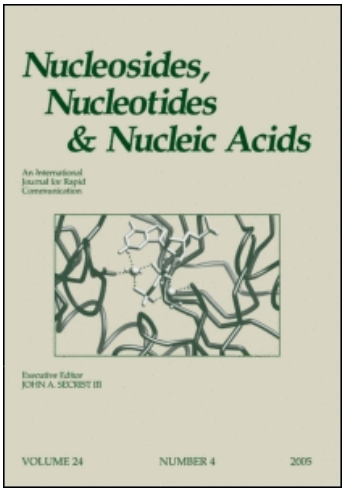

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Preparative Syntheses of Guanylate-Rich Oligonucleotides Using the Phosphotriester Method in Solution

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PREPARATIVE SYNTHESES OF GUANYLATE-RICH OLIGONUCLEOTIDES USING THE PHOSPHOTRIESTER METHOD IN SOLUTION

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Abstract: The guanylate-rich fragments: 150 mg $d(G_4T_4)$, 180 mg $d(T_4G_4)$, 350 mg $d(T_4G_4T_4)$, 30 mg $d(G_4T_4G_4)$, 85 mg $d(T_4G_4T_4G_4)$, were synthesized using the triester method. By enzymatic ligation of aliquots the 36mer $d(G_4T_4G_4T_4G_4T_4G_4T_4G_4)$ is obtained.

The short DNA molecules of the macronucleus of hypotrichous ciliates are terminated [1] with: $5'\text{-}G_4T_4G_4T_4G_4T_4G_4T_4G_4\text{-}3'$.
$C_4A_4C_4A_4C_4$

We previously have synthesized [2] the cytidylate-rich 20mer and now the guanylate-rich fragments were synthesized in three different ways, applying differently protected guanylate monomer units.

On the one hand, we protected the guanine residue only in its 2-N-position using the isobutyryl group. On the other hand, we blocked the nucleobase in the 2-N-position by the propionyl group as well as in the 6-OH-position by the diphenyl carbamoyl group [3]. The 5'-hydroxyl groups of the protected dG or those of the unprotected dT were blocked with monomethoxy- as well as with dimethoxytrityl groups. The 3'-hydroxyl groups of the tritylated and protected dG and of only tritylated dT were phosphorylated using two different approaches. In the first approach phosphorylation was performed with bis(1,2,4-triazolyl)2-chlorophenyl phosphate. In the second approach S,S-diphenyl phosphorodithioate was used which phosphorylated the 3'-hydroxyl group in the presence of 2,4,6-triisopropylbenzene sulfonyl chloride (TPS)/N-methylimidazole (MeIm) [3]. The condensations were carried out in anhydrous pyridine at room temperature using mixtures which consisted of TPS and tetrazole or of 2,4,6-trimethylbenzene sulfonyl chloride/MeIm. The condensation products were fractionated on silica gel columns.

The good yields of the condensation reactions were not remarkably influenced by both the different phosphate protecting groups and condensating agents. The yields obtained in condensating guanylate monomer units, which have been protected in their 2-N-position only decreased in the synthesis of the dimers by 5-20%, in the tetramer synthesis by 12-23% and in the octamer synthesis by 16% in comparison to the yields resulting from the condensations of double protetced guanylate monomer units.

Serious difficulties arise during the chromatographic purification of the deblocked oligonucleotides. The preparative chromatography of guanylate-rich oligonucleotides, employing DEAE-cellulose, QAE-Sephadex, Nucleosil C_{18}, Partisil 10-SAX can be performed only with considerable losses of oligonucleotides. Remarkably, the deprotected oligonucleotides can directly be used in enzymatic ligation avoiding any previous purification.

In our opinion there is no need for considerable improvements in the strategy of the oligonucleotide synthesis, but there is a great demand for more efficient separation methods for purifying the deprotetced oligonucleotides without major losses.

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