

REGIOSELECTIVE SYNTHESIS OF D-GLUCAL DERIVATIVES USING LIPASES

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ABSTRACT

Free lipases from different sources were tested in the regioselective hydrolysis of 3,4,6-tri-O-acetyl-D-glucal in phosphate buffer and in the acetylation of D-glucal with vinyl acetate. The conversion and selectivity of these reactions depended strongly on the lipase used. In the hydrolysis of 3,4,6-tri-O-acetyl-D-glucal, the lipases from *P. cepacia* and *P. fluorescens* hydrolyzed mainly the 3 position (>99% of conversion) while the *C. rugosa* gave the hydrolysis only in position 6 (65% of conversion). In the acetylation reaction of D-glucal, *C. antarctica* lipase B (CALB) gave the highest conversion in diacetylated product, at the position 3 and 6. While *C. rugosa* lipase catalyzed the formation of the monoacetylated product, at the position 6, in 60% conversion.

Keywords: Lipases. Keyword 2. D-glucal. Keyword 4. Keyword 5.

1 INTRODUCTION

Rare sugars are frequently found in complex mixtures or closely linked with other molecules in nature, making their extraction a laborious and ineffective process. As a result, there is a greater need than ever to advance synthesis techniques for valuable sugars. This is mostly because it can be difficult to get sufficient quantities of pure and useful sugar products from natural sources¹. This demand highlights the importance of developing innovative synthesis methods. Over the last 60 years, a specific type of sugar, hex-2-ene pyranoses, also known as glycals, have been fundamental to advances in synthesis and understanding the mechanisms of carbohydrate chemistry². Their distinct enol-ether moiety on a pyranose ring confers remarkable reactivity, establishing them as one of the most versatile monosaccharide derivatives with functionalization potential to date. Its remarkable versatility is evidenced in various reactions, including oxidation, rearrangements, nucleophilic additions, and substitutions². In this context, many important pharmaceutical products have been synthesized from D-glucal through various synthetic routes². In the biocatalysis context, hydrolytic enzymes such as lipases, esterases and proteases, are important catalysts that are very useful in regioselective organic synthesis of polyfunctionalized molecules³. Lipases can be used in complex transformations without the need for tedious protecting and deprotecting steps due to the selectivity that these enzymes present against one among several hydroxyl groups with similar reactivity³. Furthermore, lipase-catalyzed reactions have the general advantage of mild reaction conditions and high efficiency.

2 MATERIAL & METHODS

2.1 Chemicals

tri-O-acetyl-D-glucal and vinyl acetate were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA). The lipases used are listed in Table 1.

Table 1 Lipases used in this work

Lipase	Activity (U mg ⁻¹)
<i>Aspergillus niger</i>	390
<i>Aspergillus oryzae</i>	318
<i>Candida antarctica B</i>	164
<i>Candida rugosa</i>	107
<i>Pseudomonas cepacia</i>	366
<i>Pseudomonas fluorescens</i>	307
<i>Thermomyces lanuginosus</i>	544

2.1 Hydrolysis of tri-O-acetyl-D-glucal

For the hydrolysis of peracetylated 3,4,6-tri-O-acetyl-D-glucal, 2.0 mg of the lipase was added to a solution (1.5 mL) of substrate-1 (1 mM) in 25 mM of acetate (pH 5.0) buffer. The reaction was carried out at 25° C, 24 h and 50 rpm. Samples were removed and analyzed by reverse phase HPLC (Spectra Physic SP 100, Thermo Fisher-Scientific, Waltham, MA, USA) using a Kromasil

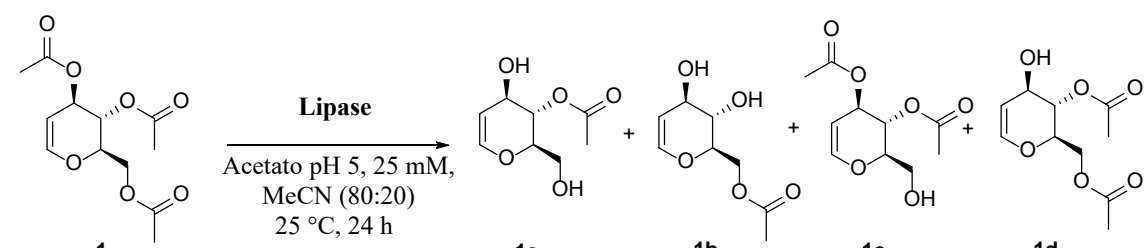
C18 column (25 cm × 0.4 cm, 5 μm-Ø) and a UV detector (Spectra Physic SP 8450, Thermo Fisher-Scientific, Waltham, MA, USA) set at 220 nm. The mobile phase utilized was acetonitrile (20%) in milli-Q water.

3 RESULTS & DISCUSSION

3.1 Hydrolysis and acetylation reactions

Different lipases in free form were used in this work to catalyze the hydrolytic deacetylation of 3,4,6-tri-O-acetyl-D-glucal (**1**). When using TLL lipase, product **1a** was obtained with 70% conversion, and product **1b** and **1d** were formed with 15% conversion, respectively. When using the *C. rugosa* lipase, product **1b** was obtained with 65% conversion. The best results were obtained with the lipases, from *P. cepacia* and *P. fluorescens*, in which product **1d** was obtained with >99% conversion. With the *A. oryzae* lipase, a mixture of hydrolysis products was obtained.

Table 2 Regioselective hydrolysis of 3,4,6-tri-O-acetyl-D-glucal (**1**) using lipases

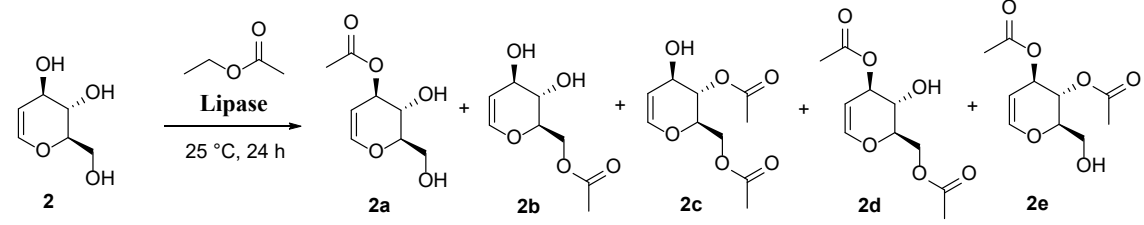


Lipase	Conversion (%)				
	1a	1b	1c	1d	
<i>Aspergillus oryzae</i>	26	8	16	19	
<i>Candida antarctica B</i>	20	nd	nd	80	
<i>Candida rugosa</i>	nd	65	nd	nd	
<i>Pseudomonas cepacia</i>	nd	nd	nd	>99	
<i>Pseudomonas fluorescens</i>	nd	nd	nd	>99	
<i>Thermomyces lanuginosus</i>	70	15	nd	15	

nd = not detected

Similarly, different free lipases were used in this work to catalyze the acetylation of D-glucal (**2**) with vinyl acetate. The best result was obtained with CALB lipase, in which the diacetylated product **2d** was obtained with 80% conversion. When using *C. rugosa* lipase, the monoacetylated product **2b** was obtained with 65% conversion. On the other hand, the diacetylated product **2c** was obtained with *P. fluorescens* lipase with 55% conversion.

Table 3 Regioselective acetylation of D-glucal (**2**) with vinyl acetate using lipases



Lipase	Conversion (%)				
	2a	2b	2c	2d	2e
<i>Aspergillus niger</i>	25	nd	nd	nd	32
<i>Candida antarctica B</i>	20	nd	nd	80	nd
<i>Candida rugosa</i>	nd	65	10	nd	<5
<i>Pseudomonas cepacia</i>	nd	23	11	nd	nd
<i>Pseudomonas fluorescens</i>	nd	21	55	18	nd

nd = not detected

4 CONCLUSION

Different lipases were tested for the regioselective hydrolysis of 3,4,6-tri-O-acetyl-D-glucal and the regioselective acetylation of D-glucal with vinyl acetate. The conversion and selectivity of the hydrolysis reaction depended strongly on the lipase used. The highest conversion of the mono-deacetylated product **1d** was >99%, at pH 7 and 25 °C using the lipases from *P. cepacia*, *P. fluorescens*. In the acetylation reaction the highest conversion of the monoacetylated product **2b** was 65% obtained with the lipase

from *C. rugosa*. For the diacetylated product the highest conversion was 80% when the *C. antarctica* lipase B (CALB) was used. This versatile strategy could also be applied to synthesize other types of glucals, producing sugars with different properties. These biocatalysts could be immobilized used to produce products that are difficult to synthesize by traditional methods. These deacetylated glycosides are valuable and fascinating building blocks for the study of carbohydrates. Thus, a variety of modified substrates combined with combinatorial biocatalysis, which uses various immobilized enzymes in different ways, allows for the production of monodeacetylated sugars at specific positions, with particular interest in the creation of novel glyco-derivatives or glycoconjugates for biological applications.

REFERENCES

- ¹ MIJAILOVIC, N., NESLER, A., PERAZZOLLI, M., AÏT BARKA E, AZIZ, A. 2021. *Molecules*. 26 (6).1720.
- ² KINFE, H. H., 2019. *Org. Biomol. Chem.* 17. 4153-4182.
- ³ FERNANDEZ-LORENTE, G., PALOMO, J. M., COCCA, J., MATEO, C., MORO, P., TERRENI, M., FERNANDEZ-LAFUENTE, R., GUIBAN, J. M., 2003, *Tetrahedron Lett.*, 59, 30, 5705-5711.

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