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Enantioselective imine reduction catalyzed by imine reductases and artificial metalloenzymes

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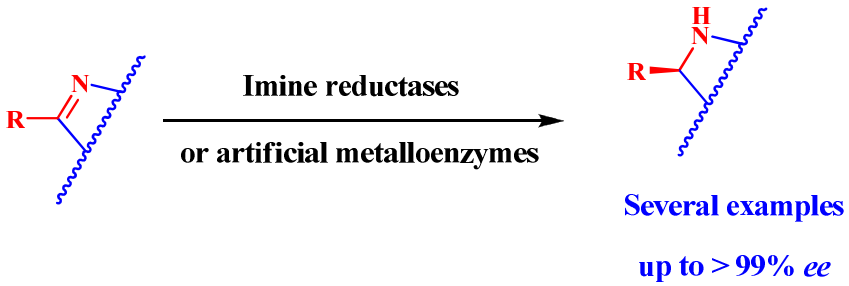
GRAPHICAL ABSTRACT



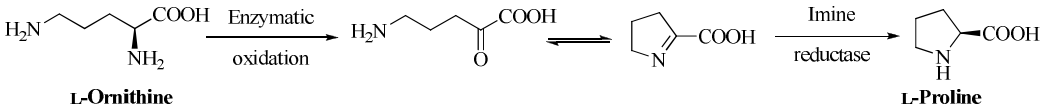
Adding value to Organic Synthesis. Novel imine reductases enable the enantioselective reduction of imines to afford chiral amines. Likewise, novel bioinspired artificial metalloenzymes can perform the same reaction as well. Remarkable recent examples are herein discussed.

1.- Introduction

Enantiopure amines are key compounds for a broad range of pharmaceuticals and agrochemicals, as well as for flavor and fragrance industries.[1] The development of selective synthetic approaches to afford these building blocks has been matter of intense research, covering enzymatic,[2] chemical[3] or mixed chemoenzymatic[4] strategies. Among these options, biocatalysis play a central role involving many different enzyme types (*e.g.* hydrolases, monoamine oxidases, transaminases, etc.) and strategies for the synthesis of enantiopure amines, even with examples at commercial scale.[2, 5] Moreover, recent trends involving highly active transaminase variants,[6] or the synthetic assessment of novel enzymes such as nitrile reductases[7] or engineered NADH-dependent L-amino acid dehydrogenases[8] are being continuously reported within this dynamic research field.

Despite the tremendous developments using oxidoreductases for the enantioselective reduction of ketones,[5] an analogous approach, namely the biocatalytic imine reduction to afford chiral amines in one step, has been scarcely developed. The lower stability of (acyclic) imines in aqueous solutions – traditional conventional media for enzymes – compared to ketones, might be a reason for that. Remarkably, many imine reductases from different sources have been biochemically described and characterized for decades now. In all these natural cases are cyclic imines which are involved, presumably due to its higher stability compared to their acyclic counterparts, which are typically prone to be hydrolyzed to the corresponding carbonylic compound in aqueous solutions. As examples, dihydrofolate reductase (DHF reductase, EC 1.5.1.3),[9] or thiomorpholine-carboxylate dehydrogenase (ketimine reductase, EC 1.5.1.25)[10] can be mentioned. Likewise, the biosynthesis of pipecolic acid or amino acids like proline have been suggested to involve imine

reductases such as Δ^1-piperideine-2-carboxylate reductase (Pip2C reductase, EC 1.5.1.21),[11] or Δ^1-pyrroline-2-carboxylate reductase (Pyr2C reductase, EC 1.5.1.1)[12] For proline biosynthesis the enzymatic oxidation of L-ornithine renders the corresponding α-keto acid, which forms equilibrium with its more thermodynamically stable imine cyclic form, which is subsequently enzymatically reduced (Scheme 1).[12]



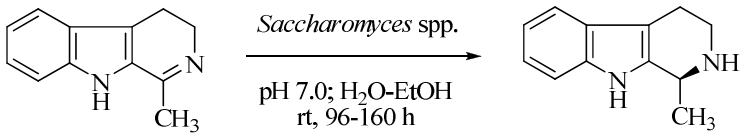
Scheme 1. Postulated biosynthetic pathway for the synthesis of L-proline, involving (dotted square) an imine reductase.[10b, 12]

On the basis of these *in vivo* enzymatic pathways, the possibility of using biocatalytic enantioselective imine reductions for synthetic purposes has been considered through different diverse strategies by several research groups. Especially over the last years the topic has started to gain importance with recent remarkable examples. In the following sections, the field is discussed in depth.

2.- Imine reductases for biocatalysis

Inspired by the above-discussed biochemical pathways for imine reduction, an expected approach was to assess whether imine reductases could be used for biocatalytic reactions. In this respect, by screening a library of imine substrates in a biphasic water-tetradecane solvent system, new imine reductase activity was described in the anaerobic bacterium *Acetobacterium woodii*.[13] Furthermore, several research groups reported on other (wild-type) whole-cell-catalyzed imine reductions. For instance, Δ^1-pyrroline-2-carboxylic acid (Pyr2C), dihydrofolic acid (DHF) and (*E*)-*N*-(1-phenylethylidene)aniline were described as active substrates for *Candida*

parapsilosis ATCC 7330, yielding moderate yields and excellent enantioselectivities along the imine reductions (up to *ees* > 99%).[14] Likewise, different *Saccharomyces* spp. strains were evaluated in the bioreduction of β-carboline imines.[15] In that work the scope of reduction of imine **1** mediated by different *Saccharomyces* was investigated using a methodology based on the use of *Saccharomyces* species employed in the fermentation of grape sugar for the reduction of dihydro-β-carbolines (Scheme 2).



1 **2**

Scheme 2. Bioreduction of β-carboline imines by *Saccharomyces* spp.[15]

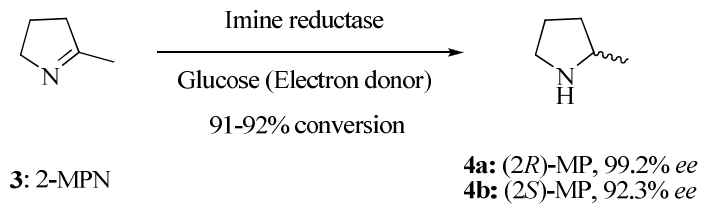
Four strains of *S. cerevisiae* and four of *S. bayanus* were screened in an ethanol-fructose-distilled water solution at 37 °C using imine **1** as substrate, affording moderate yields and high-to-excellent enantioselectivities. The best results according to both enantioselectivity (>91% *ee*) and yields (63-78%) were obtained with *S. bayanus* strains. Moreover, other imines were evaluated for the reduction with *S. bayanus* strains, affording enantiomeric excesses from 50 to 97% *ee*. According to the obtained results, the imine reduction was suggested to occur through hydrogen transfer to the *re*-face of the corresponding prochiral imine when the methyl group in **1** is substituted by other small aliphatic groups such as ethyl, halomethyl, isopropyl, etc., to yield the corresponding (*S*)-amine. Additionally, other substituents such as C_1-C_{11} aliphatic chains afforded amines with (*S*)-configuration as well. However, C_{15}-C_{17} aliphatic and aromatic substituents in the β-carboline imines led to an inversion

yielding (*R*)-amine products. More recently, cell-free extracts of earthworms *Eisenia foetida* have shown imine reductase activities as well.[16]

Besides these various proof-of-concepts using wild-type whole cells, several research groups have focused on the isolation and in-depth biocatalytic characterization of the actual imine reductases. Herein, the structure of a NADPH-dependent reductase Q1EQE0 from *Streptomyces kanamyceticus* was recently reported, and evaluated its activity towards different imine substrates.[17] The structure of Q1EQE0 in its native form, and complexed with NADPH – solved and refined to a resolution of 2.7 Å –, showed a dimer in which the monomer consist of an *N*-terminal Rossman-fold motif attached to a helical *C*-terminal domain through a helix of 28 amino acids. The structure seems to be related to those known of β-hydroxy-acid dehydrogenases, except that the essential lysine – which serves as an acid/base in the (de)protonation of the formed alcohols in those enzymes –, is replaced by an aspartate residue, Asp187 in Q1EQE0. Mutations of Asp187 to either asparagines or alanine resulted in inactive variants. Regarding substrate specificity, the enzyme catalyzed the asymmetric reduction of 2-methyl-1-pyrroline (2-MPN) **3** to the corresponding (*R*)-amine **4a** with >99% *ee* and 23% conversion in the presence of NADPH, glucose-6-phosphate and glucose-6-phosphate dehydrogenase as enzymatic system for cofactor regeneration. On the other hand, low conversions (<5%) were observed when other substrates such as 3,4-dihydroisoquinoline and 2-methyl-3,4-dihydroisoquinolin-2-ium triflate were used.[17]

In analogous lines, recently novel NADPH-dependent (*R*)- and (*S*)-imine reductases from *Streptomyces* sp. GF3587 and GF3546 were found to be highly selective in a screening using **3** as substrate.[18] These whole-cell catalysts produced either (2*R*)-methylpyrrolidine (2*R*-MP) **4a** or (2*S*)-methylpyrrolidine (2*S*-MP) **4b** with

excellent enantioselectivities (99.2% and 92.3% *ee* respectively) at high conversions (91-92%) (Scheme 3).



Scheme 3. Enantioselective reduction of 2-methyl-1-pyrroline **3** with whole-cells of *Streptomyces* sp. GF3587 and GF3546, affording (2*R*)- and (2*S*)-methylpyrrolidine, **4a** and **4b** respectively.[18]

Subsequently both enzymes have been cloned, over expressed, purified and characterized.[19] The (*R*)-imine reductase was found to be a homodimer consisting of 32 kDa subunits, with a rather narrow substrate specificity restricted only to 2-methylpyrrolydine. Conversely, the (*S*)-imine reductase – also a homodimeric protein with subunits of 30.5 kDa –, not only catalyzed the reduction of 2-methylpyrrolydine but also 1-methyl-3,4-dihydroisoquinoline and 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline were accepted as substrates, with moderate or low conversions but with excellent enantioselectivities (92 - 99% *ee*). Quite remarkably, the substrate scope of this (*S*)-imine reductase has been further extended recently by Turner's group, together with several preliminary process set-up options for biocatalysis.[20] Thus, *E. coli* whole-cells over expressing (*S*)-imine reductase can be used as biocatalysts for the reduction of a broad range of cyclic imines – involving five-, six- and seven membered substrates, and even iminium forms **5 - 10** –, to afford amines with excellent enantioselectivities in several cases (Figure 1).

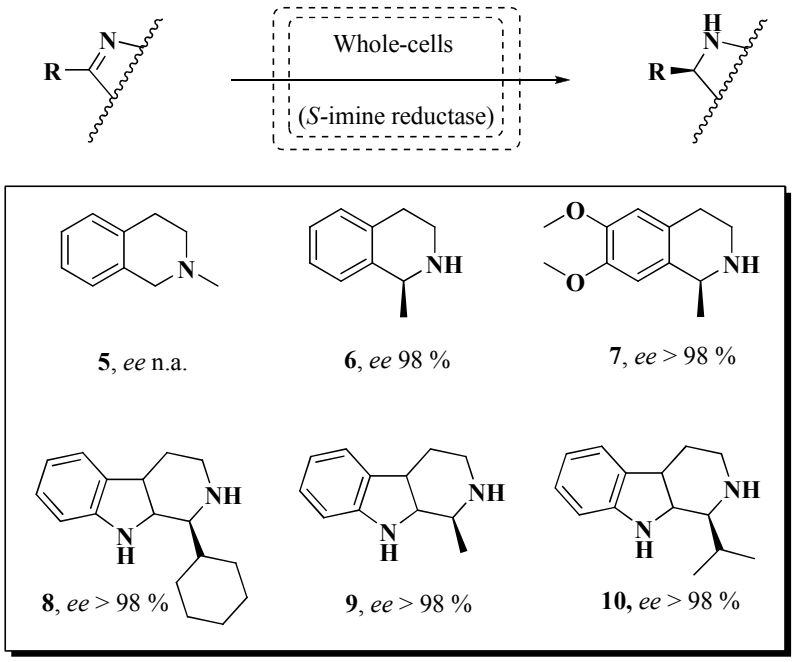
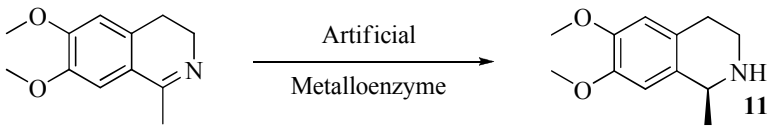


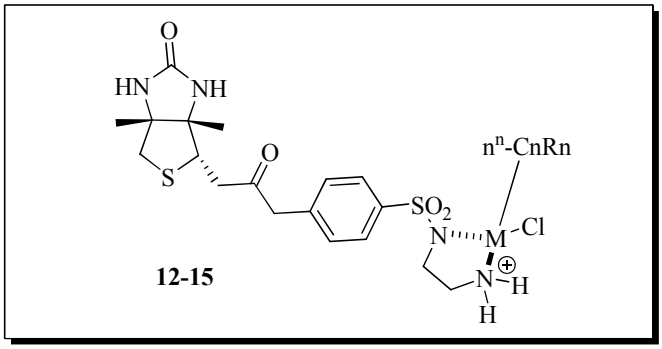
Figure 1. Imine reductions performed using (*S*)-imine reductase over expressed in *E. coli* whole cells.[20]

3.- Design of artificial metalloenzymes for imine reduction

Apart from the above-discussed examples of imine reductases, tailored artificial metalloenzymes have been assessed for the enantioselective reduction of imines. Behind these efforts lays the concept of combining chemocatalytic centers with peptide scaffolds to generate promising microenvironments for catalysis.[21] Ward *et al*. screened d^5 and d^6 piano stool complexes bearing the biotinylated aminosulfonamide ligand (Biot-*p*-L) combined with wild-type streptavidin for the production of (*R*)-salsolidine **11** (Scheme 4).[22] The complexes were also evaluated in the reduction of acetophenone to 1-phenylethanol, and, in most cases, the enantiomeric behaviour of the catalyst was coincident for both substrates. Likewise, artificial metalloenzymes enable the engineering of the reaction microenvironment of the active metal catalyst through the genetic modification of the surrounding host

protein. The optimization of an artificial imine reductase (ATHase) based on biotin-streptavidin technology was reported subsequently by Ward and co-workers as well.[23] By introducing lipophilic amino acid residues around the active site, an 8-fold increase in catalytic efficiency compared with the wild-type artificial imine reductase was achieved with moderate enantioselectivities (*ees* up to 70%). Whereas substrate inhibition was encountered for the free cofactor and the wild type ATHase, two engineered systems exhibited classical Michaelis-Menten kinetics.





M	n^n-CnRn	Abbreviation	*ee* (%); [conv. 11 (%)]
Ru	n^6-*p*-cymene	**12**: [(*p*-cymene)Ru(Biot-*p*-L)Cl]	22; [97]
Ru	n^6-benzene	**13**: [(benzene)Ru(Biot-*p*-L)Cl]	12; [76]
Rh	n^5-C_5Me_5	**14**: [Cp*Rh(Biot-*p*-L)Cl]	53; [94]
Ir	n^5-C_5Me_5	**15**: [Cp*Ir(Biot-p-L)Cl	57; [quant]

Scheme 4. Artificial metalloenzymes based on the biotin-streptavidin technology for the ATH imines.[22-23]

Finally, it must be also mentioned that the combination of artificial transfer hydrogenases have proven to be compatible with, and complementary to natural enzymes, allowing efficient concurrent tandem catalyzed reactions.[24] Herein, the general strategy was to combine the ATHase with various NADH- FAD- dependent

and haem-enzymes, resulting in orthogonal redox cascades. One of the examples is the integration of the catalytic system for the reduction of prochiral imines with subsequent deracemization of cyclic amines.[24]

4.- Concluding remarks

Several biocatalytic and bioinspired imine reduction strategies have been reported at the proof-of-concept level by several groups, adding a promising value to the existing portfolio of enzymatic strategies. Albeit the exact mechanism of the enzymatic imine reduction remains to be elucidated – with a still narrow substrate specificity –, it may be expected that more knowledge in the area will lead to novel applications in the near future. The recent observation that several oxidoreductases may efficiently catalyze reactions in non-aqueous neat substrates[25] may open novel possibilities for the otherwise water-sensitive imines, as the biocatalytic reduction of these substrates in water-free environments might then be feasible for synthetic purposes.

5.- Acknowledgements

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