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Article

Molecular Sieves Mediated Green Per-*O*-Acetylation of Carbohydrate Templates and Lipase Catalyzed Regioselective Alcoholysis of 2,3,5-Tri-*O*-Acetyl-D-Ribonolactone

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The per-*O*-acetylation of D-ribono-1,4-lactone and representative carbohydrates through the combination of acetic anhydride and molecular sieves under solvent-free conditions is demonstrated. The use of 13X/KCl molecular sieves as the heterogeneous catalyst was found to be more efficient than the excess of pyridine normally employed in the conventional method, giving high yields of the expected peracetylated product after 3 h at 25 °C or 1 h at 50 °C. The transformation can be carried out in gram scale and in an open flask. Additionally, the catalyst is readily separated from the reaction medium and can be reutilized without significant loss of activity. This green procedure for acetylation was extended to D-ribonolactone derivatives and natural carbohydrates. To demonstrate the synthetic utility of the method, 2,3,5-tri-*O*-acetyl-D-ribonolactone was selected as the substrate for the regioselective alcoholysis of acetyl group catalyzed by *Candida antarctica* lipase B in EtOH to selectively produce 2,3-di-*O*-acetyl-D-ribonolactone in gram scale.

Keywords: D-ribonolactone, per-*O*-acetylation, regioselective alcoholysis, CAL-B, molecular sieves

Introduction

Carbohydrates are considered as appealing chiral starting materials for asymmetric synthesis not only because they possess several functionalities and stereogenic centers that are amenable to a variety of transformations but also due to their relatively low cost and availability from sustainable feedstocks.[1] Nevertheless, one of the main drawbacks associated with the use of natural sugars as building blocks for the synthesis of more elaborated compounds is the possible coexistence of different isomers in solution, including cyclic α- and β-lactols and also ring-opening structures.[2] On the other hand, the oxidation of the anomeric center in pentoses and hexoses to give the corresponding lactones is an elegant strategy to solve the problem of dealing with several isomers in solution. Therefore, D-ribose (**1**, Figure 1) can be oxidized by a variety of methods[3-5] to produce D-ribono-1,4-lactone (**2**), which has been widely used as a versatile chiral building block[6,7] for the synthesis of several natural products[8-10] as well as compounds relevant to medicinal chemistry and/or chemical biology, including nucleotides and *C*-ribonucleosides as antivirals.[11-13]

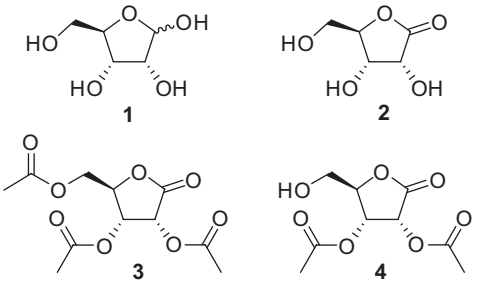

Figure 1. Structures of D-ribose (**1**), D-ribono-1,4-lactone (**2**), 2,3,5-tri-*O*-acetyl-D-ribonolactone (**3**) and 2,3-di-*O*-acetyl-D-ribonolactone (**4**).

Another drawback related to the widespread use of carbohydrates as synthetic building blocks is the presence of hydroxyl groups of similar reactivity, which frequently requires the application of protocols for hydroxyl protection

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and deprotection.[14] Per-*O*-acetylation of carbohydrates is a very usual approach to this end.[15-17] In fact, fully acetylated sugars are versatile intermediates for the synthesis of many naturally occurring glycosides, oligosaccharides and glycoconjugates. Pyridine is largely used as catalyst in acylation reactions. However, long-term exposure to this reagent can cause sterility in males.[18] Triethylamine in combination with 4-(dimethylamino)pyridine (DMAP) and acetonitrile (ACN) is an important alternative for pyridine in the per-*O*-acetylation.[19] Nevertheless, the required excess of reagents and solvents creates further environmental and economic concerns and also demands aqueous work-up. Thus, new synthetic methodologies must be developed to decrease the use of pyridine and its derivatives in organic synthesis.

Heterogeneous catalysis is being adopted as an alternative methodology for acylation due to major advantages related to its use, including the simple separation of the heterogeneous catalyst from the reaction flask by filtration at the end of the process. Also, it can be employed in a new batch without any or minimal purification/reactivation steps, which obviously represents economic and environmental advances. Therefore, molecular sieves (MS), which have several catalytic sites inside their structures, have been successfully employed to promote acylation of carbohydrates,[15] nucleosides,[20] alcohols and phenols.[21,22] The basic character of some MS is usually associated with the existence of negatively charged oxygen atoms covalently bonded to the Si-Al lattice.[23]

Lipase B from *Candida antarctica* (CAL-B) is a widely used lipase in biocatalyzed transformations exhibiting a high degree of substrate selectivity and also having the potential for use in hydrolysis and alcoholysis reactions[24] involving acylated derivatives of carbohydrates[25-28] and nucleosides.[29,30] Similar to MS, this heterogeneous biocatalyst can be readily separated from the reaction medium and also reutilized for many cycles.

As part of our interest in developing synthetic methodologies to bypass protocols of protection and deprotection of carbohydrate derivatives,[14] allied with the development of chemical transformations that do not require harmful reagents or complex purification techniques and thus avoid the generation of toxic waste,[20,21,23,31,32] we present the solvent-free and pyridine-free per-*O*-acetylation of D-ribonolactone **2** (Figure 1) and a set of representative pentoses, as well as hexoses, through the reaction with acetic anhydride mediated by MS. In addition, we developed the regioselective alcoholysis of 2,3,5-tri-*O*-acetyl-D-ribonolactone (**3**) catalyzed by CAL-B to selectively produce 2,3-di-*O*-acetyl-D-ribonolactone (**4**, Figure 1).

Results and Discussion

Peracetylation of **2** and representative carbohydrates

Initially, 2,3,5-tri-*O*-acetyl-D-ribonolactone (**3**) was prepared under conventional conditions[33] in 65% yield by treating D-ribonolactone **2** with acetic anhydride using pyridine as the catalyst at 25 °C for 3 h (Table 1, entry 1). In addition to the toxicity related to pyridine,[18] it is also important to mention that preparation of **3** by this method (or using DMAP instead of pyridine)[33] requires aqueous work-up which not only generated large volumes of residues but also led to considerable mass losses[34] of the product with negative impact in the chemical yield of the process.

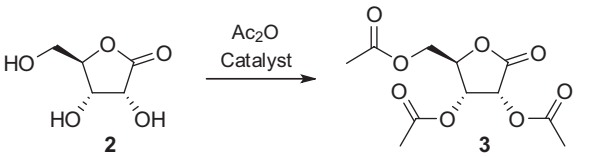
To find a suitable heterogeneous catalyst for the acetylation of D-ribonolactone **2** with acetic anhydride, the reaction was screened in the presence of four commercial and two modified MS, which were dried under vacuum for 24 h at 100 °C prior to use. After 2 h, the reactions using 3A, 4A, and 5A MS presented many new spots on the thin layer chromatography (TLC) plate (Table 1, entries 2-5). The less polar spot could be ascribed to the per-*O*-acetylated product **3**, while the more polar spots might be related to the mono- and/or diacetylated products. Attempts to separate these partially acylated D-ribono derivatives by column chromatography did not result in pure products, possibly due to fast 1,2-acyl migration in solution.[35,36] On the other hand, the use of 13X MS or the potassium-exchanged 13X (hereby denominated as 13X/KCl) led to promising results after 2 h (Table 1, entries 6 and 7, respectively). Overwhelmingly, running the reaction for an additional hour (3 h in entries 8 and 9 instead of 2 h in entries 2-7) led to an improved formation of the peracetylated lactone **3** when 13X MS was employed as the catalyst (entry 8), while the use of 13X/KCl under the same conditions was even more effective and gave **3** in quantitative yield and high purity (entry 9).

The acetylation was also performed by heating the reaction at 50 °C, with the starting material **2** being consumed in 1 h (Table 1, entry 10). However, the final yield of 80% was not as high as that obtained by conducting the reaction at 25 °C (entry 9), possibly due to the sensitivity of the D-ribonolactone **2** (as well as their acetylated products) to elevated temperatures, which might result in some *in situ* polymerization (caramelizing reaction) that was eventually separated in the work-up process. As expected, the reaction carried out in the absence of any catalyst led to negligible conversions to the acetylated product (entry 11).

Thus, the present methodology for acetylation using 13X/KCl as the catalyst can be considered as an excellent replacement for the conventional conditions using toxic

pyridine. Noteworthy, the benefits are not only in terms of environmental and economic issues, but also in the relative reaction rates (Table 1, entry 1 *versus* 8 and 9).

Table 1. Screening a variety of MS as basic catalysts for the per-*O*-acetylation reaction of D-ribonolactone **2** with acetic anhydride[a]



entry	Catalyst	Temperature / °C	time / h	Yield / %
1	Pyridine	25	3	65
2	3A	25	2	[b]
3	4A	25	2	[b]
4	4A 1000	25	2	[b]
5	5A	25	2	[b]
6	13X	25	2	61
7	13X/KCl	25	2	65
8	13X	25	3	80
9	13X/KCl	25	3	99
10	13X/KCl	50	1	80
11	None	25	2	–

[a]Reaction was followed by TLC using 1:1 EtOAc/hexanes; product **3** stains in vanillin; [b]three to four spots were observed in the TLC plate, which might be related to a mixture of acetylated derivatives of D-ribonolactone **2**.

Because catalyst reutilization is a desirable feature for industrial processes, the possibility of reusing the 13X/KCl catalyst was determined by running the peracetylation of D-ribonolactone **2** with the same catalyst for four cycles. After each batch, EtOAc was added to the reaction and the catalyst was separated by filtration. The catalytic activity of reused 13X/KCl was completely maintained even after four cycles and the product **3** was consistently obtained in high yields (89-93%) after every cycle.

Acetylation of D-ribonolactone derivatives

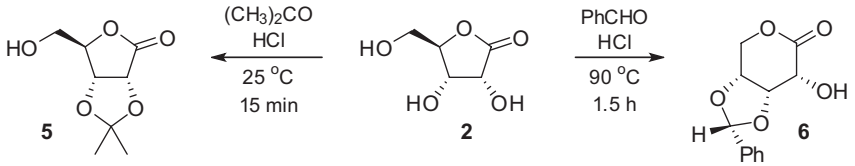
Inspired by the above results obtained for the 13X/KCl catalyst, we decided to extend the method of acetylation to some derivatives of D-ribonolactone. Thus, 2,3-*O*-isopropylidene-D-ribonolactone[3,4,7] (**5**) and 3,4-*O*-benzylidene-D-ribonolactone[7] (**6**, Zinner's lactone) were readily synthesized by the treatment of **2** with acetone (25 °C, 15 min) or benzaldehyde (90 °C, 1.5 h) in the presence of concentrated HCl (Scheme 1).

Proceeding with the acetylation of **5** and **6** with acetic anhydride using pyridine as the catalyst, at 25 °C, gave the corresponding products **7** and **8** in yields of 75% and 70%, respectively (Table 2, entries 1 and 4). Switching pyridine by 13X/KCl and applying the previously established reaction conditions furnished **7** and **8** in yields of 60% and 38%, respectively (Table 2, entries 2 and 5). The low yield observed for the acetylation of the Zinner's lactone **6** at 25 °C might be related to its low solubility in most common solvents at 25 °C. In fact, increasing the temperature improved considerably the reaction yields in both cases, with the monoacetylation being quantitatively achieved after only 1 h to give derivatives **7** and **8** (Table 2, entries 3 and 6).

Acetylation of natural carbohydrates

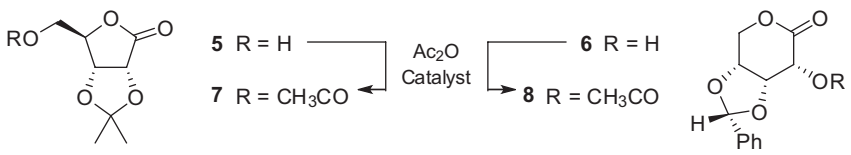
After the success observed for the acetylation of D-ribonolactone **2** and its derivatives **5** and **6**, the methodology was applied to the acetylation of a set of natural carbohydrates (Table 3). Preliminary TLC studies showed that the peracetylation of these representative carbohydrates demand longer periods to complete. Therefore, treatment of three hexoses (D-glucose, D-galactose and D-mannose) and two pentoses (D-ribose and D-xylose) with an excess (20 equiv.) of acetic anhydride in the presence of pyridine at 25 °C for 24 h furnished the corresponding per-*O*-acetylated products as a mixture of α- and β-anomers in 58-66% yield (Table 3, entries 1, 4, 7, 10, and 13). Surprisingly, the per-*O*-acetylated carbohydrates were obtained in lower yields when pyridine was replaced by 13X/KCl, under the same conditions (Table 3, entries 2, 5, 8, 11, and 14).

Because all natural carbohydrates under study presented low solubility in acetic anhydride at 25 °C, this observation might explain the limited reaction outcome observed when 13X/KCl was used as the catalyst (in comparison with



Scheme 1. Synthesis of 2,3-isopropylidenelactone **5** and Zinner's lactone **6**.

Table 2. Acetylation of the D-ribonolactone derivatives **5** and **6**



entry	Substrate	Catalyst	Temperature / °C	time / h	Product	Yield / %
1	**5**	Pyridine	25	24	**7**	75
2	**5**	13X/KCl	25	24	**7**	60
3	**5**	13X/KCl	50	1	**7**	> 99
4	**6**	Pyridine	25	24	**8**	70
5	**6**	13X/KCl	25	24	**8**	38
6	**6**	13X/KCl	80	1	**8**	> 99

pyridine, which ultimately play the role of a cosolvent). Therefore, we tested a variety of conditions to improve the substrate solubility, including the use of ultrasound bath and the addition of twice the original amount of acetic anhydride. Unfortunately, no significant improvement in the reaction rates or yields was noticed after 24 h at 25 °C under these modified conditions (results not shown). Regarding the formation of α- and β-diastereoisomers in the product mixture, with the exception of the per-*O*-acetylglucose all D-carbohydrates tested gave similar amounts of α- and β-anomers when pyridine was used as catalyst (Table 3, entry 1 *versus* 4, 7, 10). Conversely, employing 13X/KCl as the catalyst, at 80 °C, led to the selective (or even exclusive) formation of the β-anomer, with the exception of the per-*O*-acetylmannose, which gave preferentially the α-isomer (Table 3, entries 3, 6, 12, 15 *versus* 9). Surprisingly, the α-anomers of the peracetylated-D-ribose were not identified by ^{1}H nuclear magnetic resonance (NMR) at the end of the reaction using 13X/KCl as the catalyst (Table 3, entry 15).

Finally, attempts to peracetylate a ketose, namely, D-fructose, using either pyridine or the present method with 13X/KCl, at 25 °C, did not result in any consumption of the starting material by TLC (Table 3, entries 16, 17). On the other hand, conducting the transformation at high temperature led to the formation of a complex mixture of products by ^{1}H NMR (Table 3, entry 18).

Selective deacetylation of **3**

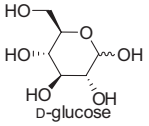
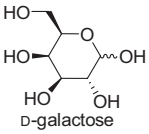
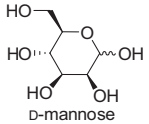
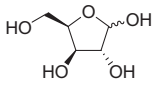
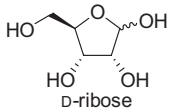
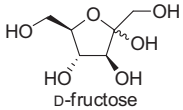
As a synthetic application for this mild and green method to prepare 2,3,5-tri-*O*-acetyl-D-ribonolactone (**3**) and other acetylated carbohydrates, we envisioned that a chemoenzymatic process should be useful to selectively produce deacetylated products. Several studies involving the selective hydrolysis of the 5-*O*-acetyl group from per-*O*-acetylated carbohydrates[25,26,34,42-44] and nucleosides[29,45-47] were reported, in which the best results were obtained in phosphate buffer. Therefore, experiments were carried out to evaluate the CAL-B catalyzed hydrolysis of **3** at physiological conditions (pH 7.0 at 35 °C). While the TLC supported the formation of a new species after 24 h, the product was very soluble in the buffer medium and attempts to extract it with CH_2Cl_2 or EtOAc failed. As an alternative work-up, water was previously removed from the buffer solution by a rotary evaporator, but attempts to separate out the possible product from the crude residue with a variety of solvents were all unsuccessful.

The regioselective lipase-catalyzed[28,34,45] alcoholysis of the acetyl group in peracetylated α- and β-D-ribofuranosides was previously demonstrated; therefore, we moved our attention to alcoholysis conditions instead of hydrolysis, and CAL-B was again the enzyme selected to conduct the experiments. Initially, to determine the minimal amount of enzyme necessary to catalyze the alcoholysis in a reasonable time, test reactions starting from 0.5 mmol of substrate **3** were run in EtOH by adding different quantities of CAL-B (Table 4).

While the conversion rates to the 2,3-di-*O*-acetyl-D-ribonolactone (**4**) were high for reactions using 280 or 140 mg of the commercial enzyme (Table 4, entries 1 and 2), decreasing the amount of CAL-B slows down the conversion to **4** under the same conditions (Table 4, entries 3, 4, and 5). In all cases, the formation of D-ribonolactone **2** and 3-*O*-acetyl-D-ribonolactone (**9**, Scheme 2) were also noted in the crude NMR due to extensive ethanolysis of the acetyl group.

To evaluate the role of the alcoholic solvent, 2,3,5-tri-*O*-acetyl-D-ribonolactone (**3**) was then submitted to alcoholysis in the presence of CAL-B (35 °C, 150 rpm)

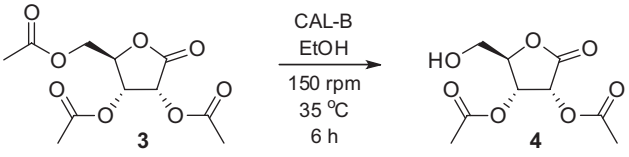
Table 3. Per-*O*-acetylation of natural carbohydrates[a]

entry	Substrate	Catalyst	Temperature / °C	Yield / %	α:β / %[b]	Ref.
1	 D-glucose	Pyridine	25	65	72:28	37
2		13X/KCl	25	26	nd[c]	–
3		13X/KCl	80	80	21:79	–
4	 D-galactose	Pyridine	25	62	58:42	38,39
5		13X/KCl	25	34	nd[c]	–
6		13X/KCl	80	78	17:83	–
7	 D-mannose	Pyridine	25	66	46:54	40
8		13X/KCl	25	53	nd[c]	–
9		13X/KCl	80	79	72:28	–
10	 D-xylose	Pyridine	25	58	57:43	41
11		13X/KCl	25	35	nd[c]	–
12		13X/KCl	80	84	19:81	–
13	 D-ribose	Pyridine	25	64	nd[c]	–
14		13X/KCl	25	20	nd[c]	–
15		13X/KCl	80	61	0:100[d]	33
16	 D-fructose	Pyridine	25	–	nd[c]	–
17		13X/KCl	25	–	nd[c]	–
18		13X/KCl	80	[e]	nd[c]	–

[a]All reactions were performed for 24 h and followed by TLC using 1:1 EtOAc/hexanes; products stain in vaniline; [b]H-α and H-β chemical shifts were assigned based on the literature (references 33, 37-41); [c]nd = not determined; [d]product obtained as a 57:47 mixture of β-D-ribonopyranose and β-D-ribonofuranose; the presence of α-anomer was not observed; [e]complex mixture of products was obtained.

using MeOH, EtOH, *i*-PrOH, and *n*-BuOH as solvent/reagent. After the time indicated in Table 5, a clear liquid aliquot was carefully removed from the reaction flask, the solvent evaporated to dryness in the rotary evaporator and the residue analyzed by ^{1}H NMR.

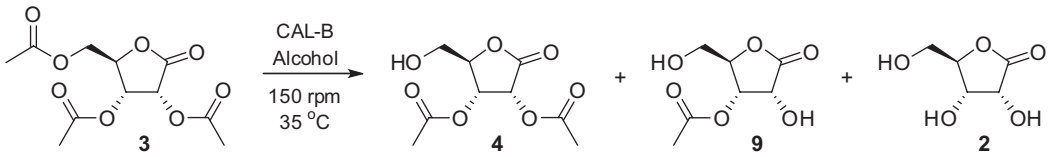
Table 4. Optimization of the amount of CAL-B for the ethanolysis of **3** in 6 h[a]



entry	CAL-B / mg	Conversion to **4** / %
1	280	93
2	140	93
3	70	81
4	35	62
5	18	44

[a]Reaction was run in a 0.5 mmol scale. Conversion was determined by ^{1}H NMR integration (400 MHz, DMSO-d_6) of the triplets at 4.88 and 4.68 ppm assigned as the H-4 of **3** and H-4 of **4**, respectively.

The ^{1}H NMR spectrum of each sample along the time presents the decrease of the triplet at 4.88 ppm, which is assigned to the H-4 from the starting peracetylated **3**, and the appearance of a triplet at 4.68 ppm, related to the H-4 from 2,3-di-*O*-acetyl-D-ribonolactone (**4**) formed as the product. The conversion rate of 2,3,5-tri-*O*-acetyl-D-ribonolactone (**3**) to 2,3-di-*O*-acetyl-D-ribonolactone (**4**) is quite similar in MeOH, *i*-PrOH, and *n*-BuOH. For instance, less than half of the starting material **3** was consumed after 6 h in *i*-PrOH (Table 5). The complete deacetylation of both **3** and **4** in *i*-PrOH ultimately furnished D-ribonolactone **2** as the exclusive product of solvolysis after 18 h (Scheme 2). On the other hand, the use of EtOH as the solvent/reagent led to fast and selective reactions, with the conversion of **3** to **4** reaching 75% after only 4 h without the competitive formation of



Scheme 2. CAL-B catalyzed alcoholysis of 2,3,5-tri-*O*-acetyl-D-ribonolactone (**3**).

Table 5. Consumption of **3** (%) through solvolysis in an added alcohol to give **4**[a]

Alcohol	0.5 h	1 h	2 h	4 h	6 h
MeOH	16	19	25	33	37
EtOH	50	54	59	75	93[b]
i-PrOH	13	14	22	31	43
n-BuOH	11	19	20	32	52

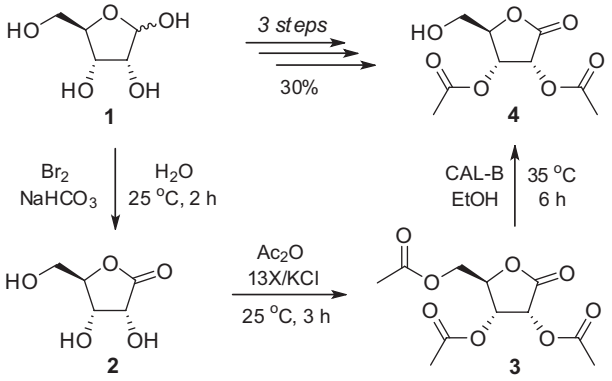
[a]Values obtained by integration of the triplets at 4.88 and 4.68 ppm assigned as the H-4 of **3** and H-4 of **4**, respectively; [b]the competitive formation of 3-*O*-acetyl-D-ribonolactone (**9**) was also observed.

any side products. However, longer reaction time (6 h) enabled the formation of 3-*O*-acetyl-D-ribonolactone (**9**) in non-negligible amounts, based on the appearance of triplets at 4.45 and 4.39 ppm corresponding to H-2 and H-4 in **9** (Scheme 2).

The reusability of immobilized enzymes can determine the economic viability of a biosynthetic process. Thus, CAL-B was employed as the catalyst for the alcoholysis of **3** in five subsequent cycles. At the end of each batch, CAL-B was removed from the reaction, rinsed with MeOH and ACN and used in the next batch. However, the reaction rate for the formation of the expected product **4** was not maintained from cycle to cycle and gradually slowed down (data not shown). This observation is possibly related to an extensive inhibition of the lipase activity caused by prolonged exposure to EtOH, which might be able to remove the aqueous micro-layer from the enzyme and thus alter the pH in the catalytic site.[48]

Scaling up: three-step synthesis of 2,3-di-*O*-acetyl-D-ribonolactone (**4**) from D-ribose (**1**)

After determining the best conditions for the per-*O*-acetylation of D-ribonolactone **2** followed by the regioselective ethanolysis of per-*O*-acetylated **3** to give 2,3-di-*O*-acetyl-D-ribonolactone (**4**), the route was adapted for a gram scale starting from D-ribose (**1**, Scheme 3). Accordingly, 2,3,5-tri-*O*-acetyl-D-ribonolactone (**3**) was prepared by first oxidizing D-ribose (**1**) to D-ribonolactone **2** using molecular bromine[5,49] followed by 13X/KCl catalyzed peracetylation reaction with acetic anhydride (ca. 29% yield, two steps). Subsequent CAL-B catalyzed ethanolysis (35 °C, 150 rpm, 6 h) cleanly produced 2,3-di-*O*-acetyl-D-ribonolactone (**4**) after purification by flash chromatography on silica gel using EtOAc/hexanes 1:3 (570 mg, 48% yield; 14% overall yield for the three steps).



Scheme 3. Three-step synthesis of **4** from **1**.

Conclusions

We have disclosed the use of 13X/KCl as an inexpensive and green catalyst to substitute toxic pyridine in the per-*O*-acetylation of D-ribonolactone **2**. Besides promoting faster acetylation reactions than pyridine without the need for aqueous work-up, the heterogeneous catalyst can be efficiently reutilized in subsequent cycles. In addition, the method was extended to the acetylation of two protected D-ribonolactone derivatives and also the per-*O*-acetylation of several natural carbohydrates, but in the latter cases it is necessary to warm the reaction to obtain good yields after 24 h. To further explore the synthetic utility of the peracetylated carbohydrates, we developed the regioselective CAL-B catalyzed enzymatic alcoholysis of 2,3,5-tri-*O*-acetyl-D-ribonolactone (**3**) to produce 2,3-di-*O*-acetyl-D-ribonolactone (**4**) in good yield under mild and green conditions (EtOH as the solvent/reagent, 35 °C). Finally, to demonstrate the synthetic utility of the peracetylation/selective deacetylation procedure, a simple and efficient three-step route was developed to prepare gram quantities of 2,3-di-*O*-acetyl-D-ribonolactone (**4**) starting from D-ribose (**1**), in 14% overall yield and only one chromatography step, indicating the feasibility of scaling up the process.

Experimental

General methods

CAL-B [Novozym 435, 10,000 propyl laurate units (PLU) mg^{-1} solid] was donated by Novozymes A/S (Brazil). The enzyme was used without any further treatment or purification. D-ribose (**1**) was acquired from Now™ Sports, D-ribono-1,4-lactone (**2**), as well as, 3A, 4A, 5A, and 13X MS were purchased from Sigma-Aldrich. 13X/KCl was prepared by the diffusion method using aqueous KCl as previously reported.[20,21] Thermally-modified 4A-1000 catalyst was prepared by heating 4A MS at 1000 °C for 1 h, according to the literature.[32] Acetic anhydride and pyridine were dried and distilled prior to use. All other chemicals were of reagent grade and used as received. Melting points were determined by a Büchi 510 melting point apparatus and are uncorrected. Infrared spectra were acquired with a FTIR spectrometer using KBr for solid samples (range 4000-400 cm^{-1}). ^1H NMR spectra were recorded at 200 or 400 MHz and ^{13}C NMR spectra at 100 MHz. Chemical shifts were recorded in parts *per* million (ppm, δ) relative to tetramethylsilane at 0.00 ppm or solvent ($CDCl_3$ at 7.26 ppm or DMSO-d_6 at 2.50 ppm for ^1H NMR, and $CDCl_3$ at 77.2 ppm or DMSO-d_6 at 39.5 ppm for ^{13}C NMR) as the internal standard.

Preparation of 2,3,5-tri-*O*-acetyl-D-ribonolactone (**3**)[14]

A mixture of D-ribonolactone **2** (148 mg, 1.0 mmol), Ac_2O (1.9 mL, 20 mmol), and 13X/KCl (600 mg) was stirred at 25 °C for 3 h. The catalyst was filtered off through a short pad of Celite® and then rinsed with 2-3 mL of EtOAc. The organic phase was concentrated under reduced pressure to give 273 mg (99% yield) of the desired product **3** as a light yellow oil.

Recyclability of the 13X/KCl catalyst

D-Ribonolactone **2** (50 mg, 0.34 mmol), Ac_2O (10 mmol) and 13X/KCl (300 mg) were added in a conic bottle flask coupled with a filter. The reaction was left at 25 °C under N_2 atmosphere. After 3 h, the mixture was filtered off under a positive pressure of N_2. The white solid catalyst was then rinsed with 5 mL of EtOAc, dried, and the mixture filtered again. The system was flushed with nitrogen for 5 min to remove excess solvent and new reagents were added in the flask. This process was repeated for another 2 times.

Preparation of 2,3-*O*-isopropylidene-D-ribonolactone (**5**)[3,4,7]

D-Ribonolactone **2** (148 mg, 1.0 mmol) and acetone (1.5 mL) were added into a 10 mL round-bottomed flask with a magnetic stirring bar and then 0.1 mL (2 drops) of 12 mol L^{-1} HCl was added at 25 °C. After 15 min, 0.5 mL of CH_2Cl_2 was poured to dissolve the white solid formed during the course of the reaction. Then the white mixture was quenched by pouring 300 mg of powdered $NaHCO_3$ in small portions (5 min, until CO_2 effervescence ceases), which turned the mixture into a yellow color. The liquid phase was decanted and a second portion of CH_2Cl_2 (0.5 mL) was added to the reaction residue to extract additional white solid. This process was repeated a second time and the combined CH_2Cl_2 extracts were evaporated to dryness using a rotary evaporator to give **5** as a colorless solid (80 mg, 42% yield).

Preparation of 3,4-*O*-benzylidene-D-ribonolactone (**6**)[7]

Benzaldehyde (0.58 mL, 0.57 mmol) was added into a 25 mL round-bottomed flask followed by D-ribonolactone **2** (85 mg, 0.57 mmol). The temperature was raised to 50 °C for 10 min until a milky mixture was formed. Then, 0.05 mL (1 drop) of 12 mol L^{-1} HCl was added and the mixture was left reacting at 90 °C for 1.5 h. After cooling down to 25 °C, 7 mL of Et_2O was added over the pinkish/yellowish crude mixture and the insoluble solid was separated by filtration and washed with 3 mL of 5% $NaHCO_3$, 3 mL of H_2O, and 3 mL of Et_2O to give **6** as a white solid (94 mg, 50% yield).

Preparation of 5-*O*-acetyl-2,3-*O*-isopropylideneribonic-1,4-lactone (**7**) and 2-*O*-acetyl-3,4-*O*-benzylidene-D-ribonolactone (**8**)[50]

D-ribonolactone derivative **5** or **6** (0.18 mmol), Ac_2O (0.95 mL, 10 mmol), and 13X/KCl (300 mg) were stirred at 80 °C for 1 h. After cooling down to 25 °C, the mixture was diluted with 3 mL of EtOAc and then filtered through a short pad of celite. The filtrate was coevaporated with toluene in the rotary evaporator to give a white solid, which was diluted in EtOAc and filtrated again to remove traces of the catalyst to give the desired product after evaporation of the solvent. Yield for **7**: 33 mg (99%); yield for **8**: 42 mg (99%).

General preparation of peracetylated carbohydrates using 13X/KCl

A mixture of the carbohydrate (0.50 mmol), Ac_2O (10 mmol), and 13X/KCl (300 mg) was stirred at 80 °C for 24 h. After cooling down to 25 °C, the catalyst was filtered off through a short pad of Celite® and then rinsed with 2-3 mL of EtOAc. The organic phase was concentrated under reduced pressure to give the desired product.

Pentaacetyl-D-glucose,[37] pentaacetyl-D-galactose,[38,39] pentaacetyl-D-mannose,[40] tetraacetyl-D-xylose,[41] and tetraacetyl-D-ribose[33] showed physical and spectral data in accordance with their expected structure and by comparison with spectral data in literature.

General preparation of peracetylated carbohydrates using pyridine

Per-*O*-acetylation of natural carbohydrates catalyzed by pyridine followed literature procedure, with slight modifications.[33] A mixture of the carbohydrate (1.0 mmol), Ac_2O (1.9 mL; 20 mmol), and pyridine (3 mL) was stirred at 25 °C for 24 h. The reaction mixture was diluted with 5 mL of CH_2Cl_2 and the organic phase was washed with 1 mol L^{-1} HCl (3 × 5 mL), saturated $NaHCO_3$ (3 × 5 mL), and brine (5 mL). The solvent was dried with anhydrous Na_2SO_4 and evaporated to furnish the per-*O*-acetylated carbohydrate derivatives. Peracetylated carbohydrates where characterized as above and showed physical and spectral data in accordance with literature.[37-41]

Determination of the catalyst amount for the alcoholysis of **3**

The optimal amount of CAL-B was determined by reacting 2,3,5-tri-*O*-acetyl-D-ribonolactone (**3**, 0.5 mmol) in EtOH (5.0 mL) using 18, 35, 70, 140, and 280 mg of the biocatalyst in a temperature-controlled incubator shaker (150 rpm, 35 °C). After 6 h, each supernatant solution was decanted, the enzyme was washed with 3 mL of MeOH followed by 3 mL of ACN and the solvent removed under reduced pressure. The light yellow oil obtained was then analyzed by ^{1}H NMR to determine the amount of 2,3-di-*O*-acetyl-D-ribonolactone (**4**) produced in each case (Table 5).

Enzyme catalyzed solvolysis in different alcohols

Experiments of biocatalyzed alcoholysis were performed by adding CAL-B (140 mg) to a suspension of the 2,3,5-tri-*O*-acetyl-D-ribonolactone (**3**, 50 mg, 0.18 mmol) in 5.0 mL of an alcohol and shaking the resulting mixtures in a temperature-controlled incubator shaker (150 rpm, 35 °C). After 0.5, 1.0, 2.0, 4.0, and 6.0 h (Table 6), biotransformations were stopped by filtering off the enzyme and washing the biocatalyst with 3 mL of MeOH followed by 3 mL of ACN. The recyclability of CAL-B was determined by applying the previously used supported enzyme in a new cycle.

Experiments involving the hydrolysis of **3** catalyzed by CAL-B were carried out according to the procedure described above, replacing the alcohol by the same volume of potassium phosphate buffer (30 mmol L^{-1}, pH 7). Control experiments carried out in the absence of the enzyme showed no reaction of compound **3**.

Three-step preparation of 2,3-di-*O*-acetyl-D-ribonolactone (**4**)

D-ribose (**1**, 10.0 g, 67.0 mmol), sodium bicarbonate (11.2 g, 130 mmol), and water (60 mL) were added in a 125 mL three-necked round-bottomed flask with an internal thermometer, a pressure-equalizing addition funnel and stirring bar. The mixture was stirred at room temperature for 15 min. Then, the flask was immersed in an ice-water bath. Bromine (1.12 g, 70 mmol) was added to the vigorously stirred aqueous solution through the addition funnel at a rate such that the reaction temperature was kept under 5 °C. After the addition, the funnel was replaced with a stopper and the mixture stirred for another 50 min. Sodium bisulfite (0.65 g, 6.25 mmol) was added and the orange color solution turned transparent. Then, the aqueous solution was transferred to a 250 mL flask and evaporated on a rotary evaporator to give a wet slurry. Absolute EtOH (40 mL) and toluene (10 mL) were added to the cloudy suspension and the solvent removed by rotary evaporation to provide a damp solid. Absolute EtOH (40 mL) was poured to the crude and the mixture heated on a steam bath for 30 min. The hot ethanolic suspension was filtered and the solids rinsed with hot absolute EtOH (10 mL). After refrigerating overnight, white crystals were formed, filtered, rinsed with cold absolute EtOH (10 mL) followed by Et_2O (10 mL) to give, after drying under vacuum, 8.16 g of a white solid (it is expected 35-45% of NaBr).[3] Part of D-ribonolactone from the previous procedure (2.20 g), 13X/KCl molecular sieves (3.0 g) and Ac_2O (20 mL) were stirred at 25 °C for 3 h. The reaction was quenched with EtOAc (20 mL) and filtered through a short pad of Celite®. The solvent was azeotropically removed using toluene (2 × 5 mL) as co-solvent in rotary evaporator to give 1.46 g of the crude acylated product **3** (> 95% pure by ^{1}H NMR) that was used in the next step without further purification. To a mixture of 2,3,5-tri-*O*-acetyl-D-ribonolactone (**3**, 1.46 g, 4.80 mmol) in EtOH (50 mL) was added CAL-B (730 mg) and the reaction was left in a temperature-controlled incubator shaker (180 rpm, 35 °C) for 6 h. Next, the supernatant solution was decanted and the enzyme washed with MeOH (10 mL) followed by ACN (10 mL). The combined solvents were removed under reduced pressure to give a light yellow oil after purification by flash column chromatography using 1:1 EtOAc/hexanes to give 570 mg of 2,3-di-*O*-acetyl-D-ribonolactone (**4**). Overall yield 14% from **1**. $[\alpha]_D^{25}$ +5.5 (c 5, EtOH); IR (film) ν_{max}/cm^{-1} 3495 (OH), 2950, 1796

(lactone), 1750 (ester), 1377, 1247, 1083; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.83 (d, 1H, *J* 6.3 Hz, H-2), 5.54 (d, *J* 6.3 Hz, 1H, H-3), 4.57-4.59 (m, 1H, H-4), 4.01 (dd, 1H, *J* 12.1 Hz, CH_2), 3.95 (d, 1H, *J* 12.1 Hz, CH_2), 2.16 (3H, s), 2.13 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.2, 20.5, 61.4, 67.1, 70.6, 83.5, 169.4, 170.0, 171.5; ESI-HRMS (CH_3OH) calcd.: $C_9H_{13}O_7$ $[M + H]^+$: 233.0656; found: 233.0662.

Supplementary Information

Supplementary information (^{1}H NMR for all compounds synthesized and ^{13}C NMR for compounds **4**, **5**, **7**, **8**, and peracetylated carbohydrates) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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