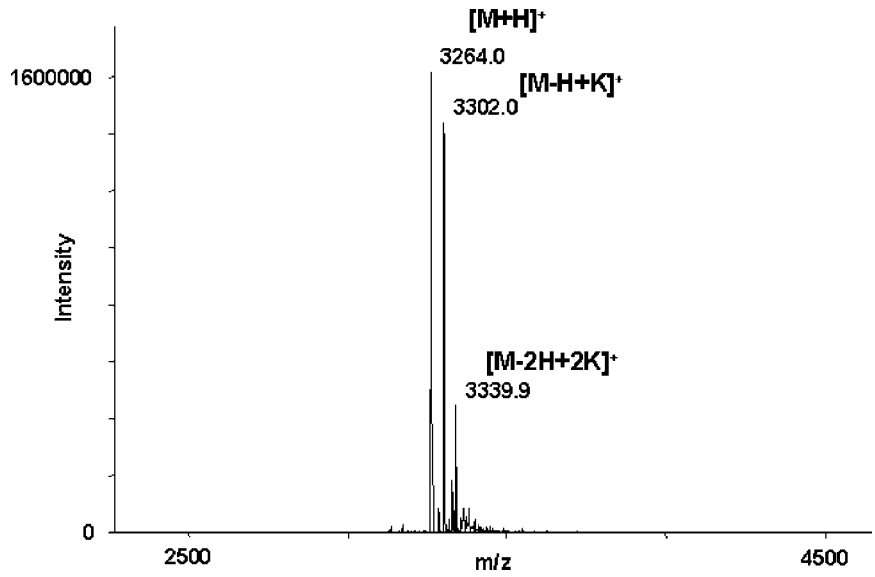

Figure 1. MALDI-TOF MS spectrum of the purified compound $HS(CH_2)$OpTTTGGGAAAC (calcd. 3263.4, found 3263.0).

reactions (residual protected oligonucleotide can be separated after conjugation, if required). Figure 1 shows the MALDI-TOF MS spectrum of the 14 min peak of TMTr-seq 4 [$HS(CH_2)_6$OpTTTGGGAAAC] purified by the standard Poly-Pak™ method (calcd. 3263.4, found: 3263.0).

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