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Rearrangements in the mechanisms of the indole alkaloid prenyltransferases*

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Abstract: The indole prenyltransferases are a family of metal-independent enzymes that catalyze the transfer of a prenyl group from dimethylallyl diphosphate (DMAPP) onto the indole ring of a tryptophan residue. These enzymes are remarkable in their ability to direct the prenyl group in either a "normal" or "reverse" fashion to positions with markedly different nucleophilicity. The enzyme 4-dimethylallyltryptophan synthase (4-DMATS) prenylates the non-nucleophilic C-4 position of the indole ring in free tryptophan. Evidence is presented in support of a mechanism that involves initial ion pair formation followed by a reverse prenylation at the nucleophilic C-3 position. A Cope rearrangement then generates the C-4 normal prenylated intermediate and deprotonation rearomatizes the indole ring. The enzyme tryprostatin B synthase (FtmPT1) catalyzes the normal C-2 prenylation of the indole ring in brevianamide F (*cyclo*-L-Trp-L-Pro). It shares high structural homology with 4-DMATS, and evidence is presented in favor of an initial C-3 prenylation (either normal or reverse) followed by carbocation rearrangements to give product. The concept of a common intermediate that partitions to different products via rearrangements can help to explain how these evolutionarily related enzymes can prenylate different positions on the indole ring.

Keywords: alkaloids; carbocation rearrangement; Cope rearrangement; enzymes; indole alkaloids; mechanism; prenyltransferase.

INTRODUCTION

The indole alkaloids are a diverse family of natural products that are derived from the amino acid tryptophan (Fig. 1). Many of these compounds possess potent biological activities and have long been used as human therapeutics, poisons, and psychedelic drugs. In many cases, the indole core has been prenylated to give the isoprenoid indole alkaloids [1–3]. Perhaps the best known of these compounds is ergotamine, which is produced from the ergot fungus, *Claviceps purpurea*. The ingestion of grain products that are contaminated with this fungus and contain ergot alkaloids leads to a gangrenous poisoning called ergotism, or Saint Anthony's fire. Taken in controlled doses, however, ergotamine acts as a vasoconstrictor and can be used in the treatment of migraine headaches. Finally, a semi-synthetic version of an ergot alkaloid, lysergic acid diethylamide or LSD, has been used as a recreational psychedelic drug.

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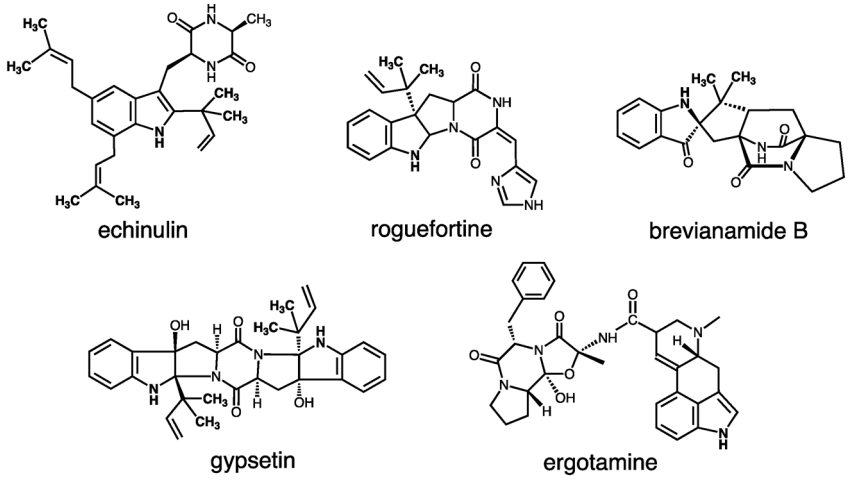

Fig. 1 Examples of prenylated indole alakloids.

While many of the advanced steps in the biosynthesis of the complex indole alkaloids are still poorly understood, great advances in outlining the early steps have recently been reported. This is clearly the case with the isoprenoid indole alkaloids where the Li group has recently identified a large family of fungal prenyltransferases responsible for the prenylation of the indole ring [2,4,5]. While all of these enzymes share sequence similarities and a common ancestry, they display a remarkable diversity in the regiochemistry of the alkylation reaction. The five-carbon isoprene unit may be attached via the primary carbon to give a "normal" prenylated product or via the tertiary carbon to give a "reverse" prenylated product. In addition, examples of enzymes that are able to catalyze prenylation (either normal, reverse, or both) at each of the seven potential positions on the indole ring have now been identified (Fig. 2) [6]. Many of these enzymes act on free tryptophan, while others act on cyclic dipeptides or benzodiazopinediones containing Trp residues.

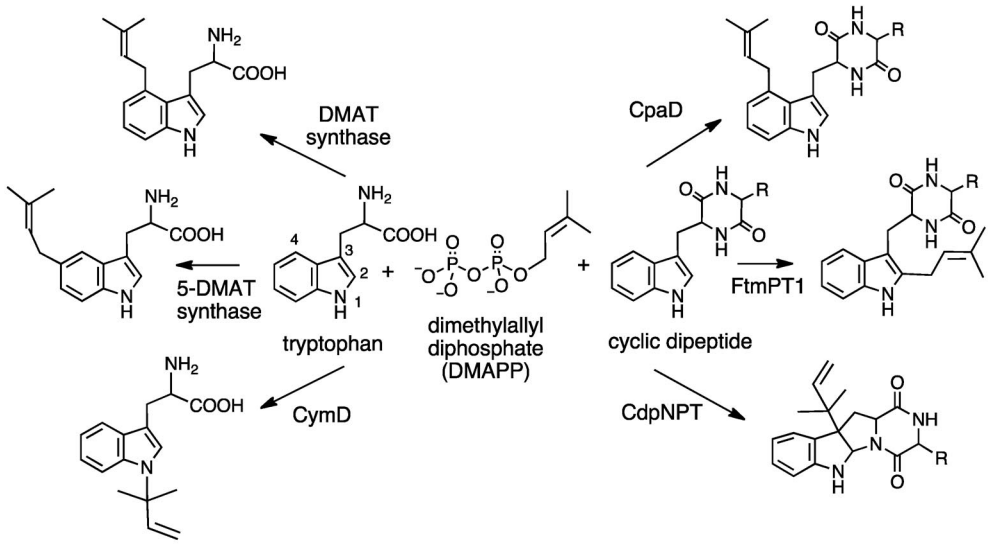

Fig. 2 Examples of indole prenyltransferases that act on either free L-tryptophan or on cyclic dipeptides.

An interesting aspect of these reactions is that the nucleophilicity of the various positions around the indole ring varies quite dramatically, yet Nature has discovered how to modify each of them selectively. The prenyl group donor is dimethylallyl diphosphate (DMAPP), which can ionize to give pyrophosphate and the dimethylallyl cation. This cation could participate in an electrophilic aromatic substitution reaction directly at the site of substitution. For a reverse prenylation, the aromatic ring would attack the tertiary carbon, and for a normal prenylation it would attack the primary carbon. The control over regioselectivity would be dictated by a careful positioning of the dimethylallyl cation within the active site. In order to promote prenylation at the least nucleophilic positions, the cation would have to be kept distant from the more nucleophilic positions (such as the indole C-3). While such a mechanism could be proposed for each of these enzymes, recent structural and mechanistic studies suggest that in some cases the carbocation adds to the nucleophilic C-3 position, and a subsequent rearrangement then occurs to migrate the prenyl group to an alternate position. Two such enzymes will be described in the following sections.

A POTENTIAL COPE REARRANGEMENT IN THE REACTION CATALYZED BY DIMETHYLALLYLTRYPTOPHAN SYNTHASE

The first of the indole prenyltransferases to be identified, and the most extensively studied from a mechanistic point of view, is 4-dimethylallyltryptophan synthase (4-DMATS) [7–9]. This enzyme converts DMAPP and tryptophan into 4-dimethylallyltryptophan during the first step in the biosynthesis of the ergot alkaloids (Fig. 3) [10,11]. The enzyme is of interest from a mechanistic point of view since the C-4 position is one of the most weakly nucleophilic positions on the indole ring. Unlike most prenytransferases, this enzyme was reported to be active in metal-free buffers. Early mechanistic studies by Floss and co-workers demonstrated that the reaction proceeded with an inversion of stereochemistry at the methylene carbon of DMAPP [12]. In addition, the Poulter group showed that fluorination of either the indole ring, or the methyl groups of DMAPP, slowed the reaction, consistent with an electrophilic addition mechanism [13]. Thus, it was proposed that the DMAPP first ionizes to give the dimethylallyl

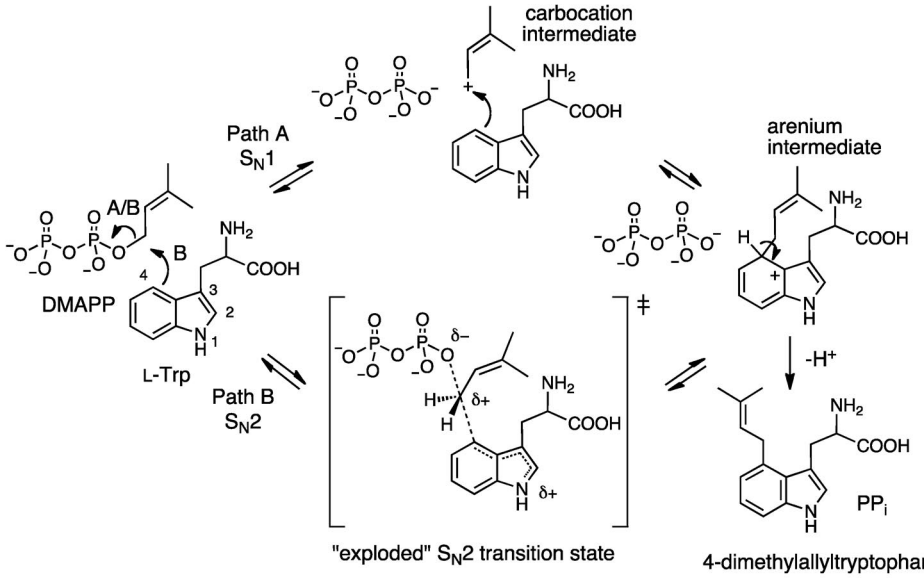

Fig. 3 Proposed mechanisms for the 4-DMATS reaction involving a direct prenylation at C-4. Path A is an S_N1-like mechanism involving a discrete carbocation intermediate. Path B is an S_N2-like mechanism involving an "exploded" transition state and no distinct carbocation intermediate.

carbocation and a subsequent electrophilic addition to the C-4 position of the indole ring gives an arenium ion intermediate (Fig. 3, Path A). Deprotonation of the arenium ion causes rearomatization and product formation.

Further studies on this enzyme were hampered by difficulties in producing recombinant protein on a large scale. A breakthrough came in the mid-2000s when the Li group demonstrated that 4-DMATS from the fungus *Aspergillus fumigatus* (FgaPT2) could be overproduced in *Escherichia coli* [7,14]. By this point, it was clear that this enzyme was a member of a larger family of indole prenyltransferases that operated in a metal-independent fashion and that lacked the characteristic (N/D)DXXD diphosphate binding site of other prenyltransferases [2,4,5]. The ability to obtain these recombinant enzymes, many for the first time, has rejuvenated interest in mechanistic studies on the indole prenyltransferases and in their potential use in the synthesis of unusual alkaloids.

The S_N1-like mechanism shown in Fig. 3, Path A predicts that the 4-DMATS reaction is initiated by the ionization of DMAPP to form a discrete dimethylallyl carbocation intermediate. This is similar to the accepted mechanism for farnesyl pyrophosphate synthase where ionization precedes attack by a poorly nucleophilic alkene [15,16]. An alternative possibility is that the indole nucleophile directly attacks DMAPP in an S_N2 fashion and displaces pyrophosphate without carbocation formation (Fig. 3, Path B). This is similar to the mechanism proposed for protein farnesyltransferase where an excellent nucleophile (a cysteine thiolate) is prenylated by farnesyl diphosphate [17,18]. In order to provide evidence for the existence of a dimethylallyl carbocation intermediate in this reaction a positional isotope exchange (PIX) reaction was performed (Fig. 4) [19]. In this experiment a sample of isotopically labeled DMAPP is synthesized in which an ^{18}O isotope is positioned in a "bridging" position between the prenyl group and the phosphorus atom (see darkened atoms in Fig. 4). This material is treated with 4-DMATS and L-Trp, and the reaction is allowed to proceed until approximately 70 % of the labeled DMAPP has been consumed. Analysis of the recovered DMAPP is performed in order to determine if any of the ^{18}O label has scrambled from a bridging position into a nonbridging position. This is readily done using ^{31}P NMR spectroscopy as an ^{18}O isotopic substitution causes a chemical shift in the phosphorus signal and the magnitude of that shift is dependent on the magnitude of the P–O bond order (greater in the nonbridging material) [20]. In the case of 4-DMATS, this analysis indicated that approximately 15 % of the recovered starting material contained isotopic label that had scrambled into a nonbridging position, indicating that a reversible P–O bond cleavage event was occurring. While the

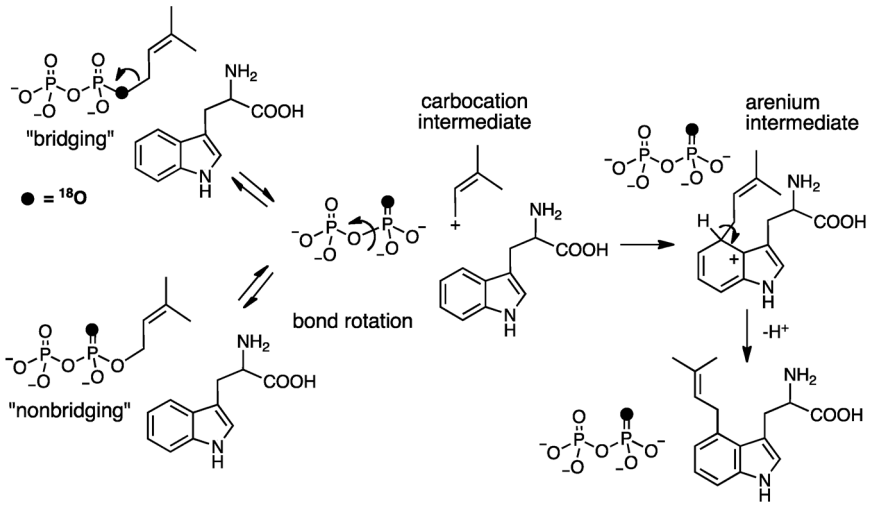

Fig. 4 The PIX experiment shown with the S_N1-like mechanism involving a discrete carbocation intermediate. Darkened atoms represent ^{18}O isotopic labels.

reversible nature of this step is readily explained by the formation of a carbocation intermediate in an S_N1 process, it is conceivable that a reversible S_N2 mechanism could also lead to isotopic scrambling. For this reason, kinetic isotope effects were used to demonstrate that C–O bond cleavage and C–C bond formation were both partially rate-determining steps of catalysis [19]. This argues strongly against a reversible S_N2 mechanism and leads one to conclude that the observation of PIX is best explained by formation of a dimethylallyl carbocation intermediate. This represents the first time that PIX has been observed with a prenyltransferase, likely because the vast majority of prenyltransferases utilize divalent metal ions during catalysis and coordination of pyrophosphate to the metal prevents bond rotation during the lifetime of any carbocation intermediate [21,22].

In order to provide further evidence for carbocation formation, the substrate analog 6-fluorotryptophan was used in the PIX experiments with isotopically labeled DMAPP [19]. It was reasoned that the electron-withdrawing fluorine substituent would decrease the nucleophilicity of the indole ring and that the initial ionization would be more readily reversible, causing an increase in PIX. When this compound was tested as a substrate, no detectable product was observed under normal reaction conditions. However, when the PIX experiment was performed with this compound, a statistical 2:1 mixture of nonbridging to bridging DMAPP was obtained. The observation of 100 % PIX without product formation indicates that ionization to form the dimethylallyl cation/pyrophosphate ion pair was still occurring, but that collapse back to starting material was greatly favored over attack by the poorly nucleophilic fluorinated indole ring.

A significant advancement in the understanding of the indole prenyltransferase family came when the first structure of 4-DMATS was reported [23]. The enzyme was found to possess a rare β/α barrel fold (or PT fold) that had previously been observed in the distantly related aromatic prenyltransferases (ABBA PTs) [24]. The authors were able to crystallize the enzyme in a complex with L-Trp and the unreactive DMAPP analog, dimethylallyl *S*-thiolodiphosphate (DMSPP, in which a sulfur replaces the oxygen bridging the dimethylallyl and pyrophosphate groups). This gives a beautiful snapshot of the Michaelis complex prior to reaction (Fig. 5). The structure reveals that the dimethylallyl and indole moieties are oriented in a coplanar fashion. Carbocation formation is likely facilitated via π-cation interactions with the indole ring and a neighboring Tyr residue. Two residues, Glu 89 and Lys 174, were implicated as playing roles as acid/base catalysts. Glu 89 forms a hydrogen bond to the indole NH and

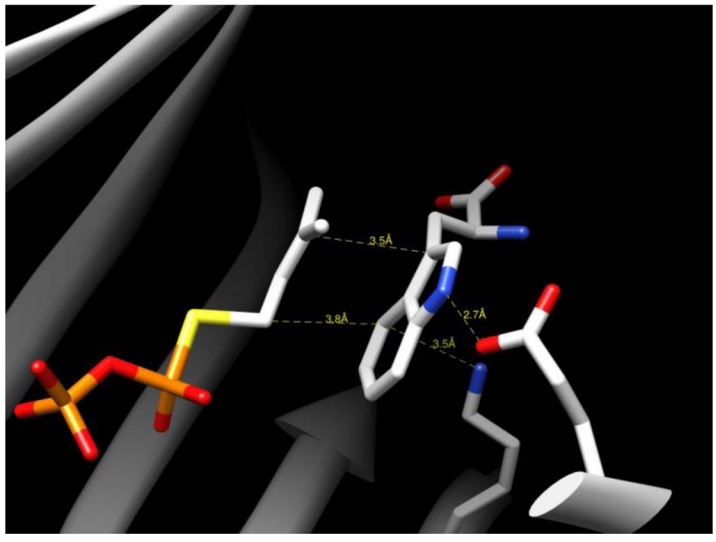

Fig. 5 The orientation of L-Trp, DMSPP, and key active site residues in the 4-DMATS active site. The side chains of Glu 89 and Lys 174 are shown. Data was taken from PDB 314X.

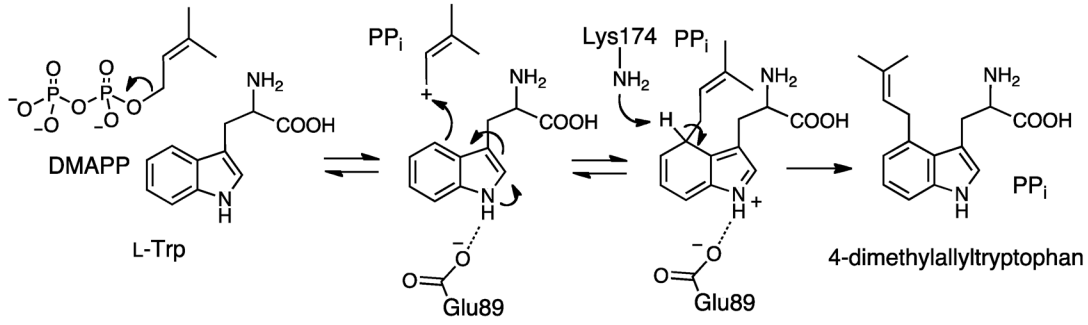

Fig. 6 The proposed roles of Glu 89 and Lys 174 in the S_N1-like 4-DMATS mechanism.

most likely serves to increase the nucleophilicity of the indole either by deprotonation or via formation of a charged hydrogen bond (Fig. 6). Lys 174 is situated in an appropriate position to serve as the base that deprotonates the arenium ion in the final rearomatization step of the pathway (Fig. 6).

In order to probe the role of these residues during catalysis, site-directed mutagenesis was employed [23,25]. Mutation of Glu 89 to either glutamine or alanine resulted in a dramatic drop in the value of k_{cat}, consistent with its proposed role in catalysis. Surprisingly, mutation of Lys 174 to glutamine only resulted in a modest 17 % depression in the value of k_{cat}. This may be understood when one considers that deprotonation of the arenium ion is accompanied by rearomatization and therefore the proton has a very low pK_a. Even a poor base such a glutamine side chain may facilitate this process. When Lys 174 was mutated to alanine a more noticeable 20-fold drop in the value of k_{cat} was observed. In each case the value of %PIX/%rxn was increased when compared to the wild-type (WT) enzyme. The increased amount of isotopic scrambling would be expected for mutations that affect steps occurring subsequent to ion pair formation. In order to probe whether the mutations resulted in loss of control of the carbocation intermediate and quenching by water to give allylic alcohols, the products of the reaction were analyzed by ^{1}H NMR spectroscopy. The Glu89Gln, Glu89Ala, and Lys174Gln mutants all gave dimethylallyltryptophan as the sole product; however, the Lys174Ala produced an unusual product, compound **1** (Fig. 7). It was immediately apparent that this new product was reverse prenylated

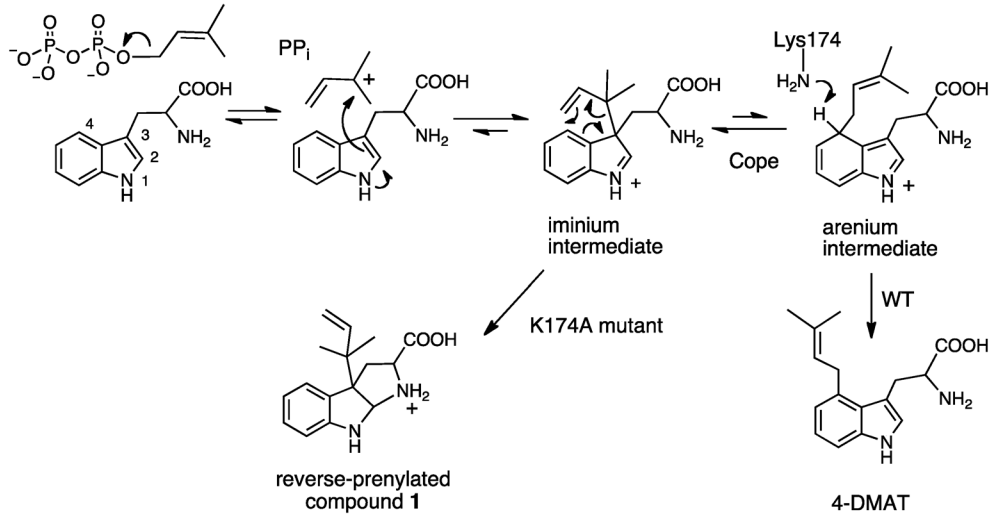

Fig. 7 A proposed Cope rearrangement mechanism for 4-DMATS and the structure of compound **1**.

from the pattern of alkene proton signals in the spectrum. Furthermore, the signal for the indole C-2 proton was shifted upfield to 5.4 ppm, indicating that a cyclization had occurred to generate a hexahydropyrroloindole core. Ultimately, it was possible to prove the identity and stereochemistry of compound **1** via synthesis [25,26].

The formation of compound **1** by the Lys174Ala mutant can be interpreted as evidence that the 4-DMATS mechanism involves an initial reverse prenylation at C-3 [25]. Such a mechanism is shown in Fig. 7, where the reverse C-3 prenylated intermediate undergoes a reversible Cope rearrangement and equilibrates with the normal C-4 prenylated arenium ion. Deprotonation of the arenium ion at C-4 then drives the equilibrium towards the product dimethylallyltryptophan. In the case of the Lys174Ala mutant, there is no proton acceptor and the equilibrium would be expected to lie towards the reverse C-3 prenylated intermediate. Ultimately a ring closure involving the α-amine would occur to give compound **1**. An examination of the 4-DMATS structure in complex with L-Trp and DMSPP supports this mechanism (Fig. 5) [23]. The coplanar arrangement of the dimethylallyl and prenyl moieties is such that if DMAPP ionization occurred without significant reorientation, both modes of attack (reverse C-3 prenylation or normal C-4 prenylation) are equally feasible from a geometric prospective. Given the much greater nucleophilicity of the indole C-3 position [27–30], it is reasonable to expect that this attack would predominate. Such a Cope rearrangement mechanism for 4-DMATS was first proposed by Arigoni and Seiler but was not published in the primary literature [11,31]. It was invoked as a way of explaining how the enzyme can alkylate the more non-nucleophilic position of the indole ring in preference to the C-3 position. The formation of compound **1**, as well as the structural analysis of the Michaelis complex, provides the first experimental evidence in support of such a mechanism.

The suggestion that the 4-DMATS mechanism involves a Cope rearrangement step is of interest as few enzymes utilize pericyclic rearrangements during catalysis. This is likely because it is difficult for an enzyme active site to stabilize a concerted transition state with little charge build-up. The prototypical example of this is found in chorismate mutase that catalyzes a Claisen rearrangement (or oxy-Cope) [32–35]. Other examples include isochorismate pyruvate lyase [36,37], and the Diels–Alderases [38–40].

Questions that arise when invoking a pericyclic rearrangement in an enzymatic mechanism are the extent to which the enzyme must accelerate this reaction and whether the non-enzymatic rearrangement occurs at any measurable rate. An inability to observe the migration of an allyl group from C-3 to C-4 in model compounds led Arigoni's Cope mechanism for 4-DMATS to fall into disfavor [11,31,41]. More recently however, non-enzymatic studies have provided evidence that such a rearrangement may be kinetically feasible (Fig. 8). Studies by Voûte et al. show that refluxing compound **2** in toluene first

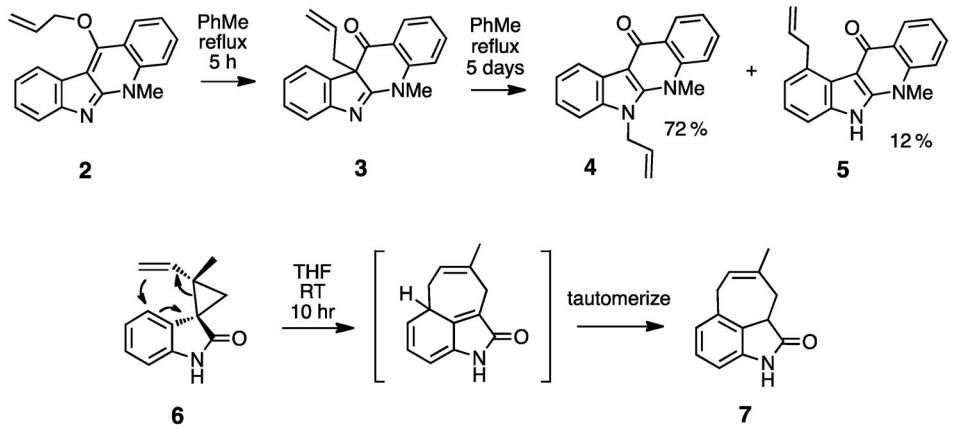

Fig. 8 Non-enzymatic reactions involving a Cope rearrangement onto the C-4 position of an indole.

leads to a Claisen rearrangement to give compound **3** and then over the course of five days gives a mixture of compounds **4** (72 %) and **5** (12 %) [42]. Compounds **4** and **5** are presumably generated by Cope rearrangements onto either the indole N-1 or C-4 positions, respectively. Quite recently, a study by Schwartzer et al. showed that compound **6** will spontaneously rearrange at room temperature to give compound **7** [43]. This presumably occurs via a Cope rearrangement onto the indole C-4 position followed by tautomerization, and was forwarded as evidence in support of the Arigoni mechanism for 4-DMATS. In a related sense, *O*-allyl phenols and *N*-allyl anilines will undergo oxy- or aza-Cope rearrangements to give ortho-allyl phenols and anilines, respectively [44–46]. Such a rearrangement is operative in prenyltransferases such as LynF that prenylate tyrosine residues within peptides at the carbon ortho to the hydroxyl group (Fig. 9) [47]. It has recently been shown that this enzyme actually catalyzes a reverse prenylation on the phenolic hydroxyl group and that a non-enzymatic Claisen rearrangement generates the "normal" prenylated product.

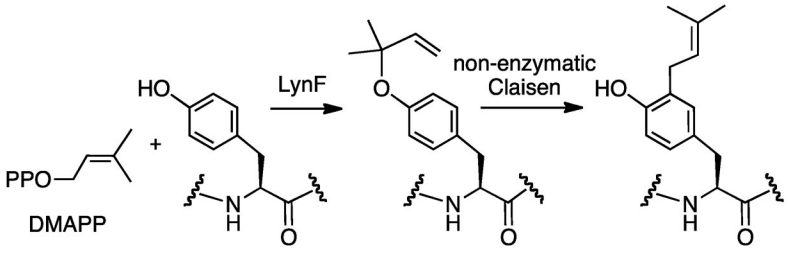

Fig. 9 The reaction catalyzed by LynF and the subsequent non-enzymatic Claisen rearrangement.

While further studies are required to fully elucidate the 4-DMATS mechanism, the observed formation of compound **1** and the analysis of the structural data combined with an understanding of the intrinsic reactivity of indoles suggests that the Cope rearrangement mechanism may likely be at play with this enzyme. At the time of submission of this manuscript a report by Rudolf et al. appeared online that probed the 4-DMATS reaction with tryptophan analogs containing substituents at the 4-position of the indole ring [48]. They found that when 4-methyl-L-tryptophan was employed as a substrate there was a low level of catalytic activity (0.3 %). The major product was reverse C-3 prenylated, which is consistent with release of the proposed intermediate formed in the Cope mechanism (Fig. 10). A minor product was normal N-1 prenylated, which could be formed from an aza-Cope reaction of the reverse C-3 prenylated compound as was the case in compound **3** converting to compound **4** (Fig. 8). A second minor product was normal C-3 prenylated, indicating that some motion of the dimethylallyl cation was

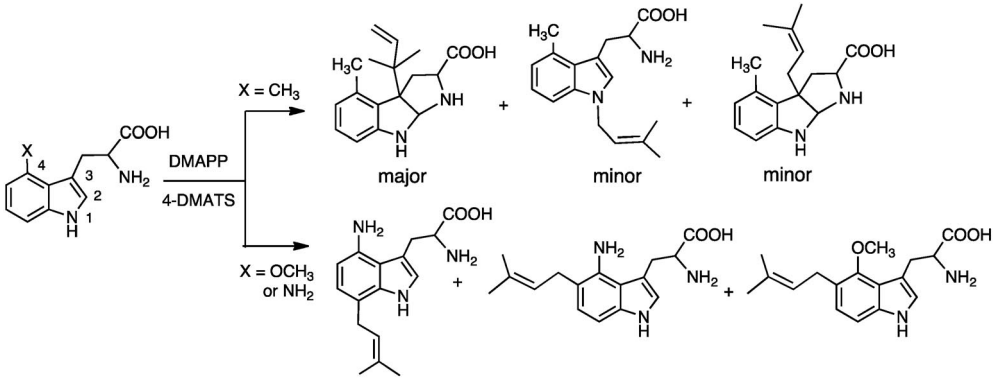

Fig. 10 Products obtained from the reaction of 4-substituted tryptophan analogs with 4-DMATS.

possible when a C-4 substituent is introduced. Perhaps more surprisingly, when 4-methoxy- or 4-amino-L-tryptophan were tested, products were isolated that were normal prenylated at the C-5 and C-7 positions. This was presented as evidence against the Cope mechanism since these products were prenylated on the phenyl ring, yet they could not have arrived from a C-3 reverse prenylated intermediate. While this study clearly shows that substitutions on the indole ring can cause motion in the active site and prenylation at alternate positions, it should be noted that the 4-methoxy and 4-amino substrates are dramatically activated towards electrophilic aromatic substitution at the C-5 and C-7 sites. Since L-Trp is not activated in such a fashion, the Cope mechanism remains an attractive possibility for the normal 4-DMATS mechanism.

CARBOCATION REARRANGEMENTS IN THE REACTION CATALYZED BY TRYPROSTATIN B SYNTHASE (FTMPT1)

Another recently studied indole prenyltransferase that may utilize rearrangements during catalysis is tryprostatin B synthase or FtmPT1 [49]. This enzyme catalyzes a normal C-2 prenylation of the indole ring of brevianamide F (*cyclo*-L-Trp-L-Pro) to give tryprostatin B (Fig. 11). It is involved in the biosynthesis of the fumitremorgin B which is a tremorgenic mycotoxin.

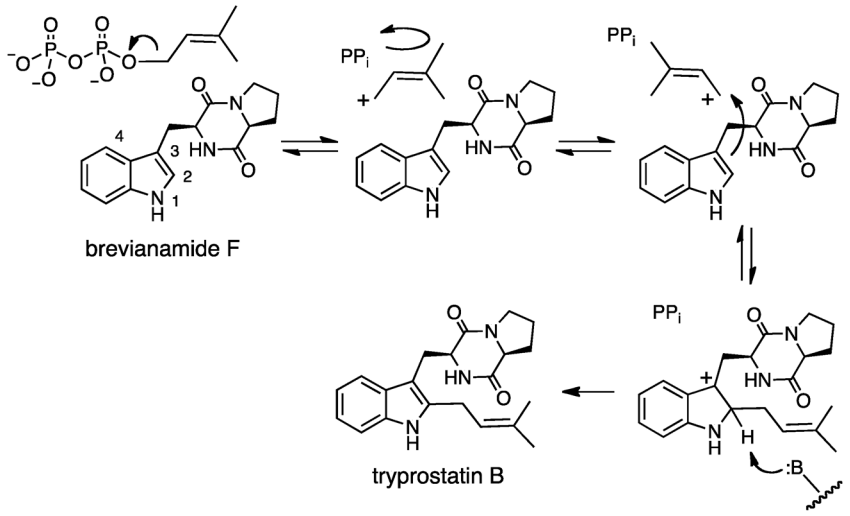

Fig. 11 A direct C-2 prenylation mechanism for the reaction catalyzed by tryprostatin B synthase (FtmPT1).

While the C-2 position of C-3 substituted indoles is reasonably nucleophilic, the recently published structure of FtmPT1 in complex with brevianamide F and DMSPP raises questions as to the mechanism that is employed by this enzyme [50]. The overall fold and active site architecture is remarkably similar to that of 4-DMATS. Most importantly, the relative orientation of the prenyl and indole moieties is also very similar with the exception that the indole ring is tilted slightly in FtmPt1 (Fig. 12). This is somewhat unexpected as the C-1 position of the prenyl group is more than 5 Å away from the C-2 position of the indole ring. In order to explain how this bond is formed, the authors invoke a mechanism involving a rotation of the dimethylallyl carbocation so that the primary and tertiary carbons exchange positions (Fig. 11). Given the high reactivity of the dimethylallyl cation and its proximity to the nucleophilic C-3 position of the indole ring, such a rotation seems unlikely. Instead, it is possible that an initial alkylation occurs at C-3 and a subsequent carbocation rearrangement leads to the C-2 normal prenylated product.

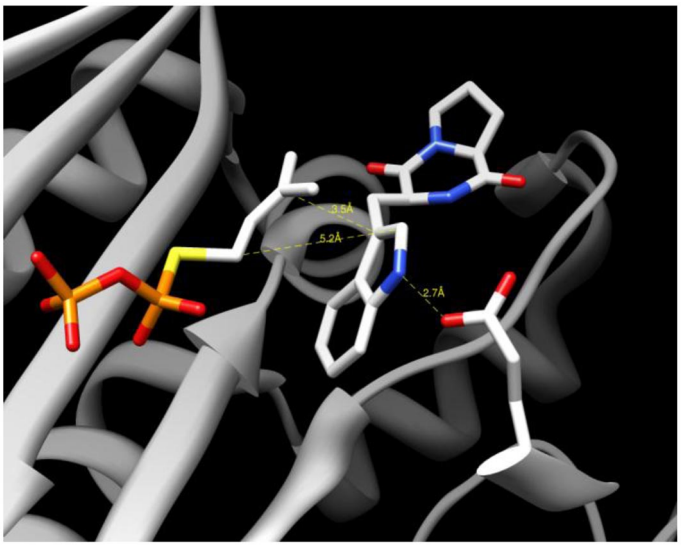

Fig. 12 The orientation of brevianamide F, DMSPP, and a key active site residue in the FtmPT1 active site. The side chain of Glu102 is shown. Data was taken from PDB 302K.

Several lines of evidence point towards either an initial C-3 *reverse* prenylation or an initial C-3 *normal* prenylation as a first step in catalysis. The former scenario is supported by the structural analysis in which the distance between the C-3 position of the indole and the C-3 (tertiary) position of the prenyl group is only 3.5 Å (Fig. 12) [50]. Furthermore, it was reported that the Gly115Thr mutant of FtmPT1 generates compound **8** as the major reaction product (Fig. 13a) [50]. This is a similar observation to that reported for the formation of compound **1** by the Lys174Ala mutant of 4-DMATS [25]. The Gly 115 residue is located proximal to the indole C-7 position, and it was mutated in an attempt to convert FtmPT1 into a 4-DMATS as this was one of the few differences between the two active sites. The increased steric bulk from this mutation could have mis-positioned the indole and allowed an unnatural C-3 reverse prenylation to occur. Alternatively, it is possible that C-3 reverse prenylation is normally the first step in catalysis and that the mutation interfered with a subsequent rearrangement so that compound **8** represents a ring-closed form of a true intermediate that was released into solution. A second piece of evidence supporting a reverse C-3 prenylation was recently reported by Chen et al. [51]. The treatment of indolylbutenone with DMAPP and FtmPT1 gave a compound that was prenylated on a non-aromatic carbon (Fig. 13b). The can be explained by an initial C-3 reverse prenylation followed by a Cope rearrangement that migrates the prenyl group off of the indole core. Finally, a third observation involves the reaction of unmodified L-Trp with DMAPP and FtmPT1. This was originally reported to produce a reverse *N*-prenylated product [52], however, we have recently shown that the true product of this reaction is compound **1** (the same species produced by the 4-DMATS Lys174Ala mutant) (Fig. 13c) [25, 53]. This demonstrates that it is possible to generate a reverse C-3 prenylated product with the WT enzyme, and further strengthens the notion that such an attack could be involved in catalysis with brevianamide F.

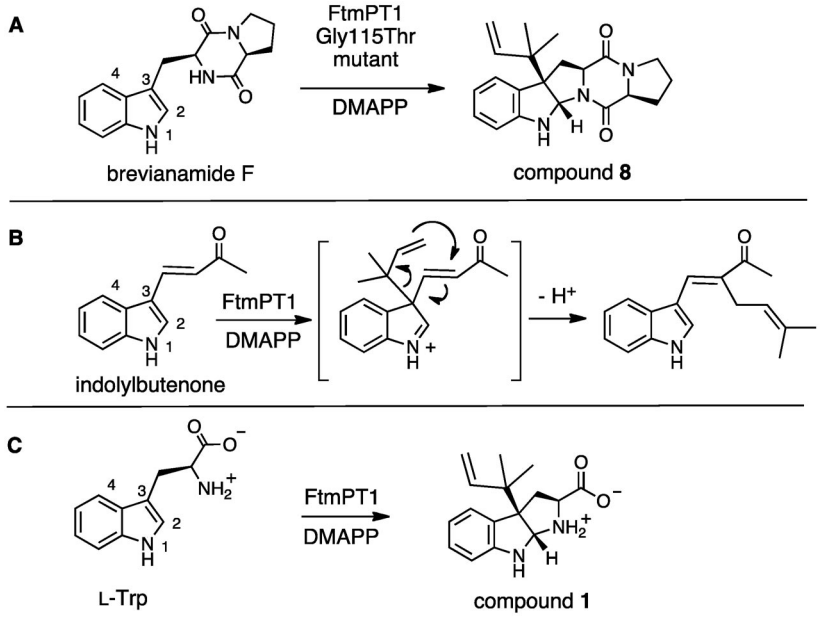

Fig. 13 Evidence in support of reverse C-3 prenylation with FtmPT1. (A) The reaction of brevianamide F catalyzed by the Gly115Thr mutant of FtmPT1. (B) The reaction of indolylbutenone catalyzed by FtmPT1. (C) The reaction of L-Trp catalyzed by FtmPT1.

A recent report from the Li group provides evidence in support of the latter scenario in which a mechanism involving an initial C-3 normal prenylation is at play [54]. They have shown that treatment of FtmPT1 with DMAPP and a series of cyclic dipeptides give mixtures of the expected C-2 normally prenylated products and the unexpected C-3 normally prenylated products (Fig. 14). These included *cyclo*-L-Trp-L-Tyr, *cyclo*-L-Trp-L-Ala, *cyclo*-L-Trp-L-His, as well as some examples containing D-amino acids. The formation of the C-3 normal prenylated products could arise from the premature release of the corresponding intermediates in a mechanism involving C-3 normal prenylation as the first step of catalysis.

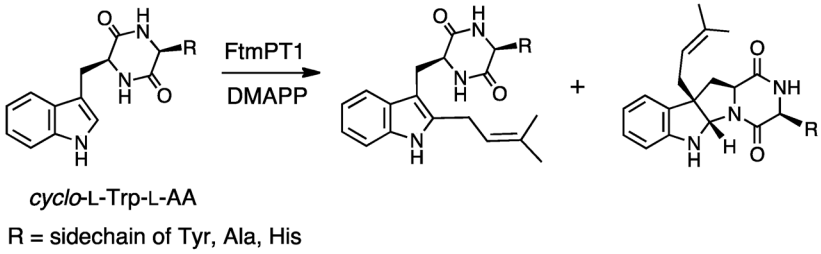

Fig. 14 Evidence in support of normal C-3 prenylation with FtmPT1. AA = amino acid.

From the studies above it is quite clear that modifications to the substrate or active site can result in either normal or reverse prenylation at C-3. This could simply be due to loss of control of the reactive dimethylallyl cation so that aberrant alkylations can occur. Alternatively, the C-3 prenylated products may be true intermediates in the normal reaction pathway that are released before subsequent rearrangements can occur. A potential mechanism involving an initial C-3 reverse prenylation is shown

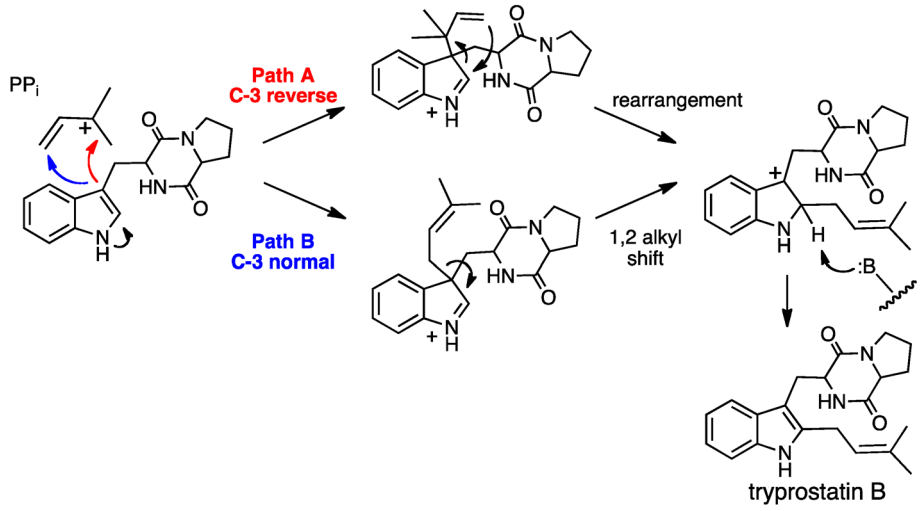

Fig. 15 Potential mechanisms for the FtmPT1 reaction involving initial C-3 prenylation. Path A involves an initial reverse C-3 prenylation. Path B involves an initial normal C-3 prenylation.

in Fig. 15, Path A. The first-formed iminium ion must rearrange to give the C-2 normal prenylated benzylic cation. This is unlikely to be a concerted process as it would constitute a [3s,5s] sigmatropic shift which is forbidden by selection rules [55,56]. Instead, it could occur in a stepwise process via a secondary cyclopentyl cation (not shown). Deprotonation of the benzylic cation gives tryprostatin B. This mechanism is strongly supported by the crystallographic data that shows the proximity of the indole C-3 to the tertiary position of the incipient cation. It is also supported by the formation of C-3 reverse prenylated products either with brevianamide F and the Gly115Thr mutant, or with L-Trp and WT enzyme. A potential mechanism involving an initial C-3 normal prenylation is shown in Fig. 15, Path B. In this case, one would have to invoke a translational motion of the prenyl cation upon ionization in order to position it properly for normal prenylation at C-3. The subsequent rearrangement is a simple 1,2 alkyl shift moving the prenyl group from C-3 to C-2. These alkyl shifts are well known to occur in indole chemistry and have been termed "Plancher rearrangements" [57–59]. In fact, such a rearrangement has been employed in the synthesis of tryprostatin B [60]. This mechanism is supported by the recent observation of C-3 normal prenylated side-products with a variety of unnatural cyclic dipeptide substrates. While further work is required to establish the mechanism of FtmPT1, the structural and product studies indicate that an initial C-3 prenylation followed by a rearrangement cannot be ruled out as a possibility.

CONCLUSIONS

The indole prenyltransferases are a large family of enzymes that have evolved to prenylate all seven positions of the indole ring in either a reverse or normal fashion [6]. In most cases they do so with high regioselectivity when acting on their preferred substrates. The ability to control the reactivity of the dimethylallyl cation and direct it to positions on the indole ring that have markedly different nucleophilicity represents a remarkable achievement of catalysis. Three of these enzymes that catalyze prenylation at the C-4, C-3, and C-2 positions of the indole ring have now been structurally characterized and all show a notably similar orientation of the bound substrates [23,50,61]. This suggests that certain family members may catalyze a common initial C-3 prenylation, and that subsequent rearrangements could lead to the final products. This is consistent with the observation that the use of unnatural substrates and/or mutant enzymes leads to the formation of C-3 prenylated products.

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