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REGIOSELECTIVE DEACETYLATION OF SECONDARY ACETYL ESTERS IN PERACETYLATED METHYL GLYCOPYRANOSIDES BY *ASPERGILLUS NIGER* LIPASE

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Summary : Peracetylated methyl β,α-D-glucopyranosides (**1,2**), methyl β,α-D-galactopyranosides (**3,4**) and methyl α-D-mannopyranoside (**5**) were transformed into the corresponding triacetates with one secondary hydroxyl group and diacetates with two secondary hydroxyl groups by the regioselective hydrolysis of *A. niger* lipase in 0.1M phosphate buffer/acetone solution.

Partially acetylated monosaccharides are very important and versatile in many ways. They are used as the intermediates in the synthesis of oligosaccharides and the preparation of *O*-substituted derivatives[1]; and are also as chiral building blocks in synthetic organic chemistry[2] and as reference compounds for the analysis of polysaccharides[3]. Direct chemical methods of deacylation of peracetylated monosaccharides were reported to possess low regioselectivity and the procedures were often necessary to involve either low-temperature operations or vigorous conditions and long reaction time[4]. Lipases have been widely used for the regioselective deacylation of polyhydroxyl molecules such as carbohydrates, steroids and glycols[5]. The enzymes have also been used for the deacylation and acylation of primary hydroxyl position of monosaccharides with high yields[6]. However, the secondary esters of peracylated methyl glycopyranosides hydrolyzed by lipases has not been investigated seriously.

In this communication, our major interest is to prepare partially protected monosaccharides with only one secondary hydroxyl group which can be used as glycosyl donors in the synthesis of oligosaccharides. Therefore, the deacylation of peracetylated methyl glycopyranosides **1**, **2**, **3**, **4**, and **5** by enzymatic hydrolysis was studied and all of the products were carefully analyzed. After screening various commercial lipases by HPLC analysis[7], *Aspergillus niger* lipase (Amano, Japan) showed a good biocatalyst for the study .

As previously reported, *A. niger* lipase generally cleaved the anomeric acetyl group of fully acetylated monosaccharides and had the capability to acylate the primary as well as secondary hydroxyl groups of glucose. *A. niger* lipase selectively deacetylate the anomeric position of 1,2,3,5-*O*-acetyl-β-D-ribofuranose and 1,2,3,5-*O*-acetyl-β-D-xylofuranose in a mixed solution

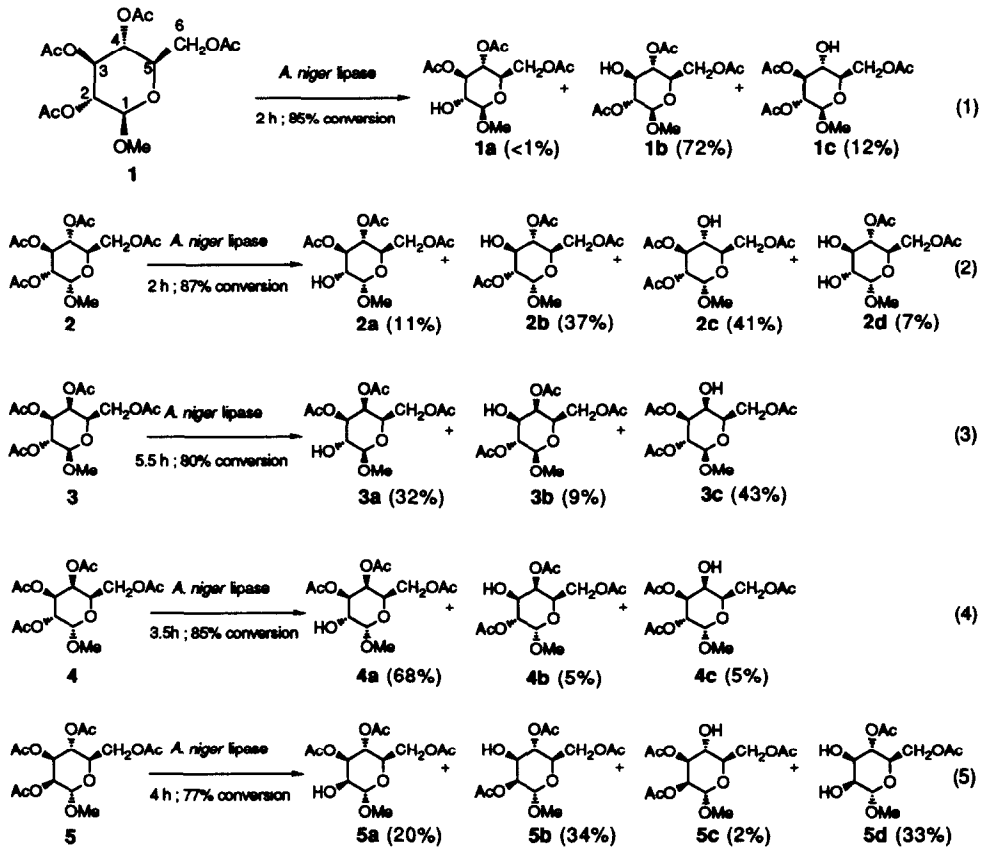
(DMF:0.1M phosphate buffer=1:10)[6b]. Also, *A. niger* lipase could convert 1,2,3,4,6-*O*-acetyl-β-D-glucopyranose to 2,3,4,6-*O*-acetyl-D-glucopyranose in 0.5 M phosphate buffer with high yield[8]. In acylation, *A. niger* lipase selectively acylate the 3- and 6-hydroxyl groups of n-octyl β-D-glucopyranoside[6c].

The deacylation experiments were performed at room temperature by dissolving substrates (1.5 g) and *A. niger* lipase (1 g) in a mixture (50 mL) of phosphate buffer (0.1 M)/acetone (9:1). The reaction was kept at pH 7.0 with 1 N NaOH(aq). After the substrate was consumed (checked by TLC using 5% MeOH in Et_2O as the developing solvent), the reaction mixture was terminated by extraction with EtOAc. Then, all the products were purified by silical gel column or by preparative HPLC.

All the structures of products were determined from the 2D ^1H-^1H COSY as well as 2D ^1H-^{13}C COSY NMR spectra.

Scheme 1 shows the results. *A. niger* lipase cleaved secondary esters exclusively. The 2-OH, 3-OH and 4-OH derivatives were generated from the hydrolysis of substrates (**1**, **2**, **3**, **4** and **5**). Due to the different configuration, each individual substrate produced different ratio of 2-OH, 3-OH and 4-OH derivatives. The reaction of **1** had high regioselectivity and obtained two products, 4-OH triacetate (**1c**) as minor one and 3-OH triacetate (**1b**) as major one. Compared with **1**, the hydrolysis of **2** was less selective and two major products, 3-OH triacetate (**2b**) and 4-OH triacetate (**2c**), were obtained in almost 1:1 ratio with less amount of 2-OH triacetate (**2a**) and 2,3-OH diacetate (**2d**). As for the hydrolysis of **3**, 2-OH triacetate (**3a**) and 4-OH triacetate (**3c**) were the major products with 3-OH triacetate (**3b**) as the minor product. Also, 2-OH triacetate (**4a**) was accumulated more than 3-OH triacetate (**4b**) and 4-OH triacetate (**4c**) in the deacetylation of **4**. *A. niger* lipase preferred to cleave the esters of C-2 and C-3 of **5** and gave 2-OH triacetate (**5a**), 3-OH triacetate (**5b**) and 2,3-OH diacetate (**5d**) with little 4-OH triacetate (**5c**).

Based on the results shown in **Scheme 1.**, the orientation of 1-methoxy and acetyl esters in the peracetylated methyl glycopyranosides had little influence on the deacetylation rate, but had large effect on regioselectivity. Unlike most lipases, *A. niger* lipase preferred to cleave the more hindered secondary esters of peracetylated methyl glycopyranosides. It indicated that *A. niger* lipase might possess unusual shape in active site that resulted in particularly different regioselectivity.



Scheme 1. Deacetylation of compounds **1-5** by *A. niger* lipase-catalyzed hydrolysis. The yields of the products were based on the conversion of substrates.

In conclusion, from the preparative point of view, *A. niger* lipase is quite suitable for the regioselective deacetylation of the 3-acetyl ester of **1** and the 2-acetyl ester of **4** to produce triacetates **1b** and **4a** with high yield, respectively. On the contrary, in the cases of **2**, **3**, and **5**, two or three triacetates are formed nearly quantitatively without remarkable regioselectivity. To enhance the regioselectivity of the enzymatic reaction by changing the reaction medium and modifying the acyl ester groups is under investigation.

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