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Research review paper

Nature-inspired enzymatic cascades to build valuable compounds

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ABSTRACT

Biocatalysis currently is focusing on enzymatic and multi-enzymatic cascade processes instead of single steps imbedded into chemical pathways. Alongside this scientific revolution, this review provides an overview on multi-enzymatic cascades that are responsible for the biosynthesis of some terpenes, alkaloids and polyethers, which are important classes of natural products. Herein, we illustrate the development of studies inspired by multi- and chemo-enzymatic approaches to build the core moieties of polyethers, polypeptide alkaloids, piperidines and pyrrolidines promoted by the joint action of oxidoreductases, hydrolases, cyclases, transaminases and imine reductases.

Contents

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1. Introduction

The attention of the biotechnology community has been attracted by the emerging availability of new biocatalysts for the synthesis of complex molecules (Turner and Truppo, 2013a). The idea of coupling one or more enzymes to promote biotransformation in a cascade of reactions is a strategy of choice when searching for environmentally benign and sustainable chemical processes (Oroz-Guinea and García-Junceda, 2013). The synergistic action of several enzymes can currently be achieved by applying protein engineering and the logical enzymatic combinations present in genome sequences (Davids et al., 2013). However, the complexity of life and chemical processes makes the enzymatic cascades a large challenge for the multigram-scale production of highly valuable chiral compounds. To overcome these issues, nature's efficiency proved unbeatable and has been used to inspire pathways to synthesize terpenes, alkaloids, fatty acids, sugars and other secondary metabolites (Conrado et al., 2008).

Herein, we illustrate multi- and chemoenzymatic approaches to build the core moieties of polyethers, polypeptide alkaloids, piperidines and pyrrolidines promoted by the joint action of oxidoreductases, hydrolases, cyclases, transaminases and imine reductases.

We will first focus on the piperidine and pyrrolidine alkaloids, which are secondary metabolites and ubiquitous to a wide diversity of plants and insects. These compounds are well described in the literature, mainly due to their biological activities and enantiopure alkaloids are highly valued. Moreover, information generated during the past decade make it possible to apply genome-encoded sequences to build molecules of higher complexity such as the polyethers and polypeptide alkaloids, which are discussed in the last section of this review.

2. Piperidine and pyrrolidine alkaloids

Alkaloids are a large group of secondary metabolites found in microorganisms, plants and animals. Their diverse biological activities are related to survival and communication (Cushnie et al., 2014).

Despite their structural diversity, alkaloids share many physical and chemical properties. They are derived from amino acids, possess at least one basic heterocyclic amine which makes them readily accessible for hydrogen bonds within proteins, enzymes and receptors.

The discovery of alkaloids in the 19th century led to a great surge of interest in natural product chemistry, and, to date, more than 18,000 alkaloids have been discovered. Some of the most important alkaloids, such as piperidine, pyrrolidine, indolizidine, quinolizidine and pyrrolizidine, have piperidine or pyrrolidine core moieties. These rings are present in many structurally complex alkaloids, although simple substituted piperidines and pyrrolidines are often biologically active compounds (Aniszewski, 2007).

Examples of such compounds include the following: piperine, which is responsible for the spicy flavors of peppers; nicotine and anabasine, which are found in tobacco; coniine, the highly toxic alkaloid that is present in *Conium maculatum* and is responsible for the poisoning and death of the famous philosopher Socrates; and ant venom pseudoalkaloids from the *Solenopsis* genus (popularly known as fire ants) (Fig. 1).

2.1. Biosynthetic pathways

True alkaloids, by definition, have amino acids as their natural precursors, thus piperidine and pyrrolidine are derived from L-lysine and L-ornithine respectively (Cordell, 2013), while pseudo piperidine alkaloids acquire their nitrogen atoms *via* transamination (Aniszewski, 2007). The general biosynthetic pathways of these alkaloids will be further discussed in the next sections.

2.1.1. Piperidine alkaloids

Piperidine alkaloids derived from lysine are denominated "true alkaloids" because their nitrogen atoms come from an amino acid. Only a few amino acids act as precursors in alkaloid biosynthesis, and L-lysine is one of the most significant. During biosynthetic processes, L-lysine is engaged in the biosynthesis of at least three alkaloidal core moieties: piperidine (C_5N), indolizine (C_5NC_3) and quinolizidine (C_5NC_4) (Aniszewski, 2007; Gupta et al., 1969; Szoke et al., 2013).

In the piperidine biosynthetic pathway, L-lysine (6) is first decarboxylated to cadaverine (7) in the presence of pyridoxal phosphate (PLP). Cadaverine (7) thus undergoes an oxidative deamination catalyzed by diamine oxidase, producing the corresponding amino aldehyde (8). The amino aldehyde 8 undergoes cyclization, resulting in the cyclic imine Δ^1-piperideine (9), which is the key intermediate in piperidine alkaloid biosynthesis (Scheme 1). Alternatively, Δ^1-piperideine can also be obtained by cyclization of the cadaverine-PLP decarboxylated complex (Szoke et al., 2013).

Δ^1-piperideine may undergo a series of sequential reactions to produce a myriad of piperidine alkaloids. For example, the reduction of Δ^1-piperideine and coupling with piperic acid (piperic acid-CoA ester, 10) produces piperine (1), an *N*-substituted piperidine alkaloid (Scheme 2) (Geisler and Gross, 1990). Δ^1-piperideine may also undergo nucleophilic addition to provide 2-substituted piperidine alkaloids, such as anabasine (3) and pelletierine (11) (Scheme 3). An intramolecular Mannich reaction of *N*-methyl pelletierine (12) produces pseudopelletierine (13), the main alkaloid found in the root-bark of the pomegranate tree (*Punica granatum*). Moreover, the intermolecular Mannich reaction of *N*-methyl pelletierine with another molecule of Δ^1-piperideine produces anaferine (14) (Szoke et al., 2013).

2.1.2. Pyrrolidine alkaloids

L-Ornithine (15) is the intermediate precursor to pyrrolidine (C_4N), pyrrolizidine (C_4NC_3) and tropane (C_4N+) alkaloids in plants. This

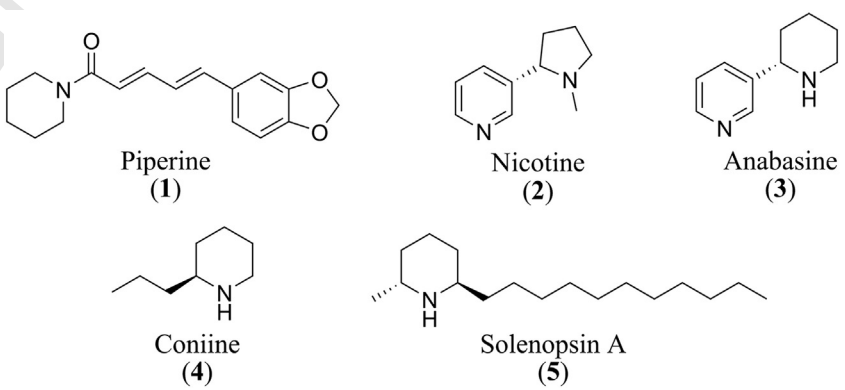

Fig. 1. Examples of piperidine and pyrrolidine alkaloids.

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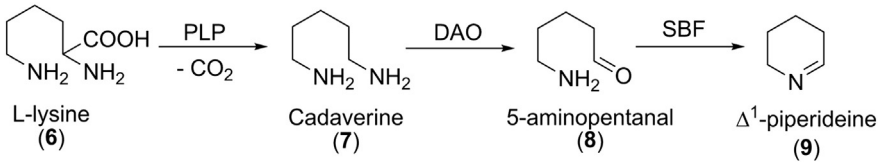
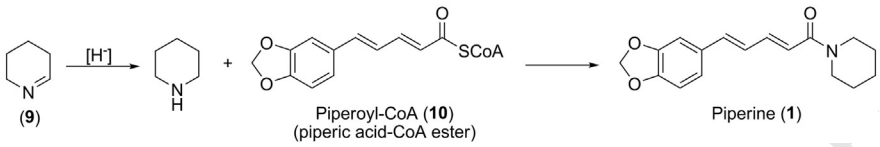
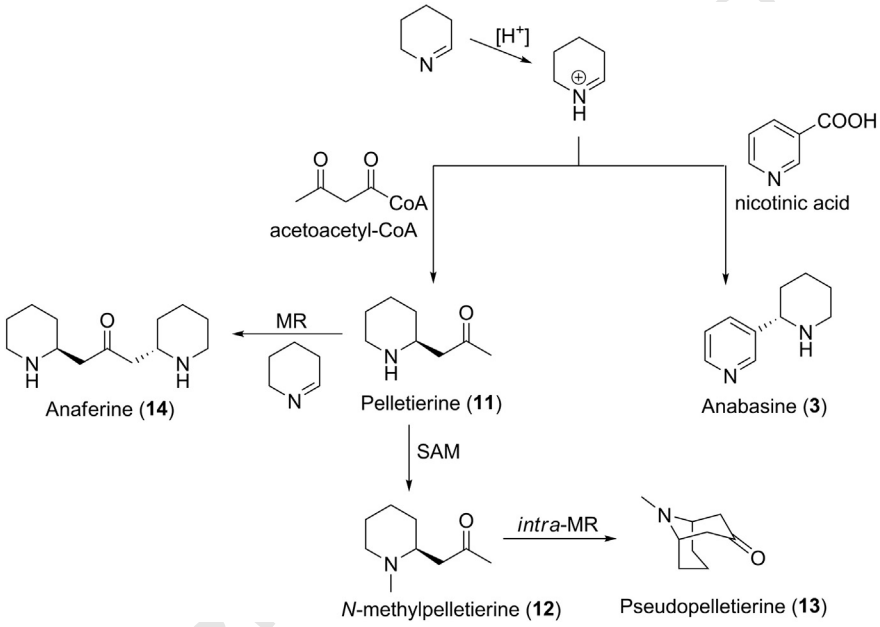


Scheme 1. Concise biosynthetic pathway to Δ^1-piperideine (9), the key intermediate in piperidine alkaloid biosynthesis. PLP: pyridoxal phosphate; DAO: diamine oxidase; SBF: Schiff base formation.



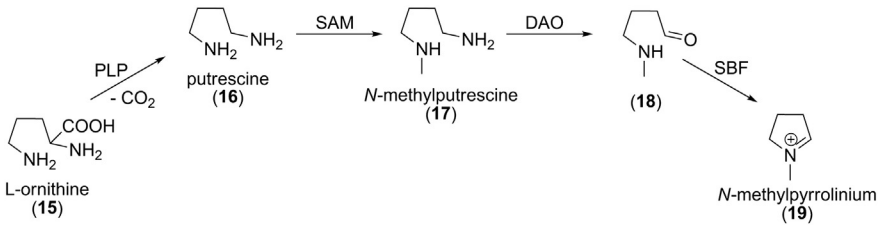
Scheme 2. Biosynthetic pathway to piperine (1): Δ^1-piperideine (9) and piperic acid coupling (10).



Scheme 3. Concise biosynthetic pathways to anabasine (3), pelletierine (11), pseudopelletierine (13) and anaferine (14): nucleophilic addition to Δ^1-piperideine and Mannich reactions. SAM: *S*-adenosyl methionine; MR: Mannich reaction.

non-protein amino acid is derived from L-glutamic acid and initiates the biosynthesis through a PLP-mediated decarboxylation, producing putrescine (16). Thus, putrescine is methylated by *S*-adenosyl methionine (SAM) to give *N*-methylputrescine (17), which is oxidized to the amino aldehyde 18 by diamine oxidase and cyclizes to the *N*-methyl-1-pyrrolinium cation (19). The *N*-methyl-1-pyrrolinium cation (19) is the key intermediate in pyrrolidine alkaloid biosynthesis (Scheme 4). Alternatively, this key intermediate may also be obtained through methylation, followed by cyclization, of the putrescine-PLP decarboxylated complex (Aniszewski, 2007; Philipov and Doncheva, 2013).

The *N*-methyl-1-pyrrolinium cation (19) is then readily converted to hygrine (20) by acetylation. Hygrine is the first alkaloid in this biosynthetic route, and many tropane alkaloids are synthesized from hygrine by way of acetyl CoA, hydrolysis and intramolecular Mannich reactions. These alkaloids include cuscohygrine (21), tropinone (21) and cocaine (23), which present outstanding biological activities, particularly as neurotransmitters (Hoye et al., 2000; Philipov and Doncheva, 2013).



Scheme 4. General biosynthetic pathway to *N*-methyl-1-pyrrolinium cation (19), the key intermediate in pyrrolidine alkaloid biosynthesis. PLP: pyridoxal phosphate; SAM: *S*-adenosyl methionine; DAO: diamine oxidase; SBF: Schiff base formation.

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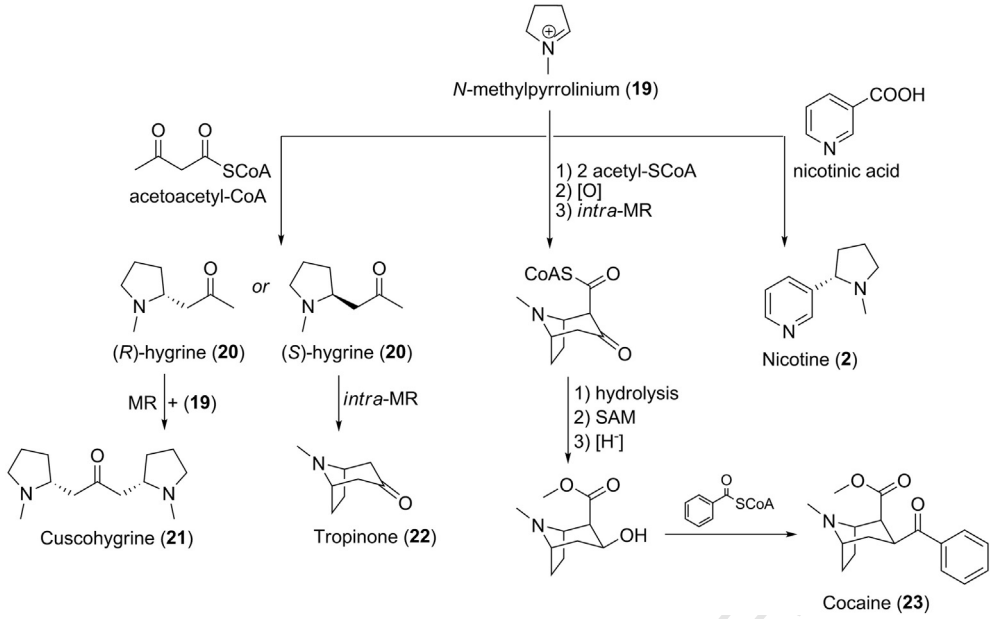


Scheme 5. Concise biosynthetic pathways to hygrine (20), cuscohygrine (21), tropinone (22), cocaine (23) and nicotine (2). MR: Mannich reaction; SAM: *S*-adenosyl methionine.

Additionally, other monosubstituted pyrrolidine alkaloids, such as nicotine (2), are obtained by nucleophilic addition to 19 (Scheme 5) (Bush et al., 1999).

2.1.3. Piperidine pseudoalkaloids

Some piperidine-containing alkaloids are not derived from amino acids and are thus classified as pseudoalkaloids. They commonly have a biosynthetic origin from acetate, and the nitrogen atom is inserted *via* transamination. The best-known examples are pinidine (24), coniine (4) and solenopsin alkaloids (5).

The proposed biosynthetic pathway for these alkaloids (Binev, 2013; Leclercq et al., 1996; Leete and Juneau, 1969) involves the cyclization of diketones or ketoaldehydes derived from the linear combination of acetate units. The introduction of an amino group with a subsequent intramolecular cyclization and imine reduction yields the piperidine alkaloids (Scheme 6).

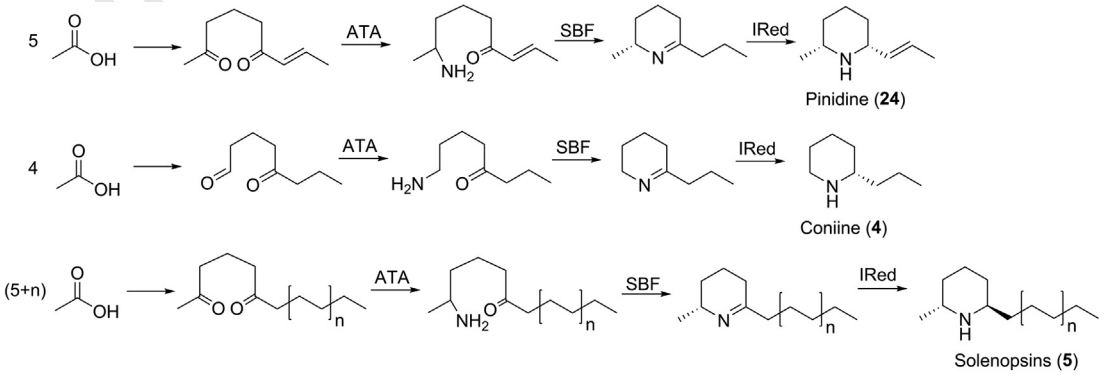
Therefore, it is clear that cyclization plays an important role in the biosynthesis of piperidine and pyrrolidine true alkaloids, which leads to the key intermediates Δ^1-piperideine (9) and *N*-methyl-1-pyrrolinium cation (19). Moreover, the piperidine pseudoalkaloid biosynthetic pathway shows that aminoaldehydes or aminoketones, the common precursors of 9 and 19, respectively, can also have an acetate origin instead of an amino acid origin.

Furthermore, the biosynthetic pathways presented here should inspire several chemo- and multi-enzymatic processes for the *in lab* production of piperidine and pyrrolidine alkaloids, which are discussed further in the next sections.

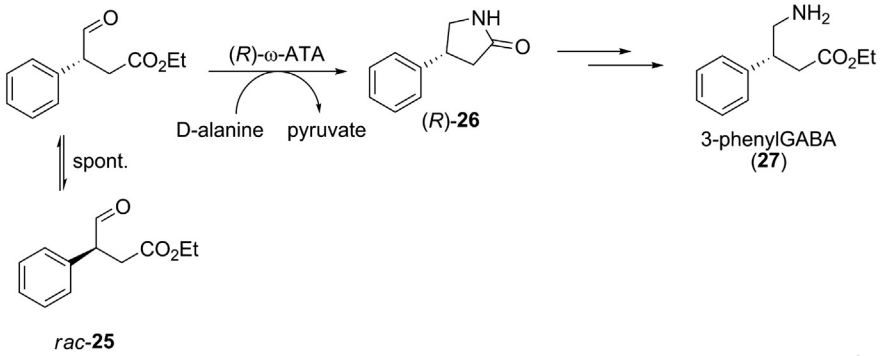
2.2. Biocatalytic approach to the synthesis of piperidine and pyrrolidine alkaloids

The asymmetric syntheses of piperidine and pyrrolidine alkaloids have been performed using chiral chemical catalysts, leading to enantiomerically pure products in appreciable yields (Abrunhosa-Thomas et al., 2013; Amat et al., 2003; Bhat and Tilve, 2013; Buffat, 2004; Mix and Blechert, 2007; Vu et al., 2013). However, enzymatic processes have gained popularity due to the requirements of mild reaction conditions and high stereoselectivity, which follow the 12 Principles of Green Chemistry.

The key step in piperidine and pyrrolidine alkaloid biosynthetic pathways involves the spontaneous cyclization of aminoketones or aminoaldehydes. Biotechnologically, these compounds can be readily obtained by enzymatic transamination of the respective diketones or ketoaldehydes using ω-transaminases. Moreover, the transamination of keto or aldehyde esters provides piperidones and pyrrolidones, which can be further converted into their respective piperidines and pyrrolidines (Kohls et al., 2014; Simon et al., 2014).



Scheme 6. Concise biosynthetic pathways to pinidine (24), coniine (4) and solenopsin alkaloids (5). ATA: transaminase; SBF: Schiff base formation; IRed: imine reductase.



Scheme 7. Synthesis of enantiomerically enriched (*R*)-4-phenylpyrrolidin-2-one (26).

The dynamic kinetic resolution of 4-oxo-3-phenylbutyric acid ethyl ester (25), applying a ω-transaminase, was the first reported biocatalytic synthesis based on the principles above (Koszelewski et al., 2009). The enzymatic transamination, followed by spontaneous cyclization of 25, produces the enantiomerically enriched (*R*)-4-phenylpyrrolidin-2-one (26). The hydrolysis of 26 provides the corresponding acyclic γ-aminobutyric acid (GABA) analog (27) (Scheme 7).

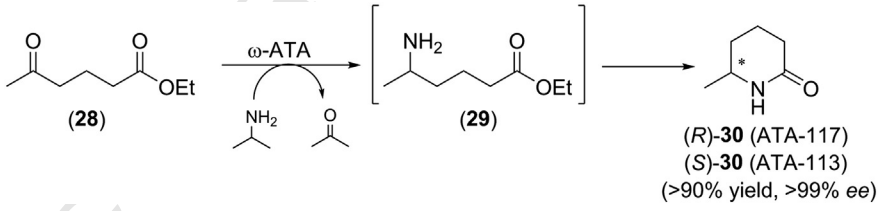
Truppo et al. (2010) applied a similar approach aimed at solving the irreversibility and product inhibition of reactions involving ω-transaminases. Ethyl 4-acetylbutyrate (28) was chosen as a model substrate. Upon transamination, the aminoester product (29) spontaneously cyclizes to 6-methyl-2-piperidone (30), driving the reaction to completion (Scheme 8).

Applying the same approach, a ω-transaminase (ATA-117) was used to produce piperidone 33 by the reductive amination of ketodiester 31 and the spontaneous cyclization of aminodiester 32 (Scheme 9). The piperidone 33 is a key intermediate in the production of an orexin receptor antagonist (34), which is a promising candidate for sleep regulation preclinical tests (Girardin et al., 2013).

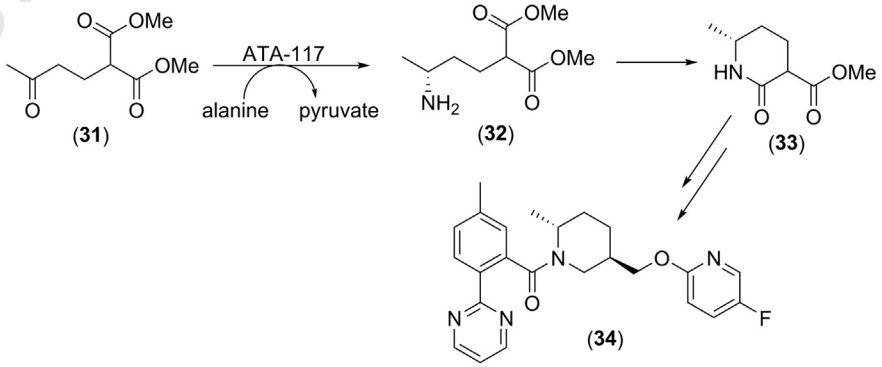
A similar methodology was also applied as the key step in the synthesis of niraparib (35), an orally active poly(ADP-ribose)polymerase inhibitor with potential anticancer activity (Chung et al., 2013). In this case, transamination was obtained with aldehyde 36 and ATA-301, resulting in the lactam 37 in 82% yield and >99% *ee*. However, aldehyde 36 is quite unstable, and this methodology was adapted to the use of a bisulfite adduct (38), which releases the parent aldehyde *in situ* under the basic reaction conditions (Scheme 10).

However, the first example of diketone monoamination followed by spontaneous cyclization, leading to a Δ^1-piperideine intermediate, was described by Simon et al. (2012). In their work, the regio- and stereoselective monoamination of several 1,5-diketones (39a–e, Scheme 11) were evaluated using six enantioselective whole-cell transaminases. The authors reported that the transamination occurs preferentially in the less hindered carbonyl moiety (methyl ketone) producing piperideines 42a–e (Scheme 11). Moreover, the reactions were, in most cases, perfectly enantioselective (>99% *ee*) and the product stereochemistry can be easily controlled by using transaminases of different enantioselectivities.

Furthermore, the enantiopure piperideines (42a–e) obtained were readily hydrogenated (H_2, Pd/C), yielding *cis*-piperidines with high diastereoselectivity (>99% *de*). The preparative synthetic potential of this method was demonstrated in the synthesis of the natural alkaloid



Scheme 8. Transamination of ethyl 4-acetylbutyrate (28) followed by spontaneous cyclization to 6-methyl-2-piperidone (30), using isopropylamine as the amine donor.



Scheme 9. Enzymatic synthesis of piperidine 33 by transamination and spontaneous cyclization of ketodiester 31.

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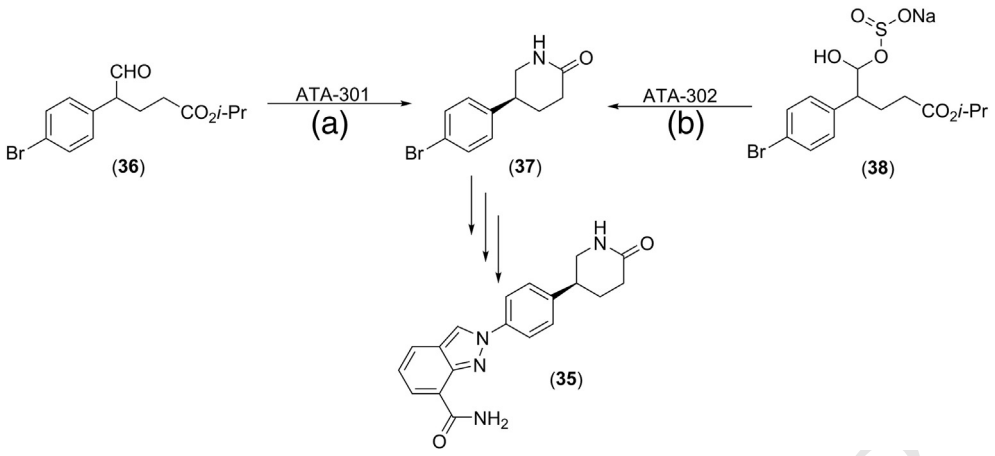
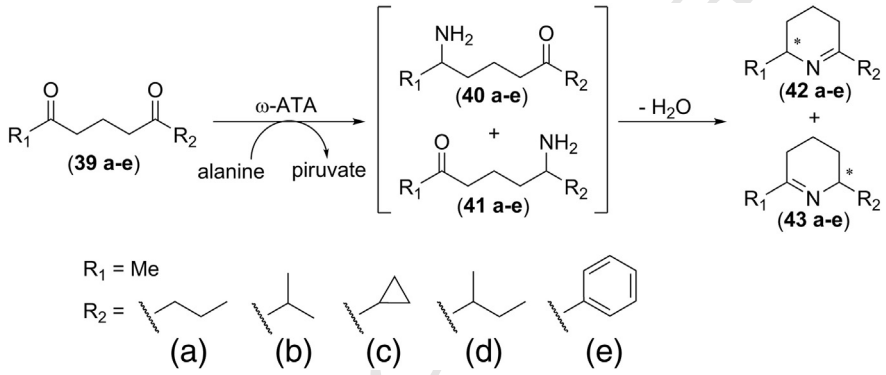


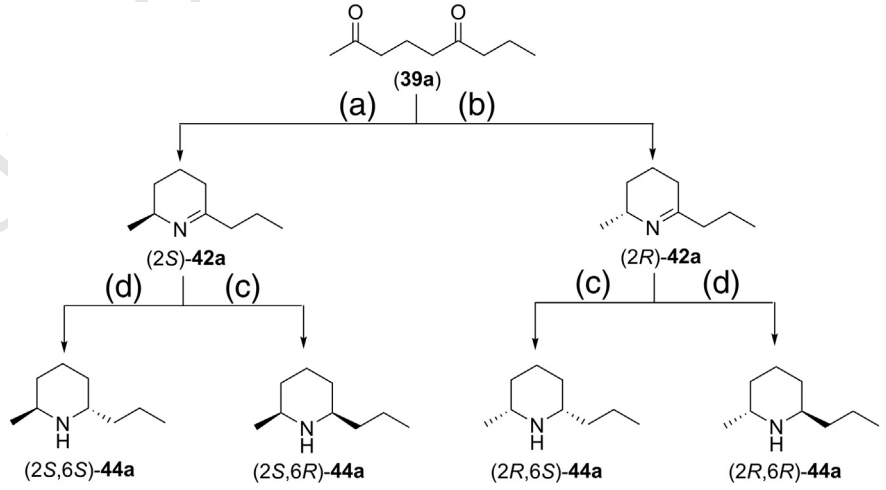
Scheme 10. Biocatalytic approach for the enantioselective synthesis of niraparib (35). Reaction conditions: (a) ATA-301 (50 wt.%), PLP, *i*-PrNH$_2$, pH 10.5 buffer, DMSO, 45 °C, 42 h. (b) Same conditions as in (a); however, using ATA-302 (35 wt.%).



Scheme 11. Regioselective monoamination of 1,5-diketones (39a–e) catalyzed by ω-transaminases (ω-ATA).

(+)-dihydropinidine ((2*S*,6*R*)-44a), a potential antifeedant against the pine weevil *Hylobius abietis*, and its enantiomer (2*R*,6*S*)-44a, which were obtained in 94% and 85% yield, respectively, over two steps (Scheme 12, reaction condition c).

Ant venom alkaloids, such as solenopsins and isosolenopsins, are an important group of natural products with outstanding biological activities (Fox et al., 2012; Pianaro et al., 2012). In this context, the above chemoenzymatic methodology was also applied to isosolenopsin (47)



Scheme 12. Chemoenzymatic synthesis of all four stereoisomers of the alkaloids dihydropinidine and *epi*-dihydropinidine 44a. Reagents and conditions: a) diketone 39a, ω-TA from *C. violaceum*, PLP, NAD^+, L-alanine, ammonium formate, FDH, AlaDH; 26 h, 30 °C, 120 rpm; b) conducted as for (a), but with (*R*)-ω-TA from *Arthrobacter* sp. and D-alanine; c) Pd/C, H_2, 4 h, 22 °C; d) Et_3Al, $LiAlH_4$, THF, 78 °C, 2 h.

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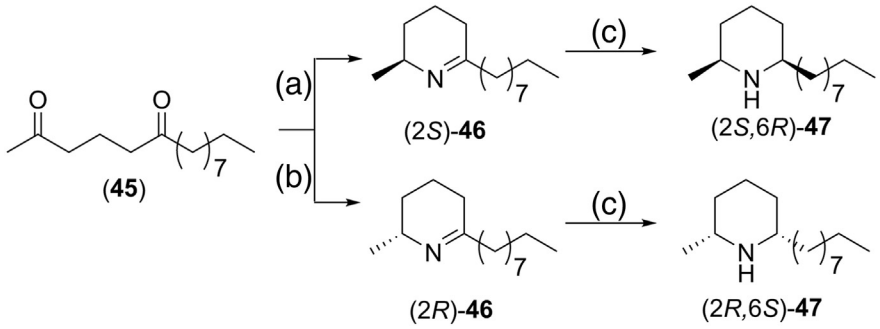


Scheme 13. Chemoenzymatic synthesis of both enantiomers of isosolenopsins (2*S*,6*R*)-47 and (2*R*,6*S*)-47. Reagents and conditions: (a) diketone 45 dissolved in DMF (20 vol.-%), ω-TA from *Arthrobacter citreus*, PLP, NAD^+, L-alanine, ammonium formate, FDH, AlaDH, 48 h, 40 °C, 700 rpm; (b) same as for (a), but with the ω-TA from (*R*)-*Arthrobacter* sp. at 30 °C with D-alanine and 20 vol.-% *N*-heptane, 5 vol.-% DMF; (c) Pd/C, H_2 (1 atm.), 4 h, 22 °C.

production from pentadecane-2,6-dione (45) (Simon et al., 2013a). The initial low conversions were overcome by optimizing the biocatalytic reaction conditions through medium engineering (temperature, pH and co-solvent). This strategy afforded isosolenopsin (47) in optically pure form, with an overall yield of 64–65% (Scheme 13).

Moreover, a new chemoenzymatic process for obtaining the *trans* enantiomers of 2,6-dialkylpiperidines ((2*S*,6*S*)-44a and (2*R*,6*R*)-42a) has been published (Simon et al., 2013b). The key step of this methodology involves the piperideine conformational change during the reduction step mediated by a Lewis acid (Scheme 12, reaction condition d).

So far, none of the methodologies addressed here, with the exception of those that remove the keto product derived from the amino donor, apply cascade reactions that involve two or more enzymes.

In this context, O' Reilly et al. (2014) developed a multi-enzymatic cascade process, involving transaminases and monoamine oxidases (MAO-N), to obtain 2,5-disubstituted pyrrolidines (50) from the corresponding 1,4-diketones (48) (Scheme 14). The use of a highly selective ω-transaminase, coupled with a diastereoselective MAO-N/$NH_3 \cdot BH_3$ chemoenzymatic cascade, enables reactions to proceed with excellent enantio- and diastereoselectivities (>94% *ee*; >98% *de*).

The success of this methodology relies on the complementary regio- and stereoselectivity displayed by both enzymes: ω-TA is highly selective for the less sterically demanding ketone (methyl or ethyl), whereas MAO-N prefers the bulkier portion of the corresponding pyrrolidine (phenyl moiety). Moreover, the compatibility of the two enzymes allows a one-pot reaction design.

Despite the one-pot multi-enzymatic approach, the step involving MAO requires a prior non-selective chemical reduction of the pyrroline intermediate for the subsequent enzymatic oxidation in a classical chemoenzymatic dynamic kinetic resolution process (Scheme 14).

Therefore, a "pure" multi-enzymatic process can only be achieved using reductases, such as imine reductases (IRED), which are responsible for imine reduction and depend on NAD(P)H as a cofactor. These enzymes would be perfectly suited to piperidine and pyrrolidine alkaloid syntheses because they prefer to catalyze piperideine and pyrroline reductions.

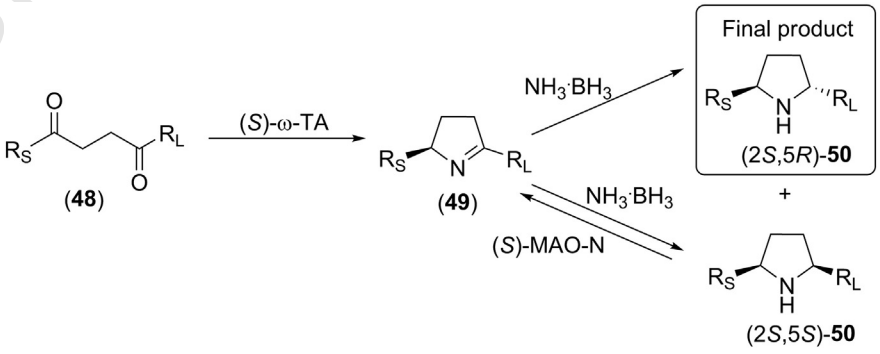
Several studies have pointed to the successful reduction of cyclic imines using imine reductases (Scheme 15) (Gand et al., 2014; Huber et al., 2014; Leipold et al., 2013; Mitsukura et al., 2010, 2011, 2013; Rodríguez-Mata et al., 2013; Scheller et al., 2014). However, multi-enzymatic processes involving transaminase and imine reductase cascades have not yet been reported.

Therefore, the biocatalytic processes inspired by the piperidine and pyrrolidine alkaloid biosynthetic pathways involve transaminases as the most important enzymes and spontaneous cyclization as the key step to produce piperideine and pyrroline intermediates. Moreover, monoamine oxidases and imine reductases can also be applied to modify the piperideine and pyrroline intermediates to their respective piperidine and pyrrolidine alkaloids in a chemo- or multi-enzymatic approach. The general catalytic properties of these three enzymes are discussed further in the next sections.

2.3. Enzymes related to the proposed alkaloid biocatalytic synthesis

2.3.1. Transaminases

Transaminases belong to the family of pyridoxal phosphate-dependent enzymes (PLP) and are ubiquitous to all organisms, primarily participating in amino acid metabolism, but not exclusively. This enzyme class can be divided into four subgroups: I, II, III and IV. The subgroups I, III, and IV encompass enzymes that transfer, exclusively, the amino group from the α-carbon of an amino acid and are named α-transaminases. However, subgroup II transaminases are capable of transferring the amino group from a non-α-carboxylic carbon and are conventionally named ω-transaminases (ω-ATA) (Turner and Truppo, 2013b).



Scheme 14. Chemoenzymatic process for the synthesis of 2,5-disubstituted pyrrolidines (50), using an (*S*)-selective ω-transaminase ((*S*)-ω-ATA) and (*S*)-selective monoamine oxidases ((*S*)-MAO-N). R_S = methyl or ethyl, R_L = phenyl derivatives.

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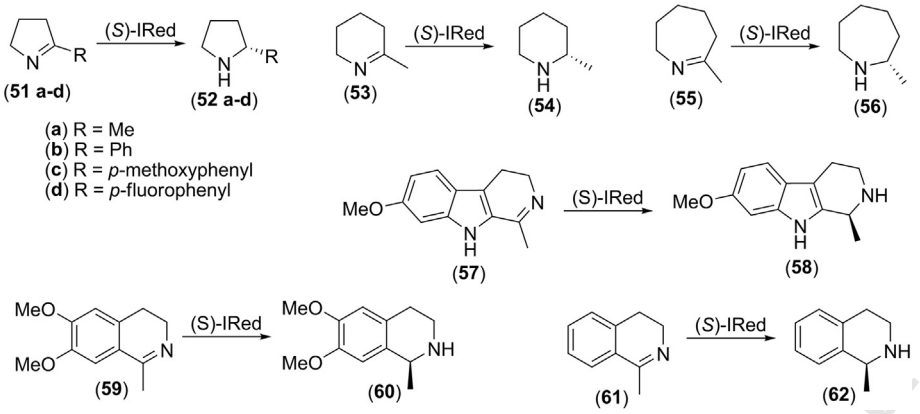


Scheme 15. Examples of cyclic imine reductions catalyzed by an (*S*)-selective imino reductase from *Streptomyces* sp. GF3546. Leipold et al., 2013.

The transaminase catalytic mechanism consists of two half-reactions in a ping-pong format (Scheme 16). Initially, the internal aldimine formed between a lysine residue of the protein and the aldehyde group of PLP is replaced by an external aldimine formed between an amino donor and PLP. A sequence of rearrangements culminates in the formation and hydrolysis of the pyridoxamine intermediate (PMP). The subsequent reaction of the PMP with an amino acceptor starts the second half of the reverse reaction, regenerating PLP bound to the enzyme and releasing the amino product (Turner and Truppo, 2013b; Willies, 2012).

Biotechnologically, the ω-ATA enzymes are more versatile than the α-ATA enzymes and are applied to the resolution of racemic amines and the asymmetric synthesis of amines from prochiral ketones (Scheme 17). The latter application has received special attention and is currently the preferred path leading to the production of chiral amines on preparative scale. Industrially, transaminases have been mainly used for the production of amino acids from ketoacids. However, the main goal of recent research in this area has been the application of these enzymes to produce chiral amines from the respective prochiral ketones, exploring structural diversity at its full extent (Malik et al., 2012; Mathew and Yun, 2012).

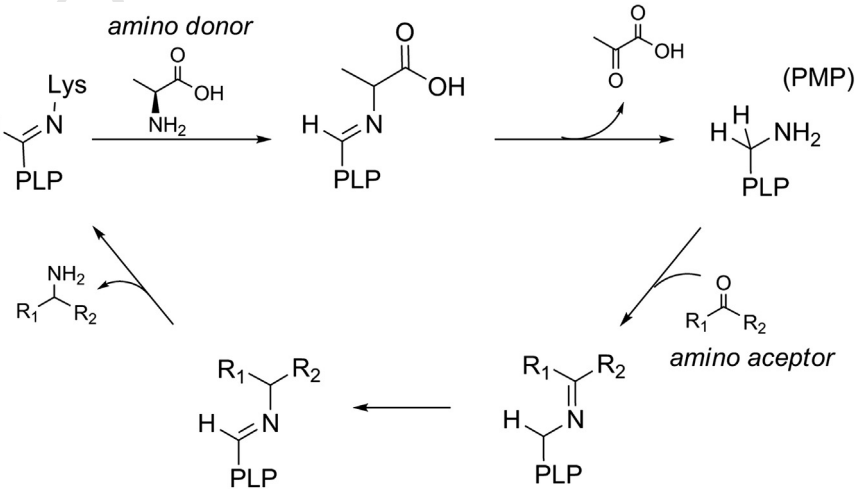
A notable recent example of a biotechnological process involving transaminases was developed by Merck and Codexis and applies a genetically improved transaminase for industrial production of the antidiabetic drug sitagliptin (64) (Savile et al., 2010). A variant of (*R*)-ATA117 (from *Arthrobacter* sp.) with 27 accumulated mutations was capable of converting the ketone precursor prositagliptin (63, 200 g L^{-1}) to sitagliptin, with an enantiomeric excess greater than 99.9% and in a 92% yield in 50% DMSO (Scheme 18). Compared to the chemical process, this biocatalytic process increased the overall yield by 10–13% and the productivity by 53%. This process reduced the total waste generated by 19% and avoided the use of metallic catalysts.

2.3.2. Imine reductases

Imine reductases (IRed) catalyze the reduction of imines to their respective amines, using NAD(P)H as a cofactor (Scheme 19a). The catalytic mechanism of these enzymes is not fully elucidated because there are only a few structural studies focused on them. However, Rodríguez-Mata et al. (2013) proposed a simple mechanism for *Streptomyces kanamyceticus* IRed based on proton transfer from an aspartic acid residue to an *N*-imine, which activates the substrate for NAD(P)H hydride transfer (Scheme 19b).

The enzymatic reduction of imines receives little attention due to the lack of known enzymes with this specific activity. However, recent studies involving asymmetric reduction of imines by *Streptomyces*, *Streptosporangium*, *Paenibacillus* and *Pseudomonas* whole cells or recombinant enzymes, as well as their structural characterization, have been published (Gand et al., 2014; Huber et al., 2014; Leipold et al., 2013; Mitsukura et al., 2010, 2011, 2013; Rodríguez-Mata et al., 2013; Scheller et al., 2014).

In addition to the scarce occurrence of imine reductases, the main challenges to applying IREDs in biocatalysis include their low activity



Scheme 16. General catalytic mechanism of transaminases.

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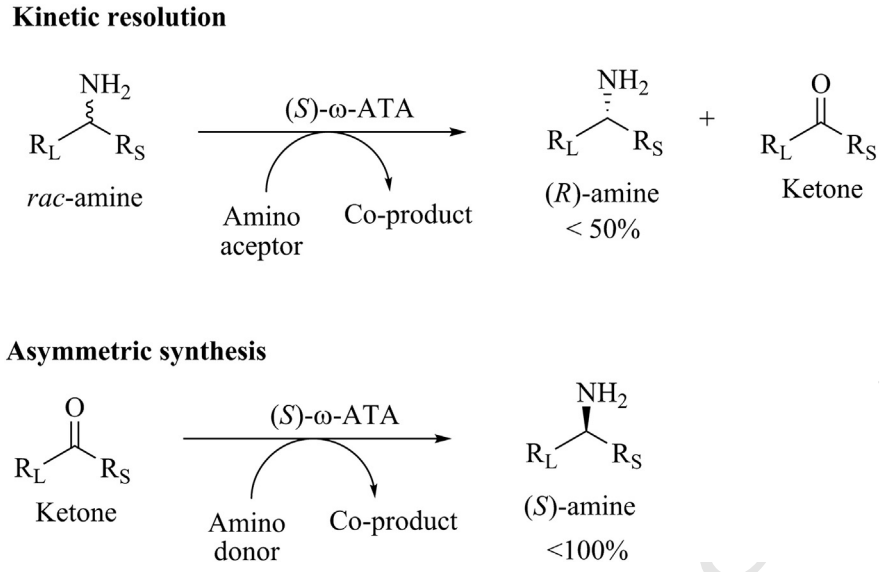
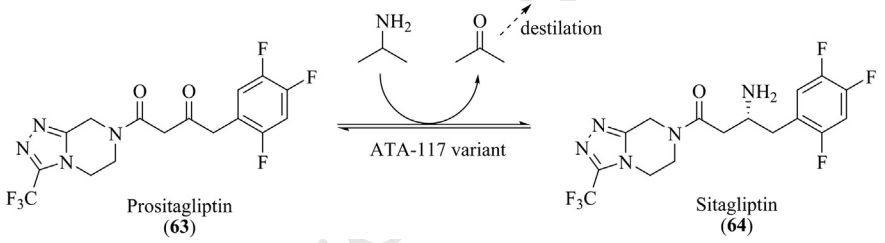
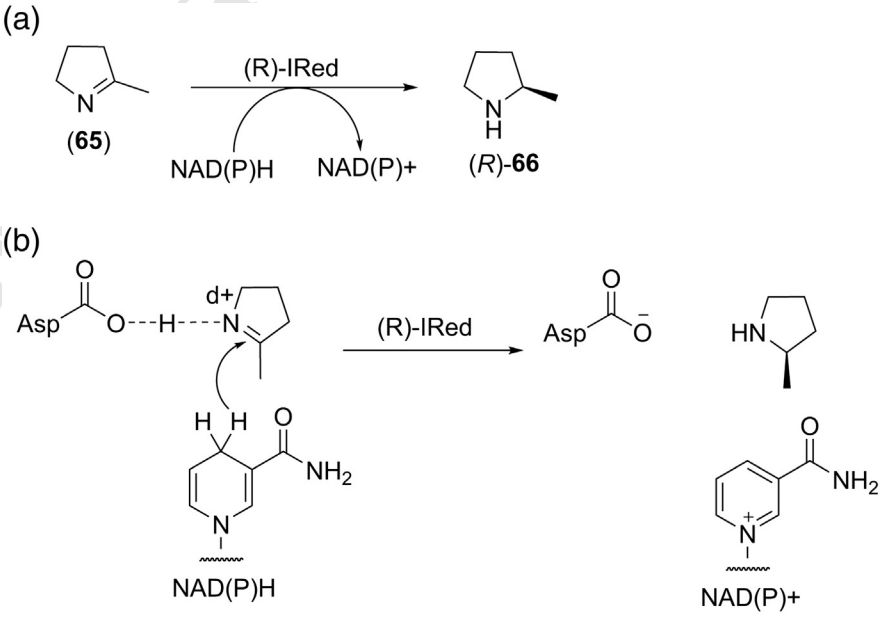


Scheme 17. Enzymatic systems applying (*S*)-ω-transaminases. In a kinetic resolution process, (*S*)-ω-ATA converts, preferentially, the (*S*)-amine to the corresponding ketone, as the (*R*)-amine remains in solution. However, the process results in the enantiopure amine with a maximum yield of 50%. In the asymmetric synthesis, (*S*)-ω-ATA may convert the prochiral ketone to an enantiomerically pure amine with a yield up to 100%, depending on the equilibrium conditions.



Scheme 18. Asymmetric synthesis of sitagliptin (64) from the prochiral ketone 63 using a (*R*)-ATA117 variant.



Scheme 19. Imine reductases. (a) Overall reaction for 2-methyl-1-pyrroline (65) reduction catalyzed by an (*R*)-selective imine reductase. (b) Catalytic mechanism proposed for the *Streptomyces kanamyceticus* (*R*)-IRed.

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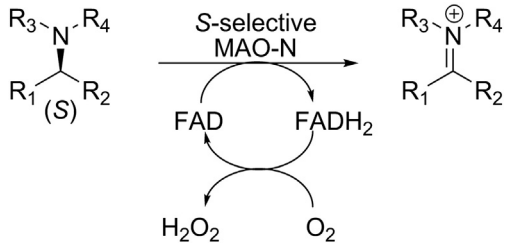


Scheme 20. General catalytic reaction of monoamine oxidases.

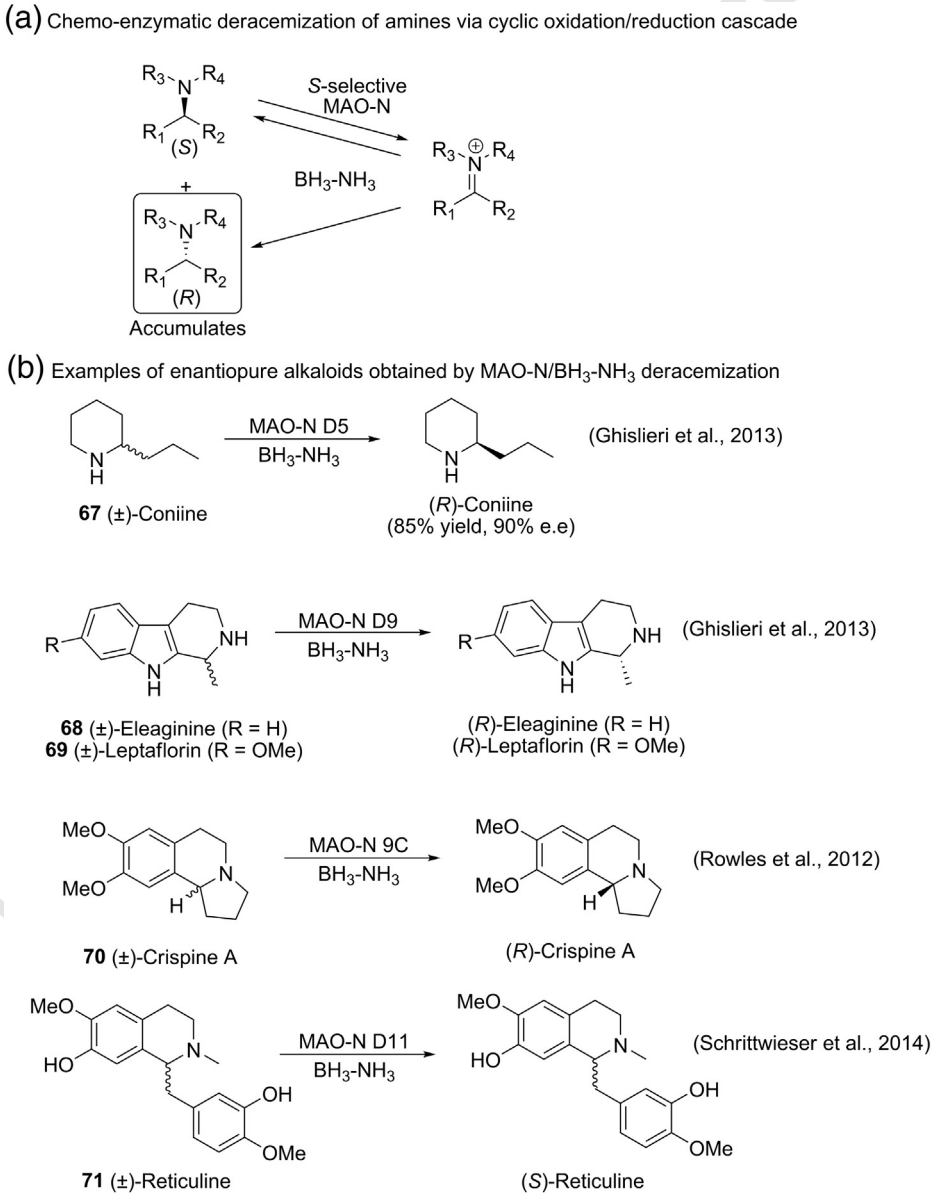
and narrow substrate specificity. These features were primarily attributed to a structure divergence between the evaluated imines and the 'true' natural substrate, which has not yet been identified, or to the fact that imine reduction might occur in a promiscuous manner. Additionally, cyclic imine reduction is the main biotechnological application of IREDs, and only a few studies have reported the reduction of acyclic imines (Gand et al., 2014; Huber et al., 2014).

2.3.3. Monoamine oxidases

Monoamine oxidases (MAO, EC 1.4.3.4) are flavin-dependent enzymes that catalyze the oxidation of C–N to C═N. The catalytic mechanism involves the flavin-oxidized form (FAD), which mediates the electron-transfer process *via* its reduced form ($FADH_2$). The oxidized cofactor is regenerated by molecular oxygen at the expense of releasing hydrogen peroxide (Scheme 20) (Turner, 2012).

Monoamine oxidase N from *Aspergillus niger* (MAO-N) is the most studied MAO for biotechnological purposes. This enzyme was first identified in 1995 by Schilling and Lerch (1995a, 1995b); however, the wild-type enzyme only catalyzes the oxidation of simple amines, such as butylamine, amylamine and benzylamine. Therefore, aiming to improve its substrate tolerance, MAO-N has been subjected to several rounds of directed evolution and screening to identify variants that possess the desirable activities.

Biotechnologically, MAO-N variants are applied to the deracemization of both acyclic and cyclic amines in a chemoenzymatic approach (Turner, 2011). The coupling of an enantioselective MAO-N with a nonselective chemical reducing agent enables the production of several chiral amines



Scheme 21. Monoamine oxidases. a) Chemoenzymatic deracemization of amines using an *S*-selective MAO-N and BH_3–NH_3 as a nonselective chemical reducing agent. b) Deracemization of alkaloids by the MAO-N/BH_3–NH_3 cyclic cascade.

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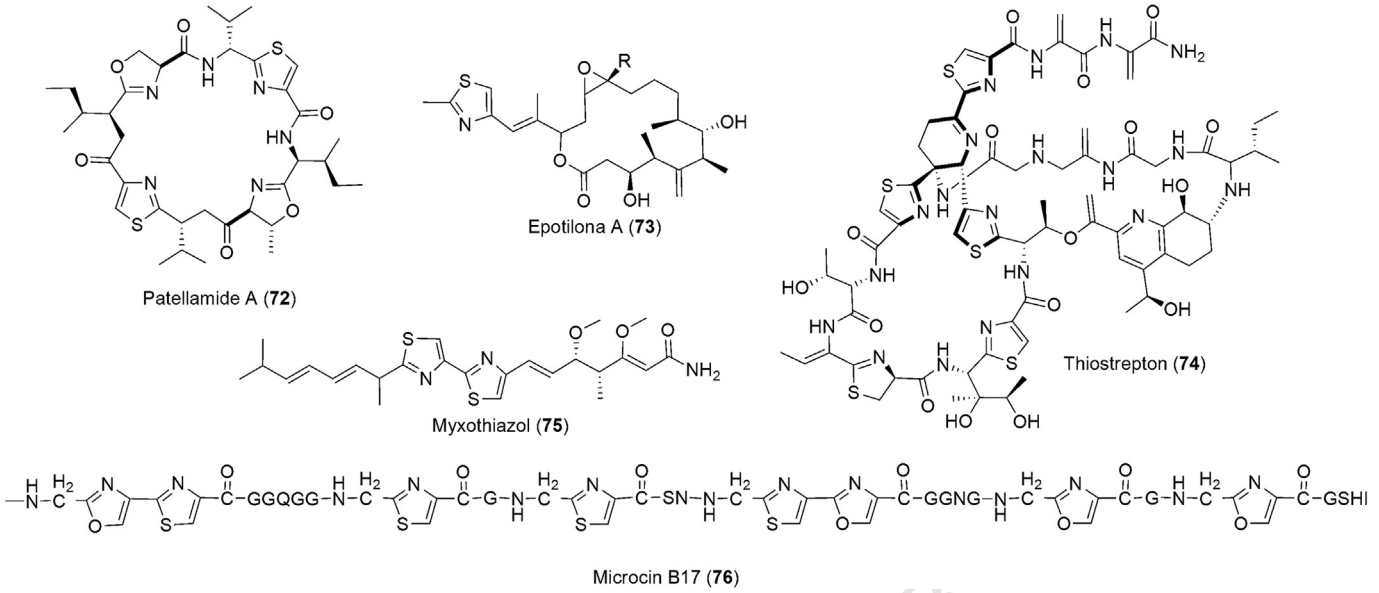


Fig. 2. Examples of nitrogen heterocycles with diverse pharmacological activities.

(Scheme 21a), including many alkaloids (Bechi et al., 2014; Foulkes et al., 2011; Ghislieri et al., 2013; Köhler et al., 2012; Rowles et al., 2012; Schrittwieser et al., 2014a, 2014b). Some examples are coniine (67), eleaginine (68), leptaflorin (69), crispine A (70) and reticuline (71) (Scheme 21b).

3. Azole-containing peptides

Azole rings are highly spread throughout natural products that contain peptide scaffolds (Cragg and Newman, 2013; Fisch, 2013; Nolan and Walsh, 2009). They are produced ubiquitously and comprise the heterocyclic peptide family, which possesses thiazole, oxazole and methylthiazole (and their precursor reduced partners thiazoline, oxazoline and methylthiazoline) moieties.

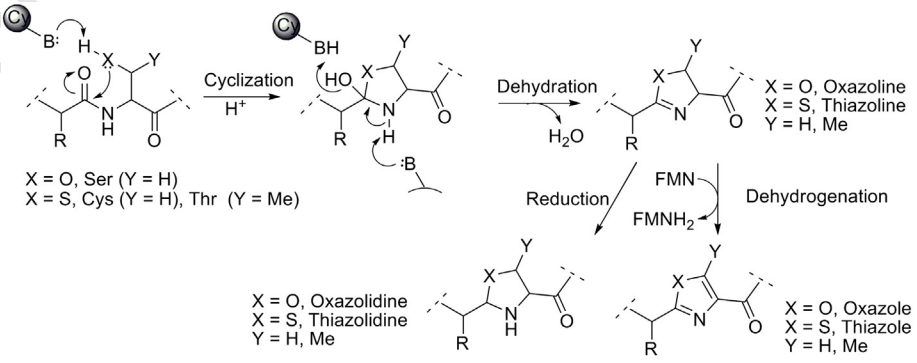
Oxazole and thiazole alkaloids are widely known for their potential pharmacological activity, including antitumor agents, such as epothilone A (73) and bleomycin (Fischbach and Walsh, 2006; Gerth et al., 1996; Schneider et al., 2003); antibiotics, such as patellamide A (72), thiostrepton (74) and the microcins (76) (Fischbach and Walsh, 2006; Fu et al., 1998; Nolan and Walsh, 2009); competitive inhibitors, such as myxothiazol (76) (Silakowski et al., 1999); and antifungals, such as micafungin (Kathiravan et al., 2012), among others (Fig. 2).

3.1. General biosynthetic pathway

Oxazole and thiazole alkaloids are nitrogen heterocycles classified as azoles. Azoles can contain additional elements, such as sulfur and oxygen. The natural products containing peptide alkaloids are biosynthesized by multi-modular non-ribosomal peptide synthetases (NRPS) and hybrid PKS-NRPS (Nolan and Walsh, 2009). In addition to the nonribosomal peptides, ribosomally synthesized and posttranslationally modified peptides (RiPPs) are a powerful source of thiopeptides and linear azole-containing peptides (LAPs), which include cyanobactins and microcins (Banala et al., 2013; Nolan and Walsh, 2009).

The primary structure of a nonribosomal peptide is dictated by the organization of the modular NRPS (Schwarzer and Marahiel, 2001). Optional domains lead to modification of the anchored amino acids, and the heterocyclization promoted by cyclization (Cy) domains generate structural variation in the peptide backbone. Conversely, the RiP peptides are products of the maturation of ribosomal protein precursors.

The NRPs and RiPPs containing cysteine (Cys), serine (Ser) and threonine (Thr) residues are ideal substrates for enzymatic heterocyclization, with Cys-heterocyclization occurring faster than Ser- and Thr-heterocyclization due to the nucleophilicity of sulfur (Belshaw et al., 1998; Nolan and Walsh, 2009). The cyclization is instantly followed by dehydration of the hydroxyl-containing intermediate, resulting in an



Scheme 22. Proposed mechanism for the enzymatic cyclodehydration of serine and cysteine/threonine residues to oxazolines or thiazolines. FMN-dependent dehydrogenation results in oxazole/thiazole five-membered rings, whereas reduction results in oxazolidines/thiazolidines.

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oxazoline (Ser/Thr) or thiazoline (Cys) ring. Subsequently, azoline oxidation to the azole is carried out by an FMN-containing oxidase. Alternatively, a reductase can promote azol(idin)e formation (Scheme 22).

Microcin B17 (76) was the first azole-containing peptide surveyed to provide insight into the heterocyclization biosynthetic pathway (Li et al., 1996; Melby et al., 2011; Nolan and Walsh, 2009). Other studies related to thiazole/oxazole heterocyclization (Crosa and Walsh, 2002; Kelly et al., 2009; Liao et al., 2009) and other azole structures (Braunshausen and Seebeck, 2011; Seebeck, 2010) helped elucidate the reaction mechanism, identify the genes responsible for the enzyme machinery and obtain new biologically active compounds (Baltz, 2006; Kwon et al., 2012; Patel and Walsh, 2001; Roy et al., 1999; Walsh et al., 2001, 2012).

Because naturally the individual catalytic domains involved in heterocyclizaton have autonomy to promote amide bond formation and cyclization of the newly formed amide, they are excellent candidates to be employed in the synthesis of valuable and complex building blocks through enzymatic cascades (Burkart and Tao, 2008; Chotani et al., 2000; Lopez-Gallego and Schmidt-Dannert, 2010). Some successful designs will be discussed in the next section.

3.2. In vitro cascade processes leading to peptide alkaloids

Studies to elucidate the assembly line for piochelin (77) led to the identification of four genes (PchD, E, F and G) that encode the enzymes required for the complete synthesis of the peptide in a modular NRPS of *Pseudomonas aeruginosa* (Reimmann et al., 1997, 1998, 2001). The *in vitro* reconstruction of piochelin from salicylate and two molecules of L-cysteine was successfully promoted in a one-pot reaction by Patel and Walsh (2001). In this cascade, 11 catalytic domains participate in at least 13 chemical steps involving a multi-enzymatic approach.

The biosynthetic pathway involves the formation of two thiazoline rings *via* Cys-cyclodehydration catalyzed by Cy domains in PchE and PchF modules. The subsequent reduction of one five-membered ring, mediated by a NADPH-dependent thiazoline reductase (PchG), led to a thiazole-thiazolidine compound. For the final steps, a methyltransferase (MT) domain from PchF promotes *N*-methylation and cooperates with a TE domain to release piochelin (Scheme 23).

A hybrid modular system containing cyclization (Cy) and oxidation (Ox) domains was designed by Duerfahrt et al. (2004) to evaluate the cascade reaction and the activity of individual modules in thiazol(in)e/oxazol(in)e heterocycle formation.

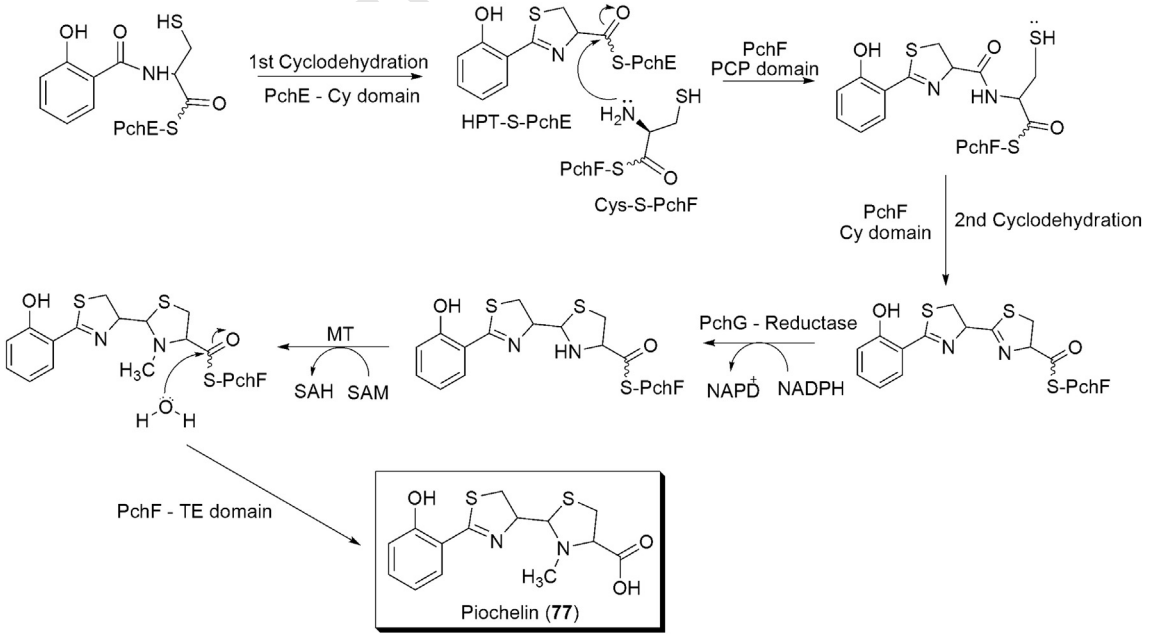
To evaluate the bimodular system (I), modules 1 (Ile-PCP) and 2 (Cy-Cys-PCP) from the bactracin biosystem were fused with a TE domain from tyrocidine C synthetase (BacA1-2-Te). The bimodular NRPS was incubated with ATP, isoleucine and cysteine, and the expected dipeptide IleCysThiazoline (78) was successfully obtained. Moreover, two hybrid bimodular fusions (II and III), one containing BacA1 (Ile-PCP) and the cyclization (Cy-Thr-PCP) module from mycobactin and the other one containing BacA1 (Ile-PCP) and the cyclization-oxidation (Cy-Cys-Ox-PCP) module from myxothiazol, were designed to exploit the potential to combine Cy domain-containing modules (Scheme 24). Both hybrid synthetases were capable of promoting product accumulation, paving the way for the rational engineering of hybrid synthetases to produce unprecedented peptide alkaloids.

4. Cyclic ethers from complex polyketides

Cyclic ether moieties are structural features of many bioactive natural products. Cyclic ethers containing 5 to 9 members are found particularly in polycyclic ethers, which comprise the polyether ionophores and the marine polyether ladders (Gallimore, 2009). Both classes of compounds contain oxygen-rich cores with low flexibility. The ionophores are capable of chelating cations, forming lipid soluble complexes capable of crossing biological membranes (Russell and Houlihan, 2003). The marine polyethers are highly toxic molecules with distinct ladder structures. Maitotoxin is the largest and the most toxic known nonpolymeric natural product, and it consists of four extended fused-ring ladder systems (Gallimore, 2009; Gallimore and Spencer, 2006).

4.1. General biosynthetic pathway

The biosynthesis of polyethers reveals important aspects on how nature builds ether rings. It is known that, biosynthetically, an intriguing cascade involving an oxidative cyclization process occurs to generate THP, THF and *O*-cyclic rings in polyether ionophores. A stereoselective oxidation promoted by a free-standing flavin monooxygenase and followed by an epoxide ring opening promoted by hydrolases- cyclases



Scheme 23. Multi-enzymatic cascade reaction for the biosynthesis of piochelin (77). MT: methyltranferase; TE: thioesterase; Cy: cyclization; SAM: *S*-adenoylmethionine; NADPH: β-nicotinamide adenine dinucleotide phosphate.

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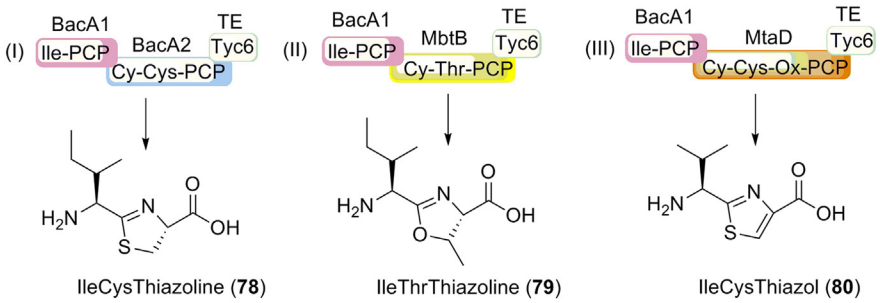

Scheme 24. Hybrid bimodular systems obtained by fusing modules from different enzymes: (I) bimodular original system; (II) hybrid bimodule containing a Cy domain from the mycobactin biosynthetic gene that catalyzes oxazoline formation; (III) hybrid bimodule containing a Cy from the myxothiazol biosynthetic gene and an Ox domain that catalyzes thiazole formation.

(epoxide hydrolases) modifies an unsaturated precursor to form the characteristic ether rings. This set of enzymes was shown to exert strong control over the stereochemical course of epoxide ring opening *in vivo* (Smith et al., 2008; Tosin et al., 2011) and *in vitro* (Minami et al., 2012; Shichijo et al., 2008).

Both terrestrial and marine polyethers originated from the classic polyketide pathway that evolves from the fatty acid biosynthetic pathway. Polyketides are assembled from monomeric acyl-CoA subunits by multimodular megasynthases. In a later step, the linear intermediates can be modified by oxidation, reduction, alkylation, halogenation, cyclization and microcyclization, among other transformations (Burkart and Tao, 2008).

4.1.1. Polyether ionophores

All polyether ionophores contain THF or THP saturated rings that are connected as spiroketal systems or separated by at least one single bond. The most extensively studied polyether ionophores are monensin (81 and 82) (Bhatt et al., 2005; Leadlay et al., 2001; Oliynyk et al., 2003) and lasalocid (83) (Westley et al., 1974a, 1974b) (Fig. 3). In monensin biosynthesis, a linear triene intermediate is oxidized to a triepoxide, which then participates in a cascade of epoxide opening and ring closure, leading to the polycyclic structure containing a [5,6]-spirosystem that in turn contains two THF rings and one THP ring. MonC was identified as the oxidase, and MonBI and MonBII are broad acceptance hydrolase cyclases (Oliynyk et al., 2003). It was shown that both subunits of MonB act synergistically (Sato et al., 2011). Lasalocid biosynthesis involves a linear diene intermediate, which is oxidized by LasC, and the hydrolysis-cyclization step is promoted by a didomain enzyme, which corresponds to a heterodimer where analogous subunits of MonBI and MonBII are connected by a link (Minami et al., 2012). Other ionophore gene clusters were found to carry similar epoxidase

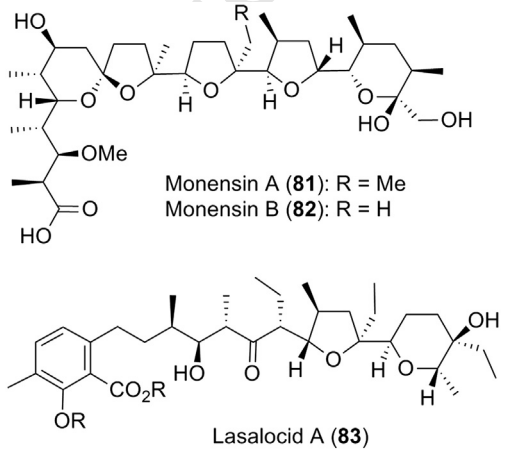

Fig. 3. Polyether ionophores monensin and lasalocid.

(FMO) and epoxide hydrolases genes that direct the oxidative cyclization process.

4.1.2. Marine polyethers

Marine polyethers, however, are highly distinctive compared with ionophore polyethers. They feature five- to nine-membered rings, and they all have contiguous fused ring systems, which confer a ladder-like appearance. Brevetoxin (84) (Lin et al., 1981; Shimizu and Chou, 1986), maitotoxin (85) (Murata et al., 1994), and gymnocins A (86) and B (87) (Satake et al., 2002, 2005) are just a few representatives of the known ladder polyethers (Fig. 4). A similar model involving a linear precursor, oxidation and hydrolysis, analogous to the antibiotic polyethers, is believed to be involved in the oxidative cyclization process for marine ladder polyethers (Gallimore, 2009; Vilotijevic and Jamison, 2010). The fused ether rings in all known ladder polyethers follow a rule of stereochemical uniformity that assumes that, in a polyepoxide precursor of a given ladder polyether, all epoxides must have identical absolute stereochemistry. This attribute is directly reflected in the *trans-syn-trans* arrangement observed in the ring fusion (Gallimore, 2009; Gallimore and Spencer, 2006; Vilotijevic and Jamison, 2010).

4.1.3. Regioselectivity in polyether cyclyzation

An interesting aspect observed during the lasalocid biosynthesis is the regioselective step involved in the opening of the polyepoxide, affording the THF-THP cyclic ethers (Matsuura et al., 2010). The empirical guidelines dictated by Baldwin's rules are used to predict the outcome of the ring forming process (Baldwin, 1976). According to Baldwin's rules, the epoxide opening reaction of hydroxyepoxides should favor the formation of the 5-*exo-tet* product, which corresponds to the smaller cyclic ethers. The 6-*endo-tet* cyclization results in an energetically disfavored process. LasB and other B enzymes that lead to the formation of THP over THF cyclic rings have the ability to promote the stabilization of the disfavored transition state (Scheme 25) (Matsuura et al., 2010; Smith et al., 2008). Similarly, the pre-brevetoxin polyepoxide undergoes nine disfavored *endo-tet* closures, suggesting that an enzyme is involved during the ring closure process to direct the cyclization process (Vilotijevic and Jamison, 2010).

4.2. In vitro stereoselective polyether ring formation

Matsuura et al. (2010) explored several lasalocid epoxide analogs in the hydrolysis reaction mediated by LasB and observed that when the hydrolysis reaction of the bisepoxide intermediate is performed in the presence of LasB, a THF-THP lasalocid-type product is formed as a result of the *endo-tet* mechanism, whereas the treatment of this intermediate under acidic conditions led to the THF–THF *exo-tet* cyclic compound (Scheme 26). LasB has been shown to be highly influenced by the epoxide stereochemistry. When diastereomeric epoxides were tested in the enzyme-promoted cyclization, they observed THF formation for the 18*R*,19*R*- and 18*S*,19*S*-epoxide units. However, in the second

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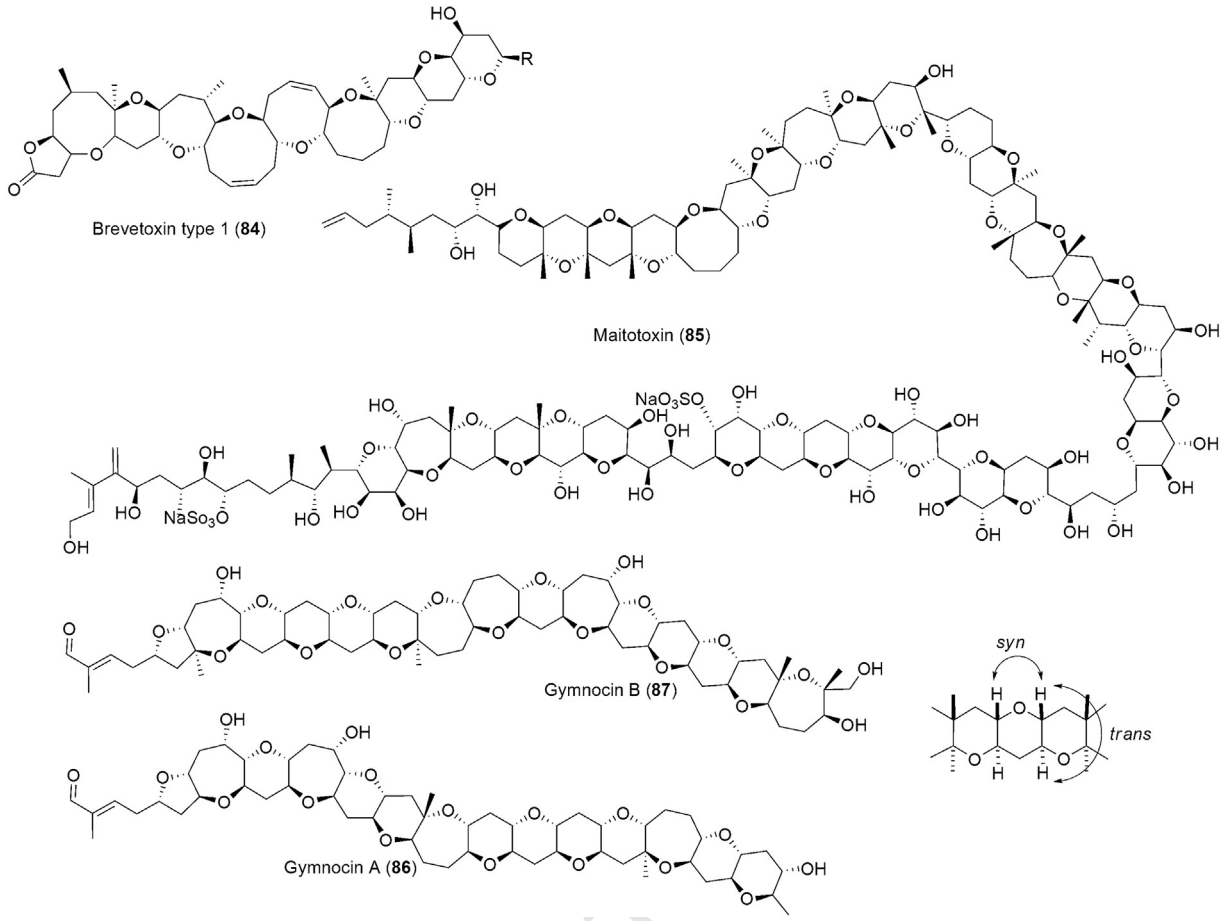
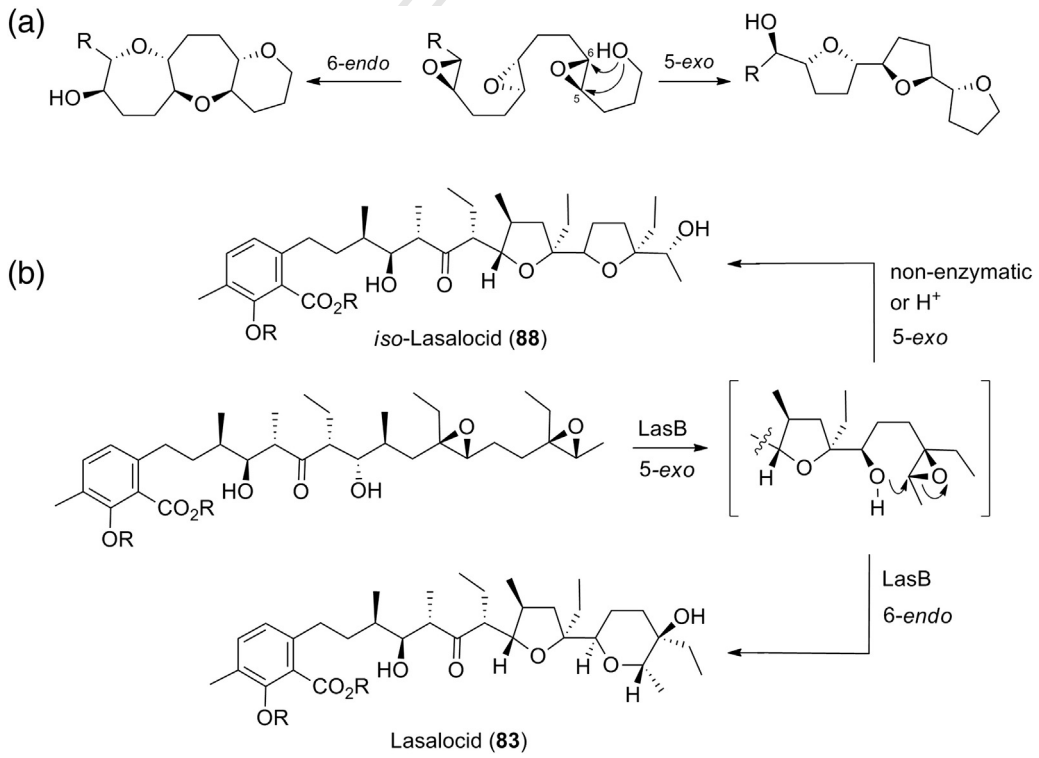
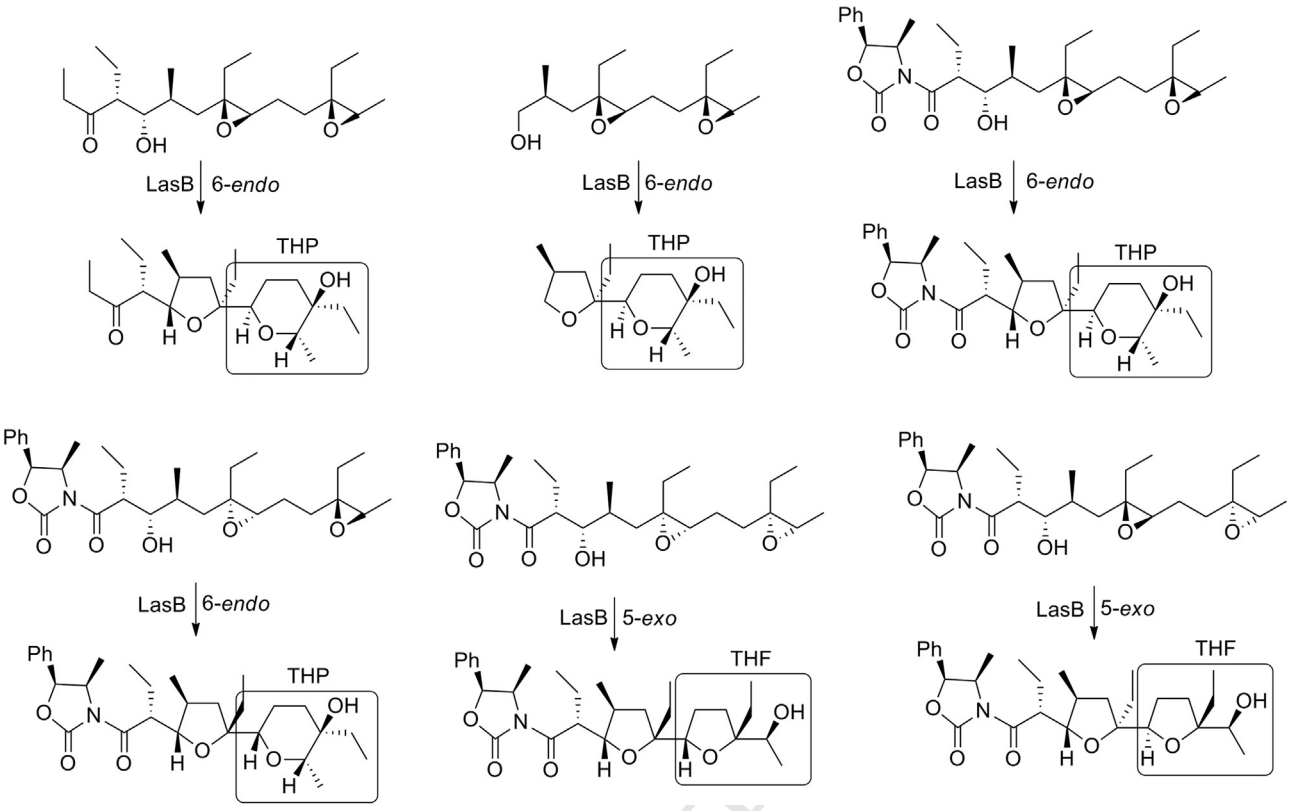


Fig. 4. Structures and structural features of a marine polyether ladder: the *trans-syn-trans* arrangement is observed in all ring fusions.



Scheme 25. The 6-*endo-tet* cyclization predominates over 5-*exo-tet* in the ring formation of marine ladders (a). A THF-THP resulting from both mechanisms is observed in the lasalocid structure (b).



Scheme 26. Remarkable selectivity exerted by LasB during the ring closure process.

cyclization, THP formation was observed for the 22*R*,23*R*-epoxide, following the same course observed for the natural product, whereas THF formation was observed for the 22*S*,23*S*-epoxide. These results reveal a remarkable selectivity exerted by the enzyme in the recognition of the precursor and in the promotion of regioselective ring closure. The group also provided evidence that individual domains of LasB catalyze the epoxide opening cascade independently (Oikawa et al., 2011). Additionally, relaxed substrate specificity was observed for this enzyme.

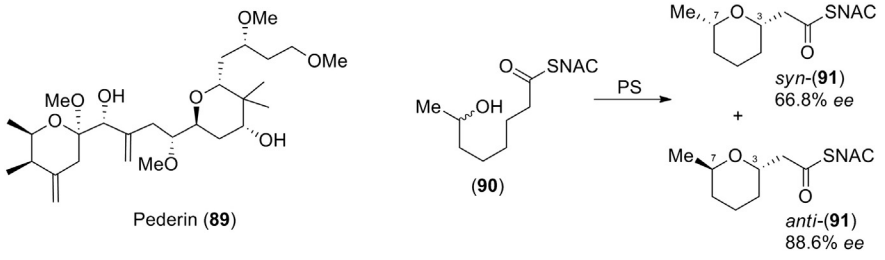
More recently, a sequential enzymatic epoxidation-hydrolysis-cyclization experiment (Minami et al., 2012) showed that the epoxidase LasC promotes two half-epoxidation reactions in the polyene precursor. In the next step, LasB or a non-enzyme-promoted reaction led to the formation of a THF–THP or THF–THF bicyclic system, respectively. All together, the results suggest that only a few epoxidases and epoxide hydrolases are required to install multiple chiral centers in the biosynthesis of polyether natural products.

Another very interesting feature of both FMN and epoxide hydrolases, and other cyclases from polyethers is the relaxed substrate acceptance, which distinguishes them from counterpart enzymes that present narrow substrate acceptance. This constitutes a limitation to their broad application in organic synthesis and to the development of industrial processes using B and C enzymes from polyethers as new tools for stereoselective synthesis.

5. Miscellaneous

5.1. Pyrane synthases

As an additional tool in the establishment of THP rings in polyether and non-polyether polyketides, Pöplau et al. (2013) reported a PKS component, termed pyrane synthase (PS) domain, that is capable of promoting ring closure *via* an oxa-conjugate cyclization. All PS domains known to date share the same overall architecture, suggesting their presence as a strategy used by PKS to install cyclic moieties. The PS from the pederin (89) biosynthetic pathway was hypothesized to participate in the generation of the THP ether moiety. The thioester (90) was predicted as a possible substrate for biotransformation. Enzyme assays with the putative substrate led to the formation of the expected cyclization product (91). Because thioester (91) was used as a racemate in the assay, a mixture of the *syn*-(91) and *anti*-(91) diastereomers in 88.6% *ee* and 66.8% *ee*, respectively, was obtained (Scheme 27). The absolute stereochemistry of C3 was determined to be *S*, consistent with



Scheme 27. Stereoselective ring closure *via* oxa-conjugate cyclization promoted by a pyrane synthase.

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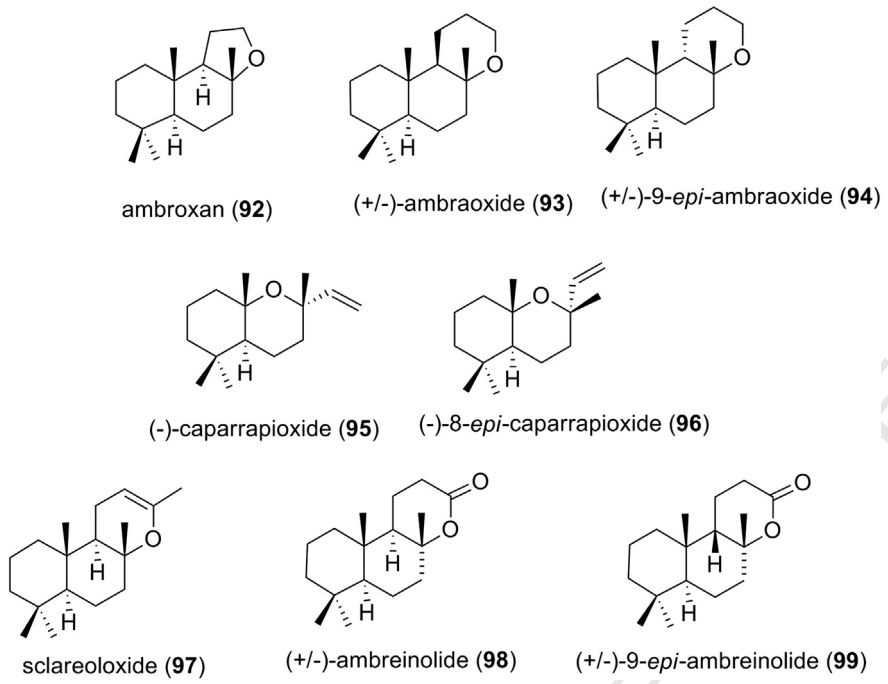

Scheme 28. Stereospecific synthesis of bi- and triheterocyclic terpenes promoted by ZmoSHC1 and AacSHC squalene–hopene cyclases.

that observed in pederin. This result shows that PS has a relaxed specificity for the outgoing C7 configuration. Additionally, it appears that PS domains are capable of converting complex precursors into 5- and 6-membered cyclic ethers. Because the PS domain does not require other PKS counterparts to be active, it represents an attractive tool for chemoenzymatic synthesis.

5.2. Terpene synthases

Terpenoids are originated from polyisoprene precursors, with the polycyclization being promoted by carbocation formation. The flexible substrate is stabilized by terpene synthases that trigger the stereoselective carbon–carbon formation (Hammer et al., 2013). Class I terpene synthases participate in the assembly of the diterpenoid taxol and the antimalarial agent artemisinin. These cyclases operate in phosphorylated substrates, whereas class II terpene synthases promote catalysis by the protonation of the terminal isoprene unit with a Brønsted acid (Siedenburg and Jendrossek, 2011).

Squalene-hopene cyclases are class II terpene cyclases known to possess high substrate tolerance, from C15 to C35 non-natural polyprenoids. However, the possibility of promoting mono- or polyheterocyclization is a most interesting and challenging process. In this context, Seitz et al. (2013) explored the SHC ZmoSHC1 from *Zymomonas mobilis* and SHC AacSHC from *Alicyclobacillus acidocaldarius* to stereoespecifically produce lactones and cyclic enol ethers.

The primary alcohols homofarnesol and bishomofarnesol and the tertiary alcohol nerolidol were converted to ambroxan (92), (±)-ambraoxide (93) and (±)-9-*epi*-ambraoxide (94), and (−)-caparrapioxide (95) and (−)-8-*epi*-caparrapioxide (96), respectively. Farnesylacetone produced sclareoloxide (97), and bishomofarnesoic acid produced a mixture of (±)-ambreinolide (98) and (±)-9-*epi*-ambreinolide (99) (Scheme 28).

The noteworthy stereoselectivity, combined with the promiscuity and evolvability of these synthases, supports them as a group of enzymes to be applied in the formation of C-X building blocks.

6. Conclusions

The multi-enzymatic approach to building complex molecules of interest is a long-standing dream shared by academia and industry, which is now ripe for consideration as an efficient tool for the preparation of chiral building blocks.

The past knowledge of natural product chemistry and biosynthetic pathways has inspired numerous biocatalytic processes. Currently, the logical approach encountered in nature is merging with current advances in protein engineering and moving the applied biocatalysis field towards the rational production of complex chiral building blocks.

The application of biocatalytic cascade processes will soon be an additional tool to introduce chirality in the total synthesis of molecules with unprecedented complexities.

7. Uncited reference

Q7

Oikawa et al., 2008

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References

Abrunhosa-Thomas I, Plas A, Vogrig A, Kandepedu N, Chalard P, Troin Y. Access to 2,6-disubstituted piperidines: control of the diastereoselectivity, scope, and limitations. applications to the stereoselective synthesis of (−)-solenopsine A and alkaloid (+)-241D. J Org Chem 2013;31:2511–26.

Amat M, Llor N, Hidalgo J, Escolano C, Bosch J. Enantioselective synthesis of piperidine, indolizidine, and quinolizidine alkaloids from a phenylglycinol-derived δ-lactam. J Org Chem 2003;68:1919–28.

Aniszewski T. Alkaloid chemistry. Alkaloids — Secrets Life. Elsevier Science; 2007. p. 61–139 [First].

Baldwin J. Rules for ring closure. J Chem Soc Chem Commun 1976;734:734–6.
Baltz RH. Molecular engineering approaches to peptide, polyketide and other antibiotics. Nat Biotechnol 2006;24(12):1533–40.
Banala S, Ensle P, Süssmuth RD. Total synthesis of the ribosomally synthesized linear azole-containing peptide plantazolicin A from *Bacillus amyloliquefaciens*. Angew Chem Int Ed 2013;52:9518–23.
Bechi B, Herter S, McKenna S, Riley C, Leimkühler S, Turner NJ, et al. Catalytic bio–chemo and bio–bio tandem oxidation reactions for amide and carboxylic acid synthesis. Green Chem R Soc Chem 2014;16:4524–9.
Belshaw PJ, Roy RS, Kelleher NL, Walsh CT. Kinetics and regioselectivity of peptide-to-heterocycle conversions by microcin B17 synthetase. Chem Biol 1998;5(7):373–84.
Bhat C, Tilve SG. Henry–Nef reaction: a practical and versatile chiral pool route to 2-substituted pyrrolidine and piperidine alkaloids. Tetrahedron 2013;69:6129–43.
Bhatt A, Stark CBW, Harvey BM, Gallimore AR, Demydchuk Ya, Spencer JB, et al. Accumulation of an *E, E, E*-triene by the monensin-producing polyketide synthase when oxidative cyclization is blocked. Angew Chem Int Ed 2005;44:7075–8.
Binev R. Alkaloids derived by amination reaction: acetate-derived (coniine). In: Ramawat KG, Mérillon J-M, editors. Nat Prod. Stara Zegora: Springer Berlin Heidelberg; 2013. p. 883–907.
Braunshausen A, Seebeck FP. Identification and characterization of the first ovothiol biosynthetic enzyme. J Am Chem Soc 2011;133:1757–9.
Buffat MGP. Synthesis of piperidines. Tetrahedron 2004;60:1701–29.
Burkart MD, Tao J. Multimodular synthases as tools of the synthetic chemist. In: Garcia-Junceda E, editor. Multi-step Enzym Catal Biotransformations Chemoenzymatic Synth. Weinheim: WILEY-VCH Verlag GmbH & Co. KGaA; 2008. p. 137–56.
Bush L, Hempfling WP, Burton H. Biosynthesis of nicotine and related compounds. In: Gorrod JW, Jacob III P, editors. Anal Determ Nicotine Relat Compd their Metab. Amsterdam: ElsevierScience B.V.; 1999. p. 13–44. [First].
Chotani G, Dodge T, Hsu A, Kumar M, LaDuca R, Trimbur D, et al. The commercial production of chemicals using pathway engineering. Biochim Biophys Acta 2000;1543(2):434–55.
Chung CK, Bulger PG, Kosjek B, Belyk KM, Rivera N, Scott ME, et al. Process development of C–N cross-coupling and enantioselective biocatalytic reactions for the asymmetric synthesis of niraparib. Org Process Res Dev 2013;18(1):215–27.
Conrado RJ, Varner JD, DeLisa MP. Engineering the spatial organization of metabolic enzymes: mimicking nature's synergy. Curr Opin Biotechnol 2008;19:492–9.
Cordell Ga. Fifty years of alkaloid biosynthesis in phytochemistry. Phytochemistry, 91. Elsevier Ltd; 2013. p. 29–51.
Cragg GM, Newman DJ. Natural products: a continuing source of novel drug leads. Biochim Biophys Acta, 1830. Elsevier B.V.; 2013. p. 3670–95.
Crosa JH, Walsh CT. Genetics and assembly line enzymology of siderophore biosynthesis in bacteria genetics and assembly line enzymology of siderophore biosynthesis in bacteria. Microbiol Mol Biol Rev 2002;66(2):223–49.
Cushnie TPT, Cushnie B, Lamb AJ. Alkaloids: an overview of their antibacterial, antibiotic-enhancing and antivirulence activities. Int J Antimicrob Agents 2014;44:377–86.
Davids T, Schmidt M, Böttcher D, Bornscheuer UT. Strategies for the discovery and engineering of enzymes for biocatalysis. Curr Opin Chem Biol 2013;17:215–20.
Duerfahrt T, Eppelmann K, Mu R, Marahiel MA, Forschung B. Rational design of a bimodular model system for the investigation of heterocyclization in nonribosomal peptide biosynthesis. Chem Biol 2004;11:261–71.
Fisch KM. Biosynthesis of natural products by microbial iterative hybrid PKS–NRPS. RSC Adv 2013;3:18228–47.
Fischbach Ma, Walsh CT. Assembly-line enzymology for polyketide and nonribosomal peptide antibiotics: logic, machinery, and mechanisms. Chem Rev 2006;106:3468–96.
Foulkes JM, Malone KJ, Coker VS, Turner NJ, Lloyd JR. Engineering a biometallic whole cell catalyst for enantioselective deracemization reactions. ACS Catal 2011;1:1589–94.
Fox EGP, Pianaro A, Solis DR, Delabie JHC, Vairo BC, Machado EDA, et al. Intraspecific and intracolonial variation in the profile of venom alkaloids and cuticular hydrocarbons of the fire ant solenopsis saevissima Smith (Hymenoptera: Formicidae). Psyche (Stuttg) 2012:1–10.
Fu X, Do T, Schmitz F. New cyclic peptides from the ascidian *Lissoclinum patella*. J Nat Prod 1998;61:1547–51.
Gallimore AR. The biosynthesis of polyketide-derived polycyclic ethers. Nat Prod Rep 2009;26:266–80.
Gallimore AR, Spencer JB. Stereochemical uniformity in marine polyether ladders—implications for the biosynthesis and structure of maitotoxin. Angew Chem Int Ed 2006;45(27):4406–13.
Gand M, Müller H, Wardenga R, Höhne M. Characterization of three novel enzymes with imine reductase activity. J Mol Catal B Enzym, 110. Elsevier B.V.; 2014. p. 126–32.
Geisler JG, Gross GG. The biosynthesis of piperine in *Piper nigrum*. Phytochemistry 1990;29(2):489–92.
Gerth K, Bedorf N, Hflen G, Reichenbach H. Epothilons A and B: antifungal and cytotoxic compounds from *Sorangium cellulosum* (myxobacteria) production, physico-chemical and biological properties. J Antibiot 1996;49(6):560–3.
Ghislieri D, Green AP, Pontini M, Willies SC, Rowles I, Frank A, et al. Engineering an enantioselective amine oxidase for the synthesis of pharmaceutical building blocks and alkaloid natural products. J Am Chem Soc 2013;135:10863–9.
Girardin M, Ouellet SG, Gauvreau D, Moore JC, Hughes G, Devine PN, et al. Convergent kilogram-scale synthesis of dual orexin receptor antagonist. Org Process Res Dev 2013;17:61–8.
Gupta RN, Spenser ID, Emistry CI. Biosynthesis of the piperidine nucleus. J Biol Chem 1969;244(1):88–94.
Hammer SC, Syrén P-O, Seitz M, Nestl BM, Hauer B. Squalene hopene cyclases: highly promiscuous and evolvable catalysts for stereoselective C–C and C–X bond formation. Curr Opin Chem Biol 2013;17(2):293–300.
Hoye TR, Bjorklund JA, Koltun DO, Renner MK. N-methylputrescine oxidation during cocaine biosynthesis: study of prochiral methylene hydrogen discrimination using the remote isotope method. Org Lett 2000;2(1):3–5.
Huber T, Schneider L, Präg A, Gerhardt S, Einsle O, Müller M. Direct reductive amination of ketones: structure and activity of S-selective imine reductases from streptomyces. ChemCatChem 2014;6:2248–52.
Kathiravan MK, Salake AB, Chothe AS, Dudhe PB, Watode RP, Mukta MS, et al. The biology and chemistry of antifungal agents: a review. Bioorg Med Chem 2012;20:5678–98.
Kelly W, Pan L, Li C. Thiostrepton biosynthesis: prototype for a new family of bacteriocins. J Am Chem Soc 2009;131:4327–34.
Köhler V, Wilson YM, Dürrenberger M, Ghislieri D, Churakova E, Quinto T, et al. Synthetic cascades are enabled by combining biocatalysts with artificial metalloenzymes. Nat Chem 2012;5:93–9.
Kohls H, Steffen-Munsberg F, Höhne M. Recent achievements in developing the biocatalytic toolbox for chiral amine synthesis. Curr Opin Chem Biol 2014;19:180–92.
Koszelewski D, Clay D, Faber K, Kroutil W. Synthesis of 4-phenylpyrrolidin-2-one via dynamic kinetic resolution catalyzed by ω-transaminases. J Mol Catal B: Enzym 2009;60:191–4.
Kwon SJ, Mora-Pale M, Lee M-Y, Dordick JS. Expanding nature's small molecule diversity via *in vitro* biosynthetic pathway engineering. Curr Opin Chem Biol 2012;16:186–95.
Leadlay P, Staunton J, Oliynyk M. Engineering of complex polyketide biosynthesis—insights from sequencing of the monensin biosynthetic gene cluster. J Ind Microbiol Biotechnol 2001;27:360–7.
Leclercq S, Braekman J, Daloze D. Biosynthesis of the solenopsins, venom alkaloids of the fire ants. Naturwissenschaften 1996;83:222–5.
Leete E, Juneau K. Biosynthesis of pinidine. J Am Chem Soc 1969;91(20):5614–8.
Leipold F, Hussain S, Ghislieri D, Turner NJ. Asymmetric reduction of cyclic imines catalyzed by a whole-cell biocatalyst containing an (S)-imine reductase. ChemCatChem 2013;5:3505–8.
Li Y, Milne J, Madison L, Kolter R, Walsh C. From peptide precursors to oxazole and thiazole-containing peptide antibiotics: microcin B17 synthase. Science 1996;274:1188–93.
Liao R, Duan L, Lei C, Pan H, Ding Y, Zhang Q, et al. Thiopeptide biosynthesis featuring ribosomally synthesized precursor peptides and conserved posttranslational modifications. Chem Biol 2009;16:141–7.
Lin Y-Y, Risk M, Ray SM, Van Engen D, Clardy J, Golik J, et al. Isolation and structure of brevetoxin B from the "Red Tide" dinoflagellate ptychodiscus brevis (*Gymnodinium breve*). J Am Chem Soc 1981;103(22):6773–5.
Lopez-Gallego F, Schmidt-Dannert C. Multi-enzymatic synthesis. Curr Opin Chem Biol 2010;14:174–83.
Malik MS, Park E-S, Shin J-S. Features and technical applications of ω-transaminases. Appl Microbiol Biotechnol 2012;94:1163–71.
Mathew S, Yun H. ω-Transaminases for the production of optically pure amines and unnatural amino acids. ACS Catal 2012;2:993–1001.
Matsuura Y, Shichijo Y, Minami A, Migita A, Oguri H, Watanabe M, et al. Intriguing substrate tolerance of epoxide hydrolase Lsd19 involved in biosynthesis of the ionophore antibiotic lasalocid A. Org Lett 2010;12(10):2226–9.
Melby JO, Nard NJ, Mitchell DA. Thiazole/oxazole-modified microcins: complex natural products from ribosomal templates. Curr Opin Chem Biol 2011;15(3):369–78.
Minami A, Shimaya M, Suzuki G, Migita A, Shinde SS, Sato K, et al. Sequential enzymatic epoxidation involved in polyether lasalocid biosynthesis. J Am Chem Soc 2012;134:7246–9.
Mitsukura K, Suzuki M, Tada K, Yoshida T, Nagasawa T. Asymmetric synthesis of chiral cyclic amine from cyclic imine by bacterial whole-cell catalyst of enantioselective imine reductase. Org Biomol Chem 2010;8:4533–5.
Mitsukura K, Suzuki M, Shinoda S, Kuramoto T, Yoshida T, Nagasawa T. Purification and characterization of a novel (R)-imine reductase from *Streptomyces* sp. GF3587. Biosci Biotechnol Biochem 2011;75:1778–82.
Mitsukura K, Kuramoto T, Yoshida T, Kimoto N, Yamamoto H, Nagasawa T. A NADPH-dependent (S)-imine reductase (SIS) from *Streptomyces* sp. GF3546 for asymmetric synthesis of optically active amines: purification, characterization, gene cloning, and expression. Appl Microbiol Biotechnol 2013;97:8079–86.
Mix S, Blechert S. Diastereoselective synthesis of 2,6-trans-disubstituted piperidines via sequential cross-metathesis–cationic cyclisation. Adv Synth Catal 2007;349:157–60.
Murata M, Yasumoto T, Naoki H, Matsunaga S, Satake M. Structure and partial stereochemical assignments for maitotoxin, the most toxic and largest natural non-biopolymer. J Am Chem Soc 1994;116:7098–107.
Nolan EM, Walsh CT. How nature morphs peptide scaffolds into antibiotics. ChemBioChem 2009;10(1):34–53.
O' Reilly E, Iglesias C, Ghislieri D, Hopwood J, Galman JL, Lloyd RC, et al. A regio- and stereoselective ω-transaminases/monoamine oxidase cascade for the synthesis of chiral 2,5-disubstituted pyrrolidines. Angew Chem Int Ed 2014;53:2447–50.
Oikawa H, Oguri H, Shichijo Y, Migita A, Watanabe M, Tokiwano T, et al. Epoxide hydrolase Lsd19 for polyether formation in the biosynthesis of lasalocid A: direct experimental evidence on polyene-polyepoxide hypothesis in polyether biosynthesis. J Am Chem Soc 2008;130:12230–1.
Oikawa H, Minami A, Migita A, Inada D, Hotta K, Watanabe K, et al. Enzymatic epoxide-opening cascades catalyzed by a pair of epoxide hydrolases in the ionophore polyether biosynthesis. Org Lett 2011;13(7):1638–41.
Oliynyk M, Stark CBW, Bhatt A, Jones M a, Hughes-Thomas Z a, Wilkinson C, et al. Analysis of the biosynthetic gene cluster for the polyether antibiotic monensin in *Streptomyces cinnamonensis* and evidence for the role of monB and monC genes in oxidative cyclization. Mol Microbiol 2003;49(5):1179–90.
Oroz-Guinea I, García-Junceda E. Enzyme catalysed tandem reactions. Curr Opin Chem Biol 2013;17:236–49.

Please cite this article as: Sigrist R, et al, Nature-inspired enzymatic cascades to build valuable compounds, Biotechnol Adv (2015), http://dx.doi.org/10.1016/j.biotechadv.2015.03.010

Patel HM, Walsh CT. *In vitro* reconstitution of the pseudomonas aeruginosa nonribosomal peptide synthesis of pyochelin: characterization of backbone tailoring thiazoline reductase and N-methyltransferase activities. Biochemistry 2001;40:9023–31.

Philipov S, Doncheva T. Alkaloids derived from ornithine: tropane alkaloids. In: Ramawat KG, Mérillon JM, editors. Nat Prod. Berlin Heidelberg: Springer-Verlag; 2013. p. 343–58.

Pianaro A, Fox EGP, Bueno OC, Marsaioli AJ. Rapid configuration analysis of the solenopsins. Tetrahedron: Asymmetry, 23. Elsevier Ltd; 2012. p. 635–42.

Pöplau P, Frank S, Morinaka BI, Piel J. An enzymatic domain for the formation of cyclic ethers in complex polyketides. Angew Chem Int Ed 2013;52:13215–8. [Dec 9].

Reimmann C, Beyeler M, Latifi A, Winteler H, Foglino M. The global activator GacA of *Pseudomonas aeruginosa* PAO positively controls the production of the autoinducer *N*-butyryl-homoserine lactone and the formation of the virulence factors pyocyanin, cyanide, and lipas. Mol Microbiol 1997;24(2):309–19.

Reimmann C, Serino L, Beyeler M, Haas D. Dihydroaeruginoic acid synthetase and pyochelin synthetase, products of the pchEF genes, are induced by extracellular pyochelin in *Pseudomonas aeruginosa*. Microbiology 1998;144:3135–48.

Reimmann C, Patel HM, Serino L, Walsh CT, Haas D, Barone M. Essential PchG-dependent reduction in pyochelin biosynthesis of *Pseudomonas aeruginosa*. J Bacteriol 2001; 183(3):813.

Rodríguez-Mata M, Frank A, Wells E, Leipold F, Turner NJ, Hart S, et al. Structure and activity of NADPH-dependent reductase Q1EQE0 from *Streptomyces kanamyceticus*, which catalyses the r-selective reduction of an imine substrate. ChemBioChem 2013;14:1372–9.

Rowles I, Malone K, Etchells L, Willies SC, Turner NJ. Directed evolution of the enzyme monoamine oxidase (MAO-N): highly efficient chemo-enzymatic deracemisation of the alkaloid (±)-crispine A. ChemCatChem 2012;4:1259–61.

Roy RS, Gehring AM, Milne JC, Belshaw PJ, Walsh CT. Thiazole and oxazole peptides: biosynthesis and molecular machinery. Nat Prod Rep 1999;16:249–63.

Russell JB, Houlihan AJ. Ionophore resistance of ruminal bacteria and its potential impact on human health. FEMS Microbiol Rev 2003;27:65–74.

Satake M, Shoji M, Oshima Y, Naoki H, Fujita T, Yasumoto T. Gymnocin-A, a cytotoxic polyether from the notorious red tide dinoflagellate, *Gymnodinium mikimotoi*. Tetrahedron Lett 2002;43:5829–32.

Satake M, Tanaka Y, Ishikura Y, Oshima Y, Naoki H, Yasumoto T. Gymnocin-B with the largest contiguous polyether rings from the red tide dinoflagellate, *Karenia* (formerly *Gymnodinium*) *mikimotoi*. Tetrahedron Lett 2005;46:3537–40.

Sato K, Minami A, Ose T, Oguri H, Oikawa H. Remarkable synergistic effect between MonBI and MonBII on epoxide opening reaction in ionophore polyether monensin biosynthesis. Tetrahedron Lett, 52. Elsevier Ltd; 2011. p. 5277–80.

Savile CK, Janey JM, Mundorff EC, Moore JC, Tam S, Jarvis WR, et al. Biocatalytic asymmetric synthesis of chiral amines from ketones applied to sitagliptin manufacture. Science 2010;329:305–9.

Scheller PN, Fademrecht S, Hofelzer S, Pleiss J, Leipold F, Turner NJ, et al. Enzyme toolbox: novel enantiocomplementary imine reductases. ChemBioChem 2014;15:2201–4.

Schilling B, Lerch K. Amine oxidases from aspergihs: identification of a novel flavin-dependent enzyme. Biochim Biophys Acta 1995a;1243:529–37.

Schilling B, Lerch K. Cloning, sequencing and heterologous expression of the monoamine oxidase gene from *Aspergillus niger*. Mol Gen Genet MGG 1995b;247:430–8.

Schneider TL, Shen B, Walsh CT. Oxidase domains in epothilone and bleomycin biosynthesis: thiazoline to thiazole oxidation during chain elongation. Biochemistry 2003; 42:9722–30.

Schrittwieser JH, Groenendaal B, Resch V, Ghislieri D, Wallner S, Fischereder E-M, et al. Deracemization by simultaneous bio-oxidative kinetic resolution and stereoinversion. Angew Chem Int Ed 2014a;53:3731–4.

Schrittwieser JH, Groenendaal B, Willies SC, Ghislieri D, Rowles I, Resch V, et al. Deracemisation of benzylisoquinoline alkaloids employing monoamine oxidase variants. Catal Sci Technol 2014b;4:3657–64.

Schwarzer D, Marahiel M a. Multimodular biocatalysts for natural product assembly. Naturwissenschaften 2001;88(3):93–101.

Seebeck FP. *In vitro* reconstitution of mycobacterial ergothioneine biosynthesis. J Am Chem Soc 2010;132:6632–3.

Seitz M, Syrén P-O, Steiner L, Klebensberger J, Nestl BM, Hauer B. Synthesis of heterocyclic terpenoids by promiscuous squalene–hopene cyclases. ChemBioChem 2013;14: 436–9.

Shimizu Y, Chou H. Structure of brevetoxin A (GB-1 toxin), the most potent toxin in the Florida red tide organism *Gymnodinium breve* (*Ptychodiscus brevis*). J Am Chem Soc 1986;108:515–6.

Siedenburg G, Jendrossek D. Squalene–hopene cyclases. Appl Environ Microbiol 2011; 77(12):3905–15.

Silakowski B, Schairer HU, Ehret H, Kunze B, Weinig S, Nordsiek G, et al. New lessons for combinatorial biosynthesis from myxobacteria. J Biol Chem 1999;274(52):37391–9.

Simon RC, Grischek B, Zepeck F, Steinreiber A, Belaj F, Kroutil W. Regio- and stereoselective monoamination of diketones without protecting groups. Angew Chem Int Ed 2012;51:6713–6.

Simon RC, Fuchs CS, Lechner H, Zepeck F, Kroutil W. Concise chemoenzymatic three step total synthesis of isosolenopsin through medium engineering. Eur J Org Chem 2013a: 3397–402.

Simon RC, Zepeck F, Kroutil W. Chemoenzymatic synthesis of all four diastereomers of 2,6-disubstituted piperidines through stereoselective monoamination of 1,5-diketones. Chem Eur J 2013b;19:2859–65.

Simon RC, Richter N, Busto E, Kroutil W. Recent developments of cascade reactions involving ω-transaminases. ACS Catal 2014;4:129–43.

Smith L, Hong H, Spencer JB, Leadlay PF. Analysis of specific mutants in the lasalocid gene cluster: evidence for enzymatic catalysis of a disfavoured polyether ring closure. ChemBioChem 2008;9:2967–75.

Szoke É, Lemberkovics É, Kursinszki L. Alkaloids derived from lysine: piperidine alkaloids. In: Ramawat KG, Mérillon J-M, editors. Nat Prod. Berlin, Heidelberg: Springer Berlin Heidelberg; 2013. p. 303–41.

Tosin M, Smith L, Leadlay PF. Insights into lasalocid A ring formation by chemical chain termination in vivo. Angew Chem Int Ed 2011;50. [11930-11833].

Truppo M, Rozzell J, Turner N. Efficient production of enantiomerically pure chiral amines at concentrations of 50 g/L using transaminases. Org Process Res Dev 2010;14:234–7.

Turner NJ. Enantioselective oxidation of C–O and C–N bonds using oxidases. Chem Rev 2011;111:4073–87.

Turner NJ. Oxidation of C–N bonds. In: Drauz K, Groger H, Oliver M, editors. Enzym Catal Org Synth. Wiley-VCH Verlag GmbH & Co. KGaA; 2012. p. 1535–52. [Third].

Turner NJ, Truppo MD. Biocatalysis enters a new era. Curr Opin Chem Biol, 17. Elsevier Ltd; 2013a. p. 212–4.

Turner NJ, Truppo MD. Synthesis of chiral amines using transaminases. In: Dunn PJ, Hii KK (Mimi), Krische MJ, Williams MT, editors. Sustain Catal Challenges Pract Pharm Fine Chem Ind. John Wiley & Sons, Inc.; 2013b. p. 63–74. [First].

Vilotijevic I, Jamison TF. Synthesis of marine polycyclic polyethers via endo-selective epoxide-opening cascades. Mar Drugs 2010;8:763–809.

Vu VH, Jouanno L-A, Cheignon A, Roisnel T, Dorcet V, Sinbandhit S, et al. Modified fry cyanation of a chiral pyridinium salt: asymmetric syntheses of (−)-coniine and (−)-solenopsin A. Eur J Org Chem 2013:5464–74.

Walsh CT, Chen H, Keating TA, Hubbard BK. Tailoring enzymes that modify nonribosomal peptides during and after chain elongation on NRPS assembly lines. Curr Opin Chem Biol 2001;5:525–34.

Walsh CT, Malcolmson SJ, Young TS. Three ring posttranslational circuses: insertion of oxazoles, thiazoles, and pyridines into protein-derived frameworks. ACS Chem Biol 2012;7:429–42.

Westley JW, Benz W, Donahue J, Evans RHJ, Scott CG, Stempel A, et al. Biosynthesis of lasalocid III — Isolation and structure determination of four homologs of lasalocid A. J Antibiot 1974a;27(10):744–53.

Westley JW, Blount JF, Evans RHJ, Stempel A, Berger J. Biosynthesis of lasalocid II — X-ray analysis of naturally occurring isomer of lasalocid A. J Antibiot 1974b;27(8):597–604.

Willies S. Reductive amination. In: Whittall J, Sutton PW, editors. Pract Methods Biocatal Biotransformations 2. Chichester: John Wiley & Sons, Ltd; 2012. p. 61–86. [First].

Please cite this article as: Sigrist R, et al, Nature-inspired enzymatic cascades to build valuable compounds, Biotechnol Adv (2015), http://dx.doi.org/10.1016/j.biotechadv.2015.03.010