Journal of Molecular Catalysis B: Enzymatic 62 (2010) 225–229

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

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

Enzymatic regioselective and complete deacetylation of two arabinonucleosides

María B. Sabaini[a], María A. Zinni[a], Martina Mohorčič[b], Jožefa Friedrich[b], Adolfo M. Iribarren[a,c], Luis E. Iglesias[a,*]

[a] Department of Science and Technology, Universidad Nacional de Quilmes, Roque Sáenz Peña 352, 1876 Bernal, Provincia de Buenos Aires, Argentina
[b] Department of Biotechnology, National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia
[c] INGEBI (CONICET), Vuelta de Obligado 2490, 1428 Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 4 September 2009
Received in revised form 16 October 2009
Accepted 27 October 2009
Available online 31 October 2009

Keywords:
Lipase
Regioselectivity
Nucleosides
Prodrugs
Keratinase

ABSTRACT

Candida antarctica lipase B (CAL-B)-catalysed regioselective deacetylation of 2′,3′,5′-tri-*O*-acetyl-1-β-D-arabinofuranosyluracil (**1**) and 2′,3′,5′-tri-*O*-acetyl-9-β-D-arabinofuranosyladenine (**2**) was studied. The choice of the reaction medium allowed the regioselective formation of products bearing different degree of acetylation: in isopropanol, CAL-B catalysed the formation of the corresponding 2′-*O*-acetylated arabinonucleosides, while hydrolyses afforded the 2′,3′-di-*O*-acetylated products. In particular, the procedure herein described allows a simple and efficient preparation of the reported vidarabine prodrug 2′,3′-di-*O*-acetyl-9-β-D-arabinofuranosyladenine, avoiding the utilisation of protective groups. Moreover, to achieve full deacetylation of the assayed substrates, a set of commercial hydrolases and fungal keratinases from *Doratomyces microsporus* (DMK) and *Paecilomyces marquandii* (PMK) were tested. While only PMK and DMK catalysed the quantitative complete deacetylation of **1**, DMK accomplished full deacetylation of **2** in shorter time than the other assayed enzymes.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Nucleoside analogues are at present well recognised therapeutical compounds [1]; among them, arabinonucleosides have found an important place as antitumoral [2] and antiviral [3–5] drugs. However, in order to get agents with better therapeutic efficacy, the delivery of nucleosides can be improved by the use of prodrugs, which provide derivatives with better pharmacokinetic properties and increased metabolical stability [6–12]. To obtain lipophilic prodrugs, regioselective derivatisation of the furanosic hydroxyls is required, a goal traditionally achieved in organic synthesis through protective groups [6,12]. In contrast to this methodology, hydrolases are employed nowadays to obtain regioselectively acylated or alkoxycarbonylated nucleosides [13,14]; in spite of this, only few among these works deal with hydrolase-catalysed transformation of arabinonucleosides [7,8,15–18].

In our laboratory we have been studying *Candida antarctica* B lipase (CAL-B)-catalysed alcoholysis of acylated nucleosides [19–22], which provides a regioselective access to 2′,3′-di-*O*-acylribonucleosides [19–21]. Thus, diacylated nucleosides, which are used as prodrugs and as intermediates in the preparation of modified nucleosides [6,23,24], can be obtained by CAL-B-catalysed alcoholysis through a simple and regioselective procedure. With this in mind, we applied this enzymatic deacylation to 6-azauridine and 3-deazauridine [25] and keeping this aim, now we extended it to arabinonucleosides. In the present work we studied CAL-B-catalysed deacetylation of 2′,3′,5′-tri-*O*-acetyl-1-β-D-arabinofuranosyluracil (**1**, Scheme 1), a model of pyrimidine arabinonucleoside, and the purine arabinonucleoside 2′,3′,5′-tri-*O*-acetyl-9-β-D-arabinofuranosyladenine (**2**, Scheme 1), the triacetylated derivative of vidarabine (9-β-D-arabinofuranosyladenine, Ara-A, **8**, Scheme 1). Vidarabine was the first antiviral nucleoside analogue licensed for the treatment of herpes virus in humans. It is also active against a wide variety of viruses, such as adenovirus causing haemorragic cystitis, poxviruses and certain rhadboviruses, hepadnarviruses and RNA tumor viruses [4,5,12]. We report herein the results obtained during enzymatic deacetylation of the above mentioned arabinonucleosides **1** and **2**.

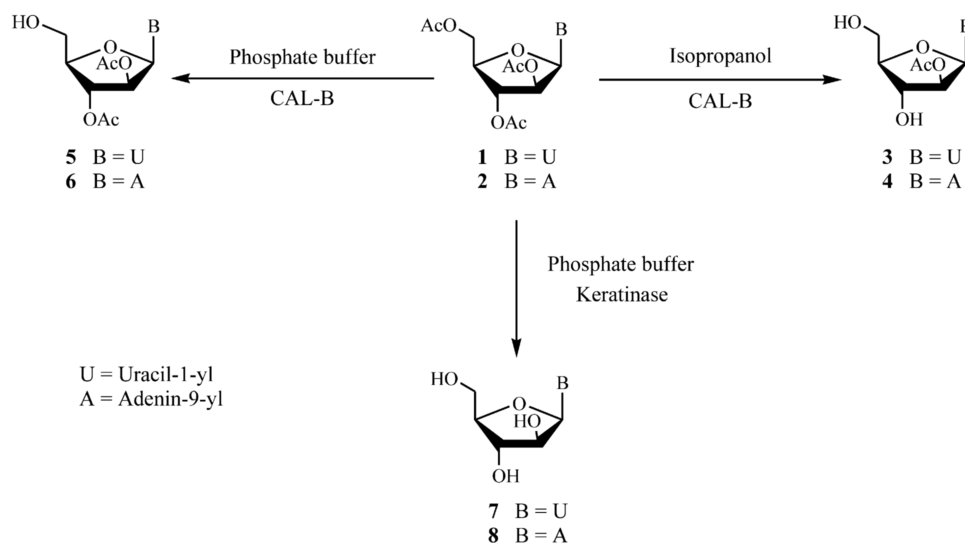
2. Experimental

2.1. General

All employed reagents and solvents were of analytical grade and obtained from commercial sources.

* Corresponding author. Tel.: +54 11 4365 7182; fax: +54 11 4365 7182.
E-mail address: leiglesias@unq.edu.ar (L.E. Iglesias).

1381-1177/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2009.10.011



Scheme 1.

TLC was performed on Silicagel 60 F_{254} plates (Merck) using dichloromethane/methanol mixtures and column chromatography was carried out using silica C18 Amicon Corporation (pore diameter: 100 Å, particle size: 20 μm).

HPLC analyses were carried out using a C18 column (length: 150 mm; internal diameter: 4.6 mm; particle size: 5 μm) with detection at 254 nm. For the analysis of aliquots from biotransformations of **1**, a 30 min gradient of water/acetonitrile from 95:5 (v/v) to 60:40 (v/v) at a flow rate of 0.9 ml min^{-1} was employed. Samples involving **2** were analysed by a 26 min gradient of the same solvent mixture from 90:10 (v/v) to 60:40 (v/v) at the same flow rate.

NMR spectra were recorded on a Bruker AC-500 spectrometer in DMSO-d_6, at 500 MHz for ^{1}H and 125 MHz for ^{13}C.

2.2. Enzymes

Enzymatic reactions were carried out in a temperature-controlled incubator shaker (Sontec OS 11, Argentina) at 200 rpm and 30 °C. Control experiments carried out in the absence of enzyme showed no conversion of the substrates.

2.2.1. Assayed commercial hydrolases

The following enzymes were purchased from Sigma–Aldrich Co.: lipases from *Candida rugosa* (CRL, Sigma Type VII, 875 U/mg solid), *Pseudomonas* sp. (PSL, Sigma Type XIII, 21 U/mg solid) and *Pseudomonas cepacia* (PCL, 90 U/g solid); esterase from pig liver (PLE, 19 U/mg solid); proteases from *Tritirachium album* (proteinase K, PK, 6.1 U/mg solid) and *Bacillus licheniformis* (subtilisin, Sigma Type VIII, 11 U/mg solid) was purchased from ICN. 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).

Enzymes were used straight without any further treatment or purification.

2.2.2. Keratinolytic enzymes from Paecilomyces marquandii (PMK) and Doratomyces microsporus (DMK)

Keratinases of the filamentous fungi *Doratomyces microsporus* (DMK) and *Paecilomyces marquandii* (PMK) were prepared by aerobic submerged fermentation followed by partial purification as reported by Gradišar et al. [26] and stored as freeze-dried powder at −20 °C. The obtained enzyme powders of DMK and PMK showed activities of 7.8 U mg^{-1} and 23.0 U mg^{-1}, respectively, measured on keratin from human stratum corneum as a substrate as previously described [27], and were used for deacetylation experiments without further treatment.

2.3. Preparation of substrates **1** and **2**

Substrates **1** and **2** (Scheme 1) were prepared by reaction of the corresponding free arabinonucleoside **7** and **8** (1 mmol) with acetic anhydride (3.6 mmol) in acetonitrile (15 ml) containing triethylamine (4 mmol) and 4-dimethylaminopyridine (0.1 mmol), according to a previously reported protocol [28]. 2′,3′,5′-Tri-*O*-acetyl-1-β-D-arabinofuranosyluracil (**1**) was purified by recrystallisation from isopropanol and 2′,3′,5′-tri-*O*-acetyl-9-β-D-arabinofuranosyladenine (**2**) by silicagel column chromatography eluting with dichloromethane/methanol 90:10 (v/v). Both substrates afforded satisfactory NMR data.

2.4. Preparation of reference samples of 2′-O-acetyl-1-β-D-arabinofuranosyluracil (**3**) and 2′-O-acetyl-9-β-D-arabinofuranosyladenine (**4**)

Reference samples of **3** and **4** were prepared through respective PLE-catalysed hydrolysis of **1** and **2** according to [7] and isolating the resulting products using C18 flash chromatography. Following the HPLC conditions for **1** reported in Section 2.1, **3** gave t_r = 7.17 min, while a t_r = 5.05 min was obtained for **4** according to the conditions described for **2**.

2.5. General procedure for the enzymatic alcoholysis of **1** and **2**

Typically, experiments of enzymatic alcoholyses were performed by adding CAL-B (12 mg) to a solution of the substrate (0.04 mmol) in the assayed alcohol at the indicated A/S (alcohol/substrate ratio) (10.4 mmol for A/S = 260 or 40 mmol for A/S = 1000) and shaking the resulting mixtures at 200 rpm and 30 °C. Aliquots from the biotransformations were withdrawn at different times and after removal of the enzyme, analysed by TLC and HPLC.

For preparation of monoacetylated nucleosides **3** and **4**, the above described protocol for alcoholysis was employed as follows.

2.5.1. 2′-O-Acetyl-1-β-D-arabinofuranosyluracil (**3**)

A mixture of **1** (0.24 mmol), isopropanol (18.6 ml, A/S = 1000) and CAL-B (72 mg) were shaken for 16 days. The biotransformations were then stopped by filtering off the enzyme and washing it with

methanol and dichloromethane. Vacuum evaporation of the filtrate gave **3**, which provided satisfactory spectral data: ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.95 (s, 3H, –CH_3), 3.63 (m, 2H, H-5′), 3.80 (m, 1H, H-4′), 4.12 (t, J = 5.0 Hz, 1H, H-3′), 5.16 (t, J = 5.0 Hz, 1H, H-2′), 5.64 (d, J = 8.1 Hz, 1H, H-5), 6.15 (d, J = 5.0 Hz, 1H, H-1′), 7.71 (d, J = 8.1 Hz, 1H, H-6), 11.35 (s, 1H, H-3). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 20.71 (–CH_3), 62.51 (C-5′), 72.76 (C-3′), 77.46 (C-2′), 82.85 (C-4′), 84.02 (C-1′), 101.30 (C-5), 141.60 (C-6), 150.51 (C-2), 163.45 (C-4), 169.51 (CO).

2.5.2. 2′-O-Acetyl-9-β-D-arabinofuranosyladenine (4)

Analogously, **2** (0.24 mmol), isopropanol (18.6 ml, A/S = 1000) and CAL-B (72 mg) were shaken for 12 days. The enzyme was filtered off, washed with methanol and dicholoromethane and the filtrates evaporated *in vacuo*. C18 flash chromatography of the crude (elution solvent: water/acetonitrile 85:15 v/v) gave **4**, affording satisfactory spectral data: ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.71 (s, 3H, –CH_3), 3.74 (m, 2H, H-5′), 3.88 (m, 1H, H-4′), 4.46 (t, J = 5.8 Hz, 1H, H-3′), 5.30 (t, J = 5.8 Hz, 1H, H-2′), 6.45 (d, J = 5.7 Hz, 1H, H-1′), 7.33 (s, 2H, NH_2), 8.15 (s, 1H, H-2), 8.28 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 20.43 (–CH_3), 62.51 (C-5′), 71.93 (C-3′), 77.93 (C-2′), 81.66 (C-4′), 83.40 (C-1′), 118.73 (C-5), 140.07 (C-8), 149.63 (C-2), 153.14 (C-4), 156.41 (C-6), 169.59 (CO).

2.6. General procedure for the enzymatic hydrolysis of 1 and 2

Experiments of lipase-catalysed hydrolysis were carried out by adding the assayed hydrolase (300 mg $mmol^{-1}$ substrate) to a mixture of the substrate (0.04 mmol) in sodium phosphate buffer (30 mM, pH 7 and 8; 4 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, which was separated by decantation. The resulting aliquots were monitored by TLC and HPLC.

This protocol was applied to prepare diacetylated products **5** and **6**, as follows:

2.6.1. 2′,3′-Di-O-acetyl-1-β-D-arabinofuranosyluracil (5)

A mixture of **1** (0.20 mmol), sodium phosphate buffer 30 mM pH 8 (20 ml) and CAL-B (60 mg) were shaken for 12 h at 30 °C. The reaction was then stopped, the buffer evaporated *in vacuo* and the resulting crude purified by C18 flash chromatography, employing water/acetonitrile 95:5 v/v as the elution solvent, to give **5**: ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.95 (s, 3H, –CH_3), 2.09 (s, 3H, –CH_3), 3.63 (m, 2H, H-5′), 4.10 (m, 1H, H-4′), 5.16 (t, J = 4.9 Hz, 1H, H-2′), 5.34 (dd, J_1 = 4.9 Hz, J_2 = 3.3 Hz, 1H, H-3′), 5.66 (d, J = 8.0 Hz, 1H, H-5), 6.19 (d, J = 5.0 Hz, 1H, H-1′), 7.75 (d, J = 8.1 Hz, 1H, H-6). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 20.64, 21.01 (–CH_3s), 63.63 (C-5′), 72.65 (C-3′), 78.67 (C-2′), 79.97 (C-4′), 85.96 (C-1′), 100.84 (C-5), 142.48 (C-6), 151.08 (C-2), 164.03 (C-4), 170.20, 170.71 (COs).

2.6.2. 2′,3′-Di-O-acetyl-9-β-D-arabinofuranosyladenine (6)

Analogously, **2** (0.20 mmol), sodium phosphate buffer 30 mM pH 7 (20 ml) and CAL-B (72 mg) were shaken for 24 h at 30 °C. The reaction was then stopped, the buffer evaporated *in vacuo* and the resulting crude subsequently purified by C18 column chromatography, eluting with water/acetonitrile 85:15 (v/v), to give **6**: ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.75 (s, 3H, –CH_3), 2.11 (s, 3H, –CH_3), 3.69 (dd, J_1 = 4.3 Hz, J_2 = 12.2 Hz, 1H, H-5′$_a$), 3.76 (dd, J_1 = 4.3 Hz, J_2 = 12.1 Hz, 1H, H-5′$_b$), 4.11 (m, 1H, H-4′), 5.52 (m, 2H, H-2′ and H-3′), 6.51 (d, J = 4.6 Hz, 1H, H-1′), 7.35 (s, 2H, NH_2), 8.16 (s, 1H, H-2), 8.31 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 20.42, 21.11 (–CH_3s), 60.73 (C-5′), 74.93 (C-3′), 75.52 (C-2′), 81.59 (C-4′), 82.29 (C-1′), 118.67 (C-5), 140.08 (C-8), 149.57 (C-2), 153.21 (C-4), 156.45 (C-6), 169.21, 170.14 (COs).

3. Results and discussion

Based on our previous results of CAL-B-catalysed deacetylation of peracetylated nucleosides [19–22,25,29], first experiments of enzymatic deacetylation of **1** and **2** were assayed by applying alcoholysis and using a high excess of alcohol (alcohol/nucleoside ratio, A/N). In this way, several alcohols (ethanol, butanol and isopropanol) were tested at an A/N = 260 and 1000.

Under the assayed experimental conditions, **1** and **2** did not afford diacetylated products in good yields because at the stage of monodeacetylation, aliquots from biotransformations contained also unreacted and further deacetylated substrate. However, 2′-monoacetylated products **3** and **4** were respectively obtained in excellent yields (Scheme 1; Entries 1 and 2, Table 1). The structure of each product was determined by NMR and HPLC, comparing with reference samples of **3** and **4**; it can also be mentioned that by HPLC analysis the different monoacetylated regioisomers can be clearly differentiated. The CAL-B-catalysed alcoholyses herein reported afforded the 2′-monoacetylated products involving an easier work up of the reaction than by applying PLE [7], since CAL-B is an immobilised biocatalyst.

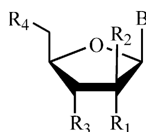
CAL-B-catalysed hydrolysis of **1** and **2** at pH 7 and 8 was then tested and the 2′,3′-di-*O*-acetylated arabinonucleosides **5** and **6** could be obtained in good yields (Scheme 1); Table 1 (Entries 3 and 4) reports the best experimental conditions found to obtain these products. Compound **6** has been described as a useful topical prodrug of vidarabine for treatment of herpes virus infections [23,30]. Its reported three-steps synthesis [23] involved protection of the 5′ hydroxyl group with a tert-butyldimethylsilyl derivative, subsequent acetylation and removal of the silyl protecting group. In this way, the enzymatic procedure herein reported provides a shorter and more efficient preparation of this product.

The regioselectivity observed in the monodeacetylation of **1** and **2** agrees with the preference towards 5′ displayed by CAL-B in acylation and deacetylation of ribo- [10,13,14,19,21,25], 2′-deoxy [13,14,22] and arabinonucleosides [8,15–17]. However, and interestingly, the behaviour of **1** and **2** contrasts to the performance of acetylated ribonucleosides in CAL-B-catalysed deacylations [19–21,25]: the hydrolysis of the latter gave non-selective mixtures of partially acylated products. In the case of herein studied arabinonucleosides **1** and **2**, different products could be regioselec-

Table 1
Enzymatic deacetylation of arabinonucleosides **1** and **2** (Scheme 1).

Entry	Substrate	Product	Yield (%)[a]	*t* (h)	Enzyme	Deacetylation medium[b]
1	**1**	**3**	100	384	CAL B	Isopropanol
2	**2**	**4**	96	288	CAL B	Isopropanol
3	**1**	**5**	72	12	CAL B	Phosphate buffer pH 8
4	**2**	**6**	83	24	CAL B	Phosphate buffer pH 7
5	**1**	**7**	100	216	PMK	Phosphate buffer pH 8
6	**2**	**8**	100	168	DMK	Phosphate buffer pH 8

[a] Determined by HPLC (see Section 2.1).
[b] See Sections 2.5 and 2.6.



9 $R_1 = R_3 = R_4 = OAc$, $R_2 = H$, B = Uracil-1-yl
10 $R_1 = R_3 = R_4 = OH$, $R_2 = H$, B = Uracil-1-yl
11 $R_1 = R_3 = R_4 = OAc$, $R_2 = H$, B = Adenin-9-yl
12 $R_1 = R_3 = R_4 = OH$, $R_2 = H$, B = Adenin-9-yl

Chart 1.

tively and satisfactorily obtained in both reaction media (alcohol or buffer). These results show the influence of nucleoside furanosic moiety on CAL-B recognition.

Besides, the regioselectivity provided by CAL-B on **2** is complementary to that recently obtained in *Candida rugosa* lipase (CRL)-catalysed hydrolysis of **2**, which gave the product deacetylated at 3′ [18].

Quantitative complete deacylation of nucleosides under mild reaction conditions is a valuable tool in modified nucleoside synthesis, since it provides a useful deprotection procedure for base-labile nucleoside derivatives. With this aim, we have studied model acylated ribonucleosides [19,20] and applied it to two chlorinated base-labile nucleosides [20] and to nucleoside carbonates and carbamates [31]. Following with this idea, we studied quantitative full deacetylation of **1** and **2**, as models of arabinonucleosides. At reaction times longer than those reported in Entries 1 and 2 (Table 1), no quantitative full deacetylation of **1** and **2** was observed in CAL-B-catalysed alcoholyses. Under the hydrolysis conditions, CAL-B catalysed the complete full deacetylation of **2** after 14 days at pH 7, but **1** was not quantitatively fully deacetylated at the assayed pHs.

Several commercial hydrolases were then tested: lipases (CRL, PSL, PCL), an esterase (PLE) and the protease subtilisin. Keratinases are a group of mostly extracellular serine-proteases that hydrolyse keratins, highly resistant scleroproteins, and are produced by some insects and mainly by microorganisms [32]. Two nonpathogenic fungi, *Doratomyces microsporus* and *Paecilomyces marquandii* were selected for the production of the active keratinolytic serine-proteases DMK [26,27,33,34] and PMK [26], respectively, which hydrolyse different keratinous-, non-keratinous proteins [26,27,33,34] and synthetic peptidic substrates [26,27]. In addition of the set of commercial hydrolases above mentioned for the hydrolysis of **1** and **2**, we tested then PMK, DMK and also proteinase K (PK), a commercially available serine-protease displaying keratinolytic activity.

Assays of **1** and **2** hydrolysis were carried out at pH 8 for PMK and DMK and at pH 7 and 8 for all the other hydrolases. Except for PMK and DMK, none of the above listed assayed enzymes allowed **1** quantitative complete deacetylation; PMK (Entry 5, Table 1) required shorter reaction time than DMK (12 days). Full complete deacetylation of **2** could be reached by DMK in 7 days (Entry 6); CRL and PCL required longer reaction times (13 days) and the other assayed enzymes, including PMK, did not give quantitative deacetylation. None of the tested hydrolases catalysed the formation of regioselectively acetylated products, except for PLE, which gave the 2′-monoacetylated derivatives **3** and **4**, as previously reported [7].

DMK and PMK were also assayed in the hydrolysis of **9** and **11** (Chart 1), the corresponding ribo counterparts of **1** and **2**. Although **9** was not quantitatively full deacetylated to **10** by these keratinases, **11** was completely deacetylated to adenosine (**12**) by PMK, in much shorter time (48 h) than the arabino substrates. These results also show the influence of nucleoside structure on these keratinases recognition; as far as we know, DMK and PMK had not been previously tested with non-peptidic substrates.

In summary, in this work we reported CAL-B-catalysed regioselective deacetylation of arabinonucleosides **1** and **2** through good or high yield reactions. The choice of the reaction media allowed the regioselective formation of products bearing different degree of acetylation: in isopropanol, CAL-B-catalysed alcoholyses gave the 2′-*O*-acetylated derivatives **3** and **4**, while hydrolyses afforded the 2′,3′-di-*O*-acetylated products **5** and **6**. In particular, the procedure herein described allows a simple and efficient preparation of the reported vidarabine prodrug **6**, avoiding the utilisation of protective groups. Moreover, the application of two keratinases allowed the quantitative complete deacetylation of **1** and **2**.

Acknowledgements

We thank UNQ and SECyT (Scientific Cooperation Project ES/PA05E02 Argentina-Slovenia), as well as the Slovenian Research Agency, for partial financial support. AMI and LEI are research members of CONICET. We are grateful to Novozymes (Brazil) for the generous gift of CAL-B.

References

[1] E. De Clercq, Antiviral Res. 67 (2005) 56–75.
[2] A. Matsuda, T. Sasaki, Cancer Sci. 95 (2004) 105–111.
[3] E. De Clercq, J. Clin. Virol. 22 (2001) 73–89.
[4] H.J. Field, E. De Clercq, Microbiol. Today 31 (2004) 58–61.
[5] K. Kurosaki, N. Miwa, Y. Yoshida, M. Kurokawa, M. Kurimoto, S. Endo, K. Shiraki, Antiviral Chem. Chemother. 15 (2004) 281–285.
[6] L.A. Jones, A.R. Moorman, S.D. Chamberlain, P. de Miranda, D.J. Reynolds, C.L. Burns, T.A. Krenitsky, J. Med. Chem. 35 (1992) 56–63.
[7] S. Manfredini, P.G. Baraldi, R. Bazzanini, F. Bortolotti, S. Vertuani, N. Ashida, H. Machida, Antiviral Chem. Chemother. 9 (1998) 25–31.
[8] M. Mahmoudian, J. Eaddy, M. Dawson, Biotechnol. Appl. Biochem. 29 (1999) 229–233.
[9] S. Manfredini, P.G. Baraldi, E. Durini, L. Porcu, A. Angusti, S. Vertuani, N. Solaroli, E. De Clercq, A. Karlsson, J. Balzarini, Bioorg. Med. Chem. Lett. 11 (2001) 1329–1332.
[10] M. Tamarez, B. Morgan, G.S.K. Wong, W. Tong, F. Bennett, R. Lovey, J.L. McCormick, A. Zaks, Org. Process. Res. Dev. 7 (2003) 951–953.
[11] N. Ciliberti, S. Manfredini, A. Angusti, E. Durini, N. Solaroli, S. Vertuani, L. Buzzoni, M.C. Bonache, E. Ben-Shalom, A. Karlsson, A. Saada, J. Balzarini, Bioorg. Med. Chem. 15 (2007) 3065–3081.
[12] W. Shen, J.S. Kim, P.E. Kish, J. Zhang, S. Mitchell, B.G. Gentry, J.M. Breitenbach, J.C. Drach, J. Hilfinger, Bioorg. Med. Chem. Lett. 19 (2009) 792–796.
[13] M. Ferrero, V. Gotor, Chem. Rev. 100 (2000) 4319–4347.
[14] D. Kadereit, H. Waldmann, Chem. Rev. 101 (2001) 3367–3396.
[15] F. Morís, V. Gotor, Tetrahedron 49 (1993) 10089–10098.
[16] X.F. Li, M.H. Zong, H. Wu, W.Y. Lou, J. Biotechnol. 124 (2006) 552–560.
[17] X.F. Li, M.H. Zong, R.D. Yang, J. Mol. Catal. B: Enzym. 38 (2006) 48–53.
[18] T. Bavaro, S. Rocchietti, D. Ubiali, M. Filice, M. Terreni, M. Pregnolato, Eur. J. Org. Chem. (2009) 1967–1975.
[19] L.E. Iglesias, M.A. Zinni, M. Gallo, A.M. Iribarren, Biotechnol. Lett. 22 (2000) 361–365.
[20] D.I. Roncaglia, A.M. Schmidt, L.E. Iglesias, A.M. Iribarren, Biotechnol. Lett. 23 (2001) 1439–1443.
[21] M.A. Zinni, L.E. Iglesias, A.M. Iribarren, Biotechnol. Lett. 24 (2002) 979–983.
[22] M.A. Zinni, S.D. Rodríguez, R.M. Pontiggia, J.M. Montserrat, L.E. Iglesias, A.M. Iribarren, J. Mol. Catal. B: Enzym. 29 (2004) 129–132.
[23] W.H. Shannon, G. Arnett, D.C. Baker, S.D. Kumar, W.I. Higuchi, Antimicrob. Agents Chemother. 24 (1983) 706–712.
[24] T.F. Wu, K.K. Ogilvie, J. Org. Chem. 55 (1990) 4717–4724.

[25] M.A. Zinni, L.E. Iglesias, A.M. Iribarren, J. Mol. Catal. B: Enzym. 47 (2007) 86–90.
[26] H. Gradišar, J. Friedrich, I. Križaj, R. Jerala, Appl. Environ. Microbiol. 71 (2005) 3420–3426.
[27] H. Gradišar, S. Kern, J. Friedrich, J. Appl. Microbiol. Biotechnol. 53 (2000) 196–200.
[28] A. Matzuda, M. Shinozaki, M. Suzuki, K. Watanabe, T. Miyasaka, Synthesis (1986) 385–386.
[29] M.A. Zinni, E. Aljinovic, L.E. Iglesias, A.M. Iribarren, Quim. Nova 27 (2004) 496–498.
[30] D.C. Baker, A.K. Hebbler, Nucleic Acid Chem. (1991) 206–210.
[31] M. Capello, M. González, S.D. Rodríguez, L.E. Iglesias, A.M. Iribarren, J. Mol. Catal. B: Enzym. 36 (2005) 36–39.
[32] R. Gupta, P. Ramnani, Appl. Microbiol. Biotechnol. 70 (2006) 21–33.
[33] J. Friedrich, S. Kern, J. Mol. Catal. B: Enzym. 21 (2003) 35–37.
[34] J. Friedrich, H. Gradišar, M. Vrecl, A. Pogačnik, Enzyme Microb. Technol. 36 (2005) 455–460.