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Regioselective Hydrolysis of Peracetylated α-D-Glucopyranose Catalyzed by Immobilized Lipases in Aqueous Medium. A Facile Preparation of Useful Intermediates for Oligosaccharide Synthesis.

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Abstract: Penta-*O*-acetyl-α-D-Glucopyranose was selectively deacetylated in aqueous media by lipases from *Candida cilindracea* (CCL) adsorbed on octyl-agarose support. Enzymatic hydrolyses was regioselective at the 4-position under neutral pH and towards the 6 position under acidic conditions. This enzymatic approach allows the one step synthesis of 1,2,3,6-tetra-*O*-acetyl-α-D-glucopyranoses **1**, a useful intermediate in oligosaccharide synthesis.

Keywords: Glycosides; esters; hydrolysis; enzyme reaction; regiocontrol;

Pure regio-isomers of tetra-*O*-acetyl glucopyranoses (TAG) may be used as key intermediates for the synthesis of a large number of glycoderivatives (oligosaccharides, sugar esters, glycopeptides, etc.).[1,2] TAG intermediates could be readily and selectively modified at their free hydroxyl group and are soluble in most organic solvents. Moreover, the protected final products, can be easily deacetylated by very mild chemical or enzymatic processes.

1,2,3,6-Tetra-*O*-acetyl-α-D-glucopyranose **1**[3,4] and related compounds, may be of particular interest due to the presence of 1,4 glycosidic bonds in natural and pharmaceutical oligosaccharides. However, the preparation of TAG with one free secondary hydroxyl group require multi-step synthesis and may pose environmental problems in large scale productions, due to the use of organic solvents and toxic reagents. To the best of our knowledge, only two papers concerning suitable chemical syntheses of **1** have been published.[3,5]

The 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose (PAG) can be used as an economic pool in regioselective deacetylation for large scale preparation of TAG. Although a large number of procedures have been reported, suitable processes for chemical hydrolysis of PAG only afford the deacetylation at the anomeric position.[6]

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The use of enzymatic catalysts such as lipases or esterases could be considered an important tool for TAG's preparation, but the enzymatic deacylations of PAG already described in literature present some drawbacks.[2,7] In most cases, reactions are very slow and proceed with poor selectivity and yield. In addition these reactions often afford a very complex mixture of tetra-, tri-, di-, and monoacetates, as well as free glucose.

The lipase catalyzed hydrolyses of PAG have been reported and good yields were obtained by deacetylation at the anomeric or 6- positions.[8,9] Regioselective hydrolysis at the C-6 position could in fact be obtained by protecting the C-1 hydroxyl group as alkyl glicoside. The regioselective deacetylation of PAG in position others than anomeric or C-6 have never been reported.

In this report we present a regioselective biocatalytic processes for PAG deacetylation at different positions, in order to obtain different regioisomers of TAG in high yields. New lipase derivatives prepared by hydrophobic absorption on octyl agarose gel,[10] have been used as catalysts for regioselective hydrolysis of PAG in aqueous medium.[11] The immobilized derivatives of six microbial lipases (*Pseudomonas. fluorescens, Candida cilindracea, Humicola lanuginosa, Candida antarctica, Aspergillus oryzae and Mucor. javanicus*) have been tested, with the aim of finding highly active and selective catalysts for the hydrolysis of PAG in aqueous media. The different lipases utilized and the results obtained in the enzymatic hydrolyses are summarized in **table 1**.

In a preliminary approach, tetra- tri-di- and monoacetates have been quantified by gas chromatography mass spectrometry analysis, but the different regioisomers of TAG have not been isolated and identified. With the lipases from *H. lanuginosa, C. antarctica, A. oryzae and M. javanicus* hydrolyses were very slow allowing only 30% of the initial substrate to be hydrolyzed after 24 hours. In addition, a small accumulation of different regioisomers of TAG was observed with the yield less then 10%; the initial substrate seems being quickly hydrolyzed to tri-, di-, or monoacetyl esters. For instance, the hydrolysis catalyzed by the lipase from *H. lanuginosa* only arise to 4% yield of TAG, because more than 20% of PAG was already hydrolyzed to tri-, di- and monoesters.

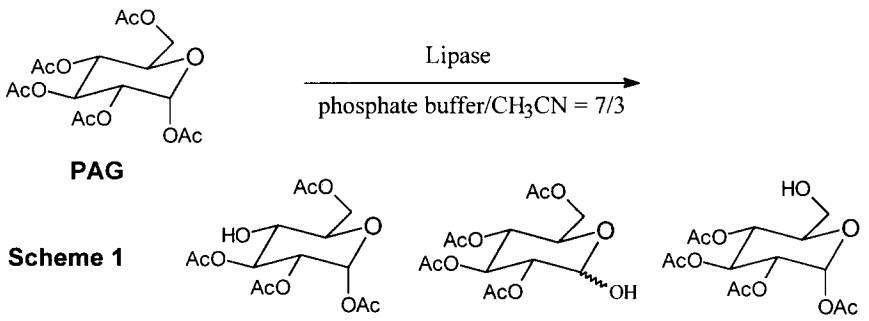
Table 1: enzymatic hydrolysis of 1,2,3,4,6 penta-*O*-acetyl glucopyranose in aqueous medium and catalyzed by different immobilized lipases.

Lipase	Time (hr)	PAG %	TAG %
P. fluorescens	3	0	98
C. cilindracea	3	0	78
H. lanuginosa	24	72	4
C. antarctica	24	76	11
A. oryzae	24	69	11
M. javanicus	24	76	10

These results indicate that PAG is a bad substrate for most of the lipase derivatives studied, while tetra, tri, di, and mono acetyl derivatives become much better substrates and the reactions proceed towards the complete hydrolysis of the initial substrate to free glucose. On the contrary, lipases from *Pseudomonas fluorescens* (PFL) and *Candida cilindracea* (CCL) were the only catalysts which gave a rapid and quantitative accumulation of different regioisomers of TAG. In these cases, PAG seems to be the best enzyme substrate compared to the less acetylated glucopyranose derivatives. After 3 hours, PFL allowed 98% of global yield of tetra acetyl esters without further hydrolysis, while using CCL a yields of 78% was achieved.

The different regioisomers of TAG obtained by catalytic hydrolysis with PFL and CCL, were separated by flash chromatography and analyzed by ^{1}H and ^{13}C NMR. The structure of the different isomers has been confirmed by COSY NMR. According to **scheme 1**, at neutral conditions PFL was completely regioselective towards the anomeric position and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose **2** was obtained in 80% yield after purification. The lipase from CCL showed a good selectivity towards the 4 position affording to a 4:1 mixture of compound **1** and 1,2,3,4-tetra-*O*-acetyl-α-D-glucopyranose **3**, respectively. Acetyl group migration from 6 to 4 position has not been observed in the reaction conditions adopted for the enzymatic hydrolysis, and the most abundant regioisomer **1** was isolated in 50% yield.[12]

The influence of the reaction conditions on the regioselectivity of PFL and CCL has also been evaluated. When PFL was used at acidic conditions, variation in the selectivity have not been observed, whereas CCL showed a dramatic inversion of regioselectivity. At pH 5 this enzyme exclusively recognized the 6-position and the hydrolysis of PAG allowed pure **3** in 75% yield after purification.[13]



PAG

Scheme 1

Lipase	pH	yield	**1**	**2(α/β)**	**3**	**(others)**
PFL	7	98%	1	91 (7/3)	-	(8)
CCL	7	78%	71	-	24	(5)
PFL	5	98%	1	94 (7/3)	-	(4)
CCL	5	80%	4	-	96	-

By using new derivatives of PFL and CCL, we have been able to get a rapid and regioselective deacetylation of PAG in aqueous media, with accumulation of TAG's and without further hydrolysis to less acetylated compounds. In addition to the synthesis in almost quantitative yield of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose **2**, the selective cleavage in 4- or 6-positions was also achieved. This set of pure regioisomers of TAG could be important intermediates for the synthesis of complex glucopyranose derivatives.

Compared with the methods previously reported for the synthesis of 1,2,3,6-tetra-*O*-acetyl-α-D-glucopyranose **1** our synthetic approach have the following advantages: *1)* good yield of isolated product was obtained in only one step, by a very simple enzymatic hydrolysis; *2)* the use of toxic reagents and organic solvents is avoided; *3)* the very stable and active enzymatic catalyst employed[10] allows high product concentration to be obtained in a short reaction time.

The use of enzyme derivatives of PFL and CCL obtained by adsorption on octyl agarose resulted very important in order to improve the yield and the regioselectivity of these lipases in the hydrolysis of peracetylated glucopyranoses. The influence of the immobilization strategy on the lipases activity and regioselectivity will be matter of forthcoming publications.

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11. 7 gr. of immobilised lipase derivative were added to 100 mL of 10 mM glucose pentacetate solution in a mixture phosphate buffer-acetonitrile 15%. The reactions were run at pH 7, 25°C, and followed potentiometrically by using a pH-stat.
12. **1,2,3,6-tetra-*O*-acetyl-α-D-glucopyranoses (1)**. ^{1}H NMR in $CDCl_3$ (δ = ppm): 1.95-2.2 (4s; CH_3; 12H), 3.61 (t; J=9 Hz; 1H-*4*); 3.97 (ddd; J=9Hz; J=2.5 Hz; J=4 Hz; 1H-*5*), 4.25 and 4.56 (AB part of ABX system; $J^{1,3}$=2.5 Hz; $J^{1,3}$=4 Hz; $J^{1,2}$=12 Hz; 2H-*6*), 5.03 (dd; J=4 Hz; J=10 Hz; 1H-*2*), 5.34 (dd; J=10 Hz; J=9 Hz; 1H-*3*) 6.31 (d; J=4 Hz; 1H-*1*).
13. **1,2,3,4-tetra-*O*-acetyl-α-D-glucopyranoses (3)**. ^{1}H NMR in $CDCl_3$ (δ = ppm): 2.00-2.25 (4s; CH_3; 12H), 3.61 and 3.76 (AB part of ABX system; $J^{1,3}$=2.5 Hz; $J^{1,3}$=4 Hz; $J^{1,2}$=13 Hz; 2H-*6*) 3.95 (ddd; J=10 Hz; J=2.5 Hz; J=4 Hz; 1H-*5*) 5.05 (dd; J=3.5 Hz; J=10 Hz; 1H-*2*) 5.14 (t; J=10 Hz; 1H-*4*); 5.56 (t; J=10 Hz; 1H-*3*) 6.31 (d; J=3.5 Hz; 1H-*1*).