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Complete and regioselective deacetylation of peracetylated uridines using a lipase

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Abstract

Lipase-catalysed alcoholysis and hydrolysis of 2′,3′,5′-tri-*O*-acetyluridine (**1a**) and 2′,3′,5′-tri-*O*-acetyl-2′-*C*-methyluridine (**1b**) were studied. Conditions for full and regioselective deacetylation of **1a** and **1b** are shown in the present work. New compound 2′,3′-di-*O*-acetyl-2′-*C*-methyluridine (**3b**) was prepared by regioselective lipase-catalysed deacetylation.

Introduction

Over the last decade much effort has been devoted to the synthesis of nucleoside analogues (Hanrahan & Hutchinson 1992) since most of the antiviral agents used at present against extensive viral affections belong to this family of compounds (Perigaud *et al.* 1992). In the synthesis of modified nucleosides, esters are currently used to protect ribose hydroxyl groups. As their subsequent removal is carried out by treatment with ammonia or methoxide (Jones 1985), reactive groups present either in the ribose or in the base may interfere (Brown & Weliky 1953, Conde *et al.* 1998, Ritzmann *et al.* 1975), thus, a mild procedure for removal of acyl groups is an alternative route of synthetic value.

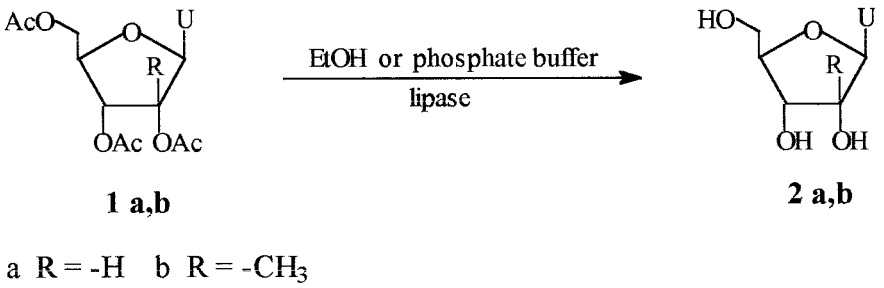
Although enzymatic techniques appear to be very attractive for such purpose, due to their well-known mildness and efficiency (Faber 1995), only little attention has been paid to hydrolase-catalysed deacylation of ribonucleosides. To our knowledge, the only available reports regarding this kind of compounds deal with protease (Singh *et al.* 1993) and lipase (Ciuffreda *et al.* 1999) catalysed regioselective hydrolysis. In the case of modified nucleosides, enzymatic regioselective and full deacylation of acyclovir derivatives have been reported (Conde *et al.* 1998). However, there are no data of enzymatic full deacylation of ribonucleosides, despite the potential usefulness of this procedure. This prompted us to study the lipase-catalyzed total deacetylation of 2′,3′,5′-tri-*O*-acetyluridine (**1a**) and 2′,3′,5′-tri-*O*-acetyl-2′-*C*-methyluridine (**1b**).

Materials and methods

General

Candida rugosa lipase (CRL, formerly *Candida cylindracea*, 1010 units mg^{-1} solid) and porcine pancreatic lipase (PPL, 43 units mg^{-1} solid) were respectively purchased from Sigma and ICN Chemicals. Lipases from *Candida antarctica* (CAL, Novozyme 435, 9500 PLU mg^{-1} solid; PLU: Propyl Laurate Units) and from *Rhizomucor miehei* (LIP, Lipozyme IM 20, 25 BIU g^{-1} solid; BIU: Batch Interesterification Units) were a generous gift from Novo Nordisk. Enzymes were used straight from the bottle without any further treatment or purification.

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



1 a,b **2 a,b**

a R = -H b R = -CH_3

Scheme 1.

Compounds **1a,b** were respectively prepared by reaction of uridine and 2′-methyluridine with an excess of acetic anhydride and triethylamine in acetonitrile. 2′-Methyluridine was synthesised through a procedure recently developed in our laboratory (M. Gallo *et al.*, unpublished results). Enzymatic reactions were carried out in a temperature-controlled incubator shaker (Sontec OS 11, Argentina) at 200 rpm and at the temperatures indicated in the text. TLC was performed on Silica gel 60 F_{254} plates (Merck) and column chromatography was carried out using silica gel Merck 60.

Proton magnetic resonance spectra were recorded on a Bruker AC-200 spectrometer in DMSO-d_6.

*Enzymatic deacetylation of nucleosides **1a,b***

(a) Enzymatic hydrolysis

In a typical experiment, lipase (CAL: 300 mg $mmol^{-1}$ substrate) was added to a suspension of the substrate (**1a,b**, 0.5 mmol) in potassium phosphate buffer (30 mM, pH 8.0, 8 ml) and the resulting mixture was shaken at 200 rpm and 28 °C. Samples of the reaction mixture were withdrawn at various times, dissolved in ethanol and dimethylformamide, and after removal of the biocatalyst, analyzed by TLC using dichloromethane/methanol (85:15, v/v) as the mobile phase. When TLC showed a single spot (R_f = 0.1 for **2a** and 0.2 for **2b**), indicating complete deacetylation, the reaction was stopped, the enzyme filtered off and washed with ethanol and a little amount of dimethylformamide. Vacuum evaporation of the filtrate afforded products **2a,b**.

(b) Enzymatic alcoholysis

Similarly to the above described procedure, CAL (300 mg $mmol^{-1}$ substrate) was added to a suspension of **1a,b** (1 mmol) in absolute ethanol (2.0 ml, 35 mmol) and the resulting mixture was shaken at 200 rpm and 28 °C.

When alcoholysis was performed to prepare the diacetylated products **3a,b**, 75 ml ethanol (1.3 mol) were employed. In this instance, samples of the reaction mixture taken at different times were analyzed by both TLC and HPLC, and after a convenient time was reached, the enzyme was filtered off and washed with ethanol and chloroform. Products **3a,b**, isolated by column chromatography using dichloromethane/methanol (95:5, v/v) as the eluent, afforded satisfactory spectral data (^{1}H and ^{13}C NMR).

Control experiments, carried out by incubation of **1a**,**b** in buffer or ethanol, showed no appreciable reaction.

HPLC analysis

During the enzymatic regioselective alcoholysis, samples were withdrawn and analyzed by HPLC (with detection at 254 nm) using a C-18 column. A gradient from water to water/acetonitrile (60:40, v/v; 20 min) was employed as the mobile phase at a flow rate of 0.4 ml min^{-1}.

Results and discussion

Two enzymatic procedures were investigated to achieve full deacetylation of nucleosides **1a,b** (Scheme 1): lipase-catalyzed alcoholysis and hydrolysis. Four lipases were tested: PPL, LIP, CRL and CAL (see Materials and methods).

Although hydrolase-catalyzed alcoholysis of esters is a valuable alternative to enzymatic hydrolysis (Drauz & Waldmann 1995), to our knowledge, it has not been applied so far to ribonucleosides. Then, lipase-catalyzed ethanolysis of **1a** was first studied in order to find suitable conditions to perform full deacetylation, since compound **1b** is a less available substrate, obtained through a complex synthetic route.

Table 1. Candida antarctica lipase-catalysed alcoholysis of 2′,3′,5′-tri-*O*-acetyluridine (**1a**) and 2′,3′,5′-tri-*O*-acetyl-2′-*C*-methyluridine (**1b**)[a].

Entry	Substrate	Ethanol/nucleoside ratio	Cosolvent	*t* (h)	Conversion[b] (%)	Yield[b] (%)	
						3	**2**
1	**1 a**	15	1,4-Dioxane[c]	168[d]	99	16	31
2			Chloroform[c]	48[d]	100	64	12
3			DMF[c]	24[d]	0	0	0
4	**1 a**	35	None	24	100	29	22
5			None	48	100	13	52
6			None	96[d]	100	0	100
7	**1 a**	35[e]	None	48[d]	99	44	11
8	**1 b**	35	None	144[d]	91	76	5
9	**1 a**	120	None	144[d]	100	5	84
10	**1 b**	120	None	144[d]	95	70	10
11	**1 a**	> 1000	None	29	81	74	0
12			None	48[d]	90	71	0
13	**1 b**	> 1000	None	96	92	92	0
14			None	120[d]	93	93	0

[a]Typical procedure: see Materials and methods.
[b]Determined by HPLC (see Materials and methods).
[c]7 ml solvent mmol^{-1} substrate was added.
[d]No further conversion was observed after this time.
[e]*n*-Butanol as nucleophile and solvent.

Only CAL, a lipase with well-documented high performance for the transformation of a wide variety of non-natural organic substrates (Kazlauskas & Bornscheuer 1998), showed activity in the alcoholysis and no reaction of nucleoside **1a** was observed using PPL, CRL and LIP. Results collected in Table 1 show that both the ethanol/nucleoside (E/N) ratio and the solvent had a dramatic influence on the enzymatic alcoholysis. At a E/N ratio = 15, no quantitative full deacetylation of **1a** could be achieved with the tested solvents. Entries 1 and 2 (Table 1) show typical data for these reactions, in which a non selective process led to a diacetylated product, identified by HPLC as compound **3a**, a mixture of monoacylated products and the free nucleoside (**2a**). Similar reaction profiles were observed at 60 °C (data not shown).

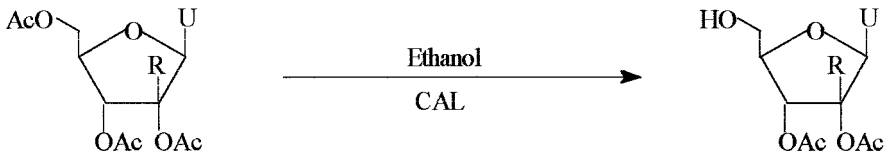
Total conversion of **1a** into the free nucleoside **2a** was accomplished within 96 h by increasing the E/S ratio to 35 (Entries 4–6). No complete full deacetylation of **1a** could be reached either at 60 °C (data not shown) or by using *n*-butanol as nucleophile (Entry 7).

Under the best conditions found to carry out total removal of acetates groups from **1a** (Entry 6), **1b** reacted slower (Entry 8) and no total conversion into **2b** could be performed, a behaviour that may be explained taking into account the steric effect of the bulky tertiary acetate of **1b**.

By further increasing the E/N ratio (Entries 9–14) a remarkable decrease in **2a,b** production was observed. In a very high excess of ethanol (E/N >1000) the reactions proceeded slower and no formation of free nucleosides **2a,b** was observed, but this condition provided a convenient method to obtain regioselectively the 2′,3′-di-*O*-acetyl derivatives **3a,b** (Entries 11–14; Scheme 2).

It is worth noting that performing the alcoholysis at a E/N ratio = 5 in dioxane and chloroform, the alcoholysis did not proceed regioselectively, even when such ratio might be expected to promove regioselectivity (Conde *et al.* 1998, Sanfilippo *et al.* 1997). Both compounds, **3b**, whose existence has not been described in literature, and **3a** would require a three step process if prepared through non enzymatic synthetic procedures.

By comparison with lipase-catalyzed alcoholysis, the course of the enzymatic hydrolysis of **1a,b** (Table 2) exhibited some differences. Total conversion of **1a** to **2a** was reached in 48 h in phosphate buffer at 60 °C (Table 2, Entry 5), faster than through alcoholysis (Table 1, Entry 6). No full deacetylation could be performed when reactions presented in Ta-



1 a,b **3 a,b**

a R = -H b R = -CH_3

Scheme 2.

Table 2. Candida antarctica lipase-catalysed hydrolysis of 2′,3′,5′-tri-*O*-acetyluridine (**1a**) and 2′,3′,5′-tri-*O*-acetyl-2′-*C*-methyluridine (**1b**)[a].

Entry	pH	*T* (°C)	Substrate	*t* (h)[b]
1	7	28	**1a**	96
2	8	28	**1a**	72
3	8	28	**1b**	24[c]
4	8	28	**1b**	288
5	8	60	**1a**	48
6	8	60	**1b**	48[d]

[a]Typical procedure: see Materials and methods.
[b]Time required to achieve quantitative formation of **2a,b**.
[c]**3b** was formed quantitatively.
[d]No quantitative full deacetylation was achieved and **3b** was formed as the main product (61% by HPLC).

ble 2 were carried out using dimethylformamide and 1,4-dioxane as solvents. Although disappearence of **1a** was complete with CCL and LIP at 28 °C and pH 8, no quantitative formation of **2a** could be achieved after 7 days of reaction, while PPL allowed only little reaction of **1a** after 9 days. Contrasting to the enzymatic alcoholysis, product **3a** was never selectively produced in the studied hydrolysis, which showed by TLC a non selective pattern of products consisting of mixtures of di- and monoacetylation compounds.

Nucleoside **1b** reacted very slowly and **2b** was formed quantitatively only after 12 days of reaction (Table 2, Entry 4), indicating again a possible unfavourable influence of the bulky tertiary acetate. At 60 °C, **3b** was the main product of a mixture also containing monoacetylated nucleosides and **2b** (Entry 6). However, **3b** was quantitatively obtained within 24 h (Entry 3), faster than through alcoholysis (Table 1, Entries 13, 14).

In summary, we found simple and mild enzymatic transformations for full removal of acetates as well as regioselective conditions for the production of compounds **3a,b** in good yields. The procedures presented herein are at present being extended to the synthesis of other ribonucleosides analogues.

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