Selective biocatalytic deacylation studies on furanose triesters: a novel and efficient approach towards bicyclonucleosides†‡

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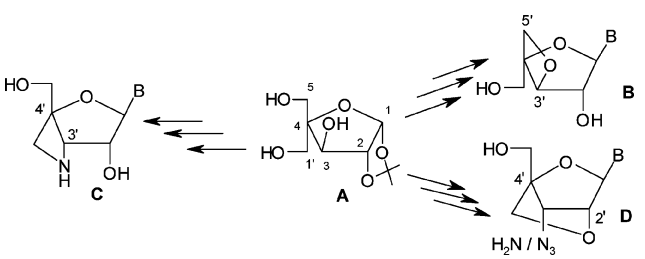
Lipozyme® TL IM catalyses the deacylation of 4-*C*-acyloxymethyl-3,5-di-*O*-acyl-1,2-*O*-(1-methylethylidene)-β-L-*threo*-pentofuranose to form 3,5-di-*O*-acyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-α-D-*xylo*-pentofuranose in a highly selective and efficient manner. The rate of lipase-catalyzed deacylation of tributanoyl furanose is 2.3 times faster than the rate of deacylation of the triacetyl furanose derivative. In order to confirm the structure of the lipase-catalyzed deacylated product, it was converted to a bicyclic sugar derivative, which can be used for the synthesis of bicyclic nucleosides of importance in the development of novel antisense and antigene oligonucleotides. Further, it has been established that the monohydroxy product of the lipase-catalyzed reaction is the result of selective deacylation of the 4-*C*-acyloxymethyl function in the substrate and not of any acyl migration process.

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,[2–12] and for their direct utilization as anti-tumor or antiviral compounds.[13–17] In recent studies for development of ideal and practical antisense molecules, oligonucleotide analogues containing non-genetic 2′,5′-phosphodiester linkages have been found to be good candidates due to their RNA-selective hybridization properties and resistance towards enzymatic degradation.[18–25] A novel class of 2′,5′-linked oligonucleotide analogs containing 3′-*O*,4′-*C*-methylene bridged ribonucleosides—*i.e.* an oxetane-fused ribofuranoside ring system along with normal 3′,5′-linked oligonucleotide analogs containing 2′-*O*,4′-*C*-methylene bridged ribonucleosides—commonly known as locked nucleic acids (LNAs), have been known to possess favorable features towards development of antisense and/or antigene candidates.[26–31]

One of the major problems emphasized in the synthesis of modified nucleosides is the presence of multiple functionalities of nearly identical reactivity which are difficult to protect and deprotect selectively.[32,33] Further, synthetic routes involving various protection and/or deprotection steps reduce the overall yields of the desired products and make the whole process tedious, time-consuming and inefficient.[33,34] It is at this juncture that nature's catalysts, enzymes, come into the picture. Recent advances in enzyme-assisted organic synthesis have allowed the preparation of structurally well-defined molecules in high yields and greater selectivity. The added advantages of the application of enzymes in organic synthesis are that they work under mild reaction conditions and are often environmentally benign. Among the different biocatalytic processes, lipase-catalyzed selective acylation/deacylation reactions represent an important class of enzymatic transformations in organic synthesis, which is mainly attributed to the low cost of lipases and their wide tolerance towards a variety of reaction conditions and substrates.[35,36] Enzymes are being recognized as efficient catalysts for many of the stereospecific and regioselective reactions necessary for carbohydrate modifications and nucleoside synthesis.[37–47]

4-*C*-Hydroxymethyl-1,2-*O*-(1-methylethylidene)-β-L-*threo*-pentofuranose (**A**) is an important precursor for the synthesis of different types of bicyclonucleosides, *i.e.* 3′-*O*,5′-*C*-methylene bridged nucleoside **B**, 3′-*N*,4′-*C*-methylene bridged nucleoside **C** and 2′-*O*,4′-*C*-methylene bridged 3′-azido-/3′-aminonucleoside **D** (Scheme 1). For the synthesis of these bicyclonucleosides, discrimination between the two primary hydroxyl groups of the trihydroxyfuranose precursor sugar **A** is highly desired. Chemical



Scheme 1 Compound **A**—a key precursor for the synthesis of various bicyclonucleosides.

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methods for the manipulation of two such primary hydroxyl groups in sugar precursors as well as in nucleosides are reported, but they are non-selective and lead to the formation of isomeric mixtures. For example, an effort towards selective benzylation of 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-α-D-*erythro*-pentofuranose led to the formation of the desired 3,5-di-*O*-benzyl-4-*C*-hydroxymethylpentofuranose product in 59% yield only together with other benzylated compounds.[48] Similarly, selective tosylation of one out of the two primary hydroxyl functions of 2′,3′-*O*-cyclohexylidene-4′-hydroxymethyluridine afforded a mixture of 4′-tosyloxymethylated- and 5′-*O*-tosylated derivatives.[49] We have earlier reported the asymmetrization of the two diastereotopic diols *viz.* 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-β-L-*threo*-pentofuranose and 3-azido-3-deoxy-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-α-D-*erythro*-pentofuranose by lipase-catalyzed acylation reactions in organic solvents as part of our chemosynthetic approach targeted towards the formation of modified nucleosides.[38,40] Recently, Kumar and Gross[50] have used the trihydroxy pentofuranose derivative **A** as a suitable scaffold for building well-defined macromolecules. They used different lipases for the strict control of the three-dimensional arrangement of substituents on the carbohydrate core.

In the present study, we have developed a highly efficient chemoenzymatic route for the diastereo- and regioselective deacylation of one out of the three acyloxy groups of **2a–2c** (Scheme 2), derived from two primary and one secondary hydroxyl groups of the potentially useful triol **A**, and subsequent reactions on the novel diacetyl derivative **3a** leading to the formation of the bicyclic nucleoside sugar precursor **8**.

Results and discussion

The trihydroxy sugar derivative **A** was synthesized starting from D-glucose following the modified procedure[1] of Youssefyeh *et al.* and others[51,52] *via* the furanose derivative **1** (Scheme 2).

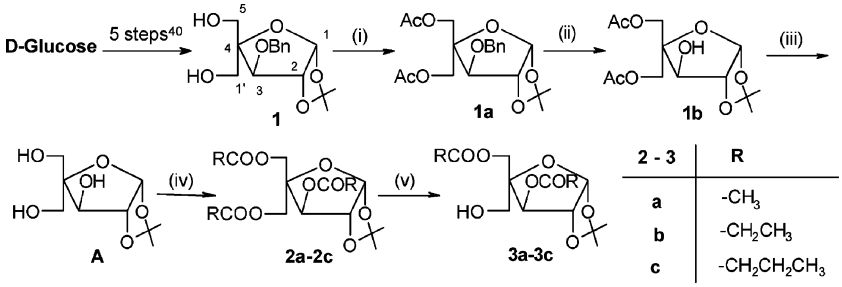
The trihydroxy sugar derivative **A** was converted in yields of 80–85% to its triacylated derivatives, 4-*C*-acetoxymethyl-3,5-di-*O*-acetyl-1,2-*O*-(1-methylethylidene)-β-L-*threo*-pentofuranose (**2a**), 3,5-di-*O*-propanoyl-1,2-*O*-(1-methylethylidene)-4-*C*-propanoyloxymethyl-β-L-*threo*-pentofuranose (**2b**) and 4-*C*-butanoyloxymethyl-3,5-di-*O*-butanoyl-1,2-*O*-(1-methylethylidene)-β-L-*threo*-pentofuranose (**2c**) using acetic anhydride, propanoic anhydride and butanoic anhydride, respectively, in the presence of a catalytic amount of 4-(dimethylamino)pyridine (DMAP) (Scheme 2). Based on our experience of biocatalytic acylation/deacylation reactions, we chose to use the enzymes *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 immobilized on accurel (CAL B–L(A)) for selective deacylation of the triacylated pentofuranose derivatives **2a–2c** 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 efficient biocatalyst for the selective deacylation of compounds **2a–2c**. The other four lipases, *i.e.* Novozyme-435, PPL, CRL and CAL B–L(A) did not accept any of the acylated sugar derivatives as substrates.

In a typical reaction, a solution of 4-*C*-acetoxymethyl-3,5-di-*O*-acetyl-1,2-*O*-(1-methylethylidene)-β-L-*threo*-pentofuranose (**2a**) in toluene containing a small amount of *n*-butanol was agitated with Lipozyme® TL IM in an incubator shaker at 40–42 °C. On completion of the reaction, as indicated by TLC examination, the 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 compound **3a**, with lower R_f value than the starting compound **2a** in 98% yield (Scheme 2, Table 1). The structure of the diacetylated compound **3a** was established as 3,5-di-*O*-acetyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-α-D-*xylo*-pentofuranose by a detailed study of its IR, ^{1}H and ^{13}C NMR, HRMS and ^{1}H NOE data, and comparison of its ^{1}H NMR spectrum with that of the starting triacetate **2a**.[1]

Table 1 Diastereoselective deacylation study of 4-*C*-acyloxymethyl-3,5-di-*O*-acyl-1,2-*O*-(1-methylethylidene)-β-L-*threo*-pentofuranose **2a–2c** and the mixed esters 4-*C*-acyloxymethyl-3,5-di-*O*-acetyl-1,2-*O*-(1-methylethylidene)-β-L-*threo*-pentofuranose **9a–9d** catalyzed by Lipozyme® TL IM in toluene in the presence of *n*-butanol[a]

Substrate	Reaction time/h	Product	Yield (%)
2a	9	**3a**	98
2b	7	**3b**	88
2c	4	**3c**	93
9a	8.5	**3a**	95
9b	8	**3a**	94
9c	10	**3a**	92
9d	96	No Reaction	0

[a] All these reactions, when performed under identical conditions but without adding the lipase Lipozyme® TL IM, did not yield any product.

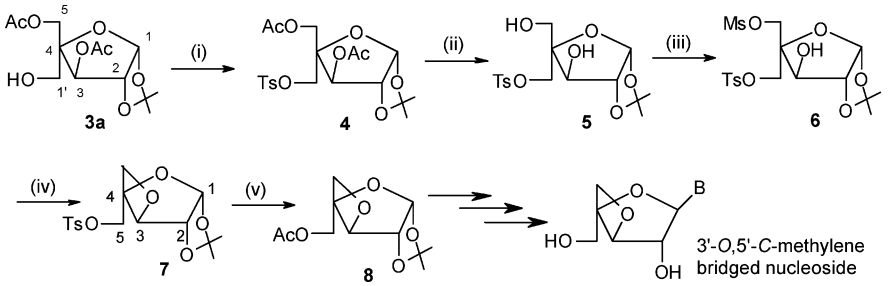


Scheme 2 Synthesis and lipase catalysed selective deacylation studies on compounds **2a–2c**. *Reagents and conditions*: (i) Ac_2O, DMAP; (ii) H_2/Pd-C, EtOAc; (iii) K_2CO_3, MeOH; (iv) excess of $(RCO)_2O$, DMAP; (v) Lipozyme® TL IM, toluene, *n*-butanol.

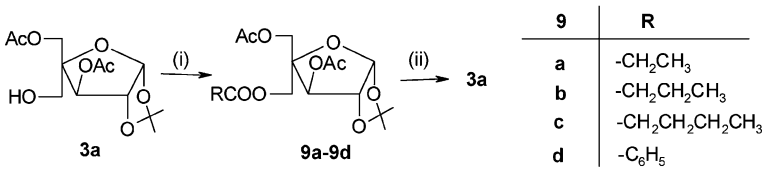
The structure of the deacetylated compound obtained in the lipase-mediated selective deacetylation of **2a** was further confirmed as 3,5-di-*O*-acetyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-α-D-*xylo*-pentofuranose (**3a**) by its chemical transformation. Treatment of compound **3a** with *p*-toluenesulfonyl chloride in the presence of base led to the tosylation of the free hydroxyl function. The tosylated sugar derivative on treatment with aqueous methanolic potassium carbonate formed a diol. Mesylation of this diol afforded a monomesyl derivative (due to preferential mesylation of the primary hydroxyl function), which on treatment with sodium hydride in DMF led to the formation of the novel tricyclic compound **3**-*O*,4-*C*-methylene-1,2-*O*-(1-methylethylidene)-5-*O*-(*p*-toluenesulfonyl)-β-L-*arabino*-pentofuranose (**7**), the product of a series of reactions which are possible only when the hydroxyl group in compound **3a** is at the C-1′ position (Scheme 3). The tosyloxymethylfuranose **7** was further transformed into the novel bicyclic compound 5-*O*-acetyl-3-*O*,4-*C*-methylene-1,2-*O*-(1-methylethylidene)-β-L-*arabino*-pentofuranose (**8**) on treatment with potassium acetate and 18-crown-6 ether in dioxane. This series of reactions demonstrates that the compound **3a**, obtained through a highly selective biocatalytic route using a novel silica gel-supported fungal lipase, can be used as a suitable precursor for the synthesis of bicyclic nucleosides (Scheme 3).

To see the effect of different acyl moieties, biocatalytic deacylation study was extended to 3,5-di-*O*-propanoyl-1,2-*O*-(1-methylethylidene)-4-*C*-propanoyloxymethyl-β-L-*threo*-pentofuranose (**2b**) and 4-*C*-butanoyloxymethyl-3,5-di-*O*-butanoyl-1,2-*O*-(1-methylethylidene)-β-L-*threo*-pentofuranose (**2c**). Lipozyme® TL IM-catalyzed deacylation of compounds **2b** and **2c** afforded the corresponding diacylated compounds, 3,5-di-*O*-propanoyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-α-D-*xylo*-pentofuranose (**3b**) and 3,5-di-*O*-butanoyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-α-D-*xylo*-pentofuranose (**3c**), respectively (Scheme 2, Table 1). It was observed that the increase in the chain length of the acyl moiety increases the rate of lipase-catalyzed deacylation reaction (Table 1). Thus, the rate of deacylation of propanoylated sugar derivative **2b** is about 1.3 times faster than the rate of deacylation of acetylated sugar derivative **2a**. Accordingly, the rate of deacylation of butanoylated sugar derivative **2c** is about 2.3 and 1.8 times faster than the rate of deacylation of acetylated and propanoylated sugar derivatives **2a** and **2b**, respectively. The structures of all the novel compounds obtained in this study (**1b**, **2a–2c**, **3a–3c** and **4–8**) were unambiguously established on the basis of their spectral (IR, ^{1}H and ^{13}C NMR, HRMS and NOE) analysis. The structure of compound **3a** was further confirmed by its chemical transformation to stereochemically restricted bicyclic sugar derivative **8**. Structures of known compounds mentioned in Scheme 2 were further confirmed by comparison of their physical and/or chemical data with those reported in the literature. It should be noted that though compound **1a** is known,[38] its physical and spectral data were not reported earlier. All deacylation reactions, when performed under identical conditions but without adding any lipase, did not proceed to any extent.

It has been observed that monoacyl derivatives of certain 1,2-diol systems isomerize by acyl migration, and the problem of acyl migration has also been reported in partially acylated nucleosides.[53–55] Thus the selectively deacylated 3,5-di-*O*-acyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-α-D-*xylo*-pentofuranose products **3a–3c** may in theory also arise due to deacylation of other acyloxy functions at C-5/C-3 in **2a–2c**, followed by acyl migration. To confirm that the isolated compounds **3a–3c** are formed in the enzyme-catalyzed reaction solely due to the deacylation of the 4-*C*-acyloxymethyl function and not due to partial or complete acyl moiety migration, the mixed esters 3-*O*-acetyl-4-*C*-acetoxymethyl-1,2-*O*-(1-methylethylidene)-5-*O*-propanoyl-β-L-*arabino*-pentofuranose (**9a**), 3-*O*-acetyl-4-*C*-acetoxymethyl-5-*O*-butanoyl-1,2-*O*-(1-methylethylidene)-β-L-*arabino*-pentofuranose (**9b**), 3-*O*-acetyl-4-*C*-acetoxymethyl-1,2-*O*-(1-methylethylidene)-5-*O*-pentanoyl-β-L-*arabino*-pentofuranose (**9c**) and 3-*O*-acetyl-4-*C*-acetoxymethyl-5-*O*-benzoyl-1,2-*O*-(1-methylethylidene)-β-L-*arabino*-pentofuranose (**9d**) were synthesized from **3a** using propanoic anhydride, butanoic anhydride, valeric anhydride or benzoic anhydride in dichloromethane (DCM) in the presence of a catalytic amount of DMAP to afford the corresponding mixed triesters in 93–95% yields (Scheme 4). The mixed triesters **9a–9d** were subjected to Lipozyme® TM IL-catalyzed deacylation reactions in toluene in the presence of *n*-butanol as acyl acceptor. It was observed that the lipase-catalyzed deacylation of all the mixed esters resulted in the exclusive formation of 3,5-di-*O*-acetyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-α-D-*xylo*-pentofuranose (**3a**), except in the case of deacylation of 3-*O*-acetyl-4-*C*-acetoxymethyl-5-*O*-benzoyl-1,2-*O*-(1-methylethylidene)-β-L-*arabino*-pentofuranose (**9d**), which is not a substrate for Lipozyme® TM IL (Table 1). The formation of just one product in all the enzyme-catalyzed reactions showed that it is obtained by selective deacylation of the 4-*C*-acyloxymethyl function of **9a–9c** only, and not through deacylation of any other acyloxy function, followed by acyl migration. Furthermore, the results of Lipozyme® TM



Scheme 3 Conversion of selectively deacetylated compound **3** into a bicyclic sugar derivative. *Reagents and conditions*: (i) *p*-TsCl, pyridine; (ii) K_2CO_3, MeOH–H_2O; (iii) MsCl, CH_2Cl_2, Et_3N; (iv) NaH, DMF; (v) CH_3COOK, dioxane, 18-crown-6 ether.



Scheme 4 Lipase-catalyzed selective deacylation studies on mixed triacylated pentofuranoses **9a–9d**. *Reagents and conditions*: (i) $(RCO)_2O$, DCM, DMAP; (ii) Lipozyme® TL IM, toluene, *n*-butanol.

IL-mediated selective deacylation of mixed esters **9a–9c** indicates that the increase in the chain length of the acyloxymethyl function at C-4 does not alter the selectivity of the lipase.

Conclusion

Lipozyme® TL IM discriminated between three ester functions derived from two primary hydroxyls and a secondary hydroxyl group in novel sugar derivatives. There are ways to differentiate between primary and secondary hydroxyl groups, but discrimination between two primary hydroxyl groups or their derivatives is in general not possible by purely classical chemical methods. The very efficient and convenient enzymatic method discovered for the discrimination of primary hydroxyl groups of furanose derivatives herein may find applications in 'green' synthesis of bicyclonucleosides, important precursors for the preparation of state-of-the-art antisense or antigene oligonucleotides.

Experimental section

General procedures

Reactions were conducted under an atmosphere of nitrogen when anhydrous solvents were used. Column chromatography was carried out using silica gel (100–200 mesh). Melting points were determined using a H_2SO_4 bath and are uncorrected. The ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance spectrometer at 300 and at 75.5 MHz, respectively. The chemical shift values are reported as δ ppm relative to TMS used as internal standard and the coupling constants (J) are measured in Hz. The FAB-HRMS spectra of all the compounds were recorded on a JEOL JMS-AX505W high-resolution mass spectrometer in positive mode using the matrix HEDS (bishydroxyethyldisulfide) doped with sodium acetate. The *Candida antarctica* lipase (CAL B or Novozyme-435), *Candida antarctica* lipase-B immobilized on accurel, *i.e.* CAL B–L(A), and *Thermomyces lanuginosus* lipase immobilized on silica (Lipozyme® TL IM) were gifts from Novozymes A/S (Copenhagen, Denmark), whereas *C. rugosa* lipase (CRL) and porcine pancreatic lipase (PPL) were purchased from Sigma Chemical Co. (USA). All the enzymes were dried over P_2O_5 under vacuum for 24 h prior to use. Toluene was distilled over P_2O_5 and stored over Na wire prior to use.

4-*C*-Acetoxymethyl-5-*O*-acetyl-3-*O*-benzyl-1,2-*O*-(1-methylethylidene)-β-L-*threo*-pentofuranose (1a)[38]

To a solution of the diol **1**[40] (20.15 g, 65 mmol) in acetic anhydride (1.1 equiv.) and pyridine (2.0 equiv.) was added a catalytic amount of 4-*N*,*N*-dimethylaminopyridine and the reaction mixture was stirred at 25–28 °C. On completion (analytical TLC), pyridine was removed under reduced pressure, ice-cold water (200 ml) was added to the residue and the product was extracted with ethyl acetate (2 × 150 ml). The combined organic phase was washed with sodium bicarbonate solution (200 ml) and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was subjected to column chromatography on silica gel. The diacetylated furanose **1a** was obtained as a colourless oil (21 g; 82%) using ethyl acetate–petroleum ether (1 : 4) as eluent. ^1H NMR ($CDCl_3$, 300 MHz): δ 7.35–7.32 (m, 5H), 5.96 (d, J = 4.0 Hz, 1H), 4.75–4.72 (m, 2H), 4.51 (d, J = 11.7 Hz, 1H), 4.37 (d, J = 11.7 Hz, 1H), 4.20–4.04 (m, 4H), 2.04 (s, 3H), 1.96 (s, 3H), 1.54 (s, 3H), 1.35 (s, 3H); ^{13}C NMR ($CDCl_3$, 75.5 Hz): δ 169.91, 169.81, 136.56, 128.12, 127.82, 127.69, 127.41, 113.08, 104.80, 85.40, 85.08, 83.37, 71.98, 63.15, 62.73, 26.75, 26.37, 20.50, 20.27; IR (thin film) 2941, 1747, 1729, 1497, 1455, 1374, 1236, 1164, 1046, 862, 749, 700, 604 cm^{-1}.

4-*C*-Acetoxymethyl-5-*O*-acetyl-1,2-*O*-(1-methylethylidene)-β-L-*threo*-pentofuranose (1b)

To a solution of the benzylated furanose **1a** (19.7 g, 50 mmol) in ethyl acetate (350 ml), 10% Pd/charcoal (4.5 g) was added and the reaction mixture was degassed with H_2 under reduced pressure. After stirring the contents at RT under an atmosphere of H_2 gas for 28 h, the reaction mixture was passed through a pad of silica gel and washed with hot chloroform. The combined filtrate was concentrated under reduced pressure and the residue thus obtained was subjected to column chromatography over silica gel using ethyl acetate–petroleum ether (3 : 7) as eluent to afford the furanose **1b** as a white solid (10.26 g; 67.5%). Mp 74–76 °C. ^1H NMR ($CDCl_3$, 300 MHz): δ 5.92 (d, J = 4.0 Hz, 1H), 5.35 (s, 1H), 4.60 (dd, J = 1.0 and 4.0 Hz, 1H), 4.36 (d, J = 11.5 Hz, 1H), 4.29 (d, J = 11.5 Hz, 1H), 4.27 (d, J = 11.5 Hz, 1H), 4.10 (d, J = 11.5 Hz, 1H), 2.08 (s, 3H), 2.06 (s, 3H), 1.60 (s, 3H), 1.33 (s, 3H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 175.52, 175.42, 117.29, 110.22, 92.92, 91.94, 81.21, 68.30, 32.08, 31.68, 26.21, 26.10; HRMS m/z calculated for $[C_{13}H_{20}O_8Na]^+$ 327.1056, observed 327.1083.

General procedure for acylation of 4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-β-L-*threo*-pentofuranose (A); preparation of triesters 2a–2c

To a mixture of 4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-β-L-*threo*-pentofuranose (**A**, 1.98 g, 9.0 mmol) and the corresponding acid anhydride (acetic/propanoic/butanoic anhydride, 3.3 equiv.) was added a catalytic amount of DMAP and the reaction mixture was stirred for 4–5 h at 28 °C. On completion (analytical TLC), the reaction mixture was poured over ice water and the product triesters were extracted with ethyl acetate (2 × 60 ml). The combined organic extract was washed with saturated

aqueous $NaHCO_3$ (80 ml), dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The residue thus obtained was purified by column chromatography on silica gel using ethyl acetate–petroleum ether (1 : 3) as eluent to afford the triacylated products **2a–2c** (80–85%). (See ESI for the characterization data of **2b,c**.‡)

4-*C*-Acetoxymethyl-3,5-di-*O*-acetyl-1,2-*O*-(1-methylethylidene)-β-L-*threo*-pentofuranose (2a)

Obtained as a white solid (2.6 g; 85%). Mp 45–46 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.98 (d, $J = 4.1$ Hz, 1H), 5.35 (s, 1H), 4.61 (dd, $J = 1.0$ and 4.1 Hz, 1H), 4.36 (d, $J = 11.5$ Hz, 1H), 4.29 (d, $J = 11.5$ Hz, 1H), 4.27 (d, $J = 11.6$ Hz, 1H), 4.10 (d, $J = 11.6$ Hz, 1H), 2.10 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 1.60 (s, 3H), 1.33 (s, 3H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 170.60, 170.54, 169.67, 113.95, 105.70, 86.18, 77.78, 63.69, 62.88, 27.19, 26.76, 21.22, 21.16, 21.03; IR (KBr) 2993, 2948, 1751, 1736, 1461, 1432, 1391, 1373, 1235, 1166, 1117, 1079, 1026, 903, 851 cm^{-1}; HRMS *m/z* calculated for $[C_{15}H_{22}O_9Na]^+$ 369.1162, observed 369.1165.

General procedure for lipase-catalyzed selective deacylation of 4-*C*-acyloxymethyl-3,5-di-*O*-acyl-1,2-*O*-(1-methylethylidene)-β-L-*threo*-pentofuranose 2a–2c

To a solution of the triacylated furanose **2a–2c** (3.0 mmol) in anhydrous toluene (30 ml) was added *n*-butanol (1.2 equiv) followed by the addition of Lipozyme® TL IM (500 mg). The reaction mixture was stirred with shaking at 40–42 °C in an incubator and the progress of the reaction was monitored periodically by TLC. On completion, the reaction was quenched by filtering off the lipase, the solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel using ethyl acetate–petroleum ether (2 : 3) as eluent to afford the deacylated products **3a–3c** (88–98%). (See ESI for the characterization data of **3b,c**.‡)

3,5-Di-*O*-acetyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-α-D-*xylo*-pentofuranose (3a)

Obtained as a white solid (894 mg; 98%). Mp 95–96 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.97 (d, $J = 4.1$ Hz, 1H), 5.29 (s, 1H), 4.64 (dd, $J = 0.9$ and 4.1 Hz, 1H), 4.24 (d, $J = 11.5$ Hz, 1H), 4.13 (d, $J = 11.5$ Hz, 1H), 3.82 (dd, $J = 5.43$ and 11.91 Hz, 1H), 3.73 (dd, $J = 8.26$ and 11.91 Hz, 1H), 2.42–2.38 (m, 1H), 2.08 (s, 6H), 1.57 (s, 3H), 1.33 (s, 3H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 170.25, 169.50, 113.17, 104.75, 87.73, 85.89, 77.33, 62.46, 62.08, 26.75, 26.22, 20.67, 20.49; IR (KBr) 3439, 2938, 1754, 1718, 1379, 1270, 1216, 1163, 1039, 887 cm^{-1}; HRMS *m/z* calculated for $[C_{13}H_{20}O_8Na]^+$ 327.1056, observed 327.1083.

4-*C*-Acetoxymethyl-3-*O*-acetyl-1,2-*O*-(1-methylethylidene)-5-*O*-(*p*-toluenesulfonyl)-β-L-*arabino*-pentofuranose (4)

To a solution of 3,5-di-*O*-acetyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-α-D-*xylo*-pentofuranose (**3a**, 760 mg, 2.5 mmol) in anhydrous pyridine (25 ml) was added *p*-toluenesulfonyl chloride (715 mg, 3.75 mmol) pinchwise during 20 min at 0 °C, and the reaction mixture was stirred for 10 h at 28 °C. On completion (analytical TLC), the reaction mixture was poured into ice-cold dilute hydrochloric acid (50 ml) and extracted with ethyl acetate (2 × 50 ml). The combined organic layer was dried (Na_2SO_4), evaporated to dryness under reduced pressure and the residue was subjected to column chromatography on silica gel using ethyl acetate–petroleum ether (2 : 3) as eluent to afford the furanose **4** as a white solid (1.1 g; 92%). Mp 116 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.80 (d, $J = 7.8$ Hz, 2H), 7.35 (d, $J = 7.8$ Hz, 2H), 5.91 (brs, 1H), 5.24 (s, 1H), 4.52 (brs, 1H), 4.26–4.20 (m, 3H), 4.07 (d, $J = 11.7$ Hz, 1H), 2.44 (s, 3H), 2.08 (s, 3H), 1.95 (s, 3H), 1.39 (s, 3H), 1.26 (s, 3H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 170.32, 169.40, 145.44, 133.06, 132.90, 130.26, 128.54, 113.56, 105.85, 85.87, 85.65, 77.41, 67.75, 62.20, 26.61, 26.20, 21.97, 20.92; IR (KBr) 2991, 1767, 1745, 1600, 1456, 1364, 1242, 1217, 1181, 1100, 1072, 1047, 1013, 977, 923, 843, 816, 726, 667, 556, 541 cm^{-1}; HRMS *m/z* calculated for $[C_{20}H_{26}O_{10}SNa]^+$ 481.1144, observed 481.1164.

4-*C*-Hydroxymethyl-1,2-*O*-(1-methylethylidene)-5-*O*-*p*-toluenesulfonyl-β-L-*arabino*-pentofuranose (5)

To a solution of the furanose **4** (687 mg, 1.5 mmol) in methanol (20 ml) was added an aqueous solution of potassium carbonate (414 mg in 10 ml water, 3 mmol), and the reaction was stirred for 1 h at 28 °C. On completion (analytical TLC), the reaction mixture was neutralized with dilute hydrochloric acid and extracted with ethyl acetate (2 × 40 ml). The combined organic phase was washed with water (50 ml), dried (Na_2SO_4), evaporated to dryness under reduced pressure, and the residue was subjected to column chromatography on silica gel using ethyl acetate–petroleum ether (3 : 2) as solvent to afford the diol **5** as a white solid (342 mg; 61%). Mp 145 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.79 (d, $J = 8.2$ Hz, 2H), 7.35 (d, $J = 8.2$ Hz, 2H), 5.95 (d, $J = 3.6$ Hz, 1H), 4.58 (d, $J = 3.7$ Hz, 1H), 4.28 (s, 1H), 4.23 (d, $J = 10.0$ Hz, 1H), 4.08 (d, $J = 10.0$ Hz, 1H), 3.94 (d, $J = 11.9$ Hz, 1H), 3.73 (d, $J = 11.9$ Hz, 1H), 2.45 (s, 3H), 1.35 (s, 3H), 1.27 (s, 3H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 145.62, 132.73, 130.37, 128.45, 113.07, 105.92, 87.82, 87.56, 78.00, 69.77, 63.26, 26.83, 26.26, 22.02; IR (KBr) 3529, 2975, 2932, 1596, 1451, 1421, 1358, 1261, 1224, 1179, 1068, 1010, 960, 864, 836, 812, 790, 665, 557, 534 cm^{-1}; HRMS *m/z* calculated for $[C_{16}H_{22}O_8SNa]^+$ 397.0933, observed 397.0957.

4-*C*-Methanesulfonyloxy-1,2-*O*-(1-methylethylidene)-5-*O*-(*p*-toluenesulfonyl)-β-L-*arabino*-pentofuranose (6)

To a solution of the diol **5** (300 mg, 0.8 mmol) in anhydrous CH_2Cl_2 (25 ml), were added triethylamine (121 mg, 1.2 mmol) and methanesulfonyl chloride (110 mg, 0.96 mmol) at 0 °C. The temperature of the reaction mixture was allowed to attain 28 °C and it was stirred at this temperature for 5 h. On completion (analytical TLC), the reaction mixture was neutralized with dilute hydrochloric acid and extracted with CH_2Cl_2 (2 × 40 ml). The combined organic phase was washed with water (50 ml), dried (Na_2SO_4), evaporated to dryness under reduced pressure, and the residue was subjected to column chromatography on silica gel using ethyl acetate–petroleum ether (1 : 1) as eluent to afford the furanose **6** as an oil (228 mg; 63%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.80 (d, $J = 8.0$ Hz, 2H), 7.36 (d, $J = 8.0$ Hz, 2H), 5.93 (d, $J = 3.5$ Hz, 1H), 4.57 (d, $J = 3.9$ Hz, 1H), 4.35 (brs, 3H), 4.25–4.16 (m, 2H), 3.01 (s, 3H), 2.45 (s, 3H), 1.36 (s, 3H), 1.26

(s, 3H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 145.19, 129.89, 127.99, 112.84, 105.59, 105.03, 86.79, 86.21, 77.30, 67.36, 66.91, 37.29, 29.56, 26.08, 25.63, 21.51; IR (KBr) 3435, 2924, 1376, 1333, 1175, 1036, 1011, 982, 966, 821, 774, 553 cm^{-1}; HRMS *m/z* calculated for $[C_{17}H_{24}O_{10}S_2Na]^+$ 475.0709, observed 475.0739.

3-*O*,4-*C*-Methylene-1,2-*O*-(1-methylethylidene)-5-*O*-(*p*-toluenesulfonyl)-β-L-*arabino*-pentofuranose (7)

To a solution of the furanose **6** (181 mg, 0.4 mmol) in anhydrous DMF (8 ml) was added sodium hydride (12 mg, 0.5 mmol) portion-wise during 10 min at 0 °C and the contents were stirred for 12 h at 28 °C. On completion (analytical TLC), the reaction mixture was poured into ice-cold water (25 ml) and the compound was extracted with CH_2Cl_2 (2 × 25 ml). The combined organic layer was dried over Na_2SO_4, evaporated to dryness under reduced pressure, and the residue was subjected to column chromatography on silica gel using ethyl acetate–petroleum ether (2 : 3) as eluent to afford the pure furanose derivative **7** as a white solid (91 mg; 64%). Mp 80–82 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.81 (d, J = 7.9 Hz, 2H), 7.36 (d, J = 7.9 Hz, 2H), 6.19 (s, 1H), 4.98 (s, 1H), 4.67–4.62 (m, 2H), 4.29 (d, J = 7.6 Hz, 1H), 4.19 (brs, 2H), 2.45 (s, 3H), 1.40 (s, 3H), 1.33 (s, 3H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 145.61, 132.73, 130.33, 128.47, 115.25, 108.40, 87.55, 86.11, 84.44, 78.70, 68.31, 27.97, 27.03, 22.01; IR (KBr) 2929, 1597, 1454, 1368, 1176, 831 cm^{-1}; HRMS *m/z* calculated for $[C_{16}H_{20}O_7SNa]^+$ 379.0827, observed 379.0832.

5-*O*-Acetyl-3-*O*,4-*C*-methylene-1,2-*O*-(1-methylethylidene)-β-L-*arabino*-pentofuranose (8)

To a solution of the furanose derivative **7** (71 mg, 0.2 mmol) in dioxane (8 ml) was added potassium acetate (40 mg, 0.4 mmol) and 18-crown-6 ether (63 mg, 0.24 mmol), and the resulting mixture was refluxed for 12 h. On completion, the contents were cooled to RT, poured into ice-cold water (10 ml) and extracted with ethyl acetate (2 × 20 ml). The combined organic phase was dried over Na_2SO_4, evaporated to dryness under reduced pressure, and the residue was subjected to column chromatography on silica gel using ethyl acetate–petroleum ether (3 : 7) as eluent to afford the acetylated furanose derivative **8** as an oil (35 mg; 71%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.18 (s, 1H), 4.96 (s, 1H), 4.62–4.59 (m, 2H), 4.32 (d, J = 6.9 Hz, 1H), 4.27–4.21 (m, 2H), 2.04 (s, 3H), 1.46 (s, 3H), 1.30 (s, 3H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 169.54, 113.88, 106.75, 86.52, 85.21, 83.28, 78.13, 62.67, 26.87, 25.76, 19.74; IR (thin film) 2956, 1746, 1372, 1229, 1162, 1094, 1070, 1047, 1012, 961, 890, 850 cm^{-1}; HRMS *m/z* calculated for $[C_{11}H_{16}O_6Na]^+$ 267.0845, observed 267.0863.

General procedure for acylation of 3,5-di-*O*-acetyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-α-D-*xylo*-pentofuranose (3a)

To a solution of **3a** (400 mg, 1.3 mmol) in DCM (10 mL) was added the corresponding acid anhydride (propanoic/butanoic/pentanoic and benzoic anhydride, 1.2 equiv.) and a catalytic amount of DMAP, and the reaction mixture was stirred for 4–5 h at room temperature. On completion (analytical TLC), the reaction mixture was poured over ice water and the mixed triester product was extracted with chloroform (2 × 60 ml). The combined organic extract was washed with saturated aqueous $NaHCO_3$ (2 × 50 ml), dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The residue thus obtained was purified by column chromatography on silica gel using ethyl acetate–petroleum ether (1 : 3) as eluent to afford the pure mixed triesters **9a–9d** in 93–95% yields. (See ESI for the characterization data of **9b–9d**.‡)

3-*O*-Acetyl-4-*C*-acetoxymethyl-1,2-*O*-(1-methylethylidene)-5-*O*-propanoyl-β-L-*arabino*-pentofuranose (9a)

Obtained as a viscous oil (445 mg; 95%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.98 (d, J = 4.1 Hz, 1H), 5.36 (s, 1H), 4.62 (d, J = 3.6 Hz, 1H), 4.33 (d, J = 12.6 Hz, 2H), 4.25 (d, J = 11.7 Hz, 1H), 4.09 (d, J = 11.6 Hz, 1H), 2.36 (q, J = 7.2 Hz, 2H), 2.11 (s, 3H), 2.08 (s, 3H), 1.60 (s, 3H), 1.33 (s, 3H), 1.14 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 173.90, 170.40, 169.54, 113.67, 105.45, 85.94, 77.50, 63.21, 62.68, 27.52, 26.90, 26.44, 21.00, 20.81, 9.13; IR (thin film) 2993, 2948, 1751, 1736, 1461, 1432, 1391, 1373, 1235, 1166, 1117, 1079, 1026, 903, 851 cm^{-1}; HRMS *m/z* calculated for $[C_{16}H_{24}O_9Na]^+$ 383.1313, observed 383.1312.

General procedure for lipase-catalyzed selective deacylation of 3-*O*-acetyl-4-*C*-acetoxymethyl-5-*O*-acyl-1,2-*O*-(1-methylethylidene)-β-L-*arabino*-pentofuranose 9a–9d

The Lipozyme® TL IM-catalyzed deacylation reaction on mixed triesters **9a–9d** was performed in the same way as that on compounds **2a–2c**. The products formed due to incubation of triesters **9a–9c** were found to be identical to 3,5-di-*O*-acetyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-α-D-*xylo*-pentofuranose (**3a**). Incubation of 3-*O*-acetyl-4-*C*-acetoxymethyl-5-*O*-benzoyl-1,2-*O*-(1-methylethylidene)-β-L-*arabino*-pentofuranose (**9d**) did not lead to the formation of any product.

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