CHAPTER

Applied biocatalysis in deep eutectic solvents

16

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16.1 Introduction

Deep eutectic solvents (DES) have emerged over the last two decades as nonconventional solvents. For sustainability, natural DES (NADES) have been designed and are considered to mimic the aqueous environment for an enzyme [1,2]. DES are attractive for their inexpensive and simple preparation (mixing and stirring), tunable properties, and inherent biodegradability. DES are composed of a mixture of quaternary ammonium salts acting as a hydrogen bond acceptor (HBA, Table 16.1), and a counter compound, such as a sugar, performing as hydrogen bond donor (HBD). The properties of a DES depends on the intermolecular interactions between its HBA and HBD components, with the nature of its hydrogen-bonding lowering its overall melting point, leading to a liquid eutectic solvent mixture without further processing or purification needed [3]. With this hydrogen-bonding network, varying the molar ratio of the HBA and HBD enables tuning of the freezing point, such that the solid salt choline chloride (ChCl, Table 16.1) mixed with urea (U) with a molar ratio of 1:2 ChCl:U leads to a freezing point of 12°C. On the other hand, a molar ratio of 1:2 for ChCl:glycerol (Gly) gives a freezing point of −40°C.

DES can reproduce in vivo cell environments [4], such that a protein structure may be better preserved than in organic solvents. DES have thus been increasingly applied in biocatalysis [5−7].

This chapter essentially focuses on the use of DES in relevant biocatalytic reactions, with an emphasis on performance measured by reaction conversions or yields that have clear synthetic applications. The reader is directed to further extensive literature that already addresses other aspects such as changes in activity and stability [8−13]. We begin our overview by showcasing interesting single reactions catalyzed by hydrolases in DES and DES-aqueous medium mixtures, in particular, lipases, which have been fully explored due to their easy handling and lack of cofactor requirements. Next, the use of oxidoreductases, lyases and transferases will be discussed offering a wide number of possibilities for the production of organic compounds in a selective and sustainable manner. Finally, the development of multicatalytic transformations will be covered by combining enzymes with organocatalysts or metal species, which allow the design of multistep chemoenzymatic cascades.

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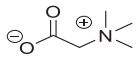
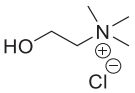
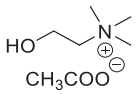
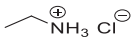
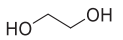
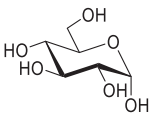
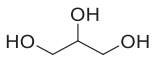
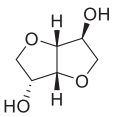
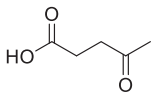
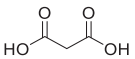
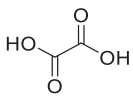
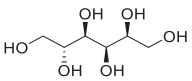
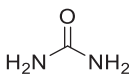
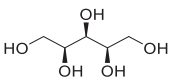
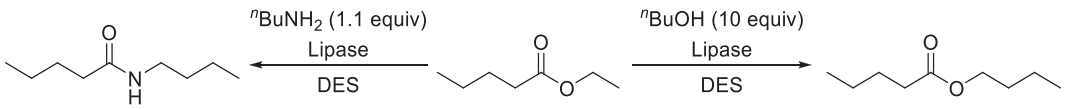
Table 16.1 Selected list of hydrogen bond acceptor (HBAs) and hydrogen bond donor (HBDs) leading to deep eutectic solvents described in this chapter, including their abbreviation, name and structure.

HBAs					
Be	Betaine		ChCl	Choline chloride	
ChAc	Choline acetate		EACl	Ethylammonium chloride	
HBDs					
EG	Ethylene glycol		Glc	Glucose	
Gly	Glycerol		Iso	Isosorbide	
LA	Levulinic acid		MA	Malonic acid	
Ox	Oxalic acid		So	Sorbitol	
U	Urea		Xyl	Xylitol	

16.2 Hydrolases

Their simplicity of use and lack of cofactor requirements for their correct action has motivated the great interest for the development of hydrolase-catalyzed reactions in organic synthesis, including a wide variety of enzymes such as lipases, esterases, amidases, nitrilases, proteases and epoxide hydrolases (EHs), among others. While most of these biocatalysts have been employed for hydrolytic processes, lipases have attracted especial attention due to their ability to react with very different nucleophiles (alcohols, amines, ammonia, hydrazines, thiols or hydrogen peroxide) to favor synthetic reactions over the competitive hydrolytic processes. Without any doubt, the advances on enzyme immobilization techniques have paved the way for the production of multiple organic compounds including for instances esters, amides, hydrazides, thioesters and peracids. Since the pioneer work developed by Kazlauskas and coworkers in 2008 [14], the use of DES has grown in an exponential manner [15,16]. This research group reported for the first time the use of lipases for the transesterification between ethyl valerate and 1-butanol using DES as solvents (Scheme 16.1),



SCHEME 16.1

Lipase-catalyzed transesterification and aminolysis of ethyl valerate with *n*-butanol and *n*-butylamine, respectively, in DES.

finding, in some cases and depending on the DES components, similar or superior conversion values with *Candida antarctica* lipase B (CAL-B), *C. antarctica* lipase A and *Pseudomonas cepacia* lipase, in comparison with the reactions carried out in a hydrophobic solvent such as toluene, traditionally used in lipase-catalyzed reactions. Remarkably, competitive reactions were observed in the transesterification reaction when the DES contained alcohol components, while the aminolysis side reaction with ethylammonium chloride (EACl) was not found in any extension. Additionally, the benefits of using DES as additives in lipase- and EH-catalyzed reactions were demonstrated when using *p*-nitrophenyl acetate and styrene oxide as substrates, respectively, achievements that have been widely expanded in recent years for synthetic goals as described in a recent review by Erol and Hollmann [17].

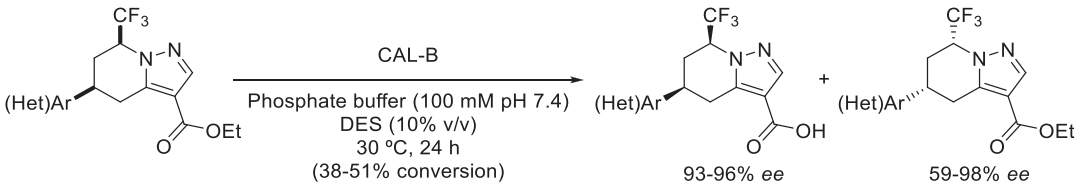
From this starting point, the use of DES as reaction media in biotransformations have exponentially grown, presenting these neoteric solvents as environmentally friendly solvents for multiple applications. Next, the use of hydrolases in DES will be described, dividing their applications depending on the reaction types, which means starting from classical hydrolytic reactions to later move toward synthetic application using a series of nucleophiles.

16.2.1 Hydrolases and deep eutectic solvents in hydrolytic reactions

Hydrolysis is the natural reaction of the hydrolase family, and the ability of multiple enzymes were demonstrated in conventional aqueous media, but also when using organic solvents as solvents and water as reactive nucleophile. In this case, the use of DES as cosolvents helps the solubility of organic compounds and are compatible with the use of a wide family of hydrolases including cellulases, EHs, glucosidases or lipases. Next, practical examples will be discussed where conversion and/or yields have been reported.

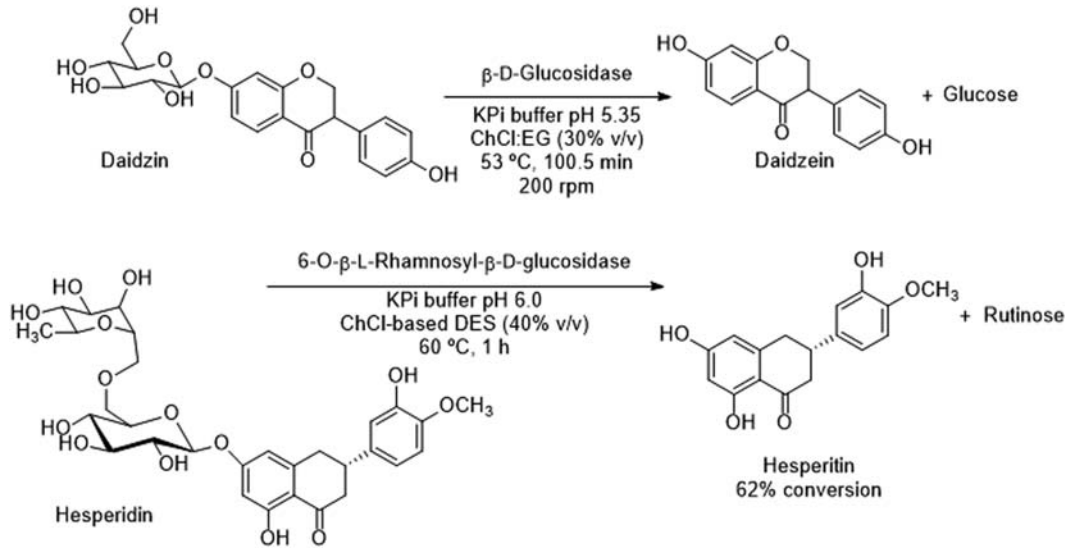
The stereoselective hydrolysis of racemic ethyl *cis* − 5-substituted-7-(trifluoromethyl) − 4,5,6,7-tetrahydropyrazolo[1,5-a]pyridine-3-carboxylates was developed using ChCl-based DES (Gly, U and Xyl, 10% vol) as additives (Scheme 16.2) [18]. Despite the DES did not fully dissolve the substrates, in contrast with the perfect solubility achieved when employing dimethyl sulfoxide, the kinetic resolution of three substrates was conducted with excellent enantioselectivities using CAL-B (38%−51% conversion, $E > 82$).

The flavonoid chemistry is usually challenging due to the low solubility of this type of substrates in traditional organic solvents, so the use of DES might be a useful solution to solve this problem. Cheng and Zhang studied the hydrolysis of daidzin to prepare daidzein using the almond β-D-glucosidase (Scheme 16.3 *top*), and after optimization of different reaction parameters such as DES components, reaction time, temperature and solvent system, the best conversion was found



SCHEME 16.2

Kinetic resolution of racemic tetrahydropyrazolo[1,5-α]pyrimidine derivatives in buffer using DES as cosolvents.



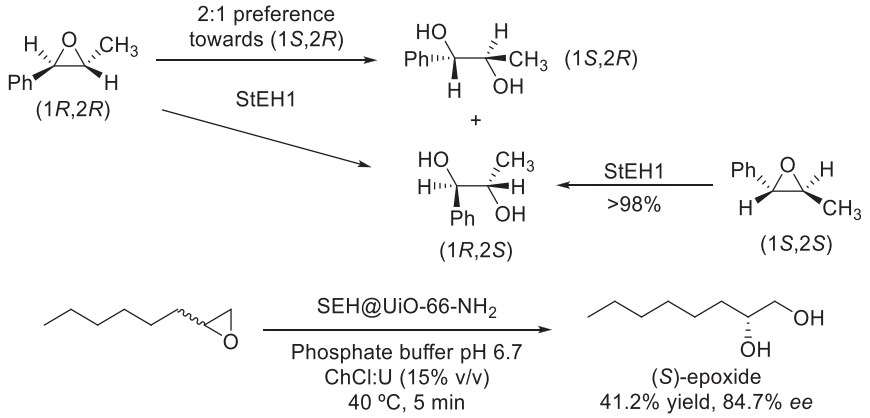
SCHEME 16.3

Enzymatic-catalyzed hydrolysis of flavonoid substrates in DES/buffer media.

using the ChCl:EG (1:2 mol/mol) in a 30% v/v, yielding a 97.5% of daizden with good purity [19]. Interestingly, the enzyme was recycled and reused six times with a final conversion above 50%.

Following with flavonoid chemistry, the enzymatic hydrolysis of hesperidin to produce hesperitin was achieved using 6-*O*-α-L-rhamnosyl-β-D-glucosidase as biocatalysts (Scheme 16.3 *bottom*) [20]. The use of DES helped in the solubilization of hesperidin in up to 90 mM, employing ChCl-based solvents containing U, Gly and EG as hydrogen-bond donors in different ratios, observing a complete deactivation of the enzyme when the DES content was over 80% v/v. Satisfyingly, the conversion reached a 62% after 1 h at 60°C using the ChCl:Gly-buffer (40:60 v/v) system, which was a better result than the reaction in pure aqueous medium (26%).

EHs allow the production of vicinal diols and epoxides with excellent regio- and stereoselective levels in aqueous medium, with the use of DES improving enzymatic activities. In 2010, Widersten



SCHEME 16.4

Epoxide hydrolase-catalyzed hydrolysis reactions using buffer/DES systems.

and coworkers described the combination of buffer and DES for the hydrolysis of 1,2-*trans* − 2-methylstyrene oxide enantiomers as substrates using potato EH from *Solanum tuberosum* (StEH1) as biocatalyst (Scheme 16.4) [21]. Interestingly, the use of certain DES as cosolvents such as ChCl:EG, ChCl:Gly and ChCl:U (1:2 mol/mol) improved the reaction kinetics and the regioselectivity of the process toward the epoxide ring opening at the benzylic carbon. In addition, it was calculated that all DES dissolved around 1.5 times more substrate than when using the pure buffer, achieving complete conversion after only 4 h for the hydrolysis of the (1*S*,2*S*)-epoxide. Lou and coworkers have also reported the applicability of using a buffer/DES mixture containing of 15% v/v ChCl:U, achieving the stereoselective hydrolysis of 1,2-epoxyoctane to (*R*) − 1,2-octanediol (81% *ee*) in 41% yield through immobilization of the soybean EH [22].

16.2.2 Hydrolase-catalyzed nonhydrolytic conventional reactions

The use of lipases for synthetic applications has allowed the production of several families of compounds with high chemo-, regio- and stereoselectivity. Among the different biotransformations catalyzed by lipases, probably esterification, aminolysis and transesterifications processes have gained the major attention, the use of DES appearing as an alternative to traditional conventional organic solvents.

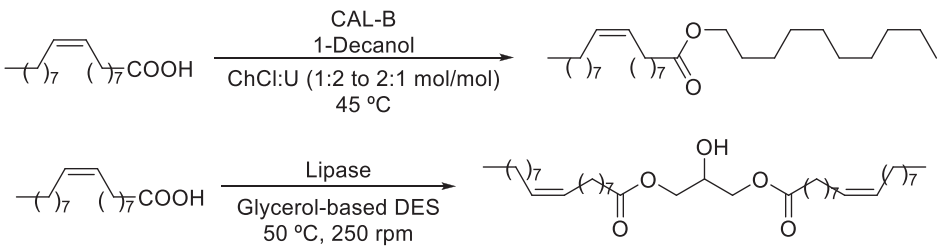
16.2.2.1 Esterification reactions

The reaction between carboxylic acids and alcohols represents the more straightforward approach to produce esters as main products, forming water as by-product. Generally, this reaction can be easily developed using nonenzymatic approaches involving the formation of reaction chloride acid intermediates, although the use of lipases allows the development of environmentally friendly reactions. In this context, the use of DES has been described, for instance in the esterification of oleic acid with 1-decanol as nucleophile as reported by Kleiner and Schörken using CAL-B as soluble

biocatalyst (Scheme 16.5 *top*) [23]. A two-phase reaction system was employed, the CAL-B catalyzed esterification occurring in the interface and shifting the equilibrium toward decyl oleate production by entrapment of the water molecules formed as reaction by-product, the use of DES such as ChCl:Gly and ChCl:U, the latest leading to the desired ester with excellent selectivity.

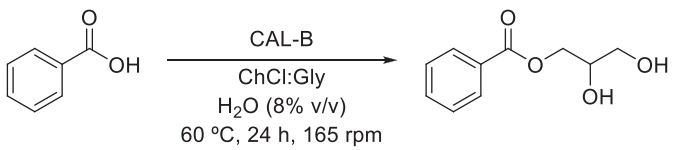
Zeng and coworkers reported later the lipase-catalyzed esterification of oleic acid with glycerol [24]. Five lipases from *C. antarctica*, *Thermomyces lanuginosus*, *Rhizomucor miehei*, *Penicillium camemberti* and *Rhizopus oryzae* (ROL) were tested using the glycerol as substrate but also as part of the DES solvent in combination with ChCl and betaine as quaternary ammonium salts (hydrogen-bond acceptors, HBAs), and the influence of the HBA:HBD ratio (1 to 1−2.5, HBD defined as hydrogen-bond donor), water content (0%−4%) and reaction time were explored. Best results were found with the ChCl:Gly (1:2 mol/mol) in the presence of molecular sieves and immobilized CAL-B (Novozyme 435) at 50°C, achieving a 43% conversion to the corresponding 1,3-diacylglycerol after only 1 h (Scheme 16.5 *bottom*).

The use of water has a key influence in enzymatic and process, and recently the synthesis of α-monobenzoate glycerol has been reported starting from benzoic acid and glycerol using commercially available CAL-B (Novozyme 435). Four different DES were tested (ChCl:HBD 1:2 mol/mol), satisfyingly the use of ChCl:Gly and water (8%−20% v/v) leading to full conversions and complete selectivity after 24 h at 60°C (Scheme 16.6) [25]. The water has a key role in the reaction medium, highly decreasing its viscosity so interestingly, percentages of water below 5% led to lower conversion values to the α-monobenzoate glycerol (40%−80%), while the use of a 30% water content stop the ester formation around 40% due to the appearance of byproducts caused by the hydrolytic action of the enzyme. In spite of the observed significant decrease of the conversion



SCHEME 16.5

Lipase-catalyzed esterification of oleic acid with glycerol-based DES.



SCHEME 16.6

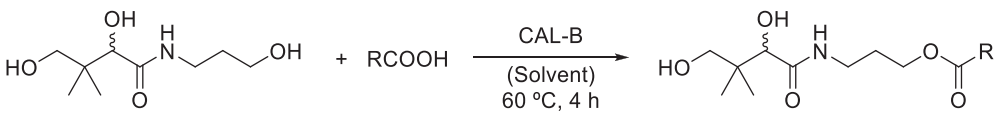
CAL-B catalyzed esterification of benzoic acid with glycerol in DES-water binary mixtures.

during the first recycling experiment, from 100% to 63%, later reuses do not lead to a dramatic loss of activity (52%, 48% and 47% conversion, respectively) in the reactions at 60°C for 24 h.

Compared to the 13 g α-monobenzoate glycerol/Ld achieved with Novozyme 435, the same authors reported the use of different immobilized CAL-B preparations, for example, via cross-linked enzyme aggregates (CAL-B CLEA), describing a more stable enzyme that can be reused for six cycles with any loss of activity, and attaining a higher productivity under similar reaction conditions (35 g α-monobenzoate glycerol/Ld) [26]. In addition, the double immobilization of CAL-B by encapsulation of the CAL-B CLEA in Lentikats provided a stable and active catalyst, successfully producing α-monobenzoate glycerol in DES by using batch and continuous mode processes [27], which expand the possibilities of CAL-B in the monobenzoylation of glycerol in flow mode using DES-water mixtures [28]. More recently, the use of *Pseudomonas stutzeri* lipase immobilized as CLEAs has been reported as an alternative to CAL-B for the production of α-monobenzoate glycerol, although attaining a low yield, around 20% conversion, after 24 h at 60°C when using ChCl: Gly (1:2 mol/mol) and 10% of water as phosphate buffer pH 7.0 [29].

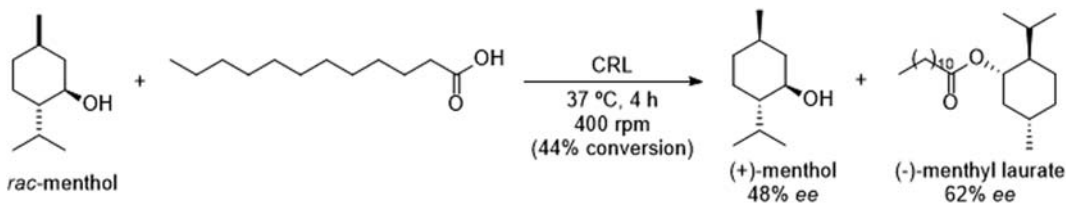
Lozano and coworkers have expanded the potential of ionic liquids (ILs) and DES by describing the esterification of six fatty acids (capric, lauric, linoleic, myristic, palmitic and linoleic acids) to prepare the corresponding panthenyl monoacyl esters (Scheme 16.7) [30]. Reactions were carried out by stirring the fatty acids with solid panthenol (1−3 equiv) for 4 h at 60°C in the presence of CAL-B and molecular sieves, selecting the production of the panthenyl monolaurate for the study of the enzyme recycling that was possible for seven operation cycles with conversions over 80% and excellent selectivities.

Finally in this section, the unique example of a stereoselective transformation through an esterification reaction in DES is described [31], this is the case of the esterification of lauric acid with racemic menthol by simply mixed and stirred both components in the presence of *Candida rugosa* lipase (Scheme 16.8). After optimization of the menthol: acid ratio, the best conditions were found



SCHEME 16.7

CAL-B catalyzed esterification of panthenol with fatty acids through in situ DES formation.



SCHEME 16.8

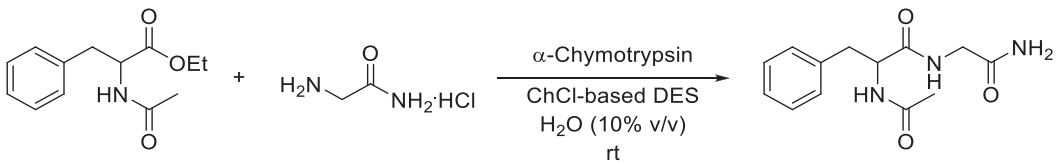
Kinetic resolution of racemic menthol by reaction with lauric acid.

when using 0.5 equiv of the lauric acid, reaching a 44% conversion after 3 h at 37°C, and recovering substrate and product with moderate optical purity: (−)-menthyl laurate (62% *ee*) and (+)-menthol (48% *ee*).

16.2.2.2 Aminolysis reactions and peptide synthesis

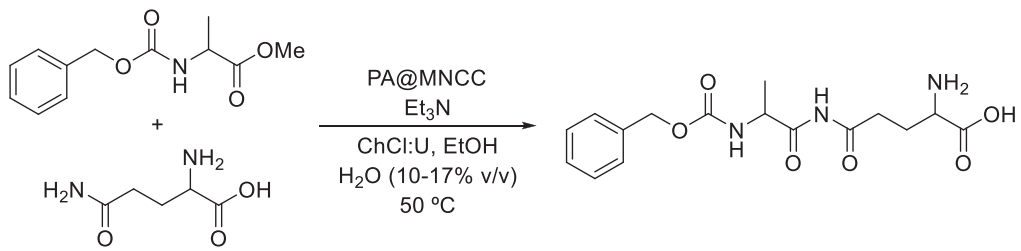
The replacement of alcohols by amines as nucleophiles give direct access to amides when starting from carboxylic acid or esters, the use of lipases and proteases in hydrophobic solvents being highly explored in the last three decades. However, DES is a good candidate as the reaction solvent, and this is the case of the reaction between racemic *N*-acetyl-L-phenylalanine ethyl ester with glycinamide hydrochloride (0.5 equiv) using α-chymotrypsin and DES solvents (Scheme 16.9) [32]. The influence of the water content, enzyme loading and reagent concentrations was explored employing four different ChCl-based DES, finding ChCl:Gly (1:2 mol/mol) as ideal media for the production of the corresponding peptide with excellent conversions at 10%−25% water percentage, while increasing to a 50% water content led to a significant formation of the corresponding carboxylic acid due to a competitive hydrolysis reaction. Interestingly, the reusability of the enzyme was studied, observing a significant loss of activity after the four cycle, moving from quantitative conversion to a value under 40% of the reaction with 10% water content and after 2 h at room temperature.

Cao and coworkers reported the synthesis of *N*-(benzyloxycarbonyl)-alanyl-glutamine dipeptide (Z-Ala-Gln) using the Z-L-alanine methyl ester (Z-Ala-OMe) as acyl donor and glutamine (Gln, 2.06 equiv) as nucleophile in ChCl:U (1.2 mol/mol), while a papain (PA) immobilized onto magnetic nanocrystalline cellulose (MNCC) was selected as enzyme (Scheme 16.10) [33]. The authors



SCHEME 16.9

Chymotrypsin-catalyzed peptide synthesis between *N*-acetyl-L-phenylalanine ethyl ester with glycinamide hydrochloride in a water/ChCl-based DES mixture.



SCHEME 16.10

Enzymatic synthesis of *N*-(benzyloxycarbonyl)-alanyl-glutamine in DES catalyzed by a PA@MNCC nanobiocatalyst.

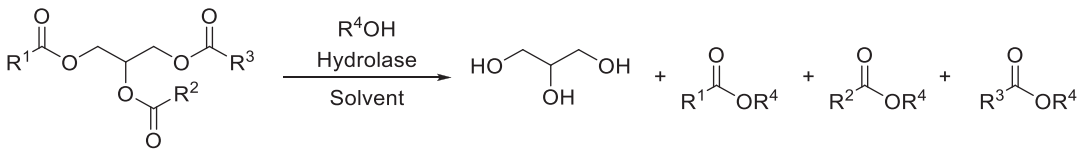
explored different reaction variables including the influence of water content, ester:amide ratio, temperature and the comparison between the use of free or immobilized papain, disclosing a selective peptide synthesis with a maximum 71.5% conversion value. The use of an organic base such as triethylamine was a key item for the complete solubility of the reagents, its presence not causing a detriment in the operational stability of the biocatalyst since more than 88% of its activity was retained after reusing it for five times.

16.2.2.3 Transesterification and transphosphatidylation reactions

Excluding the development of hydrolytic processes, the transesterification reaction between esters and alcohols is probably the most widely explored transformation using lipases as biocatalysts. In this context, the use of hydrophobic solvents has received great attention, appearing in the last two decades ILs as alternative sustainable solvents including more recently the use of DES. The use of these neoteric solvents in transesterification reactions can be mainly accomplished in three different ways: (1) as unique solvents; (2) in combination with lower amounts of water or isopropanol (<10% v/v) as cosolvent that interferes in the hydrogen-bond network between the DES and the reactants, normally increasing the reactivity of the catalytic system but without preferentially favoring the concomitant hydrolytic reaction; (3) in combination with traditional organic solvents to provide a higher enzyme stability.

Biocatalyzed biodiesel production consists in the reaction between natural triglycerides and aliphatic alcohols such as methanol or ethanol (Scheme 16.11), the use of hydrolases in different reaction media (organic solvents and neoteric solvents) being extremely helpful for synthetic purposes [34]. Yang and coworkers reported the reaction between the crude oil extracted from *Millettia pinnata* seeds with methanol in ChAc:Gly (1:2 mol/mol) at 50°C, finding immobilized CAL-B and *T. lanuginosus* lipase (TLL) as the more suitable enzymes reaching after 48 h 55% and 45% conversion, respectively [35] Yellow horn seed oil has also served for the biodiesel production using CAL-B under microwave irradiation [36]. From the eleven tested DES, ChCl:Gly (1:2 mol/mol) gave the best results and after studying the influence of the enzyme loading, methanol/oil ratio and microwave parameters, a 95% conversion was achieved after 120 h using MeOH (6 equiv) at 50°C. Interestingly, the enzyme was successfully reused with gradual deactivation of the enzyme until 70% conversion after the sixth cycle.

The use of low water contents has been demonstrated to have a beneficial impact in the production of biodiesel, as described by Zhao and coworkers in 2011 [37]. Therefore, the transesterification of the triglyceride Miglyol oil 812 with methanol and CAL-B in DES formed by ChAc or ChCl as ABD and glycerol as HBD led to high conversions (82%–97%) in short reaction times (1–3 h) at low contents of water (1% v/v) and under optimized reaction conditions, these are 50°C,

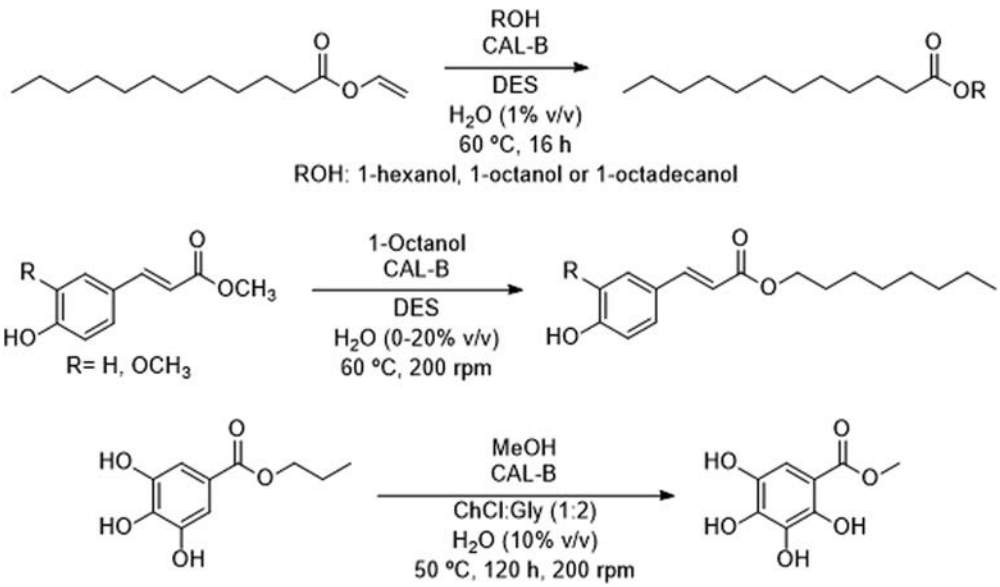


SCHEME 16.11

Lipase-catalyzed production of biodiesel through transesterification reactions.

ChAc:Gly (1:1.5 mol/mol) and MeOH (20% v/v). Under similar conditions with methanol as nucleophile, but starting from soybean oil [38], CAL-B has also been found as a suitable enzyme to produce biodiesel. In this case, after 24 h at 50°C the use of ChCl:Gly (1:2 mol/mol) allowed 81 or 88% conversion when using 1% or 0.2% of water, respectively. At the lowest water concentration, the reusability of the enzyme was successfully demonstrated for four cycles (79%−88% conversion).

The transesterification of vinyl laurate with different alcohols such as 1-butanol, 1-octanol or 1-octadecanol (6 equiv) have been successfully achieved using CAL-B and DES at lower water contents (1%, Scheme 16.12 *top*) [39]. The influence of the DES HBD was analyzed, finding that with ChCl:U and ChCl:Gly quantitative conversions to the desired esters were attained in all cases after 16 h at 60°C. However, the DES containing malonic acid (MA), oxalic acid (Ox), or ethylene glycol (EG) led to lower conversions (5%−41%) and selectivities (25%−100%) due to the reactivity of these components and the formation of byproducts with the concomitant destruction of the DES over the time. The same research group reported the use of DES (ChCl:U and ChCl:Gly) in the transesterification of phenolic esters such as methyl *p*-coumarate and methyl ferulate with CAL-B (Scheme 16.12 *middle*) [40]. For instance, the reaction between methyl *p*-coumarate and 1-octanol (6 equiv) was scaled-up to 3 g of substrate, yielding after 72 h a 93% isolated yield of the octyl ester (97% conversion). In these transesterification reactions, a great benefit was observed when using water as cosolvent, moving from very low conversions (<2% after 4 days) in pure DES to quantitative conversions at 8%−10% of water, without observing significant ester hydrolysis. The



SCHEME 16.12

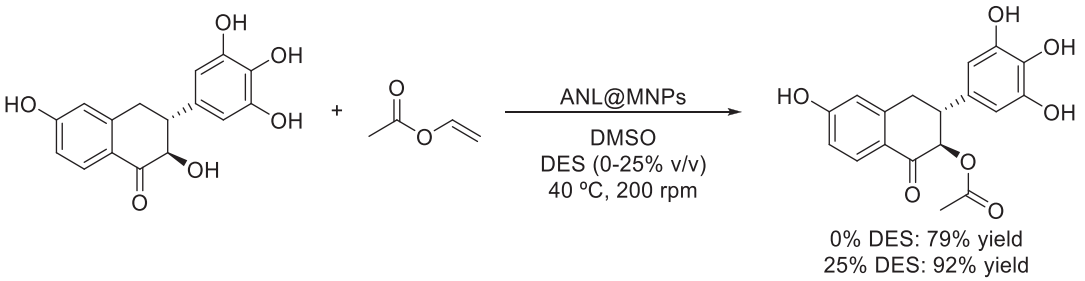
Transesterification of vinyl laurate and phenolic esters with aliphatic alcohols in the presence of CAL-B and low water contents.

competitive hydrolytic reaction occurred with significant extension when higher water contents were used (10%−20%).

CAL-B has also efficiently catalyzed the transesterification of propyl gallate using methanol as nucleophile (Scheme 16.12 *bottom*), overcoming the negligible conversion attained when exploring the corresponding esterification of gallic acid in methanol, 2-butanone or DES, probably caused by phenolic acid inhibition [41]. The transesterification process was studied at different enzyme concentrations (10−80 g/L), methanol concentrations (1−8 equiv), temperatures (35°C−60°C) and agitation speeds (75−250 rpm), finding around 60% conversion at 50°C and 55°C with almost exclusive ester formation when using 40 mM substrate concentration, 6 equiv of methanol, ChCl:Gly (1:2 mol/mol), water (10%) and 200 rpm. The reactivity seriously decreased at lower (35°C and 45°C) and higher temperatures (60°C).

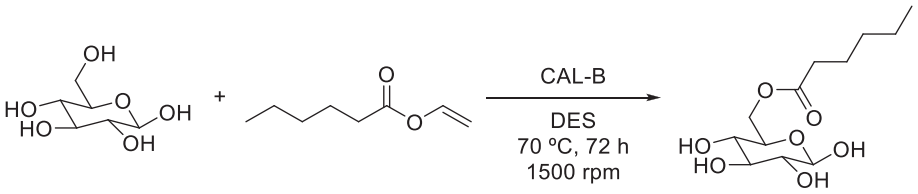
Interestingly, these transesterification reactions can be performed in a chemo-, regio- and/or stereoselective manner, the use of DES helping to favor the solubility of highly polar compounds or improving the stability of the enzyme in the reaction medium. This is the case of the monoacetylation of dihydromyricetin (DMY, 20 mM), a natural flavanol displaying antibacterial, antiinflammatory and antitumoral activities, that was reacted with vinyl acetate (10 equiv) using the *Aspergillus niger* lipase (ANL) immobilized onto magnetic nanoparticles in a high polar solvent such as dimethylsulfoxide (DMSO) [42]. The monoacetate at the C-16 position was obtained in 79% yield in the absence of DES, while the use of a 25% of ChCl:Gly improved the conversion until 92% (Scheme 16.13).

Regarding the development of regioselective transformations, Pöhnlein and coworkers reported in 2015 the selective acylation of glucose using vinyl hexanoate (2 equiv) and CAL-B in pure DES (Scheme 16.14) [43]. The best results were found in ChCl:U (1:2 mol/mol) and ChCl:Glc (1:1 mol/mol), obtaining glucose-6-*O*-hexanoate as the major product after 3 days at 70°C. Hollenbach and coworkers reported the synthesis of glucose monodecanoate in a hydrophobic DES formed by (−)-menthol and decanoic acid catalyzed by CAL-B [44], attaining superior results to the ones previously obtained in the same reaction but using a traditional hydrophilic DES such as ChCl:U (1:2 mol/mol) with a 5% of water (11% yield after 24 h vs <1%) [45]. More recently, Ortega and coworkers reported the use of a ChCl:Gly/DMSO/*tert*-butanol (1:1:3 v/v/v) system to overcome the mass transfer limitations in pure DES, yielding almost a 25% conversion in the regioselective reaction between glucose and vinyl laurate using different hydrolases [46].



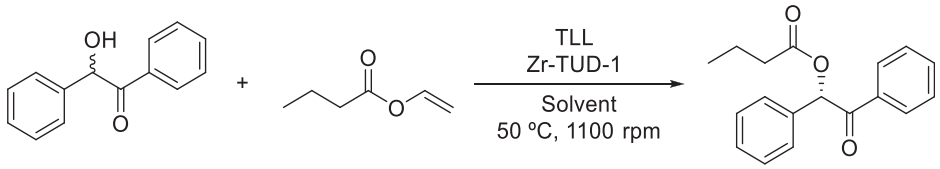
SCHEME 16.13

Lipase-catalyzed acetylation of DMY using a DMSO/DES (3:1 v/v) mixture.



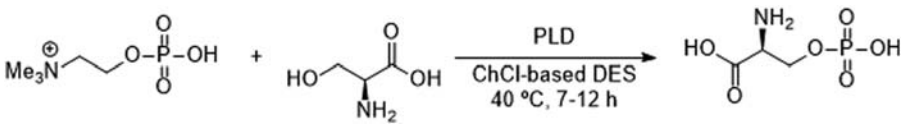
SCHEME 16.14

CAL-B catalyzed acylation of glucose with vinyl hexanoate in DES.



SCHEME 16.15

Lipase-catalyzed dynamic kinetic resolution of racemic benzoin in organic solvents and DES using vinyl butyrate as acyl donor.



SCHEME 16.16

Phospholipase-catalyzed transphosphatidylation reaction between phosphatidylcholine and L-serine for the synthesis of phosphatidylserine.

Regarding the use of hydrolases in stereoselective processes, Kara and coworkers studied the dynamic kinetic resolution of racemic benzoin (Scheme 16.15), using vinyl butanoate (6 equiv) as acyl donor in both organic solvents (toluene, 2-methyltetrahydrofuran and cyclopentyl methyl ether) and DES (EACl:Gly, ChCl:Gly, ChCl:Iso, ChCl:LA, ChCl:Ox and ChCl:U; 1:1.5−2.0 mol/mol) [47]. TLL was selected as stereoselective enzyme and combined with a heterogeneous zirconium-based catalyst (Zr-TUD-1) to achieve the racemization of the unreacted benzoin. Although the use of organic solvents led to much better results in terms of conversion, the use of ChCl:Iso (1:2 mol/mol) provides an elegant alternative for downstream processing, improving also the poor solubility of the benzoin, especially when some amount of isopropanol (10% v/v) was added as cosolvent.

Finally, the unique example of a transphosphatidylation reaction is covered in this section, which occurred between phosphatidylcholine and L-serine (6 equiv) in DES to produce phosphatidylserine, which is a phospholipid with interesting properties to rejuvenate brain cell membranes and increase acetylcholine brain levels (Scheme 16.16) [48]. The reaction was catalyzed by the

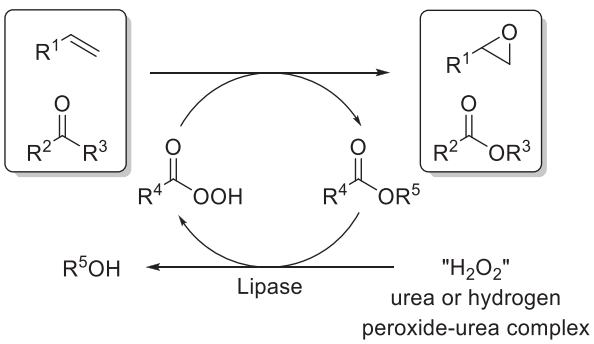
phospholipase D (PLD) using a 0.5% of water in both ChCl:Gly (1:2 mol/mol) and ChCl:Gly (1:2 mol) at 40°C, obtaining the phosphatidylserine in 90% and 92% yield after 7 and 12 h, respectively. Remarkably, the PLD seemed to be highly stable and active along the time, conserving 81% of its original activity after 9 reuses under optimized conditions in the ChCl:EG system.

16.2.3 Nonconventional biotransformations using hydrolases as catalysts

The ability of the enzyme active site to catalyze unexpected reactions, different from its natural reaction, is known as biocatalytic promiscuity, and from the wide number of biocatalysts, lipases have appeared in the last two decades as versatile catalysts for synthetic transformations, for instance for the formation of C−C and C−heteroatom bonds. This is an area of relevant interest since it can provide synthetic alternatives usually in a simple manner. However, it is important to investigate the reaction pathway since the catalytic center of the enzyme is not always responsible for the studied reaction, and this approach is known as pseudopromiscuity [49,50]. Classical examples are the lipase-mediated epoxidations or the Baeyer−Villiger reactions where the enzyme is only responsible of the formation of a reactive peracid intermediate, which subsequently reacts with the corresponding epoxide or ketone, respectively (Scheme 16.17). Therefore, the global reaction occurs in a tandem mode, first the lipase-catalyzed ester perhydrolysis of the starting carboxylic acid or ester using a hydrogen peroxide source to form the peracid, and then the chemical reaction of this active specie with the olefin or carbonyl compound to form the desired oxygenated compound.

16.2.3.1 Tandem oxidative reactions mediated by lipases in deep eutectic solvents

Without any doubt, CAL-B has been the most employed enzyme for this type of transformations, displaying a good reactivity in DES as described in previous sections of this contribution with more conventional reactions. Among the epoxidation reactions, a series of examples can be highlighted, for instance Wang and coworkers described the epoxidation of aliphatic alkenes and styrenes using an aqueous solution of H_2O_2 (2 equiv) and octatonic acid as peracid precursor (1 equiv) in phosphate

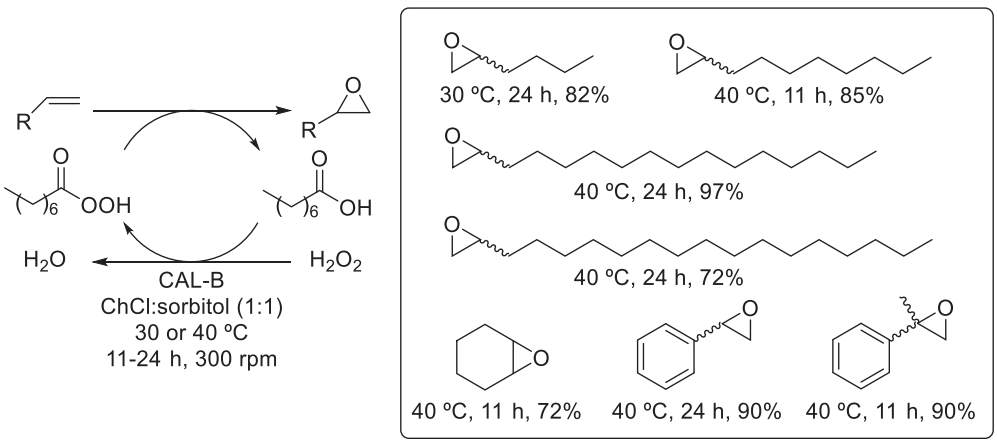


SCHEME 16.17

General scheme for lipase-mediated epoxidation or Baeyer−Villiger reactions via formation of peracid intermediates.

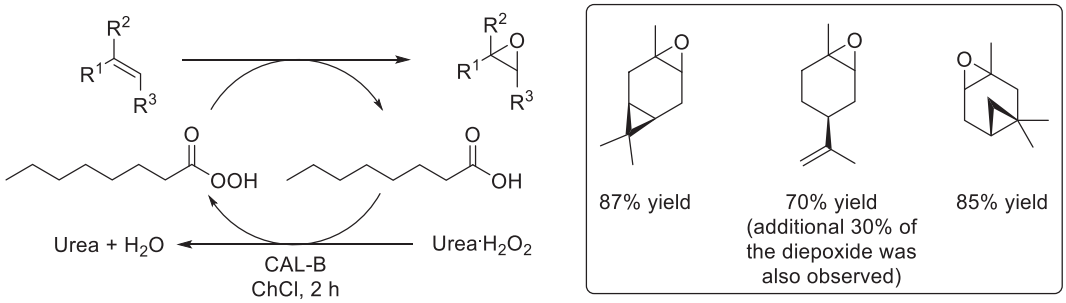
buffer pH 6.0 and nine different ChCl-based DES (Scheme 16.18) [51]. The best results were found in ChCl:So (1:1 mol/mol) leading to the corresponding epoxides in 72%−97% conversion by GC analyses after 11−24 h at 30°C or 40°C.

CAL-B and octanoic acid have resulted to be also a good system for the epoxidation of monoterpenes such as 3-carene, limonene and α-pinene in DES (Scheme 16.19) [52]. The DES mixtures were prepared in situ by mixing ChCl and the urea-hydrogen peroxide (2 equiv), achieving quantitative conversions to the epoxides after 2 or 3 h, which were easily recovered with good to high yields (77%−87%) after a simple extraction protocol with water and ethyl acetate. Interestingly, the valorization of agricultural wastes has been recently demonstrated through the epoxidation of limonene from orange peels using a ChCl:1,2-propanediol:H_2O in an equimolar ratio or ChCl:EG (1:1 mol/mol) and a series of enzymes including lipases (CAL-B, RML or TLL) or the choline



SCHEME 16.18

CAL-B-mediated epoxidation of alkenes and styrene using octanoic acid and hydrogen peroxide.



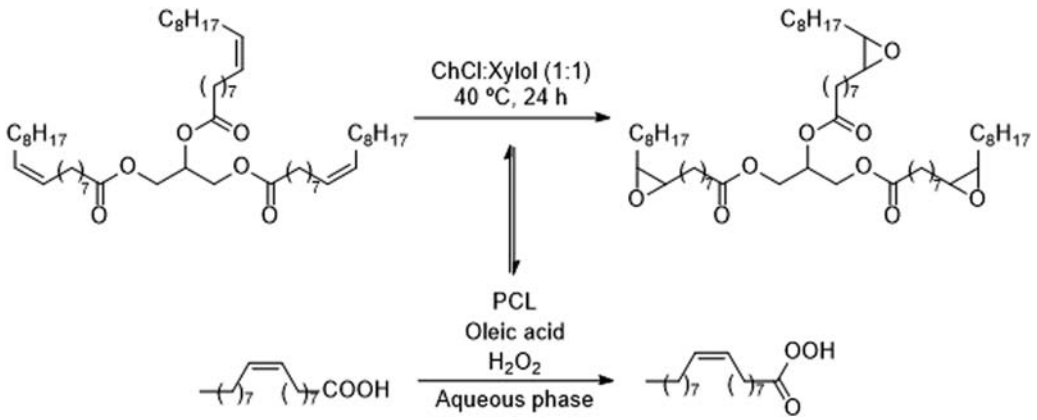
SCHEME 16.19

CAL-B-mediated epoxidation of monoterpenes using in situ generated ChCl-based DES.

oxidase isolated from the soil bacterium *Arthrobacter nicotianae*, obtaining mixtures of the mono- and diepoxide as also occurred in the previous examples [53].

The potential of other lipases in epoxidation reactions in DES has also been demonstrated. This is the case of the lipase G from *P. camembertii* (PCL), which displayed even better results than CAL-B in the oxidation of glyceryl trioleate, since PCL avoids the formation of side hydrolytic products observed in the reaction with CAL-B (Scheme 16.20) [54]. Reactions were carried out in buffer and five different DES, finding the best conditions with the ChCl:Xyl (1:1 mol/mol) when using hydrogen peroxide (3 equiv) and 40°C. In this case, the external addition of the peracid precursor was not needed since it was present in the vegetable oil samples used as starting materials. PCL has also been found to be a suitable enzyme for the formation of epoxides using the soybean oil as source of triglycerides, finding in this case the best results using a biphasic system composed by the oil and water, while the ChCl:So (1:1 mol/mol) served to form a micro emulsion enabling the surface tension lowering of hydrophobic organic phases in aqueous medium [55].

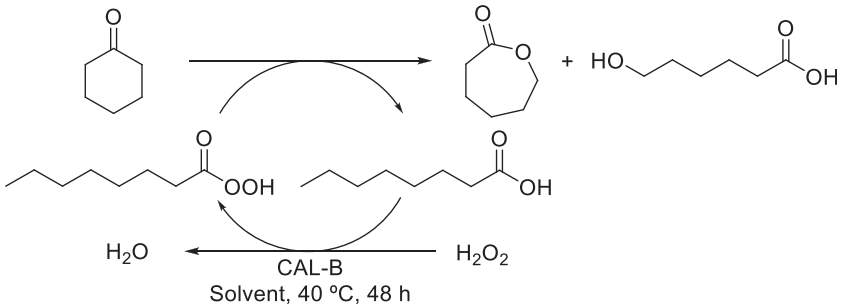
CAL-B-mediated Baeyer–Villiger have also attracted recent attention, such as the case of the transformation of cyclobutanone, cyclopentanone, cyclohexanone and 4-pentanone in the corresponding lactones [56]. In this case, the reactivity of the CAL-B wild-type and the engineered Ser105Ala mutant was compared using an aqueous solution of hydrogen peroxide (30% H_2O_2) and octanoic acid as peracid precursor using cyclohexanone as benchmark substrate (Table 16.2). In hexane-water (2:1 v/v) as solvent, the CAL-B wild-type highly favored the formation of the hydroxyl acid formed through lipase-catalyzed hydrolysis of the lactone (2:1 hydroxy acid vs lactone, 55% conversion), while the mutant predominantly led to the formation of the ε-caprolactone (1:2.5 ratio, 24% conversion). From the five DES tested, higher selectivities toward the Baeyer–Villiger products were found when the reactions were carried out in a DES systems such as ChCl:So (1:1 mol/mol), attaining for the Baeyer–Villiger oxidation of cyclohexanone a 92% conversion (46% selectivity) with the wild-type and a 47% conversion (99% selectivity) with the mutant.



SCHEME 16.20

Lipase G from PCL-mediated epoxidation of glyceryl trioleate.

Table 16.2 Baeyer–Villiger oxidation of ketones using CAL-B wild type or its Ser105Ala mutant and H_2O_2 (2 equiv) in ChCl:So (1:1) after 48 h at 40°C.

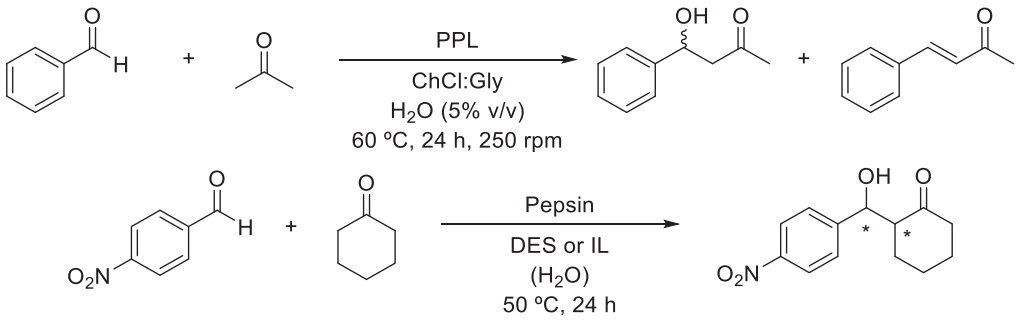


		CAL-B wild type		CAL-B Ser105Ala mutant	
Entry	**Ketone**	**Conv. (%)**	**Selectivity (%)[a]**	**Conv. (%)**	**Selectivity (%)[a]**
1	Cyclobutanone	99	93	99	100
2	Cyclopentanone	95	48	51	97
3	Cyclohexanone	92	46	47	99

[a]*The selectivity consists in the percentage between the lactone (Baeyer–Villiger product) and the hydroxyl acid formation (lactone hydrolysis).*

Among the C–C bond formation transformations, aldol reactions represent elegant examples to provide access to hydroxy carbonyl compounds with high selectivity. Traditionally, aldolases have efficiently catalyzed these biotransformations in a highly stereoselective manner, although in recent years the nonconventional action of lipases has been reported. For instance, the use of porcine pancreas lipase (PPL) could catalyze the aldol reaction between a series of substituted benzaldehydes and aliphatic aldehydes with acetone and cyclic ketones in DES [57]. Selecting as model reaction the one between benzaldehyde and acetone, and after optimization of the reaction conditions including aldehyde:ketone ratio, PPL loading and ChCl:Gly composition, full conversions were achieved toward the formation of the 4-hydroxy-4-phenylbutan-2-one after 24 h at 60°C (Scheme 16.21 *top*). The addition of some water to the DES (5%–20%) allowed the minimization of the side-product formation derived from the aldol-dehydration sequence, being able to develop synthetically useful reaction at high benzaldehyde concentrations (1–6 M) using 5 equiv of acetone. Finally, the enzyme recycling was studied for the reaction between 4-nitrobenzaldehyde and cyclohexanone (2 equiv), finding a significant drop of activity after the first recycling (around 33% of the enzyme activity) probably caused by the severe reaction conditions (60°C). The asymmetric version of this latest reaction has been described by Wang et al. using pepsin in ILs and DES (Scheme 16.21 *bottom*), optimizing the reaction conditions in DES in terms of deionized water composition (20%–95%) for the reaction in ChCl:Gly (1:2 mol/mol), and achieving the best results with the system ChCl:Gly/H_2O (3:7 v/v) for a 79.7% yield, 28:72 *anti*/*syn* diastereoselectivity and 60% *ee* of the major *syn* enantiomer [58].

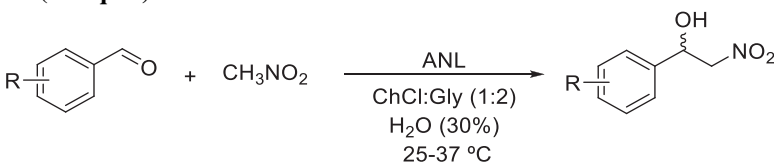
Another interesting C–C bond formation transformation is the Henry reaction, the ANL being able to catalyze the process between nitromethane and different benzaldehydes in pure DES [59].



SCHEME 16.21

Hydrolase-catalyzed aldol reactions in neoteric solvents.

Table 16.3 ANL-catalyzed Henry reaction between a series of benzaldehydes (214 mM) and nitromethane (15 equiv).



		100% H_2O		ChCl:Gly (1:2 mol/mol)/30% H_2O	
1	**R**	**Time (h)**	**Yield (%)**	**Time (h)**	**Yield (%)**
1	H	120	–	48	–
2	2-NO_2	10	81	4	87.7
3	3-NO_2	10	85	4	88.5
4	4-NO_2	10	87	4	92.2
5	2,4-$(NO_2)_2$	10	70	4	91.7
6	4-F	120	46[a]	48	12.3
7	4-Cl	120	80[a]	24	62
8	4-Br	120	91	24	89
9	4-OMe	120	37[a]	48	9.6
10	4-Me	120	14[a]	48	––

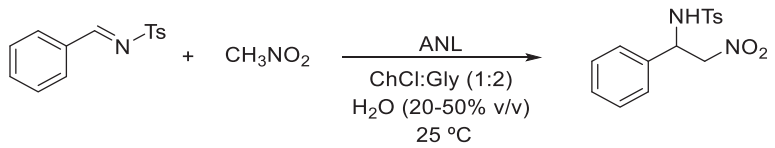
[a]*Reactions carried out at 37°C instead of 25°C.*

The biotransformation between 4-nitrobenzaldehyde and nitromethane was studied as benchmark reaction using various ChCl-based DES (Gly, EG and U as HBD in different ratios), water contents, reagents concentrations, enzyme loadings and temperatures, finding the highest conversion values for the those developed in ChCl:Gly/water (7:3 v/v, Table 16.3). Then, the study was extended to other substituted benzaldehydes comparing its reactivity with the reactions carried out in aqueous medium, observing a high influence of the pattern substitution at the phenyl ring.

In the same contribution, ANL was able to catalyze the aza-Henry reaction between (*E*)-*N*-[(4-methylbenzene-1-sulfonyl)oxy] − 1-phenylmethanimine and nitromethane (15 equiv) in ChCl:Gly (1:2 mol/mol), leading to the aza-Henry product in 35.5%−38.7% yield after 40 h at 25°C using a 20%−50% water content (Scheme 16.22), while lower amounts of water or the reaction in pure water led to poorer results.

Moving to C−heteroatom bond formation, Dandekar and coworkers reported the methylation of chitosan using dimethyl carbonate as methylating agent, and the lipase from *Burkholderia* species (Amano lipase PS) as biocatalyst (Fig. 16.1) [60], representing an environmentally friendly strategy to replace traditional transformation with methyl iodide in alkaline conditions. ChCl:Gly and ChCl:U (1:2 mol/mol) were used in combination with aqueous systems or/and dimethylformamide (DMF), finding PS as a highly active enzyme for the synthesis of *N*- and *O*-methylated chitosan derivatives, while no reaction was observed with CAL-B.

Multicomponent reactions are straightforward strategies to provide access to complex molecules, the use of lipases representing a clean an efficient way to perform this type of transformations. For instance, the Biginelli reaction has been successfully reported for the synthesis of pyrimidine derivatives combining the lipase from ROL with the use of aqueous, organic (methanol, 1,4-dioxane or DMF) and DES (ChCl:U (1:2 mol/mol)) solvents [61]. Seven dihydropyrimidin-2(1*H*)-one derivatives were prepared starting from an aromatic aldehyde, a dicarbonyl compound (ethyl acetoacetate or pentane-2,4-dione, 1.05 equiv) and urea or thiourea (1.1. equiv) using the ChCl:U (Scheme 16.23). In particular, the acceleration of the reaction between



SCHEME 16.22

ANL-catalyzed aza-Henry reaction between a *N*-tosyl imine and nitromethane in a DES/water system.

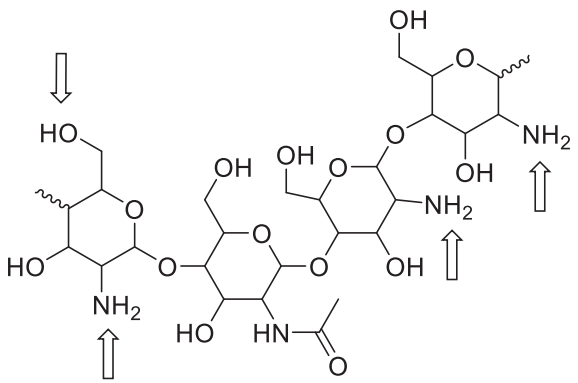
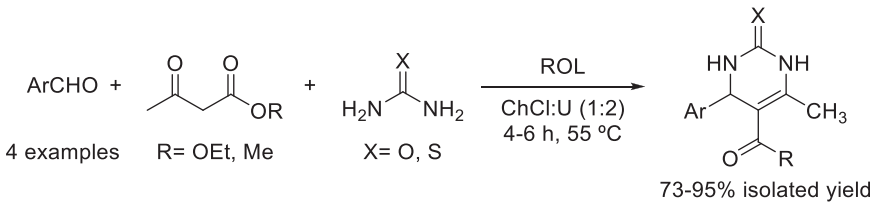


FIGURE 16.1

Chitosan positions susceptible of reaction through lipase-catalyzed methylation.



SCHEME 16.23

Lipase-catalyzed multicomponent Biginelli reaction accelerated by ROL in DES.

6-methoxynaphthalene-2-carbaldehyde, ethyl acetoacetate, and urea due to the enzyme action was significant, since in the absence of the ROL only 20% yield was reached after 7.5 h, while a 95% after 4 h was attained in its presence. The recyclability of the DES and lipase system was also demonstrated in this reaction, obtaining 75%–95% yields after five consecutive experiments.

16.3 Redox enzymes

The use of oxidoreductases (EC 1) in DES-water systems have been widely explored in the last decade, whether to enhance conversion or selectivity [62–64]. From this class of enzymes, alcohol dehydrogenases (ADHs) in reductive transformations, laccases and peroxidases for oxidation purposes, and flavin-dependent monooxygenases in the selective oxygenation of organic molecules, are without a doubt the biocatalysts of choice when considering DES as cosolvents. The main differences with previously studied hydrolases reside in their action mechanism and cofactor dependency, whether on inorganic metals such as copper for laccases, or on relatively small organic molecules such as nicotinamide adenine dinucleotides (NAD or NADP derivatives) for ADHs. This section has been classified depending on the type of reaction, moving from stereoselective bioreductions to nonselective oxidations and oxygenation transformations.

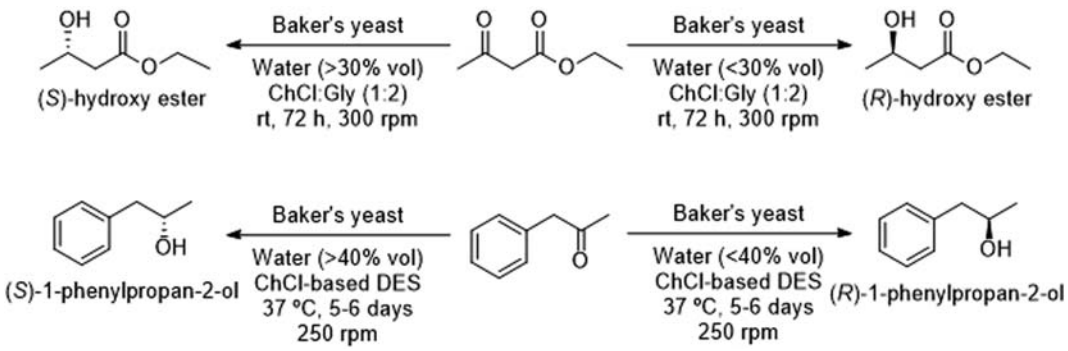
16.3.1 Reductions

The biocatalytic reduction of carbonyl groups is the most studied redox reaction in DES-water systems, employing mainly whole cell biocatalysts for stereoselective transformation [62,63]. The use of these enzyme preparations provide some advantages over isolated purified enzymes, such as the lack of external cofactor addition and the reduced of the system complexity since cofactor recycling is not required, which leads to a reduction of the overall cost of the process. In this context, the use of DES as cosolvents enable the work under higher substrate concentration compared to total aqueous medium, at the same time that the enzyme preserves its integrity. Nevertheless, the use of whole cells is sometimes limited by the presence of other enzymatic activities, which can produce a decrease in the yield and optical purity of the products caused by the occurrence of competitive enzymatic reactions.

Baker's yeast (*Saccharomyces cerevisiae*) has been the enzyme most commonly employed for reduction processes in mixtures of water and DES. Thus, Maugeri and Domínguez de María

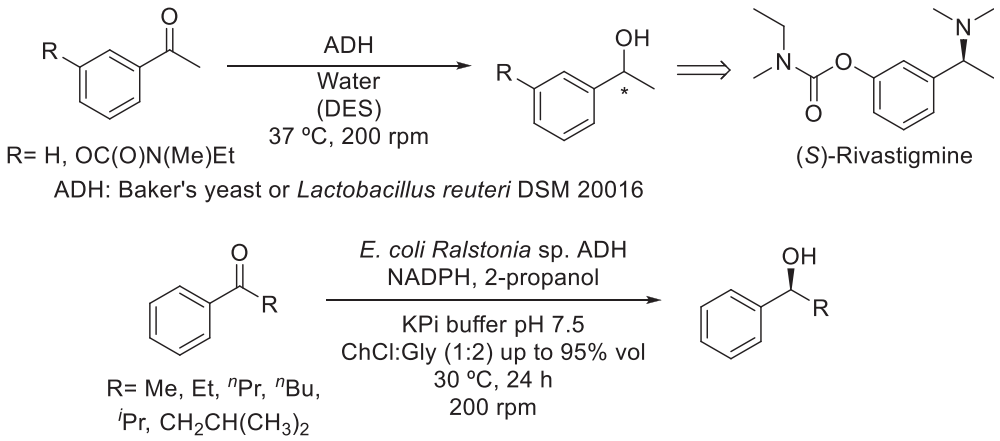
reported a pioneer work exploring the bioreduction of ethyl acetoacetate (Scheme 16.24 *top*) [65]. Complete conversion toward the formation of the ethyl (*S*) − 3-hydroxybutanoate was achieved in pure water after 72 h, while the addition of DES invert the selectivity toward the (*R*)-hydroxy ester, obtaining a virtually racemic alcohol at 30% v/v of DES and a total enantioselectivity in neat ChCl (1:2 mol/mol and remnant 1% weight of water determined by Karl-Fischer method). The achievement of this stereoinversion can be rationalized attending to the potential inhibitory effect of DES on (*S*)-oxidoreductases present in the BY whole cell preparation. Interestingly, the enzyme was still active for reaction times over 200 h when attempting reactions at both 10% and 50% of water. Redovnioković and coworkers deeply explored the same reaction employing a series of ChCl-based DES with different HBDs and ratios compared to the HBAs such as Gly, EG, Glc, Fru, Xyl, MA, Ox or U. [66] Selectivities and conversion values were highly depending on the type of HBD and the DES:water ratio, finding NADES involving sugars as HBDs (ChCl:Glc and Xyl) as suitable cosolvents (10% vol) for synthetic purposes, leading to the ethyl (*S*) − 3-hydroxybutanoate in high yield and selectivity (>90% conversion and >90% *ee*) as occur in pure water.

The inhibition of the Baker's yeast (*S*)-selectivity was also observed by Capriati and coworkers in the bioreduction of 1-phenylpropan-2-one (Scheme 16.24 *bottom*) [67]. The preferentially formation of the corresponding (*R*)-alcohol was observed at high DES contents (over 60% vol), the structure of the DES (Gly, Fru, Glc and U) playing a key role in the enantioselectivity value. For instance, the use of a mixture composed by 90% ChCl:Gly (1:2 mol/mol) and 10% water led to the (*R*) − 1-phenylpropan-2-ol in 96% *ee*, while the (S)-alcohol was obtained in also 96% *ee* in the absence of any DES. The use of other arylpropanones demonstrated the high influence of the substitution patterns at the aromatic ring in the product yield and selectivity. The same authors described the use of Baker's yeast resting cells in the bioreduction of acetophenone and 3-acetylphenyl ethyl(methyl)carbamate to the corresponding (*S*)-alcohols, the latter a precursor of the active pharmaceutical ingredient (*S*)-rivastigmine, a cholinesterase inhibitor used for the treatment of patients with moderate Alzheimer's disease (Scheme 16.25 *top*) [68]. However, better results were found when using the (*R*)-selective *Lactobacillus reuteri* DSM 20016 whole cells, although the use of DES was not reported in these reactions. Other aromatic ketones have also been



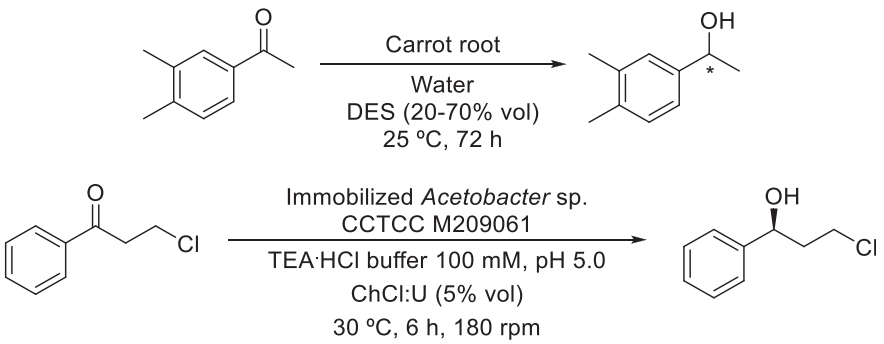
SCHEME 16.24

Change in the stereoselectivity in the bioreduction of prochiral ketones with Baker's yeast whole cells due to the presence of DES.



SCHEME 16.25

Stereoselective bioreduction of aromatic ketones in DES-water media.



SCHEME 16.26

Stereoselective bioreduction of aromatic ketones in DES-water media.

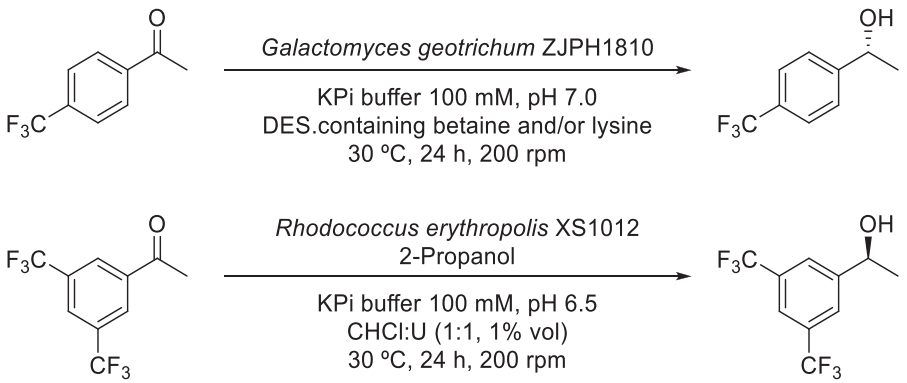
effectively reduced using recombinant overexpressing whole cells ADHs such as horse liver ADH (HLADH) and *Ralstonia* sp. (*Ras*ADH) using isopropanol for nicotinamide cofactor recycling purposes (Scheme 16.25 *bottom*) [69]. Special attention was paid to the use of *Ras*ADH that still remain active at 95% vol of ChCl:Gly (1:2 mol/mol), observing the highest enantiomeric excess at 90%−95% DES ratio.

Following with the reduction of aromatic ketones, 1-(3,4-dimethylphenyl)ethanone was used as substrate by Redovniković and coworkers with carrot roots as biocatalyst (Scheme 16.26 *top*) [70]. Starting from a 91% conversion and 96% *ee* of the (*S*)-alcohol in pure water, the use of five different DES composed by ChCl with EG (1:2), Glc (1:1) Gly (1:2), Xyl (2:1) and Xylol (5:2) were attempted finding. A dramatic decrease in activity was observed at higher DES contents

(20%−70% of DES), since conversion did not overcome 55%, observing an inversion of the enantioselectivity to the preferentially formation of the (*R*)-alcohol when only 30% of water was employed (33%−75% *ee*). Unfortunately, lower percentages of water led to poor conversion values (<10%) due to the high viscosity of the DES.

Enzyme immobilization can provide some advantages as occurred in the reduction of 3-chloropropiophenone to (*S*) − 3-chloro-1-phenylpropanol using *Acetobacter* sp. CCTCC M209061 (Scheme 16.26 *bottom*) [71]. The whole cells enzyme was immobilized on polyvinyl alcohol-sodium sulfate, studying its activity by combining a series of DES with TEA.HCl buffer pH 5.0. The ChCl:U (1:2 mol/mol) provided the best results, achieving the enantiopure (*S*)-alcohol in 82% isolated yield after 6 h when developing a 500 mL-scale reaction under optimal reaction conditions.

DES with variable HBAs, such as betaine, L-proline or L-carnitine, were prepared and used in the bioreduction of 1-[4-(trifluoromethyl)phenyl]ethanone (Scheme 16.27 *top*) [72]. These novel cosolvents (1% vol) enhanced the catalytic efficiency of *Galactomyces geotrichum* ZJPH1810 compared with traditional ChCl-based DES. In addition, the synthesis and application of lysine-based NADES was described, displaying also certain ability to enhance the regeneration of coenzyme were reported. Interestingly, the 500 mL-scale reactions at 400 mM substrate concentration using the betaine:Lys DES, occurred in 92.4% yield compared with the 78.4% yield achieved in phosphate buffer system, and maintaining the complete selectivity. The same research group has explored the reduction of 3,5-bis(trifluoromethyl)acetophenone using *Rhodococcus erythropolis* XS1012 for the synthesis of (*S*) − 3,5-bis(trifluoromethyl)phenylethanol, a key pharmaceutical intermediate of the NK-1 receptor antagonist (Scheme 16.27 *bottom*) [73]. In this case, ChCl:U (1:1 mol/mol) gave the best results with only a 1% vol, yielding the enantiopure alcohol with 91.9% conversion at 150 mM substrate concentration after 24 h at 30°C, which overcome the results obtained in buffer (82.6% yield). Similarly, the bioreduction of 2-chloro-1-(3,4-difluorophenyl)ethanone to produce (*S*) − 2-chloro-1-(3,4-difluorophenyl)ethanol was successfully achieved in a choline acetate/lysine (ChAc:Lys)-containing medium [74]. This DES strengthen coenzyme regeneration and improving cell membrane permeability during the bioreduction, leading to 87.0%



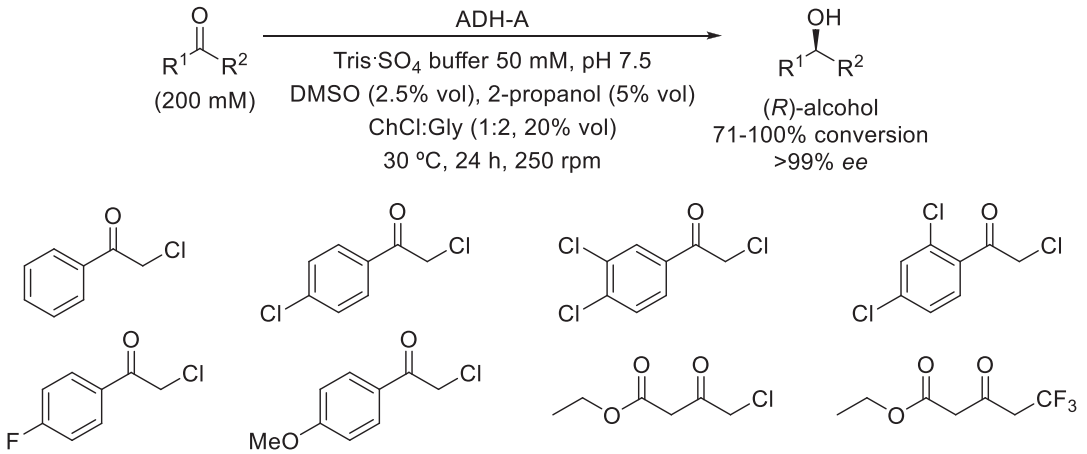
SCHEME 16.27

Bioreduction of trifluoromethyl ketones using DES as cosolvents.

conversion with complete selectivity, and increasing in 3.threefold the ketone concentration compared to the reaction in aqueous medium.

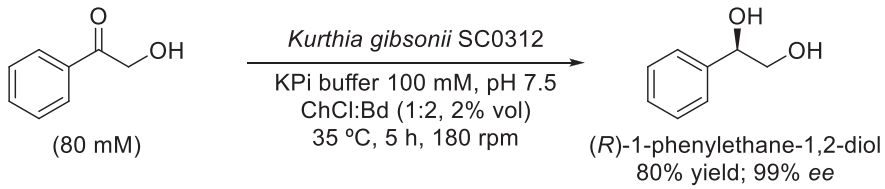
α-Haloketones are versatile precursors in organic synthesis because they can produce chiral halohydrins through bioreduction products, which in turn are immediate precursors of epoxides and azidoalcohols, among other interesting organic compounds. The use of DES in the bioreduction of α-haloketones have been recently reported using two stereocomplementary enzymes, the ADH from *Lactobacillus brevis* (*Lb*ADH) and the one from *Rhodococcus ruber* (ADH-A, Scheme 16.28) [75]. The main advantage of using DES up to 50% v/v, for instance ChCl:Gly (1:2 mol/mol), resides in the possibility to work at very high substrate concentrations (300−400 mM).

(*R*) − 1-Phenylethane-1,2-diol is a synthetic precursor of chiral drugs such as β-adrenergic blocking agents, and it can be straightforward accessed by stereoselective reduction of 2-hydroxyacetophenone (Scheme 16.29). The effect of five ChCl-based DESs on the reaction catalyzed by *Kurthia gibsonii* SC0312 were investigated by Lou and coworkers [76], the use of ChCl:1,4-butanediol (ChCl:Bd 1:4 mol/mol) in 2% vol increasing the catalytic rate of the enzyme



SCHEME 16.28

Bioreduction of α-haloketones in buffer-DES mixtures.



SCHEME 16.29

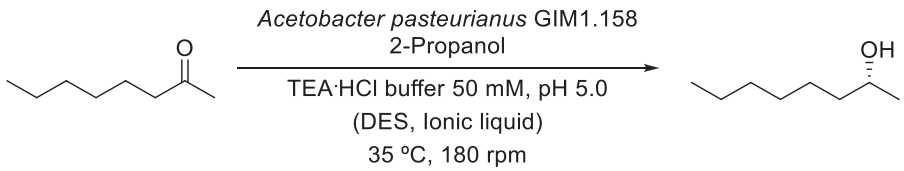
Asymmetric bioreduction of 2-hydroxyacetophenone in the presence of ChCl:Bd (2% vol).

by 22%. The reaction at 80 mM 2-hydroxyacetophenone concentration allowed the recovery of the (*R*)-alcohol in 80% yield and enantiomerically pure form.

The bioreduction of aliphatic ketones has also been reported in the literature, for instance 2-octanone was reduced by *Acetobacter pasteurianus* GIM1.158 cells (Scheme 16.30) using TEA. HCl buffer-ChCl-based DES mixtures (HBDs: U, Gly, EG, Ox, MA or Im) [77]. ChCl:EG (1:2 mol/mol) gave the best results in terms of reaction yield, finding a 40% ChCl:EG (1:2 mol/mol) ratio as optimal to obtain (*R*) − 2-octanol. The use of a biphasic system including the DES, the buffer and an imidazole-based IL (1-butyl-3-methylimidazolium hexafluorophosphate) was found to be an ideal media for the reduction of 2-octanone at high substrate concentration (1.5 M).

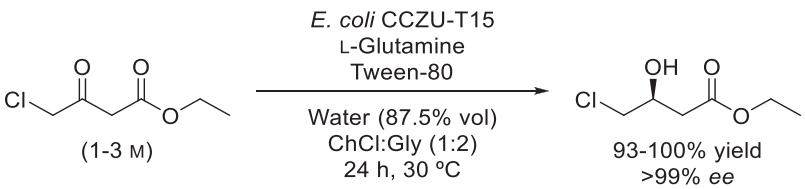
Ketoesters are also good substrates for bioreduction processes to obtain optically active hydroxyesters, as described earlier in this chapter [65,66]. Ethyl 4-chloro-3-oxobutanoate was converted to ethyl (*S*) − 4-chloro-3-hydroxybutyrate, a versatile precursor of chiral pharmaceuticals, using whole cells of *Escherichia coli* CCZU-T15 in a mixture of ChCl:Gly (1:2 mol/mol, 15% vol) and water (Scheme 16.31) [78]. Reaction conditions were optimized, finding a great improvement by adding: (1) the Tween-80 surfactant for a better dispersion of the substrate in the reaction medium; (2) L-glutamine to facilitate the cofactor synthesis. Thus the (*S*)-hydroxyester was obtained in enantiopure form when developing the reaction at a 2 M substrate concentration.

Structurally related to hydroxyesters, α-acetylbutyrolactone was reduced to α' − 1'-hydroxyethyl-γ-butyrolactone antistereoisomers (Scheme 16.32) using both growing and resting culture of seven different yeast strains [79]. After testing organic solvents (ethanol, glycerol, hexane and isopropanol) and ChCl:Gly (1:2 mol/mol) to help the substrate solubility, the DES (10%−50% vol) in combination with *Candida viswanathi* AM120, led to faster processes, also slightly improving the enantio- and diastereoselectivity in comparison with the reaction in buffer pH 7.0, especially when using 10 or 25% of DES.



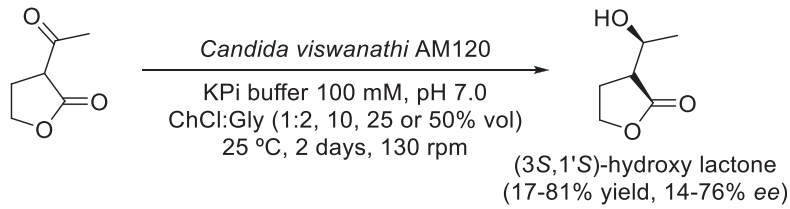
SCHEME 16.30

Stereoselective bioreduction of aliphatic ketones using ADHs in DES-water media.



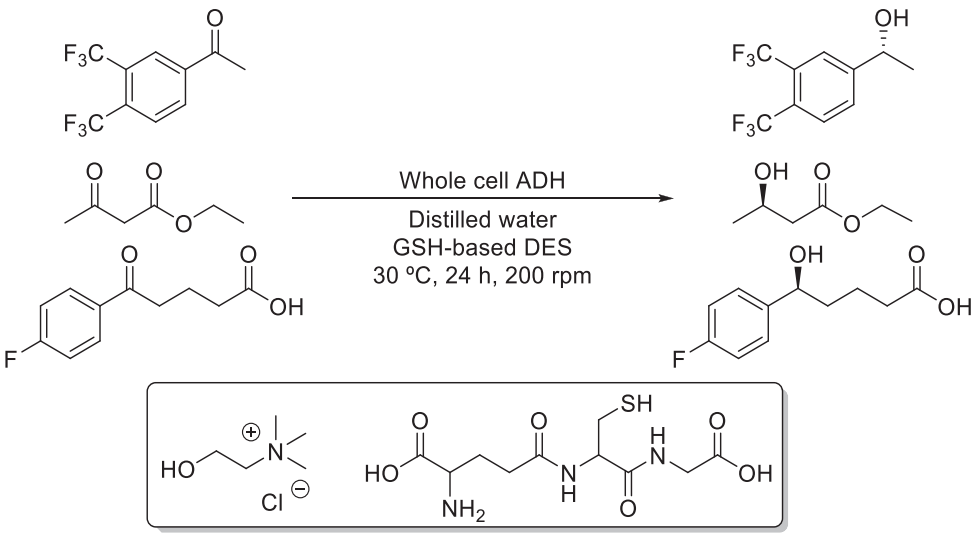
SCHEME 16.31

Stereoselective bioreduction of 4-chloro-3-oxobutanoate at high substrate concentrations using a water-DES mixture in the presence of a surfactant.



SCHEME 16.32

Stereoselective bioreduction of α-acetylbutyrolactone in DES-aqueous medium.



SCHEME 16.33

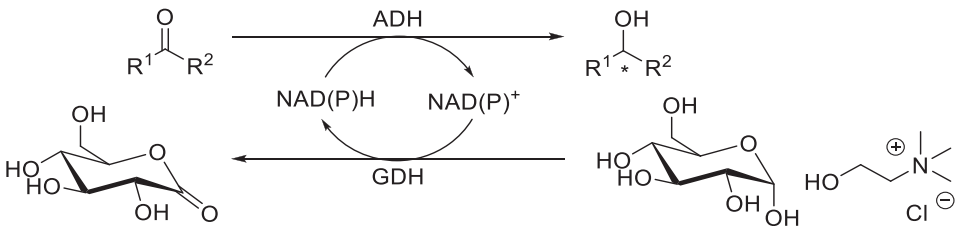
Bioreductions of carbonyl compounds using DES using GSH-based DES as cosolvents.

In the search for more environmentally friendly solvents, the use of a novel oligopeptide-based DES as cosolvent has been reported in the stereoselective enzyme-catalyzed reductions of thee selected substrates, including an arylketone, a ketoester and a ketoacid [80]. This DES contains ChCl and glutathione (GSH, comprised of Glu, Cys, and Gly, Scheme 16.33) and helped in the reduction of 3,5-bis(trifluoromethyl)acetophenone, 5-(4-fluorophenyl) − 5-oxopentanoic acid and ethyl acetoacetate, especially at higher substrate concentrations. Although it was only used in low ratios (0.5%−1.5% vol), the use of DES allowed the achievement of higher conversions in shorter reactions times, maintaining the enzyme selectivity. These achievements were explained based on the fact that the GSH forms hydrogen bonds with ChCl, as well as with the carbonyl group oxygen of the ketone, increasing its electrophilicity, thus facilitating proton acceptance from the reduced cofactor and promoting coenzyme regeneration.

Glucose dehydrogenase is an enzyme usually employed for reduced nicotinamide cofactor purposes at the expense of D-glucose oxidation into D-glucono-1,5-lactone that later spontaneously

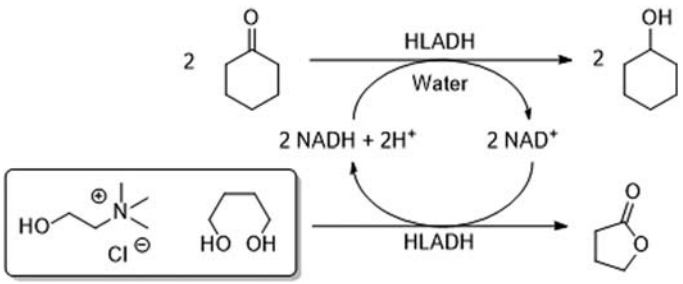
hydrolyzes toward the formation of D-gluconic acid. The possibility to use D-glucose as a HBD in a designer NADES has been reported, offering solutions for the bioreduction of ketones (Scheme 16.34) [81]. Thus, a higher solubility of ketones and a more efficient cofactor regeneration system is possible when combining an aqueous buffer (50 mM Tris.HCl, pH 7.5) and ChCl:Glc (1.5:1 mol/mol), Glc serving as a cosubstrate for several ADHs including *Lb*ADH, *Ras*ADH and ADH from *Thermoanaerobacter* sp. (ADH-T) in the bioreduction acetophenone, propiophenone and 2-octanone, respectively. The beneficial effect of this system was demonstrated at variable DES concentrations (10%−30%) and substrate concentrations (25−200 mM), finding great improvements at 50 and 100 mM ketones concentration compared to the reactions in buffer (83%−100% vs 59%−81% conversion), and maintaining the complete stereoselectivity.

Kara and coworkers were very active in the design of designer DES for bioreduction process, going through molecular dynamics simulations to quantify the molecular flexibility, hydration layer, and intraprotein hydrogen bonds of the HLADH [82]. The benchmark reaction considered was the transformation of cyclohexanone into cyclohexanol coupled with butane-1,4-diol (BD) as "smart cosubstrate" for cofactor regeneration (Scheme 16.35), which allows shifting the thermodynamic equilibrium to the alcohol side. The synthetic application was expanded to other substrates, and although benzaldehyde and ethyl 4-chloro-3-oxobutanoate did not lead to any conversion due to solubility issues, cinnamaldehyde was reduced to cinnamyl alcohol, a relevant compound in the aroma industry, in low extension (25%) after 48 h [83].



SCHEME 16.34

Use of glucose-based DES as designer cosolvents in the bioreduction of ketones.



SCHEME 16.35

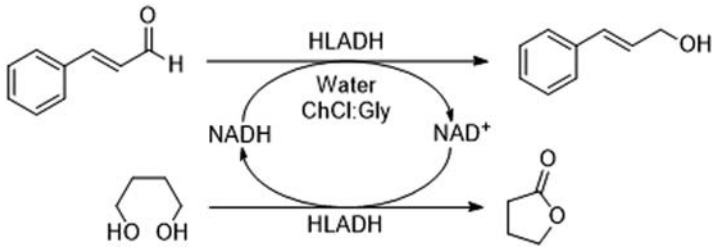
Use of 1,4-butanediol-based DES as designer cosolvents in the bioreduction of ketones.

The reduction of cinnamaldehyde to cinnamyl alcohol catalyzed by HLADH using 1,4-butanediol as cosubstrate for NADH recycling in varying mixtures of ChCl:Gly-water has been recently studied in depth (Scheme 16.36) [84]. Conversions led to up to 40 mM cinnamyl alcohol in the ChCl:Gly (1:9 mol/mol) mixture containing 20% vol of water within 8 h, compared with less than 10 mM in the when ChCl:Gly (1:2 mol/mol) was attempted, the higher glycerol content seemed to promoted higher conversions for HLADH.

Much less explored is the reduction of carbon−carbon double bonds using ene-reductases, this is the case of the Δ1,2-dehydrogeneration of cortisone acetate to prednisone acetate by using an immobilized version of the *Arthrobacter simplex* (Scheme 16.37) [85]. Three ChCl-based DES were used as cosolvents (4%−6% vol, HBD: Gly, EG and U, 32%−92% conversion), ChCl:U providing a higher conversion in comparison with the reaction in buffer (68%). The main advantages of using DES are: (1) the achievement of a better solubility of the cortisone acetate, as occurred with ethanol as cosolvent, running the reaction at 5 g/L substrate concentration; and (2) the possibility to reuse the enzyme, which was possible during 5 cycles, leading to 82%−93% conversion range.

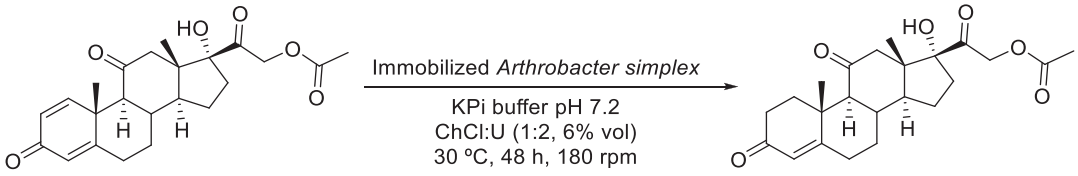
16.3.2 Oxidations

Reduction processes have been previously presented as ideal transformations to afford chiral alcohols in aqueous-DES mixtures. Next, their reverse reactions, alcohol oxidation for the formation of carbonyl compounds, are here described involving ADHs, catalases, laccases and oxidases.



SCHEME 16.36

Reduction of cinnamaldehyde catalyzed by HLADH using 1,4-butanediol as cosubstrate for NADH recycling in varying mixtures of DES-water.



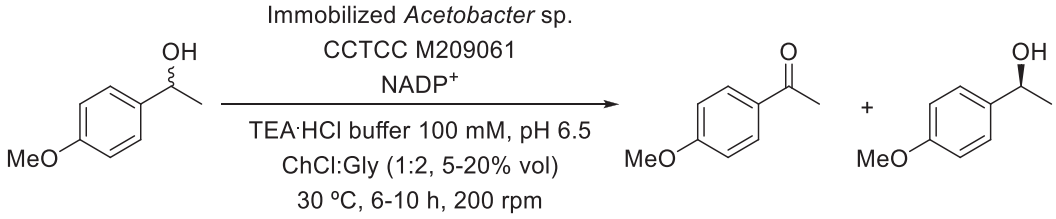
SCHEME 16.37

Bioreduction of cortisone acetate to prednisone acetate using DES as cosolvents.

However, despite the increasing literature in the field of oxidative transformations in neoteric solvents [86,87], the oxidation of alcohol in DES media remains nowadays in a premature stage because most of the reported works lacks organic synthetic applications in terms of reaction yields and product isolation, just focusing on kinetic and stability studies.

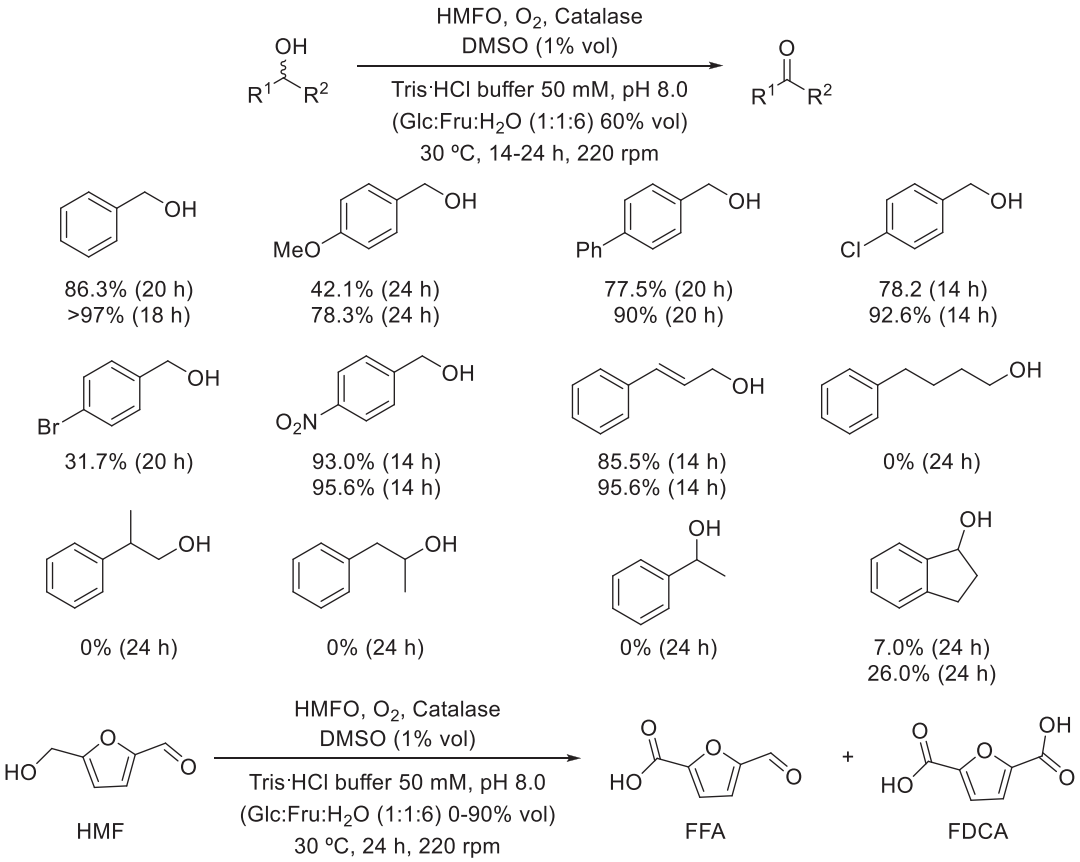
Kinetic resolution of secondary alcohols using oxidative enzymes constitutes an elegant manner to access to optically active alcohols, although the maximum conversion into an enantiomerically pure product is limited to 50%. Free and immobilized whole cells of *Acetobacter* sp. CCTCC M209061 were used in the resolution of 1-(4-methoxyphenyl)ethanol using a combination of TEA. HCl buffer and six different DES (Scheme 16.38) [88]. Selecting the immobilized biocatalysts, ChCl:Gly (1:2 mol/mol) in a 20% vol led to the best results, reaching a 49.4% conversion into the 1-(4-methoxyphenyl)ethanone, and recovering the (*S*) − 1-(4-methoxyphenyl)ethanol in 98.7% *ee*. Optimization of the reaction conditions were explored in terms of: (1) DES concentration, finding the optimal conditions at 5% vol for a 50% conversion after 9 h at 30°C; and (2) substrate concentration, increasing from 30 to 55 mM for the 500 mL preparative scale transformation, obtaining a 51.5% conversion after 7 h, and isolating the (*S*)-alcohol in enantiomerically pure form, while higher alcohol concentrations (60 and 65 mM) led to a decrease in the conversion (48.3−49.2%) and substrate enantiomeric excess (94.5%−97.8%). The same authors further investigated this oxidative resolution mixing the buffer with different ILs (12%−30% vol) to improve the productivity of the system [89]. Best results were found with 1-butyl-3-methylimidazolium hexafluorophosphate [bmimPF_6, 20% vol], obtaining a significant improvement when also the ChCl:Gly (8% vol) was also added, especially in terms of substrate concentration that was successfully increased until 80 mM (51.3% conversion, >99% *ee* of (*S*)-alcohol after 7 h at 30°C and 220 rpm).

The beneficial effect of DES as cosolvents has been recently reported in the nonselective oxidation of a series of primary and secondary alcohols using the 5-hydroxymethylfurfural oxidase (HMFO) in comparison with the use of plain buffer or in the presence of DMSO (1% vol) [90]. After testing a series of (natural) DES, in some cases higher conversions into the corresponding carbonyl compounds were obtained (Scheme 16.39). Best results were found using a Glc:Fru:H_2O (1:1:6 v/v/v) mixture, particularly studying the oxidation of 5′-hydroxymethyl furfural (HMF) into 5-formylfuran-2-carboxylic acid (FFA) and furan-2,5-dicarboxylic acid (FDCA), the latter platform molecule for the preparation of biobased polymers. Interestingly, when the reaction was performed in buffer only, the formation of FFA was highly favored (84%), whereas 16% of the FDCA was obtained. Complete conversions were also found at 30% and 60% of DES, finding the highest formation of FDCA (31%) at 60% DES after 24 h.



SCHEME 16.38

Kinetic oxidative resolution of racemic 1-(4-methoxyphenyl)ethanol.

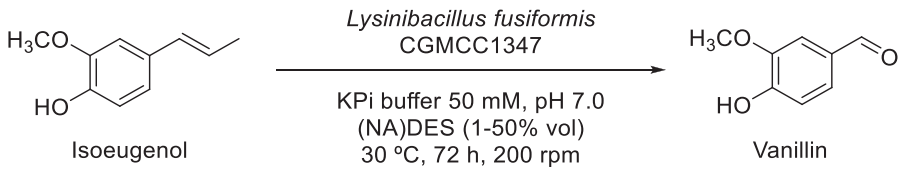


SCHEME 16.39

HMFO-catalyzed oxidation or primary and secondary alcohols in buffer-NADES mixtures. Top row corresponds with the reaction in the absence of DES, while in those cases reported, the bottom row corresponds to the reaction in the presence of DES.

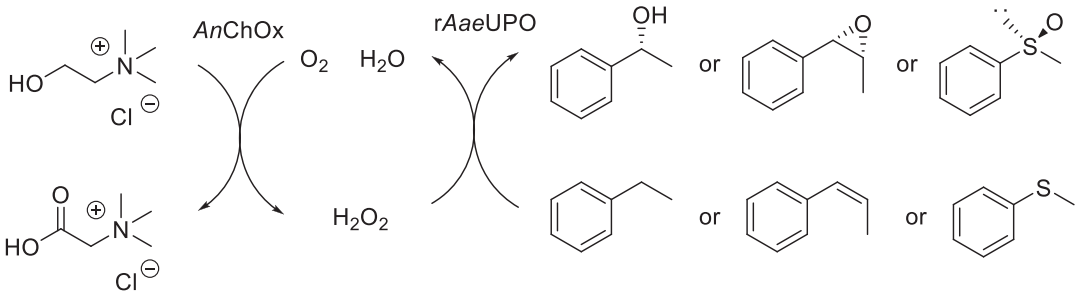
16.3.3 Hydroxyfunctionalization and dioxygenation reactions

The last section of the use of redox enzymes in DES media deals with the insertion of oxygen atoms or molecules using different enzyme classes. Enzymatic alkene oxidative-cleavage has been reported using *Lysinibacillus fusiformis* cells CGMCC1347 in whole cells form, converting isoeugenol to vanillin (Scheme 16.40) [91]. A total of 24 DES and 21 NADES were employed as cosolvents, finding some trends, for instance: (1) ChAc-based DES reached higher conversions than the ones attained with ChCl-based ones; and (2) Sugar DES provided better results than alcohol and organic acid HBD components. A 20% vol of NADES provide a beneficial effect, improving the yields especially with ChCl:lactose (4:1) and ChCl:raffinose (11:2), affording 132% and 131% higher conversions when compared to buffer only. Immobilization of the cells on poly(vinyl



SCHEME 16.40

Enzymatic alkene oxidative-cleavage of isoeugenol to vanillin.



SCHEME 16.41

Hydroxyfunctionalization of benzenes and alkenes using designers DES.

alcohol)-alginate beads led to higher conversion in both DES and NADES., observing the best results with ChAc-based DES that retained for at least 13 cycles its activity in 72 h reactions, displaying good operational stability. The same authors reported an experimental and mechanistic study of the same transformation using a lipoxygenase (EC 1.13.11.12), non heme, iron-containing dioxygenase [92]. The biotransformation was developed in borate buffer (200 mM, pH 9.0) and the presence of different additives including surfactants (6), organic solvents (10), DES (8) and NADES (13). Best results were obtained in DES and NADES, obtaining slightly better results in the reaction with 20% of ChCl:So (5:2 mol/mol) than in the reaction with pure buffer.

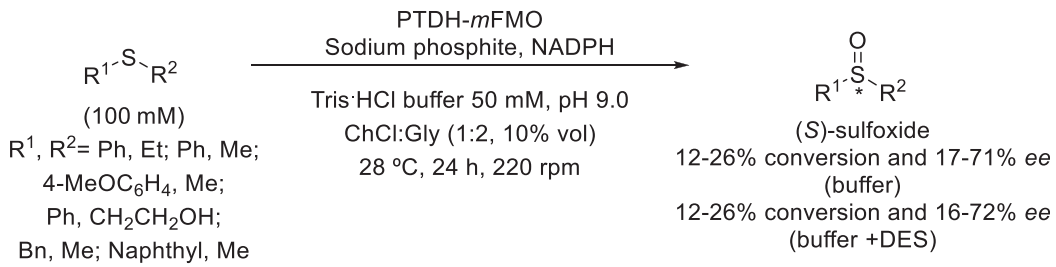
As occurred with the use of designer DES for bioreduction processes, their application as more than cosolvents and stabilizers have been also recently reported in oxygenation reactions, where the ChCl-based DES act as sacrificial electron donor for the in situ H_2O_2 generation (Scheme 16.41) [93]. Thus, the transformation of ethyl benzene to 1-phenyethanol, or *cis*-β-methylstyrene into epoxides is possible by in situ H_2O_2 generation using the choline oxidase from *A. nicotianae* (*An*ChOx) that converts choline into betaine, and the concomitant generation of 2 equiv of H_2O_2 to drive the corresponding peroxygenase-catalyzed oxyfunctionalization using the recombinant evolved peroxygenase from *Agrocybe aegerita* (r*Aae*UPO). In this manner, the situ hydrogen peroxide formation is leveled in the presence of the unspecific peroxygenase, avoiding oxidative inactive of the biocatalyst. Reactions were carried out in phosphate buffer pH 7.0 employing different (NA) DES sources and concentrations (up to 50%), and seem to be applicable in to other type of transformation such as sulfoxidation reactions as described below.

Finally, examples of sulfoxidation reactions involving DES have been included, since (chiral) sulfoxides are important organic compounds with wide applications in medicinal and organic chemistry [94]. Very recently, Hollmann and coworkers extended the potential of choline-based DES in oxygenation reactions (Scheme 16.41), by studying their participation in the sulfoxidation of methyl phenyl sulfide (35 mM), also known as thioanisole [95]. The best results were found using equal volumes of phosphate buffer and (NA)DES when considering urea as HBD and a temperature of 30°C.

Finally, the benefits of using NADES in the asymmetric sulfoxidation of six sulfides have been reported by de Gonzalo (Scheme 16.42) [96], employing the flavin-containing monooxygenase from *Methylophaga* sp. strain SK1 (mFMO) fused to phosphite dehydrogenase for cofactor regeneration purposes (PTDH-mFMO). Ethyl phenyl sulfide was considered as a benchmark substrate for the optimization of the reaction conditions and analyze the effect of the addition of DES in the reaction medium, which was mostly negative in most of the cases at variable substrate concentrations (10−200 mM). Only the use of ChCl:EG (1:2 mol/mol) or ChCl:Gly (1:2 mol/mol) up to 10% v/v led to comparable reaction results as for the one carried out in pure buffer, and a similar trend was observed with other sulfides, for which the use of NADES has only minimum benefits at high substrate concentrations.

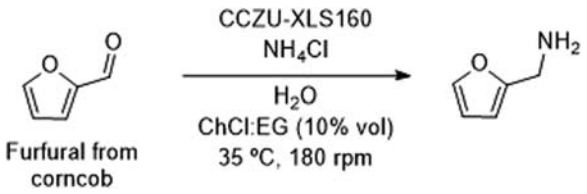
16.4 Lyases and transferases in single transformations using deep eutectic solvents as solvents

Apart from oxidoreductases (EC.1) and hydrolases (EC.3), transferases (EC.2) and lyases (EC.4) provide sustainable solutions for synthetic transformations, although their applications in DES has been scarcely reported in the literature. Inside the transferase class, there is only example dealing with individual transformations developed in DES consisting in the production of furfurylamine from biomass containing furfural (Scheme 16.43) [97]. For that purpose, a recombinant *E. coli* CCZU-XLS160 whole cells harboring ω-transaminase and L-alanine dehydrogenase was constructed and used in combination with inexpensive ammonium chloride as amine source. Thus, the transaminase was able to catalyze the furfural amination in ChCl:EG-water (1:9 v/v) at 35°C, while the L-alanine dehydrogenase was responsible for the transformation of the formed pyruvate into



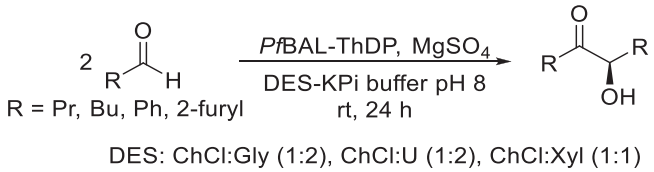
SCHEME 16.42

Enzymatic asymmetric sulfoxidation in buffer and DES-buffer systems.



SCHEME 16.43

Transaminase-catalyzed amination of furfural derived from biomass to furfurylamine.



SCHEME 16.44

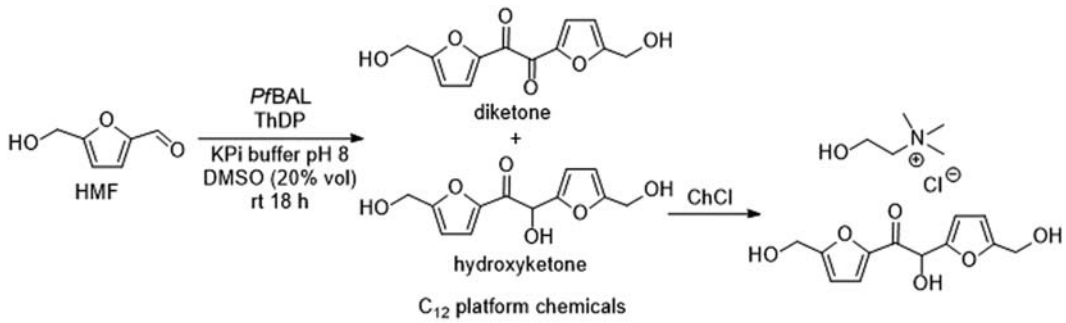
BAL-catalyzed self-condensation of aliphatic or aromatic aldehydes in DES-buffer mixtures.

L-alanine. Overall, 92.3 mM of furfural derived from corncob was entirely transformed to furfurylamine with a productivity of 0.39 g of amine/g xylan in corncob.

Inside the lyase class, the use of benzaldehyde lyases (BALs) and phenolic acid decarboxylases (PADs) has been reported. For instance, Domínguez de María and coworkers reported the use of a thiamine-diphosphate-dependent BAL from *Pseudomonas fluorescens* (*Pf*BAL) in DES-water mixtures for the carboligation of a series of aldehydes (Scheme 16.44) [98]. The reactions were explored in three different ChCl-based DES including Gly (1:2 mol/mol), U (1:2) or Xyl (1:1) as HBDs and variable amount of water (0%−100%), obtaining high to excellent conversions for self-condensation of two aldehydes molecules using ChCl:Gly (1:2)-phosphate buffer (6:4 v/v), and also variable selectivity levels toward the corresponding (*R*)-enantiomers after 24 h at room temperature: butyraldehyde (96% conversion, 52% *ee*), valeraldehyde (98% conversion, 27% *ee*), benzaldehyde (>99% conversion, >99% *ee*), and 2-furaldehyde (75% conversion, 63% *ee*). A decrease of the enzyme activity was found at higher ChCl:Gly percentages, being almost negligible at 90% DES content. However, a full conversion was achieved in the reaction with valeraldehyde when changing to the ChCl:U (1:2 mol/mol) in a 70% ratio.

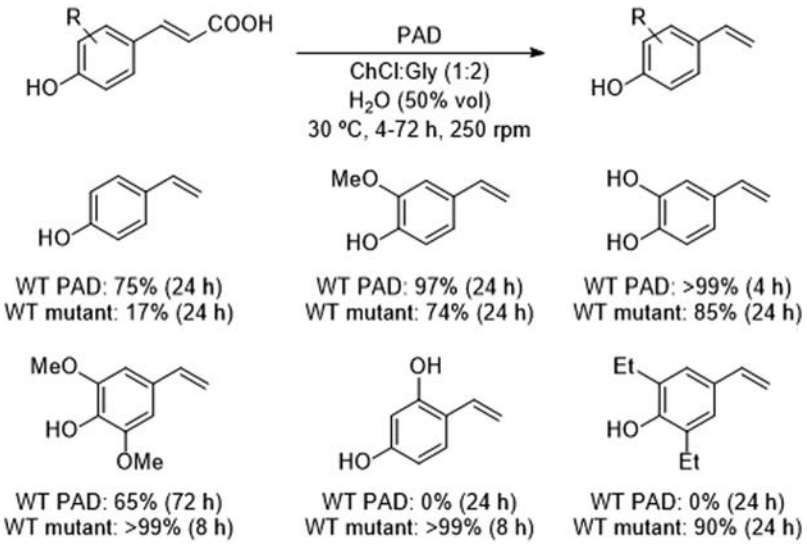
The same research group has reported the use of the *Pf*BAL for the umpolung carboligation of furfural, hydroxymethylfurfural (HMF) and mixtures of them in pure aqueous systems, providing a new C_{10}−C_{12} platform of chemicals (Scheme 16.45) [99]. The so-obtained hydroxyl ketones were later applied as HBDs for the synthesis of novel DES, although their application in enzyme-catalyzed transformations was not tested.

More recently, the use of a phenolic acid decarboxylase from *Bacillus subtilis* (*Bs*PAD) wild-type (PAD WT) and its I85A mutant (PAD mutant) has been explored for the conversion of *p*-hydroxycinnamic acid derivatives to the corresponding *p*-hydroxystyrenes using ChCl-based DES (U, So and Gly as HBDs)-water binary systems (Scheme 16.46) [100]. The DES helps to solubilize



SCHEME 16.45

BAL-catalyzed carboligation of HMF, and later mixing of the hydroxyl ketone with ChCl for the formation of novel DES.



SCHEME 16.46

Conversion values of the *Bs*PAD-catalyzed decarboxylation of *p*-hydroxycinnamic acids performed at 300 mM substrate concentration, except for the 2,6-diethyl-4-vinylphenol formation, that was performed at 10 mM substrate concentration and with addition of DMSO (1%) to favor the solubility of the starting cinnamic acid.

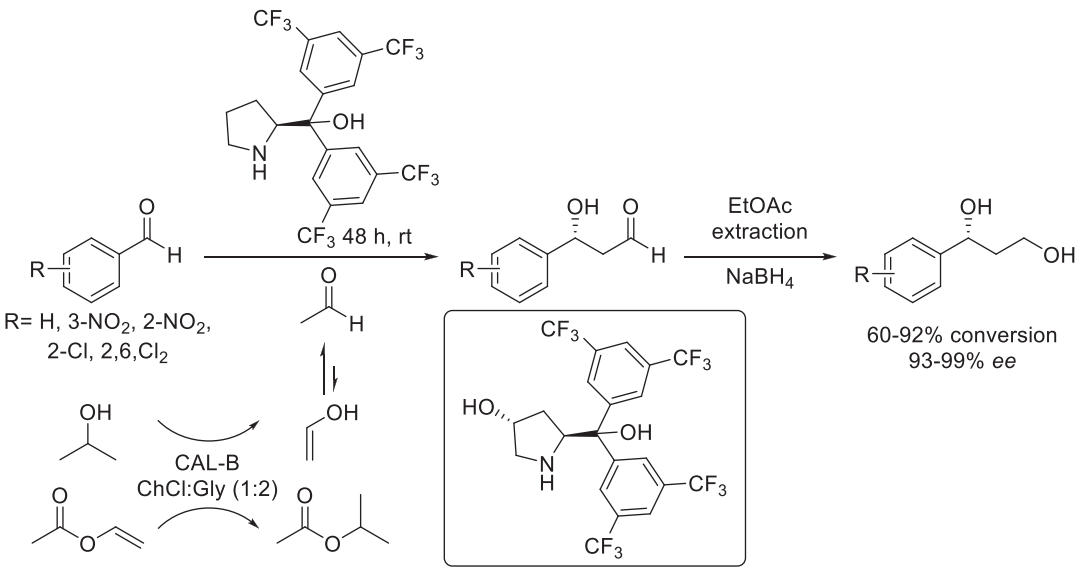
these substrates making possible reactions with substrate concentrations up to 300 mM, studying the influence of water in different percentage (0%−50%). Although, the reactivity of the six tested cinnamic acids was very different, a general methodology was disclosed attaining good to quantitative conversions either with the wild type or with the evolved PAD variant.

16.5 Multicatalytic transformations

The use of single enzyme transformations in DES systems and also in some cases multienzymatic transformations to shift the reaction equilibrium or allowing the cofactor recycling is disclosed, presenting the combination of both enzymes and DES as ideal catalytic systems for practical applications. Next, examples of cascade process developed in a sequential or concurrent manner will be discussed, demonstrating that enzymes can be also used together with organocatalysts and metal species.

16.5.1 Combination of enzymes and organocatalysts

The use of organocatalysts in DES has attracted recent attention mainly for aldol and Michael-type additions [101,102], the combination with enzymes being possible through cascade process, until now when using hydrolases for determined transformations. Domínguez de María and coworkers developed the in situ formation of acetaldehyde through the CAL-B catalyzed transesterification of 2-propanol (3 mmol) with vinyl acetate (3 mmol) in ChCl:Gly (1:2 mol/mol) at room temperature (Scheme 16.47), which subsequently reacted with 4-nitrobenzaldehyde (1 mmol, 1 M) in the presence of a highly substituted proline derivative (0.2 mmol) [103]. Thus, 1-(4-nitrophenyl)propane-1,3-diol was obtained in 92% conversion (70% isolated yield after liquid-liquid extraction with ethyl acetate, EtOAc) and 95% *ee* after reduction of the aldehyde intermediate with sodium borohydride (6 mmol). Recycling experiments of the DES and CAL-B system were performed, after



SCHEME 16.47

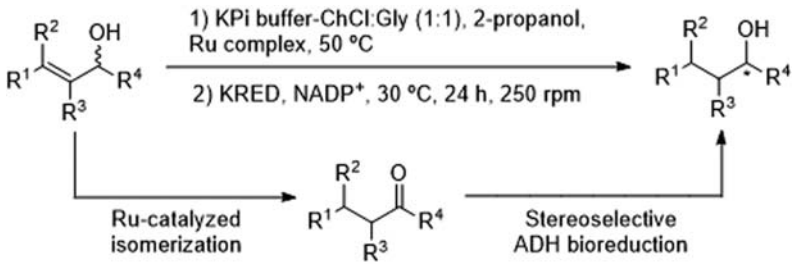
Use of lipase-diaryl prolinol catalytic systems for cross-aldol reactions between aromatic aldehydes and in situ generated acetaldehyde.

recovering the desired hydroxy aldehyde by liquid-liquid extraction with EtOAc, without requiring the addition of the organocatalyst for two cycles. The methodology resulted to be general when applied to other aromatic aldehydes (60%−85% conversion and 93%−99% *ee*), although it failed when attempting an heteroaromatic aldehyde or cinnamaldehydes (<42% conversion). The same research group proposed the use of a structurally similar organocatalyst containing an extra hydroxyl group at the C-3 position of the pyrrolidine ring (boxed in Scheme 16.47) [104], which allows a stronger interaction with the DES, thus improving the conversion and recycling possibilities of the catalytic system for the tandem reaction between 2-propanol, vinyl acetate and 4-nitrobenzaldehyde accelerated by CAL-B in ChCl:Gly (1:2 mol/mol).

16.5.2 Combination of enzymes and metal species

Biocatalysis and metal catalysis are pivotal strategies for the synthesis of organic compounds, usually requiring very different reaction conditions for the correct action of both types of catalysis. In the search for ideal reaction conditions to pair the exquisite selective displayed by biological catalyst with the broad range of applications that offer chemical catalysts, DES has emerged as environmentally friendly reaction medium, or at least part of it in combination with water [105]. Next, a series of cascade metal−enzyme transformations will be discussed including the use of metal complexes derived from palladium or ruthenium, and enzymes such as ADHs, amine transaminases and phenolic acid decarboxylases.

The first application of DES as cosolvents for the combination of biological and metal catalysts consisted of the ruthenium-catalyzed isomerization of racemic allylic alcohols followed by stereoselective bioreduction of the resulting unsaturated ketone intermediates (Scheme 16.48) [106]. Prior to studying the one-pot two-step approach, each of the two steps were studied individually with five ChCl-based DES, finding the best results with the mixture ChCl:Gly (1:2 mol/mol). When applying the multicatalytic approach, the proper selection of ADH allowed the production of both alcohol enantiomers with excellent stereoselection (93%−>99% *ee*) and variable isolated yield depending on the substrate structure (65%−100% conversion) in the one-pot sequential approach. The concurrent strategy was also successfully accomplished when using the commercially available KRED-P2-C11 (68%−98% conversion, >99% *ee*), although failed for the *Lactobacillus kefir* ADH



SCHEME 16.48

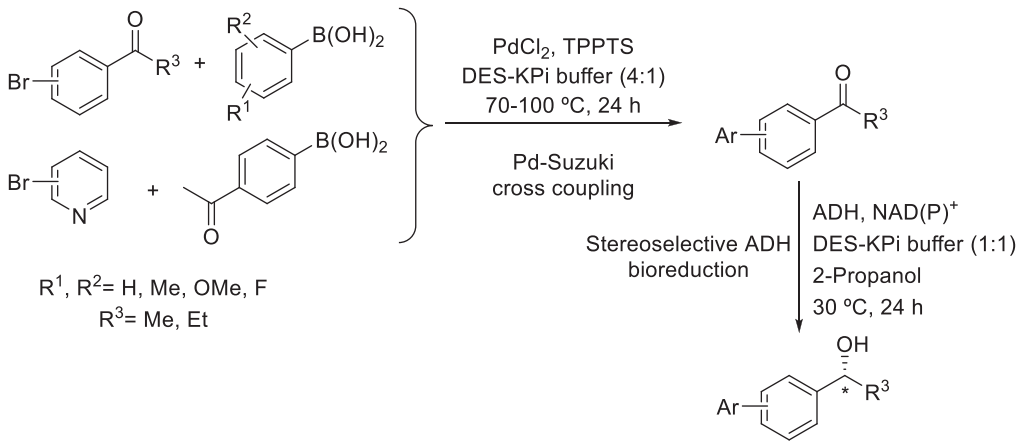
Chemoenzymatic one-pot isomerization-bioreduction sequence developed in a DES-buffer system.

heterologously expressed in *E. coli* since only 21% of the enantiopure (*R*) − 1-phenylpropan-1-ol was formed.

Another metal-catalyzed transformation combined with the stereoselective action of ADHs using DES as part of the solvent is the one consisting in the palladium-catalyzed Suzuki-cross coupling of (het)aryl bromides with arylboronic acids (Scheme 16.49) [107]. The use of DES such as ChCl:Gly (1:2 mol/mol) allowed to work with high substrate concentrations for the overall process (200 mM for the initial Suzuki-cross coupling and then adjusted to 75 mM for the bioreduction step), yielding a series of chiral biaryl compounds in enantiopure form. Apart from the different ideal substrate concentrations for both steps, the approach was developed in a sequential manner because of the high temperatures required for the Suzuki-cross coupling (70°C or 100°C) that are incompatible with the correct ADH action, *L. kefir* to provide access to the (*R*)-alcohols and ADH-A from *R. ruber* for the (*S*)-enantiomers.

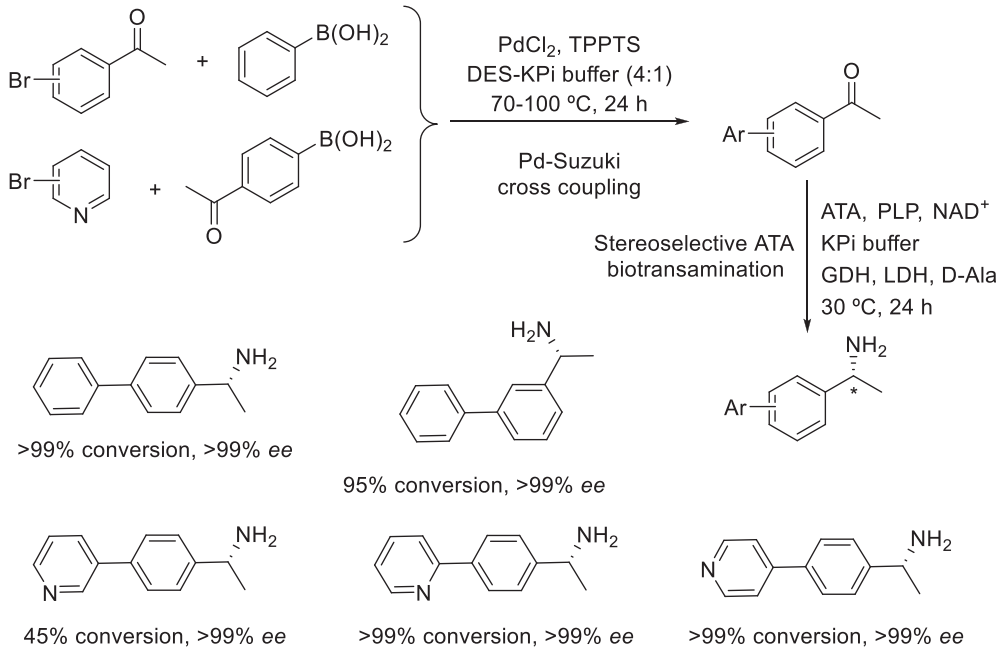
Remarkably and following a similar strategy, the combination of palladium chloride and an amine transaminase was successfully developed by the same authors in a sequential manner for the production of a series of (hetero)aromatic chiral amines, the biotransamination of the biaryl ketone intermediates being possible using D-alanine as amine donor and *Exophiala xenobiotica* wild-type ω-transaminase (EX-ω-TA) or its T273S mutant (EX-STA) as enzyme (Scheme 16.50) [108].

Very recently, a mixture of ChCl-based DES and water has been used first in batch and later in continuous flow for the development of an enzyme-metal sequence to produce the (*E*) − 4-hydroxy-stilbene (Scheme 16.51) [109]. After optimization of the reaction conditions, the continuous mode approach involves the use of an immobilized phenolic acid decarboxylase (PAD) for the decarboxylation of *p*-coumaric acid (30 min for a space-time-yield of 4.8 g/Lh) and subsequent Pd-catalyzed Heck cross coupling with iodobenzene (45 min for a space-time-yield of 0.52 g/Lh). Importantly, the use of DES allowed the development of the tandem enzymatic decarboxylation/Heck coupling in a 20 mM substrate concentration, compared with a maximum 5% achieved in



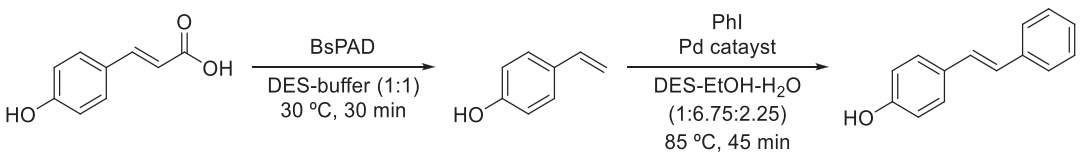
SCHEME 16.49

Chemoenzymatic sequence involving a Suzuki-cross coupling followed by bioreduction of the intermediate biaryl ketones developed in a DES-buffer system.



SCHEME 16.50

Chemoenzymatic sequence involving a Suzuki-cross coupling followed by biotransamination of the intermediate biaryl ketones developed in a DES-buffer system.

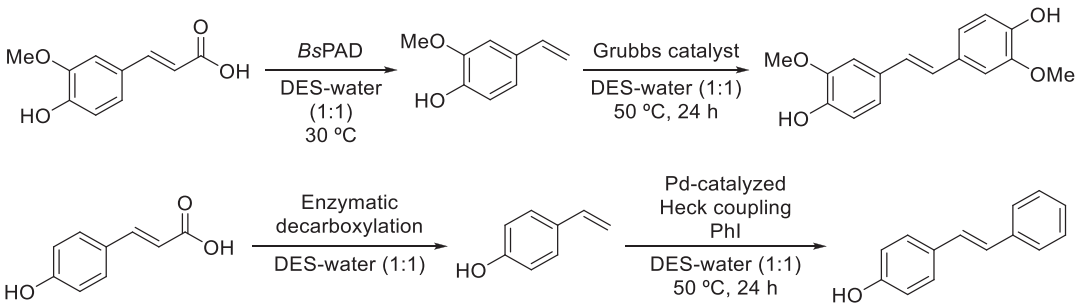


SCHEME 16.51

Chemoenzymatic synthesis of (*E*) − 4-hydroxy-stilbene under flow conditions via PAD-catalyzed decarboxylation of *p*-coumaric acid followed by Pd-catalyzed Heck cross coupling of 4-vinylphenol intermediate with iodobenzene.

water, tackling the incompatibility between both catalysts, and avoiding the isolation of reaction intermediates that are prone to polymerize, thus obtaining a higher yield (20%) than initially expected.

Finally, two independent strategies have been recently explored involving the combination of *Bs*PAD with palladium and ruthenium complexes for the synthesis of biaryl derivatives in DES-water systems (1:1 v/v) [110]. On one hand, the enzymatic decarboxylation of ferulic acid was followed by ruthenium-catalyzed metathesis of the resulting olefin using the Grubbs catalyst,



SCHEME 16.52

Synthesis of stilbenes via enzyme-metal cascades developed in a sequential manner using DES-water systems.

obtaining the corresponding stilbene in only 15% yield (Scheme 16.52 *top*). On the other hand, the aforementioned enzymatic decarboxylation reaction was coupled to a Heck-type C−C coupling using iodobenzene (Scheme 16.52 *bottom*). In this case, the PAD was immobilized onto a solid support and activated with tertiary amine groups (PAD@EC-TEA), giving a lower specific activity than the soluble enzyme but exhibiting and excellent decarboxylation activity in the ChCl:Gly (1:2 mol/mol):water (1:1) system, achieving full conversion after 2 h at a 200 mM substrate concentration. Then after enzyme filtration, the Heck coupling with iodobenzene was developed but no conversion was found possibly due to the partial elution of the *Bs*PAD by the DES, as no problems were observed when water was used as solvent for the first step, leading to a 60% overall yield.

16.6 Conclusions and perspectives

In this chapter, we have focused on showcasing reaction yields or conversions of biocatalytic reactions performed in pure DES and DES-aqueous media mixtures to highlight their applicability in organic synthesis. DES provide advantages as alternatives to organic solvents by enhancing enzyme activity and stability, yield, stereopreference, with higher substrate solubility, while remaining innocuous for the biocatalyst, thus leading to higher yields and a good perspective for synthetic applications. The synergy between biocatalysts and DES contributes to the sustainable production of various industrially relevant compounds with high yields and selectivities, transformations that can be developed in a single transformation or by means of successive steps through chemoenzymatic multicatalytic cascades. Future studies on exploring and exploiting the use of DES as the substrate source itself would be attractive to further implement.

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