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A mild and quantitative procedure for the removal of nucleoside alkoxycarbonyl groups using pig liver esterase or *Candida antarctica* B lipase

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Abstract

A set of eight mono-, di-, tri- and tetraalkoxycarbonylated nucleosides was tested in order to assess their enzymatic hydrolysis. All the alkoxycarbonyl groups of the assayed substrates, from both carbonate and carbamate functions, were quantitatively hydrolysed using pig liver esterase (PLE) at pH 7 and 60 °C, regardless of the nucleoside base. Quantitative full alkoxycarbonyl groups removal was also reached by *Candida antarctica* B lipase (CAL B) under mild conditions, but in this case, longer reaction times were required. Thus, PLE appears as an useful catalyst for the mild and quantitative deprotection of nucleoside carbonates and carbamates in the synthesis of modified nucleosides.

Keywords: Carbonates; Carbamates; Deprotection; Hydrolases; Nucleosides

1. Introduction

Carbonates display a wide range of applications in synthetic, polymer and medicinal chemistry [1]. While in the former field carbonates are employed as protective groups for diols and poliols [2], in the latter, the lipophilic nature of carbonates can afford prodrugs of pharmacological active compounds [3]. Therefore, the use of carbonates in nucleoside chemistry offers an interesting potential. However, the use of alkyl carbonates as protective groups of nucleosides has been very restricted because their removal requires strong basic medium, frequently not compatible with the complex structure of such molecules. To avoid this limitation, carbonates usually bear a second substituent that provides an easier deprotection strategy [2,4].

Traditional reagents employed to obtain carbonates can be replaced nowadays by using biocatalysts and according to this trend, nucleosides can be alcoxycarbonylated regioselectively by oxime carbonates using lipases [5–8]. In spite of these antecedents, as far as we know, hydrolases have not been applied to nucleoside carbonates removal, apart from a work related to the field of solid phase chemistry [9]. Besides, in general, enzymatic hydrolysis of carbonates [10–20] has been reported in a lesser extent than ester enzymatic hydrolysis.

Taking into account these facts and our previous results on the enzymatic deacylation of acylated nucleosides [21–24], we thought of interest to apply the mild reactions provided by hydrolases to the deprotection of nucleoside alkyl carbonates and in this paper we report the quantitative enzymatic hydrolysis of compounds **1**–**8** (Scheme 1).

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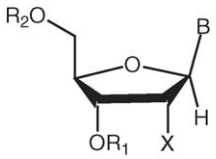

1: X = OH, R_1 = H, R_2 = $CO_2CH_2CH_3$, B = Uracil-1-yl
2: XR_1 = OCO, R_2= H, B = Uracil-1-yl
3: X = $OCO_2CH_2CH_3$, R_1= R_2 = $CO_2CH_2CH_3$, B = Uracil-1-yl
4: X = $OCO_2CH_2CH_3$, R_1= R_2 = $CO_2CH_2CH_3$, B = Hypoxanthin-9-yl
5: X = $OCO_2CH_2CH_3$, R_1= R_2 = $CO_2CH_2CH_3$, B = Guanin-9-yl
6: X = $OCO_2CH_2CH_3$, R_1= R_2 = $CO_2CH_2CH_3$, B = *N*-6-Ethoxycarbonyladenin-9-yl
7: X = $OCO_2CH_2CH_3$, R_1= R_2 = $CO_2CH_2CH_3$, B = *N*-4-Ethoxycarbonylcytosin-1-yl
8: X = H, R_1= R_2 = $CO_2CH_2CH_3$, B = Thymin-1-yl

Scheme 1.

2. Experimental

2.1. 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. Enzymatic reactions were carried out in a temperature-controlled incubator shaker (Sontec OS 11, Argentina) at 250 rpm and 30 °C or 60 °C.

All employed reagents and solvents were of analytical grade and obtained from commercial sources. Dioxane 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, mixtures of dichloromethane–methanol were used as the mobile phase.

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.

2.2. Preparation of the substrates *1*–*8* (Scheme 1)

5′-*O*-Ethoxycarbonyluridine (**1**) was prepared by CAL B-catalysed reaction of uridine with diethyl pyrocarbonate in dioxane, following a protocol described by Morís and Gotor for the reaction of thymidine and dimethyl pyrocarbonate [6].

To obtain uridine 2′,3′-carbonate (**2**), uridine first reacted with 4,4′-dimethoxytrityl chloride following a described protocol [25], to give 5′-*O*-(4,4′-dimethoxytrityl)uridine. Subsequent reaction with 1,1′-carbonyldiimidazole in dioxane at 20 °C [26] afforded 5′-*O*-(4,4′-dimethoxytrityl)uridine-2′,3′-carbonate. Deprotection of the latter in acetic acid afforded compound **2**.

2′,3′,5′-Tri-*O*-ethoxycarbonyluridine (**3**), 2′,3′,5′-tri-*O*-ethoxycarbonylinosine (**4**), 2′,3′,5′-tri-*O*-ethoxycarbonylguanosine (**5**), *N*-6-ethoxycarbonyl-2′,3′,5′-tri-*O*-ethoxycarbonyladenosine (**6**), *N*-4-ethoxycarbonyl-2′,3′,5′-tri-*O*-ethoxycarbonylcytosine (**7**) and 3′,5′-di-*O*-ethoxycarbonylthymidine (**8**) were obtained through reaction of the corresponding free nucleosides with an excess of ethyl chloroformate in pyridine using a modified standard procedure [27]. A higher excess of ethyl chloroformate allowed the preparation of **6** and **7**. In all cases, products **1**–**8** were purified by silicagel column chromatography, affording satisfactory NMR data.

2.3. General procedure for the enzymatic hydrolysis of *1*–*8*

Hydrolyses were performed by adding the commercially available hydrolases PLE or CAL B (15 mg) to a suspension of **1**–**8** (0.050 mmol) in phosphate buffer 30 mM pH 7 (1 ml) and shaking at 250 rpm at the temperatures reported in Table 1. Aliquots from the biotransformations were withdrawn and after centrifugation of the enzyme, analysed by TLC until disappearance of both the substrates and the mixtures of partially alkoxycarbonylated products. In all cases, when quantitative full removal of alkoxycarbonyl groups was reached, only one spot corresponding to the free nucleoside was observed. To provide an example, uridine (from substrates **1**–**3**) gave an R_f = 0.1 using silicagel plates and dichloromethane–methanol 90:10 (v/v) as the mobile phase, while mixtures of uridine monoethoxycarbonylated products exhibited R_f = 0.2–0.3; the diethoxycarbonylated products and the substrate presented higher mobilities. Quantitative full hydrolysis was reported (for uridine, Entries 1–3, 9–11 and 17–19 in Table 1) when spots at R_f = 0.2–0.3 disappeared. This behaviour was observed for all the assayed substrates. Aliquots corresponding to full quantitative deprotection were also co-chromatographied with reference samples of the respective free nucleosides.

Moreover, aliquots from PLE-catalysed hydrolysis of **3** at 60 °C were also analysed by HPLC using a C-18 column and water/acetonitrile 68:32 (v/v) as the mobile phase (0.9 ml min^{-1}), with detection at 254 nm. The aliquot giving the sole spot at R_f = 0.1 by TLC (Entry 3) afforded only one

Table 1
Enzymatic hydrolysis of nucleoside alkyl carbonates **1–8** (Scheme 1)[a]

Entry	Substrate	Enzyme	T (°C)	t (h)[b]
1	**1**	PLE[c]	60	2
2	**2**	PLE	60	4
3	**3**	PLE	60	4
4	**4**	PLE	60	72
5	**5**	PLE	60	5
6	**6**	PLE	60	6
7	**7**	PLE	60	5
8	**8**	PLE	60	5
9	**1**	PLE	30	24
10	**2**	PLE	30	24
11	**3**	PLE	30	48
12	**4**	PLE	30	120
13	**5**	PLE	30	312
14	**6**	PLE	30	24
15	**7**	PLE	30	24
16	**8**	PLE	30	24
17	**1**	CAL B[d]	60	24
18	**2**	CAL B	60	22
19	**3**	CAL B	60	24
20	**4**	CAL B	60	96
21	**5**	CAL B	60	120
22	**6**	CAL B	60	48
23	**7**	CAL B	60	48
24	**8**	CAL B	60	96

[a] Typical procedure: see Section 2.3.
[b] Full quantitative hydrolysis yielding the corresponding free nucleoside was reached at the reported time.
[c] PLE: pig liver esterase.
[d] CAL B: *Candida antarctica* B lipase.

signal at $t_r = 1.96$ min, corresponding to the t_r of a uridine reference sample, as expected.

It is worth mentioning that the obtained full deprotected nucleosides are stable at the employed experimental conditions and give no further reaction in presence of the assayed hydrolases.

Control experiments showed that no reaction of **1**–**8** took place without hydrolase.

3. Results and discussion

Hydrolyses were assayed at pH 7, and two hydrolases, pig liver esterase (PLE) [28] and *C. antarctica* B lipase (CAL B) [28], were employed. Among other uses, PLE has been employed for the hydrolysis of acylated nucleosides [7,29–31] and of carbonates structurally different to nucleosides [12,16,20], while CAL B has been described to catalyse the synthesis of 5′-*O*-alkoxycarbonyl derivatives of nucleosides [7,8] and of carbonate intermediates for the synthesis of (*S*)-(+)-zopiclone [18,19].

Table 1 shows that both enzymes allow the quantitative hydrolysis under mild reaction conditions of all the tested substrates to afford the corresponding free nucleosides. It is interesting noting that carbamates of **6** and **7** were also hydrolysed during the biotransformations; this result is rather unexpected since carbamates are not substrates for many serine hydrolases and some of them are inhibitors of these enzymes, especially of lipases [32]. As far as we know, no previous reports have been published about PLE or CAL B catalysed hydrolyses of carbamates.

The best results were reached with PLE at 60 °C, which affords the quantitative removal of all carbonates (Entries 1–8, Table 1) and carbamates (Entries 6, 7) in shorter reaction times than at 30 °C (Entries 9–16). CAL B-catalysed biotransformations at 60 °C (Entries 17–24) proceeded slower if compared with PLE and even much longer reaction times were required at 30 °C (3–15 days, data not shown). No quantitative hydrolysis of **1** could be reached using porcine pancreatic lipase at pH 7.

Before full quantitative alkoxycarbonyl groups removal, the biotransformations reported in this work proceeded unselectively, giving mixtures of partially alkoxycarbonylated nucleosides. Regarding to this behaviour it can be mentioned that in our previous experiments of ribonucleoside deacylation, the enzymatic hydrolysis did not proceed selectively [22,23].

In summary, results in Table 1 show that the tested hydrolases remove all the alkoxycarbonyl groups of substrates **1**–**8**, regardless of the nucleoside base. The results reached with PLE at pH and 60 °C suggest that this enzyme may have potential applications for the mild and quantitative deprotection of alkoxycarbonyl groups in the synthesis of modified nucleosides.

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