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SHORT PUBLICATION

An improved regioselective preparation of methyl 2,3-di-O-acetyl-α,β-D-xylofuranoside

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Abstract
Methyl 2,3-di-*O*-acetyl-α,β-D-xylofuranoside was prepared as the sole regioisomer in 63–72% yield, according to the applied mass of substrate, through a *Candida antarctica* lipase B catalysed alcoholysis of methyl 2,3,5-tri-*O*-acetyl-α,β-D-xylofuranoside. The product is a potential synthetic precursor for 5-modified xylofuranosides and 5′-modified xylonucleosides.

Keywords: *Candida antarctica lipase B, regioselectivity, methyl 2,3-di-O-acetyl-α,β-D-xylofuranoside*

Introduction

Regioselectively acylated carbohydrates are useful synthetic precursors of many natural product analogues. Although at present biocatalysis provides convenient methodologies to prepare acylated carbohydrate derivatives, most of the products are hexopyranoses, while pentofuranoses have received less attention (Kadereit & Waldmann 2001; Filice et al. 2010; Fernández-Lorente et al. 2003; Chien & Chern 2004; Jun et al. 2005; D'Antona et al. 2005; Mastihubová et al. 2006; Prasad et al. 2007).

In our laboratory, we have been studying the preparation of regioselectively acetylated furanoses carrying 5-hydroxyl groups through *Candida antarctica* B lipase (CAL-B) catalysed deacetylation of the corresponding 5-acetylated compounds. Deacetylation can be achieved by either hydrolysis or alcoholysis; however, the alcoholysis of methyl 2,3,5-tri-*O*-acetyl-α-D-ribofuranoside provided regioselectively methyl 2,3-di-*O*-acetyl-α-D-ribofuranoside, while its hydrolysis afforded a non regioselective mixture of products (Iñigo et al. 2005).

Further experiments on acetylated alkyl α,β-D-ribofuranosides alcoholysis showed that the anomeric substituent affects CAL-B regioselectivity (Gudiño et al. 2010). Regarding furanose stereochemistry other than ribose, CAL-B catalysed alcoholysis of some 2-deoxy- and arabinofuranoses allowed the regio- and stereoselective preparation of methyl 3-*O*-acetyl-2-deoxy-α-D-ribofuranoside, 1,3-di-*O*-acetyl-2-deoxy-α-D-ribofuranose and 1,2,3-tri-*O*-acetyl-α-D-arabinofuranose in de = 100% from the anomeric mixtures of the corresponding 5-*O*-acetylated compounds; methyl 2,3,5-tri-*O*-acetyl-α,β-D-arabinofuranoside reacted with poor regio- and stereoselectivity (Gudiño et al. 2009).

Methyl 2,3-di-*O*-acetyl-α,β-D-xylofuranoside (**2**) can be used as a synthetic intermediate in the preparation of 5-modified-2,3-di-*O*-acetylated xylofuranosides, such as the corresponding diacetylated xylofuronic acid (Epp & Widlanski 1999; Lederkremer & Marino 2003; Devine & Scammells 2008), and further employed for the synthesis of 5-modified xylonucleosides, through glycosidation reactions (Devine & Scammells 2008; Maity et al. 2008).

In spite of this, only a few studies report the satisfactory preparation of regioselectively acetylated xylofuranosides. In the field of biocatalysis, most of the references above, involving pentofuranoses (Kadereit & Waldmann 2001; Filice et al. 2010; Fernández-Lorente et al. 2003; Chien & Chern 2004; Jun et al. 2005; D'Antona et al. 2005;

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Mastihubová et al. 2006; Prasad et al. 2007) deal with ribo and arabino moieties. Concerning xylofuranosides, Wong and co-workers (Hennen et al. 1988) have reported the only previous synthesis of methyl 2,3-di-*O*-acetyl-α-D-xylofuranoside (**2**α), based on a *Candida rugosa* (formerly *Candida cylindracea*) lipase (CRL) catalysed hydrolysis at pH 7.0 of methyl 2,3,5-tri-*O*-acetyl-α,β-D-xylofuranoside (**1**). However, the biotransformation gave a 5:3 mixture of methyl 2,3-di-*O*-acetyl-α-D-xylofuranoside (50%) and methyl 2,5-di-*O*-acetyl-β-D-xylofuranoside. In a previous paper from us applying *Musa sapientum* as the biocatalyst (Taverna-Porro et al. 2007), **1** afforded a complex mixture of products. In this paper, we report an efficient regioselective preparation of methyl 2,3-di-*O*-acetyl-α,β-D-xylofuranoside (**2**) through a CAL-B catalysed alcoholysis of **1** (Scheme 1).

Materials and methods

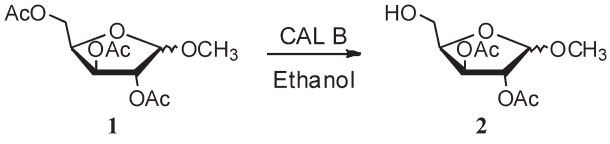
General methods

NMR spectra were recorded on a Bruker AC-500 spectrometer in $CDCl_3$, at 500 MHz for ^1H and 125 MHz for ^{13}C using TMS and $CDCl_3$ as internal standards, respectively. Coupling constants ($\mathcal{J}$) are reported in hertz and the chemical shifts (δ) in parts per million. Assignments were done on the basis of 2D HSQC.

All reagents and solvents employed were of analytical grade and obtained from commercial sources. The alcohols and pyridine were dried and distilled prior to use. Absolute ethanol was used for the enzymatic alcoholysis.

TLC was performed on Silicagel 60 F_{254} plates (Merck) and the resulting plates were developed using ethanol–sulphuric acid 80:20 v/v with heating. Silicagel Merck 60 was used for flash column chromatography.

Lipase B from *Candida antarctica* (CAL-B, Novozym 435, 10,000 PLU/mg solid; PLU: Propyl Laurate Units) was a generous gift from Novozymes (Brazil) and *Candida rugosa* lipase (CRL, 875 units activity/mg solid) was purchased from Sigma Chemical Co. Both enzymes were used straight without any further treatment or purification.



1 **2**

Scheme 1. *Candida antarctica* B lipase (CAL-B) catalysed regioselective preparation of methyl 2,3-di-*O*-acetyl-α,β-D-xylofuranoside (**2**).

Enzymatic reactions were carried out in a temperature-controlled incubator shaker (Sontec OS 11, Argentina).

Methyl 2,3,5-tri-*O*-acetyl-α,β-D-xylofuranoside (**1**) was prepared through standard protocols (Shi et al. 2002). Purification of the crude product by column chromatography employing dichloromethane–methanol 98:2 v/v as the eluent afforded the desired substrate (58%), which afforded satisfactory NMR data.

Analytical procedure for CAL-B catalysed deacetylation of 1

In a typical analytical protocol, the substrate (10 mg) was dissolved in the alcohol at the alcohol/substrate molar (A/S) ratio = 1200, 660 or 120 and CAL-B (300 mg $mmol^{-1}$ substrate) was added. When assaying a co-solvent, it was added in such a volume that it represented 10% of the final reaction volume. In the case of the reactions conducted in a low excess of alcohol (A/S = 3), the final reaction volume contained 99.3% of the solvent.

The resulting reaction mixtures were shaken at 200 rpm and 30°C or 45°C; samples were taken at different times and after removal of the enzyme, monitored by TLC using dichloromethane–methanol 95:5 v/v as the mobile phase.

Control experiments carried out in the absence of the enzyme showed no appreciable reaction.

Preparative procedure: methyl 2,3-di-O-acetyl-α,β-D-xylofuranoside (2)

According to the above described protocol, for preparative purposes the substrate (100 mg, 0.31 mmol) was dissolved in tert-butylmethylether (7.7 ml) and ethanol (0.053 ml, A/S = 3) and CAL-B (93 mg) was added. The reaction mixture was shaken at 200 rpm and 45°C for 24 hours. Then the lipase was filtered off, washed with dichloromethane and the resulting filtrate evaporated; the crude product was purified by silicagel column chromatography using dichloromethane–methanol 98:2. Isolated compound **2** (72% yield; α.β – 1.0/1.4) gave satisfactory NMR data consistent with those previously reported for **2**α (Hennen et al. 1988): ^1H NMR ($CDCl_3$, 500 MHz): 2.11, 2.12, 2.13 (3s, 12H, $COCH_3$s, 2α and 2β), 3.40 (s, 3H, OCH_3, 2α), 3.45 (s, 3H, OCH_3, 2β), 3.66–3.72 (2m, 4H, H-5 and H-5′, 2α and 2β), 4.35–4.38 (m, 1H, H-4, 2β), 4.47–4.51 (m, 1H, H-4, 2α), 4.88 (d, 1H, $\mathcal{J}_{1,2}$ 1.1, H-1, 2α), 5.05 (dd, 1H, $\mathcal{J}_{2,3}$ 6.0, $\mathcal{J}_{2,1}$ 4.7, H-2, 2β), 5.15 (d, 1H, $\mathcal{J}_{1,2}$ 4.7, H-1, 2β), 5.19 (dd, 1H, $\mathcal{J}_{2,3}$ 2.7, $\mathcal{J}_{2,1}$ 1.1, H-2, 2α), 5.30 (dd, 1H, $\mathcal{J}_{3,4}$ 6.6, $\mathcal{J}_{3,2}$ 2.7, H-3, 2α), 5.52 (dd,

1H, $J_{3,4}$ 7.1, $J_{3,2}$ 6.0, H-3, 2β). ^{13}C NMR ($CDCl_3$, 125 MHz): 20.64, 20.70, 20.74, 20.78 ($COCH_3$s, 2α and 2β), 55.56, 55.59 (OCH_3s, 2α and 2β), 60.85 (C-5, 2β), 61.44 (C-5, 2α), 75.06 (C-3, 2β), 75.38 (C-3, 2α), 75.66 (C-4, 2β), 76.94 (C-2, 2β), 80.84 (C-2, 2α), 80.89 (C-4, 2α), 99.53 (C-1, 2β), 106.84 (C-1, 2α), 170.36, 170.55, 170.64, 170.65 (COs, 2α and 2β).

Application of the above preparative procedure to 500 mg and 1 g of the substrate produced **2** in 65% and 63% yield, respectively.

Results and discussion

Previous studies by us showed that the alcohol/substrate (A/S) ratio affects the regioselectivity of CAL-B catalysed alcoholysis, a better regioselectivity have been reached at very high A/S ratios (A/S >100) in the deacetylation of furanoses (Iñigo et al. 2005; Gudiño et al. 2009; Gudiño et al. 2010) and nucleosides (Zinni et al. 2007; Sabaini et al. 2010; Ferrero & Gotor 2000a, 2000b, Li et al. 2010). Thus, we tested CAL-B catalysed alcoholysis of **1** at A/S = 1200, 660 and 120; the former ratio gave a poor conversion of the substrate, while the latter two did not provide regioselective reactions. Replacement of ethanol by alcohols, such as propanol, butanol and isopropanol, at the mentioned A/S ratios, did not afford better results. In an attempt to improve regioselectivity, a solvent was added to the reaction mixtures; thus, petroleum ether, tert-butylmethylether (TBME), dichloromethane, acetone, dioxane and acetonitrile were tested. Since again no satisfactory results were obtained, we decided to carry out the reactions in a low alcohol (ethanol, 1-butanol) content medium (A/S = 3) containing the solvents listed above. The best results were found with ethanol–TBME, which allowed the preparation of **2** in 72% yield. It should be mentioned that in 1-butanol–TBME, CAL-B has been reported (D'Antona et al. 2005) to recognise the anomers of the hexose 1,2,3,4,6-penta-*O*-acetyl-D-fructofuranose differentially, the α-anomer affording 1,2,3,4-tetra-*O*-acetyl-α-D-fructofuranose and the β one, 2,3,4,6-tetra-*O*-acetyl-β-D-fructofuranose. In our case, the enzymatic alcoholysis producing **2** occurred with similar recognition for both anomers: the ratio of α,β-epimers was 1.0/1.1 for **1** and 1.0/1.4 for **2**, as determined by integration of NMR H-1 signals. Compound **2**β has not previously been reported in the literature.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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