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Deacylation studies on furanose triesters using an immobilized lipase: Synthesis of a key precursor for bicyclonucleosides†

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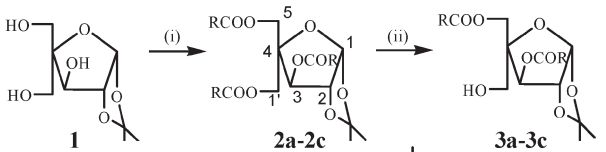
Lipozyme® TL IM immobilized on silica catalyses the deacylation of 4-*C*-acyloxymethyl-3,5-di-*O*-acyl-1,2-*O*-(1-methylethylidene)-*β*-L-*threo*-pentofuranose to form 3,5-di-*O*-acyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-*α*-D-*xylo*-pentofuranose in a highly selective and efficient manner.

The 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,[1–4] and for their direct utilization as anti-tumor or anti-viral compounds.[5,6] 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.[7,8]

In the present study, we have developed a highly efficient chemo-enzymatic route for the diastereo- and regioselective deacylation of one out of the three acyloxy groups of **2a–2c** (Scheme 1), derived from the two primary and one secondary hydroxyl groups of the potentially useful triol **1**, which is an important precursor for the synthesis of different types of bicyclonucleosides.

The trihydroxy sugar derivative **1** was synthesized, starting from D-glucose, by following a modified procedure of Youssefyeh *et al.*;[9] debenzylation of 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-*β*-L-*threo*-pentofuranose (**4**)[10] resulted in the formation of pentofuranose **1** in very poor yield (Scheme 2). In our modified procedure, the two primary hydroxyl groups of **4** were first acetylated to give its diacetyl derivative **5**,[11] which upon debenzylation furnished the novel diacetate 4-*C*-acetyloxymethyl-5-*O*-acetyl-1,2-*O*-(1-methylethylidene)-*β*-L-*threo*-pentofuranose (**6**). The diacetate **6**, on treatment with K_2CO_3–methanol, led to the formation of target compound **1** in an overall yield of 9% from D-glucose (Scheme 2). The trihydroxy sugar derivative **1** was converted to its triacylated derivatives **2a**, **2b** and **2c** using acetic anhydride, propanoic anhydride and butanoic anhydride, respectively, in the presence of a catalytic amount of DMAP in yields of 80–85% (Scheme 1).



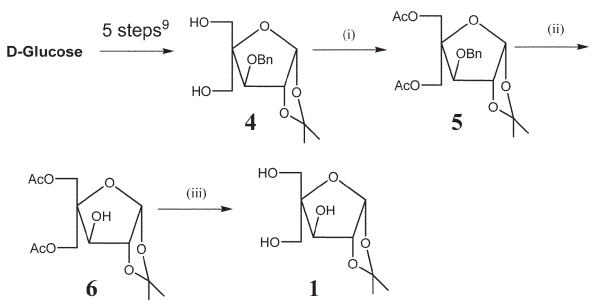
2-3	R
a	-CH_3
b	-CH_2CH_3
c	-$CH_2CH_2CH_3$

Reagents and conditions:
(i) $(RCO)_2O$, DMAP;
(ii) CAL-L(A), toluene, *n*-butanol

Scheme 1 Lipase-catalyzed selective deacylation studies on triacylated pentofuranoses **2a–2c**.

Based on our experience of biocatalytic transacylation reactions on polyhydroxy compounds and their peracylated derivatives, 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), *Theremomyces lanuginosus* lipase immobilized on silica (Lipozyme® TL IM) and the *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 deacylation of compounds **2a–2c** (Scheme 1, Table 1). The other lipases, *i.e.* Novozyme-435, [CAL B-L(A)], PPL and CRL, did not accept any of the acylated sugar derivatives as substrates.

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 on the basis of its IR, ^{1}H NMR, ^{13}C NMR, HRMS and ^{1}H NOE experiments, and comparison of its ^{1}H NMR spectrum with that of the starting triacetate **2a**. Thus,



Reagents and conditions: (i) Ac_2O, DMAP; (ii) H_2/ Pd-C, EtOAc; (iii) K_2CO_3, MeOH

Scheme 2 Synthesis of compound **1** from glucose.

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

Substrate	Product	Reaction time/h	Percentage yield
4-*C*-Acetoxymethyl-3,5-di-*O*-acetyl-1,2-*O*-(1-methylethylidene)-*β*-L-*threo*-pentofuranose (**2a**)	3,5-Di-*O*-acetyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-α-D-*xylo*-pentofuranose (**3a**)	9	98
3,5-Di-*O*-propanoyl-1,2-*O*-(1-methylethylidene)-4-*C*-propanoyloxymethyl-*β*-L-*threo*-pentofuranose (**2b**)	3,5-Di-*O*-propanoyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-α-D-*xylo*-pentofuranose (**3b**)	7	88
4-*C*-Butanoyloxymethyl-3,5-di-*O*-butanoyl-1,2-*O*-(1-methylethylidene)-*β*-L-*threo*-pentofuranose (**2c**)	3,5-Di-*O*-butanoyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-α-D-*xylo*-pentofuranose (**3c**)	4	93

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

the C-3 proton in product **3a** and in the starting triacetate **2a** resonated at practically the same δ value, *i.e.* at 5.29 and 5.35, respectively, which indicated that the acetoxy function at the C-3 position, present in the starting triester **2a**, is intact in the product **3a**. Furthermore, two pairs of doublets, resonating at δ 4.36 and 4.29, and at 4.27 and 4.10, were observed due to C–1′H and C–5H in the ^{1}H NMR spectrum of the starting triacetate **2a**; one pair of these doublets, resonating at δ 4.36 and 4.29, shifted upfield to δ 3.82 and 3.73 in the ^{1}H NMR spectrum of the product diacetate **3a**.

The formation of a single monodeacetylated product indicates that the lipase selectively catalysed the deacetylation of the acetoxy function, either at the C-5 or C-1′ position, in compound **2a**. The NOE study carried out on product diacetate **3a** proved that the acetoxy function at the C-1′ position in **2a** is deacetylated in the presence of the lipase. Thus, the irradiation of the upfield-shifted signals of C–1′H, *i.e.* those resonating at δ 3.82 and 3.73 in compound **3a**, exhibited appreciable NOE (4%) on the C-3 proton resonating at δ 5.29. If the upfield-shifted signals at δ 3.82 and 3.73 had been due to the C-5 protons, their irradiation would have not affected the signal of the C-3 proton because they are not in close proximity.

It has been observed that increasing the chain length of the acyl moiety increases the rate of the 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 (**2a–2c**, **3a–3c** and **6**) were unambiguously established on the basis of their spectral (IR, ^{1}H NMR, ^{13}C NMR, HRMS and NOE) analyses. The structures of the known compounds mentioned in Scheme 2 were further confirmed by comparison of their physical and/or spectral data with those reported in the literature. All deacylation reactions, when performed under identical conditions but without adding any lipase, did not proceed to any extent.

Lipozyme® TL IM discriminated between the three ester functions derived from two primary hydroxyl groups and a secondary hydroxyl group in novel sugar derivatives. Ourselves and others have previously demonstrated the effect of solvent,[12] temperature[13,14] and acylating agents[11] on the selectivity of lipases. The present study demonstrates the selectivity of lipases due to their immobilization on different solid supports. This may be because of the fact that the protein adopts different conformations and geometries on different support surfaces. Also, 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 classical chemical methods alone. The very efficient and convenient enzymatic method discovered for the discrimination between two primary hydroxyl groups of the furanose derivatives herein may find applications in the 'green' synthesis of bicyclonucleosides, important precursors for the preparation of antisense or antigene oligonucleotides.[15]

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