Recent Advances in the Chemoenzymatic Synthesis of Carbohydrates and Carbohydrate Mimetics

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Contents

I. General Introduction

Enzymes are increasingly recognized as useful catalysts for organic synthesis. One of the main attractions for the use of enzymes is their ability to perform reactions in a stereoselective way. Additionally, the use of enzymes has several advantages over chemical methods. Because of the mild conditions in enzymatic reactions and the regioselectivity displayed by enzymes, protective group chemistry can be reduced to a minimum. Since most enzymes operate at room temperature in aqueous solution around pH 7, their reactions are often compatible with each other. This makes it possible to combine several enzymes in a one-pot, multistep reaction sequence. Their use in aqueous solution and their biodegradability make enzymes also an excellent environmentally acceptable option. The high regio- and stereoselectivity and catalytic efficiency make enzymes especially useful for the synthesis of complex, highly functionalized molecules like carbohydrates.

Carbohydrates have become a major focus of current biological research. Through coupling of several multifunctional monosaccharides with different stereochemistry, an immense variety of complex structures can be assembled, and in which vast amounts of information can be stored. Nature has made use of this information source, since more and more complex carbohydrates and conjugates have been found to play an important role in various types of biochemical recognition. These include growth, development, immune response, infection, cell adhesion, metastasis, and numerous signal transduction events. The availability of these compounds and their mimetics through synthesis will provide insight into their biological role and might lead to the rational design of new generations of therapeutic agents. The synthesis of such highly functionalized, complex molecules via chemical methods often requires many selective protection and deprotection steps which can be avoided using enzymes. The synthesis of monosaccharides and related compounds via enzymatic aldol addition reaction catalyzed by aldolases has been proven to be very useful. The coupling of monosaccharides to form oligosaccharides via specific, glycosidic linkages can be catalyzed by glycosidases or glycosyltransferases. In this review we will focus mainly on the recent progress in the enzymatic or chemoenzymatic synthesis using aldolases and glycosyltransferases. Special attention will be given to the use of these enzymes in multistep synthesis of complex molecules. In addition, the synthesis of carbohydrate mimetics is a promising development which can lead to new therapeutic agents which are more stable and structurally less complicated than their carbohydrate counterparts. Several examples will be given to illustrate the chemoenzymatic synthesis of some interesting carbohydrate mimetics.

II. Aldol Addition with Aldolases

1. Introduction

The aldol condensation has been an effective method in organic synthesis for the formation of carbon−carbon bonds.[1] Much effort has been directed toward making these reactions proceed in a stereoselective way. The use of chiral auxiliaries has been successful in achieving high stereoselectivity; however, in most cases stoichiometric quantities of these chiral auxiliaries are needed.[1] The use of a catalytic amount of auxiliary would of course be preferable. In recent years several successful examples of chemical catalytic asymmetric aldol reactions have been described.[2] Various chiral Lewis acids or metal complexes have been employed as catalysts with high stereoselectivity. In general the donors in these reactions are

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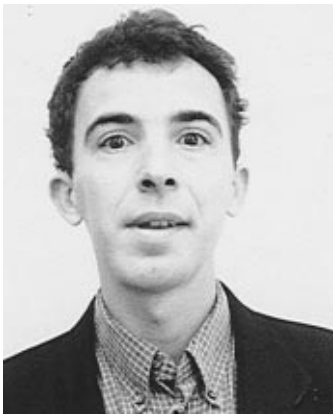
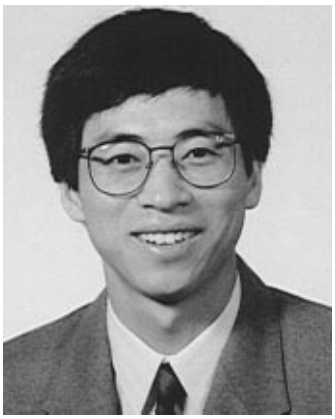


Wolfgang Fitz was born in 1965 in Hohenems, Austria. He received his B.S. degree at ETH Zürich, Switzerland, where he also obtained his Ph.D. in Chemistry under the supervision of Professor Duilio Arigoni (1994). Currently, he is a Swiss National Foundation postdoctoral fellow with Professor Wong at The Scripps Research Institute. His research interests include enzyme mechanisms, the design and synthesis of enzyme inhibitors and the use of enzymes in organic synthesis.



Lei Qiao (born in 1964) received his B.S. in medicinal chemistry from Beijing Medical University at Beijing, China, and his Ph.D. in chemistry with Professor John C. Vederas in 1994 from the University of Alberta at Edmonton, Canada. In the same year, he joined the research group of Professor Wong at The Scripps Research Institute as a postdoctoral fellow. His research interests include the development of new chemoenzymatic methodology and synthesis of inhibitors of specific enzymes involved in carbohydrate metabolism.

Professor Chi-Huey Wong received his B.S. and M.S. degreees from National Taiwan University, and Ph.D. 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 he has been Professor and Ernest W. Hahn Chair in Chemistry at The Scripps Research Institute. He is also Head of the Frontier Research Program on Glycotechnology at RIKEN (Institute of Physical and Chemical Research) in Japan. His current interests are in the areas 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.

reactive nucleophiles like silyl enol ethers (Mukaiyama aldol reaction), nitromethane (nitroaldol reaction), or isocyanoacetic esters (Knoevenagel reaction). Frequently large amounts of catalyst (up to 20 mol %) and low temperatures are required, and the stereoselectivity is highly dependent on the structure of the substrates used.

Complementary to these chemical methods is the use of aldolases, which in general are known to induce high stereoselectivity. In the beginning of this century a class of enzymes was recognized that catalyzed the reversible formation of hexoses from their three-carbon components via an aldol condensation.[3] Originally named zymohexase, the enzymes capable of catalyzing an aldol condensation are now known as aldolases. Several of these aldolases have been explored for use as catalysts in organic synthesis. For recent reviews, see refs 4−10. In this section the different types of aldolases and their recent applications in organic synthesis will be discussed in detail.

Aldolases are part of a large group of enzymes called lyases and are present in all organisms. They are mainly involved in the metabolism of carbohydrates, but also of amino acids and hydroxy acids. Although their function *in vivo* is often related to the degradative cleavage of metabolites, the reactions are reversible and by choosing the proper conditions, C−C bond formation can become favored. For example, using an excess of one of the reactants often shifts the equilibrium to the synthesis direction. Over 30 aldolases have been identified and isolated so far, the majority of which catalyzes the reversible stereospecific addition of a ketone donor to an aldehyde acceptor. Mechanistically, two distinct classes can be recognized (Scheme 1).[11] Type I aldolases form a Schiff base intermediate in the active site with the donor substrate, which subsequently adds ste-

Scheme 1

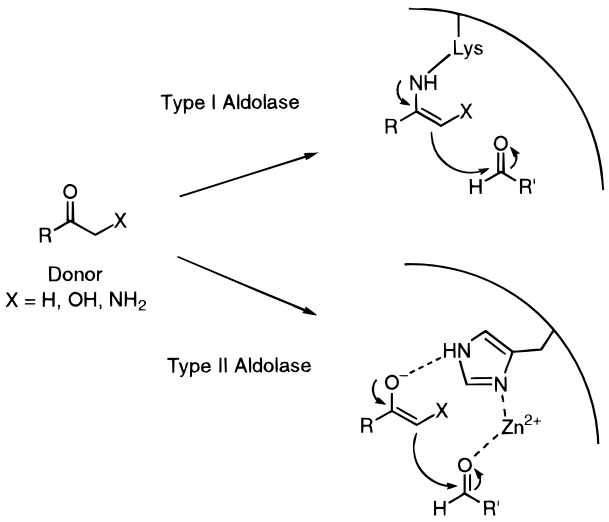
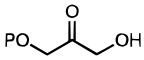
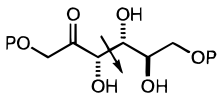
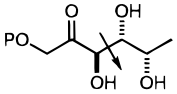
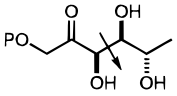
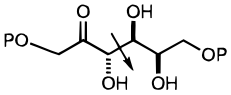
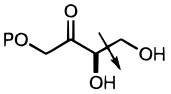
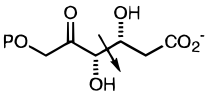


Table 1. DHAP-Dependent Aldolases and *in Vivo* Aldol Products

Donor: 	Ref.
Fructose 1,6-diphosphate (FDP) aldolase (EC 4.1.2.13) 	
L-Fuculose 1-phosphate (Fuc 1-P) aldolase (EC 4.1.2.17) 	
L-Rhamnulose 1-phosphate (Rha 1-P) aldolase (EC 4.1.2.19) 	
Tagatose 1,6-diphosphate (TDP) aldolase 	
Ketotetrose phosphate aldolase (EC 4.1.2.2) 	(12)
Phospho-5-keto-2-deoxy-gluconate aldolase (EC 4.1.2.29) 	(13)

reospecifically to the acceptor. Type II aldolases use a Zn^{2+} cofactor, which acts as a Lewis acid in the active site. Type I aldolases are primarily found in animals and higher plants, type II aldolases are predominantly encountered in bacteria and fungi. In general, type II aldolases are more stable than type I aldolases. Both types of enzymes are rather specific for the donor substrate but exhibit a more relaxed specificity for the acceptor substrate. The stereoselectivity of the aldol reaction is in general controlled by the enzyme and does not depend on the structure or stereochemistry of the substrate, making the stereochemistry of the products highly predictable. There are, however, some exceptions to this, especially if thermodynamically controlled reaction condi-

Table 2. Pyruvate- and Phosphoenolpyruvate-Dependent Aldolases and *in Vivo* Aldol Products

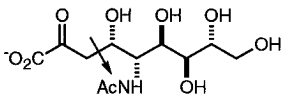
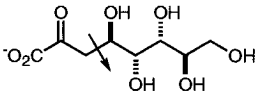
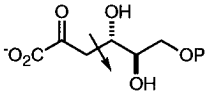
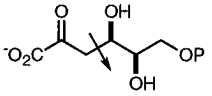
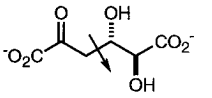
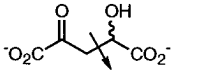
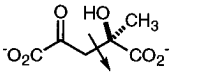
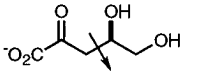
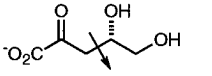
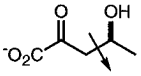
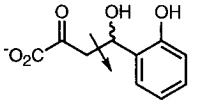
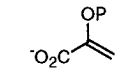
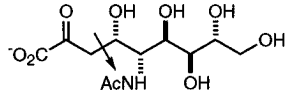
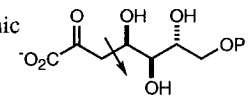
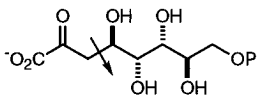
Donor: 	Ref.
N-Acetylneuraminic (NeuAc) aldolase (EC 4.1.3.3) 	
3-Deoxy-D-*manno*-octulosonate (KDO) aldolase (EC 4.1.2.23) 	
2-Keto-3-deoxy-6-phosphogluconate (KDPG) aldolase (EC 4.1.2.14) 	(14)
2-Keto-3-deoxy-6-phospho galactonate aldolase (EC 4.1.2.21) 	(15)
2-Keto-3-deoxy-D-glucarate (KDG) aldolase (EC 4.1.2.20) 	(16)
2-Keto-4-hydroxyglutarate (KHG) aldolase (EC 4.1.2.31) 	(17)
4-Hydroxy-2-keto-4-methyl glutarate aldolase (EC 4.1.3.17) 	(14)
2-Keto-3-deoxy-D-xylonate aldolase (EC 4.1.2.28) 	(14)
2-Keto-3-deoxy-L-arabonate aldolase (EC 4.1.2.18) 	(18)
4-Hydroxy-2-ketovalerate aldolase 	(19)
2'-Hydroxybenzalpyruvate aldolase 	(20)
Donor: 	
N-Acetylneuraminic (NeuAc) synthetase (EC 4.1.3.19) 	
3-Deoxy-D-*arabino*-2-heptulosonic acid 7-phosphate (DAHP) synthetase (EC 4.1.2.15) 	
3-Deoxy-D-*manno*-octulosonate (KDO 8-P) synthetase (EC 4.1.2.16) 	

Table 3. Acetaldehyde-Dependent Aldolases and *in Vivo* Aldol Products

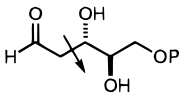
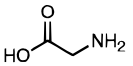
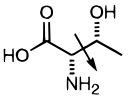
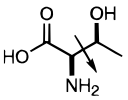
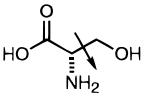
	Donor: 
2-Deoxyribose 5-phosphate aldolase (DERA) (EC 4.1.2.4)	

Table 4. Glycine-Dependent Aldolases and *in Vivo* Aldol Products

	Donor: 
L-Threonine aldolase (EC 4.1.2.5)	
D-Threonine aldolase	
Serine hydroxymethyl transferase (EC 2.1.2.1)	

tions are used, as for example in neuraminic acid aldolase reactions.

The aldolases that have been investigated for use in synthesis can be divided into four main groups, on the basis of the structure of the donor substrate and their products. The first group uses dihydroxyacetone phosphate (DHAP) as the donor substrate, resulting in the formation of a ketose 1-phosphate after reaction with an aldehyde (Table 1). The second group of aldolases uses pyruvate or phosphoenol pyruvate as donor, to form 3-deoxy-2-keto acids as products (Table 2). The third group consists of one aldolase, 2-deoxyribose 5-phosphate aldolase, which uses acetaldehyde as donor to give a 2-deoxy aldehyde as product (Table 3). The last group uses glycine as a donor to form different α-substituted amino acids (Table 4). In sections 2−5 the different groups will be discussed in detail.

Apart from these main groups, several aldolases are known which have not been investigated in terms of their use in synthesis or their substrate specificity. For completeness these are grouped together in Table 5.

Aldolases can be isolated from different organisms in enough quantity to be experimentally useful. In order to obtain sufficient quantities for larger scale reactions, several aldolases have been cloned and

Table 5. Other Aldolases and the Reactions They Catalyze *in Vivo*

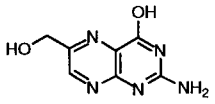
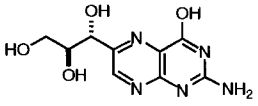
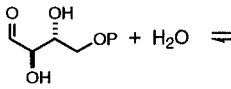
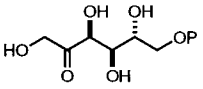
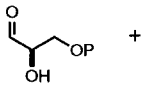
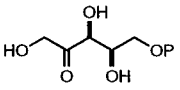
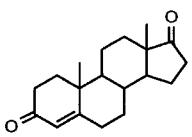
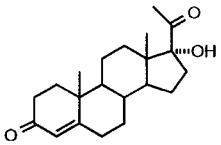
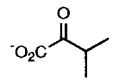
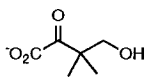
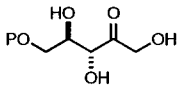
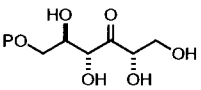
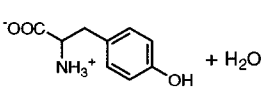
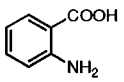
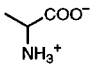
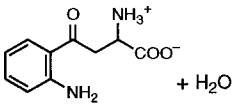
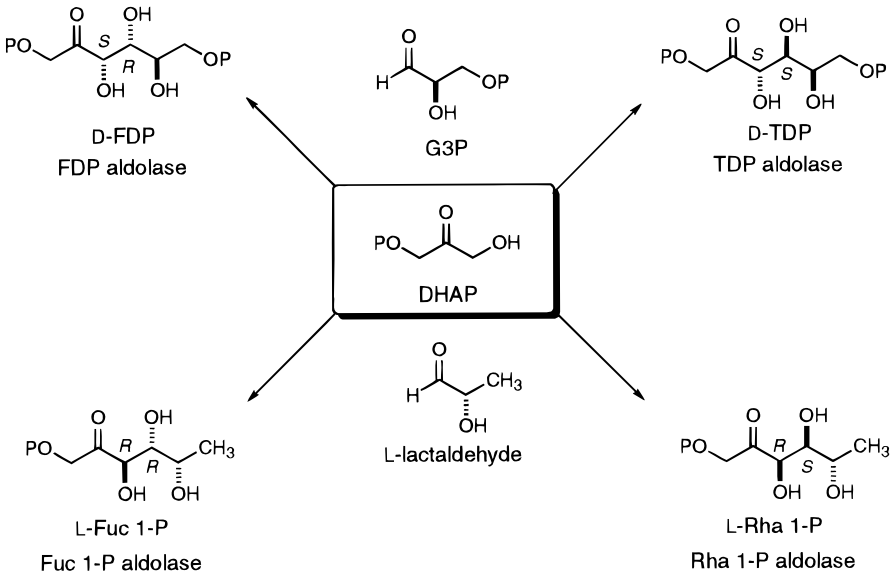
aldolase	donor	acceptor	product	Ref.
Dihydroneopterin aldolase (EC 4.1.2.25)				(21)
Fructose 6-phosphate phosphoketolase (EC 4.1.2.22)				(22)
Phosphoketolase (EC 4.1.2.9)				(23)
17 α-Hydroxyprogesterone aldolase (EC 4.1.2.30)				(24)
Ketopantoaldolase (EC 4.1.2.12)				(25)
3-Hexulose phosphate synthase (EC 3.7.1.3)				(26)
Tyrosinephenol lyase (EC 4.1.99.2)				(27)
L-Kynurenine hydrolase (EC 3.7.1.3)				(28)

Table 6. Commercially Available Aldolases

aldolase	supplier	source
fructose-1,6-diphosphate aldolase (type I)	Boehringer, Calbiochem, Fluka, Serva, Sigma	rabbit muscle, trout muscle
fructose-1,6-diphosphate aldolase (type II)	Boehringer, Fluka, Sigma	*S. aureus, T. thermophilus*
fuculose-1-phosphate aldolase	Boehringer[a]	*E. coli*
rhamnulose-1-phosphate aldolase	Boehringer[a]	*E. coli*
N-acetylneuraminic acid aldolase	Toyobo	microorganisms
	Sigma	*E. coli*
		C. perfringens
	Boehringer	*E. coli*

[a] Only available in synthesis grade purity.

Scheme 2



overexpressed, and the strains are mostly available from ATCC. These include fructose 1,6-diphosphate aldolase (ATCC 77472), fuculose 1-phosphate aldolase (ATCC 86984), rhamnulose 1-phosphate aldolase (ATCC 86983), tagatose 1,6-diphosphate aldolase (ATCC 87025) and 2-deoxyribose 5-phosphate aldolase (ATCC 86963). At present several aldolases are commercially available. Their suppliers and sources are given in Table 6.

2. DHAP-Dependent Aldolases

2.1. General Background

In vivo, four DHAP-dependent aldolases catalyze the reversible asymmetrical aldol addition of DHAP to D-glyceraldehyde 3-phosphate (G3P) or L-lactaldehyde, and each reaction generates one unique product whose stereochemistry at C-3 and C-4 is complementary to the others (Scheme 2). FDP aldolase condenses DHAP and G3P to form D-fructose 1,6-diphosphate (D-FDP) with 3*S*/4*R* stereochemistry. By using the same substrates, tagatose 1,6-diphosphate (TDP) aldolase produces 3*S*/4*S* D-tagatose 1,6-diphosphate. Fuculose 1-phosphate aldolase and rhamnulose 1-phosphate aldolase catalyze the condensation between DHAP and L-lactaldehyde to give L-fuculose 1-phosphate and L-rhamnulose 1-phosphate, with 3*R*/4*R* and 3*R*/4*S* stereochemistry, respectively.

To date, FDP aldolase has been most extensively studied. Both type I and type II enzymes are known and they have been isolated from several mammalian and selected microbial sources (for a comprehensive list see refs 4 and 5). For synthesis, the type I enzyme from rabbit muscle (RAMA) has been used most often. This enzyme is not particularly air sensitive, although there is an active site thiol group. The half-life of the free enzyme, which is ca. 2 days in aqueous solution at pH 7.0,[29,30] can be lengthened by immobilization or by enclosure in a dialysis membrane. Recently, the monomeric type I FDP aldolase from *Staphylococcus carnosus* has been shown to be much more stable and has also been used in synthesis.[31] Type II FDP aldolases were found to be more stable; for example, the enzyme from *E. coli* has no thiol group in the active site and has a half-life of ca. 60 days in 0.3 mM Zn^{2+} at pH 7.0.[30] Despite the small degree of homology in primary sequence between the enzymes from rabbit muscle (type I) and *E. coli* (type II), studies have shown that they possess almost the same substrate specificity.[32]

Fuc 1-P aldolase and Rha 1-P aldolase have only been found as type II enzymes in several microorganisms.[33] They have been cloned and overexpressed and subsequently purified.[34−36] TDP aldolase, a type I aldolase involved in the galactose metabolism of *cocci*, has also been isolated from several sources[37] and has been cloned and overexpressed recently.[36]

All four types of DHAP-dependent aldolases have been explored for synthetic application. Extensive studies have demonstrated that, while being quite specific for the donor substrate DHAP, these enzymes accept a wide range of aldehydes as the acceptor substrates. A detailed description of the substrate specificity of these enzymes has appeared recently.[4]

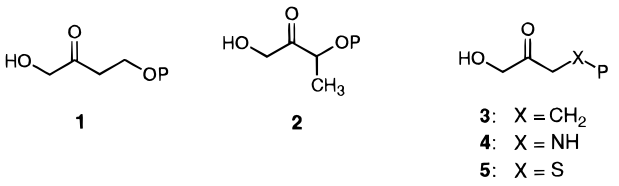
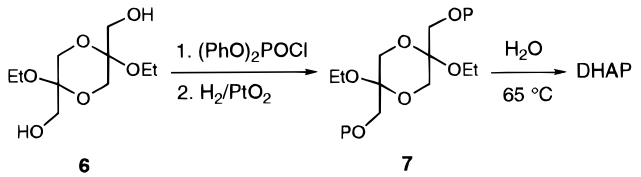


Figure 1.

In general, unhindered aliphatic aldehydes, α-heteroatom-substituted aldehydes, and monosaccharides and their derivatives are suitable acceptors. For FDP aldolase, phosphorylated aldehydes react at enhanced rates relative to their unphosphorylated species due to the resemblance to its natural substrate G3P. Aromatic aldehydes, sterically hindered aliphatic and α,β-unsaturated aldehydes are not substrates in general. The enzyme has however been reported to accept aromatic aldehydes and α-branched aldehydes, when it is used in the form of cross-linked enzyme crystals.[30b] The specificity for the donor substrate is much more stringent. Among many of the DHAP analogs tested, only compounds **1** and **2** were found to be weak substrates for RAMA (ca. 10% of the activity of DHAP)[29,38] (Figure 1). Recently, compounds **3**−**5** were reported to be accepted by Rha aldolase as DHAP analogs; however, the aldol adducts were not isolated and only partially characterized with a mixture of other reaction components.[39]

Since DHAP-dependent aldolases are very specific for the donor substrate, the availability of DHAP has been an important issue for the development of the practical usage of these enzymes. Although commercially available, DHAP is too expensive for enzymatic synthesis on a large scale; hence, several chemical and enzymatic methods for generating DHAP have been developed.[39−46] Among these methods, chemical synthesis of DHAP provides the cleanest product. The use of relatively pure DHAP also favors the formation of the aldol adducts and simplifies the purification process. Phosphorylation of dihydroxyacetone dimer **6** with $(PhO)_2POCl$[45b] followed by reductive cleavage of the phenyl esters gives DHAP dimer precursor **7**, which can be hydrolyzed to give DHAP in 61% overall yield (Scheme 3).[46]

Scheme 3



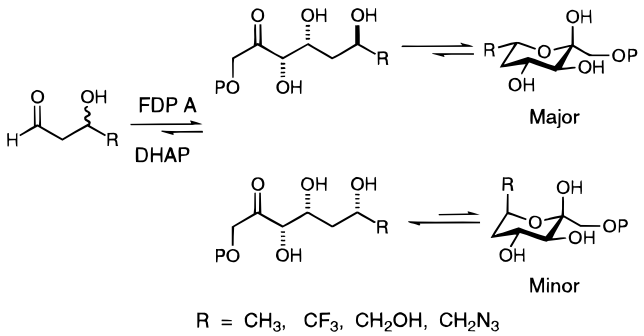
Phosphorylation can also be achieved by using $(PhCH_2O)_2PNEt_2$[44] or $POCl_3$;[45a] however, lower overall yields (55% and 35%, respectively) and less pure products were obtained.

The aldol reactions with unnatural substrates catalyzed by these aldolases generate ketose phosphates as the initial products. The charged phosphate group facilitates the product separation; it also can be easily removed by enzymatic hydrolysis with acid phosphatase (EC 3.1.3.2). The stereochemistry of the vicinal diols produced usually follows closely to those of the natural substrates. The configurations of C-3 are invariably conserved; however, depending on the enzymes and the substitution pattern of the aldehydes, the stereoselectivity at C-4 is less consistent. In almost all the cases tested, FDP aldolase produces the products with the stereochemistry precisely following that of FDP, namely the D-*threo* configuration. Fuc 1-P and Rha 1-P aldolase generate vicinal diols with D-*erythro* and L-*threo* configurations, respectively. However, for a few substrates, the stereoselectivity at C-4 is somewhat diminished.[35] Sterically unhindered 2-hydroxyaldehydes normally give very high diastereoselectivities. TDP aldolase, on the other hand, yields diastereomeric mixtures in all cases investigated so far.[36,47] Only with the natural substrate D-glyceraldehyde does the major product (D-TDP) have the tagatose (D-*erythro*) configuration. Due to the lack of stereoselectivity, TDP aldolase is not yet synthetically useful; however, with suitable protein engineering, this stereoselectivity may be improved in the future, and this improvement would allow the generation of all four possible C3/C4 diastereomeric adducts by these four aldolases.

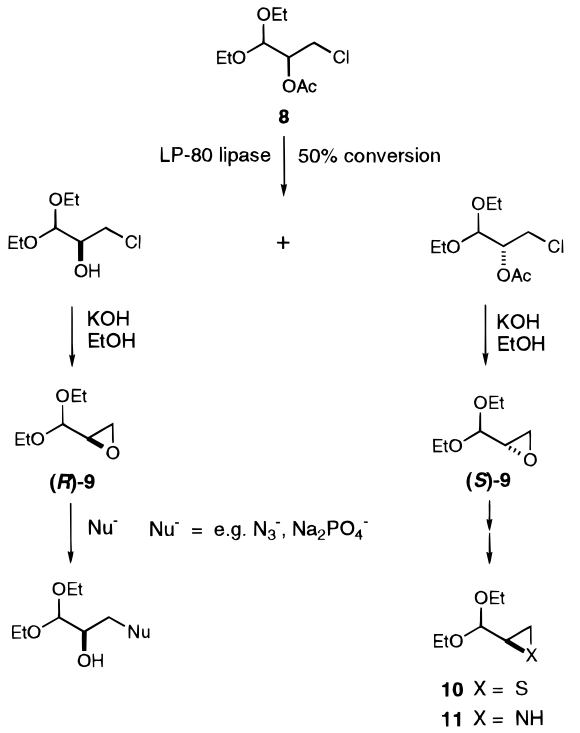
The aldehyde substrates can be used as racemic mixtures. The diastereoselectivity exhibited by aldolases was found to be dependent on the reaction conditions. In a kinetically controlled experiment, the D-enantiomer of G3P was accepted by FDP aldolase with a 20:1 preference over the L-enantiomer.[29] A lower enantioselectivity was observed for the unphosphorylated aldehyde. Fuc aldolase and Rha aldolase, on the other hand, show significant kinetic preference for the L-enantiomer of 2-hydroxy aldehydes (>95:5).[48] Therefore, the kinetic resolution of racemic mixtures of aldehyde substrates can be achieved and a single diastereomeric adduct can be obtained if the reaction is stopped before reaching the equilibrium.

Differentiation between racemic aldehydes can also be achieved under thermodynamically controlled conditions. In the cases wherein the aldol products can cyclize to form pyranoses, the products with the least 1,3-diaxial interaction will predominate after equilibration due to the reversible nature of the aldol reaction. This is illustrated by the aldol condensation of a series of 2- or 3-substituted 3-hydroxypropanals with DHAP catalyzed by FDP aldolase (Scheme 4).[29,49] All reactions provide predominantly the thermodynamically more stable compounds. With 2-methyl-3-hydroxypropanal as substrate, the reaction produced two diastereomers in a 97:3 ratio in favor of the product with the methyl group at the equatorial position. This resolution approach is, however, lim-

Scheme 4



Scheme 5



ited to the instances in which the desired products are significantly more stable than the undesired ones.

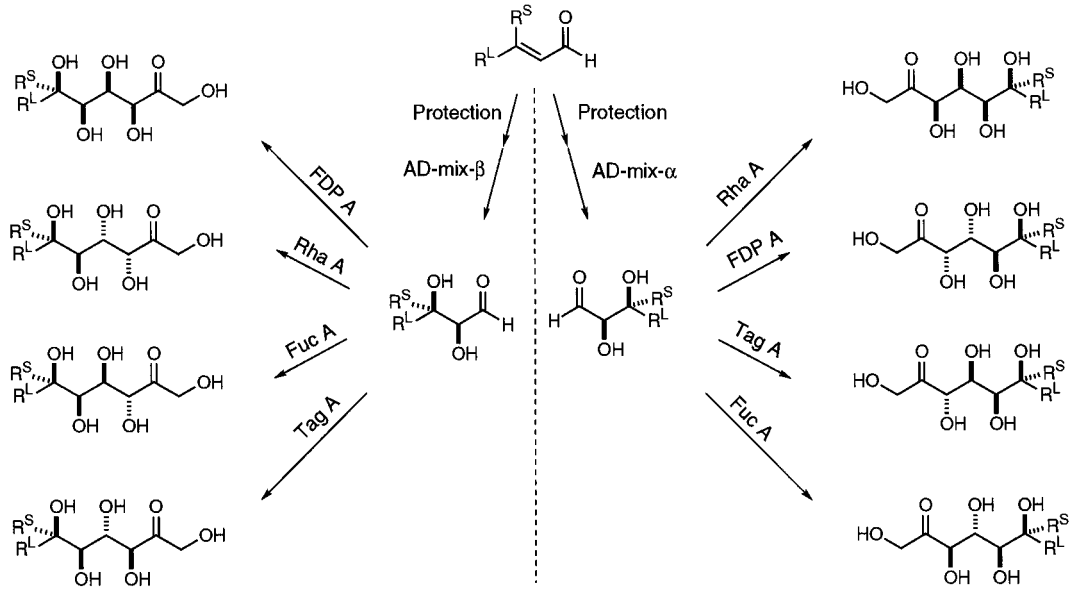
2.2. Synthetic Applications

Owing to their broad substrate tolerance and defined product stereochemistry, DHAP-dependent aldolases have been employed for the synthesis of a large number of monosaccharides from simple precursors. These include ^{13}C-labeled sugars,[40,50,51] heterosubstituted sugars, deoxy sugars, fluoro sugars,[52] and high-carbon sugars.[41,53,54] Many of these monosaccharides were subsequently converted into other interesting derivatives, such as aza sugars, thio sugars, and carbocyclic compounds.

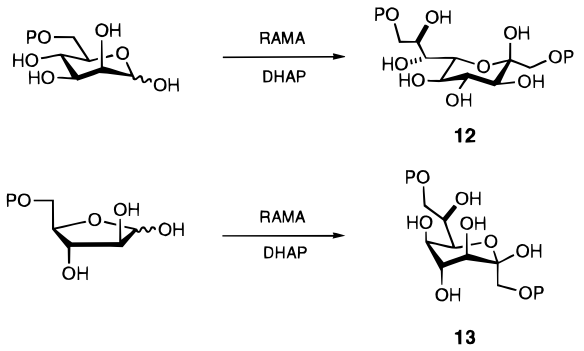
2.2.a. Monosaccharides. More than 100 aldehydes have been used as the acceptor substrates for DHAP-dependent aldolases to prepare monosaccharides. For an extensive listing, see a recent review.[4] The aldehyde substrates can be used in racemic form; however, use of single enantiomers avoids the separation of diastereomeric products. To prepare optically active aldehydes, lipase-catalyzed resolution of racemic aldehyde precursors has been a very effective approach. Examples are represented by the asymmetric synthesis of 3-substituted 2-hydroxy aldehydes (Scheme 5).[55] Enantioselective hydrolysis of 3-chloro-2-acetoxypropanal diethyl acetal (**8**) with LP-80 lipase followed by base treatment of the resolved materials generates the corresponding (*R*)- and (*S*)-glycidaldehyde acetal (**9**). Regioselective ring opening by various nucleophiles then introduces the substituents. The epoxides can also be easily converted to the corresponding thiirane **10** and aziridine **11** with inversion of configuration. Further derivatization provides other types of enantiomerically pure aldehydes. Several examples of the use of these and other aldehydes obtained via lipase resolution will be given further in this review.

Preparation of enantiomerically pure aldehydes from achiral precursors can also be accomplished by chemical methods. In this regard, Sharpless asymmetrical epoxidation[56] and dihydroxylation[57] have been widely used. Tandem use of the osmium-catalyzed asymmetric dihydroxylation (AD) and aldolase-catalyzed condensation allows quick and facile synthesis of carbohydrates with complete stereocontrol (Scheme 6).[58] AD of protected α,β-unsaturated aldehydes followed by deprotection provides chiral dihydroxyaldehydes. Subsequent aldol condensation with DHAP catalyzed by aldolases gives diastereomeric 6-substituted hexuloses. By suitable choice of alkenal, AD-mix, and aldolases, numerous carbohydrate derivatives and their stereoisomers are potentially accessible. The D-and L-forms of fructose and their respective enantiomeric derivatives were synthesized accordingly with Rha aldolase and FDP aldolase as catalyst, respectively. This approach clearly demonstrates the complementary feature of these enzymes and their potential application to the

Scheme 6



Scheme 7

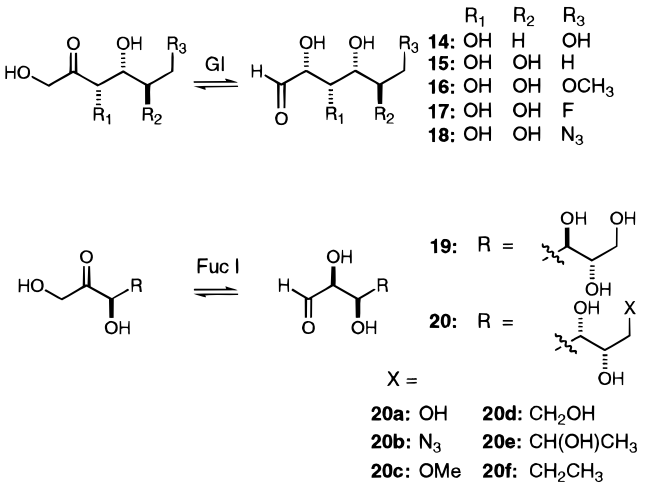


construction of diverse combinatorial carbohydrate libraries.

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 centers. This provides a new route to novel high-carbon sugars which are difficult to obtain from either chemical syntheses or natural sources. A number of these compounds have been synthesized, including analogs of sialic acid and KDO (e.g. **12**, **13**) (Scheme 7).[54]

As shown by the examples above, DHAP-dependent aldolases generate several types of ketose monosaccharides. However, most of the important naturally occurring carbohydrates are aldoses. One strategy to convert aldolase-generated ketoses to aldoses is the application of isomerases. Glucose isomerase[59] [GI, or xylose isomerase (EC 5.3.1.5)] catalyzes the isomerization of fructose to glucose and is used in the food industry for the production of high fructose corn syrup. GI also accepts fructose analogs that are modified at the 3, 5, and 6 position. Various FDP aldolase products can be isomerized to a mixture of the ketose and aldose and subsequently separated. A series of 6-substituted glucoses (**14**−**18**) were synthesized using this FDP aldolase/GI methodology (Scheme 8).[49,53] Fuc isomerase (Fuc I, EC 5.3.1.3) and

Scheme 8



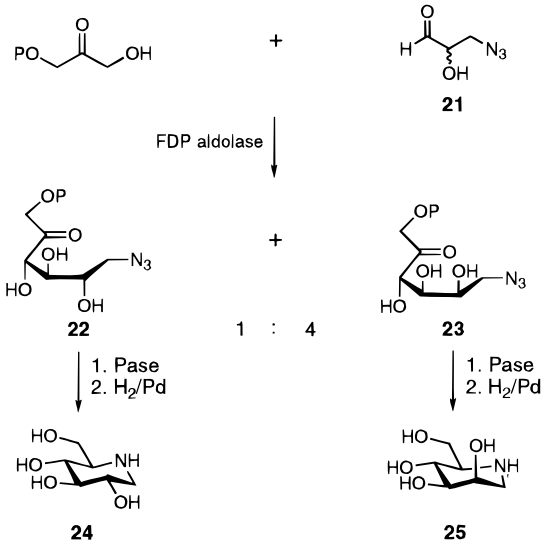
Rha isomerase (Rha I; EC 5.3.1.14) have also been cloned and overexpressed.[48] Fuc isomerase has been used to prepare L-glucose (**19**), L-galactose (**20a**), and various L-galactose and L-fucose derivatives, such as 6-azido-6-deoxy-L-galactose (**20b**), 6-*O*-methyl-L-galactose (**20c**), 6-hydroxymethyl-L-fucose (**20d**), 6-hydroxy-6-methyl-L-fucose (**20e**), and 6-methyl-L-fucose (**20f**) from the corresponding L-glyceraldehyde derivative and DHAP by combination with Fuc-1-P aldolase and Rha aldolase, respectively.[60]

Another method for conversion of ketoses to aldoses is through the so-called "inversion strategy".[61] Monoprotected dialdehydes were used as substrates for aldolase to generate protected aldehyde ketoses, which were then stereoselectively reduced, either enzymatically[41,62] or chemically.[61] Subsequent deprotection of the aldehyde afforded the aldoses.

2.2.b. Aza and Thio Sugars. Aza sugars have become increasingly important targets due to their potential value as enzyme inhibitors and therapeutical agents.[63] The direct extension of the aldolase strategy has led to one of the most effective and practical routes for the synthesis of aza sugars.[64] Ketoses containing a suitable amine synthon such as an azido group can be generated by enzymatic aldol condensation. Unmasking of the amine functions followed by reductive amination by a palladium-mediated hydrogenation results in the production of aza sugars. A detailed study on the use of various amine synthons containing aldehydes for FDP aldolase demonstrated that the best substrates are those containing an azido group.[65] Besides DHAP-dependent aldolases, other aldolases, such as 2-deoxyribose 5-phosphate aldolase and pyruvate dependent aldolases, can also be used. On the basis of this general strategy, a variety of aza sugars is now readily available. Slight modification of this general strategy leads to the preparation of thio sugars.

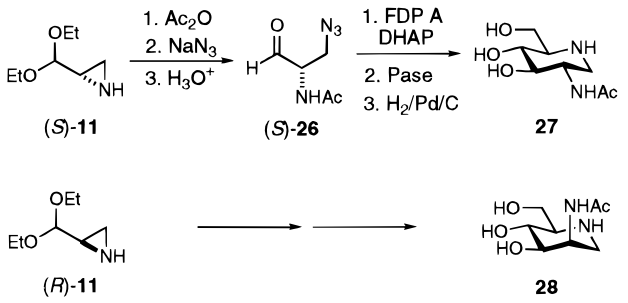
Deoxynojirimycin (**24**) and deoxymannojirimycin (**25**) are potent glycosidase inhibitors. They were readily prepared with RAMA-catalyzed C−C bond formation as the key step.[66,67] Aldol condensation of racemic 3-azido-2-hydroxypropanal (**21**) and DHAP gave diastereomeric 6-azido ketoses **22** and **23** (Scheme 9). Subsequent removal of phosphate by

Scheme 9



acid phosphatase followed by reductive amination gave the products **24** and **25** in a 1:4 ratio in favor of the manno derivative. This result is consistent with

Scheme 10

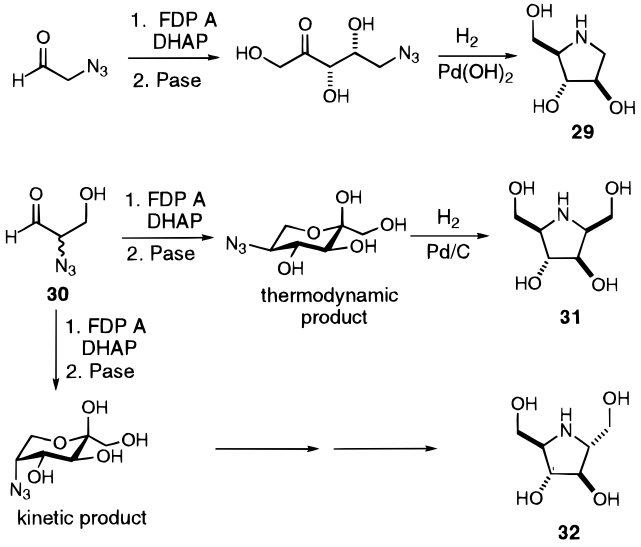


the observation that D-aldehydes are better substrates than L-aldehydes for FDP aldolase. Aza sugars **24** and **25** were also prepared stereoselectively with the respective optically pure azido aldehydes which were obtained in >98% ee by LP-80-catalyzed resolution of racemic acetal precursor.[30]

Aza sugars **27** and **28** corresponding to *N*-acetylglucosamine and *N*-acetylmannosamine have been prepared analogously by a RAMA-catalyzed aldol condensation/reductive amination procedure (Scheme 10).[68] The required substrates, (*S*)- and (*R*)-3-azido-2-acetamidopropanal (**26**), were synthesized from (*S*)- or (*R*)-aziridine (**11**) derived from lipase-resolved (*R*)- and (*S*)-3-azido-2-hydroxypropanal diethyl acetal. Acetylation of the nitrogen followed by regioselective ring opening with sodium azide yielded the required aldehyde skeleton.

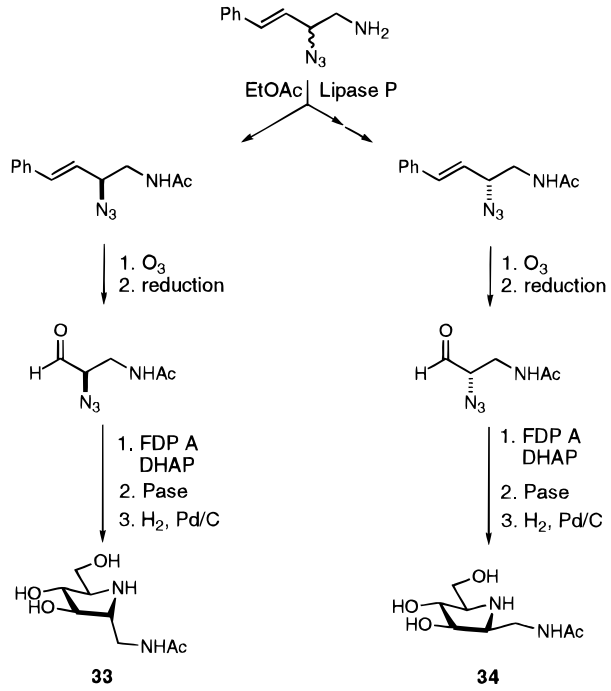
With 2-azido aldehydes as substrates, a number of polyhydroxylated pyrrolidines were consequently synthesized.[65,66,69] 1,4-Dideoxy-1,4-imino-D-arabinitol (**29**) was prepared from Cbz-protected α-aminoacetaldehyde[66] or azidoacetaldehyde,[65] and both (2*S*,5*R*)- and (2*R*,5*R*)-bis(hydroxymethyl)-(3*R*,4*R*)-dihydroxypyrrolidine (**31** and **32**, respectively) were synthesized from racemic 2-azido-3-hydroxypropanal (**30**) (Scheme 11).

Scheme 11

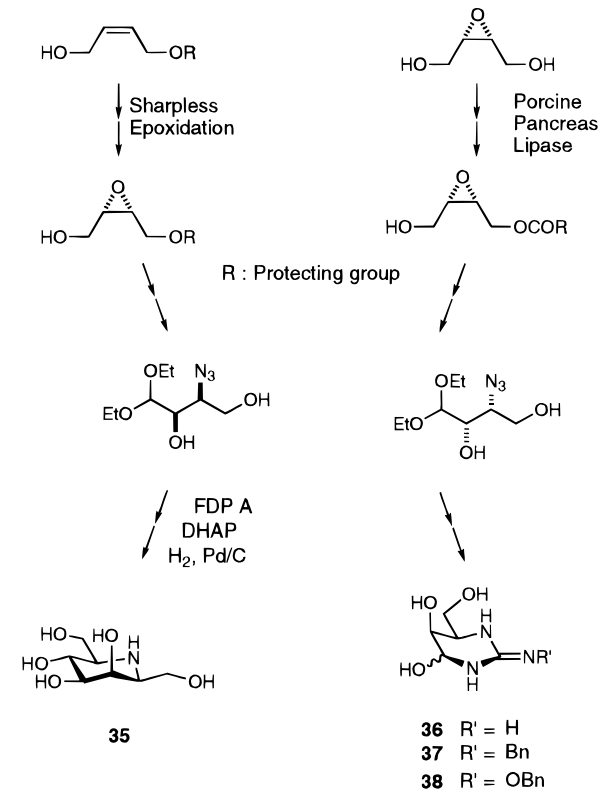


In the latter case, the thermodynamic product of the aldol addition was converted to the (2*S*,5*R*)-pyrrolidine **31**,[65] while the kinetic product gave the (2*R*,5*R*) stereoisomer **32**.[69] The pyrrolidines **33** and **34** structurally related to *N*-acetylglucosamine were prepared stereoselectively by similar transformation from lipase resolved aldehyde precursors (Scheme 12).[70]

Scheme 12



Scheme 13



Use of 3-azido-4-hydroxy aldehydes results in the formation of homoaza sugars (e.g. **35**) (Scheme 13).[71] The optically pure aldehydes can be obtained either by Sharpless epoxidation of the olefins[71b] or enzymatic resolution of the epoxides.[72] The lipase-resolved material was also used to prepare another class of glycosyl cation mimics, the tetrahydropyrimidines (**36**−**38**).[73,74] These compounds exist in equilibrium with their guanidinotetrose forms which are predominant at low pH. The tetrahydropyrimidine forms are potent inhibitors of α-galactosidase

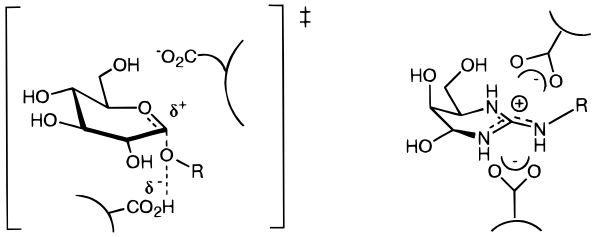


Figure 2.

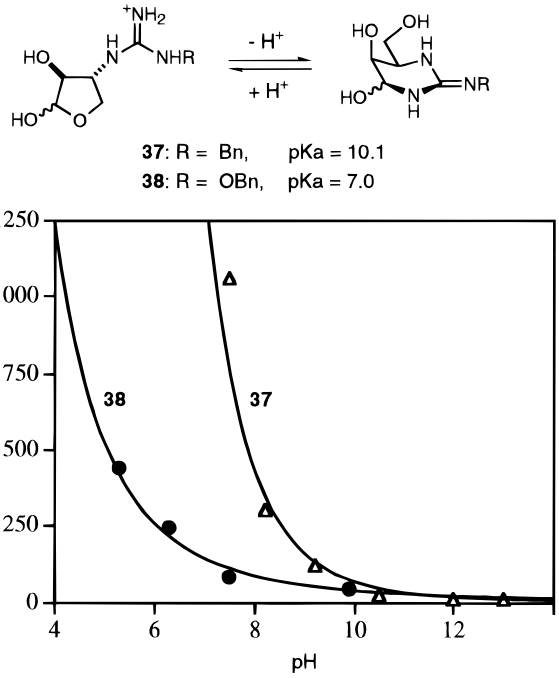


Figure 3.

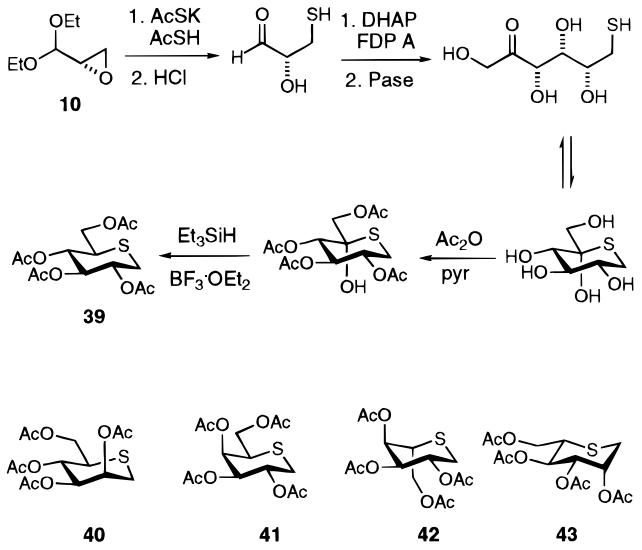
due to their close resemblance to the half-chair conformation of the transition state present in the galactosidase reaction (Figure 2). Interestingly, a newly developed inhibitor **38** with an OBn group attached to the nitrogen has a much lower pK_a and inhibits the enzyme near physiological pH, in contrast to the others which require higher pH for similar inhibition (Figure 3).[74]

In an attempt to prepare seven-membered aza sugars with 4-azido aldehydes, the reaction produced a six-membered amino sugar instead.[75] Direct reductive amination of the aldol products prior to removal of the phosphate group leads to the formation of 6-deoxy aza sugars and their analogs.[68] Reduction is thought to proceed through the imine 6-phosphate intermediate instead of reduction of the aza sugar 6-phosphate; glucose 6-phosphate was not reduced under the same conditions.

The Pd-catalyzed reductive amination of azido ketose is stereoselective. The hydrogen is delivered to the presumed imine intermediate from the face opposite to the axial-OH groups based on steric reasons, or from the face that affords the least torsional strain upon rehybridization of the sp^2 center. The hydroxyl-directing effect usually takes precedence.

Similar to the synthesis of aza sugars, a series of deoxy thio sugars was prepared by aldol condensation of thioaldehydes and DHAP followed by reduction of the resulting thioketoses[76] (Scheme 14). Regioselective ring opening of the (*S*)-glycidaldehyde diethyl acetal (**10**) with potassium thioacetate introduced the thio function. RAMA-catalyzed aldol condensation followed by dephosphorylation gave the corresponding thioketose,[77] which was then acetylated and reduced to the 1-deoxy-5-thio-D-glucopyranose peracetate (**39**).[76] Similarly, 1-deoxy-5-thio-D-mannopyranose (**40**) was obtained from the other aldehyde enantiomer, while Fuc-1-P aldolase-catalyzed reaction provided 1-deoxy-5-thio-D-galactopyranose (**41**) and 1-deoxy-5-thio-L-altropyranose (**42**), and Rha aldolase catalyzed-reaction produced 1-deoxy-5-thio-L-mannopyranose (**43**).[76]

Scheme 14



2.2.c. Other Chemoenzymatic Applications. The products from the FDP aldolase-catalyzed reaction have also been used to synthesize a variety of other compounds. These include the α-keto acid sugar 3-deoxy-D-*arabino*-heptulosonic acid (DAHP) (**44**),[78] the beetle pheromone (+)-*exo*-brevicomin (**45**),[79] the homo-C-nucleoside **46**,[80] the C-glycosides **47**[81,82] and **48**,[83] and the cyclitol **49** (Figure 4). The aldolase was used in the key step to establish the desired chirality in the target molecules. Although the synthesis of all the compounds in Figure 4 is based

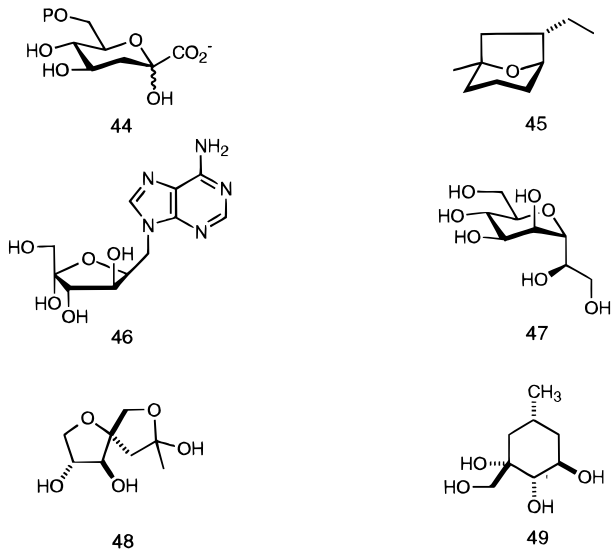
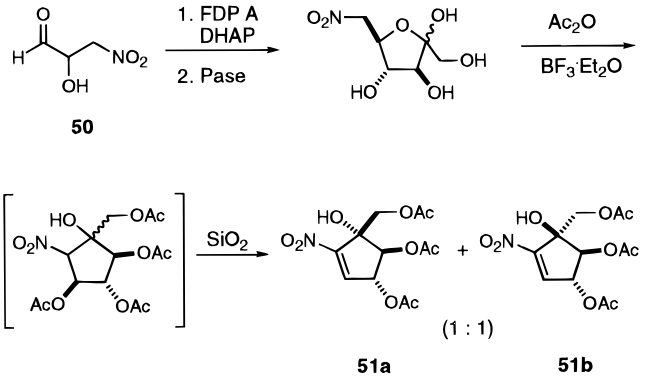


Figure 4.

Scheme 15



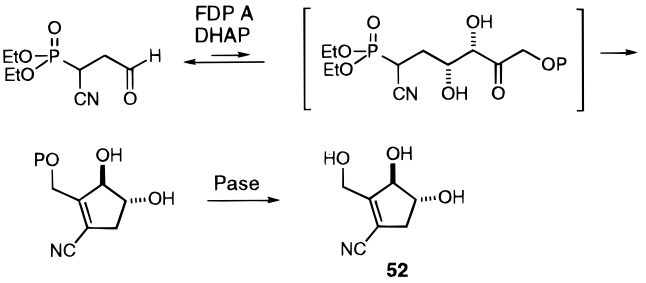
50 51a 51b

on aldol reactions catalyzed by FDP aldolase, stereoisomeric derivatives can be obtained by using other DHAP-dependent aldolases.

Cyclitols are an interesting class of biologically active compounds and the use of aldolases provides a chemoenzymatic strategy to their synthesis.[84] An example is the synthesis of nitrocyclitols **51a** and **51b**, which was accomplished by FDP aldolase-catalyzed reaction with nitroaldehyde **50**, followed by a nonenzymatic intramolecular nitro−aldol reaction (Scheme 15).[84a]

Recently, a one-pot synthesis of the cyclitol **52** was reported, involving a FDP aldolase-catalyzed reaction between a phosphonate aldehyde and DHAP. The aldol product cyclized *in situ* via an intramolecular Horner−Wadsworth−Emmons olefination to give the polyhydroxylated cyclopentene **52** (Scheme 16).[84b] Using this approach, different functionalized cyclitols may become easily accessible.

Scheme 16



52

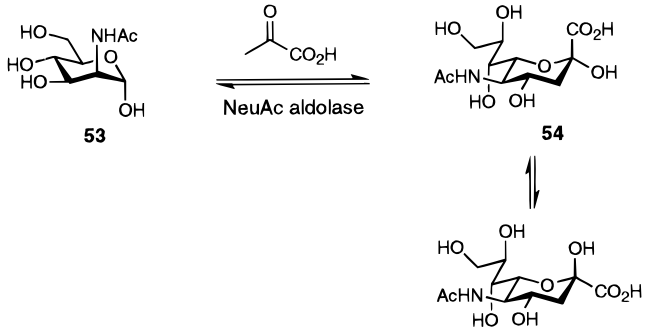
3. Pyruvate- and Phosphoenolpyruvate-Dependent Aldolases

For thermodynamic reasons, pyruvate-dependent aldolases have catabolic functions *in vivo*, whereas their counterparts employing phosphoenolpyruvate as the donor substrate are involved in the biosynthesis of keto acids. Both classes of enzymes can be used to prepare similar keto acid products *in vitro* and they are discussed jointly in the following for the sake of simplicity.

3.1. N-Acetylneuraminic Acid Aldolase and NeuAc Synthetase

In vivo, the enzyme *N*-acetylneuraminic acid (NeuAc) aldolase (EC 4.1.3.3), also named sialic acid aldolase, catalyzes the reversible aldol reaction of *N*-acetyl-D-mannosamine [ManNAc (**53**)] and pyruvate.[85] The α anomer of ManNAc is the substrate

Scheme 17



53 54

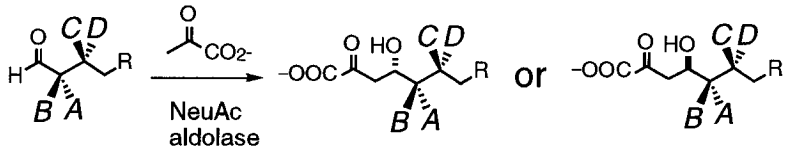
and the less stable α anomer of *N*-acetyl-5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (NeuAc, **54**, a sialic acid) is released after the enzymatic reaction (Scheme 17).[86]

The retro-aldol reaction is favored with an equilibrium constant of 12.7 M^{-1}, as the enzyme has a catabolic function *in vivo*. When the aldolase is used for synthetic purposes, an equilibrium favoring the condensation product is usually induced by providing pyruvate in excess.[87] NeuAc aldolase has been isolated from bacteria and mammals, and in both cases, it is a Schiff base-forming type I aldolase. The imidazole ring of a histidine residue is presumed to protonate the aldehyde group of the acceptor substrate.[88] NeuAc aldolase can be used in solution, enclosed in a dialysis membrane[89] or in immobilized forms.[90] The enzyme is active between pH 6 and 9, has a temperature optimum of 37 °C, and is stable under oxygen.[87,91] The enzymes from *Clostridia* and *E. coli* are now commercially available (Toyobo), and the enzyme from *E. coli* has been cloned and overexpressed.[92]

Synthetic studies have shown that a high conversion (>80%) of ManNAc (**53**) to NeuAc (**54**) was achieved using the isolated enzyme, although several equivalents of pyruvate were required. Purification of the free sialic acid product can best be achieved by decomposing excess pyruvate with pyruvate decarboxylase.[93] For reactions performed at small scale a work-up procedure involving acid-catalyzed esterification of the products followed by simple silica gel chromatography of the resulting sialic acid methyl ester has been proposed.[94] Alternatively, the need for employing an excess of pyruvate can be avoided by coupling the NeuAc synthesis to a second step, which leads to a thermodynamically more stable product. For example, NeuAc aldolase reactions were coupled with a sialyltransferase reaction to produce sialylsaccharides.[95] A variant of this method started from a mixture of ManNAc and GlcNAc, whereby GlcNAc was epimerized to ManNAc chemically[96] or enzymatically with *N*-acetylglucosamine 2-epimerase.[97] Recently, it has also been demonstrated that the equilibrium can be shifted toward product formation, if the reactions are run at high substrate concentrations.[97]

With regard to its substrate specificity, NeuAc aldolase has been shown to be specific for pyruvate as the donor substrate. Acetylphosphonate, 3-fluoropyruvate, 3-hydroxybutanoate, 2-oxobutyrate, and 3-bromopyruvate are not accepted.[98] The enzyme is remarkably flexible to a variety of acceptor substrates, including hexoses, pentoses and tetroses, in

Table 7. Reactivity and Stereochemical Outcome in NeuAc Aldolase-Catalyzed Reactions



Product of *si* face attack Product of *re* face attack

A	*B*	*C*	*D*	examples	reactivity[a]	face of attack
NHAc	H	OH	H	*N*-acetyl-D-mannosamine	+++	*si*
				6-*O*-acetyl-*N*-acetyl-D-mannosamine	+++	*si*
				6-*O*-lactyl-*N*-acetyl-D-mannosamine	+++	*si*
				6-*O*-(dimethylphosphinyl)-*N*-acetyl-D-mannosamine	+++	*si*
				6-deoxy-6-fluoro-*N*-acetyl-D-mannosamine	+++	*si*
				6-deoxy -6-azido-*N*-acetyl-D-mannosamine	+++	*si*
				5-deoxy -*N*-acetyl-D-mannosamine	+++	*si*
				5-*O*-methyl-*N*-acetyl-D-mannosamine	+	*si*
OH	H	OH	H	D-mannose	+++	*si*
				6-*O*-acetyl-D-mannose	+++	n.d.
				L-gulose	+++	*si*
				D-talose	+++	*si*
				L-allose	+++	*si*
				D-rhamnose	+++	*si*
				5-deoxy -D-mannose	+++	*si*
				4-deoxy -D-mannose	+++	*si*
				4,6-dideoxy-4,6-difluoro-D-talose	+++	*si*
				D-lyxose	++	*si*
				L-erythrose	++	*si*
H	H	OH	H	2-deoxy -D-glucose	+++	*si*
				2-deoxy -D-galactose	+++	n.d.
				2,6-dideoxy-D-glucose	+++	*si*
NHCbz	H	OH	H	*N*-Cbz-D-mannosamine	+++	*si*
N_3	H	OH	H	2-azido-2-deoxy -D-mannose	+++	*si*
Ph	H	OH	H	2-deoxy -2-*C*-phenyl-D-mannose	+++	*si*
OH	H	H	H	3-deoxy -D-mannose	+	
OH	H	N_3	H	3-azido-3-deoxy -D-mannose	-	
OH	H	NH_2	H	3-amino-3-deoxy -D-mannose	-	
OH	H	Br	H	3-bromo-3-deoxy -D-mannose	-	
N_3	H	N_3	H	2,3-diazido-2,3-dideoxy-D-mannose	-	
N_3	H	Cl	H	2-azido-3-chloro-2,3-dideoxy-D-mannose	-	
H	OH	OH	H	D-glucose	++	*si*
				L-fucose	++	n.d.
				L-arabinose	++	*si*
				D-xylose	++	*si*
				L-threose	+	n.d.
H	F	OH	H	2-deoxy -2-fluoro-D-glucose	++	*si*
H	NHAc	OH	H	*N*-acetyl-D-glucosamine	-	
H	OH	Br	H	3-bromo-3-deoxy -D-glucose	-	
OH	H	H	OH	D-altrose	++	*re*[b]
				D-arabinose	++	*re*[b]
				L-xylose	+	*re*[b]
				D-threose	++	*re*
H	OH	H	OH	L-mannose	++	*re*
				L-rhamnose	++	*re*
				D-allose	+	n.d.
				L-talose	++	*re*
				D-gulose	++	*re*[b]
				D-ribose	++	*re*[b]
				D-erythrose	+	n.d.
H	NHAc	H	OH	*N*-acetyl-L-mannosamine	++	*re*
H	N_3	H	OH	2-azido-2-deoxy-L-mannose	+	*re*[b]
H	H	H	OH	2-deoxy -L-glucose	++	*re*
				2-deoxy -L-rhamnose	++	*re*

[a] +++, good substrate; ++, weak substrate; +, very weak substrate; -, no substrate. [b] Partial *re* attack only. n.d., not determined. (Ref. 90,93,94,95,98,99.)

both D- and L-forms. Over 60 aldoses have to date been demonstrated to be accepted by the enzyme (see Table 7).[90,93−95,99] Substitutions at C(4), C(5) and C(6) of ManNAc (**53**) are allowed with a slight preference for the absolute stereochemistry to be the same as in **53**. The C(2) substituent is critical both in terms of size and stereochemistry (see Table 7) and a free hydroxyl at C(3) is a prerequisite for successful aldol condensation.[94]

In contrast to the strict enzymatic control of the stereochemical course usually encountered with aldolases, the stereochemical outcome of NeuAc aldolase-catalyzed reactions depends on the structure of the substrate (Table 7). With most substrates [e.g. the natural substrate D-ManNAc(**53**)] the carbonyl group is attacked from the *si* face, which results in the formation of a new stereogenic center with *S* configuration. With some substrates [e.g. L-mannose (**55**), D-altrose, L-xylose], on the other hand, the stereochemical course of the reaction can be reversed: the enamine attacks the carbonyl component from the *re* face and the C(4) center of the resulting sialic acid has the *R* configuration. The stereochemical outcome of the reaction seems to be under thermodynamic control. In all cases, where *re* attack has been shown to occur partially or exclusively, the resulting *R* configurated product is the thermodynamically more stable species with the hydroxy group at C(4) in the equatorial position. In general, reactions involving *si* face attack (the normal case) proceed faster than those involving *re* face attack. ^{1}H NMR studies of the product distribution during the course of the reactions also indicate that *re* face attack is kinetically disfavored. In cases, where the product resulting from *re* face attack is the more stable compound, the product distribution changes during the course of the reaction. With L-mannose (**55**), for example, the ratio C(4)-*S*-product versus C(4)-*R*-product has been demonstrated to change from 3:5 after 1.5 h to 1:10 after 7 days.[93] A recently proposed active-site model of the enzyme rationalizes the observed kinetic behavior by invoking the occurrence of boatlike intermediates in reactions giving C(4)-*R*-products.[94]

Several biologically interesting L-sugars, including L-NeuAc (**56**), L-KDO (**58**), and L-KDN (**57**), were synthesized by taking advantage of the stereochemical preferences of the enzyme (Figure 5). With C_5-aldoses usually mixtures of C(4)-*S*- and C(4)-*R*-products were obtained, as for example in the NeuAc aldolase-catalyzed synthesis of KDO (**60**) from D-arabinose (**59**) as the acceptor substrate.[99d] When an excess of D-arabinose was provided, the equilibrium was shifted toward formation of the C(4)-*R*-product **60**.[99i] Reactions with the C_4-aldoses L-erythrose (**61**) and D-threose (**62**) as substrates, on the other hand, were stereospecific (Figure 5).[94] Recently, a sequential aldol addition sequence was developed, which involved the use of DERA to generate aldehydes, which were then further transferred into C(4)-*R*-keto acids using NeuAc aldolase (see section 4).[100]

Due to the biological importance of sialic acids and the commercial availability of NeuAc aldolase, various NeuAc derivatives were synthesized enzymatically. For example, an efficient synthesis of 9-*O*-acetyl-NeuAc was developed by regioselective irreversible acetylation of ManNAc catalyzed by subtilisin followed by NeuAc aldolase-catalyzed condensation of the resulting 6-*O*-acetyl-ManNAc with pyruvate.[98] This two-step enzymatic synthesis provided 9-*O*-acetyl-NeuAc in ca. 80% yield. A similar procedure was applied in the preparation of 9-*O*-lactyl-NeuAc.[98,99f] Also involving the use of subtilisin and NeuAc aldolase, the 9-*O*-glycyl-NeuAc derivative **63** was prepared, which was then converted into the fluorescent sialic acid **64** (Scheme 18).[101]

Other examples for NeuAc aldolase-mediated syntheses of biologically interesting sialic acids include the synthesis of the α-methyl ketoside of an *N*-unprotected NeuAc for use in the preparation of *N*-substituted NeuAc derivatives.[99c] Also, polyacrylamides with attached α-sialoside groups[102] and polymers of *C*-linked sialosides were prepared, which strongly inhibit agglutination of erythrocytes by influenza virus.[103] Aza sugars were also prepared with NeuAc aldolase: condensation of pyruvate with *N*-Cbz-D-mannosamine (**65**), followed by a reductive

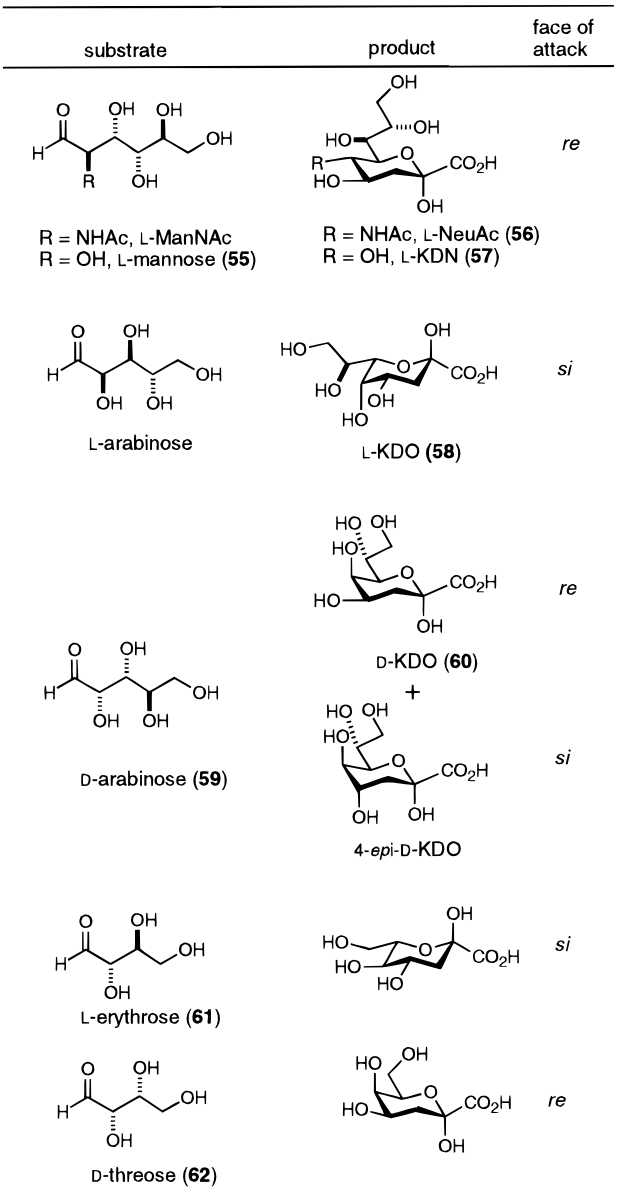
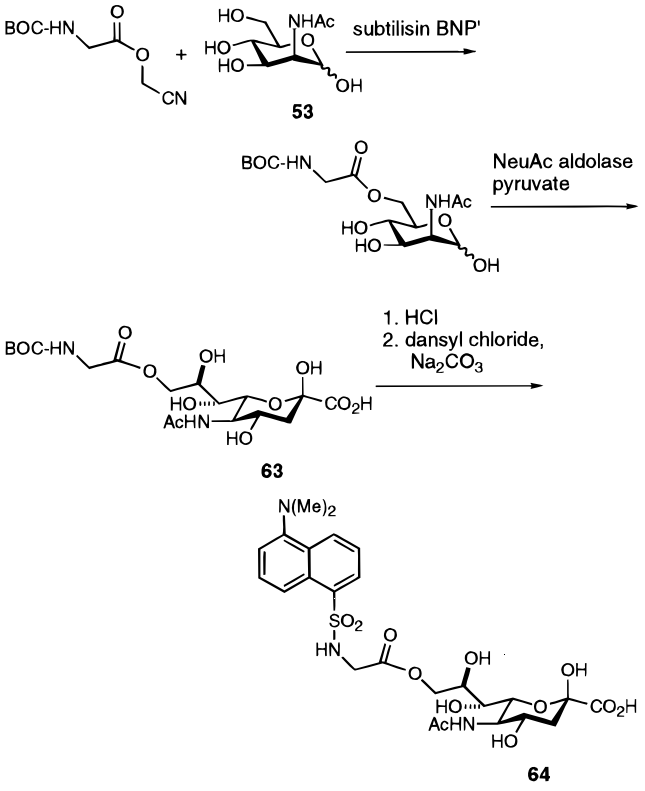


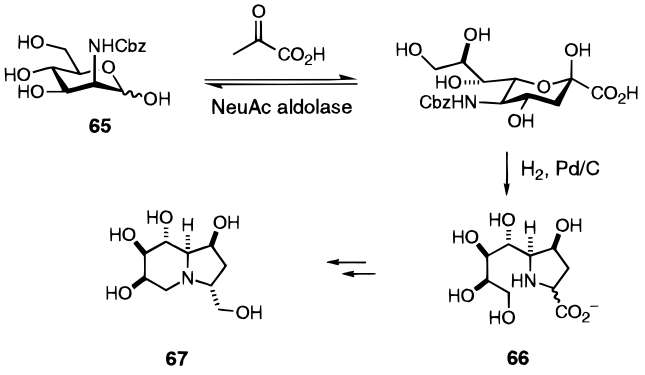
Figure 5.

Scheme 18



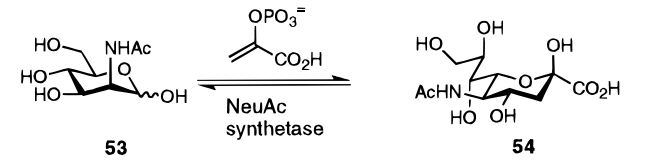
amination of the aldolase product gave the pyrrolidine **66**, which was further converted to 3-(hydroxymethyl)-6-epicastanospermine (**67**) (Scheme 19).[99h]

Scheme 19



The synthesis of NeuAc (**54**) *in vivo* is accomplished by NeuAc synthetase (EC 4.1.3.19) through the irreversible condensation of PEP and *N*-acetylmannosamine (**53**) (Scheme 20).[91] This enzyme has not

Scheme 20

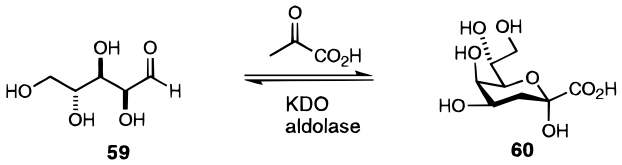


yet been isolated and characterized; it might nevertheless prove synthetically useful in the future, as the forward reaction is favored thermodynamically.

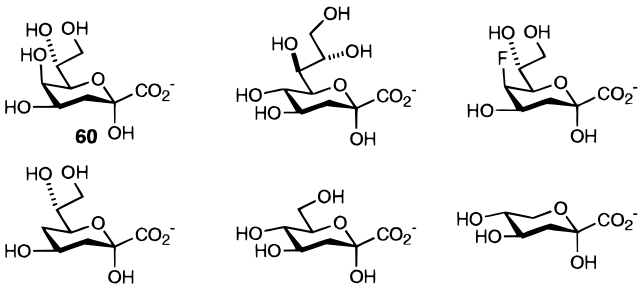
3.2. 3-Deoxy-D-manno-2-octulosonate (KDO) Aldolase and 3-Deoxy-D-manno-2-octulosonate 8-Phosphate (KDO-8-P) Synthetase

3-Deoxy-D-*manno*-2-octulosonate aldolase (EC 4.1.2.23), also named 2-keto-3-deoxyoctanoate (KDO) aldolase, is the enzyme responsible for the degradation of KDO *in vivo*. Since KDO (**60**) and its activated form CMP-KDO are key intermediates in the biosynthesis of the outer membrane lipopolysaccharide (LPS) of gram-negative bacteria,[104] analogs of KDO may inhibit LPS biosynthesis or interact with the LPS binding protein.[105] KDO aldolase catalyzes the reversible condensation of pyruvate with D-arabinose (**59**) to form KDO with an equilibrium constant in the cleavage direction of 0.77 M^{-1} (Scheme 21).

Scheme 21

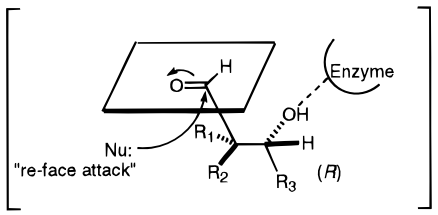


Compounds synthesized on preparative-scale using KDO aldolase:



The enzyme has been isolated and purified from *E. coli* [106] and *Aerobacter cloacae*.[107] Preliminary investigations of the substrate specificity indicated a high specificity for KDO in the direction of the cleavage. The condensation reaction, on the other hand, proceeded with some flexibility: various unnatural substrates, namely D-ribose, D-xylose, D-lyxose, L-arabinose, D-arabinose 5-phosphate, and *N*-acetylmannosamine, were found to be accepted by the aldolase, albeit with relative rates of <5% compared to D-arabinose.[106] More recent studies on the substrate specificity showed that the KDO aldolase from *Aureobacterium barkerei*, strain KDO-37-2, accepted an even wider variety of aldose substrates, including hexoses, pentoses, tetroses, and trioses, among them pentoses and tetroses being the best substrates.[108] The enzyme was found to be specific for substrates having a *R*-configuration at C(3), whereas the stereochemical requirements at C-2 were less stringent. Under kinetic control, the C(2)-*S*-configuration is favored while the C(2)-*R*-configuration is thermodynamically favored (Figure 6).

Various aldol addition reactions were conducted on a preparative scale including the synthesis of the parent compound KDO, which was obtained in 67% yield.[108] In all cases observed so far, pyruvate attacked the aldose substrate stereoselectively on the *re* face of the carbonyl group.

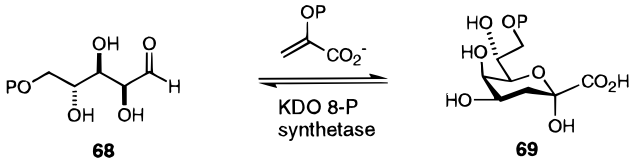


R_1 = OH; R_2 = H (*S*); kinetically favored
R_1 = H; R_2 = OH (*R*); thermodynamically favored

Figure 6.

3-Deoxy-D-*manno*-2-octulosonate 8-phosphate synthetase (EC 4.1.2.16), also named phospho-2-keto-3-deoxyoctanoate (KDO 8-P) synthetase, participates in the biosynthesis of bacterial lipopolysaccharides by catalyzing the irreversible aldol reaction of PEP and D-arabinose 5-phosphate (**68**) to give KDO 8-P (**69**) (Scheme 22).[109]

Scheme 22



68 **69**

The enzyme has been isolated from *E. coli* B[110] and *Pseudomonas aeruginosa*.[111] The *E. coli* enzyme has been cloned and overexpressed in *E. coli* and *Salmonella typhimurium*.[112] Little is known about the substrate specificity of KDO 8-P synthetase, but initial studies suggest that this enzyme is highly specific for its natural substrates. A preparative scale synthesis of KDO 8-P itself has been reported, which involved the use of KDO 8-P synthetase.[110] The required starting material, D-arabinose 5-phosphate, can be prepared either by an isomerase-catalyzed isomerization of D-ribose 5-phosphate[106] or by the hexokinase-mediated phosphorylation of arabinose.[111]

3.3. Other Pyruvate- and Phosphoenolpyruvate-Dependent Aldolases

A variety of other keto acids producing aldolases has not been extensively investigated for their use in synthesis to date, but nevertheless hold some promise for future applications. These enzymes include 3-deoxy-D-arabino-2-heptulosonic acid 7-phosphate (DAHP) synthetase (EC 4.1.2.15), 2-keto-4-hydroxyglutarate (KHG) aldolase (EC 4.1.2.31), 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase (EC 4.1.2.14), and 2-keto-3-deoxy-D-gluconate (KDG) aldolase (EC 4.1.2.20); their *in vivo* reactions are illustrated in Table 2.

DAHP synthetase has been employed for the straightforward synthesis of DAHP (**72**) on preparative scale (Scheme 23).[113] The multienzyme system contained transketolase, which generated D-erythrose 4-phosphate (**71**) from Fru 6-P (**70**) in the presence of D-ribose 5-phosphate. Fru 6-P, in turn, was generated from D-fructose and ATP through a hexokinase-catalyzed reaction in the presence of an ATP regeneration system. This efficient one-pot synthesis was subsequently even more simplified by the results of further studies indicating that it was more efficient and economical to use whole cells containing a DAHP synthetase plasmid.[114] Such a system conveniently provides all the enzymes required for the synthesis of DAHP.

KHG aldolase displays a somewhat atypical substrate specificity, when compared to other aldolases, in that various pyruvate derivatives are accepted as donor substrates by this enzyme.[115]

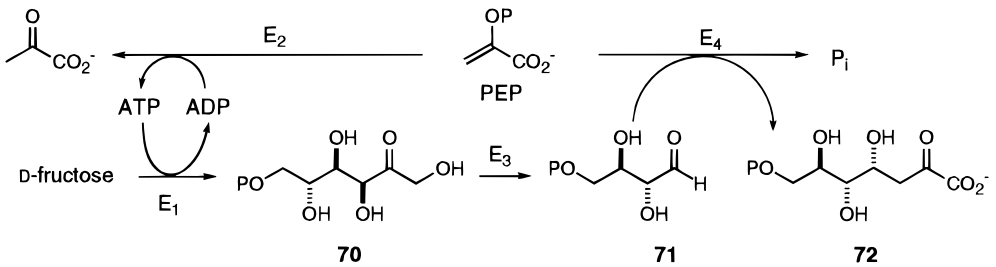
KDPG aldolase from *P. fluorescens* was shown to accept various unnatural aldehydes, albeit at slow rates (<5%) when compared to the natural substrate.[116] The new stereocenter at C(4) consistently had the *S*-configuration.

By using KDG aldolase from *Aspergillus niger*, 2-keto-3-deoxy-D-gluconate was synthesized on preparative scale with a de of 92.[117]

4. 2-Deoxyribose-5-phosphate Aldolase

In vivo 2-deoxyribose-5-phosphate aldolase (DERA, EC 4.1.2.4) catalyzes the reversible aldol reaction of acetaldehyde and D-glyceraldehyde 3-phosphate (G3P) to form 2-deoxyribose 5-phosphate (**73**) (Scheme 24). DERA is a type I aldolase which has been isolated from animal tissues[118] and microorganisms.[119] It has been overexpressed in *E. coli* and large quantities of the enzyme can easily be obtained.[120−122] Under reaction conditions with a temperature of 25 °C and pH 7.5 the enzyme is fairly stable, retaining 70% of its activity after 10 days. In addition to acetaldehyde, DERA is also able to accept propanal, acetone, and fluoroacetone as donor substrates, albeit at a much slower rate. Due to the high specific activity of the overexpressed enzyme[121] products from slowly reacting substrates can still be rapidly obtained. The acceptor substrates have very little structural requirements. 2-Hydroxyaldehydes appear to react the fastest, with D-isomers being preferred over L-isomers, making selective formation of products from a racemic mixture of acceptor substrates possible. The

Scheme 23



70 **71** **72**

E_1) Hexokinase; E_2) Pyruvate kinase; E_3) Transketolase + D-ribose 5-P; E_4) DAHP synthetase

Scheme 24

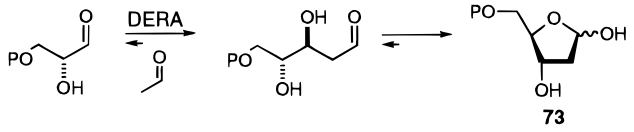
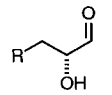
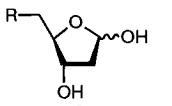
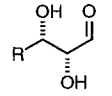
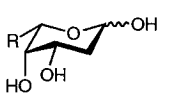
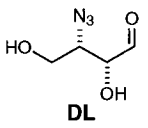
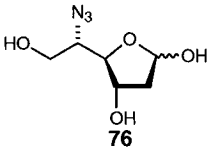
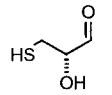
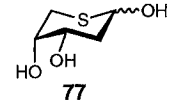
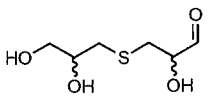
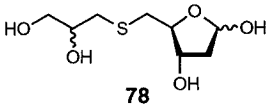
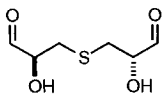
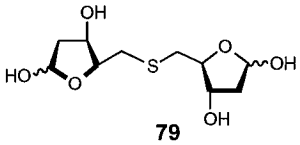
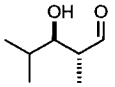
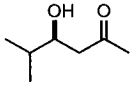
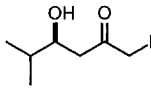
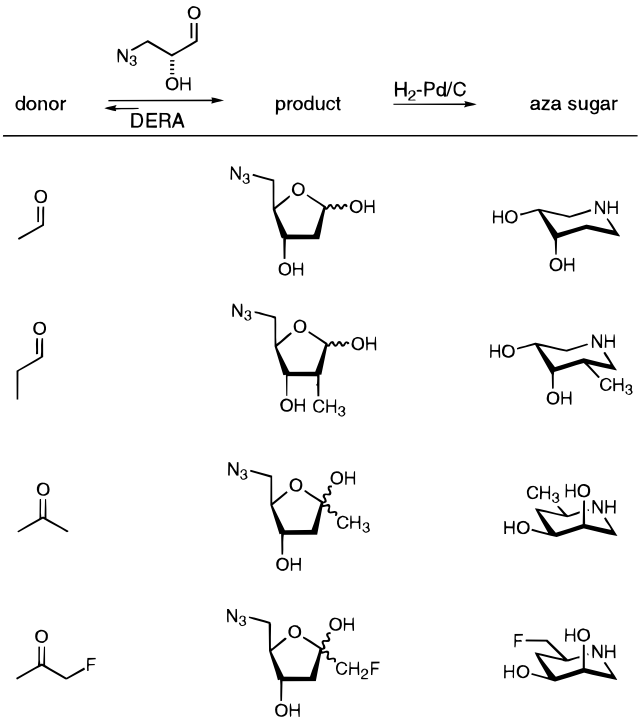


Table 8. Reactions Catalyzed by DERA

acceptor	donor	product
		 **74** R = H R = Br; R = F R = OH; R = Cl R = CH_3
		 **75** R = CH_3; R = CH_2OH; R = Ph
 **DL**		 **76**
		 **77**
		 **78**
		 **79**
		
		
		

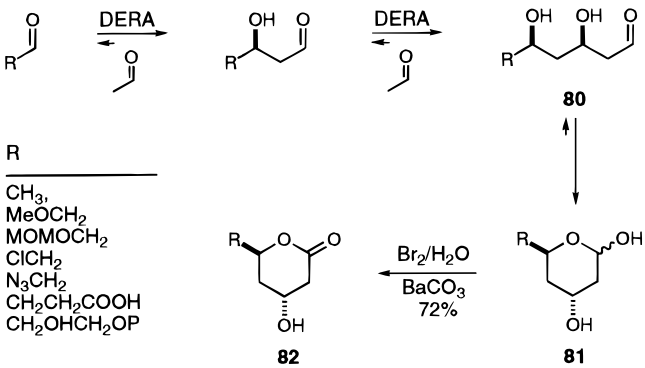
stereochemistry of the newly generated chiral center is strictly determined by the enzyme, in general giving products with the *S*-configuration. In Table 8 a variety of products are shown that have been prepared via DERA-catalyzed aldol condensation. These include 2-deoxy sugar analogs **74**−**76**, thio sugars **77** and **78**, and the glycolipid precursor **79**. DERA-catalyzed aldol condensation of 2,3-dihydroxybutyraldehyde with acetaldehyde gave 2-deoxy-L-fucose (**75**, R = CH_3), a constituent of several antibiotics. Other alkylated D-*threo* 2,3-dihydroxy aldehydes, which can be easily prepared via Sharpless asymmetric dihydroxylation of the corresponding α,β-unsaturated aldehydes, lead to 6-alkylated 2-deoxyfucose derivatives. 2-Deoxy-L-fucose and derivatives might be useful analogs of L-fucose, which is a terminal monosaccharide in many biologically active carbohydrates and conjugates. In Table 9 several aza sugars are given which have been prepared with DERA.

Table 9. Aza Sugars Prepared via DERA-Mediated Synthesis



When acetaldehyde is used as the donor, the products from the DERA-catalyzed reaction are aldehydes, capable of being an acceptor substrate for a second aldol condensation. This was first observed for DERA itself.[123] When α-substituted acetaldehydes were used containing functionality that will not cyclize to a hemiacetal after the first aldol reaction, the products reacted with a second molecule of acetaldehyde to form 2,4-dideoxyhexoses **80** (Scheme 25). These products cyclize to a stable hemiacetal **81**, thus stopping the polymerization after two se-

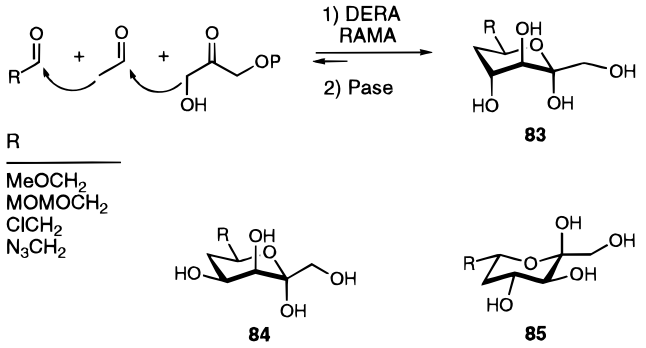
Scheme 25



quential aldol reactions. The products can be converted to derivatives of the lactone moiety of mevinic acids, which are active as cholesterol-lowering agents. This two-step synthesis provides the shortest route toward these chiral lactone functionalities **82** and uses extremely simple starting materials. The best substrate for the DERA-catalyzed sequential reaction appeared to be succinic semialdehyde (R = CH_2CH_2-COOH) which has a carboxylic acid group, mimicking the phosphate group in the natural substrate G3P.[122]

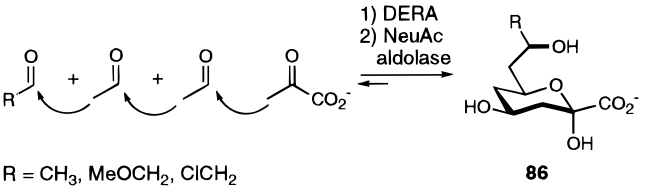
Similar one-pot sequential aldol reactions were performed by combining DERA with FDP-aldolase (Scheme 26).[100,124] The products of these reactions

Scheme 26



are 5-deoxy ketoses **83** with three substituents in axial position. Due to the formation of these thermodynamically unfavored products and the long reaction times for these reactions, some inversion of the usual stereochemistry of both DERA and FDP-aldolase was observed, leading to products of type **84** and **85**. Combination of DERA and NeuAc-aldolase gave sialic acid derivatives of type **86** (Scheme 27).[100]

Scheme 27



In this case, however, synthesis of **86** was not possible using a one-pot system, due to the incompatibility of the reaction conditions for the two enzymes.

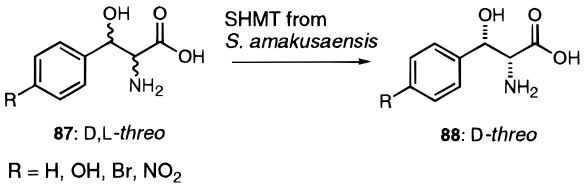
5. Glycine-Dependent Aldolases

The glycine-dependent aldolases are pyridoxal 5-phosphate-dependent enzymes which catalyze the reversible aldol reaction of glycine with an aldehyde acceptor to form a β-hydroxy-α-amino acid.[125] Two types of glycine-dependent aldolases have been found, the serine hydroxymethyltransferases (SHMT) and the threonine aldolases. *In vivo* SHMT (EC 2.1.2.1) catalyzes the reversible aldol reaction between glycine and formaldehyde to give L-serine. For this reaction the cofactor tetrahydrofolate (THF) is required, which binds nonenzymatically with formaldehyde to form N^5,N^{10}-methylenetetrahydrofolate which is then accepted by the enzyme.[125a] SHMT accepts several other aldehydes as substrate for which THF is not required. With acetaldehyde as acceptor the product L-allothreonine is formed, originally thought to be catalyzed by L-allothreonine aldolase (EC 4.1.2.6). SHMT, or L-allothreonine aldolase, has been used for the resolution of racemic *erythro* β-hydroxy α-amino acids to produce pure D-*erythro* isomers.[126] SHMT, overexpressed in *Klebsiella aerogenes*, has been successfully used for the preparation of L-serine.[127] By carefully controlling the formaldehyde concentration, an 88% conversion of glycine to serine could be achieved to give a solution containing 450 g of serine/L.[128] The synthesis of other β-hydroxy α-amino acids with SHMT has been less successful. The enzyme is selective for the L-configuration at the α-center, but in general displays poor *erythro*−*threo* discrimination. In small scale reactions the SHMT from corn and rabbit liver gave primarily compounds with the L-*erythro* configuration.[129] The enzyme from pig liver has been used in larger, preparative scale reactions; however, diastereomeric mixtures of *erythro* and *threo* compounds were obtained.[130] Due to the long reaction times, necessary to achieve reasonable yields, the kinetic preference for the L-*erythro* isomers disappeared, because of the formation of equally or more stable *threo* isomers.

Threonine aldolases catalyze the reversible aldol reaction between glycine and acetaldehyde to give threonine and both D- and L-threonine aldolases have been reported. The substrates for the L-threonine aldolases (EC 4.1.2.5) are also substrates for L-SHMT (*vide supra*) and the difference between SHMT, allothreonine and threonine aldolases is often vague. Many threonine aldolases also accept allothreonine derivatives as substrate, sometimes even preferably over compounds with the *threo* configuration.[131,132]

Threonine aldolases have been extensively used for the resolution of racemic β-hydroxy α-amino acids.[133,134] For example with a L-threonine aldolase isolated from *Streptomyces amakusaensis* several racemic mixtures of 3-(*p*-substituted-phenyl)serines **87** were resolved to give the enantiomers **88** with the D-*threo* stereochemistry in >95% ee. (Scheme 28).[135,136]

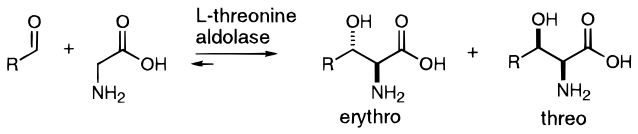
Scheme 28

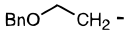
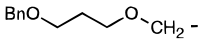
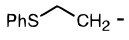
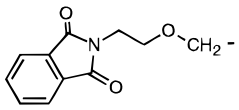


Only a few examples are known of the use of threonine aldolases in bond-forming reactions. Patents describe the use of D-threonine aldoase from *Arthrobacter* DK.19 for the synthesis of several D-β-hydroxy α-amino acids in low yield.[137] L-threonine aldolase (EC 4.1.2.5) from *Candida humicola* has been crystallized[138] and has been investigated for use in condensation reactions.[132] The enzyme accepted a broad range of aldehydes, but in general mixtures of L-*erythro* and L-*threo* products were obtained, with the L-*erythro* configuration preferred (Table 10).

Hydroxy aldehydes gave complex product mixtures because of their interaction with free amino acids. This could be prevented by the protection of the

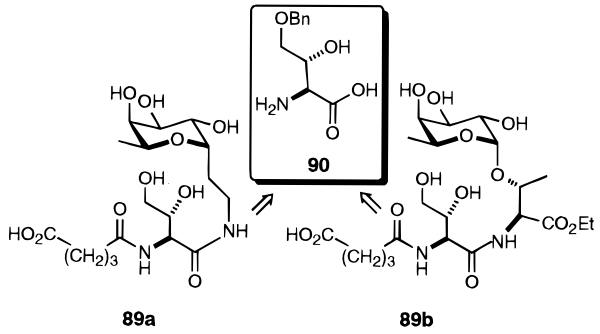
Table 10. L-Threonine Aldolase-Catalyzed Synthesis of L-β-Hydroxy α-Amino Acids



	R	Yield (%)	Ratio erythro : threo
1	CH_3 -	38	93 : 7
2	Ph -	87	60 : 40
3	N_3CH_2 -	45-75	70 : 30 to 100 : 0
4	$BnOCH_2$ -	78	92 : 8
5		53	53 : 47
6		45	92 : 8
7		80	50 : 50
8		10	86 : 14

hydroxyl groups and in this way several L-threonine and L-allothreonine derivatives with a protected hydroxyl group at C4 were prepared. Acceptor aldehydes with an oxygen functionality at the α-position of the aldehyde gave in general high *erythro/threo* ratios (entries 4, 6, and 8). The ratio was reduced when the oxygen was in the β-position (entry 5). Although α,β-unsaturated aldehydes were in general not accepted as substrates, several thiophenol derived aldehydes, e.g. **7**, were accepted, providing a route toward unsaturated amino acids. One of the best substrates was (benzyloxy)acetaldehyde (entry 4). The product of this reaction, the 4-hydroxy-L-allothreonine derivative **90**, has been used as a key intermediate in the synthesis of the potent sialyl Le^x mimetics **89a** and **89b**[139] (Scheme 29).

Scheme 29



III. Enzymatic Glycosidation

The complexity of oligosaccharide structures is a prerequisite for the participation of these compounds in finely tuned cellular recognition phenomena. To chemists working in the field, the synthesis of these complex structures has remained a challenge. One of the difficulties encountered in oligosaccharide synthesis is the task to couple building blocks in a stereoselective manner. This is in contrast to the synthesis of other naturally occurring oligomers such as peptides and oligonucleotides, where the stereochemical information is stored in the building blocks and the couplings do not involve the formation of new chiral centers. A second difficulty encountered in oligosaccharide synthesis originates from the polyfunctionality of these compounds, which necessitates the use of elaborate protective group chemistry, if complex carbohydrates are to be synthesized through conventional methods. Although considerable progress has been made recently toward the development of more sophisticated synthetic methodologies for the synthesis of glycoconjugates,[140] the chemical synthesis of biologically interesting complex carbohydrates on >1 mmol scale still appears to be a problem not yet solved. Alternatively, the enzymatic approach offers a viable alternative especially in this area, which is devoid of some of the problems encountered in chemical synthesis. Two classes of enzymes are primarily involved in carbohydrate metabolism in Nature. Glycosidases[141] participate in the catabolism of glycoconjugates, and glycosyltransferases[142] are involved in the biosynthesis of oligosaccharides. Both types of enzymes have been extensively exploited for synthesis.[143]

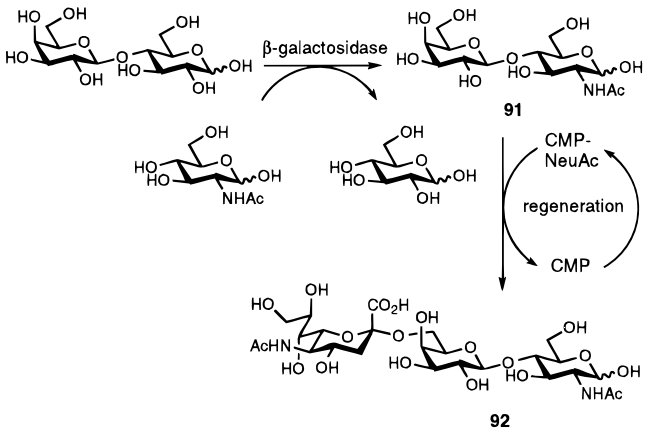
1. Glycosidases and Transglycosidases

Glycosidases cleave glycosidic bonds *in vivo*, but they can be employed as synthetic catalysts under appropriate conditions. They are readily available and inexpensive. Glycosidase-mediated synthesis of glycosides can be performed either under thermodynamically or under kinetically controlled conditions. The equilibrium approach, involving the direct reversal of the natural function of the enzymes, is conceptually simple and some examples have been reported in the early part of this century.[144] The formation of glycosides from not activated precursors is an endergonic process ($\Delta G = +4$ kcal/mol) and reaction conditions must be chosen, which drive the reaction toward glycoside production. This may be achieved to some extent either through using the substrates in high concentration[145] or through the addition of water-miscible organic cosolvents.[146] Nevertheless, the equilibrium approach suffers from poor yields, usually not exceeding 15%, and of the formation of side products. Both features may render the isolation and purification of the desired products difficult. Glycosidase-catalyzed glycoside synthesis under kinetically controlled conditions, on the other hand, relies on the formation of a reactive intermediate from an activated glycosyl donor.[146,147] The reactive species is then trapped with exogenous nucleophiles to form a new glycosidic bond. Suitable glycosyl donors for this transglycosylation reaction include di- or oligosaccharides, aryl glycosides, and glycosyl fluorides. The reactive intermediate must be trapped more rapidly by the glycosyl acceptor than by water. Indeed, enhanced rates of glycosidase-

catalyzed glycosyl cleavage have been observed in the presence of alcohols.[148] This effect has been ascribed to more effective binding of the alcohol relative to water in the active site. Another proposed rationalization is that the mechanism involves a solvent separated ion pair toward which an alcohol is a better nucleophile than water.[148] Although glycoside formation may be favored kinetically under certain circumstances, hydrolysis always remains favored thermodynamically. The reaction must therefore be carefully monitored and stopped, when the glycosyl donor is consumed, in order to minimize glycoside hydrolysis. The kinetically controlled approach has primarily been applied to the retaining glycosidases, but using glycosyl fluorides as glycosyl donors, inverting glycosidases have been used to afford products having the configuration at the anomeric position which was opposite to that of the donor.[149]

From the vast amount of glycosidase-catalyzed glycosidations reported so far (see a recent review[143b] for an extensive tabulation) it is obvious that the reactions are stereoselective processes, in contrast to many chemical methods. The regioselectivity, however, is not necessarily absolute or predictable. In general, the primary hydroxy group of the acceptor reacts preferentially over secondary hydroxy groups, resulting in a 1,6-glycosidic linkage. Some control of selectivity could be achieved by the selection of an appropriate donor/acceptor combination.[147,150] A second possibility to control the regioselectivity involves the use of glycosidases from different species. For example, the β-galactosidase from testes catalyzes the formation of β-1,3-linkages.[151] The minor products produced in this preparation were then hydrolyzed by the *E. coli* β-galactosidase which preferentially hydrolyzes β-1,6-linked galactosyl residues. The overall yield of the β-1,3-linked disaccharides was around 10−20%. *Bacillus circulans* β-galactosidase prefers 1,4-glycosidic bond formation and has been used in the large scale synthesis of *N*-acetyllactosamine (**91**). Coupled with a α-2,6-sialyltransferase, the disaccharide **91** was converted to the sialyldisaccharide **92** which is no longer subject to the glycosidase-catalyzed hydrolysis, thus improving the yield (Scheme 30).[152]

Scheme 30

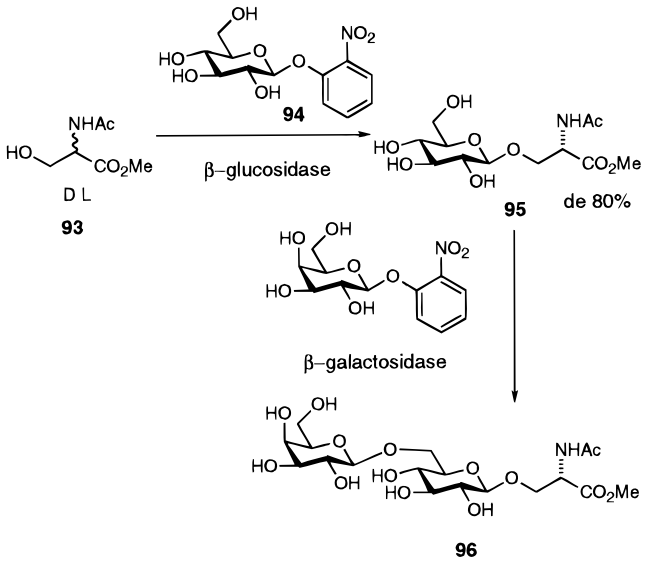


Polysaccharides have also been synthesized using kinetically controlled glycosidase reactions, as exemplified by the cellulase-catalyzed polymerization of β-cellobiosyl fluoride to form cellulose.[153]

Glycosidase-mediated glycosidation of nonsugar acceptors has also been achieved.[154] These reactions are especially interesting with chiral, racemic or meso alcoholic acceptors, as moderate to excellent diastereoselectivity were obtained.

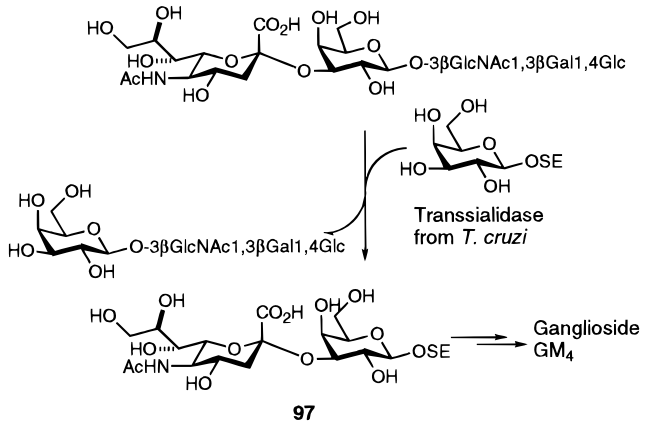
For example, using β-glucosidase with racemic AcNH-Ser-OMe (**93**) as the acceptor and β-Glc-OPh-*o*-NO_2 (**94**) as the donor, the obtained glucoside **95** was shown to have a de of 80%.[155] After β-galactosidase-mediated glycosidation, the desired glycopeptide fragment **96** was obtained (Scheme 31).

Scheme 31



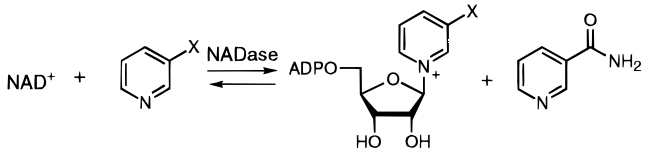
Similar to glycosidases, transglycosidases also cleave glycosidic linkages. These enzymes, however, transfer the glycosyl moiety to another acceptor with a minimal amount of hydrolysis. A transsialidase from *Trypanosoma cruzi* has been shown to reversibly transfer sialic acid to and from the 3-position of terminal β-Gal residues (Scheme 32).[156] Chains

Scheme 32



terminating in α-linked galactose are not substrates. A number of oligosaccharides containing the NeuAcα2,3Galβ-substructure (e.g. **97**) have been synthesized using this transsialidase.[157] Additionally, this enzyme has been shown to resialylate the terminal galactose units of the cell-surface glycoproteins and glycolipids of sialidase-treated erythrocytes.[158] Thus, the *T. cruzi* transsialidase potentially provides a

Scheme 33



X = -$CONH_2$, -$CSNH_2$, -$CONHNH_2$, -CO_2CH_3 (reversible reaction)
X = -$COCH_3$, -CHO, -CO_2H, -CN, I, H, NH_2 (irreversible reaction)

useful alternative to α-2,3-sialyltransferase, since its substrate specificity is less limited compared to the transferase.

A β-fructofuranosidase from *Antherobacter* sp. K-1 has been used to transfer fructose from sucrose to the 6-position of the glucose residues of stevioside and rubusoside.[159] A sucrase from *Bacillus subtilis* catalyzes the reversible transfer of fructose from sucrose to the 6-hydroxyl of a fructose unit at the nonreducing end of a levan chain.[160] Several unnatural sucrose derivatives have been prepared by taking advantage of this process.[160c]

A related class of enzymes are *N*-transribosylases which catalyze the transfer of ribose or 2-deoxyribose between two purine bases (type I) or any two bases (type II). These enzymes are stereospecific for the β-anomer of the nucleoside product and have been employed in the synthesis of nucleoside analogs.[161] Thymidine and 2′-deoxycytidine are the best glycosyl donors, and a fair amount of variation in the acceptor bases is tolerated. Examples of nucleoside analogs prepared via transribosylases are the antileukemic and immunosuppressive nucleoside 2-chloroadenosine,[161c] and the anticancer agent 2-chlorodeoxyadenosine.[161d]

NAD^+ glycohydrolase has been employed in an exchange reaction for the preparation of NAD^+ analogs.[162] The enzyme accepts nicotinamide analogs with modifications at the amide functionality as substrates. The reactions may be either reversible or irreversible, depending on the structures of the nicotinamide analogs used (Scheme 33). NADH and its 6-hydroxyl derivative are not accepted by the enzyme. With 4-amino, 4-(methylamino)-, or 4-(dimethylamino)nicotinamide or -nicotinate as substrate, the product NAD analog was obtained as the 1,4-dihydro-type tautomer.[163]

2. Glycosyltransferases

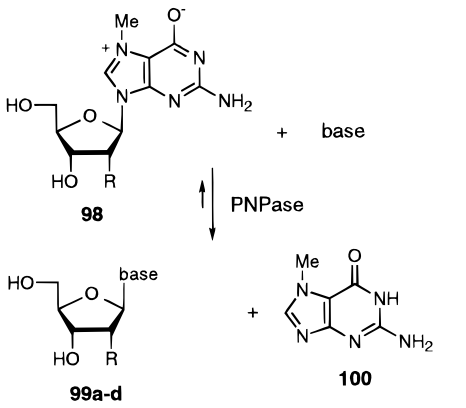
Glycosyltransferases are responsible for the biosynthesis of oligosaccharides. They can be divided into two classes according to the type of glycosyl donor they accept. The enzymes of the Leloir pathway, are named after the Argentinean biochemist, who discovered them and investigated their properties and functions.[142a] They utilize as glycosyl donors monosaccharides, which are activated as glycosyl esters of nucleoside mono- or diphosphates. They are responsible for the synthesis of most *N*- and *O*-linked glycoproteins and glycoconjugates in mammals. A diverse array of monosaccharides (e.g. xylose, arabinose, KDO, deoxy sugars) and oligosaccharides is also present in microorganisms, plants, and invertebrates.[142d,e,164] The enzymes responsible for their biosynthesis, however, have not been extensively investigated for synthesis, although they follow the same principles as do those in mammalian systems. Non-Leloir transferases, on the other hand, typically use glycosyl phosphates as glycosyl donors. In general, the Leloir-type enzymes display higher stereo- and regioselectivities than their glycosyl phosphate-transferring relatives, but both classes of enzymes have been demonstrated to be useful for the synthesis of oligosaccharides.

2.1. Non-Leloir Glycosyltransferases

Non-Leloir transferases have been used to prepare a variety of oligosaccharides and polysaccharides. Sucrose phosphorylase and trehalose phosphorylase have been employed in the synthesis of sucrose and trehalose, respectively.[165] Potato phosphorylase (EC 2.4.1.1) has been employed for the preparation of maltose oligomers[166] and of a family of linear, star- and comb-shaped polymers.[167] Cyclodextrin α-1,4-glucosyltransferase (EC 2.4.1.19) from *Bacillus macerans* can be used *in vitro* to cyclize oligomaltose to form α-, β-, and δ-cyclodextrin, and to form oligosaccharides by transferring sugars from cyclodextrin to a variety of acceptors,[168] including aza sugars such as deoxynojirimycin or *N*-substituted derivatives thereof.[169]

Of particular interest are the nucleoside phosphorylases (PNPases), catalyzing the reversible formation of a purine or pyrimidine nucleoside from ribose-1-phosphate (R-1-P) and a purine or pyrimidine base. The equilibrium lies in favor of nucleoside formation. Similar to the *N*-transribosylases,[161] these enzymes have been used for the preparation of unnatural nucleosides.[161f] The method relies on the transfer of the ribose moiety of a readily available nucleoside to a different purine or pyrimidine base or analogs through the intermediacy of R-1-P. The exchange reaction may either involve the isolation of R-1-P, which is prepared in good yield from a nucleoside in the presence of a high concentration of phosphate[170] and is then used as the glycosyl donor in an enzymatic coupling reaction with *N*-heterocycles, or can be performed in a one-pot exchange of one base for another in the presence of a catalytic amount of inorganic phosphate without isolation of R-1-P. The latter approach usually suffers from the formation of a mixture of the substrate and the product nucleosides. Sometimes the natural purine or pyrimidine base released from the glycosyl donor is a potent competitive inhibitor versus the purine or pyrimidine analog, for which the enzyme has a lower affinity.

These problems have recently been overcome through a new approach using activated purine derivatives as the ribosyl donors.[171] The activated purine derivatives (e.g. **98**) were prepared by 7-*N*-methylation of inosine and guanosine to provide derivatives that are excellent substrates for phosphorolytic cleavage by PNPase (Figure 7). The cleaved 7-*N*-methylpurines (e.g. **100**) are insoluble and do not show any measurable product inhibition; the equilibrium of the reaction greatly favors product formation. The effectiveness of this approach was demonstrated by the one-pot synthesis of virazole (**99b**) from 1,2,4-triazole-3-carboxamide and 7-*N*-



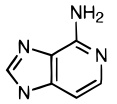
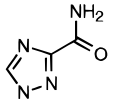
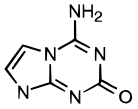
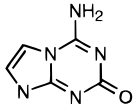
Compound	R	base	yield (%)	ref.
99a	OH		53	171
99b	OH		44	171
99c	OH		22	172
99d	H		54	172

Figure 7.

methylinosine (**98**).[171] Recently, this method was applied to the synthesis of the unnatural deoxyribonucleoside **99d** (Figure 7).[172]

2.2. Leloir-type Glycosyltransferases

Due to their high selectivity in terms of both regio- and stereochemistry, the use of Leloir-type glycosyltransferases is particularly promising for synthetic purposes. The Leloir enzymes are responsible for the synthesis of most glycoproteins and other glycoconjugates in mammalian systems. The Leloir pathway[142] begins with the activation of a monosaccharide (D-glucose, D-galactose, D-mannose, or *N*-acetyl-D-glucosamine) as its sugar 1-phosphate which is subsequently converted into the corresponding nucleoside diphosphate sugar (UDP-D-glucose, UDP-D-galactose, GDP-D-mannose, and UNP-*N*-acetyl-D-glucosamine). Enzymatic modification of these nucleoside diphosphate sugars yields UDP-D-glucuronic acid, UDP-*N*-acetyl-D-galactosamine, and GDP-L-fucose. In addition, CMP-*N*-acetylneuraminic acid is formed directly from *N*-acetylneuraminic acid and CTP. The eight activated monosaccharides are the substrates for the glycosyltransferases in mammals in combination with a wide range of glycosyl acceptors. In microorganisms, additional nucleotide sugars are widely distributed. An example is CMP-KDO, which is biosynthesized from KDO and CTP.

Table 11. Cloned Glycosyltransferases of the Leloir Pathway

enzyme	source	ref
UDP-glucuronosyltransferase	murine liver	173
	rat liver	174
α-1,2-mannosyltransferase	yeast	175
α-2,3-sialyltransferase	porcine submaxillary gland	176
	human placenta	177
	murine/rat brain	178
α-2,6-sialyltransferase	rat liver	179
	chicken embryo	180
	chicken testes	181
α-2,8-sialyltransferase	human melanoma cells	182
	murine brain	183
β-1,4-galactosyltransferase	bovine placenta	184
	bovine mammary gland	185
	murine mammary gland	186
	bovine liver	187
	murine F9 cells	188
	bovine kidney epithelial cells	189
	murine testes	190
	human placenta	191
	human milk	192
α-1,3-galactosyltransferase	murine F9 cells	193
	new world monkey	194
α-1,2-fucosyltransferase	human A431 cells	195
α-1,3/1,4-fucosyltransferase	human A431 cells	196
α-1,3-fucosyltransferase IV−VII	human A431 cells	197
polypeptide *N*-acetyl-galactosaminyltransferase	bovine colostrum	198
β-1,2-*N*-acetyl-glucosaminyltransferase	rabbit	199
	murine F9 cells	200
β-1,4-*N*-acetyl-glucosaminyltransferase	murine 14-7fd cells	201
β-1,6-*N*-acetyl-glucosaminyltransferase	rat kidney	202

Each glycosyl donor is accepted by various glycosyltransferases mediating the transfer of the glycosyl group onto different acceptors. *In vivo*, these enzymes are in general specific for the given glycosyl donor, the type of linkage to be formed, the structure of the acceptor and a specific hydroxyl group of the acceptor. The observed specificity is required to permit the synthesis of information-containing biomolecules without a template; however, glycosyltransferases display some flexibility with respect to their substrate requirements under *in vitro* conditions, i.e. when the substrates are provided in high concentration.

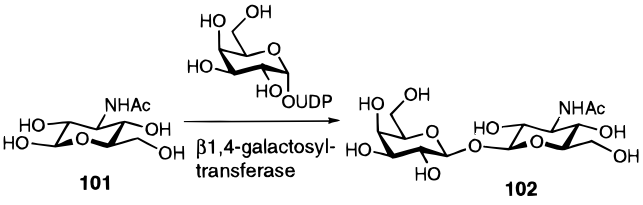
A practical use of glycosyltransferases in the chemical synthesis of oligosaccharides has in the past been limited by the restricted availability of the enzymes and the expensive synthesis of the required activated monosaccharide building blocks. Three transferases, namely α-2,3-sialyltransferase from porcine liver, α-2,6-sialyltransferase from rat liver and β-1,4-galactosyltransferase from bovine and human milk, are commercially available to date (Sigma). Due to the flourishing cloning and overexpression of new transferases, the synthetic potential is being continously widened. Over 30 transferases have been cloned so far (Table 11).[173−202]

Other glycosyltransferases can be obtained from readily available tissue sources in at least milliunit amounts, which is sufficient for milligram-scale synthesis. These are tabulated in a recent review.[143d]

Leloir-type glycosyltransferases have been widely used for the synthesis of oligosaccharides and glycoconjugates. An extensive tabulation of the numerous reported glycosyltransferase-catalyzed reactions is presented in a recent review.[143b] It is obvious that many biologically important oligosaccharides and analogs can be prepared, if the required glycosyltransferases are available. Some recent synthetic applications of this will be given at the end of this section.

Due to its availability, β-1,4-galactosyltransferase (EC 2.4.1.22)[203] is the most extensively studied glycosyltransferase of the Leloir-pathway with regard to its substrate specificity. The enzyme catalyzes the transfer of galactose from UDP-Gal to the 4-position of β-linked GlcNAc residues to produce the Galβ1,-4GlcNAc substructure. In the presence of lactalbumin, however, glucose is the preferred acceptor, resulting in the formation of lactose, Galβ1,4Glc. Galactosyltransferase utilizes as acceptor substrates *N*-acetylglucosamine and glucose and β-glycosides thereof, 2-deoxyglucose, D-xylose, 5-thioglucose, *N*-acetylmuramic acid, and myoinositol.[203a−c] Modifications at the 3- or 6-position of the acceptor GlcNAc are also tolerated.[204] Both α- and β-glycosides of glucose are acceptable, but the presence of lactalbumin is required for galactosyl transfer onto α-glycosides. A particularly interesting example is the β,β-1,1-linked disaccharide **102**, in which the anomeric hydroxyl of 3-acetamido-3-deoxyglucose (**101**) served as the acceptor moiety (Scheme 34).[205] The formation

Scheme 34



of β,β-1,1-linked products has subsequently been observed with other substrates, and an active-site model of the enzyme has been deduced from the information gained in these experiments.[206]

With regard to the donor substrate, galactosyltransferase also transfers various galactose analogs from their respective UDP derivatives, thus providing an enzymatic route to oligosaccharides which terminate in β-1,4-linked residues other than galactose.[207] Although the rate of the enzyme-catalyzed transfer of many of these unnatural donor substrates is rather slow, this method is useful for milligram-scale synthesis.

Both α-2,6- and α-2,3-sialyltransferases have been used for oligosaccharide synthesis.[208] Sialyltransferases generally transfer *N*-acetylneuraminic acid to either the 3- or 6-position of terminal Gal or GalNAc residues. Some sialyltransferases have been shown to accept CMP-NeuAc analogs which are derivatized at the 9-position of the sialic acid side chain.[209] Analogs of the acceptors Galβ1,4GlcNAc and Galβ1,3GalNAc, in which the acetamido function was replaced by other functional groups, were also accepted by the enzymes.[210] Recently, α-2,8-linked homopolymers of sialic acid have been prepared using α-2,8-sialyltransferase.[211]

Several fucosyltransferases have been isolated and used for *in vitro* synthesis. The Lewis A α-1,-4fucosyltransferase has been shown to transfer unnatural fucose derivatives in form of their GDP esters.[212] This enzyme also transfers a fucose residue, which can be substituted on C-6 by a sterically demanding structure.[213] The enzyme α-1,3-fucosyltransferase has been used to L-fucosylate the 3-position of the GlcNAc of *N*-acetyllactosamine and of sialyl α-2,3-*N*-acetyllactosamine.[214] Several acceptor substrates with modifications in the GlcNAc residue could also be fucosylated.[215] A related enzyme, α-1,3/4-fucosyltransferase, has been used on preparative scale to fucosylate the GlcNAc 3-position of Galβ1,-4GlcNAc and the GlcNAc 4-position of Galβ1,-3GlcNAc.[214b,216] The corresponding sialylated substrates have also been employed as acceptors.[214b]

N-acetylglucosaminyl transferases I−VI catalyze the addition of GlcNAc residues to the core pentasaccharide of asparagine glycoproteins,[217] thus controlling the branching pattern of *N*-linked glycoproteins *in vivo*. Each of the enzymes transfers a β-GlcNAc residue from the donor UDP-GlcNAc to a mannose or other acceptor. GlcNAc transferases have been utilized to transfer nonnatural residues onto oligosaccharides.[218]

Different mannosyltransferases have been shown to transfer mannose and 4-deoxymannose from their respective GDP adducts to acceptors.[219] α-1,2-Mannosyltransferase was employed to transfer mannose to the 2-position of various derivatized α-mannosides and α-mannosyl peptides to produce the Manα1,-2Man structural unit.[185b,c] A recent report indicates that mannosyltransferases from pig liver accept GlcNAcβ1,4GlcNAc phytanyl pyrophosphate, an analog in which the dolichol chain of the natural substrate is replaced by the phytanyl moiety.[220]

Some studies on the substrate specificities of sucrose synthetase[221] and oligosaccharyltransferase[143b,222] have been performed, but these enzymes have not been used widely for synthesic purposes so far.

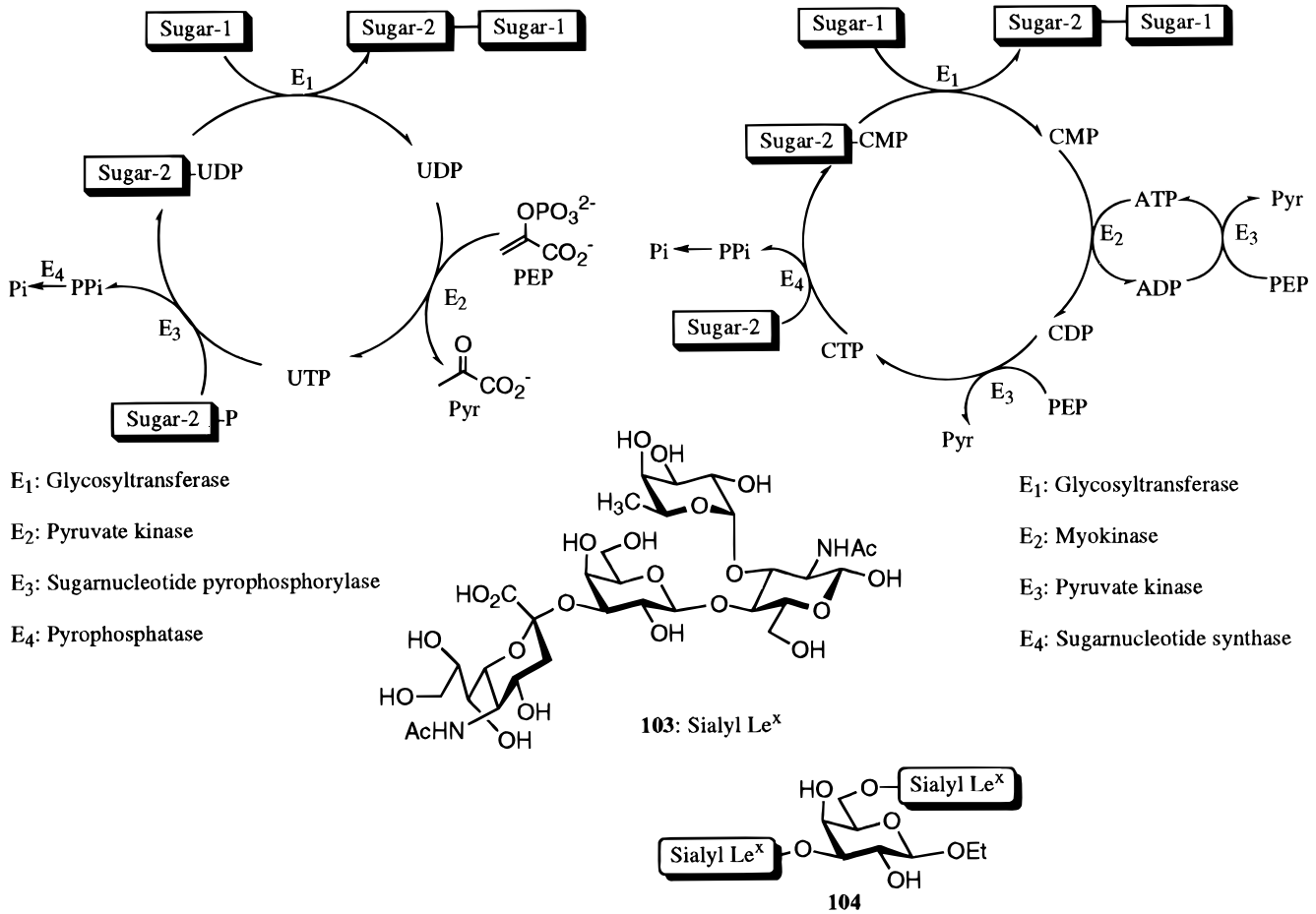
In most glycosyltransferase reactions sugar nucleotides are used stoichiometrically and the availability of these sugar nucleotides is of major importance for the successful application of glycosyltransferases. Efficient enzymatic or chemoenzymatic syntheses of the eight common nucleoside (di)phosphate sugars have been developed (Table 12).[209,223−232] The required nucleoside triphosphates can be prepared either chemically via the condensation of an activated nucleoside, usually in the form of a phosphoramidate, or enzymatically starting from nucleoside monophosphates using kinases.[143b]

The use of presynthesized sugar nucleotides is restricted to analytical and small-scale reactions due to their high cost. In addition the released nucleoside mono- or diphosphates cause product inhibition and complicate the isolation of the final product. Some of the sugar nucleotides are not very stable in solution[212,234,235] as was demonstrated for CMP-KDO, which has a half-life time of 34 min at pH 7.5, 25 °C.[235] These problems can be avoided by the use of a strategy in which the sugar nucleotide is regener-

Table 12. Synthesis of the Nucleoside (Di)phosphate Cofactors

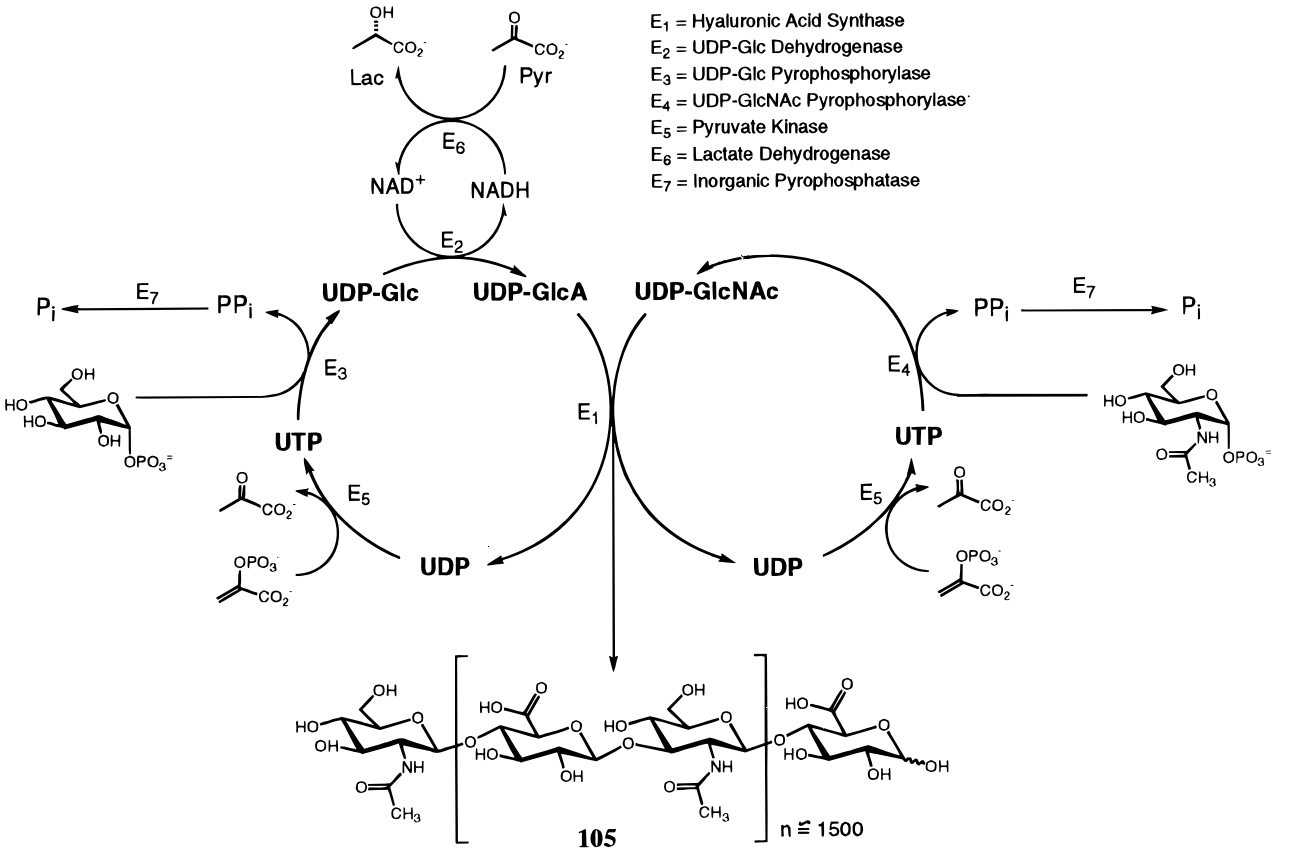
cofactor	method	ref(s)
UDP-Glc	from Glc-1-P and UTP with UDP-glucose pyrophosphorylase	223
UDP-Gal	from Gal-1-P and UTP with UDP-galactose pyrophosphorylase	142d,e
	from UDP-glucose with UDP-glucose epimerase	223b
	from Gal-1-P and UDP-Glc with UDP-galactose uridyl transferase	224
	from UMP and galactose using cells of *Torulopsis candida*	223c
	by chemical synthesis	225
UDP-GlcNAc	from GlcNAc-1-P and UTP with UDP-GlcNac pyrophosphorylase	212c
	from GlcNH2−1-P and UTP with UDP-GlcNac pyrophosphorylase followed by *N*-acetylation	226
UDP-GalNAc	from UDP-Glc and Gal-1-P with UDP-glucose:galactosylphosphate uridyl-transferase	142d,e,224,227
GDP-Man	from Glc and GMP with Bakers' yeast	228
	from Man-1-P and GTP with GDP-Man pyrophosphorylase	223a
GDP-Fuc	from GDP-Man with crude a crude enzyme preparation from *Agrobacterium radiobacter*	229
	from Fuc-1-P and GTP with GDP-Fuc pyrophosphorylase	214a
	by chemical synthesis	212,225b,230
UDP-GlcA	by oxidation of UDP-Glc with UDP-Glc dehydrogenase from bovine liver or guinea pig liver	223a,231
CMP-NeuAc	from NeuAc and CMP with CMP-NeuAc synthetase	96,208b,c,232
	by chemical synthesis	233

Scheme 35



ated *in situ* from the released nucleoside mono- or diphosphate. In that case only a catalytic amount of nucleoside mono- or diphosphate and a stoichiometric amount of the donor monosaccharide can be used as starting material. Sugar nucleotides are regenerated via sugar nucleotide pyrophosphorylases or sugar nucleotide synthases. The required nucleoside triphosphates for this process can be regenerated from the corresponding mono- and diphosphates using different kinases. General regeneration systems are represented in Scheme 35 for glycosyl transferases which use UDP-glycosides and CMP-glycosides. Such regeneration systems have been developed for UDP-Gal,[204a,b,236] CMP-NeuAc,[237] UDP-GLcNAc,[238] GDP-Man,[185b] GDP-Fuc,[214a] and UDP-GlcA.[231] The necessary enzymes for these systems are becoming increasingly accessible through cloning and overexpression of their respective genes. A recent example is CMP-KDO synthetase, overexpressed in *E. coli*.[239] The UDP-Gal and CMP-NeuAc regeneration systems have been combined in a one-pot reaction and applied to the synthesis of sialyl oligosaccharides. An example of the strength of this methodology is the synthesis of sialyl Le^x (**103**) where four monosaccharides are coupled using subsequently β-1,4-galactosyltransferase, α-2,3-sialyltransferase and α-1,3-fucosyltransferase with regeneration systems of the necessary sugar nucleotides for all three

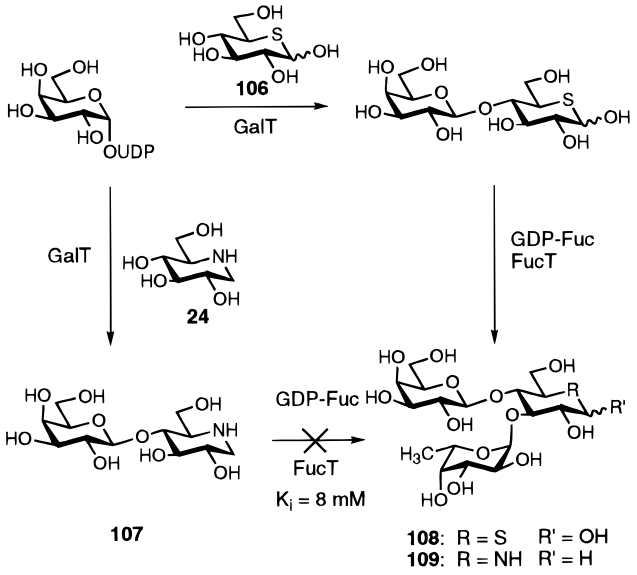
Scheme 36



steps.[214a] Chemical synthesis of sialyl Le^x requires multiple protection and deprotection steps, making the process difficult for large scale production. The enzymatic synthesis based on glycosyltransferases proceeds regio- and stereoselectively in aqueous solution without protecting groups, and kilogram quantities of sialyl Le^x are prepared this way. Glycosyltransferases can also be used to produce a variety of active analogs of sialyl Le^x. Starting with two GlcNAc molecules, attached to each other via various spacers, application of the three sequential glycosyltransferases gave a variety of bivalent sialyl Le^x compounds.[240] All the synthesized dimers had similar or higher activities than the sialyl Le^x tetrasaccharide **103**. The galactose anchored dimer **104** binds about 5-fold better than sialyl Le^x to the E-selectin receptor, suggesting a possible multivalent ligand−receptor interaction.[241]

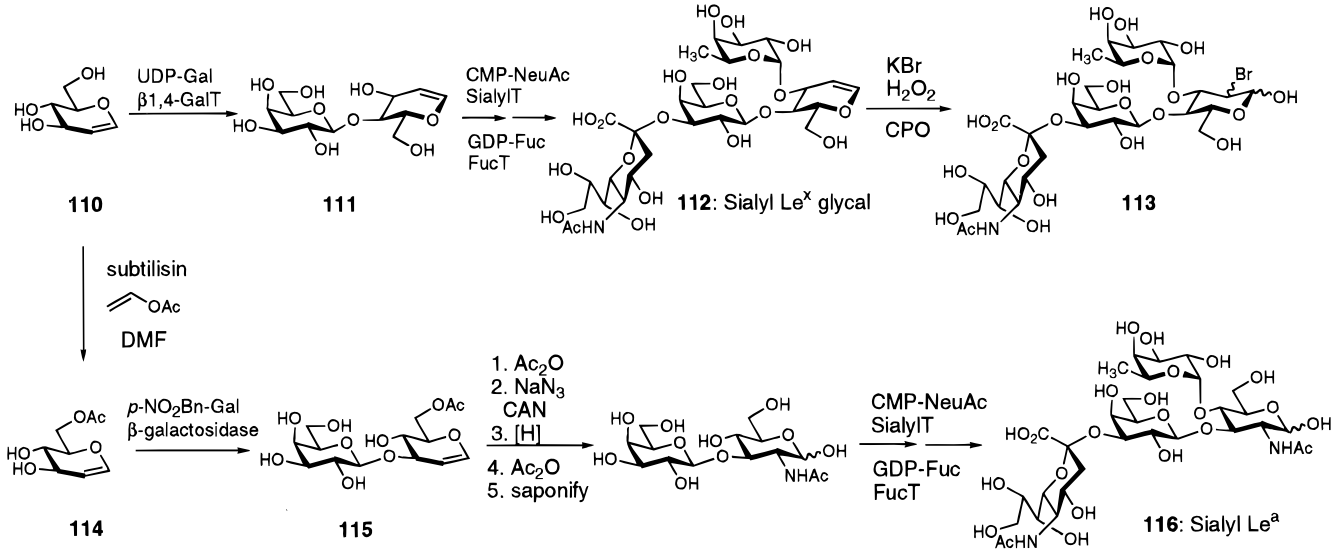
The use of glycosyltransferases is not confined to the formation of a single glycosidic bond. Sequential reaction of the sugar nucleotide donor with the growing oligosaccharide will give a polysaccharide.[211] This was demonstrated in the synthesis of hyaluronic acid (HA, **105**),[242] an important biopolymer used in medicine. HA with a molecular weight of $\sim 5.5 \times 10^5$ has been prepared from UDP-GlcNAc and UDP-GlcA using hyaluronic acid synthetase coupled with regeneration of the sugar nucleotides (Scheme 36). Two key enzymes used in the cofactor regeneration, *i.e.* UDP-GlcNAc pyrophosphorylase and UDP-glucose dehydrogenase were overexpressed in *E. coli*. This procedure also demonstrates the utility of sugar nucleotide regeneration in the enzymatic synthesis of high molecular weight polysaccharides.

Scheme 37



By using glycosides linked to non-carbohydrate moieties as acceptors for the glycosyltransferase reaction several glycoconjugates can be prepared. Recent examples are glycopeptides,[243] glycosylated alkaloids,[244] and glycosphingolipids.[245] Because of the hydrophobicity of the ceramide part of glycosphingolipids they are poor acceptor substrates for the glycosyltransferases. By using a less hydrophobic sphingosine derivative, which can be converted chemically into the desired ceramide, glycosyltransferase reactions can take place more easily and in this way

Scheme 38



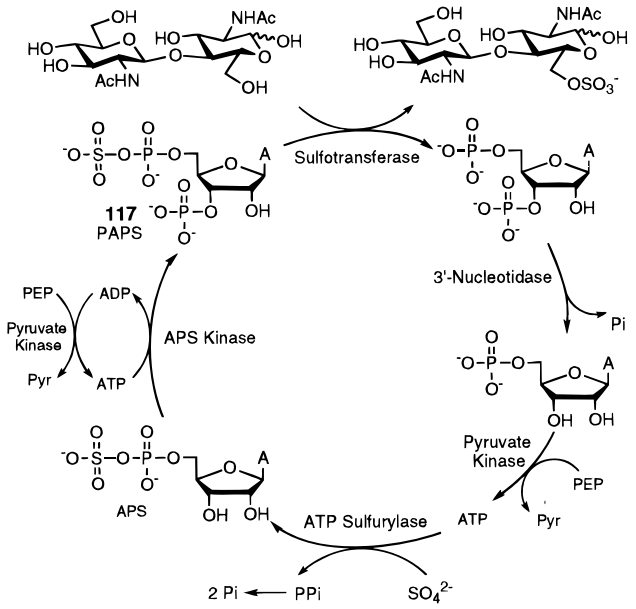
a variety of gangliosides,[246] e.g. ganglioside GM_3,[247] 3′-nLM_1, and 6′-nLM_1,[248] were synthesized.

By varying the substrates for the glycosyltransferases, analogs of naturally occurring oligosaccharides can be produced. In this way the thio sugar **106** has been incorporated in the Lewis X synthesis to give 5-thio-GlcLe^x (**108**) (Scheme 37).[214a] Deoxynojirimycin (**24**), synthesized via aldolase catalysis (Scheme 9), was coupled to galactose with galactosyltransferase to give Gal-β-1,4-deoxynojirimycin (**107**).[214a] Subsequent coupling to a fucose unit to obtain **109** was not possible because of inhibition of fucosyltransferase by **107**. The chemoenzymatic synthesis of the trisaccharide moiety of ganglioside GM_{1b} is another example of the combination of aldolase and glycosyltransferase catalyzed synthesis.[249]

When a glycosyltransferase is not available for the desired glycosidic bond formation, the coupling may be accomplished with a glycosidase. An example is the use of glucal (**110**) as acceptor substrate for glycosidic bond formation (Scheme 38). The β-1,4-lactal **111** was prepared via β-1,4-galactosyltransferase catalyzed reaction.[204c] The β-1,3-lactal **115** was prepared via regioselective acetylation of glucal with an engineered subtilisin in high concentration of DMF to give **114**, followed by β-galactosidase reaction.[150c,238] The latter product was converted into sialyl Le^a (**116**) via azidonitration followed by further enzymatic glycosylation.[150c] Similar glycosylation of β-1,4-lactal **111** led to the sialyl Le^x terminal glycal **112** which, like sialyl Le^a (**116**), has similar inhibition activity for E-selectin binding as sialyl Le^x.[214a] Glycal **112** can also be converted to other sialyl Le^x derivatives. An example is the chloroperoxidase (CPO)-catalyzed regioselective bromohydration of **112** to give 2-deoxy-2-bromosialyl Le^x **113**.[250]

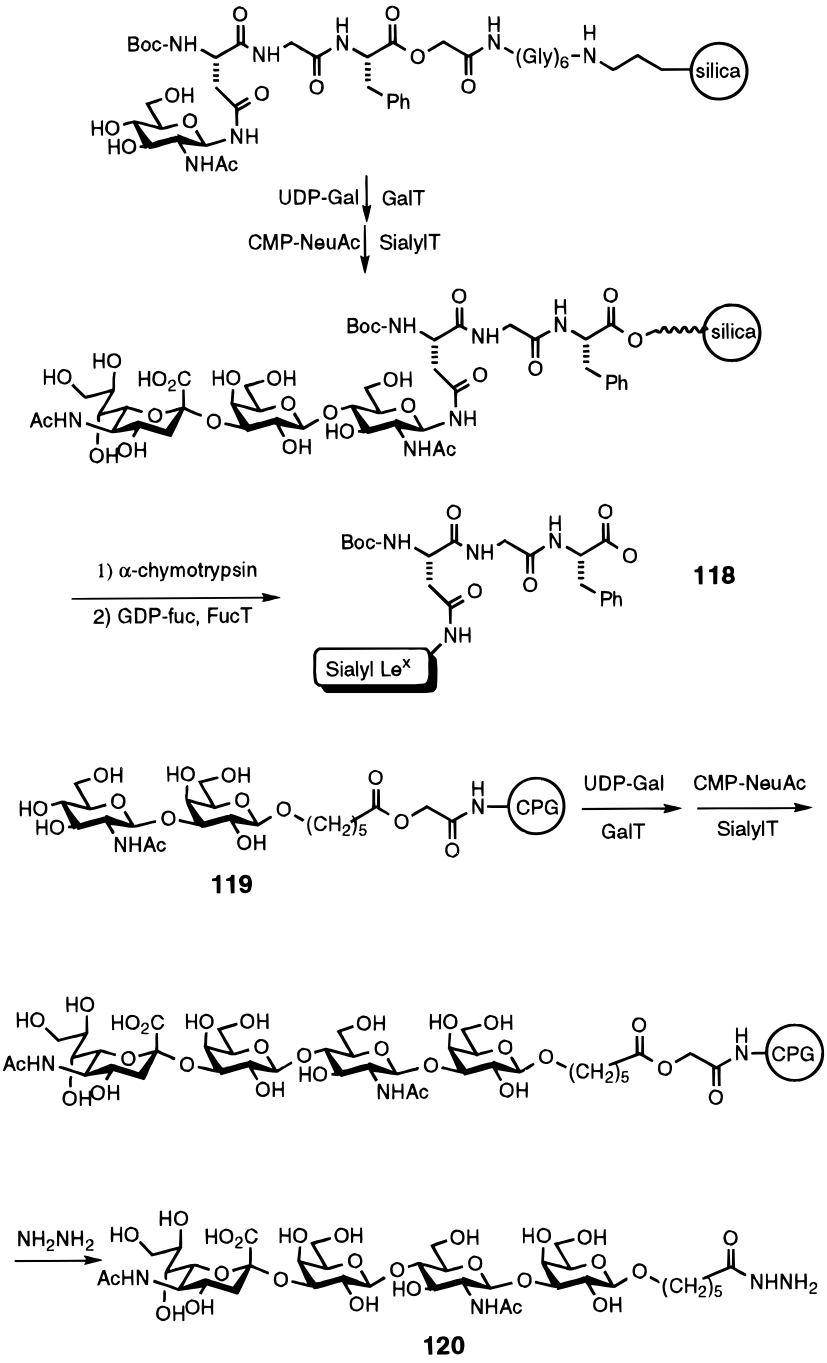
Another interesting modification of the initially formed oligosaccharides is the sulfation of hydroxyl groups. For example, heparin contains several sulfate groups and Le^x Gal-3′-O-sulfate is a strong mimic for sialyl Le^x.[251] Enzymatic sulfation of hydroxyl groups is catalyzed by sulfotransferases. The universal donor of the sulfate group for these enzymes is 3′-phosphoadenosine 5′-phosphate sulfate (**117**) (PAPS). Similar to the sugar nucleotides, one of the limitations for the use of sulfotransferases is the availability of PAPS. It is expensive, chemically labile, and causes inhibition problems. Recently a multienzyme regeneration system was developed for PAPS (Scheme 39),[252] making enzymatic sulfation more feasible in the near future. The *in situ* regeneration of PAPS has been used in combination with an overexpressed sulfotransferase for the synthesis of saccharide sulfates. This is exemplified by the synthesis of *N,N*′-diacetylchitobiose 6-sulfate, as illustrated in Scheme 39.

Scheme 39



Similar to protein- and nucleic acid-related chemistry and biochemistry, the development of solid-phase synthesis of oligosaccharides would greatly benefit the research in carbohydrate-related chemistry. The availabiliy of solid-phase methodology for the synthesis of oligosaccharides is however limited due to the lack of effective differential protection/

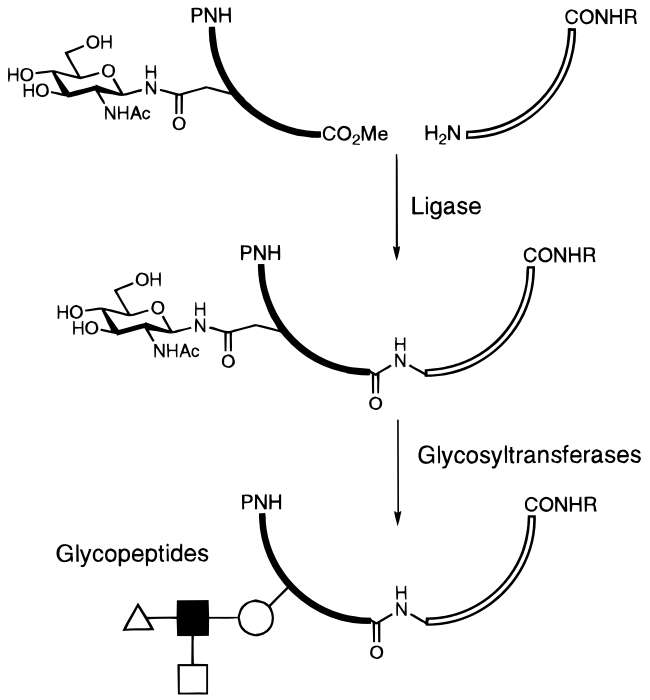
Scheme 40



deprotection strategies and high-yield stereoselective coupling of multifunctional carbohydrate donors and acceptors.[253] Due to the high regio- and stereoselectivity of glycosyltransferases, they are potentially useful in solid-phase synthesis.[254,255] One strategy has been developed where glycosyltransferases are used to couple glycosides to a monosaccharide unit which is attached to a silica bound peptide spacer.[256] Glycopeptides can be obtained via enzymatic cleavage of the peptide spacer from the solid support with α-chymotrypsin. This is illustrated by the synthesis of the sialyl Le^x glycopeptide **118** (Scheme 40).[256] The system should also enable the synthesis of oligosaccharides as the peptide moiety can be removed enzymatically using endoglycosidases. In another example of the use of glycosyltransferase in oligosaccharide synthesis, the tetrasaccharide **120** was synthesized starting from the disaccharide **119**, attached to controlled pore glass (CPG) via a spacer group containing an ester bond.[257] The conversion for both glycosylation steps was found to be >98%. Glycosyltransferases have also been used for the synthesis of glycopeptides bound to PEGA resin[258] and for oligosaccharides or glycopeptides linked to water-soluble polymers.[242b,259]

The protease subtilisin BPN′ has been altered via site-directed mutagenesis to create a thermostable thiosubtilisin variant which was able to ligate peptide segments in aqueous solution.[260] Combination of such a ligase with glycosyltransferases will provide another route to glycopeptides as depicted in Scheme 41.

Scheme 41



IV. Concluding Remarks

Despite the recent progress in the chemical synthesis of carbohydrate-based compounds, the preparation of carbohydrates via enzymatic routes has gained broad recognition and has become a valuable addition to the existing conventional methods. Presently, aldolases are the most useful group of biocatalysts for the preparation of rare and unnatural monosaccharides and related molecules. Glycosyltransferases are becoming readily available and the development of practical synthetic routes to their required sugar nucleotides combined with regeneration systems for these cofactors will make glycosyltransferases the catalysts of choice for the synthesis of oligosaccharides. Other enzymes, e.g. lipases, proteases, and oxidoreductases, can be useful to prepare the necessary chiral starting materials or to catalyze specific functional group transformations. Cloning and overexpression will provide an ever increasing number of synthetically useful enzymes in quantities sufficient for preparative synthesis. As the structures of the enzymes become better defined, site-directed mutagenesis and chemical modifications may be used to tailor the stability, regioselectivity, and substrate specificity of the enzymes.

Through enzymatic and chemoenzymatic approaches, complex carbohydrates and carbohydrate mimetics will become increasingly accessible. This will lead to a better understanding of important processes in glycobiology such as receptor-mediated recognition and glycoprotein processing, which can subsequently lead to the development of new therapeutics and diagnostics.

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