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Perspective

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Chemoenzymatic Dynamic Kinetic Resolution: A Powerful Tool for the Preparation of Enantiomerically Pure Alcohols and Amines

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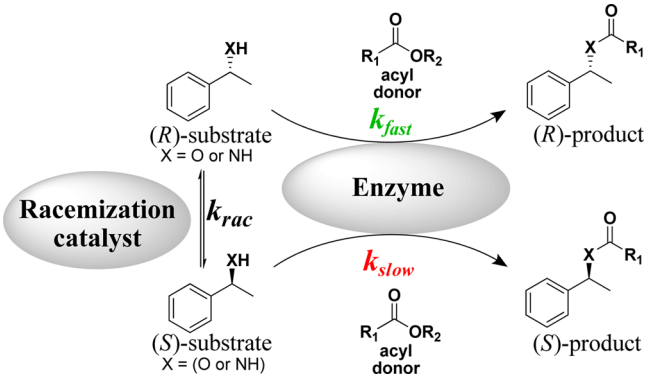
ABSTRACT: Chemoenzymatic dynamic kinetic resolution (DKR) constitutes a convenient and efficient method to access enantiomerically pure alcohol and amine derivatives. This Perspective highlights the work carried out within this field during the past two decades and pinpoints important avenues for future research. First, the Perspective will summarize the more developed area of alcohol DKR, by delineating the way from the earliest proof-of-concept protocols to the current state-of-the-art systems that allows for the highly efficient and selective preparation of a wide range of enantiomerically pure alcohol derivatives. Thereafter, the Perspective will focus on the more challenging DKR of amines, by presenting the currently available homogeneous and heterogeneous methods and their respective limitations. In these two parts, significant attention will be dedicated to the design of efficient racemization methods as an important means of developing milder DKR protocols. In the final part of the Perspective, a brief overview of the research that has been devoted toward improving enzymes as biocatalysts is presented.

1. INTRODUCTION

Enantiomerically pure alcohols and amines constitute important synthetic building blocks and key targets in the manufacturing of a wide range of chemical products, such as agrochemicals, food additives, fragrances, and pharmaceuticals.[1,2] Consequently, significant efforts have been dedicated to the enantioselective synthesis of these compounds, including catalytic protocols for carbon-heteroatom bond formation,[3−5] hydrogenations of ketones/imines,[1,6,7] nucleophilic addition to carbonyl compounds,[8−11] and kinetic resolution (KR).[12−17] Of these methods, enzymatic KR of racemic mixtures is the most common way to access enantiomerically pure alcohols and amines on an industrial scale, owing to its high performance in terms of activity and selectivity.[17] For the KR of these compounds, the process can be made either (*R*)- or (*S*)-selective depending on whether a lipase or a serine protease is chosen as the enzymatic resolving agent. In most of the reported KR protocols of alcohols and amines, the enzyme resolves the racemic substrate through selective acylation of one of its enantiomers, which allows for the isolation of the enantiopure alcohol or amine using conventional purification techniques. The acyl group being transferred to substrate by the enzyme comes from a so-called acyl donor, which is added to the reaction in at least equimolar amounts in regard to the substrate. Since this transesterification process is fully reversible, highly activated esters or enol esters are commonly employed as acyl donors to push the reactions toward the formation of the acylated product.[18,19]

Unfortunately, enzymatic KR, as all other resolution methods, suffers from the limitation that the maximum theoretical yield is only 50%. An efficient way to overcome this drawback and achieve a theoretical yield of 100% is to combine the resolution process with *in situ* racemization in a so-called dynamic kinetic resolution (DKR) (Scheme 1). To date, a variety of protocols for the racemization of alcohols and amines have been developed, and these involve for example acid/base catalysts, transition-metal complexes, metal nanoparticles, or enzymes.[2,20−23]

Scheme 1. Example of a (*R*)-Selective Chemoenzymatic DKR of Secondary Alcohols and Primary Amines



However, the design of a successful DKR system is far from simple, given that the following requirements must be fulfilled: (i) the KR must display a sufficient enantioselectivity (*E* value[24] = $k_{fast}/k_{slow} \geq 20$); (ii) the enzyme and the racemization catalyst must be compatible with one another; (iii) the rate of racemization (k_{rac}) must be at least 10 times faster than the enzyme-catalyzed reaction of the slow reacting enantiomer (k_{slow}); and (iv) the racemization catalyst must not react with the product formed from the resolution. Among these requirements, the compatibility between the enzyme and racemization catalyst is generally the critical issue, since these catalysts often operate optimally under very different condition.[25] It is also common that the racemization catalyst interferes with the enzymatic resolution or that the enzyme and its accompanying additives (e.g., surfactants and stabilizers) have an inhibitory effect on the racemization catalyst. As a result of this compatibility issue, the identification of reaction conditions that enable both high

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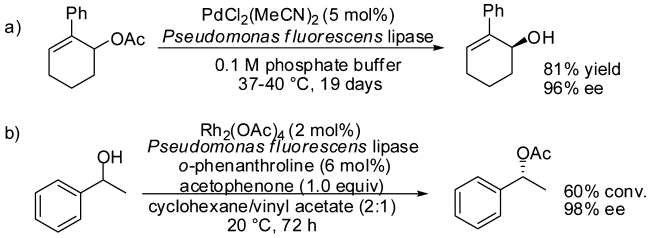
enantioselectivity of the KR and efficient racemization has been a reoccurring challenge within the field of DKR.

This Perspective summarizes the key features of the extensive research that has been dedicated to the chemoenzymatic DKR of alcohols and amines during the past two decades. The aim is to cover both the biological and chemical aspects of the DKR, by discussing topics ranging from the design of enzyme-compatible racemization catalysts to enzyme engineering. Further, we wish to point out the current state-of-the-art DKR protocols and their respective limitations, in an attempt to highlight novel avenues for future research.

2. EARLY COMBINATIONS OF METAL CATALYSTS AND ENZYMES FOR THE DKR OF SECONDARY ALCOHOLS

In 1996, Williams reported the DKR of an allylic acetate derivative by the combinative use of *Pseudomonas fluorescens* lipase and $PdCl_2(MeCN)_2$ to give the corresponding allylic alcohol in 81% yield and 96% ee after 19 days (Scheme 2a).[26] In

Scheme 2. Early Chemoenzymatic DKR Systems of Alcohol Derivatives Developed by the Williams Group



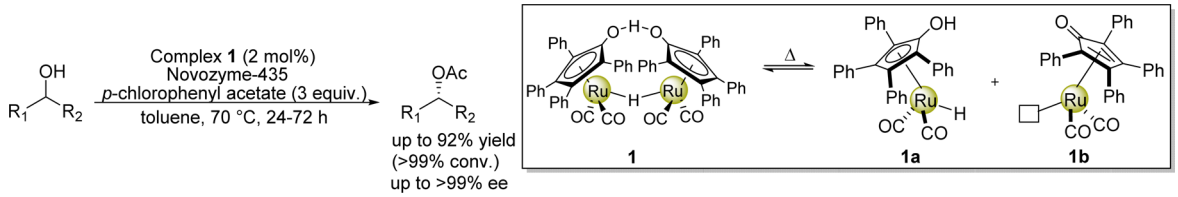
this reaction, the acetylated alcohol was deracemized by the coupling of a lipase-catalyzed ester hydrolysis to a racemization proceeding via palladium(II)-mediated 1,3-acetate shift. Although, the reaction proceeded at an impractical rate, this seminal study was an important first step that demonstrated the possibility of combining transition metal catalysis with enzyme catalysis for achieving DKR. In a subsequent study, Williams and co-workers developed a method for the DKR of secondary alcohols lacking adjacent C=C double bonds by utilizing racemization catalysts operating through a reversible hydrogen-transfer mechanism.[27] Among the studied catalysts, $Rh_2(OAc)_4$ gave the best results and was combined with lipase-catalyzed transesterification, affording (*R*)-1-phenylethanol with 60% conversion and 98% ee (Scheme 2b). Unfortunately, this method suffers from several critical drawbacks, such as a low conversion of the overall DKR and the necessity of both *o*-phenanthroline and acetophenone as additives for efficient racemization. However, despite the disadvantages of this particular DKR system, this study was important since it demonstrated the potential of using metal catalysts operating via transfer hydrogenation mechanisms as a general method for racemizing alcohols. This work became a great source of inspiration for subsequent catalyst design, triggering the development of a great number of racemization catalysts based on different transition metals, which all functioned through different transfer hydrogenation mechanisms (*vide infra*). Unfortunately, it is beyond the scope and purpose of this Perspective to provide an in-depth mechanistic discussion for these transfer hydrogenative racemization processes, and thus we kindly refer interested readers to some recent reviews that cover this topic.[1,20,21,28]

Following the pioneering work of Williams, the group of Bäckvall developed the first practical system for the DKR of secondary alcohols,[29,30] which involved *Candida antarctica* lipase B (CALB) immobilized on acrylic resin (also known under the trade name Novozyme-435) and Shvo's dimeric ruthenium complex **1** (Scheme 3).[31,32] This protocol was found to be compatible with a wide range of aliphatic and benzylic alcohols, providing the corresponding (*R*)-acetates in high yields and ee's. A drawback of this DKR system is that the Shvo complex **1** requires 70 °C to efficiently split into the two monomeric species **1a** and **1b**, which mediates racemization through an outer sphere redox mechanism.[31,32] Because of this heat activation, the Shvo complex **1** can only be combined with thermostable lipases, which limits the number of enzymes that can be used in the DKR. For example, sensitive serine proteases that exhibit (*S*)-selectivity according to Kazlauskas' rule[33] cannot be used together with **1.**

Another issue of the Shvo complex **1** was that it had to be used together with activated aryl esters such as *p*-chlorophenyl acetate, since simpler alkenyl acetates as acyl donors were found to interfere with the racemization and lead to substantial formation of ketone side products. Despite these limitations, complex **1** has been successfully combined with several lipases for the DKR of *β*-azido alcohols,[34] benzoins,[35] *β*-halo alcohols,[36] heteroaryl ethanols,[37] hydroxylalkanephosphonates,[38] *γ*-hydroxy amides,[39] hydroxyl acid esters,[40−43] hydroxyl aldehydes,[42] *β*-hydroxy alkyl sulfones,[44] and *β*-nitrile alcohols.[45] In addition, complex **1** has been applied as racemization catalyst in the DKR of *β*-substituted primary alcohols, where racemization occurs through enolization of the intermediate aldehydes.[46]

Subsequent research aimed at discovering more active catalysts that could efficiently racemize alcohols under milder reaction conditions, enabling the use of a wider range of enzymes for DKR. The group of Park reported on (η^5-indenyl)RuCl-$(PPh_3)_2$, **2**, as an efficient racemization catalyst for alcohols at room temperature, which unlike **1** only produced negligible amounts of ketone byproduct.[47] However, a severe drawback of complex **2** is that it requires KOH to display catalytic activity, which is detrimental for applications in DKR as the base can hydrolyze the product acetates and also cause enzyme deactivation. It was later found that complex **2** could instead be activated by O_2 and Et_3N, but unfortunately a higher reaction temperature (60 °C) was needed for efficient racemization in this case. In the latter study, complex **2** was successfully combined

Scheme 3. DKR of Secondary Alcohols by Shvo's Dimeric Ru Complex 1 and Novozyme-435



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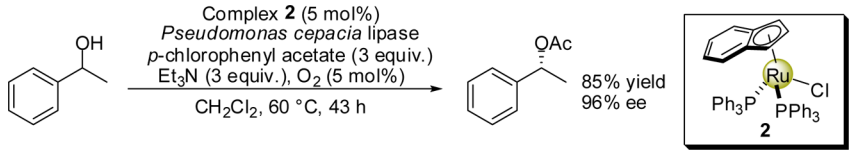
Scheme 4. DKR of Secondary Alcohols by Complex 2 and *Pseudomonas cepacia* Lipase



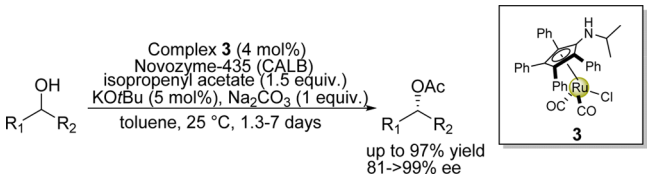
with *Pseudomonas cepacia* lipase (PS-C) for the DKR of a small scope of simple secondary alcohols (Scheme 4).[48]

3. DEVELOPMENT OF MORE PRACTICAL PROTOCOLS FOR THE DKR OF MORE FUNCTIONALIZED ALCOHOLS

The initial success achieved by combining enzymes with ruthenium-based complexes strongly influenced the subsequent research within the field of DKR, which resulted in the development of a number of protocols utilizing different transfer hydrogenation-type ruthenium complexes as the racemization catalyst.[49−51] However, the first main breakthrough came in 2002 when the group of Park prepared the monomeric ruthenium aminocyclopentadienyl complex **3** and demonstrated that it could efficiently racemize secondary alcohols at room temperature.[52,53] Unlike complex **1**, this racemization catalyst did not require any heat activation but was activated by KO*t*Bu. In contrast to KOH, KO*t*Bu shows a higher compatibility with most DKR systems commonly used.

Complex **3** was successfully paired with Novozyme-435 for the synthesis of a variety of functionalized aliphatic and benzylic (*R*)-acetates in high yields and ee's at room temperature (Scheme 5).[52,53] An advantage of this DKR protocol was that the cheap

Scheme 5. DKR of Secondary Alcohols by Complex 3 and Novozyme-435



and readily available isopropenyl acetate could be used as the acyl donor instead of activated esters, such as *p*-chlorophenyl acetate. Unfortunately, the DKR reactions were found to progress slowly, requiring reaction times of up to 7 days, which is in sharp contrast to the separate racemization and KR reactions, which were generally complete within a few hours. This significant difference in efficiency between the DKR and the separate reactions suggests that complex **3** and CALB are not fully compatible with one another, leading to partial deactivation of both catalysts.

Because of its good racemization activity at room temperature, complex **3** could also be combined with the more sensitive protease subtilisin Carlsberg, which opened up for (*S*)-selective DKR protocols of secondary alcohols.[54] In addition, the group of Kim and Park has in a recent study demonstrated that complex **3** can be used together with ionic surfactant-stabilized *Burkholderia cepacia* lipase for the DKR of allylic secondary alcohols at room temperature in excellent yields and ee's.[55]

Shortly after the development of **3**, the group of Bäckvall prepared a related monomeric ruthenium pentaarylcyclopentadiene complex **4**,[20] which proved to be a highly efficient catalyst that managed to fully racemize enantiomerically pure 1-phenylethanol within 10 min, even at catalyst loadings as low as 0.5 mol %.[56] This racemization catalyst displayed many similarities to complex **3**, both in terms of structure and activation method; however, the absence of an amino-functionality in the cyclopentadienyl ligand resulted in an improved compatibility with enzymes. Thus, combination of complex **4** with CALB (Novozym-435) afforded a fast DKR of secondary alcohols, e.g., 1-phenylethanol was transformed to its acetate in high yield and >99% ee in 3 h.[57,58] Complex **4** paved the way for a new generation of DKR and dynamic asymmetric transformation (DYKAT) protocols involving several different enzymes, many of which had not been possible to incorporate previously. DKR protocols utilizing complex **4** have been applied to the deracemization of a wide range of functionalized secondary alcohols in excellent yields and ee's, including aliphatic alcohols,[57,58] allylic alcohols,[59−62] chlorohydrins,[63] diols,[64−67] homoallylic alcohols,[58,68] and N-heterocyclic 1,2-amino alcohols[69] (Figure 1). In the case of the DKR of 1-phenylethanol, a large scale reaction with only 0.05 mol % of complex **4** was carried out on a 1 mol-scale to furnish 159 g (97% yield) of the corresponding (*R*)-acetate in 99.8% ee.[70] DKR and DYKAT systems involving complex **4** have also been employed in the synthesis of several biologically relevant molecules[64,68,69,71−73] and pharmaceuticals.[74−79] As with complex **3**, the racemization activity of **4** at room temperature allowed it to be combined with subtilisin Carlsberg for (*S*)-selective DKR protocols.[60,61,80]

For some chlorohydrins[63] and for alcohols containing a distant olefin group, such as homoallylic alcohols[68] and 5-hexen-2-ol,[81] racemization occurs significantly slower with ruthenium-based catalysts such as **4**, which calls for increased reaction temperatures in the DKRs. For these substrates, further research into more efficient racemization protocols is warranted to enable DKRs incorporating sensitive proteases. One promising way to achieve a more efficient racemization of chlorohydrins could perhaps be to match the electronic properties of catalyst and substrate as recently reported by the group of Bäckvall.[82] In this study, it was found that a highly electron-deficient analogue of complex **4** gave a 10−30 times faster racemization of chlorohydrins than the standard catalyst. The authors ascribed the improved racemization rate to the higher efficiency of the electron-deficient catalyst in abstracting the hydride from this electron-deficient class of substrates.

In recent years, several analogous enzyme-compatible racemization catalysts based on the cyclopentadienyl ruthenium core have been synthesized (Figure 2). Particularly, the group of Kim and Park has made several key contributions to the field of alcohol DKR by developing ruthenium-based racemization catalysts exhibiting improved stability and broader scope. One of these catalysts is the benzyloxy derivative **5** that has been used as a racemization catalyst in the DKR of a number of aliphatic and benzylic secondary alcohols under air atmosphere.[83] The possibility to run the reactions open to air constitutes a significant practical improvement compared to the previous systems involving catalysts **3** and **4**, which both require the use of dry and inert conditions to prevent catalyst deactivation. Another

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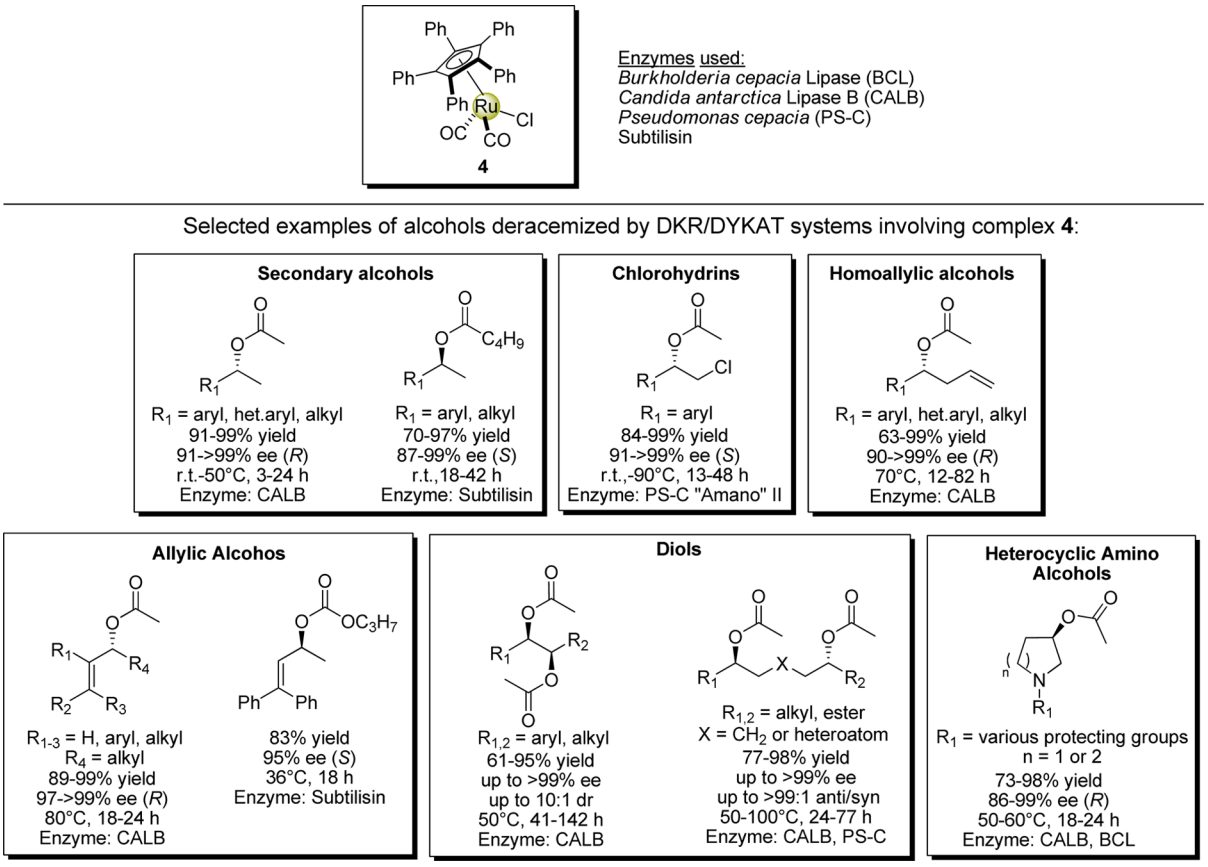

Figure 1. Scope of the DKR systems involving complex **4** and various enzymes.

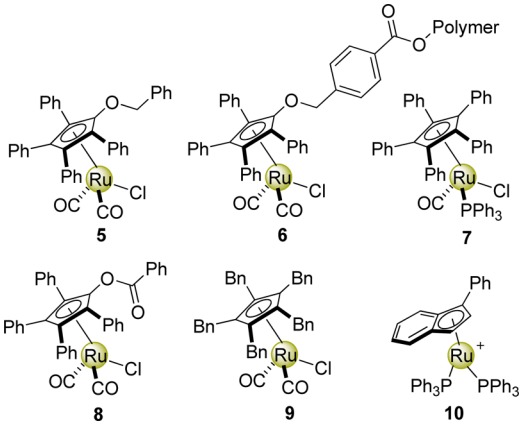

Figure 2. Ruthenium cyclopentadienyl-type racemization catalysts for DKR of secondary alcohols.

advantage of the benzyloxy motif was that it could be exploited as a handle for linking **5** onto polystyrene to create a heterogeneous version.[83] Interestingly, the DKRs involving the polymer-bound catalyst **6** gave comparable results in terms of yield and ee to those employing homogeneous **5**, demonstrating that heterogenization of the catalyst had a negligible effect on the racemization activity. Moreover, catalyst **6** exhibited good recyclability that allowed it to be reused three times in the DKR of 1-phenylethanol, where the (*R*)-acetate could be obtained in ≥95% yield and 99% ee over all cycles. In a subsequent study, catalyst **6** was employed in the key step of the synthesis of the enantiomerically pure pharmaceutical (−)-rivastigmine.[84] Kim, Park, and co-workers have also developed another air-stable analogue of complex **4** by replacing one of its carbon monoxide ligands with PPh_3.[85] The resulting catalyst **7** could be activated at room temperature by Ag_2O and used together with Novozyme-435 for the DKR of a small set of aliphatic and benzylic alcohols in excellent ee's.[85]

Recently, the group of Kim and Park reported on an interesting ruthenium complex **8** containing an acyl substituted cyclopentadienyl ligand. This catalyst allowed for a significant extension of the scope of enantiomerically pure secondary alcohols that can be accessed through chemoenzymatic DKR.[86] By combining catalyst **8** with ionic surfactant-coated *Burkholderia cepacia* lipase, the DKR of a variety of secondary alcohols was accomplished at 25−60 °C, including α-arylpropargyl alcohols, B(pin)-substituted benzylic alcohols, γ-chloro alcohols, and TMS-propargyl alcohols.[55,87]

The groups of Leino and Kanerva reported on the preparation of the related pentabenzylcyclopentadienyl ruthenium complex **9**, which displayed comparable activity and scope of utility to that of complex **4**.[88−90] However, an advantage of **9** is that its benzyl-substituted ligand can be conveniently synthesized on a large scale from the simple and cheap starting materials cyclopentadiene and benzyl alcohol. This can be compared to the syntheses of complexes **1** and **3**-**8**, which require the significantly more expensive precursor tetraphenylcyclopentadienone.

Another useful racemization catalyst, which does not require the use of strong alkoxide bases, was very recently reported by Nolan and co-workers.[91] The cationic ruthenium indenyl complex **10** is efficiently activated by the mild base, K_2CO_3, and was successfully combined with Novozyme-435 for the DKR of a variety of secondary alcohols in high yields and ee's at room temperature.[91]

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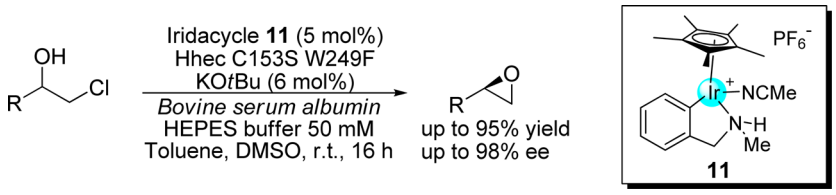
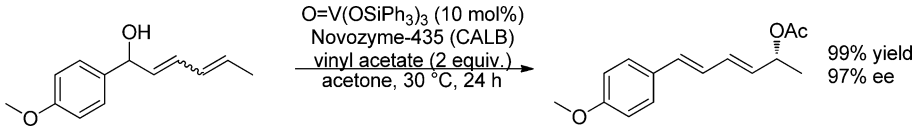
Scheme 6. Synthesis of Enantiomerically Pure Epoxides by Iridacycle 11 and a Haloalcohol Dehalogenase



Scheme 7. Asymmetric Synthesis of a Dienyl Acetate by $[VO(OSiPh_3)_3]$ and Novozyme-435



Recently, the group of Martín-Matute showed that a ruthenium catalyst, formed *in situ* from the readily available complex $[Ru(p\text{-cymene})Cl_2]_2$ and the ligand 1,4-bis-(diphenylphosphino)butane, could be employed in combination with lipase TL from *Pseudomonas stutzeri* for the efficient DKR of α-hydroxy ketones at room temperature.[92] The DKR of these substrates provides straightforward access to a variety of functionalized molecules, such as enantiomerically pure amino alcohol and diol derivatives.

So far this Perspective has mainly described protocols employing ruthenium-based racemization catalysts, but it is important to also highlight the work on secondary alcohol DKR that involves other metals. For instance, Feringa, De Vries, and co-workers have developed a procedure for synthesizing enantiomerically pure epoxides in one step from the corresponding chlorohydrins, by utilizing the cationic iridacycle **11** together with a doubly mutated haloalcohol dehalogenase (Hhec) in a biphasic system comprising toluene and 50 mM HEPES buffer (Scheme 6).[93] As with the monomeric ruthenium-based racemization catalysts **3**-**9**, iridacycle **11** was activated by KO*t*Bu, enabling efficient DKR at room temperature. Iridacycle **11** displayed an intriguing complementary reactivity to the ruthenium systems by exhibiting a significantly higher racemization activity and selectivity toward chlorohydrins compared to conventional benzylic secondary alcohols. Another iridium-catalyzed protocol for the base-free DKR of non-functionalized aliphatic and benzylic secondary alcohols was disclosed by Marr et al. This DKR utilized a series of "piano-stool"-type iridium NHC complexes together with CALB.[94]

A general topic of concern regarding ruthenium- and iridium-based catalytic systems for racemization is the relatively high cost and low natural availability of these metals. Therefore, efforts have been made to develop more cost-effective and readily accessible metal catalysts. An example addressing this requirement is the $AlMe_3$/binol/CALB system designed by Berkessel et al., which was used for the DKR of both aliphatic and benzylic alcohols at room temperature.[95] In addition, a number of vanadium-based catalytic protocols for the DKR of alcohols have been developed during the past decade. Akai and co-workers demonstrated that the oxyvanadium(V) complex $[VO(OSiPh_3)_3)]$ could racemize secondary allylic alcohols through 1,3-transposition of the hydroxyl group under mild reaction conditions. Accordingly, this catalyst was found to be compatible with several lipases, such as *Burkholderia cepacia* lipase, Novozyme-435 and *Pseudomonas fluorescens* lipase, which allowed for DKR of a wide range of linear and cyclic allylic secondary alcohols.[96,97] Moreover, the developed methodology could be used to transform a stereoisomeric mixture of dienols into a single dienyl acetate product in excellent yield and ee (Scheme 7).[97] The authors also prepared heterogeneous analogues of this oxyvanadium(V) catalyst, which were immobilized on both a polymer[97] and a mesoporous silica.[98] The latter heterogeneous catalyst proved to be recyclable over six cycles without any loss in activity, and furthermore, it was capable of racemizing benzylic, heteroaromatic and propargylic alcohols. The ability of this catalyst to mediate the racemization of substrates lacking the allylic alcohol motif indicates that it can also operate through a more general dehydrative mechanism proceeding via a carbocation intermediate. Another heterogeneous protocol for the DKR of secondary alcohols involving vanadium catalysis was reported by Wuyts et al.[99] In this system, $VOSO_4$ was combined with Novozyme-435 to achieve deracemization of several benzylic alcohols in octane at 80 °C.

There are also a number of reports on the use of heterogeneous acids and zeolites as racemization catalysts together with lipases for DKR of secondary alcohols.[100−105] However, the major limitation of most of these protocols is that they can only racemize alcohols through a dehydration mechanism, which limits their scope to substrates that can form stable carbocations. Furthermore, many of these systems suffer from reduced yields of the desired DKR products due to substantial formation of elimination side products.

In sharp contrast to secondary alcohols, tertiary alcohols are a significantly more cumbersome class of substrates for which there exist no practical DKR protocols. Although, there are a few enzymes that can resolve tertiary alcohols,[106−108] it has proven difficult to couple these KR processes to *in situ* racemization. Since the quaternary stereocenter of tertiary alcohols lacks a hydride substituent, it is not possible to utilize any of the transfer hydrogenation-type racemization catalysts. Thus, the list of available racemization catalysts for tertiary alcohols is primarily limited to those operating through dehydrative mechanisms (e.g., Lewis acids or vanadium catalysts) proceeding via the formation of a tertiary carbocation. The latter carbocation intermediate is formed much more readily than the corresponding secondary one, which should facilitate the racemization of tertiary alcohols. The development of a general and practical DKR protocol for tertiary alcohols would be considered as an important milestone within the field of asymmetric synthesis, given the high prevalence of this structural motif in natural products and pharmaceuticals.[108]

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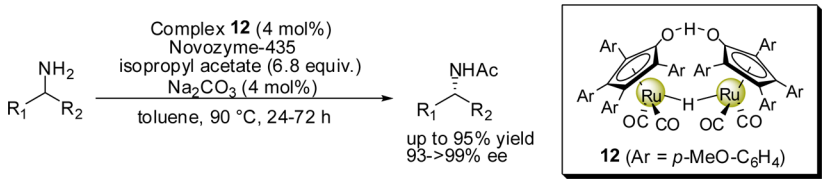
Scheme 8. DKR of Primary Amines by Complex 12 and Novozyme-435



4. THE MORE CHALLENGING DKR OF AMINES

As with alcohols, there are a variety of efficient methods for obtaining enantiomerically pure amines by the use of enzymatic KR.[14,16,17] However, the available DKR protocols are drastically fewer in number due to the lack of efficient amine racemization catalysts. The main reason for the difficulty of racemizing amines is that they can act as strong coordinating ligands, which may lead to inhibition or even complete deactivation of the metal catalysts. Thus, high temperatures are generally required to disrupt this undesired coordination and promote the racemization reaction. As previously discussed, the use of elevated reaction temperatures is undesirable from a DKR perspective, since it restricts the set of enzymes that can be employed. An additional challenge associated with the racemization of amines is that the generated imine intermediate is highly reactive and can thus take part in several side reactions, which reduces the yield of the desired DKR product. For example, the imine is prone to undergo hydrolysis into the corresponding ketone in the presence of water. The imine intermediate can also be subject to nucleophilic attack by another amine molecule to produce an aminal, which upon elimination of an ammonium ion forms a secondary imine that can be further reduced to a secondary amine byproduct. It has been found that both of these side reactions are usually favored by an elevated temperature, which further highlights the importance of efficient and mild amine racemization protocols.

The first DKR of an amine was reported by Reetz and Schimossek in 1996, where resolution of 1-phenylethylamine was accomplished by coupling CALB-catalyzed amine acylation to Pd/C-catalyzed racemization.[109] Unfortunately, the DKR reaction, which was performed in triethylamine at 50−55 °C using ethyl acetate as the acyl donor, was found to proceed slowly, and despite a reaction time of 8 days, it only gave a moderate conversion of 60%. Following this work, the group of Bäckvall demonstrated that the Shvo dimer **1** can be used as an efficient racemization catalyst for primary amines at 110 °C.[110] As a result of the high temperature, the enzymatic resolution was run separately at a lower temperature, and therefore the racemization and resolution had to be done stepwise. However, this problem was later circumvented by changing to the methoxy-substituted Shvo analogue **12**, which enabled efficient racemization at 90 °C. By using complex **12** together with Novozyme-435, the one-pot DKR of several aliphatic and benzylic primary amines was achieved in high yields and excellent ee's (Scheme 8).[111]

This protocol enabled the DKR of 1-phenylethylamine to be performed on a multigram scale, with a low catalytic loading (1.25 mol %) and with a substrate concentration of up to 0.9 M, affording the corresponding (*R*)-amide in good isolated yield and 98% ee.[112]

A noteworthy feature of the DKR protocol involving **12** was that isopropyl acetate could be used as the acyl donor. Although, this acyl donor may seem as the ideal choice given its low price and high availability, the use of carboxylic esters as acylating agents is generally undesired in amine DKR as they give an amide product that requires harsh reaction conditions to be reconverted back to the amine. Commonly, strong acids and elevated temperatures are required to cleave the stable amide bond, which may be detrimental for substrates containing sensitive functional groups. To address this issue, Bäckvall and co-workers developed an improved procedure for the DKR of both aliphatic and benzylic primary amines involving complex **12** and CALB that worked efficiently with dibenzyl carbonate as the acyl donor.[113] In contrast to the amide functionality, the installed benzyloxy carbonyl group can be easily removed under mild reaction conditions through Pd-catalyzed hydrogenolysis.

Together, the broad substrate scope involving both aliphatic and benzylic primary amines, the possibility of using carbonate-based acyl donors and the scalability make this catalytic protocol one of the most practical methods for the DKR of primary amines available to date. The group of Bäckvall has also demonstrated that complex **12** can be used in combination with the related enzyme *Candida antarctica* lipase A (CALA) immobilized on siliceous mesocellular foam (MCF) for the DKR of β-amino esters.[114]

Inspired by the seminal findings of Reetz and Schimossek, several research groups continued to study heterogeneous racemization protocols based on palladium for application in amine DKR. The first steps toward a practical DKR method for amines using this strategy were taken by Jacobs and co-workers with their investigation on how alkaline earth supports affected the racemization activity of immobilized Pd particles.[115,116] Among the tested catalysts, Pd on $BaSO_4$ was found to exhibit the highest activity and selectivity. The DKR with this racemization catalyst was performed at 70 °C under 0.1 bar of H_2, using Novozyme-435 as the resolving agent and either ethyl acetate or isopropyl acetate as the acyl donor. Under these reaction conditions, a range of benzylic primary amines were converted into the corresponding (*R*)-amides in high yields and ee's within 24−72 h (Scheme 10).[115,116] Andrade et al. later demonstrated that this protocol can also be applied for the DKR of selenium-containing benzylic primary amines with good results.[117] However, a significant drawback of this DKR system is that it is limited mainly to benzylic amines, while aliphatic primary amines generally react too slowly. The only aliphatic amine that was tolerated by this system was 1-methyl-3-phenylpropylamine, which contained a distant aryl group that was most likely the reason for the success. Despite, the presence of an aromatic moiety in the structure, this aliphatic amine was found to racemize significantly more slowly than the benzylic substrates and thus the corresponding DKR required both elevated temperatures and longer reaction times to give satisfactory results.[116]

In a subsequent study, the group of De Vos demonstrated that the activity and selectivity of the $Pd/BaSO_4$ and $Pd/CaCO_3$ catalysts in the racemization of primary amines could be improved by using microwave irradiation as an alternative heating method.[118] The reason for this phenomenon is that metal clusters are capable of efficiently absorbing microwave

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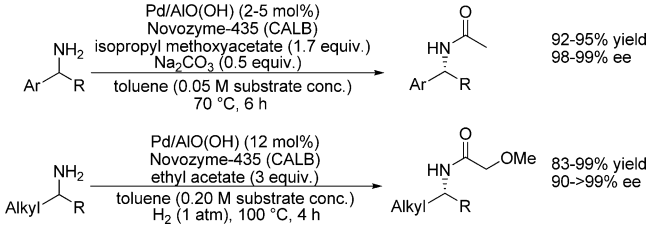
irradiation, which results in the generation of so-called "hot-spots" that can reach a temperature that exceeds that of the surrounding reaction media. The use of microwave irradiation was also found to lead to faster DKRs, although the ee's of these reactions were generally lower than those performed with conventional heating in an oil bath, because of a more facile background chemical acylation under the employed microwave conditions.

A useful DKR method involving heterogeneous palladium has been reported by Kim, Park and co-workers (Scheme 9).[119,120] In

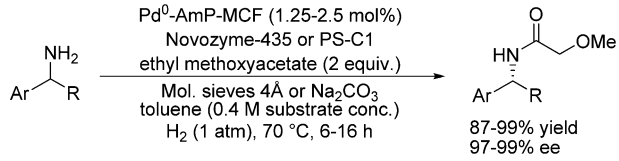
Scheme 9. DKR of Aliphatic and Benzylic Primary Amines by Pd/AlO(OH) and Novozyme-435



this protocol, nanoparticulate Pd immobilized on AlO(OH) is employed as the racemization catalyst together with Novozyme-435. This catalyst combination proved effective in the DKR of a range of benzylic primary amines, enabling the preparation of the corresponding (*R*)-amide products in high yields and excellent ee's. However, in line with the protocol developed by Jacobs and co-workers, this catalytic system required significantly harsher reaction conditions for the DKR of aliphatic substrates (12 mol % Pd, 100 °C and 1 atm H_2). Interestingly, both the Pd nanocatalyst and the enzyme could be recycled eight times in the DKR of 1-methyl-3-phenylpropylamine without any observable decrease in either conversion or ee. In a subsequent study, the group of Kim and Park extended the scope of this method to also include α-amino amides.[121] In addition, Bäckvall and co-workers have applied the Pd/AlO(OH) catalyst in combination with CALA-MCF for the DKR of β-amino esters.[122]

Xu et al. reported on the preparation of a heterogeneous racemization catalyst based on Pd immobilized on a layered double-hydroxide-dodecyl sulfate anion support and demonstrated that it could be used with Novozyme-435 for the DKR of benzylic primary amines at 55 °C.[123] Unfortunately this protocol suffered from several drawbacks, such as high catalyst loadings, dilute substrate concentrations, and the need of the activated ester 4-chlorophenyl valerate as acyl donor. Recently, the group of Bäckvall also developed a palladium-based heterogeneous racemization catalyst, consisting of 1.5−3.0 nm-sized Pd nanoparticles immobilized on aminopropyl-functionalized MCF (AmP-MCF).[124] This Pd nanocatalyst (Pd^0-AmP-MCF) exhibited high activity in the racemization of 1-phenylethylamine, and moreover it displayed good enzyme-compatibility that allowed it to be used in DKR. The Pd^0-AmP-MCF catalyst was combined with Novozyme-435 for the DKR of a range of primary benzylic amines at 70 °C, producing the corresponding (*R*)-amides in high yields and excellent ee's (Scheme 10).[125] Furthermore, by increasing the catalytic amount of palladium from 1.25 to 5.0 mol % it was possible to maintain an efficient racemization even at 50 °C, which allowed the catalyst to be used in a DKR of 1-phenylethylamine with the sensitive enzyme Amano Lipase PS-C1 (*Burkholderia cepacia* lipase immobilized on ceramic beads). Remarkably, this is the first time that Amano

Scheme 10. DKR of Benzylic Primary Amines by Pd^0-AmP-MCF and Novozyme-435/Amano Lipase PS-C1



Lipase PS-C1 has been successfully utilized in a DKR of an amine. It is also important to highlight the fact that the amount of Pd nanocatalyst used in these DKR reactions with reasonably short reaction times is lower than previously reported for primary amines and that the reactions are run at a substrate concentration of 0.4 M, which is significantly higher than that used in previously reported systems.[119,120,123] Other practical advantages of the Pd^0-AmP-MCF were that it displayed high stability and low leaching, which allowed it to be recycled up to four times in the DKR of 1-phenylethylamine without any observable decrease in performance. As with other palladium-based systems, this DKR system does not work well for aliphatic amines.

The group of Li has also studied this system for the DKR of primary amines;[126] however, they used a slightly different version of the Pd^0-AmP-MCF catalyst that was impregnated with K_2CO_3 and contained a lower palladium loading than the one used by Bäckvall and co-workers (2.0 versus 8.0 wt % Pd). Even though this alternative protocol allowed for an efficient and selective DKR of several amines, it is difficult to compare its performance to the system published by the group of Bäckvall, as it was studied under very different reaction conditions involving increased enzyme loadings and significantly lower substrate concentrations.

The MCF material that was employed by the group of Bäckvall and Li to support the Pd nanoparticles has also been used to immobilize CALA.[114,122] With this versatility of the MCF in mind, the group of Bäckvall explored the possibility of co-immobilizing Pd nanoparticles and an enzyme into the cavities of this support. This was done by first preparing the Pd^0-AmP-MCF catalyst with a moderate loading of palladium to leave a number of free aminopropyl groups for the enzyme, then functionalizing the free aminopropyl groups of the support with glutaraldehyde, and finally exploiting the aldehyde groups as linkers for the anchoring of CALB.[127] By this co-immobilization strategy, a metalloenzyme-resembling bifunctional catalyst was obtained that can perform both racemization and KR (Figure 3). This hybrid catalyst was evaluated in the DKR of 1-phenylethylamine using ethyl methoxy acetate as the acyl donor under 1 atm. of H_2 at 70 °C. Under these conditions the desired (*R*)-amide product was obtained in 99% yield and 99% ee within 16 h. Interestingly, the reaction involving the hybrid catalyst was found to proceed faster than that of separately supported Pd(0)-AmP-MCF and CALB-MCF, highlighting that the close proximity of the two catalysts increases the rate of the DKR. The hybrid catalyst could be recycled, but unfortunately it was found to exhibit diminished activity from the third cycle as a result of partial enzyme denaturation caused by the hydrophilic silica support surface.

It is not only palladium- and ruthenium-based racemization catalysts that have been employed in amine DKR systems. The group of De Vos showed that both Raney Ni and Raney Co catalysts could be combined with Novozyme-435 for the DKR of primary amines.[128] Unfortunately, these DKRs were found to proceed slowly, and even though they were performed at 70−80 °C for 2−5 days, the conversions and ee's were generally low. On

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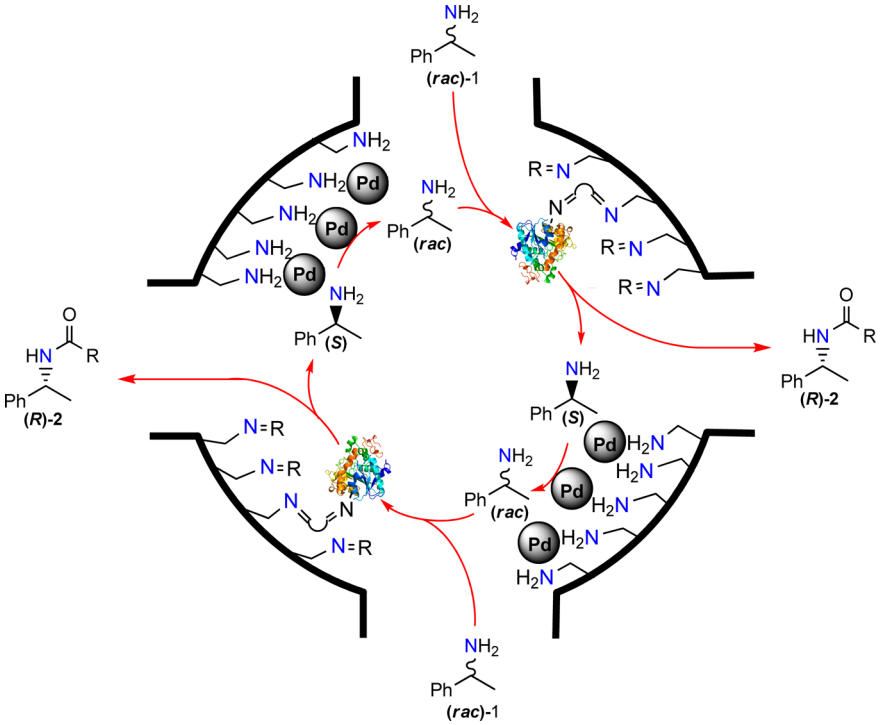
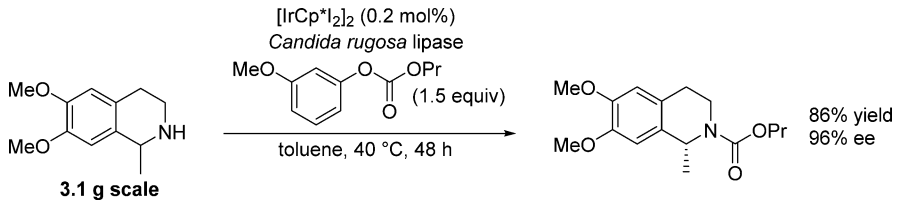


Figure 3. DKR of an amine with a bifunctional biomimetic catalyst in which Pd nanoparticles and a lipase (CALB) are co-immobilized in MCF. (Reprinted from ref 127 with permission from the publisher. Copyright 2013 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim).

Scheme 11. DKR of a Secondary Amine by $[IrCp^*I_2]_2$ and *Candida rugosa* Lipase



the other hand, this catalytic system displayed an interesting preference for aliphatic primary amines, allowing for a faster DKR of these substrates. Surprisingly, by following the ee of the starting material throughout the reaction, it was established that the long reaction time was due to a rate-limiting KR process. Normally, the enzymatic KR of primary amines should proceed fast at these high temperatures. These results therefore suggest that the Raney metal catalysts have an inhibitory effect on the enzyme. It was found that enzyme poisoning was caused by leaching of cobalt and nickel ions and that this problem could be circumvented by performing the KR and racemization in separate pots in a successive manner. However, this greatly diminished the practical utility of this DKR protocol.

Another interesting DKR system based on homogeneous $[IrCp^*I_2]_2$ and *Candida rugosa* lipase was reported by Page and co-workers and was used for the deracemization of a secondary amine on a multigram scale.[129,130] The DKR of secondary amines is significantly more challenging, because the extra substituent on the nitrogen brings additional steric bulk that prevents the substrate from being accepted by the enzyme. Despite the fact that several secondary amines could be efficiently racemized by the iridium catalyst, the authors only managed to construct a working DKR for one isoquinoline derivative (Scheme 11).

In nature, the stereoinversion of some amino acids is carried out by a family of racemases that utilize pyridoxal phosphate as the catalytically active prosthetic group.[131] These enzymes operate through a so-called Schiff base-type mechanism, where the catalytically active pyridoxal phosphate group reacts with an amino acid to produce an imine intermediate, from which racemization occurs via a base-mediated enamine-imine interconversion. Inspired by this class of natural racemases, Felten et al. developed a synthetic active-site analogue by complexating $Zn(OTf)_2$ to picolinaldehyde. This racemization catalyst was successfully combined with the enzyme alacase (another name for subtilisin Carlsberg) for the DKR of a small series of γ-branched amino acids in high enantioselectivity at room temperature.[132]

Recently, several reports on metal-free methods for the DKR of amines have appeared. The group of Gil and Bertrand has shown that racemization of amines can be achieved by the use of *in situ* generated sulfanyl radicals and that it is possible to couple this process to enzymatic acylation.[133−136] Although this method has so far only been applied to the DKR of simple primary amines containing no or little functionality, it is promising since the racemization occurs under mild reaction conditions, which enables the use of sensitive proteases. Another well-established method to racemize amino acid derivatives containing acidic α-protons is to employ a base that is sufficiently strong to deprotonate these substrates.[137] For example, Tessaro and co-workers have successfully utilized the organic base 1,8-

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diazabicycloundec-7-ene (DBU) as a racemization catalyst in the mild DKR of several amino acid derivatives by combining it with subtilisin-catalyzed thioester hydrolysis.[138−140]

5. METHODS FOR IMPROVING ENZYMES AS BIOCATALYSTS

So far, this Perspective has focused almost entirely on the design of efficient racemization catalysts as a means to broaden the scope of the DKR methodology. However, it is important not to overlook the considerable amount of work that has been done to improve enzymes as biocatalysts. The properties of an enzyme can be improved by immobilization, cross-linking, surfactant-stabilization, or enzyme engineering/directed evolution. The latter topic has been greatly propelled by advances in the field of molecular biology and genetic engineering, which has led to the development of new recombinant technologies that makes it possible to incorporate tailor-made DNA fragments into organisms, such as *Escherichia coli* and *Pichia pastoris*, and use them as hosts for the expression of mutant enzymes with novel properties. Unfortunately, it is beyond the scope of this Perspective to summarize all key contributions to this vast research area, and therefore we kindly refer interested readers to a number of excellent recent reviews that cover this topic thoroughly.[141−145] Instead, our aim is to describe some selected techniques that we believe have the potential of making an impact on the field of alcohol and amine DKR.

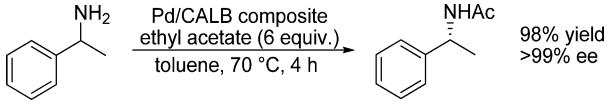
A classical way to improve the thermostability of enzymes and thus make them available for a wider range of DKR protocols is to immobilize them on a heterogeneous support.[144,146,147] In fact, the majority of the commercially available enzymes that are used for DKR today are already supported on various types of carriers, including ceramic beads, diatomaceous earth, ionic liquids, resins, and silicas. In addition to improving the thermostability, the immobilization of enzymes also lead to several practical advantages, such as easier handling, simpler separation, and possibilities of recycling.

Another intriguing method to improve the general performance of enzymes is to polymerize them into so-called cross-linked enzyme aggregates (CLEAs) by the use of a bifunctional cross-linking agent.[148−150] Typically, glutaraldehyde is used for this purpose as it can react with free lysine residues on the surface of two neighboring enzyme molecules and covalently link them together through stable Schiff-base-type bonds. This cross-linking leads to the formation and precipitation of large insoluble enzyme aggregates, which can be easily separated by either centrifugation or filtration. Remarkably, these CLEAs often display comparable catalytic activity to that of the free enzyme, suggesting that the enzyme is locked in its active conformation in the aggregate and that diffusion of substrate into the enzyme's active site is not significantly hindered. Furthermore, this aggregation strategy has been shown to lead to dramatic improvements of the stability of the enzyme toward elevated temperatures, hostile solvents, and autoproteolysis. These improvements are a direct consequence of the decrease in flexibility, which suppresses deactivation through denaturation. An additional advantage of the CLEA methodology is that it combines the processes of enzyme purification and immobilization into a single operation. Consequently, it is possible to apply this method directly on crude extracts instead of pure enzyme solutions.

Today, a substantial number of CLEAs based on lipases and proteases have been developed and successfully used for the preparation of enantiomerically pure alcohols and amines.[107,140,151−156] Despite these achievements, there is to the best of our knowledge only one group, who has so far studied CLEA for applications in DKR.[137−139] Tessaro and co-workers studied the advantages of the CLEA methodology in DKRs of amino acid derivatives involving subtilisin Carlsberg and DBU.[138,139] Here, subtilisin Carlsberg was found to exhibit a higher tolerance to DBU when it was turned into a CLEA, which enabled a more efficient DKR. The promising results from this work suggest that the CLEA methodology could find applications in other DKR protocols as well. For example, in the case of amine DKR, where the racemization catalysts require elevated reaction temperatures, there is a need for thermostable enzymes and here the use of CLEAs could be advantageous.

An alternative approach to prepare heterogeneous enzyme composites reminiscent of the CLEA methodology was recently reported by the group of Zare.[157] In this method, flower-shaped protein-inorganic hybrid nanostructures could be generated upon addition of Cu(II) ions to enzymes, such as CALA, carbonic anhydrase, laccase, and α-lactalbumin. As in the case with the CLEAs, the nanoflowers were shown to exhibit a significantly higher thermostability than the free enzymes. Interestingly, the nanoflowers also exhibited significantly enhanced catalytic activities, which were ascribed to the high surface area and the confinement of the enzymes in the nanoflowers. Inspired by this study, Filice et al. synthesized a similar protein-inorganic hybrid by mixing CALB with $Pd(OAc)_2$ in aqueous media.[158] In this reaction, the CALB acts as a reducing agent for the Pd(II) ions, which leads to the formation of small Pd nanoparticles within the emerging polymeric enzyme composite. The Pd/CALB composite was shown to display both acylation and racemization activity, which allowed it to be employed as a bifunctional catalyst in the DKR of 1-phenylethylamine in excellent yield and ee (Scheme 12).

Scheme 12. DKR of 1-Phenylethylamine by a Bifunctional Pd/CALB Composite



For most DKR applications, it is crucial that the enzyme operates efficiently in organic solvents since most substrates and racemization catalysts are not soluble in aqueous media. Many lipases work well in dry organic solvents, whereas proteases do not. One way to improve the activity and stability of a protease in organic solvents is to coat it with a lipid or surfactant before lyophilization. This treatment generates a reversed micelle around the enzyme with concomitant solubilization of small amounts of water, which provides the protease with a stable aqueous microenvironment that is maintained even when it is suspended in an organic solvent. Several examples on the successful use of surfactants to stabilize enzymes and enable DKRs have already been presented in this Perspective.[54,55,80,87,135]

Alternative ways of obtaining enzyme mutants with improved properties involve rational enzyme design[141,159,160] and directed evolution.[160−164] In the rational design approach, amino acid residues that are anticipated to play a key role for the function of the enzymes are first identified by the means of for example X-ray crystallography, homology studies, or computational models. These amino acids are then selectively replaced by other amino

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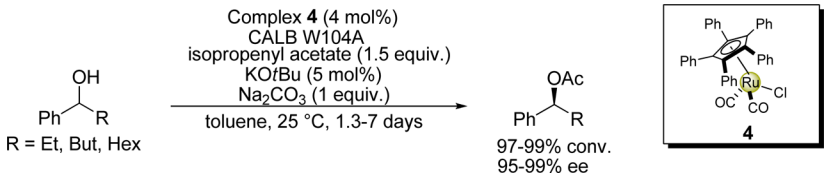
Scheme 13. DKR of Sterically-Demanding 1-Phenylalkanols by Complex 4 and CALB W104A



acid residues that are expected to yield a mutant variant displaying the desired properties. Unfortunately, the structural information on the enzyme that is needed to guide such efforts is in most cases limited, which imposes a severe restriction on when this methodology can be utilized. In fact, even with this knowledge in hand, it is often difficult to predict what structural modifications that should be incorporated in order to improve the performance of the enzyme. This is because our understanding of the chemical principles that govern the function and stability of enzymes is still very limited.

Despite this issue, rational design has been successfully used at multiple occasions for improving the performance of lipases.[160] For example, Hult and co-workers used a rational design approach to create a CALB mutant that exhibited reverse enantioselectivity (*S*) as well as an improved substrate tolerance toward bulky secondary alcohols.[165,166] Interestingly, this dramatic alteration of the catalytic properties was achieved by exchanging a single amino acid residue in the so-called stereospecificity pocket of the enzyme. In the wild-type CALB, the fast-reacting enantiomer places its medium-sized group in the stereoselectivity pocket and its large group toward the entrance of the active site. The access of the large group to the stereoselectivity pocket is effectively prevented by three sterically demanding amino acid residues: Thr42, Ser47 and Trp104. The authors identified that a mutant with fundamentally different substrate preference could be generated by changing the sterically demanding Trp104 to a smaller alanine residue. Subsequently, Bäckvall and co-workers used this enzyme variant, denoted as CALB W104A, together with complex **4** for the (*S*)-selective DKR of a series of bulky 1-phenylalkanols in high yields and ee's (Scheme 13).[167] Following this study, the group of Bäckvall and Hult explored CALB W104 as the resolving enzyme for diarylmethanols; however, satisfactory *E* values were only obtained for substrates where the two aryl substituents differed significantly in size.[168] As a result, the development of a DKR protocol for the latter substrate class was never pursued. The group of Kim and Park recently solved the DKR of this substrate class by using ruthenium complex **8** together with activated lipoprotein lipase.[169]

Recently, Ema, Sakai, and co-workers redesigned *Burkholderia cepacia* lipase by introducing two alterations, I287F and I290A, into the catalytically active site using a rational design approach.[170,171] This double mutant removed a substantial part of the steric congestion in the active site, which enabled this enzyme variant to accept a wide range of extremely bulky secondary alcohols. Furthermore, it was found that the phenylalanine residue introduced at position 287 could participate in an additional C−H/π-interaction with the substrate alcohol, which helped to stabilize the transition state of the acylation reaction and led to an improved (*R*)-selectivity of the enzyme. Although, the authors only evaluated this *Burkholderia cepacia* lipase variant for KR purposes, it is reasonable to envision that a mild DKR protocol could be constructed by combining this enzyme with any of the available ruthenium-based racemization catalysts.

In comparison to rational design, generation of large enzyme libraries with subsequent screening and selection (e.g., directed evolution) is a more useful method for accessing enzyme mutants with improved properties. In this directed evolution approach, natural evolution is artificially mimicked under laboratory settings to create a Darwinian-type selection process that will favor emergence of a desired mutant. In practice, this is done by performing iterative cycles of: (i) generation of gene libraries from the parent wild-type enzyme by the use of various mutagenesis techniques;[172−174] (ii) expression of the corresponding enzymes from the gene libraries; (iii) screening of the enzyme mutants for a desired property using various high-throughput methods;[175,176] and (iv) selecting an improved mutant as a template for the next round of mutagenesis/expression/screening (Figure 4). To date, the directed evolution

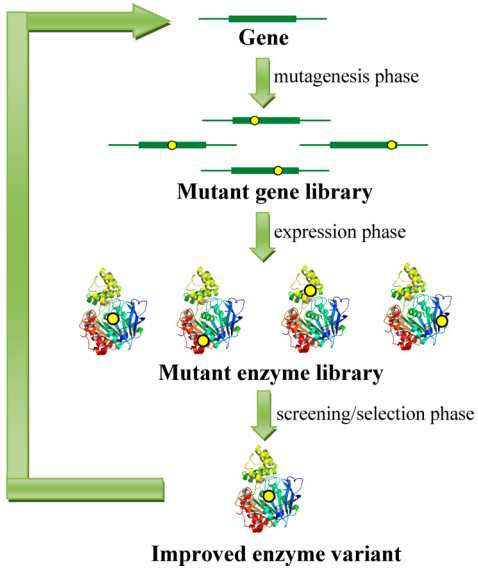


Figure 4. Schematic representation of the directed evolution methodology, where an iterative number of mutagenesis, expression, screening and selection cycles is conducted until an enzyme mutant with desired properties has been obtained.

methodology has been successfully used to modulate several properties of enzymes, including solvent tolerance,[177−179] thermostability,[180−184] and higher enantioselectivity for a broader scope of substrates.[106,185−190]

6. SUMMARY AND OUTLOOK

The field of alcohol DKR has certainly advanced significantly during the past two decades and reached a high level of maturity. Today, a wide range of functionalized primary and secondary alcohols can be efficiently resolved by the use of chemoenzymatic DKR. The key to this progress has been the successful design of

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several racemization catalysts, particularly those based on ruthenium, that can racemize alcohols under mild reaction conditions, thereby enabling the use of an increased number of enzymes. Ultimately, it is the enzyme component of the DKR that determines what types of substrates that can be resolved and which enantiomer of the product that is favored. Therefore, it is essential to have access to DKR protocols that involve a variety of enzymes. So far, lipases have been the enzymes of choice for DKRs, owing to their high activity and selectivity. Moreover, these enzymes are associated with a number of practical advantages, including high commercial availability, high thermostability, and good tolerance toward organic reaction media. However, as mentioned previously most naturally occurring lipases preferentially give (*R*)-selective resolution of secondary alcohols, and this imposes a limitation for the DKR, since the (*S*)-product cannot be accessed directly. It is therefore important to have access to (*S*)-selective enzymes so that the (*S*)-product can be prepared directly by DKR. Examples of (*S*)-selective enzymes in KR of alcohols are serine proteases, but they are unfortunately not very thermostable. For most secondary alcohols, racemization can be accomplished in reasonable times at room temperature thanks to the most recently developed ruthenium catalysts, which enable DKR systems involving serine proteases. However, for certain challenging substrate classes, such as chlorohydrins and alcohols containing distant olefin groups, the performance of the available ruthenium catalysts is not sufficient to allow for mild DKR's, and here there exists an opportunity for new catalyst design. Another important topic of research within the field of alcohol DKR is to develop efficient racemization protocols for tertiary alcohols, which are compatible with the currently available enzymatic KR processes.

In contrast to alcohols, the available DKR systems for amines are significantly fewer in number due to challenges associated with the racemization of these substrates. Despite the considerable amount of research that has been dedicated to amine DKR, most of the reported protocols still involve racemization catalysts that require high reaction temperatures to function efficiently, which greatly restrict the set of enzymes that can be employed. Moreover, the majority of these DKR protocols have only been successful with substrates that are readily racemized, such as α-amino acid derivatives and benzylic amines. When it comes to aliphatic amines, the available DKR protocols are significantly fewer in number and generally involve harsh reaction conditions. Here, the recently developed metal-free methods to racemize amines by the use of sulfanyl radicals show great promise and might hold the key to mild DKR of both aliphatic and benzylic amines. However, a major concern regarding the racemization by sulfanyl radicals is that it has so far only been combined with a limited number of enzymes, and it is still unclear how widely applicable this method is for DKR.

Research efforts dedicated toward improving the enzyme component will also play an important role in advancing the field of alcohol and amine DKR. With available molecular biological techniques, chemists now have access to methods for improving and expanding the portfolio of enzymes provided by nature. In particular, evolution of enzymes via generation of large libraries with subsequent screening and selection is a highly useful method for obtaining new enzyme variants with improved properties. Until very recently, all screening studies on lipase libraries for increased enantioselectivity had dealt with the hydrolysis of esters in an aqueous medium. However, most DKRs of alcohols and amines are carried out as transacylations in an organic solvent. Recently, a method was reported that enables evolution of a lipase for transacylation of secondary alcohols in organic solvent, and it was demonstrated that CALA gave a double mutant (CALAY93L/L367I) with a significantly improved *E* value, 100 vs 3, in the transacylation of 1-phenylethanol in isooctane.[191] This method is promising and may provide new improved enzymes for the DKR of alcohols and amines.

Another promising technique to improve the thermostability of enzymes is the CLEA methodology, where enzymes are converted into heterogeneous aggregates through treatment with a bifunctional cross-linking agent. It is expected that the CLEA methodology will find future applications in DKR of alcohols and amines.

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Notes

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