Chemistry & Biology
Review

Biosynthetic Manipulation of Tryptophan in Bacteria: Pathways and Mechanisms

Lona M. Alkhalaf[1] and Katherine S. Ryan[1,*]
[1]Department of Chemistry, University of British Columbia, Vancouver, BC, V6T 2K9, Canada
*Correspondence: ksryan@chem.ubc.ca
http://dx.doi.org/10.1016/j.chembiol.2015.02.005

Tryptophan, the most chemically complex and the least abundant of the 20 common proteinogenic amino acids, is a biosynthetic precursor to a large number of complex microbial natural products. Many of these molecules are promising scaffolds for drug discovery and development. The chemical features of tryptophan, including its ability to undergo enzymatic modifications at almost every atom in its structure and its propensity to undergo spontaneous, non-enzyme catalyzed chemistry, make it a unique biological precursor for the generation of chemical complexity. Here, we review the pathways that enable incorporation of tryptophan into complex metabolites in bacteria, with a focus on recently discovered, unusual metabolic transformations.

Introduction

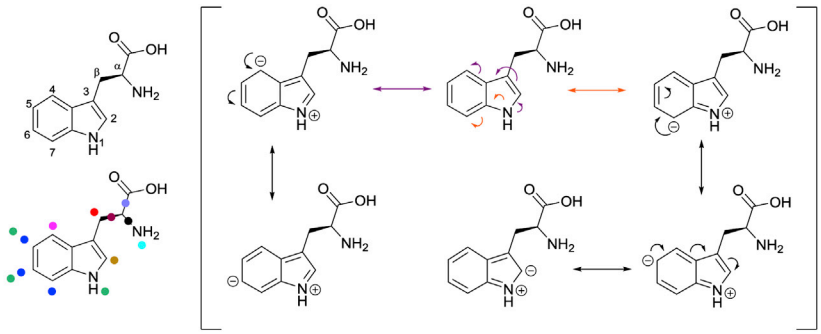
Tryptophan (Trp) is an unusual member of the 20 canonical amino acids. It is the largest by molecular weight and is the least abundant, both in terms of its use in proteins (Santiveri and Jiménez, 2010) and as found free in cells (Bennett et al., 2009). Structural biologists refining crystallographic data at moderate resolutions can usually first place the bulky indole side chain of a Trp into initial electron density as a way to begin building more detailed maps (Waldrop et al., 1994). This bulky side chain is not just a useful marker for crystallographers, however; the rarity of Trp generally means it is positioned meaningfully in a protein backbone, playing a key role in the protein (Wallace and Janes, 1999). Indeed, evolutionary biologists find that Trp is the most conserved among the amino acids, whereas other amino acids are found to be more readily exchanged over evolutionary time (Dayhoff et al., 1978). The important role of Trp is also exemplified by the observation that random mutations in the codon for Trp (as well as those for Cys) are most likely to cause disease in humans compared to other amino acids (Vitkup et al., 2003).

Trp also plays an important role as a building block for chemical diversity. Here, the reactivity of Trp comes to the forefront. The electron-rich indole can undergo electrophilic aromatic substitution to give a variety of modified derivatives. Trp can also be activated by oxidation to indolepyruvate or by conversion to an iminium ion, poising it for construction of more complex structures. In microbes, there are a large number of Trp-derived natural products. These range from small molecules, such as the antifungal pyrrolnitrin, to large polypeptides, such as the antibacterial cyclomarin. In this review, we discuss a number of routes that enable conversion of Trp to more complex structures, focusing specifically on pathways in bacteria. We begin with a brief overview of the chemistry of Trp. Next, we discuss a variety of enzymatic manipulations that have been identified for the Trp substrate. Then we delve into the details of a number of biosynthetic pathways: how Trp is built up and used to make new heterocycles, how Trp is destroyed to generate dramatically altered structures, and how Trp is dimerized to give bisindoles. We end with a brief description on the incorporation of Trp into natural product peptides. Our focus throughout is on recently discovered, unusual metabolic transformations.

Chemistry and Biochemistry of Tryptophan

Tryptophan is an amino acid with the β carbon connected to the 3-position of an indole group. This electron-rich indole is very susceptible to electrophilic substitution such as alkylation, nitration, and halogenation (Sundberg, 1970). In indole itself, the 3-position is most reactive, but this reactivity is decreased when the position is alkylated as in Trp. Thus, electrophilic substitution reactions can occur at many positions of Trp, due to the presence of resonance forms obtained from feeding electron density from the indole nitrogen into the pi system (Figure 1). Alkylation also commonly occurs at the indole nitrogen in synthetic reactions, and the nitrogen requires protection before alkylation at other positions can be achieved (Dhanak and Reese, 1986). Overall, the delocalization of electron density from the nitrogen makes it a very weak base—the pK_a of protonated indole is −3.5 (Hinman and Lang, 1964)—and causes Trp to be easily oxidized (Simat and Steinhart, 1998). These interesting chemical properties mean that, despite being the least chemically abundant proteinogenic amino acid, Trp is incorporated by bacteria into a large array of bioactive natural products, which act as antibiotics, anticancer agents, herbicides, anti-inflammatories, and antifungals, among others (Figure 2). Of course, all amino acids are incorporated into bioactive molecules in bacteria via the action of both ribosomal and non-ribosomal pathways (Arnison et al., 2013; Hur et al., 2012), and Trp is also incorporated into these pathways. However, the enhanced reactivity and oxidative nature of Trp mean it also undergoes other interesting biochemical transformations.

Trp biosynthesis begins with the shikimic acid pathway, and the final step is the coupling of L-serine with indole by the pyridoxal phosphate (PLP)-dependent Trp synthase (Bentley, 1990). Metabolic engineering efforts have been made to improve Trp production. For instance, the Wittmann group set out to produce violacein, a Trp-derived alkaloid,



Reaction	Reaction site	Enzymes	Enzyme Class	Natural Product class
Nitration	C4	TxtE[i]	Cytochrome P450	Thaxtomin
Chlorination	C5	*ClaH[ii]/ *AbeH[iii]	Flavin-dependant halogenase	Cladoniamide/ BE-54017
		PyrH[iv]		Pyrroindomycin
	C6	SttH[v]		Unknown
		*BorH[vi]		Borregomycin
		ThdH[vii]		Thienodoline
	C7	RebH[viii]		Rebeccamycin
		PrnA[ix]		Pyrollnitrin
		*AtmH[x]		AT2433
Oxidation	β-C	Qui15/homologs[xi]	Cytochrome P450	Quinoxaline containing
Prenylation	N1	CymD[xii]	N-prenyl transferase	Cyclomarin/ cyclomarazines
	C5	SCO7467[xiii]	Indole prenyltransferase	Prenylated indole derivatives
	C6	IptA[xiv]		
Methylation	C2	TsrA[xv]	SAM-dependant methyl transferase	Thiostrepton
Decarboxylation	CO_2	**PsmH[xvi]	PLP-dependant decarboxylase	Physostigmine
		AADC[xvii]		Bacillamides
Isonitrile synthesis	-	Amb1-3/ homologs[xviii]	Isonitrile synthase/oxygenase	Hapalindoles
		IsnA-B[xix]		*trans*-Isonitrile indole
Oxidative deamination	NH_2	NPr1276[xx]	Dehydrogenase	Scytonemin
		MarG[xxi]	PLP-dependant transaminase	Maremycin
		RebO/homologs[xxii]	Amino acid oxidase	Rebeccamycin/ bisindoles
		*StnP2[xxiii]		Streptonigrin
Cleavage	-	NosL[xxiv]	Radical SAM	Nosiheptide
		NocL[xxv]		Nocathiacin

Figure 1. Enzymatic Modification of Trp

(Top) Numbering of Trp and resonance forms. (Bottom) Enzymes involved in the formation of Trp analogs. *These enzymes have not been directly characterized; functions are inferred from genetic data. **Utilizes 5-OH-Trp as a substrate. i, Barry et al., 2012; Yu et al., 2013; ii, Du and Ryan, 2015; iii, Chang and Brady, 2011; iv, Zehner et al., 2005; v, Zeng and Zhan, 2011; vi, Chang and Brady, 2013; vii, Milbredt et al., 2014; viii, Yeh et al., 2005; ix, Dong et al., 2005; x, Gao et al., 2006; xi, Zhang et al., 2013; xii, Schultz et al., 2010; xiii, Ozaki et al., 2013; xiv, Takahashi et al., 2010; xv, Pierre et al., 2012; xvi, Liu et al., 2014; xvii, Yuwen et al., 2013; xviii, Hillwig et al., 2014a; xix, Brady and Clardy, 2005; xx, Balskus and Walsh, 2008; xxi, Zou et al., 2013; xxii, Nishizawa et al., 2005; xxiii, Xu et al., 2013; xxiv, Zhang et al., 2011a; xxv, Zhang et al., 2011b.

through heterologous expression of the gene cluster in *Escherichia coli*. By deleting the repressor of the Trp operon, eliminating degradation pathways of Trp and serine, removing feedback control of the anthranilate synthase gene *trpE*, and increasing the production of the precursors chorismate, erythrose 4-phosphate, and serine, they were able to achieve increased production of L-Trp. This precursor pool, in turn, facilitated violacein titers of 710 mg/l (Rodrigues et al., 2013), a dramatic improvement over prior efforts. More recently this was further improved to allow use of the much cheaper glycerol as a carbon source and improved production of the related metabolite deoxyviolacein to 1.6 g/l (Rodrigues et al., 2014). A different approach to exploit Trp production was undertaken by the Goss group: by feeding in haloindoles to an *E. coli* strain overexpressing a Trp synthase gene, they were able to obtain L-halotryptophans, including L-7-I-Trp (Smith et al., 2014). This progress in improving Trp and analog production sets the standard for large-scale production of Trp-containing secondary metabolites.

Interestingly, many Trp-derived natural product biosynthetic gene clusters have embedded *trp* genes that are additional to the *trp* genes elsewhere in the genome, perhaps reflecting the relative scarcity of the free amino acid for diversion to secondary metabolism. These additional *trp* gene-containing clusters are found associated with the calcium-dependent antibiotic cluster in *Streptomyces coelicolor* (Xie et al., 2003a), and in several cyanobacterial gene clusters (Micallef et al., 2014; Xie et al., 2003b). The embedded *trp* operon in the scytonemin cluster in *Nostoc punctiforme* is transcriptionally activated by UV light, along with the known biosynthetic genes, strongly suggesting that these additional *trp* genes are dedicated to provision of the Trp precursor for scytonemin production (Sorrels et al., 2009).

Enzymatic Manipulation of Tryptophan

The inherent reactivity of Trp means it is harnessed as the substrate of a number of enzymes that halogenate, oxidize, methylate, and decarboxylate to produce analogs of increased chemical diversity (Figure 1). These analogs can then be incorporated into biosynthetic pathways. One of the most interesting modifications in bacterial natural product biosynthesis is chlorination. As can be seen in Figure 1, a number of different enzymes are capable of catalyzing chlorination at C5, C6, and C7 of Trp in the biosynthetic pathways to bisindoles, thienodolin, pyrroindomycin, and pyrrolnitrin. Characterized Trp chlorinases are $FADH_2$-dependant halogenases. Structural and mechanistic

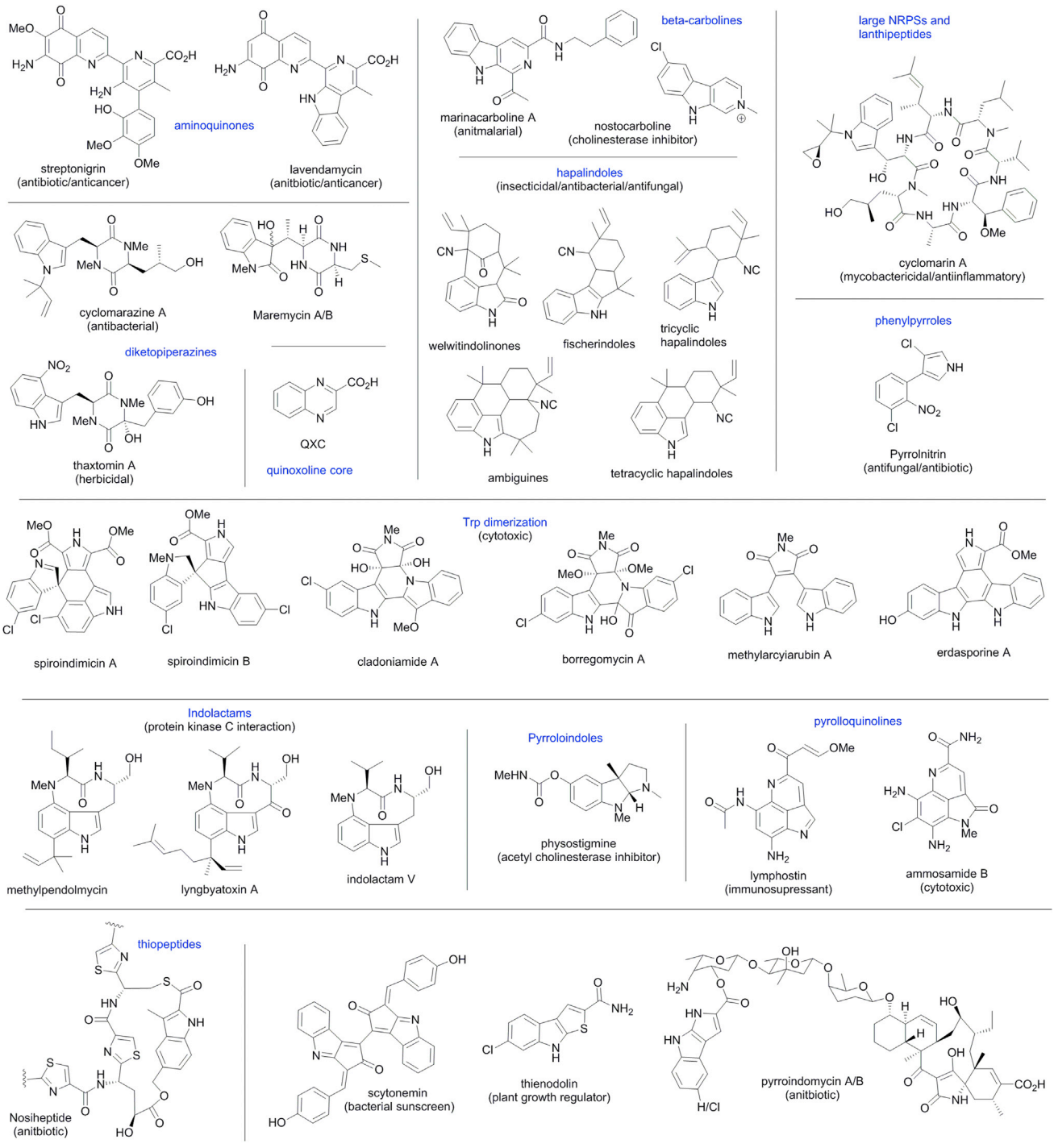


Figure 2. Examples of the Diverse Natural Products Produced by Bacteria that Incorporate Trp.

investigations of the Trp-7-chlorinases PrnA (Dong et al., 2005) and RebH (Yeh et al., 2007) have revealed that the mechanism of this reaction is likely to involve creation of HOCl in the flavin-binding domain via chloride attack of a flavin-peroxide intermediate. This highly reactive intermediate is then transferred from the flavin-binding domain to the substrate-binding domain, where chlorination is proposed to occur via electrophilic attack. A Glu and Lys have been implicated in increasing the electrophilicity of HOCl and maintaining it in the correct orientation for C7 halogenation (Flecks et al., 2008; Yeh et al., 2007). Interestingly, no Trp-4-chlorinase has yet been observed, although 4-Cl-Trp has been isolated from a plant source (Sakagami et al., 1993). It is possible that the isolation of genes for chlorination at the C5, C6, and C7 positions reflects the fact that the lysine residue

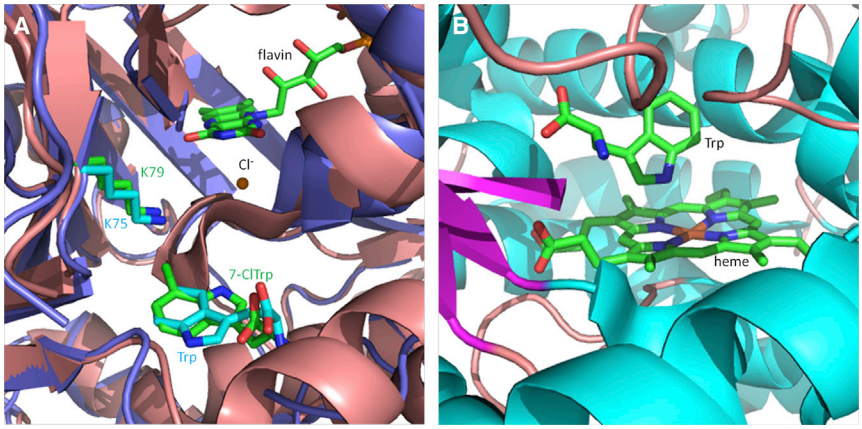


Figure 3. X-Ray Crystal Structures of Oxidative Enzymes Utilizing Trp
(A) $FADH_2$-dependent halogenases PrnA (PDB 2AR8, pink ribbon and green sticks) (Dong et al., 2005) and PyrH (2WEU, purple ribbon and blue sticks) (Zhu et al., 2009), showing "flipped" Trp responsible for halogenations at the 7- and 5-positions, respectively.
(B) Cytochrome P450 TxtE (PDB 4TPO), with bound Trp, responsible for nitration of Trp in thaxtomin biosynthesis (Dodani et al., 2014).

in Trp halogenases is positioned in a way that makes positions 5, 6, and 7 of Trp accessible by "flipping" of the Trp within the active site (Zhu et al., 2009) (Figure 3A), whereas chlorination at C4 would require complete repositioning of Trp.

Another common way to manipulate Trp is prenylation, which is known to occur at seven sites on the indole ring, particularly in fungal pathways (Yu et al., 2012). From bacterial systems, CymD catalyzes the reverse prenylation of Trp at N1 in cyclomarin biosynthesis (Schultz et al., 2010), and IptA catalyzes normal prenylation at C6 (Takahashi et al., 2010). Recently, a gene cluster that is widely distributed among actinomycetes was shown to encode SCO7467, which catalyzes the prenylation of Trp at C5 (Ozaki et al., 2013). Mechanistically, isotope labeling experiments on CymD have indicated that ionization of dimethylallyl pyrophosphate must occur before prenylation and that deprotonation of the indole occurs before or during direct attack of the carbocation generated (Qian et al., 2012). This is in contrast to the prenylation of Trp at C4 by 4-dimethylallyl Trp synthase, a fungal enzyme, where initial attack of the carbocation occurs at the more reactive 3-position, then subsequent intramolecular Cope rearrangement is proposed to place the prenyl group at the required position (Luk et al., 2011). In addition, although not directly acting on a Trp precursor, prenyl transferases are also responsible for adding chemical diversity to indolactams, following biosynthesis of the core structure, via reverse prenylation at the 7-position (Edwards and Gerwick, 2004).

A less common modification is nitration. The only enzyme found to nitrate Trp is TxtE, involved in biosynthesis of thaxtomins (Barry et al., 2012). This enzyme utilizes nitric oxide, which is produced by a nitric oxide synthase, TxtD, to regioselectively nitrate at C4 of Trp. This reaction is particularly remarkable given that nitric oxide usually acts as an inhibitor to cytochromes P450 (CYPs). Recent studies of the substrate tolerance of TxtE identified that a number of modifications could be made to the indole ring while still retaining enzyme activity, suggesting a potential future role for this enzyme as a nitrating biocatalyst (Dodani et al., 2014). Although crystal structures of this CYP have been solved (Dodani et al., 2014; Yu et al., 2013) (Figure 3B), the mechanism of the reaction, specifically how it differs from a usual CYP reaction, remains unknown.

Despite the common occurrence of methylation reactions within biosynthetic pathways, the only known methylation reaction to directly utilize Trp as a substrate is C2 methylation by TsrA during thiostrepton biosynthesis (Pierre et al., 2012). This reaction has a unique mechanism by which the methyl group is transferred from *S*-adenosyl methionine (SAM) to a cobalamine cofactor, and then to Trp, utilizing a [4Fe-4S] center. Other C2-methyltransferases include StnQ2 from streptonigrin biosynthesis (Xu et al., 2013) and MarI from maremycin biosynthesis (Zou et al., 2013), but these enzymes react with indolepyruvate rather than Trp itself.

Another modification is hydroxylation. One enzyme, Qui15, catalyzes hydroxylation at the β carbon of thioester-tethered Trp to create an intermediate, which is later incorporated into quinoxaline containing natural products. Interestingly, Qui15 only acts on the thiotemplated Trp (Zhang et al., 2013) but, following hydroxylation, the product is hypothesized to be cleaved from the enzyme, indicating that Trp is only loaded onto the carrier protein in order for hydroxylation to occur (Watanabe et al., 2006). Physostigmine has also been shown to derive from hydroxylated Trp; however, the proposed Trp-5-hydroxylase is not contained within the biosynthetic gene cluster (Liu et al., 2014).

A number of transformations also occur on Trp to disrupt the amino acid and create alternative functional groups. For example, the PLP-dependent aromatic L-amino acid decarboxylase (AADC) is responsible for decarboxylating Trp to give tryptamine in the biosynthesis of baccilamide C (Yuwen et al., 2013). A similar enzyme, PsmH, carries out an analogous reaction on 5-OH-Trp to give 5-OH-tryptamine, which acts as an intermediate in physostigmine biosynthesis (Liu et al., 2014). The precursor for the hapalindoles is *cis*-isonitrile indole, synthesized by AmbI1-3 from Trp, ribulose-5-phosphate, and α-ketoglutarate (α-KG), although AmbI2 appears to be functionally redundant in vitro (Hillwig et al., 2014a). This set of enzymes was identified by comparison with the known isonitrile synthase system in *E. coli*, which consists of an isonitrile synthase IsnA and an α-KG-dependent oxygenase IsnB that converts Trp to *trans*-isonitrile indole (Brady and Clardy, 2005). Radical SAM-dependent rearrangement of the α-β C-C bond of Trp by NosL in nosiheptide biosynthesis, and by NocL in nocathiacin biosynthesis, completely removes the amino acid moiety to produce 3-methyl-2-indolic acid (Zhang et al., 2011a, 2011b).

Finally, a common modification is oxidative deamination, which occurs to convert the amine group of the amino acid into a ketone. There are three routes to achieving this transformation. In maremycin biosynthesis, a PLP-dependent aminotransferase is responsible for the conversion (Zou et al., 2013). In

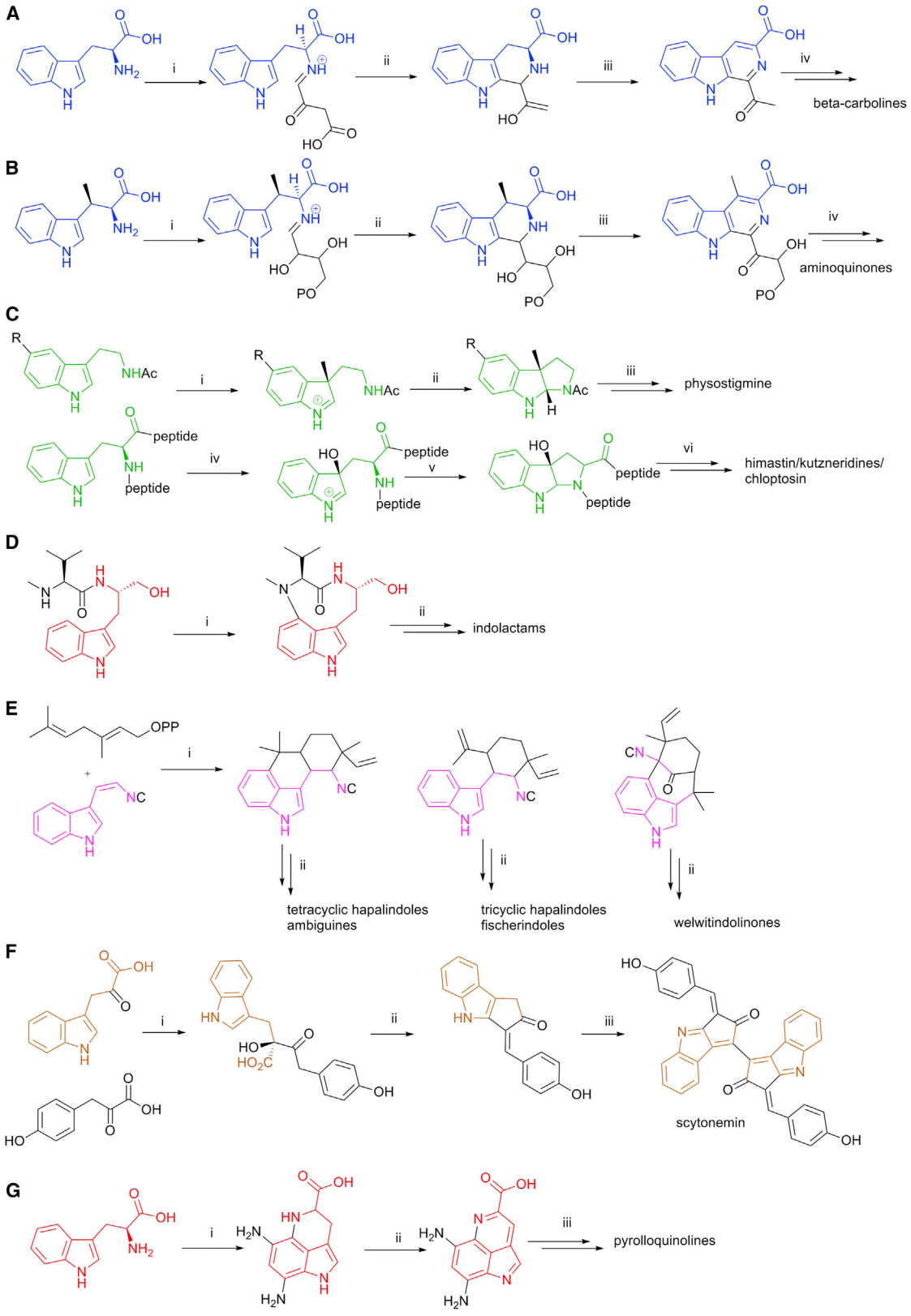


Figure 4. Formation of Novel Heterocycles

Blue, cyclization promoted by coupling to form a reactive intermediate; green, cyclization promoted by nucleophilic addition; red, cyclization promoted by oxidation; purple, intermolecular cyclization; brown, cyclization not requiring cofactors.

(legend continued on next page)

scytonemin biosynthesis, a dedicated dehydrogenase ScyB is responsible for production of indole-3-pyruvate (IPA) from Trp (Balskus and Walsh, 2008). The transformation in rebeccamycin biosynthesis is carried out preferentially on 7-Cl-Trp by RebO, an amino acid oxidase, to give 7-Cl-IPA (Nishizawa et al., 2005). Overall this large body of work shows the range of modifications made to Trp, to provide a diverse array of starting materials for incorporation into biosynthetic pathways.

New Heterocycles from Cyclization of Tryptophan Precursors

Many tryptophan-derived natural products contain heterocycles in addition to the indole. The cyclization reactions that form these additional heterocycles are often preceded by enzymatic steps that increase the reactivity of the Trp precursor. Here, we describe pathways that lead to (1) β-carbolines and aminoquinones, (2) pyrroloindoles, (3) indolactams, (4) hapalindoles, and (5) pyrroloquinolines.

The β-carbolines, containing a pyrido[3,4-*b*]indole ring, include bacterially derived molecules marinacarbolines, saframycin, and lavendamycin. Remarkably, in marinacarboline biosynthesis, a single enzyme McbB has been linked to construction of the β-carbolines. Biosynthetic investigations suggest that McbB catalyzes an initial Pictet-Spengler condensation between Trp and oxaloacetaldehyde, followed by decarboxylation and desaturation (Chen et al., 2013), with spontaneous oxidative transformations potentially playing a critical role (Yang et al., 2006). Following the reaction of McbB, the β-carboline is thought to be coupled to decarboxylated analogs of Trp, Tyr, or Phe to afford a range of marinacarbolines (Figure 4A). A similar pathway is proposed in the initial stages of aminoquinone biosynthesis, in which coupling of the amino nitrogen of a β-Me-Trp to an aldehyde of a phosphosugar promotes the Pictet-Spengler cyclization (Xu et al., 2013) (Figure 4B).

The major ring-closing step in pyrroloindole biosynthesis involves creation of a reactive iminium ion, which is subsequently attacked by the amine of the amino acid. In physostigmine this iminium ion is formed by methylation at C3 of the indole (Liu et al., 2014), whereas in himastatin it might be formed via hydroxylation at C3 (Ma et al., 2011). Following ring closure, this intermediate can be incorporated into non-ribosomal peptide synthetase (NRPS) assembly systems to give himastatin, and possibly to give related molecules such as kutznerides (Fujimori et al., 2007), chloptosin (Umezawa et al., 2000), and NW-GO1 (Guo et al., 2009) (Figure 4C).

Oxidation at C4 of indole is also a common method of achieving cyclization of new heterocycles from Trp precursors. For indolactams, the biosynthesis begins with NRPS coupling of Trp to Val, with an *N*-methylation domain catalyzing methylation of the Val amine and a reductase domain catalyzing reduction of the Trp carboxylic acid to give an alcohol. Oxidation by a CYP promotes cyclization at C4 of the indole to give the distinctive indolactam structure (Figure 4D). A prenyltransferase then adds diversity by alkylating at the C7 position of the indole (Edwards and Gerwick, 2004). The same set of reactions with Ile instead of Val gives rise to methylpendolmycin (Ma et al., 2012).

Another mechanism for formation of a new ring system is intermolecular cyclization. Hapalindole-type natural products are terpenoid indole alkaloids with a characteristic isonitrile group. These metabolites, such as the ambiguines and welwitindolinones, are isolated from cyanobacteria. Biochemical investigations have established the origins of the isocyanovinyl-indole precursor from L-Trp and ribulose-5-phosphate through the action of AmbI1 and AmbI3, as discussed previously. However, the central steps, specifically those involved in coupling isocyanovinyl-indole with geranyl pyrophosphate to form the different hapalindole ring structures, have not been demonstrated in vitro (Figure 4E). Following construction of the tri- or tetracyclic ring, oxidases, prenyltransferases, and chlorinases add further diversity to the hapalindoles (Hillwig et al., 2014a, 2014b; Hillwig and Liu, 2014).

Another complex natural product that derives from cyclization of a Trp-derived precursor is the cyanobacterial sunscreen scytonemin, which is biosynthesized from Trp and *p*-hydroxyphenylpyruvic acid. The initial step involves oxidative deamination of Trp by ScyB to form indole-3-pyruvate (IPA). IPA is then coupled to *p*-hydroxyphenylpyruvic acid by ThDP-dependent ScyA to produce a β-keto acid product (Balskus and Walsh, 2008). The subsequent step, cyclization and decarboxylation to form the cyclopentyl[*b*]indole, is catalyzed by ScyC, an apparently cofactor-independent enzyme of no known class (Balskus and Walsh, 2009) (Figure 4F). Following production of the monomer, oxidative dimerization must occur to produce the final scytonemin structure, but the mechanism for this reaction is unclear.

(A) β-Carboline marinocarboline. i, McbB catalyzes coupling of Trp to oxaloacetaldehyde; ii, decarboxylation and Pictet-Spengler cyclization forms a new heterocycle; iii, spontaneous oxidation forms the β-carboline structure; iv, the free carboxylic acid can be coupled to different amino acid-derived amines to form a variety of β-carbolines (Chen et al., 2013).
(B) Aminoquinone streptonigrin. i, StnI is proposed to couple β-Me-Trp to a phosphosugar; ii, Pictet-Spengler cyclization forms a new heterocycle; iii, enzyme catalyzed or spontaneous oxidation creates the aminoquinone precursor; iv, numerous enzyme-catalyzed steps transform this precursor into aminoquinones (Xu et al., 2013).
(C) Pyrroloindole physostigmine (above) and himastatin (below). i, SAM-dependent methylation is catalyzed by PsmD, creating an iminium ion; ii, attack of the iminium by the amine creates a novel heterocycle; iii, methylation, deacetylation, and a further methylation generates physostigmine (Liu et al., 2014); iv, hydroxylation by CYP HmtT might form an iminium ion; v, attack of the iminium by the amine creates a novel heterocycle (Ma et al., 2011); vi, further modifications create a range of depsipeptides such as himastatin, kutzneridines, and chlopostin.
(D) Indolactam lyngbyatoxin. i, NRPS-catalyzed synthesis of the dipeptide oxidation by CYP LtxB and cyclization generates the indolactam; ii, prenylation by LtxC (Edwards and Gerwick, 2004).
(E) Hapalindoles. i, Coupling between isocyanovinyl-indole and geranyl pyrophosphate, proposed to be catalyzed by AmbP1 and homologs, generates three possible products (Hillwig et al., 2014a); ii, a variety of Reiske-type oxygenases have been implicated in transforming the simple scaffolds into more complex hapalindoles (Hillwig et al., 2014b).
(F) Scytonemin. i, Oxidized Trp is coupled to p-hydroxyphenylpyruvic acid by ScyA (Balskus and Walsh, 2008); ii, cyclization and decarboxylation by ScyC (Balskus and Walsh, 2009); iii, dimerization via an unknown mechanism.
(G) The biosynthetic pathway to pyrroloquinolines is unknown but might proceed via i, oxidation of Trp at C5 followed by cyclization; ii, oxidation to generate the pyrroloquinoline; iii, further modifications to generate a variety of pyrroloquinolines.

 ©2015 Elsevier Ltd All rights reserved

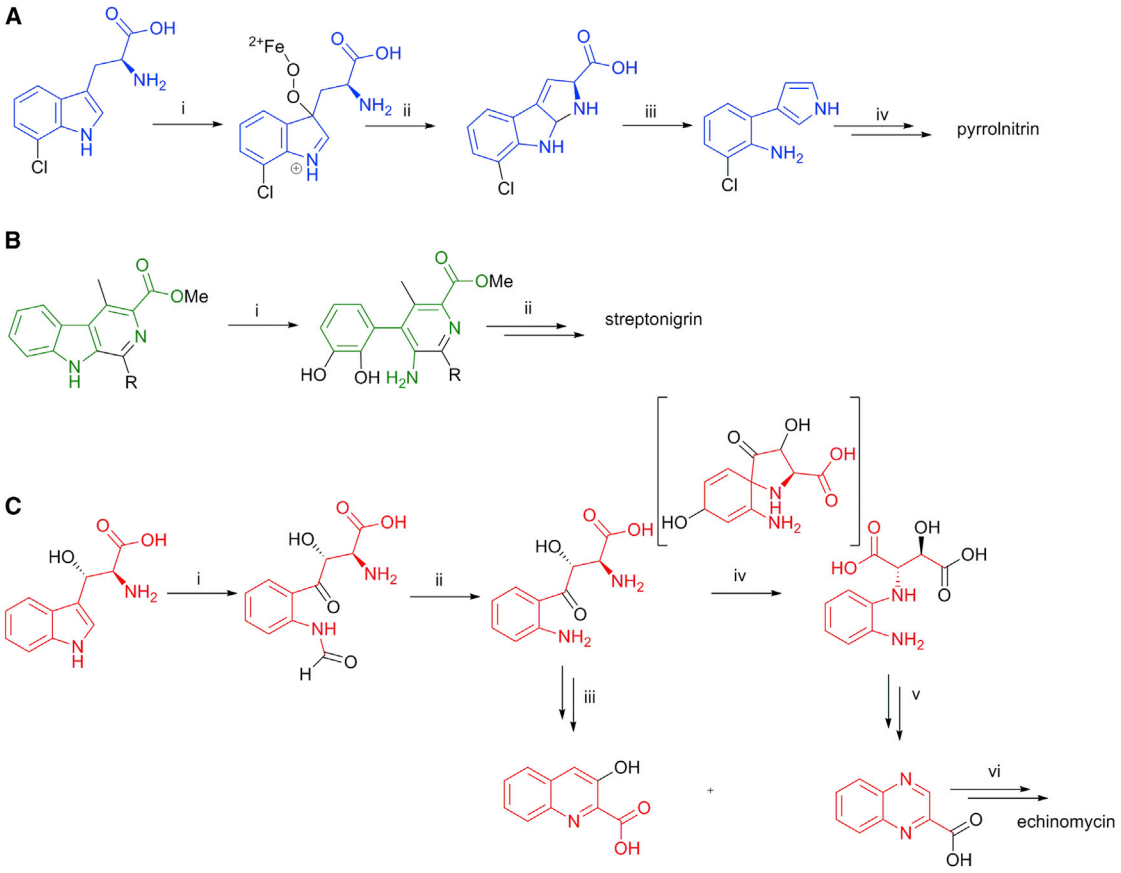


Figure 5. Disguised Trp-Derived Natural Products Involving C-N Bond Breaking of the Indole

(A) Pyrrolnitrin. i, Oxidation at C3 by PrnB (heme-dependent dioxygenase) causes formation of a reactive iminium; ii, iminium attack by the amine leads to formation of a new 5-membered ring; iii, decarboxylation and ring opening generates monodechloro-amino pyrrolnitrin (Zhu et al., 2010); iv, PrnC (FAD-dependent chlorinase) catalyzes a second chlorination, and PrnD (oxygenase) causes oxidation of the amine to a nitro group and generates pyrrolnitrin (Lee et al., 2006).

(B) Streptonigrin. i, Oxidative ring opening by StbB1-3; ii, a number of additional enzymes are utilized to generate aminoquinones such as streptonigrin (Xu et al., 2013).

(C) Echinomycin. i, Oxidative ring opening by Trp 2,3-dioxygenase (Zhang et al., 2013); ii, arylformidase activity by an unknown enzyme; iii, in SW-163D biosynthesis, Swb1 (aminotransferase) and Swb2 (oxidoreductase) generate 3-hydroxyquinaldic acid; iv, in echinomycin biosynthesis, Ecm4 (oxidoreductase) causes rearrangement of the ring-opened product via a 5-membered ring; v, oxidation by Ecm3 (dehydrogenase) is followed by spontaneous decarboxylation, cyclization, and dehydration to generate quinoxaline-2-carboxylic acid; vi, these intermediates are incorporated into NRPSs to produce quinoxaline- and quinolone-type antibiotics such as echinomycin and SW-163D (Hirose et al., 2011).

Although many of the enzymes involved in cyclization have been characterized, there are several natural products in which the responsible enzymes are unknown. Thienolodin, for example, has an interesting thiol-containing ring. Although the gene cluster for thienolodin production has been identified and both the initial and final enzymes in the pathway have been characterized, neither the mechanism for sulfur incorporation nor for cyclization has been determined (Milbredt et al., 2014). Likewise, although the pyrroloquinoline lymphostin has been shown to be derived from Trp, and the nitrogens at C5 and C7 have been shown by ^{15}N feeding experiments to derive from glutamine, the mechanism for incorporation of these precursors and for cyclization at C4 is unknown (Miyanaga et al., 2011) (Figure 4G).

Disguising Tryptophan in Natural Products

In all the previously discussed cases the metabolic origins of the natural product are fairly easily determined, due to retention of the basic indole scaffold throughout the biosynthetic pathways. Other natural products have been isolated that are not immediately characterizable as tryptophan derived, since C-C or C-N bond breaking within the indole has disrupted this distinguishing feature. It is the susceptibility of Trp to oxidation that promotes these reactions.

In pyrrolnitrin biosynthesis, indole cleavage occurs after Trp chlorination via a heme-dependent dioxygenase PrnB. This reaction is proposed, based on crystallographic evidence, to start via iminium formation through oxidation at C3, with subsequent attack by the primary amino acid amine. Elimination of the hydroxide at C3 and decarboxylation then breaks the original N1-C2 bond and forms the pyrrole (Zhu et al., 2010). Following this key ring-opening step, a second chlorinase, PrnC, catalyzes chlorination at the pyrrole ring and a Reiske oxygenase oxidizes the remaining amine to a nitro group to give pyrrolnitrin (Lee et al., 2006) (Figure 5A).

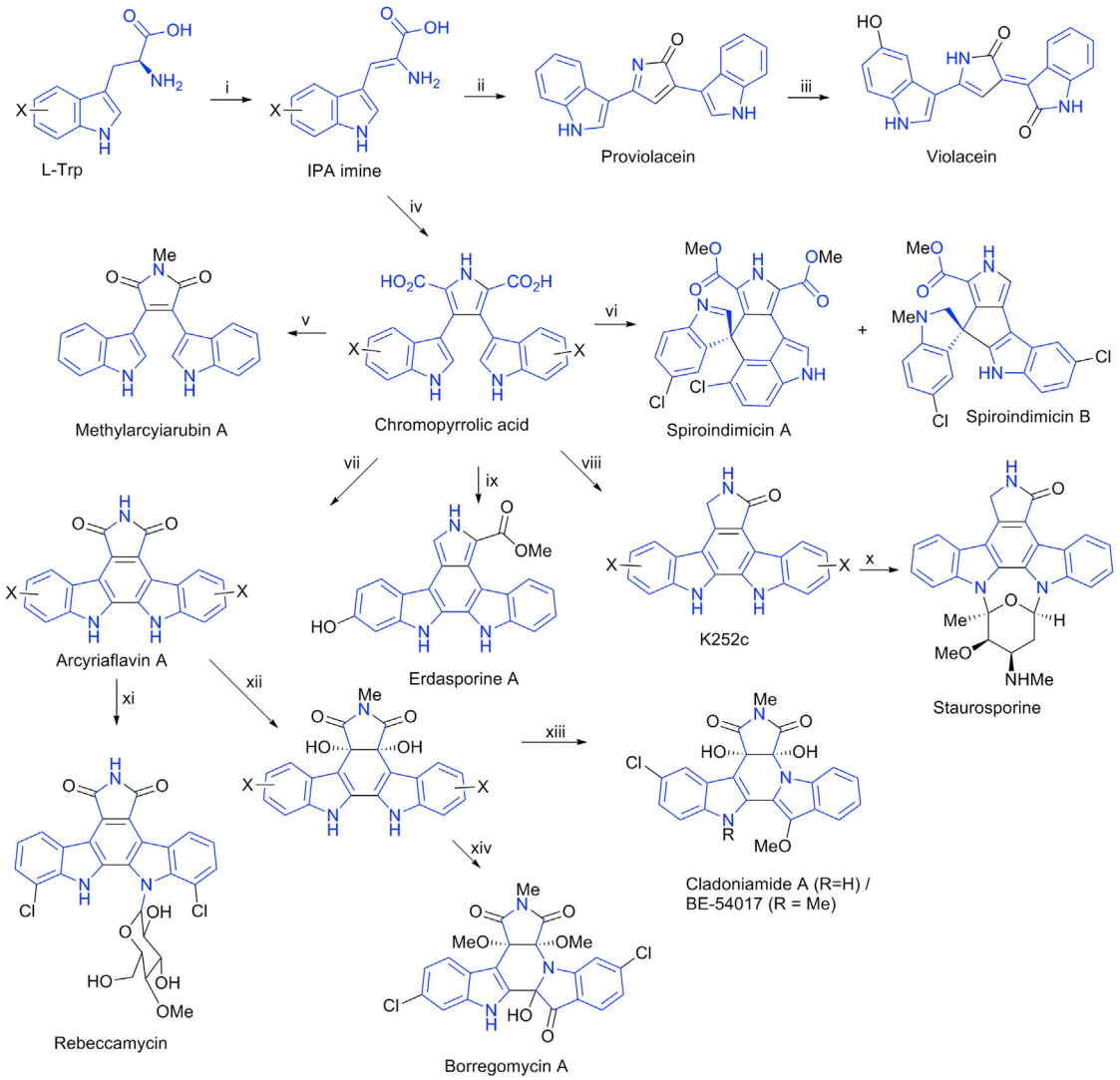


Figure 6. Trp Dimerization Pathways

i, Conversion of Trp to the IPA imine by amino acid oxidase (Nishizawa et al., 2005); ii, proviolacein forms from the action of chromopyrrolic acid (CPA) synthase and violacein biosynthetic enzyme VioE (Sánchez et al., 2006b); iii, conversion to violacein by flavin-dependent oxygenases VioC and VioD (Balibar and Walsh, 2006); iv, formation of the central intermediate CPA from two molecules of the IPA imine (Asamizu et al., 2012); v, formation of methylarcyriarubin A by MarC (Chang and Brady, 2014); vi, CPA is the proposed precursor of the spiroindimicins (Zhang et al., 2012); vii, 8-electron oxidative decarboxylation by RebC leads to arcyriaflavin; viii, 4-electron oxidative decarboxylation by StaC leads to K252C (Asamizu et al., 2011; Goldman et al., 2012; Groom et al., 2011); ix, formation of erdasporine by EspM and EspX (Chang et al., 2013); x, glycosylation of K252C by StaG, followed by attachment of the sugar to the other indole nitrogen by StaN and methylation by StaMA and StaMB forms staurosporine (Onaka et al., 2002); xi, glycosylation of arcyriaflavin A by RebG and methylation by RebM leads to rebeccamycin (Zhang et al., 2006); xii, epoxidation and hydrolysis, followed by *N*-methylation, and xiii, oxidation followed by rotation of the "right-hand" indole, recyclization, and methylation produces cladoniamide A and BE-54017 (Chang and Brady, 2011; Du et al., 2014); xiv, borregomycin A derives from oxidation of the intermediate by BorX2, followed by rotation of the "right-hand" indole and recyclization, putatively followed by action of additional borregomycin enzymes (Chang and Brady, 2013).

Aminoquinone streptonigrin is unique in that it first undergoes a Pictet-Spengler cyclization to add an extra ring to its structure, as discussed above for the aminoquinones, then subsequently undergoes ring opening to disrupt the indole. As with pyrrolnitrin biosynthesis the ring opening is initiated by oxidation, although in this case by the StnB1-3 system (Xu et al., 2013) (Figure 5B).

Quinoxaline- and quinolone-containing natural products, such as echinomycin and triostin A, and SW-163C and UK-63598, respectively, are perhaps the least obviously identifiable as being derived from Trp. The transformation from Trp to quinoxaline and quinoline begins with the unusual hydroxylation, described above, in which Trp is first loaded onto an NRPS adenylation domain, hydroxylated, and then putatively cleaved. The hydroxylated product is then oxidized by a Trp 2,3 dioxygenase, which breaks the C2-C3 bond (Zhang et al., 2013). Arylformidase activity then leads to an intermediate that can undergo a transamination/cyclization reaction, followed by a reduction, to give quinolone. Alternatively, this molecule can instead first undergo an oxidative rearrangement in which the amide nitrogen attacks the benzene ring to form a five-membered heterocycle. This

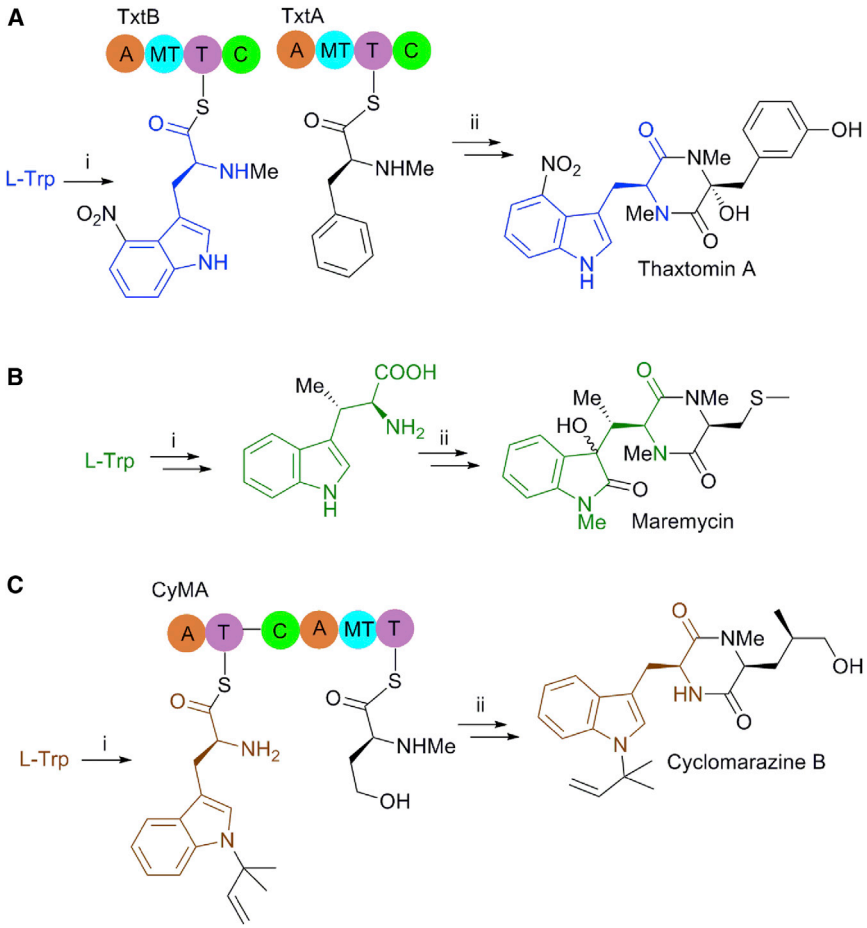


Figure 7. Pathways to Generate Diketopiperazines

(A) Thaxtomins. i, Trp is nitrated, loaded onto TxtB (NRPS), and *N*-methylated; ii, condensation of 4-nitro-N-Me-Trp with N-Me-Phe by condensation domains in TxtB and TxtA form the DKP, and TxtC (P450) is proposed to catalyze dihydroxylation to generate thaxtomin A (Barry et al., 2012).

(B) Maremycins. i, MarG (aminotransferase) and MarI (methyltransferase) generate β-methyl-Trp from Trp, with MarH (cupin-fold) acting as a stereochemical-switch protein (Zou et al., 2013); ii, proposed NRPS coupling with methionine would generate maremycin.

(C) Cyclomarazine A. i, Trp is prenylated by CymD (prenyl transferase) (Schultz et al., 2010) and loaded onto the first module of CymA (NRPS); ii, condensation of prenylated Trp and N-Me-Met by CymA generates cyclomarazine B (Schultz et al., 2008).

heterocycle can undergo ring opening by water to regenerate the aromatic ring and leave an *N*-linked side chain. Decarboxylation and cyclization of this rearranged product are believed to be spontaneous, and result in production of quinoxaline (Hirose et al., 2011) (Figure 5C).

Dimerization Pathways

The dimerization pathways of Trp, including the biosynthesis of violacein, rebeccamycin, staurosporine, and K252a, have largely been covered by previous reviews (Ryan and Drennan, 2009; Sánchez et al., 2006a), but in recent years several novel natural products have been identified with alternative scaffolds (Figure 6). In an interesting example of exploiting a key biosynthetic gene to find new pathways and compounds, degenerate primers specific for the chromopyrrolic acid (CPA) synthase gene *staD* have been used to screen microbial isolates and eDNA sources for new *staD*-containing gene clusters. Through this approach, the Zhang group has discovered the spiroindimicins (Zhang et al., 2012) and indimicins (Zhang et al., 2014). Chang and Brady have screened an impressive number of eDNA libraries for *staD*-containing clusters, and have found gene clusters for BE-54017, borregomycins, erdasporines, and methylarcyriarubin A, and have demonstrated that these molecules derive from CPA (Chang and Brady, 2011, 2013, 2014; Chang et al., 2013). In addition, they identified a unique target for BE-54017/cladoniamide as the proteolipid subunit of the vacuolar ATPase (Chang et al., 2014). Both BE-54017/cladoniamide and borregomycins are distinguished from the other bisindoles by oxidation of the central double bond, which results in a *cis*-bishydroxide and disruption of the aromatic ring. A further oxidation is also thought to occur, which allows for rotation of one of the indole groups and produces the unique indolotryptoline scaffold (Du et al., 2014). Methylation reactions are believed to play an important role in the stability of these reactive natural products. In borregomycin biosynthesis, premature methylation of the bishydroxide is proposed to inhibit the subsequent oxidative steps and prevent formation of the indolotryptoline (Chang and Brady, 2013), and in cladoniamide biosynthesis, genetic elimination of the methyltransferase responsible for the final *O*-methylation reroutes intermediates to xenocladoniamides (Du et al., 2013).

Incorporation into Peptides

A common route for the incorporation of all amino acids into complex natural products is through peptide bond formation. Indeed, both NRPS products, such as daptomycin and calcium-dependent antibiotics, and ribosomally synthesized lanthipeptides, such as planosporocin and actagardine, have Trp residues in their final structures. Here we focus on diketopiperazines (DKPs), which are commonly formed by NRPSs in which, following loading by two modules, the condensation domain of the second module catalyzes the formation of a second peptide bond between the two amino acids. This mechanism has been reviewed elsewhere (Gu et al., 2013), but there are noteworthy modifications to some Trp-containing DKPs, including maremycins, thaxtomins, and cyclomarazines. In thaxtomins, this modification takes the form of a regiospecific nitration at the 4-position of Trp, discussed previously. The biosynthesis is completed by NRPS coupling and cyclization to Phe, *N*-methylation, and proposed hydroxylation by a bifunctional CYP, TxtC (King and Calhoun, 2009) (Figure 7A).

Analogously, cyclomarazine is prenylated at the indole nitrogen before NRPS manipulation, which carries out both condensation reactions and methylation at the amide (Schultz et al., 2010). Cyclomarazine is unique, as it is a shunt product of the much larger NRPS system used to make cyclomarin (Schultz et al., 2008) (Figure 7B). The initial steps of maremycin biosynthesis involve a unique three-enzyme pathway of an aminotransferase, a methyltransferase, and an unusual cupin fold-containing enzyme that directs the stereochemical outcome, ultimately producing β-Me-Trp (Zou et al., 2013) (Figure 7C). A more unusual route to DKPs, including Trp-containing structures, is by the action of cyclodipeptide synthases (CDPS), which utilize aminoacyl tRNAs in an ATP-independent fashion to directly form peptide bonds. The cyclized products can then be modified via oxidation, methylation, and dehydrogenation (Giessen et al., 2013).

Conclusion

The pathways described herein show the many different ways in which Trp can be incorporated into biosynthetic pathways. The inherent reactivity of Trp encourages oxidative reactions, electrophilic aromatic substitutions, and alkylation reactions, which produce a diverse array of starting materials. These intermediates can then be incorporated into different scaffolds via multiple mechanisms: intramolecular and intermolecular cyclization reactions, reactions which break the core indole ring of Trp, dimerization of two Trp moieties, and Trp incorporation into common peptide pathways. Further ''decoration'' of these scaffolds then adds extra diversity.

The growing body of work investigating the ways in which Trp can be manipulated and transformed into useful natural products has also informed studies on synthetic biology and channeling these pathways into alternative products. Enzymatic halogenation, for example, is a promising biosynthetic tool. The O'Connor group showed that both RebH and PyrH could be introduced into a plant system, leading to 7-Cl-Trp and 5-Cl-Trp incorporation into plant alkaloids (Runguphan et al., 2010). The stability and productive efficiency of RebH have also been vastly improved, both in isolation, to allow production of 7-Cl-Trp on a gram scale (Frese and Sewald, 2014), and to utilize the enzyme on non-natural substrates as a synthetic biocatalyst (Payne et al., 2013). These are just examples of what has been accomplished with one enzyme that acts on Trp. What can be further accomplished from the plethora of additional pathways and enzymes described here remains to be seen.

REFERENCES

Arnison, P.G., Bibb, M.J., Bierbaum, G., Bowers, A.A., Bugni, T.S., Bulaj, G., Camarero, J.A., Campopiano, D.J., Challis, G.L., Clardy, J., et al. (2013). Ribosomally synthesized and post-translationally modified peptide natural products: overview and recommendations for a universal nomenclature. Nat. Prod. Rep. *30*, 108–160.

Asamizu, S., Shiro, Y., Igarashi, Y., Nagano, S., and Onaka, H. (2011). Characterization and functional modification of StaC and RebC, which are involved in the pyrrole oxidation of indolocarbazole biosynthesis. Biosci. Biotechnol. Biochem. *75*, 2184–2193.

Asamizu, S., Hirano, S., Onaka, H., Koshino, H., Shiro, Y., and Nagano, S. (2012). Coupling reaction of indolepyruvic acid by StaD and its product: implications for biosynthesis of indolocarbazole and violacein. ChemBioChem *13*, 2495–2500.

Balibar, C.J., and Walsh, C.T. (2006). In vitro biosynthesis of violacein from L-tryptophan by the enzymes VioA-E from *Chromobacterium violaceum*. Biochemistry *45*, 15444–15457.

Balskus, E.P., and Walsh, C.T. (2008). Investigating the initial steps in the biosynthesis of cyanobacterial sunscreen scytonemin. J. Am. Chem. Soc. *130*, 15260–15261.

Balskus, E.P., and Walsh, C.T. (2009). An enzymatic cyclopentyl[*b*]indole formation involved in scytonemin biosynthesis. J. Am. Chem. Soc. *131*, 14648–14649.

Barry, S.M., Kers, J.A., Johnson, E.G., Song, L., Aston, P.R., Patel, B., Krasnoff, S.B., Crane, B.R., Gibson, D.M., Loria, R., et al. (2012). Cytochrome P450–catalyzed L-tryptophan nitration in thaxtomin phytotoxin biosynthesis. Nat. Chem. Biol. *8*, 814–816.

Bennett, B.D., Kimball, E.H., Gao, M., Osterhout, R., Van Dien, S.J., and Rabinowitz, J.D. (2009). Absolute metabolite concentrations and implied enzyme active site occupancy in *Escherichia coli*. Nat. Chem. Biol. *5*, 593–599.

Bentley, R. (1990). The shikimate pathway—a metabolic tree with many branches. Crit. Rev. Biochem. Mol. Biol. *25*, 307–384.

Brady, S.F., and Clardy, J. (2005). Cloning and heterologous expression of isocyanide biosynthetic genes from environmental DNA. Angew. Chem. Int. Ed. Engl. *117*, 7225–7227.

Chang, F.Y., and Brady, S.F. (2011). Cloning and characterization of an environmental DNA-derived gene cluster that encodes the biosynthesis of the antitumor substance BE-54017. J. Am. Chem. Soc. *133*, 9996–9999.

Chang, F.-Y., and Brady, S.F. (2013). Discovery of indolotryptoline antiproliferative agents by homology-guided metagenomic screening. Proc. Natl. Acad. Sci. USA *110*, 2478–2483.

Chang, F.-Y., and Brady, S.F. (2014). Characterization of an environmental DNA-derived gene cluster that encodes the bisindolylmaleimide methylarcyriarubin. ChemBioChem *15*, 815–821.

Chang, F.-Y., Ternei, M.A., Calle, P.Y., and Brady, S.F. (2013). Discovery and synthetic refactoring of tryptophan dimer gene clusters from the environment. J. Am. Chem. Soc. *135*, 17906–17912.

Chang, F.-Y., Kawashima, S.A., and Brady, S.F. (2014). Mutations in the proteolipid subunits of the vacuolar H(+)-ATPase provide resistance to indolotryptoline natural products. Biochemistry *53*, 7123–7131.

Chen, Q., Ji, C., Song, Y., Huang, H., Ma, J., Tian, X., and Ju, J. (2013). Discovery of McbB, an enzyme catalyzing the β-carboline skeleton construction in the marinacarboline biosynthetic pathway. Angew. Chem. Int. Ed. Engl. *52*, 9980–9984.

Dayhoff, M.O., Schwartz, R.M., and Orcutt, B.C. (1978). A model of evolutionary change in proteins. In Atlas of Protein Sequence and Structure, B.C. Orcutt, ed. (National Biomedical Research Foundation), pp. 345–352.

Dhanak, D., and Reese, C.B. (1986). Studies in the protection of pyrrole and indole derivatives. J. Chem. Soc. Perkin Trans. *1*, 2181–2186.

Dodani, S.C., Cahn, J.K.B., Heinisch, T., Brinkmann-Chen, S., McIntosh, J.A., and Arnold, F.H. (2014). Structural, functional, and spectroscopic characterization of the substrate scope of the novel nitrating cytochrome P450 TxtE. ChemBioChem *15*, 2259–2267.

Dong, C., Flecks, S., Unversucht, S., Haupt, C., van Pee, K.H., and Naismith, J.H. (2005). Tryptophan 7-halogenase (PrnA) structure suggests a mechanism for regioselective chlorination. Science *309*, 2216–2219.

Du, Y.-L., and Ryan, K.S. (2015). Expansion of bisindole biosynthetic pathways by combinatorial construction. ACS Synth. Biol. http://dx.doi.org/10.1021/sb5003218.

Du, Y.-L., Ding, T., and Ryan, K.S. (2013). Biosynthetic *O*-methylation protects cladoniamides from self-destruction. Org. Lett. *15*, 2538–2541.

Du, Y.-L., Williams, D.E., Patrick, B.O., Andersen, R.J., and Ryan, K.S. (2014). Reconstruction of cladoniamide biosynthesis reveals nonenzymatic routes to bisindole diversity. ACS Chem. Biol. *9*, 2748–2754.

Edwards, D.J., and Gerwick, W.H. (2004). Lyngbyatoxin biosynthesis: sequence of biosynthetic gene cluster and identification of a novel aromatic prenyltransferase. J. Am. Chem. Soc. *126*, 11432–11433.

 ©2015 Elsevier Ltd All rights reserved

Flecks, S., Patallo, E.P., Zhu, X., Ernyei, A.J., Seifert, G., Schneider, A., Dong, C., Naismith, J.H., and van Pée, K.H. (2008). New insights into the mechanism of enzymatic chlorination of tryptophan. Angew. Chem. Int. Ed. Engl. *47*, 9533–9536.

Frese, M., and Sewald, N. (2014). Enzymatic halogenation of tryptophan on a gram scale. Angew. Chem. Int. Ed. Engl. *54*, 298–301.

Fujimori, D.G., Hrvatin, S., Neumann, C.S., Strieker, M., Marahiel, M.A., and Walsh, C.T. (2007). Cloning and characterization of the biosynthetic gene cluster for kutznerides. Proc. Natl. Acad. Sci. USA *104*, 16498–16503.

Gao, Q., Zhang, C., Blanchard, S., and Thorson, J.S. (2006). Deciphering indolocarbazole and enediyne aminodideoxypentose biosynthesis through comparative genomics: insights from the AT2433 biosynthetic locus. Chem. Biol. *13*, 733–743.

Giessen, T.W., von Tesmar, A.M., and Marahiel, M.A. (2013). A tRNA-dependent two-enzyme pathway for the generation of singly and doubly methylated ditryptophan 2,5-diketopiperazines. Biochemistry *52*, 4274–4283.

Goldman, P.J., Ryan, K.S., Hamill, M.J., Howard-Jones, A.R., Walsh, C.T., Elliott, S.J., and Drennan, C.L. (2012). An unusual role for a mobile flavin in StaC-like indolocarbazole biosynthetic enzymes. Chem. Biol. *19*, 855–865.

Groom, K., Bhattacharya, A., and Zechel, D.L. (2011). Rebeccamycin and staurosporine biosynthesis: insight into the mechanisms of the flavin-dependent monooxygenases RebC and StaC. ChemBioChem *12*, 396–400.

Gu, B., He, S., Yan, X., and Zhang, L. (2013). Tentative biosynthetic pathways of some microbial diketopiperazines. Appl. Microbiol. Biotechnol. *97*, 8439–8453.

Guo, Z., Shen, L., Ji, Z., Zhang, J., Huang, L., and Wu, W. (2009). NW-G01, a novel cyclic hexadepsipeptide antibiotic, produced by *Streptomyces albofla-vus* 313: I. Taxonomy, fermentation, isolation, physicochemical properties and antibacterial activities. J. Antibiot. *62*, 201–205.

Hillwig, M.L., and Liu, X. (2014). A new family of iron-dependent halogenases acts on freestanding substrates. Nat. Chem. Biol. *10*, 921–923.

Hillwig, M.L., Zhu, Q., and Liu, X. (2014a). Biosynthesis of ambiguine indole alkaloids in cyanobacterium *Fischerella ambigua*. ACS Chem. Biol. *9*, 372–377.

Hillwig, M.L., Fuhrman, H.A., Ittiamornkul, K., Sevco, T.J., Kwak, D.H., and Liu, X. (2014b). Identification and characterization of a welwitindolinone alkaloid biosynthetic gene cluster in the Stigonematalean cyanobacterium *Hapalosi-phon welwitschii*. ChemBioChem *15*, 665–669.

Hinman, R.L., and Lang, J. (1964). The protonation of indoles basicity studies. The dependence of acidity functions on indicator structure. J. Am. Chem. Soc. *86*, 3796–3806.

Hirose, Y., Watanabe, K., Minami, A., Nakamura, T., Oguri, H., and Oikawa, H. (2011). Involvement of common intermediate 3-hydroxy-L-kynurenine in chromophore biosynthesis of quinomycin family antibiotics. J. Antibiot. *64*, 117–122.

Hur, G.H., Vickery, C.R., and Burkart, M.D. (2012). Explorations of catalytic domains in non-ribosomal peptide synthetase enzymology. Nat. Prod. Rep. *29*, 1074.

King, R.R., and Calhoun, L.A. (2009). The thaxtomin phytotoxins: sources, synthesis, biosynthesis, biotransformation and biological activity. Phytochemistry *70*, 833–841.

Lee, J.-K., Ang, E.-L., and Zhao, H. (2006). Probing the substrate specificity of aminopyrrolnitrin oxygenase (PrnD) by mutational analysis. J. Bacteriol. *188*, 6179–6183.

Liu, J., Ng, T., Rui, Z., Ad, O., and Zhang, W. (2014). Unusual acetylation-dependent reaction cascade in the biosynthesis of the pyrroloindole drug physostigmine. Angew. Chem. Int. Ed. Engl. *53*, 136–139.

Luk, L.Y.P., Qian, Q., and Tanner, M.E. (2011). A Cope rearrangement in the reaction catalyzed by dimethylallyltryptophan synthase? J. Am. Chem. Soc. *133*, 12342–12345.

Ma, J., Wang, Z., Huang, H., Luo, M., Zuo, D., Wang, B., Sun, A., Cheng, Y.-Q., Zhang, C., and Ju, J. (2011). Biosynthesis of himastatin: assembly line and characterization of three cytochrome P450 enzymes involved in the post-tailoring oxidative steps. Angew. Chem. Int. Ed. Engl. *50*, 7797–7802.

Ma, J., Zuo, D., Song, Y., Wang, B., Huang, H., Yao, Y., Li, W., Zhang, S., Zhang, C., and Ju, J. (2012). Characterization of a single gene cluster responsible for methylpendolmycin and pendolmycin biosynthesis in the deep sea bacterium *Marinactinospora thermotolerans*. ChemBioChem *13*, 547–552.

Micallef, M.L., Sharma, D., Bunn, B.M., Gerwick, L., Viswanathan, R., and Moffitt, M.C. (2014). Comparative analysis of hapalindole, ambiguine and welwitindolinone gene clusters and reconstitution of indole-isonitrile biosynthesis from cyanobacteria. BMC Microbiol. *14*, 213–231.

Milbredt, D., Patallo, E.P., and van Pée, K.-H. (2014). A tryptophan 6-halogenase and an amidotransferase are involved in thienodolin biosynthesis. ChemBioChem *15*, 1011–1020.

Miyanaga, A., Janso, J.E., McDonald, L., He, M., Liu, H., Barbieri, L., Eustáquio, A.S., Fielding, E.N., Carter, G.T., Jensen, P.R., et al. (2011). Discovery and assembly-line biosynthesis of the lymphostin pyrroloquinoline alkaloid family of mTOR inhibitors in *Salinispora* bacteria. J. Am. Chem. Soc. *133*, 13311–13313.

Nishizawa, T., Aldrich, C.C., and Sherman, D.H. (2005). Molecular analysis of the rebeccamycin L-amino acid oxidase from *Lechevalieria aerocolonigenes* ATCC 39243. J. Bacteriol. *187*, 2084–2092.

Onaka, H., Taniguchi, S., Igarashi, Y., and Furumai, T. (2002). Cloning of the staurosporine biosynthetic gene cluster from *Streptomyces* sp. TP-A0274 and its heterologous expression in *Streptomyces lividans*. J. Antibiot. *55*, 1063–1071.

Ozaki, T., Nishiyama, M., and Kuzuyama, T. (2013). Novel tryptophan metabolism by a potential gene cluster that is widely distributed among actinomycetes. J. Biol. Chem. *288*, 9946–9956.

Payne, J.T., Andorfer, M.C., and Lewis, J.C. (2013). Regioselective arene halogenation using the FAD-dependent halogenase RebH. Angew. Chem. Int. Ed. Engl. *52*, 5271–5274.

Pierre, S., Guillot, A., Benjdia, A., Sandström, C., Langella, P., and Berteau, O. (2012). Thiostrepton tryptophan methyltransferase expands the chemistry of radical SAM enzymes. Nat. Chem. Biol. *8*, 957–959.

Qian, Q., Schultz, A.W., Moore, B.S., and Tanner, M.E. (2012). Mechanistic studies on CymD: a tryptophan reverse N-prenyltransferase. Biochemistry *51*, 7733–7739.

Rodrigues, A.L., Trachtmann, N., Becker, J., Lohanatha, A.F., Blotenberg, J., Bolten, C.J., Korneli, C., de Souza Lima, A.O., Porto, L.M., Sprenger, G.A., et al. (2013). Systems metabolic engineering of *Escherichia coli* for production of the antitumor drugs violacein and deoxyviolacein. Metab. Eng. *20*, 29–41.

Rodrigues, A.L., Becker, J., de Souza Lima, A.O., Porto, L.M., and Wittmann, C. (2014). Systems metabolic engineering of *Escherichia coli* for gram scale production of the antitumor drug deoxyviolacein from glycerol. Biotechnol. Bioeng. *111*, 2280–2289.

Runguphan, W., Qu, X., and O'Connor, S.E. (2010). Integrating carbon-halogen bond formation into medicinal plant metabolism. Nature *468*, 461–464.

Ryan, K.S., and Drennan, C.L. (2009). Divergent pathways in the biosynthesis of bisindole natural products. Chem. Biol. *16*, 351–364.

Sakagami, Y., Manabe, K., Aitani, T., Thiruvikraman, S.V., and Marumo, S. (1993). L-4-chlorotryptophan from immatum of *Pisum sativum* and reassignment of the absolute stereochemistry of N-malonyl-4-chlorotryptophan. Tetrahedron Lett. *34*, 1057–1060.

Sánchez, C., Méndez, C., and Salas, J.A. (2006a). Indolocarbazole natural products: occurrence, biosynthesis, and biological activity. Nat. Prod. Rep. *23*, 1007–1045.

Sánchez, C., Braña, A.F., Méndez, C., and Salas, J.A. (2006b). Reevaluation of the violacein biosynthetic pathway and its relationship to indolocarbazole biosynthesis. ChemBioChem *7*, 1231–1240.

Santiveri, C.M., and Jiménez, M.A. (2010). Tryptophan residues: scarce in proteins but strong stabilizers of β-hairpin peptides. Biopolymers *94*, 779–790.

Schultz, A.W., Oh, D.C., Carney, J.R., Williamson, R.T., Udwary, D.W., Jensen, P.R., Gould, S.J., Fenical, W., and Moore, B.S. (2008). Biosynthesis and structures of cyclomarins and cyclomarazines, prenylated cyclic peptides of marine actinobacterial origin. J. Am. Chem. Soc. *130*, 4507–4516.

Schultz, A.W., Lewis, C.A., Luzung, M.R., Baran, P.S., and Moore, B.S. (2010). Functional characterization of the cyclomarin/cyclomarazine prenyltransferase CymD directs the biosynthesis of unnatural cyclic peptides. J. Nat. Prod. *73*, 373–377.

Simat, T.J., and Steinhart, H. (1998). Oxidation of free tryptophan and tryptophan residues in peptides and proteins. J. Agric. Food. Chem. *46*, 490–498.

Smith, D.R.M., Willemse, T., Gkotsi, D.S., Schepens, W., Maes, B.U.W., Ballet, S., and Goss, R.J.M. (2014). The first one-pot synthesis of L-7-iodotryptophan from 7-iodoindole and serine, and an improved synthesis of other L-7-halotryptophans. Org. Lett. *16*, 2622–2625.

Sorrels, C.M., Proteau, P.J., and Gerwick, W.H. (2009). Organization, evolution, and expression analysis of the biosynthetic gene cluster for scytonemin, a cyanobacterial UV-absorbing pigment. Appl. Environ. Microbiol. *75*, 4861–4869.

Sundberg, R.J. (1970). The Chemistry of Indoles. (Academic Press).

Takahashi, S., Takagi, H., Toyoda, A., Uramoto, M., Nogawa, T., Ueki, M., Sakaki, Y., and Osada, H. (2010). Biochemical characterization of a novel indole prenyltransferase from *Streptomyces* sp. SN-593. J. Bacteriol. *192*, 2839–2851.

Umezawa, K., Ikeda, Y., Uchihata, Y., Naganawa, H., and Kondo, S. (2000). Chloptosin, an apoptosis-inducing dimeric cyclohexapeptide produced by *Streptomyces*. J. Org. Chem. *65*, 459–463.

Vitkup, D., Sander, C., and Church, G.M. (2003). The amino-acid mutational spectrum of human genetic disease. Genome Biol. *4*, R72.

Waldrop, G.L., Rayment, I., and Holden, H.M. (1994). Three-dimensional structure of the biotin carboxylase subunit of acetyl-CoA carboxylase. Biochemistry *33*, 10249–10256.

Wallace, B.A., and Janes, R.W. (1999). Tryptophans in membrane proteins. X-ray crystallographic analyses. Adv. Exp. Med. Biol. *467*, 789–799.

Watanabe, K., Hotta, K., Praseuth, A.P., Koketsu, K., Migita, A., Boddy, C.N., Wang, C.C.C., Oguri, H., and Oikawa, H. (2006). Total biosynthesis of antitumor nonribosomal peptides in *Escherichia coli*. Nat. Chem. Biol. *2*, 423–428.

Xie, G., Keyhani, N.O., Bonner, C.A., and Jensen, R.A. (2003a). Ancient origin of the tryptophan operon and the dynamics of evolutionary change. Microbiol. Mol. Biol. Rev. MMBR *67*, 303–342.

Xie, G., Bonner, C.A., Brettin, T., Gottardo, R., Keyhani, N.O., and Jensen, R.A. (2003b). Lateral gene transfer and ancient paralogy of operons containing redundant copies of tryptophan-pathway genes in *Xylella* species and in heterocystous cyanobacteria. Genome Biol. *4*, R14.

Xu, F., Kong, D., He, X., Zhang, Z., Han, M., Xie, X., Wang, P., Cheng, H., Tao, M., Zhang, L., et al. (2013). Characterization of streptonigrin biosynthesis reveals a cryptic carboxyl methylation and an unusual oxidative cleavage of a N-C bond. J. Am. Chem. Soc. *135*, 1739–1748.

Yang, M.-L., Kuo, P.-C., Damu, A.G., Chang, R.-J., Chiou, W.-F., and Wu, T.-S. (2006). A versatile route to the synthesis of 1-substituted β-carbolines by a single step Pictet-Spengler cyclization. Tetrahedron *62*, 10900–10906.

Yeh, E., Garneau, S., and Walsh, C.T. (2005). Robust in vitro activity of RebF and RebH, a two-component reductase/halogenase, generating 7-chlorotryptophan during rebeccamycin biosynthesis. Proc. Natl. Acad. Sci. USA *102*, 3960–3965.

Yeh, E., Blasiak, L.C., Koglin, A., Drennan, C.L., and Walsh, C.T. (2007). Chlorination by a long-lived intermediate in the mechanism of flavin-dependent halogenases. Biochemistry *46*, 1284–1292.

Yu, X., Liu, Y., Xie, X., Zheng, X.-D., and Li, S.-M. (2012). Biochemical characterization of indole prenyltransferases: filling the last gap of prenylation positions by a 5-dimethylallyltryptophan synthase from *Aspergillus clavatus*. J. Biol. Chem. *287*, 1371–1380.

Yu, F., Li, M., Xu, C., Wang, Z., Zhou, H., Yang, M., Chen, Y., Tang, L., and He, J. (2013). Structural insights into the mechanism for recognizing substrate of the cytochrome P450 enzyme TxtE. PLoS One *8*, e81526.

Yuwen, L., Zhang, F.-L., Chen, Q.-H., Lin, S.-J., Zhao, Y.-L., and Li, Z.-Y. (2013). The role of aromatic L-amino acid decarboxylase in bacillamide C biosynthesis by *Bacillus atrophaeus* C89. Sci. Rep. *3*, 1753–1763.

Zehner, S., Kotzsch, A., Bister, B., Süssmuth, R.D., Méndez, C., Salas, J.A., and van Pée, K.-H. (2005). A regioselective tryptophan 5-halogenase is involved in pyrroindomycin biosynthesis in *Streptomyces rugosporus* LL-42D005. Chem. Biol. *12*, 445–452.

Zeng, J., and Zhan, J. (2011). Characterization of a tryptophan 6-halogenase from *Streptomyces toxytricini*. Biotechnol. Lett. *33*, 1607–1613.

Zhang, C., Albermann, C., Fu, X., Peters, N.R., Chisholm, J.D., Zhang, G., Gilbert, E.J., Wang, P.G., Van Vranken, D.L., and Thorson, J.S. (2006). RebG- and RebM-catalyzed indolocarbazole diversification. ChemBioChem *7*, 795–804.

Zhang, Q., Li, Y., Chen, D., Yu, Y., Duan, L., Shen, B., and Liu, W. (2011a). Radical-mediated enzymatic carbon chain fragmentation-recombination. Nat. Chem. Biol. *7*, 154–160.

Zhang, Q., Chen, D., Lin, J., Liao, R., Tong, W., Xu, Z., and Liu, W. (2011b). Characterization of NocL involved in thiopeptide nocathiacin I biosynthesis: a [4Fe-4S] cluster and the catalysis of a radical S-adenosylmethionine enzyme. J. Biol. Chem. *286*, 21287–21294.

Zhang, W., Liu, Z., Li, S., Yang, T., Zhang, Q., Ma, L., Tian, X., Zhang, H., Huang, C., Zhang, S., et al. (2012). Spiroindimicins A-D: new bisindole alkaloids from a deep-sea-derived actinomycete. Org. Lett. *14*, 3364–3367.

Zhang, C., Kong, L., Liu, Q., Lei, X., Zhu, T., Yin, J., Lin, B., Deng, Z., and You, D. (2013). In vitro characterization of echinomycin biosynthesis: formation and hydroxylation of L-tryptophanyl-S-enzyme and oxidation of (2S,3S) β-hydroxytryptophan. PLoS One *8*, e56772.

Zhang, W., Ma, L., Li, S., Liu, Z., Chen, Y., Zhang, H., Zhang, G., Zhang, Q., Tian, X., Yuan, C., et al. (2014). Indimicins A-E, bisindole alkaloids from the deep-sea-derived *Streptomyces* sp SCSIO 03032. J. Nat. Prod. *77*, 1887–1892.

Zhu, X., De Laurentis, W., Leang, K., Herrmann, J., Ihlefeld, K., van Pée, K.-H., and Naismith, J.H. (2009). Structural insights into regioselectivity in the enzymatic chlorination of tryptophan. J. Mol. Biol. *391*, 74–85.

Zhu, X., van Pee, K.-H., and Naismith, J.H. (2010). The ternary complex of PrnB (the second enzyme in the pyrrolnitrin biosynthesis pathway), tryptophan, and cyanide yields new mechanistic insights into the indolamine dioxygenase superfamily. J. Biol. Chem. *285*, 21126–21133.

Zou, Y., Fang, Q., Yin, H., Liang, Z., Kong, D., Bai, L., Deng, Z., and Lin, S. (2013). Stereospecific biosynthesis of β-methyltryptophan from L-tryptophan features a stereochemical switch. Angew. Chem. Int. Ed. Engl. *52*, 12951–12955.

 ©2015 Elsevier Ltd All rights reserved