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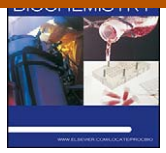

Regioselective acylation of D-ribono-1,4-lactone catalyzed by lipases

Damianni Sebrão, Marcus M. Sá, Maria da Graça Nascimento*

Departamento de Química, Universidade Federal de Santa Catarina, Florianópolis, SC 88040-900, Brazil

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ABSTRACT

Lipases from ten different sources and two mycelium-bound lipases isolated from Amazonian fungi were screened as biocatalysts in the acylation reaction of D-ribono-1,4-lactone with a variety of acyl donors in non-aqueous media. Several reaction parameters were evaluated including the type and amount of enzyme, acyl donor, and organic solvent, as well as the influence of water and the recyclability of the catalyst. When *Candida antarctica* lipase (CAL-B) was used, the acylation was highly regioselective and the corresponding 5-acyl-D-ribono-1,4-lactones were observed as the sole product. The best conversion (>99%) into 5-acetyl-D-ribono-1,4-lactone was obtained through the combination of vinyl acetate as the acetyl donor and 10 mg (100 U) of CAL-B in dry acetonitrile after 24 h. However, lipases from *Burkholderia cepacia* (PSL-C and PSL-D), *Pseudomonas fluorescens* (AK) and *Thermomyces langinosus* (Lipozyme TL-IM) gave mixtures of mono-, di- and tri-acetylated products in lower conversions. CAL-B maintained its catalytic activity during five cycles of repeated use when decanoic and dodecanoic acids were employed as acyl donors in the acylation of D-ribono-1,4-lactone.

1. Introduction

Carbohydrates are valuable sources for the production of synthetic compounds of general relevance [1–3]. For example, sugar fatty acid esters are an important class of biodegradable non-ionic surfactants with widespread application in the food, cosmetic and pharmaceutical industries [4]. The acylation of an alcohol to produce an ester is amongst the most fundamental organic reactions, and acylation of monosaccharides is often involved in the multistep synthesis of complex carbohydrates [5–7]. While the acylation of simple alcohols can be readily accomplished by base-catalyzed transesterification under relatively simple conditions, the regioselective synthesis of acyl esters of sugars is much more difficult and unpredictable due to the presence of multiple hydroxyl groups. Therefore, an efficient multistep synthesis of carbohydrate derivatives frequently requires the introduction of protective groups and their subsequent removal [8–10].

On the other hand, enzymatically catalyzed sugar fatty acid esterification reactions are, in general, reasonably specific and the regioselective acylation of several carbohydrates with hydrolases such as lipases, esterases and proteases has been reported [11–14]. Lipases (EC 3.1.1.3) are the most used for this purpose, with recent reports describing efficient protocols for the selective lipase-catalyzed acylation of monosaccharide derivatives and polymeric prodrugs of 5-fluorouridine [15]. It is also known that the source of the lipase, as well as many of the reaction conditions, is responsible for dramatic differences in the reaction outcome, and these parameters can be fine tuned in order to generate the desired regioisomeric product.

Chemical transformations which do not require harmful reagents or complex purification techniques and do not generate toxic waste represent a fundamental target of contemporary organic synthesis [16–18]. The high selectivity of enzymes can simplify industrial processes by increasing the chemical yields and reducing the generation of by-products, and biocatalysis is thus an important tool in the development of green chemistry [19,20]. D-Ribono-1,4-lactone (**1**) is an inexpensive and abundant sugar derivative that is commercially available from renewable resources [21,22]. Because of its high functionality with contiguous chiral centers, **1** has been widely used as a versatile chiral building block for the construction of a variety of natural products [23–25]. In principle, the acetylation of D-ribono-1,4-lactone (**1**) may lead to seven distinct acetylated products (three mono-, three di- and one triacetylated derivatives, Scheme 1). However, many other unexpected products can also be formed in one simple reaction involving carbohydrates due to the possible presence of different species co-existing in equilibrium under certain reaction conditions and also to processes ranging from rearrangements to functional group migration [26–28].

As part of our research interest in lipase-catalyzed transformations [29–31], we studied the regioselective acylation of the primary and secondary hydroxyl groups of D-ribono-1,4-lactone (**1**) catalyzed by lipases from different sources under a variety of reaction conditions, and the results are presented herein.

* Corresponding author. Tel.: +55 48 37216844; fax: +55 48 37216850.
E-mail address: graca@qmc.ufsc.br (M.G. Nascimento).

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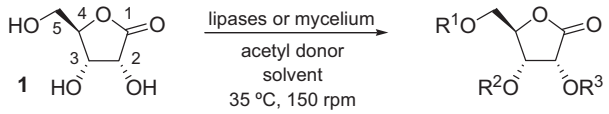


	R^1	R^2	R^3		R^1	R^2	R^3
2	H	H	Ac	**6**	Ac	H	Ac
3	H	Ac	H	**7**	Ac	Ac	H
4	H	Ac	Ac	**8**	Ac	Ac	Ac
5	Ac	H	H				

Scheme 1. Acetylation of D-ribono-1,4-lactone (**1**) catalyzed by lipases.

2. Experimental

2.1. Materials

All chemicals were of reagent grade and were used as received. *Candida antarctica* lipase B (CAL-B, Novozym 435, 10,000 PLU/g) and *Thermomyces langinosus* (Lipozyme TL-IM, 250 IUN/g) were donated by Novozymes A/S. Lipases from *Burkholderia cepacia* (PSL 30,000 U/g; PSL-C "Amano" I, 1638 U/g; and PSL-D "Amano" I, 744 u/g), *Pseudomonas fluorescens* (AK, 26,600 U/g) and *Rhizopus oryzae* (F-AP15, 150 u/mg) were donated by Amano Enzymes Inc. *Candida rugosa* lipase (CRL, ≥700 unit/mg solid) was purchased from Sigma–Aldrich. Native lipases from *Aspergillus niger* AC-54 (ANL, 19.4 U/mL) and *Rhizopus oligosporus* (ROL, 14.9 U/mL) were kindly donated by Professor Patrícia de Oliveira Carvalho from Universidade de São Francisco (USF), Bragança Paulista, SP, Brazil and were isolated from microorganisms collected in the region of Bueno Brandão, MG, Brazil. The mycelium-bound lipases isolated from Amazonian fungi UEA_53 (*Astrocaryum aculeatum*) and UEA_115 (Amazon wood) were kindly donated by Professor Sandra Patrícia Zanotto from Universidade Estadual do Amazonas (UEA), Manaus, AM, Brazil. D-Ribono-1,4-lactone and hexanoic, octanoic, decanoic, gallic, mandelic, phenylacetic and octadecanoic acids were purchased from Sigma–Aldrich. Butanoic, dodecanoic and hexadecanoic acids, ethyl acetate, ethyl acetoacetate and acetic anhydride were provided by Vetec. Vinyl acetate, isopropenyl acetate and tetradecanoic acid were obtained from Fluka Chemika. Acetic and propanoic acids were provided from Reagen. Benzoic acid, glycine, D,L-alanine and L-cysteine were obtained from Merck.

2.2. Analytical methods

Infrared spectra were acquired with a Perkin-Elmer FTIR 1600 spectrometer using KBr for solid samples (range 4000–400 cm^{-1}). ^1H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, with a Varian 400 Mercury Plus spectrometer, using DMSO-d_6 as the solvent and TMS as the internal standard. Elemental analyses were conducted in a Carlo Erba CHNS EA-1110 instrument at Central Analítica, UFSC, Florianópolis, SC, Brazil.

2.3. General enzymatic procedure for the acylation of D-ribono-1,4-lactone (*1*)

The selected lipase was added to a solution containing the lactone **1** (0.5 mmol) and an acyl donor (1.5 mmol) in 10.0 mL of an anhydrous solvent and the reaction mixture was incubated in a shaker at 35 °C and 150 rpm. After the allotted time for each reaction, 1.0 mL aliquots were withdrawn and the reaction was stopped by filtering off the lipase. Conversions (%) were determined by ^1H NMR by comparing the signals of the initial lactone **1** with the corresponding signals of the acylated products. Control experiments were also conducted without lipases (free or immobilized) under similar reaction conditions, and no product was formed.

2.4. Synthesis of 5-O-acetyl-D-ribono-1,4-lactone (*5*) catalyzed by CAL-B

The reaction was initiated by dissolving D-ribono-1,4-lactone **1** (74.0 mg, 0.5 mmol) and vinyl acetate (0.14 mL, 1.5 mmol) in anhydrous acetonitrile (10.0 mL) followed by the addition of CAL-B (10.0 mg). The mixture was shaken at 35 °C and 150 rpm for 24 h. The reaction was stopped by filtering off the lipase. Finally, solvent was evaporated and **5** was obtained as a white solid after recrystallization in acetone (94% yield); m.p. 140-143 °C (lit.[10] 148 °C); IR: 3476, 3289, 1761, 1749, 1429 and 1385 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.03 (s, 3H), 4.10-4.17 (m, 2H), 4.24 (dd, 3.6 Hz, 12.4 Hz, 1H), 4.38-4.43 (m, 2H), 5.57 (d, 4.0 Hz, 1H, D_2O exchange) and 5.87 (d, 7.6 Hz, 1H, D_2O exchange); ^{13}C NMR (DMSO-d_6): δ 21.1 (CH_3), 63.6 (CH_2), 68.9 (CH), 69.4 (CH), 82.7 (CH), 171.0 (C=O), 177.0 (C=O). Anal. calcd. for $C_7H_{10}O_6$: C, 44.2; H, 5.3. Found: C, 44.1; H, 5.3.

2.5. Synthesis of 5-O-lauryl-D-ribono-1,4-lactone (*14*) catalyzed by CAL-B

Anhydrous acetonitrile (10.0 mL) was added to a mixture of D-ribono-1,4-lactone **1** (459 mg, 3.1 mmol) and lauric acid (621 mg, 3.1 mmol) followed by addition of CAL-B (70.0 mg). The final mixture was stirred at 150 rpm and 35 °C for 24 h. The solid product that precipitated out was separated by filtration (together with the insoluble enzyme). Re-dissolution of the cake in a 1:1 ethanol/ethyl ether mixture followed by filtration to separate the catalyst gave **14** as a crystalline solid after evaporation of the solvent and recrystallization in ethanol/ethyl ether (68% yield); m.p. 123–125 °C; IR: 3497, 3304, 1765, 1754, 1244 and 1160 cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.85 (m, 3H), 1.24 (m, 16H), 1.49 (m, 2H), 2.32 (t, 7.2 Hz, 2H), 4.11–4.20 (m, 2H), 4.24 (dd, 3.6 Hz, 12.0 Hz, 1H), 4.40–4.45 (m, 2H), 5.60 (d, 3.6 Hz, 1H, D_2O exchange), 5.92 (d, 7.6 Hz, 1H, D_2O exchange); ^{13}C NMR (DMSO-d_6): δ 14.6 (CH_3), 22.8 (CH_2), 25.0 (CH_2), 29.1 (CH_2), 29.2 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 32.0 (CH_2), 33.9 (CH_2), 63.6 (CH_2), 69.0 (CH), 69.5 (CH), 82.6 (CH), 173.0 (C=O), 176.0 (C=O). Anal. calcd. for $C_{17}H_{30}O_6$: C, 61.8; H, 9.2. Found: C, 60.7; H, 9.4.

3. Results and discussion

3.1. Screening of enzymes

In the first approach, ten commercially available lipases, in free or immobilized forms, and two mycelium-bound lipases isolated from native Amazon plants (Brazil) [32] were screened for their efficiency in the acetylation of lactone **1** with vinyl acetate. The relative composition of acetylated products was based on analysis of the ^1H NMR spectra of the crude reaction products after certain time periods (6 and 24 h) as shown in Table 1.

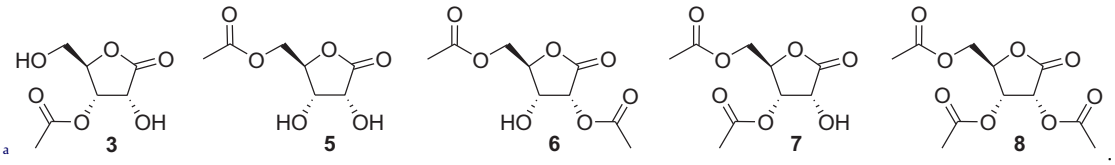
From the data presented in Table 1 it is clear that the conversion degree and product distribution were dependent on the source of the biocatalyst. Poor activities were observed for PSL, F-AP15, CRL, ROL, ANL lipases and UEA_53 mycelium, with no product being detected even after 72 h reaction (results not shown). On the other hand, much better reaction profiles were obtained with lipase B from *Candida antarctica* (CAL-B) as the biocatalyst, furnishing the 5-monoacetylated derivative **5** as the sole product in a quantitative conversion (>99%) after 24 h, although high conversions were also observed in shorter times (Table 1, entry 1). With the use of Lipozyme TL-IM, a low-cost biocatalyst previously employed for the regioselective acylation of 5-fluorouridine analogs [15], the conversions into the monoacetylated products **3** and **5** were slow and not regioselective under the standard conditions (10 mg catalyst, entry 2). However, the conversion to **5** could be reasonably increased to 85% with complete regioselectivity (**5**:**3** ratio >99:1) using larger amounts of the catalyst (entry 3).

For the other lipases studied (Table 1, entries 4, 5 and 7), very poor regioselectivities were observed under our standard conditions or in the presence of larger amounts of the catalyst (entries 6 and 8), which accelerates the reaction at the expense of the selectivity. In all but one case (entry 7), the product mixture was composed of 5-*O*-acetyl-D-ribonolactone (**5**) as the main component, together with 3-*O*-acetyl- (**3**), 2,5-*O*-diacetyl- (**6**), 3,5-*O*-diacetyl- (**7**) and/or 2,3,5-*O*-triacetyl-D-ribonolactone (**8**) as recurrent minor by-products. The competitive formation of mono- and di-acetylated compounds was inferred from the NMR spectra of the crude products since it is relatively easy to differentiate between a carbinolic (CHOH) hydrogen nucleus and its *O*-acetylated derivative (CHOAc). Also, the spectral data are consistent with the expected structure and with the data for model compounds [10,15,25,33,34].

Finally, interesting results were found for the UEA_115 mycelium, which catalyzed the regioselective 5-monoacetylation of ribonolactone **1** to produce **5**, in spite of the slow reaction rate (41% conversion after 72 h) using a large amount of catalyst (Table 1, entries 9 and 10).

Thus, with regard to the selectivity observed for CAL-B and Lipozyme TL-IM, the preferential acetylation at the less-hindered primary 5-hydroxy group leading to the regioselective formation of 5-acetylated product **5** is in agreement with similar studies in the literature [13,15,34–38], although this was not the case for all of the other enzymes tested. Considering the above results, CAL-B was selected for the following studies.

Table 1
Acetylation of **1** with vinyl acetate using different biocatalysts



[a]

	Lipase	M_{cat} (mg)	Conversion (%)[b]							
			6 h				24 h			
			3	**5**	**6**	**7**	**3**	**5**	**6**	**7**
1	CAL-B	10	–[c]	85	–	–	–[c]	>99	–	–
2	Lipozyme TL-IM	10	7	16	–	–	5	24	–	–
3	Lipozyme TL-IM	40	nd	nd[d]	nd	nd	–	85	–	–
4	AK	10	8	10	–	6	8	12	–	8
5	PSL-C "Amano" I	10	14	19	–	11	17	28	11	16
6	PSL-C "Amano" I[e]	60	14	32	13	14	–	46	17	23
7	PSL-D "Amano"	10	7	10	–	–	15	13	–	–
8	PSL-D "Amano"	40	nd	nd	nd	nd	23	29	7	11
9	Mycelium UEA_115	10	–	–	–	–	–	8	–	–
10	Mycelium UEA_115	40	nd	nd	nd	nd	–	41[f]	–	–

[a] Reaction conditions: **1** (0.5 mmol), vinyl acetate (1.5 mmol), lipase or mycelium, acetonitrile (10 mL), 35 °C, 150 rpm.
[b] Determined by ^{1}H NMR (400 MHz, DMSO-d_6).
[c] Negligible conversion (<1%).
[d] Not determined.
[e] Triacetylated product **8** (14%) was also obtained after 24 h reaction.
[f] Conversion after 72 h reaction.

3.2. Effect of organic solvent and presence of water

It is well established that enzyme activity can be dramatically affected by the choice of the organic solvent, and good correlations between the reaction rate and the polarity of the solvent have been achieved [39–41]. Log *P* (logarithm of the partition coefficient of the solvent for the standard octanol/water two-phase system) is the most useful parameter to classify the solvents for biocatalytic reactions. Accordingly, solvents with log $P \leq 2$ are more hydrophilic and tend to strip away the water molecules present on the surface of the enzyme, reducing the catalytic activity. Conversely, solvents with high log P (≥ 4) are hydrophobic and stabilize the enzymes, being the most suitable for biocatalytic processes [39]. In order to study the solvent effect, a series of polar aprotic solvents were used for the CAL-B-catalyzed acetylation reaction of lactone **1**, which is not soluble in solvents of low polarity. Acetonitrile, acetone, tetrahydrofuran (THF), dioxane and *N*,*N*-dimethylformamide (DMF) were selected for the acetylation of **1** with vinyl acetate (Table 2).

The data given in Table 2 show that there was no clear relationship between log *P* and the conversion degrees. Using the less polar solvents (log $P \geq -0.49$) such as acetone, THF and acetonitrile, the 5-monoacetylated derivative **5** was selectively obtained with conversion degrees ranging from 61% to 72% (entries 1–3). On the other hand, in the case of dioxane (log $P = -1.1$) there was not only a considerably lower conversion to the expected product **5** but also a decrease in the regioselectivity (entry 4), whereas DMF (log $P = -1.0$) completely inhibited the formation of any product (entry 5).

Table 2
Solvent effects on the CAL-B-catalyzed acetylation of **1**.

Entry	Solvent	Log *P*[a]	Conversion to **5** (%)[b]
1	Acetonitrile	−0.33	72
2	THF	−0.49	68
3	Acetone	−0.23	61
4	Dioxane[c]	−1.1	44
5	DMF	−1.0	<1

[a] Ref. [39].
[b] Determined by ^{1}H NMR (400 MHz, DMSO-d_6).
[c] Mixture of compounds: **5** (44%), **3** (9%) and **7** (6%).

It is important to observe that all the solvents suitable for the regioselective acetylation of **5** (Table 2, entries 1–3) possess reduced toxicity and also have low boiling points, which make the work-up and purification steps easier in comparison with other methodologies that employ expensive and high-boiling point chemicals, such as pyridine, dimethyl sulfoxide or DMF.

Another parameter which needs to be evaluated is the water content, which plays an important role in controlling the enzyme performance in organic media. It is well described that lipases possess a water monolayer at their surface, or clusters around the charged groups of the protein, which maintains the native conformation even in organic solvents. The optimal amount of water required depends on several parameters, such as the type of solvent, substrate, and solid support, the polarity of the enzyme active site, and the reaction conditions [42]. However, the presence of water in the reaction medium can decrease the conversion degrees in lipase catalyzed acylations, shifting the equilibrium towards ester hydrolysis to give the corresponding carboxylic acids [43].

Therefore, CAL-B-catalyzed acetylation of **1** with vinyl acetate was also studied with the addition of 27.0 μL (3 equiv.) of water. The influence of the reaction time was then evaluated in this reaction (Fig. 1). While the degree of conversion to the product **5** (~50% after 24 h) was lower than that obtained using dry acetonitrile (>99%, 24 h), the regioselectivity of the process was entirely maintained. The results presented in Fig. 1 also show that the conversion to **5** in dry acetonitrile rapidly increased in the first 6 h (up to 85%), and from this point onwards it formed a plateau reaching a quantitative conversion to **5** after 24 h. Thus, considering the results obtained herein, dry acetonitrile as the solvent and a reaction time of 6 h were selected for the subsequent studies involving the acetylation of ribonolactone **1**.

3.3. Effect of CAL-B loading

The influence of the amount of CAL-B on the regioselective acetylation of **1** with vinyl acetate in dry acetonitrile was then evaluated, in the range of 0–50 mg (0–500 Units). The results, presented in Fig. 2, show that high conversions to the 5-monoacetylated product **5** were achieved with as little as 10 mg (100 U) CAL-B in 6 h.

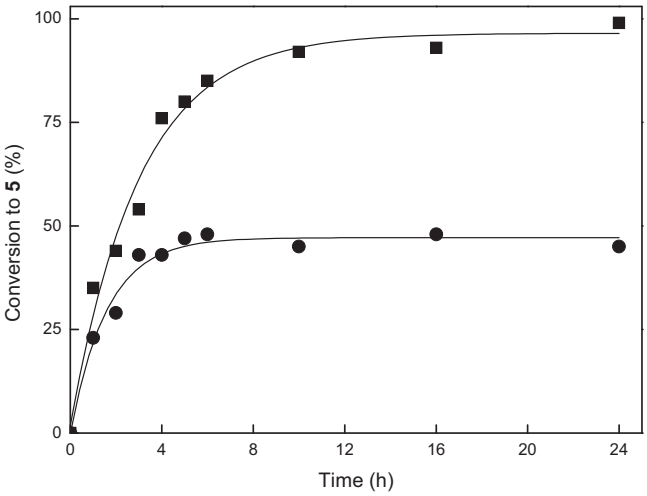


Fig. 1. Influence of time on the regioselective acetylation of **1** in dry acetonitrile (■) and in acetonitrile + water (27.0 μL; 1.5 mmol) (●). Reaction conditions: **1** (0.5 mmol), vinyl acetate (1.5 mmol), CAL-B (10 mg, 100 Units), acetonitrile (10 mL), 35 °C, 150 rpm.

Therefore, the substrate/catalyst ratio employed in the subsequent experiments was set at 0.5 mmol/10 mg.

3.4. Influence of amount and type of acyl donor

Next, the type and relative amount of acyl donor (carboxylic acid, anhydride or ester) were evaluated for the regioselective acylation of lactone **1** using CAL-B as the biocatalyst. Two methods were designed for carrying out the transformations: in the first, a 3:1 molar ratio of acyl donor/substrate was used with acetonitrile as the solvent; in the second, a large excess of the acyl donor (200 equiv.) was employed as both the reactant and solvent. The observed conversions to the 5-monoacetylated product **5** are given in Table 3.

Good conversion degrees and regioselective formation of **5** were obtained for most of the acetyl donors screened. The highest conversion degree was achieved with vinyl acetate, for both methods studied (Table 3, entry 1). On the other hand, isopropenyl acetate is only useful in high excess (entry 2). Interestingly, using ethyl acetate, which is of very low cost, as the reactant/solvent gave an acceptable 53% conversion to the 5-monoacetyl derivative **5**

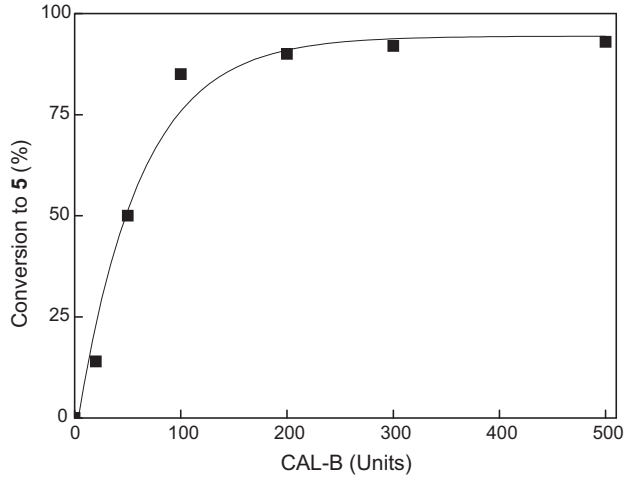


Fig. 2. Influence of CAL-B units on the acetylation of **1**. Reaction conditions: **1** (0.5 mmol), vinyl acetate (1.5 mmol), CAL-B (0–50 mg; 0–500 Units), dry acetonitrile (10 mL), 35 °C, 150 rpm, 6 h.

Table 3
Influence of acetyl donor on the acetylation of **1**.

	Acetyl donor	Conversion (%)[a]	
		3:1[b,c]	200:1[b,d]
1	Vinyl acetate	85	>99
2	Isopropenyl acetate	28	75
3	Ethyl acetate	23	53
4	Acetic anhydride	52	16[e]
5	Acetic acid	<5%	<5%

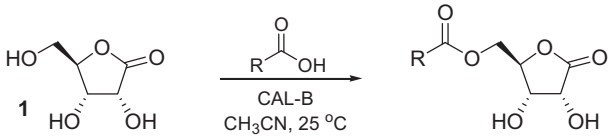
[a] Determined by ^{1}H NMR (400 MHz, DMSO-d_6).
[b] Molar ratio of the acyl donor to the substrate **1**.
[c] Reaction conditions: **1** (0.5 mmol), acyl donor (1.5 mmol), acetonitrile (10 mL), CAL-B (10 mg), 35 °C, 150 rpm, 6 h.
[d] Reaction conditions: **1** (0.5 mmol), acyl donor (100 mmol), CAL-B (10 mg), 35 °C, 150 rpm, 6 h.
[e] Mixture of products: **5** (16%), **3** (32%) and **7** (27%).

after 6 h reaction (entry 3). Acetic anhydride was also studied as the acetyl donor, but its more pronounced reactivity (even in the absence of any catalyst) [44] precluded its use in great excess due to the competitive formation of acetylated by-products **3** and **6** (entry 4). Nevertheless, the method employing a combination of acetic anhydride/**1** (3:1) in CH_3CN reestablished the regioselectivity towards the exclusive formation of **5** in moderate conversion. As expected, acetic acid was very ineffective as the acyl donor and no detectable product was formed under the test conditions (entry 5).

It has been stated that some carboxylic acids, such as acetic and propionic, act as potent inhibitors of lipase activity by removing its aqueous micro-layer and by altering the pH in the catalytic site, contributing to the observed decrease in enzyme activity [45].

Due to the biological importance associated with carbohydrate fatty acid esters [4,46], the CAL-B-catalyzed acylation of ribonolactone **1** was extended to a series of saturated carboxylic acids (Scheme 2). The results showed the total regioselectivity of CAL-B-catalyzed acylations, providing exclusively the corresponding 5-acylated sugar derivatives. Low to good conversions were obtained depending on the alkyl chain length of the acyl donor (Fig. 3). As previously discussed, no product was formed when acetic acid was employed. Better results were observed when propionic and butyric acids were used, with conversions of 28% and 38% being obtained for the corresponding esters **9** and **10**, respectively. Higher conversion degrees were obtained for linear carboxylic acids with six, eight, ten, twelve and fourteen carbons, giving the 5-acyl derivatives **11–15** in the range of 50–67%. However, a decrease in the conversion degrees was observed when palmitic (C16) and stearic (C18) acids were employed, forming the corresponding products **16** and **17** in 41% and 46%, respectively. In these cases, the lower conversion degrees may be due to stereo-electronic effects as well as a reduced solubility of acyl donors in acetonitrile. These results are in agreement with previously reported data, wherein CAL-B was described as highly active for short and medium chain length carboxylic acids and esters, but of decreased activity for long-chain fatty acids and esters [31,47–49].

Therefore, the variety of acyl donors that are compatible with CAL-B makes this biocatalyst a versatile tool for regioselective acylations. Moreover, using carboxylic acids as the acyl donor the only



Scheme 2. Regioselective acylation of D-ribono-1,4-lactone (**1**) catalyzed by CAL-B.

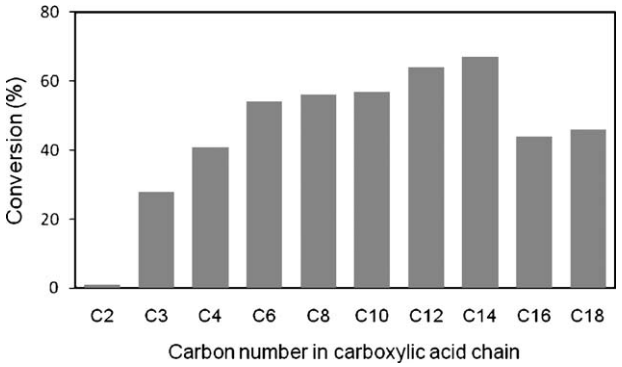


Fig. 3. Effect of chain length on the regioselective acylation of **1**. Reaction conditions: **1** (0.5 mmol), acyl donor (1.5 mmol), acetonitrile (10 mL), CAL-B (10 mg), 35 °C, 150 rpm, 24 h.

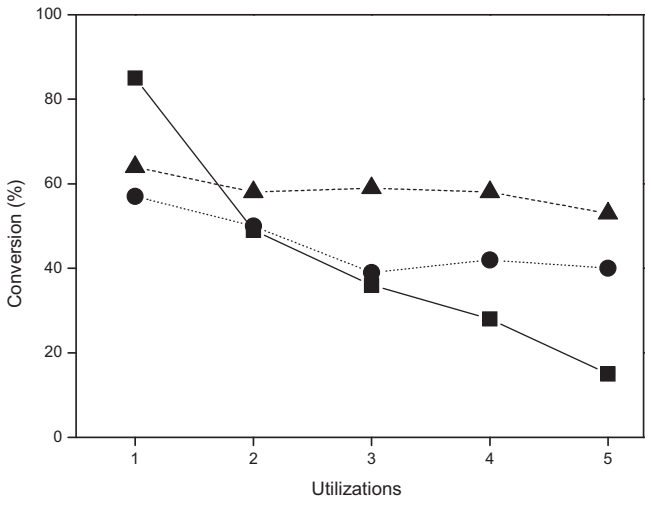


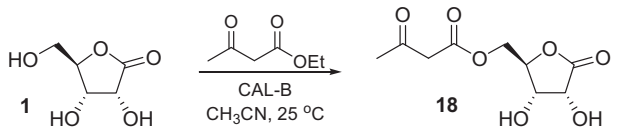
Fig. 4. Effect of catalyst reuse on the acylation of **1** with vinyl acetate (■) in 6 h reaction, and with decanoic (●) and dodecanoic (▲) acids in 24 h. Reaction conditions: **1** (0.5 mmol), acyl donor (1.5 mmol), CAL-B (10 mg), acetonitrile (10.0 mL), 35 °C, 150 rpm.

by-product generated was water, which is in line with a clean and environmentally friendly processes [16–20]. However, attempts to acylate ribonolactone **1** with functionalized carboxylic acids such as benzoic, phenylacetic, galic and mandelic acids, as well as some amino acids including glycine, D,L-alanine and L-cysteine, under the experimental reaction conditions described above, did not lead to any detectable product in the crude reaction after 48 h. One possible reason is that the presence of polar groups negatively influences the reaction profile, since it is known that CAL-B is not a suitable catalyst for the esterification of sugars with unsaturated or arylaliphatic acids like cinnamic acid and its derivatives, benzoic acids, phenolic derivatives and α-substituted carboxylic acids, with some exceptions [50].

An interesting way to install a functional group moiety in the 5-position of the ribonolactone framework is through a transesterification reaction using an excess of ethyl acetoacetate as the acyl donor. A three-fold excess of ethyl acetoacetate led to the 5-acetoacetyl-D-ribonolactone derivative **18** in a conversion degree of 28% after 24 h. However, the conversion could be increased to 50% by employing a considerable excess (100:1) of ethyl acetoacetate (Scheme 3). Although the conversion was relatively low, given that this one-step transformation is clean and offers simplicity it compared very favorably with the previous preparation of **18** from **1** which involves three steps (protection/deprotection protocols), gave a low overall yield, and requires toxic reagents such as diketene, pyridine and benzene [51]. Since the 5-acetoacetyl-D-ribonolactone derivative **18** is a precursor of biologically active compounds, this CAL-B-catalyzed transesterification reaction could have useful applications in synthetic organic chemistry, and further studies are underway.

3.5. Recyclability of the catalyst

The reusability of immobilized enzymes is an important property of any catalyst and can determine the economic viability of a biosynthetic process. In this regard, CAL-B was employed in five subsequent cycles in the acylation of **1** with vinyl acetate, decanoic and dodecanoic acids under the experimental conditions described above for each acyl donor (Table 3 and Fig. 3). At the end of each batch, the immobilized lipase was removed from the reaction medium by filtration and washed with acetonitrile in order to remove any substrate or product retained in the catalyst surface. In the next step, the substrates and the recuperated catalyst were added to a new reaction medium and at the end of each reaction, the conversion degrees were determined. The results are presented in Fig. 4.



Scheme 3. Regioselective acylation of D-ribono-1,4-lactone (**1**) with ethyl acetoacetate catalyzed by CAL-B.

With the use of vinyl acetate the conversion to **5** decreased after each reutilization, reaching a value of 15% in the fifth cycle. Although vinyl acetate was shown to be a suitable acetyl donor for the acetylation of **1**, the presence of significant amounts of acetaldehyde (generated as the by-product in the transesterification reaction) led to inactivation of the lipase by the condensation reaction with essential amino groups of the enzyme to give Schiff bases [52], thus preventing the application of the catalyst for consecutive cycles. Another possible cause of enzyme deactivation with vinyl acetate as the acyl donor could be related to its slow hydrolysis generating small amounts of acetic acid, which is also harmful to the biocatalyst as discussed above.

However, the use of decanoic acid as the acyl donor led to the maintenance of a reasonable level of catalytic activity after each reutilization and the conversion degrees were in the range of 57–39% after five cycles. Dodecanoic acid gave slightly better conversions after each batch, in the range of 64–53%. Besides generating water as the by-product, these long-chain carboxylic acids are not toxic to the enzymes and, therefore, can be employed without any additional precautions.

4. Conclusions

The results showed that CAL-B was the most versatile biocatalyst for the regioselective acylation of D-ribono-1,4-lactone (**1**). However, many parameters influence the reaction outcome, including the source of the enzyme, the nature of the acyl donor and the organic solvent, the amount of biocatalyst and the presence of water. CAL-B could be reused in at least five cycles without a significant loss in the activity employed decanoic and dodecanoic acids as acyl donors and producing water as the sole by-product. In contrast to the multistep chemical process, the enzymatic acylation of D-ribono-1,4-lactone furnished, in most cases, only the 5-acylated derivative under mild conditions.

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