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REVIEW

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Deracemisation Processes Employing Organocatalysis and Enzyme Catalysis

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Dedicated to Prof. Alicia Peñeñory on the occasion of her retirement.

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Abstract. Deracemisation methods have demonstrated their importance in the preparation of chiral compounds in the last years. In order to resolve a racemic mixture in a dynamic sense, one enantiomer of the starting material can be converted to the other through a deracemisation procedure, that can be achieved by different mechanisms based on stereoinversion or enantioconvergence, often involving two-opposite half reactions, being at least one of the reactions enantioselective enough to finally obtain an enantioenriched chiral compound.
The focus of this comprehensive review will be the application of deracemisation procedures in the present century in order to obtain optically active valuable compounds when employing non-metallic catalysts. Thus, the review will mainly focus on the use of different enzymatic preparations (purified enzymes, cell-free extracts or whole cell systems) and organocatalysts for deracemisation of racemic mixtures.

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Keywords: Deracemisation; Biocatalysis; Organocatalysis; Enantioselectivity; Chiral amines

1 Introduction

One of the most employed methodologies for the preparation of optically pure compounds at large scale includes the classical and kinetic resolution of a racemic mixture. Unfortunately, these methods present a maximum theoretical yield of 50% of the desired compounds, whereas the other 50% of the starting material is discarded.[1] In order to overcome this limitation, several strategies have been developed to transform a racemic compound into a single enantiomer with a 100% theoretical yield. Most of the described methodologies lead to the conversion of both enantiomers of the substrate into a single enantiomer of a product with high yield and optical purity, in a dynamic kinetic resolution (DKR) or in a dynamic kinetic asymmetric transformation (DYKAT) process.[2] However, these processes lead to an enantioenriched but a different chemical entity than the starting racemic compound, and a further reaction is required to obtain the enantiopure starting material.
A racemic compound can be completely converted into a single enantiomer of the same compound through a deracemisation process. Deracemisation is a very efficient procedure to obtain optically active molecules, as there is no need for further steps in order to obtain the desired compound, thus increasing the atom economy of the processes. Deracemisation procedures consist in two half-reactions that are opposite in reacting direction and completely distinct in mechanistic pathways, provided that at least one of these two reactions must be enantioselective to ensure a successful process. Redox processes are the most employed approaches in order to destroy and then create a stereocenter in a molecule for its deracemisation. The catalyst or the system of catalysts involved in the racemization should be stable enough at the conditions of both the oxidation

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and the reduction. Redox deracemisations have been mainly used for the synthesis of chiral alcohols and amines, as these compounds present stable oxidized intermediates (ketones and imines, respectively).

Deracemisations employing different strategies have been widely studied, due to the intrinsic advantages of this type of processes.[3] Apart from those in which metal catalysts are involved, with a borderline exception,[4] we will be focusing in the last advances performed for the development of deracemisation methods using enzymes and/or organocatalysts. The application of both biological systems (free enzymes or whole cells),[5] and (relatively) small organic molecules, respectively, as catalysts in organic synthesis,[6] has gained an increasing interest in the recent years, being able to carry out the preparation of valuable compounds under mild and environmentally benign conditions. Both methodologies have been successfully combined in order to develop novel synthetic routes.[7]

Deracemisation protocols have been gathered in four groups, according to the process taking place for the preparation of the chiral compound, i.e. 1) Deracemisation by stereoinversion (DbI); 2) Linear deracemisation (LD); 3) Cyclic deracemisation (CyD); and 4) Enantioconvergent process (EC), as shown in Figure 1.

We have divided this survey in two main sections: enzymatic deracemisations and organocatalysed deracemisations. Some of the described procedures employed both type of catalysts. In this case, we have considered the catalyst that imparts selectivity as the main one.

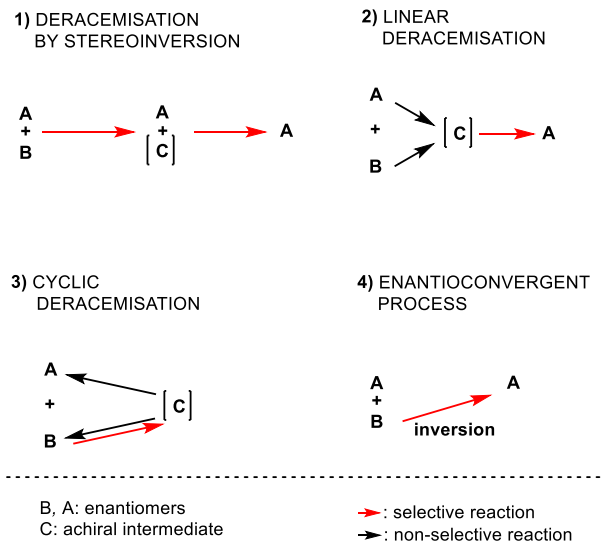


Figure 1. Pathways involved in deracemisation procedures.

Carmen Aranda studied Pharmacy in the University of Seville and received her Master's degree in biotechnology from Autonomous University of Madrid in 2014. Currently, she is a PhD student in the IRNAS Research Institute in Seville. Her research is focused on biocatalysis using fungal peroxygenases and more recently on engineering such enzymes for improving their activity and selectivity.

Gabriela Oksdath.Mansilla obtained her degree in Chemistry (2006) at Córdoba National University (Argentina). Then, she joined Prof Alicia Peñeñory's group as a Ph.D. CONICET-fellow, dealing with Photoinduced Electron Transfer processes (2010). In 2008 she spent a two months in the Bergische Universität, Gesamthochschule Wuppertal, Physikalische Chemie (Germany), under the supervision of Prof. Ian Barnes. In 2017 she spent one month in the Faculty of Chemistry and Pharmacy, University of Regensburg, under the supervision of Prof. Burkhard König. In 2019 she spent eight months in the Institute for Nanoscale Science and Technology, College of Science and Engineering; Flinder University, Australia under the supervision of Prof. Colin Raston and Prof. Justin Chalker. Since 2013 she is a researcher at the National Research Council of Argentina, Córdoba National University, Argentina, where she is currently developing new synthetic strategies using continuous-flow technology. Her research interest is the development of greener, scalable and innovative approach for preparing organosulfur- and organoselenium-containing compounds of interest.

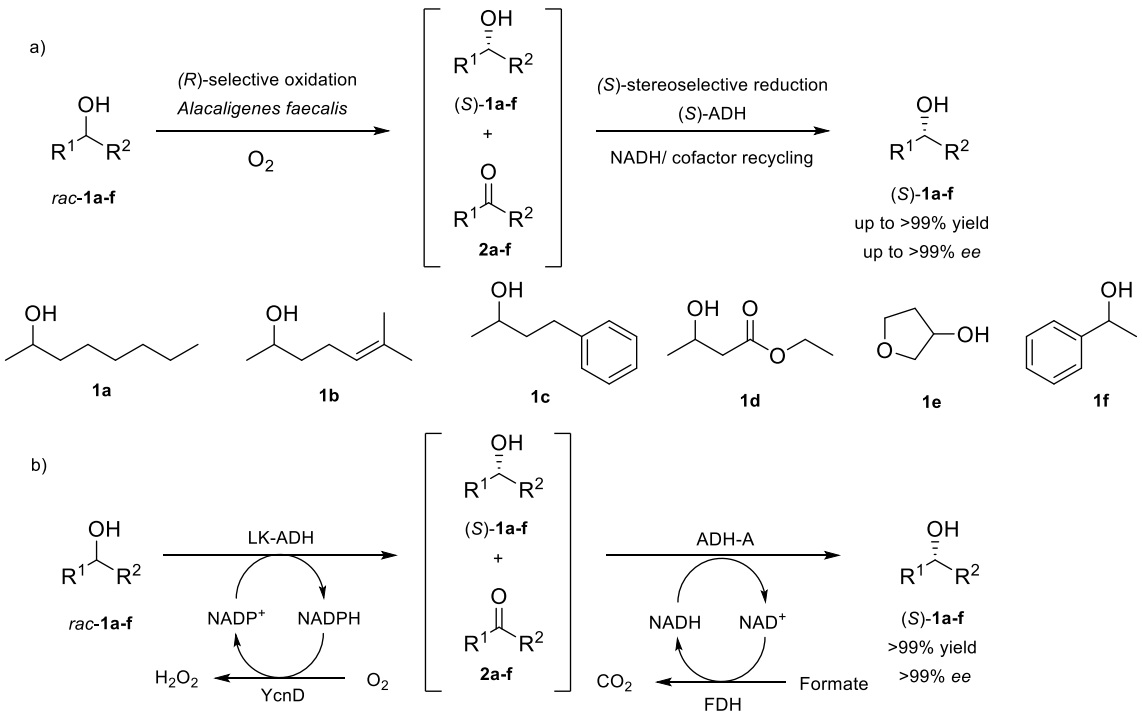
Fabricio R. Bisogno obtained his degree in Biochemistry (2004) at San Luis National University (Argentina). In 2007, he spent half a year at the Weizmann Institute of Sciences (Israel, Prof. Meir Lahav) involved in research on the origin of homochirality. Then, he pursued PhD studies at University of Oviedo (Spain, Prof. Iván Lavandera and Prof. Vicente Gotor), dealing with biocatalytic redox processes (2010). Besides, in 2012 he obtained another PhD degree at San Luis National University (Argentina, Prof. Marcela Kurina-Sanz) working on fungal biotransformation of bioactive compounds. After postdoc periods in Oviedo and Córdoba (Argentina), in 2013 he joined the Prof Alicia Peñéñory's group as researcher at Córdoba National University and INFIQC-CONICET (Argentina). His research interest comprises combination of biocatalysis with metal- and organocatalysis for the construction of cooperative systems, along with exploration of novel reactivities for organosulfur and –selenium containing compounds in enzymatic or biomimetic processes.

Gonzalo de Gonzalo obtained his Ph.D. in 2003 at the University of Oviedo, working on the field of biocatalysis employing lipases and oxynitrilases. He spent his postdoctoral research at Consiglio Nazionale delle Ricerche (ICRM, Milano, Italy), moving back to University of Oviedo with a Juan de la Cierva Fellowship. After a one-year postdoctoral stage at University of Groningen (The Netherlands) working in the research of novel oxidative biocatalysts, he spent two years at the R&D Department of the pharmaceutical company Antibióticos S.A.U. (León, Spain). He obtained a Ramón y Cajal (MINECO, Spain) contract at the Universidad de Sevilla and he is currently an Associate Professor at this university. His research is focused on asymmetric synthesis by using different approaches, including biocatalytic and organocatalytic procedures, as well as the development of concurrent chemo- and biocatalytic reactions.

2 Enzymatic deracemisation procedures

2.1 Deracemisation by stereoinversion

The deracemisation by stereoinversion using different biocatalysts is an oxidation/reduction process in which one enantiomer of the racemic starting material is oxidized to a prochiral intermediate, which can be detected in small amounts in the reaction medium.[8] This prochiral compound is then selectively reduced by a catalyst with the opposite enantiopreference, leading to one enantiomer of the starting material with high yields and optical purities. Most of the examples of deracemisation by stereoinversion have been developed with secondary alcohols, compounds widely employed in different fields as pharmaceutical industry, flavour and fragrances, food and material sciences. Initially, some examples were successfully developed in presence of different ruthenium catalysts.[9] Regarding the use of biocatalysts, initial studies were developed in two-step processes. Thus, racemic lactate was converted into D-lactate coupling the L-specific oxidation of the racemate with L-lactate oxidase from *Aerococcus viridans* with the non-selective reduction of pyruvate to racemic lactate with sodium borohydride.[10] Another example was shown in the deracemisation of racemic 2-hydroxyacids into the (*R*)-enantiomers developed with high yields and enantioselectivities by combining the selective oxidation of the starting materials with molecular oxygen catalysed by the glycolate oxidase from spinach (*Spinacia oleracea*), with the non-selective reduction of the 2-oxo acids by the D-lactate dehydrogenase from *Lactobacillus leichmannii*.[11]



Scheme 1. Biocatalysed stereoinversion of racemic alcohols employing alcohol dehydrogenases (ADHs).[12,13]

In 2008 it was described the one-pot deracemisation of secondary alcohols using two biocatalysts in a tandem reaction sequence using molecular oxygen and a cofactor recycling system as additional reagents

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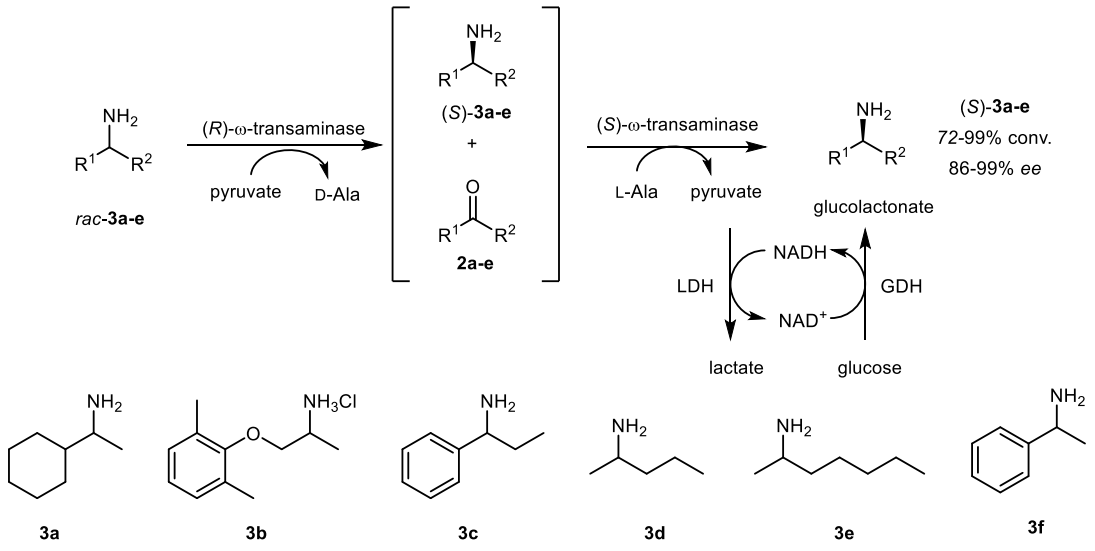
(Scheme 1a).[12] Initial experiments with different microorganisms showed promising results when employing both lyophilized and resting cells of *Alcaligenes faecalis* DSM13975. Thus, enantiopure (*S*)-2-octanol (**1a**) was obtained from racemic 2-octanol after 22 hours, with only a 29-41% of 2-octanone, indicating that this excellent selectivity was not only due to kinetic resolution. Freshly harvested cells of *Alcaligenes faecalis* DSM13975, which catalyse the selective alcohol oxidation, were coupled with the *S*-selective ADH-A from *Rhodococcus ruber* DSM44541, which reduces selectively the ketone formed, in presence of the cofactor recycling system glucose dehydrogenase (GDH)/glucose. After 4 hours, excellent conversions and enantiomeric excesses were achieved for both (*S*)-2-octanol (**1a**) and (*S*)-sulcatol (**1b**), with no presence of the respective ketones. This fact indicates that the reduction is the fastest step of the process. The use of RE-ADH (ADH from *Rhodococcus erythropolis*) or 2-propanol as cofactor recycling system led to the same excellent results. The system was extended to several secondary alcohols, recovering the (*S*)-alcohols with excellent yields and no trace of the ketones after 8-16 hours. Optical purities depend on the substrate structure, being possible to work at substrate concentrations of 100 g L^{-1}. The deracemisation was applied to obtain the (*R*)-alcohols by using an anti-Prelog ADH as the *Lactobacillus kefir* (LK-ADH) in the reduction step. No ketone was observed for all the alcohols tested, being possible to achieve better selectivities for some substrates when compared with ADH-A. This methodology was carried out in a preparative scale starting from 485 mg of (±)-4-phenyl-2-butanol (**1c**), which was treated in presence of *Alcaligenes faecalis* DSM13975, RE-ADH and GDH/glucose. After 16 hours at 30ºC, enantiopure (*S*)-**1c** was recovered in 91% yield without any other product in the reaction. The enzymes involved in the *Alcaligenes faecalis* DSM13975-catalysed oxidation of alcohols to ketones are an (*R*)-alcohol oxidase together with NADH oxidase to regenerate the cofactor at the expense of molecular oxygen. This fact allowed to develop a more general process for the deracemisation of racemic secondary alcohols.[13] Thus, a cell free extract of *A. faecalis* was combined with a NADPH-dependent (*S*)-selective ADH (from *Thermoanaerobium brockii*) coupled with formate dehydrogenase (FDH)/formate for cofactor recycling. At these conditions, racemic **1a** was completely converted to the (*S*)-enantiomer with 48% *ee* after 16 hours. It was observed that two cofactor-recycling systems could be employed in solution with different purposes. One system was used to recycle the oxidize NAD^+, whereas the other one to reduce NADPH. In view of this, a novel system with purified ADHs was developed, as shown in Scheme 1b. The oxidation step was catalysed by the (*R*)-selective LK-ADH, selecting the FMN-dependent YcnD from *Bacillus subtilis* as NADPH oxidase to regenerate this cofactor. For the reduction, the NADH-dependent (*S*)-selective ADH-A was employed together with a NADH-specific FDH. At these conditions, a wide variety of racemic alcohols were converted into their (*S*)-enantiomer with complete enantioselectivity and yield in times ranging from 6 to 24 hours. The ADHs can be exchanged by related biocatalysts with a different enantiopreference in order to obtain the (*R*)-alcohols. Thus, when the NADPH-dependent (*S*)-selective ADH from *Thermoanaerobium brockii* was combined with the NADH-dependent (*R*)-selective ADH-PR2 in presence of YcnD and FDH for the cofactor regeneration, the corresponding (*R*)-alcohols were achieved with high yields and optical purities. The deracemisation of 1-phenylethanol (**1f**) was performed at 50 mg scale using LK-ADH and ADH-A. After 16 hours at 30ºC, enantiopure (*S*)-**1f** was recovered in 90% isolated yield.

Resting cells of *Candida albicans* were able to catalyse the deracemisation of 1-phenylethanol (**1f**) by combining the oxidative kinetic resolution of the racemic alcohol to acetophenone (**2f**), with the bioreduction of **2f** catalysed by different anti-Prelog alcohol dehydrogenases.[14] *Lactobacillus brevis* CCT 3745 (LBADH) was shown to be the best catalyst, as (*R*)-**1f** was achieved from acetophenone with 90% yield and 99% *ee* after 24 hours. When *C. albicans* cells immobilized onto calcium alginate beads were used in the oxidation of racemic **1f** in presence of acetone to regenerate the NAD^+ cofactor, and after this, free *L. brevis* cells were added with 2-propanol to regenerate the NADH required for the reductive step, enantiopure (*R*)-**1f** was obtained with complete conversion after 17 hours. In a similar fashion, the deracemisation using *L. brevis* cells for the oxidation step and *C. albicans* cells for the reduction one was also studied to yield (*S*)-alcohol, but with lower *ee* than for the (*R*)-enantiomer. The use of the immobilized cells during the first step of the process simplify the work-up of the reactions, as these cells can be removed from the medium before the reduction step and reused in further four oxidative steps.

In 2016 it was described the one-pot deracemisation of racemic 1-phenyl-1,2-ethanediol to its (*R*)-enantiomer by employing a *de novo* multi-enzymatic system.[15] After testing more than 20 oxidoreductases, it was found than the ketoreductase from *Candida parapsilosis* CCTCC M203011 (SCR1) was the best biocatalyst in the enantioselective bioxidation of (*S*)-1-phenyl-1,2-ethanodiol to 2-hydroxyacetophenone in presence of $NADP^+$. This ketone was selectively reduced by KRD from *Zygosaccharomyces rouxii* ATCC14462 using NADPH as cofactor, in order to obtain (*R*)-1-phenyl-1,2-ethanediol with high optical purity. Optimization of reaction parameters as temperature, pH, biocatalysts ratio and $NADP^+$/NADPH, afforded the (*R*)-alcohol with 91% yield and 99% *ee* after 8 hours when combining both enzymes. Reactions were carried out at 30ºC in aqueous buffer pH 7.0 with an activity ratio of SCR1/KRD 1:4 and a molar ratio of $NADP^+$/NADPH 3:1.

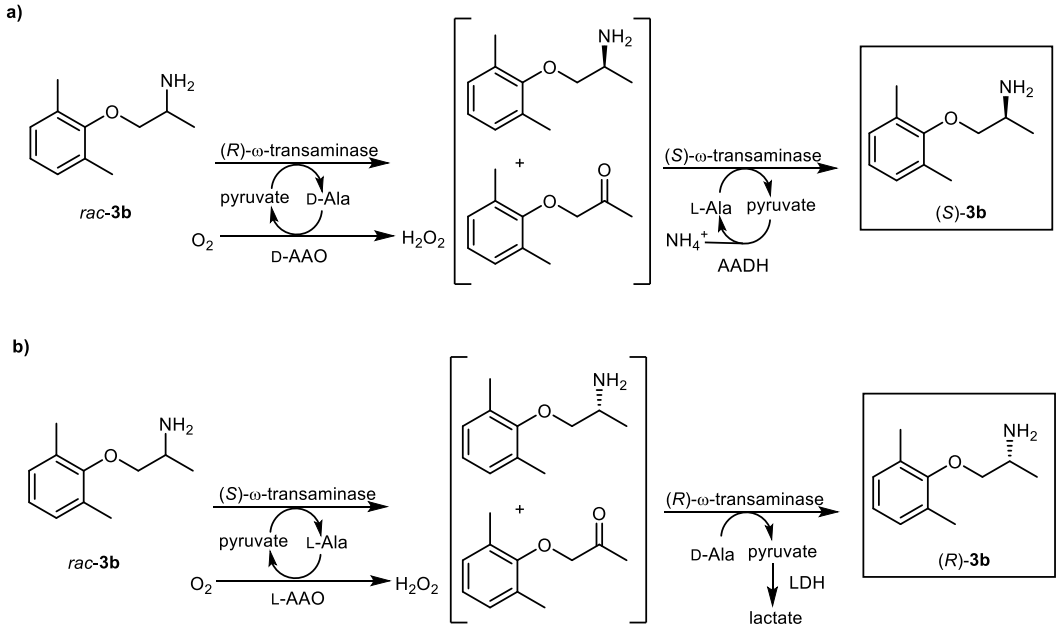
Stereoinversions have been also applied to carry out the deracemisation of racemic amines, by combining two ω-transaminases (ω-TAs) for the synthesis of these optically pure compounds.[16] In 2009, the deracemisation of racemic primary amines in a one-pot two-step process was described, combining the kinetic resolution of the starting amine with a reductive amination step of the formed ketone (Scheme 2).[17] The kinetic resolution of the racemic amines was tested in presence of different transaminases with pyruvate as amine acceptor. Three commercial enzymes (ATA-113, ATA-114 and ATA-117) were selected, achieving the enantiopure amines after 24 hours with conversions close to 50%. ATA-1134 and ATA-114 led to the (*S*)-amines, whereas ATA-117 afforded the (*R)*-amine. This kinetic resolution was coupled with the selective amination of the ketone formed in the first step employing a second ω-transaminase (with opposite enantiopreference to the first one) and L-alanine as amine donor. In order to shift the ketone-amine equilibrium, the pyruvate formed in this step was removed using lactate dehydrogenase (LDH) in a coupled reaction system. The optical purity of the amines formed was moderate, due to a partial catalysis of the amination step by the first ω-transaminase. To overcome this issue, after completion of the kinetic resolution, the reaction was heated up to 75ºC for 30 minutes to inactivate the first biocatalyst and then the second biocatalyst was added. Despite the enzyme lost using this method, enantiopure (*R*)- or (*S*)-amines were obtained with high conversions, depending on the addition order of the enzymes. The reaction was scaled up to 50 mg (20 mM concentration) for the deracemisation of 1-cyclohexylethan-1-amine (**3a**). After 48 hours, the enantiopure (*S*)-**3a** was obtained with 98% conversion (92% isolated yield). When starting from racemic 1-(2,6-dimethylphenoxy)-2-propanamine (**3b**), enantiopure (*S*)-**3b** was recovered with 95% yield. In a further development to avoid the need of inactivate the ω-TA employed in the kinetic resolution, two opposite enantioselective ω-TAs were used in the one-pot one-step deracemisation of racemic primary amines.[18] In the first step, the (*S*)-enantiomer of the amine was converted to the ketone by a (*S*)-ω-TA in presence of an amino acceptor. In the second step, the ketone is converted to the (*R*)-amine using a (*R*)-selective ω-TA and an amino donor. As a requisite, the (*R*)-ω-TA has to show a very low activity toward the amino acceptor used in the first step. For this process, the (*R*)-selective ω-TAs from *Mycobacterium vanbaalenii* (ω-TAMV) and from *Neosartorya fischeri* (ω-TANF) and the (*S*)-selective from *Polaromonas* sp. JS666 (ω-TAPO) and *Vibrio fluvialis* JS17 (ω-TAVF), were studied. The specificity of these enzymes on different amine acceptors (seven ketoacids and two ketoesters) was tested, being observed that the use of 2-oxopentanedioic acid was the best choice, as it is inexpensive and showed a high activity for the *Polaromonas* sp. JS666 transaminase and no activity for the (*R*)-selective biocatalysts. In addition, the side product glutamate can be easily removed from the reaction. After separately optimization of the two reaction steps, namely the kinetic resolution and the amination; deracemisation of α-methylbenzylamine (**3f**, 50 mM) was performed in presence of pyridoxal phosphate (PLP) as cofactor and LDH for removing 2-oxopentanedioic acid. After 48 hours, enantiopure (*R*)-methyl benzyl amine was recovered with almost complete conversion when using both ω-TAPO/ω-TAMV and ω-TAPO/ω-TANF. This system was extended to the deracemisation of other aromatic amines, obtaining the enantiopure (*R*)-enantiomers with excellent conversions.



Scheme 2. Stereoinversion of racemic primary amines (**3a-e**) employing ω-transaminases.[17]

By the same year, a similar method was carried out for the effective deracemisation of mexiletine (**3b**, Scheme 3),[19] a chiral amine clinically used in cardiac disorders, currently employed as racemate. Besides, the (*S*)-enantiomer has attracted attention due to the potential use in neuromuscular disorders.[20] Authors developed a one-pot two-steps procedure featuring a kinetic resolution followed by a stereoselective amination. This strategy lies on the sequential activity of two enantiocomplementary ω-transaminases, thus leading to any **3b** enantiomer (depending on the addition order of the ω-transaminases) with the concomitant recycling of the cosubstrate (pyruvate) needed by the action of an amino acid oxidase (AAO). Thus, in the first step a (*R*)-selective ω-transaminase was applied to selectively transform the (*R*)-enantiomer into the corresponding ketone using pyruvate/D-AAO catalytic system in the presence of O_2. In a second step, a (*S*)-selective ω-transaminase catalysed the formal reductive amination of the formed ketone into enantiopure (*S*)-mexiletine using ammonium cation released in the previous step and L-alanine in catalytic amounts (see Scheme 3a). This L-alanine was recycled from pyruvate using an L-alanine dehydrogenase (AADH). In a similar fashion, the use of a (*S*)-selective ω-transaminase in the first step together with a (*R*)-selective one for the reductive amination of the ketone led to enantiopure (*R*)-**3b**, as shown in Scheme 3b. On a bigger scale, this procedure was successfully applied to the preparation of 100 mg of each enantiomer of mexiletine in 48 h working at 28 mM substrate concentration.

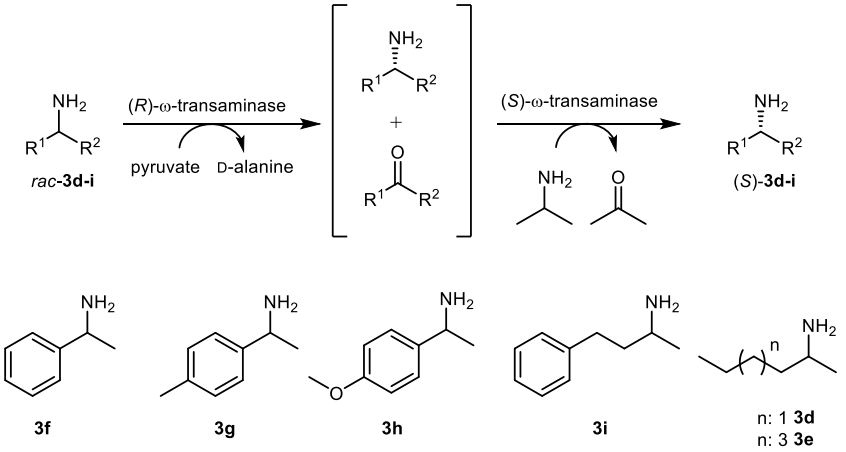
A further development for the application of two enantiocomplementary ω-transaminases in the synthesis of optically amines has been recently published by applying an engineered metabolic pathway.[21] Thus, the deracemisation of several aromatic and aliphatic racemic amines to the (*S*)-enantiomers has been carried out by combining the deamination catalysed by the (*R*)-selective ω-TA from *Arthrobacter* sp. (ARTA) in presence of pyruvate as cofactor, with the deamination of the ketones formed catalysed by the (*S*)-selective ω-TA from *Ochrobactrum anthropi* (OATA), using isopropylamine as amine source, as shown in Scheme 4. Due to the fact that pyruvate and isopropylamine are substrates for both ARTA and OATA, a number of secondary undesired reactions can occur during the deracemisation process. These secondary reactions have been suppressed by carrying out process and protein engineering strategies, controlling the reaction pathway to achieve only the desired reactions. The combination of thermodynamic and kinetic control afforded the (S)-amines with excellent results both *in vivo* and *in vitro*. The processes have been improved by using an ARTA mutant with improved activity, whereas the metabolic removal of acetone form the reaction medium supresses some undesired side reactions. Both ω-TAs have been expressed in *E. coli* cells where native glycolysis or reinforced deamination of L-threonine has been exploited to supply α-keto acid to the system. As an example, (*S*)-α-methylbenzylamine can be achieved with 95% reaction yield and 99% *ee* after 7 hours.



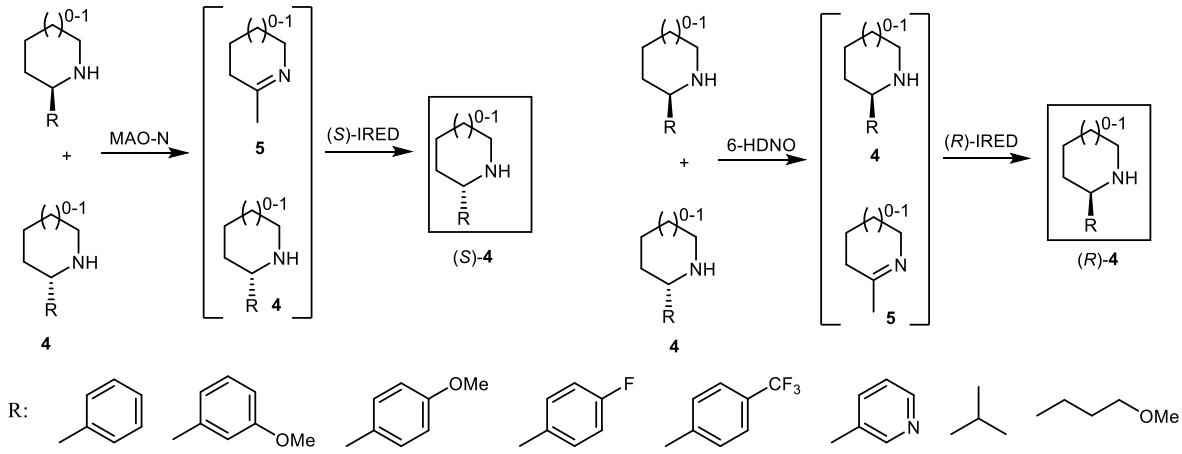
Scheme 3. Deracemisation of mexiletine (**3b**) employing ω-transaminases.[19]

In a different approach, the combination of engineered amine oxidases (AOs) and imine reductases (IREDs)[22] in a one-pot reaction, allowed the deracemisation by stereoinversion of cyclic amines, affording enantioenriched 2-substituted pyrrolidines (**4**) with high yields and *ee* values

(Scheme 5). In this approach, starting from the racemic amine, either the (*S*)-selective monoamine oxidase from *Aspergillus niger* (MAO-N)[23] or the (*R*)-selective 6-hydroxy-D-nicotine oxidase (E350L/E352D variant)[24] from *Arthrobacter nicotinovorans* (6-HDNO),[25] selectively oxidizes one enantiomer of amine **4** to the imine **5**, whereas the antipode remains intact. Then, the IRED stereoselectively reduces the formed imine into the amine but of opposite handedness. In this way, half of the starting material remains untouched while the other half undergoes stereoinversion. In order to obtain the highest enzymatic activity for both 6-HDNO/MAO-N and IRED enzymes, different reaction conditions were screened, such as pH, buffer concentration and cosubstrate (glucose). It must be emphasized that the selectivity of both MAO and IRED should match and has to be carefully checked for each substrate. With the optimized conditions, the one-pot system was scaled up. Thus, (*S*)-2-phenylpiperidine was produced on a 0.25 g scale starting from the racemic amine. Finally, a substantial improvement in terms of atom economy and a time reduction of 2-4 h in the deracemisation process was observed using IRED (DbI) instead of ammonia borane ($NH_3 \cdot BH_3$), as can be observed in section 2.3.



Scheme 4. Deracemisation of racemic amines employing two ω-transaminases with metabolic pathway control.[21]

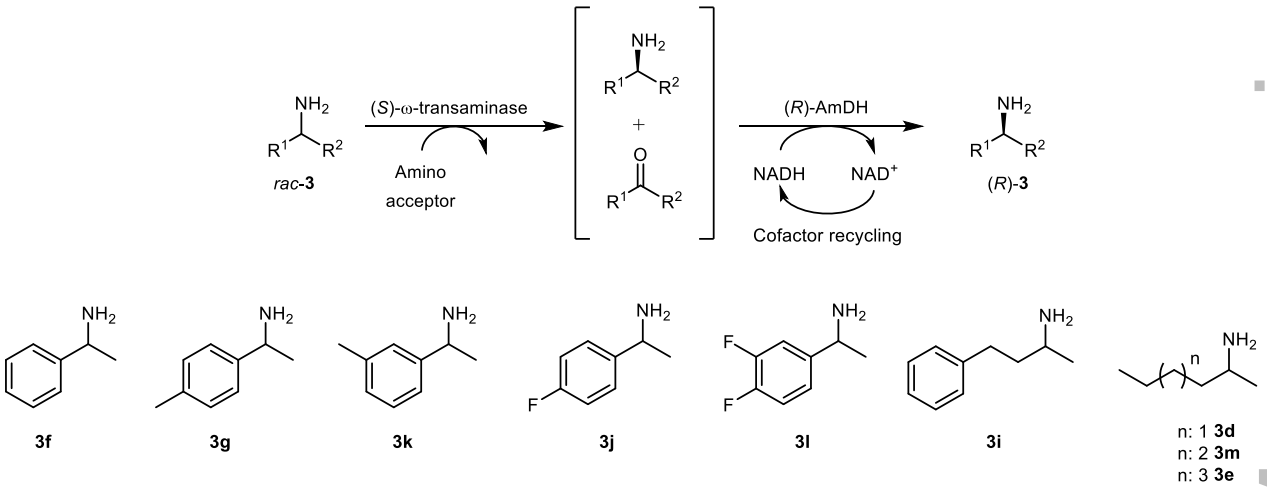


Scheme 5. Stereoinversion of 2-substituted pyrrolidines and piperidines by combining monoamine oxidases and imine reductases.[25]

In 2019 it has been described the deracemisation of a set of racemic amines to the corresponding (*R*)- or (*S*)-enantiomers by combining ω-TAs and amine dehydrogenases(AmDHs) in a biocatalytic cascade process.[26] In order to obtain the (*R*)-amines, the initial step consist in the selective deamination of racemic amines catalysed by different (*S*)-selective ω-TAs, as shown in Scheme 6. Best results were achieved by whole-cells of the ω-TA from *Polaromonas* sp. JS666 (ω-TAPO). The second step was the (*R*)-selective amination of the ketones obtained, catalysed by L-phenylalanine dehydrogenase of *Rhodococcus* sp. (*Rs*-AmDH) or a chimeric AmDH (Chi-AmDH). Processes were optimized at pH 9.5 employing formate dehydrogenase (FDH) from *Pseudomonas* sp. and glucose dehydrogenase (GDH) from *Bacillus subtilis* for NADH regeneration. Having both the AmDH and

the ω-TA whole-cell processes optimized, they were tested in tandem for the deracemisation of different racemic aromatic and aliphatic amines. After optimizing the use of 15% v/v of DMSO as cosolvent and modifying the vector system for the expression of the AmDHs, high conversions were obtained for almost all the amines tested (80-99%) with excellent enantioselectivities (*ee*>99%).

The same approach was further employed for the preparation of the enantiopure (*S*)-amines. Thus, the enantioselective deamination of racemic amines was catalysed by (*R*)-selective AmDHs, followed by the enantioselective amination by (*S*)-selective ω-TAs. Unfortunately, the equilibrium of both processes was highly unfavourable. In order to obtain synthetically valuable reactions, benzylamine was employed as amino donor as in the ω-TA-catalysed aminations. Benzaldehyde formed in the deamination of benzyalmine is used by the aldehyde reductase (AHR) from *Synechocystis* sp. to give benzyl alcohol and regenerate NAD^+ cofactor for AmDH. The efficiency of this system when coupling AmDH with the ω-TA from *Vibrio fluvialis* JS17 was tested in the deracemisation of 4-phenylbutan-2-amine to its (*S*)-enantiomer with complete conversion and 95% *ee* after 24 hours in presence of 20 mM benzylamine and 0.2 mM benzaldehyde. A set of 13 racemic amines was tested, achieving the corresponding (*S*)-amines with high conversions and enantioselectivities.



Scheme 6. Deracemisation of racemic amines to the (*R*)-enantiomers combining ω-TAs and amine dehydrogenases(AmDHs).[26]

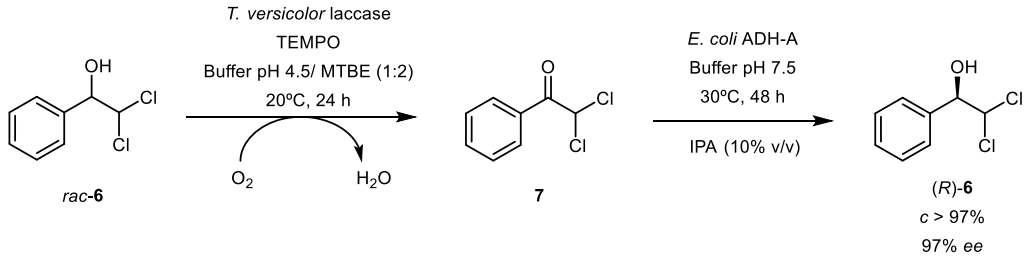
2.2 Linear deracemisation employing biocatalysts

Linear deracemisations are slightly different to the stereoinversion protocols, as in this case, both enantiomers of the starting material are oxidized to a prochiral intermediate, which is selectively reduced to a single enantiomer of the starting material with high yields.

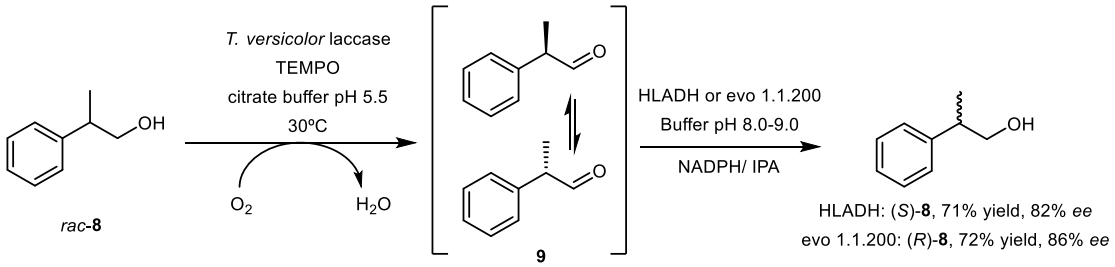
This methodology has been also applied to the preparation of chiral secondary alcohols. Thus, the deracemisation of 2,2'-dichloro-1-phenylethanol (**6**) was performed through linear deracemisation employing an iridium catalyst for the oxidation of the alcohol to the ketone, and an ADH for the selective bioreduction of the ketone in a one-pot process.[27] But this deracemisation occurred with a moderate selectivity, being recovered (*R*)-**6** with 40% *ee*. In order to improve this result, a novel methodology was described in 2014. Thus, the one-pot alcohol **6** oxidation catalysed by the *Trametes versicolor* laccase /TEMPO system, was followed by the selective reduction of ketone **7** employing *E. coli* ADH-A cells (Scheme 7).[28] After 24 hours of the laccase/TEMPO oxidation at pH 4.5 in presence of *tert*-butyl methyl ether (MTBE) at 33% v/v as cosolvent, pH was adjusted to 7.5 by addition of Tris and HCl, in order to inactivate the laccase. Then *E. coli* ADH-A and *iso*-propanol as hydrogen donor (10% v/v) was added. After 48 hours at 30ºC, (*R*)-**6** was recovered with complete conversion and 97% *ee*.

An interesting example in which two different enzymes are used in the oxidation/reduction process has been shown in the linear deracemisation of profenol (2-phenyl-1-propanol, **8**), the key core of NSAID drugs.[29] A laccase/TEMPO system was employed for the oxidation of the alcohol to form a racemic aldehyde which is then selectively reduced by ADHs through a dynamic kinetic resolution (DKR) process (Scheme 8). The *Trametes versicolor* laccase/TEMPO system was able to catalyse the oxidation of the starting alcohol at 30 mM concentration with 85% conversion after 2 hours. A 15% of the overoxidation product (carboxylic acid) was observed, so the substrate concentration was increased to 90 mM to reduce this byproduct. ADH from *E. coli* (ADH-P) and Horse Liver ADH (HLADH) were used for the bioreduction to obtain the (*S*)-alcohol whereas evo-1.1.200 was employed for the (*R*)-enantiomer. A stepwise process was

studied starting from the racemic alcohol. After the laccase catalysed oxidation, the bioreduction was carried out without isolation of the aldehyde. For all the ADHs, high conversions (>95%) and good enantioselectivities (86-87% *ee*) were obtained. The deracemisation was also performed in a one-pot process at 150 mg of starting material. The laccase/TEMPO catalysed oxidation of the alcohol during 3.5 hours in citrate buffer pH 5.5 led to the aldehyde. Then, the reaction was diluted with phosphate buffer adjusting the pH to 8.0 for HLADH and 9.0 for evo.1.1.200 in order to inactivate the laccase. Then NADH and *iso*-propanol (IPA, 5% v/v) were added, obtaining the (*S*)- or (*R*)-enantiomer of the product depending on the chosen ADH, with yields around 70% and optical purities of 82-86%. Given that stereocenters at the alpha position of an aldehyde carbonyl are readily epimerized through tautomerisation, a DKR process took place. The decrease in the optical purity of the product observed in comparison with the single bioreduction reaction was attributed to side oxidation reactions.



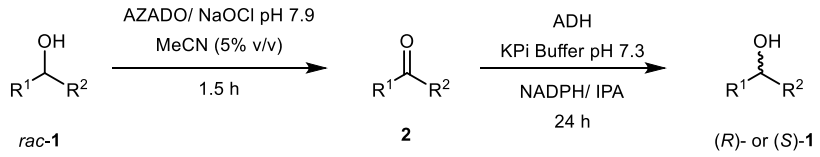
Scheme 7. Deracemisation of 2,2'-dichloro-1-phenylethanol (**6**) employing laccases and alcohol dehydrogenases.[28]



Scheme 8. Deracemisation of profenol (**8**) combining a non-stereoselective oxidation process followed by an enantioselective bioreduction under dynamic conditions.[28]

In the last few years, some sequential deracemisation examples of racemic alcohols have been described including an initial step of non-selective organocatalytic oxidation of the starting alcohol to a prochiral ketone, followed by an enantioselective reduction step catalysed by different enzymes. This strategy was followed for the preparation of chiral primary and secondary alcohols, in which the oxidation process was performed by using organocatalysts such as TEMPO or AZADO (2-azaadamantane *N*-oxyl) and the bioreduction was carried out in presence of alcohol dehydrogenases. Thus, a set of aromatic and aliphatic secondary racemic alcohols were converted into the pure enantiomers in one-pot procedure, in which the nonselective oxidation using TEMPO/iodine was followed by the bioreduction of the prochiral ketone formed using ADH with different selectivities.[30] After studying different oxidants, it was observed that best results for the alcohol oxidation in aqueous buffer were obtained TEMPO/iodine system, as this catalyst allowed the system to work in one-pot, since the activity of the ADH remained unaltered. Nevertheless, an inorganic reductant ($Na_2S_2O_3$) was required to quench the excess of iodine used in the first step, prior to the bioreduction. The optimum conditions for the complete oxidation of 1-phenylethanol into acetophenone were found in buffer Tris/HCl pH 10.0 using 0.2 equivalents of TEMPO and 1.5 equivalents of iodine at 30ºC after sonication of the mixture for 1 hour. This method could be extended to other aromatic and aliphatic racemic alcohols. For most of the substrates tested, complete conversions were achieved after 1 hour. The oxidation system was combined with the bioreduction catalysed by the Prelog *E.coli*/ADH-A, without the need of an external cofactor. Enantiopure (*S*)-alcohols were recovered after 24 hours with conversions higher than 95% for a set of aromatic and aliphatic substrates. When the anti-Prelog NADH-dependent ADHs as LBADH or evo-1.1.200 were employed, the (*R*)-alcohols (>99% *ee*) could be produced from racemates with conversions over 90%. A similar approach has been recently developed in the one-pot deracemisation of a wide variety of racemic aliphatic and aromatic *sec*-alcohols (Scheme 9). In this procedure, AZADO was employed as oxidative organocatalyst along with stoichiometric

NaOCl in combination with different alcohol dehydrogenases.[31] Commercial ADHs from Codexis© were able to selectively reduce all the ketones tested to the (*S*)- or (*R*)-enantiopure alcohols with complete conversions in presence of IPA as cosubstrate in a substrate coupled approach for the regeneration of the nicotinamide cofactor. AZADO, combined with NaOCl, shows a high catalytic potential and can be employed in the oxidation of hindered secondary alcohols. In order to effectively coupling the AZADO-catalysed oxidation with the bioreduction employing the ADHs, the main parameter to control was the salt concentration reached after the oxidation (NaCl from the NaOCl and carbonate species form the $NaHCO_3$ used to lower the pH from the commercial NaOCl solution). At the standard conditions, this concentration was very high, which reduces the solubility of the resulting ketone and leads to an incomplete reduction. To overcome this issue, addition of $NaHCO_3$ in the first step was suppressed controlling the pH of the 1-(4-trifluoromethyl)-phenylpropan-2-ol oxidation (250 mM) with phosphate buffer. After 1.5 hours of AZADO/NaOCl catalysed oxidation, IPA, $NADP^+$, the ADH and phosphate buffer pH 7.0 were added to reach a 100 mM alcohol concentration, leading to a complete conversion of (*S*)-alcohol after 24 hours. This method was extended to other alcohols, being observed that for some of them, conversions were not complete. A further dilution process in the reduction step was necessary, in order to work at ketone concentrations of 50 or 25 mM to ensure a complete deracemisation.



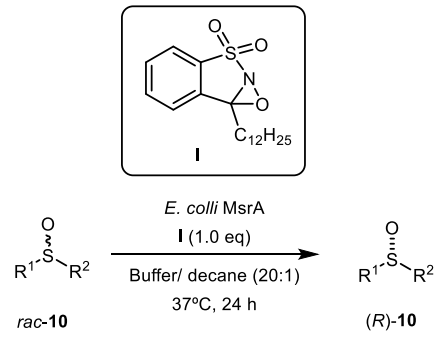
R^1: Ph, 3-MeO-C_6H_4, 3-NO_2-C_6H_4, 4-Cl-C_6H_4, cyclohexyl.
R^2: Me, CH_2Cl.

Scheme 9. Deracemisation of secondary alcohols in one-pot by the non-selective oxidation reaction with AZADO organocatalyst and the enantioselective reduction by alcohol dehydrogenases.[31]

2.3 Cyclic deracemisations using biocatalysts

A process in which a selective biocatalysed process in one redox direction is combined with a non-selective reaction in the opposite direction that, after a certain number of redox cycles, furnish an optically enriched product starting from its racemate is often called cyclic deracemisation (CyD). The idea behind this system is that the non-reactive enantiomer accumulates during the process whereas the reactive one is converted into the achiral intermediate, provided that one biocatalyst is enantioselective enough. The most common approach makes use of selective oxidative enzyme along with a non-selective reductant. In this way, several *sec*-alcohols and amines have been deracemised through the corresponding achiral keto- or imino- (iminium-) derivative, respectively. Although far less explored, the joint use of a selective reductive enzyme and a non-selective oxidant can be set up, as the example show below for the deracemisation of sulfoxides.

Chiral sulfoxides can be also obtained through redox deracemisation procedures.[32] Methionine sulfoxide reductase A (MsrA) was employed, as this biocatalyst is excellent for the synthesis of chiral sulfoxides by kinetic resolution, reducing selectively one of the enantiomers of the starting sulfoxide to the sulfide (Scheme 10).[33] This enzyme accepts a wide range of aromatic methyl and ethyl sulfoxides. In order to carry out effective kinetic resolutions in presence of *E. coli* cells expressing MsrA, a 5% v/v of an organic cosolvent as *n*-decane was required as reservoir of the formed sulfides. The development of this biphasic system allowed looking for a lipophilic oxidant able to oxidize the sulfide back to the racemic sulfoxide and returning to the catalytic cycle in a cyclic deracemisation process. Oxidant optimization showed that the easy-to-synthesize oxaziridine **I** was the best choice for performing the deracemisation, as it was compatible with the *E. coli* cells and showed a good reactivity. Thus, racemic *p*-bromophenyl methyl sulfoxide (**10a**) was treated with the *E. coli* cells overexpressing MsrA in buffer containing the oxidant and 5% v/v *n*-decane. After 24 hours, a 79% yield of (*R*)-**10a** was isolated with >99% *ee*. In view of these results, different sulfoxides were deracemised, recovering the enantiopure (*R*)-enantiomers with yields from moderate to high (52-90%). This procedure was also applied to the deracemisation of the anti-inflammatory drug sulindac. Due to its low water solubility, 1.0 equivalent of β-cyclodextrin was added. After 24 hours, a 61% of (*R*)-sulindac with 97% *ee* was recovered.

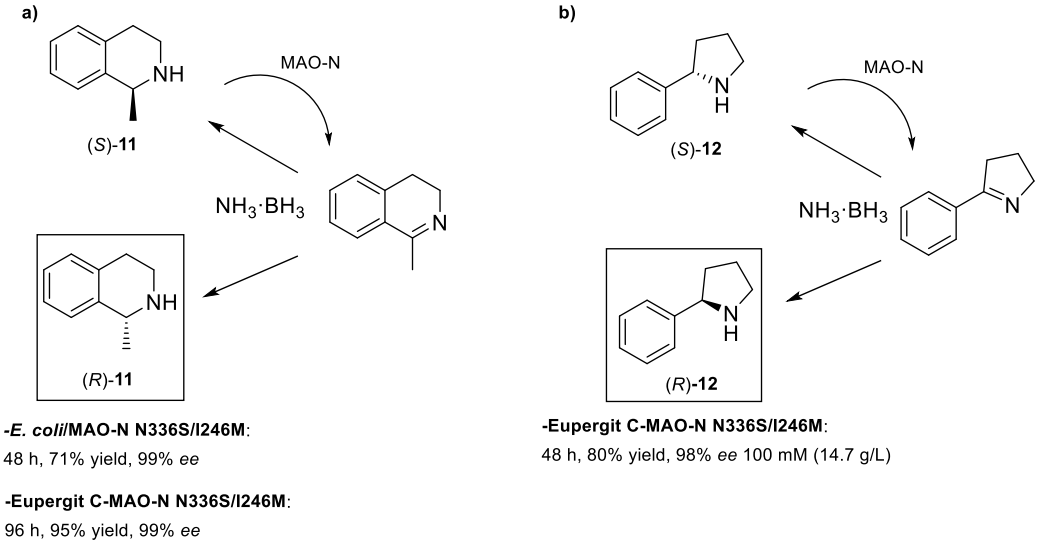


R^1: Ph, 4-OMe-C_6H_4, 4-Me-C_6H_4, 4-Br-C_6H_4, 4-OH-C_6H_4, $PhCH_2$, $PhCH_2CH_2$,
R^2: Me, Et

Scheme 10. Cyclic deracemisation for the preparation of optically active sulfoxides.[32]

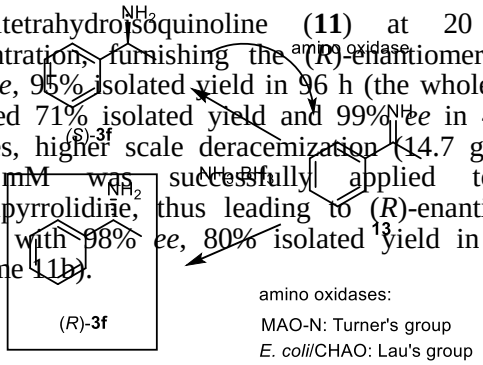
Resting cells of *Candida albicans* CCT 076 are able to selectively oxidize (*S*)-1-phenylethanol (**1f**) to acetophenone (**2f**), which was then reduced by a partially (*S*)-selective enzyme.[14] In this way, (*R*)-**1f** was accumulating after several redox cycles in a cyclic deracemisation, in which the oxidation step was highly selective and fast, whereas the reduction was slower and partially selective. After 6 days, chiral alcohol can be obtained with 82% isolated yield and 98% *ee*.

In a pioneering work, an extremely selective monoamino oxidase variant, MAO-N, was obtained through directed evolution by using a wild type MAO isolated from *Aspergillus niger* as starting point. After ca.150000 colonies screening, the authors were able to find and characterize this improved mutant (Asn336Ser), not only in terms of activity but also in terms of enantioselectivity (from 17:1 to >100:1 towards *rac*-α-methyl benzylamine **3f**) With this biocatalyst in hand, a CyD process was envisaged as shown in Scheme 9 and therefore, a set of readily available reductants were screened ($NaBH_4$, Pd/C-HCOONa, $NH_3{\cdot}BH_3$) being amine borane complex best suited for this CyD process.[34] Thus, the whole system was challenged towards *rac*-α-methyl benzylamine (1 mM) and (*R*)-**3f** was isolated in 77% yield and 93% *ee*.



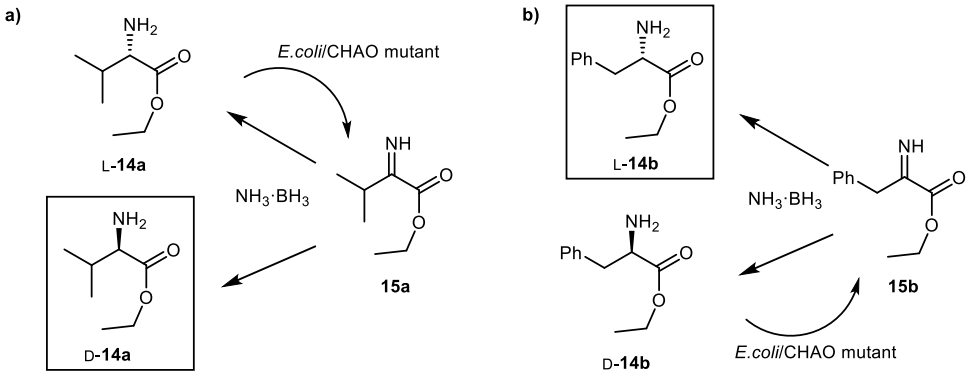
Scheme 11. CyD of racemic secondary amines employing amine oxidase and a chemical reductant.[35]

2005, an improvement of the previously reported amine CyD was achieved by random mutagenesis and screening over the MAO isolated from *Aspergillus niger*.[35] In this report, the authors were able to identify and characterise a MAO-N variant that readily accepted and efficiently deracemised secondary amines when combined with a typical non selective reducing agent $NH_3{\cdot}BH_3$. Firstly, *E. coli* whole cells overexpressing the double mutant MAO-N Asn336Ser/Ile246Met was used in analytical scale to screen possible substrates with different structural features. With the best accepted substrates, preparative scale CyD reactions where performed at 20 mM substrate concentration (Scheme 11a). The enzyme was purified and covalently immobilised onto Eupergit C resin, affording a robust catalyst that facilitated purification and improved isolated yields, albeit at a lower rate. Best results were obtained in the deracemization of racemic 1-methyltetrahydroisoquinoline (**11**) at 20 mM concentration, furnishing the (*R*)-enantiomer with 99% *ee*, 95% isolated yield in 96 h (the whole cells afforded 71% isolated yield and 99% *ee* in 48 h). Besides, higher scale deracemization (14.7 g/L) at 100 mM was successfully applied to 2-phenylpyrrolidine, thus leading to (*R*)-enantiomer, (*R*)-**12** with 98% *ee*, 80% isolated yield in 48 h (Scheme 11b).



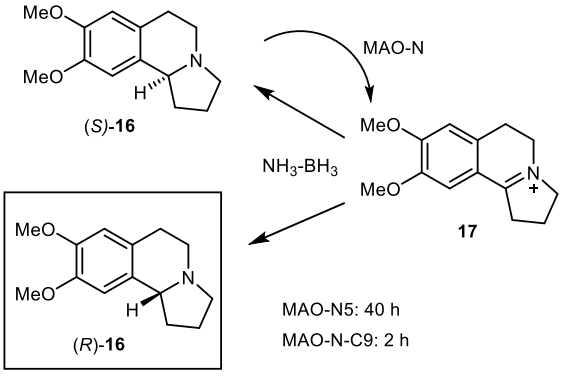
Scheme 12. CyD of racemic primary amines employing amine oxidases and a chemical reductant.[36]

In 2012 (Scheme 12) a related work was reported dealing with either whole cell biocatalyst (*E. coli* overexpressing cyclohexylamine oxidase CHAO derived from *Brevibacterium oxydans* IH-35A) or crude enzymatic preparation instead of fungal or mammalian-derived enzymes.[36] Employing such bacterial biocatalysts together with ammonia-borane complex for the efficient CyD of *rac*-α-methyl benzylamine, enantiopure (*R*)-**3f** could be isolated.



Scheme 13. Cyclic deracemisation of amino esters by employing cyclohexylamine oxidase and $NH_3 \cdot BH_3$.[37]

A recent application of these CHAO enzymes has been shown in the CyD of amino esters (**14a-b**). A set of mutations was performed in the entrance of the active site to widen the substrate acceptance.[37] In this line, the CHAO mutants were able to resolve *rac*-valine and *rac*-phenylalanine as ethyl esters derivatives, among other amino acids. By combining *E. coli*/CHAO mutant (Y321I/M226T) and $NH_3 \cdot BH_3$ at pH 6.5 and 30 °C, an efficient CyD was accomplished. After acid hydrolysis, a 95% yield was obtained for D-valine (**14a**, Scheme 13a). Interestingly, for *rac*-phenylalanine ethyl ester (**14b**, Scheme 13b), a complete reversal of enantioselectivity was observed as compared with the wild type CHAO, thus affording the unexpected L-**14b.** Crispine A (**16**) is an alkaloid isolated from *Carduus crispus*, for which there are several enantioselective syntheses reported. In particular, a CyD approach was developed employing MAO-N5 variant and amine-borane complex, affording **16** in overall 48% yield and >97% *ee* after 40 hours of deracemisation process (Scheme 14).[38]



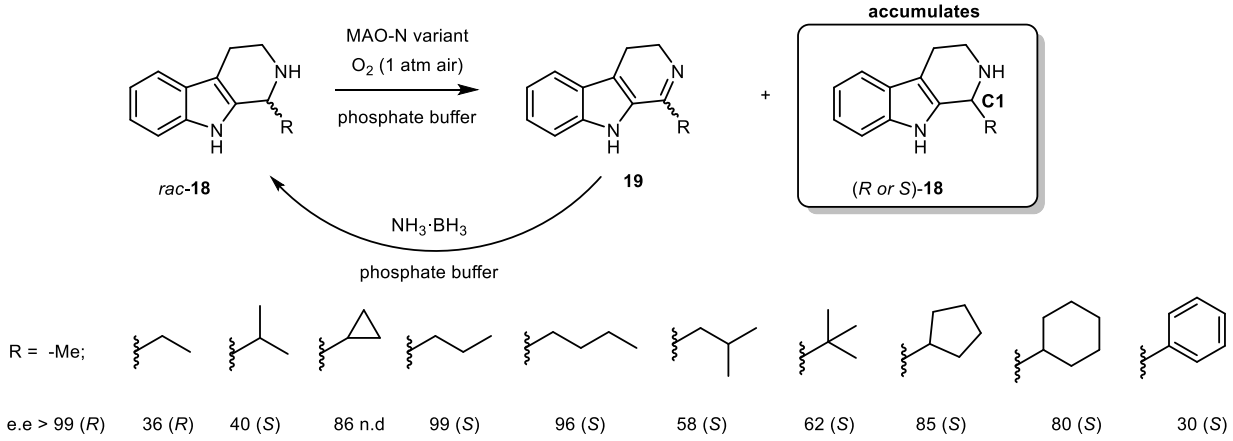
Scheme 14. Cyclic deracemisation of crispine A (**16**) employing MAO-N5 and its mutant.[38]

The authors noticed that a similar compound lacking the two methoxy groups in the aromatic ring, afforded a similar result after only 6 hours. Thus, in a further publication, a rational redesign of the MAO-N5 was conducted in order to fix the steric limitations exerted by these two methoxy groups. To do so, mutant libraries replacing key amino acids were constructed and screened. The more active mutant was selected for further rounds of optimization. In this way, a 990-fold increase in the specific activity was achieved for MAO-N-9C, a mutant featuring four mutated residues (F210L, L213T, M242Q and M246T9) from MAO-N5. This mutant allowed the deracemisation of crispine A in only two hours.[39]

In the field of natural product asymmetric synthesis, Turner´s group developed a CyD strategy for the asymmetric synthesis of a series of tetrahydro-β-carbolines (THBCs, **18**). This kind of heterocyclic compounds, with complex molecular architectures, is an important family of bioactive alkaloids and constituted a source of inspiration for many synthetic organic chemists. In this work, the authors carried out the successful CyD of 1-substituted β-carbolines derivatives employing MAO-N variants (Scheme 15).[40]

In this way, using a variant of MAO-N in combination with $NH_3 \cdot BH_3$ as a non-selective chemical reducing agent, the deracemisation of these compounds was accomplished thus achieving the β-carboline derivatives with high optical purity in most cases (from 30% to > 99%). Specifically, two

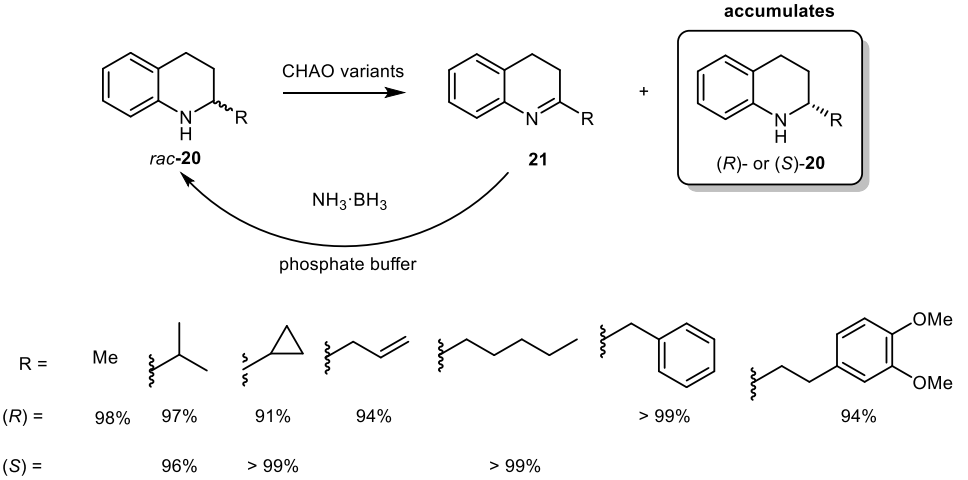
different variants of the monoamine oxidase from *Aspergillus niger* (MAO-N) were evaluated. Furthermore, an influence of the C1-substituent of the β-carbolines derivatives was observed. Thus, by increasing the size and lipophilicity of this substituent, an increase in the optical purity of the deracemised product was observed. For both enzyme variants, a switch in enantiopreference was observed, regarding to the nature of this substituent. For bulkier substrates, the MAO-N variants exhibit (*R*)-selectivity. The factors that influence this enantioselectivity were explored by docking simulation of both MAO-N variants examining the different binding modes of the substrates. As expected, these studies showed that the steric issues exerted by C1 substituent into the active site, is a key factor that influences the selectivity displayed by MAO-N variants.



Scheme 15: CyD of 1-substituted β-carbolines derivatives using the MAO-N/ $NH_3 \cdot BH_3$ system.[40]

More recently, this protocol was expanded to other challenging cyclic amines such as 2-substituted piperidines, morpholines, thiomorpholines and piperazines. Two enanticomplementary MAO enzymes were successfully applied for the CyD of these substrates, *id est*, MAO-N variants and 6-hydroxy-D-nicotine oxidase E350L/E352D. Thus, by using this CyD protocol, both enantiomers were easily accessible in up to 99% *ee* and high conversion.[41]

Recently, in the quest for biocatalysts with broader substrate spectrum, Zhu, Lau and co-workers engineered cyclohexylamine oxidase (CHAO) and applied it in the deracemisation of secondary amines. Taking into account that CHAO has the advantage high substrate specificity on alicyclic amines and *sec*-alkylamines; rounds of saturation mutagenesis in active sites residues were performed. The activity of CHAO variants was evaluated in the deracemisation of 2-substituted tetrahydroquinolines (THQs, **20**), valuable alkaloids with a broad spectrum of biological activities. In this way, comparative studies of enzymatic activity between wild type CHAO (*wt* CHAO) and mutant CHAOs were carried out. The results suggest a much higher activity of the mutant CHAOs towards secondary amines than *wt* CHAO, which prefers primary amines.[42] Furthermore, steric factors are responsible of a reduced enzyme activity of the mutant CHAOs toward 2-subtituted THQs, observing higher conversion for methyl group over allyl>benzyl>phenyl substitution. On the other hand, the mutant CHAOs resulted more selective for the (*S*)-enantiomers. Finally, a preparative scale for the deracemisation of 2-methyl-THQ with mutant CHAO in combination with $NH_3 \cdot BH_3$ was performed, affording the corresponding (*R*)-2-methyl-THQ after 6 h with 76% of isolated yield and 98% *ee* (Scheme 16).



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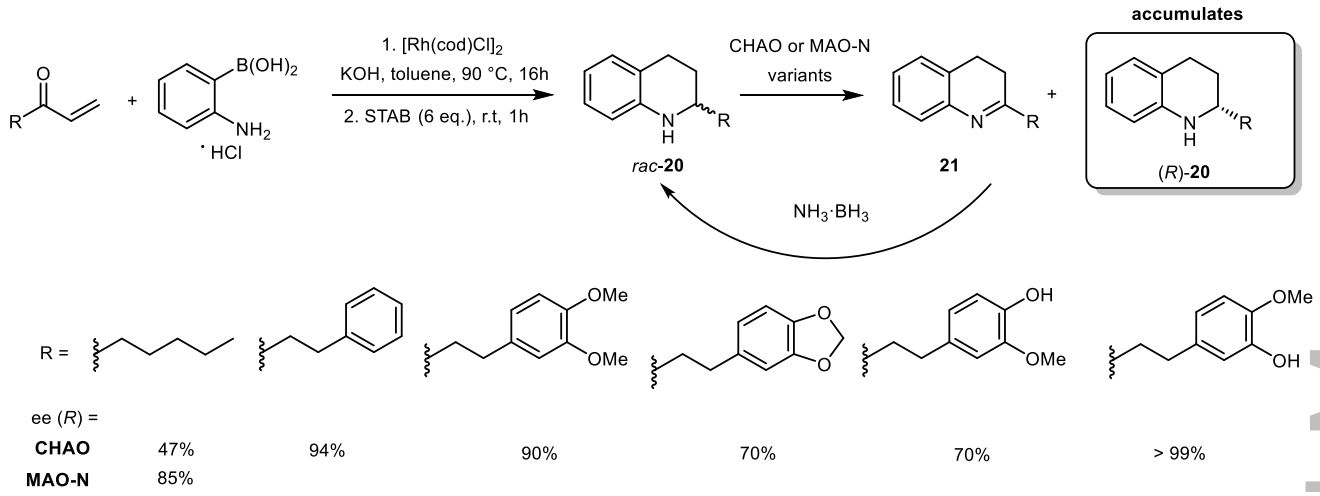
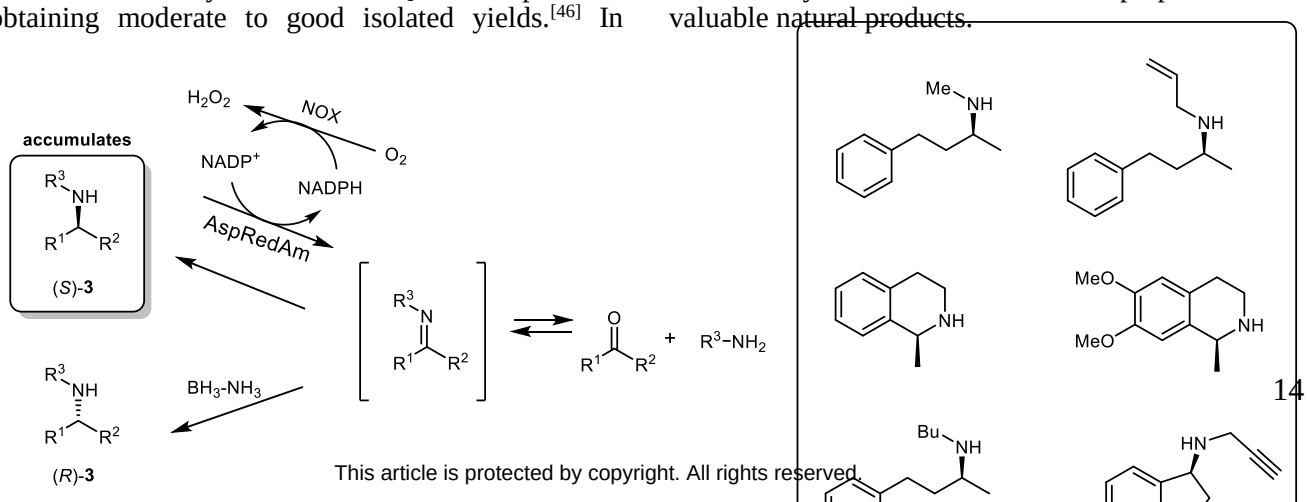
Scheme 16: Deracemisation of 2-substituted-tetrahydroquinolines (THQs, **18**) using CHAO variants.[42]

As an extension of the CyD, Zhu, Wu, Lau and co-workers developed a new library of CHAO mutants in order to evaluate the deracemisation process towards a family of 2-subtitued-THQs.[43] For all the substrates evaluated, high *ee* values and isolated yields were obtained (>91% and >57%, respectively). Furthermore, the protein engineering of CHAO based on the modification of a series of amino acid residues located in the vicinity of the active site of the enzyme, allowed access, in some cases, to the enantiocomplementary 2-subtitued-THQs. Specifically, for 2-isopropyl and 2-cyclopropyl-THQ the (*S*)-enantiomer was preferred for one of the CHAO mutants. To explain this observation, molecular dynamics (MD) were carried out, evaluating representative structures of the substrate coordinating to *N*-atom of FAD. In all the cases, a correct distance (< 2.5 Å) between the N-atom of FAD and the H-atom of the stereogenic carbon for the (*S*)-enantiomer, was observed. Theoretical studies together with engineering enzymatic work provide a set of new tools, which can be potentially applied to asymmetric synthesis.



Scheme 17. Synthesis of 2-substituted-THQs through Rh-catalysed formal [4+2] cycloaddition followed by CyD employing amine oxidases.[46]

By the same time, Marsden, Turner, and co-workers developed a sequential process for the asymmetric synthesis of 2-substituted THQs), previously isolated from *Galipea officinalis*, with antimalarial activity.[44] Such an approach consists in the efficient application of a chemo- and biocatalytic sequential process. Using the same strategy previously reported by Marsden,[45] the nitrogen heterocycles could be obtained through a rhodium-catalysed formal [4+2] cycloaddition of *ortho*-aminophenylboronic acids with Michael acceptors. This methodology was extended to the synthesis of THQ natural products obtaining moderate to good isolated yields.[46] In order to complete the asymmetric synthesis of THQ, deracemisation process was carried out, evaluating tree different AOx enzyme variants (MAO-N D9, CHAO and 6-HDNO) (Scheme 17). For all the substrates, CHAO showed good activity, giving optical purities of 47-99%. Only 2-pentenyl-THQ, was substrate of all tested enzymes, being a suitable substrate for the MAO-N D9 with an 85% *ee* after 96 h. Finally, preparative scale was examined, demonstrating the potential application of these rather deracemisation process in combination with chemocatalysis for the efficient preparation of valuable natural products.



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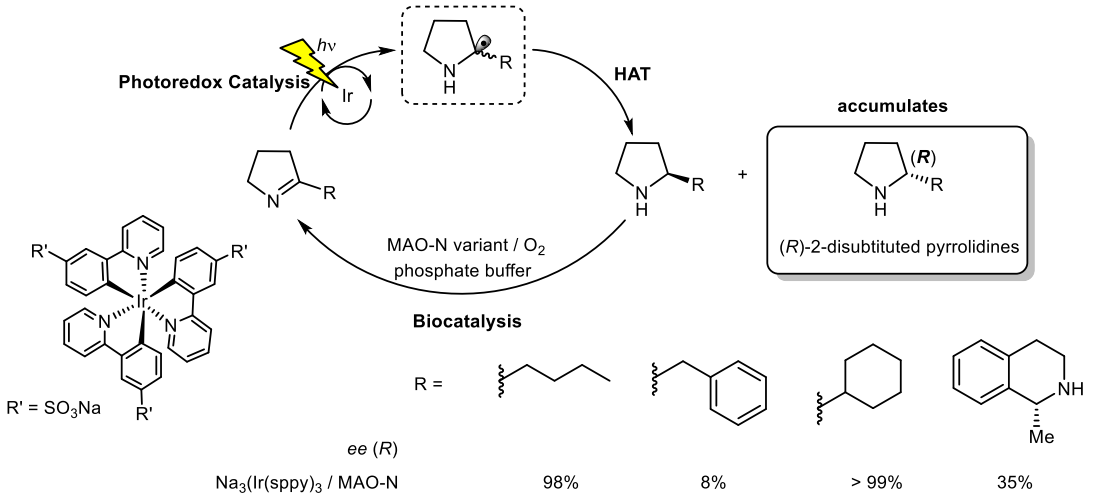
Scheme 18. CyD of primary and secondary amines using the AspRedAm/$NH_3{\cdot}BH_3$ system.[47]

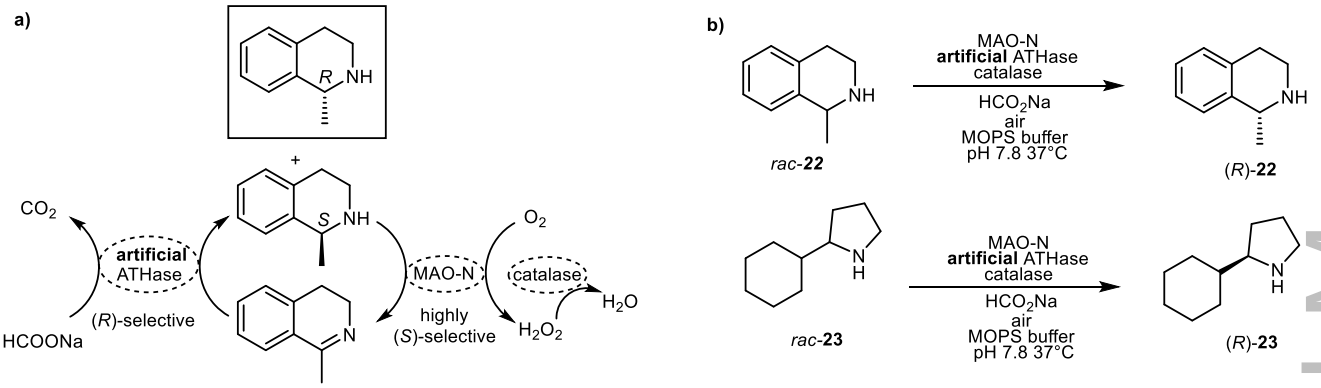
Recently, an elegant approach was reported by Turner and co-workers, which involves the use of NADP(H)-dependent reductive aminase (AspRedAm) from *Aspergillus oryzae* in an oxidative way, in replacement of MAO-N system. In this case, in order to recycling the $NADP^+$ co-factor, the combination with a NADPH oxidase (NOX) was employed. Finally, applying a non selective chemical reducing agent ($NH_3{\cdot}BH_3$), a complete deracemization can be attained. After 24h of reaction, a series of amines can be obtained with high conversion (74-98 %) and selectivity (93-99% *ee*) of the desired enantiomer by using enantiocomplementary biocatalysts (Scheme 18). Additionally, the employed AspRedAm variant allowed access to novel amine products, which are not substrate of the MAO-N variants, thus showing the complementarity this alternative system.[47]

It is clear that the strategic combination of different catalytic processes, has gained attention for the synthetic community and, nowadays, interesting applications can be found in the literature. In this context, the recent work reported by Wenger, Ward and co-workers explores the possibility of merging photoredox and biocatalysis toward the asymmetric synthesis of amines.[48] Visible-light photoredox catalysis has emerged as an attractive methodology to generate radical intermediates in mild and controlled conditions. Besides, biocatalysis brings about high selectivity and efficiency in a simple way. With this in mind, the authors proposed a cyclic amine deracemisation by the joint action of a monoamine oxidase (MAO-N-9), which catalyses the selective oxidation of one of the enantiomers to the corresponding amine, followed by the generation of α-amino alkyl radicals *via* photo-reduction of the corresponding cyclic imine and subsequent hydrogen atom transfer (HAT) process. Under these conditions, enantioenriched amines can be successfully obtained by a CyD process (Scheme 19). In order to allow both catalytic processes to run concurrently, a novel water-soluble trisulfonated variant of *fac*-[$Ir(ppy)_3$] photocatalyst was prepared. Additionally, ascorbic acid ($AscH_2/AscH^-$) was used to drive the HAT process. Kinetic studies revealed that the biocatalytic oxidation is the rate determining step in the overall reaction system. Finally, photobiocatalysis was evaluated for 2-subtistituted cyclic amine, attaining high yields and high *ee* (>95% and >98 %, respectively) for the butyl or cyclohexyl groups. However, for benzyl substituent, the process failed in the biocatalytic step, obtaining only 8% of the corresponding (*R*)-isomer. Likewise, when 1-methyl-3,4-dihydroisoquinoline was evaluated, a racemic product was obtained. This observation could be explained by the higher stabilization of the α-amino radical, leading to a less exergonic HAT pathway. For these cases, the use of a more polar hydrogen donor may accelerate the HAT process, obtaining a 35% *ee* for 1-methyl-3,4-dihydroisoquinoline when 4-mercaptophenylacetic acid or 3-mercaptopropionic acid derivatives, were used. Although the exploration of others cyclic amines was limited, this work demonstrates the fruitful cooperation between photocatalysis and biocatalysis, opening the possibility of novel processes design to get access to molecules with higher complexity in a simple way.



Scheme 19: Combining photoredox and biocatalysis toward the asymmetric synthesis of amines in a CyD procedure.[48]

A very elegant approach for cyclic amines deracemisation has been recently achieved by successfully encompassing the action of evolved *S*-selective MAO-N from *Aspergillus. niger* and artificial metalloenzymes (Scheme 20), in this case the so-called 'artificial transfer hydrogenase' (ATHase).[49] Thus, the use of borane-based reductant has been replaced by a catalytic system based on an Ir complex attached to biotin in order to ensure the binding to streptavidin (or its mutants) in a rather specific and favourable fashion. Remarkably, since the Ir complex is buried into the streptavidin scaffold, the biocatalysts are protected from mutual inactivation. Another aspect to be highlighted is the need of consuming as soon as formed the H_2O_2 (released as by-product of the MAO-N) which inactivates the ATHase. To get rid of this oxidant, catalase was efficiently incorporated into the system. In this way, two structurally different compounds, α-methyl tetrahydroisoquinoline (**22**) and 2-cyclohexyl pyrrolidine (**23**) were successfully deracemised (99 % conversion and >99 % *ee* for the *R*-enantiomer in both cases) employing these three enzyme concurrent process that consumes O_2 and formate as oxidant and hydrogen-donor, respectively. Protein engineering played a crucial role in this work, since both MAO-N and streptavidin (Sav) mutants were screened and combined in order to maximise the final enantioselectivity. Authors pointed out that the stereochemical outcome of the overall system is governed by the MAO-N. Considering that the ATHase stereoselectivity is not perfect, the process is closer to a CyD than to a deracemisation by stereoinversion.



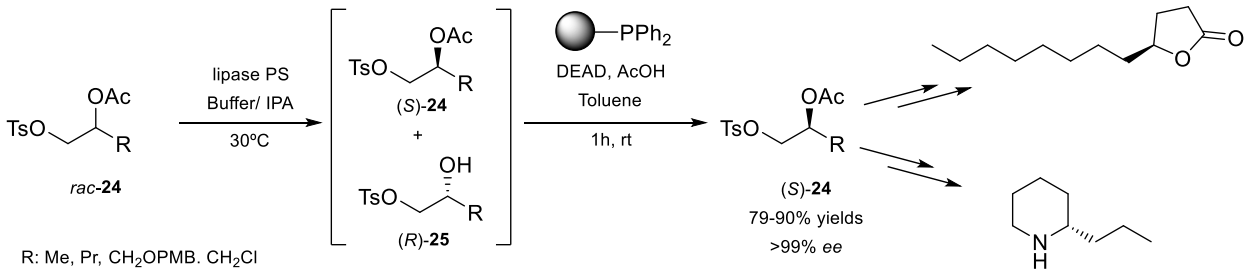
Scheme 20. Cyclic deracemisation of racemic secondary amines **22**-**23** employing MAO-N and artificial ATHase.[49]

2.4 Enantioconvergent processes

A less explored strategy for the efficient deracemisation of valuable chiral compounds are the so-called enantioconvergent processes, in which both enantiomers of the starting substrate undergo different reaction pathways that finally converge into the same enantiomer (or homochiral pair of similarly reactive compounds). In a broad sense, enantioconvergent processes can be regarded as deracemisation by stereoinversion, since one enantiomer will keep the original stereochemistry while the antipode will undergo inversion in its handedness. However, the DbI can be distinguished from EC because in former there is a destruction-recreation sequence in the stereochemical information [for instance (*S*)-sp^3-sp^2-(*R*)-sp^3] whereas in EC processes the stereoinversion takes place by S_N2 mechanism, as in epoxide hydrolase- or sulfatase-catalysed deracemizations.[50] Similar processes have been deeply studied in the hydrolysis of racemic epoxides by using jointly both retaining- and inverting-epoxide hydrolases, sulfatases and dehalogenases.[51]

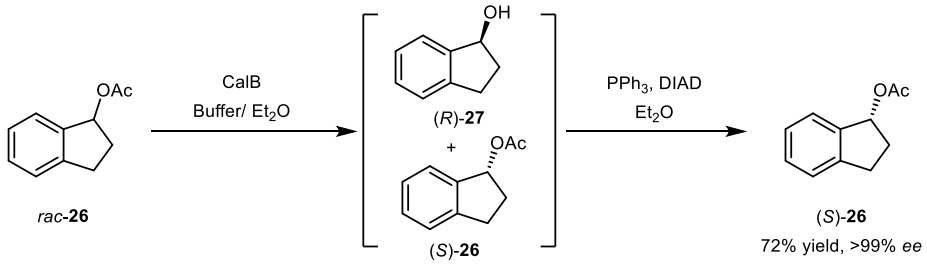
Apart from the biocatalysed deracemisation strategies using redox enzymes, some other examples have been described employing hydrolases. Some of these hydrolase-catalysed deracemisations include the selective enzymatic hydrolysis of an ester followed by a Mitsunobu inversion reaction onto the formed alcohol. Overall, this strategy comprising kinetic resolution and Mitsunobu inversion, can be considered an ER. Thus, in 2009 it was performed the effective preparation of optically active 1,2-diol monotosylate *O*-acetyl-derivatives (**24**) using different lipases and polymer-bound triphenylphosphine for the Mitsunobu reaction (Scheme 21).[52] Lipase PS was able to selectively hydrolyse a set of 2-acetoxy tosylates in phosphate buffer pH 6.5 at 30ºC containing 10% of diiso-propyl ether (DIPE), leading to the (*R*)-alcohols and (*S*)-esters with an excellent enantioselectivities (*E*>100). For the Mitsunobu inversion reaction, the use of a polystyrene-bound triphenylphosphine in presence of

DEAD and acetic acid facilitated the product separation by filtration and allowed for smoothly reaction, recovering the (*S*)-enantiomer of the ester from the (*R*)-alcohol in 1 hour with complete conversion and 99% *ee*. The sequential deracemisation combining the two steps led to the final 2-acetoxy-1-tosylated diols with excellent optical purities (93-98% *ee*) and high yields (73-91%), depending on the substrate structure. Such a protocol was succesfully applied for the preparation of valuable heterocyclic compounds such as alkaloids and insect pheromones as well as fragrance compounds.[53]



Scheme 21. Deracemisation of 1,2-diol monotosylates derivatives (**24**) by the combination of selective lipase-mediated hydrolysis and the Mitsunobu inversion.[52]

The same procedure was employed by Bouzemi and co.[54] in the deracemisation of a set of arylalkylcarbinol derivatives (**26**) employing the lipase B from *Candida antarctica* (CalB) and the Mitsunobu inversion. CalB was able to catalyse the selective hydrolysis of the racemic starting material to the (*R*)-alcohols and (*S*)-acetates with high selectivity.[55] Lipase amount is a key parameter in the enantioselectivity of the kinetic resolution. Thus, decreasing the amount of lipase improves both reactivity and selectivity. After CalB removal by filtration, the crude mixture of unreacted (*S*)-**26** and (*R*)-**27** were treated with triphenylphosphine (PPh_3), diisopropylazadicarboxylate (DIAD) and acetic acid at 0 °C to convert the formed (*R*)-alcohols into the corresponding (*S*)-acetates. Both the inversion selectivity and the yield depended on the substrate aromatic substituents. CalB presented (*R*) enantiopreference, so the (*R*)-acetates were hydrolysed by the enzyme and converted into (*S*)-acetates by Mitsunobu inversion in a biphasic system (Scheme 22). The (*S*)-acetates were obtained in 71%–99% *ee* and 76%–89% yields.



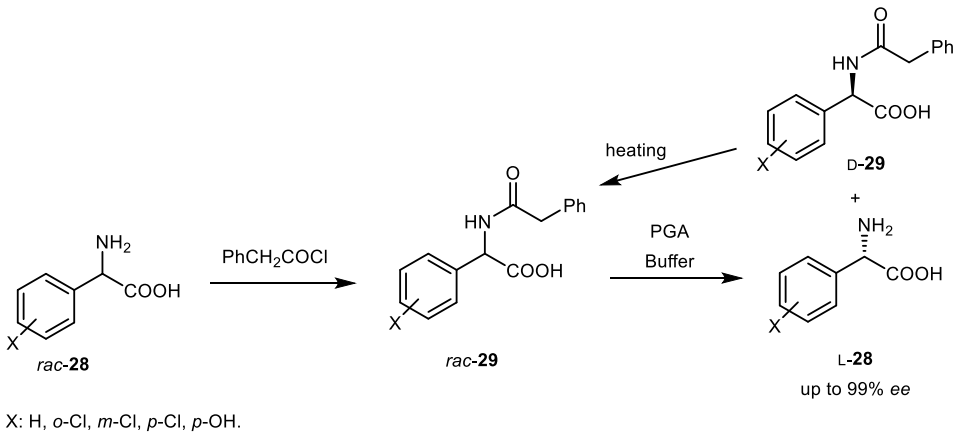
Scheme 22. Deracemisation of arylalkylcarbinols using CalB and Mitsunobu inversion.[54]

2.5. Other enzymatic deracemisation protocols

A different deracemisation pathway using hydrolytic reactions was developed in the synthesis of non-natural L-2-aryl-amino acids (**28**) by a chemoenzymatic route using a highly active and stereoselective recombinant penicillin G acylase (PGA).[56] The *N*-phenylacetyl derivatives (**29**), were selectively hydrolysed by the PGA to the L-enantiomer, whereas the unreacted D-enantiomer could be racemised by heating for recycling in a sequential procedure (Scheme 23). The key step of

this procedure was the biocatalyst screening in order to find a PGA with high stereoselectivity and activity towards the racemic substrates. The overexpressed recombinant PGA from the fermentation broth of *B. subtilis* WB800/pPZW103-PGA, was able to selectively hydrolysed *N*-phenylacetyl derivatives of the 2-arylamino acids with excellent enantioselectivity (*E*>200) and conversions close to 50% after short reaction times. The position and type of the substrate substituents influenced the PGA activity but selectivity was not affected.

When the hydrolysis catalysed by the recombinant PGA of **28a** (X: *o*-Cl) was carried out at multimilligram scale, a conversion close to 50% was achieved after 4 hours in a process with excellent selectivity and a catalyst productivity (grams of product per gram of catalyst) of 58.7 g/g. After the enzymatic hydrolysis, the supernatant was acidified with HCl in order to precipitate the unreacted D-**29a** and phenylacetic acid. The pH of the aqueous solution was adjusted to 7.0 to recover L-**28a**. The mixture of the recovered D-enantiomer and phenylacetic acid was heated at 170 °C for 15 min and then cooled. After this treatment, the D-compounds were completely racemised and employed as substrates in a new reaction cycle, being possible to achieve complete conversions of the enantiopure L-enantiomer.

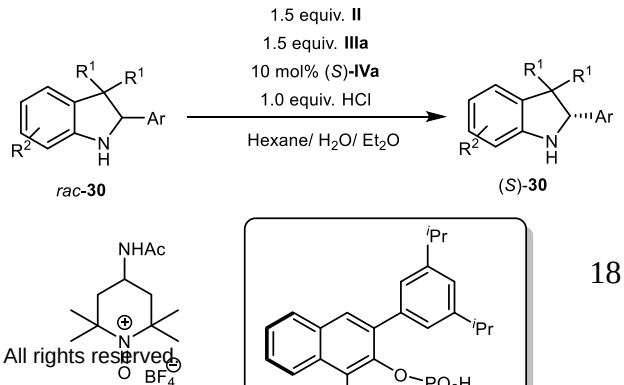


Scheme 23. Synthesis of enantiopure non-natural L-2-arylamino acids by chemoenzymatic deracemisation using PGA.[56]

3. Organocatalysed deracemisations

Even when there are fewer examples than by using biocatalysts, some deracemisation protocols using organocatalysts have been developed in the last years.[6] Most of these examples have been performed using redox catalysts, whereas others are based in acid/base catalysis to ensure a proper deracemisation of the starting materials..

In 2013 it was described the CyD of different 3*H*-indolines (**30**) through their oxidation to the corresponding 3*H*-indoles using an oxopiperidinium salt (Bobbitt's salt) in toluene under anionic phase-transfer conditions, and the selective reduction of the 3*H*-indoles generated by employing a Hantzsch ester in presence of a chiral phosphoric acid catalyst,[57] as shown in Scheme 24.[58] The process was carried out in a biphasic system, in which the substrate is dissolved in organic solvent, the oxidant and the reductant are solids and water can be also present in the reaction media. As the selectivity of the oxidation step is very low, the high selectivity of the reduction step allows the effective deracemisation, as observed for linear deracemisation methodologies. The reaction optimization showed that the best conditions were found by using as solvent a mixture *n*-hexane/Et_2O/water in proportion 9:1:10, using 1.0 equivalent of hydrochloric acid as additive in presence of 1.5 equiv. of oxopiperidinium **II**, the Hantzsch ester **IIIa** (1.55 equiv.) and 10 mol% of the chiral phosphoric acid (*S*)-TRIP (**IVa**). 3*H*-Indolines presenting several substitution pattern were tested to this deracemisation procedure. 2-Aryl substituted substrates led to the (*S*)-**30** with good yields and high optical purities. Similar conditions were employed for the deracemisation of substituted tetrahydroquinolines in order to obtain the (*R*)-enantiomers with high yields and enantiomeric excesses, being required 3.0 or more equivalents of oxidant and reductant due to a second oxidation from the dihydroquinoline intermediate to the quinolone.



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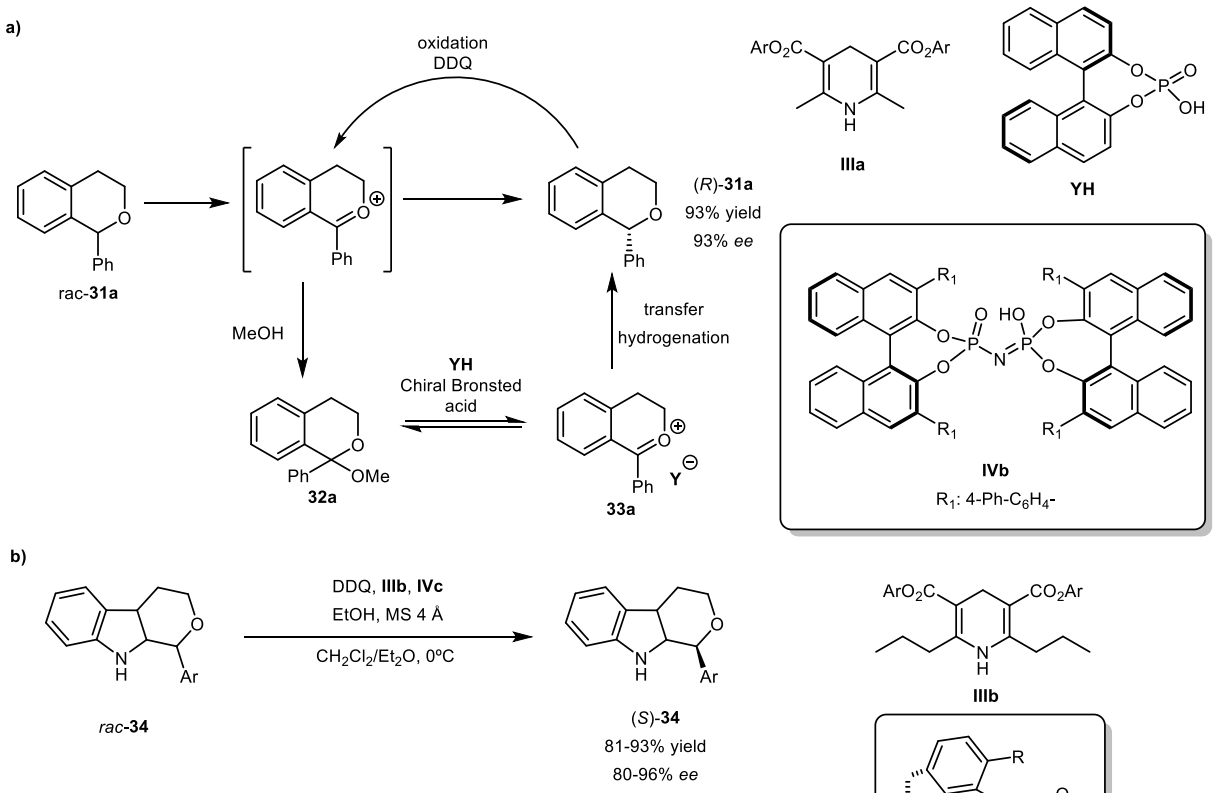
Scheme 24. Deracemisation of racemic 3*H*-indolines employing organocatalysts.[58]

The redox deracemisation of cyclic ethers could occur through an oxocarbenium intermediate that then has to be selectively reduced.[59] In order to overcome the stability issues of the oxocarbenium intermediates, the addition of protic additives able to react with them, is required. This additive makes the conversion of the racemic ether **31** into the acetal **32**, which in presence of a chiral Brønsted acid catalyst would lead to the chiral ion pair **33**, and then asymmetric hydrogen transfer through chiral anion catalysis would afford the desired (*R*)-**31**. Thus, initial experiments were performed in the deracemisation of 6*H*-benzo[*c*]chromene (**31a**, Scheme 25a), a motif occurring in the structure of different valuable compounds.[60] Hantzsch ester **IIIa** and a chiral phosphoric acid were employed in the asymmetric hydrogenation. Different oxidants were tested in absence of additive, but very poor (if any) enantioselectivities were achieved, being required higher amounts of oxidant. The addition of protic additives led to complete oxidation even with 1.05 equivalents of oxidant, obtaining (*R*)-chromene with the highest enantioselectivity in presence of DDQ and methanol with molecular sieves. The best phosphoric acid for this process was **IVb**, whereas the solvent optimization afforded the chiral compound in 93% yield and 93% *ee* in a mixture of MTBE and DCM. Mechanistic studies confirm that an acetal intermediate is involved in the deracemisation process and that the presence in this acetal of a methoxy group as leaving group has a positive effect in terms of a selective asymmetric transfer hydrogenation. By this reason, methanol was selected among other additives.

These optimized conditions were then extended to other 6*H*-benzo[*c*]chromenes. For most of the compounds tested, a substituent in the arene ring led to good results, achieving the (*R*)-compounds with good yields and optical purities up to 99%. Chromenes presenting aryl and heteroaryl groups at the α-position also led to the optically active substrates with high selectivities. Reactions can also be performed with starting materials containing α-alkyl, -allyl, -alkenyl and -alkynyl groups with excellent yields and high optical purities. This linear deracemisation protocol was also tested in the deracemisation of isochromans with high yields and optical purities. For these substrates, Na_2CO_3 was required as additive instead of MeOH, in order to avoid undesired reactions that occur between the reaction intermediate and some DDQ species generated in the medium. Using Na_2CO_3 (0.5 equiv.), the chiral isochroman was recovered with 90% *ee*. Different substituted 1*H*-isochromenes were tested, being observed that all the α-aryl and α-alkyl derivatives were deracemised under the standard conditions with enantiomeric excesses up to 95%.



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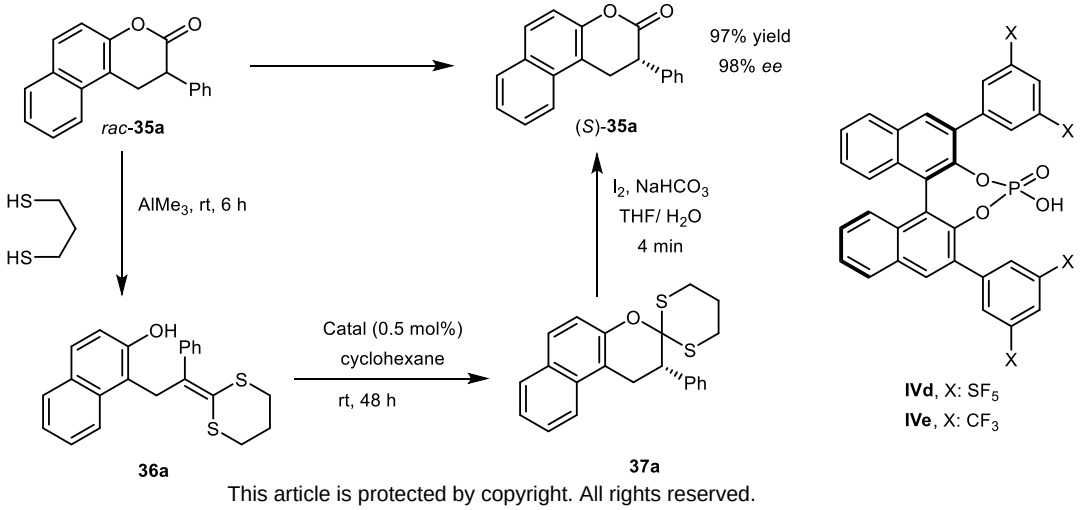
Scheme 25. a) Organocatalysed deracemisation of 6*H*-benzo[*c*]chromene, b) Deracemisation of 1,3,4,9-tetrahydropyrano[3,4-b]indoles using DDQ and a chiral phosphoric acid.[60,62]

Chiral 1,3,4,9-tetrahydropyrano[3,4-b]indoles (**34**, THPIs) are valuable structures present in many biologically active compounds, but there are very few examples of their enantioselective synthesis.[61] In 2018 the redox deracemisation of a set of α-substituted THPIs was proposed employing DDQ as oxidant and a chiral phosphoric acid for the asymmetric hydrogenation through chiral anion catalysis (Scheme 25b).[62] Compound **34a** was selected as model substrate, being observed that the presence of a protic additive led to a completion in the oxidation step, and the highest selectivity was achieved in presence of a stoichiometric amount of EtOH. Process optimization led to the SPINOL-chiral phosphoric derivative **IVc** as the best catalysts in presence of a DCM/Et_2O mixture at 0ºC containing 4.0 Å molecular sieves. Once obtained the best conditions for the CyD, a set of α-aryl substituted THPIs were subjected to the deracemisation, being recovered (*S*)-**34** with high yields and enantiomeric excesses (80-96% *ee*), with no effect of the electronic properties of the aryl group at α-position. 1-Naphthyl, 2-furyl and 2-thienyl groups were also successfully deracemised. The use of indoles with different groups in the aromatic ring also led to the enantioenriched starting material with yields higher than 90% and excellent optical purities, whereas no reaction was observed for the α-methyl derivative. The positive effect of EtOH as additive was studied, being established that in the presence of DDQ and EtOH a ketal intermediate was formed in the deracemisation, which in presence of the chiral phosphoric acid afforded the contact ion pair formed by the oxocarbenium and the chiral phosphate. The last step is the selective reduction of the tight ion pair by Hantzsch ester **IIIb**.

Apart from the redox approach to perform organocatalysed deracemisation, some other methodologies have been developed. Thus, in 2012, List *et al*. achieved the effective deracemisation of α-aryl hydrocoumarins (**35**), compounds with biologically active properties, by using a three-step procedure that comprises the dithioketal protection of the starting material, the asymmetric Brønsted acid-catalysed cyclization of the ketene dithioketal to the corresponding *ortho* ester and the final hydrolysis to obtain the enantiopure α-substituted lactone.[63] The asymmetric catalytic cyclization of the ketene **36** was carried out in cyclohexane at room temperature in presence of different chiral phosphoric acids. The use of the commercially available compound **IVd** at 10 mol% led to a 95% conversion and an 80% *ee* after 2 hours. Catalyst loading can be reduced even to 0.5%, being achieved a 93% yield after 2.5 days and 87% *ee*. Good results could be also obtained with **IVe** at 5 mol%, with an excellent selectivity (90% *ee*), but a lower activity (99% after 1 week). The use of **IVd,e** (5 mol%) was extended to other ketene dithioketals with high yields and enantioselectivities regardless the electronic nature of the ketene. Once optimized this process, the deracemisation of 1.9 g of hydrocoumarin **35a** was performed, as shown in Scheme 26. First, this compound was converted in the achiral ketene dithioketal **36a** with 1,3-propanedithiolate and trimethylaluminum, and isolated by precipitation in diethyl ether. The dithioketal was dissolved in cyclohexane at room temperature in presence of 0.5 mol% of catalyst **IVd** to yield **37a**, which was treated after 48 hours with I_2/$NaHCO_3$ to obtain enantiopure (*S*)-**35a** with quantitative yield. According to the Figure 1, this methodology resembles a linear deracemisation.



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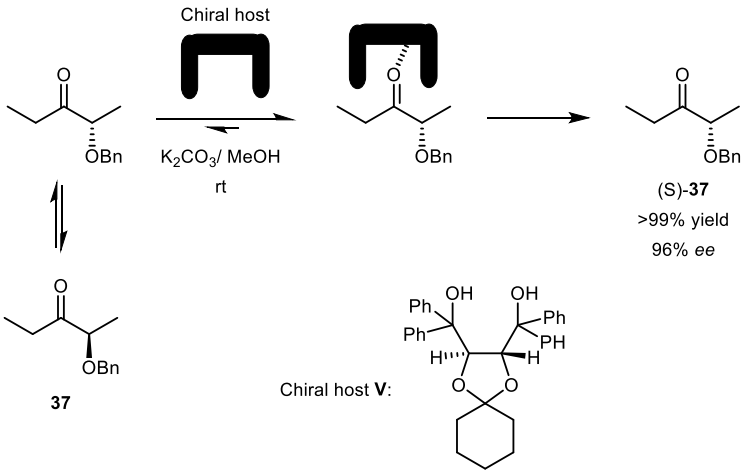
Scheme 26. Organocatalysed deracemisation of α-aryl hydrocomoumarins in a three-step procedure.[63]

Optically active sulfones are present in a wide range of biologically active compounds, being developed several methodologies for their synthesis.[64] A method to obtain these compounds consist in performing an enantioselective β-elimination reaction in order to generate an olefinic product, whereas the unreactive enantiomer of the racemic sulfone is recovered.[65] This kinetic resolution was successfully performed by treating 0.1 mmol/mL of starting sulfone with a chiral BINOL-derived polyether (10 mol%) containing a 3,3`-diiodo-substitution pattern in the aromatic ring, using 1,4-dioxane as solvent and 1.5 equivalents of KF as base. These conditions were extended to several (hetero)aryl sulfones with good results. In order to overcome the limitation of the kinetic resolutions, the formed α,β-unsaturated ketone was converted into the starting β-sulfonyl ketones using sulfinic acid. Thus, after the first kinetic resolution, the enone was quantitavely converted in racemic starting material and then another kinetic resolution was carried out. After three of these cycles, chiral (*R*)-sulfonyl ketone was achieved with 85% yield and 97% *ee*. Overall, this procedure can be regarded as an organocatalised CyD.

4. Other metal-free deracemisations

Despite the extensive use of biocatalysts for the deracemisation of compounds in absence of metals along with the organocatalysed processes, there are other few procedures in which metal free deracemisations are accomplished.

The host-guest inclusion complexation technique, which has been usually employed in the kinetic resolution of racemates,[66] has been successfully applied for the deracemisation of α-substituted cyclic ketones under basic conditions. This approach can be also used in the deracemisation of α-hydroxyketones (Scheme 27).[67] Thus, racemic 2-benzyloxy-3-pentanone was tested as substrate in the base-catalysed racemization combined with the asymmetric inclusion complexation in order to obtain the chiral starting material. The racemization of the starting material worked well with different inorganic bases, being selected K_2CO_3 due its mild basicity. As chiral host, the (-)-TADDOL derivative **V** was employed in stoichiometric amounts. When the reaction was performed in a mixture MeOH/H_2O 1:1 at room temperature, the chiral ketone was obtained with 97% and 49% *ee* after three days, whereas the chiral host was recovered quantitatively. The increase in the substrate concentration allowed obtaining a much higher optical purity of ketone (*S*)-**1**, achieving this compound with 96% *ee*. This methodology was extended to other acyclic α-hydroxy ketones, being necessary to modify the substrate concentration and the base in order to optimize the results for each substrate. In general, excellent yields and moderate to high selectivities were measured.



Scheme 27. Deracemisation of α-hydroxyketones by host-guest inclusion complexation.[67]

The Viedma Ripening is a method that allows the conversion of a racemic mixture of a solid substance into a single enantiomer with a theoretically 100% yield.[68] This process consists in the grinding of a

suspension of racemic conglomerate crystals in combination with racemization in solution, thus resulting in the complete deracemisation in the solid phase. There are some requirements in order to perform the Viedma Ripening: First, the target molecule must undergo racemization in solution, and second, the enantiomers of the molecule must crystallize as separate crystals, forming a racemic conglomerate. Most of the examples in which compounds have been deracemised by this technique include amino acids and derivatives, as well as organometallic compounds.[69] In 2017, the deracemisation of several allylic sulfoxides was studied, as these compounds are able to racemise at temperatures close to 50ºC.[70] From all the sulfoxides tested, only allyl 4-nitrophenylsulfoxide was able to crystallize as a racemic conglomerate, so it was employed for the Viedma ripening experiments. This compound racemised at room temperature in toluene and diethyl ether. Using toluene at room temperature led to a 12% *ee* of the sulfoxide after one month, and this result was employed for further optimization. Thus, employing refluxing diethyl ether as solvent, and creating an initial bias, a complete deracemisation of the compound was observed after 8 days.

5. Conclusions and Future Perspectives

In the last ten years, a huge number of deracemisation protocols have been developed in order to obtain a chiral compound starting from its racemic mixture. Most of the examples have been described using biocatalysts, whereas the use of organocatalysts for this purpose is still in its infancy. Excellent results can be achieved specially in the preparation of chiral alcohols and amines using stereoinversion or cyclic deracemisation through redox processes, involving the formation of an oxidized compound and the following reduction to the chiral starting material. Once optimized, these methodologies present the advantage that there is no need of further reactions in order to obtain the target molecules, something required in other approaches as the dynamic resolutions, whereas the theoretical yields can be up to 100%, impossible in the classical kinetic resolutions.

By these reasons, deracemisation processes have become an attractive alternative to the traditional strategies such as kinetic resolution and dynamic processes. The enormous variety of (bio)catalysts that (bio)chemists have been lately developing, with broader substrate acceptance profile, raised thermostability and robustness, increases the possibilities of novel deracemisation designs with the combination of selective and efficient catalysts in harmonic fashion. It seems obvious that the state-of-the-art technologies such as continuous flow systems, photochemistry, preparative electrochemistry and mechanochemistry, to name just a few, will be more integrated to complex deracemisation systems, thereby providing a better use of resources and a remarkable improvement of the overall process sustainability.

Although the biocatalysis and organocatalysis has reached a high level of sophistication, not only novel reactions but also known reactions running through a novel mechanism are reported in the literature every day.[71] It seems obvious in this line and owing to their intrinsic advantages, deracemisation protocol designs shall involve such ever-growing source of elements to develop efficient systems. Thus, deracemisation will become a sustainable alternative to traditional methodologies currently employed to obtain highly demanded enantioenriched compounds.

Acknowledgements

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REVIEW

Deracemisation processes employing organocatalysis and enzyme catalysis

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