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Review

# In vitro synthesis of artificial polysaccharides by glycosidases and glycosynthases

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**Abstract**—Artificial polysaccharides produced by in vitro enzymatic synthesis are new biomaterials with defined structures that either mimic natural polysaccharides or have unnatural structures and functionalities. This review summarizes recent developments in the in vitro polysaccharide synthesis by *endo*-glycosidases, grouped in two major strategies: (a) native retaining *endo*-glycosidases under kinetically controlled conditions (transglycosylation with activated glycosyl donors), and (b) glycosynthases, engineered glycosidases devoid of hydrolase activity but with high transglycosylation activity. Polysaccharides are obtained by enzymatic polymerization of simple glycosyl donors by repetitive condensation. This approach not only provides a powerful methodology to produce polysaccharides with defined structures and morphologies as novel biomaterials, but is also a valuable tool to analyze the mechanisms of polymerization and packing to acquire high-order molecular assemblies.


*Keywords:* Glycosidases; Glycosynthases; Polysaccharides; Enzymatic synthesis; Biomaterials

**Contents**



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## 1. Introduction

Oligosaccharides and polysaccharides are key biomolecules in essentially all living organisms. They have multiple functions including serving as structural components of cell walls, energy storage, cell recognition, regulation of signaling, cell differentiation, cell proliferation, and immune responses.[1–4] The biosynthesis of naturally occurring oligo- and polysaccharides is a complex process that involves formation of glycosidic bonds between their constituent monosaccharide units and side chain modifications to produce specific functional group derivatizations. Glycosyltransferases, transglycosidases, and phosphorylases are the enzymes responsible for glycosyl bond formation, whereas accessory enzymes such as estearases, epimerases, and sulfotransferases modify the carbohydrate structures to produce the functional biomolecules.[4–7]

Polysaccharides are highly diverse in structure and biological functions. They are essential for many fields of research, for example, for biochemical studies in glycobiology, as potential drugs directed to enzymes or receptors involved in their function and metabolism, and as advanced materials due to their biocompatibility, structure-forming capacity, and environmentally benign properties.[8–13] The development of efficient synthetic methodologies for their preparation has therefore been in high demand. Methods for both chemical and enzymatic syntheses have experienced notable advances in the last decade with the aim of producing either polysaccharides resembling the natural products or novel polysaccharide mimetics for biomedical applications and for biomaterials development.

Chemical synthesis has evolved greatly because improved glycosyl donors and advanced synthetic methodologies have been developed (recent reviews[14–18]). However, perfect control of the regio- and stereochemistry of glycosylation is still a difficult problem, and the synthesis of complex oligo- and polysaccharides is often limited to the milligram scale and difficult to scale-up. Enzymatic synthesis is an alternative that overcomes these limitations because of their high catalytic activity, lack of undesirable side-reactions, mild reaction conditions, and high regio- and stereoselectivity.[19–22]

In nature, glycosyltransferases (GT) are responsible for glycoside bond formation. They catalyze the transfer of a monosaccharide from a sugar nucleotide donor (in Leloir GTs) to an acceptor, acting processively in homopolysaccharide biosynthesis or in combination with other GTs to produce heteropolysaccharides. Although the use of glycosyltransferases is still limited as a general tool for in vitro carbohydrate synthesis, a number of recombinant enzymes are available and different methodologies are being developed for large-scale synthesis. Good examples are the synthesis of hyaluran using the hyaluran synthase from *Streptococcus equisimilis* via an enzymatic process with coupled regeneration of the sugar nucleotide[23] or the use of engineered *Bacillus subtilis* cells expressing the hyaluran synthase enzyme and producing hyaluronic acid in the 1 MDa range.[24]

Glycosidases (GH) are degrading enzymes that catalyze the hydrolysis of glycosidic bonds, but their normal hydrolytic reaction can be reversed under appropriate conditions.[25] Therefore, glycosidases have been extensively studied as biocatalysts for oligo- and polysaccharide synthesis. They are stable enzymes, easy to produce, and a large number of enzymes from different organisms and with different specificities are available. In addition, the glycosyl donors required are cheap compounds and easy to obtain in a multigram scale.

Retaining glycosidases follow a double-displacement reaction mechanism via the formation and hydrolysis of a glycosyl-enzyme intermediate.[26–29] The canonical mechanism involves two steps with general acid–base catalysis: in the first step (*glycosylation*) the amino acid residue acting as a general acid protonates the glycosidic oxygen, while the deprotonated carboxylate functioning as a nucleophile attacks the anomeric center with concomitant C–O breaking of the scissile glycosidic bond leading to a covalent glycosyl-enzyme intermediate. The second *deglycosylation* step involves the attack by a molecule of water assisted by the conjugate base of the general acid residue, which renders the free sugar with overall retention of configuration, and the enzyme returns to its initial protonation state (Scheme 1). Under conditions that favor reversal of their normal hydrolytic reaction, retaining glycosidases have been extensively used as catalysts in oligosaccharide synthesis. This may be achieved either by displacing the equilibrium toward glycosidic bond formation (*thermodynamically controlled condensation*) or by using activated glycosyl donors (*kinetically controlled transglycosylation*).

This review focuses on recent developments on the enzymatic synthesis of polysaccharides by retaining *endo*-glycosidases. This refers to enzymatic polymerization of simple glycosyl donors by repetitive condensation (transglycosylation) to produce synthetic polysaccharides resembling either the natural products or mimetics with unnatural structures or functionalities. In vitro enzymatic synthesis not only provides a powerful methodology to produce polysaccharide derivatives with defined structures, but is also a valuable tool to analyze the mechanisms of polymerization and packing to acquire high-order molecular assemblies.

Two general strategies are currently under development:

(a) The use of wild-type glycosidases under kinetically controlled conditions (transglycosylation). The competing hydrolase activity is reduced by modifying the reaction conditions, and the insolubility of