

Regioselective Enzymatic Acylation of Multi-hydroxyl Compounds in Organic Synthesis

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Abstract With current developments in enzyme-catalyzed reactions and techniques available for rational redesign of natural biocatalysts, the enzymatic biosynthesis can become one of the most valuable synthetic methods. Enzymatic regioselective catalysis in organic media has played a key role in pursuing asymmetric synthesis for active chiral compounds. Here, we shortly describe some historical issues of the rapidly growing area, enzymatic catalysis in synthetic organic chemistry and then review researches that have been carried out in the regioselective enzymatic catalysis for the past two decades. An application of this technology to the modification of some potential target drug compound will be also presented.

Keywords: enzymatic synthesis, regioselective synthesis, sugar acylation, glycoside, drug modification, jineol

INTRODUCTION

Enzymes occupy a unique position in synthetic chemistry and have been widely used as catalysts in organic synthesis. The stereoselectivity and regioselectivity, together with available techniques for low cost production of enzymes and rational alteration of their properties, make enzymes attractive as synthetic catalysts. Environmental concerns and the regulatory constraints imposed on chemical and pharmaceutical industries have also stimulated the use of biological methods, which may offer clean and mild synthetic processes. In the first part the present article shortly summarizes historical aspects of enzymatic catalysis in synthetic organic chemistry, focusing on the use of cell-free enzymatic catalysts in organic solvents.

A dominant use of biocatalysis in organic media has involved stereoselective transformation, primarily for the synthesis of enantiopure chiral drugs over the past two decades. Chiral drugs continue to be a significant force in the global pharmaceutical market place because the efficient and economical building of enantiopure compounds is most important in the pharmaceutical industry [1]. Hydrolytic enzymes have been widely applied for the preparation of enantiopure compounds from racemic pairs, prochiral- or meso-compounds, or diastereomeric mixtures [2-6]. The general premise behind enzymatic resolution is that the enzyme esterifies (or hydrolyzes) only a single enantiomer of racemic substrates, which provides a means of separation [7,8].

Along with stereoselectivity, enzymes also possess unparallel regioselectivity. Particularly the enzymatic regioselective acylation of sugars offers an alternative to the poor selectivity of chemical synthesis due to the abundance of hydroxyl groups in sugar molecules and the similar reactivity of most of them [9-16]. The present article will review and analyze enzymatic regioselective transformations in organic synthesis. We will limit our discussion to regioselective acylation of multi-hydroxyl compounds such as sugars catalyzed by enzymes in organic solvents and will highlight selected applications for drug modifications. The application of this technology to a promising target drug compound will be also presented.

History of Enzymatic Catalysis in Organic Solvents

The use of enzymes in organic media is now very common, but the recognition of these systems is relatively quite recent. The root of this research field lies in the early 1980s of the renowned papers by Zaks and Klivanov [17,18]. Even though the field took off rapidly in the 1980s, the earliest reports already appeared over 100 years ago [19]. In the 1930s various aspects of lipase catalysis in organic media were investigated by a Polish scientist Ernest Aleksander Sym [20,21]. However, no industrial applications of enzymes in organic media were achieved until the 1980s [22,23]. Recently, Halling and Kvittingen [24] pointed out these facts and raised a question "why did biocatalysis not take off in the 1930s?" and immediately Klivanov [25] has endeavored to answer the question. In Klivanov's summary, he persisted that the biocatalysis in organic media did not take off in the 1930s by proposing the following two reasons in answer to the title's question: (1) 'the enabling scienti-

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Table 1. Enzymes commonly used in organic synthesis (Adapted from Koeller & Wong 2001 [40])

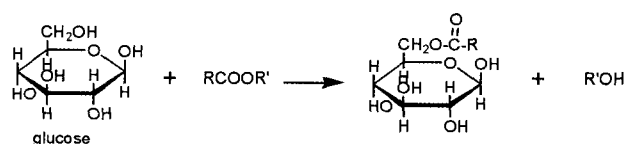
Enzymes	Reactions
Esterase, lipase	Ester hydrolysis, formation
Amidases (proteases, acylases)	Amide hydrolysis, formation
Dehydrogenases	Oxidoreduction of alcohols and ketones
Oxidases (mono- and dioxygenases)	Oxidation
Peroxidases	Oxidation, epoxidation, halohydrate
Kinases	Phosphorylation (ATP-dependent)
Aldolases, transketolases	Aldol reaction (C-C bond)
Glycosidases, glycosyltransferases	Glycosidic bond formation
Phosphorylases, phosphatases	Formation and hydrolysis of phosphate
Sulphotransferase	Formation of sulphate esters
Transaminases	Amino acid synthesis (C-N bond)
Hydrolases	Hydrolysis
Isomerases, lyases, hydratases	Isomerization, addition, elimination, replacement

fic advance of the mid-1980s that typical, ordinary enzymes can function in organic media was not made. Those lipases can do so must have been seen as a mere extension of their natural abnormal reaction habitat (lipid-water interfaces); and (2) 'at that time there was no demand for enantiopure compounds'.

No matter whose argument is more supported by historical evidence, both the key scientific breakthrough and the compelling practical need appeared in the mid-1980s, thus the enzyme action in organic and other low-water media has now become a very popular area of researches. Besides the need for enantiopure compounds, the technological demand for regioselective catalysis urged the technology to be established as it is. Therefore, there are many enzymes commonly used in organic synthesis as shown in Table 1.

Regioselective Transformations Catalyzed by Enzymes in Organic Solvents

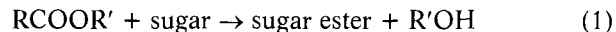
Enzymes are remarkable catalysts with unparalleled positional (regio-) selectivities. As a result, biocatalysts can be used in both simple and complex transformations without the need for tedious blocking and deblocking steps common in regioselective organic synthesis. Such a high selectivity also affords the efficient reactions with few by-products, thereby making enzymes an environmentally friendly alternative to the conventional chemical catalysts.

**Fig. 1.** Regioselective acylation of monosaccharide at primary hydroxyl group.

Regioselective Acylation of Sugars

Modification of only one out of several identical functional groups in a molecule is one of the central themes of modern organic synthesis. A representative and important example is the regioselective acylation of hydroxyl groups in sugars [9-16] because even discrimination between primary and secondary hydroxyls usually involves multistep procedures with conventional synthetic scheme.

Enzymatic transesterifications in organic solvents have proven to be extremely powerful for regioselective transformations, and many lipases and proteases have been successfully utilized for a wide range of regioselective transformations of sugar compounds. The widely employed strategy is enzymatic transesterification as shown in equation 1, where R is an aliphatic or aromatic moiety and R'OH is usually trichloroethanol [9,11,13] or trifluoroethanol [14,26,27].



Vinyl esters have been also found to be useful as acylating agents for enzyme-catalyzed regioselective acylation of a number of hydroxyl compounds [12,28,29]. The alcohol freed from the transesterification rapidly tautomerizes to volatile acetaldehyde or acetone, making the process irreversible and simpler for product isolation.

Many researchers [9,11,16] established that various unprotected monosaccharides could be acylated in anhydrous organic solvents at the primary hydroxyl group in a regioselective manner as illustrated for glucose in Fig. 1. Wong's group [12] confirmed this enzymatic regioselectivity in the acylation of sugars, including numerous furanose and pyranose derivatives.

Regioselective acylation of secondary hydroxyl groups in sugars was also demonstrated by Therisod and Klibanov [10]. Several commercially available lipases catalyzed transesterification reactions between activated ester and monosaccharides with blocked C-6 hydroxyl groups (enzymatically acylated or chemically alkylated) in organic solvents. Lipases exhibited a remarkable selectivity by discriminating among the four available secondary hydroxyl groups in C-6 protected glucose, galactose, and mannose. The C-2 or C-3 hydroxyl group was exclusively acylated depending on the enzyme. This positional specificity of lipases was used for either fully enzymatic or chemoenzymatic preparative synthesis of C-2 or C-3 monoesters of glucose, not easily attainable by chemical methods. The resultant C-2 and C-3 esters of C-6 protected sugars could afford C-2 and C-3 monoesters fol-

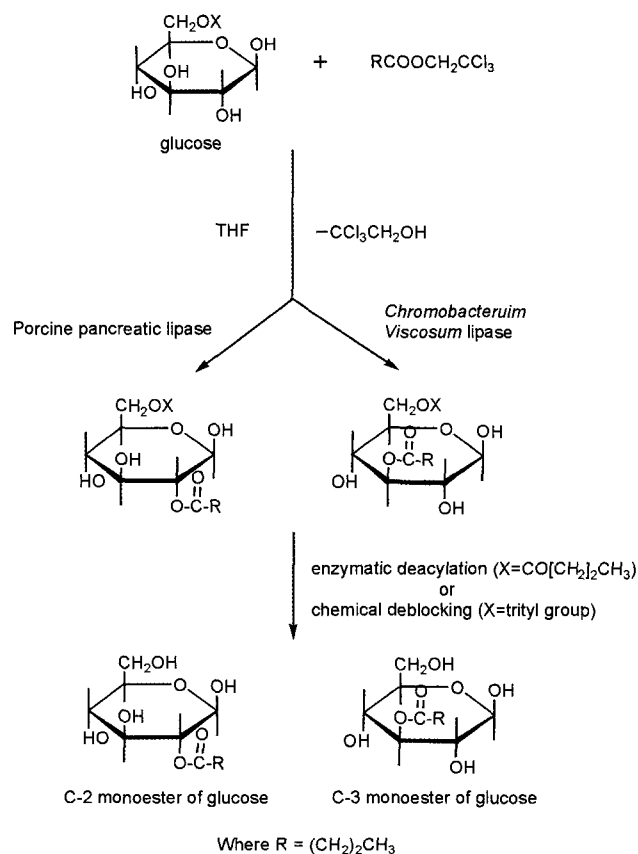


Fig. 2. Enzymatic or chemoenzymatic synthesis of C-2 or C-3 monoesters of glucose.

lowing either enzymatic or chemical deprotection as shown for glucose in Fig. 2. Using a similar strategy with the lipase from *Pseudomonas cepacia*, various 3-*O*-acyl derivatives of pyranose compound were prepared by Pulido and Gotor [15].

By combining the regioselective acylation of disaccharide and subsequent enzymatic hydrolysis of the disaccharide ester product, the monosaccharide ester with its ester bond in a specific position could be prepared [14]. Fig. 3 shows the strategy for the synthesis of 1-*O*-fructose using subtilisin and yeast α -glucosidase as catalysts.

Monoesters or diesters of disaccharides such as sucrose have been also prepared by enzymatic transesterification reactions. Taking sucrose as an example, preferential acylation of sugar primary hydroxyls over secondary hydroxyls was observed and among 3 different primary hydroxyls (1'-OH, 6'-OH, and 6-OH), enzymes most preferably acylate at the 1'-position [11, 14,27,28,29]. Distinctly from chemical acylation, enzymes often differ in their regioselectivities. For example, many enzymes including subtilisin Carlsberg [11], Optimase M-440 (an alkaline protease from *Bacillus licheniformis*) [28], and Proleather (an alkaline protease from *Bacillus* sp.) [27] preferentially acylates sucrose at the 1'-OH whereas lipase from *Pseudomonas cepacia* [29] acylates primarily at the 6-OH (Fig. 4). For the subsequent acylation of the sucrose

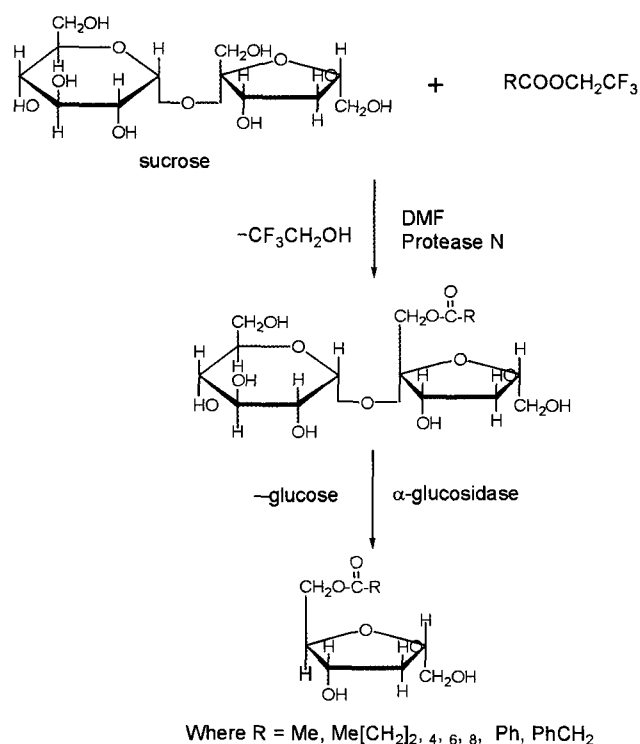


Fig. 3. Enzymatic synthesis of 1-*O*-fructose ester.

monoester, the second acylation site might be different depending on the enzyme used. Optimase M-440 yielded the diester mixture of sucrose 6,1' diester and 6',1' diester whereas Proleather [27] gave only sucrose 6,1' diester. In addition to enzyme itself, control over enzymatic regioselectivity could be achieved by rational choices of substrate and solvent [29].

The regioselective acylation of sugar alcohols provides more uses of these common and abundant raw materials for the synthesis of biosurfactants, sweeteners, food ingredients, and chemical and pharmaceutical intermediates. For instance, primary monoesters of sugar alcohols and fatty acids were prepared from transesterification or esterification reactions catalyzed by some lipases. These enzymatically prepared sugar alcohol esters have been found to be excellent surfactants [30].

(Chemo)enzymatic Synthesis of Sugar-containing Polymers

Enzymatic regioselective acylation could be used for the synthesis of sugar-containing polymers [26,27,31,32]. For instance, mono-acryl derivatives of sugar compounds have been synthesized using enzymes as site-specific catalysts. Such products could be used as sugar-containing monomers polymerizable through well-established free radical methods [31]. The final polymers have the sugar moiety as a side chain attached to their backbone.

The high degree of positional selectivity provided by the enzyme also enables sugars such as sucrose to react as if it were a diol compound. Recently, we have reported the

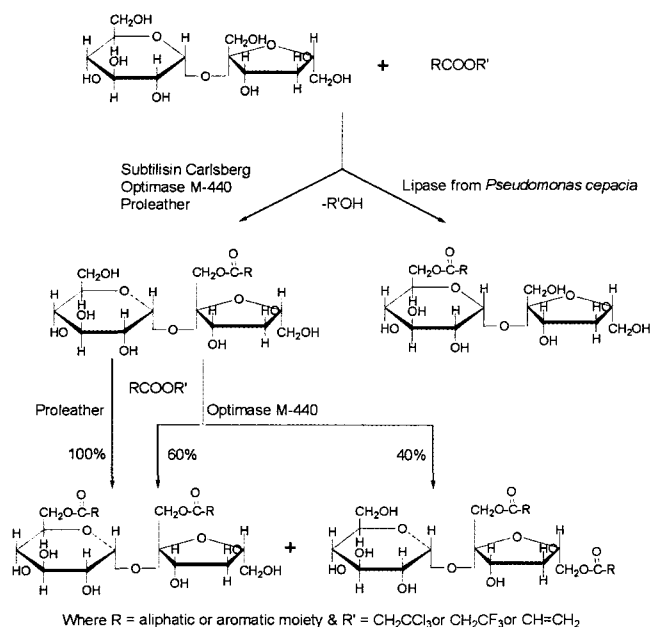


Fig. 4. Regioselective synthesis of sucrose monoester and sucrose diester.

synthesis of sugar-containing aromatic polymers by a chemoenzymatic method [26] using an alkaline protease from *Bacillus licheniformis* (Fig. 5). The key step in the chemoenzymatic method for the synthesis of sugar-containing aromatic polymers is a highly selective enzymatic transesterification of a sugar with aromatic diesters and the protease was found to efficiently catalyze the transesterification of aromatic diesters and sugar alcohols with a high regioselectivity. In this way, the regioselectivity of the enzyme can be used for the construction of sugar-based polymers having highly regular structures. The incorporation of sugars into traditional polymers such as polyesters [26,27,32], polyamides, phenolic polymers [33], and polyacrylates [31], may significantly extend the application of such materials into areas such as catalysts for organic synthesis and hydrogels. Also, polymers bearing sugar residues might be of great importance for pharmacological applications where the sugar groups play an important role.

Enzymatic Modification of Natural Glycoside Compounds

Glycosides of various classes of natural products are widely distributed in nature, where they are often esterified with aliphatic and aromatic acids at specific OH's of their sugar moieties. Many of these compounds are pharmacologically important molecules or possess other interesting properties. For instance, ginsenosides (Fig. 6) are therapeutic oligoglycosides isolated from the water-soluble portion of dried roots and leaves of *Panax ginseng* C. A. Meyer, a plant widely used in traditional Korean and Chinese medicine. Recently, a careful examination of white ginseng extracts has revealed that some

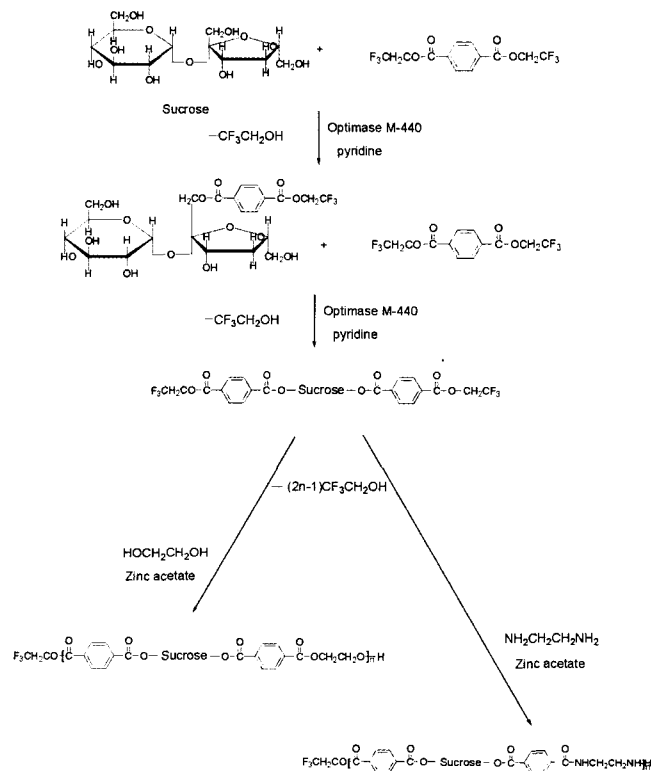


Fig. 5. Chemoenzymatic synthesis of sucrose-containing aromatic polymers.

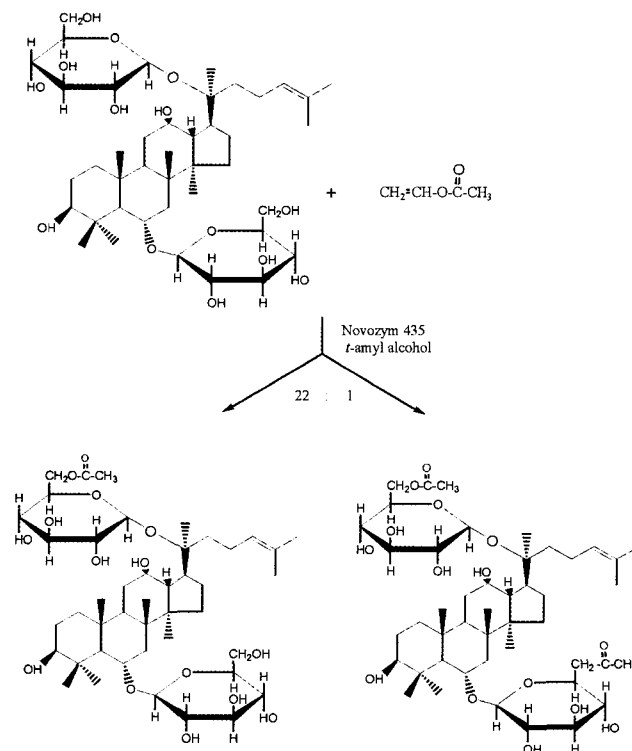


Fig. 6. Enzymatic modification of ginsenoside Rg₁.

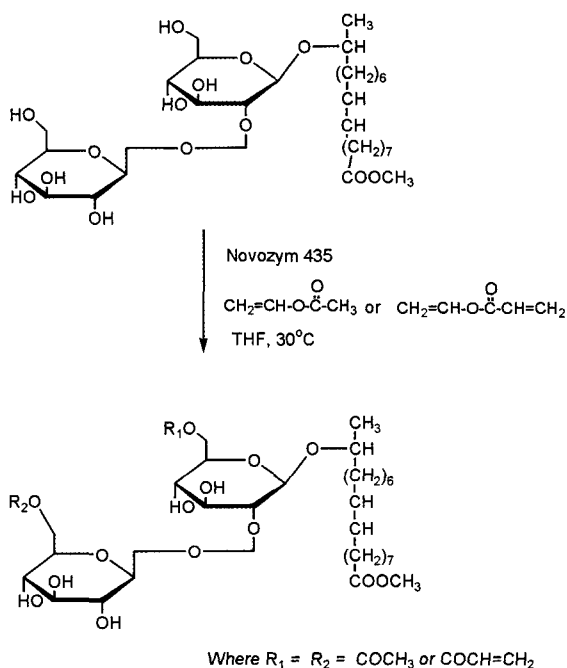


Fig. 7. Regioselective acylation of sophorolipids.

ginsenosides are present as monoesters of malonic acid, with the acylation site occurring invariably at one of the primary hydroxyls of the sugar moiety. These carboxy-acetyl ginsenosides behave as acidic saponins and, besides being more soluble in water than the ordinary glycosides, they also cause a remarkable increase of the solubility of the other ginsenosides [34].

Riva *et al.* [35] examined the behavior of some of these glycosides towards enzymatic acylation. For example, the lipase B from *Candida antarctica* (Novozym 435) efficiently acylated ginsenoside Rg₁ with vinyl acetate in *t*-amyl alcohol. The two products were identified as 6'-*O*-acetyl- and 6',6''-*O*-diacetyl-ginsenoside Rg₁, respectively.

Recently, enzyme-mediated regioselective acylations of glycolipid was also reported by Kaplan *et al.* [36]. The same lipase was shown to be an efficient catalyst for acylation of sophorolipids esters. Sophorolipids are microbial extracellular surface-active glycolipids. The alkyl esters of sophorolipids were subjected to Novozym 435 catalyzed acylation in dry tetrahydrofuran (THF) with vinyl acetate and vinyl acrylate to diacyl derivatives. The reactions were highly regioselective, and exclusive acylation of the hydroxyl groups on C-6' and C-6'' took place (Fig. 7). The existing data on the use of glycolipids to treat very severe immune disorders is certainly promising and the enzymatically synthesized sophorolipid analogues would lead to a new family of glycolipids that may be important immunomodulators.

Other natural glycoside compounds with pharmacological properties include digitonin, colchicoside, and flavonoid glycosides, which could be also acylated in a regioselective manner using enzymes [34]. In addition to sugar or glycoside compounds, there are many challeng-

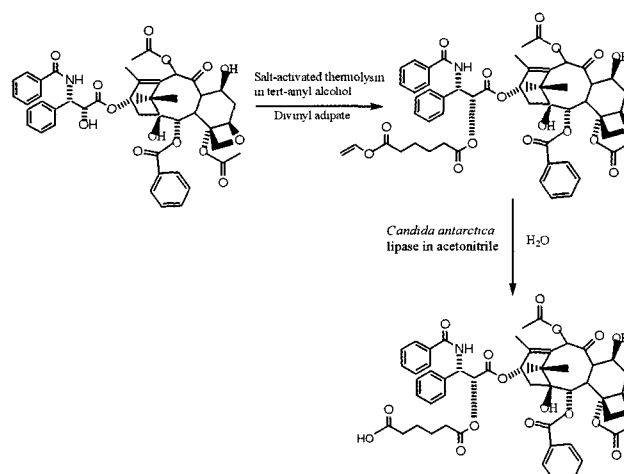


Fig. 8. Enzymatic synthesis of paclitaxel (taxol) derivatives.

ing hydroxy compounds including glycols, glycerols and steroids for regioselective acylation.

Enzymatic Regioselective Acylation for Drug Modifications

Enzymatic regioselective acylation could be profitably applied for the drug derivatives to improve the pharmacokinetic properties of drugs. One good example is the synthesis of water-soluble paclitaxel derivatives by enzymatic acylation [37]. Paclitaxel is a powerful antimetabolic agent that acts on promoting tubulin assembly into stable aggregated structures. Although paclitaxel has shown tremendous potential as an anticancer compound, its use as an anticancer drug is compromised by its poor water solubility. Therefore, a number of water-soluble paclitaxel prodrugs have been synthesized that contain hydrophilic or charged functionalities attached to specific sites on the paclitaxel molecule.

It was reported that acylation at the 2' position could be a very effective strategy for improving the water solubility of paclitaxel and the bacterial protease thermolysin was found to acylate selectively the 2'-hydroxyl of taxol in *t*-amylalcohol (Fig. 8). Yields of the 2'-acyl derivatives approached 100% using KCl-activated thermolysin. For the specific acylation with divinyladipate, a taxol 2'-vinyladipate was generated, which served as the acyl donor for *Candida antarctica* lipase-catalyzed hydrolysis of the terminal vinyl ester. The resulting taxol 2'-adipic acid derivative was nearly 1,700 times more soluble in water than the native taxol. In this case, taxol (not sugar compound) was served as a multi-hydroxyl acyl acceptor for the site-specific acylation.

Enzymatic Regioselective Glycosylation for Drug Modification

Like paclitaxel, some drug compounds have a major disadvantage of their limited solubility in an aqueous solution. Therefore, several attempts have been made to

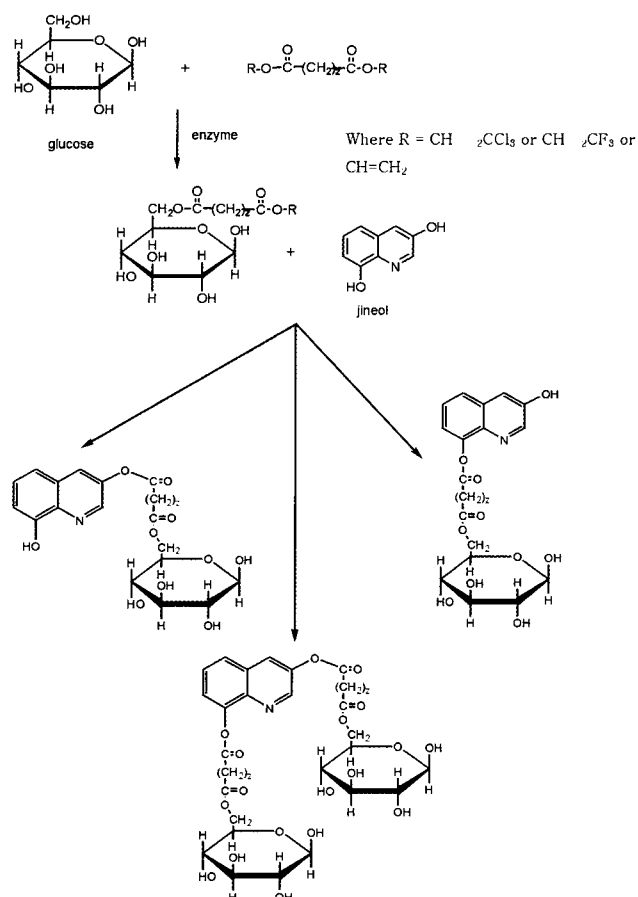


Fig. 9. Strategy for the regioselective synthesis of Jineol glycosides.

overcome this problem by modifying the drug compounds. One way to increase the water solubility of drugs is to conjugate the sugar moiety into the drugs in such a way that the sugar part serves as a hydrophilic component [38]. Moreover, the incorporation of sugar compounds into drugs could lead to reduction of the toxic effects of the drugs.

One of the promising and suitable target drug compounds for the regioselective synthesis of drug-glycoside, might be Jineol. Jineol is a quinoline compound, which has been recently found and can be obtained from *Scolopendra subspinipes* by conventional solvent extraction methods [39]. Jineol is found to have anticancer and anti-inflammatory activity. However, it has some limited water-solubility for wide clinical usage and enzymatic glycosylation can be designed for the synthesis of Jineol-glycoside. Jineol has two hydroxyl groups (3-OH & 8-OH) and the first thing to be done is to determine the pharmacophore of Jineol and screen or develop the enzyme with desirable site selectivity. The possible strategy is to glycosylate Jineol at the specific hydroxyl group that is not involved in pharmacophore of Jineol. Taking glucose as a model sugar compound, enzymatic site-specific transesterifications of Jineol are illustrated in Fig. 9, in

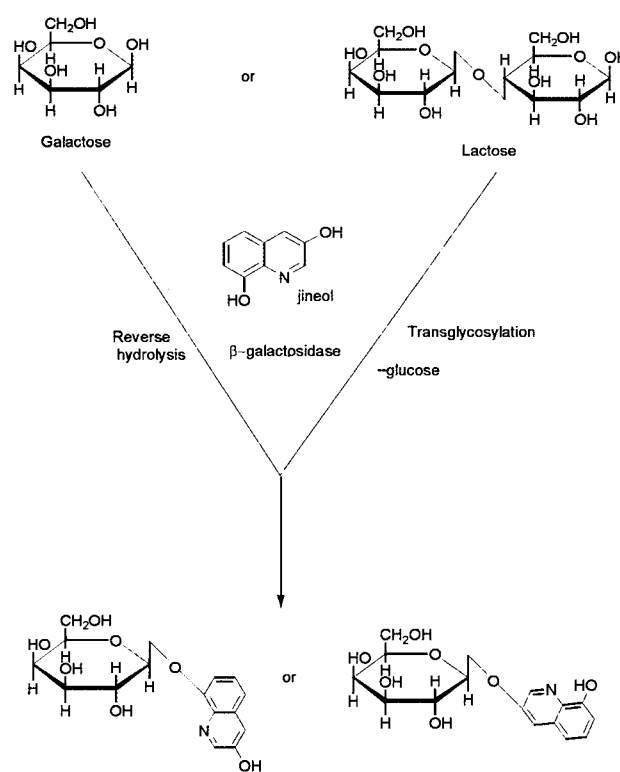


Fig. 10. β -galactosidase-catalyzed synthesis of Jineol glycosides.

which protease or lipases are used as catalysts. When both hydroxyl groups are proved not to be involved in the drug activity, both of them could be acylated for the dramatic improvement of the water-solubility. For this strategy, direct glycosylation or transglycosylation of Jineol can be also designed using other classes of enzyme such as glycosidase and glycosyl transferase (Fig. 10).

Besides, drug glycosides can be designed to reduce the side effect by site specific targeting with glycoside or insertion of some bonds such as ester susceptible to enzymatic cleavage may represent one approach to make the action of a drug more restricted to the site of application.

CONCLUSION

As understanding of enzyme-catalyzed reactions and related techniques continues to grow, the enzymes will expand the repertoire of organic synthesis. Especially, their special attractiveness in the areas of regioselective organic synthesis has become more apparent and they are expected to find predominant application in very highly value-added products such as pharmaceutically applicable agents such as anticancer and antiviral drugs. Furthermore, a well-designed combination of classical chemistry and enzymatic catalysis would significantly extend the synthetic utility of enzymes with their remarkable regioselectivity.

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