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Biocatalysis in Non-Conventional Media: Unlocking the Potential for Sustainable Chiral Amine Synthesis

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The application of biocatalysis has become essential in both academic and industrial domains for the asymmetric synthesis of chiral amines, and it serves as an alternative tool to transition-metal catalysis and complements traditional chemical methods. It relies on the swift expansion of available processes, primarily as a result of advanced tools for enzyme discovery, combined with high-throughput laboratory evolution techniques for optimizing biocatalysts. This concept paper explores the utilization of non-conventional media such as ether-type solvents, deep eutectic solvents, and micellar catalysis to enhance biocatalytic reactions for chiral amine synthesis. Each section focuses on the unique properties of these media, including their ability to stabilize enzymes, alter substrate solubility, and modulate enzyme selectivity. The paper aims to provide insights into how these innovative media can overcome traditional limitations, offering new avenues for sustainable and efficient chiral amine production through biocatalytic processes.

Introduction

The synthesis of chiral amines holds immense significance across diverse domains, ranging from natural products and pharmaceuticals to small-molecule biological probes. A striking observation underscores the prominence of α-stereogenic amines and resulting amides, constituting nearly half of the active pharmaceutical ingredients (APIs) currently available on the drug market. Most syntheses rely on chemical approaches centred on the asymmetric hydrogenation of imines and enamines,[1] and on the reductive amination of pro-chiral ketones.[2] However, their utilization poses significant challenges, necessitating harsh reaction conditions and relying on expensive and sensitive transition-metal complexes. Additionally, many of these catalysts fail to achieve high enantiomeric purity, which presents issues in their application to pharmaceutical production, especially since the "chiral switch" of 1997 in the pharmaceutical industry heightened the demand for methodologies producing chiral amines with high optical purity.[3] In response to these challenges, biocatalysis offers a complementary approach owing to the intrinsic high stereocontrol of enzymes, enabled by various enzymatic engineering technologies such as directed evolution[4] and site-directed mutagenesis,[5] which rapidly generate libraries of enzymes tailored to specific transformations. In this scenario, the biocatalytic preparation of α-stereogenic amines can be achieved through different enzymes,[6] as lipases,[7] amine dehydrogenases (AmDHs),[8] monoamine oxidases (MAOs),[9] transaminases (TAs),[10] and the recently discovered imine reductases (IREDs).[11] An emblematic example of the application of enzymes in the preparation of chiral amines is the case of Sitagliptin phosphate (Januvia™) by Merck, a drug for type 2 diabetes.[12] Its chemical synthesis from ketoamide **1** entails the asymmetric hydrogenation of an enamine under high pressure, employing a rhodium-based chiral catalyst.[13] However, this process faces challenges with inadequate stereoselectivity and contamination of the product with rhodium. Consequently, additional purification steps are required (a carbon treatment to remove metal traces and a recrystallization), which enhance both enantiomeric excess and chemical purity, but at the same time compromise the final yield. Through the utilization of a transaminase scaffold and diverse protein engineering techniques, Savile and coworkers devised in 2010 a catalyst and a process that markedly enhance the efficiency of Sitagliptin manufacturing (Scheme 1).[14] Compared to the chemical process, the biocatalytic approach offers a 10–13% increase in overall yield, an improved ee and a 19% reduction in total waste, which implies a decrease in total manufacturing costs. For this reason, the process received the Presidential Green Chemistry Challenge Award (Greener Reaction Conditions Award) from the U.S. Environmental Protection Agency (EPA).

Recently, there has been recognition of how replacing the traditional reaction media employed in biocatalysis with non-conventional solvents could further improve the sustainability of biocatalysis and address some issues arising from its application to large-scale productions. This review highlights the most significant developments in the application of such non-conventional media in biocatalysis, stepping up the production of chiral amines, leading to high yields and enantiomeric excesses, and enabling chemo-enzymatic cascades. More specifically, each section is dedicated to green

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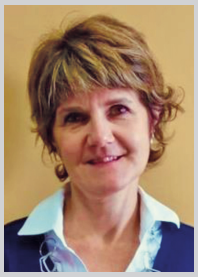
solvents, Deep Eutectic Solvents and biocatalysis in micellar environment respectively. Preceding them, we have included a concise explanation of what are presently recognized as non-conventional solvents and how their implementation into biocatalysis can have a deep impact.

Exploring Non-Conventional Reaction Media in Biocatalysis: Opportunities and Advantages

In the context of biocatalysis, the term "non-conventional" generally refers to non-aqueous media (organic solvents, biphasic systems, Deep Eutectic Solvents, Ionic Liquids) or aqueous solutions doped with additives not commonly found in buffer solutions, thus being unconventional in biocatalysis. The replacement of traditional media offers two main benefits. First, while enzymes have naturally evolved to function in aqueous environments to maximize their activity and stability, water's high polarity often conflicts with hydrophobic substrates, necessitating the use of poorly water-soluble chemicals at low molarity (5.0–50 mM). The use of non-conventional media is advantageous for enhancing substrate concentration, reducing reaction times, and therefore increasing the productivity of the overall transformation. Additionally, sustainability is a key factor. As water has been considered an excellent green solvent, this prospective must change when we think to industrial scale production where downstream processing negatively affects the greenness of the process.[15] Indeed, the use of non-conventional solvents result in the generation of limited volumes of wastewater after the biocatalysed processes and facilitate their scale-up, as well as the integration of chemo-enzymatic cascades into existing syntheses.[16]

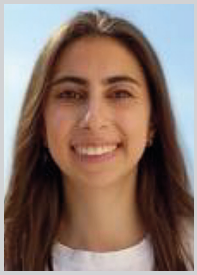
Exploration of biotransformations in non-aqueous media began in the 1980s, initially focusing on lipases in organic solvents,[17] and later expanding to include ionic liquids (ILs),[18] and supercritical liquids.[19] In this sense, the pioneering work carried out by Nobel Prize winner, Frances Arnold, has opened the way to new possibilities. By utilizing directed evolution techniques on enzymes, Arnold demonstrated that it is possible to enhance the properties of existing enzymes, making them more tolerant to the presence of organic solvents such as acetonitrile, dimethyl sulfoxide and dimethylformamide, thereby enabling more efficient chemical reactions.[20] Moreover, her



Prof. Cristina Prandi is Full Professor of Organic Chemistry at Chemistry Department of the University of Torino where she is also Vice Rector for Research; her main interests are related to organometallic chemistry, target oriented synthesis and synthetic methodologies in unconventional solvents in line with sustainability and green chemistry principles. She has carried out research on the synthesis of bioactive phytohormones analogues focusing on SAR (Structure Activity Relationship) studies and design of active derivatives. Her research interest is now focused on chemo-enzymatic cascade reactions in non-conventional solvents.

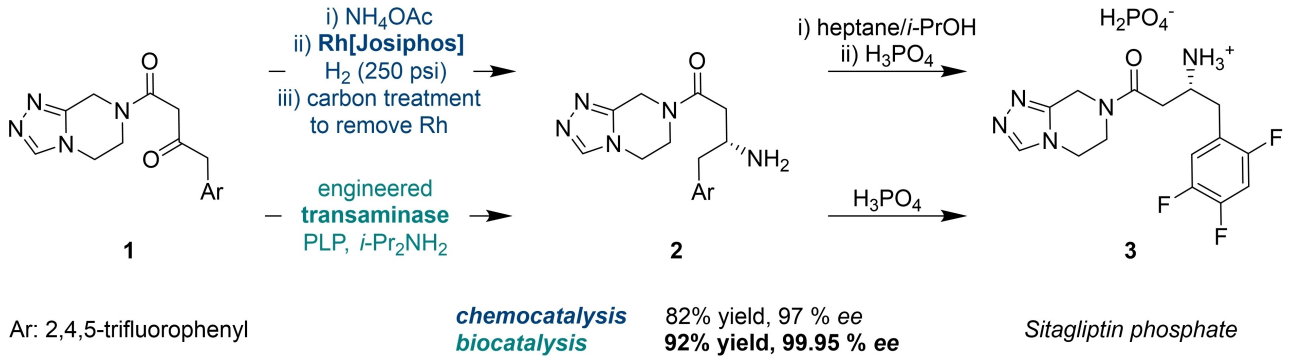
Stefano Parisotto is assistant professor at the University of Torino, where he obtained his PhD in Chemical and Material Sciences in 2019. He completed postdoctoral research at Stockholm University under Prof. Abraham Mendoza, focusing on C-H functionalization of alkanes with redox-active carbenes, and at the University of Torino with Prof. Annamaria Deagostino, specializing in the synthesis of boronated compounds for medical applications. In 2023, he started his academic career in the group of Prof. Cristina Prandi, where his current research centers on developing novel photo-enzymatic reactions for stereoselective C-N and C-O bond formation.

Marco Blangetti received his PhD in Organic Chemistry (2010) from the University of Turin (IT). He held a postdoctoral position at the University College Dublin (IE) with Prof. Donal F. O'Shea (2011–2013) and he worked as postdoc in medicinal chemistry at the University of Turin (2013–2017). He has been appointed Research Associate (2017), Assistant Professor (2020), and from 2023 is Associate Professor in Organic Chemistry at the University of Turin (IT). His current research interests focus on the advancement of novel synthetic strategies involving polar organometallic reagents under non-conventional bench-type aerobic conditions and on (bio)catalysed cascade processes.



Federica De Nardi graduated in 2021 from the University of Turin with a MSc in Chemistry. She is currently a third-year PhD student in Chemical and Material Sciences under the supervision of Prof. Cristina Prandi. Her doctoral studies focus on developing sustainable methodologies, exploring the use of green solvents and enzymatic catalysis in chemical transformations.

Stefania Cananà graduated from the University of Turin with a master's degree in industrial biotechnologies in 2021. She is currently conducting her PhD studies at the University of Turin under the supervision of Prof. Cristina Prandi. Her research activity focuses on biocatalytic transformations in unconventional green solvents.



Scheme 1. Chemo- and biocatalytic preparation of Sitagliptin phosphate from ketoamide **1**.

work has opened new avenues to produce useful molecules through enzyme engineering, and to perform specific chemical reactions with a higher degree of efficiency. However, concerns about the sustainability of organic solvents have led to the exploration of other options. Indeed, there is now an incumbent need to move towards more sustainable non-conventional options, such as green and possibly biogenic, solvents, Deep Eutectic Solvents, and by the exploitation of micellar catalysis with surfactants.

Green Ether-Type Solvents

Among the highly hydrophobic ethers, 2-methyltetrahydrofuran (2-MeTHF) and cyclopentyl methyl ether (CPME) are the most employed in combination with biocatalysis.[21] In addition to their green features, they have also become available on a larger scale, thanks to their wider use, which has contributed in lowering their price and making them competitive with respect to "standard organic solvents" (Table 1).

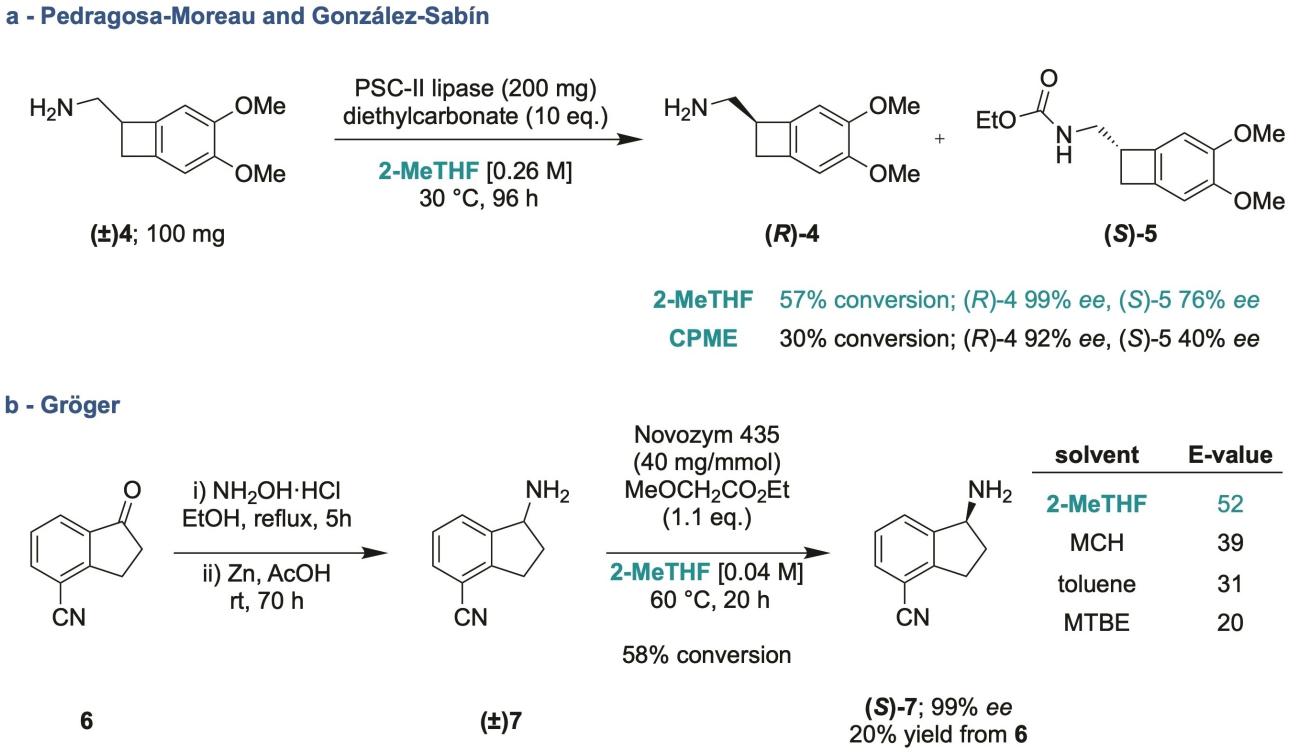
2-MeTHF is a volatile cyclic ether generated by the chemocatalytic treatment of furfural or levulinic acid derived from biomass. Compared with THF, 2-MeTHF shows a lower water miscibility, higher stability towards strong organolithium bases, and lower volatility. Also, preliminary studies have shown that 2-MeTHF displays lower toxicity, and it has been approved for use in pharmaceutical chemical processes.[22] In the last years, its use in biocatalysis as (co)solvent has significantly increased with applications for hydrolases, oxidoreductases and lyases.[23] The use of CPME as an eco-friendly solvent has also gained attention in recent years. Although the industrial synthesis of CPME remains reliant on petrochemicals, specifically utilizing cyclopentene and methanol to achieve excellent atom economy, its biogenic production from substrates such as cyclopentanol or cyclopentanone, derived from furfural or bio-based adipic acid respectively would significantly enhance the sustainability of CPME, thereby closing the loop for eco-friendly solvents. Despite this, it is considered eco-friendly thanks to its manageable boiling point (106 °C) and low solubility in water. Furthermore, it exerts low toxicity, negligible peroxide formation rate, a narrow explosion range, and remains stable under strong acidic and basic conditions.[24] Overall, these features confer much promise for its use as a solvent or cosolvent in many fields of chemistry. With respect to biocatalysis it is mainly employed with lipases. The utilization of 2-MeTHF and CPME in biocatalysis hinges upon the stability of enzymes and their formulation. Depending on these factors, they may be employed either with undiluted solvents, in conjunction with water to preserve enzymatic function, or immobilized on various solid supports. Among enzymes, lipases stand out for their remarkable stability and selective hydrolytic capabilities, even in pure organic solvents, rendering them invaluable for the stereospecific acylation of secondary alcohols and amines. Moreover, their facile immobilization on solid supports enhances their appeal, facilitating enzyme recovery and recycling for multiple catalytic cycles.

Commonly used *Candida antarctica* lipases B and A (CAL–B and CAL–A) have been extensively applied to the resolution of α-stereogenic amines. On the contrary, processes involving amines whose amino group is remote from the stereocenter, are rarely reported. In this context, Pedragosa-Moreau and González-Sabín exploited a supported lipase from *Pseudomonas cepacia* (PSC-II) for the kinetic resolution of racemic α-substituted methylamine **(±)-4** through alkoxycarbonylation with diethyl carbonate in 2-MeTHF (Scheme 2a).[25] The resultant (*S*)-carbamate **(S)-5** served as a chiral building block for the synthesis of the heart rate-lowering drug Ivabradine, yielding an overall 30% isolated yield. The crucial focus of the investigation was the search for the optimal enzyme. Furthermore, considering the substantial enzyme loading employed

Table 1. Comparison of solvent prices

Solvent	Cost (€/L)[a]
2-Methyltetrahydrofuran (2MeTHF)	60
Cyclopentyl methyl ether (CPME)	125
Tetrahydrofuran (THF)	71
Diethyl ether (Et_2O)	36
Toluene (PhMe)	40
Acetonitrile (MeCN)	58

[a] All costs tabulated for EMPLURA ® grade solvents on Sigma-Aldrich website.



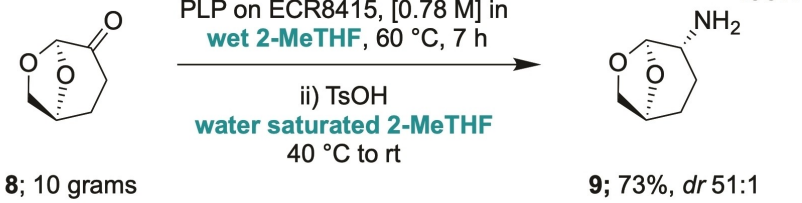
Scheme 2. Lipase-catalysed resolution of primary amines in 2-MeTHF.

(100% w/w of enzyme/substrate), efforts were made to enhance process sustainability. The authors demonstrated that the recovered enzyme could be recycled up to five times without any loss of activity, achieved through a simple filtration and extraction with methyl *tert*-butyl ether (MTBE). In their investigations, the authors also explored the use of CPME as non-conventional solvent for the kinetic resolution of **(±)-4**, albeit with slightly lower conversion and enantioselectivity. A recent patent by Gröger outlines another instance of lipase-catalysed kinetic resolution employing 2-MeTHF, aimed at synthesizing an enantiopure precursor crucial to produce Ozanimod (Zeposia®), an immunomodulatory drug recently approved for the treatment of relapsing multiple sclerosis.[26] Here, the focus of the work was the kinetic resolution of racemic 1-indenylamine **(±)-7**, obtained from indanone **6**. This process involved *N*-acylation with ethyl 2-methoxyacetate in 2-MeTHF, utilizing commercial Novozym 435 as the catalyst (Scheme 2b).

Noteworthy, the solvent deeply affects the efficiency of the kinetic resolution of amine **(±)7**. Compared to the other solvents tested (methyl *tert*-butyl ether, methylcyclohexane (MCH) and toluene), 2-MeTHF showed the highest E-value. This study is a valuable example of how the application of this specific green ether can improve the sustainability of biocatalysis not only thanks to its biogenic properties, but also by improving the efficiency of the desired transformation.

Integrating enzymes and reaction engineering methodologies offers alternative approaches to address synthetic strategies for amine synthesis. In this context, the use of immobilized enzymes represents a significant advantageous strategy for preserving the activity of more delicate enzymes. Indeed, immobilization enables the utilization of enzymes in diverse environments beyond the typical buffered aqueous solution, thanks to the protection provided by the carrier to which they are attached. This is particularly important for the class of transaminase enzymes, which are part of the pyridoxal 5′-phosphate (PLP)-dependent enzyme family. These enzymes catalyse the transfer of an amino group from a donor amine to a pro-chiral ketone, yielding the chiral amine and a carbonyl compound as the sole by-product. An exemplary illustration of this concept is the research conducted by Soto, Forstater, and their colleagues at Merck.[27] They utilized an immobilized amino transferase for the amination of the dihydrolevoglucosenone (Cyrene™) **8**, an intermediate in the synthesis of Nemtabrutinibis, an inhibitor of Bruton's tyrosine kinase (BTK). Through protein engineering and enzyme evolution, they identified the optimal enzyme not only in terms of activity and selectivity, but also for thermostability and compatibility with organic solvents. The authors employed their ATA-492 immobilized on the amino resin ECR8415 in a SpinChem reactor using various organic solvent/water systems. Water-saturated 2-MeTHF (water saturation prevents enzyme deactivation as observed by Truppo *et al.*),[28] was found to be the optimal solvent, yielding the desired product in 95% (Scheme 3). Among the tested solvents, ethyl acetate gave excellent yields as well, but with lower diastereoselectivity (93.5%, *dr* 14.6:1), on the contrary, 2-propanol induced a good stereoselectivity, but with a modest yield (48.6%, *dr* 18.0:1). 2-MeTHF was capable of providing for

Soto, Forstater and Merck



8; 10 grams

9; 73%, *dr* 51:1

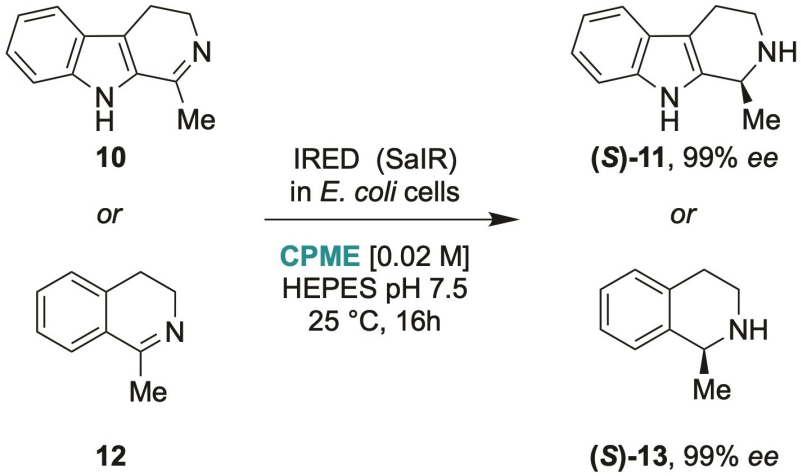
solvent	yield (%)	*dr* (x:1)
2-MeTHF	94.6	17.5
IPAc	84.3	5.2
IPA	48.6	18.0
MTBE	82.9	9.3
toluene	74.5	14.5
EtOAc	93.5	14.6
DMSO	0.4	-

Scheme 3. Biocatalytic preparation of amine **9** from dihydrolevoglucosenone.

both high yield and diasteroselectivity (94.6%, *dr* 17.5:1). The process was easily performed at a 10 grams scale with a minor drop in the yield (73%) and the diastereomeric ratio could be further improved from 22:1 to 51:1 by crystallization affording >99% pure *para*-toluenesulfonate salt **9**.

Another non-conventional approach to the enzymatic synthesis of chiral amines involves the utilization of whole-cell catalysis, particularly with NADPH-dependent imine reductases (IREDs). For instance, lyophilized *E. coli* cells overexpressing IREDs were employed in a micro-aqueous environment, defined as a system with a single liquid phase where water content is minimized, and saturation levels are reached in the presence of a hydrophobic organic solvent.[29] Water is added to maintain enzymatic activity, mobility, and solubility of additional cofactors. Due to the low water content, whole-cell catalysis is commonly employed in micro-aqueous systems, benefiting from the co-existence of enzymes and cofactor recycling systems inside the cells, ensuring their optimal environment. In 2016, Rother and Maugeri investigated the use of whole-cell catalysis for the bioreduction of imines in micro-aqueous systems.[30] The authors utilized IREDs from various organisms in the form of lyophilized whole cells, also containing glucose dehydrogenases (GDH) necessary for cofactor recycling, in the reduction of β-carboline **10** and isoquinoline **12**. They assessed selected IREDs with a panel of five organic solvents with differing properties, using only a 10% of HEPES buffer to solubilize the lyophilized enzymes. Low conversions were anticipated due to the denaturing effects of organic solvents on the cell membrane and enzymes, yet methyl isobutyl ketone (MIBK) and CPME yielded promising results, despite the modest conversions (Scheme 4). A comprehensive investigation of the solvent system revealed that in the presence of MIBK, cell clumping occurred with increasing quantities of HEPES buffer (5–15% v/v), whereas with CPME this phenomenon was absent, ensuring good cell distribution. After finding the best solvent, the authors tested several IREDs in CPME with 10% v/v HEPES buffer at a 20 mM loading of substrates and reported moderate to high conversions with both (*R*)-selective and (*S*)-selective enzymes, yielding products **(*S*)-11** and **(*S*)-13** with excellent selectivity.

Maugeri, Rother



10

or

12

(*S*)-11, 99% *ee*

or

(*S*)-13, 99% *ee*

preliminary solvent screening

solvent	conversion (%)
CH_2Cl_2	-
2-MeTHF	-
EtOAc	-
MIBK	34
CPME	25

Scheme 4. Whole-cell catalysis with IREDs in micro-aqueous reaction system.

Deep Eutectic Solvents

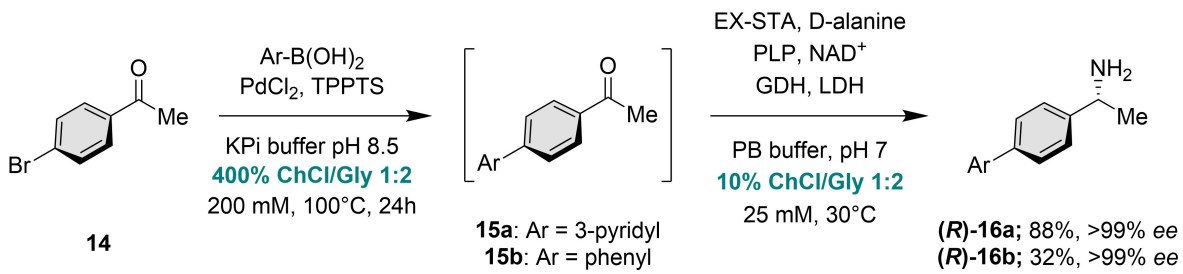
Deep eutectic solvents (DES) have emerged as promising alternatives to conventional organic media in various chemical processes due to their low toxicity, biodegradability, and low cost. These solvents are typically composed of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD), which form a eutectic mixture with a melting point lower than that of the individual components. In addition, natural deep eutectic solvents (NADES) are a subset of DES derived from naturally occurring compounds such as sugars, amino acids, and organic acids. NADES offer additional advantages, including sustainability and eco-friendliness, as they are sourced from renewable materials. Both DES and NADES exhibit unique solvent properties and can be tailored for specific applications by adjusting the composition of the mixture.[31] Their tunability, combined with their green credentials, makes them attractive candidates for various biocatalytic reactions, including enzyme-catalysed amine synthesis.[32] Moreover, the similarity of (NA)DES components to cytoplasmic constituents is intriguing. This medium holds the potential to stabilize enzyme structures by mimicking a more natural environment, thereby influencing enzyme activity.

In a recent publication by González-Sabín, Gröger and co-workers, various transaminases were successfully employed in a chemoenzymatic process to access biaryl-substituted amines (Scheme 5).[33] Following their previous integration of palladium catalysis with ketoreductases in Deep Eutectic Solvents, where the unique properties of such neoteric mixtures enabled to reach high substrate concentration for the overall process,[34] in this work the activity of different transaminases was compared in various DES. Mostly, they investigated the engineered (*R*)-selective amine transaminase from *Exophiala xenobiotica* (EX-ωTA), which naturally converts biaryl ketones to the corresponding amines with high conversion and excellent enantioselectivity.[35]

Accordingly, the biocatalytic transamination of ketone **15a** was investigated as a model reaction with the variant EX-STA (amino acid exchange T273S) to evaluate the enzymatic activity in such neoteric solvents. Alanine was used as amino donor, combined with the LDH/GDH (lactate dehydrogenase/glucose dehydrogenase) recycling system, and supplemented with choline chloride (ChCl)/glycerol (Gly) (1:2) deep eutectic system. Ketone amination remained efficient with almost quantitative conversions up to 15% of DES. An increase to 25% DES decreased the enzymatic performance, at 50% the activity of the ATA was completely suppressed. EX-STA exhibited perfect enantioselectivity toward (***R***)-**16a** regardless of the solvent's ratio, producing the resulting amine in >99% *ee* in all cases. Once having assessed the potential inhibitory effects of the reagents from the Suzuki coupling step, they identified a compatibility window for the one-pot chemoenzymatic cascade performed in a sequential mode. With these premises, the coupling of equimolar amounts of 4′-bromoacetophenone **14** and phenylboronic acid was efficiently conducted at 100°C in DES-water 4:1 (200 mM). Upon completion, the reaction mixture containing 4′-phenylacetophenone (**15b**) was diluted to 75 mM with the buffer for the bioamination and supplemented with EX-STA, LDH, GDH, glucose, related cofactors, and the amino donor. After incubation, the benzylic amine (**16b**) was produced with a conversion of 15%. The authors attributed such low conversion to the high DES fraction. As a matter of fact, the dilution of the DES-water 4:1 mixture from 200 mM to 75 mM resulted in a medium still containing ~30% DES, but further dilution to 25 mM, resulting in a final 10% of DES, led to an optimal 45% conversion. Despite the scope of the tandem protocol being limited, the paper reports a remarkable enzymatic activity of transaminases in non-conventional media and is an excellent proof of concept of the practical value of biorenewable solvents for biocatalysis.

Deep Eutectic Solvent-buffer mixtures were also successfully used by Prandi and co-workers as sustainable reaction media to produce a panel of chiral 2-aryl substituted cyclic *N*-heterocycles **18** from imines **17**.[36] The reduction of the model substrate 5-phenyl-3,4-dihydro-2*H*-pyrrole **17a** with commercial IREDs and a GDH-based cofactor recycling system was efficiently performed in 50% v/v DES-buffer mixture (DES=Choline chloride (ChCl)/Glycerol (Gly) 1:2 v/v) with substrate loadings up to 100 mM. Under these conditions (*S*)-2-phenylpyrroline **18a** was produced with >99% *ee* in 62% yield. Further increasing of substrate loading to 200 mM remained compatible with the biocatalyst, but a slight decrease in the yield was observed due to enzyme inhibition. In comparison, no conversion was detected at the same substrate concentration in pure phosphate buffer, highlighting the benefit of using DESs-buffer mixtures. Additionally, the authors investigated the role of the single DES components and concluded that an equimolar mixture of phosphate buffer and glycerol was found to be as effective as the DES, without the need for choline chloride. The applicability of the methodology was tested on 5-, 6-, and 7-

Gröger, González-Sabín



Scheme 5. One-pot synthesis of biaryl-substituted amines by combination of Suzuki coupling with transaminases in DES.

membered cyclic imines **17**, isolating the corresponding chiral amines **18** in moderate to good yields with excellent stereoselectivity toward the *S* enantiomer (Scheme 6).

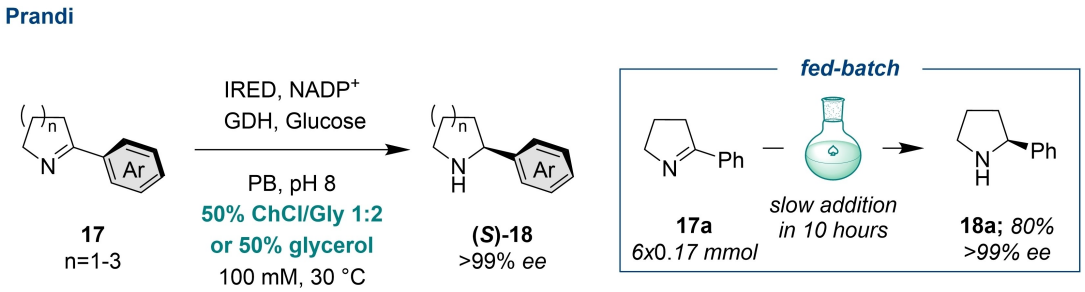
As mentioned before, the use of organic co-solvents or non-conventional reaction media can solve the issue associated with the low solubility in water of the organic compounds, but working at high concentrations can be detrimental, due to an overload of the substrate which can lead to enzyme inhibition. To avoid this problem, fed-batch processes can be helpful. The gradual addition of a solution of the substrate into the reactor over time, results in a continuously increasing reaction volume, thus tackling the solubility challenges mentioned earlier but also ensuring that substrate concentration is maintained below inhibitory levels, thereby boosting enzyme Turnover Number (TON). In this sense, the authors devised a fed-batch protocol, slowly feeding the substrate to a single load of the enzymatic cocktail (IRED-44, GDH, cofactors and glucose) in a 50% v/v PB/Gly solution. This approach kept the substrate concentration within the optimal range for enzyme catalytic activity, allowing for a seven-fold scale-up without requiring catalyst recovery or product removal between formal reaction cycles. As a result, the fed-batch strategy achieved the conversion of 1 millimol of substrate **17a** in 80% yield and >99% *ee*.

Micellar Solutions

The addition of surfactants to aqueous solution is a key modification of the reaction media to ensure low levels of enzyme inhibition and facilitating tandem chemo-enzymatic cascades. Indeed, compartmentalization formed by a micellar system creates a natural hydrophobic environment for organic molecules, enabling the compatibility of two systems that otherwise would not be used in the same medium. This allows the more efficient conversion of poorly water-soluble substrates. In 2022 Lipshutz and Dussart-Gautheret presented the first study in which an aqueous micellar media was successfully employed with transaminases in a chemoenzymatic transformation.[37] In this pioneering example several ketones **20** (aromatic, cyclic, aliphatic) were subjected to biocatalytic transamination exploiting the non-ionic surfactant TPGS-750-M **19**, producing the corresponding primary amine **21** in high yields and excellent enantioselectivity (Scheme 7).

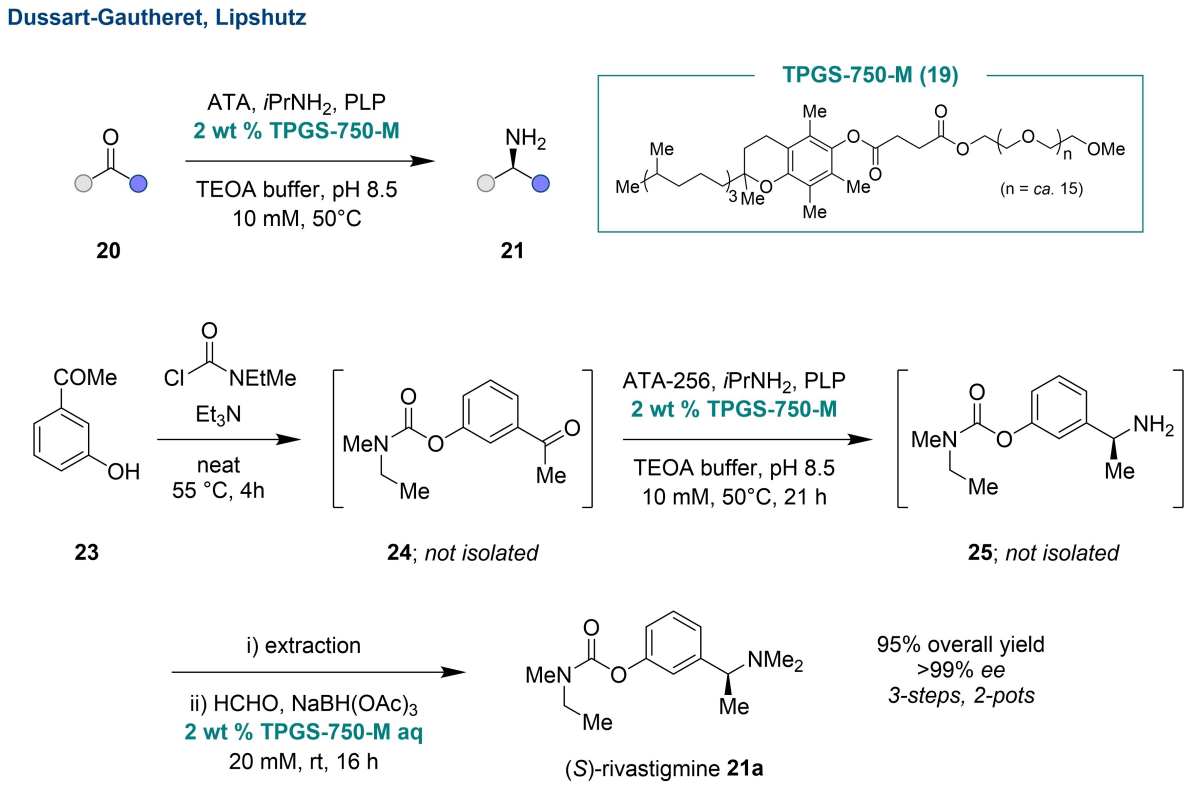
Noteworthy, a mixed sequence of chemo- and biocatalytic reactions involving ATAs resulted in the first synthesis of the cholinesterase inhibitor (*S*)-rivastigmine **21a**, a widely employed drug for the treatment of dementia. Not only this approach is the most efficient process to date, but it was performed in the complete absence of waste-generating organic solvents as reaction media. Exploiting the same surfactant in more steps, the final product was isolated in >99% *ee* and 95% yield, starting from ketone **23**, without any need to isolate or purify intermediates **24** and **25**. This suggests a promising avenue for further advancements in biocatalysis in aqueous micellar system, with potential implications for diverse applications in pharmaceuticals, fine chemicals, and beyond. In this sense, in 2023 Lipshutz used non-ionic surfactants to improve the conversion of various imines using IREDs.[38] The selected commercial IREDs showed increased conversion up to 40% in the presence of 2 wt% TPGS-750-M surfactant depending on the substrates. This increase in the enzymatic activity was clearly evident in the formal reductive amination of cyclohexanone **26a** and 4-acetylanisole **26b** with benzylamine **27a** and cyclopropylamine **27b** respectively. These findings were applied to a diverse library of ketones **26** and amines **27**, employing either *in situ* formed or isolated imines **28**. This resulted in the synthesis of secondary amines **29** with yields ranging from 60 to 99% and excellent enantioselectivity in some cases (Scheme 8).

Again, to highlight how the incorporating a surfactant into aqueous enzymatic reactions offers the advantage of facilitating tandem chemo-enzymatic cascades, the authors demonstrated the feasibility of telescoping reductive amination with palladium-

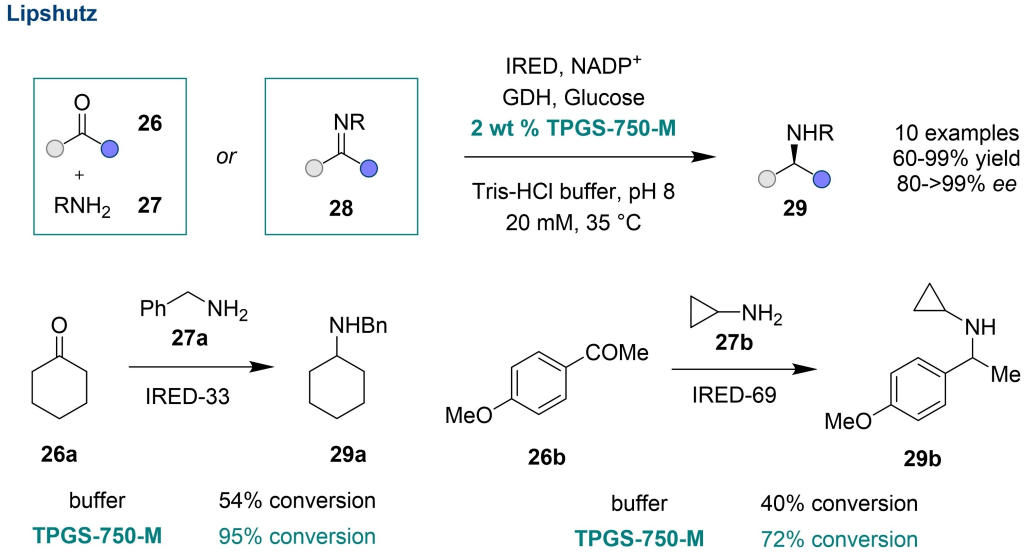


solvent	yield (%) 5 mM	25 mM	50 mM	100 mM	200 mM
PB	68	28	4	-	-
PB/(ChCl/Gly 1:2) 50/50	66	50	55	62	49
PB/Gly 50/50	92	68	73	68	45

Scheme 6. Asymmetric imine reduction with IREDs in Deep Eutectic Solvent.



Scheme 7. Amine synthesis *via* micellar catalysis using ATAs and multistep chemo-enzymatic route to rivastigmine.



Scheme 8. amine synthesis via micellar catalysis using IREDs.

catalysed arylation, vinylation, or alkynylation to access structurally relevant amines to demonstrate easy the incorporation of chemo- and enzymatic approaches toward high valuable products.

Conclusions

In conclusion, the field of the biocatalytic synthesis of chiral amines presents promising avenues for addressing the increasing demand for these compounds in various applications, particularly in pharmaceuticals. The limitations and challenges associated with conventional synthetic methods, including the use of toxic reagents and sensitive transition-metal complexes, underscore the

importance of exploring sustainable alternatives. Biocatalysis, with its inherent stereocontrol and sustainability features, emerges as a viable solution. The ongoing efforts to improve enzyme stability, activity, and general applicability align with the broader goal of achieving sustainability in biocatalytic processes and result in tremendous advances mostly in protein engineering. Still, as research continues to push boundaries and overcome existing limitations, an approach within everyone's reach like medium engineering, not limited by the need for specific expertise and equipments for enzyme engineering, can be a valuable solution for fulfilling the potential of biocatalysis for large-scale production of chiral amines as well as for the laboratory scale preparations, thus making it fully comparable with the more affordable transition metal catalysis or organocatalysis. Presently the utilization of neoteric solvents in biocatalysis shows encouraging results, though it remains in its early stages of exploration. There are various pertinent considerations surrounding their application that necessitate comprehensive examination. Among these, understanding their influence on enzyme activity and evaluating their recyclability are paramount. As research advances, we anticipate significant strides and refinements in this domain, potentially leading to enhanced efficacy and sustainability in biocatalytic applications. In addition, it is important to emphasize that the use of non-conventional media can further hamper the environmental impact of biocatalysis, by waste reduction, for examples by conducting processes at higher concentration or by combining consecutive steps in chemo- and photo-enzymatic cascades not easily performed in pure aqueous media. Indeed, given the crucial role of chiral amines in the pharmaceutical industry, adopting sustainable approaches in all phases of the synthesis process, including the choice of the reaction medium, is essential. Ultimately, we believe that through an integrated approach involving both the selection of effective biocatalysts and the use of non-conventional solvents it will be possible to achieve a breakthrough towards eco-friendlier and efficient large-scale syntheses.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: biocatalysis · deep eutectic solvent · enzymes · non-conventional media · chiral amines

[1] A. Cabré, X. Verdaguer, A. Riera, *Chem. Rev.* **2021**, *122*, 269–339.
[2] Y. Tian, L. a. Hu, Y.-Z. Wang, X. Zhang, Q. Yin, *Org. Chem. Front.* **2021**, *8*, 2328–2342.
[3] I. Agranat, H. Caner, J. Caldwell, *Nat. Rev. Drug Discov.* **2002**, *1*, 753–768.
[4] P. A. Romero, F. H. Arnold, *Nat. Rev. Mol. Cell Bio.* **2009**, *10*, 866–876.
[5] A. Sharma, G. Gupta, T. Ahmad, S. Mansoor, B. Kaur, *Food Rev. Int.* **2021**, *37*, 121–154.
[6] D. Ghislieri, N. J. Turner, *Top. Catal.* **2014**, *57*, 284–300.
[7] H. Ismail, R. M. Lau, F. van Rantwijk, R. A. Sheldon, *Adv. Synt. Catal.* **2008**, *350*, 1511–1516.
[8] J. Liu, W. Kong, J. Bai, Y. Li, L. Dong, L. Zhou, Y. Liu, J. Gao, R. T. B. Allen, N. J. Turner, *Chem Catal.* **2022**, *2*, 1288–1314.
[9] M. D. Patil, G. Grogan, A. Bommarius, H. Yun, *ACS Catal.* **2018**, *8*, 10985–11015.
[10] A. Gomm, E. O'Reilly, *Curr. Opin. Chem. Biol.* **2018**, *43*, 106–112.
[11] G. Grogan, N. J. Turner, *Chem. Eur. J.* **2016**, *22*, 1900–1907.
[12] a) D. M. Kendall, R. M. Cuddihy, R. M. Bergenstal, *Am. J. Med. Stud.* **2009**, *122*, S37–S50; b) D. Williams-Herman, S. S. Engel, E. Round, J. Johnson, G. T. Golm, H. Guo, B. J. Musser, M. J. Davies, K. D. Kaufman, B. J. Goldstein, *BMC Endocrine Disorders* **2010**, *10*, 1–21.
[13] K. B. Hansen, Y. Hsiao, F. Xu, N. Rivera, A. Clausen, M. Kubryk, S. Krska, T. Rosner, B. Simmons, J. Balsells, N. Ikemoto, Y. Sun, F. Spindler, C. Malan, E. J. J. Grabowski, J. D. Armstrong, *J. Am. Chem. Soc.* **2009**, *131*, 8798–8804.
[14] C. K. Savile, J. M. Janey, E. C. Mundorff, J. C. Moore, S. Tam, W. R. Jarvis, J. C. Colbeck, A. Krebber, F. J. Fleitz, J. Brands, *Science* **2010**, *329*, 305–309.
[15] R. A. Sheldon, J. M. Woodley, *Chem. Rev.* **2018**, *118*, 801–838.
[16] N. Zhang, P. Domínguez de María, S. Kara, *Catalysts* **2024**, *14*, 84.
[17] A. Zaks, A. M. Klibanov, *J. Biol. Chem.* **1988**, *263*, 3194–3201.
[18] R. Madeira Lau, F. Van Rantwijk, K. Seddon, R. Sheldon, *Org. Lett.* **2000**, *2*, 4189–4191.
[19] S. V. Kamat, E. J. Beckman, A. J. Russell, *Crit. Rev. Biotechnol.* **1995**, *15*, 41–71.
[20] a) T. Seng Wong, F. H. Arnold, U. Schwaneberg, *Biotechnol. Bioeng.* **2004**, *85*, 351–358; b) K. Chen, F. H. Arnold, *PNAS* **1993**, *90*, 5618–5622
[21] M. Miele, L. Ielo, V. Pace, A. R. Alcántara, *in Greener Synthesis of Organic Compounds*, CRC Press, **2022**, 89–117.
[22] V. Antonucci, J. Coleman, J. B. Ferry, N. Johnson, M. Mathe, J. P. Scott, J. Xu, *Org. Process Res. Dev.* **2011**, *15*(4), 939–941.
[23] A. R. Alcantara, P. Dominguez de María, *Curr. Green Chem.* **2018**, *5*, 86–103.
[24] G. de Gonzalo, A. R. Alcántara, P. Domínguez de María, *ChemSusChem* **2019**, *12*, 2083–2097.
[25] S. Pedragosa-Moreau, A. Le Flohic, V. Thienpondt, F. Lefoulon, A.-M. Petit, N. Ríos-Lombardía, F. Morís, J. González-Sabín, *Adv. Synt. Catal.* **2017**, *359*, 485–493.
[26] a) Löwe, J., Uthoff, F., Lepp, C., Gröger, H., Donsbach, K., WO2019197571A1 **2019**; b) F. Uthoff, J. Löwe, C. Harms, K. Donsbach, H. Gröger, *J. Org. Chem.* **2019**, *84*, 4856–4866.
[27] C. K. Prier, K. Camacho Soto, J. H. Forstater, N. Kuhl, J. T. Kuethe, W. L. Cheung-Lee, M. J. Di Maso, C. M. Eberle, S. T. Grosser, H.-I. Ho, E. Hoyt, A. Maguire, K. M. Maloney, A. Makarewicz, J. P. McMullen, J. C. Moore, G. S. Murphy, K. Narsimhan, W. Pan, N. R. Rivera, A. Saha-Shah, D. A. Thaisrivongs, D. Verma, A. Wyatt, D. Zewge, *ACS Catal.* **2023**, *13*, 7707–7714.
[28] M. D. Truppo, H. Strotman, G. Hughes, *ChemCatChem* **2012**, *4*, 1071–1074.
[29] M. M. van Schie, J.-D. Spöring, M. Bocola, P. D. de María, D. Rother, *Green Chem.* **2021**, *23*, 3191–3206.
[30] Z. Maugeri, D. Rother, *Adv. Synth. Catal.* **2016**, *358*, 2745–2750.
[31] A. Mannu, M. Blangetti, S. Baldino, C. Prandi, *Materials* **2021**, *14*, 2494.
[32] D. Arnodo, E. Maffeis, F. Marra, S. Nejrotti, C. Prandi, *Molecules* **2023**, *28*, 516.
[33] J. Paris, A. Telzerow, N. Ríos-Lombardía, K. Steiner, H. Schwab, F. Morís, H. Gröger, J. González-Sabín, *ACS Sustain. Chem. Eng.* **2019**, *7*, 5486–5493.
[34] J. Paris, N. Ríos-Lombardía, F. Morís, H. Gröger, J. González-Sabín, *ChemCatChem* **2018**, *10*, 4417–4423.

[35] A. Telzerow, J. Paris, M. Håkansson, J. González-Sabín, N. Ríos-Lombardía, M. Schurmann, H. Gröger, F. Morís, R. Kourist, H. Schwab, *ACS Catal.* **2018**, *9*, 1140–1148.
[36] D. Arnodo, F. De Nardi, S. Parisotto, E. De Nardo, S. Cananà, F. Salvatico, E. De Marchi, D. Scarpi, M. Blangetti, E. G. Occhiato, C. Prandi, *ChemSusChem* **2024**, e202301243.
[37] J. Dussart-Gautheret, J. Yu, K. Ganesh, G. Rajendra, F. Gallou, B. H. Lipshutz, *Green Chem.* **2022**, *24*, 6172–6178.
[38] X. Li, Y. Hu, J. D. Bailey, B. H. Lipshutz, *Org. Lett.* **2024**, *26*, 2778–2783.

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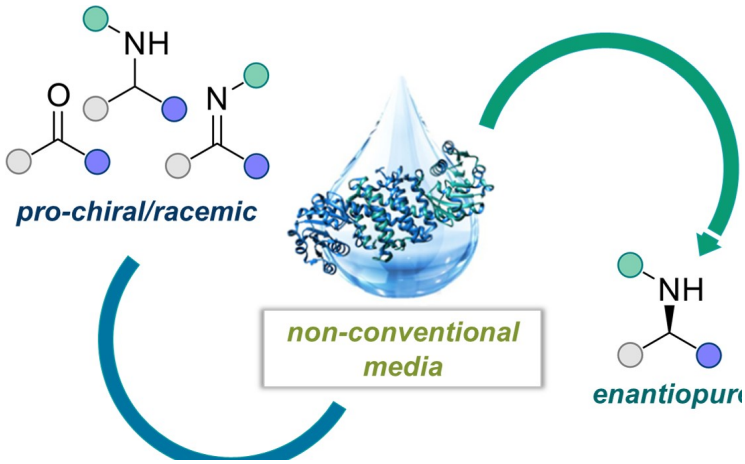

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Replacing traditional aqueous reaction media with non-conventional solvents shows great potential for enhancing the sustainability of enzyme catalysis and addressing issues in large-scale production applications. This review highlights significant advancements in using non-conventional media, such as organic green solvents and Deep Eutectic Solvents, to produce chiral amines with high yields and enantiomeric excesses, as well as to enable chemoenzymatic cascades.