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Extension of the terpene chemical space: the very first biosynthetic steps

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Abstract: The structural diversity of terpenes is particularly notable and many studies are carried out to increase it further. In the terpene biosynthetic pathway this diversity is accessible from only two common precursors, *i.e.* isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Methods recently developed (*e.g.* the Terpene Mini Path) allow to obtain DMAPP and IPP from a two-steps enzymatic conversion of industrially available isopentenol (IOH) and dimethylallyl alcohol (DMAOH) into their corresponding diphosphates. Easily available IOH and DMAOH analogs then offer a quick access to modified terpenoids avoiding thus the tedious chemical synthesis of unnatural diphosphates. The aim of this mini-review is to cover the literature devoted to the use of these analogs for widening the accessible terpene chemical space.

1. Introduction

Terpenes constitute the largest class of natural compounds with more than 80000 different structures described [1]. Their physical and chemical properties as well as their biological activities have not escape humanity attention. Their use as perfumes, dyes, material, anticancer, antimalarial or antimicrobial agents, anti-oxidants, etc. is well known. The structure of their biosynthetic pathway is unique among natural products. Indeed, all the terpenes biosynthesized on earth are produced from only two precursors, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), themselves derived from glucose via one of the two pathways known as the mevalonate pathway (MEV) and the methyl erythritol pathway (MEP), depending on the producing organism or organelle. These two universal precursors are combined by aliphatic prenyl transferases into homologous diphosphates such as geranyl diphosphate (C₁₀, GPP), farnesyl diphosphate (C₁₅, FPP), geranylgeranyl diphosphate (C₂₀, GGPP) and geranylgeranyl diphosphate (C₂₅, GFPP). These acyclic diphosphates are the respective precursors of monoterpenes, sesquiterpenes and triterpenes, diterpenes and tetraterpenes and sesterterpenes. The extraordinary structural diversity of terpenes appears in the following stages of their biosynthesis. First, these acyclic diphosphates are transformed into hydrocarbons, alcohols or ethers by a myriad of terpene synthases/cyclases. A second diversity layer is then added when each of the initially obtained structures is modified by decorative enzymes such as Cyt-P450, transferases, oxidoreductases, etc. The ability of some prenyl transferases to catalyze the prenylation of natural non-terpene

compounds using DMAPP, GPP, FPP and GGPP as prenylating agents, provides another layer of diversity by enabling the generation of natural compounds of mixed biosynthetic origin. Despite the tremendous structural diversity of natural terpenes, many studies have been carried out to further expand their diversity in order to find molecules with new properties. The aim of this mini-review is to explore what has been done in previous years to diversify the structure of the terpene precursors IPP and DMAPP, either chemically or enzymatically, and how the terpene landscape has thus been enlarged. This mini-review complements two recently published reviews describing the substrate promiscuity of terpene synthases/cyclases [2,3] that readers are encouraged to consult for a general overview of the enzymatic generation of unnatural terpenes. It also highlights the potential of the recently developed Terpene Mini-Path (TMP) to easily access non-canonical terpenes enzymatically instead of chemically, either *in vivo* or *in vitro* (see also [3] in this context). Here we will first focus on the substrate promiscuity of enzymes involved in the synthesis or modification of C₅ diphosphate precursors, then on the substrate promiscuity of prenyl transferases (aromatic or aliphatic) and finally on the biosynthesis and chemo-enzymatic synthesis of terpenes using non-canonical prenylated diphosphates.

Gilles Iacazio is Professor of Chemistry and Biochemistry at Aix-Marseille University (France). His research focuses on the use of enzymes, either *in vitro* or *in vivo*, to access chemicals. He recently develops the Terpene Mini-Path (TMP), a very simplified and artificial enzymatic cascade to generate the universal terpene precursors isopentenyl diphosphate and dimethylallyl diphosphate. His interest is now on the use and applications of the TMP to produce natural and non-natural terpenes and terpenoids.

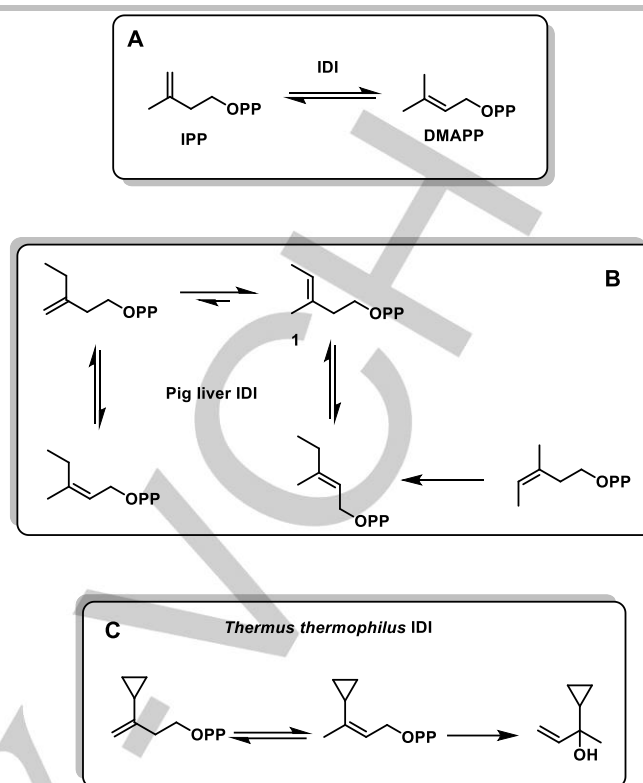


MINIREVIEW

Katia Duquesne is associate Professor of Molecular Biology and Biochemistry at Aix-Marseille University (France). She's particularly interested in tuning ratio of enzymes of biosynthetic cascades, playing with plasmids and genetics tools. She was part of the very exciting TMP project. Now her interests are directed towards using the optimized TMP for *in vivo* applications, either to access large amounts of chemical of interest, or to generate new innovative molecules.



Julie Couillaud graduated from Aix-Marseille University (France) as a MSc in chemistry with a specialization in chemistry applied to biological systems. During her studies she first worked as a research student in physical chemistry for the characterization of an unusual iron-sulfur protein from giant viruses. Passionate about the valorization of the use of biological systems such as enzymes for the production of natural compounds, she joined the Biosciences team at the Institute of Molecular Sciences of Marseille in 2017 as a PhD student. Her work focused on the *in vitro* development of the TMP and its optimized 2.0 version for the production of various terpenoids through an original enzymatic cascade.



Scheme 1. A Reaction catalyzed by IDI. B Reaction catalyzed by pig liver IDI with C₆ IPP homologs (adapted from [9,10]). C Reaction catalyzed by *Thermus thermophilus* IDI with a cyclopropyl IPP homolog (adapted from [11,12]).

2. Enzymes involved in the synthesis or modification of C₅-diphosphates and precursors

2.1. Isopentenyl diphosphate isomerase (IDI)

IDIs play a key role in terpene biosynthesis by balancing the ratio between IPP and DMAPP, the substrates of numerous prenyl transferases and the precursors of all terpenes [4,5,6]. There exist two different types of IDIs catalyzing the reversible isomerization of IPP into DMAPP (Scheme 1A). Type I IDIs are zinc metalloproteins belonging to the NUDIX superfamily and are found in all types of organisms while type II IDIs are flavoenzymes (FMN) belonging to the TIM barrel protein family and are restricted to Archaea and Bacteria [4,5,6]. Both types need a divalent cation (Mg²⁺) for catalysis and are considered to first protonate their substrates, thus favoring the isomerization of the double bond, followed by de-protonation through an antarafacial 1,3 allylic rearrangement for type I [7] and a suprafacial 1,3-proton addition/elimination for type II [8]. The reaction transforms IPP into DMAPP a highly electrophilic molecule which is the basic building block of the biosynthesis of aliphatic prenyl diphosphate such as GPP, FPP, GGPP and higher homologs. Interestingly, some studies have been performed on both types of IDI with artificial substrates. Using type I IDI from pig liver, Koyama *et al.* studied 5 homologous artificial C₆ substrates [9,10] and showed that from any of these substrates, (*E*)-3-methyl-3-pentenyl-diphosphate (1) was the major end product of the reaction (Scheme 1B).

Observations gained from the studies of pig liver IDI were of interest in relation to the biosynthesis of natural terpene compounds such as faranalin and juvenile insect hormones (*vide infra*). Indeed, these compounds carry additional methyl groups on their carbon backbone, potentially deriving from C₆ IPP and DMAPP homologs.

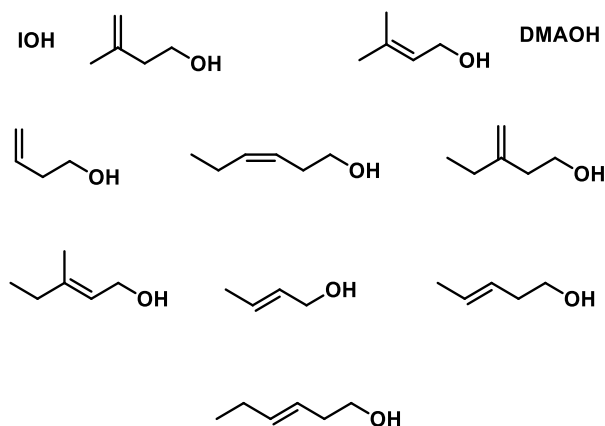
During studies carried out to determine the mechanism of action of the *Thermus thermophilus* IDI-II, Poulter's group synthesized cyclopropyl and epoxy analogues of IPP [11,12]. While the latter has been shown to be an irreversible time-dependent inhibitor of the enzyme, the cyclopropyl analogue of IPP is first reversibly isomerized to the cyclopropyl equivalent of DMAPP, which then spontaneously hydrolyzes to a tertiary vinyl alcohol (Scheme 1C).

2.2. Prenol kinases

In 2019 four different groups reported independently the development of a simplified biochemical access to terpenoids, either *in vitro* or *in vivo*, starting from isopentenol (IOH) and dimethylallyl alcohol (DMAOH) and using a two enzymes cascade to access the universal precursors IPP and DMAPP. In all cases the second phosphorylation involved an isopentenyl phosphate kinase (IPK) from an archaeon (*vide infra*) and the first phosphorylation was either realized by a choline kinase from *Saccharomyces cerevisiae* [13], an acid phosphatase (AP) from *Shigella flexneri* [14], an AP mutant (V78L) from *Salmonella enterica* or an AP from *Xanthomonas translucens* [15] or the hydroxyethylthiazole kinase from *Escherichia coli* [16]. This novel biosynthetic pathway was either called the Isopentenol Utilization Pathway IUP [13], the Alcohol-Dependent Hemiterpene pathway

MINIREVIEW

ADH [14], the Terpene Mini-Path TMP [15] or the Isoprenoid Alcohol (IPA) pathway [16]. The recently discovered substrate promiscuity of *E. coli* hydroxyethylthiazole kinase (*Ec*ThiM), toward IOH and DMAOH [17,16] was further extended by Allemann's group with IOH/DMAOH analogs [18] and from their own words, they found some "good substrates" for the *Ec*ThiM kinase (Scheme 2).

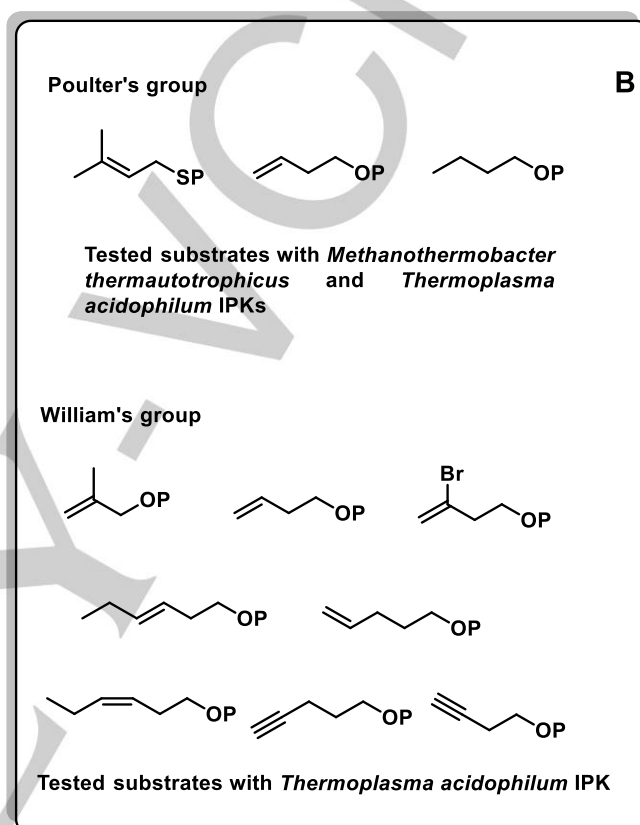
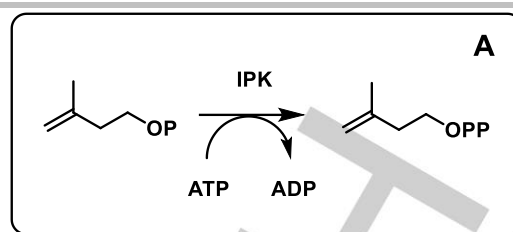


Scheme 2. Substrates shown to be "good substrates" for the ATP dependent monophosphorylation catalyzed by *Ec*ThiM kinase [18].

Finding a kinase able to monophosphorylate various IOH/DMAOH analogs is of course of interest in the context of the newly developed TMP. Indeed, if it can be coupled to an IPK with similar substrate promiscuity the construction of a non-natural terpene mini-path could be envisioned (*vide infra*).

2.3. Isopentenyl phosphate kinases (IPKs)

IPKs are part of a modified mevalonate pathway found in archaeobacteria [19]. They catalyze the ATP dependent phosphorylation of isopentenyl phosphate, arising from the decarboxylation of either mevalonate 5-phosphate for most archaea [20] or mevalonate 3,5-diphosphate for extreme acidophiles [21], and leading to IPP (Scheme 3A). IPK is also the second kinase used in the various terpene mini-paths developed recently [13,14,15,16].



Scheme 3. A Reaction catalyzed by IPKs. B Non-natural substrates of *Methanothermobacter thermautotrophicus* and *Thermoplasma acidophilum* IPKs.

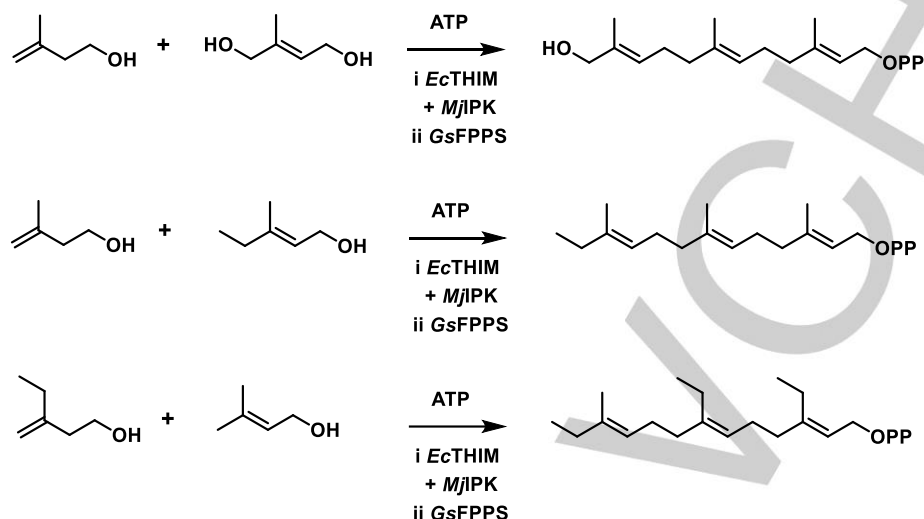
During the study of two Archean IPKs (from *Methanothermobacter thermautotrophicus* and *Thermoplasma acidophilum*), Poulter's group [22] tested IP, DMAP, isopentenyl thiolphosphate, 1-butyl phosphate, 3-buten-1-yl phosphate and geranyl phosphate (GP) as potential substrates of both IPKs (Scheme 3B). All compounds were substrates of the two enzymes with comparable kinetic parameters except GP, found to be a poor substrate. In the context of the newly developed TMP (*vide supra*), Williams' group [23] extended the repertoire of unnatural substrates of the *Thermoplasma acidophilum* IPK by testing a library of 17 monophosphates as potential substrates in addition to IP, DMAP, GP, neryl phosphate and farnesyl phosphate (FP). Five monophosphates were not substrates, seven gave less than 25% conversion and eight gave more than 50% conversion in addition to IP and DMAP (Scheme 3B).

The ability of *Ec*ThiM and IPK from *Methanocaldococcus jannaschii* (*Mj*IPK) to be used together to transform homologs of IOH/DMAOH into their corresponding diphosphate was

MINIREVIEW

recently tested [18]. The former two enzymes were combined in a two enzymes cascade to generate homologs of DMAPP and IPP. The later were tested as substrates of the farnesyl diphosphate synthase from *Geobacillus stearothermophilus* (GsFPPS), the

formation of FPP homologs being used as a read-out. The enzymatic sequence allowed the formation of 3 FPP homologs starting from 3 different IOH/DMAOH homologs (Scheme 4).



Scheme 4. Two steps enzymatic access to FPP homologs combining the TMP and a FPPS [18].

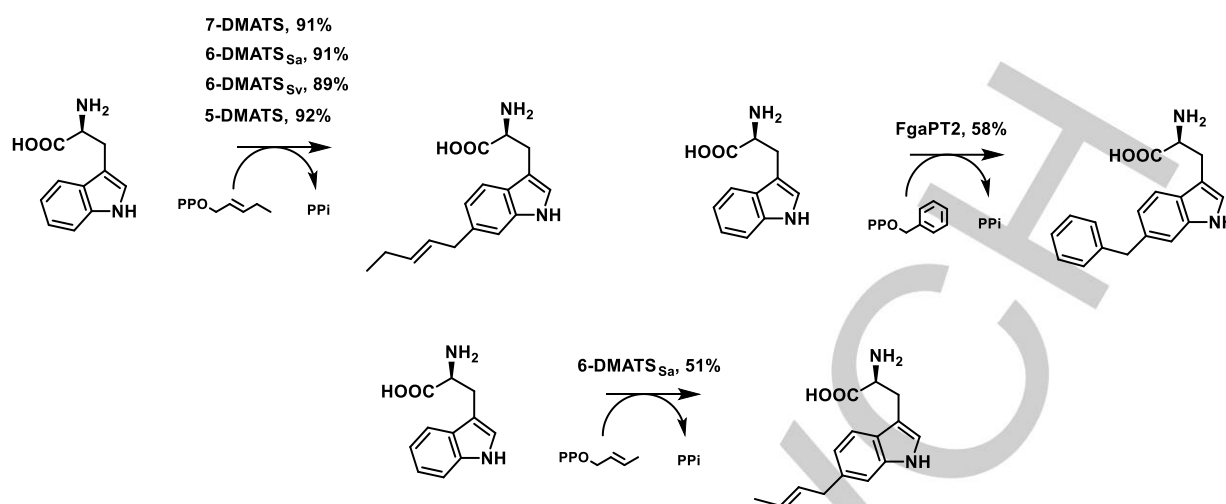
The substrate promiscuity associated with *EcTimM* and *IPK* is particularly interesting in the context of the newly developed TMP since the synthesis of unnatural terpenes could now be considered biochemically, something difficult in the case of the use of the MEV or MEP pathways. In addition, avoiding the rather restrictive chemical synthesis of diphosphates, the TMP can offer a very interesting means of testing unnatural diphosphates, provided that the non-canonical alcohol and its monophosphate are substrates of the two kinases of the mini-path.

3. Substrate promiscuity of prenyl transferases

3.1. Aromatic prenyl transferases

Aromatic prenyl transferases (Ar-PTases) catalyze the Friedel-Crafts reaction between an aromatic acceptor and prenyl donors of different lengths (DMAPP, GPP, FPP, GGPP) leading to *C*-prenylation, but also catalyze the *N*- or *O*-prenylation, depending on the enzymes and the two substrates used [24,25,26]. The prenylation can be “normal” the prenyl donor being linked to the

aromatic substrate through its *C*-1 carbon atom or “reversed” when the link involves the *C*-3 carbon atom. Three main classes of Ar-PTases are recognized, the *UbiA* superfamily [27], the *DMATS* superfamily [28] and the *ABBA* family [29,30], the latter 2 families being constituted of soluble enzymes, the former of intramembrane proteins. It is recognized that the prenylation of aromatic compounds increases their biological activities [31] probably by increasing their interaction with bio-membranes, making prenylated aromatic compounds targets of interest for the pharmaceutical industry [32]. The exquisite regioselectivity of Ar-PTases prompted the development of the biocatalytic synthesis of these compounds both *in vitro* and *in vivo* [33,34]. In addition, some studies have shown that Ar-PTases are promiscuous enzymes capable of using various aromatic substrates but also various non-canonical alkenyl diphosphate donors. In a series of pioneering articles [35,36,37,38,39], S.-M. Li's group tested three analogs of DMAPP (2-butenyl diphosphate, 2-pentenyl diphosphate and benzyl diphosphate) with various Ar-PTases whose natural substrates or best substrates are tyrosine, tryptophan and cyclodipeptides. These three substitutes were found to be substrates of the eleven enzymes tested and in some cases a yield greater than 50% was obtained (Scheme 5).

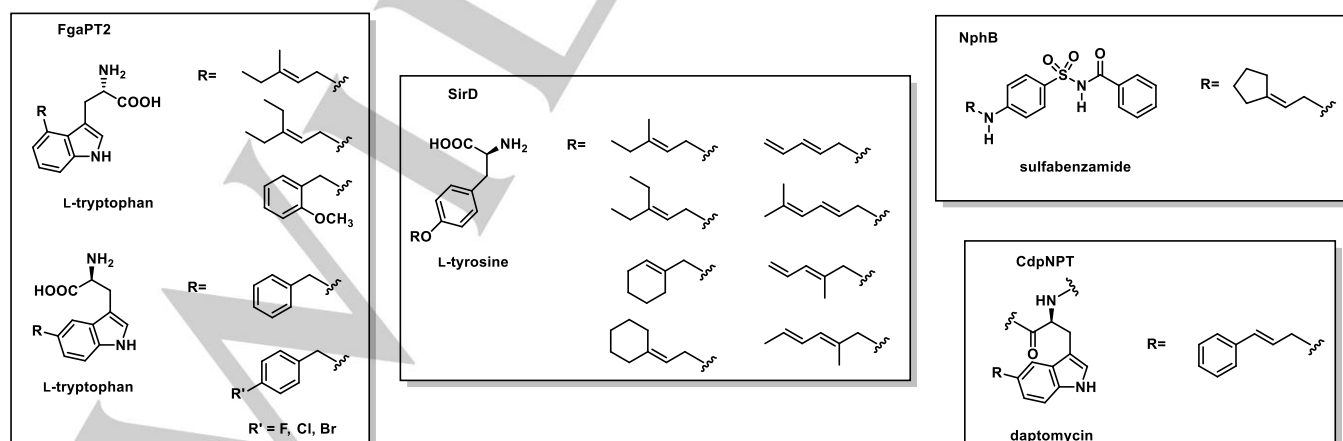


Scheme 5. Transformations reaching more than 50% yield using non-natural diphosphates (2-butenyl diphosphate, 2-pentenyl diphosphate and benzyl diphosphate) and L-tryptophan as substrates of different A-PTases [35,36,37,38,39].

These results demonstrate the substrate promiscuity of the Ar-PTases with respect to the diphosphate donor in addition to their already described promiscuity with respect to the aromatic substrate. From these studies two remarks have been made, first the regioselectivity of the enzyme is often modified especially with tryptophan and derivatives thereof [35,36,37,39] and in some cases the prenylation change from normal to reverse [39].

In a series of results published very recently, Singh's group studied, with different libraries of alkenyl- and aryl-diphosphates of ever-increasing size (up to 66 diphosphate compounds tested), the substrate promiscuity of various Ar-PTases [40,41,42]. Using SirD, a tyrosine *O*-prenyl transferase [40], eight out of twenty diphosphate analogs led to the corresponding *O*-alkenyl-1-tyrosine derivatives with a yield greater than 50% after a reaction time of 16 h (Scheme 6). Using FgaPT2, a normal *C*-4 prenyl transferase acting on L-tryptophan, seven out of thirty-three unnatural diphosphates tested resulted in the formation of a single product with *C*-4 or *C*-5 alkylation of tryptophan [41] and a yield

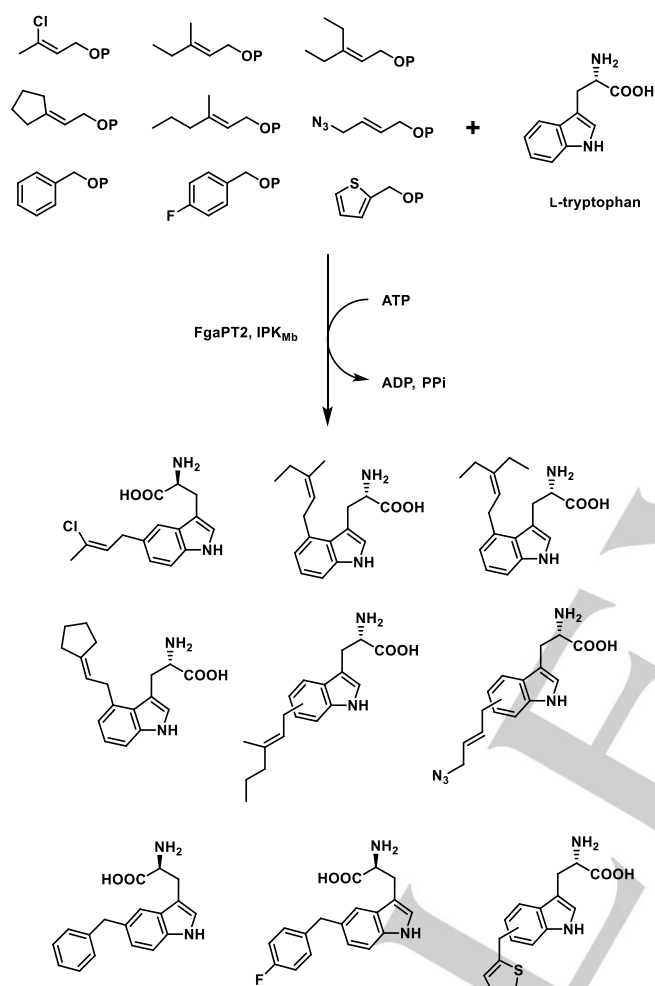
greater than 50% after 16 h of reaction time (Scheme 6). NphB is an Ar-PTase from *Streptomyces* sp. CL190 involved in the biosynthesis of naphterpin [43] and catalyzing the *C*-4 alkylation of 1,3,6,8-tetrahydroxy naphthalene with geranyl diphosphate as prenyl donor. When NphB was tested on a sulfabenzamide with an impressive library of sixty-six natural and unnatural alkenyl and aryl diphosphates [42], a 100% conversion was obtained with the cyclopentyl derivative of DMAPP (Scheme 6). Finally, the tryptophan residue of the last resort lipodepsipeptide antibiotic daptomycin has also been targeted [44] with a library of 39 alkenyl- and aryl-diphosphates using CdpNPT, a prenyl transferase from *Aspergillus fumigatus*, already reported for catalyzing the DMAPP dependent prenylation of daptomycin [45] on a tryptophan residue. Depending on the non-natural diphosphate used, various positions of the tryptophan residue were prenylated except in one case, for which a single product with a yield greater than 50% was obtained (Scheme 6).



Scheme 6. Transformations reaching more than 50% yield and giving only one alkenyl or aromatic adduct thanks to the use of unnatural diphosphates and various aromatic compounds as substrates of Ar-PTases SirD, FgaPT2, NphB and CdpNPT. R = H for the various substrates before reaction [40,41,42,44].

MINIREVIEW

In a step forward to develop a “non-natural” terpene mini-path, a library of 58 alkenyl or aryl monophosphates was first tested with 5 different archaeal IPKs [46]. The five enzymes showed a very large substrate promiscuity towards tested monophosphates and nine of them were selected to conduct a two steps cascade involving *Methanosarcina barkeri* IPK and an aromatic prenyl transferase FgaPT2 acting on L-tryptophan. Both enzymes showed sufficient substrate promiscuity to generate the corresponding alkylated L-tryptophan derivatives in more than 75% yield (Scheme 7).



Scheme 7. DMAP analogs, tested as alkylating agents during coupled IPK-FgaPT2 catalysis and alkylated L-tryptophan derivatives obtained thereof through the two enzymes cascade (adapted from ref [46]).

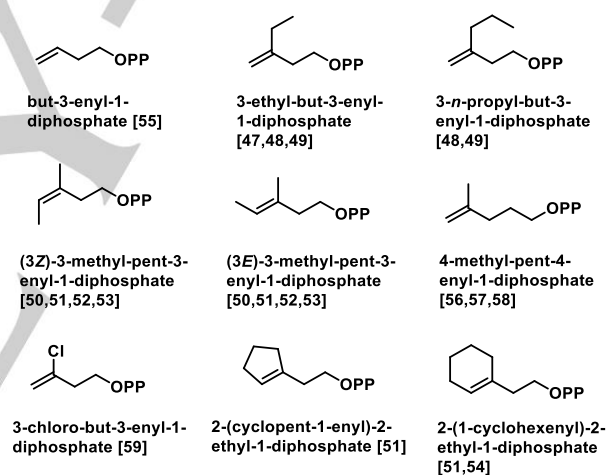
These two sets of experiments conducted by the Li's and Singh's groups clearly demonstrated that the substrate promiscuity of Ar-PTases is not limited to the aromatic acceptor, but that many diphosphates are also accepted. In view of the numerous documented cases these characteristics look to be well shared among this class of enzymes.

3.2. Aliphatic prenyl transferases

Aliphatic prenyl transferases (Al-PTases) use a starter unit and one or more extension units to produce acyclic diphosphates such as GPP, FPP, GGPP and GFPP. Generally, the starting units are, depending on the length of the final product, provided by allylic diphosphates such as DMAPP, GPP or FPP. These compounds are then treated with Al-PTases, which add one or more extension units (IPP) to the starter one. Various Al-PTases have been tested with respect to their substrate promiscuity both for starter and extending units, farnesyl diphosphate synthase (FPPS) being by far the most popular. We highlight here the described results that make sense from a synthetic point of view. The interested readers can find more information in the cited references, especially for poor Al-PTases substrates.

FPPS with DMAPP and GPP as starter units

Numerous IPP homologues lacking a methyl group, bearing an extra methyl group on various positions, a chlorine atom or a cycle built around the double bond have been chemically synthesized and tested as substrates of various FPPS in combination with DMAPP or GPP as starter units (Scheme 8).

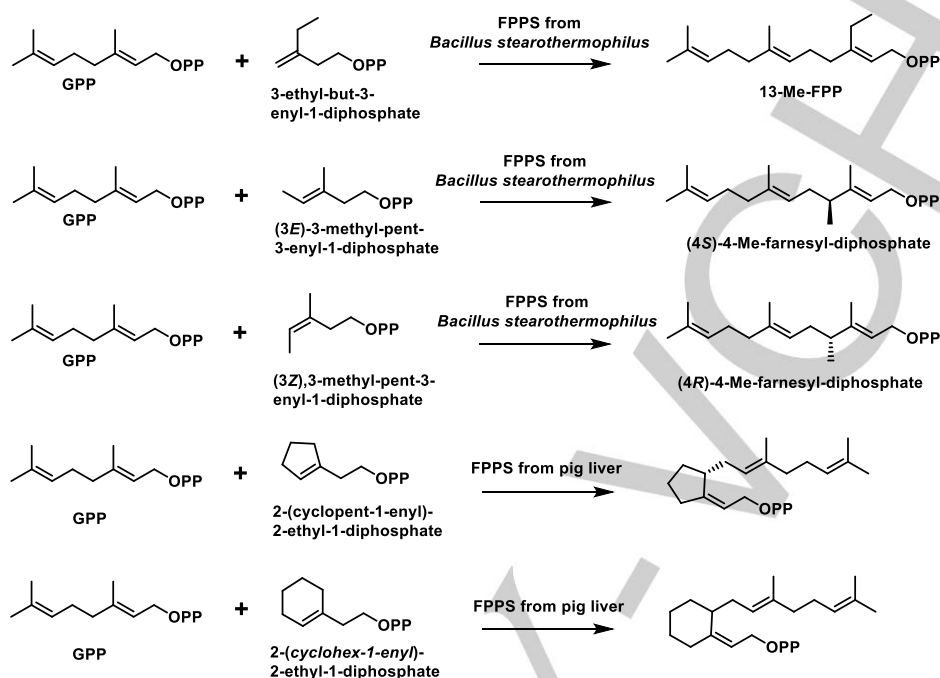


Scheme 8. Panel of IPP analogs, tested as extension units during FPPS catalysis and associated references.

Using 3-ethyl-but-3-enyl-1-diphosphate as a substrate [47,48,49], the FPPS of the thermotolerant bacterium *Bacillus stearothermophilus* was able to generate, with GPP as a starting unit and a good yield (Scheme 9), the FPP homolog carrying a C-3 ethyl substituent [49]. The same enzyme was found to be superior [50] to pig liver FPPS [51,52] in terms of final yield when tested with (3Z)-3-methyl-pent-3-enyl-1-diphosphate and (3E)-3-methyl-pent-3-enyl-1-diphosphate. The characteristic of these two compounds is that if they act as FPPS substrates, the product formed is chiral. Starting from the *E* isomer and using GPP as the starting unit, (4S)-4-methyl-farnesyl diphosphate was obtained from both enzymes while from the *Z* isomer, the (4R)-4-methyl-farnesyl diphosphate enantiomer was obtained (Scheme 9). In the case of the enzyme from *B. stearothermophilus*, the two enantiomers were obtained in an enantiomerically pure form [53]. Interestingly, the pig liver FPPS proved to be superior in terms of final yield to its bacterial counterpart when tested with a cyclic

MINIREVIEW

equivalent of IPP [51,54]. Starting from GPP, two C₅ and C₆ cyclic derivatives of FPP were obtained, the structure of which somewhat resembles that of prostaglandins (Scheme 9).

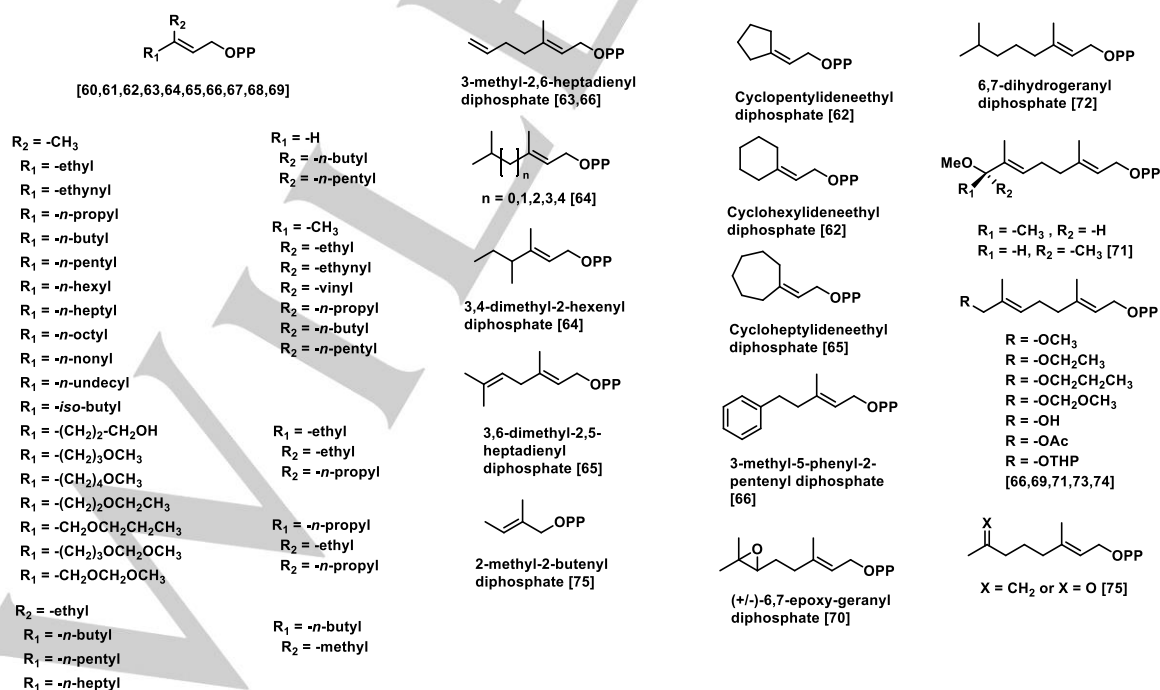


Scheme 9. Reactions of synthetic interest catalyzed by FPPS on IPP analogs tested as extender units.

FPPS with IPP as extender unit

Since the late sixties numerous homologs of DMAPP and GPP have been chemically synthesized and tested as starter units in

combination with IPP as extender unit during FPPS catalysis (Scheme 10).

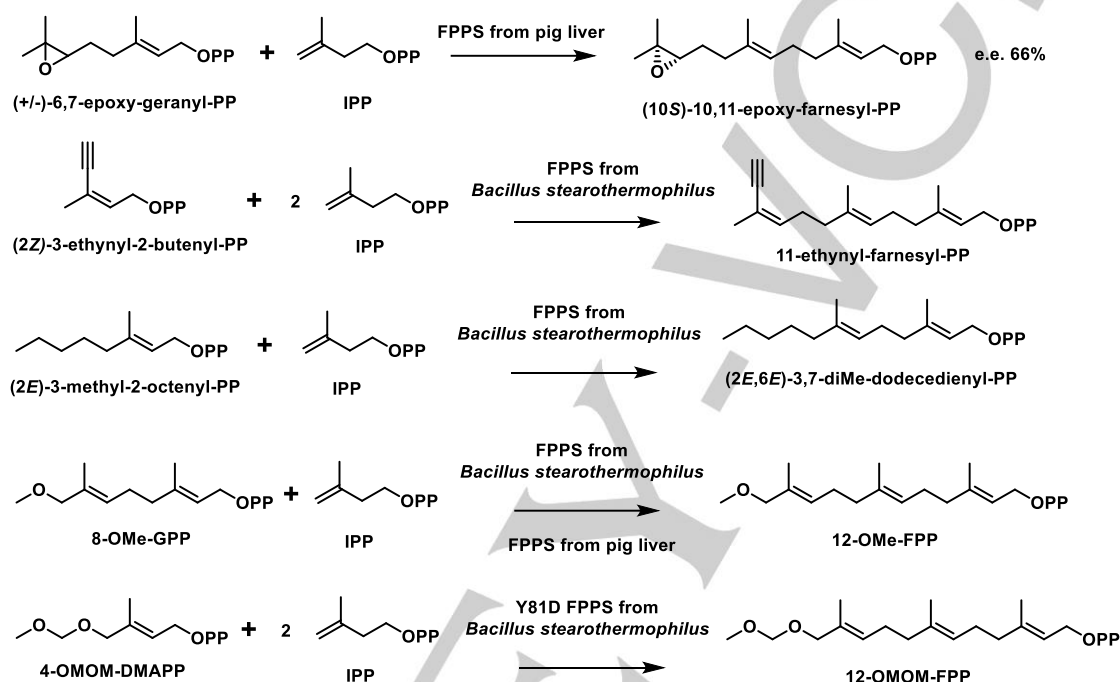


Scheme 10. DMAPP/GPP analogs tested as starter units during FPPS catalysis.

MINIREVIEW

Nearly 60 chemically synthesized homologs of DMAPP and GPP have been tested as starter units with various (*E*)-FPPSs from pig liver, pumpkin, *Bacillus stearothermophilus* (wild type and mutants) and a (*Z*)-FPPS from *Thermobifida fusca*. Some results can be highlighted from this wealth of information (Scheme 11). When (+/-)-6,7-epoxy-geranyl diphosphate was used as substrate of pig liver FPPS, the corresponding (*S*)-3-epoxy-farnesyl-diphosphate was obtained with an enantiomeric excess of 66% but albeit in low yield (16%). The 3-ethynyl derivative of DMAPP can be doubly condensed with IPP by *Bacillus stearothermophilus*

FPPS leading to the 11-ethynyl-farnesyl-diphosphate in 89% relative yield as compared to FPP (taken as 100%). Some compounds proved to be even better substrates than natural ones. This is the case for example for the (*E*)-*n*-pentyl equivalent of DMAPP (191% relative activity as compared to GPP with *Bs*FPPS), for the 8-methoxy derivative of GPP (299 and 189% relative activity as compared to GPP with respectively pig liver FPPS and *Bs* FPPS) and the MOM protected 4-OH-DMAPP (134% relative activity as compared to DMAPP with *B. stearothermophilus* Y81D mutant FPPS).



Scheme 11. Reactions of synthetic interest catalyzed by FPPS on DMAPP and GPP analogs tested as starter units.

Other aliphatic prenyl transferases

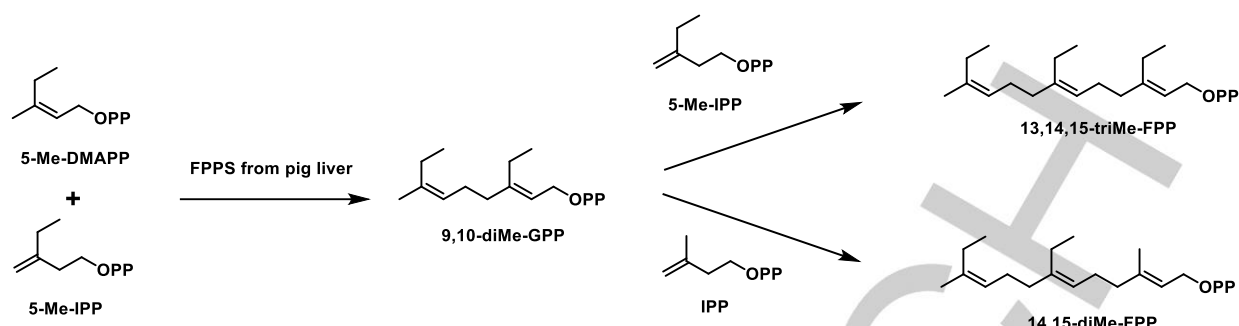
The substrate promiscuity of other Al-PTases such as the geranylgeranyl diphosphate synthase (GGPPS), hexaprenyl, heptaprenyl, undecaprenyl and solanesyl diphosphate synthases have also been studied. The best results, in term of final yield, were recorded when using GGPPS with some of the DMAPP/GPP equivalents described in the section FPPS and especially with 3-methyl-2-alkenyl and 3-methyl-2-iso-alkenyl diphosphate of various length. The interested reader is referred to the following articles regarding GGPPS [76,77,78,58,79] as well as other aliphatic prenyl transferases [80,81,58,82,83,84,85,86] for more information on tested substrates, formed products and yields.

4. Synthesis and biosynthesis of non-canonical terpenes

4.1. Synthesis

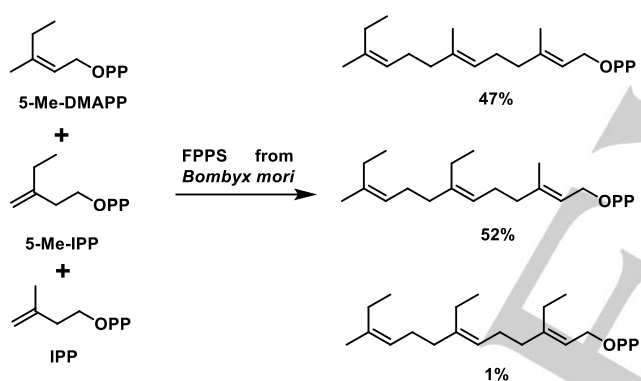
Based on the demonstrated substrate promiscuity of pig liver FPPS (*vide supra*), the carbon skeleton of two juvenile insect hormones (JH), JH0 and JH1 (Scheme 12) were obtained using 5-Me-IPP or IPP and 5-Me-DMAPP or 9,10-diMe-GPP as substrates [87]. When 5-Me-DMAPP and 5-Me-IPP were submitted to pig liver FPPS catalysis, 13,14,15-triMe-FPP (the carbon skeleton of insect JH0) and 9,10-diMe-GPP were obtained, the latter being an intermediate of the reaction.

MINIREVIEW



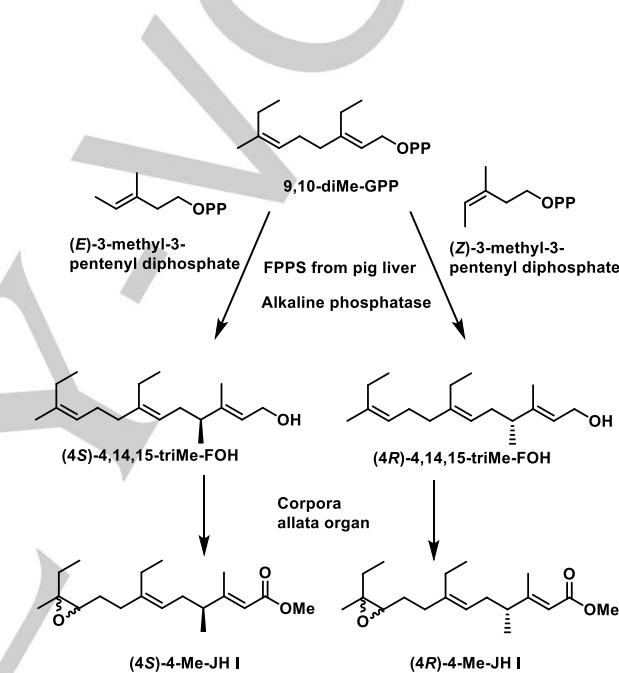
Scheme 12. Use of pig liver FPPS in the synthesis of the carbon skeleton of two juvenile insect hormones in the form of 14,15-diMe- and 13,14,15-triMe-FPP.

To access 14,15-diMe-FPP (the carbon skeleton of the juvenile insect hormone JHI), 9,10-diMe-GPP was chemically synthesized and condensed with IPP using FPPS from pig liver. Later on, the same group, used a mixture of 5-Me-DMAPP, 5-Me-IPP and IPP and the purified FPPS II from larvae of silkworm *Bombyx mori* in a single experiment [88]. They thus accessed the carbon skeleton of JHI, JHII and JH0 with a 47/52/1 proportion (Scheme 13).



Scheme 13. Use of *Bombyx mori* FPPS II in the synthesis of the carbon skeleton of JHI, JHII and JH0.

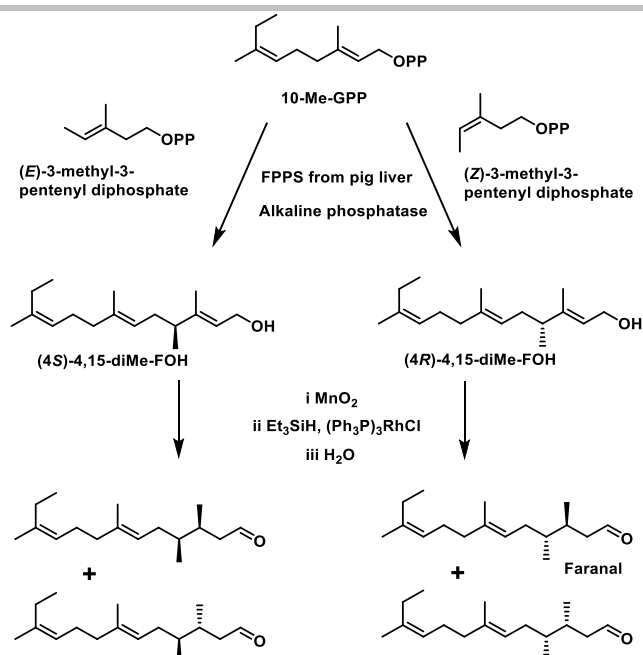
These first two studies laid the foundations for the enzymatic synthesis of the juvenile natural insect hormone 4-Me JHI [89]. Using pig liver FPPS, alkaline phosphatase, chemically synthesized 9,10-diMe-GPP and either (*E*)- or (*Z*)-3-methyl-3-pentenyl diphosphate, (*4S*)- and (*4R*)-4,14,15-triMe farnesol were both generated. The two compounds were then incubated with 10 pairs of adult female *Manduca sexta* corpus allata, resulting in two diastereomers of 4-MeJH I and thus establishing the absolute (*4S*) configuration for natural 4-MeJH I (Scheme 14).



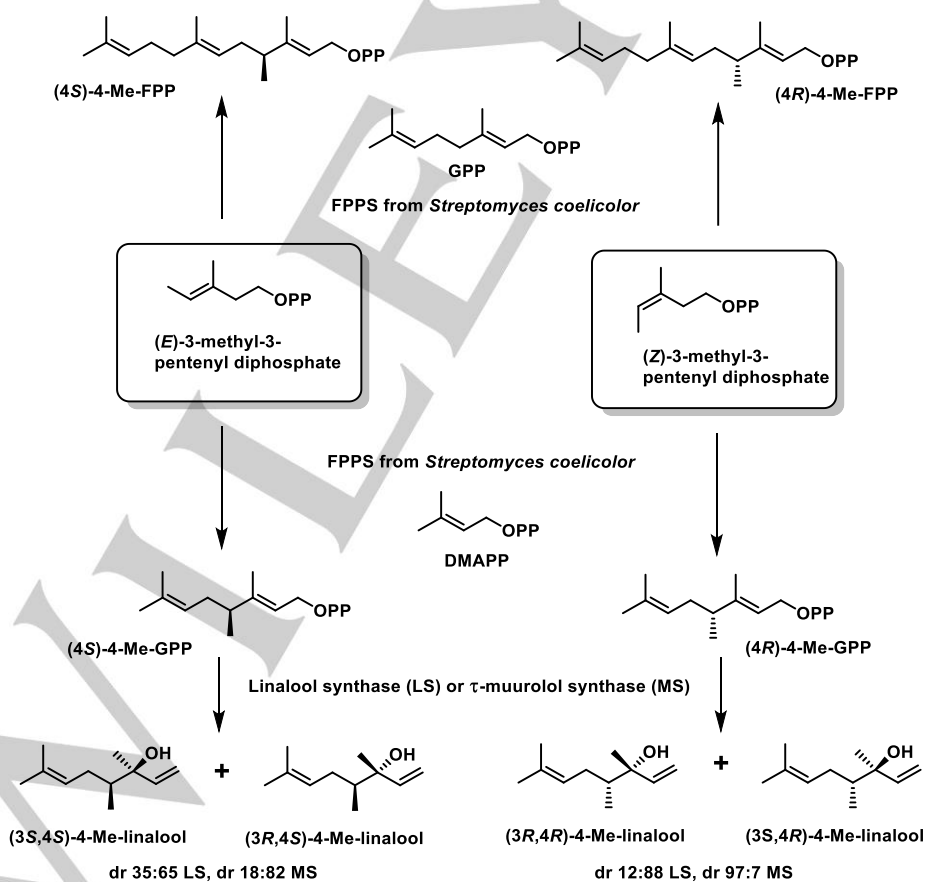
Scheme 14. (Bio)synthesis of (*4R*)- and (*4S*)-4Me-JHI.

A similar reaction scheme was used for the synthesis of the trail pheromone of the pharaoh ant, *Monomorium pharaonic*, whose structure is reminiscent of the one of 4-MeJH I [90]. In this case, 15-Me-GPP was obtained either chemically or enzymatically and then similarly condensed with either (*E*)- or (*Z*)-3-methyl-3-pentenyl diphosphate using the FPPS from pig liver, followed by treatment with an alkaline phosphatase. The alcohols thus obtained were then chemically oxidized to the corresponding aldehydes and the conjugated double bond reduced, resulting in each case in a separable mixture of two diastereomers (Scheme 15). The four diastereomers of faranal thus obtained were then tested and the configuration of the natural pheromone was established as being (*3S,4R*) by a biological test.

MINIREVIEW



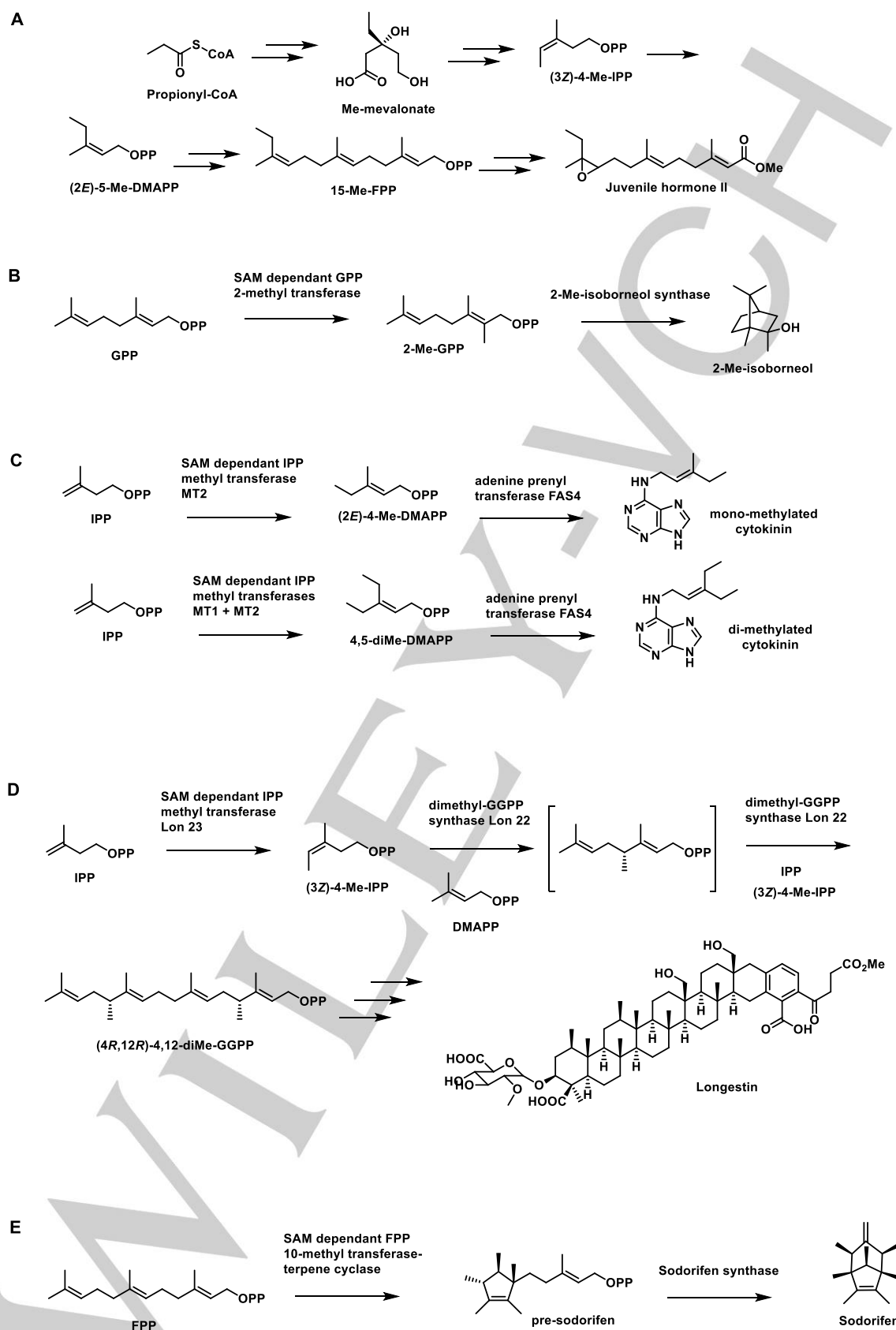
Scheme 15. (Bio)synthesis of the four stereoisomers of the trail pheromone faranal.



Scheme 16. Enzymatic synthesis of 4-Me derivatives of farnesol, geraniol and linalool.

Using the same (bio)synthetic methodology, some faranal derivatives were also obtained and a bioassay revealed that the configuration of the 10,11 double bonds could be either *Z* or *E* but the methyl group at position seven could not be replaced by an ethyl group without loss of activity [91].

Very recently, by using the FPPS from *Streptomyces coelicolor*, Dickschat's group produced each of the two enantiomers of 4-Me-geranyl diphosphate and 4-Me-farnesyl diphosphate starting from (*E*)- and (*Z*)-3-methyl-3-pentenyl diphosphate and from DMAPP and GPP respectively (Scheme 16).



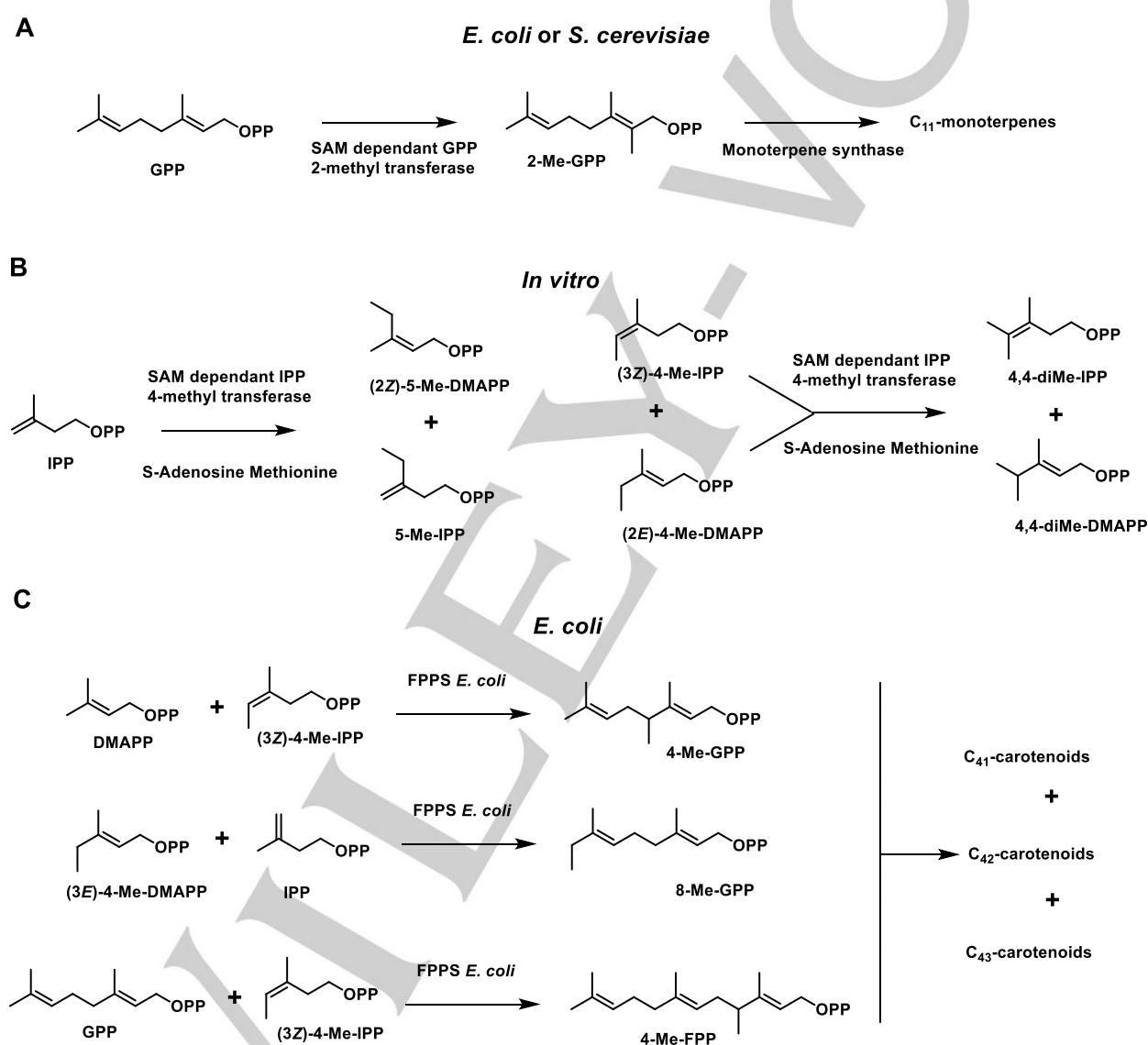
Scheme 18. Examples of various methylated-prenyl diphosphates involved in the biosynthesis of some terpenoids. The juvenile hormone biosynthetic path is proposed.

MINIREVIEW

4.3. *In vivo* use of methylated prenyl diphosphate precursors

Recently, *in vivo* studies have taken advantage of some of the various prenyl diphosphate methyl transferases identified in the biosynthesis of non-canonical terpenoids, in order to extend the terpene chemical space. The GPP 2-methyl transferases from *Streptomyces coelicolor* and *Pseudanabaena limnetica* involved in the biosynthesis of 2-methylisoborneol were respectively introduced in *E. coli* [99] and *S. cerevisiae* [100] to produce various C-11 methylated-monoterpenes (Scheme 19A). On the other hand, a newly discovered *Streptomyces monomykini* IPP 4-

methyl transferase proved to catalyze the formation of various mono- and di-methylated diphosphates from IPP (Scheme 19B) [101]. When expressed in *E. coli* engineered toward high IPP and DMAPP production, 4- and 8-Me-geraniol as well as 4-Me-farnesol were detected in the culture medium, suggesting that endogenous FPPS from *E. coli* could accommodate 4-Me-IPP and 4-Me-DMAPP formed by the methyl transferase. Furthermore, when combined with various carotenoid pathways, mono-, bis- and tris methylated carotenoids were detected proving the ability of these path to generate non-canonical terpenes (Scheme 19C).



Scheme 19. Use of prenyl diphosphate methyl transferases to access non-canonical terpenoids. **A** *In vivo* access to non-natural C₁₁-monoterpenes using a GPP 2-methyl transferase. **B** IPP derivatives obtained through *in vitro* action of *Streptomyces monomykini* IPP 4-methyl transferase. **C** *In vivo* access to non-natural C₄₁-, C₄₂- and C₄₃-carotenoids using *Streptomyces monomykini* IPP 4-methyl transferase.

5. Conclusion

Throughout this mini-review we underlined that numerous enzymes involved in the early steps of terpene biosynthesis possess a large substrate promiscuity allowing the generation of numerous homologs of classical terpene precursors such as DMAPP, IPP, GPP and FPP. While the demonstration of this substrate promiscuity was originally conditioned to the chemical synthesis of the corresponding diphosphates, the development of the TMP allows now to limit the chemical synthesis to non-canonical alcohols (homologs of DMAOH or IOH), leaving the generation of the corresponding diphosphate derivatives to the 2 kinases of the mini-path [18]. Such a simplified access to non-canonical diphosphates highlights the interest of the TMP for extending the terpenoid chemical space beyond its capacity to simplify the biotechnological access to natural terpenoids. Another recent development in the biotechnological production of terpenes is the discovery and use of various SAM-dependent methyl transferases that can modify already formed diphosphates such as DMAPP, IPP or GPP. When used in conjunction with late terpene biosynthetic pathways such as the monoterpene pathway or the carotenoid pathway, new natural compounds can be obtained in this way, opening a new and great avenue for the biotechnological production of non-natural terpene compounds. Such opportunities are strengthened by the nearly indefinite source of novel enzymes that can be tapped from the biodiversity as well as by the probably even greater capacity of protein engineering to generate fine-tuned enzymes for very specialized transformations. Combining such diversity with the demonstrated ability of the TMP to function both *in vitro* and *in vivo* hold great promises in the biotechnological production of natural as well as non-natural terpenoid compounds.

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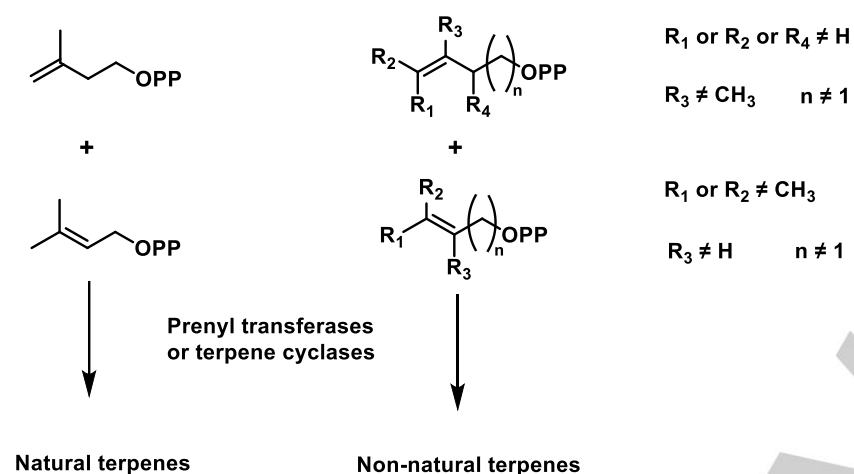
Keywords: IPP analogs • DMAPP analogs • non-natural terpenes • prenyl transferases • terpene mini-path

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Entry for the Table of Contents



Due to a unique biosynthetic scheme, the diversification of terpene structure could be envisioned through the structural modification of the two universal common terpene precursors IPP and DMAPP. This mini-review covers the synthesis, the use as prenyl transferase substrates and the synthetic application of IPP and DMAPP homologs developed to diversify the terpene chemical space.