

Recent Advances in Aldolase-Catalyzed Asymmetric Synthesis

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Abstract: This review is focused on advances over the last several years in the application of aldolases to organic synthesis. Several new technologies have been implemented to increase the scope and practicality of aldolases as tools for the synthetic chemist. These include directed evolution, discovery of new classes of aldolases in nature and the laboratory, and substrate and reaction engineering.

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1 Introduction

The aldol reaction has long been recognized as one of the most useful tools for the synthetic chemist. The ability to form carbon-carbon bonds and generate up to two new stereogenic centers allows the organic chemist to fashion a broad range of both natural and novel poly-hydroxylated compounds. Excellent control of the stereochemical outcome of the aldol reaction has been achieved by the stoichiometric use of chiral auxiliaries,^[1] however, catalytic asymmetric aldol reactions are desired whenever possible. Such catalytic methods for the aldol reaction include the use of chiral Lewis acids,^[2,3] organocatalysts^[4] and catalytic antibodies.^[5] This review highlights recent advances in the use of aldolases for preparative asymmetric synthesis.

Aldolases have evolved to catalyze the metabolism and catabolism of highly oxygenated metabolites, and are found in many biosynthetic pathways of carbohydrates, keto acids, and some amino acids. The aldolase family of enzymes is divided into two classes on a mechanistic basis. The class I aldolases activate their donor substrates by the formation of a Schiff base with a strictly conserved active site lysine. The enamine tautomer formed in the active site then attacks with high selectivity the appropriate face of the bound acceptor aldehyde. The enzyme-bound imine is then hydrolyzed, releasing the product. In the class II aldolases, a metal co-factor is bound in the enzyme active site by histidine residues. This bound metal ion, usually Zn²⁺ (although Co²⁺ or Fe²⁺ can also be

active), acts as a Lewis acid to activate the bound donor substrate. Figure 1 illustrates these reaction mechanisms.

Aldolases bind their respective donor substrates with high specificity and generally will not accept any other donors, even if their structures are similar to the natural donor. This strict donor specificity provides a functional basis for classifying aldolases. One important class of aldolases utilizes dihydroxyacetone phosphate (DHAP) as the donor substrate, and in contrast will catalyze reactions with a relatively broad range of different acceptor aldehydes. Well-known members of this class include fructose 1,6-diphosphate (FDP) aldolase and L-rhamnulose 1-phosphate (RhaD) aldolase. The synthetic utility of these DHAP-dependent aldolases has been thoroughly demonstrated with a wide array of novel acceptor aldehydes.^[2,6] DHAP-dependent aldolases have been used to synthesize ¹³C-labeled sugars, deoxy sugars, fluoro sugars and iminocyclitols. However, the requirement for the very expensive DHAP donor is a major drawback of these enzymes. Although there have been several methods for generating DHAP reported in the literature,^[7] recent efforts have been directed towards eliminating the need for DHAP, either by directed evolution, reaction engineering, or exploitation of newly discovered enzymes.

The pyruvate/phosphoenol pyruvate class of aldolases is a large family of enzymes that have been used to prepare various α -keto acids. One member of this class, *N*-acetylneuraminic acid (NeuAc) aldolase (or sialic acid aldolase) has been particularly well studied.

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He started his independent career as Assistant Professor of Chemistry at Texas A&M University in 1983, became Associate Professor in 1986 and Professor in 1987. He was Professor and Ernest W. Hahn Chair in Chemistry at the Scripps Research Institute (1989–2006) and Director of the Genomics Research Center at Academia Sinica, Taipei (2003–2006). Since October 2006, he has been Professor of Chemistry at The Scripps Research Institute and President of Academia Sinica. Professor Wong is a recipient of The Searle Scholar Award in Biomedical Sciences (1985), the Presidential Young Investigator Award in Chemistry (1986), the American Chemical Society A. C. Cope Scholar Award (1993), the Roy Whistler Award of the International Carbohydrate Organization (1994), the American Chemical Society Harrison Howe Award in Chemistry (1998), the American Chemical Society Claude S. Hudson Award in Carbohydrate Chemistry (1999), the International Enzyme Engineering Award (1999), the Presidential Green Chemistry Challenge Award (2000), and the American Chemical Society Award for Creative Work in Synthetic Organic Chemistry (2005). He is a member of Academia Sinica, Taipei (1994), the American Academy of Arts and Sciences (1996), and the U.S. National Academy of Sciences (2002).

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This aldolase is particularly interesting because the stereochemical outcome of the aldol reaction is determined by the substrate. This has been exploited in the synthesis of various D- and L-sialic acid analogues.^[8]

The family of acetaldehyde-dependent aldolases contains only one member: 2-deoxy-D-ribose-5-phosphate aldolase (DERA). It is the only known aldolase that catalyzes the aldol reaction between two aldehydes, glyceraldehyde 3-phosphate and acetaldehyde. DERA is capable of the sequential addition of two acetaldehyde molecules to the acceptor aldehyde.^[9] As with many other aldolases, DERA is capable of utilizing many unnatural acceptor aldehydes.^[10] This has been used to great effect in the preparation of

chiral synthons for use in a synthesis of epothilones A and C.^[11] A synthesis of the key fragment of atorvastatin catalyzed by DERA^[12] has attracted attention to this aldolase in recent years.

The final family of aldolases is the glycine-dependent aldolases. These enzymes catalyze the reversible formation of the hydroxylated amino acids D- and L-threonine and serine. The glycine-dependent aldolases have not been studied as synthetic catalysts to the extent as some of the other aldolases. L-Threonine aldolase has, however, been used with various acceptor aldehydes.^[13]

Table 1 shows some important representatives of these four types of aldolases. As more than 30 aldo-

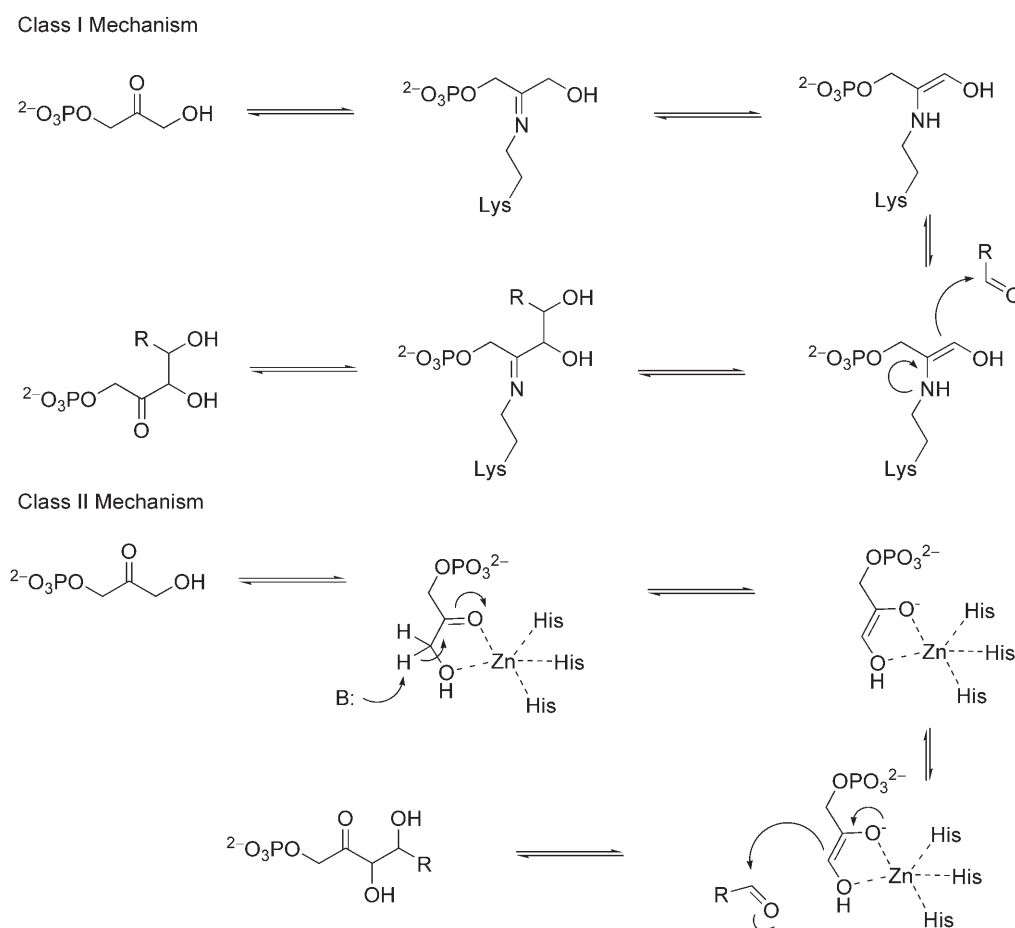


Figure 1. Mechanisms of class I and II aldolases.

aldolases are known and our focus is on the most recent advances in the biocatalytic aldol reaction, the reader is referred to previous reviews^[2,6,14] for more information.

The advantages of using aldolases, namely very high stereospecificity, environmentally benign reaction conditions, and sidestepping the need for protection/deprotection strategies, are accompanied by some notable disadvantages. Not all substrates are readily soluble in aqueous solvent, and enzymes and their substrates (such as DHAP) can be costly and unstable. Also, the inherent specificity of aldolases leads to a limited number of substrates and stereochemical outcomes being available. The very specificity that defines enzymes has prevented aldolases from becoming broad-based, general catalysts for asymmetric synthesis. A variety of non-enzymatic, asymmetric aldol technologies have been developed, as summarized below. Recent approaches to tackling the limitations to practical use of aldolases will be reviewed in the following section.

A very active area in the field of chemical catalysis is the chiral Lewis acid-catalyzed asymmetric aldol reaction. The coordination of a chiral Lewis acid to an

acceptor aldehyde creates an asymmetric environment. This activated aldehyde is attacked by a nucleophilic species, such as a silyl enol ether, from the less-hindered face. The majority of this research has been conducted on complexes of tin, titanium and boron although other metals are being investigated. As the subject of this review is biocatalytic aldol reactions, the reader is referred to the number of excellent reviews on this topic.^[2,3]

Recent years have seen an increasing interest in organocatalysis. In organocatalytic asymmetric aldol reactions, a chiral imine intermediate is formed by the condensation of a chiral amine catalyst, such as proline or a proline derivative, with a donor. The activated chiral enamine then selectively attacks the acceptor and the imine is hydrolyzed to release the aldol reaction product and free the catalyst to react again. This mechanism is similar to that of the class I aldolases. Since the initial reports of the L-proline catalyzed aldol reaction,^[15] many applications and improvements have been reported.^[4,5]

Finally, catalytic antibodies have also been generated that catalyze asymmetric aldol reactions. In creating catalytic antibodies, the massive diversity-generat-

Table 1. The four divisions of aldolases as classified by their donor substrates.

Donor	Acceptor	Product	Aldolase
			fructose 1,6-bisphosphate aldolase
			rhamnulose 1-phosphate aldolase
			N-acetylneuraminic acid aldolase
			2-deoxyribose 5-phosphate aldolase
			L-threonine aldolase

ing power of the immune system is used to access an immense library of potential catalysts. By immunizing against a hapten designed to mimic the transition state of the aldol reaction, the first catalytic antibody capable of an aldol reaction was reported in 1995.^[16] Further improvements were realized through a method called reactive immunization.^[17] In this more sophisticated technique a β -diketone that reacts with the active site lysine of a catalytic antibody forms a vinylogous amide that can be detected using UV spectroscopy. This was used in the discovery of the aldolase antibody 38C2,^[18] an antibody with an extremely wide substrate scope. This catalyst is now commercially available.

2 Directed Evolution and Enzyme Engineering

An integral component to the optimization and application of aldolases as catalysts for the asymmetric aldol reaction is that the structural and functional information of the enzyme is encoded in the DNA sequence of the aldolase gene. This allows the researcher to affect changes in the catalyst's structure through either informed, rational means, or by random sam-

pling without any information about the enzyme's structure. Many researchers have used mutagenesis techniques to help abrogate the disadvantages of enzyme catalysis by widening the scope of possible acceptor substrates, allowing for novel stereochemical outcomes, and enhancing enzyme stability to temperature and organic solvent.

There are a number of superb reviews on the subject of protein engineering and directed evolution and screening methodologies.^[19] Rather than delve into these topics, here we illustrate the means by which researchers have altered aldolases to achieve new, useful properties.

2.1 DHAP-Dependent Aldolases

With the aim of enhancing its scope, Williams et al. modified the stereochemical course of an aldolase-catalyzed reaction.^[20] Specifically, tagatose 1,6-bisphosphate (TBP) aldolase was mutated over several rounds of directed evolution to become a fructose 1,6-bisphosphate (FBP) aldolase. It must be noted that the substrates for the two enzymes, FBP aldolase and TBP aldolase are exactly the same: DHAP and D-glyceraldehyde 3-phosphate (Figure 2). It is the opposite stereochemical outcome of the carbon-carbon

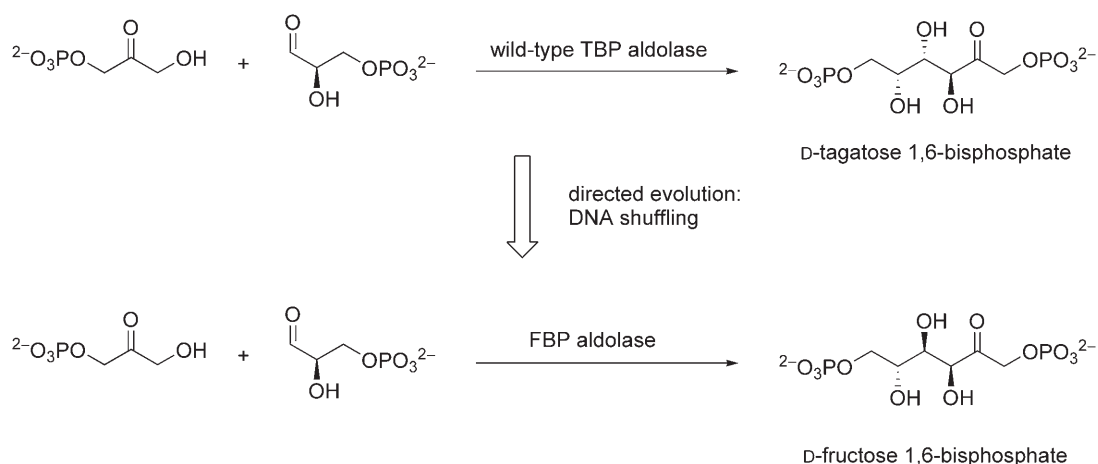


Figure 2. Alteration of the stereochemical outcome of an aldolase-catalyzed reaction by directed evolution.

bond forming reaction that leads to the production of TBP *versus* FBP.

Initially, a library of randomly mutated aldolases was generated using DNA shuffling.^[21] This method resulted in an average of one amino acid substitution per gene. *E. coli* cells harboring the mutant aldolase genes were grown in 96-well microtiter plates, lysed, and the cleared lysate was used for screening by a colorimetric coupled enzyme assay measuring the production of glyceraldehyde 3-phosphate by retroaldol reaction. Activity for both TBP and FBP was measured and the re-confirmed hits were pooled and subjected to another round of DNA shuffling. The next generation mutants were screened once more, using a lower concentration of substrate. This process was reiterated and produced a third generation mutant exhibiting an 80-fold improvement in $k_{\text{cat}}/K_{\text{m}}$ for FBP and a change in diastereoselectivity from >99:<1 preference for TBP to a 4:1 preference for FBP.

This third generation mutant possessed a total of four mutations. Intriguingly, three of these mutations occur in residues completely conserved in all members of FBP and TBP aldolases while the fourth occurs in a semi-conserved residue. The authors go on to suggest these mutations affect subtle changes in the metal and phosphate binding sites of this class II aldolase.

A thermostable aldolase variant was also produced by directed evolution studies in the Berry lab.^[22] Once again using DNA shuffling, in this case using FBP aldolase genes from *E. coli* and *Ed. ictaluri*, a library of mutants was produced. Lysates were assayed for activity in the standard coupled-enzyme assay after heat treatment for 10 min. A large number of variants, 5000, were screened in the first generation resulting in two improved variants. These were crossed in another DNA shuffling experiment giving rise to a second generation library. Screening continued at a higher temperature. After four rounds of screening, a

fourth generation mutant was discovered with a ~190-fold improvement in $t_{1/2}$ at 53 °C over the *E. coli*-derived parent.

Since it has been observed that an increase in thermostability can result in increased compatibility with organic solvents, Hao and Berry probed the activity of this fourth generation FBP aldolase in various organic solvents. Both activity in the presence of organic solvent and activity after incubation in organic solvent were measured. In all cases the thermostable mutant outperformed its *Ed. ictaluri* and *E. coli* parents. It was especially resistant to irreversible inactivation by organic solvent. This report shows that it is possible to obtain aldolases with increased stability using directed evolution.

2.2 Pyruvate-Dependent Aldolases

The earliest application of directed evolution to an aldolase was reported by this laboratory in 2000.^[23] *E. coli* D-2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase, a pyruvate-dependent aldolase, was subjected to rounds of error-prone PCR and DNA shuffling in an effort to alter its substrate specificity. By screening for mutants with enhanced activity for unphosphorylated KDG, improved activity for unphosphorylated acceptors resulted. An increase in relative activity for D- and L-glyceraldehyde was observed and used to synthesize the L-sugar 3-deoxy-L-threo-2-hexulosonic acid, **1** (Figure 3).

Woodhall et al. applied structurally-guided mutagenesis to a pyruvate-dependent aldolase to synthesize sialic acid mimetics.^[24] Instead of the natural *N*-acetyl-D-mannosamine (D-ManNAc) acceptor, the researchers sought a new aldolase capable of accepting substituted amides. Analyzing the X-ray crystal structure of sialic acid aldolase (*N*-acetylneuraminic acid

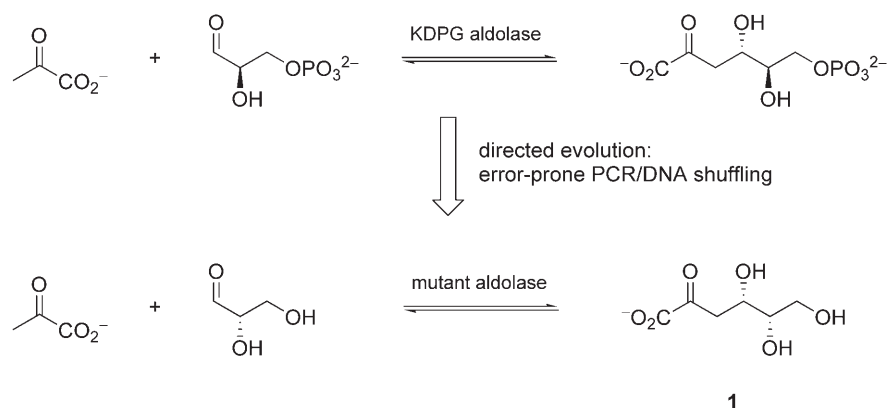


Figure 3. Altered substrate specificity of KDPG aldolase by directed evolution.

aldolase) with a bound inhibitor 4-oxosialic acid, they selected three residues responsible for interactions with the C-7/C-9 portion of the molecule. These three residues were separately targeted by saturation mutagenesis and screened against the *N,N*-di-*n*-propylamide, **2**, shown in Figure 4. By developing an aldolase capable of accepting large amide substituents, it was hypothesized that such an aldolase could also accept smaller ones.

The libraries of mutants from saturated mutagenesis were screened in the retroaldol direction in the standard enzyme-coupled assay, following the consumption of NADH by lactate dehydrogenase as it reduces pyruvate released by the aldolase. A mutant, E192N, was identified from these libraries with a 50-fold higher $k_{\text{cat}}/K_{\text{m}}$ for the screening substrate than the wild-type enzyme.

The new aldolase could accept various tertiary amides in 37–66% yields, with a diastereoselectivity of about 4:1. Both yield and diastereoselectivity de-

creased for secondary amides with bulky substituents, and the reaction time had to be increased to 14 days.

In a continuation of these studies, an effort was made to enhance the diastereoselectivity of this sialic acid aldolase variant.^[25] Using error-prone PCR on the E192N mutant gene and screening for selectivity against both diastereomers in the retroaldol direction led to the discovery of variants with improved selectivity for each of the two epimers **2** and **3**. Saturation mutagenesis of active site amino acid residues resulted in an *S*-selective aldolase [$k_{\text{cat}}/K_{\text{m}}(R)/k_{\text{cat}}/K_{\text{m}}(S) < 0.02$] possessing only two mutations from wild type, E192N and T167G, and an *R*-selective aldolase [$k_{\text{cat}}/K_{\text{m}}(R)/k_{\text{cat}}/K_{\text{m}}(S) = 48$] containing three mutations E192N, T167V and S208V.

Interestingly, both of these selective aldolases exhibited decreased activity compared to the parent enzyme E192N. The authors suggest that this is due to the screening protocol which selected hits based solely on improved selectivity, not activity.

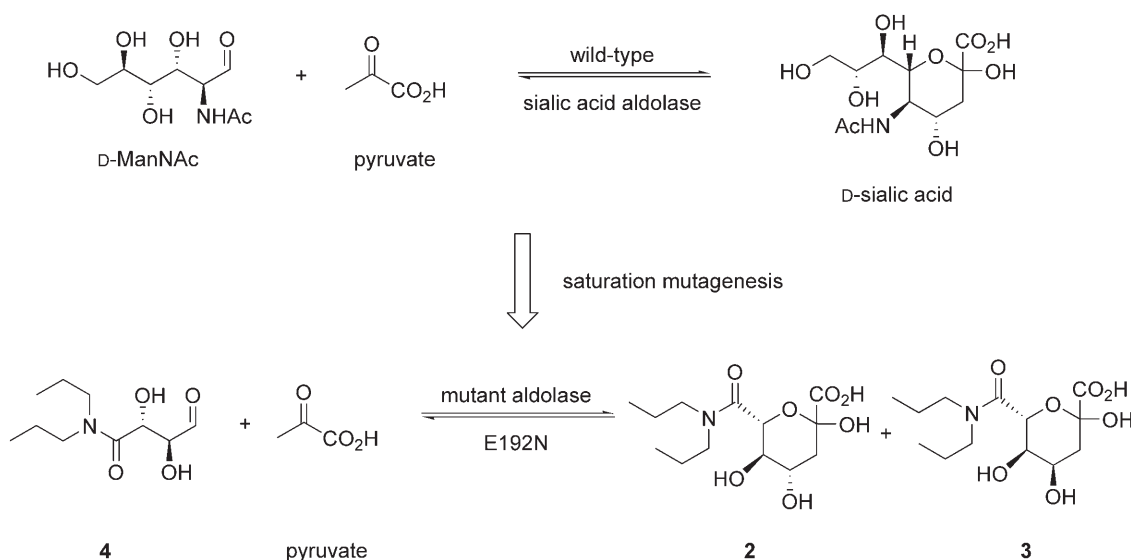


Figure 4. Synthesis of sialic acid mimics by a rationally designed aldolase mutant.

In recent work from our laboratory, directed evolution was applied to *N*-acetylneuraminic acid aldolase to create a new enzymatic function, L-3-deoxymanno-2-octulosonic acid (L-KDO) aldolase.^[26] This required an enzyme accepting L-arabinose as opposed to the natural D-ManNAc (Figure 5). Libraries of mutants were generated by error-prone PCR and screened for the consumption of L-KDO by monitoring the decrease in NADH fluorescence in the standard enzyme-coupled assay. Confirmed hits were subjected to further rounds of error-prone PCR. Eventually, a fifth-generation aldolase with an approximate 24-fold improvement in $k_{\text{cat}}/K_{\text{m}}$ for L-KDO compared to the wild-type aldolase was discovered. This mutant had eight amino acid changes, all of which were outside of the active site. The ability of the variants generated in this study to synthesize other unusual sugars was also explored. For instance, activity relative to D-ManNAc was increased for D-mannosamine, L-mannose, D-arabinose and D-gulose. The increased acceptance of D-mannosamine is particularly useful as this free amine can be used to generate new sialic acid acyl analogues.

The creation of large, diverse libraries of mutants is done routinely. The greatest challenge now lies in screening such libraries. The enzyme-coupled NADH consumption/production-based assays discussed thus far are useful, but sensitivity and throughput are limited. *In vivo* selection schemes can enhance screening capabilities by several orders of magnitude. With this in mind, Griffiths et al. developed an *in vivo* selection system for pyruvate-dependent aldolases and used this system to evolve a 2-keto-3-deoxy-6-phosphoglucuronate (KDPG) aldolase capable of accepting the unnatural 2-keto-4-hydroxyoctanoate (KHO)^[27]. They utilized a known *E. coli* cell line PB25, which is auxo-

trophic for pyruvate during growth on ribose. The addition of 50 μM pyruvate rescues the auxotroph, as does the addition of 2-keto-4-hydroxy-4-(2'-pyridyl)-butyrate, a known substrate for this KDPG aldolase. Thus, rescue can be achieved by a mutant aldolase that catalyzes a retroaldol reaction with KHO to release pyruvate. As illustrated in Figure 6, a library of KDPG mutants generated by error-prone PCR was screened for KHO activity using minimal medium supplemented with KHO. Three mutants capable of growing on this KHO medium were identified.

2.3 Acetaldehyde-Dependent Aldoases

Rational, structure-guided mutagenesis has been used to improve the acceptance of novel substrates by 2-deoxy-D-ribose 5-phosphate aldolase (DERA). While DERA does accept aldehydes other than D-glyceraldehyde 3-phosphate, the enzyme prefers the presence of the phosphate group. As phosphorylated substrates are expensive or difficult to obtain, and the phosphate group must be removed from the product by a phosphatase, a mutant capable of improved acceptance of non-phosphorylated D-glyceraldehyde was desired.

With a high resolution X-ray crystallographic structure of DERA in hand,^[28] DeSantis et al. chose to mutate specific residues in the active site responsible for binding this phosphate group.^[29] To change the electrostatic environment of the active site, five amino acid residues were changed to glutamic or aspartic acid by site-directed mutagenesis. After expression and purification of these five mutants, enzyme kinetics were measured for the retroaldol reaction with 2-deoxy-D-ribose 5-phosphate and 2-deoxy-D-ribose. The mutant S238D exhibited a 2.5-fold improvement

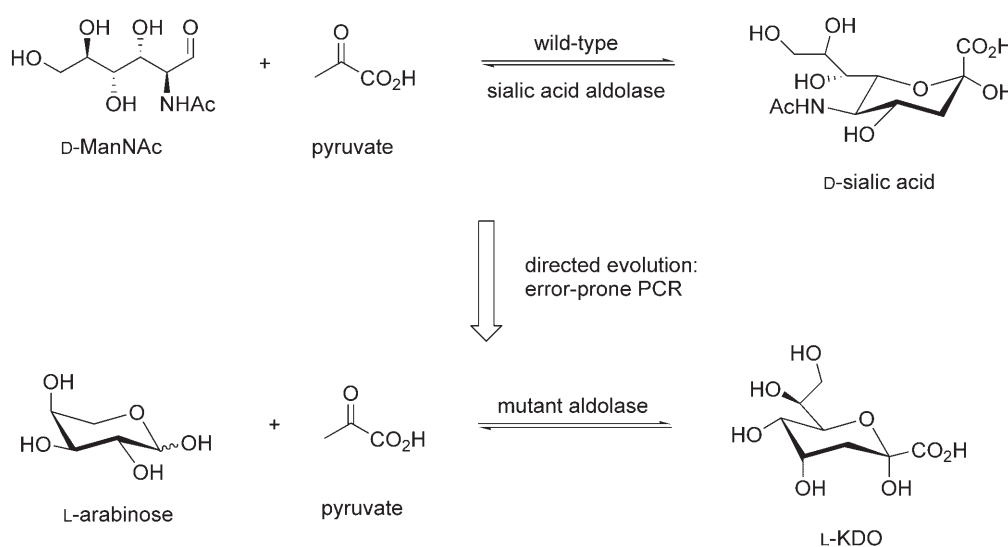


Figure 5. Directed evolution of an L-KDO aldolase.

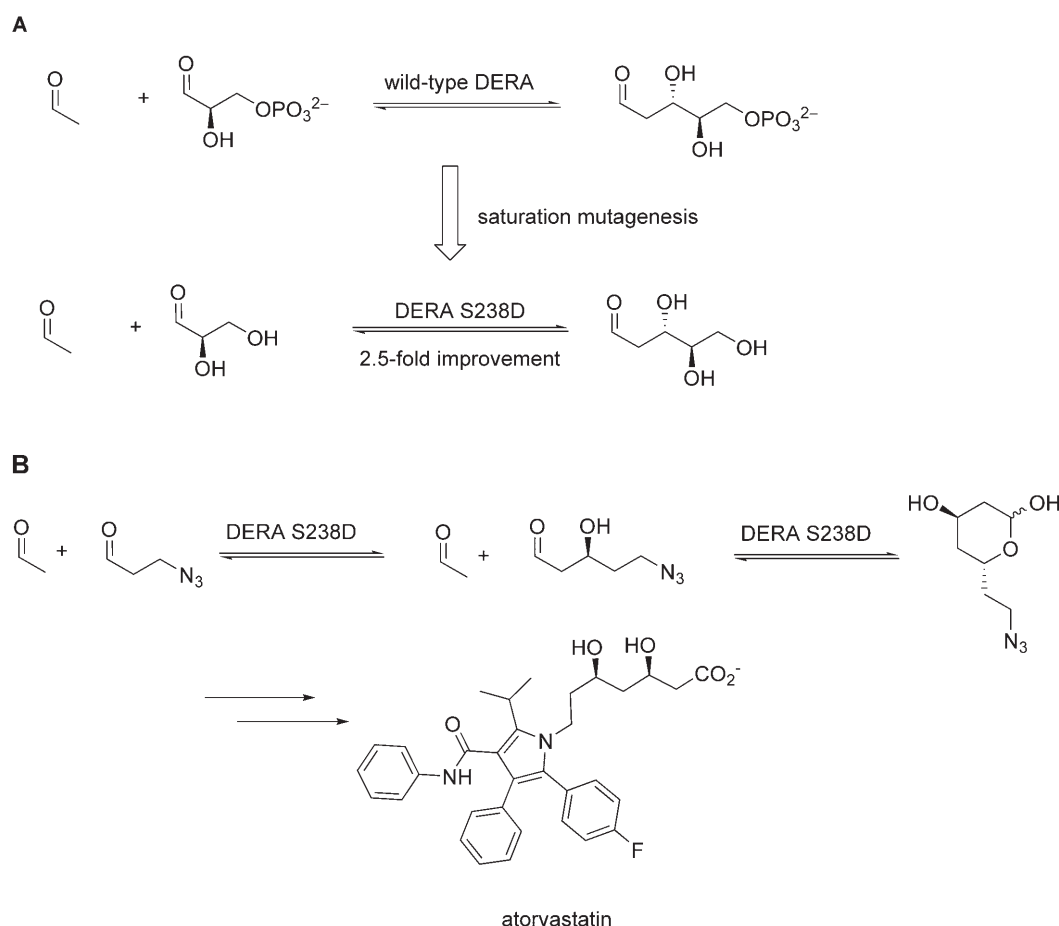


Figure 7. A: Saturation mutagenesis leads to a DERA mutant S238D with a 2.5-fold improvement in $k_{\text{cat}}/K_{\text{m}}$ for synthesis of unphosphorylated 2-deoxy-D-ribose. **B:** Application of DERA S238D in a synthesis of atorvastatin.

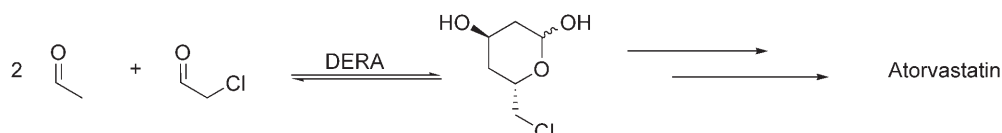


Figure 8. Tandem reactions of DERA in the synthesis of an atorvastatin intermediate.

Quite recently Castillo et al. demonstrated the potential of this enzyme for asymmetric synthesis.^[32] In their report, FSA was used in a facile two-step synthesis of D-fagomine, a naturally occurring imino sugar that inhibits glycosidases. Shown in Figure 9, the aldol reaction catalyzed by FSA between the DHA donor and *N*-carboxybenzoyl-3-aminopropanol took place with 89% yield and a diastereomeric ratio of 93:7 before purification. D-Fagomine is accessed by reductive amination with an overall yield of 51% and 99% *de*. It is likely that FSA will find broad utility in the future.

A novel aldolase has also been engineered from another enzyme. By a single active site mutation Seebek et al. created an aldolase from a PLP-dependent

alanine racemase.^[33] It was thought that His166 of *Geobacillus stearothermophilus* could act in the key proton abstraction step on the PLP-bound substrate as a similar histidine residue does in threonine aldolases. His166, however, is involved in a hydrogen bond with Tyr265. Thus, tyrosine was removed and the point mutant Y265A was found to exhibit a $k_{\text{cat}}/K_{\text{m}}$ for the retroaldol reaction with β -phenylserine 2.3×10^5 -fold higher than the wild-type alanine racemase. A 4×10^3 -fold decrease in alanine racemase activity was also observed.

In a follow-up study, the retroaldol reaction of this new aldolase was tested with α -methyl- β -phenylserine.^[34] Both the *3R* and *3S* diastereomers of α -methyl- β -phenylserine were accepted with even greater effi-

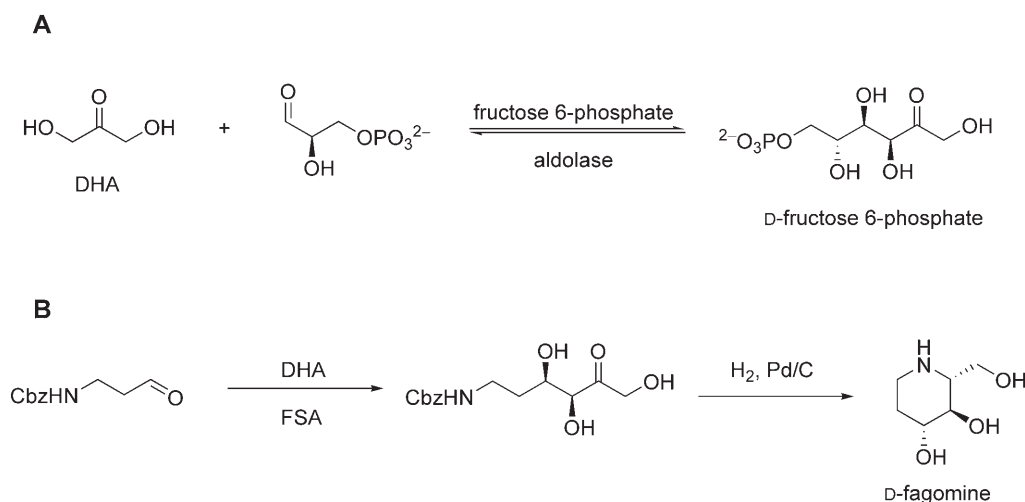


Figure 9. **A:** Wild-type reaction of FSA aldolase. **B:** D-Fagomine synthesis by FSA.

ciency than β -phenylserine. While further optimization is necessary, this novel aldolase is a promising potential biocatalyst.

It is almost a certainty that microorganisms with their tremendous metabolic diversity will afford more aldolases. In particular, work on elucidating the metabolic pathways of xenobiotic degradation by microbes has yielded new aldolases which have yet to be explored by biocatalysis researchers. A recent review by Samland and Sprenger^[14] concludes with some thoughts on novel aldolases and where they may come from and the reader is directed to this review and the work cited therein.

4 Reaction and Substrate Engineering

As powerful as directed evolution and protein engineering are, these methods are not always practical or necessary. Screening protocols can be difficult to develop and time consuming to put into practice. *In vivo* selections are undeniably powerful, but their development is quite challenging. For the industrial researcher in particular, the iterative gain in a useful function that is characteristic of directed evolution is not feasible when faced with short timelines and cost limitations. It is often the case, however, that simple alterations to an acceptor substrate or changes to reaction conditions can lead to improved processes. This can be illustrated in the case of DERA. This enzyme accepts D-glyceraldehyde 3-phosphate in preference to the L-enantiomer. In situations when the C-2 substituent is changed from a hydroxy group to a methyl or methoxy group this preference is inverted, allowing for the formation of products with new stereochemistries. An X-ray crystal structure was used to rationalize this inversion of stereoselectivity. The presence of a small hydrophobic pocket inside the

active site on the opposite side of the natural substrate can be invoked as the source of the inversion of specificity.^[35]

Along similar lines, Lamble et al. reported on observations with a thermostable KDGA aldolase from the extremophile *S. solfataricus*.^[36] This enzyme exhibits high promiscuity and is capable of synthesizing all four possible diastereomers of 3-deoxy-2-hexulose starting from racemic glyceraldehyde. However, upon addition of an acetonide group to the glyceraldehyde substrates (Figure 10), greatly enhanced selectivity was observed. For both *R*- and *S*-glyceraldehydes, the stereochemistry of the newly formed C–C bond is always *S*. The authors suggest that the steric bulk of the acetonide protecting group likely blocks the aldehyde from binding in a manner that would allow pyruvate to be delivered from the *Re* face.

Researchers at Diversa used reaction engineering to improve the DERA-catalyzed production of a statin intermediate.^[37] First, an improved DERA was identified by screening of environmental DNA libraries. A new DERA enzyme was discovered from an unknown organism, which was superior to *E. coli* DERA with respect to catalyst load. As discussed in the above example from DSM, DERA is inhibited by chloroacetaldehyde, a potent electrophile. To overcome this, a fed-batch process was developed wherein the substrates chloroacetaldehyde and acetaldehyde were added slowly to the reaction mixture. Thus chloroacetaldehyde was consumed as it was added and did not reach inhibitory concentrations. Through a combination of discovery of a new DERA and the fed-batch process, much higher concentrations of product were obtained while at the same time using less catalyst. Additionally, other groups have also developed aldolase-dependent processes for the preparation of statin intermediates.^[38]

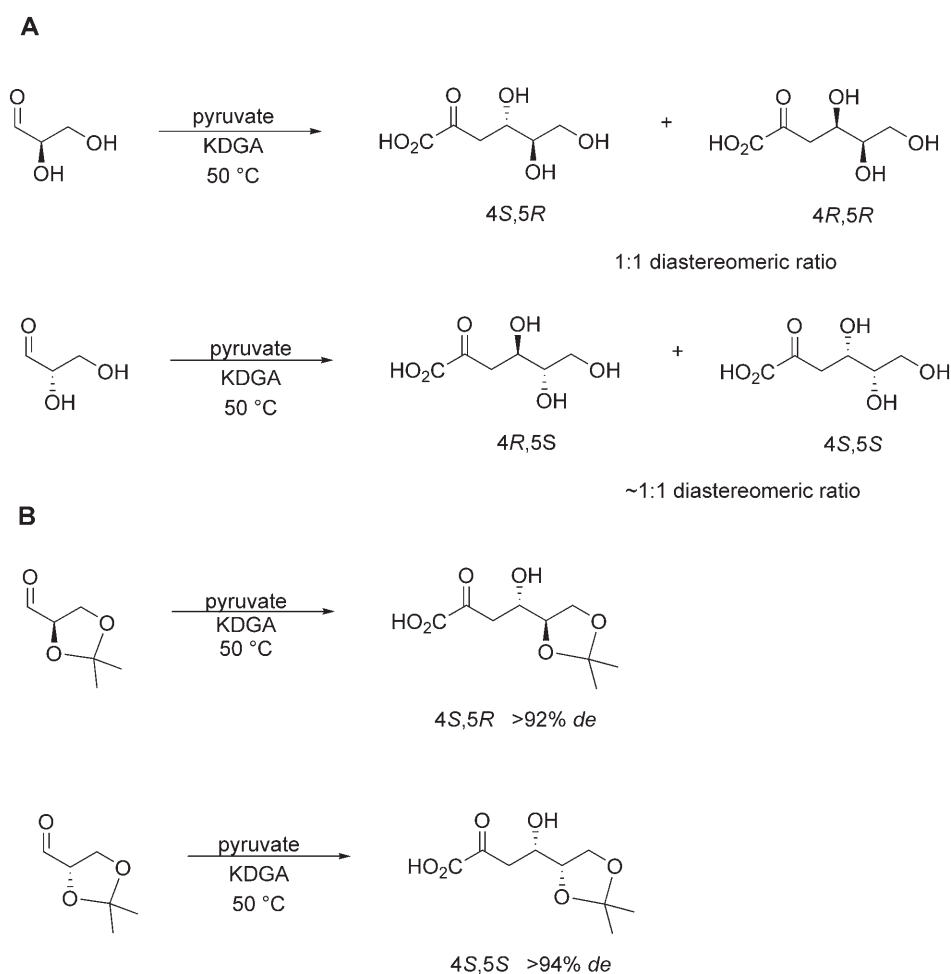


Figure 10. Substrate engineering. **A:** Poor selectivity of *S. solfataricus* KDGA aldolase. **B:** Enhanced stereoselectivity by acetonide protection of glyceraldehyde substrates.

As discussed earlier, one of the drawbacks to using aldolases in asymmetric catalysis is the limited number of donor substrates that these enzymes may use. Reaction engineering approaches have been explored as a means to allow the use of DHA as a donor by DHAP-dependent aldolases. Previously, these efforts have involved *in situ* formation of arsenate or vanadate esters,^[39] which mimic the natural phosphate ester of DHAP. Given the unwanted redox activity of vanadate and the toxicity of arsenate, this solution is not ideal. Recent work in our laboratory has explored the use of inexpensive borate as a phosphate ester mimic.^[40] It was discovered that the DHAP-dependent rhamnulose 1-phosphate aldolase (RhaD) accepts DHA when reactions are carried out in borate buffer, whereas no product is observed in other buffers. This was exploited in a high-yielding synthesis of L-fructose from DHA and glyceraldehyde. Various azidoaldehyde acceptors were also reacted with DHA in the presence of borate, and after reductive cyclization yielded iminocyclitols (Figure 11).

A mechanistic explanation of the borate-mediated reaction was suggested by monitoring the reverse reaction. No retroaldol activity was observed for L-fructose in the presence of borate buffer. It is hypothesized that stable complexes between borate and L-fructose form under the reaction conditions and prevent productive binding of L-fructose to the aldolase. Thus the equilibrium is driven in the synthetic direction by trapping of the product.

5 Future Prospects and Challenges

In this review the scope and limitations of using aldolases to catalyze asymmetric aldol reactions are discussed. Work in recent years has been focused on overcoming limitations through directed evolution and reaction engineering. More work remains to be done in broadening the scope of available substrates and conditions that are tolerated, including cosolvents, pH and temperature. Activity at high substrate

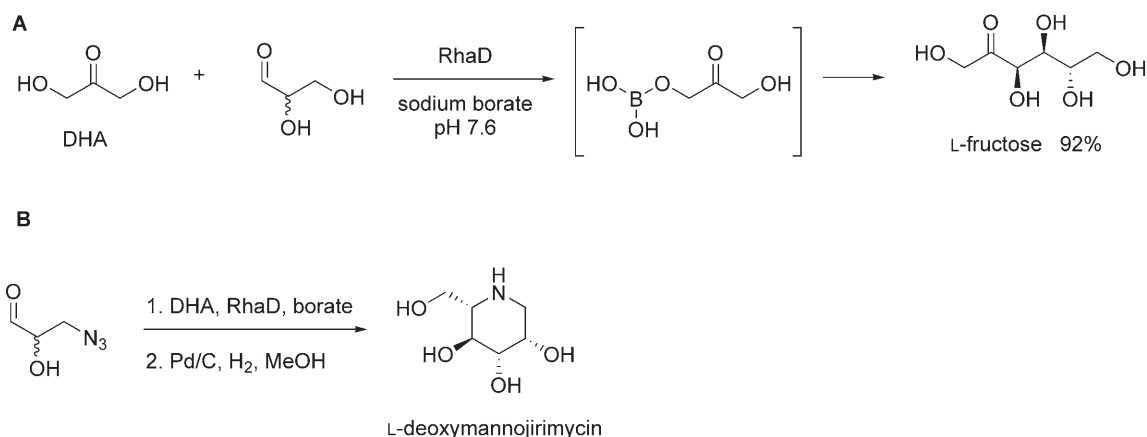


Figure 11. A: Synthesis of L-fructose by RhaD using DHA and borate buffer. **B:** Two-step synthesis of L-deoxymannojirimycin.

concentrations is also important for viable industrial processes.

Directed evolution and reaction engineering will continue to be applied to these problems, although thus far the improvements have been by relatively small increments. As these technologies become more advanced, more dramatic improvements are expected. It is likely that more aldolases will be discovered from genome sequence data, metabolite degradation pathways, and extreme environments, and that these novel aldolases will increase the breadth of molecules accessible by enzymatic catalysis.

A major challenge in the application of aldolases for synthesis, and for biocatalysis in general, is not technical, but rather philosophical. Biocatalysis is a multidisciplinary field, and progress requires the integration of chemical and biological techniques. Future discoveries and more widespread application of biocatalysis require chemists to consider enzymes as part of their synthetic toolbox, and likewise for biologists to consider the chemical applications of enzymes. For instance, in many industrial processes, enzymes have been employed only as a last resort to effect transformations that proved to be challenging by traditional methods. However, in cases where there is a dedicated biocatalysis group and strong communication between chemistry and biology, biocatalysis may be considered as an option in designing synthetic strategies at the outset. In these cases the potential value of aldolases and other enzymes as catalysts for organic synthesis can truly be realized.

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