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From a Racemate to a Single Enantiomer: Deracemization by Stereoinversion

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Abstract: The stereoinversion of one enantiomer into its mirror-image counterpart within a racemate furnishes a single stereoisomeric product in 100% theoretical yield. This extremely efficient type of deracemization, whereby substrate and product possess an identical chemical structure, can be achieved by using bio- or chemo-catalysts or combinations thereof and is applicable to secondary alcohols, amines and α-substituted carboxylic acids. Special emphasis is devoted to the theoretical background of the one-pot, single-step deracemization of *sec*-alcohols.

Keywords: alcohols; amines; amino acids; biotransformations; deracemization; stereoinversion

1 Introduction

The release of the general guidelines by the FDA in 1992 regarding the use of stereoisomeric bioactive compounds in pharmaceutical and agricultural applications[1,2] has dramatically increased the efforts devoted to the development of methods for the production of chiral compounds in non-racemic form. The majority of processes employed to date for the preparation of enantiopure compounds rely on asymmetric catalysis.[3,4] These processes can be classified into the following categories (Scheme 1).

(i) Desymmetrization of a prochiral- or *meso*-compound (**M**) through 'enantiomeric' pathways leads to the formation of a single enantiomeric product (**P** or **Q**) in 100% theoretical yield. The stereochemical outcome and the enantiomeric composition is determined by the relative magnitude of rate constants k_P and k_Q.[5–7]

(ii) On the contrary, kinetic resolution of a racemate (consisting of enantiomers **A** and **B**) leads to the formation of a pair of product and (remaining non-converted) substrate enantiomers (**P**/**B** or **Q**/**A**, respectively), each in 50% theoretical yield.[8–10] Since in general only one enantiomer is needed for synthesis, and there is little (or no) use for the other, it has to be regarded as 'waste' and thus represents a considerable economic burden for the process.

At a first glimpse, the overall efficiency of desymmetrization is by far superior to that of kinetic resolution and it appears as an anachronistic fact that, for biotransformation processes, the vast majority (approx. 80%) consists of the less efficient kinetic resolutions.[11] This puzzling fact can be explained by taking into account that the (theoretically possible) number of racemates will always be higher than that of prochiral or *meso*-compounds (Scheme 1). In prochiral and *meso*-compounds, only three functional elements can be varied due to the presence of an internal symmetry plane, whereas four groups are available for permutations in racemic compounds bearing a central element of chirality. As a consequence, significantly more racemates can be synthesized than prochiral and *meso*-compounds and therefore have to be considered as possible starting materials.

As a result of the combinatorial consequences of the relative number of functional groups as outlined above, the question is not *how to avoid* racemic starting materials by choosing prochiral or *meso*-compounds, but *how to improve* the economic balance of

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Kurt Faber, born 1953 in Klagenfurt (Carinthia/Austria), studied chemistry at the Karl-Franzens University in Graz, where he received his Ph.D. in 1982. From 1982–1983 he was in St. John's (Canada) for a post-doctoral fellowship and continued his career at the University of Technology (Graz), where he became associate professor in 1997. The following year he was appointed full professor at the University of Graz, where he heads a research group devoted to the use of biocatalysts for the synthetic transformation of non-natural compounds. He was a visiting scientist at University of Tokyo (1987/1988), Exeter University (1990), University of Trondheim (1994), Stockholm University (2001) and University of Minnesota (2005).

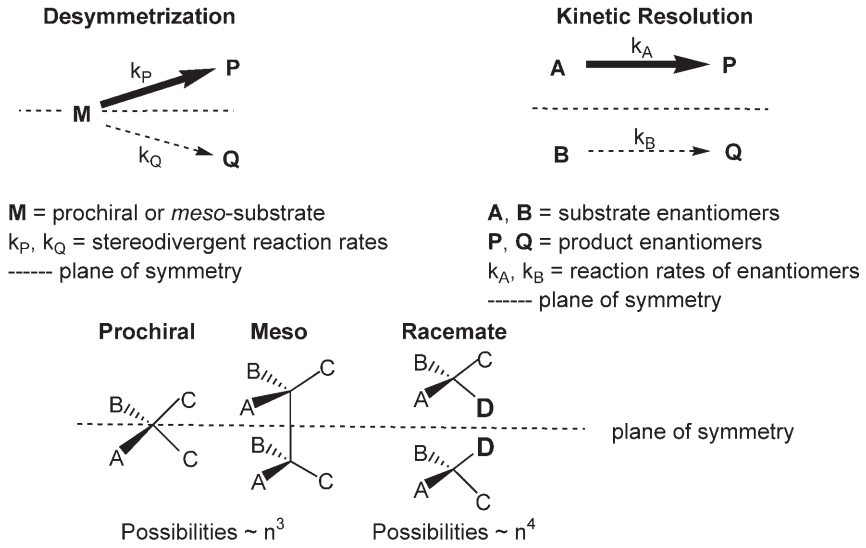
Iván Lavandera was born in 1976 and graduated in Chemistry in 1998 at the University of Oviedo, where he completed his Ph.D. in 2003 with Prof. Vicente Gotor. During his doctoral graduation, he spent three months in 2002 at the Department of Chemistry of McGill University (Montreal) under the supervision of Prof. Romas Kazlauskas. He continued in Prof. Gotor's group as a researcher until 2005, when he moved to the University of Graz as a postdoctoral researcher with Prof. Wolfgang Kroutil. His research interests are focused on biocatalysis, especially lipases and oxidoreductases, and the study of enzyme reactivity by means of computational methods.

Wolfgang Kroutil (born in 1972 in Graz, Austria) received his undergraduate training in chemistry at the University of Technology in Graz (Austria) and completed his graduate studies in Exeter (UK) and Graz. After his Ph.D. he gained two years of industrial experience in the biocatalysis research group at Syngenta (formerly Novartis CP) in Basel (Switzerland) and in the R&D department of Krems Chemie Chemical Services (Austria). In 2000 he joined the research group of Prof. Faber at the Department of Chemistry at the Karl-Franzens-University Graz as assistant professor. After his habilitation he became associate professor in 2004. He was a visiting scientist at the University of Stuttgart/Germany (2003) and the University of Cambridge/UK (2004).

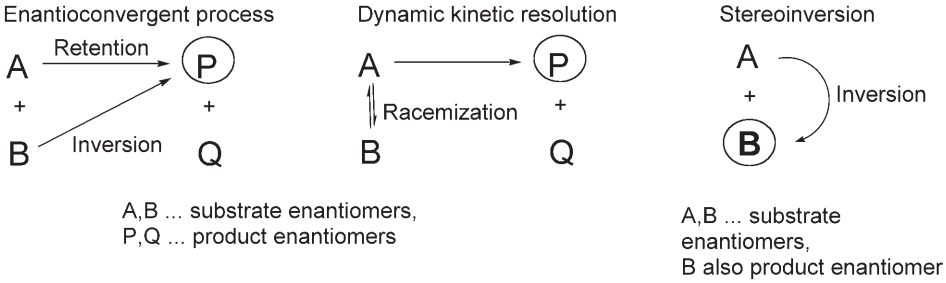
kinetic resolution by breaking the 50% yield threshold of a single enantiomer. In this respect, several strategies were recently devised which allow the complete transformation of both enantiomers into a single stereoisomeric product in 100% theoretical yield. These techniques are generally referred to as 'deracemization' processes.[12]

The majority of deracemization protocols rely on the transformation of both substrate enantiomers **A** and **B** into a single product of different structure (i.e., **P** or **Q**) *via* independent enantioconvergent pathways through retention and inversion of configuration (Scheme 2, enantioconvergent process).[12–14] Alternatively, the non-reacting substrate enantiomer may be racemized (*in situ*) to furnish a dynamic kinetic resolution (DKR) (Scheme 2).[15–20]

In contrast, deracemization by enantioselective stereoinversion[20] of enantiomer **A** yields substrate enantiomer **B** as the sole product. This process is sometimes also denoted as enantiomerization.[22] However, the term 'enantiomerization' has been more frequently used in the context of the interconversion of chiral-



Scheme 1. Strategies for the preparation of non-racemic compounds through asymmetric catalytic processes.



Scheme 2. Deracemization techniques. For enantioconvergent processes and dynamic kinetic resolutions, substrate (**A** + **B**) and product (**P**) are not identical, whereas stereoinversion of enantiomer **A** leads to its mirror-image counterpart **B**.

ly labile compounds, resembling racemization rather than deracemization.

Upon closer inspection, stereoinversion appears superior to the enantioconvergent process and DKR, since in theory only half of the starting material (**A**) has to undergo a chemical transformation (i.e., inversion) while the other half already represents the desired enantiomeric final product (**B**). In contrast, both enantiomers have to be transformed in enantioconvergent processes and DKRs, which at least doubles the amount of reactants needed. Therefore the economy of the latter is inferior to stereoinversion/enantiomerization.

Since the first comprehensive review by Carnell[23] on deracemization by stereoinversion, an impressive number of reports appeared recently and several recent reviews have touched this topic in part.[12,24–28]

Of the various possibilities to perform deracemization by stereoinversion, a one-pot, single-step protocol is certainly the most challenging. In certain cases, one-pot stereoinversion can only be achieved through a two-step (oxidation-reduction) sequence. In a one-pot, single-step process these redox reactions have to take place at the same time and the use of chemical redox reagents has not been described yet. All single-step stereoinversion processes described so far involve biocatalysts.

This review, devoted to deracemization by stereoinversion, is subdivided according to the type of substrate, namely secondary alcohols, α-aryl-/α-aryloxy-carboxylic acids, chiral amines and amino acids. Other techniques are discussed in the last section.

2 Secondary Alcohols

2.1 Single-Step Deracemization

2.1.1 Single Microorganism

Chiral non-racemic secondary alcohols are widely used in many sectors including the agrochemical and pharmaceutical industry, flavor and fragrances, food applications and in material sciences, such as liquid crystals. Racemic *sec*-alcohols are attractive starting materials, since they are often more easily accessible

than the corresponding ketones, although ketones are frequently reduced in an asymmetric fashion using chemical[29,30] or biocatalytic methods.[25,31–33] For instance, *rac*-pantolactone is easier to synthesize than ketopantoyl lactone. In order to break the 50% yield limit of kinetic resolution employing, e.g., lipase-catalyzed acyl-transfer[34] or enantioselective oxidation,[35–39] alternatives are desirable.

The ability of a microbial cell to selectively interconvert one enantiomer into its mirror-image counterpart within a given racemic substrate is not obvious, because these transformations do not represent common pathways found in Nature. As a consequence, the first report on the microbial stereoinversion of a *sec*-alcohol was not concentrated on its stereoinversion. It was an intellectual sideline when the complex pathways of the sequential asymmetric reduction of a 1,2-diketone (1,2-diphenylethanedione/benzil) *via* the corresponding α-hydroxy ketone (1,2-diphenyl-2-hydroxyethanone/benzoin) to finally furnish the *vic*-diol (1,2-diphenylethane-1,2-diol/hydrobenzoin) was investigated using *Saccharomyces uvarum*.[40] However, almost at the same time, the first intended stereoinversion of an α-hydroxylactone (pantolactone) using resting cells of *Rhodococcus erythropolis* IFO 12540 was described.[41] After these seminal papers, it took quite a while until microbial stereoinversion was more widely investigated and finally turned out as a general method for the preparation of single enantiomers from racemates.

Although the number of examples for the single-step deracemization of *sec*-alcohols employing whole microbial or plant cells has been steadily increasing over the past years, only speculations about the actual driving force of the stereoinversion as well as its mechanism and the enzyme(s) involved exist (for a detailed discussion see Section 2.1.2.). Table 1 gives an overview of compounds bearing a *sec*-hydroxy group which have been successfully deracemized by stereoinversion employing various whole-cell biocatalysts; such as bacteria, fungi, yeasts, and even plant cells. As can be deduced from the table, the applicability of this stereoinversion protocol is remarkably broad and encompasses α-hydroxy esters/lactones, β-hydroxy esters, benzylic, propargylic and aliphatic alcohols as well as *vic*-diols bearing a single chiral center. The concept of microbial stereoinversion has also successfully applied to diols bearing two chiral centers. These cases are not included in this review since the underlying kinetics involve the occurrence of chiral intermediates and thus are fundamentally different.[23,42–44]

Upon close inspection, one severe drawback of the majority of deracemizations reported so far is the long reaction time, i.e., up to several days. It was only recently that Chadha and co-workers reduced this period to hours.[60–62,64] Additionally, the substrate concentration is generally below preparative significance. On the other hand, making both enantiomers accessible by choosing the appropriate organism or by switching the reaction conditions, such as the pH value, has been accomplished.

2.1.2 Mechanism of Microbial Deracemization

It is a puzzling fact that, in spite of the remarkable body of experimental data on the microbial stereoinversion of *sec*-alcohols available to date, the exact mechanism of this intriguing biotransformation and the nature of its driving force are still unknown. In the following section we attempt to draw a first line into this direction.

Already in early reports it was speculated that the microbial deracemization of secondary alcohols in an 'one-pot, single-step' system occurred *via* an oxidation-reduction sequence involving the corresponding prochiral ketone as intermediate, which was often detected in small amounts. Unambiguous proof was provided by Nakamura et al.,[50] who subjected the labeled alcohol *rac*-1-*d*-phenylethanol to microbial deracemization (Scheme 3) and showed that the deuterium on the chiral center from the reacting enantiomer being inverted was exchanged by hydrogen from 'outside', while the mirror-image remained unaffected. Therefore, it was concluded that at least two redox enzymes must be involved.

The most obvious explanation of these facts involves two (or more) nicotinamide-dependent alcohol dehydrogenases (ADHs) possessing opposite stereopreference.[23,49,52,59,60,64] It is concluded that if one alcohol dehydrogenase shows Prelog preference, the other one has to show 'anti-Prelog' preference (Scheme 4).[26,53] While one enantiomer from the racemate is selectively oxidized by a dehydrogenase, the ketone thus formed is reduced back again by a different ADH displaying opposite stereopreference. This reduction step is supposed to be irreversible, however, this is not generally accepted due to the expected reversibility of a catalyst.

Avoiding an irreversible process in the proposed mechanism, deracemization by *Cunninghamella echinulata*[46] was shown to occur by sequential highly selective oxidation of the (*S*)-enantiomer followed by partially (*S*)-selective reduction of the ketone intermediate. Although oxidation and reduction show the *same* stereopreference, the amount of the (*R*)-enantiomer increases due to the incomplete stereoselectivity of the reduction. On the contrary, the (*S*)-enantiomer is gradually depleted due to the perfect (*S*)-enantioselectivity of the oxidation. In other words, the desired (*R*)-enantiomer derives from a 'mistake' in the asymmetric reduction step. The reaction Scheme resembles Scheme 4, but with the difference that the en-

Table 1. Deracemization of *rac-sec*-alcohols bearing a single stereocenter by microbial stereoinversion.

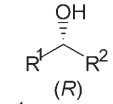
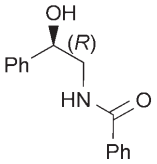
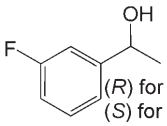
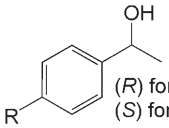
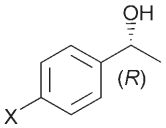
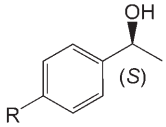
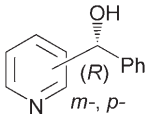
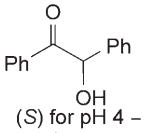
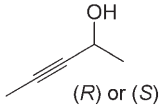
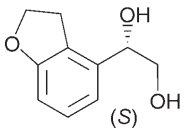
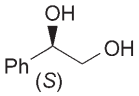
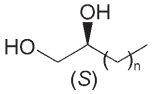
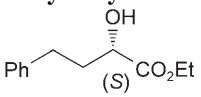
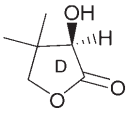
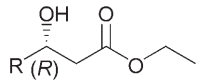
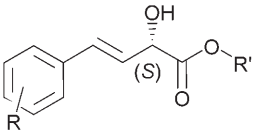
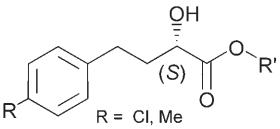
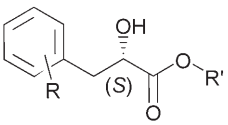
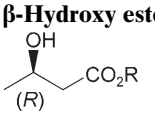
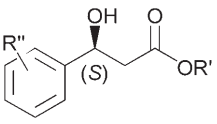
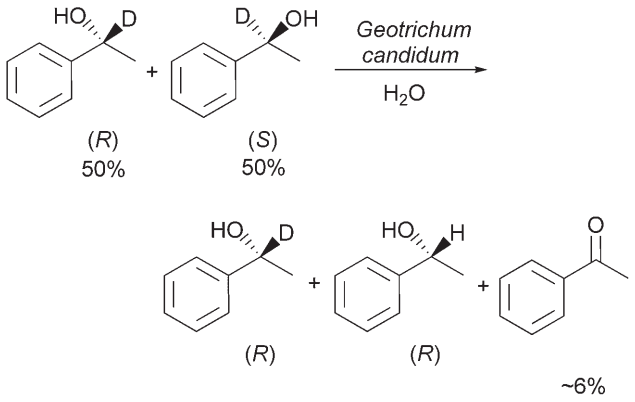
Product	Organism	*ee* max [%]	Yield [%]	Conc. [g/L]	Time	Ratio[a] [g g^{-1}]	Ref.
Aliphatic alcohols							
 (*R*) R^1 = Ar, Het-Ar, Ar-CH_2- R^2 = Me, Et, *n*-Bu, *c*-hexyl, vinyl	*Sphingomonas paucimobilis*	99	90	~1	4–6 d	n.g.	[45]
Benzylic alcohols							
	*Cunninghamella echinulata*	92	57	~0.1	6 d	n.g.	[46]
 (*R*) for 3320 (*S*) for 4038	*Aspergillus terreus* CCT 3320 and CCT 4083	>99	59	~0.4	1–8 d	250	[47]
 (*R*) for 3320 (*S*) for 4038 R = NO_2, Br, Cl	*Aspergillus terreus* CCT 3320 and CCT 4083	>99	up to 86[b]	~0.4	1–17 d	250	[48]
 X = H, Cl, OMe	*Geotrichum candidum*	up to 99	55-99[b]	~3.3	24 h	45	[49,50]
 R = H, Br, Me, NO_2, SeMe	*Arracacia xanthorrhiza* (arracacha) *Polymnia sonchifolia* (yacon) *Zingiber officinale* (ginger)	97 93 98	12-98[b] 99[b] 99[b]	~1.25	3–6 d	200	[51]
 *m*-, *p*-	*Catharanthus roseus* (periwinkle)	up to >99	92–99	~0.44	9–17 d	150	[52,53]
 (*S*) for pH 4 – 5 (*R*) for pH 7.5 – 8	*Rhizopus oryzae*	97 (*R*) 85 (*S*)	73–76 71	~1	21 d 15 d	n.g.	[54]
Propargylic alcohols							
 (*R*) or (*S*)	(*R*): *Nocardia fusca*, *N. globerula*, *N. erythropolis*; (*S*): *Nocardia pseudosporangifera*	100 (*R*) 98 (*S*)	83[b] 70[b]	~5	1–5 d	30	[55,56]
***vic*-Diols**							
 (*S*)	*Candida boidinii* *Pichia methanolica* *Hansenula polymorpha*	up to 100 up to 100 up to 60	72[b] 88[b] 99[b]	~0.2	2–7 d	1500	[57]

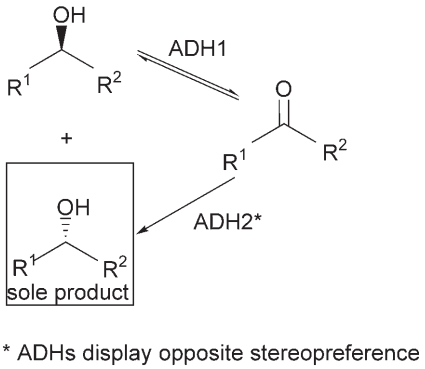
Table 1. (Continued)

Product	Organism	*ee* max [%]	Yield [%]	Conc. [g/L]	Time	Ratio[a] [g g^{-1}]	Ref.
	*Candida parapsilosis*	98	92	8	48 h	6.3	[58]
	*Candida parapsilosis*	100, $n=2,3$ 79, $n=1$	99	3–10	1–3 d	n.g.	[59]
α-Hydroxy esters/lactones							
	*Candida parapsilosis*	>99	85–90	~3	1 h	67	[60]
	*Rhodococcus erythropolis*	94	70	18	4 d	4	[41]
 R = Ph, *o*-MeC$_6$H$_4$, *p*-MeC$_6$H$_4$ *p*-ClC$_6$H$_4$, *p*-NO$_2$C$_6$H$_4$, *o*-ClC$_6$H$_4$, PhCH=CH	*Candida parapsilosis* (immobilized)	>99	28–68	~0.25	6 h	800	[61]
 R = H, *p*-Cl, *p*-Me, *o*-Cl, *m*-NO$_2$ 2,4-di-Cl, 2,5-di-MeO R' = Me, Et, CH$_2$Ph	*Candida parapsilosis*	98	52–99	~3	1.5 h	100	[62]
 R = Cl, Me R' = Me, Et	*Candida parapsilosis*	95	69–70	~3	1.5 h	100	[62]
 R = H, *p*-Me, 2,4-di-Cl R' = Me, Et	*Candida parapsilosis*	60	62–70	~3	1.5 h	100	[62]
β-Hydroxy esters							
	*Geotrichum candidum*	96	75[b]	9	70 h	1	[63]
 R' = Me, Et R'' = H, *p*-MeO, *p*-NO$_2$ *p*-Me, *o*-Me	*Candida parapsilosis*	99	62–75	~0.25	6 h	400	[64]

[a] Ratio = substrate:wet cells (w/w).
[b] GC or HPLC yield; n.g. = not given.



Scheme 3. Deracemization of labeled 1-*d*-phenylethanol by whole cells of *Geotrichum candidum*.



Scheme 4. Redox-mediated stereoinversion of an alcohol *via* its prochiral ketone.

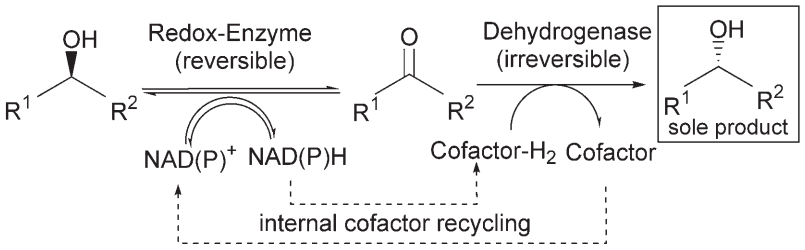
zyme(s) involved have the same apparent enantiopreference and most importantly, none of the reactions need to be irreversible. From a kinetic point of view, this is a more sophisticated case of the processes described below using non-stereoselective chemical reducing agents (see Sections 2.1.4 and 4.1) combined with highly selective oxidation.

Due to the involvement of a consecutive oxidation-reduction reaction, the net redox balance of this process is zero and (in an ideal case) no external cofactor recycling is necessary, since the redox equivalents, such as NAD(P)H, are being recycled internally between both steps (Scheme 5).

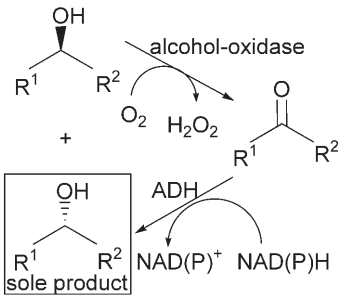
In order to provide a driving force to push the system from the racemate in the desired uphill direction towards one of the enantiomers, several suggestions have been proposed so far.

(i) Although not proven in detail, it has been speculated that one of the dehydrogenases is 'unable to catalyze the back-reaction,'[42,59,65] which is difficult to bring in line with the principles of catalysis. (ii) Another explanation suggests the dependence of the two ADHs on different cofactors, i.e., NADH and NADPH.[23,42,45] For example, if NAD^+ is required for the oxidation, NADPH would be required for the reductive half-reaction. As a consequence, an independent cofactor recycling system for each cycle would be required. Such a system depending on both NADH and NADPH cofactors was found to operate in the deracemization of 1,2-pentanediol with *Candida parapsilosis*[59] using a cell-free extract. Inhibitor-based studies showed that NAD^+/NADH was responsible for the selective oxidoreduction of the (*R*)-diol, while (*S*)-selective ketone reduction was NADPH-dependent. Thus, the following mechanism was proposed for the stereoinversion: firstly, reversible oxidoreduction between (*R*)-pentane-1,2-diol and 1-hydroxy-2-pentanone by an NAD^+-linked dehydrogenase and secondly, irreversible reduction of the latter to the (*S*)-diol by an NADPH-linked dehydrogenase. Alternatively, it was suggested that the process involves reversible oxido-reduction catalyzed by an NAD^+-dependent, (*S*)-specific dehydrogenase and reversible reduction catalyzed by NADPH-dependent (*R*)-specific dehydrogenase from *Nocardia fusca*,[55,56] while the opposite cofactor requirement was found for *Candida parapsilosis* CCTCC M203011.[58] After all of these studies, the problem of 'irreversibility' remains unsolved.

(iii) Further attempts to provide an explanation took additional reactions into account, which would provide energy from an 'external' driving force to shift the equilibrium. Along this line, it was proposed that molecular oxygen might be involved in cofactor regeneration, because stereoinversion was faster under aerobic conditions,[28,50,65] which would suggest the involvement of an alcohol oxidase rather than an alcohol dehydrogenase (Scheme 6). The required reducing equivalents to provide NAD(P)H for the re-



Scheme 5. Internal cofactor recycling in the deracemization of *sec*-alcohols.



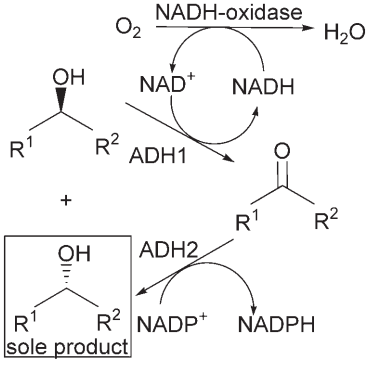
Scheme 6. Deracemization *via* an alcohol oxidase and an alcohol dehydrogenase.

duction step might be obtained from the added sugar or alcohols, such as ethanol,[57,60,61,64] which was occasionally supplied as co-solvent to increase the solubility of the substrate and was not considered to participate in the redox reaction. In this context, the remarkably short reaction times reported by Chadha and co-workers,[60–62,64] suggest that the co-solvent most likely is involved in the overall process.

Along this line, Allan and Carnell[45] went in search for a suspected alcohol oxidase[62,66] in the deracemizing strain *Sphingomonas paucimobilis*, but none could be identified.

Based on the data available to date, it appears more likely that deracemization of *sec*-alcohols follows different mechanisms in different microorganisms, and the presence of a single unique mechanism for all deracemization processes seems rather unlikely.

In addition to the processes proposed above, the involvement of an NAD(P)H oxidase[67–72] can be envisaged to provide the driving force for the deracemization (Scheme 7). For instance, if an NAD^+-dependent ADH would catalyze the oxidation and an NADPH-specific ADH possessing opposite stereopreference would be responsible for the reduction, an NADH-specific oxidase[69] could provide the required irreversibility of the system.



Scheme 7. Deracemization *via* an NADH-oxidase and an alcohol dehydrogenase.

Considering the redox equivalents required for the processes depicted in Scheme 6 and 7, it is clear that the overall energy balance is not zero anymore, as in the case of internal cofactor recycling (Scheme 5). Overall, a minimum of 0.5 equivalents of reagents are required for each of the oxidation and reduction steps.

Since the ΔH of the interconversion of enantiomers is zero, deracemization by stereoinversion is purely depending on entropy. Consequently, by theory, the minimum Gibbs enthalpy to shift the racemate as the energetic minimum in solution towards a single enantiomer requires (according to Eliel) $\Delta G = 0.40$ kcal mol^{-1} at 20 °C,[73] which is a rather low amount of energy, and obviously much lower than the energy required for the processes discussed above.

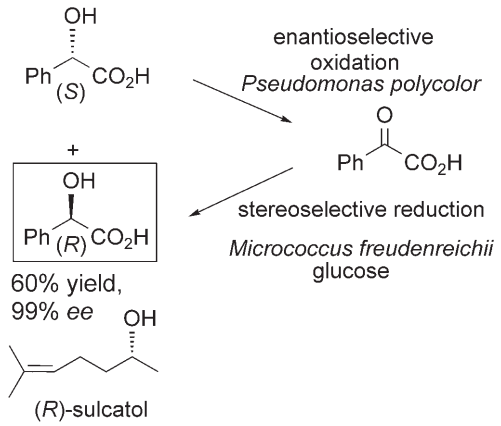
Summarizing on the mechanism, the elucidation of the exact mechanism(s) involved in deracemizations and the identification of the origin of the redox equivalents remains a considerable challenge. An even more challenging task will be to design a process which requires as least energy (and therefore reagents) as possible to approximate the minimum ΔG of 0.40 kcal mol^{-1}.

2.1.3 Two Microorganisms

Alternatively, a 'one-pot, single-step' deracemization of *sec*-alcohols has been achieved in a single reaction vessel by employing two different microorganisms. However, the number of examples of this type is limited and oxidation and reduction are usually done in separate steps in a 'one-pot, two-step' procedure (Section 2.2.2).

For instance, racemic mandelic acid (300 mM) was deracemized in the presence of whole cells of *Pseudomonas polycolor* IFO 3918 and *Micrococcus freudenreichii* FERM-P 13221 in a two-liter flask for 24 h by feeding glucose.[74] Separate experiments showed that *Pseudomonas polycolor* was responsible for oxidation, while *Micrococcus freudenreichii* was needed for reduction of the corresponding α-keto acid (benzoyl formate). After 24 h, (*R*)-mandelic acid was isolated in 60 % yield and 99 % *ee* (Scheme 8).

In analogy, *Bacillus stearothermophilus,* displaying Prelog specificity, was used for the oxidation of (*S*)-sulcatol, while simultaneous microbial reduction of the corresponding ketone was achieved by *Yarrowia lipolytica,* displaying anti-Prelog activity, to furnish the (*R*)-alcohol in 82 % yield and 90 % *ee*.[75] However, improved yields were obtained when each step was performed separately.



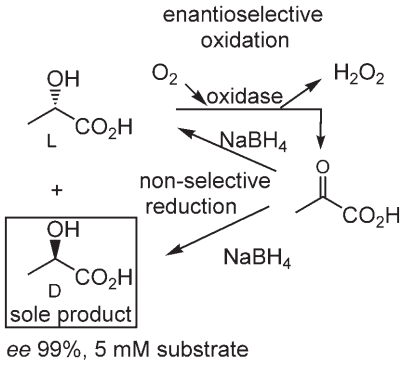
Scheme 8. Deracemization of mandelic acid and sulcatol *via* simultaneous oxidation and reduction using two microorganisms.

2.1.4 Combining Biocatalytic Oxidation with (Electro)chemical Reduction

Due to the incompatibility of reagents, the combination of two chemical methods, one for oxidation and one for reduction in a single flask for the deracemization of hydroxy compounds is very difficult to imagine and (to the best of our knowledge) this protocol has not been verified so far. However, the combination of chemical reduction with a matching biocatalytic oxidation has been reported.

'One-pot, single-step' deracemization of lactate was achieved by combining enantioselective biocatalytic oxidation of the L-isomer with non-selective reduction of pyruvate thus obtained using sodium borohydride (Scheme 9). Complete conversion of the racemate to the D-enantiomer was reached within 90 min at a low substrate concentration (5 mM).[76]

Although this process appears rather inefficient on a first glimpse, a detailed look into its kinetics reveals its merits. For the sake of clarity, cyclic deracemization is explained by regarding oxidation and reduction



Scheme 9. Deracemization of lactate by combining biocatalytic oxidation (L-lactate oxidase LOX II from *Aerococcus viridans*) and non-selective reduction ($NaBH_4$).

as separate sequential steps. If the oxidation is highly L-selective, L-lactate is entirely converted to pyruvate in 50% yield, while 50% of the material (D-lactate) remains untouched. In the second step of the first cycle, non-selective reduction of pyruvate yields L- and D-lactate (each 25%). The latter is added to the untouched 50% D-lactate to yield 75% D-lactate, i.e., the ratio of L-/D-lactate is now 25/75 after the first cycle (Figure 1). After the second cycle, this ratio is

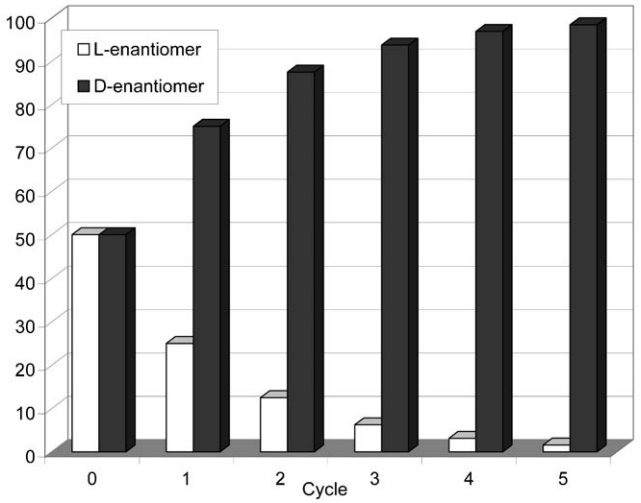


Figure 1. Deracemization of *sec*-alcohols or amines *via* a cyclic oxidation-reduction sequence.

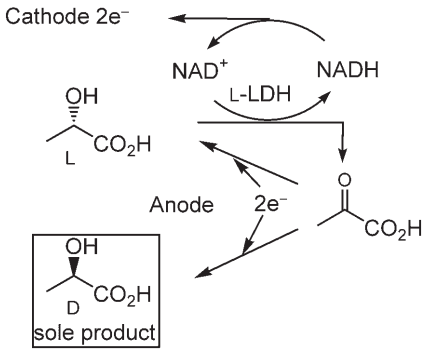
12.5/87.5, etc. Although an infinite number of cycles is required to reach enantiopure material (in theory), simple five-fold repetition of this oxidation-reduction sequence[66] leads to an *ee* of 97%. Overall, the non-reactive enantiomer of the starting material serves as the 'sink' of the system, where all of the material will eventually end after a certain number of oxidation-reduction cycles. In practice, biocatalytic enantioselective oxidation and non-selective chemical reduction run in parallel.

However, enantioselectivities for oxidation are often ranging below *E* values of 100. For these cases, the enantioselectivity determines two crucial factors of the system, (i) the maximum obtainable *ee* after an infinite number of cycles and (ii) the number of cycles required to reach a certain desired *ee*. The merits and limits of cyclic deracemization systems have been described based on the underlying kinetics.[66]

Considering the amount of reagents needed for oxidation and reduction, one molar equivalent of oxidant as well as reducing agent, are required (perfect enantioselectivity assumed). Thus, the non-selective reduction is compensated by the requirement of a double amount of oxidation and reducing agent.

The above mentioned protocol is not only applicable to the deracemization of racemates, but also to

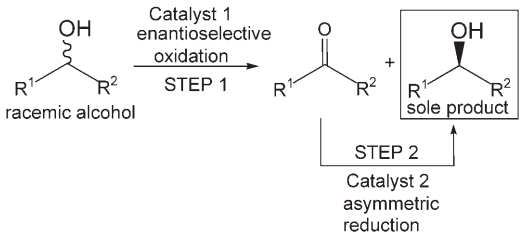
the selective stereoinversion of a single enantiomer into its mirror-image counterpart. Thus, when L-lactate was oxidized by L-lactate dehydrogenase at the expense of NAD^+, the pyruvate formed was reduced electrochemically to *rac*-lactate with simultaneous cofactor-recycling. Cyclic simultaneous repetition yielded D-lactate as the sole isomer (Scheme 10).[77]



Scheme 10. Stereoinversion of L-lactate (580 μmol) *via* (i) enantioselective oxidation employing L-lactate dehydrogenase (L-LDH), (ii) electrochemical reduction of pyruvate, and (iii) electrochemical oxidation of the cofactor NADH.

2.2 Two-Step Deracemization

Alternatively, deracemization of *sec*-alcohols *via* oxidation-reduction can be performed in a stepwise manner using two different organisms or isolated enzymes (Scheme 11, Sections 2.2.1 and 2.2.2) or by using a single chemical catalyst which displays low selectivity for oxidation and high selectivity for reduction (Section 2.2.3).

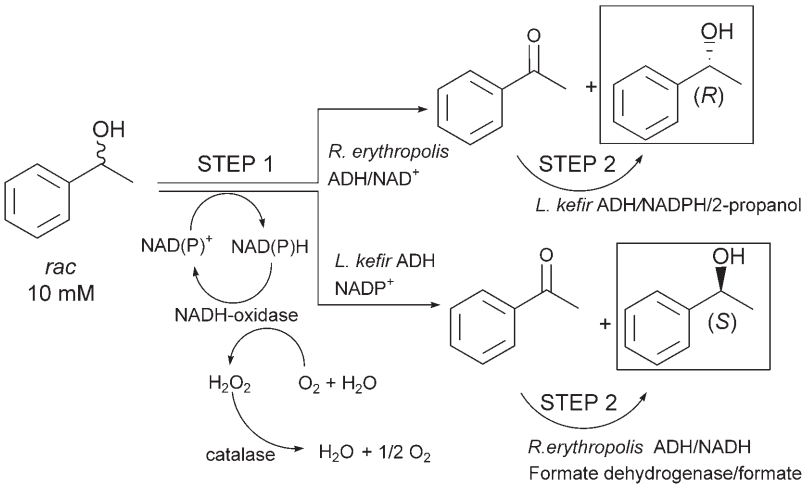


Scheme 11. Deracemization of *sec*-alcohols *via* two independent separate oxidation-reduction steps.

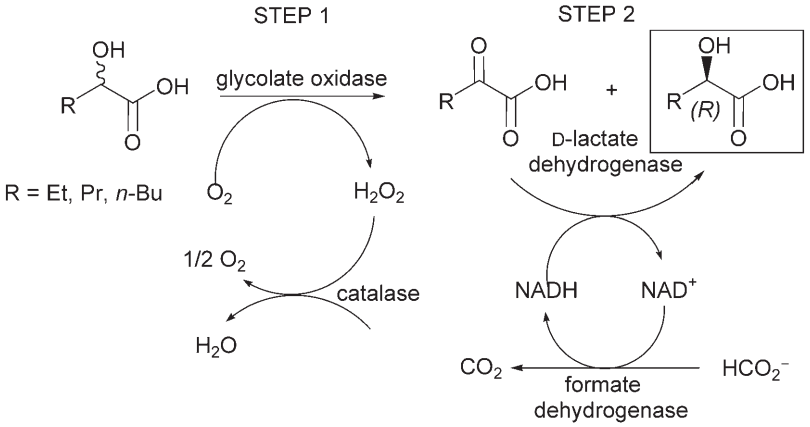
2.2.1 Employing Enzymes

A stepwise route to enantiomerically pure alcohols from the corresponding racemates was achieved by employing two stereocomplementary alcohol dehydrogenases, namely the (*R*)-specific NADPH-dependent ADH from *Lactobacillus kefir* and the (*S*)-specific NADH-dependent ADH from *Rhodococcus erythropolis*.[78] The flexibility of this strategy was demonstrated by the fact that both enantiomers were accessible by the simple switch of the order of usage of the ADHs (Scheme 12). In the first step, oxidative kinetic resolution yielded 50% of the desired alcohol and 50% of the ketone. In the second separate step, the latter was subsequently reduced by the matching ADH, yielding the desired pure enantiomer in 100% yield.

A related two-step deracemization procedure employed an oxidase (glycolate oxidase), which transformed the (*S*)-enantiomer of an α-hydroxy acid to the α-keto acid, which in turn was reduced in a second separate step by lactate dehydrogenase to the (*R*)-enantiomer obtaining enantiopure (*R*)-α-hydroxy acids (Scheme 13).[79,80] Although the process appears elegant and bears a certain potential to be combined



Scheme 12. Enantiocomplementary deracemization of *sec*-alcohols *via* an oxidation-reduction sequence employing two purified alcohol dehydrogenases in a stepwise manner.



Scheme 13. Stepwise deracemization of *rac*-α-hydroxycarboxylic acids *via* enzymatic two-step oxidation-reduction sequence.

in a single-step/one-pot procedure due to the compatibility of both enzymatic redox steps, analogous processes for other *sec*-alcohols were never described, most likely due to the lack of readily available *sec*-alcohol oxidases.

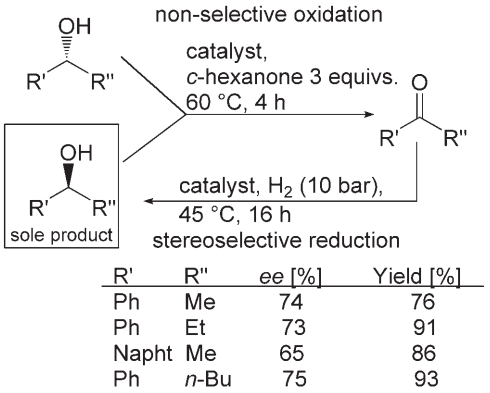
2.2.2 Employing two Microorganisms

Although biocatalysts show higher compatibility with each other than chemical catalysts and are therefore expected to be better suited for single-step deracemization *via* oxidation-reduction sequences in the same reactor (see Section 2.1.3), problems were encountered for certain microorganisms. For instance, the enantioselective oxidation of (+)-mandelic acid from the racemate by *Alcaligenes bronchisepticus* required aerobic conditions, while cell-free extract of *Streptococcus faecalis* IFO 12964 employed for the reduction of the intermediate benzoyl formate was inactivated under these conditions.[81] As a consequence, both of these steps had to be performed in a stepwise manner.

In analogy, for the deracemization of pantoyl lactone, a precursor of the water soluble vitamin B_5 (pantothenic acid) and a constituent of coenzyme A, two organisms – *Nocardia asteroides* and *Candida parapsilosis* – were used in tandem.[82]

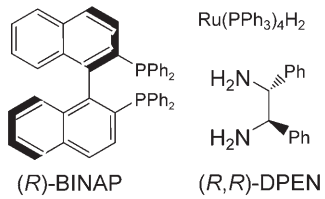
2.2.3 Chemical Catalyst(s)

Very recently, a novel method was proposed, in which the main advantage is to use a 'single' catalyst for both oxidation and reduction steps. In the group of Williams it was noticed[83] that a certain Ru-BINAP-diphenylethyldiamine (DPEN) catalyst showed high stereoselectivity for the asymmetric hydrogenation of ketones,[29] while it was rather unselective for the corresponding reduction *via* hydrogen transfer using 2-propanol as hydrogen-source.[84] In order to exploit this 'weakness' for deracemization purposes, it was envisaged to use this catalyst for the non-selective oxidation of *rac*-*sec*-alcohols *via* hydrogen transfer, followed by highly stereoselective reduction under hydrogenation conditions using the same catalyst. Although the first attempts were not successful, various *sec*-alcohols were successfully resolved by using a mixture of $Ru(PPh_3)_4H_2$, (*R*)-BINAP and DPEN as a non-defined catalyst (Scheme 14). After non-selective hydrogen transfer oxidation using cyclohexanone as hydrogen acceptor, the reaction was pressurized with hydrogen thereby switching to the stereoselective reduction mode.



R'	R''	*ee* [%]	Yield [%]
Ph	Me	74	76
Ph	Et	73	91
Napht	Me	65	86
Ph	*n*-Bu	75	93

Catalyst: combination of

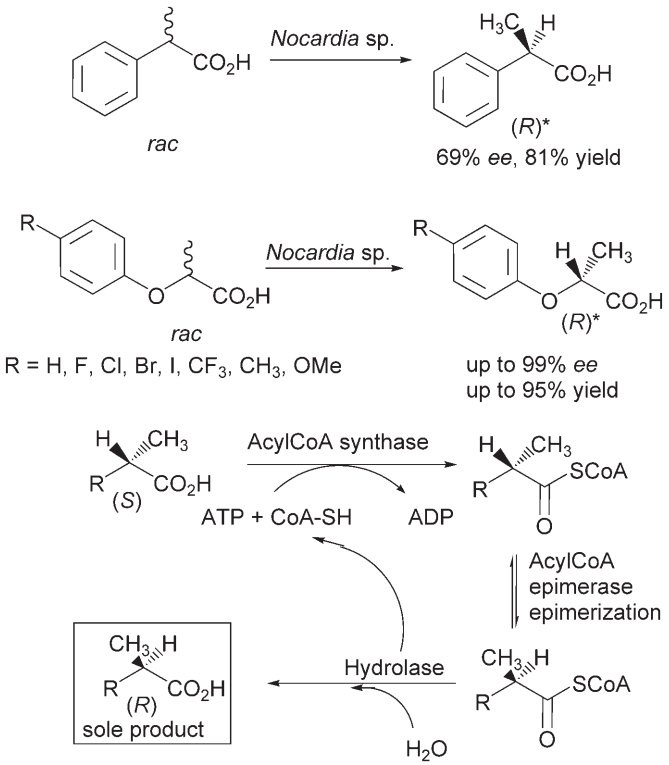


(*R*)-BINAP (*R*,*R*)-DPEN

Scheme 14. Deracemization of *sec*-alcohols *via* a non-selective oxidation/selective reduction sequence employing a combination of chemical catalysts.

3 α-Aryl- and α-Aryloxycarboxylic Acids

α-Aryl- and α-aryloxy-substituted propionic acids are important anti-inflammatory agents (e.g., naproxen, ibuprofen) and agrochemicals (e.g., diclofop, fluazifop), where the majority of the bioactivity resides in the (*S*)- (α-aryl) or (*R*)-enantiomer (α-aroyloxy), respectively. A 'one-pot, single-step' deracemization of these compounds was accomplished *via* biocatalytic stereoinversion using fermenting or resting cells of *Nocardia diaphanozonaria* JCM 3208.[85,86] Thus, α-aryl- and *para*-substituted 2-aryloxypropanoic acids were obtained in high yields and excellent *ee*s from the corresponding racemates (Scheme 15).[87]



Scheme 15. Microbial deracemization of α-phenyl- and *p*-substituted α-aryloxypropionic acids by *Nocardia diaphanozonaria* JCM 3208. * Note the switch in CIP sequence priority.

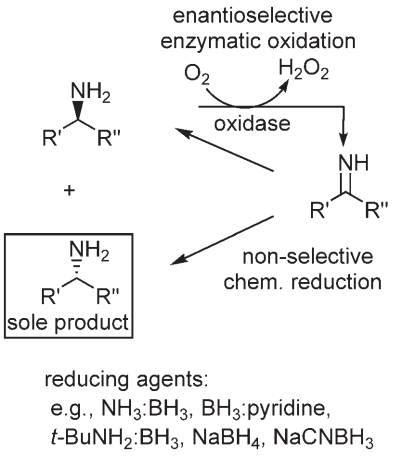
Careful elucidation of the enzymatic pathways showed that this microbial stereoinversion takes place *via* the following three-step sequence: (i) formation of an 'activated' acyl-CoA derivative of the (*S*)-acid, followed by (ii) epimerization of the latter to yield the (*R*)-isomer, and finally (iii) hydrolysis of the (*R*)-acyl-CoA ester. Thus, deracemization does not act through an oxidation-reduction sequence as for *sec*-hydroxy groups, but involves an acyl-CoA synthetase, an epimerase and a hydrolase, all of which occur in common fatty acid biosynthesis/degradation pathways. The competing β-oxidation of the substrate could be eliminated by addition of acyl-CoA dehydrogenase inhibitors.[88] The driving force for this deracemization draws its energy from ATP, which is required in stoichiometric amounts to form the acyl-CoA intermediate. Since the requirement of molar amounts of ATP (even when recycled) is expensive, preparative-scale deracemization using isolated enzymes becomes unlikely, but is feasible by using resting/viable cells, which provide the energy for CoA ester formation.

4 Chiral Amines and Amino Acids

4.1 Combining an Oxidase with a Chemical Reducing Agent

Deracemization of compounds bearing a chiral amino group can be achieved in a similar fashion as described for secondary alcohols in a 'one-pot, single-step' process (see Section 2.1.4). Thus, (enzyme-mediated) enantioselective oxidation of a *rac*-amine *via* kinetic resolution yields the prochiral imine. In a subsequent step, the latter is (chemically) reduced in a non-selective fashion yielding an enantioenriched amine. Cyclic combination of both steps leads to a highly versatile deracemization technique (Scheme 16). Depending on the type of substrate, care has to be taken to suppress the undesired hydrolysis of the intermediate imine by adjustment of the reaction conditions, such as pH and temperature.

First approaches in the deracemization of amino compounds were directed towards the synthesis of enantiopure α-amino acids starting from the corresponding racemates.[22] Soda and co-workers designed a procedure for the synthesis of L-proline from the racemate using a coupled system of D-amino acid oxidase and sodium borohydride as reducing agent.[89]



Scheme 16. Deracemization of amino acids or amines *via* a cyclic oxidation-reduction sequence.

Thus, D-proline was enantioselectively oxidized to form the corresponding cyclic imino acid (Δ^1-pyrroline-2-carboxylic acid), which was chemically reduced to furnish DL-proline again. Finally, enantiopure L-proline was obtained as the sole product in quantitative yield. In a similar way, DL-pipecolic acid was converted to L-pipecolic acid.[90] However, the use of sodium borohydride, which slowly decomposes in aqueous solution causes a significant rise in the pH value, which has to be controlled.

This problem was addressed by Turner and his group,[91] who have shown that milder (and thus more water-stable) reducing agents than sodium borohydride can be used in combination with a suitable oxidase for the deracemization of amino acids and primary or secondary amines.[92,93] Thus, using L-amino acid oxidase from *Proteus myxofaciens* and various amine-borane complexes,[94] or D-amino acid oxidase from porcine kidney and sodium cyanoborohydride,[95] the preparation of several natural and non-natural enantiopure D- and L-amino acids was achieved, respectively. In a more recent report, several β- and γ-substituted α-amino acids were deracemized using D-amino acid oxidase from *Trigonopsis variabilis* and sodium cyanoborohydride or sodium borohydride.[96]

In order to extend the applicability of this elegant procedure to chiral amines (which are not oxidized by amino acid oxidases), an amine oxidase from *Aspergillus niger* was identified as a suitable biocatalyst. Since the wild-type enzyme showed insufficient activity, directed evolution of this biocatalyst was performed to furnish a novel amine oxidase possessing not only a wider substrate spectrum, but also enhanced activity and enantioselectivity (Figure 2).[97–99]

The Asn336Ser variant of the amine oxidase showed highest activity towards substrates bearing a methyl substituent and a bulky alkyl/aryl group adjacent to the amino-carbon atom. For instance, its activity on L-α-methylbenzylamine was 47-fold higher than that of the wild-type enzyme,[97] and it also accepted secondary amines.[98] In all cases examined so far, the enzyme mutant was enantioselective for the (*S*)-isomer of the *rac*-amine substrate. For this deracemization system, ammonia-borane was chosen as the reducing agent. In continuation of this work, a 'secondary amine oxidase' was obtained by directed evolution from the Asn336Ser variant.[100] Thus, a new double mutant (Asn336Ser/Ile246Met) showed enhanced activity towards chiral secondary amines (e.g., 5-fold higher than the original mutant towards 1-methyltetrahydroisoquinoline). The double mutant was used in immobilized form (on Eupergit C) for the preparative-scale deracemization of *rac*-2-phenylpyrrolidine in combination with ammonia-borane complex as reducing agent. This concept was very recently extended to the deracemization of *tertiary* amines, such as *N*-methyl-2-phenylpyrrolidine, using a new variant of this monoamine oxidase containing five mutations (Ile246Met/Asn336Ser/Met348Lys/Thr384Asn/Asp385Ser, 'MAO-*N*-5') which was obtained after several rounds of directed evolution.[101]

4.2 Multienzyme System

In order to avoid the non-selective chemical reducing agent, 'one-pot, single-step' deracemization of α-amino acids was achieved by using microorganisms or multienzyme systems which catalyzed both the oxidation and the reduction step in a stereocomplementary fashion. The first report on such a system appeared about 40 years ago by Chibata et al.[102] They showed that a cell suspension of *Pseudomonas fluorescens* or *Pseudomonas miyamizu* was able to deracemize DL-phenylalanine to L-phenylalanine within 24–48 h of incubation. Detailed studies suggested that this process was not catalyzed by a single enzyme, but it proceeded *via* the following two-step system. Oxidative degradation of D-phenylalanine by D-amino acid oxidase furnished phenylpyruvic acid, which was converted to L-phenylalanine by transamination or reductive amination (Scheme 17). This type of microbial deracemization was later proven to be of general use and it was successfully applied to obtain the L-isomers of

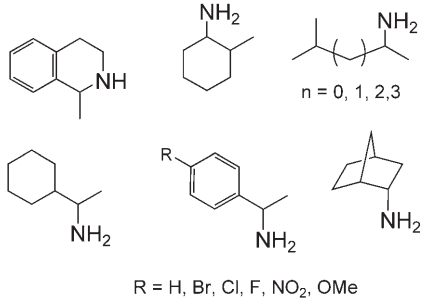
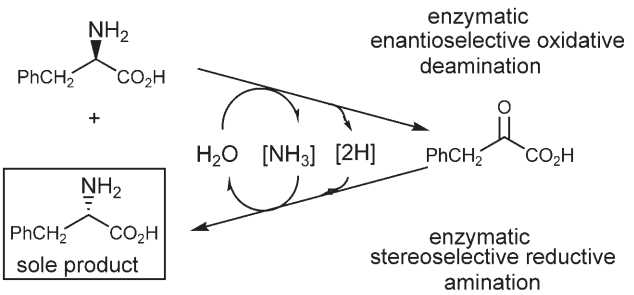


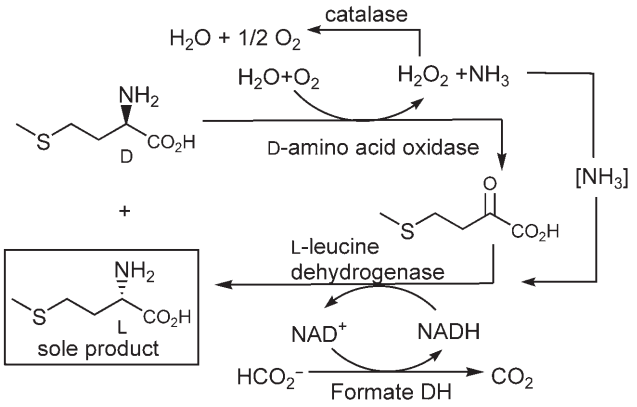
Figure 2. Selection of chiral amines which were enantioselectively oxidized by the Asn336Ser mutant of the amine oxidase from *Aspergillus niger*.



Scheme 17. Deracemization of DL-phenylalanine by whole cells of *Pseudomonas* sp. *via* two stereoselective enzyme-catalyzed redox steps.

several α-amino acids from the corresponding racemates using various microorganisms.[103] Since the corresponding α-keto acids were observed as intermediates in these biotransformations, it was shown that these processes generally occurred *via* the combination of D-selective deamination and L-selective transamination.

In a related approach, DL-methionine was efficiently deracemized to obtain the L-enantiomer using a multienzyme system consisting of a D-amino acid oxidase, a catalase, leucine dehydrogenase and formate dehydrogenase (Scheme 18).[104]



Scheme 18. Deracemization of DL-methionine to L-methionine by a multienzyme system.

Aiming at the development of more efficient whole-cell biocatalysts, deracemization of 4-chlorophenylalanine was reported using a 'designer bug,' i.e., *Escherichia coli* cells expressing an L-amino acid aminotransferase from *Sinorhizobium meliloti* ATCC 51124. The enantiopure L-α-amino acid was obtained in high optical yield *via* the tandem action of D-amino acid dehydrogenase from the *E. coli* host cell (induced by L-alanine in the medium), and the cloned L-amino acid aminotransferase from *Sinorhizobium meliloti*.[105]

5 Chemical Methods

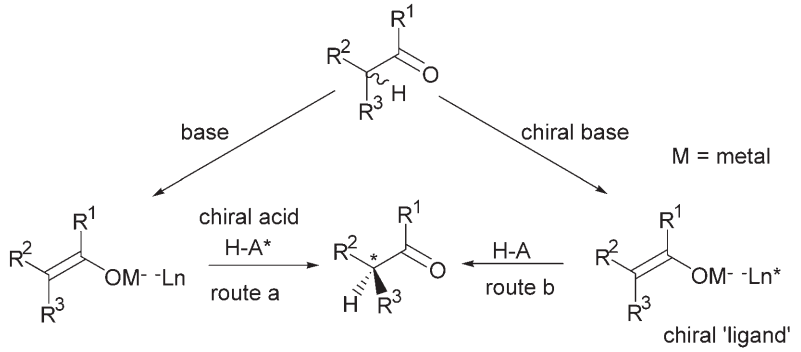
5.1 Enantioselective Protonation

Despite the large number of deracemizations based on biocatalytic methods, several protocols have been developed based on chemo-catalysis alone. One of the most widely employed systems is the two-step enantioselective protonation of a prochiral enolate derived from a C–H acidic substrate like ketones, esters, and amides using chiral protonating agents (Scheme 19, route a), or chiral aggregates of achiral acids (Scheme 19, route b). This procedure represents a 'one-pot, two-step' methodology and has been widely used employing a stoichiometric or catalytic amount of the chiral protonating/deprotonating agent. Since this technique has been recently reviewed,[106] we refer to this publication for further information.

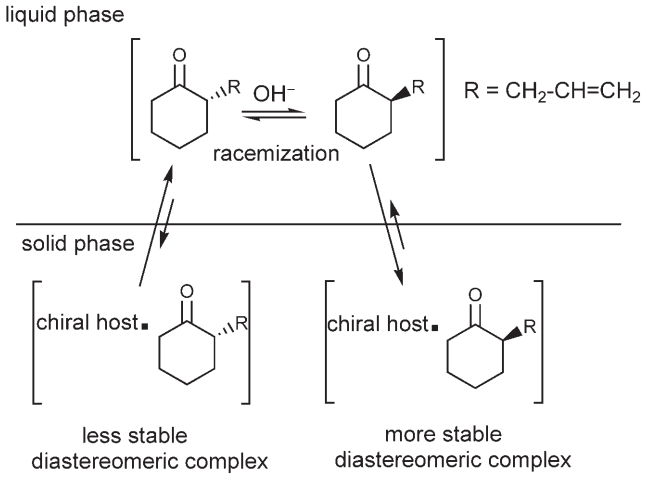
5.2 Chiral Environment

An intriguing method termed 'thermodynamically controlled deracemization' was employed by Tsunoda and co-workers to deracemize 2-allylcyclohexanone to furnish a single enantiomer in 93% *ee* using a solid-liquid two-phase system (Scheme 20).[107] While the chiral solid phase formed diastereomeric host-guest complexes with the substrate enantiomers, thereby preferentially 'trapping' one of the enantiomers, the substrate was racemized under basic conditions in the liquid phase. Since this system was thermodynamically controlled, it took three days for recovering the solid containing the complexed (*R*)-2-allylcyclohexanone.

Close inspection reveals that this method is a two-step procedure: formation of the diastereomeric substrate-host complex in the first step is followed by the liberation of the substrate in a second step. The same is true for the following example from a recent paper (Scheme 21).[108] Deracemization of an α,α-disubstituted hydantoin derivative was achieved by treatment



Scheme 19. Deracemization of C–H acidic compounds (ketones, esters, amides) *via* an achiral enolate intermediate using a chiral protonating agent (route a) or using an achiral protonating agent on a chiral aggregate (route b).



Scheme 20. Deracemization of 2-allylcyclohexanone using a chiral solid environment (host) which forms a more stable complex with one of the two enantiomers.

with brucine to affect precipitation of the brucine-(*S*)-hydantoin complex, while in situ racemization took place under basic conditions *via* a (presumed) achiral quinone intermediate. After decomplexation by neutralization with HCl, the enantiopure (−)-(*S*)-hydantoin derivative was obtained.

A similar system as the above, was first reported by Havinga as early as 1954.[109] Spontaneous resolution of enantiomers by crystallization as described already by Pasteur was combined with in situ racemization of the enantiomers, similar to the example given in Scheme 20. Controlled crystallization of *rac*-*N*-ethyl-*N*-allyl-*N*-methylanilinium iodide from chloroform, possessing one asymmetric nitrogen, led to an enantioenriched product.

6 Conclusions

Due to the fact that the transformation of a racemate into a single stereoisomer *via* stereoinversion requires remarkably little energy and therefore is a process with a (theoretical) atom efficiency[110] of 100%, it is to be expected that increasing attention will be paid to the development of such techniques in general. This review summarizes state-of-the-art concepts for deracemization of compounds possessing a single chiral *sec*-alcohol or amino group *via* stereoinversion. More specifically, the intriguing mechanisms of the microbial deracemization of *sec*-alcohols are analyzed in detail.

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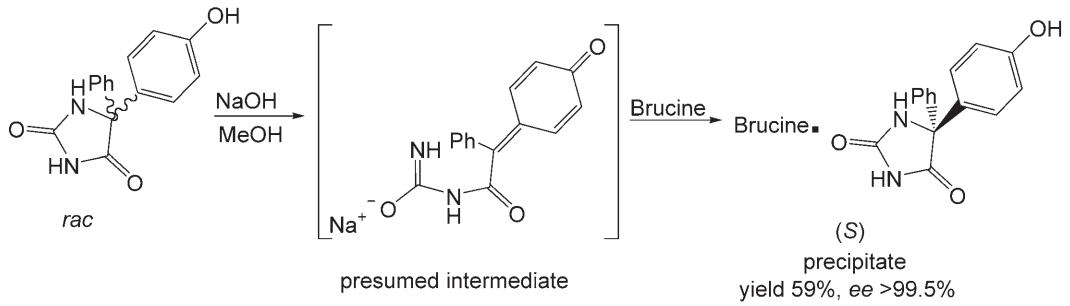

Scheme 21. Deracemization of an α,α-disubstituted hydantoin *via* diastereomeric complex formation under basic conditions.

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