Synthesis and Biocatalytic Conversion of Natural and Artificial Isoprenoid Diphosphates

Dissertation

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vorgelegt von

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“A tidy lab means a lazy chemist.”

Jöns Jakob Berzelius

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1. Introduction

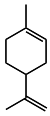
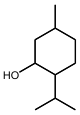
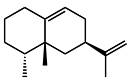
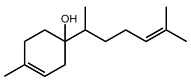
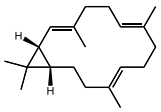
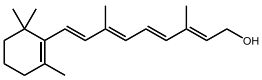
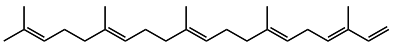
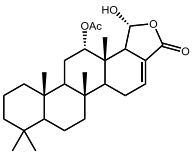
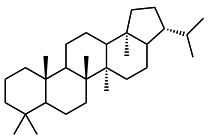
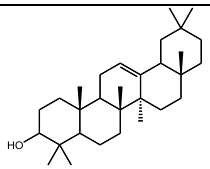
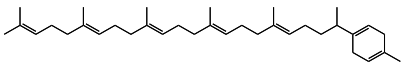
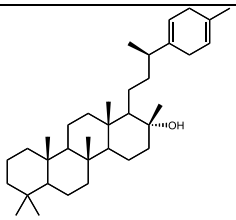
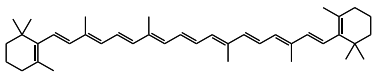
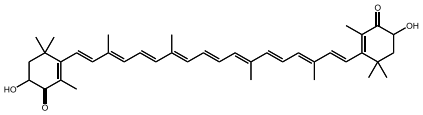
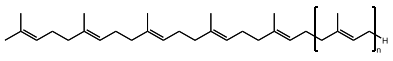
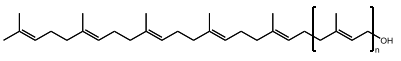
1.1 Isoprenoids

Isoprenoids, also called terpenoids, represent a highly diverse natural product class derived from isoprene. Following the IUPAC, the more than 30,000 known examples[1] are divided in two groups.[2] Whereas isoprenes (terpenes) represent pure hydrocarbons derived from isoprene units, the isoprenoids (terpenoids) contain heteroatoms like oxygen due to structural modifications. Furthermore they are classified into hemi- (C_5), mono- (C_{10}), sesqui- (C_{15}), di- (C_{20}), sester- (C_{25}), tri- (C_{30}), sesquar- (C_{35}), tetra- (C_{40}) and polyterpenes ($C_{>40}$) according to their number of carbon atoms (see **Tab. 1**).

Isoprenoids are an important class of both primary and secondary metabolites in plants, animals, fungi and bacteria. As part of the primary metabolism in plants, gibberellins which are derived from the diterpene *ent*-gibberellane, act as hormones responsible for growth regulation and other developmental processes.[3] The triterpene lanosterol serves as precursor for the biosynthesis of steroids that regulate a vast number of biological functions in animals, plants fungi and even some prokaryotic species.[4,5] As secondary metabolites isoprenoids play a significant role in the defense against different pathogens. Due to these versatile biological functions, isoprenoids offer a large variety of valuable properties making them an appreciated resource for industrial as wells as medicinal purposes. Volatile terpenes can keep away natural enemies due to their characteristic scent.[6,7] At the same time this scent is the reason why a lot of monoterpenes are widely applied in the flavor and fragrance industry.[8] Several terpenes show an antimicrobial activity while others inhibit the growth of fungi.[9-11] A well-known example is the production of the antifungal diterpene casbene by *Ricinus communis* plants which is triggered by a previous fungal infection.[12]

The essential oils of plants serve as key sources for most of the isoprenoids used in industry.[13] The sesquiterpenoid Artemisinin from *Artemisia annua* and the diterpenoid paclitaxel from *Taxus brevifolia* are the most popular examples for a well-established medicinal use of this natural product class. While the former represents one of the most potent inhibitors of different *Plasmodium* species causing Malaria the latter one is successfully used in the treatment of various types of cancer.[14,15]

Tab. 1 – Classification of terpenes/terpenoids by the number of carbon atoms including one example of each class and subgroup.

carbons	terpene class	terpene example	terpenoid example
5	hemiterpenes	 isoprene	 tiglic acid
10	monoterpenes	 limonene	 menthol
15	sesquiterpenes	 5-*epi*-aristolochene	 β-bisabolol
20	diterpenes	 casbene	 retinol
25	sesterterpenes	 geranylfarnesene	 scalarine
30	triterpenes	 hopane	 β-amyrin
35	sesquarterpenes	 tetraprenyl-β-curcumene	 sporulenol
40	teraterpenes	 β-carotene	 astaxanthin
>40	polyterpenes	 gutta-percha (n ≈ 100)	 dolichol (n = 15)

1.2 Biosynthesis

In 1953 Leopold Ružička discovered that "the carbon skeleton of terpenes is composed of isoprene units linked in a regular or irregular arrangement".[16] A few years later this "isoprene rule" was proven to be true when several workgroups revealed that, despite their complexity, isoprenoids are derived from only two simple C_5 precursors, namely dimethylallyl diphosphate (DMADP) and isopentenyl diphosphate (IDP).[17-21] These diphosphates are biosynthesized via two different biological pathways. Whereas the methylerythritol phosphate (MEP) pathway uses pyruvate and glyceraldehyde 3-phosphate as starting material the mevalonate (MVA) pathway bases on the conversion of acetyl coenzyme A (see **Fig. 1**).[22-27]

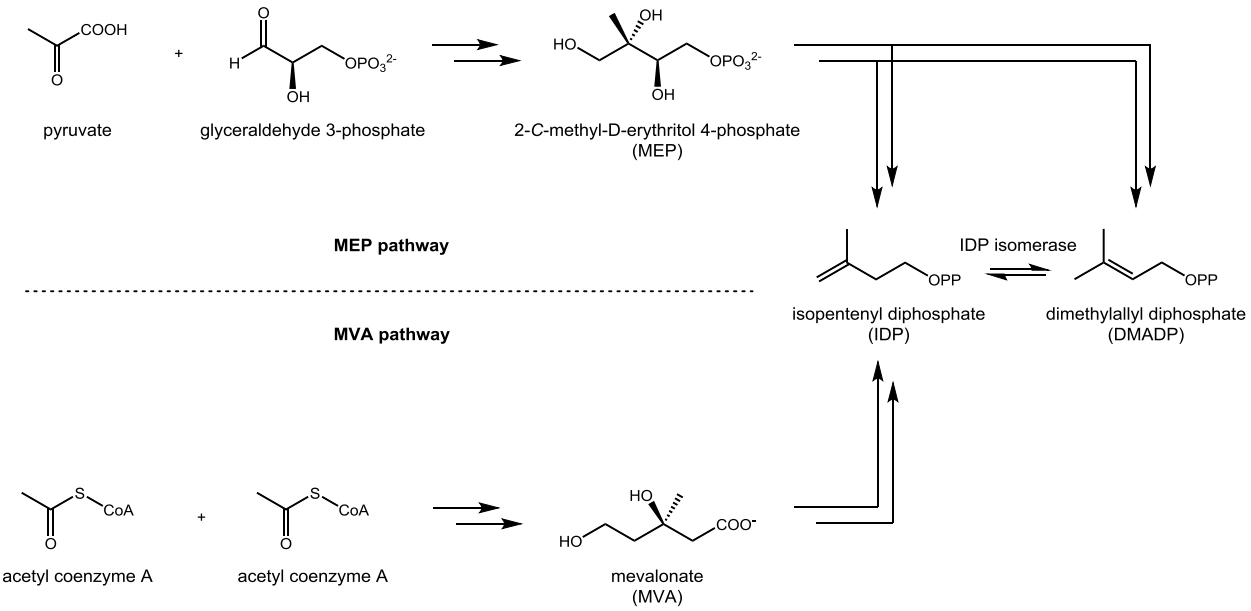


Fig. 1 – Illustration of the MEP and MVA pathways leading to the biosynthesis of isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMADP).

The enzyme IDP isomerase is then establishing an equilibrium of the produced homoallylic IDP and the allylic DMADP making both molecules accessible for further biological pathways.[28]

The next important step in the terpene biosynthesis is the chain elongation catalyzed by the enzyme class of prenyl transferases. In these reactions, an allylic starter unit (e.g. DMADP) is elongated by one or more homoallylic IDP units.[29] As shown in **Fig. 2**, this enables the synthesis of longer chain isoprenoid diphosphates like geranyl diphosphate (GDP, C_{10}), farnesyl diphosphate (FDP, C_{15}) and geranylgeranyl diphosphate (GGDP, C_{20}). The elongation

with a larger number of IDP units leads to the formation of polyprenyl diphosphates like undecaprenyl diphosphate (UDP, C_{55}). This molecule consists of eleven C_5 units and plays an essential role in the bacterial cell wall formation.[30,31] The class of polyterpenes also comprises natural rubber that is among others produced by the rubber tree (*Hevea brasiliensis*).[32] In contrast to the (*E*)-configured gutta-percha from *Palaquium gutta* the double bonds of natural rubber show a (*Z*)-configuration resulting in different mechanical properties.[33]

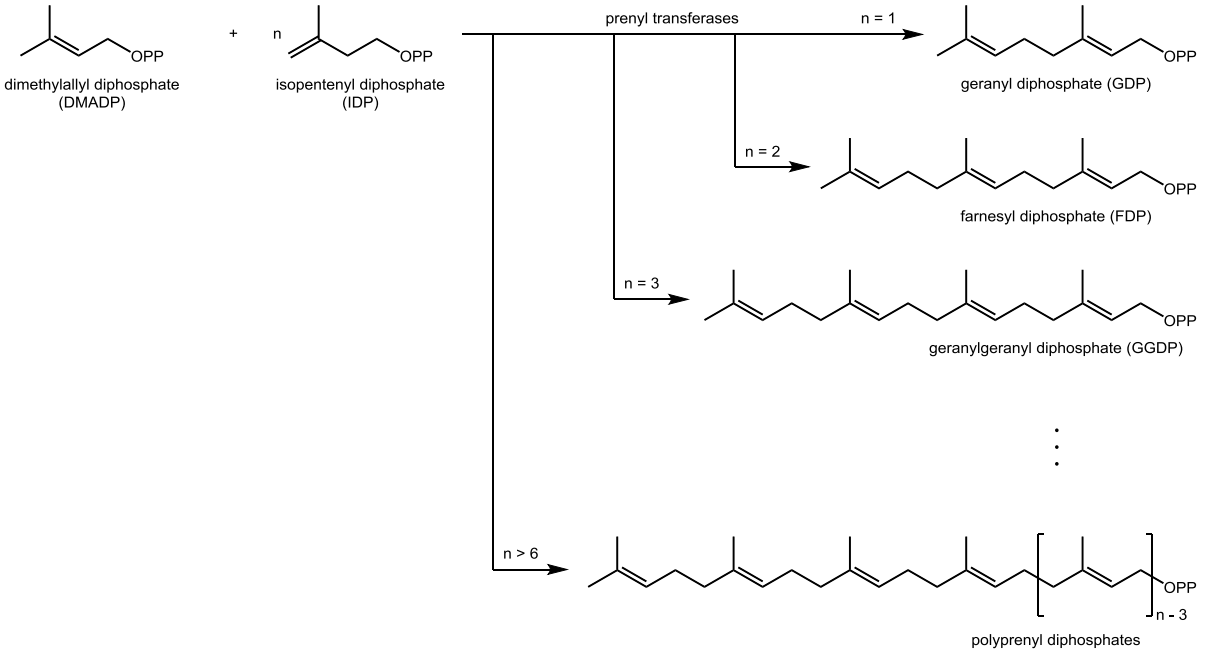


Fig. 2 – Biosynthesis of longer chain prenyl diphosphates by prenyl transferases starting from the C5 precursors DMADP and IDP.

The elongated isoprenoid diphosphates can then act as substrates for the magnesium dependent enzyme class of terpene synthases.[34,35] As many of their catalyzed reactions comprise cyclizations, they are also called terpene cyclases. Interestingly the triterpene and tetraterpenes synthases are not directly accepting the corresponding elongated diphosphates as substrates. Instead squalene and oxidosqualene, biosynthesized from the dimerization of FDP, represent the key substrates for triterpene synthases whereas lycopene, originating from the dimerization of geranylgeranyl diphosphate, acts as substrate for tetraterpene synthases responsible for the biosynthesis of carotenoids.[36,37] The different biosynthetic pathways of terpenes and terpenoids are summarized in **Fig. 3**.

Dependent on the product formed, the nomenclature used in the classification of terpenes is also applied to terpene synthases. As an example, the limonene synthase used in this work is

classified as monoterpene synthase due to the monoterpene product limonene that is formed in course of its catalyzed conversion reaction starting from GDP. This does in turn not mean that terpene synthases are limited to the conversion or production of one single compound. In the past it could be shown that terpene synthases are liberal with respect to their product spectra.[38]

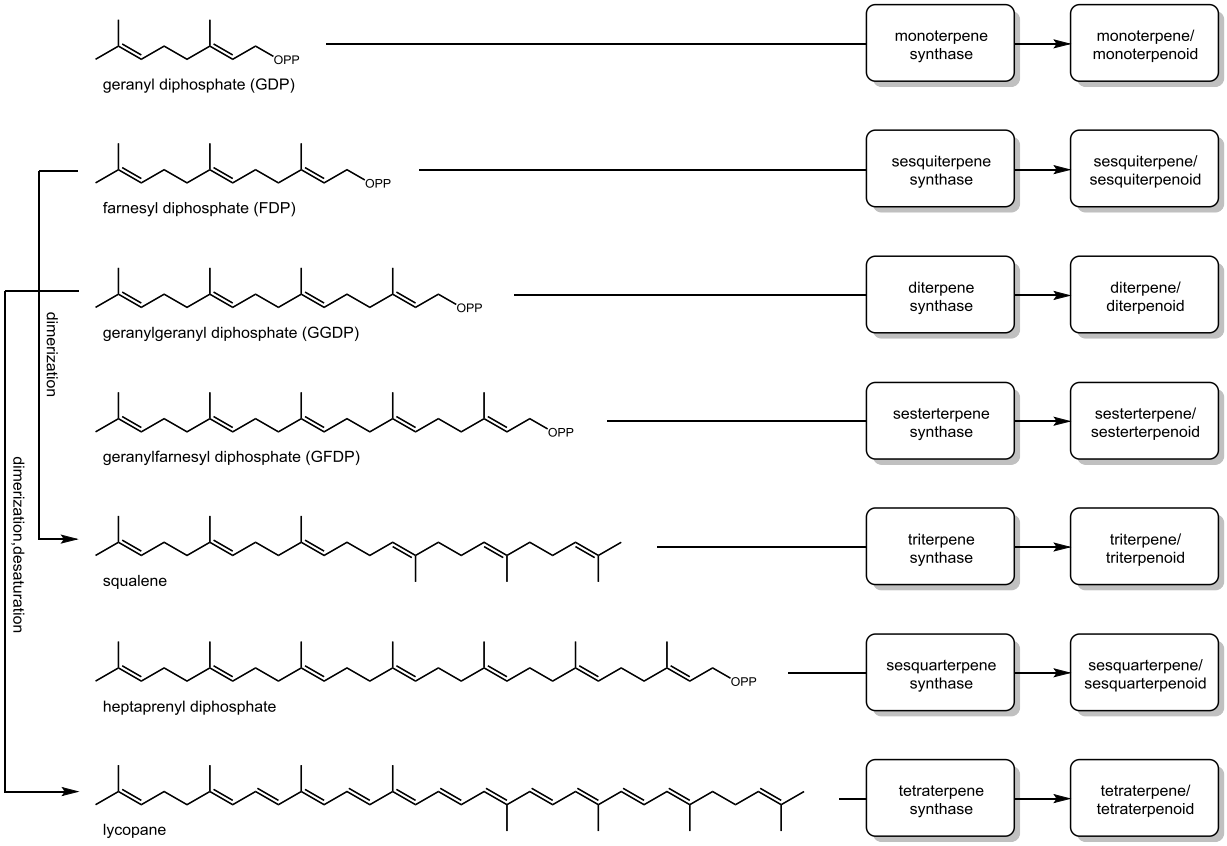


Fig. 3 – Biosynthesis of terpenes/terpenoids from their corresponding precursors by terpene synthases. All precursors are exemplary shown in all-*trans*-configuration.

1.3 The Role of (*Z*)-Configured Terpene Precursors in Nature

In the field of medium- and long-chain prenyl transferases, the formation of *cis*-configured double bonds is well known and understood. In contrast, only little is known about the relevance of *cis*-configured intermediates in the terpene biosynthesis. All of the exemplary terpene precursors shown in **Fig. 3** feature an all-*trans*-configuration and for a long time this was believed to be the whole truth.

In 1970 Heinstein *et al.* investigated the biosynthesis of gossypol in *Gossypium hirsutum* (Upland Cotton). They could show that among all four farnesyl diphosphate isomers (*Z*,*E*)-FDP and (*Z*,*Z*)-FDP are the most effective substrates in the biocatalytic production of hemigossypol.[39] In addition, they could identify an enzyme from the same plant that is able

to produce all four FDP isomers.[40,41] In 1993 Akhila *et al.* could finally confirm that gossypol from *Thespesia populnea* (Portia Tree) is indeed produced from (*Z*,*E*)-FDP and (*Z*,*Z*)-FDP. Both isomers are hereby converted via different cyclization pathways leading to the same final product. This was the first evidence that not all terpenes are biosynthesized from all-*trans*-configured precursors.

A few years later Schulbach *et al.* discovered the existence of a new FDP synthase in *Mycobacterium tuberculosis*. It accepts the (*E*)-configured geranyl diphosphate (GDP) as well as the (*Z*)-configured neryl diphosphate (NDP) as a substrate resulting in the formation of both (*Z*,*E*)-FDP and (*Z*,*Z*)-FDP.[42,43]

In 2009 Schimiller *et al.* identified a NDP synthase from *Solanum lycopersicum* (Cultivated Tomato). Moreover they could show that the monoterpenes known to be produced by this tomato species are biosynthesized from NDP rather than GDP.[44] At the same time Sallaud *et al.* found two interesting synthases from *Solanum habrochaites* (Wild Tomato). The first one is able to synthesize NDP and (*Z*,*Z*)-FDP from the C_5 precursors DMADP and IDP while the second one catalyzes the conversion of (*Z*,*Z*)-FDP to various santalene and bergamotene species.[45] While Jones *et al.* could confirm that the santalene-bergamotene synthase from *Santalum album* also accepts (*Z*,*Z*)-FDP as a substrate[46] two further examples of (*Z*,*Z*)-FDP synthases from bacteria were characterized by the group of Sagami.[47,48]

All of these findings suggest that (*Z*)-configured isoprenoid diphosphates could play a more important role in the biosynthesis of terpenes than initially assumed. Despite all of the mentioned studies their real relevance remains unclear to date and is therefore further investigated within the context of this thesis.

1.4 The Role of Methyl Side Chains in the Conversion of Isoprenoid Diphosphates

Several studies on prenyl transferases could show that the methyl group at position 3 of the allylic substrate is crucial for a successful enzymatic conversion.[49-51] Artificial substrates that are lacking this methyl group are often still bound but poorly, if at all, converted by the corresponding enzymes. As a consequence, this kind of substrates is investigated as a potential class of inhibitors.

In the field of terpene synthases nothing is yet reported about the relevance of this methyl group. Hence the influence of an absent methyl group at position 3, next to the diphosphate moiety, is also analyzed as a part of this thesis.

2. Objectives

2.1 Modular and Stereospecific Synthesis of Isoprenoid Diphosphates

Since a large variety of different but structurally related isoprenoid diphosphates shall be used within the context of the biocatalytic conversions a suitable synthesis method represents a mandatory prerequisite. Hence, a modular and stereospecific method for the fast and efficient synthesis of isoprenoid diphosphates shall be established. This method should allow the synthesis of all geometric isomers of geranyl diphosphate (GDP), farnesyl diphosphate (FDP) and geranylgeranyl diphosphate (GGDP) as well as a set of corresponding 3-desmethyl derivatives.

2.2 Conversion of All Geometric Isomers of GDP, FDP and GGDP by Terpene Synthases

The biocatalytic conversion of natural and artificial isoprenoid diphosphates by three terpene synthases, namely limonene synthase (CsTPS1) from *Cannabis sativa*, aristolochene synthase (TEAS) from *Nicotiana tabacum* and casbene synthase (RcCAS) from *Ricinus communis,* shall be investigated. The focus regarding the used diphosphate precursors shall lie on all geometric isomers of the corresponding natural substrates. The resulting products shall be analyzed via GC-MS experiments expecting new insights regarding the influence of the substrates double bond configuration on the observed product spectra.

2.3 Conversion of 3-Desmethyl Derivatives of GDP, FDP and GGDP by Terpene Synthases

The biocatalytic conversion of artificial isoprenoid diphosphates by three terpene synthases, namely limonene synthase (CsTPS1) from *Cannabis sativa*, aristolochene synthase (TEAS) from *Nicotiana tabacum* and casbene synthase (RcCAS) from *Ricinus communis,* shall be investigated. The focus regarding the used diphosphate precursors shall lie on 3-desmethyl derivatives of the corresponding natural substrates. The resulting products shall be analyzed via GC-MS experiments expecting new insights regarding the influence of 3-methyl group on the biocatalytic conversion of isoprenoid diphosphate to terpenes.

2.4 Synthesis of Isopentenyl Diphosphate (IDP) Derivatives

Within the scope of an industry cooperation the proof of concept for the biocatalytic production of rubber derivatives using medium-chain *cis*-prenyl transferases shall be demonstrated. For this purpose a set of isopentenyl diphosphate (IDP) derivatives featuring modifications at position 3 shall be synthesized.

3. Modular and Stereospecific Synthesis of Isoprenoid Alcohols

3.1 Introduction

The first major objective of this thesis is the establishment of a modular method for the synthesis of a variety of structurally related isoprenoid alcohols. In this way, desired isoprenoids can be easily synthesized by the linkage of different building blocks from a corresponding library. Since the target isoprenoids contain multiple double bonds the syntheses have to be performed in a stereospecific manner. Furthermore the method has to be fast and reliable to be widely applicable. Due to the tough requirements, the search for a suitable method turned out to be challenging.

3.2 Strategy

The idea is to build up the desired molecules from C_5 building blocks, following nature's example. **Fig. 4** shows the resulting retrosynthetic analysis of (*E*,*Z*)-farnesol. This C_{15} isoprenoid can be formed by a coupling reaction of a C_{10} and a C_5 isoprenoid whereas the C_{10} compound can be formed of two C_5 isoprenoids. Unfortunately the allylic position of the reactive groups turned out to be problematic. While there are a lot of different and well established reaction methods for the coupling of sp^2- and sp^3-hybridized carbons,[52-56] the number of sp^3/sp^3-cross-coupling reactions, that retain the configuration and position of the corresponding β,γ-double bonds, turned out to be limited.

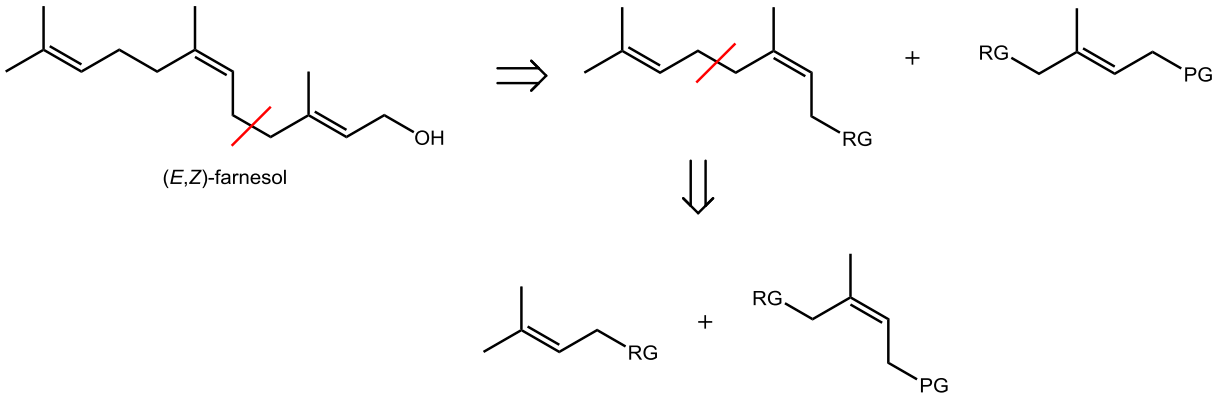


Fig. 4 – Retrosynthetic analysis of (*E*,*Z*)-farnesol. RG: reactive group, PG: protective group

Three selected publications served as basis for experiments investigating the stereospecificity of such reactions.[57-59] In a first attempt, following the procedure of Hall *et al.*, iron powder was used as catalyst in the coupling reaction of two allylic halides.[57] It represents an easy to handle method with good yields but a complete lack of

stereospecificity, proven by GC and NMR analysis of the obtained product mixtures. A promising procedure of Weix *et al.,* using nickel(II) complexes as catalyst in the coupling reaction of allylic acetates and allylic halides, did not lead to sufficient yields.[58] The first successful attempt based on a procedure published by Yamamoto *et al.* in 1991.[59] Using elemental barium as catalyst, it was possible to dimerize geranyl chloride in a stereospecific manner with a yield of 57 %. Unfortunately the reaction turned out to lack reliability and was therefore barely reproducible. Besides, it is not suitable for cross-coupling reactions of two different building blocks because both of the reactants are of the same type.

After these unsatisfactory results, the solution to this complex issue appeared in the form of a publication by J. F. Biellmann and J. B. Ducep.[60] They suggest a stereoselective cross-coupling reaction of an allylic phenyl sulfide and an allylic halide, initiated by the addition of *n*-butyllithium. This results in a deprotonation at the α-position of the corresponding allylic phenyl sulfide. The formed anion is highly stabilized by the phenyl sulfide, fixing it in the α-position. This prevents side reactions by making a mesomerization of the allylic anion (see **Fig. 5**) energetically unfavorable. In case of the methods described before, using iron, nickel and barium catalysts, most of these undesired side products could be identified by NMR analysis.

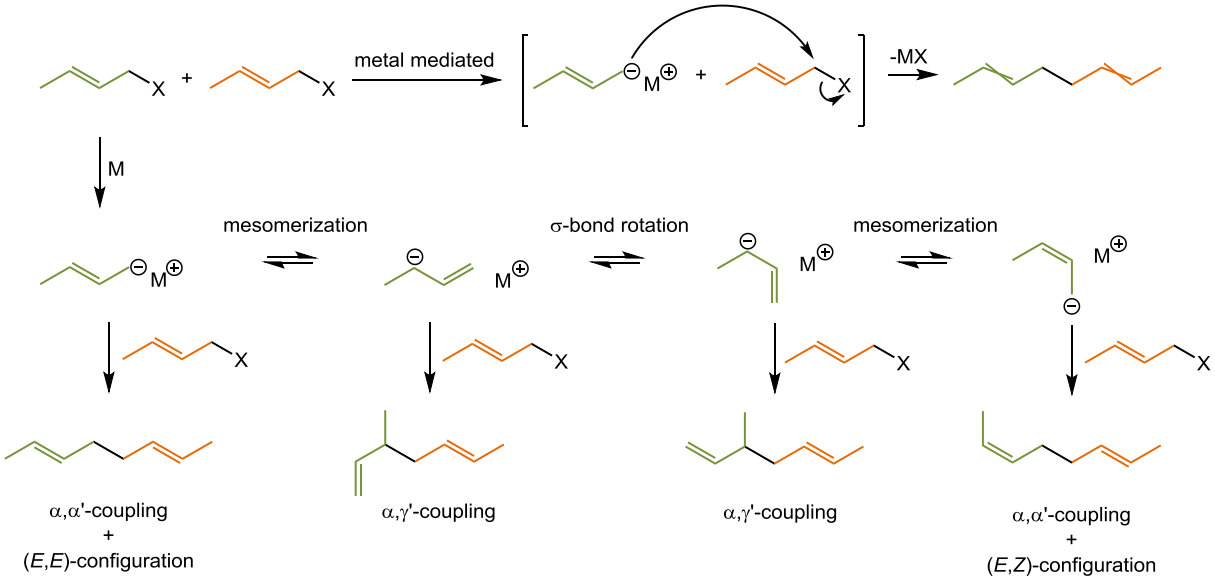


Fig. 5 – Illustration of possible side reactions using the example of a metal mediated homocoupling reaction of 2-butenyl chloride. top: general reaction scheme; bottom: possible side reactions and their resultant products

The product of the Biellmann-Ducep reaction is finally formed by an S_N2 reaction, started by the attack of the anion of the allylic phenyl sulfide at the α-carbon of the allylic halide. This

promising method was tested in the synthesis of (*E*,*Z*)-farnesol, following the retrosynthetic analysis (see **Fig. 4**). Due to the commercial availability of nerol, the synthesis step for the formation of this C_{10} isoprenoid could be skipped. In the first reaction step nerol was transferred into neryl phenyl sulfide, using a mixture of tri-*n*-butylphosphine and diphenyl disulfide. Subsequently the reaction product was coupled to an allylic halide in the mentioned Biellmann-Ducep cross-coupling reaction. The benzyl protective group as well as the thiophenyl moiety were finally cleaved under Birch conditions, yielding the desired (*E*,*Z*)-farnesol.

The following chapters contain detailed information about the used methods including reaction mechanisms and yields of the resulting products. The used reaction conditions were based on an advancement of the originally described procedure, published by Nakatani *et al.* in 2007.[61] This implies the use of the controversial hexamethylphosphoramide (HMPA). On the one hand it has the ability to break up *n*-butyllithium clusters, resulting in a much higher activity of this reagent. On the other hand it has been shown to cause nasal cancer in rats.[62] Although the nose epithelium of rats is known to be extremely sensitive to carcinogenic substances, the compound must be handled with particular care. Remaining quantities of hexamethylphosphoramide should be properly destroyed before disposal. The hydrolysis to less critical dimethylamine and phosphoric acid can be achieved by refluxing it in concentrated hydrochloric acid.[63]

3.3 Modular Three Step Elongation of Allylic Alcohols

3.3.1 Activation Step – Synthesis of Allylic Phenyl Sulfides

The synthesis of an allylic phenyl sulfide can be seen as activation step, transferring an isoprenoid alcohol into a form that is able to react specifically in the following cross-coupling step. The use of 1.1 – 1.5 equivalents of a mixture of tri-*n*-butylphosphine and diphenyl disulfide in THF at room temperature results in an Appel reaction-like mechanism shown in **Fig. 6**. The attack of the phosphorus atom of tri-*n*-butylphosphine on one of the sulfur atoms results in the heterolytic fission of the disulfide bond. The formed thiophenolate anion is then deprotonating the allylic alcohol going along with its activation. The increased nucleophilicity of the alkoxide allows an attack on the positively charged phosphorus atom releasing the other thiophenolate moiety. In the final step, this thiolate forms the desired allylic phenyl sulfide in a S_N2 reaction with the phosphonium intermediate. The formation of

the phosphorous-oxygen double bond of the side product tri-*n*-butylphosphine oxide represents the driving force of such a reaction.

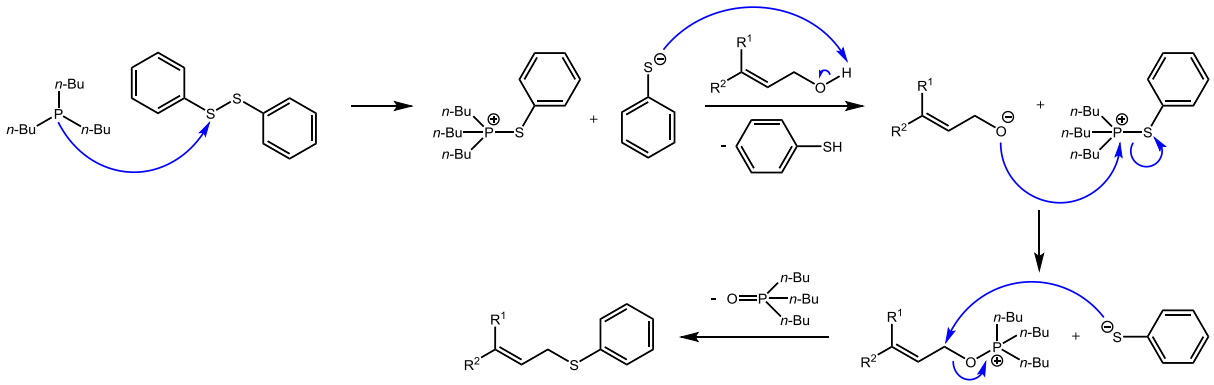
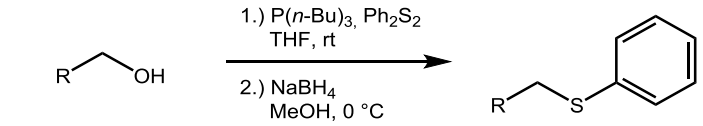


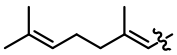
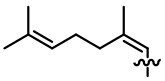
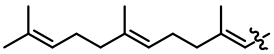
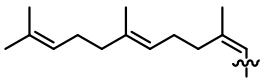
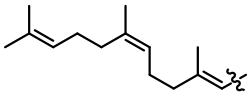
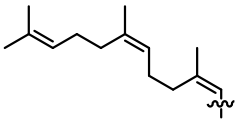
Fig. 6 – Synthesis of allylic phenyl sulfides, following an Appel reaction-like mechanism.

Unreacted diphenyl disulfide is removed by the addition of an equivalent of sodium borohydride at 0 °C reducing it to thiophenol, another side product of the previous reaction. Prior to this reduction the solvent is replaced by methanol, resulting in a higher reaction rate compared to the use of THF. A small quantity of added 2 M sodium hydroxide solution can increase the stability of the sodium borohydride within the eight hours reaction time. The progress can be followed by the color of the reaction mixture. It will change from yellow to colorless, making it easy to determine the completion of the transformation. Afterwards 2 M sodium hydroxide solution is added, leading to a conversion of thiophenol to water soluble sodium thiophenolate. The subsequent extraction with *n*-hexane allows the selective extraction of the allylic sulfide and tri-*n*-butylphosphine oxide. This is finally removed by column chromatography on silica, using a mixture of *n*-hexane and ethyl acetate (20:1) as a mobile phase. Most of the products were obtained as colorless oils. However, small impurities of remaining diphenyl disulfide can result in a pale yellow color. As these impurities will not cause problems during the next step, they can be ignored without any concerns. Noteworthy are the relatively high melting points of the products. All compounds with an alkyl chain of at least ten carbon atoms turn into white solids when cooled to -30 °C. The yields showed to be good to excellent with a range from 79 to 95 %. **Tab. 2** contains all of the compounds synthesized by this method.

Tab. 2 – Synthesized phenyl sulfides including numbering, molecular weights and yields.



prenol, **4b, 5b, 8b-11b** → **1e, 4e, 5e, 8e-11e**

R	number (educt)	number (product)	M_w (g mol^{-1})	yield (%)
	prenol	**1e**	178.29	85
	**4b**	**4e**	246.41	93
	**5b**	**5e**	246.41	87
	**8b**	**8e**	314.53	91
	**9b**	**9e**	314.53	94
	**10b**	**10e**	314.53	95
	**11b**	**11e**	314.53	79

3.3.2 Cross-Coupling Step – The Biellmann-Ducep Reaction

The cross-coupling reaction represents the key step in the presented synthesis of isoprenoid alcohols. Due to the extremely poor separability of corresponding stereoisomers it needs to be highly stereoselective to overcome this problem. To investigate this feature, neryl phenyl sulfide (**5e**) was coupled to an (*E*)-configured C5-building block (**25**). The reaction mechanism is shown in **Fig. 7**. The addition of *n*-butyllithium leads to a deprotonation in α-position. The formed carbanion is stabilized by the neighboring sulfur atom, preventing a mesomerization to a γ-carbanion. The nature of this stabilization and the acidifying effect on hydrogen atoms in α-position to sulfur atoms, respectively, was topic of research for many years. Numerous effects have been discussed, for example an electron acceptor conjugation of the sulfur with the α-carbanion.[64,65] More recent publications are supporting another major influence, the strong polarizability effect of sulfur.[66,67]

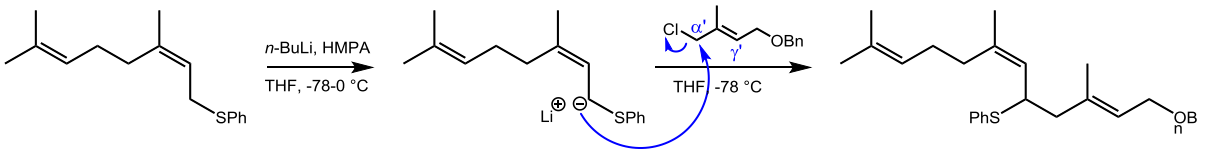


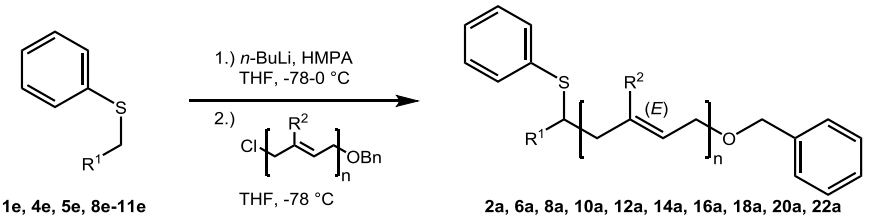
Fig. 7 – Mechanism of the stereospecific cross-coupling reaction of an allylic phenyl sulfide to an allylic choride initiated by the addition of *n*-BuLi and HMPA

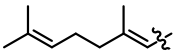
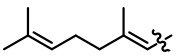
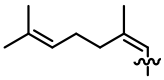
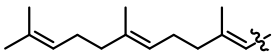
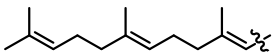
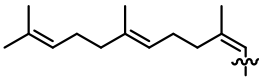
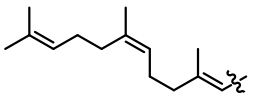
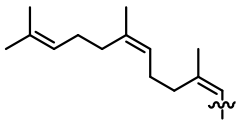
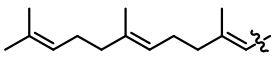
The carbanion in α-position attacks the corresponding halide building block at the α'-position. The resulting S_N2 reaction leads to a α,α'-coupling without isomerization of the adjacent double bonds. Products of an attack on the γ'-position of the halide building block could not be detected. The stereo- and regiospecificity could be proven by NMR analysis of the resultant products. In all cases only one single stereo- and regioisomer could be detected.

Several color changes during the performance of this reaction represent another helpful advantage. The colorless solution of the allylic phenyl sulfide in THF gets yellow by addition of *n*-butyllithium. This displays the characteristic color of sulfur stabilized carbanions. While *n*-butyllithium exists as tetramers and dimers in THF, added HMPA breaks up these clusters resulting in a higher activity of the organolithium reagent.[68] This shifts the equilibrium to the deprotonated form of the starting material, giving rise to another color change to red. Even at -78 °C the reaction rate is so fast that the reaction is almost behaving like a titration. Therefore the red color is changing back to yellow upon addition of exactly one equivalent of the corresponding halide building block. This is quite helpful for recognizing the endpoint of the reaction and avoids the addition of too much or too little of the allylic halide. As a result, the subsequent purification gets easier because the starting material as well as the halide building block show a similar polarity compared to the desired product, making it hard to remove these impurities by column chromatography. Upon addition of saturated ammonium chloride solution during workup, the reaction mixture gets colorless again.

All coupled products were obtained as colorless oils. Their solubility in methanol decreases with increasing chain length. For this reason, a few drops of ethyl acetate had to be added to dissolve some of the products in methanol in order to perform the required ESI-MS analyses. With a range of 73 to 94 % the yield of the coupling reactions to (*E*)-configured building blocks was good to excellent in all cases. All of the performed reactions are summarized in **Tab. 3**. Cross-coupling reactions to the corresponding (*Z*)-configured building blocks showed similar yields ranging from 75 to 92 %. These reactions are outlined in **Tab. 4**.

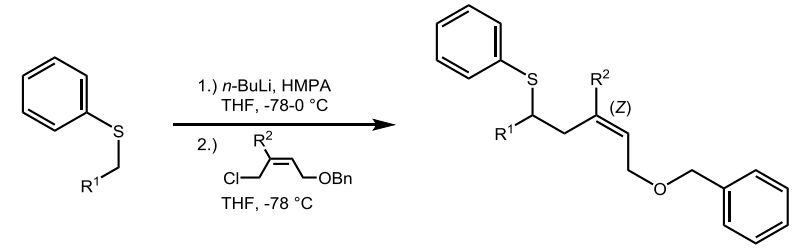
Tab. 3 – Synthesized (2*E*)-configured coupling products including numbering, molecular weights and yields.



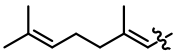
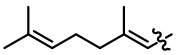
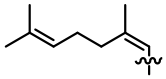
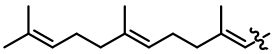
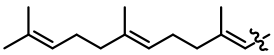
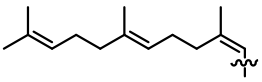
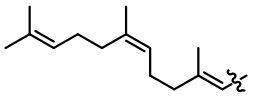
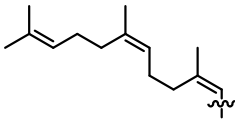
R^1	R^2	n	number (educt)	number (product)	M_w (g mol^{-1})	yield (%)
	H	1	**1e**	**2a**	338.51	73
	H	1	**4e**	**6a**	406.63	94
	Me	1	**4e**	**8a**	420.66	86
	Me	1	**5e**	**10a**	420.66	83
	H	1	**8e**	**12a**	474.75	87
	Me	1	**8e**	**14a**	488.77	88
	Me	1	**9e**	**16a**	488.77	85
	Me	1	**10e**	**18a**	488.77	78
	Me	1	**11e**	**20a**	488.77	82
	Me	2	**8e**	**22a**	556.89	76

The achieved results underline the good reliability of the used method. It shows to be independent of the double bond configuration as well as the chain length of the involved building blocks. To investigate the possibility to use longer-chained halide building blocks under these reactions conditions, (*E*,*E*)-farnesyl phenyl sulfide (**8e**) was coupled to an allylic halide featuring two prenyl units (**28**), yielding the (*E*,*E*,*E*,*E*)-geranylfarnesyl intermediate (**22a**) in a yield of 76 %. This is in compliance with the yields using halides with only one prenyl unit.

Tab. 4 – Synthesized (2*Z*)-configured coupling products including numbering, molecular weights and yields.



1e, 4e, 5e, 8e-11e — 3a, 7a, 9a, 11a, 13a, 15a, 17a, 19a, 21a

R^1	R^2	number (educt)	number (product)	M_w (g mol^{-1})	yield (%)
	H	**1e**	**3a**	338.51	80
	H	**4e**	**7a**	406.63	79
	Me	**4e**	**9a**	420.66	84
	Me	**5e**	**11a**	420.66	92
	H	**8e**	**13a**	474.75	81
	Me	**8e**	**15a**	488.77	89
	Me	**9e**	**17a**	488.77	82
	Me	**10e**	**19a**	488.77	75
	Me	**11e**	**21a**	488.77	82

As a result, the Biellmann-Ducep reaction showed to be a powerful and reliable method for the cross-coupling of two allylic building blocks. On the one hand an allylic phenyl sulfide as starter unit and on the other hand an allylic chloride serving as elongation unit. All performed reactions lead stereospecifically to one single stereoisomer. No products of any double bond isomerization process could be detected. Therefore it proves to be the method of choice for the establishment of the intended fast and modular synthesis method for isoprenoid alcohols.

3.3.3 Reduction Step – Synthesis of Allylic Alcohols under Birch Conditions

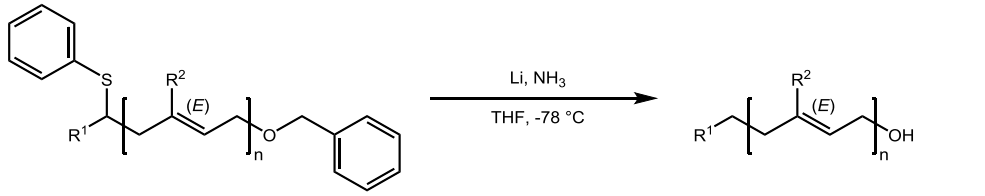
In the Biellmann-Ducep reaction described in chapter 3.3.2 benzyl protected chain elongation building blocks are used, leading to the formation of benzyl protected isoprenoid alcohols. Moreover the products feature a phenylthio moiety originating in the use of phenyl sulfides as starter units. Both of these functional groups have to be removed to produce the corresponding free isoprenoid alcohols. The original publications of Biellmann and Ducep mention the possibility to remove the phenylthio group under Birch conditions.[69] Also other workgroups adopted the method to remove this moiety subsequent to Biellmann-Ducep like cross-coupling procedures.[61,70,71] Fortunately benzyl groups are also known to be cleaved under these conditions.[72,73]

Hence both of the unwanted functional groups can be cleaved at the same time in one single reaction step using a mixture of lithium and ethylamine. Mixing these (dry) reactants results in a blue color, arising from the presence of free electrons. This can be seen as a consequence of the dissociation of elemental lithium to lithium cations and single electrons. The reaction mechanism of a Birch reduction is quite complicated due to the occurrence of a lot of different reaction paths initiated by single electron transfers to the phenyl rings of the starting material. Due to its highly speculative nature the mechanism is not explained in further detail.

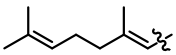
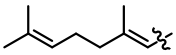
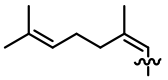
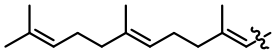
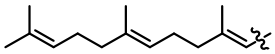
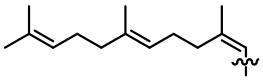
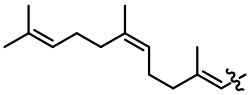
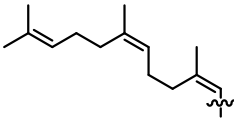
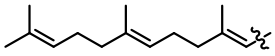
Because of the easier handling, ethylamine was replaced with gaseous ammonia in this work. This was first condensed using an acetone/dry ice bath and then mixed with ten equivalents of elemental lithium. During the whole reaction time the cooling bath was left in place to avoid unwanted evaporation of excess ammonia. In a first try, no conversion of the starting material (**10a**) could be detected. The addition of a small amount of THF solved the problem, identifying it as solubility issue. So it appeared that the extremely hydrophobic products of the cross-coupling steps are completely insoluble in liquid ammonia. THF acts as solubilizer allowing a complete conversion of the starting material within minutes. The end of the reaction is indicated by a persisting deep blue color of the reaction mixture. By the addition of saturated ammonium chloride solution the mixture turns colorless again and residual lithium is decomposed within minutes. Extraction of the reaction mixture with diethyl ether and subsequent evaporation of the solvent leads to a colorless and oily residue that can be purified by column chromatography on silica using a mixture of hexane and ethyl acetate as a mobile phase. The corresponding solvent ratio can be easily adjusted to the

polarity of the achieved alcohols. All of them were obtained as colorless oils in yields ranging from moderate 54 % to good 90 %. While the syntheses of 2-(*E*)-configured alcohols are summarized in **Tab. 5** the reactions yielding 2-(*Z*)-configured alcohols are shown in **Tab. 6**.

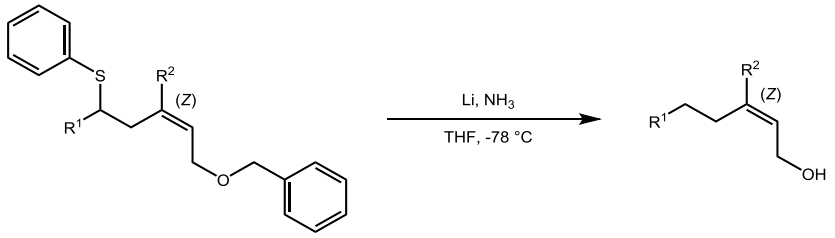
Tab. 5 – Synthesized (2*E*)-configured isoprenoid alcohols including numbering, molecular weights and yields.



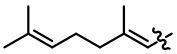
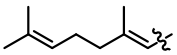
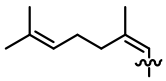
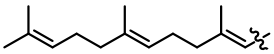
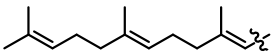
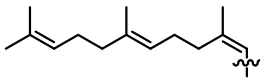
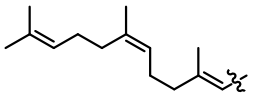
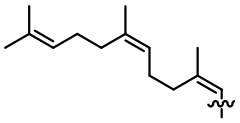
2a, 6a, 8a,10a,12a, 14a, 16a, 18a, 20a, 22a **2b, 6b, 8b,10b,12b, 14b, 16b, 18b, 20b, 22b**

R^1	R^2	n	**number** (educt)	**number** (product)	M_w (g mol^{-1})	**yield** (%)
	H	1	**2a**	**2b**	140.23	90
	H	1	**6a**	**6b**	208.35	73
	Me	1	**8a**	**8b**	222.37	68
	Me	1	**10a**	**10b**	222.37	82
	H	1	**12a**	**12b**	276.46	76
	Me	1	**14a**	**14b**	290.49	69
	Me	1	**16a**	**16b**	290.49	57
	Me	1	**18a**	**18b**	290.49	69
	Me	1	**20a**	**20b**	290.49	72
	Me	2	**22a**	**22b**	358.61	78

Tab. 6 – Synthesized (2*Z*)-configured isoprenoid alcohols including numbering, molecular weights and yields.



3a, 7a, 9a, 11a, 13a, 15a, 17a, 19a, 21a — 3b, 7b, 9b, 11b, 13b, 15b, 17b, 19b, 21b

R^1	R^2	number (educt)	number (product)	M_w (g mol^{-1})	yield (%)
	H	**3a**	**3b**	140.23	65
	H	**7a**	**7b**	208.35	54
	Me	**9a**	**9b**	222.37	86
	Me	**11a**	**11b**	222.37	70
	H	**13a**	**13b**	276.46	68
	Me	**15a**	**15b**	290.49	59
	Me	**17a**	**17b**	290.49	69
	Me	**19a**	**19b**	290.49	72
	Me	**21a**	**21b**	290.49	68

The stereospecificity of the reaction was determined by NMR analysis. All products show a complete retention of the double bond configuration present in the corresponding starting material. **Fig. 8** contains exemplary proton and carbon spectra of **10b** recorded in $CDCl_3$, proving that the product consists of only one single stereoisomer. All chemical shifts are in good compliance with literature data.[74].

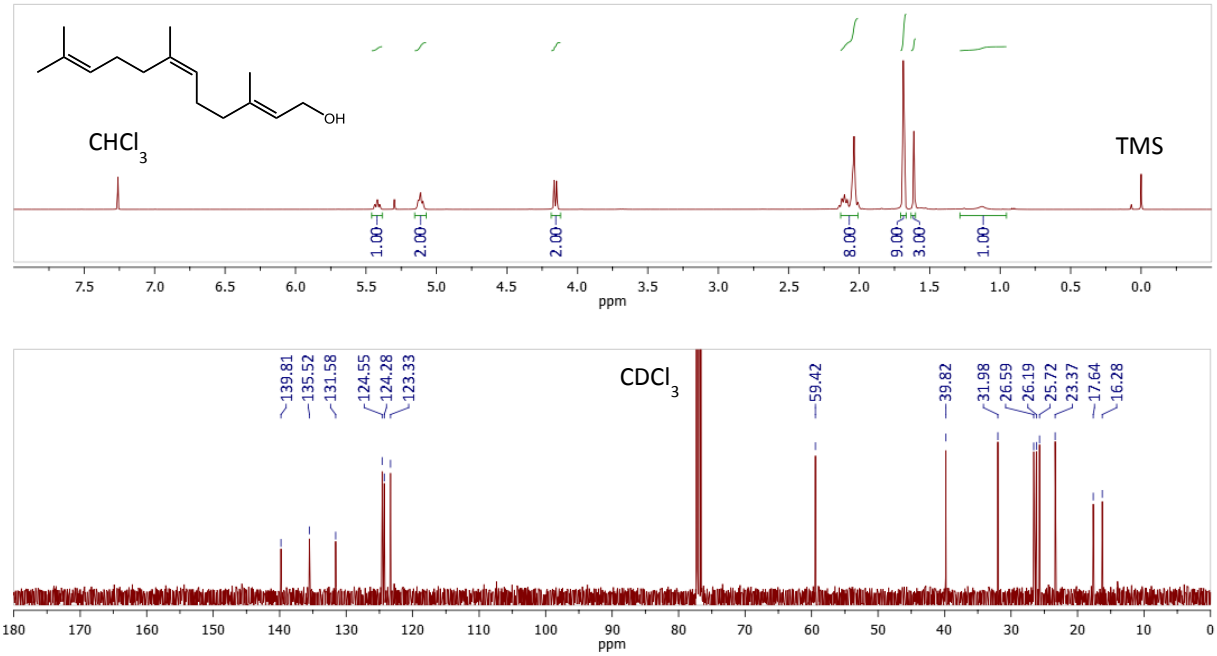


Fig. 8 – Proton (top) and carbon (bottom) NMR spectra of (*E*,*Z*)-farnesol **(10b)** recorded in $CDCl_3$

3.4 Iterative Synthesis of Isoprenoid Alcohols

In the previous chapters it could be shown that the Biellmann-Ducep reaction is suitable for the stereospecific cross coupling of allylic prenyl building blocks. Combined with a previous activation step and a subsequent reduction step it allows the conversion of allylic alcohols to the corresponding elongated allylic alcohols over three steps. Due to the use of bifunctional chain elongation building blocks, the product of this elongation process is of the same kind as the starting material and can therefore deal as basis for another chain elongation. This enables an iterative process, allowing the fast step by step synthesis of complex molecules from the desired building blocks based on commercially available substances. **Fig. 9** summarizes the performed syntheses of all farnesol and geranylgeraniol double bond isomers as well as geranylfarnesol using the described method.

All substances have been synthesized from the commercially available geraniol and nerol as well as from three different C_5/C_{10} building blocks in (*E*)- and (*Z*)-configuration (**25**/**28**/**35**), respectively. The yields over three steps from alcohol to alcohol ranged from 44 % to 67 %, while the yields over 6 steps ranged from 22 % to 36 %. These small variations underline the good reliability and reproducibility of the presented iterative synthesis method. All target alcohols were obtained as pure single isomers in quantities up to 6.36 g in case of compound **9b**.

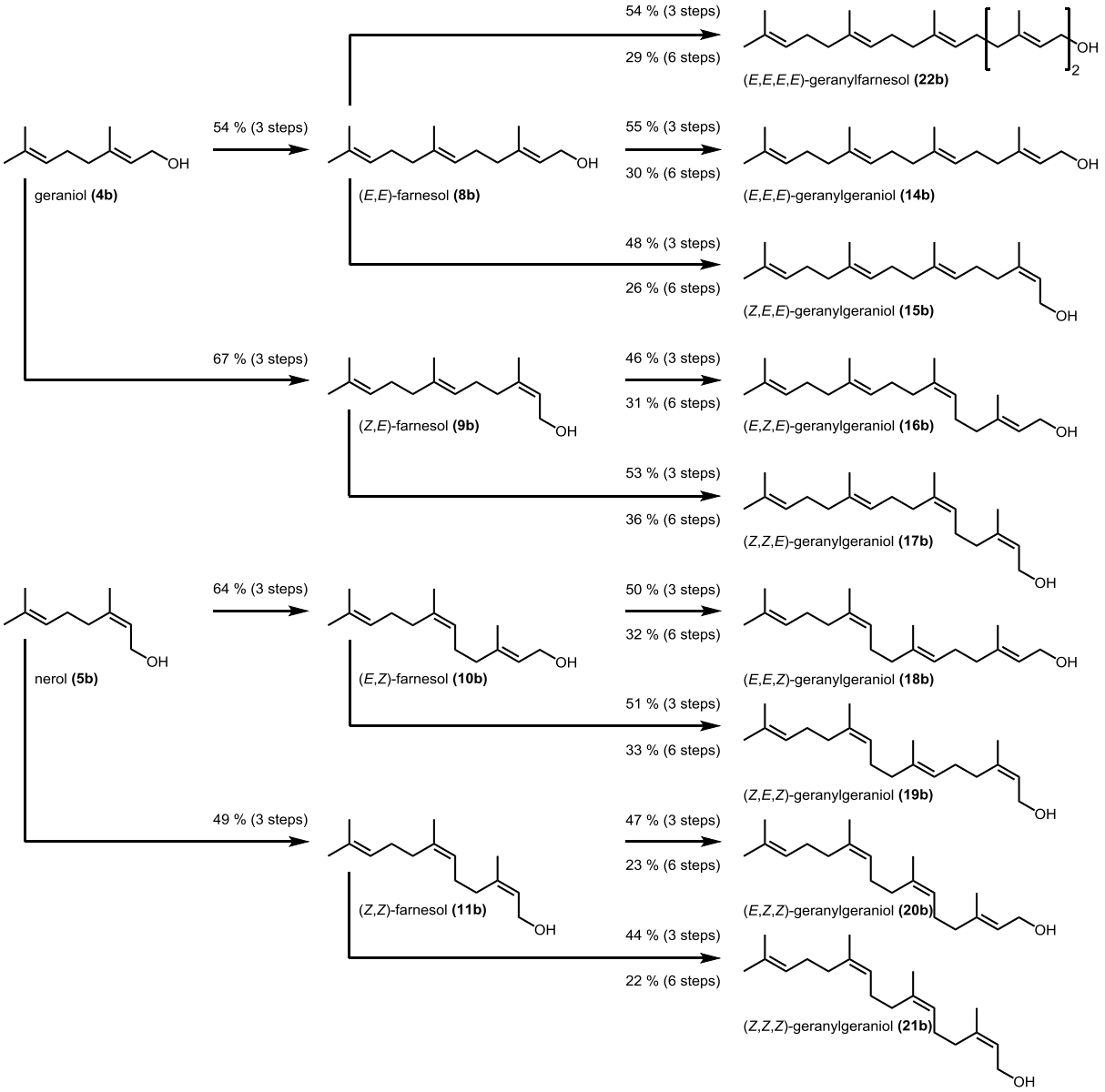


Fig. 9 – Synthesis of all farnesol and geranylgeraniol double bond isomers as well as geranylfarnesol using the established method. Yields are given for the three step reactions from alcohol to alcohol and for the total yield over six steps in case of the geranylgeraniol and geranylfarnesol derivatives.

In addition to these double bond isomers, alcohols lacking the methyl group at position 3 have been synthesized. The syntheses were carried out using corresponding (*E*)- and (*Z*)-configured C_4 building blocks (**38**/**40**). The yields over 3 steps, ranging from 40 to 64 %, are in good compliance with the elongations using C_5 building blocks. **Fig. 10** illustrates the synthesis of six different 3-norisoprenoid alcohols.

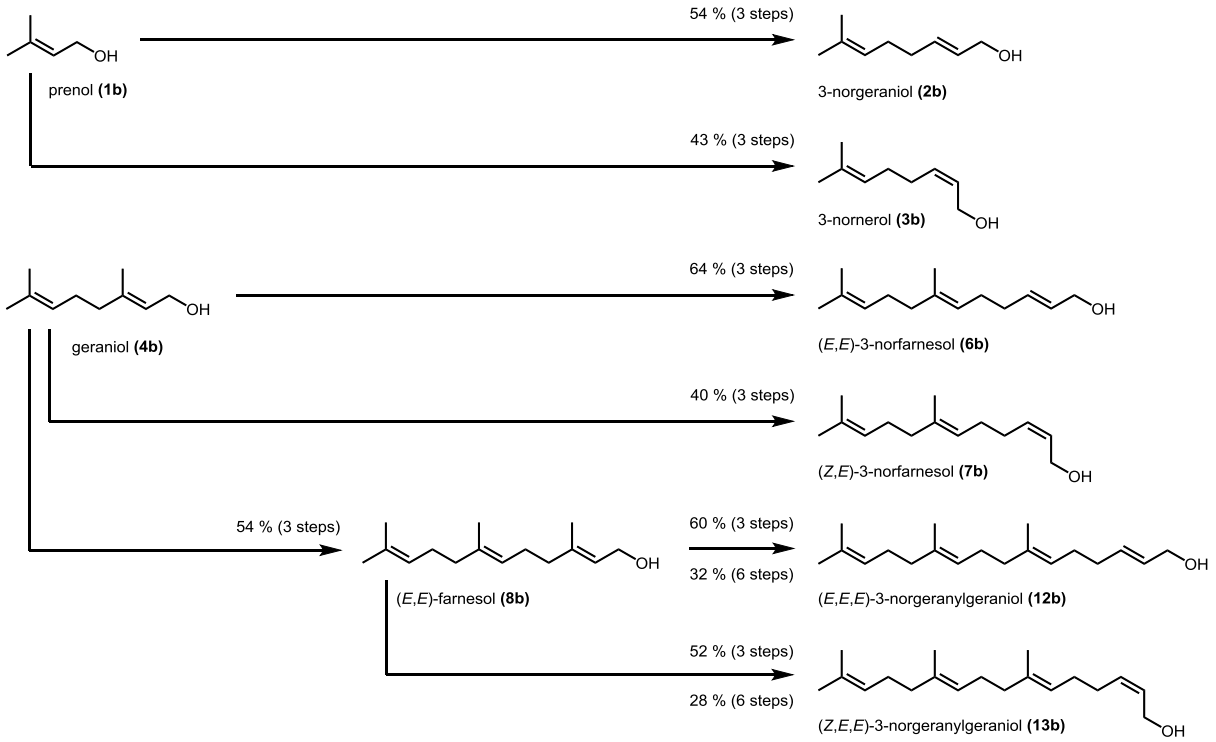


Fig. 10 – Synthesis of isoprenoid alcohols lacking a methyl group at position 3 using the established method. Yields are given for the three step reactions from alcohol to alcohol and for the total yield over six steps in case of the geranylgeraniol derivatives.

3.5 Synthesis of Allylic Halide Building Blocks for Chain Elongation

Beside the allylic phenyl sulfide an allylic halide is needed in the Biellmann-Ducep cross-coupling reaction. To expand the space of possible products a variety of different chain elongation building blocks is desirable. For this reason five different allylic chlorides have been synthesized. The first examples represent (*E*)-configured C_5- and C_{10}-building blocks, respectively. Initially more carbon atoms are coupled to the phenyl sulfide because of the attached benzyl group. However, this group is cleaved in the following step resulting in an elongation of the starter unit by 5 or 10 carbon atoms. **Fig. 11** shows the related syntheses.

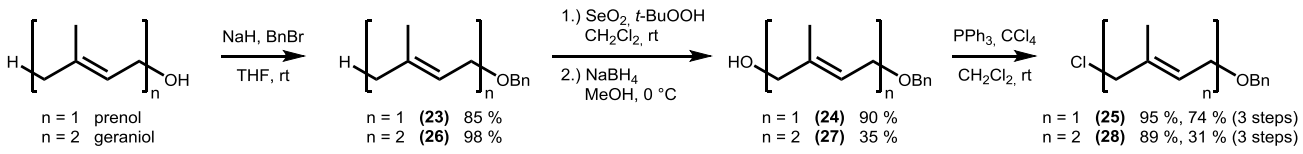


Fig. 11 – Syntheses of the (*E*)-configured C5- and C10-building blocks (**25**/**28**).

In a first step the benzyl group was introduced to the alcohol (prenol/geraniol) serving as starting material. The addition of sodium hydride causes a deprotonation of the hydroxyl group. The resulting alkoxide attacks the α-position of benzyl bromide, forming the benzyl

ether (**23**/**26**) in an S_N2 reaction.[75] The terminal (*E*)-methyl group of the product is then oxidized using a mixture of selenium dioxide and *tert*-butyl hydroperoxide in a Riley oxidation.[76] Unfortunately, the method results in a partial overoxidation to the corresponding aldehyde. A subsequent reduction using sodium borohydride in methanol converts the aldehyde back to the desired alcohol (**24**/**27**). In a last step, the allylic chloride (**25**/**28**) is formed in an Appel reaction making use of a mixture of triphenylphosphine and tetrachloromethane.[77] This method was chosen because of its product specificity. Compared to many other halogenation methods it doesn't lead to the formation of linalyl like isomers as side product.[78]

The synthesis of the corresponding (*Z*)-configured C_5-building block showed to be more challenging. The route for the compounds **25** and **28** could not be used because the Riley-oxidation is primarily resulting in a hydroxylation of the methyl group in (*E*)-position. Another way to form a (*Z*)-configured and methylated double bond is the stereospecific addition of a methyl group to an alkyne function. Around this key step, a reaction route (see **Fig. 12**) for the synthesis of the building block could be established. Four crucial steps are based on a publication by Jirgensons *et al.* from 2011.[79]

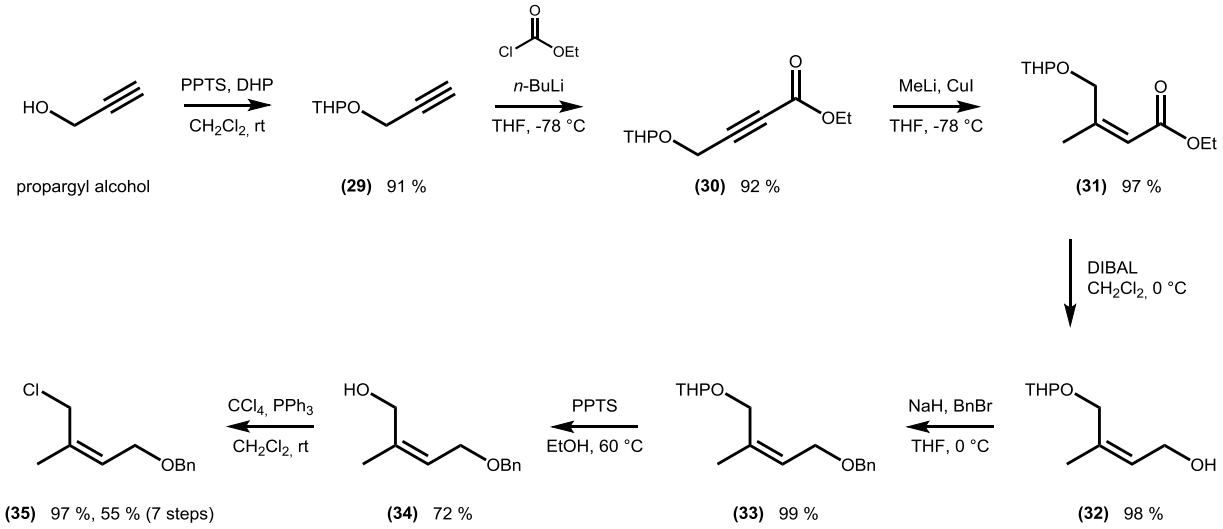


Fig. 12 – Synthesis of the (*Z*)-configured C_5-building block **(35)** starting from propargyl alcohol.

In a first step, propargyl alcohol was THP-protected using pyridinium *p*-toluenesulfonate and 3,4-dihydropyrane.[80] The resulting product (**29**) was then deprotonated with *n*-butyllithium. The subsequent addition of ethyl chloroformate yielded the desired ester (**30**). A Gilman cuprate, formed by the mixture of methyllithium and copper(I)-iodide in THF, allowed the

regio- and stereoselective addition of a methyl group in β-position to the carbonyl function resulting in the desired (*Z*)-configuration of the double bond (**31**). An allylic alcohol (**32**) was obtained in a reduction of the carbonyl group with diisobutylaluminium hydride.[79] The product was then transformed into a benzyl ether (**33**) using sodium hydride and benzyl bromide in THF.[75] Subsequent acid catalyzed deprotection of the THP group gave another allylic alcohol (**34**) which was then halogenated in an Appel reaction to the desired chloride (**35**).[77,79] Most of the yields in these reactions showed to be surprisingly good, resulting in excellent 55 % over seven steps.

The synthesis of an (*E*)-configured C_4 building block, lacking the methyl group compared to compound **25** turned out to be less time consuming. **Fig. 13** shows the synthesis, starting from the commercially available 1,4-but-2-ynediol.

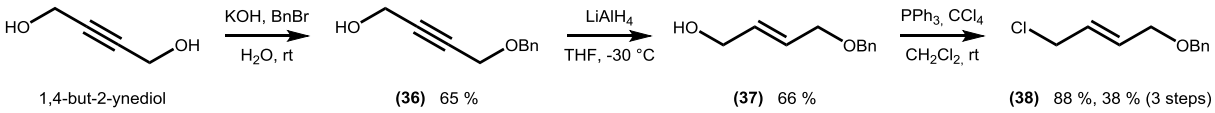


Fig. 13 – Synthesis of an (*E*)-configured C4-building block starting from 1,4-butynediol.

Originally, the starting material was deprotonated followed by addition on benzyl bromide. Due to the higher acidity of the hydroxyl groups, compared to a corresponding alkene, potassium hydroxide is a sufficient base in this reaction.[81] The formed benzyl ether (**36**) was then stereospecifically hydrogenated to the (*E*)-configured allylic alcohol (**37**) using lithium aluminium hydride.[82] Final halogenation via an Appel reaction yielded the desired (*E*)-configured C_4 building block (**38**) in a yield of 38 % over three steps.[77]

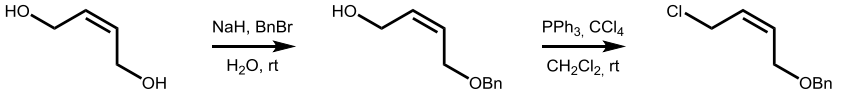


Fig. 14 – Synthesis of a (*Z*)-configured C_4-building block starting from 1,4-but-2-enediol.

Due to the commercial availability of (*Z*)-1,4-but-2-enediol the (*Z*)-configured C_4-building block could be obtained in a short two step synthesis shown in **Fig. 14**. (*Z*)-1,4-But-2-enediol was transformed into a benzyl ether (**39**), using sodium hydride and benzyl bromide.[75] The final halogenation led to the desired (*Z*)-configured C4 building block (**40**) in a yield of 74 % over two steps.[77]

3.6 Competitive Methods

A method published by Gibbs *et al.* in 1999 can be seen as direct competitor of the method established in the course of this thesis.[83,84] In these reactions an allylic alcohol is transformed into the corresponding bromide which is then converted to a β-ketoester via the addition of a lithium/sodium dienolate. The subsequent addition of a strong base forms the enolate of the ß-ketone. By simultaneous triflation the molecule can be trapped in this enolate state. The configuration of the resulting α,β-double bond is determined by the solvent used in this key step. While the use of THF leads to the formation of an (*E*)-configured double bond the replacement with DMF results in a (*Z*)-configuration. Unfortunately the reaction is not completely stereospecific in the former case, which can lead to severe problems in the purification of the desired isomer. In addition, two more steps are necessary to obtain the target compound resulting in a total number of five steps from educt alcohol to product alcohol. Combined with overall lower yields this method is inferior in all single aspects.

Three years later Corey *et al.* suggested a method based on the cross-coupling of isoprene acetals with organosilanes or organostannanes.[85,86] The former deal as starting units and can be synthesized from the corresponding alcohols in two steps whereas the coupling reaction itself is catalyzed by boron trifluoride. The remaining side chains and protective groups are then removed in two further steps. Featuring better yields the method shows to be superior to the one reported by Gibbs. On the other hand it still takes five steps to obtain the elongated alcohol.

In the same year Negishi *et al.* came out with another procedure based on the well-known cross-coupling reaction named after him.[87] However, since a vast number of steps are necessary to build up the desired isoprenoid alcohols using this method, it is mentioned rather for the sake of completeness.

A more recent procedure reported by Cheng *et al.* in 2008 is also taking advantage of the Biellmann-Ducep reaction in the cross-coupling step, solving the problem of stereospecificity.[88] At the same time the phenyl sulfide intermediate is unnecessarily replaced by the corresponding phenylsulfonyl compound synthesized in a two-step reaction from the initial alcohol. Instead of a benzyl protective group they use a *tert*-butyldiphenylsilyl group within the chain elongation unit. As the cleavage of this moiety requires different conditions compared to the cleavage of the phenylsulfonyl residue, one

more reaction step needs to be performed resulting again in a total number of five reactions steps from educt alcohol to product alcohol. In terms of stereospecificity and yields this method can compete with the one established as a part of this thesis. Since it comprises two more steps per chain elongation Chengs procedure lacks efficiency and shows to be more time-consuming and not least more costly with regard to the required reagents.

3.7 Further Applications

Besides the use as precursors in the synthesis of organic diphosphates the resulting alcohols can be used for several further purposes. On the one hand they can serve as starting material in subsequent chemical syntheses; on the other hand they can be used as analytical standards. In the course of this thesis both of these possibilities were combined to generate an appropriate liquid chromatography standard for the analysis of products resulting from biocatalytic conversion reactions catalyzed by prenyl transferases (not part of this thesis). In the corresponding experiments a geranyl diphosphate derivative featuring a fluorescent tag was elongated by a but-3-enyl unit resulting in the formation of a new (*Z*)-configured double bond (see **Fig. 15**). A subsequent dephosphorylation using an alkaline phosphatase yielded compound **44**.

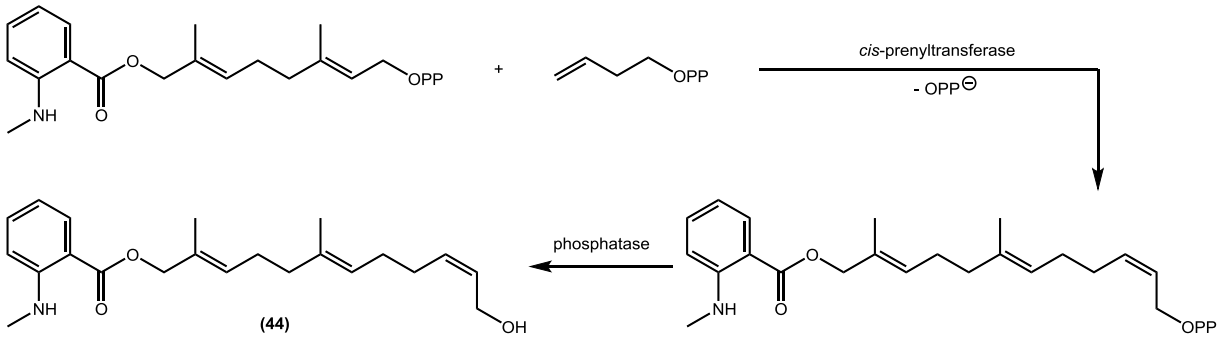


Fig. 15 – Biocatalytic synthesis of **44** in two steps. In the first step a tagged geranyl diphosphate derivative is elongated by but-3-enyl diphosphate in a reaction catalyzed by a cis-prenyltransferase. In a second step the diphosphate moiety is cleaved by a phosphatase yielding the corresponding alcohol **44**.

The product distribution is then analyzed by HPLC. To prove that the desired product shows the right double bond configuration a chemical standard is indispensable.

Therefore the compound **44** was chemically synthesized basing on the already described synthesis of (*Z*,*E*)-3-norfarnesol (**7b**). As shown in **Fig. 16** the starting alcohol was THP-protected using 3,4-dihydropyran and pyridinium *p*-toluenesulfonate. The terminal methyl group of the resulting ether (**41**) was then hydroxylated in a Riley oxidation. The

resulting alcohol (**42**) was esterified using *N*-methylisatoic anhydride forming the fluorescent ester (**43**). The removal of the protecting group yielded the desired alcohol (**44**) in a total yield of 4 % over seven steps. The supposed double bond configuration was proven NMR analysis.

Finally the presented method for the stereospecific synthesis of isoprenoid alcohols could be successfully applied for the generation of a valuable liquid chromatography standard.

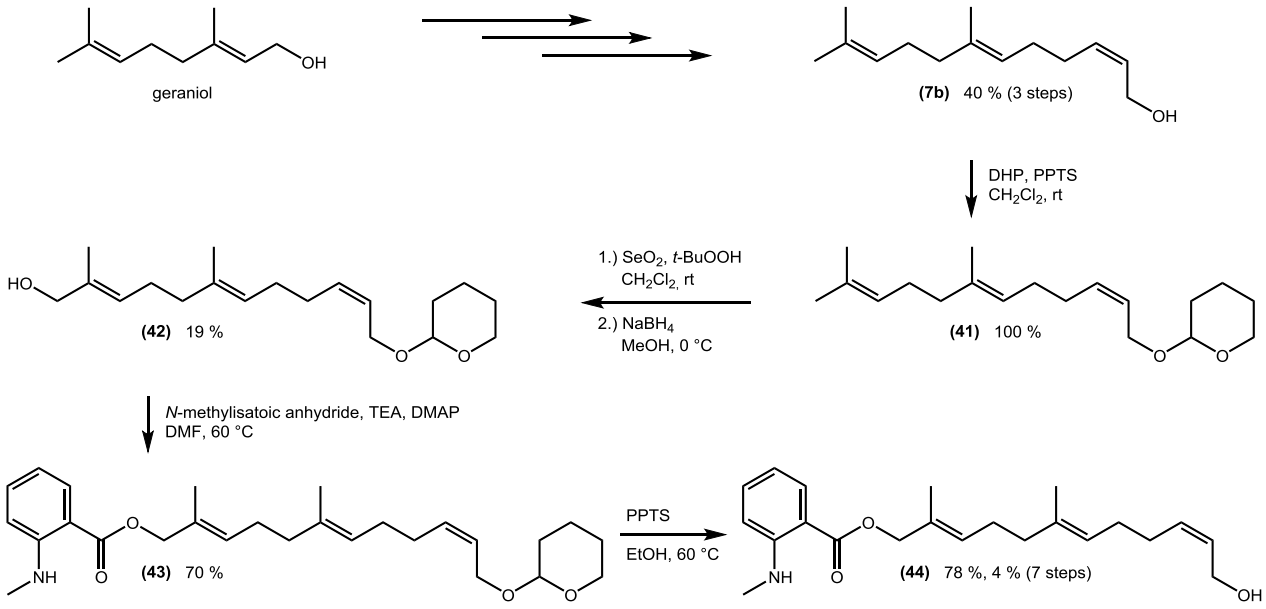


Fig. 16 – Chemical synthesis of **44** in three steps starting from (*Z*,*E*)-3-norfarnesol (**7b**).

3.8 Summary

Within the context of this work a modular method for the synthesis of allylic isoprenoid alcohols could be established. Its outstanding reliability and stereospecificity could be proven by NMR analysis. A cross-coupling method reported by Biellmann and Ducep in 1971 represents the key step of this method.[60] Combined with a previous activation step as well as a subsequent reduction step, allylic alcohols can be elongated to more complex allylic alcohols in only three steps. The use of bifunctional building blocks for chain elongation allows an iterative process resulting in an extremely fast and easy synthesis of a large variety of different products. Starting from the commercially available alcohols prenol, geraniol and nerol, 19 different allylic isoprenoid alcohols have been synthesized using only five different chain elongation building blocks whereas twelve of these alcohols have been formed by the use of only two elongation units.

Finally, the presented method is proven to be superior to all similar methods described in

literature. As a result it represents the new gold standard for the modular and stereospecific synthesis of isoprenoid alcohols. In a future perspective the scope of possible products can be expanded easily by the synthesis of new chain elongation building blocks as well as the use of different starter units.

4. Synthesis of Isoprenoid Diphosphates

4.1 Introduction

In 1958 the synthesis of organic diphosphates was suddenly arousing interest among the scientific community due to the discovery that isopentenyl diphosphate (IDP) and farnesyl diphosphate (FDP) represent important intermediates in the biosynthesis of isoprenoids like squalene.[18,19] As a result, several synthetic methods were developed within the following years. The resulting availability of radiolabeled allylic and homoallylic diphosphates paved the way for the discovery of several biosynthetic pathways. Despite small improvements, the method of Cramer *et al.* published in 1959 turned out to serve as standard for more than twenty years.[89] In this reaction, two phosphate units were sequentially linked to the parent alcohol after activation via trichloroacetonitrile. Unfortunately the method is not specific towards the synthesis of organic diphosphates, resulting in the formation of corresponding mono- and oligophosphates as side products. A solution to this problem was published by Dixit *et al.* in 1981.[90] In contrast to the electrophilic introduction of two single phosphate units they described the specific and nucleophilic introduction of a diphosphate unit starting from allylic halides and tetra-*n*-butylammonium salts. Both methods have in common that the crude product has to be subjected to a final cation exchange chromatography to obtain the desired trisammonium salt which can then be used in a large variety of biological assays. In 2002 Dessoy *et al.* developed a new diphosphorylation method in the Wessjohann lab.[91,92] Here the diphosphate group is also introduced as one single unit in a nucleophilic substitution reaction. The advancement is represented by the used diphosphorylation agent, a fully TMS-protected diphosphate moiety. In course of the reaction only one single position gets deprotected resulting in the formation of a suitable nucleophile that can attack an allylic halide or homoallylic tosylate. The remaining protective groups of the organic diphosphate intermediate are cleaved by addition of concentrated ammonia solution during workup. As a side effect, the product is automatically transformed into the desired trisammonium salt making a cation exchange redundant. Hence, this state-of-the-art method was used in all diphosphorylation reactions covered by this thesis.

4.2 Allylic Isoprenoid Diphosphates

4.2.1 Synthesis of Allylic Bromides

Many different methods for the conversion of allylic alcohols to the corresponding bromides have been published.[93] Unfortunately, some of them cause an isomerization leading to linalyl like bromides as side product (see **Fig. 17**). Affected is for instance the conversion by hydrogen bromide and phosphorus tribromide whereas the reaction under Appel conditions leads to a specific transformation to the desired product.[78]

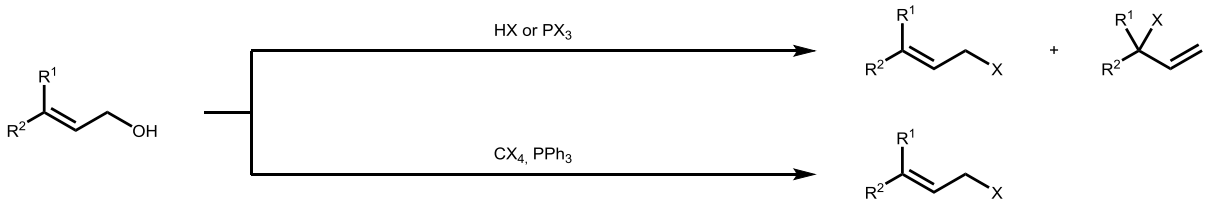


Fig. 17 – Synthesis of allylic bromides. While the conversion by HX or PX_3 leads to linalyl like side products, the conversion under Appel conditions avoids isomerization. X = Cl, Br, I; R^1, R^2 = H, alkyl.

Therefore the Appel reaction became the method of choice for the bromination of allylic alcohols.[94] In order to perform the reaction the alcohol was dissolved in dichloromethane followed by the addition of tetrabromomethane. Afterwards the reaction mixture was cooled to 0 °C followed by the slow and careful addition of triphenylphosphine. Even at this temperature the reaction is done within minutes. That's why fast addition of triphenylphosphine in larger scale reactions can result in boiling of the solvent regardless of the used cooling.

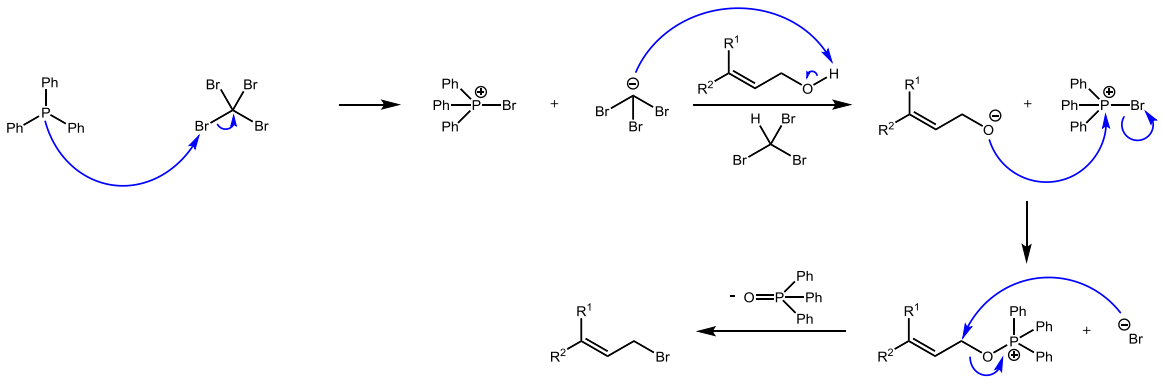


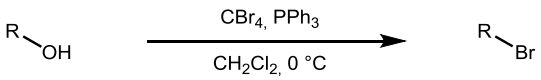
Fig. 18 – Mechanism for the synthesis of allylic bromides from allylic alcohols under Appel conditions.

According to the mechanism shown in **Fig. 18** the formation of the strong phosphorous-oxygen double bound is the driving force in this reaction. As a result, triphenylphosphine oxide represents a major side product. Due to its low solubility in *n*-hexane it can be

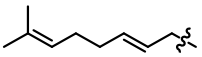
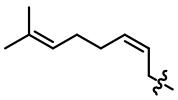
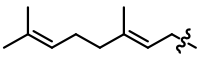
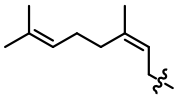
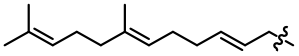
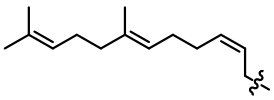
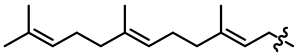
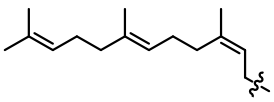
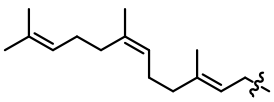
precipitated by the addition of an excess of this nonpolar solvent after the end of the reaction. A subsequent filtration will remove most of the side product resulting in a more effective column chromatography.

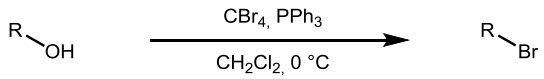
While the use of a mixture of *n*-hexane and ethyl acetate as a mobile phase can result in decomposition of the desired products during column chromatography, they showed to be stable if the latter one is replaced by methyl *tert*-butyl ether. All bromides were obtained as colorless oils that were turning slightly yellow after some hours. Due to the limited stability of allylic bromides all products were freshly prepared prior to use. **Tab. 7** contains all synthesized bromides including numbering, molecular weight and yields. The yields in these reactions usually ranged from excellent 91 % to 100 %. Only in case of **5c**, **15c** and **17c,** lower yields were obtained due to partial decomposition during column chromatography.

Tab. 7 – Synthesized allylic bromides including numbering, molecular weights and yields.

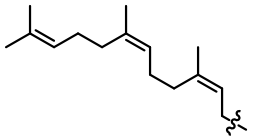
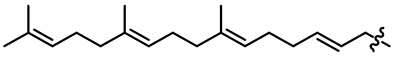
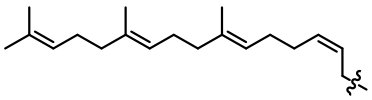
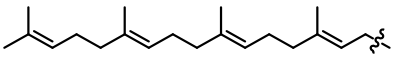
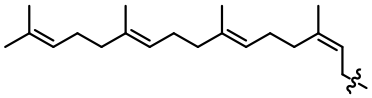
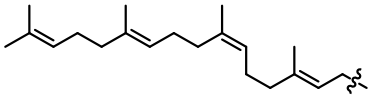
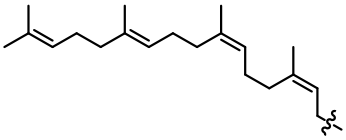
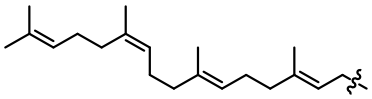
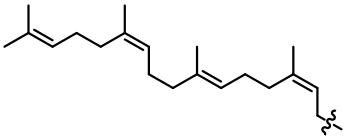
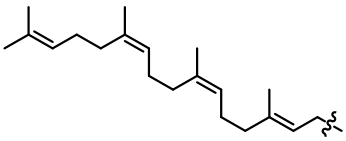
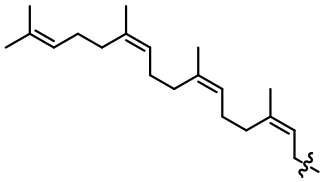
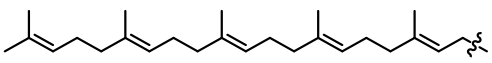


2b, **3b**, geraniol, nerol, **6b-22b** → **2c-22c**

R	number (educt)	number (product)	M_w (g mol^{-1})	yield (%)
	**2b**	**2c**	203.12	100
	**3b**	**3c**	203.12	99
	geraniol	**4c**	217.15	98
	nerol	**5c**	217.15	55
	**6b**	**6c**	271.24	98
	**7b**	**7c**	271.24	97
	**8b**	**8c**	285.27	94
	**9b**	**9c**	285.27	92
	**10b**	**10c**	285.27	96



2b, **3b**, geraniol, nerol, **6b-22b** — **2c-22c**

R	number (educt)	number (product)	M_w (g mol^{-1})	yield (%)
	**11b**	**11c**	285.27	91
	**12b**	**12c**	339.36	97
	**13b**	**13c**	339.36	99
	**14b**	**14c**	353.39	94
	**15b**	**15c**	353.39	77
	**16b**	**16c**	353.39	90
	**17b**	**17c**	353.39	74
	**18b**	**18c**	353.39	99
	**19b**	**19c**	353.39	96
	**20b**	**20c**	353.39	96
	**21b**	**21c**	353.39	99
	**22b**	**22c**	421.51	91

4.2.2 Synthesis of Allylic Diphosphates

The syntheses of allylic isoprenoid diphosphates were performed using a method developed by Dessoy *et al.* at the Wessjohann lab.[91,92,95] It allows the conversion of corresponding precursor bromides with tetrakis(trimethylsilyl) diphosphate as diphosphorylation agent. This reagent can be formed by full protection of disodium dihydrogen diphosphate in a reaction with trimethylsilyl chloride.[95] **Fig. 19** shows the mechanism of the diphosphate ester formation.

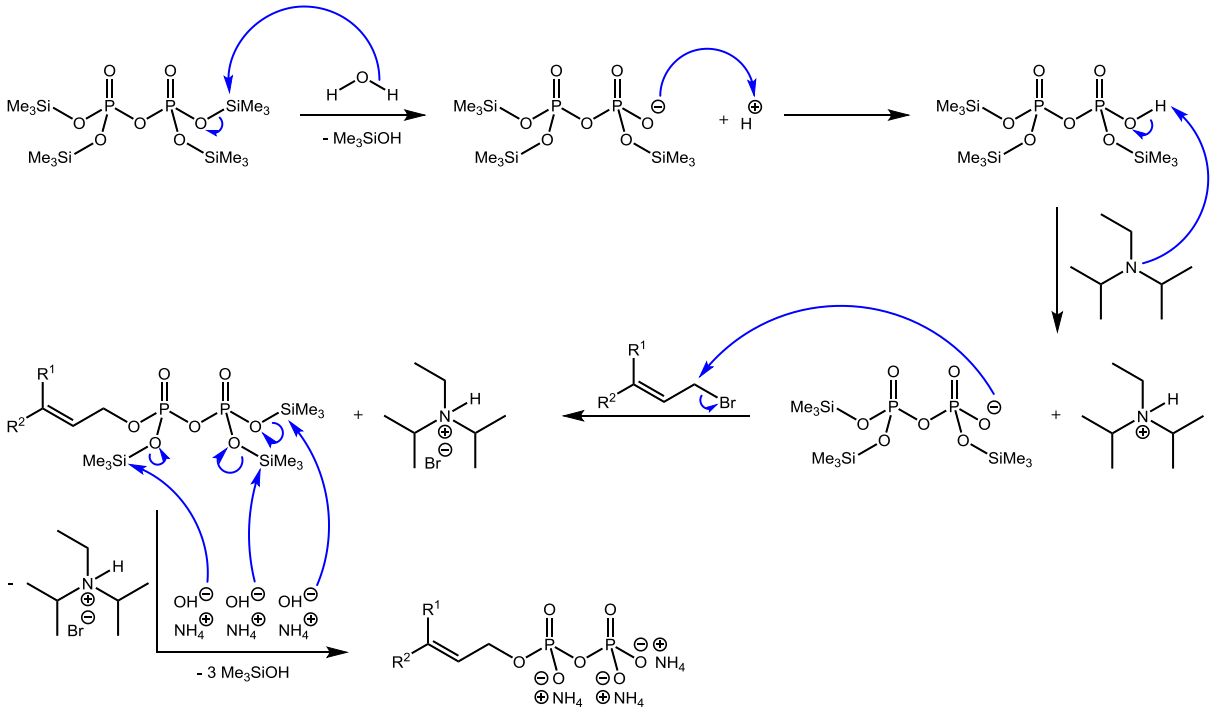


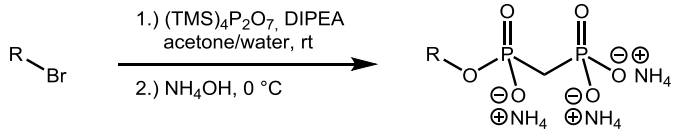
Fig. 19 – Mechanism for the synthesis of allylic diphosphates from allylic bromides using tetrakis(trimethylsilyl) diphosphate as diphosphorylation agent.

In a first step the diphosphorylation agent is partly deprotected by a small amount of water. To avoid a multiple deprotection, which could for example lead to the formation of organic diesters, an excess of the diphosphate is used. The resulting hydroxyl group is then deprotonated by *N*,*N*-diisopropylethylamine, leading to the diphosphorylation agent in the proper sense. The diphosphate is then formed in an S_N2 reaction with an allylic bromide. After the reaction is complete, 6 M ammonium hydroxide solution is added to remove the remaining protective groups from the diphosphate moiety. This step represents the major advantage of this method, because it directly leads to the desired trisammonium salt without the need of a time consuming cation exchange chromatography.

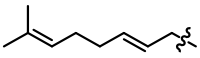
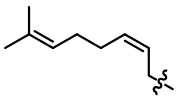
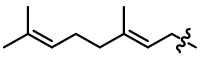
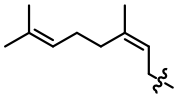
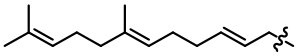
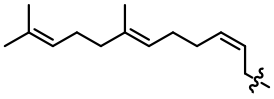
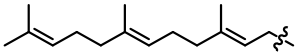
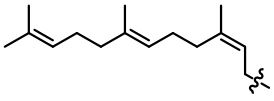
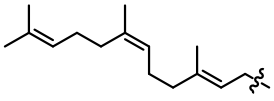
The extraction of the crude reaction mixture with diethyl ether removes unreacted starting

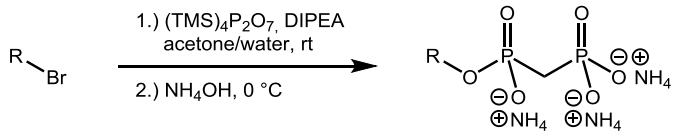
material as well as nonpolar side products of the reaction. In case of longer-chained diphosphates it might be necessary to speed up the subsequent separation of the two phases by centrifugation. The aqueous phase is then separated and mixed with an excess of ethanol resulting in the precipitation of inorganic phosphates. These unwanted impurities are filtered off and the filtrate is concentrated to some milliliters under reduced pressure. Afterwards the pH value has to be set to 12/13 using 6 M ammonium hydroxide solution. The addition of an excess of acetonitrile leads to the precipitation of the desired product. It is filtered off and dissolved in a small amount of ammonium hydroxide solution. A final lyophilization yields the allylic diphosphate as white fluffy solid. In **Tab. 8** all performed allylic diphosphorylation reactions are listed.

Tab. 8 – Synthesized allylic diphosphates including numbering, molecular weights and yields.

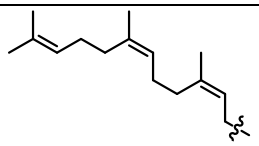
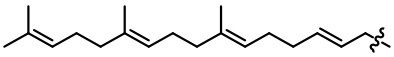
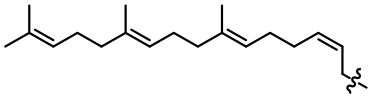
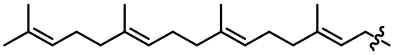
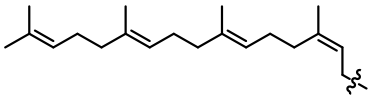
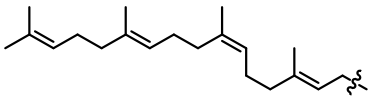
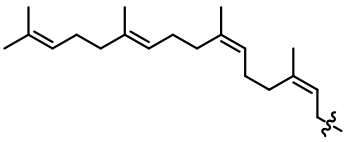
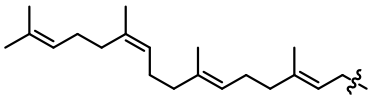
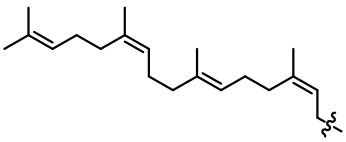
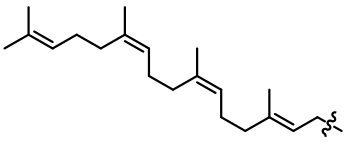
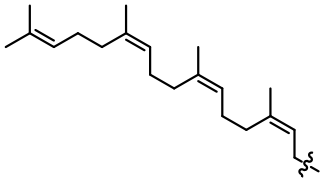
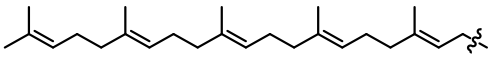


2c-22c **2d-22d**

R^1	**number** (educt)	**number** (product)	M_w (g mol^{-1})	**yield** (%)
	**2c**	**2d**	351.28	21
	**3c**	**3d**	351.28	20
	**4c**	**4d**	365.30	72
	**5c**	**5d**	365.30	23
	**6c**	**6d**	419.40	30
	**7c**	**7d**	419.40	41
	**8c**	**8d**	433.42	32
	**9c**	**9d**	433.42	44
	**10c**	**10d**	433.42	35



2c-22c **2d-22d**

R^1	**number** (educt)	**number** (product)	**M_w** (g mol^{-1})	**yield** (%)
	**11c**	**11d**	433.42	20
	**12c**	**12d**	487.51	23
	**13c**	**13d**	487.51	21
	**14c**	**14d**	501.54	31
	**15c**	**15d**	501.54	21
	**16c**	**16d**	501.54	8
	**17c**	**17d**	501.54	7
	**18c**	**18d**	501.54	42
	**19c**	**19d**	501.54	27
	**20c**	**20d**	501.54	65
	**21c**	**21d**	501.54	41
	**22c**	**22d**	569.66	16

Unfortunately, the obtained yields turned out to be low. They ranged from disappointing 7 % in case of **17d** to good 72 % in case of **4d**. Most of the desired products were obtained in

yields of 20-40 %. On the one hand this is significantly limiting the amount of available product in the last step, on the other hand only low milligram quantities of the organic diphosphates are needed for the subsequent biological studies. Therefore more than enough material was produced for the desired application.

4.3 Homoallylic Isoprenoid Diphosphates

4.3.1 Synthesis of Homoallylic Alcohols

As mentioned, one objective was to synthesize short-chained homoallylic diphosphates with different substituents at position 3. Unfortunately only two suitable alcohol precursors, namely but-3-enol and 3-bromobut-3-enol, were commercially available. Therefore several methods were used to extend the scope of available alcohols.

Two further compounds have been synthesized by carbonyl-ene reactions using 2-substituted propene derivatives as starting material. The use of paraformaldehyde and diethylaluminium chloride as catalyst resulted in the formation of the corresponding homoallylic alcohols.[96] **Fig. 20** shows the mechanism of these reactions. Diethylaluminium chloride appeared to be a good catalyst in such reactions. On the one hand it initiates the reaction by activation of the carbonyl compound, on the other hand it avoids side reactions because the intermediate ene adduct-Et_2AlCl complex is able to give ethane and the more stable aluminium alkoxide in a further reaction. This prevents proton catalyzed rearrangements as well as solvolysis.[97]

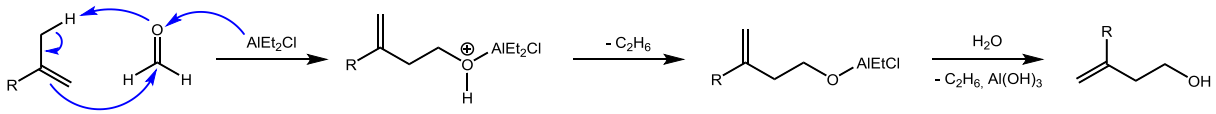


Fig. 20 – Mechanism for the synthesis of homoallylic alcohols from 3-substituted propenes in a carbonyl-ene reaction. R = Cl, Ph

While 3-phenylbut-3-enol was obtained in a yield of 65 %, the yield of 3-chlorobut-3-enol was not determined due to its low boiling point. Even the careful concentration of the resulting column chromatography fractions under reduced pressure showed to be challenging and went along with losses of the desired product. Therefore a solution in a mixture of *n*-hexane and ethyl acetate was used in the next step.

3-(Trifluoromethyl)but-3-enol was synthesized in five steps according to a publication by Liu *et al.* from 2011.[98] The related reaction scheme is shown in **Fig. 21**.

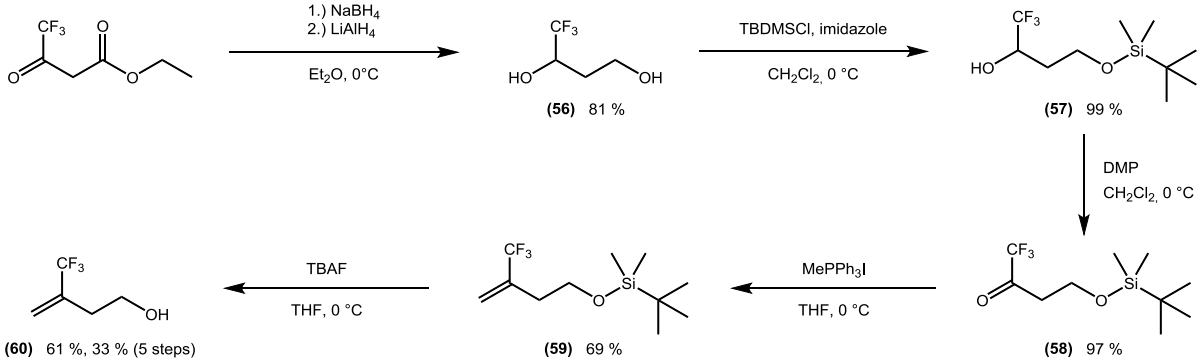


Fig. 21 – Synthesis of 3-(Trifluoromethyl)but-3-enol starting from ethyl 4,4,4-trifluoro-3-oxobutanoate.

In the first step ethyl 4,4,4-trifluoro-3-oxobutanoate was consecutively reduced by sodium borohydride and lithium aluminium hydride. The primary hydroxyl group of the resulting dialcohol (**56**) was then protected by a *tert*-butyldimethylsilyl group. The secondary hydroxyl function of the protected alcohol (**57**) was then oxidized to the ketone (**58**) using Dess-Martin periodinane. The terminal alkene (**59**) was obtained in a Wittig reaction using methyltriphenylphosphonium iodide. Final deprotection of the silyl protective group by tetra-*n*-butylammonium fluoride led to the desired homoallylic alcohol (**60**) in a total yield of 33 %.

3-(Thiomethyl)but-3-enol was obtained in two steps starting from 2-(methylthio)ethanol (see **Fig. 22**). Methyl vinyl sulfide (**63**) was formed by dripping of the starting material to neat potassium hydroxide at 150 °C.[99] The immediately forming product was distilled directly from the reaction flask, resulting in a yield of 77 %. In the next step it was potassiated using Schlosser's base. The homoallylic alcohol (**64**) is then formed in a ring opening reaction initiated by the attack of the nucleophilic intermediate on ethylene oxide.[100] This represents an elegant method for the synthesis of alcohols with a simultaneous elongation of the starting material by two carbon atoms. At the same time ethylene oxide is known to be carcinogenic and should therefore be handled with care.[101]

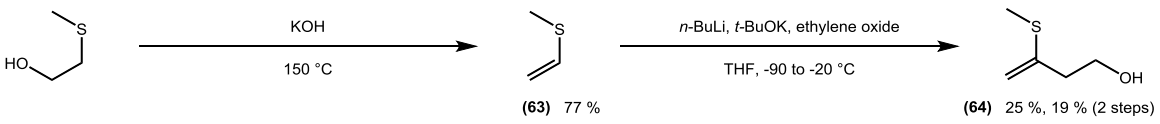
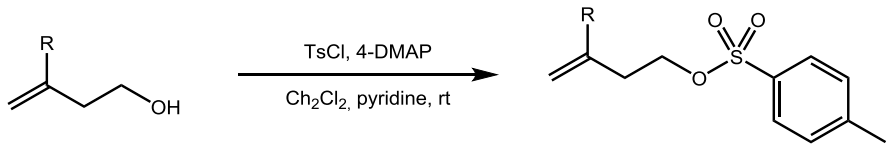


Fig. 22 – Synthesis of 3-(methylthio)but-3-enol starting from 2-(methylthio)ethanol.

4.3.2 Synthesis of Homoallylic Tosylates

The synthesis of homoallylic tosylates using 4-toluenesulfonyl chloride and pyridine or 4-dimethylaminopyridine as a base is a well-established method.[102] In the present work, six homoallylic alcohols with different substituents at position 3 have been converted to the corresponding tosylates. Purification was performed by column chromatography on silica. The use of a mixture of *n*-hexane and ethyl acetate showed to be inappropriate. The replacement of the latter one by dichloromethane results in a better separation of excess 4-toluenesulfonyl chloride and the desired products. The yields in these reactions seem to depend on the electronic properties of the substituents at position 3. It turned out to be quite low (65 %) in the case of the slightly electron donating phenyl substituent and even lower (39 %) in the case of the strong electron donating thiomethyl substituent. Electron withdrawing substituents as well as the hydrogen substituent led to good yields in the range of 87 to 95 %. All products were obtained as colorless oils. **Tab. 9** summarizes all of the performed reactions.

Tab. 9 – Synthesized homoallylic tosylates including numbering, molecular weights and yields.



but-3-enol, 3-bromobut-3-enol, **48, 53, 60, 64** — **46, 49, 51, 54, 61, 65**

R	**number** (educt)	**number** (product)	M_w (g mol^{-1})	**yield** (%)
H	but-3-enol	**46**	226.29	89
Cl	**48**	**49**	260.73	ND
Br	3-bromobut-3-enol	**51**	305.19	87
Ph	**53**	**54**	302.29	65
CF_3	**60**	**61**	294.29	95
SMe	**64**	**65**	272.38	39

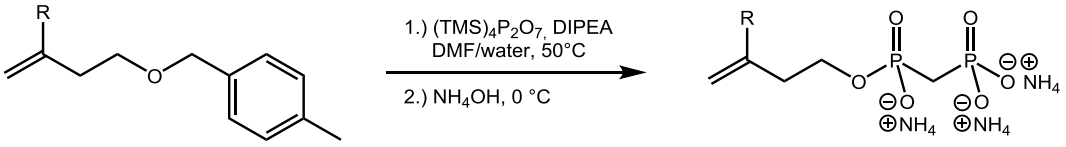
4.3.3 Synthesis of Homoallylic Diphosphates

The method for the synthesis of allylic diphosphates (see 4.2.2) was also used or the synthesis of homoallylic diphosphates. Unfortunately, the use of acetone as solvent led to a negligible conversion of the starting material. The replacement with DMF could solve the

problem resulting in acceptable conversion rates. An increase of the reaction temperature to 50 °C could improve the yields even further. Despite these two minor changes the reaction mechanism stays the same as in **Fig. 19**, except for the leaving group in the nucleophilic substitution reaction, which is in this case represented by a tosyl moiety.

Another change concerns the purification of the resultant products. While the allylic counterparts were purified by fractional precipitation, this method turned out to be unreliable in case of the homoallylic diphosphates. This is mainly caused by the short C_4 alkyl chains of the synthesized products making them extremely polar. Therefore the solubility difference between inorganic phosphates and organic diphosphates becomes too small to perform the fractional precipitation method in a reliable and reproducible manner. As a result, the homoallylic diphosphates were purified by column chromatography on silica using a mixture of isopropanol and concentrated ammonia solution as a mobile phase. This method bases on a publication by Keller *et al.* in 1993.[103] Final lyophilization yielded the desired homoallylic diphosphates as white fluffy solids. The yields were overall low ranging from 10 to 28 %. In contrast to the case of homoallylic tosylates, the yields seem to benefit from an increasing electron donating nature of the substituent at position 3. All of the synthesized homoallylic diphosphates, as shown in **Tab. 10**, play an significant role within the dissertation thesis of Dr. Jeanette Keim.[104]

Tab. 10 – Synthesized homoallylic diphosphates including numbering, molecular weights and yields.



46, 49, 51, 54, 61, 65 **47, 50, 52, 55, 62, 66**

R	**number** (educt)	**number** (product)	**M_w** (g mol^{-1})	**yield** (%)
H	**46**	**47**	281.19	14
Cl	**49**	**50**	315.63	10
Br	**51**	**52**	360.08	11
Ph	**54**	**55**	357.28	28
CF_3	**61**	**62**	349.18	15
SMe	**65**	**66**	327.27	24

4.4 Summary

All in all 21 allylic diphosphates and 6 homoallylic diphosphates were synthesized using the described method. All products were obtained as white solids representing the corresponding ammonium salts. The allylic compounds were purified by fractional precipitation leading to an average yield of only 30 %. The reason for this can be found in the incomplete precipitation of the product. The precipitation from a smaller amount of solvent could increase the yield but would also decrease the purity. Since only small quantities of the synthesized substrates were required for the following enzyme assays, purity was given priority. The homoallylic counterparts were purified by column chromatography with an even smaller average yield of 17 %. The reason was not found in the purification process but in the bad conversion rates of the starting material. In all cases, most of the tosylate could be recovered during workup. Unfortunately, this problem could not be solved using higher temperatures or longer reaction times. However, the obtained quantities showed to be more than sufficient to perform all desired bioassays.

Compared to the well-established method, using tetrakis(tetrabutylammonium) diphosphate as diphosphorylation agent, the in-house method is equal regarding the yields. With respect to the workup of isoprenoid diphosphates it showed to be superior because the crude product is already obtained as the desired trisammonium salt. Therefore the cation exchange step can be skipped, saving time and money.

5. Biocatalytic Conversion of Isoprenoid Diphosphates by Terpene Synthases

5.1 Introduction

Terpene Synthases catalyze the conversion of a limited number of isoprenoid precursors to the large variety of terpenes. As mentioned, two important types of precursors can be converted by this enzyme class. Triterpene synthases convert the hydrocarbon squalene or its oxidized form oxidosqualene whereas the reactions of tetraterpene synthases are based on the highly unsaturated hydrocarbon lycopene. At the same time mono-, sesqui-, di-, sester- and sesquarterpene synthases use isoprenoid diphosphates as substrates. All of their catalyzed reactions are initiated by the formation of a highly reactive carbocation intermediate. Dependent on its formation, terpene synthases are divided into two groups. In case of class I terpene synthases the carbocation is formed by heterolytic fission of the C-O bond resulting in the cleavage of the anionic diphosphate moiety. The class II terpene synthases catalyze its formation through protonation of a certain double bond. As squalene, oxidosqualene and lycopene do not contain diphosphate moieties the tri- and tetraterpene synthases necessarily belong to class II. This does in turn not mean that all enzymes that are able to convert isoprenoid diphosphates are following a class I mechanism.

The terpene synthases used in the course of this thesis (limonene synthase (CsTPS1)[105] from *Cannabis sativa*, aristolochene synthase (TEAS)[106,107] from *Nicotiana tabacum* and casbene synthase (RcCAS)[108] from *Ricinus communis*) all show a class I activity (see **Tab. 11**). As a consequence, all of these enzymes share common structural features responsible for the binding of three divalent metal ions: the DDXXD/E and (N,D)D(L,I,V)X(S,T)XXXE motifs.[109] These metal ions, in this case represented by Mg^{2+}, are responsible for the binding and cleavage of the diphosphate moiety present within the isoprenoid precursors.

Tab. 11 – Properties of the enzymes used for the biocatalytic transformations in the course of this thesis.

name	(-)-limonene synthase (CsTPS1)	5-*epi*-aristolochene synthase (TEAS)	casbene synthase (RcCAS)
origin	*Cannabis sativa*	*Nicotiana tabacum*	*Ricinus communis*
type	class I monoterpene synthase	class I sesquiterpene synthase	class I diterpene synthase
natural substrate	geranyl diphosphate (GDP)	farnesyl diphosphate (FDP)	geranylgeranyl diphosphate (GGDP)
natural product	(-)-limonene	(+)-5-*epi*-aristolochene	casbene

5.2 Biocatalytic Conversion Reactions

5.2.1 Conversion of C_{10} Isoprenoid Diphosphates by Limonene Synthase (CsTPS1)

In a first experiment the natural substrate GDP (**4d**) as well as its geometric isomer NDP (**5d**) was tested. According to literature, the outcome of such experiments depends on the ability of the used enzyme to form corresponding geranyl or neryl cation intermediates.[110-112] Whereas the formation of the transoid geranyl cation leads to acyclic products, the formation of the cisoid neryl cation results in the formation of cyclic products. Some terpene synthases are able to convert both cationic species into each other via a linalyl diphosphate intermediate (see **Fig. 24**).[112] If limonene synthase (CsTPS1) should also catalyze this isomerization of the 2,3-double bond, the conversion of GDP (**4d**) and NDP (**5d**) would lead to the same product species.

Indeed, the related GC-MS analyses (see **Fig. 23**) revealed that the same products are formed in similar yields, regardless of the used C_{10} substrate. This confirms that limonene synthase (CsTPS1) catalyzes the isomerization of the 2,3-double bond resulting in the simultaneous presence of a transoid geranyl cation as well as a cisoid neryl cation, enabling the formation of monoterpenes across both pathways.

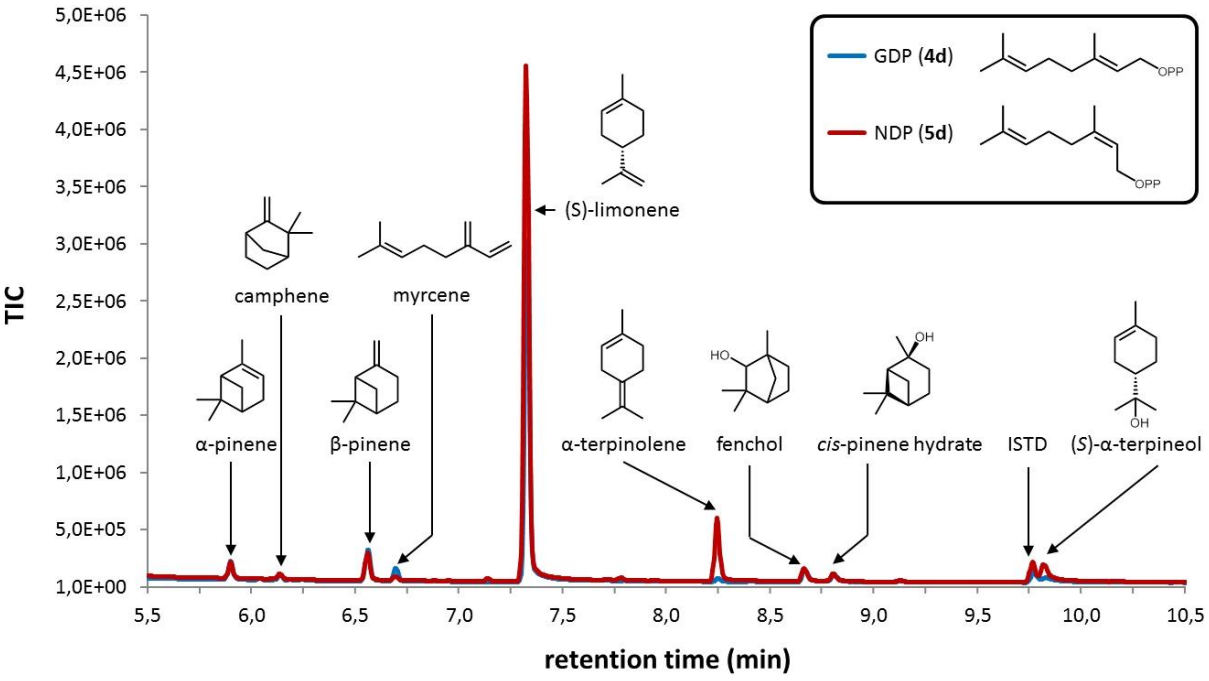


Fig. 23 – Stacked GC-Chromatograms of the products formed by the conversion of GDP (**4d**) and NDP (**5d**) by limonene synthase (CsTPS1). Identification via comparison of retention indices and EI mass spectra using the NIST11 and FFNSC databases. ISTD – internal standard (naphthalene).

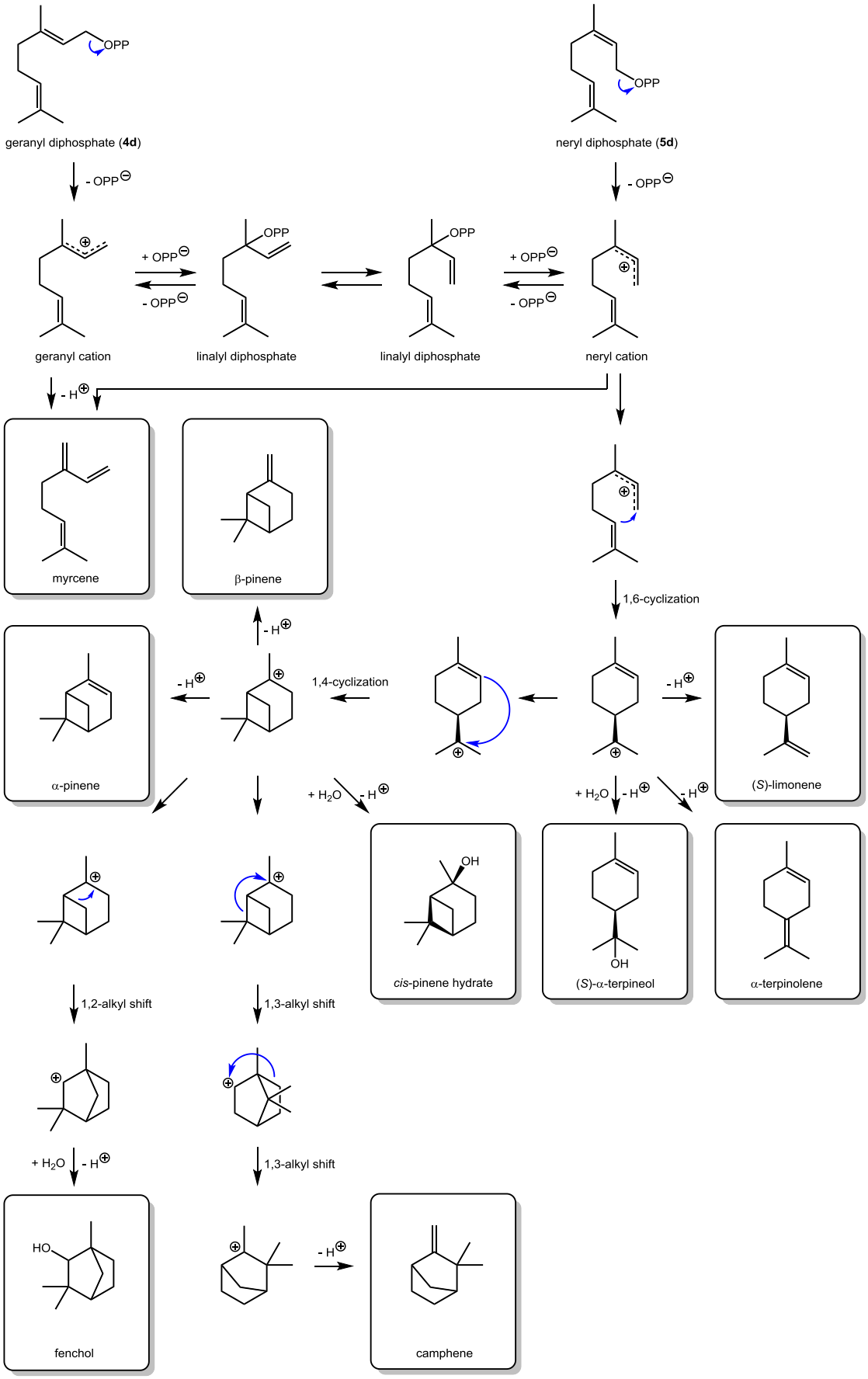


Fig. 24 – Biocatalytic conversion of GDP (**4d**) and NDP (**5d**) by limonene synthase (CsTPS1). Mechanism of the 2,3-double bond isomerization (top) and the proposed conversion reactions (middle to bottom) leading to the observed products (framed).

All of the major products could be identified by comparison of the retention indices and EI mass spectra using the NIST11 and FFNSC database. (*S*)-limonene represents the main product with a share of 77 % in case of both GDP (**4d**) and NDP (**5d**) as substrate. Furthermore the cisoid pathway led to the formation of the cyclic monoterpes α-pinene (GDP: 4 %; NDP 2 %), β-pinene (7 %; 4 %), camphene (1 %; 1 %) and α-terpinolene (1 %; 9 %) plus the monoterpenoid alcohols (*S*)-α-terpineol (1 %; 2 %), fenchol (3 %; 2 %) and *cis*-pinene hydrate (2 %; 1 %). The latter were formed by the reaction of water with one of the highly reactive cation intermediates. The only compound which can be directly produced via the transoid pathway was the acyclic monoterpene myrcene (3 %; 1 %). The mechanisms related to all of the mentioned products are summarized in **Fig. 24**.

The product ratios showed to be barely dependent on the used C_{10} substrate. Only α-terpinolene (1 %; 9 %) is produced in a significantly higher amount when the natural substrate GDP (**4d**) is replaced by NDP (**5d**). Therefore the 2,3-double bond isomerization is supposed to be faster than the product formation via deprotonation or cyclization reactions. The observed product profiles are in good compliance with the results obtained by GC-MS analyses of *Cannabis sativa* trichomes.[105] As a consequence both GDP (**4d**) and NDP (**5d**) could represent the enzymes natural substrate. A detailed quantification of the observed products can be found in the appendix (see chapter 10.3.1).

5.2.2 Conversion of C_9 Isoprenoid Diphosphates by Limonene Synthase (CsTPS1)

The corresponding C_9 substrates, lacking a methyl group at position 3, were poorly accepted by CsTPS1. According to **Fig. 25** the conversion of norGDP (**2d**) led to a barely detectable amount of 4-isopropenylcyclohexane (100 %) whereas the conversion of norNDP (**3d**) yielded a significantly higher amount of this compound (68 %) in addition to 1-methylene-3-(1-methylethylidene)cyclopentane (23 %) and miscellaneous unidentified species (9 %).

Both products are supposed to originate from the formation of an α-norterpinyl cation intermediate via the cisoid pathway (see **Fig. 26**). Its deprotonation leads to the stable 4-isopropenylcyclohexane (norlimonene) as well as to α-norterpinolene. The latter seems to be unstable compared to α-terpinolene resulting in a rearrangement to the observed cyclopentane derivative. It could not be clarified whether the rearrangement occurs spontaneously during the enzyme assays or as a result of high temperatures during the GC measurements.

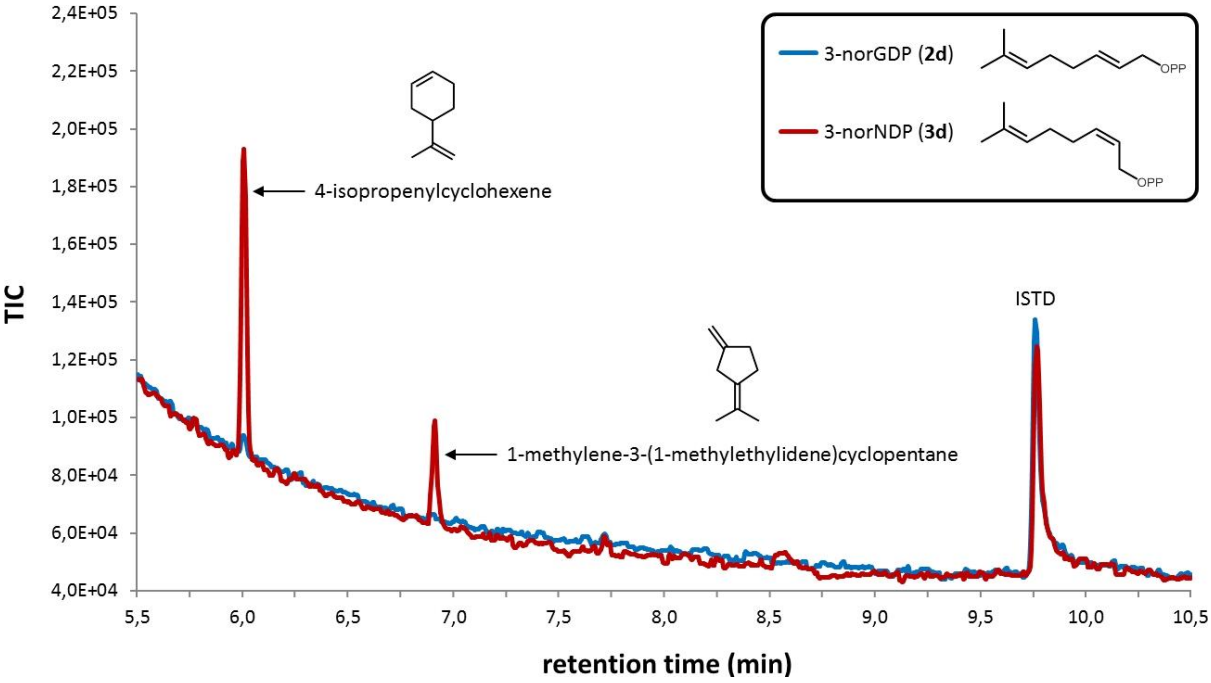


Fig. 25 – Stacked GC-Chromatograms of the products formed by the conversion of norGDP (**2d**) and norNDP (**3d**) by limonene synthase (CsTPS1). Identification via comparison of retention indices and EI mass spectra using the NIST11 and FFNSC databases. ISTD – internal standard (naphthalene).

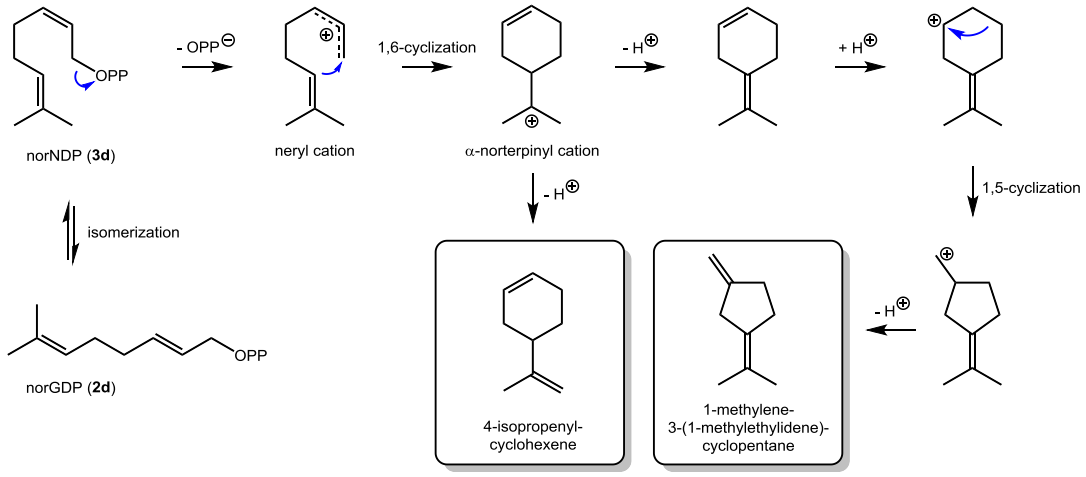


Fig. 26 – Biocatalytic conversion of norGDP (**2d**) and norNDP (**3d**) by limonene synthase (CsTPS1). Proposed mechanism of the conversion reactions, via the cisoid pathway, leading to the observed products (framed).

This time the (*Z*)-configuration of the 2,3-double bond is an important prerequisite for an occurring product formation. Therefore the situation shows to be completely different compared to the conversion of C_{10} substrates (see 5.2.1). The lacking methyl group seems to considerably affect the degree of double bond isomerization via linalyl like intermediates. Such an intermediate is supposed to be instable, making an isomerization energetically less favorable. The fact that no corresponding norlinalyl phosphates are described in literature supports this hypothesis. However, since a small amount of norlimonene is still formed by

the conversion of the (*E*)-configured norGDP (**2d**), the isomerization pathway seems not to be fully disabled.

The results show that the removal of the 3-methyl group leads to significant changes in the enzymatic conversion reactions catalyzed by limonene synthase (CsTPS1). Due to the less favorable formation of norlinalyl intermediates, the isomerization reaction is outpaced by the product formation via an initial 1,6-cyclization. This increases the product specificity with respect to the initial double bond configuration of the used substrate. Moreover the conversion rates show to be considerably lower. This can be explained by two major reasons. On the one hand the missing methyl group results in a smaller size of the substrate which leads to a non-optimal fit regarding the enzymes active site. On the other hand the missing electron donating effect of the methyl group decreases the electron density of the 2,3-double bond resulting in a lower stability of the formed carbocation. A detailed quantification of the observed products can be found in the appendix (see chapter 10.3.1).

5.2.3 Conversion of C_{15} Isoprenoid Diphosphates by Aristolochene Synthase (TEAS)

All of the tested FDP isomers (**8d**-**11d**) were converted by aristolochene synthase (TEAS). In case of the 2-(*E*)-isomers (**8d**, **10d**), a quite specific conversion to one dominating product could be observed. While most of the natural substrate (*E*,*E*)-FDP (**8d**) was converted to the expected product (+)-5-*epi*-aristolochene (86 %), the use of (*E*,*Z*)-FDP (**10d**) as a substrate in the enzymatic conversion reaction was mainly yielding the corresponding germacrene A species (97 %) (see **Fig. 27**). As the double bond configurations are supposed to be retained in the cyclization reactions, this product should represent (*E*,*Z*)-germacrene A.

The conversion of 2-(*Z*)-isomers of FDP (**9d**, **11d**) proceeded in a surprisingly unspecific manner. In both cases a variety of different products was observed (see **Fig. 28**). The product mix resulting from the conversion of (*Z*,*E*)-FDP (**9d**) was in good compliance with a publication by Coates *et al.* from 2010.[113] These experiments yielded the main product prezizaene (51 %) as well as α-cedrene/α-funebrene (18 %), β-curcumene (10 %), (*Z*)-γ-bisabolene (2 %) and various unidentified products (19 %). Since the EI fragmentation patterns of α-cedrene and α-funebrene are almost identical, it is hard to say which one exactly is formed. However, in the mentioned publication the corresponding product was identified as α-cedrene by NMR analysis. The conversion of (*Z*,*Z*)-FDP (**11d**) gave a huge variety of 10 identified products. With a share of 20 % α-cedrene/α-funebrene here represents the most prominent product followed by amorpha-4,11-diene (18 %), β-cedrene

(13 %), (*E*)-γ-bisabolene (8 %), γ-curcumene (7 %), β-curcumene (7 %), (*Z*)-γ-bisabolene (6 %), acoradiene (5 %), β-bisabolene (4 %) and most likely (*Z*,*Z*)-germacrene A (1 %) and miscellaneous unidentified products (11 %).

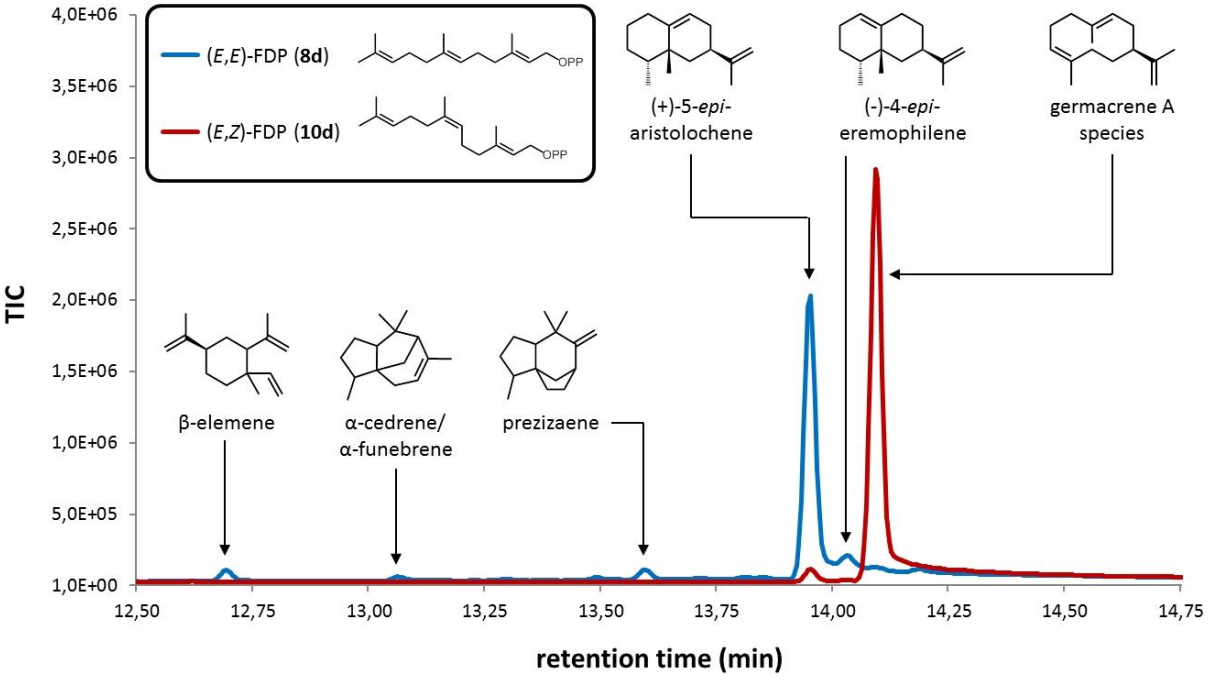


Fig. 27 – Stacked GC-Chromatograms of the products formed by the conversion of (*E*,*E*)-FDP (**8d**) and (*E*,*Z*)-FDP (**10d**) by aristolochene synthase (TEAS). Identification via comparison of retention indices and EI mass spectra using the NIST11 and FFNSC databases. ISTD – internal standard (naphthalene).

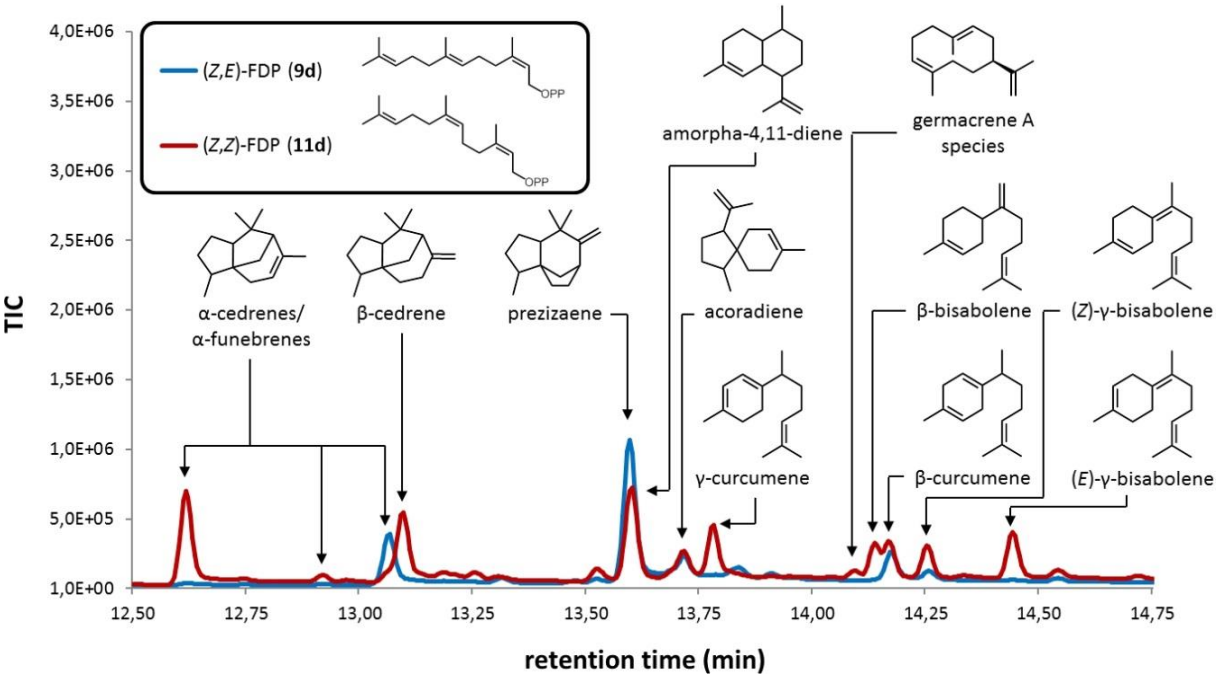


Fig. 28 – Stacked GC-Chromatograms of the products formed by the conversion of (*Z*,*E*)-FDP (**9d**) and (*Z*,*Z*)-FDP (**11d**) by aristolochene synthase (TEAS). Identification via comparison of retention indices and EI mass spectra using the NIST11 and FFNSC databases. ISTD – internal standard (naphthalene).

In general, the assigned products originate from two different pathways. The first one is initiated by a 1,10-cyclization followed by a deprotonation to germacrene A as shown in **Fig. 29**. The further protonation of the former double bond at position 6 is then enabling a second cyclization step resulting in a subsequent 1,2-alkyl shift of the methyl group at position 7. A final deprotonation forms either (+)-5-*epi*-aristolochene or (-)-4-*epi*-eromophilene. The right double bond configuration of the germacrene A intermediate seems to be crucial for the second cyclization step as an (*E*,*E*)-configuration in case of the natural substrate allows this step whereas an (*E*,*Z*)-configuration in case of **10d** does not lead to products of a second cyclization reaction. The reason can be most likely found in the different ring conformations of these germacrene A intermediates. Moreover the conversion of the natural substrate (**8d**) leads to β-elemene, a product that at the first glance doesn't seem to be structurally related to the other products of this pathway. However, it represents the product of a cope rearrangement of the germacrene A intermediate. As expected, the conversion of 2-(*E*)-isomers of FDP (**8d**, **10d**) almost exclusively led to the formation of products related to this pathway.

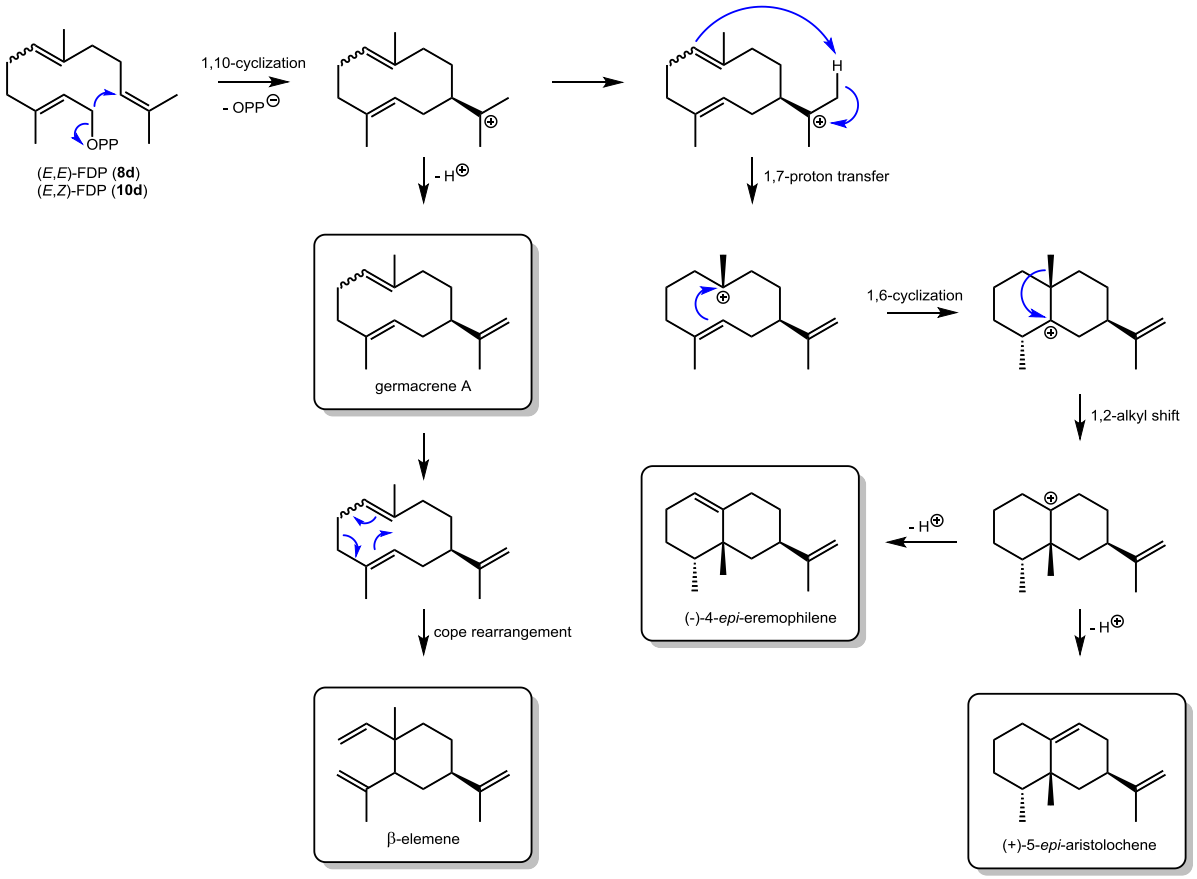


Fig. 29 – Biocatalytic conversion of (*E*,*E*)-FDP (**8d**) and (*E*,*Z*)-FDP (**10d**) by aristolochene synthase (TEAS). Proposed mechanism for the formation of the observed products (framed) resulting from an initial 1,10-cyclization.

The second pathway is initiated by a 1,6-cyclization to the bisabolyl cation (see **Fig. 30**). A deprotonation of this species, also in combination with a possible 1,2-hydride shift, leads to a variety of bisabolene and curcumene type products while a second 1,5-cyclization generates the acoradienyl cation. Its simple deprotonation is yielding acoradiene.

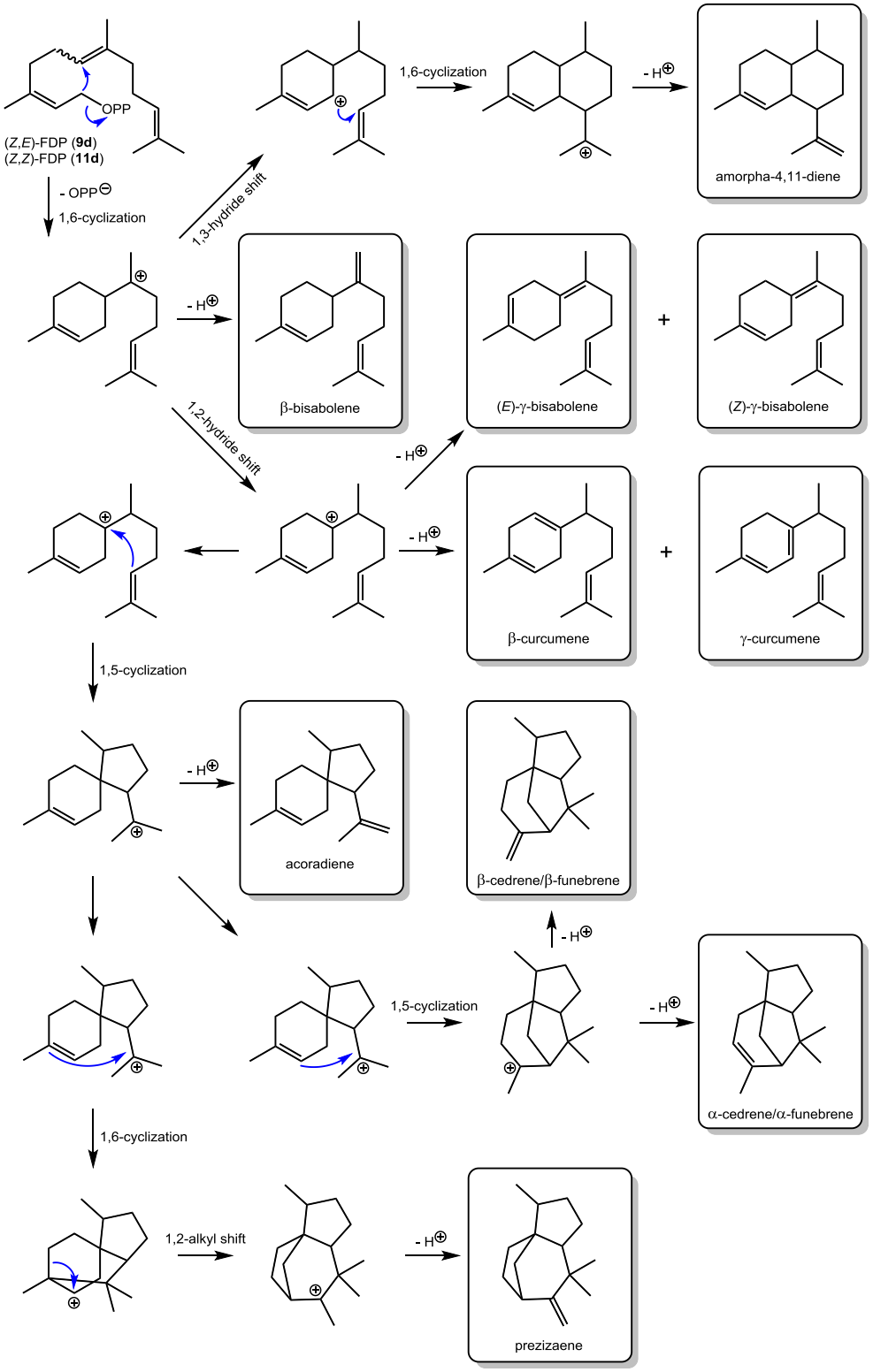


Fig. 30 – Biocatalytic conversion of (*Z*,*E*)-FDP (**9d**) and (*Z*,*Z*)-FDP (**11d**) by aristolochene synthase (TEAS). Proposed mechanism for the formation of the observed products (framed) resulting from an initial 1,6-cyclization.

More interesting is the possibility of a third cyclization reaction which can proceed in two different ways. A 1,5-cyclization followed by the corresponding deprotonation paves the way to α-cedrene and α-funebrene type products which only differ in means of stereochemistry. At the same time, a 1,6-cyclization mechanism including a 1,2-alkyl shift as well as the final deprotonation yields prezizaene. Since substrates featuring a 2-(*Z*)-configuration (**9d**, **11d**) can easily perform the initial 1,6-cyclization, almost all of their resulting products can be derived from this pathway.

All in all the conversion of FDP isomers (**8d**-**11d**) leads to a huge variety of different products. The product composition is strongly dependent on the used substrate. Furthermore, different configurations of the 2,3-double bond result in predominantly different products. However, since there are some product overlaps, aristolochene synthase (TEAS) seems to be able to isomerize this double bond. A strong indicator is the formation of products from the 1,6-cyclization pathway using 2-(*E*)-configured substrates (**8d**, **10d**) since a 2-(*Z*)-configuration is indispensable to enable the necessary 1,6-cyclization (see **Fig. 30**). Compared to the cyclization reactions, the double bond isomerization seems to be considerably slower. Otherwise the resulting product composition of substrates that only vary in the configuration of the 2-double bond would not differ that much. Instead it would remind of the situation observed in case of limonene synthase (CsTPS1). Interestingly the geometric isomers **9d**-**11d** were converted to a higher extent compared to the natural substrate (*E*,*E*)-FDP (**8d**). A detailed quantification of the observed products can be found in the appendix (see chapter 10.3.2).

5.2.4 Conversion of C_{14} Isoprenoid Diphosphates by Aristolochene Synthase (TEAS)

None of the tested 3-norFDP substrates (**6d**-**7d**) was accepted as a substrate by aristolochene synthase (TEAS). The corresponding GC-chromatograms (see **Fig. 31**) do not show any compound beside the internal standard naphthalene. Again, this can be explained by the lacking 3-methyl group resulting in a smaller size of the substrate as well as a more electron-poor 2,3-double bond (see 5.2.2).

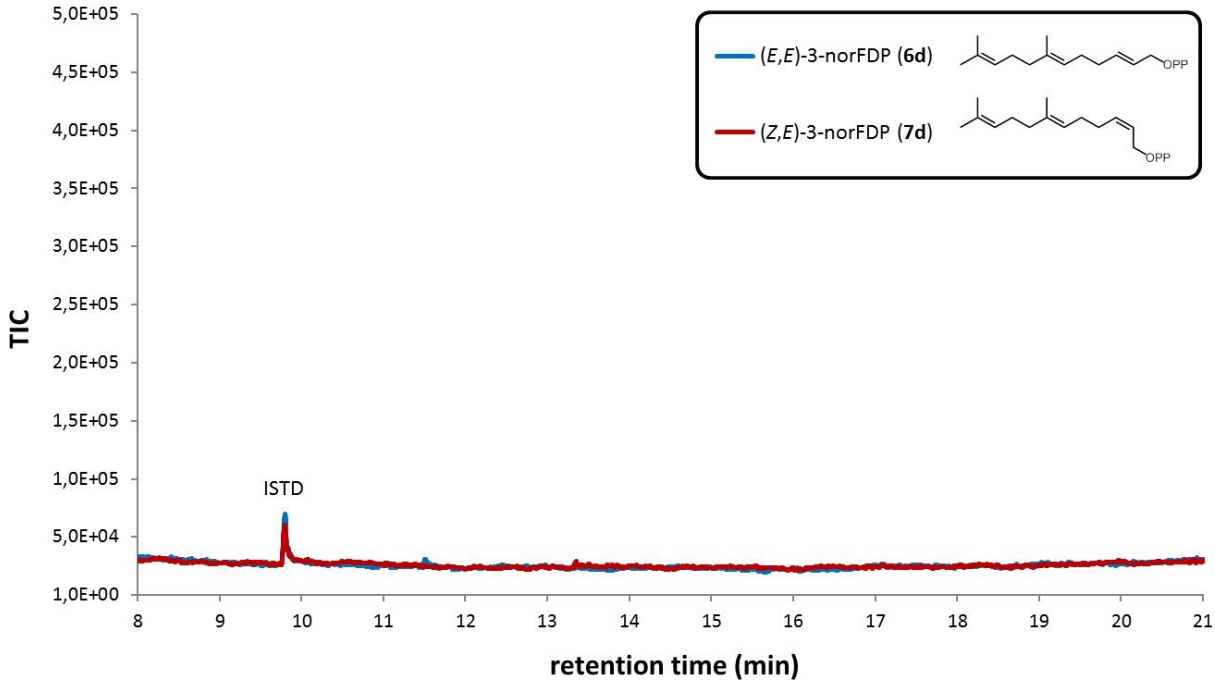


Fig. 31 – Stacked GC-Chromatograms of the products formed by the conversion of (*E*,*E*)-norFDP (**6d**) and (*Z*,*E*)-norFDP (**7d**) by aristolochene synthase (TEAS). Identification via comparison of retention indices and EI mass spectra using the NIST11 and FFNSC databases. ISTD – internal standard (naphthalene).

5.2.5 Conversion of C_{20} Isoprenoid Diphosphates by Casbene Synthase (RcCAS)

Seven out of the eight geometric isomers of GGDP (**14d**-**21d**) were accepted as a substrate by casbene synthase (RcCAS). Only (*E*,*Z*,*Z*)-GGDP (**20d**) was not converted. This can hardly be explained by its geometry since all other isomers are accepted. However, a more conclusive explanation for this observation could not be found in the course of this work. Five (**15d**-**19d**) out of seven geometric isomers showed a higher conversion compared to the natural substrate (*E*,*E*,*E*)-geranylgeranyl diphosphate (**14d**). Only (*Z*,*Z*,*Z*)-GGDP (**21d**) was converted to a lower extent.

While the conversion by limonene synthase (CsTPS1) and aristolochene synthase (TEAS) often lead to numerous products, casbene synthase (RcCAS) showed to be more specific in this concern. In most of the cases only one or two major products were observed (see **Fig. 32**). Due to the lack of fitting diterpene entries within the NIST11 and FFNSC databases, the assignment of the products turned out to be challenging. As a consequence only a limited number of products could be assigned to a certain structure.

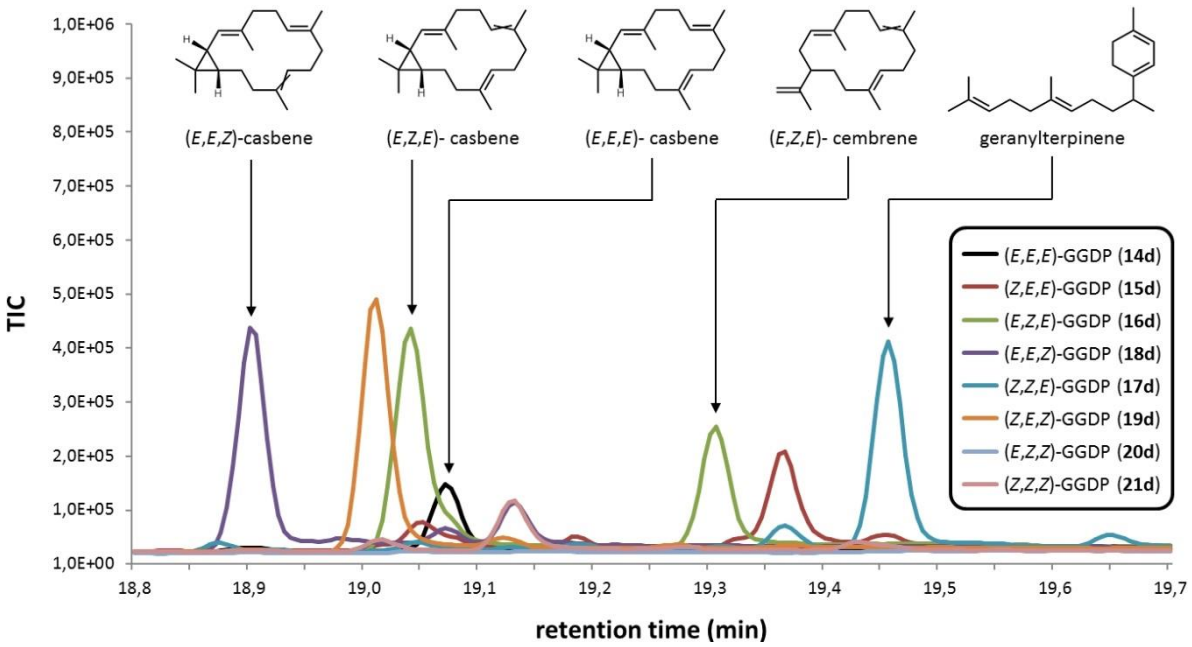


Fig. 32 – Stacked GC-Chromatograms of the products formed by the conversion of GGDP (**14d**-**21d**) by casbene synthase (RcCAS). Identification via comparison of retention indices and EI mass spectra using the NIST11 and FFNSC databases. Crossed double bonds represent a (*Z*)-configuration.

As expected, the conversion of (*E*,*E*,*E*)-GGDP (**14d**) leads to the main product casbene with a share of 87 %. The corresponding side product (13 %) could not be identified. The biocatalytic transformation of (*E*,*Z*,*E*)-GGDP (**16d**) and (*E*,*E*,*Z*)-GGDP (**18d**) led to products that show an EI fragmentation pattern identical to that of casbene. Therefore they are supposed to represent the corresponding geometric isomers of the natural (*E*,*E*,*E*)-configured casbene. In addition to the mentioned casbene isomer (68 %) the conversion of (*E*,*Z*,*E*)-GGDP (**16d**) resulted in a product with an EI fragmentation identical to that of cembrene A (32 %). This is not surprising as the biosynthesis of these two compounds is related (see **Fig. 33**). A first 1,14-cyclization of the corresponding GGDP isomer leads to a formation of a cembrenyl A cation. Whereas a deprotonation results in the formation of cembrene A, a second cyclization to a characteristic cyclopropane ring including a subsequent deprotonation forms casbene. The slightly different retention times of the three assigned casbene isomers are caused by different configurations of the involved double bonds. These are supposed to be retained during the cyclization process.

Another product that could be identified is geranylterpinene yielded by the conversion of (*Z*,*Z*,*E*)-GGDP (**17d**). It is formed from an initial 1,6-cyclization followed by a 1,2-hydride shift and a final deprotonation (see **Fig. 33**). Unfortunately the remaining products could not be identified due to lack of suitable references. However, the obtained data show that, in

contrast to the limonene synthase (CsTPS1) and aristolochene synthase (TEAS), the conversion of 2,3-double bond isomers doesn't lead to any detectable product overlaps. Hence, the existence of a substantial 2,3-double bond isomerization process can be excluded. A detailed quantification of the observed products can be found in the appendix (see chapter 10.3.3).

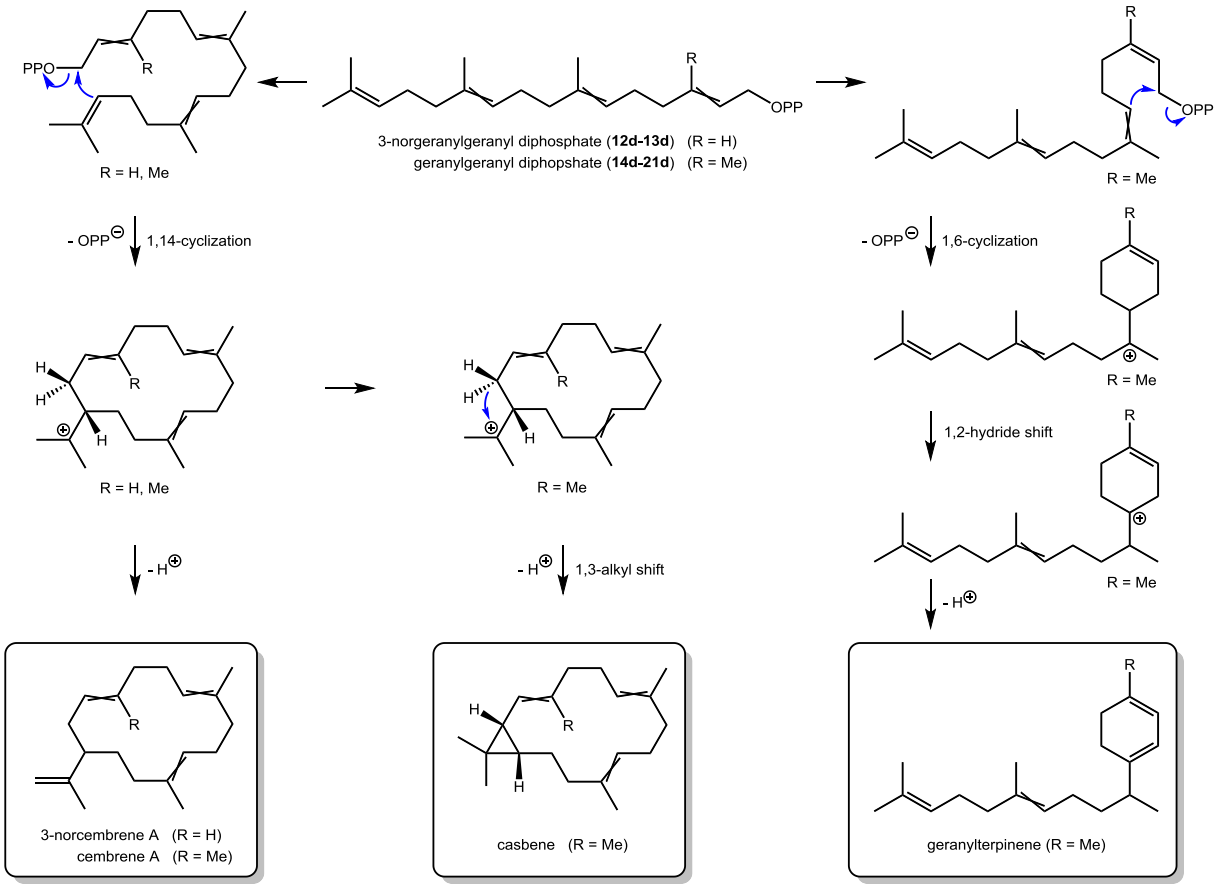


Fig. 33 – Biocatalytic conversion of GGDP (**14d**-**21d**) by casbene synthase (RcCAS). Proposed mechanism for the formation of the observed products (framed) resulting from an initial 1,6- or 1,14-cyclization. Crossed double bonds represent an undefined configuration.

5.2.6 Conversion of C_{19}/C_{25} Isoprenoid Diphosphates by Casbene Synthase (RcCAS)

(*Z*,*E*,*E*)-norGGDP (**13d**) and (*E*,*E*,*E*,*E*)-GFDP (**22d**) were not accepted as a substrate by casbene synthase (RcCAS). The corresponding GC-chromatograms (see **Fig. 34**) do not show any compound beside the internal standard naphthalene. In case of (*Z*,*E*,*E*)-norGGDP (**13d**) this can be once again explained by the lacking 3-methyl group resulting in a smaller size of the substrate as well as a more electron-poor 2,3-double bond (see 5.2.2). In contrast (*E*,*E*,*E*,*E*)-GFDP (**22d**) seems to be too bulky to fit into the active site of the enzyme.

At the same time (*E*,*E*,*E*)-norGGDP (**12d**) is poorly accepted as a substrate and hence converted to one single product (see **Fig. 34**). The corresponding EI mass spectrum shows a

molecular ion at *m/z* = 258 and a fragmentation pattern almost identical to that of cembrene A (see 10.3.3). Therefore this product most likely represents 3-norcembrene A. This C_{19} isoprenoid is supposed to be formed in a 1,14-cyclization reaction exactly as its natural C_{20} counterpart (see **Fig. 33**). Interestingly no product of a further cyclization, namely 3-norcasbene, could be detected. A detailed quantification of the observed products can be found in the appendix (see chapter 10.3.3).

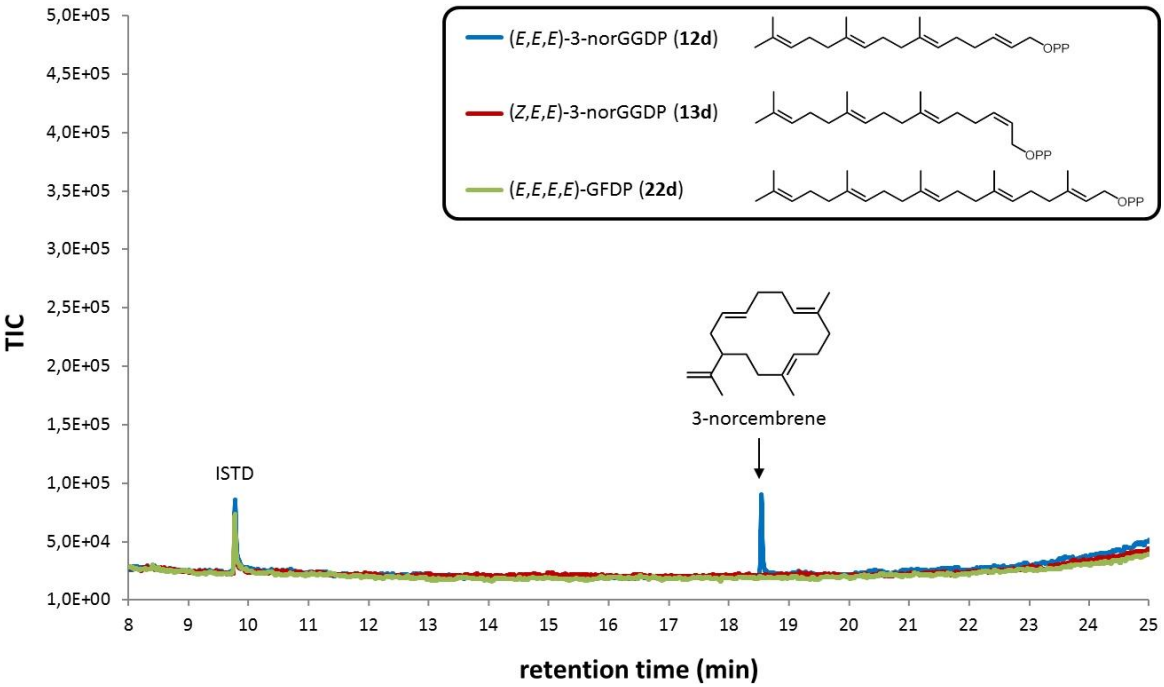


Fig. 34 – Stacked GC-Chromatograms of the products formed by the conversion of (*E*,*E*,*E*)-norGGDP (**12d**), (*Z*,*E*,*E*)-norGGDP (**13d**) and (*E*,*E*,*E*,*E*)-GFDP (**22d**) by casbene synthase (RcCAS). Identification via comparison of retention indices and EI mass spectra using the NIST11 and FFNSC databases. ISTD – internal standard (naphthalene).

5.3 Summary

The supposed promiscuity of the used terpene synthases could be confirmed in the course of the biocatalytic conversion reactions. Almost all geometric isomers of the enzymes natural isoprenoid diphosphate precursors were accepted as substrates, leading to the formation of a large variety of products.

In case of limonene synthase (CsTPS1), the double bond configuration of the used C_{10} substrate barely influenced the observed product distribution. This indicates that the 2,3-double bond isomerization process has to be considerably faster than the product formation. In contrast to this, the isomerization becomes less important for the conversion reactions catalyzed by (+)-5-*epi*-aristolochene synthase (TEAS). This results in a strong

dependence of the product distribution on the particular substrate. Surprisingly only (*E*,*Z*,*Z*)-GGDP (**20d**) was not converted by casbene synthase (RcCAS). Since no product overlaps for the conversion of 2,3-double bond isomers with casbene synthase (RcCAS) can be detected, an isomerization process does not seem to exist for this enzyme at all.

The experiments show that all of the tested enzymes are able to handle the conversion of geometric isomers of their "supposed" natural substrates. Since most of them are converted to an even higher extent, it cannot be ruled out that at least some of the used artificial substrates could play a role in their natural environment. This would represent an elegant method for living organisms to expand their scope of available terpenes. Furthermore it can be assumed that many other terpene synthases will behave in a similar manner. The conversion of geometric isomers of their natural substrates could lead to the discovery of further novel terpenes and terpenoids as well as interesting product distributions valuable for industrial purposes.

Substrates lacking a methyl group at position 3 were barely, if at all, converted by the corresponding enzymes. The reason can be found in a non-optimal fit of these substrates into the active site of the protein as well in a more electron-poor double bond resulting in a lower stability of the formed carbocation intermediates.

6. Abstract

Within the first major part of the present thesis a method for the modular and stereospecific synthesis of allylic isoprenoid alcohols was established. The Biellmann-Ducep reaction represents the key step of this method. The combination with a previous activation step as well as a subsequent reduction step allows the elongation of allylic alcohols to more complex alcohols in only three steps. The use of bifunctional building blocks for chain elongation allows an iterative variation, resulting in an extremely fast and easy synthesis of a large variety of different products. Starting from the commercially available alcohols prenol, geraniol and nerol, 19 different isoprenoid alcohols have been synthesized using only five different chain elongation building blocks whereas twelve of these alcohols have been formed by the use of only two elongation units. Compared to similar methods reported in literature, the described procedure shows to be superior in all cases. As a result it represents the new gold standard for the modular and stereospecific synthesis of isoprenoid alcohols. In a future perspective, the scope of possible products can be expanded easily by the synthesis of new chain elongation building blocks as well as the use of different starter units.

In the second part of the thesis the resulting alcohols were diphosphorylated using a novel method which was also developed at the Leibniz Institute of Plant Biochemistry. Instead of the well-established tetrakis(tetrabutylammonium) diphosphate it makes use of tetrakis(trimethylsilyl) diphosphate as a potent diphosphorylation agent. Whereas the in-house method shows to be equal regarding the yields, it is superior with respect to the workup of isoprenoid diphosphates because the crude product is already obtained as the desired trisammonium salt. Therefore the cation exchange step can be skipped saving time and money. All in all, 21 allylic diphosphates and 6 homoallylic diphosphates were synthesized in a high purity using the described method. All products were obtained as white solids representing the corresponding ammonium salts. While the allylic diphosphates were purified by fractional precipitation, their homoallylic counterparts were subjected to column chromatography. Basing on the mentioned modular synthesis of allylic alcohols, all geometric isomers of geranylgeranyl diphosphate as well as geranylfarnesyl diphosphate could be chemically synthesized for the first time.

Within the final part of the thesis the obtained allylic diphosphates were used as substrates for three different terpene synthases namely limonene synthase (CsTPS1, monoterpene synthase) from *Cannabis sativa*, aristolochene synthase (TEAS, sesquiterpene synthase) from

Nicotiana tabacum and casbene synthase (RcCAS, diterpene synthase) from *Ricinus communis*. GC-MS analyses revealed that most of the tested 21 diphosphates were accepted as substrates by the corresponding enzymes, resulting in the formation of a large variety of both known and novel terpenes and terpenoids. Even more remarkable is the finding that most of the geometric isomers were converted to a higher extent compared to the enzymes natural substrates.

One one hand the experiments showed that the double bond configuration of the substrate does not influence the product composition in case of limonene synthase. On the other hand the replacement of the natural substrates by their geometric isomers leads to a significant alteration of the product species formed by aristolochene and casbene synthase. This can be explained by more or less distinct double bond isomerization processes well known in the field of terpene synthase. Substrates lacking a methyl group at position 3 were barely, if at all, converted by the above mentioned enzymes. The reason can be found in a non-optimal fit of these substrates into the active site of the protein as well in a more electron-poor double bond resulting in a lower stability of the formed carbocation intermediates.

Finally, the once again proven promiscuity of terpene synthases combined with the establishment of a powerful method for the fast and modular synthesis of a large variety of different substrates paved the way for further complex investigations of this enzyme class. The work done in this thesis represents a solid basis providing tools for more comprehensive investigations. Future experiments could give new insights into the mechanistic details of terpene synthases. Furthermore the conversion of geometric isomers or other analogues of corresponding natural substrates could lead to the discovery of highly interesting product species and compositions using wild type enzymes.

7. Zusammenfassung

Innerhalb des ersten Teils der vorliegenden Arbeit wurde eine Methode für die modulare und stereospezifische Synthese von allylischen Isoprenoidalkoholen etabliert. Den Schlüsselschritt stellt hierbei eine Biellmann-Ducep-Reaktion dar. Die Kombination mit einem vorrausgehenden Aktivierungsschritt sowie einer nachfolgenden Reduktion erlaubt in nur drei Reaktionsschritten die Verlängerung allylischer Alkohole zu komplexeren Alkoholen. Werden bifunktionale Module zur Kettenverlängerung verwendet, kann die Methode auch iterativ ausgeführt werden, was wiederum die extrem schnelle und einfache Synthese einer Vielzahl unterschiedlicher Verbindungen ermöglicht. Ausgehend von den kommerziell erhältlichen Alkoholen Prenol, Geraniol und Nerol konnten durch die Nutzung von nur fünf unterschiedlichen Modulen zur Kettenverlängerung bereits 19 verschiedene Isoprenoidalkohole synthetisiert werden, wovon zwölf dieser Produkte sogar nur die Nutzung zweier Verlängerungseinheiten erforderten. Im Vergleich mit literaturbekannten Methoden zeigt sich die hier vorgestellte Möglichkeit stets überlegen und stellt somit den neuen Goldstandard für die modulare und stereospezifische Synthese von Isoprenoidalkoholen dar. Die Bandbreite möglicher Produkte kann dabei in Zukunft auf einfache Weise durch Synthese neuer Ausgangsverbindungen sowie Verlängerungseinheiten erweitert werden.

Im zweiten Teil der Arbeit wurden die erhaltenen Alkohole, mittels einer Methode die ebenfalls am Leibniz-Institut für Pflanzenbiochemie entwickelt wurde, diphosphoryliert. Statt Tetrakis(tetrabutylammonium)diphosphat wird dabei Tetrakis(trimethylsilyl)diphosphat als potentes Diphosphorylierungsreagenz verwendet. Während diese Methode ähnliche Ausbeuten liefert, stellt sie sich hinsichtlich der Aufarbeitung als überlegen dar, da die Rohprodukte bereits als Ammoniumsalze erhalten werden. Aus diesem Grund kann auf einen anschließenden Kationenaustausch verzichtet werden was wiederum Zeit und Geld spart. Unter Verwendung dieser Methode wurden im Rahmen der Arbeit insgesamt 21 verschiedene allylische Diphosphate sowie 6 homoallylische Diphosphate in einer hohen Reinheit synthetisiert. Alle Produkte wurden dabei in Form von weißen Festoffen als entsprechende Ammoniumsalze erhalten. Während die allylischen Diphosphate durch fraktionierte Fällung gereinigt wurden, erfolgte die Reinigung der homoallylischen Diphosphate durch Säulenchromatographie. Basierend auf der modularen Synthese der Vorläufer-Alkohole konnten erstmal alle geometrischen Isomere des

Geranylgeranyldiphosphates sowie Geranylfarnesyldiphosphat synthetisiert werden.

Im abschließenden Teil der Arbeit wurden dann die erhaltenen allylischen Diphosphate als Substrate für drei verschiedene Terpensynthasen genutzt: die Limonensynthase (CsTPS1, Monoterpensynthase) aus *Cannabis sativa*, die 5-*epi*-Aristlochensynthase (TEAS, Sesquiterpensynthase) aus *Nicotiana tabacum* sowie die Casbensynthase (RcCAS, Diterpensynthase) aus *Ricinus communis*. GC-MS-Analysen zeigten dass die meisten der 21 getesteten Substrate von den jeweiligen Enzymen als solche akzeptiert wurden. Dies führte zur Bildung einer Vielzahl von sowohl bekannten als auch neuartigen Terpenen und Terpenoiden. Dabei hatte die Doppelbindungskonfiguration der Substrate im Fall der Limonensynthase kaum Einfluss auf die beobachteten Produktzusammensetzungen. Wurden jedoch die natürlichen Substrate der 5-*epi*-Aristlochensynthase und Casbensynthase durch entsprechende Isomere ersetzt, konnte ein erheblicher Einfluss auf die Zusammensetzung der Produktspektren nachgewiesen werden. Dies kann auf einen unterschiedlich stark ausgeprägten Einfluss von, im Feld der Terpensynthasen wohlbekannten, Isomerisierungs-prozessen zurückgeführt werden. Substrate die keine Methylgruppe an Position 3 aufwiesen wurden, wenn überhaupt, nur sehr schlecht umgesetzt. Die Begründung hierfür liegt zum einen in einem nicht optimalen Sitz des Substrates im aktiven Zentrum des Enzyms und zum anderen in einer elektronenärmeren Doppelbindung, die zu einer schlechteren Stabilisierung des intermediär entstehenden Carbokations führt.

Zusammenfassend bereitet die erneut bestätigte Promiskuität der Terpensynthasen, in Kombination mit der Etablierung einer eleganten Methode für die schnelle und modulare Synthese einer Vielzahl unterschiedlicher Substrate, den Weg für weitere und tiefergehende Untersuchungen dieser Enzymklasse. Der Inhalt dieser Arbeit stellt dafür, durch die Etablierung verschiedener Methoden und Werkzeuge, einen soliden Ausgangspunkt dar. Zukünftige Experimente könnten weitere Details zu den Mechanismen von Terpensynthasen ans Licht fördern. Außerdem könnte die Umsetzung geometrischer Isomere, oder sonstiger Derivate von natürlichen Substraten, unter Nutzung von Wildtyp-Enzymen zur Entdeckung hochinteressanter Produktspezies und -zusammensetzungen führen.

8. Materials and Methods

8.1 Materials

Chemicals

While all chemicals were purchased from Sigma-Aldrich (St. Louis, USA) all solvents were purchased from Merck KGaA (Darmstadt, Germany). The latter were distilled prior to use. Deuterated solvents were acquired from Deutero GmbH (Kastellaun, Germany). The water used in the biochemical experiments was purified using a Milli-Q Biocel water purification system (Millipore, Billerica, USA).

Flash chromatography

Flash chromatography was performed on Merck silica gel 60 (40 – 63 µm). All solvents were distilled prior to use.

8.2 Analytical Methods

Thin layer chromatography (TLC)

Thin layer chromatography was performed on Merck TLC Silica gel 60 F_{254} sheets. UV-sensitive compounds were visualized by UV light (254/366 nm) using a Camag UV cabinet. All other compounds were visualized by one of the following stains.

Stain A: ethanol (94 mL), sulfuric acid (5 mL), 4-anisaldehyde (1 mL)

Stain B: water (470 mL), sulfuric acid (30 mL), phosphomolybdic acid (12.5 g), cerium(IV) sulfate (5 g)

Stain C: water (200 mL), sodium hydroxide solution (10 %, 1.5 mL), potassium carbonate (10 g), potassium permanganate (1.5 g)

The retention factors of several compounds showed to be highly dependent on the amount of substance put on the sheets. In order to avoid any confusion, no explicit values are stated.

NMR spectroscopy

The NMR spectra were obtained from either a 600 MHz Agilent VNMRS 600 NMR spectrometer or a 400 MHz Agilent DD2 400 NMR spectrometer. Chemical shifts were referenced to internal TMS (^{1}H spectra recorded in deuterated organic solvents), solvent residual signals (^{13}C spectra recorded in deuterated organic solvents), internal TMSP-d4 ($^{1}H/^{13}C$ spectra recorded in D_2O), and external phosphoric acid (^{31}P spectra recorded in D_2O).

The data were evaluated by Mestrelab Research S.L. MestReNova 6.0.2 software.

ESI mass spectrometry

The positive and negative ion ESI mass spectra were obtained from a API3200 Triple Quadrupole mass spectrometer (AB Sciex, Framingham, Massachusetts, USA) equipped with an electrospray ion source (spray voltage 5.5 kV/-4.5 kV; source temperature 400 °C). The sample solutions were introduced via an Agilent 1200 HPLC. The data were evaluated by the Analyst® software 1.6.3.

High resolution mass spectrometry

The positive and negative ion high resolution ESI mass spectra marked with "*" were obtained from an Orbitrap Elite mass spectrometer (Thermofisher Scientific, Bremen, Germany) equipped with an HESI electrospray ion source (spray voltage 4.0 kV; capillary temperature 275 °C, source heater temperature 40 °C; FTMS resolution 60.000). Nitrogen was used as sheath gas. The sample solutions were introduced continuously via a 500 µL Hamilton syringe pump with a flow rate of 5 µL min^{-1}. The instrument was externally calibrated by the Pierce® LTQ Velos ESI positive ion calibration solution (product number 88323) and Pierce® ESI negative ion calibration solution (product number 88324) from Thermofisher Scientific, Rockford, IL, 61105 USA). The data were evaluated by the Xcalibur software 2.7 SP1.

The positive and negative ion high resolution ESI mass spectra marked with "**" were obtained from a Bruker Apex III Fourier transform ion cyclotron resonance (FTICR) mass spectrometer (Bruker Daltonics, Billerica, USA) equipped with an Infinity® cell, a 7.0 Tesla superconducting magnet (Bruker, Karlsruhe, Germany), an RF-only hexapole ion guide and an external electrospray ion source. Nitrogen was used as drying gas at 150 °C. The sample solutions were introduced continuously via a syringe pump with a flow rate of 120 µL h^{-1}. All data were acquired with 512 k data points and zero filled to 2048 k by averaging 32 scans. The data were evaluated by the Bruker XMASS 7.0.8 software.

GC/EI-MS analysis

The GC/EI-MS analyses were obtained from a GCMS-QP2010 Ultra (Shimadzu, Duisburg, Germany). The EI-MS measurements were performed with an electron energy of 70 eV and a source temperature of 200 °C. The gas chromatography was performed using a Zebron

ZB-5MS column (Phenomenex, Aschaffenburg, Germany). Helium was used as carrier gas with a flow rate of 1.1 mL min^{-1} (splitless injection, injector temperature 220 °C, interface temperature 300 °C, injection volume 1 mL). The column temperature program started at 40 °C and was increased to 300 °C with a heating rate of 10 °C min^{-1}. The Kovats retention indices were calculated according to **Eq. 1** after calibration using a C_8-C_{20} *n*-alkane standard mixture and *n*-docosane (C_{22}).[114] Identification of the observed compounds was done by comparison of the retention indices as well as the EI-MS data with mass spectral databases (NIST11, FFNSC) and literature data. The data were evaluated by the Shimadzu GCMSsolution and OpenChrom 0.9.0 software.

$$RI = 100 \cdot c + 100\left(\frac{(t_R)_x - (t_R)_c}{(t_R)_{c+1} - (t_R)_c}\right)$$

Eq. 1 – Calculation of Kovats retention indices. *RI* – retention index, *c* – number of carbon atoms in the smaller *n*-alkane, $(t_r)_x$ – retention time of the unknown substance, $(t_r)_c$ – retention time of the smaller *n*-alkane, $(t_r)_{c+1}$ – retention time of the larger *n*-alkane

8.3 Biochemical Methods

Protein Expression and Purification

All used terpene synthases, namely limonene synthase (CsTPS1), 5-*epi*-aristolochene synthase (TEAS) and casbene synthase (RcCAS) were expressed and purified by Dr. Jeanette Keim at the Leibniz Institute of Plant Biochemistry (IPB Halle).[104] **Tab. 12** contains selected parameters of these experiments.

Tab. 12 – Parameters for the expression and purification of three terpene synthases by Dr. Jeanette Keim.

enzyme	**CsTPS1**	**TEAS**	**RcCAS**
origin	*Cannabis sativa*	*Nicotiana tabacum*	*Ricinus communis*
plasmid	pET101/D-TOPO-CsTPS1	pET28b(+)-TEAS	pET32-RcCAS
antibiotic resistance	ampicillin	kanamycin	ampicillin
supplier	Dr. Nils Günnewich, IPB Halle (Germany)[105,115]	Prof. Chappell, University of Kentucky (USA)[116]	gene synthesis[108] via Eurofins Genomics, Ebersberg (Germany)

enzyme	CsTPS1	TEAS	RcCAS
***E. coli* strain**	BL21(DE3)		
genotype	F$^-$ *ompT hsdS*$_B$ ($r_B^- m_B^-$) *gal dcm* (DE3)		
supplier	Invitrogen, Carlsbad (USA)		
growth medium	LB medium (lysogeny broth medium)	TB medium (terrific broth medium)	LB medium (lysogeny broth medium)
composition	1 % tryptone 0.5 % yeast extract 1 % NaCl	1.2 % tryptone 2.4 % yeast extract 0.4 % (v/v) glycerol 72 mM K_2HPO_4 17 mM KH_2PO_4	1 % tryptone 0.5 % yeast extract 1 % NaCl
pH value	7.0	7.5	7.0
Induction reagent	IPTG	IPTG	IPTG
concentration	1.0 mM	0.1 mM	0.4 mM
temperature	28 °C	37 °C	20 °C
duration	6 h	6 h	7 h
purification	IMAC (Ni^{2+})	IMAC (Co^{2+})	IMAC (Ni^{2+})

Biocatalytic Conversion of Isoprenoid Diphosphates by Limonene Synthase (CsTPS1)

The enzymatic conversion reactions were performed in capped glass tubes in a volume of 500 µL CsTPS1-assay buffer (10 mM MOPSO/NaOH, 20 mM $MgCl_2$, 1 mM DTT, pH 7) containing 100 µg mL^{-1} CsTPS1 and 0.2 mM of a C9/C10 isoprenoid diphosphate. The resulting mixture was carefully overlayed with 200 µL of a mixture of *n*-hexane and *n*-heptane (1:1, v/v) containing naphthalene (25 µM) as internal standard. After three hours of incubation at 30 °C the formed products were extracted by vortexing for 30 s. Subsequently the organic phase was analyzed by GC/EI-MS. The assay was performed in triplicates with an additional blank without enzyme.

Biocatalytic Conversion of Isoprenoid Diphosphates by 5-*Epi*-aristolochene Synthase (TEAS)

The enzymatic conversion reactions were performed in capped glass tubes in a volume of 500 µL TEAS-assay buffer (50 mM Hepes/NaOH, 100 mM NaCl, 20 mM $MgCl_2$, 1 mM DTT, pH 7.5) containing 100 µg mL^{-1} TEAS and 0.2 mM of a C14/C15 isoprenoid diphosphate. The resulting mixture was carefully overlayed with 200 µL of a mixture of *n*-hexane and *n*-heptane (1:1, v/v) containing naphthalene (25 µM) as internal standard. After three hours of incubation at 22 °C the formed products were extracted by vortexing for 30 s. Subsequently the organic phase was analyzed by GC/EI-MS. The assay was performed in triplicates with an additional blank without enzyme.

Biocatalytic Conversion of Isoprenoid Diphosphates by Casbene Synthase (RcCAS)

The enzymatic conversion reactions were performed in capped glass tubes in a volume of 500 µL RcCAS-assay buffer (50 mM Tris/HCl, 5 mM $MgCl_2$, 10 % glycerol (v/v), pH 8) containing 200 µg mL^{-1} RcCAS and 0.2 mM of a C19/C20/C25 isoprenoid diphosphate. The resulting mixture was carefully overlayed with 200 µL of a mixture of *n*-hexane and *n*-heptane (1:1, v/v) containing naphthalene (25 µM) as internal standard. After three hours of incubation at 30 °C the formed products were extracted by vortexing for 30 s. Subsequently the organic phase was analyzed by GC/EI-MS. The assay was performed in triplicates with an additional blank without enzyme.

8.4 Synthetic Methods

Method 1 – Synthesis of Allylic Isoprenoid Diphosphates

DIPEA (3.3 eq.) and a mixture of acetone (3.5 eq.) and water (1.6 eq.) is added to tetrakis(trimethylsilyl) diphosphate (5 eq.) at 0 °C. After 10 minutes of stirring, an allylic bromide (1 eq.) is added and the reaction mixture is left for additional 24 hours at room temperature without stirring. Subsequently, the liquid phase is separated from the formed crystals and is added to a stirred ammonium hydroxide solution (6 M) at 0 °C. The pH value has to be around 12-13 to make sure that the hydrolysis is complete. The resulting solution is washed three times with diethyl ether. The aqueous phase is separated and mixed with an excess of ethanol resulting in the precipitation of inorganic phosphates. The resulting dispersion is filtered and the filtrate is concentrated to a few milliliters under reduced pressure. The pH value is set to 12-13 using ammonium hydroxide solution (6 M). The addition of an excess of acetonitrile leads to the precipitation of the desired product. It is

filtered off and dissolved in a small amount of ammonium hydroxide solution. A final lyophilization yields the allylic isoprenoid diphosphate as trisammonium salt.

Method 2 – Synthesis of Homoallylic Isoprenoid Diphosphates

DIPEA (3.3 eq.) and a mixture of DMF (3.5 eq.) and water (1.6 eq.) is added to tetrakis(trimethylsilyl) diphosphate (5 eq.) at 0 °C. After 10 minutes of stirring, a homoallylic tosylate (1 eq.) is added and the reaction is stirred for additional 24 hours at 50 °C. Subsequently the reaction mixture is added to a stirred ammonium hydroxide solution (6 M) at 0 °C. The pH value has to be around 12-13 to make sure that the hydrolysis is complete. The resulting solution is washed three times with diethyl ether. Afterwards the aqueous phase is lyophilized and purified by flash chromatography on silica with a mixture of isopropanol, conc. ammonia solution and water as a mobile phase, yielding the corresponding homoallylic prenyl diphosphate as trisammonium salt.

Method 3 – Synthesis of Homoallylic Isoprenoid Tosylates

A homoallylic alcohol (1 eq.) is dissolved in dry dichloromethane. Then pyridine (2 eq.), 4-toluenesulfonyl chloride (2 eq.) and DMAP (cat.) is added and the solution is stirred overnight at room temperature. The reaction mixture is washed with aqueous HCl (1M), saturated sodium bicarbonate solution and brine. The organic phase is dried (Na_2SO_4) and concentrated under reduced pressure. The residue is purified by flash chromatography on silica (*n*-hexane/dichloromethane), yielding the corresponding homoallylic prenyl tosylate.

Method 4 – Synthesis of Benzyl Ethers

Benzyl bromide (1 eq.) and sodium hydride (1.3 eq.) is added to a solution of an alcohol (1 eq.) in THF at 0 °C. The resulting solution is warmed to room temperature and stirred for 15 hours. After the addition of water, the reaction mixture is extracted with *n*-hexane. The combined organic layers are washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue is purified by flash chromatography on silica (*n*-hexane/EtOAc), yielding the corresponding benzyl ether.

Method 5 – Oxidative Synthesis of Allylic Alcohols

Selenium dioxide (0.2 eq.), salicylic acid (0.2 eq.) and *tert*-butyl hydroperoxide (70 % in water, 3 eq.) is added to a solution of a corresponding alkene in dichloromethane. After 36 hours of stirring, the reaction mixture is extracted with ethyl acetate. The combined organic

layers are washed successively with saturated sodium bicarbonate solution, saturated copper(II) sulfate solution, saturated sodium thiosulfate solution, water and brine. The organic phase is dried (Na_2SO_4), filtered and concentrated under reduced pressure. The resulting residue is dissolved in methanol. Sodium borohydride (1 eq.) is added and the solution is stirred for one hour at room temperature. After concentration under reduced pressure and the addition of water, the mixture is extracted witch ethyl acetate. The combined organic layers are washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue is purified by flash chromatography on silica (*n*-hexane/EtOAc), yielding the corresponding allylic alcohol.

Method 6 – Synthesis of Allylic Phenyl Sulfides

Diphenyl disulfide (1.1 – 1.5 eq.) and tributylphosphine (1.1 – 1.5 eq.) is added to a solution of an allylic alcohol (1 eq.) in THF at 0 °C. After eight hours of stirring at room temperature, sodium hydroxide solution (2 M, 100 mL) is added and the resulting mixture is extracted with *n*-hexane. The combined organic layers are washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue is dissolved in a mixture of methanol (90 mL), sodium hydroxide solution (2 M, 10 mL) and sodium borohydride (1 eq.) is added at 0 °C. After eight hours of stirring at this temperature, aqueous sodium hydroxide solution (2M, 50 mL) is added and the reaction mixture is extracted with *n*-hexane. The combined organic layers are washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue is purified by flash chromatography on silica (*n*-hexane/EtOAc), yielding the corresponding allylic phenyl sulfide.

Method 7 – Synthesis of Allylic Chlorides

Tetrachloromethane (1.0 – 1.5 eq.) and triphenylphosphine (1.0 – 1.5 eq.) is added to a solution of an allylic alcohol in dichloromethane at 0 °C. After 30 minutes of stirring, the reaction mixture is allowed to warm to room temperature and is stirred overnight. After addition of *n*-hexane the precipitate is filtered off and the filtrate is concentrated under reduced pressure. The residue is dissolved in a small amount of *n*-hexane and is stored at -30 °C overnight. The precipitate is filtered off and the filtrate is concentrated under reduced pressure, yielding the corresponding allylic chloride. If necessary the residue is further purified by flash chromatography on silica (*n*-hexane/*t*BME).

Method 8 – Synthesis of Allylic Bromides

Tetrabromomethane (1.0 – 1.5 eq.) and triphenylphosphine (1.0 – 1.5 eq.) is added to a solution of an allylic alcohol in dichloromethane at 0 °C. After one hour of stirring, *n*-hexane is added. The precipitate is filtered off and the filtrate is concentrated under reduced pressure. The residue is dissolved in a small amount of *n*-hexane and is stored at -30 °C overnight. The precipitate is filtered off and the filtrate is concentrated under reduced pressure at 80 °C to remove the entire bromoform, yielding the corresponding allylic bromide. If necessary the residue is further purified by flash chromatography on silica (*n*-hexane/*t*BME).

Method 9 – Cross Coupling of Allylic Chlorides and Allylic Sulfides

n-Butyllithium (2.5 M solution in hexane, 1.1 eq.) is added to a solution of an allylic sulfide (1 eq.) in THF at -78 °C. The resulting yellow solution is stirred for 30 minutes at this temperature and for additional 30 minutes at 0 °C. The reaction is cooled to -78 °C and HMPT (1.5 eq.) is added. Subsequently an allylic chloride (1 – 1.2 eq.) is added to the red solution and the mixture is allowed to warm to room temperature over 30 minutes. Saturated ammonium chloride solution is added and the reaction mixture is extracted with diethyl ether. The combined organic layers are washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue is purified by flash chromatography on silica (*n*-hexane/EtOAc), yielding the corresponding cross coupling product.

Method 10 – Reductive Synthesis of Allylic Alcohols

Lithium (10 eq.) is added to a mixture of liquid ammonia and THF (1:1, excess) at -78 °C. The blue solution is stirred for 30 minutes at this temperature. The cross coupling product (1 eq., see procedure 9) is added and the reaction mixture is stirred for 15 minutes at -78 °C. Methanol is added until complete dissipation of the blue color. Saturated ammonium chloride solution is added at room temperature and the reaction mixture is extracted with diethyl ether. The combined organic layers are washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue is purified by flash chromatography on silica (*n*-hexane/EtOAc), yielding the corresponding allylic alcohol.

Method 11 – Introduction of a THP Protective Group

Pyridinium *p*-toluenesulfonate (0.05 eq.) and 3,4-dihydro-2*H*-pyran (1.5 eq.) is added to a solution of an alcohol (1 eq.) in dichloromethane. After four hours of stirring at room

temperature, the solution is concentrated under reduced pressure and the residue is dissolved in diethyl ether. The organic layer is washed with saturated sodium bicarbonate solution, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue is purified by distillation or flash chromatography on silica (*n*-hexane/EtOAc), yielding the corresponding THP protected alcohol.

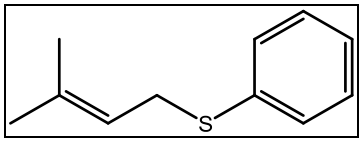
Method 12 – Cleavage of a THP Protective Group

Pyridinium *p*-toluenesulfonate (0.1 eq.) is added to a solution of a THP-protected alcohol (1 eq.) in ethanol. After 8 hours of stirring at 60 °C saturated sodium bicarbonate solution is added and the resulting mixture is extracted with diethyl ether. The combined organic layers are washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue is purified by distillation or flash chromatography on silica (*n*-hexane/EtOAc), yielding the corresponding alcohol.

8.5 Syntheses

Phenyl prenyl sulfide (1e)

According to the **synthetic method 6**, prenol (6.08 mL, 60.00 mmol), diphenyl disulfide (14.41 g, 66.00 mmol), tributylphosphine (16.28 mL, 66.00 mmol), sodium borohydride (2.27 g, 60.00 mmol) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding phenyl prenyl sulfide (9.12 g, 51.15 mmol, 85 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33 (m, 2H), 7.26 (m, 2H), 7.17 (m, 1H), 5.30 (t, *J* = 7.7 Hz, 1H), 3.54 (d, *J* = 7.7 Hz, 2H), 1.71 (s, 3H), 1.58 ppm (s, 3H);

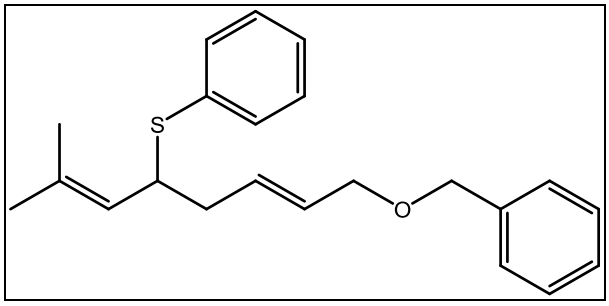
^{13}C NMR (101 MHz, $CDCl_3$): δ = 136.82, 136.31, 129.67, 128.67, 125.92, 119.29, 32.20, 25.62, 17.64 ppm;

MS/ESI *m/z* = 177.3 ($[M-H]^+$, 100 %);

HRMS/ESI* calcd for $C_{11}H_{13}S$: 177.0732 $[M-H]^+$, found: 177.0734.

(2*E*)-1-(Benzyloxy)-7-methyl-5-(phenylthio)octa-2,6-diene (2a)

According to the **synthetic method 9**, phenyl prenyl sulfide (3.57 g, 20 mmol), (2*E*)-4-(benzyloxy)but-2-enyl chloride (4.72 g, 24 mmol), *n*-butyllithium (2.5 M in hexane, 8.8 mL, 22 mmol), HMPT (5.27 mL, 30 mmol) and THF (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*E*)-1-(benzyloxy)-7-methyl-5-(phenylthio)octa-2,6-diene (4.92 g, 14.53 mmol, 73 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.45 – 7.20 (m, 10H), 5.75 – 5.58 (m, 2H), 5.05 (d, *J* = 10.0 Hz, 1H), 4.48 (s, 2H), 3.97 (d, *J* = 5.8 Hz, 2H), 3.90 (ddd, *J* = 10.0, 8.3, 5.5 Hz, 1H), 2.44 (m, 1H), 2.32 (m, 1H), 1.66 (s, 3H), 1.39 ppm (s, 3H);

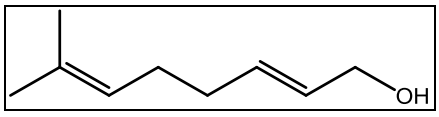
^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.36, 134.87, 134.68, 133.65, 131.06, 128.69, 128.51, 128.32, 127.76, 127.52, 127.22, 125.01, 71.67, 70.53, 47.07, 38.20, 25.57, 17.98 ppm;

MS/ESI *m/z* = 356.5 ($[M+NH_4]^+$, 35 %), 361.1 ($[M+Na]^+$, 100 %), 699.5 ($[2M+Na]^+$, 48 %);

HRMS/ESI* calcd for $C_{22}H_{26}NaOS$: 361.1597 $[M+H]^+$, found: 361.1588.

(2*E*)-7-Methylocta-2,6-dienol (2b)

According to the **synthetic method 10**, lithium (922 mg, 132.90 mmol), (2*E*)-1-(benzyloxy)-7-methyl-5-(phenylthio)octa-2,6-diene (4.5 g, 13.29 mmol), ammonia (25 mL) and THF (25 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 4:1 → 1:1), yielding (2*E*)-7-methylocta-2,6-dienol (1.67 g, 11.91 mmol, 90 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.76 – 5.60 (m, 2H), 5.11 (m, 1H), 4.08 (d, *J* = 5.1 Hz, 2H), 2.12 – 2.02 (m, 4H), 1.69 (s, 3H), 1.60 (s, 3H), 1.52 ppm (s, 1H);

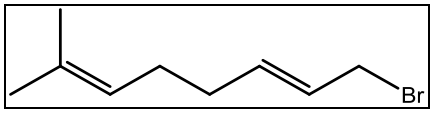
^{13}C NMR (101 MHz, $CDCl_3$): δ = 132.98, 131.89, 129.02, 123.70, 63.74, 32.38, 27.64, 25.63, 17.69 ppm;

MS/ESI *m/z* = 163.6 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_9H_{17}O$: 141.1274 $[M+Na]^+$, found: 141.1270.

(2*E*)-7-Methylocta-2,6-dienyl bromide (2c)

According to the **synthetic method 8**, (2*E*)-7-methylocta-2,6-dienol (1.67 g, 11.91 mmol), tetrabromomethane (4.74 g, 14.29 mmol), triphenylphosphine (3.75 g, 14.29 mmol) and dichloromethane (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 6:1), yielding (2*E*)-7-methylocta-2,6-dienyl bromide (2.42 g, 11.91 mmol, 100 %) as a colorless oil.

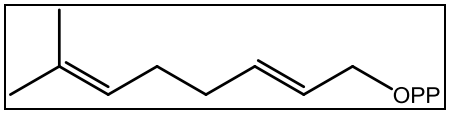


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.82 – 5.65 (m, 2H), 5.09 (m, 1H), 3.95 (d, *J* = 7.2 Hz, 2H), 2.14 – 2.02 (m, 4H), 1.69 (s, 3H), 1.60 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 136.26, 132.24, 126.46, 123.36, 33.56, 32.27, 27.37, 25.67, 17.75 ppm.

(2*E*)-7-Methylocta-2,6-dienyl diphosphate (2d)

According to the **synthetic method 1**, DIPEA (6.35 mL, 37.36 mmol), acetone (2.91 mL, 39.62 mmol), water (326 µL, 18.11 mmol), tetrakis(trimethylsilyl) diphosphate (26.42 g, 56.60 mmol) and (2*E*)-7-methylocta-2,6-dienyl bromide (2.30 g, 11.32 mmol) was used. Purification was done by precipitation, yielding (2*E*)-7-methylocta-2,6-dienyl diphosphate (857 mg, 2.43 mmol, 21 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.88 (dt, *J* = 15.4, 6.3 Hz, 1H), 5.70 (dt, *J* = 15.4, 6.3 Hz, 1H), 5.25 (m, 1H), 4.39 (dd, *J* = 6.7, 6.3 Hz, 2H), 2.17 – 2.08 (m, 4H), 1.71 (s, 3H), 1.64 ppm (s, 3H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 138.16, 136.66, 128.78 (d, *J* = 8.0 Hz), 126.95, 69.49 (d, *J* = 5.3 Hz), 34.58, 29.67, 27.66, 19.86 ppm;

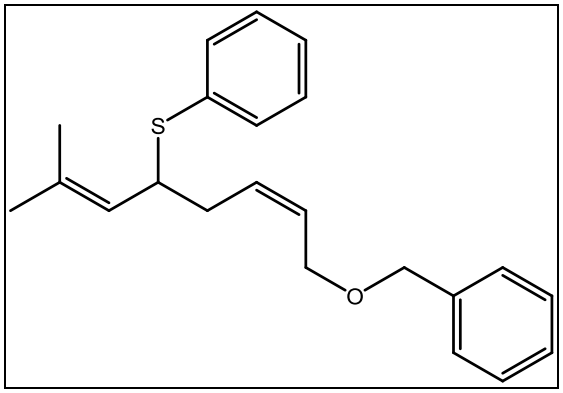
^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.87 (d, *J* = 21.9 Hz), -10.40 ppm (dt, *J* = 21.9, 6.7 Hz);

MS/ESI *m/z* = 299.1 ($[M\text{-}2NH_3\text{-}NH_4]^-$, 100 %), 599.3 ($[2M\text{-}5NH_3\text{-}NH_4]^-$, 26 %);

HRMS/ESI* calcd for $C_9H_{18}NaO_7P_2$: 323.0420 $[M\text{-}3NH_3+Na]^+$, found: 323.0408.

(2*Z*)-1-(Benzyloxy)-7-methyl-5-(phenylthio)octa-2,6-diene (3a)

According to the **synthetic method 9**, phenyl prenyl sulfide (2.50 g, 14.02 mmol), (2*Z*)-4-(benzyloxy)but-2-enyl chloride (3.31 g, 16.82 mmol), *n*-butyllithium (2.5 M in hexane, 6.17 mL, 15.42 mmol), HMPT (3.69 mL, 21.03 mmol) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*Z*)-1-(Benzyloxy)-7-methyl-5-(phenylthio)octa-2,6-diene (3.79 g, 11.20 mmol, 80 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42 – 7.22 (m, 10H), 5.71 – 5.58 (m, 2H), 5.03 (d, *J* = 10.1 Hz, 1H), 4.47 (s, 2H), 4.04 – 4.00 (m, 2H), 3.88 (ddd, *J* = 10.1, 8.5, 5.3 Hz, 1H), 2.45 (m, 1H), 2.27 (m, 1H), 1.65 (s, 3H), 1.40 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.29, 135.11, 134.65, 133.58, 129.82, 128.54, 128.35,

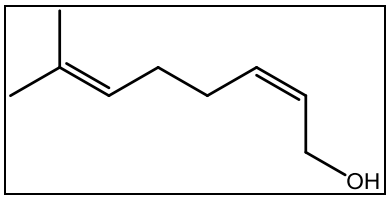
128.06, 127.72, 127.56, 127.25, 124.83, 72.19, 65.87, 47.16, 33.37, 25.58, 17.97 ppm;

MS/ESI *m/z* = 361.4 ($[M+Na]^+$, 100 %), 699.5 ($[2M+Na]^+$, 3 %);

HRMS/ESI* calcd for $C_{22}H_{26}NaOS$: 361.1597 $[M+H]^+$, found: 361.1583.

(2*Z*)-7-Methylocta-2,6-dienol (3b)

According to the **synthetic method 10**, lithium (718 mg, 103.40 mmol), (2*Z*)-1-(Benzyloxy)-7-methyl-5-(phenylthio)octa-2,6-diene (3.50 g, 10.34 mmol), ammonia (50 mL) and THF (50mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 4:1), yielding (2*Z*)-7-methylocta-2,6-dienol (939 mg, 6.70 mmol, 65 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.66 – 5.51 (m, 2H), 5.11 (t, *J* = 6.6 Hz, 1H), 4.18 (d, *J* = 6.6 Hz, 2H), 2.15 – 2.01 (m, 4H), 1.70 (s, 3H), 1.61 (s, 3H), 1.43 ppm (s, 1H);

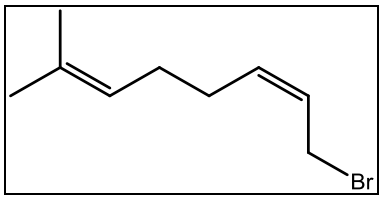
^{13}C NMR (101 MHz, $CDCl_3$): δ = 132.67, 132.40, 128.63, 123.61, 58.50, 27.89, 27.62, 25.66, 17.73 ppm;

MS/ESI *m/z* = 179.5 ($[M+K]^+$, 100 %);

HRMS/ESI* calcd for $C_9H_{17}O$: 141.1274 $[M+Na]^+$, found: 141.1267.

(2*Z*)-7-Methylocta-2,6-dienyl bromide (3c)

According to the **synthetic method 8**, (2*Z*)-7-methylocta-2,6-dienol (937 mg, 6.68 mmol), tetrabromomethane (2.66 g, 8.02 mmol), triphenylphosphine (2.01 g, 8.02 mmol) and dichloromethane (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 20:1), yielding (2*Z*)-7-methylocta-2,6-dienyl bromide (1.34 g, 6.60 mmol, 99 %) as a colorless oil.

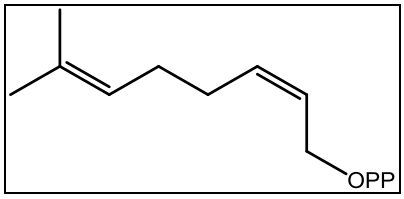


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.73 (m, 1H), 5.61 (m, 1H), 5.12 (t, *J* = 6.9 Hz, 1H), 4.00 (d, *J* = 8.3 Hz, 2H), 2.21 – 2.04 (m, 4H), 1.70 (s, 3H), 1.61 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 135.46, 132.51, 125.47, 123.35, 27.48, 27.34, 27.18, 25.68, 17.75 ppm.

(2*Z*)-7-Methylocta-2,6-dienyl diphosphate (3d)

According to the **synthetic method 1**, DIPEA (2.21 mL, 13.00 mmol), acetone (1.01 mL, 13.79 mmol), water (142 µL, 7.88 mmol), tetrakis(trimethylsilyl) diphosphate (9.19 g, 19.70 mmol) and (2*Z*)-7-methylocta-2,6-dienyl bromide (800 mg, 3.94 mmol) was used. Purification was done by precipitation, yielding (2*Z*)-7-methylocta-2,6-dienyl diphosphate (270 mg, 769 µmol, 20 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.74 – 5.62 (m, 2H), 5.24 (t, *J* = 6.4 Hz, 1H), 4.39 (t, *J* = 6.5 Hz, 2H), 2.22 – 2.06 (m, 4H), 1.71 (s, 3H), 1.64 ppm (s, 3H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 137.10, 136.82, 128.61 (d, *J* = 8.0 Hz), 126.84, 64.57 (d, *J* = 5.3 Hz), 30.12, 29.86, 27.69, 19.90 ppm;

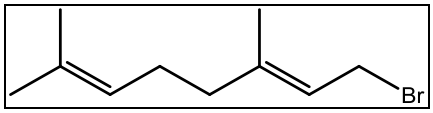
^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.72 (d, *J* = 21.8 Hz), -10.34 ppm (dt, *J* = 21.8, 6.5 Hz);

MS/ESI *m/z* = 299.4 ($[M\text{-}2NH_3\text{-}NH_4]^-$, 100 %), 599.3 ($[2M\text{-}5NH_3\text{-}NH_4]^-$, 28 %);

HRMS/ESI* calcd for $C_9H_{17}O_7P_2$: 299.0455 $[M\text{-}2NH_3\text{-}NH_4]^-$, found: 299.0448.

Geranyl bromide (4c)

According to the **synthetic method 8**, geraniol (5.00 g, 32.41 mmol), tetrabromomethane (11.82 g, 35.65 mmol), triphenylphosphine (9.35 g, 35.65 mmol) and dichloromethane (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 10:1), yielding geranyl bromide (6.89 g, 31.73 mmol, 98 %) as a light yellow oil.

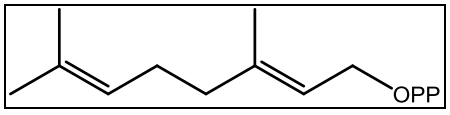


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.53 (t, *J* = 8.4 Hz, 1H), 5.07 (m, 1H), 4.02 (d, *J* = 8.4 Hz, 2H), 2.15 – 2.03 (m, 4H), 1.73 (s, 3H), 1.68 (s, 3H), 1.60 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 143.54, 131.94, 123.50, 120.51, 39.50, 29.63, 26.18, 25.64, 17.67, 15.94 ppm.

Geranyl diphosphate (4d)

According to the **synthetic method 1**, DIPEA (7.76 mL, 45.61 mmol), acetone (3.56 mL, 48.37 mmol), water (398 µL, 22.11 mmol), tetrakis(trimethylsilyl) diphosphate (32.24 g, 69.10 mmol) and geranyl bromide (3.00 g, 13.82 mmol) was used. Purification was done by precipitation, yielding geranyl diphosphate (3.62 g, 9.91 mmol, 72 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.47 (t, *J* = 6.6 Hz, 1H), 5.22 (t, *J* = 6.6 Hz, 1H), 4.48 (dd, *J* = 6.6, 6.1 Hz, 2H), 2.21 – 2.07 (m, 4H), 1.73 (s, 3H), 1.70 (s, 3H), 1.64 ppm (s, 3H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 145.48, 136.50, 126.94, 122.74 (d, *J* = 8.6 Hz), 65.32 (d, *J* = 5.2 Hz), 41.61, 28.43, 27.66, 19.80, 18.42 ppm;

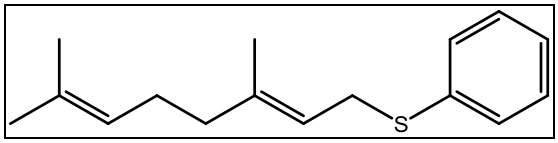
^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.50 (d, *J* = 21.7 Hz), -10.16 ppm (dt, *J* = 21.7, 6.1 Hz);

MS/ESI *m/z* = 313.2 ($[M-2NH_3-NH_4]^-$, 100 %), 627.4 ($[2M-5NH_3-NH_4]^-$, 25 %);

HRMS/ESI** calcd for $C_{10}H_{19}O_7P_2$: 313.0611 $[M-2NH_3-NH_4]^-$, found: 313.0601.

Geranyl phenyl sulfide (4e)

According to the **synthetic method 6**, geraniol (6.17 g, 40.00 mmol), diphenyl disulfide (13.10 g, 60.00 mmol), tributylphosphine (14.80 mL, 60.00 mmol), sodium borohydride (1.51 g, 40.00 mmol) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding geranyl phenyl sulfide (9.18 g, 37.25 mmol, 93 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33 (m, 2H), 7.25 (m, 2H), 7.16 (m, 1H), 5.30 (t, *J* = 7.6 Hz, 1H), 5.06 (m, 1H), 3.54 (d, *J* = 7.6 Hz, 2H), 2.09 – 1.96 (m, 4H), 1.67 (s, 3H), 1.59 (s, 3H), 1.57 ppm (s, 3H);

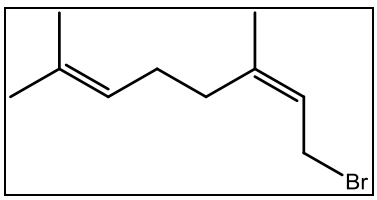
^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.79, 136.76, 131.56, 129.84, 128.63, 125.94, 123.89, 119.24, 39.53, 32.16, 26.42, 25.63, 17.65, 15.98 ppm;

MS/ESI *m/z* = 269.0 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{16}H_{23}S$: 247.1515 $[M+H]^+$, found: 247.1525.

Neryl bromide (5c)

According to the **synthetic method 8**, nerol (2 mL, 11.37 mmol), tetrabromomethane (5.66 g, 17.06 mmol), triphenylphosphine (4.47 g, 17.06 mmol) and dichloromethane (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 20:1), yielding neryl bromide (1.37 g, 6.31 mmol, 55 %) as a light yellow oil.

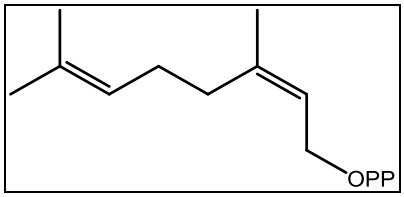


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.53 (t, *J* = 8.5 Hz, 1H), 5.12 (m, 1H), 4.01 (d, *J* = 8.5 Hz, 2H), 2.19 – 2.07 (m, 4H), 1.78 (s, 3H), 1.69 (s, 3H), 1.62 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 143.36, 132.35, 123.48, 121.36, 31.77, 29.38, 26.22, 25.68, 23.54, 17.68 ppm;

Neryl diphosphate (5d)

According to the **synthetic method 1**, DIPEA (1.29 mL, 7.60 mmol), acetone (592 µL, 8.06 mmol), water (66 µL, 3.68 mmol), tetrakis(trimethylsilyl) diphosphate (5.37 g, 11.51 mmol) and neryl bromide (500 mg, 2.30 mmol) was used. Purification was done by flash chromatography on silica (isopropanol/conc. ammonia/water, 12:6:2), yielding neryl diphosphate (196 mg, 0.54 mmol, 23 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.49 (t, *J* = 7.2 Hz, 1H), 5.22 (m, 1H), 4.47 (dd, *J* = 7.2, 6.4 Hz, 2H), 2.22 – 2.10 (m, 4H), 1.78 (s, 3H), 1.71 (s, 3H), 1.64 ppm (s, 3H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 145.43, 136.74, 126.85, 123.84 (d, *J* = 8.3 Hz), 65.04 (d, *J* = 5.2 Hz), 34.11, 28.88, 27.71, 25.45, 19.85 ppm;

^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.30 (d, *J* = 22.2 Hz), -10.29 ppm (dt, *J* = 22.2, 6.4 Hz);

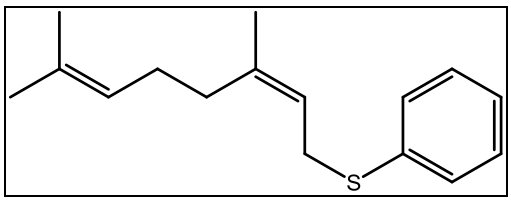
MS/ESI *m/z* = 313.4 ($[M\text{-}2NH_3\text{-}NH_4]^-$, 100 %), 627.4 ($[2M\text{-}5NH_3\text{-}NH_4]^-$, 39 %);

HRMS/ESI** calcd for $C_{10}H_{19}O_7P_2$: 313.0611 $[M\text{-}2NH_3\text{-}NH_4]^-$, found: 313.0608.

Neryl phenyl sulfide (5e)

According to the **synthetic method 6**, nerol (6.93 mL, 40.00 mmol), diphenyl disulfide (13.10 g, 60.00 mmol), tributylphosphine (14.80 mL, 60.00 mmol), sodium borohydride

(1.51 g, 40.00 mmol) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding neryl phenyl sulfide (8.59 g, 34.86 mmol, 87 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32 (m, 2H), 7.25 (m, 2H), 7.15 (m, 1H), 5.32 (t, *J* = 7.7 Hz, 1H), 5.09 (m, 1H), 3.55 (d, *J* = 7.7 Hz, 2H), 2.06 – 2.00 (m, 4H), 1.71 (s, 3H), 1.68 (s, 3H), 1.60 ppm (s, 3H);

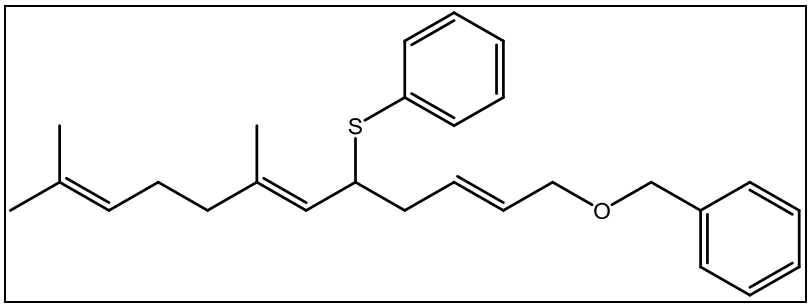
^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.95, 137.02, 131.90, 129.35, 128.68, 125.81, 123.87, 119.82, 31.90, 31.85, 26.52, 25.66, 23.32, 17.65 ppm;

MS/ESI *m/z* = 269.2 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{16}H_{23}S$: 247.1515 $[M+H]^+$, found: 247.1515.

(2*E*,6*E*)-1-(Benzyloxy)-7,11-dimethyl-5-(phenylthio)dodeca-2,6,10-triene (6a)

According to the **synthetic method 9**, geranyl phenyl sulfide (4.93 g, 20.00 mmol), (2*E*)-4-(benzyloxy)but-2-enyl chloride (3.93 g, 20.00 mmol), *n*-butyllithium (2.5 M in hexane, 8.80 mL, 22.00 mmol), HMPT (5.27 mL, 30.00 mmol) and THF (150 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*E*,6*E*)-1-(benzyloxy)-7,11-dimethyl-5-(phenylthio)dodeca-2,6,10-triene (7.62 g, 18.74 mmol, 94 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 – 7.38 (m, 2H), 7.35 – 7.30 (m, 4H), 7.30 – 7.19 (m,

4H), 5.76 – 5.58 (m, 2H), 5.09 – 4.99 (m, 2H), 4.48 (s, 2H), 3.96 (d, *J* = 5.7 Hz, 2H), 3.91 (ddd, *J* = 9.9, 8.4, 5.5 Hz, 1H), 2.44 (m, 1H), 2.32 (m, 1H), 2.06 – 1.88 (m, 4H), 1.66 (s, 3H), 1.57 (s, 3H), 1.40 ppm (s, 3H);

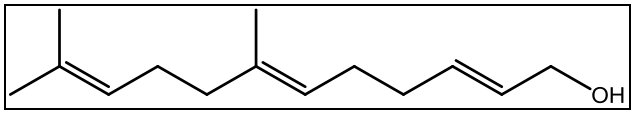
^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.32, 138.26, 134.62, 133.64, 131.44, 130.99, 128.68, 128.48, 128.28, 127.70, 127.47, 127.18, 125.02, 123.96, 71.69, 70.51, 46.88, 39.51, 38.22, 26.46, 25.63, 17.64, 16.32 ppm;

MS/ESI *m/z* = 429.3 ($[M+Na]^+$, 100 %), 835.5 ($[2M+Na]^+$, 48 %);

HRMS/ESI* calcd for $C_{27}H_{34}NaOS$: 429.2223 $[M+Na]^+$, found: 429.2217.

(2*E*,6*E*)-7,11-Dimethyldodeca-2,6,10-trienol (6b)

According to the **synthetic method 10**, lithium (1.18 g, 169.69 mmol), (2*E*,6*E*)-1-(benzyloxy)-7,11-dimethyl-5-(phenylthio)dodeca-2,6,10-triene (6.90 g, 16.97 mmol), ammonia (100 mL) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 4:1), yielding (2*E*,6*E*)-7,11-dimethyldodeca-2,6,10-trienol (2.58 g, 12.38 mmol, 73 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.75 – 5.60 (m, 2H), 5.13 (t, *J* = 6.7 Hz, 1H), 5.09 (t, *J* = 6.9 Hz, 1H), 4.08 (d, *J* = 4.9 Hz, 2H), 2.12 – 1.94 (m, 8H), 1.68 (s, 3H), 1.60 (s, 6H), 1.43 ppm (br, 1H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 135.53, 133.01, 131.29, 129.04, 124.27, 123.59, 63.77, 39.66, 32.40, 27.55, 26.68, 25.65, 17.65, 16.02 ppm;

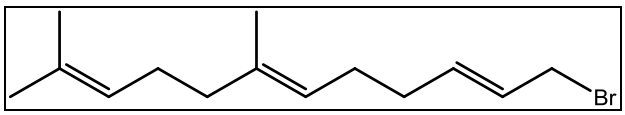
MS/ESI *m/z* = 231.2 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{14}H_{25}O$: 209.1900 $[M+H]^+$, found: 209.1901.

(2*E*,6*E*)-7,11-Dimethyldodeca-2,6,10-trienyl bromide (6c)

According to the **synthetic method 8**, (2*E*,6*E*)-7,11-dimethyldodeca-2,6,10-trienol (920 mg, 4.42 mmol), tetrabromomethane (1.76 g, 5.30 mmol), triphenylphosphine (1.39 g,

5.30 mmol) and dichloromethane (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 20:1), yielding (2*E*,6*E*)-7,11-dimethyldodeca-2,6,10-trienyl bromide (1.17 g, 4.31 mmol, 98 %) as a colorless oil.

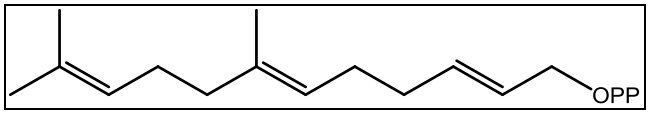


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.83 – 5.65 (m, 2H), 5.11 (t, *J* = 6.9 Hz, 1H), 5.09 (t, *J* = 6.9 Hz, 1H), 3.95 (d, *J* = 7.2 Hz, 2H), 2.14 – 1.95 (m, 8H), 1.68 (s, 3H), 1.60 (s, 3H), 1.59 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 136.27, 135.86, 131.34, 126.46, 124.28, 123.23, 39.67, 33.56, 32.27, 27.25, 26.68, 25.70, 17.69, 16.08 ppm.

(2*E*,6*E*)-7,11-Dimethyldodeca-2,6,10-trienyl diphosphate (6d)

According to the **synthetic method 1**, DIPEA (2.42 mL, 14.22 mmol), acetone (1.11 mL, 15.09 mmol), water (124 µL, 6.90 mmol), tetrakis(trimethylsilyl) diphosphate (10.06 g, 21.55 mmol) and (2*E*,6*E*)-7,11-dimethyldodeca-2,6,10-trienyl bromide (1.17 g, 4.31 mmol) was used. Purification was done by precipitation, yielding (2*E*,6*E*)-7,11-dimethyldodeca-2,6,10-trienyl diphosphate (550 mg, 1.31 mmol, 30 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.88 (m, 1H), 5.70 (m, 1H), 5.24 (m, 1H), 5.18 (t, *J* = 6.5 Hz, 1H), 4.39 (t, *J* = 5.9 Hz, 2H), 2.18 – 1.99 (m, 8H), 1.69 (s, 3H), 1.62 ppm (s, 6H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 139.39, 138.14, 135.87, 128.75 (d, *J* = 8.0 Hz), 127.28, 126.99, 69.44 (d, *J* = 5.4 Hz), 41.79, 34.73, 29.76, 28.78, 27.79, 19.89, 18.21 ppm;

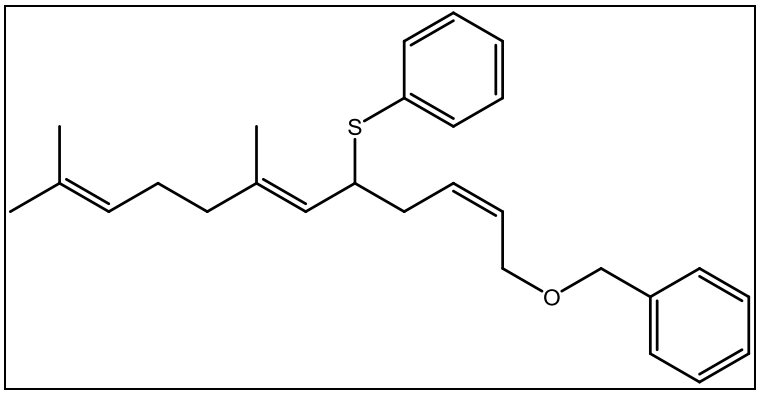
^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.35 (br), -10.03 ppm (br);

MS/ESI *m/z* = 366.9 ($[M\text{-}2NH_3\text{-}NH_4]^-$, 100 %), 736.2 ($[2M\text{-}5NH_3\text{-}NH_4]^-$, 29 %);

HRMS/ESI** calcd for $C_{14}H_{25}O_7P_2$: 367.1081 $[M-2NH_3-NH_4]^-$, found: 367.1078.

(2*Z*,6*E*)-1-(Benzyloxy)-7,11-dimethyl-5-(phenylthio)dodeca-2,6,10-triene (7a)

According to the **synthetic method 9**, geranyl phenyl sulfide (4.00 g, 16.23 mmol), (2*Z*)-4-(benzyloxy)but-2-enyl chloride (3.83 g, 19.48 mmol), *n*-butyllithium (2.5 M in hexane, 7.14 mL, 17.85 mmol), HMPT (4.28 mL, 24.35 mmol) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*Z*,6*E*)-1-(benzyloxy)-7,11-dimethyl-5-(phenylthio)dodeca-2,6,10-triene (5.22 g, 12.84 mmol, 79 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40 (m, 2H), 7.33 (m, 4H), 7.30 – 7.20 (m, 4H), 5.72 – 5.57 (m, 2H), 5.06 – 5.00 (m, 2H), 4.48 (s, 2H), 4.07 – 3.95 (m, 2H), 3.89 (ddd, *J* = 9.8, 8.7, 5.3 Hz, 1H), 2.47 (m, 1H), 2.27 (m, 1H), 2.07 – 1.88 (m, 4H), 1.66 (s, 3H), 1.58 (s, 3H), 1.40 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.57, 138.27, 134.58, 133.64, 131.52, 129.84, 128.52, 128.33, 127.98, 127.70, 127.54, 127.25, 124.84, 123.94, 72.19, 65.86, 46.98, 39.53, 33.39, 26.43, 25.63, 17.65, 16.30 ppm;

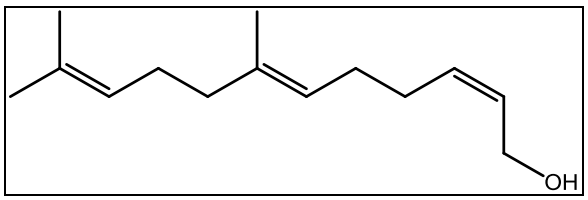
MS/ESI *m/z* = 429.3 ($[M+Na]^+$, 100 %), 835.4 ($[2M+Na]^+$, 19 %);

HRMS/ESI* calcd for $C_{27}H_{34}NaOS$: 429.2223 $[M+Na]^+$, found: 429.2232.

(2*Z*,6*E*)-7,11-Dimethyldodeca-2,6,10-trienol (7b)

According to the **synthetic method 10**, lithium (904 mg, 130.3 mmol), (2*Z*,6*E*)-1-(benzyloxy)-7,11-dimethyl-5-(phenylthio)dodeca-2,6,10-triene (5.30 g, 13.03 mmol), ammonia (100 mL) and THF (100 mL) was used. Purification was done by flash chromatography on silica

(*n*-hexane/EtOAc, 4:1), yielding (2*Z*,6*E*)-7,11-dimethyldodeca-2,6,10-trienol (1.46 g, 7.01 mmol, 54 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.72 – 5.50 (m, 2H), 5.12 (t, *J* = 6.9 Hz, 1H), 5.09 (t, *J* = 7.0 Hz, 1H), 4.18 (d, *J* = 6.3 Hz, 2H), 2.17 – 1.95 (m, 8H), 1.68 (s, 3H), 1.60 (s, 6H) 1.52 ppm (br, 1H);

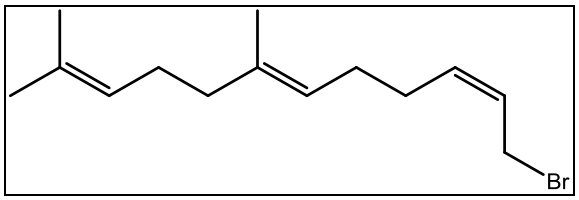
^{13}C NMR (101 MHz, $CDCl_3$): δ = 135.97, 132.68, 131.37, 128.59, 124.22, 123.44, 58.54, 39.68, 27.80, 27.62, 26.66, 25.66, 17.66, 16.05 ppm;

MS/ESI *m/z* = 231.5 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{14}H_{24}NaO$: 231.1719 $[M+Na]^+$, found: 231.1729.

(2*Z*,6*E*)-7,11-Dimethyldodeca-2,6,10-trienyl bromide (7c)

According to the **synthetic method 8**, (2*Z*,6*E*)-7,11-dimethyldodeca-2,6,10-trienol (950 mg, 4.56 mmol), tetrabromomethane (1.81 g, 5.47 mmol), triphenylphosphine (1.43 g, 5.47 mmol) and dichloromethane (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 20:1), yielding (2*Z*,6*E*)-7,11-dimethyldodeca-2,6,10-trienyl bromide (1.20 g, 4.42 mmol, 97 %) as a colorless oil.



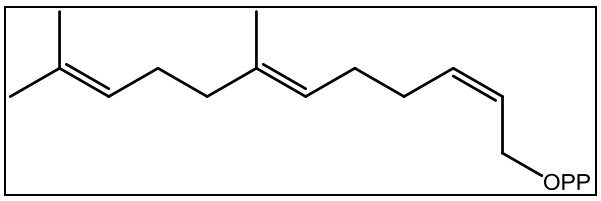
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.79 – 5.57 (m, 2H), 5.13 (t, *J* = 6.9 Hz, 1H), 5.09 (t, *J* = 6.9 Hz, 1H), 4.00 (d, *J* = 8.3 Hz, 2H), 2.22 – 1.95 (m, 8H), 1.68 (s, 3H), 1.61 ppm (s, 6H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 136.14, 135.51, 131.38, 125.43, 124.26, 123.19, 39.69,

27.37, 27.35, 27.17, 26.68, 25.69, 17.69, 16.09 ppm.

(2*Z*,6*E*)-7,11-Dimethyldodeca-2,6,10-trienyl diphosphate (7d)

According to the **synthetic method 1**, DIPEA (2.48 mL, 14.59 mmol), acetone (1.14 mL, 15.47 mmol), water (127 µL, 7.07 mmol), tetrakis(trimethylsilyl) diphosphate (10.31 g, 22.10 mmol) and (2*Z*,6*E*)-7,11-dimethyldodeca-2,6,10-trienyl bromide (1.20 g, 4.42 mmol) was used. Purification was done by precipitation, yielding (2*Z*,6*E*)-7,11-dimethyldodeca-2,6,10-trienyl diphosphate (756 mg, 1.80 mmol, 41 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.75 – 5.61 (m, 2H), 5.24 (t, *J* = 6.2 Hz, 1H), 5.20 (t, *J* = 6.3 Hz, 1H), 4.53 (t, *J* = 6.5 Hz, 2H), 2.23 – 2.00 (m, 8H), 1.70 (s, 3H), 1.63 ppm (s, 6H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 139.78, 137.05, 136.21, 128.57 (d, *J* = 7.9 Hz), 127.29, 126.92, 64.52 (d, *J* = 5.1 Hz), 41.72, 30.11, 29.84, 28.67, 27.73, 19.84, 18.19 ppm;

^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.31 (d, *J* = 21.8 Hz), -10.09 ppm (dt, *J* = 21.8, 6.5 Hz);

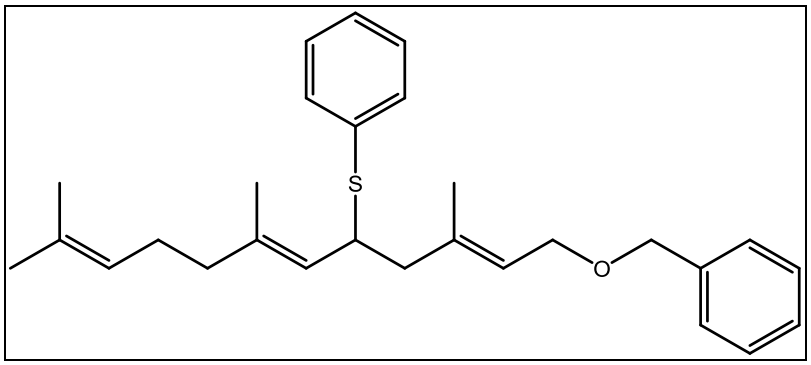
MS/ESI *m/z* = 367.2 ($[M-2NH_3-NH_4]^-$, 100 %), 735.6 ($[2M-5NH_3-NH_4]^-$, 25 %);

HRMS/ESI** calcd for $C_{14}H_{25}O_7P_2$: 367.1081 $[M-2NH_3-NH_4]^-$, found: 367.1078.

(2*E*,6*E*)-1-(Benzyloxy)-3,7,11-trimethyl-5-(phenylthio)dodeca-2,6,10-triene (8a)

According to the **synthetic method 9**, geranyl phenyl sulfide (500 mg, 2.03 mmol), (2*E*)-4-(benzyloxy)-2-methylbut-2-enyl chloride (514 mg, 2.44 mmol), *n*-butyllithium (2.5 M in hexane, 892 µL, 2.23 mmol), HMPT (536 µL, 3.05 mmol) and THF (20 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding

(2*E*,6*E*)-1-(Benzyloxy)-3,7,11-trimethyl-5-(phenylthio)dodeca-2,6,10-triene (731 mg, 1.74 mmol, 86 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.45 – 7.19 (m, 10H), 5.43 (t, *J* = 6.5 Hz, 1H), 5.05 – 4.97 (m, 2H), 4.47 (s, 2H), 4.05 (ddd, *J* = 10.0, 9.1, 5.7 Hz, 1H), 4.01 (d, *J* = 6.5 Hz, 2H), 2.44 (dd, *J* = 13.7, 5.7 Hz, 1H), 2.26 (dd, *J* = 13.7, 9.1 Hz, 1H), 2.01 – 1.87 (m, 4H), 1.65 (s, 3H), 1.62 (s, 3H), 1.57 (s, 3H), 1.35 ppm (s, 3H);

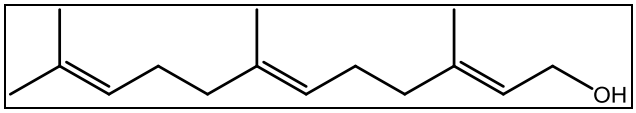
^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.49, 137.98, 137.26, 134.69, 133.94, 131.45, 128.47, 128.29, 127.77, 127.47, 127.27, 125.52, 124.02, 123.77, 71.71, 66.26, 45.71, 45.45, 39.53, 26.53, 25.63, 17.65, 16.55, 16.17 ppm;

MS/ESI *m/z* = 438.3 ($[M+NH_4]^+$, 43 %), 443.4 ($[M+Na]^+$, 100 %), 858.7 ($[2M+NH_4]^+$, 20 %), 863.8 ($[2M+Na]^+$, 49 %);

HRMS/ESI* calcd for $C_{28}H_{36}NaOS$: 443.2379 $[M+H]^+$, found: 443.2386.

(2*E*,6*E*)-Farnesol (8b)

According to the **synthetic method 10**, lithium (96 mg, 15 mmol), (2*E*,6*E*)-1-(Benzyloxy)-3,7,11-trimethyl-5-(phenylthio)dodeca-2,6,10-triene (631 mg, 1.50 mmol), ammonia (10 mL) and THF (10 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 4:1), yielding (2*E*,6*E*)-farnesol (227 mg, 1.02 mmol, 68 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.42 (t, *J* = 6.9 Hz, 1H), 5.11 (t, *J* = 6.9 Hz, 1H), 5.09 (t, *J* =

7.0 Hz, 1H), 4.15 (d, *J* = 6.9 Hz, 2H), 2.16 – 1.94 (m, 8H), 1.68 (s, 6H), 1.60 (s, 6H), 1.33 ppm (s, 1H);

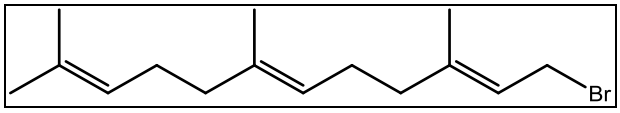
^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.78, 135.33, 131.30, 124.29, 123.75, 123.32, 59.38, 39.67, 39.52, 26.70, 26.28, 25.66, 17.66, 16.25, 15.98 ppm;

MS/ESI *m/z* = 245.3 ($[M+Na]^+$, 100 %), 261.3 ($[M+K]^+$, 17 %);

HRMS/ESI* calcd for $C_{15}H_{26}NaO$: 245.1876 $[M+Na]^+$, found: 245.1872.

(2*E*,6*E*)-Farnesyl bromide (8c)

According to the **synthetic method 8**, (2*E*,6*E*)-farnesol (250 mg, 1.12 mmol), tetrabromomethane (444 mg, 1.34 mmol), triphenylphosphine (351 mg, 1.34 mmol) and dichloromethane (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 20:1), yielding (2*E*,6*E*)-farnesyl bromide (300 mg, 1.05 mmol, 94 %) as a light yellow oil.

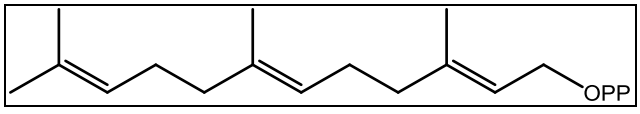


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.53 (t, *J* = 8.4 Hz, 1H), 5.12 – 5.05 (m, 2H), 4.02 (d, *J* = 8.4 Hz, 2H), 2.16 – 1.94 (m, 8H), 1.73 (s, 3H), 1.68 (s, 3H), 1.60 ppm (s, 6H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 143.52, 135.55, 131.25, 124.27, 123.33, 120.51, 39.62, 39.47, 29.62, 26.65, 26.04, 25.66, 17.66, 16.01, 15.94 ppm.

(2*E*,6*E*)-Farnesyl diphosphate (8d)

According to the **synthetic method 1**, DIPEA (3.70 mL, 21.76 mmol), acetone (1.70 mL, 23.12 mmol), water (187 µL, 10.38 mmol), tetrakis(trimethylsilyl) diphosphate (15.20 g, 32.57 mmol) and (2*E*,6*E*)-farnesyl bromide (2.05 g, 6.51 mmol) was used. Purification was done by precipitation, yielding (2*E*,6*E*)-farnesyl diphosphate (910 mg, 2.01 mmol, 32 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.46 (t, *J* = 6.6 Hz, 1H), 5.22 – 5.11 (m, 2H), 4.47 (t, *J* = 6.6 Hz, 2H), 2.20 – 1.97 (m, 8H), 1.73 (s, 3H), 1.68 (s, 3H), 1.62 (s, 3H), 1.61 ppm (s, 3H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 145.28, 138.80, 135.08, 127.28, 127.03, 122.77 (d, *J* = 8.7 Hz), 65.24 (d, *J* = 5.2 Hz), 42.02, 41.98, 29.03, 28.85, 27.93, 19.99, 18.62, 18.27 ppm;

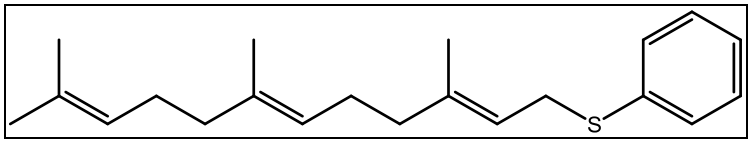
^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.36 (d, *J* = 21.2 Hz), -10.11 ppm (dt, *J* = 21.2, 6.6 Hz);

MS/ESI *m/z* = 381.1 ($[M\text{-}2NH_3\text{-}NH_4]^-$, 100 %), 763.6 ($[2M\text{-}5NH_3\text{-}NH_4]^-$, 33 %);

HRMS/ESI** calcd for $C_{15}H_{27}O_7P_2$: 381.1237 $[M\text{-}2NH_3\text{-}NH_4]^-$, found: 381.1237.

(2*E*,6*E*)-Farnesyl phenyl sulfide (8e)

According to the **synthetic method 6**, (2*E*,6*E*)-farnesol (10 g, 44.97 mmol), diphenyl disulfide (14.73 g, 67.46 mmol), tributylphosphine (16.64 mL, 67.46 mmol) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*E*,6*E*)-farnesyl phenyl sulfide (12.81 g, 40.73 mmol, 91 %) as a light yellow oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33 (d, *J* = 7.4 Hz, 2H), 7.25 (t, *J* = 7.4 Hz, 2H), 7.16 (t, *J* = 7.4 Hz, 1H), 5.31 (t, *J* = 7.7 Hz, 1H), 5.12 – 5.04 (m, 2H), 3.54 (d, *J* = 7.7 Hz, 2H), 2.10 – 1.93 (m, 8H), 1.68 (s, 3H), 1.60 (s, 3H), 1.58 (s, 3H), 1.57 ppm (s, 3H);

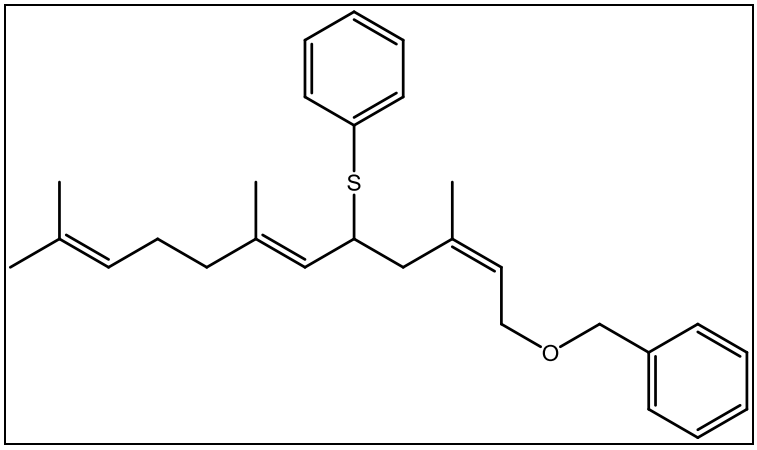
^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.82, 136.78, 135.21, 131.18, 129.80, 128.61, 125.91, 124.32, 123.74, 119.21, 39.64, 39.53, 32.15, 26.70, 26.33, 25.65, 17.64, 15.99, 15.96 ppm;

MS/ESI *m/z* = 337.3 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{21}H_{31}S$: 315.2141 $[M+H]^+$, found: 315.2144.

(2*Z*,6*E*)-1-(Benzyloxy)-3,7,11-trimethyl-5-(phenylthio)dodeca-2,6,10-triene (9a)

According to the **synthetic method 9**, geranyl phenyl sulfide (9.86 g, 40.00 mmol), (2*Z*)-4-(benzyloxy)-2-methylbut-2-enyl chloride (8.43 g, 40.00 mmol), *n*-butyllithium (2.5 M in hexane, 17.60 mL, 44.00 mmol), HMPT (10.54 mL, 60.00 mmol) and THF (200 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*Z*,6*E*)-1-(benzyloxy)-3,7,11-trimethyl-5-(phenylthio)dodeca-2,6,10-triene (14.17 g, 33.69 mmol, 84 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 – 7.38 (m, 2H), 7.35 – 7.31 (m, 4H), 7.31 – 7.20 (m, 4H), 5.46 (t, *J* = 6.7 Hz, 1H), 5.04 – 4.95 (m, 2H), 4.46 (s, 2H), 4.07 – 3.91 (m, 3H), 2.44 (dd, *J* = 13.5, 5.5 Hz, 1H), 2.30 (dd, *J* = 13.5, 9.3 Hz, 1H), 2.02 – 1.85 (m, 4H), 1.75 (s, 3H), 1.66 (s, 3H), 1.57 (s, 3H), 1.34 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.39, 138.16, 137.38, 134.61, 133.93, 131.43, 128.47, 128.28, 127.69, 127.46, 127.32, 125.25, 124.09, 123.93, 72.19, 66.46, 46.21, 39.50, 37.94, 26.35, 25.60, 23.82, 17.62, 16.07 ppm;

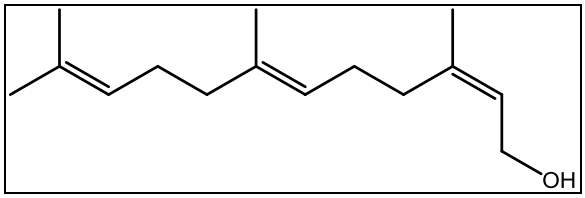
MS/ESI *m/z* = 443.6 ($[M+Na]^+$, 100 %), 863.9 ($[2M+Na]^+$, 12 %);

HRMS/ESI* calcd for $C_{28}H_{36}NaOS$: 443.2379 $[M+Na]^+$, found: 443.2378.

(2*Z*,6*E*)-Farnesol (9b)

According to the **synthetic method 10**, lithium (2.31 g, 332.81 mmol), (2*Z*,6*E*)-1-(benzyloxy)-3,7,11-trimethyl-5-(phenylthio)dodeca-2,6,10-triene (14.00 g, 33.28 mmol), ammonia (100 mL) and THF (100 mL) was used. Purification was done by flash chromatography on

silica (*n*-hexane/EtOAc, 4:1), yielding (2*Z*,6*E*)-farnesol (6.36 g, 28.60 mmol, 86 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44 (t, *J* = 7.2 Hz, 1H), 5.14 – 5.06 (m, 2H), 4.10 (d, *J* = 7.2 Hz, 2H), 2.15 – 1.95 (m, 8H), 1.75 (s, 3H), 1.68 (s, 3H), 1.60 ppm (s, 6H);

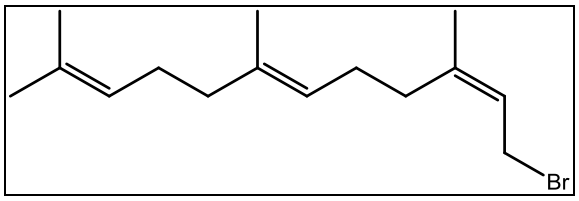
^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.86, 135.87, 131.36, 124.36, 124.18, 123.53, 58.95, 39.64, 31.92, 26.60, 26.46, 25.63, 23.39, 17.62, 15.94 ppm;

MS/ESI *m/z* = 245.3 ($[M+Na]^+$, 100 %), 261.3 ($[M+K]^+$, 6 %);

HRMS/ESI* calcd for $C_{15}H_{26}NaO$: 245.1876 $[M+Na]^+$, found: 245.1884.

(2*Z*,6*E*)-Farnesyl bromide (9c)

According to the **synthetic method 8**, (2*Z*,6*E*)-farnesol (100 mg, 450 µmol), tetrabromomethane (224 mg, 676 µmol), triphenylphosphine (177 mg, 676 µmol) and dichloromethane (20 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 20:1), yielding (2*Z*,6*E*)-farnesyl bromide (118 mg, 414 µmol, 92 %) as a light yellow oil.

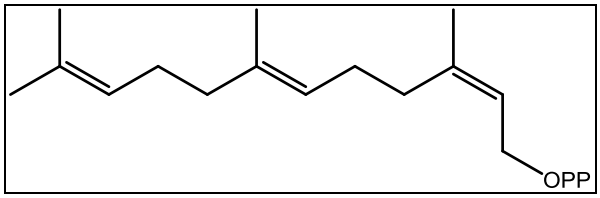


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.53 (t, *J* = 8.5 Hz, 1H), 5.13 (t, *J* = 6.9 Hz, 1H), 5.09 (t, *J* = 6.9 Hz, 1H), 4.01 (d, *J* = 8.5 Hz, 2H), 2.19 – 1.95 (m, 8H), 1.78 (s, 3H), 1.68 (s, 3H), 1.62 (s, 3H), 1.60 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 143.43, 135.98, 131.37, 124.25, 123.29, 121.36, 39.68, 31.75, 29.39, 26.66, 26.13, 25.69, 23.55, 17.68, 16.03 ppm.

(2*Z*,6*E*)-Farnesyl diphosphate (9d)

According to the **synthetic method 1**, DIPEA (296 µL, 1.74 mmol), acetone (136 µL, 1.85 mmol), water (15 µL, 0.84 mmol), tetrakis(trimethylsilyl) diphosphate (1.23 g, 2.64 mmol) and (2Z,6E)-farnesyl bromide (150 mg, 0.53 mmol) was used. Purification was done by flash chromatography on silica (isopropanol/conc. ammonia/water, 14:6:2), yielding (2*Z*,6*E*)-farnesyl diphosphate (100 mg, 0.23 mmol, 44 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.49 (t, *J* = 6.8 Hz, 1H), 5.26 – 5.17 (m, 2H), 4.47 (t, *J* = 6.8 Hz, 2H), 2.23 – 2.09 (m, 6H), 2.07 – 2.01 (m, 2H), 1.78 (s, 3H), 1.70 (s, 3H), 1.63 ppm (s, 6H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 145.63, 139.72, 136.37, 127.33, 126.97, 123.70 (d, *J* = 8.1 Hz), 65.03 (d, *J* = 5.2 Hz), 41.69, 34.13, 28.97, 28.64, 27.72, 25.58, 19.84, 18.13 ppm;

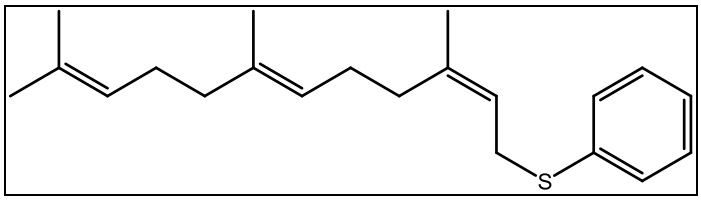
^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.06 (d, *J* = 22.0 Hz), -9.97 ppm (dt, *J* = 22.0, 6.8 Hz);

MS/ESI *m/z* = 381.3 ($[M\text{-}2NH_3\text{-}NH_4]^-$, 100 %), 763.4 ($[2M\text{-}5NH_3\text{-}NH_4]^-$, 40 %);

HRMS/ESI** calcd for $C_{15}H_{27}O_7P_2$: 381.1237 $[M\text{-}2NH_3\text{-}NH_4]^-$, found: 381.1234.

(2*Z*,6*E*)-Farnesyl phenyl sulfide (9e)

According to the **synthetic method 6**, (2*Z*,6*E*)-farnesol (6.20 g, 27.88 mmol), diphenyl disulfide (7.91 g, 36.24 mmol), tributylphosphine (8.35 mL, 33.46 mmol), sodium borohydride (1.055 g, 27.88 mmol) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*Z*,6*E*)-farnesyl phenyl sulfide (8.22 g, 26.13 mmol, 94 %) as a light yellow oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32 (d, *J* = 7.7 Hz, 2H), 7.25 (t, *J* = 7.4 Hz, 2H), 7.15 (t, *J* = 7.2 Hz, 1H), 5.32 (t, *J* = 7.7 Hz, 1H), 5.13 – 5.06 (m, 2H), 3.55 (d, *J* = 7.7 Hz, 2H), 2.11 – 1.94 (m, 8H), 1.72 (s, 3H), 1.68 (s, 3H), 1.60 ppm (s, 6H);

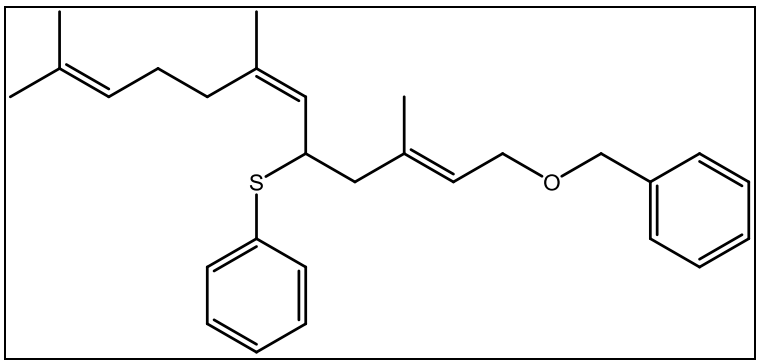
^{13}C NMR (101 MHz, $CDCl_3$): δ = 140.00, 137.02, 135.52, 131.26, 129.35, 128.67, 125.80, 124.29, 123.66, 119.78, 39.66, 31.87, 31.86, 26.66, 26.41, 25.66, 23.34, 17.66, 16.00 ppm;

MS/ESI *m/z* = 337.3 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{21}H_{31}S$: 315.2141 $[M+H]^+$, found: 315.2148.

(2*E*,6*Z*)-1-(Benzyloxy)-3,7,11-trimethyl-5-(phenylthio)dodeca-2,6,10-triene (10a)

According to the **synthetic method 9**, neryl phenyl sulfide (9.86 g, 40.00 mmol), (2*E*)-4-(benzyloxy)-2-methylbut-2-enyl chloride (8.43 g, 40.00 mmol), *n*-butyllithium (2.5 M in hexane, 17.60 mL, 44.00 mmol), HMPT (10.54 mL, 60.00 mmol) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*E*,6*Z*)-1-(benzyloxy)-3,7,11-trimethyl-5-(phenylthio)dodeca-2,6,10-triene (13.90 g, 33.04 mmol, 83 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40 (m, 2H), 7.32 (m, 4H), 7.29 – 7.20 (m, 4H), 5.32 (tq, *J* = 6.7, 1.3 Hz, 1H), 5.05 – 4.99 (m, 2H), 4.47 (s, 2H), 4.05 (ddd, *J* = 10.0, 8.7, 6.0 Hz, 1H), 4.01 (d, *J* = 6.7 Hz, 2H), 2.40 (dd, *J* = 13.6, 6.0 Hz, 1H), 2.25 (dd, *J* =

13.6, 8.7 Hz, 1H), 1.94 – 1.83 (m, 3H), 1.79 – 1.70 (m, 1H), 1.66 (s, 3H), 1.64 (d, *J* = 1.3 Hz, 3H), 1.62 (s, 3H), 1.56 ppm (s, 3H);

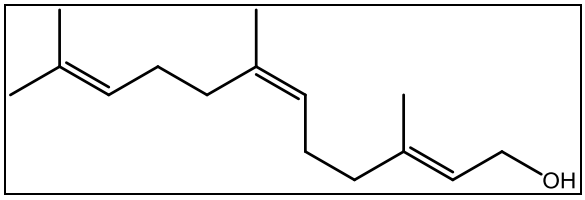
^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.49, 138.15, 137.16, 134.67, 133.80, 131.69, 128.50, 128.28, 127.77, 127.47, 127.26, 126.05, 124.04, 123.96, 71.67, 66.26, 45.78, 45.39, 32.13, 26.37, 25.67, 23.08, 17.64, 16.54 ppm;

MS/ESI *m/z* = 438.7 ($[M+NH_4]^+$, 39 %), 443.2 ($[M+Na]^+$, 100 %), 858.8 ($[2M+NH_4]^+$, 13 %), 863.9 ($[2M+Na]^+$, 57 %);

HRMS/ESI* calcd for $C_{28}H_{36}NaOS$: 443.2379 $[M+Na]^+$, found: 443.2395.

(2*E*,6*Z*)-Farnesol (10b)

According to the **synthetic method 10**, lithium (804 mg, 115.8 mmol), (2*E*,6*Z*)-1-(benzyloxy)-3,7,11-trimethyl-5-(phenylthio)dodeca-2,6,10-triene (4.87 g, 11.58 mmol), ammonia (100 mL) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 4:1), yielding (2*E*,6*Z*)-farnesol (2.11 g, 9.49 mmol, 82 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.42 (t, *J* = 6.9 Hz, 1H), 5.15 – 5.08 (m, 2H), 4.16 (d, *J* = 6.9 Hz, 2H), 2.15 – 1.99 (m, 8H), 1.70 – 1.67 (m, 9H), 1.61 ppm (s, 3H);

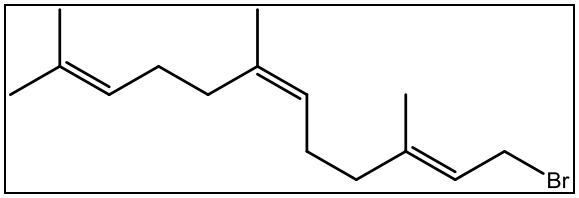
^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.74, 135.49, 131.55, 124.54, 124.26, 123.33, 59.39, 39.81, 31.96, 26.57, 26.17, 25.70, 23.35, 17.62, 16.26 ppm;

MS/ESI *m/z* = 245.2 ($[M+Na]^+$, 100 %), 261.2 ($[M+K]^+$, 25 %);

HRMS/ESI* calcd for $C_{15}H_{26}NaO$: 245.1876 $[M+Na]^+$, found: 245.1880.

(2*E*,6*Z*)-Farnesyl bromide (10c)

According to the **synthetic method 8**, (2*E*,6*Z*)-farnesol (449 mg, 2.02 mmol), tetrabromomethane (1.00 g, 3.03 mmol), triphenylphosphine (795 mg, 3.03 mmol) and dichloromethane (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 20:1), yielding (2*E*,6*Z*)-farnesyl bromide (553 mg, 1.94 mmol, 96 %) as a light yellow oil.

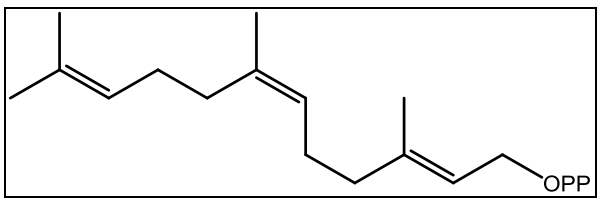


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.53 (t, *J* = 8.4 Hz, 1H), 5.15 – 5.05 (m, 2H), 4.01 (d, *J* = 8.4 Hz, 2H), 2.15 – 1.99 (m, 8H), 1.72 (s, 3H), 1.69 (s, 3H), 1.68 (s, 3H), 1.61 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 143.54, 135.71, 131.57, 124.23, 124.20, 120.52, 39.79, 31.96, 29.63, 26.55, 25.98, 25.71, 23.34, 17.63, 15.96 ppm.

(2*E*,6Z)-Farnesyl diphosphate (10d)

According to the **synthetic method 1**, DIPEA (983 µL, 5.78 mmol), acetone (451 µL, 6.13 mmol), water (50 µL, 2.80 mmol), tetrakis(trimethylsilyl) diphosphate (4.08 g, 8.75 mmol) and (2*E*,6*Z*)-farnesyl bromide (500 mg, 1.75 mmol) was used. Purification was done by precipitation, yielding (2*E*,6*Z*)-farnesyl diphosphate (269 mg, 0.62 mmol, 35 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.47 (t, *J* = 7.0 Hz, 1H), 5.27 – 5.18 (m, 2H), 4.48 (t, *J* = 6.4 Hz, 2H), 2.21 – 2.05 (m, 8H), 1.73 (s, 3H), 1.70 (s, 6H), 1.64 ppm (s, 3H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 145.33, 139.64, 136.19, 127.87, 127.17, 122.89 (d, *J* = 8.7 Hz), 65.27 (d, *J* = 5.1 Hz), 42.08, 34.09, 28.77, 28.42, 27.82, 25.39, 19.83, 18.45 ppm;

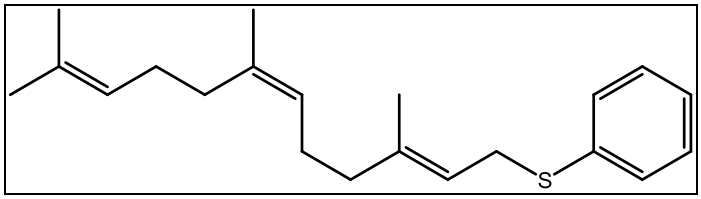
^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.52 (br), -10.29 ppm (br);

MS/ESI *m/z* = 381.4 ($[M-2NH_3-NH_4]^-$, 100 %), 763.6 ($[2M-5NH_3-NH_4]^-$, 41 %);

HRMS/ESI** calcd for $C_{15}H_{27}O_7P_2$: 381.1237 $[M-2NH_3-NH_4]^-$, found: 381.1234.

(2*E*,6*Z*)-Farnesyl phenyl sulfide (10e)

According to the **synthetic method 6**, (2*E*,6*Z*)-farnesol (5.20 g, 23.38 mmol), diphenyl disulfide (6.64 g, 30.39 mmol), tributylphosphine (7.01 mL, 28.06 mmol), sodium borohydride (884 mg, 23.38 mmol) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*E*,6*Z*)-farnesyl phenyl sulfide (6.96 g, 22.13 mmol, 95 %) as a light yellow oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34 (d, *J* = 7.3 Hz, 2H), 7.25 (t, *J* = 7.5 Hz, 2H), 7.16 (t, *J* = 7.3 Hz, 1H), 5.31 (t, *J* = 7.7 Hz, 1H), 5.11 (m, 1H), 5.07 (t, *J* = 7.0 Hz, 1H), 3.54 (d, *J* = 7.7 Hz, 2H), 2.09 – 1.96 (m, 8H), 1.68 (s, 3H), 1.67 (s, 3H), 1.61 (s, 3H), 1.57 ppm (s, 3H);

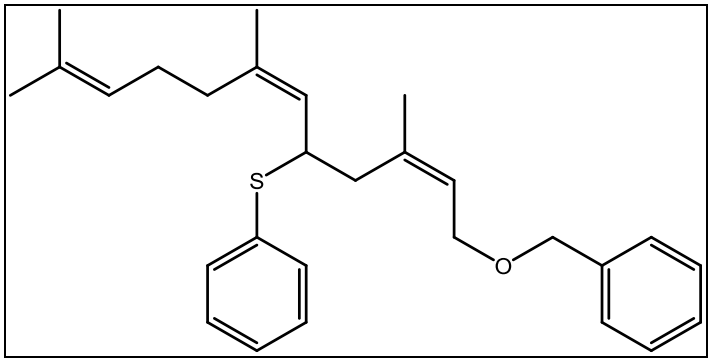
^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.81, 136.71, 135.37, 131.50, 129.91, 128.64, 125.98, 124.57, 124.29, 119.26, 39.83, 32.19, 31.96, 26.57, 26.23, 25.71, 23.33, 17.62, 16.00 ppm;

MS/ESI *m/z* = 337.4 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{21}H_{31}S$: 315.2141 $[M+H]^+$, found: 315.2146.

(2*Z*,6*Z*)-1-(Benzyloxy)-3,7,11-trimethyl-5-(phenylthio)dodeca-2,6,10-triene (11a)

According to the **synthetic method 9**, neryl phenyl sulfide (9.44 g, 30.00 mmol), (2*Z*)-4-(benzyloxy)-2-methylbut-2-enyl chloride (6.32 g, 30.00 mmol), *n*-butyllithium (2.5 M in hexane, 13.20 mL, 33.00 mmol), HMPT (7.91 mL, 45.00 mmol) and THF (150 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*Z*,6*Z*)-1-(benzyloxy)-3,7,11-trimethyl-5-(phenylthio)dodeca-2,6,10-triene (11.63 g, 27.65 mmol, 92 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42 – 7.38 (m, 2H), 7.36 – 7.30 (m, 4H), 7.30 – 7.20 (m, 4H), 5.46 (t, *J* = 6.7 Hz, 1H), 5.04 – 4.96 (m, 2H), 4.45 (s, 2H), 4.04 – 3.89 (m, 3H), 2.41 (dd, *J* = 13.5, 5.5 Hz, 1H), 2.27 (dd, *J* = 13.5, 9.1 Hz, 1H), 1.94 – 1.81 (m, 3H), 1.77 – 1.68 (m, 1H), 1.74 (s, 3H), 1.65 (s, 3H), 1.63 (s, 3H), 1.56 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.46, 138.40, 137.21, 134.64, 133.87, 131.70, 128.56, 128.33, 127.73, 127.50, 127.39, 125.89, 124.35, 124.00, 72.21, 66.53, 46.02, 38.32, 32.05, 26.42, 25.66, 23.94, 23.10, 17.66 ppm;

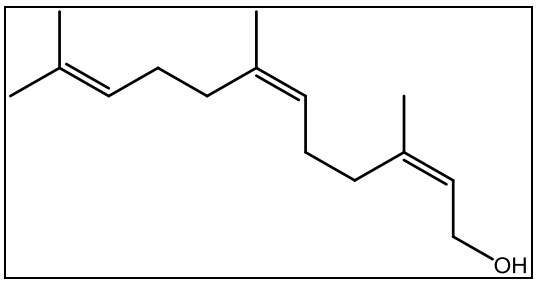
MS/ESI *m/z* = 443.6 ($[M+Na]^+$, 100 %), 863.9 ($[2M+Na]^+$, 53 %);

HRMS/ESI* calcd for $C_{28}H_{36}NaOS$: 443.2379 $[M+Na]^+$, found: 443.2369.

(2*Z*,6*Z*)-Farnesol (11b)

According to the **synthetic method 10**, lithium 1.98 mg, 285.27 mmol), (2*Z*,6*Z*)-1-(benzyloxy)-3,7,11-trimethyl-5-(phenylthio)dodeca-2,6,10-triene (12.00 g, 28.53 mmol), ammonia (50 mL) and THF (100 mL) was used. Purification was done by flash

chromatography on silica (*n*-hexane/EtOAc, 4:1), yielding (2*Z*,6*Z*)-farnesol (4.41 g, 19.83 mmol, 70 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.45 (t, *J* = 7.2 Hz, 1H), 5.15 – 5.06 (m, 2H), 4.09 (d, *J* = 7.2 Hz, 2H), 2.14 – 1.99 (m, 8H), 1.75 (s, 3H), 1.69 (s, 6H), 1.61 ppm (s, 3H);

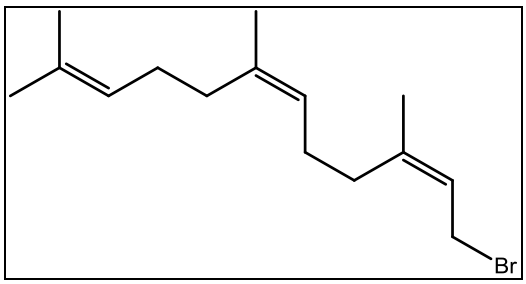
^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.90, 136.14, 131.64, 124.49, 124.43, 124.17, 59.00, 32.22, 31.92, 26.62, 26.31, 25.70, 23.45, 23.31, 17.63 ppm;

MS/ESI *m/z* = 245.3 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{15}H_{26}NaO$: 245.1876 $[M+Na]^+$, found: 245.1872.

(2*Z*,6*Z*)-Farnesyl bromide (11c)

According to the **synthetic method 8**, (2*Z*,6*Z*)-farnesol (985 mg, 4.43 mmol), tetrabromomethane (2.21 g, 6.65 mmol), triphenylphosphine (1.74 g, 6.65 mmol) and dichloromethane (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 20:1), yielding (2*Z*,6*Z*)-farnesyl bromide (1.15 g, 4.03 mmol, 91 %) as a light yellow oil.

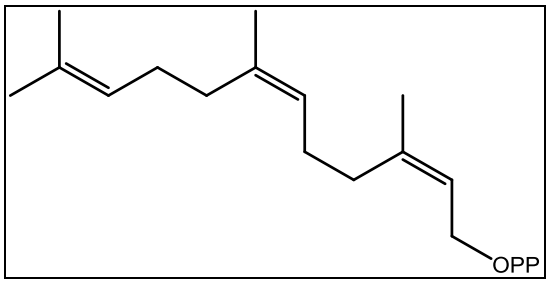


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.53 (t, *J* = 8.5 Hz, 1H), 5.16 – 5.08 (m, 2H), 4.00 (d, *J* = 8.5 Hz, 2H), 2.21 – 1.97 (m, 8H), 1.77 (s, 3H), 1.69 (s, 6H), 1.61 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 143.29, 136.07, 131.59, 124.19, 124.17, 121.39, 32.05, 31.94, 29.33, 26.59, 26.01, 25.72, 23.57, 23.34, 17.65 ppm.

(2*Z*,6*Z*)-Farnesyl diphosphate (11d)

According to the **synthetic method 1**, DIPEA (2.17 mL, 12.74 mmol), acetone (993 µL, 13.51 mmol), water (111 µL, 6.18 mmol), tetrakis(trimethylsilyl) diphosphate (9.01 g, 19.30 mmol) and (2*Z*,6*Z*)-farnesyl bromide (1.10 g, 3.86 mmol) was used. Purification was done by precipitation, yielding (2*Z*,6*Z*)-farnesyl diphosphate (335 mg, 0.77 mmol, 20 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.49 (t, *J* = 7.1 Hz, 1H), 5.26 – 5.17 (m, 2H), 4.46 (t, *J* = 6.8 Hz, 2H), 2.22 – 2.06 (m, 8H), 1.78 (s, 3H), 1.70 (s, 6H), 1.64 ppm (s, 3H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 145.27, 140.01, 136.48, 127.75, 127.21, 123.85 (d, *J* = 8.2 Hz), 65.03 (d, *J* = 5.2 Hz), 34.35, 34.02, 28.76, 28.75, 27.75, 25.51, 25.32, 19.80 ppm;

^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.25 (br), -10.35 ppm (br);

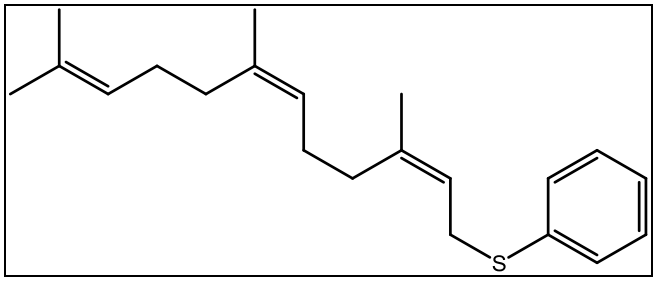
MS/ESI *m/z* = 381.5 ($[M\text{-}2NH_3\text{-}NH_4]^-$, 100 %), 763.6 ($[2M\text{-}5NH_3\text{-}NH_4]^-$, 40 %);

HRMS/ESI** calcd for $C_{15}H_{27}O_7P_2$: 381.1237 $[M\text{-}2NH_3\text{-}NH_4]^-$, found: 381.1232.

(2*Z*,6*Z*)-Farnesyl phenyl sulfide (11e)

According to the **synthetic method 6**, (2*Z*,6*Z*)-farnesol (4.41 g, 19.83 mmol), diphenyl disulfide (6.50 g, 29.75 mmol), tributylphosphine (7.43 mL, 29.75 mmol), sodium borohydride (750 mg, 19.83 mmol) and THF (100 mL) was used. Purification was done by

flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*Z*,6*Z*)-farnesyl phenyl sulfide (4.93 g, 15.67 mmol, 79 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32 (d, *J* = 7.4 Hz, 2H), 7.25 (t, *J* = 7.4 Hz, 2H), 7.15 (t, *J* = 7.4 Hz, 1H), 5.32 (t, *J* = 7.7 Hz, 1H), 5.14 – 5.08 (m, 2H), 3.55 (d, *J* = 7.7 Hz, 2H), 2.09 – 1.99 (m, 8H), 1.71 (s, 3H), 1.69 (s, 3H), 1.67 (s, 3H), 1.60 ppm (s, 3H);

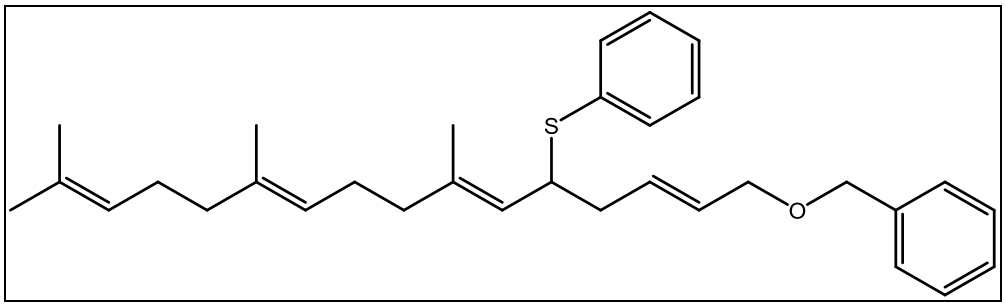
^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.90, 137.02, 135.66, 131.49, 129.32, 128.67, 125.79, 124.55, 124.24, 119.85, 32.18, 31.93, 31.83, 26.61, 26.31, 25.68, 23.37, 23.34, 17.62 ppm;

MS/ESI *m/z* = 337.3 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{21}H_{31}S$: 315.2141 $[M+H]^+$, found: 315.2144.

(2*E*,6*E*,10*E*)-1-(Benzyloxy)-7,11,15-trimethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (12a)

According to the **synthetic method 9**, farnesyl phenyl sulfide (1.50 g, 4.77 mmol), (2*E*)-4-(benzyloxy)but-2-enyl chloride (1.12 g, 5.72 mmol), *n*-butyllithium (2.5 M in hexane, 2.10 mL, 5.25 mmol), HMPT (1.26 mL, 7.16 mmol) and THF (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*E*,6*E*,10*E*)-1-(benzyloxy)-7,11,15-trimethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (1.98 g, 4.17 mmol, 87 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44 – 7.20 (m, 10H), 5.76 – 5.59 (m, 2H), 5.12 – 5.02 (m, 3H), 4.48 (s, 2H), 3.96 (d, *J* = 5.8 Hz, 2H), 3.92 (ddd, *J* = 9.9, 8.4, 5.6 Hz, 1H), 2.44 (m, 1H), 2.32 (m, 1H), 2.10 – 1.90 (m, 8H), 1.68 (s, 3H), 1.59 (s, 3H), 1.57 (s, 3H), 1.40 ppm (s, 3H);

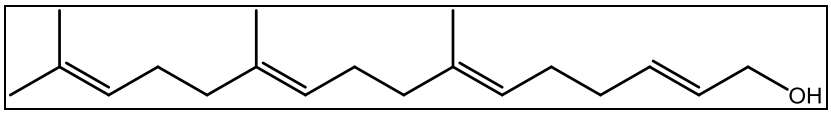
^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.35, 138.33, 135.13, 134.64, 133.65, 131.21, 131.00, 128.68, 128.49, 128.29, 127.71, 127.48, 127.19, 124.98, 124.30, 123.81, 71.69, 70.52, 46.91, 39.66, 39.54, 38.23, 26.71, 26.46, 25.66, 17.65, 16.37, 15.97 ppm;

MS/ESI *m/z* = 497.3 ($[M+Na]^+$, 100 %), 971.8 ($[2M+Na]^+$, 25 %);

HRMS/ESI* calcd for $C_{32}H_{42}NaOS$: 497.2849 $[M+Na]^+$, found: 497.2856.

(2*E*,6*E*,10*E*)-7,11,15-Trimethylhexadeca-2,6,10,14-tetraenol (12b)

According to the **synthetic method 10**, lithium (260 mg, 37.49 mmol), (2*E*,6*E*,10*E*)-1-(benzyloxy)-7,11,15-trimethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (1.78 g, 3.75 mmol), ammonia (50 mL) and THF (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 4:1), yielding (2*E*,6*E*,10*E*)-7,11,15-trimethylhexadeca-2,6,10,14-tetraenol (792 mg, 2.86 mmol, 76 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.74 – 5.59 (m, 2H), 5.16 – 5.06 (m, 3H), 4.07 (d, *J* = 5.0 Hz, 2H), 2.12 – 1.94 (m, 12H), 1.68 (s, 3H), 1.60 (s, 9H), 1.52 ppm (br, 1H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 135.51, 134.90, 132.95, 131.20, 129.03, 124.34, 124.12, 123.59, 63.72, 39.68, 39.64, 32.40, 27.56, 26.72, 26.56, 25.64, 17.62, 16.01,

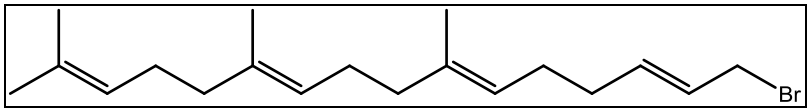
15.96 ppm;

MS/ESI *m/z* = 299.5 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{19}H_{32}NaO$: 299.2345 $[M+Na]^+$, found: 299.2346.

(2*E*,6*E*,10*E*)-7,11,15-Trimethylhexadeca-2,6,10,14-tetraenyl bromide (12c)

According to the **synthetic method 8**, (2*E*,6*E*,10*E*)-7,11,15-trimethylhexadeca-2,6,10,14-tetraenol (750 mg, 2.71 mmol), tetrabromomethane (1.35 g, 4.07 mmol), triphenylphosphine (1.07 g, 4.07 mmol) and dichloromethane (25 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 20:1), yielding (2*E*,6*E*,10*E*)-7,11,15-trimethylhexadeca-2,6,10,14-tetraenyl bromide (896 mg, 2.64 mmol, 97 %) as a light yellow oil.

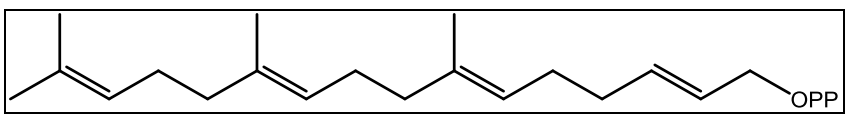


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.82 – 5.64 (m, 2H), 5.15 – 5.05 (m, 3H), 3.94 (d, *J* = 7.2 Hz, 2H), 2.16 – 1.94 (m, 12H), 1.68 (s, 3H), 1.60 ppm (s, 9H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 136.20, 135.84, 134.93, 131.21, 126.46, 124.36, 124.13, 123.23, 39.71, 39.65, 33.48, 32.27, 27.25, 26.75, 26.56, 25.68, 17.67, 16.08, 16.01 ppm.

(2*E*,6*E*,10*E*)-7,11,15-Trimethylhexadeca-2,6,10,14-tetraenyl diphosphate (12d)

According to the **synthetic method 1**, DIPEA (1.48 mL, 8.71 mmol), acetone (679 µL, 9.24 mmol), water (76 µL, 4.22 mmol), tetrakis(trimethylsilyl) diphosphate (6.16 g, 13.20 mmol) and (2*E*,6*E*,10*E*)-7,11,15-trimethylhexadeca-2,6,10,14-tetraenyl bromide (896 mg, 2.64 mmol) was used. Purification was done by precipitation, yielding (2*E*,6*E*,10*E*)-7,11,15-trimethylhexadeca-2,6,10,14-tetraenyl diphosphate (296 mg, 607 µmol, 23 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + CD_3OD + ND_4OD): δ = 5.86 (m, 1H), 5.68 (m, 1H), 5.19 – 5.06 (m, 3H), 4.38 (t, *J* = 6.5 Hz, 2H), 2.14 – 1.91 (m, 12H), 1.65 (s, 3H), 1.61 (s, 3H), 1.58 ppm (s, 6H);

^{13}C NMR (101 MHz, D_2O + CD_3OD + ND_4OD): δ = 138.50, 137.96, 137.59, 133.77, 128.76 (d, *J* = 8.0 Hz), 127.32, 127.16, 126.76, 69.37 (d, *J* = 4.2 Hz), 42.46, 42.41, 35.19, 30.27, 29.45, 29.45, 28.17, 20.16, 18.55, 18.54 ppm;

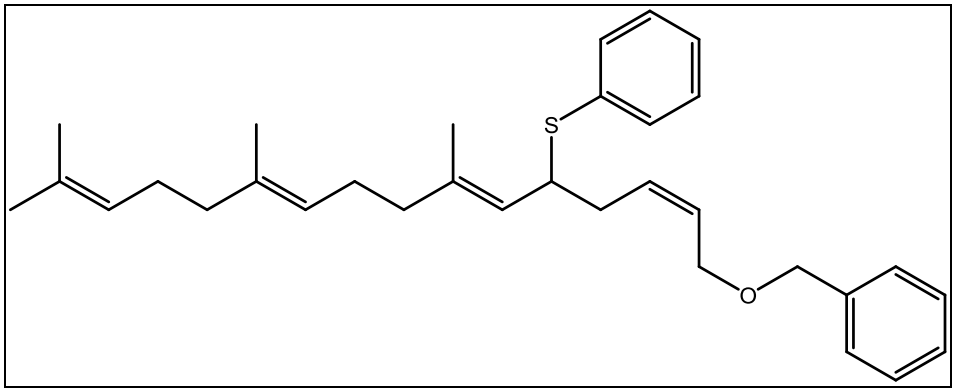
^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.63 (br), -10.30 ppm (br);

MS/ESI *m/z* = 435.3 ($[M-2NH_3-NH_4]^-$, 100 %), 872.0 ($[2M-5NH_3-NH_4]^-$, 38 %);

HRMS/ESI* calcd for $C_{19}H_{33}O_7P_2$: 435.1707 $[M-2NH_3-NH_4]^-$, found: 435.1711.

(2*Z*,6*E*,10*E*)-1-(Benzyloxy)-7,11,15-trimethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (13a)

According to the **synthetic method 9**, farnesyl phenyl sulfide (2.00 g, 6.36 mmol), (2*Z*)-4-(benzyloxy)but-2-enyl chloride (1.50 g, 7.63 mmol), *n*-butyllithium (2.5 M in hexane, 2.80 mL, 7.00 mmol), HMPT (1.68 mL, 9.54 mmol) and THF (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*Z*,6*E*,10*E*)-1-(benzyloxy)-7,11,15-trimethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (2.44 g, 5.14 mmol, 81 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 – 7.20 (m, 10H), 5.71 – 5.57 (m, 2H), 5.13 – 5.00 (m, 3H), 4.47 (s, 2H), 4.05 – 3.99 (m, 2H), 3.89 (ddd, *J* = 9.9, 8.7, 5.4 Hz, 1H), 2.46 (m, 1H), 2.27 (m, 1H), 2.12 – 1.89 (m, 8H), 1.68 (s, 3H), 1.60 (s, 3H), 1.58 (s,

3H), 1.41 ppm (s, 3H);

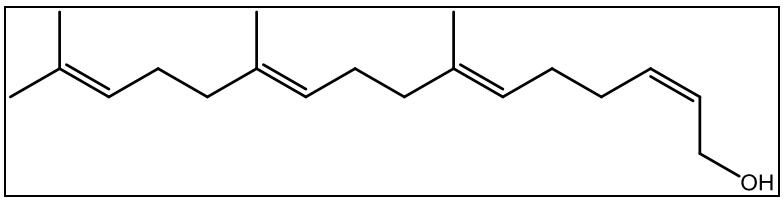
^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.67, 138.28, 135.20, 134.61, 133.64, 131.26, 129.84, 128.54, 128.34, 128.01, 127.71, 127.56, 127.25, 124.79, 124.31, 123.78, 72.19, 65.87, 47.01, 39.67, 39.56, 33.40, 26.72, 26.43, 25.68, 17.67, 16.36, 16.00 ppm;

MS/ESI *m/z* = 492.5 ($[M+NH_4]^+$, 38 %), 497.4 ($[M+Na]^+$, 100 %), 966.9 ($[2M+NH_4]^+$, 4 %), 971.9 ($[2M+Na]^+$, 22 %);

HRMS/ESI* calcd for $C_{32}H_{42}NaOS$: 497.2849 $[M+Na]^+$, found: 497.2842.

(2*Z*,6*E*,10*E*)-7,11,15-trimethylhexadeca-2,6,10,14-tetraenol (13b)

According to the **synthetic method 10**, lithium (321 mg, 46.30 mmol), (2*Z*,6*E*,10*E*)-1-(benzyloxy)-7,11,15-trimethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (2.20 g, 4.63 mmol), ammonia (50 mL) and THF (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 5:1), yielding (2*Z*,6*E*,10*E*)-7,11,15-trimethylhexadeca-2,6,10,14-tetraenol (869 mg, 3.14 mmol, 68 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.66 – 5.51 (m, 2H), 5.16 – 5.06 (m, 3H), 4.18 (d, *J* = 6.2 Hz, 2H), 2.18 – 1.94 (m, 12H), 1.68 (s, 3H), 1.60 (s, 9H), 1.34 ppm (s, 1H);

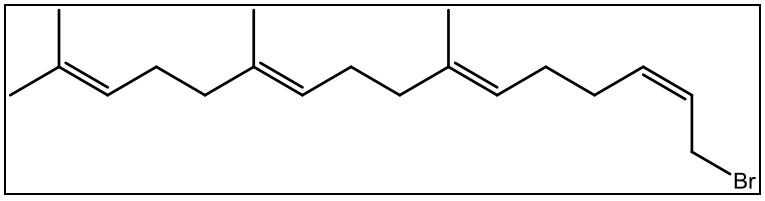
^{13}C NMR (101 MHz, $CDCl_3$): δ = 136.01, 135.01, 132.69, 131.25, 128.59, 124.36, 124.09, 123.45, 58.55, 39.70, 39.68, 27.83, 27.64, 26.74, 26.57, 25.67, 17.66, 16.08, 15.99 ppm;

MS/ESI *m/z* = 299.8 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{19}H_{33}O$: 277.2526 $[M+H]^+$, found: 277.2538.

(2*Z*,6*E*,10*E*)-7,11,15-Trimethylhexadeca-2,6,10,14-tetraenyl bromide (13c)

According to the **synthetic method 8**, (2*Z*,6*E*,10*E*)-7,11,15-trimethylhexadeca-2,6,10,14-tetraenol (861 mg, 3.11 mmol), tetrabromomethane (1.55 g, 4.67 mmol), triphenylphosphine (1.22 g, 4.67 mmol) and dichloromethane (25 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 20:1), yielding (2*Z*,6*E*,10*E*)-7,11,15-trimethylhexadeca-2,6,10,14-tetraenyl bromide (1.05 g, 3.08 mmol, 99 %) as a light yellow oil.

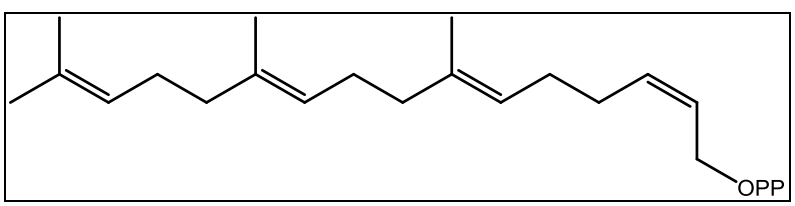


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.73 (m, 1H), 3.62 (dt, *J* = 10.6, 7.2 Hz, 1H), 5.16 – 5.06 (m, 3H), 3.99 (d, *J* = 8.3 Hz, 2H), 2.22 – 1.94 (m, 12H), 1.68 (s, 3H), 1.61 (s, 3H), 1.60 ppm (s, 6H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 136.15, 135.48, 134.99, 131.23, 125.44, 124.36, 124.11, 123.19, 39.71, 39.67, 27.38, 27.30, 27.18, 26.75, 26.58, 25.69, 17.68, 16.11, 16.01 ppm.

(2*Z*,6*E*,10*E*)-7,11,15-Trimethylhexadeca-2,6,10,14-tetraenyl diphosphate (13d)

According to the **synthetic method 1**, DIPEA (1.66 mL, 9.74 mmol), acetone (759 µL, 10.33 mmol), water (85 µL, 4.72 mmol), tetrakis(trimethylsilyl) diphosphate (6.88 g, 14.75 mmol) and (2*Z*,6*E*,10*E*)-7,11,15-trimethylhexadeca-2,6,10,14-tetraenyl bromide (1.00 g, 2.95 mmol) was used. Purification was done by precipitation, yielding (2*Z*,6*E*,10*E*)-7,11,15-trimethylhexadeca-2,6,10,14-tetraenyl diphosphate (308 mg, 632 µmol, 21 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.71 – 5.57 (m, 2H), 5.18 (t, *J* = 6.7 Hz, 1H), 5.15

– 5.06 (m, 2H), 4.51 (m, 2H), 2.20 – 1.93 (m, 12H), 1.65 (s, 3H), 1.60 (s, 3H), 1.58 (s, 3H), 1.57 ppm (s, 3H);

^{13}C NMR (101 MHz, D_2O + CD_3OD + ND_4OD): δ = 138.84, 137.60, 136.42, 133.77, 128.73 (d, *J* = 6.7 Hz), 127.32, 127.19, 126.63, 64.47 (d, *J* = 4.6 Hz), 42.46, 42.41, 30.52, 29.99, 29.47, 29.45, 28.16, 20.15, 18.61, 18.53 ppm;

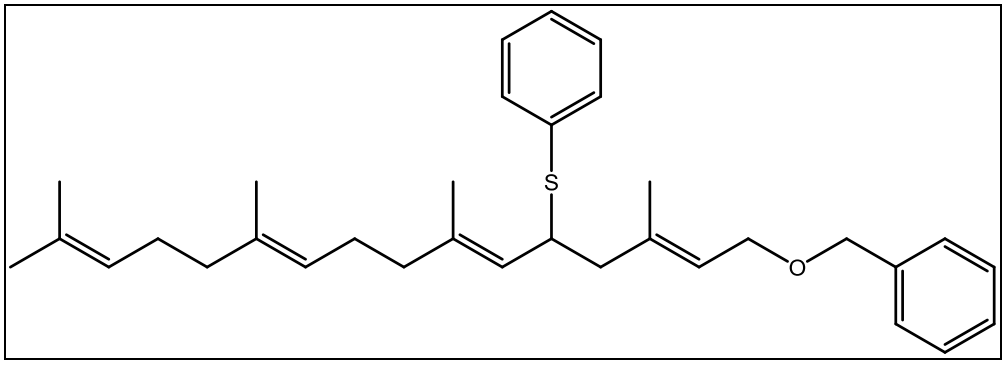
^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.63 (br), -9.89 ppm (br);

MS/ESI *m/z* = 435.5 ($[M-2NH_3-NH_4]^-$, 100 %), 871.8 ($[2M-5NH_3-NH_4]^-$, 31 %);

HRMS/ESI* calcd for $C_{19}H_{33}O_7P_2$: 435.1707 $[M-2NH_3-NH_4]^-$, found: 435.1702.

(2*E*,6*E*,10*E*)-1-(Benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (14a)

According to the **synthetic method 9**, farnesyl phenyl sulfide (638 mg, 2.03 mmol), (2*E*)-4-(benzyloxy)-2-methylbut-2-enyl chloride (514 mg, 2.44 mmol), *n*-butyllithium (2.5 M in hexane, 892 µL, 2.23 mmol), HMPT (536 µL, 3.05 mmol) and THF (20 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*E*,6*E*,10*E*)-1-(benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (877 mg, 1.79 mmol, 88 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44 – 7.21 (m, 10H), 5.43 (t, *J* = 6.7 Hz, 1H), 5.12 – 4.99 (m, 3H), 4.47 (s, 2H), 4.04 (ddd, *J* = 9.6, 9.0, 5.8 Hz, 1H), 4.01 (d, *J* = 6.7 Hz, 2H), 2.44 (dd, *J* = 13.8, 5.8 Hz, 1H), 2.26 (dd, *J* = 13.8, 9.0 Hz, 1H), 2.09 – 1.88 (m, 8H), 1.68 (s, 3H), 1.62 (s, 3H), 1.60 (s, 3H), 1.57 (s, 3H), 1.36 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.51, 138.07, 137.27, 135.15, 134.72, 133.94, 131.27,

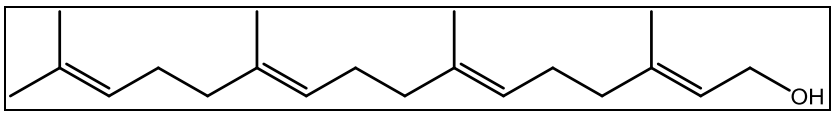
128.48, 128.30, 127.77, 127.48, 127.27, 125.50, 124.33, 123.89, 123.78, 71.70, 66.26, 45.73, 45.46, 39.68, 39.57, 26.74, 26.55, 25.69, 17.68, 16.57, 16.23, 15.96 ppm;

MS/ESI *m/z* = 506.5 ($[M+NH_4]^+$, 33 %), 511.1 ($[M+Na]^+$, 100 %), 994.9 ($[2M+NH_4]^+$, 12 %), 999.6 ($[2M+Na]^+$, 47 %);

HRMS/ESI* calcd for $C_{33}H_{44}NaOS$: 511.3005 $[M+H]^+$, found: 511.3002.

(2*E*,6*E*,10*E*)-Geranylgeraniol (14b)

According to the **synthetic method 10**, lithium (96 mg, 15 mmol), (2*E*,6*E*,10*E*)-1-(Benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (733 mg, 1.50 mmol), ammonia (10 mL) and THF (10 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 4:1), yielding (2*E*,6*E*,10*E*)-geranylgeraniol (302 mg, 1.04 mmol, 69 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.42 (t, *J* = 6.9 Hz, 1H), 5.15 – 5.06 (m, 3H), 4.15 (d, *J* = 6.9 Hz, 2H), 2.16 – 1.94 (m, 12H), 1.68 (m, 6H), 1.60 ppm (s, 9H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.72, 135.33, 134.92, 131.21, 124.35, 124.14, 123.75, 123.31, 59.34, 39.69, 39.66, 39.53, 26.73, 26.60, 26.30, 25.65, 17.64, 16.24, 15.98, 15.97 ppm;

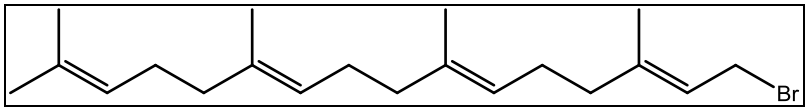
MS/ESI *m/z* = 313.1 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{20}H_{34}NaO$: 313.2502 $[M+Na]^+$, found: 313.2500.

(2*E*,6*E*,10*E*)-Geranylgeranyl bromide (14c)

According to the **synthetic method 8**, geranylgeraniol (233 mg, 802 µmol), tetrabromomethane (319 mg, 962 µmol), triphenylphosphine (252 mg, 962 µmol) and dichloromethane (50 mL) was used. Purification was done by flash chromatography on silica

(*n*-hexane/*t*BME, 20:1), yielding (2*E*,6*E*,10*E*)-geranylgeranyl bromide (265 mg, 751 µmol, 94 %) as a light yellow oil.

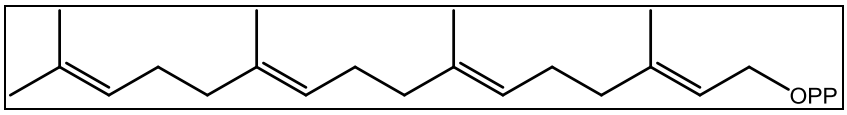


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.53 (t, *J* = 8.4 Hz, 1H), 5.13 – 5.06 (m, 3H), 4.01 (d, *J* = 8.4 Hz, 2H), 2.16 – 1.94 (m, 12H), 1.73 (s, 3H), 1.68 (s, 3H), 1.60 ppm (s, 9H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 143.49, 135.56, 134.88, 131.16, 124.34, 124.12, 123.34, 120.51, 39.68, 39.62, 39.48, 29.58, 26.72, 26.55, 26.06, 25.67, 17.67, 16.04, 15.99, 15.95 ppm.

(2*E*,6*E*,10*E*)-Geranylgeranyl diphosphate (14d)

According to the **synthetic method 1**, DIPEA (3.86 mL, 22.69 mmol), acetone (1.77 mL, 24.07 mmol), water (198 µL, 11.00 mmol), tetrakis(trimethylsilyl) diphosphate (16.05 g, 34.38 mmol) and (2*E*,6*E*,10*E*)-geranylgeranyl bromide (2.43 g, 6.88 mmol) was used. Purification was done by flash chromatography on silica (isopropanol/conc. ammonia/water, 16:6:2), yielding (2*E*,6*E*,10*E*)-geranylgeranyl diphosphate (1.08 g, 2.15 mmol, 31 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.45 (t, *J* = 6.4 Hz, 1H), 5.18 – 5.06 (m, 3H), 4.46 (t, *J* = 6.4 Hz, 2H), 2.17 – 1.90 (m, 12H), 1.72 (s, 3H), 1.65 (s, 3H), 1.61 (s, 3H), 1.58 ppm (s, 6H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 145.01, 138.27, 137.51, 133.67, 127.36, 127.19, 126.91, 122.78 (d, *J* = 8.4 Hz), 65.23 (d, *J* = 5.4 Hz), 42.55, 42.51, 42.38, 29.55, 29.53, 29.34, 28.23, 20.21, 18.86, 18.60, 18.57 ppm;

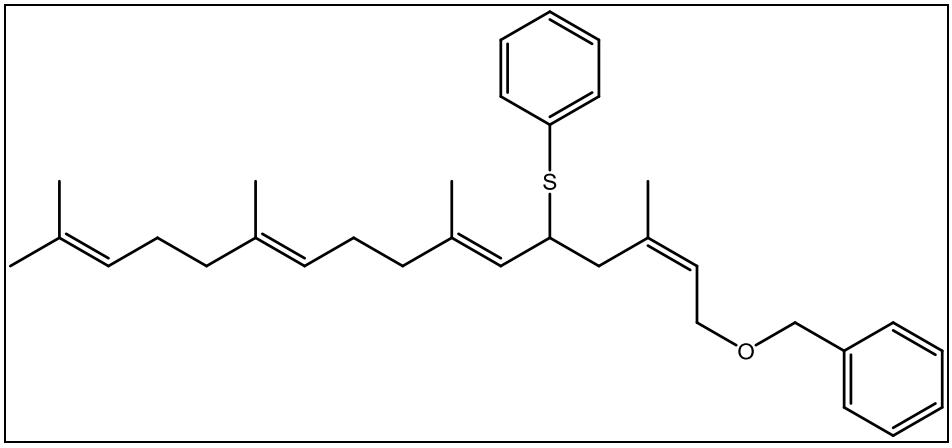
^{31}P NMR (162 MHz, D_2O + ND_4OD): -5.76 (d, *J* = 20.6 Hz), -9.69 ppm (dt, *J* = 20.6, 6.4 Hz);

MS/ESI *m/z* = 449.3 ($[M-2NH_3-NH_4]^-$, 100 %), 898.6 ($[2M-5NH_3-NH_4]^-$, 21 %);

HRMS/ESI** calcd for $C_{20}H_{35}O_7P_2$: 449.1864 $[M-2NH_3-NH_4]^-$, found: 449.1861.

(2*Z*,6*E*,10*E*)-1-(Benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (15a)

According to the **synthetic method 9**, farnesyl phenyl sulfide (1.50 g, 4.77 mmol), (2*Z*)-4-(benzyloxy)-2-methylbut-2-enyl chloride (1.21 g, 5.72 mmol), *n*-butyllithium (2.5 M in hexane, 2.10 mL, 5.25 mmol), HMPT (1.26 mL, 7.16 mmol) and THF (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*Z*,6*E*,10*E*)-1-(benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (2.08 g, 4.26 mmol, 89 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 – 7.20 (m, 10H), 5.46 (t, *J* = 6.7 Hz, 1H), 5.09 (t, *J* = 7.0 Hz, 1H), 5.04 (t, *J* = 6.9 Hz, 1H), 4.99 (d, *J* = 10.2 Hz, 1H), 4.46 (s, 2H), 4.04 – 3.91 (m, 3H), 2.44 (dd, *J* = 13.6, 5.5 Hz, 1H), 2.30 (dd, *J* = 13.6, 9.2 Hz, 1H), 2.10 – 1.87 (m, 8H), 1.75 (s, 3H), 1.68 (s, 3H), 1.60 (s, 3H), 1.57 (s, 3H), 1.34 ppm (s, 3H);

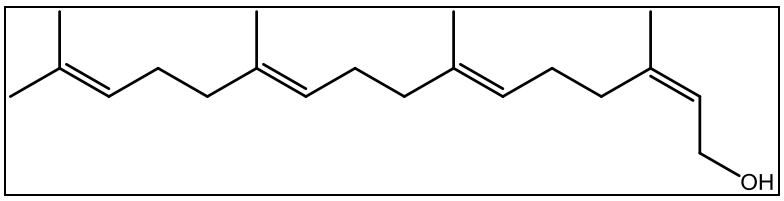
^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.39, 138.23, 137.37, 135.11, 134.64, 133.93, 131.18, 128.47, 128.27, 127.68, 127.45, 127.31, 125.22, 124.29, 124.10, 123.78, 72.19, 66.46, 46.22, 39.64, 39.51, 37.95, 26.70, 26.38, 25.65, 23.84, 17.64, 16.13, 15.93 ppm;

MS/ESI *m/z* = 511.5 ($[M+Na]^+$, 100 %), 1000.0 ($[2M+Na]^+$, 25 %);

HRMS/ESI* calcd for $C_{33}H_{44}NaOS$: 511.3005 $[M+Na]^+$, found: 511.3008.

(2*Z*,6*E*,10*E*)-Geranylgeraniol (15b)

According to the **synthetic method 10**, lithium (295 mg, 42.56 mmol), (2*Z*,6*E*,10*E*)-1-(benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (2.08 g, 4.26 mmol), ammonia (50 mL) and THF (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 4:1), yielding (2*Z*,6*E*,10*E*)-geranylgeraniol (730 mg, 2.51 mmol, 59 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44 (t, *J* = 7.1 Hz, 1H), 5.15 – 5.06 (m, 3H), 4.10 (d, *J* = 7.1 Hz, 2H), 2.14 – 1.94 (m, 12H), 1.75 (s, 3H), 1.68 (s, 3H), 1.60 (s, 9H), 1.34 ppm (br, 1H);

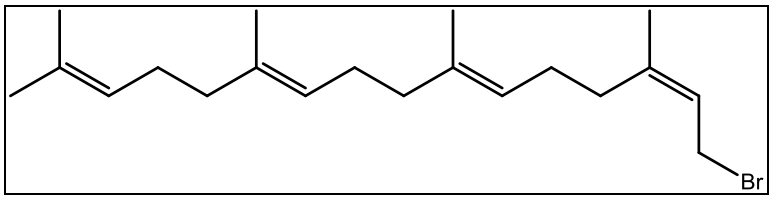
^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.86, 135.91, 134.99, 131.19, 124.36, 124.33, 124.04, 123.54, 58.95, 39.67, 39.64, 31.95, 26.71, 26.53, 26.48, 25.64, 23.39, 17.62, 15.96, 15.94 ppm;

MS/ESI *m/z* = 313.4 ($[M+Na]^+$, 100 %), 329.5 ($[M+K]^+$, 13 %);

HRMS/ESI* calcd for $C_{20}H_{34}NaO$: 313.2502 $[M+Na]^+$, found: 313.2502.

(2*Z*,6*E*,10*E*)-Geranylgeranyl bromide (15c)

According to the **synthetic method 8**, (2*Z*,6*E*,10*E*)-geranylgeraniol (700 mg, 2.41 mmol), tetrabromomethane (1.20 g, 3.62 mmol), triphenylphosphine (949 mg, 3.62 mmol) and dichloromethane (25 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 20:1), yielding (2*Z*,6*E*,10*E*)-geranylgeranyl bromide (658 mg, 1.86 mmol, 77 %) as a light yellow oil.

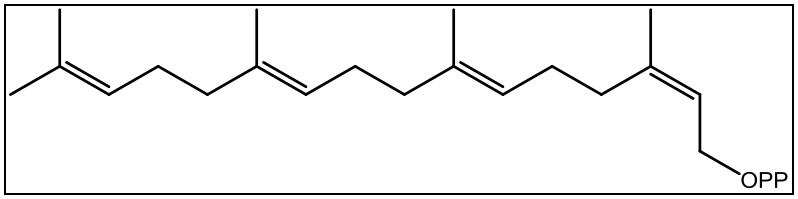


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.53 (t, *J* = 8.5 Hz, 1H), 5.15 – 5.06 (m, 3H), 4.00 (d, *J* = 8.5 Hz, 2H), 2.21 – 1.92 (m, 12H), 1.78 (s, 3H), 1.68 (s, 6H), 1.60 ppm (s, 6H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 143.38, 136.00, 135.00, 131.21, 124.37, 124.11, 123.29, 121.37, 39.72, 39.68, 31.77, 29.32, 26.71, 26.58, 26.15, 25.69, 23.55, 17.68, 16.06, 16.01 ppm.

(2*Z*,6*E*,10*E*)-Geranylgeranyl diphosphate (15d)

According to the **synthetic method 1**, DIPEA (1.04 mL, 6.14 mmol), acetone (479 µL, 6.51 mmol), water (54 µL, 2.98 mmol), tetrakis(trimethylsilyl) diphosphate (4.34 g, 9.30 mmol) and (2*Z*,6*E*,10*E*)-geranylgeranyl bromide (658 mg, 1.86 mmol) was used. Purification was done by precipitation, yielding (2*Z*,6*E*,10*E*)-geranylgeranyl diphosphate (192 mg, 383 µmol, 21 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.48 (m, 1H), 5.18 – 5.06 (m, 3H), 4.47 (m, 2H), 2.20 – 1.91 (m, 12H), 1.77 (s, 3H), 1.65 (s, 3H), 1.61 (s, 3H), 1.58 (s, 3H), 1.57 ppm (s, 3H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 144.75, 138.62, 137.68, 133.85, 127.34, 127.24, 126.98, 124.05, 64.94, 42.40, 42.37, 34.51, 29.71, 29.44, 29.38, 28.16, 26.27, 20.15, 18.52, 18.51 ppm;

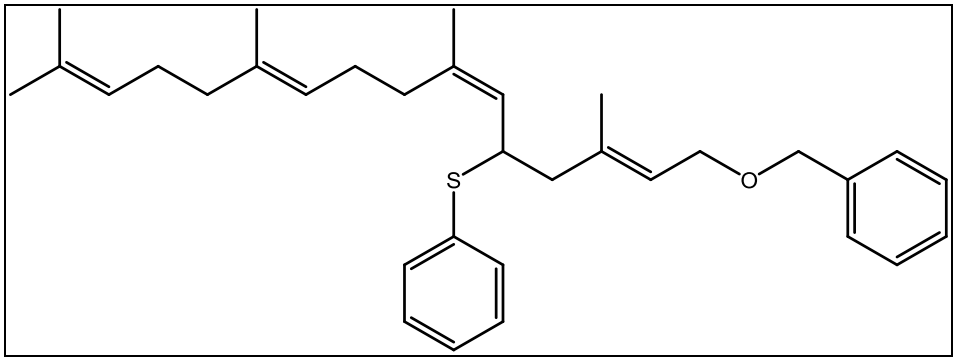
^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.51 (br), -9.91 ppm (br);

MS/ESI *m/z* = 449.1 ($[M\text{-}2NH_3\text{-}NH_4]^-$, 100 %), 899.7 ($[2M\text{-}5NH_3\text{-}NH_4]^-$, 48 %);

HRMS/ESI** calcd for $C_{20}H_{35}O_7P_2$: 449.1864 $[M-2NH_3-NH_4]^-$, found: 449.1865.

(2*E*,6*Z*,10*E*)-1-(Benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (16a)

According to the **synthetic method 9**, (2*Z*,6*E*)-farnesyl phenyl sulfide (2.74 g, 8.70 mmol), (2*E*)-4-(benzyloxy)-2-methylbut-2-enyl chloride (1.83 g, 8.70 mmol), *n*-butyllithium (2.5 M in hexane, 3.83 mL, 9.57 mmol), HMPT (2.29 mL, 13.05 mmol) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*E*,6*Z*,10*E*)-1-(benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (3.60 g, 7.37 mmol, 85 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 – 7.39 (m, 2H), 7.35 – 7.29 (m, 4H), 7.29 – 7.19 (m, 4H), 5.43 (t, *J* = 6.8 Hz, 1H), 5.11 – 5.00 (m, 3H), 4.47 (s, 2H), 4.06 (ddd, *J* = 10.2, 9.2, 6.1 Hz, 1H), 4.01 (d, *J* = 6.8 Hz, 2H), 2.41 (dd, *J* = 14.0, 6.1 Hz, 1H), 2.26 (dd, *J* = 14.0, 9.2 Hz, 1H), 2.10 – 1.83 (m, 8H), 1.68 (s, 3H), 1.64 (s, 3H), 1.62 (s, 3H), 1.59 (s, 3H), 1.55 ppm (s, 3H);

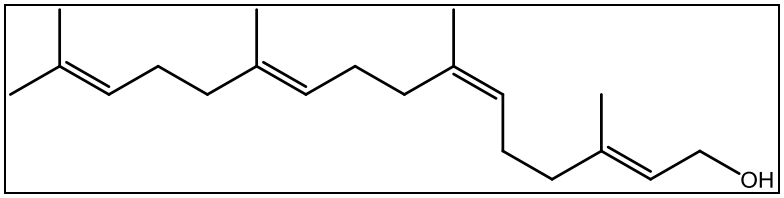
^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.48, 138.25, 137.14, 135.31, 134.68, 133.79, 131.27, 128.50, 128.28, 127.76, 127.46, 127.27, 125.99, 124.25, 123.97, 123.91, 71.66, 66.25, 45.79, 45.37, 39.66, 32.13, 26.66, 26.37, 25.67, 23.14, 17.66, 16.55, 15.95 ppm;

MS/ESI *m/z* = 506.4 ($[M+NH_4]^+$, 12 %), 511.4 ($[M+Na]^+$, 100 %), 994.9 ($[2M+NH_4]^+$, 4 %), 999.9 ($[2M+Na]^+$, 60 %);

HRMS/ESI* calcd for $C_{33}H_{44}NaOS$: 511.3005 $[M+H]^+$, found: 511.3010.

(2*E*,6*Z*,10*E*)-Geranylgeraniol (16b)

According to the **synthetic method 10**, lithium (483 mg, 69.56 mmol), (2*E*,6*Z*,10*E*)-1-(benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (3.40 g, 6.96 mmol), ammonia (50 mL) and THF (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 4:1), yielding (2*E*,6*Z*,10*E*)-geranylgeraniol (1.16 g, 3.99 mmol, 57 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.42 (t, *J* = 6.9 Hz, 1H), 5.16 – 5.07 (m, 3H), 4.15 (d, *J* = 6.9 Hz, 2H), 2.16 – 1.95 (m, 12H), 1.70 – 1.67 (m, 9H), 1.61 (s, 3H), 1.60 (s, 3H), 1.29 ppm (br, 1H);

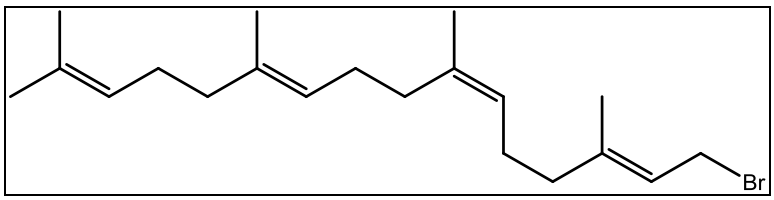
^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.66, 135.53, 135.16, 131.27, 124.50, 124.32, 124.05, 123.35, 59.35, 39.80, 39.71, 31.93, 26.68, 26.48, 26.16, 25.65, 23.36, 17.64, 16.25, 15.95 ppm;

MS/ESI *m/z* = 313.7 ($[M+Na]^+$, 100 %), 329.5 ($[M+K]^+$, 11 %);

HRMS/ESI* calcd for $C_{20}H_{34}NaO$: 313.2502 $[M+Na]^+$, found: 313.2507.

(2*E*,6*Z*,10*E*)-Geranylgeranyl bromide (16c)

According to the **synthetic method 8**, (2*E*,6*Z*,10*E*)-geranylgeraniol (1.19 g, 4.10 mmol), tetrabromomethane (1.63 g, 4.92 mmol), triphenylphosphine (1.29 g, 4.92 mmol) and dichloromethane (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 20:1), yielding (2*E*,6*Z*,10*E*)-geranylgeranyl bromide (1.30 g, 3.68 mmol, 90 %) as a colorless oil.

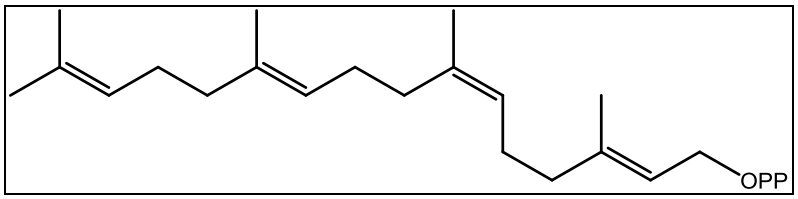


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.53 (t, *J* = 8.4 Hz, 1H), 5.16 – 5.05 (m, 3H), 4.02 (d, *J* = 8.4 Hz, 2H), 2.15 – 1.95 (m, 12H), 1.73 (s, 3H), 1.69 (s, 3H), 1.68 (s, 3H), 1.61 (s, 3H), 1.60 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 143.56, 135.80, 135.23, 131.31, 124.33, 124.17, 124.04, 120.54, 39.82, 39.74, 31.96, 29.64, 26.71, 26.49, 26.00, 25.69, 23.38, 17.69, 15.99, 15.98 ppm.

(2*E*,6*Z*,10*E*)-Geranylgeranyl diphosphate (16d)

According to the **synthetic method 1**, DIPEA (2.06 mL, 12.14 mmol), acetone (947 µL, 12.88 mmol), water (106 µL, 5.89 mmol), tetrakis(trimethylsilyl) diphosphate (8.59 g, 18.40 mmol) and (2*E*,6*Z*,10*E*)-geranylgeranyl bromide (1.30 g, 3.68 mmol) was used. Purification was done by precipitation, yielding (2*E*,6*Z*,10*E*)-geranylgeranyl diphosphate (151 mg, 301 µmol, 8 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.45 (t, *J* = 6.5 Hz, 1H), 5.16 (m, 1H), 5.15 (m, 1H), 5.10 (m, 1H), 4.46 (m, 2H), 2.17 – 1.93 (m, 12H), 1.70 (s, 3H), 1.67 (s, 3H), 1.65 (s, 3H), 1.59 (s, 3H), 1.58 ppm (s, 3H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 144.48, 138.40, 137.86, 133.99, 127.65, 127.28, 127.13, 123.37 (d, *J* = 8.4 Hz), 65.20 (d, *J* = 5.1 Hz), 42.42, 42.39, 34.59, 29.35, 29.25, 28.57, 28.14, 25.95, 20.14, 18.47, 18.44.

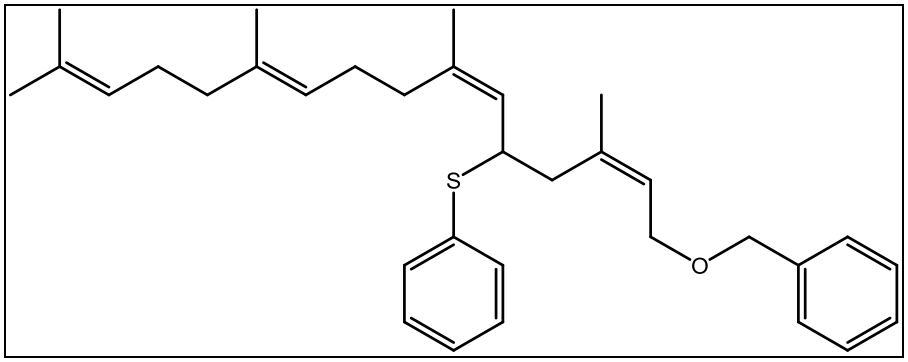
^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.43 (br), -9.97 ppm (br);

MS/ESI *m/z* = 448.7 ($[M\text{-}2NH_3\text{-}NH_4]^-$, 100 %), 899.5 ($[2M\text{-}5NH_3\text{-}NH_4]^-$, 48 %);

HRMS/ESI** calcd for $C_{20}H_{35}O_7P_2$: 449.1864 $[M\text{-}2NH_3\text{-}NH_4]^-$, found: 449.1858.

(2*Z*,6*Z*,10*E*)-1-(Benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (17a)

According to the **synthetic method 9**, (2*Z*,6*E*)-farnesyl phenyl sulfide (2.74 g, 8.70 mmol), (2*E*)-4-(benzyloxy)-2-methylbut-2-enyl chloride (1.83 g, 8.70 mmol), *n*-butyllithium (2.5 M in hexane, 3.83 mL, 9.57 mmol), HMPT (2.29 mL, 13.05 mmol) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*Z*,6*Z*,10*E*)-1-(benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (3.49 g, 7.14 mmol, 82 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42 – 7.38 (m, 2H), 7.35 – 7.31 (m, 4H), 7.31 – 7.21 (m, 4H), 5.46 (t, *J* = 6.5 Hz, 1H), 5.08 (t, *J* = 6.9 Hz, 1H), 5.02 (t, *J* = 6.6 Hz, 1H), 4.99 (d, *J* = 10.5 Hz, 1H), 4.45 (s, 2H), 4.04 – 3.89 (m, 3H), 2.41 (dd, *J* = 13.5, 5.4 Hz, 1H), 2.28 (dd, *J* = 13.5, 9.1 Hz, 1H), 2.09 – 1.82 (m, 8H), 1.74 (s, 3H), 1.68 (s, 3H), 1.63 (s, 3H), 1.59 (s, 3H), 1.55 ppm (s, 3H);

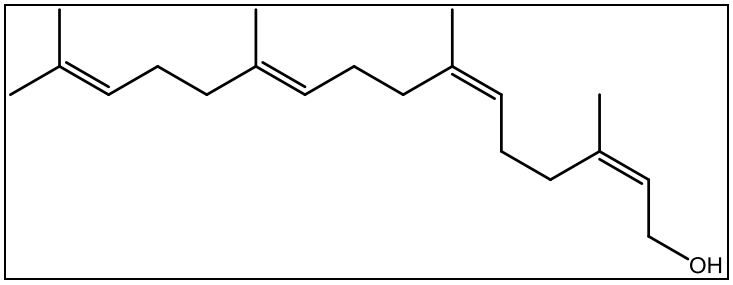
^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.50, 138.45, 137.19, 135.32, 134.64, 133.85, 131.28, 128.56, 128.32, 127.71, 127.49, 127.38, 125.81, 124.35, 124.26, 123.88, 72.21, 66.51, 45.99, 39.66, 38.32, 32.05, 26.67, 26.42, 25.68, 23.94, 23.16, 17.67, 15.95 ppm;

MS/ESI *m/z* = 511.5 ($[M+Na]^+$, 100 %), 994.8 ($[2M+NH_4]^+$, 2 %), 999.9 ($[2M+Na]^+$, 35 %);

HRMS/ESI* calcd for $C_{33}H_{44}NaOS$: 511.3005 $[M+H]^+$, found: 511.3018.

(2*Z*,6*Z*,10*E*)-Geranylgeraniol (17b)

According to the **synthetic method 10**, lithium (483 mg, 69.56 mmol), (2*Z*,6*Z*,10*E*)-1-(benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (3.40 g, 6.96 mmol), ammonia (50 mL) and THF (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 4:1), yielding (2*Z*,6*Z*,10*E*)-geranylgeraniol (1.39 g, 4.79 mmol, 69 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.45 (t, *J* = 7.2 Hz, 1H), 5.15 – 5.06 (m, 3H), 4.09 (d, *J* = 7.2 Hz, 2H), 2.13 – 1.94 (m, 12H), 1.75 (s, 3H), 1.70 (s, 3H), 1.68 (s, 3H), 1.61 (s, 3H), 1.60 (s, 3H), 1.20 ppm (br, 1H);

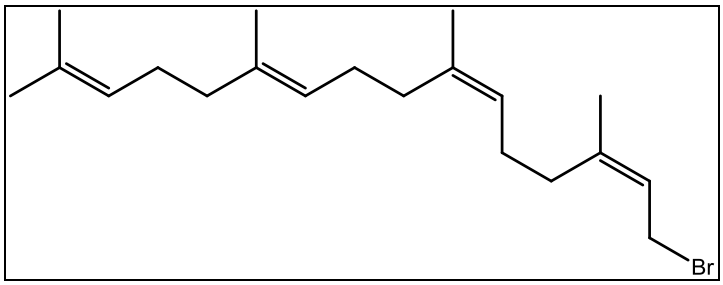
^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.82, 136.19, 135.25, 131.30, 124.45, 124.44, 124.30, 123.99, 58.98, 39.71, 32.22, 31.90, 26.69, 26.55, 26.31, 25.66, 23.43, 23.34, 17.65, 15.94 ppm;

MS/ESI *m/z* = 313.6 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{20}H_{34}NaO$: 313.2502 $[M+Na]^+$, found: 313.2500.

(2*Z*,6*Z*,10*E*)-Geranylgeranyl bromide (17c)

According to the **synthetic method 8**, (2*Z*,6*Z*,10*E*)-geranylgeraniol (1.64 g, 5.65 mmol), tetrabromomethane (2.25 g, 6.78 mmol), triphenylphosphine (1.78 g, 6.78 mmol) and dichloromethane (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 20:1), yielding (2*Z*,6*Z*,10*E*)-geranylgeranyl bromide (1.47 g, 4.16 mmol, 74 %) as a colorless oil.

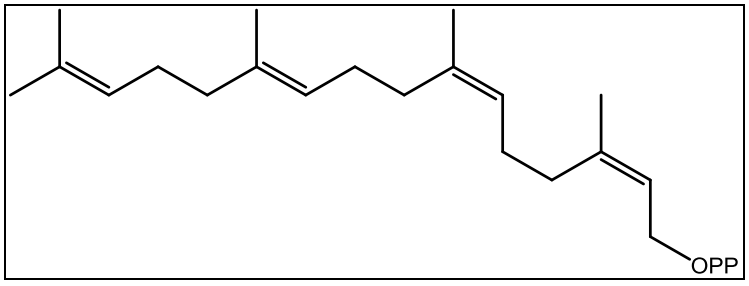


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.53 (t, *J* = 8.5 Hz, 1H), 5.16 – 5.06 (m, 3H), 4.00 (d, *J* = 8.5 Hz, 2H), 2.16 – 1.93 (m, 12H), 1.77 (s, 3H), 1.70 (s, 3H), 1.69 (s, 3H), 1.62 (s, 3H), 1.61 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 143.33, 136.19, 135.25, 131.28, 124.33, 124.13, 124.03, 121.40, 39.70, 32.08, 31.95, 29.36, 26.68, 26.64, 26.56, 25.69, 23.59, 23.39, 17.69, 16.00 ppm.

(2*Z*,6*Z*,10*E*)-Geranylgeranyl diphosphate (17d)

According to the **synthetic method 1**, DIPEA (2.34 mL, 13.73 mmol), acetone (1.07 mL, 14.56 mmol), water (120 µL, 6.66 mmol), tetrakis(trimethylsilyl) diphosphate (9.71 g, 20.80 mmol) and (2*Z*,6*Z*,10*E*)-geranylgeranyl bromide (1.47 g, 4.16 mmol) was used. Purification was done by precipitation, yielding (2*Z*,6*Z*,10*E*)-geranylgeranyl diphosphate (152 mg, 303 µmol, 7 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.48 (m, 1H), 5.17 (m, 1H), 5.14 (m, 1H), 5.10 (m, 1H), 4.45 (m, 2H), 2.21 – 1.93 (m, 12H), 1.75 (s, 3H), 1.69 (s, 3H), 1.65 (s, 3H), 1.60 (s, 3H), 1.57 ppm (s, 3H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 144.19, 138.77, 137.88, 133.94, 127.59, 127.29, 127.16, 124.27 (d, *J* = 7.8 Hz), 64.90 (m), 42.39, 34.64, 34.53, 29.37, 29.29, 28.91, 28.13, 25.81, 25.64, 20.14, 18.47;

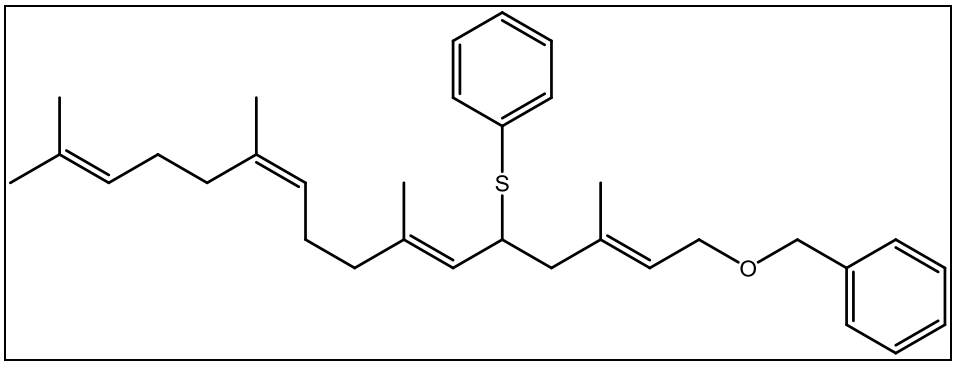
^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.31 (br), -9.86 ppm (br);

MS/ESI *m/z* = 449.3 ($[M-2NH_3-NH_4]^-$, 100 %);

HRMS/ESI** calcd for $C_{20}H_{35}O_7P_2$: 449.1864 $[M-2NH_3-NH_4]^-$, found: 449.1860.

(2*E*,6*E*,10*Z*)-1-(Benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (18a)

According to the **synthetic method 9**, (2*E*,6*Z*)-farnesyl phenyl sulfide (2.74 g, 8.70 mmol), (2*E*)-4-(benzyloxy)-2-methylbut-2-enyl chloride (1.83 g, 8.70 mmol), *n*-butyllithium (2.5 M in hexane, 3.83 mL, 9.57 mmol), HMPT (2.29 mL, 13.05 mmol) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*E*,6*E*,10*Z*)-1-(benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (3.30 g, 6.75 mmol, 78 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 – 7.38 (m, 2H), 7.34 – 7.29 (m, 4H), 7.29 – 7.20 (m, 4H), 5.43 (t, *J* = 6.7 Hz, 1H), 5.11 (m, 1H), 5.06 – 4.99 (m, 2H), 4.47 (s, 2H), 4.03 (ddd, *J* = 9.8, 9.0, 5.8 Hz, 1H), 4.01 (d, *J* = 6.7 Hz, 2H), 2.45 (dd, *J* = 13.8, 5.8 Hz, 1H), 2.26 (dd, *J* = 13.8, 9.0 Hz, 1H), 2.09 – 1.85 (m, 8H), 1.69 (s, 3H), 1.66 (s, 3H), 1.62 (s, 3H), 1.61 (s, 3H), 1.34 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.48, 137.90, 137.26, 135.20, 134.65, 133.99, 131.46, 128.44, 128.27, 127.75, 127.45, 127.27, 125.56, 124.67, 124.28, 123.76, 71.69, 66.22, 45.74, 45.42, 39.83, 31.94, 26.55, 26.30, 25.71, 23.31, 17.63, 16.54, 16.13 ppm;

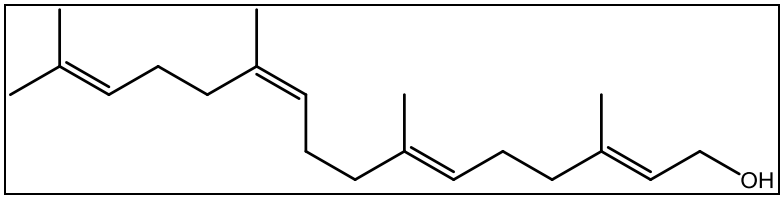
MS/ESI *m/z* = 506.6 ($[M+NH_4]^+$, 46 %), 511.5 ($[M+Na]^+$, 100 %), 995.0 ($[2M+NH_4]^+$,

13 %), 1000.0 ([2M+Na]$^+$, 38 %);

HRMS/ESI* calcd for $C_{33}H_{44}NaOS$: 511.3005 [M+H]$^+$, found: 511.3006.

(2*E*,6*E*,10*Z*)-Geranylgeraniol (18b)

According to the **synthetic method 10**, lithium (398 mg, 57.29 mmol), (2*E*,6*E*,10*Z*)-1-(benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (2.80 g, 5.73 mmol), ammonia (50 mL) and THF (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 4:1), yielding (2*E*,6*E*,10*Z*)-geranylgeraniol (1.15 g, 3.96 mmol, 69 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.42 (t, *J* = 6.9 Hz, 1H), 5.15 – 5.08 (m, 3H), 4.15 (d, *J* = 6.9 Hz, 2H), 2.16 – 1.94 (m, 12H), 1.69 (s, 3H), 1.68 (s, 3H), 1.68 (s, 3H), 1.61 (s, 3H), 1.60 (s, 3H), 1.31 ppm (br, 1H);

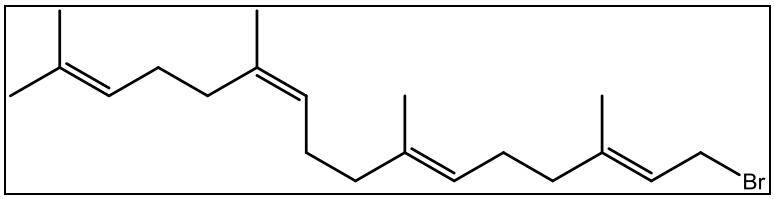
^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.69, 135.30, 135.06, 131.45, 124.96, 124.34, 123.76, 123.34, 59.33, 39.95, 39.52, 31.96, 26.59, 26.49, 26.28, 25.68, 23.34, 17.59, 16.24, 15.97 ppm;

MS/ESI *m/z* = 313.7 ([M+Na]$^+$, 100 %), 329.4 ([M+K]$^+$, 21 %);

HRMS/ESI* calcd for $C_{20}H_{34}NaO$: 313.2502 [M+Na]$^+$, found: 313.2501.

(2*E*,6*E*,10*Z*)-Geranylgeranyl bromide (18c)

According to the **synthetic method 8**, (2*E*,6*E*,10*Z*)-geranylgeraniol (1.15 g, 3.96 mmol), tetrabromomethane (1.97 g, 5.94 mmol), triphenylphosphine (1.56 g, 5.94 mmol) and dichloromethane (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 10:1), yielding (2*E*,6*E*,10*Z*)-geranylgeranyl bromide (1.38 g, 3.91 mmol, 99 %) as a colorless oil.

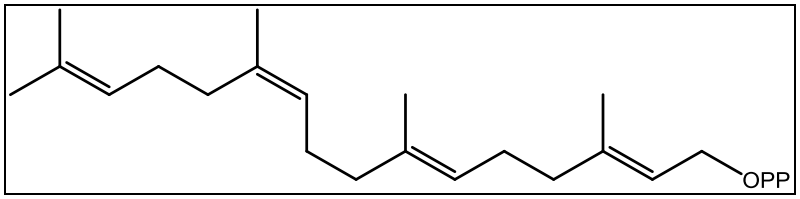


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.53 (t, *J* = 8.4 Hz, 1H), 5.15 – 5.05 (m, 3H), 4.02 (d, *J* = 8.4 Hz, 2H), 2.16 – 1.94 (m, 12H), 1.73 (s, 3H), 1.69 (s, 3H), 1.68 (s, 3H), 1.61 (s, 3H), 1.59 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 143.58, 135.61, 135.10, 131.48, 124.99, 124.37, 123.39, 120.56, 39.96, 39.52, 31.99, 29.64, 26.63, 26.50, 26.09, 25.72, 23.39, 17.64, 16.05, 15.97 ppm.

(2*E*,6*E*,10*Z*)-Geranylgeranyl diphosphate (18d)

According to the **synthetic method 1**, DIPEA (2.06 mL, 12.14 mmol), acetone (947 µL, 12.88 mmol), water (106 µL, 5.89 mmol), tetrakis(trimethylsilyl) diphosphate (8.59 g, 18.40 mmol) and (2*E*,6*E*,10*Z*)-geranylgeranyl bromide (1.30 g, 3.68 mmol) was used. Purification was done by precipitation, yielding (2*E*,6*E*,10*Z*)-geranylgeranyl diphosphate (770 mg, 1.54 mmol, 42 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.45 (m, 1H), 5.19 – 5.06 (m, 3H), 4.46 (m, 2H), 2.17 – 1.91 (m, 12H), 1.72 (s, 3H), 1.66 (s, 6H), 1.60 (s, 3H), 1.59 ppm (s, 3H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 145.03, 138.20, 137.68, 134.17, 128.06, 127.23, 127.05, 122.84 (d, *J* = 8.2 Hz), 65.22 (d, *J* = 4.4 Hz), 42.71, 42.28, 34.63, 29.30, 29.21, 29.20, 28.23, 25.92, 20.11, 18.76, 18.44;

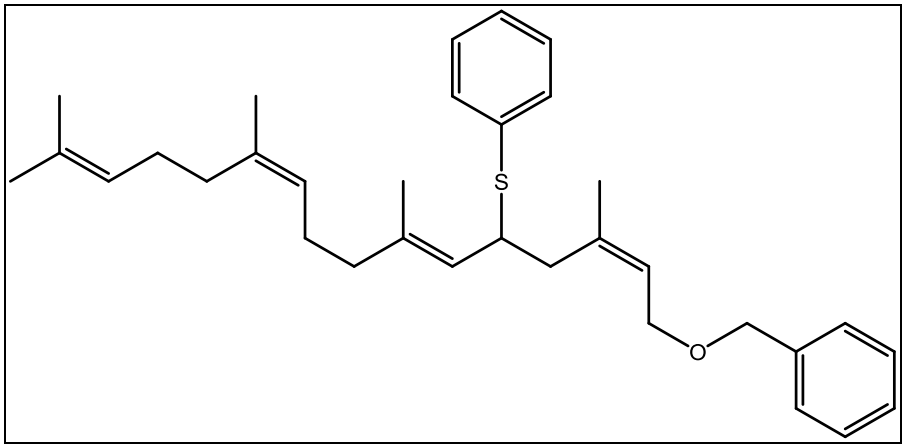
^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.35 (br), -9.99 ppm (br);

MS/ESI *m/z* = 449.0 ($[M-2NH_3-NH_4]^-$, 100 %), 899.6 ($[2M-5NH_3-NH_4]^-$, 34 %);

HRMS/ESI** calcd for $C_{20}H_{35}O_7P_2$: 449.1864 $[M\text{-}2NH_3\text{-}NH_4]^-$, found: 449.1853.

(2*Z*,6*E*,10*Z*)-1-(Benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (19a)

According to the **synthetic method 9**, (2*E*,6*Z*)-farnesyl phenyl sulfide (2.74 g, 8.70 mmol), (2*Z*)-4-(benzyloxy)-2-methylbut-2-enyl chloride (1.83 g, 8.70 mmol), *n*-butyllithium (2.5 M in hexane, 3.83 mL, 9.57 mmol), HMPT (2.29 mL, 13.05 mmol) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*Z*,6*E*,10*Z*)-1-(benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (3.17 g, 6.49 mmol, 75 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42 – 7.38 (m, 2H), 7.34 – 7.32 (m, 4H), 7.32 – 7.21 (m, 4H), 5.46 (t, *J* = 6.7 Hz, 1H), 5.11 (m, 1H), 5.02 (t, *J* = 6.5 Hz, 1H), 4.98 (d, *J* = 10.2 Hz, 1H), 4.46 (s, 2H), 4.04 – 3.91 (m, 3H), 2.44 (dd, *J* = 13.5, 5.6 Hz, 1H), 2.30 (dd, *J* = 13.5, 9.3 Hz, 1H), 2.09 – 1.86 (m, 8H), 1.75 (s, 3H), 1.69 (s, 3H), 1.66 (s, 3H), 1.61 (s, 3H), 1.33 ppm (s, 3H);

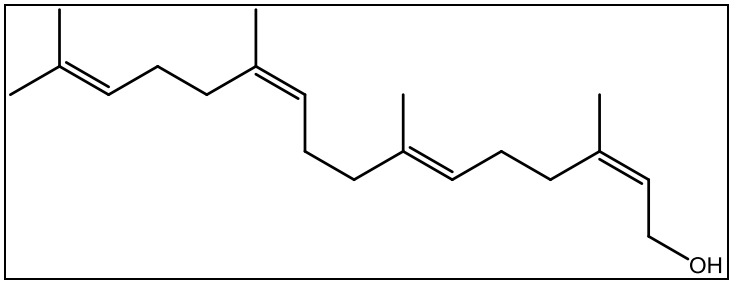
^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.41, 138.13, 137.41, 135.24, 134.60, 134.00, 131.46, 128.48, 128.30, 127.70, 127.47, 127.35, 125.31, 124.61, 124.27, 124.11, 72.21, 66.47, 46.26, 39.82, 37.94, 31.94, 26.55, 26.16, 25.71, 23.87, 23.31, 17.63, 16.06 ppm;

MS/ESI *m/z* = 511.5 ($[M+Na]^+$, 100 %), 1000.0 ($[2M+Na]^+$, 43 %);

HRMS/ESI* calcd for $C_{33}H_{44}NaOS$: 511.3005 $[M+H]^+$, found: 511.3003.

(2*Z*,6*E*,10*Z*)-Geranylgeraniol (19b)

According to the **synthetic method 10**, lithium (398 mg, 57.29 mmol), (2*Z*,6*E*,10*Z*)-1-(benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (2.80 g, 5.73 mmol), ammonia (50 mL) and THF (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 4:1), yielding (2*Z*,6*E*,10*Z*)-geranylgeraniol (1.19 g, 4.10 mmol, 72 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44 (t, *J* = 7.1 Hz, 1H), 5.16 – 5.07 (m, 3H), 4.10 (d, *J* = 7.1 Hz, 2H), 2.15 – 1.94 (m, 12H), 1.75 (s, 3H), 1.69 (s, 3H), 1.68 (s, 3H), 1.61 (s, 3H), 1.60 (s, 3H), 1.25 ppm (br, 1H);

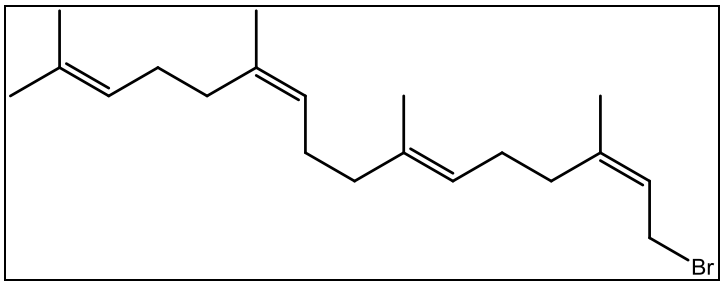
^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.91, 135.87, 135.16, 131.47, 124.88, 124.36, 124.34, 123.58, 58.99, 39.95, 31.96, 31.95, 26.59, 26.50, 26.43, 25.69, 23.42, 23.35, 17.60, 15.96 ppm;

MS/ESI *m/z* = 313.7 ($[M+Na]^+$, 100 %), 329.6 ($[M+K]^+$, 15 %);

HRMS/ESI* calcd for $C_{20}H_{34}NaO$: 313.2502 $[M+Na]^+$, found: 313.2501.

(2*Z*,6*E*,10*Z*)-Geranylgeranyl bromide (19c)

According to the **synthetic method 8**, (2*Z*,6*E*,10*Z*)-geranylgeraniol (1.15 g, 3.96 mmol), tetrabromomethane (1.97 g, 5.94 mmol), triphenylphosphine (1.56 g, 5.94 mmol) and dichloromethane (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 10:1), yielding (2*Z*,6*E*,10*Z*)-geranylgeranyl bromide (1.34 g, 3.79 mmol, 96 %) as a colorless oil.

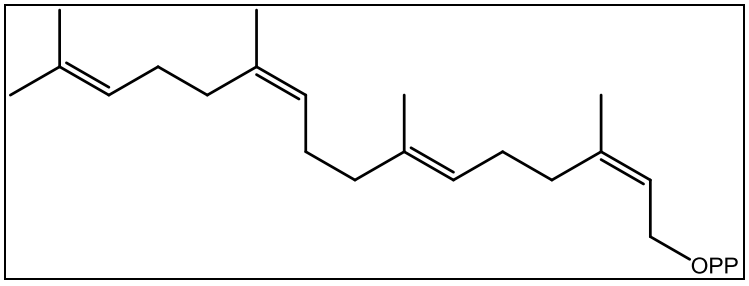


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.53 (t, *J* = 8.5 Hz, 1H), 5.16 – 5.07 (m, 3H), 4.01 (d, *J* = 8.5 Hz, 2H), 2.19 – 1.95 (m, 12H), 1.78 (s, 3H), 1.69 (s, 6H), 1.62 ppm (s, 6H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 143.44, 136.00, 135.19, 131.51, 124.94, 124.36, 123.30, 121.36, 39.98, 32.00, 31.75, 29.41, 26.63, 26.47, 26.13, 25.73, 23.56, 23.39, 17.65, 16.05 ppm.

(2*Z*,6*E*,10*Z*)-Geranylgeranyl diphosphate (19d)

According to the **synthetic method 1**, DIPEA (2.06 mL, 12.14 mmol), acetone (947 µL, 12.88 mmol), water (106 µL, 5.89 mmol), tetrakis(trimethylsilyl) diphosphate (8.59 g, 18.40 mmol) and (2*Z*,6*E*,10*Z*)-geranylgeranyl bromide (1.30 g, 3.68 mmol) was used. Purification was done by precipitation, yielding (2*Z*,6*E*,10*Z*)-geranylgeranyl diphosphate (499 mg, 995 µmol, 27 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.48 (m, 1H), 5.22 – 5.07 (m, 3H), 4.46 (m, 2H), 2.19 – 1.95 (m, 12H), 1.77 (s, 3H), 1.66 (s, 6H), 1.60 (s, 3H), 1.59 ppm (s, 3H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 144.72, 138.51, 137.86, 134.27, 128.12, 127.26, 127.10, 124.08 (d, *J* = 8.0 Hz), 64.94 (d, *J* = 3.6 Hz), 42.61, 34.59, 34.42, 29.53, 29.26, 29.13, 28.18, 26.10, 25.85, 20.08, 18.36;

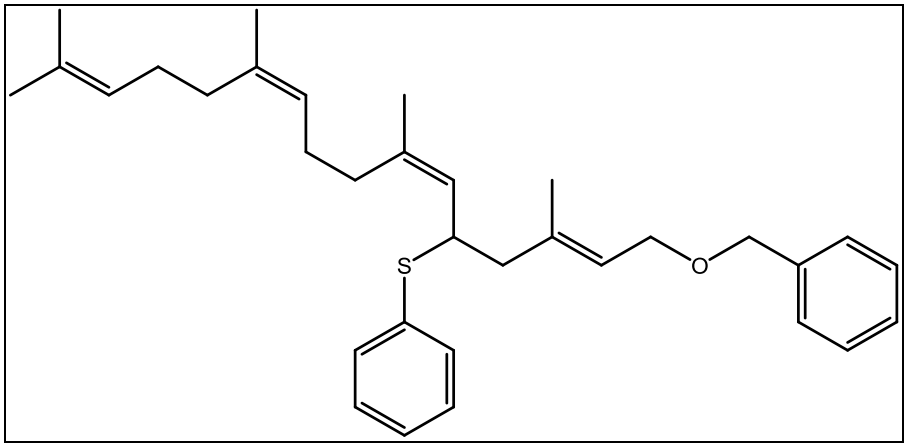
^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.33 (br), -9.85 ppm (br);

MS/ESI *m/z* = 449.3 ($[M-2NH_3-NH_4]^-$, 100 %), 899.4 ($[2M-5NH_3-NH_4]^-$, 53 %);

HRMS/ESI** calcd for $C_{20}H_{35}O_7P_2$: 449.1864 $[M-2NH_3-NH_4]^-$, found: 449.1856.

(2*E*,6*Z*,10*Z*)-1-(Benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (20a)

According to the **synthetic method 9**, (2*Z*,6*Z*)-farnesyl phenyl sulfide (2.40 g, 7.63 mmol), (2*E*)-4-(benzyloxy)-2-methylbut-2-enyl chloride (1.61 g, 7.63 mmol), *n*-butyllithium (2.5 M in hexane, 3.36 mL, 8.39 mmol), HMPT (2.01 mL, 11.45 mmol) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*E*,6*Z*,10*Z*)-1-(benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (3.04 g, 6.22 mmol, 82 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42 – 7.38 (m, 2H), 7.34 – 7.31 (m, 4H), 7.29 – 7.20 (m, 4H), 5.43 (t, *J* = 6.7 Hz, 1H), 5.12 – 5.00 (m, 3H), 4.47 (s, 2H), 4.05 (ddd, *J* = 10.1, 8.6, 6.0 Hz, 1H), 4.01 (d, *J* = 6.7 Hz, 1H),2.40 (dd, *J* = 13.7, 6.0 Hz, 1H), 2.26 (dd, *J* = 13.7, 8.6 Hz, 1H), 2.07 – 1.83 (m, 8H), 1.67 (s, 3H), 1.66 (s, 3H), 1.64 (s, 3H), 1.62 (s, 3H), 1.59 ppm (s, 3H);

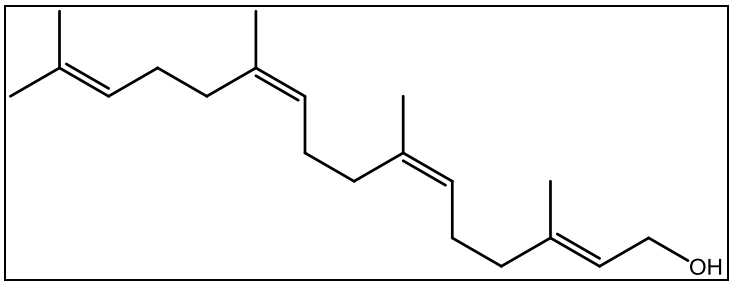
^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.49, 138.06, 137.13, 135.49, 134.65, 133.83, 131.48, 128.48, 128.28, 127.76, 127.46, 127.26, 126.09, 124.70, 124.21, 123.99, 71.67, 66.26, 45.79, 45.34, 32.38, 31.90, 26.60, 26.09, 25.69, 23.36, 23.09, 17.63, 16.50 ppm;

MS/ESI *m/z* = 511.5 ($[M+Na]^+$, 100 %), 999.8 ($[2M+Na]^+$, 52 %);

HRMS/ESI* calcd for $C_{33}H_{44}NaOS$: 511.3005 $[M+H]^+$, found: 511.3006.

(2*E*,6*Z*,10*Z*)-Geranylgeraniol (20b)

According to the **synthetic method 10**, lithium (426 mg, 61.38 mmol), (2*E*,6*Z*,10*Z*)-1-(benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (3.00 g, 6.14 mmol), ammonia (50 mL) and THF (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 4:1), yielding (2*E*,6*Z*,10*Z*)-geranylgeraniol (1.28 g, 4.41 mmol, 72 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.42 (t, *J* = 6.9 Hz, 1H), 5.16 – 5.08 (m, 3H), 4.15 (d, *J* = 6.9 Hz, 2H), 2.16 – 1.98 (m, 12H), 1.69 (s, 6H), 1.68 (s, 3H), 1.67 (s, 3H), 1.61 (s, 3H), 1.33 ppm (br, 1H);

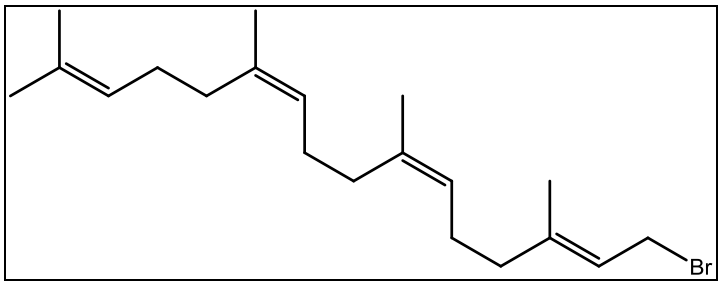
^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.64, 135.43, 135.30, 131.49, 124.93, 124.56, 124.29, 123.35, 59.34, 39.79, 32.23, 31.93, 26.65, 26.33, 26.16, 25.67, 23.38, 23.37, 17.60, 16.23 ppm;

MS/ESI *m/z* = 313.4 ($[M+Na]^+$, 100 %), 329.0 ($[M+K]^+$, 11 %);

HRMS/ESI* calcd for $C_{20}H_{34}NaO$: 313.2502 $[M+Na]^+$, found: 313.2502.

(2*E*,6*Z*,10*Z*)-Geranylgeranyl bromide (20c)

According to the **synthetic method 8**, (2*E*,6*Z*,10*Z*)-geranylgeraniol (1.25 g, 4.30 mmol), tetrabromomethane (2.14 g, 6.45 mmol), triphenylphosphine (1.69 g, 6.45 mmol) and dichloromethane (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 10:1), yielding (2*E*,6*Z*,10*Z*)-geranylgeranyl bromide (1.46 g, 4.13 mmol, 96 %) as a colorless oil.

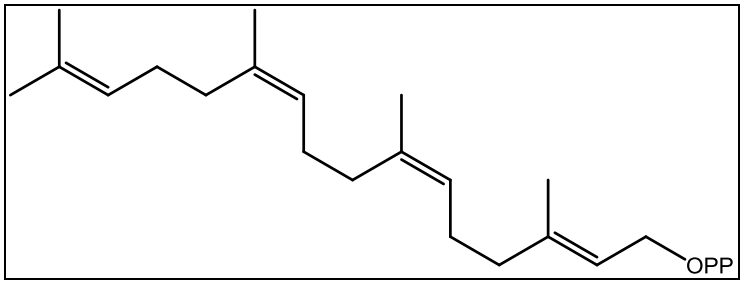


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.53 (t, *J* = 8.4 Hz, 1H), 5.16 – 5.06 (m, 3H), 4.02 (d, *J* = 8.4 Hz, 2H), 2.15 – 1.99 (m, 12H), 1.72 (s, 3H), 1.69 (s, 9H), 1.61 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 143.54, 135.69, 135.37, 131.52, 124.93, 124.30, 124.23, 120.54, 39.80, 32.25, 31.96, 29.62, 26.67, 26.33, 25.99, 25.72, 23.41, 23.39, 17.65, 15.97 ppm.

(2*E*,6*Z*,10*Z*)-Geranylgeranyl diphosphate (20d)

According to the **synthetic method 1**, DIPEA (2.22 mL, 13.07 mmol), acetone (1.02 mL, 13.86 mmol), water (114 µL, 6.34 mmol), tetrakis(trimethylsilyl) diphosphate (9.24 g, 19.80 mmol) and (2*E*,6*Z*,10*Z*)-geranylgeranyl bromide (1.40 g, 3.96 mmol) was used. Purification was done by precipitation, yielding (2*E*,6*E*,10*Z*)-geranylgeranyl diphosphate (1.30 g, 2.59 mmol, 65 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.46 (m, 1H), 5.20 – 5.08 (m, 1H), 4.46 (m, 2H), 2.17 – 1.99 (m, 12H), 1.71 (s, 3H), 1.67 (s, 9H), 1.59 ppm (s, 3H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 144.63, 138.16, 138.04, 134.29, 127.99, 127.86, 127.20, 123.24 (d, *J* = 8.3 Hz), 65.18 (d, *J* = 4.8 Hz), 42.45, 34.83, 34.56, 29.31, 29.00, 28.72, 28.17, 25.88, 25.87, 20.10, 18.50;

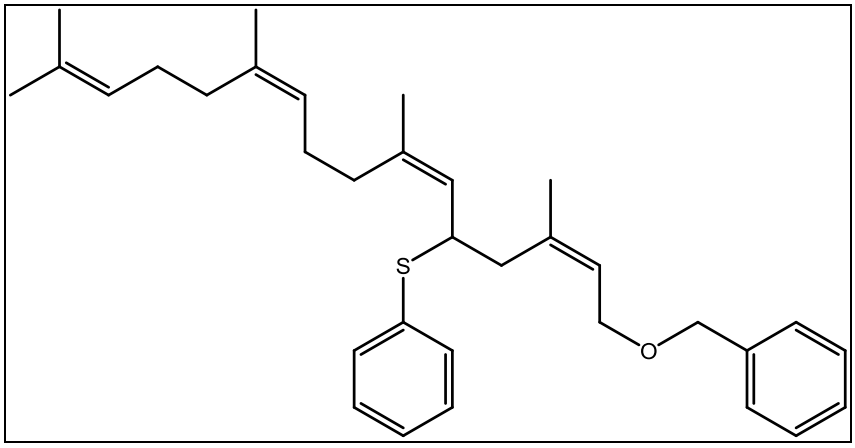
^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.29 (br), -9.98 ppm (br);

MS/ESI *m/z* = 448.8 ([M-2NH_3-NH_4]$^-$, 100 %), 899.4 ([2M-5NH_3-NH_4]$^-$, 45 %);

HRMS/ESI** calcd for $C_{20}H_{35}O_7P_2$: 449.1864 [M-2NH_3-NH_4]$^-$, found: 449.1855.

(2*Z*,6*Z*,10*Z*)-1-(Benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (21a)

According to the **synthetic method 9**, (2*Z*,6*Z*)-farnesyl phenyl sulfide (2.40 g, 7.63 mmol), (2*Z*)-4-(benzyloxy)-2-methylbut-2-enyl chloride (1.61 g, 7.63 mmol), *n*-butyllithium (2.5 M in hexane, 3.36 mL, 8.39 mmol), HMPT (2.01 mL, 11.45 mmol) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*Z*,6*Z*,10*Z*)-1-(benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (3.05 g, 6.24 mmol, 82 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42 – 7.37 (m, 2H), 7.35 – 7.30 (m, 4H), 7.30 – 7.20 (m, 4H), 5.46 (t, *J* = 6.7 Hz, 1H), 5.09 (m, 1H), 5.02 (m, 1H), 4.99 (d, *J* = 10.2 Hz, 1H), 4.45 (s, 2H), 4.04 – 3.90 (m, 3H), 2.41 (dd, *J* = 13.5, 5.5 Hz, 1H), 2.28 (dd, *J* = 13.5, 9.1 Hz, 1H), 2.08 – 1.83 (m, 8H), 1.74 (s, 3H), 1.67 (s, 3H), 1.66 (s, 3H), 1.62 (s, 3H), 1.59 ppm (s, 3H);

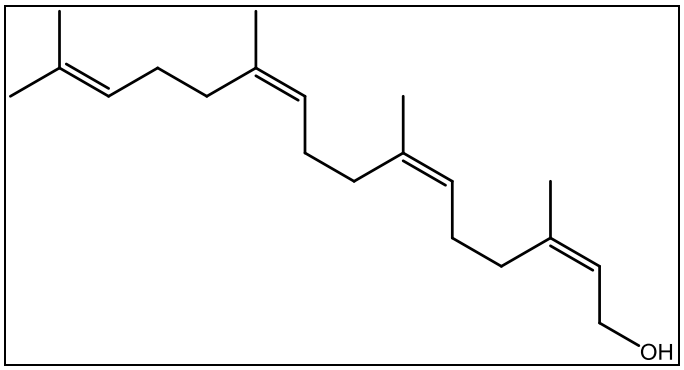
^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.44, 138.29, 137.16, 135.45, 134.59, 133.86, 131.46, 128.52, 128.29, 127.69, 127.46, 127.35, 125.90, 124.62, 124.33, 124.20, 72.18, 66.49, 45.93, 38.27, 32.26, 31.89, 26.58, 26.10, 25.68, 23.88, 23.33, 23.09, 17.61 ppm;

MS/ESI *m/z* = 511.1 ([M+Na]$^+$, 100 %), 999.8 ([2M+Na]$^+$, 55 %);

HRMS/ESI* calcd for $C_{33}H_{44}NaOS$: 511.3005 $[M+H]^+$, found: 511.3006.

(2*Z*,6*Z*,10*Z*)-Geranylgeraniol (21b)

According to the **synthetic method 10**, lithium (426 mg, 61.38 mmol), (2*Z*,6*Z*,10*Z*)-1-(benzyloxy)-3,7,11,15-tetramethyl-5-(phenylthio)hexadeca-2,6,10,14-tetraene (3.00 g, 6.14 mmol), ammonia (50 mL) and THF (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 4:1), yielding (2*Z*,6*Z*,10*Z*)-geranylgeraniol (1.21 g, 4.17 mmol, 68 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44 (t, *J* = 7.2 Hz, 1H), 5.15 – 5.08 (m, 3H), 4.09 (d, *J* = 7.2 Hz, 2H), 2.13 – 1.99 (m, 12H), 1.75 (s, 3H), 1.69 (s, 9H), 1.61 (s, 3H), 1.28 ppm (br, 1H);

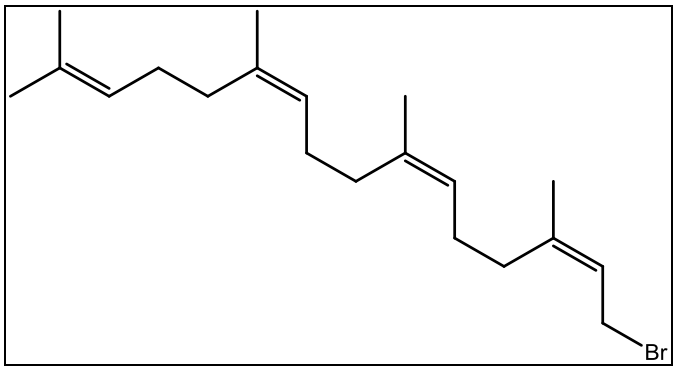
^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.76, 136.04, 135.39, 131.51, 124.83, 124.49, 124.45, 124.26, 58.96, 32.19, 32.18, 31.93, 26.64, 26.36, 26.29, 25.68, 23.41, 23.36, 23.33, 17.61 ppm;

MS/ESI *m/z* = 313.6 ($[M+Na]^+$, 100 %), 329.4 ($[M+K]^+$, 10 %);

HRMS/ESI* calcd for $C_{20}H_{34}NaO$: 313.2502 $[M+Na]^+$, found: 313.2501.

(2*Z*,6*Z*,10*Z*)-Geranylgeranyl bromide (21c)

According to the **synthetic method 8**, (2*Z*,6*Z*,10*Z0*)-geranylgeraniol (1.15 g, 3.96 mmol), tetrabromomethane (1.97 g, 5.94 mmol), triphenylphosphine (1.56 g, 5.94 mmol) and dichloromethane (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 10:1), yielding (2*Z*,6*Z*,10*Z*)-geranylgeranyl bromide (1.39 g, 3.93 mmol, 99 %) as a colorless oil.

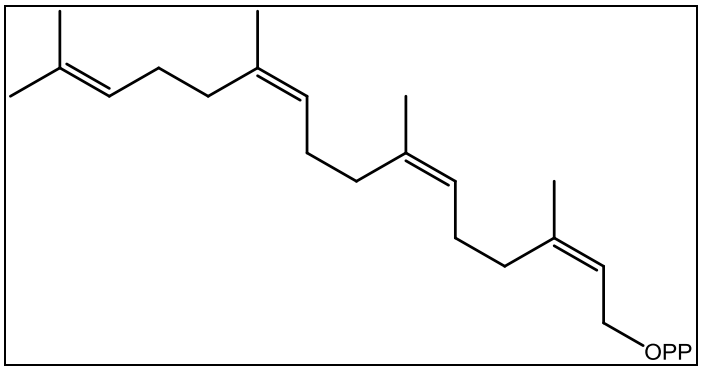


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.53 (t, *J* = 8.5 Hz, 1H), 5.15 – 5.09 (m, 3H), 4.00 (d, *J* = 8.5 Hz, 2H), 2.16 – 2.00 (m, 12H), 1.77 (s, 3H), 1.69 (s, 9H), 1.61 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 143.32, 136.07, 135.42, 131.53, 124.87, 124.30, 124.20, 121.40, 32.23, 32.04, 31.98, 29.37, 26.68, 26.37, 26.02, 25.72, 23.57, 23.42, 23.39, 17.65 ppm.

(2*Z*,6*Z*,10*Z*)-Geranylgeranyl diphosphate (21d)

According to the **synthetic method 1**, DIPEA (2.06 mL, 12.14 mmol), acetone (947 µL, 12.88 mmol), water (106 µL, 5.89 mmol), tetrakis(trimethylsilyl) diphosphate (8.59 g, 18.40 mmol) and (2*Z*,6*Z*,10*Z*)-geranylgeranyl bromide (1.30 g, 3.68 mmol) was used. Purification was done by precipitation, yielding (2*Z*,6*Z*,10*Z*)-geranylgeranyl diphosphate (758 mg, 1.51 mmol, 41 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.48 (m, 1H), 5.23 – 5.05 (m, 3H), 4.45 (m, 2H), 2.21 – 1.95 (m, 12H), 1.75 (s, 3H), 1.68 (s, 3H), 1.66 (s, 6H), 1.59 ppm (s, 3H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 144.21, 138.43, 137.85, 134.17, 128.01, 127.79,

127.17, 124.25 (d, *J* = 7.9 Hz), 64.89 (d, *J* = 4.0 Hz), 34.84, 34.56, 34.53, 29.34, 29.00, 28.99, 28.17, 25.92, 25.78, 25.70, 20.09;

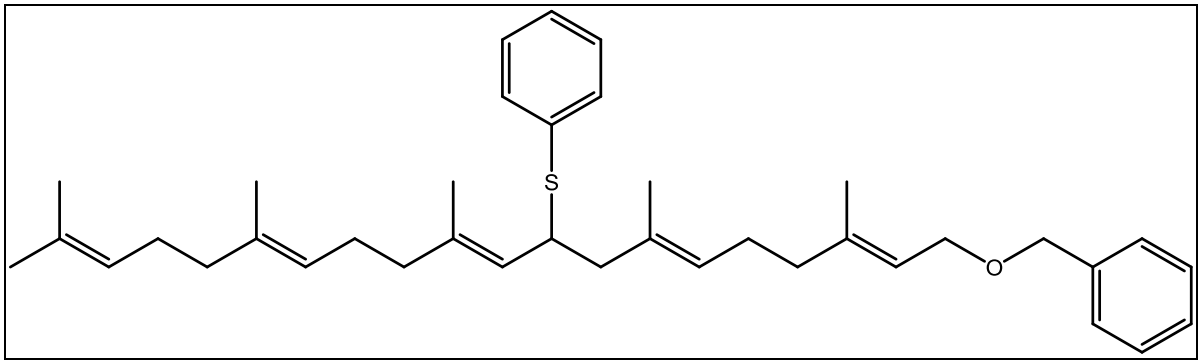
^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.30 (br), -9.86 ppm (br);

MS/ESI *m/z* = 448.7 ($[M-2NH_3-NH_4]^-$, 100 %), 899.6 ($[2M-5NH_3-NH_4]^-$, 72 %);

HRMS/ESI** calcd for $C_{20}H_{35}O_7P_2$: 449.1864 $[M-2NH_3-NH_4]^-$, found: 449.1858.

(2*E*,6*E*,10*E*,14*E*)-1-(Benzyloxy)-3,7,11,15,19-pentamethyl-5-(phenylthio)icosa-2,6,10,14,18-pentaene (22a)

According to the **synthetic method 9**, farnesyl phenyl sulfide (3.00 g, 9.54 mmol), (2*E*)-8-(benzyloxy)-2,6-dimethylocta-2,6-dienyl chloride (3.19 g, 11.45 mmol), *n*-butyllithium (2.5 M in hexane, 4.20 mL, 10.49 mmol), HMPT (2.51 mL, 14.31 mmol) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding (2*E*,6*E*,10*E*,14*E*)-1-(benzyloxy)-3,7,11,15,19-pentamethyl-5-(phenylthio)icosa-2,6,10,14,18-pentaene (4.04 g, 7.25 mmol, 76 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 – 7.17 (m, 10H), 5.40 (t, *J* = 6.8 Hz, 1H), 5.16 (t, *J* = 6.8 Hz, 1H), 5.11 – 5.03 (m, 2H), 4.99 (d, *J* = 10.0 Hz, 1H), 4.49 (s, 2H), 4.01 (m, 1H), 4.02 (d, *J* = 6.8 Hz, 2H), 2.38 (dd, *J* = 13.6, 5.7 Hz, 1H), 2.18 (dd, *J* = 13.6, 9.0 Hz, 1H), 2.13 – 1.87 (m, 12H), 1.68 (s, 3H), 1.63 (s, 3H), 1.59 (s, 3H), 1.58 (s, 3H), 1.57 (s, 3H), 1.35 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 140.20, 138.53, 137.62, 135.04, 135.02, 133.69, 132.28, 131.21, 128.38, 128.28, 127.75, 127.45, 127.04, 126.81, 125.80, 124.32,

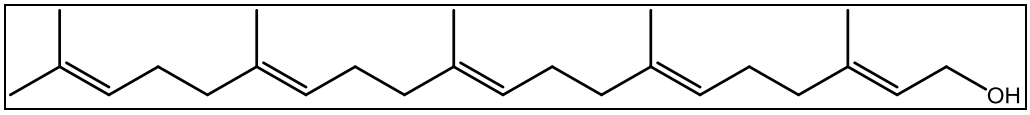
123.95, 120.83, 71.98, 66.54, 45.85, 45.55, 39.66, 39.54, 39.36, 26.72, 26.58, 26.34, 25.66, 17.66, 16.46, 16.18, 16.06, 15.95 ppm;

MS/ESI *m/z* = 579.5 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{38}H_{52}NaOS$: 579.3631 $[M+Na]^+$, found: 579.3624.

(2*E*,6*E*,10*E*,14*E*)-Geranylfarnesol (22b)

According to the **synthetic method 10**, lithium (474 mg, 68.24 mmol), (2*E*,6*E*,10*E*,14*E*)-1-(benzyloxy)-3,7,11,15,19-pentamethyl-5-(phenylthio)icosa-2,6,10,14,18-pentaene (3.80 g, 6.82 mmol), ammonia (50 mL) and THF (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 4:1 → 2:1), yielding (2*E*,6*E*,10*E*,14*E*)-geranylfarnesol (1.90 g, 5.30 mmol, 78 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.42 (t, *J* = 6.9 Hz, 1H), 5.15 – 5.06 (m, 4H), 4.15 (d, *J* = 6.9 Hz, 2H), 2.17 – 1.93 (m, 16H), 1.68 (s, 6H), 1.60 ppm (s, 12H);

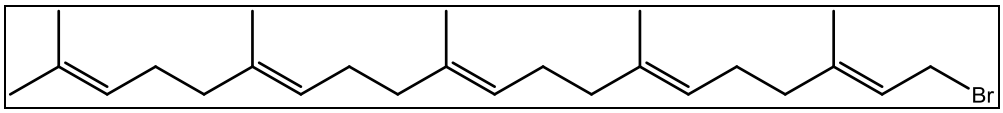
^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.78, 135.37, 134.96, 134.88, 131.22, 124.38, 124.23, 124.17, 123.74, 123.31, 59.37, 39.70, 39.69, 39.68, 39.54, 26.75, 26.64, 26.63, 26.31, 25.66, 17.65, 16.26, 16.00, 15.99, 15.97 ppm;

MS/ESI *m/z* = 381.1 ($[M+Na]^+$, 100 %), 397.4 ($[M+K]^+$, 12 %);

HRMS/ESI* calcd for $C_{25}H_{42}NaO$: 381.3128 $[M+Na]^+$, found: 381.3117.

(2*E*,6*E*,10*E*,14*E*)-Geranylfarnesyl bromide (22c)

According to the **synthetic method 8**, geranylfarnesol (1.85 g, 5.16 mmol), tetrabromomethane (2.05 g, 6.19 mmol), triphenylphosphine (1.62 g, 6.19 mmol) and dichloromethane (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/*t*BME, 20:1), yielding geranylfarnesyl bromide (1.99 g, 4.72 mmol, 91 %) as a light yellow oil.

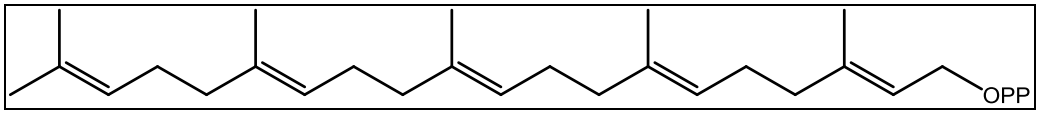


^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.53 (t, *J* = 8.4 Hz, 1H), 5.16 – 5.04 (m, 4H), 4.02 (d, *J* = 8.4 Hz, 2H), 2.16 – 1.94 (m, 16H), 1.73 (s, 3H), 1.68 (s, 3H), 1.60 ppm (s, 12H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 143.57, 135.63, 134.96, 134.88, 131.21, 124.39, 124.22, 124.17, 123.36, 120.53, 39.71, 39.70, 39.67, 39.52, 29.63, 26.76, 26.65, 26.61, 26.09, 25.68, 17.67, 16.05, 16.01, 16.00, 15.96 ppm.

(2*E*,6*E*,10*E*,14*E*)-Geranylfarnesyl diphosphate (22d)

According to the **synthetic method 1**, DIPEA (2.40 mL, 14.09 mmol), acetone (1.10 mL, 14.95 mmol), water (154 µL, 8.54 mmol), tetrakis(trimethylsilyl) diphosphate (9.96 g, 21.35 mmol) and geranylfarnesyl bromide (1.80 g, 4.27 mmol) was used. Purification was done by precipitation, yielding geranylfarnesyl diphosphate (379 mg, 665 µmol, 16 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + CD_3OD + ND_4OD): δ = 5.43 (m, 1H), 5.19 – 5.00 (m, 4H), 4.46 (m, 2H), 2.22 – 1.81 (m, 16H), 1.71 (s, 3H), 1.63 (s, 3H), 1.59 (s, 3H), 1.57 (s, 3H), 1.56 ppm (s, 6H);

^{13}C NMR (101 MHz, D_2O + CD_3OD + ND_4OD): δ = 144.05, 137.85, 137.24, 137.07, 133.20, 127.28, 127.15, 127.12, 126.80, 123.13 (m), 65.23 (d, *J* = 5.3 Hz), 42.70, 42.60, 42.54, 42.49, 29.78, 29.58, 29.56, 29.49, 28.22, 20.15, 18.81, 18.57, 18.54, 18.48 ppm;

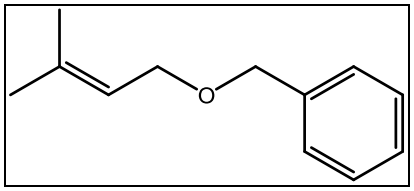
^{31}P NMR (162 MHz, D_2O + CD_3OD + ND_4OD): -6.79 (br), -10.10 ppm (br);

MS/ESI *m/z* = 517.6 ($[M\text{-}2NH_3\text{-}NH_4]^-$, 100 %), 1035.0 ($[2M\text{-}5NH_3\text{-}NH_4]^-$, 21 %);

HRMS/ESI* calcd for $C_{25}H_{43}O_7P_2$: 517.2489 $[M\text{-}2NH_3\text{-}NH_4]^-$, found: 517.2481.

Benzyl prenyl ether (23)

According to the **synthetic method 4**, prenol (10 mL, 100 mmol), sodium hydride (60 % dispersion in mineral oil, 5.2 g, 130 mmol), benzyl bromide (11.88 mL, 100 mmol) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 40:1), yielding benzyl prenyl ether (14.98 g, 84.50 mmol, 85 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36 – 7.21 (m, 5H), 5.40 (t, *J* = 6.9 Hz, 1H), 4.49 (s, 2H), 4.00 (d, *J* = 6.9 Hz, 2H), 1.75 (s, 3H), 1.64 ppm (s, 3H);

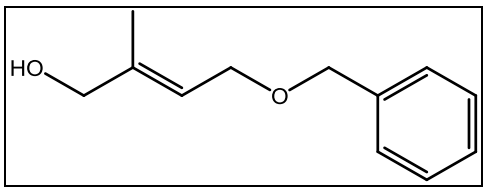
^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.54, 137.03, 128.25, 127.72, 127.42, 121.07, 71.97, 66.51, 25.72, 17.96 ppm;

MS/ESI *m/z* = 199.2 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{12}H_{16}NaO$: 199.1093 $[M+Na]^+$, found: 199.1095.

(2*E*)-4-(benzyloxy)-2-methylbut-2-enol (24)

According to the **synthetic method 5**, benzyl prenyl ether (1.00 g, 5.67 mmol), selenium dioxide (94 mg, 0.85 mmol), salicylic acid (117 mg, 0.85 mmol), *tert*-butyl hydroperoxide (70 % in water, 2.34 mL, 17.01 mmol) and sodium borohydride (214 mg, 5.67 mmol) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 4:1 → 1:2), yielding (2*E*)-4-(benzyloxy)-2-methylbut-2-enol (980 mg, 5.10 mmol, 90 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36 – 7.24 (m, 5H), 5.65 (t, *J* = 6.5 Hz, 1H), 4.50 (s, 2H), 4.07 (d, *J* = 6.5 Hz, 2H), 3.98 (s, 2H), 2.16 (br, 1H), 1.65 ppm (s, 3H);

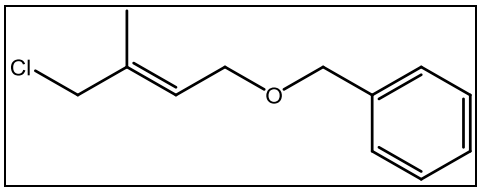
^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.26, 138.24, 128.32, 127.75, 127.57, 121.29, 72.29, 67.90, 66.15, 13.82 ppm;

MS/ESI *m/z* = 215.1 ($[M+Na]^+$, 62 %), 231.3 ($[M+K]^+$, 100 %), 407.4 ($[2M+Na]^+$, 22 %), 423.1 ($[2M+K]^+$, 16 %);

HRMS/ESI* calcd for $C_{12}H_{16}NaO_2$: 215.1043 $[M+Na]^+$, found: 215.1044.

(2*E*)-4-(benzyloxy)-2-methylbut-2-enyl chloride (25)

According to the **synthetic method 7**, (2*E*)-4-(benzyloxy)-2-methylbut-2-enol (7.24 g, 37.66 mmol), tetrachloromethane (5.50 mL, 56.49 mmol), triphenylphosphine (14.82 g, 56.49 mmol) and dichloromethane (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 5:1 → 3:1), yielding (2*E*)-4-(benzyloxy)-2-methylbut-2-enyl chloride (7.53 g, 35.73 mmol, 95 %) as a colorless oil.



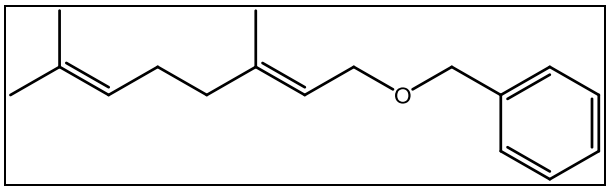
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38 – 7.23 (m, 5H), 5.75 (t, *J* = 6.5 Hz, 1H), 4.51 (s, 2H), 4.06 (d, *J* = 6.5 Hz, 2H), 4.01 (s, 2H), 1.75 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.05, 135.34, 128.36, 127.76, 127.65, 126.66, 72.38, 66.21, 51.28, 14.54 ppm;

MS/ESI *m/z* = 233.1 ($[M+Na]^+$, 100 %);

Benzyl geranyl ether (26)

According to the **synthetic method 4**, geraniol (10 g, 64.83 mmol), sodium hydride (60 % dispersion in mineral oil, 3.37 g, 84.28 mmol), benzyl bromide (7.70 mL, 64.83 mmol) and THF (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 40:1 → 10:1), yielding benzyl geranyl ether (15.49 g, 63.39 mmol, 98 %) as a yellowish oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37 – 7.23 (m, 5H), 5.40 (t, *J* = 6.6 Hz, 1H), 5.10 (t, *J* = 6.7 Hz, 1H), 4.50 (s, 2H), 4.03 (d, *J* = 6.6 Hz, 2H), 2.16 – 2.01 (m, 4H), 1.68 (s, 3H), 1.64 (s, 3H), 1.60 ppm (s, 3H);

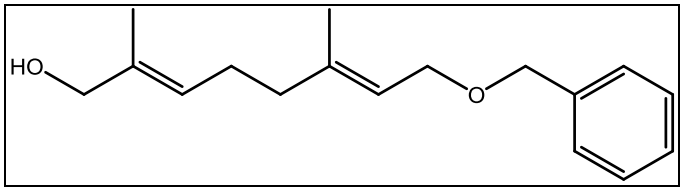
^{13}C NMR (101 MHz, $CDCl_3$): δ = 140.36, 138.59, 131.61, 128.31, 127.80, 127.47, 123.99, 120.81, 71.92, 66.56, 39.59, 26.36, 25.67, 17.66, 16.46 ppm;

MS/ESI *m/z* = 267.6 ($[M+Na]^+$, 100 %), 283.7 ($[M+K]^+$, 39 %);

HRMS/ESI* calcd for $C_{17}H_{24}NaO$: 267.1719 $[M+Na]^+$, found: 267.1713.

(2*E*)-8-(Benzyloxy)-2,6-dimethylocta-2,6-dienol (27)

According to the **synthetic method 5**, benzyl geranyl ether (15.00 g, 61.38 mmol), selenium dioxide (1.36 g, 12.28 mmol), salicylic acid (1.70 g, 12.28 mmol), *tert*-butyl hydroperoxide (70 % in water, 25.49 mL, 184.14 mmol) and sodium borohydride (2.32 g, 61.38 mmol) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 4:1 → 1:1), yielding (2*E*)-8-(benzyloxy)-2,6-dimethylocta-2,6-dienol (5.55 g, 21.32 mmol, 35 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37 – 7.26 (m, 5H), 5.40 (t, *J* = 6.7 Hz, 1H), 5.38 (t, *J* = 6.7 Hz, 1H), 4.50 (s, 2H), 4.02 (d, *J* = 6.7 Hz, 2H), 3.98 (s, 2H), 2.22 – 2.13 (m, 2H), 2.12 – 2.05 (m, 2H), 1.66 (s, 3H), 1.65 (s, 3H), 1.55 ppm (br, 1H);

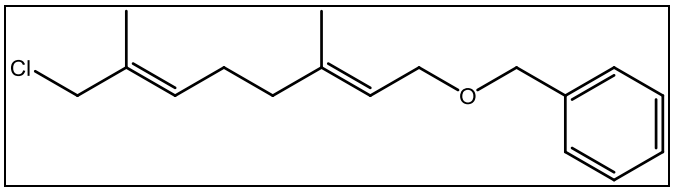
^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.90, 138.47, 135.10, 128.33, 127.80, 127.53, 125.59, 121.13, 72.07, 68.91, 66.53, 39.11, 25.77, 16.43, 13.67 ppm;

MS/ESI *m/z* = 283.4 ($[M+Na]^+$, 100 %), 299.5 ($[M+K]^+$, 14 %);

HRMS/ESI* calcd for $C_{17}H_{24}NaO_2$: 283.1669 $[M+Na]^+$, found: 283.1664.

(2*E*)-8-(Benzyloxy)-2,6-dimethylocta-2,6-dienyl chloride (28)

According to the **synthetic method 7**, (2*E*)-8-(benzyloxy)-2,6-dimethylocta-2,6-dienol (5.40 g, 20.74 mmol), tetrachloromethane (3.33 mL, 31.11 mmol), triphenylphosphine (8.16 g, 31.11 mmol) and dichloromethane (100 mL) was used. The product was used without further purification, yielding (2*E*)-8-(benzyloxy)-2,6-dimethylocta-2,6-dienyl chloride (5.13 g, 18.40 mmol, 89 %) as a colorless oil.

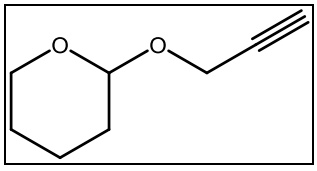


^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39 – 7.22 (m, 5H), 5.51 (t, *J* = 6.7 Hz, 1H), 5.41 (t, *J* = 6.7 Hz, 1H), 4.50 (s, 2H), 4.03 (d, *J* = 6.7 Hz, 2H), 4.00 (s, 2H), 2.22 – 2.14 (m, 2H), 2.22 – 2.05 (m, 2H), 1.73 (s, 3H), 1.64 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.53, 138.49, 131.94, 130.20, 128.33, 127.78, 127.52, 121.34, 72.07, 66.53, 52.39, 38.73, 26.26, 16.46, 14.13 ppm;

2-(Prop-2-ynyloxy)tetrahydro-2*H*-pyran (29)

According to the **synthetic method 11**, 2-propynol (34.62 mL, 600 mmol), pyridinium *p*-toluenesulfonate (7.54 g, 30 mmol) and 3,4-Dihydro-2*H*-pyran (81.41 mL, 900 mmol) was used. Purification was done by distillation (64-66 °C, 19 mbar), yielding 2-(prop-2-ynyloxy)tetrahydro-2*H*-pyran (76.93 g, 548.79 mmol, 91 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.82 (t, *J* = 3.3 Hz, 1H), 4.29 (dd, *J* = 15.7, 2.4 Hz, 1H), 4.23 (dd, *J* = 15.7, 2.4 Hz, 1H), 3.84 (m, 1H), 3.54 (m, 1H), 2.43 (t, *J* = 2.4 Hz, 1H), 1.90 – 1.69 (m, 2H), 1.68 – 1.49 ppm (m, 4H);

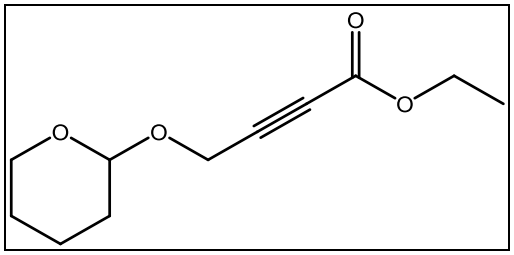
^{13}C NMR (101 MHz, $CDCl_3$): δ = 96.68, 79.67, 73.88, 61.83, 53.85, 30.10, 25.23, 18.88 ppm;

MS/ESI *m/z* = 163.3 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_8H_{12}NaO_2$: 163.0730 $[M+Na]^+$, found: 163.0728.

Ethyl 4-[(tetrahydro-2*H*-pyran-2-yl)oxy]but-2-ynoate (30)

n-Butyllithium (2.5 M solution in hexane, 220 mL, 550 mmol) was added to a solution of 2-(prop-2-ynyloxy)tetrahydro-2*H*-pyran (70.09 g, 500 mmol) in THF (300 mL) at -78 °C. After one hour of stirring at this temperature ethyl chloroformate (57.12 mL, 600 mmol) was added and the resulting mixture was allowed to warm to room temperature over 3 hours. Saturated ammonium chloride solution (250 mL) was added and the mixture was stirred for more 30 minutes at room temperature. The mixture was extracted with diethyl ether and the combined organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by distillation (125-128 °C, 6 mbar), yielding ethyl 4-[(tetrahydro-2H-pyran-2-yl)oxy]but-2-ynoate (91.14 g, 457.68 mmol, 92 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.81 (t, *J* = 3.2 Hz, 1H), 4.24 (q, *J* = 7.1 Hz, 2H), 4.38 (s, 2H), 3.82 (m, 1H), 3.55 (m, 1H), 1.88 – 1.70 (m, 2H), 1.68 – 1.50 (m, 4H), 1.31 ppm (t, *J* = 7.1 Hz, 3H);

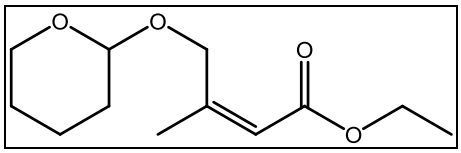
^{13}C NMR (101 MHz, $CDCl_3$): δ = 153.10, 97.05, 83.40, 77.51, 61.99, 61.86, 53.55, 29.97, 25.18, 18.71, 13.92 ppm;

MS/ESI *m/z* = 235.6 ($[M+Na]^+$, 100 %), 251.6 ($[M+K]^+$, 10 %), 447.4 ($[2M+Na]^+$, 29 %);

HRMS/ESI* calcd for $C_{11}H_{16}NaO_4$: 235.0941 $[M+Na]^+$, found: 235.0941.

Ethyl (*Z*)-3-methyl-4-[(tetrahydro-2*H*-pyran-2-yl)oxy]but-2-enoate (31)

Methyl lithium (1.6 M in diethyl ether, 316.25 mL, 506 mmol) was added to a suspension of copper(I) iodide (48.18 g, 253 mmol) in THF (250 mL) at 0 °C. The mixture was stirred for 30 minutes at this temperature and was then cooled to -78 °C. Ethyl 4-[(tetrahydro-2H-pyran-2-yl)oxy]but-2-ynoate (48.82 g, 230 mmol) was added and the resulting mixture was stirred for three hours at this temperature. Saturated ammonium chloride solution (250 mL) was added and the mixture was allowed to warm to room temperature. The resulting solution was filtered and extracted with diethyl ether. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo, yielding ethyl (2*Z*)-3-methyl-4-[(tetrahydro-2H-pyran-2-yl)oxy]but-2-enoate (50.75 g, 222.31 mmol, 97 %) as a light yellow oil. The product could be used in the next step without further purification.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.73 (q, *J* = 1.0 Hz, 1H), 4.75 (d, *J* = 14.5 Hz, 1H), 4.72 (d, *J* = 14.5 Hz, 1H), 4.62 (dd, *J* = 4.1, 3.0 Hz, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 3.87 (m, 1H), 3.53 (m, 1H), 2.00 (d, *J* = 1.0 Hz, 3H), 1.89 – 1.70 (m, 2H), 1.65 – 1.49 (m, 4H), 1.27 ppm (t, *J* = 7.1 Hz, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 165.81, 156.73, 116.89, 98.63, 66.43, 62.27, 59.70, 30.54, 25.37, 21.80, 19.48, 14.21 ppm;

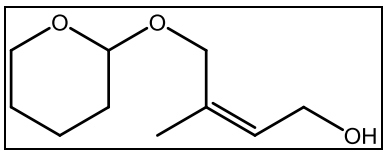
MS/ESI *m/z* = 251.0 ($[M+Na]^+$, 100 %), 479.2 ($[2M+Na]^+$, 63 %);

HRMS/ESI* calcd for $C_{12}H_{20}NaO_4$: 251.1254 $[M+Na]^+$, found: 251.1247.

(2*Z*)-3-Methyl-4-[(tetrahydro-2*H*-pyran-2-yl)oxy]but-2-enol (32)

Diisobutylaluminium hydride (1.0 M solution in dichloromethane, 444.62 mL, 444.62 mmol) was added to a solution of (2*Z*)-3-methyl-4-[(tetrahydro-2H-pyran-2-yl)oxy]but-2-enoate (50.75 g, 222.31 mmol) in dichloromethane (200 mL) at 0 °C. After one hour of stirring at this temperature potassium sodium tartrate (1.0 M solution in water, 200 mL) was carefully added and the resulting mixture was allowed to warm to room temperature over 2 hours. The mixture was extracted with dichloromethane (3 x 300 mL) and the combined organic

layers were dried (Na_2SO_4), filtered and concentrated in vacuo, yielding (2*Z*)-3-Methyl-4-[(tetrahydro-2H-pyran-2-yl)oxy]but-2-enol (40.72 g, 218.63 mmol, 98 %) as a light yellow oil. The product could be used in the next step without further purification.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.71 (t, *J* = 7.1 Hz, 1H), 4.65 (t, *J* = 3.2 Hz, 1H), 4.22 – 4.05 (m, 4H), 3.85 (m, 1H), 3.55 (m, 1H), 2.41 (br, 1H), 1.81 (s, 3H), 1.78 – 1.47 ppm (m, 6H);

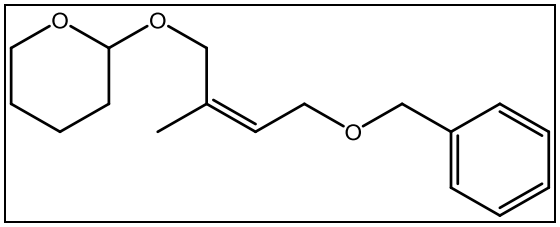
^{13}C NMR (101 MHz, $CDCl_3$): δ = 135.76, 128.55, 96.70, 65.09, 61.80, 58.09, 30.31, 25.29, 21.80, 19.01 ppm;

MS/ESI *m/z* = 209.5 ($[M+Na]^+$, 100 %), 395.4 ($[2M+Na]^+$, 92 %);

HRMS/ESI* calcd for $C_{10}H_{18}NaO_3$: 209.1148 $[M+Na]^+$, found: 209.1148.

(2*Z*)-1-Benzyloxy-3-methyl-4-[(tetrahydro-2*H*-pyran-2-yl)oxy]but-2-ene (33)

According to the **synthetic method 4**, (2*Z*)-3-Methyl-4-[(tetrahydro-2H-pyran-2-yl)oxy]but-2-enol (30 g, 161.07 mmol), sodium hydride (60 % dispersion in mineral oil, 8.37 g, 209.39 mmol), benzyl bromide (19.13 mL, 161.07 mmol) and THF (200 mL) was used. Concentration of the combined organic layers was yielding (2*Z*)-1-Benzyloxy-3-methyl-4-[(tetrahydro-2*H*-pyran-2-yl)oxy]but-2-ene (44.24 g, 160.00 mmol, 99 %) as a colorless oil. The product could be used in the next step without further purification.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40 – 7.24 (m, 5H), 5.60 (t, *J* = 6.8 Hz, 1H), 4.56 (t, *J* = 3.4 Hz, 1H), 4.50 (s, 2H), 4.16 – 4.03 (m, 4H), 3.83 (m, 1H), 3.48 (m, 1H), 1.83 (s, 3H), 1.82 – 1.45 ppm (m, 6H);

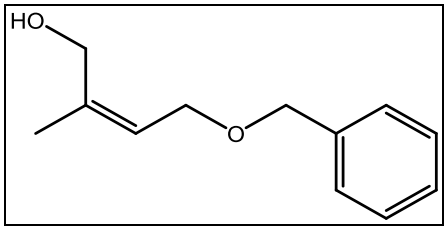
^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.35, 136.67, 128.30, 127.75, 127.51, 125.47, 97.51, 72.17, 65.91, 65.28, 62.05, 30.54, 25.42, 21.67, 19.38 ppm;

MS/ESI *m/z* = 299.7 ($[M+Na]^+$, 100 %);

HRMS/ESI* n.d.

(2*Z*)-4-(benzyloxy)-2-methylbut-2-enol (34)

According to the **synthetic method 12**, (*Z*)-1-Benzyloxy-3-methyl-4-[(tetrahydro-2*H*-pyran-2-yl)oxy]but-2-ene (44 g, 159.20 mmol), pyridinium *p*-toluenesulfonate (4 g, 15.92 mmol) and ethanol (200 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 4:1 → 1:2), yielding (2*Z*)-4-(benzyloxy)-2-methylbut-2-enol (22.16 g, 115.26 mmol, 72 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38 – 7.24 (m, 5H), 5.56 (t, *J* = 6.8 Hz, 1H), 4.51 (s, 2H), 4.08 (s, 2H), 4.03 (d, *J* = 6.8 Hz, 2H), 2.15 (s, 1H), 1.83 ppm (s, 3H);

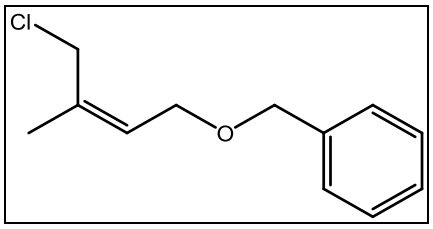
^{13}C NMR (101 MHz, $CDCl_3$): δ = 140.83, 137.92, 128.40, 127.84, 127.71, 123.58, 72.38, 65.63, 61.81, 21.56 ppm;

MS/ESI *m/z* = 215.4 ($[M+Na]^+$, 100 %), 407.5 ($[2M+Na]^+$, 30 %);

HRMS/ESI* calcd for $C_{12}H_{16}NaO_2$: 215.1043 $[M+Na]^+$, found: 215.1038.

(2*Z*)-4-(benzyloxy)-2-methylbut-2-enyl chloride (35)

According to the **synthetic method 7**, (2*Z*)-4-(benzyloxy)-2-methylbut-2-enol (22.00 g, 114.43 mmol), tetrachloromethane (16.71 mL, 171.65 mmol), triphenylphosphine (45.02 g, 171.65 mmol) and dichloromethane (200 mL) was used. The product was used without further purification, yielding (2*Z*)-4-(benzyloxy)-2-methylbut-2-enyl chloride (23.48 g, 111.44 mmol, 97 %) as a colorless oil.

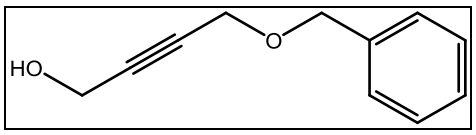


^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38 – 7.24 (m, 5H), 5.61 (tq, *J* = 6.9, 0.9 Hz, 1H), 4.51 (s, 2H), 4.06 (d, *J* = 6.9 Hz, 2H), 4.05 (s, 2H), 1.89 ppm (d, *J* = 0.9 Hz, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.03, 136.02, 128.41, 127.80, 127.70, 126.87, 72.37, 65.57, 43.04, 21.61 ppm;

4-(Benzyloxy)but-2-ynol (36)

Potassium hydroxide (28.06 g, 500 mmol) was added to a solution of 2-butyne-1,4-diol (43.05 g, 500 mmol) in water (100 mL). After 10 minutes of stirring at room temperature, benzyl bromide (14.85 mL, 125 mmol) was added and the resulting solution was stirred for more 24 hours. After addition of saturated ammonium chloride solution (50 mL), the reaction mixture was extracted with ethyl acetate. The combined organic layers were washed with saturated sodium bicarbonate solution and brine. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography on silica (*n*-hexane/EtOAc, 4:1 → 1:4), yielding 4-(Benzyloxy)but-2-ynol (14.25 g, 80.87 mmol, 65 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37 – 7.25 (m, 5H), 4.59 (s, 2H), 4.29 (t, *J* = 1.8 Hz, 2H), 4.20 (t, *J* = 1.8 Hz, 2H), 2.34 ppm (s, 1H);

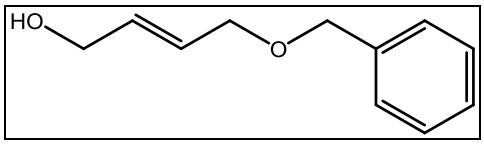
^{13}C NMR (101 MHz, $CDCl_3$): δ = 137.21, 128.35, 127.98, 127.82, 84.88, 81.41, 71.62, 57.32, 50.86 ppm;

MS/ESI *m/z* = 199.3 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{11}H_{12}NaO_2$: 199.0730 $[M+Na]^+$, found: 199.0728.

(2*E*)-4-(benzyloxy)but-2-enol (37)

Lithium aluminium hydride (2.65 g, 69.80 mmol) was added to a stirred solution of 4-(benzyloxy)but-2-ynol (12.30 g, 69.80 mmol) in THF (150 mL) at -30 °C. The solution was allowed to warm to room temperature over 3 hours. Subsequently water (4 mL), sodium hydroxide solution (2 M, 12 mL) and water (4 mL) was slowly added. The precipitated solid was filtered off and rinsed with THF (100 mL). The resulting filtrate was washed with water and brine. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography on silica (*n*-hexane/EtOAc, 5:1 → 1:3), yielding (2*E*)-4-(benzyloxy)but-2-enol (8.23 g, 46.18 mmol, 66 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36 – 7.24 (m, 5H), 5.92 – 5.77 (m, 2H), 4.11 (d, J = 5.1 Hz, 2H), 4.02 (d, J = 5.1 Hz, 2H), 2.24 (s, 1H);

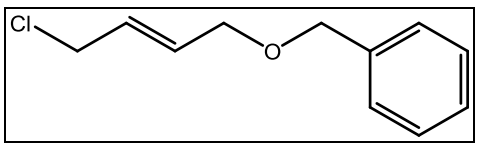
^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.04, 132.28, 128.29, 127.66, 127.55, 127.47, 72.16, 70.00, 62.68 ppm;

MS/ESI *m/z* = 201.3 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{11}H_{14}NaO_2$: 201.0886 $[M+Na]^+$, found: 201.0884.

(2*E*)-4-(benzyloxy)but-2-enyl chloride (38)

According to the **synthetic method 7**, (2*E*)-4-(benzyloxy)but-2-enol (8.00 g, 44.89 mmol), tetrachloromethane (6.56 mL, 67.34 mmol), triphenylphosphine (17.66 g, 67.34 mmol) and dichloromethane (100 mL) was used. The product was used without further purification, yielding (2*E*)-4-(benzyloxy)but-2-enyl chloride (7.79 g, 39.61 mmol, 88 %) as a colorless oil.

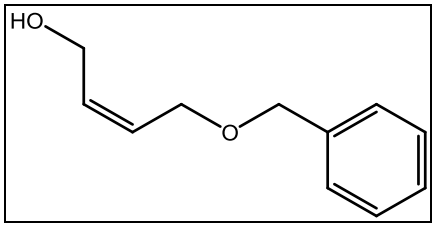


^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38 – 7.25 (m, 5H), 5.96 – 5.84 (m, 2H), 4.52 (s, 2H), 4.06 (m, 2H), 4.04 (m, 2H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 137.98, 131.16, 128.38, 128.29, 127.70, 127.66, 72.36, 69.41, 44.32 ppm;

(2*Z*)-4-(benzyloxy)but-2-enol (39)

Sodium hydride (60 % dispersion in mineral oil, 5.2 g, 130 mmol) was carefully added to a solution of *cis*-2-butene-1,4-diol (41.1 mL, 500 mmol) in THF (100 mL) at 0 °C. After 30 minutes of stirring at this temperature, benzyl bromide (14.85 mL, 125 mmol) was added. The resulting solution was allowed to warm to room temperature and was stirred for 4 hours. After addition of saturated ammonium chloride solution (50 mL), the reaction mixture was extracted with diethyl ether. The combined organic layers were washed with saturated sodium bicarbonate solution and brine. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography on silica (*n*-hexane/EtOAc, 2:1 → 1:3), yielding (2*Z*)-4-(benzyloxy)but-2-enol (18.8 g, 105.48 mmol, 84 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37 – 7.24 (m, 5H), 5.82 – 5.66 (m, *2*H), 4.50 (s, 2H), 4.12 (d, *J* = 6.2 Hz, 2H), 4.07 (d, *J* = 6.1 Hz, 2H), 2.41 ppm (s, 1H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 137.76, 132.35, 128.35, 127.94, 127.75, 127.69, 72.36, 65.54, 58.46 ppm;

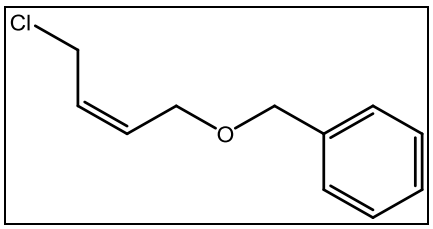
MS/ESI *m/z* = 201.1 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{11}H_{14}NaO_2$: 201.0886 $[M+Na]^+$, found: 201.0887.

(2*Z*)-4-(benzyloxy)but-2-enyl chloride (40)

According to the **synthetic method 7**, (2*Z*)-4-(benzyloxy)but-2-enol (15 g, 84.16 mmol), tetrachloromethane (12.29 mL, 126.24 mmol), triphenylphosphine (33.11 g, 126.24 mmol) and dichloromethane (100 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 5:1 → 3:1), yielding (2*Z*)-4-(benzyloxy)but-2-enyl chloride (14.61 g,

74.29 mmol, 88 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40 – 7.23 (m, 5H), 5.85 – 5.74 (m, 2H), 4.52 (s, 2H), 4.16 – 4.03 ppm (m, 4H);

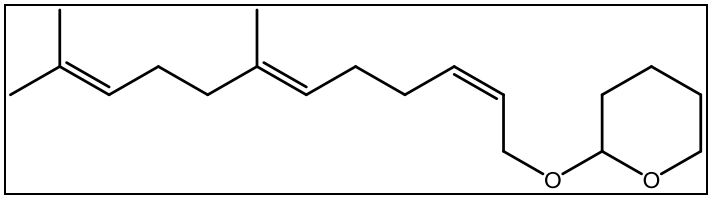
^{13}C NMR (101 MHz, $CDCl_3$): δ = 137.85, 130.71, 128.41, 128.40, 127.76, 127.74, 72.40, 65.07, 3j9.14 ppm;

MS/ESI *m/z* = 219.2 ($[M+Na]^+$, 100 %);

HRMS/ESI* n.d.

2-{[(2*Z*,6*E*)-7,11-dimethyldodeca-2,6,10-trienyl]oxy}tetrahydro-2H-pyran (41)

According to the **synthetic method 11**, (2*Z*,6*E*)-7,11-dimethyldodeca-2,6,10-trienol (1.00 g, 4.80 mmol), pyridinium *p*-toluenesulfonate (60 mg, 0.24 mmol) and 3,4-Dihydro-2*H*-pyran (651 µL, 7.20 mmol) was used. Removal of the solvent under reduced pressure was yielding 2-{[(2*Z*,6*E*)-7,11-dimethyldodeca-2,6,10-trienyl]oxy}tetrahydro-2*H*-pyran (1.40 g, 4.80 mmol, 100 %) as a colorless oil. The product was used in the next step without further purification.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.64 – 5.53 (m, 2H), 5.12 (t, *J* = 6.9 Hz, 1H), 5.09 (t, *J* = 7.0 Hz, 1H), 4.63 (m, 1H), 4.26 (dd, *J* = 12.1, 4.9 Hz, 1H), 4.08 (dd, *J* = 12.1, 6.0 Hz, 1H), 3.88 (m, 1H), 3.51 (m, 1H), 2.17 – 1.94 (m, 8H), 1.90 – 1.78 (m, 1H), 1.76 – 1.70 (m, 1H), 1.68 (s, 3H), 1.64 – 1.47 (m, 4H), 1.60 ppm (s, 6H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 135.61, 133.23, 131.23, 125.97, 124.27, 123.57, 97.82, 62.72, 62.14, 39.67, 30.66, 27.88, 27.73, 26.66, 25.63, 25.46, 19.48, 17.63,

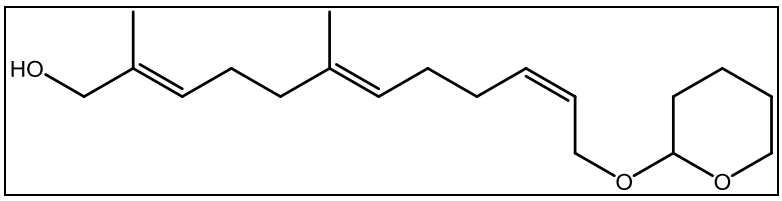
16.01 ppm;

MS/ESI *m/z* = 315.5 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{19}H_{32}NaO_2$: 315.2295 $[M+Na]^+$, found: 315.2298.

2-{[(2*Z*,6*E*,10*E*)-7,11-dimethyl-12-hydroxydodeca-2,6,10-trienyl]oxy}tetrahydro-2*H*-pyran (42)

According to the **synthetic method 5**, 2-{[(2*Z*,6*E*)-7,11-dimethyldodeca-2,6,10-trienyl]oxy}tetrahydro-2*H*-pyran (4.66 g, 15.92 mmol), selenium dioxide (354 mg, 3.19 mmol), *tert*-butyl hydroperoxide (70 % in water, 6.61 mL, 47.77 mmol), sodium borohydride (602 mg, 15.92 mmol) and dichloromethane (50 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 4:1), yielding 2-{[(2*Z*,6*E*,10*E*)-7,11-dimethyl-12-hydroxydodeca-2,6,10-trienyl]oxy}tetrahydro-2*H*-pyran (950 mg, 3.08 mmol, 19 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.63 – 5.52 (m, 2H), 5.38 (t, *J* = 6.9 Hz, 1H), 5.13 (t, *J* = 6.7 Hz, 1H), 4.64 (t, *J* = 3.3 Hz, 1H), 4.25 (dd, *J* = 12.4, 4.4 Hz, 1H), 4.07 (dd, *J* = 12.4, 5.9 Hz, 1H), 3.98 (s, 2H), 3.88 (m, 1H), 3.51 (m, 1H), 2.18 – 1.99 (m, 8H), 1.94 (br, 1H), 1.89 – 1.77 (m, 1H), 1.76 – 1.70 (m, 1H), 1.70 – 1.48 (m, 4H), 1.66 (s, 3H), 1.60 ppm (s, 3H);

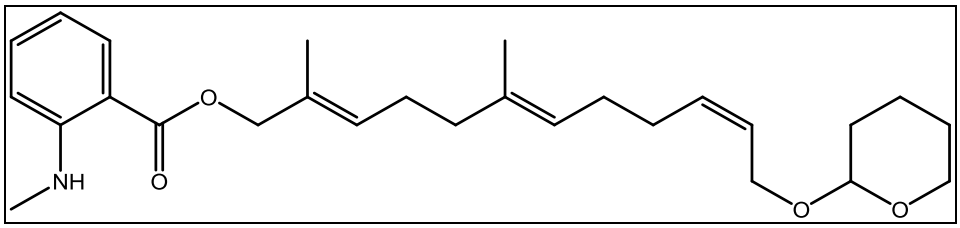
^{13}C NMR (101 MHz, $CDCl_3$): δ = 135.17, 134.71, 133.13, 125.95, 125.58, 123.80, 97.75, 68.66, 62.67, 62.06, 39.18, 30.55, 27.76, 27.63, 26.00, 25.37, 19.36, 15.93, 13.57 ppm;

MS/ESI *m/z* = 331.5 ($[M+Na]^+$, 14 %), 347.7 ($[M+K]^+$, 100 %);

HRMS/ESI* calcd for $C_{19}H_{32}NaO_3$: 331.2244 $[M+Na]^+$, found: 331.2232.

(2*E*,6*E*,10*Z*)-2,6-Dimethyl-12-[(tetrahydro-2*H*-pyran-2-yl)oxy]dodeca-2,6,10-trienyl 2-(methylamino)benzoate (43)

N-Methylisatoic anhydride (1.02 g, 5.77 mmol), triethylamine (805 µL, 5.77 mmol) and 4-dimethylaminopyridine (71 mg, 577 µmol) was added to a solution of 2-{[(2*Z*,6*E*,10*E*)-7,11-dimethyl-12-hydroxydodeca-2,6,10-trienyl]oxy}tetrahydro-2*H*-pyran (890 mg, 2.89 mmol) in DMF (10 mL). After 18 hours of stirring at 60 °C, ethyl acetate brine (50 mL) was added and the resulting mixture was extracted with ethyl acetate. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 15:1), yielding (2*E*,6*E*,10*Z*)-2,6-dimethyl-12-[(tetrahydro-2*H*-pyran-2-yl)oxy]dodeca-2,6,10-trienyl 2-(methylamino)benzoate (890 mg, 2.02 mmol, 70 %) as a light yellow oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.92 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.78 (br, 1H), 7.37 (ddd, *J* = 8.6, 7.2, 1.6 Hz, 1H), 6.67 (dd, *J* = 8.6, 1.2 Hz, 1H), 6.59 (ddd, *J* = 8.0, 7.2, 1.2 Hz, 1H), 5.63 – 5.53 (m, 2H), 5.51 (t, *J* = 7.0 Hz, 1H), 5.14 (t, *J* = 7.0 Hz, 1H), 4.64 (s, 2H), 4.63 (t, *J* = 3.2 Hz, 1H), 4.25 (dd, *J* = 12.4, 4.4 Hz, 1H), 4.07 (dd, *J* = 12.4, 5.9 Hz, 1H), 3.88 (ddd, *J* = 10.0, 6.6 ,2.5 Hz, 1H), 3.51 (m, 1H), 2.90 (s, 3H), 2.21 – 2.00 (m, 8H), 1.83 (m, 1H), 1.72 (s, 3H), 1.71 (m, 1H), 1.64 – 1.47 (m, 4H), 1.61 ppm (s, 3H);

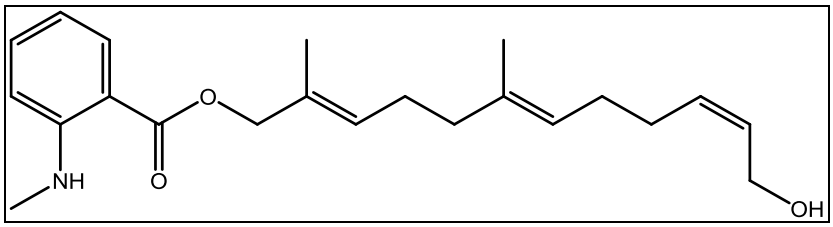
^{13}C NMR (101 MHz, $CDCl_3$): δ = 168.41, 151.90, 135.13, 134.53, 133.14, 131.51, 130.24, 128.99, 126.06, 124.02, 114.40, 110.77, 110.15, 97.85, 69.80, 62.73, 62.16, 39.08, 30.67, 29.59, 27.89, 27.70, 26.34, 25.46, 19.49, 16.02, 14.01 ppm;

MS/ESI *m/z* = 464.4 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{27}H_{39}NNaO_4$: 464.2771 $[M+Na]^+$, found: 464.2754.

(2*E*,6*E*,10*Z*)-12-Hydroxy-2,6-dimethyldodeca-2,6,10-trienyl 2-(methylamino)benzoate (44)

According to the **synthetic method 12**, (2*E*,6*E*,10*Z*)-2,6-dimethyl-12-[(tetrahydro-2*H*-pyran-2-yl)oxy]dodeca-2,6,10-trienyl 2-(methylamino)benzoate (890 mg, 2.01 mmol), pyridinium *p*-toluenesulfonate (76 mg, 302 µmol) and ethanol (20 mL) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 6:1 → 2:1), yielding (2*E*,6*E*,10*Z*)-12-hydroxy-2,6-dimethyldodeca-2,6,10-trienyl 2-(methylamino)benzoate (560 mg, 1.57 mmol, 78 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.92 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.37 (ddd, *J* = 8.6, 7.1, 1.5 Hz, 1H), 6.66 (dd, *J* = 8.5, 1.0 Hz, 1H), 6.58 (ddd, *J* = 8.0, 7.1, 1.0 Hz, 1H), 5.63 – 5.53 (m, 2H), 5.51 (t, *J* = 6.3 Hz, 1H), 5.13 (t, *J* = 7.0 Hz, 1H), 4.63 (s, 2H), 4.16 (d, *J* = 6.4 Hz, 2H), 2.89 (s, 3H), 2.20 – 2.00 (m, 8H), 1.72 (s, 3H), 1.60 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 168.39, 151.93, 135.26, 134.50, 132.34, 131.46, 130.22, 128.86, 128.68, 123.91, 114.28, 110.67, 110.02, 69.75, 58.43, 38.99, 29.49, 27.76, 27.53, 26.18, 15.96, 13.97 ppm;

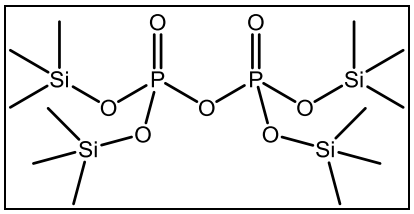
MS/ESI *m/z* = 380.6 ($[M+Na]^+$, 100 %);

HRMS/ESI* calcd for $C_{22}H_{31}NNaO_3$: 380.2196 $[M+Na]^+$, found: 380.2179.

Tetrakis(trimethylsilyl) diphosphate (45)

Pyridine (4.84 mL, 60 mmol) and trimethylsilyl chloride (33.58 mL, 264 mmol) is added to a dispersion of disodium dihydrogen diphosphate (13.31 g, 60 mmol) in formamide (30 mL) at 0 °C. After 4 hours of vigorous stirring the reaction mixture is allowed to warm to room temperature and is extracted with petroleum ether (3 x 30 mL). The combined organic phases are concentrated under reduced pressure yielding tetrakis(trimethylsilyl)

diphosphate (26.64 g, 57.08 mmol, 95 %) as a colorless oil. The product was used in the diphosphorylation reactions without further purification.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.24 (s, 36H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 0.60 ppm;

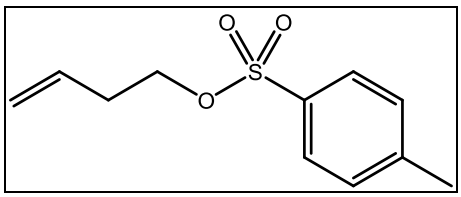
^{29}Si NMR (99 MHz, $CDCl_3$): δ = 24.60 ppm;

^{31}P NMR (162 MHz, $CDCl_3$): δ = -30.76 ppm;

HRMS/ESI** calcd for $C_{12}H_{37}O_7P_2Si_4$: 467.1086 $[M+H]^+$, found: 467.1086.[91]

But-3-enyl tosylate (46)

According to the **synthetic method 3**, 4-toluenesulfonyl chloride (26.44 g, 138.68 mmol), pyridine (11.19 mL, 138.68 mmol), but-3-enol (5.00 g, 69.34 mmol), and DMAP (cat.) was used. Purification was done by flash chromatography on silica (*n*-hexane/dichloromethane, 5:1 → 1:1), yielding but-3-enyl tosylate (14.03 g, 62.00 mmol, 89 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.79 (d, *J* = 8.1 Hz, 2H), 7.35 (d, *J* = 8.1 Hz, 2H), 5.67 (ddt, *J* = 17.1, 10.3, 6.7 Hz, 1H), 5.07 (dq, *J* = 17.1, 1.5 Hz, 1H), 5.05 (dq, *J* = 10.3, 1.5 Hz, 1H), 4.06 (t, *J* = 6.7 Hz, 2H), 2.45 (s, 3H), 2.39 ppm (qt, *J* = 6.7, 1.5 Hz, 2H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 144.70, 132.89, 132.30, 129.74, 127.76, 118.08, 69.34, 33.00, 21.52 ppm;

MS/ESI *m/z* = 249.1 ($[M+Na]^+$, 100 %), 475.1 ($[2M+Na]^+$, 52 %);

HRMS/ESI** calcd for $C_{11}H_{14}NaO_3S$: 249.0556 $[M+Na]^+$, found: 249.0557.

But-3-enyl diphosphate (47)

According to the **synthetic method 2**, DIPEA (7.44 mL, 43.75 mmol), DMF (3.57 mL, 46.40 mmol), water (382 µL, 21.21 mmol), tetrakis(trimethylsilyl) diphosphate (30.94 g, 66.29 mmol) and but-3-enyl tosylate (3.00 g, 13.26 mmol) was used. Purification was done by flash chromatography on silica (isopropanol/conc. ammonia/water, 8:6:2), yielding but-3-enyl diphosphate (540 mg, 1.91 mmol, 14 %) as a white solid.

^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.91 (ddt, *J* = 17.2, 10.4, 6.7 Hz, 1H), 5.19 (ddd, *J* = 17.2, 3.0, 1.5 Hz, 1H), 5.11 (ddt, *J* = 10.4, 3.0, 1.5 Hz, 1H), 3.99 (q, *J* = 6.7 Hz, 2H), 2.42 ppm (qt, *J* = 6.7, 1.5 Hz, 2H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 135.52, 116.93, 65.26 (d, *J* = 5.7 Hz), 34.50 ppm (d, *J* = 7.5 Hz);

^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.33 (d, *J* = 21.8 Hz), -10.30 ppm (dt, *J* = 21.8, 6.7 Hz);

MS/ESI *m/z* = 231.0 ($[M-2NH_3-NH_4]^-$, 100 %), 463.0 ($[2M-5NH_3-NH_4]^-$, 42 %);

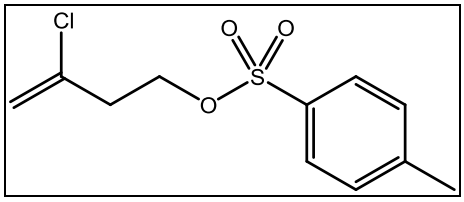
HRMS/ESI** calcd for $C_4H_9O_7P_2$: 230.9829 $[M-2NH_3-NH_4]^-$, found: 230.9826.

3-Chlorobut-3-enyl tosylate (49)

Paraformaldehyde (1.50 g, 50 mmol), 2-chloropropene (4.26 mL, 50 mmol) and diethylaluminium chloride (1 M in *n*-hexane, 50 mL, 50 mmol) was dissolved in dry dichloromethane (50 mL) at -5 °C. After 24 hours of stirring at this temperature, saturated ammonium chloride solution (50 mL) was added and the reaction mixture was stirred at room temperature for more 10 minutes. The mixture was extracted with dichloromethane (3 x 50 mL) and the combined organic layers were dried (Na_2SO_4), filtered and carefully concentrated under reduced pressure. The intermediate was purified by flash

chromatography on silica (*n*-hexane/EtOAc, 2:3). The eluent could not be completely removed due to the high volatility of the obtained compound. Therefore a colorless solution of 3-chlorobut-3-enol **(48)** (in *n*-hexane/EtOAc, 12.4 mL) was used in the following step.

According to the **synthetic method 3**, 3-chlorobut-3-enol (solution in *n*-hexane/EtOAc, 12.4 mL), 4-toluenesulfonyl chloride (19.07 g, 100 mmol), pyridine (8.08 mL, 100 mmol) and DMAP (cat.) was used. Purification was done by flash chromatography on silica (*n*-hexane/dichloromethane, 5:1 → 1:1), yielding 3-chlorobut-3-enyl tosylate (5.10 g, 19.56 mmol, 39 % over 2 steps) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.79 (d, *J* = 8.1 Hz, 2H), 7.35 (d, *J* = 8.1 Hz, 2H), 5.24 (d, *J* = 1.6 Hz, 1H), 5.21 (dt, *J* = 1.6, 1.0 Hz, 1H), 4.21 (t, *J* = 6.3 Hz, 2H), 2.67 (td, *J* = 6.3, 1.0 Hz, 2H), 2.46 ppm (s, 3H);

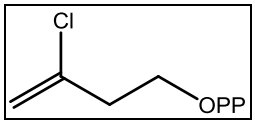
^{13}C NMR (101 MHz, $CDCl_3$): δ = 144.92, 136.62, 132.73, 129.83, 127.91, 115.57, 66.62, 38.62, 21.63 ppm;

MS/ESI *m/z* = 283.1 ($[M+Na]^+$, 100 %), 543.2 ($[2M+Na]^+$, 14 %);

HRMS/ESI** calcd for $C_{11}H_{13}ClNaO_3S$: 283.0166 $[M+Na]^+$, found: 283.0166.

<u>3-Chlorobut-3-enyl diphosphate (50)</u>

According to the **synthetic method 2**, DIPEA (1.10 mL, 6.45 mmol), DMF (527 µL, 6.85 mmol), water (56 µL, 3.13 mmol), tetrakis(trimethylsilyl) diphosphate (4.56 g, 9.78 mmol) and 3-chlorobut-3-enyl tosylate (510 mg, 1.96 mmol) was used. Purification was done by flash chromatography on silica (isopropanol/conc. ammonia/water, 8:6:2), yielding 3-chlorobut-3-enyl diphosphate (65 mg, 0.20 mmol, 10 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.40 (d, *J* = 1.2 Hz, 1H), 5.33 (d, *J* = 1.2 Hz, 1H),

4.14 (dt, *J* = 7.1, 6.2 Hz, 2H), 2.73 ppm (t, *J* = 6.2 Hz, 2H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 141.53, 117.42, 65.64 (d, *J* = 5.5 Hz), 42.39 ppm (d, *J* = 7.4 Hz);

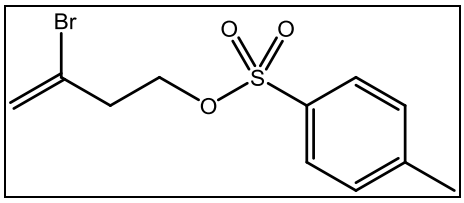
^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.00 (d, *J* = 21.9 Hz), -10.16 ppm (dt, *J* = 21.9, 7.1 Hz);

MS/ESI *m/z* = 229.3 ($[M-2NH_3-NH_4-HCl]^-$, 25 %), 264.9 ($[M-2NH_3-NH_4]^-$, 100 %), 531.1 ($[2M-5NH_3-NH_4]^-$, 26 %);

HRMS/ESI** calcd for $C_4H_8ClO_7P_2$: 264.9439 $[M-2NH_3-NH_4]^-$, found: 264.9436.

3-Bromobut-3-enyl tosylate (51)

According to the **synthetic method 3**, 4-toluenesulfonyl chloride (2.53 g, 13.25 mmol), pyridine (1.07 mL, 13.25 mmol), 3-bromobut-3-enol (1.00 g, 6.62 mmol), and DMAP (cat.) was used. Purification was done by flash chromatography on silica (*n*-hexane/dichloromethane, 4:1 → 1:2), yielding 3-bromobut-3-enyl tosylate (1.76 g, 5.77 mmol, 87 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.80 (d, *J* = 8.2 Hz, 2H), 7.36 (d, *J* = 8.2 Hz, 2H), 5.66 (dt, *J* = 2.0, 1.0 Hz, 1H), 5.49 (d, *J* = 2.0 Hz, 1H), 4.19 (t, *J* = 6.2 Hz, 2H), 2.75 (td, *J* = 6.2, 1.0 Hz, 2H), 2.46 ppm (s, 3H);

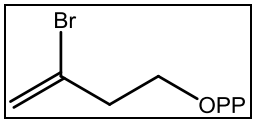
^{13}C NMR (101 MHz, $CDCl_3$): δ = 144.93, 132.73, 129.85, 127.95, 127.53, 120.19, 67.17, 40.72, 21.65 ppm;

MS/ESI *m/z* = 326.9 ($[M+Na]^+$, 100 %), 631.2 ($[2M+Na]^+$, 40 %);

HRMS/ESI** calcd for $C_{11}H_{13}BrNaO_3S$: 326.9661 $[M+Na]^+$, found: 326.9660.

3-Bromobut-3-enyl diphosphate (52)

According to the **synthetic method 2**, DIPEA (1.84 mL, 10.81 mmol), DMF (882 µL, 11.47 mmol), water (94 µL, 5.24 mmol), tetrakis(trimethylsilyl) diphosphate (7.65 g, 16.38 mmol) and 3-bromobut-3-enyl tosylate (1000 mg, 3.28 mmol) was used. Purification was done by flash chromatography on silica (isopropanol/conc. ammonia/water, 9:6:2), yielding 3-bromobut-3-enyl diphosphate (132 mg, 0.36 mmol, 11 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.83 (d, *J* = 1.9 Hz, 1H), 5.58 (d, *J* = 1.9 Hz, 1H), 4.12 (dt, *J* = 7.4, 6.2 Hz, 2H), 2.81 ppm (t, *J* = 6.2 Hz, 2H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 132.81, 122.03, 66.16 (d, *J* = 5.5 Hz), 44.54 ppm (d, *J* = 7.4 Hz);

^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.07 (d, *J* = 22.1 Hz), -10.32 ppm (dt, *J* = 22.1, 7.4 Hz);

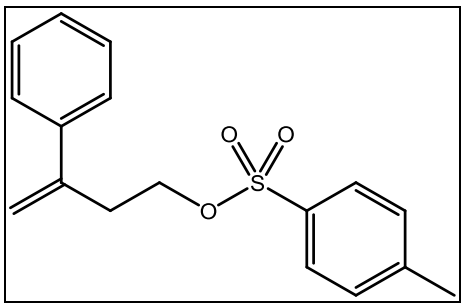
MS/ESI *m/z* = 229.0 ($[M\text{-}2NH_3\text{-}NH_4\text{-}HBr]^-$, 74 %), 309.0 ($[M\text{-}2NH_3\text{-}NH_4]^-$, 100 %), 619.3 ($[2M\text{-}5NH_3\text{-}NH_4]^-$, 17 %);

HRMS/ESI** calcd for $C_4H_8BrO_7P_2$: 308.8934 $[M\text{-}2NH_3\text{-}NH_4]^-$, found: 308.8930.

3-Phenylbut-3-enyl tosylate (54)

Paraformaldehyde (6.0 g, 200 mmol), 2-phenylpropene (6.50 mL, 50 mmol) and diethylaluminium chloride (1 M in *n*-hexane, 50 mL, 50 mmol) was dissolved in dry dichloromethane (50 mL) at -5 °C. After 30 hours of stirring at this temperature, saturated ammonium chloride (50 mL) was added and the reaction mixture was stirred at room temperature for other 10 minutes. The mixture was extracted with dichloromethane (3 x 50 mL) and the combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The intermediate was purified by flash chromatography on silica (*n*-hexane/EtOAc, 2:3), yielding 3-phenylbut-3-enol **(53)** (4.55 g, 30.70 mmol, 61 %) as a colorless oil.

According to the **synthetic method 3**, 3-phenylbut-3-enol (3.85 g, 25.98 mmol), 4-toluenesulfonyl chloride (9.91 g, 51.96 mmol), pyridine (4.19 mL, 51.96 mmol), and DMAP (cat.) was used. Purification was done by flash chromatography on silica (*n*-hexane/dichloromethane, 5:1 → 1:5), yielding 3-phenylbut-3-enyl tosylate (5.10 g, 16.87 mmol, 65 %, 40 % over 2 steps) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.72 (d, *J* = 8.2 Hz, 2H), 7.29 (d, *J* = 8.2 Hz, 2H), 7.28 – 7.24 (m, 5H), 5.34 (d, *J* = 1.0 Hz, 1H), 5.08 (q, *J* = 1.0 Hz, 1H), 4.10 (t, *J* = 7.1 Hz, 2H), 2.86 (td, *J* = 7.1, 1.0 Hz, 2H), 2.43 ppm (s, 3H);

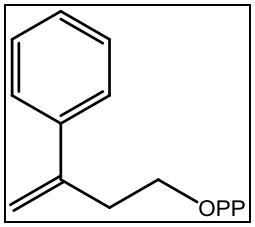
^{13}C NMR (101 MHz, $CDCl_3$): δ = 144.64, 142.69, 139.61, 132.94, 129.74, 128.40, 127.81, 127.72, 125.88, 115.28, 68.62, 34.68, 21.60 ppm;

MS/ESI *m/z* = 325.4 ($[M+Na]^+$, 100 %), 627.1 ($[2M+Na]^+$, 36 %);

HRMS/ESI** calcd for $C_{17}H_{18}NaO_3S$: 325.0869 $[M+Na]^+$, found: 325.0870.

3-Phenylbut-3-enyl diphosphate (55)

According to the **synthetic method 2**, DIPEA (3.71 mL, 21.83 mmol), DMF (1.78 mL, 23.15 mmol), water (191 µL, 10.58 mmol), tetrakis(trimethylsilyl) diphosphate (15.43 g, 33.07 mmol) and 3-phenylbut-3-enyl tosylate (2.00 g, 6.61 mmol) was used. Purification was done by flash chromatography on silica (isopropanol/conc. ammonia/water, 10:6:2), yielding 3-phenylbut-3-enyl diphosphate (654 mg, 1.82 mmol, 28 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 7.59 (m, 2H), 7.45 (m, 2H), 7.38 (m, 1H), 5.49 (d, *J* = 1.1 Hz, 1H), 5.29 (d, *J* = 1.1 Hz, 1H), 4.08 (q, *J* = 7.1 Hz, 2H), 2.92 ppm (t, *J* = 7.1 Hz, 2H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 147.50, 143.34, 131.56, 130.76, 128.98, 117.13, 67.27 (d, *J* = 5.6 Hz), 38.27 ppm (d, *J* = 7.4 Hz);

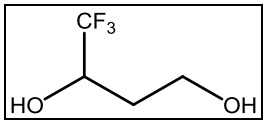
^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.31 (d, *J* = 21.8 Hz), -10.37 ppm (dt, *J* = 21.8, 7.1 Hz);

MS/ESI *m/z* = 307.3 ($[M-2NH_3-NH_4]^-$, 100 %), 615.2 ($[2M-5NH_3-NH_4]^-$, 46 %);

HRMS/ESI** calcd for $C_{10}H_{13}O_7P_2$: 307.0142 $[M-2NH_3-NH_4]^-$, found: 307.0138.

2,4-Dihydroxy-1,1,1-trifluorobutane (56)

Sodium borohydride (2.70 g, 71.46 mmol) was added to a solution of ethyl 4,4,4-trifluoro-3-oxobutanoate (11.96 g, 64.96 mmol) in dry diethyl ether (150 mL) at 0 °C. After 30 minutes of stirring the solution was allowed to warm to room temperature and was stirred for additional 16 hours. The reaction mixture was cooled to 0 °C and hydrochloric acid (1 M, 100 mL) was added carefully. The formed solid was removed by filtration and the filtrate was extracted with diethyl ether (3 x 50 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. The obtained colorless oil was added to a stirred suspension of lithium aluminium hydride (6.18 g, 162.95 mmol) in dry diethyl ether (100 mL) at 0 °C. After one hour the solution was allowed to warm to room temperature and was stirred for more 24 hours. The reaction mixture was cooled to 0 °C and hydrochloric acid (1 M, 100 mL) was added carefully. The reaction mixture was extracted with diethyl ether (3 x 50 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by distillation (b.p. 62-65 °C, 6 mbar), yielding 2,4-dihydroxy-1,1,1-trifluorobutane (7.56 g, 52.47 mmol, 81 % over 2 steps) as a colorless oil.



^{1}H NMR (400 MHz, CD_3OD): δ = 4.09 (dqd, *J* = 10.2, 7.2, 2.7 Hz, 1H), 3.73 (dd, *J* = 7.9, 4.7 Hz, 2H), 1.87 (dtd, *J* = 14.2, 7.9, 2.7 Hz, 1H), 1.70 ppm (ddt, *J* = 14.2, 10.2,

4.7 Hz, 1H);

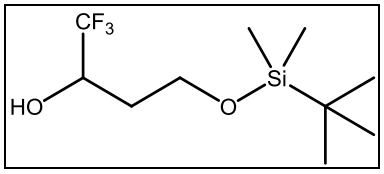
^{13}C NMR (101 MHz, CD_3OD): δ = 127.11 (q, *J* = 281.2 Hz), 67.71 (q, *J* = 31.0 Hz), 58.26, 33.59 ppm (q, *J* = 1.4 Hz);

^{19}F NMR (376 MHz, CD_3OD): -81.51 ppm (d, *J* = 7.2 Hz);

MS/ESI *m/z* = 142.9 ($[M-H]^-$, 100 %);

4-[(tert-butyldimethylsilyl)oxy]-1,1,1-trifluorobutan-2-ol (57)

Tert-Butyldimethylsilyl chloride (7.48 g, 49.62 mmol) was added to a solution of 2,4-dihydroxy-1,1,1-trifluorobutane (6.50 g, 45.11 mmol) and imidazole (6.14 g, 90.22 mmol) in dry dichloromethane (50 mL) at 0 °C. After one hour of stirring, the solution was allowed to warm to room temperature. After 15 more hours of stirring, brine (40 mL) was added and the reaction mixture was extracted with dichloromethane (3 x 50 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding 4-[(tert-butyldimethylsilyl)oxy]-1,1,1-trifluorobutan-2-ol (11.51 g, 44.55 mmol, 99 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.19 (dqdd, *J* = 9.0, 7.0, 4.0, 3.1 Hz, 1H), 3.99 (d, *J* = 4.0 Hz, 1H), 3.96 (ddd, *J* = 10.1, 7.3, 4.1 Hz, 1H), 3.86 (ddd, *J* = 10.1, 7.3, 4.1 Hz, 1H), 1.93 (dddd, *J* = 14.5, 7.3, 4.1, 3.1 Hz, 1H), 1.85 (dddd, *J* = 14.5, 9.0, 7.3, 4.1 Hz, 1H), 0.91 (s, 9H), 0.10 ppm (s, 6H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 124.99 (q, *J* = 281.1 Hz), 70.01 (q, *J* = 31.4 Hz), 60.73, 31.15 (q, *J* = 1.8 Hz), 25.70, 18.03, -5.71 ppm;

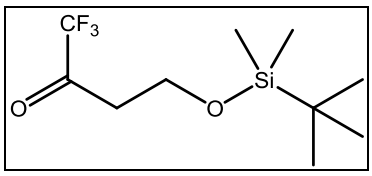
^{19}F NMR (376 MHz, $CDCl_3$): --79.97 ppm (d, *J* = 7.0 Hz);

MS/ESI *m/z* = 259.2 ($[M+H]^+$, 49 %), 281.2 ($[M+Na]^+$, 100 %);

HRMS/ESI** calcd for $C_{10}H_{22}F_3O_2Si$: 259.1336 $[M+H]^+$, found: 259.1338; calcd for $C_{10}H_{21}F_3NaO_2Si$: 281.1155 $[M+Na]^+$, found: 281.1157.

4-[(tert-butyldimethylsilyl)oxy]-1,1,1-trifluorobutan-2-one (58)

Dess-Martin periodinane (16.75 g, 39.48 mmol) was added to a solution of 4-[(tert-butyldimethylsilyl)oxy]-1,1,1-trifluorobutan-2-ol (8.50 g, 32.90 mmol) in dry dichloromethane (100 mL) at 0 °C. After 15 minutes of stirring the solution was allowed to warm to room temperature and was stirred for more 24 hours. The solvent was removed in vacuo and the residue was purified by flash chromatography on silica (*n*-hexane/EtOAc, 10:1), yielding 4-[(tert-butyldimethylsilyl)oxy]-1,1,1-trifluorobutan-2-one (8.22 g, 32.07 mmol, 97 %) as a colorless oil.



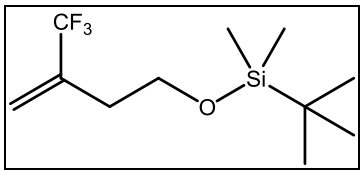
^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.97 (t, *J* = 6.0 Hz, 2H), 2.91 (t, *J* = 6.0 Hz, 2H), 0.87 (s, 9H), 0.06 ppm (s, 6H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 189.99 (q, *J* = 35.8 Hz), 115.35 (q, *J* = 291.6 Hz), 57.08, 39.64, 25.66, 18.12, -5.64 ppm;

^{19}F NMR (376 MHz, $CDCl_3$): -79.95 ppm.

Tert-butyldimethyl((3-(trifluoromethyl)but-3-en-1-yl)oxy)silane (59)

n-Butyllithium (2.5 M in *n*-hexane, 8.19 mL, 20.48 mmol) was added to a solution of methyltriphenylphosphonium iodide (8.87 g, 21.84 mmol) in dry THF (50 mL) at 0 °C. After one hour of stirring 4-[(tert-butyldimethylsilyl)oxy]-1,1,1-trifluorobutan-2-one (3.50 g, 13.65 mmol) was added and the reaction mixture was stirred for more three hours. Water (50 mL) was added and the mixture was extracted with ethyl acetate (3 x 50 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc, 20:1), yielding tert-butyldimethyl((3-(trifluoromethyl)but-3-en-1-yl)oxy)silane (2.39 g, 9.40 mmol, 69 %) as a colorless oil.



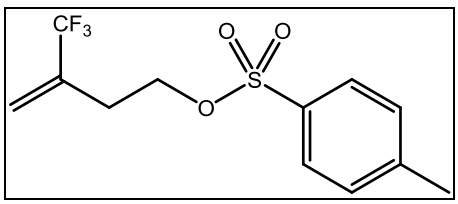
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.71 (q, *J* = 1.4 Hz, 1H), 5.41 (q, *J* = 1.4 Hz, 1H), 3.76 (t, *J* = 6.7 Hz, 2H), 2.41 (t, *J* = 6.7 Hz, 2H), 0.89 (s, 9H), 0.05 ppm (s, 6H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 135.37 (q, *J* = 29.6 Hz), 123.74 (q, *J* = 273.2 Hz), 119.57 (q, *J* = 5.9 Hz), 61.05, 32.96, 25.83, 18.24, -5.46 ppm;

^{19}F NMR (376 MHz, $CDCl_3$): -68.89 ppm.

3-(Trifluoromethyl)but-3-enyl tosylate (61)

Tert-butyldimethyl((3-(trifluoromethyl)but-3-en-1-yl)oxy)silane (2.10 g, 8.26 mmol) was added to a solution of tetra-*n*-butylammonium fluoride (1 M in THF, 10 mL, 10 mmol) in THF (25 mL) at 0 °C. After one hour of stirring the solvent was removed in vacuo and the intermediate was purified by flash chromatography on silica (*n*-pentane/diethyl ether, 1:1), yielding 3-(trifluoromethyl)but-3-enol **(60)** (0.70 g, 5.00 mmol, 61 %) as a colorless oil.
According to the **synthetic method 3**, 4-toluenesulfonyl chloride (1.90 g, 9.99 mmol), pyridine (807 µL, 9.99 mmol), 3-(trifluoromethyl)but-3-enol (700 mg, 5.00 mmol), and DMAP (cat.) was used. Purification was done by flash chromatography on silica (*n*-hexane/dichloromethane, 5:1 → 1:1), yielding 3-(Trifluoromethyl)but-3-enyl tosylate (1.40 g, 4.76 mmol, 95 %, 58 % over two steps) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.78 (d, *J* = 8.2 Hz, 2H), 7.36 (d, *J* = 8.2 Hz, 2H), 5.76 (q, *J* = 1.4 Hz, 1H), 5.42 (sext, *J* = 1.4 Hz, 1H), 4.17 (t, *J* = 6.6 Hz, 2H), 2.57 (td, *J* = 6.6, 1.4 Hz, 2H), 2.46 ppm (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$): δ = 145.04, 133.07 (q, *J* = 30.3 Hz), 132.75, 129.89, 127.87, 123.15 (q, *J* = 273.4 Hz), 121.16 (q, *J* = 5.7 Hz), 67.09, 29.22, 21.60 ppm;

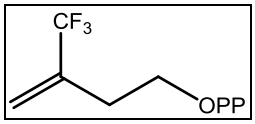
^{19}F NMR (376 MHz, $CDCl_3$): -68.81 ppm;

MS/ESI *m/z* = 317.2 ($[M+Na]^+$, 100 %), 611.1 ($[2M+Na]^+$, 13 %);

HRMS/ESI** calcd for $C_{12}H_{13}F_3NaO_3S$: 317.0430 $[M+Na]^+$, found: 317.0431.

3-(Trifluoromethyl)but-3-enyl diphosphate (62)

According to the **synthetic method 2**, DIPEA (1.72 mL, 10.09 mmol), DMF (824 µL, 10.70 mmol), water (88 µL, 4.89 mmol), tetrakis(trimethylsilyl) diphosphate (7.14 g, 15.29 mmol) and 3-(trifluoromethyl)but-3-enyl tosylate (900 mg, 3.06 mmol) was used. Purification was done by flash chromatography on silica (isopropanol/conc. ammonia/water, 9:6:2), yielding 3-(trifluoromethyl)but-3-enyl diphosphate (156 mg, 0.44 mmol, 15 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.85 (m, 1H), 5.65 (m, 1H), 4.13 (q, *J* = 6.8 Hz, 2H), 2.61 ppm (t, *J* = 6.8 Hz, 2H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 136.88 (q, *J* = 29.4 Hz), 126.65 (q, *J* = 272.7 Hz), 123.21 (q, *J* = 5.9 Hz), 66.14 (d, *J* = 5.4 Hz), 32.61 ppm (d, *J* = 7.7 Hz);

^{19}F NMR (376 MHz, D_2O + ND_4OD): -68.36 ppm;

^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.39 (d, *J* = 21.8 Hz), -10.76 ppm (dt, *J* = 21.8, 7.0 Hz);

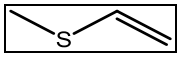
MS/ESI *m/z* = 299.2 ($[M-2NH_3-NH_4]^-$, 100 %), 599.3 ($[2M-5NH_3-NH_4]^-$, 47 %), 899.2 ($[3M-8NH_3-NH_4]^-$, 8 %);

HRMS/ESI** calcd for $C_5H_8F_3O_7P_2$: 298.9703 $[M-2NH_3-NH_4]^-$, found: 298.9700.

Methyl vinyl sulfide (63)

2-(Methylthio)ethanol (4.72 mL, 54.25 mmol) was added dropwise to potassium hydroxide (12.18 g, 217 mmol), which was heated to 150 °C. Direct distillation (b.p. 65-67 °C, normal

pressure) of the formed product was yielding methyl vinyl sulfide (3.09 g, 41.72 mmol, 77 %) as a colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.44 (dd, *J* = 16.5, 10.2 Hz, 1H), 5.19 (d, *J* = 10.2 Hz, 1H), 4.96 (d, *J* = 16.5 Hz, 1H), 2.25 ppm (s, 3H);

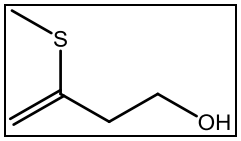
^{13}C NMR (101 MHz, $CDCl_3$): δ = 132.84, 108.31, 13.46 ppm;

MS/ESI *m/z* = 112.9 ($[M+K]^+$, 100 %), 187.0 ($[2M+K]^+$, 59 %);

HRMS/ESI** calcd for C_3H_7S: 75.0263 $[M+H]^+$, found: 75.0261.

3-(Thiomethyl)but-3-enol (64)

n-Butyllithium (2.5 M in *n*-hexane, 16.19 mL, 40.46 mmol) and methyl vinyl sulfide (3.00 g, 40.46 mmol) was added to a solution of potassium *tert*-butoxide (4.54 g, 40.46 mmol) in THF (50 mL) at -90 °C. The reaction mixture was warmed to -60 °C and ethylene oxide (2.85 g, 64.74 mmol) was added. The resulting solution was stirred for 30 minutes at -20 °C. After the addition of water (50 mL), the reaction mixture was extracted with diethyl ether (3 x 50 mL) and the combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification was done by distillation (b.p. 40-42 °C, 3 mbar), yielding 3-(thiomethyl)but-3-enol (1.20 g, 10.15 mmol, 25 %) as a colorless oil.



^{1}H NMR (400 MHz, pyridine-d5): δ = 6.20 (tt, *J* = 5.6, 1.0 Hz, 1H), 5.25 (s, 1H), 4.74 (s, 1H), 4.09 (td, *J* = 6.9, 5.6 Hz, 2H), 2.74 (td, *J* = 6.9, 1.0 Hz, 2H), 2.14 ppm (s, 3H);

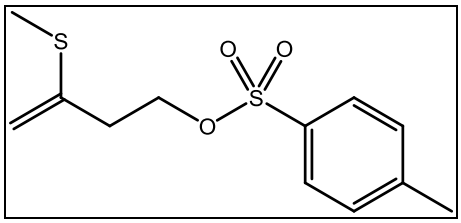
^{13}C NMR (101 MHz, pyridine-d5): δ = 144.63, 105.54, 61.48, 41.86, 14.46 ppm;

MS/ESI *m/z* = 157.1 ($[M+K]^+$, 26 %), 275.4 ($[2M+K]^+$, 100 %);

HRMS/ESI** calcd for $C_5H_{11}OS$: 119.0525 $[M+H]^+$, found: 119.0522.

3-(Thiomethyl)but-3-enyl tosylate (65)

According to the **synthetic method 3**, 4-toluenesulfonyl chloride (1.61 g, 8.46 mmol), pyridine (683 µL, 8.46 mmol), 3-(methylthio)but-3-enol (500 mg, 4.23 mmol), and DMAP (cat.) was used. Purification was done by flash chromatography on silica (*n*-hexane/EtOAc/triethylamine, 80:20:2), yielding 3-(methylthio)but-3-enyl tosylate (451 mg, 1.66 mmol, 39 %) as a colorless oil.



^{1}H NMR (400 MHz, pyridine-d5): δ = 7.95 (d, *J* = 8.2 Hz, 2H), 7.29 (d, *J* = 8.2 Hz, 2H), 5.09 (s, 1H), 4.68 (s, 1H), 4.33 (t, *J* = 6.6 Hz, 2H), 2.61 (t, *J* = 6.6 Hz, 2H), 2.25 (s, 3H), 2.07 ppm (s, 3H);

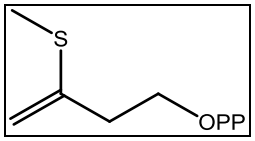
^{13}C NMR (101 MHz, pyridine-d5): δ = 145.20, 141.88, 133.66, 130.28, 128.29, 107.35, 69.31, 36.96, 21.30, 14.45 ppm;

MS/ESI *m/z* = 295.1 ($[M+Na]^+$, 100 %), 567.1 ($[2M+Na]^+$, 62 %);

HRMS/ESI** calcd for $C_{12}H_{16}NaO_3S_2$: 295.0433 $[M+Na]^+$, found: 295.0431.

3-(Thiomethyl)but-3-enyl diphosphate (66)

According to the **synthetic method 2**, DIPEA (927 µL, 5.45 mmol), DMF (445 µL, 5.78 mmol), water (48 µL, 2.64 mmol), tetrakis(trimethylsilyl) diphosphate (3.86 g, 8.26 mmol) and 3-(thiomethyl)but-3-enyl tosylate (450 mg, 1.65 mmol) was used. Purification was done by flash chromatography on silica (isopropanol/conc. ammonia/water, 9:6:2), yielding 3-(thiomethyl)but-3-enyl diphosphate (131 mg, 0.40 mmol, 24 %) as a white solid.



^{1}H NMR (400 MHz, D_2O + ND_4OD): δ = 5.27 (s, 1H), 4.87 (s, 1H), 4.10 (q, *J* = 7.0 Hz, 2H), 2.63 (t, *J* = 6.7 Hz, 2H), 2.29 ppm (s, 3H);

^{13}C NMR (101 MHz, D_2O + ND_4OD): δ = 145.54, 109.82, 67.23 (d, *J* = 5.5 Hz), 40.43 (d, *J* = 7.4 Hz), 16.60 ppm;

^{31}P NMR (162 MHz, D_2O + ND_4OD): -6.30 (d, *J* = 22.2 Hz), -10.50 ppm (dt, *J* = 22.2, 7.0 Hz);

MS/ESI *m/z* = 277.2 ($[M\text{-}2NH_3\text{-}NH_4]^-$, 100 %), 555.4 ($[2M\text{-}5NH_3\text{-}NH_4]^-$, 24 %);

HRMS/ESI** calcd for $C_5H_{11}O_7P_2S$: 276.9706 $[M\text{-}2NH_3\text{-}NH_4]^-$, found: 276.9706.

9. Bibliography

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10. Appendix

10.1 List of Abbreviations

CsTPS1	limonene synthase
DIPEA	*N*,*N*-diisopropylethylamine
DMADP	dimethylallyl diphosphate
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMP	Dess-Martin periodinane
DTT	dithiothreitol
EI	electron impact
ESI	electrospray ionization
EtOAc	ethyl acetate
FDP	farnesyl diphosphate
FFNSC	Flavour and Fragrance Natural and Synthetic Compounds
FTICR	fourier transform ion cyclotron resonance
GC	gas chromatography
GDP	geranyl diphosphate
GFDP	geranylfarnesyl diphosphate
GGDP	geranylgeranyl diphosphate
HMPA	hexamethylphosphoramide
HPLC	high-performance liquid chromatography
HRMS	high resolution mass spectrometry
IDP	isopentenyl diphosphate
IMAC	immobilized metal ion affinity chromatography
IPTG	Isopropyl β-D-1-thiogalactopyranoside
ISTD	internal standard
IUPAC	International Union of Pure and Applied Chemistry
LB	lysogeny broth
MEP	2-*C*-methyl-D-erythritol 4-phosphate
MOPSO	3-morpholino-2-hydroxypropanesulfonic acid
MS	mass spectrometry
MVA	mevalonate

M_W	molecular weight
NDP	neryl diphosphate
NIST	National Institute of Standards and Technology
NMR	nuclear magnetic resonance
OPP	diphosphate moiety
ppm	parts per million
RcCAS	casbene synthase
RI	retention index
RT	retention time
S_N2	nucleophilic substitution type 2
TB	terrific broth
TBAF	tetra-*n*-butylammonium fluoride
TBDMSCl	*tert*-butyldimethylsilyl chloride
*t*BME	methyl *tert*-butyl ether
TEAS	5-*epi*-aristolochene synthase
THF	tetrahydrofuran
THP	tetrahydropyran
TIC	total ion current
TLC	thin layer chromatography
TMS	tetramethylsilane or trimethylsilyl-
TMSP	3-(trimethylsilyl)propanoic acid
UDP	undecaprenyl diphosphate
UV	ultraviolet

10.2 List of Synthesized Organic Diphosphates

number	chemical name	CAS number (free acid)	CAS number (trisammonium salt)	chemical synthesis published
2d	3-norgeranyl diphosphate	192512-68-8	NA	yes
3d	3-norneryl diphosphate	NA	NA	no
4d	geranyl diphosphate	763-10-0	NA	yes
5d	neryl diphosphate	16751-02-3	NA	yes
6d	(2*E*,6*E*)-3-norfarnesyl diphosphate	59681-04-8	NA	no
7d	(2*Z*,6*E*)-3-norfarnesyl diphosphate	61252-12-8	NA	no
8d	(2*E*,6*E*)-farnesyl diphosphate	372-97-4	116057-57-9	yes
9d	(2*Z*,6*E*)-farnesyl diphosphate	40716-68-5	1221271-46-0	yes
10d	(2*E*,6*Z*)-farnesyl diphosphate	27248-38-0	NA	yes
11d	(2*Z*,6*Z*)-farnesyl diphosphate	27248-37-9	NA	yes
12d	(2*Z*,6*E*,10*E*)-3-norgeranylgeranyl diphosphate	273933-82-7	NA	yes
13d	(2*E*,6*E*,10*E*)-3-norgeranylgeranyl diphosphate	336621-61-5	NA	yes
14d	(2*E*,6*E*,10*E*)-geranylgeranyl diphosphate	6699-20-3	313263-08-0	yes
15d	(2*Z*,6*E*,10*E*)-geranylgeranyl diphosphate	64732-91-8	NA	yes
16d	(2*E*,6*Z*,10*E*)-geranylgeranyl diphosphate	178357-98-7	NA	yes
17d	(2*Z*,6*Z*,10*E*)-geranylgeranyl diphosphate	NA	NA	no
18d	(2*E*,6*E*,10*Z*)-geranylgeranyl diphosphate	905720-43-6	NA	yes
19d	(2*Z*,6*E*,10*Z*)-geranylgeranyl diphosphate	NA	NA	no
20d	(2*E*,6*Z*,10*Z*)-geranylgeranyl diphosphate	NA	NA	no
21d	(2*Z*,6*Z*,10*Z*)-geranylgeranyl diphosphate	1563176-32-8	NA	no
22d	(2*E*,6*E*,10*E*,14*E*)-geranylfarnesyl diphosphate	15493-60-4	NA	no
47	but-3-enyl diphosphate	104072-25-5	NA	yes
50	3-chlorobut-3-enyl diphosphate	1274917-95-1	NA	yes
52	3-bromobut-3-enyl diphosphate	96555-68-9	NA	yes
55	3-phenylbut-3-enyl diphosphate	1579961-26-4	NA	no
62	3-(trifluoromethyl)but-3-enyl diphosphate	45202-54-8	NA	yes
66	3-(methylthio)but-3-enyl diphosphate	1346447-65-1	NA	yes

10.3 Supplemental Data for the Biocatalytic Conversion Assays

10.3.1 Limonene Synthase (CsTPS1) Assays

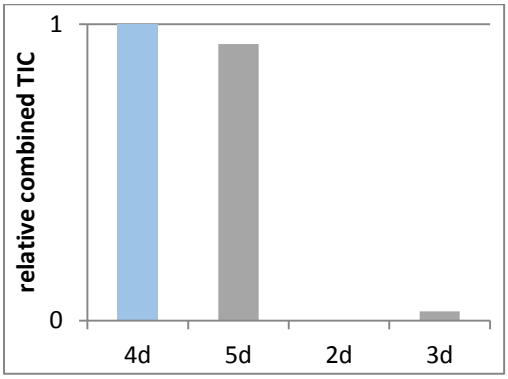
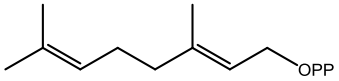


Fig. 35 – Relative combined total ion currents (TICs) of all products obtained from the biocatalytic conversion of C_9/C_{10} substrates by limonene synthase (CsTPS1). Comparison to the combined total ion currents (TIC) of all products obtained from the conversion of the enzymes natural substrate geranyl diphosphate (**4d**). Other substrates: neryl diphosphate (**5d**), 3-norgeranyl diphosphate (**2d**), 3-norneryl diphosphate (**3d**).

Geranyl diphosphate (4d)



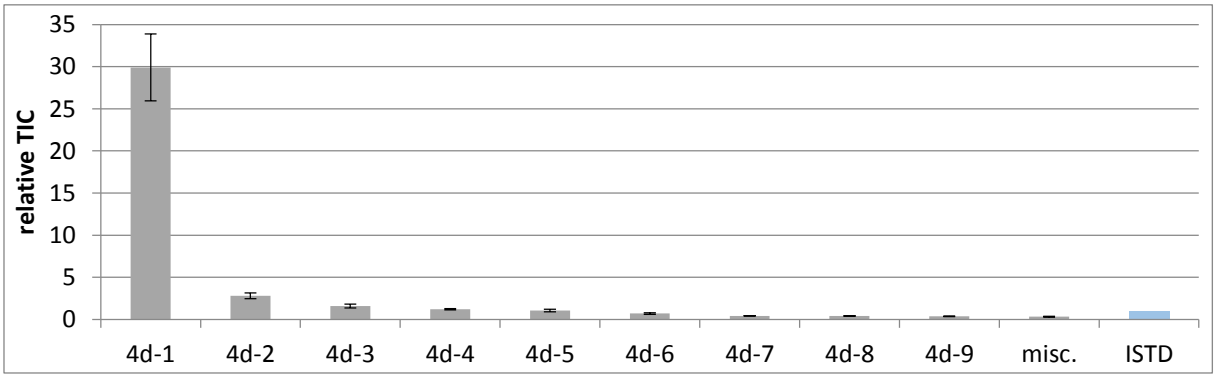
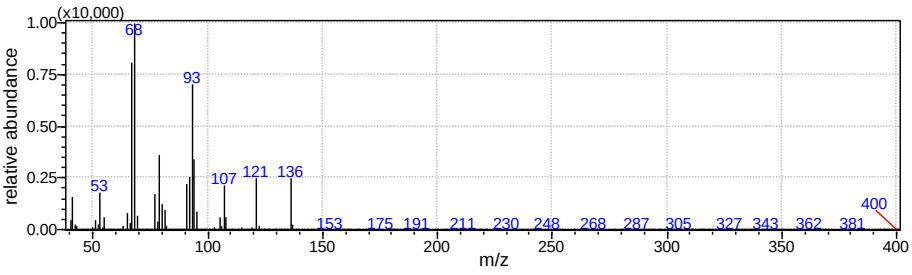


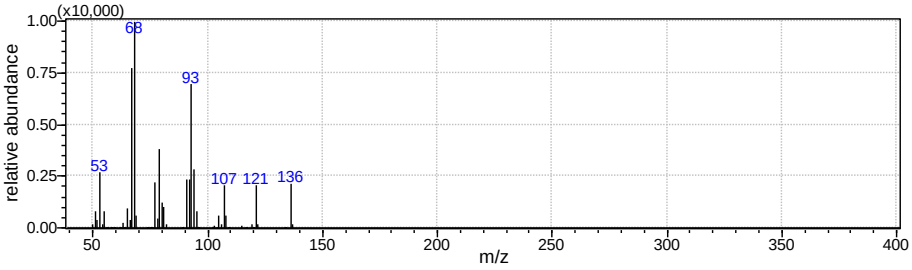
Fig. 36 – Relative total ion currents (TIC) of the observed products compared to the internal standard (ISTD).

4d-1

experimental EI spectrum RI = 1034, RT = 7.324 min

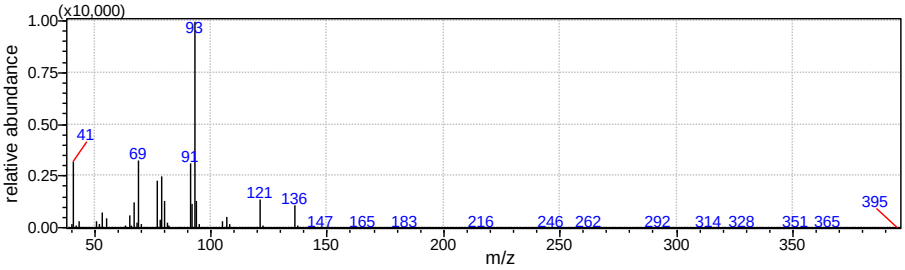


database entry: (*S*)-limonene RI = 1018

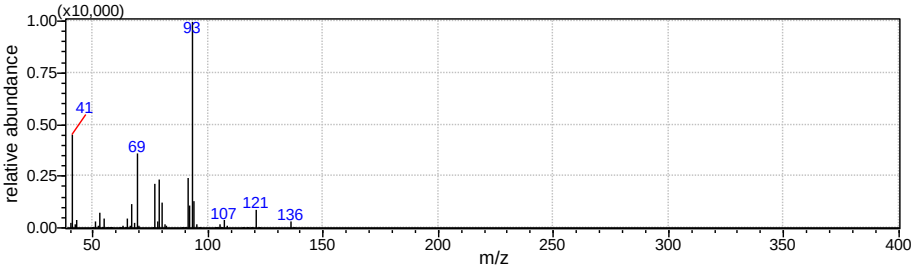


4d-2

experimental EI spectrum RI = 984, RT = 6.558 min

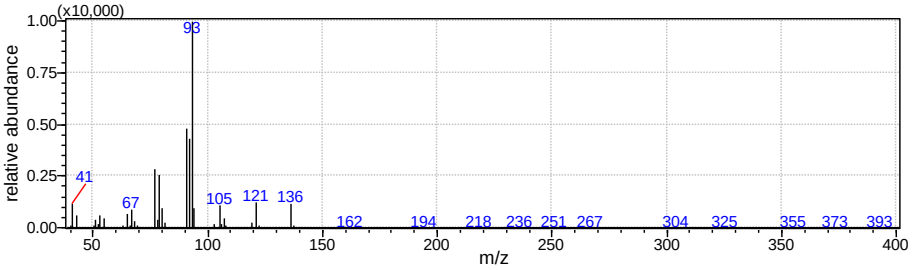


database entry: β-pinene RI = 943

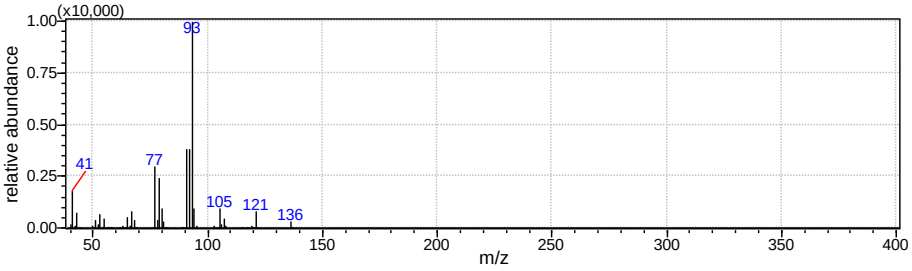


4d-3

experimental EI spectrum RI = 939, RT = 5.897 min

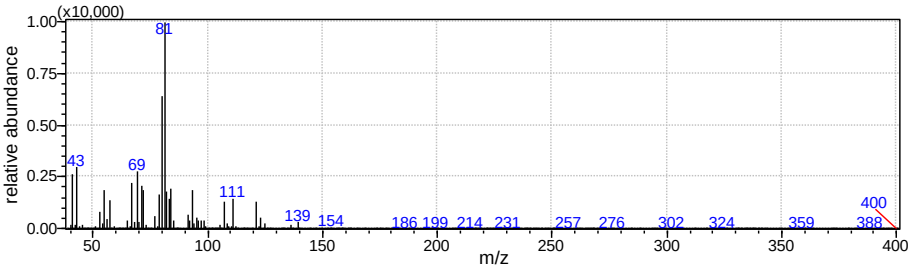


database entry: α-pinene RI = 933

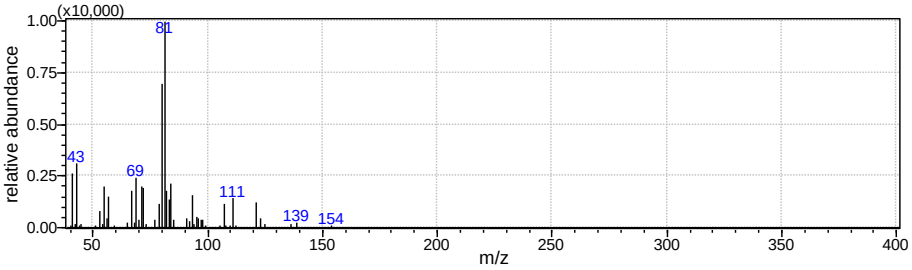


4d-4

experimental EI spectrum RI = 1121, RT = 8.662 min

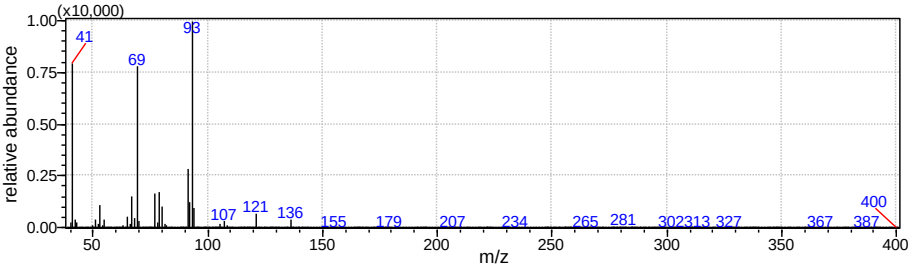


database entry: fenchol RI = 1123

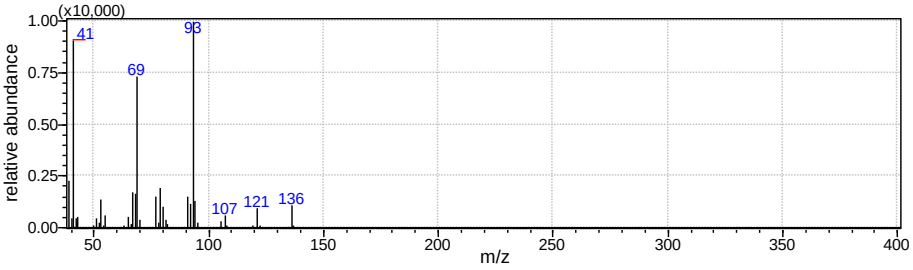


4d-5

experimental EI spectrum RI = 992, RT = 6.692 min

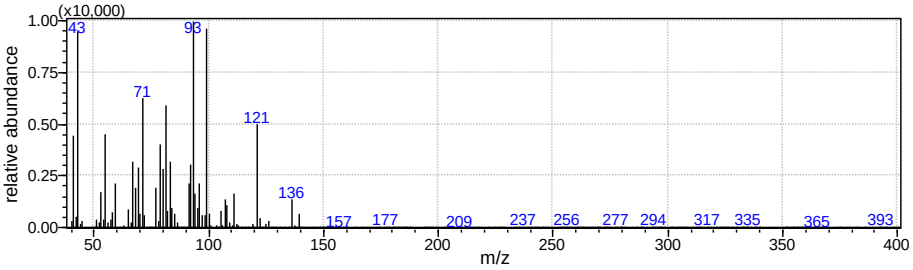


database entry: myrcene RI = 991

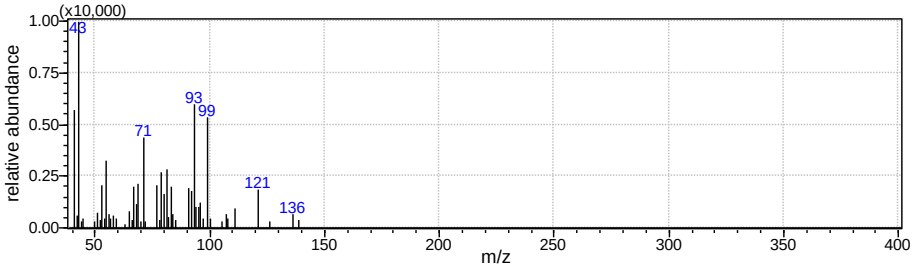


4d-6

experimental EI spectrum RI = 1131, RT = 8.803 min

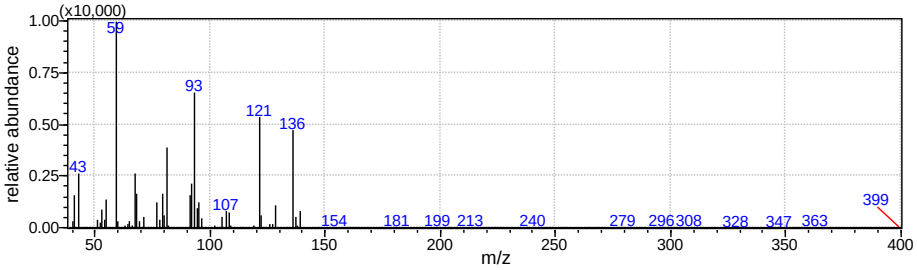


database entry: *cis*-pinene hydrate RI = 1144

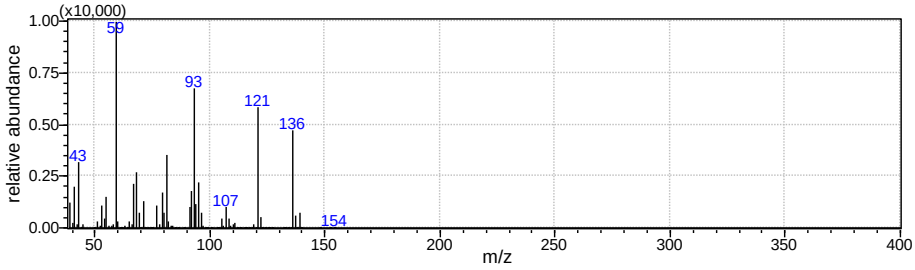


4d-7

experimental EI spectrum RI = 1199, RT = 9.829 min

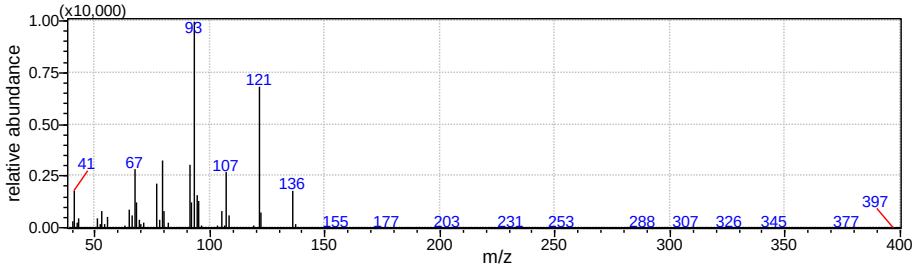


database entry: (*S*)-α-terpineol RI = 1143

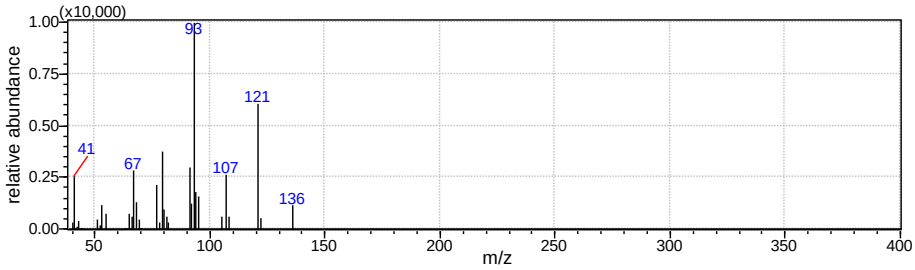


4d-8

experimental EI spectrum RI = 955, RT = 6.134 min

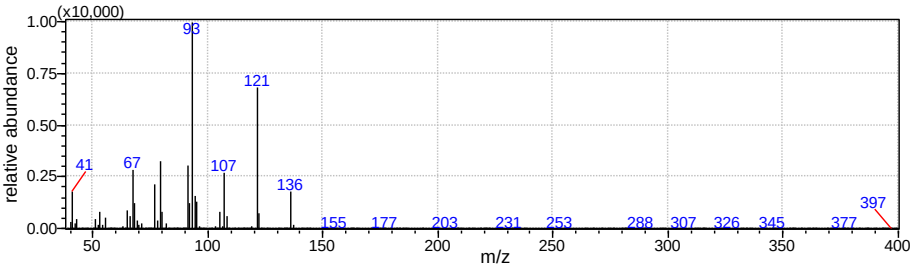


database entry: camphene RI = 953

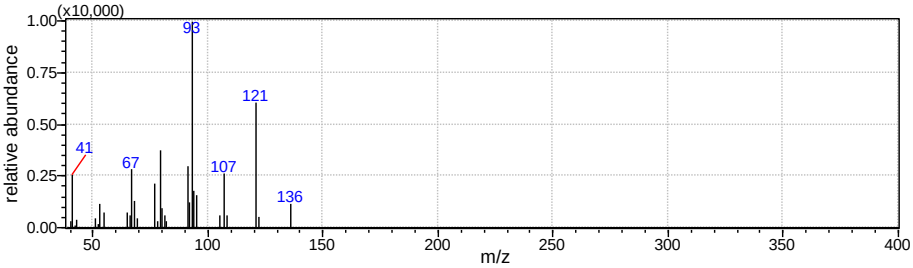


4d-9

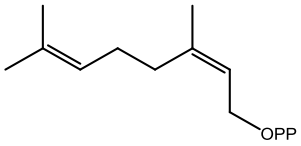
experimental EI spectrum RI = 1093, RT = 8.246



database entry: α-terpinolene RI = 1086



Neryl diphosphate (5d)



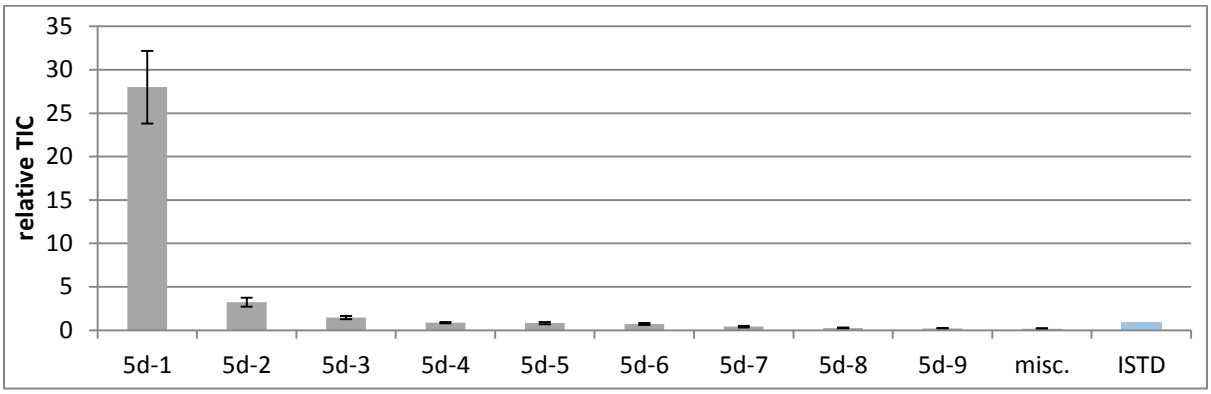
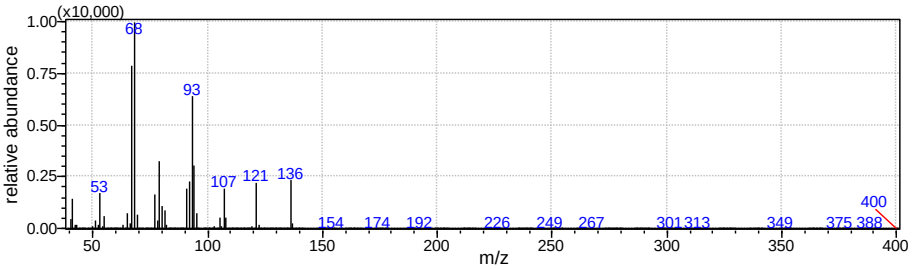


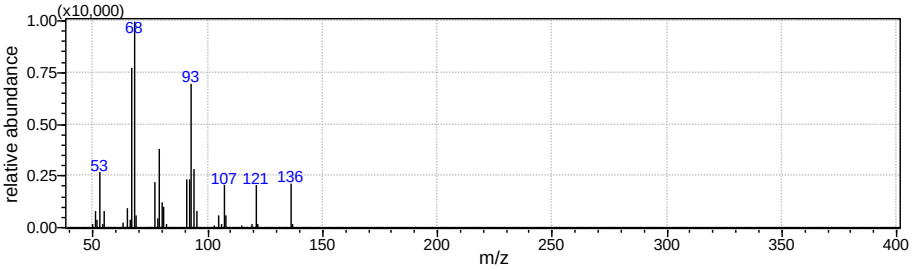
Fig. 37 – Relative total ion currents (TIC) of the observed products compared to the internal standard (ISTD).

5d-1

experimental EI spectrum RI = 1033, RT = 7.322 min

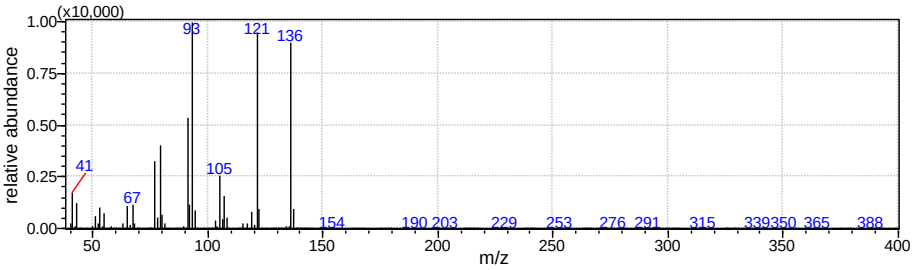


database entry: (*S*)-limonene RI = 1018

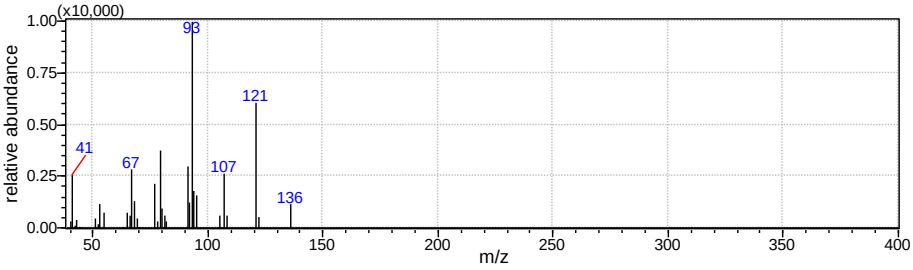


5d-2

experimental EI spectrum RI = 1093, RT = 8.242

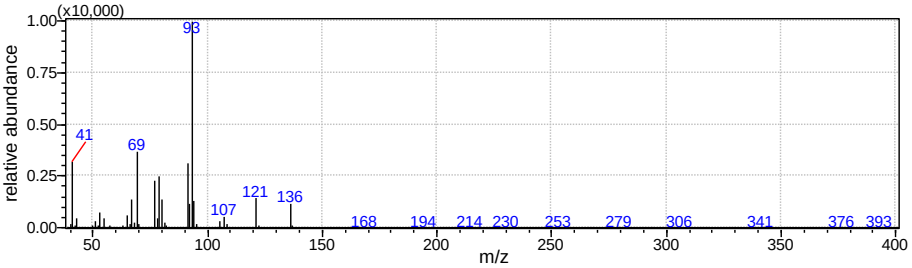


database entry: α-terpinolene RI = 1086

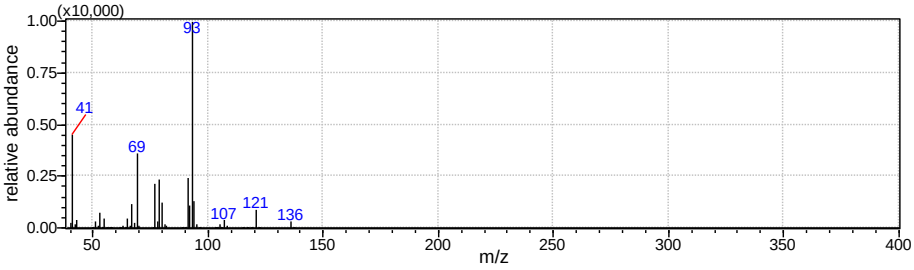


5d-3

experimental EI spectrum RI = 983, RT = 6.556 min

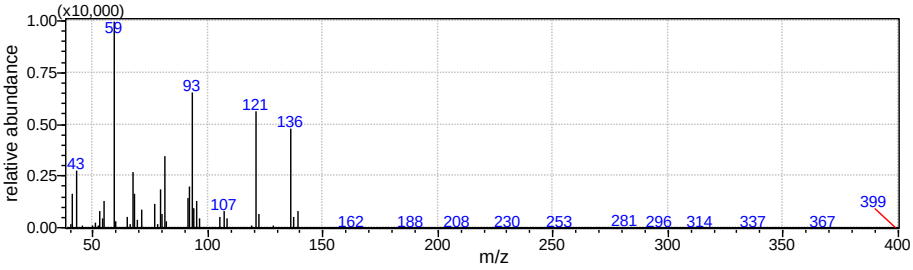


database entry: β-pinene RI = 943

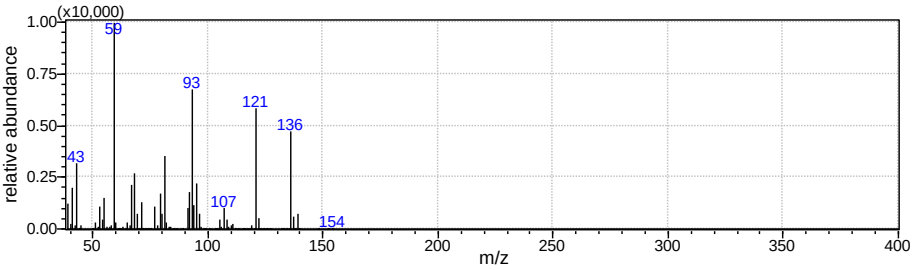


5d-4

experimental EI spectrum RI = 1198, RT = 9.819 min

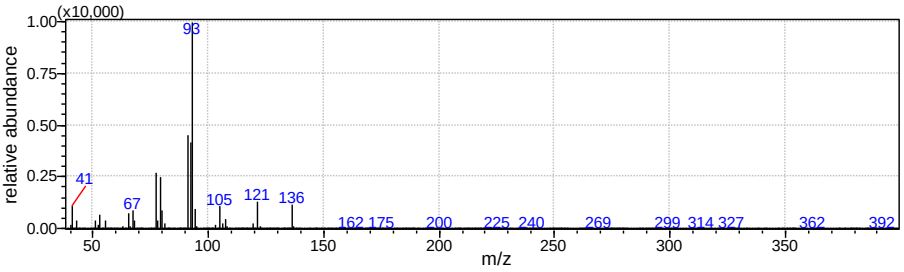


database entry: (*S*)-α-terpineol RI = 1143

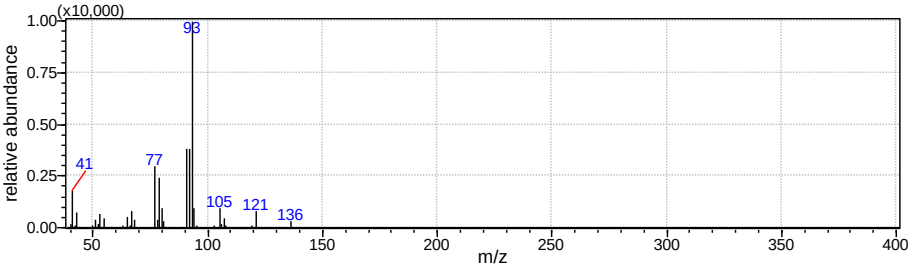


5d-5

experimental EI spectrum RI = 939, RT = 5.895 min

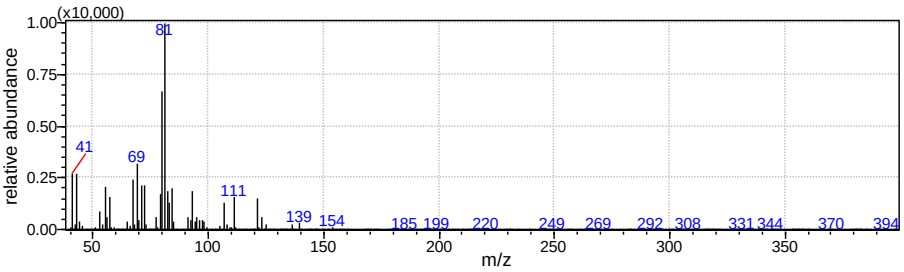


database entry: α-pinene RI = 933

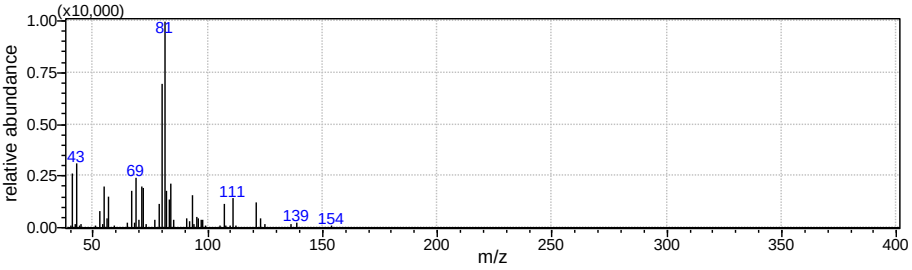


5d-6

experimental EI spectrum RI = 1121, RT = 8.662 min

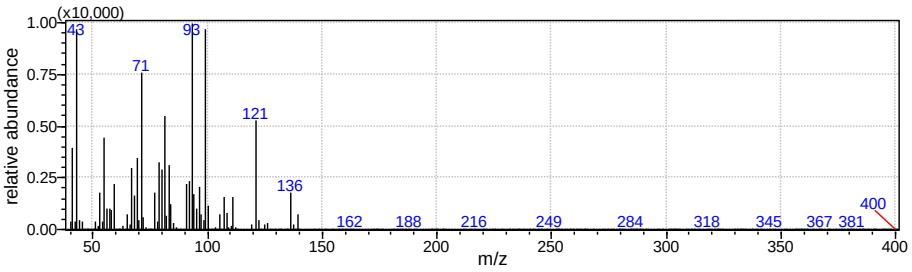


database entry: fenchol RI = 1123

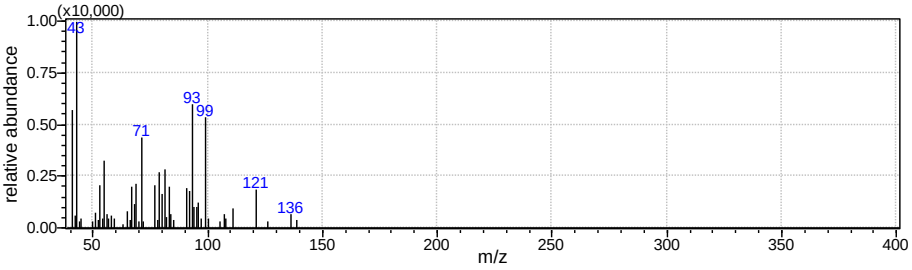


5d-7

experimental EI spectrum RI = 1131, RT = 8.803 min

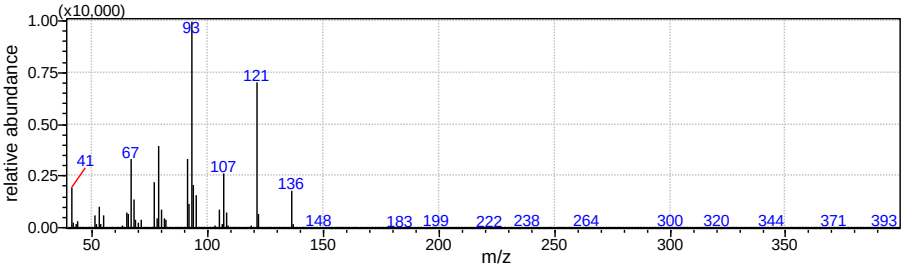


database entry: *cis*-pinene hydrate RI = 1144

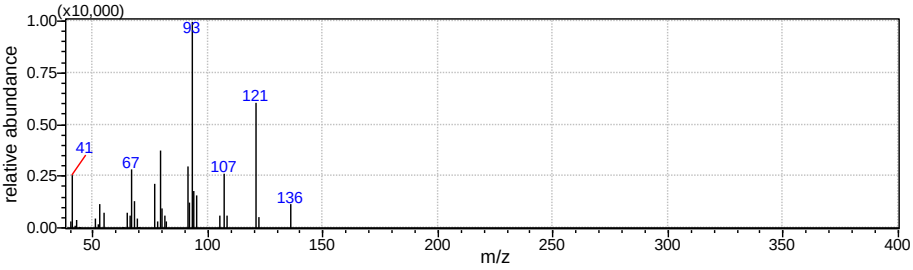


5d-8

experimental EI spectrum RI = 955, RT = 6.132 min

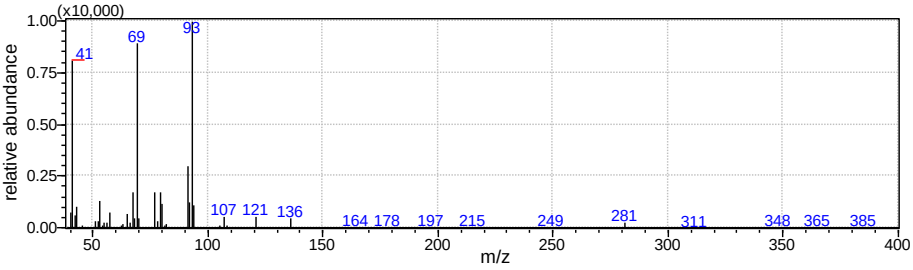


database entry: camphene RI = 953

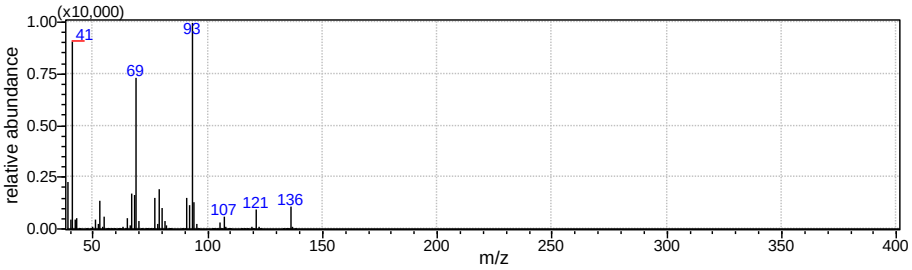


5d-9

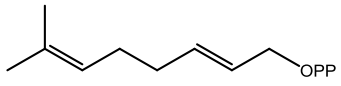
experimental EI spectrum RI = 992, RT = 6.690 min



database entry: myrcene RI = 991



3-Norgeranyl diphosphate (2d)



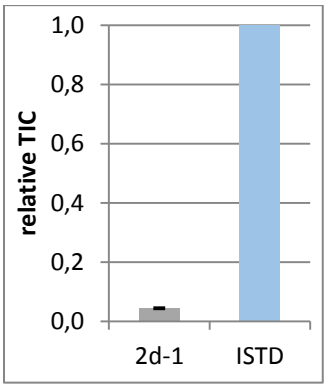
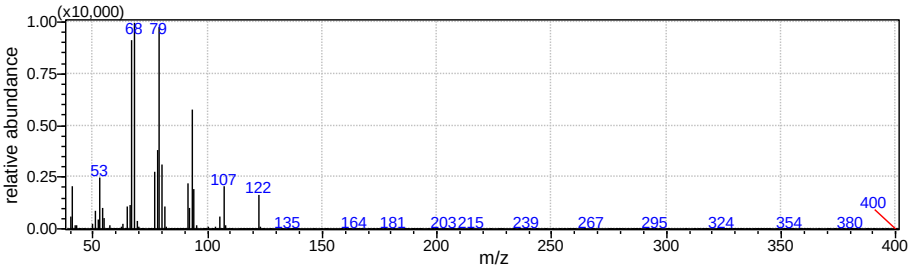


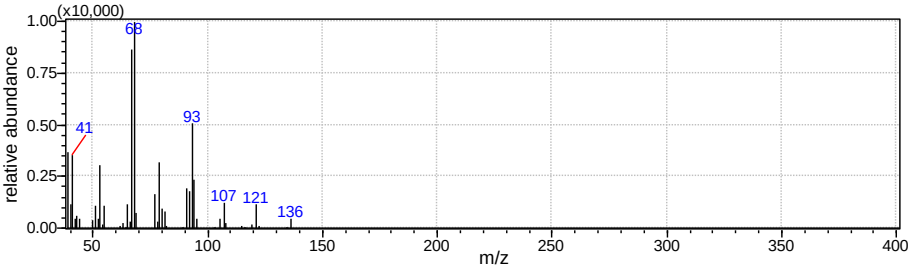
Fig. 38 – Relative total ion currents (TIC) of the observed products compared to the internal standard (ISTD).

2d-1

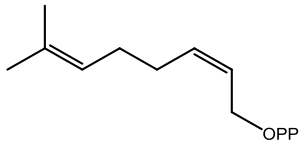
experimental EI spectrum RI = 946, RT = 5.999 min



database entry: (*S*)-limonene RI = 991



3-Nerneryl diphosphate (3d)



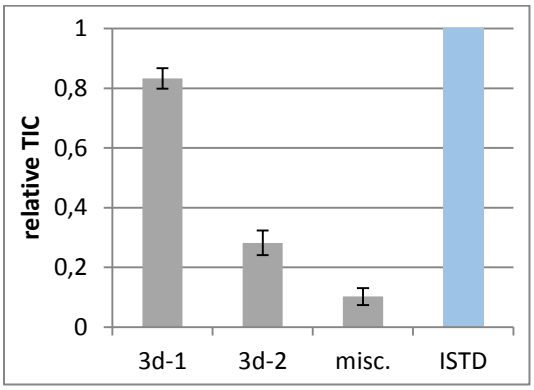
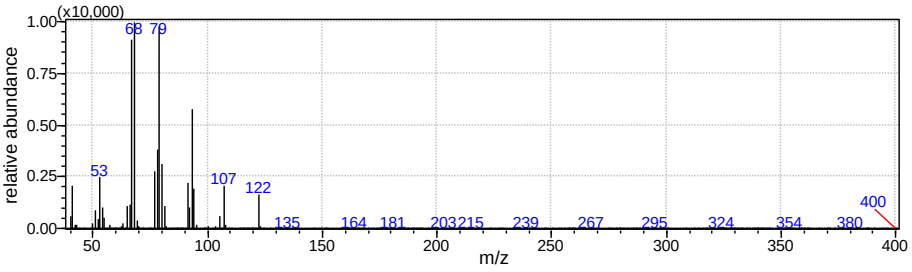


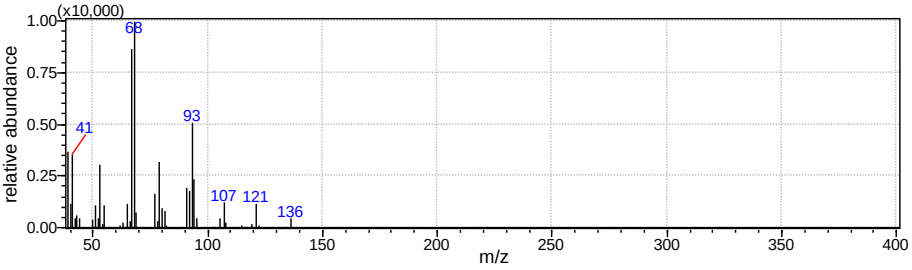
Fig. 39 – Relative total ion currents (TIC) of the observed products compared to the internal standard (ISTD).

3d-1

experimental EI spectrum RI = 946, RT = 6.001 min

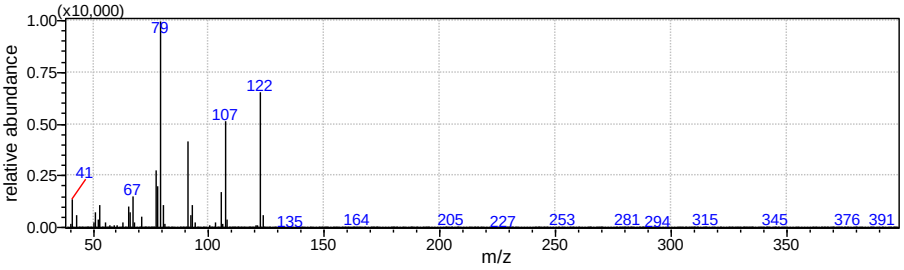


database entry: (*S*)-limonene RI = 991

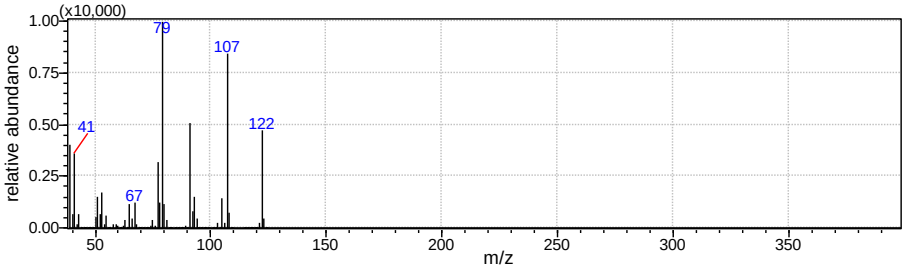


3d-2

experimental EI spectrum RI = 1006, RT = 6.907 min



database entry: 1-methylene-3-(1-methylethylidene)cyclopentane RI = 927



10.3.2 5-*epi*-Aristolochene Synthase (TEAS) Assays

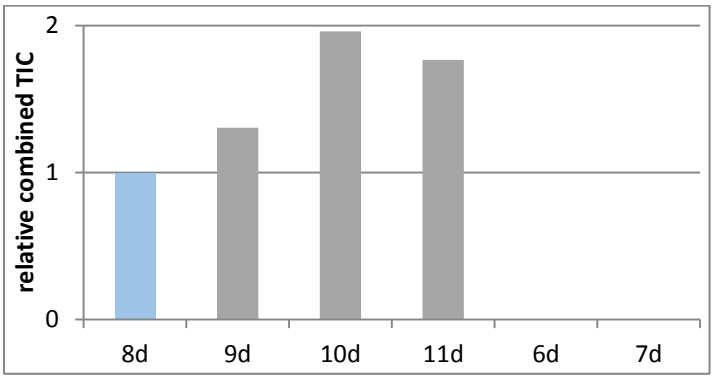
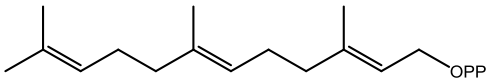


Fig. 40 – Relative combined total ion currents (TICs) of all products obtained from the biocatalytic conversion of C_{14}/C_{15} substrates by 5-*epi*-aristolochene synthase (TEAS). Comparison to the combined total ion currents (TIC) of all products obtained from the conversion of the enzymes natural substrate (*E*,*E*)-farnesyl diphosphate (**8d**). Other substrates: (*Z*,*E*)-farnesyl diphosphate (**9d**), (*E*,*Z*)-farnesyl diphosphate (**10d**), (*Z*,*Z*)-farnesyl diphosphate (**11d**), (*E*,*E*)-3-norfarnesyl diphosphate (**6d**), (*Z*,*E*)-3-norfarnesyl diphosphate (**7d**). No conversion in case of **6d** and **7d**.

(*E*,*E*)-Farnesyl diphosphate (8d)



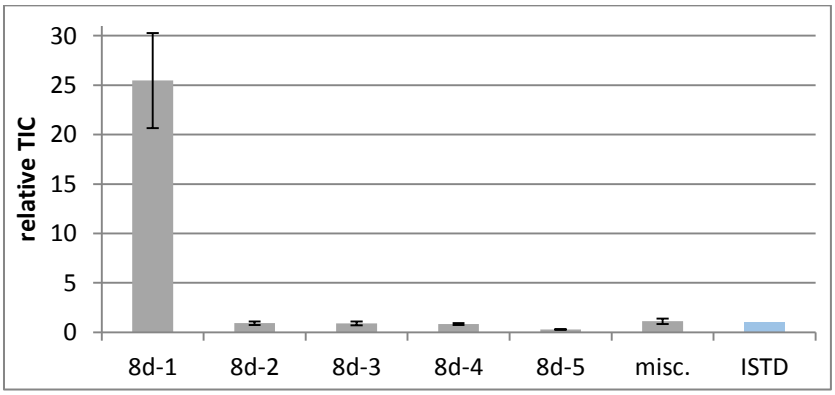
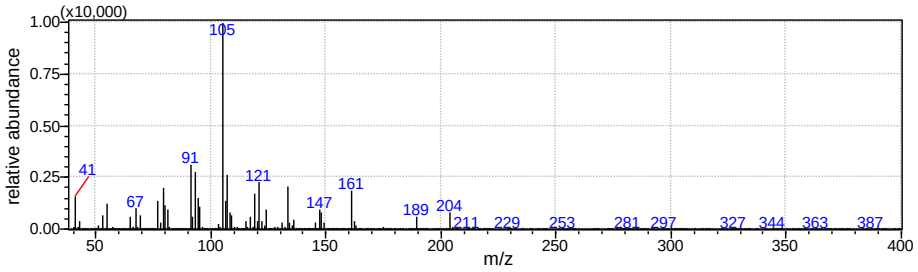


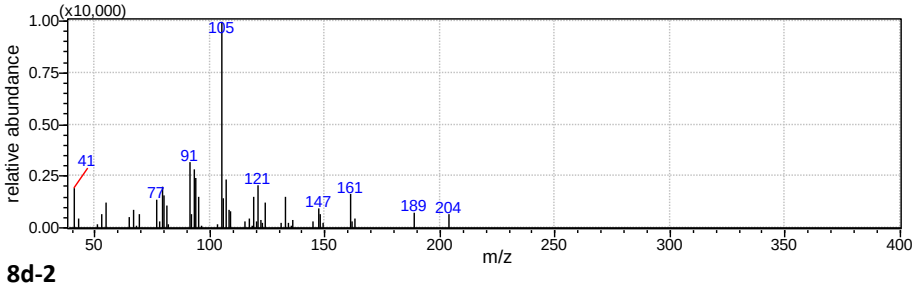
Fig. 41 – Relative total ion currents (TIC) of the observed products compared to the internal standard (ISTD).

8d-1

experimental EI spectrum RI = 1505, RT = 13.948 min

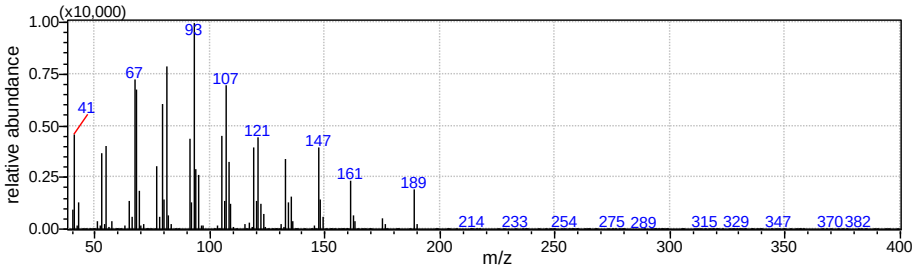


database entry: (+)-5-*epi*-aristolochene RI = 1473

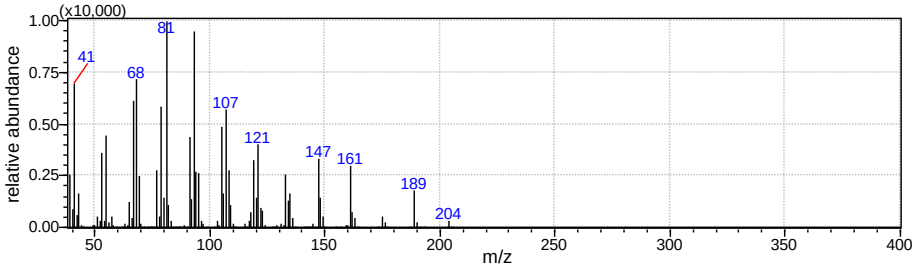


8d-2

experimental EI spectrum RI = 1406, RT = 12.688 min

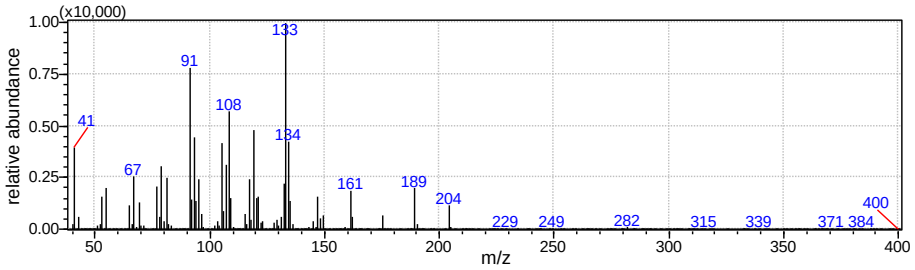


database entry: β-elemene RI = 1398

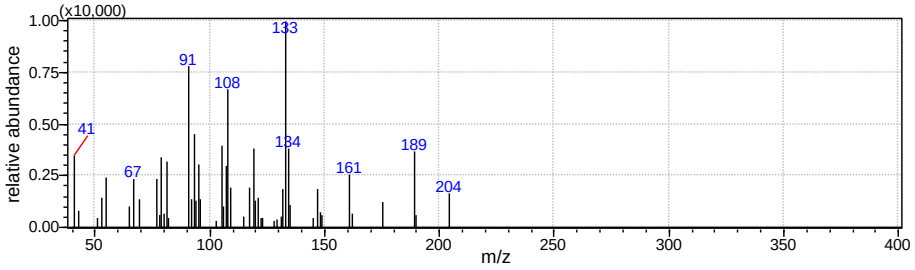


8d-3

experimental EI spectrum RI = 1476, RT = 13.592 min

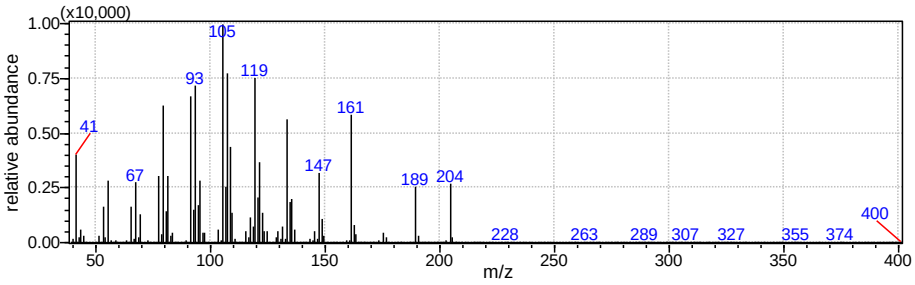


database entry: prezizaene RI = 1457

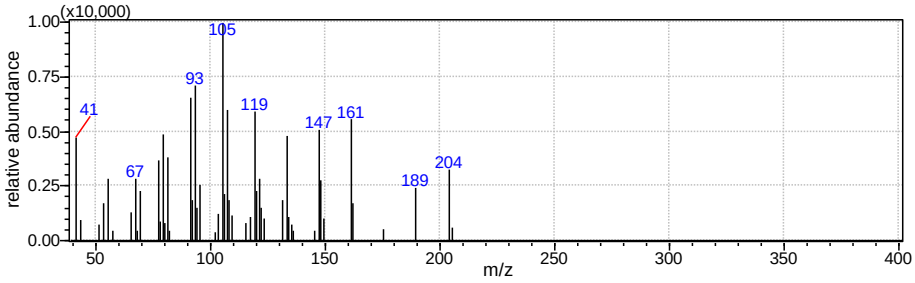


8d-4

experimental EI spectrum RI = 1511, RT = 14.026 min

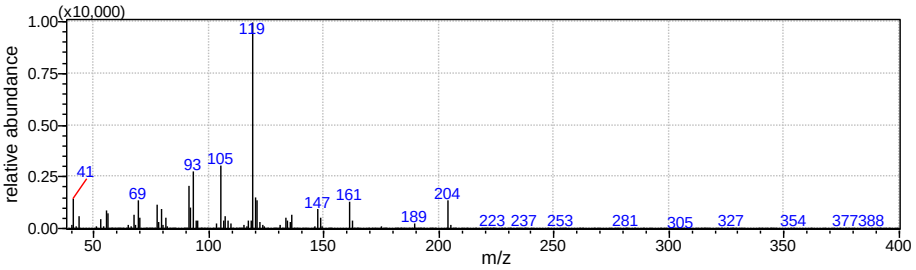


database entry: (-)-4-*epi*-eremophilene RI = 1474

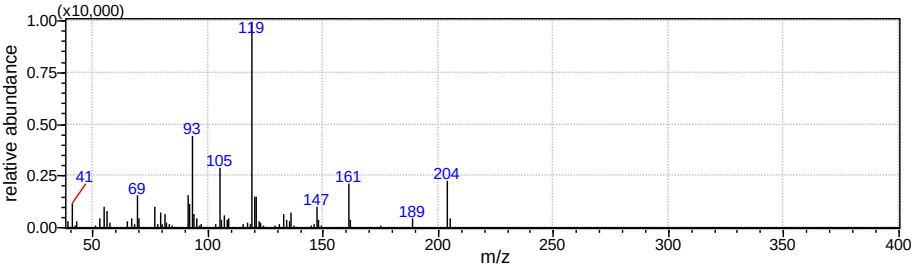


8d-5

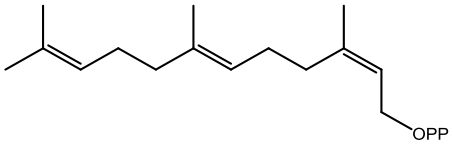
experimental EI spectrum RI = 1435, RT = 13.062 min



database entry: α-cedrene RI = 1403



(*Z*,*E*)-Farnesyl diphosphate (9d)



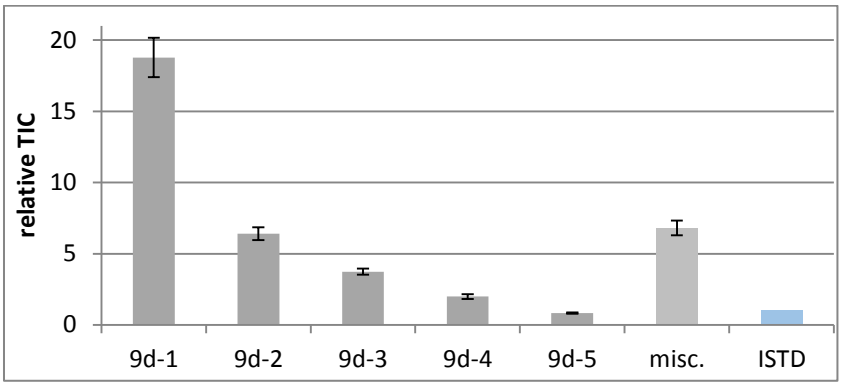
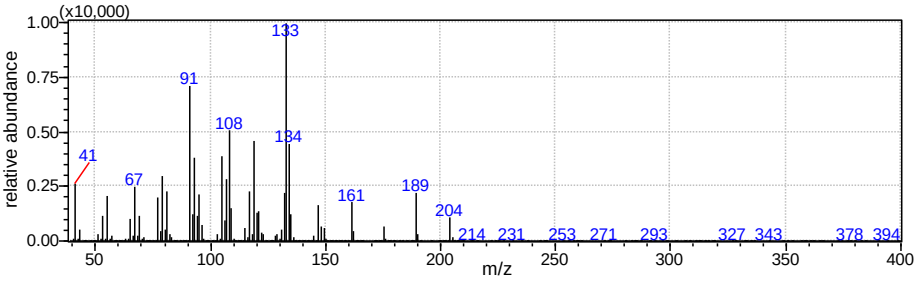


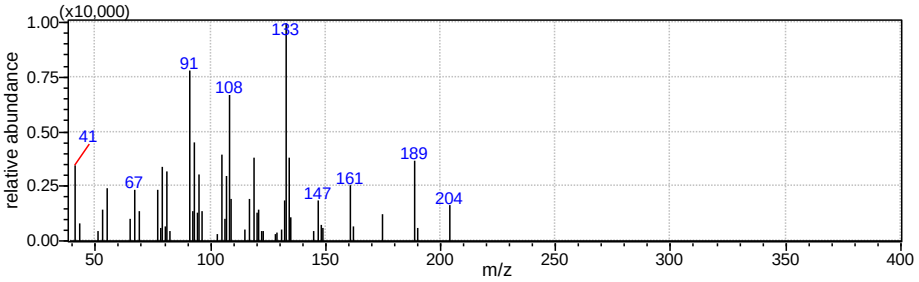
Fig. 42 – Relative total ion currents (TIC) of the observed products compared to the internal standard (ISTD).

9d-1

experimental EI spectrum RI = 1477, RT = 13.596 min

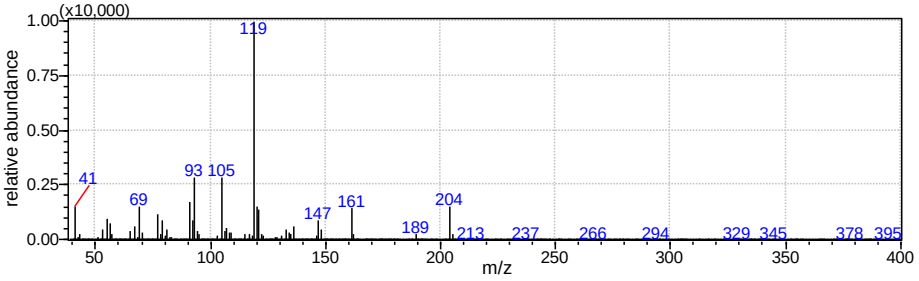


database entry: prezizaene RI = 1457

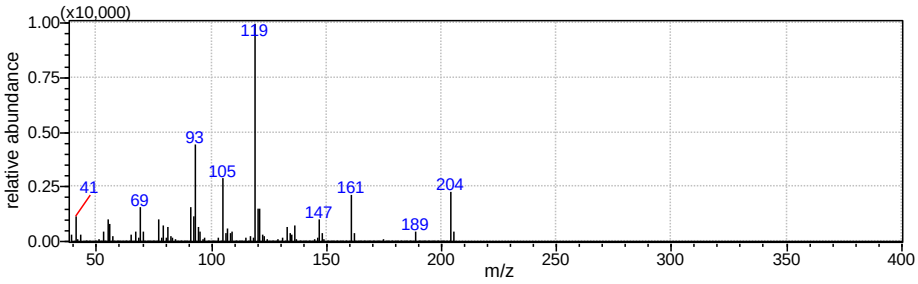


9d-2

experimental EI spectrum RI = 1435, RT = 13.063 min

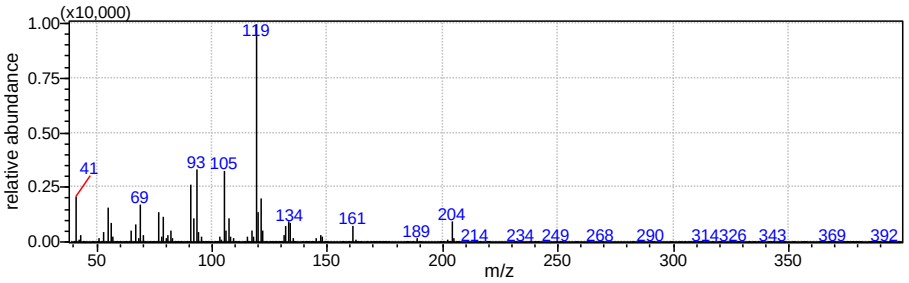


database entry: α-cedrene RI = 1403

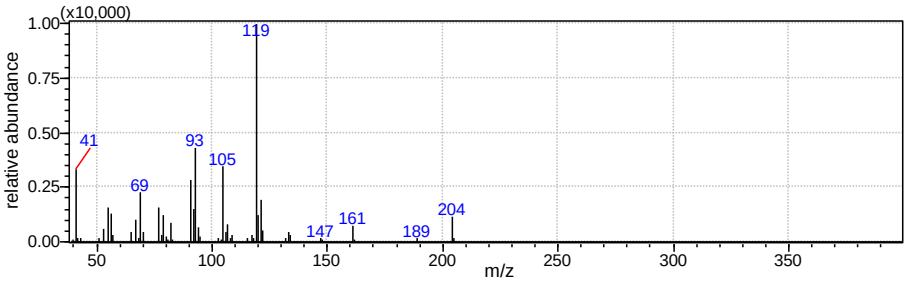


9d-3

experimental EI spectrum RI = 1523, RT = 14.174 min

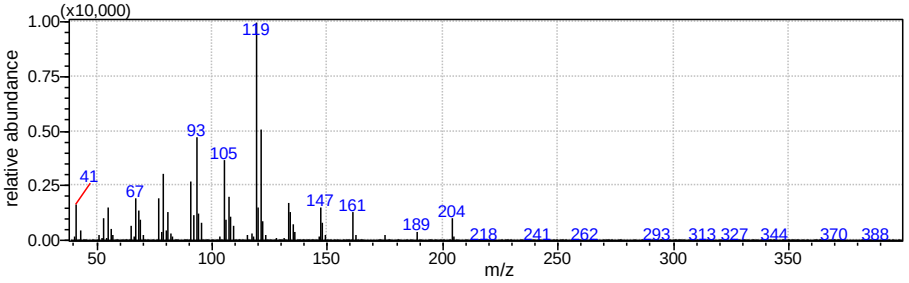


database entry: β-curcumene RI = 1511

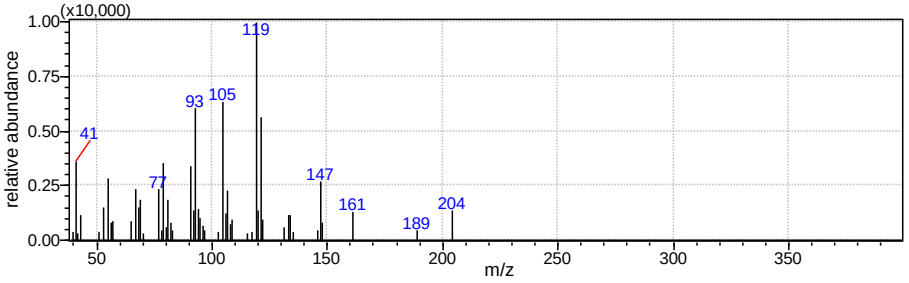


9d-4

experimental EI spectrum RI = 1486, RT = 13.716 min

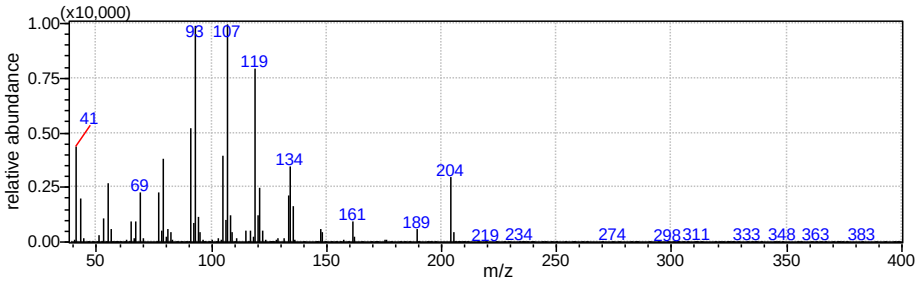


database entry: acoradiene RI = 1471

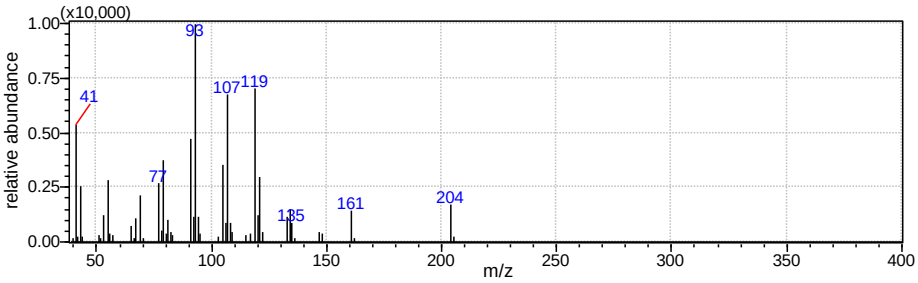


9d-5

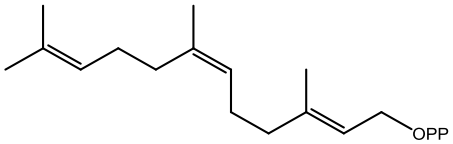
experimental EI spectrum RI = 1530, RT = 14.256 min



database entry: (*Z*)-γ-bisabolene RI = 1511



(*E*,*Z*)-Farnesyl diphosphate (10d)



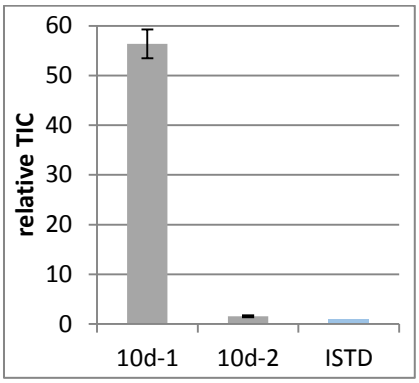
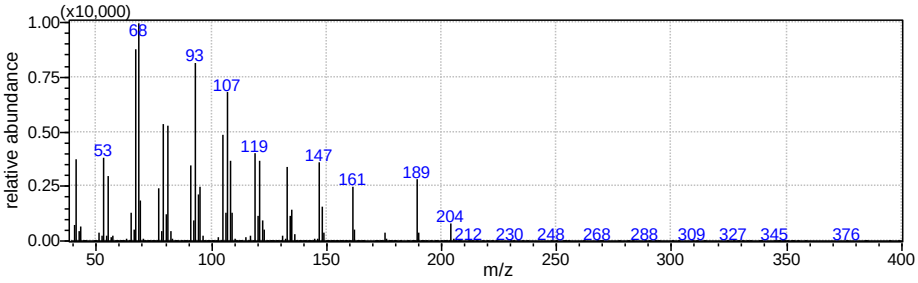


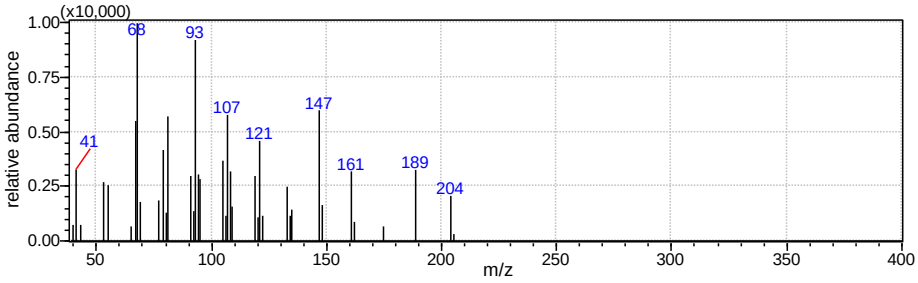
Fig. 43 – Relative total ion currents (TIC) of the observed products compared to the internal standard (ISTD).

10d-1

experimental EI spectrum RI = 1516, RT = 14.092 min

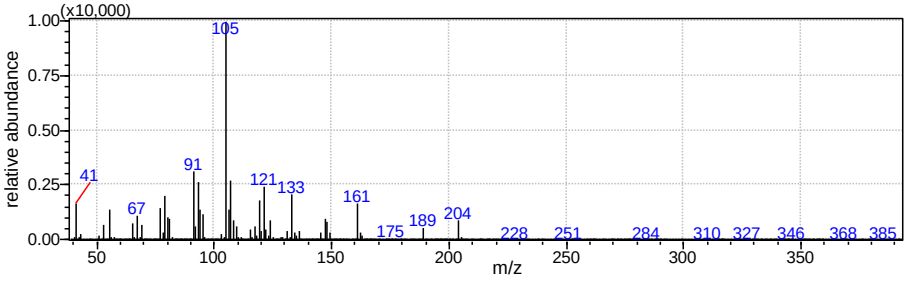


database entry: germacrene A RI = 1570

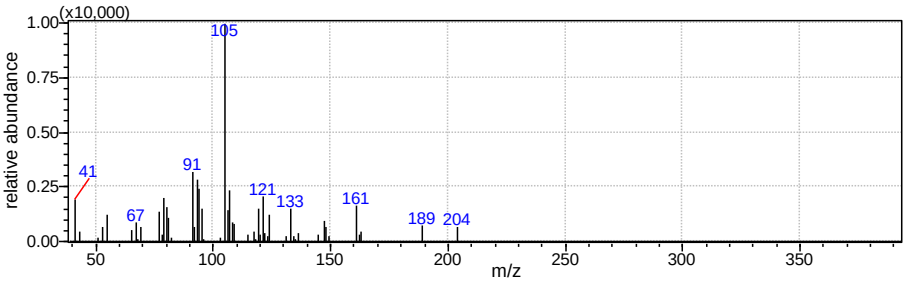


10d-2

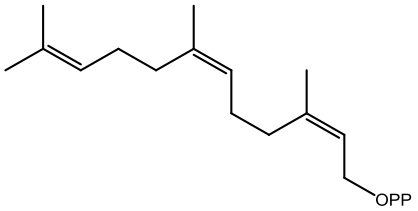
experimental EI spectrum RI = 1505, RT = 13.951 min



database entry: (+)-5-*epi*-aristolochene RI = 1473



(*Z*,*Z*)-Farnesyl diphosphate (11d)



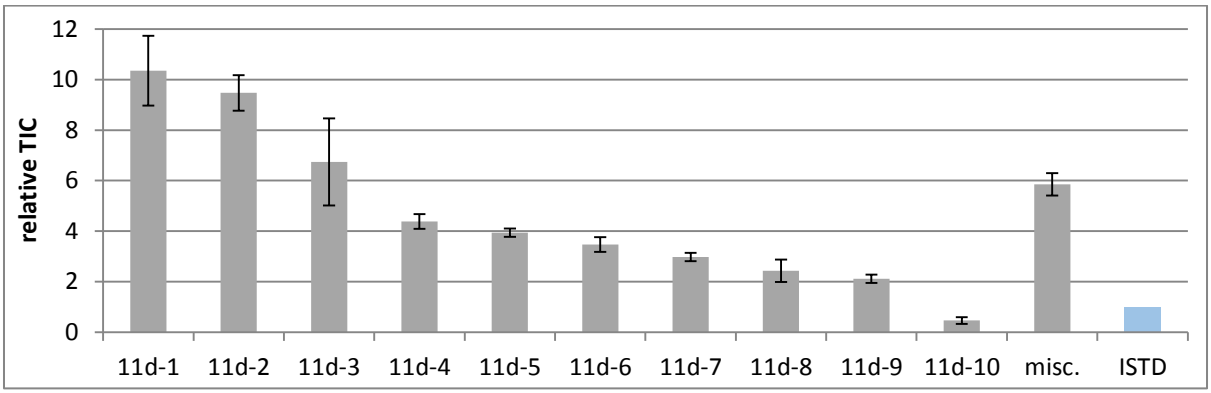
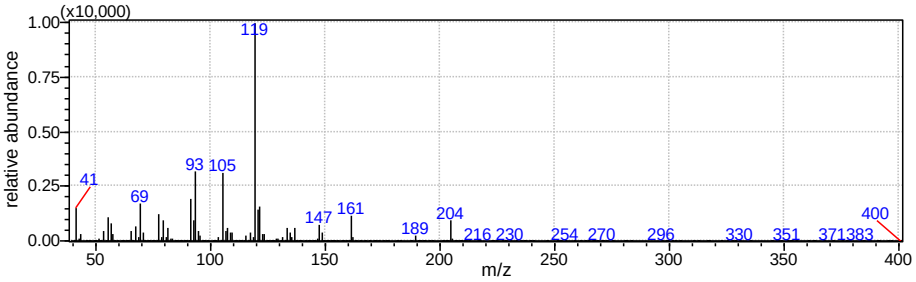


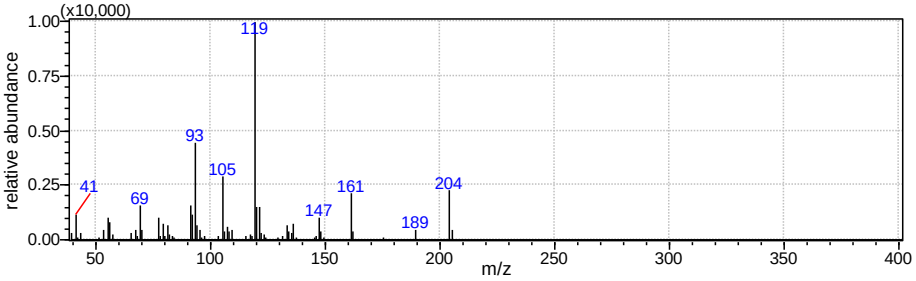
Fig. 44 – Relative total ion currents (TIC) of the observed products compared to the internal standard (ISTD).

11d-1

experimental EI spectrum RI = 1400, RT = 12.616 min

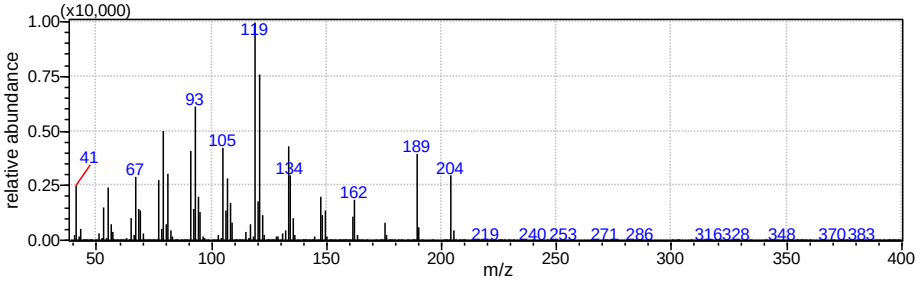


database entry: α-cedrene RI = 1414

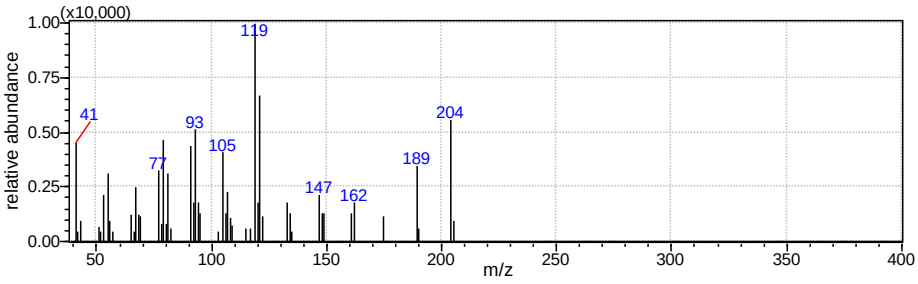


11d-2

experimental EI spectrum RI = 1477, RT = 13.599 min

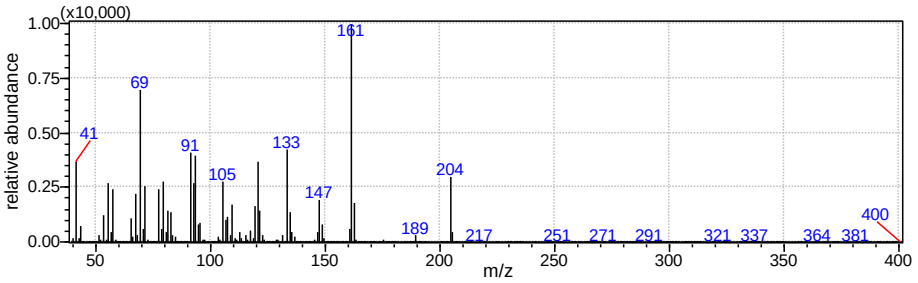


database entry: amorpha-4,11-diene RI = 1451

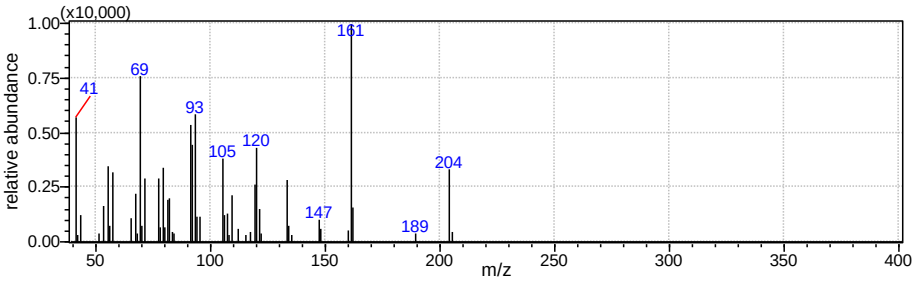


11d-3

experimental EI spectrum RI = 1438, RT = 13.094 min

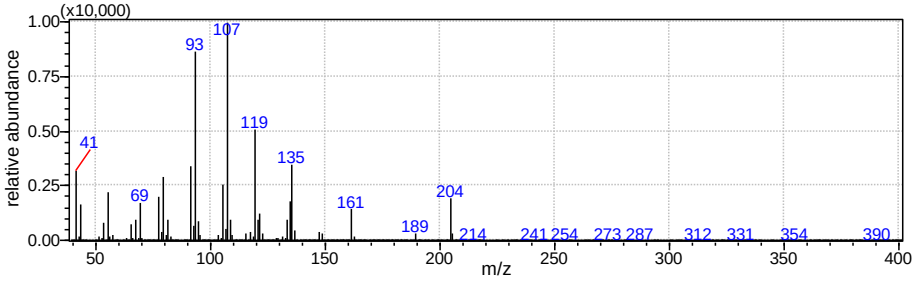


database entry: β-cedrene RI = 1423

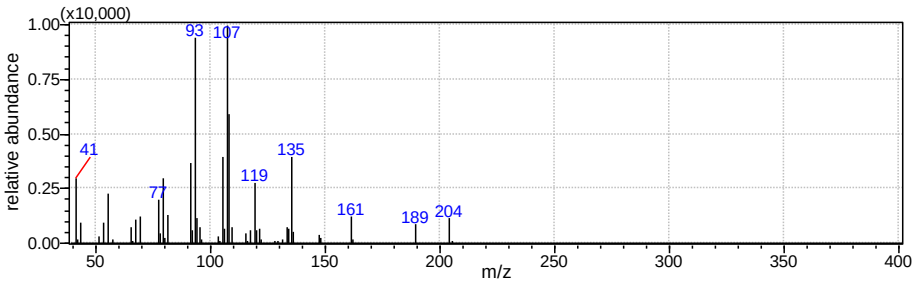


11d-4

experimental EI spectrum RI = 1545, RT = 14.440 min

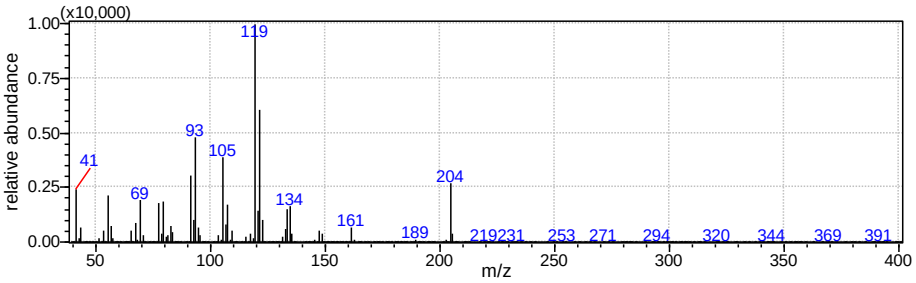


database entry: (*E*)-γ-bisabolene RI = 1528

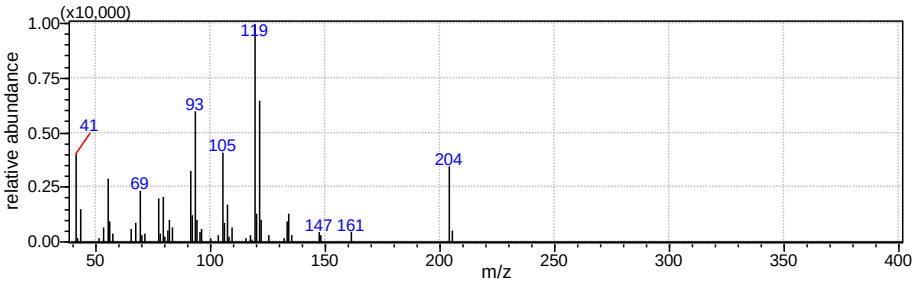


11d-5

experimental EI spectrum RI = 1491, RT = 13.779 min

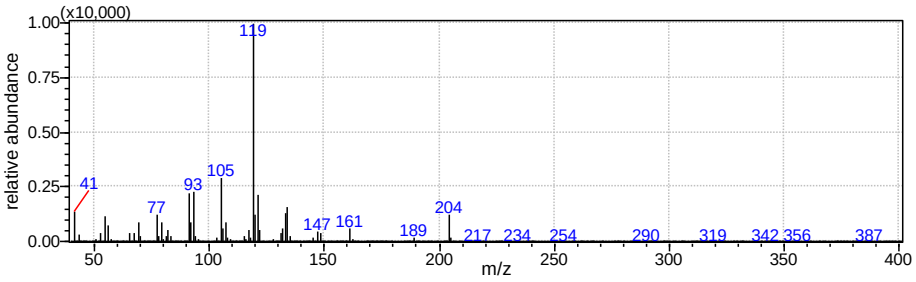


database entry: γ-curcumene RI = 1482

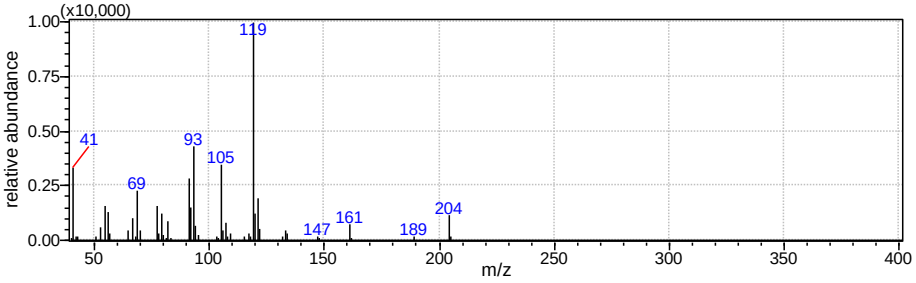


11d-6

experimental EI spectrum RI = 1522, RT = 14.167 min

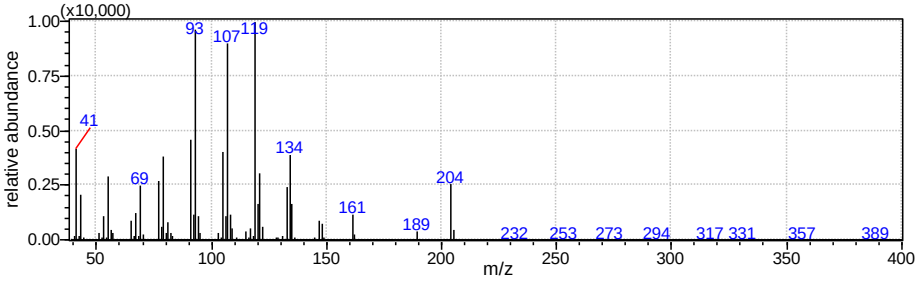


database entry: β-curcumene RI = 1511

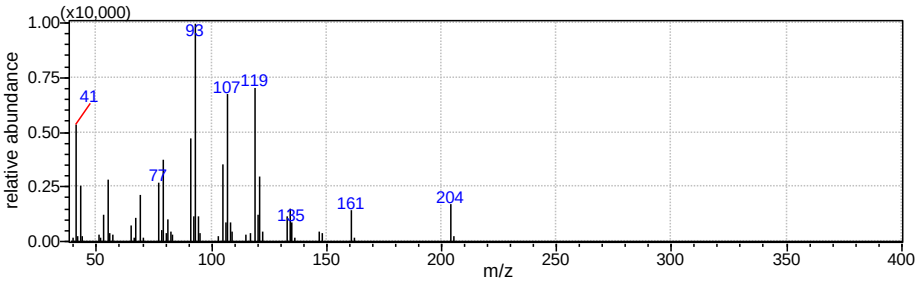


11d-7

experimental EI spectrum RI = 1529, RT = 14.252 min

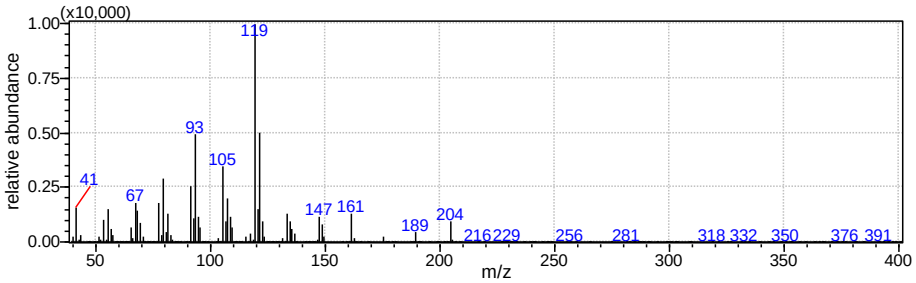


database entry: (*Z*)-γ-bisabolene RI = 1511

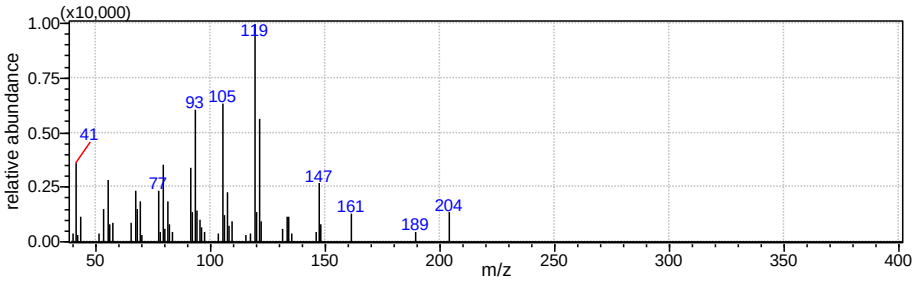


11d-8

experimental EI spectrum RI = 1486, RT = 13.714 min

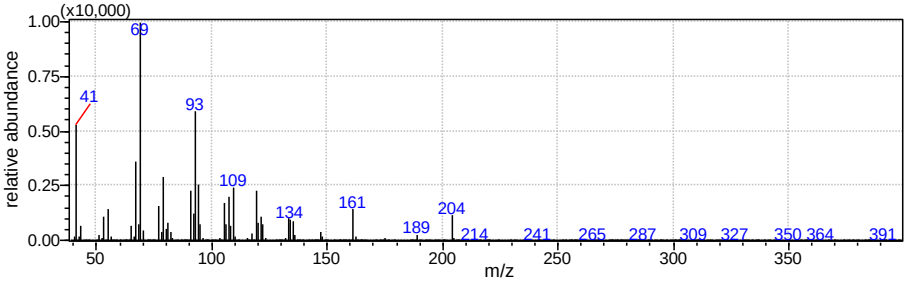


database entry: acoradiene RI = 1471

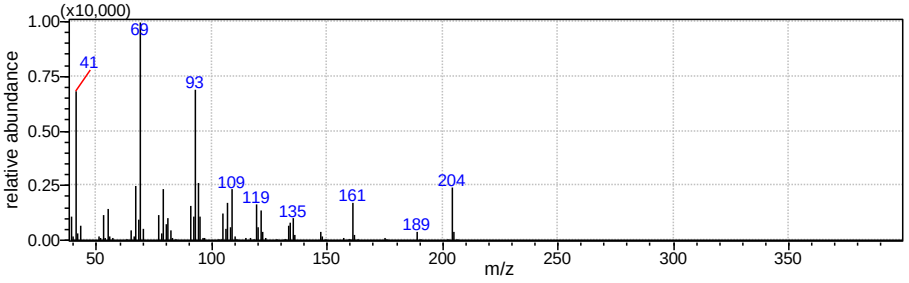


11d-9

experimental EI spectrum RI = 1520, RT = 14.136 min

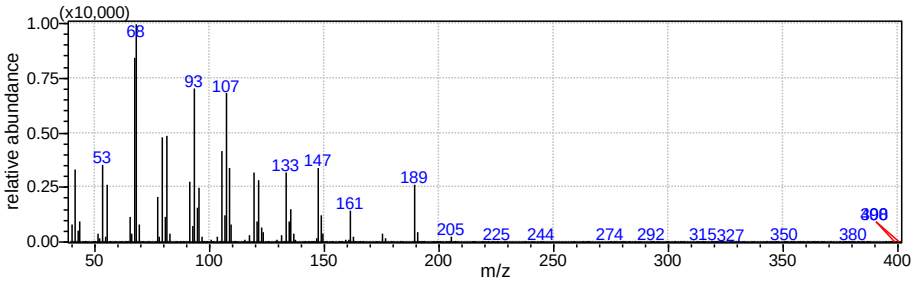


database entry: β-bisabolene RI = 1500

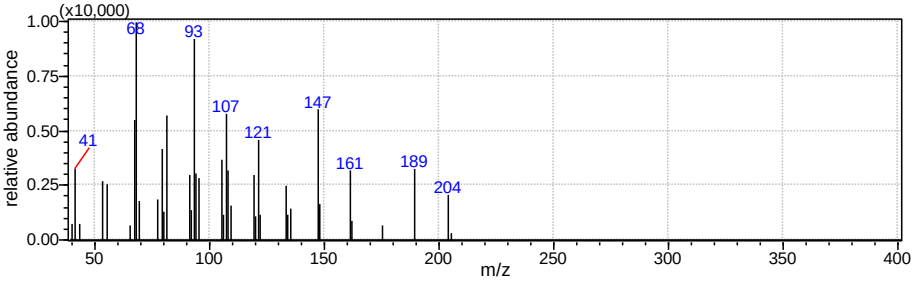


11d-10

experimental EI spectrum RI = 1516, RT = 14.091 min



database entry: germacrene A RI = 1570



10.3.3 Casbene Synthase (RcCAS) Assays

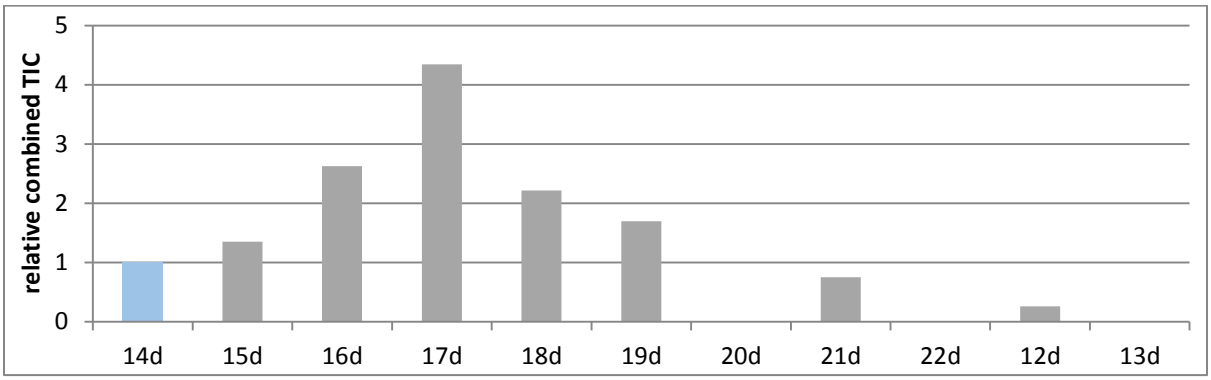
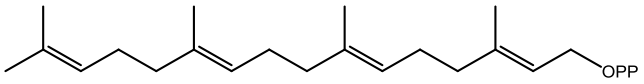


Fig. 45 – Relative combined total ion currents (TICs) of all products obtained from the biocatalytic conversion of $C_{19}/C_{20}/C_{25}$ substrates by casbene synthase (RcCAS). Comparison to the combined total ion current (TIC) of all products obtained from the conversion of the enzymes natural substrate (*E*,*E*,*E*)-geranylgeranyl diphosphate (**14d**). Other substrates: (*Z*,*E*,*E*)-geranylgeranyl diphosphate (**15d**), (*E*,*Z*,*E*)-geranylgeranyl diphosphate (**16d**), (*Z*,*Z*,*E*)-geranylgeranyl diphosphate (**17d**), (*E*,*E*,*Z*)-geranylgeranyl diphosphate (**18d**), (*Z*,*E*,*Z*)-geranylgeranyl diphosphate (**19d**), (*E*,*Z*,*Z*)-geranylgeranyl diphosphate (**20d**), (*Z*,*Z*,*Z*)-geranylgeranyl diphosphate (**21d**), (*E*,*E*,*E*,*E*)-geranylfarnesyl diphosphate (**22d**), (*E*,*E*,*E*)-3-norgeranylgeranyl diphosphate (**12d**), (*Z*,*E*,*E*)-3-norgeranylgeranyl diphosphate (**13d**), No conversion in case of **13d**, **20d** and **22d**.

(*E*,*E*,*E*)-Geranylgeranyl diphosphate (14d)



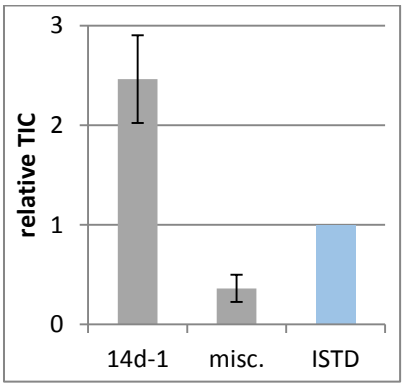
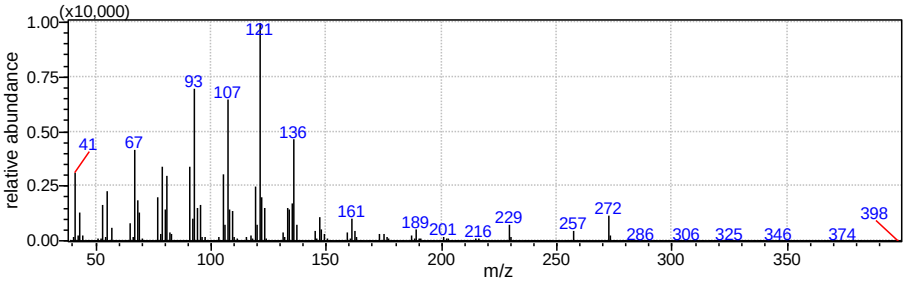


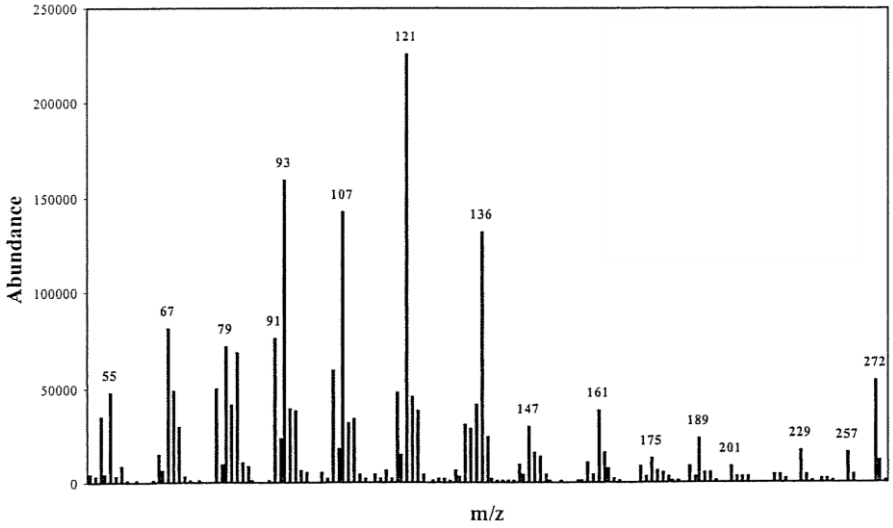
Fig. 46 – Relative total ion currents (TIC) of the observed products compared to the internal standard (ISTD).

14d-1

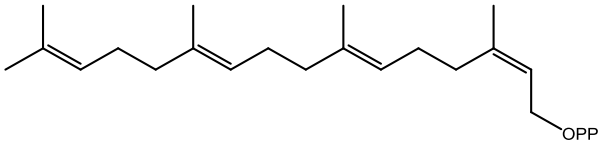
experimental EI spectrum RI = 1969, RT = 19.072 min



literature: casbene[117]



(*Z*,*E*,*E*)-Geranylgeranyl diphosphate (15d)



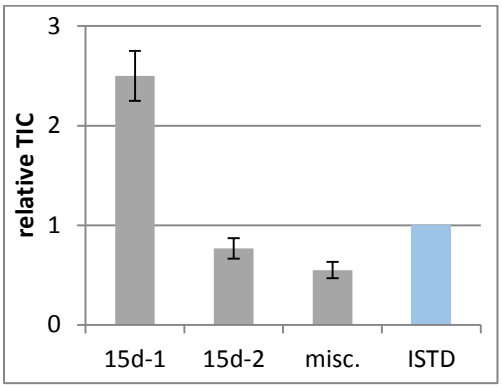
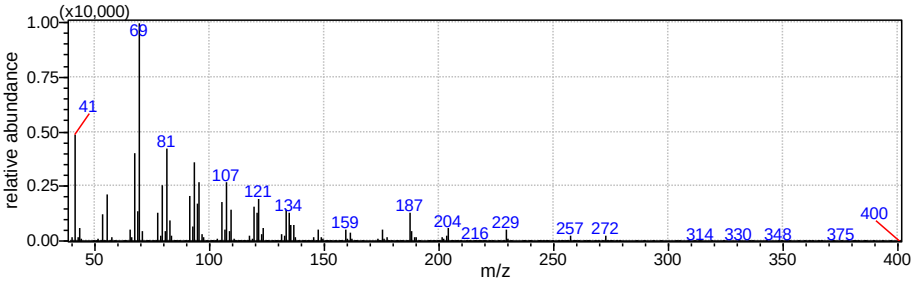


Fig. 47 – Relative total ion currents (TIC) of the observed products compared to the internal standard (ISTD).

15d-1

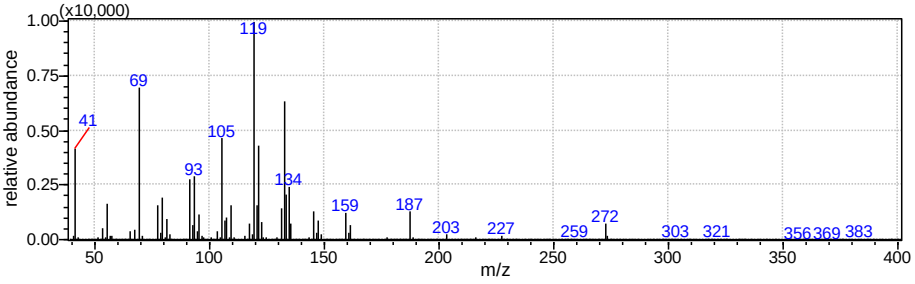
experimental EI spectrum RI = 1998, RT = 19.360 min



database entry: no hit

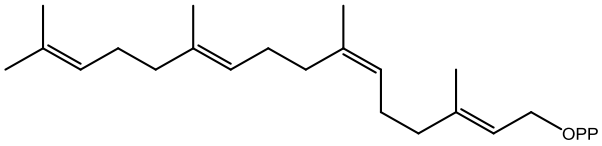
15d-2

experimental EI spectrum RI = 1966, RT = 19.047 min



database entry: no hit

(*E*,*Z*,*E*)-Geranylgeranyl diphosphate (16d)



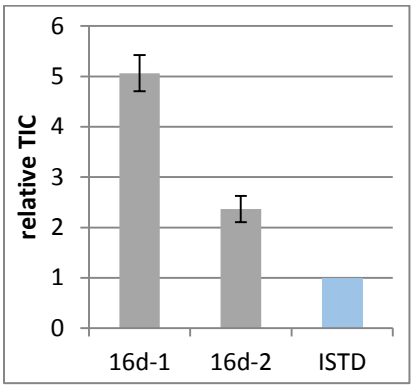
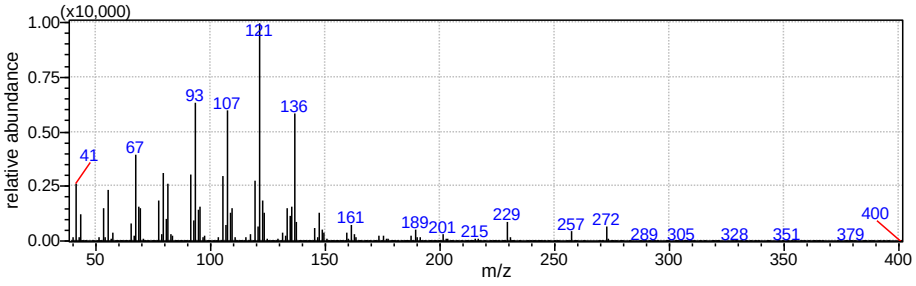


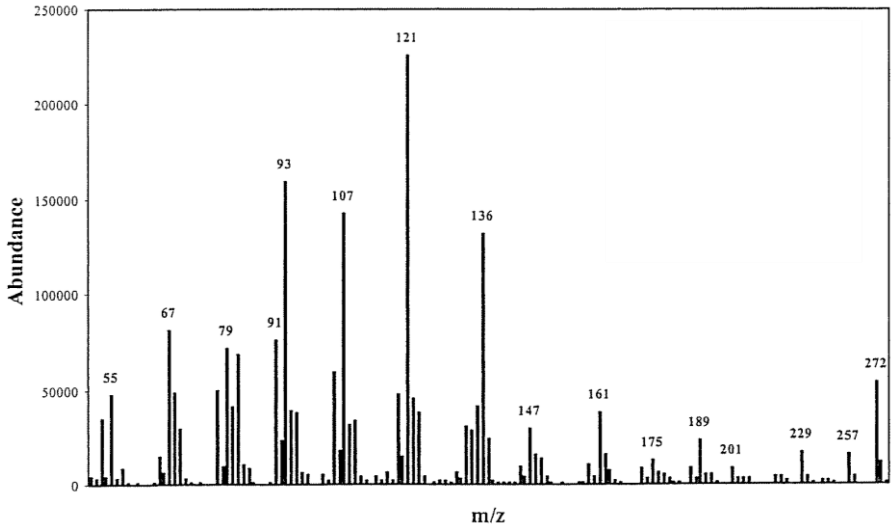
Fig. 48 – Relative total ion currents (TIC) of the observed products compared to the internal standard (ISTD).

16d-1

experimental EI spectrum RI = 1966, RT = 19.039 min

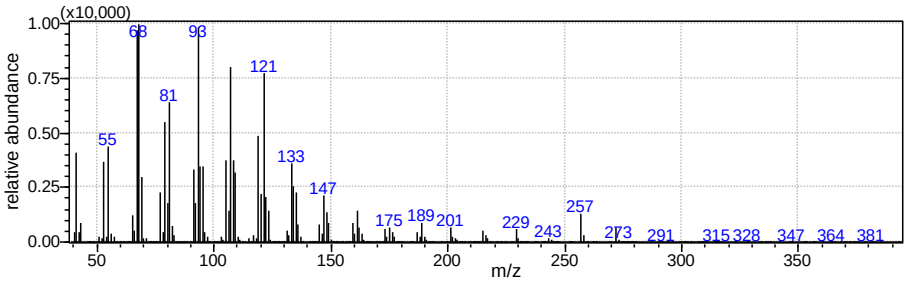


literature: casbene[117]

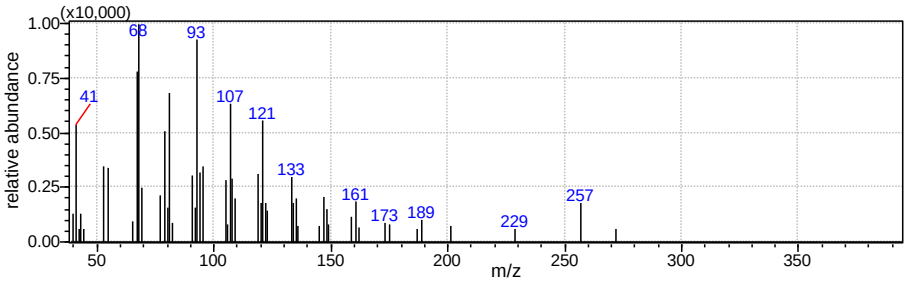


16d-2

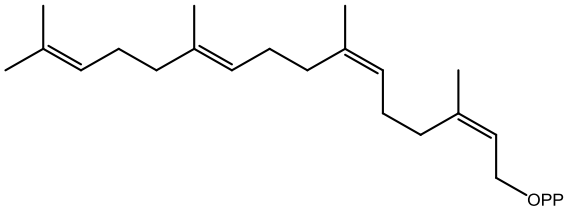
experimental EI spectrum RI = 1993, RT = 19.304 min



database entry: cembrene A RI = 1960



(*Z*,*Z*,*E*)-Geranylgeranyl diphosphate (17d)



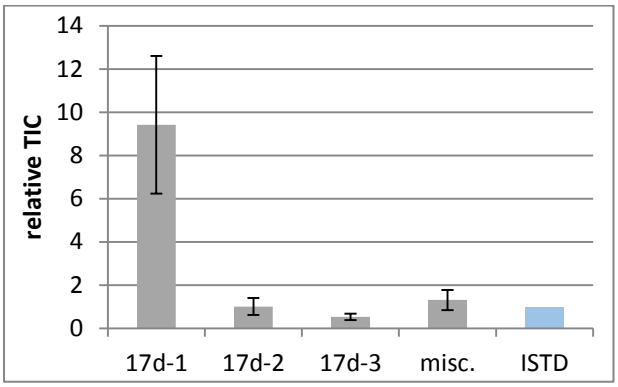
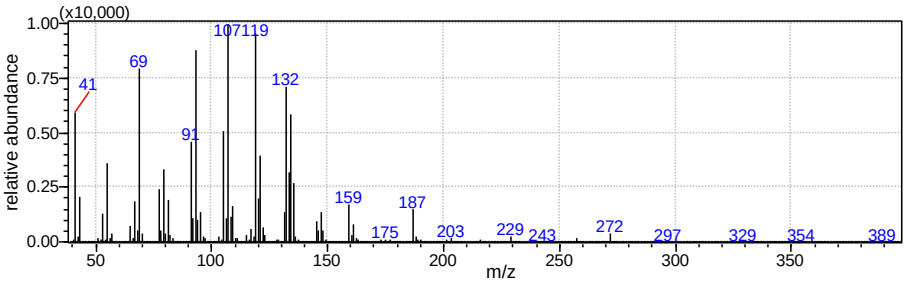


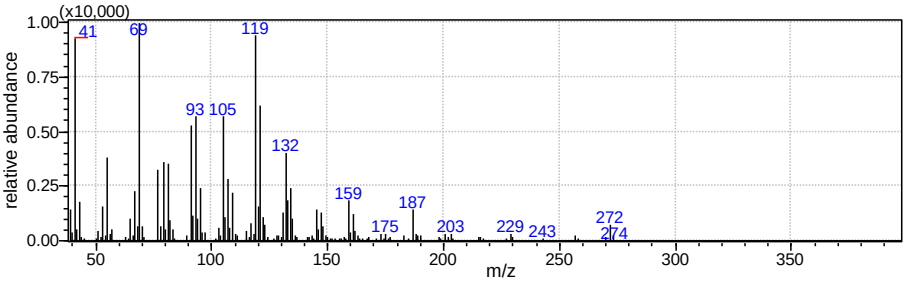
Fig. 49 – Relative total ion currents (TIC) of the observed products compared to the internal standard (ISTD).

17d-1

experimental EI spectrum RI = 2008, RT = 19.454 min

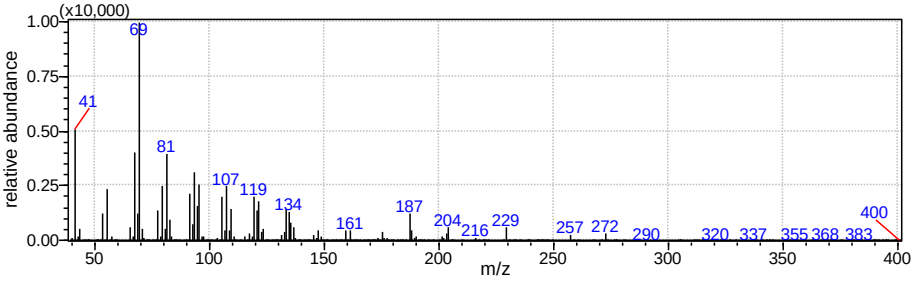


database entry: geranylterpinene RI = 1962



17d-2

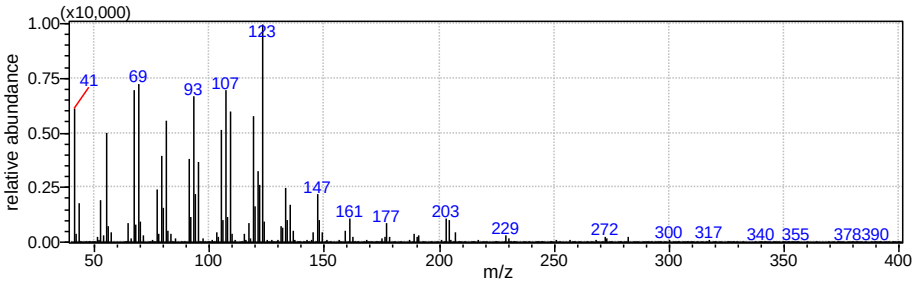
experimental EI spectrum RI = 1999, RT = 19.365 min



database entry: no hit

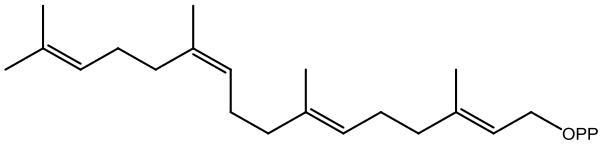
17d-3

experimental EI spectrum RI = 2029, RT = 19.645 min



database entry: no hit

(*E*,*E*,*Z*)-Geranylgeranyl diphosphate (18d)



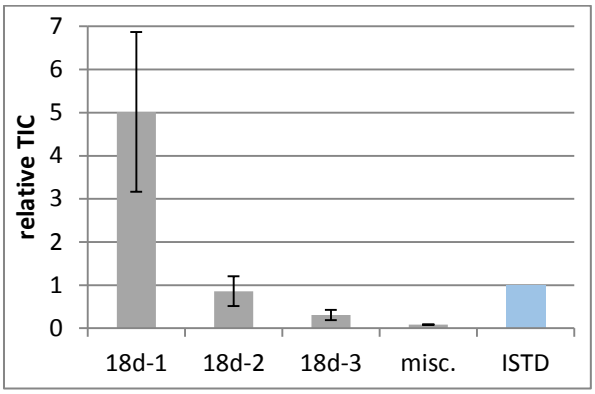
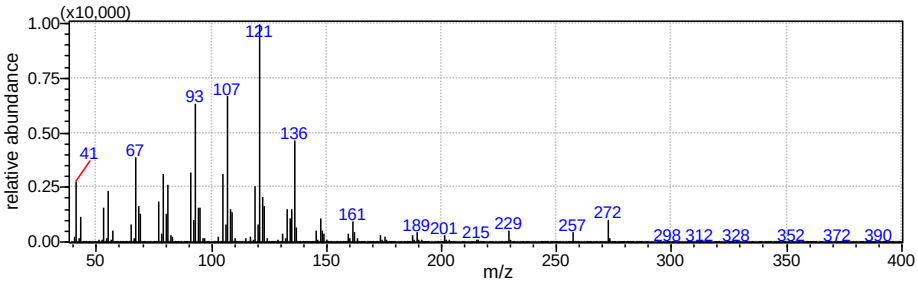


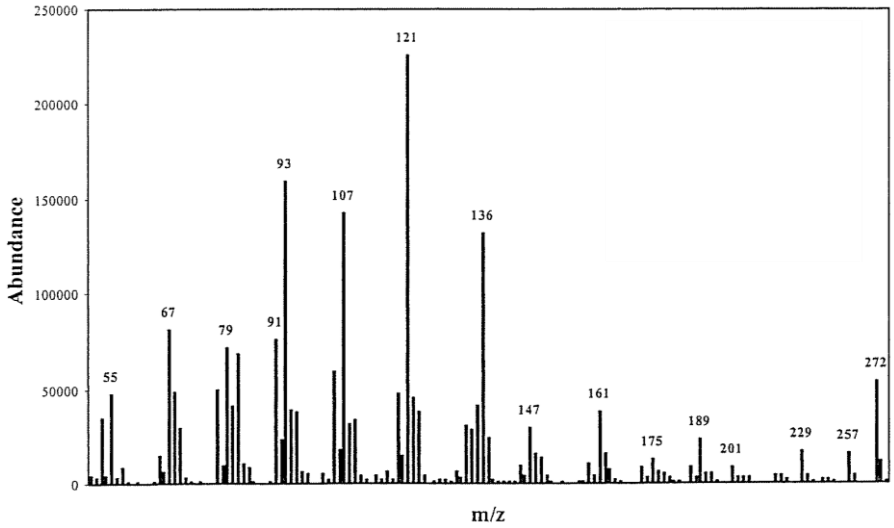
Fig. 50 – Relative total ion currents (TIC) of the observed products compared to the internal standard (ISTD).

18d-1

experimental EI spectrum RI = 1952, RT = 18.903 min

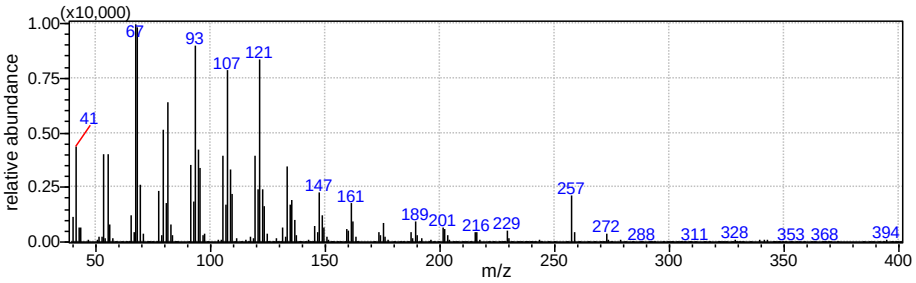


literature: casbene[117]



18d-2

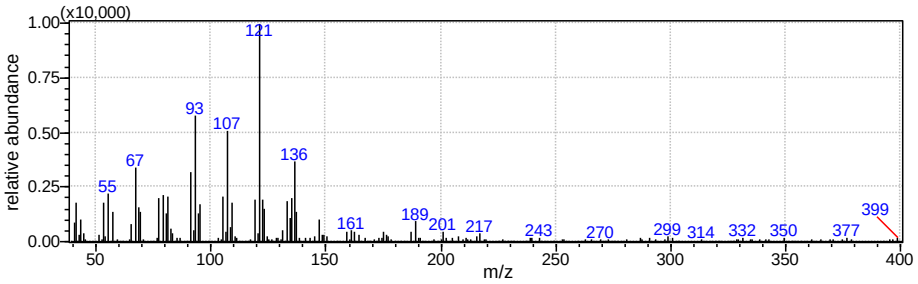
experimental EI spectrum RI = 1975, RT = 19.132 min



database entry: no hit

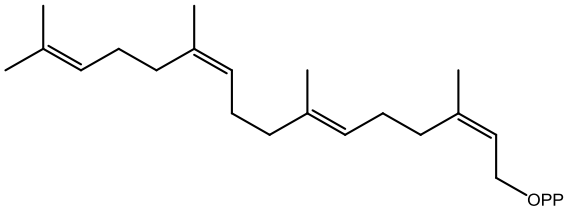
18d-3

experimental EI spectrum RI = 1969, RT = 19.071 min



database entry: no hit

(*Z*,*E*,*Z*)-Geranylgeranyl diphosphate (19d)



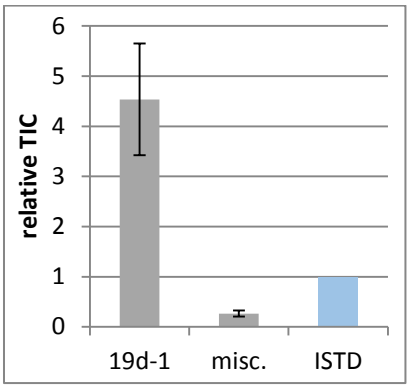
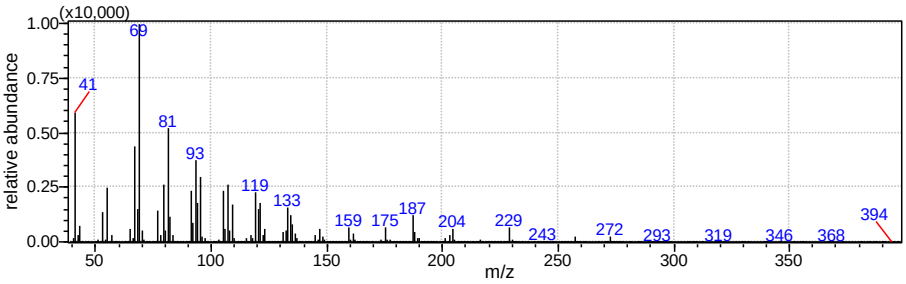


Fig. 51 – Relative total ion currents (TIC) of the observed products compared to the internal standard (ISTD).

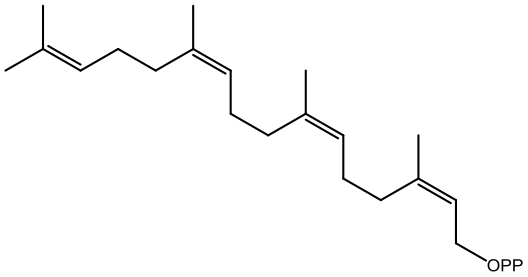
19d-1

experimental EI spectrum RI = 1963, RT = 19.010 min



database entry: no hit

(*Z*,*Z*,*Z*)-Geranylgeranyl diphosphate (21d)



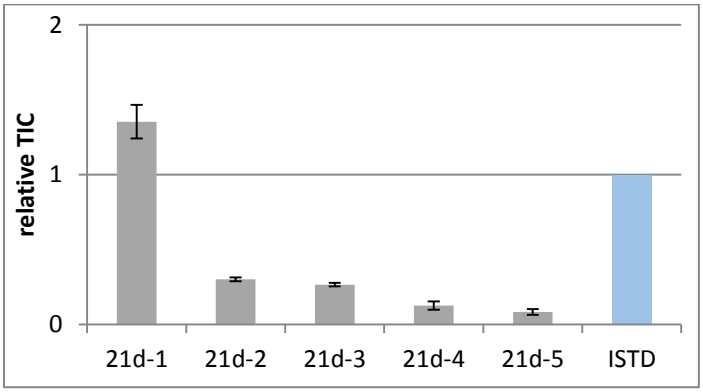
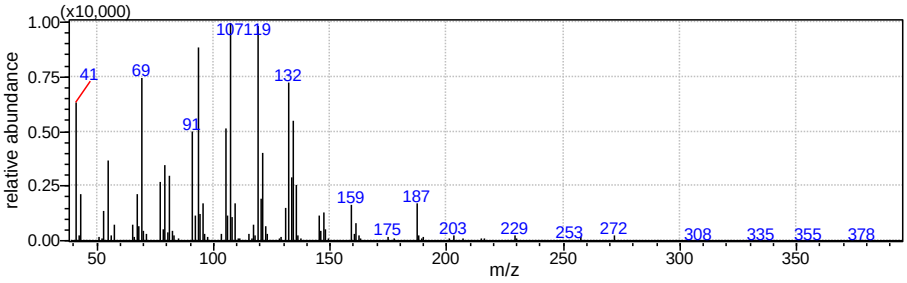


Fig. 52 – Relative total ion currents (TIC) of the observed products compared to the internal standard (ISTD).

21d-1

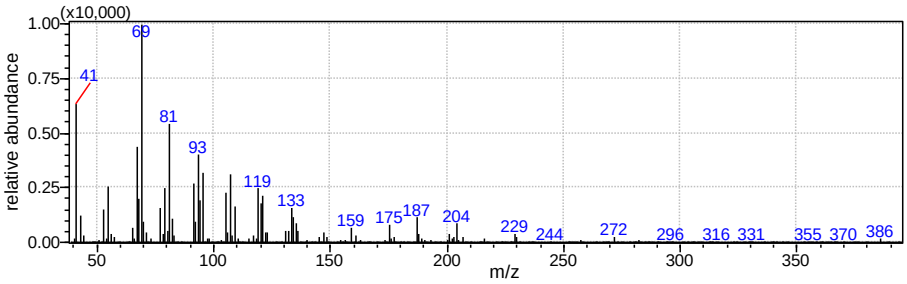
experimental EI spectrum RI = 1975, RT = 19.129 min



database entry: no hit

21d-2

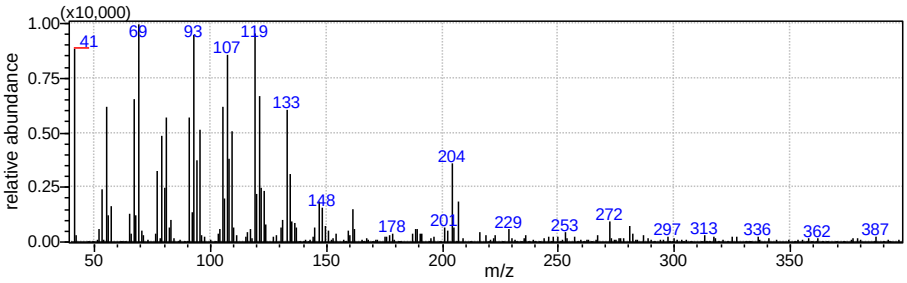
experimental EI spectrum RI = 1963, RT = 19.015 min



database entry: no hit

21d-3

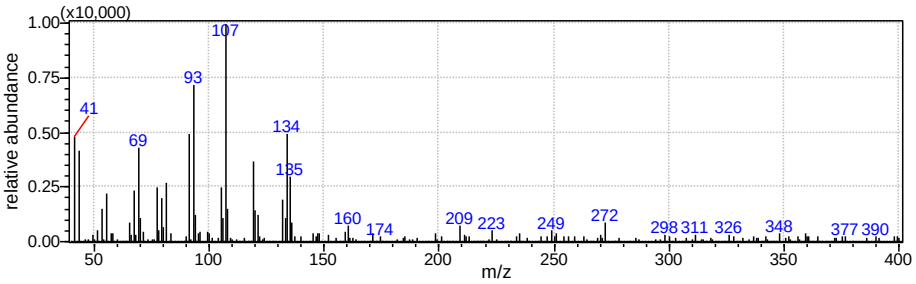
experimental EI spectrum RI = 2005, RT = 19.429 min



database entry: no hit

21d-4

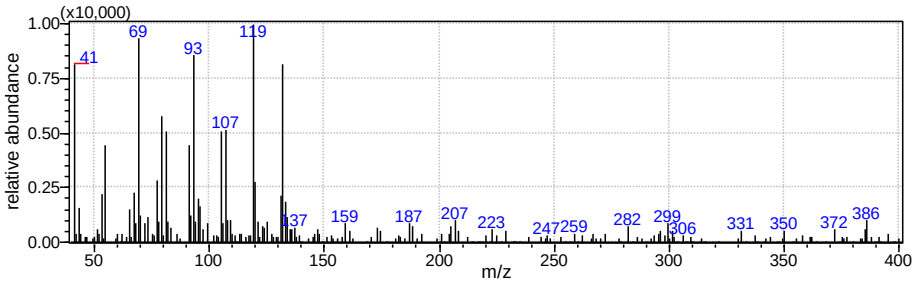
experimental EI spectrum RI = 1988, RT = 19.271 min



database entry: no hit

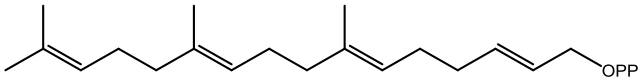
21d-5

experimental EI spectrum RI = 1921, RT = 18.597 min



database entry: no hit

(*E*,*E*,*E*)-3-Norgeranylgeranyl diphosphate (12d)



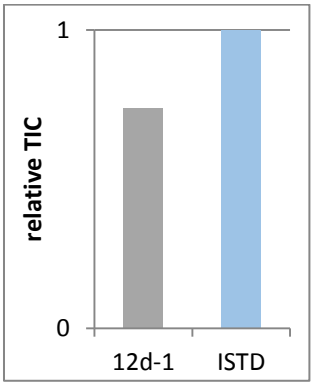
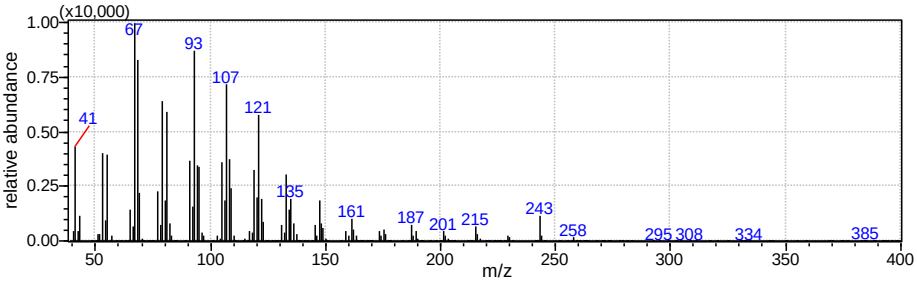


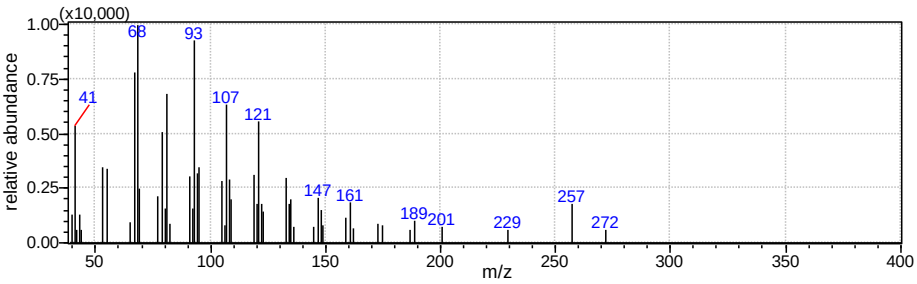
Fig. 53 – Relative total ion currents (TIC) of the observed products compared to the internal standard (ISTD).

12d-1

experimental EI spectrum RI = 1915, RT = 18.539 min



database entry: cembrene A RI = 1960



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I thank Benjamin Weigel for all the help concerning the GC-MS measurements.

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I thank all the people who have been part of our uncounted and breathtaking foosball matches.

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Curriculum Vitae

Personal Information	
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nationality	German
Professional Experience	
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Doctorate	
11/2011 – 10/2015	**Scientific Assistant** Leibniz Institute of Plant Biochemistry, Halle (Saale) Department of Bioorganic Chemistry, Prof. Dr. Ludger Wessjohann Topic: *"Synthesis and Biocatalytic Conversion of Natural and Artificial Isoprenoid Diphosphates"*
University Education	
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01/2011 – 7/2011	**Master Thesis** Martin Luther University Halle-Wittenberg, Halle (Saale) Institute of Organic Chemistry, Prof. Dr. René Csuk Topic: *„Synthesis and Characterization of Biologically Active Maslinic Acid Derivatives"* Grade: *„A"* (1.0)
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08/2005 – 04/2006	Malteser Hilfsdienst e.V. Köthen (Anhalt)

Secondary Education

09/1996 – 07/2005	Burggymnasium Aken (Elbe), Abitur Majoring in German and Mathematics grade: *„B“* (2.1)

Conferences

07/2014	ICOS-20, 20th International Conference on Organic Chemistry Budapest, Hungary Talk: *“Synthesis and Biocatalytic Conversion of New Artificial Isoprenoids”*
05/2014	47th Ph.D. Workshop on Natural Products Halle (Saale), Germany Talk: *“A Modular System for the Stereospecific Synthesis of Isoprenoids”*
06/2013	TERPNET 2013, 11th International Meeting on Biosynthesis, function and biotechnology of isoprenoids in terrestrial and marine organisms Kolymvari, Greece Poster: *“A Biocatalytic Approach Towards Artificial Terpenoid Skeletons”*
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R. Nagel, C. Bernholz, E. Vranová, J. Košuth, N. Bergau, **S. Ludwig**, L. Wessjohann, J. Gershenzon, A. Tissier, A. Schmidt *The Plant Journal* **2015**, *84*, 847.

J. Keim, **S. Ludwig**, H. F. Schreckenbach, L. Wessjohann, C. Dreisbach, T. Früh *European Patent Application* EP 2848693.

Halle (Saale), 15.12.2016

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Statutory Declaration / Eidesstaatliche Erklärung

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