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Diastereoselective enzymatic preparation of acetylated pentofuranosides carrying free 5-hydroxyl groups

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ABSTRACT

Methyl 3-*O*-acetyl-2-deoxy-α-D-ribofuranoside, 1,3-di-*O*-acetyl-2-deoxy-α-D-ribofuranose and 1,2,3-tri-*O*-acetyl-α-D-arabinofuranose were diastereoselectively prepared (de = 100%) from anomeric mixtures of the corresponding 5-acetylated compounds through *Candida antarctica* B lipase (CAL B)-catalysed alcoholysis.

1. Introduction

It is well known that stereoselective reactions are powerful transformations in organic synthesis, since they avoid undesired products and difficult separation procedures; consequently, they are valuable tools in the chemistry of polyhydroxylated compounds such as carbohydrates. Among these compounds, partially acetylated derivatives of anomerically pure sugars are important as synthetic precursors of oligosaccharides, glycopeptides and nucleosides.[1]

Nowadays, the efficient synthesis of partially acylated carbohydrates can be achieved by the use of hydrolytic enzymes such as lipases, which catalyse regioselective acylation and deacylation reactions.[2–4] Most of the acylated carbohydrates obtained through this methodology are derived from hexopyranose moieties;[3–6] in contrast, pentofuranoses[3,4,7–13] and in particular, the stereoselective enzymatic acylation and deacylation of their anomeric mixtures,[3,7,11] have been reported to a lesser extent.

In our laboratory it was found that the *Candida antarctica* B lipase (CAL B)-catalysed alcoholysis of peracetylated nucleosides regioselectively affords the corresponding 5′-O-deacylated products;[14–17] we extended the scope of this biotransformation to pentofuranosides. In a previous paper,[18] we reported the different behaviours of methyl 2,3,5-tri-*O*-acetyl-α-D-ribofuranoside **1a** (Scheme 1) and methyl 2,3,5-tri-*O*-acetyl-β-D-ribofuranoside **1b** in CAL B-catalysed alcoholysis. While the enzymatic deprotection of the former proceeded regioselectively to afford methyl 2,3-di-*O*-acetyl-α-D-ribofuranoside **7a** in high yield, the alcoholysis of the β-diastereomer was less selective, leading to fully deacetylated methyl β-D-ribofuranoside. Recently, in work aimed at the synthesis of nucleosides from furanosides,[19] we have reported that CAL B-catalysed deacetylation of 1,3,5-tri-*O*-acetyl-2-deoxy-α, β-D-ribofuranose **4a**,**b** and methyl 2,3,5-tri-*O*-acetyl-α, β-D-arabinofuranoside **5a**,**b** afforded the corresponding 5-O-deacetylated furanosides **10** and **11**.

These results prompted us to study in more detail the stereoselectivity of these enzymatic deacetylations; herein we report the results obtained (Scheme 2).

2. Results and discussion

Experiments of CAL-B-catalysed alcoholysis of 2-deoxy **3a**,**b** and **4a**,**b** and arabino substrates **5a**,**b** and **6a**,**b** were studied at alcohol/substrate ratios (A/S) = 1200, 120 and 3; a high excess was assessed since it provided regioselectivity in the CAL B-catalysed alcoholysis of acylated nucleosides[14–17] and methyl 2,3,5-tri-*O*-acetyl-α,β-D-ribofuranoside.[18] Under these conditions, Table 1 shows the A/S ratios and times allowing maximum production of monodeacetylated diastereomerically pure products **9a**, **10a** and **12a** from the corresponding anomeric substrates **3a**,**b**, **4a**,**b** and **6a**,**b**. No significant further conversion of the 5-O-monodeacetylated product was observed after the times indicated in Table 1.

Since alcoholysis of **4a**,**b** at the high A/S ratio employed for **3a**,**b** (entry 1) afforded low substrate conversion, other experimental conditions were tested; at a lesser alcohol excess in dichloromethane (entry 2) a better yield of **10a** could be obtained; replacement of dichloromethane by acetonitrile, petroleum ether or dioxane gave no appreciable reaction. With the tetraacetylated arabinofuranose **6a**,**b** the best conditions for the formation of the free 5-hydroxyl derivative **12a** involved ethanol at A/S = 120 and dimethylformamide as a cosolvent (entry 3).

In every case, products **9**, **10** and **12** consisted only of the 5-O-deacetylated regioisomer, without removal of the more reactive anomeric acetate of **4** and **6**. Their ^{1}H NMR analysis showed H-5 signals at ca. 3.7–3.9 ppm (Section 4.3.2); this as well as a shift towards higher fields of ca. 0.5–0.6 ppm in comparison with H-5 of substrates

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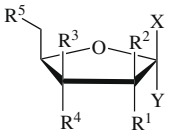
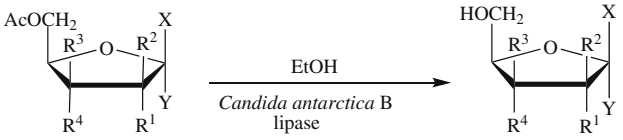


1a X = H, Y = OCH_3 ; $R^1 = R^4 = R^5$ = OAc ; $R^2 = R^3$ = H
1b X = OCH_3, Y = H ; $R^1 = R^4 = R^5$ = OAc ; $R^2 = R^3$ = H
2a X = H, Y = OAc; $R^1 = R^4 = R^5$ = OAc ; $R^2 = R^3$ = H
2b X = OAc, Y = H; $R^1 = R^4 = R^5$ = OAc ; $R^2 = R^3$ = H
3a X = H , Y = OCH_3; $R^4 = R^5$ = OAc; $R^1 = R^2 = R^3$ = H
3b X = OCH_3 , Y = H; $R^4 = R^5$ = OAc; $R^1 = R^2 = R^3$ = H
4a X = H , Y = OAc; $R^4 = R^5$ = OAc; $R^1 = R^2 = R^3$ = H
4b X = OAc , Y = H; $R^4 = R^5$ = OAc; $R^1 = R^2 = R^3$ = H
5a X = H , Y = OCH_3; $R^2 = R^4 = R^5$ = OAc; $R^1 = R^3$ = H
5b X = OCH_3, Y = H; $R^2 = R^4 = R^5$ = OAc; $R^1 = R^3$ = H
6a X = H, Y = OAc; $R^2 = R^4 = R^5$ = OAc; $R^1 = R^3$ = H
6b X = OAc, Y = H; $R^2 = R^4 = R^5$ = OAc; $R^1 = R^3$ = H

7a X = H, Y = OCH_3 ; $R^1 = R^4$ = OAc ; $R^2 = R^3$ = H; R^5 = OH
7b X = OCH_3, Y = H ; $R^1 = R^4$ = OAc ; $R^2 = R^3$ = H; R^5 = OH
8a X = H, Y = OAc; $R^1 = R^4$ = OAc ; $R^2 = R^3$ = H; R^5 = OH
8b X = OAc, Y = H; $R^1 = R^4$ = OAc ; $R^2 = R^3$ = H; R^5 = OH
9a X = H , Y = OCH_3; R^4 = OAc; $R^1 = R^2 = R^3$ = H; R^5 = OH
10a X = H , Y = OAc; R^4 = OAc; $R^1 = R^2 = R^3$ = H; R^5 = OH
11a X = H , Y = OCH_3; $R^2 = R^4$ = OAc; $R^1 = R^3$ = H; R^5 = OH
11b X = OCH_3, Y = H; $R^2 = R^4$ = OAc; $R^1 = R^3$ = H; R^5 = OH
12a X = H, Y = OAc; $R^2 = R^4$ = OAc; $R^1 = R^3$ = H; R^5 = OH

Scheme 1.



3,4,6 a,b **9,10,12 a**

3a, 9a X = H , Y = OCH_3; R^4 = OAc; $R^1 = R^2 = R^3$ = H
3b X = OCH_3 , Y = H; R^4 = OAc; $R^1 = R^2 = R^3$ = H
4a, 10a X = H , Y = OAc; R^4 = OAc; $R^1 = R^2 = R^3$ = H
4b X = OAc , Y = H; R^4 = OAc; $R^1 = R^2 = R^3$ = H
6a, 12a X = H, Y = OAc; $R^2 = R^4$ = OAc; $R^1 = R^3$ = H
6b X = OAc, Y = H; $R^2 = R^4$ = OAc; $R^1 = R^3$ = H

Scheme 2. CAL B-catalysed diastereoselective deacetylation of pentofuranosides **3**, **4** and **6**.

(data not shown) is consistent with primary alcohol methylene hydrogens, indicating that the deacetylation occurred at the 5-acetate.

^{1}H NMR analysis allowed us to evaluate the stereoselectivity of CAL B-catalysed deacetylation of the anomeric mixtures studied. Products **9a**, **10a** and **12a** were obtained exclusively as the α-anomer, indicating a high diastereoselectivity of CAL B in the deacetylation of these substrates. The results suggest that pentose substitution at carbon 2 affects the observed stereoselectivity, since **2a**,**b** and **5a**,**b**, which share the same 3-substitution pattern as **3-**, **4-** and **6a**,**b**, reacted with lower diastereoselectivity. However, compounds **8** and **11**, and the corresponding 5-O-deacetylated products from **2** and **5**, were enriched in the α-anomer (for **2**, β/α ratio = 2.6/1.0, for **8**, β/α ratio = 1.0/3.0, at a A/S = 1200; for **5**, β/α ratio = 1.1/1.0, for **11**, β/α ratio = 1.0/2.5 at a A/S = 120, using acetone as a cosolvent).

In addition to the yield of products reported in Table 1, calculated by taking into account the mass of each substrate as anomeric mixtures, a yield based on the mass of the α-anomer content in the substrate and in the product was determined (% α-anomer recovery, Table 1). This value shows that CAL B-catalysed alcoholysis allows the recovery of the α-anomer from anomeric mixtures in moderate yields. In this way, this enzymatic procedure provides not only an access to regioselectively 5-O-monodeacetylated products, but also a simple method to resolve pentofuranoside anomeric mixtures, which are usually difficult to separate through traditional chromatographic procedures.

Concerning the stereoselectivity of CAL B in the catalysis of the carbohydrate anomers, some papers have reported differences in the behaviour of pyranose anomers.[3,5,20,21] In CAL B-catalysed acylations, the alkyl pyranoside anomers displayed different regioselectivities;[5,21] in alcoholyses, differential recognition of pentaacetylated glucosamine epimers by CAL B has been described.[20] However, with regard to furanoses, apart from work reporting the regioselective alcoholysis of the hexose 1,2,3,4,6-penta-*O*-acetyl-α,β-D-fructofuranose,[11] in which a different regioselectivity in the monodeacetylation of each anomer was observed, to the best of our knowledge no other work on the stereoselective CAL B-catalysed reaction of furanoses has been reported. In the field of nucleosides, CAL B-catalysed hydrolysis of an α/β mixture of *p*-chlorobenzoyl-protected thymidine afforded the 5′-deacylated α anomer.[22]

3. Conclusion

In conclusion, this work describes a simple procedure for a highly stereoselective preparation of three free 5-hydroxyl-acety-

Table 1
CAL B-catalysed diastereoselective preparation of **9a**, **10a** and **12a** (Scheme 2)

Entry	Substrate	Substrate β/α ratio[a]	*T* (°C)	*t* (h)	Alcohol	A/S[b]	Product (yield, %)[c]	de[d] (%)	α-Anomer recovery[e] (%)
1	**3a**,**b**	1.1/1.0	30	24	Ethanol	1200	**9a** (32)	100	67
2	**4a**,**b**	1.2/1.0	30	1.5	Ethanol[f]	3	**10a** (24)	100	58
3	**6a**,**b**	1.3/1.0	30	24	Ethanol[g]	120	**12a** (30)	100	69

[a] Anomeric ratio, determined by ^{1}H NMR.
[b] Alcohol/substrate molar ratio.
[c] Yield determined after isolation by silica gel column chromatography.
[d] Diastereomeric excess, determined by ^{1}H NMR.
[e] Yield calculated on the basis of α-anomer content in isolated product and in the substrate.
[f] Dichloromethane was employed as solvent.
[g] Dimethylformamide was employed as cosolvent.

lated pentofuranosides, through an enzymatic alcoholysis. By using this methodology, these products can be prepared in two steps from the corresponding free D-pentofuranose, avoiding an additional step involved in traditional synthetic procedures when applying protecting groups. Moreover, it provides a simple procedure for the separation of anomeric mixtures of these pentofuranosides. In this way, the results herein described widen the applications of CAL B in the field of pentofuranoses.

4. Experimental

4.1. General

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; δ are indicated in ppm and J, in hertz. Optical rotations were measured on a Perkin Elmer Polarimeter 343.

All employed reagents and solvents were of analytical grade and obtained from commercial sources. For the enzymatic alcoholyses, absolute ethanol was employed and the solvents tested were dried and distilled prior to use.

TLC was performed on Silica Gel 60 F_{254} plates (Merck) and the resulting plates were developed using ethanol–sulfuric acid 80:20 v/v with heating. Column chromatography was carried out using silica Gel Merck 60 (0.040–0.063 mm).

Lipase B from *C. antarctica* (CAL B, Novozym 435, 10,000 PLU/mg solid; PLU:Propyl Laurate Units), a generous gift from Novozymes (Brazil), was used without any further treatment or purification.

Enzymatic reactions were carried out in a temperature-controlled incubator shaker (Sontec OS 11, Argentina) at 200 rpm and 30 °C.

4.2. Preparation of the substrates

Substrate **3** was prepared via standard protocols.[18] 2-Deoxy-D-ribose in an excess of methanol containing sulfuric acid and copper sulfate afforded methyl 2-deoxyriboside, which by subsequent treatment with acetic anhydride and dimethylaminopyridine in pyridine gave the triacetylated compound. Purification of the crude product by column chromatography afforded methyl 3,5-di-*O*-acetyl-2-deoxy-α, β-D-ribofuranoside **3a**,**b**, which gave satisfactory NMR data.

Since direct peracetylation of the corresponding D-pentose with acetic anhydride in pyridine produces mixtures of furanoses and pyranoses, in order to prepare **4** and **6**, the pentose was first treated with acetic anhydride in dry tetrahydrofurane and CAL B,[23] then further acylated with acetic anhydride in pyridine, following the previously described protocols.[23] Application of this procedure gave, after purification of the crude products by column chromatography, the substrates 1,3,5-tri-*O*-acetyl-2-deoxy-α, β-D-ribofuranose **4a**,**b** and 1,2,3,5-tetra-*O*-acetyl-α, β-D-arabinofuranose **6a**,**b**.

4.3. CAL B-catalysed deacetylation of substrates 3, 4 and 6

4.3.1. Analytical procedure

In a typical analytical protocol, the substrate (10 mg) was dissolved in the nucleophilic alcohol at an alcohol/substrate molar ratio (A/S) = 1200 or 120 and CAL B (300 mg $mmol^{-1}$ substrate) was added.

When a cosolvent was assayed, the substrate was dissolved in a mixture of alcohol and 10 % (v/v) of the solvent tested, maintaining the above reported A/S ratios. An A/S = 3 was also assayed; in this case, a 0.7 % (v/v) mixture of alcohol and solvent was employed.

The resulting reaction mixtures were shaken at 200 rpm and 30 °C. Samples were taken at different times and, after removal of the enzyme, monitored by TLC using dichloromethane–methanol 95:5 v/v as the mobile phase.

Control experiments carried out in the absence of the enzyme showed no appreciable reaction.

4.3.2. Preparative procedure

4.3.2.1. Methyl 3-*O*-acetyl-2-deoxy-α-D-ribofuranoside 9a. According to the analytical procedure, substrate **3a**,**b** (0.31 mmol) was dissolved in ethanol at an A/S = 1200 (21.6 mL) and CAL B (93 mg) was added. The reaction mixture was shaken at 200 rpm and 30 °C for 24 h. Then the lipase was filtered off, washed with dichloromethane, the resulting filtrates evaporated and the crude product subsequently purified by silicagel column chromatography using dichloromethane–methanol 98:2, affording **9a** (32%), $[\alpha]_D^{20} = +129.4$ (*c* 0.06, CH_3CH_2OH), ^1H NMR (500 MHz, $CDCl_3$): 2.03 (ddd, 1H, J = 14.6, 8.7, 5.3 Hz, H-2), 2.09 (s, 3H, CH_3), 3,40 (s, 3H, OCH_3), 3.76 (dd, 1H, J = 11.8, 3.8 Hz, H-5), 3.82 (dd, 1H, J = 11.8, 3.8 Hz, H-5′), 4.11 (pq, 1H, J = 3.8, 3.8 Hz, H-4), 5.07-5.09 (m, 1H, H-3), 5.10 (d, 1H, J = 5.3 Hz, H-1). ^{13}C NMR (125 MHz, $CDCl_3$): 21.15 (CH_3), 39.26 (C-2), 55.08 (OCH_3), 62.59 (C-5), 74.27 (C-3), 83.54 (C-4), 104.96 (C-1), 171.40 (CO).

4.3.2.2. 1,3-Di-*O*-acetyl-2-deoxy-α-D-ribofuranose 10a. Following the above reported protocol, **4a**,**b** (0.31 mmol) was dissolved in ethanol (A/S = 3, 0.053 mL) and dichloromethane (7.5 mL) and the mixture shaken for 1.5 h. After the described work up, **10a** (24%) was obtained: $[\alpha]_D^{20} = +89.4$ (*c* 0.04, CH_3CH_2OH), ^1H NMR (500 MHz, $CDCl_3$): 2.08, (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 2.50 (ddd, 1H, J = 15.0, 7.9, 5.3 Hz, H-2), 3.74–3.83 (m, 2H, H-5, H-5′), 4.29 (dd, 1H, J = 6.7, 3.6 Hz, H-4), 5.16-5.19 (m, 1H, H-3), 6.39 (d, 1H, J = 5.3 Hz, H-1). ^{13}C NMR (125 MHz, $CDCl_3$): 21.04, 21.29 (CH_3s), 38.57 (C-2), 62.42 (C-5), 74.00 (C-3), 86.09 (C-4), 98.34 (C-1), 170.48, 171.08 (COs).

4.3.2.3. 1,2,3-Tri-*O*-acetyl-α-D-arabinofuranose 12a. At first, **6a**,**b** (0.31 mmol) was dissolved in ethanol at an A/S = 120 (2.20 mL) and 0.20 mL of DMF and the mixture shaken for 24 h. Application of the above protocol and the involved work up gave **12a** (30%): $[\alpha]_D^{20} = -13.3$ (*c* 0.12, CH_3CH_2OH), ^1H NMR (500 MHz, $CDCl_3$): 2.12, 2.13, 2.14 (9H, CH_3s), 3.78–3.93 (m, 2H, H-5, H-5′), 4.21–4.25 (m, 1H, H-4), 5.12 (dd, 1H, J = 5.1, 1.8 Hz, H-3), 5.25 (d, 1H, J = 1.8, H-2), 6.18 (s, 1H, H-1). ^{13}C NMR (125 MHz, $CDCl_3$): 20.59, 20.61, 20.62 (CH_3s), 61.23 (C-5), 74.10 (C-3), 76.83 (C-2), 82.50 (C-4), 93.78 (C-1), 169.82, 171.10, 170.14 (COs).

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