Enzymatic Routes to Enantiomerically Pure Aromatic α-Hydroxy Carboxylic Acids: A Further Example for the Diversity of Biocatalysis

Harald Gröger*

Degussa AG, Project House Biotechnology, Rodenbacher Chaussee 4, 63457 Hanau-Wolfgang, Germany
Fax: (+49) 6181–59–2961, e-mail: harald.groeger@degussa.com

Received June 6, 2001; Accepted July 10, 2001

Abstract: An important class of compounds which can be produced by means of enzymatic routes are enantiomerically pure aromatic α-hydroxy carboxylic acids, in particular mandelic acid and derivatives thereof. Numerous different types of enantioselective biocatalytic approaches to these target molecules have been developed. Among them are chiral enzymatic resolution processes using racemic precursors as well as asymmetric catalytic methods starting from prochiral compounds. Regarding the resolution processes, nitrilase-catalyzed hydrolysis and dehydrocyanation of racemic cyanohydrins as well as stereocontrolled ester cleavage of *O*-acetylcyanohydrins and α-hydroxy carboxylic acid esters, respectively, have been developed. Main contributions in the field of asymmetric catalytic concepts are the asymmetric reduction of α-keto acids as well as the asymmetric hydrocyanation of aldehydes. In the latter case, a subsequent chemical hydrolysis gives the desired products. In the following, these different concepts for the biocatalytic preparation of aromatic α-hydroxy carboxylic acid are discussed showing that biocatalysis can provide the organic chemist with many versatile synthetic pathways to obtain chiral aromatic α-hydroxy carboxylic acids.

Keywords: asymmetric catalysis; asymmetric synthesis; carboxylic acids; chiral resolution; cyanohydrins

1 Introduction

Biocatalysts are not only known for their broad variety of reactions they are able to catalyze, but also for their ability to work well even on a large scale.[1] An important class of compounds which can be produced by means of enzymatic routes are aromatic α-hydroxy carboxylic acids (some selected compounds are shown in Figure 1).[2] Among them, enantiomerically pure mandelic acid and substituted derivatives thereof are regarded as the most important representatives from a commercial point of view. Numerous applications have been reported from the life science industry. For example, (*R*)-mandelic acid (*R*)-**1** is both a versatile intermediate for pharmaceuticals (e.g., semisynthetic β-lactam antibiotics) and a resolving agent in chiral resolution processes. The production scale of (*R*)-mandelic acid (*R*)-**1** is in the range of at least several hundred metric tons per year. Enantiomerically pure chloro- and *O*-substituted mandelic acid derivatives also play an important role in the synthesis of pharmaceuticals. Furthermore, aromatic

  1615-4150/01/34306–7-547–558 $ 17.50-.50/0

Harald Gröger, born in 1968 in Erlangen (Germany), studied Chemistry at the University of Erlangen-Nürnberg and the University of Oldenburg, where he received his Ph.D. under the direction of Professor J. Martens in 1997. From 1997 to 1998 he was a postdoctoral fellow in the group of Professor M. Shibasaki at the University of Tokyo working on new asymmetric catalytic syntheses. In 1998 Harald Gröger joined SKW Trostberg AG, and held an R&D position as a research scientist in the speciality and fine chemicals division until 2001. After the recent merger between SKW Trostberg AG and Degussa-Hüls AG in 2001, he became a project manager in the Project House Biotechnology of the new Degussa AG. Currently he is focusing on the development of asymmetric biocatalytic methods feasible on technical scale. Harald Gröger has authored 35 publications, 3 book contributions, and several patents and patent applications. His research interests include all aspects of asymmetric catalysis by means of biocatalysts and chiral synthetic catalysts, as well as the chemistry of heterocycles.

α-hydroxy carboxylic acids are versatile intermediates in the preparation of other type of chiral compounds, e.g., chiral diols.

Due to the importance of enantiomerically pure aromatic α-hydroxy carboxylic acids, it is not surprising that tremendous efforts have been made to establish enantioselective routes for their production, in particular with respect to (*R*)-mandelic acid. Highly efficient asymmetric methods to these compounds or chiral precursors thereof have been realized using metal-based complexes or organic catalysts, respectively. For examples, important contributions in this field have been made by Shibasaki et al.,[3a,3b,3c,3d,3e] Inoue et al.,[3f,3g] Carpentier et al.,[4a] Agbossou et al.,[4b] and many other groups.[5] In addition, the asymmetric formation of aromatic α-hydroxy carboxylic acids can be also realized by choosing a biocatalytic process as a synthetic key step. As mentioned above, also enzymes are highly valuable catalysts, and allow the manufacture of chiral chemicals on an industrial scale with high enantioselectivity, yield, volumetric productivity and little waste. Many enzymatic approaches to enantiomerically pure aromatic α-hydroxy carboxylic acid have been developed by several groups applying a broad range of concepts. These concepts are based on completely different

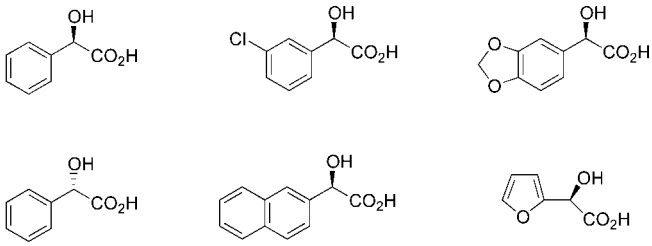


Figure 1. Selected examples of chiral aromatic α-hydroxy carboxylic acids.

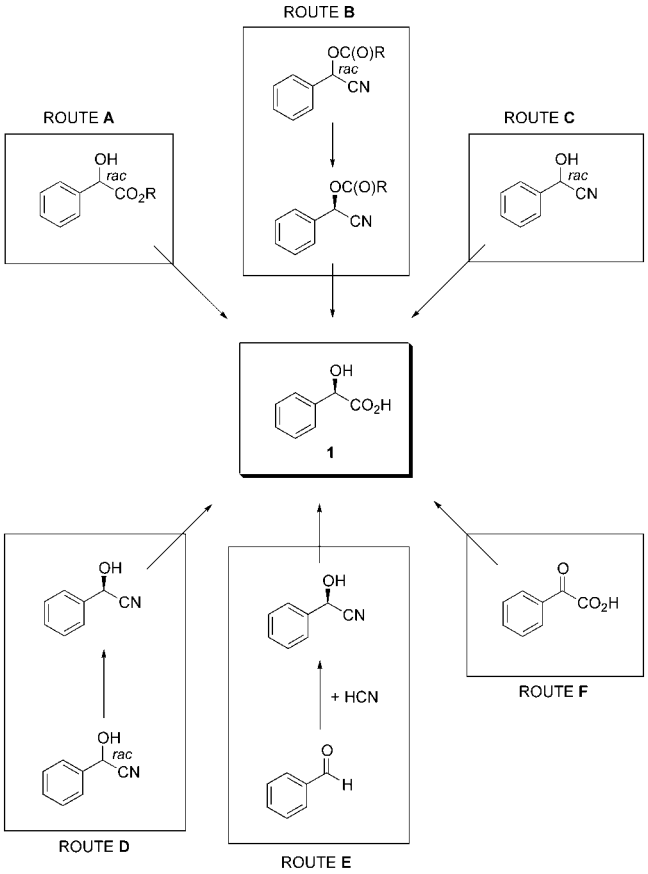
types of biotransformations covering chiral resolution processes as well as asymmetric syntheses starting from prochiral compounds. In the following these concepts for the biocatalytic preparation of aromatic α-hydroxy carboxylic acid are discussed showing that biocatalysis can provide the organic chemist with many synthetic tools to synthesize the target molecules. A main focus will be on the asymmetric manufacture of (*R*)-mandelic acid [(*R*)-**1**] and substituted derivatives thereof.

2 Overview: Biocatalytic Routes to Aromatic α-Hydroxy Carboxylic Acids

An enzymatic access to enantiomerically enriched aromatic α-hydroxy carboxylic acids can be realized by several reactions using different type of biotransformations as a key step. In Scheme 1 the basic principles of those synthetic routes are shown, exemplified by the preparation of (*R*)-mandelic acid (*R*)-**1**.

As a "typical enzymatic" way resolution processes are well-known. Starting from a racemic mandelic acid ester as a substrate a selective ester cleavage gives the desired free carboxylic acid (route **A**). Instead of a *rac*-mandelic acid ester also other types of racemic precursors, in particular *rac*-mandelonitrile or a derivative thereof, can function as a substrate (routes **B**, **C**, and **D**). Due to the bifunctionality of mandelonitrile, the hydroxy or cyano group can be modified enantioselectively during such a process. Thus, using racemic *O*-acylated substrates allows an enantioselective deacetylation under formation of the desired product (*R*)-**1** (route **B**). In addition, an access to the desired chiral mandelic acid can be realized by resolving the cyano group enantiospecifically (route **C**). The biocatalytic enantioselective degradation of *rac*-mandelonitrile represents a further pathway to the enantiomerically enriched cyanohydrin which can be easily converted to the corresponding mandelic acid (route **D**).

An interesting alternative to resolution processes are asymmetric conversions (routes **E** and **F**). Start-

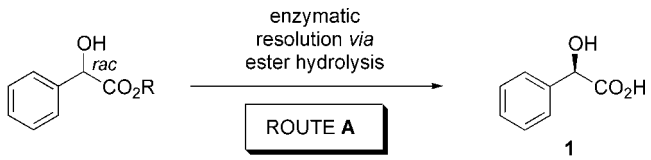


Scheme 1.

ing from a prochiral substrate theoretically quantitative conversions can be achieved. Such types of conversions are very economical and have also been applied to the synthesis of (*R*)-mandelic acid. As suitable prochiral starting materials aromatic aldehydes have been chosen in enzymatic hydrocyanation reactions (route **E**). The resulting (*R*)-mandelonitrile (derivatives) can be converted into the desired (*R*)-mandelic acid *via* hydrolysis in a subsequent step. A second possibility to design an enzymatic asymmetric access to (*R*)-mandelic acid is based on a biocatalytic reduction of arylglyoxylic acids (route F).

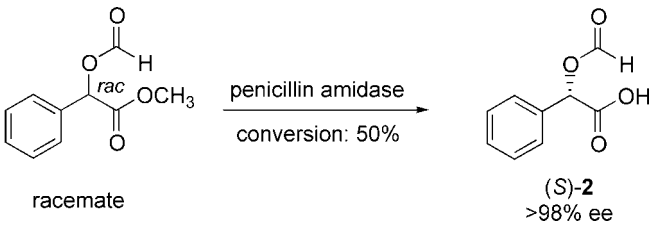
3 Route A: Enzymatic Hydrolysis of an Ester Group

The enzymatic hydrolysis of racemic aromatic α-hydroxy esters according to route **A** has been proven to represent a simple and practical approach to the desired target molecules **1** (see Scheme 1, route **A**, and Equation 1). Starting from racemic mandelic acid esters as a substrate an enantioselective hydrolysis furnishes the enantiomerically enriched carboxylic acids.



(1)

The penicillin amidase was found to act as a versatile biocatalyst for such a resolution of *O*-protected mandelic acid esters.[6] For example, a complete resolution of formylated mandelic acid methyl ester (with 50% conversion) led to the corresponding (*S*)-carboxylic acid (*S*)-**2** with an enantioselectivity of >98% ee (Scheme 2).

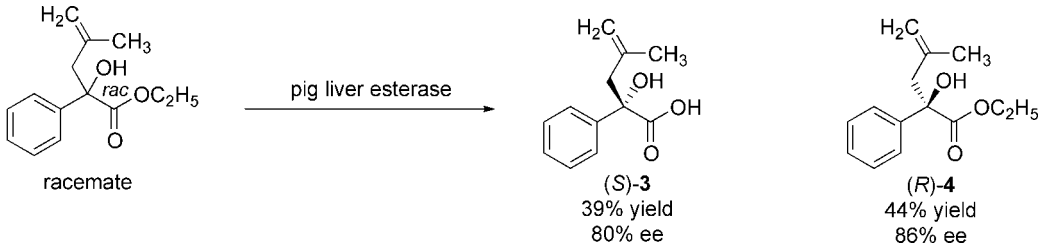


Scheme 2.

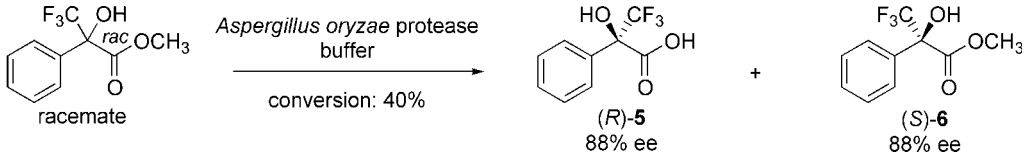
Wong et al. reported a resolution of *rac*-mandelic acid methyl ester in the presence of a "free", non-protected hydroxy group.[7] The desired (*R*)-mandelic acid can be produced directly from the methyl ester, however with moderate enantioselectivity (40% ee). Nevertheless, in principle such a process offers the advantage of less reaction steps since acylation of racemic mandelic acid as well as deacylation of the enantiomerically enriched (*R*)-mandelic acid do not have to be carried out.

Interestingly, even carboxylic acid esters with a quaternary stereogenic carbon center are suitable substrates for an enzymatic hydrolytic resolution. It is noteworthy that a protection of the hydroxy group is not essential for this reaction, too. When a pig liver esterase is applied as a biocatalyst the (*S*)-carboxylic acid was isolated in 39% yield and with 80% ee, and the non-converted (*R*)-ester could be recovered in 44% yield (86% ee; Scheme 3).[8] Enantiomerically pure (*S*)-carboxylic acid (*S*)-**3** could be obtained after one crystallization.[9]

The resolution of bulky esters by proteases has been also reported by Griengl, Faber and coworkers. In the presence of *Aspergillus oryzae* protease as a biocatalyst, the racemic α-trifluoromethyl mandelic ester was converted into the corresponding (*R*)-carboxylic acid with an enantioselectivity of 88% ee (conversion: 40%; Scheme 4).[10] A single recrystallization led to enantiomerically pure material. This carboxylic acid (*R*)-**5** is a precursor of Mosher's acid which represents a powerful chiral auxiliary for the



Scheme 3.

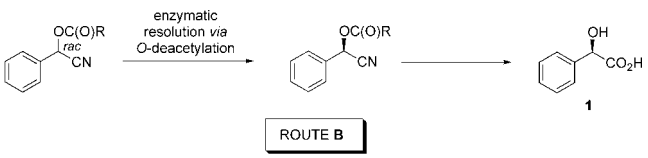


Scheme 4.

determination of the optical purity of alcohols and amines, respectively. The protease derived from *Aspergillus oryzae* has been shown to be useful for the resolution of several other types of sterically hindered trisubstituted carboxylates as a substrate, too.[11] In contrast, "traditional" proteases such as subtilisin gave non-satisfactory results.

4 Route B: Biocatalytic Transesterification – Enzymatic Hydrolysis of the *O*-Acetyl Group

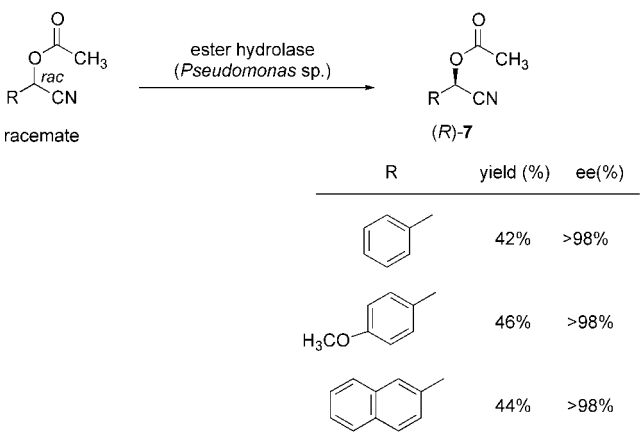
An interesting intermediate for aromatic carboxylic acids is given by a chiral cyanohydrin since it can be easily converted into the corresponding carboxylic acid without loss of optical purity. Since the cyanohydrins can be regarded as an alcohol bearing a cyano group in the α-position, it is not surprising that also *O*-acetylated derivatives were used in enzymatic hydrolytic resolution processes. This process represents the key step in route **B** (see Scheme 1 and Equation 2). In general, the enzymatic hydrolysis of acetylated alcohols is a well-known practical protocol for the preparation of enantiomerically enriched and "free" alcohols and *O*-acetylated derivatives thereof.[12]



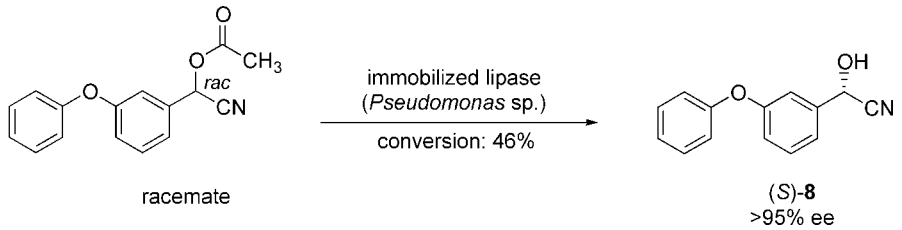
(2)

In the presence of an ester hydrolase (*Pseudomonas* sp.) one enantiomer of a racemic cyanohydrin acetate can be hydrolyzed selectively.[13] As shown in Scheme 5, the optically active acetates **7** have been obtained in good chemical yields (usually >40%) and with high enantioselectivities (>98% ee) for the majority of substrates. The resulting free, non-acylated cyanohydrins, however, show a low ee due to racemization. It has been shown that this reaction can be carried out with a broad range of substituted mandelonitriles as starting material. A comparable reaction using butanoates (instead of acetates) has also been reported with enantioselectivities of up to 97% ee.[14]

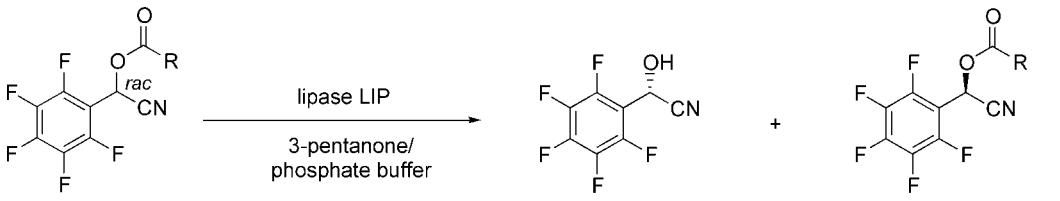
A protocol developed by Mitsuda et al. describes the hydrolysis of cyanohydrin esters using *Arthrobacter* sp. lipase.[15,16] Excellent enantioselectivities in the range of >99% ee have been obtained in the synthesis of (*S*)-α-cyano-3-phenoxybenzyl alcohol. The suitability of this reaction as a highly economical method for the manufacture of (*S*)-α-cyano-3-phenoxybenzyl alcohol from a technical point of view has been demonstrated by Fishman et al. recently.[17] It is noteworthy that the reaction can be carried out when operating at a high substrate concentration of up to 20% (w/w). In addition, an interesting continuous process with an immobilized lipase has been already developed on the gram scale. Furthermore, nearly full conversion, a high enantioselectivity of >96% ee, and the



Scheme 5.



Scheme 6.



R	Yield (%)	ee (%)	Yield (%)	ee (%)
CH_3	38	97	46	92
C_2H_5	32	89	43	96

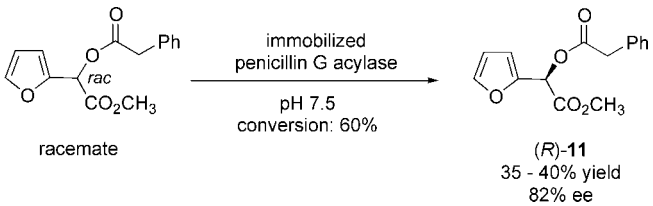
Scheme 7.

racemization of the undesired enantiomer were reported. The authors applied this efficient process as a key step in a chemoenzymatic process for the synthesis of (*S*)-α-cyano-3-phenoxybenzyl alcohol [(*S*)-**8**] (Scheme 6) which is an important intermediate for the production of pyrethroids.

An extension of the enzymatic transesterification concept towards the synthesis of perfluorophenyl-containing cyanohydrin products has been reported by Sakai and coworkers.[18] These types of fluorinated compounds are of interest since the perfluorophenyl group shows some unique features. The lipase-catalyzed transesterification of the racemic *O*-acylated cyanohydrin led to the formation of both products, (*S*)-**9** and (*R*)-**10**, in good yields and high enantioselectivities (Scheme 7).

The application of the enzymatic *O*-acetyl cleavage to the synthesis of chiral 2-furylcarbinols has been reported by Waldmann.[19] Use of Pen G acylase (immobilized on Eupergit C) as an enzyme led to the unhydrolyzed esters **11** in good yields of 35 – 40%. As shown in Scheme 8 the reaction proceeds with an enantioselectivity of 82% ee.

It is noteworthy that also the reverse reaction can be carried out in an enantioselective manner. When using lipases in an organic solvent the enantioselective ester formation led to the formation of enantiomerically enriched *O*-acylated cyanohydrins (up to 98% ee).[14,20,21] Interestingly, also a dynamic kinetic resolution has been developed which quantitatively converts a racemic cyanohydrin in one enantiomer.[22,23,24] The *O*-acylated compounds can be deacylated again subsequently without loss of enantio-



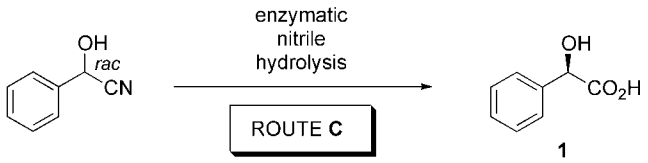
Scheme 8.

selectivity. The selective esterification has been also applied to the synthesis of perfluorophenyl-containing products. This reaction, which represents a synthetic alternative to the corresponding reverse enzymatic deacylation,[25] was carried out with vinyl acetate and led to a quantitative conversion (50%). In the presence of the lipase LIP as a biocatalyst a high enantioselectivity of 98% ee and 96% ee was obtained for both types of products (acylated and non-acylated).

5 Route C: Enzymatic Hydrolysis of the Cyano Group

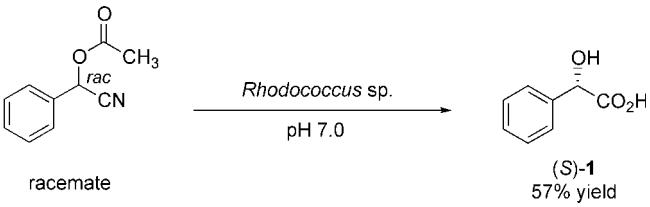
An enantioselective hydrolysis of the cyano group of a racemic cyanohydrin represents another attractive method for the preparation of chiral aromatic α-hydroxy carboxylic acids. This reaction can be also carried out efficiently using enzymes (see Scheme 1, route C, and Equation 3). This concept has the advantage that the desired aromatic carboxylic acid is directly formed in one step starting from a racemic compound, namely the racemic cyanohydrin. Thus,

the asymmetric key step represents the final reaction step, and a further derivatization of the chiral product is not required.



(3)

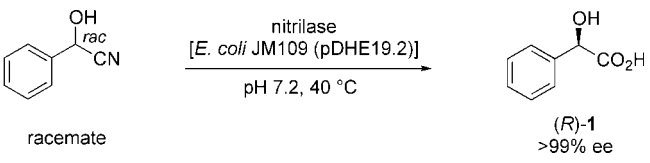
Choosing an *O*-acylated mandelonitrile as a substrate, a stereocontrolled biotransformation with *Rhodococcus* sp. AJ270 at pH 7.0 gave enantiomerically enriched (*S*)-mandelic acid [(*S*)-**1**] directly in 57% yield (Scheme 9).[26]



Scheme 9.

Recently it has been shown that the hydroxy group does not have to be protected as an *O*-acetyl group. Thus, a non-modified "free" mandelonitrile can be used directly as a substrate which makes this reaction more economical. A broad range of aromatic α-hydroxy carboxylic acid esters can be synthesized in the presence of microorganisms.[27,28]

The enzymatic, stereoselective conversion of racemic nitriles into carboxylic acids has been recently extended by BASF to an industrial resolution process for the manufacture of (*R*)-mandelic acid (Scheme 10).[29] A nitrilase [*E. coli* JM109 (pDHE 19.2)] efficiently catalyzes the hydrolysis of the (*R*)-enantiomer with high conversion and excellent enantioselectivities of up to >99% ee. The undesired (*S*)-cyanohydrin can be converted into the racemate during the reaction process. With this nitrilase-based technology in hand, the production of (*R*)-mandelic acid is carried out on a multi-ton scale at BASF.

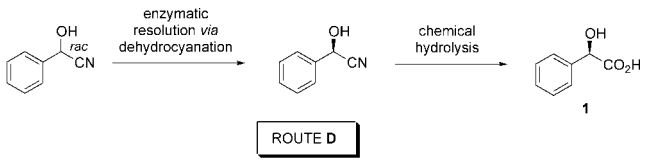


Scheme 10.

The hydrolysis of nitriles for the stereoselective manufacture of fine chemicals has also been reported by Matcham and coworkers.[30]

6 Route D: Enzymatic Cleavage of *rac*-Cyanohydrins

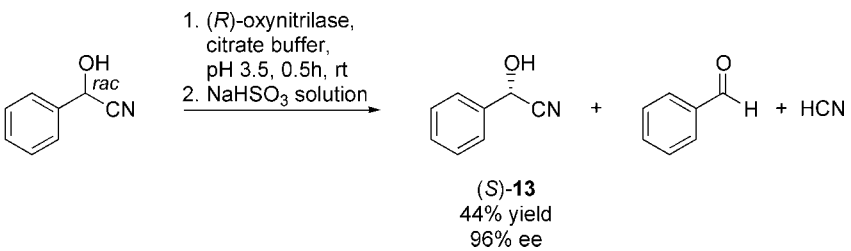
The cleavage of a racemic cyanohydrin under formation of the corresponding aldehyde and HCN can also proceed under stereocontrol when using oxynitrilases, since these enzymes are not only able to catalyze the formation of cyanohydrins (see route **E**) but also the reverse cleavage reaction. Combining this type of kinetic resolution with a subsequent hydrolysis of the chiral cyanohydrin results in a further type of (two-step) formation of α-hydroxy carboxylic acids (see Scheme 1, route **D**, and Equation 4).



(4)

A variety of oxynitrilases has been used for the resolution of aromatic cyanohydrins.[31,32] Since the cyanohydrin cleavage reaction is reversible, the removal of the reaction by-products (HCN and aldehyde) is desirable to obtain good yields. Thus, several methodologies for the removal of these by-products have been developed.[32,33]

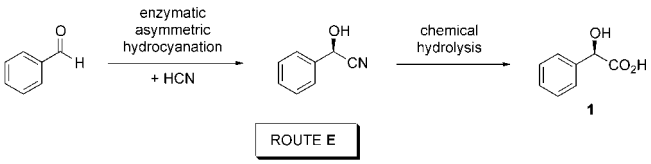
A remarkable contribution was reported by the Effenberger group.[34] Capturing the formed aldehyde with hydrogen sulfite led to an efficient (*R*)-oxynitrilase-based resolution process which gave (*S*)-mandelonitrile [(*S*)-**13**] in 44% yield and with 96% ee (Scheme 11).



Scheme 11.

7 Route E: Enantioselective Biocatalytic Hydrocyanation of Aromatic Aldehydes

The probably most popular enantioselective biocatalytic synthetic route to aromatic α-hydroxy carboxylic acids is based on a two-step process starting from an aldehyde as a substrate. At first, an enzymatic asymmetric hydrocyanation of the aldehyde gives the required chiral cyanohydrin. This biocatalytic step represents the key step. The enantiomerically enriched cyanohydrins are subsequently converted into the α-hydroxy carboxylic acids *via* hydrolysis (see Scheme 1, route **E**, and Equation 5).



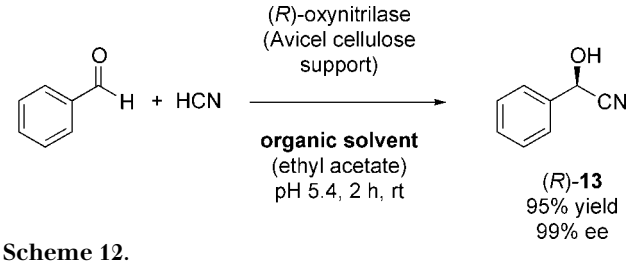
(5)

Numerous contributions on the field of enantioselective biocatalytic hydrocyanation of aromatic aldehydes have been published, and are already reviewed by several authors. These reviews about the asymmetric synthesis of cyanohydrins as well as synthetic applications thereof give a detailed overview about the state of the art and the contributions in this field.[35,36,37] Thus, in the following section, the main focus is on a brief summary of the pioneering work as well as recently reported improvements with respect to large scale applications.

Interestingly, the biocatalytic hydrocyanation of an aromatic aldehyde, namely benzaldehyde, under formation of enantiomerically pure mandelonitrile belongs to one of the first revealed enantioselective syntheses. This reaction was discovered by Rosenthaler in 1908.[38] For a long time the synthetic applicability was limited due to low enantioselectivities caused by the undesired formation of the racemic cyanohydrins.[39] Intensive investigation of the synthetic potential of the enzymatic hydrocyanation started at the end of the 1980's. Two main findings about how to suppress the formation of the racemic product were connected with a remarkable increase of the enantioselectivity (see below).

7.1 Enzymatic Enantioselective Hydrocyanation in Organic Media

In 1987 Effenberger et al. reported that carrying out the asymmetric oxynitrilase-catalyzed hydrocyanation in an organic system at pH 5.4 led to the desired cyanohydrins in high yields and with excellent enantioselectivities of up to >99% ee (Scheme 12).[40] This was the first example of a highly enantioselective hydrocyanation reaction using oxynitrilases as a biocatalyst.



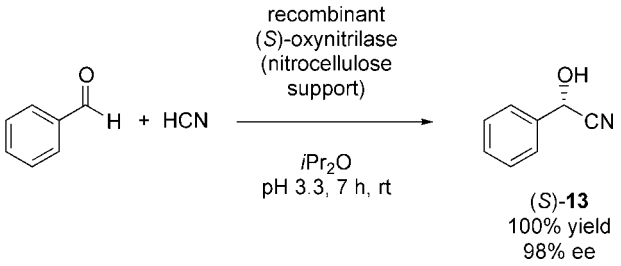
Scheme 12.

Under these reaction conditions the undesired chemical and non-stereoselective addition of HCN to the aldehyde giving racemic product is suppressed. The cyanohydrin formation in organic media offers several advantages: The reaction can be carried out at pH 5.4 which represents the pH optimum of the enzyme's activity. In addition, high enantioselectivities are obtained and many substrates are tolerated due to their solubility in the organic phase.

The first experiments reported by Effenberger et al. were carried out in ethyl acetate,[40] but other types of organic solvents have also been used.[41] Further investigation showed that the efficiency of the reaction can be improved when diisopropyl ether functions as a solvent.[42,43] Not only have high enantioselectivities been obtained but the activity of the enzyme remained unchanged for several weeks. It appeared that the low water content of diisopropyl ether has a beneficial effect.

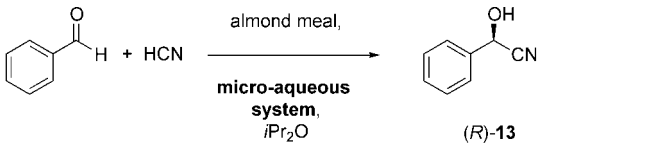
For the reactions carried out in organic solvents immobilization has proven to be also advantageous. As a biocatalyst, an (*R*)-oxynitrilase immobilized on a Avicel-cellulose support has been applied.[40] It is noteworthy that immobilizing the enzyme on a crystalline cellulose support like Avicel not only gives high enantioselectivities but also makes it possible to retain the enzyme activity for at least 80 hours. Thus, a continuous process can be carried out efficiently in an enzyme membrane reactor (EMR).[42]

The solid support nitrocellulose was found by Effenberger et al. as a preferred immobilizate when using the first recombinant (*S*)-oxynitrilase as an enzyme.[44] In the presence of this immobilized biocatalyst, a highly efficient process was realized which works well with a broad variety of aldehydes. A representative example is given in Scheme 13. In contrast to cellulose which led only to moderate enantioselectivities in organic solvents, nitrocellulose-bound (*S*)-oxynitrilase gave high enantioselectivities in most cases. Furthermore, a broad variety of other types of solid supports has been proven to work well. Among them are several cellulose-based supports,[45] silica gel,[46] Eupergit C,[45] and Celite,[43,47] as well as liquid crystals.[48]



Scheme 13.

It has been additionally shown that not only isolated enzymes but also almond meal itself can be used as a biocatalyst in organic media. Numerous groups contributed to this method, and excellent results have been obtained with respect to enantioselectivity and yield.[49,50] Recently Lin et al. developed a remarkable extension of this method by means of a so-called "micro-aqueous" organic reaction system.[51,52] The organic phase serves as a big reservoir of substrate and product, and the enzyme meal retains the essential (small) amount of water under these conditions. Using diisopropyl ether as a solvent, the water content is in the range of 0.14 – 0.32% (v/v). This "micro-aqueous" system has been shown to perform the hydrocyanation reaction efficiently, and high enantioselectivities and yields have been obtained even at a reaction temperature of 30 °C (Scheme 14).

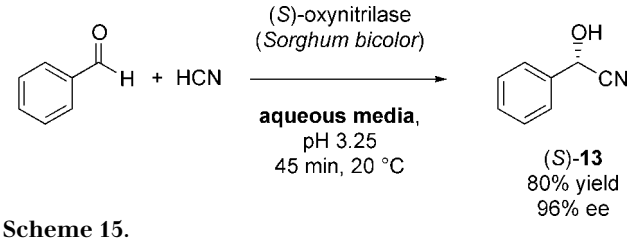


T [°C]	Yield [%]	ee [%]
4	100	99
30	98	97

Scheme 14.

7.2 Enzymatic Enantioselective Hydrocyanation in Aqueous Media

A second way to prevent the undesired non-asymmetric side reaction under formation of racemic products was found by Kula et al. when applying an (*S*)-oxynitrilase in an aqueous solution at a low pH of <4.[53] Under these reaction conditions a highly enantioselective cyanohydrin formation with ee values of up to >99% has been observed (Scheme 15). The non-stereochemical addition of HCN to the C=O bond does not occur under these reaction conditions. This reaction has been applied successfully to the synthesis of (*S*)-cyanohydrins using (*S*)-oxynitrilases.

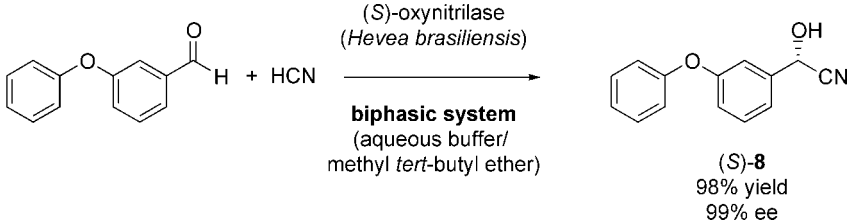


Scheme 15.

This reaction works very well when using water-soluble aldehydes as a substrate. From a technical point of view it is an advantage of this procedure that the use of highly toxic, pure HCN can be avoided by using an aqueous solution thereof [however, aqueous HCN solutions are still very toxic and must be handled with extreme caution; safety instructions are given in the material safety data sheet (MSDS) of HCN. For further safety information about HCN, see the international chemical safety card of HCN (ICSC0492)]. Due to its efficiency the reaction can also be carried out as a continuous process in the enzyme membrane reactor (EMR) as reported by Kula and Wandrey and coworkers.[54] A high space-time yield of 2.4 kg/(L·d) and a conversion rate of 90% was obtained for the hydrocyanation using (*R*)-oxynitrilases.

7.3 Enzymatic Enantioselective Hydrocyanation in Biphasic Solvent Systems

For industrial applications, biphasic solvent systems consisting of an aqueous phase and a water-immiscible phase are often advantageous. The chemistry of (*S*)-oxynitrilase-catalyzed hydrocyanation in such a solvent system has been intensively investigated by Griengl et al., in particular with respect to the synthesis of (*S*)-*m*-phenoxybenzaldehyde cyanohydrin which represents a commercially required intermediate for the manufacture of pyrethroids.[55,56,57,58,59,60] For example, as a biocatalyst an enzyme derived from the plant *Hevea brasiliensis* which has been cloned and overexpressed in a microbial host organism was used. In the presence of this biocatalyst the desired products were obtained with high enantioselectivities (Scheme 16).[58]



Scheme 16.

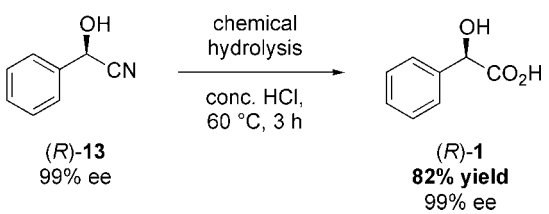
In the meantime this oxynitrilase-catalyzed hydrocyanation has been already extended to a commercial process. For the production of (*S*)-*m*-phenoxybenzaldehyde cyanohydrin DSM established an enzymatic hydrocyanation process on an industrial scale based on the above-mentioned efficient protocol by the Griengl group.

The biphasic solvent system has also been shown to represent a suitable media for the enzymatic manufacture of (*R*)-mandelonitrile on an industrial scale. Loos et al. reported a detailed optimization study of the asymmetric hydrocyanation of benzaldehyde in the presence of (*R*)-oxynitrilases. A commercially highly feasible method based on a two-phase system was developed with an impressive space-time-yield of 2.1 mol/(L·h).[61] In addition, a high chemical yield (98%) and an excellent enantioselectivity of 98% ee was obtained. As a two-phase system, a mixture consisting of methyl *tert*-butyl ether and an aqueous buffer was used. Interestingly, the aqueous oxynitrilase-containing layer can be re-used for (at least) 5 times without loss of efficiency of the reaction.

The advantages of immobilization have been also recognized for the biotransformation in a biphasic solvent system. Very recently, an alternative cheap method has been developed for the immobilization of oxynitrilases by Vorlop, Capan and Gröger et al., namely the two-step-preparation of cross-linked and polyvinyl alcohol-entrapped (*R*)-oxynitrilases.[62] In a first step, a cross-linking process was carried out, and subsequently this cross-linked enzyme was entrapped in a polyvinyl alcohol-based hydrogel matrix. In this step a lens-shaped catalyst with a well-defined particle diameter in the mm range is produced. These lens-shaped hydrogels are elastic and flexible towards mechanical treatment, show no catalyst leaching, and can be recycled efficiently. Applying the entrapped (*R*)-oxynitrilases in a biphasic solvent system gave similarly good results as compared with the free enzymes.

7.4 Chemical Hydrolysis of Enantiomerically Enriched Cyanohydrins

Based on the enantiomerically pure cyanohydrins, Effenberger et al. developed efficient strategies for the subsequent hydrolytic steps (Scheme 17).[63,64] Hydrolyzing the chiral cyanohydrin in concentrated hydrochloric acid at 60 °C for 3 h led to the desired α-hydroxy carboxylic acid in high yields. It is noteworthy that this step proceeds without racemization.

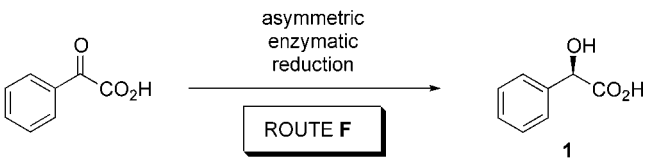


Scheme 17.

In conclusion, the asymmetric hydrocyanation of aldehydes represents a highly efficient biocatalytic process, not only due to the broad variety of contributions by numerous groups. At present – as mentioned by Effenberger – the "preparation of (*R*)- and (*S*)-2-hydroxycarboxylic acids, respectively, *via* hydrolysis of the readily accessible chiral cyanohydrins is currently the most general approach to this important class of compounds".[65]

8 Route F: Enantioselective Reduction of α-Keto Acids and Esters Thereof

The biotransformation of an α-keto ester into the corresponding α-hydroxy derivatives can also represent the key step in the synthesis of aromatic α-hydroxy carboxylic acid derivatives (see Scheme 1, route F, and Equation 6).

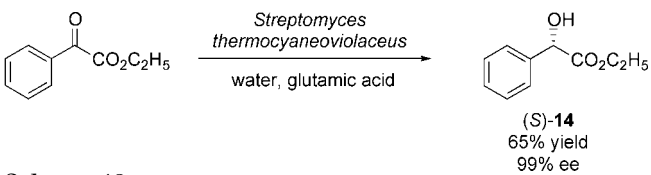


(6)

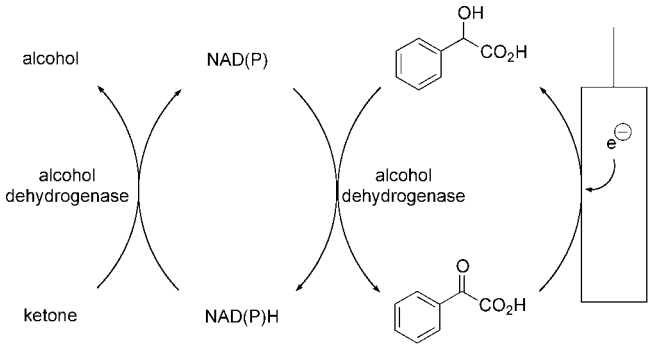
In the presence of baker's yeast (*R*)-mandelate was obtained from an α-keto phenylacetic ester by a fermentation process. The yields have been high and good enantioselectivities of up to 100% ee have been reported.[66] Another approach represents the application of cheap and easy available baker's yeast as a biocatalyst using an α-keto acid ethyl ester as starting material.[67] The baker's yeast has been immobilized on chrysoltile. The yield was in a moderate range of 50%.

For the same reaction immobilized carrot cells as well as *Streptomyces thermocyaneoviolaceus*, respectively, have been proven to be suitable biocatalysts (with yields of 35 and 65%, respectively, for the ethyl ester (*S*)-**14**; see Scheme 18).[68,69]

It is noteworthy that also "free" carboxylic acids can serve as a substrate when using an alcohol dehydrogenase (ADH) as a biocatalyst in a combined elec-



Scheme 18.



Scheme 19.

trochemical and biocatalytic process.[70] This concept is based on a combination of an electrochemical reduction of a ketone with an enantioselective enzymatic oxidation of the formed (racemic) alcohol using ADH. The principle of this process is shown in Scheme 19. The electrochemical reduction furnishes racemic alcohols from ketones whereas the ADH selectively catalyzes the oxidation of one enantiomer. In addition, the ADH is needed for the coenzyme regeneration.

This process was carried out in a *t*-BuOH/water mixture as a solvent and has been shown to work well for many alcohol derivatives. The chemical reduction of benzoylformic acid as a substrate, however, gave only racemic mandelic acid with ADH and NADPH, suggesting that it is difficult for ADH (from *T. brockii*) to recognize the difference in the sterical sizes between phenyl and carboxylic acid groups. Nevertheless, this process is interesting since it allows – in principle – the quantitative conversion of a racemate (*via* its corresponding ketone) into an enantiomerically pure form.

An alternative efficient enantioselective synthesis of (*R*)-mandelic acid *via* enzymatic reduction of the corresponding α-keto acid has been developed by Hummel and Kula et al. in 1988.[71] As a biocatalyst, an (*R*)-mandelic acid dehydrogenase from *Lactobacillus curvatus* was found. Based on this enzyme, a breakthrough in the field of an industrially feasible asymmetric reduction process by means of such a dehydrogenases-route has been disclosed by Wandrey, Hummel, Kula and coworkers.[72]

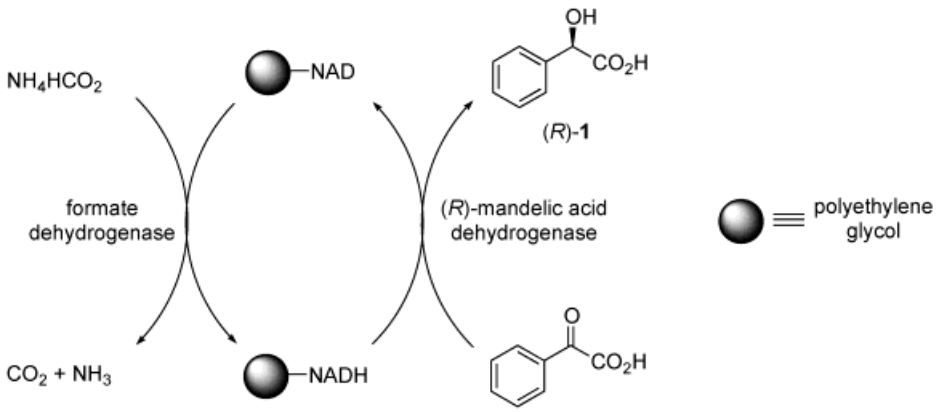
A continuous conversion of phenylglyoxylic acid to (*R*)-mandelic acid was performed in an enzyme membrane reactor (EMR) with simultaneous co-factor regeneration. A mandelate dehydrogenase and a formate dehydrogenase have been used as biocatalysts. This two-enzyme process allowed the production of (*R*)-mandelic acid with high space-time yields of 700 g (L·d) at a low enzyme consumption. The concept of this continuous (*R*)-mandelic acid production is shown in Scheme 20. Without doubt, this type of biotransformation represents a highly efficient access to enantiomerically pure mandelic acid, and is suitable for applications on a large scale.

9 Summary and Outlook

A broad variety of different biocatalytic concepts towards the synthesis of chiral α-hydroxy carboxylic acids has been developed. Among them are chiral enzymatic resolution processes as well as asymmetric catalytic syntheses. For several of those concepts large-scale operations have been established indicating the diversity of biocatalysis and the broad variety of highly efficient biocatalysts.

Acknowledgements

The author would like to thank Prof. Dr. Masakatsu Shibasaki, Ass.-Prof. Dr. Motomu Kanai, Ass.-Prof. Yoshitaka Hamashima, Prof. Dr. Klaus-Dieter Vorlop, Dipl.-Chem. Emine Capan, Dr. Björn Antelmann, Dr. Benedikt Hammer, Dr. Sylvia Huber, Dr. Jürgen Sans, Dr. Stefan Weiss, Dr. Klaus Griesar, Dr. Jürgen Roos, Dr. Andreas Karau, Dr. Oliver May, Dr. Stefan Verseck and Prof. Dr. Karlheinz Drauz for many intensive, fruitful and stimulating discussions on asymmetric synthetic concepts for the synthesis of α-hydroxy carboxylic acids and chiral precursors thereof. In addition, thanks are due to Dr. Michael Schwarm for many helpful comments and suggestions during preparation of the manuscript.



Scheme 20.

References and Notes

[1] For a comprehensive overview about biotransformations in general, see: (a) H.-J. Rehm, G. Reed, A. Pühler, P. Stadler, D. R. Kelly, *Biotechnology: Biotranformations I and II*, Vols. 8a and 8b, 2nd Edn., Wiley-VCH, Weinheim, **1998**; (b) K. Faber, *Biotransformations in Organic Chemistry*, Springer, Berlin, **1997**; (c) A. Liese, K. Seelbach, C. Wandrey, *Industrial Biotransformations*, Wiley-VCH, Weinheim, **2000**.

[2] For a comprehensive overview about the chemistry of chiral α-hydroxy carboxylic acids in general, see: G. Coppola, H. Schuster, α-*Hydroxy Acids in Enantioselective Synthesis*, Wiley-VCH, Weinheim, **1997**.

[3] For the asymmetric synthesis of chiral cyanohydrins (as precursors) *via* metal-catalysts and organocatalysts, see: (a) Y. Hamashima, D. Sawada, M. Kanai, M. Shibasaki, *J. Am. Chem. Soc.* **1999**, *121*, 2641; (b) D. Sawada, M. Shibasaki, *Angew. Chem.* **2000**, *112*, 215; *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 209; (c) M. Kanai, Y. Hamashima, M. Shibasaki, *Tetrahedron Lett.* **2000**, *41*, 2405; (d) Y. Hamashima, M. Kanai, M. Shibasaki, *J. Am. Chem. Soc.* **2000**, *122*, 7412; (e) Y. Hamashima, M. Kanai, M Shibasaki, *Tetrahedron Lett.* **2001**, *42*, 691; (f) K. Tanaka, A. Mori, S. Inoue, *J. Org. Chem.* **1990**, *55*, 181; (g) A. Mori, H. Nitta, M. Kudo, S. Inoue, *Tetrahedron Lett.* **1991**, *32*, 4333.

[4] An asymmetric approach to aromatic α-hydroxy carboxylic acid derivatives *via* metal-based asymmetric catalytic hydrogenation of keto acids has been developed by: (a) J.-F. Carpentier, A. Mortreux, *Tetrahedron: Asymmetry* **1997**, *8*, 1083; (b) C. Pasquier, S. Naili, L. Pelinski, J. Brocard, A. Mortreux, F. Agbossou, *Tetrahedron: Asymmetry* **1998**, *9*, 193.

[5] Several metal-catalytic asymmetric routes for the synthesis of aromatic α-hydroxy carboxylic acids or chiral precursors thereof are discussed in: (a) E. N. Jacobsen, A. Pfaltz, H. Yamamoto (Eds.), *Comprehensive Asymmetric Catalysis I-III*, Springer, Berlin, **1999**; (b) I. Ojima (Ed.), *Catalytic Asymmetric Synthesis*, 2. Ed., Wiley-VCH, New York, **2000**.

[6] C. Fuganti, C. M. Rosell, S. Servi, A. Tagliani, M. Terreni, *Tetrahedron: Asymmetry* **1992**, *3*, 383.

[7] C. H. Wong, S.-T. Chen, W. J. Hennen, J. A. Bibbs, Y.-F. Wang, J. L.-C. Liu, M. W. Pantoliano, M. Whitlow, P. N. Bryan, *J. Am. Chem. Soc.* **1990**, *112*, 945.

[8] H. Moorlag, R. M. Kellogg, *Tetrahedron: Asymmetry* **1991**, *2*, 705.

[9] H. Moorlag, R. M. Kellogg, M. Kloosterman, B. Kaptein, J. Kamphuis, H. E. Schoemaker, *J. Org. Chem.* **1990**, *55*, 5878.

[10] C. Feichter, K. Faber, H. Griengl, *J. Chem. Soc., Perkin Trans. I* **1991**, 653.

[11] B. Berger, A. de Raadt, H. Griengl, W. Hayden, P. Hechtberger, N. Klempier, K. Faber, *Pure Appl. Chem.* **1992**, *64*, 1085.

[12] For an general overview, see ref.[1b], p. 59ff.

[13] A. van Almsick, J. Buddrus, P. Hönicke-Schmidt, K. Laumen, M. P. Schneider, *J. Chem. Soc., Chem. Commun.* **1989**, 1391.

[14] F. Effenberger, B. Gutterer, T. Ziegler, E. Eckhardt, R. Aichholz, *Liebigs Ann. Chem.* **1991**, 47.

[15] S. Mitsuda, S. Nabeshima, H. Hirohara, *Appl. Microbiol. Biotechnol.* **1989**, *31*, 334.

[16] S. Mitsuda, H. Yamamoto, T. Umemura, H. Hirohara, S. Nabeshima, *Agric. Biol. Chem.* **1990**, *54*, 2907.

[17] A. Fishman, M. Zviely, *Tetrahedron: Asymmetry* **1998**, *9*, 107.

[18] T. Sakai, Y. Miki, M. Nakatani, T. Ema, K. Uneyama, M. Utaka, *Tetrahedron Lett.* **1998**, *39*, 5233.

[19] H. Waldmann, *Tetrahedron Lett.* **1989**, *30*, 3057.

[20] Y. F. Wang, S. T. Chen, K. K. C. Liu, C. H. Wong, *Tetrahedron Lett.* **1989**, *30*, 1917.

[21] S. H. Hsu, S. S. Wu, Y. F. Wang, C. H. Wong, *Tetrahedron Lett.* **1990**, *31*, 6403.

[22] M. Inagaki, J. Hiratake, T. Nishioka, J. Oda, *J. Am. Chem. Soc.* **1991**, *113*, 9360.

[23] M. Inagaki, A. Hatanaka, M. Mimura, J. Hiratake, T. Nishioka, J. Oda, *Bull. Chem. Soc. Jpn.* **1992**, *65*, 111.

[24] M. Inagaki, J. Hiratake, T. Nishioka, J. Oda, *J. Org. Chem.* **1992**, *57*, 5643.

[25] T. Sakai, Y. Miki, M. Tsuboi, H. Takeuchi, T. Ema, K. Uneyama, M. Utaka, *J. Org. Chem.* **2000**, *65*, 2740.

[26] M.-X. Wang, G. Lu, G.-J. Ji, Z.-T. Huang, O. Meth-Cohn, J. Colby, *Tetrahedron: Asymmetry* **2000**, *11*, 1123.

[27] Y. Hashimoto (Nitto Chemical Industry), *EP* 666320, **1995**.

[28] K. Tamura (Nitto Chemical Industry), *EP* 711836, **1996**.

[29] M. Ress-Löschke, T. Friedrich, B. Hauer, R. Mattes, D. Engels (BASF), *WO* 0023577, **2000**.

[30] G. Matcham, A. Bowen, S. Lee, P. Pienkos, A. Zeitlin, *Proceedings of Chiral'94 USA Symposium*, Spring Innovations Ltd., **1994**, 55.

[31] C.-H. Mao, L. Anderson, *Phytochemistry* **1967**, *6*, 473.

[32] P. van Eikeren, *US Patent* 5241087, **1993**.

[33] E. Menendez, R. Brieva, F. Rebolledo, V. Gotor, *J. Chem. Soc., Chem. Commun.* **1995**, 989.

[34] F. Effenberger, A. Schwämmle, *Biocatal. Biotransform.* **1997**, *14*, 167.

[35] F. Effenberger, *Angew. Chem.* **1994**, *106*, 1609; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1555.

[36] R. J. H. Gregory, *Chem. Rev.* **1999**, *99*, 3649.

[37] M. Schmidt, H. Griengl, *Top. Curr. Chem.* **1999**, *200*, 193.

[38] L. Rosenthaler, *Biochem. Z.* **1908**, *14*, 238.

[39] W. Becker, H. Freund, E. Pfeil, *Angew. Chem. Int. Ed. Engl.* **1965**, *4*, 1079.

[40] F. Effenberger, T. Ziegler, S. Förster, *Angew. Chem.* **1987**, *99*, 491; *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 458.

[41] P. Zandbergen, J. van der Linden, J. Brussee, A. van der Gen, *Synth. Commun.* **1991**, *21*, 1387.

[42] B. Bauer, H. Strathmann, F. Effenberger, *DE* 4041896, **1991**.

[43] F. Wehtje, P. Adlercreutz, B. Mattiason, *Biotechnol. Bioeng.* **1990**, *36*, 39.

[44] S. Förster, J. Roos, F. Effenberger, H. Wajant, H. Sprauer, *Angew. Chem.* **1996**, *108*, 493; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 437.

[45] B. Hörsch, *Ph. D. Thesis*, University of Stuttgart, **1990**.

[46] E. Wehtje, P. Adlercreutz, B. Mattiason, *Appl. Microbiol. Biotechnol.* **1988**, *29*, 419.

[47] E. Wehtje, P. Adlercreutz, B. Mattiason, *Biotechnol. Bioeng.* **1994**, *41*, 171.

[48] P. Miethe, M.-R. Kula, I. M. Stuertz, C. Wandrey, U. Kragl, *US Patent* 5,122,462, **1992**.
[49] P. Zandbergen, J. van der Linden, J. Brussee, A. van der Gen, *Synth. Commun*. **1991**, *21*, 1386.
[50] T. T. Huuhtanen, L. T. Kanerva, *Tetrahedron: Asymmetry* **1992**, *3*, 1223.
[51] S.-Q. Han, G.-Q. Lin, Z.-Y. Li, *Tetrahedron: Asymmetry* **1998**, *9*, 1835.
[52] G. Lin, S. Han, Z. Li, *Tetrahedron* **1999**, *55*, 3531.
[53] U. Niedermeyer, M. R. Kula, *Angew. Chem.* **1990**, *102*, 423; *Angew. Chem. Int. Ed. Engl*. **1990**, *29*, 386.
[54] U. Kragl, U. Niedermeyer, M.-R. Kula, C. Wandrey, *Ann. N. Y. Acad. Sci*. **1990**, *613*, 167.
[55] N. Klempier, H. Griengl, M. Hayn, *Tetrahedron Lett.* **1993**, *34*, 4769.
[56] N. Klempier, U. Pichler, H. Griengl, *Tetrahedron: Asymmetry* **1995**, *6*, 845.
[57] M. Hasslacher, M. Schall, M. Hayn, H. Griengl, S. D. Kohlwein, H. Schwab, *J. Biol. Chem.* **1996**, *271*, 5884.
[58] H. Griengl, A. Hickel, D. V. Johnson, C. Kratky, M. Schmidt, H. Schwab, *Chem. Commun*. **1997**, 1933.
[59] M. Schmidt, S. Hervé, N. Klempier, H. Griengl, *Tetrahedron* **1996**, *52*, 7833.
[60] M. Bauer, H. Griengl, W. Steiner, *Enzyme Microb. Technol.* **1999**, *24*, 514.
[61] W. T. Loos, H. W. Gelluk, M. M. Ruijken, C. G. Kruse, J. Brussee, A. van der Gen, *Biocatal. Biotransform*. **1995**, *12*, 255.
[62] H. Gröger, E. Capan, A. Barthuber, K.-D. Vorlop, *Org. Lett*. **2001**, *3*, 1969–1972.
[63] F. Effenberger, B. Hörsch, S. Förster, T. Ziegler, *Tetrahedron Lett.* **1990**, *31*, 1249.
[64] T. Ziegler, B. Hörsch, F. Effenberger, *Synthesis* **1990**, 575.
[65] F. Effenberger, in *Stereoselective Biocatalysis* (Ed.: R. N. Patel), Marcel Dekker Inc., New York, **2000**, chapter 12, p. 332.
[66] B. S. Deol, D. D. Ridley, G. W. Simpson, *Aust. J. Chem*. **1976**, *29*, 2459.
[67] R. Wendhausen, Jr., P. J. S. Moran, I. Joekes, J. A. R. Rodrigues, *J. Mol. Catal. B: Enzym*. **1998**, *5*, 69.
[68] Y. Naoshima, Y. Akakabe, M. Takahashi, T. Saika, M. Kamezawa, H. Tachibana, T. Ohtani, Takehiko, *Recent Res. Dev. Phytochem*. **1998**, *2*, 11.
[69] K. Ishihara, H. Yamaguchi, H. Hamada, N. Nakajiman, K. Nakamura, *J. Mol. Catal. B: Enzym*. **2000**, *10*, 429.
[70] R. Yuan, S. Watanabe, S. Kuwabata, H. Yoneyama, *J. Org. Chem*. **1997**, *62*, 2494.
[71] W. Hummel, H. Schütte, M.-R. Kula, *Appl. Microbiol. Biotechnol*. **1988**, *28*, 433.
[72] D. Vasic-Racki, M. Jonas, C. Wandrey, W. Hummel, M.-R. Kula, *Appl. Microbiol. Biotechnol*. **1989**, *31*, 215.