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NOVEL SELECTIVE BIOCATALYTIC DEACYLATION STUDIES ON KEY PRECURSORS FOR BICYCLONUCLEOSIDES

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□ *Immobilized Candida antarctica lipase and Thermomyces lanuginosus lipase catalyze the deacylation of precursors of LNA analogs, 4′-C-acyloxymethyl-2′,3′,5′-tri-O-acyl-β-L-threo-pentofuranosylthymine and 4-C-acyloxymethyl-3,5-di-O-acyl-1,2-O-(1-methylethylidene)-β-L-threo-pentofuranose, respectively in a highly selective and efficient manner.*

Keywords LNA analogs; bicyclonucleosides

INTRODUCTION

The synthesis of novel nucleoside analogues is gaining importance because of their applications as key intermediates in the development of antisense and/or antigene oligonucleotides to regulate targeted gene expression,[1] and for their direct utilization as anti-tumor or antiviral compounds.[2] A novel class of 3′,5′-linked oligonucleotide analogs containing 2′-*O*,4′-*C*-methylene bridged ribonucleosides, commonly known as

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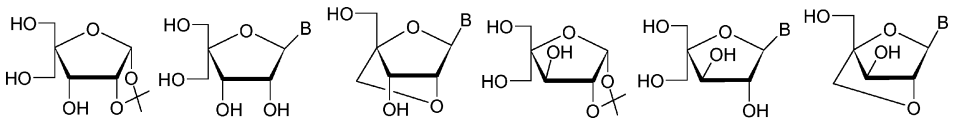


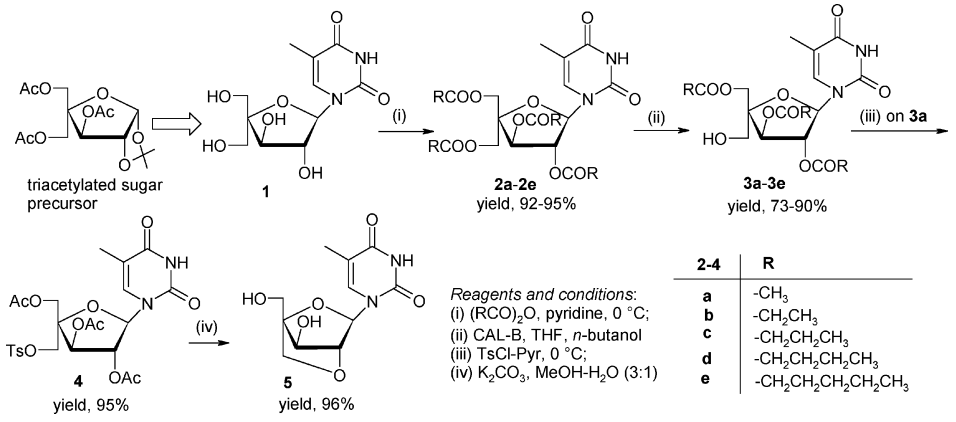
FIGURE 1 Bicyclonucleosides and their precursors.

locked nucleic acids (LNAs) have been known to possess favorable features towards development of antisense and/or antigene candidates.[3] One of the crucial steps in the synthesis of LNA/LNA analogs is the discrimination between two primary hydroxy groups of almost identical reactivity in 4/4′-hydroxymethylated sugar or nucleoside precursors (Figure 1).

Enzymes are being recognized as efficient catalysts for many of the stereospecific and regioselective reactions necessary for carbohydrate modifications and nucleoside synthesis.[4] In the present study, we have developed a highly efficient enzymatic route for diastereoselective deacylation of one of the two acyloxy groups involving primary hydroxyl functions in 4′-*C*-acyloxymethyl-2′,3′,5′-tri-*O*-acyl-β-L-*threo*-pentofuranosyl thymine and 4-*C*-acyloxymethyl-3,5-di-*O*-acyl-1,2-*O*-(1-methylethylidene)-β-L-*threo*-pentofuranose, precursors of bicyclic nucleosides.

DEACYLATION STUDIES ON 4′-*C*-ACYLOXYMETHYL-2′,3′,5′-TRI-*O*-ACYL-β-L-*THREO*-PENTOFURANOSYLTHYMINE

The nucleoside 4′-*C*-hydroxymethyl-*threo*-pentofuranosylthymine (**1**) was synthesized from triacetylated sugar precursor 4-*C*-acetoxymethyl-3,5-di-*O*-acetyl-1,2-*O*-(1-methylethylidene)-β-L-*threo*-pentofuranose[5] in 3 steps in an overall yield of 64% and quantitatively converted into its peracylates **2a–2e** using corresponding acid anhydride in pyridine (Scheme 1). Three



SCHEME 1

lipases, *i.e*. Novozyme-435, *Candida rugosa* lipase (CRL) and *Candida antarctica* lipase-B immobilized on accurel [CAL-L(A)] were screened for the selective deacylation of the tetraacylated nucleosides **2a–2e** in different organic solvents in the presence of *n*-butanol as an acyl acceptor. Novozyme-435 in tetrahydrofuran was found to be the most suitable combination for the deacylation of nucleosides **2a–2e** at 50–55°C.

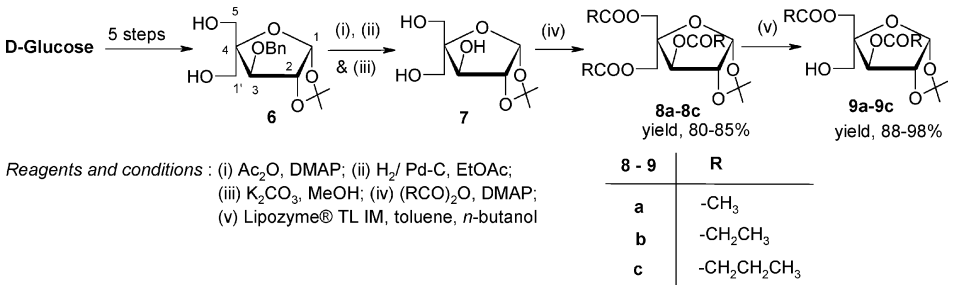
In a typical reaction, a solution of 4′-*C*-acyloxymethyl-2′,3′,5′-tri-*O*-acyl-β-L-*threo*-pentofuranosylthymine **2a–2e** in tetrahydrofuran containing a small amount of *n*-butanol was incubated with Novozyme-435 (50% w/w of the **2a–2e**) in an incubator shaker at 50–55°C. On completion of the reaction, as indicated by TLC examination, enzyme was filtered off and the solvent removed under reduced pressure. The crude product thus obtained was passed through a small silica gel column to afford the pure deacylated compounds **3a–3e**, with lower R_f values than the corresponding starting compounds **2a–2e** in 73–90% yields (Scheme 1).

The structures of enzymatically deacylated nucleosides **3a–3e** was established as 4′-*C*-hydroxymethyl-2′,3′,5′-tri-*O*-acyl-β-L-*threo*-pentofuranosylthymine on the basis of their IR, ^{1}H NMR, ^{13}C NMR, HRMS data and by comparison with the ^{1}H- and ^{13}C NMR spectral data of similar compounds. The structure of 4′-*C*-hydroxymethyl-2′,3′,5′-tri-*O*-acetyl-β-L-*threo*-pentofuranosylthymine (**3a**) was further confirmed by conversion of this selectively deacetylated compound to the bicyclic nucleoside **5** via tosylation of its hydroxyl function, followed by hydrolysis of the three acetoxyl functions with simultaneous cyclisation due to detosylation with the 2′-hydroxyl group under aqueous-methanolic potassium carbonate condition (Scheme 1). Conversion of enzymatically prepared hydroxynucleoside **3a** into bicyclic nucleoside **5**, unambiguously established the structure of other selectively deacylated nucleosides **3b–3e** as 4′-*C*-hydroxymethyl-pentofuranosylthymine acylates.

DEACYLATION STUDIES ON 4-*C*-ACYLOXYMETHYL-3,5-DI-*O*-ACYL-1,2-*O*-(1-METHYLETHYLIDENE)-β-L-*THREO*-PENTOFURANOSES 8a–8c

The trihydroxy sugar **7** (synthesized starting from D-glucose following the modified procedure of Youssefyeh et al.[6]) was converted to its triacylated derivatives **8a–8c** using acetic anhydride, propanoic anhydride and butanoic anhydride, respectively in the presence of catalytic amount of DMAP in 80–85% yields (Scheme 2).[5]

Four lipases, i.e. *Candida antarctica* lipase-B immobilized on polyacrylate (Lewatit), commonly known as Novozyme-435, porcine pancreatic lipase (PPL), *Candida rugosa* lipase (CRL), *Thermomyces lanuginosus* lipase immobilized on silica (Lipozyme TL IM) and *Candida antarctica* lipase-B

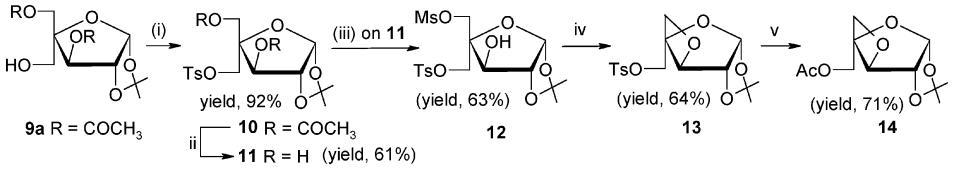


SCHEME 2

immobilized on accurel [CAL-L(A)] were screened for the selective deacylation of the triacylated pentofuranose derivatives **8a–8c** in different organic solvents in the presence of *n*-butanol as the acyl acceptor. Lipozyme TL IM in toluene was found to be the most suitable combination for the deacylation of compounds **8a–8c**.

In a typical reaction, a solution of 4-*C*-acyloxymethyl-3,5-di-*O*-acyl-1,2-*O*-(1-methylethylidene)-*β*-L-*threo*-pentofuranose **8a–8c** in toluene containing a small amount of *n*-butanol was incubated with Lipozyme TL IM in an incubator shaker at 40–42°C. On completion of the reaction, as indicated by TLC examination, enzyme was filtered off and the solvent removed under reduced pressure. The crude product thus obtained was passed through a small silica gel column to afford the pure deacylated compounds **9a–9c**, with lower R_f value than the corresponding starting compounds **8a–8c** in 88–98% yields (Scheme 2).[5]

The structures of enzymatically deacylated compounds **9a–9c** were established as 3,5-di-*O*-acyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-*α*-D-*xylo*-pentofuranoses on the basis of their IR, ^{1}H NMR, ^{13}C NMR, HRMS and ^{1}H nOe experiments, and comparison of their ^{1}H NMR spectrum with those of the starting triacylates **8a–8c**. The structure of one of the deacylated compound, viz., **9a** was further confirmed as 3,5-di-*O*-acetyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-*α*-D-*xylo*-pentofuranose by chemical transformation as shown in Scheme 3. The formation of the bicyclic



SCHEME 3

compounds **13** and **14** finally confirmed that the hydroxyl group in compound **9a**, and so as in **9b** and **9c** is at the C-1′ position (Scheme 3).

CONCLUSION

Highly efficient and convenient enzymatic method discovered for the discrimination between two primary hydroxyl groups of sugar and nucleoside precursors, herein may find applications in "green" synthesis[7] of bicyclonucleosides, important precursors for the preparation of antisense or antigene oligonucleotides.

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