Enantioselective Enzyme-Catalysed Synthesis of Cyanohydrins

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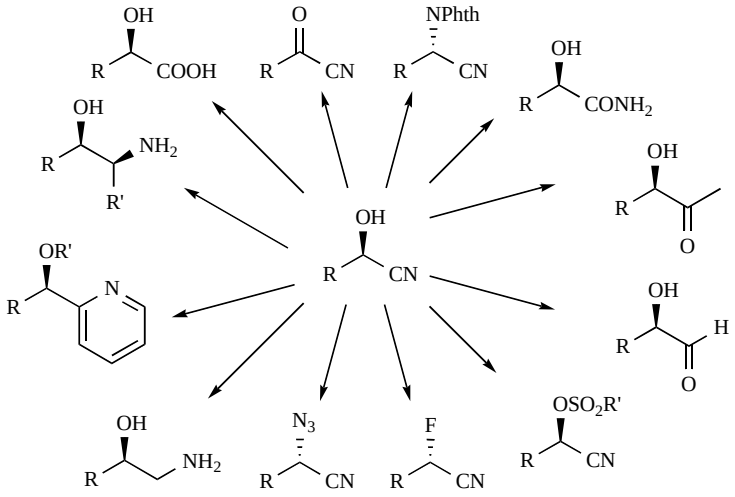
Abstract: Cyanohydrins are valuable intermediates in organic synthesis. They enable an extensive chemistry that can be implemented starting either from the nitrile or from the hydroxy functionality. A number of challenges relevant to the enzyme-catalysed synthesis of chiral cyanohydrins have been addressed in recent years. In this review we will discuss three topics: (1) The enantioselective synthesis of cyanohydrins catalysed by Hydroxynitrile Lyases (Oxynitrilases); (2) The preparation of enantiopure cyanohydrins catalysed by hydrolases/lipases *via* (dynamic) kinetic resolutions and (3) the (*in situ*) conversion of the enantiopure cyanohydrins, extending the use of cyanohydrins as building blocks in organic synthesis.

Key Words: Cyanohydrin, hydroxynitrile lyase, oxynitrilase, lipase, kinetic resolution, dynamic kinetic resolution.

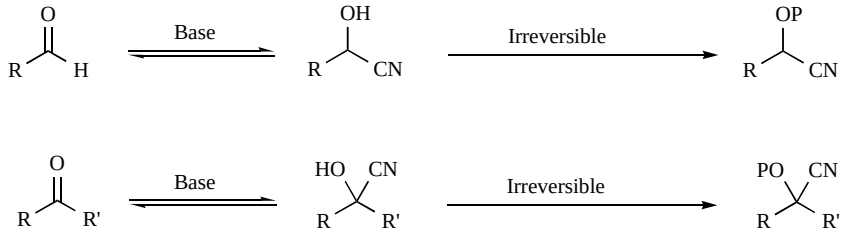
1. INTRODUCTION

Cyanohydrins are versatile building blocks in organic synthesis (Scheme **1**). Their synthesis from prochiral aldehydes and ketones by the addition of HCN has therefore always attracted attention [1-6]. As early as 1908 the first enzyme-catalysed enantioselective synthesis was described [7]. Ever since enzymes have been among the most versatile tools for the enantioselective synthesis of cyanohydrins [8-10].

The formation of cyanohydrins is a base-catalysed equilibrium reaction (see Scheme **2**) [11]. The nucleophilic cyanide ion attacks the electrophilic carbonyl group yielding the three dimensional cyanohydrin. When starting with aldehydes the equilibrium tends to lie on the product side. However, when ketones are employed yields are low. This is mainly due to the considerable steric congestion in the product, a tertiary alcohol with three other bulky substituents. This can be circumvented by using a large excess of HCN,



Scheme 1. Possible applications of cyanohydrins.



Scheme 2. The formation of cyanohydrins derived from aldehydes and ketones is an equilibrium reaction. P = protective group.

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which is not desirable. Alternatively, two-phase systems have been used, with a catalyst containing aqueous layer and an organic layer, possibly pure starting material. Yet another alternative is to perform the reaction in organic solvents and to render it irreversible by an *in situ* protection. In many transition metal-catalysed cyanohydrin syntheses TMSCN is used to achieve this aim [1-4]. This is however not possible with enzymes [5-8].

During the last years many new enantioselective cyanohydrin syntheses have been developed and an equally rich new follow-up chemistry has explored novel routes for their application in total synthesis. Here we present the application of different types of enzymes for the enantioselective cyanohydrin synthesis in a way that is accessible for organic chemists. These enzymes are readily available and can be used with standard equipment under standard conditions – that is to say, often in organic solvents. The emphasis is on the enantioselective synthesis and not on the enzyme - which in fact is just yet another catalyst – just like the many other catalysts that organic chemists use. In the final part of this review we describe the many successful conversions of cyanohydrins, demonstrating how versatile a building block they are. Virtually all of these follow-up reactions are not enzyme-catalysed, indeed several are not catalytic.

2. HYDROXYNITRILE LYASE CATALYSED SYNTHESIS OF CYANOHYDRINS

Hydroxynitrile Lyases (HNL's, also known as Oxynitrilases) are enzymes that catalyse the release of HCN from natural cyanohydrins in several higher plants [5, 6, 9]. This reaction is a defense mechanism of these plants against several herbivores, commonly known as cyanogenesis. Since the discovery of these versatile enzymes, much effort has been put into research on their application as catalysts for the reverse reaction, the enantioselective synthesis of cyanohydrins.

2.1. Sources of HNL's, their Substrate Ranges and Selectivities

The HNL's from *Prunus amygdalus* (*Pa*HNL), *Sorghum bicolor* (*Sb*HNL), *Manihot esculenta* (*Me*HNL) and *Linum usitatissimum* (*Lu*HNL) are all commercially available from the major chemicals suppliers. They are currently the four major HNL's that are being used in asymmetric synthesis of enantiopure cyanohydrins derived from several aldehydes and some ketones. The catalytic mechanism of *Hb*HNL is shown in Scheme **3** [13]. This class of HNL's (also including *Me*HNL [19]) is closely related to hydrolases. *Sb*HNL represents a second class which is similar to carboxypeptidases [14], while *Lu*HNL belongs to yet another class which is similar to medium chain alcohol dehydrogenases. One characteristic of *Lu*HNL, different from the other HNL's, is that it requires a zinc ion for catalysis [20]. The *Pa*HNL is closely related to FAD dependent redox enzymes [14]. The price of all the different HNL's is comparable to that of enantiopure Bi(2-naphthol) (BINOL), the chiral backbone of many chiral catalysts (see Table **2**). BINOL then needs to be converted *via* several steps into either Shibasaki's catalyst or Najera and Saa's catalyst. Both of these catalysts contain $AlCl_3$ as the Lewis acidic species. The expensive TMSCN had to be used instead of KCN that is used in enzyme catalyzed reactions [4].

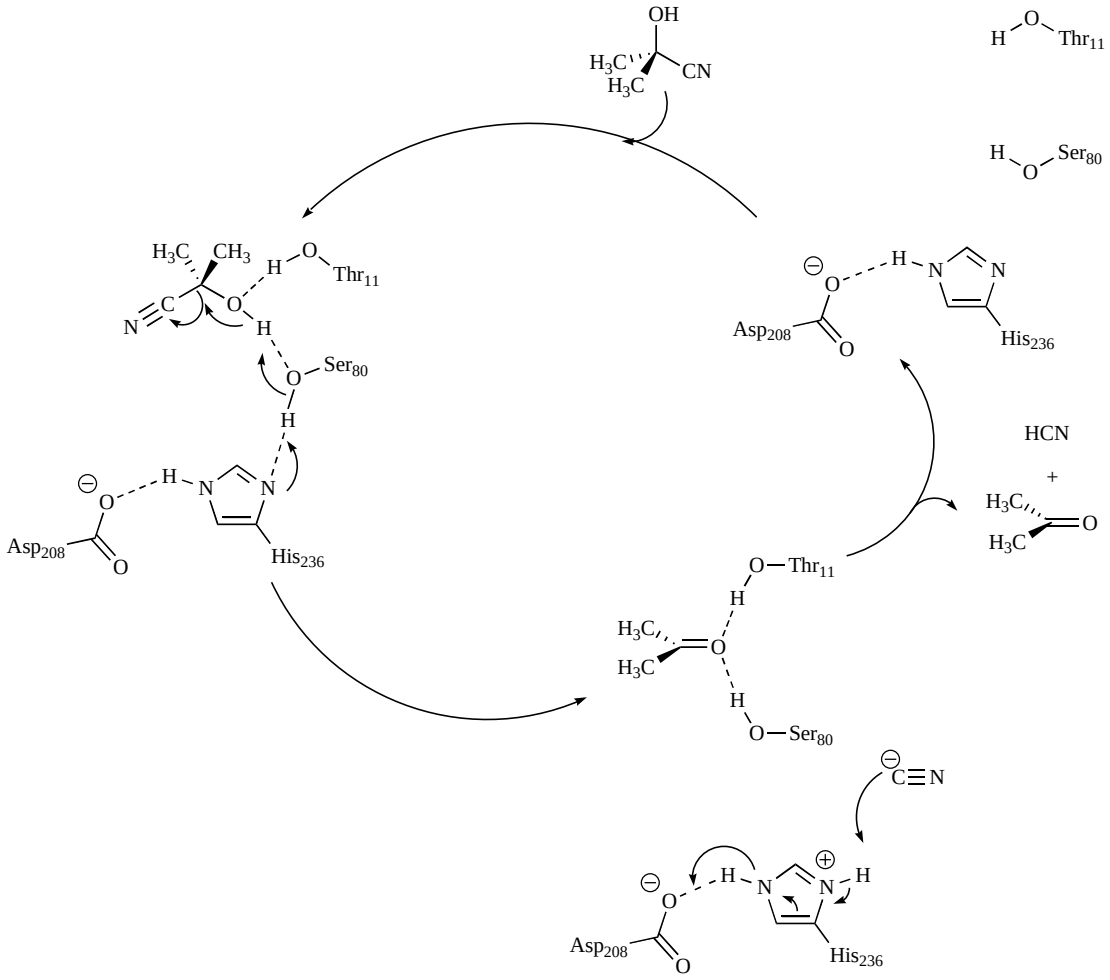
Table 2. Comparison of Prices for HNL's and BINOL

Chemical	Amount	Price
(*S*)-(-)-/(*R*)-(+)-1,1'-Bi(2-naphthol)	10 g	121.60 €
TMSCN	25 g	96.80 €
KCN	25 g	19.50 €
*Pa*HNL	1000 U	131.50 €
*Me*HNL	1 ml (3000 U)	103.00 €
*Pa*HNL-CLEA/*Me*HNL-CLEA	50 mg	125.00 €

*Pa*HNL can be found in almonds, *Hb*HNL comes from the rubber tree, *Sb*HNL from cane like grass, *Me*HNL is found in cassava and *Lu*HNL in flax (see Table **1**). Out of these five HNL's, *Pa*HNL and *Lu*HNL are (*R*)-selective. The three other HNL's are all (*S*)-selective. The accessible amount of the respective HNL's depends on the natural sources they are isolated from. Some sources have been shown to contain large amounts of HNL's, while others only contain a small amount of the wanted enzyme. Lately, the production of some HNL's, especially *Me*HNL and *Hb*HNL, has been optimized by cloning and over-expressing them in different bacterial and yeast strains, such as *Escherichia coli* and *Pichia pas-*

Table 1. Overview of Various Sources of HNL's, their Substrate Ranges and Selectivities

HNL Source	Substrate Specificity	Selectivity	Ref.
Hevea brasiliensis (rubber tree)	Aliphatic/aromatic/heteroaromatic/α,β-unsaturated	(*S*)	[12-14]
Manihot esculenta (cassava)	Aliphatic/aromatic/heteroaromatic/α,β-unsaturated	(*S*)	[12, 14]
Sorghum bicolor (cane like grass)	Aromatic/heteroaromatic	(*S*)	[14]
Prunus amygdalus (almond)	Aliphatic/aromatic/heteroaromatic/α,β-unsaturated	(*R*)	[7, 9]
Linum usitatissimum (flax)	Aliphatic/aromatic/α,β-unsaturated	(*R*)	[12]
Malus communis (apple)	Aromatic	(*R*)	[24]
Prunus species (apricot/cherry/plum/peach)	Aliphatic/aromatic/heteroaromatic/α,β-unsaturated	(*R*)	[9]
Arabidopsis thaliana (mouse-ear cress)	Aliphatic/aromatic/heteroaromatic/α,β-unsaturated	(*R*)	[21]
Sambucus nigra (black elderberry)	Aromatic	(*S*)	[23]
Eriobotrya japonica L. (loquat fruit)	Aliphatic/aromatic/heteroaromatic/α,β-unsaturated	(*R*)	[25]
Aleurodiscus amorphous (crust fungus)	Oxidative degradation of cyanohydrins	(*S*)	[22]



Scheme 3. The catalytic mechanism of the α,β fold Hydrolase derived HNL's (here shown for *Hb*HNL).

toris. Thus a constant supply of highly purified (*S*)- and (*R*)-selective enzyme at reasonable prices is ensured [9, 25, 26].

2.1.1. Immobilization of HNL's

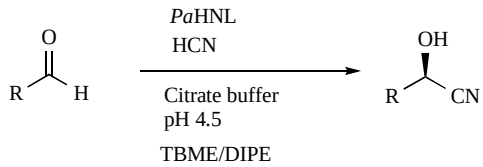
Enzyme immobilization is another technology that has enabled a large step forward for the use of HNL's in organic chemistry. Immobilizing the different HNL's onto various supports (such as cellulose) or as CLEA's (Cross-Linked Enzyme Aggregates) not only allows easier handling of these enzymes for organic chemists. It also gives the possibility of recycling of the enzymes [15-18].

CLEA's of HNL's, especially, have shown a high degree of stability and activity, even after several runs. One advantage of the immobilization as CLEA is that it combines purification and immobilization in a single step. Thus, a highly pure enzyme is not necessary, in contrast to other enzyme immobilization processes. The CLEA made from the (*R*)-selective *Pa*HNL proved to be highly active in the asymmetric cyanation of various aldehydes. It was recycled ten times without any loss of activity. In a similar manner, CLEA's of the (*S*)-selective *Me*HNL and *Hb*HNL were prepared. The most important advantage with CLEA's is that they can be used in organic solvents. Higher enantioselectivities compared to the free HNL's have been observed, simply because the competing reaction, non-enzymatic cyanation, is suppressed [27-32].

2.2. HNL-Catalysed Addition of HCN to Aldehydes and Ketones

The HNL-catalysed synthesis of various cyanohydrins from aldehydes has been studied for a long time, indeed the first example dates back to 1908 [7]. A broad range of aliphatic as well as aromatic aldehydes have been converted into their corresponding cyanohydrins with excellent enantiopurities. However, in some cases, lower yields and enantioselectivities were found (see Table **3**).

These limitations were overcome by performing the reactions in a biphasic system (see Scheme **4**) [33-36]. This approach gave an easy access to cyanohydrins in both high yield and enantiopurity. Today this is the method that is commonly used. Only very recently the immobilization of HNL's as CLEA's has enabled their application in organic solvents, opening up new possibilities [32].



Scheme 4. A biphasic system for the formation of enantiopure cyanohydrins.

The low enantioselectivities are often due to the base-catalysed racemic chemical background reaction. By performing the HNL-catalysed reaction at a relatively low pH (4-5) this racemic reaction can be suppressed. If, however, the pH is lowered too far the HNL is deactivated [37].

The asymmetric addition of cyanide to ketones has proven to be more difficult, due to the higher degree of steric hindrance related

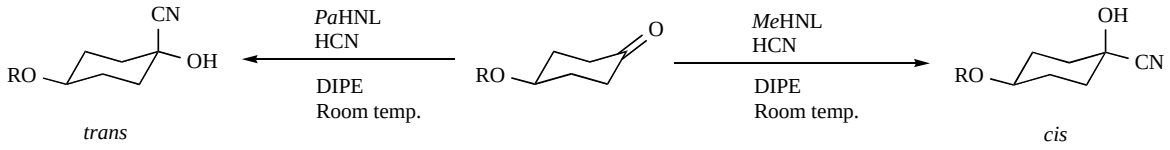
Table 3. Overview of Various Cyanohydrins Derived from Aldehydes Prepared with HNL's

R	HNL	Stereoselectivity	Enantiopurity	Ref.
(*E*)-PhCH=CH	*Hb*HNL	*S*	99%	[33]
3-Furyl	*Hb*HNL	*S*	98%	[33]
3-Thienyl	*Hb*HNL	*S*	99%	[33]
$CH_3(CH_2)_4$	*Hb*HNL	*S*	98%	[33]
(*E*)-$CH_3(CH_2)_4CH$=CH	*Hb*HNL	*S*	99%	[33]
CH_2=$CHCH_2$	*Hb*HNL	*S*	93%	[33]
CH_2=$C(CH_3)CH_2$	*Hb*HNL	*S*	97%	[33]
$(CH_3)_2C$=$CHCH_2$	*Hb*HNL	*S*	95%	[33]
CH_3CH_2CH=$CHCH_2$	*Hb*HNL	*S*	87%	[33]
2-Furyl	*Pa*HNL	*R*	99%	[18]
3-Thienyl	*Pa*HNL	*R*	99%	[18]
CH_3CH=CH	*Pa*HNL	*R*	95%	[18]
$CH_3S(CH_2)_2$	*Pa*HNL	*R*	96%	[18]
$CH_3CH_2CH_2$	*Pa*HNL	*R*	98%	[18]
$(CH_3)_3$	*Pa*HNL	*R*	83%	[18]

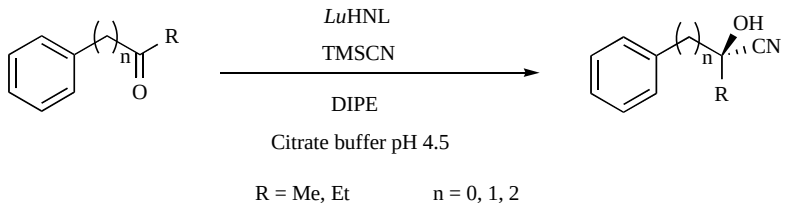
to these types of compounds [11]. Over the past few years, several papers describing the synthesis of enantiopure cyanohydrins derived from aliphatic methyl ketones and cyclic ketones were published (Scheme **5**). Good yields were obtained by using large excesses of HCN [38].

The synthesis of cyanohydrins derived from aromatic ketones, on the other hand, has been less studied until now. Recently, various aromatic ketones, such as acetophenone, phenylacetone, benzylacetone and propiophenone have been used in asymmetric cyanation with *Lu*HNL as catalyst [39]. An important result was that this HNL gave products with (*S*)-configuration (Scheme **6**). This was in contrast with earlier observations using *Lu*HNL, which gave (*R*)-cyanohydrins with aliphatic aldehydes and ketones.

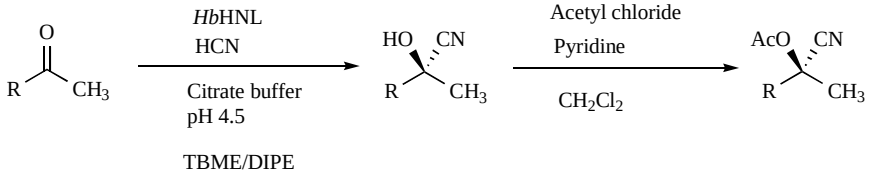
Similar substrates were also converted by *Hb*HNL (Scheme **7**) [33]. The resulting cyanohydrins were prepared with good to high enantiopurities.



Scheme 5. Cyanohydrins from cyclohexanones.



Scheme 6. *Lu*HNL-catalysed synthesis of cyanohydrins derived from aromatic ketones.



Scheme 7. *Hb*HNL-catalysed synthesis of cyanohydrins in a biphasic system.

An elegant approach to overcome the limitations imposed on the reaction by the equilibrium is the *in situ* protection of the cyanohydrin [40]. Thereby the product is removed from the equilibrium and yields of 100% of the protected cyanohydrin should become possible. Ethyl cyanoformate was used as cyanide donor and protecting agent (Scheme **8**). Unfortunately not all of the mandelonitrile formed was converted into the desired product, but this strategy holds much potential for the future.

Recently a two-phase approach to circumvent thermodynamic limitations in the formation of ketone based cyanohydrins was published. However, only those cyanohydrins in which the hydroxyl group formed an intramolecular hydrogen bond were isolated in yields higher than 25% (see Fig. **1**) [41].

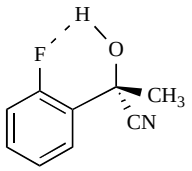


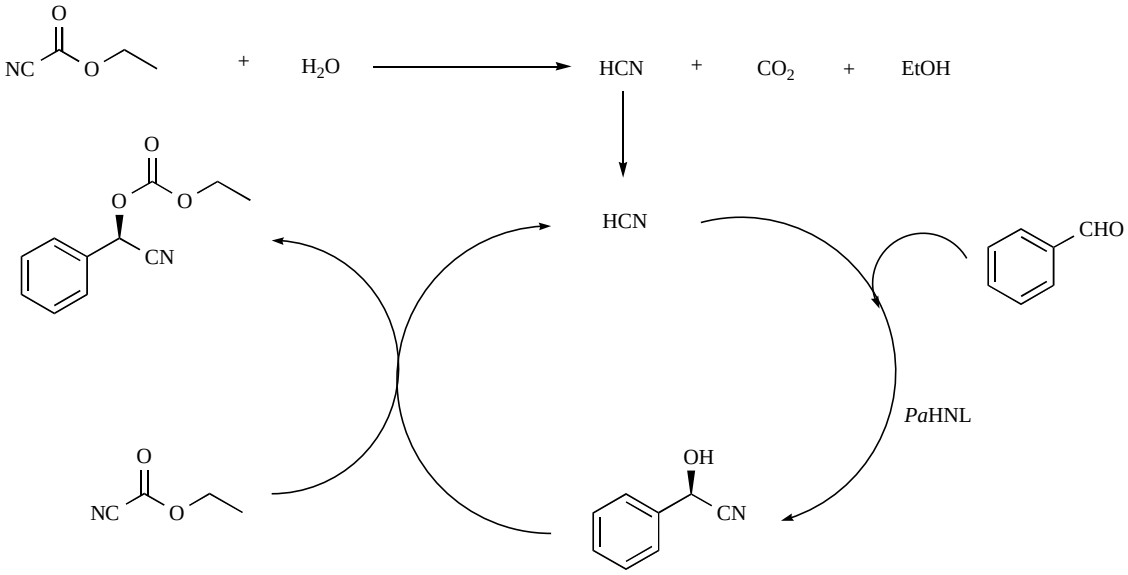
Fig. (1). A possible hydrogen bond formation in cyanohydrins from substituted methyl ketones.

2.3. Applications of HNL's in Organic Synthesis

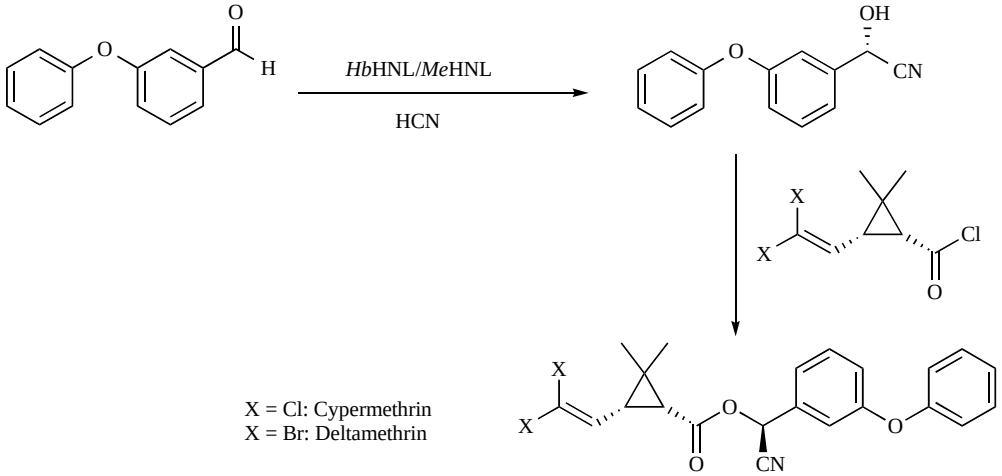
The industrial syntheses of enantiopure cyanohydrins are based on HNL's. This underlines that HNL's are versatile, stable and economically attractive catalysts [9, 16, 26].

Cyanohydrin esters have been and are well known for their applications as insecticides, especially the esters formed between enantiopure cyanohydrins and chiral pyrethrum acids. These compounds, known as pyrethroids, are one of the most important classes of insecticides today. They normally contain (*S*)-3-phenoxybenzaldehyde cyanohydrin as the alcohol moiety [42-44]. This is now made on an industrial scale using either *Hb*HNL or *Me*HNL (Scheme **9**) [5, 9]. The large-scale synthesis of (*R*)-2-amino-1-(2-furyl)ethanol is another example of the industrial application of HNL's [45].

Another example is the production of the blockbuster Clopidogrel, also known as Plavix (Scheme **10**). In this case the starting aldehyde is 2-chlorobenzaldehyde. (*R*)-2-Chlorobenzaldehyde cyanohydrin is formed through asymmetric cyanide addition using almond meal or genetically modified *Pa*HNL [46]. Facile follow-up chemistry of both the nitrile (Pinner reaction) and the hydroxyl group gives the final compound, the (*S*)-enantiomer of Clopidogrel, with excellent enantioselectivity. A differently genetically modified



Scheme 8. A one-pot chemoenzymatic synthesis of protected cyanohydrins.



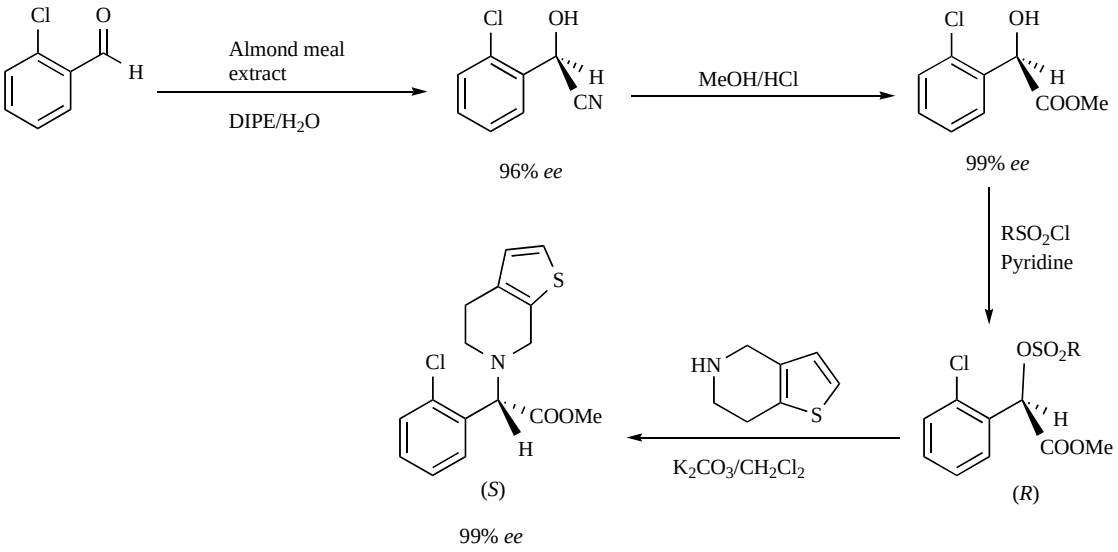
Scheme 9. Synthesis of pyrethroids with insecticidal activities using HNL's.

*Pa*HNL gives highly enantioenriched intermediates for the synthesis of Enalapril (Scheme **11**) [47].

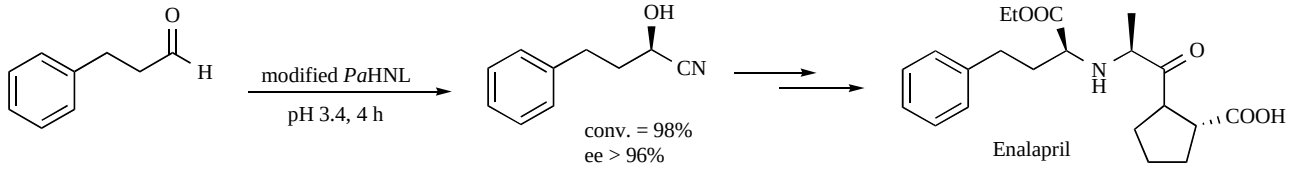
Another important class of compounds in organic chemistry are the 1,2-aminoalcohols. They are known to possess a broad range of biological activity. They are either classified as adrenalin-like compounds, with one stereocenter in C-1, or as ephedrine-like compounds, which on the other hand possess two stereocenters (C-1 and C-2).

Ephedrine can easily be formed from the TMS-ether of mandelonitrile. In three simple steps, a Grignard reaction, transimination and reduction, ephedrine is synthesised as the final compound with both excellent enantioselectivity and diastereoselectivity (see Scheme **12**) [5, 6].

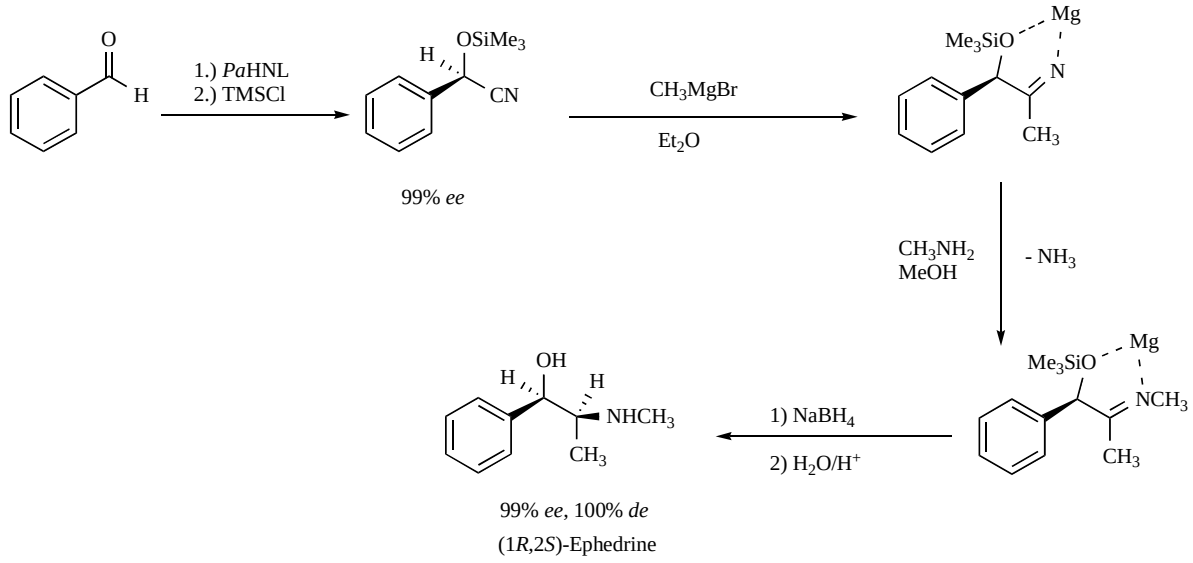
Cyanohydrins of protected 4-hydroxycyclohexanones have proven to be excellent starting materials for the preparation of Rengyol and Isorengyol, respectively. These compounds are important



Scheme 10. Stereoselective synthesis of the blockbuster drug Clopidogrel (Plavix).



Scheme 11. HNL-catalysed synthesis of Enalapril.



Scheme 12. Synthesis of Ephedrine.

natural products with anti-inflammatory, antibacterial and antiemetic properties. In the case of (*R*)-*Pa*HNL-catalysed addition of HCN, *trans*-selectivity was observed. *cis*-Selectivity was observed when addition of HCN was catalysed by the (*S*)-selective *Me*HNL [38]. The benzyl protection group was chosen as it gave the highest *cis*-/*trans*-stereoselectivity with both HNL's (2:98 for *Pa*HNL and 82:18 for *Me*HNL, respectively). The separation of the isomers was achieved at a later stage in the synthesis. Reduction with DIBAL gave the corresponding aldehydes which were subjected to a Wittig reaction. The resulting olefins were treated with diborane (hydroboration) and a reduction gave Rengyol and Isorengyol as the final products (see Scheme **13**).

The aldehydes used for the formation of cyanohydrins can be very unstable due to their susceptibility to oxidation or isomerisation. One such aldehyde is 3-butenal. In order to avoid isomerisation it was prepared *in situ* and used after the removal of the oxidation catalyst. In this manner the (*S*)-enantiomer of 3-butenal cyanohydrin could be prepared (Scheme **14**) [48]. It was then used for the preparation of deoxysugars, building blocks for Mureidomycin A [48a]. The (*R*)-enantiomer had been synthesized earlier with *Pa*HNL as a catalyst. It was then submitted to reduction, transamination and later a metathesis reaction (see Scheme **52**).

3. ENANTIOPURE CYANOHYDRINS *VIA* HYDROLASE/ LIPASE-CATALYSED (DYNAMIC) KINETIC RESOLUTION

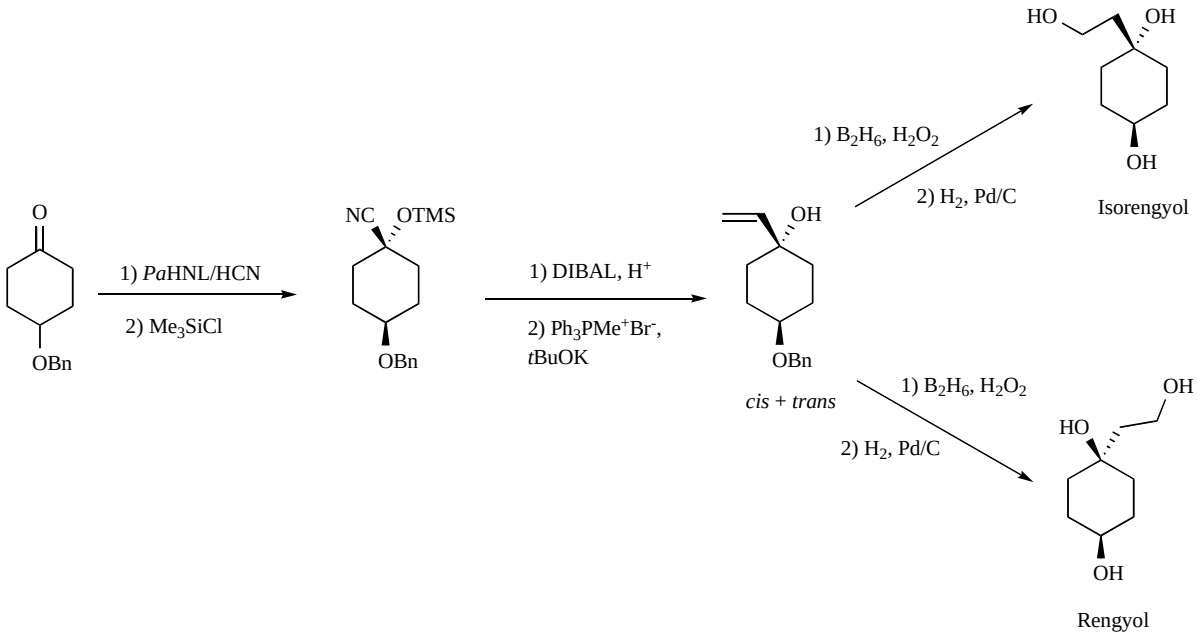
A kinetic resolution of a (racemic) mixture of an enantiomer pair is based on the difference in speed of their conversion in the chosen reaction. Hydrolases and in particular lipases catalyse the hydrolysis and formation of esters. Their enantioselectivity can be exploited for the kinetic resolution of countless alcohols, typically secondary alcohols, but also of tertiary alcohols. The esterification of the alcohol as well as the hydrolysis of its ester is also a suitable reaction for a kinetic resolution (Scheme **15**). If the kinetic resolution is combined with an *in situ* synthesis or racemisation of the enantiomer pair then the procedure is called a dynamic kinetic resolution and instead of the maximum yield of 50% of a kinetic resolution a dynamic kinetic resolution might yield 100% product (Scheme **15**) [49-51].

3.1. Cyanohydrins Derived from Aldehydes

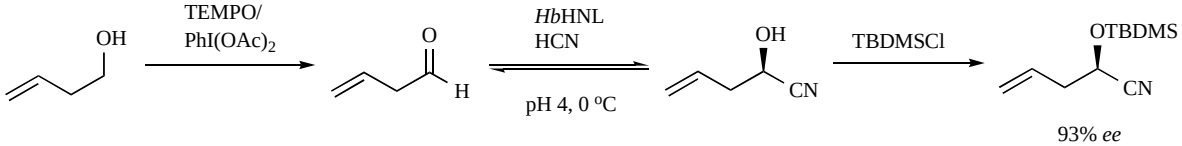
3.1.1. Lipase-Catalysed Kinetic Resolution of Aldehyde-Based Cyanohydrins

Cyanohydrins from aldehydes are secondary alcohols, the ideal substrates for lipases [49]. A racemic cyanohydrin ester is hydrolysed using a (*S*)-specific lipase to give the (*R*)-ester and the (*S*)-cyanohydrin and *vice versa*. These two compounds can easily be separated from each other and the undesired enantiomer can be racemised by base treatment and can be recycled. As mentioned above the yield of an enzymatic hydrolysis is limited to 50%. Another disadvantage with the lipase-catalysed hydrolysis of cyanohydrin esters is that the product, which is formed during the reaction, the free cyanohydrin, can be unstable in water (Scheme **16**). Indeed, the formation and decomposition of unprotected cyanohydrins is a base-catalysed reaction and can already proceed under neutral conditions.

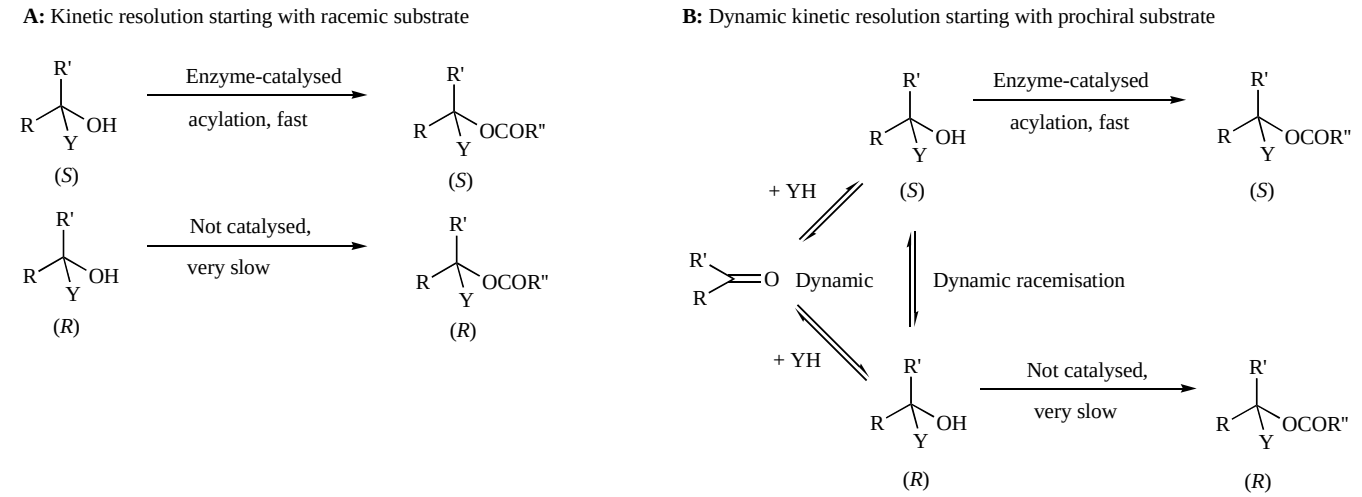
A wide range of hydrolases is used for the enantioselective hydrolysis or formation of cyanohydrin esters (Scheme **17**). Most of these enzymes are readily available *via* standard suppliers at reasonable prices, they are very stable, can be used in dry organic solvents and are straightforward to handle [49]. The most common hydrolases and their enantioselectivity are listed in Tables **4** and **5**.



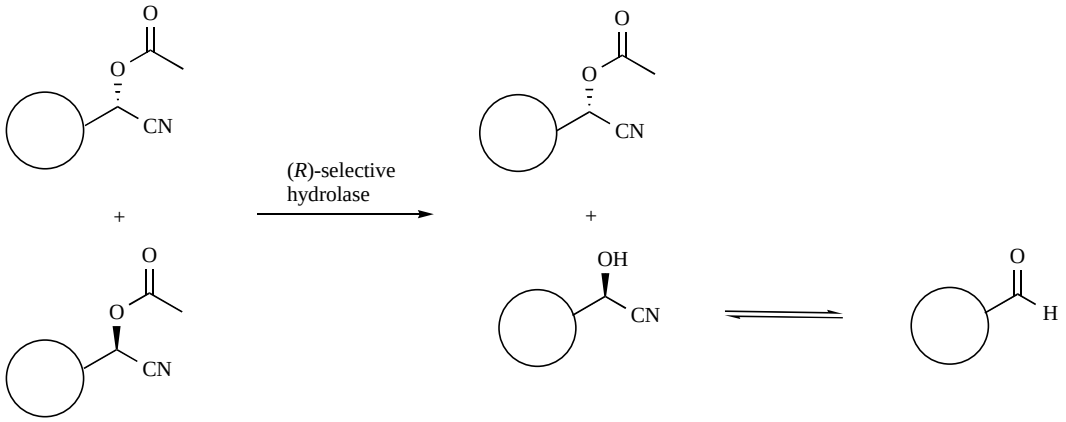
Scheme 13. Synthesis of Rengyol and Isorengyol.



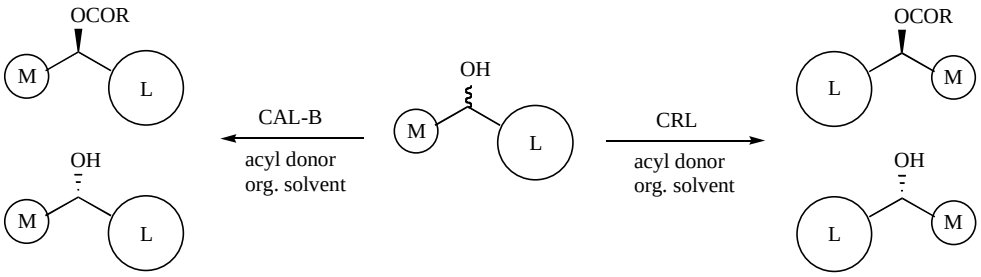
Scheme 14. A combined oxidation-hydrocyanation protocol used in the preparation of γ,δ-unsaturated cyanohydrins.



Scheme 15. Overview of kinetic and dynamic kinetic resolutions.



Scheme 16. Kinetic resolution of a cyanohydrin ester catalysed by a (*R*)-specific hydrolase.

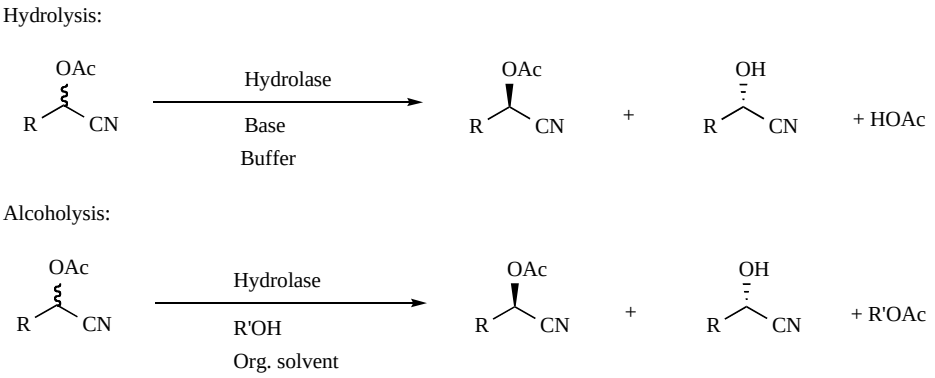


Scheme 17 CAL-B and CRL show opposite enantioselectivities for secondary alcohols.

The kinetic resolution can be performed as the hydrolysis of the racemic ester or as the alcoholysis of the racemic esters (Scheme **18**).

Heterocyclic ring systems are frequently found in medicines today, mainly because they provide such a large and diverse range of interactions. When a cyanohydrin is part of a heterocyclic scaffold, a broad range of biologically active compounds can be prepared. In this way, chiral cyanohydrins fulfil the criteria for many drugs such as for Clopidogrel. The quest for high enantiopurity is the most important one, and also the most challenging. Enzymatic kinetic resolutions are attractive tools for achieving this goal. Several important compounds have been made using this methodology, such as phenylfuran-based cyanohydrin esters [55], furylbenzothiazole-based cyanohydrin acetates [58] and phenothiazine-based cyanohydrin acetates [56].

Cyanohydrin esters derived from phenylfurans were made enantiomerically pure by using *Burkholderia cepacia* lipase [BCL, also known as *Pseudomonas cepacia* lipase (PCL) and *Pseudomonas fluorescens*, lipase Amano, Amano PS] in toluene (the optimal solvent) through a kinetic resolution (E = 15). *Candida antarctica* lipase B (CAL-B) was found to be less active, giving an E-value of 10. With *Candida antarctica* lipase A (CAL-A) however, a different situation arose. It showed no selectivity and racemic butanoate was obtained in close to quantitative yield. Two other lipases [lipase AK from *Pseudomonas fluorescens* and lipase F from *Rhizopus oryzae* (ROL)] tested were found to be inactive towards



Scheme 18 Kinetic resolution by hydrolysis or alcoholysis.

the substrate. The other enantiomer, the (*S*)-cyanohydrin butanoate, could be obtained through an alcoholysis reaction catalysed by BCL (Scheme **20**). This traditional kinetic resolution proceeded with very high enantioselectivity (E = 100) [55].

Table 4. Kinetic Resolution by Hydrolysis or Alcoholysis

R	Enzyme	Enantioselectivity	Ref.
n-C_4H_9	PPL	*S*	[52]
n-C_7H_{13}	PPL	*S*	[52]
Cyclohexyl	PPL	*S*	[52]
Ph	CAL-B	*S*	[53, 54]
4-Chlorophenyl	CAL-B	*S*	[53]
3-Phenoxyphenyl	CAL-B	*S*	[53]
Phenylfuranyl	BCL	*R*[a]	[55]
Phenothiazinyl	CAL-A	*R*[a]	[56]
n-C_7H_{13}	CRL	*S*	[57]
Cyclohexyl	CRL	*S*	[57]
Ph	BCL	*S*	[57]
3-Phenoxyphenyl	BCL	*S*	[42]

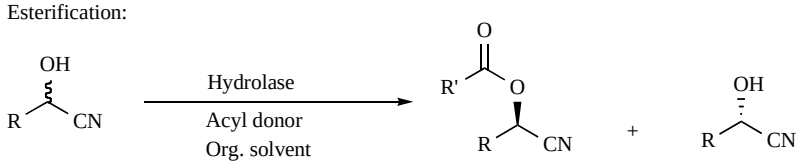
PPL = Porcine Pancreas Lipase; CAL-B = *Candida antarctica* lipase B; BCL = *Burkholderia cepacia* lipase; CAL-A = *Candida antarctica* lipase A; CRL = *Candida rugosa* lipase.
[a] Reverse in enantioselectivity due to CIP rules.

The methodology mentioned above has also been used on a similar class of compounds, namely the furylbenzothiazole-based cyanohydrins [58]. CAL-A was the best enzyme for these compounds. Other potential lipases were shown to be inactive. Reaction with CAL-A and vinyl acetate as acyl donor in acetonitrile as sol-

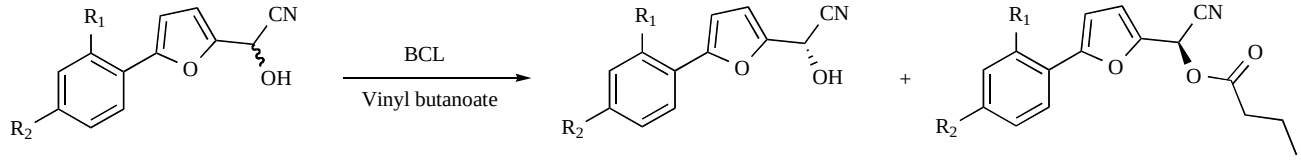
Table 5. Kinetic Resolution by Esterification

R	Enzyme	Enantioselectivity	Ref.
Furylbenzothiazolyl	CAL-A	*R*[a]	[58]
Phenothiazinyl	CAL-A	*R*[a]	[56]
Phenylfuranyl	BCL	*R*[a]	[55]
n-C_7H_{13}	CRL	*R*	[52]
Phenyl	CRL	*R*	[57]
n-propyl	CRL	*R*	[57]
Cyclohexyl	CRL	*R*	[52]
Phenyl	BCL	*S*	[57]
n-propyl	BCL	*S*	[57]
n-C_4H_9	PPL	*S*	[52]
n-C_7H_{13}	PPL	*S*	[52]

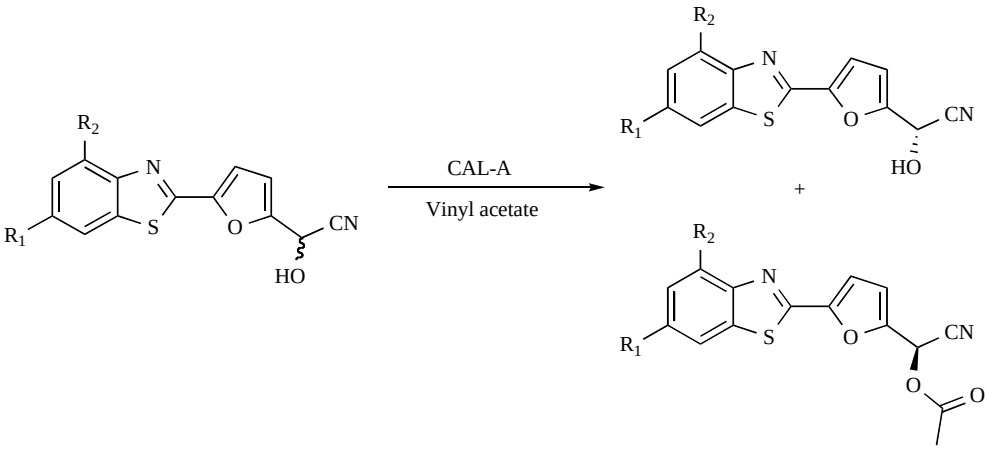
CAL-A = *Candida antarctica* lipase A; BCL = *Burkholderia cepacia* lipase; CRL = *Candida rugosa* lipase; PPL = Porcine Pancreas Lipase.
[a] Reverse in enantioselectivity due to CIP rules.



Scheme 19. Kinetic resolution by esterification.



Scheme 20. Kinetic resolution of phenylfuran cyanohydrins [55].



Scheme 21. Kinetic resolution of furylbenzothiazole cyanohydrins [58].

vent afforded the acylated cyanohydrins with a high degree of enantioselectivity (84-96% *ee*). This lipase showed preference towards the (*R*)-enantiomer of these substrates (Scheme **21**).

Drugs based on the phenothiazine structure have been shown to possess both antipsychotic and antimicrobial properties [56]. Today they are mainly being used in the treatment of diseases in the central nervous system, such as Creutzfeldt-Jakob disease. CAL-A was found to be the most suitable catalyst for the resolution of the phenothiazine building block, giving an E of 48. Other lipases, such as CAL-B (E = 13) and BCL (E = 8), were less enantioselective towards these substrates. Solvent optimisation was performed, which led to an increase in the E to more than 100. Enzymatic alcoholysis afforded both enantiomers with very high enantioselectivities (99%, Scheme **22**).

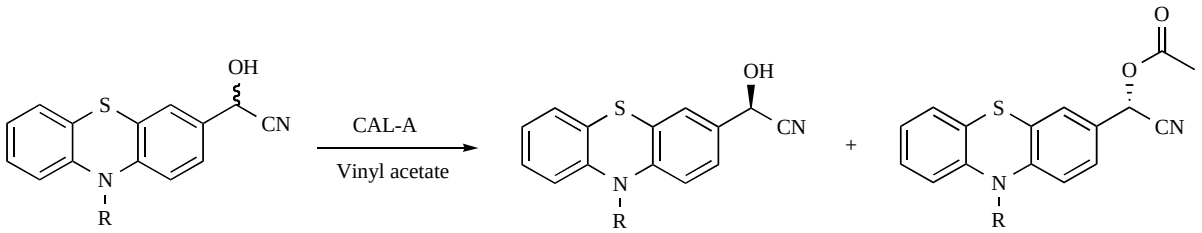
(*R*)-Cyanohydrins derived from aliphatic aldehydes can be prepared using almond meal as a catalyst. The (*S*)-enantiomers, however, have proven to be difficult to access. This is because aliphatic aldehydes are not readily accepted by the corresponding (*S*)-HNL's. Lipases, on the other hand, have been found to give good results with aliphatic cyanohydrins. The lipases from *Candida rugosa* and *Burkholderia cepacia* and porcine pancreas lipase were the best enzymes, giving E-values from 3 - 44 in esterification reactions and 15 – 35 in the alcoholysis reactions [52, 57].

A general problem of the kinetic resolution is that one enantiomer of the cyanohydrin is left unprotected. This problem has been overcome in a straightforward manner. Once the kinetic resolution in dry toluene was complete, vinyl butyrate was added. CAL-B that first catalysed the kinetic resolution now catalysed the protection of the free cyanohydrin as a butanoate. Overall a racemic acetate was converted in a one-pot two-step sequence into enantiopure (*R*)-cyanohydrin acetates and (*S*)-cyanohydrin butanoates, all catalysed by one enzyme. These could be separated by straightforward column chromatography [59].

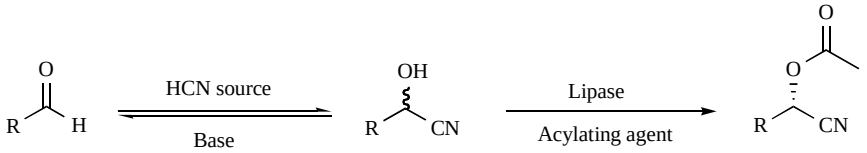
3.1.2 Lipase-Catalysed Dynamic Kinetic Resolution

When a kinetic resolution is combined with the reversible synthesis of the racemic cyanohydrin from a prochiral carbonyl compound then the desired cyanohydrin enantiomer can be obtained in 100% yield (Scheme **15**) [50, 51]. This overcomes the low yields of kinetic resolutions. Instead, the reaction now is essentially a bond-forming reaction, which can form part of an enantioselective synthesis. A catalyst that allows the fast formation and decomposition of the racemic cyanohydrin ensures that the hydrolase is always confronted with (almost) racemic cyanohydrin. The decomposition is equally important, since the undesired enantiomer of the cyanohydrin has to be broken down to the carbonyl compound again enabling the formation of racemic cyanohydrin (Scheme **23**).

As early as 1991 it was shown that such a dynamic kinetic resolution was viable. Extensive screening revealed alkaline Amberlite as a suitable catalyst for the formation of racemic cyanohydrins. Equally important it proved to be a good racemisation catalyst for aromatic cyanohydrins. Coupled with a BCL catalysed kinetic resolution many different aldehydes were converted into cyanohydrins



Scheme 22. Kinetic resolution of phenothiazine cyanohydrins.



Scheme 23. Dynamic kinetic resolution of cyanohydrin esters.

with good enantioselectivities and yields [60-62]. This technology was also applied to the kinetic resolutions described above in Schemes 20-22. When the reaction is performed using basic Amberlite rapid interconversion of the two enantiomers of the free cyanohydrin occurs. The lipases were all immobilised on Celite and catalysed the dynamic kinetic resolutions with excellent selectivities and yields [55, 56, 58] (Scheme **24**).

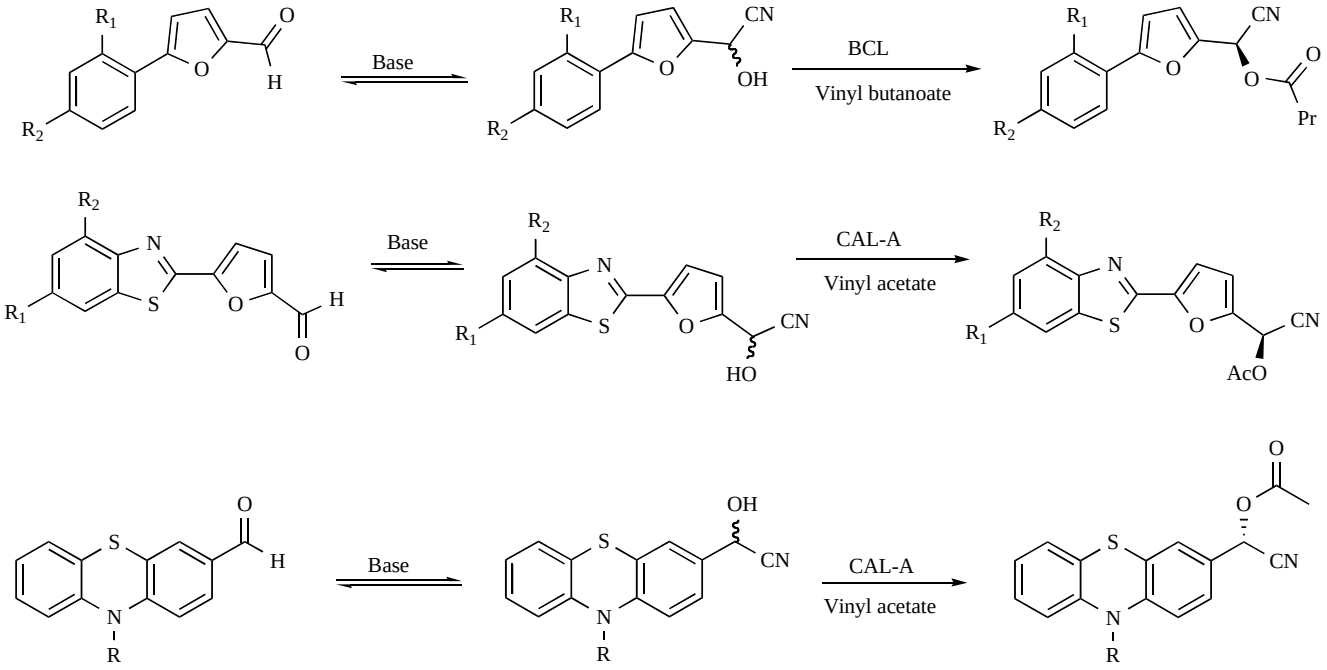
All of the dynamic kinetic resolutions were, however, not very fast and could take up to one week. A thorough investigation of the different parameters that influence the reaction revealed that traces of acid formed, due to the hydrolysis of the acyl donor, severely hampered the reaction [63]. This hydrolysis was directly linked to the carrier material of the enzyme. When the lipase is immobilised on Celite R 633 the dynamic kinetic resolution starting with aromatic aldehydes was successful. The (*S*)-enantiomer of mandelonitrile acetate has been made *via* such a dynamic kinetic resolution catalysed by CAL-B. The product was obtained in 96% yield with an *ee* of 98%. This is an improvement of this reaction in comparison to using conventional metal transition catalysts [64]. This methodology was also applicable to other aromatic aldehydes [65] (Scheme **25a**). Recently it was described that Amberlite could be replaced by silica-supported benzyltrimethylammonium hydroxide in this reaction [65a].

Cyanohydrins prepared from aliphatic aldehydes are more stable than those prepared from aromatic aldehydes. Therefore the basic Amberlite was not strong enough to realize the rapid synthesis and racemisation of these cyanohydrins. When, however, NaCN was employed as a base the dynamic kinetic resolution proceeded smoothly [66] (Scheme **25b**).

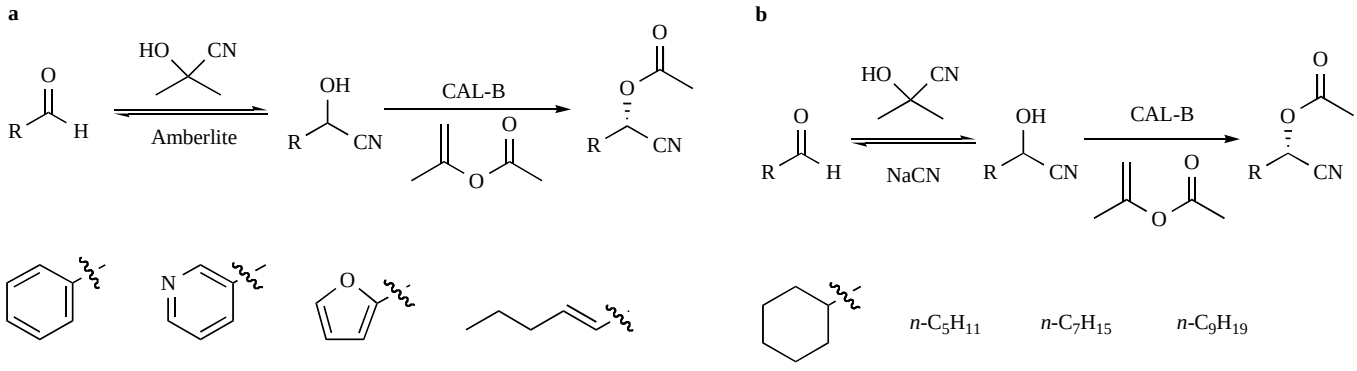
Given the wealth of hydrolases with (*R*)- and (*S*)-selectivity (see Tables **4** and **5**) the applicability of the dynamic kinetic resolution as a tool in organic synthesis is virtually unlimited. Since this type of reaction converts an aldehyde into a chiral and protected cyanohydrin it is equivalent to many chemical cyanohydrin syntheses [2-4].

3.1.3. Other Applications

Next to the countless synthetic applications of namely lipases and esterases, hydrolases offer many other opportunities. Over the past few years, high-throughput experimentation has become increasingly important. Efficient high-throughput screening (HTS) methods are an essential part of this research. Colorimetric and



Scheme 24. Successful dynamic kinetic resolutions for the preparation of drug candidates.

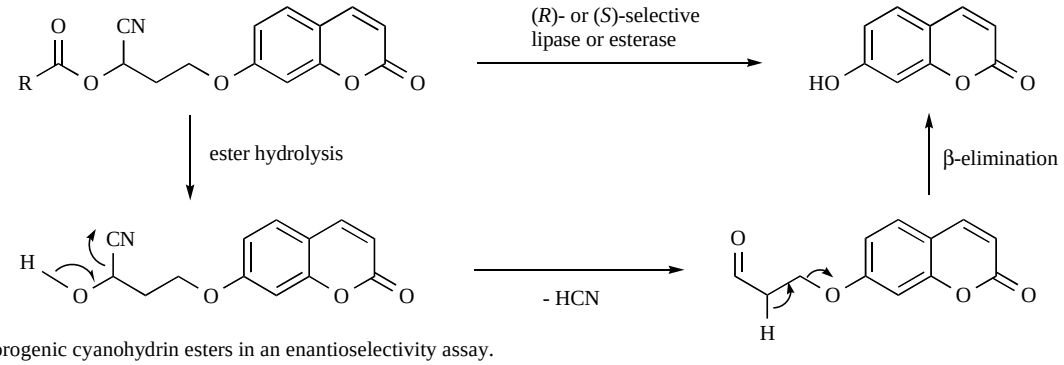


Scheme 25. CAL-B catalysed dynamic kinetic resolutions: (**a**) aromatic aldehydes are converted with basic Amberlite; (**b**) aliphatic aldehydes are converted with NaCN as base.

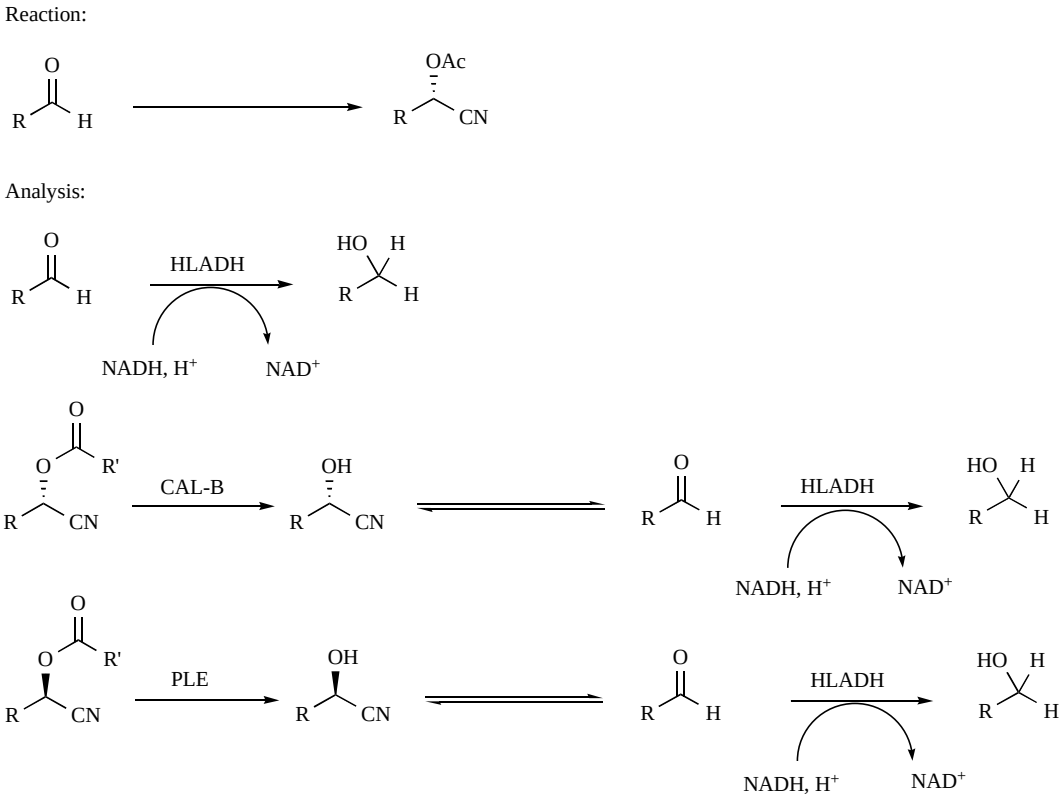
fluorimetric assays to follow the reactions are especially popular, because they only require simple reagents, rather than special instrumentation. Chromogenic and fluorogenic ester substrates, pH-indicators and enzyme-coupled assays are examples of these.

It has been shown that chiral fluorogenic cyanohydrin esters can be used as probes for determining enantioselectivity of lipases and esterases [67]. The product of the ester hydrolysis, the free cyanohydrin, is not by itself fluorescent. By an *in situ* transformation, first hydrolysis with the concomitant loss of HCN, and then β-elimination, gives umbelliferone as a product. This compound is fluorescent, and is widely used in biological assays (Scheme **26**). These fluorogenic cyanohydrin esters are simple probes for screening enzyme activity on chiral secondary esters. They also have very low spontaneous reactivity, which eliminates the possibility for autocatalysis.

It has been shown that the enantioselectivity, yield and conversion of a reaction, as demonstrated with the Lewis acid or Lewis base catalysed addition of acetyl cyanides to benzaldehyde, can all be determined using a combination of enzymes [68, 69]. Only one enzyme needs to be enantioselective, while the other enzymes should be not enantioselective. The principle is that an enantiomeric mixture of different chemical species is converted by using chemoselective enzymatic transformations. This gives the opportunity to measure the enantioselectivity by simple chemical analyses. First horse liver alcohol dehydrogenase (HLADH) is added to the reaction mixture to reduce any aldehyde that was not converted. Then highly enantioselective CAL-B hydrolyses the (*S*)-acetate of the formed cyanohydrin acetate. The cyanohydrin released decomposes and liberates benzaldehyde which again is reduced by HLADH. This reaction is followed photospectroscopically. Then the unselective pig liver esterase is added which hydrolyses the (*R*)-acetate of the cyanohydrin. Again benzaldehyde is liberated and reduced and the reaction is quantified. Thus the concentrations of (*S*)- and (*R*)-acetate are known and the *ee* can be calculated. This method is reliable when compared with chiral GC, and has the significant advantage of being faster and easier to perform. Good results have been obtained with a broad variety of compounds, ranging from aliphatic to aromatic and heterocyclic acetylated cyanohydrins (Scheme **27**).



Scheme 26. Fluorogenic cyanohydrin esters in an enantioselectivity assay.



Scheme 27. High-throughput synthesis and analysis of acylated cyanohydrins.

3.2. Cyanohydrins Derived from Ketones

Much work has been performed on lipase-catalysed kinetic resolutions and dynamic kinetic resolutions of cyanohydrins derived from aldehydes. Cyanohydrins derived from ketones, however, have been scarcely investigated [70-72]. This is mainly due to their low reactivity because they are in fact tertiary alcohols. Tertiary alcohols are known to be problematic substrates for hydrolases/lipases. Nonetheless several direct and indirect approaches for the kinetic resolution of cyanohydrins from ketones have been developed.

3.2.1. Lipase-Catalysed Kinetic Resolution of Ketone-Based Cyanohydrins

Until a short time ago virtually no examples of kinetic resolutions of ketone-based cyanohydrins existed [70-73]. Recently, a systematic study on the activity of hydrolases towards cyanohydrin esters derived from a range of aromatic ketones was published [73]. A series of arylaliphatic cyanohydrin acetates were screened as substrates in the enzymatic hydrolysis using commercially available hydrolases. Several lipases [CAL-A, CAL-B, *Candida rugosa* lipase (CRL), also known as *Candida cylindracea* lipase (CCL), BCL, porcine pancreas lipase (PPL), *Rhizomucor miehei* lipase (RML)], proteases [Subtilisin A (SubA), α-chymotrypsin] and esterases (PLE) were tested on the cyanohydrin acetates mentioned above. Even the *Me*HNL was included in the screening because it resembles hydrolases by having a catalytic triad in its active site. Unfortunately, it showed no activity.

CAL-A, normally the hydrolase of choice towards the esters of bulky alcohols, did surprisingly not show any activity at all. However, CRL and BCL, respectively, were found to be active. In accordance with the literature, these lipases showed preference for the (*R*)-enantiomer of the cyanohydrin acetates. CRL was found to be the most selective of the two (E = 8 – 13), while BCL only showed a small degree of enantioselectivity (E = 6) with a rather narrow substrate range.

Surprisingly, SubA, a protease, proved to be a good catalyst for the hydrolysis of ketone-based cyanohydrin acetates. Another advantage using SubA was that it gave the opposite enantiomer. This reversal in stereopreference between lipases and subtilisin has been known for a long time for secondary alcohols. Subtilisin A showed good enantioselectivity for all substrates towards the (*S*)-enantiomer with an E-value of 7 – 58 (Scheme **28**, Table **6**). This enabled a straightforward approach towards one type of enantiopure protected tertiary alcohols, which so far have been difficult or impossible to obtain through traditional catalysis using different metal catalysts, as well as by enzymatic methods.

Table 6. Hydrolases Used in the Preparation of Enantiopure Cyanohydrin Esters Derived from Ketones [73]

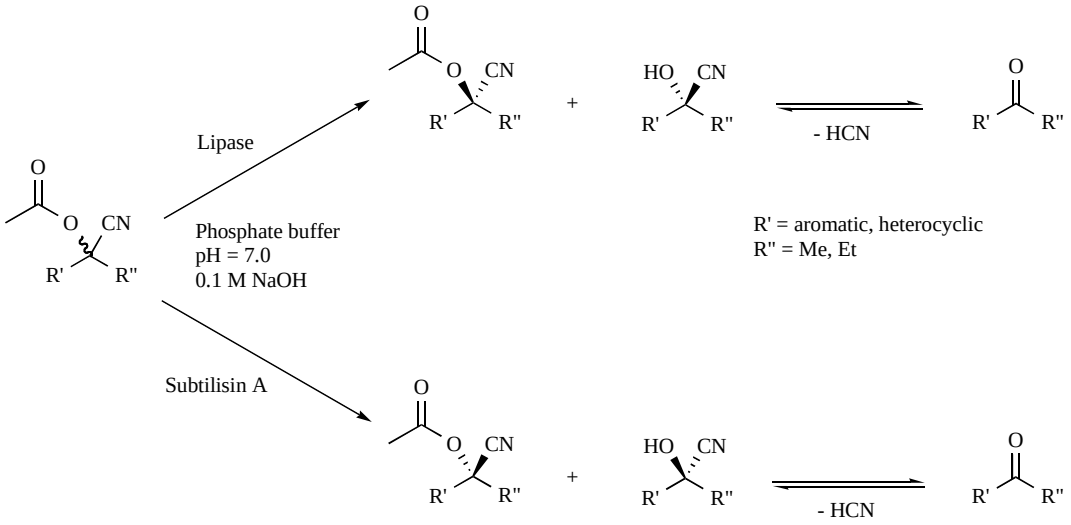
Enzyme	Cyanohydrin Ester		Enantioselectivity
CRL	R' = Ph	R" = Me	*R*
	R' = Thiophenyl	R" = Me	*R*
BCL	R' = Ph	R" = Me	*R*
SubA	R' = Ph	R" = Et	*S*
	R' = Thiophenyl	R" = Me	*S*
	R' = 4-Methoxyphenyl	R" = Me	*S*

3.2.2. Alternative Routes

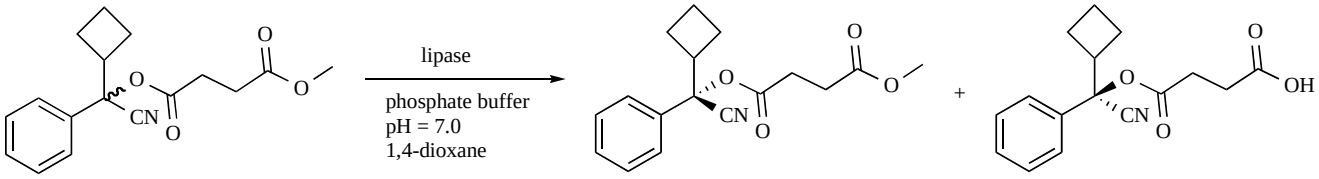
Since the direct resolution of tertiary alcohols using enzymatic methods is such a challenge to the organic chemist alternatives have been developed. This problem can for instance be circumvented by substrate modification. One elegant approach to this has been used in the resolution of 1-cycloalkyl-1-hydroxy-1-phenylcyanohydrins [74]. These compounds are important building blocks since they are the key precursors in the total synthesis of (*S*)-oxybutynin and its derivatives, a type of muscarinic receptor antagonists that are being used in the treatment of urinary tract disorders. Preliminary trials on the lipase-catalysed resolutions with the tertiary alcohol did not give any conversion.

When extending the substrate with an alkyl chain and performing an enzyme-catalysed hydrolysis on the methyl ester down to the corresponding acid five bonds remote from the stereocenter of the substrate, some enantioselectivity was observed (E = 2 – 6), although it was not very high (Scheme **29**).

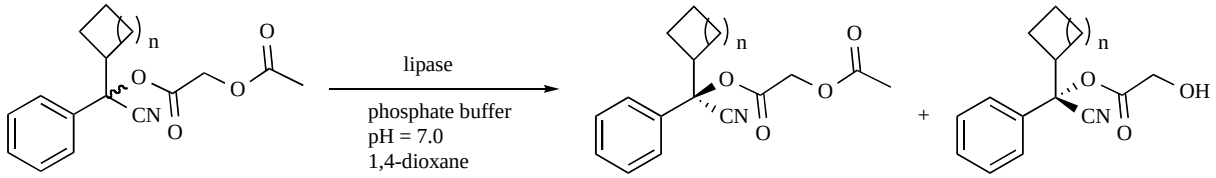
Acetoxyacetylated cyanohydrins gave good rates of reaction (16 hours) and very high enantioselectivities using CAL-B and BCL (E > 200). Moreover, the two employed lipases showed opposite enantioselectivity, CAL-B being (*R*)-selective, while BCL afforded the (*S*)-enantiomer. Increasing the ring size from cyclobutyl to cyclopentyl and cyclohexyl, however, resulted in lower enantioselectivities (E = 1 – 7, Scheme **30**).



Scheme 28. Enzymatic hydrolysis of ketone-based cyanohydrin acetates.



Scheme 29. Kinetic resolution of hindered cyanohydrins.

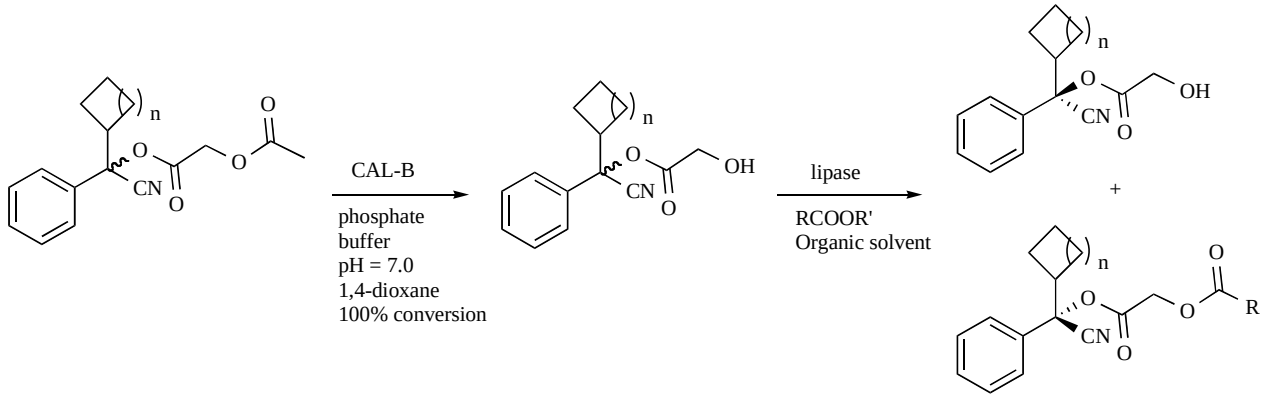


Scheme 30. Kinetic resolution of hindered cyanohydrins.

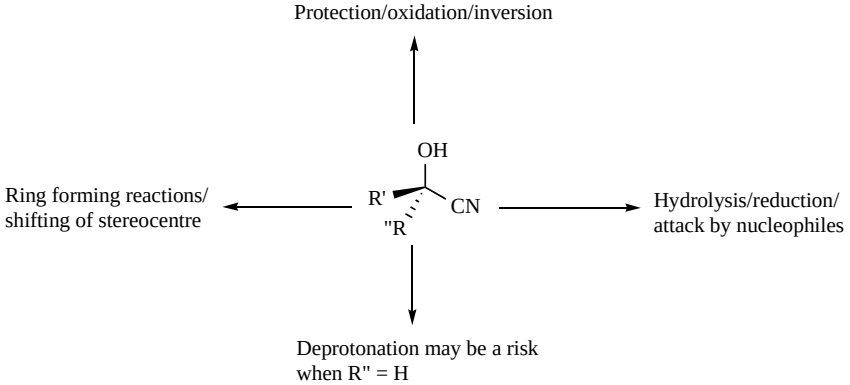
The length of the spacer has been shown to be crucial, as shortening or lengthening it with one atom did not give any result. Longer chain esters (butyrate, decanoate, laurate and benzoate) gave better enantioselectivities with BCL (Scheme **31**). This is a good example of how it is possible to affect an enzymes stereo/regio-selectivity through remote interactions [74].

4. FOLLOW-UP CHEMISTRY ON CYANOHYDRINS

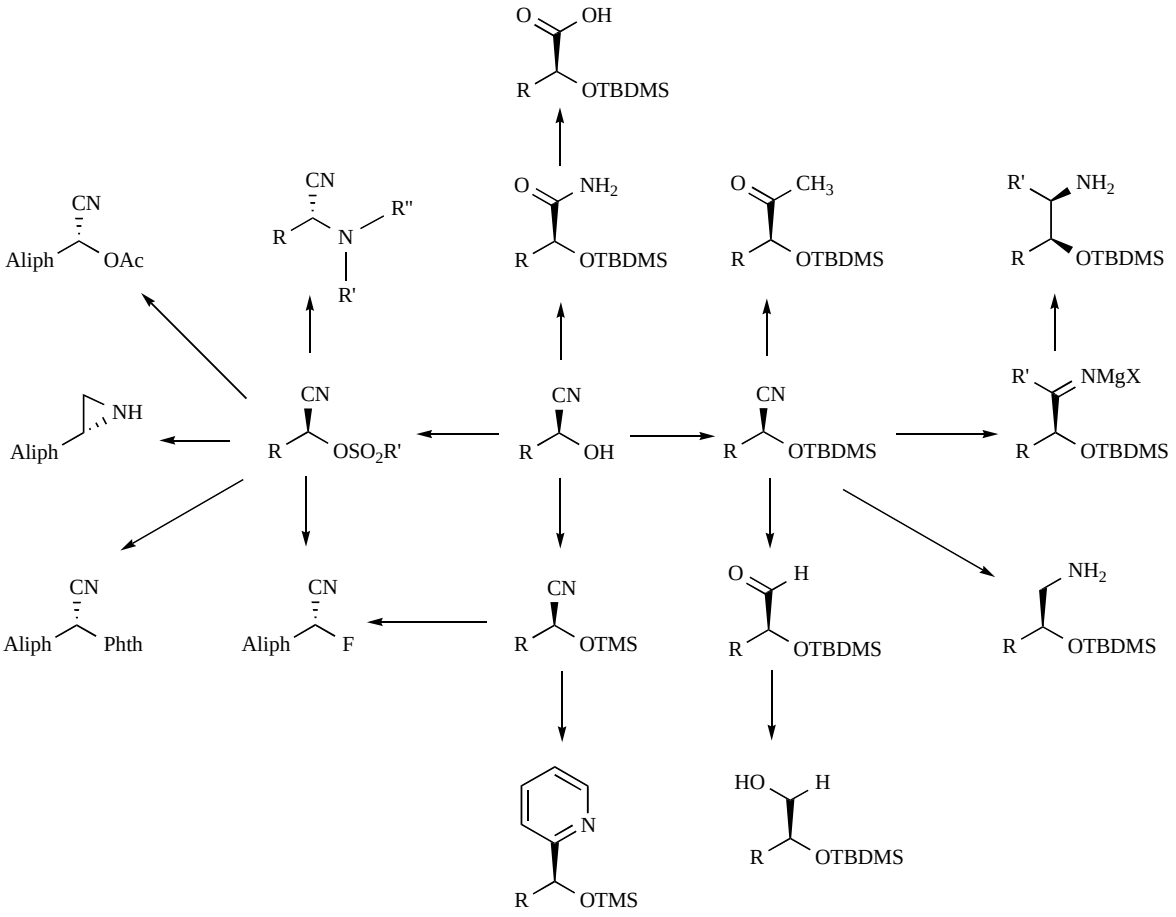
The chemistry that can be performed on cyanohydrins is extensive. The two highly versatile functional groups that they consist of, the hydroxyl and nitrile group respectively, offer many opportunities to the organic chemist (Scheme **32**). Conversions that destroy the chirality, such as oxidation and base-catalysed deprotonation/racemisation, will not be considered here. In addition to the modification of the hydroxyl and the nitrile group different side groups attached to the cyanohydrins further enriches their chemistry. A summary of the chemistry possible to date is given in Scheme **33**. It demonstrates that chiral cyanohydrins are a very good starting point for preparing important and attractive building blocks for a large range of compounds ranging from pharmaceuticals and natural products to those important in the cosmetics, flavor, fragrance and agrochemical industry. In this part, recently reported transformations of cyanohydrins and their applications will be described.



Scheme 31. Kinetic resolution of hindered cyanohydrins.



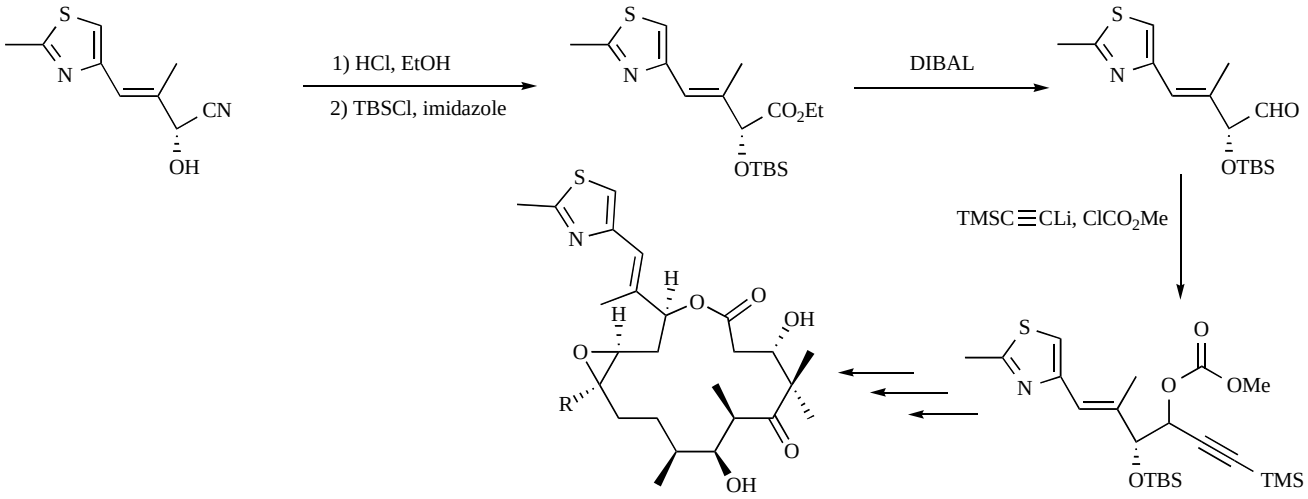
Scheme 32. Possible follow-up chemistry on cyanohydrins.



Scheme 33. Follow-up chemistry on cyanohydrins.

4.1. Hydrolysis of the Nitrile Group

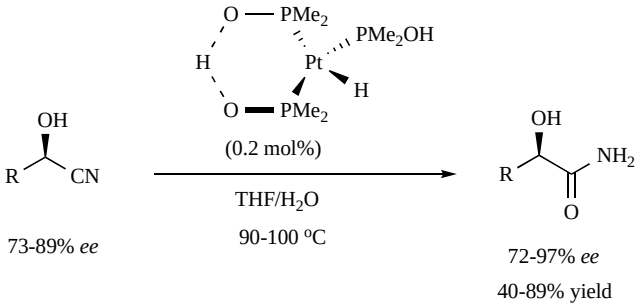
The hydrolysis of the nitrile group of cyanohydrins yields amides or acids. The reaction can be performed with concentrated acids or bases, bases however, might racemise the cyanohydrins [1, 5, 6, 18, 34]. Concentrated acids lead in the first instance to the amide, but the selectivity is not always high enough and complete hydrolysis to the acid might occur. If the acid is generated *in situ* by hydrolysis of TMSCl the reaction will stop at the amide stage [74a]. The concentrated acids can be replaced by gentle chemical catalysts or biocatalysts ensuring mild reaction conditions and the possibility to obtain only the amide, if this is desired. Both approaches will be briefly described. If the reaction is performed in dry alcohols the Pinner reaction leads to the esters. This has been applied in a recent total synthesis of epothilones A and B (Scheme **34**).



Epothilones A (R = H) and B (R = Me)

Scheme 34. Use of asymmetric cyanation in the total synthesis of epothilones A and B.

Epothilones are compounds with antitumor activities. They act in the same way as taxol, through binding and stabilizing microtubules. Because of this, they are considered as promising drug candidates. Asymmetric cyanation of an aldehyde is one of the key transformations in the approach described here. The resulting cyanohydrin is then subjected to the HCl-catalysed Pinner reaction to give the corresponding ethyl ester. Reduction with DIBAL, carbamate formation and another reduction gave the final compound which was used in the synthesis of fragment A of epothilones A and B [75].



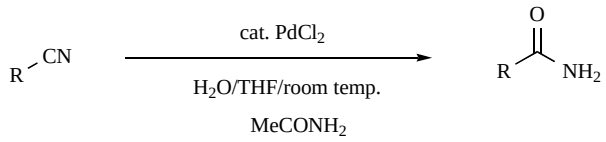
R = Ph, 4-OMeC$_6$H$_4$, 4-CF$_3$C$_6$H$_4$, PhC=C, Cyclohexyl, C$_8$H$_{17}$

Scheme 35. Synthesis of α-acetoxy amides by nitrile hydrolysis.

4.1.1. Amide Formation with Transition-Metal Catalysts

Amides can be made from the corresponding nitriles by the use of a platinum(II) phosphinito catalyst. It has been shown to convert various cyanohydrin acetates derived from aldehydes, into their corresponding α-acetoxy amides in a chemoselective manner under neutral conditions not causing any racemisation (Scheme **35**) [76]. Enantiopure cyanohydrin acetates were converted into enantiomerically pure amides. The reaction, however, is rather slow and required elevated temperatures.

Recently, a new method for nitrile hydrolysis to the corresponding amide was reported (Scheme **36**) [77]. The main advantage with this procedure is that commercially available palladium(II)chloride is used as a catalyst, in contrast to the platinum(II) phosphinito catalyst that has to be synthesized in two steps. Good yields of the amides are obtained with aqueous acetonitrile as solvent. Acetamide is added to ensure that the reverse reaction, dehydration of amides to nitriles, is inhibited. This method is a viable alternative to the platinum-catalysed reaction because of its simplicity and its mild reaction conditions (room temperature).



R = Ph, naphthyl, 4-Ph-1-butyl, aliphatic

Scheme 36. Hydrolysis of nitriles to amides catalysed by $PdCl_2$.

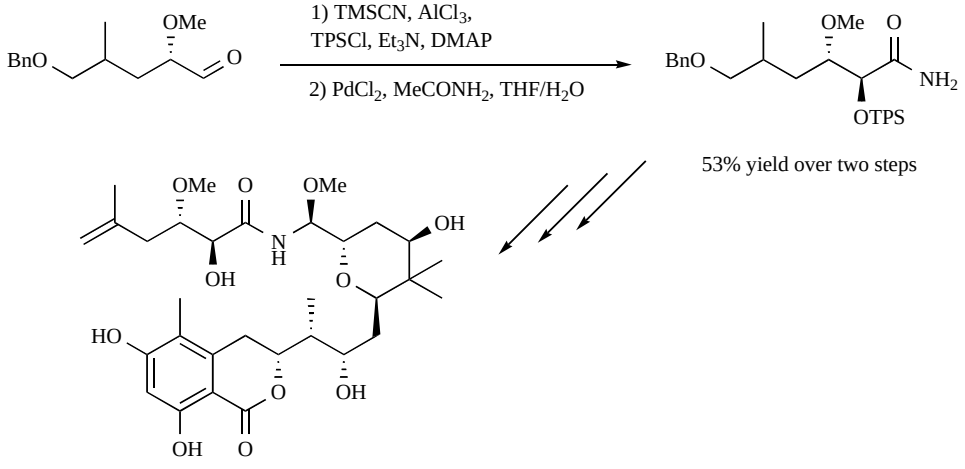
The acyclic side chain of the cytotoxin Psymberin 1 has been prepared using this methodology [78]. The final step in the synthesis of the acyclic side chain is the formation of a protected cyanohydrin from the corresponding aldehyde, and subsequent hydrolysis to the amide. This transformation proceeds with 53% yield for both steps (see Scheme **37**).

4.1.2. Amide and Acid Formation with Enzymes

A significant advantage of employing enzymes for the hydrolysis of cyanohydrins, is their high selectivity and the possibility to exclusively obtain amides under very mild conditions [79, 80]. Nitrilases hydrolyse the nitrile group to an acid, while nitrile hydratases will stop at the amide stage (Scheme **38**). It has recently been demonstrated that *Rhodococcus erythropolis* NCIMB 11540 is an important biocatalyst, which contains a highly active nitrile hydratase and amidase again yielding the acid as the product. They have been cloned in *Escherichia coli*. Moreover, when the nitrile hydratase is separated from its amidase, amides rather than the free carboxylic acids are formed during the reaction. A wide range of substrates can be accepted (Scheme **38**), and have given amides in high yield (82%) with a high degree of enantiopurity (99%) [81].

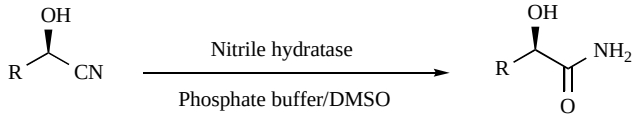
When the amidase is added to the amide formed, the second step of the hydrolysis is accelerated. The final carboxylic acids are then obtained as products without any loss of enantiopurity (Scheme **39**) [81].

Application of combi-CLEA's (consisting of two or more enzymes) has been successfully employed in the one-pot synthesis of enantiopure (*S*)-mandelic acid starting from benzaldehyde [82]. The CLEA was composed of the (*S*)-selective *Me*HNL and a non-



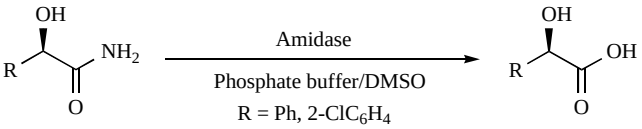
Scheme 37. Synthesis of the acyclic side chain of Psymberin 1 with a $PdCl_2$ catalysed nitrile hydrolysis.

selective nitrilase (Nlase) from *Pseudomonas fluorescens* (Scheme **40**). As mentioned above nitrilases hydrolyse nitrile groups directly to acids.

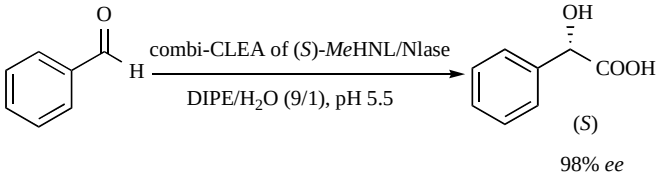


R = $PhCH_2CH_2$, 2-ClC_6H_4, 4-$CH_3C_6H_4$, 3-OPh-C_6H_4

Scheme 38. Synthesis of amides by nitrile hydrolysis with a nitrile hydratase.

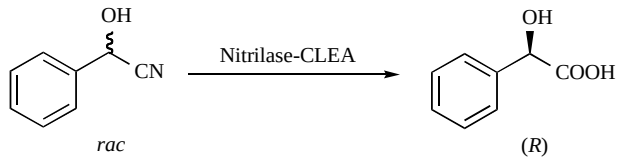


Scheme 39. Formation of carboxylic acids by amide hydrolysis using an amidase.



Scheme 40. Use of a combi-CLEA to prepare (*S*)-mandelic acid.

Another example recently reported, also describes the use of CLEA's. In this case a CLEA of the nitrilase from *Alcaligenes faecalis* was prepared [83]. This enzyme was able to convert 100% racemic mandelonitrile to the (*R*)-enantiomer of mandelic acid (see Scheme **41**). This is due to the fact that the reaction actually is a dynamic kinetic resolution. The initially unconverted (*R*)-mandelonitrile is racemised under the reaction conditions.



Scheme 41. Hydrolysis of racemic mandelonitrile to (*R*)-mandelic acid by an enantioselective nitrilase.

4.2. Reduction of the Nitrile Group

4.2.1. Catalytic Hydrogenation: Formation of Amino-Alcohols

Chiral amino-alcohols are important building blocks. They are indeed present in many β-blockers. *N*-acylated β-amino alcohols can readily be transformed into β-*sec* amino alcohols, which are found in pharmaceuticals, such as etilefrine, bamethane and denopamine. Normally, they are made through reduction of free cyanohydrins with subsequent acylation of the amino group and a second reduction. When enantiopure cyanohydrins are used, racemisation can occur as these compounds are known to be relatively unstable. Their corresponding esters, however, are stable and do not racemise.

Recently, a novel catalytic approach towards enantiopure *N*-acyl β-amino alcohols was developed [84]. In contrast to earlier methodologies, this route proceeds *via* catalytic hydrogenation of cyanohydrin esters with a following intramolecular migration of the acyl group (see Scheme **42**). Optimal conditions for this reaction cascade were found using a Design of Experiment approach (DoE). Nickel immobilised on alumina in dioxane as solvent gave the best results. The cascade yields up to 90% for aliphatic cyanohydrins and up to 50% for aromatic substrates. When enantiopure aliphatic substrates are employed their stereochemistry remains unaffected, while aromatic substrates show some degree of racemisation due to the low stability of the benzyl proton.

4.2.2. Reductive Amination

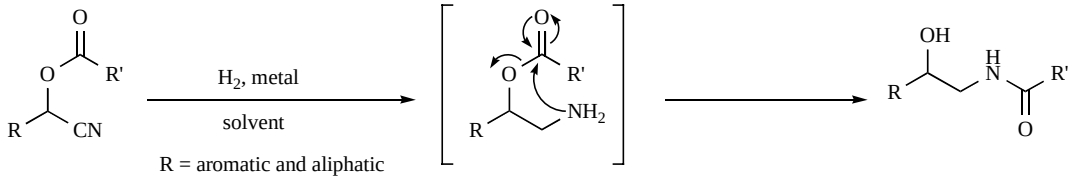
A biocatalytic approach towards enantiopure piperidones, versatile building blocks for the preparation of biologically active compounds, has been investigated [85]. The stereocentre established in the HNL-catalysed step induces, together with the diamine, the stereochemistry of the aminal. Due to the stable cyclic aminal, no imine is formed and the reduction cannot proceed to the amine stage. This remarkable catalytic reduction gave access to the final products in good yields (Scheme **43**).

4.2.3. Reduction of Cyanohydrins with Hydrides

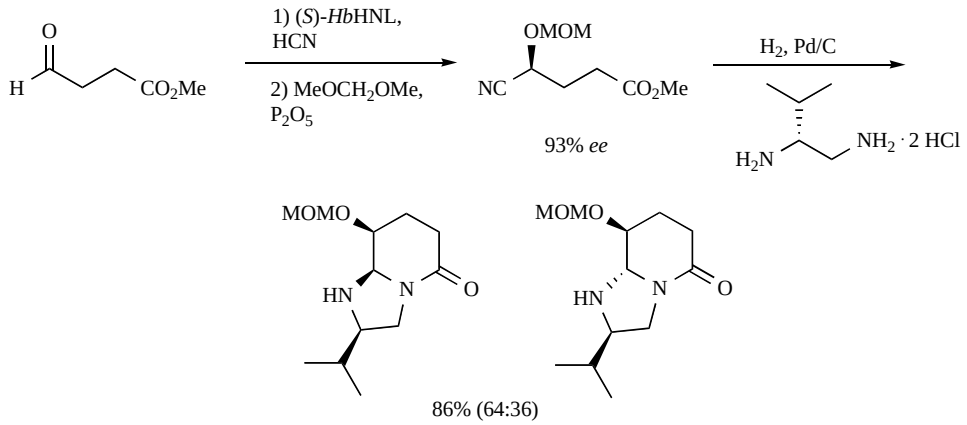
The non-catalytic reduction of cyanohydrins with hydrides is well established and proceeds smoothly [1, 3, 5, 6, 18]. With DIBAL it is possible to stop at the imine/aldehyde stage, while more powerful reagents such as $LiAlH_4$ allow the complete reduction (see Scheme **33**). Novel methods of chemo-selective reduction have been reported and demonstrated in the synthesis of fully orthogonal protected sialidase inhibitors. The β-lactam ring can be seen as a valuable building block for making more complex compounds. By combining this moiety with a cyanohydrin, many interesting alternatives arise. When a β-lactam cyanohydrin was subjected to $NaBH_4$ in the presence of a nickel salt, the nitrile group was selectively reduced to the corresponding amine. Changing the reducing agent to $LiBH_4$, it was found that the β-lactam ring was reduced to the corresponding β-amino-δ-hydroxy nitrile in high yield (82%); i.e. the nitrile group remains unaltered (see Scheme **44**). When sodium methoxide is employed for ring opening (yield 90-98%), the $NaBH_4$/nickel salt combination as reducing agent yields the piperidones (not shown) and later piperidines (after $LiAlH_4$ reduction of the amide) are formed in good yields. Finally, alkylation of the amine group with methyl bromoacetate under basic conditions gives the *N*-methoxycarbonylmethyl piperidines, which are used as sialidase inhibitors [86].

4.2.4. Addition of Grignard Reagents

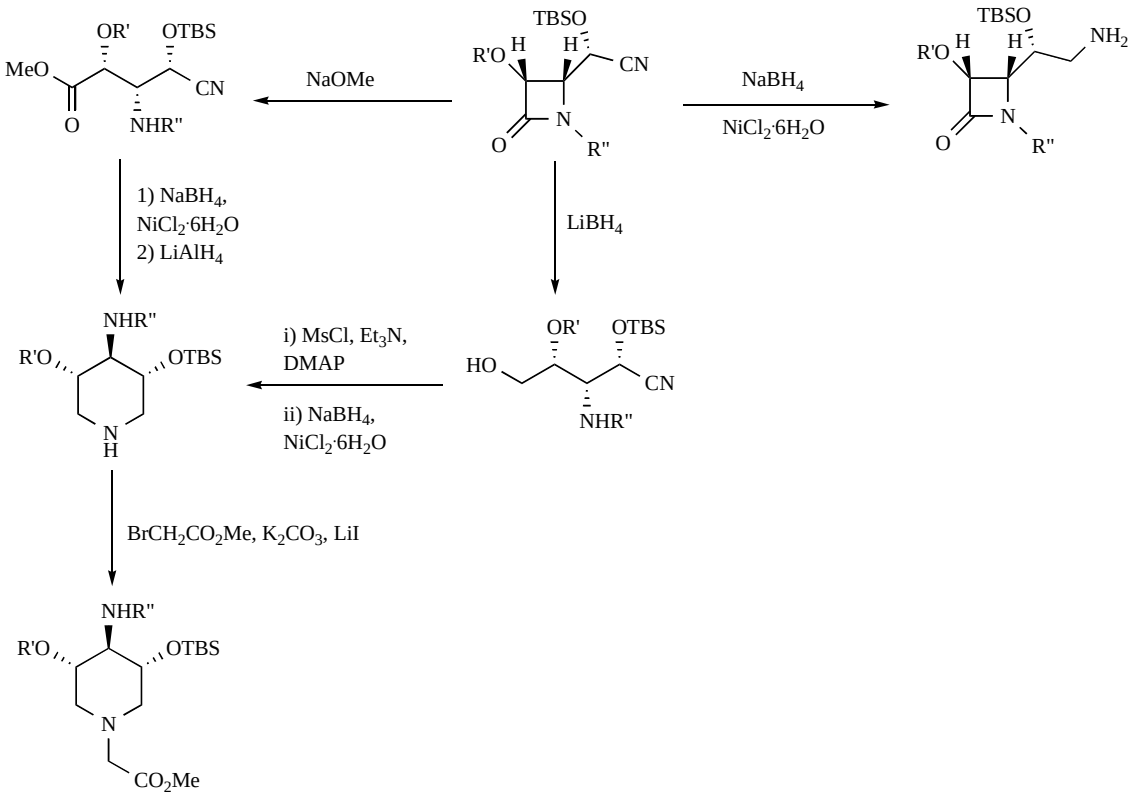
Next to the reduction of cyanohydrins with hydrides they can also be treated with carbon nucleophiles, such as Grignard com-



Scheme 42. Catalytic hydrogenation and acyl migration of cyanohydrin acetates.



Scheme 43. Piperidones through reductive amination.



Scheme 44. Chemo-selective reduction of β-lactam cyanohydrins.

pounds. Most of this chemistry is well investigated and reviewed (see Scheme **12**) [1, 3, 5, 6, 18]. The basic principle is that after the first Grignard addition a relatively stable intermediate is obtained. This can either be reduced with $NaBH_4$ or treated with an amine, allowing transimination before the reduction (see Scheme **12**) [87].

4.2.5. Blaise Reaction

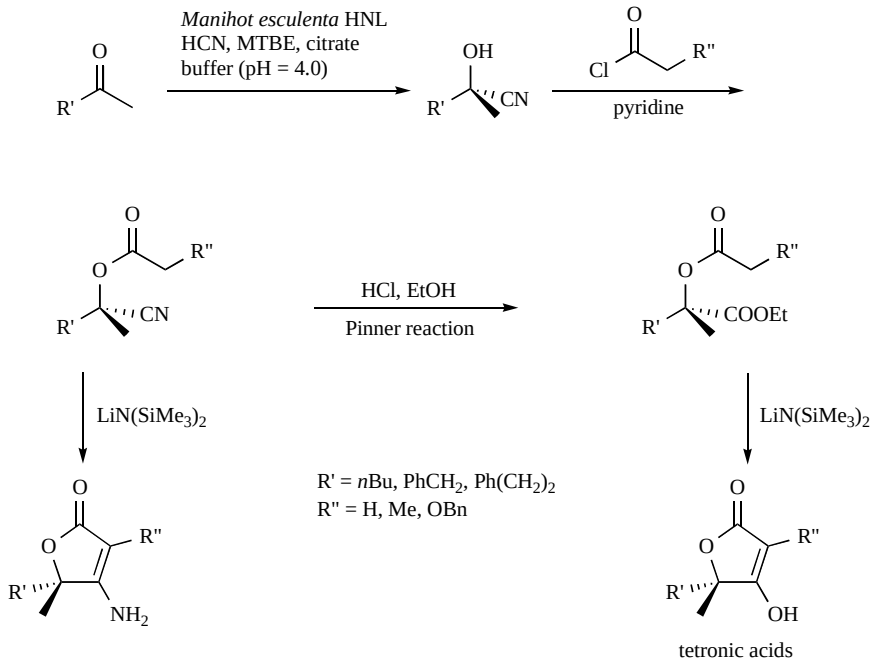
In the ketone-based cyanohydrins, no acidic hydrogen on the α-carbon is present allowing a cyclisation reaction. The hydroxyl group needs first to be protected as an ester. This ester can then be deprotonated in its α-position, enabling a nucleophilic intramolecular attack on the cyano group, the Blaise reaction [88]. The cyclic intermediate can be hydrolysed to a tetronic acid (Scheme **45**).

4.3. Cyclotrimerization: Formation of Pyridyl Alcohols

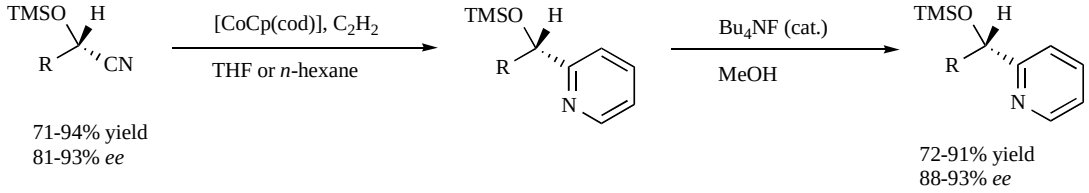
Very recently a well-established catalytic pyridine synthesis was for the first time performed with a protected chiral cyanohydrin as starting material (Scheme **46**) [89]. The pyridine ring is formed *via* a cobalt(I)-catalysed cyclotrimerization reaction with acetylene. The reaction works best with the TMS-ethers of the cyanohydrins, while the corresponding acetates gave very poor yields. The stereocentres are retained throughout the reaction, no racemisation occurred. This opens up an entirely new line of transition metal-catalysed modification of cyanohydrins.

4.4. Protection of the Hydroxyl Group

In many of the transition metal-catalysed syntheses of cyanohydrins they are formed as a TMS-ether, while in the enzymatic dynamic kinetic resolutions typically the acetates are obtained. Since cyanohydrins are rather sensitive they normally need to be protected. As racemisation occurs rather easily under basic conditions care has to be taken when they are protected in the presence of a base. But the silyl protection groups can readily be introduced un-



Scheme 45. The Blaise reaction.



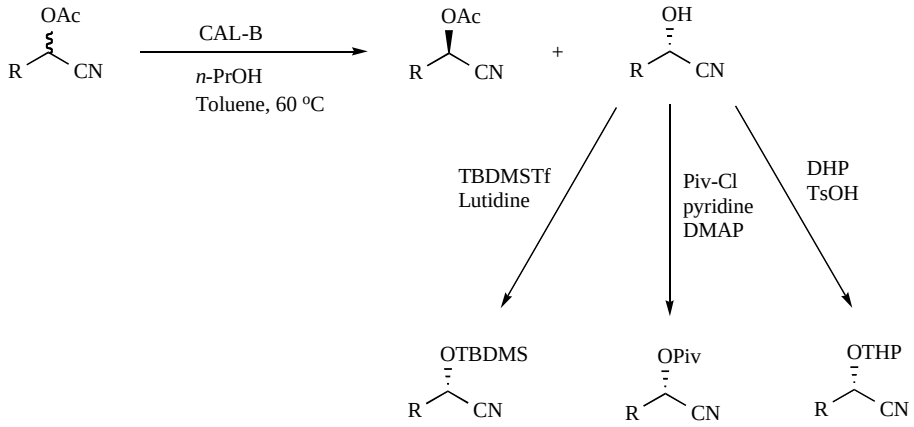
Scheme 46. Formation of chiral pyridyl alcohols through cyclotrimerization.

der these conditions and so can many ester protection groups. Under acidic conditions the THP ethers are formed and also the MIP and PIP ethers [5, 6].

An interesting example of the introduction of these types of protection groups has been described in combination with an enzyme-catalysed kinetic resolution in dry toluene. The enzyme is removed by filtration and the protecting reagents are added to the dry reaction mixture, yielding the enantiomerically pure (*R*)-cyanohydrin acetates and the TBDMS, Piv or THP protected (*S*)-cyanohydrins (Scheme **47**) [59].

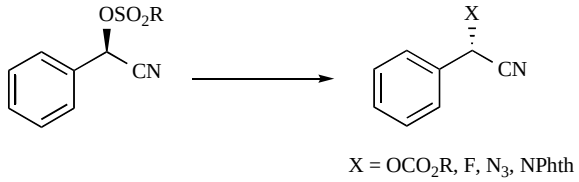
4.5. Conversion into a Leaving Group: Inversion of Configuration

The substitution of the hydroxy group is of great interest as it allows the introduction of many other groups. In order to retain the stereochemical information the substitution must occur with complete inversion. Enantiopure α-sulfonyl oxynitriles have been used for the inversion of configuration of cyanohydrins (Scheme **48**) [90]. In particular for aliphatic substrates this strategy has been successful. For aromatic cyanohydrins the risk of racemisation is, however great. It is important to keep in mind that aromatic cyano-



Scheme 47. Protection of enantiopure cyanohydrins.

hydrins tend to be configurationally less stable due to the high acidity of the benzylic hydrogen. Therefore their sulfonyloxynitriles are unstable and decompose easily. As the example of Clopidogrel (Scheme **10**) shows, this problem can be overcome. An alternative is the Mitsunobu reaction [91] that proceeds smoothly with aromatic cyanohydrins (see Scheme **49**).



Scheme 48. Inversion of configuration using α-sulfonyl oxynitriles.

4.6. Other Transformations

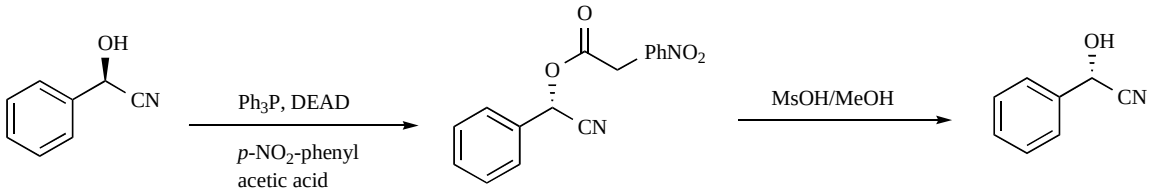
If the cyanohydrin contains other functional groups, these can be utilized for further modifications. Of particular interest are those reactions that allow transferring the chirality locked in the cyanohydrin functional groups to another part of the molecule.

4.6.1. Allylic Substitution and Alkylation Reactions

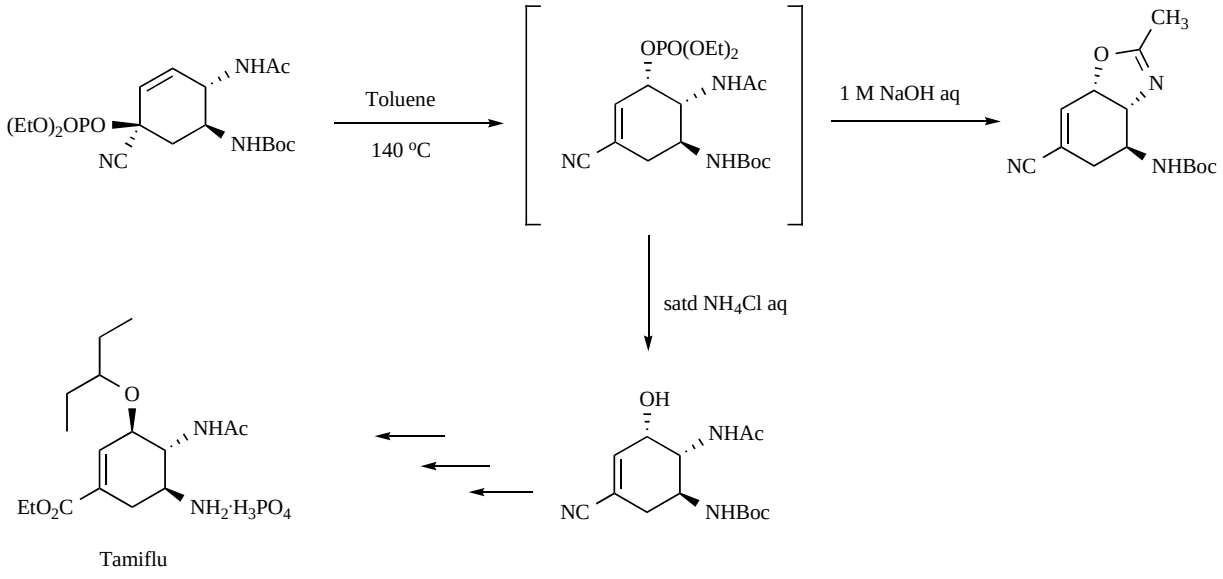
Allylic substitution has been employed in the total synthesis of the anti-influenza drug Tamiflu [92]. An allylic oxygen function and a one-carbon unit on the double bond were introduced by formation of the cyanophosphate from the corresponding enone (using diethyl phosphoryl cyanide and LiCN as catalyst) and an allylic substitution using an oxygen nucleophile. The first attempt of the allylic substitution under thermal conditions gave the cyclic carbamate as main product with the alcohol as a byproduct when work-up was done with sodium hydroxide. However, by changing to ammonium chloride in the work-up, the allylic alcohol was formed exclusively in high yield (78%) (Scheme **50**). This allowed for an efficient synthesis of Tamiflu.

Other types of nucleophiles have also been used in allylic substitution reactions. In these cases, especially allylic cyano-*O*-phosphates, but also cyano carbonates, were shown to be extremely good substrates for this kind of reaction [93]. γ-Functionalised *α,β*-unsaturated nitriles were obtained as the products, both with high diastereoselectivity and enantioselectivity. Substitution takes place with retention (or double inversion) of configuration when the (*E*)-nitriles are formed. They are the major products of this transformation. The minor (*Z*)-nitriles are formed with inversion of stereochemistry (see Scheme **51**). The reactions are either palladium- or iridium-catalysed. Palladium is normally chosen, first of all because it is cheaper than iridium, but also because it gives better diastereo/enantio-selectivities and little racemisation. The selectivity of the reaction is explained by the fact that the affinity of palladium towards the nitrile group is favored, and gives the products with (*E*)-configuration.

Alkylation of allylic cyanohydrin-*O*-phosphates catalysed by organocuprates [94] is an alternative to the palladium-/iridium-catalysed reactions described above. One advantage with this approach is that hard nucleophiles such as Grignard reagents and organozinc reagents can be used, in contrast to the palladium-catalysed version, which only allows the use of soft nucleophiles. The results are the same though, the main products being those with (*E*) configurations (Scheme **51**). Regioselectivity in the reactions is determined by the properties of the starting material (the cyanohydrin), the leaving group and type of organocuprate used. The appli-



Scheme 49. Inversion of configuration of cyanohydrins *via* the Mitsunobu reaction.



Scheme 50. Allylic substitution of a cyanohydrin, a key step in the synthesis of Tamiflu.

cations for the substituted α,β-unsaturated nitriles are numerous. For example, they can be converted into their esters by acidic hydrolysis. Further reduction of the ester with $LiAlH_4$ gives the corresponding alcohol. The final product is the enantiomer of the sex pheromone of the yellow mealworm *Tenebrio molitor* L (see Scheme **51**).

4.7. Ring-Forming Reactions

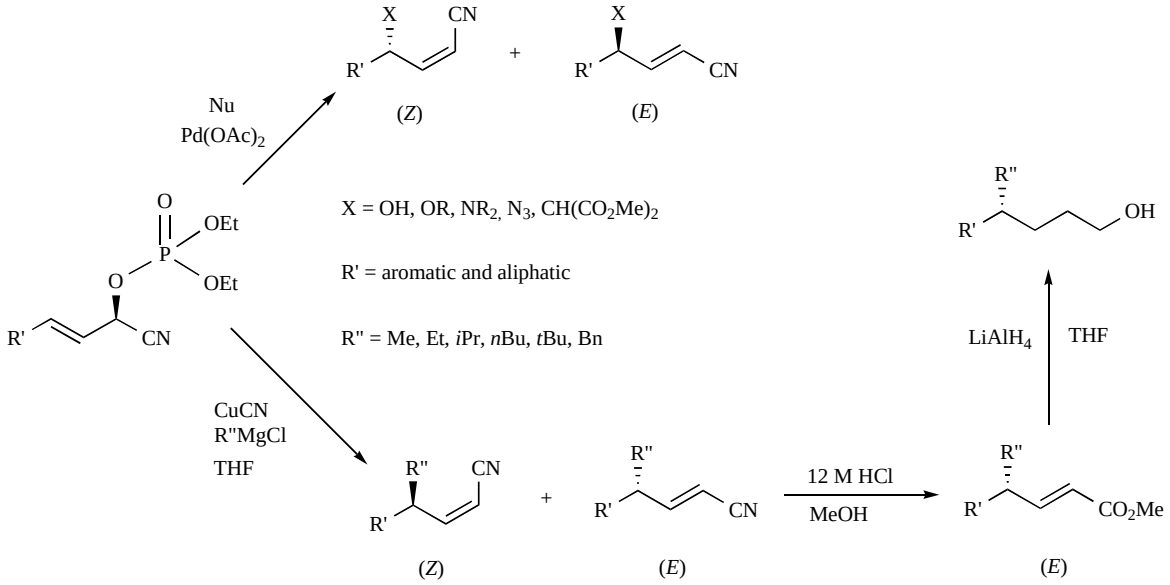
4.7.1. Ring-Closing Metathesis: Easy Access to Novel Ring Systems

Since its discovery, ring-closing metathesis with the Grubbs catalyst (which is now readily available), has been shown to be an extremely powerful method and has opened up endless possibilities towards novel ring syntheses. Cyanohydrins have been shown to be excellent substrates for the formation of a broad range of these ring systems, ranging from cyclic unsaturated 1,2-ethanolamines to unsaturated nitrogen-containing heterocycles.

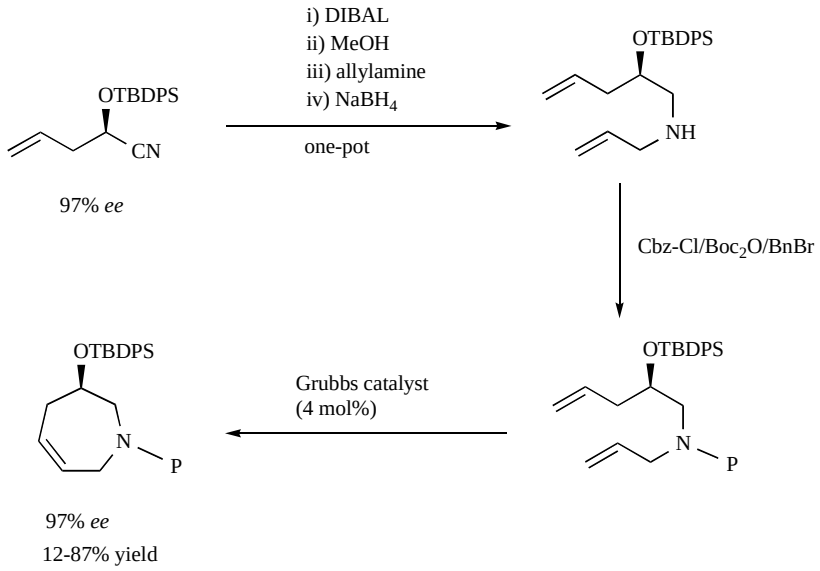
Tetrahydroazepinols were made from cyanohydrins using a combined reduction-ring-closing metathesis reaction sequence [95]. The starting point was γ,δ-unsaturated TBDPS-protected cyanohydrins (see also Scheme **14**). These compounds were converted into secondary amines through a DIBAL reduction-transimination (using allylamine)-$NaBH_4$ reduction sequence in a "one-pot" reaction in quantitative yield. Then, these secondary amines were protected using different protecting groups (Cbz, Boc and Bn respectively), before they were subjected to ring-closing metathesis using Grubbs catalyst (4 mol %). All substrates underwent ring-closure to give the heterocyclic product in good yields (38-88 %) (Scheme **52**) [95]. When starting with enantiopure cyanohydrins (97-99% *ee*), the enantiopurity was retained in the final products (99% *ee*).

5. CONCLUSION AND OUTLOOK

The application of enzymes for the enantioselective synthesis of cyanohydrins is now one hundred years old and still developing.



Scheme 51. Alkylation with organocuprates or palladium-catalysed substitution of allylic cyanohydrins.



Scheme 52. Formation of tetrahydroazepinols derivatives by ring-closing metathesis.

The formation of both enantiomers of the cyanohydrins can be catalysed by readily available enzymes. With the current development of biochemistry and genetics it can be expected that the number of enzymes at dispense to the organic chemist will continue to rise. Many of these enzymes can be used in two-phase systems or in pure organic solvents in a straightforward manner. The versatility of these bio-catalysts is reflected by their application in several industrial processes and in many organic syntheses.

The real challenge in the area of the enantioselective enzyme-catalysed cyanohydrin synthesis is the same that the transition metal catalysts face: The efficient, high yielding and highly enantioselective conversion of ketones. Here much has to be expected from the application of hydrolases in dynamic kinetic resolutions and of the application of HNL's in dry organic solvents, to enable *in situ* derivatisation.

The application of chiral cyanohydrins is relying too often on non-catalytic, rather wasteful reactions. It can be expected that the current trend towards catalytic conversions, such as the transition metal-catalysed hydrolysis and the Ni-catalysed hydrogenation of the nitrile group will be continued. Fundamentally the only limitation to the enantioselective preparation and conversion of cyanohydrins is the imagination of the synthetic organic chemist.

ACKNOWLEDGEMENT

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