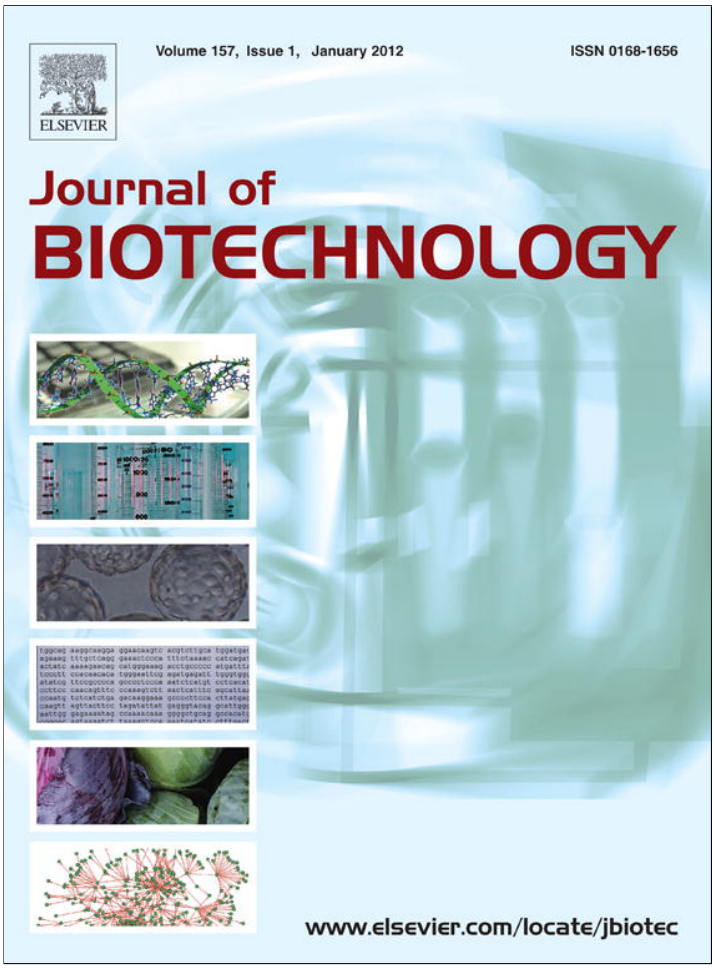

Journal of Biotechnology 165 (2013) 99–101

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Short communication

An efficient and mild access to *N*-acetyl protected purine nucleosides based on a chemoselective enzymatic hydrolysis

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ARTICLE INFO

Article history:
Received 16 October 2012
Received in revised form 1 March 2013
Accepted 6 March 2013
Available online xxx

Keywords:
Chemoselectivity
Candida antarctica lipase B
Acylase I
Nucleobase *N*-protection
Oligonucleotide building blocks

ABSTRACT

N-Monoacetylated derivatives of ribo- (adenosine, guanosine) and 2′-deoxyribonucleosides (2′-deoxyadenosine and 2′-deoxyguanosine), useful as oligonucleotide building blocks, were obtained in 88–100% by enzymatic chemoselective hydrolysis of the corresponding peracetylated nucleosides. Among the tested hydrolases, most satisfactory results were found with acylase I from *Aspergillus melleus* and *Candida antarctica* lipase B. For acylase I, the observed chemoselectivity towards ester hydrolysis, without amide reaction, broadens the information about the selectivity of the enzyme and its synthetic applications in the field of nucleosides.

1. Introduction

The biological and therapeutic properties of nucleosides and oligonucleotides have prompted the development of improved synthetic procedures to obtain these molecules. In particular, there is much interest in oligonucleotide synthesis, owing to the therapeutical potential of these molecules in gene therapy and their applications in molecular biology and diagnostics; in addition, the synthesis of oligoribonucleotides still needs to be improved (Beaucage and Reese, 2009).

The success of a synthetic route to natural and modified nucleosides and oligonucleotides depends to a large extent on highly chemo- and/or regioselective recognition of the different nucleophilic groups of similar reactivity. Consequently, selective acylation/deacylation of hydroxyl and amino groups is a key transformation that has been dealt with different strategies. In addition to chemical procedures based on the application of protecting groups, enzymatic catalysis is recognized nowadays to provide useful tools in organic synthesis (Faber, 2004; Kadereit and Waldmann, 2001) due to the outstanding selectivity and mild reaction condition of enzymes. Synthetic applications of hydrolases in the field of nucleosides have been reviewed over the last two decades, first by Ferrero and Gotor (2000a, 2000b) and recently by Zong and coworkers (Li et al., 2010). Many of the reported biotransformations are integrated into chemoenzymatic procedures aiming at varied goals, such as the synthesis of non traditional oligonucleotides building blocks from 2′-deoxynucleosides and 2′-*O*-alkylribonucleosides (Díaz-Rodríguez et al., 2006). As above outlined, chemoselective *N*- versus *O*-acylation of nucleosides is key in nucleoside chemistry, since the amino exocyclic groups of adenosine, guanosine and cytidine must be protected before oligonucleotide synthesis; this protection can be performed directly only for cytosine nucleosides (Blat et al., 1989). Selective *N*-acylation cannot be accomplished directly for adenine and guanine nucleosides, and it is usually carried out by two procedures. One involves transient protection (Jones, 1985), a three steps and laborious procedure that involves silylation of the hydroxyl groups, *N*-acylation and subsequent desilylation; the other procedure (Jones, 1985; Pitsch et al., 2001) is peracylation of the nucleoside, followed by chemoselective *O*-deacylation using strong basic reagents, which promote undesirable side reactions if the modified nucleoside bears base-labile substituents, such as in the case of 8-halopurine nucleosides.

In this paper we present an alternative approach to *N*-acetylated purine nucleosides, useful as building blocks for oligonucleotide synthesis, based on enzymatic chemoselective deacetylation of the peracetylated ribo- (**1** and **2**, Fig. 1) and 2′-deoxyribonucleosides (**3** and **4**). We have focused on the acetyl as the *N*-protecting group since it has already proved to be useful in the synthesis of natural and modified oligonucleotide building blocks (Pitsch et al., 2001; Reddy et al., 1997); after oligonucleotide synthesis, *N*-acetamides can be quantitatively removed under short-time deprotection conditions (Pitsch et al., 2001; Reddy et al., 1997).

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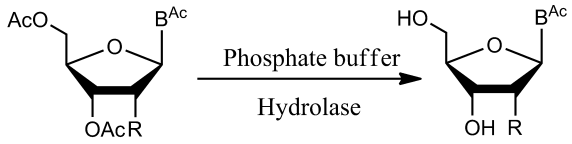
http://dx.doi.org/10.1016/j.jbiotec.2013.03.004



1-4 **5-8**

1 R = OAc, B^{Ac} = *N*-2-Acetylguanin-9-yl
2 R = OAc, B^{Ac} = *N*-6-Acetyladenin-9-yl
3 R = H, B^{Ac} = *N*-2-Acetylguanin-9-yl
4 R = H, B^{Ac} = *N*-6-Acetyladenin-9-yl
5 R = OH, B^{Ac} = *N*-2-Acetylguanin-9-yl
6 R = OH, B^{Ac} = *N*-6-Acetyladenin-9-yl
7 R = H, B^{Ac} = *N*-2-Acetylguanin-9-yl
8 R = H, B^{Ac} = *N*-6-Acetyladenin-9-yl

Fig. 1. Enzymatic chemoselective full *O*-deacetylation of peracetylated nucleosides **1**–**4**.

2. Assayed hydrolytic enzymes

The following enzymes were purchased from Sigma–Aldrich Co.: lipase from *Candida rugosa* (Sigma Type VII, 875 units/mg solid); proteases from *Tritirachium album* (proteinase K, 6.1 units/mg solid), *Bacillus licheniformis* (subtilisin A, Sigma Type VIII, 11 units/mg solid), *Rhizopus sp* (6.1 units/mg solid), *Aspergillus oryzae* (3.6 units/mg solid), *Aspergillus sojae* (Sigma Type XIX, 0.37 units/mg solid); acylase I from *Aspergillus melleus* (AMA, 0.72 units/mg solid), from *Aspergillus sp* (ASA, immobilized on Eupergit, 0.102 units/mg solid), grade I from porcine kidney (PKA-I, 1503 units/mg solid), grade II from porcine kidney (PKA-II, 541 units/mg solid) and esterase from pig liver (PLE, 19 units/mg solid). Lipase B from *Candida antarctica* (CAL-B, Novozym 435, 9500 PLU/mg solid; PLU: propyl laurate units) was a generous gift from Novozymes (Brazil). Enzymes were used straight without any further treatment or purification.

3. General procedure for the enzymatic hydrolysis of 1–4

Enzyme-catalyzed hydrolysis was tested by adding the hydrolase (enzyme/substrate (E/S) ratio: 1120 mg/mmol for PKA-I and ASA; 1360 mg/mmol for AMA; 1300 mg/mmol for PKA-II; 1200 mg/mmol for the other enzymes; MWs of substrates (g/mol): **1**: 451; **2**: 435; **3**: 393; **4**: 377) to a suspension of the substrate (0.04 mmol) in potassium phosphate buffer (30 mM; pH 7 for CAL-B and PLE; pH 8 for the other enzymes; 2.1 ml). The resulting reaction mixtures were shaken at 200 rpm at 30 °C. Samples were taken at different times and the enzyme removed by centrifugation, except for CAL-B and ASA, which were separated by decantation. The resulting aliquots were monitored by TLC and HPLC.

The above protocol was carried out to prepare products **5–8**, which afforded satisfactory NMR data in agreement to those previously published; the preparative procedure for **8** is described below, along with its not previously reported NMR data:

4. N-6-Acetyl-2′-deoxyadenosine (8)

Potassium phosphate buffer 30 mM pH 7 (16.4 ml), **4** (0.18 mmoles) and CAL-B (227 mg) were shaken for 120 h at 30 °C. Then, the buffer was evaporated *in vacuo* and the resulting crude purified by column chromatography. Elution with dichloromethane/methanol 80:20 v/v gave **8** (69%). ^{1}H-NMR (DMSO-d_6, 500 MHz): 2.09 (s, 3H, –CH_3), 2.35 (ddd, J_1 = 13.2 Hz, J_2 = 6.3 Hz, J_3 = 3.4 Hz, 1H, H-2′$_a$), 2.78 (ddd, J_1 = 13.3 Hz, J_2 = 7.3 Hz, J_3 = 6.3 Hz, 1H, H-2′$_b$), 3.55 (dd, J_1 = 11.8 Hz, J_2 = 4.5 Hz, 1H, H-5′$_a$), 3.64 (dd, J_1 = 11.8 Hz, J_2 = 4.6 Hz, 1H, H-5′$_b$), 3.91 (m, 1H, H-4′), 4.45 (m, 1H, H-3′), 6.46 (t, J = 6.3 Hz, 1H, H-1′), 8.31 (s, 1H, H-2), 8.66 (s, 1H, H-8), 10.70 (br, 1H, NH). ^{13}C-NMR (DMSO-d_6, 125 MHz): 24.26 (–CH_3), 30.58 (C-2′), 61.52 (C-5′), 70.60 (C-3′), 83.53 (C-4′), 87.91 (C-1′), 123.59 (C-5), 142.51 (C-8), 149.44 (C-4), 151.34 (C-2), 151.43 (C-6), 168.90 (CO).

5. Results and Discussion

To attain the chemoselective formation of products **5–8** (Fig. 1), the previously listed set of hydrolases, involving lipases, proteases, acylases and one esterase, was tested in the hydrolysis of peracetylated substrates **1**–**4**. This screening allowed the selection of the most useful enzymes, shown in Table 1, which catalyzed the quantitative conversion of three substrates into the corresponding *N*-monoacetylated products (Entries 1–3, 6–10); product **6** was obtained in 88% (Entry 4). ^{1}H-NMR of the isolated monoacetylated products showed no signals at the expected chemical shifts for ester groups, indicating the chemoselective full removal of *O*-acetyl groups.

As a general tendence, the assayed enzymes not included in Table 1 gave mixtures of partially acetylated products. The best results of the screening were found by assaying the acylase I (EC 3.5.1.14) from *Asp. melleus* (AMA) and the lipase B from *C. antarctica* (CAL-B); the esterase from pig liver (PLE) catalyzed the quantitative formation of only one of the four target products (Entry 3). Although over the last years some works have reported lipase- (Simons et al., 2008; Torres-Gavilán et al., 2006) and esterase- (Simons et al., 2008) catalyzed amide hydrolysis, the chemoselectivity displayed by CAL-B and PLE towards **1–4** is in agreement with the generally accepted behaviour of lipases and esterases, which are assumed to catalyze ester and not amide hydrolysis (Bornscheuer and Kazlauskas, 2006).

Regarding acylase I, less studied if compared to lipases, its most frequently reported activity concerns the enantioselective hydrolysis of *N*-acylated-α-aminoacids (Simons et al., 2008; Chenault et al., 1989). Only very few works have reported the chemo- and regioselectivity of this enzyme (Liljeblad and Kanerva, 1999; Liljeblad et al.,

Table 1
Enzymatic chemoselective full *O*-deacetylation of peracetylated nucleosides **1**–**4** (Fig. 1).

Entry	Substrate[a]	Product	Conversion (%)[b]	*t* (day)	Enzyme
1	**1**	**5**	100	2	AMA
2	**1**	**5**	100	3	CAL-B
3	**1**	**5**	100	7	PLE
4	**2**	**6**	88	2	AMA
5	**2**	**6**	79	8	CAL-B
6	**3**	**7**	100	5	AMA
7	**3**	**7**	100	11	PKA-II
8	**3**	**7**	100	1	CAL-B
9	**4**	**8**	100	3	AMA
10	**4**	**8**	100	2	CAL-B

[a] Prepared by reaction of the corresponding free nucleoside with an excess of acetic anhydride (Pitsch et al., 2001).
[b] Determined by HPLC.

2000, 2001), showing results that contrast with the performance of substrates **1**–**4**, since no *N*-deacetylation was observed. Acylase I-catalyzed ester hydrolysis reported in literature is limited to methyl esters, and as far as we know, no previous reports describe acylase I-catalyzed hydrolysis of esters bearing complex alcohol moieties, which would allow a more pertinent comparison. The chemoselectivity of acylase I observed by us was found in the alcoholysis of esters of *N*-acylated-α-aminoacids, where the more natural amide function for AMA remained unreacted (Liljeblad and Kanerva, 1999; Liljeblad et al., 2000, 2001). As stated in the literature, predictability for acylase I selectivity seems limited on the basis of the present knowledge (Liljeblad and Kanerva, 2006).

In summary, the present paper provides a simple and efficient access to *N*-acetyl protected ribo- and 2′-deoxyribopurine nucleosides, based on chemoselective hydrolyses of the corresponding peracetylated nucleosides. In contrast to the chemical procedures already reported for such *N*-protected nucleosides, this approach involves mild reaction conditions, allowing also the preparation of base-labile *N*-monoacetylated derivatives. Among the tested hydrolases, most satisfactory results were found by using acylase I from *Asp. melleus* and from porcine kidney and by *C. antarctica* B lipase. For the acylase I enzymes, the observed chemoselectivity towards ester hydrolysis widens the information about the selectivity and the scope of their synthetic applications.

Acknowledgements

We thank UNQ for financial support. AMI and LEI are members of the Scientific Researcher Career of CONICET (Argentina). We are grateful to Novozymes (Brazil) for the generous gift of CAL-B.

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