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Asymmetric Reduction of Cyclic Imines by Imine Reductase Enzymes in Non-Conventional Solvents

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The first enantioselective reduction of 2-substituted cyclic imines to the corresponding amines (pyrrolidines, piperidines, and azepines) by imine reductases (IREDs) in non-conventional solvents is reported. The best results were obtained in a glycerol/phosphate buffer 1:1 mixture, in which heterocyclic amines were produced with full conversions (>99%), moderate to good yields (22–84%) and excellent *S*-enantioselectivities (up to >99% *ee*). Remarkably, the process can be performed at a 100 mM substrate loading, which, for the model compound, means a concentration of 14.5 g L^{-1}. A fed-batch protocol was also developed for a convenient scale-up transformation, and one millimole of substrate **1a** was readily converted into 120 mg of enantiopure amine (*S*)-**2a** with a remarkable 80% overall yield. This aspect strongly contributes to making the process potentially attractive for large-scale applications in terms of economic and environmental sustainability for a good number of substrates used to produce enantiopure cyclic amines of high pharmaceutical interest.

Introduction

Biocatalysis is nowadays considered as a green and sustainable technology for transformation processes. Although water and phosphate buffers have been used as solvents for biocatalyzed reactions for years, limitations arising from the low solubility of organic compounds in such media significantly restrict the applications of biocatalysts in organic synthesis. Moreover, the low substrate (and product) concentrations in biocatalysis largely consume the benefits of water as unproblematic solvent. Increasing reagent concentration, therefore, represents a major current challenge in biocatalysis, enabling it to fulfil its green promise and become an attractive alternative in preparative scale organic synthesis procedures.[1] An ideal solvent for biotransformation should be non-toxic, biocompatible, biodegradable and sustainable while supporting high enzyme activity and stability. Enzymes have also been demonstrated to be active and stable in non-aqueous media.[2] Among hydrophilic solvents, glycerol can mimic the effect of water and is reported to preserve enzyme structure.[3] However, recent reports indicate that the stabilizing effect is not universally applicable to all class of enzymes.[4] Various methodologies reporting the use of non-conventional media for biocatalyzed processes have been extensively reviewed.[5] Deep Eutectic Solvents (DESs) are eutectic mixtures commonly obtained by mixing, heating and stirring a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) in specific molar ratios. The term "Deep Eutectic Solvents" (DESs) was firstly introduced in 2003 by Abbot and co-workers to describe the behavior of eutectic mixtures formed by quaternary ammonium salts and urea.[6] DESs find application in organometallic chemistry[7] and in transformations catalyzed by bio-,[8] metal-[9] and organocatalysts.[10] In the field of biocatalysis, the first report about the use of DESs in the presence of an enzyme was by Kazlauskas and co-workers in 2008.[11] Since then, several protocols have been developed for biotransformations catalysed by both isolated enzymes and whole cells in DESs and DES-buffer mixtures.[5b,7,12] Many enzymes including lipases,[8a,13] proteases,[8c] epoxide hydrolases,[14] lyases,[8d] and oxidoreductases[5c,12b,15] have already been used in DESs. However, to the best of our knowledge, the use of DESs in bioreduction processes with isolated enzymes has been limited to ketoreductases (KREDs)[8f] and alcohol dehydrogenases (ADHs).[8j] Drawing on our interest in both biocatalysis[16] and the use of non-conventional solvents,[5a,7a,9a,16a,h,17] we decided to investigate the efficiency of an emergent class of NADPH-dependent enzymes, namely imine reductases (IREDs), in the reduction of cyclic imines using non-conventional solvents.[18] This family of oxidoreductases was discovered in 2010 by Mitsukura and co-workers[19] and has since gained increasing attention because it offers a promising biocatalytic approach to obtaining primary, secondary and

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tertiary chiral amines, which are key intermediates in the synthesis of several biological active compounds (Figure 1).[20] Despite asymmetric reduction of imines represents a significant challenge for enzymatic reductions, due to the aqueous lability of C=N bond, IREDs have shown great potential for the biocatalytic asymmetric reduction of cyclic and linear imines as well as reductive amination reactions.[21] In this work, we report the development of the optimal conditions for using this class of enzymes in non-conventional solvents at elevate substrate concentration of up to 100 mM, particularly when employing cyclic imines as substrates. We are delighted to present the first successful asymmetric bioreduction of such imines by commercially available imine reductases (IREDs) in non-conventional media.

Results and Discussion

Optimization of the reaction conditions

We initiated our investigation by selecting 5-phenyl-3,4-dihydro-2*H*-pyrrole **1 a** (Figure 2) as the model imine substrate. This compound is increasingly utilized as key pro-chiral intermediate

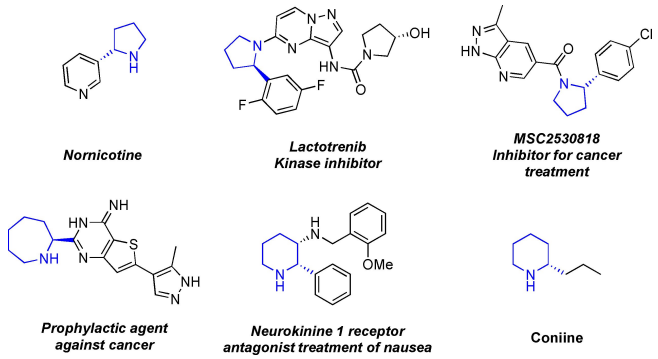


Figure 1. Natural and synthetic compounds showing chiral pyrrolidine, piperidine and azepine as structural cores.

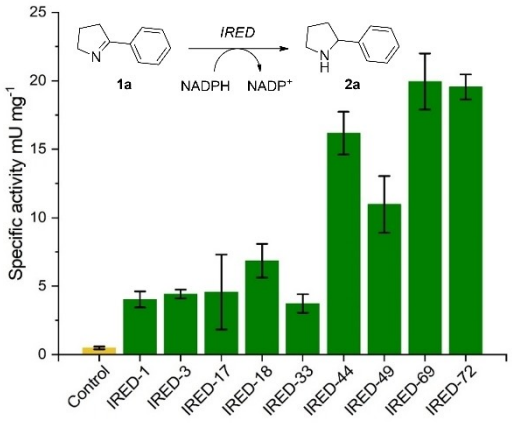


Figure 2. IREDs specific activity (mU mg^{-1}) with substrate **1 a**. Reaction conditions: 30 °C, substrate **1 a** (0.5 mM) and NADPH (0.1 mM) in PB (100 mM, pH 8). "Control" performed without enzyme (see Supporting Information).

in the syntheses of potential drugs,[22] such as selective KV1.5 blocker BMS394136[23] and *k*-opioid receptor antagonist LY2456302.[24] Consequently, efforts have already been directed toward developing procedures employing IREDs for imine reduction.[25] Turner and co-workers have extensively reported on the asymmetric reduction of cyclic imines using IREDs,[18a,25c,26] including a chemoenzymatic alkylation of tetrahydroquinolines.[27] Following the procedure established by Turner and co-workers,[25b] we initially conducted a screening of the commercial IRED collection[28] for the reduction of imine **1 a** in potassium phosphate buffer. The IRED-catalyzed reduction involves the combined use of glucose dehydrogenase from *Bacillus subtilis* (GDH) and D-glucose as a cofactor recycling system. This is necessary because NADPH is consumed, resulting in the formation of $NADP^+$ during the IRED-catalyzed step. Subsequent GDH-catalyzed regeneration of NADPH leads to the formation of D-gluconolactone. To identify the most suitable enzymes to be subsequently used in optimizing the reaction conditions, we initially evaluated the activity of the commercial IRED collection in terms of NADPH consumption during the reduction of the model substrate **1 a** in phosphate buffer (PB) (Figure 2, see Supporting Information (SI) for details).

As shown in Figure 2, enzymes IRED-44, -69 and -72 catalyzed the reduction of imine **1 a** to corresponding amine (*S*)-**2 a**[25c] with significant activity. Among these, IRED-44 gave the highest *ee* (> 99%). Consequently, these enzymes were selected for further investigation to explore the feasibility of using non-conventional media as solvents in the IRED-catalysed bioreduction of prochiral imines. We started with a eutectic mixture (DES) composed of choline chloride (ChCl) and glycerol (Gly), in a 1:2 stoichiometric ratio, along with IRED-44 as the enzyme. Notably, ChCl/Gly 1:2 mixture possesses lower viscosity compared to other DESs, allowing to work at lower temperatures and with moderate stirring. This minimizes the risk of enzyme inactivation or degradation under harsher conditions.[29] Initially, imine **1 a** was incubated with IRED-44 in pure DES ChCl/Gly 1:2 at 30 °C, both at 5 mM and 100 mM concentration. In both cases, we were unable to recover the reduced amine **2 a** (see SI for details). Therefore, we decided to use a DES percentage of 50% (v/v in PB), which also corresponds to the optimal relative amount of DESs used for KRED-catalysed reduction in DES.[8e,j]

The first parameter we evaluated in our investigation was the impact of substrate concentration on the transformation. As reported in Figure 3, we studied the behavior of IRED-44 both in PB (blue line) and in PB/DES (red line) at various substrate concentrations. At 5 mM IRED-44 produced the reduced amine **2 a** in good yield in both solvents. However, the behavior diverges as the concentration of **1 a** increases. At 100 mM in PB the reaction was ineffective, and we only recovered starting material **1 a**. Conversely, at the same concentration in PB/DES the yield of **2 a** was 62% and with >99% *ee*. These studies revealed that the optimal conditions for performing IRED-catalyzed bioreductions of prochiral imines in a ChCl/Gly 1:2 DES 50%+PB 50% (v/v) mixture involve working at a final imine **1 a** concentration of 100 mM. However, even at 150 and 200 mM, the obtained yields of 49% and 51% respectively, may

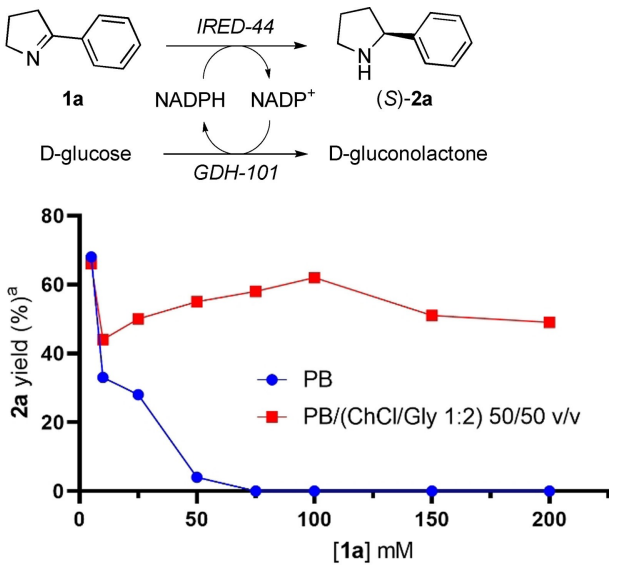


Figure 3. Initial study for the asymmetric reduction of **1 a** in PB (blue line) and in PB/(ChCl/Gly, red line) at selected concentrations. Reaction conditions: D-glucose (100 mg), $NADP^+$ (6.4 mg), GDH-101 (6.0 mg), IRED-44 (12.0 mg), **1 a** (0.14 mmol, 20.3 mg) added in DMF (30 μL), stirred (700 rpm) at 30 °C for 16 h in the selected solvent system [phosphate buffer (PB): potassium phosphate buffer (100 mM, pH 8)]. Yields of [**2 a**] were determined by quantitative ^{1}H NMR analysis. %*ee* values (> 99%) were determined by Chiral-HPLC. Absolute configurations were determined by α_D values measured at 25 °C (see Supporting Information).

be of interest in a scaled-up process. When comparing these results to those obtained in pure PB, the most significant outcome consists in the possibility of working at higher concentrations, with consequent reduction of solvent volumes. Encouraged by these results, we continued our study by optimizing the IRED-catalyzed bioreduction using IRED-44, with the goal of further improving the results by fine-tuning the reaction conditions (Table 1).

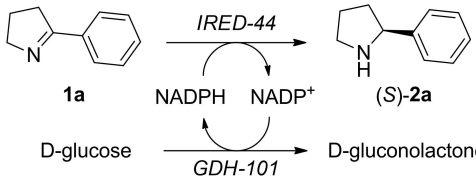
Reference reaction conditions obtained by initial screening in PB/DES (Figure 3) are presented in Table 1, entry 1. In the optimization study, we assessed several parameters as *i.* the behaviour of various DESs (entries 2–8) in combination with PB; *ii.* the role of the solvent components (entries 9–12), *iii.* the amount of enzyme (entries 13 and 14), *iv.* the reaction time (entry 15), *v.* the role of each individual cofactors (entries 16–18). First, we examined the behaviour of various DESs in 50% v/v with PB. Lower yields were obtained with ChCl/urea 1:2 (38%, entry 2), ChCl/H_2O 1:2 (44%, entry 3), TBABr/Gly 1:2 (< 3%, entry 5), ChCl/D-fructose 2:1 (18%, entry 6). A good yield was obtained with ChCl/D-glucose 2:1 (57%, > 99%*ee*, entry 4). Remarkably, in this case, the external addition of D-glucose can be avoided as the D-glucose component of the DES participates in the redox system. Finally, CPME alone or in combination with PB (entries 7 and 8), proved to be ineffective in this reaction. We then investigated the role of each individual component of DES. To this end, we conducted the reaction in PB and choline chloride only (entry 9), in PB and glycerol (entry 10), and in PB by sequentially adding choline chloride and glycerol as independent components (entry 11). As shown in Table 1, the mixture of PB and ChCl (entry 9) was ineffective, while PB and glycerol (entry 10, 67%) yielded results in terms of conversion comparable to those with DES (entry 1, 62%). For this reason we decided to continue our investigation in PB/glycerol 50:50 v/v.[30] Next, we evaluated the optimal enzyme loading. Reducing the catalyst loading to 5.6 mg (entry 13), resulted in a yield drop to 15%, and no conversion of the substrate was observed with 2.8 mg (entry 14). From these studies it became evident that the best conditions to perform IRED-catalyzed bioreductions of prochiral imines is to use a 12 mg of enzyme loading. We also optimized the reaction time by monitoring the reaction progress, and we found that a higher yield (70%) was achieved after only 4 h (entry 15). Thus, we decided to proceed with the optimization study keeping 4 h as the standard reaction time. To complete this optimization study, we examined the role of the redox system components. As expected, the absence of the IRED enzyme itself (entry 16), or $NADP^+$ (entry 17), or GDH (entry 18) prevented the reduction to occur. Additionally, a dedicated protocol was optimized for the work-up procedure to avoid gelification issues that occurred upon basic quenching of the reaction crude (see Supporting Information for the optimized work-up). In conclusion, as both PB/(ChCl/D-glucose) and PB/glycerol are suitable reaction media for reduction of cyclic imines with IREDs at 100 mM concentration and beyond, we decided to move forward with the evaluation of the reaction scope using PB/glycerol as the most feasible solvent system.

IRED screening

In light of the new optimized reaction conditions and to demonstrate the scope of cyclic imine reduction by IRED in phosphate buffer/glycerol solvent, we applied the IRED-catalyzed reduction to a panel of cyclic imines, including six- and seven-membered ring substrates.[31] For this purpose, we conducted a screening of the IRED collection for the reduction of 5-phenyl-3,4-dihydro-2*H*-pyrrole **1 a** as the model compound for five membered rings, 6-phenyl-2,3,4,5-tetrahydropyridine **3 a** as the model compound for six-membered rings and on 7-phenyl-3,4,5,6-tetrahydro-2*H*-azepine **5 a** for seven-membered rings. With substrate **1 a**, as shown in Figure 4, IRED-17, -69 and -72 produced good results in terms of yield, but IRED-44 exhibited excellent performance with the highest yield (70%) and > 99%*ee* (*S*-enantioselectivity).

With 6-phenyl-2,3,4,5-tetrahydropyridine **3 a**, as reported in Figure 4, IRED-1, -17, -18, -33, -44, -49, -69 and -72 catalyzed the reduction with satisfactory substrate conversion and enantioselectivity. Among the IREDs active on substrate **3 a**, we decided to continue our investigation using IRED-72, which gave the best results in terms of enantioselectivity (see Supporting Information). The same investigation was carried out for seven-membered imine 7-phenyl-3,4,5,6-tetrahydro-2*H*-azepine **5 a**, as shown in Figure 4. In this case only IRED-44, -69 and -72 provided good conversion (and excellent enantioselectivity) to the expected amine **6 a**. Based on this screening, the scope of the reaction was investigated using IRED-44 for five-membered

Table 1. Enzymatic reduction of **1a** under different reaction conditions.[a]



entry	Solvent (% v/v)[b]	IRED-44 (mg)	$NADP^+$ (mg)	GDH (mg)	D-glucose (mg)	*t* [h]	**1a** (%)[c]	**2a** Yield (%)[c]	%*ee* (*R*/*S*)[d,e]
1	PB/(ChCl/Gly 1:2) 50:50	12.0	6.4	6.0	100	16	<3	62	>99, *S*
Solvent system									
2	PB/(ChCl/urea 1:2) 50:50	12.0	6.4	6.0	100	16	33	38	>99, *S*
3	PB/(ChCl/H_2O 1:2) 50:50	12.0	6.4	6.0	100	16	<3	44	>99, *S*
4	PB/(ChCl/D-glucose 2:1) 50:50	12.0	6.4	6.0	0	16	<3	57	>99, *S*
5	PB/(TBABr/Gly 1:2) 50:50	12.0	6.4	6.0	100	16	75	<3	–
6	PB/(ChCl/D-fructose 2:1) 50:50	12.0	6.4	6.0	100	16	<3	18	–
7	PB/CPME 50:50	12.0	6.4	6.0	100	16	57	9	–
8	CPME	12.0	6.4	6.0	100	16	74	<3	–
DES components									
9	PB/ChCl 50:50	12.0	6.4	6.0	100	16	67	6	–
10	PB/Gly 50:50	12.0	6.4	6.0	100	16	<3	68	>99, *S*
11	PB/(ChCl + Gly 1/2) 50:50	12.0	6.4	6.0	100	16	<3	61	>99, *S*
12	Gly	12.0	6.4	6.0	100	16	<3	<3	–
Catalyst loading									
13	PB/Gly 50:50	5.6	2.9	2.8	46.7	16	43	15	–
14	PB/Gly 50:50	2.8	1.5	1.4	23.2	16	78	<3	–
Time									
15	**PB/Gly 50:50**	**12.0**	**6.4**	**6.0**	**100**	**4**	**<3**	**70**	**>99, *S***
Control									
16	PB/Gly 50:50	–	6.4	6.0	100	4	70	<3	–
17	PB/Gly 50:50	12.0	–	6.0	100	4	75	10	–
18	PB/Gly 50:50	12.0	6.4	–	100	4	81	<3	–

[a] Reaction conditions: D-glucose, $NADP^+$, GDH-101 and IRED-44 (selected amounts), **1a** (0.14 mmol, 20.3 mg, 100 mM) added in DMF (30 μL), stirred (700 rpm) at 30 °C in the selected solvent system (PB: potassium phosphate buffer 100 mM, pH 8) for the selected time. [b] 1.4 mL total. [c] Determined by quantitative ^1H NMR analysis. [d] %*ee* values determined by Chiral-HPLC (see Supporting Information). [e] Absolute configuration determined by α_D values measured at 25 °C.

imines, and with IRED-72 for six- and seven-membered imines, respectively.

Reaction scope

In Scheme 1, the results of the reaction scope are reported. Good yields and excellent enantioselectivities were achieved for dihydropyrroles **1a**–**e**, tetrahydropyridines **3a**–**c** and for tetrahydroazepines **5a** and **5c**. However, **5b** afforded the corresponding azepane **6b** only in moderate yield. Instead, more sterically hindered substituents, such as naphthyl and *t*-butyl in 2-position of the pyrrolidine ring prevented the reduction reaction. Notably, the methodology also worked well with 2-alkyl piperidines. The procedure was successfully applied to 6-propyl-2,3,4,5-tetrahydropyridine **3d** for the enantioselective synthesis of the piperidine alkaloid (*R*)-(−)-coniine. In this case, after a quick screening, IRED-18 proved to be the most effective biocatalyst. This is consistent with the results reported by Turner on the reduction of 6-alkyl-2,3,4,5-tetrahydropyridine with (*R*)-IRED[25b] which shares a 70.9% sequence similarity to the IRED-18.[21b] Indeed, the reduction carried out with IRED-18 in glycerol successfully yielded (*R*)-(−)-coniine (**4d**, Scheme 1) in 66% yield and complete (*R*)-enantioselectivity. Remarkably, a study on the relative potencies of the two enantiomers of coniine on cells expressing human fetal nicotinic neuromuscular receptors demonstrated higher activity for the same enantiomer (*R*)-(−)-coniine.[32]

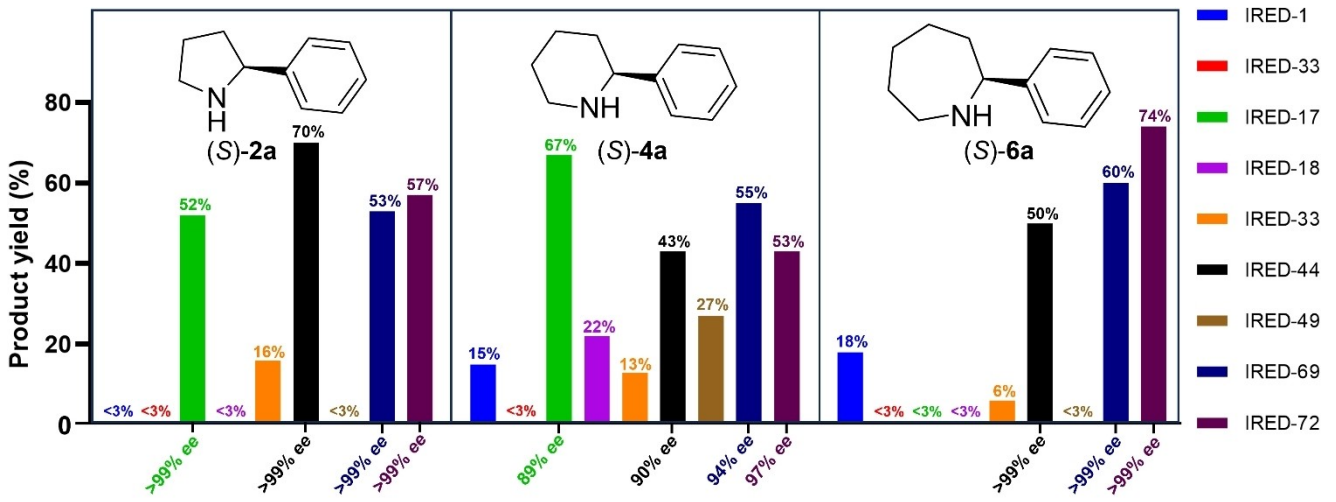
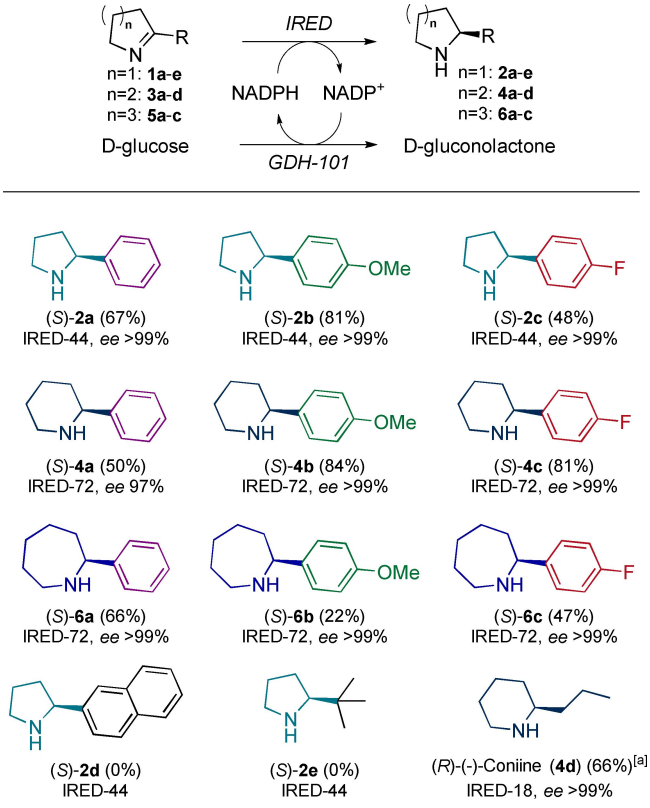


Figure 4. IRED screening for the asymmetric reduction of substrates **1 a**, **3 a** and **5 a**. Reaction conditions: D-glucose (100 mg), $NADP^+$ (6.4 mg), GDH-101 (6.0 mg) selected IRED (12.0 mg), imine substrate (0.14 mmol, 100 mM) added in DMF (30 μL), stirred (700 rpm) at 30 °C for 4 h in PB/Gly 50 : 50 (1.4 mL) (PB: potassium phosphate buffer 100 mM, pH 8). Yields determined by quantitative ^{1}H NMR analysis. %*ee* values (from 89% to >99%) were determined by Chiral-HPLC analysis when the reaction showed appropriate conversion (see Supporting Information). Absolute configurations were determined by α_D values measured at 25 °C.



Scheme 1. Reaction scope. Yields refer to the isolated product. Conditions: D-glucose (100 mg), $NADP^+$ (6.4 mg), GDH-101 (6.0 mg) and selected IRED (12.0 mg), substrate (0.14 mmol) added in DMF (30 μL), stirred (700 rpm) at 30 °C in PB/Gly 50 : 50 (1.4 mL), 4 h. %*ee* values determined by Chiral-HPLC analysis (See Supporting Information). Absolute configuration determined by α_D values measured at 25 °C. [a] Reaction was performed over 16 h; Chiral-HPLC analyses were performed after derivatization with TsCl.

Scale-up fed-batch protocol

In the realm of homogeneous biocatalysis, the employment of enzymes for the production of APIs in industrial processes such as transaminases,[33] dehydrogenases[34] and hydrolases[35] is well established.[36] However, the same cannot be stated for IREDs, primarily due to their recent discovery.[19] Nevertheless, some recent studies on reductive aminations[37, 21b] and dihydroquinolines reduction[38] have been reported with a special focus on the scalability of these transformations. Among the numerous approaches to move from the sub-millimolar scale, typical for biochemical investigations, to (semi)-preparative synthesis, enzyme immobilisation and recycling,[39] flow-techniques[40] or fed-batch protocols[41] are the most common approaches. Given the challenges associated with enzyme recovery in homogeneous biocatalysis,[42] mainly related to the need for enzyme denaturation to fully isolate the desired product, we opted to scale up our imine asymmetric reduction by fed-batch strategy for its practicality. In this regard, we designed a straightforward fed-batch protocol (Figure 5) in which imine **1 a** was periodically added to the reaction mixture until no further conversion was detected. Considering its fundamental role, we initiated by evaluating the effect the initial **1 a** concentration in PB/glycerol 50 : 50 on the reaction efficiency (See Supporting Information, Table S1). The resulting yield/[**1 a**] profile resembled that observed with PB/DES (Figure 3). Consequently, we started with the first addition of **1 a** at 10 mM and proceeded with five more additions every 2 h. Operating within the range of suitable concentrations (10-100 mM) where IRED-44 displayed the highest catalytic efficiency allowed us the possibility to find the best compromise between solvent volume and overall yield of **2 a**. After 24 h we quenched the reaction mixture and proceeded with the optimized workup procedure followed by purification and isolation of product (*S*)-**2 a**.

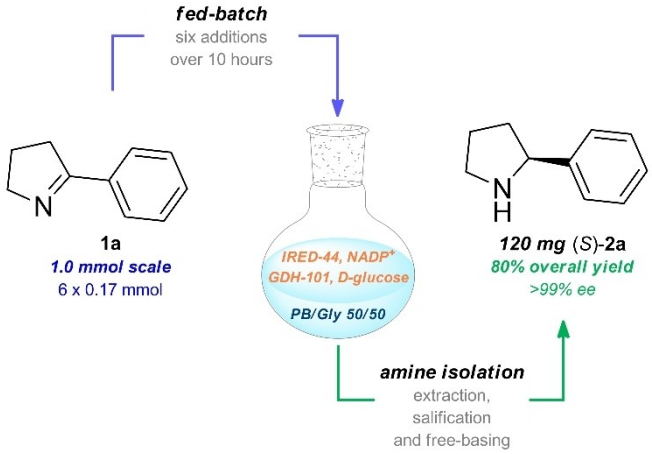


Figure 5. Fed-batch protocol for the scale-up enzymatic reduction of **1a**.

This fed-batch protocol enabled a convenient seven-fold scale-up transformation, where one millimole of substrate **1a** was readily converted into 120 mg of enantiopure amine (*S*)-**2a** with an 80% overall yield. In addition, this procedure exploits a formal recycle of the catalytic system, wherein a single batch of IRED-44, $NADP^+$ and GDH-101 can reduce six batches of substrate **1a** without requiring catalysts recovery or continuous product removal. Green metrics calculations demonstrate a 2.5-fold reduction in the E-factor using the fed-batch strategy (see Supporting Information for the E- factor evaluation).

Conclusions

We have successfully developed the first enantioselective conversion of several 2-substituted cyclic imines into the corresponding amines (pyrrolidines, piperidines, and azepines) through the reduction of the corresponding cyclic imines catalyzed by IREDs, utilizing both glycerol/phosphate buffer and DES/phosphate buffer mixtures. Glycerol belongs to the biomass-derived solvents, which are emerging as greener alternatives to volatile organic compounds (VOCs) in organic synthesis. As a major by-product of the biodiesel industry, glycerol is a particularly appealing green solvent, due to its low cost and renewable feedstock. We have also demonstrated that ChCl/D-glucose/phosphate buffer mixture can serve as a valid alternative as an active solvent in the reduction process, containing the necessary glucose for the redox cycle within the medium itself. The methodology we propose allows for the production of heterocyclic amines with full conversions, good yields and excellent enantioselectivities. It is worth to remark that the process can be performed at 100 mM substrate loading and up to 200 mM with a slight decrease in yield, which, specifically for **1a**, corresponds to a concentration of 14.5 $g\,L^{-1}$. We have also established a fed-batch methodology that enables the production of 120 mg of enantiopure amine from 1 mmol of substrate using a single batch of IRED-44, NADP and GDH-101, achieving a formal recycle of the catalytic system. This aspect significantly contributes to making the process potentially attractive for large-scale applications in the context of economic and environmental sustainability for a specific set of substrates, leading to the production of enantiopure heterocyclic amines of high pharmaceutical interest.

Experimental Section

General procedure for asymmetric enzymatic reduction in PB/Gly 50:50 v/v. All reactions were performed under air. In an open screw cap 7 mL vial D-glucose (100 mg), $NADP^+$ (6.4 mg), GDH-101 (6.0 mg) and the selected IRED (12.0 mg) were added consecutively in this order to PB/Gly 50:50 v/v (1.4 mL). The mixture was allowed to homogenize for 5 min at 30 °C. A solution of substrate (0.14 mmol) in 30 μL of DMF was then added to the mixture. The reaction was stirred (700 rpm) at 30 °C for 4 h. 1.5 mL of 1 M NaOH was then added, and the mixture shaken vigorously. The mixture was transferred in a 10 mL Erlenmeyer flask and 2 mL of Et_2O added. The heterogeneous mixture was stirred for 5 min to allow the denaturation and gelification of the enzymes. The mixture was filtered through a cotton plug *in vacuo*. The clear heterogeneous mixture was then transferred to a separating funnel and the aqueous phase extracted twice with Et_2O (2 mL). The combined organic phases were washed with 1 M NaOH (5 mL), dried with Na_2SO_4 and the solvent removed under reduced pressure. Crude products were purified by flash column chromatography and enantiomeric excesses (%*ee*) were determined by Chiral-HPLC analysis. Polarimetric analyses were performed to determine α_D values at 25 °C. (*S*)-**2-Phenylpyrrolidine (2a)**: general procedure with IRED-44 starting from **1a**. Purification by flash column chromatography (PE/Et_3N 98:2 v/v) gave **2a** as a colorless oil (13.8 mg, 67%, R_f = 0.16 PE/Et_3N 98:2 v/v). %*ee*: > 99. $[\alpha]_{25}^{D}$: −35.3 (c = 0.4, $CHCl_3$). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.38–7.34 (m, 2H), 7.33–7.29 (m, 2H), 7.25–7.21 (m, 1H), 4.12 (t, *J* = 8.0 Hz, 1H), 3.21 (ddd, *J* = 10.2, 7.8, 5.3 Hz, 1H), 3.02 (ddd, *J* = 10.2, 8.4, 6.6 Hz, 1H), 2.24–2.16 (m, 1H) superimposed to 2.16 (br s, 1H), 1.98-1.81 (m, 2H), 1.73-1.64 (m, 1H). **^{13}C{^{1}H} NMR** (150 MHz, $CDCl_3$): δ 144.7, 128.5, 127.0, 126.7, 62.8, 47.1, 34.4, 25.7.[25c] (*R*)-(−)-**Coniine (4d)**: general procedure with IRED-18 starting from **3d**. Reaction was performed over 16 h. Purification by flash column chromatography (EtOAc/MeOH 98:2 v/v + 1.0% Et_3N) gave **4d** as a colorless oil (11.8 mg, 66%, R_f = 0.12, EtOAc/MeOH 98:2 v/v + 1.0% Et_3N). %*ee*: > 99. $[\alpha]_{25}^{D}$: −4.1 (c = 1.0, $CHCl_3$). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 3.00 (d, *J* = 11.5 Hz, 1H), 2.56 (td, *J* = 11.8, 2.7 Hz, 1H), 2.41–2.34 (m, 1H), 1.71 (d, *J* = 11.1 Hz, 1H), 1.59 (d, *J* = 13.3 Hz, 1H), 1.52 (d, *J* = 14.0 Hz, 1H) superimposed to 1.49 (br s, 1H), 1.37-1.21 (m, 6H), 1.06–0.94 (m, 1H), 0.85 (t, *J* = 5.5 Hz, 3H). **^{13}C{^{1}H} NMR** (150 MHz, $CDCl_3$): δ 56.7, 47.3, 39.8, 33.1, 26.7, 25.0, 19.1, 14.3.[43]

Supporting Information

The authors have cited additional references within the Supporting Information.[44–50]

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

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