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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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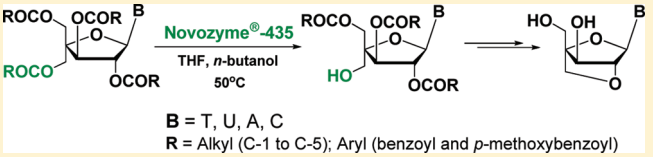
Supporting Information

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

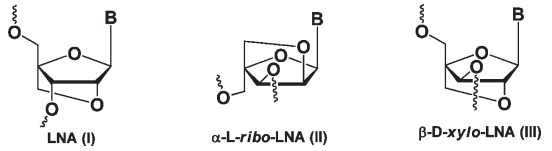
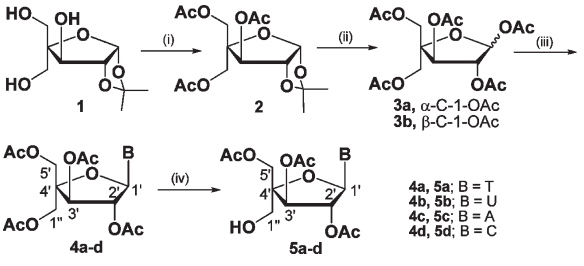


Figure 1. Structures of LNA, α-L-*ribo*-LNA, and β-D-*xylo*-LNA.

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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Table 1. Novozyme-435 Catalyzed Deacylation of 4a−d, 7a−f, and 9a−d in THF and *n*-Butanol[a]

entry	substrate	reacn time (h)	product	yield (%)[b]
1	**4a**	20	**5a**	87
2	**4b**	11	**5b**	92
3	**4c**	14	**5c**	82
4	**4d**	16	**5d**	93
5	**7a**	12	**8a**	85
6	**7b**	8	**8b**	95
7	**7c**	20	**8c**	81
8	**7d**	22	**8d**	73
9	**7e**	48	nr[c]	
10	**7f**	48	nr	
11	**9a**	10	**5a**	85
12	**9b**	8	**5a**	90
13	**9c**	15	**5a**	83
14	**9d**	48	nr	

[a] All these reactions, when performed under identical conditions but without adding Novozyme-435, did not yield any product. [b] Isolated yield. [c] nr = no reaction.

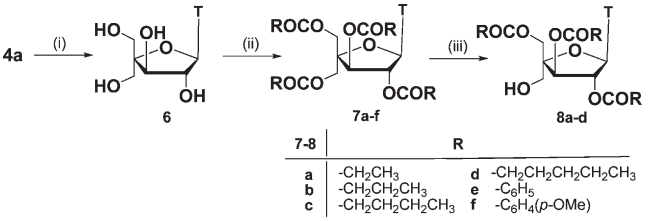
and efficiently deacetylated the C1″-acetoxy group over other acetoxyl groups in compound **4a** to afford the monodeacetylated nucleoside **5a** in 87% yield (Scheme 1). The rate of the Novozyme-435 catalyzed deacetylation reaction in THF was much faster than that of Lipozyme TL IM in THF, whereas in toluene both reactions were relatively slower as compared to the reactions in THF. The rate of Novozyme-435 catalyzed reactions in other solvents was too slow to be of any practical application. Other lipases, i.e. *Amano* PS, CRL, and PPL, did not show any selectivity during deacetylation reactions studied on **4a** in any of the organic solvents. The progress of the Novozyme-435 and Lipozyme TL IM catalyzed deacetylation reactions in THF on nucleoside **4a** was monitored by HPLC. It was observed that the rate of acceleration of the conversion of the reactant **4a** to the product **5a** gradually decreases with time.

Other nucleosides **4b**−**d** were also subjected to the Novozyme-435 catalyzed deacetylation reaction in THF using a small amount of *n*-butanol to obtain the expected monodeacetylated compounds **5b**−**d** in 92, 82, and 93% yields, respectively (Scheme 1, Table 1). The structures of all tetraacetylated nucleosides **4a**−**d** and monodeacetylated nucleosides **5a**−**d** were unambiguously established on the basis of their IR and ^{1}H, ^{13}C, COSY, and HSQC NMR spectra and HRMS data analysis.

To understand the effect of different acyl groups on the catalytic efficiency of Novozyme-435, six different acylated nucleosides **7a**−**f** were synthesized by acylation of 4′-*C*-hydroxymethyl-β-D-*xylo*furanosylthymine (**6**) with propanoic, butanoic, pentanoic, hexanoic, benzoic, and *p*-methoxybenzoic anhydrides.[10] The nucleoside **6** in turn was obtained by deacetylation of compound **4a** using ammoniacal methanol.[11] The acylated nucleosides **7a**−**f** were incubated with Novozyme-435 in THF in the presence of *n*-butanol to obtain the monodeacylated compounds **8a**−**d**[12] (Scheme 2, Table 1).

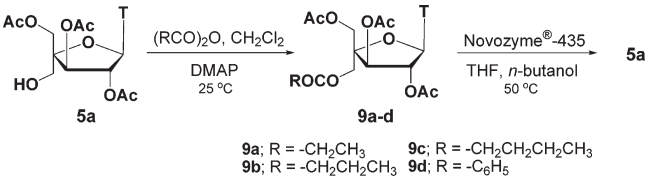
The results compiled in Table 1 reveal that the rate of the deacylation reaction decreases on an increase of acyl chain length from four to six carbons as in entries 6−8, whereas the rate of the reaction increases with the increase of acyl chain length from two to four carbons as in entries 1, 5, and 6. Thus, 4′-*C*-butanoyloxymethyl-2′,3′,5′-tri-*O*-butanoyl-β-D-*xylo*furanosylnucleoside (**7b**)

Scheme 2. Novozyme-435 Catalyzed Deacylation Studies on Peracylated Nucleosides 7a−f: Effect of Acyl Chain Length [a]



[a] Reaction conditions: (i) saturated methanolic ammonia, 25 °C; (ii) $(RCO)_2O$, CH_2Cl_2, pyridine, 25 °C; (iii) Novozyme-435, THF, *n*-butanol, 50 °C.

Scheme 3. Novozyme-435 Catalyzed Deacylation Reaction on Mixed Esters 9a−d



was found to be the best substrate for selective deacylation reactions catalyzed by Novozyme-435. The enhancement in the rate of biocatalytic debutanoylation reaction of **7b** was found to be in accordance with the "Kazlauskas rule".[13] Nucleosides **7e**,**f** containing benzoyl and *p*-methoxybenzoyl instead of acyl groups, were not found to be substrates for Novozyme-435.

The problem of acyl migration has been encountered in 1,2-/1,3-diol systems as well as in partially acylated nucleosides.[14,15] Thus, there exists a possibility of formation of **5a**−**d** and **8a**−**d** via initial C5′ or C3′ deacetylation catalyzed by Novozyme-435 and subsequent acyl migration from C1″- to C5′/C3′-positions.

To ascertain the route of formation of compounds **5a**−**d** and **8a**−**d** during enzyme-catalyzed deacylation reactions on **4a**−**d** and **7a**−**d**, the mixed esters **9a**−**d** of monodeacetylated nucleosides **5a** were synthesized in 89−94% yields using propanoic, butanoic, pentanoic, and benzoic anhydrides in CH_2Cl_2 in the presence of a catalytic amount of DMAP (Scheme 3).[16] The mixed tetraesters **9a**−**d** were then subjected to Novozyme-435 catalyzed deacylation reactions in THF, using *n*-butanol as an acyl acceptor and the previously described reaction conditions. It was observed that the biocatalytic deacylation reaction of the three mixed esters **9a**−**c** resulted in the formation of identical products, i.e. nucleoside **5a** in 85, 90, and 83% yields, respectively (Scheme 3, Table 1). The mixed ester **9d**, containing the benzoyl group, was not found to be a substrate for Novozyme-435. The results obtained from the deacylation studies on mixed esters clearly indicated that the formation of monodeacylated product during biocatalytic deacylation reactions is solely due to the removal of the acyl group from the C4′-acyloxymethyl function of the starting peracylated compounds.

The synthesis of *xylo*-LNA monomers T, U, and A **11a**−**c** from monodeacetylated nucleosides **5a**−**c** was successfully achieved by tosylation of the C-1″ hydroxyl group to produce **10a**−**c** followed by deacetylation and concomitant cyclization of the resultant hydroxyl nucleosides with a large excess of K_2CO_3 in methanol/water (3/1), in 91−96% yields (Scheme 4).

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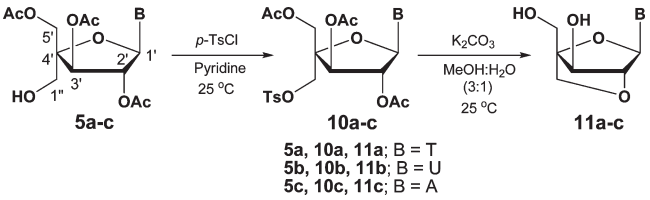
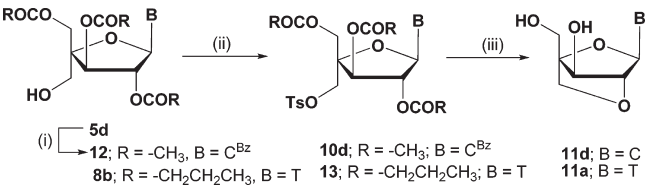
Scheme 4. Synthesis of *xylo*-LNA Monomers 11a−c from Novozyme-435 Catalyzed Monodeacetylated Nucleosides 5a−c



Scheme 5. Synthesis of *xylo*-LNA-C from Compound 5d and of *xylo*-LNA-T from Compound 8b[a]



[a] Reaction conditions: (i) Bz_2O, DMF, 25 °C; (ii) *p*-TsCl, pyridine, 25 °C; (iii) K_2CO_3, MeOH/H_2O (3/1), 25 °C.

During the synthesis of 1-(2′-*O*,4′-*C*-methylene-*xylo*furanosyl)cytosine (**11d**; *xylo*-LNA-C), tosylation of monodeacetylated nucleoside **5d** resulted in the formation of a mixture of products, perhaps due to involvement of the exocyclic amino group. To overcome this problem, the cytosine NH_2 group in compound **5d** was selectively benzoylated using benzoic anhydride in DMF to afford nucleoside **12**, which on tosylation (to afford compound **10d**) and a subsequent one-pot deacetylation−cyclization reaction afforded the *xylo*-LNA-C **11d** in 86% yield (Scheme 5).

The debutanoylated nucleoside **8b** obtained from Novozyme-435 catalyzed monodebutanoylation of nucleoside **7b** was also converted into *xylo*-LNA thymine monomer **11a**, in an overall yield of 83% (Scheme 5). The structures of compounds **6**, **7a−f**, **8a−d**, **9a−d**, **10a−d**, **11a−d**, **12**, and **13** have been unambiguously established on the basis of their IR and ^1H and ^{13}C NMR spectra and HRMS data analysis. The structure of the known compound **11a**[3a] was further confirmed on the basis of comparison of its spectral data with those reported in the literature, whereas *xylo*-LNA monomers U, A, and C have been synthesized and reported in the literature for the first time.

In summary, highly efficient Novozyme-435 catalyzed biocatalytic routes for the selective deacylation of the C-1″ acyloxy function 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 have been developed for the first time. The biocatalytic methodology has been used for the efficient synthesis of *xylo*-LNA monomers T, U, A, and C in excellent yields. The developed environmentally friendly biocatalytic methodology may find application for the synthesis of *xylo*-LNA/LNA monomers to be used for the synthesis of modified oligonucleotides for therapeutic and diagnostic applications.

EXPERIMENTAL SECTION

General Procedure for the Synthesis of 1-(4′-*C*-Acetoxymethyl-2′,3′,5′-tri-*O*-acetyl-β-D-*xylo*furanosyl) Nucleosides 4a, b,d. To stirred solutions of penta-O-acetylated sugar derivatives **3a,b** (2.5 g, 6.40 mmol) and thymine (1.21 g, 9.61 mmol)/uracil (1.44 g, 12.82 mmol) or cytosine (1.07 g, 9.62 mmol) in anhydrous acetonitrile (60 mL), was added *N,O*-bis(trimethylsilyl)acetamide (6.4−11.9 mL) dropwise. The reaction mixture was stirred at reflux for 1 h and then cooled to 0 °C. To the cooled reaction mixture was added trimethylsilyltrifluoromethanesulfonate (2.0 mL, 10.90 mmol) dropwise with stirring, and the solution was heated at 70−80 °C for 4−6 h. The reaction was quenched with a cold saturated aqueous solution of sodium hydrogen carbonate (100 mL), and extraction was performed with dichloromethane (3 × 100 mL). The combined organic phase was washed with saturated solutions of $NaHCO_3$ (2 × 100 mL) and brine (2 × 100 mL) and was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the residue thus obtained was purified by silica gel column chromatography using MeOH/$CHCl_3$ as eluent to afford nucleosides **4a,b,d** in 65−72% yields.

1-(4′-C-Acetoxymethyl-2′,3′,5′-tri-O-acetyl-β-D-xylofuranosyl)thymine (***4a***). Obtained as an off-white solid (2.04 g, 70%). R_f = 0.6 (5% MeOH/$CHCl_3$). Mp: 130−132 °C. $[\alpha]_D^{23}$ = −51.6° (*c* 0.1, MeOH). ^1H NMR ($CDCl_3$, 400 MHz): δ 1.95 (3H, s), 2.08, 2.09, 2.12, 2.14 (12H, 4s), 4.00 (1H, d, *J* = 11.8 Hz), 4.25 (2H, d, *J* = 6.6 Hz), 4.52 (1H, d, *J* = 12.4 Hz), 5.39 (1H, t, *J* = 6.6 Hz), 5.53 (1H, d, *J* = 5.9 Hz), 6.18 (1H, d, *J* = 6.6 Hz), 7.37 (1H, s), 8.71 (1H, br s). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 14.4, 22.4, 22.5, 22.8, 64.0, 65.8, 76.7, 79.8, 84.6, 86.6, 114.1, 136.4, 151.9, 164.8, 171.1, 171.4, 171.6, 172.1. IR (thin film) ν_{max}: 3207, 2918, 2849, 1750, 1698, 1459, 1374, 1227, 1107, 1053, 916 cm^{-1}. HR-ESI-TOF-MS: *m/z* 457.1490 ($[M + H]^+$), calcd for $[C_{19}H_{24}N_2O_{11} + H]^+$ 457.1458.

1-(4′-C-Acetoxymethyl-2′,3′,5′-tri-O-acetyl-β-D-xylofuranosyl)uracil (***4b***). Obtained as a white sticky solid (1.84 g, 65%). R_f = 0.6 (5% MeOH/$CHCl_3$). $[\alpha]_D^{22}$ = −4.3° (*c* 0.1, MeOH). ^1H NMR ($CDCl_3$, 300 MHz): δ 2.10, 2.17, 2.18, 2.21 (12H, 4s), 4.11 (1H, d, *J* = 12.0 Hz), 4.31−4.36 (2H, m), 4.59 (1H, d, *J* = 12.0 Hz), 5.48 (1H, t, *J* = 6.0 Hz), 5.62 (1H, d, *J* = 5.8 Hz), 5.91 (1H, d, *J* = 8.1 Hz), 6.27 (1H, d, *J* = 6.2 Hz), 7.65 (1H, d, *J* = 8.1 Hz), 9.81 (1H, br s). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 19.2, 19.3, 19.5, 19.6, 61.0, 62.6, 73.9, 76.7, 82.1, 83.9, 102.5, 137.4, 149.2, 161.7, 168.2, 168.5, 168.7, 168.9. IR (thin film): ν_{max} 3206, 2918, 2849, 1750, 1698, 1459, 1374, 1227, 1107, 1053, 916 cm^{-1}. HR-ESI-TOF-MS: *m/z* 465.1116 ($[M + Na]^+$), calcd for $[C_{18}H_{22}N_2O_{11} + Na]^+$ 465.1116.

1-(4′-C-Acetoxymethyl-2′,3′,5′-tri-O-acetyl-β-D-xylofuranosyl)cytosine (***4d***). Obtained as a white solid (2.10 g, 72%). R_f = 0.4 (10% MeOH/$CHCl_3$). Mp: 142−144 °C. $[\alpha]_D^{22}$ = +13.2° (*c* 0.1, MeOH). ^1H NMR ($CDCl_3$, 300 MHz): δ 2.08, 2.10, 2.13, 2.14 (12H, 4s), 4.08 (1H, d, *J* = 11.9 Hz), 4.30 (2H, dd, *J* = 11.7 and 9.4 Hz), 4.50 (1H, d, *J* = 11.9 Hz), 5.43 (1H, t, *J* = 5.4 Hz), 5.50 (1H, d, *J* = 5.2 Hz), 5.70 (1H, br s), 5.89 (1H, d, *J* = 7.4 Hz), 6.26 (1H, d, *J* = 5.6 Hz), 7.59 (1H, d, *J* = 7.5 Hz), 7.80 (1H, br s). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 20.4, 20.5, 20.6, 20.7, 62.1, 63.5, 75.3, 78.5, 83.4, 86.4, 95.7, 139.9, 155.5, 165.6, 169.2, 169.6, 169.8, 170.1. IR (thin film): ν_{max} 3357, 3188, 1751, 1647, 1601, 1476, 1425, 1370, 1227, 1049 cm^{-1}. HR-ESI-TOF-MS: *m/z* 442.1490 ($[M + H]^+$), calcd for $[C_{18}H_{23}N_3O_{10}+H]^+$ 442.1462.

Synthesis of 9-(4′-*C*-Acetoxymethyl-2′,3′,5′-tri-*O*-acetyl-β-D-*xylo*furanosyl)adenine (4c). To stirred solutions of penta-*O*-acetylated sugar derivatives **3a,b** (2.5 g, 6.40 mmol) and adenine (0.87 g, 6.40 mmol) in anhydrous acetonitrile (60 mL) was added $SnCl_4$ (3.2 mL, 27.2 mmol) dropwise. The reaction mixture was stirred at 25 °C for 4 h. The reaction was quenched with a cold saturated aqueous solution of sodium hydrogen carbonate (100 mL), the reaction mixture was passed through a Celite pad, and extraction was performed with ethyl acetate (3 × 100 mL). The combined organic phase was washed with saturated aqueous solutions of $NaHCO_3$ (2 × 100 mL) and brine (2 × 100 mL) and was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography using MeOH/$CHCl_3$ as eluent to give nucleoside **4c** as a white solid (2.23 g, 75%). R_f = 0.5 (5% MeOH/$CHCl_3$). Mp: 106−108 °C. $[\alpha]_D^{23}$ = −37.1° (*c* 0.1, MeOH). ^1H NMR ($CDCl_3$, 300 MHz): δ 2.08, 2.11, 2.12, 2.13 (12H, 4s), 4.19 (1H, d,

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J = 12.0 Hz), 4.33 (2H, s), 4.56 (1H, d, J = 12.0 Hz), 5.65 (1H, d, J = 5.6 Hz), 5.91 (2H, br s), 6.05 (1H, t, J = 5.5 Hz), 6.26 (1H, d, J = 5.8 Hz), 8.10 (1H, s), 8.35 (1H, s). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 20.6, 20.7, 20.8, 62.5, 63.8, 76.6, 78.3, 83.8, 84.9, 119.6, 138.4, 149.9, 153.4, 155.6, 169.5, 169.9, 170.2. IR (KBr) ν_{max}: 3300, 2924, 1747, 1640, 1377, 1213, 1041 cm^{-1}. HR-ESI-TOF-MS: m/z 466.1605 ([M + H]$^+$), calcd for [$C_{19}H_{23}N_5O_9$ + H]$^+$ 466.1574.

General Procedure for Novozyme-435 Catalyzed Deacetylation Reaction on Peracetylated Nucleosides 4a−d: Preparation of Monodeacetylated Nucleosides 5a−d. To solutions of tetraacetylated nucleosides **4a**−**d** (1.09 mmol) in dry THF (30 mL) was added *n*-butanol (1.30 mmol), followed by the addition of Novozyme-435 (50% w/w of the peracetylated nucleosides **4a**−**d**). The reaction mixture was stirred at 50 °C in an incubator shaker, and the progress of the reaction was monitored periodically by TLC. On completion, the reaction was quenched by filtering off the Novozyme-435, the solvent was removed under reduced pressure and the residue thus obtained was purified by silica gel column chromatography using MeOH/$CHCl_3$ to afford the monodeacetylated nucleosides **5a**−**d** in 82−93% yields.

*1-(4′-C-Hydroxymethyl-2′,3′,5′-tri-O-acetyl-β-D-xylofuranosyl)thymine (**5a**).* Obtained as a white sticky solid (0.35 g, 87%). R_f = 0.3 (5% MeOH/$CHCl_3$). $[\alpha]_D^{23}$ = −49.5° (c 0.1, MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.95 (3H, s), 2.08, 2.10, 2.18 (9H, 3s), 3.83 (1H, s), 4.00 (1H, d, J = 12.1 Hz), 4.54 (1H, d, J = 12.1 Hz), 5.43 (1H, t, J = 6.9 Hz), 5.69 (1H, d, J = 5.2 Hz), 6.29 (1H, d, J = 7.0 Hz), 7.48 (1H, s), 9.70 (1H, br s). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 12.7, 20.6, 21.0, 62.7, 63.4, 74.6, 77.2, 83.7, 84.8, 112.4, 134.5, 151.0, 163.6, 169.5, 171.0. IR (thin film): ν_{max} 3447, 2918, 2849, 1749, 1696, 1461, 1376, 1230, 1052 cm^{-1}. HR-ESI-TOF-MS: m/z 437.1169 ([M + Na]$^+$), calcd for [$C_{17}H_{22}N_2O_{10}$ + Na]$^+$ 437.1172.

*1-(4′-C-Hydroxymethyl-2′,3′,5′-tri-O-acetyl-β-D-xylofuranosyl)uracil (**5b**).* Obtained as a white solid (0.40 g, 92%). R_f = 0.35 (5% MeOH/$CHCl_3$). Mp: 116−118 °C. $[\alpha]_D^{23}$ = +8.4° (c 0.1, MeOH). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.09, 2.12, 2.16 (9H, 3s), 3.70 (1H, br s), 3.74 (2H, br s), 3.98 (1H, d, J = 12.2 Hz), 4.51 (1H, d, J = 12.2 Hz), 5.44 (1H, s), 5.68 (1H, d, J = 6.8 Hz), 5.84 (1H, d, J = 8.1 Hz), 6.24 (1H, d, J = 6.9 Hz), 7.66 (1H, d, J = 8.1 Hz), 9.65 (1H, br s). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 19.4, 19.7, 61.4, 62.1, 73.3, 76.2, 82.9, 83.9, 102.7, 137.5, 149.6, 161.8, 168.7, 169.0. IR (thin film): ν_{max} 3447, 2918, 2849, 1749, 1696, 1461, 1376, 1230, 1052, 913 cm^{-1}. HR-ESI-TOF-MS: m/z 423.0999 ([M + Na]$^+$), calcd for [$C_{16}H_{20}N_2O_{10}$ + Na]$^+$ 423.1010.

*9-(4′-C-Hydroxymethyl-2′,3′,5′-tri-O-acetyl-β-D-xylofuranosyl)adenine (**5c**).* Obtained as a white solid (0.38 g, 82%). R_f = 0.3 (5% MeOH/$CHCl_3$). Mp: 130−132 °C. $[\alpha]_D^{24}$ = −62.3° (c 0.1, MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.01, 2.10, 2.14 (9H, 3s), 3.80 (2H, br s), 4.12 (1H, d, J = 12.0 Hz), 4.56 (1H, d, J = 12.0 Hz), 5.87 (1H, d, J = 6.7 Hz), 6.14 (1H, t, J = 6.6 Hz), 6.28 (1H, d, J = 6.8 Hz), 6.38 (2H, br s), 8.13 (1H, s), 8.27 (1H, s). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 19.3, 19.4, 19.7, 61.7, 62.0, 74.1, 77.2, 83.8, 85.4, 118.5, 138.6, 148.9, 152.7, 155.3, 169.8, 170.0, 170.2. IR (thin film): ν_{max} 3352, 2926, 1750, 1646, 1602, 1478, 1429, 1371, 1228, 1051, 908 cm^{-1}. HR-ESI-TOF-MS: m/z 446.1279 ([M + Na]$^+$), calcd for [$C_{17}H_{21}N_5O_8$ + Na]$^+$ 446.1288.

*1-(4′-C-Hydroxymethyl-2′,3′,5′-tri-O-acetyl-β-D-xylofuranosyl)cytosine (**5d**).* Obtained as a white solid (0.40 g, 93%). R_f = 0.4 (10% MeOH/$CHCl_3$). Mp: 155−157 °C. $[\alpha]_D^{22}$ = −4.6° (c 0.1, MeOH). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.01, 2.05, 2.10 (9H, 3s), 3.62−3.74 (3H, br s), 4.02 (1H, d, J = 12.0 Hz), 4.47 (1H, d, J = 12.0 Hz), 5.45 (1H, t, J = 6.7 Hz), 5.64 (1H, d, J = 6.5 Hz), 5.99 (1H, d, J = 7.4 Hz), 6.30 (1H, d, J = 6.8 Hz), 6.80 (1H, br s), 7.63 (1H, d, J = 7.5 Hz), 8.20 (1H, br s). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 20.97, 21.0, 21.3, 63.1, 63.5, 75.3, 77.0, 78.3, 85.2, 97.1, 140.3, 156.5, 166.1, 170.3, 170.5, 171.1. IR (thin film): ν_{max} 3451, 3251, 1752, 1698, 1638, 1575, 1373, 1283, 1225, 1087, 687 cm^{-1}. HR-ESI-TOF-MS: m/z 422.1198 ([M + Na]$^+$), calcd for [$C_{16}H_{21}N_3O_9$ + Na]$^+$ 422.1175.

Synthesis of 1-(4′-*C*-hydroxymethyl-β-D-*xylo*furanosyl)thymine (6).[11] Obtained as a white solid (1.70 g, 90%). R_f = 0.35 (25% MeOH/$CHCl_3$). Mp: 132−134 °C. $[\alpha]_D^{25}$ = −57.2° (c 0.1, H_2O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.78 (3H, s), 3.17 (1H, d, J = 3.5 Hz), 3.41−3.45 (1H, m), 3.60 (1H, d, J = 3.3 Hz), 4.06−4.13 (3H, m), 4.61 (1H, br s), 4.84 (1H, br s), 5.25 (1H, br s), 5.48 (1H, d, J = 4.7 Hz), 5.77 (1H, d, J = 4.1 Hz), 7.88 (1H, s), 11.17 (1H, br s). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 20.3, 69.8, 70.7, 83.4, 86.6, 93.5, 94.1, 117.5, 144.8, 159.0, 171.8. IR (KBr) ν_{max}: 3401, 2929, 1694, 1476, 1265, 1150, 917 cm^{-1}. HR-ESI-TOF-MS: m/z 311.0822 ([M + Na]$^+$), calcd for [$C_{11}H_{16}N_2O_7$ + Na]$^+$ 311.0850.

General Procedure for the Synthesis of 1-(4′-*C*-acyloxymethyl-2′,3′,5′-tri-*O*-acyl-β-D-*xylo*furanosyl)thymines 7a−f.[10] The complete physical and spectral data of acylates **7a**−**f** are given below.

*1-(4′-C-Propanoyloxymethyl-2′,3′,5′-tri-O-propanoyl-β-D-xylofuranosyl)thymine (**7a**).* Obtained as a colorless viscous oil (0.8 g, 90%). R_f = 0.6 (2% MeOH/$CHCl_3$). $[\alpha]_D^{24}$ = −28.5° (c 0.1, MeOH). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.09−1.25 (12H, m), 1.96 (3H, s), 2.33−2.47 (8H, m), 4.02 (1H, d, J = 12.1 Hz), 4.29 (2H, d, J = 3.6 Hz), 4.57 (1H, d, J = 12.1 Hz), 5.45 (1H, t, J = 6.3 Hz), 5.59 (1H, d, J = 6.1 Hz), 6.24 (1H, d, J = 6.5 Hz), 7.43 (1H, s), 9.44 (1H, br s). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 7.8, 7.9, 8.08, 8.15, 11.8, 26.26, 26.30, 26.5, 26.7, 61.5, 63.2, 74.2, 76.6, 82.1, 83.5, 111.3, 133.5, 149.6, 162.7, 172.0, 172.4, 172.6. IR (thin film): ν_{max} 3206, 1747, 1696, 1465, 1272, 1152, 1040, 978, 888 cm^{-1}. HR-ESI-TOF-MS: m/z 535.1902 ([M + Na]$^+$), calcd for [$C_{23}H_{32}N_2O_{11}$ + Na]$^+$ 535.1898.

*1-(4′-C-Butanoyloxymethyl-2′,3′,5′-tri-O-butanoyl-β-D-xylofuranosyl)thymine (**7b**).* Obtained as a colorless viscous oil (0.9 g, 92%). R_f = 0.5 (30% EtOAc in petroleum ether). $[\alpha]_D^{24}$ = −48.4° (c 0.1, $CHCl_3$). HR-ESI-TOF-MS: m/z 591.2548 ([M + Na]$^+$), calcd for [$C_{27}H_{40}N_2O_{11}$ + Na]$^+$ 591.2524.

*1-(4′-C-Pentanoyloxymethyl-2′,3′,5′-tri-O-pentanoyl-β-D-xylofuranosyl)thymine (**7c**).* Obtained as a colorless viscous oil (0.97 g, 90%). R_f = 0.6 (20% EtOAc in petroleum ether). $[\alpha]_D^{24}$ = +14.5° (c 0.1, $CHCl_3$). HR-ESI-TOF-MS: m/z 647.3158 ([M + Na]$^+$), calcd for [$C_{31}H_{48}N_2O_{11}$ + Na]$^+$ 647.3150.

*1-(4′-C-Hexanoyloxymethyl-2′,3′,5′-tri-O-hexanoyl-β-D-xylofuranosyl)thymine (**7d**).* Obtained as a colorless viscous oil (1.11 g, 94%). R_f = 0.5 (10% EtOAc in petroleum ether). $[\alpha]_D^{24}$ = −15.60° (c 0.1, $CHCl_3$). HR-ESI-TOF-MS: m/z 703.3778 ([M + Na]$^+$), calcd for [$C_{35}H_{56}N_2O_{11}$ + Na]$^+$ 703.3776.

*1-(4′-C-Benzoyloxymethyl-2′,3′,5′-tri-O-benzoyl-β-D-xylofuranosyl)thymine (**7e**).* Obtained as an off-white solid (1.13 g, 93%). R_f = 0.7 (20% EtOAc in petroleum ether). Mp: 108−110 °C. $[\alpha]_D^{24}$ = +6.1° (c 0.1, $CHCl_3$). HR-ESI-TOF-MS: m/z 727.1893 ([M + Na]$^+$), calcd for [$C_{39}H_{32}N_2O_{11}$ + Na]$^+$ 727.1898.

*1-(4′-C-p-Anisyloxymethyl-2′,3′,5′-tri-O-p-anisyl-β-D-xylofuranosyl)thymine (**7f**).* Obtained as an off-white solid (1.34 g, 94%). R_f = 0.7 (20% EtOAc in petroleum ether). Mp: 98−100 °C. $[\alpha]_D^{24}$ = −16.9° (c 0.1, $CHCl_3$). HR-ESI-TOF-MS: m/z 847.2315 ([M + Na]$^+$), calcd for [$C_{43}H_{40}N_2O_{15}$ + Na]$^+$ 847.2321.

General Procedure for Novozyme-435 Catalyzed Deacylation Reaction on Acylated Nucleosides 7a−f: Preparation of Monodeacylated Nucleosides 8a−d.[12] The complete physical and spectral data of monodeacylated nucleosides **8a**−**d** are given below.

*1-(4′-C-Hydroxymethyl-2′,3′,5′-tri-O-propanoyl-β-D-xylofuranosyl)thymine (**8a**).* Obtained as a white sticky solid (0.42 g, 85%). R_f = 0.7 (5% MeOH/$CHCl_3$). $[\alpha]_D^{23}$ = −32.3° (c 0.1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.07−1.24 (9H, m), 1.95 (3H, s), 2.34−2.45 (6H, m), 3.62 (1H, br s), 3.74 (2H, br s), 3.97 (1H, d, J = 12.1 Hz), 4.53 (1H, d, J = 12.1 Hz), 5.49 (1H, t, J = 6.8 Hz), 5.69 (1H, d, J = 6.7 Hz), 6.31 (1H, d, J = 7.0 Hz), 7.50 (1H, s), 9.48 (1H, br s). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 8.6, 8.62, 8.9, 12.5, 27.0, 27.1, 27.5, 62.6, 63.3, 74.4, 77.0, 83.5, 84.7, 112.3, 134.4, 150.8, 163.4, 173.2, 173.6. IR (thin film): ν_{max} 3454, 2960, 1745, 1694, 1469, 1158, 1093, 1039, 930 cm^{-1}. HR-ESI-TOF-MS: m/z 479.1629 ([M + Na]$^+$), calcd for [$C_{20}H_{28}N_2O_{10}$ + Na]$^+$ 479.1636.

*1-(4′-C-Hydroxymethyl-2′,3′,5′-tri-O-butanoyl-β-D-xylofuranosyl)thymine (**8b**).* Obtained as a colorless viscous oil (0.52 g, 95%). R_f = 0.4

(50% EtOAc in petroleum ether). $[\alpha]_D^{24} = -38.9°$ (c 0.1, $CHCl_3$). HR-ESI-TOF-MS: m/z 521.2097 ($[M + Na]^+$), calcd for $[C_{23}H_{34}N_2O_{10} + Na]^+$ 521.2106.

*1-(4′-C-Hydroxymethyl-2′,3′,5′-tri-O-pentanoyl-β-D-xylofuranosyl) thymine (**8c**).* Obtained as a colorless viscous oil (0.48 g, 81%). $R_f = 0.5$ (35% EtOAc in petroleum ether). $[\alpha]_D^{25} = -68.3°$ (c 0.1, $CHCl_3$). HR-ESI-TOF-MS: m/z 563.2587 ($[M + Na]^+$), calcd for $[C_{26}H_{40}N_2O_{10} + Na]^+$ 563.2575.

*1-(4′-C-Hydroxymethyl-2′,3′,5′-tri-O-hexanoyl-β-D-xylofuranosyl) thymine (**8d**).* Obtained as a colorless viscous oil (0.46 g, 73%). $R_f = 0.4$ (20% EtOAc in petroleum ether). $[\alpha]_D^{25} = -39.9°$ (c 0.1, $CHCl_3$). HR-ESI-TOF-MS: m/z 605.3051 ($[M + Na]^+$), calcd for $[C_{29}H_{46}N_2O_{10} + Na]^+$ 605.3045.

General Procedure for the Synthesis of Mixed Esters Tetra-*O*-acylated Nucleosides 9a−d.[16] The complete physical and spectral data of the model mixed ester **9a** are given below. The structures of the three other mixed esters, 1-(4′-*C*-butanoyloxymethyl-2′,3′,5′-tri-*O*-acetyl-α-L-*arabino*furanosyl)thymine (**9b**), 1-(4′-*C*-pentanoyloxymethyl-2′,3′,5′-tri-*O*-acetyl-α-L-*arabino*furanosyl)thymine (**9c**), and 1-(4′-*C*-benzoyloxymethyl-2′,3′,5′-tri-*O*-acetyl-α-L-*arabino*furanosyl)thymine (**9d**), were unambiguously established on the basis of their IR, ^1H and ^{13}C NMR, and HRMS data analysis.

*1-(4′-C-Propanoyloxymethyl-2′,3′,5′-tri-O-acetyl-α-L-arabinofuranosyl) thymine (**9a**).* Obtained as a colorless viscous oil (100 mg, 89%). $R_f = 0.6$ (5% MeOH/$CHCl_3$). $[\alpha]_D^{22} = -70.40°$ (c 0.1, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 1.19 (3H, t, J = 7.3 Hz), 1.98 (3H, s), 2.11, 2.17 (9H, 2s), 2.45 (2H, q, J = 7.3 Hz), 4.02 (1H, d, J = 11.8 Hz), 4.28 (2H, d, J = 8.1 Hz), 4.56 (1H, d, J = 11.7 Hz), 5.42 (1H, t, J = 6.6 Hz), 5.58 (1H, d, J = 6.6 Hz), 6.21 (1H, d, J = 6.6 Hz), 7.41 (1H, d, J = 1.5 Hz), 8.95 (1H, br s). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 10.8, 14.6, 22.36, 22.43, 22.7, 29.2, 64.3, 65.8, 77.0, 79.5, 84.8, 86.4, 114.1, 136.3, 152.3, 165.5, 171.3, 171.6, 171.9, 175.4. IR (thin film): ν_{max} 3216, 2984, 1748, 1464, 1372, 1225, 1057, 896, 756 cm^{-1}. HR-ESI-TOF-MS: m/z 493.1425 ($[M + Na]^+$), calcd for $[C_{20}H_{26}N_2O_{11} + Na]^+$ 493.1429.

General Procedure for Novozyme-435 Catalyzed Deacetylation Reaction on Mixed Esters 9a−d.[17]

General Procedure for the Synthesis of 10a−c.[18] The complete physical and spectral data of tosylated nucleosides **10a−c** are given below.

*1-(4′-C-p-Toluenesulfonylmethyl-2′,3′,5′-tri-O-acetyl-α-L-arabinofuranosyl)thymine (**10a**).* Obtained as a white solid (0.54 g, 95%). $R_f = 0.5$ (5% MeOH/$CHCl_3$). Mp: 155−156 °C. $[\alpha]_D^{23} = -40.4°$ (c 0.1, MeOH). ^1H NMR ($CDCl_3$, 300 MHz): δ 1.94 (3H, s), 2.08 and 2.09 (9H, 2s), 2.45 (3H, s), 4.01 (1H, d, J = 12.1 Hz), 4.21 (2H, d, J = 3.7 Hz), 4.49 (1H, d, J = 12.1 Hz), 5.40 (1H, t, J = 5.9 Hz), 5.48 (1H, d, J = 5.8 Hz), 6.05 (1H, d, J = 6.0 Hz), 7.29 (1H, s), 7.37 (2H, d, J = 8.0 Hz), 7.82 (2H, d, J = 8.0 Hz), 9.07 (1H, br s). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 13.0, 20.8, 20.9, 21.1, 22.0, 62.3, 69.0, 75.5, 76.99, 83.2, 85.8, 112.5, 128.5, 130.4, 132.4, 134.9, 145.8, 150.6, 163.6, 169.6, 169.9, 170.2. IR (thin film): ν_{max} 3207, 2923, 1754, 1693, 1462, 1376, 1216, 1175, 1050, 981 cm^{-1}. HR-ESI-TOF-MS: m/z 591.1261 ($[M + Na]^+$), calcd for $[C_{24}H_{28}N_2O_{12}S + Na]^+$ 591.1261.

*1-(4′-C-p-Toluenesulfonylmethyl-2′,3′,5′-tri-O-acetyl-α-L-arabinofuranosyl)uracil (**10b**).* Obtained as an off-white solid (0.51 g, 92%). R_f = 0.5 (5% MeOH/$CHCl_3$). Mp: 136−138 °C. $[\alpha]_D^{23} = -34.1°$ (c 0.1, MeOH). ^1H NMR ($CDCl_3$, 300 MHz): δ 2.04, 2.07, 2.08 (9H, 3s), 2.45 (3H, s), 4.02 (1H, d, J = 12.2 Hz), 4.21 (2H, d, J = 2.4 Hz), 4.45 (1H, d, J = 12.1 Hz), 5.37 (1H, t, J = 5.6 Hz), 5.47 (1H, d, J = 5.4 Hz), 5.79 (1H, d, J = 8.0 Hz), 6.02 (1H, d, J = 5.8 Hz), 7.37 (2H, d, J = 8.1 Hz), 7.47 (1H, d, J = 8.0 Hz), 7.82 (2H, d, J = 8.1 Hz), 9.05 (1H, br s). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 22.8, 23.1, 24.0, 64.1, 70.8, 77.3, 80.2, 85.6, 88.3, 105.9, 130.4, 132.4, 134.6, 141.1, 147.9, 152.3, 164.9, 171.6, 171.9, 172.1. IR (thin film): ν_{max} 3205, 2923, 2854, 1751, 1696, 1460, 1376, 1222, 1176, 1053, 983, 916 cm^{-1}. HR-ESI-TOF-MS: m/z 577.1078 ($[M + Na]^+$), calcd for $[C_{23}H_{26}N_2O_{12}S + Na]^+$ 577.1099.

*9-(4′-C-p-Toluenesulfonylmethyl-2′,3′,5′-tri-O-acetyl-α-L-arabinofuranosyl)adenine (**10c**).* Obtained as a light yellow solid (0.54 g, 93%). $R_f = 0.5$ (5% MeOH/$CHCl_3$). Mp: 128−130 °C. $[\alpha]_D^{24} = -69.6°$ (c 0.1, MeOH). ^1H NMR ($CDCl_3$, 300 MHz): δ 2.03, 2.05, 2.09 (9H, 3s), 2.43 (3H, s), 4.15 (1H, d, J = 12.1 Hz), 4.25 (2H, br s), 4.52 (1H, d, J = 12.1 Hz), 5.61−5.63 (1H, m), 5.79 (2H, br s), 6.11−6.12 (2H, br s), 7.34 (2H, d, J = 8.1 Hz), 7.83 (2H, d, J = 8.1 Hz), 7.98 (1H, s), 8.35 (1H, s). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 20.51, 20.54, 20.7, 21.7, 62.3, 68.8, 75.2, 76.6, 83.4, 85.3, 119.8, 128.1, 130.0, 132.3, 138.7, 145.4, 149.9, 153.3, 155.5, 169.4, 169.8. IR (KBr) ν_{max}: 3325, 3157, 2926, 1752, 1647, 1600, 1370, 1224, 1177, 1055, 996, 904 cm^{-1}. HR-ESI-TOF-MS: m/z 600.1370 ($[M + Na]^+$), calcd for $[C_{24}H_{27}N_5O_{10}S + Na]^+$ 600.1371.

General Procedure for the Synthesis of the *xylo*-LNA Monomers 11a−c. Solutions of tosylated nucleosides **10a−c** (1.0 mmol) and potassium carbonate (6.0 equiv) in methanol/water (3/1, 15 mL) were stirred at 25−28 °C for 8 h. On completion of the reaction (analytical TLC), the solvent was removed under reduced pressure. The residue thus obtained was purified by silica gel column chromatography using MeOH/$CHCl_3$ as eluent to afford nucleosides **11a−c** in 91−96% yields.

*1-(2′-O,4′-C-methylene-xylofuranosyl)thymine (**11a**).*[3a] Obtained as a white solid (0.26 g, 96%). R_f = 0.3 (10% MeOH/$CHCl_3$). Mp: 122−124 °C. $[\alpha]_D^{24} = +4.9°$ (c 0.1, MeOH). The spectral data were found to be identical with those reported in the literature.

*1-(2′-O,4′-C-methylene-xylofuranosyl)uracil (**11b**).* Obtained as a white solid (0.24 g, 93%). R_f = 0.3 (10% MeOH/$CHCl_3$). Mp: 216−218 °C. $[\alpha]_D^{23} = +73.7°$ (c 0.1, MeOH). ^1H NMR (DMSO-d_6, 300 MHz): δ 3.73 (1H, d, J = 8.2 Hz), 3.83 (2H, m), 3.96 (1H, d, J = 8.2 Hz), 4.08 (1H, s), 4.22 (1H, br s), 4.98 (1H, br s), 5.47−5.50 (2H, m), 5.66 (1H, br s), 7.69 (1H, d, J = 8.1 Hz), 11.23 (1H, br s). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 56.0, 71.2, 72.1, 76.8, 87.6, 88.9, 98.0, 140.5, 149.4, 162.7. IR (Nujol) ν_{max}: 3491, 2922, 1694, 1463, 1305, 1276, 1175, 1098, 1044, 1014, 986, 901, 886 cm^{-1}. HR-ESI-TOF-MS: m/z 279.0591 ($[M + Na]^+$), calcd for $[C_{10}H_{12}N_2O_6 + Na]^+$ 279.0588.

*9-(2′-O,4′-C-methylene-xylofuranosyl)adenine (**11c**).* Obtained as a white solid (0.23 g, 91%). R_f = 0.3 (10% MeOH/$CHCl_3$). Mp: 236−238 °C. $[\alpha]_D^{24} = -72.1°$ (c 0.1, MeOH). ^1H NMR (DMSO-d_6, 300 MHz): δ 3.81−3.84 (3H, m), 4.05 (1H, d, J = 8.1 Hz), 4.18 (1H, s), 4.44 (1H, s), 5.01 (1H, br s), 5.84 (1H, br s), 6.01 (1H, s), 7.20 (2H, br s), 8.13 (1H, s), 8.19 (1H, s). ^{13}C NMR ($CDCl_3$+DMSO-d_6, 75.5 MHz): δ 57.7, 73.1, 73.9, 79.2, 87.2, 89.6, 119.2, 140.8, 149.8, 153.3, 156.6. IR (KBr) ν_{max}: 3332, 3153, 2929, 1665, 1607, 1475, 1419, 1375, 1335, 1301, 1207, 1057, 992, 895, 851, 795 cm^{-1}. HR-ESI-TOF-MS: m/z 280.1028 ($[M + H]^+$), calcd for $[C_{11}H_{13}N_5O_4 + H]^+$ 280.1040.

Synthesis of 1-(4′-*C*-Hydroxymethyl-2′,3′,5′-tri-*O*-acetyl-β-D-*xylo*furanosyl)-N^4-benzoylcytosine (12). Compound **5d** (100 mg, 0.26 mmol) and benzoic anhydride (71 mg, 0.313 mmol) were dissolved in 5 mL of dry DMF. and the reaction mixture was stirred for 5 h at 25 °C. After completion of the reaction, the excess DMF was removed under reduced pressure and the crude product thus obtained was purified by using silica gel column chromatography to yield **12** as a white solid (115 mg, 88%). R_f = 0.6 (10% MeOH/$CHCl_3$). Mp: 96−98 °C. $[\alpha]_D^{22} = -12.74°$ (c 0.1, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 2.09, 2.11, 2.17 (9H, 3s), 3.80 (2H, d, J = 2.2 Hz), 4.08 (1H, d, J = 11.7 Hz), 4.58 (1H, d, J = 11.7 Hz), 5.49 (1H, t, J = 6.6 Hz), 5.67 (1H, d, J = 5.9 Hz), 6.39 (1H, d, J = 5.8 Hz), 7.50−7.54 (4H, m), 7.60−7.62 (1H, m), 7.92 (1H, d, J = 7.3 Hz), 8.14 (1H, d, J = 7.3 Hz). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 20.58, 20.63, 20.9, 62.5, 63.2, 75.0, 78.9, 86.0, 97.6, 127.7, 129.9, 132.7, 133.3, 143.4, 162.5, 169.8, 170.0, 170.1. IR (thin film): ν_{max} 3228, 3019, 1753, 1698, 1665, 1620, 1561, 1450, 1431, 1371, 1315, 1241, 1109, 1056, 1003, 914 cm^{-1}. HR-ESI-TOF-MS: m/z 504.1617 ($[M + H]^+$), calcd for $[C_{23}H_{25}N_3O_{10}+H]^+$ 504.1613.

Synthesis of 1-(4′-*C*-*p*-Toluenesulfonylmethyl-2′,3′,5′-tri-*O*-acetyl-β-D-*xylo*furanosyl)-N^4-benzoylcytosine (10d). Obtained according to the procedure given for the tosylation of nucleosides **5a−c** as

a white solid in 93% yield. R_f = 0.5 (5% MeOH/$CHCl_3$). Mp: 93−95 °C. $[\alpha]_D^{22}$ = −18.49° (*c* 0.1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.07, 2.08, 2.10 (9H, 3s), 2.44 (3H, s), 4.08 (1H, d, *J* = 12.4 Hz), 4.27 (2H, s), 4.55 (1H, d, *J* = 12.5 Hz), 5.41 (1H, m), 5.48 (1H, d, *J* = 4.4 Hz), 6.11 (1H, d, *J* = 4.4 Hz), 7.37 (2H, d, *J* = 8.0 Hz), 7.51−7.53 (2H, m), 7.60−7.62 (2H, m), 7.83 (2H, d, *J* = 8.8 Hz), 7.90 (2H, d, *J* = 7.3 Hz), 7.98 (1H, d, *J* = 8.0 Hz), 9.04 (1H, br s). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 20.4, 20.7, 21.6, 61.4, 67.9, 74.9, 78.9, 84.4, 87.7, 127.6, 128.0, 129.0, 130.0, 133.3, 143.4, 145.4, 162.5, 169.0, 169.4, 169.6. IR (thin film): ν_{max} 3320, 3021, 1755, 1674, 1627, 1557, 1485, 1372, 1313, 1238, 1178, 1096, 1057, 916 cm^{-1}. HR-ESI-TOF-MS: *m*/*z* 658.1687 ([M + H]$^+$), calcd for $[C_{30}H_{31}N_3O_{12}S + H]^+$ 658.1701.

Synthesis of 1-(2′-*O*,4′-*C*-Methylene-*xylo*furanosyl)cytosine (11d). Obtained according to the procedure given for the synthesis of **11a**−**c** as a white solid in 86% yield. R_f = 0.3 (30% MeOH/$CHCl_3$). Mp: 200−203 °C dec. $[\alpha]_D^{19}$ = +25.44° (*c* 0.1, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.71 (1H, d, *J* = 8.0 Hz), 3.77 (1H, d, *J* = 11.7 Hz), 3.84 (1H, d, *J* = 11.7 Hz), 3.95 (1H, d, *J* = 8.1 Hz), 4.03 (1H, d, *J* = 2.2 Hz), 4.15 (1H, d, *J* = 2.2 Hz), 5.47 (1H, s), 5.61 (1H, d, *J* = 8.0 Hz), 6.96 (1H, br s), 7.07 (1H, br s), 7.67 (1H, d, *J* = 8.1 Hz). ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 56.8, 72.2, 72.8, 77.4, 88.8, 89.0, 91.6, 142.0, 155.1, 165.8. IR (KBr): ν_{max} 3405, 1645, 1494, 1196, 1050, 994, 790 cm^{-1}. HR-ESI-TOF-MS: *m*/*z* 278.0740 ([M + Na]$^+$), calcd for $[C_{10}H_{13}N_3O_5 + Na]^+$ 278.0747.

Synthesis of Tosylated Compound 13. Obtained according to the procedure given for the tosylation of nucleosides **5a**−**c** as a white solid in 93% yield. R_f = 0.5 (30% EtOAc in petroleum ether). Mp: 102−104 °C. $[\alpha]_D^{22}$ = −55.04° (*c* 0.1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.87−0.94 (9H, m), 1.55−1.67 (6H, m), 1.92 (3H, s), 2.24−2.30 (6H, m), 2.43 (3H, s), 3.93 (1H, d, *J* = 11.7 Hz), 4.12−4.20 (2H, m), 4.50 (1H, d, *J* = 11.7 Hz), 5.37 (1H, m), 5.46 (1H, d, *J* = 5.9 Hz), 6.03 (1H, d, *J* = 6.6 Hz), 7.30−7.35 (3H, m), 7.79 (2H, d, *J* = 8.8 Hz), 8.49 (1H, br s). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 15.0, 15.7, 15.8, 15.9, 20.3, 20.4, 20.5, 24.0, 37.79, 37.85, 38.3, 64.1, 70.9, 77.0, 79.7, 84.9, 87.2, 114.4, 130.3, 132.3, 134.4, 136.8, 147.8, 152.4, 165.6, 174.2, 174.6, 174.8. IR (thin film): ν_{max} 3200, 2967, 2877, 1749, 1697, 1459, 1368, 1250, 1190, 1177, 1096, 987, 814 cm^{-1}. HR-ESI-TOF-MS: *m*/*z* 675.2184 ([M + Na]$^+$), calcd for $[C_{30}H_{40}N_2O_{12}S + Na]^+$ 675.2194.

Preparation of *xylo*-LNA-T 11a from Compound 13. Obtained from the tosylated compound **13** according to the procedure given for the synthesis of *xylo*-LNA monomers **11a**−**c** as a white solid in 90% yield.

■ ASSOCIATED CONTENT

Supporting Information. Figures giving ^{1}H and ^{13}C NMR spectra of compounds **3**, **4a**−**d**, **5a**−**d**, **6**, **7a**−**f**, **8a**−**d**, **9a**−**d**, **10a**−**d**, **11a**−**d**, **12**, and **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Lipase Novozyme-435 and Lipozyme TL IM were obtained as gifts from Novozyme A/S Denmark. CRL and PPL was purchased from Sigma-Aldrich Chemical Co., and *Amano* PS was purchased from Amano Pharmaceuticals.

(10) Peracylated nucleosides **7a**−**f** were synthesized by addition of pyridine (10 mL) to a mixture of tetrahydroxy nucleoside **6** (1.74 mmol) and the corresponding acid anhydride (propanoic, butanoic, valeric, hexanoic, benzoic, or *p*-anisyl anhydride, 7.66 mmol), followed by stirring of the reaction mixture at 25 °C for 4−5 h in 90−94% yields.

(11) Compound **6** was synthesized by stirring a solution of the corresponding peracetylated analogue **4a** (6.57 mmol) in half-saturated methanolic ammonia (60 mL) at 25 °C for 10 h, followed by purification with the help of CC using MeOH/$CHCl_3$ as eluent in 90% yield.

(12) The procedure used for the Novozyme-435 catalyzed deacetylation of peracetylated nucleosides **4a**−**d** was followed for the deacylation of peracylated nucleosides **7a**−**f** to afford **8a**−**d** in 73−95% yields. Incubation of nucleosides **7e,f** with Novozyme-435 did not yield any product.

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(16) To a solution of compound **5a** (0.24 mmol) in 5 mL of dichloromethane was added the corresponding acid anhydride (propanoic, butanoic, pentanoic, or benzoic anhydride, 0.29 mmol) in the presence of a catalytic amount of DMAP, and the reaction mixture was stirred at 25 °C to afford compounds **9a**−**d** in 89−94% yields after aqueous workup and CC purification using MeOH/$CHCl_3$.

(17) The procedure used for the Novozyme-435 catalyzed deacetylation of peracetylated nucleosides **4a**−**d** was followed for the deacylation of

mixed esters **9a**−**d** to afford the identical product **5a** in 85, 90, and 93% yields, respectively, in all cases except **9d**. Incubation of nucleoside **9d** with Novozyme-435 did not yield any product.

(18) Solutions of compounds **5a**−**c** (1.0 mmol) and *p*-toluenesulfonyl chloride (1.5 mmol) in anhydrous pyridine (10 mL) were stirred at 25−28 °C for 10 h. On completion the reaction mixture was poured over 10% ice-cold hydrochloric acid solution and the product was extracted with chloroform (2 × 100 mL). The combined organic extract was washed with saturated aqueous $NaHCO_3$ (2 × 100 mL) and dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The residue thus obtained was purified by silica gel column chromatography using MeOH/$CHCl_3$ as eluent to afford the tosylated nucleosides **10a**−**c** in 92−95% yields.

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