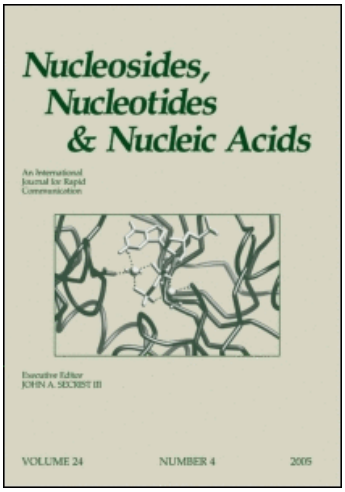

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Syhthesis of Oligonucleotides Containing 4-0-Ethylthymidine

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SYNTHESIS OF OLIGONUCLEOTIDES CONTAINING 4-O-ETHYLTHYMIDINE

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ABSTRACT : Use of p-nitrophenylethyl base protecting groups together with phosphite-triester solid-phase methodology allows the rapid and efficient preparation of oligonucleotides bearing the mutagenic base 4-O-ethylthymine, while standard base protecting groups are not compatible with the presence of this base.

The carcinogenicity of N-nitroso alkylating agents such as nitrosoureas and nitrosoamines is believed to be mediated by some products of alkylation of the nucleobases of DNA in particular 6-O-alkylguanines and 4-O-alkylthymines (1).

In order to study the biological and structural role of 4-alkylthymines in DNA, it is important to develop an efficient method to incorporate these analogues in synthetic DNA. It is well known that these compounds are difficult to incorporate into synthetic DNA because they are sensitive to nucleophiles like ammonia or amines or thiols used for the deprotection of synthetic DNA (2). These problems have been recently solved for the preparation of oligonucleotides bearing some O-alkylthymines (3, 4) but the proposed methods are not useful for the rest of the O-alkylthymines.

In this communication, we will describe for the first time a method for the preparation of 4-O-ethylthymines that could be easily adapted for the synthesis of oligonucleotides containing any O-alkylthymine and, in general, other nucleophile-sensitive base analogues.

The method is based on the use of p-nitrophenylethyl type groups (NPE, NPEOC) as base protecting groups. These groups were described by Pfleiderer and coworkers (5) and

they are removed through a beta-elimination reaction with a strong non-nucleophilic base like DBU (1,8-diazabiciclo [5.4.0] undec-7-ene). In such conditions 4-O-alkylthymines are stable. We have prepared the 2-cyanoethyl-N,N-diisopropyl phosphoramidites of the NPE,NPEOC protected nucleosides (A^{NPE}, C^{NPE} and $G^{NPE,NPEOC}$) and the amidite of 4-O-ethylthymine. Afterwards, we have synthesized, using standard phosphite-triester solid-phase methods, the oligonucleotides 5'TTCT^{Et}CTT3', 5'T^{Et}T^{Et}CGACTAGT3', and 5'GGGTT^{Et}TTCCG3'. After the synthesis we used a two-step deprotection protocol. First, a 6 hours treatment of the oligonucleotide-resin with 0.5 M DBU in pyridine in order to remove the major part of the protecting groups and a second treatment with a 0.5 M DBU solution in ethanol / pyridine (1:1) to cleave the oligonucleotide from the resin. Using this protocol we have prepared 10 mg of a decamer containing 4-O-ethylthymine. Structural studies are presently under way in order to stablish the base pairing properties of 4-O-ethylthymine.

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