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Biocatalytic Deacylation Studies on Tetra-*O*-acyl-β-D-*xylo*furanosyl Nucleosides: Synthesis of *xylo*-LNA Monomers

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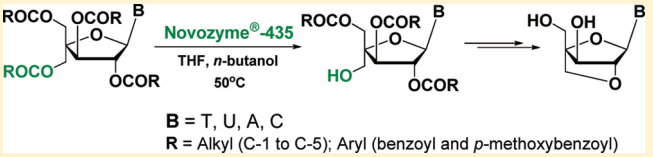
Supporting Information

ABSTRACT: A Novozyme-435 catalytic methodology has been developed for selective deacylation of one of the acyloxy functions involving a primary −OH group over the other acyloxy functions involving primary and secondary −OH groups in 4′-*C*-acyloxymethyl-2′,3′,5′-tri-*O*-acyl-β-D-*xylo*furanosyl nucleosides. Optimization of the biocatalytic reaction revealed that tetra-*O*-butanoyl-β-D-*xylo*furanosyl nucleosides are the best substrates for the enzyme. The possibility of acyl migration during enzymatic deacylation reactions has been ruled out by carrying out biocatalytic deacylation reactions on mixed esters of 4′-*C*-hydroxymethyl-2′,3′,5′-tri-*O*-acetyl-β-D-*xylo*furanosyl nucleosides. The developed methodology has been used for the efficient synthesis of *xylo*-LNA monomers T, U, A, and C in good yields.



Recently, research has been focused on restricting the conformation of the furanose ring either in C-2′ endo or in C-3′ endo conformations, primarily to develop antisense oligonucleotides with striking target affinity and biological stability. This has resulted in the synthesis of a large number of chemically modified nucleotides/oligonucleotides, among which the locked nucleic acid (LNA, I) has attracted extensive attention.[1] In this series fully modified oligonucleotides α-L-*ribo*-LNA (II) and β-D-*xylo*-LNA (III) hybridize to both DNA and RNA with high affinity (Figure 1). The experiments toward slightly mismatched RNA targets showed that hybridization of β-D-*xylo*-configured LNA toward RNA is selective.[2]

One of the major problems in the synthesis of LNA and its diastereomeric analogues is the discrimination between two primary hydroxyl groups, as in 4′-*C*-acetoxymethyl-2′,3′,5′-tri-*O*-acetyl-β-D-*xylo*furanosyl nucleosides (**4**), which is the key precursor for the synthesis of *xylo*-LNA.[3] Herein we report an environmentally friendly biocatalytic[4−6] one-pot synthesis of 4′-*C*-hydroxymethyl-2′,3′,5′-tri-*O*-acyl-β-D-*xylo*furanosyl nucleosides from their corresponding peracylated derivatives and conversion of the synthesized monohydroxy nucleosides into *xylo*-LNA in an overall yields of 65−79%.

The trihydroxy furanoside **1** was synthesized from D-glucose following the procedure described by Moffatt et al.[7] Peracetylation of compound **1** followed by acetolysis of the resulted triacetate **2**[4c] afforded pentaacetoxy furanoside **3** in an overall yields of 89%. The Vorbrüggen coupling[8] of **3** with thymine, uracil, adenine, and cytosine afforded the corresponding peracetylated nucleosides **4a**−**d** in 65−75% yields (Scheme 1).

In a model biocatalytic reaction, five different lipases,[9] viz. Novozyme-435 or CAL-B, Lipozyme TL IM, *Amano* PS, CRL, and PPL, were screened for the selective deacetylation of nucleoside **4a** in five different organic solvents, i.e. THF, toluene, DIPE, acetonitrile, and acetone, using *n*-butanol as the acetyl acceptor at

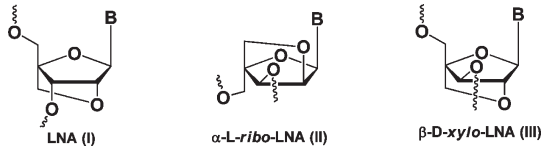
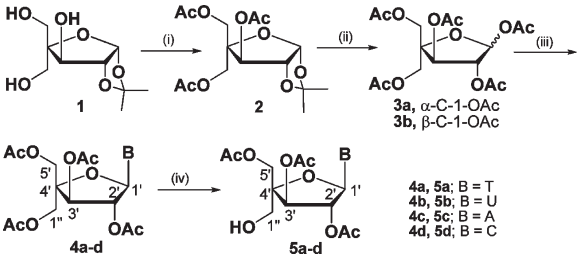


Figure 1. Structures of LNA, α-L-*ribo*-LNA, and β-D-*xylo*-LNA.

Scheme 1. Synthesis and Biocatalytic Deacetylation Studies on Tetra-O-acetylated Nucleosides 4a−d[a]



[a] Reaction conditions: (i) Ac_2O, DMAP (cat.), CH_2Cl_2, 25 °C; (ii) Ac_2O, AcOH, H_2SO_4 (100/10/0.1), 0 °C; (iii) for T, U, and C *N*,*O*-bis(trimethylsilyl)acetamide, trimethylsilyltrifluoromethane sulfonate in acetonitrile, 70−80 °C, and for A $SnCl_4$ in acetonitrile, 25 °C; (iv) Novozyme-435, THF, *n*-butanol, 50 °C.

40, 50, and 60 °C and at 200 rpm in an incubator shaker. Among the different lipases, Novozyme-435 and Lipozyme TL IM (50% w/w of the substrate) in THF and toluene at 50 °C showed selectivity

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