Chiral Amines in Total Synthesis: The Biocatalytic Approach

Procedures for the efficient production of enantiopure amines are highly desired reactions in total synthesis in lab-scale as well for industrial applications. Recently several biocatalytic methods matured to a level that offers straightforward preparative formation of chiral amines.

Introduction

Aliphatic amines are considered ubiquitous functional groups in chemical space, with applications as dyes, surfactants and coatings as well as in fine chemicals such as agrochemicals and active pharmaceutical ingredients (APIs) [1]. However, according to recent estimations, 40% of APIs and 20% of agrochemicals contain a chiral amine moiety, which has to be prepared in a stereoselective fashion [2].

Despite the demand for stereochemically pure amines and the huge industry behind the production of aliphatic amines, methods for the direct and selective production of single enantiomers still suffer from limitations, such as cumbersome work with protecting/activating groups or the use of costly and potentially toxic transition metal catalysts [2b]. Since nature provides amines in manifold forms, ranging from amino acids to polypeptides and alkaloids, most chiral amines originate from nature’s chiral pool. Besides that, amines are usually prepared as racemic mixture of both enantiomers, which are subsequently separated by diastereomeric crystallization or by selective enzymatic transformation of one of the enantiomers using hydrolases. However, these kinetic resolutions imply the loss of 50% of the product consisting of the “wrong” stereo-configuration [1].

An emerging field in asymmetric synthesis, fit to overcome all the aforementioned objectives, is the field of biocatalysis. Enzymes in general are regarded efficient, highly stereoselective and green catalysts [3]. Whereas hydrolases already have found wide applications in chemical industry, e.g. for the resolution of racemic amines [1, 4], several elegant enzymatic protocols for the formation of amines as single enantiomers in high purity were only recently established and applied to synthesis [2, 4-5]. Herein a selection of these processes will be highlighted.
ω-Transaminase-catalysed production of chiral piperidines

Enantioselective reductive aminations are regarded highly important reactions in asymmetric organic synthesis. Enzymes from the class of ω-transaminases (ω-TA) perform the biocatalytic equivalent of this reaction, by transferring the amine moiety of a donor molecule [a commonly used donor is isoproylamine (IPA)] onto the carbonyl of the acceptor molecule, selectively in (R)- or (S)-form, depending on the enzyme (Scheme 1, A). The industrial value of ω-TAs was demonstrated in the production of sitagliptine, which could be performed on the impressive scale of 200 g/L. However, since the reaction is reversible, different strategies had to be developed in order to shift the equilibrium towards the desired amine. A simple and widely applied approach is the use of an excess of IPA. An elegant example is also the use of catalytic amounts of L-alanine with ammonium formate and the enzymes alanine dehydrogenase (AlaDH) and formate dehydrogenase (FDH). Together with an ω-TA, this sophisticated combination of enzymes in one pot resembles a direct reductive amination with the sole use of ammonium formate [2b, 4-5].

Recently, the highly regio- and stereoselective transformation of the sterically less hindered ketone of diketones 2 was described to yield chiral cyclic imines 3 (Scheme 1, B) [6]. Via subsequent diastereoselective reduction, stereochemically pure cyclic secondary amines (4) could be produced (Scheme 1, C). As the spontaneous cyclisation shifts the reaction equilibrium towards the product side, conversions of > 99% were achieved. By careful selection of the biocatalyst and the reduction conditions, all four enantiomers of 2,6-disubstituted piperidines 4 could be reached.[6-7] Using this methodology, the synthetic route towards dihydropinidine [(2S,6R)-4a], a potential antifeedant for the pine weevil Hylobius anietis could be shortened from up to 14 steps to a three step sequence, starting from 1 [6]. Similarly, its diastereomer (-)-epi-dihydropinidine [(2S,6S)-4a], which is active against the eastern spruce budworm, could be produced in high yield and
good diastereoselectivity [7a]. Furthermore, the applicability of the system towards sterically demanding substrates was demonstrated in the three-step synthesis of isosolenopsin [(2S,6R)-4b] and its diastereomers [7b].

**Monoamine oxidase (MAO) catalysed formation of alkaloids**

MAO-N from Aspergillus niger was started to be investigated as a biocatalyst at the beginning of 2000 [8]. The enzyme was engineered to an efficient catalyst during the last 15 years via the systematic generation of variants with increased activity and selectivity as well as expanded substrate-scope allowing the synthesis of natural products and APIs [2b]. The enzyme catalyses the oxidation of amines (primary, secondary and tertiary) into imines accompanied by the simultaneous reduction of molecular oxygen to hydrogen peroxide. The potential of the enzyme has been demonstrated in numerous deracemization processes, where the enantioselective oxidation of an amine is followed by a non-selective reduction of the imine, thereby allowing the accumulation of one enantiomer over repeated oxidation-reduction cycles (Scheme 2, A) [9]. A particularly elegant example of the use of MAO-N is the asymmetric synthesis of the alkaloid natural product (R)-harmicine [(R)-7, Scheme 2, B] [10]. The enzyme (MAO-N D9 variant) is involved in two steps: first it generates a reactive iminium ion (6) from 5, which undergoes a non-stereoselective Pictet-Spengler reaction forming racemic harmicine (rac-7). The latter is then deracemized with the enzyme using BH$_3$-NH$_3$ as a chemical reducing agent affording (R)-harmicine [(R)-7] in 99% ee and 83% conversion. MAO-N has been also coupled with other enzymes (e.g. ω-TAs and berberine bridge enzyme) allowing the preparation of valuable chiral amines.[11]

**Biocatalytic imine reduction**

The toolbox of enzymes giving access to chiral amines is being extended with the growing number of imine reducing enzymes. The strategies followed to find such catalytic systems involve (i) the creation of artificial metalloenzymes, (ii) rational engineering of amino acid dehydrogenases and (iii) seeking after naturally occurring novel NAD(P)H-dependent imine reductases. All strategies have provided successful examples but the field is still in its infancy and needs further efforts in order to be implemented in synthesis.[2a]

**Conclusion**
The application of enzymes in the synthesis of chiral fine chemicals has been widely developed in the past decades. The selective formation of amines using biocatalysts is a topic that was introduced only recently but has already demonstrated its feasibility in numerous studies and applications.

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