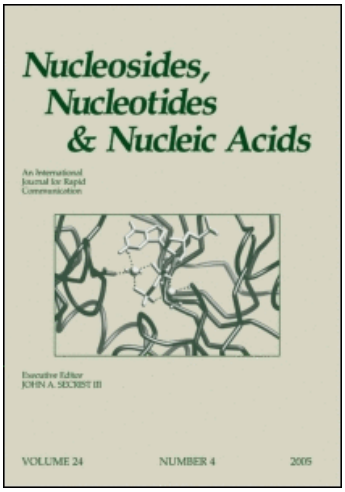

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Direct Regioselective Enzymatic Acylation of Nucleosides: Building-Blocks for the Solution Phase Synthesis of Oligonucleotides

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ABSTRACT

The synthesis of 3′- and 5′-*O*-levulinyl nucleosidic monomers through enzymatic acylation with acetonoxime levulinate is demonstrated. The acylation process takes place in one-step and use of expensive reagents, such as DMTrCl is avoided. The regioselectivity of the procedure makes it very convenient for acylated monomers required for solution phase synthesis of oligonucleotides.

INTRODUCTION

The potential of antisense oligonucleotides for the treatment of a variety of diseases, through sequence-specific modulation of gene expression has been well recognized.[1] Chemical modifications in oligonucleotides[2] have resulted in enhanced

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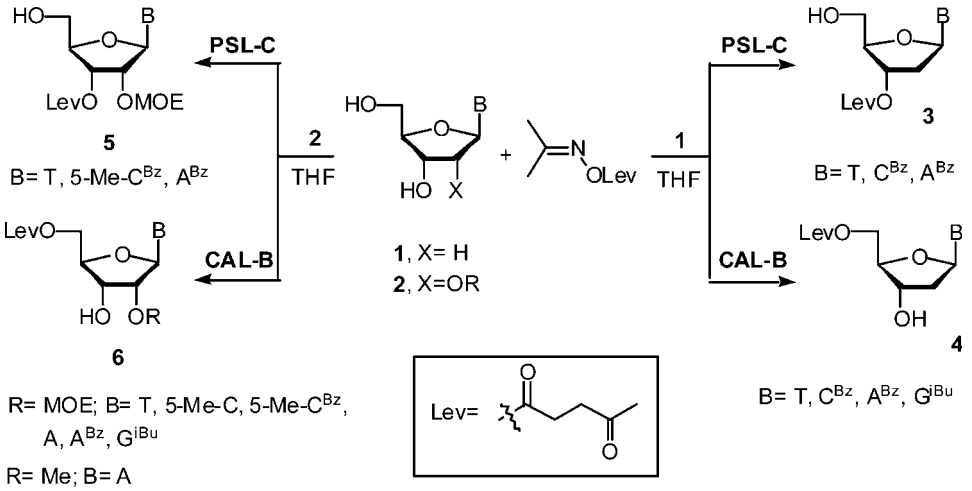
nuclease resistance, cellular uptake, and appropriate hybridization to mRNAs or target genes. With the recent success of various antisense oligonucleotides undergoing human clinical trials and possibility of their commercial launch in the near future may require very large quantities of therapeutically useful oligonucleotides. Particularly when multi-kilogram quantities of oligonucleotides are required, solution-phase synthesis appears to be an alternative method of choice instead of traditional solid-phase synthesis.[3] The key building blocks for the solution-phase oligonucleotide synthesis are 3′- and/or 5′-*O*-levulinyl nucleosidic monomers.[4] The levulinyl group is frequently chosen because it is stable to coupling conditions and can be selectively cleaved, without adversely impacting other protecting groups in the molecule. Until recently, the preparation of these building blocks has been carried out through multiple protection/deprotection steps. For the manipulation of protecting groups, application of biocatalysts in organic synthesis has become an attractive alternative to the conventional chemical methods.[5]

Recently, we have reported a short and convenient synthesis of 3′- and 5′-*O*-levulinylnucleosides from the corresponding 3′,5′-di-*O*-levulinyl derivatives by regioselective enzymatic hydrolysis, avoiding several tedious chemical protection/deprotection steps.[4] During hydrolysis process, base-protected cytidine and adenosine derivatives were not good substrates for the enzyme *Candida antarctica* lipase B (CAL-B), which afforded the 3′-*O*-levulinylnucleosides. To overcome this limitation of our earlier work, herein we report for the first time a regioselective enzymatic acylation of various base-protected nucleosides with acetonoxime levulinate using biocatalysts.

RESULTS AND DISCUSSION

For the enzymatic acylation, acetonoxime levulinate was used as acylating agent due to higher selectivity compared with levulinic anhydride. CAL-B catalyzes the



Scheme 1.

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acylation of the 5′-OH of nucleosides **1** and **2** with excellent regioselectivity affording 5′-*O*-levulinyl derivatives **4** and **6**, respectively, in high yields (Sch. 1). In contrast to the hydrolysis process[4] base-protected cytidine and adenosine derivatives were adequate substrates for CAL-B during acylation. Nevertheless, when *Pseudomonas cepacia* lipase (PSL-C) is employed, 3′-OH is acylated with high selectivity furnishing 3′-*O*-levulinylnucleosides **3** and **5**. As observed with the hydrolysis process, the selectivity of PSL-C was lost when acylation of base unprotected or 2′-*O*-methyl nucleosides was attempted. Furthermore, PSL-C did not shown selectivity during acylation of guanosine derivatives. Such nucleosides could be prepared by enzymatic hydrolysis, where CAL-B hydrolyzed selectively the 5′-*O*-levulinyl group to afford the 3′-*O*-levulinyl derivatives.

In summary, we have developed an efficient method for the synthesis of 3′- and 5′-*O*-levulinyl protected nucleosides through regioselective enzymatic acylation of the parent nucleosides. It is noteworthy that preparation requires a single step, enzymes can be recycled several cycles, and expensive reagent such as DMTrCl is eliminated. These advantages make the acylation procedure very convenient from an industrial point-of-view. In addition, base-protected cytidine and adenosine derivatives, not available through the hydrolysis process, were now accessible. Both hydrolysis and acylation procedures are complementary to each other and offer an excellent alternative to the conventional method. We are in process of scaling-up these reactions for large-scale applications.

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