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Lipase-Catalyzed Regioselective One-Step Synthesis of Penta-*O*-acetyl-3-hydroxylactal

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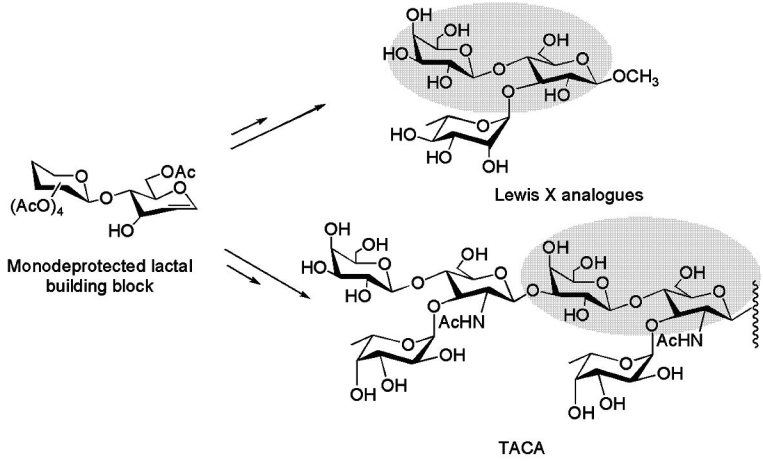
A highly efficient regioselective enzymatic preparation of penta-*O*-acetyl-1,5-anhydro-2-deoxy-3-hydroxy-4-*O*-β-galactopyranosyl-D-arabinohex-1-enitol has been successfully performed for the first time. This product was obtained in >99 % conversion (>95 % overall yield) by hydrolysis of per-*O*-acetylated lactal catalyzed by the lipase from *Rhizomucor miehei* immobilized on octylagarose. This molecule could be a potential building block for efficient synthesis of oligosaccharides or glycoconjugates.

Introduction

Many biologically active small molecules derive their activities from sugar substituents. Changes in the structures of these sugars can have a profound impact on the biological properties of the parent compounds.[1,2]

Different methods for "improving" natural product structures have succeeded in generating focused libraries of structurally related analogues with enhanced pharmaceutical properties.[3]

In this way, pure regioisomers of *O*-acetyllactal presenting a unique free hydroxy group – e.g., obtained from per-*O*-acetylated lactal – could be excellent building blocks for the synthesis of different biological active oligosaccharides, especially with the 3-OH position free in the glucal moiety for the synthesis of sialyl Lewis X analogues or the hexasaccharide $_{dim}Le^{x}$-tumor-associated carbohydrate antigen (TACA)[4] (Scheme 1).



Scheme 1. Synthetic applications of C-3 monohydroxy acetylated lactal in oligosaccharides synthesis. The lactal skeleton is marked by a grey circle.

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Enzymes – especially lipases – represent an alternative to the chemical methods – in some cases more tedious and with low overall yields[5] – for the regioselective deprotection of carbohydrates. Lipases are promiscuous enzymes with a high versatility being able to recognize a broad range of substrates with high regio- and enantioselectivity.[6,7] The properties of these enzymes have been greatly modulated by using different immobilization strategies,[8] due to the great flexibility of their active center, causing drastic conformational changes.[9] This methodology has been successfully applied in racemic mixture resolutions,[8] asymmetric reactions[8] or regioselective deprotection of monosaccharides.[10]

Here we present for the fist time a highly efficient regioselective hydrolysis of per-*O*-acetylated lactal by immobilized preparations of a lipase from *Rhizomucor miehei*.

Results and Discussion

Several lipases from different sources were purified by a well-described method to avoid possible effects of contaminant enzymes.[11] After that, they were immobilized on CNBr-activated agarose by covalent attachment.[12] Then, they were tested in the hydrolysis of per-*O*-acetylated lactal (**1**) (Table 1). Lipases from *Candida antarctica B* (CAL-B), from *Candida rugosa* (CRL) or even the phospholipase LECITASE-ULTRA (LECI) have been described as quite interesting enzymes for monosaccharide hydrolysis,[10] but in this reaction they were poorly active. The lipase from *Rhizomucor miehei* (RML) was identified as the most active enzyme in the hydrolysis of per-*O*-acetylated lactal **1** (Table 1), more than 15-fold compared to LECI or 10-fold compared to CAL-B. Furthermore, other immobilized lipases like lipase from *Pseudomonas fluorescens* (PFL) or from *Aspergillus oryzae* (AOL) also exhibited much lower specific activity.

Table 1. Hydrolytic activity of different lipases immobilized on CNBr-agarose in the hydrolysis of **1**.[a]

Entry	Enzyme	Reaction rate[b]
1	PFL	5
2	LECI	3
3	RML	50
5	AOL	5
6	CAL-B	5
7	CRL	10

[a] **1** (0.01 mmol), biocatalyst (0.4 g), CH_3CN/acetate buffer (20:80) (2 mL), pH 5.00, 25 °C. [b] Initial rate in $\mu mol \times mg_{prot}^{-1} \times h^{-1} \times 10^{-3}$. It was calculated at 10–15% yield.

Therefore, RML was chosen from this preliminary screening as the optimal enzyme to further develop these studies. Next, the enzyme was immobilized by using different protocols, since this method has proven to be very successful for modulating the lipase properties in aqueous media.[8]

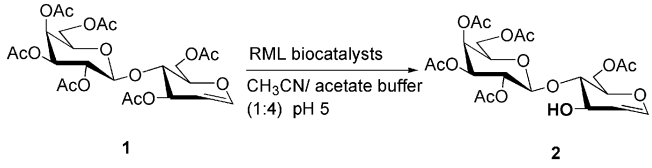
The immobilization of RML on octyl-agarose beads by interfacial activation on the hydrophobic surface[11] gave the most active catalyst for the hydrolysis of **1**, 40-fold higher than the activity observed with CNBr-RML (Table 2). The use of other carriers to immobilize RML as DEAE-agarose beads or Eupergit-C gave biocatalysts less than 5-fold as active as octyl-RML.

Table 2. Specificity and regioselectivity of different immobilized preparations of RML in the hydrolysis of **1**.[a]

Entry	Support	Initial rate[b]	Reaction time (h)	Conversion (%)	**2** (%)[c]
1	Octyl	800	24	100	99
2	CNBr	50	48	100	99
3	DEAE	195	24	100	92
4	EupergitC	160	36	100	80

[a] **1** (0.01 mmol), biocatalyst (0.4 g), CH_3CN/acetate buffer (20:80) (2 mL), pH 5.00, 25 °C. [b] Initial rate in $\mu mol \times mg_{prot}^{-1} \times h^{-1} \times 10^{-3}$. It was calculated at 10–15% yield. [c] Yield of 3-OH monohydroxy acetylated product (**2**).

All RML preparations were quite regioselective towards the deprotection of the acetyl group in C-3 of **1**[13] (Scheme 2). However, the final yield of the product depended on the biocatalyst used. The octyl-RML and CNBr-RML catalysts produced 3-OH monodeprotected product **2** with 99% yield at 100% conversion; whereas the DEAE-RML and EupergitC-RML preparations gave 92% and 80% yield, respectively (producing some by-products) (Table 2).



Scheme 2. Regioselective hydrolysis at the C-3 position of per-*O*-acetylated lactal **1** catalyzed by different RML-immobilized preparations.

Finally, the reaction was scaled up to produce **2** starting from 8 g/L of **1**[14] dissolved in sodium acetate buffer (25 mM) with (20%, v/v) acetonitrile at pH 5 and by using octyl-RML as catalyst. Complete consumption of substrate **1** was achieved after 24 h (Figure 1) with 99% conversion of **2**. The activity and regioselectivity of the catalyst was preserved even after 5 cycles of reaction (see Supporting

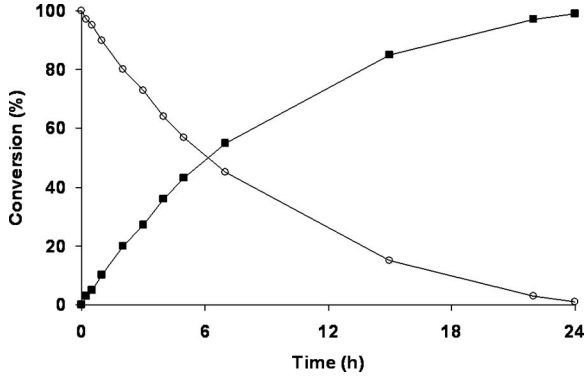


Figure 1. Reaction course of the hydrolysis of **1** catalyzed by the octyl-RML preparation; empty circles: **1**; squares: **2**.

Information). The new product **2** was synthesized by this protocol in high purity without further purification (Figure 2) – just simple extraction (see Supporting Information) – with 95% overall yield. ^{13}C NMR spectrum, FAB mass spectrum, elemental analysis, melting point and optical rotation were performed to complete the product characterization.[15]

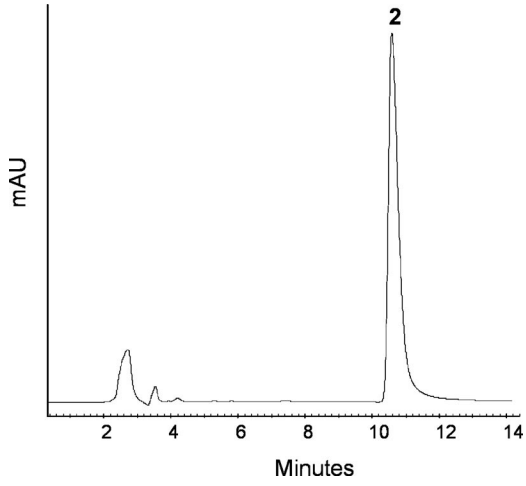


Figure 2. HPLC trace of **2** after hydrolysis catalyzed by octyl-RML.

Conclusions

We have described for the first time a simple and efficient enzymatic approach for the regioselective hydrolysis of hexa-*O*-acetyllactal to obtain penta-*O*-acetyl-3-hydroxylactal by hydrolysis catalyzed by the octyl-RML preparation. The correct selection of the lipase source and the immobilization protocol were the key point for these very good results. The product was obtained without any further purification – just by simple extraction with ethyl acetate – after the biocatalytic reaction in 95% overall yield.

Therefore, a building block with great potential applications has been synthesized by this one-step enzymatic method with possible orthogonal modifications of the free OH group and of the double bond[16] to prepare oligosaccharides or glycoconjugates in an efficient manner. Furthermore, the high hydrolytic rate achieved for this biocatalyst in the hydrolysis of peracetylated lactal could make possible the application of this process at laboratory as well as at industrial scale.

Supporting Information (see footnote on the first page of this article): Experimental procedures.

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[12] All the lipase-immobilized preparations were containing 4 mg of pure lipase per gram of solid. For more details, see Supporting Information.
[13] **2**: ^1H NMR (500 MHz, $CDCl_3$): δ = 6.33 (d, J = 5.90 Hz, 1 H, 1-H), 5.38 (d, J = 3.39 Hz, 1 H, 4′-H), 5.25 (t, J = 8.37 Hz, 1 H, 2′-H), 5.00 (dd,1 H, 3′-H), 4.76 (dd, 1 H, 2-H), 4.59 (d, J = 8.0 Hz, 1 H, 1′-H), 4.43 (m, 1 H, 3-H), 4.20–4.10 (m, 2 H, 6a-,6′b-H), 4.13–4.07 (m, 2 H, 6′a-,6′b-H), 4.08 (m, 1 H, 5′-H), 3.98 (m, 1 H, 5-H), 3.63 (m, 1 H, 4-H), 2.15–2.09 (5 s, 15 H, 5 CH_3) ppm. 2D-COSY was used to assign the signals.
[14] For the scale-up of the reaction to 8 g/L, 0.71 mmol of **1** was hydrolyzed in 50 mL of a solution of 50 mM sodium acetate (80%) and acetonitrile (20%) at pH 5 by using 5 g of octyl-RML preparation.
[15] ^{13}C NMR (300 MHz,$CDCl_3$): δ = 170.58, 170.03, 169.60, 143.75, 102.66, 102.19, 81.96, 73.77, 71.51, 70.82, 68.70, 68.26, 66.98, 62.58, 62.10, 20.79, 20.59, 20.55, 20.45 ppm. $[\alpha]_D^{25}$ = +60 (c = 0.48, CH_2Cl_2). M.p. 56.2–57.5 °C. MS (FAB): calcd. for $C_{22}H_{30}O_{14}$ 518.1636; found 541.1541 [M + Na]. $C_{22}H_{30}O_{14}$ (518.16): calcd. C 50.96, H 5.83, O 43.20; found C 49.55, H 5.72, O 42.80.
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