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Enzymatic alcoholysis of 3′,5′-di-*O*-acetyl-2′-deoxynucleosides

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Abstract

Candida antarctica-B (CAL-B) lipase-catalysed alcoholysis of a set of 3′,5′-di-*O*-acetyl-2′-deoxynucleosides (**1a**–**e**) gave the corresponding 3′-*O*-acetyl-2′-deoxy-nucleosides (**2a**–**e**) in yields ranging from 50 to 96%. The alcohol employed in the biotransformation affected the rate of the enzymatic reaction and the yield of the 3′-*O*-acetylated product, but in all cases only this regioisomer was formed. The obtained results are in agreement with the regioselectivity displayed by CAL-B lipase in previously reported biotransformations of nucleosides. CAL-B catalysed alcoholysis of 2′,3′,5′-tri-*O*-acetyl-cytidine and 4-*N*-acetyl-2′,3′,5′-tri-*O*-acetylcytidine was also studied, affording with the same regioselectivity the corresponding free 5′-hydroxyl nucleosides.

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

1. Introduction

Biotransformations catalysed by hydrolytic enzymes provide convenient methods to achieve regioselective reactions in the sugar moiety of nucleosides. In addition to the usefulness of the described and reviewed enzymatic regioselective acylation and alkoxycarbonylation of nucleosides [1,2], the hydrolase-catalysed deacylation of nucleosides has also been studied through enzymatic hydrolysis of peracylated deoxynucleosides [3–7] and ribonucleosides [6,8,9].

Over the last years we have been studying the enzymatic deacylation of peracylated ribonucleosides through enzymatic alcoholysis and we have found that *Candida antarctica*-B (CAL-B) lipase catalyses efficiently the formation of the corresponding 2′,3′-di-*O*-acylribo-nucleosides [10–12].

Taking into account these results, we considered of interest to study the behaviour of 3′,5′-di-*O*-acetyl-2′-deoxynucleosides (**1a**–**e**, Scheme 1) in the CAL-B catalysed alcoholysis (Scheme 2) and herein we report the results of these biotransformations.

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2. Experimental

2.1. Materials

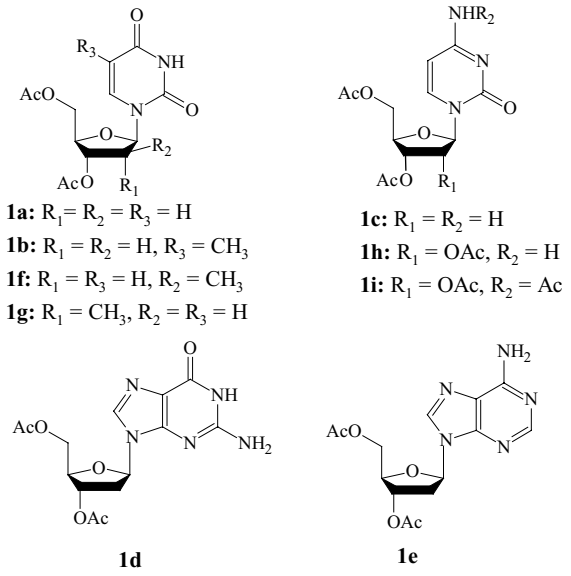
2.1.1. General

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). 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. (2′*S*)-2′-deoxy-2′-*C*-methyluridine (**1f**, Scheme 1) and (2′*R*)-2′-deoxy-2′-*C*-methyluridine (**1g**) were prepared following a previously reported protocol developed by us [13].

2.1.2. Preparation of the substrates *1a–i* (Scheme 1)

3′,5′-di-*O*-acetyl-2′-deoxyuridine (**1a**), 3′,5′-di-*O*-acetyl-thymidine (**1b**), 3′,5′-di-*O*-acetyl-2′-deoxycytidine (**1c**), 3′,5′-di-*O*-acetyl-2′-deoxyguanosine (**1d**), 3′,5′-di-*O*-acetyl-2′-deoxyadenosine (**1e**), (2′*S*)-3′,5′-di-*O*-acetyl-2′-deoxy-2′-*C*-methyluridine (**1f**), (2′*R*)-3′,5′-di-*O*-acetyl-2′-deoxy-2′-*C*-methyluridine (**1g**) and 2′,3′,5′-tri-*O*-acetylcytidine (**1h**) were prepared in high yields from the corresponding free nucleoside. According to a procedure described for the preparation of (**1d**) [14], the nucleoside was treated at room



Scheme 1. Assayed substrates in the CAL-B lipase-catalysed alcoholysis.

temperature with acetic anhydride in acetonitrile containing triethylamine and 4-dimethylaminopyridine. After 2 h of reaction the crude products were purified by recrystallisation from isopropanol for **1b**, **d**, **e**, affording the corresponding products; products **1a**, **c**, **f**–**h** were isolated by silica gel column chromatography.

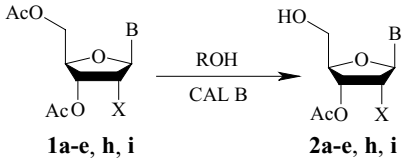
To obtain 4-*N*-acetyl-2′,3′,5′-tri-*O*-acetylcytidine (**1i**), the acetylating mixture was heated in a bath at 50 °C for 2 h and the product isolated after column chromatography.

Compounds **1a**–**i** afforded satisfactory NMR data.

2.2. Enzymatic reactions

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

Following a previously reported protocol [10–12], experiments of biocatalysed alcoholyses were performed by adding



R = Et, Pr, Bu
a: B = Uracil, X = H
b: B = Thymine, X = H
c: B = Cytosine, X = H
d: B = Guanine, X = H
e: B = Adenine, X = H
h: B = Cytosine, X = OAc
i: B = *N*-acetylcytosine, X = OAc

Scheme 2. CAL-B lipase-catalysed alcoholysis of 3′, 5′-di-*O*-acetyl-2′-deoxynucleosides (**1a**–**e**) and ribocytidines (**1h**, **i**).

CAL (150 mg) to a suspension of the nucleoside (0.5 mmol) in the alcohol (0.65 mol) and shaking the resulting mixtures at 200 rpm and 30 °C during the times indicated in the text. Aliquots of the biotransformations were withdrawn at different times, dissolved in a mixture of methanol–acetonitrile and after removal of the enzyme, analysed by TLC and HPLC. When convenient times were reached for preparative purposes, the biotransformations were stopped by filtering off the enzyme and washing it with methanol and acetonitrile.

As an example of a typical preparative procedure, 172 mg of CAL-B was added to a suspension of 3′,5′-di-*O*-acetyl-2′-deoxyguanosine (**1d**, 200 mg, 0.57 mmol) in 1-butanol (68 ml, 0.744 mol) and the resulting mixture was shaken for 48 h. The enzyme was then filtered off, washed with chloroform and the filtrates evaporated in vacuum. The product, 3′-*O*-acetyl-2′-deoxyguanosine (**2d**), provided satisfactory spectral data: ^{1}H NMR (DMSO-d_6, 500 MHz): δ 2.08 (s, 3H, –CH_3), 2.39 (m, 1H, H-2′$_a$), 2.77 (m, 1H, H-2′$_b$), 3.57 (m, 2H, H-5′), 4.01 (m, 1H, H-4′), 5.32 (m, 1H, H-3′), 5.39 (s$_a$, 1H, OH), 6.13 (dd, J_1 = 6 Hz, J_2 = 9 Hz, 1H, H-1′), 6.47 (s$_a$, 2H, NH_2), 7.95 (s, 1H, H-8), 10.63 (s$_a$, 1H, NH). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 20.83 (CH_3), 36.61 (C-2′), 61.50 (C-5′), 74.98 (C-3′), 82.56 (C-1′*), 84.87 (C-4′*), 128.50 (C-5), 135.12 (C-8), 153.59 (C-2*), 153.69 (C-4*), 156.63 (C-6), 169.91 (CO).

2.3. Analytical methods

TLC was performed on Silicagel 60 F_{254} plates (Merck) and column chromatography was carried out using silicagel Merck 60. In TLC analyses dichloromethane–methanol mixtures (98:2 v/v for aliquots involving **1f**, **g**, 90:10 v/v for aliquots involving **1a**, **b**, **e**, **h**, **i** and 85:15 v/v for those related to **1c**, **d**) were used as the mobile phase.

Nuclear magnetic resonance spectra were recorded on a Bruker AC-500 spectrometer in DMSO-d_6 or $CDCl_3$. ^{13}C NMR chemical shift assignations bearing an asterisk may be interchangeable.

HPLC analyses were conducted by using a C-18 column with detection at 254 nm. For samples involving **1a**–**c** the mobile phase was water/acetonitrile 75:25 (v/v); for those involving **1d**, **e**, **h**, **i**, water/acetonitrile 80:20 (v/v), in all cases at a flow rate 0.9 ml min^{-1}.

3. Results and discussion

Taking into account our previous results on CAL-B catalysed alcoholysis of peracylated ribonucleosides [10–12], which showed that the best regioselectivity could be reached by carrying out the biotransformations in a very high excess of ethanol, experiments of enzymatic alcoholysis of the diacetylated substrates **1a**–**i** were performed using a similar nucleoside/alcohol ratio (see Experimental).

Table 1
CAL-B lipase-catalysed alcoholysis of 3′, 5′-di-*O*-acetyl-2′-deoxynucleosides **1a**–**e**[a] (Scheme 2)

Entry	Substrate	Alcohol	*t* (h)	Conversion (%)[b]	**2**[c] (%)[b]
1	**1a**	Ethanol	24	52	41
2	**1**[a]	1-Propanol	28	50	35
3	**1**[a]	1-Butanol	120	80	50
4	**1**[a]	1-Butanol	168	91	42
5	**1b**	Ethanol	48	77	41
6	**1b**	1-Propanol	24	62	61
7	**1b**	1-Butanol	2	45	23
8	**1b**	1-Butanol	24	93	11
9	**1c**	Ethanol	48	60	32
10	**1c**	1-Propanol	48	71	67
11	**1c**	1-Butanol	24	0	0
12	**1d**	Ethanol	48	87	58
13	**1d**	1-Propanol	24	49	49
14	**1d**	1-Propanol	48	61	49
15	**1d**	1-Butanol	48	100	96
16	**1e**	Ethanol	48	69	53
17	**1e**	1-Butanol	24	88	60

[a] See Section 2.
[b] Determined by HPLC (see Section 2).
[c] 3′-*O*-acetylated product (see Scheme 2); the differences between conversion and **2** yields correspond to free 2′-deoxynucleoside, which was also formed.

Under these conditions, only one monoacetylated product was formed, giving 3′-*O*-acetyl-2′-deoxynucleosides **2a**–**e** (Table 1). This behaviour agrees with the regioselectivity displayed by CAL-B towards the 5′-moiety in the enzymatic acylation [1,2,6,15,16] and deacylation [1,2,6–8,10–12] of nucleosides. In all experiments, in addition to the 3′-*O*-acetylated products, the corresponding 2′-deoxynucleoside was also formed; although higher substrate conversions were obtained at longer reaction times, reported data (Table 1) refer to maximum **2a**–**e** yields. As the degree of conversion of the biotransformation increased, higher amounts of the corresponding diol were formed (typical data are given in Entries 3–4, 7–8, 13–14); however, in most cases no quantitative full deacetylation of the substrates was observed. The enzymatic butanolysis of 3′, 5′-di-*O*-acetyl-2′-deoxyguanosine (**1d**) afforded the best result for a preparative purpose: after 48 h at 30 °C, 3′-*O*-acetyl-2′-deoxyguanosine (**2d**) was formed in 96% (Table 1, Entry 15). The choice of the alcohol had effect on the rate and the yield of the biotransformation.

The structural determination of products **2a**–**e** was carried out by NMR spectroscopy. In ^{1}H NMR, H-5′ shows a shift to higher fields up to ca. 0.7 ppm compared to the substrates. Moreover, ^{13}C NMR signals of C-5′ present a downfield shift of ca. 2 ppm referred to C-5′ in the diacetylated substrates, indicating that 5′-hydroxyl is free. Spectroscopy data of products **2a**–**e** agreed with those previously reported [16,17].

3′-*O*-acetylated compounds **2a**–**e** have been previously prepared enzymatically by direct acetylation of the corresponding 2′-deoxynucleosides using oxime esters [16,17], but in such cases lipase from *P. cepacia* (PSL) was employed as the biocatalyst, due to the regioselectivity exhibited by this lipase towards the 3′-hydroxyl of 2′-deoxynucleosides.

Table 2
CAL-B lipase-catalysed alcoholysis of some acetylated cytosine nucleosides (**1c**, **h**, **i**)[a] (Scheme 2)

Entry	Substrate	Alcohol	*t* (h)	Conversion (%)[b]	**2** (%)[b]
1	**1c**	1-Propanol	48	71	67[c]
2	**1h**	Ethanol	20	92	85[d]
3	**1i**	Ethanol	72	84	67[e]
4	**1i**	1-Butanol	48	54	49[e]

[a] See Section 2.
[b] Determined by HPLC (see Section 2). The differences between conversion and **2** yields correspond to free nucleoside, which was also formed.
[c] 3-*O*-acetylcytidine (Table 1, Entry 10).
[d] 2′,3′-di-*O*-acetylcytidine (see Scheme 2).
[e] 4-*N*-acetyl-2′,3′-di-*O*-acetylcytidine (see Scheme 2).

When (2′*S*)-3′,5′-di-*O*-acetyl-2′-deoxy-2′-*C*-methyluridine (**1f**) and (2′*R*)-3′,5′-di-*O*-acetyl-2′-deoxy-2′-*C*-methyluridine (**1g**) were tested in the CAL-B catalysed ethanolysis, under the same experimental conditions employed for the diacetylated 2′-deoxyuridine (**1a**), no reaction occurred after 24 h. Although this behaviour could be explained at first in terms of the bulkier nature of **1f**, **g** compared with **1a** it is remarkable that 2′,3′,5′-tri-*O*-acetyl-2′-*C*-methyluridine was a substrate for this biotransformation, affording the corresponding free 5′-hydroxyl product regioselectively and in very good yield [10]. These results show a difference in performance between a ribonucleoside and its corresponding 2′-deoxynucleoside, a behaviour that Gotor and coworkers described for the PSL-catalysed acylation and alkoxycarbonylation of ribonucleosides and 2′-deoxynucleosides [1,2,15,16].

In order to complete our study of the enzymatic alcoholysis of peracetylated ribonucleosides, we also examined the CAL-B catalysed deacetylation of two acetylated cytidine derivatives (**1h**, **i**, Scheme 1), whose enzymatic alcoholysis had not been previously reported. Table 2 (Entries 2–4) show that both substrates afforded regioselectively the corresponding 2′,3′-di-*O*-acetylated nucleosides (**2h**, **i**, Scheme 2); even when in all cases some amounts of monoacetylated and free nucleosides were detected, no formation of other regioisomer of **2h**, **i** occurred, showing again the preference of CAL-B towards the cleavage of the 5′-*O*-acetate of nucleosides.

4. Conclusions

CAL-B lipase-catalysed alcoholysis of 3′,5′-di-*O*-acetyl-2′-deoxynucleosides gave the corresponding 3′-*O*-acetyl-2′-deoxynucleoside in yields ranging from 50 to 96%. In most cases, lower product yields were obtained compared to those afforded by CAL-B lipase-catalysed alcoholysis of peracetylated ribonucleosides [10–12]. The choice of the

alcohol affected the rate of the enzymatic reaction and the yield of the 3′-*O*-acetylated product, but in all cases only this regioisomer was formed. This fact agrees with the regioselectivity displayed by CAL-B lipase towards the ribose moiety of nucleosides. The regioselective deacetylation of the 5′-*O*-acetate was further observed in the enzymatic alcoholysis of two acetylated cytidines.

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