Enantioselective C–C bond synthesis catalysed by enzymes

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The enantioselective synthesis of C–C bonds is often the pivotal step of a synthesis. Nature has made a variety of versatile enzymes available that catalyse this type of reaction very selectively under mild conditions. Cyanohydrins, acyloins (α-hydroxy ketones), α-hydroxy acids and aldols (β-hydroxy ketones) are very efficiently synthesised enantioselectively with the aid of C–C bond forming enzymes, which we discuss in this *tutorial review*. In the case of the α-hydroxy acids the applications of nitrilases in a synthetic dkr even allows a disconnection that has no enantioselective chemical equivalent.

1. Introduction

C–C bond forming reactions are one of the mainstays of organic chemistry. Indeed, they are the key steps in most syntheses and it is an essential part of the training of every chemist to obtain a thorough knowledge of C–C bond synthesis. Current organic chemistry textbooks consequently give detailed descriptions on how to construct complex carbon frameworks. Surprisingly they do not mention enzymes, and this although nature is the master builder of complex structures. Possibly due to this exclusion their application in academic research laboratories, and in particular in total syntheses, is still rather underdeveloped. The enzymatic C–C bond forming reactions are often highly chemo-, regio-, and enantioselective, they proceed under very mild conditions and the amount of waste generated—in the form of chiral auxiliaries, protection groups and other side reactions (isomerisations, rearrangements *etc.*)—is negligible. With the intention to compensate this omission of organic chemistry textbooks, we aim to give a concise overview of nature's versatile tools and their application in organic synthesis. We concentrate on C–C bond forming enzymes that are readily available and straightforward to use for every chemist.

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1.1. A few words about enzymes

Wherever there is life, enzymes can be found. Vast numbers of these natural catalysts exist that can work in the most extreme environments. They catalyse a huge variety of different transformations and can accelerate reactions up to 10^{17} fold.[1] However startling the origins of some of the enzymes may sound, they are straightforward to use once one is accustomed to a few differences to "normal" catalysts. Numerous of these properties are actually very welcome. Unlike many chemical reagents, enzymes are not moisture or air sensitive. Enzymes are proteins and tend to be soluble in water but insoluble in organic solvents. This is an advantage too, since they can often be used in organic solvents and after the reaction they can be removed by a simple filtration. A great number of the commercially available enzymes are immobilised on carriers

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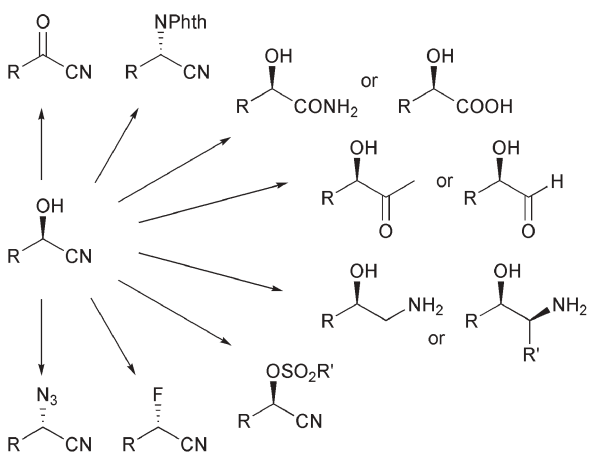
to aid their removal. Most enzymes are active under mild conditions, *i.e.* at room temperature and near neutral pH. In addition to all these practical aspects they are environmentally benign and completely biodegradable. The main difference from chemical catalysts is that they are applied in units and not in moles. *Via* an activity test, the number of units per weight (solid enzymes) or volume (enzyme solution) of the enzyme is determined with a standard reaction. Even when some loss of activity is observed due to aging, the results are just as reproducible as in the case of any other catalyst if the same activity/number of units is added to the reaction. Similar to chemical catalysts, the right conditions for each enzyme need to be found too, and many can actually be used under extreme conditions, at high and low temperatures and pH values. Overall, enzymes are very selective and specific catalysts, that act under mild conditions and are straightforward to apply. Consequently, many enzyme-based reactions have been established on an industrial scale.

A large number of enzymes for the formation of C–C bonds is available. Many of them are lyases and, as we will show, hydrolases. These classes of enzymes were designed by nature for breaking down molecules. By reversing the equilibrium, towards the natural substrates rather than destroying them, these enzymes can be applied in the synthesis of C–C bonds. As degrading enzymes, they are robust but not very substrate specific. They are, however, specific for the functional group they destroy in nature and generate in the laboratory. Equally important, they are very stereoselective. Thus these enzymes are ideal for application in organic synthesis.[2,3]

2. Cyanohydrins

Cyanohydrins are versatile building blocks in organic synthesis; their enantioselective formation has therefore attracted considerable attention (Scheme 1).[2,4–6] The addition of HCN to carbonyl compounds is a reversible reaction and hence the starting material and the product are in equilibrium. The racemic addition of HCN to aldehydes and ketones is base catalysed. In order to suppress this racemic background-reaction during the enantioselective synthesis, neutral or possibly acidic reaction conditions need to be applied. Both chemical and enzymatic syntheses are comparable. The formation of cyanohydrins from aldehydes proceeds readily. However, the equilibrium for ketones tends to lie on the side of the starting materials. Therefore these reactions can only be performed successfully, by either bio- or chemo-catalysis, when an excess of HCN is used or when the product is constantly removed from the equilibrium. The unfavourable equilibrium can fortunately also be turned into an advantage. The liquid acetone cyanohydrin (**25**) can replace the volatile HCN, releasing it *in situ* during the synthesis of an aldehyde based cyanohydrin; thereby significantly improving safety in the laboratory. Alternative methods for the safe handling of cyanides on a laboratory scale are, for instance, to use cyanide salts in solution. These solutions can be acidified and used as the aqueous layer in two-phase systems or the HCN can be extracted into the organic layer with the desired solvent for reactions in an organic phase. After the reaction excess cyanide can readily be destroyed with iron(II) sulfate or bleach. In any case, all work involving cyanide (enzyme-catalysed or not) must be performed in a fume hood and an HCN detector should always be at hand.[5]



Scheme 1 Cyanohydrins as building blocks.

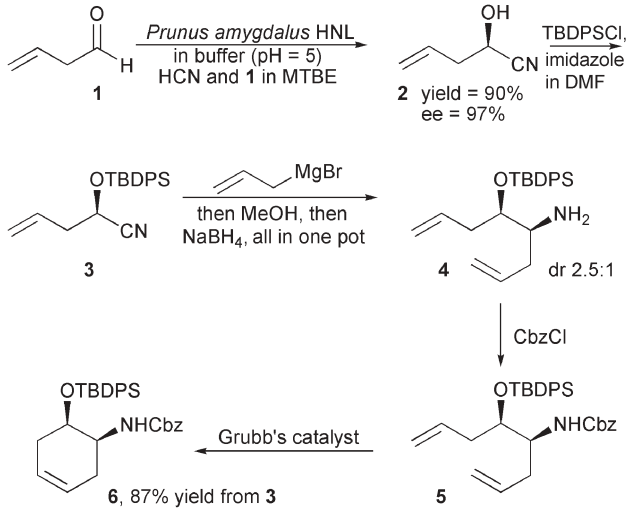
2.1. Hydroxynitrile lyases

Nature provides the chemist with *R*- and *S*-selective enzymes that catalyse the formation of cyanohydrins. They are known as hydroxynitrile lyases (HNL) or oxynitrilases.[4–6] Their natural function is to degrade natural cyanohydrins, such as mandelonitrile (**33**) in almonds. This reaction occurs if a predator injures the plant-tissue. The HCN released acts as a deterrent. In the case of almonds, the benzaldehyde (**32**) that is released at the same time is ironically the flavour that attracts humans to eat almonds. Since HCN is a common deterrent in the plant kingdom, the number of HNLs available is large. Structurally they can be very different and consequently their exact mechanisms of action can be different. This has, however, very little influence on their practical applicability. Especially since all the HNLs utilise acid–base catalysis, no cofactor needs to be added, nor are any of the HNLs metalloenzymes.[7] Given their generally low substrate specificity, combined with a very high stereoselectivity, it is possible to find a suitable biocatalyst for almost any synthetic problem (Table 1). Moreover they can easily be employed and are stable in two-phase systems, emulsions and some of them in pure organic solvents.

Due to the very positive properties of HNLs they have been applied in the syntheses of many very different compounds. *Prunus amygdalus* HNL has already been used for almost 100 years. It has successfully been employed in the synthesis of both aromatic and aliphatic cyanohydrins. After optimisation of the reaction conditions, **2** was prepared in excellent yield and enantioselectivity. The subsequent Grignard reaction of the protected cyanohydrin was quenched with methanol and *in situ* reduction yielded **4**. The diastereoselectivity of the reduction (dr = 2.5:1) was induced by the stereocentre that was established with the aid of the HNL.[8] Protection and ring closing metathesis then gave the unsaturated cyclic aminoalcohol **6** in a very good yield (Scheme 2).

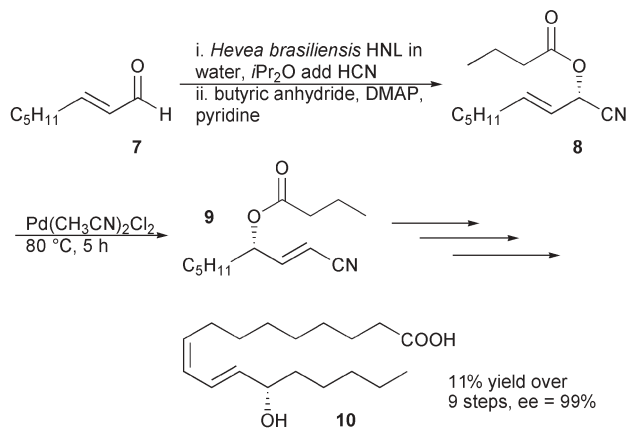
Table 1 Successfully applied hydroxynitrile lyases (HNLs)

Name and origin	Natural substrate	Stereoselectivity
Prunus amygdalus HNL, almonds	(*R*)-Mandelonitrile (**33**)	*R*
Linum usitatissimum HNL, flax-seedlings	(*R*)-Butanone cyanohydrin and acetone cyanohydrin (**25**)	*R*
Hevea brasiliensis HNL, rubber-tree leaves	Acetone cyanohydrin (**25**)	*S*
Sorghum bicolor HNL, millet-seedlings	(*S*)-4-Hydroxy-mandelonitrile	*S*
Manihot esculenta HNL, manioc leaves	Acetone cyanohydrin (**25**)	*S*



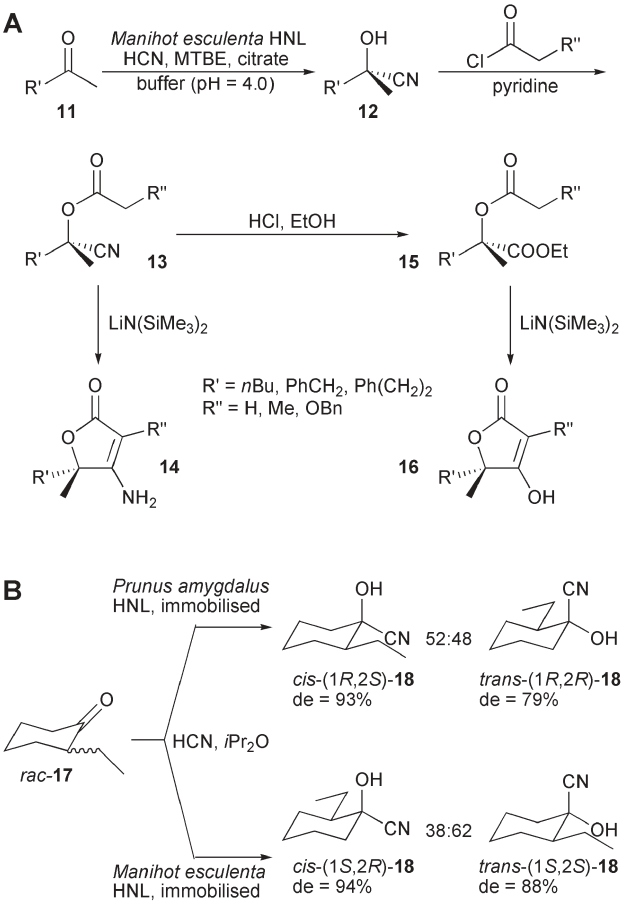
Scheme 2 *Prunus amygdalus* HNL catalysed synthesis of **6**.

Utilising the *S*-selective *Hevea brasiliensis* HNL and a palladium catalysed [3,3]-sigmatropic rearrangement it was possible to synthesise coriolic acid (**10**), a linoleic acid metabolite with important biological activities. Again the new C–C bond was formed and the stereocentre was established with an excellent yield and enantioselectivity (Scheme 3). This is not only a very efficient synthesis but also an example of how the stereocentre of the cyanohydrin can readily be transferred to a different part of the molecule.[9] Hereby the scope of the cyanohydrins as building blocks is expanded significantly.



Scheme 3 *Hevea brasiliensis* HNL based synthesis of coriolic acid (**10**).

Although the formation of cyanohydrins from ketones is difficult (due to the unfavourable equilibrium) it can be achieved. When methyl ketones **11** were treated with 1.5 equivalents of HCN in an emulsion of citrate buffer (pH = 4.0) and MTBE, *Manihot esculenta* HNL catalysed the synthesis of the corresponding cyanohydrins **12** with good yields (85–97%) and enantioselectivities (69–98%). Subsequent transformation into the corresponding esters **13** allowed a second C–C bond forming reaction. Since these cyanohydrins have no α-hydrogen, they cannot be deprotonated adjacent to the nitrile group. The newly formed ester however, could be deprotonated and then a ring closing attack on the nitrile function yielded the unsaturated lactones (**14**, Scheme 4A). When the nitrile function was first converted *via* a Pinner reaction into an ester **15**, the intramolecular Claisen reaction gave chiral tetronic acids (**16**).[10] In an attempt to widen the application of HNLs towards more demanding ketones,



Scheme 4 HNL-catalysed cyanohydrin syntheses from ketones.

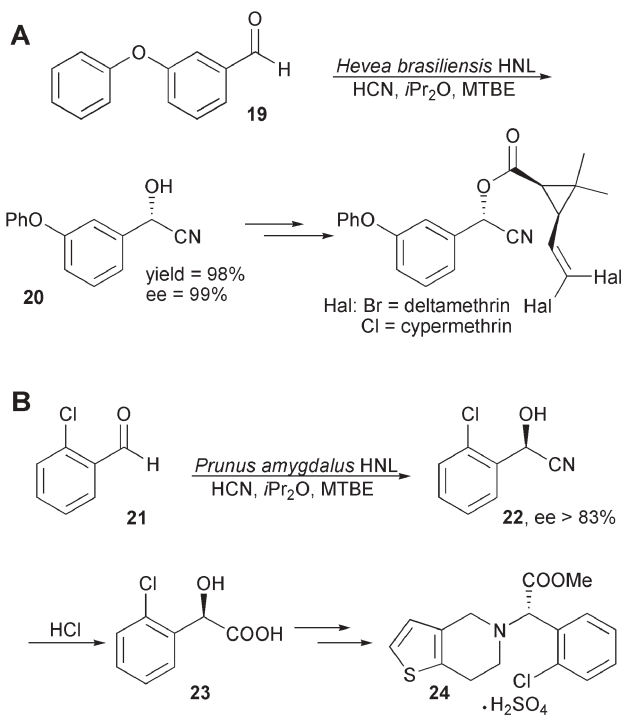
substituted cyclohexanones were studied as substrates. Both enzymes, *Prunus amygdalus* HNL and *Manihot esculenta* HNL, displayed very high enantioselectivity. That is to say the enzymes catalysed the formation of an (*R*)-cyanohydrin (*Prunus amygdalus* HNL) or an (*S*)-cyanohydrin (*Manihot esculenta* HNL). Contrary to chemical catalysis very little to no *cis*:*trans* selectivity is observed. Basically the transformation of the racemic starting material led to a resolution of the enantiomers into diastereoisomers. The higher the stereoselectivity of the enzyme for the formation of an *R*- or and *S*-stereocentre, *i.e.* the α-C of the cyanohydrin, the closer the *cis*:*trans* ratio was to 50:50 (Scheme 4B). This also implies that close to quantitative yields of the desired alkyl substituted cyclohexanone cyanohydrin can be obtained if enantiopure starting materials and the right enzyme are employed. The conversions that were described are excellent, however they could only be obtained with a significant excess of HCN (4:1).[11]

Industrially *Hevea brasiliensis* HNL is applied for the large-scale production of (*S*)-*m*-phenoxy-mandelonitrile (**20**), an intermediate for insecticides such as deltamethrin and cypermethrin (Scheme 5A). *Prunus amygdalus* HNL is employed for the bulk production of (*R*)-*o*-chloro-mandelonitrile (**22**), an intermediate for clopidogrel (**24**), an antithrombotic drug (Scheme 5B).[12]

2.2. Lipase-based synthetic dynamic kinetic resolution

The base catalysed racemic synthesis of cyanohydrins is normally an undesired background reaction. However, one can also turn this dynamic equilibrium-reaction into an advantage. For instance, by combining it with the irreversible and enantioselective synthesis of a cyanohydrin ester. For this enantioselective acylation, lipases are used as catalysts. Lipases hydrolyse lipids and, together with esterases and amidases, are also called hydrolases. As in the case of the hydroxynitrile lyases they can be employed to catalyse the reverse reaction; in the case of the lipases the enantioselective synthesis of esters. This ester synthesis can be performed irreversibly when the appropriate acyl donors are used.[13] The overall combination of a dynamic equilibrium (here of aldehyde **26** and cyanohydrin **27**) and a kinetic resolution (the fast conversion of one enantiomer, here *S*-**27**, while the other enantiomer does not react) in one pot is called dynamic kinetic resolution (dkr). If a prochiral starting material is used, here the aldehyde **26**, and a new bond is formed, it is a synthetic dkr. Summarising, this one-pot reaction sequence is an example of a lipase-catalysed formation of a new C–C bond (Scheme 6A).

Based on this methodology many heterocyclic cyanohydrin acetates such as **31** were prepared in high yields and with excellent enantioselectivities, *Candida antarctica* lipase A (CAL-A) being the lipase of choice (Scheme 6B).[14] A detailed study of the reaction conditions revealed that the carrier on which the lipase is immobilised is important. With celite R-633 as support for *Candida antarctica* lipase B (CAL-B), mandelonitrile acetate (*S*-**34**) could be synthesised in 97% yield and 98% *ee* (Scheme 6C). This is not only a significant improvement on earlier results for the synthetic dkr, but also better than the best chemical catalyst (yield of **34** = 88%, *ee* = 90%).[15] In all cases alkaline amberlite was employed as a base and acetone cyanohydrin (**25**) was used as the HCN source; since it is a tertiary alcohol the lipases cannot convert it into an ester.

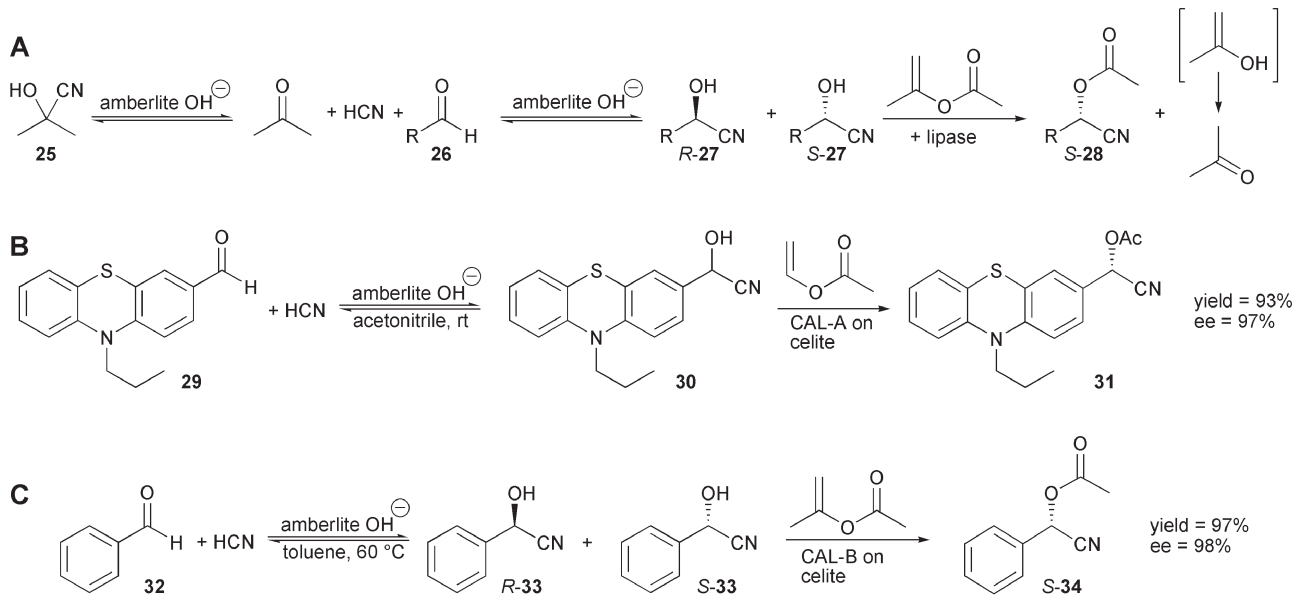


Scheme 5 Industrial applications of HNLs.

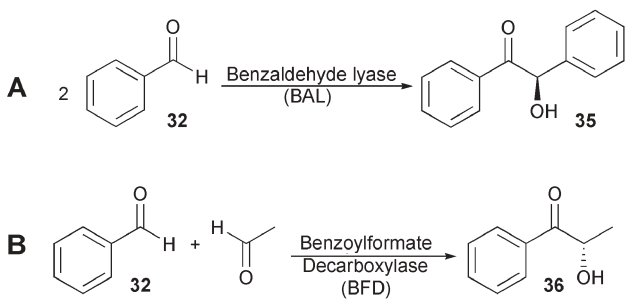
3. α-Hydroxy ketones (acyloins)

Acyloins are valuable intermediates for synthesis. Their bifunctional nature and the presence of a stereocentre make them amenable to further synthetic transformations. There are two classical chemical syntheses for these α-hydroxy ketones: the acyloin condensation and the benzoin condensation. In the acyloin condensation a new C–C bond is formed under reductive conditions. In the benzoin condensation a new C–C bond is formed too, however, this is accompanied by the breaking of another C–C bond in one of the starting materials, the cyanohydrin (used to achieve the necessary umpolung). Similar to these two approaches a number of enzymes catalyse this type of reaction, however, the reaction conditions are considerably milder. Enzymes such as benzaldehyde lyase (BAL, Scheme 7A) catalyse the formation of a new C–C bond enantioselectively. But also transketolases (TK) and decarboxylases, such as benzoylformate decarboxylase (BFD), catalyse acyloin formation efficiently. Similar to the chemical benzoin reaction they break a C–C bond while forming a new one. This making and breaking of C–C bonds involves a decarboxylation in both the transketolase and the decarboxylase-catalysed reactions. When transketolase is applied it might also involve the discarding of a ketone, *i.e.* of several carbon atoms. In contrast to TK, BFD can also catalyse the formation of new C–C bonds without breaking others (Scheme 7B).[16]

All of the enzymes that catalyse the C–C bond formation between two carbonyl groups leading to α-hydroxy ketones,



Scheme 6 Lipase-catalysed syntheses of cyanohydrin acetates, based on a synthetic dynamic kinetic resolution.



Scheme 7 A: BAL catalyses acyloin formation from two aldehydes; B: BFD catalyses the formation of a new C–C bond. When one of the aldehydes is aliphatic the enzyme is *S*-selective.

rely on a cofactor: thiamin diphosphate (**37**). The unphosphorylated thiamin is better known as vitamin B_1, and deficiency thereof is the cause of the disease beriberi. Thiamin diphosphate has a similar function as the cyanide in the benzoin condensation. After deprotonation, the resulting ylide (**38**) attacks the carbonyl group of the aldehyde (**32**). Shifting of the negative charge from the alcoholate (**39**) to the carbanion (**40**) initiates an umpolung of the carbonyl group. This umpolung enables the formation of the new C–C bond. The carbanion (**40**) can attack a second aldehyde, stereoselectively forming the new C–C bond of the α-hydroxy ketones. A shift of the alcoholate ion and elimination releases the acyloin (**36**) and the ylide (**38**). The enzyme is now ready for another catalytic cycle (Scheme 8A).[16,17]

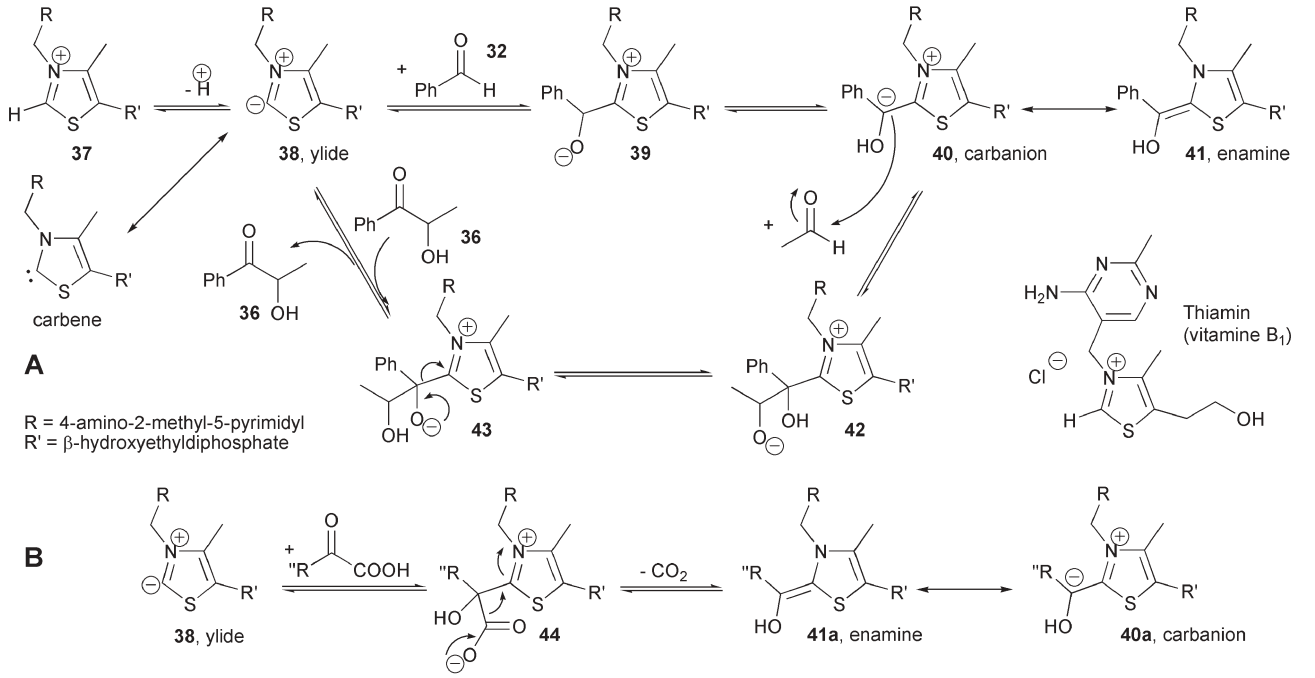
In the case of the decarboxylase- and transketolase-catalysed reactions starting with α-keto acids, the ylide (**38**) attacks the keto function to form an intermediate carboxylate ion (**44**). Decarboxylation, *i.e.* C–C bond fragmentation, leads to the reactive carbanion (**40a**, Scheme 8B), that can then enantioselectively form the new C–C bond of the desired α-hydroxy ketone, as shown in Scheme 8A. TK requires two cofactors, Mg^{2+} as well as **37**.[17,18]

The enantioselective synthesis of α-hydroxy ketones *via* a C–C bond forming reaction has received a considerable impulse during the last years. Both chemical[19] and enzymatic[16] approaches have only been fully developed for a short time. Consequently the full potential of either approach has not yet been explored. Four different enzymes are commonly used for this reaction. With these four enzymes, BAL, BFD, pyruvate decarboxylase (PDC)[16] and TK,[18] many different compounds can be prepared, however, not every stereoisomer is accessible yet.

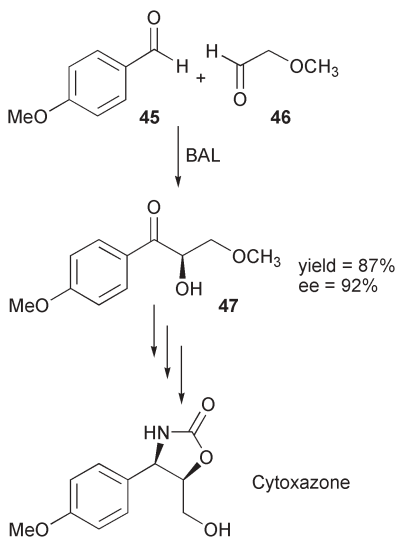
BAL is an *R*-selective enzyme that accepts either two aromatic aldehydes (Scheme 7A) or an aliphatic and an aromatic aldehyde. This enables an enantioselective access to a variety of biologically active compounds and natural products such as *Streptomyces* sp. metabolite cytoxazone (Scheme 9).[20] If formaldehyde is used instead of acetaldehyde or its derivatives, hydroxyacetophenones are obtained.[21]

Of particular interest is that not only BAL, but also BFD, can be applied in the *R*-selective cross coupling reactions between two aromatic aldehydes (Scheme 10). While their stereoselectivity in these reactions is identical, their substrate specificity is different, widening the scope of this cross coupling.[22] (*S*)-Acyloins of two aromatic aldehydes are, so far, enzymatically only accessible *via* a kinetic resolution of the racemic mixture. In this context it is particularly remarkable that BFD is *R*-selective only for the coupling of two aromatic aldehydes. In contrast to this observation, BFD is *S*-selective for the coupling of two aliphatic aldehydes or of aliphatic aldehydes with aromatic aldehydes (Scheme 7B). Thus, for the coupling reaction between an aromatic aldehyde and aliphatic aldehyde, BAL and BFD complement each other's stereoselectivity.[23]

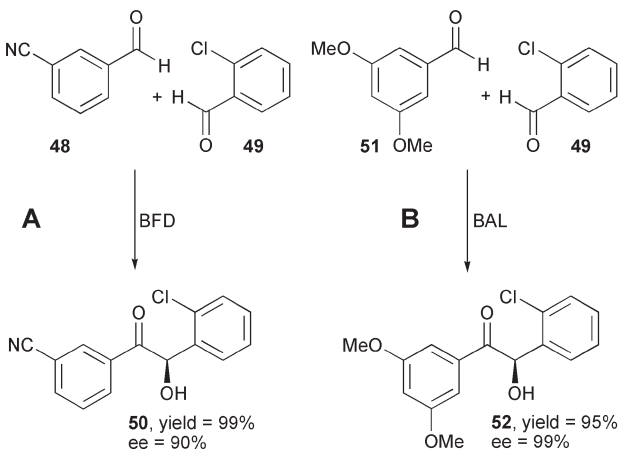
With PDC, an acyloin that cannot be prepared using either BAL or BFD comes into reach. In the above-described



Scheme 8 After deprotonation, **37** initiates an umpolung of the carbonyl group, thereby enabling the C–C bond formation between two carbonyl groups.



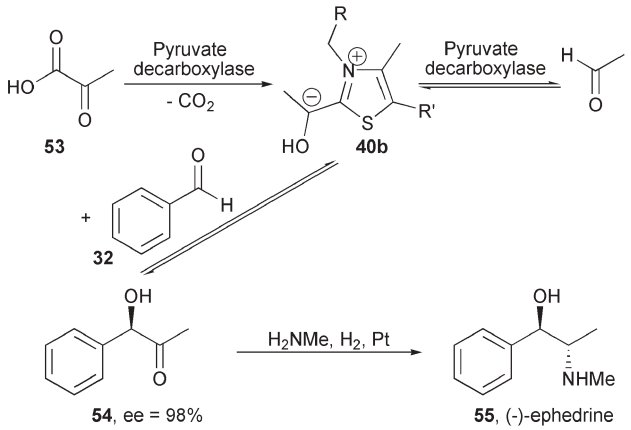
Scheme 9 Application of BAL in enantioselective synthesis.



Scheme 10 *R*-Selective cross coupling reactions between two different aromatic aldehydes catalysed by BAL and BFD.

reactions between an aromatic and an aliphatic aldehyde the keto group of the product is always adjacent to the benzene ring (Scheme 7B and 9). In the PDC catalysed reaction of pyruvate (**53**) and benzaldehyde (**32**), the C–C bond formation is coupled to a decarboxylation, *i.e.* the breaking of a C–C bond. Instead of pyruvate, acetaldehyde can be employed, thus avoiding the loss of a carbon atom from the starting material. The (*R*)-phenylacetylcarbinol (**54**) obtained from these reactions is converted on a commercial scale into (−)-ephedrine (**55**, Scheme 11).[16,24] Unfortunately the research on PDC has very much been focused on this industrial application and the full synthetic potential of this enzyme has not yet been realised.

The transketolase class (TK) of enzymes plays a vital role in sugar metabolism. TK transfers an α-hydroxy carbonyl fragment from D-xylulose 5-phosphate to D-ribose 5-phosphate, yielding D-sedoheptulose 7-phosphate and D-glyceraldehyde 3-phosphate (Scheme 12A). This reaction is an equilibrium reaction and thus not very versatile for organic synthesis. Fortunately TK also accepts unphosphorylated substrates and instead of xylulose pyruvate (**53**) can be employed. Under these modified circumstances carbon dioxide and not D-glyceraldehyde 3-phosphate is the leaving group. Since this is a gas it escapes from the reaction mixture and the overall process becomes irreversible.[18] This versatile variation has been used for the synthesis of ulosonic acid analogues that are studied as inhibitors of the bacterial cell-wall biosynthesis



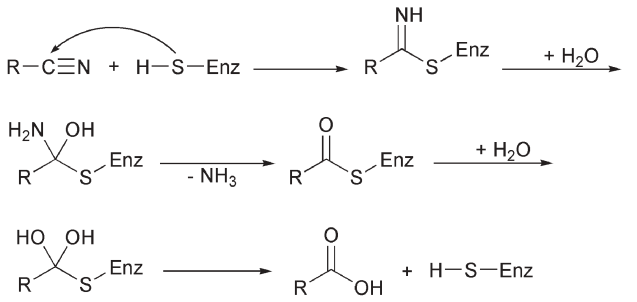
Scheme 11 Application of PDC in the synthesis of (−)-ephedrine (**55**).

(Scheme 12B).[25] It is noteworthy that TK displays stereoselectivity towards the aldehyde (**56**), accepting just one enantiomer. This selectivity of TK is normally limited to the α-C. It can be a drawback, since it narrows the number of substrates that can be converted by TK.

4. α-Hydroxy acids

The synthesis of α-hydroxy acids proceeds *via* a synthetic dynamic kinetic resolution. This disconnection is based on a synthetic dynamic equilibrium, during which the new C–C bond is formed as described in section 2.2. By combining the synthetic equilibrium with an irreversible kinetic resolution, the overall reaction sequence furnishes the desired products in excellent yields and selectivities. Here nitrilases are applied as enantioselective catalysts. Nitrilases are hydrolytic enzymes, *i.e.* they too can be classified as hydrolases, just like the lipases. Their natural function is the hydrolysis of nitrile groups directly to acids (Scheme 13).[26,27] In the synthetic dkr they catalyse the reaction for which nature developed them.

Again, an aldehyde is the prochiral starting material, which is in a base-catalysed equilibrium with a racemic cyanohydrin.



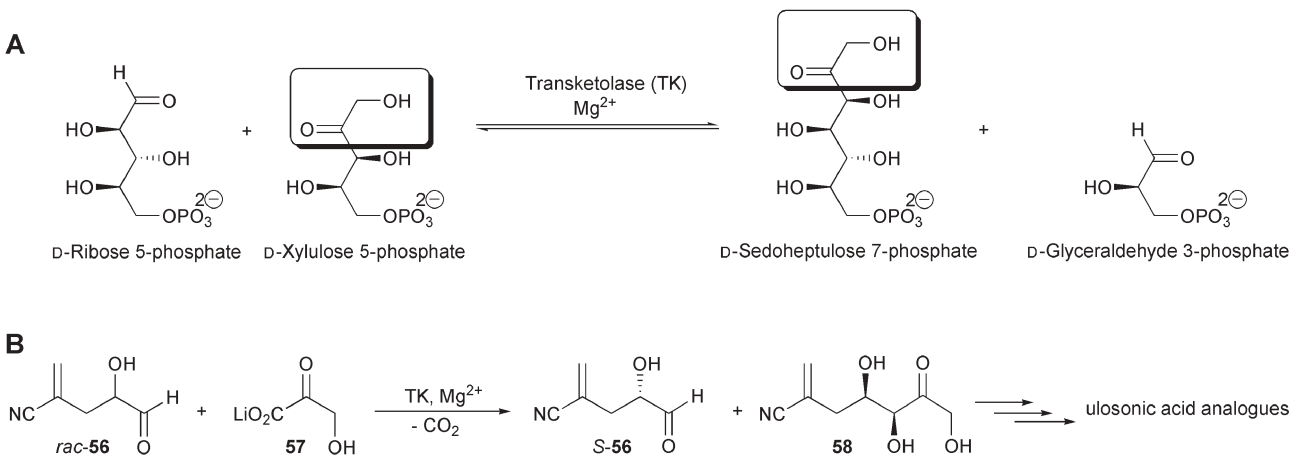
Scheme 13 Nitrilases catalyse the direct conversion of nitriles into carboxylic acids and ammonia.

By adding an enantioselective nitrilase the racemic cyanohydrin is irreversibly converted into the desired α-hydroxy acid. Nature provides a vast variety of *R*- and *S*-selective nitrilases that accept aliphatic and aromatic substrates, ensuring a large scope for this disconnection (Scheme 14).[28] However, nitrilases tend not to recognize cyanohydrins that are prepared from ketones as substrates. Based on this synthetic dkr several companies produce (*R*)-mandelic acid and analogues on a multi ton scale.[12]

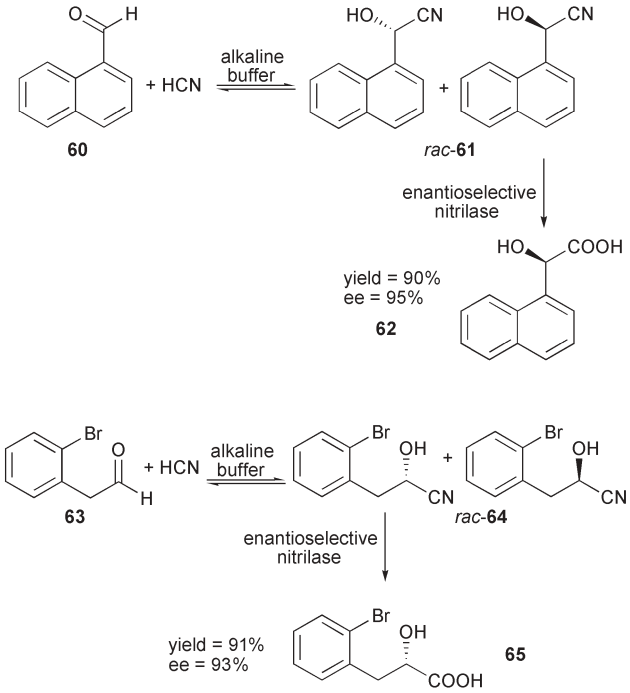
This reaction cascade has no direct enantioselective chemocatalytic equivalent and is a welcome addition to synthetic organic chemistry. Moreover, it can potentially also be performed with nitrile hydratases (enzymes that convert nitriles into amides), expanding the scope of the reaction even further. An alternative approach is to enantioselectively prepare the cyanohydrins with the help of HNLs (see 2.1.) and convert them with the help of unselective nitrilases into the desired carboxylic acids. This can be either done in two separate steps[29] or integrated into a single process.[30]

5. Aldols (β-hydroxy-carbonyl compounds)

The aldol reaction is one of the most important reactions to introduce a new C–C bond and it is also highly atom-economic. In the case of the aldol reaction a significant difference between the chemically catalysed and the enzyme



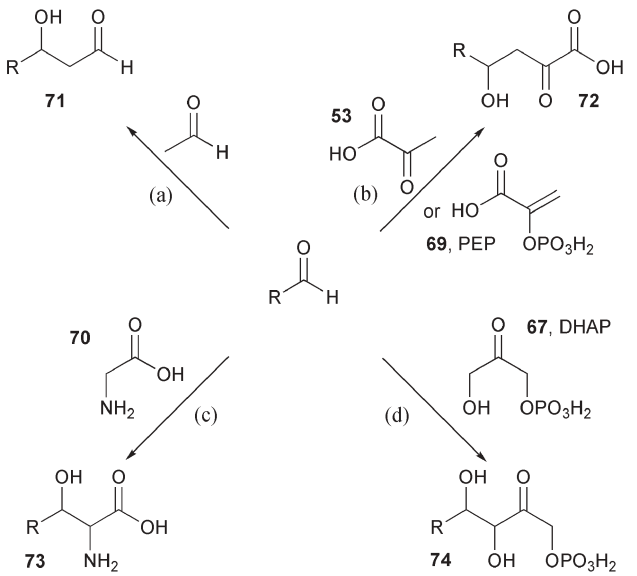
Scheme 12 Transketolase (TK) catalyses the transfer of α-hydroxy carbonyl fragments: A: TK plays a vital part in sugar metabolism. B: In combination with a pyruvate derivative (**57**), TK irreversibly catalyses the formation of a new C–C bond.



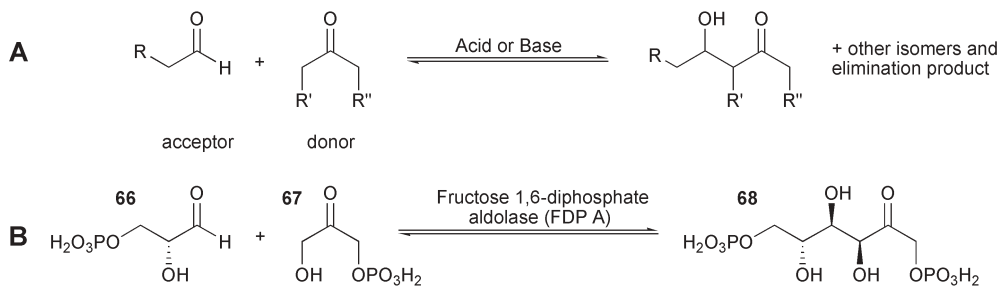
Scheme 14 Enantioselective synthesis of α-hydroxy acids *via* a nitrilase-catalysed synthetic dkr.

catalysed reaction exists.[31] The acid or base catalysed aldol reaction is performed with starting materials that tend to have no substituents in the α-position. The product is a β-hydroxy carbonyl compound, with the classical 1,3-functionality (Scheme 15A). Care has to be taken to direct the reaction in such a manner that only one product is obtained, *i.e.* that only one molecule acts as a donor and the other as an acceptor, and that no elimination takes place. The aldolases that catalyse the aldol reaction in nature are often obtained from the sugar metabolic cycle. Consequently they need highly substituted substrates and the products often have functional groups in the 1-, 2-, 3-, and 4-position. Unlike the chemical reaction, they almost always yield just one product (Scheme 15B), *i.e.* they display an excellent control over which molecule is the acceptor and which the donor. This has, however, one drawback: they employ only a very limited number of carbonyl compounds as donors. Of course, the chemical reaction has successfully been converted into a selective reaction. So far only few chiral catalysts are known, proline and its derivatives being among the exceptions,[32] which can be used directly with aldehydes and ketones. In most cases chiral auxiliaries and a plethora of other tools have to be applied, introducing extra steps and generating much waste.

From a synthetic point of view, the chemically catalysed and the aldolase-catalysed reactions complement each other. Both can be utilised to synthesise compounds that are difficult to obtain with the other type of catalyst. Aldolases have an excellent control over the regiochemistry and accept a wide variety of acceptor molecules. As mentioned above they allow only few donor molecules. The aldolases that are commonly used activate four different donor molecules and are classified according to them (Scheme 16). The reactions that these four groups of aldolases allow are the disconnections that they enable for a retrosynthetic analysis.[2,33] Other aldolases are known, however, their application for synthesis has so far been very limited and they will therefore not be discussed here.



Scheme 16 Aldolases are grouped according to the donor molecule that they utilise: (a) acetaldehyde-dependent aldolases, (b) phosphoenolpyruvate (PEP, **69**) and pyruvate (**53**) dependent aldolases, (c) glycine-dependent aldolases and (d) dihydroxyacetone phosphate (**67**, DHAP) dependent aldolases.

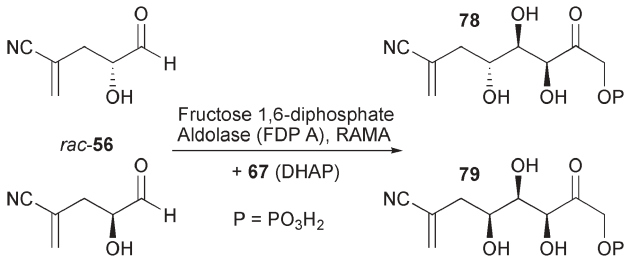


Scheme 15 A: Chemically, the aldol reaction can be catalysed by acids and bases; product control can be difficult. B: Aldolases commonly catalyse the conversion of highly functionalised starting materials; they are very regio- and stereoselective.

5.1. DHAP-dependent aldolases

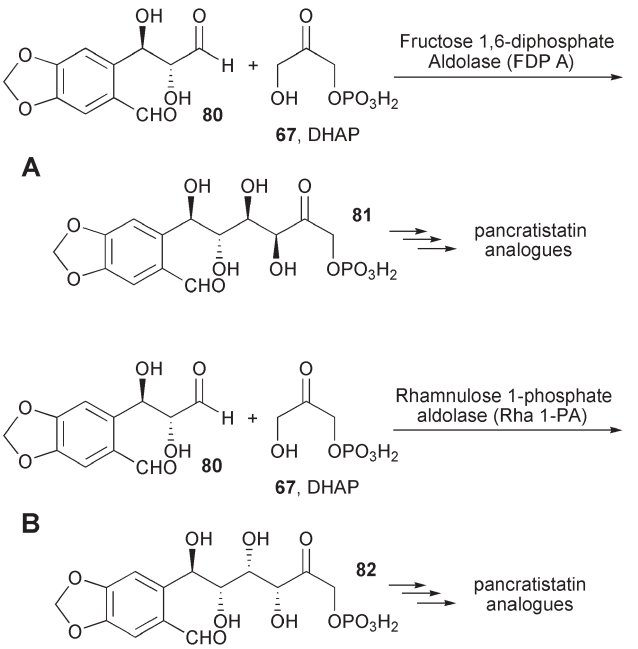
DHAP-dependent aldolases come under the class lyases, just like the hydroxynitrile lyases (see 2.1.).[2,31,33] Like the HNLs, no cofactors need to be added to the reaction mixture and these aldolases are straightforward to use. There are two types of aldolases: Type I aldolases are primarily found in higher plants and animals. They work by an enamine mechanism, which incorporates the active site lysine residue of the enzyme. The amino group of the lysine residue forms a Schiff base with the carbonyl group of the donor, dihydroxyacetone phosphate (**67**, DHAP), and this activates the DHAP. The imine (**75**) thus formed, tautomerises to the enamine (**76**), and the enamine adds stereoselectively to the acceptor molecule (Scheme 17A). Type II aldolases are found in fungi and bacteria; they contain a Zn^{2+} ion in the active site of the enzyme. This is thought to act as a Lewis acid that polarises the carbonyl group of the donor and forms the enediolate (**77**). This nucleophile then adds to the acceptor aldehyde stereoselectively. In this case, a glutamate residue (Enz–COO^-) and a tyrosine residue (Enz–OH), present in the active site of the enzyme, assist in the removal and donation of protons respectively (Scheme 17B). Both types of aldolases control the stereochemistry of the reaction, it is virtually independent of the structure of the acceptor and many different acceptors can be employed with a high predictability of the stereochemical outcome of the synthesis. It is important to mention that both Type I and Type II aldolases catalyse the same reactions although they have completely different modes of action and are obtained from different organisms.

In the DHAP-dependent aldolase-catalysed C–C bond formations, two new stereocentres are established. Consequently, four different stereoisomers can be formed. Enantioselective aldolases that selectively catalyse the formation of just one of each of the stereoisomers are available and have been utilised with great success. In particular, the fructose 1,6-diphosphate aldolase (FDP A, Scheme 15B) that catalyses the formation of the D-*threo* stereochemistry, has been employed in many syntheses. One such FDP A that can be isolated from rabbit muscles is better known as RAMA (**Ra**bbit **M**uscle **A**ldolase). It has been utilised for the synthesis of ulosonic acid analogues, complementing TK which was also used for this purpose (Scheme 12B). Unlike TK, RAMA converts both enantiomers of the racemic aldehyde **56** giving access to the diastereomers **78** and **79** (Scheme 18).[25]

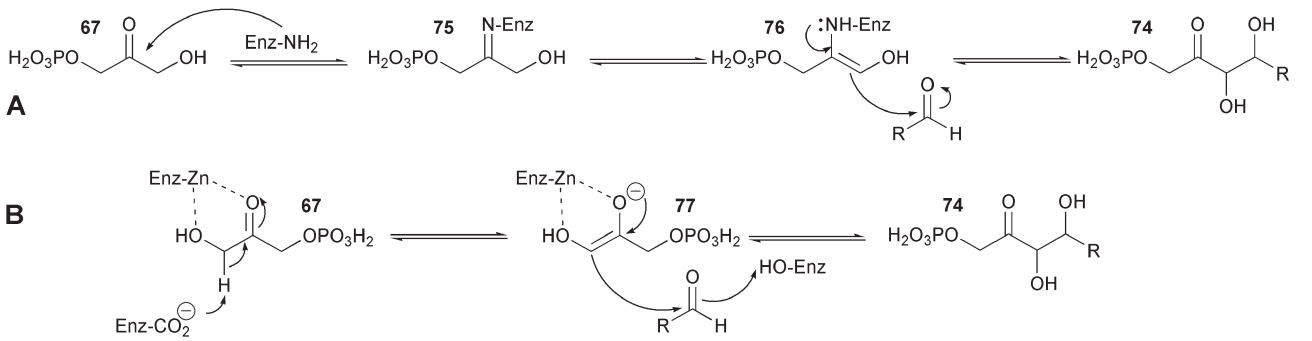


Scheme 18 The FDP aldolase RAMA enantioselectively catalysed the formation of a new C–C bond independent of the stereochemistry of the acceptor, here *rac*-**56**.

In a study of pancratistatin analogues, FDP A was employed to catalyse the formation of **81** with D-*threo* stereochemistry (Scheme 19A). When rhamnulose 1-phosphate aldolase (Rha 1-PA) was employed, the diastereomer **82**, with L-*threo* stereochemistry, was obtained with excellent selectivity (Scheme 19B).[34] These two enzymes allow the stereoselective



Scheme 19 D- and L-*threo*-specific aldolases were applied for the synthesis of pancratistatin analogues.



Scheme 17 A: Mechanism of Type I aldolase. B: Mechanism of Type II aldolase.

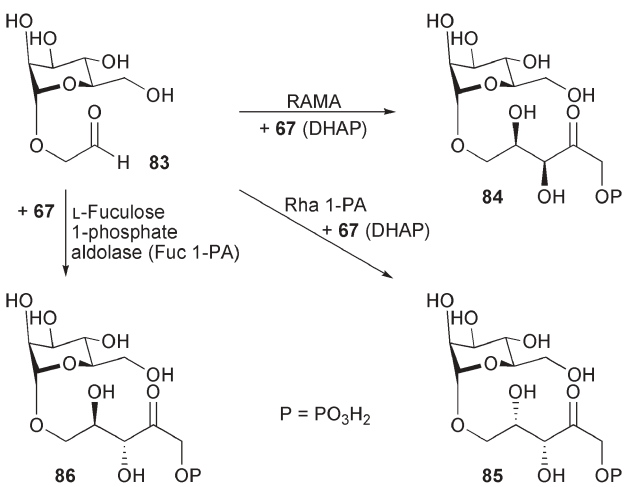
synthesis of the two *threo*-stereoisomers. They were also used successfully for the synthesis of different diastereoisomers of sialyl Lewis X mimetics as selectin inhibitors. In addition to the two *threo*-selective aldolases RAMA and Rha 1-PA, the D-*erythro*-selective L-fuculose 1-phosphate aldolase (Fuc 1-PA) was utilised. It was thus possible to synthesise three of the four diastereoisomers enantioselectively (Scheme 20).[35] The only remaining diastereoisomer that was not prepared has the L-*erythro* stereochemistry. Unfortunately the aldolase that might catalyse its formation, tagatose 1,6-diphosphate aldolase (TDP A), is not very stereoselective and therefore often yields mixtures of diastereoisomers.

A significant drawback of the DHAP-dependent aldolases is that dihydroxyacetone (**87**) is not a substrate and DHAP has to be used. DHAP is expensive and labile at neutral and basic pHs. It can be synthesised chemically or by enzyme-catalysis. A variety of procedures exist and some of them can be performed in the presence of the aldolase, allowing a one-pot procedure. Recently a dihydroxyacetone kinase (DHAK) that catalyses the phosphorylation of **87** was cloned. The ATP that supplies the phosphate can be recycled *in situ* with the aid of an acetate kinase (AK), ensuring the need for only a cheap phosphate source, acetylphosphate (**88**). These two enzyme steps were integrated with a Fuc 1-PA-catalysed step, yielding the desired aldol products **89** in good to excellent yield (Scheme 21).[36] It can be assumed that when this phosphorylation procedure is coupled with other aldolases, such as FDP A and Rha 1-PA, the other stereoisomers will also be accessible.

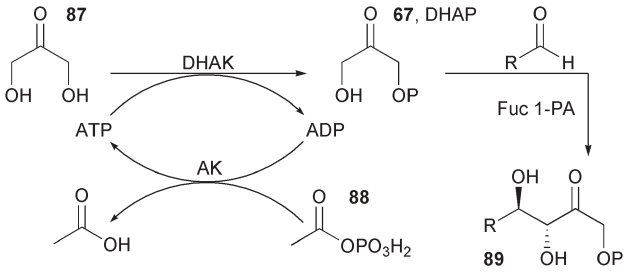
Another problem of the DHAP-dependent aldolases is that the product is phosphorylated, *i.e.* the aldol reaction has to be followed by a dephosphorylation step. For this purpose several enzymes are available and the reaction normally proceeds under mild conditions.

5.2. PEP- and pyruvate-dependent aldolases

Pyruvate (**53**) dependent aldolases catalyse the breaking of a C–C bond in nature. This reaction can, however, be reversed if an excess of **53** is used. In the product one new stereocentre is



Scheme 20 RAMA, Rha 1-PA and Fuc 1-PA allow the synthesis of three of the four possible diastereoisomers that can be prepared with DHAP-dependent aldolases.



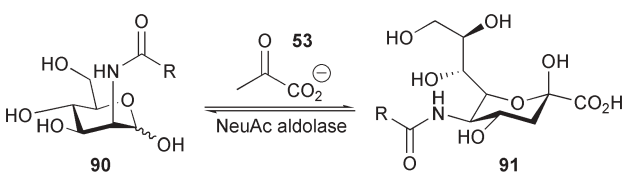
Scheme 21 Multi-enzyme system for the one-pot aldol reaction with *in situ* generation of DHAP.

established. The natural function of PEP (**69**) dependent aldolases on the other hand is to catalyse the synthesis of α-keto acids. Since PEP is a very reactive, unstable substrate they are not commonly used in synthesis and will not be discussed here.

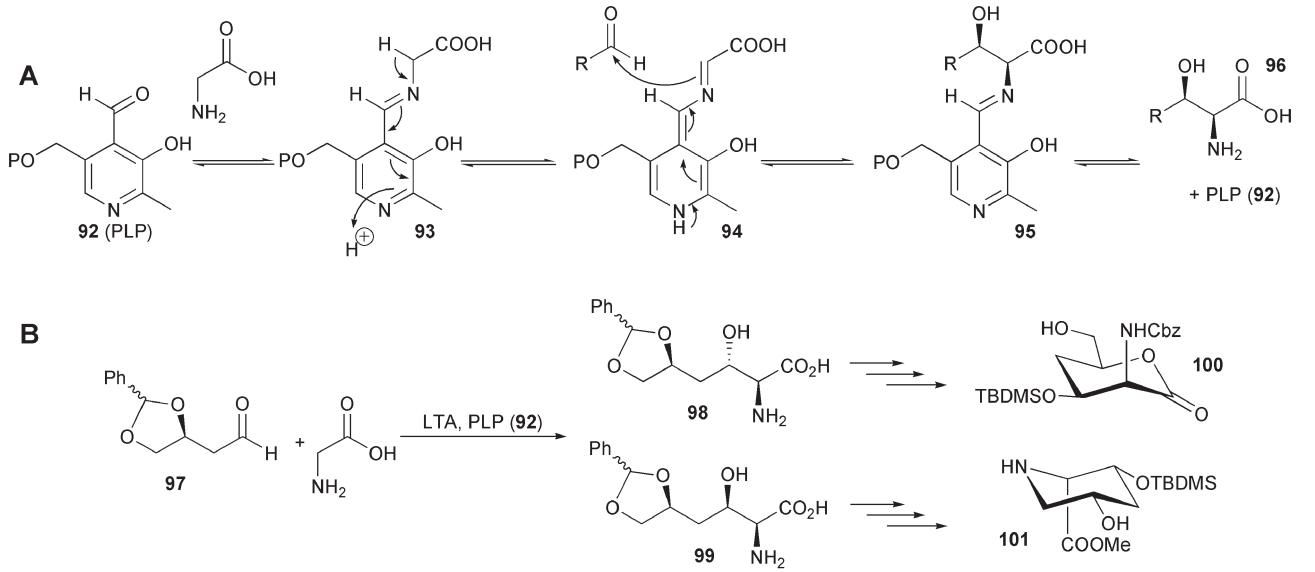
N-Acetylneuraminic acid aldolase (NeuAc aldolase) is commercially available and has been the subject of much study.[2,31,33] Although other pyruvate-dependent aldolases have been used in synthesis, they are not readily available and thus not a subject of this review. NeuAc aldolase catalyses the aldol reaction between pyruvate (**53**) and mannose or mannose derivatives. The enzyme activates the donor **53** as an enamine, similar to the Type I aldolase described in section 5.1. (Scheme 17A). The stereochemistry of the NeuAc aldolase catalysed reactions is governed by the structure of the substrate. This is in contrast to the DHAP-dependent aldolases where the stereochemistry is mainly governed by the enzyme. It has been used for the synthesis of aza sugars and various sialic acid derivatives and it is therefore also known as sialic acid aldolase. Various chemo-enzymatic syntheses of sialic acid derivatives have been developed over the years. Acylated mannosamine derivatives **90** could be converted into sialic acids with a variety of substituents at C-5 (**91**, Scheme 22).[37] For *N*-acetylmannosamine (**90**, R = Me) this reaction has been scaled up to an industrial level.[38]

5.3. Glycine-dependent aldolases

The glycine-dependent aldolases contain a PLP (**92**) cofactor, to which the glycine binds. Subsequent deprotonation allows the reaction with an aldehyde. Two new stereocentres are established during the C–C bond formation (Scheme 23A).[33] However, only L-threonine aldolase (LTA) is commonly used. Although one would expect LTA to exclusively catalyse the formation of the *threo* products (**96**, **99**) it does show a preference for the *erythro* products **98** and often both are obtained as mixtures (Scheme 23B).[39]



Scheme 22 Synthesis of sialic acid derivatives.



Scheme 23 A: Glycine-dependent aldolases utilise PLP as a cofactor for the activation of glycine. B: LTA has only a limited enantioselectivity and often *erythro*- and *threo*-products are obtained.

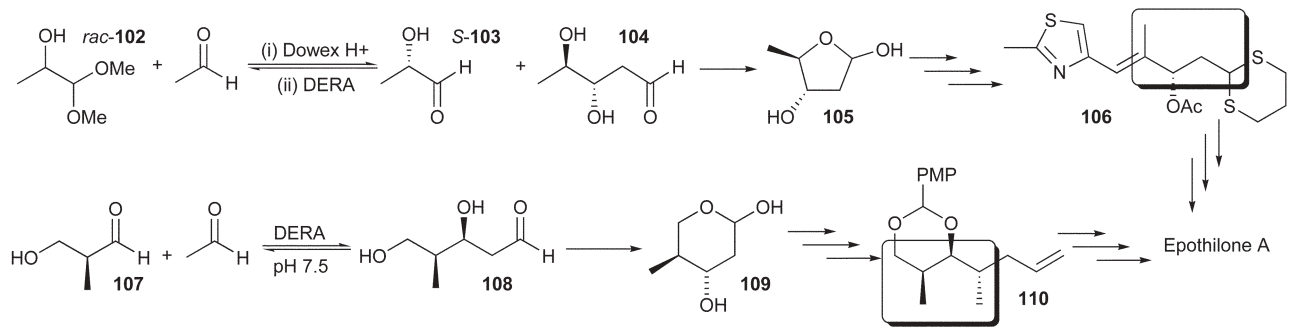
Serine hydroxymethyl transferase (SHMT) is another glycine-dependent enzyme. Its natural function is to transfer a formaldehyde equivalent onto glycine, it can however, also accept other aldehydes. Its stereoselectivity is not always sufficient and its application so far has been limited.

5.4. Acetaldehyde-dependent aldolases

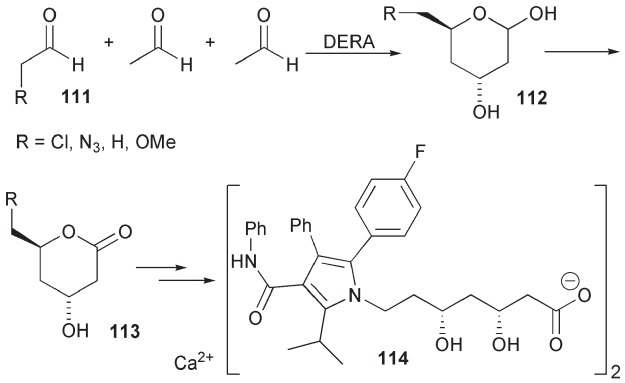
To date only one acetaldehyde-dependent aldolase has extensively been applied in organic synthesis: the 2-deoxyribose 5-phosphate aldolase (DERA).[33] DERA is a Type I aldolase that activates the donor acetaldehyde as an enamine (Scheme 17A). Like the DHAP-dependent aldolases, DERA accepts a broad range of acceptors; additionally it shows some flexibility for different donors. The stereochemistry at the α-carbon of the acceptor, as well as the nature of the substituent (polar/unpolar), determines whether the acceptor is a good substrate for DERA. If racemic aldehydes are used, DERA often catalyses the conversion of only one enantiomer, similar to TK (Scheme 12B). However, if the "wrong" enantiomer of the acceptor is used and is optically pure, DERA does occasionally convert it too.

DERA has been used with great success in the synthesis of epothilone A. Two of the seven stereocentres in epothilone A were established by DERA. When racemic aldehyde **103** was generated *in situ* from **102**, DERA converted the *R*-enantiomer into **104**. This combined kinetic resolution and C–C bond formation yielded a building block with two chiral centres. The optical information obtained from the kinetic resolution was lost later, since the alcohol was oxidised. Thus, for the overall yield it would have been better if DERA had displayed no stereoselectivity for the acceptor. In the DERA-catalysed synthesis of another part of epothilone A, DERA is again highly stereoselective. Fortunately its preference is for *S*-**107**, the aldehyde that has to be submitted to the C–C bond formation in order to obtain the desired **108**. Both DERA-catalysed reactions yield open chain products that form stable hemiacetals (**105** and **109**). This ensures that the equilibrium reactions are shifted towards the desired products. Further synthetic manipulations converted intermediates **105** and **109** into epothilone A (Scheme 24).[40]

A particularly elegant application of DERA is the sequential synthesis of hemiacetal **112** (Scheme 25).[41,42] Two DERA-catalysed aldol reactions convert one equivalent of **111** and



Scheme 24 Application of DERA in the synthesis of epothilone A.



Scheme 25 Sequential DERA-catalysed aldol reactions in one pot.

two equivalents of acetaldehyde into the stable **112**. Subsequent oxidation yielded the lactone **113** in excellent optical purity, proving the great versatility of this class of enzymes. Lactone **113** (R = N_3) is an intermediate in the synthesis of atorvastatin (**114**), a cholesterol-lowering drug.

6. Conclusion and outlook

The application of enzymes in the enantioselective synthesis of C–C bonds is well established. The *R*- and *S*-selective synthesis of cyanohydrins can be performed with great ease on laboratory and industrial scale. The HNLs that catalyse these carbon bond formations need no cofactors and are straightforward to use. The same holds true for the preparation of (*S*)-cyanohydrin acetates **28** *via* the synthetic dkr. Although no (*R*)-cyanohydrin acetates have been prepared in this manner, this approach has great potential for organic synthesis. This is revealed in the enantioselective synthesis of the α-hydroxy acids *R*-**62** and *S*-**65** *via* a disconnection otherwise not available to chemists. Since the synthetic dkr can be performed with any reversible C–C bond forming reaction that can be coupled to an irreversible hydrolase-catalysed reaction, many enzyme-catalysed reactions are waiting to be discovered. The aldol reaction is one of the possible candidates for such a dkr.[13]

The thiamin diphosphate (**37**) dependent enzymes, BAL, BFD, TK and PDC have opened a new avenue for the enantioselective synthesis of α-hydroxy ketones. This disconnection has long been neglected, but has been reinvigorated by these enzymes. The chemical counterparts to these enzymes that have been developed recently are less enantioselective. Even though a cofactor is needed for the BAL-, BFD-, TK-, and PDC-catalysed reactions, this is a minor drawback in view of the fact that the carbenes used for the chemical reaction are not commercially available and have to be synthesised.

The aldolases have opened an entirely new approach towards highly substituted target molecules. In particular the three DHAP-dependent aldolases, Rha 1-PA, Fuc 1-PA and FDP A, have enabled reactions that were not possible earlier. Although they require phosphorylated starting materials and the actual C–C bond-forming reaction has to be combined with at least two other steps, these enzymes have proven their value. Moreover it has to be expected that straightforward solutions to the phosphorylation/dephosphorylation will soon become standard (see for instance Scheme 21). The other outstanding aldolase that has enriched organic synthesis and is straightforward to use is DERA. The cascade reactions that have recently been described powerfully demonstrate the full potential of the C–C bond forming enzymes. In addition many other enzymes, especially from the polyketide and terpene biosynthetic pathways, will become widely available, further enriching the choice of synthetic tools that the organic chemist has. With all this knowledge in mind and many enzymes within reach, these gentle natural catalysts should shape the future of synthetic organic chemistry.

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