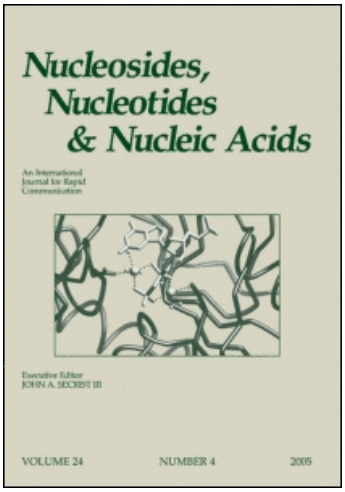

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Synthesis of Oligonucleotide Prodrugs Bearing *N*-Acetyl Nucleobases

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ABSTRACT

N-Acetyl oligonucleotides and their prodrugs were synthesized on photolabile solid support. Tm studies showed a decrease of hydridization for *N*-acetyl A and G and an increase for *N*-acetyl C. In cells extract, acetyl groups were hydrolysed.

Key Words: Protecting group; Enzymolabile; Base-sensitive.

We have been applying a prodrug concept to oligonucleotides (pro-oligos), to overcome their instability in biological fluids and enhance their low cellular uptake.[1] In pro-oligos, the majority of phosphate are transitorily masked with the carboxyesterase-labile S-Acetyl-2-ThioEthyl (Me-SATE) group. To validate this approach we have shown on pro-thymidine models that there are efficiently and rapidly taken up by cells[2] and the Me-SATE groups are hydrolysed in cells extract.[3]

To synthesize hetero pro-oligos, we evaluated the acetyl group[4] as a permanent group of the nucleobases.

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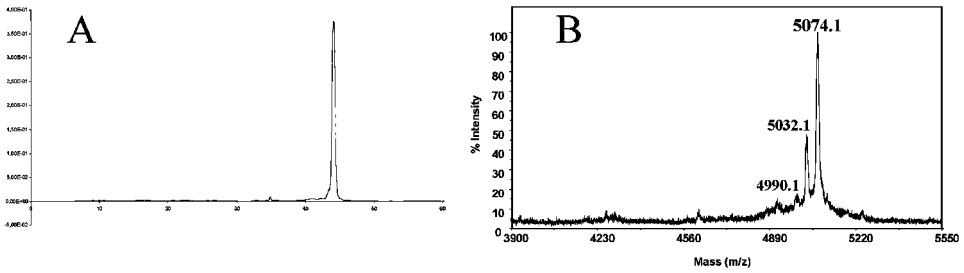

Figure 1. HPLC and MALDI-TOF MS of the *N*-acetyl GCGACCCAACAC pro-oligo bearing 8 Me-SATE and 4 diester linkages targeted against the IRES site.

First, the 5′-DMTr of A^{Ac}, C^{Ac}, G^{Ac} and T were synthesized and converted into the corresponding Me-SATE phosphoramidite and H-phosphonate derivatives. Then, we synthesized three *N*-acetylated dodecanucleotide models each bearing 4 of one *N*-acetylated nucleobases and 8 thymidines. We noticed some deacetylation on A and C during their work-up while on G acetyls were stable. Tm studies showed a slight increase for oligo bearing C^{Ac} ($\approx +1°C$/mod) and a dramatic decrease for oligos bearing G^{Ac} and A^{Ac} (-7.5 and $\approx -7°C$ /mod respectively).

Incubation of these *N*-acetyl oligos in CEM cells extract showed by MALDI-TOF mass spectrometry that the acetyl groups were rapidly hydrolysed on A and C while on G the hydrolysis was slower.

Since acetyl groups could be removed by an enzymatic way, we decided to use them on Me-SATE pro-oligos. Two *N*-Acyl Me-SATE pro-oligos targeted against the internal ribosome entry sequence (IRES) of HCV of a plasmid construct were synthesized using the phosphoramidite and H-phosphonate chemistries on a photolabile solid support. These pro-oligos presented the same sequence (GCGACCCAACAC) but a different number of Me-SATE on each wing of 3 and 4 with a central window of phosphodiester linkages of 6 and 4 respectively, in order to gain molecules of different lipophilicity. Indeed, we have shown that lipophilicity is an important factor for their uptake, with a better uptake when the lipophilicity increased. After synthesis the both *N*-acyl Me-SATE pro-oligos were purified by reverse phase C_{18} HPLC and characterized by MALDI-TOF MS (Fig. 1). The MALDI-TOF spectra showed a slight deacetylation of the pro-oligo with signals spaced by 42 Da corresponding to a loss of one acetyl group (Fig. 1B).

Thanks to *N*-acetyl protection, hetero MeSATE pro-oligos were synthesized. In a near future their capacity to inhibit gene expression would be evaluated.

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