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Deprotection of peracetylated methyl D-ribosides through enzymatic alcoholysis: Different recognition of the anomers

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Abstract

The anomers methyl 2,3,5-tri-*O*-acetyl-α-D-ribofuranoside and methyl 2,3,5-tri-*O*-acetyl-β-D-ribofuranoside showed a different behaviour in the *Candida antarctica* B lipase-catalysed alcoholysis. While the enzymatic deprotection of the former proceeded regioselectively affording methyl 2,3-di-*O*-acetyl-α-D-ribofuranoside in 81% yield in 3 h at 45 °C showing no further transformation, the alcoholysis of the β-diasteromer was less selective. For this anomer, mixtures of partially acetylated products were formed, but contrasting to the α epimer, full deacetylated methyl β-D-ribofuranoside was quantitatively formed at long reaction times (5 days).

Keywords: Deacetylation; Enzymatic alcoholysis; Lipases; Regioselectivity; Ribosides

1. Introduction

Partially acylated carbohydrates are key intermediates in the preparation of oligosaccharides, glycopeptides and modified nucleosides [1,2]; in particular, sugar monoesters are important as biodegradable surfactants [3]. Due to the polyhydroxylated nature of sugars, the preparation of partially acylated derivatives has been traditionally carried out using protection–deprotection procedures, usually yielding mixtures of products.

At present, hydrolases are well-recognized biocatalysts for the regioselective transformation of polyhydroxylated compounds and therefore, they provide an useful access to partially acylated carbohydrates [4–6]. Even when the literature describes many examples of the regioselective modification of carbohydrates through enzymatic acylation or deacylation of peracylated derivatives, most of them involve hexopyranose rings; in contrast, the enzymatic regioselective transformation of pentofuranoses has been studied in a lesser extent [5–11]. Among the enzymatic deacetylations related to pentofuranosides, *Candida rugosa* lipase (CRL)-catalysed hydrolysis of methyl 2,3,5-tri-*O*-acetyl-D-ribofuranoside [9,10] and 1,2,3,5-tetra-*O*-acetyl-ribofuranose [11] afforded the corresponding 5-hydroxyl free derivatives.

During the last years we have been studying the *Candida antarctica* B lipase (CAL B)-catalysed alcoholysis of peracylated nucleosides and this procedure provided a simple and efficient access of 2′,3′-di-*O*-acetyl ribonucleosides [12,13], 2′,3′-di-*O*-acyl ribonucleosides carrying lipophilic acyl groups [14] and 3′-*O*-acetyl-2′-deoxy nucleosides [15]. As far as we know, no results have been published on CAL B-catalysed deacetylation of carbohydrate pentofuranoside derivatives, the only work being a paper dealing with pyranosides [16]. In this work we report the different

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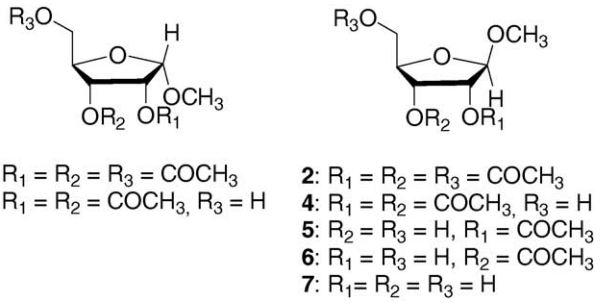

1: $R_1 = R_2 = R_3 = COCH_3$
3: $R_1 = R_2 = COCH_3$, $R_3 = H$
2: $R_1 = R_2 = R_3 = COCH_3$
4: $R_1 = R_2 = COCH_3$, $R_3 = H$
5: $R_2 = R_3 = H$, $R_1 = COCH_3$
6: $R_1 = R_3 = H$, $R_2 = COCH_3$
7: $R_1 = R_2 = R_3 = H$

Scheme 1. .

behaviour exhibited by the diasteromers methyl 2,3,5-tri-*O*-acetyl-α-D-ribofuranoside (**1**, Scheme 1) and methyl 2,3,5-tri-*O*-acetyl-β-D-ribofuranoside (**2**, Scheme 1) in CAL B-catalysed alcoholysis.

2. Experimental

2.1. General

Lipase B from *C. antarctica* (CAL B, Novozym 435, 10,000 PLU/mg solid; PLU: propyl laurate units) was a generous gift from Novozymes (Brazil). The enzyme was used straight without any further treatment or purification. All employed reagents and solvents were of analytical grade and obtained from commercial sources. Methanol and pyridine were dried and distilled prior to use.

TLC was performed on Silicagel 60 F_{254} plates (Merck) and column chromatography was carried out using Silicagel Merck 60. For TLC analyses, dichloromethane–methanol 95:5 (v/v) was used as the mobile phase. TLC plates were revealed using ethanol–sulphuric acid 80:20 (v/v) with heating.

NMR spectra were recorded on a Bruker AC-500 spectrometer in $CDCl_3$, at 500 MHz for ^{1}H and 125 MHz for ^{13}C using TMS and $CDCl_3$ as internal standards, respectively.

Enzymatic reactions were carried out in a temperature-controlled incubator shaker (Sontec OS 11, Argentina) at 200 rpm and 30 or 45 °C.

2.2. Preparation of methyl 2,3,5-tri-O-acetyl-α-D-ribofuranoside (1) and methyl 2,3,5-tri-O-acetyl-β-D-ribofuranoside (2)

Substrates **1** and **2** were prepared by modification of previously described methods [17]. Reaction of ribose in methanol containing H_2SO_4 and $CuSO_4$ afforded methyl α,β-D-ribofuranoside; subsequent reaction with acetic anhydride and dimethylaminopyridine in pyridine gave a mixture of peracetylated anomers. Isolation of each anomer was carried out by silica gel column chromatograpy, affording methyl 2,3,5-tri-*O*-acetyl-α-D-ribofuranoside (**1**) and methyl 2,3,5-tri-*O*-acetyl-β-D-ribofuranoside (**2**). Both epimers afforded satisfactory NMR data.

2.3. General procedure for CAL B-catalysed deacetylation of 1 and 2

Typically, **1** or **2** (20 mg, 0.069 mmol) was dissolved in ethanol (0.48 mL, 8.28 mmol) and CAL B (20 mg) was added. The reaction mixture was shaken at 200 rpm at 30 or 45 °C. Samples were taken at different times and monitored by TLC.

Experiments of CAL B-catalysed hydrolysis were carried out according to the above-described protocol replacing the alcohol by the same volume of potassium phosphate buffer (30 mM, pH 7).

Control experiments carried out in the absence of the enzyme showed no reaction of **1** and **2**.

2.4. Preparative procedure for methyl 2,3-di-O-acetyl-α-D-ribofuranoside (3)

Compound **1** (50 mg, 0.17 mmol) was dissolved in ethanol (1.25 mL) and CAL B (52 mg) was added. The reaction mixture was shaken at 45 °C and 200 rpm for 3 h; then the lipase was filtered off, washed with dichloromethane and the resulting filtrates evaporated. The crude product was purified by silica gel column chromatography using dichloromethane–methanol 98:2, yielding **3** (35 mg, 81% yield) as a syrup that provided NMR data according to previously reported spectra [9].

2.5. CAL B-catalysed alcoholysis of 2

2.5.1. At 6 h

Compound **2** (51 mg, 0.18 mmol) was dissolved in ethanol (1.25 mL) and CAL B (52 mg) was added. The reaction mixture was shaken at 30 °C and 200 rpm for 6 h and then, the biotransformation was stopped, the enzyme filtered off and the ethanol evaporated. The crude obtained residue (42 mg) was analysed by ^{1}H NMR.

2.5.2. At 24 h

Following the above-described procedure, the biotransformation was carried out for 24 h. The resulting crude was analysed by ^{1}H NMR, revealing a mixture of methyl 2-*O*-acetyl-β-D-ribofuranoside and methyl 3-*O*-acetyl-β-D-ribofuranoside in a 1.5:1 ratio. The residue was then purified by silica gel column chromatography using diclorometane–methanol 98:2 (v/v) affording the mixture of monoacetylated products in 45% yield.

2.5.3. At 7 days

In a similar way, the biotransformation was conducted for 7 days. At this time, TLC analysis of an aliquot of the biotransformation showed a sole spot coinciding with the R_f of a patron of methyl β-D-ribofuranoside, indicating full quantitative deacetylation.

3. Results and discussion

First, CAL B-catalysed alcoholysis of methyl 2,3,5-tri-*O*-acetyl-α-D-ribofuranoside (**1**) was studied. Since a very high ethanol/substrate ratio (E/S = 1300) at 30 °C provided conditions that favoured the regioselective CAL B-catalysed deprotection of 5′-*O*-acyl groups of peracylated nucleosides [12–15], such experimental conditions were initially employed. As only low conversion of the substrate was observed after 48 h, the alcoholysis was carried out at a minor ethanol/substrate ratio (E/S = 120). In this case, the regioselective formation of a sole product was observed at 3 h; after purification by column chromatography, it was isolated in 65% yield and its structure determined as methyl 2,3-di-*O*-acetyl-α-D-ribofuranoside (**3**, Scheme 2; Entry 1, Table 1). The ^{13}C NMR spectrum of **3** showed two pairs of related signals at 20.52 and 20.83 ppm and at 170.02 and 170.79 ppm corresponding to the methyls and carbonyl groups of the acetyls. A signal at 3.73–3.81 ppm in the ^{1}H NMR spectrum of **3** was consistent with the chemical shifts of the 5-hydrogens attached to a carbon bearing a primary free alcohol.

The regioselectivity showed by CAL B in the alcoholysis of **1** is in agreement with the selectivity displayed by this enzyme in the direct acylation of furanoses [18,19]: in all cases, a preference towards the primary hydroxyl group is observed.

CAL-B alcoholysis of **1** was also studied at very long times (21 days), showing no remarkable further reaction; only traces of three products were formed, assessed as monoacetylated derivatives and methyl α-D-ribofuranoside, due to their TLC mobility. When the biotransformation was studied at 45 °C, using the E/S ratio = 120, the qualitative reaction profile agreed with that obtained at 30 °C, but **3** could be isolated in 81% yield at 3 h (Entry 2, Table 1).

Compared to **1**, methyl 2,3,5-tri-*O*-acetyl-β-D-ribofuranoside (**2**, Scheme 1) showed a lesser conversion at an E/S ratio = 120 and 30 °C, but even at short reaction times the formation of a diacetylated and monoacetylated products was observed by TLC. Therefore, the diacetylated product could not be obtained in good yield even at the time of its maximal formation (6 h). At this time, the reaction was stopped and after removal of the lipase and the ethanol, the crude reaction mixture was analysed by NMR without further purification. The ^{1}H NMR spectrum of the mixture showed signals at 3.58–3.66 and 3.72–3.80 ppm, typical chemical shifts of hydrogens attached to a carbon bearing a free primary hydroxyl group, in an equal ratio. This would suggest that the diacetylated product in the mixture was methyl 2,3-di-*O*-acetyl-β-D-ribofuranoside (**4**, Scheme 1) and would indicate the same regioselectivity of the lipase towards the removal of the first acetyl group in both anomers (Entry 3, Table 1). As in the CAL B-catalysed butanolysis of pentaacetylated glucosamine epimers [16], the α epimer afforded more regioselective results and the reaction did not further proceed.

At 24 h, a different behaviour was again observed for the alcoholysis of **2** if compared with **1**. The reaction mixture consisted of monoacetylated products and methyl β-D-ribofuranoside (**7**, Scheme 1) and only minor amounts of **2** and **4** were detected. The crude reaction mixture was purified by column chromatography, isolating the mixture of monoacetylated compounds in 45% yield (Entry 4, Table 1), identified as methyl 2-*O*-acetyl-β-D-ribofuranoside (**5**, Scheme 1) and methyl 3-*O*-acetyl-β-D-ribofuranoside (**6**, Scheme 1) on the basis of bidimensional heteronuclear and ^{1}H homodecoupling experiments (data not shown).

However, at longer reaction times (7 days) **7** was quantitatively formed (Entry 5, Table 1). At 45 °C the biotransformation of **2** proceeded with a similar profile, but the time required to achieve full quantitative deacetylation was slightly shortened (5 days; Entry 6).

Experiments were also carried out to evaluate the CAL B-catalysed hydrolysis of **1** and **2** (pH 7 at 30 °C), finding again differences in the behaviour of the anomers. Although the performance of each diasteromer agreed qualitatively with the results obtained in the alcoholysis, a less regioselective profile was observed for the hydrolysis of **1**; moreover, after the first deacetylation step the reaction proceeded further affording a mixture of monoacetylated products and methyl riboside. In this way, the alcoholysis appears as a better procedure for the preparative obtention of **3** than the hydrolysis; similarly,

Table 1
C. antarctica B (CAL B)-catalysed alcoholysis of methyl 2,3,5-tri-*O*-acetyl-α-D-ribofuranoside (**1**) and methyl 2,3,5-tri-*O*-acetyl-β-D-ribofuranoside (**2**)[a]

Entry	Substrate	*t* (h)	*T* (°C)	Product
1	**1**	3	30	Methyl 2,3-di-*O*-acetyl-α-D-ribofuranoside[b]
2	**1**	3	45	Methyl 2,3-di-*O*-acetyl-α-D-ribofuranoside[c]
3	**2**	6	30	Methyl 2,3-di-*O*-acetyl-β-D-ribofuranoside[d]
4	**2**	24	30	Methyl 2-*O*-acetyl-β-D-ribofuranoside + methyl 3-*O*-acetyl-β-D-ribofuranoside[e]
5	**2**	168	30	Methyl β-D-ribofuranoside[f]
6	**2**	120	45	Methyl β-D-ribofuranoside[f]

[a] Typical procedure: See Section 2.3.
[b] Isolated in 65% yield after column chromatography. No further appreciable conversion of this product was observed at longer reaction times.
[c] Isolated in 81% yield after column chromatography. No further appreciable conversion of this product was observed at longer reaction times.
[d] Monoacetylated products were also formed.
[e] Isolated as a mixture in 45% yield, after column chromatography.
[f] Quantitative formation (determined by tlc).

in the experiments of the enzymatic deprotection of acylated nucleosides, we found the alcoholysis to be more selective than the hydrolysis [12–15]. It is worth noting that when Wong and coworkers studied the CRL-catalysed hydrolysis of **1** and **2** at pH 7 in DMF at 37 °C [9], no remarkable difference in the performance of each anomer was found and **3** and **4** were respectively isolated in high yields.

4. Conclusions

The anomers methyl 2,3,5-tri-*O*-acetyl-α-D-ribofuranoside (**1**) and methyl 2,3,5-tri-*O*-acetyl-β-D-ribofuranoside (**2**) exhibited a different behaviour in the *C. antarctica* B lipase-catalysed alcoholysis. The enzymatic deprotection of the former gave regioselectively methyl 2,3-di-*O*-acetyl-α-D-ribofuranoside (**3**) with no further remarkable transformation, affording this product in 81% yield at 3 h and 45 °C. In contrast, the alcoholysis of the β-diasteromer was less selective. For this diasteromer, mixtures of partially acetylated products were obtained; however, full deacetylation of the substrate was obtained, yielding quantitatively methyl β-D-ribofuranoside at longer reaction times (5 days at 45 °C).

Since the differences in the enzymatic recognition of **1** and **2** afford products with different chromatographic mobility, the alcoholysis could also provide an useful and simple procedure for the separation of anomeric mixtures. At present we are extending this study to other carbohydrate derivatives.

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