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2598 *Current Organic Chemistry,* **2012**, *16*, **2598-2612**

New Trends in Organic Synthesis with Oxidative Enzymes

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Abstract: Oxidative enzymes constitute privileged catalysts in organic synthesis. Environmentally benign reaction conditions along with high selectivity are the most appealing characteristic shown by these biocatalysts in contrast to classical metal-based reagents. The present review surveys new perspectives and concepts derived from oxidative enzymatic processes, comprising oxidative C-C bond forming reactions, atroposelective oxidations, oxidative dynamic processes, interconnected reactions, cyclic deracemizations, oxidative desymmetrizations and artificial oxidative enzymes. Selected examples taken from the recent literature are discussed, highlighting relevant aspects from a synthetic point of view. Thus, application of these biocatalyzed reactions in the preparation of chiral high-added value compounds is also outlined. Finally, future perspectives for the development of novel oxidative enzymatic processes and further applications of well settled ones are presented.

Keywords: Dynamic kinetic resolution, Cyclic deracemization, Desymmetrization, Oxidative enzymes, Artificial enzymes, Cofactor engineering.

INTRODUCTION

Researchers from industry and academia are facing new challenges almost every day in organic synthesis. It becomes obvious that addressing those challenges goes in hand with the development of new technologies, leading to a tremendous advance in the field. Therefore, as pointed out by Noyori, Organic Synthesis must pursue a practical elegance, that is, not only the development of fancy processes and complex devices, but also the chemist must address a practical problem [1]. In this line, biocatalytic processes have gained momentum and nowadays there are many ton-scale syntheses based on enzymatic processes [2,3]. This fact is due, at least partially, to the outstanding selectivity shown by enzymes and the intrinsic sustainability of such processes [4,5]. In the last few years, the field of biocatalysis has witnessed, in the authors' opinion, a change in the focus of its efforts. Among other scenarios, previous enzymatic works attempted mainly to tackle problems of enantioselectivity displayed by non-enzymatic reactions and, to a lesser extent, situations in which labile functional groups should be preserved when traditional reagents/catalysts proved to be not suitable or not enough chemoselective. In turn, new concepts have arisen and previous ones reached an impressive level of development nowadays, overcoming scale-, availability- and substrate spectrum-related limits. Besides, new chemical reactions and process designs are being realized with great success. The aim of the present revision is not an exhaustive survey of oxidative enzymatic processes, for such an approach the reader may consult excellent recent reviews on this topic [6,7]. Indeed, the authors have undertaken a selection of representative recently appeared examples, comprising oxidative enzymatic reactions that might be of great interest from a synthetic point of view.

Perhaps, within the strategies that have attracted more attention, are dynamic protocols that enable access to 100% theoretical yield in a chemical transformation [8]. In enzymatic dynamic processes, most of the reports deal with hydrolases, with a strong predominance of lipases [9]. In the last few years redox enzymes are gaining relevance [10], providing new possibilities and, thus, paving the way to novel oxidative dynamic processes. In a similar way, enzymatic desymmetrization reactions permit 100% theoretical yield as well and in the past decade have demonstrated to be powerful strategies to define one or more chiral centers, not only by the enantiopurity and chemical yields of the resulting products but also for the simplicity and versatility of this procedure, in sharp contrast with conventional methods [11]. Furthermore, very recently, another sort of oxidative enzymatic transformation, namely the oxidative C-C bond forming reaction has emerged. It must be emphasized this type of C-H activation is still a challenge for organo- and metal catalysis [12,13], either by polar or radical processes when working in aqueous reaction media [14]. In spite of its early stage, the mentioned reaction represents a starting point for new discoveries in C-C enzymatic forming reactions.

It is not surprising that researchers from across the field of Organic Chemistry get inspired by biosynthetic machineries and try to mimic them in a flask. Therefore, a flourishing research area deals with advantages of both worlds, *i.e.* the robustness of metal catalysis and the fine tuning of the second coordination sphere of the central metal by the careful adjustment of the weak interactions with the amino acid backbone, thus providing a suitable chiral environment for the reaction to take place [15]. Not only metal catalysis combines with macromolecules to engender versatile hybrid cata-

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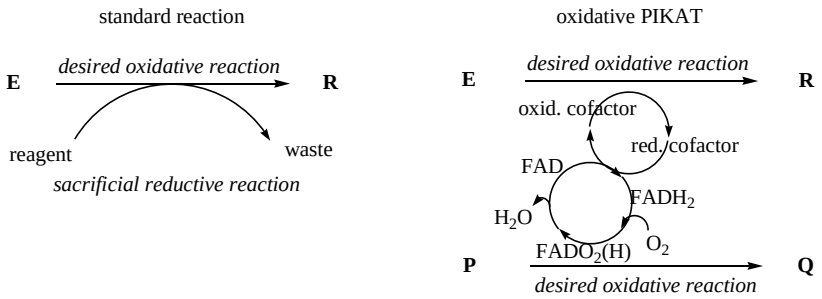
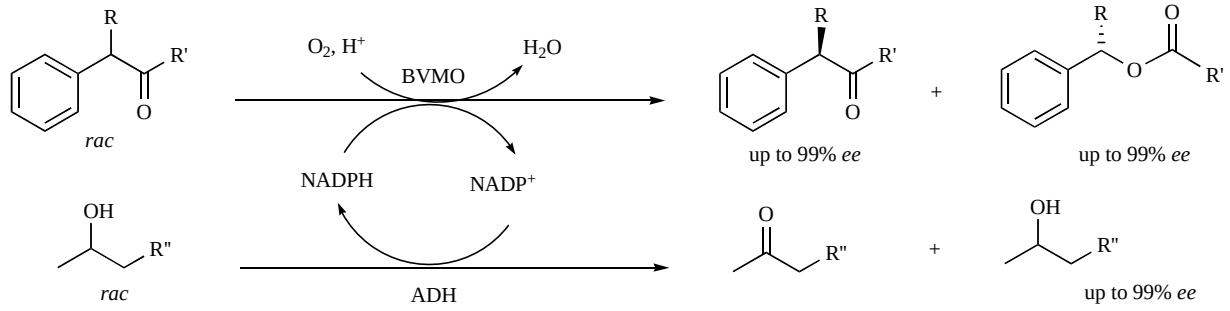


Scheme 1. Comparison between standard redox couple and oxidative PIKAT system.



Scheme 2. Parallel Interconnected Kinetic Asymmetric Transformations (PIKAT) of racemic ketones and alcohols catalyzed by BVMOs and ADHs.

lyst, but also organocatalysis is being exploited for such a purpose [16]. Thus, new generation artificial biocatalysts are being designed by taking advantage of flavin (and related active molecules), and their binding affinities for proteins and cyclodextrins, with promising oxidative reactivities toward a broad range of substrates.

Parallel Interconnected Kinetic Asymmetric Transformations (PIKAT) with Oxidative Enzymes

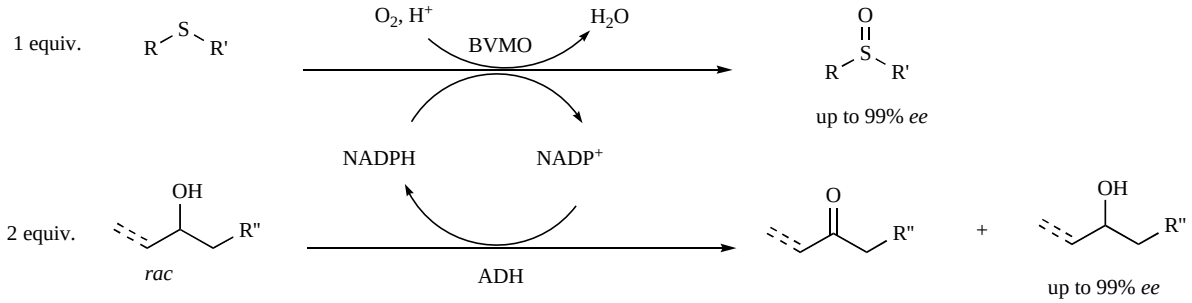
In the frame of the redox economy concept [17], one should minimize oxidative and reductive reactions and keep an effective balance of them along the synthetic sequence. It might be assumed that in a given redox reaction, the redox equivalents are provided by a sacrificial reaction. For instance, in a simple redox transformation such as the reduction of a keto group to form an alcohol, the redox equivalents come from the $NaBH_4$, rendering the desired alcohol plus the borate salts. Hence, this oxidative hemireaction is not synthetically relevant but necessary to power up the reductive one.

Some biocatalytic protocols have nicely coupled two opposite redox synthetically relevant reactions to build up a redox neutral overall process [18,19], turning the sacrificial hemireaction into a productive one. Recently, it was reported the development of a biocatalytic system by which two oxidative reactions, namely the oxidation of a racemic alcohol along with the oxidation of a racemic ketone work strictly in parallel (Scheme **1**). The trick is the use of an oxidative transformation that consumes the cofactor in its reduced form, (*i.e*., for the reduction of flavin coenzyme and further reaction with dioxygen) rendering the desired oxidized product plus the oxidized cofactor and, in this way, the coupled reaction makes use of the latter, recycling it internally, thus working concurrently by performing two oxidations in interconnected fashion [20].

Likewise, the system has been effectively orchestrated by exhaustive optimization of reaction parameters, since cross-reactivity of the selected enzymes must be avoided. Besides, pH, reaction temperature, and chemical nature of substrates are critical parameters for the process success so, kinetic measurement of each enzyme with the substrate candidates must be taken into account in advance.

It was clearly demonstrated that the use of a Baeyer-Villiger monooxygenase (BVMO) in combination with an alcohol dehydrogenase (ADH), resulted in an effective approach to perform two oxidative enantioselective kinetic resolutions in one pot, maximizing the redox economy of the overall process. As a subclass of the monooxygenases, BVMOs perform the insertion of one atom of oxygen from molecular oxygen into a substrate, being reduced the remaining oxygen atom into water [21,22]. BVMOs are flavin-dependent monooxygenases that require nicotinamide cofactors [NAD(P)H] as electron source. These enzymes are able to catalyze the Baeyer-Villiger oxidation, a key reaction in organic synthesis, as well as the boron atom oxidation and the oxygenation of different heteroatoms as nitrogen, sulfur, selenium, iodine and phosphorous. Indeed, some epoxidations have been described catalyzed by BVMOs. These BVMO-catalyzed processes often display high regio- and/or enantioselectivities, characteristics difficult to match by conventional methodologies, while using mild and environmental friendly conditions. Likewise, the well known ADHs are useful biocatalysts either to reduce carbonyl groups or to oxidize alcohols. These efficient enzymes are pyridine-cofactor dependent proteins, able to catalyze redox reactions over a wide spectrum of substrates with high regio- and stereo- selectivity [23]. Besides, in account for its robustness, it has been demonstrated that they tolerate non-conventional reaction conditions such as high organic solvent concentration, the presence of ionic liquids, and pHs above 10 [24]. These characteristics make these oxidoreductases versatile biocatalysts for multienzymatic systems.

The use of different BVMOs (phenylacetone monooxygenase (PAMO) from *Thermobifida fusca* [25], its M446G mutant (M446G PAMO) [26], and 4-hydroxyacetophenone monooxygenase (HAPMO) [27], from *Pseudomonas fluorescens* ACB) and ADHs (LBADH from *Lactobacillus brevis* [28], and ADH-T from *Thermoanaerobacter* sp.[29]) allowed the preparation of a series of α-substituted ketones, esters and *sec*-alcohols with outstanding enantioselectivities (up to 99% *ee*, Scheme **2**). Hence, in this approach,



Scheme 3. PIKAT system combining the asymmetric oxidation of prochiral sulfides with the kinetic resolution of racemic *sec*-alcohols.

the PIKAT system affords three optically enriched products at once in only 6 h reaction time.

Moreover, the procedure could be extended to other oxidative transformation such as sulfide oxidation. Enantioenriched (hetero)aryl alkyl and dialkylsulfoxides, together with *sec*-alcohols with up to >99% *ee* in both cases, were obtained (Scheme **3**). Besides, the stereochemistry of the resulting products can be tuned by the proper choice of the involved biocatalysts, giving rise to all the possible product combinations in terms of configuration at the stereocenter [30].

As a proof of concept, it was demonstrated that the interconnected system was still productive even at cofactor concentration as low as 1 μM. This is truly noteworthy since intracellular NADPH concentration in bacteria is in the same order of magnitude. In order to evaluate the catalytic efficiency, total turnover number were calculated focusing on the cofactor and, in most cases, reached TON of > 5500 (calculated for the formation of a single resulting product, thus, if the two oxidation reactions are considered, the TON value is exactly the double). It must be emphasized that this protocol is not restricted to the above mentioned sort of enzymes; other biocatalysts may fit into this design, thereby enabling new perspectives about their scope and limitations.

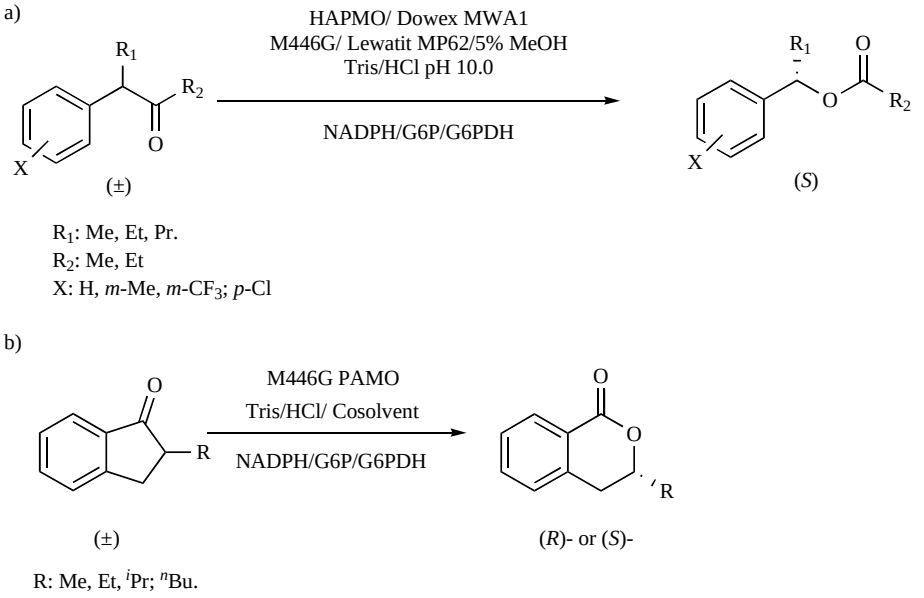
Enzymatic Oxidative Dynamic Kinetic Resolutions (DKRs)

As above mentioned, one of the main approaches for the synthesis of enantiomerically pure compounds is the kinetic resolution of racemic substrates, a technique that is usually straightforward and satisfactory in terms of optical purity, but it presents the intrinsic limitation of the 50% maximum yield for an enantiomer. Strategies to increase the yield are, therefore, of great importance. Thus, Dynamic Kinetic Resolutions (DKRs) have emerged in order to overcome the limitation of the classical kinetic resolutions [31,32]. In a DKR process, the resolution step is coupled with the *in situ* substrate racemization in order to obtain theoretically a single enantiomeric product in 100% yield. If the kinetic resolution is selective and the racemization fast enough, DKR can dramatically improve the synthetic efficiency for the desired enantiomer. Substrate racemization can be performed by a standard non-enzymatic catalyst, as for example inorganic salts, organometallic compounds or aldehydes. Besides, substrates can also be racemized by a S_N2 reaction, redox reactions or by using ionic exchange resins. Alternatively, thermal racemization, catalyzed by acids or bases or by using biocatalysts (racemases), may be applied. DKRs protocols have been widely applied in biocatalysis, directed mainly to the preparation of enantioenriched chiral alcohols or amines, by combining the enzyme-catalyzed kinetic resolution of these substrates using a hydrolase with the substrate racemization catalyzed by a metal-based reagent [33]. In a lesser extent, DKRs have been described using oxidoreductases. However, most of the examples involve the use of ADHs and base-catalyzed racemization for the preparation of chiral alcohols with high yields and optical purities [34]. Regarding DKRs employing oxidative enzymes, most of the examples involve Baeyer-Villiger monooxygenases and base-catalyzed racemization. This is a well-known method that has been extensively utilized and especially applicable to compounds with a stereocenter bearing an acidic proton.

The first DKR process employing BVMOs was performed by the Furstoss group on a racemic α-substituted cyclopentanone, namely (±)-2-benzyloxymethylcyclopentanone, a ketone that can suffer a spontaneous pH-dependent racemization *via* a keto-enol tautomerization favored by its acidic alpha hydrogen [35]. Complete racemization was observed after 7 hours at pH 9.0. Thus, the oxidation performed at pH 8.5 employing whole cells of *E. coli* TOP10[pQR239] overexpressing CHMO from *Acinetobacter* sp. led to the formation of the (*R*)-6-benzyloxymethyl-tetrahydropyran-2-one with 96% *ee* and an analytical yield of 85% after 24 hours. However, some bottlenecks such as the partial loss of enzymatic activity at the high pH and the low substrate concentration required to avoid inhibition or toxicity effects, hampered the preparative application of this process. These drawbacks were further circumvented by the addition of anion exchange resins that allowed racemization to occur at neutral pH [36]. The oxidation of (±)-2-benzyloxymethylcyclopentanone catalyzed by *E. coli* $CHMO_{Acineto}$ cells was performed in presence of different resins, obtaining the best results with the macroporous weak anionic resins Lewatit MP62 and MP64, bearing tertiary amines as functional group. Optimization of the experimental conditions permitted, when working at pH 7.5, a three-fold increase in substrate concentration (1.0 g L^{-1}) in presence of the resins, being obtained the enantiopure (*R*)-lactone with a preparative isolated yield higher than 84%.

More recently, isolated BVMOs have also been employed in the DKR of racemic ketones. The first example was shown by Gotor *et al.* [37] by performing the selective oxidation of (±)-α-acetylphenylacetonitrile catalyzed by PAMO. When the Baeyer-Villiger oxidation was carried out at pH 8.0, no final product was observed. As previously proven, this BVMO maintains its catalytic activity in the presence of different concentrations of organic cosolvents with modified and/or improved biocatalytic properties [38], thus, ketone oxidation was conducted in these media, leading to (*R*)-acetoxyphenylacetonitrile with excellent optical purities. PAMO-catalyzed oxidation of the racemic ketone in buffer with 5% (v/v) ethyl acetate afforded enantiopure (*R*)-lactone with 58% conversion after 72 hours, while 28% of the starting material was recovered in a racemic fashion, being performed a PAMO-base-catalyzed DKR process.

Likewise, 4-hydroxyacetophenone monooxygenase has been employed with success in the kinetic resolution of racemic benzyl



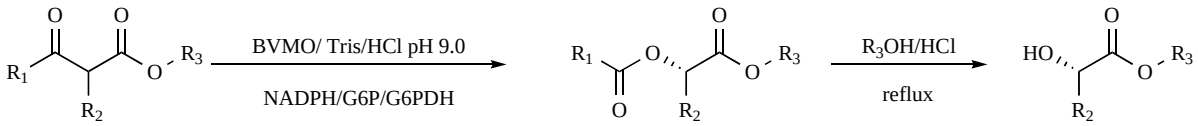
Scheme 4. BVMO-catalyzed DKRs of racemic benzylketones and 2-alkyl-1-indanones.

ketones, leading to the (*S*)-esters and the remaining (*R*)-ketones with moderate conversions and high enantioselectivities (Scheme **4a**) [39]. This resolution was strongly dependent on pH, leading to higher conversions but lower enantioselectivities when working at more basic pHs. Due to the acidic character of the α-hydrogen of the benzylketones, a pH-dependent racemization could be expected. However, when (±)-3-phenylbutan-2-one was dissolved in buffer at pH 8.0, no racemization was observed, while this process was sluggish at pH 10.0. The use of anion exchange resins afforded a higher ketone racemization rate [40], which allowed the development of a DKR process. Based on these results, biooxidation of (±)-3-phenylbutan-2-one was catalyzed by HAPMO at pH 10.0 in the presence of a set of anion exchange resins. The use of strong resins provided a fast racemization of the starting material, together with a high deactivation the enzymatic activity. In contrast, ketone racemization in the presence of different weak anion resins was slower when compared with strong ones but these materials did not induce a significant HAPMO deactivation, being possible to recover the (*S*)-enantiomer with conversions higher than 50% and good optical purities. The best result was achieved in presence of Dowex MWA-1, leading to 86% of the final product with 84% *ee*. Biooxidations in presence of Dowex MWA-1 were extended to the preparation of a set of (*S*)-benzylesters with conversions higher than 65% in almost all the compounds and good optical purities, depending on the substrate structure. Thus, the ketone 3-(3-trifluoromethylphenyl) butan-2-one was oxidized with 88% conversion after 96 hours and 79% *ee* while a ketone bearing longer alkyl chains as 4-phenyl-3-heptanone was oxidized with 50% conversion after 144 hours with a modest optical purity (*ee*=65%).

The cloned and overexpressed mutant M446G of phenylacetone monooxygenase (M446G PAMO) has been also employed in the DKR of a set of racemic benzylketones in presence of anionic exchange resins, as shown in Scheme **4a** [41]. Best results were achieved with the weak exchange resin Lewatit MP62, affording the (*S*)-benzylesters with high yields and moderate to good optical purities. Oxidations were performed in buffer at pH 10.0 containing 5% (v/v) methanol, a solvent that activates this BVMO. After 96 hours, (*S*)-1-phenylethyl acetate was recovered with 75% conversion and 79% *ee*, remaining 3-phenylbutan-2-one with a very low optical purity. *m*-Methyl- and *p*-nitro- derivatives of 3-phenylbutan-2-one were the best substrates for this procedure, with optical purities around 90% *ee* and high conversions after 120 hours.

Naturally occurring and synthetic 3,4-dihydroisocoumarins and their derivatives are key intermediates in the synthesis of biologically active molecules, presenting themselves interesting activities as antifungal, antiulcer, antimalarial or antiallergenic agents [42]. A wide set of procedures has been described in order to obtain these interesting compounds, but all the methodologies suffer from some drawbacks as harsh reaction conditions, multistep procedures or low yields. Recently, a very elegant biocatalytic approach was designed for the preparation of these compounds with high yields and optical purities through a DKR protocol [43]. M446G-PAMO was employed as biocatalyst in the oxidation of (±)-2-methyl-1-indanone at pH 10.0 and 40ºC to obtain the corresponding (*R*)-3-methyl-3,4-dihydroisocoumarin with 79% *ee* and 76% conversion after 48 hours (Scheme **4b**). It is interesting to note that the use of this biocatalyst led to the formation of the so-called "unexpected" lactone, the product not predicted by the well-known established regioselectivity of the Baeyer-Villiger oxidation, which makes this biotransformation of high synthetic interest. In order to optimize both conversion and optical purity, oxidation was performed in the presence of 5% (v/v) of different organic co-solvents, observing that methanol increased biocatalyst activity with a negative effect in the selectivity, while the use of hexane afforded higher selectivities with no significant changes in the activity. Oxidations performed at lower pHs or temperatures led to an important loss in the conversions, but accompanied with an increase in the enzymatic selectivity when using 5% (v/v) methanol. The biocatalytic procedure was extended to other racemic 2-alkyl-1-indanones, recovering the optically active 3-alkyl-3,4-dihydroisocoumarins with excellent selectivities (≥90% *ee*) and good conversions. Higher yields were obtained with those ketones bearing short alkyl chains (methyl or ethyl), while the oxidation of (±)-2-butyl-1-indonane required longer times to achieve the final lactone with 80% conversion and 92% *ee*.

Isolated BVMOs have been also employed as chiral biocatalyst in the oxidation of racemic α-alkyl-β-ketoesters, as shown in Scheme **5** [44]. Interestingly, under the reaction conditions, sponta-



BVMO: HAPMO, PAMO
R_1: Me, Et
R_2: Me, Et, CH_2Ph, $CH_2CH=CH_2$
R_3: Me, Et, iPr, CH_2Ph

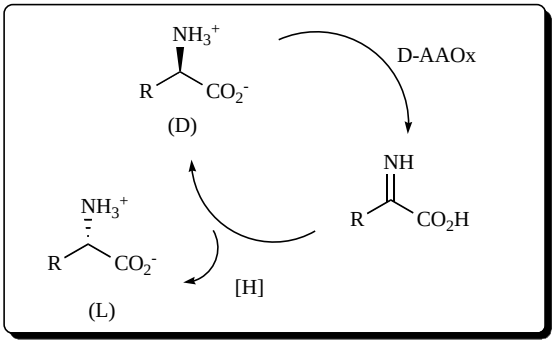
Scheme 5. Chemoenzymatic synthesis of (*S*)-α-hydroxyesters from racemic α-alkyl-β-ketoesters through a BVMO-catalyzed Dynamic Kinetic Resolution.

neous substrate racemization was observed, leading to an effective DKR in order to provide a set of α-*O*-acylated hydroxy esters, valuable compounds in organic synthesis. PAMO, its M446G mutant and HAPMO were the biocatalysts chosen for this purpose. Initially, the BVMO-catalyzed oxidation of racemic methyl 2-methyl-3-oxobutanoate was carried out in Tris/HCl 50 mM at pH 8.0. Oxidations in the presence of PAMO or HAPMO led to enantiopure (*S*)-diester. Although the conversions were lower than 30%, the racemic β-ketoester was recovered after 48 hours, showing that under these reaction conditions substrate racemization is feasible. As PAMO has shown higher activities at high pH values [39], a fast substrate racemization was ensured and, therefore, it was possible to obtain 62% of enantiopure product after 48 hours by working at pH 9.0. Same reaction conditions were applied to the oxidation of other α-methyl-β-ketoesters with different alkyl moieties, being observed for PAMO that, by enlarging the alkyl chain to isopropyl, faster oxidations were produced with complete selectivity leading in every case to enantiopure (*S*)-diester. The positive effect of organic co-solvents in PAMO-biocatalyzed processes was also shown in the DKR of racemic 2-methyl-3-oxopentanoate, for which the presence of 5% (v/v) ^{t}BuOMe led to a higher conversion and to a slightly higher optical purity for the final product. The presence of this co-solvent also increased the substrate optimal concentration in the biooxidation of racemic isopropyl 2-acetoxybutanonate. PAMO also showed a complete selectivity and conversion in the oxidation of both methyl and ethyl 2-acetoxybutanonate. Bulkier ketoesters such as racemic ethyl 2-acetylpent-4-enoate and phenyl 2-acetoxypropanoate were completely oxidized by both HAPMO and PAMO with excellent enantioselectivity (*ee*≥99%). Biooxidations were scaled up, being isolated the α-acylated hydroxyesters with moderate to high yields. These compounds were converted with good yields (60-82%) to the enantiopure (*S*)-α-hydroxyesters by the proper acid-catalyzed alcoholysis.

Selected Enzymatic Oxidative Cyclic Deracemizations

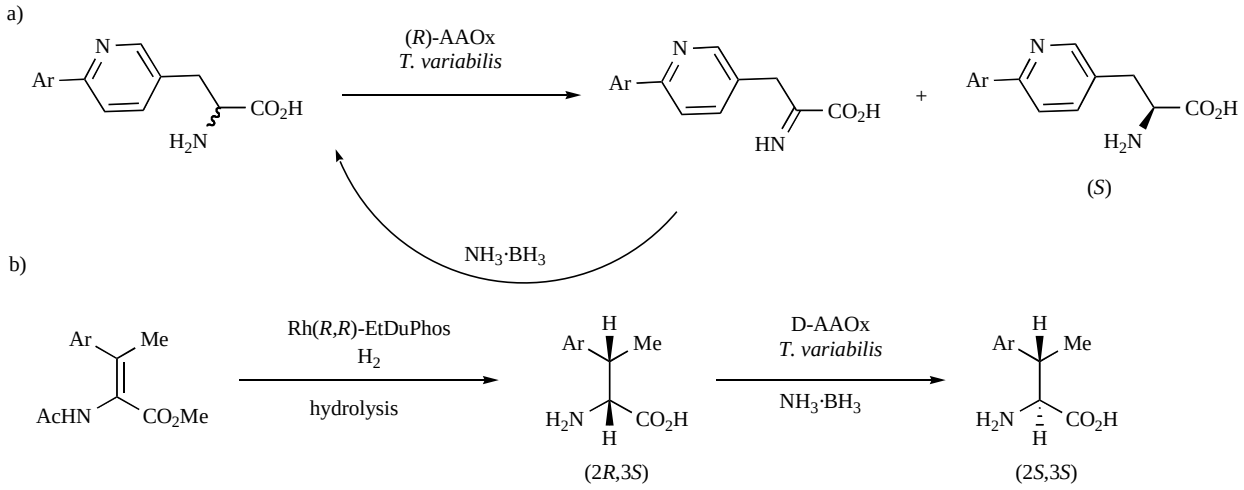
Similar to DKR, Cyclic Deracemization (CyD) is a powerful strategy to overcome the inherent 50% maximum yield of conventional Kinetic Resolution [45]. It is noteworthy that the combination of non-enzymatic reagents with biocatalysts in the same reaction vessel is a novel trend that is gaining relevance, nowadays. In CyD, a stereoselective oxidative transformation works concurrently with a non-selective reductive reaction, allowing (after a certain number of cycles) the desired product in enantioenriched form and close to 100% theoretical yield.

Oxidases have been used for deracemization of amines and amino acids by employing a cyclic oxidation and reduction sequence. Although less explored by synthetic chemists, oxidases catalyze the oxidation of substrates employing the cheap and clean molecular oxygen as electron acceptor, which is reduced to water or hydrogen peroxide [7]. In the first step of the catalytic cycle, one enantiomer of the starting material is selectively oxidized by the biocatalyst to the achiral imine or imino acid intermediate [46]. In the second step, the C=N of these compounds is reduced in a non-stereoselective manner to regenerate the amine, as shown in Scheme **7**. Since the seminal work in this field forty years ago, performing the CyD of L-alanine and L-leucine with porcine kidney D-amino acid oxidase (D-AAOx) and sodium borohydride [47], several amino acids deracemizations have been developed. Alternative reducing agents as sodium cyanoborohydride, amine-borane complexes and catalytic transfer hydrogenation with formate as hydrogen source and a metal catalyst have been employed, in order to circumvent the use of sodium borohydride, unstable at the neutral pHs in which oxidases present their highest activities [48]. Even an electrochemical procedure for performing CyDs has been described in the deracemization of racemic leucine with the D-AAOx from *Trigonopsis variabilis* performed in a batch reactor containing a graphite electrode at -1.5 V vs Ag/AgCl. Using this approach, 3.5 mmol L^{-1} d^{-1} of L-leucine were obtained with 91% *ee* [49].



Scheme 6. General mechanism for the CyD catalyzed by amino acid oxidases.

Recently, the CyD of *rac*-2-amino-3-(6-*o*-tolylpyridin-3-yl)propanoic acid catalyzed by the Celite®-immobilized (*R*)-amine oxidase from *T. variabilis* expressed in *E. coli* has been described (Scheme **7a**). The selective oxidation in presence of the biocatalyst was combined with a borane-ammonia complex-non selective reduction [50]. The (*R*)-oxidase was able to oxidize the (*R*)-enantiomer of the starting amine to the corresponding imine, leaving unaffected the (*S*)-amino acid. Before the imine hydrolyzes into the ketoacid, the borane-ammonia complex was able to reduce this compound to the racemic amino acid in a CyD process. The (*S*)-amino acid was obtained with the highest yields (around 80%) by working at pH 6.0-7.0 using 10 equivalents of the reduction complex, while a complete enantioselectivity (*ee*≥99%) was observed at pHs 6.0-8.0.



Scheme 7. CyDs processes biocatalyzed by AAOx to synthesize enantiopure amino acids.

Enantiopure β-branched α-amino acids are building blocks in organic synthesis [51]. A number of methods have been described for their synthesis, but the preparation of the four possible steroisomers results complicated with the classical methodologies. As shown in Scheme **7b**, Turner *et al*. have developed a chemoenzymatic methodology to synthesize all four steroisomers of a range of enantiomerically pure β-methyl-β-arylalanine analogues, by combining an amino acid oxidase-based deracemization with the chemocatalytic asymmetric hydrogenation of dehydroamino acids [52]. Thus, using a three step procedure starting from L-threonine methyl ester, a range of (*Z*)-didehydroamino acids were prepared. These compounds led to the corresponding (2*R*,3*S*)- and (2*S*,3*R*)-amino acids in presence of chiral rhodium-DuPhos catalyst, followed by hydrolysis of the amide and ester protecting groups. Hydrogenation processes were highly selective, achieving optical purities higher than 93% *ee* for all the compounds. (2*R*,3*S*)-Amino acids were subjected to the D-amino acid oxidase of *T. variabilis* in combination with the ammonia-borane complex, being recovered the (2*S*,3*S*)-diastereomers with excellent stereoselectivity and good yields (68-82%). The corresponding (2*S*,3*R*)-β-arylphenylalanine analogues were converted to the (2*R*,3*R*)-diastereomers with high yields (80-92%) and excellent selectivity using snake venom L-amino acid oxidase and the borane complex. The stereoinversion of (2*R*,3*R*)-β-methylphenylalanine to the (2*S*,3*R*)-diastereomer was performed on preparative scale using the D-AAOx from *T. variabilis*, affording the product in 55% isolated yield after a simple enzyme filtration, evaporation and recrystallization from ethanol/ethyl acetate.

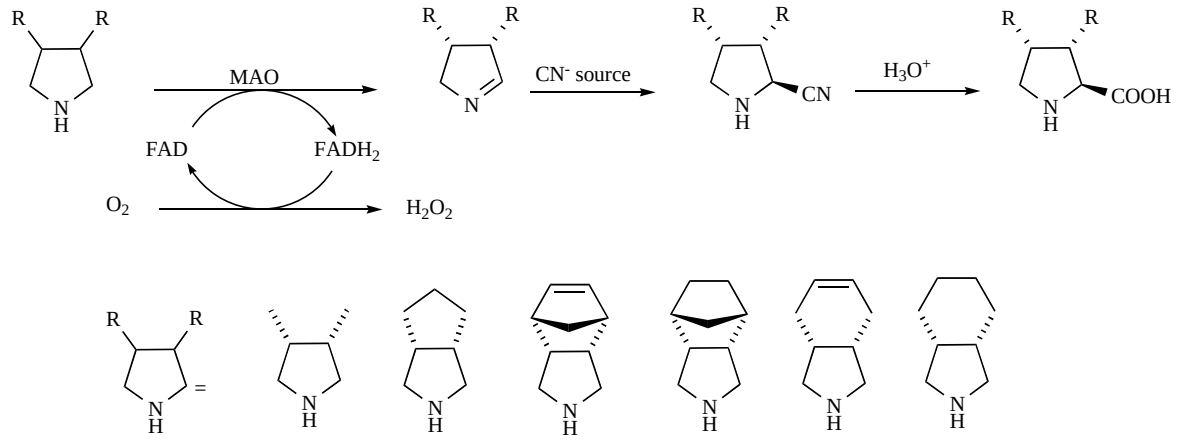
Tertiary amines are valuable building blocks in organic synthesis, especially in enantiopure form. They are widely employed as chiral auxiliaries and catalysts. Differently to primary and secondary amines, there are not so many preparation methods of these compounds. An improved variant of monoamine oxidase from *Aspergillus niger* (MAO-N5) has been used in the oxidation of a range of tertiary amines using molecular oxygen as oxidant [53]. The biocatalyst showed the highest activity for those substrates containing a pyrrolidine ring linked to bulk groups as (±)-*N*-methyl-2-phenylpyrrolidine or 3-(*N*-methylpyrrolidin-2-yl)pyridine, while good activities were achieved in the oxidation of 2-(methoxymethyl)-*N*-methylpyrrolidine and 1,2-dimethyl-1,2,3,4-tetrahydroisoquinoline. When (±)-*N*-methyl-2-phenylpyrrolidine was treated in presence of MAO-N-5 and borane-ammonia complex, (*R*)-amine was recovered with complete selectivity and 75% conversion after 24 hours.

Selected Enzymatic Oxidative Desymmetrizations

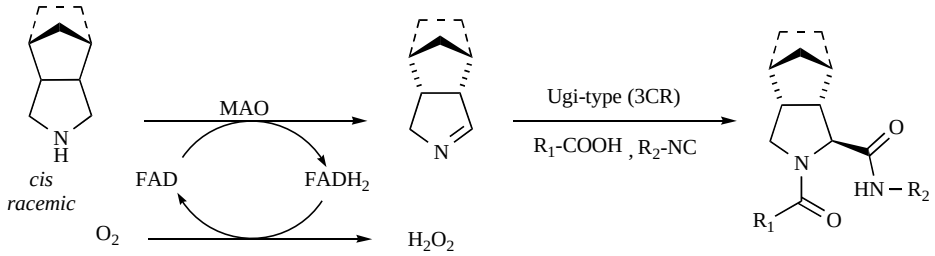
As already mentioned, desymmetrization processes are powerful tools to reach a theoretical 100% yield. Usually, the most employed strategy is the enzymatic transformation of prochiral compounds, such as ketones or sulfides with a huge number of examples. These desymmetrization processes will not be covered in the present review because they have been recently comprehensively surveyed [11]. Another type of desymmetrization is the biotransformation of *meso* compounds, such as diols, diesters, etc., being the hydrolase-catalyzed desymmetrization the most used protocol. A special sort of substrates for desymmetrization comprises atropoisomers. Such stereoisomerism may be found in molecules in which free rotation about a single chemical bond is restricted and both possible conformers can be isolated. In other words, the rotation energetic barrier is high enough to avoid the spontaneous rotation, leading to two possible (and isolable) isomeric forms. These atropoisomers are often denoted as *M* (from "minus", by the analogy with the sense of helicity, for those with a *Ra* stereochemistry) and *P* (from "plus", likewise for those with a *Sa* stereochemistry) [54]. It must be emphasized that just a few reports dealing with enzymatic atroposelectivity have appeared so far and the key step is accomplished through a lipase/esterase catalyzed process either over allenic [55], and biaryl substrates [56].

-Meso Cyclic Amine Desymetrization

Regarding recently developed desymmetrization of *meso* compounds, a very elegant approach was developed by Turner *et al* [57], as depicted in Scheme **8**. Thus, employing *meso* cyclic amines, mostly derived from pyrrolidine, a desymetrization oxidative process was catalyzed by MAO-N5 from *A. niger* using dioxygen as electron acceptor and FAD as coenzyme, furnishing Δ^1-pyrrolines bearing a *cis*-3,4-disubstitution pattern with excellent *ee*s (94->99%). As an application, those enantioenriched pyrrolines were reacted with cyanide affording the *trans*-addition products, namely the 2-cyanopyrrolidines. The latter are precursors of non natural proline derivatives which can be found in more complex structures, such as peptidic drugs (*e.g.* telaprevir and boceprevir) employed in antiviral therapies. In view to this, some of the obtained 3,4-disubstituted 2-cyanopyrrolidines were hydrolyzed in the



Scheme 8. MAO-N5 catalyzed desymmetrization of *meso*-pyrrolidines.



Scheme 9. Combining MAO-N5-catalyzed desymmetrization and Ugi-type 3CR.

presence of acid, giving rise to the non natural amino acids with very high *ee* (94%) and diastereomeric ratio (95:5), and rather acceptable overall yield of 51%.

This methodology has tremendous synthetic potential, as demonstrated by the contributions from Orru *et al.* and Ruitjer *et al.*, in which the *meso*-cyclic amine desymmetrization plays a pivotal role as source of enantioenriched cyclic imines, further employed in multicomponent reactions for the asymmetric synthesis of linear peptides and diketopiperazines (DKPs) as polycyclic alkaloid mimics. Hence, as recently reported [58], a series of prolyl peptides was synthesized departing from enantiopure cyclic imines biocatalytically desymmetrized by means of MAO catalyzed process (Scheme **9**). In this case, an Ugi-type three component reaction (3CR), employing the cyclic imine, an isocyanide and a carboxylic acid, was applied to obtain substituted prolyl peptides. In order to show an application of such non-natural peptides, one of them was employed as organocatalyst to carry out conjugate addition of an enolizable aliphatic aldehyde on nitroolefins, to efficiently generate two adjacent chiral centers in *syn* relative configuration with good selectivity.

Further, Ruitjer *et al*. [59], moved one step beyond and taking advantage of the chiral enantioenriched imine obtained by MAO-catalyzed desymmetrization, developed a reaction sequence in which after the biocatalytic step, an Ugi-type 3CR comprising the imine, an α-keto acid and an isonitrile followed by a Pictec-Spengler reaction takes place. These authors coined the acronym MUPS from MAO-N oxidation/Ugi MCR/Pictet–Spengler-type cyclization. This methodology afforded several DKPs alkaloid mimics, defining up to five chiral centers along the reaction sequence and displaying the strategy's versatility as a source of chemical diversity.

The same group established a combined convergent strategy for the synthesis of telaprevir, a peptidic drug that behaves as a protease inhibitor of hepatitis C virus and is currently at phase III clinical trials [60]. Thus, the protocol combines an enzymatic step with two different 3CRs, namely an Ugi-type reaction and a Passerini reaction. The synthesis of the central prolyl analogue by enzymatic desymmetrization, defines two stereocenters and governs the stereochemical outcome of the 3CRs. It must be emphasized that this strategy allows the synthesis of telaprevir in 11 steps (compared to 24 of the original synthesis) in a 45% yield. Besides, since the chiral disubstituted imine may bear different substituents in positions 3 and 4, the methodology offers the opportunity to get access to telaprevir analogues and, thus, showing its potential for the preparation of second generation drugs.

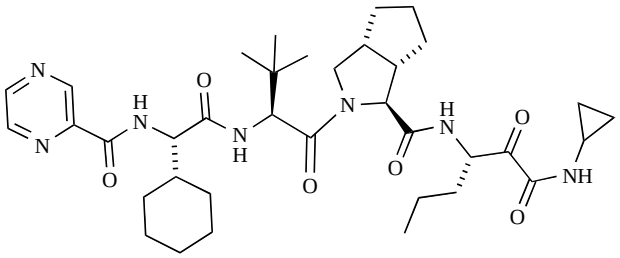
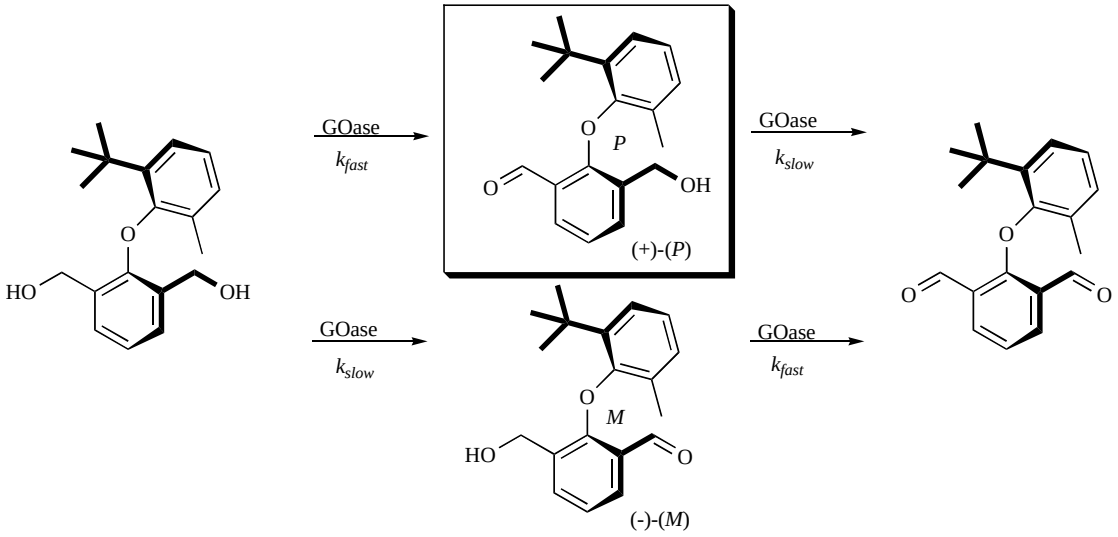


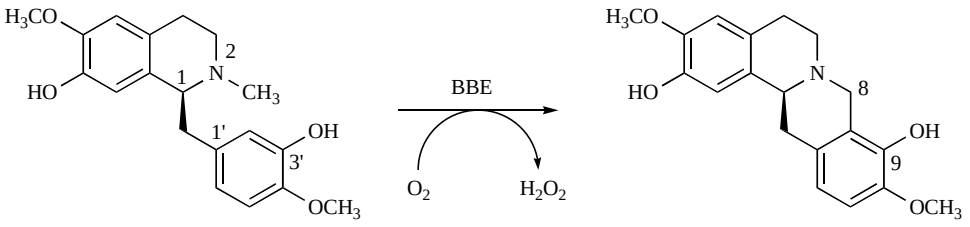
Fig. (1). Structure of telaprevir, a hepatitis C virus protease inhibitor.

- Atroposelective Oxidative Enzymatic Desymmetrization

In the last three decades, atropoisomers have gained enormous relevance in catalysis, either in transition metal-catalyzed reactions where non-racemic C2-symmetric compounds are used as ligand or in organocatalysis. This fact is easily illustrated by the Nobel Prize awarded to Prof. R. Noyori in 2001 for his developments in asymmetric hydrogenation reactions, mostly catalyzed by Rh-BINAP complexes. As mentioned by Noyori, it took more than four years to reach a reliable method for the preparation of enantiopure BINAP [61], thus showing that atroposelectivity in organic synthesis



Scheme 10. GOase-catalyzed desymmetrization of diaryl ether affording optically enriched atropoisomers.



(*S*)-Reticuline (*S*)-Scoulerine

Scheme 11. Oxidation of (*S*)-reticuline into (*S*)-scoulerine catalyzed by BBE in nature.

becomes, indeed, a synthetic goal in itself. Besides, biaryl single atropoisomers are present in several highly bioactive natural products such as the antibiotic vancomycin and the nerve growth-promoting agent mastigophorene A, among others. This fact prompted researchers to deepen in the synthesis of atropoisomeric interesting motifs, as could be appreciated in the past decade [62].

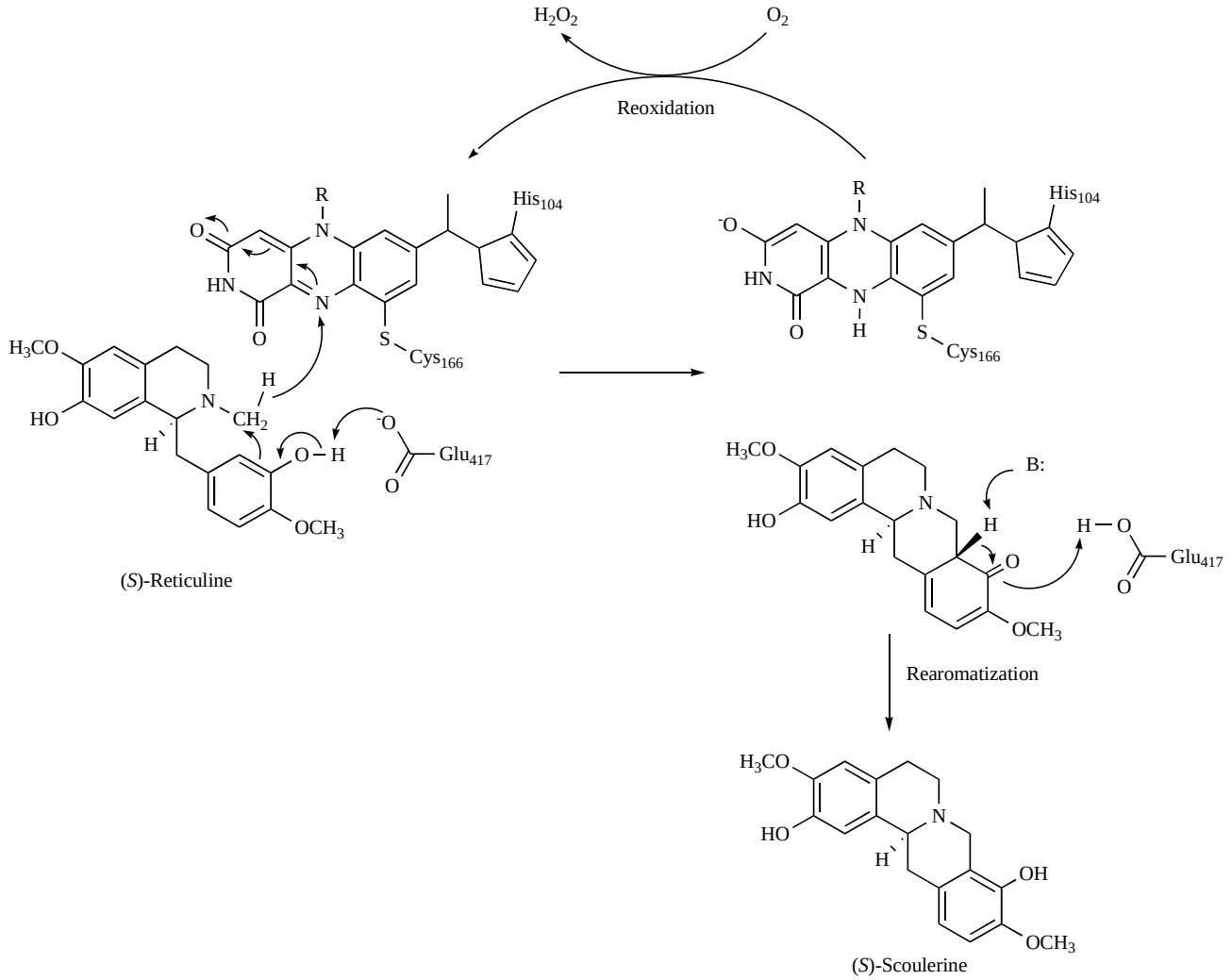
Concerning atroposelective enzymatic oxidative desymmetrization, the first example has been recently reported [63]. An achiral diaryl ether was effectively desymmetrized by means of an evolved galactose oxidase (GOase), among other non-oxidative enzymatic processes (Scheme **10**). Thus, the starting symmetric ether possesses two *prim*-alcohol moieties suitable for oxidation by this GOase. It must be noted that the achieved atroposelectivity was very high, affording the (+)-(*P*) atropoisomer with 94% *ee*. However, the final *ee* is a consequence of the action of the enzyme on the diol, rendering the (+)-(*P*) hydroxy aldehyde atropoisomer as a major product in 88 % *ee* and a subsequent highly selective oxidation of the minor formed product, namely (-)-(*M*) hydroxy aldehyde, towards the corresponding dialdehyde. This latter oxidation accounts for the improvement in (+)-(*P*) optical purity, albeit it decreases its overall yield. To prove this, the authors demonstrated that when starting from racemic hydroxy aldehyde, the GOase furnished the dialdehyde and the remaining hydroxy aldehyde was the (+)-(*P*)-atropoisomer with 99% *ee*.

It is must be taken into account that rotation energy barriers should be high enough in order to prevent racemization at room temperature. Thus, substituent in the aryl moiety must exert sufficient steric bulkiness and, of course, be suitable in size for the enzyme to be accepted as substrate. In this report, authors claim that a *tert*-butyl group was appropriate, but an *iso*-propyl led to racemic product, showing the strict steric control of the process.

Synthetically Relevant Enzymatic Oxidative C-C Bond Forming Reactions

The structural complexity of many secondary metabolites only found in plants makes assume that these organisms must contain exclusive enzymes able to catalyze the reactions leading to these compounds. The difficulty of working with plant tissues and the purification of their proteins, delayed for years the actual exploitation of plant enzymes for biotechnological purposes. However, during the last two decades, the outstanding progress in the molecular biology field has allowed amending that situation, opening to new possibilities to explore the plant enzymology. One of the most recent and relevant examples is the study of berberine bridge enzyme (BBE) from *Eschscholzia californica* (California poppy) for the enantioselective oxidative C-C bond forming on non-natural tetrahydroisoquinolines.

BBE [(*S*)-reticuline: oxygen oxidoreductase (methylene-bridge-forming), EC 1.21.3.3] is a vesicular plant enzyme that belongs to a recently discovered family of flavoproteins in which the flavin cofactor (FAD) is covalently attached to two amino acid residues: cysteine and histidine [64]. It plays a central role in the biosynthesis of protoberberine, protopine and benzophenanthridine alkaloids by catalyzing the formation of the berberine bridgehead carbon of (*S*)-scoulerine from the *N*-methyl carbon of (*S*)-reticuline.This specific reaction does not have an equivalent in synthetic organic chemistry or in nature (Scheme **11**). These alkaloids are phytoalexins which are accumulated in certain species of Ranunculales in response of



Scheme 12. Concerted mechanism for the stereoselective oxidation of (*S*)-reticuline catalyzed by BBE.

plants to herbivores and pathogenic attack [65,66]. BBE was first cloned and heterologously expressed in a catalytically active form in *Saccharomyces cerevisiae* [66] and later in *Pichia pastoris* [67].

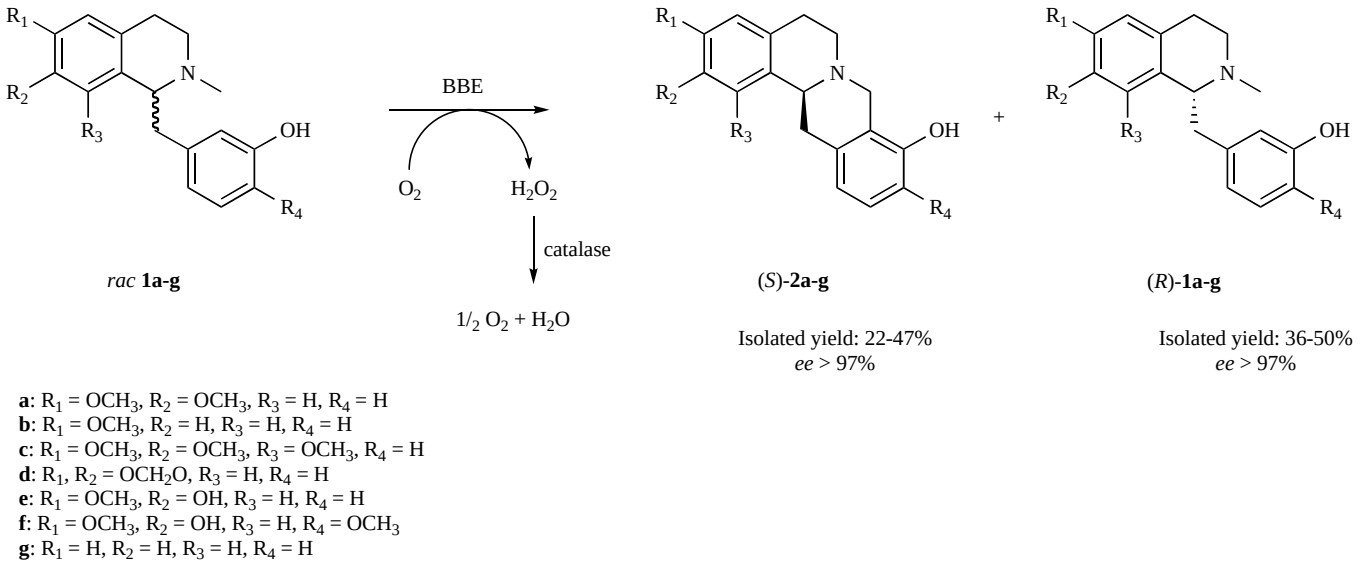
Winkler *et al*. elucidated the three-dimensional structure of BBE from *E. californica* by X-ray crystal analysis. The complex with the natural substrate (*S*)-reticuline allowed them identifying the amino acids involved in the active site of the enzyme. Based also on directed mutagenesis studies, it was possible to elucidate their function and propose an unprecedented concerted mechanism for the BBE-catalyzed oxidation [68]. The reaction is initiated by the oxidation of the *N*-methyl group of reticuline via the transfer of a hydride ion to the N-5 position of the isoalloxazine ring of FAD which undergoes a reversible two-electron reduction. Concurrently the proton of the hydroxyl moiety on the position C-3' of the substrate is abstracted by the carboxyl group of Glu-417 allowing the stereoselective ring closure through a concerted S_N2-type attack onto the *N*-methyl group (Scheme **12**).

The biotechnological potential (application) of BBE has been recently recognized and successfully exploited by the Kroutil's group [69-71]. Through different chemical strategies, they synthesized *rac*-reticuline and six other *rac*-benzylisoquinolines, which were used as substrates for BBE-catalyzed kinetic resolution (Scheme **13**). This enzyme is quite robust maintaining its activity even in the presence of high concentration of different organic solvents and exhibiting a high substrate tolerance by accepting a large variety of non-natural substances (substrate promiscuity). The enantioselective oxidative C-C bond formation was demonstrated on a preparative scale (500 mg) leading to optically pure products (*ee* > 97%) [70].

Recent Developments on Artificial Oxidative Enzymes

Synthetic organic community was historically reluctant to adopt biocatalysts in their benches. However, the exquisite selectivity along with rate enhancements in the order of 10^8-10^{12} is always appealing. Thus, it is conceivable that the robustness of a metal catalyzed reaction in combination with the chiral environment provided by a biomacromolecule may nicely work. This assumption was brought to real facts around 30 years ago.

Although initial reports on artificial metalloenzymes date back to the late 1970s with the pioneering works of Whitesides' group [72], in the last few years there has been an increasing interest in the creation and application of artificial (metallo)enzymes [73,74]. For this reason, covalent, supramolecular and dative anchoring procedures have been employed to link a catalytically active moiety to a chiral macromolecule scaffold, which can be based on carbohydrate, DNA or peptide. Using these artificial metalloenzymes, enantioselective processes such as ester hydrolysis, hydrogenation, allylic alkylation, Diels-Alder and Michael additions, have been

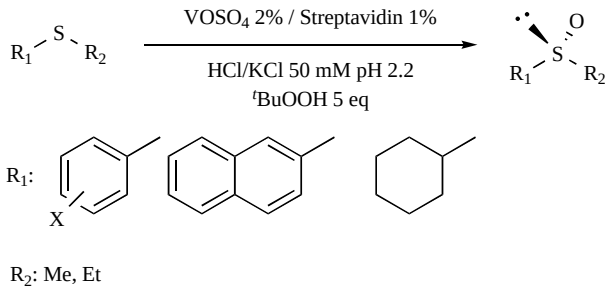


a: $R_1 = OCH_3$, $R_2 = OCH_3$, $R_3 = H$, $R_4 = H$
b: $R_1 = OCH_3$, $R_2 = H$, $R_3 = H$, $R_4 = H$
c: $R_1 = OCH_3$, $R_2 = OCH_3$, $R_3 = OCH_3$, $R_4 = H$
d: R_1, $R_2 = OCH_2O$, $R_3 = H$, $R_4 = H$
e: $R_1 = OCH_3$, $R_2 = OH$, $R_3 = H$, $R_4 = H$
f: $R_1 = OCH_3$, $R_2 = OH$, $R_3 = H$, $R_4 = OCH_3$
g: $R_1 = H$, $R_2 = H$, $R_3 = H$, $R_4 = H$

Scheme 13. BBE-catalyzed oxidative kinetic resolution via C-C bond formation of non-natural *rac*-benzylisoquinolines.

designed. These catalysts have been also employed in oxidative processes *e.g.* enantioselective hydroxylations, epoxidations or sulfoxidations. In general, in early reports, such methodologies displayed moderate selectivity, but some recent examples have shown that it is possible to carry out oxidations with good results.

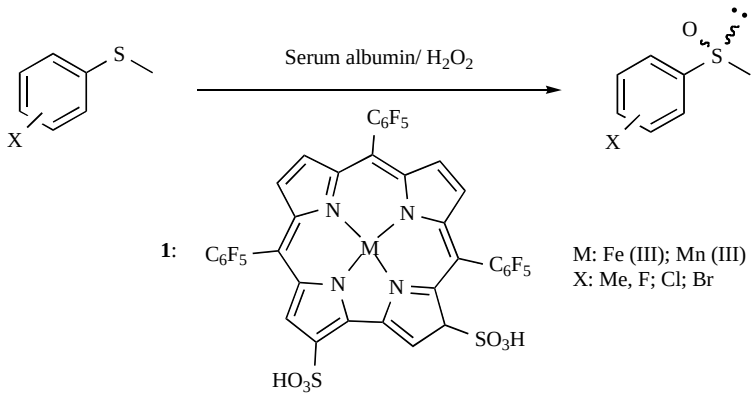
The potential of biotin-avidin technology to create artificial metalloenzymes has been widely studied, due to the high supramolecular affinity of biotin and derivatives for avidin or streptavidin [75]. The incorporation of biotinylated organometallic moieties with (strept)avidin led to novel catalysts employed in several enantioselective reactions. One recent development of this technology was described by the Ward's group, employing a vanadyl-loaded streptavidin catalyst in order to perform enantioselective sulfoxidations of prochiral substrates [76] (Scheme **14**). Oxidation of thioanisole was performed by using HCl/KCl buffer and ^{t}BuOOH (5 equivalents) as oxidizing agent. Under the optimized conditions of 2 mol % vanadium and 1 mol % protein, (*R*)-methyl phenyl sulfoxide was recovered with 94% conversion and moderate *ee* (46%). These conditions were extended to other aromatic sulfides as 4-methyl- and 4-methoxythioanisole, ethyl phenyl sulfide and methyl 2-naphthyl sulfide. All these compounds were oxidized with good to excellent conversions and high optical purities (*ee* around 90%), being achieved the (*R*)-sulfoxides with very low amounts of the overoxidation product, sulfone. This catalyst allowed the preparation of (*R*)-benzyl methyl sulfoxide with quantitative yield and good *ee* while the non aromatic (*R*)-cyclohexyl methyl sulfoxide was recovered with 61% yield and 86% *ee*.



Scheme 14. Synthesis of chiral sulfoxides employing as catalyst a vanadium source combined with streptavidin.

The covalent attachment of an achiral manganese salen complex into sperm whale myoglobin apoprotein by methane thiosulfonate groups, led to the preparation of a sulfoxidation catalyst which presents good activity (51% conversion) but poor selectivity (*ee*=12%) in the oxidation of thioanisole [77]. This result was assumed to be due to the multiple orientations of the Mn(salen) complex into the protein. An improved catalyst was prepared by a precise control of the placement of the manganese complex. Two mutations (L72C and Y103C) were made in order to selectively anchor the compound into the myoglobin. Experiments with the new catalyst led to a significant increase in the enzymatic activity and selectivity, recovering (*S*)-methyl phenyl sulfoxide with a moderate optical purity (*ee*=52%).

Since its first preparation in 1999, triarylcorroles have reached a great development in chemistry, due to their unique properties in catalysis, sensors and medicine-oriented research [78]. Corrole **1** iron and manganese complexes have been linked to five different serum albumins in order to create novel sulfoxidation biocatalysts [79]. Reactions on different aryl methyl sulfides were performed at room temperature in aqueous medium and with hydrogen peroxide as oxidant (Scheme **15**). Enzymatic activities and optical purities of the sulfoxides obtained were strongly dependent on the substrate, the protein and the metallic moiety. Thus, albumin source has a great effect on the optical purities of the final products, being achieved for both metallocorroles the highest selectivities with the bovin (BSA) and the rabbit (RSA) serum albumin (*ee* average for the sulfides tested 51% and 45%, respectively). BSA and pig serum albumin (PSA) led in all the cases to the (*S*)-sulfoxides, while the (*S*)- or the (*R*)-enantiomer can be afforded while using RSA, human (HSA) or sheep serum albumin (SSA). Metal source of the corrole is also important, being achieved, in general, better optical purities for the manganese-corrole than for the iron-coordinated one. For instance, (*S*)-*o*-chlorophenyl methyl sulfoxide could be obtained with higher stereoselectivity (around 70% *ee*), while the *o*-bromo derivative was achieved with *ee*=74% and *m*-bromophenyl methyl sulfide was oxidized with 30% conversion and 61% *ee*. For most of the sulfides tested with RSA, HSA and SSA, an opposite behavior on the sulfoxide selectivity was observed depending on the metal associated to the corrole. The effect of *para*-aryl substitution was alike for those groups similar in size to methyl and chloro substitu-



Scheme 15. Sulfoxidation processes catalyzed by iron and manganese corroles coupled to serum albumin.

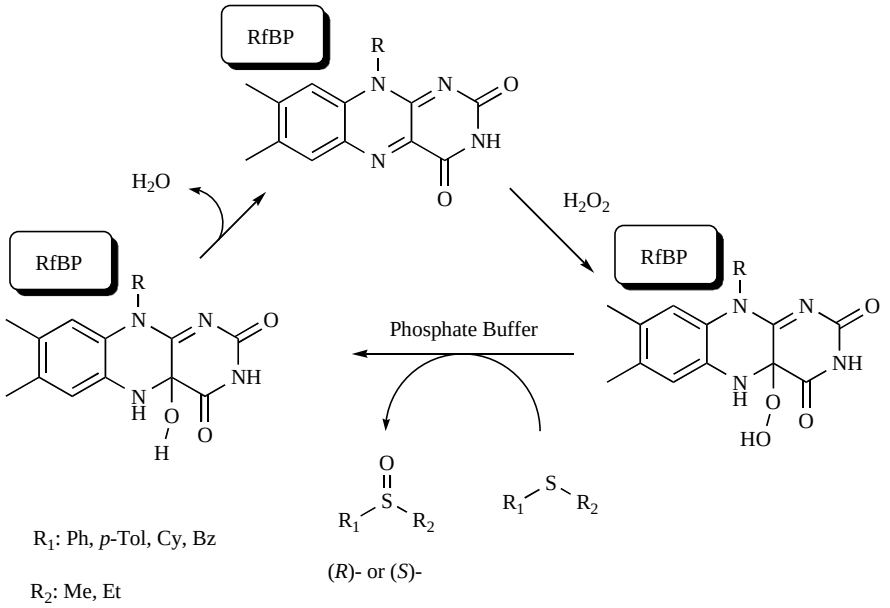
ents. However, regardless the albumin source and the catalyst, the highest *ee* were obtained for *meta*- and/or *ortho*-substitution, suggesting that the substrate effect is due to steric issues.

Carbonic anhydrase (CA) is a zinc-containing enzyme that catalyzes the reversible hydration of carbon dioxide [80]. This enzyme has been employed in the enantioselective hydrolysis of methyl mandelate and several *N*-acetyl amino acid methyl esters. Some years ago, carbonic anhydrase was employed as host of manganese in order to create a novel catalyst with peroxidase activity to perform selective epoxidations [81], due to the well-known ability of manganese ion to catalyze such reactions. By incubating bovin carbonic anhydrase apoenzyme with manganese the novel catalyst was prepared, which was able to selectively oxidize styrenes with hydrogen peroxide and bicarbonate. This process was sensitive to the reaction medium, obtaining the best performance in terms of selectivity using triethanolamine or BES buffers. In general, the enantioselectivity of the CA-[Mn]-epoxidation of styrenes was moderate, but comparable or even better than the achieved by natural peroxidases. CA-[Mn] presented other two advantages towards peroxidases: a broader substrate range and the lack of aldehyde side products. This metalloenzyme catalyzed for instance the epoxidation of *trans*-β-methyl styrene with an optical purity of 46% *ee*, but this compound was not oxidized by the chloroperoxidase from *Caldariomyces fumago* (CPO). Epoxidation of *p*-chlorostyrene led to 13% of the (*R*)-epoxide with the highest optical purity (*ee*=67%), while the reaction of 4-bromo-1-butene occurred with 45% *ee* and very low conversion. A similar study was performed by using both human and bovin carbonic anhydrase linked to manganese in order to perform the oxidation of styrene with hydrogen peroxide and sodium bicarbonate [82]. Again, results were modest in terms of biocatalytic parameters. Best selectivity was obtained in the formation of 10% of (*R*)-2-phenyloxirane (52% *ee*) when using the bovine enzyme and hydrogen peroxide. The human enzyme led to higher yield and slightly lower enantioselectivity. Bulkier aromatic alkenes were oxidized with modest results, probably due to the narrow cavity of the active site of this catalyst. Structural information of carbonic anhydrase allowed preparing four mutants in the 199 position, where a threonine plays a fundamental role in the catalysis. Two out of the four mutants, namely T199A and T199S, led to similar conversions but much lower *ee* with respect to the wild type enzyme, indicating that this residue is essential for chiral recognition.

As mentioned, the catalytic possibilities of both RNA and DNA have been recognized in the last few years, by using natural RNA enzymes (ribozymes) as well as by laboratory-evolution of catalytic DNAs (DNAzymes) [83,84]. One recent example has been described by Sen and co-workers by combining different guanine-rich RNAs and DNAs that fold to form guanine quadruplexes with Fe(III) heme. These metalloenzymes show a robust catalytic activity by transferring an oxygen atom from hydrogen peroxide to a variety of substrates [85]. Thus, one 18-nucleotide DNAzyme, called PS2.M, was activated (in the presence of Fe(III) heme, DNA, and thioanisole) with hydrogen peroxide, leading to the formation of methyl phenyl sulfoxide. Reaction was catalyzed with lack of stereoselectivity, being recovered the racemic final product. These sulfoxidations were extended to some thioanisole analogues, observing activity for all the substrates, while the thioanisole oxidation was performed by other guanidine quadruplexes. The enzymatic systems were also tested in the oxidation of indole. Solutions of this compound were incubated with Fe(III) heme and PS2.M and activated with hydrogen peroxide, leading to the formation of a complex mixture of products in which the major compounds found were isatin, 2-oxoindole, indigo and indigoid products. PS2.M catalyzed the oxidation of styrene into styrene oxide, being formed as secondary product phenylacetaldehyde by a hydrogen rearrangement. Kinetic analysis revealed that PS2.M is a superior catalyst for thioanisole and styrene oxidation when compared with classical peroxidases.

Besides, numerous flavin derivatives have been synthesized and applied in the last few decades. While natural flavin cofactors as FMN or FAD are unstable in their peroxy form, N^5-alkylated flavins can form stable 4α-peroxyflavins upon reaction with hydrogen peroxide, as shown by Bruice *et al*. in initial studies [86]. Subsequent researches have demonstrated that *N*-alkylated flavins are powerful oxidating catalysts that have been widely employed in the Baeyer-Villiger reaction, the oxidation of different heteroatoms, hydroxylation reactions and other oxidative processes [87]. Stereodiscrimination in oxidation reactions catalyzed by flavin derivatives has been induced so far by weak π-π interaction between the flavin and an aromatic substrate, but in the last few years further developments have been achieved [88].

The asymmetric sulfoxidation of different thioanisole derivatives has been described using an enzymatic-like catalytic approach, which makes use of a chiral substrate-binding scaffold to control the oxidation selectivity. Cyclodextrins have been widely employed as enzyme-mimics able to increase the reaction rate of different transformations and providing chiral environment to achieve enantioselective processes [89]. Thus, a set of activated N^5-ethylflavins were conjugated with β-cyclodextrins in order to create novel catalysts for enantioselective oxidations [90]. Reactions were carried



Scheme 16. General method for the enzymatic sulfoxidation of prochiral sulfides by cofactor engineering employing modified flavins linked to riboflavin-binding protein from *Gallus gallus*.

out using hydrogen peroxide as oxidant in aqueous medium. By adjusting the catalyst it was possible to achieve quantitative conversions after 10 minutes and high enantioselectivities (around 75% *ee*) when oxidizing electron-rich substrates as *p*-hydroxy- or *p*-methoxythioanisole. Methyl phenyl sulfoxide was prepared with 64% *ee* while derivatives with electron-withdrawing groups afforded moderate selectivities. Very low catalyst loadings (0.2 mol%) were required to reach a TON of 395. These preliminary results were extended by preparing and testing four novel alloxazine-cyclodextrin conjugates [91], *i.e.*, through the linkage of two N^5-ethylflavins with α- or β-cyclodextrins by a spacer of variable length. *n*-Alkyl methyl sulfides were oxidized by both types of cyclodextrins with high conversions and good selectivities depending on the linker employed. Bulkier substrates as benzyl, phenyl, cyclohexyl or *tert*-butyl gave higher conversions and enantioselectivities with β-cyclodextrin-containing catalysts, being possible to recover the *tert*-butyl methyl sulfoxide with quantitative conversion and 91% *ee*, while for the aromatic sulfoxides optical purities of 70% *ee* were reached. Overall, the sulfoxidations carried out with hydrogen peroxide as oxidant were very efficient with the flavin-catalysts tested, allowing good to excellent conversions after 1 hour in buffer medium employing only 1.0 mol % of the organocatalyst. No overoxidation of the obtained sulfoxides was observed.

Riboflavin-binding protein (RfBP) from *Gallus gallus* is a protein with no catalytic activity but with high binding capacity for riboflavin and a wide range of flavin derivatives [92]. Taking advantage of this affinity, the apo form of this binding protein was prepared and reconstituted with a concise library of alkylated riboflavin and lumiflavin derivatives, all of them presenting the flavin N^5-ethylated [93]. Incorporation of the reduced forms of the flavins to the riboflavin-binding protein was successful as it could be observed by the colored protein as well as by the fluorescence tritations performed. These artificial flavoproteins were tested for their ability to carry out peroxide-driven stereoselective sulfoxidations at low temperature in presence of EDTA using 1% w/w of biocatalyst (Scheme **16**). All the created flavoproteins were active in sulfoxidation processes. The reaction of different alkyl aryl or dialkyl sulfides using the N^5-ethylated riboflavin linked to RfBP proceeded with moderate conversions (37-55%) and low to moderate optical purities (10-30% *ee*) in the formation of the (*R*)-sulfoxides. Three N^5-ethylated lumiflavin derivatives were also linked to RfBP and employed in sulfoxidations. Again, optical purities for the three biocatalysts were low to moderate while conversions remained moderate, but in this case, (*S*)-sulfoxides were achieved. When using the demethylated form of flavin 1,3,5-triethylalloxazine, again a competent peroxide-driven monooxygenase was obtained, giving rise preferentially to (*R*)-sulfoxides with moderate selectivities. Thus, the enantioselectivity of the created flavoenzymes is strongly dependent on the type of non-natural flavin cofactor employed. This is a very attractive feature as it allows the selective preparation of both enantiomers of the product. By simple cofactor redesign it was possible to build up novel stereocomplementary biocatalysts able to perform enantioselective oxidations affording either *R*- or *S*- enantiomer.

SUMMARY AND OUTLOOK

A vast body of work has been done to conduct catalytic reactions in a more sustainable fashion. Enzymatic reactions encompass tremendous selectivity with high reaction rate along with functioning in aqueous media and mild conditions. It is not surprising that every day more industrial processes are adopting enzymatically-catalyzed transformations as key steps.

From a synthetic point of view, enzymatic oxidation is a powerful tool due to its exquisite stereo- and enantioselectivity and, perhaps even more prominently, because of the great chemo- and regioselectivity [94]. Besides, oxidative enzymes employ safe and economic oxidizing agents, such as dioxygen or hydrogen peroxide, thus resulting in generally innocuous reduced byproducts. Moreover, the advance in protein rational design [95] and several strategies comprising mutagenesis methods [96], allows the development of a biocatalyst with practically any desired characteristic, often making enzymes first-choice catalysts in organic synthesis.

As shown in this review, settled strategies such as DKRs have reached an impressive extent of development to obtain products with practically perfect selectivities and toward a broad range of substrates. Other protocols permitting 100% maximum theoretical yield such as desymmetrizations may rival DKRs depending on the availability of either the racemic or prochiral starting material, respectively. In this line, taking into consideration the importance of axially chiral compounds in catalysis, atroposelective oxidative transformations are emerging as promising new methods to further developments. Combinations of metal catalysis and atroposelective processes in one-pot procedures are appealing challenges to be developed in the forthcoming years.

Concurrent oxidative transformations cannot be neglected, if atom and redox economy are considered. Mimicking nature, these approaches allow the optimization of resources' fate by connecting parallel transformations and, thus, better chemical profits could be obtained in terms of space-time yields [97]. More synthetically useful oxidative enzymatic processes taking place in interconnected fashion to be employed either in solution or co-immobilized on a solid inert support or compartmentalized in nanoreactors, are expected to appear in the near future [98]. Today, carbon-carbon bond formation represents one of the golden targets for organic synthesis. As already highlighted [99], besides BBE, there are some other novel oxidoreductases that catalyze stereoselective oxidocyclization through a C-C coupling reaction, which have attracted a great deal of attention, such as Δ^1-tetrahydrocannabinolic acid (THCA) synthase and cannabidiolic acid (CBDA) synthase from *Cannabis sativa*. Their importance lies in the fact that they can be potentially used for the synthesis of pharmacologically active compounds derived from the cannabinoid skeleton. Both enzymes were cloned and expressed in insect cell cultures or tobacco hairy roots, but further studies will be necessary for their application in biocatalysis [100]. In this field, the newly discovered enzymes taken from plants open up a myriad of new possibilities for challenging oxidative couplings and other sort of valuable reactions, rather difficult to fulfil with conventional catalysts. Efforts are being spent in this promising area.

Perhaps one of the more fertile area for interdisciplinary work is the design and building-up of artificial (metallo)enzymes, which have proven to be fruitful catalysts for both already known and novel enzymatic reactivities. With the impact of computational methods and quantum calculations applied to characterize and predict transition state stabilization interactions [95], novel and powerful hybrid catalysts shall appear and rival existing enzymes, providing new opportunities for the synthetic chemist.

Although scarcely explored, photochemical [101], and electrochemical [102] approaches could be integrated to biocatalytic reactions in order to power up oxidative processes, be they of polar or radical nature. Besides, the use of co-immobilization of different biocatalyst onto a conducting polymeric support may provide an opportunity to build up artificial enzymatic networks.

CONFLICT OF INTEREST

The author(s) confirm that this article has no conflicts of interest.

AKNOWLEDGEMENTS

AAO acknowledges Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Universidad Nacional de San Luis (UNSL) for financial support. FRB is thankful to CONICET and Universidad Nacional de Córdoba (UNC) for financial support.

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Received: April 05, 2012 Revised: August 02, 2012 Accepted: August 07, 2012