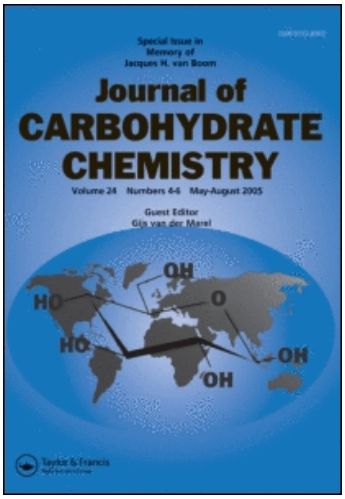

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ENZYMATIC TRANSESTERIFICATION OF ALKYL 2,3,4-TRI-*O*-ACYL-β-D-XYLOPYRANOSIDES

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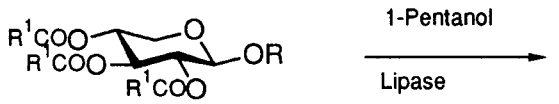
ABSTRACT

The transesterification of some alkyl 2,3,4-tri-*O*-acyl-β-D-xylopyranosides catalysed by *Pseudomonas cepacia* lipase in organic solvents has been studied. In all the cases a unique regioisomeric product was obtained, the alkyl 2,3-di-*O*-acyl-β-D-xylopyranoside. The 2,3-di-*O*-acetyl derivative (**2b**) was obtained in 93% yield. These compounds thus obtained are useful intermediates in the synthesis of 4-*O*-β-D-galactopyranosyl-D-xylose.

INTRODUCTION

Acylation and deacylation reactions catalysed by lipases have become a common tool in organic synthesis.[1] These enzymes have the advantages that many of them are commercially available, they are inexpensive and work efficiently in organic solvents.[2] In multi-acylated sugar derivatives the lipase-catalysed reactions may give regioselectivities that in some cases are difficult to obtain by chemical methods.[3] We have now carried out the lipase-catalysed transesterification of alkyl tri-*O*-acyl-β-D-xylopyranosides **1a-d** which has provided partially protected xylose derivatives, useful intermediates for the synthesis of 4-*O*-β-D-galactopyranosyl-D-xylose (deacetylated **3**). This



1a R = CH_3 ; R^1 = $CH_3(CH_2)_4$
1b R = CH_3 ; R^1 = CH_3
1c R = $CH_3(CH_2)_7$; R^1 = $CH_3(CH_2)_4$
1d R = $CH_3(CH_2)_7$; R^1 = CH_3

2a R = CH_3 ; R^1 = $CH_3(CH_2)_4$
2b R = CH_3 ; R^1 = CH_3
2c R = $CH_3(CH_2)_7$; R^1 = $CH_3(CH_2)_4$
2d R = $CH_3(CH_2)_7$; R^1 = CH_3

Enzyme	PPL	CCL	PSL	GCL	RAL	ANL	React. 0
Unreacted **1a** (%)	93.98	83.32	1.83	98.13	98.07	97.6	100

Figure 1. Screening of lipases. GLC data

disaccharide is present in the carbohydrate-protein linkage region of proteoglycans[4] and has also been used to evaluate the *in vivo* activity of intestinal lactase.[5]

RESULTS AND DISCUSSION

To study the enzymatic transesterification of **1a-d,** we first carried out a screening of lipases. Among six different lipases we tested,[6] the *Pseudomonas cepacia* lipase (lipase PS) was significantly the most active in the transesterification of **1a** (10 mM) with 1-pentanol (50 mM) in isooctane in a 1 day incubation with 20 mg/mL of lipase (Figure 1). No conversion was observed in absence of enzyme (reaction O). We then checked the influence of the solvent and the nature of the substituents in the β-D-xylopyranoside substrate on the yield and regioselectivity of the reaction using lipase PS.

For the six organic solvents used in the transesterification of **1a** with 1-pentanol we always obtained methyl 2,3-di-*O*-hexanoyl-β-D-xylopyranoside (**2**a) as the only reaction product; neither other regioisomers nor products of higher degree of deacylation were observed after two days incubation. We selected six organic solvents displaying different degrees of hydrophobicity,[7] from hydrophilic acetonitrile (logP= -0.33) to isooctane (logP= 4.5). As

Table 1. Conversion after two days incubation of **1a** (20 mM), 1-pentanol (48 mM) and lipase PS (10 mg / mL) in different solvents

Solvent[a]	Conversion of **1a** (%)[b]
Acetonitrile	11
Triethylamine[c]	21
Benzene	5
Trichloroethane	9
tert-Amyl alcohol	59
Isooctane	58

a. All solvents were dried, distilled and stored over molecular sieves 4Å before use.
b. Determined by GLC.
c. No deacylation was observed under these anhydrous conditions in a blank reaction without enzyme.

expected, the rate of the reaction varies with the solvent, although there is not a clear correlation between rate and hydrophobicity.[8] The highest rates were found in *tert*-amyl alcohol and isooctane (Table 1).

It has been shown that the regioselectivity of the transesterification catalysed by this lipase in organic solvents may be influenced by the presence of hydrophobic substituents in the substrate.[9] We have not observed this effect in the transesterification reaction of xylopyranosides **1a-d**, having acyl and alkyl substituents of different chain length, with 1-pentanol in *tert*-amyl alcohol. We obtained again the xylosyl derivatives **2a-d** with the HO-4 free as the only product. Nevertheless, the reaction was faster with methyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**1b**) (Table 2). The high selectivity observed could be ascribed to steric factors, since the C(4)-C(5)-O(5) of the pyranoid ring has a smaller volume than the C(1)-C(2)-C(3) side. From a synthetic point of view this high regioselectivity has practical applications. Compound **2b** could be obtained in 93 % yield after a 4 days incubation and was subsequently galactosylated following a previously described method[10] to give the precursor methyl 2,3-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-xylopyranoside **3** (Figure 2), illustrating the utility of this enzymatic reaction in the synthesis of oligosaccharides.

Table 2. Conversion after two days incubation of **1a-d** (20 mM). 1-pentanol (48 mM) and lipase PS (10 mg / mL) in tert-amyl alcohol

Substrate	Conversion of **1a-d** (%)[a]
1 a	58
1 b	75 (93)[b]
1 c	45
1 d	42

a. Determined by GLC
b. After 4 days incubation

EXPERIMENTAL

Material and methods. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Column chromatography was performed on silica gel Merck (70-230). Gas-liquid chromatography was carried out using a Hewlett-Packard capillary gas chromatograph equipped with a fused SE-54 column (10 m) with a 0.3 i.d. and 0.15 mm film. ^{1}H NMR spectra were recorded using a Varian XL-300 (300 MHz) spectrometer. Optical rotations were determined with a Perkin-Elmer 141 polarimeter.

Alkyl 2,3,4-tri-*O*-acyl-β-D-xylopyranosides (1a-d). Compounds **1a-d** were prepared by conventional acylation of methyl and octyl β-D-xylopyranosides using acetic anhydride or hexanoic anhydride in pyridine and catalytic amounts of 4-dimethylaminopyridine.

1a: oil, $[\alpha]_D$ - 26.1° (*c* 0.9, chloroform). *Anal.* Calcd for $C_{24}H_{42}O_8$: C, 62.88; H, 9.17. Found: C, 62.93; H, 8.89.

1b, mp 98-100 °C, $[\alpha]_D$ -60.0 ° (*c* 1, chloroform); lit.[11] $[\alpha]_D$ -60.8°. *Anal.* Calcd for $C_{12}H_{18}O_8$: C, 49.65; H, 6.20. Found: C, 49.81; H, 6.36.

1c, oil, $[\alpha]_D$ -13.4 ° (*c* 1, chloroform). No accurate elemental analysis could be obtained.

1d, oil, $[\alpha]_D$ -43.8 ° (*c* 0.9, chloroform); lit.[12] $[\alpha]_D$ -53.4 °. *Anal.* Calcd for $C_{19}H_{32}O_8$: C, 58.76; H, 8.24. Found: C, 58.57; H, 8.10.

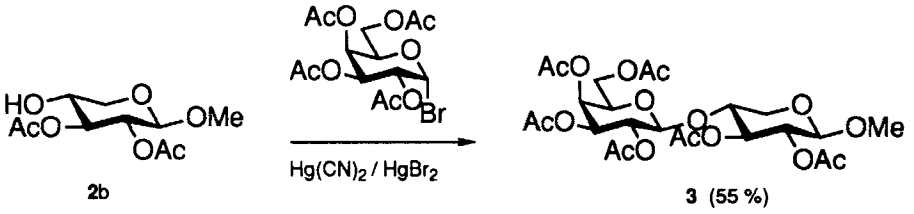


Figure 2

Enzymatic reactions. Transesterification of **1a** is representative of the general procedure: A mixture of substrate **1a** (2 g, 22 mM), 1-pentanol (868 µL, 40 mM) and lipase PS (4 g) in the isooctane (200 mL) was incubated in an orbital shaker at room temperature. At various intervals of time the reaction mixture was analysed by GLC - an aliquot (20 µL) was treated with acetic anhydride (20 µL) and pyridine (40 µL) for **1a** and **1c**, or hexanoic anhydride (20 µL) and pyridine (40 µL) for **1b** and **1d**, kept at room temperature for 1h and the resulting solution injected (1 µL) into the GL chromatograph. The reaction was stopped by removing the enzymes by filtration. The filtrate was concentrated and the reaction products were purified from the resulting residue following the methods described below. Product structures were determined by ^{1}H NMR. The data of ^{1}H NMR spectra of **2a-d** are summarised in Table 3.

2a.- The residue was eluted on a silica gel column (hexane-AcOEt 4:1); oil, 89%. $[\alpha]_D$ -39.8 ° (*c* 1, chloroform). *Anal.* Calcd for $C_{18}H_{32}O_7$: C, 60.00; H, 8.88. Found: C, 59.96; H, 8.95.

2b.- Reaction was carried out in *tert*-amyl alcohol. The residue was extracted with boiling ethanol. On cooling, the product precipitated as a syrup, 84%. $[\alpha]_D$ -69.0 ° (*c* 1, chloroform). *Anal.* Calcd for $C_{10}H_{16}O_7$: C, 48.38; H, 6.45. Found: C, 47.98; H, 6.60.

2c.- Eluted on a silica gel column (hexane-AcOEt 12:1); oil, 87%. $[\alpha]_D$ -11.9 ° (*c* 1.2, chloroform). *Anal.* Calcd for $C_{25}H_{46}O_7$: C, 65.50; H, 10.04. Found: C, 65.87; H, 10.38.

2d.- Reaction was carried out in *tert*-amyl alcohol. Eluted on a silica gel column (hexane-ethyl ether 2:3); oil, 60%. $[\alpha]_D$ -41.79 ° (*c* 0.48, chloroform). *Anal.* Calcd for $C_{17}H_{30}O_7$: C, 58.95; H, 8.82. Found: C, 59.20; H, 8.89.

Methyl 2,3-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-xylopyranoside (3). A solution of **2b** (0.11 g, 0.44

Table 3. ^{1}H NMR data for compounds **2a-d**

	Chemical shifts, δ						Coupling constants, Hz			
	H-1	H-2	H-3	H-4	H-5a	H-5e	$J_{1,2}$	$J_{4,5a}$	$J_{4,5e}$	$J_{5a,5a}$
2a	4.30	4.86	4.86	3.75	3.33	4.09	6.9	8.8	4.9	11.7
2b	4.31	4.83	4.83	3.74	3.29	4.02	6.8	8.8	5.0	11.8
2c	4.40	4.85	4.85	3.76	3.31	4.02	6.7	8.8	4.8	11.7
2d	4.40	4.88	4.88	3.75	3.32	4.03	6.8	8.7	5.3	12.0

mmol), mercuric cyanide (0.1 g, 0.4 mmol) and mercuric bromide (0.015 g, 0.04 mmol) in acetonitrile (1 mL) was stirred at room temperature under argon for 15 min. Then, a solution of acetobromogalactose (0.18 g, 0.45 mmol) in acetonitrile (0.25 mL) was added. After 2 h, more acetobromogalactose in acetonitrile (same amount) was added, and the stirring was continued overnight. The reaction mixture was diluted with aqueous 10% potassium iodide, extracted with chloroform, the organic phase dried (Na_2SO_4) and concentrated. The residue was eluted (hexane-ethyl acetate 1:1) to give, first, 1,2,3,4,6-penta-*O*-acetyl-α,β-D-galactopyranose. Next eluted was methyl 2,3-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-xylopyranoside (0.07 g, 28 %). Last eluted was compound **3** (0.143 g, 55%). ^{1}H NMR data ($CDCl_3$) for **3**: δ 5.35 (dd, 1 H, $J_{3',4'}$=3.4 Hz, H-4'), 5.11 (t, 1H, $J_{2,3} = J_{3,4}$=8.0 Hz, H-3), 5.09 (dd, 1 H, $J_{1',2'}$=7.7 Hz, $J_{2',3'}$=10.4 Hz, H-2'), 4.97 (dd, 1 H, H-3'), 4.82 (dd, 1 H, $J_{1,2}$=6.9 Hz, $J_{2,3}$=8.5 Hz, H-2), 4.49 (d, 1 H, H-1'), 4.36 (d, 1 H, H-1'), 4.20 (m, 2 H, H-6'a, -6'e), 4.09 (dd, 1 H, $J_{4,5e}$=6.4 Hz, $J_{5e,5a}$=9.2 Hz, H-5e), 3.94 (dt, 1 H, H-5'), 3.82 (ddd, 1 H, $J_{4,5a}$=7.9 Hz, H-4), 3.45 (s, 3H, OMe), 3.37 (dd, 1H, H-5a), 2.14, 2.05, 2.04, 2.03, 2.01 and 1.99 (6s, each 3H, 6 MeCO).

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