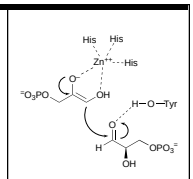
Enzymes in organic synthesis: recent developments in aldol reactions and glycosylations



CHEMICAL SOCIETY REVIEWS

Shuichi Takayama, Glenn J. McGarvey and Chi-Huey Wong*

Department of Chemistry and the Skaggs Institute of Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

Carbohydrates have not been as accessible as other biomolecules such as proteins and nucleic acids and are the least exploited. As a result of their highly asymmetric and densely functionalized nature, carbohydrates are difficult to synthesize using conventional chemistry. Enzymatic synthesis, however, with its high selectivity and mild reaction conditions is very useful for the preparation of carbohydrates. This review gives a brief overview of recent developments in the application of enzymatic aldol reactions and glycosylations to carbohydrate synthesis.

1 Introduction

Advances in efficient production of biomolecules have an impact on numerous fields of science. Among the available technologies, PCR (polymerase chain reaction), solid phase synthesis, over expression of proteins in microorganisms, and protein display have allowed scientists to routinely access nucleic acids and proteins. This has led not only to a deeper understanding of these classes of molecules, but also to new developments in various fields from chemistry and biology to medicine, materials science and even computing. A third class of biomolecules, the carbohydrates have proved less accessible and are far less explored. Though there has been much progress in the area of chemical carbohydrate synthesis,[1–3] it is still difficult to synthesize these highly asymmetric and densely functionalized compounds on a routine or large-scale basis using conventional chemistry. There are also no PCR equivalent replication systems or template-based expression systems available for carbohydrates. Enzymatic synthesis, however, with its high selectivity and mild reaction conditions is very useful for the preparation of carbohydrates. Advances in recombinant DNA technology has made it possible to express almost any enzyme including enzymes useful for carbohydrate synthesis and many of these enzymes are now commercially available for routine use in any laboratory. These developments are making carbohydrate structures more accessible, contributing to a deeper understanding of carbohydrate recognition and opening new opportunities in various fields of science. This review gives a brief overview of recent developments in the use of these enzymes in aldol reactions and glycosylations. Also included is the application to synthesis of bioactive compounds.

2 Aldolase-catalyzed synthesis of novel sugars

Over 30 aldolases have been identified and isolated so far, the majority of which catalyze the reversible stereospecific addition of a ketone donor to an aldehyde acceptor.[4–7] Mechanistically, two distinct classes can be recognized (Fig. 1). Type I aldolases form a Schiff-base intermediate in the active site with the donor

Professor Chi-Huey Wong received his BS and MS degree from National Taiwan University, and his PhD in Chemistry with George M. Whitesides from Massachusetts Institute of Technology. He then moved along with Professor Whitesides to Harvard University as a postdoctoral fellow for another year. He taught at Texas A&M University for six years and since 1989 has held the Ernst W. Hahn Chair in Chemistry at The Scripps Research Institute. His current interests lie in the area of bioorganic and synthetic chemistry, especially the development of new synthetic chemistry based on enzymatic and chemoenzymatic reactions and the rational development of mechanism-based inhibitors of enzymes and receptors.

Chi-Huey Wong

Professor Glenn J. McGarvey received his BS in Chemistry from the University of California at Santa Barbara then completed his PhD in Chemistry at the University of California at Davis under the direction of R. Bryan Miller. This was followed by two years of postdoctoral research at the California Institute of Technology in the laboratories of Professor Robert E. Ireland. He then took his present position on the faculty of the Department of Chemistry at the University of Virginia where his research interests to date have been broadly based in synthetic organic chemistry. Professor McGarvey is presently on leave from UVa and is a visiting scientist at The Scripps Research Institute.

Glenn J. McGarvey

Shuichi Takayama

Shuichi Takayama received his BS and MS degree from Tokyo University then entered the Graduate Program in Chemistry at the Scripps Research Institute for his PhD studies. He has been a graduate student in Professor Chi-Huey Wong's group since 1994. His research interest is in understanding the phenomena of life on a molecular level using chemical and enzymatic organic synthesis.

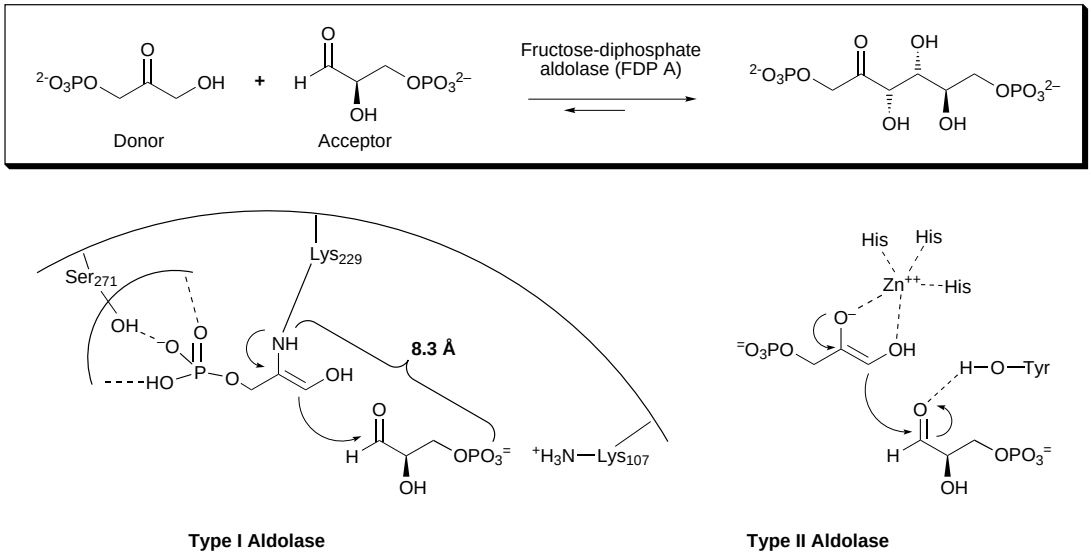


Fig. 1 Mechanism of type I and type II aldolases

substrate, which subsequently adds stereospecifically to the acceptor.[8,9] Type II aldolases use a Zn^{2+} cofactor, which acts as a Lewis acid in the active site.[10,11]

Although aldolases are quite specific for the nucleophilic donor component, a high degree of flexibility is allowed for the acceptor aldehyde component permitting the synthesis of various unnatural sugars. Aldolases can also be classified according to the donor component utilized [dihydroxyacetone phosphate (DHAP), pyruvate, acetaldehyde, or glycine]. Four dihydroxyacetone phosphate-dependent aldolases having complementary stereoselectivities have been cloned and overexpressed (Fig. 2), and three of them are commercially available (Table 1).

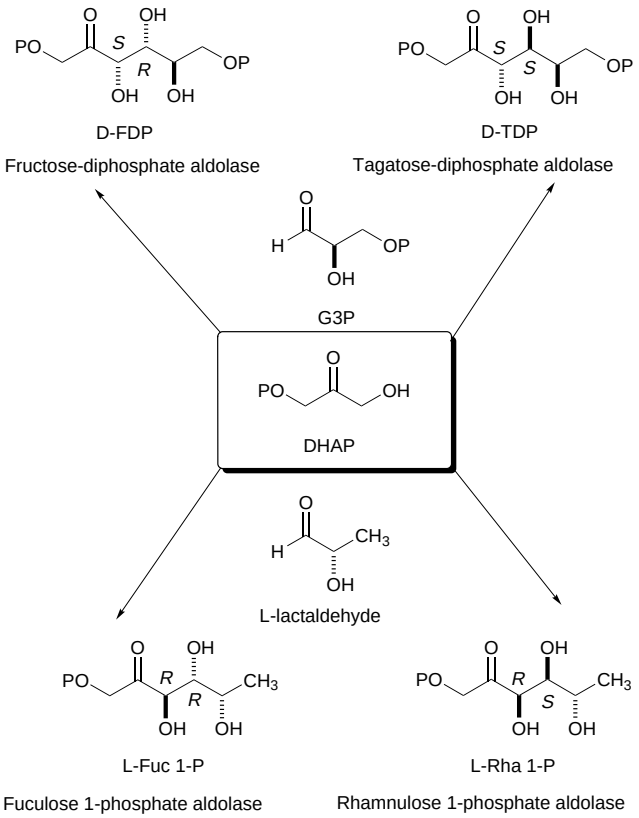


Fig. 2 Product stereochemistries generated by the four complementary DHAP aldolases

Table 1 Some commercial sources of aldolases, glycosyltransferases, and glycosidases[a]

Aldolase type enzymes	Supplier
Fructose-1,6-diphosphate aldolase Type I	Boehringer, Calbiochem, Fluka, Serva, Sigma, Worthington
Fructose-1,6-diphosphate aldolase Type II	Boehringer, Fluka, Sigma
Fuculose-1-phosphate aldolase	Boehringer
Rhamnulose-1-phosphate aldolase	Boehringer
N-Acetylneuraminic acid aldolase	Toyobo, Sigma, Boehringer
Transaldolase	Sigma
Transketolase	Sigma, Fluka
Glycosyltransferases	**Supplier**
β1,4-Galactosyltransferase	Boehringer, Calbiochem, Fluka, Sigma, Oxford GlycoSystems, Worthington
α2,6-Sialyltransferase	Boehringer, Calbiochem, Sigma
α2,3-Sialyltransferase	Calbiochem
α1,3-Fucosyltransferase	Calbiochem, Oxford Glycosystems
α1,2-Mannosyltransferase	Calbiochem
Glycosidases	

[a] Many variety of glycosidases from various sources (too many to list here) are available from the suppliers listed above as well as some other suppliers. Refer to the references for suppliers of specific glycosidases.

More than 100 aldehydes have been used as acceptor substrates for DHAP dependent aldolases to prepare monosaccharides.[4–7] DHAP dependent aldolases also catalyze the condensation of pentose and hexose phosphates with DHAP, consequently extending the sugar chain by three carbons while introducing two new stereogenic centres. This provides a route to novel high-carbon sugars which are difficult to obtain from either chemical synthesis or natural sources. A number of these compounds have been synthesized, including analogs of 2-keto-3-deoxyoctulosonate (KDO) [Fig. 3(*a*)]. When an appropriate dialdehyde is the substrate, *C*-disaccharide mimetics where two sugars are linked by carbon instead of oxygen, can be prepared by enzymatic tandem aldol reactions [Fig. 3(*b*)].[12] Pyruvate-dependent aldolases, such as neuraminic acid aldolase (NeuAc aldolase), have also been used extensively in synthesis.[4–7] An example is illustrated in the synthesis of analogs of *N*-acetyl neuraminic acid, a biologically important sugar involved in cell adhesion processes and viral infection [Fig. 3(*c*)].[13,14] These

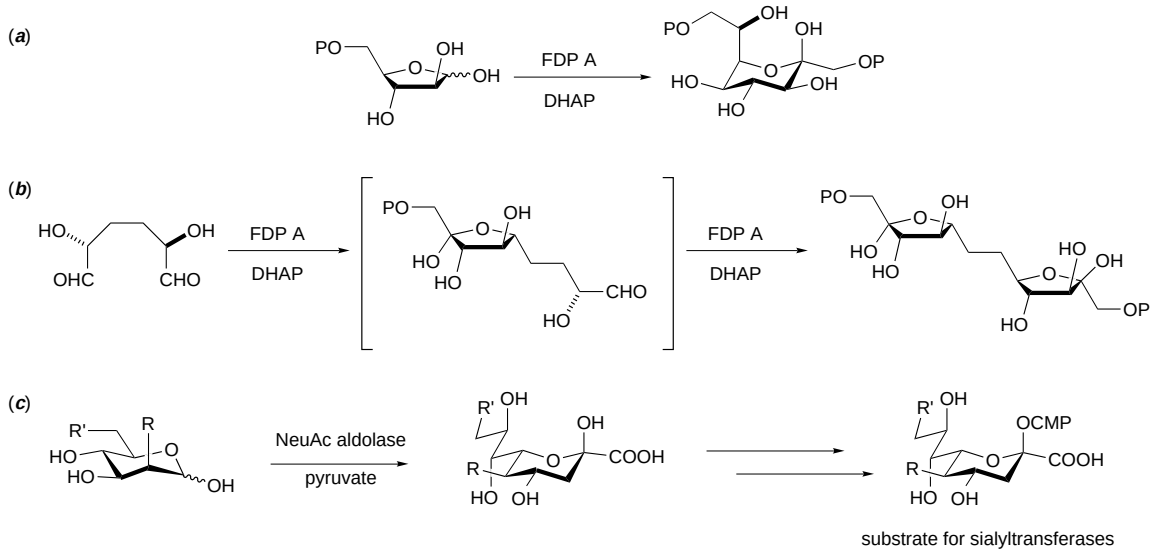


Fig. 3 Aldolase-catalyzed synthesis of novel high-carbon sugars

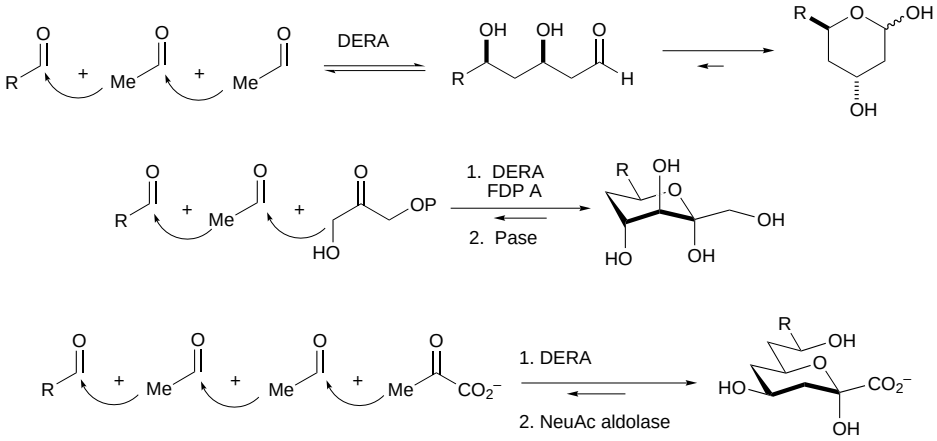


Fig. 4 Sequential aldol reactions catalyzed by DERA

unnatural neuraminic acids can be converted to their cytidine-5′-monophospho-derivatives (CMP-derivatives) that sialyltransferases accept as a substrate, providing a route to modified sialyloligosaccharides [Fig. 3(*c*)].[14,15]

Further study of enzymatic aldol reactions has led to the development of new sequential aldol reactions resulting in the combination of three or four substrates in one pot (Fig. 4).[16] The key to this reaction is the use of deoxyribose-5-phosphate aldolase (DERA) which gives aldehyde products that can serve as a substrate for further enzymatic aldol reactions. With the increasing understanding of the specificity of various aldolases, these sequential aldolase reaction processes are expected to find use in the synthesis of various uncommon monosaccharides. The enzymatic aldol reaction of azido-aldehydes have been used to prepare imino-sugars [Fig. 5(*a*)].[4–7,17] Azido-sugars prepared by aldolase-catalyzed reactions can be converted to cyclic-imine sugars by reduction under strongly acidic conditions followed by neutralization, or to iminocyclitols by Pd, Pt, or Rh-catalyzed hydrogenation in which the reduction of azide to amine, rearrangement to imine, and reductive amination takes place in one pot.[17] Thio-aldehydes have been used to prepare deoxythiosugars [Fig. 5(*b*)].[4] When nitroaldehydes are used as substrates, the enzymatic products undergo an intramolecular nitroaldol reaction to give nitrocyclitols [Fig. 5(*c*)].[4] When phosphonate-containing aldehydes are used as substrates, the products spontaneously undergo a Horner–Wadsworth–Emmons olefination to give another type of cyclitol [Fig. 5(*d*)].[4]

Transketolases and transaldolases are commercially available enzymes that have also been used for enzymatic aldol type reactions.[6,7] Highly efficient overexpression and large-scale synthesis of carbohydrates have been reported with transketolase.[18] There are other enzymes that are not classified as aldolases but that catalyze aldol type reactions (often classified as synthases and transferases). These enzymes are increasingly important but will not be mentioned in this article because of space limitations and because they have not been used very much in carbohydrate synthesis. Antibodies that catalyze aldol reactions have also been made.[19]

3 Enzymatic synthesis of oligosaccharides, glycopeptides and glycoproteins

3.1 Glycosyltransferases

In vivo, oligosaccharides are synthesized sequentially, in a one-linkage one-enzyme fashion, by a variety of glycosyltransferases that catalyze the transfer of sugars from an activated species, such as sugar nucleotides, to a growing oligosaccharide chain. This contrasts with the formation of proteins and nucleic acids which are synthesized by a single biocatalytic machinery that forms all linkages according to a template. Due to advances in recombinant DNA technology, many of the glycosyltransferases are now available in large quantities for the *in vitro* synthesis of various oligosaccharides. Coupled with the regeneration of sugar nucleotides, these

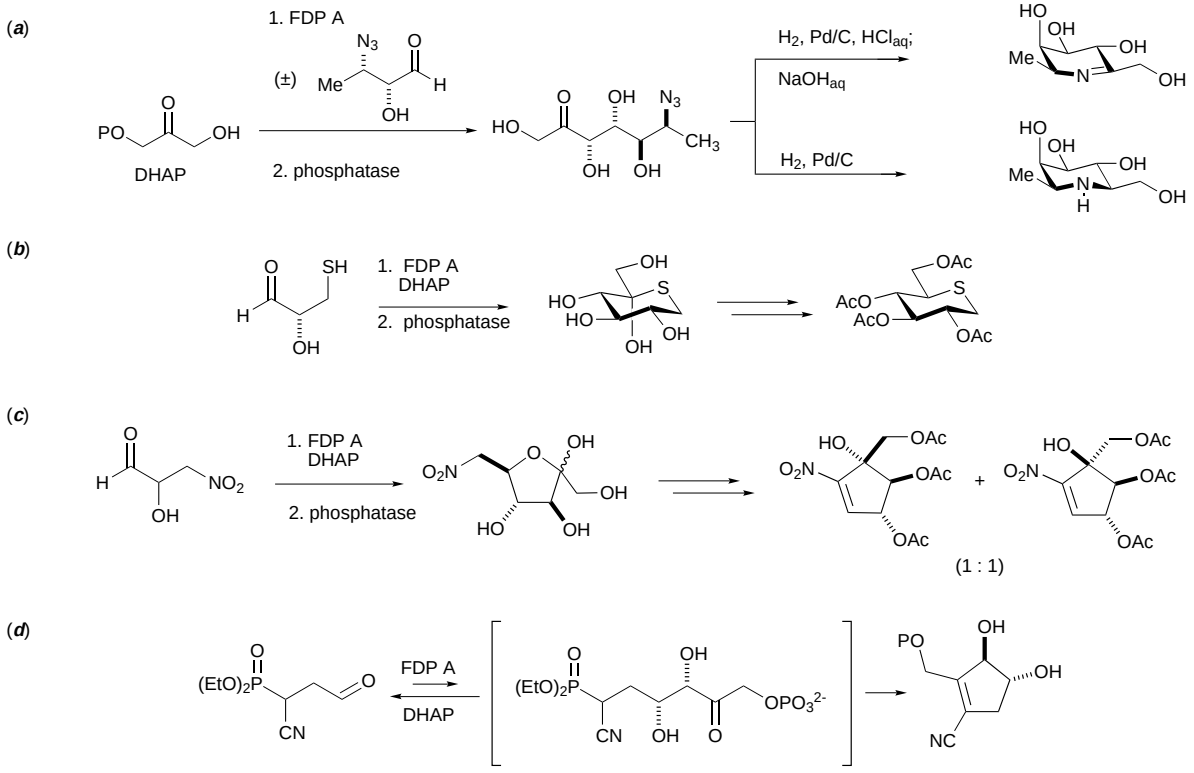


Fig. 5 Synthesis of imino-sugars, deoxythiosugars, and cyclitols using aldolases

enzymes have been developed for large-scale synthesis. The cofactor regeneration scheme not only reduces the cost of sugar nucleotides, but also lessens the problem of product inhibition caused by the resulting nucleoside phosphates.[20] This enzymatic strategy of oligosaccharide synthesis has been applied to the kilogram-scale synthesis of the oligosaccharide sialyl Lewis^x (SLe^x) which is in clinical trials as a new anti-inflammatory agent for the treatment of reperfusion injury [Fig. 6(*a*)]. Other regeneration schemes have also been developed. Recently, a novel regeneration system for UDP-Gal has been used in combination with α- and β-galactosyltransferases to afford a xenotransplantation antigen [Fig. 6(*b*)].[21]

Glycosyltransferases have also been used in the solid-phase and solution-phase synthesis of glycopeptides.[22,23] More recently, glycosyl transferases in combination with the protease, subtilisin, have been used for the synthesis of novel glycoproteins. Ribonuclease B (RNase B) with a heterogeneous carbohydrate composition was remodeled to a homogeneous species *via* enzymatic removal of the heterogeneous saccharide units, followed by addition of new sugars, including unnatural sugars such as mercury containing sugars, with glycosyltransferases (Fig. 7).[24] Combined with the enzymatic ligation of peptide fragments to proteins, this strategy provides a powerful method for the preparation of glycoproteins. The mild reaction conditions and biocompatibility of the enzymatic method for synthesis of glycopeptides and glycoproteins are complementary to the solution- and solid-phase chemical approaches and may be more suitable for the synthesis of large and complex structures. Indeed, the principles of enzymatic aldol-reactions and glycosylations have been applied to systems as complex as that of engineering cell surfaces.[25]

Some glycosyl transferases catalyze the formation of sugar polymers. Sequential reaction of sugar nucleotide donors with a growing oligosaccharide will give a polysaccharide. This was demonstrated in the synthesis of a medicinally important biopolymer hyaluronic acid (HA).[26] HA with a molecular mass of ~500 000 has been prepared from UDP–GlcNAc and UDP–glucuronic acid (UDP–GlcA) using haluronic acid synthetase coupled with regeneration of the sugar nucleotides (Fig. 8).

3.2 Glycosidases

Glycosidases are enzymes that activate sugars towards hydrolysis of glycosidic bonds *in vivo*. Under appropriate conditions, however, the activated intermediates can be intercepted by other sugars to form new glycosidic bonds. This is especially useful when a glycosyltransferase is not available or difficult to obtain. Glycosidases also have the advantage of not requiring expensive sugar nucleotides as the sugar donor. An example is the synthesis of sialyl Lewis^a, in which the 1,3-linked *N*-acetyllactosamine was prepared *via* a β-galactosidase reaction followed by enzymatic glycosylation using sialyl and fucosyl transferases [Fig. 9(*a*)].[4] By sequentially using two different galactosidases, a xenotransplantation antigen was also synthesized [Fig. 9(*b*)].[27,28] The carbohydrate polymer cellulose has been prepared using cellulase.[29] Catalytic antibodies with glycosidase activities have been prepared and may also become useful for synthesis in the future.[30]

One of the drawbacks of glycosidase mediated glycosylations has been the low yield associated with the hydrolytic nature of the enzyme. Various strategies have been used to overcome this problem. Some of the novel strategies are depicted in Fig. 10. Glycosidase-catalyzed synthesis of disaccharides, for example, can be coupled *in situ* with a glycosyltransferase reaction to improve the overall yield [Fig. 10(*a*)].[4] Another interesting way to improve the yield and facilitate product isolation of glycosidase catalyzed glycosidation reactions has been demonstrated in the galactosidase-catalyzed synthesis of *N*-acetyl lactosamine, one of the intermediates in the synthesis of SLe^x.[31] The key was the use of 6-oxo *p*-nitrophenyl galactose, prepared by enzymatic oxidation of the corresponding galactose derivative with galactose oxidase, as the glycosyl donor. The 6-oxo derivatives are less prone to hydrolysis resulting in improved yields of the 6′-oxo disaccharide. Reduction of the aldehyde with sodium borohydride afforded the desired product and also facilitated isolation due to formation of a boron complex [Fig. 10(*b*)]. Chitinase, which normally works to hydrolyze the polymer, chitin, has been used to synthesize artificial chitin in quantitative yield.[32] The key to this polymerization reaction is the use of a transition state analog substrate and performing the

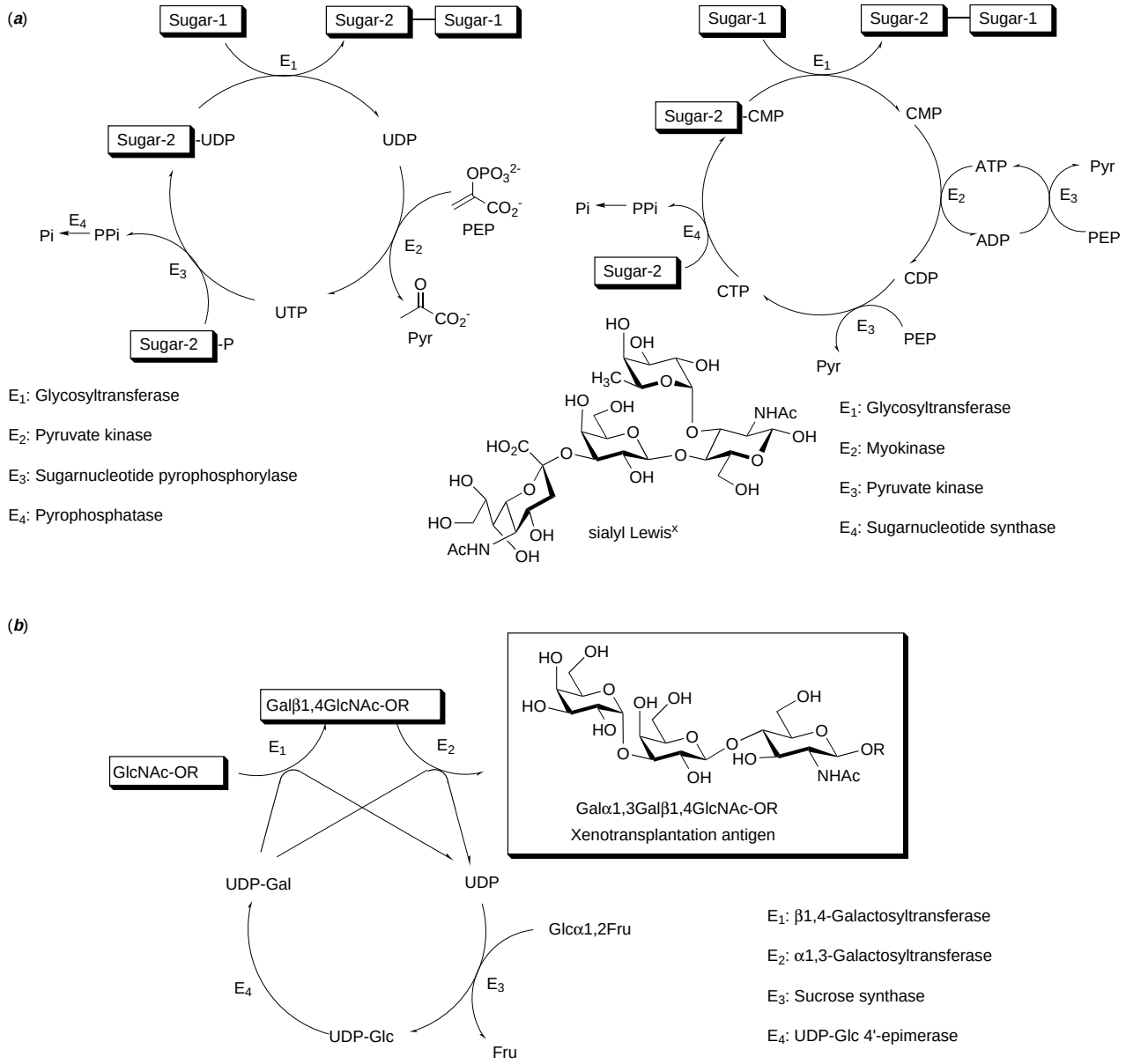


Fig. 6 Multiple enzyme systems used in the large-scale synthesis of oligosaccharides

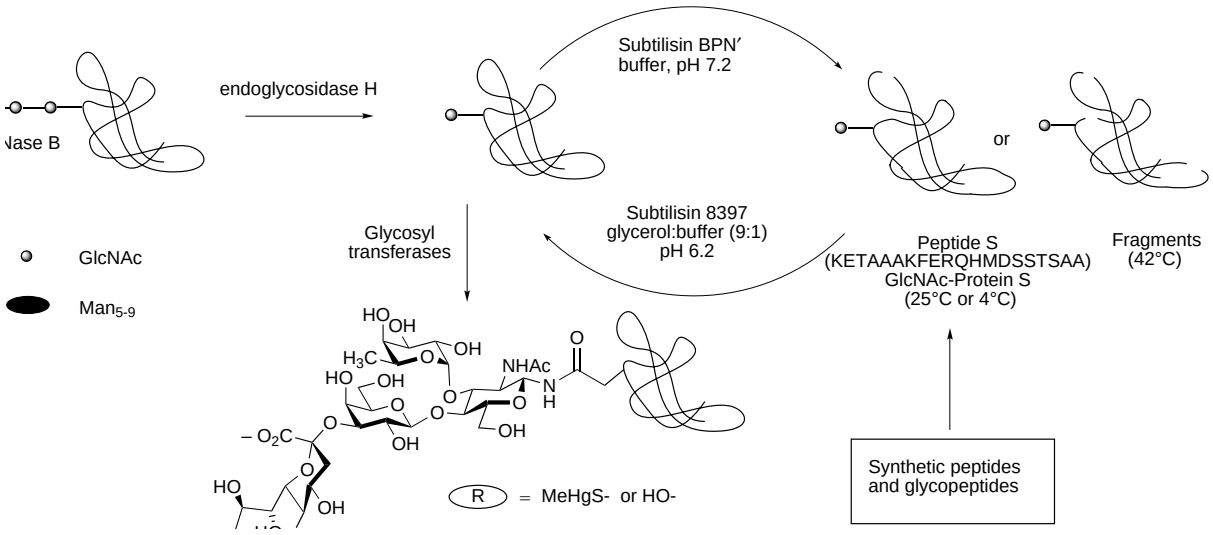


Fig. 7 Enzymatic synthesis of glycoproteins: Synthesis of a ribonuclease B glycoform containing sialyl Lewis^x

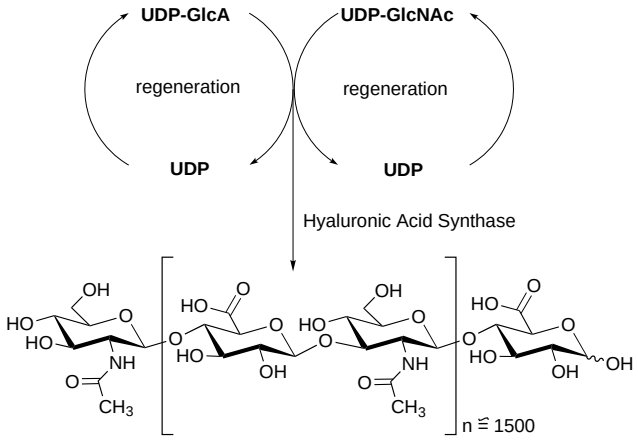


Fig. 8 Enzymatic synthesis of hyaluronic acid with regeneration of sugar nucleotides

reaction at a high pH where the enzyme can activate the substrate but cannot hydrolyze product [Fig. 10(*c*)].

4 Some applications in glycobiology

4.1 Synthesis of sialyl Lewis^x mimetics

Though carbohydrates themselves are not always suitable as drugs—they are too unstable and orally inactive—understanding the mechanism of carbohydrate recognition opens the way for new concepts and strategies in drug development, such as designing carbohydrate mimics with better pharmacological properties to intervene with carbohydrate mediated processes. This has been the case with sialyl Lewis^x (SLe^x), a carbohydrate moiety involved in cell adhesion processes. The chemistry developed for the synthesis of SLe^x and related structures has led to important discoveries in structure–function relationships [Fig. 11(*a*)].[33,34] These discoveries, together with the conformation of SLe^x determined by NMR [Fig. 11(*a*)],[20,33,34] have led to the rational development of SLe^x mimetics which may be comparable to or even better than the natural ligand as inhibitors of selectin recognition events. Several groups have been

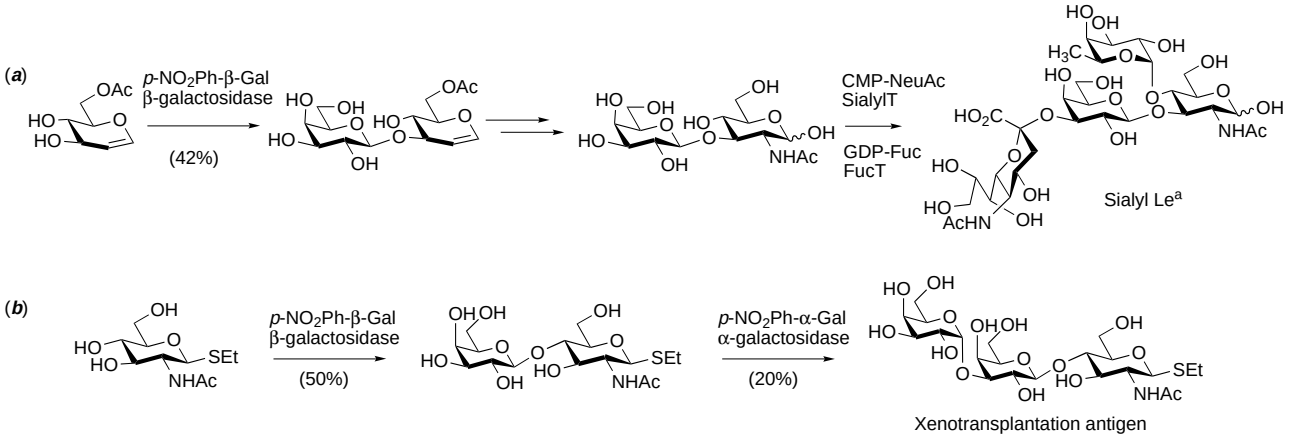


Fig. 9 Use of galactosidases in the synthesis of sialyl Lewis^a and a xenotransplantation antigen

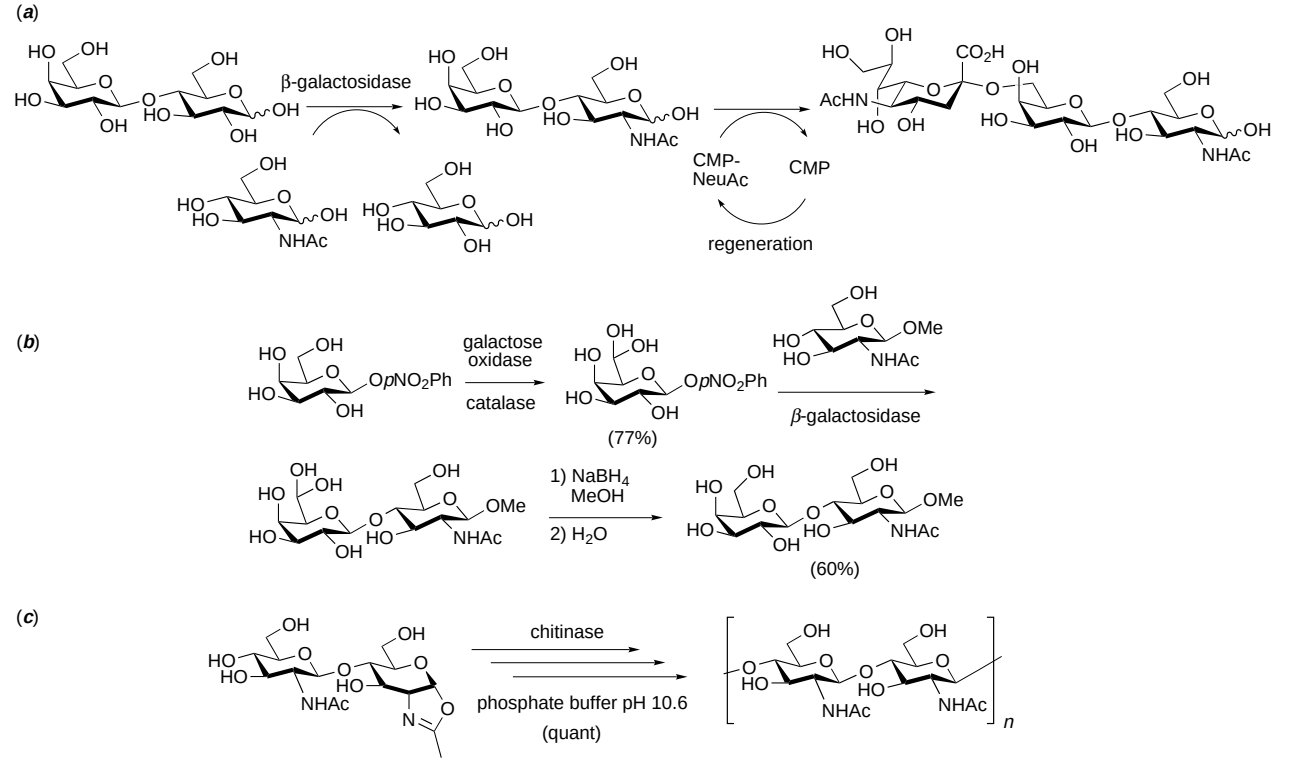


Fig. 10 Strategies used to improve the yields of glycosidase catalyzed glycosylations

actively engaged in this effort, and several SLe^x mimetics developed [Fig. 11(*b*)] have been shown to have affinities for selectins increased from the millimolar range for SLe^x to the micromolar range for the mimetics.[34] Some key reactions used in the development of these mimetic compounds are the enzymatic aldol reactions. A key component used in the synthesis of some of the potent mimetics is (2*S*,3*R*)-2-amino-3,4-dihydroxybutanoic acid (L-hydroxythreonine), which can be easily prepared *via* a threonine aldolase-catalyzed addition reaction.[35] The fructose-diphosphate aldolase catalyzed aldol reaction of DHAP and a mannose derivative gave a phosphorylated compound which had especially good inhibition against P-selectin binding.[34]

4.2 Synthesis of glycosidase and glycosyltransferase inhibitors

Inhibition of the enzymes associated with carbohydrate biosynthesis is also biologically and medicinally important. Both glycosidases and glycosyltransferases are important enzymes involved in the processing and synthesis of oligosaccharides and are therefore obvious targets for intervention. The reactions catalyzed by these enzymes are thought to proceed through similar transition states in which substantial sp^2 character and positive charge develop at the anomeric centre of the reacting sugar [Fig. 12(*a*)]. This mechanistic rationale has led to the development of transition-state analog inhibitors of glycosidases and glycosyltransferases. Various 5-, 6-, and 7-membered ring imino-sugars have been synthesized using aldolases [Fig. 12(*b*)].[4–6,17,36] These nitrogen-containing heterocycles are potent inhibitors of glycosidases and have also been used as key components in the synthesis of glycosyltransferase inhibitors. An inhibitor of α-1,3-fucosyltransferase, for example, has been prepared by the attachment of the *N*-acetyllactosamine moiety to an iminocyclitol-type α-fucosidase inhibitor [Fig. 12(*c*)].[37] Combined with new high throughput assays, the nitrogen heterocycles can also serve as core structures for combinatorial approaches to the development of glycoprocessing enzyme inhibitors.[17]

5 Conclusion and future prospects

As we have briefly reviewed, enzymatic aldol reactions and glycosylations have made access to carbohydrate molecules

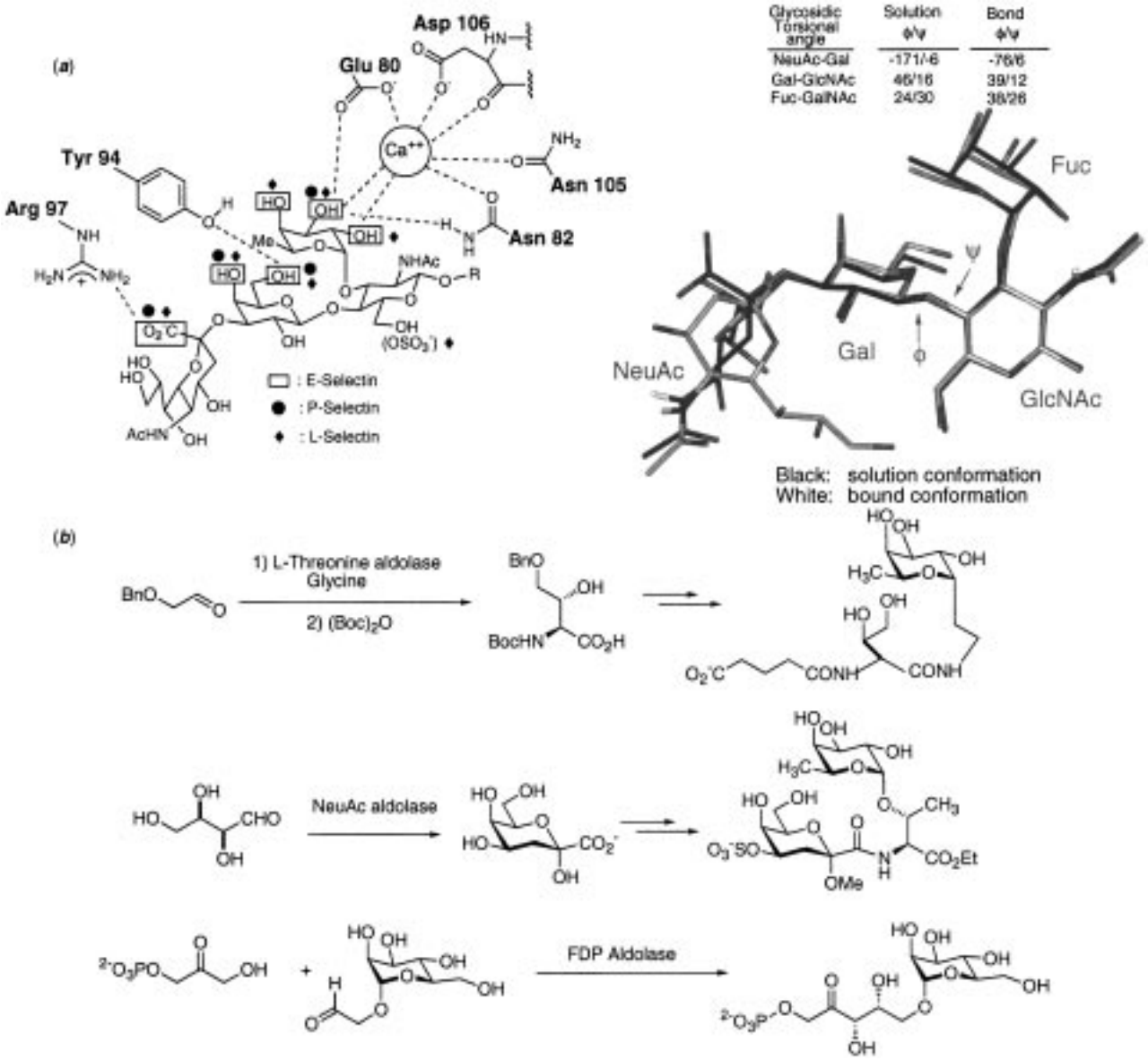


Fig. 11 (*a*) Sialyl Lewis^x (SLe^x) functional groups crucial for selectin binding and NMR structure of SLe^x. (*b*) Chemo-enzymatic synthesis of SLe^x mimetics

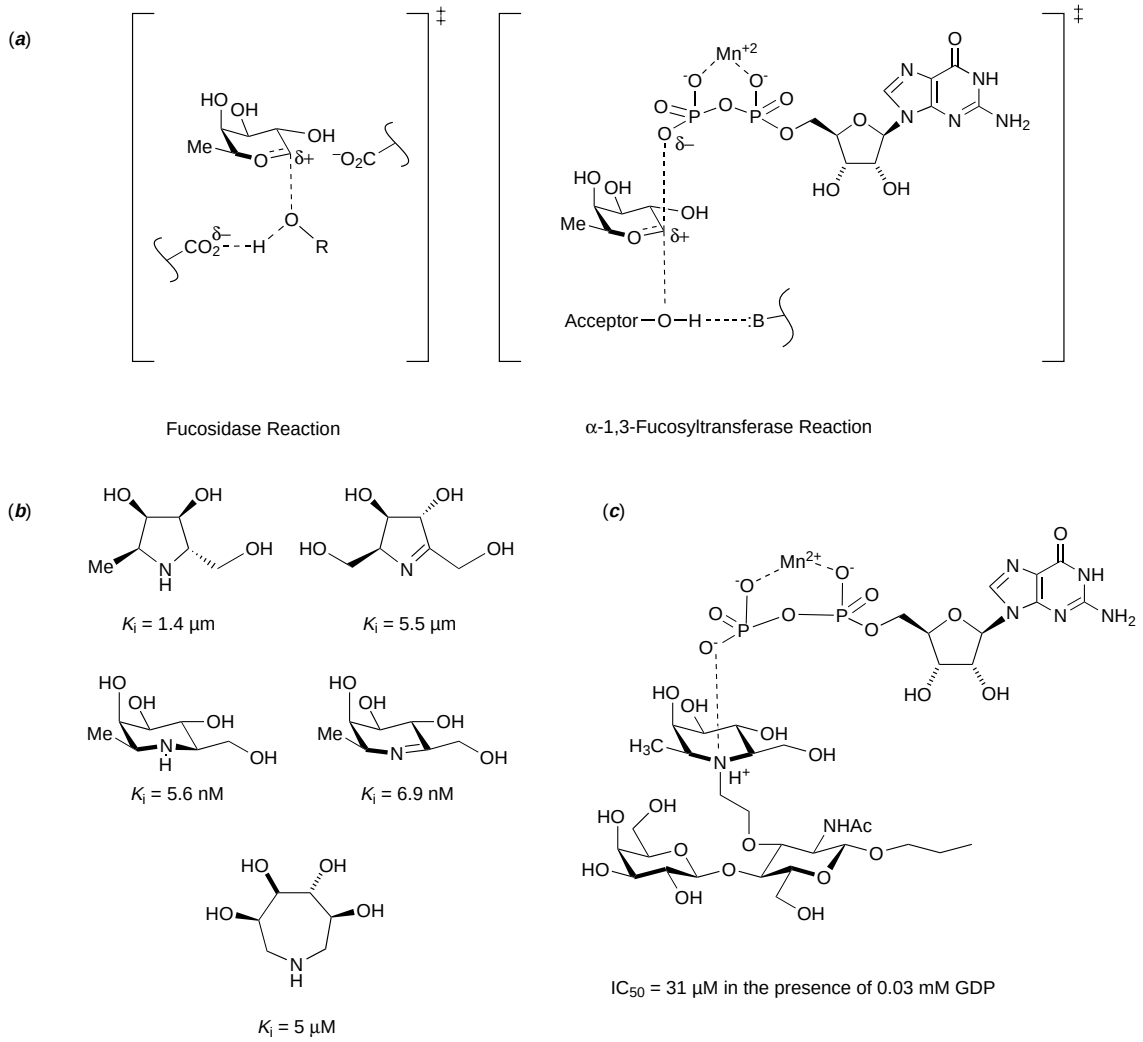


Fig. 12 (*a*) Transition-state of glycosidases and glycosyltransferases. (*b*) Transition-state analog inhibitors of fucosidase. (*c*) An azatrisaccharide fucosyltransferase inhibitor shown as a complex with GDP.

more routine and available on a large-scale basis. Many glycoprocessing enzymes useful for synthesis are already available and new ones are becoming accessible at an ever increasing rate. Indeed, it is believed that any oligosaccharide in the mammalian system can be prepared in large quantities based on the glycosyltransferase methodology as methods for the regeneration of all relevant sugar nucleotides have been developed.[6] In cases where an enzyme that catalyzes a certain reaction is difficult to obtain or non-existent, the use of alternative biocatalysts (for example, glycosidases and catalytic antibodies) or novel substrates (for example, Fig. 5) is useful. These enzymatic tools are accelerating research in glycobiology and medicine as well as provide opportunities to open new doors in other fields of science. With an increasing number of these enzymes becoming commercially available (Table 1), it is anticipated that use of enzymatic aldol reactions and glycosylations will become more and more routine and find many applications in various fields, as was the case with proteins and nucleic acids.

6 Acknowledgement

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