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# Regioselective Reactions of Monosaccharides and Disaccharides by Enzymes

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Peracetylated mono- and disaccharides were regioselectively hydrolyzed by hydrolytic enzymes such as lipases and proteases; thus many partially acetylated mono- and disaccharides could be prepared by this way.

## INTRODUCTION

Carbohydrates contain multiple hydroxyl groups and stereocenters, and provide an abundant and inexpensive source of synthons for various applications in organic synthesis.[1,2] So far, many natural products and their unnatural analogues have been synthesized by using carbohydrate derivatives as chiral synthons. The preparation of carbohydrate derivatives requires selective manipulation of the multiple hydroxyl groups. Recently, hydrolytic enzymes mainly for lipases and proteases have been applied to the regioselective acylations and deacylations of readily available carbohydrates and their derivatives.[3]

## REGIOSELECTIVE ACYLATION

The enzymatic synthesis of triglycerides was reported.[4,5] Lipases which could hydrolyze or synthesize triglycerides was expected to show activity to synthesize carbohydrate esters. In 1984, the enzymatic acylation of sugars such as sucrose, glucose, fructose and sorbitol was reported; sugar esters were obtained from sugars and fatty acids by microbial lipase-catalyzed esterification in a buffer solution, but gave low yields.[6] Since then, enzymatic acylation of sugars was investigated carefully in varied conditions to enhance yields.[7-10] In order to suppress the equilibrium toward hydrolysis, enzymatic acylation was carried out in dry organic solvents with activated acyl donors such as 2,2,2-trihaloethyl esters.[7,10] Solvents with higher polarity have more dissolving ability toward sugar, but generally make enzymes inactive. Tetrahydrofuran, dimethylformamide (DMF), ethyl acetate and pyridine were found to be suitable for this reaction.[7-11] Enol esters such as vinyl acetate are excellent acyl donors for the displacement of the reaction toward products due to isomerization of the released enol to the unreactive ketone or aldehyde.[12] Most furanoses and pyranoses have highly regioselective acylation at primary hydroxyl groups which might be the least sterically hindered. C-2 or C-3 hydroxyl groups could also be acylated, if primary hydroxyl groups of monosaacharides were blocked.[8] Protease N (subtilisin) is active in anhydrous DMF. With the high dissolving potency of DMF, the primary hydroxyl groups of disaccharides, riboflavin, salicin, adenosine and uridine were regioselectively acylated.[9]

## REGIOSELECTIVE DEACYLATION

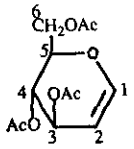
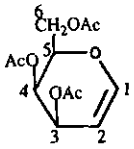
Enzymatic hydrolysis of polyacylated sugars yielded a mixture of products.[13-15] As these results gave a hint of poor regioselectivity of enzymatic hydrolysis reactions of this type were scarcely attempted for some years. Since that time, hydrolytic enzymes particularly for microbial lipases have been widely used in the preparation of chiral synthons and optically active compounds.[16,17] Furthermore, enzymatic hydrolysis of polyacylated sugars was reinvestigated. Obviously different from previous results, reactions of this type conducted by different enzymes showed high regioselectivity. The results are described in what follows:

### Enzymatic Hydrolysis of Peracylated Glycals

The deacylation of tri-*O*-acetyl-D-glucal by lipase-catalyzed ester cleavage in buffered solution afforded 4,6-di-*O*-acetyl-D-glucal in 90% yield.[10] In the case of tri-*O*-acetyl-D-galactal, the formation of two compounds, 4,6-di-

This review is invited to commemorate the sixtieth anniversary of the Chinese Chemical Society.

Table 1. Enzymatic Hydrolysis of Tri-*O*-acetyl-D-glucal and Tri-*O*-acetyl-D-galactal

| Substrates | Enzyme[a] | Reaction Solution | Site of Cleavage | Yield/% |
|---|---|---|---|---|
|  | lipase AK | phosphate buffer | 3-OH | 95 |
| | PPL | phosphate buffer | 3-OH | 35 |
| | | | 4-OH | 45 |
| | lipase GC | phosphate buffer | 3-OH | 60 |
|  | lipase AP6 | phosphate buffer | 3-OH | 10 |
| | | | 4-OH | 80 |
| | lipase FAP-15 | phosphate buffer | 3-OH | 10 |
| | | | 4-OH | 80 |

[a] The lipases were obtained from Amano, Japan; lipase AK (*Pseudomonas sp.*), lipase GC (*Geotrichum candidum*), lipase FAP-15 (*Rhizopus oryzae*) and lipase AP6 (*Aspergillus niger*). Porcine pancreas lipase (PPL) was from Sigma, USA.

*O*-acetyl-D-galactal and 3,6-di-*O*-acetyl-D-galactal was observed (Table 1). These two compounds rapidly interconverted with the migration of acetyl groups between C-3 and C-4 through a five-membered ring transition structure (Fig. 1). Because the C-3 and C-4 hydroxyl groups have the trans configuration, glucal derivatives suffer no such migration.[18] The results indicated that allylic esters were the main cleavage sites of peracylated glycals in lipase-catalyzed hydrolysis.

**Enzymatic Hydrolysis of Peracylated Furanoses**

According to the previous report,[11] several peracylated pentoses and peracylated methyl pentosides were hydrolyzed by lipases. Among the lipases tested, *Aspergillus niger* lipase (AP6 from Amano company; Japan) and *Candida cylindracea* lipase (*Candida* lipase from Sigma, US) provided large rates of hydrolysis leading to monodeacylated products (Table 2). The hydrolysis of methyl 3,5-di-*O*-acetyl-2-deoxy-$\alpha$-D-ribofuranoside by *Candida* lipase was not selective; both methyl 3-*O*-acetyl-2-deoxy-$\alpha$-D-ribofuranoside and methyl 5-*O*-acetyl-2-deoxy-$\alpha$-D-ribofuranoside were obtained in a 4:5 ratio. All other furanose derivatives tested showed 100% regioselectivity at C-1 (anomeric site) for peracylated furanoses and at C-5 (primary site) for peracylated methyl furanosides.

**Enzymatic Hydrolysis of Peracylated Pyranoses**

Regioselectivity in the hydrolysis of peracylated methyl pyranoside and peracylated pyranose by enzymatic hydrolysis is also shown mainly at primary esters (C-6) and anomeric ester (C-1), respectively.[11,19,20] In order to investigate the deacylation site of pyranose other than at the C-1 ester, 1-methyl peracetyl pyranoses such as methyl $\beta$-D-glucopyranoside (1), methyl $\alpha$-D-glucopyranoside (2), methyl $\beta$-D-galactopyranoside (3), methyl $\alpha$-D-galactopyranoside (4) and methyl $\alpha$-D-mannopyranoside (5) were introduced as substrates for the hydrolysis of different lipases. Among the various commercial lipases, lipase OF (*Candida cylindracea* from Meito Sangyo Company; Japan) and lipase AP6 have high rates of hydrolysis toward the substrates. Compounds **2**, **4** and **5** were regioselectively deacetylated by lipase OF to give the 6-OH derivatives as major products (> 90%) and 4-OH and 4,6-OH as minor products (about 3%) (Table 3). The generation of 4-OH and 4,6-OH derivatives does not mean that the enzyme directly cleaved the ester at C-4. Rather the acetyl group probably migrated from C-4 to C-6 with further hydrolysis of the newly formed ester at C-6 (Fig. 2). Although the C-6

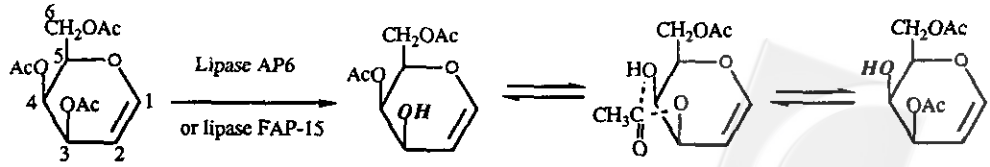
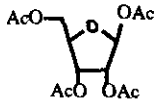
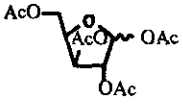
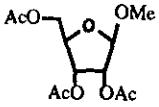
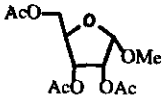
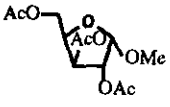
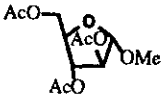
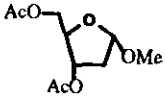
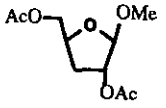



Fig. 1. The interconversion of 4,6-di-*O*-acetyl-D-galactal and 3,6-di-*O*-acetyl-D-galactal by the acetyl migration through a five-membered ring mechanism.

Table 2. Enzymatic Hydrolysis of Peracetyl Methylpentosides and Peracetyl Pentosides

| Substrates | Enzyme | Reaction Solution | Site of Cleavage | Yield/% |
|---|---|---|---|---|
|  | lipase AP6 | DMF/0.1 M phosphate buffer (1:10) | 1-OH | 63 |
|  | lipase AP6 | DMF/0.1 M phosphate buffer (1:10) | 1-OH | 50 |
|  | *Candida* lipase | DMF/0.1 M phosphate buffer (1:10) | 5-OH | 85 |
|  | *Candida* lipase | DMF/0.1 M phosphate buffer (1:10) | 5-OH | 96 |
|  | *Candida* lipase | DMF/0.1 M phosphate buffer (1:10) | 5-OH | 98 |
|  | *Candida* lipase | DMF/0.1 M phosphate buffer (1:10) | 5-OH | 98 |
|  | *Candida* lipase | DMF/0.1 M phosphate buffer (1:10) | 5-OH<br>3-OH | 40<br>50 |
|  | *Candida* lipase | DMF/0.1 M phosphate buffer (1:10) | 5-OH | 63 |

ester of **1** and **2** (or **3** and **4**) was the major cleavage site by lipase OF, **2** (or **4**) had not only a greater rate of hydrolysis but also greater regioselectivity than those of **1** (or **3**). The results showed that -$OCH_3$ in the $\beta$-configuration may somewhat hinder the ester of C-6 from entering the catalytic site of the enzyme to decrease the rate of hydrolysis. The low regioselectivity of **1** and **3** revealed that the rate of deacetylation in C-6 and the rate of acetyl migration C-4 → C-6 determine the amount of 6-OH, 4-OH and 4,6-OH derivatives in the reaction mixture.

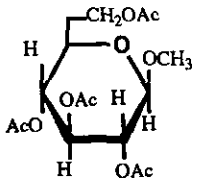
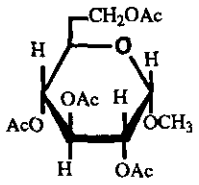
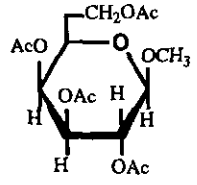
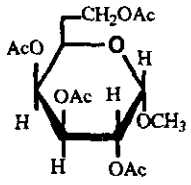
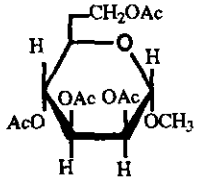
Unlike the regioselectivity of lipase OF, lipase Ap6 showed exclusive cleavage at secondary esters. The 2-OH, 3-OH and 4-OH derivatives were generated from hydrolysis of the five substrates (Table 3). Due to the varied configuration of the substrates, each individual substrate has a specific ratio of 2-OH, 3-OH and 4-OH derivatives produced from lipase AP6-catalyzed hydrolysis. The two lipases have complementary regioselectivity which could provide a efficient way to prepare partially acetylated monosaccharides.

**Enzymatic Hydrolysis of Peracylated Disaccharides**

**(a) Sucrose**

Octa-*O*-acetylsucrose contains three primary esters and five secondary esters but has no anomeric ester. The compound was reported to be subjected to chemical and enzymatic deacylation.[21-26] However, these methods generally deacylate the ester of C-4′, C-1′ and C-6′. The compound was regioselectively hydrolyzed by the lipase AK (*Pseudomonas sp*) in aqueous buffer and two hepta-*O*-acetylsucroses and two hexa-*O*-acetylsucroses were obtained by column purification.[27] After analysis of

Table 3. The Hydrolysis of Methyl β-D-Glucopyranoside (1), Methyl α-D-Glucopyranoside (2), Methyl β-D-Galactopyranoside (3), Methyl α-D-Galactopyranoside (4) and Methyl α-D-Mannopyranoside (5) Catalyzed by Lipase OF and AP6

| Substrates | Enzyme | Reaction Period/h | Site of Cleavage | Yield/% |
|---|---|---|---|---|
|  1 | lipase OF | 12 | 6-OH | 30 |
| | | | 4-OH | 11 |
| | | | 4,6-OH | 58 |
| | lipase AP6 | 2 | 4-OH | 12 |
| | | | 3-OH | 72 |
|  2 | lipase OF | 1.5 | 6-OH | 91 |
| | | | 4-OH | 3 |
| | | | 4,6-OH | 3 |
| | lipase AP6 | 2 | 4-OH | 41 |
| | | | 3-OH | 37 |
| | | | 2-OH | 11 |
| | | | 2,3-OH | 7 |
|  3 | lipase OF | 8 | 6-OH | 79 |
| | | | 4-OH | 9 |
| | | | 4,6-OH | 8 |
| | lipase AP6 | 5.5 | 4-OH | 43 |
| | | | 3-OH | 9 |
| | | | 2-OH | 32 |
|  4 | lipase OF | 2 | 6-OH | 92 |
| | | | 4-OH | 3 |
| | | | 4,6-OH | 3 |
| | lipase AP6 | 3.5 | 4-OH | 5 |
| | | | 3-OH | 5 |
| | | | 2-OH | 68 |
|  5 | lipase OF | 2.5 | 6-OH | 94 |
| | | | 4-OH | 3 |
| | | | 4,6-OH | 2 |
| | lipase AP6 | 4 | 4-OH | 2 |
| | | | 3-OH | 34 |
| | | | 2-OH | 20 |
| | | | 2,3-OH | 33 |

NMR spectra, the products were shown to be 3,4,6,1′,3′,4′,6′-hepta-*O*-acetyl-sucrose, 2,3,4,6,1′,3′,6′-hepta-*O*-acetylsucrose, 3,4,6,1′,3′,6′-hexa-*O*-acetylsucrose and 2,3,4,6,3′,6′-hexa-*O*-acetylsucrose. This was the first deacylation of the ester of C-2′ (Fig. 3).

In the hydrolysis of octa-*O*-acetylsucrose with lipase OF,[28] the major products at various durations of reaction were isolated. Their structures were analyzed; the pathway of the reaction appears in Fig. 4. With the different regioselectivity of lipase OF toward octa-*O*-acetylsucrose, the products showed that the position of deacylation were all in the glucosyl moiety. Lipase OF preferentially cleaved 6-ester to form 2,3,4,1′,3′,4′,6′-hepta-*O*-acetylsucrose and then convert to 2,3,6,1′,3′,4′,6′-hepta-*O*-acetylsucrose through 4 ⟶ 6 acetyl migration. The enzyme repeatedly cleaved the 6-ester to produce 2,3,1′,3′,4′,6′-hexa-*O*-

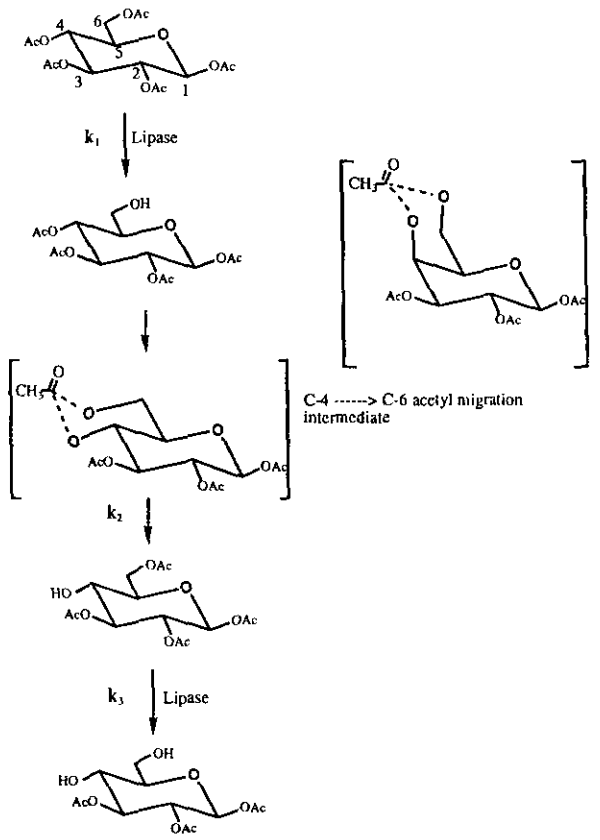



Fig. 2. Proposed mechanism of formation of 6-OH, 4-OH and 4,6-OH derivatives in lipase-catalyzed hydrolysis.

acetylsucrose. A trace of 2,6,1′,3′,4′,6′-hexa-*O*-acetylsucrose accompanied by 2,3,1′,3′,4′,6′-hexa-*O*-acetylsucrose was found. As there was no acetyl migration between C-3 and C-6, the formation of 2,6,1′,3′,4′,6′-hexa-*O*-acetylsucrose appeared to be produced directly from deacylation of the 3-ester of 2,3,6,1′,3′,4′,6′-hepta-*O*-acetylsucrose. Finally, the major pentaacetate, 2,1′,3′,4′,6′-penta-*O*-acetylsucrose was obtained by deacylation of the 6-ester of 2,6,1′,3′,4′,6′-hexa-*O*acetylsucrose or the 3-ester of 2,3,1′,3′,4′,6′-hexa-*O*-acetylsucrose. The yields of these products are varied, depending on the reaction period.

These results indicate that enzymatic deacylation of octa-*O*-acetylsucrose was versatile in regioselectivity. Several hydrolytic enzymes were introduced for the deacylation reaction;[29] the results are shown in Fig. 5. Deacetylation of octa-*O*-acetylsucrose with alcalase or protease N (subtilisin Carlsberg) gave the 2,3,4,6,3′,4′,6′-hepta-*O*-acetate as the initial major product with the 2,3,4,6,3′,4′-hexa-*O*-acetate as the subsequent main product. 2,3,4,1′,3′,4′,6′-Hepta-*O*-acetate was obtained by the action of lipase OF or lipase AP6, and the 2,3,4,6,1′,3′,6′- and 2,3,4,6,1′,3′,4′-hepta-*O*-acetate by the action of *Candida* lipase and chymotrypsin, respectively. The 2,3,6,1′,3′,4′,6′-hepta-*O*-acetate was formed from 2,3,4,1′,3′,4′,6′-hepta-*O*-acetate by acyl migration. The lipase of *Candida cylindracea* from various sources have

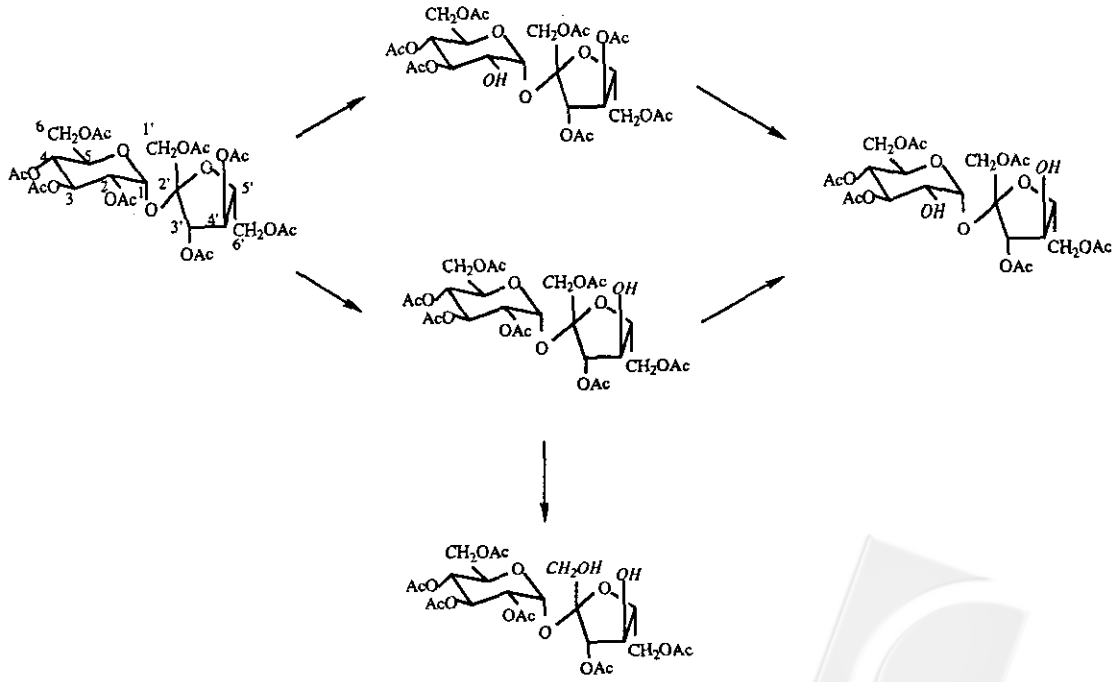


Fig. 3. Lipase AK-catalyzed hydrolysis of octa-*O*-acetylsucrose.

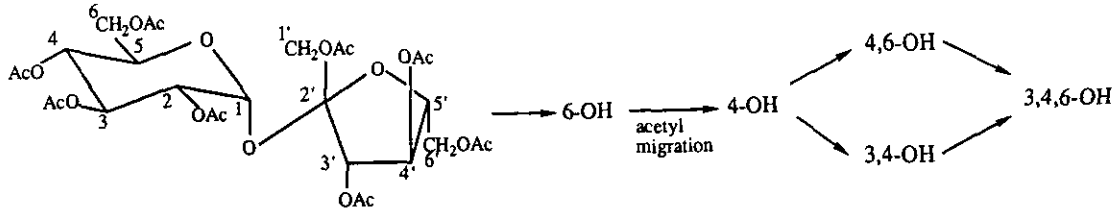



Fig. 4. Lipase OF-catalyzed hydrolysis of octa-*O*-acetyl sucrose.

variable regioselectivity.

**(b) Maltose/Cellobiose**

More than ten enzymes were examined for use in the hydrolysis of octa-*O*-acetyl maltose and octa-*O*-acetyl cellobiose.[30] Although the enzymes showed varied hydrolytic rates, all the enzymes tested had the same cleavage site on the anomeric esters of both compounds (Fig. 6). In order to demonstrate further the enzymatic cleavage on 1-ester, each product was reacted with *tert*-butyldimethylsilyl chloride. Because of steric hindrance, the protecting group tended to introduce into the equatorial position, which had less steric hindrance, to give 1-*tert*-butyldimethylsilyl-2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-maltose or 1-*tert*-butyldimethylsilyl-2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-cellobiose.

Partly because of greater accessibility and partly because of the anomeric effect, anomeric esters in the monosaccharides and disaccharide are the first target site for attack by enzymes.

## APPLICATION AND PERSPECTIVES

Partially acylated mono- and disaccharides are useful compounds in various fields. For example, sucrose esters are potentially important as emulsifiers in cosmetics, medicines and food preservatives.[31] Furthermore, naturally occurring sucrose esters secreted from the glandular trichomes of the foliage of many wild potato species have special biological functions to entrap arthropod pests.[32] Glucose derivatives are converted chemically to other useful compounds such as 6-deoxy-6-fluoro-α-D-glucose and 6-*O*-metylglucose and also can be suitable monosaccharides units for synthesis of oligosaccharides[20].

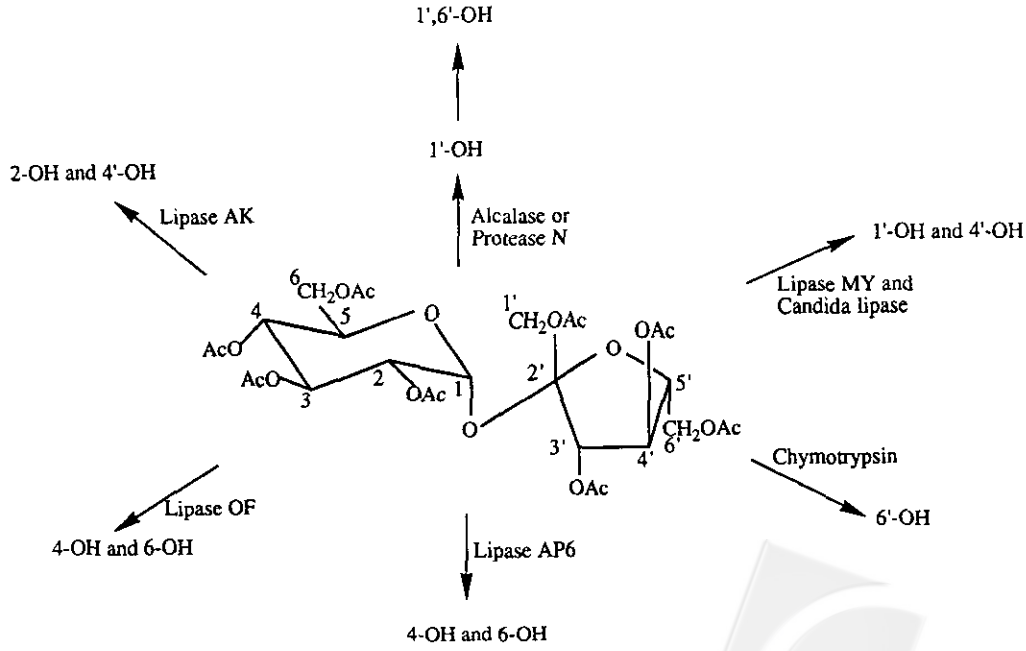



Fig. 5. Location of acetyl groups cleaved by various enzymes in the hydrolysis of octa-*O*-acetylsucrose.

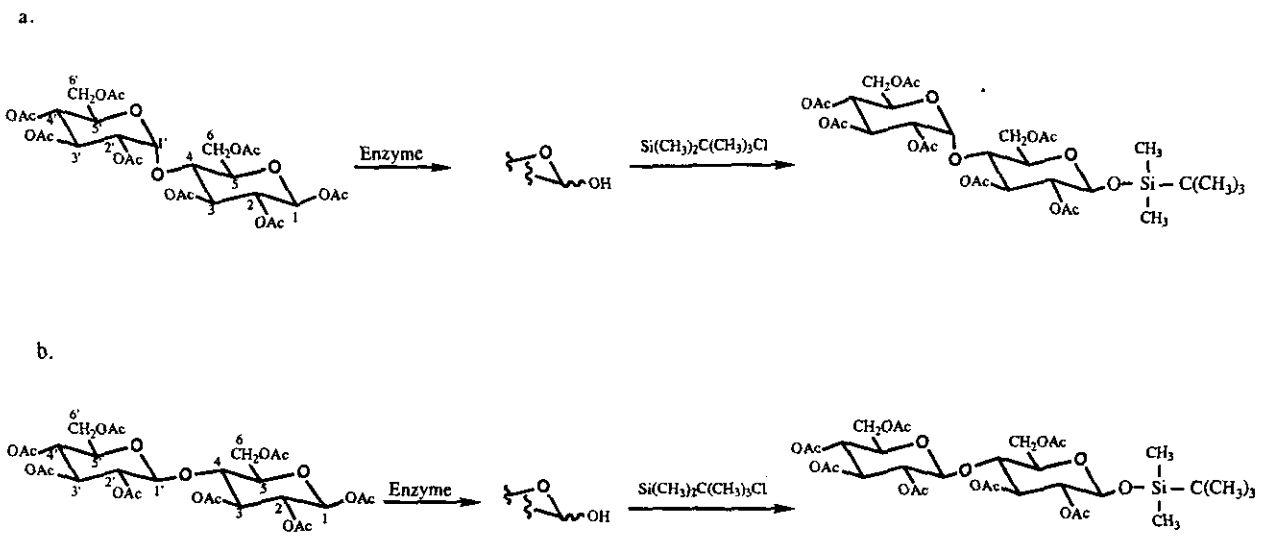



Fig. 6. Enzymatic hydrolysis of maltose octaacetate (a) and cellobiose octaacetate (b).

2,3,6,3',4'-Penta-*O*-acetyl sucrose, the precursor of sucralose, was prepared from the sequential hydrolysis of sucrose octaacetate by alcalase and lipase AP6[33] (Fig. 7).

Partially acylated sugars were prepared by several chemical approaches. They were synthesized from trityl ethers and acetals of sugars as precursors or alkaline treatment of peracetylated sugars.[23-26] Relative to the chemical methods, enzymatic methods have several advantages: due to the greater regioselectivity of enzymatic reactions, mixtures of products are less complex; the products are more readily purified and obtained in better yields; the procedures are simpler and more efficient. As described above, enzymatic acylation and deacylation is a practical method to prepare certain carbohydrate derivatives which are difficult to prepare chemically.

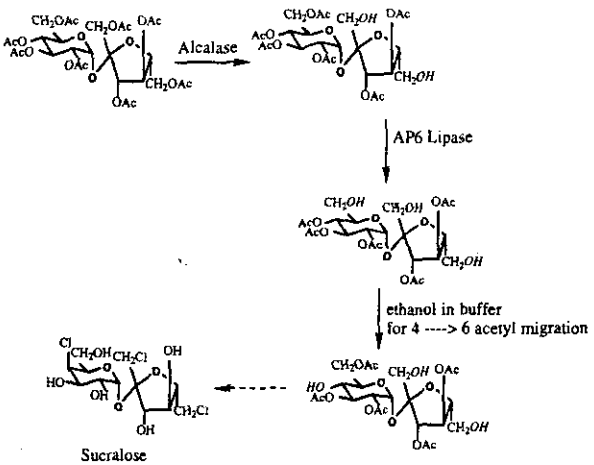



Fig. 7. Preparation of 2,3,6,3',4'-penta-*O*-acetyl sucrose, the precursor of sucralose, from sequential hydrolysis of sucrose octaacetate by alcalase and lipase AP6.

For enzymatic deacylation, substrates with longer acyl groups were generally hydrolyzed more rapidly by lipases and organic cosolvents such as DMF enhanced selectivity and improved the solubility of the hydrophobic substrates. However, it is not well understood how the solvent influences the regioselectivity of enzymes and the intramolecular migration of acetyl groups.

Shaw and Liaw reported that replacement of water by alcohols for enzymatic alcoholysis gave improved yields and enhanced regioselectivity although the reaction was somewhat slower.[34] Therefore, changing the reaction conditions, particularly adding solvents to increase regioselectivity and yields, deserves to be studied carefully. From a practical point of view, the screening of new microbial lipases which possess higher regioselectivity and stability in aqueous-organic cosolvent solution for use in the regioselective hydrolysis of acylated glycosides is also worth pursuing.

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**Key Words**

Enzymatic hydrolysis; Regioselectivity and Sugar derivatives.

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