**Synthesis of Chiral Amines using Redox Biocatalysis**

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**Abstract**

Chiral amines feature in a large number of small molecule pharmaceuticals, and thus methods for their asymmetric synthesis are of considerable interest. Biocatalytic approaches have come to the fore in recent years as these offer advantages of superior atom economy, mild reaction conditions and excellent stereoselectivity. Advances in redox cofactor process technology have meant that oxidoreductase enzymes in particular now have growing potential as industrial catalysts for amine formation. In this review we survey recent developments in the discovery and application of oxidoreductase enzymes for amine production, including monoamine oxidases (MAOs), engineered and natural amine dehydrogenases (AmDHs), Imine Reductases (IREDs) and Reductive Aminases (RedAms), in addition to their application in enzyme cascades.

**Introduction**

Chiral amines feature in many of the world’s leading small-molecule pharmaceuticals, and there is significant interest therefore in developing asymmetric methods for their synthesis. In addition to transition metal-based catalysts [1-3], enzymes have also been the focus of attention, with considerable research focused on hydrolytic enzymes, such as lipases and *N-*acylases [4]which are readily available and simple to apply in acylation of racemic amines, or the hydrolysis of racemic acyl amines. Transaminase (TA)-catalyzed processes, in which the amine from a donor molecule, such as alanine, is transferred *via* the prosthetic group pyridoxal-5’ phosphate to an aldehyde or ketone substrate, have also been the subject of intense research in recent years [5-7]. The knowledge accumulated on these enzymes has permitted extensive protein engineering experiments that look to broaden their substrate specificity and process suitability, most famously for the production of the antidiabetic agent sitagliptin [8] and very recently for the amination of bulky ketones [9].

Despite their many advantages, hydrolases and TAs are limited to the production of primary amines, and so interest in complementary enzymes for amine synthesis has been ongoing. In particular, interest in oxidoreductases for the production of chiral amines has grown, as in many cases they catalyze the synthesis of enantio-enriched primary, but also secondary amines from racemic amine substrates or prochiral ketones. They can also be exploited in enzyme cascades in which multiple enzymes are combined to convert even simpler precursors. This survey focuses on recent reports of the discovery and the synthetic application of oxidoreductases for chiral amine production.

**Monoamine Oxidases (MAOs)**

Flavin-dependent Monoamine Oxidases (MAOs) catalyze the oxidation of amines to imines, with concomitant reduction of an FAD cofactor and the generation of hydrogen peroxide. MAO-N from *Aspergillus niger* has been extensively engineered by the Turner group to give variants with complementary substrate specificities [10]. A lysate of *E. coli* cells expressing the ‘MAO-N-D9’ variant of MAO-N was applied in a chemocatalytic process for the production of 3,4-dihydroisoquinolin-1-(*2H*)-one (DHIO, **3** **Figure 1A**) from 1,2,3,4-tetrahydroisquinoline (THIQ, **1**) [11]. In this process, MAO-N furnished the imine **2**, followed by oxidation to the lactam product **3** by copper (I) iodide and hydrogen peroxide in a one-pot, two-step process, generating **3** in 69.4% yield. A serendipitous discovery revealed that the mutant MAO-N-D5 catalyzed an aza-Friedel-Crafts reaction of *meso*-pyrrolidines [12•]. In this reaction substrate **4** was converted to 2-pyrrolylpyrrolidine **5** in 25% yield and with 70% e.e., through the *retro*-Diels-Alder (*r*-DA) reaction of the imine product, followed by the attack of the resultant pyrrole at the electrophilic carbon of the imine (**Figure 1B**). Conditions were optimised using the saturated analog **6**, which could not undergo the *r*-DA reaction, and a 67% yield of the product **7** was obtained with 95% e.e. MAO-N and a related enzyme, 6-Hydroxy-D-Nicotine Oxidase (6-HDNO) were also reported to catalyze the aromatization of 3-pyrrolines such as **9** into pyrroles **10** [13]. Moreover, MAO-N-D5 could be combined in one pot with a GII Grubbs catalyst for the sequential ring-closing metathesis (RCM) of diallylanilines such as **8** and **11**, followed by aromatization of the pyrroline products **9** and **12**, to give pyrroles **10** and **13** in up to 84% isolated yield (**Figure 1C**).

When MAOs are applied to a racemic amine substrate, and coupled with a reducing agent, a catalytic cycle is formed wherein the amine enantiomer that is disfavoured by the MAO is enriched in a series of oxidation-reduction cycles [10]. In a recent example, the MAO Cyclohexylamine Oxidase from *Brevibacterium oxydans* (CHAO) was evolved to yield mutants of improved and complementary specificity towards primary and secondary amines [14]. Mutant Y321I was applied to the preparative deracemisation of 1-(4-methoxybenzyl)-1,2,3,4,5,6,7,8-octahydroisoquinoline **14** with ammonia borane as the reductant, to give the deracemised amine (*S*)-**14**, a precursor for dextromethorphan, in 78% yield and with 99% e.e (**Figure 1D**).



**Figure 1**. New transformations that exploit the oxidative activity of Monoamine Oxidases (MAOs).

**Engineering Amino Acid Dehydrogenases (AADHs) to make Amine Dehydrogenases (AmDHs)**

Amino acid dehydrogenases (AADHs) catalyze the NAD(P)H dependent formation of amino acids from keto acids in the presence of ammonia [15]. Pioneering work by the Bommarius group had previously shown that variants of Leucine Dehydrogenase from *Bacillus stearothermophillus* (BsLeuDH) [16] and Phenylalanine Dehydrogenase from *Bacillus badius* (BbPheDH) [17] could be engineered to accept ketones as substrates, forming Amine Dehydrogenases (BsAmDH and BbAmDH), if mutations were made within the carboxylate recognition pockets of the enzymes. Following this work, Li and co-workers engineered a PheDH from *Rhodococcus* sp. M4 for the amination of phenylacetone **16** and 4-phenyl-2-butanone **18** through the creation of a triple mutant K66Q/S149G/N262C modelled on the BbPheDH AmDH variant [18]. (*R*)-amphetamine **17** and (*R*)-1-methyl-phenylpropylamine **19,** were produced with 95% conversion and each with >98% e.e. in reactions in which NADH was recycled using glucose dehydrogenase (GDH) (**Figure 2A**). The performance of this AmDH was enhanced through immobilisation on magnetic nanoparticles (MNPs), and these could also be re-used, with up to 81% residual activity after a third application [19•]. Mutti and co-workers replaced GDH with formate dehydrogenase (FDH), and performed the amination of 50 mM *para*-methoxy phenylacetone to give an 82% yield of the (*R*)-amine product with >99% e.e., in a wide-ranging study of AmDH activity against a range of aromatic and aliphatic ketones [20]. Further improvements in the process suitability of AmDHs were achieved through the engineering of a homolog of PheDH from the thermophile *Caldalkalibacillus thermarum* (*Cal*-AmDH)[21•]. *Cal-*AmDH displayed a melting temperature (Tm) of 83.5 °C, 27 °C higher than that exhibited by BbAmDH. Moreover, *Cal-*AmDH catalyzed the preparative amination of phenoxy-2-propanone at a concentration of 400 mM, in a two-phase system that incorporated 25% (v/v) isoamyl acetate, giving the (*R*)-amine product in 96% yield and with a space-time yield of 60g L-1 d-1. Turner and co-workers applied engineered BbAmDH in a cascade reaction with alcohol dehydrogenases (ADHs) for the sequential transformation of racemic alcohols into chiral amines, in a hydrogen–borrowing cascade with in-loop cofactor recycling [22••]. BbAmDH was coupled to either an (*R*)- or an (*S*)-selective ADHfor the transformation of (*R*)- or (*S*)- aromatic secondary alcohols to amines with up to 96% conversion and with e.e.s of >99% in most cases, with the addition of only 5% NADH relative to substrate. The cascade was also performed with a mixture of (*R*-)- and (*S*)-selectiveADHs with BbAmDH, to give 81% conversion of racemic 1-phenyl, 2-propanol **20** (**Figure 2B**) to the amine (*R*)-**17** with >99% e.e. A similar cascade was reported by Xu and co-workers, although in that case, an engineered LeuDH from *Exiguobactertium sibiricum* was employed with an ADH from *Streptomyces coelicolor* that displayed comparable activities for the oxidation of both enantiomers of the substrate alcohol [23]. A series of racemic alcohols was converted to chiral amines with 2-pentanol converted to (*R*)-2-pentanamine with 94% conversion and >99% e.e.

**A Natural AmDH**

The potential of engineered AmDHs has prompted a search for naturally occurring enzymes that display equivalent activity. Using the sequences of L-erythro-3,5-diaminohexanoate dehydrogenase (3,5-DAHDH) and (2*R*,4*S*)-2,4-diaminopentanoate dehydrogenase (2,4-DAPDH) as search models, Mayol and co-workers identified genes encoding enzymes competent for the NADPH-dependent amination of 4-oxo pentanoic acid **21** (**Figure 2C**) [24••]. One homolog, AmDH4 from *Petrotoga mobilis*, catalyzed the preparative conversion of this substrate to 4-(*S*)-aminopentanoic acid **22** with an isolated yield of 88% and an e.e. of >99%, in a system that employed FDH for cofactor recycling.



**Figure 2**. Transformations by engineered and natural amine dehydrogenases AmDHs.

**Imine Reductases (IREDs)**

The discovery of IREDs by the Mitsukura group [25,26] has stimulated considerable research into these enzymes, which catalyze the NADPH-dependent asymmetric reduction of prochiral cyclic imines [27-30]. Hussain and co-workers applied cells of *E. coli* expressingthe (*R*)-selective IRED from Streptomyces sp. GF3587 to the reduction of model compound **23** and other 5- 6- (**Table 1**, entries (**a**),(**b**)) and also 7-membered cyclic imines and iminium ions [31]. Substrates with aliphatic and aromatic substituents in the 2-position of the ring were transformed with excellent conversions and, for the most part, excellent e.e.s, and a gram scale reduction of 2-*n*-propyl piperideine **25** yielded the bioactive alkaloid (*R*)-coniine **26** with 90% yield and 98% e.e. Li and co-workers performed a screen of fifty IRED sequences, yielding an enzyme from *Paenibacillus lactis* that catalyzed the reduction of indoline amines such as **27** and structurally-related iminium ionsto (*S*)-amine products such as **28**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Entry | Enzyme | Substrate | Product | Yield (%) | e.e. | Reference |
| (a) | (*R*)-IRED from *Streptomyces* sp. GF3587 | **23** | (*R*)-**24** | >98 | >98 | [22], [28] |
| (b) | (*R*)-IRED from *Streptomyces* sp. GF3587 | **25** | (*R*)-**26**, | >90 | >98 | [28] |
| (c) | (*S*)-IRED from *P*. *lactis* | **27** | (*S*)-**28** | 75 | >99 | [29] |
| (d) | (*S*)-IRED from *N. halophila* | **29** | (*S*)-**30** | 86 | >99 | [30] |
| (e) | (*S*)-IRED from *N. halophila* | **25** | (*R*)-**26** | 90 | 82 | [30] |
| (f) | IR\_14 from *N. cyriacigeorgica* | **31** | (*S*)-**32** | 71 | 87 | [31] |
| (g) | IR\_14 from *N. cyriacigeorgica* | **33** | (*R*)-**34** | 42 | 67 | [31] |
| (h) | IRED from *A.orientalis*(whole-cells) | **31** | (*S*)-**32** | n.d. | 85 | [32] |
| (i) | IRED from *A.orientalis*(pure enzyme) | **31** | (*R*)-**32** | n.d. | 98 | [32] |

**Table 1**. Asymmetric reductions of prochiral imines by Imine Reductases IREDs.

with high enantioselectivity (**Table 1**, entry (**c**)) [32]. Other IREDs possessed more complex stereochemical behaviour. While an IRED from *Bacillus cereus* (BcIRED) catalyzed predominantly the (*S*)-selective reduction of cyclic imines, a homolog from *Nocardiopsis halophila* (NhIRED) displayed an inversion of selectivity when transforming 2-methyl piperideine **29** or 2-*n*-propyl piperideine **25** (**Table 1**, entries (**d**)and (**e**)) [33]. This abrupt switch in enantioselectivity in some IREDs was confirmed by researchers at Roche, who showed that, from a library of twenty IREDs, ‘IR\_14’ from *Nocardia cyriacigeorgica* GUH-2 converted **31** to the (*S*)-amine **32** and **33** to the (*R*)-product **34**, with the hydride delivered to the prochiral imine from the opposite face of the substrate (**Table 1**, entries (**f**) and (**g**)) [34•]. 100 mg scale conversions of **31** by ‘IR\_20’ from *Streptomyces tsukubaensis* gave 99% conversion to the amine product (*R*)-**32**. A more striking example was observed with the IRED from *Amycolatopsis orientalis* (*Ao*IRED), which displayed a switch in enantiopreference for the reduction of 1-methyldihydroquinoline **31** dependent on the nature (whole cells, lysate or purified enzyme) and age of the biocatalyst (**Table 1**, entries (**h**) and (**i**)) [35]. Höhne and co-workers engineered the (*R*)-selective IRED from Streptomyces sp. GF3587 to improve its recognition of the cheaper, non-phosphorylated cofactor NADH through a single mutation of lysine 40 to alanine, and applied this variant to the enantioselective reduction of **23** on an 800 mg scale, with NADH recycling using GDH [36]. As with AmDHs, IREDs have also been applied in cascade reactions. In the first example, IREDs have been applied as the reducing agent in MAO-catalyzed deracemisation cycles, leading to enhanced enantioselectivity in the production of piperidine and pyrrolidine substrates [37]. In the second, a complex cascade comprising carboxylic acid reductase (CAR), TA and IRED was constructed for the conversion of keto acid substrates to piperidines and pyrrolidines through aldehyde and cyclic imine intermediates [38••].

**Reductive Aminations using IREDs**

The discovery of IREDs also prompted researchers to investigate whether the enzymes could be applied to the reductive amination of ketones, with the objective of forming chiral secondary amines. A study by Huber and co-workers had demonstrated that 4-phenyl-2-butanone **35** was converted to (*S*)-2(methylamino)-4-phenylbutane **36** with 8.8% conversion and an e.e. of 76%, in the presence of a large excess of both enzyme and methylammonium buffer (**Table 2,** Entry (**a**)) [39]. Scheller and co-workers then demonstrated that, with a 50-fold excess of methylamine, benzaldehyde was transformed to methylbenzylamine with 73% conversion by (*R*)-IRED-Sr from *Streptosporangium roseus*. Furthermore, the enzyme catalyzed the formation of acetophenone **37** to the (*R*)-amine product **38** with 39% conversion and 87% e.e. at pH 9, which favoured imine formation (**Table 2,** Entry (**b**)) [40]. Following these reports, the Roche group applied their library of 28 IREDs to the reductive amination of ketones including acetophenone, 2-hexanone and (*R*)-3-methylcyclohexanone [41••] with ammonia, methylamine and butylamine as amine partners. In these cases, a 12.5-fold excess of amine and a pH of 9.3 was used. The aminations of **39** and **41** with ammonia or methylamine were performed on 100 mg scale using ‘IR\_20’, giving chiral amine products with 55% yield and 96% e.e. in the second case (**Table 2,** Entry (**d**)). Höhne followed these experiments with a focus on the production of the anti-Parkinson’s agent rasagiline **44** through the amination of indanone **43** using propargylamine in the presence of IREDs. In the presence of ‘IR\_14’ from the Roche library, **43** was converted to the (*R*)-enantiomer of **44** in 58% yield and with 90% e.e., whereas an equivalent

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Entry | Enzyme | Substrate Ketone | Amine and equivalents | Product | Yield (%) | e.e. | Ref. |
| (a) | (*S*)-IRED from *Streptomyces* sp. GF3546 | **35** | MeNH2Buffer (in large excess) | (*S*)-**36** | 8.8 | 76 | [36] |
| (b) | IRED from*S. roseus* | **37** | MeNH2(50X) | (*R*)-**38** | 39 | 87 | [37] |
| (c) | IR\_20 from *S. tsukubaensis* | (*R*)-**39** | NH3(12.5X) | (1*S*, 3*R*)-**40** | 50 | 94 (d.e.) | [38] |
| (d) | IR\_20 from *S. tsukubaensis* | **41** | MeNH2(12.5X) | (*R*)*-***42** | 55 | 96 | [38] |
| (e) | IR\_14 from*N. cyriacigeorgica* | **43** | Propargylamine(40x) | (*R*)-**44** | 58 | 90 | [39] |
| (f) | IR\_SipFrom *S. ipomoeae* | **43** | Propargylamine(40x) | (*S*)-**44** | 81 | 72 | [39] |
| (g) | *Asp*RedAmFrom *A. oryzae* | 45 | Allylamine(1x) | **46** | 73 | n.a. | [40] |
| (h) | *Asp*RedAmFrom *A. oryzae* | **45** | Propargylamine(1x) | **47** | 94 | n.a. | [40] |

**Table 2**. IREDs applied to the reductive aminations of prochiral ketones.

reaction using ‘IR-Sip’ from *Streptomyces ipomoeae* 91-03 gave the (*S*)-enantiomer of **44** in 81% yield and 72% e.e. [42•] (**Table 2,** Entries (**e**) and (**f**)). In these cases a 40-fold excess of amine was employed at a pH of 9.5. In each if the above cases it was clear both from the large excess of amine donor and the operating pH, that amine production was dependent on factors that favoured the abiotic formation of imines, suggesting that the IREDs catalyze the reduction of pre-formed imines only. In recent work Aleku and co-workers have discovered an IRED homolog from the fungus *Aspergillus oryzae,* which catalyzes both imine formation and imine reduction for a range of ketone and amine partners supplied in a stoichiometric ratio. Thus cyclohexanone **45** was coupled with allylamine and propargylamine to give amines **46** and **47** with yields of 73 and 94% respectively [43••] (**Table 2,** Entries (**g**) and (**h**). In addition this ‘reductive aminase’ (*Asp*RedAm) was able to mediate the formation of chiral amines at lower amine:ketone ratios than previously described and at a pH of 7.0. The discovery of this RedAm points to an important new direction for investigations on IREDs and their homologs for the atom efficient production of chiral secondary amines from ketones.

**Conclusion**

The last two years have seen significant advances in enzyme discovery and engineering directed towards the implementation of oxidoreductases in chiral amine formation. These developments look to make the use of such enzymes as commonplace as that of ketoreductases (KREDs) in an industrial context. The application of these emergent activities will depend upon further research into their catalytic characteristics, including mechanism, substrate range and stability, and also their further engineering for process suitability.

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