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Preparation of potential 3-deazauridine and 6-azauridine prodrugs through an enzymatic alcoholysis

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Abstract

A set of 3-deazauridine and 6-azauridine peracylated derivatives were regioselectively deacylated through *Candida antarctica* B lipase (CAL B) catalysed alcoholysis. This biotransformation provided an access to six new 2′,3′-di-*O*-acylated derivatives of 3-deazauridine and 6-azauridine carrying acetyl, butanoyl or hexanoyl moieties, which were obtained in 80–99% yield. The regioselectivity displayed by CAL B towards 5′-*O*-acyl group removal agrees with the previously reported behavior of this enzyme in the acylation and deacylation of nucleosides. Log *P*, aqueous solubility and aqueous stability of these new diacylated compounds were determined, suggesting that the dibutanoylated and the dihexanoylated derivatives of 3-deazauridine and 6-azauridine could potentially act as prodrugs of the parent pharmacological active nucleosides.

Keywords: Enzymatic alcoholysis; Lipase; Nucleosides; Prodrugs; Regioselectivity

1. Introduction

Nucleoside analogues are recognised pharmacological agents for the treatment of different diseases, finding important applications in antiviral [1–3] and anticancer [4] therapies. Among pharmacological active analogues of uridine, 3-deazauridine (**1**, Scheme 1) is a potent and specific CTP synthetase inhibitor showing antitumor activity [5], while 6-azauridine (**2**, Scheme 1) exhibits antiviral activity against a wide range of viruses responsible for diseases such as dengue [6] and is also used as an anti-psoriasis and anti-neoplastic agent [7]. In the latter case, 2′,3′,5′-tri-*O*-acetyl-6-azauridine (**6a**, azaribine, Scheme 2) was employed since nucleosides are polar molecules and more lipophilic derivatives can act as prodrugs with improved pharmacokinetic profile and therapeutic efficacy [8–13]. The preparation of such prodrugs may involve the regioselective derivatization of the furanosic hydroxyls; although this goal is not achieved satisfactorily through traditional synthetic transformations, monoacylated or alkoxycarbonylated nucleosides have been obtained by hydrolase-catalysed regioselective biotransformations [14,15].

In our laboratory, we have been applying the *Candida antarctica* B lipase (CAL B) catalysed alcoholysis to acylated nucleosides [16–19] and this procedure has shown to be a regioselective, simple and efficient access to 2′,3′-di-*O*-acylnucleosides [16–18]. Since some diacylated nucleosides have been described as prodrugs and their synthesis using traditional acylating agents usually yields poor regioselectivity and consequently, mixtures of partially acylated compounds [8], we decided to extend the study of the CAL B-catalysed alcoholysis in order to obtain a new set of potential prodrugs of **1** and **2** (**3–8b**, Scheme 2).

2. Experimental

2.1. General

Lipase B from *C. antarctica* (CAL B, Novozym 435, 10,000 propyl laurate units (PLU)/mg solid) 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

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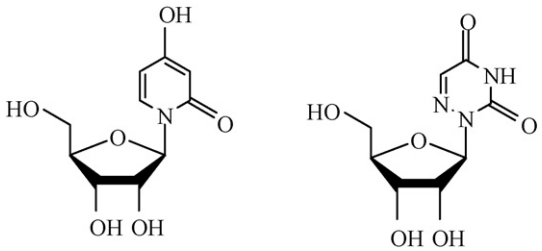


Scheme 1.

(Sontec OS 11, Argentina) at 250 rpm and 30 °C. No conversion took place in absence of lipase.

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

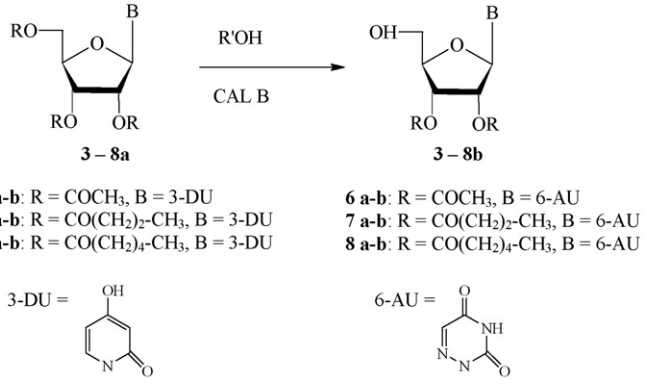
TLC was performed on Silicagel 60 F_{254} plates (Merck) and column chromatography was carried out using silicagel Merck 60.

HPLC analyses were carried out using a C-18 column with detection at 254 nm; in all cases, the mobile phase was water/acetonitrile 75:25 (v/v) at a flow rate of 1 ml min^{-1}.

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

2.2. Preparation of the substrates *3–8a*

As a general procedure, peracylated derivatives of **1** and **2** (**3–5a** for **1**; **6–8a** for **2**, Scheme 2) were prepared by reaction of the free nucleoside (1 mmol) with the corresponding acid anhydride (4 mmol) in acetonitrile (15 ml) containing triethylamine (4 mmol) and 4-dimethylaminopyridine (0.12 mmol), according to a previously reported protocol [20]. Under these conditions, no acylation of 3-deazauridine enolic hydroxyl took place; this can be explained considering the lesser nucleophicity of this aromatic hydroxyl if compared with the ribosidic hydroxyls. In all cases, products **1–8** were purified by silicagel column chromatography, affording satisfactory NMR data.



Scheme 2.

Table 1
Candida antarctica B lipase (CAL B)-catalysed alcoholysis of 3-deazauridine and 6-azauridine peracylated derivatives **3–8a**[a] (Scheme 2)

Entry	Substrate	Product **3–8b** (%)[b]	*t* (h)	Alcohol	*A*/*S*[c]
1	**3a**	78	24	Ethanol	1000
2	**3a**	80	8	Ethanol	260
3	**3a**	80	48	1-Butanol	1000
4	**4a**	90	24	Ethanol	1000
5	**4a**	99	7	Ethanol	260
6	**4a**	92	24	1-Butanol	1000
7	**5a**	75	24	Ethanol	1000
8	**5a**	90	6	Ethanol	260
9	**5a**	80	24	1-Butanol	1000
10	**6a**	77	24	Ethanol	1000
11	**6a**	96	0.5	Ethanol	260
12	**6a**	99	3.5	1-Butanol	1000
13	**7a**	78	24	Ethanol	1000
14	**7a**	88	7	Ethanol	260
15	**7a**	66	48	1-Butanol	1000
16	**8a**	50	24	Ethanol	1000
17	**8a**	80	4	Ethanol	260
18	**8a**	65	24	1-Butanol	1000

[a] Typical procedure (see Section 2.3).
[b] Determined by HPLC (see Section 2.1).
[c] *A*/*S* = alcohol/substrate molar ratio.

2.3. General procedure for the enzymatic alcoholysis of *3–8a*

Typically, experiments of enzymatic alcoholyses were performed by adding CAL (10 mg) to a suspension of **3–8a** (0.030 mmol) in the alcohol (7.80 mmol for *A*/*S* = 260 or 30 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, dissolved in acetonitrile and after removal of the enzyme, analysed by TLC and HPLC.

For preparative purposes, the above-described protocol was followed using **3–8a** (0.5 mmol) and ethanol (130 mmol, *A*/*S* = 260). When times reported in Table 1 were reached, the biotransformations were stopped by filtering off the enzyme and washing it with methanol. For each case, vacuum evaporation of the filtrate gave a crude residue that was subsequently purified by silicagel column chromatography (elution solvent and yield are given between brackets) to afford 2′,3′-di-*O*-acylated products **3–8b**: **3b** (dichloromethane–methanol 96:4, v/v, 71%), **4b** (dichloromethane–methanol 96:4, v/v, 85%), **5b** (dichloromethane–methanol 94:6, v/v, 70%), **6b** (dichloromethane–methanol 95:5, v/v, 75%), **7b** (dichloromethane–methanol 95:5, v/v, 65%), **8b** (dichloromethane–methanol 95:5, v/v, 50%).

2.4. Determination of the aqueous solubility of compounds *1, 2, 3–8a, 3–8b*

An excess of each compound (3–12 mg for **4a**, **5a–b**, **7a** and **8a**; 25–75 mg for **3a–b**, **4b**, **6a–b**, **7b**, and **8b**) was shaken in phosphate buffer 30 mM pH 7 (2 ml) at 37 °C for 2 h. The resulting mixtures were then centrifuged for 10 min at 10,000 rpm, and the supernatants filtered through 0.2-μm cellulose filtres. The

Table 2
^{1}H NMR of 3-deazauridine acylated derivatives[a] (Scheme 2)

Compound	H6	H5	H3	H1′	H2′	H3′	H4′	H5′$_a$	H5′$_b$	Acyl moieties
3a	7.45 (d, J = 8 Hz)	6.10 (dd, J_1 = 8 Hz, J_2 = 2 Hz)	5.96 (d, J = 2 Hz)	6.26 (d, J = 4 Hz)	5.29–5.32 (m, 2H)			4.33–4.41 (m, 3H)		2.14 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H)
3b	7.58 (d, J = 8 Hz)	6.05 (dd, J_1 = 8 Hz, J_2 = 2 Hz)	5.83 (d, J = 2 Hz)	6.09 (d, J = 5 Hz)	5.53 (t, J = 5 Hz)	5.46 (t, J = 5 Hz)	4.21 (m)	3.96 (dd, J_1 = 12 Hz, J_2 = 2 Hz)	3.79 (dd, J_1 = 12 Hz, J_2 = 2 Hz)	2.09 (s, 3H), 2.08 (s, 3H)
4a	7.47 (d, J = 8 Hz)	6.09 (dd, J_1 = 8 Hz, J_2 = 2 Hz)	6.00 (d, J = 2 Hz)	6.31 (d, J = 4 Hz)	5.30–5.36 (m, 2H)			4.34–4.38 (m, 3H)		2.34 (m, 6H), 1.65 (m, 6H), 0.93 (m, 9H)
4b	7.56 (d, J = 8 Hz)	6.04 (dd, J_1 = 8 Hz, J_2 = 2 Hz)	5.83 (d, J = 2 Hz)	6.07 (d, J = 5 Hz)	5.54 (t, J = 5 Hz)	5.47 (t, J = 5 Hz)	4.20 (m)	3.96 (dd, J_1 = 12 Hz, J_2 = 2 Hz)	3.80 (dd, J_1 = 12 Hz, J_2 = 2 Hz)	2.30 (m, 4H), 1.62 (m, 4H), 0.92 (m, 6H)
5a	7.47 (d, J = 8 Hz)	6.07–6.09 (m, 2H)		6.31 (d, J = 4 Hz)	5.30–5.35 (m, 2H)			4.34–4.37 (m, 3H)		2.36 (m, 6H), 1.63 (m, 6H), 1.31 (m, 12H), 0.89 (m, 9H)
5b	7.53 (d, J = 8 Hz)	6.05 (dd, J$_1$ = 8 Hz, J$_2$ = 2 Hz)	5.86 (d, J = 2 Hz)	6.27 (d, J = 5 Hz)	5.55 (t, J = 5 Hz)	5.48 (t, J = 5 Hz)	4.20 (m)	3.98 (dd, J_1 = 12 Hz, J_2 = 2 Hz)	3.81 (dd, J_1 = 12 Hz, J_2 = 2 Hz)	2.33 (m, 4H), 1.60 (m, 4H), 1.29 (m, 8H), 0.91 (m, 6H)

[a] 500 MHz, $CDCl_3$; chemical shifts (δ) are expressed in ppm.

Table 3
^{1}H NMR of 6-azauridine acylated derivatives[a] (Scheme 2)

Compound	H3	H5	H1′	H2′	H3′	H4′	H5′$_a$	H5′$_b$	Acyl moieties
6a	n.o.[b]	7.47 (s)	6.29 (d, J = 4 Hz)	5.63 (dd, J_1 = 6 Hz, J_2 = 4 Hz)	5.42 (t, J = 6 Hz)	4.33 (dt, J_1 = 6 Hz, J_2 = 4 Hz)	4.17 (dd, J_1 = 12 Hz, J_2 = 6 Hz)	4.37 (dd, J_1 = 12 Hz, J_2 = 4 Hz)	2.10 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H)
6b	9.70 (s)	7.51 (s)	6.25 (d, J = 4 Hz)	5.65 (dd, J_1 = 5 Hz, J_2 = 4 Hz)	5.48 (t, J = 5 Hz)	4.23 (dt, J_1 = 5 Hz, J_2 = 3 Hz)	3.87 (dd, J_1 = 13 Hz, J_2 = 3 Hz)	3.72 (dd, J_1 = 13 Hz, J_2 = 3 Hz)	2.10 (s, 3H), 2.09 (s, 3H)
7a	n.o.[b]	7.49 (s)	6.23 (d, J = 5 Hz)	5.66 (t, J = 5 Hz)	5.48 (t, J = 5 Hz)	4.21 (m)	3.87 (dd, J_1 = 13 Hz, J_2 = 3 Hz)	3.72 (dd, J_1 = 13 Hz, J_2 = 3 Hz)	2.31 (m, 6H), 1.64 (m, 6H), 0.95 (m, 9H)
7b	9.72 (s)	7.45 (s)	6.23 (d, J = 5 Hz)	5.66 (t, 5Hz)	5.49 (t, J = 5 Hz)	4.22 (m)	3.72 (dd, J_1 = 13 Hz) J_2 = 3 Hz)	3.87 (dd, J_1 = 13 Hz, J_2 = 3 Hz)	2.32 (m, 4H), 1.65 (m, 4H), 0.95 (m, 6H)
8a	9.48 (s)	7.48 (s)	6.26 (d, J = 4 Hz)	5.63 (dd, J_1 = 6 Hz, J_2 = 4 Hz))	5.39 (t, J = 6 Hz)	4.32 (m)	4.36 (dd, J_1 = 12 Hz, J_2 = 4 Hz)	4.17 (dd, J_1 = 12 Hz, J_2 = 4 Hz)	2.36 (m, 6H), 1.63 (m, 6 H), 1.31 (m, 12 H), 0.89 (m, 9H)
8b	9.70 (s)	7.50 (s)	6.22 (d, J = 5 Hz)	5.65 (t, J = 5 Hz)	5.47 (t, J = 5 Hz))	4.21 (m)	3.72 (dd, J_1 = 12 Hz, J_2 = 3 Hz)	3.87 (dd, J_1 = 12 Hz, J_2 = 3 Hz)	2.33 (m, 4H), 1.61 (m, 4H), 1.31 (m, 8H), 0.88 (m, 6H)

[a] 500 MHz, $CDCl_3$; chemical shifts (δ) are expressed in ppm.
[b] n.o.: unobserved.

Table 4
^{13}C MNR of 3-deazauridine acylated derivatives[a] (Scheme 2)

Compound	C2	C3	C4	C5	C6	C1′	C2′	C3′	C4′	C5′	Acyl moieties
3a	169.06	99.27	164.68	103.77	133.09	87.63	74.04	69.70	79.41	62.88	170.38, 169.68, 169.60 (COs), 20.80, 20.53, 20.48
3b	168.92	99.36	164.97	103.63	135.20	89.36	74.01	70.38	82.96	61.30	170.28, 170.04, (COs) 20.65, 20.55
4a	169.03	99.32	164.66	103.76	132.97	87.42	73.93	69.52	79.64	62.75	173.00, 172.26, 172.13 (COs), 36.00, 35.72, 35.66, 18.36, 18.31, 18.25, 13.68, 13.62, 13.59
4b	168.95	99.03	164.95	103.67	135.13	89.72	73.91	70.14	83.07	61.38	172.85, 172.52 (COs), 35.86, 35.74, 18.29, 18.26, 13.65, 13.59
5a	168.81	99.50	164.60	103.66	133.55	87.44	73.92	69.58	79.66	62.80	173.20, 172.43, 172.29 (COs), 34.12, 34.01, 33.85, 33.78, 31.29, 31.25, 31.24, 31.20, 24.55, 24.46, 24.43, 24.42, 22.33, 22.30,13.90, 13.89
5b	168.96	99.15	164.92	103.68	134.15	87.55	73.87	70.08	83.08	61.43	172.99, 172.60 (COs), 33.96, 33.89, 31.26, 31.20, 24.49, 24.46, 22.32, 22.30

[a] 125 MHz, $CDCl_3$; chemical shifts (δ) are expressed in ppm.

UV spectra of the filtrates were recorded and the absorbance at the λ_{max} employed to determine the concentration of the saturated solution. Measurements were carried out by triplicate.

2.5. Determination of log P of compounds *1*, *2*, *3–8a*, *3–8b*

The octanol–water partition coefficients (log *P*) were determined by the shake flask method. Solutions of each compound (0.05–0.08 mM) in phosphate buffer 30 mM pH 7 (5 ml) were shaken at 200 rpm and 37 °C for 2 h. Then, the same volume of 1-octanol was added and the mixture further shaken for 24 h under the same experimental conditions. The two layers were separated and the amount of the compound in the aqueous layer determined by UV absorbance, taking into account the absorbance values previously determined in buffer. Log *P* was determined from the formula:

$$\log P = -\log\left[\frac{A_0 - A_f}{A_f}\right]$$

where A_0 is the initial absorbance of the aqueous layer and A_f the absorbance of the aqueous layer after equilibration.

3. Results and discussion

First, the enzymatic alcoholysis of **3–8a** was studied (Table 1) employing a large alcohol/substrate molar ratio ($A/S = 1000$), since in previous experiments of CAL B-catalysed alcoholysis of nucleosides we found that a high excess of the alcohol gave regioselectively diacylated products in good yields. Moreover, an $A/S = 260$ was also assayed taking into account that this ratio, calculated from a experimental design methodology, improved the yield of 2′,3′-di-*O*-hexanoyluridine obtained through enzymatic alcoholysis [21]. Data presented in Table 1 show that diacylated products **3–8b** could be obtained in 80–99% yield; in most cases, the best results were reached by using ethanol at an $A/S = 260$.

New compounds **3–8b** were identified as the 2′,3′-di-*O*-acylated derivatives of the corresponding nucleosides, according to NMR data. ^{1}H NMR spectra (Tables 2 and 3) showed for each product two signals at 3.7–3.9 ppm, a chemical shift consistent with methylene hydrogens of primary alcohols (H5′$_a$ and 5′$_b$). Moreover, ^{13}C NMR (Tables 4 and 5) revealed that while C5′ chemical shift did not show a significant difference

Table 5
^{13}C RMN of 6-azauridine acylated derivatives[a] (Scheme 2)

Compound	C2	C4	C5	C1′	C2′	C3′	C4′	C5′	Acyl moieties
6a	148.06	156.02	136.60	88.35	72.70	70.68	79.57	63.27	170.53, 169.65, 169.57 (COs), 20.72, 20.49, 20.47
6b	147.81	155.51	136.85	88.72	72.89	70.89	83.33	62.02	170.01, 169.82 (COs), 20.64, 20.52
7a	148.05	156.01	136.70	88.92	72.74	70.78	79.48	64.11	172.78, 172.56 (COs), 35.82, 35.69, 30.89, 18.31, 18.23, 13.62, 13.55
7b	147.72	155.45	136.72	88.97	72.76	70.75	83.47	62.10	172.58, 172.38 (COs), 35.83, 35.7, 18.32, 18.24, 13.63, 13.57
8a	148.07	156.02	136.66	88.57	72.75	70.76	79.37	63.32	172.52, 172.25 (COs), 34.22, 34.11, 33.85, 33.68, 31.29, 31.25, 31.24, 31.20, 24.55, 24.46, 24.43, 24.42, 22.33, 22.30,13.90, 13.89
8b	147.73	155.55	136.74	88.99	72.77	70.73	83.43	62.07	172.75, 172.54 (COs), 33.94, 33.83, 31.25, 31.18, 24.50, 24.41, 22.29, 22.25, 13.86, 13.84

[a] 125 MHz, $CDCl_3$; chemical shifts (δ) are expressed in ppm.

Table 6
Maximal wavelength, partition coefficient (log *P*) and aqueous solubility of compounds **1**, **2**, **3**–**8a** and **3**–**8b** (Scheme 2)

Entry	Compound	λ_{max}[a], nm (ε, cm^{-1} mM^{-1})	Log *P*[b]	Solubility[c] (mg/ml)
1	**1**	258.0 (5.82)	−1.75	34.98
2	**3a**	257.6 (11.76)	0.12	12.55
3	**3b**	252.7 (6.51)	−1.32	22.89
4	**4a**	257.6 (16.33)	3.15	0.44
5	**4b**	256.5 (7.52)	0.7	9.38
6	**5a**	252.7 (1.14)	4.01	0.13
7	**5b**	256.5 (6.21)	1.77	5.02
8	**2**	255.6 (7.69)	−2.37	198.75
9	**6a**	255.8 (2.08)	−0.05	21.15
10	**6b**	254.9 (6.83)	−1.09	32.24
11	**7a**	255.0 (5.77)	2.57	1.08
12	**7b**	256.1 (7.11)	1.05	14.44
13	**8a**	256.9 (1.02)	3.08	0.59
14	**8b**	253.3 (4.83)	1.97	8.39

[a] In 30 mM phosphate buffer (see Section 2.4).
[b] The 1-octanol/water partition coefficient was determined using the shake flask method (see Section 2.5).
[c] Determined in 30 mM phosphate buffer (pH 7) at 37 °C (see Section 2.4).

in comparison with C5′ of the triacylated compounds **3**–**8a** (ca. 1.5 ppm), a shift towards lower fields was observed for C4′ in the 2′,3′-di-*O*-acylated derivatives (ca. 3.5 ppm).

The regioselectivity displayed in these experiments by CAL B towards 5′-*O*-acyl group removal agrees with the behavior previously observed in the acylation and deacylation of nucleosides using this lipase [14–19].

In order to characterize some properties of **3**–**8b** as potential prodrugs, their aqueous solubility (Section 2.4) and their partition coefficient (Section 2.5) were determined and compared with those of **1**, **2** and **3**–**8a** (Table 6). The obtained data suggest that the 2′,3′-di-*O*-butanoylated (**4b**, Entry 5; **7b**, Entry 12) and the 2′,3′-di-*O*-hexanoylated derivatives (**5b**, Entry 7; **8b**, Entry 14) could be considered as potential prodrugs for enteral and intramuscular preparations.

Table 7
Aqueous stability of nucleosides **3**–**8a** and **3**–**8b**[a] (Scheme 2)

Compound	Recovery (%)[b] after 45 days at 4 °C	Recovery (%)[b] after 24 h at 37 °C
3a	99	98
3b	98	94
4a	97	95
4b	97	98
5a	97	96
5b	97	94
6a	98	98
6b	98	96
7a	97	99
7b	98	97
8a	96	96
8b	97	95

[a] In 30 mM phosphate buffer (pH 7).
[b] Determined by HPLC (see Section 2.1).

Moreover, the stability of these acylated compounds in aqueous medium was studied at 4 and 37 °C (Table 7). In the former case, only losses below 4% of the potential prodrugs were found after 45 days of incubation and in the latter one, no more than 6% of the compound was hydrolysed after 24 h of incubation. This behavior indicates that these compounds have appropriate storage stability and do not undergo appreciable spontaneous chemical hydrolysis.

In summary, in this work we have prepared in high yields a set of new 3-deazauridine and 6-azauridine diacylated derivatives through the application of a regioselective enzymatic alcoholysis. Some of these diacylated nucleosides have aqueous solubilities and lipophilicity that could render them useful as potential prodrugs of the parent drugs.

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References

[1] E. Ichikawa, K. Kato, Curr. Med. Chem. 8 (2001) 385.
[2] H.H. Balfour, New Engl. J. Med. 340 (2004) 1255.
[3] E. De Clercq, Antivir. Res. 67 (2005) 56.
[4] C.R. Wagner, V.V. Lyer, E.J. Mclintee, Cancer Res. 59 (2000) 2944.
[5] S. Hatse, E. De Clercq, J. Balzarini, Biochem. Pharmacol. 58 (1999) 539.
[6] J.M. Crance, N. Scaramozzino, A. Jouan, D. Garin, Antivir. Res. 58 (2003) 73.
[7] J. Zhou, C.M. Riley, R.L. Schowen, J. Pharm. Biomed. Anal. 26 (2001) 701.
[8] 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.
[9] S. Manfredini, P.G. Baraldi, R. Bazzanini, F. Bortolotti, S. Vertuani, N. Ashida, H. Machida, Antivir. Chem. Chemother. 9 (1998) 25.
[10] M. Mahmoudian, J. Eaddy, M. Dawson, Biotechnol. Appl. Biochem. 29 (1999) 229.
[11] R.L. Hanson, Z. Shi, D.B. Brzozowski, A. Banerjee, T.P. Kissick, J. Singh, A.J. Pullockaran, J.T. North, J. Fan, J. Howell, S.C. Durand, M.A. Montana, N.R. Kronenthal, R.H. Mueller, R.N. Patel, Bioorg. Med. Chem. 8 (2000) 2681.
[12] 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.
[13] N. Wang, Z.C. Chen, D.S. Lu, X.F. Lin, Bioorg. Med. Chem. Lett. 15 (2005) 4064.
[14] M. Ferrero, V. Gotor, Chem. Rev. 100 (2000) 4319.
[15] D. Kadereit, H. Waldmann, Chem. Rev. 101 (2001) 3367.
[16] L.E. Iglesias, M.A. Zinni, M. Gallo, A.M. Iribarren, Biotechnol. Lett. 22 (2000) 361.
[17] D.I. Roncaglia, A.M. Schmidt, L.E. Iglesias, A.M. Iribarren, Biotechnol. Lett. 23 (2001) 1439.
[18] M.A. Zinni, L.E. Iglesias, A.M. Iribarren, Biotechnol. Lett. 24 (2002) 979.
[19] 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.
[20] A. Matzuda, M. Shinozaki, M. Suzuki, K. Watanabe, T. Miyasaka, Synthesis (1986) 385.
[21] M.A. Zinni, E. Aljinovic, L.E. Iglesias, A.M. Iribarren, Quim. Nova 27 (2004) 496.