Chemobiosynthese von neuen Terpenoiden

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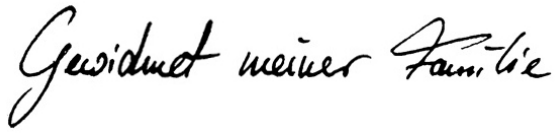

“Life is like going the wrong way on a moving walkway.
Stand still and you go backwards.
Walk and you stay put.
To get ahead, you have to hustle.”

– Casey Neistat/Farrelly Brothers

ABSTRACT

Cong Duc Tran

CHEMOBIOSYNTHESE VON NEUEN TERPENOIDEN

CHEMO-BIOSYNTHESIS OF NOVEL TERPENOIDS

Keywords: terpenes, terpenoids, sesquiterpenes, biotransformation, sesquiterpene cyclases, unnatural diphosphates, synthesis

Terpenes represent the largest and structurally most diverse group of natural products. Since ancient times, humans have used terpenes contained in essential oils as medicinal agents, preservatives, and fragrances in their everyday life. Nowadays they are used in many scientific, industrial, and medical fields for their diverse properties. In the perfume and food industries, for example, sesquiterpenes in particular are used for their characteristic fragrances and flavors.

In the ongoing search for new terpenes with sensory properties and biological activity, enzyme-catalyzed biotransformations represent an exciting and efficient approach. Synthetically accessed, non-natural linear terpenoids can be used as precursors for terpene cyclases to obtain various cyclic products. By using different enzymes, it is also possible to obtain multiple different products starting from the same substrate.

In this dissertation, the synthesis of nine non-natural linear farnesyl pyrophosphate derivatives and their subsequent application as substrates in biotransformations with eight selected heterologously expressed sesquiterpene cyclases is presented. The generated novel cyclization products were isolated, purified, and their structure elucidated. The information obtained from these results was used to investigate the cyclization mechanisms and substrate flexibility of the enzymes used.

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ABBREVIATIONS

4-DMAP	4-Dimethylaminopyridine
9-BBN	9-Borabicyclo(3.3.1)nonane
Ac	Acetyl
APS	Ammonium persulfate
aq	Aqueous
A_{total}	Proportion/Percentage of total
BcBot2	Presilphiperfolan-8-β-ol synthase
Bn	Benyzl
bp	Boiling point
brsm	Based on recovered starting material
Bu	Butyl
cat	Catalyst
conc	Concentrated, concentration
Cop4	Cubebol synthase
COSY	Correlation spectroscopy
Cp	Cyclopentadienyl
CV	Column volume
Cyc1	*Epi*-isozizaene synthase
DHP	3,4-Dihydro-2H-pyran
DIAD	Di*iso*propyl azodicarboxylate
DIBAL-H	Di*iso*butylaluminium hydride
DIPA	Di*iso*propylamine
DIPEA	N,N-Di*iso*propylethylamine
DMAPP	Dimethylallyl pyrophosphate
DME	Dimethoxyethane, ethylene glycol
DMF	Dimethyl formamide
DMP	Dess-Martin periodinane
DMS	Dimethyl sulfide
DMSO	Dimethyl sulfoxide
dppf	1,1′-Bis(diphenylphosphino)ferrocene
DTT	Dithiothreitol
EA	Ethyl acetate
eq	Equivalents
ESI	Electron spray ionisation
Et	Ethyl
Et_2O	Diethyl ether
FPP	Farnesyl pyrophosphate
GC	Gas chromatography
GcoA	Caryolan-1-ol synthase
gen	Generation
GPP	Geranyl pyrophosphate
h	Hours
HEPES	2-[4-(2-Hydroxyethyl)piperazin-1-yl]ethane-1-sulfonic acid
His-tag	Histidine tag
HSQC	Heteronuclear multiple-bond correlation spectroscopy
Hvs1	Vetispiradiene synthase
HWE	Horner-Wadsworth-Emmons
IMAC	Immobilized metal affinity chromatography

IPP	Isopentenyl pyrophosphate
IPTG	Isopropyl β-D-1-thiogalactopyranoside
IUPAC	International Union of Pure and Applied Chemistry
LB	Lysogeny broth
LDA	Lithium di*iso*propylamide
M	Mass
m/z	Mass-to-charge ratio
*m*CPBA	*meta*-Chloroperoxybenzoic acid
Me	Methyl
min	Minutes
MS	Mass spectrometry
Ms	Mesyl/methanesulfonyl
MWCO	Molecular weight cut-off
n-BuLi	*n*-Butyllithium
NCS	*N*-chlorosuccinimide
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser effect spectroscopy
nr	Number
NTA	Nitrilotriacetic acid
OD	Optical density
OPP	Ammonium diphosphate, $-OP_2O_6(NH_4)_3$
oXs	Over X steps (X = natural number)
PE	Petroleum ether
PenA	Pentalenene synthase
PPase	Pyrophosphatase
Pr	Propanol
*p*TsOH	*p*-toluenesulfonic acid
R_f	Retardation factor
RI	Retention index
rpm	Revolutions per minute
rt	Room temperature
sat	Saturated
SDS-PAGE	Sodium dodecyl sulphate–polyacrylamide gel electrophoresis
SM	Starting material
S_N	Nucleophilic substitution
SOB	Super optimal broth
SOC	SOB with catabolite repression
STC	Sesquiterpene cyclase
TBAF	Tetra-*n*-butylammonium fluoride
TBS	*tert*-Butyldimethylsilyl
t-BuLi	*tert*-Butyllithium
TC	Terpene cyclcase
TEAP	Bis-triethylammonium phosphate
TEMED	Tetramethylethylenediamine
Tf	Trifluoromethylsulfonyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
THP	Tetrahydropyran
TIC	Total ionic count
TIPS	Tri*iso*propylsilyl
TLC	Thin-layer chromatography

TMEDA	Tetramethylethylendiamine
TMS-Cl	Trimethylsilyl chloride
Tps32	Viridiflorene synthase
t_R	Retention time
Tri5	Trichodiene synthase
TRIS	Tris(hydroxymethyl)aminomethane
Ts	Tosyl
Vis	Visible
wt	Weight
XD	X Dimensional

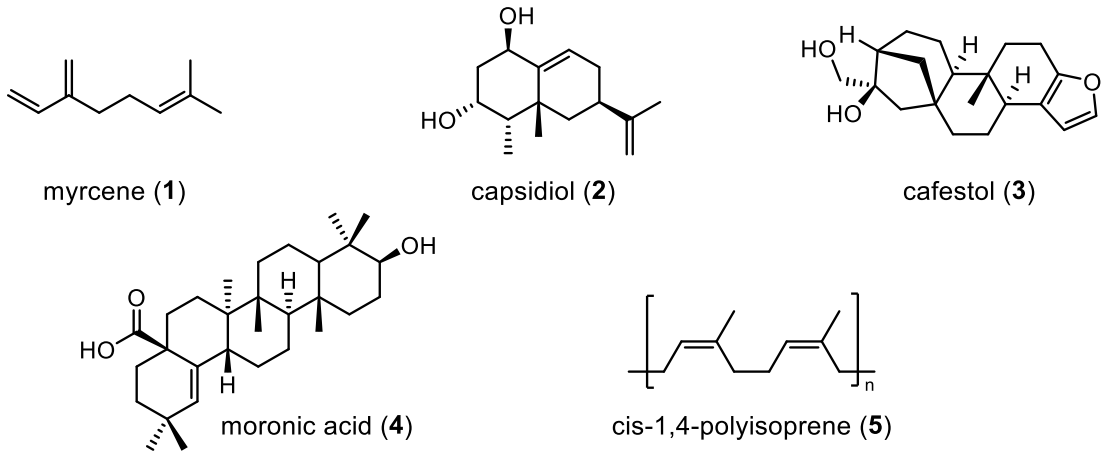
PRELIMINARY REMARKS

- The following terms and designations are used interchangeably in this dissertation:
    - “terpene”, “terpenoid”, and “isoprenoid”
    - “-OPP” and “-$OP_2O_3(NH_4)_3$”. The notation depends on the available space in the respective scheme/figure
    - “qualitative biotransformation” and “analytical *in vitro* enzyme tests”
    - “diphosphate” and “pyrophosphate”
    - “unnatural substrate” and “non-natural substrate”.
- In general, two decimal digits are used. Zeros after the decimal point are omitted in certain cases: “1.00 mL” is indicated as “1 mL”.
- Figure and scheme captions are written in justified format with insertion of 1 cm to the left and right text border.
- The atom numbering of molecules is not based on IUPAC regulations, but on the position in the carbon skeleton.

1 INTRODUCTION

1.1 TERPENES

Terpenes are chemical compounds with a wide variety of structural motifs and represent the largest class of natural products with more than 80 000 known compounds found in all kingdoms of life.[1] The term is derived from the tree resin turpentine (*Greek: terebinthine*), which is mainly isolated from pine trees and has a strong odor. Derivatives containing heteroatoms are called terpenoids. In a broader sense, the term also refers to natural degradation products such as ionones, as well as natural and synthetic derivatives including terpene alcohols, aldehydes, esters, epoxides, ketones, and similar.[2,3] The class of terpenes includes small fragrance and aroma molecules as well as larger bioactive compounds such as vitamins and steroids. Representatives of this class are often responsible for the smell, taste, or medicinal effect of roots, flowers, herbs, citrus fruits, and the like.[4] To demonstrate the structural diversity of terpenes, selected representatives with different structural motifs are presented (Figure 1).[1,4,5,6]



C_n	Class	Compound	Natural occurrence	Properties and uses
10	mono	**1**	hops and cannabis	food additive, anti-inflammatory,
15	sesqui	**2**	chili pepper	antimicrobial
20	di	**3**	coffea arabica	skin care, anticarcinogenic
30	tri	**4**	sumac, mistletoe	anti-viral
n	poly	**5**	natural rubber	latex

Figure 1: Selected examples of terpenes and terpenoids with different structural motifs and characteristics. All presented compounds are found in nature and are used by humans for their various properties.

Despite their structural complexity and diversity, terpenes can be constructed from a small number of acyclic precursors. In 1887, the German Nobel Prize laureate Wallach was the first to recognize that all terpenes can be traced back to C_5-isoprene (**7**) subunits, which is why terpenes are also called isoprenoids.[7] On this basis, Ruzicka established the isoprene rule in 1953, in which he described linear allyl diphosphates as the basic building blocks of terpenes.[8]

In nature, isoprene units are embodied by the hemiterpenes dimethylallyl pyrophosphate (DMAPP, **8**) and isopentenyl pyrophosphate (IPP, **6**). In this context, the dimethyl end is known as the head and the pyrophosphate end is called the tail. The formation of longer mono-, sesqui-, di-, and sesterterpenes proceeds via head ⟶ tail coupling of IPP (**6**) and DMAPP (**8**). These linking reactions are catalyzed by prenyltransferases. For the formation of larger terpenes, tail ⟶ tail linkages between geranyl pyrophosphate (GPP, **9**) or farnesyl pyrophosphate (FPP, **10**) precursors are also observed (Figure 2).[9]

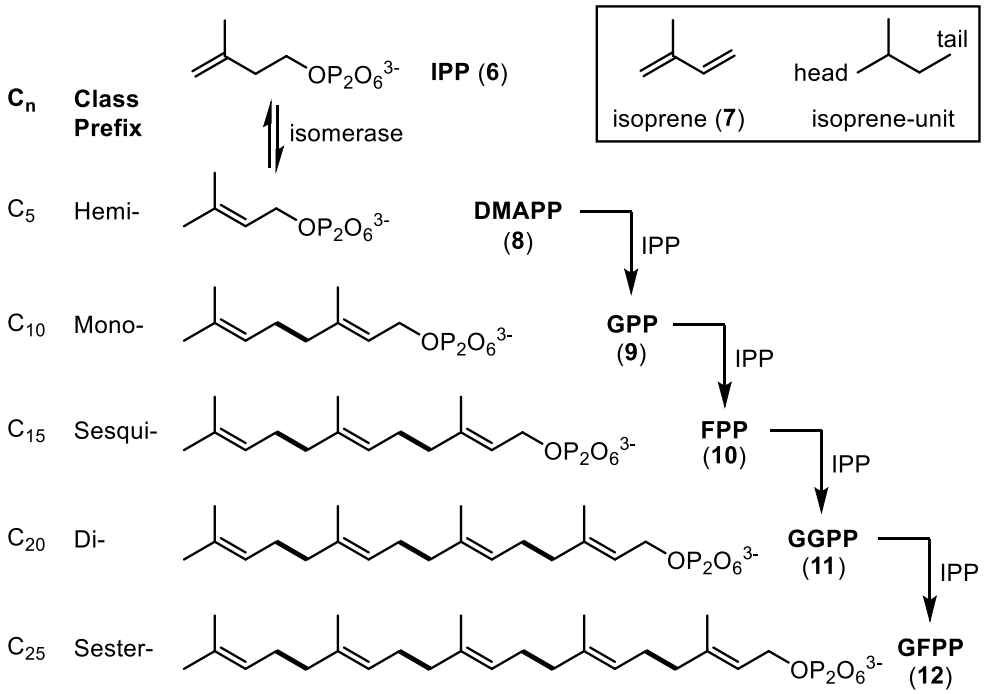
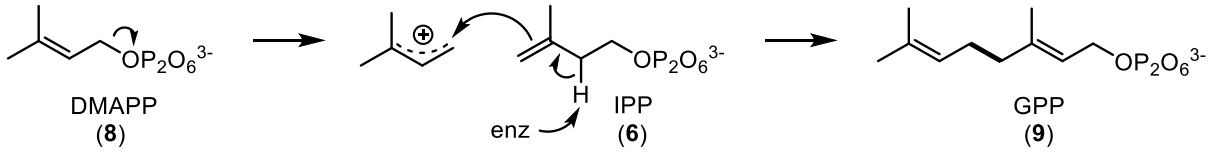


Figure 2: The carbon skeleton of terpenes and terpenoids is constructed from isoprene units. Depending on the number of these units (C_n), they are divided into subclasses (higher classes not shown). Generated bonds between isoprene units are highlighted in bold. DMA = dimethylallyl, I = isoprenyl, G = geranyl, F = farnesyl, PP = pyrophosphate.

Chemically, elongation initiates with the cleavage of the diphosphate function of DMAPP (**8**), converting it to an allyl cation. The reactive intermediate formed can then be nucleophilically targeted by a base-activated IPP (**6**) to form GPP (**9**). This elongation reaction is catalyzed by prenyltransferases and can result in the formation of larger diphosphates upon repetition. [1,3,6,9]



Scheme 1: Biosynthetic chain extension of acyclic terpenes catalyzed by prenyltransferases. A head ⟶ tail coupling of DMAPP (**8**) and IPP (**6**) yields GPP (**9**).

Using the isoprene rule, the formation of most linear terpenes can be well reconstructed. However, the deconstruction of complex cyclic terpenes into individual isoprene units is not

always straightforward. In these cases, it must always be taken into account that although cyclic terpenes can be formed from acyclic starting materials, the subsequent reactions can lead to deviations of the isoprene rule.

Although terpenes are found everywhere in nature, they are commonly used by plants for various physiological functions. Not only are they utilized as fungicides and attractants, but also as hormones and light-absorbing pigments. The microbial world is another place where isoprenoids can be found in abundance. A wide variety of complex terpenes including antibiotics, antitumor compounds, mycotoxins, and phytohormones have been isolated from fungi and marine organisms. Marine isoprenoids in particular are known to contain substituents that are rarely found in other areas of nature. Terpenes also play a major part in the animal kingdom. Insects convert isoprenoids, which are predominantly ingested through the consumption of plants, into hormones or pheromones. Humans have been using terpenes found in essential oils as medicines, preservatives, and fragrances in their everyday life since ancient times. Nowadays, they are mainly used in the medical field, as fragrances in the cosmetics industry, as ingredients in the rubber industry, and as preservatives and flavor enhancers in the food industry.[3,4,9,10]

1.2 ENZYMES AND BIOTRANSFORMATIONS

Enzymes are catalytically active proteins that perform highly specific and important functions in the metabolism of all living organisms. When the German physiologist Kühne first used the word “enzyme” in 1878, he was referring to the ability of yeast to produce alcohol from sugar. The name is derived from the Greek words “within” (*en*) and “yeast” (*zume*). Enzymes are considered biological catalysts (or biocatalysts) because they enable and accelerate biochemical reactions in living organisms. They keep the metabolism going, cause the build-up and breakdown of important cell components, pass on genetic information, and convert those into gene products. They are responsible for their own synthesis and are overall essential for many life processes. In addition to their biological functions, enzymes can be extracted from cells and then used to catalyze numerous scientific and industrial processes. For example, they are used in detergents and for the production of sweeteners also. They also play an important role in agriculture, food chemistry, the leather industry, and the production of antibiotics. Apart from these industrial examples, biocatalysts are also used in scientific research and the medicinal field due to their high efficiency (Table 1).[11–14]

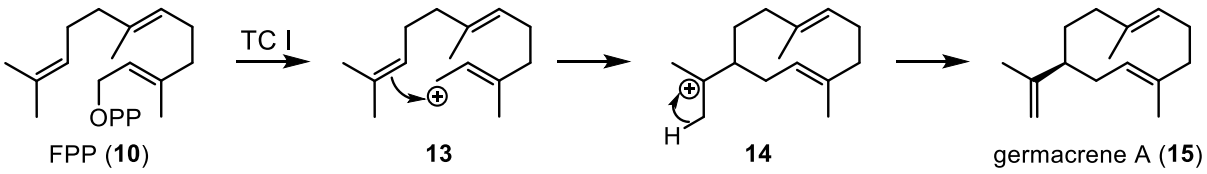
Table 1: Examples for the application of enzymes in different industries and the medical field. Recently, terpenoids have been used in the development of advanced biofuels, as properties have been rediscovered that make them suitable candidates to be used in fuels.[11,15]

Industry	Enzyme	Source	Application
food industry	α-amylase	fungal	clarification of fruit juice, beer brewing
	protease	animal fungal, plants	coagulation of milk, meat tenderization
	pectinase	fungal	coffee bean fermentation
pharmaceutical	lipase cellulase	animal fungal	digestive aids
	glucose oxidase	fungal plant	test strips for diabetics
wine	pectinase	fungal	pressing, clarification, filtration
photography	protease	bacterial	silver recovery from spent film
textile	amylase	bacterial, malt, animals	desizing of fabrics

The process in which enzymes are used as a biological system to facilitate structural changes to a chemical compound that is not the natural substrate is called biotransformation. The difference to biosynthesis is that biotransformations can also take place outside their natural environment. Compared to traditional chemical catalysis, enzymatically catalyzed biotransformations have numerous advantages. First, reactive functionalities do not need to be protected prior to biotransformation and non-reactive positions do not need to be activated. In addition, biocatalysts are often found to have higher selectivity and efficiency compared to conventional metal catalysis. They often exhibit not only high regio- and chemocontrol but also enantiocontrol, which even allows the generation of chiral products from racemic mixtures. Biotransformations are also often considered a more environmentally friendly alternative compared to conventional metal catalysis, as no toxic heavy metals are needed and because reactions can often be performed under milder conditions. For example, most enzymes show optimal activity at ambient temperature (20–40 °C), atmospheric pressure, and neutral pH values (pH 5–8). Under these conditions, undesirable side reactions that can occur at higher temperatures, such as in chemical catalysis, can be prevented. However, reactions outside the ideal physiological conditions easily affect the enzymatic stability, resulting in a higher susceptibility to proteolysis or inactivation of the enzyme. For this reason, enzymes in the industry are often immobilized on support materials to increase their longevity, facilitate catalyst separation, and enable recycling.[13,16]

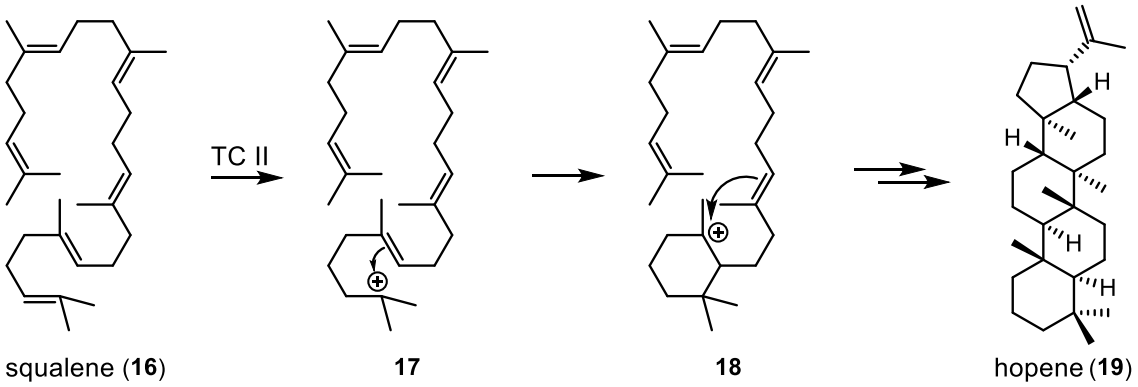
1.3 TERPENE CYCLASES

Terpene cyclases are enzymes specialized in converting linear terpenoid precursors into complex cyclic/polycyclic products via a cationic reaction cascade. These cyclases not only enable complex chemical transformations but also stabilize the highly reactive intermediates and facilitate the stereoselective formation of complex structures. Depending on the enzyme structure and the method used to initiate the reaction cascade, terpene cyclases are divided into two classes.



Scheme 2: Mechanism of the initiation of a cyclization cascade by class I terpene cyclases. Starting from FPP (**10**), enzymatic cleavage of the diphosphate group leads to the formation of the cationic intermediate **13**, which then triggers a series of cyclization reactions leading to the formation of germacrene A (**15**). TC = Terpene cyclase.

The secondary structure of class I terpene cyclases consists of α-helices and their active pocket is located in the middle of this α-helix cluster. At the entrance of the active site are two conserved amino acid sequences responsible for the coordination of a trinuclear metal cluster. This in turn is responsible for the coordination, alignment, and subsequent activation of a diphosphate substrate by cleavage of the diphosphate group. With the formation of the allyl cation **13**, the first step in the reaction cascade is also initiated (Scheme 2). Compared to prenyltransferases, class I terpene cyclases have similar structural and mechanistic features, suggesting a common evolutionary background.[1,17–20]

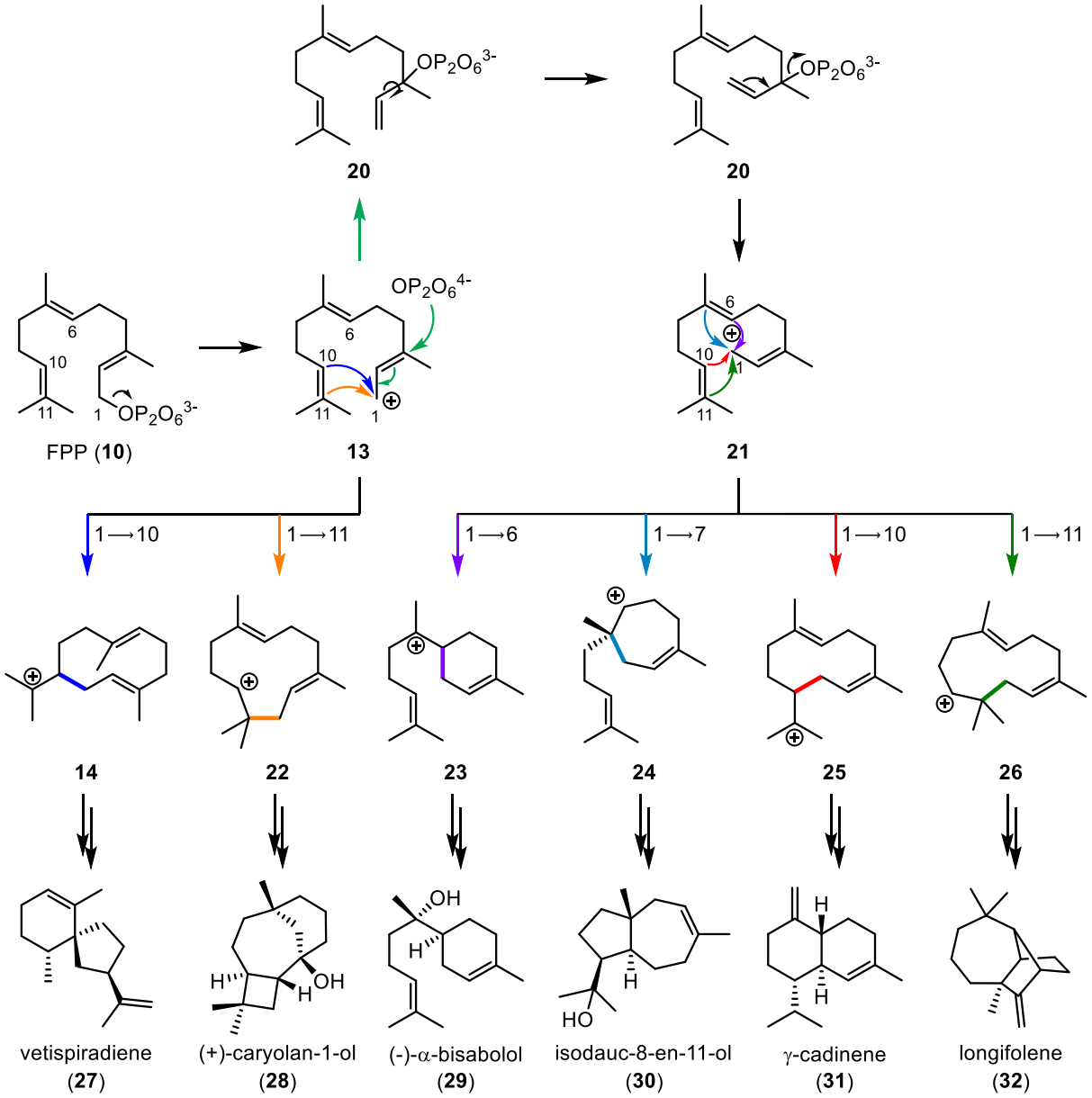


Scheme 3: Mechanism of initiation of a cyclization cascade by class II terpene cyclases. Starting from the triterpene squalene (**16**), acid-catalyzed deprotonation leads to the formation of cationic intermediate **17**, which then triggers a series of cyclization reactions leading to the formation of hopene (**19**). TC = Terpene cyclase.

In contrast, the active site of class II terpene cyclases is located at the intersection of two α-helical domains. The formation of a carbocation occurs through the activation of acyclic

terpenes without a pyrophosphate leaving group. This acid-catalyzed process is initiated with the protonation of a double bond or an epoxide function (Scheme 3).

After the formation of a carbocation, a series of follow-up reactions is triggered. This reaction cascade is governed by the structure of the active pocket of the enzyme, which serves as a template. Both the substrate and its corresponding intermediates are directed by specific amino acids within the active pocket, resulting in the selective formation of cyclic terpenes.[1,17–19,21] The options for the follow-up cyclizations are presented using FPP (**10**) and sesquiterpene cyclases as representative examples (Scheme 4).[20,22–27]



Scheme 4: Examples of possible follow-up cyclization reactions found in cyclization cascades of sesquiterpene cyclases. After the initial ionization of FPP (**10**), the formed carbocation **13** can undergo isomerization and various cyclizations to form different cationic intermediates (**14, 22–26**). After a cascade of follow-up reactions, including Wagner-Meerwein rearrangements and hydride shifts, the formation of new complex cyclic products (**27–32**) is finalized via proton abstraction or trapping of the carbocation with water.

With the cleavage of the diphosphate function and the resulting formation of farnesyl cation **13**, the possibilities for two direct macrocyclizations open up. Through a 1⟶10 cyclization, (*E*,*E*)-germacrane dienyl cation **14** is generated, and through a 1⟶11 cyclization, (*E*,*E*)-humulyl cation **22** is formed. From here, various cyclic products are formed via follow-up reactions. Starting from cyclodecane **14**, for example, vetispiradiene (**27**) can be generated and starting from cycloundecane **22**, caryolan-1-ol (**28**) can be obtained. Returning to the farnesyl cation **13**, a 1⟶6 or 1⟶7 cyclization via the central double bond could be feasible at first glance. However, these cyclizations are inhibited at this point by the (*E*)-configuration of the C2-C3 double bond. Initiated by a nucleophilic attack of the previously cleaved diphosphate anion, isomerization of the C2-C3 double bond occurs. The resulting farnesyl cation **21** can now undergo four cyclizations. A 1⟶6 cyclization yields bisabolyl cation **23** and a 1⟶7 cyclization affords cycloheptaene cation **24**. In addition, a 1⟶10 or 1⟶11 cyclization leads to the formation of (*E*,*Z*)-germacradienyl cation **25** or (*E*,*Z*)-humulyl cation **26** respectively. Starting from these four intermediates (**23–26**), various corresponding natural products (**29-32**) can be obtained via a series of subsequent reactions. These follow-up reactions often include protonations, Wagner-Meerwein rearrangements, hydride shifts, methyl shifts, water additions, and deprotonations. Each of the last two reactions terminate the cascade and release the cyclization product from the active pocket.[22–25,27]

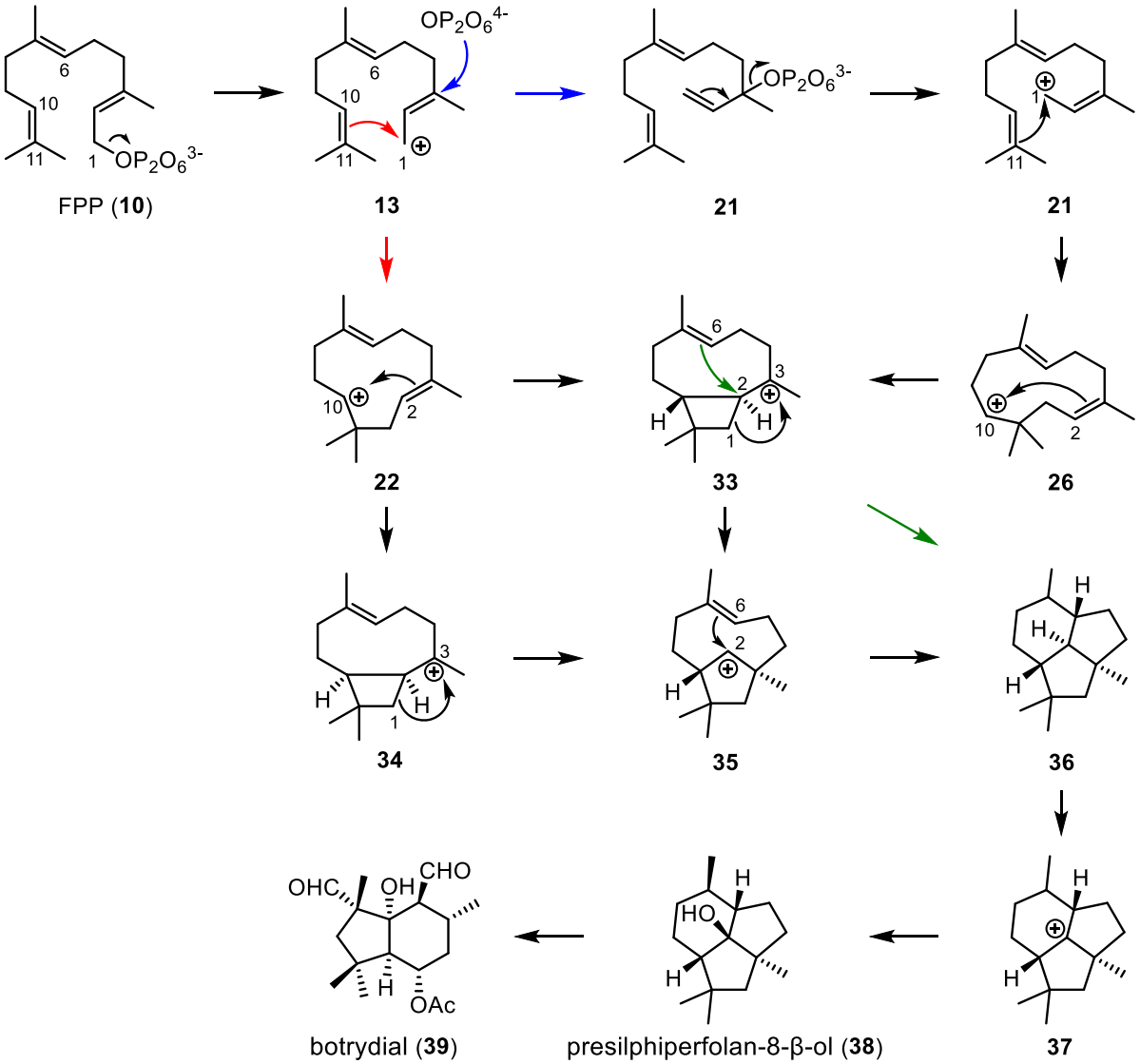
These follow-up reactions are presented in more detail in the following chapters, in which selected sesquiterpene cyclases from fungal, bacterial, and plant sources will be discussed.

1.3.1 PRESILPHIPERFOLAN-8-B-OL SYNTHASE – BCBOT2

The sesquiterpene cyclase BcBot2 was isolated from the gray mold *Botrytis cinerea*. Its gene was discovered as part of a gene cluster for the biosynthesis of the phytotoxin botrydial (**39**). With this toxin, *Botrytis cinerea* causes the gray mold disease, which is known to affect over 200 plant species. It causes leaf-spot diseases, rotting of berries, and powdery mildew on lettuce and tomatoes.[28] Since this fungus can cause economic damage by attacking both ornamental and agricultural crops, research on the biosynthesis of its phytotoxic sesquiterpenes is of special interest to the agricultural sector.[29,30]

To date, several proposals for the mechanism of the biosynthetic pathway have been published. In the following, the proposals of Hanson[31], Cane[30,32], and Tantillo[33] are presented in greater detail. According to Hanson, 1⟶11 macrocyclization occurs immediately after the formation of farnesyl carbocation **13** by initial cleavage of the diphosphate. The resulting (*E*,*E*)-humulyl cation **22** is then converted to caryophyllene cation **33** via a 2⟶10 cyclization. The ring-expanded product **35** obtained after Waagner-Meerwein rearrangement, yields tricyclic intermediate **36** through 6⟶2 cyclization. Up to this point, Cane's mechanistic proposal differs only slightly from Hanson's. After the 2⟶10 cyclization of intermediate **22**, Cane assumes that caryophyllene cation **34**, with the cyclobutyl group in one plane, is formed. The subsequent

reactions are analogous to those in Hanson's proposal and also lead to the formation of tricyclic intermediate **36**. Based on quantum mechanical ring-closing calculations, Tantillo assumes that the initial 1⟶11 cyclization occurs only after isomerization of the C2-C3 double bond. The generated (*E*,*Z*)-humulyl cation **26** then provides caryophyllene intermediate **33** via 2⟶10 cyclization. In contrast to the other two proposals, cyclization and ring extension occur simultaneously in the next step, yielding tricyclic intermediate **36**. From here, all three proposals suggest a 1,3-hydride shift followed by water addition for the formation of presilphiperfolan-8-β-ol (**38**) (Scheme 5).

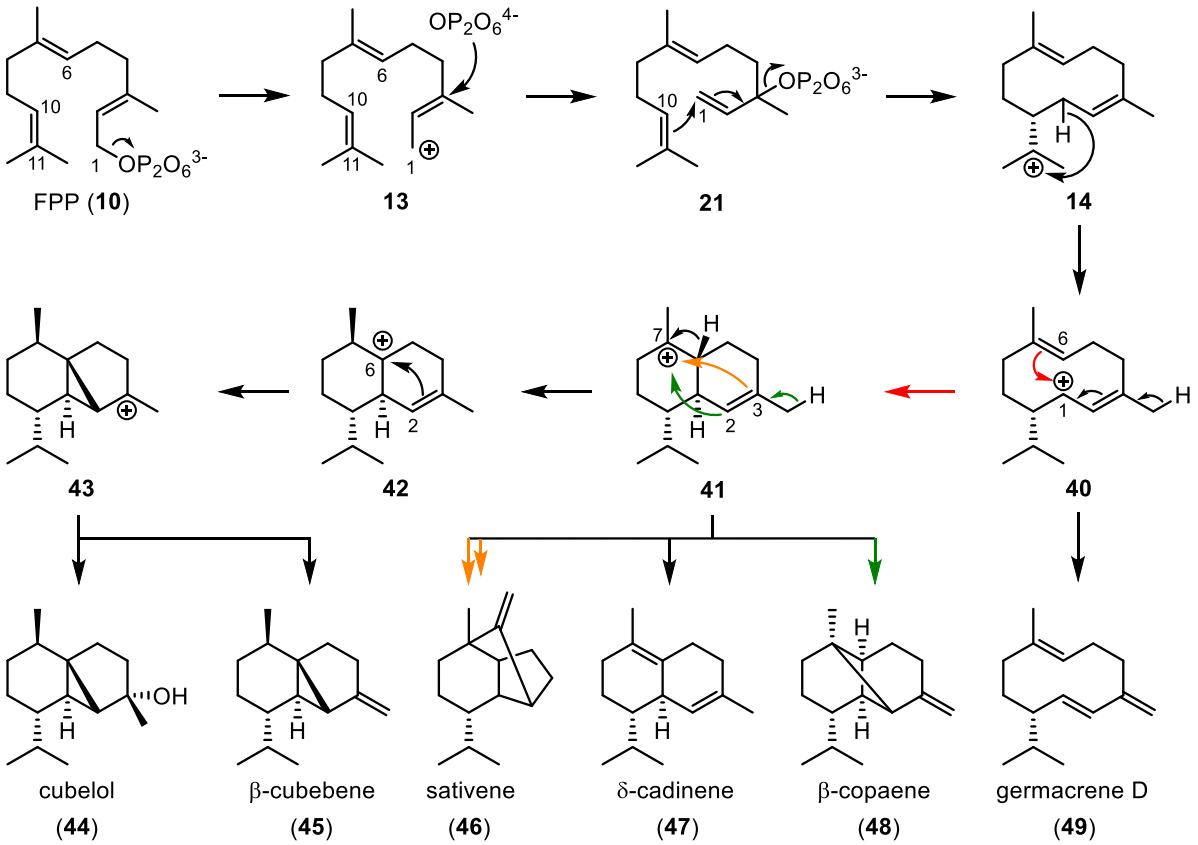


Scheme 5: Mechanistic proposals of Hanson, Cane and Tantillo for the BcBot2-catalyzed biosynthesis of presilphiperfolan-8-β-ol (**38**). The tricyclic compound was found to function an intermediate in the biosynthesis of the phytotoxin botrydial (**39**). Mechanistic proposals were formulated based on studies with isotope-labeled FPP analogs, modeling experiments, and isolated intermediates.

1.3.2 CUBEBOL SYNTHASE – COP4

The sesquiterpene cyclase Cop4 was isolated from the mushroom gray shag (*Coprinopsis cinerea*). Its gene was discovered when scientists searched for homologous sequences to the sesquiterpene cyclases trichodiene, aristolochene, and presilphiperfolan-8-β-ol synthase in fungal genomes. After heterologous expression in *E. coli* and subsequent characterization, Schmidt-Dannert[34–36] and co-workers isolated several cyclization products (**44–49**) upon biotransformation of Cop4 with FPP (**10**).

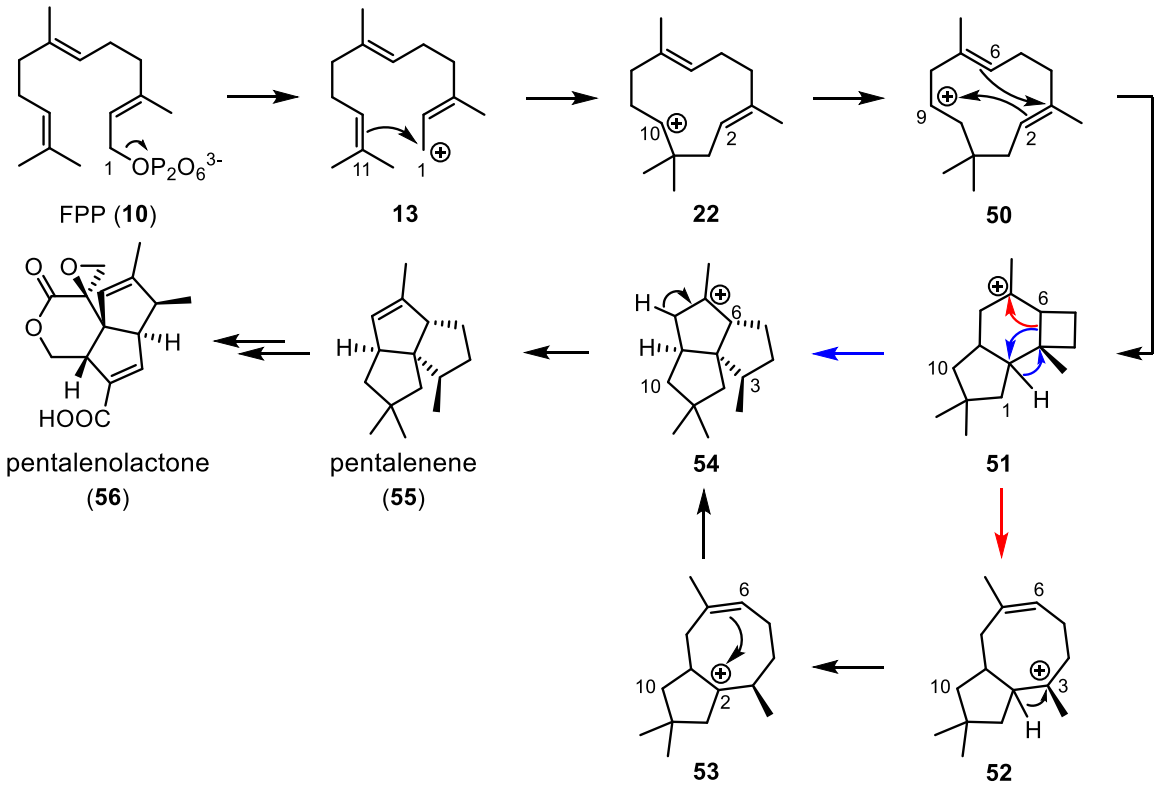
After cleavage of the pyrophosphate function, the farnesyl cation formed (**13**) undergoes isomerization of the C2-C3 double bond. The resulting nerolidyl diphosphate (**21**) is then converted to germacrane dienyl cation **40** via a 1⟶10 cyclization followed by a 1,3-hydride shift. From here, follow-up reactions such as cyclizations, Waagner-Meerwein rearrangements, and hydride shifts lead to the formation of various structurally diverse products (**44–49**) (Scheme 6).[34,35]



Scheme 6: Mechanism proposed by Schmidt-Dannert and co-workers for the Cop4-catalyzed biosynthesis of various cyclic sesquiterpenes (**44–49**). Based on homology modeling, Cop4 is assumed to have a large active pocket. This allows the substrate and its intermediates to adopt different conformations, which would explain the formation of a large number of cyclization products.

1.3.3 PENTALENENE SYNTHASE – PENA

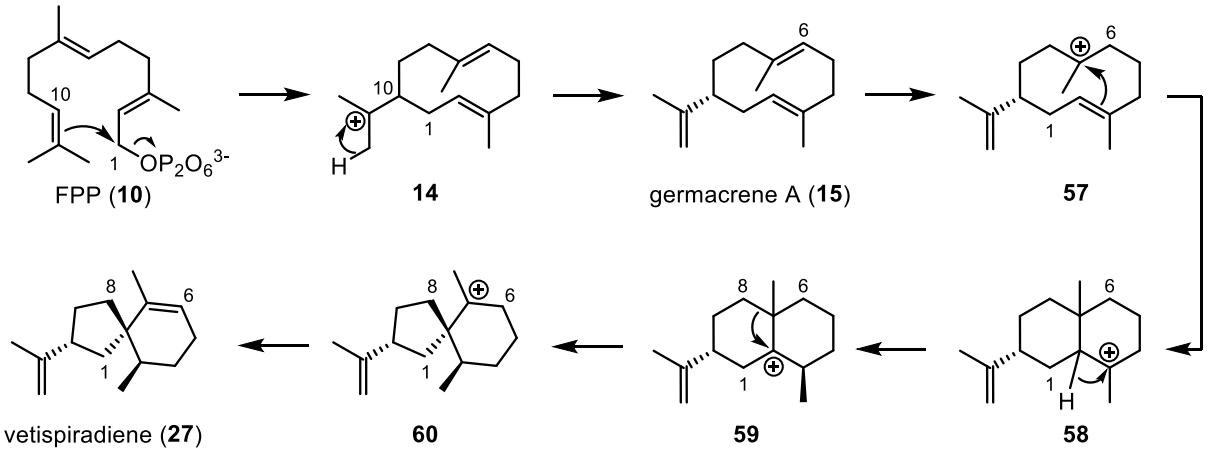
The bacterial sesquiterpene cyclase PenA was isolated from *Streptomyces* and is one of the first terpene cyclases to be identified. In a study published in 1983, Cane and Tillman[37] reported the successful conversion of FPP (**10**) to pentalenene (**55**) using an extract from *Streptomyces exfoliatus*. PenA is part of a gene cluster responsible for the biosynthesis of pentalenolactone (**56**). This antibiotic natural product is used in human and veterinary medicine as well as in the agricultural sector.[23,38] Based on experiments with isotope-labeled FPP derivatives and quantum mechanical calculations by Cane[39] and Tantillo[40,41], the biosynthesis begins with a 1⟶11 cyclization after cleavage of the diphosphate function of FPP (**10**). The resulting humulyl cation **22** is then converted to the 7-protoilludyl cation (**51**) via a 1,2-hydride shift followed by a concerted rearrangement. From here, Pentalenene (**55**) can be formed via two plausible mechanistic pathways. First, the cationic intermediate **54** can be formed by a simple ring extension. In the second option, the cyclobutane ring is first opened, resulting in the rearrangement of the positive charge. After subsequent 1-2 hydride shift and cyclization, the cationic intermediate **54** is also formed. From here, the final deprotonation leads to the formation of pentalenene (**55**), which serves as a precursor for the synthesis of **56** (Scheme 7).



Scheme 7: Mechanism proposed by Cane and Tantillo for the PenA-catalyzed biosynthesis of pentalenene (**57**), an intermediate in the biosynthesis of pentalenolactone (**56**). The latter is a sesquiterpene lactone that exhibits antibiotic activity.

1.3.4 VETISPIRADIENE SYNTHASE – HVS1

The plant-based sesquiterpene cyclase Hvs1 was isolated from Egyptian henbane (*Hyoscyamus muticus*). It was characterized in a publication by Back and Chappell in 1996.[42] After successful heterologous expression in *E. coli* and isolation of the enzyme, the researchers performed mainly kinetic and mechanistic studies. The formation of vetispiradiene starts with a 1⟶10 cyclization after cleavage of the pyrophosphate function of FPP (**10**). Subsequent deprotonation leads to the formation of germacrene A (**15**) as a neutral intermediate. Protonation of the C6-C7 double bond, followed by a 2⟶7 cyclization, leads to the formation of bicyclic intermediate **58**. Vetispiradiene (**27**) was formed via a 1,2-hydride shift followed by a Waagner-Meerwein rearrangement and final deprotonation (Scheme 8).[43]



Scheme 8: Mechanism proposed by Whitehead and co-workers for the Hvs1-catalyzed biosynthesis of vetispiradiene (**27**). The bicyclic sesquiterpene belongs to the group of phytoalexins, which exhibit antimicrobial properties.

1.4 BIOTRANSFORMATIONS OF NON-NATURAL SUBSTRATES USING TERPENE CYCLASES

Because of their high efficiency and selectivity in converting natural substrates via complex reaction mechanisms, it is often assumed that enzymes exhibit little tolerance towards non-natural substrates. However, many enzymes have been found to exhibit substrate promiscuous features allowing them to metabolize compounds that are not their natural substrates and for which they are not specialized. The unnatural substrate is thereby converted to potentially new products, while the catalyzed reaction is maintained. According to current scientific understanding, most of today's enzymes possess this property as a result of mutation and natural selection originating from a few ancestral enzymes that exhibited broad selectivity and specificity. Consequently, this enzyme property may serve as an important starting point for the synthesis of new complex cyclic terpenes.[9,12,16,44,45]

In the research field of terpenes, cyclases have usually been studied to determine their structure and elucidate their complex reaction mechanisms. For this purpose, natural linear precursors or isotope-labeled derivatives were mostly used.[46] This stands in contrast to the rare cases in which unnatural linear substrates were utilized. Here, unnatural precursors were used to inhibit cyclases in order to study the corresponding reaction mechanism.[47–49] For example, Christianson[50] and co-workers were able to use fluorinated FPP derivatives (**70**–**71**) as competitive inhibitors to perform conformational analyses on aristolochene synthase. Additionally, Cane[51] and co-workers also succeeded in gaining many insights into the biosynthetic mechanism of trichodiene synthase using dihydro, cyclopropyl, and elongated FPP derivatives (**61**–**65**) (Figure 3).

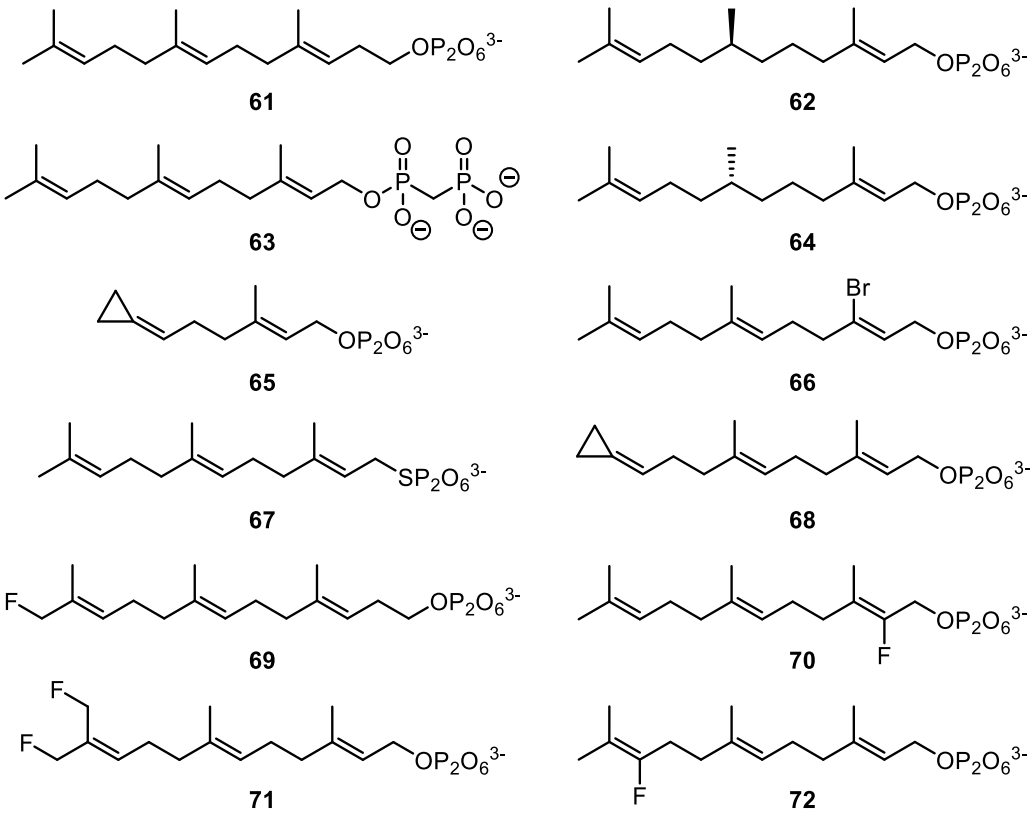
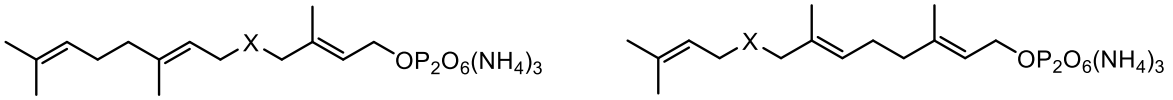


Figure 3: Examples of unnatural linear terpenes synthesized to be used as inhibitors. The results of biotransformation with these unnatural substrates were used to elucidate biosynthetic mechanisms and enzyme structures.[44,45,47–51,52]

In contrast to the many studies that have used unnatural substrates as inhibitors, the Kirschning group was among the first to deliberately synthesize unnatural substrates in an effort to obtain new cyclic terpenoids with interesting structural motifs that are more difficult to access synthetically. In a recent publication, Kirschning[53] and co-workers reported the synthesis of six novel heteroatom-modified FPP derivatives (**73–78**) to be used as substrates in biotransformations with sesquiterpene cyclases (Figure 4).

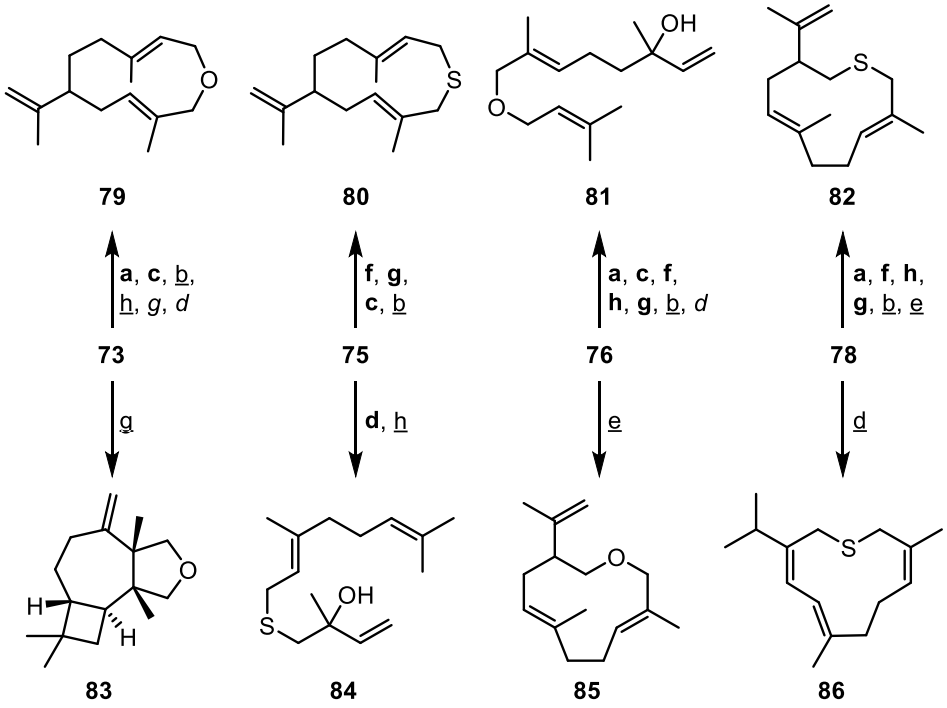


73 (X= O)
74 (X= NMe)
75 (X= S)

76 (X= O)
77 (X= NMe)
78 (X= S)

Figure 4: Unnatural linear terpenoids containing oxygen, nitrogen, and sulfur (**73–78**) synthetically accessed by the Kirschning group.

In addition to obtaining new cyclic products, the substrate flexibility of sesquiterpene cyclases toward heteroatom-modified derivatives was to be evaluated. For the biotransformations, eight selected recombinant and purified sesquiterpene cyclases from fungal, bacterial, and plant sources were used (Scheme 9).



Scheme 9: After synthesizing various unnatural heteroatom-modified FPP derivatives (**73–78**), biotransformation with eight selected sesquiterpene cyclases yielded eight structurally diverse products (**79–86**). Enzymes, plant origin: a) Pts = patchoulol synthase, b) Tps32 = viridiflorene synthase, c) Hvs1 = vetispiradiene synthase; bacterial origin: d) GcoA = caryolan-1-ol synthase, e) TmS = T-muurolol synthase, f) PenA = pentalenene synthase; fungal origin: g) BcBot2 = presilphiperfolan-8-b-ol synthase, h) Cop4 = cubebol synthase; enzymes in **bold**: main product; enzymes underlined: used for preparative scale; enzymes in *italic*: traces of product formed.

The unnatural FPP derivatives (**73–78**) employed were shown to be effective substrates. In a single biotransformation step, new complex macrocyclic products with defined stereocenters were obtained (**79–86**). Some of the isolated compounds were also found to have moderate potential as olfactory stimulants (Scheme 9). The highlighted methods and results demonstrate that enzyme-catalyzed biotransformations with unnatural substrates provide efficient and convenient access to complex terpenoids. Therefore, these multidisciplinary synthetic approaches provide a suitable alternative to classical chemical synthesis.

2 AIM OF THIS DISSERTATION

Due to the great diversity of their properties, terpenes are used for a wide range of applications in science, medicine, and industry. For example, they are of high interest to the cosmetics and perfume industry due to their special olfactory properties. Complex cyclic terpenoid products can be obtained from more accessible acyclic terpenes via enzyme-catalyzed biotransformation. Many challenges of classical chemical synthesis, such as stereoselectivity and chemoselectivity, can be more easily overcome with enzyme catalysis. In addition, enzymes often accept derivatives of the natural precursor substrates and convert them into novel compounds. By using different enzymes, it is also possible to obtain several different products from the same starting material.

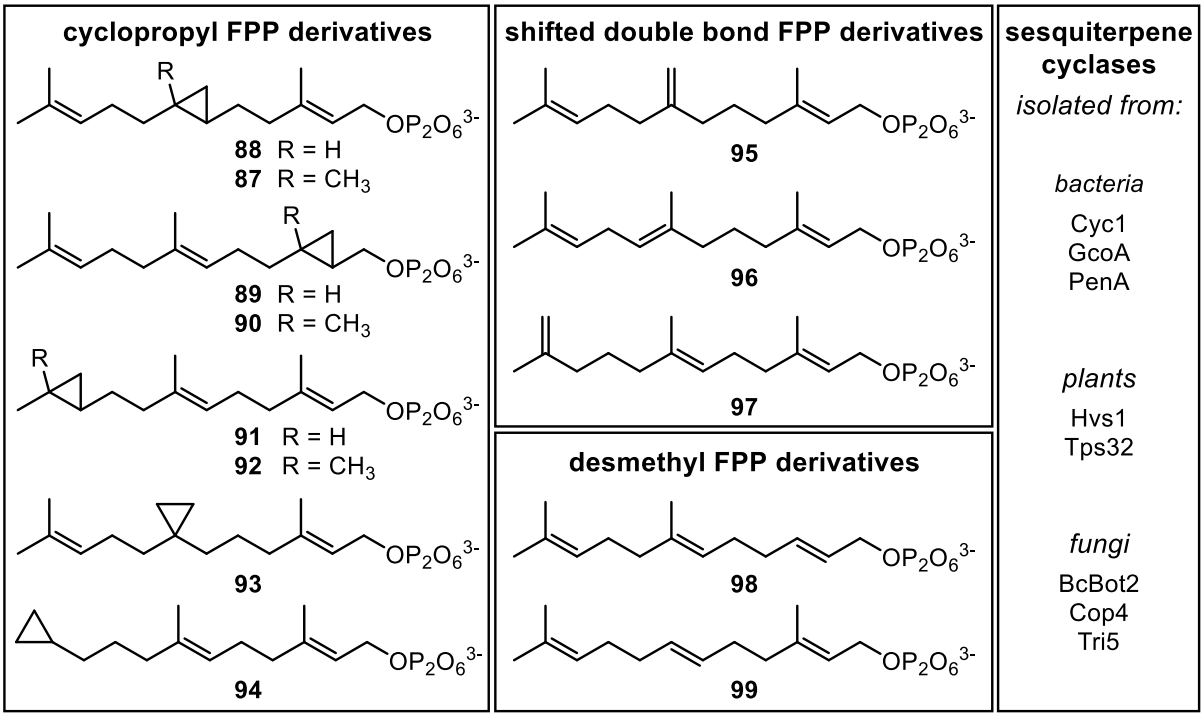
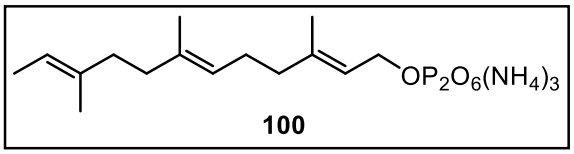


Figure 5: Unnatural FPP derivatives to be synthesized in this work and subsequently utilized as substrates for eight selected sesquiterpene cyclases of different origins. The aim is to produce new cyclic terpenoids and to gain new insights into enzymatic mechanisms. BcBot2 = presilphiperfolan-8-β-ol synthase; Cop4 = cubebol synthase; Cyc1 = *epi*-isozizaene synthase; GcoA = caryolan-1-ol synthase; Hvs1 = vetispiradiene synthase; PenA = pentalenene synthase; Tps32 = viridiflorene synthase; Tri5 = trichodiene synthase.

The aim of this thesis is the synthesis of unnatural linear farnesyl diphosphate derivatives for the subsequent enzymatic conversion with sesquiterpene cyclases (Figure 5). For this purpose, eight selected cyclases from fungal, bacterial, and plant sources are intended to be heterologously expressed, isolated, and purified. The activity of the enzymes obtained will be determined by positive control experiments with the natural substrate FPP (**10**). Since unnatural FPP derivatives with various heteroatoms and shifted methyl groups have been extensively studied by the Kirschning group in the past,[53] this work will focus on the synthesis of cyclopropyl, desmethyl and double bond shifted FPP derivatives (**87**–**99**). One goal of these

experiments is to obtain novel structurally complex cyclic sesquiterpenes that are difficult to access chemically. Additionally, the results will be used to gain further insights into the biosynthetic mechanisms of terpene cyclases, thereby supporting or refuting existing mechanistic proposals.



100

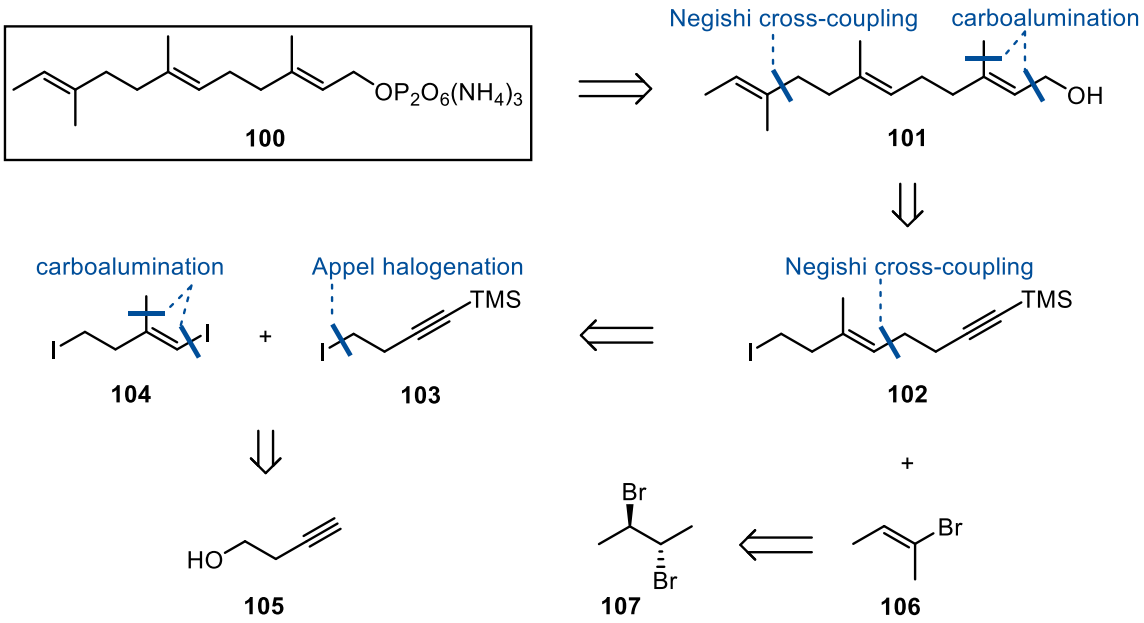
Figure 6: Methyl-shifted FPP derivative **100**. In initial qualitative biotransformations, the substrate was readily accepted by the cyclases used.

Before starting the synthesis of the unnatural pyrophosphates described (**87**–**99**), methyl-shifted FPP derivative **100** is to be synthesized first (Figure 6). This diphosphate already delivered promising results in initial analytical enzyme tests in the past. The synthesis was developed and carried out by Schröder[54] at that time. The goal now is to synthesize diphosphate **100** in larger quantities for subsequent use in biotransformations on a semi-preparative scale.

3 RESULTS AND DISCUSSION - CHEMICAL SYNTHESES

3.1 METHYL SHIFTED FPP DERIVATIVE 100

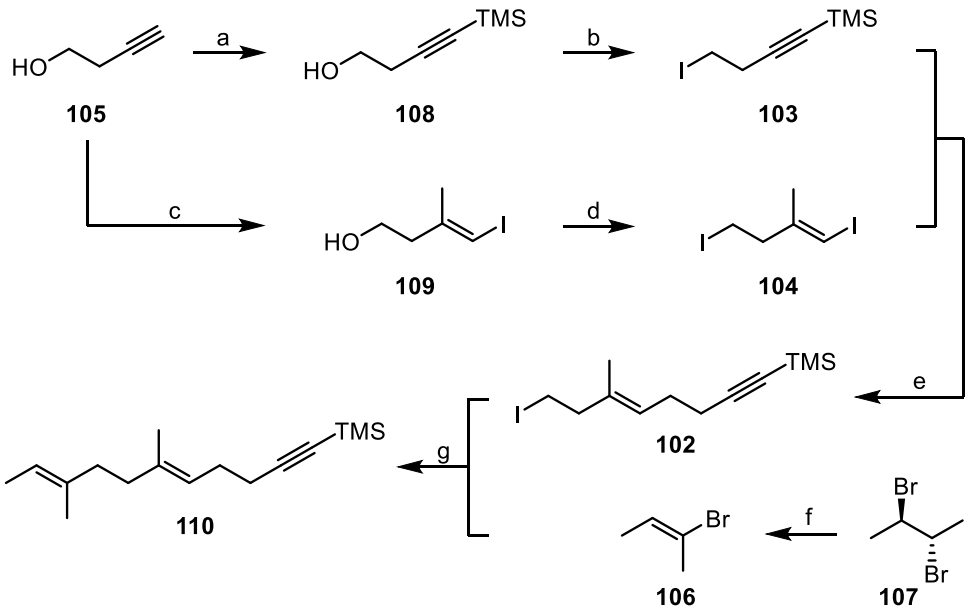
The first objective of this dissertation was the synthesis of pyrophosphate **100** according to a synthesis strategy previously established by Schröder.[54] The desired FPP derivative **100** has a shifted methyl group compared to the natural substrate FPP (**10**). Diphosphate **100** was planned to be obtained via a two-step pyrophosphorylation procedure by Poulter and co-workers[55,56] starting from alcohol **101**. The latter was to be obtained from iodide **102** via carboalumination and an sp^2-sp^3 Negishi[57] cross-coupling reaction with bromide **106**. For the synthesis of iodide **102**, a second Negishi coupling was planned to connect iodides **103** and **104**, both of which were to be prepared from 3-butyne-1-ol (**105**) (Scheme 10).



Scheme 10: Retrosynthetic approach by Schröder for the preparation of methyl shifted FPP derivative **100**. Starting from commercially available *meso*-2,3-dibromobutane (**107**) and 3-butyne-1-ol (**105**), the preparation of alcohol **101** was planned to be achieved via two Negishi cross-coupling reactions as the key steps.

The forward synthesis was initiated with the preparation of iodide **103** and vinyl iodide **104** starting from the same parent building block. After silylation of the alkyne function and subsequent halogenation under Appel conditions, 3-butyne-1-ol (**105**) was converted to iodide **103** in two steps and excellent yield (Scheme 11).[57] Using the same starting material, the synthesis of vinyl iodide **104** was achieved with a zirconium catalyzed carboalumination followed by a halogenation reaction under Appel conditions.[58] Following a protocol by Negishi[57] and co-workers, the two iodides obtained (**103**, **104**) were coupled via a palladium-catalyzed sp^2-sp^3 cross-coupling reaction to afford the homoallyl iodide **102** in good yield. Subsequently, efforts were made to convert homoallyl iodide **102** and vinyl bromide **106**, which

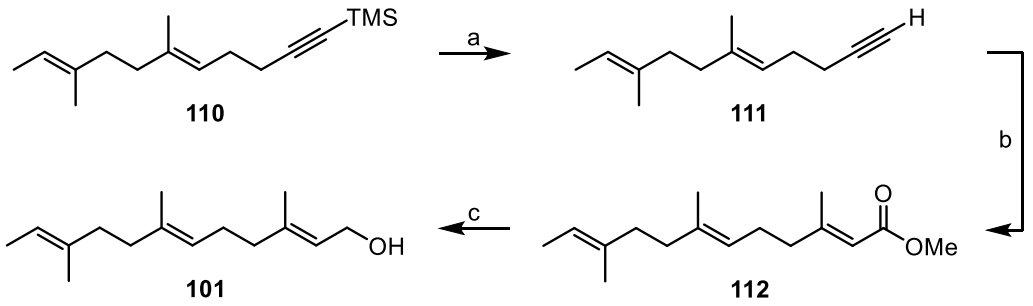
was obtained from *meso*-2,3-dibromobutane (**107**) via an E2 elimination reaction,[59] into the desired coupling product **110** by utilizing a second Negishi coupling reaction (Scheme 11).



Scheme 11: Synthesis of compound **110** starting from *meso*-2,3-dibromobutane (**107**) and 3-butyne-1-ol (**105**). a) *n*-BuLi, THF, -78 °C → rt, 1 h *then* TMS-Cl, -78 °C → rt, 2 h, quant.; b) I_2, PPh_3, imidazole, CH_2Cl_2, 0 °C, 2 h, 93 %; c) Cp_2ZrCl_2, $AlMe_3$, CH_2Cl_2, -25 °C, 10 min *then* H_2O, 10 min, -25 °C *then* **105** (previously stirred with $AlMe_3$ in CH_2Cl_2 at 0 °C), -25 °C → rt, 18 h *then* I_2 in Et_2O, -25 °C, 2 h, 79 %; d) I_2, PPh_3, imidazole, CH_2Cl_2, 0 °C, 2 h, 91 %; e) **103**, *t*-BuLi, Et_2O, -78 °C, 30 min *then* $ZnBr_2$ in THF, -78 °C → 0 °C, 30 min *then* **104** and Pd(dppf)Cl_2·(CH_2Cl_2) in THF, 0 °C → rt, 3 h, 74 %; f) KOH, $(CH_2OH)_2$, 120 °C, 1 h, 81 %; g) *t*-BuLi, Et_2O, -78 °C, 30 min *then* $ZnBr_2$ in THF, -78 °C → 0 °C, 30 min *then* **106** and Pd(dppf)Cl_2·(CH_2Cl_2) in THF, 0 °C → rt, 4 h, 59 %.

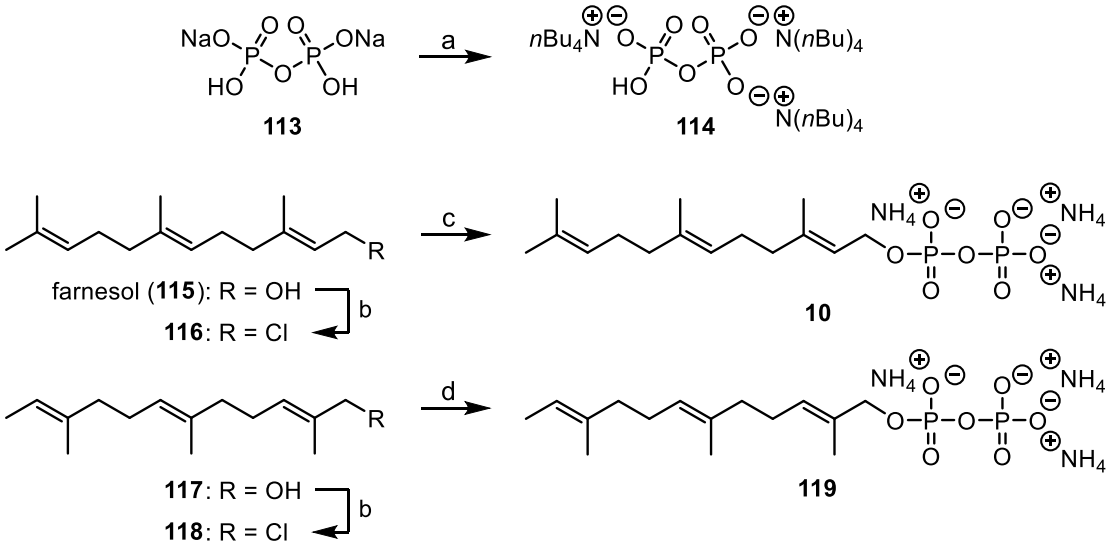
Surprisingly, the second cross-coupling reaction to produce **110** initially proved to be quite challenging. Although the reaction conditions were analogous to those of the first coupling reaction, which worked well and gave good yields, no or very little product was obtained after the second coupling. Initially, it was assumed that the highly sensitive reaction might be inhibited by oxygen or water impurities. But even after all reagents used were newly acquired, immediately stored in a glove box, and thoroughly dried before use, no satisfactory results could be obtained when probing the second Negishi coupling. It was later found that, for unknown reasons, the reaction only worked with zinc bromide from a particular supplier (see EXPERIMENTAL SECTION, p. 65).

After coupling product **110** was finally obtained in sufficient amounts, treatment with potassium hydroxide led to the formation of alkyne **111**. Subsequent zirconium-catalyzed carboalumination followed by termination of the reaction with methyl chloroformate led to the generation of α, β-unsaturated ester **112** in good yield. The obtained ester was then reduced with di*iso*butylaluminium hydride to afford the desired alcohol **101** (Scheme 12).[54]



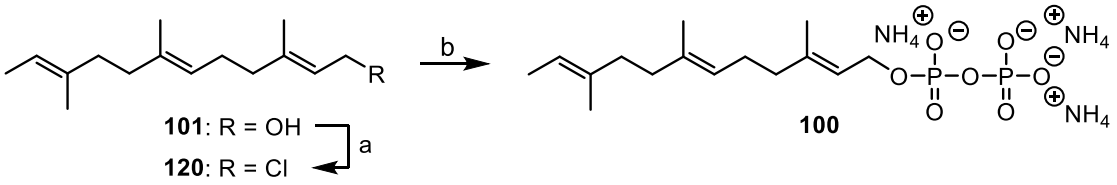
Scheme 12: Final reactions for the synthesis of alcohol **101** starting from the coupling product **110**. a) KOH, MeOH/H_2O, rt, 4 h; b) Cp_2ZrCl_2, $AlMe_3$, CH_2Cl_2, 0 °C, 10 min *then* **111**, 0 °C → rt, 20 h *then* $ClCO_2Me$, rt, 16 h, 77 % o2s; c) DIBAL-H, CH_2Cl_2, 0 °C, 2 h, 80 %.

With alcohol **101** in hand, the next step was the synthesis of the corresponding diphosphate. For this purpose, a two-step procedure developed by Poulter[56,60] and co-workers was planned to be used. In this protocol, the employed alcohol is first converted into a more reactive halide intermediate which is then transformed into the corresponding pyrophosphate with the addition of a diphosphate salt. The crude mixture obtained is then passed through an ion-exchange column to yield the desired diphosphate as an ammonium salt. Since this is not a standard chemical synthesis process, the individual steps were not yet familiar. Therefore, the method was first practiced with more easily accessible substrates in order to gain more confidence and avoid substrate losses due to execution errors. Following the presented procedure by Poulter, the required pyrophosphate salt **114** was initially prepared. From here, two allyl diphosphates were synthesized starting from the corresponding allyl alcohols (Scheme 13).



Scheme 13: Preparation of diphosphate salt **114** used for phosphorylation and synthesis of two diphosphates to practice the reaction procedure. Unnatural substrate **117** was provided by Schröder. a) NH_4OH, DOWEX ion-exchange column (H^+ form) *then* nBu$_4$NOH, 97 %; b) DMS, NCS, CH_2Cl_2, -30 °C, 20 min *then* allyl alcohol, CH_2Cl_2, -40 °C ⟶ rt, 2 h; c) $P_2O_7H(n$Bu$_4$N$)_3$ (**114**), CH_3CN, rt, 16 h. *then* DOWEX ion-exchange column, 72 % o2s; d) $P_2O_7H(n$Bu$_4$N$)_3$ (**114**), CH_3CN, rt, 16 h. *then* DOWEX ion-exchange column, 33 % o2s.

With the successful synthesis of two diphosphates, the handling of the pyrophosphate salt and the ion-exchange column was more familiar, therefore the phosphorylation of alcohol **101** was carried out. Using the previously presented procedure and reaction conditions, diphosphate **100** was successfully obtained as a colorless foamy solid (Scheme 14).

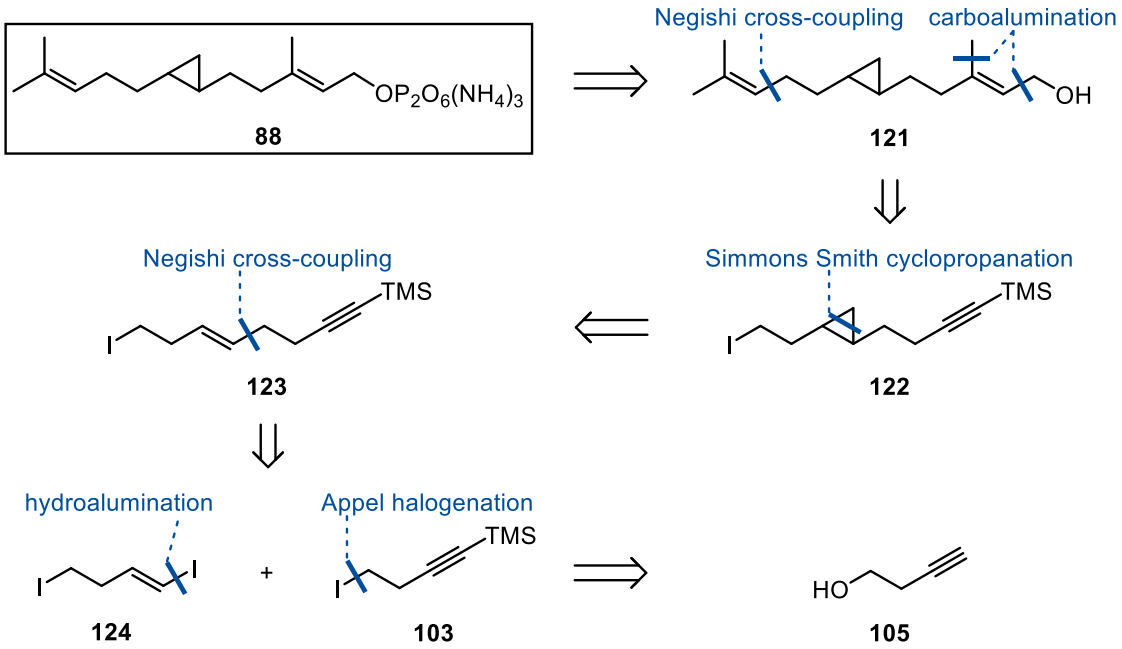


Scheme 14: Two-step synthesis of methyl shifted FPP derivative **100** starting from the previously afforded alcohol **101**. a) DMS, NCS, CH_2Cl_2, -30 °C, 20 min *then* allyl alcohol, CH_2Cl_2, -40 °C ⟶ rt, 2 h; c) $P_2O_7H(nBu_4N)_3$ (**114**), CH_3CN, rt, 16 h. *then* DOWEX ion-exchange column, 75 % o2s.

3.2 CYCLOPROPYL DIPHOSPHATE 88 AND DESMETHYL FPP DERIVATIVE 99

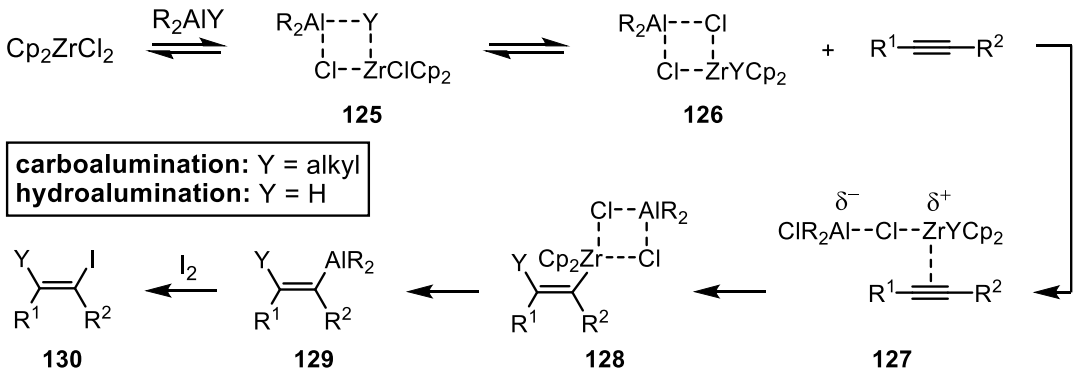
3.2.1 FIRST SYNTHESIS STRATEGY FOR THE PREPARATION OF 88

The first synthesis strategy to obtain cyclopropyl diphosphate **88** was strongly based on the previously performed synthesis of methyl shifted FPP derivative **100**. Starting from cyclopropyl iodide **122** as the key intermediate, the final reactions for the preparation of **88** were planned to be adopted from the synthesis of **100**. Simmons-Smith[61] cyclopropanation was envisioned to lead to the formation of cyclopropyl iodide **122**, starting from iodide **123**. The latter was to be obtained via a second Negishi coupling of iodides **124** and **103**, which were to be prepared from the same alcohol (**105**) (Scheme 15).



Scheme 15: Retrosynthesis of cyclopropyl diphosphate **88**. For the construction of the carbon backbone, two Negishi cross-couplings and a Simmons-Smith cyclopropanation were planned to be used as key steps. Most reactions were adopted from Schröder's strategy for the synthesis of methyl shifted FPP derivative **100**.[54]

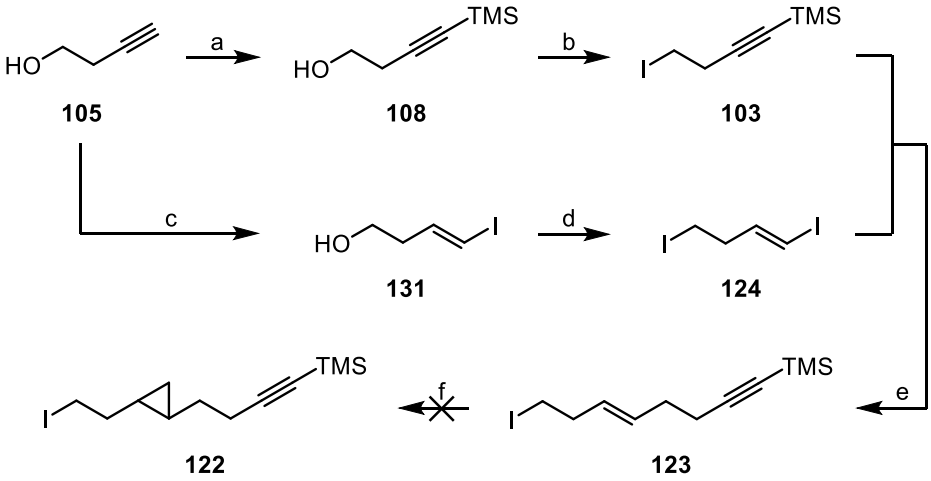
The first step of this synthesis was the preparation of the iodides **103** and **124**. The former was synthesized over two steps as described above and was obtained in excellent yield. For diiodide **124**, zirconium-catalyzed hydroalumination[62] was used. The difference to carboalumination[58] reactions lies in the activation of the Zr catalyst, which is not activated with trimethylaluminium but with di*iso*butylaluminium hydride. The latter transfers a hydride to the active Zr species instead of a methyl group, which would be transferred when trimethylaluminium had been used (Scheme 16).[63]



Scheme 16: Comparison of the mechanisms for hydro- and carboalumination. After activation of the Zr catalyst by an organoaluminium species, insertion to an alkyl function occurs. The regioselective addition is primarily sterically driven. Upon addition of iodide, the active metal species is displaced and a vinyl iodide is obtained.

After addition of the *in situ* generated Schwartz[64] reagent (Cp_2ZrHCl) to alkyne **105**, treatment with iodine resulted in metal-halogen exchange yielding vinyl iodide **131**. Subsequent

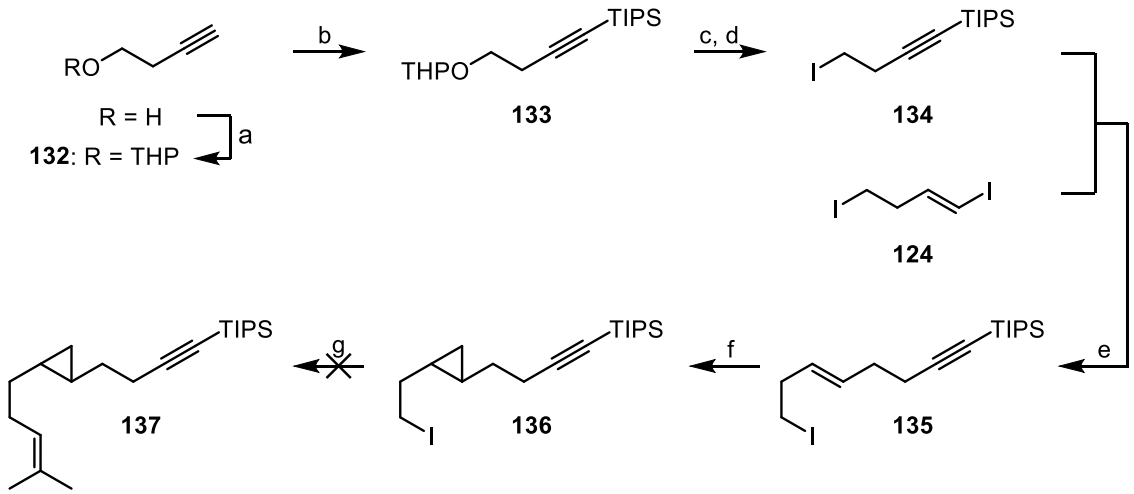
halogenation under Appel conditions delivered diiodide **124** in good yield. The two fragments (**103** and **124**) were then coupled under the Negishi cross-coupling conditions as previously described to yield iodide **123** (Scheme 17).[57]



Scheme 17: Synthesis of iodide **123** starting from commercially available 3-butyne-1-ol (**105**). A Negishi cross-coupling reaction was used as the key step to construct the carbon backbone. a) *n*-BuLi, THF, -78 °C → rt, 1 h *then* TMS-Cl, -78 °C → rt, 2 h, quant.; b) I_2, PPh_3, imidazole, CH_2Cl_2, 0 °C, 2 h, 93 %; c) Cp_2ZrCl_2, DIBAL-H, THF, 0 °C, 30 min *then* **105** , 0 °C ⟶ rt, 90 min, *then* I_2 in THF, -78 °C ⟶ rt, 18 h, 43 %; d) I_2, PPh_3, imidazole, CH_2Cl_2, 0 °C ⟶ rt, 80 min, 58 %; e) **103**, *t*-BuLi, Et_2O, -78 °C, 30 min *then* $ZnBr_2$ in THF, -78 °C → 0 °C, 30 min *then* **104** and $Pd(dppf)Cl_2 \cdot (CH_2Cl_2)$ in THF, 0 °C → rt, 2.5 h, 66 %, 88 % *brsm*; f) Et_2Zn, TFA, CH_2Cl_2, 0 °C, 1 h *then* CH_2I_2, CH_2Cl_2, 0 °C, 1 h *then* **123**, CH_2Cl_2, 0 °C ⟶ rt, 16 h.

For the introduction of a cyclopropyl group as the key element, a Shi[61] variation of the Simmons-Smith reaction was carried out. In this variation, a modified Zn species is used, which increases the efficiency of the reaction and allows the use of milder conditions. Unfortunately, all efforts failed to achieve any satisfactory results (Scheme 17). Based on NMR analysis of the purified reaction mixtures, it was assumed that the TMS group was cleaved under the deployed reaction conditions. It was therefore decided to replace the relatively unstable TMS group with a TIPS group, which is more resistant to cleavage under the conditions employed.

For this purpose, 3-butyne-1-ol (**105**) was initially converted to THP ether **132** under mildly acidic conditions. After the introduction of a TIPS group to afford **133**,[65] the desired iodide **134** was obtained after cleavage of the dihydropyran group and subsequent halogenation (Scheme 18).



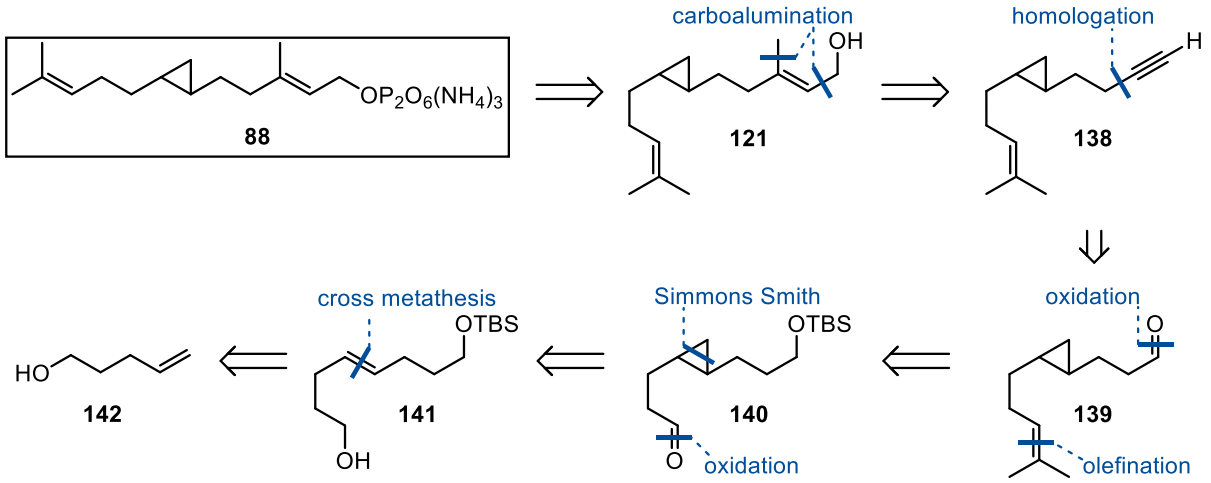
Scheme 18: Four-step synthesis of iodide **134** and subsequent Negishi coupling with vinyl iodide **124** to obtain coupling product **135**. After the subsequent Simmons-Smith cyclopropanation successfully led to the formation of **136**, efforts to extend the carbon chain via a second Negishi coupling were unsuccessful. a) DHP, *p*TsOH, CH_2Cl_2, rt, 1 h; b) *n*-BuLi, THF, -78 °C, 1 h *then* TIPS-Cl, -78 °C ⟶ rt, 18 h; c) *p*TsOH, CH_3OH, rt; d) I_2, PPh_3, imidazole, CH_2Cl_2, 0 °C, 20 min *then* **133**, 0 °C ⟶ rt, 2 h, 81 % o4s; e) *t*-BuLi, **134**, Et_2O, -78 °C, 30 min *then* $ZnBr_2$ in THF, -78 °C → 0 °C, 30 min *then* **124** and Pd(dppf)Cl_2·(CH_2Cl_2) in THF, 0 °C → rt, 4 h, 62 %; f) Et_2Zn, TFA, CH_2Cl_2, 0 °C, 30 min *then* CH_2I_2, CH_2Cl_2, 0 °C, 30 h *then* **135**, CH_2Cl_2, 0 °C ⟶ rt, 1 h, 56 %; g) *t*-BuLi, **136**, Et_2O, -78 °C, 30 min *then* $ZnBr_2$ in THF, -78 °C → 0 °C, 30 min *then* 1-bromo-2-methyl-1-propene and Pd(dppf)Cl_2·(CH_2Cl_2) in THF, 0 °C → rt, 16 h.

With both iodide **134** and vinyl iodide **124** in hand, coupling under the previously presented Negishi conditions afforded iodide **135** in good yield. Unlike the last experiments with TMS-protected alkyne **123**, introduction of the key cyclopropyl moiety to iodide **135** via Simmons-Smith cyclopropanation led to the desired iodide **136**. However, subsequent attempts to extend the carbon skeleton via Negishi coupling with commercially available 1-bromo-2-methyl-1-propene remained fruitless (Scheme 18). Since the challenges with the now established sp^2-sp^3 Negishi cross-coupling conditions had already led to many time-consuming problems in the past, it was decided to develop a new synthetic strategy.

3.2.2 SECOND SYNTHESIS STRATEGY FOR THE PREPARATION OF 88

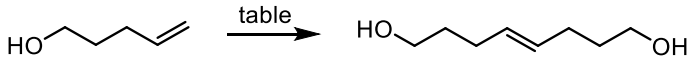
In contrast to the initial idea of coupling two different substrates, the second synthesis approach aims towards the preparation of a symmetric dimer to construct the carbon backbone of cyclopropyl diphosphate **88**.

The FPP derivative **88** was intended to be synthesized from alkyne **138** via carboalumination[58] and esterification followed by reduction and phosphorylation. The latter was to be obtained from aldehyde **139** via a Corey-Fuchs[66] style homologation. It was assumed that **139** should be accessible via Wittig[67] olefination, desilylation, and subsequent oxidation from aldehyde **140**. The cyclopropyl moiety was planned to be introduced via Simmons-Smith cyclopropanation of alcohol **141**, which should be accessible via cross-metathesis[68–70] and subsequent monosilylation of 4-penten-1-ol (**142**) (Scheme 19).



Scheme 19: Second retrosynthetic approach towards the synthesis of cyclopropyl diphosphate **88**. Cross-metathesis with an (*E*)-selective catalyst was planned to be the key step in the construction of the carbon backbone.

The presented synthesis commences with a (*E*)-selective dimerization of pent-4-en-1-ol (**142**). For this purpose, several Grubbs[71] catalysts were first screened in order to maximize the yield and selectivity. The results of the initial catalyst screenings indicated that a choice had to be made between yield and selectivity, as all experiments yielded similarly mediocre results (Scheme 20, entry 1–4). However, after testing not only different catalysts but also reaction conditions and addition protocols, a suitable process for the cross-metathesis was found (Scheme 20, entry 5–7). To promote intermolecular reactions, metathesis was performed without or with the minimum amount of solvent required. In addition, a constant gas flow was provided to remove forming ethylene gas, which would cause decomposition of the product via ethenolysis. Moreover, the catalyst was added in portions, since the catalyst activity decreases over time. These adjustments to the reaction process led to an significant increase of the yield of diol **143** and consequently reduced the required amount of the expensive catalyst (Scheme 20, entry 7).

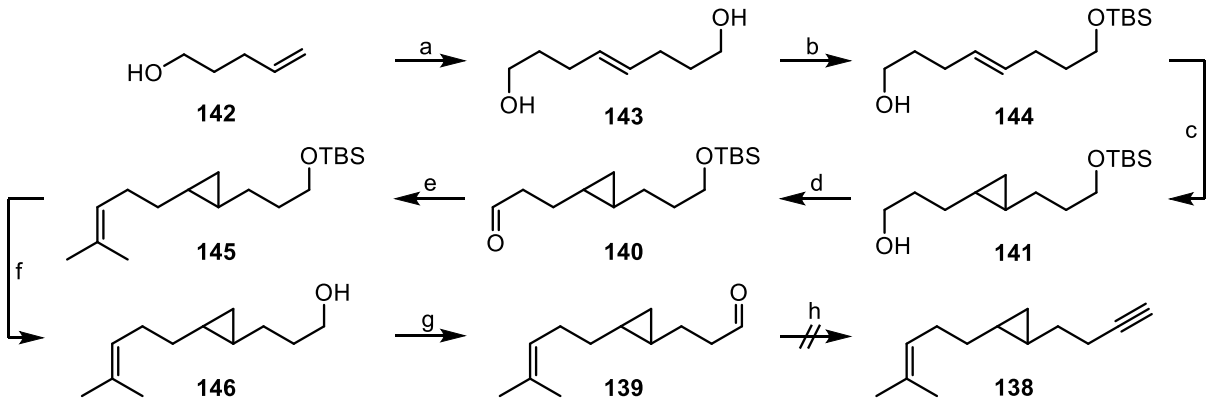


142 **143**

Entry	Catalyst	Conditions and process	Result
1	Grubbs I	SM in CH_2Cl_2 (1 M) + cat[B], rt, 3 d	traces
2	Hoveyda-Grubbs	SM[A] + cat[B], rt, 18 h	45 %, *E*:*Z* = 4:1
3	Grubbs I	SM[A] + cat[B], rt, 18 h	24 %, *E*:*Z* = 10:1
4	Grubbs II	SM[A] + cat[B], rt, 18 h	53 %, *E*:*Z* = 4:1
5	Grubbs I	SM[A] + cat[B], rt, 18 h *then* reflux, 9 h	50 %, 82 % *brsm*, *E*:*Z* = 4:1
6	Grubbs I	SM neat + cat[C], rt, 18 h, no light, constant Ar flow	86 %, *E*:*Z* = 4:1
7	Grubbs I	SM neat + cat[D], rt, 18 h, no light, constant Ar flow	75 %, *E*:*Z* = 9:1

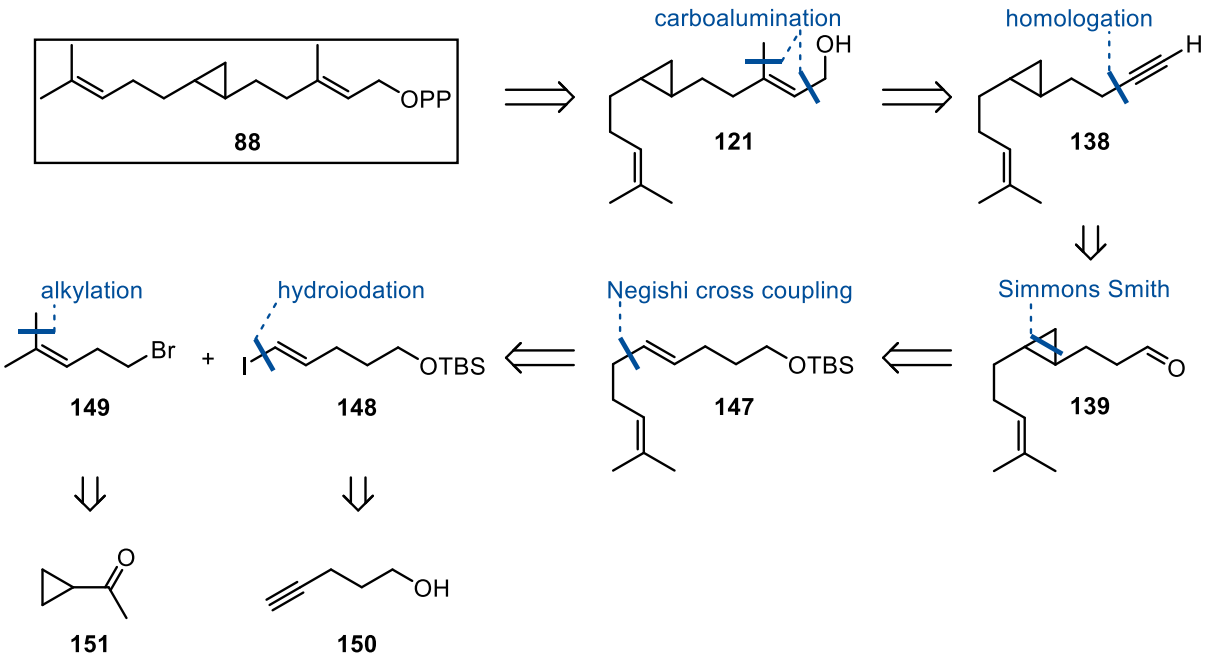
Scheme 20: Overview of the conducted optimization experiments for (*E*)-selective cross-coupling of pent-4-en-1-ol (**142**). A) Dissolved in degassed CH_2Cl_2 (1 M); B) Added in one portion; C) Saturated catalyst solution (CH_2Cl_2), dropwise addition over 18 h; D) Added in three portions over 18 h.

With diol **143** in hand, cyclopropyl alcohol **141** was obtained via monosilylation followed by Simmons-Smith cyclopropanation. Aldehyde **140** obtained from the subsequent Swern[72] oxidation was then converted to alkene **145** via a Wittig[73] reaction. From here, acidic desilylation followed by DMP[74] oxidation resulted in the formation of aldehyde **139**. Unfortunately, subsequent homologation reactions using various methods (Corey-Fuchs[66], TMS-CHN_2[75], Bestmann-Ohira[76]) failed to produce alkyne **138** in acceptable yields.



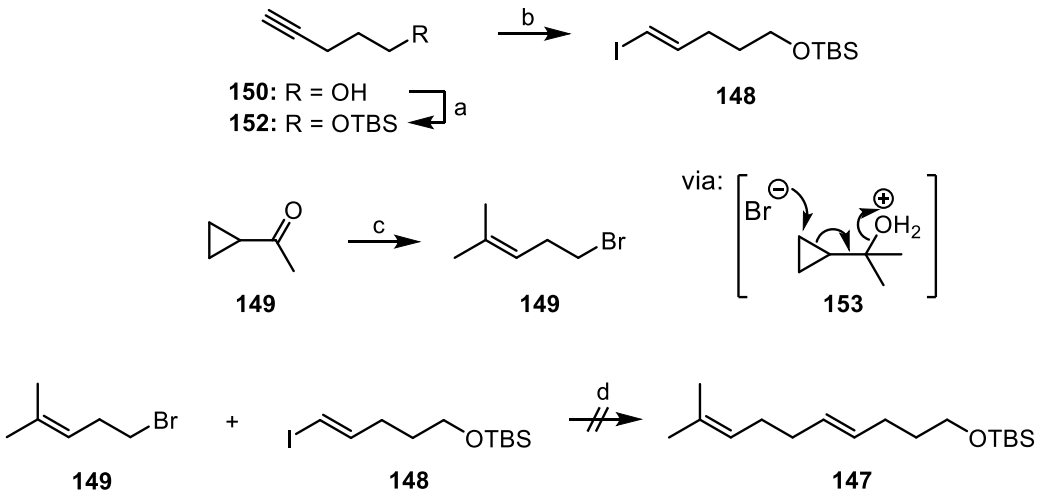
Scheme 21: Initial reactions steps of the second synthesis strategy for the preparation of cyclopropyl diphosphate **88**. a) Grubbs I, rt, 15 h, 86 %, *E*:*Z* = 10:1; b) NaH, THF, 0 °C, 1.5 h *then* TBS-Cl, THF, 0 °C → rt, 45 min, 55 %, 75 % *brsm*; c) Et_2Zn, TFA, CH_2Cl_2, 0 °C, 30 min *then* CH_2I_2, CH_2Cl_2, 0 °C, 30 h *then* **144**, CH_2Cl_2, 0 °C ⟶ rt, 1 h, 80 %; d) $(COCl)_2$, DMSO, CH_2Cl_2, -78 °C, 0.5 h *then* **141**, CH_2Cl_2, -78 °C, 0.5 h *then* Et_3N, -78 °C ⟶ rt, 2 h, 81 %; e) *n*-BuLi, *iso*propyltriphenyl-phosphonium iodide, Et_2O, 0 °C ⟶ rt, 1.5 h *then* **140** in Et_2O, rt, 30 min, 41 %; f) HCl, THF, rt, 30 min; g) DMP, CH_2Cl_2, rt, 2 h, 62 % o2s.

Simultaneously to the strategy presented above, an alternative route towards the preparation of aldehyde **139** was pursued. In this alternative pathway, aldehyde **139** was envisioned to be obtained after Simmons-Smith cyclopropanation and oxidation of diene **147**. The latter was planned to be obtained via Negishi[57] sp^2-sp^3 cross-coupling of bromide **149** and iodide **148**, which were first to be prepared from 4-pentyn-1-ol (**150**) and 1-cyclopropylethan-1-one (**151**) (Scheme 22).



Scheme 22: Second retrosynthetic approach towards the synthesis of cyclopropyl diphosphate **88** with a different route to aldehyde **139**. A Negishi sp^2-sp^3 cross-coupling and a homologation reaction for the elongation of the carbon backbone were envisioned to be the key steps.

After the silylation of commercially available 4-pentyn-1-ol (**150**), the subsequent Zr-catalyzed hydroiodation of the generated alkyne **152** led to the formation of iodide **148**.[62] For the synthesis of bromide **149**, methylmagnesium bromide was used to introduce a methyl group to 1-cyclopropylethan-1-one (**151**). Under acidic conditions, the resulting hydroxy group was protonated, making it a suitable leaving group (**153**). The subsequent attack of a nucleophilic bromide opened the cyclopropane ring, leading to the formation of bromide **149**.[77] Unfortunately, all further efforts to couple the afforded halides via a Negishi cross-coupling were unsuccessful (Scheme 23).

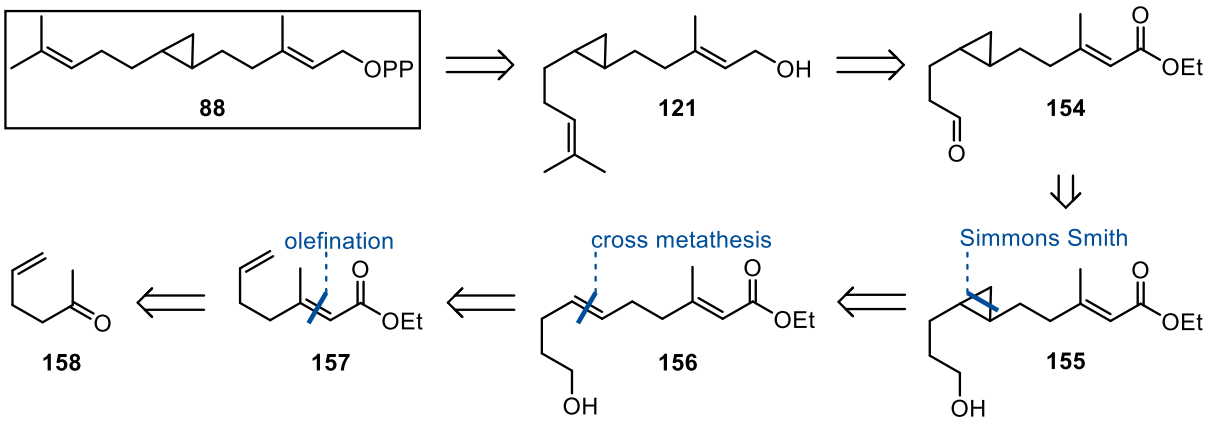


Scheme 23: Initial steps of the alternative synthesis of aldehyde **139**. a) TBS-Cl, imidazole, CH_2Cl_2, 0 °C, 16 h; b) Cp_2ZrCl_2, DIBAL-H, THF, 0 °C, 30 min *then* **152**, THF, 0 °C ⟶ rt, 1.5 h *then* I_2, THF, -78 °C ⟶ rt, 18 h, 74 % o2s; c) CH_3MgBr, THF, 50 °C, 1 h *then* H_2SO_4/H_2O, 10 °C ⟶ rt, 30 min, 78 %; d) *t*-BuLi, **148**, Et_2O, -78 °C, 30 min *then* $ZnBr_2$, THF, -78 °C ⟶ 0 °C, 30 min *then* **149**, Pd(dppf)Cl_2, THF, 0 °C ⟶ rt, 18 h.

Since the synthesis approaches presented so far remained unsuccessful, two completely new strategies were developed and tested simultaneously. First, a route was chosen in which the challenging key steps were to be performed directly at the beginning of the synthesis. This would allow a quick determination of whether the synthetic strategy is viable. The second route focused on minimizing the use of risky and unusual reactions. In this case, established and well-performing reactions were mainly selected for the construction of the carbon backbone.

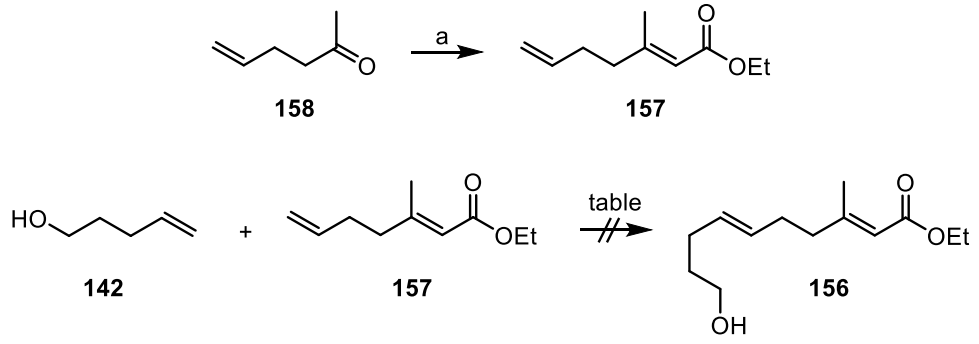
3.2.3 THIRD SYNTHESIS STRATEGY FOR THE PREPARATION OF 88

The third synthetic strategy for the preparation of cyclopropyl diphosphate **88** relied on an (*E*)-selective olefination and a cross-metathesis as the key steps to construct the carbon backbone. Before final phosphorylation to obtain **88**, alcohol **121** was intended to be prepared from ester **154** via Wittig olefination and subsequent ester reduction. The introduction of the key cyclopropyl moiety was planned to be achieved via Simmons-Smith cyclopropanation of alcohol **156**, which was to be prepared from hex-5-en-2-one (**158**) by olefination and cross-metathesis (Scheme 24).



Scheme 24: Third generation retrosynthetic approach towards the synthesis of cyclopropyl diphosphate **88**. The synthesis was designed to begin with two (*E*)-selective key steps to construct the carbon skeleton.

Starting from commercially available hex-5-en-2-one (**158**), the initial (*E*)-selective olefination under HWE-conditions successfully delivered ester **157**.[78] For the synthesis of alcohol **156**, 4-penten-1-ol (**142**) and diene **157** were treated with various Ru-catalysts under different conditions.[71] Unfortunately, initial coupling attempts yielded primarily dimerization side-products and not the desired dienol **156**. Further optimization efforts also failed to deliver satisfying results (Scheme 25). Therefore, this synthesis route was quickly abandoned and the focus was directed to the fourth synthetic strategy, which was carried out in parallel to this route.

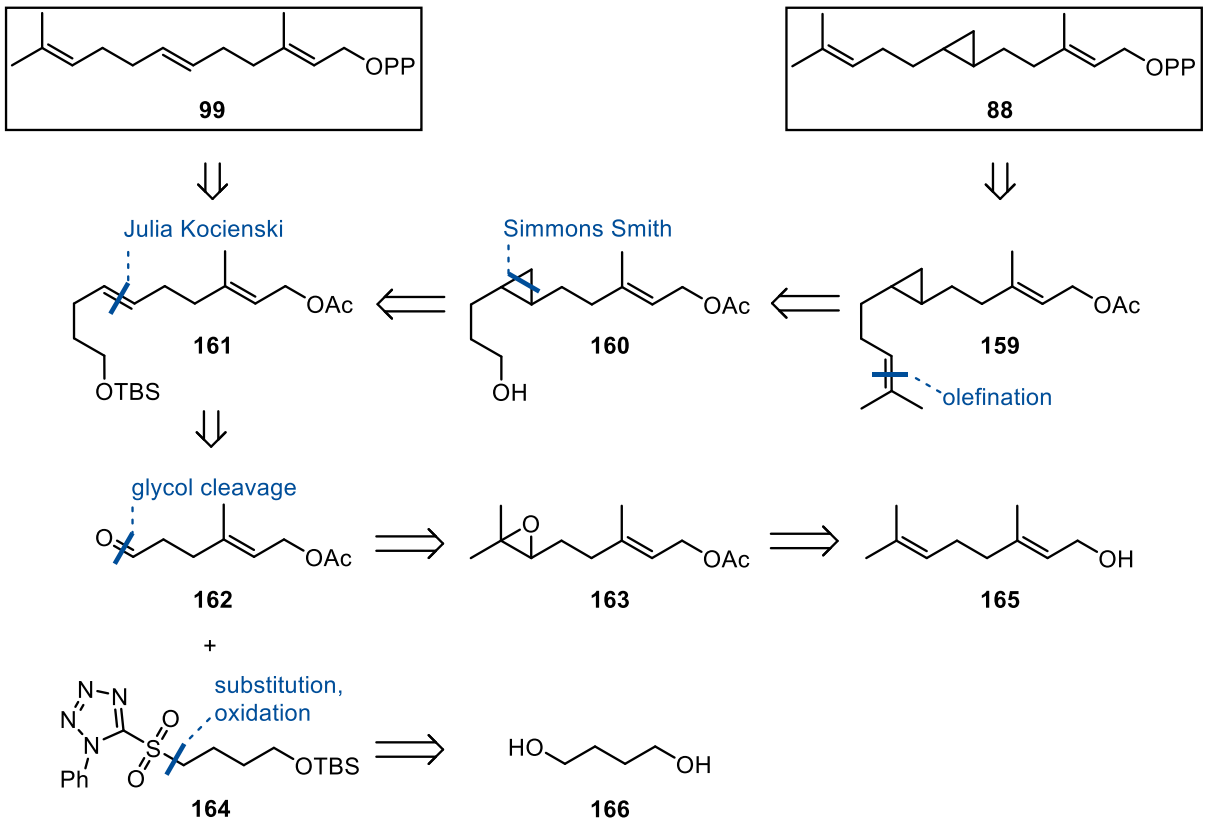


Entry	Catalyst	Conditions	Result
1	Grubbs I	neat, rt	only side products
2	Grubbs II	neat, rt	traces
3	Hoveyda-Grubbs	neat, rt	traces
4	Grubbs II	CH_2Cl_2, rt	traces

Scheme 25: HWE-olefination yielding **157** and deployed cross-coupling conditions for the synthesis of **156**. a) Ethyl 2-diethoxyphosphorylacetate, NaH, THF, 0 °C, 2 h *then* ketone, THF, 0 °C ⟶ rt, 20 h, 44 % (*E*:*Z* = 3.7:1).

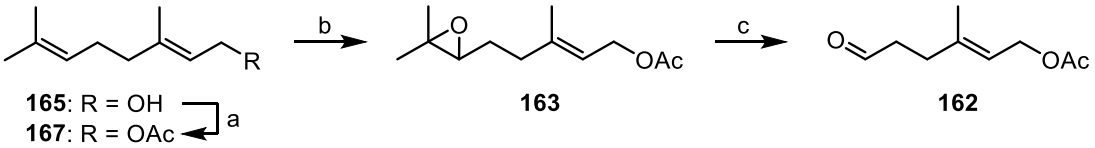
3.2.4 DESMETHYL FPP DERIVATIVE 99 AND FOURTH SYNTHESIS STRATEGY FOR THE PREPARATION OF CYCLOPROPYL DIPHOSPHATE 88

As previously discussed, a fourth synthetic strategy was pursued parallel to the third-generation route. Originally, the synthesis was planned to yield only the desmethyl FPP derivative **99**. Later, the route was extended to include the synthesis of cyclopropyl diphosphate **88** (Scheme 26). The key intermediate for the synthesis of both diphosphates is diene **161**. After desilylation and oxidation to the corresponding aldehyde, subsequent Wittig olefination and phosphorylation should deliver desmethyl FPP derivative **99**. The only modification for the synthesis of **88** is a Simmons-Smith cyclopropanation, which was planned to be performed before oxidation of the alcohol function. For the construction of the (*E*)-double bond of **161**, Julia-Kocienski reagent[79] **164** should be used, which was planned to be obtained from commercially available butane-1,4-diol (**166**). The first steps of this synthesis strategy were directed towards the preparation of aldehyde **162** starting from geraniol (**165**) (Scheme 26).



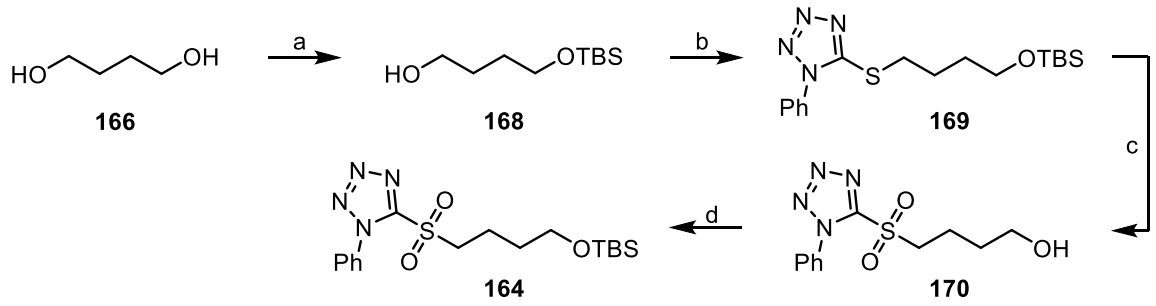
Scheme 26: Retrosynthetic approach towards the synthesis of desmethyl FPP derivative **99**. The route was later expanded to include the synthesis of cyclopropyl diphosphate **88**. For the construction of the carbon backbone, a regioselective Julia-Kocienski olefination was planned to be used as the key step.

The first step of this fourth-generation synthesis was the conversion of commercially available geraniol (**165**) to the corresponding acetate **167**. After chemoselective epoxidation with *m*CPBA, glycol cleavage of the resulting epoxide **163** using periodic acid successfully delivered aldehyde **162** (Scheme 27).[80]



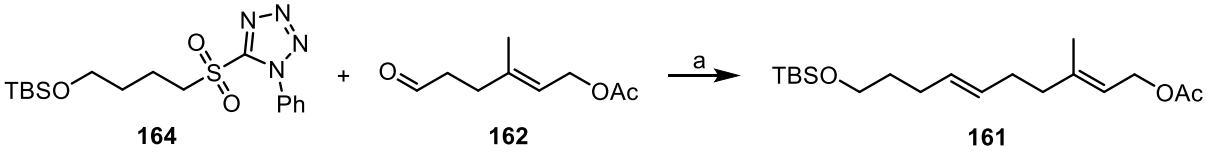
Scheme 27: Three-step synthesis of aldehyde **162** starting from commercially available geraniol (**165**). After acetylation, epoxidation, and subsequent glycol cleavage, the desired product was obtained. a) Ac_2O, 4-DMAP, Et_3N, CH_2Cl_2, 0 °C, 30 min, 98 %; b) *m*CPBA, CH_2Cl_2, 0 °C, 1.5 h, 54 %; c) H_5IO_6, THF/H_2O, 0 °C, 1 h, 54 %.

After obtaining aldehyde **162** as the western fragment for the planned olefination, Julia-Kocienski reagent **164** needed to be synthesized starting from commercially available butane-1,4-diol (**166**). After monosilylation of the diol, a tetrazole moiety was introduced via a Mitsunobu reaction to give **169**. Then, oxidation with H_2O_2 and ammonium heptamolybdate lead to the formation of alcohol **170**. Unfortunately, the acidic conditions used for the oxidation led to the cleavage of the silyl group. Therefore, it had to be reinstalled in the last step to obtain the Julia-Kocienski reagent **164** in four steps (Scheme 28).[79,81]



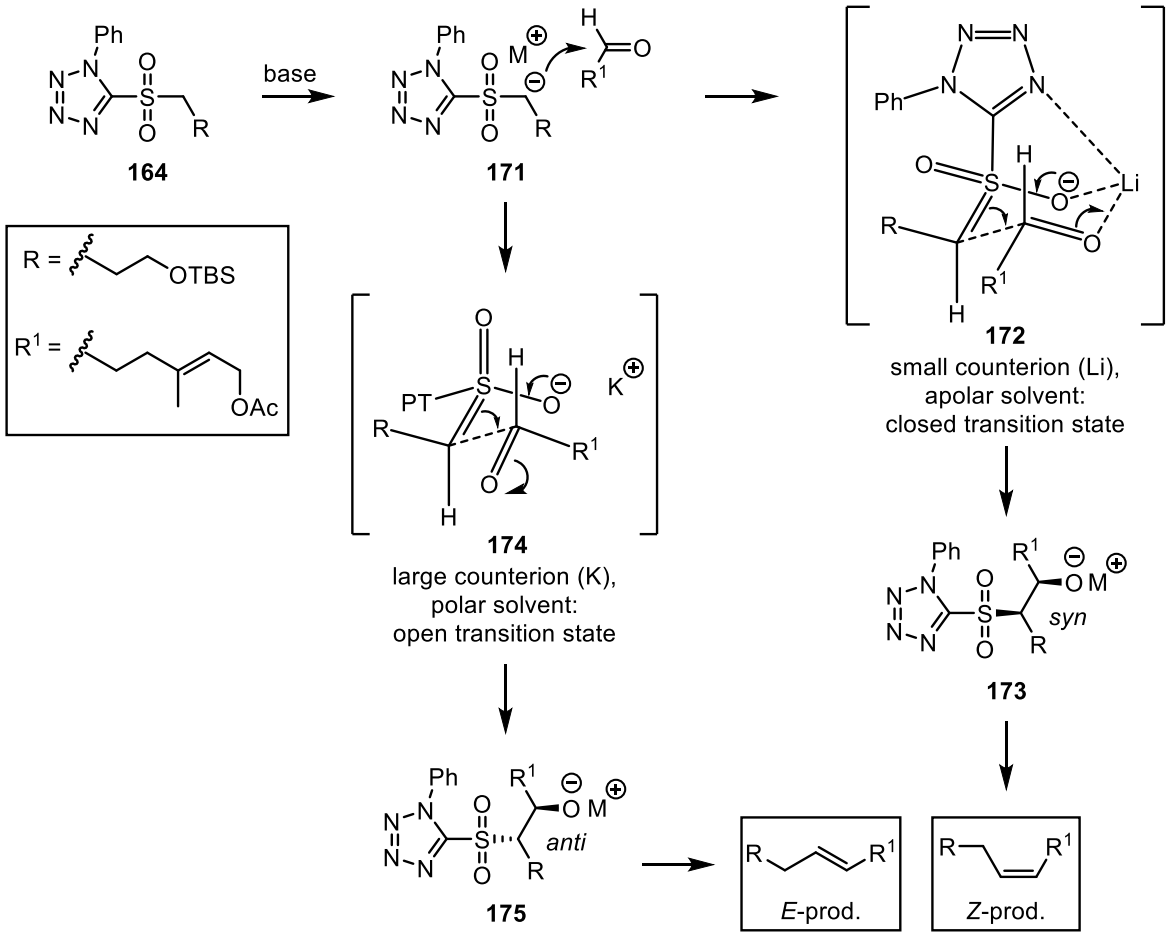
Scheme 28: Four-step synthesis of Julia-Kocienski reagent **164** starting from commercially available butane-1,4-diol (**166**). a) TBS-Cl, imidazole, CH_2Cl_2, 0 °C, 2 h, 94 %; b) DIAD, PPh_3, 1-phenyl-1*H*-tetrazole-5-thiol, THF, rt *then* **168**, THF, 0 °C → rt, 2 h, 86 %; c) $(NH_4)_6Mo_7O_{24}\times4$ H_2O, H_2O_2, EtOH, 0 °C → rt, 18 h, 84 %; d) TBS-Cl, imidazole, CH_2Cl_2, 0 °C, 16 h, quant. crude.

With both aldehyde **162** and tetrazole **164** in hand, the key (*E*)-selective olefination to construct the central olefinic double bond was carried out (Scheme 29).[82] Surprisingly, NMR analysis of the olefination product indicates the formation of only the desired isomer. One reason for this fortunate result could have been the choice of reaction parameters.



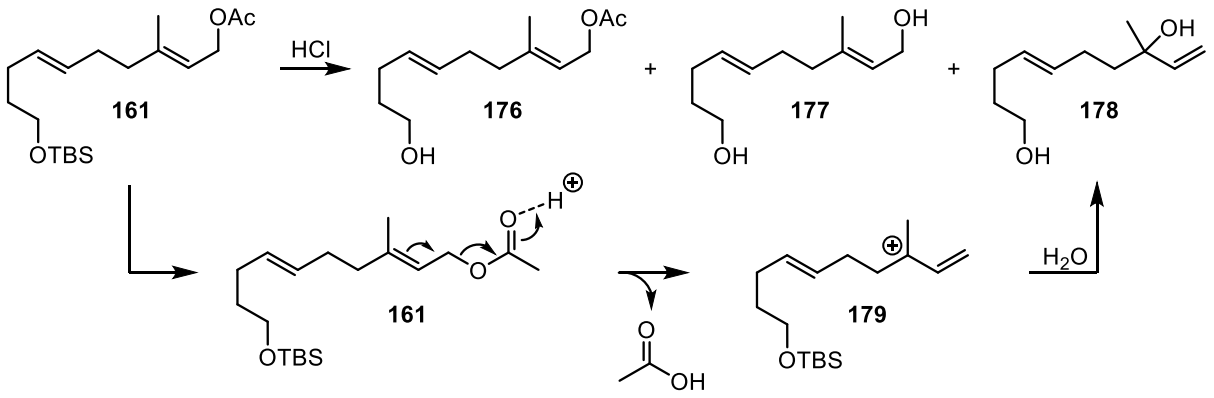
Scheme 29: Julia-Kocienski olefination as the key step towards the synthesis of pyrophosphates **99** and **88**. a) KHMDS, **164**, DME, -78 °C *then* **162**, DME, -78 °C → rt, 18 h, 57 %.

For the olefination, a bulky base with a large cation (K^+) and a polar solvent were chosen to favor the open transition state of the intermediate **174**, which in turn guides the selectivity towards the desired (*E*)-product (Scheme 30).[82,83]



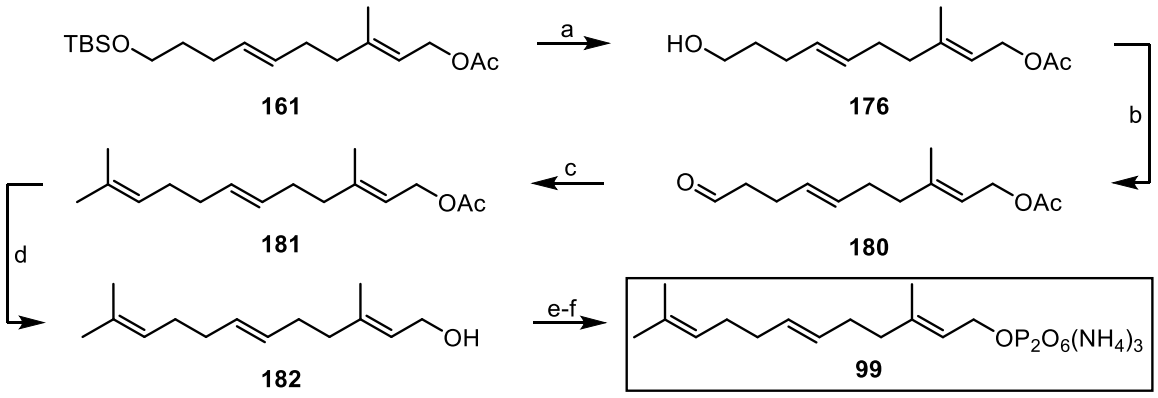
Scheme 30: Proposed mechanism for the Julia-Kocienski olefination. The generation of the regioisomers can be influenced by changing the reaction conditions such as the base or the solvent; PT = phenyl tetrazole.

With the successful synthesis of diene **161**, the next step was the removal of the silyl group. Initial experiments under acidic conditions resulted in mixtures with various products. After separation of the products by column chromatography, further analysis showed that two by-products were obtained in addition to the desired alcohol (**176**–**178**). A possible mechanism for the formation of these products is proposed below (Scheme 31).



Scheme 31: Acidic desilylation of **161** afforded a mixture of different alcohols (**176**–**178**). Proposed mechanism for the generation of **178**: After acidic activation of the acetyl group and subsequent elimination of acetic acid, the forming tertiary carbocation **179** is intercepted by water resulting in the tertiary alcohol **178**.

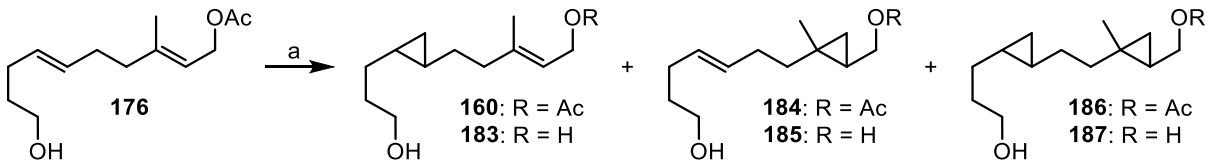
The results suggest that the acetyl group is too unstable under the acidic conditions employed. Therefore, desilylation was carried out using TBAF, which gave the desired alcohol **176** in quantitative yield (Scheme 32). With the preparation of alcohol **176**, the key intermediate for the synthesis of desmethyl FPP derivative **99** and cyclopropyl diphosphate **88** was obtained. Since the respective routes diverge from this point, the synthesis of **99** was continued first. Starting from alcohol **176**, triene **181** was obtained via DMP-oxidation followed by Wittig olefination. After subsequent basic deacetylation, the resulting allyl alcohol **182** was phosphorylated in two steps to obtain the desired diphosphate **99** (Scheme 32).



Scheme 32: Final steps in the synthesis of desmethyl diphosphate **99** starting from olefination product **161**. a) TBAF, THF, rt, 16 h, quant.; b) DMP, CH_2Cl_2, rt, 2.5 h; c) *n*-BuLi, *iso*propyltriphenylphosphonium iodide, Et_2O, 0 °C ⟶ rt, 1.5 h *then* **180** in Et_2O, rt, 70 min; d) KOH, THF/MeOH, rt, 18 h, 30 % o4s; e) DMS, NCS, CH_2Cl_2, -30 °C, 20 min *then* **182**, CH_2Cl_2, -40 °C ⟶ rt, 18 h; f) $P_2O_7H(nBu_4N)_3$ (**114**), CH_3CN, rt, 16 h. *then* DOWEX ion-exchange column, quant. o2s.

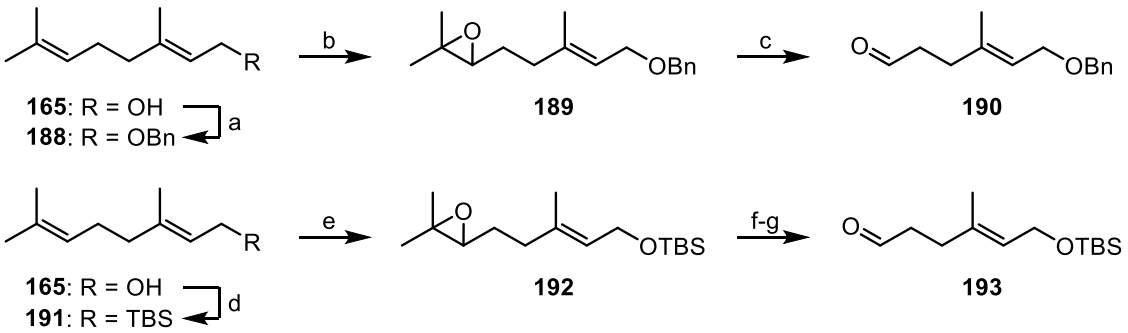
With the successful synthesis of desmethyl FPP derivative **99**, the focus was shifted to the remaining steps to synthesize cyclopropyl diphosphate **88**. Starting from alcohol **176**, several Simmons-Smith cyclopropanation reactions were performed. Unfortunately, the efforts to introduce the key cyclopropyl moiety remained fruitless. NMR analysis of the crude mixtures

showed cyclopropanation of the allylic double bond and di-cyclopropane products, indicating acetate cleavage under the conditions used (Scheme 33).



Scheme 33: Efforts to install a cyclopropyl group to alcohol **176** via Simmons-Smith cyclopropanation resulted in mixtures of cyclopropanated products (**160**, **183**–**187**). a) Et_2Zn, TFA, CH_2Cl_2, 0 °C, 30 min *then* CH_2I_2, CH_2Cl_2, 0 °C, 30 h *then* **176**, CH_2Cl_2, 0 °C ⟶ rt, 1 h.

Therefore, it was decided to synthesize the allylic western fragment with alternative protecting groups. Analogous to the synthesis of **162**, aldehydes **190** and **193** were prepared in three steps starting from geraniol (**165**) (Scheme 34).[84–86]

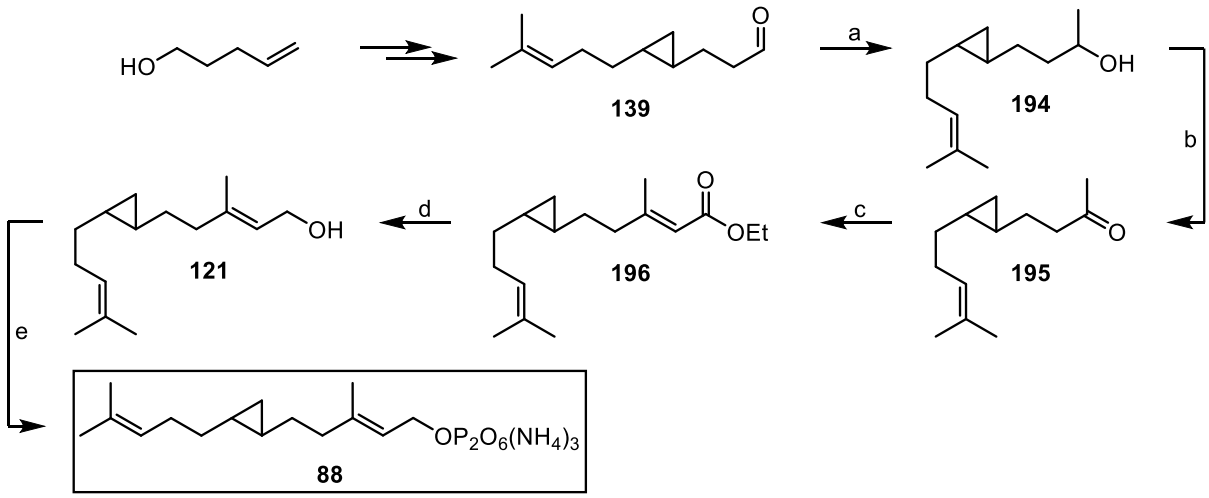


Scheme 34: Synthesis of the aldehydes **190** and **193** starting from geraniol (**165**). For the three-step syntheses, conditions analogous to those described above were used. Only minor adjustments were made depending on the protecting group used. a) NaH, THF, 0 °C, 20 min *then* BnBr, THF, 0 °C ⟶ rt, 1 h *then* reflux, 5 h, 82 %; b) *m*CPBA, CH_2Cl_2, 0 °C, 3.5 h, 79 %; c) H_5IO_6, THF/Et_2O, 0 °C, 1 h, 29 %; d) TBS-Cl, imidazole, CH_2Cl_2, rt, 3.5 h, 99 %; e) *m*CPBA, CH_2Cl_2, 0 °C, 3 h, 69 %; f) H_5IO_6, THF/H_2O, 0 °C, 1 h; g) TBS-Cl, imidazole, CH_2Cl_2, 0 °C ⟶ rt, 45 min, 53 % o2s.

After the preparation of the desired aldehydes (**190** and **193**), the respective elongation steps via Julia-Kocienski olefination were planned to be performed. However, before these reactions were initialized, the desired cyclopropyl diphosphate **88** could be obtained via a variation of the second-generation synthesis approach.

3.2.5 FINAL SYNTHESIS STRATEGY FOR THE PREPARATION OF 88

The synthetic strategy that finally provided the desired cyclopropyl diphosphate **88** is a modified version of the second-generation approach. Since this failed only in the final steps, no alterations were made until the synthesis of aldehyde **139**. From here, it has not been possible to obtain the desired diphosphate by homologation and carboalumination. Therefore, it was decided to only modify these last reaction steps. For the elongation of the carbon backbone, aldehyde **139** was first converted into the corresponding methyl ketone **195** by Grignard addition followed by oxidation. The resulting ketone was then converted into ester **196** using a standard HWE protocol. After reduction of the resulting ester to the corresponding alcohol **121**, cyclopropyl diphosphate **88** was obtained through phosphorylation with bis-triethylammonium phosphate (TEAP) (Scheme 35).[87]

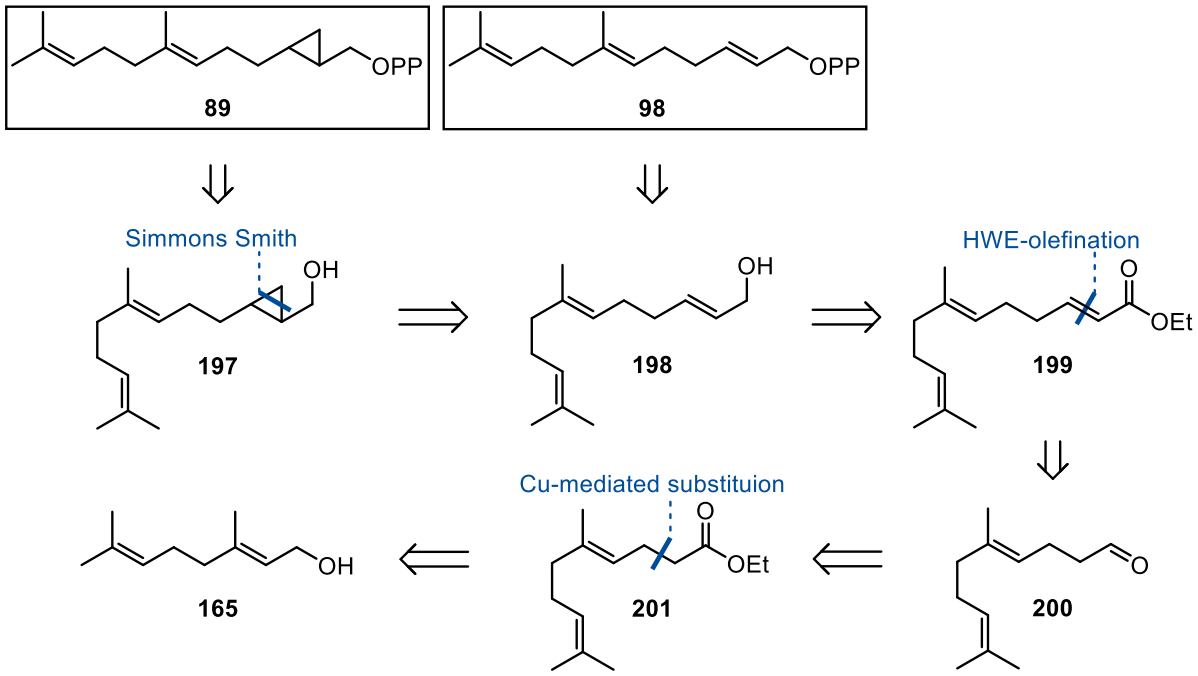


Scheme 35: Final steps in the synthesis of cyclopropyl diphosphate **88** starting from aldehyde **139**, which was prepared according to the second-generation synthesis strategy. a) CH_3MgBr, Et_2O, 0 °C, 1.5 h; b) DMP, CH_2Cl_2, rt, 2 h, 64 % o2s; c) Triethyl phosphonoacetate, NaH, THF, 0 °C, 2 h *then* **194** in THF, 0 °C ⟶ rt, 24 h, quant. (*E*:*Z* = 4.6:1); d) DIBAL-H, Et_2O, 0 °C, 3 h, 66 %; e) Cl_3CCN, TEAP, CH_3CN, rt, 15 min, 62 %.

3.3 DESMETHYL FPP DERIVATIVE 98 AND CYCLOPROPYL DIPHOSPHATES 89S/R AND 90S/R

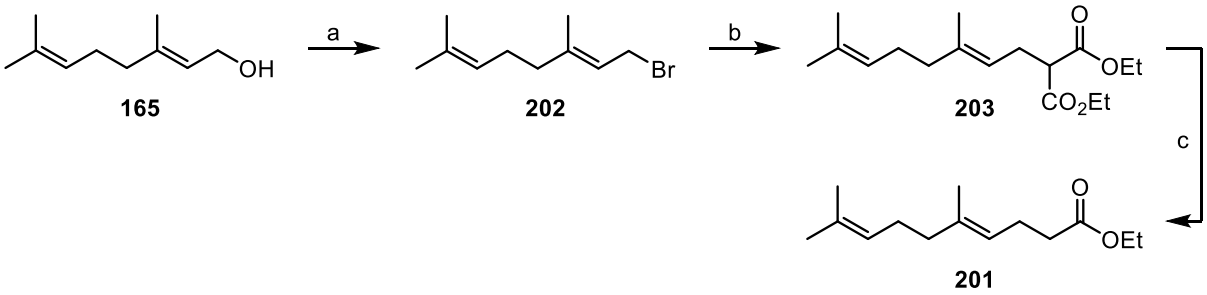
3.3.1 ALCOHOL 198 AS THE KEY INTERMEDIATE FOR 98 AND 89

Both pyrophosphates **98**, as well as **89**, were planned to be synthesized using alcohol **198** as the key intermediate. The carbon backbone of the desired products was intended to be constructed starting from commercially available geraniol (**165**) through a combination of homologation and olefination reactions (Scheme 36).



Scheme 36: Retrosynthetic approach towards the synthesis of pyrophosphates **98** and **89**. An HWE-olefination and an organocopper mediated substitution were planned to serve as the key elongation reactions.

The first step of the envisioned synthesis was the conversion of geraniol (**165**) into the corresponding bromide **202** under Appel conditions. Subsequently, the carbon chain was extended via S_N2 reaction with diethyl malonate serving as the nucleophile. The obtained diester **203** was then stirred under refluxing conditions to achieve decarboxylation leading to the desired ester **201** (Scheme 37).[55,88]

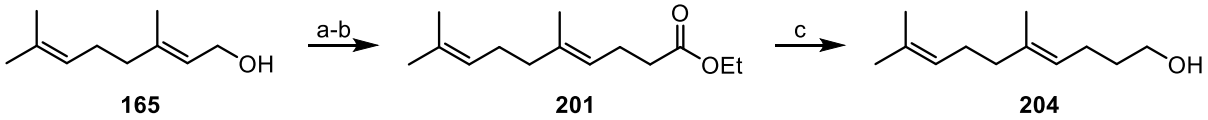


Scheme 37: Bromination of geraniol (**165**) via an Appel reaction followed by substitution and decarboxylation for the elongation of the carbon chain. a) PBr_3, THF, 0 °C, 45 min; b) diethyl malonate, K_2CO_3, DMF, rt, 20 h; c) LiCl, H_2O, DMSO, 160 °C, 20 h, 26 % o3s.

Even though the implemented sequence of substitution and decarboxylation did deliver the desired ester **201**, the overall yield had to substantially improve. It was therefore decided to search for an alternative synthesis route. The demonstrated extension reactions were replaced by a procedure reported by Dickschat and co-workers.[87]

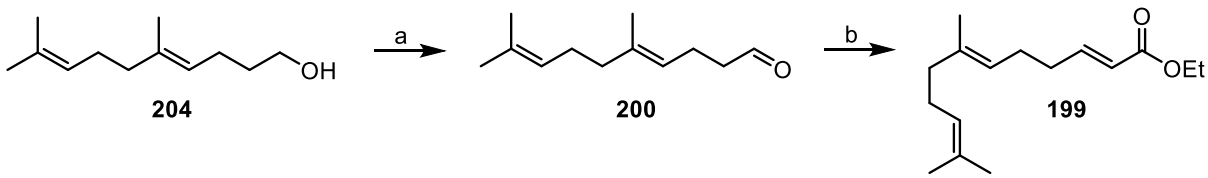
Geraniol (**165**) was first converted to the corresponding bromide **202**. Then, copper-mediated deprotonation of ethyl acetate with *in situ* generated LDA led to the formation of a nucleophilic

copper enolate. Reaction with bromide **202** resulted in elongation of the carbon skeleton through substitution. After subsequent reduction with DIBAL-H, alcohol **204** was obtained in three steps (Scheme 38).



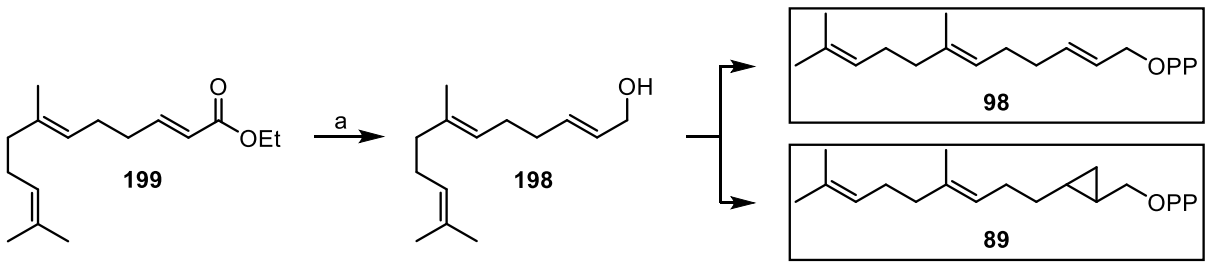
Scheme 38: Preparation of alcohol **204** via bromination, copper-mediated elongation, and reduction. a) PBr_3, THF, 0 °C, 45 min; b) DIPA, *n*-BuLi, THF, 0 °C, 1 h *then* ethyl acetate, CuI, THF, -110 °C ⟶ -50 °C, 1.5 h *then* **202**, THF**,** -30 °C, 2 h; c) DIBAL-H, Et_2O, -78 °C ⟶ rt, 18 h, 41 % o3s.

After the subsequent oxidation of alcohol **204** under Swern conditions, the obtained volatile aldehyde **200** was immediately converted to ester **199** by Wittig olefination (Scheme 39).



Scheme 39: Swern oxidation of alcohol **204** and subsequent Wittig reaction to obtain ester **199**. a) $(COCl)_2$, DMSO, CH_2Cl_2, -78 °C, 1.5 h *then* **204**, CH_2Cl_2, -78 °C, 2 h *then* Et_3N, -78 °C ⟶ rt, 14 h, 80 %; b) Ph_3PCHCO_2Et, toluene, rt, 18 h, 67 %.

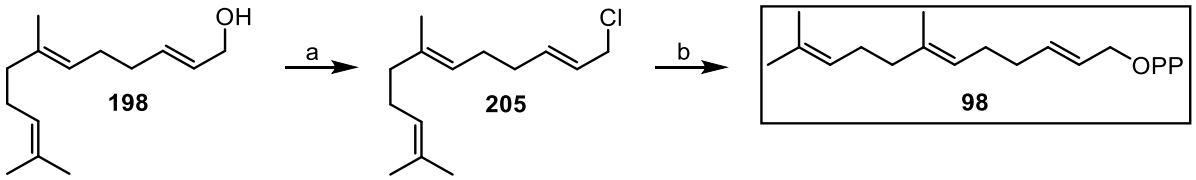
Ester **199** was then reduced with DIBAL-H to afford the desired key intermediate **198**. From here, the allyl alcohol could either be converted directly into the corresponding pyrophosphate **98** or first undergo cyclopropanation and subsequent pyrophosphorylation to give **89** (Scheme 40).[55]



Scheme 40: DIBAL-H reduction of ester **199** to obtain alcohol **198** and diverging routes towards the synthesis of FPP derivatives **98** and **89**. a) DIBAL-H, Et_2O, -78 °C, 18 h, 71 %.

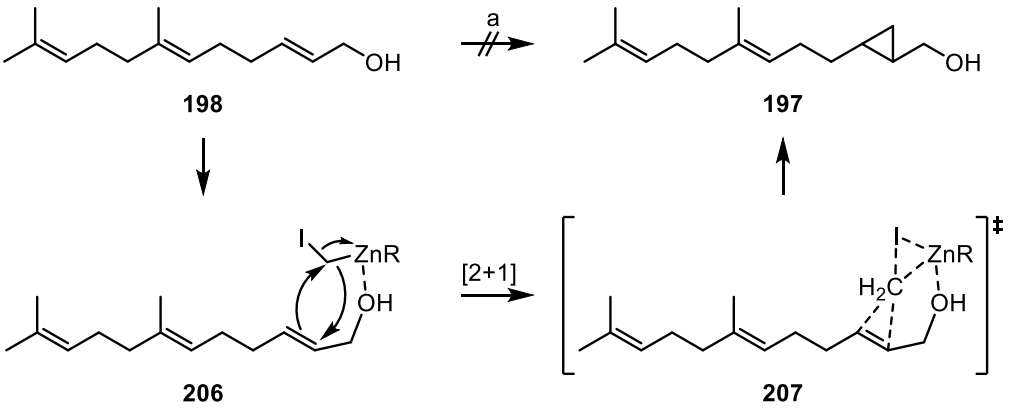
3.3.2 DESMETHYL FPP DERIVATIVE 98 STARTING FROM ALCOHOL 198

With alcohol **198** in hand, the next step was its conversion to the corresponding diphosphate. After halogenation of the allyl alcohol, the resulting chloride **205** was transformed into the unnatural desmethyl pyrophosphate **98** under the established conditions (Scheme 41).[56,60]



Scheme 41: Final steps in the synthesis of desmethyl pyrophosphate **98**. Halogenation and subsequent substitution using pyrophosphate salt **114**. a) NCS, DMS, CH_2Cl_2, -30 °C ⟶ 0 °C, 20 min *then* **198**, CH_2Cl_2, -40 °C ⟶ rt, 2 h; b) $P_2O_7H(nBu_4N)_3$ (**114**), CH_3CN, rt, 16 h *then* DOWEX ion exchange column, quant. o2s.

With the successful synthesis of desmethyl pyrophosphate **98**, the next goal was the preparation of cyclopropyl diphosphate **89**. For this purpose, a cyclopropane moiety needs to be introduced. Since choosing the correct reaction conditions was the priority at this stage, stereocontrol was not further considered. The Shi variation of the Simmons-Smith cyclopropanation reaction was used to convert allyl alcohol **198** into the corresponding cyclopropyl alcohol. Theoretically, the hydroxy group should act as a directing group guiding the organozinc species towards the allylic double bond (**206**–**207**), resulting in chemoselective cyclopropanation (Scheme 42).[61]

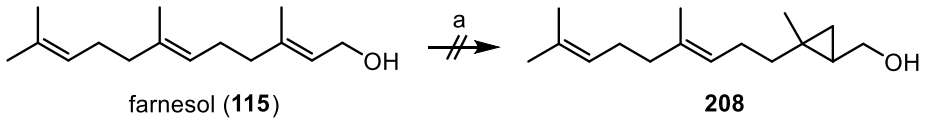


Scheme 42: Proposed mechanism for the substrate-directed chemoselective Simmons-Smith cyclopropanation of alcohol **198**. a) Et_2Zn, TFA, CH_2Cl_2, 0 °C, 20 min *then* CH_2I_2, CH_2Cl_2, 0 °C, 20 min *then* **198**, CH_2Cl_2, 0 °C ⟶ rt, 4 h; R = CO_2CF_3.

Unfortunately, the first cyclopropanation test reactions failed to yield any product. Therefore, further experiments were carried out with commercially available farnesol (**115**) in order to conserve the limited amount of alcohol **198** available.

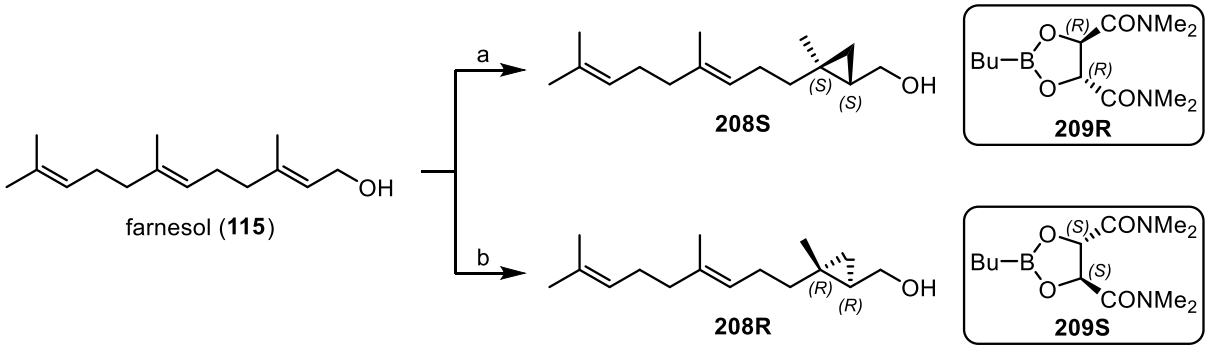
3.3.3 SIMMONS-SMITH TEST REACTIONS WITH FARNESOL

Since the last attempt at cyclopropanation with a mild variant of the Simmons-Smith reaction was unsuccessful, more forcing conditions were next tested on the test substrate farnesol (**115**). Both the equivalents of the highly reactive reactants, as well as the reaction time were significantly increased.



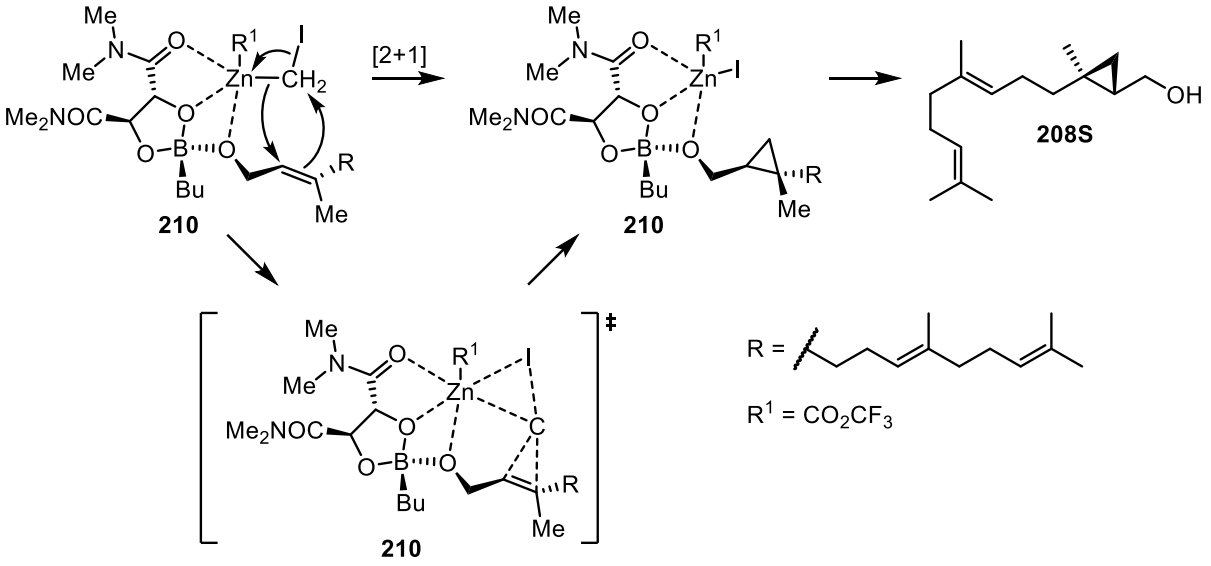
Scheme 43: Cyclopropanation test reaction of farnesol (**115**) using more aggressive Simmons-Smith conditions a) Et_2Zn, TFA, CH_2Cl_2, 0 °C, 1 h *then* CH_2I_2, CH_2Cl_2, 0 °C, 1 h *then* **115**, CH_2Cl_2, 0 °C ⟶ rt, 16 h.

Unfortunately, even these reaction conditions did not produce the desired results. After further experiments with varying reaction conditions remained unsuccessful, the initial idea of racemic cyclopropanation was skipped and asymmetric transformations were directly attempted. Following a method published by Charette and co-workers, stereoselective cyclopropanation reactions using chiral organoboron ligands were carried out (Scheme 44).[89,90]



Scheme 44: Asymmetric Simmons-Smith cyclopropanations of farnesol (**115**) using organoboron ligands. a) Et_2Zn, DME, CH_2I_2, CH_2Cl_2, 0 °C, 30 min *then* **115**, **209R**, CH_2Cl_2, 0 °C ⟶ rt, 18 h, quant >92 % *ee*; b) Et_2Zn, DME, CH_2I_2, CH_2Cl_2, 0 °C, 30 min *then* **115**, **209S**, CH_2Cl_2, 0 °C ⟶ rt, 18 h, 94 %, 94 % *ee*.

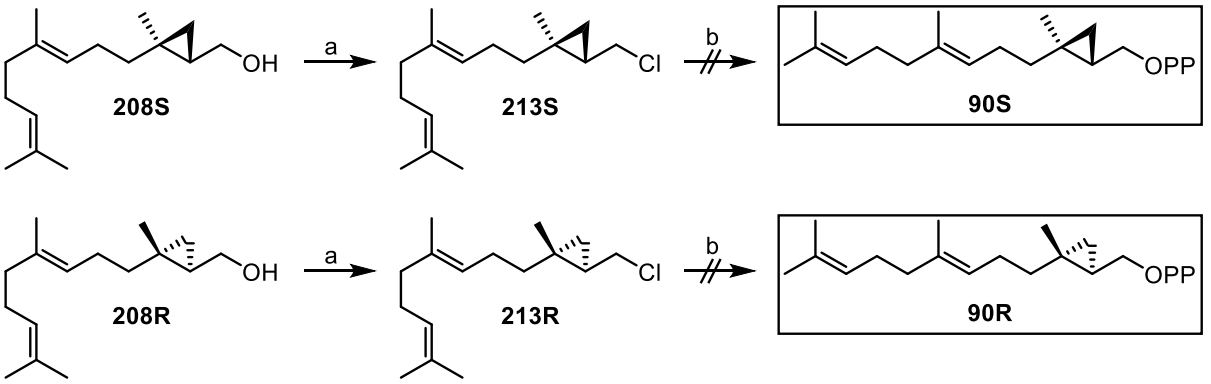
The use of the boron ligands resulted not only in the formation of the desired cyclopropanes **208R** and **208S**, but the protocol also provided excellent stereocontrol as determined by chiral GC. The results suggest, that the utilization of the chiral ligands increases the reactivity of the organometal complex. A plausible mechanism for the asymmetric nature of this reaction is presented below (Scheme 45).[89,91]



Scheme 45: Proposed mechanism for the asymmetric Simmons-Smith reaction with a chiral boron ligand. Usage of the (*R*)-ligand (**209R**) leads to the formation of the (*S*)-product and *vice versa*.

In general, the asymmetric Simmons-Smith reaction is a cyclopropanation in which a Zn-carbenoid reacts with a double bond to form a cyclopropane with high facial selectivity. Utilizing different chiral ligands, the stereoselectivity of the reaction can be directed. Initially, two oxygen atoms of the dioxaborolane ligand, as well as the hydroxy group of the alkene, coordinate to the Zn atom. This guides the active Zn-species into a position inside the complex, in which the formation of one stereoisomer via [2+1] addition is preferred (Scheme 45).[91]

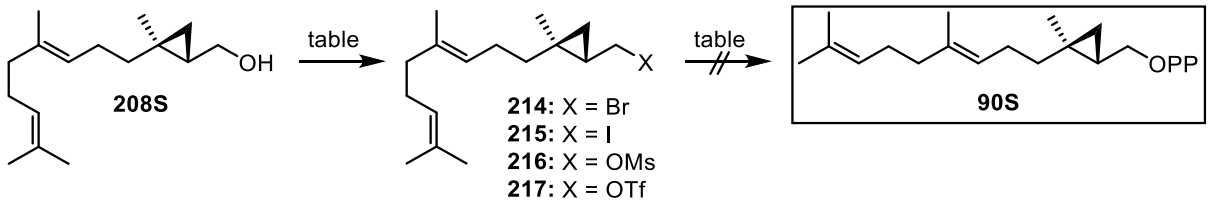
With both isomers of alcohol **208** in hand, halogenation and subsequent transformation into the corresponding pyrophosphates (**90S, 90R)** were conducted (Scheme 46).



Scheme 46: Successful halogenation of **208S** and **208R** and subsequent phosphorylations that yielded no products. a) DMS, NCS, CH_2Cl_2, -30 °C, 20 min *then* alcohol, CH_2Cl_2, -40 °C ⟶ rt, 2 h; b) $P_2O_7H(nBu_4N)_3$ (**114**), CH_3CN, rt, 16 h.

After the successful conversion of alcohols **208S** and **208R** to their corresponding chlorides, the final pyrophosphorylation step failed to yield any product. The deployed chlorides were fully recovered, which suggests that no reaction occurred. The results point to the fact that chlorides **213R** and **213S** were too unreactive towards the bulky nucleophile. Therefore, the

hydroxy group of **208S** was substituted with better leaving groups. It was hypothesized that the increased reactivity of the corresponding intermediates could lead to the formation of the desired pyrophosphate **90** (Scheme 47).



Entry	Conditions (rt + 60 °C)	Intermediate	Result phosphorylation[A]
1	PPh_3, CBr_4, CH_2Cl_2, rt, 30 min	**214**	no rct., **214** recovered
2	PPh_3, imidazole, I_2, CH_2Cl_2, 0 °C, 1 h	**215**	no rct., **215** recovered
3	MsCl, Et_3N, DMF, 0 °C, 1.5 h	**216**	no rct., **216** recovered
4	Tf_2O, DIPEA, CH_2Cl_2, 0 °C, 1 h	**217**	no rct., **217** recovered

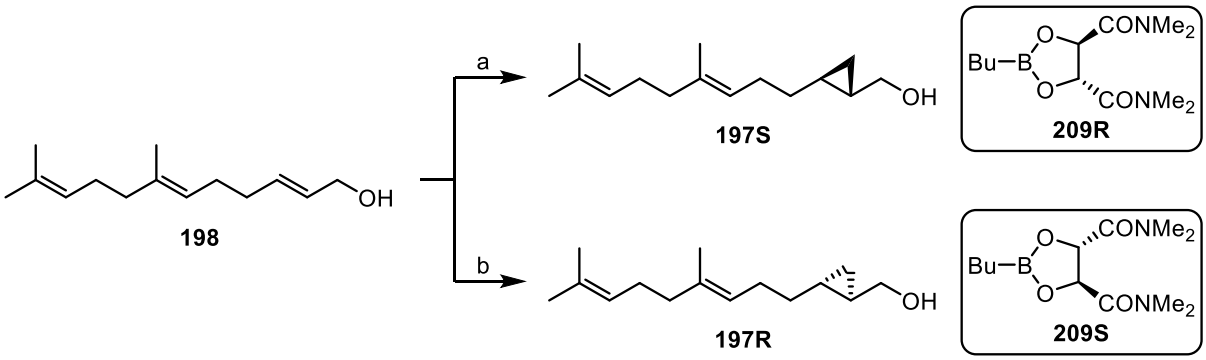
Scheme 47: Deployed conditions for the introduction of various leaving groups to alcohol **208S** and subsequent diphosphorylation. All intermediates were used in their crude form without further purification. Alcohol **208S** was chosen over the other isomer because the respective ligand required for the production was more inexpensive. A) Conditions phosphorylation: $P_2O_7H(nBu_4N)_3$ (**114**), CH_3CN, rt, 16 h.

All transformations of alcohol **208S** successfully delivered the corresponding products (**214**–**217**), as judged by NMR spectroscopy and TLC of the crude mixture. However, it was not possible to convert any of the intermediates into the desired cyclopropyl diphosphate **90**. Further increasing reaction times and temperatures did not lead to any improvements (Scheme 47).

A plausible explanation for the difficulties in the final substitution could be the steric hindrance caused by the cyclopropane ring. Since all previous substitutions with alcohol **208** proceeded without complications, the substrate-induced steric hindrance can only be part of the problem. Therefore, it is assumed that the problems associated with the last step are caused by the lack of reactivity due to the steric hindrance of the cyclopropane ring and the bulkiness of the nucleophile.

3.3.4 FINAL STEPS IN THE SYNTHESIS OF 89 STARTING FROM ALCOHOL 198

Since the applied reaction conditions for the asymmetric Simmons-Smith reaction of farnesol (**115**) delivered satisfying results, they were adopted for the cyclopropanation of alcohol **198**. Utilizing the reported ligands, both isomers of **197** were successfully obtained in good yields (Scheme 48).



Scheme 48: Stereoselective cyclopropanation of **198** using the presented asymmetric Simmons-Smith reaction. a) Et_2Zn, DME, CH_2I_2, CH_2Cl_2, 0 °C, 30 min *then* **198**, **209R**, CH_2Cl_2, 0 °C ⟶ rt, 18 h, 89 %; b) Et_2Zn, DME, CH_2I_2, CH_2Cl_2, 0 °C, 30 min *then* **198**, **209S**, CH_2Cl_2, 0 °C ⟶ rt, 18 h, 73 %.

Although the challenges of pyrophosphorylation in the presence of an adjacent cyclopropyl group were known at this point, the final steps to obtain pyrophosphates **89S** and **89R** were still carried out (Scheme 49). This was done because of assumptions about the reactivity of **218**, which were based on structural calculations. Comparing the calculated 3D structures of chlorides **218R** and **213R**, it was assumed that **218R** induces less substrate-driven steric hindrance due to the missing methyl group in the γ-position to the leaving group. Since S_N2-type nucleophilic attacks always occur from the backside of the leaving group, the missing methyl group could lead to increased reactivity of **218** compared to **213R** (Figure 7).

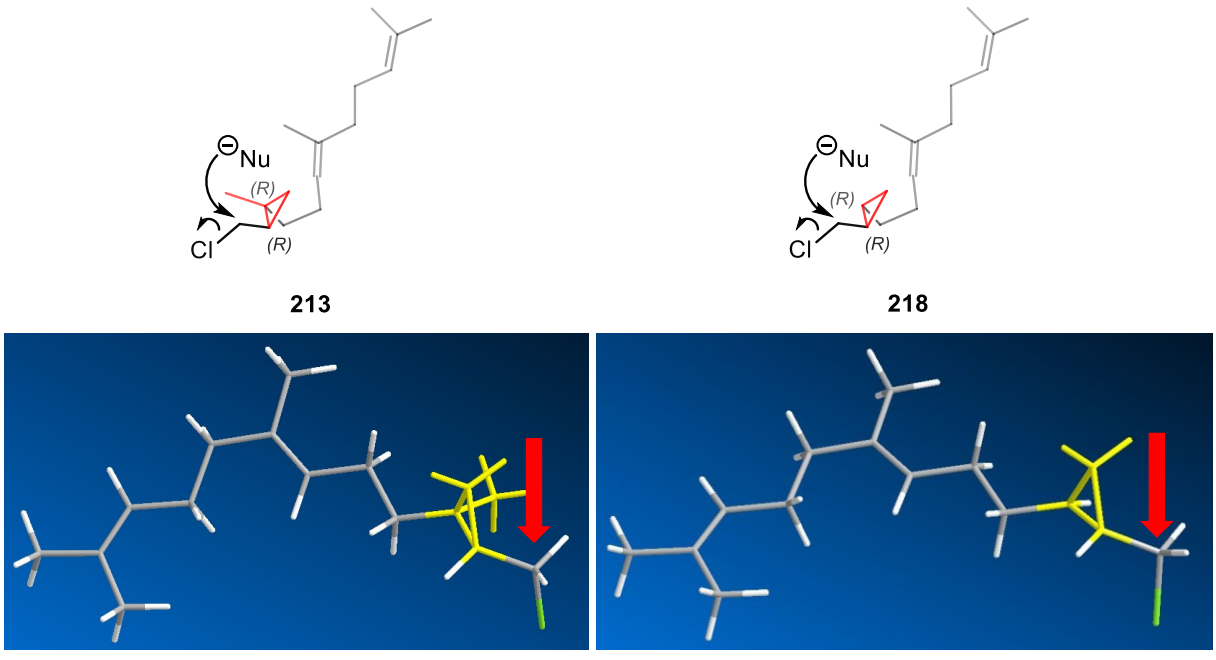
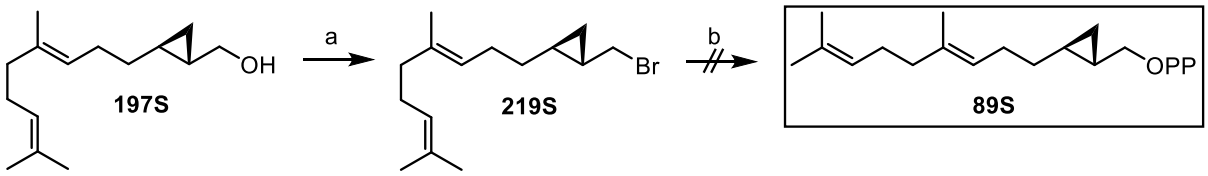


Figure 7: 3D structures of **213R** and **218R** illustrated with CHEMDRAW (top) and CHEMDRAW3D (bottom) by PERKINELMER. For the structures on the bottom, the local energy minima were calculated. Atoms and bonds likely to cause steric hindrance are highlighted in red (top) and yellow (bottom). Red arrow: assumed attack trajectory of the bulky nucleophile.

Unfortunately, the results did not confirm the postulated theories based on the shown structural calculations. Even at elevated temperatures and longer reaction times, bromide **219S** was too inactive to produce the desired pyrophosphate **89S**.



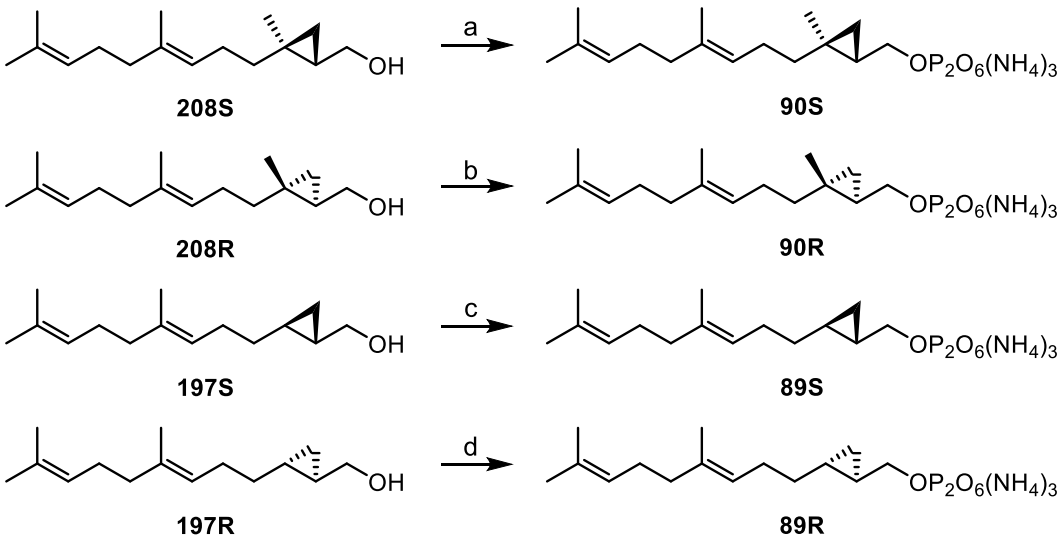
Scheme 49: Final steps towards the synthesis of **89S**. Successful halogenation to obtain **218S** and subsequent pyrophosphorylation. a) PPh_3, CBr_4, CH_2Cl_2, rt, 1 h; b) $P_2O_7H(nBu_4N)_3$ (**114**), CH_3CN, rt, 16 h.

Both the syntheses of **90S** and **90R** and those of **89S** and **89R** failed at the last phosphorylation step. Therefore an alternative phosphorylation strategy was pursued.

3.3.5 ALTERNATIVE PHOSPHORYLATION PROCEDURE FOR 89S/R AND 90S/R

The alternative method for phosphorylation utilizes *in situ* generated bis-triethylammonium phosphate (TEAP) as the active phosphorylation reagent.[92] This method has been considered inferior to the previously used method by Poulter[56,60] because diphosphates are not selectively produced. It does have the advantage of phosphorylating alcohols in one step, but mono- and triphosphates are produced alongside the desired diphosphates, resulting in a lower overall yield. In addition, the method calls for the use of a highly polar solvent mixture for column chromatographic separation of the obtained phosphates. Since initial isolation experiments with this solvent in the past did not allow separation of the phosphates used, this method was not considered viable at first.

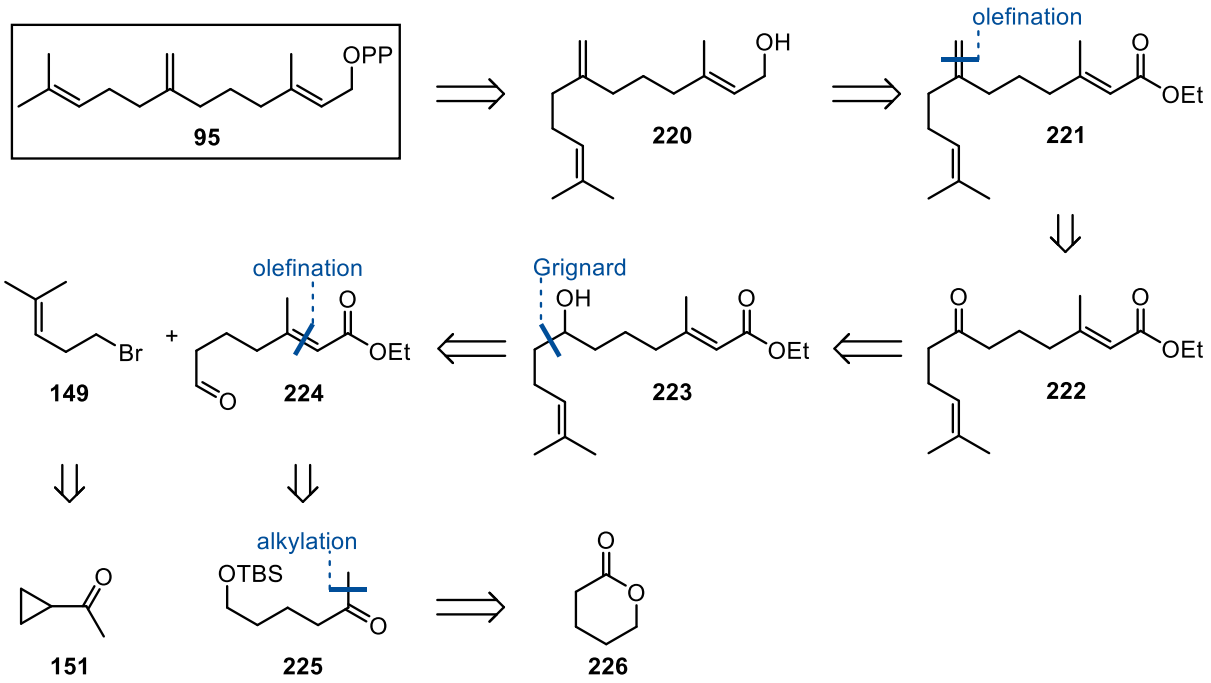
However, despite extensive optimizations to convert cyclopropyl alcohols **197S**, **197R**, **208S**, and **208R** into the corresponding diphosphates, these could not be obtained. Thus, the TEAP method was re-explored. The respective allyl alcohols were first activated with trichloroacetonitrile and then treated with freshly prepared TEAP. After subsequent column chromatographic purification, all four cyclopropyl alcohols employed were converted and the corresponding diphosphates could be collected (Scheme 50).



Scheme 50: Alternative method for the one-step phosphorylation of four cyclopropyl alcohols. a-d) Cl_3CCN, TEAP, CH_3CN, rt, 15 min, 43 % (a), 49 % (b), 24 % (c), 34 % (d).

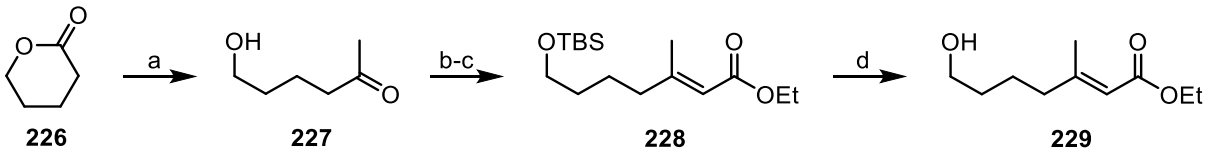
3.4 DOUBLE BOND SHIFTED FPP DERIVATIVE 95

Unlike the previous strategies, which focused mainly on the synthesis of desmethyl and cyclopropyl FPP derivatives, the latest strategy aims for the generation of derivatives with shifted double bonds. The first targeted structure (**95**) features a shifted double bond in the central position compared to farnesol (**115**). The terminal alkene was planned to be obtained from ketone **222** via Wittig or Tebbe olefination. The key step for constructing the carbon backbone was envisioned to be a Grignard reaction between aldehyde **224** and bromide **149**. Aldehyde **224** should be accessible from commercially available tetrahydro-2*H*-pyran-2-one (**226**) via alkylation of the lactone and (*E*)-selective HWE olefination (Scheme 51).



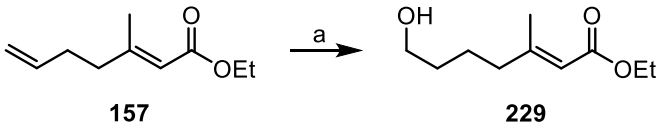
Scheme 51: Retrosynthetic approach towards the synthesis of alkene **95**. A Grignard reaction was planned to be utilized as the key reaction for the construction of the carbon backbone.

The first steps of the synthesis were directed towards the generation of alcohol **229**. After nucleophilic lactone opening using MeMgBr,[93] silylation of the formed alcohol, and subsequent HWE olefination yielded ester **228**.[78] Final desilylation under acidic conditions delivered the desired alcohol **229** in four steps (Scheme 52).



Scheme 52: Four-step synthesis of alcohol **229** starting from tetrahydro-2*H*-pyran-2one (**226**). a) MeLi, Et_2O, -78 °C, 2 h; b) TBS-Cl, imidazole, CH_2Cl_2, 0 °C ⟶ rt, 1.5 h, 97 % o2s; c) Ethyl 2-diethoxyphosphorylacetate, NaH, THF, 0 °C, 2 h *then* ketone, THF, 0 °C ⟶ rt, 48 h, 35 %, 85 % *brsm*, (*E*:*Z* = 3:1); d) conc. HCl, THF, rt, 74 %.

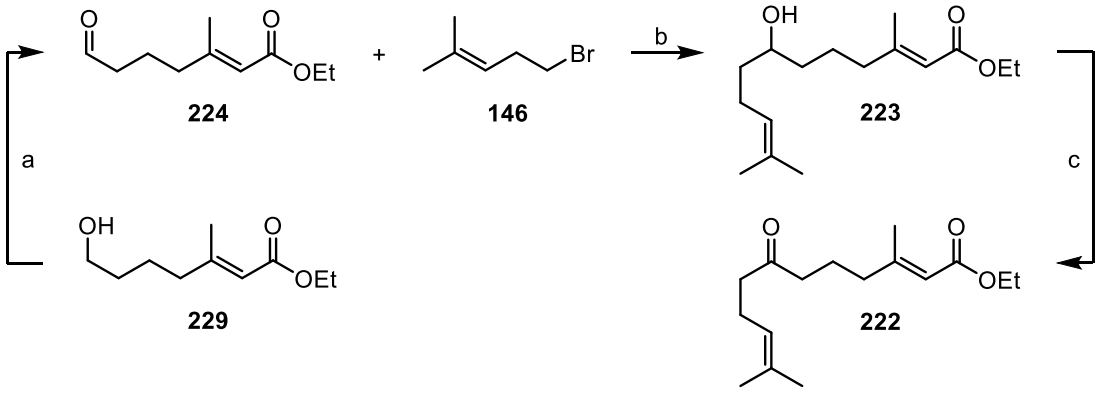
Alternatively, alcohol **229** was afforded in one hydroboration step starting from alkene **157**, which was synthesized as an intermediate during the third-generation synthesis approach towards the preparation of cyclopropyl diphosphate **88** (Scheme 53). This approach is a shorter and more efficient alternative for obtaining **229** compared to the four-step synthesis described above. [94]



157 229

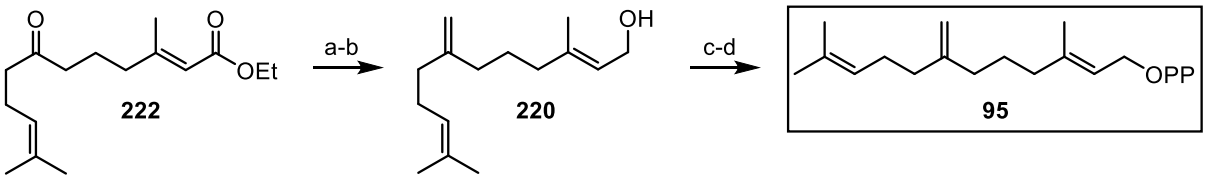
Scheme 53: Synthetic access to alcohol **229** in a single hydroboration step starting from alkene **157**. a) 9-BBN, H_2O_2, $NaHCO_3$, THF, 0 °C ⟶ rt, 61 %.

After the successful preparation of alcohol **229**, it was transformed into the corresponding aldehyde (**224**) under Swern conditions. With both aldehyde **224** and bromide **149** in hand, the fragments were connected after the bromide **146** was converted into a Grignard reagent. The magnesium employed had to be activated using a catalytic amount of iodine. Surprisingly, the formation of the desired product could not be detected by thin layer chromatography. The newly formed alcohol (**223**) was directly oxidized under Swern conditions, which finally delivered the desired ketone **222** (Scheme 54).



Scheme 54: Swern oxidation of alcohol **229** and subsequent coupling of the generated aldehyde **224** and bromide **149** under Grignard conditions as the key step for constructing the carbon backbone of **95**. a) $(COCl)_2$, DMSO, CH_2Cl_2, -78 °C, 30 min *then* **229**, CH_2Cl_2, -78 °C, 30 min *then* Et_3N, -78 °C ⟶ rt, 18 h, quant b) Mg, I_2, Et_2O, rt, 30 min *then* **149**, Et_2O, rt, 2 h *then* **224**, Et_2O, 0 °C ⟶ rt, 18 h; c) $(COCl)_2$, DMSO, CH_2Cl_2, -78 °C, 30 min *then* **223**, CH_2Cl_2, -78 °C, 100 min *then* Et_3N, -78 °C ⟶ rt, 18 h, 34 % o2s.

For the finalization of the synthesis, ketone **222** was converted to the terminal alkene **221** by a Wittig protocol. Subsequent reduction using DIBAL-H delivered alcohol **220**. Lastly, halogenation followed by pyrophosphorylation delivered double bond shifted FPP derivative **95** (Scheme 55).



Scheme 55: Final steps towards the synthesis of double bond shifted FPP-derivative **95**. a) KO*t*-Bu, Ph_3PCH_3Br, THF, 0 °C ⟶ 60 °C, 16 h; b) DIBAL-H, CH_2Cl_2, -78 °C ⟶ rt, 4.5 h, 43 % o2s; c) DMS, NCS, CH_2Cl_2, -30 °C, 20 min *then* **220**, CH_2Cl_2, -40 °C ⟶ rt, 2 h; d) $P_2O_7H(nBu_4N)_3$ (**114**), CH_3CN, rt, 16 h, 17 %.

4 RESULTS AND DISCUSSION - MICROBIOLOGICAL WORK

4.1 OVEREXPRESSION, ISOLATION, AND PURIFICATION OF SESQUITERPENE CYCLASES

Overexpression of the eight selected sesquiterpene cyclases (BcBot2, Cop4, Cyc1, GcoA, Hvs1, PenA, Tps32, Tri5) was performed in *E. coli* BL21 (DE3). The cloning and transformation of the synthetic genes to produce the first stock cultures were done by former members of the Kirschning group.[53,95,96]

Enzyme production was conducted in 2YT media with kanamycin as an additive which was inoculated with seed culture generated from cryogenic stock cultures of the respective transformed *E. coli* BL21 (DE3). After the optical density ($\lambda = 600$ nm, OD_{600}) of the main culture exceeded the required value (≥0.4), production of the respective recombinant enzyme was induced with the addition of *iso*propyl β-D-1-thiogalactopyranoside (IPTG). After further incubation at decreased temperature (16 °C), the resulting suspension was centrifuged, the medium decanted, and the resulting cell pellet stored at -20 °C (Figure 8).[53]

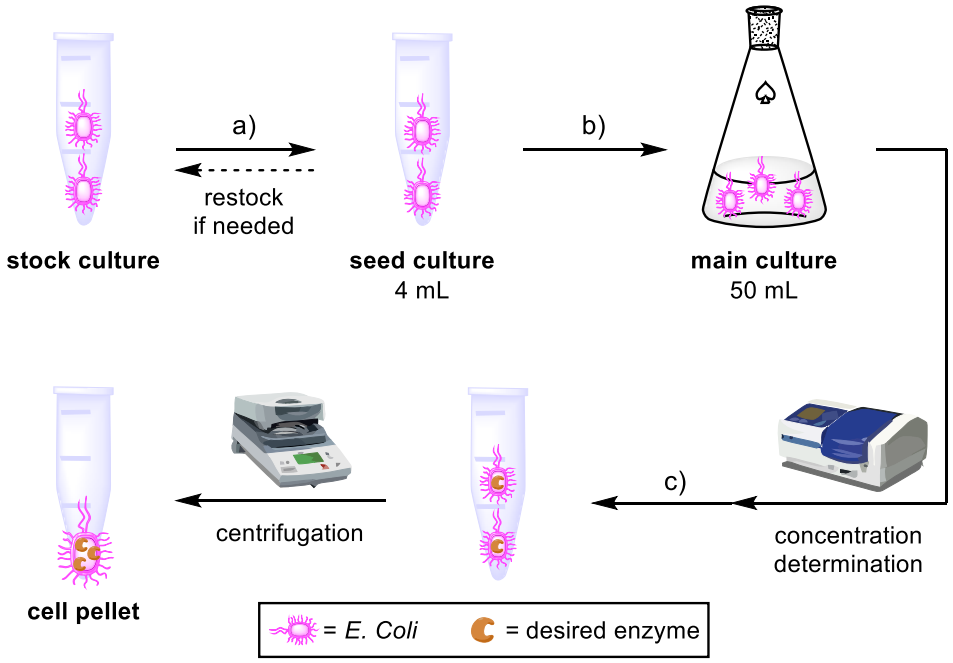


Figure 8: Schematic illustration of the procedure used to overexpress the desired sesquiterpene cyclases. a) LB-medium, kanamycin, incubation until $OD_{600} \geq 0.4$, 37 °C, 180 rpm; b) seed culture (1 mL), 2TY-medium. kanamycin, 3 h, 37 °C, 180 rpm; c) IPTG, 20–24 h, 16 °C, 180 rpm.

The cell pellets collected for each respective enzyme were then resuspended in buffer and the cells ultrasonically lysed. To remove solid cell fragments, the resulting suspension was centrifuged. The supernatant containing the enzyme was then loaded onto a Ni-NTA column and aq. imidazole solutions with increasing concentrations were used for elution. Fractions containing the desired enzymes (determined via SDS-PAGE or Bradford assay) were combined

in a concentration/membrane tube and concentrated via centrifugation. Finally, remaining imidazole and buffer salts were removed with a desalting column and the obtained enzyme stock solution was fractionated into aliquots. Enzyme concentrations of the aliquots were determined photometrically, and after dilution with preservation buffer, the protein solutions were stored at -80 °C (Figure 9).

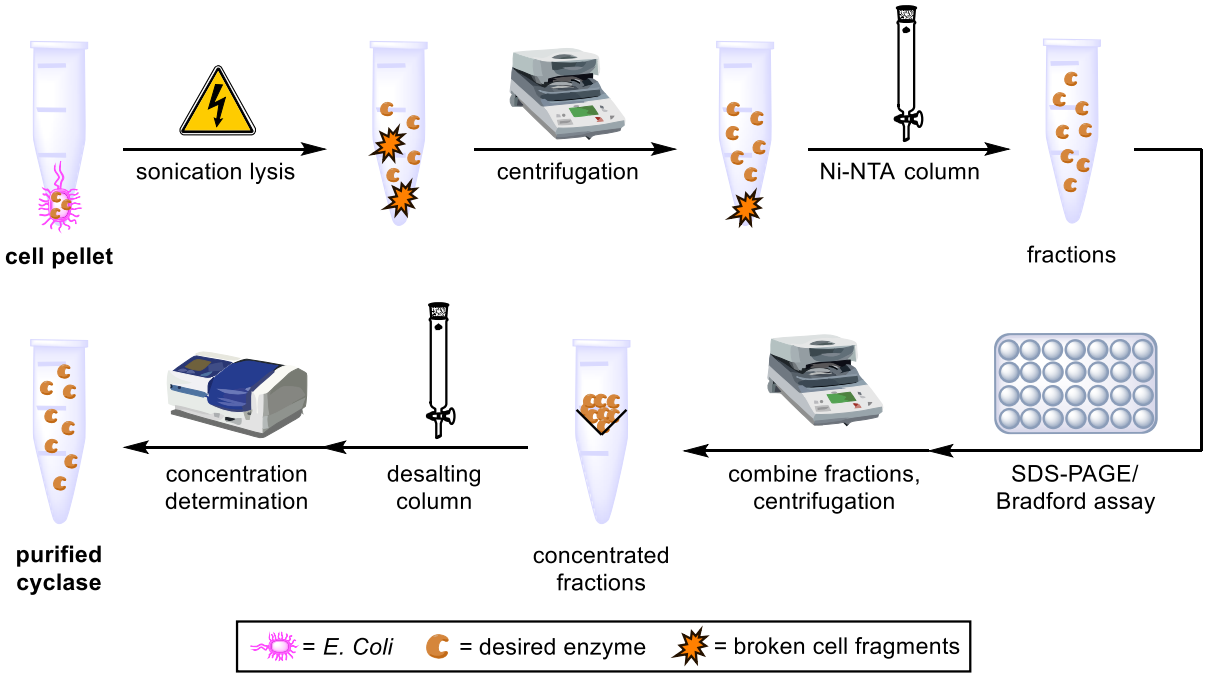


Figure 9: Schematic illustration of the isolation and purification procedure used to obtain the desired recombinant enzymes.

Using the methods described above, all eight desired sesquiterpene cyclases were successfully isolated and purified. To investigate their activity, analytical enzyme tests with the natural substrate FPP (**10**) were performed (see next chapter). All available enzyme aliquots and their respective concentrations are summarized below (Table 2).

Table 2: Summary of all obtained enzyme aliquots and their respective concentrations. In general, 200 µL portions were obtained. All listed enzymes were active and their concentrations were determined via UV-Vis spectroscopy (λ = 280 nm).

Enzyme	Aliquot concentrations [g/L]				
	1	2	3	4	5
BcBot2	0.37	0.55	3.42	6.80	10.50
PenA	3.45	3.81	2.52	9.46	
Cop4	5.89	6.03	5.29	5.08	6.64
GCoA	7.61	6.15	9.29		
Hvs1	4.20	4.57	1.92		
Cyc1	1.30	1.00			
Tps32	4.66	4.66	4.66		
Tri5	4.99	4.99	4.99		

4.2 ANALYTICAL *IN VITRO* ENZYME TESTS WITH FARNESYL PYROPHOSPHATE

4.2.1 DETERMINATION OF ENZYME ACTIVITY – POSITIVE CONTROL

To examine the activity of the previously obtained sesquiterpene cyclases, qualitative biotransformations of the natural substrate FPP (**10**) were carried out. Reactions were performed on an analytical scale (0.5 mL) under reaction conditions (see Experimental Section, p. 73) previously established by the Kirschning group.[53,95,96] These positive controls were repeated each time the cyclases were used to ensure that enzyme activity did not decrease or disappear over time.

First, all necessary reagents and solutions were mixed in the reaction vial except for the active enzyme. With the addition of the respective enzyme, the biotransformation reaction was initiated. After incubation for 20–30 min at 34 °C, the reaction mixtures were extracted with *n*-hexane (100 µL) and analyzed via GC-MS. In addition to the positive controls, nine negative controls were carried out in which only the enzymes or FPP (**10**) were incubated. With these negative controls as a comparison, impurities and products were identified more efficiently. As a representative example for the analysis performed on all enzyme assays, the chromatogram of the reaction of **10** with BcBot2 and those of the corresponding negative controls are compared below (Figure 10).

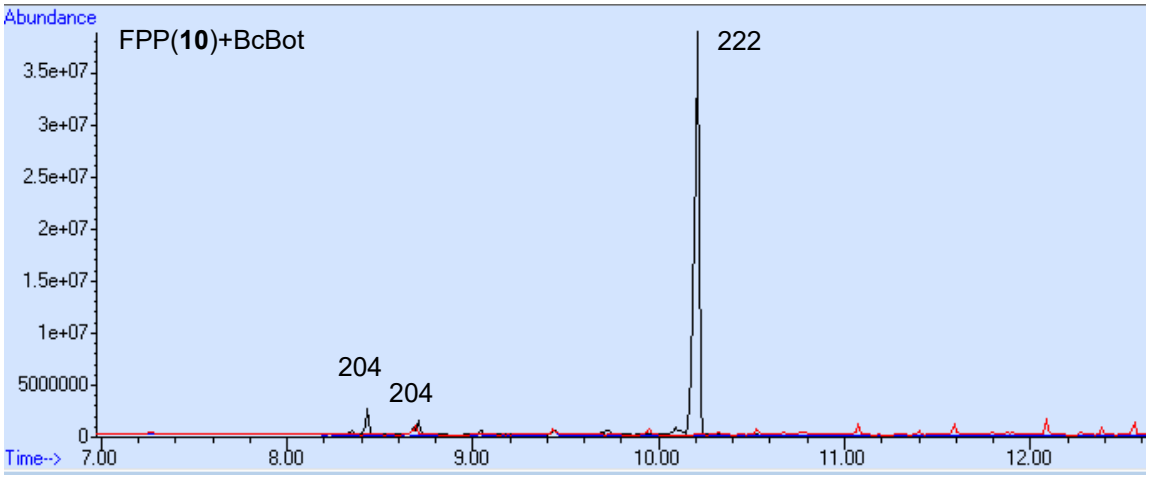
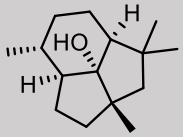
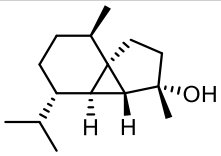
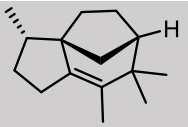
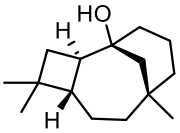
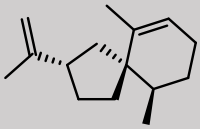
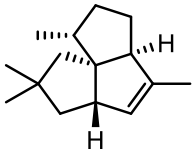
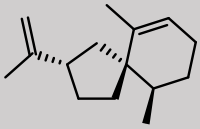
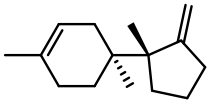


Figure 10: GC-MS chromatograms of the qualitative biotransformation of BcBot2 with FPP (**10**) and the corresponding negative controls. The TIC-trails of enzyme+substrate (black), only enzyme (red), and only substrate (blue) are overlayed for comparison. Numbers above the peaks are the corresponding m/z ratios.

Comparison of the TIC-lane of the biotransformation with those of the corresponding negative controls shows the formation of presilphiperfolan-8-β-ol (**38**) as the major product (m/z = 222, RI = 1645) along with two minor products (m/z = 204). The data suggests that the formed products were not part of the negative controls, so it can be assumed that the enzyme was active. Identification of the products was done by comparing the mass spectra, retention times (t_R), and

retention indices (RI) with the results of previous group members and the literature (if available).[32,95,96] As anticipated, biotransformation of FPP (**10**) with the isolated cyclases yielded natural terpenoid products with the expected masses (m/z = 204, 222) and retention times. An overview of the main products of the positive controls is listed below (Table 3).

Table 3: Main products of the analytical enzyme tests with FPP (**10**) and the isolated cyclases (positive controls). The listed results prove the activity of the tested enzymes.

Enzyme	Main product	Structure	t_R [min]	RI	m/z
BcBot2	presilphiperfolan-8-β-ol (**38**)		10.212	1645	222
Cop4	cubebol (**44**)		9.640	1548	222
Cyc1	*epi*-isozizaene (**230**)		9.240	1483	204
GcoA	(+)-caryolan-1-ol (**28**)		10.075	1621	222
Hvs1	vetispiradiene (**27**)		9.573	1537	204
PenA	pentalenene (**55**)		8.477	1367	204
Tps32	vetispiradiene (**27**)		9.573	1537	204
Tri5	trichodiene (**231**)		9.067	1456	204

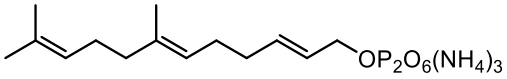
4.3 ANALYTICAL *IN VITRO* ENZYME TESTS WITH UNNATURAL FPP DERIVATIVES

Analogous to the analytical enzyme assays with FPP (**10**), qualitative biotransformations of unnatural FPP derivatives were performed under the same conditions and using the same procedure as described before. The GC-MS data collected were also analyzed using the chromatograms of the respective negative controls as a comparison. For clarity, the overlayed chromatograms are not displayed and the results are presented in tabular form (for GC-MS data see ATTACHMENT, p. 209). In addition to t_R and RI, the mass-to-charge ratio (m/z) is given. The reported area fraction (A_{total}) describes the percentage of a signal in relation to all observed signals. Depending on the termination mechanism of the cyclization cascade (deprotonation or water addition), two product masses were sought for each substrate.

4.3.1 BIOTRANSFORMATION OF DESMETHYL DIPHOSPHATES 98 AND 99

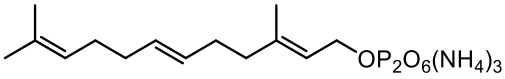
Based on the GC-MS results collected, desmethyl diphosphate **98** and **99** were both accepted by a total of six enzymes and each delivered numerous biotransformation products with the sought m/z (190, 208) for deprotonated and water addition products respectively (Figure 11). However, signal intensities are much lower (1/10–1/100) compared to the natural product signals of the positive controls, indicating very little product formation. Moreover, the compounds were obtained as mixtures with many by-products. Therefore, it was decided not to perform further scale-up and separation experiments.

In this context, however, the biotransformations of **98** with BcBot2 and **99** with Tri5 and Cyc1 are noteworthy because a major product was formed in each case. For **99**, it is assumed that both enzymes generated the same product (RI = 1381, m/z = 190). If the overall yield of these reactions can be increased in the future by optimizing the reaction conditions, the isolation of new cyclic terpenes may be possible in these cases.



98

Enzyme	t_R [min]	RI	A_{total} [%]	m/z
BcBot2	8.721	1402	15.9	190
	9.095	**1461**	**28.0**	**190**
	9.336	1497	5.6	190
Cop4	8.756	1407	2.2	190
	8.963	1440	5.0	190
	9.068	1456	10.0	190
	9.339	1498	8.9	190
	9.377	1504	4.0	190
	10.283	1657	20.6	190, 208
GcoA	8.428	1360	4.1	190
	8.591	1383	5.4	190
	8.725	1402	10.4	190
	9.097	1461	15.0	190
	9.349	1499	6.7	190
	10.055	1617	4.3	190
Tps32	8.935	1436	7.2	190
	8.989	1444	17.1	190
	9.040	1452	18.7	190
Cyc1	9.069	1457	14.4	190
	9.344	1498	6.4	190
	10.283	1657	7.2	190, 208
Tri5	8.950	1438	4.3	190
	9.063	1456	4.2	190
	9.096	1461	8.8	190
	9.353	1500	6.5	190



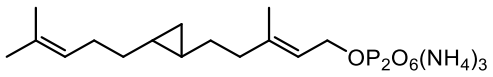
99

Enzyme	t_R [min]	RI	A_{total} [%]	m/z
BcBot2	8.194	1325	8.9	190
	8.468	1365	21.2	190
	9.136	1467	9.5	190
	9.534	1530	15.8	190, 208
PenA	9.131	1466	5.7	190
	9.528	1529	3.7	190
	9.599	1541	12.7	190
Cop4	8.053	1304	5.8	190
	8.585	1382	2.4	190
	8.848	1422	11.0	190
	9.030	1451	14.3	190
	9.136	1467	17.3	190
	9.534	1530	8.8	190, 208
Hvs1	8.052	1304	22.7	190
	8.961	1440	12.6	190
	9.135	1467	14.7	190
Cyc1	**8.577**	**1381**	**35.0**	**190**
	8.860	1424	7.5	190
	9.135	1467	7.1	190
Tri5	**8.577**	**1381**	**41.3**	**190**
	8.769	1409	4.2	190
	9.019	1449	6.0	190
	9.135	1467	9.1	190
	9.342	1498	9.2	190

Figure 11: Products of the analytical enzyme tests of **98** and **99** with the previously isolated cyclases based on GC-MS data. Relatively nonpolar deprotonation products (m/z = 190) and polar sesquiterpene alcohols (m/z = 218) are sought. Noteworthy results are highlighted in bold.

4.3.2 BIOTRANSFORMATION OF CYCLOPROPYL DIPHOSPHATES 88 AND 89S/R

Similar to the desmethyl diphosphates, cyclopropyl FPP derivative **88** was well accepted by the enzymes used. Of the eight cyclases, reaction with **88** yielded products with the sought m/z (190, 208) in seven cases (Figure 12). Unfortunately, the compounds were again obtained as mixtures with many by-products and signal intensities indicated very little product formation for this substrate as well. Although some products seem to be produced by several enzymes, the only notable result is the reaction with Tps32 which delivered a single major product (RI = 1541, m/z = 204). Isolation of a new cyclic terpene may also be possible in this case if the overall yield can be increased through optimization experiments. For these reasons, it was again decided not to perform further scale-up and separation experiments.



88

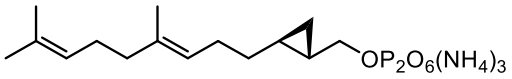
Enzyme	t_R [min]	RI	A_{total} [%]	m/z
PenA	9.585	1539	21.9	204
	9.734	1564	14.4	222
Cop4	9.596	1541	19.5	204
	9.637	1548	5.4	204
	9.746	1566	4.5	222
	9.824	1578	6.0	204
	9.885	1588	2.8	204, 222
Hvs1	9.594	1540	9.3	204
	9.739	1564	15.1	222
GcoA	9.591	1540	21.1	204
	9.639	1548	5.0	204
	9.739	1564	6.2	222
	9.820	1578	4.7	204
	9.885	1588	11.3	204, 222
Tps32	**9.599**	**1541**	**48.2**	**204**
Cyc1	9.042	1452	14.2	204
	9.591	1540	11.3	204
	9.642	1548	3.5	204
	9.739	1564	5.2	222
	9.820	1578	3.2	204, 222
	10.329	1665	2.4	204
Tri5	9.036	1451	17.2	204
	9.591	1540	8.4	204
	9.738	1564	19.6	204, 222
	9.875	1587	4.3	222

Figure 12: Products of the analytical enzyme tests with **88** and the previously isolated cyclases based on GC-MS results. Relatively nonpolar deprotonation products (m/z = 204) and polar sesquiterpene alcohols (m/z = 222) are sought. Noteworthy results are highlighted in bold.

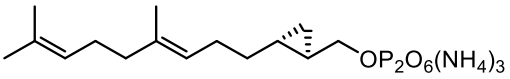
Compared to the previously presented results, cyclopropyl diphosphates **89S** and **89R** were poorly accepted by the cyclases used (Figure 13). **89S** was accepted as a substrate by BcBot2 and GcoA and delivered two different products. The reactions with both enzymes yielded one major (RI = 1771, m/z = 204, 222) and one minor product (RI = 1750, m/z = 222), each of which can be assumed to be identical. **89R** was transformed by BcBot2, Cop4, and GcoA, which resulted in three different products. Just as in the previous case, all three cyclases produced one major product. Here, BcBot2 and GcoA were assumed to produce the same cyclization product (RI = 1771, m/z = 204). Moreover, BcBot2 and GcoA appear to form the same main product regardless of the isomer.

As previously discussed, biotransformations with the highlighted enzymes could lead to new cyclization products if the overall yield can be increased through optimization experiments.

However, it was decided not to perform further scale-up and separation experiments for the same reasons as stated before.



89S



89R

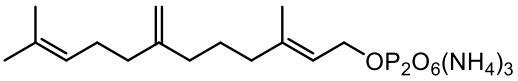
Enzyme	t_R [min]	RI	A_{total} [%]	m/z
BcBot2	10.805	1750	13.3	222
	10.917	**1771**	**39.4**	**222, 204**
GcoA	10.804	1750	13.8	222
	10.913	**1770**	**29.4**	**222, 204**

Enzyme	t_R [min]	RI	A_{total} [%]	m/z
BcBot2	**10.919**	**1771**	**40.5**	**204**
Cop4	**9.571**	**1537**	**35.2**	**204**
	9.675	1554	16.6	204
GcoA	**10.917**	**1771**	**39.1**	**204**

Figure 13: Products of the analytical enzyme tests with **89S** and **89R** based on GC-MS results. Relatively nonpolar deprotonation products (m/z = 204) and polar sesquiterpene alcohols (m/z = 222) are sought. Noteworthy results are highlighted in bold.

4.3.3 BIOTRANSFORMATION OF DOUBLE BOND SHIFTED FPP DERIVATIVE 95

In contrast to the previously presented substrates, double bond shifted diphosphate **95** was surprisingly poorly accepted by the employed enzymes and yielded only a single product with BcBot2 (RI = 1662, m/z = 222). Due to the low intensity of the corresponding signal, it was decided not to perform any further biotransformation experiments with **95** (Figure 14).



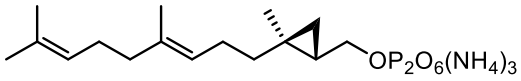
95

Enzyme	t_R [min]	RI	A_{total} [%]	m/z
BcBot2	10.312	1662	7.8	222

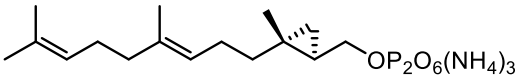
Figure 14: Products of the analytical enzyme tests with **95** and the previously isolated cyclases based on GC-MS results. Relatively nonpolar deprotonation products (m/z = 204) and polar sesquiterpene alcohols (m/z = 222) are sought.

4.3.4 BIOTRANSFORMATION OF CYCLOPROPYLMETHYL DIPHOSPHATES 90S AND 90R

Based on the GC-MS results collected, cyclopropylmethyl diphosphate **90S** was accepted by four enzymes and delivered numerous products (Figure 15). The biotransformations with BcBot2, Cop4, Cyc1, and Tri5 each yielded product mixtures with a major product, but again, the signal intensities were comparatively low. Besides some by-products formed by different enzymes, Bcbot2 and Cop4 also appear to produce the same main product (RI = 1616, m/z = 218). For the same reasons as discussed before, it was decided not to perform further scale-up and separation experiments.



molF3S



molF3R

Enzyme	t_R [min]	RI	A_{total} [%]	m/z
BcBot2	**9.451**	**1516**	**28.8**	**218**
	9.725	1562	11.4	236
	10.376	1673	23.2	236
	10.969	1780	18.1	236
Cop4	**9.453**	**1517**	**43.9**	**218**
	9.730	1563	12.5	218
	9.875	1587	12.8	218
	10.975	1781	2.9	236
Cyc1	9.447	1516	16.0	218
	9.725	1562	18.1	218
	9.835	**1580**	**41.0**	**218**
Tri5	9.450	1516	20.9	218
	9.725	**1562**	**57.9**	**236**
	9.836	1580	9.0	236

Enzyme	t_R [min]	RI	A_{total} [%]	m/z
BcBot2	9.452	1517	1.0	218
	9.640	1548	2.4	218
	9.714	1560	4.1	218
	9.953	**1599**	**15.7**	**218**
	10.932	**1773**	**24.7**	**236**
	10.982	**1782**	**33.8**	**236**
Cop4	9.448	1516	15.8	218
	9.846	1582	10.8	218
	10.885	1765	6.3	236
	10.970	**1780**	**46.8**	**236**
Cyc1	9.449	1516	20.4	218
	9.849	1582	5.1	236
	10.968	1780	11.8	236
GcoA	9.442	1515	5.0	236
	10.922	1772	6.0	236
	10.968	1780	12.8	236
Tri5	9.450	1516	20.8	218
	9.725	**1562**	**44.2**	**218**
	9.835	1580	15.8	218

Figure 15: Products of the analytical enzyme tests of **90S** and **90R** with the previously isolated cyclases based on GC-MS results. Relatively nonpolar deprotonation products (m/z = 218) and polar sesquiterpene alcohols (m/z = 236) are sought. Noteworthy results are highlighted in bold.

Lastly, cyclopropylmethyl diphosphate **90R** was accepted by five enzymes and delivered numerous products (Figure 15). The substrate was accepted by BcBot2, Cop4, Cyc1, GcoA, and Tri5 of which the reactions with Cyc1 and GcoA delivered product mixtures with no main product. Biotransformation with Tri5 resulted in a mixture with a major product (RI = 1562, m/z = 218), but the signal intensities were very weak compared to the positive control. Fortunately, **90R** was readily accepted by BcBot2 and Cop4. Both reactions yielded a mixture containing major products. The crucial factor in these cases is the strong intensity of the product signals, which are comparable to those of the positive control and therefore indicate high product formation. Comparison of the GC-MS data of these two transformations shows that the main product of Cop4 is also produced by BcBot2 (RI = 1780, m/z = 236), which also delivers two other products (Figure 16). Considering these promising results, it was decided to repeat the reaction of **90R** with BcBot2 on a larger scale.

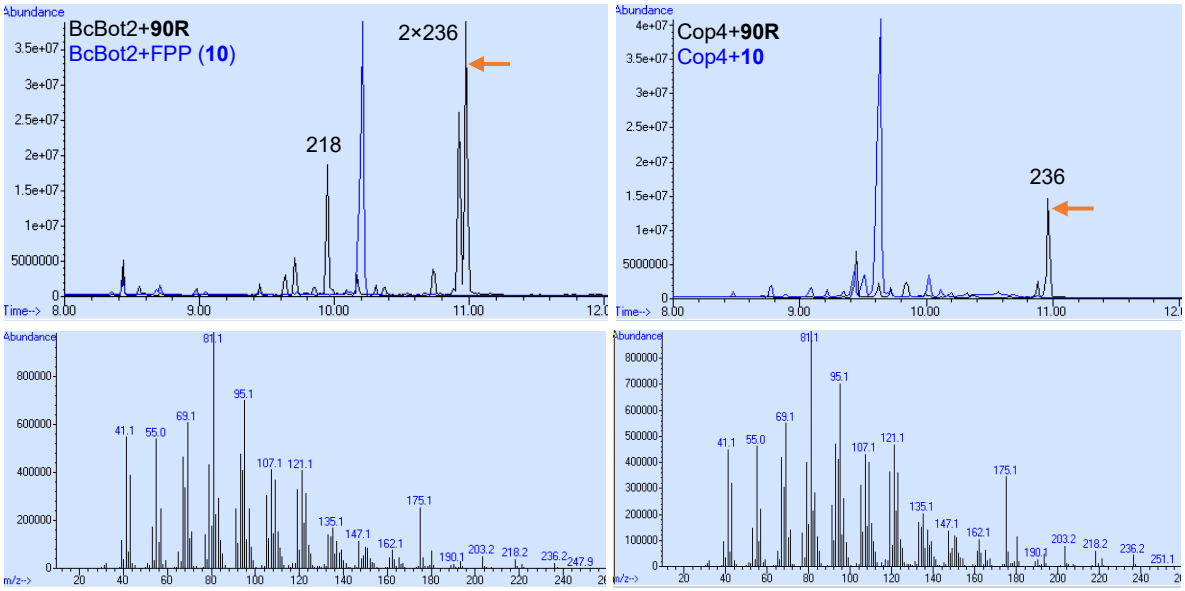
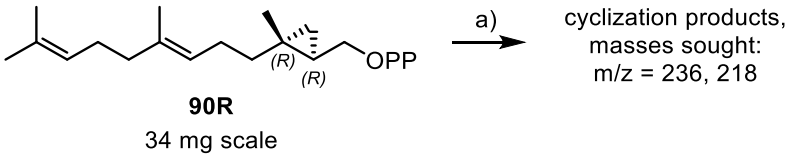


Figure 16: Top: Chromatograms of the qualitative biotransformations of BcBot2 (left) and Cop4 (right) with cyclopropyl diphosphate **90R** (black) and the respective positive controls with FPP (**10**) (blue). The TIC-trails are overlayed for comparison. Numbers above the peaks are the corresponding m/z ratios. Bottom: Mass spectra of the peaks marked by an arrow above. The fragmentation pattern indicates that the products generated by the two enzymes are identical.

4.4 SEMI-PREPARATIVE BIOTRANSFORMATION OF CYCLOPROPYL DIPHOSPHATE 90R

After analytical *in vitro* tests with the cyclopropane derivative **90R** and the cyclase BcBot2 delivered promising results, the reaction was scaled up in order to obtain sufficient material for the isolation and subsequent characterization of the cyclization products. Compared to the analytical tests, the reaction conditions and the reaction procedure were slightly adjusted for semi-preparative biotransformation. Because of the larger scale (50 mL), pyrophosphatase (PPase) was added to reduce the diphosphate anion concentration, which increases during the reaction and could lead to enzyme inhibition. Moreover, polysorbate 20 (Tween 20) was used to ensure better emulsification of the biotransformation mixture. Lastly, the method, sequence of reagent addition, and the workup procedure were also changed.



Scheme 56: Semi-preparative biotransformation of **90R** with BcBot2. a) HEPES, DTT, $MgCl_2$, tween 20, PPase, BcBot2, rt, 1.5 h *then* 34 °C, 26 h, 150 rpm.

In contrast to the analytical tests, the semi-preparative biotransformation was not initiated with the addition of the enzyme. BcBot2 was initially mixed with all required reagents and buffer solutions inside a flask. To this mixture, the first portion of the substrate (**90R**) was

continuously added using a syringe pump. Over the course of incubation (26 h, 34 °C, 150 rpm), two additional portions of the substrate were added. Thereafter, the crude mixture was extracted with *n*-hexane using a standard chemical extraction procedure. GC-MS analysis of the crude mixture provided results that were consistent with those of the analytical tests. Additionally, it was found that the assumed cyclization products were detectable by TLC with permanganate stain (Figure 17). Therefore, it was decided to carry out a silica column chromatographic purification prior to purification by preparative gas chromatography.

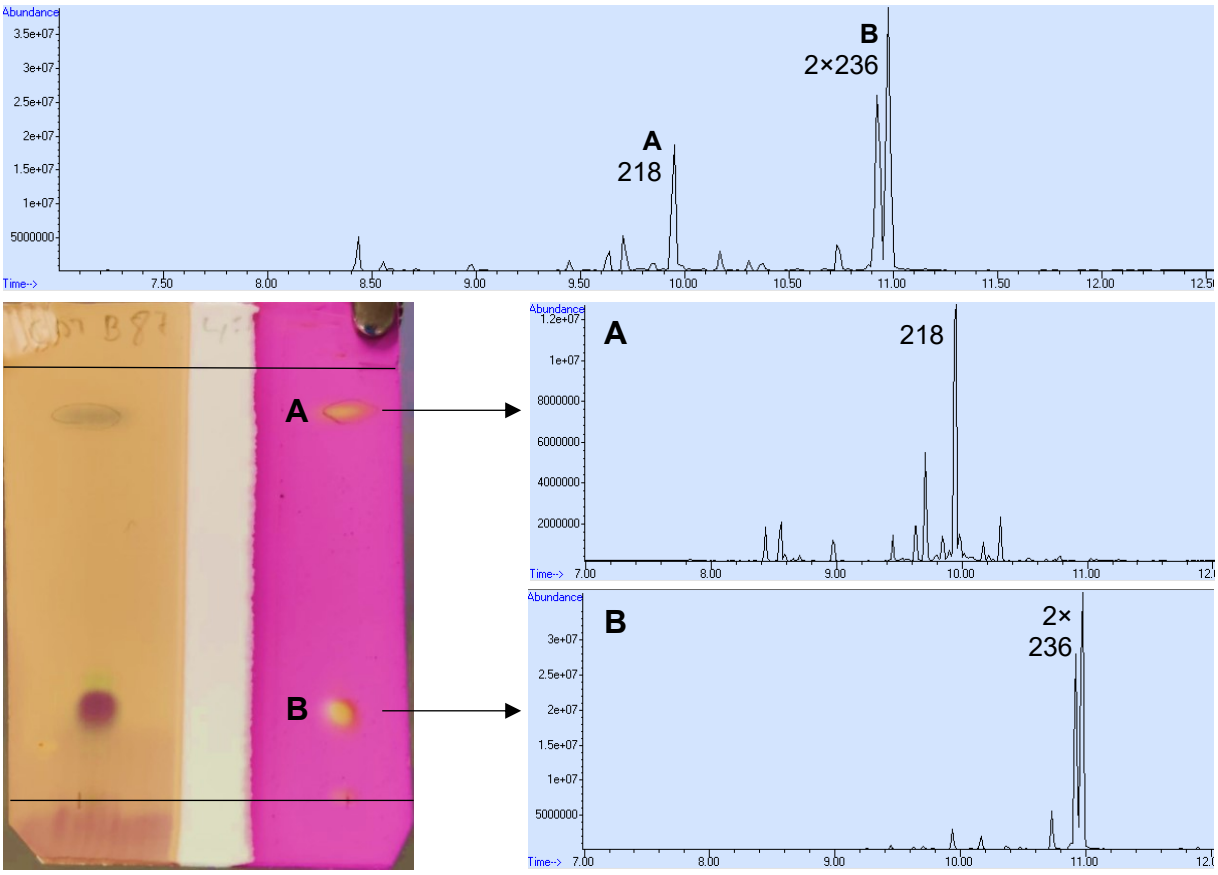


Figure 17: Top: Chromatogram of the crude extraction mixture of the semi-preparative biotransformation of **90R** with BcBot2. Bottom left: Thin-layer-chromatographic analysis of the crude extraction mixture (PE:Et_2O = 4:1). The spots were visualized with a vanillin (left) and a $KMnO_4$ (right) solution. Bottom right: The GC-MS analysis of the two fractions obtained after column chromatographic purification indicates the successful separation of the two spots (A and B). Numbers above the peaks are the corresponding m/z ratios.

Purification via column chromatography allowed the successful separation of the two major spots observed in the TLC analysis. GC-MS analysis of the obtained fractions indicates that fraction A contains a relatively nonpolar main product but also a few minor side products. Fraction B, on the other hand, had a higher overall purity but consisted of a mixture of two relatively polar main products (Table 4).

Table 4: GC-MS analysis of the two fractions obtained after column chromatographic purification of the biotransformation of **90R** with BcBot2.

Fraction	t_R [min]	RI	A_{total} [%]	m/z
A	9.946	1598	45.5	218
B	10.927	1773	37.2	236
	10.977	1782	51.4	236

Consequently, the next step would have been further purification of the obtained compounds by preparative GC. However, since part of the material is always lost during this isolation method and very little material was available, it was decided to perform further analysis experiments first.

Based on the preliminary GC-MS results, it was hypothesized that the three compounds obtained were desired cyclization products. Fraction A would thus contain one deprotonation product and fraction B would consist of two sesquiterpene alcohols. To verify the hypothesis, a series of NMR experiments for full structural elucidation was conducted. The results obtained from NMR measurements are graphically summarized below (Figure 18).

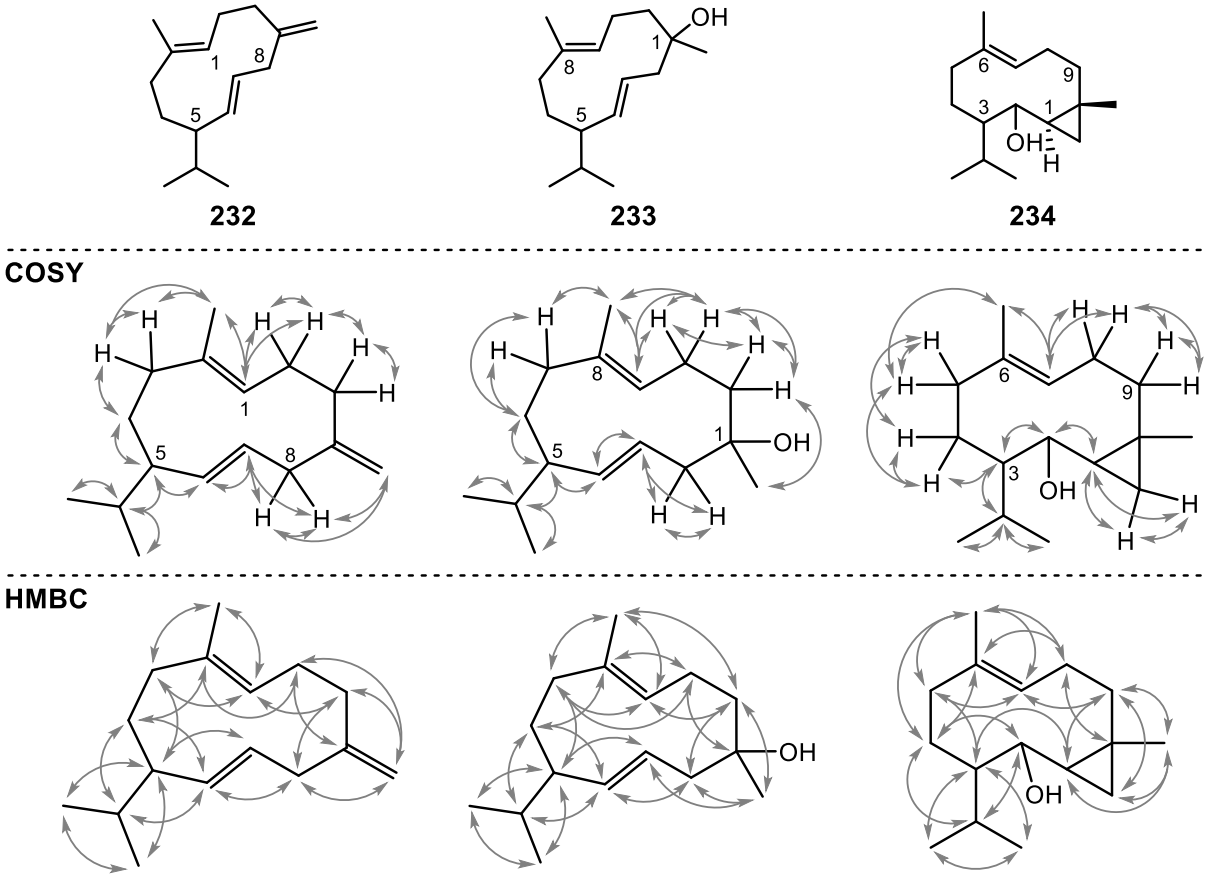
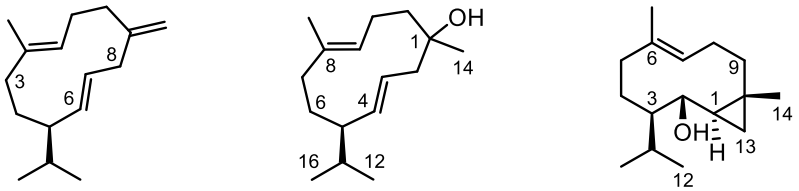


Figure 18: Proposed constitutional structures for the products (**232**–**234**) obtained from the biotransformation of **90R** and BcBot2 based on NMR-data. The 2D COSY correlations are shown in the second row and the HMBC correlations (without ^2J coupling for clarity) are shown in the third row. Carbon atom numbering is based on IUPAC nomenclature.

Using the obtained NMR data, especially the 2D spectra (COSY, HMBC, and HSQC), a proposal for the constitution of the three cyclic products was made. As assumed, fraction A contained the relatively nonpolar triene **232** and fraction B was a mixture of the cyclic alcohols **233** and **234**. With the integrals of the ^{1}H-NMR signals, the ratio of the obtained alcohols in fraction B was calculated (**233**:**234** = 2:3). Subsequently, the preferred conformation of the macrocycles and the relative stereochemistry of the substituents were studied in detail. In particular, coupling constants (*J*) and 2D NOESY correlations were thoroughly analyzed (Figure 19). Based on the high values for the coupling constants between the olefinic hydrogen atoms (^{3}J > 15 Hz), the 1,2-disubstituted double bonds of **232** and **233** are assumed to be (*E*)-configurated. In addition, the listed NOESY correlations support this conformational proposal and provide further information on the spatial orientation of the substituents. The presented NOESY correlations are obtained from the measurements of the alcohols. Unfortunately, no NOESY data could be recorded for triene **232** because the highly volatile triene evaporated from the NMR tube during the measurement. However, assuming that all three biotransformation products are formed by the same cyclization mechanism and considering all the previously reported results, a preliminary proposal for the absolute stereochemistry of the biotransformation products is presented below (Figure 19).



232 **233** **234**

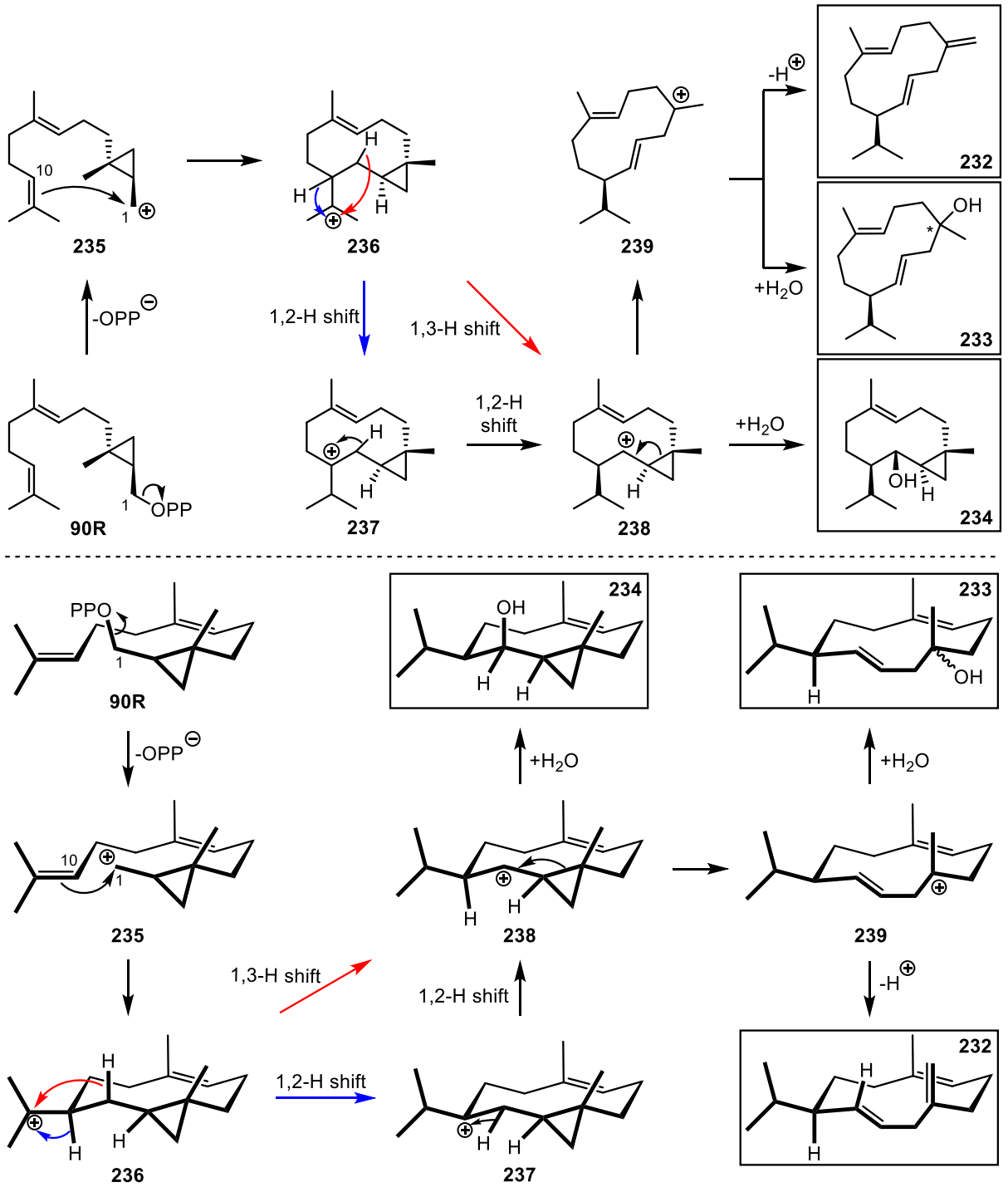
Product	Coupling constants *J* [Hz]				
232	H5-H6	H6-H7	H7-H8a	H7-H8b	H8a-H8b
	9.4	15.2	9.4	5.5	12.6
233	H2a-H2b	H2a-H3	H2b-H3	H3-H4	H4-H5
	13.0	10.0	5.4	15.4	10.0
234	H1-H2	H2-H3	H1-H13a	H1-H13b	H13a-H13b
	9.8	1.6	5.6	8.4	4.2

Product	NOESY correlations						
233	H3-H4	H3-H14	H4-H12	H4-H16	H5-H3	H9-H14	
234	H1-H2	H2-H3	H2-H12	H2-H13	H2-H14	H3-H7	H7-H14

Figure 19: Preliminary proposal for the configurations of the obtained cyclization products as well as selected coupling constants (*J*) and NOESY correlations used to determine the stereochemistry.

With the preliminary structure elucidation in hand, preparative GC was performed with the goal of separating the two alcohols, further purifying the products, and then conducting final NMR measurements. Unfortunately, all product material was lost during the purification cascade due to technical issues with the pGC instrument. Although this eliminated the possibility to measure NMR data for the individual alcohols, the spectra obtained for the mixture (**233** and **234**) were sufficient to differentiate and elucidate both products.

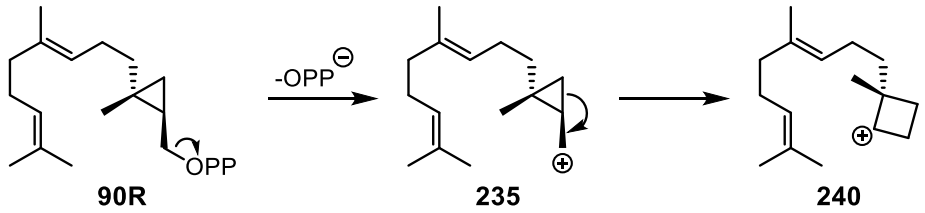
In summary, the NMR analysis of the two mixtures confirmed the initial hypothesis. The relatively nonpolar triene **232**, found in fraction A, is most likely a product resulting from the termination of the cyclization cascade by deprotonation. In contrast, the more polar cyclic alcohols **233** and **234**, found in fraction B, are likely the result of water addition to active intermediates within the cyclization cascade. To further support the proposals discussed, more detailed thought was given to possible cyclization mechanisms.



Scheme 57: Proposed cyclization mechanism for the formation of the obtained biotransformation products **232–234**. The mechanism is visualized in two different ways for better comprehension. Stereochemical proposals are based on the analysis of measured NMR data.

Based on mechanistic suggestions for similar reactions in the literature,[1,19,32,34,41] it was assumed that the three cyclization products are formed via a common macrocyclization step followed by several hydride shifts (Scheme 57).

A reasonable first step of the cationic cyclization cascade is a 1⟶10 macrocyclization initiated by the activation of the diphosphate group, which is eliminated as an anion during the cyclization process. It is important to note that this process appears to occur simultaneously, as otherwise the primary cation would most likely be trapped by a rearrangement of the cyclopropyl to a cyclobutyl group (Scheme 58).



Scheme 58: Possible cyclopropyl-cyclobutyl rearrangement to shift the highly reactive primary cation to a more stable secondary position and to reduce the ring tension. On this basis, the initial 1⟶10 macrocyclization is assumed to be a concerted reaction.

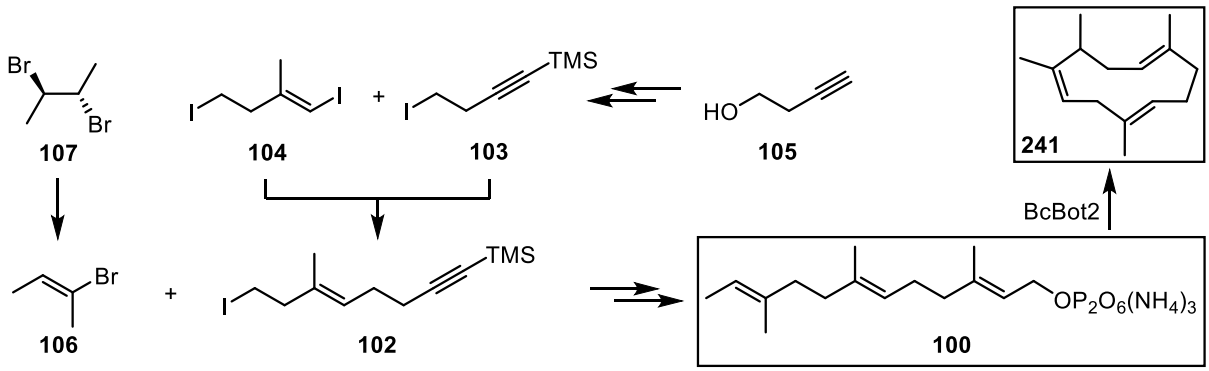
The resulting intermediate (**236**) with a tertiary cation at C11 could then either undergo two successive 1,2-H shifts or one 1,3-H shift to form a secondary cation (**238**) with the charge at C1. It is assumed that all three products derive from this key intermediate and that further reactions for the formation of the individual products diverge from here. For one, cyclopropyl alcohol **234** could be the result of water-addition to the secondary cation of **238**. A second way the reaction cascade could continue is by opening the cyclopropane ring, resulting in the shift of the secondary cation at C1 to the tertiary position C3 (**239**). From here, water addition would lead to the formation of diene **233** and deprotonation would result in the formation of triene **232**. In conclusion, the proposed mechanism logically explains the formation of all three obtained biotransformation products. These mechanistic relatives share a common macrocyclization and a series of hydride shifts, which further supports the previous proposals concerning their absolute stereochemistry (Scheme 57).

5 SUMMARY AND OUTLOOK

5.1 CHEMICAL WORK

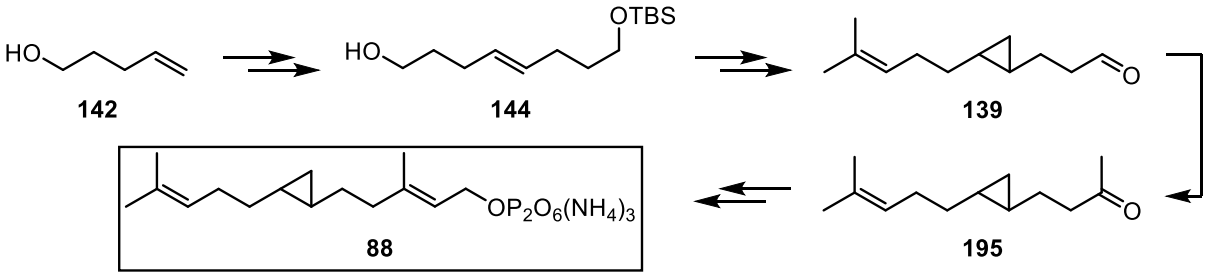
In this Ph.D. thesis, nine unnatural linear FPP derivatives were successfully synthesized and subsequently employed as substrates in biotransformations with sesquiterpene cyclases to investigate the cyclization mechanisms and substrate flexibility of the enzymes. As biocatalysts, eight sesquiterpene cyclases from plant, bacterial and fungal sources were selected. These were first heterologously expressed in *E. coli* and obtained after subsequent isolation and purification. With most unnatural substrates, the enzymatic tests yielded promising results. For the isolation of new products in the future, however, the yields must first be improved. Still, one substrate was particularly well accepted and yielded three cyclic sesquiterpenes which were isolated and characterized.

At the beginning of the work, methyl-shifted FPP derivative **100** was successfully prepared according to a synthesis strategy developed by Schröder. The desired diphosphate was obtained in twelve steps (nine linear steps) using two sp^2-sp^3 Negishi cross-couplings and two Zr-catalyzed carboaluminations as key reactions. The obtained diphosphate **100** was subsequently utilized by Harms as a substrate in biotransformations with BcBot2 and PenA. In combination with the former, humulene derivative **241** with an intense pepper scent was obtained (Scheme 59).[97]



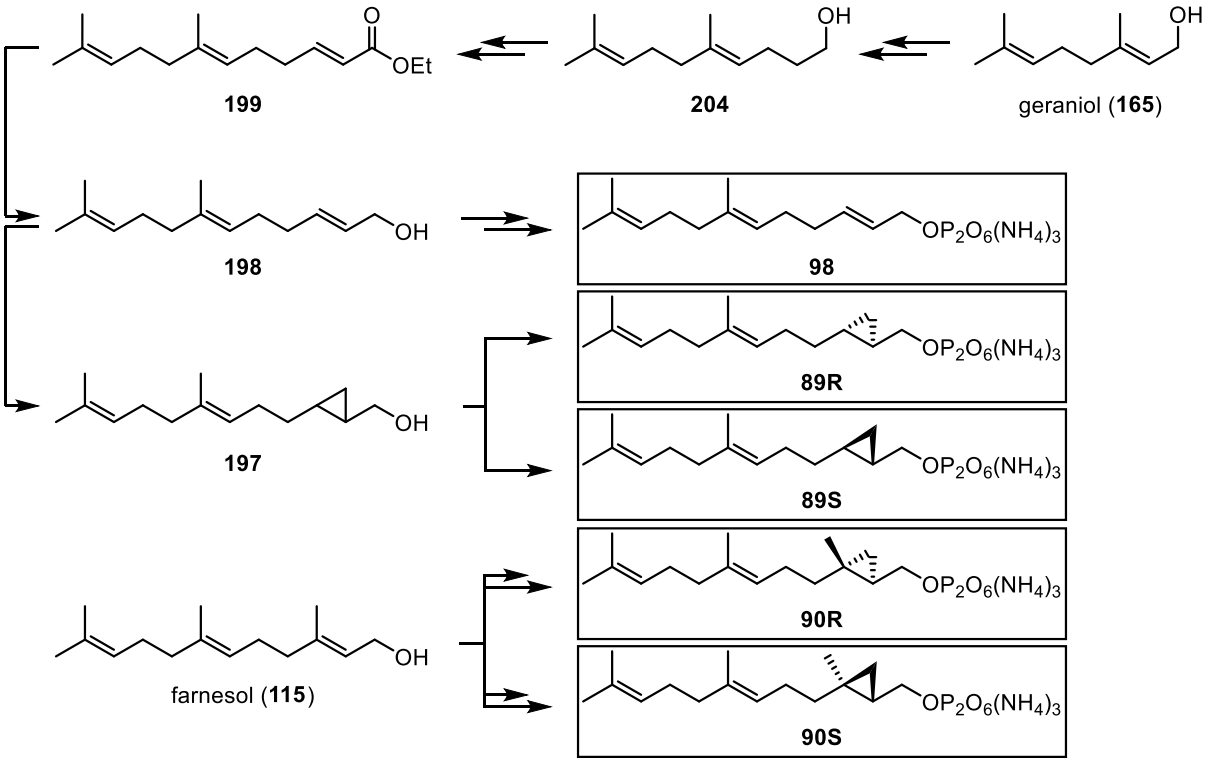
Scheme 59: Abbreviated twelve-step synthesis of methyl shifted FPP derivative **100** starting from *meso*-2,3-dibromobutane (**106**) and 3-butyne-1-ol (**105**) and subsequent biotransformation with BcBot2 to obtain a cyclic product with fragrant properties.

The next step was the synthesis of cyclopropyl diphosphate **88** starting from pent-4-en-1-ol (**142**). The desired diphosphate was obtained in twelve linear steps using cross-metathesis and olefination reactions as the key steps to construct the carbon skeleton (Scheme 60).



Scheme 60: Abbreviated twelve-step synthesis of cyclopropyl diphosphate **100** starting from pent-4-en-1-ol (**142**). The synthesis began with the dimerization of the starting material as the basis for the construction of the carbon skeleton.

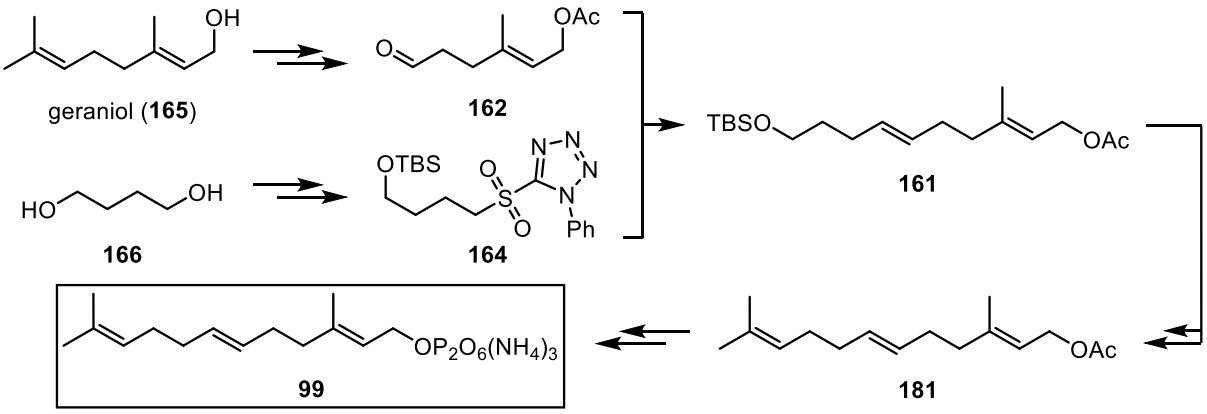
Subsequently, starting from geraniol (**165**), desmethyl farnesol derivative **198** was prepared to be used as the key intermediate in the synthesis of a series of diphosphates. Desmethyl FPP derivative **98** and both isomers of cyclopropyl diphosphate **89** were prepared from the alcohol (**198**). The latter were obtained via asymmetric Simmons-Smith cyclopropanation followed by phosphorylation with TEAP. In the same manner, two isomers of cyclopropylmethyl diphosphate **90** were synthesized starting from farnesol (**115**) (Scheme 61).



Scheme 61: Abbreviated overview of the synthesis of desmethyl diphosphate **98** and various cyclopropyl FPP derivatives. Both **98** and the two isomers of cyclopropyl diphosphate **89** were prepared in nine linear steps. cyclopropyl diphosphates **90S** and **90R** were obtained after two reactions starting from farnesol (**115**).

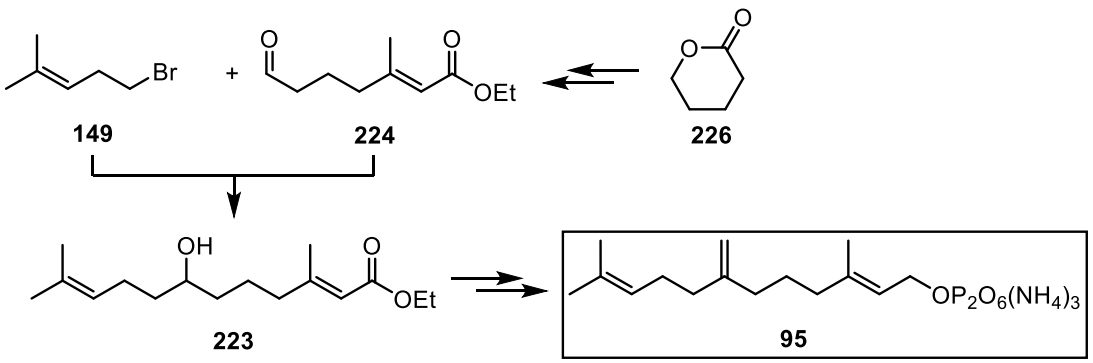
Next, another desmethyl FPP derivative with a centrally missing methyl group (**99**) was synthesized. For this purpose, two fragments were prepared starting from geraniol (**165**) and

butane-1,4-diol (**166**). After Julia-Kocienski olefination to connect the fragments, the desired product was obtained in a few final reactions (Scheme 62).



Scheme 62: Abbreviated 13-step synthesis (ten linear steps starting from **165**) of desmethyl diphosphate **99**. For the key step to elongate the carbon backbone, a Julia-Kocienski reagent (**164**) was prepared in four steps starting from butane-1,4-diol (**166**).

With the desired cyclopropyl and desmethyl diphosphates in hand, efforts shifted towards the synthesis of double bond modified FPP derivatives. The first derivative to be synthesized was diphosphate **95** with a terminal double bond in the center of the structure. A Grignard addition was employed as the key step in the construction of the carbon skeleton. The desired diphosphate **95** was obtained in eleven linear steps starting from δ-valerolactone (Scheme 63).



Scheme 63: Abbreviated eleven-step synthesis of double bond shifted FPP derivative **99**.

5.2 MICROBIOLOGICAL WORK AND OUTLOOK

After a series of unnatural substrates had been synthesized, the planned enzymatic experiments were initiated. For this purpose, the eight selected sesquiterpene cyclases were first heterologously expressed in *E. coli*, isolated, and purified. Before each application of the obtained enzymes, positive controls were performed with the natural substrate FPP (**10**) to confirm their activity (Table 5).

Table 5: All obtained sesquiterpene cyclases, the number of available aliquots, and the maximum reached concentration. All listed enzymes were active and their concentrations were determined via UV-Vis spectroscopy

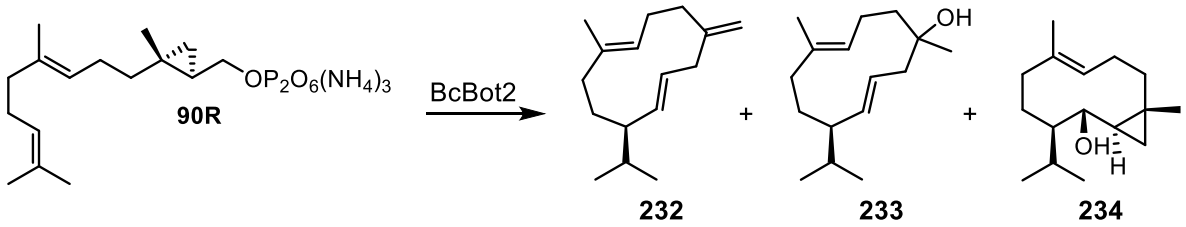
Enzyme	BcBot2	PenA	Cop4	GcoA	Tps32	Cyc1	Tri5	Hvs1
Available aliquots	5	4	5	3	3	2	3	3
Max conc. [mg/mL]	10.5	9.46	6.64	9.28	4.66	1.3	4.99	4.57

With all required components in hand, in vitro enzyme assays were performed on an analytical scale (qualitative biotransformations). Preliminary analyses showed that all substrates used were converted by at least one enzyme, resulting in numerous biotransformation products. Although many products were found, most of them were obtained in mixtures with many by-products. In addition, GC-MS analysis indicated such low product formation that scaling up the reaction for most substrates did not seem reasonable (Table 6).

Table 6: Results of the analytical in vitro enzyme tests performed with eight unnatural substrates and eight sesquiterpene cyclases. The numbers listed are the m/z detected via GC-MS. Most of the biotransformation products were produced in very small quantities. Cases in which good yields were achieved and scale-up is viable are highlighted in bold.

Substrate	BcBot2	PenA	Cop4	Hvs1	GcoA	Tps32	Cyc1	Tri5
90R	**218, 236**	/	**218, 236**	/	236	/	236	218
90S	218, 236	/	218	/	/	/	218	218, 236
89R	204	/	204	/	204	/	/	/
89S	204, 222	/	/	/	204, 222	/	/	/
88	/	204, 222	204, 222	204, 222	204, 222	204	204, 222	204, 222
98	190	/	190, 208	/	190	190	190, 208	190
99	190, 208	190	190, 208	190	/	/	190	190
95	222	/	/	/	/	/	/	/

Biotransformation of **90R** with BcBot2 yielded products in sufficient quantities. Initial GC-MS analyses also indicate that more than one major product was formed. For these reasons, the biotransformation of BcBot2 with **90R** was repeated on a larger scale (Scheme 64).



Scheme 64: Semi-preparative biotransformation of **90R** with BcBot2 yielded three new cyclic products. The constitutional structure of the terpenoids was fully elucidated with NMR data, the stereochemistry shown is a preliminary proposal based on the results of 2D-NMR experiments.

GC-MS analysis of the enzymatic conversion of **90R** with BcBot2 on a semi-preparative scale revealed the formation of three main products. In addition, TLC analysis showed that one relatively nonpolar and two more polar products had formed. Subsequent column chromatographic purification allowed the separation of the nonpolar product from the two more polar compounds. Preliminary NMR analysis of the two fractions obtained showed the successful formation of three novel cyclic terpenes (Scheme 64). The subsequent attempt to separate the two polar products by preparative GC was unfortunately not successful. Nevertheless, both the nonpolar product and the two polar products were fully characterized.

As often mentioned, many biotransformation products were found in the analytical enzyme tests with the unnatural derivatives used. With this large amount of different biotransformations (48 reactions in total), it was not possible to optimize each reaction due to time constraints, so in most cases, only very small amounts of product were detected. Nonetheless, the results are very promising, as they show that the employed substrates are in principle readily accepted by the cyclases. Therefore, optimization experiments should be performed in the future to improve the overall yield of the biotransformations shown. For example, previous group members successfully increased the efficiency of biotransformations by altering pH, temperature, and substrate and enzyme concentrations, among other factors.[95,96] If this can also be achieved for the reactions presented above, then the corresponding semi-preparative approaches offer a promising way to isolate new cyclic terpenes.

6 EXPERIMENTAL SECTION

6.1 GENERAL REMARKS

Reagents and Solvents

All commercially obtained solvents and reagents were supplied by ABCR, ACROS ORGANICS, ALFA-AESAR, CARBOLUTION, CARL ROTH, FLUOROCHEM, HONEYWELL, MERCK, NEW ENGLAND BIOLABS, SIGMA-ALDRICH, and TCI and were used as provided unless stated otherwise. Dry solvents (CH_2Cl_2, DMF, Et_2O, toluene) were taken from the SOLVENT PURIFICATION SYSTEM (SPS) by M. BRAUN, purchased (benzene, MeCN, MeOH) or freshly distilled (THF). Petroleum ether (bp.: 40–60 °C) and CH_2Cl_2 were always freshly distilled before use. All deuterated solvents for NMR measurements were purchased from DEUTERO. Amine bases were dried over KOH (triethylamine, di*iso*propylamine) or CaH_2 (di*iso*propylethylamine) and freshly distilled before use. The ZnBr (ultra-dry, 99.999%) used for Negishi coupling reactions was acquired from ABCR.

Reactions and Consumables

All reactions with reagents sensitive to air or humidity were performed in flame-dried glassware and under argon atmosphere using SCHLENK techniques. Syringes and cannulas were purchased from B. BRAUN MELSUNGEN. Polypropylene vials and pipette tips used for the microbiological work were obtained from SARSTEDT. These plastic vials were initially used as reaction vessels for analytical biotransformations but were later replaced by glass vials purchased from MACHEREY-NAGEL.

Column Chromatography

For manual column chromatography, silica gel (type: 60 M, grain size: 40–63 µm) from MACHEREY-NAGEL was used. The elution was performed under slight overpressure. For automated flash column chromatography, the flash purification system SEPACORE by BÜCHI and prepacked cartridges (CHROMABOND by MACHEREY-NAGEL and FLASHPURE by BÜCHI) were used.

Thin-Layer-Chromatography (TLC)

Silica gel-coated aluminium plates (type 60 F254) by MERCK, were used for qualitative TLC analysis. The spots were visualized with UV light (λ = 248 nm) and/or with staining solutions ($KMnO_4$, vanillin, anisaldehyde).

Mass Spectrometry

The high-resolution mass spectra (HRMS) were measured with a MICROMASS LCT spectrometer which uses a LOCKSPRAY dual ion source and a WATERS ALLIANCE 2695 system.

In addition, a WATERS QTOF PREMIER spectrometer was used in combination with a WATERS ACQUITY UPLC system. An electron spray ionizer (ESI) was used for ionization. The calculated and actual masses are given in atomic mass units.

NMR Spectroscopy

All reported NMR experiments are performed at room temperature using various BRUKER instruments, of which the ULTRASHIELD-400 and the ASCEND-400 are automated open-access devices (Table 7).

Table 7: Employed BRUKER NMR devices and the respective frequencies for ^{1}H-NMR, ^{13}C-NMR, and ^{31}P-NMR experiments.

Bruker Instrument	Frequency [MHz]		
	^{1}H	^{13}C	^{31}P
Ultrashield-400	400	100	-
Ascend-400	400	100	162
Ultrashield-500	500	125	-
Avance-600 Ascend-600	600	151	-

Chemical shifts (δ) are reported in parts per million (ppm) and were calibrated with the residual proton signals of the solvents used [$\delta(CDCl_3)$ = 7.26 ppm (^{1}H), 77.16 ppm (^{13}C); $\delta(H_2O)$ = 4.79 ppm (^{1}H)]. Coupling constants (J) are reported in Herz (Hz) and signal multiplicities are reported in abbreviated form (Table 8).

Table 8: Multiplicity of NMR signals and their abbreviation. Combinations of the acronyms are used to describe Signals.

Abbreviation	Multiplicity
s	singlet
d	doublet
t	triplet
q	quartet
qi	quintet
sex	sextet
m	multiplet
br	broad signal

The software TOPSPIN by BRUKER was used for the analysis of spectra. The number of protons in each signal was determined via integration. For the full characterization of literature-unknown structures, 2D-NMR experiments (COSY, NOESY, HMBC, HSQC) were conducted.

Optical Rotation

Specific optical rotations $\left([\alpha]_D^T\right)$ were measured using a glass cuvette (volume = 1 mL, length = 1 dm) and the PERKIN-ELMER 341/343 series spectrometer and are reported in degrees $\left(^\circ = \frac{^\circ \cdot \text{mL}}{\text{g} \cdot \text{dm}}\right)$. Concentrations (c) are reported in $10\,\frac{\text{mg}}{\text{ml}}$ and measurements were performed at varying temperatures (T) with a sodium lamp (sodium-D-line, λ = 589 nm).

Microbiological Solutions and Nutrient Media

Buffer solutions and cultivation media were all prepared with deionized water. Cultivation media were autoclaved (15 min, 121 °C, 2 bar) before use (Table 9).

Table 9: Composition of various buffers and media utilized for the microbiological work.

Cultivation/nutrient media	
LB-medium/agar	
yeast extract	0.5 g/L
tryptone	1 g/L
NaCl	0.5 g/L
agar (if necessary)	1.5 g/L
2TY-medium	
yeast extract	1 g/L
tryptone	1.6 g/L
NaCl	0.5 g/L

Protein purification	
lysis buffer (pH 8)	
TRIS-HCl	40 mM
NaCl	100 mM
washing buffer (pH 8)	
TRIS-HCl	40 mM
NaCl	100 mM
imidazole	25 mM
elution buffer (pH 8)	
TRIS-HCl	40 mM
NaCl	100 mM
imidazole	500 mM

SDS-PAGE	
Lämmli-mix	
TRIS-HCl (pH 6.8)	150 mM
SDS	6 g/L
glycerol	30 g/L
bromophenol blue	0.02 g/L
Lämmli buffer	
Lämmli-mix	80 %
DTT	100 mM
SDS	1 g/L
10x running buffer	
TRIS-base	0.25 M
SDS	1 g/L
glycerol	1.92 M
dye solution	
Coomassie Brilliant Blue P250	0.1 g/L
*iso*propanol	25 %
acetic acid	10%
decolorizing solution	
*iso*propanol	25 %
acetic acid	10 %

Enzyme test reaction buffer	
HEPES	50 mM
DTT	5 mM
if necessary	
$MgCl_2$	5 mM
substrate	0.15 mM
pH	7.5

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis was performed to mainly identify biotransformation products. For measurements, samples were dissolved in GC-grade *n*-hexane and the solution was injected splitless. Measurements were conducted using a GC-MS instrument with an autosampler (Table 10) and the total measuring time (20 min) and injection volume (1 µL) were kept constant.

Table 10: Instruments and components used for GC-MS measurements.

Manufacturer	Agilent
Instrument	GC: 7890B, MS: 5977B
Columns (length, inner diameter, film thickness)	Optima 5HT (30 m, 0.25 mm, 0.25 µm)
Mass spectrometer	Quadrupol (mass range = 30–650 amu)
Detector	flame ionization detector, MS
Ion source	electron ionization, 70 eV
Injector	KAS 4 (60 °C–300 °C, 12 °C/s)
Temperature gradient	50 °C (1 min)–300 °C (6.5 min), 20 °C/s
Carrier gas	helium (rate = 15 mL/min)

In addition to retention times (t_R), retention indices (RI) are also reported because retention times shift over time, even when using the same equipment and the same conditions. For calibration, a mixture of *n*-alkanes with distinct retention values is measured in regular intervals. To calculate the retention indices of the substances the following equation (1) is used:

$$\text{(1):} \qquad RI = 100 \left[n + \frac{\log t_R(\text{sample}) - \log t_R(s)}{\log t_R(B) - \log t_R(s)} \right]$$

RI retention index
$t_R(s)$ retention time of the alkane eluting right before the sample
$t_R(B)$ retention time of the alkane eluting right after the sample
n number of carbons of the alkane eluting right before the sample

Chiral Gas Chromatography

Chiral gas chromatography was performed to determine the enantiomeric or diastereomeric excess of substances. The samples were manually injected into a GC device with different chiral columns (Table 11). The injection volume was kept constant (5 µL) but the temperature gradient, as well as the measuring time, were optimized for each sample.

Table 11: Instruments and parts used for chiral GC measurements.

Manufacturer	Agilent
Instrument	HP 6890 Series
Columns (length, inner diameter, film thickness)	Hydrodex-β-6-tbdm (25 m, 0.25 mm, 0.25 µm), Lipodex-G (25 m, 0.25 mm, 0.25 µm)
Detector	flame ionization detector
Injector	hot injection at 300 °C
Split	1:10
Carrier gas	helium

Preparative Gas Chromatography (pGC)

For the purification of biotransformation products, preparative GC was performed. The substances were dissolved in *n*-pentane. The injection volume was kept constant (1 µL) but the temperature gradient, as well as the measuring time, were optimized for each sample. The components of the GC device are listed below (Table 12).

Manufacturer	Agilent
Instrument	HP 6890 Series
Columns (length, inner diameter, film thickness)	Zebron ZB-1 (30 m, 0.53 mm, 3 µm)
Detector	flame ionization detector
Injector	hot injection
Split	splitless
Carrier gas	hydrogen

Table 12: Instruments and parts used for pGC measurements.

Other Instruments for Biological Work

Listed below are various instruments that have been used for the microbiological experiments (Table 13).

Table 13: A list of various instruments used for microbiological experiments.

Equipment	Unit name	Manufacturer
centrifuges	Vacuum Centrifuge Concentrator 5301	Eppendorf
	Microcentrifuge 5417R	Eppendorf
	Table Centrifuge MiniStar Silverline	VWR International
	Heraeus Megafuge 16R	Thermo Fisher Scientific
incubators	Shaking Incubator: Innova 44, Excella E24	New Brunswick Scientific
	Thermomixer Comfort 5355	Eppendorf
	Heratherm Incubator	Thermo Fisher Scientific
pipettes	Pipetman P2, P10, P100, P200, P1000	Gilson
electrophoresis	PerfectBlue Gel System Midi S	Peqlab Biotechnologie
	Consort Electrophoresis Power Supply E835	Sigma Aldrich
	Consort Electrophoresis Power Supply E833	Sigma Aldrich
	Gel Doc XR+ System	Bio-Rad Laboratories
	Gel chamber system Mini-PROTEAN Tetra Cell	Bio-Rad Laboratories
	Gel chamber system ComPhor Mini	Biozym Scientific
photometer	SPECTRAmax Plus^{384}	Molecular Devices
	Multiskan GO Microplate	Thermo Scientific
	DS-11+ Spectrophotometer	DeNovix
	FoodALYT Photometer	Omnilab

6.2 GENERAL MICROBIOLOGICAL PROCEDURES

6.2.1 HETEROLOGOUS EXPRESSION

Overexpression of the eight selected sesquiterpene cyclases (BcBot2, Cop4, Cyc1, GcoA, Hvs1, PenA, Tps32, Tri5) was performed in *E. coli* BL21 (DE3). The respective genes are encoded in a pET28a(+) vector containing a kanamycin resistance, T7 polymerase, a lac-operator, and polyhistidine (His)-tags. Except for PenA, which was expressed with a His-tag on both termini, all enzymes were expressed with an N-terminal His tag. All work steps were performed under a sterile environment using a clean bench and sterile equipment and containers. In addition, all deployed stock cultures were prepared in advance and stored at -80 °C.

Initially, a preculture was prepared by mixing LB-medium (4 mL), kanamycin (50 µg/mL, 4 µL), and a stock culture (50 µL). The preculture mixture was then incubated for 4 h at 37 °C and 180 rpm. At this stage, a portion of the preculture can be mixed with an aq. glycerol (30 %) solution and stored at -80 °C to be used as stock culture. To synthesize the main culture, the preculture (1 mL) was mixed with 2TY-medium (50 mL) and kanamycin (50 µg/mL,50 µL), and incubation at 37 °C and 180 rpm was continued until the required optical density value ($\geq$ 0.4,) was achieved (2–5 h). The optical density was measured with a photometer (λ = 600 nm) using 2TY-medium as a blank. The resulting cloudy suspension was then charged with IPTG (1 M, 50 µL) to induce protein expression and incubation was continued for 20–24 h at 16 °C and 180 rpm. After centrifugation (4500 rpm, 10 min, 4 °C), the supernatant was decanted and the obtained cell pellet was stored at -20 °C.

6.2.2 CELL DISRUPTION

The obtained cell pellet after heterologous expression was resuspended in lysis buffer (1 mL/100 mg pellet) and ultrasonically disrupted (amplitude: 45 %, 10 min, pulse/pause: 4 s/6 s). To remove all solid cell components, the resulting cloudy suspension was centrifuged (10 000 rpm, 4 °C) and the cell lysate was used in the following protein purification process. All vessels containing temperature-sensitive components were cooled with ice baths throughout the procedure.

6.2.3 IMMOBILIZED METAL CHELATE AFFINITY CHROMATOGRAPHY

Immobilized metal chelate affinity chromatography (IMAC) was used to purify the previously obtained proteins. For this purpose, a nickel-nitrilotriacetic acid-agarose (Ni-NTA-agarose) column was employed. Ni-NTA-agarose is a nickel-charged affinity resin that is generally utilized for the purification of recombinant proteins containing a hexahistidine-tag. Proteins that bind to the resin can be eluted with low pH buffers or aq. imidazole/histidine solutions.

The prepacked column (PROTINO by MACHEREY-NAGEL) was first washed with distilled water [3 column volumes (CV)] and then conditioned by washing with lysis buffer (5 CV). The cell lysate obtained after cell disruption was passed through the column twice and the eluate was collected. The proteins that bound to the column were then eluted with aq. imidazole solutions (fractions: 25/50/100/250/500 mM, 5 mL each). All fractions were collected, immediately cooled (0 °C) and a small portion was used for SDS-PAGE analysis. Alternatively, a Bradford rapid assay was performed to qualitatively examine protein concentrations. All fractions containing the desired enzymes were collected and combined, fractions with no proteins were discarded. Finally, the column was rinsed with an aq. imidazole solution (1 M, 2 CV) to completely remove any remaining proteins. Before storage, the column was washed with distilled water (2 CV) and then stored over an aq. ethanol solution (30 %) at 4 °C. All vessels containing temperature-sensitive components as well as all solutions and buffers were cooled with ice baths during all work steps.

Bradford assay: A small portion of the collected fractions (10 µL) were transferred to a 96-well microplate and a dye solution (1x-ROTI-NANOQUAT by CARLROTH, 140 µL) was added. Fractions containing proteins turned blue, the concentration was visually estimated from the intensity of the blue coloration.

6.2.4 CONCENTRATION AND DESALINATION

The fractions collected from IMAC were placed in a centrifugal filter unit (AMICON ULTRA-15 by MERCK, 30 kDa MWCO) and concentrated via centrifugation (4500 rpm, 30 min, 4 °C). The total volume obtained was dependent on the result of all previous steps (0.1–1 mL). To remove excess salts and other impurities, a desalting column was used. For this purpose, a column (CYTIVA PD-10 by FISHER SCIENTIFIC) containing cross-linked dextran gel (SEPHADEX G-25 MEDIUM) was first washed with distilled water (3 CV) and enzyme reaction buffer (2 CV). The protein solution was added onto the column, conditioned (2.5 mL) and eluted (3.5 mL) with enzyme reaction buffer, and collected in a centrifugal filter unit. After centrifugation (4500 rpm, 30 min, 4 °C), the concentrated and purified enzymes were fractionated into aliquots (≤200 µL). After the protein concentrations [c_{stock}(Enz)] were determined, the aliquots were stored at -80 °C and were ready for the following enzyme tests. Alternatively, the protein concentration was determined first, then separation into aliquots was performed. Before storage, the column was washed with distilled water (3 CV) and then stored over an aq. ethanol solution (20 %) at 4 °C.

Protein concentrations of the collected fractions were determined via UV-VIS spectroscopy. For this, the absorbance of the protein solutions (1 µL) was measured ($\lambda = 280$ nm). The protein-specific extinction coefficients determined by Oberhauser[95] were used to calculate the concentration of each fraction.

Table 14: The theoretical molecular weight and the extinction coefficient of the cyclases used. With these values, the enzyme concentration was determined using the integrated program of the photometer.

Enzyme	Extinction coefficient	Mass [kDa]
BcBot2	69330	47.43
Cop4	63830	41.86
Cyc1	86860	43.55
GCoA	54430	40.07
Hvs1	93170	66.48
PenA	60390	43.07
Tps32	87210	64.53
Tri5	79300	46.16

6.2.5 DISCONTINUED SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) is a method to separate denatured proteins according to their size and charge. The technique was used to analyze the proteins obtained from the heterologous expression experiments.

One day prior to electrophoresis, the stacking and separation gels (Table 15) were cast into molds and allowed to solidify/polymerize overnight at 4 °C. The next day, the sample proteins were diluted with Lämmli-buffer (100 µL) and denatured by heating (10 min, 95 °C). The employed size standard (COLOR PRESTRAINED PROTEIN STANDARD) was commercially obtained from NEW ENGLAND BIOLABS. To concentrate the samples in the stacking gel, electrophoresis was initially performed at a lower voltage (100 V) for 20 min. For the actual separation step, a higher voltage (150 V) was applied for 1.5 h. After completion, the gel was stained using the reported dye solution (1 h) and then treated with the decolorizing solution.

Table 15: Composition of the employed stacking and separating gel. The reported values allow the casting of two gels. APS and TEMED were added to initiate polymerization.

Components	Stacking gel (5 mL)	Separation gel (10 mL)
acrylamide-bis-acrylamide solution (30 %)	0.83 mL	5 mL
tris-base (1.5 M)	0.63 mL (pH 6.8)	2.5 mL (pH 8.8)
SDS	0.05 %	0.01 %
ammonium persulfate (APS)	0.05 %	0.01 %
tetramethylethylenediamine (TEMED)	5 µL	4 µL

6.2.6 ANALYTICAL *IN VITRO* ENZYME TESTS

Analytical enzyme *in vitro* assays (qualitative biotransformations) were conducted to determine the activity of the previously obtained sesquiterpene cyclases and to test their promiscuity toward a variety of unnatural substrates. The reactions were initially conducted in plastic vials. After some time, glass vessels were used, as it was found that plastic vessels released too many plasticizers and other impurities. Before each assay, the activity of the cyclases was tested with

positive controls (**10** as substrate). Additionally, negative controls were prepared when a new substrate was examined (incubation either without enzyme or substrate). For example, for one unnatural substrate that was tested with 8 enzymes, a total of 25 biotransformations were conducted: subtrate+enzyme (8×), **10**+enzyme (8×), only enzyme/substrate (9×). The enzyme assays were performed under the reported conditions (Table 16).

Table 16: Deployed conditions for *in vitro* analytical enzyme assays and composition of solutions and buffers used. V(RB) = Reaction buffer, V_{stock}(sub) = substrate stock solution.

Conditions per vial		**V(RB) = 25 mL**	
total volume	0.5 mL	HEPES	354 mg
HEPES	50 mM	DTT	19 mg
DTT	5 mM	$MgCl_2$	12 mg
$MgCl_2$	5 mM	H_2O	25 mL
enzyme	0.1 g/L	**V_{stock}(sub) = 0.2 mL**	
substrate	0.15 mM	c_{stock}(sub)	0.05 mol/L
pH	7.5	n_{stock}(sub)	0.01 mmol
		aq. NH_4HCO_3 (10 %)	0.2 mL

First, the reaction buffer and the substrate stock solution were prepared (Table 16) and added to each reaction vial. Depending on the concentration of the respective enzyme stock solution [c_{stock}(Enz)], the added volumes [V(RB), V_{stock}(sub)] had to be calculated for each batch. Then, the biotransformations were started by adding the purified enzymes [V_{stock}(Enz)] and the reaction solutions were incubated at 34 °C for 20–30 min. Afterward, the reaction solutions were cooled to 0 °C and *n*-hexane (100 µL) was added to each vial. After extraction with a vortex mixer (30 s), the resulting suspensions were centrifuged (6000 rpm, 4 °C, 30 s) to achieve phase separation. The organic phases (60 µL) were each removed with a pipette, transferred to vials with micro glass inserts, and analyzed via GC-MS.

6.2.7 SEMI-PREPARATIVE BIOTRANSFORMATION

The conditions and procedure for semi-preparative biotransformation were slightly changed compared to the analytical tests (Table 17). The main difference is the supplemental addition of polysorbate 20 (Tween 20) as a stabilizer and emulsifier as well as pyrophosphatase (PPase). Moreover, the work-up procedure and the order and method of reagent addition were changed.

Table 17: Deployed conditions for semi-preparative biotransformation and composition of solutions and buffers used. V(RB) = Reaction buffer, V_{stock}(sub) = substrate stock solution.

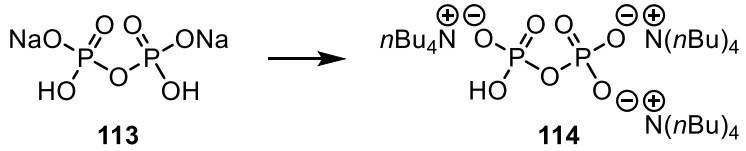
Conditions per batch		**V(RB) = 50 mL**	
total volume	50 mL	HEPES	708 mg
HEPES	50 mM	DTT	39 mg
DTT	5 mM	$MgCl_2$	71 mg
$MgCl_2$	15 mM	H_2O	50 mL
enzyme	0.2 g/L	Tween 20	5 µL
substrate	1.5 mM	PPase	1 µL
pH	7.5	**V_{stock}(sub) = 1.5 mL**	
Tween 20	0.01 %	c_{stock}(sub)	50 mmol/L
PPase	1 µL	n_{stock}(sub)	75 µmol
		aq. NH_4HCO_3 (10 %)	1.5 mL

First, the substrate stock solution and the reaction buffer were prepared and the latter was added to a round bottom flask. After adding the respective purified enzyme solution [V_{stock}(Enz), 0.1 g/L], the substrate stock solution was added continuously with a syringe pump (1 mL/h) at room temperature. After completion, the resulting colorless suspension was incubated at 34 °C and 150 rpm for 6 h. Then, another portion of enzyme solution (0.05 g/L) was added and incubation was continued for 16 h. After this time, a final portion of enzyme solution (0.05 g/L) was added and incubation was continued for 4 h. The reaction mixture was extracted with Et_2O and the phases separated. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure (≥800 mbar, 40 °C). The crude mixture was purified by column chromatography (silica, *n*-pentane:Et_2O) to yield the desired biotransformation products.

6.3 GENERAL CHEMICAL-SYNTHETIC PROCEDURES

6.3.1 TWO-STEP SYNTHESIS OF DIPHOSPHATES VIA ALLYL CHLORIDE INTERMEDIATES

Synthesis of 114: Tris(tetrabutylammonium)hydrogen pyrophosphate



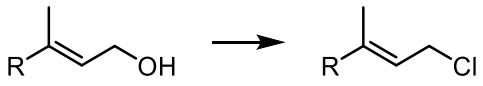
Disodium dihydrogen pyrophosphate (**113**) (8.88 g, 40.0 mmol, 1 eq.) was diluted with an aq. NH_4OH solution (10 %, 40 mL), loaded onto an ion-exchange column (DOWEX AG 50WX8 100–200 mesh, H^+ form) and eluted with H_2O until the eluate was pH-neutral. Then, the basic eluate was adjusted to pH 7 by titration with an aq. tetrabutylammonium hydroxide (40 %) solution and all solvents were removed under reduced pressure. The desired pyrophosphate salt **114** (35 g, 38.79 mol, 97 %) was obtained as a hygroscopic colorless solid and stored at -20 °C in a glove box. The analytical data agree with those reported in the literature.[53,56,60]

^{1}H-NMR (400 MHz, D_2O): δ = 3.21–3.17 (m, 24 H, Bu), 1.68–1.60 (m, 24 H, Bu), 1.35 (sex, J = 7.4 Hz, 24 H, Bu), 0.94 (t, J = 7.4 Hz, 36 H, Bu) ppm;
^{13}C-NMR (100 MHz, D_2O): δ = 58.1 (t, J = 2.8 Hz, Bu), 23.1 (Bu), 19.1 (t, J = 1.5 Hz, Bu), 12.8 (Bu) ppm;
^{31}P-NMR (160 MHz, D_2O): δ = -7.64 (s, 2 P) ppm.

Adjustment of the ion exchange column (DOWEX AG 50WX8, 100-200 mesh)

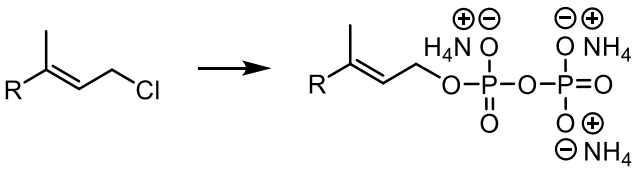
NH_4^+ form: Wash with a NH_3 solution [H_2O (200 mL) + NH_3 (conc., 50 mL)], then wash with H_2O until neutral.
H^+ form: Wash with aq. HCl (3 M), then wash with H_2O until neutral.

General Synthesis of Allyl Chlorides Starting from Allyl Alcohols



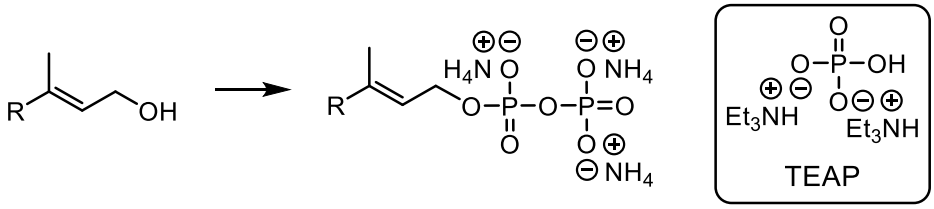
Dimethyl sulfide (2.7 eq.) was slowly added to a stirring solution of *N*-chlorosuccinimide (1 M, 2.5 eq.) in CH_2Cl_2 at -30 °C. The reaction mixture was warmed to 0 °C and stirred for 20 min. Then, after the mixture was cooled to -40 °C, the respective allyl alcohol (1 M, 1 eq.) in CH_2Cl_2 was added, and stirring at room temperature was continued until no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NaCl solution, the phases were separated, and the aqueous phase was extracted with *n*-pentane. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was used in the next step without further purification.

General Synthesis of Pyrophosphates Starting from Allyl Chlorides



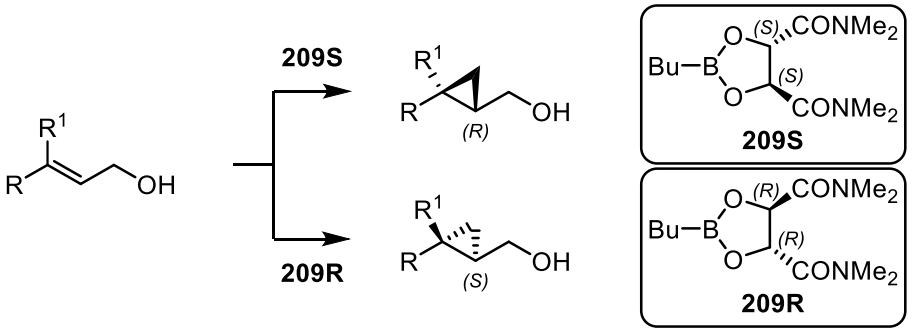
Freshly prepared allyl chloride (1 M, 1 eq.) was dissolved in CH_3CN and treated with pyrophosphate salt **114** (2 eq.) at room temperature. The reaction mixture was stirred at room temperature until no further conversion of the starting material was observed. After the solvent was removed under reduced pressure, the residue was diluted with ion-exchange buffer, loaded onto an ion-exchange column (DOWEX AG 50WX8 100-200 mesh, $NH4^+$ form), and eluted with ion-exchange buffer. The progress of the elution was monitored by TLC. After completion, the eluate was concentrated under reduced pressure and the resulting solid was dissolved in as little aq. NH_4HCO_3 (0.05 M) as possible. The mixture was transferred to a centrifugation vessel, diluted with CH_3CN:*i*-PrOH (1:1), and shaken vigorously. The resulting suspension was centrifuged (5000 rpm, 3 min), the supernatant (organic phase) was decanted, and the aqueous phase was again treated with CH_3CN:*i*-PrOH (1:1). This procedure was repeated until no precipitate formed upon the addition of the organic mixture. Finally, the combined organic phases were concentrated under reduced pressure to yield the desired salt.[56,60]

6.3.2 ONE-STEP SYNTHESIS OF DIPHOSPHATES STARTING FROM ALLYL ALCOHOLS



Before starting the phosphorylation reaction, a bis-triethylammonium phosphate (TEAP) solution had to be prepared. For this purpose, two solutions (A and B) were produced in advance. For solution A, phosphoric acid (conc., 1.5 mL) was mixed with acetonitrile (9.4 mL) resulting in a diluted phosphoric acid/acetonitrile (3 M) solution. Similarly, freshly distilled triethylamine (11 mL) was diluted with acetonitrile (10 mL) to obtain solution B (3.8 M). To prepare the desired TEAP (3.5 M) solution, solutions A (0.91 mL) and B (1.5 mL) were mixed and the mixture was stirred at 36 °C for 5–10 min. In a second flask, trichloroacetonitrile (25 eq.) was slowly added to the respective allyl alcohol (1 eq.) at room temperature. To this reaction mixture, freshly prepared TEAP (3.5 M, 27 eq.) was added in three portions at 5 min intervals. After the addition of the last portion, stirring was continued for 5 min and the crude mixture was purified by column chromatography (silica, *i*PrOH:NH_3(conc.):H_2O = 6:3:1) to yield the desired diphosphates.[92]

6.3.3 ASYMMETRIC CYCLOPROPANATION OF ALLYL ALCOHOLS

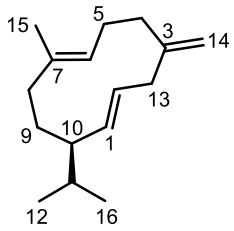


Diethylzinc (2 M, 2 eq.), DME (2.2 eq.), and CH_2Cl_2 were added to a two-neck flask and the mixture was cooled to 0 °C. (A two-neck flask with an inlet adapter was used for this reaction because diethylzinc vapors clog cannulas very quickly, which can lead to dangerous pressure increases during the reaction). A solution of diiodomethane (1 M, 2.2 eq.) in CH_2Cl_2 was added dropwise and stirring was continued for 30 min at 0 °C. In a second flask, the respective allyl alcohol (1 M, 1 eq.) and ligand (1 M, 1.5 eq.) were mixed in CH_2Cl_2 at 0 °C. The resulting solution was added quickly to the reaction flask and the mixture was allowed to warm to room temperature. Stirring was continued at room temperature until no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NH_4Cl solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were subsequently washed with aq. HCl (1 M), a sat. aq. $NaHCO_3$ and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:Et_2O = 10:1 → 4:1) to yield the desired cyclopropyl alcohol.[89,90]

6.4 SEMI-PREPARATIVE BIOTRANSFORMATION OF 90R WITH BCBOT2

Following the general procedure for semi-preparative biotransformation (p.80), three new cyclic terpenes were obtained using the sesquiterpene cyclase BcBot2 and diphosphate **90R** (33.5 mg, 75 µmol). Purification of the crude mixture via column chromatography (silica, *n*-pentane:Et_2O = 10:1 ⟶ 4:1 ⟶ 2:1) allowed separation of the triene **232** from the more polar alcohols **233** and **234**, which were obtained as a mixture (**233**:**234** = 2:3). Separation attempts via preparative GC were unsuccessful.

Biotransformation product 232: (*R*,1*E*,6*E*)-5-Isopropyl-2-methyl-9-methylenecycloundeca-1,6-diene

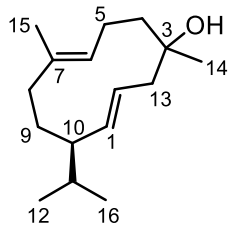


232

(*R*,1*E*,6*E*)-5-Isopropyl-2-methyl-9-methylenecycloundeca-1,6-diene (4 mg, 18 µmol, 24 %) was obtained as a highly volatile colorless oil with impurities.

R_f = 0.90 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.12–5.15 (m, 1 H, H-6), 5.11 (ddd, *J* = 15.1 Hz, 9.4 Hz, 5.5 Hz, 1 H, H-2), 5.02 (dd, *J* = 15.3 Hz, 9.5 Hz, 1 H, H-1), 4.81 (d, *J* = 8.2 Hz, 2 H, H-14), 2.73 (dd, *J* = 12.5 Hz, 5.4 Hz, 1 H, H-13), 2.48 (dd, *J* = 12.7 Hz, 9.4 Hz, 1 H, H-13), 2.28–2.32 (m, 1 H, H-5), 2.22–2.25 (m, 1 H, H-8), 2.12–2.15 (m, 1 H, H-4), 2.06–2.10 (m, 1 H, H-8), 1.94–1.96 (m, 1 H, H-4), 1.90–1.92 (m, 1 H, H-5), 1.84–1.87 (m, 1 H, H-10), 1.40–1.43 (m, 2 H, H-9), 1.49 (m, 4 H, H-11, H-15), 0.83 (d, *J* = 6.8 Hz, 3 H, H-12/16), 0.80 (d, *J* = 6.9 Hz, 3 H, H-12/16) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 150.1 (C-3) 135.2 (C-7), 133.5 (C-1), 128.9 (C-2), 127.3 (C-6), 111.2 (C-14), 53.1 (C-10), 43.4 (C-13), 41.6 (C-8), 33.6 (C-11), 34.2 (C-4), 29.6 (C-5), 20.8 (C-12), 28.2 (C-9), 15.9 (C-15), 19.0 (C-16) ppm.

Biotransformation product 233: (3*E*,5*R*,8*E*)-5-Isopropyl-1,8-dimethylcycloundeca-3,8-dien-1-ol

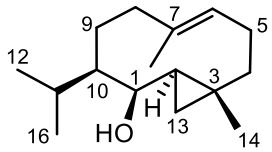


233

(3*E*,5*R*,8*E*)-5-Isopropyl-1,8-dimethylcycloundeca-3,8-dien-1-ol (**233**) (2 mg, 8.5 µmol, 11 %) was obtained as a volatile colorless oil.

R_f = 0.20 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.28 (ddd, *J* = 15.4 Hz, 10.0 Hz, 5.2 Hz, 1 H, H-2) 5.13 (dd, *J* = 15.4 Hz, 9.8 Hz, 1 H, H-1), 5.06–5.09 (m, 1 H, H-6), 2.26–2.33 (m, 1 H, H-5), 2.23–2.25 (m, 1 H, H-8), 2.18–2.19 (m, 1 H, H-13), 2.15–2.17 (m, 1 H, H-8), 2.01 (dd, *J* = 13.0 Hz, 10.1 Hz, 1 H, H-13), 1.89–1.95 (m, 1 H, H-10), 1.82 (m, 1 H, H-5), 1.80 (m, 1 H, H-4), 1.67–1.69 (m, 1 H, H-4), 1.57 (s, 3 H, H-15), 1.55 (br, 1 H, OH), 1.49–1.52 (m, 1 H, H-11), 1.36–1.40 (m, 2 H, H-9), 1.24 (m, 3 H, H-14), 0.83 (d, *J* = 6.8 Hz, 3 H, H-12/16), 0.80 (d, *J* = 6.9 Hz, 3 H, H-12/16) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 135.9 (C-1) 133.2 (C-7), 128.8 (C-2), 130.1 (C-6), 72.8 (C-3), 53.5 (C-10), 48.0 (C-13), 33.8 (C-11), 42.2 (C-8), 30.1 (C-14), 40.0 (C-4), 27.2 (C-9), 23.9 (C-5), 16.0 (C-15), 18.8 (C-12/16), 20.7 (C-12/16) ppm.

Biotransformation product 234: (1*R*,2*S*,3*R*,10*R*,*E*)-3-Isopropyl-6,10-dimethyl-bicyclo[8.1.0]undec-6-en-2-ol

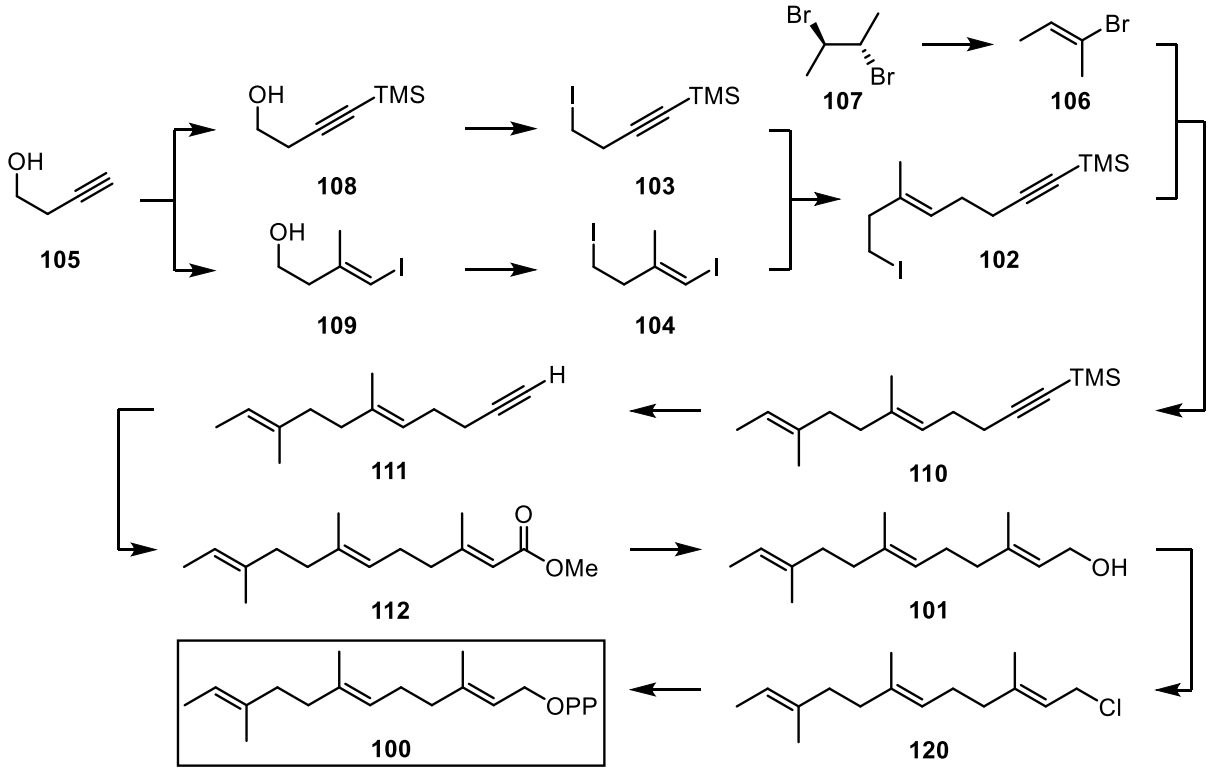


234

(1*R*,2*S*,3*R*,10*R*,*E*)-3-Isopropyl-6,10-dimethyl-bicyclo[8.1.0]undec-6-en-2-ol (**234**) (3 mg, 13 µmol, 17 %) was obtained as a volatile colorless oil.

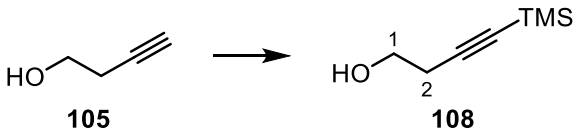
R_f = 0.20 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.32 (t, *J* = 7.6 Hz, 1 H, H-6) 3.36 (dd, *J* = 9.9 Hz, 1.7 Hz, 1 H, H-1), 2.20–2.25 (m, 2 H, H-5, H-8), 2.13–2.17 (m, 1 H, H-5), 1.84–1.88 (m, 1 H, H-4), 1.80 (m, 1 H, H-8), 1.73–1.76 (m, 1 H, H-9), 1.67–1.69 (m, 1 H, H-11), 1.64 (s, 3 H, H-15), 1.55 (br, 1 H, OH), 1.32–1.35 (m, 1 H, H-9), 1.05–1.08 (m, 1 H, H-10), 1.00 (d, *J* = 6.7 Hz, 3 H, H-12/16), 0.98 (s, 3 H, H-14), 0.93 (d, *J* = 6.6 Hz, 3 H, H-12/16), 0.85–0.87 (m, 1 H, H-4), 0.62 (ddd, *J* = 9.7 Hz, 8.3 Hz, 5.6 Hz, 1 H, H-2), 0.40 (dd, *J* = 8.3 Hz, 4.3 Hz, 1 H, H-13), 0.12 (dd, *J* = 5.5 Hz, 4.3 Hz, 1 H, H-13) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 133.2 (C-7) 124.0 (C-6), 74.7 (C-1), 48.4 (C-10), 39.2 (C-4), 38.8 (C-8), 32.2 (C-11), 30.0 (C-2), 25.6 (C-9), 24.2 (C-5), 21.7 (C-12/16), 21.3 (C-12/16), 18.8 (C-15), 18.5 (C-3), 17.3 (C-14), 17.2 (C-13) ppm.

6.5 SYNTHESIS OF METHYL SHIFTED FPP DERIVATIVE 100



Scheme 65: Overview of the forward synthesis of **100**.

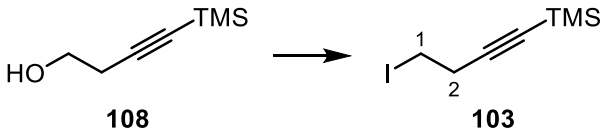
108: 4-(Trimethylsilyl)but-3-yn-1-ol



To a stirring solution of but-3-yn-1-ol (**105**) (10 g, 0.14 mol, 1 eq.) in THF (140 mL) was dropwise added *n*-butyllithium (2.5 M in hexane, 120 mL, 0.30 mol, 2.1 eq.). The mixture was warmed to room temperature and stirring was continued for 1 h. The resulting suspension was then cooled to -78 °C and trimethylsilyl chloride (40 mL, 0.30 mol, 2.1 eq.) was slowly added. After stirring for 2 h at -78 °C, no further conversion of the starting material was observed. The reaction was terminated by the addition of aq. HCl (1 M). The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 10:1) to yield the desired alcohol **108** (20.3 g, 0.14 mol, quant.) as a colorless oil.
The analytical data agree with those reported in the literature.[98]

R_f = 0.5 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 3.71 (t, J = 6.3 Hz, 2 H, H-1), 2.50 (t, J = 6.3 Hz, 2 H, H-2), 1.75 (bs, 1 H, OH), 0.16 (s, 9 H, TMS) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 103.4 (C-3), 87.2 (C-4), 61.0 (C-1), 24.4 (C-2), 0.21 (TMS) ppm.

103: (4-Iodobut-1-yn-1-yl)trimethylsilane

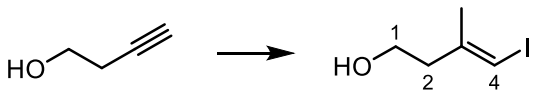


108 **103**

To alcohol **108** (3 g, 21.08 mmol, 1 eq.), triphenylphosphine (8.29 g, 31.62 mmol, 1.5 eq.), and imidazole (2.15 g, 31.62 mmol, 1.5 eq.) in CH_2Cl_2 (80 mL) was added iodine (8.03 g, 31.62 mmol, 1.5 eq.) at 0 °C and under exclusion of light. After stirring for 2 h at room temperature, no further conversion of the starting material was observed. To precipitate remaining triphenylphosphine and triphenylphosphine oxide, most of the CH_2Cl_2 was removed under reduced pressure and *n*-pentane was added. The solid impurities were removed via filtration and the filter cake was washed with *n*-pentane and Et_2O. This step was repeated until no further precipitation was observed upon addition of *n*-pentane. The combined organic phases were washed with a sat. aq. $Na_2S_2O_3$ and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE) to yield the desired iodide **103** (4.95 g, 19.64 mmol, 93 %) as a colorless oil.[99]
The analytical data are consistent with those reported in the literature.[100]

R_f = 0.35 (PE);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 3.22 (t, J = 7.5 Hz, 2 H, H-1), 2.79 (t, J = 7.5 Hz, 2 H, H-2), 0.16 (s, 9 H, TMS) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 105.2 (C-4), 86.9 (C-3), 25.2 (C-1), 1.2 (C-2), 0.1 (TMS) ppm.

109: (*E*)-4-Iodo-3-methylbut-3-en-1-ol

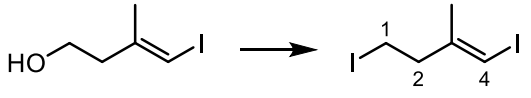


105 **109**

To zirconocene dichloride (83.4 mg, 0.29 mmol, 0.2 eq.) in CH_2Cl_2 (2 mL) was slowly added trimethylaluminium (2 M in hexane, 2.21 mL, 4.42 mmol, 3.1 eq.) at room temperature and the reaction mixture was stirred for 10 min. H_2O (0.04 mL, 2.21 mmol, 1.55 eq.) was then very carefully added at room temperature and stirring was continued for 10 min. In a separate flask, trimethylaluminium (0.25 mL, 0.49 mmol, 0.34 eq.) was slowly added to a stirring solution of 3-butyne-1-ol (**105**) (100 mg, 1.43 mmol, 1 eq.) in CH_2Cl_2 (2 mL) at 0 °C. This mixture was stirred for 20 min at 0 °C and then dropwise added to the reaction flask. After stirring for 18 h at room temperature, it was cooled to -25 °C and a solution of iodine (543 mg, 2.14 mmol, 1.5 eq.) in Et_2O (4 mL) was slowly added. Stirring was continued for 2 h at room temperature, after which no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. Na, K-tartrate solution and the resulting viscous mixture stirred for 16 h at room temperature. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. $Na_2S_2O_3$ and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 5:1) to yield the desired vinyl iodide **109** (240 mg, 1.13 mmol, 79 %) as a yellow oil.
The analytical data are consistent with those reported in the literature.[101]

R_f = 0.35 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 6.03 (s, 1 H, H-4), 3.73 (t, J = 6.2 Hz, 2 H, H-1), 2.49 (t, J = 6.2 Hz, 2 H, H-2), 1.88 (s, 3 H, CH_3) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 144.7 (C-3), 77.0 (C-4), 60.3 (C-1), 42.6 (C-2), 23.9 (CH_3) ppm.

104: (*E*)-1,4-Diiodo-2-methylbut-1-ene



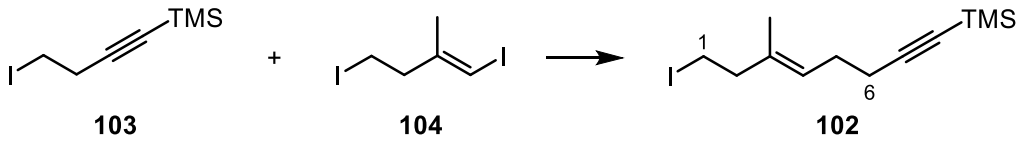
109 **104**

To vinyl iodide **109** (1 g, 4.72 mmol, 1 eq.), triphenylphosphine (1.86 g, 7.07 mmol, 1.5 eq.), and imidazole (481 mg, 7.07 mmol, 1.5 eq.) in CH_2Cl_2 (20 mL) was added iodine (1.79 g, 7.07 mmol, 1.5 eq.) at 0 °C and under exclusion of light. After stirring for 2 h at room

temperature, no further conversion of the starting material was observed. In order to precipitate remaining triphenylphosphine and triphenylphosphine oxide, most of the CH_2Cl_2 was removed under reduced pressure and *n*-pentane was added. The solid impurities were removed via filtration and the filter cake was washed with *n*-pentane and Et_2O. This step was repeated until no further precipitation was observed. The combined organic layers were washed with a sat. aq. $Na_2S_2O_3$ and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE) to yield the desired compound **104** (1.39 g, 4.32 mmol, 91 %) as a pink and light sensitive oil.
The analytical data are consistent with those reported in the literature.[57]

R_f = 0.6 (PE);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 6.07 (s, 1 H, H-4), 3.22 (t, J = 7.5 Hz, 2 H, H-1), 2.76 (t, J = 7.5 Hz, 2 H, H-2), 1.85 (s, 3 H, CH_3) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 146.1 (C-3), 77.9 (C-4), 43.4 (C-2), 23.3 (CH_3), 2.3 (C-1) ppm.

102: (*E*)-(8-Iodo-6-methyloct-5-en-1-yn-1-yl)trimethylsilane



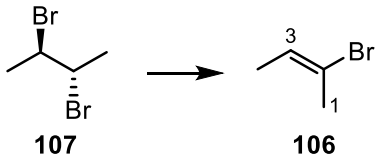
103 **104** **102**

t-Butyllithium (1.9 M in hexane, 0.76 mL, 1.44 mmol, 3.1 eq.) was dropwise added to a stirring solution of iodide **103** (176 mg, 0.70 mmol, 1.5 eq.) in Et_2O (0.7 mL) at -78 °C and stirring was continued for 30 min. Then, freshly flame-dried zinc bromide (157 mg, 0.70 mmol, 1.5 eq.) in THF (0.7 mL) was slowly added at -78 °C. The reaction mixture was warmed to 0 °C and stirring was continued for 30 min. In a separate flask, $Pd(dppf)Cl_2 \cdot (CH_2Cl_2)$ (7.6 mg, 0.01 mmol, 0.02 eq.) in THF (0.5 mL) was mixed with vinyl iodide **104** (150 mg, 0.47 mmol, 1 eq.) at room temperature. The resulting red suspension was stirred for 20 min and then added to the reaction flask. The reaction mixture was warmed to room temperature and stirred for 3°h, after which no further conversion of the starting material was observed. The reaction was terminated by the addition of H_2O, the phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE) to yield the desired iodide **102** (110 mg, 0.34 mmol, 74 %) as a light-yellow oil.
The analytical data are consistent with those reported in the literature.[57]

R_f = 0.3 (PE);

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.25 (m, 1 H, H-4), 3.22 (t, J = 7.6 Hz, 2 H, H-1), 2.54 (t, J = 7.6 Hz, 2 H, H-2), 2.25 (m, 4 H, 5-H, H-6), 1.63 (s, 3 H, CH_3), 0.14 (s, 9 H, TMS) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 135.2 (C-3), 125.7 (C-4), 107.2 (C-7), 84.7 (C-8), 43.9 (C-2), 27.5 (C-5), 20.2(C-6), 15.6 (CH_3), 4.8 (C-1), 0.3 (TMS) ppm.

106: (*E*)-2-Bromobut-2-ene

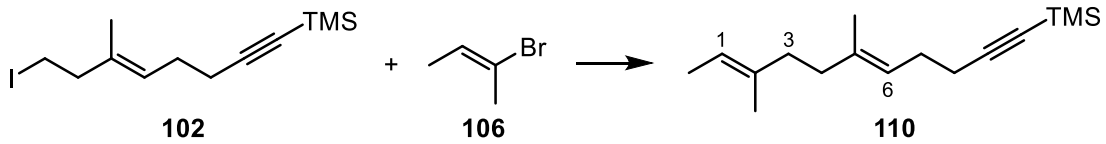


107 **106**

meso-2,3-Dibromobutane (**107**) (9.7 g, 44.93 mmol, 1 eq.) was dissolved in ethylene glycol (20 mL) and the reaction solution was heated to 120 °C. Potassium hydroxide (3.02 g, 56.11 mmol, 1.2 eq.) in ethylene glycol (50 mL) was slowly added and the reaction mixture was stirred under reflux conditions for 2 h. The product was isolated via distillation and dried over $MgSO_4$. Vinyl bromide **106** (4.9 g, 36.27 mmol, 81 %) was obtained as a highly volatile colorless oil.[59]
The analytical data are consistent with those reported in the literature.[102]

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.88 (qq, J = 7.1 Hz, 1.4 Hz, 1 H, H-3), 2.20 (qi, J = 1.2 Hz, 3 H, H-1), 1.61 (dq, J = 6.9 Hz, 1.2 Hz, 3 H, H-4) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 126.7 (C-3), 119.6 (C-2), 22.9 (C-1), 15.1 (C-4) ppm.

110: ((5*E*,9*E*)-6,9-Dimethylundeca-5,9-dien-1-yn-1-yl)trimethylsilane



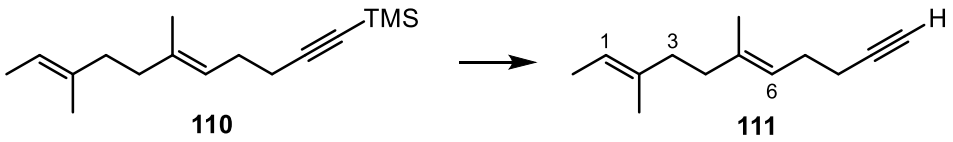
102 **106** **110**

To iodide **102** (1 g, 3.12 mmol, 1 eq.) in Et_2O (3 mL), was dropwise added *t*-butyllithium (1.9 M in hexane, 3.62 mL, 6.87 mmol, 2.2 eq.) at -78 °C and the resulting mixture stirred for 30 min. Then, freshly flame-dried zinc bromide (1.05 g, 4.68 mmol, 1.5 eq.) in THF (5 mL) was slowly added and stirring was continued for 30 min at 0 °C. In a separate flask, $Pd(dppf)Cl_2 \cdot (CH_2Cl_2)$ (127 mg, 0.16 mmol, 0.05 eq.) in THF (6 mL) was mixed with vinyl bromide **106** (843 mg, 6.24 mmol, 2 eq.) at room temperature. The resulting red suspension was

stirred for 20 min and then added to the reaction flask. The reaction mixture was warmed to room temperature and stirred for 4°h, after which no further conversion of the starting material was observed. The reaction was terminated by the addition of H_2O, the phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE) to yield the title compound **110** (458 mg, 1.84 mmol, 59 %) as a colorless oil.
The analytical data are consistent with those reported in the literature.[54]

R_f = 0.45 (PE);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.25–5.12 (m, 2 H, 1-H, H-6), 2.24–2.22 (m, 4 H, 2×CH_2), 2.08 (m, 4 H, 2×CH_2), 1.64 (m, 3 H, CH_3), 1.62 (m, 3 H, CH_3), 1.59 (d, J = 7 Hz, 3 H, CH_3-1), 0.17 (s, 9 H, TMS) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 136.9 (C-5), 135.9 (C-2), 122.6 (C-6), 118.4 (C-1), 107.6 (C-9), 84.4 (C-10), 38.5 (C-3, C-4), 27.5 (C-7), 20.5 (C-8), 16.3 (CH_3), 15.8 (CH_3), 13.5 (CH_3), 0.3 (TMS) ppm.

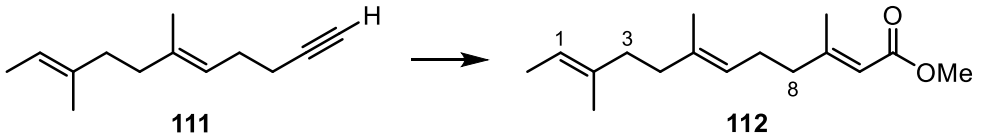
111: (5*E*,9*E*)-6,9-Dimethylundeca-5,9-dien-1-yne



110 **111**

To a stirring solution of diene **110** (50 mg, 0.20 mmol, 1 eq.) in aq. methanol (MeOH:H_2O = 9:1, 1 mL) was added potassium hydroxide (34 mg, 0.6 mmol, 3 eq.) at room temperature. After the reaction mixture was stirred for 4 h, no further conversion of the starting material was observed. The mixture was diluted with Et_2O, the phases were separated, and the aqueous phase was extracted with Et_2O. Then, the combined organic layers were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude colorless oil was used in the next step without further purification.

112: Methyl (2*E*,6*E*,10*E*)-3,7,10-trimethyldodeca-2,6,10-trienoate

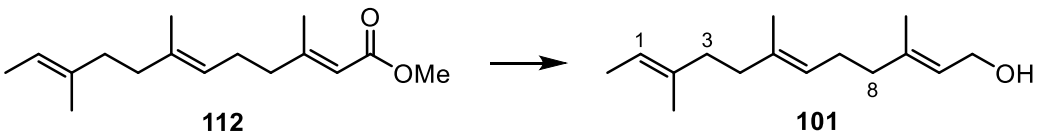


111 **112**

Trimethylaluminium (2 M in hexane, 0.2 mL, 0.4 mmol, 2 eq.) was slowly added to zirconocene dichloride (58 mg, 0.2 mmol, 1 eq.) in CH_2Cl_2 (1 mL) at 0 °C. After stirring for 10 min, alkyne **111** (35 mg, 0.2 mmol, 1 eq.) in CH_2Cl_2 (1 mL) was added at 0 °C and stirring was continued for 20 h at room temperature. Then, methyl chloroformate (0.08 mL, 0.99 mmol, 5 eq.) was slowly added and the reaction mixture was stirred for 16 h at room temperature, after which no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NH_4Cl solution. The phases were separated, the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 50:1 → 20:1) to yield ester **112** (38 mg, 0.15 mmol, 77 % o2s) as a colorless oil. The analytical data are consistent with those reported in the literature.[54]

R_f = 0.45 (PE:EtOAc = 50:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.66 (s, 1 H, H-10), 5.66 (q, J = 6.5 Hz, 1 H, H-1), 5.07 (m, 1 H, H-6), 3.68 (s, 3 H, OCH_3), 2.16–2.15 (m, 7 H, H-7, H-8, CH_3-9), 2.04 (m, 4 H, H-3, H-4), 1.59–1.58 (m, 6 H, CH_3-2, CH_3-5), 1.56 (m, 3 H, CH_3-1) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 167.4(CO_2CH_3), 160.3 (C-9), 136.5 (C-5), 135.9 (C-2), 122.9 (C-6), 118.4 (C-1), 115.3 (C-10), 50.9 (OCH_3), 41.1 (C-8), 38.5 (C-3, C-4), 38.5 (C-3, C-4), 26.1 (C-7), 19.0 (CH_3-9), 16.1 (CH_3-5), 15.8 (CH_3-2), 13.5 (CH_3-1) ppm.

101: (2*E*,6*E*,10*E*)-3,7,10-Trimethyldodeca-2,6,10-trien-1-ol

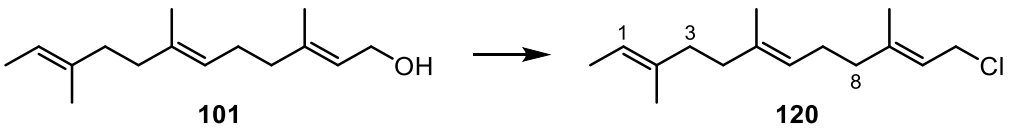


112 **101**

A stirring solution of methyl ester **112** (100 mg, 0.40 mmol, 1 eq.) in CH_2Cl_2 (2 mL) was cooled to 0 °C and slowly treated with di*iso*butylaluminium hydride (0.88 mL, 0.88 mmol, 2.2 eq.). After stirring the reaction mixture at room temperature for 2 h, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. K, Na-tartrate solution and stirring was continued for 18 h. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 10:1 → 5:1) to yield allyl alcohol **101** (70 mg, 0.31 mmol, 80 %) as a colorless oil.
The analytical data are consistent with those reported in the literature.[54]

R_f = 0.55 (PE:EtOAc = 3:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.41 (tq, *J* = 6.9 Hz, 1.3 Hz, 1 H, H-10), 5.19 (q, *J* = 6.6 Hz, 1 H, H-1), 5.10 (t, *J* = 6.7 Hz, 1 H, H-6), 4.15 (d, *J* = 6.9 Hz, 2 H, H-11), 2.13–2.01 (m, 8 H, H-3, H-4, H-7, H-8), 1.68 (m, 3 H, CH_3-9), 1.59 (m, 6 H, CH_3-2, CH_3-5), 1.56 (d, *J* = 6.6 Hz, 3 H, CH_3-1) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 140.0 (C-9), 136.0 (C-5), 135.7 (C-2), 123.8 (C-10), 123.5 (C-6), 118.4 (C-1), 115.3 (C-10), 59.6 (C-11), 39.7 (C-8), 38.6 (C-3, C-4), 38.5 (C-3, C-4), 26.4 (C-7), 16.4 (CH_3-9), 16.1 (CH_3-5), 15.8 (CH_3-2), 13.5 (CH_3-1) ppm.

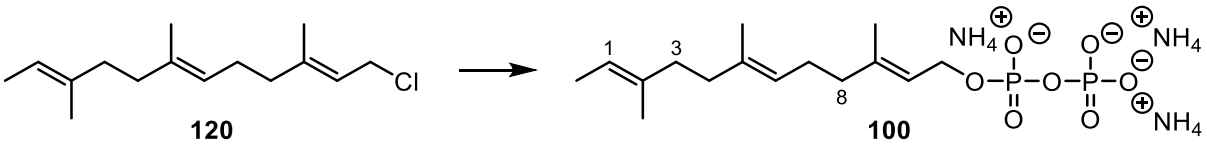
120: (2*E*,6*E*,10*E*)-1-Chloro-3,7,10-trimethyldodeca-2,6,10-triene



101 **120**

Allyl chloride **120** was prepared according to the general procedure for the synthesis of allyl chlorides (p. 76) starting from allyl alcohol **101** (60 mg, 0.27 mmol, 1 eq.) and was obtained as a yellow oil.
R_f = 0.8 (PE:EtOAc = 4:1).

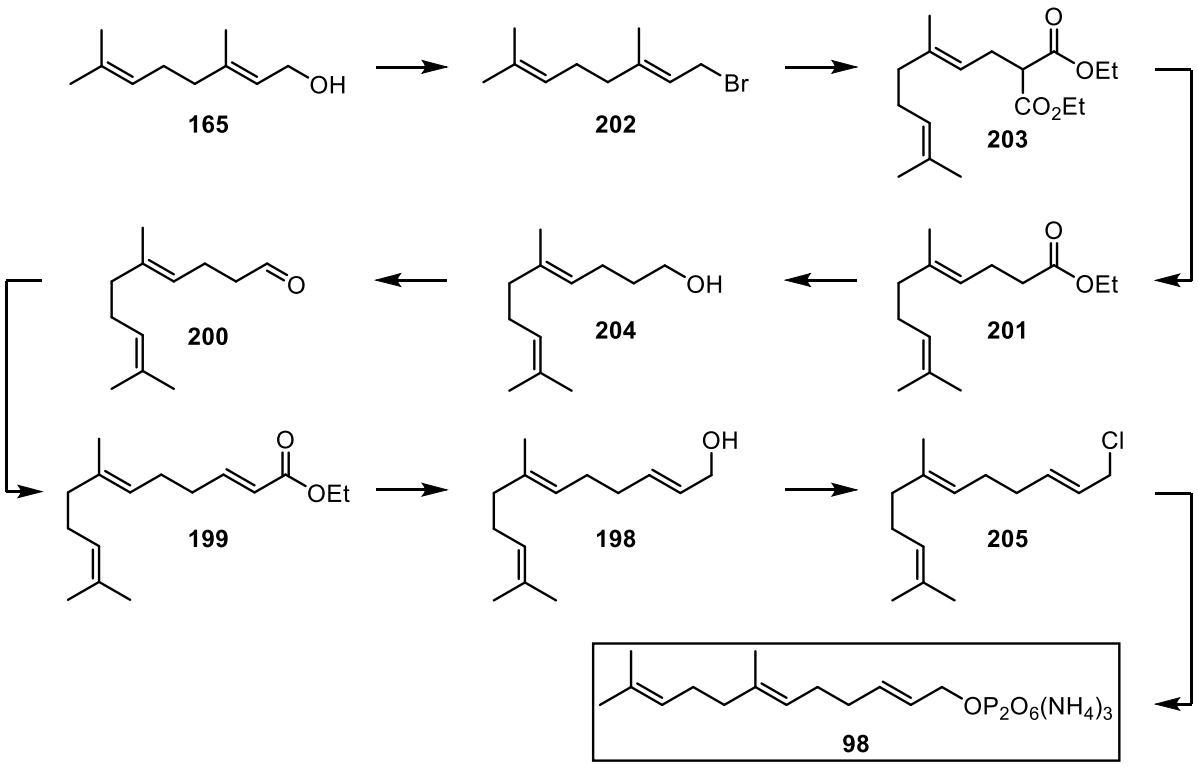
100: (2*E*,6*E*,10*E*)-3,7,10-Trimethyldodeca-2,6,10-trien-1-yl diphosphate



Allyl diphosphate **100** was prepared according to the general two-step procedure for the synthesis of pyrophosphates (p. 76) starting from allyl chloride **120** (64 mg, 0.27 mmol, 1 eq.). Pyrophosphate **100** (86 mg, 0.20 mmol, 75 % o2s) was obtained as a colorless solid with impurities of pyrophosphate salt **114**.

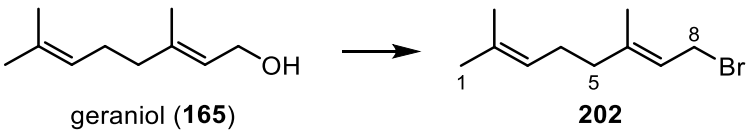
^{1}H-NMR (400 MHz, D_2O): δ = 5.38 (t, J = 6.6 Hz, 1 H, H-10), 5.21 (q, J = 6.6 Hz, 1 H, H-1), 5.14 (t, J = 6.8 Hz, 1 H, H-6), 4.39 (t, J = 6.6 Hz, 2 H, H-11), 2.12–2.05 (m, 8 H, H-3, H-4, H-7, H-8), 1.63 (m, 3 H, CH_3-9), 1.58–1.46 (m, 9 H, CH_3-1, CH_3-2, CH_3-5) ppm;
^{13}C-NMR (500 MHz, D_2O): δ = 142.7 (C-9), 137.2 (C-5), 136.8 (C-2), 124.3 (C-6), 119.9 (d, C-10), 119.0 (C-1), 62.4(d, C-10), 38.8 (C-8), 37.4 (d, C-3, C-4), 25.6 (C-7), 15.6 (CH_3-5), 15.1 (CH_3-9), 14.7 (CH_3-2), 12.6 (CH_3-1) ppm;
^{31}P-NMR (160 MHz, D_2O): δ = -6.30 (d, J = 22.2 Hz, 1 P, terminal P), -10.04 (dt, J = 22.2 Hz, 5.9 Hz, 1 P, RO-PO$_3$) ppm.

6.6 SYNTHESIS OF DESMETHYL FPP DERIVATIVE 98



Scheme 66: Overview of the forward synthesis of desmethyl FPP derivative **98** starting from **165**.

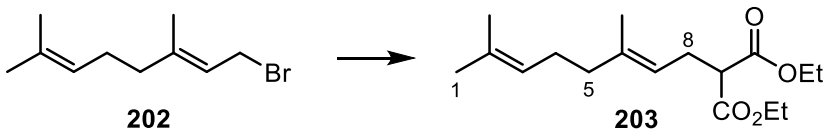
202: (*E*)-1-Bromo-3,7-dimethylocta-2,6-diene



Phosphorus tribromide (4.93 mL, 0.05 mol, 0.4 eq.) was added dropwise to a stirring solution of geraniol (**165**) (20 g, 0.13 mol, 1 eq.) in dry THF (60 mL) at 0 °C. After stirring for 1 h at 0 °C, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. $NaHCO_3$ solution. The phases were separated and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The obtained colorless oil was used in the next reaction without further purification. The analytical data are consistent with those reported in the literature.[88]

R_f = 0.8 (PE:EtOAc = 4:1);

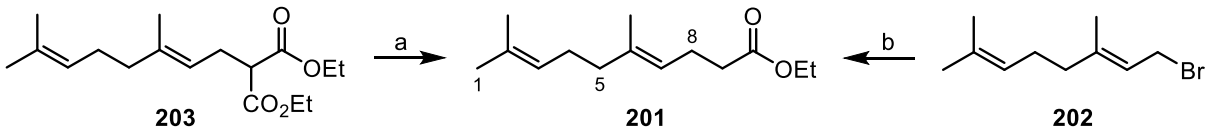
203: Diethyl (*E*)-2-(3,7-dimethylocta-2,6-dien-1-yl)malonate



To a solution of K_2CO_3 in DMF (0.5 M, 25 mL) was added diethyl malonate (2.07 g, 12.89 mmol, 1 eq.) and geranyl bromide (**202**) (2.8 g, 12.89 mmol, 1 eq.) at room temperature. After stirring for 18 h at room temperature, no further conversion of the starting material was observed. The reaction was terminated by the addition of aq. HCl (1 M). The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with H_2O and a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The obtained crude colorless oil was used in the next reaction without further purification.[88]

R_f = 0.6 (PE:EtOAc = 4:1);

201: Ethyl (*E*)-5,9-dimethyldeca-4,8-dienoate

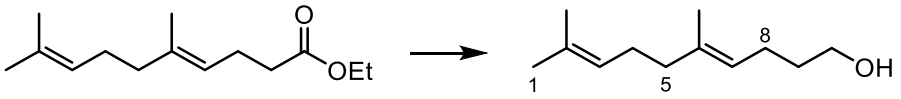


a): To a stirring solution of crude diester **203** (1.59 g, 5.36 mmol, 1 eq.) in DMSO (25 mL) was added H_2O (125.5 mg, 6.97 mmol, 1.3 eq.) and lithium chloride (0.58 g, 13.68 mmol, 2.55 eq.) at room temperature. After heating to 160 °C and stirring the reaction mixture for 18 h under refluxing conditions, no further conversion of the starting material was observed. The crude mixture was cooled to room temperature and extracted with Et_2O. The combined organic phases were washed with H_2O and a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 20:1) to yield the desired ester **201** (314 mg, 1.4 mmol, 26 % o3s) as a yellow oil.[55]

b): *n*-Butyllithium (1.6 M in hexane, 4.26 g, 66.45 mmol, 3.05 eq.) was added to freshly-distilled di*iso*propylamine (6.67 g, 66.45 mmol, 2.05 eq.) in dry THF (80 mL) at 0 °C. The mixture was stirred at the same temperature for 1 h to yield lithium di*iso*propylamide. In a second flask, EtOAc (5.85 g, 66.45 mmol, 2.05 eq.) was added to a suspension of copper(I)-iodide (24.69 g, 129.7 mmol, 4 eq.) in THF (240 mL) at -110 °C. The freshly prepared lithium di*iso*propylamide mixture was added to the second flask at -100 °C and stirring was continued for 1.5 h at -50 °C. Then, freshly prepared geranyl bromide (**202**) (7.04 g,

32.41 mmol, 1 eq.) in THF (60 mL) was added and stirring was continued for 2 h at -30 °C, after which no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NH_4Cl solution. The phases were separated and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The obtained crude yellow oil was used in the next reaction without further purification.
The analytical data are consistent with those reported in the literature.[87]
R_f = 0.45 (PE:EtOAc = 20:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.13–5.05 (m, 2 H, H-3, H-7), 4.12 (q, J = 7.1 Hz, 2 H, CO_2-CH_2), 2.32–2.30 (m, 4 H, H-8, H-9), 2.08–1.94 (m, 4 H, H-4, H-5), 1.67 (m, 3 H, H-1), 1.62-1.57 (m, 6 H, 2×CH_3), 1.25 (t, J = 7.1 Hz, 3 H, CO_2CH_2-CH_3) ppm.

204: (*E*)-5,9-Dimethyldeca-4,8-dien-1-ol

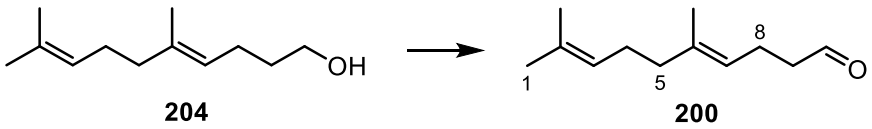


201 **204**

Di*iso*butylaluminium hydride (1 M in hexane, 10.41 g, 71.30 mmol, 2.2 eq.) was slowly added to a solution of ester **201** (7.27 g, 31.41 mmol, 1 eq., 1 M) in dry Et_2O at -78 °C. After stirring for 18 h at room temperature, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. Na, K-tartrate solution. Stirring was continued for 16 h at room temperature, after which the phases were separated and the aqueous phase extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 5:1) to yield the desired alcohol **204** (2.43 g, 13.33 mmol, 41 % o3s starting from **165**) as a colorless oil.
The analytical data are consistent with those reported in the literature.[55,87]

R_f = 0.3 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.17–5.13 (m, 1 H, H-3/7), 5.10–5.06 (m, 1 H, H-3/7), 3.65 (t, J = 6.3 Hz, 2 H, H-10), 2.11–2.05 (m, 4 H, H-4, H-5), 2.07–1.96 (m, 2 H, H-8), 1.68 (m, 3 H, H-1), 1.64–1.59 (m, 8 H, H-9, 2×CH_3), 1.41 (bs, 1 H, OH) ppm.

200: (*E*)-5,9-Dimethyldeca-4,8-dienal

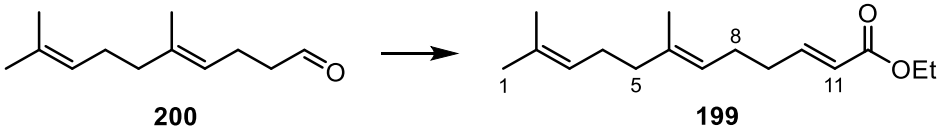


204 **200**

To a stirring solution of oxalyl chloride (0.34 mL, 3.92 mmol, 1.5 eq.) in CH_2Cl_2 (3.9 mL) was slowly added DMSO (0.56 mL, 7.83 mmol, 3 eq.) at -78 °C and stirring was continued for 1.5 h. Then, alcohol **204** (476 mg, 2.61 mmol, 1 eq.) in CH_2Cl_2 (1.3 mL) was slowly added. After stirring the mixture for 2 h at -78 °C, freshly-distilled triethylamine (1.81 mL, 13.05 mmol, 5 eq.) was slowly added. The reaction mixture was allowed to warm to room temperature and stirred for 14 h, after which no further conversion of the starting material was observed. The reaction was terminated by the addition of H_2O, the phases were separated, and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat aq. NH_4Cl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:Et_2O = 20:1 → 4:1) to yield the desired aldehyde **200** (375 mg, 2.09 mmol, 80 %) as a colorless oil.[103]
The analytical data are consistent with those reported in the literature.[87]

R_f = 0.5 (PE:EtOAc = 10:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 9.76 (t, J = 1.8 Hz, 1 H, H-10), 5.12–5.05 (m, 2 H, H-3, H-7), 2.48–2.43 (m, 2 H, H-9), 2.36–2.30 (m, 2 H, H-8), 2.07–1.96 (m, 4 H, H-4, H-5), 1.68 (m, 3 H, H-1), 1.62–1.59 (m, 6 H, 2×CH_3) ppm.

199: **Ethyl (2*E*,6*E*)-7,11-dimethyldodeca-2,6,10-trienoate**



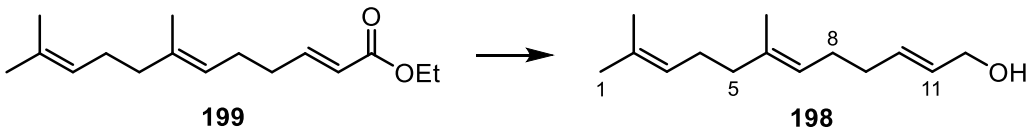
200 **199**

To a stirring solution of aldehyde **200** (187 mg, 1.04 mmol, 1 eq.) in toluene (2 mL) was added ethyl (triphenylphosphoranylidene)acetate (542 mg, 1.56 mmol, 1.5 eq.) at room temperature. After stirring for 18 h, no further conversion of the starting material was observed. The solvent was removed under reduced pressure and the crude mixture was dry loaded onto a column. After purification via column chromatography (silica, PE:Et_2O = 20:1), the desired ester **199** (174 mg, 0.69 mmol, 67 %) was obtained as a colorless oil.
The analytical data are consistent with those reported in the literature.[55]

R_f = 0.55 (PE:EtOAc = 10:1);

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 6.96 (dt, J = 15.6 Hz, 6.7 Hz, 1 H, H-10), 5.82 (d, J = 15.6 Hz, 1 H, H-11), 5.13–5.07 (m, 2 H, H-3, H-7), 4.18 (q, J = 7.1 Hz, 2 H, CO_2-CH_2), 2.26–2.14 (m, 2 H, H-8, H-9), 2.09–1.96 (m, 4 H, H-4, H-5), 1.68 (s, 3 H, H-1), 1.60 (s, 6 H, 2×CH_3), 1.28 (t, J = 7.1 Hz, 3 H, CO_2CH_2-CH_3) ppm.

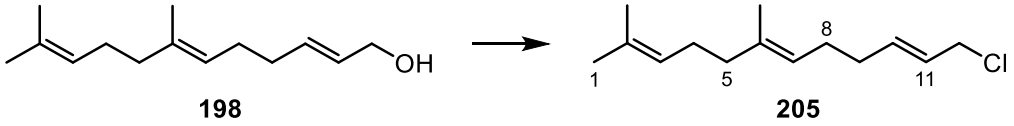
198: **(2*E*,6*E*)-7,11-Dimethyldodeca-2,6,10-trien-1-ol**



To a stirring solution of ester **199** (350 mg, 1.4 mmol, 1 eq.) in Et_2O (3.5 mL) was dropwise added di*iso*butylaluminium hydride (1 M in hexane, 3.08 mL, 3.08 mmol, 2.2 eq.) at -78 °C. Stirring was continued for 18 h at room temperature, after which full conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. Na, K-tartrate solution. After stirring the mixture for 16 h at room temperature, the phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:Et_2O = 5:1) to yield the desired alcohol **198** (207 mg, 0.99 mmol, 71 %) as a colorless oil.
The analytical data are in agreement with the literature data.[55]

R_f = 0.25 (PE:EtOAc = 10:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.73–5.62 (m, 2 H, H-10, H-11), 5.12–5.07 (m, 2 H, H-3, H-7), 4.09 (d, J = 5.1 Hz, 2 H, H-12), 2.09–1.96 (m, 8 H, H-4, H-5, H-8, H-9), 1.68 (s, 3 H, H-1), 1.60 (s, 6 H, 2×CH_3) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 135.7 (C-6), 133.3 (C-11), 131.5 (C-2), 129.2 (C-10), 124.4 (C-7), 123.8 (C-3), 64.0 (C-12), 39.8 (C-5), 32.6 (C-9), 27.7 (C-8), 26.8 (C-4), 25.8 (C-1), 17.8 (CH_3-2), 16.2 (CH_3-6) ppm.

205: (2*E*,6*E*)-1-Chloro-7,11-dimethyldodeca-2,6,10-triene

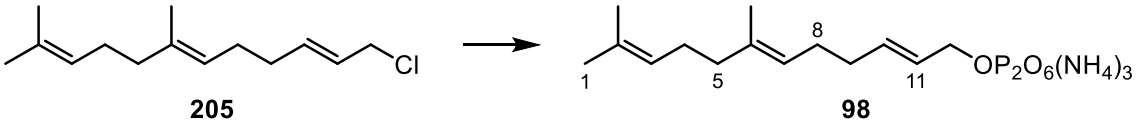


198 **205**

Allyl chloride **205** was prepared according to the general procedure for the synthesis of allyl chlorides (p. 76) starting from allyl alcohol **198** (50 mg, 0.24 mmol, 1 eq.) and was obtained as a yellow oil.

R$_f$ = 0.75 (PE:EtOAc = 4:1);

98: Ammonium (2*E*,6*E*)-7,11-dimethyldodeca-2,6,10-trien-1-yl diphosphate

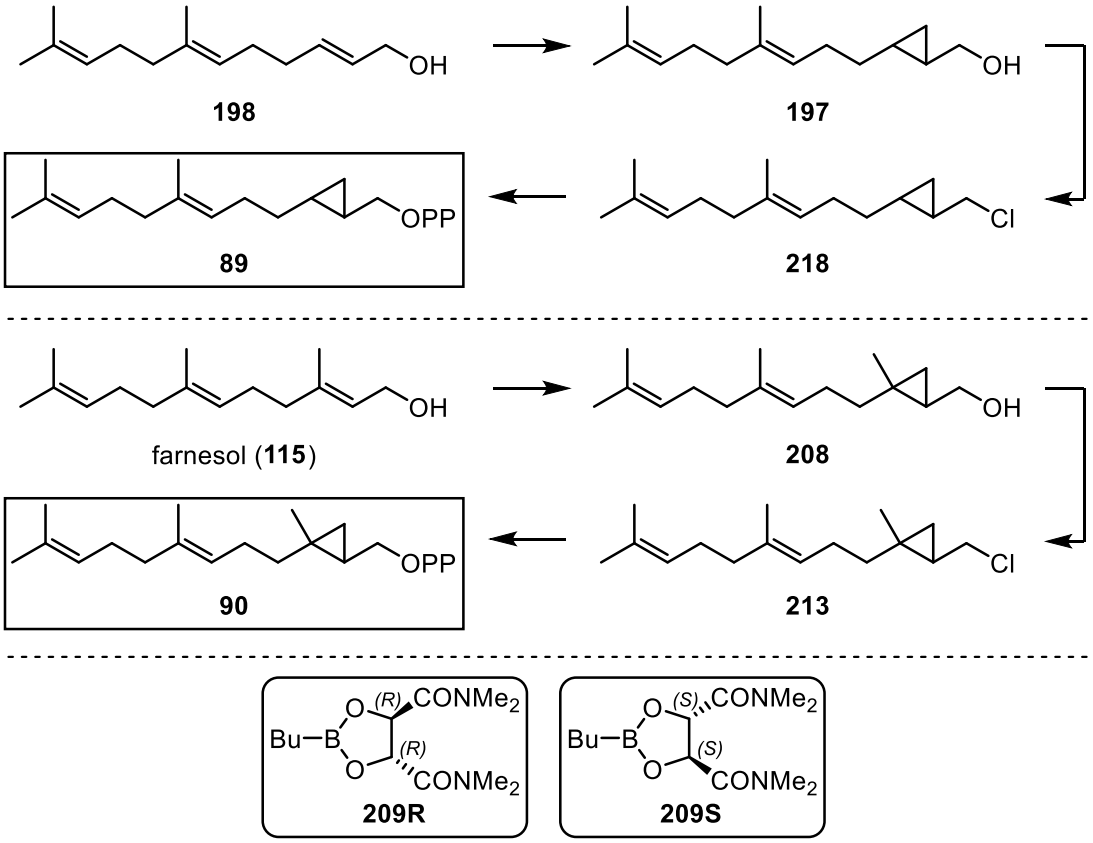


205 **98**

Allyl diphosphate **98** was prepared according to the general two-step procedure for the synthesis of pyrophosphates (p. 76) starting from allyl chloride **205** (50 mg, 0.24 mmol, 1 eq.). Pyrophosphate **98** (100 mg, 0.24 mmol, quant. o2s) was obtained as a colorless solid.

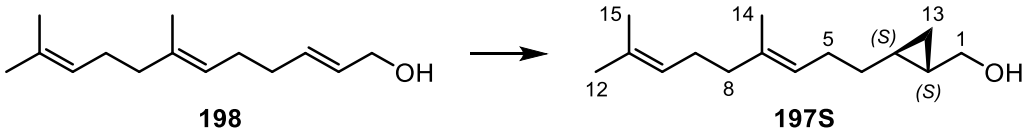
^{1}H-NMR (400 MHz, D_2O): δ = 5.77–5.74 (m, 1 H, H-10/11), 5.61–5.54 (m, 1 H, H-10/11), 5.13–5.07 (m, 2 H, H-3, H-7), 4.27 (t, *J* = 6.2 Hz, 2 H, H-12), 2.00–1.90 (m, 8 H, H-4, H-5, H-8, H-9), 1.61 (m, 9 H, 3×CH_3) ppm;
^{13}C-NMR (100 MHz, D_2O): δ = 136.8 (C-6), 135.5 (C-11), 133.5 (C-2), 125.7 (C-10), 124.4 (C-7), 124.1 (C-3), 66.8 (C-12), 38.7 (C-5), 31.7 (C-9), 26.7 (C-8), 25.7 (C-4), 24.8 (C-1), 16.9 (CH_3-2), 15.2 (CH_3-6) ppm;
^{31}P-NMR (160 MHz, D_2O): δ = -6.38 (d, *J* = 21.5 Hz, 1 P), -10.23 (d, *J* = 21.4 Hz, 1 P) ppm.

6.7 SYNTHESES OF CYCLOPROPYL DIPHOSPHATES 89S/R AND 90S/R



Scheme 67: Overview of the forward synthesis of **89** and **90**. The compounds are shown without stereo information but were synthesized asymmetrically using the ligands shown.

197S: ((1*S*,2*S*)-2-((*E*)-4,8-Dimethylnona-3,7-dien-1-yl)cyclopropyl)methanol



Starting from allyl alcohol **198** (100 mg, 0.48 mmol, 1 eq.), the desired cyclopropyl alcohol **197S** was prepared according to the general procedure for the asymmetric cyclopropanation of allyl alcohols (p. 79) using (4*R*,5*R*)-2-butyl-N^4,N^4,N^5,N^5-tetramethyl-1,3,2-dioxaborolane-4,5-dicarboxamide (**209R**, 194.5 mg, 1.06 mmol, 1.5 eq.). Purification via column chromatography (silica, PE:EtOAc = 10:1 → 7:1) delivered alcohol **197S** (92 mg, 0.41 mmol, 89 %) as a colorless oil with minor impurities.

R_f = 0.3 (PE:EtOAc = 4:1);
$[\alpha]^{24}_D$ = 7.2° (c = 1.1, $CHCl_3$);

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.15 (t, J = 13.4 Hz, 1 H, H-6), 5.09 (t, J = 6.8 Hz, 1 H, H-10), 3.45 (d, J = 7.1 Hz, 2 H, H-1), 2.11–2.04 (m, 4 H, H-5, H-9), 2.00–1.95 (m, 2 H, H-8), 1.67 (s, 3 H, H-12), 1.61 (s, 3 H, H-14), 1.60 (s, 3 H, H-15), 1.29–1.20 (m, 2 H, H-4), 0.89–0.83 (m, 1 H, H-2), 0.65–0.57 (m, 1 H, H-3), 0.39–0.30 (m, 2 H, H-13) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 135.4 (C-7), 131.5 (C-11), 124.5 (C-6), 124.4 (C-10), 67.4 (C-1), 39.9 (C-8), 33.9 (C-4), 28.1 (C-5/9), 26.8 (C-5/9), 25.8 (C-12), 21.5 (C-2), 17.8 (C-15), 17.1 (C-3), 16.1 (C-14), 10.1(C-13) ppm.

197R: ((1*R*,2*R*)-2-((*E*)-4,8-Dimethylnona-3,7-dien-1-yl)cyclopropyl)methanol

198 **197R**

Starting from allyl alcohol **198** (100 mg, 0.48 mmol, 1 eq.), the desired cyclopropyl alcohol **197R** was prepared according to the general procedure for the asymmetric cyclopropanation of allyl alcohols (p. 79) using (4*S*,5*S*)-2-butyl-N^4,N^4,N^5,N^5-tetramethyl-1,3,2-dioxaborolane-4,5-dicarboxamide (**209S**, 194.5 mg, 1.06 mmol, 1.5 eq.). Purification via column chromatography (silica, PE:EtOAc = 10:1 → 5:1) delivered alcohol **197R** (61 mg, 0.27 mmol, 73 %) as a colorless oil with minor impurities.

R_f = 0.3 (PE:EtOAc = 4:1);
$[\alpha]^{24}_D$ = -8.4° (c = 1.0, $CHCl_3$);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.15 (t, J = 13.7 Hz, 1 H, H-6), 5.09 (t, J = 6.8 Hz, 1 H, H-10), 3.43 (d, J = 7.1 Hz, 2 H, H-1), 2.11–2.04 (m, 4 H, H-5, H-9), 2.00–1.95 (m, 2 H, H-8), 1.67 (s, 3 H, H-12), 1.61 (s, 3 H, H-14), 1.60 (s, 3 H, H-15), 1.29–1.20 (m, 2 H, H-4), 0.91–0.81 (m, 1 H, H-2), 0.65–0.57 (m, 1 H, H-3), 0.39–0.30 (m, 2 H, H-13) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 135.4 (C-7), 131.5 (C-11), 124.5 (C-6), 124.4 (C-10), 67.4 (C-1), 39.9 (C-8), 33.9 (C-4), 28.1 (C-5/9), 26.8 (C-5/9), 25.8 (C-12), 21.5 (C-2), 17.8 (C-15), 17.1 (C-3), 16.1 (C-14), 10.1(C-13) ppm.

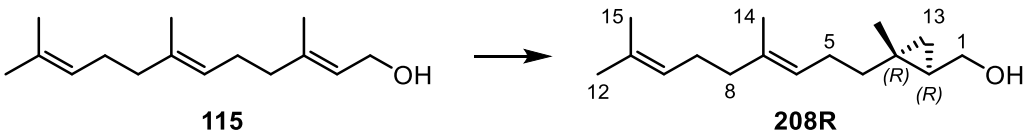
208S: ((1*S*,2*S*)-2-((*E*)-4,8-Dimethylnona-3,7-dien-1-yl)-2-methylcyclopropyl)methanol

115 **208S**

Starting from farnesol (**115**) (300 mg, 1.35 mmol, 1 eq.), the desired cyclopropyl methyl alcohol **208S** was prepared according to the general procedure for the asymmetric cyclopropanation of allyl alcohols (p. 79) using (4*R*,5*R*)-2-butyl-N^4,N^4,N^5,N^5-tetramethyl-1,3,2-dioxaborolane-4,5-dicarboxamide (**209R**, 546.6 mg, 2.02 mmol, 1.5 eq.) and CH_2I_2 (4 eq.). Purification via column chromatography (silica, PE:EtOAc = 10:1 → 4:1) delivered alcohol **208S** (318.9 mg, 1.35 mmol, quant.) as a colorless oil.
The analytical data are consistent with those reported in the literature.[89]

R_f = 0.3 (PE:EtOAc = 4:1);
$[\alpha]^{24}_D$ = 6.7° (c = 1.0, $CHCl_3$; Lit.: 5.5°, c = 3.5, $CHCl_3$);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.14–5.07 (m, 2 H, H-6, H-10), 3.71 (dd, *J* = 11.4 Hz, 6.6 Hz, 1 H, H-1), 3.50 (dd, *J* = 11.4 Hz, 8.6 Hz, 1 H, H-1), 2.10–2.03 (m, 4 H, H-5, H-9), 1.99–1.95 (m, 2 H, H-8), 1.67 (s, 3 H, H-12), 1.60 (s, 3 H, H-15), 1.60 (s, 3 H, H-16), 1.40–1.32 (m, 1 H, H-4), 1.27 (bs, 1 H, OH), 1.20–1.12 (m, 1 H, H-4), 1.10 (s, 3 H, H-14), 0.95–0.88 (m, 1 H, H-2), 0.51 (dd, *J* = 8.6 Hz, 4.8 Hz, 1 H, H-13), 0.13 (t, *J* = 4.8 Hz, 1 H, H-13) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 135.1 (C-7), 131.5 (C-11), 124.5 (d, C-6, C-10), 64.1 (C-1), 41.2 (C-4), 39.8 (C-8), 26.8 (C-5/9), 26.3 (C-5/9), 25.8 (C-12), 25.5 (C-2), 20.1 (C-3), 17.9 (C-13), 17.8 (C-16), 17.2 (C-14), 16.1 (C-15) ppm.

208R: ((1*R*,2*R*)-2-((*E*)-4,8-Dimethylnona-3,7-dien-1-yl)-2-methylcyclopropyl)methanol



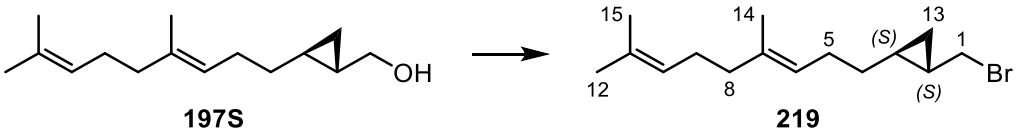
115 **208R**

Starting from farnesol (**115**) (300 mg, 1.35 mmol, 1 eq.), the desired cyclopropyl methyl alcohol **208R** was prepared according to the general procedure for the asymmetric cyclopropanation of allyl alcohols (p. 79) using (4*S*,5*S*)-2-butyl-N^4,N^4,N^5,N^5-tetramethyl-1,3,2-dioxaborolane-4,5-dicarboxamide (**209S**, 546.6 mg, 2.02 mmol, 1.5 eq.) and CH_2I_2 (4 eq.). Purification via column chromatography (silica, PE:EtOAc = 10:1 → 4:1) delivered alcohol **208R** (299 mg, 1.27 mmol, 94 %, 94 % *ee*) as a colorless oil.

R_f = 0.3 (PE:EtOAc = 4:1);
$[\alpha]^{24}_D$ = -6.6° (c = 1.0, $CHCl_3$);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.14–5.07 (m, 2 H, H-6, H-10), 3.71 (dd, *J* = 11.4 Hz, 6.6 Hz, 1 H, H-1), 3.50 (dd, *J* = 11.3 Hz, 8.6 Hz, 1 H, H-1), 2.10–2.03 (m, 4 H, H-5, H-9), 1.99–1.95 (m, 2 H, H-8), 1.67 (s, 3 H, H-12), 1.60 (s, 3 H, H-15), 1.60 (s, 3 H, H-16), 1.40–1.32 (m, 1 H, H-4), 1.20–1.15 (m, 1 H, H-4), 1.10 (s, 3 H, H-14), 0.95–0.88 (m, 1 H, H-2), 0.51 (dd, *J* = 8.6 Hz, 4.5 Hz, 1 H, H-13), 0.13 (t, *J* = 4.9 Hz, 1 H, H-13) ppm;

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 135.1 (C-7), 131.5 (C-11), 124.5 (d, C-6, C-10), 64.1 (C-1), 41.3 (C-4), 39.9 (C-8), 26.8 (C-5/9), 26.4 (C-5/9), 25.8 (C-12), 25.5 (C-2), 20.1 (C-3), 17.9 (C-13), 17.8 (C-16), 17.2 (C-14), 16.1 (C-15) ppm.

219: (1*S*,2*S*)-1-(Bromomethyl)-2-((*E*)-4,8-dimethylnona-3,7-dien-1-yl)cyclopropane

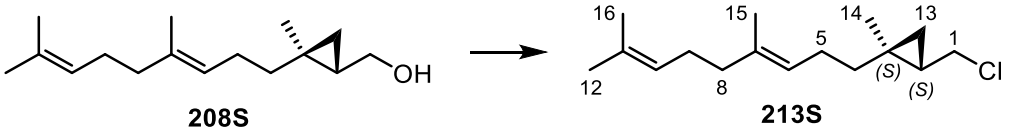


197S **219**

To alcohol **197S** (5 mg, 0.02 mmol, 1 eq.) in CH_2Cl_2 (0.7 mL) was added triphenylphosphine (7.08 mg, 0.03 mmol, 1.2 eq.) and tetrabromomethane (0.01 mL, 0.02 mmol, 1.1 eq.) at room temperature. After stirring for 1 h at room temperature, no further conversion of the starting material was observed. TLC analysis showed full conversion of the alcohol to a single new product. After adding *n*-pentane to the reaction mixture, the forming precipitate was removed using a short plug column. The crude pale-yellow oil was used in the next reaction without further purification steps.

R_f = 0.8 (PE:EtOAc = 4:1).

213S: (1*S*,2*S*)-2-(Chloromethyl)-1-((*E*)-4,8-dimethylnona-3,7-dien-1-yl)-1-methylcyclopropane

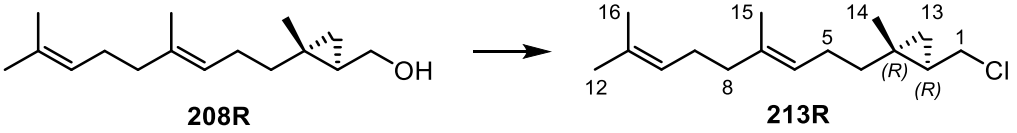


208S **213S**

Chloride **213S** was prepared according to the general procedure for the synthesis of allyl chlorides (p. 76) starting from alcohol **208S** (50 mg, 0.21 mmol, 1 eq.) and was obtained as a yellow oil.

R_f = 0.8 (PE:EtOAc = 4:1).

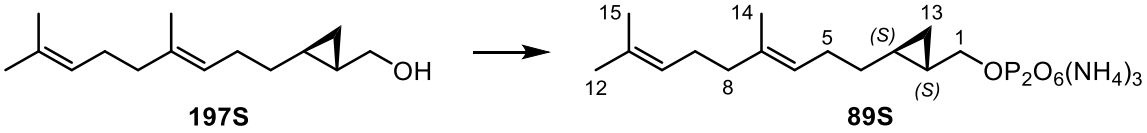
213R: (1*R*,2*R*)-2-(Chloromethyl)-1-((*E*)-4,8-dimethylnona-3,7-dien-1-yl)-1-methylcyclopropane



Chloride **213R** was prepared according to the general procedure for the synthesis of allyl chlorides (p. 76) starting from alcohol **208R** (50 mg, 0.21 mmol, 1 eq.) and was obtained as a yellow oil.

R$_f$ = 0.8 (PE:EtOAc = 4:1).

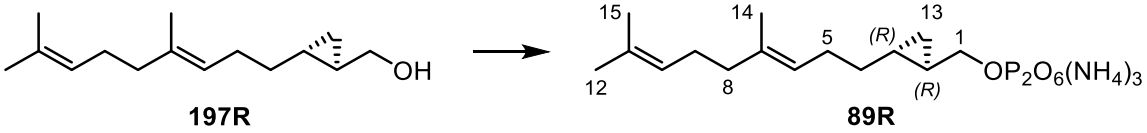
89S: Ammonium ((1*S*,2*S*)-2-((*E*)-4,8-dimethylnona-3,7-dien-1-yl)cyclopropyl)methyl diphosphate



Cyclopropyl diphosphate **89S** was prepared according to the general one-step procedure for the synthesis of diphosphates (p. 78) starting from alcohol **197S** (30 mg, 135 µmol, 1 eq.). Purification of the crude mixture via column chromatography (silica, *i*PrOH:NH_3:H_2O = 6:3:1) yielded the desired diphosphate **89S** (14 mg, 32.3 µmol, 24 %) as a colorless solid.

R$_f$ = 0.27 (*i*PrOH:NH_3:H_2O = 6:3:1);
^{1}H-NMR (400 MHz, D_2O): δ = 5.31–5.26 (m, 1 H, H-6), 5.23–5.16 (m, 1 H, H-10), 3.99–3.90 (m, 1 H, H-1), 3.72–3.60 (m, 1 H, H-1), 2.16–2.09 (m, 4 H, H-5, H-9), 2.09–2.02 (m, 2 H, H-8), 1.69 (s, 3 H, H-12), 1.64 (s, 3 H, H-14), 1.62 (s, 3 H, H-15), 1.39–1.33 (m, 2 H, H-4), 0.99–0.92 (m, 1 H, H-2), 0.79–0.71 (m, 1 H, H-3), 0.52–0.47 (m, 1 H, H-13), 0.42–0.36 (m, 1 H, H-13) ppm;
^{13}C-NMR (100 MHz, D_2O): δ = 136.2 (C-7), 133.3 (C-11), 124.9 (C-6), 124.5 (C-10), 70.9 (d, C-1), 38.8 (C-8), 33.1 (C-4), 27.3 (C-5/9), 25.8 (C-5/9), 24.8 (C-12), 18.1 (d, C-2), 17.0 (C-15), 16.7 (C-3), 15.2 (C-14), 9.8 (C-13) ppm.
^{31}P-NMR (160 MHz, D_2O): δ = -10.48 (d, *J* = 20.9 Hz, 1 P, terminal P), -10.73 (d, *J* = 20.9 Hz, 6.3 Hz, 1 P, RO-*P*O_3-) ppm.

89R: Ammonium ((1*R*,2*R*)-2-((*E*)-4,8-dimethylnona-3,7-dien-1-yl)cyclopropyl)methyl diphosphate

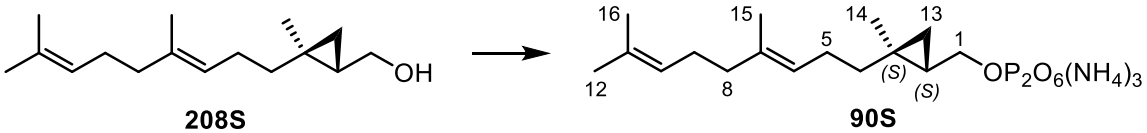


197R **89R**

Cyclopropyl diphosphate **89R** was prepared according to the general one-step procedure for the synthesis of diphosphates (p. 78) starting from alcohol **197R** (30 mg, 135 µmol, 1 eq.). Purification of the crude mixture via column chromatography (silica, *i*PrOH:NH_3:H_2O = 6:3:1) yielded the desired diphosphate **89R** (20 mg, 46.1 µmol, 34 %) as a colorless solid.

R_f = 0.27 (*i*PrOH:NH_3:H_2O = 6:3:1);
^{1}H-NMR (400 MHz, D_2O): δ = 5.29 (t, *J* = 7.3 Hz, 1 H, H-6), 5.20 (tt, *J* = 6.9 Hz, 1.3 Hz, 1 H, H-10), 4.00–3.89 (m, 1 H, H-1), 3.68–3.61 (m, 1 H, H-1), 2.16–2.09 (m, 4 H, H-5, H-9), 2.09–2.02 (m, 2 H, H-8), 1.69 (s, 3 H, H-12), 1.64 (s, 3 H, H-14), 1.63 (s, 3 H, H-15), 1.37–1.30 (m, 2 H, H-4), 0.99–0.93 (m, 1 H, H-2), 0.77–0.71 (m, 1 H, H-3), 0.51–0.46 (m, 1 H, H-13), 0.42–0.36 (m, 1 H, H-13) ppm;
^{13}C-NMR (100 MHz, D_2O): δ = 136.3 (C-7), 133.5 (C-11), 124.9 (C-6), 124.5 (C-10), 71.0 (d, C-1), 38.8 (C-8), 33.0 (C-4), 27.2 (C-5/9), 25.7 (C-5/9), 24.8 (C-12), 18.2 (d, C-2), 16.9 (C-15), 16.7 (C-3), 15.2 (C-14), 9.8 (C-13) ppm.
^{31}P-NMR (160 MHz, D_2O): δ = -10.38 (d, *J* = 20.4 Hz, 1 P, terminal P), -10.71 (dt, *J* = 20.5 Hz, 6.3 Hz, 1 P, RO-*P*O_3-) ppm.

90S: Ammonium ((1*S*,2*S*)-2-((*E*)-4,8-dimethylnona-3,7-dien-1-yl)-2-methylcyclopropyl)methyl diphosphate



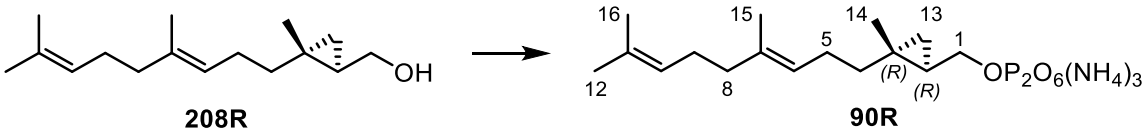
208S **90S**

Cyclopropylmethyl diphosphate **90S** was prepared according to the general one-step procedure for the synthesis of diphosphates (p. 78) starting from alcohol **208S** (10 mg, 42 µmol, 1 eq.). Purification of the crude mixture via column chromatography (silica, *i*PrOH:NH_3:H_2O = 6:3:1) yielded the desired diphosphate **90R** (8 mg, 17.9 µmol, 43 %) as a colorless solid.

R_f = 0.27 (*i*PrOH:NH_3:H_2O = 6:3:1);

^{1}H-NMR (400 MHz, D_2O): δ = 5.24 (t, J = 6.6 Hz, 1 H, H-6), 5.18 (t, J = 6.4 Hz, 1 H, H-10), 4.06–4.00 (m, 1 H, H-1), 3.91–3.84 (m, 1 H, H-1), 2.16–2.06 (m, 4 H, H-5, H-9), 2.03–2.00 (m, 2 H, H-8), 1.67 (s, 3 H, H-12), 1.62 (s, 3 H, H-15), 1.61 (s, 3 H, H-16), 1.28–1.24 (m, 2 H, H-4), 1.10 (s, 3 H, H-14), 1.01–0.99 (m, 1 H, H-2), 0.57 (dd, J = 8.4 Hz, 4.0 Hz, 1 H, H-13), 0.23 (t, J = 4.6 Hz, 1 H, H-13) ppm;
^{13}C-NMR (100 MHz, D_2O): δ = 136.0 (C-7), 133.4 (C-11), 125.2 (C-6), 124.5 (C-10), 64.2 (C-1), 40.4 (C-4), 38.8 (C-8), 25.7 (C-5/9), 24.8 (C-5/9, C-12), 23.1 (d, C-2), 20.0 (C-3), 17.1 (C-13), 16.9 (C-16), 16.4 (C-14), 15.1 (C-15) ppm.
^{31}P-NMR (160 MHz, D_2O): δ = -9.54 (d, J = 20.7 Hz, 1 P, terminal P), -10.61 (dt, J = 20.7 Hz, 5.8 Hz, 1 P, RO-PO$_3$-) ppm.

90R: Ammonium ((1*R*,2*R*)-2-((*E*)-4,8-dimethylnona-3,7-dien-1-yl)-2-methylcyclopropyl)methyl diphosphate

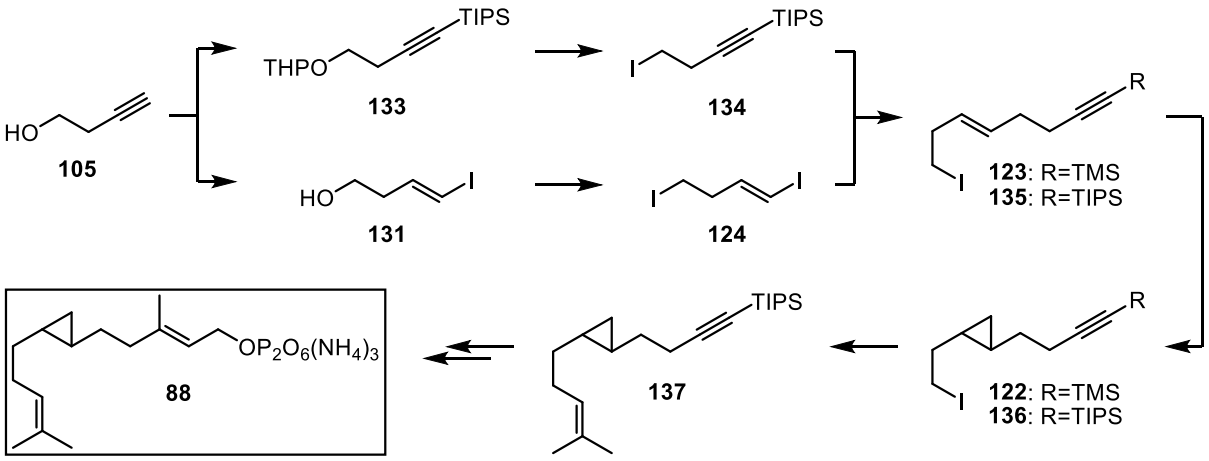


Cyclopropylmethyl diphosphate **90R** was prepared according to the general one-step procedure for the synthesis of diphosphates (p. 78) starting from alcohol **208R** (95 mg, 0.40 mmol, 1 eq.). Purification of the crude mixture via column chromatography (silica, *i*PrOH:NH_3:H_2O = 6:3:1) yielded the desired diphosphate **90R** (88 mg, 0.20 mmol, 49 %) as a colorless solid.

R_f = 0.27 (*i*PrOH:NH_3:H_2O = 6:3:1);
^{1}H-NMR (400 MHz, D_2O): δ = 5.23 (t, J = 6.9 Hz, 1 H, H-6), 5.17 (t, J = 6.6 Hz, 1 H, H-10), 4.05–3.97 (m, 1 H, H-1), 3.90–3.84 (m, 1 H, H-1), 2.16–2.06 (m, 4 H, H-5, H-9), 2.03–1.98 (m, 2 H, H-8), 1.67 (s, 3 H, H-12), 1.62 (s, 3 H, H-15), 1.61 (s, 3 H, H-16), 1.28–1.25 (m, 2 H, H-4), 1.09 (s, 3 H, H-14), 1.01–0.94 (m, 1 H, H-2), 0.56 (dd, J = 8.4 Hz, 4.2 Hz, 1 H, H-13), 0.23 (t, J = 4.8 Hz, 1 H, H-13) ppm;
^{13}C-NMR (100 MHz, D_2O): δ = 136.0 (C-7), 133.4 (C-11), 125.2 (C-6), 124.5 (C-10), 64.2 (C-1), 40.4 (C-4), 38.8 (C-8), 25.7 (C-5/9), 24.8 (C-5/9, C-12), 23.1 (d, C-2), 20.0 (C-3), 17.1 (C-13), 16.9 (C-16), 16.4 (C-14), 15.1 (C-15) ppm.
^{31}P-NMR (160 MHz, D_2O): δ = -7.55 (d, J = 21.4 Hz, 1 P, terminal P), -10.44 (dt, J = 21.5 Hz, 5.5 Hz, 1 P, RO-PO$_3$-) ppm.

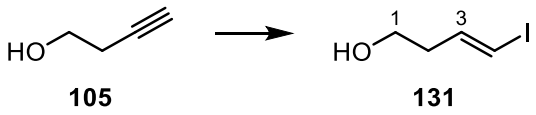
6.8 SYNTHESIS OF CYCLOPROPYL DIPHOSPHATE 88

6.8.1 FIRST-GENERATION SYNTHESIS APPROACH



Scheme 68: Abbreviated overview of the initial synthesis approach towards the preparation of cyclopropyl diphosphate **88**.

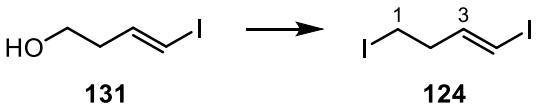
131: (*E*)-4-Iodobut-3-en-1-ol



To zirconocene dichloride (4.58 g, 15.69 mmol, 1.1 eq.) in THF (30 mL) was slowly added di*iso*butylaluminium hydride (1 M in hexane, 15.69 mL, 15.69 mmol, 1.1 eq.) at 0 °C and the resulting suspension was stirred for 30 min. Then, 3-butyne-1-ol (**105**) (1 g, 14.27 mmol, 1 eq.) in THF (15 mL) was slowly added and the mixture was stirred at room temperature for 1.5 h. A solution of iodine (4.7 g, 18.55 mmol, 1.3 eq.) in THF (20 mL) was slowly added at -78 °C and stirring was continued for 18 h at room temperature, after which no further conversion of the starting material was observed. The reaction was terminated by the addition of aq. HCl (1 M), the phases were separated, and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. $Na_2S_2O_3$ and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 5:1) to yield the desired vinyl iodide **131** (1.22 g, 6.16 mmol, 43 %) as a yellow oil.
The analytical data are consistent with those reported in the literature.[62]

R_f = 0.2 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 6.54 (dt, J = 14.5 Hz, 7.3 Hz, 1 H, H-3), 6.16 (d, J = 14.4 Hz, 1 H, H-4), 3.68 (t, J = 6.2 Hz, 2 H, H-1), 2.32 (q, J = 6.2 Hz, 2 H, H-2) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 144.8 (C-3), 77.4 (C-4), 60.5 (C-1), 39.3 (C-2) ppm.

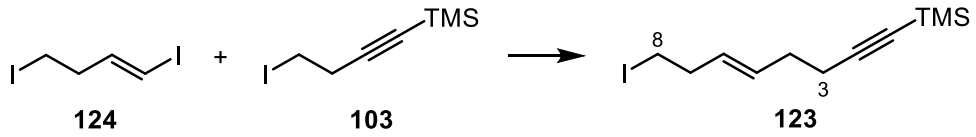
124: (*E*)-1,4-Diiodobut-1-ene



To a stirring solution of triphenylphosphine (2.09 g, 7.95 mmol, 1.05 eq.) and imidazole (542 mg, 7.95 mmol, 1.05 eq.) in CH_2Cl_2 (50 mL) was added iodine (2.02 g, 7.95 mmol, 1.05 eq.) at 0 °C. The mixture was stirred for 20 min under the exclusion of light, after which vinyl iodide **131** (1.5 g, 7.58 mmol, 1 eq.) in CH_2Cl_2 (8 mL) was slowly added. After stirring for 1 h at room temperature, no further conversion of the starting material was observed. To precipitate remaining triphenylphosphine and triphenylphosphine oxide, most of the CH_2Cl_2 was removed under reduced pressure and *n*-pentane was added. The resulting solid impurities were removed via filtration and the filter cake was washed with *n*-pentane and Et_2O. This step was repeated until no further precipitation upon addition of *n*-pentane was observed. The combined organic phases were washed with a sat. aq. $Na_2S_2O_3$ and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE) to yield the desired diiodide **124** (1.35 g, 4.39 mmol, 58 %) as a light-sensitive orange oil.
The analytical data are consistent with those reported in the literature.[104]

R_f = 0.45 (PE);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 6.49 (dt, *J* = 14.3 Hz, 7.2 Hz, 1 H, H-3), 6.23 (d, *J* = 14.4 Hz, 1 H, H-4), 3.17 (t, *J* = 7.1 Hz, 2 H, H-1), 2.65 (q, *J* = 7.2 Hz, 2 H, H-2) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 144.4 (C-3), 77.9 (C-4), 39.3 (C-2), 2.6 (C-1) ppm.

123: (*E*)-(8-Iodooct-5-en-1-yn-1-yl)trimethylsilane

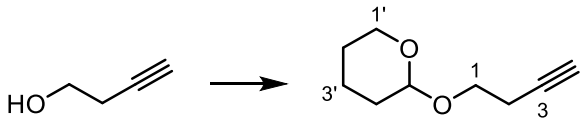


t-Butyllithium (1.9 M in hexane, 0.56 mL, 1.07 mmol, 2.2 eq.) was dropwise added to a stirring solution of iodide **103** (160 mg, 0.63 mmol, 1.3 eq.) in Et_2O (0.6 mL) at -78 °C and stirring was continued for 30 min. Then, freshly flame-dried zinc bromide (165 mg, 0.73 mmol, 1.5 eq.) in THF (1 mL) was slowly added. The reaction mixture was warmed to 0 °C and stirred for 30 min. In a separate flask, $Pd(dppf)Cl_2 \cdot (CH_2Cl_2)$ (20 mg, 0.02 mmol, 0.05 eq.) in THF (0.5 mL) was mixed with vinyl iodide **124** (150 mg, 0.49 mmol, 1 eq.). The resulting red suspension was stirred for 20 min and then added to the reaction flask. The reaction mixture was warmed to room temperature and stirred for 2.5 h, after which no further conversion of the

starting material was observed. The reaction was terminated by the addition of H_2O, the phases were separated, and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE) to yield the desired compound **123** (99 mg, 0.32 mmol, 66 %, 82 % *brsm*) as a colorless oil.[57]

R_f = 0.25 (PE);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.60–5.53 (m, 1 H, H-5), 5.47–5.39 (m, 1 H, H-6), 3.15 (t, J = 7.3 Hz, 2 H, H-8), 2.56 (dt, J = 7.0 Hz, 6.7 Hz, 2 H, H-7), 2.31–2.19 (m, 4 H, H-3, H-4), 0.15 (s, 9 H, TMS) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 131.3 (C-5), 129.9 (C-6), 106.8 (C-2), 85.1 (C-1), 36.8 (C-7), 31.8 (C-4), 20.2 (C-3), 5.8 (C-8), 0.3 (TMS) ppm.

132: 2-(But-3-yn-1-yloxy)tetrahydro-2*H*-pyran

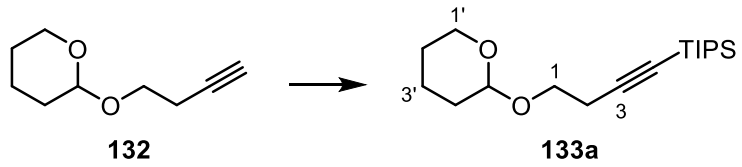


105 **132**

To a stirring solution of 3-butyne-1-ol (**105**) (1 g, 14.27 mmol, 1 eq.) and 3,4-dihydro-2H-pyran (3 g, 35.67 mmol, 2.5 eq.) in CH_2Cl_2 (50 mL) was added a catalytical amount of *p*-toluenesulfonic acid (12 mg, 0.07 mmol, 0.01 eq.) at room temperature. The resulting pink solution was stirred for 1 h at room temperature, after which no further conversion of the starting material was observed. The reaction solution was diluted with Et_2O (50 mL) and washed with a sat. aq. $NaHCO_3$ and NaCl solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was obtained as a colorless oil, which was used in the next step without further purification.[65]
The analytical data are consistent with those reported in the literature.[105]

R_f = 0.55 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 4.62 (t, J = 3.5 Hz, 1 H, H-5'), 3.88–3.78 (m, 2 H, H-1, H-1'), 3.57–3.46 (m, 2 H, H-1, H-1'), 2.46 (dt, J = 7.1 Hz, 2.7 Hz, 2 H, H-2), 1.95 (t, J = 2.7 Hz, 1 H, H-4), 1.82–1.48 (m, 6 H, H-2', H-3', H-4') ppm.

133a: Tri*iso*propyl(4-((tetrahydro-2*H*-pyran-2-yl)oxy)but-1-yn-1-yl)silane

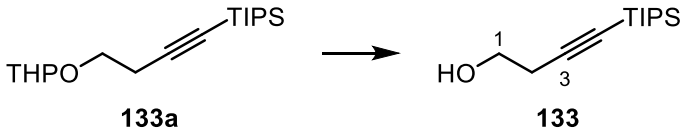


132 **133a**

To a stirring solution of alkyne **132** (2 g, 12.98 mmol, 1 eq.) in THF (13 mL) was dropwise added *n*-butyllithium (2.5 M in hexane, 6.75 mL, 16.87 mmol, 1.3 eq.) at -78 °C and stirring was continued for 1 h. Then, tri*iso*propylsilyl chloride (3.06 mL, 14.28 mmol, 1.1 eq.) was slowly added and the mixture was warmed to room temperature. After stirring for 18 h, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. $NaHCO_3$ solution and then diluted with Et_2O. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was obtained as a light-yellow oil, which was used in the next step without further purification.[65]

R_f = 0.75 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 4.66 (t, J = 3.3 Hz, 1 H, H-5'), 3.91–3.80 (m, 2 H, H-1, H-1'), 3.57–3.49 (m, 2 H, H-1, H-1'), 2.54 (t, J = 7.1 Hz, 2 H, H-2), 1.84–1.49 (m, 6 H, H-2', H-3', H-4'), 1.05 (m, 21 H, TIPS) ppm.

133: 4-(Tri*iso*propylsilyl)but-3-yn-1-ol

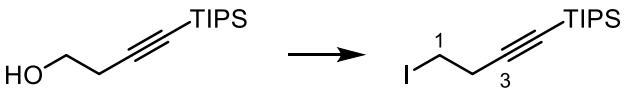


133a **133**

To alkyne **133a** (3 g, 9.66 mmol, 1 eq.) in MeOH (10 mL) was slowly added *p*-toluenesulfonic acid (1.66 g, 9.66 mmol, 1 eq.) at room temperature. The reaction solution was stirred at the same temperature and the reaction progress was monitored periodically via TLC. If necessary, a portion of *p*-toluenesulfonic acid (1 eq.) was added until the complete conversion of the starting material was observed. Then, MeOH was removed under reduced pressure and the residue was diluted with Et_2O. The reaction mixture was neutralized by addition of a sat. aq. $NaHCO_3$ solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was obtained as a brown oil, which was used in the next step without further purification.
The analytical data are consistent with those reported in the literature.[65]

R_f = 0.45 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 3.72 (t, *J* = 6.2 Hz, 2 H, H-1), 2.54 (t, *J* = 6.2 Hz, 2 H, H-2), 1.07-1.05 (m, 21 H, TIPS) ppm.

134: (4-Iodobut-1-yn-1-yl)tri*iso*propylsilane

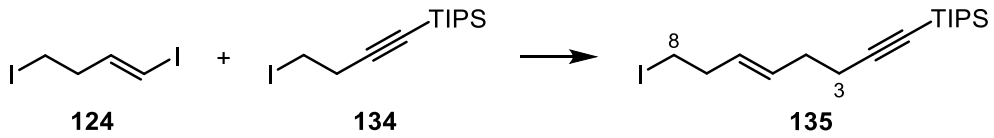


133 **134**

To a stirring solution of triphenylphosphine (3.93 g, 14.98 mmol, 1.05 eq.) and imidazole (1.02 g, 14.98 mmol, 1.05 eq.) in CH_2Cl_2 (50 mL) was added iodine (3.8 g, 14.98 mmol, 1.05 eq.) at 0 °C. Stirring was continued for 20 min at 0 °C under the exclusion of light, after which alcohol **133** (3.23 g, 14.27 mmol, 1 eq.) in CH_2Cl_2 (15 mL) was slowly added. After stirring for 2 h at room temperature, no further conversion of the starting material was observed. To precipitate remaining triphenylphosphine and triphenylphosphine oxide, most of the CH_2Cl_2 was removed under reduced pressure and *n*-pentane was added. The solid impurities were removed via filtration and the filter cake was washed with *n*-pentane and Et_2O. This step was repeated until no further precipitation upon addition of *n*-pentane was observed. The combined organic phases were washed with a sat. aq. $Na_2S_2O_3$ and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE) to yield the desired iodide **134** (3.9 g, 11.59 mmol, 81 % o4s) as a light-pink oil.
The analytical data are consistent with those reported in the literature.[106]

R_f = 0.8 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 3.24 (t, *J* = 7.3 Hz, 2 H, H-1), 2.83 (t, *J* = 7.3 Hz, 2 H, H-2), 1.07 (m, 21 H, TIPS) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 106.7 (C-4), 83.0 (C-3), 25.3 (C-1), 18.8 (TIPS), 11.3 (TIPS), 1.9 (C-2) ppm.

135: (*E*)-(8-Iodooct-5-en-1-yn-1-yl)tri*iso*propylsilane

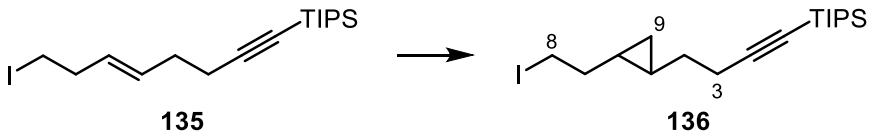


124 **134** **135**

t-Butyllithium (1.9 M in hexane, 3.76 mL, 7.15 mmol, 2.2 eq.) was dropwise added to a stirring solution of iodide **134** (1.42 mg, 4.22 mmol, 1.3 eq.) in Et_2O (4 mL) at -78 °C. After stirring for 30 min, freshly flame-dried zinc bromide (1.1 g, 4.87 mmol, 1.5 eq.) in THF (5 mL) was slowly added. The reaction mixture was warmed to 0 °C and stirred for 30 min. In a separate flask, $Pd(dppf)Cl_2 \cdot (CH_2Cl_2)$ (132 mg, 0.16 mmol, 0.05 eq.) in THF (5 mL) was mixed with vinyl iodide **124** (1 g, 3.25 mmol, 1 eq.). The resulting red suspension was stirred for 20 min and then added to the reaction flask. The reaction mixture was warmed up to room temperature and stirred for 4 h, after which no further conversion of the starting material was observed. The reaction was terminated by the addition of H_2O, the phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE) to yield the desired compound **135** (794 mg, 2.03 mmol, 62 %) as a colorless oil containing minor impurities.[57]

R_f = 0.45 (PE);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.63–5.56 (m, 1 H, H-5), 5.48–5.40 (m, 1 H, H-6), 3.14 (t, *J* = 7.3 Hz, 2 H, H-8), 2.58–2.53 (m, 2 H, H-7), 2.34–2.20 (m, 4 H, H-3, H-4), 1.06–1.03 (m, 21 H, TIPS) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 131.5 (C-5), 129.9 (C-6), 110.9 (C-2), 108.4 (C-1), 36.9 (C-7), 32.1 (C-4), 20.2 (C-3), 18.8 (TIPS), 11.4 (TIPS), 5.7 (C-8) ppm.

136: (4-(2-(2-Iodoethyl)cyclopropyl)but-1-yn-1-yl)tri*iso*propylsilane

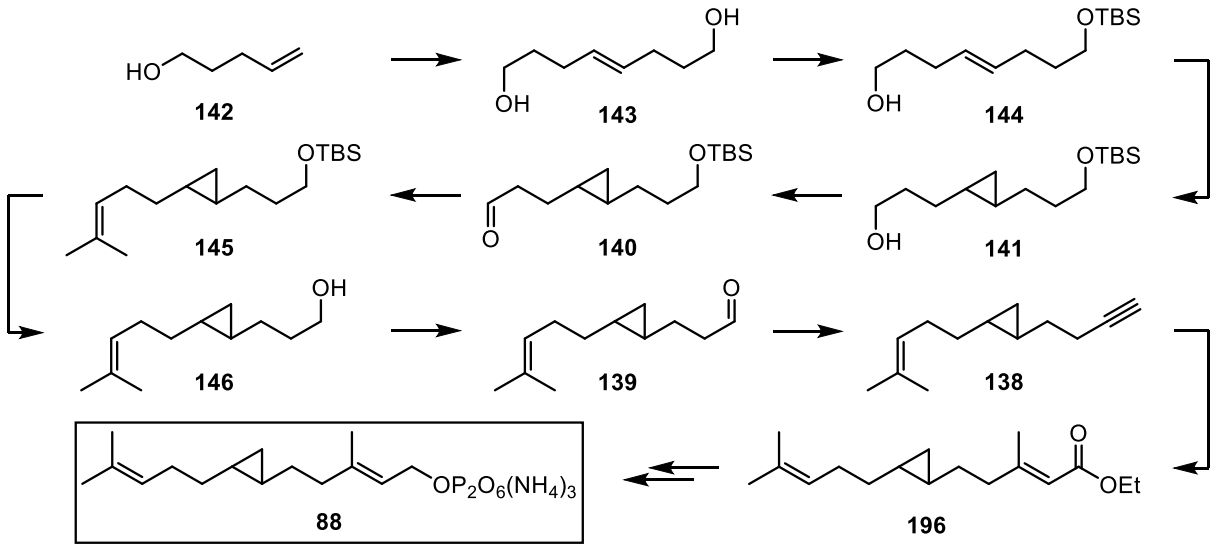


135 **136**

Diethylzinc (1 M in hexane, 1.28 mL, 1.28 mmol, 5 eq.) and CH_2Cl_2 (1.5 mL) were added to a two-neck flask and the mixture cooled to 0 °C. (For this reaction, a two-neck flask with an inlet adapter was used because diethylzinc vapors clog cannulas very quickly, which can lead to dangerous pressure increases during the reaction). A solution of freshly-distilled trifluoroacetic acid (0.1 mL, 1.28 mmol, 5 eq.) in CH_2Cl_2 (1.3 mL) was dropwise added and the resulting viscous suspension was vigorously stirred for 30 min at 0 °C. Then, a solution of diiodomethane (0.1 mL, 1.28 mmol, 5 eq.) in CH_2Cl_2 (1.3 mL) was slowly added. After 30 min of stirring, a solution of iodide **135** (0.10 g, 0.26 mmol, 1 eq.) in CH_2Cl_2 (1.3 mL) was dropwise added and the reaction mixture was allowed to warm to room temperature. After stirring was continued for 1 h, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NH_4Cl solution, the phases were separated, and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. $NaHCO_3$ and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE) to yield the desired cyclopropyl iodide **136** (58 mg, 0.14 mmol, 56 %) as a colorless oil containing minor impurities.[61]

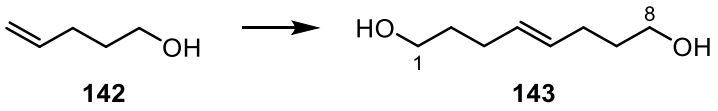
R_f = 0.45 (PE);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 3.23–3.18 (m, 2 H, H-8), 2.33 (t, J = 7.0 Hz, 2 H, H-3), 1.89–1.80 (m, 1 H, H-7), 1.74–1.65 (m, 1 H, H-7), 1.58–1.49 (m, 1 H, H-4), 1.41–1.33 (m, 1 H, H-4), 1.07–1.06 (m, 21 H, TIPS), 0.74–0.70 (m, 1 H, H-6), 0.63–0.59 (m, 1 H, H-5), 0.38–0.31 (m, 2 H, H-9) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 109.1 (C-1), 80.42 (C-2), 38.4 (C-7), 33.4 (C-4), 20.2 (C-3), 19.7 (C-5), 18.7 (TIPS), 18.0 (C-6), 11.7 (C-9), 11.4 (TIPS), 5.9 (C-8) ppm.

6.8.2 SECOND-GENERATION SYNTHESIS APPROACH



Scheme 69: Overview of the second-generation synthesis strategy towards the preparation of cyclopropyl diphosphate **88**.

143: (*E*)-Oct-4-ene-1,8-diol

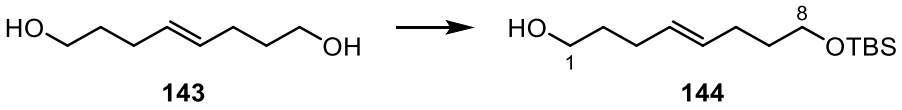


A dropping funnel was charged with Grubbs catalyst (1st gen., 956 mg, 1.16 mmol, 0.02 eq.) and freshly degassed CH_2Cl_2 (16 mL) and placed onto a two-neck flask containing pent-4-en-1-ol (**142**) (5.00 g, 58.05 mmol, 1 eq.). Over the course of 18 h, the violet catalyst suspension was dropwise added to the neat alcohol at room temperature and under the exclusion of light. (Alternatively, the catalyst was added in five portions over the couse of 15 h to the neat alcohol). To prevent ethenolysis, a constant argon flow was provided. After no further conversion of the starting material was observed, the reaction mixture was concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 1:1 → 0:1) to yield an isomeric mixture (*E*:*Z* = 10:1) of the desired diol **143** (3.59 g, 24.87 mmol, 86 %) as a yellow oil.
The analytical data are consistent with those reported in the literature.[68–70]

R_f = 0.15 (PE:EtOAc = 1:1);
ESI-MS (HRMS): calculated: $C_8H_{16}O_2$ $[M+Na]^+$: 167.1048, found: 167.1046 m/z.
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.48–5.39 (m, 2 H, H-4, H-5), 3.65 (t, J = 6.4 Hz, 4 H, H-1, H-8), 2.21–2.06 (m, 4 H, H-3, H-6), 1.63 (m, 6 H, H-2, H-7, 2×OH) ppm;

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 130.4 (C-4, C-5), 62.7 (C-1, C-8), 32.5 (C-2, C-7), 29.0 (C-3, C-6) ppm.

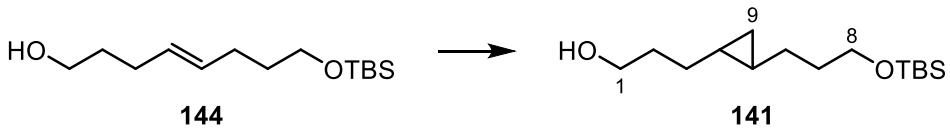
144: (*E*)-8-((*tert*-Butyldimethylsilyl)oxy)oct-4-en-1-ol



To a stirring suspension of sodium hydride (60 % in mineral oil, 1.09 g, 45.39 mmol, 1.05 eq.) in THF (68 mL) was added a solution of diol **143** (6.23 g, 43.23 mmol, 1 eq.) in THF (43 mL) at 0° C. The mixture was warmed to room temperature and stirred vigorously for 1.5 h, after which a colorless amorphous precipitate had formed. The mixture was cooled to 0 °C and a solution of *t*-butyldimethylsilyl chloride (6.84 g, 45.39 mmol, 1.05 eq.) in THF (45 mL) was added dropwise. After stirring for 45 min at room temperature, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. $NaHCO_3$ solution, the phases were separated, and the aqueous phase was extracted with Et_2O. The combined organic phases were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 10:1 → 0:1) to yield the desired alcohol **144** (6.17 g, 23.86 mmol, 55 %, 75 % *brsm*) as a colorless oil. Doubly silylated by-product (*E*)-2,2,3,3,14,14,15,15-octamethyl-4,13-dioxa-3,14-disilahexadec-8-ene (2.59 g, 6.96 mmol, 16 %) was isolated as a side product. After desilylation with aq. HCl (1 M), diol **143** was recovered in quantitative yield.
The analytical data are consistent with those reported in the literature.[107]

R_f = 0.5 (PE:EtOAc = 2:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.46–5.38 (m, 2 H, H-4, H-5), 3.65 (t, J = 6.5 Hz, 2 H, H-1/8), 3.60 (t, J = 6.4 Hz, 2 H, H-1/8), 2.14–2.01 (m, 4 H, H-3, H-6), 1.63 (qui, J = 6.9 Hz, 2 H, H-2/7), 1.57 (qui, J = 7.0 Hz, 2 H, H-2/7), 1.56 (bs, 1 H, OH), 0.89 (s, 9 H, TBS), 0.04 (s, 6 H, TBS) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 130.7 (C-4/5), 129.9 (C-4/5), 62.7 (C-1, C-8), 32.8 (C-2/7), 32.6 (C-2/7), 29.0 (C-3/6), 28.9 (C-3/6), 26.1 (TBS), -5.1 (TBS) ppm.

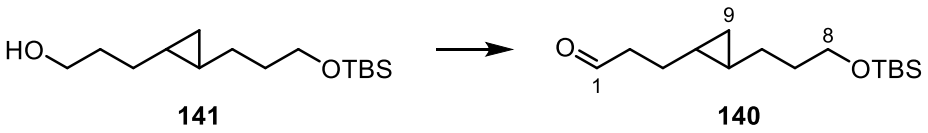
141: 3-(2-(3-((*tert*-Butyldimethylsilyl)oxy)propyl)cyclopropyl)propan-1-ol



Diethylzinc (1 M in hexane, 9.7 mL, 9.67 mmol, 2.5 eq.) and CH_2Cl_2 (10 mL) were added to a two-neck flask and the mixture was cooled to 0 °C. (For this reaction, a two-neck flask with an inlet adapter was used because diethylzinc vapors clog cannulas very quickly, which can lead to dangerous pressure increases during the reaction). A solution of freshly-distilled trifluoroacetic acid (0.74 mL, 9.67 mmol, 2.5 eq.) in CH_2Cl_2 (5 mL) was dropwise added and the resulting colorless viscous suspension stirred vigorously for 30 min at 0 °C. Then, a solution of diiodomethane (0.78 mL, 9.67 mmol, 2.5 eq.) in CH_2Cl_2 (5 mL) was slowly added. After 30 min of stirring, alcohol **144** (1 g, 3.87 mmol, 1 eq.) in CH_2Cl_2 (4 mL) was dropwise added and the mixture was allowed to warm to room temperature. After stirring for 1 h, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NH_4Cl solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. $NaHCO_3$ and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 4:1) to yield the desired cyclopropyl alcohol **141** (843.8 mg, 2.94 mmol, 80 %) as a colorless oil.[61]

R_f = 0.5 (PE:EtOAc = 2:1);
ESI-MS (HRMS): calculated: $C_{15}H_{33}O_2Si$ $[M+H]^+$: 273.2250, found: 273.2248 m/z.
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 3.69–3.60 (m, 4 H, H-1, H-8), 1.70–1.56 (m, 4 H, H-2, H-7), 1.30–1.19 (m, 4 H, H-3, H-6), 0.89 (s, 9 H, TBS), 0.42–0.39 (m, 2 H, H-4, H-5), 0.2–0.17 (m, 2 H, H-9), 0.04 (s, 6 H, TBS) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 63.2 (C-1/8), 63.0 (C-1/8), 33.0 (C-2/7), 32.9 (C-2/7), 30.6 (C-3/6), 26.1 (TBS), 18.7 (C-4/5), 18.6 (C-4/5), 12.0 (C-9), -5.1 (TBS) ppm.

140: 3-(2-(3-((*tert*-Butyldimethylsilyl)oxy)propyl)cyclopropyl)propanal

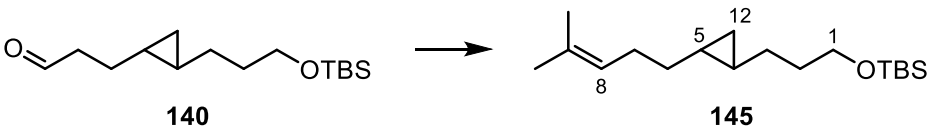


141 **140**

To a stirring solution of oxalyl chloride (1.39 mL, 16.23 mmol, 1.5 eq.) in CH_2Cl_2 (16 mL) was slowly added DMSO (2.31 mL, 32.46 mmol, 3 eq.) at -78 °C and stirring was continued for 30 min. Then, alcohol **141** (2.95 g, 10.82 mmol, 1 eq.) in CH_2Cl_2 (11.00 mL) was slowly added. After stirring for 30 min, freshly-distilled triethylamine (7.5 mL, 54.09 mmol, 5 eq.) was slowly added and the reaction mixture was allowed to warm to room temperature. After stirring was continued for 2 h, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NH_4Cl solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, *n*-pentane:Et_2O = 15:1) to obtain aldehyde **140** (2.36 g, 8.71 mmol, 81 %) as a colorless oil.

R_f = 0.7 (PE:EtOAc = 4:1);
ESI-MS (HRMS): calculated: $C_{15}H_{30}O_2SiNa$ $[M+Na]^+$: 293.1913, found: 293.1912 m/z.
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 9.80–9.78 (m, 1 H, H-1), 3.65–3.59 (m, 2 H, H-8), 2.55–2.49 (m, 2 H, H-2), 1.64–1.51 (m, 4 H, H-3, H-7), 1.27–1.20 (m, 2 H, H-6), 0.89 (s, 9 H, TBS), 0.49–0.42 (m, 2 H, H-4, H-5), 0.27–0.21 (m, 2 H, H-9), 0.04 (s, 6 H, TBS) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 202.95 (C-1), 63.1 (C-8), 44.3 (C-2), 33.0 (C-7), 30.4 (C-6), 27.1 (C-3), 26.1 (TBS), 19.0 (C-4/5), 18.3 (C-4/5), 12.2 (C-9), -5.1 (TBS) ppm.

145: *tert*-Butyldimethyl(3-(2-(4-methylpent-3-en-1-yl)cyclopropyl)propoxy)silane



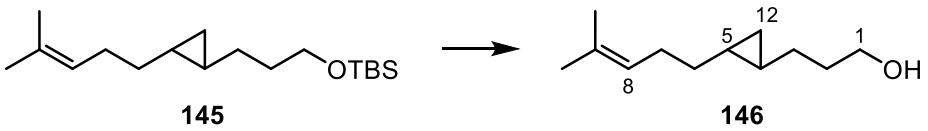
140 **145**

To a stirring suspension of *iso*propyltriphenylphosphonium iodide (1.42 g, 3.28 mmol, 1.5 eq.) in Et_2O (12 mL) was added *n*-butyllithium (1.6 M in hexane, 2.33 mL, 3.72 mmol, 1.7 eq.) at 0 °C. Within minutes, the reaction suspension turned bright red due to the formation of the phosphorous ylide. The reaction mixture was warmed up to room temperature and stirred for 1.5 h, after which a solution of aldehyde **140** (592 mg, 2.19 mmol, 1 eq.) in Et_2O (5 mL) was added. After stirring was continued for 30 min, no further conversion of the starting material

was observed. The reaction was terminated by the addition of water. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were diluted with PE, which resulted in the precipitation of triphenylphosphine oxide. The solid impurities were removed via filtration, the filtrate was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 50:1 → 20:1) to yield the desired product **145** (263 mg, 0.89 mmol, 41 %) as a colorless oil.[73]

R_f = 0.7 (PE:EtOAc = 20:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.18–5.12 (m, 1 H, H-8), 3.67–3.60 (m, 2 H, H-1), 2.04 (dt, J = 7.5, 7.5 Hz, 2 H, H-7), 1.68 (s, 3 H, H-10), 1.64–1.58 (m, 2 H, H-2), 1.61 (s, 3 H, CH_3-9), 1.31–1.13 (m, 4 H, H-3, H-6), 0.89 (s, 9 H, TBS), 0.46–0.36 (m, 2 H, H-4, H-5), 0.20–0.15 (m, 2 H, H-12), 0.04 (s, 6 H, TBS) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 131.3 (C-9), 124.9 (C-8), 63.2 (C-1), 34.8 (C-6), 33.1 (C-2), 30.6 (C-3), 28.3 (C-7), 26.1 (TBS), 25.9 (C-10), 18.81 (C-4/5), 18.79 (C-4/5), 17.8 (CH_3-9), -5.1 (TBS) ppm.

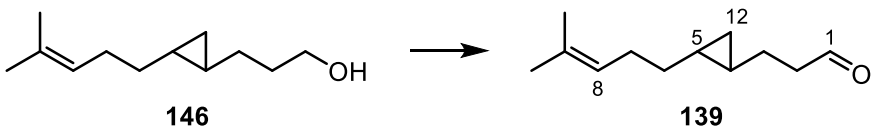
146: **3-(2-(4-Methylpent-3-en-1-yl)cyclopropyl)propan-1-ol**



145 **146**

To a stirring solution of alkene **145** (110 mg, 0.37 mmol, 1 eq.) in THF (1 mL) was added an aq. solution of HCl (1 M, 1.48 mL, 1.48 mmol, 4 eq.) at room temperature. After stirring the reaction solution for a period of 30 min, full conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. $NaHCO_3$ solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was obtained as a colorless oil, which was used in the next step without further purification.

139: **3-(2-(4-Methylpent-3-en-1-yl)cyclopropyl)propanal**

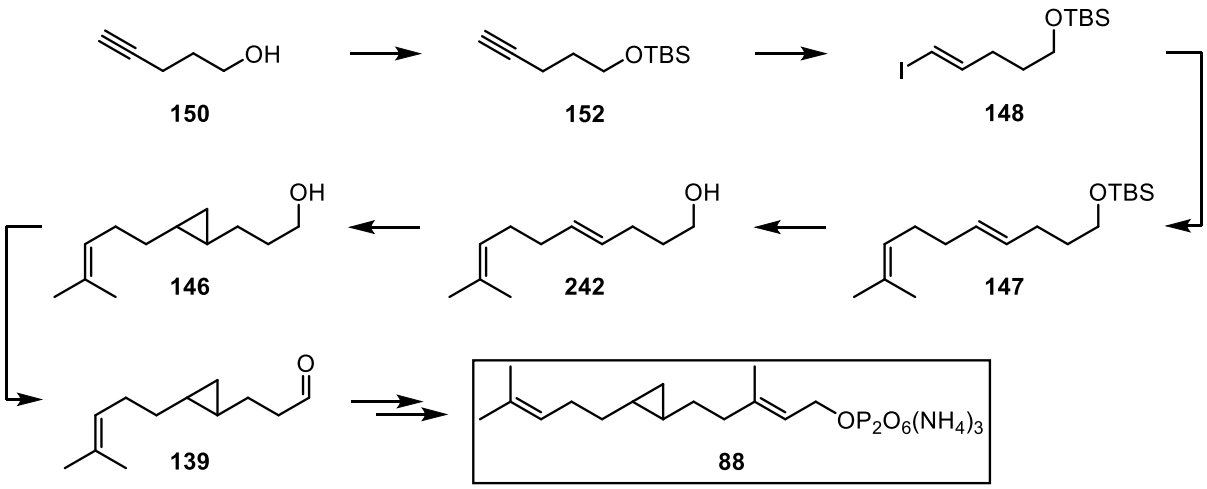


146 **139**

To a solution of alcohol **146** (60 mg, 0.33 mmol, 1 eq.) in CH_2Cl_2 (3 mL) was added Dess-Martin periodinane (279.2 mg, 0.66 mmol, 2 eq.) at room temperature. The resulting suspension was stirred for 2 h, after which no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. $NaHCO_3$ solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. $Na_2S_2O_3$ and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 10:1 → 4:1) to yield the desired aldehyde **139** (41 mg, 0.23 mmol, 62 % o2s) as a highly volatile yellow oil with a fruity/citrusy-like scent.

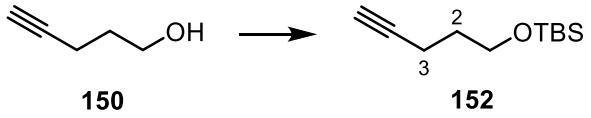
R_f = 0.6 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 9.80–9.77 (m, 1 H, H-1), 5.13–5.09 (m, 1 H, H-8), 2.53–2.48 (m, 2 H, H-2), 2.08–2.02 (m, 2 H, H-7), 1.68 (s, 3 H, H-10), 1.60 (s, 3 H, CH_3-9), 1.56–1.51 (m, 2 H, H-3), 1.30–1.16 (m, 2 H, H-6), 0.47–0.42 (m, 2 H, H-4, H-5), 0.24–0.21 (m, 2 H, H-12) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 203.0 (C-1), 131.5 (C-9), 124.6 (C-8), 44.2 (C-2), 34.5 (C-6), 28.2 (C-7), 27.1 (C-3), 25.8 (C-10), 19.0 (C-4/5), 18.4 (C-4/5), 17.8 (CH_3-9), 12.1 (C-12) ppm.

6.8.3 ALTERNATIVE SYNTHESIS ROUTE TO ALDEHYDE 139



Scheme 70: Overview of the alternative synthesis approach towards the preparation of aldehyde **139**.

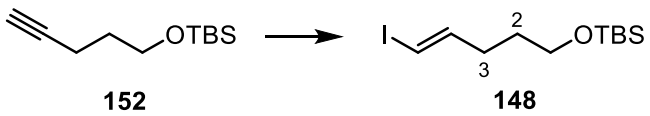
152: *tert*-Butyldimethyl(pent-4-yn-1-yloxy)silane



Pent-4-yn-1-ol (**150**) (0.5 g, 5.94 mmol, 1 eq.) and imidazole (688 mg, 10.1 mmol, 1.7 eq.) were dissolved in CH_2Cl_2 (8 mL) and cooled to 0 °C. Then, *tert*-butyldimethylsilyl chloride (1.34 g, 8.29 mmol, 1.5 eq.) in CH_2Cl_2 (4 mL) was slowly added and the reaction solution was warmed to room temperature. After stirring the mixture for 16 h, full conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NH_4Cl solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was obtained as a colorless oil, which was used in the next step without further purification.

$\mathbf{R_f}$ = 0.81 (PE:EtOAc = 4:1).

148: (*E*)-*tert*-Butyl((5-iodopent-4-en-1-yl)oxy)dimethylsilane

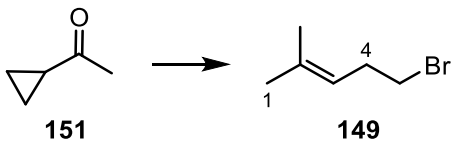


152 **148**

To zirconocene dichloride (2.26 g, 7.73 mmol, 1.3 eq.) in THF (8 mL) was slowly added di*iso*butylaluminium hydride (1 M in hexane, 7.73 mL, 7.73 mmol, 1.3 eq.) at 0 °C. After the resulting colorless suspension was stirred for 1 h at 0 °C, **152** (1.18 g, 5.94 mmol, 1 eq.) in THF (6 mL) was carefully added and the ice bath removed. The resulting yellow suspension was stirred for 1.5 h at room temperature. Then, the reaction mixture was cooled to -78 °C and a solution of iodine (1.5 g, 5.94 mmol, 1 eq.) in THF (12 mL) was added dropwise. After warming the reaction mixture up to room temperature and stirring for 16 h, no further conversion of the starting material was observed. The reaction was terminated by the addition of aq. HCl (1 M), the phases were separated, and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. $NaHCO_3$, $Na_2S_2O_3$, and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 10:1 → 4:1) to yield the desired compound **148** (1,52 g, 4.67 mmol, 79 % o2s) as a light-sensitive pale pink oil.
The analytical data are consistent with those reported in the literature.[108]

R_f = 0.81 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 6.52 (dt, J = 14.3 Hz, 7.2 Hz, 1 H, H-4), 6.00 (dt, J = 14.3 Hz, 1.5 Hz, 1 H, H-5), 3.60 (t, J = 6.1 Hz, 2 H, H-1), 2.16–2.09 (m, 2 H, H-3), 1.64–1.57 (m, 2 H, H-2), 0.89 (s, 9 H, TBS), 0.04 (s, 6 H, TBS) ppm;

149: 5-Bromo-2-methylpent-2-ene

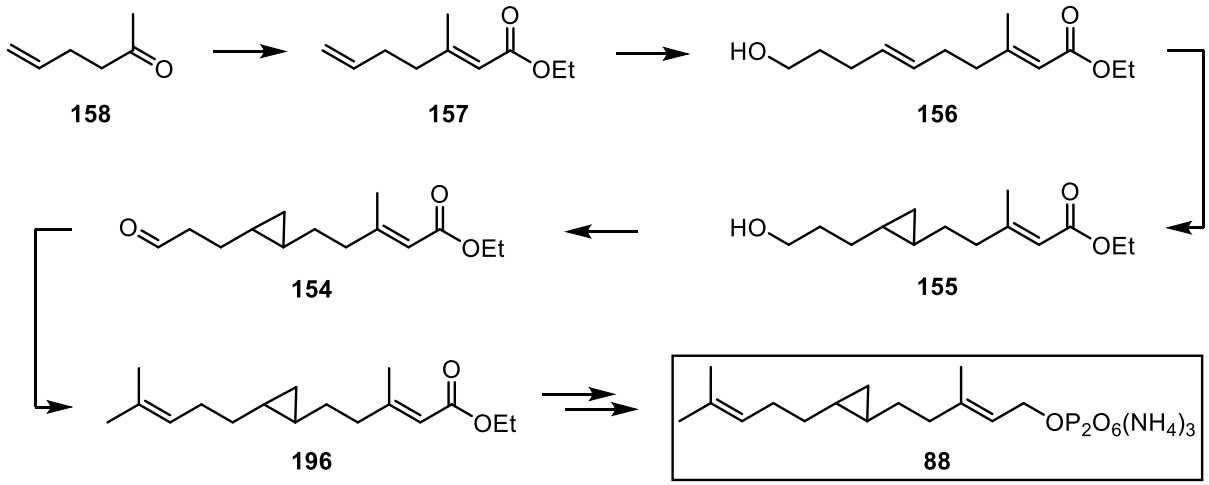


151 **149**

Bromide **149** is commercially available but can be prepared from much more inexpensive 1-cyclopropylethan-1-one (**151**). To a stirring solution of the cyclopropyl ketone (8.24 mL, 83.21 mmol, 1 eq.) in THF (11.7 mL) was slowly added methylmagnesium bromide (3 M in Et_2O, 33.3 mL, 99.85 mmol, 1.2 eq.) at room temperature. The reaction mixture was stirred under refluxing conditions for 1 h then cooled to 0 °C. An aq. sulfuric acid solution (21 mL, conc. H_2SO_4:H_2O = 1:2) was carefully added while making sure that the temperature of the reaction mixture did not exceed 10 °C. After the mixture was stirred for 30 min at 10 °C, the phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. $NaHCO_3$ and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The desired bromide **149** (10.62 g, 65.13 mmol, 78 %) was obtained as a colorless oil.[77]
The analytical data are consistent with those reported in the literature.[109]

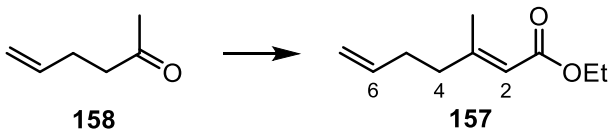
R_f = 0.8 (PE:EtOAc = 10:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.15–5.10 (m, 1 H, H-3), 3.33 (t, J = 7.3 Hz, 2 H, H-5), 2.55 (dt, J = 7.3 Hz, 7.3 Hz, 2 H, H-4), 1.71 (s, 3 H, H-1), 1.63 (m, 3 H, CH_3-2) ppm.

6.8.4 THIRD-GENERATION SYNTHESIS APPROACH



Scheme 71: Abbreviated overview of the third-generation synthesis approach towards the preparation cyclopropyl diphosphate **88**.

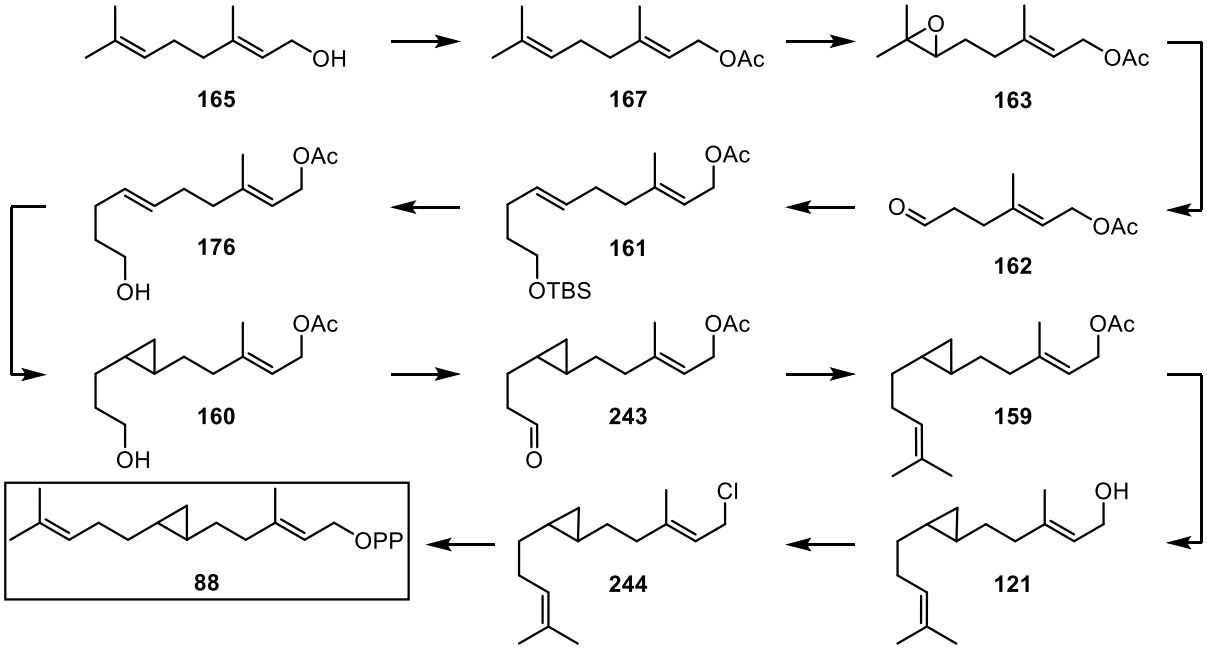
157: Ethyl (*E*)-3-methylhepta-2,6-dienoate



Ethyl 2-diethoxyphosphorylacetate (5 mL, 5.65 g, 25.2 mmol, 8.24 eq.) was added to a suspension of sodium hydride (60 % in mineral oil, 223 mg, 5.58 mmol, 1.82 eq.) in THF (5 mL) at 0 °C. The mixture was stirred for 2 h at room temperature, after which hex-5-en-2-one (**158**) (0.35 mL, 0.3 g, 3.06 mmol, 1.00 eq.) was added at 0 °C. After removing the ice bath and stirring the reaction mixture for 20 h at room temperature, no further conversion of the starting material was observed. The reaction was terminated by the addition of H_2O. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 20:1) to yield the desired alkene **157** (226 mg, 1.34 mmol, 44 %, *E*:*Z* = 3.7:1) as a colorless oil.[78] The analytical data are consistent with those reported in the literature.[110]

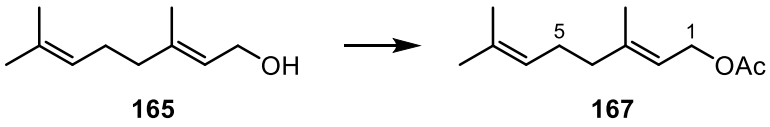
R_f = 0.57 (PE:EtOAc = 20:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.73–5.88 (m, 1 H, H-6), 5.67 (s, 1 H, H-2), 4.97–5.06 (m, 2 H, H-7), 4.14 (q, *J* = 7.1 Hz, 2 H, OEt), 2.23 (s, 4 H, H-4, H-5), 2.16 (d, *J* = 0.9 Hz, 3 H, CH_3-3), 1.27 (t, *J* = 7.2 Hz, 3 H, OEt) ppm.

6.8.5 FOURTH-GENERATION SYNTHESIS APPROACH



Scheme 72: Overview of the fourth-generation synthesis approach towards the preparation of diphosphate **88**.

167: (*E*)-3,7-Dimethylocta-2,6-dien-1-yl acetate

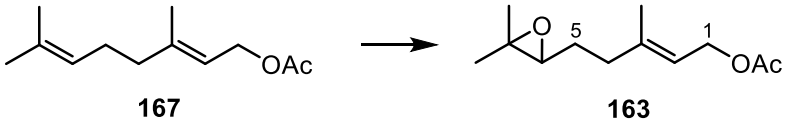


To a stirring solution of geraniol (**165**) (12.3 mL, 70.09 mmol, 1 eq.) in CH_2Cl_2 (115 mL) was added 4-dimethylaminopyridine (0.16 g, 1.31 mmol, 0.02 eq.) and triethylamine (12.7 mL, 91.12 mmol, 1.3 eq.) at room temperature. The resulting solution was cooled to 0 °C and acetic anhydride (8 mL, 84.62 mmol, 1.21 eq.) was slowly added. After stirring for 30 min at room temperature, no further conversion of the starting material was observed. The reaction was terminated by the addition of H_2O, the phases were separated, and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with aq. HCl (1 M), a sat. aq. $NaHCO_3$ and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 20:1) to yield the desired acetate **167** (13.42 g, 68.38 mmol, 98 %) as a colorless oil.
The analytical data are consistent with those reported in the literature.[111]

R_f = 0.5 (PE:EtOAc = 20:1);

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.34 (qt, J = 7.1 Hz, 1.2 Hz, 1 H, H-2), 5.1–5.06 (m, 1 H, H-6), 4.58 (d, J = 7.1 Hz, 2 H, H-1), 2.11–2.02 (m, 7 H, H-4, H-5, Ac), 1.70 (s, 3 H, CH_3-3), 1.68 (d, J = 0.9 Hz, 3 H, CH_3-7), 1.60 (s, 3 H, H-8) ppm.

163: (*E*)-5-(3,3-Dimethyloxiran-2-yl)-3-methylpent-2-en-1-yl acetate

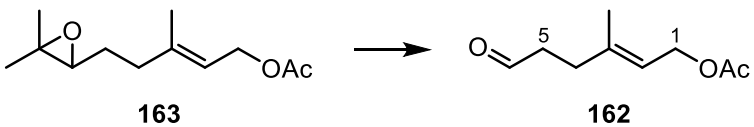


167 **163**

To a stirring solution of acetate **167** (13.4 g, 68.28 mmol, 1 eq.) in CH_2Cl_2 (115 mL) was added *meta*-chloroperoxybenzoic acid (18.53 g, 82.68 mmol, 1.2 eq.) in two portions at 0 °C. The second portion was added 15 min after the first. After stirring for 1 h at room temperature, no further conversion of the starting material was observed. The reaction was terminated by the addition of aq. NaOH (1 M), the phases were separated, and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 10:1) to yield the desired epoxide **163** (7.89 g, 37.17 mmol, 54 %) as a colorless oil with minor *meta*-chlorobenzoic acid impurities.
The analytical data are consistent with those reported in the literature.[80]

R_f = 0.24 (PE:EtOAc = 10:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.37 (t, J = 7.1 Hz, 1 H, H-2), 4.59 (d, J = 7.1 Hz, 2 H, H-1), 2.71 (t, J = 6.2 Hz, 1 H, H-6), 2.26–2.11 (m, 2 H, H-4), 2.05 (s, 3 H, Ac), 1.72 (s, 3 H, CH_3-3), 1.69–1.63 (m, 2 H, H-5), 1.30 (s, 3 H, H-8/CH_3-7), 1.26 (s, 3 H, H-8/CH_3-7) ppm.

162: (*E*)-3-Methyl-6-oxohex-2-en-1-yl acetate



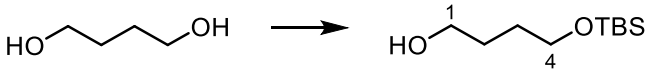
163 **162**

Periodic acid (H_5IO_6, 9.29 g, 40.62 mmol, 1.09 eq.) was added to a stirring solution of epoxide **163** (7.89 g, 37.17 mmol, 1 eq.) in THF (50 mL) at 0 °C. After stirring was continued for 1 h at room temperature, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NaCl solution, the phases were separated, and the aqueous phase was extracted with Et_2O. The combined organic phases were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by

column chromatography (silica, PE:EtOAc = 5:1) to yield the desired aldehyde **162** (3.4 g, 19.98 mmol, 54 %) as a pale-yellow oil.
The analytical data are consistent with those reported in the literature.[80]

R_f = 0.25 (PE:EtOAc = 5:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 9.77 (t, *J* = 1.6 Hz, 1 H, H-6), 5.37–5.33 (m, 1 H, H-2), 4.57 (d, *J* = 7.0 Hz, 2 H, H-1), 2.59–2.55 (m, 2 H, H-5), 2.37 (t, *J* = 7.5 Hz, 2 H, H-4), 2.04 (s, 3 H, Ac), 1.71 (s, 3 H, CH_3-3) ppm.

168: 4-((*tert*-Butyldimethylsilyl)oxy)butan-1-ol

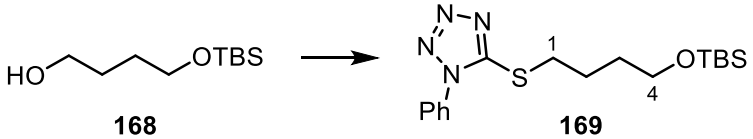


166 **168**

Butane-1,4-diol (**166**) (11.96 g, 132.7 mmol, 4 eq.) and imidazole (3.39 g, 49.76 mmol, 1.5 eq.) were dissolved in CH_2Cl_2 (90 mL) and cooled to 0 °C. *tert*-Butyldimethylsilyl chloride (5.00 g, 33.17 mmol, 1 eq.) in CH_2Cl_2 (15 mL) was slowly added to the reaction solution. After stirring for 2 h at 0 °C, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NH_4Cl solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 100:1 → 1:1) to yield the desired alcohol **168** (6.37 g, 31.19 mmol, 94 %) as a colorless oil.
The analytical data are consistent with those reported in the literature.[112]

R_f = 0.7 (PE:EtOAc = 2:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 3.65 (dt, *J* = 10.3 Hz, 5.6 Hz, 4 H, H-1, H-4), 1.70–1.61 (m, 4 H, H-2, H-3), 0.90 (s, 9 H, TBS), 0.07 (s, 6 H, TBS) ppm.

169: 5-((4-((*tert*-Butyldimethylsilyl)oxy)butyl)thio)-1-phenyl-1*H*-tetrazole



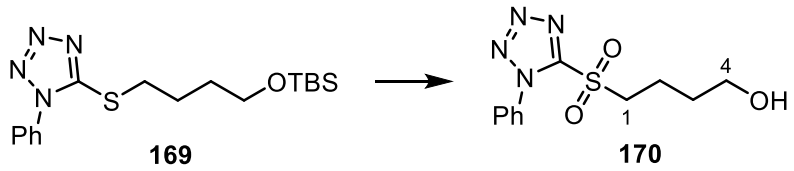
168 **169**

Di*iso*propyl azodicarboxylate (DIAD, 2.18 g, 10.76 mmol, 1.1 eq.) was added to triphenylphosphine (2.82 g, 10.76 mmol, 1.1 eq.) and 1-phenyl-1*H*-tetrazole-5-thiol (1.92 g, 10.76 mmol, 1.1 eq.) in THF (15 mL) at room temperature. To the resulting pale-yellow

suspension was slowly added alcohol **168** (2.00 g, 9.79 mmol, 1 eq.) in THF (10 mL) at 0 °C. After the reaction mixture was stirred for 2 h at room temperature, full conversion of the starting material was observed. The crude mixture was concentrated under reduced pressure and dry-loaded onto a column. Purification via column chromatography (silica, PE:EtOAc = 15:1) yielded the desired product **169** (3.08 g, 8.45 mmol, 86 %) as a colorless oil.
The analytical data are consistent with those reported in the literature.[79,81]

R_f = 0.5 (PE:Et_2O = 5:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.59–7.53 (m, 5 H, Ph), 3.64 (t, J = 6.2 Hz, 2 H, H-4), 3.43 (t, J = 7.3 Hz, 2 H, H-1), 1.90 (tt, J = 7.4 Hz, 7.4 Hz, 2 H, H-2), 1.67 (tt, J = 7.3 Hz, 6.3 Hz, 2 H, H-3), 0.87 (s, 9 H, TBS), 0.03 (s, 6 H, TBS) ppm.

170: 4-((1-Phenyl-1*H*-tetrazol-5-yl)sulfonyl)butan-1-ol

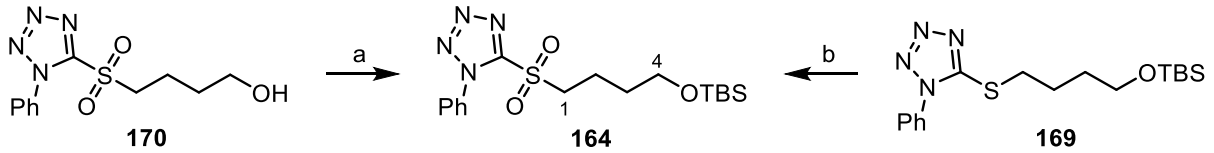


169 **170**

To a solution of **169** (1.5 g, 4.11 mmol, 1 eq.) in EtOH (4 mL) was added a mixture of ammonium heptamolybdate tetrahydrate (458 mg, 0.37 mmol, 0.09 eq.) and aq. hydrogen peroxide (35 g/L, 4.5 mL, 46.29 mmol, 11.3 eq.) at 0 °C. After stirring for 18 h at room temperature, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. $NaHCO_3$ solution. The phases were separated and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 5:1 → 1:1) to yield the desired alcohol **170** (983 mg, 3.48 mmol, 84 %) as a colorless oil.
The analytical data are consistent with those reported in the literature.[79]

R_f = 0.2 (PE:EtOAc = 1:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.73–7.60 (m, 5 H, Ph), 3.85 (m, 2 H, H-4), 3.76 (t, J = 6.0 Hz, 2 H, H-1), 2.12 (tt, J = 7.7 Hz, 7.7 Hz, 2 H, H-3), 1.80 (tt, J = 6.9 Hz, 6.7 Hz, 2 H, H-2), 1.60 (bs, 1 H, OH) ppm.

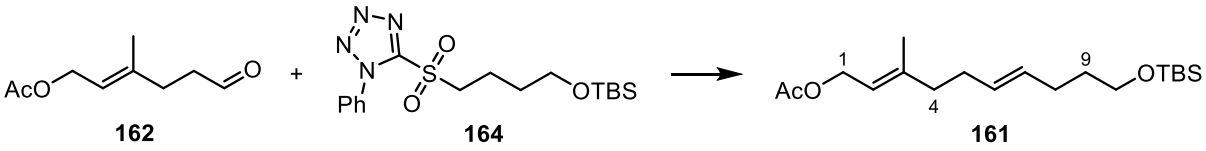
164: 5-((4-((*tert*-Butyldimethylsilyl)oxy)butyl)sulfonyl)-1-phenyl-1*H*-tetrazole



a) To a stirring solution of alcohol **170** (11.96 g, 132.7 mmol, 4 eq.) and imidazole (3.39 g, 49.76 mmol, 1.5 eq.) in CH_2Cl_2 (90 mL) was added *tert*-butyldimethylsilyl chloride (5.00 g, 33.17 mmol, 1 eq.) in CH_2Cl_2 (15 mL) at 0 °C. After stirring for 16 h at room temperature, complete conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NH_4Cl solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was obtained as a colorless crystalline solid, which was used in the next reaction without further purification.
b) To a solution of **169** (7. g, 19.2 mmol, 1 eq.) in CH_2Cl_2 (50 mL) was added sodium hydrogen carbonate (8.07 g, 96.0 mmol, 5 eq.) and *meta*-chloroperoxybenzoic acid (8.28 g, 48.0 mmol, 2.5 eq.) at 0 °C. After the reaction mixture was stirred for 4 h at room temperature, it was cooled to 0 °C and a second portion of *meta*-chloroperoxybenzoic acid (1 eq.) was added. Stirring was continued for 16 h, after which no further conversion of the starting material was observed. The reaction was terminated by the addition of H_2O, the phases were separated, and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 10:1) to yield the desired Julia-Kocienski reagent **164** (6.46 g, 16.29 mmol, 85 %) as a colorless crystalline solid.
The analytical data are consistent with those reported in the literature.[79,81]

R_f = 0.5 (PE:Et_2O = 5:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.73–7.60 (m, 5 H, Ph), 3.83 (m, 2 H, H-4), 3.69 (t, J = 5.9 Hz, 2 H, H-1), 2.12–2.05 (m, 2 H, H-3), 1.77–1.71 (m, 2 H, H-2), 0.90 (s, 9 H, TBS), 0.07 (s, 6 H, TBS) ppm.

161: (2*E*,6*E*)-10-((*tert*-Butyldimethylsilyl)oxy)-3-methyldeca-2,6-dien-1-yl acetate

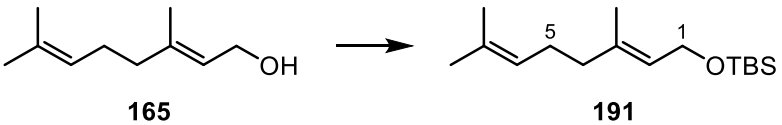


162 **164** **161**

To a stirring solution of sulfone **164** (1.28 g, 3.23 mmol, 1.1 eq.) in DME (6 mL) was added potassium bis(trimethylsilyl)amide (KHMDS, 0.5 M in toluene, 7.05 mL, 3.53 mmol, 1.2 eq.) at -78 °C. Right after, a solution of aldehyde **162** (500 mg, 2.94 mmol, 1 eq.) in DME (6 mL) was added to the reaction mixture. The resulting colorless suspension was stirred for 18 h at room temperature, after which no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NH_4Cl solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:Et_2O = 10:1) to yield the desired diene **161** (566 mg, 1.66 mmol, 57 %) as a colorless oil.[79,80]

R_f = 0.65 (PE:EtOAc = 10:1);
ESI-MS (HRMS): calculated: $C_{19}H_{36}O_3SiNa$ $[M+Na]^+$: 363.2331, found: 363.2318 m/z.
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.42–5.32 (m, 3 H, H-2 H-6, H-7), 4.58 (d, J = 7.1 Hz, 2 H, H-1), 3.59 (t, J = 6.5 Hz, 2 H, H-10), 2.13–2.00 (m, 9 H, H-4, H-5, H-8, Ac), 1.69 (s, 3 H, CH_3-3), 1.57–1.52 (m, 2 H, H-9), 0.89 (s, 3 H, TBS), 0.04 (s, 6 H, TBS) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 171.3 (Ac), 142.8 (C-3), 130.4 (C-6), 129.8 (C-7), 118.5 (C-2), 62.7 (C-10), 61.5 (C-1), 39.7 (C-4), 32.8 (C-9), 30.9 (C-5), 28.9 (C-8), 26.1 (TBS), 21.2 (Ac), 16.6 (CH_3-3), -5.1 (TBS) ppm.

191: (*E*)-*tert*-Butyl((3,7-dimethylocta-2,6-dien-1-yl)oxy)dimethylsilane



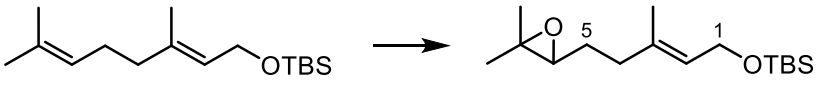
165 **191**

To a stirring solution of **165** (11.20 mL, 10.01 g, 64.91 mmol, 1 eq.) and imidazole (7.51 g, 110.31 mmol, 1.7 eq.) in CH_2Cl_2 (86 mL) was added *tert*-butyldimethylsilyl chloride (14.8 g, 98.19 mmol, 1.5 eq.) in CH_2Cl_2 (50 mL) at room temperature. After stirring the reaction solution for 3.5 h, full conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NH_4Cl solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure.

The crude product was purified by column chromatography (silica, PE:EtOAc = 1:0 → 1:1) to yield the desired compound **191** (17.18 g, 63.97 mmol, 99 %) as a colorless oil.
The analytical data are consistent with those reported in the literature.[113]

R_f = 0.18 (PE:EtOAc = 100:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.30 (t, J = 6.3 Hz, 1 H, H-2), 5.10 (t, J = 6.3 Hz, 1 H, H-6), 4.19 (d, J = 6.3 Hz, 2 H, H-1), 2.12–2.06 (m, 2 H, H-5), 2.06–1.98 (m, 2 H, H-4), 1.68 (s, 3 H, CH_3-7), 1.62 (s, 3 H, CH_3-3), 1.60 (s, 3 H, H-8), 0.90 (s, 9 H, TBS), 0.07 (s, 6 H, TBS) ppm.

192: (*E*)-*tert*-Butyl((5-(3,3-dimethyloxiran-2-yl)-3-methylpent-2-en-1-yl)oxy)dimethylsilane

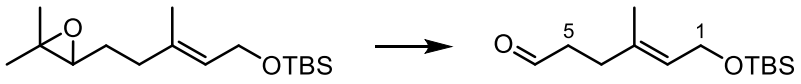


191 **192**

To **191** (15.50 g, 57.72 mmol, 1 eq.) in CH_2Cl_2 (100 mL) was added *meta*-chloroperoxybenzoic acid (77 %, 14.33 g, 63.94 mmol, 1.1 eq.) in CH_2Cl_2 (100 mL) at 0 °C. The resulting colorless suspension was stirred for 3 h, after which no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat aq. $NaHCO_3$ solution. The phases were separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 30:1) to yield the desired epoxide **192** (11.32 g, 39.77 mmol, 69 %) as a colorless oil.
The analytical data are consistent with those reported in the literature.[86]

R_f = 0.23 (PE:EtOAc = 30:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.34 (t, J = 6.3 Hz, 1 H, H-2), 4.19 (d, J = 6.3 Hz, 2 H, H-1), 2.71 (d, J = 6.3 Hz, 1 H, H-6), 2.22–2.06 (m, 2 H, H-5), 1.74–1.58 (m, 5 H, H-4, CH_3-3), 1.30 (s, 3 H, CH_3-7/H-8), 1.26 (s, 3 H, CH_3-7/H-8), 0.90 (s, 9 H, TBS), 0.07 (s, 6 H, TBS) ppm.

193: (*E*)-6-((*tert*-Butyldimethylsilyl)oxy)-4-methylhex-4-enal

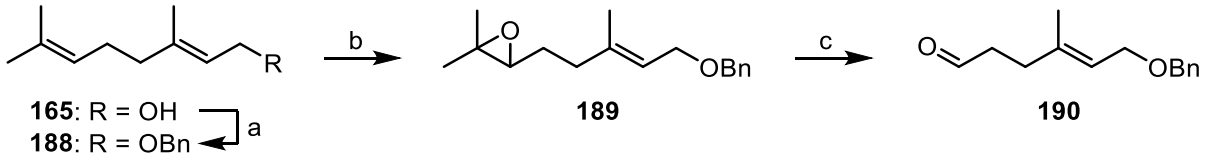


192 **193**

Periodic acid (H_5IO_6, 0.88 g, 3.88 mmol, 1.1 eq.) in H_2O (4.4 mL) was added to a solution of epoxide **192** (1.0 g, 3.51 mmol, 1 eq.) in THF (6.4 mL) at 0 °C. The mixture was stirred for 1 h, after which $NaHCO_3$ (0.66 g, 7.81 mmol, 2.22 eq.) was added. After stirring was continued for 1 h at room temperature, no further conversion of the starting material was observed. The mixture was filtered over celite and the phases were separated. The aqueous phase was extracted with Et_2O and the combined organic phases were dried over $MgSO_4$, filtered, and concentrated under reduced pressure.
The crude product was dissolved CH_2Cl_2 (7 mL) and cooled to 0 °C. To this solution were added imidazole (0.62 g, 9.5 mmol, 2.58 eq.) and *tert*-butyldimethylsilyl chloride (0.69 g, 4.58 mmol, 1.3 eq.). The reaction mixture was stirred for 45 min at room temperature after which no further conversion was observed. The reaction was terminated by the addition of a sat. aq. $NaHCO_3$ solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 10:1) to yield the desired aldehyde **193** (0.45 g, 1.86 mmol, 53 % o2s) as a colorless oil.
The analytical data are consistent with those reported in the literature.[86]

R_f = 0.43 (PE:EtOAc = 10:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 9.78 (s, 1 H, H-6), 5.32 (dt, J = 6.2 Hz, 1.2 Hz, 1 H, H-2), 4.18 (d, J = 6.2 Hz, 2 H, H-1), 2.56 (dt, J = 7.5 Hz, 1.2 Hz, 2 H, H-5), 2.30 (t, J = 7.5 Hz, 2 H, H-4), 1.60 (s, 3 H, CH_3-3), 0.90 (s, 9 H, TBS), 0.06 (s, 6 H, TBS) ppm.

190: (*E*)-6-(Benzyloxy)-4-methylhex-4-enal



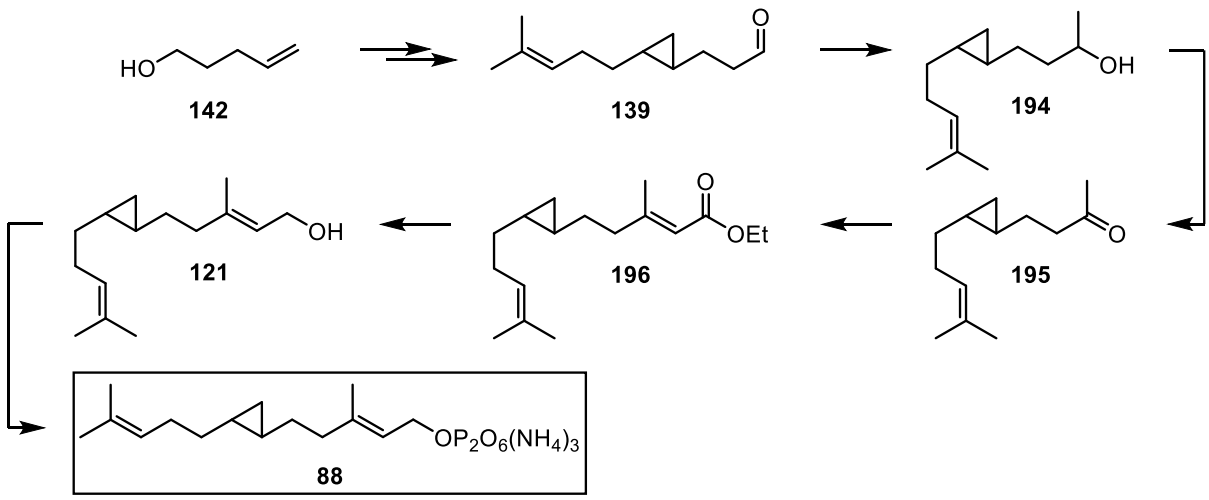
Analogous to aldehydes **162** and **193**, aldehyde **190** was synthesized in three steps starting from geraniol (**165**).
a-b) Following the procedure by McGeary et al., (3*E*)-3-(5-benzyloxy-3-methylpent-3-enyl)-2,2-dimethyloxirane **189** (4.25 g, 16.36 mmol, 63 % o2s) was synthesized in two steps and was obtained as a colorless oil.[84]

R_f = 0.1 (PE:EtOAc = 17:3);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.32–7.27 (m, 5 H, Ph), 5.45 (qt, *J* =6.7 Hz, 1.3 Hz, 1 H, H-2), 4.51 (s, 2 H, CH_2-Ph), 4.03 (d, *J* = 6.8 Hz, 2 H, H-1), 2.71 (t, *J* = 6.2 Hz, 1 H, H-6), 2.26– 2.11 (m, 2 H, H-5), 1.69–1.63 (m, 5 H, H-4/CH_3-3), 1.30 (s, 3 H, H-8/CH_3-7), 1.26 (s, 3 H, H-8/CH_3-7) ppm.

c) Starting from epoxide **189** (2.06 g, 7.91 mmol, 1 eq.), (*E*)-6-(benzyloxy)-4-methylhex-4-enal **190** (0.69 g, 3.16 mmol, 39 %) was obtained as a colorless oil.
The analytical data are consistent with those reported in the literature.[85]

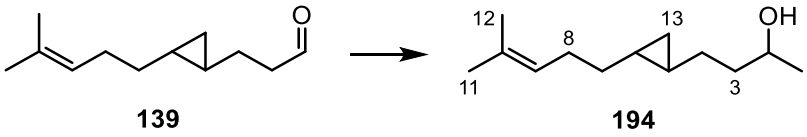
R_f = 0.13 (PE:EtOAc = 17:3);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 9.78 (t, *J* = 1.7 Hz, 1 H, H-6), 7.35–7.27 (m, 5 H, Ph), 5.45 (qt, *J* =10.0 Hz, 1.3 Hz, 1 H, H-2), 4.50 (s, 2 H, CH_2-Ph), 4.02 (d, *J* = 6.7 Hz, 2 H, H-1), 2.57–2.55 (m, 2 H, H-5), 2.37 (t, *J* =7.5 Hz, 2 H, H-4), 1.67 (s, 3 H, CH_3-3) ppm.

6.8.6 LAST-GENERATION SYNTHESIS APPROACH



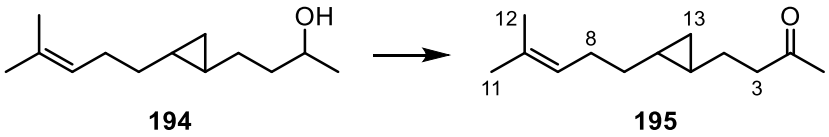
Scheme 73: Overview of the final synthesis strategy towards cyclopropyl diphosphate **88**. Aldehyde **139** was prepared in 7 steps following the second-generation strategy towards **88**.

194: 4-(2-(4-Methylpent-3-en-1-yl)cyclopropyl)butan-2-ol



To a stirring solution of aldehyde **139** (220 mg, 1.22 mmol, 1 eq,) in Et_2O (0.11 mL) was slowly added methylmagnesium bromide (3 M in Et_2O, 175 mg, 1.46 mmol, 1.2 eq.) at 0 °C. After stirring for 1.5 h, no further conversion of the starting material was observed. The reaction was terminated by addition of sat. aq. NH_4Cl solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was obtained as a colorless oil, which was used in the next step without further purification.[87]

195: 4-(2-(4-Methylpent-3-en-1-yl)cyclopropyl)butan-2-one

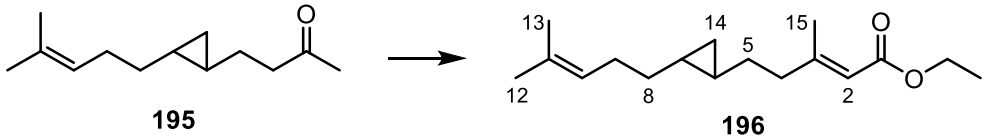


194 **195**

To a stirring solution of alcohol **194** (239 mg, 1.22 mmol, 1 eq.) in CH_2Cl_2 (1.2 mL) was added Dess-Martin periodinane (878 mg, 2.07 mmol, 1.7 eq.) in CH_2Cl_2 (6.9 mL) at room temperature. After stirring for 2 h, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. $NaHCO_3$ solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. $Na_2S_2O_3$ and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (silica, *n*-pentane:Et_2O = 10:1) to yield the desired aldehyde **195** (151 mg, 0.78 mmol, 64 % o2s) as a volatile colorless oil with light impurities.

R_f = 0.55 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.09–5.14 (m, 1 H, H-9), 2.50 (t, *J* = 7.5 Hz, 2 H, H-3), 2.14 (s, 3 H, H-1), 2.02 (dt, *J* = 7.5 Hz, 7.4 Hz, 2 H, H-8), 1.68 (s, 3 H, H-11), 1.60 (s, 3 H, H-12), 1.46 (dt, *J* = 7.3 Hz, 7.0 Hz, 2 H, H-4), 1.10–1.26 (m, 2 H, H-7), 0.40–0.44 (m, 2 H, H-5, H-6), 0.17–0.20 (m, 2 H, H-13) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 209.4 (C-2), 131.4 (C-10), 124.7 (C-9), 43.9 (C-3), 34.6 (C-7), 30.1 (C-1), 28.7 (C-4), 28.2 (C-8), 25.9 (C-11), 18.9 (C-5, C-6), 18.4 (C-12), 11.9 (C-13) ppm.

196: Ethyl (*E*)-3-methyl-5-(2-(4-methylpent-3-en-1-yl)cyclopropyl)pent-2-enoate



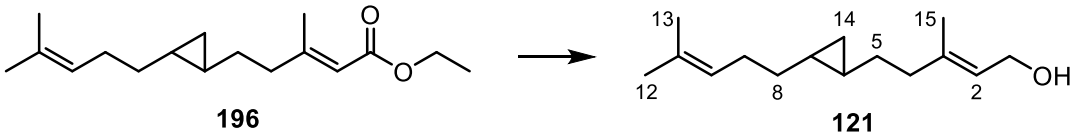
195 **196**

To a stirring solution of sodium hydride (90 % in mineral oil, 25 mg, 0.93 mmol, 1.5 eq.) in THF (0.9 mL) was dropwise added ethyl 2-(diethoxyphosphoryl)acetate (194 mg, 0.87 mmol, 1.4 eq.) at 0 °C and stirring was continued for 2 h. Then, ketone **195** (120 mg, 0.62 mmol, 1 eq.) in THF (0.6 mL) was slowly added and the mixture warmed to room temperature. After stirring for 16 h, the starting material was only partially converted. Therefore, another portion of phosphonium ylide (0.5 eq) was prepared and added to the reaction mixture. Stirring was continued for 24 h, after which no further conversion of the starting material was observed. The

reaction was terminated by the addition of H_2O, the phases were separated, and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (silica, PE:EtOAc = 10:1) to yield the desired ester **196** (164 mg, 0.62 mmol, quant, *E*:*Z* = 4.6:1) as a colorless oil.

R_f = 0.8 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.68–5.65 (m, 1 H, H-2), 5.15–5.10 (m, 1 H, H-10), 4.14 (q, *J* = 7.1 Hz, 2 H, OEt), 2.21 (t, *J* = 7.7 Hz, 2 H, H-4), 2.15–2.14 (m, 3 H, H-15), 2.07–2.00 (m, 2 H, H-9), 1.68 (s, 3 H, H-12), 1.61 (s, 3 H, H-13), 1.37 (dt, *J* = 15.1 Hz, 7.5 Hz, 2 H, H-5), 1.27 (t, *J* = 7.1 Hz, 3 H, OEt), 1.27–1.15 (m, 2 H, H-8), 0.39–0.48 (m, 2 H, H-6, H-7), 0.16–0.24 (m, 2 H, H-14) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 167.1 (C-1), 160.3 (C-3), 131.4 (C-11), 124.7 (C-10), 115.6 (C-2), 59.6 (OEt), 41.1 (C-4), 34.6 (C-8), 32.5 (C-5), 28.2 (C-9), 25.9 (C-12), 18.9 (C-6, C-7, C-15), 18.6 (C-13), 14.5 (OEt), 12.0 (C-14) ppm.

121: (*E*)-3-methyl-5-(2-(4-methylpent-3-en-1-yl)cyclopropyl)pent-2-en-1-ol

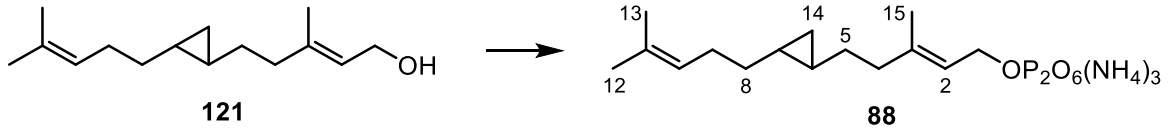


To a stirring solution of ester **196** (163 mg, 0.62 mmol, 1 eq.) in Et_2O (0.6 mL) was slowly added di*iso*butylaluminium hydride (1 M in hexane, 1.36 mL, 1.36 mmol, 2.2 eq.) at 0 °C. After stirring for 3 h at room temperature, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. Na, K-tartrate solution and the resulting viscous mixture stirred for 16 h at room temperature. The phases were separated, and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (silica, PE:EtOAc = 4:1) to yield the desired alcohol **121** (91 mg, 0.41 mmol, 66 %, *E*:*Z* = 2.5:1) as a colorless oil.

R_f = 0.3 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.42–5.39 (m, 1 H, H-2), 5.15–5.11 (m, 1 H, H-10), 4.14 (d, *J* = 6.9 Hz, 2 H, H-1), 2.09 (t, *J* = 8.0 Hz, 2 H, H-4), 2.01–2.00 (m, 2 H, H-9), 1.68 (s, 3 H, H-12), 1.66 (s, 3 H, H-15), 1.61 (s, 3 H, H-13), 1.29–1.24 (m, 2 H, H-5), 1.23–1.18 (m, 2 H, H-8), 0.46–0.35 (m, 2 H, H-6, H-7), 0.19–0.14 (m, 2 H, H-14) ppm;

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 140.3 (C-3), 131.4 (C-11), 124.8 (C-10), 123.2 (C-2), 59.6 (C-1), 39.7 (C-4), 34.7 (C-8), 32.7 (C-5), 28.3 (C-9), 25.9 (C-12), 18.8 (C-6, C-7, C-13), 16.4 (C-15), 11.9 (C-14) ppm.

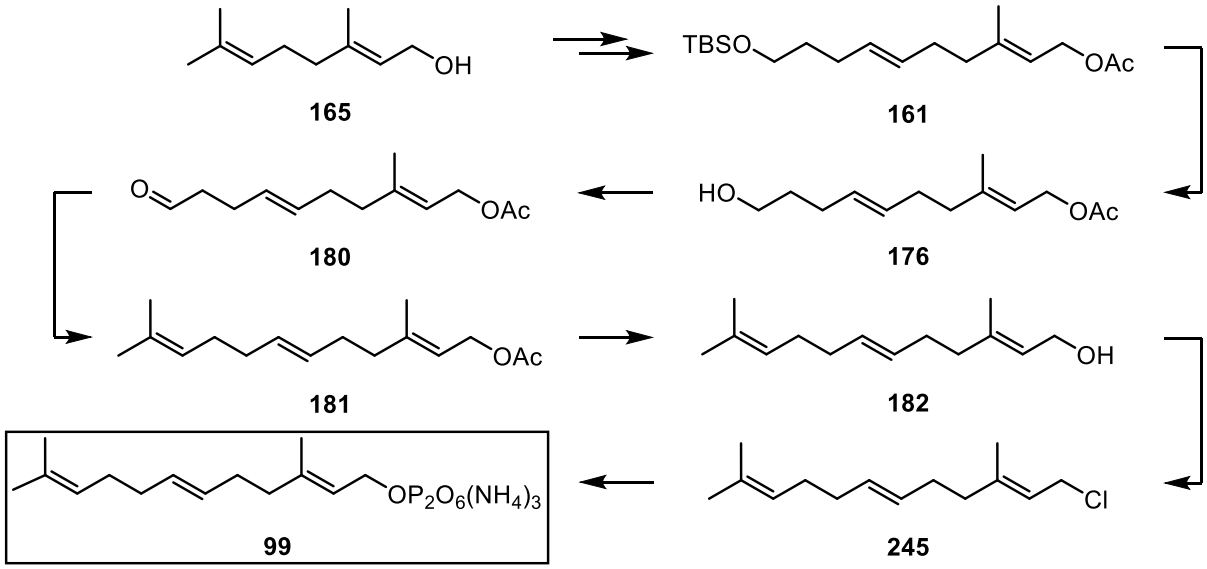
88: Ammonium (*E*)-3-methyl-5-(2-(4-methylpent-3-en-1-yl)cyclopropyl)pent-2-en-1-yl diphosphate



Cyclopropyl diphosphate **88** was prepared according to the general one-step procedure for the synthesis of diphosphates (p. 78) starting from allyl alcohol **121** (20 mg, 0.09 mmol, 1 eq.). Purification of the crude mixture via column chromatography (silica, *i*PrOH:NH_3:H_2O = 6:3:1) yielded the desired diphosphate **88** (24 mg, 55 µmol, 62 %) as a colorless solid with impurities of TEAP salt.

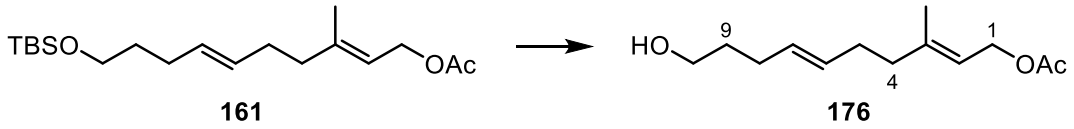
R_f = 0.27 (*i*PrOH:NH_3:H_2O = 6:3:1);
^{1}H-NMR (400 MHz, D_2O): δ = 5.49–5.42 (m, 1 H, H-2), 5.32–5.23 (m, 1 H, H-10), 4.53–4.45 (m, 2 H, H-1), 2.14 (t, *J* = 7.6 Hz, 2 H, H-4), 2.07 (dt, *J* = 7.2 Hz, 7.0 Hz, 2 H, H-9), 1.70 (s, 3 H, H-15), 1.69 (s, 3 H, H-12), 1.63 (s, 3 H, H-13), 1.29–1.24 (m, 4 H, H-5, H-8), 0.52–0.42 (m, 2 H, H-6, H-7), 0.25–0.16 (m, 2 H, H-14) ppm;
^{13}C-NMR (100 MHz, D_2O): δ = 143.8 (C-3), 133.2 (C-11), 125.2 (C-10), 119.0 (C-2), 62.9 (C-1), 38.9 (C-4), 33.8 (C-5/8), 31.8 (C-5/8), 27.5 (C-9), 24.8 (C-12), 18.0 (C-6, C-7), 16.9 (C-13), 15.6 (C-15), 11.0 (C-14) ppm.
^{31}P-NMR (160 MHz, D_2O): δ = -10.10 (d, *J* = 20.8 Hz, 1 P, terminal P), -10.41 (dt, *J* = 20.8 Hz, 5.9 Hz, 1 P, RO-*P*O_3-) ppm.

6.9 SYNTHESIS OF DESMETHYL DIPHOSPHATE 99



Scheme 74: Overview of the forward synthesis of desmethyl FPP derivative **99**. The initial steps for the preparation of **161** are described in the third synthesis strategy for cyclopropyl diphosphate **88** (p. 121).

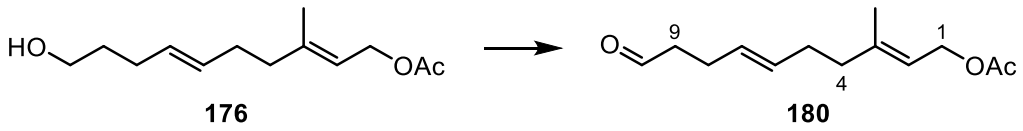
176: (2*E*,6*E*)-10-Hydroxy-3-methyldeca-2,6-dien-1-yl acetate



To a stirring solution of **161** (796 mg, 2.34 mmol, 1 eq.) in THF (11.7 mL) was added tetra-*n*-butylammonium fluoride (1 M in THF, 3.51 mL, 3.51 mmol, 1.5 eq.) at room temperature. After stirring for 1.5 h, full conversion of the starting material was observed. The reaction was terminated by the addition of H_2O, the phases were separated, and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was obtained as a colorless oil, which was used in the next reaction without further purification.

R_f = 0.15 (PE:EtOAc = 4:1);
ESI-MS (HRMS): calculated: $C_{13}H_{22}O_3Na$ $[M+Na]^+$: 249.1467, found: 249.1475 m/z.

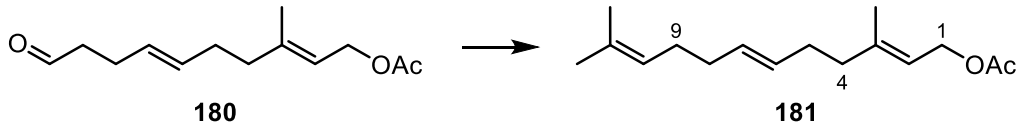
180: (2*E*,6*E*)-3-Methyl-10-oxodeca-2,6-dien-1-yl acetate



To a stirring solution of alcohol **176** (529 mg, 2.34 mmol, 1 eq.) in CH_2Cl_2 (7 mL) was added Dess-Martin periodinane (1.49 g, 3.51 mmol, 1.5 eq.) in CH_2Cl_2 (35 mL) at room temperature. After stirring for 4.5 h, full conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. $NaHCO_3$ solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. $Na_2S_2O_3$ and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was obtained as a colorless oil, which was used in the next step without further purification.

R_f = 0.4 (PE:EtOAc = 3:1);
ESI-MS (HRMS): calculated: $C_{13}H_{20}O_3Na$ $[M+Na]^+$: 247.1310, found: 247.1317 m/z.

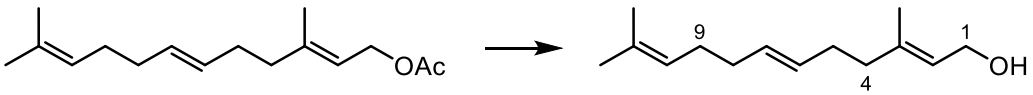
181: (2*E*,6*E*)-3,11-Dimethyldodeca-2,6,10-trien-1-yl acetate



To a stirring suspension of *iso*propyltriphenylphosphonium iodide (1.51 g, 3.5 mmol, 1.5 eq.) in Et_2O (3.5 mL) was added *n*-butyllithium (2.5 M in hexane, 1.59 mL, 3.97 mmol, 1.7 eq.) at 0 °C. Within minutes, the reaction suspension turned bright red due to the formation of the phosphorous ylide. The reaction mixture was warmed to room temperature and stirred for 1.5 h, after which a solution of aldehyde **180** (524 mg, 2.34 mmol, 1 eq.) in Et_2O (2.5 mL) was added. After stirring was continued for 2 h, no further conversion of the starting material was observed. The reaction was terminated by the addition of water, the phases were separated, and the aqueous phase was extracted with Et_2O. The combined organic phases were diluted with PE, which resulted in the precipitation of triphenylphosphine oxide. The solid impurities were removed via filtration, the filtrate was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was obtained as a colorless oil, which was used in the next step without further purification.

R_f = 0.7 (PE:EtOAc = 4:1);

182: (2*E*,6*E*)-3,11-Dimethyldodeca-2,6,10-trien-1-ol

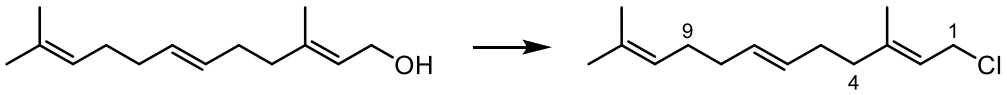


181 **182**

To a stirring solution of **181** (585 mg, 2.34 mmol, 1 eq.) in THF/MeOH (9:1, 5.5 mL) was added potassium hydroxide (180 mg, 3.21 mmol, 1.37 eq.) at room temperature. After the reaction mixture was stirred for 16 h, no further conversion of the starting material was observed. The mixture was diluted with Et_2O, the phases were separated, and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, *n*-pentane:Et_2O = 6:1 → 0:1) to yield the desired alcohol **182** (145 mg, 696 µmol, 30 % o4s) as a colorless oil.
The analytical data are consistent with those reported in the literature.[114]

R_f = 0.15 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.47–5.36 (m, 3 H, H-2, H-6, H-7), 5.11 (m, 1 H, H-10), 4.15 (d, *J* = 6.9 Hz, 2 H, H-1), 2.14–1.98 (m, 8 H, H-4, H-5, H-8, H-9), 1.69 (s, 3 H, H-12), 1.67 (s, 3 H, CH_3-3), 1.60 (s, 3 H, CH_3-11) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 139.8 (C-3), 131.8 (C-11), 130.6 (C-6/7), 129.8 (C-6/7), 124.2 (C-10), 123.6 (C-2), 59.5 (C-1), 39.7 (C-4/5/8/9), 32.9 (C-4/5/8/9), 31.0 (C-4/5/8/9), 28.3 (C-4/5/8/9), 25.8 (C-12), 19.9 (CH_3-11), 16.4 (CH_3-3) ppm.

245: (2*E*,6*E*)-1-Chloro-3,11-dimethyldodeca-2,6,10-triene

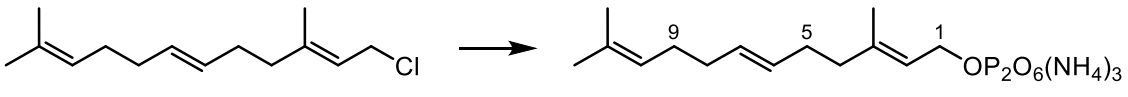


182 **245**

Chloride **molD9** was prepared according to the general procedure for the synthesis of allyl chlorides (p. 76) starting from alcohol **182** (50 mg, 0.24 mmol, 1 eq.) and was obtained as a yellow oil.

R_f = 0.95 (PE:EtOAc = 4:1);

99: Ammonium (2*E*,6*E*)-3,11-dimethyldodeca-2,6,10-trien-1-yl diphosphate

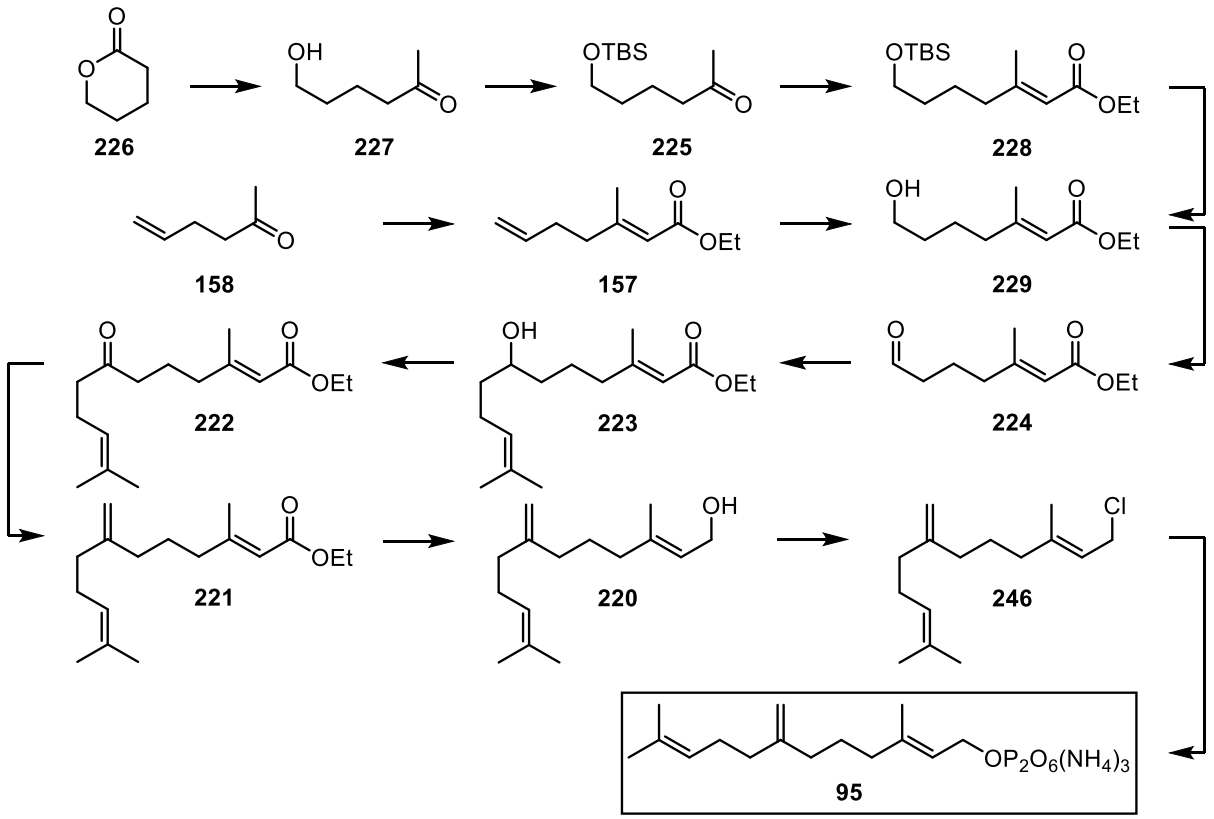


245 **99**

Desmethyl diphosphate **99** was prepared according to the general two-step procedure for the synthesis of pyrophosphates (p. 76) starting from allyl chloride **245** (53 mg, 234 µmol, 1 eq.). Pyrophosphate **99** (98 mg, 234 µmol, quant. o2s) was obtained as a colorless solid with diphosphate salt impurities (**114**).

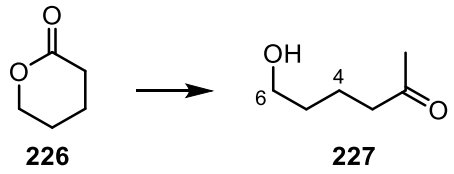
^{1}H-NMR (400 MHz, D_2O): δ = 5.53–5.40 (m, 3 H, H-2, H-6, H-7), 5.20 (m, 1 H, H-10), 4.46 (d, J = 6.6 Hz, 2 H, H-1), 2.16–1.98 (m, 8 H, H-4, H-5, H-8, H-9), 1.69 (s, 3 H, H-12), 1.68 (s, 3 H, CH_3-3), 1.60 (s, 3 H, CH_3-11) ppm;
^{13}C-NMR (100 MHz, D_2O): δ = 142.8 (C-3), 133.6 (C-11), 130.7 (C-6/7), 130.4 (C-6/7), 124.2 (C-10), 124.3 (C-2), 62.7 (C-1), 38.7 (C-4/5/8/9), 32.0 (C-4/5/8/9), 30.1 (C-4/5/8/9), 27.2 (C-4/5/8/9), 24.8 (C-12), 17.0 (CH_3-11), 15.6 (CH_3-3) ppm;
^{31}P-NMR (160 MHz, D_2O): δ = -8.45 (m, 1 P), -10.22 (m, 1 P) ppm.

6.10 SYNTHESIS OF DOUBLE BOND SHIFTED FPP DERIVATIVE 95



Scheme 75: Overview of the forward synthesis of cyclopropyl-pyrophosphate **95**. Alcohol **229** was synthesized in two ways.

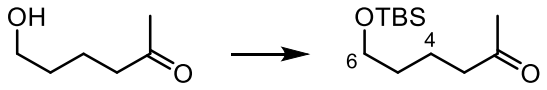
227: 6-Hydroxyhexan-2-one



Methyllithium (1.6 M in Et_2O, 483 mg, 21.97 mmol, 1.1 eq.) was added dropwise over the course of 30 min to tetrahydro-2*H*-pyran-2-one (**226**) (2.00 g, 19.98 mmol, 1 eq.) in Et_2O (20 mL) at -78 °C. After stirring for 1.5 h at -78 °C, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NH_4Cl solution, the phases were separated, and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude alcohol was obtained as a pale-yellow oil, which was used in the next reaction without further purification.[93]

R_f = 0.2 (PE:EtOAc = 4:1);

225: 6-((*tert*-Butyldimethylsilyl)oxy)hexan-2-one

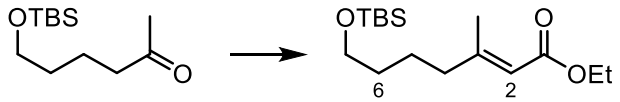


227 **225**

To a stirring solution of alcohol **227** (2.32 g, 19.98 mmol, 1 eq.) and imidazole (2.31 g, 33.96 mmol, 1.7 eq.) in CH_2Cl_2 (25 mL) was slowly added *tert*-butyldimethylsilyl chloride (4.52 g, 29.97 mmol, 1.5 eq.) in CH_2Cl_2 (15 mL) at 0 °C. After stirring for 1.5 h at 0 °C, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NH_4Cl solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 15:1) to yield the desired ketone **225** (4.47 g, 19.39 mmol, 97 % o2s) as a colorless oil.
The analytical data are consistent with those reported in the literature.[115]

R_f = 0.45 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 3.60 (t, J = 6.3 Hz, 2 H, H-6), 2.45 (t, J = 7.3 Hz, 2 H, H-3), 2.13 (s, 3 H, H-1), 1.67–1.47 (m, 4 H, H-4, H-5), 0.88 (s, 9 H, TBS), 0.04 (s, 6 H, TBS) ppm.

228: Ethyl (*E*)-7-((*tert*-butyldimethylsilyl)oxy)-3-methylhept-2-enoate

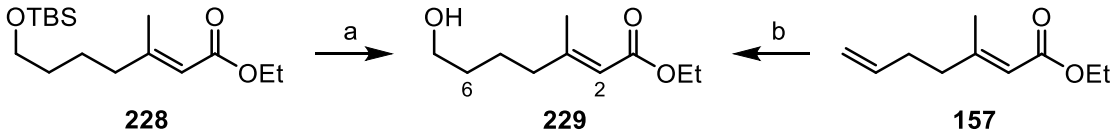


225 **228**

Ethyl 2-diethoxyphosphorylacetate (5.22 g, 23.27 mmol, 1.2 eq.) in THF (10 mL) was slowly added to a suspension of NaH (60 % in mineral oil, 558.3 mg, 23.27 mmol, 1.2 eq.) in THF (23 mL) at 0 °C. The resulting yellow suspension was stirred for 2.5 h, after which ketone **225** (4.47 g, 19.39 mmol, 1 eq.) in THF (10 mL) was slowly added and the ice bath removed. After stirring for 18 h at room temperature, no further conversion of the starting material was observed. The reaction was terminated by the addition of H_2O, the phases were separated, and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 20:1) to obtain the desired ester **228** (2.05 g, 6.81 mmol, 35 %, 84 % brsm, E:Z = 3:1) as a colorless oil.
The analytical data are consistent with those reported in the literature.[116]

R_f = 0.7 (PE:EtOAc = 9:1);
^{1}H-NMR (*E*-isomer, 400 MHz, $CDCl_3$): δ = 5.67–5.66 (m, 1 H, H-2), 4.14 (q, *J* = 7.1 Hz, 2 H, OEt), 3.61 (t, *J* = 5.9 Hz, 2 H, H-7), 2.14 (m, 5 H, H-4, CH_3-3), 1.56–1.51 (m, 4 H, H-6, H-5), 1.30 (t, *J* = 7.0 Hz, 3 H, OEt), 0.89 (s, 9 H, TBS), 0.04 (s, 6 H, TBS) ppm;
^{1}H-NMR (*Z*-isomer**,** 400 MHz, $CDCl_3$): δ = 5.67–5.66 (m, 1 H, H-2), 4.15–4.09 (m, 2 H, OEt), 3.61 (m, 2 H, H-7), 2.63 (t, *J* = 7.4 Hz, 2 H, H-4), 1.87 (d, *J* = 1.2 Hz, 3 H, CH_3-3), 1.56–1.51 (m, 4 H, H-6, H-5), 1.26 (t, *J* = 7.1 Hz, 3 H, OEt), 0.88 (s, 9 H, TBS), 0.04 (s, 6 H, TBS) ppm.

229: Ethyl (*E*)-7-hydroxy-3-methylhept-2-enoate



228 **229** **157**

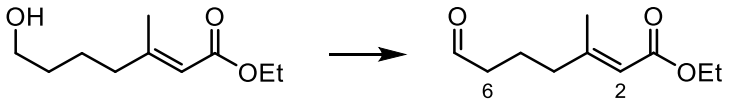
a) To a stirring solution of ester **228** (2.05 g, 6.81 mmol, 1 eq.) in THF (7 mL) was added HCl (conc., 2 mL, 23.3 mmol, 3.4 eq.) at room temperature. After stirring for 1 h, the progression of the reaction was checked via TLC. The addition of HCl (conc.) was repeated until full conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. $NaHCO_3$ solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 5:1 → 1:1) to yield the desired alcohol **229** (934 mg, 5.02 mmol, 74 %, *E*:*Z* = 3:1) as a colorless volatile oil.

b) To alkene **157** (2.51 g, 14.9 mmol, 1.00 eq.) in THF (145 mL) was added 9-borabicyclo(3.3.1)nonane (0.5 M in THF, 31.30 mL, 15.66 mmol, 1.05 eq.) at 0 °C. After the reaction solution was stirred for 17 h at room temperature, a sat. aq. $NaHCO_3$ solution was added and the mixture cooled to 0 °C. Aq. hydrogen peroxide (35 %, 5.70 mL, 66.41 mmol, 4.47 eq.) was added and the reaction mixture warmed to room temperature. After stirring was continued for 2 h, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. Na_2SO_3 solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 3:1) to yield the desired alcohol **229** (1.69 g, 9.09 mmol, 61 %, *E*:*Z* = 3:1) as a colorless volatile oil.[94]
The analytical data are consistent with those reported in the literature.[117]

R_f = 0.2 (PE:EtOAc = 4:1);

^{1}H-NMR (*E*-isomer, 400 MHz, $CDCl_3$): δ = 5.67–5.66 (m, 1 H, H-2), 4.14 (q, *J* = 7.1 Hz, 2 H, OEt), 3.65 (t, *J* = 6.2 Hz, 2 H, H-7), 2.18–2.15 (m, 5 H, H-4, CH_3-3), 1.63–1.55 (m, 4 H, H-6, H-5), 1.52 (bs, 1 H, OH), 1.27 (t, *J* = 7.1 Hz, 3 H, OEt) ppm;
^{1}H-NMR (*Z*-isomer**,** 400 MHz, $CDCl_3$): δ = 5.67–5.66 (m, 1 H, H-2), 4.12 (q, *J* = 7.1 Hz, 2 H, OEt), 3.69 (t, *J* = 6.1 Hz, 2 H, H-7), 2.63–2.59 (m, 2 H, H-4), 1.89(d, *J* = 1.4 Hz, 3 H, CH_3-3), 1.63–1.55 (m, 4 H, H-6, H-5), 1.52 (bs, 1 H, OH), 1.26 (t, *J* = 7.1 Hz, 3 H, OEt) ppm.

224: Ethyl-(*E*)-3-methyl-7-oxohept-2-enoate

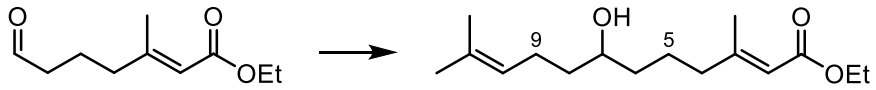


229 **224**

To a stirring solution of oxalyl chloride (0.70 mL, 1.04 g, 8.16 mmol, 1.53 eq.) in CH_2Cl_2 (8.00 mL) was slowly added DMSO (1.15 mL, 16.19 mmol, 3 eq.) at -78 °C and stirring was continued for 30 min. Then, alcohol **229** (996 mg, 5.35 mmol, 1 eq.) in CH_2Cl_2 (9.00 mL) was slowly added. After stirring for 30 min, freshly-distilled triethylamine (3.80 mL, 27.41 mmol, 5.1 eq.) was slowly added. The reaction mixture was allowed to warm to room temperature and stirred for 18 h, at which point no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NH_4Cl solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, *n*-pentane:Et_2O = 3:1) to obtain aldehyde **224** (1.38 g, 7.47 mmol, quant.) as a colorless oil.
The analytical data are consistent with those reported in the literature.[117]

R_f = 0.65 (PE:EtOAc = 30:1);
^{1}H-NMR (*E*-isomer, 400 MHz, $CDCl_3$): δ = 9.78 (t, *J* = 1.4 Hz, 1 H, H-7), 5.67–5.66 (m, 1 H, H-2), 4.15 (q, *J* = 7.1 Hz, 2 H, OEt), 2.46 (dt, *J* = 7.2 Hz, 1.4 Hz, 2 H, H-6), 2.19–2.15 (m, 5 H, H-4, CH_3-3), 1.86–1.79 (m, 2 H, H-5), 1.28 (t, *J* = 7.1 Hz, 3 H, OEt) ppm;
^{1}H-NMR (*Z*-isomer, 400 MHz, $CDCl_3$): δ = 9.79–9.78 (m, 1 H, H-7), 5.70–5.69 (m, 1 H, H-2), 4.14 (q, *J* = 7.1 Hz, 2 H, OEt), 2.67–2.63 (m, 2 H, H-4), 2.49 (dt, *J* = 7.2 Hz, 1.6 Hz, 2 H, H-6), 1.89 (d, *J* = 1.4 Hz, 3 H, CH_3-3), 1.86–1.78 (m, 2 H, H-5), 1.27 (t, *J* = 7.1 Hz, 3 H, OEt) ppm.

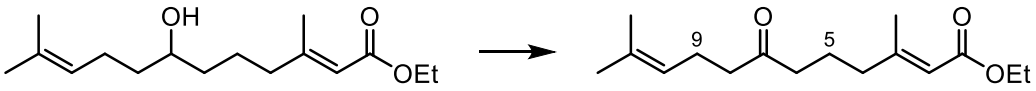
223: Ethyl (*E*)-7-hydroxy-3,11-dimethyldodeca-2,10-dienoate



224 **223**

To Mg-shavings (79 mg, 3.26 mmol, 3 eq.) in Et_2O (3 mL) was added a catalytical amount of iodine and the mixture stirred at room temperature for 30 min or until the brown color drastically decreased. Then, bromide **149** (177 mg, 1.09 mmol, 1 eq.) was added and stirring was continued for 15 min, after which a second portion of bromide **149** (177 mg, 1.09 mmol, 1 eq.) in Et_2O (1 mL) was added. After stirring for 100 min at room temperature, the reaction mixture was cooled to 0 °C, aldehyde **224** (200 mg, 1.09 mmol, 1 eq.) in Et_2O (1 mL) was dropwise added, and the ice bath removed. Stirring was continued for 18 h at room temperature, after which no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NH_4Cl solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude alcohol was obtained as a pale-yellow oil, which was used in the next reaction without further purification.

222: Ethyl-(*E*)-3,11-dimethyl-7-oxododeca-2,10-dienoate



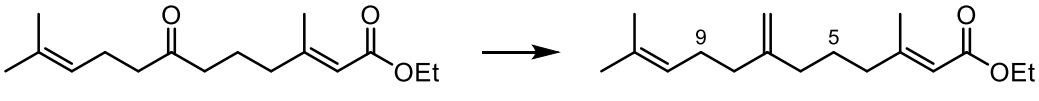
223 **222**

To a stirring solution of Dess-Martin periodinane (1.39 g, 3.27 mmol, 1.5 eq.) in CH_2Cl_2 (3.5 mL) was added crude alcohol **223** (585 mg, 2.18 mmol, 1 eq.) at room temperature. After stirring for 1.5 h, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. $NaHCO_3$ solution. The phases were separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with a sat. aq. $Na_2S_2O_3$ and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (silica, PE:EtOAc = 1:0 → 10:1) to yield the desired ketone **222** (246 mg, 0.92 mmol, 34 % o2s, *E*:*Z* = 4:1) as a colorless oil.

R_f = 0.52 (PE:EtOAc = 10:1);
ESI-MS (HRMS): calculated: $C_{16}H_{26}O_3Na$ $[M+Na]^+$: 289.1780, found: 289.1784 m/z;

^{1}H-NMR (*E*-isomer, 400 MHz, $CDCl_3$): δ = 5.65–5.64 (m, 1 H, H-2), 5.07–5.03 (m, 1 H, H-10), 4.14 (q, *J* = 7.2 Hz, 2 H, OEt), 2.47–2.37 (m, 4 H, H-8, H-6), 2.24 (dt, *J* = 7.2 Hz, 7.1 Hz, 2 H, H-9), 2.14–2.11 (m, 5 H, H-4, CH_3-3), 1.80–1.73 (m, 2 H, H-5), 1.67 (s, 3 H, H-12), 1.61 (s, 3 H, CH_3-11), 1.27 (t, *J* = 7.1 Hz, 3 H, OEt) ppm;
^{1}H-NMR (*Z*-isomer, 400 MHz, $CDCl_3$): δ = 5.67 (m, 1 H, H-2), 5.07–5.03 (m, 1 H, H-10), 4.12 (q, *J* = 7.2 Hz, 2 H, OEt), 2.63–2.59 (m, 2 H, H-4), 2.47–2.37 (m, 4 H, H-8, H-6), 2.24 (dt, *J* = 7.2 Hz, 7.1 Hz, 2 H, H-9), 1.88 (d, *J* = 1.3 Hz, 3 H, CH_3-3), 1.80–1.73 (m, 2 H, H-5), 1.67 (s, 3 H, H-12), 1.61 (s, 3 H, CH_3-11), 1.26 (t, *J* = 7.1 Hz, 3 H, OEt) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 210.4 (C-7), 166.9 (C-1), 159.1 (C-3), 132.9 (C-11), 122.8 (C-10), 116.3 (C-2), 59.7 (OEt), 43.0 (C-8), 41.9 (C-6), 40.2 (C-4), 25.8 (C-12), 22.7 (C-9), 21.3 (C-5), 18.7 (CH_3-3), 17.8 (CH_3-11), 14.5 (OEt) ppm.

221: Ethyl-(*E*)-3,11-dimethyl-7-methylenedodeca-2,10-dienoate



222 **221**

Potassium *tert*-butoxide (97 mg, 0.86 mmol, 1.56 eq.) and methyltriphenylphosphonium bromide (325 mg, 0.91 mmol, 1.64 eq.) were mixed and cooled to 0 °C. THF (3 mL) was then added and the reaction mixture was stirred for 2 h at room temperature, after which ketone **222** (148 mg, 0.56 mmol, 1 eq.) in THF (2.5 mL) was dropwise added. After the mixture was heated to 60 °C and stirred for 16 h, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NH_4Cl solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 50:1) to obtain the desired alkene **221** (97 mg, 0.37 mmol, 66 %) as a pale-yellow oil with light impurities.
The analytical data are consistent with those reported in the literature.[118]

R_f = 0.39 (PE:EtOAc = 50:1);
ESI-MS (HRMS): calculated: $C_{17}H_{28}O_2Na$ $[M+Na]^+$: 287.1987, found: 287.1989 m/z;
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.66 (m, 1 H, H-2), 5.11–5.09 (m, 1 H, H-10), 4.74–4.73 (m, 2 H, CH_2-7), 4.14 (q, *J* = 7.0 Hz, 2 H, OEt), 2.16–2.08 (m, 7 H, H-4, H-9, CH_3-3), 2.05–1.99 (m, 4 H, H-6, H-8), 1.69 (s, 3 H, H-12), 1.64–1.59 (m, 5 H, H-5, CH_3-11), 1.29–1.26 (m, 3 H, OEt) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 160.1 (C-3), 149.1 (C-7), 128.6 (C-11), 124.2 (C-10), 115.8 (C-2), 109.5 (CH_2-7), 59.6 (OEt), 40.6 (C-4), 36.1 (C-6), 35.7 (C-8), 26.6 (C-9), 25.8 (C-12), 25.6 (C-5), 18.9 (CH_3-3), 17.9 (CH_3-11), 14.5 (OEt) ppm.

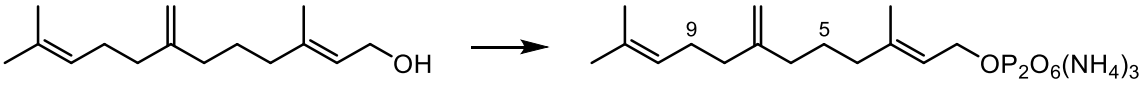
220: (*E*)-3,11-Dimethyl-7-methylenedodeca-2,10-dien-1-ol

221 **220**

Di*iso*butylaluminium hydride (1 M in hexane, 0.59 mL, 0.59 mmol, 2.22 eq.) was slowly added to a solution of ester **221** (97 mg, 0.27 mmol, 1 eq.) in dry CH_2Cl_2 (1 mL) at -78 °C. After stirring the solution for 3.5 h at room temperature, TLC analysis indicated uncomplete conversion of the ester. Therefore, another portion of di*iso*butylaluminium hydride (1 M in hexane, 0.50 mL, 0.50 mmol, 1.88 eq.) was added and the reaction solution stirred for 1 h at room temperature, at which point no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. Na, K-tartrate solution and the resulting viscous mixture stirred for 16 h at room temperature. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 3:1) to obtain the desired alcohol **220** (53 mg, 0.24 mmol, 65 %, *E*:*Z* = 2.5:1) as a pale-yellow oil.
The analytical data are consistent with those reported in the literature.[118]

R_f = 0.52 (PE:EtOAc = 3:1);
^{1}H-NMR (*E*-isomer, 400 MHz, $CDCl_3$): δ = 5.43–5.40 (m, 1 H, H-2), 5.13–5.09 (m, 1 H, H-10), 4.72 (s, 2 H, CH_2-7), 4.16 (d, *J* = 6.9 Hz, 2 H, H-1), 2.14–1.98 (m, 8 H, H-4, H-6, H-8, H-9), 1.69–1.67 (m, 6 H, H-12, CH_3-3), 1.61 (s, 3 H, CH_3-11), 1.58–1.54 (m, 2 H, H-5) ppm;
^{1}H-NMR (*Z*-isomer, 400 MHz, $CDCl_3$): δ = 5.43–5.40 (m, 1 H, H-2), 5.13–5.09 (m, 1 H, H-10), 4.72 (s, 2 H, CH_2-7), 4.12 (d, *J* = 7.0 Hz, 2 H, H-1), 2.14–1.98 (m, 8 H, H-4, H-6, H-8, H-9), 1.74 (s, 3 H, CH_3-3), 1.69–1.67 (m, 3 H, H-12), 1.61 (s, 3 H, CH_3-11), 1.58–1.54 (m, 2 H, H-5) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 149.6 (C-7), 140.1 (C-3), 131.7 (C-11), 124.3 (C-2), 123.5 (C-10), 109.1 (CH_2-7), 59.6 (C-1), 39.4 (C-4), 36.2 (C-6), 35.9 (C-8), 26.6 (C-9), 25.9 (C-12), 25.9 (C-5), 17.9 (CH_3-11), 16.4 (CH_3-3) ppm.

95: Ammonium (*E*)-3,11-dimethyl-7-methylenedodeca-2,10-dien-1-yl diphosphate



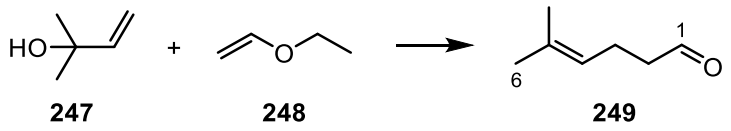
220 **95**

The double bond shifted FPP derivative **95** was prepared according to the general one-step procedure for the synthesis of diphosphates (p. 78) starting from alcohol **220** (30 mg, 135 µmol, 1 eq.). Purification of the crude mixture via column chromatography (silica, *i*PrOH:NH_3:H_2O = 6:3:1) yielded the desired diphosphate **95** (10 mg, 23 µmol, 17 %) as a colorless solid with TEAP salt impurities.
The analytical data are consistent with those reported in the literature.[118]

R_f = 0.27 (*i*PrOH:NH_3:H_2O = 6:3:1);
^{1}H-NMR (*E*-isomer, 400 MHz, D_2O): δ = 5.46–5.42 (m, 1 H, H-2), 5.22–5.16 (m, 1 H, H-10), 4.52–4.44 (m, 2 H, CH_2-7), 4.00 (qi, J = 6.2 Hz, 2 H, H-1), 2.15–2.01 (m, 8 H, H-4, H-6, H-8, H-9), 1.70–1.67 (m, 6 H, H-12, CH_3-3), 1.62 (s, 3 H, CH_3-11), 1.59–1.54 (m, 2 H, H-5) ppm;
^{13}C-NMR (100 MHz, D_2O): δ = 151.5 (C-7), 143.2 (C-3), 133.5 (C-11), 124.4 (C-10), 119.6 (d, C-2), 108.7 (CH_2-7), 64.2 (C-1), 38.5 (C-4), 35.4 (C-6), 34.9 (C-8), 25.6 (C-9), 25.1 (C-5), 24.8 (C-12), 16.9 (CH_3-11), 15.5 (CH_3-3) ppm.

6.11 MISCELLANEOUS REACTIONS

249: 5-Methylhex-4-enal

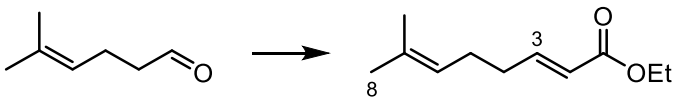


247 **248** **249**

A high-pressure seal tube was charged with ethoxyethane (**248**) (16 g, 21 mL, 0.22 mol, 1.5 eq.), 2-methyl-3-buten-2-ol (**247**) (12 g, 15 mL, 0.14 mol, eq.), and phosphoric acid (85 %, 0.17 g, 97 µL, 1.4 mmol, 0.01 eq.). The reaction mixture was heated to 150 °C and allowed to stir for 2.5 h. The pale yellow solution was cooled to room temperature and neutralized with triethylamine. Purification by fractional distillation (80⟶90 °C, 330⟶10 mbar) afforded the desired aldehyde **249** as a colorless oil (14.84 g, 132.27 mmol, 92 %).
The analytical data are consistent with those reported in the literature.[119]

R_f = 0.8 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 9.76 (t, J = 1.7 Hz, 1 H, H-1), 5.08 (m, 1 H, H-4), 2.46 (m, 2 H, H-2), 2.32 (m, 2 H, H-3), 1.68 (s, 3 H, CH_3-5), 1.62 (s, 3 H, H-6) ppm.

250: Ethyl (*E*)-7-methylocta-2,6-dienoate

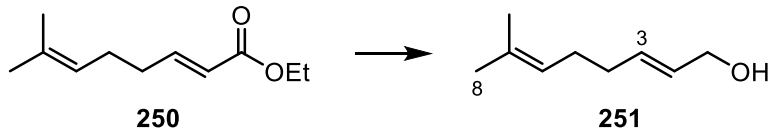


249 **250**

To a stirring solution of ethyl (triphenylphosphoranylidene) acetate (9.32 g, 26.7 mmol, 1.5 eq.) in toluene (26 mL) was added aldehyde **249** (2 g, 17.8 mmol, 1 eq.) at room temperature. After stirring was continued for 18 h, no further conversion of the starting material was observed. The reaction mixture was diluted with PE, which resulted in the precipitation of triphenylphosphine oxide. The solid impurities were removed via filtration, the filtrate was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was obtained as a colorless oil, which was used in the next step without further purification.[120]

R_f = 0.6 (PE:EtOAc = 4:1);

251: (*E*)-7-Methylocta-2,6-dien-1-ol

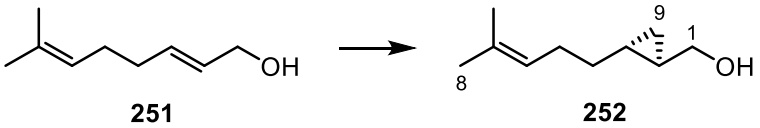


250 **251**

To a stirring solution of ester **250** (3.25 g, 17.8 mmol, 1 eq.) in Et_2O (18 mL) was dropwise added di*iso*butylaluminium hydride (1 M in hexane, 39.2 mL, 39.2 mmol, 2.2 eq.) at 0 °C. After stirring for 2.5 h at room temperature, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. Na, K-tartrate solution and the resulting viscous mixture stirred for 16 h at room temperature. The phases were separated, and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (silica, PE:EtOAc = 10:1 ⟶ 4:1) to yield the desired alcohol **251** (1.37 g, 9.78 mmol, 55 % o2s, *E*:*Z* = 15.5:1) as a colorless oil.
The analytical data are consistent with those reported in the literature.[120]

R_f = 0.25 (PE:EtOAc = 4:1);
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.75–5.62 (m, 2 H, H-2, H-3), 5.11 (m, 1 H, H-6), 4.09 (d, *J* = 5.0 Hz, 1 H, H-1), 2.07 (m, 4 H, H-4, H-5), 1.69 (s, 3 H, H-8), 1.60 (s, 3 H, CH_3-7) ppm.

252: ((1*R*,2*R*)-2-(4-Methylpent-3-en-1-yl)cyclopropyl)methanol



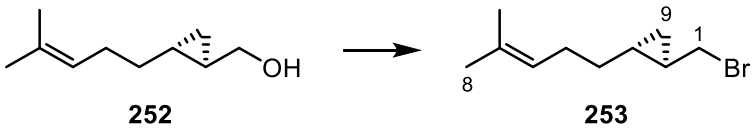
251 **252**

Starting from allyl alcohol **251** (50 mg, 0.36 mmol, 1 eq.), the desired cyclopropyl alcohol **252** was prepared according to the general procedure for the asymmetric cyclopropanation of allyl alcohols (p. 79) using (4*S*,5*S*)-2-butyl-N^4,N^4,N^5,N^5-tetramethyl-1,3,2-dioxaborolane-4,5-dicarboxamide (**209S**, 144 mg, 0.54 mmol, 1.5 eq.). Purification via column chromatography (silica, *n*-pentane:Et_2O = 10:1) delivered cyclopropyl alcohol **252** (44 mg, 0.36 mmol, 80 %) as a colorless oil with minor impurities of the double cyclopropanated product.

R_f = 0.3 (PE:EtOAc = 4:1);

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.14 (t, J = 7.1 Hz, 1 H, H-6), 3.74–3.38 (m, 2 H, H-1), 2.07 (m, 2 H, H-5), 1.69 (s, 3 H, H-8), 1.62 (s, 3 H, CH_3-7), 1.37–1.31 (m, 2 H, H-4), 0.88 (m, 1 H, H-3), 0.62 (m, 1 H, H-2), 0.40–0.29 (m, 2 H, H-9) ppm;
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 131.8 (C-7), 124.5 (C-6), 67.4 (C-1), 33.9 (C-4), 28.2 (C-5), 25.9 (C-8), 17.9 (C-2), 17.1 (CH_3-7), 14.3 (C-3), 10.0 (C-9) ppm.

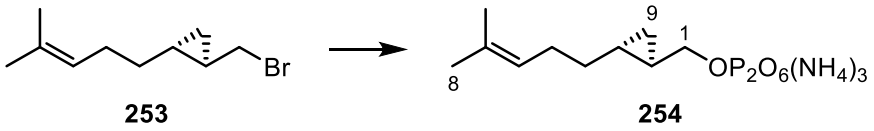
253: (1*R*,2*R*)-1-(Bromomethyl)-2-(4-methylpent-3-en-1-yl)cyclopropane



252 **253**

To a stirring solution of cyclopropyl alcohol **252** (40 mg, 0.26 mmol, 1 eq.) in CH_2Cl_2 (0.3 mL) and triphenylphosphine (156 mg, 0.60 mmol, 2.3 eq.) was added tetrabromomethane (189 mg, 0.57 mmol, 2.2 eq.) at room temperature. After stirring for 18 h, no further conversion of the starting material was observed. To remove remaining triphenylphosphine and triphenylphosphine oxide, most of the CH_2Cl_2 was removed under reduced pressure and *n*-pentane was added. The solid impurities were removed via filtration and the filter cake was washed with *n*-pentane and Et_2O. This step was repeated until no further precipitation was observed upon addition of *n*-pentane. The combined organic phases were washed with a sat. aq. $Na_2S_2O_3$ and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was obtained as a yellow oil, which was used in the next step without further purification.

254: Ammonium ((1*R*,2*R*)-2-(4-methylpent-3-en-1-yl)cyclopropyl)methyl diphosphate



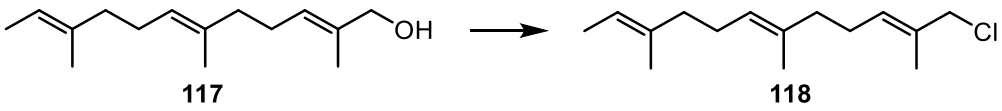
253 **254**

Cyclopropyl diphosphate **254** was prepared according to the general two-step procedure for the synthesis of pyrophosphates (p. 76) starting from allyl bromide **253** (56 mg, 259 µmol, 1 eq.). Pyrophosphate **254** (95 mg, 259 µmol, quant. o2s) was obtained as a colorless solid with diphosphate salt impurities (**114**).

^{1}H-NMR (400 MHz, D_2O): δ = 5.17 (t, J = 7.1 Hz, 1 H, H-6), 3.84–3.77 (m, 1 H, H-1), 3.58–3.49 (m, 1 H, H-1), 1.99 (m, 2 H, H-5), 1.59 (s, 3 H, H-8), 1.53 (s, 3 H, CH_3-7), 1.21–1.11 (m, 2 H, H-4), 0.84 (m, 1 H, H-3), 0.64 (m, 1 H, H-2), 0.40–0.23 (m, 2 H, H-9) ppm;

^{13}C-NMR (100 MHz, D_2O): δ = 133.3 (C-7), 124.8 (C-6), 70.8 (C-1), 33.0 (C-4), 27.3 (C-5), 24.8 (C-8), 18.1 (C-2), 16.9 (CH_3-7), 16.6 (C-3), 9.7 (C-9) ppm;
^{31}P-NMR (160 MHz, D_2O): δ = -8.40 (m, 1 P), -10.47 (m, 1 P) ppm.

118: (2*E*,6*E*,10*E*)-1-Chloro-3,7,10-trimethyldodeca-2,6,10-triene

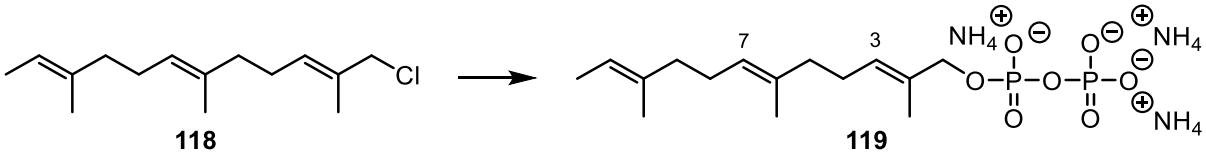


117 118

Allyl chloride **118** was prepared according to the general procedure for the synthesis of allyl chlorides (p. 76) starting from alcohol **117** (50 mg, 0.22 mmol, 1 eq.) and was obtained as a yellow oil.

R_f = 0.8 (PE:EtOAc = 4:1).

119: (2*E*,6*E*,10*E*)-3,7,10-Trimethyldodeca-2,6,10-trien-1-yl diphosphate

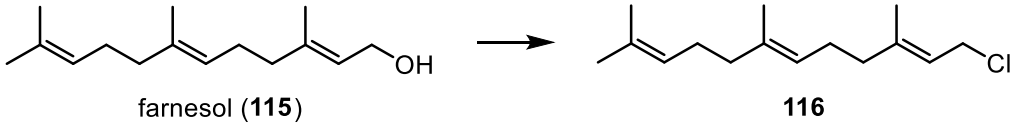


118 119

Allyl diphosphate **119** was prepared according to the general two-step procedure for the synthesis of pyrophosphates (p. 76) starting from allyl chloride **118** (54 mg, 0.22 mmol, 1 eq.). Diphosphate **119** (32 mg, 0.07 mmol, 33 % o2s) was obtained as a colorless solid with minor impurities of pyrophosphate salt **114**.
The analytical data are consistent with those reported in the literature.[54]

^{1}H-NMR (400 MHz, D_2O): δ = 5.55 (t, J = 6.7 Hz, 1 H, 2-H), 5.33–5.18 (m, 2 H, H-6, H-11), 4.32 (d, J = 5.6 Hz, 2 H, H-1), 2.21–2.00 (m, 8 H, H-4, H-5, H-8, H-9), 1.73–1.54 (m, 12 H, 4×CH_3) ppm;
^{13}C-NMR (100 MHz, D_2O): δ = 137.1 (C-10), 136.4 (C-6), 132.2 (d, C-2), 128.7 (C-3), 124.5 (C-7), 118.9 (C-11), 71.7 (C-1), 38.8 (C-5/9), 38.4 (C-5/9), 25.7 (C-4/8), 23.7 (C-4/8), 15.3 (CH_3), 14.9 (CH_3), 13.0 (CH_3), 12.6 (CH_3) ppm.

116: (2*E*,6*E*)-1-Chloro-3,7,11-trimethyldodeca-2,6,10-triene

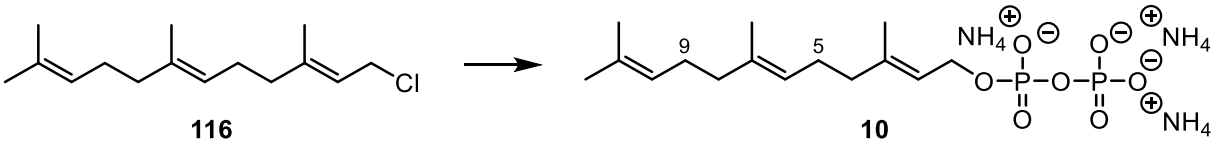


farnesol (**115**) **116**

Allyl chloride **116** was prepared according to the general procedure for the synthesis of allyl chlorides (p. 76) starting from farnesol (**115**) (300 mg, 1.35 mmol, 1 eq.) and was obtained as a yellow oil.

R$_f$ = 0.75 (PE:EtOAc = 4:1).

10: (2*E*,6*E*)-3,7,11-Trimethyldodeca-2,6,10-trien-1-yl diphosphate



116 **10**

Farnesyl pyrophosphate (**10)** was prepared according to the general two-step procedure for the synthesis of pyrophosphates (p. 76) starting from allyl chloride **116** (50 mg, 0.21 mmol, 1 eq.). Diphosphate **10** (65 mg, 0.15 mmol, 72 % o2s) was obtained as a colorless solid with minor impurities of pyrophosphate salt **114**.
The analytical data are consistent with those reported in the literature.[56]

^{1}H-NMR (400 MHz, D_2O): δ = 5.46 (t, J = 6.8 Hz, 1 H, H-2), 5.25–5.16 (m, 2 H, H-6, H-10), 4.46 (t, J = 6.5 Hz, 2 H, H-1), 2.18–1.99 (m, 8 H, H-4, H-5, H-8, H-9), 1.71 (s, 3 H, CH_3-3), 1.68 (s, 3 H, CH_3-7), 1.61 (s, 6 H, H-12, CH_3-11) ppm;
^{31}P-NMR (106 MHz, D_2O): δ = -6.45 (d, J = 22.1 Hz, 1 P), -10.00 (d, J = 21.9 Hz, 1 P) ppm.

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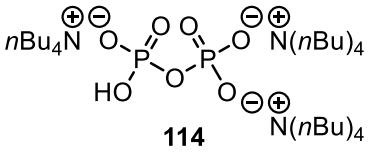
8 ATTACHMENTS

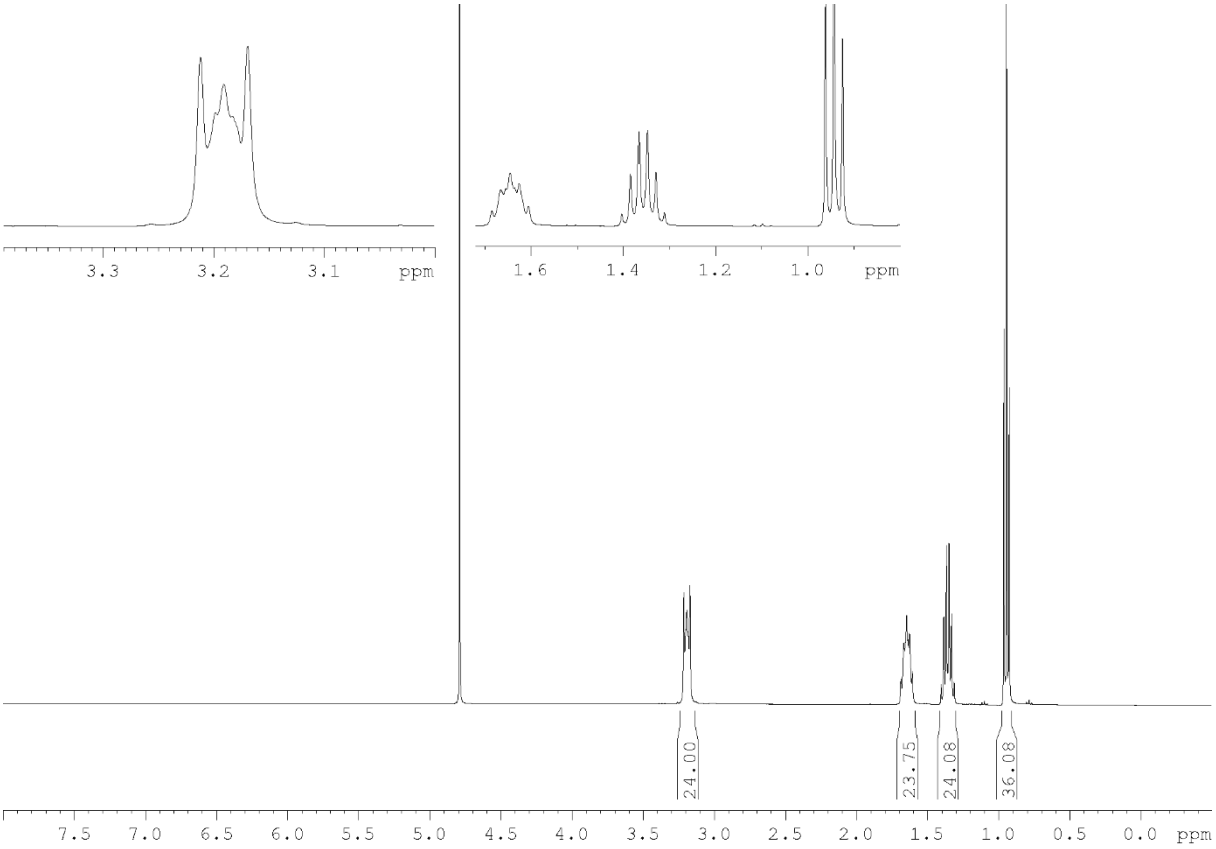
8.1 NMR DATA

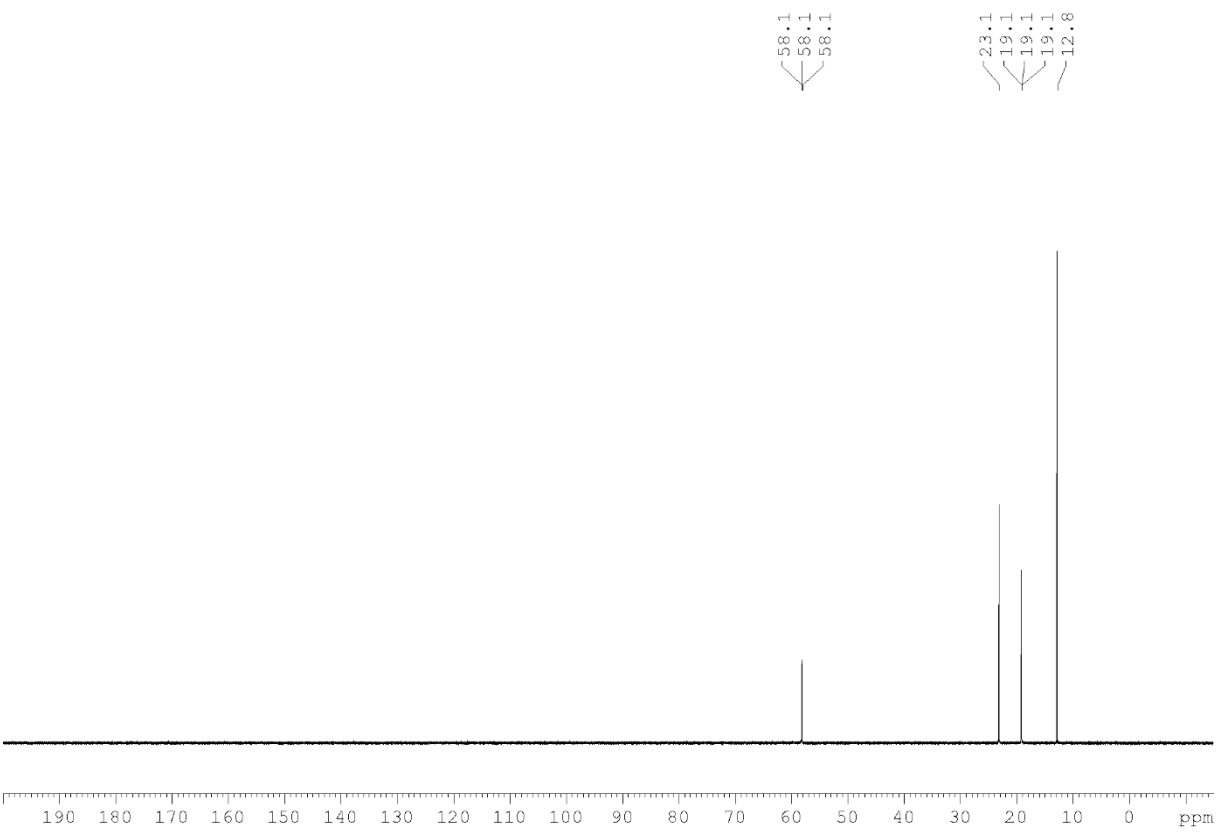
Remarks

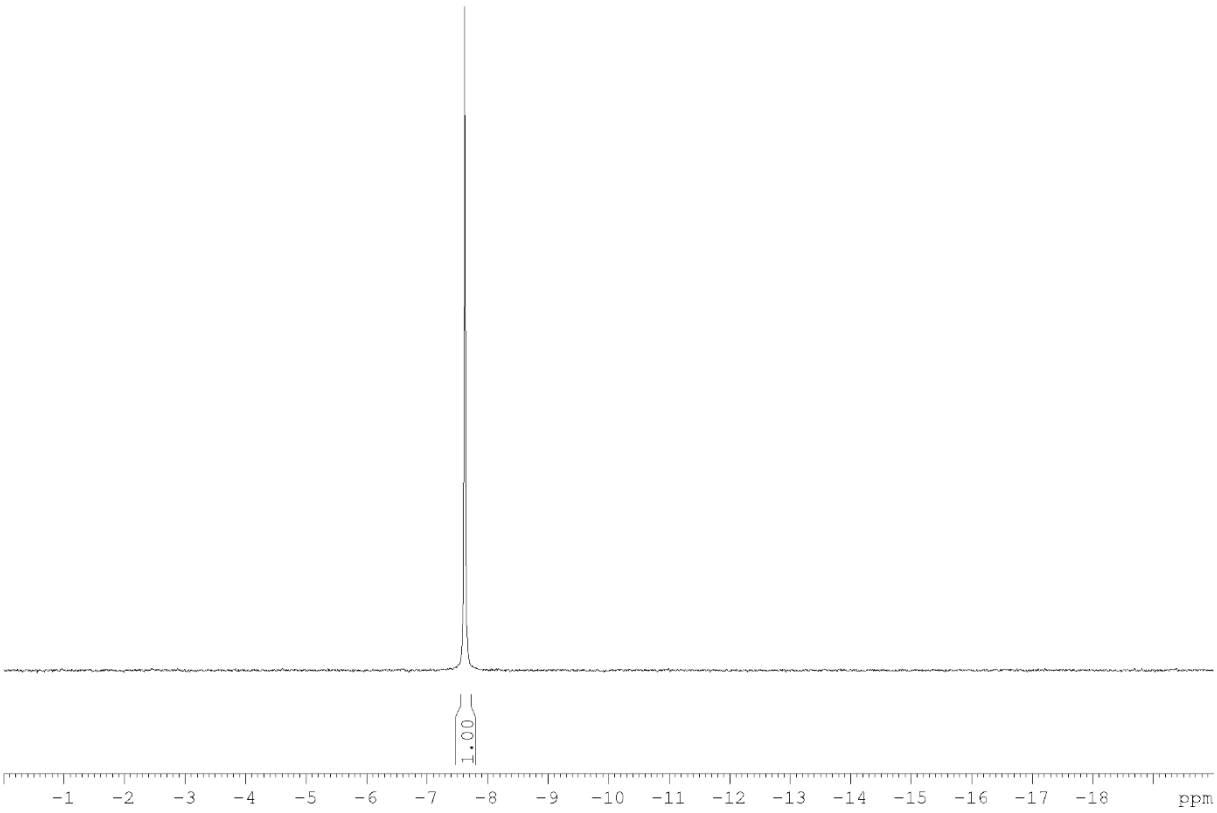
- NMR spectra of literature-unknown compounds and selected key (literature-known) intermediates are attached. For the latter, generally only ^{1}H spectra are included.
- For each molecule, the NMR spectra are reported in the following order: ^{1}H, ^{13}C, ^{31}P.

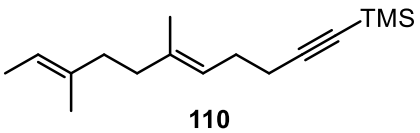


114

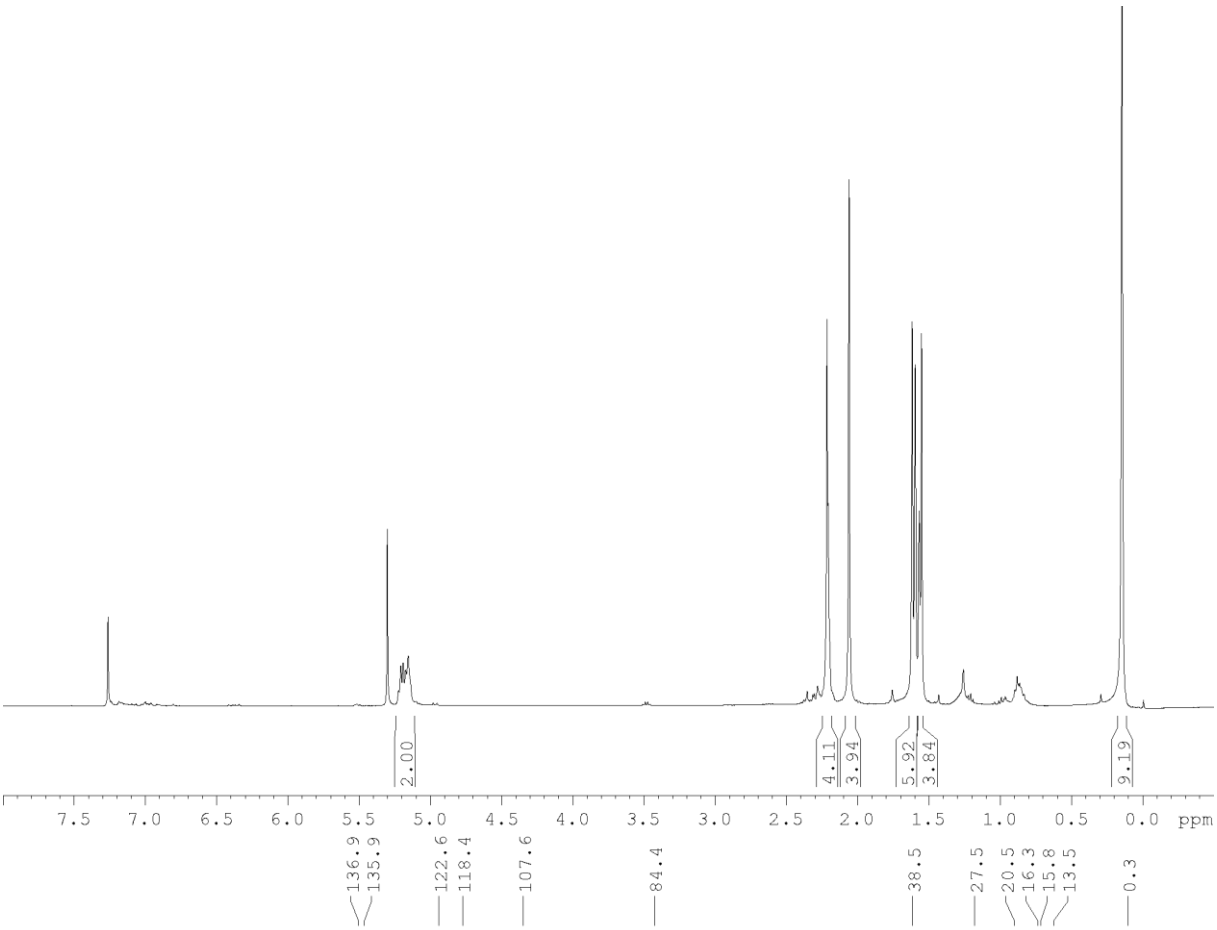



58.1
58.1
58.1
23.1
19.1
19.1
19.1
12.8
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


1.00
-1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18 ppm


TMS

110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4.11
3.94
5.92
3.84
9.19
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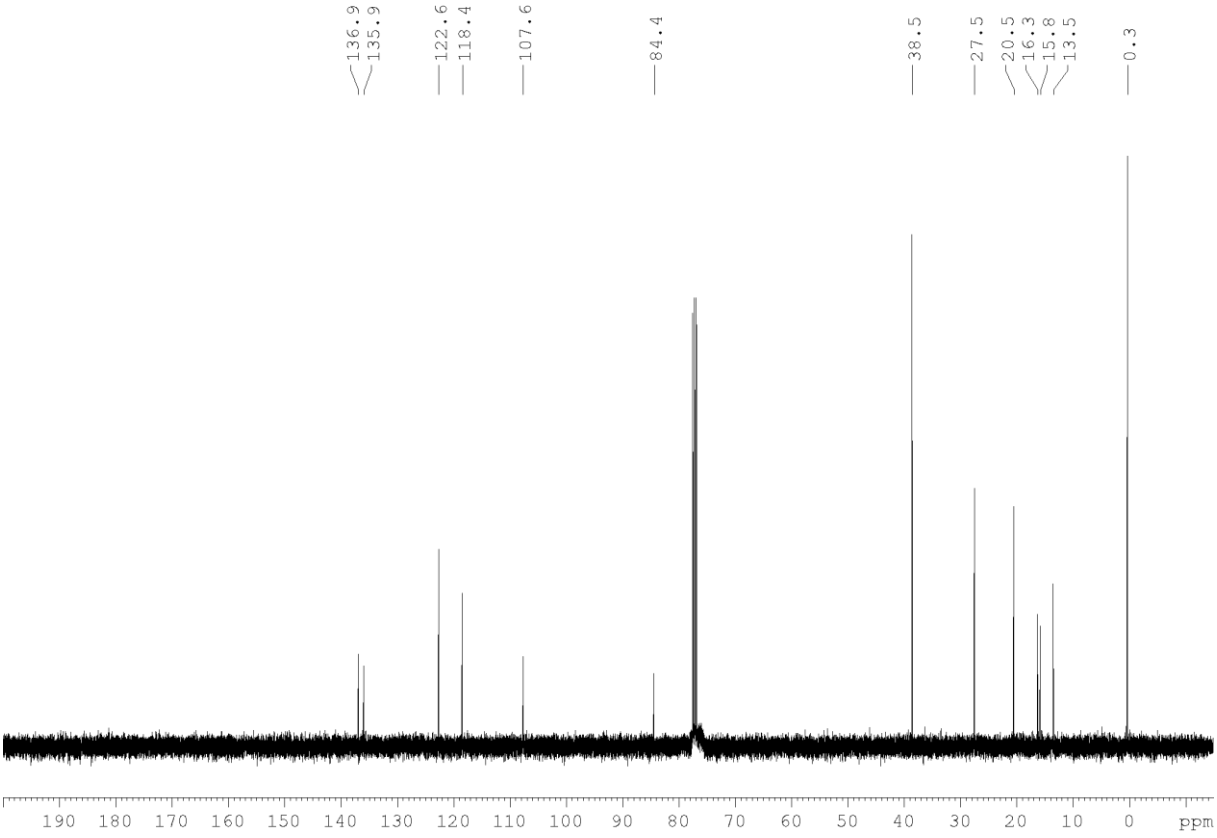
136.9
135.9
122.6
118.4
107.6
84.4
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27.5
20.5
16.3
15.8
13.5
0.3
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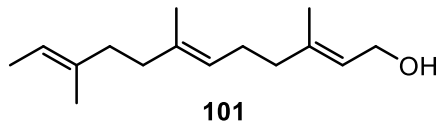
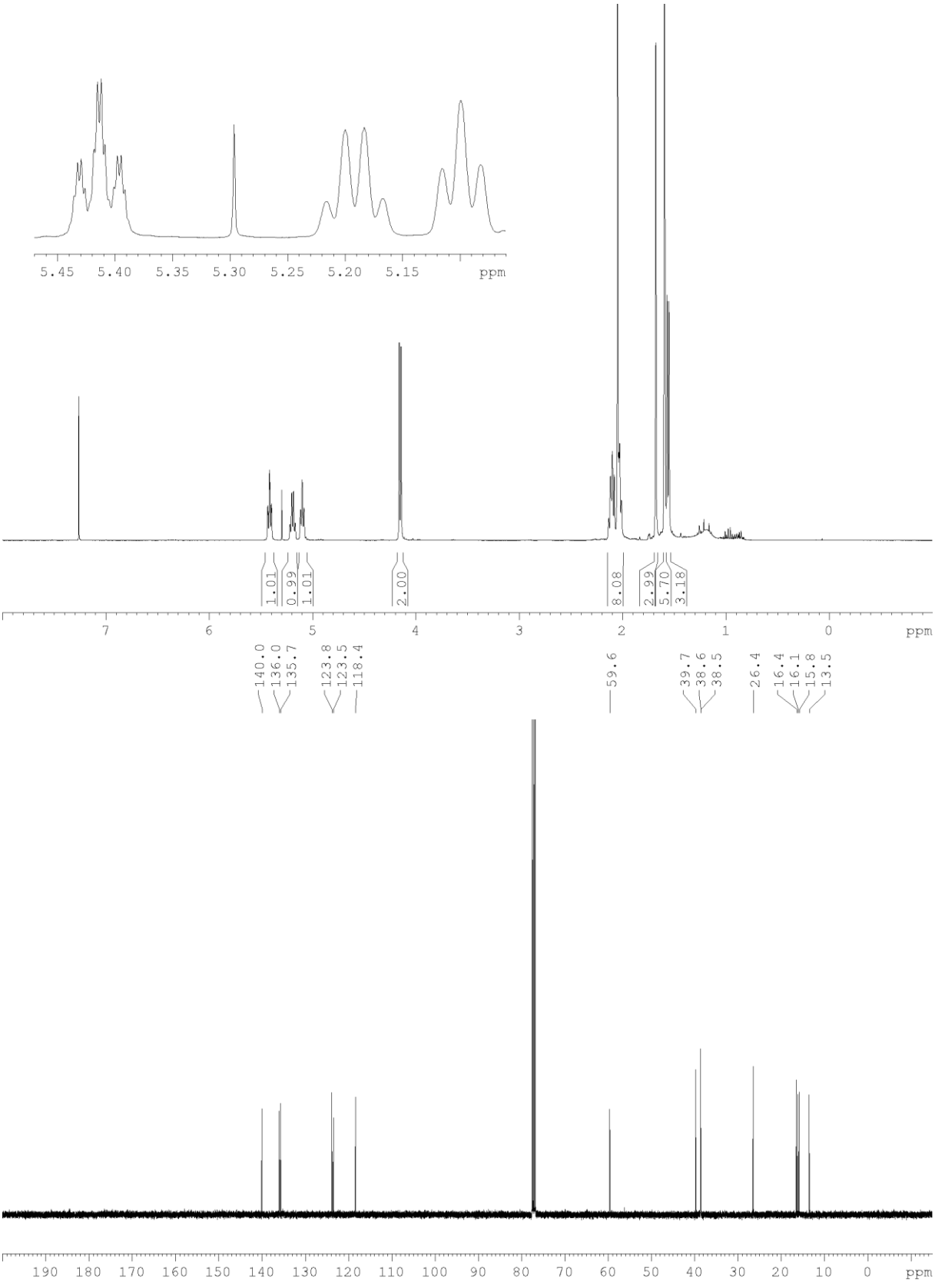
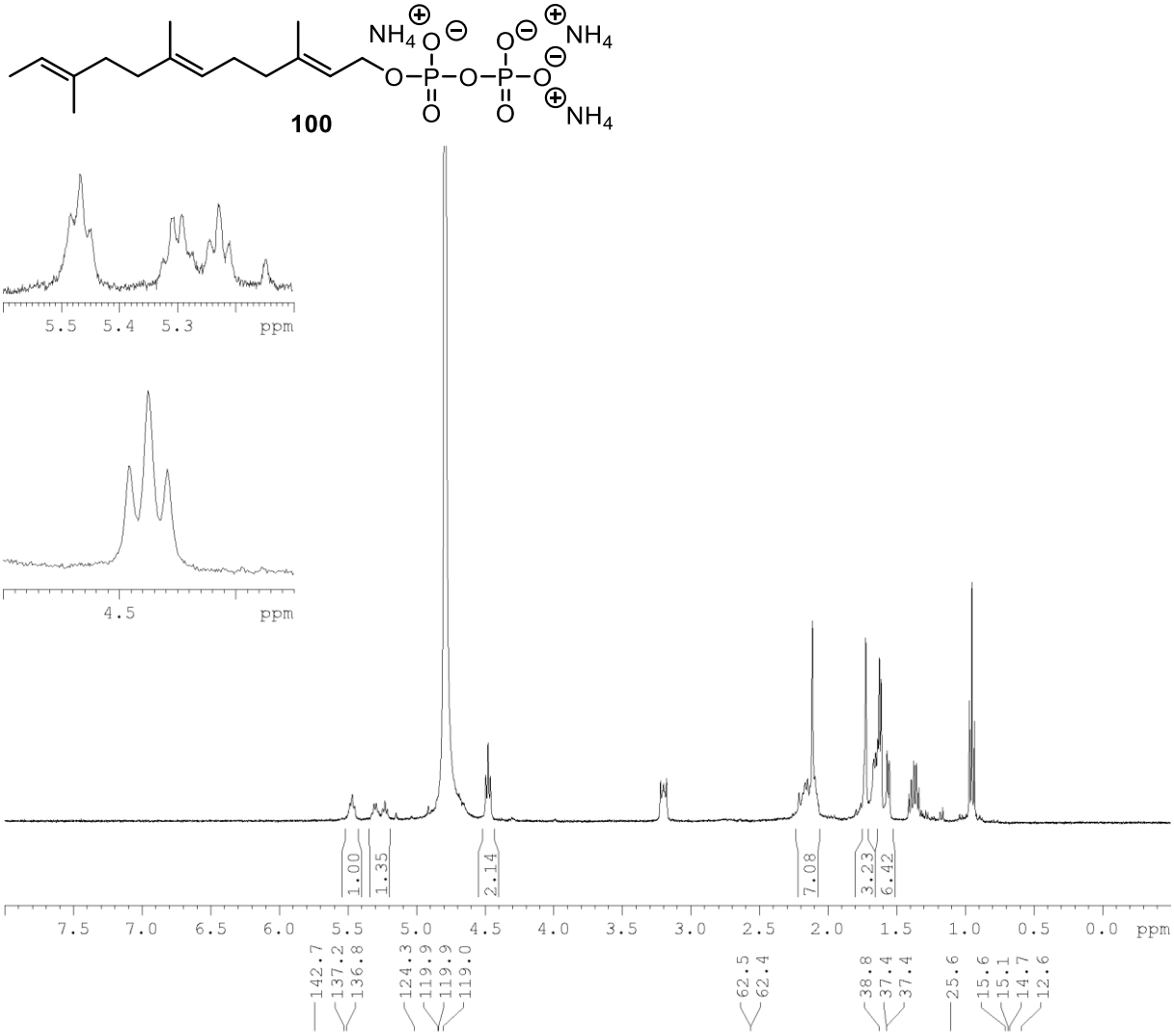
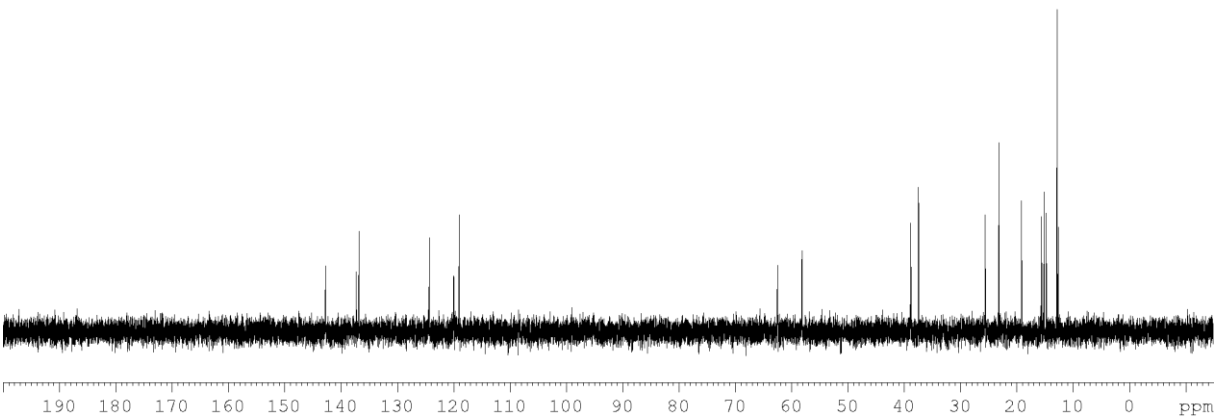
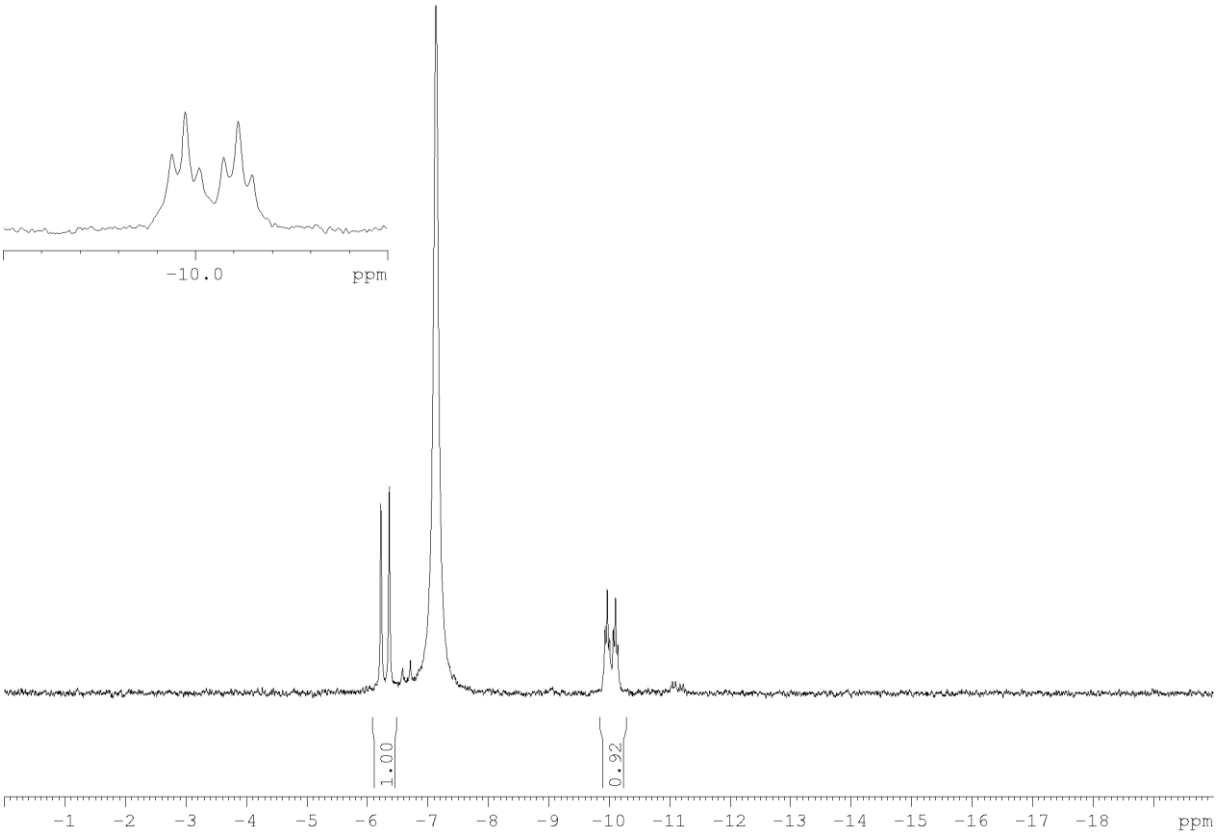

OH

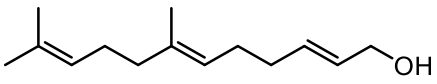
101


5.45 5.40 5.35 5.30 5.25 5.20 5.15 ppm
1.01 0.99 1.01 2.00 8.08 2.99 5.70 3.18
7 6 5 4 3 2 1 0 ppm
140.0 136.0 135.7 123.8 123.5 118.4 59.6 39.7 38.6 38.5 26.4 16.4 16.1 15.8 13.5
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

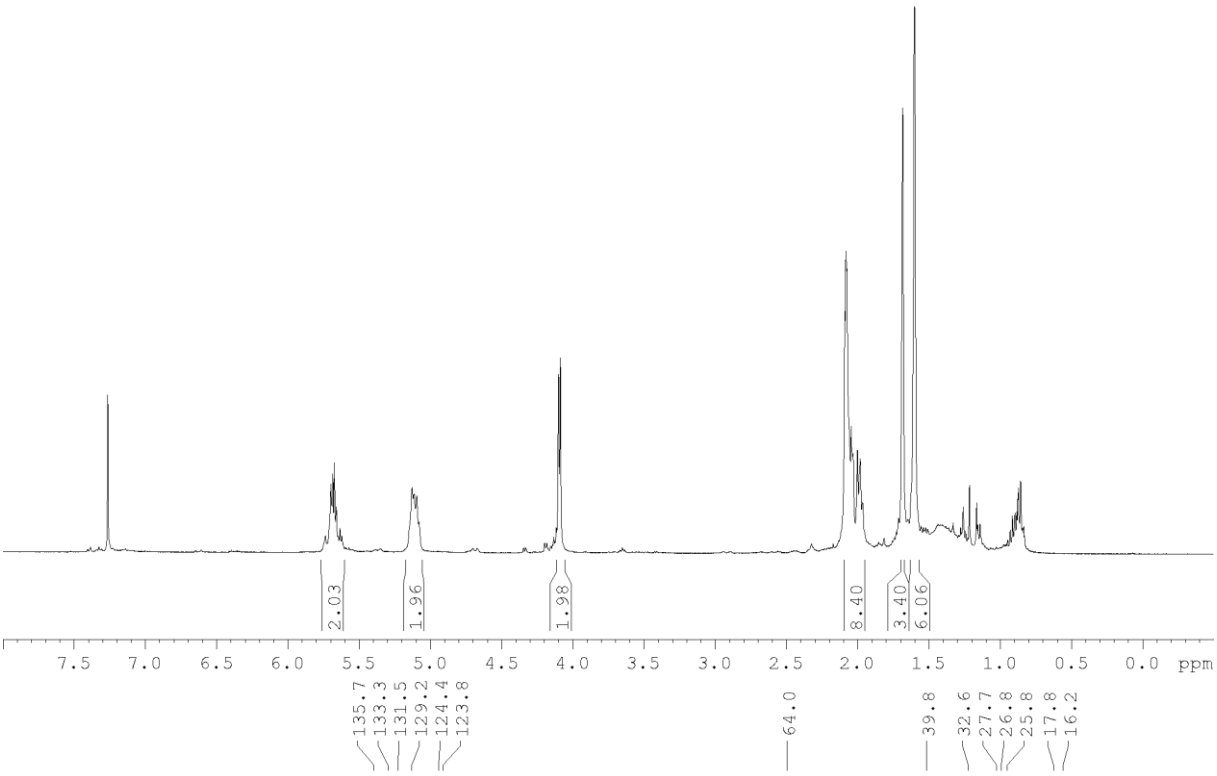

NH4
NH4
NH4
100
5.5 5.4 5.3 ppm
4.5 ppm
1.00
1.35
2.14
7.08
3.23
6.42
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
142.7
137.2
136.8
124.3
119.9
119.9
119.0
62.5
62.4
38.8
37.4
37.4
25.6
15.6
15.1
14.7
12.6

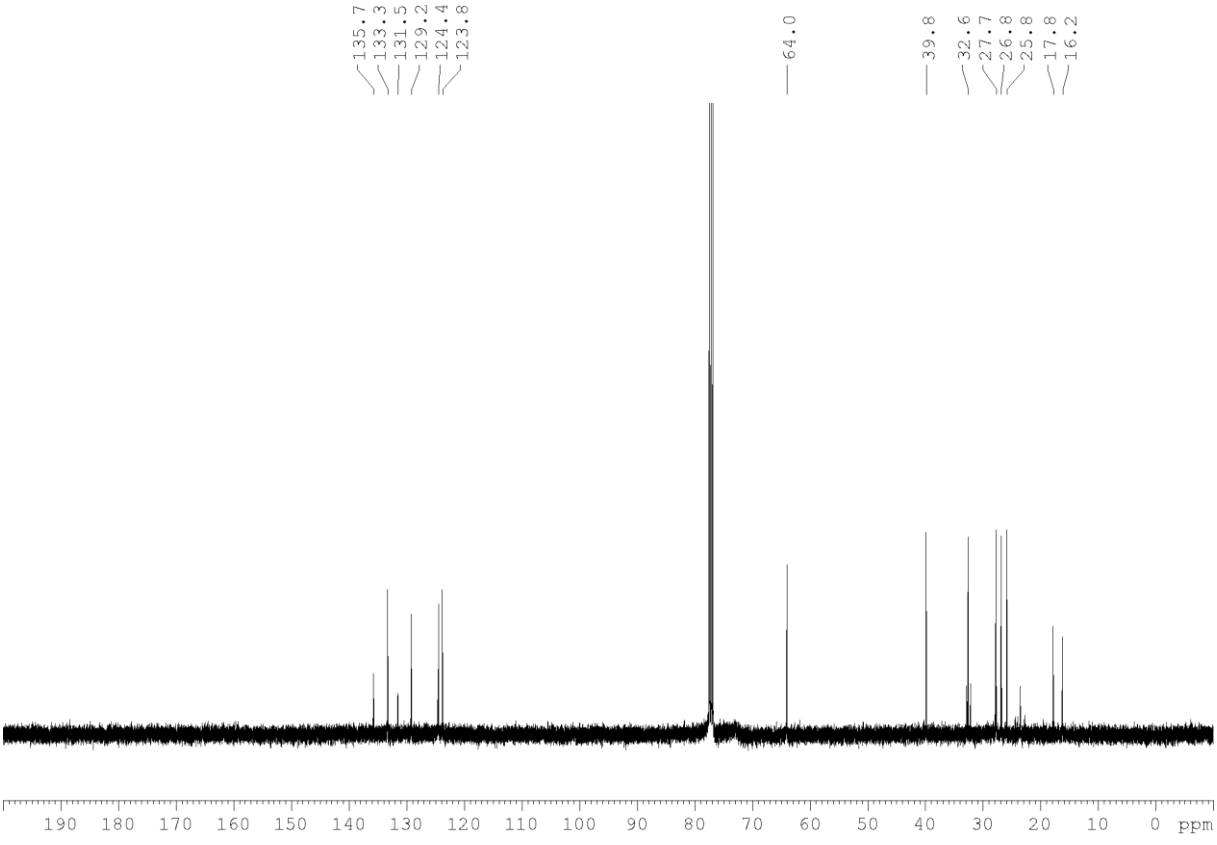

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

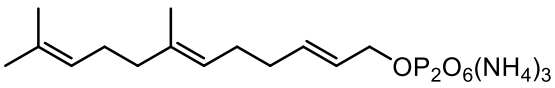

-10.0
ppm
1.00
0.92
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
-13
-14
-15
-16
-17
-18
ppm


OH

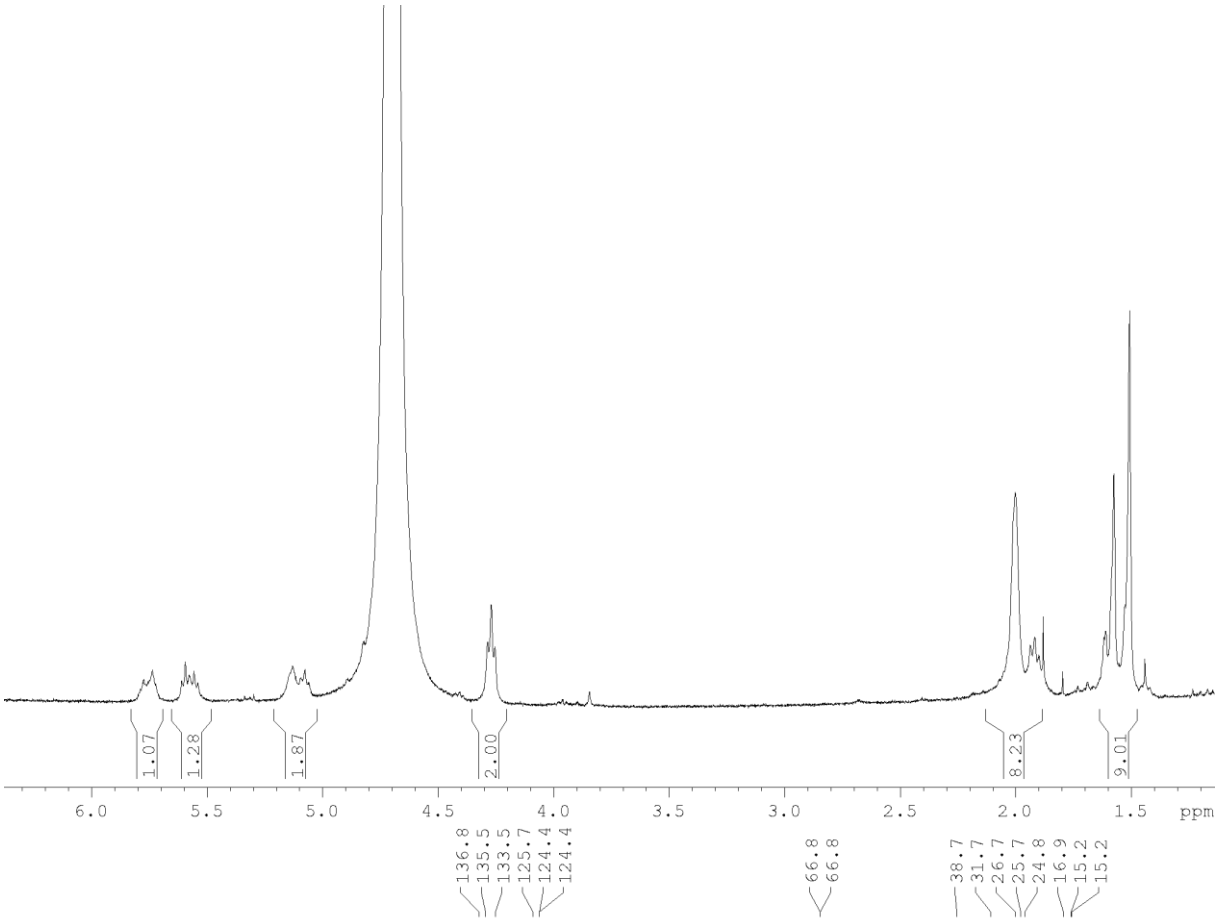
198

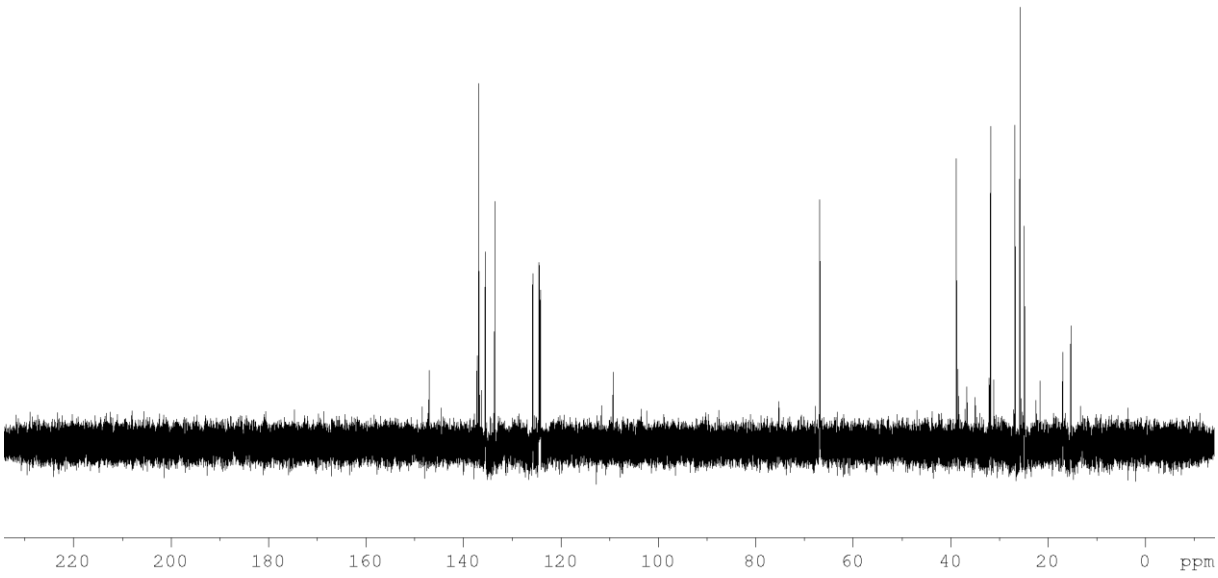

2.03
1.96
1.98
8.40
3.40
6.06
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

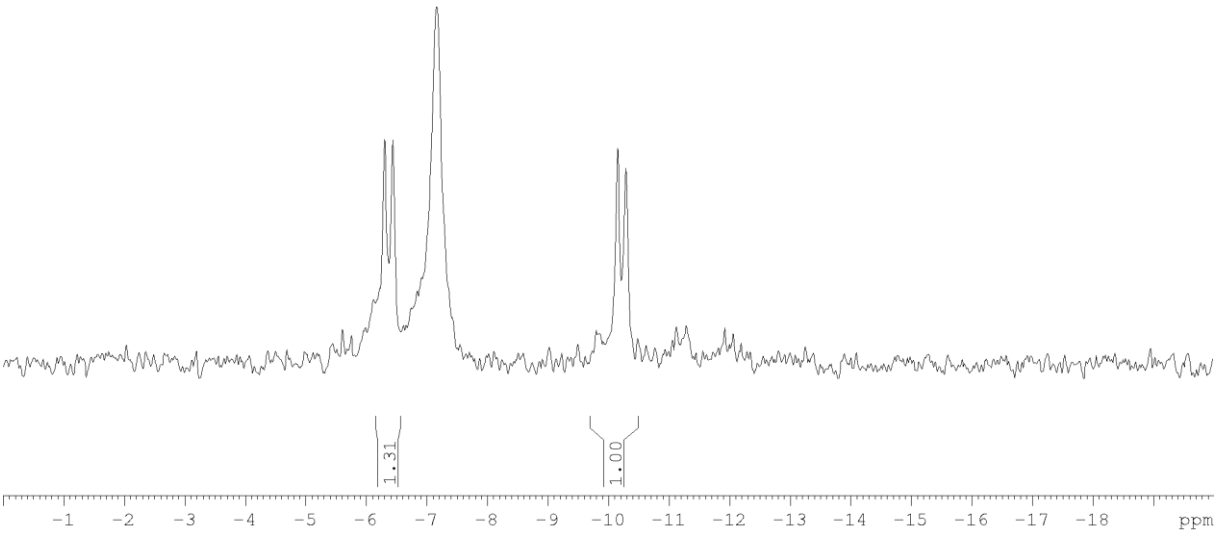

135.7
133.3
131.5
129.2
124.4
123.8
64.0
39.8
32.6
27.7
26.8
25.8
17.8
16.2
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

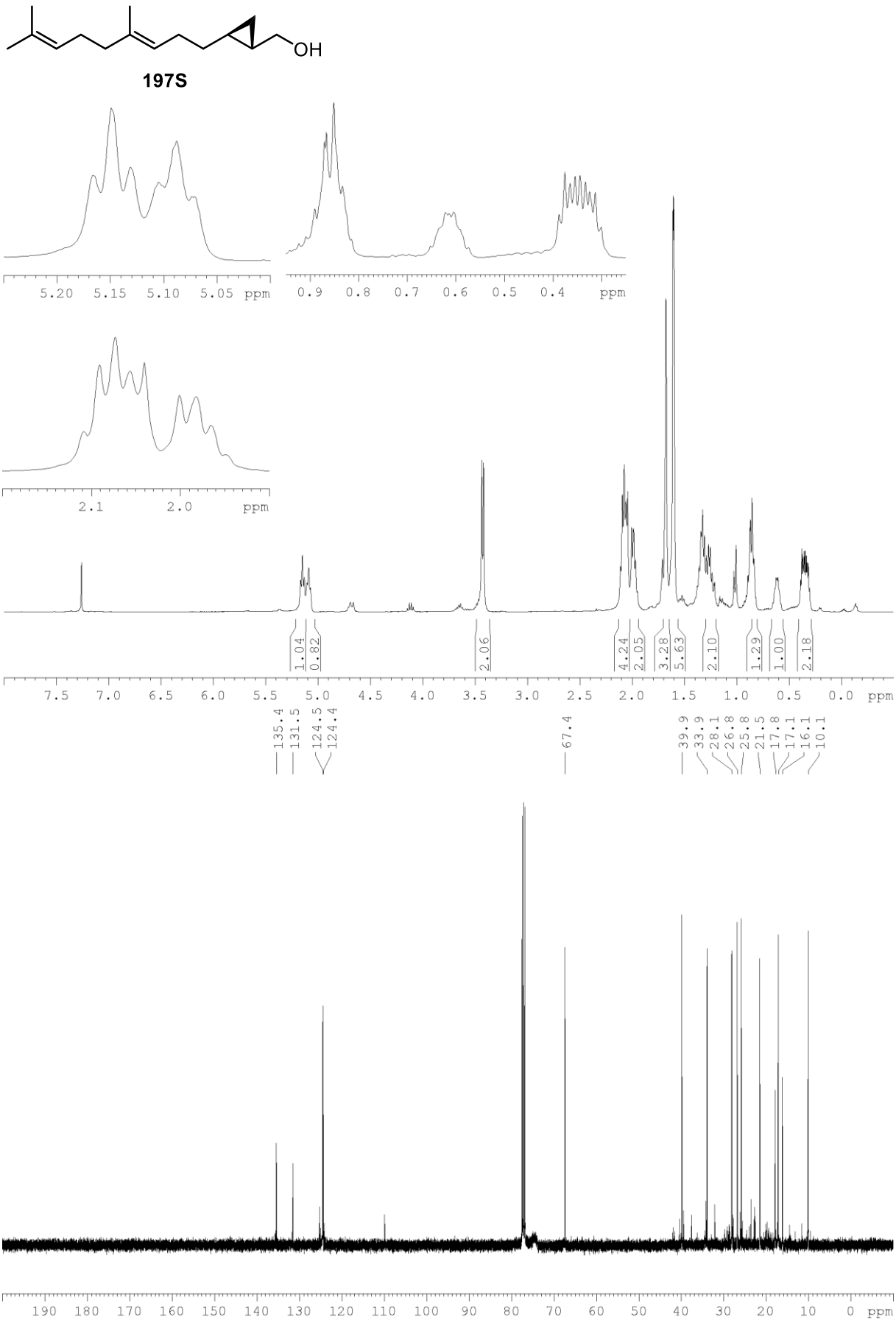

OP2O6(NH4)3

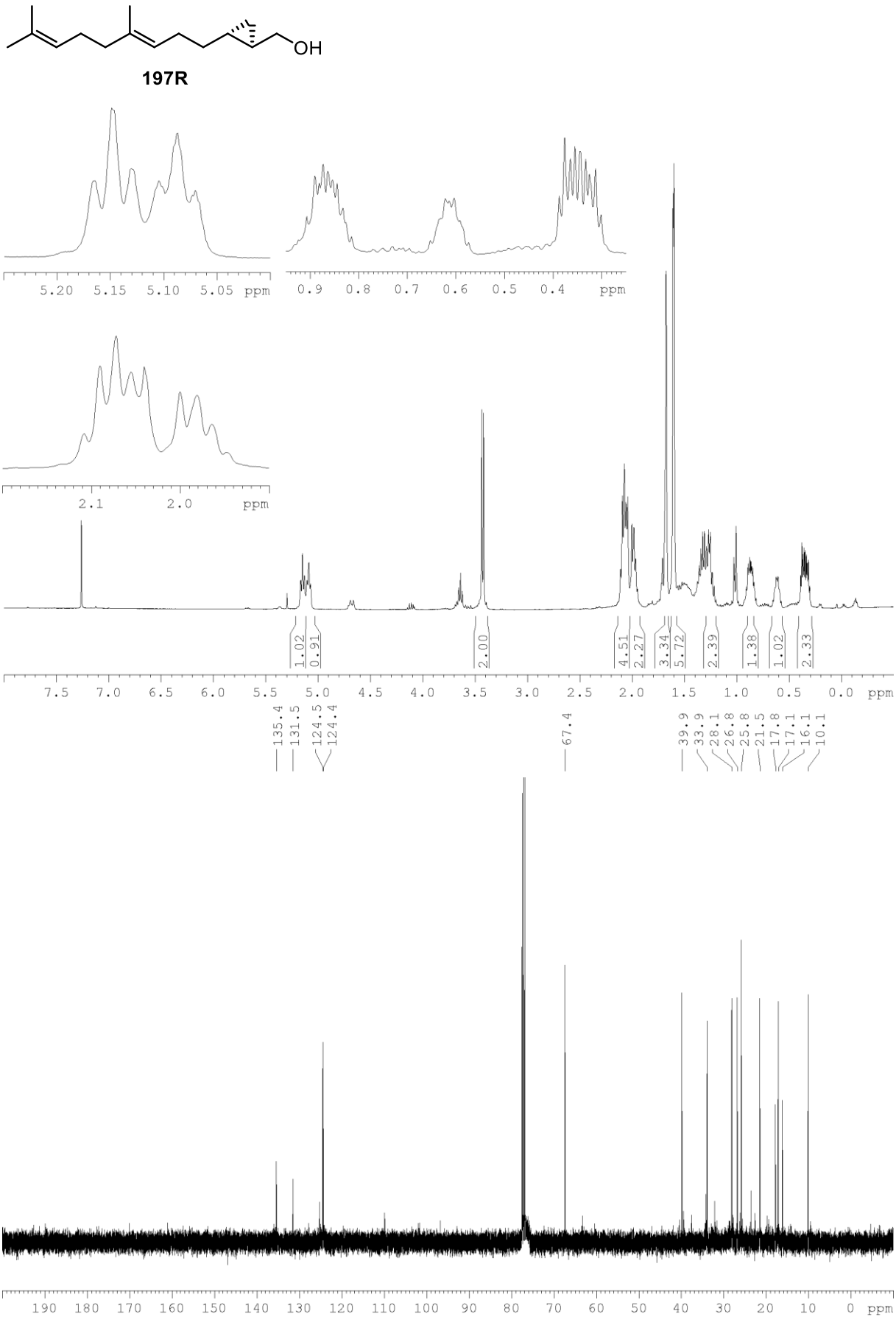
98

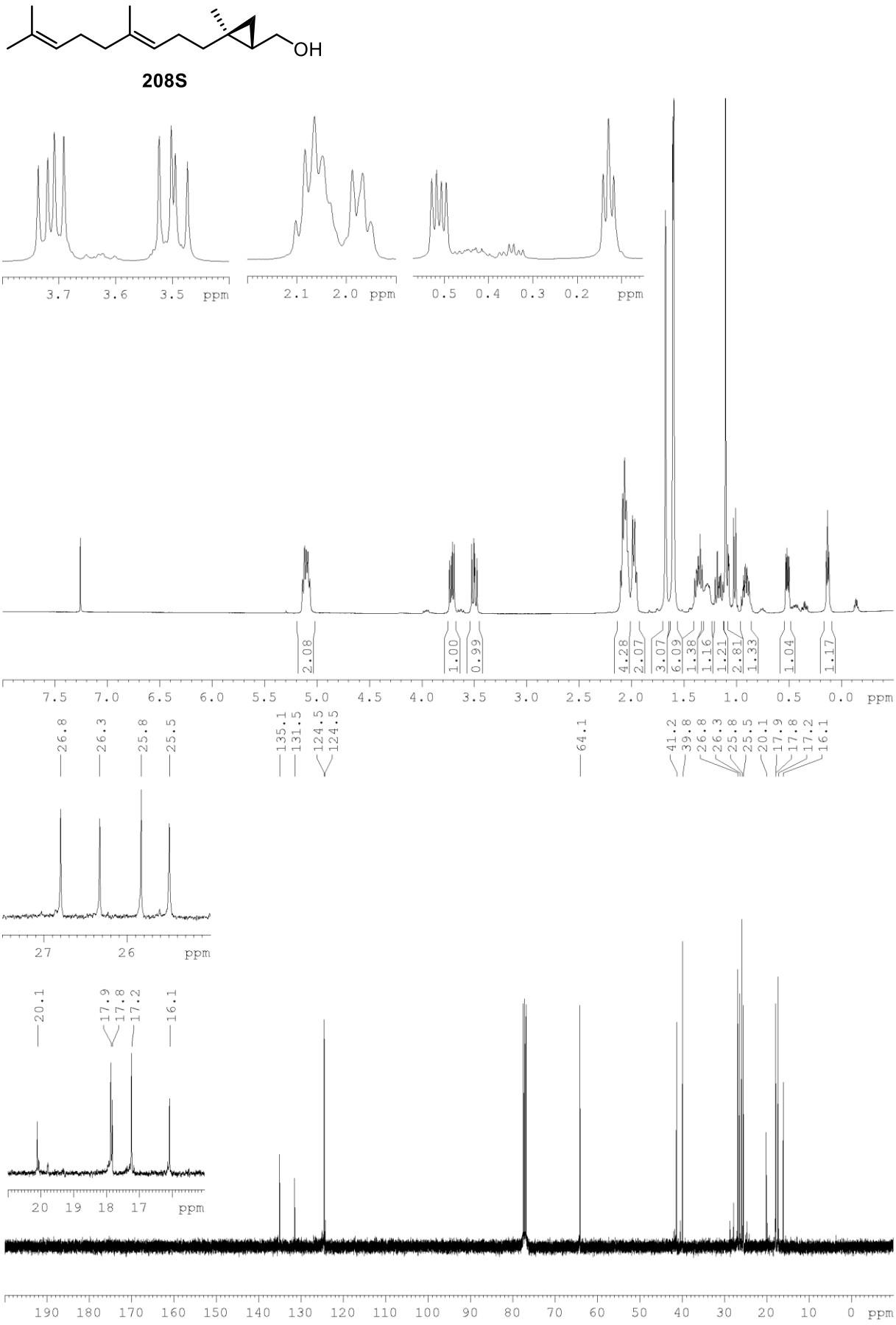

1.07
1.28
1.87
2.00
8.23
9.01
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
136.8
135.5
133.5
125.7
124.4
124.4
66.8
66.8
38.7
31.7
26.7
25.7
24.8
16.9
15.2
15.2

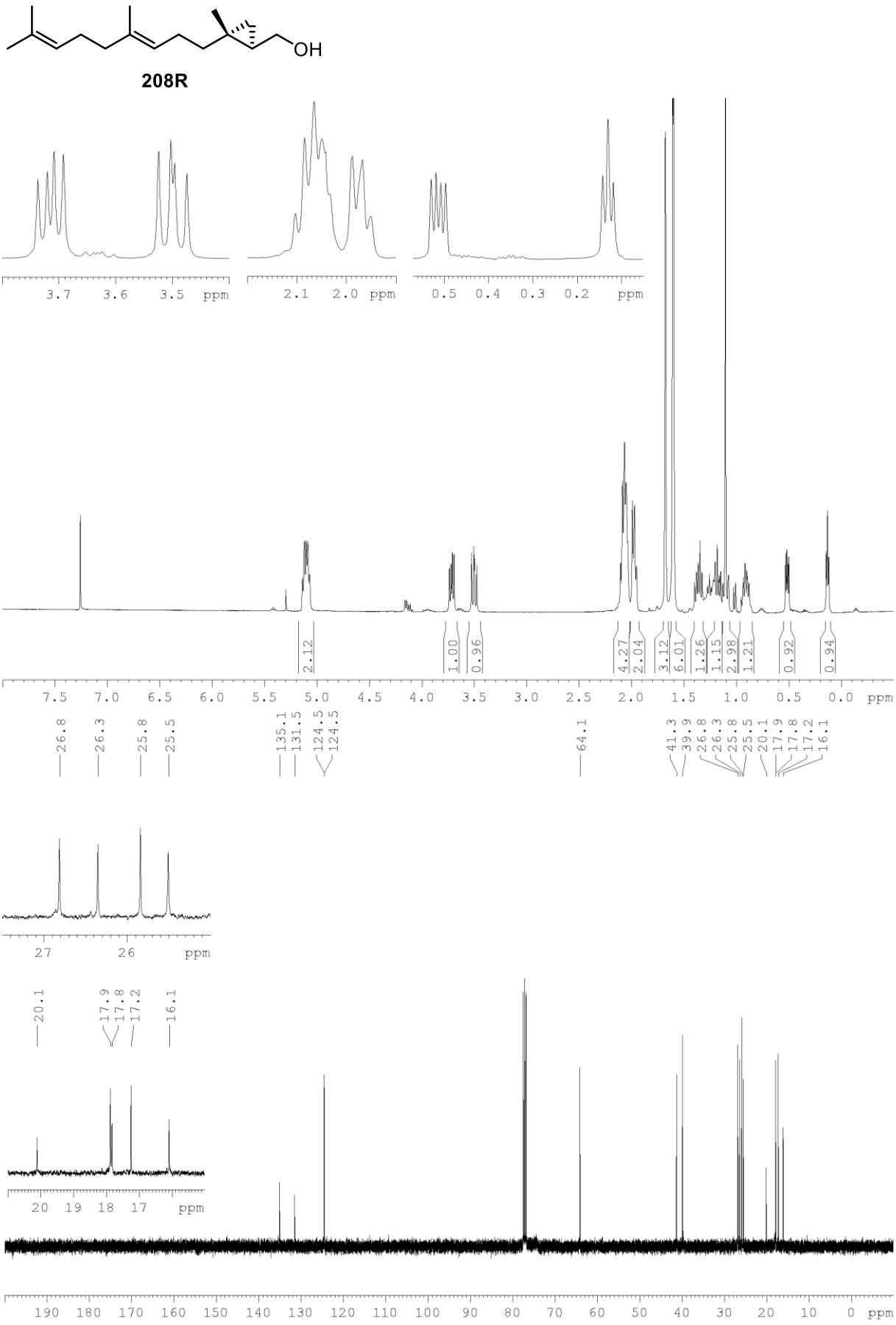

220
200
180
160
140
120
100
80
60
40
20
0
ppm

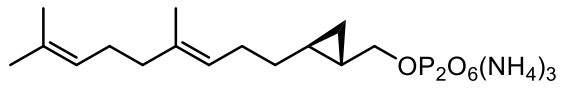

1.31
1.00
-1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18
ppm


OH
197S
5.20 5.15 5.10 5.05 ppm
0.9 0.8 0.7 0.6 0.5 0.4 ppm
2.1 2.0 ppm
1.04 0.82 2.06 4.24 2.05 3.28 5.63 2.10 1.29 1.00 2.18
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
135.4 131.5 124.5 124.4 67.4 39.9 33.9 28.1 26.8 25.8 21.5 17.8 17.1 16.1 10.1
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

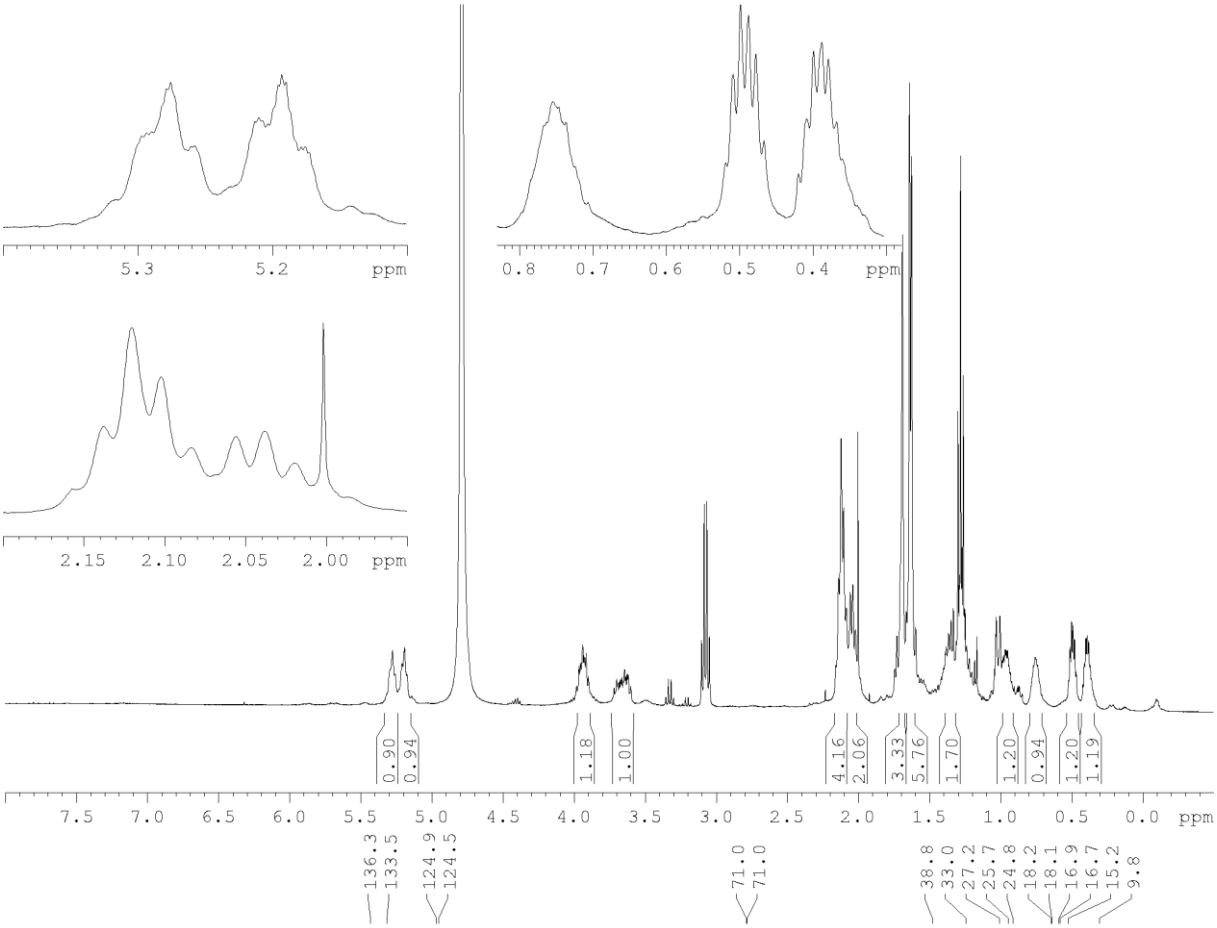

OH
197R
5.20 5.15 5.10 5.05 ppm
0.9 0.8 0.7 0.6 0.5 0.4 ppm
2.1 2.0 ppm
1.02 0.91 2.00 4.51 2.27 3.34 5.72 2.39 1.38 1.02 2.33
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
135.4 131.5 124.5 124.4 67.4 39.9 33.9 28.1 26.8 25.8 21.5 17.8 17.1 16.1 10.1
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

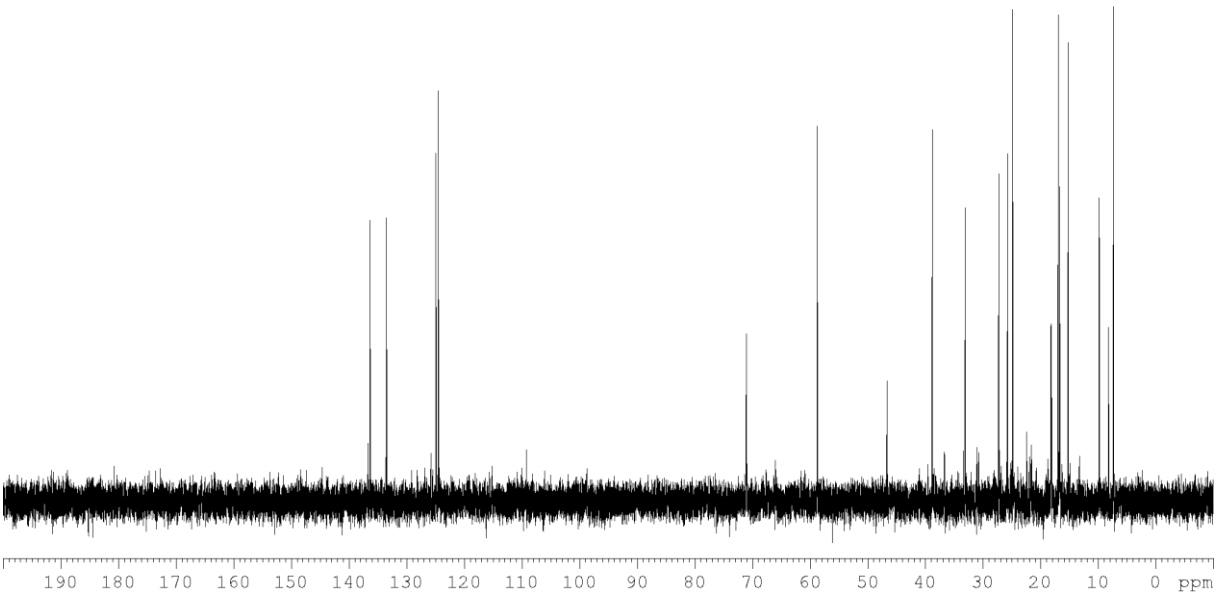

OH
208S
3.7 3.6 3.5 ppm
2.1 2.0 ppm
0.5 0.4 0.3 0.2 ppm
2.08
1.00
0.99
4.28
2.07
3.07
6.09
1.38
1.16
1.21
2.81
1.33
1.04
1.17
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
26.8
26.3
25.8
25.5
135.1
131.5
124.5
124.5
64.1
41.2
39.8
26.8
26.3
25.8
25.5
20.1
17.9
17.8
17.2
16.1
27 26 ppm
20.1
17.9
17.8
17.2
16.1
20 19 18 17 ppm
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

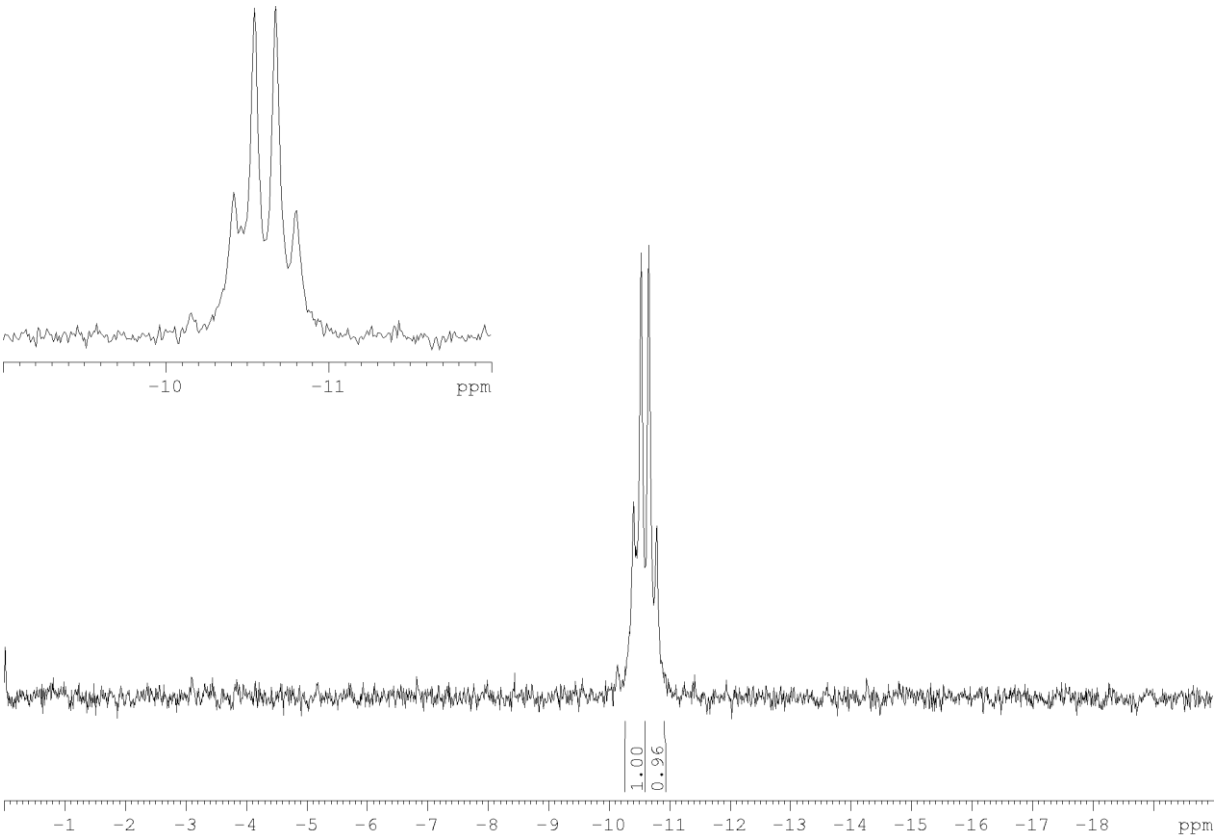

OH
208R
3.7 3.6 3.5 ppm
2.1 2.0 ppm
0.5 0.4 0.3 0.2 ppm
2.12
1.00
0.96
4.27
2.04
3.12
6.01
1.26
1.15
2.98
1.21
0.92
0.94
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
26.8
26.3
25.8
25.5
135.1
131.5
124.5
124.5
64.1
41.3
39.9
26.8
26.3
25.8
25.5
20.1
17.9
17.8
17.2
16.1
27 26 ppm
20.1
17.9
17.8
17.2
16.1
20 19 18 17 ppm
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

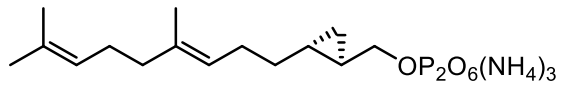

OP2O6(NH4)3

89S

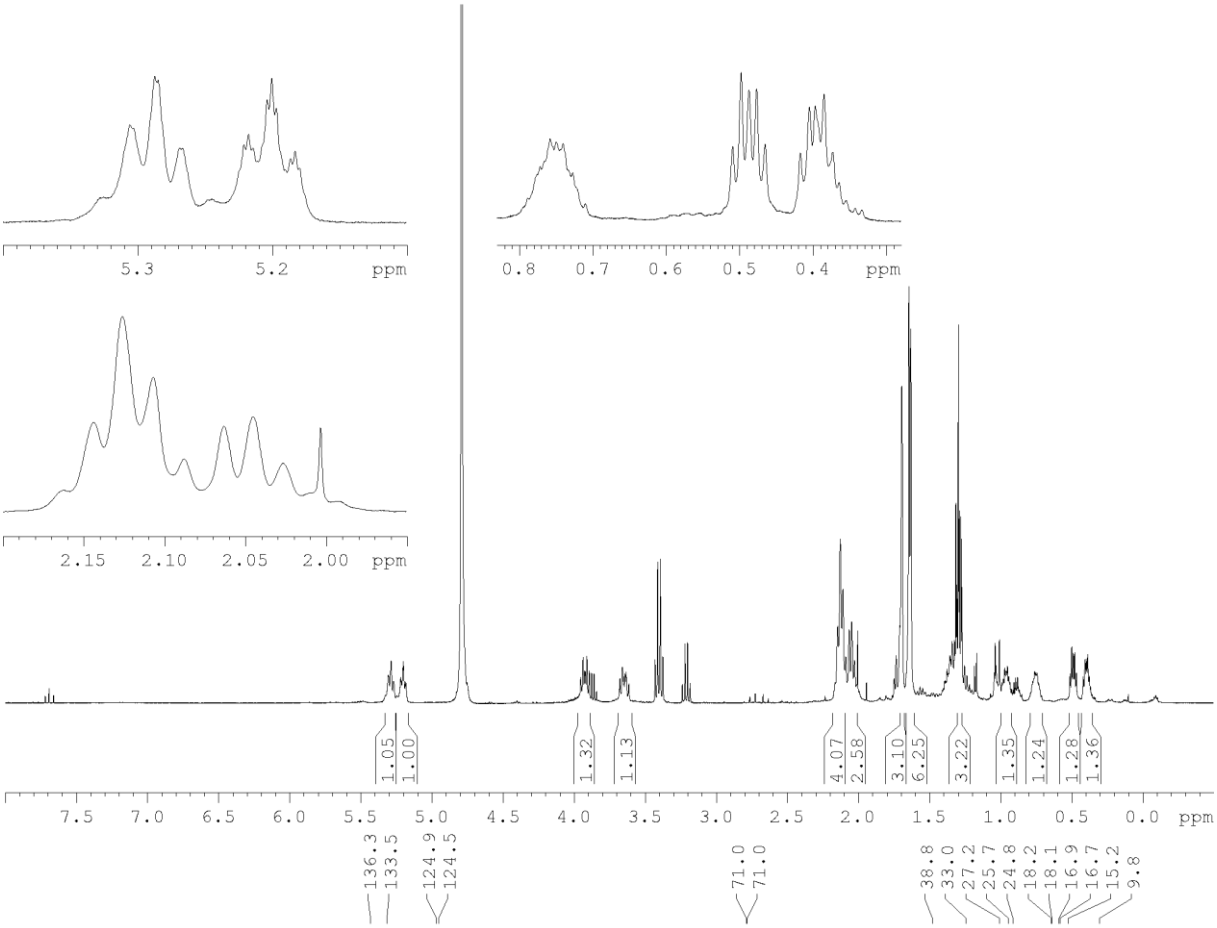

5.3
5.2
ppm
0.8
0.7
0.6
0.5
0.4
ppm
2.15
2.10
2.05
2.00
ppm
0.90
0.94
1.18
1.00
4.16
2.06
3.33
5.76
1.70
1.20
0.94
1.20
1.19
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
136.3
133.5
124.9
124.5
71.0
71.0
38.8
33.0
27.2
25.7
24.8
18.2
18.1
16.9
16.7
15.2
9.8

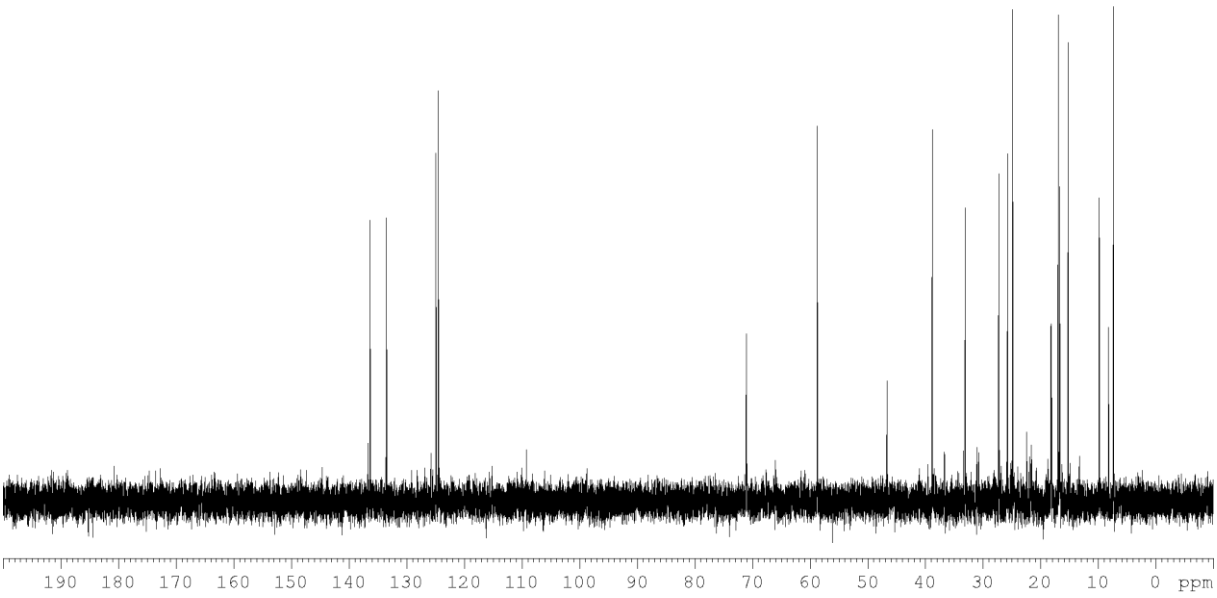

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

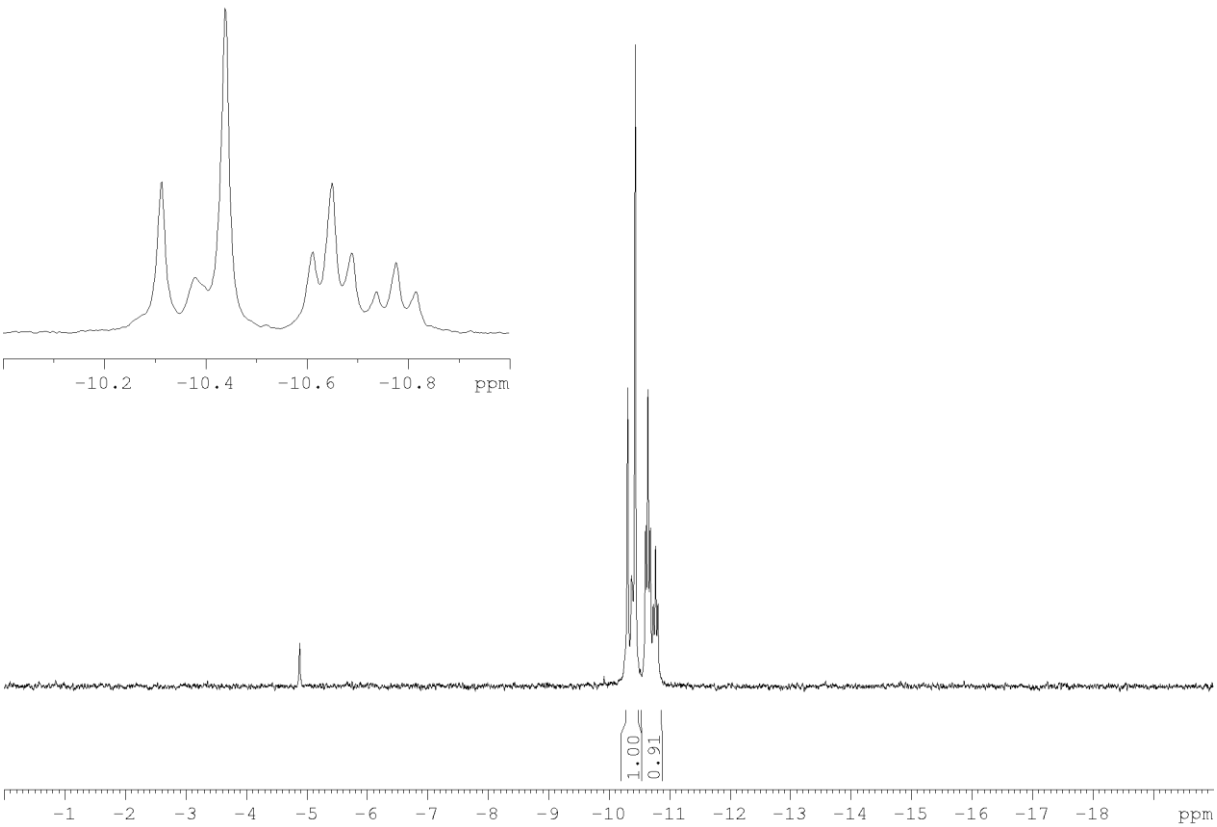

-10
-11
ppm
1.00
0.96
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
-13
-14
-15
-16
-17
-18
ppm

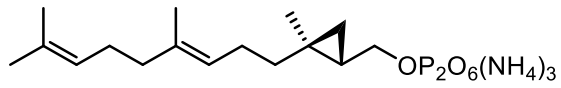

OP2O6(NH4)3

89R

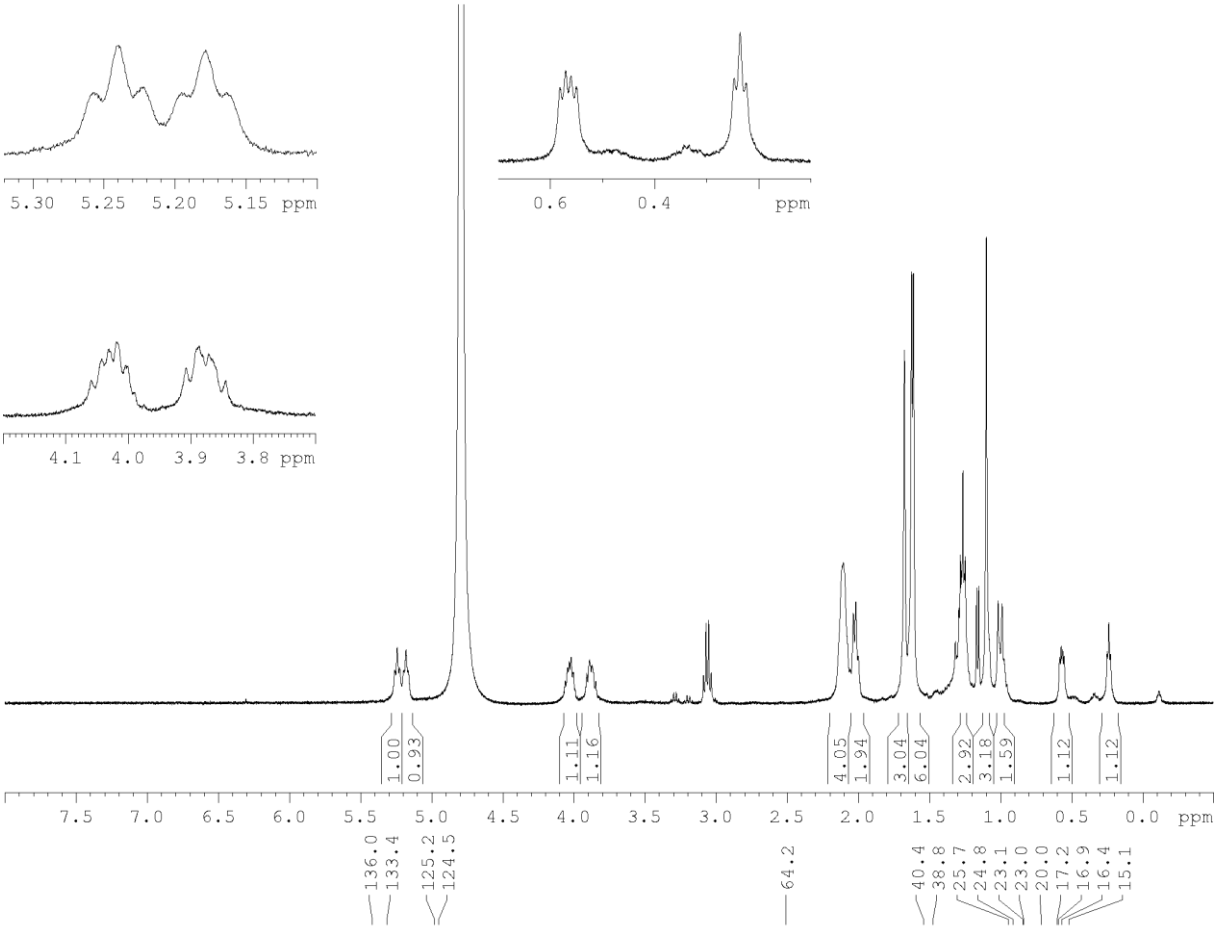

5.3
5.2
ppm
0.8
0.7
0.6
0.5
0.4
ppm
2.15
2.10
2.05
2.00
ppm
1.05
1.00
1.32
1.13
4.07
2.58
3.10
6.25
3.22
1.35
1.24
1.28
1.36
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
136.3
133.5
124.9
124.5
71.0
71.0
38.8
33.0
27.2
25.7
24.8
18.2
18.1
16.9
16.7
15.2
9.8

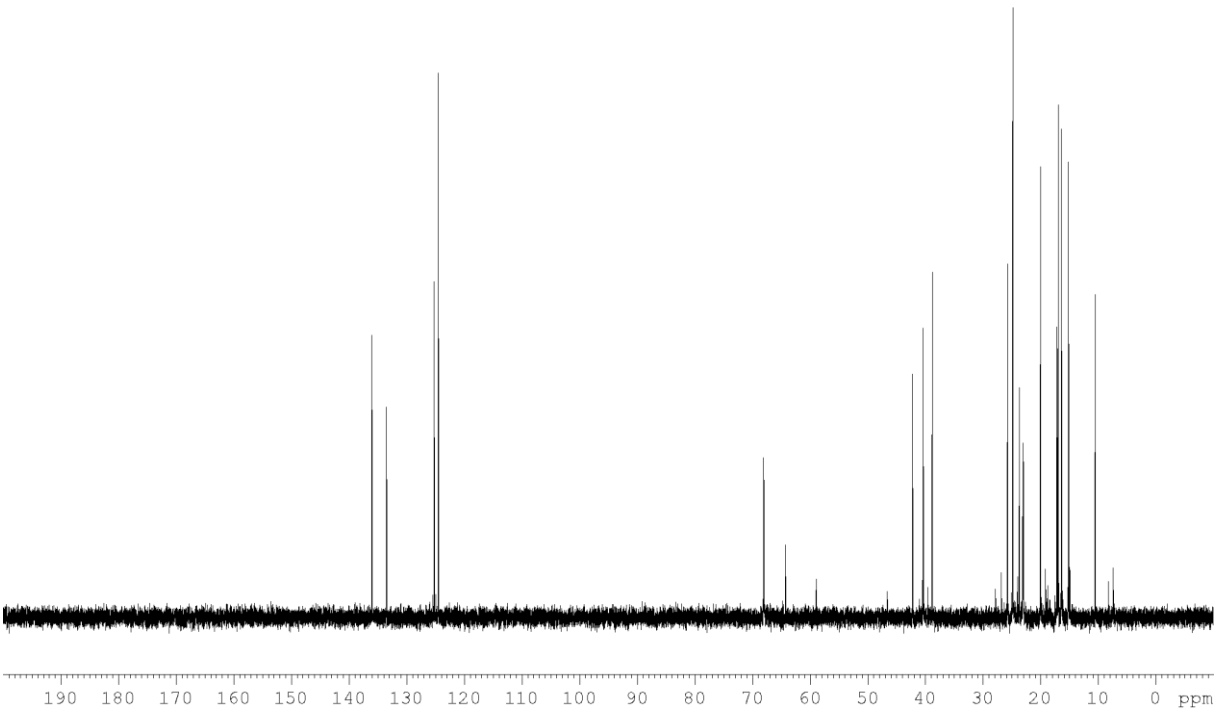

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

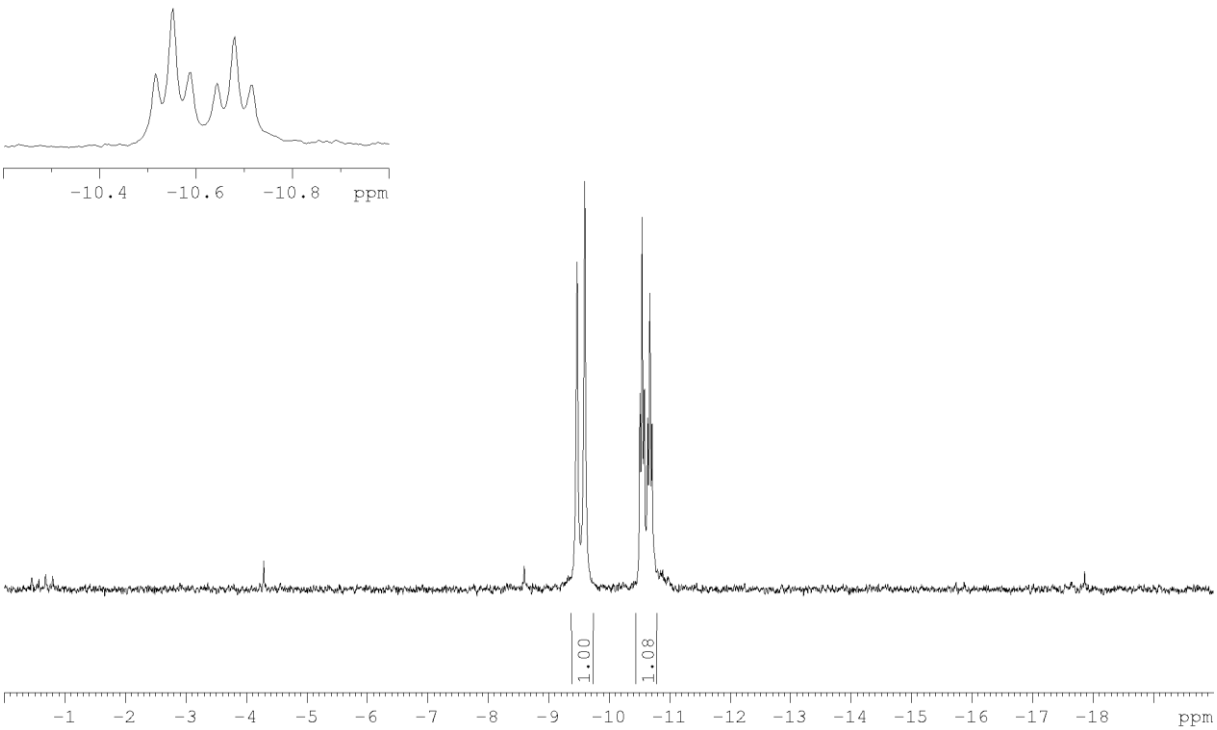

-10.2
-10.4
-10.6
-10.8
ppm
1.00
0.91
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
-13
-14
-15
-16
-17
-18
ppm

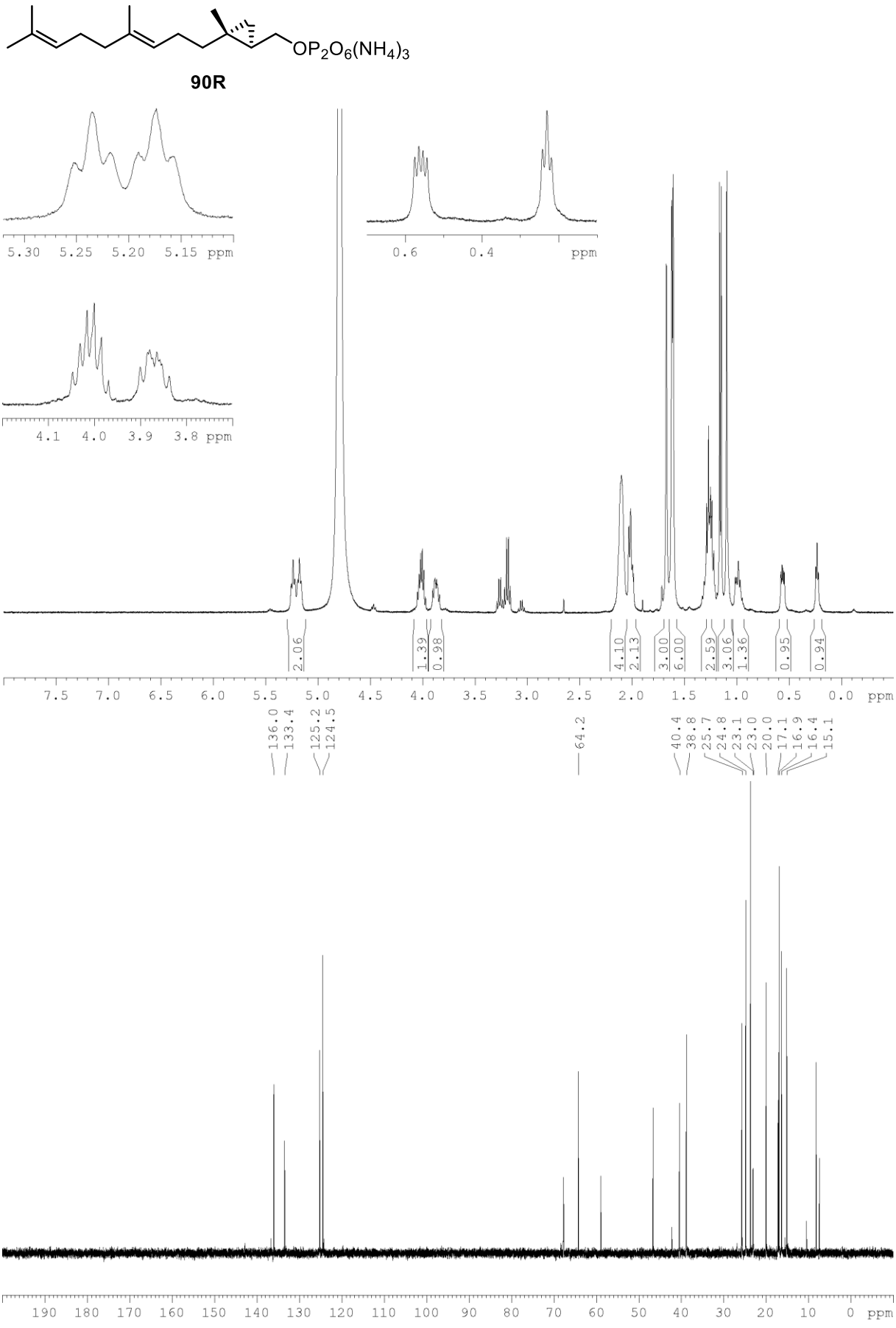

OP2O6(NH4)3

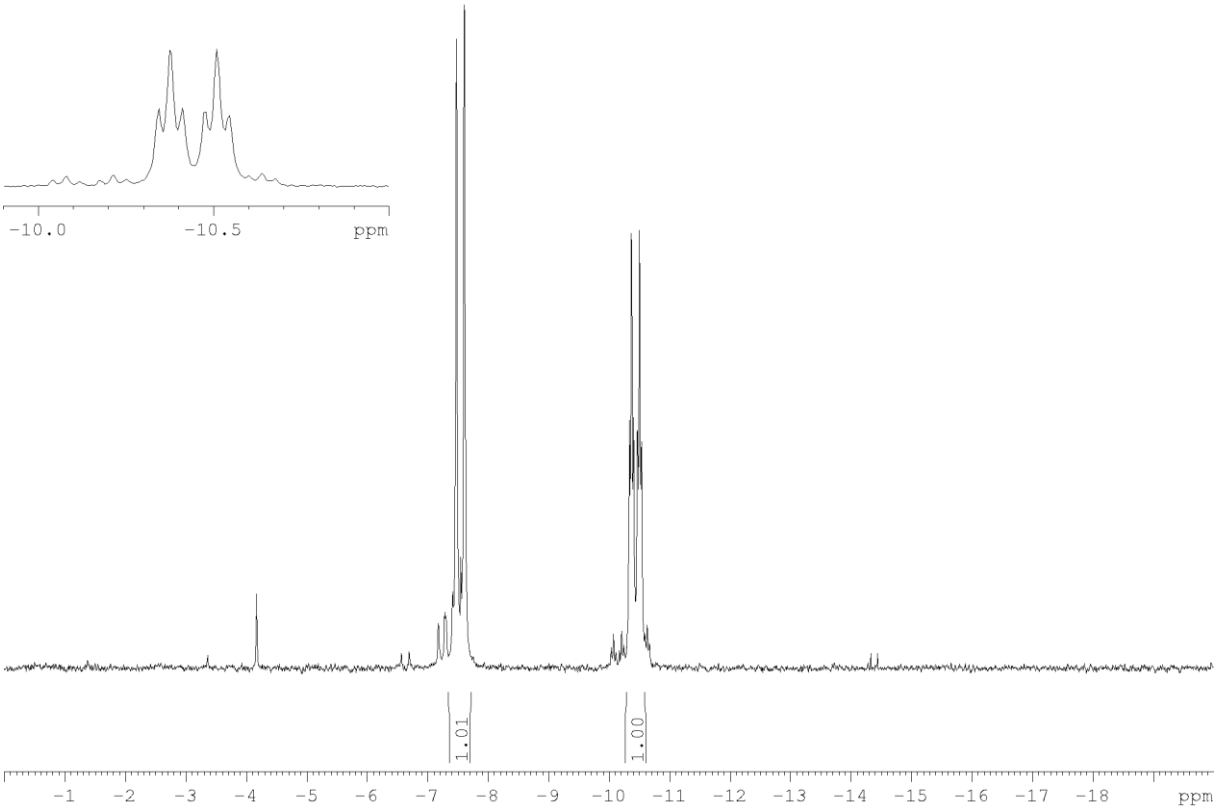
90S

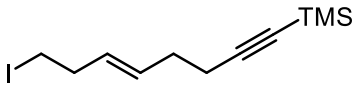

5.30 5.25 5.20 5.15 ppm
0.6 0.4 ppm
4.1 4.0 3.9 3.8 ppm
1.00 0.93 1.11 1.16 4.05 1.94 3.04 6.04 2.92 3.18 1.59 1.12 1.12
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm


136.0 133.4 125.2 124.5 64.2 40.4 38.8 25.7 24.8 23.1 23.0 20.0 17.2 16.9 16.4 15.1
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

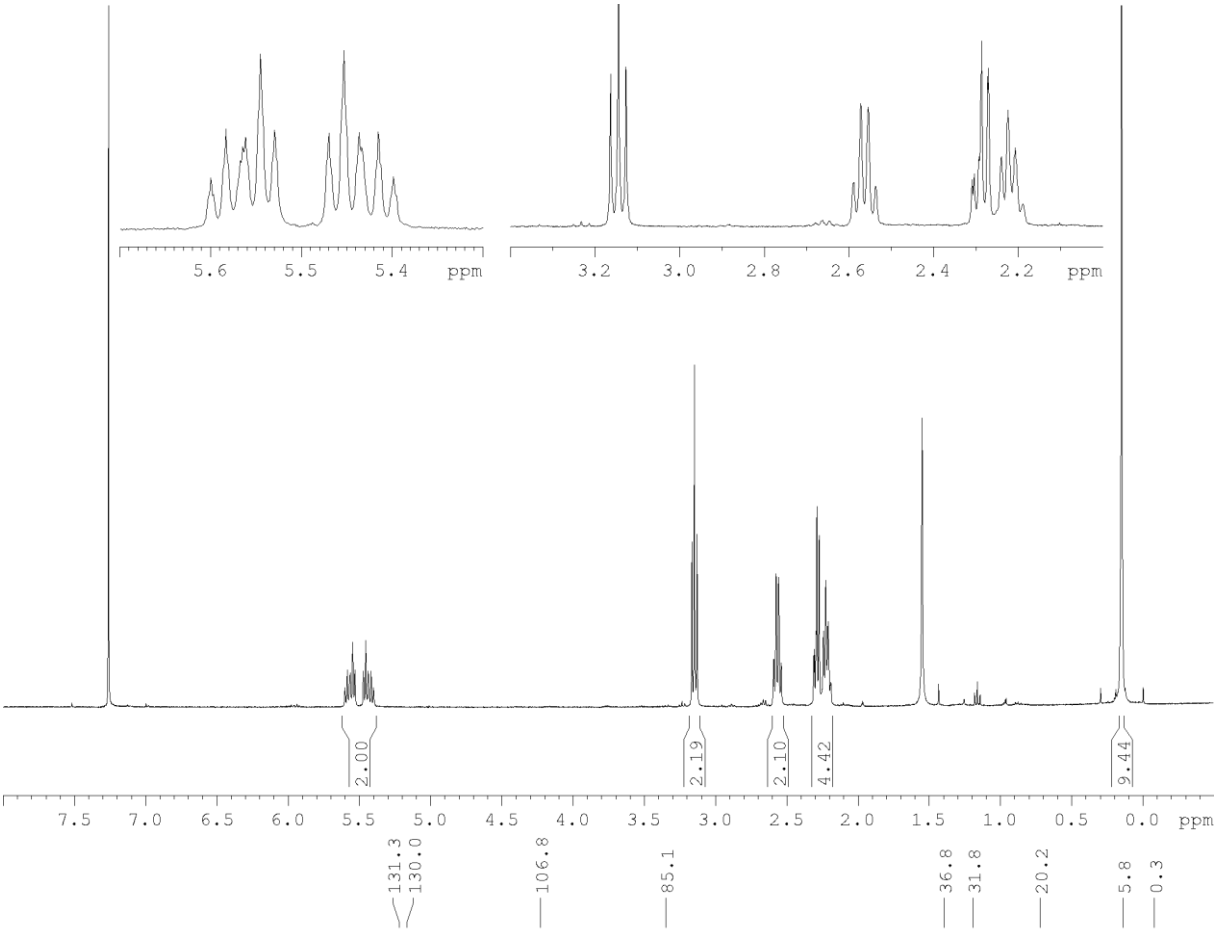

-10.4
-10.6
-10.8
ppm
1.00
1.08
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
-13
-14
-15
-16
-17
-18
ppm

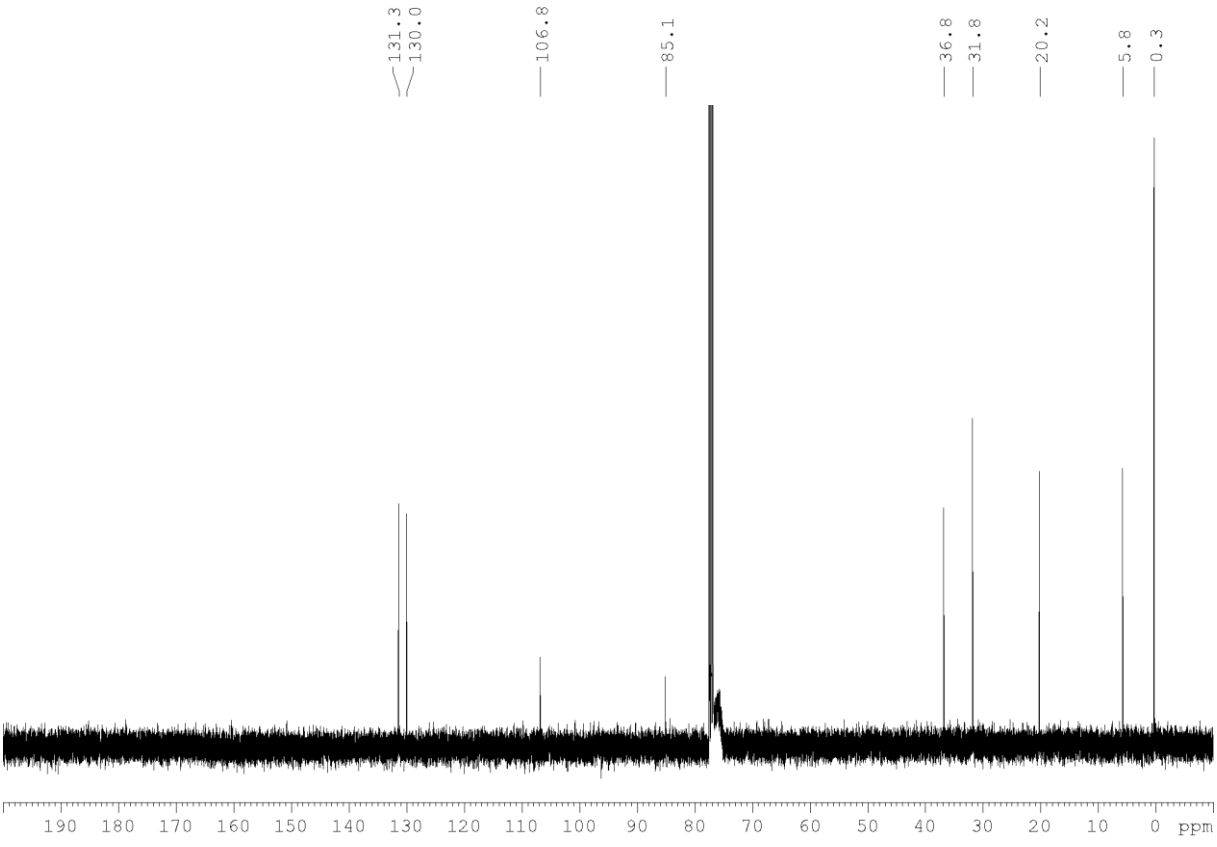

OP2O6(NH4)3
90R
5.30 5.25 5.20 5.15 ppm
0.6 0.4 ppm
4.1 4.0 3.9 3.8 ppm
2.06
1.39
0.98
4.10
2.13
3.00
6.00
2.59
3.06
1.36
0.95
0.94
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
136.0
133.4
125.2
124.5
64.2
40.4
38.8
25.7
24.8
23.1
23.0
20.0
17.1
16.9
16.4
15.1
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

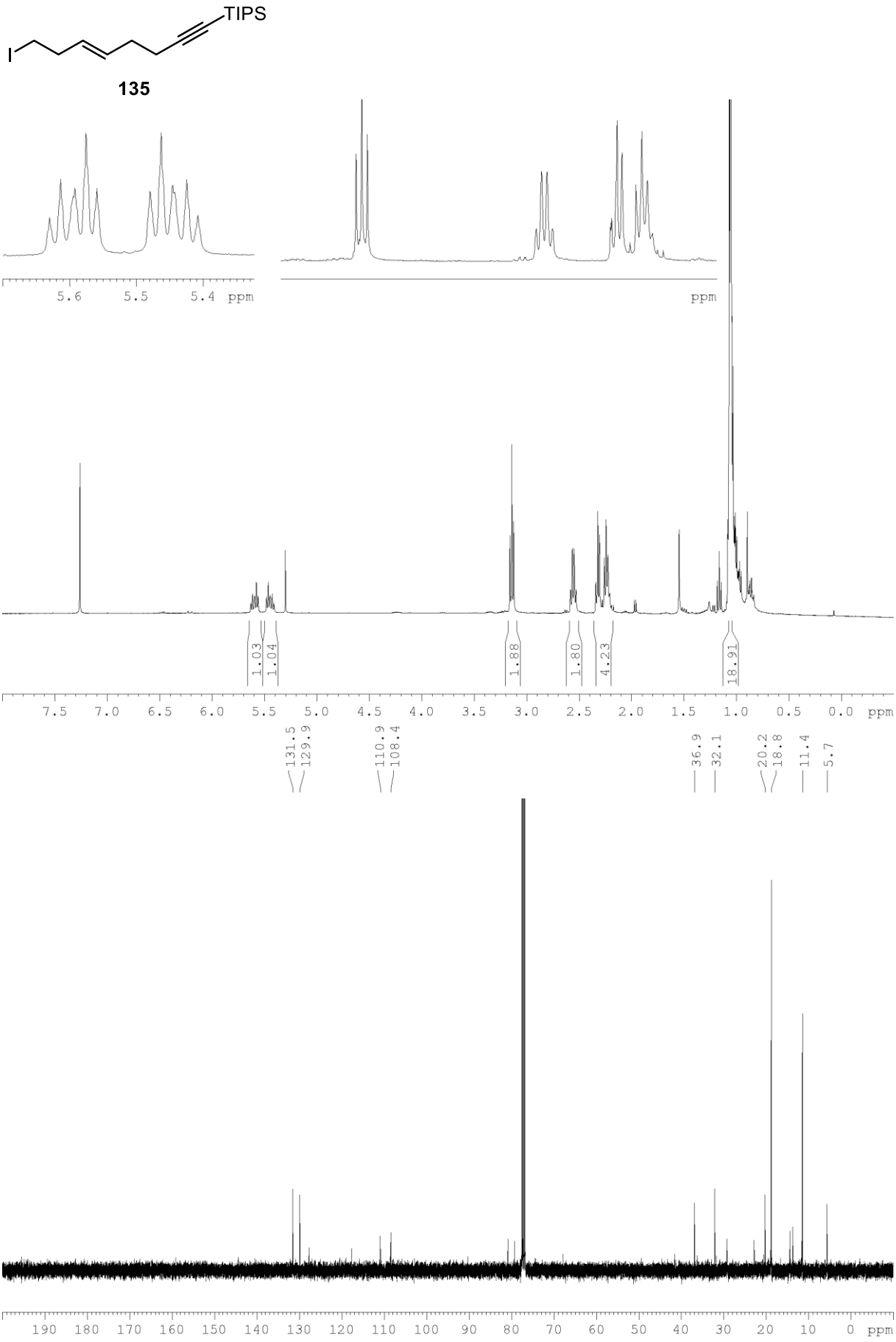

-10.0
-10.5
ppm
1.01
1.00
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
-13
-14
-15
-16
-17
-18
ppm

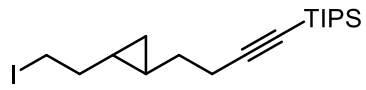

TMS
I

123


5.6
5.5
5.4
ppm
3.2
3.0
2.8
2.6
2.4
2.2
ppm
2.00
2.19
2.10
4.42
9.44
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm


131.3
130.0
106.8
85.1
36.8
31.8
20.2
5.8
0.3
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


TIPS
I
135
5.6
5.5
5.4 ppm
ppm
1.03
1.04
1.88
1.80
4.23
18.91
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
131.5
129.9
110.9
108.4
36.9
32.1
20.2
18.8
11.4
5.7
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


TIPS
I

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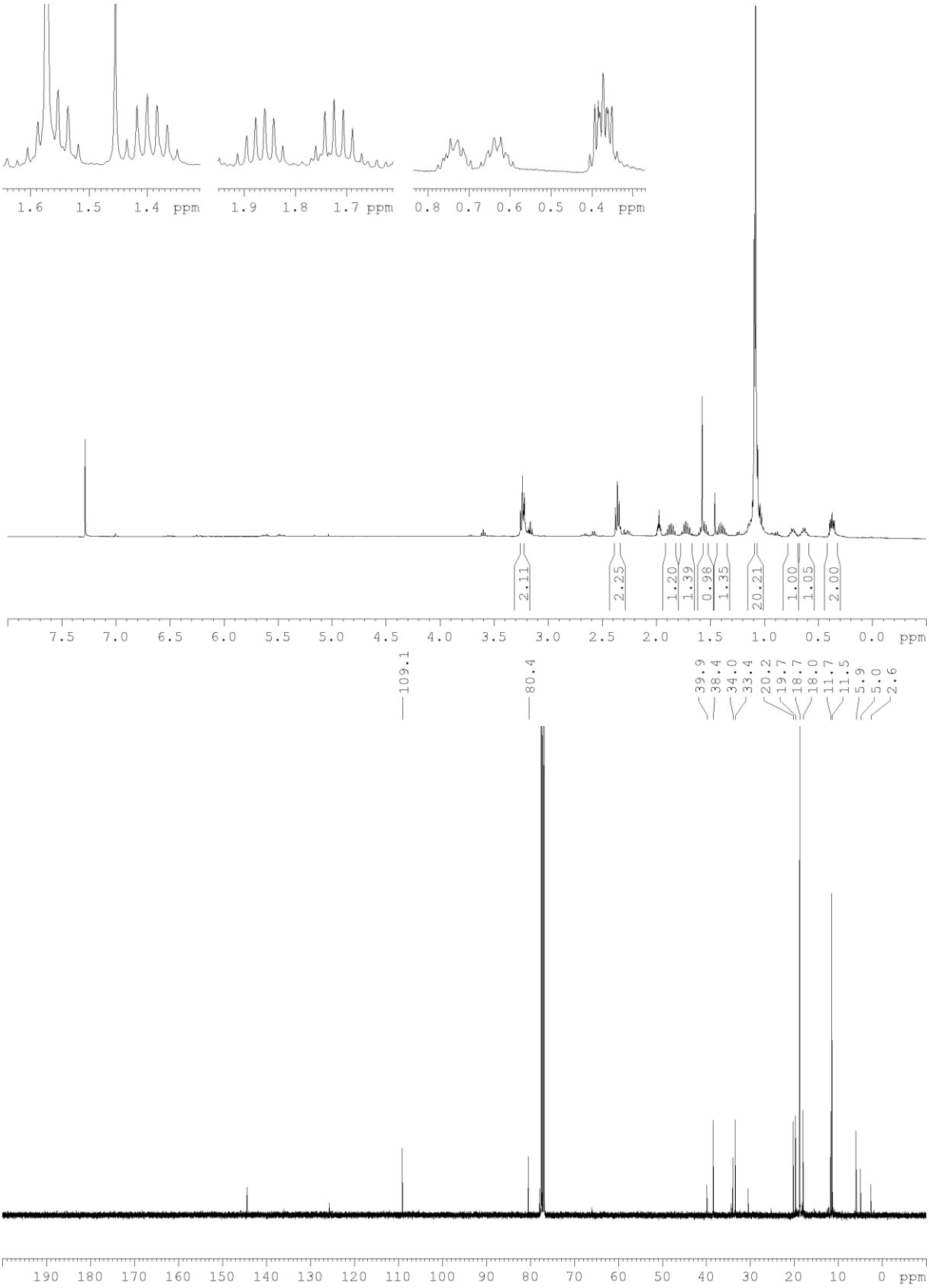
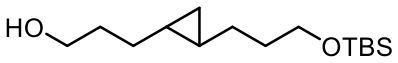
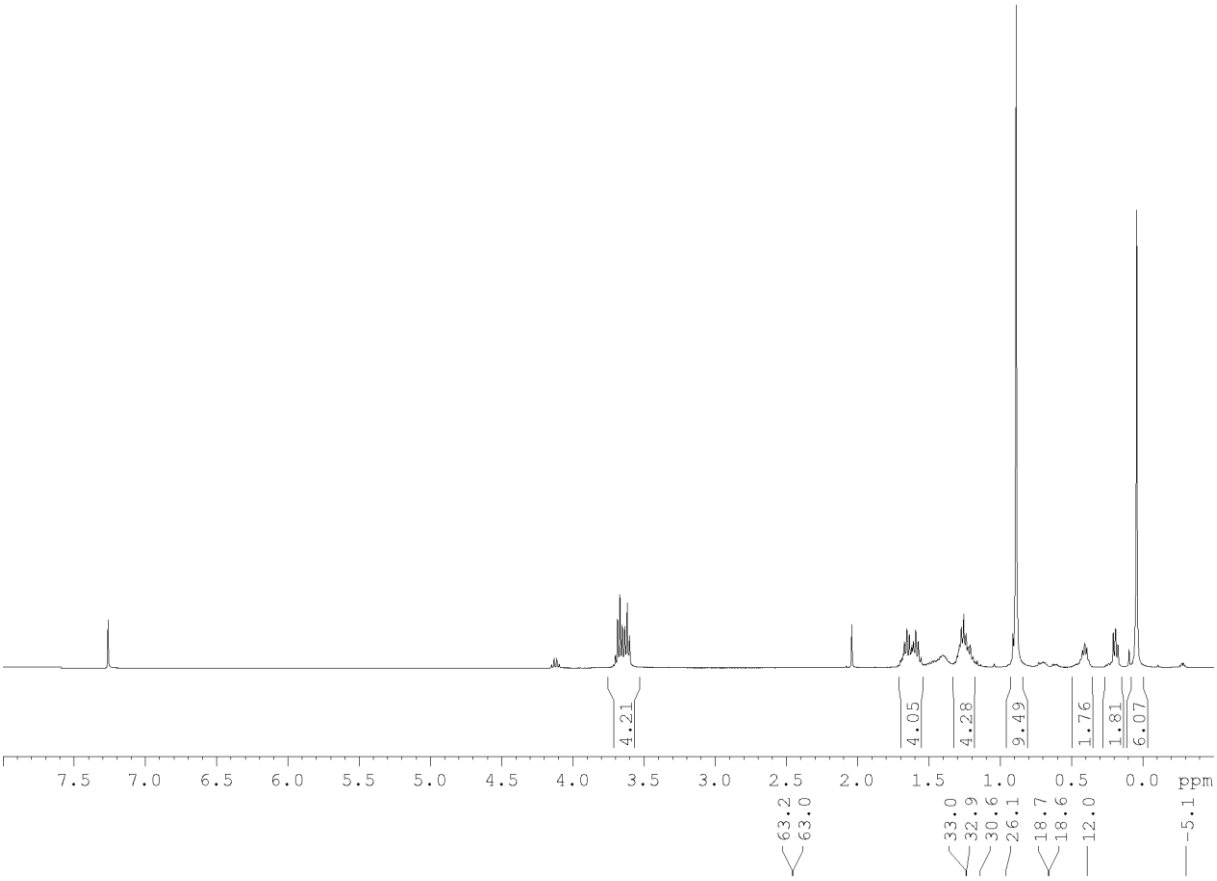
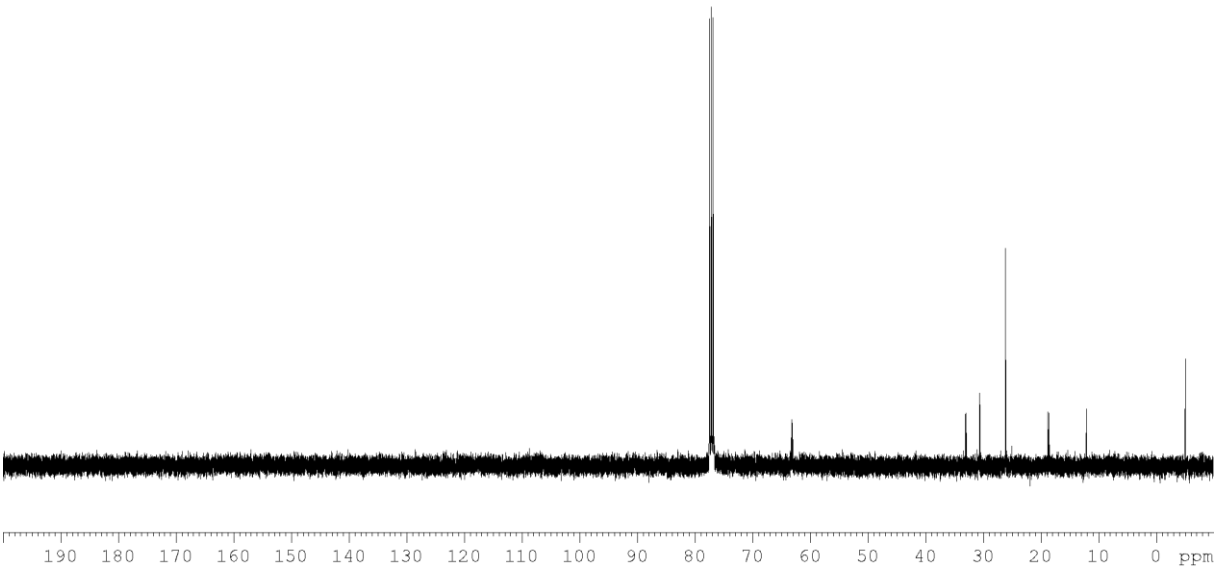
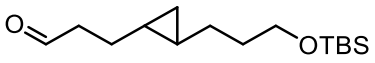

1.6 1.5 1.4 ppm
1.9 1.8 1.7 ppm
0.8 0.7 0.6 0.5 0.4 ppm
2.11
2.25
1.20
1.39
0.98
1.35
20.21
1.00
1.05
2.00
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
109.1
80.4
39.9
38.4
34.0
33.4
20.2
19.7
18.7
18.0
11.7
11.5
5.9
5.0
2.6
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


HO
OTBS

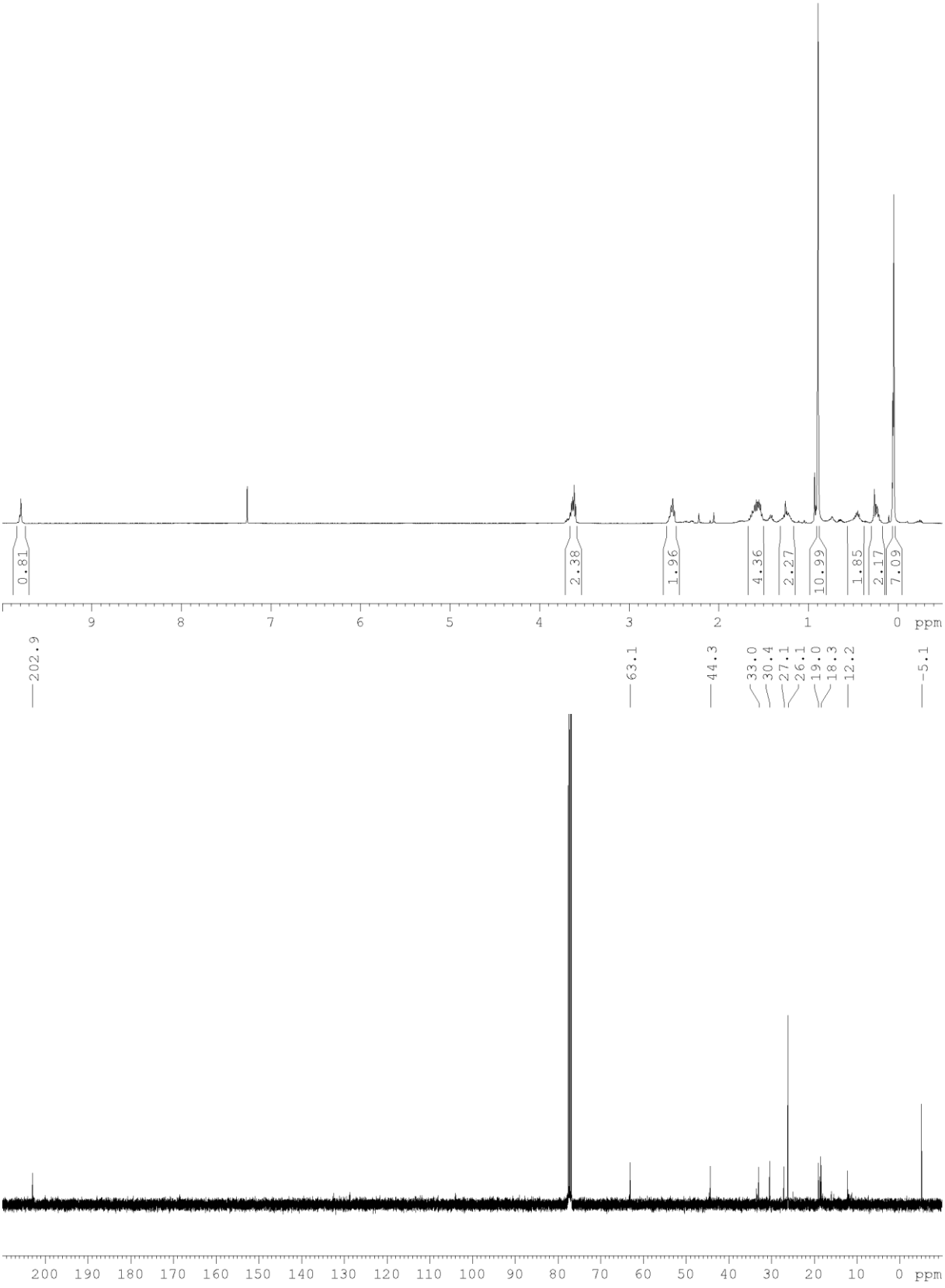
141

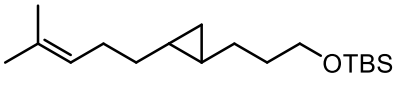

4.21
4.05
4.28
9.49
1.76
1.81
6.07
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
63.2
63.0
33.0
32.9
30.6
26.1
18.7
18.6
12.0
-5.1


190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

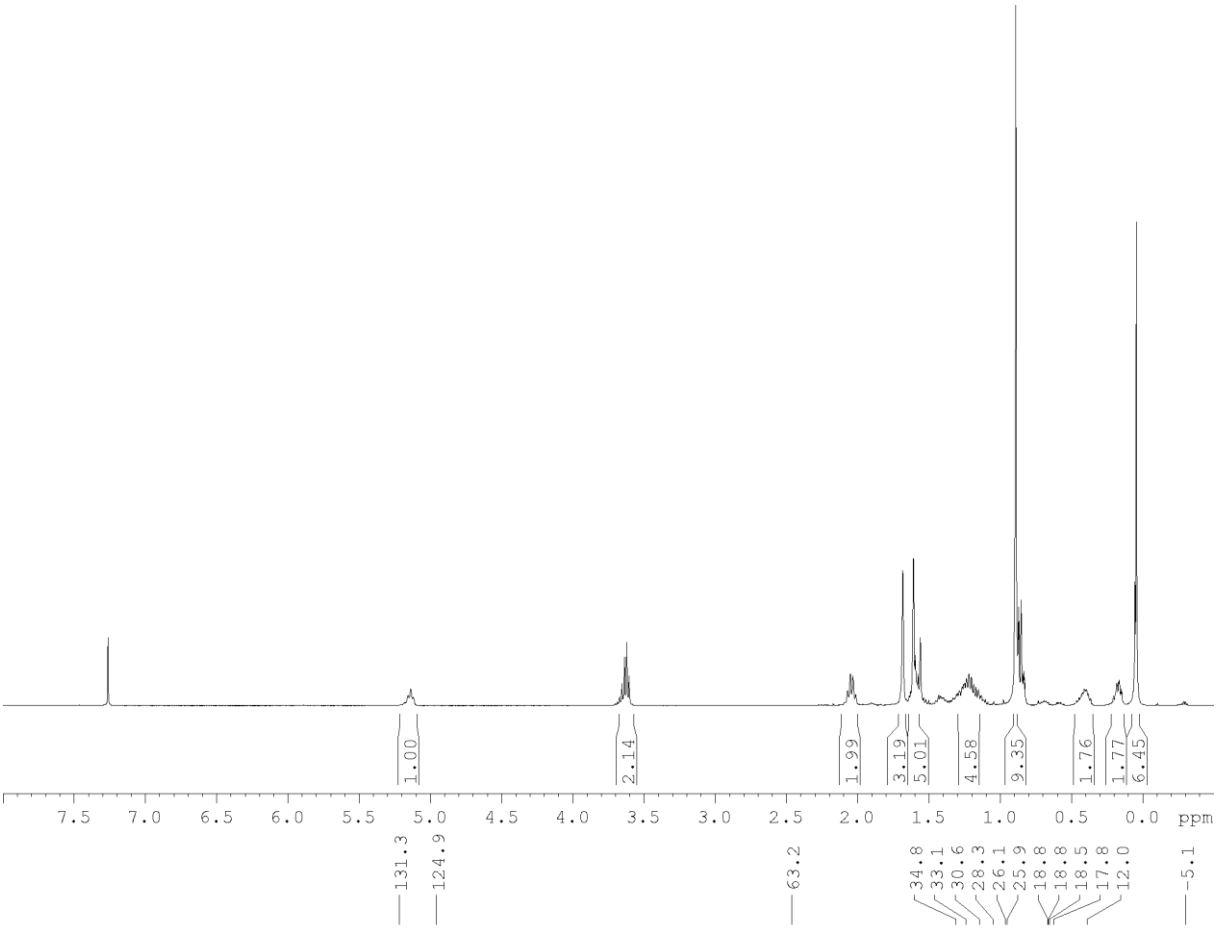

O
OTBS

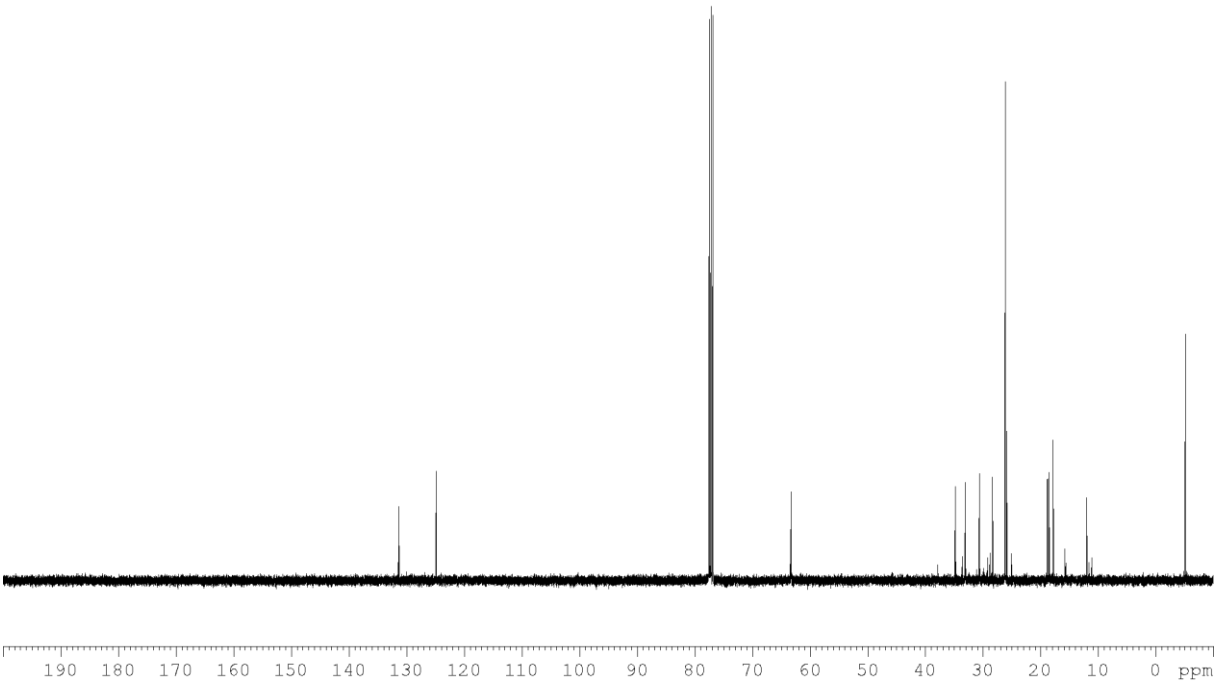
140

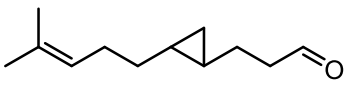

0.81
2.38
1.96
4.36
2.27
10.99
1.85
2.17
7.09
9 8 7 6 5 4 3 2 1 0 ppm
202.9
63.1
44.3
33.0
30.4
27.1
26.1
19.0
18.3
12.2
-5.1
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


OTBS

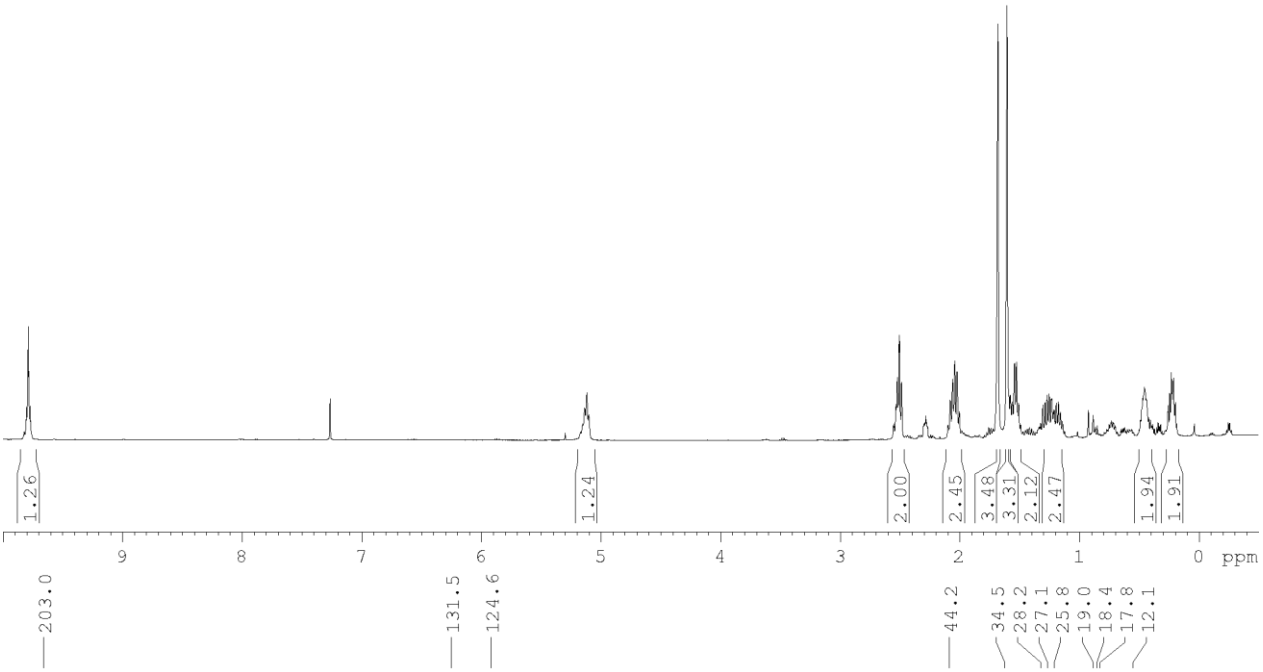
145

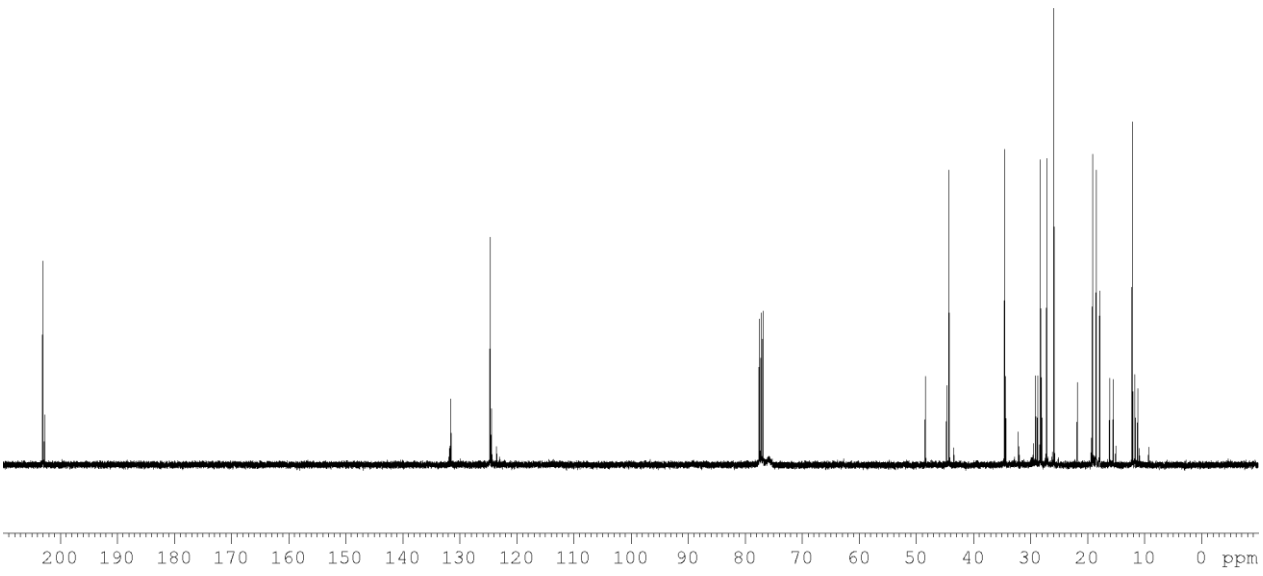

1.00
2.14
1.99
3.19
5.01
4.58
9.35
1.76
1.77
6.45
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
131.3
124.9
63.2
34.8
33.1
30.6
28.3
26.1
25.9
18.8
18.8
18.5
17.8
12.0
-5.1

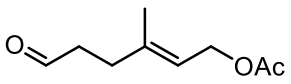

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm



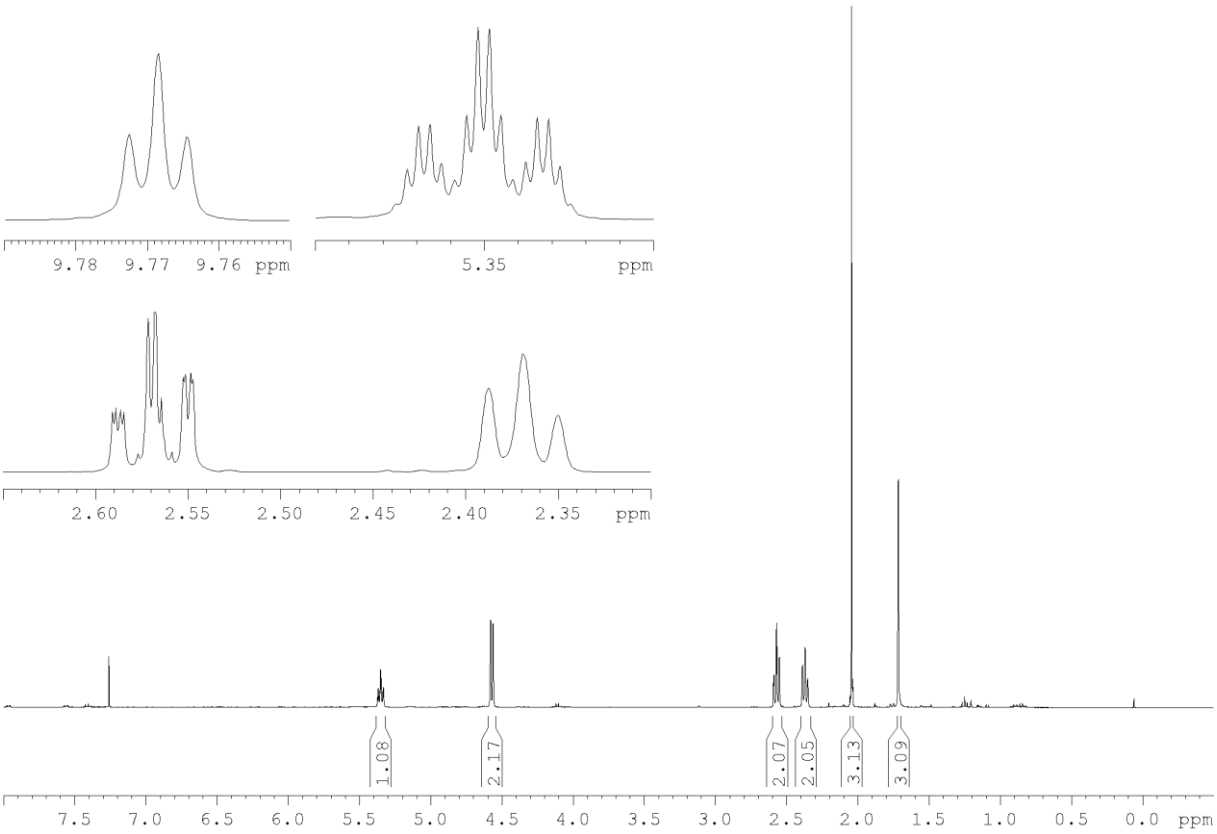
139

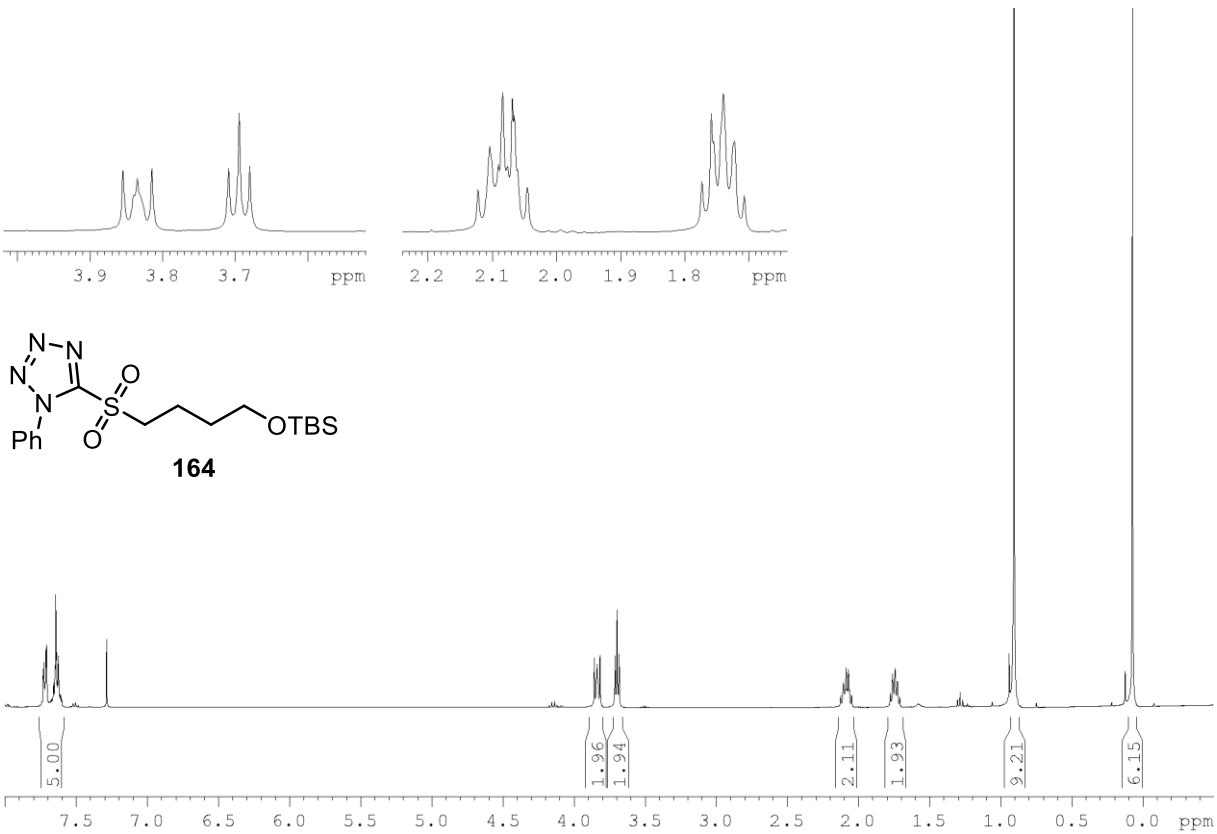

1.26
1.24
2.00
2.45
3.48
3.31
2.12
2.47
1.94
1.91
9 8 7 6 5 4 3 2 1 0 ppm
203.0
131.5
124.6
44.2
34.5
28.2
27.1
25.8
19.0
18.4
17.8
12.1


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


O
OAc

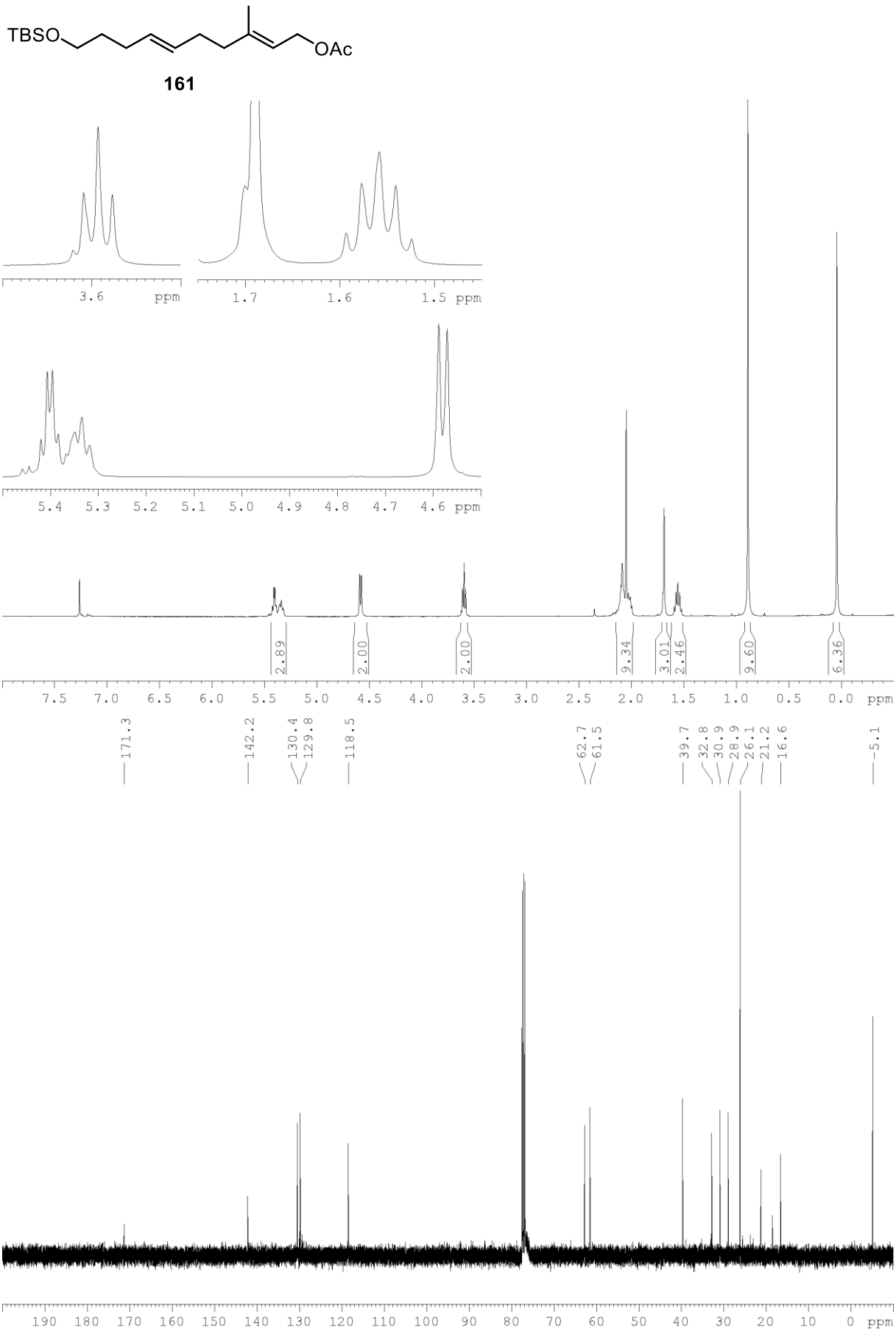
162

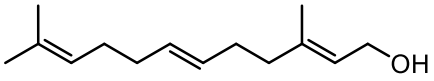

9.78 9.77 9.76 ppm
5.35 ppm
2.60 2.55 2.50 2.45 2.40 2.35 ppm
1.08
2.17
2.07
2.05
3.13
3.09
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm


3.9 3.8 3.7 ppm
2.2 2.1 2.0 1.9 1.8 ppm
5.00
1.96
1.94
2.11
1.93
9.21
6.15
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

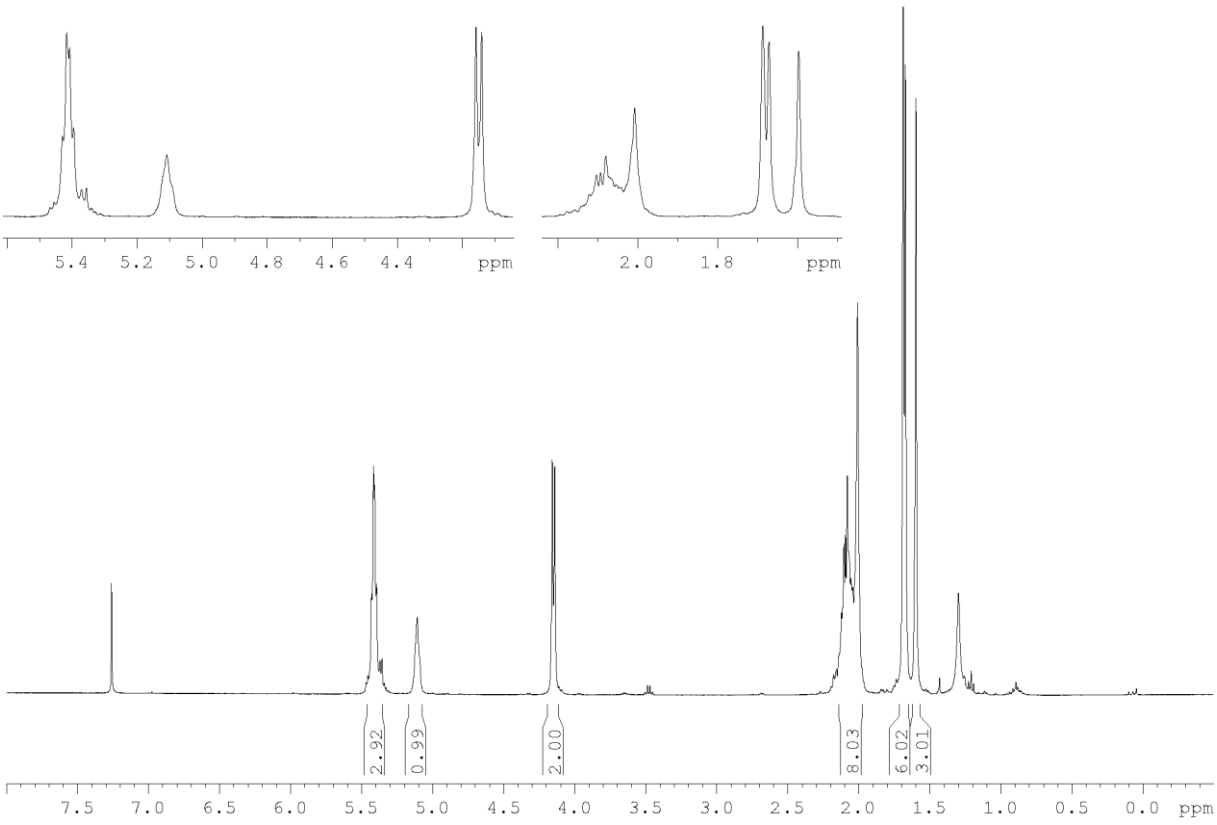
N
N
N
N
Ph
S
O
O
OTBS

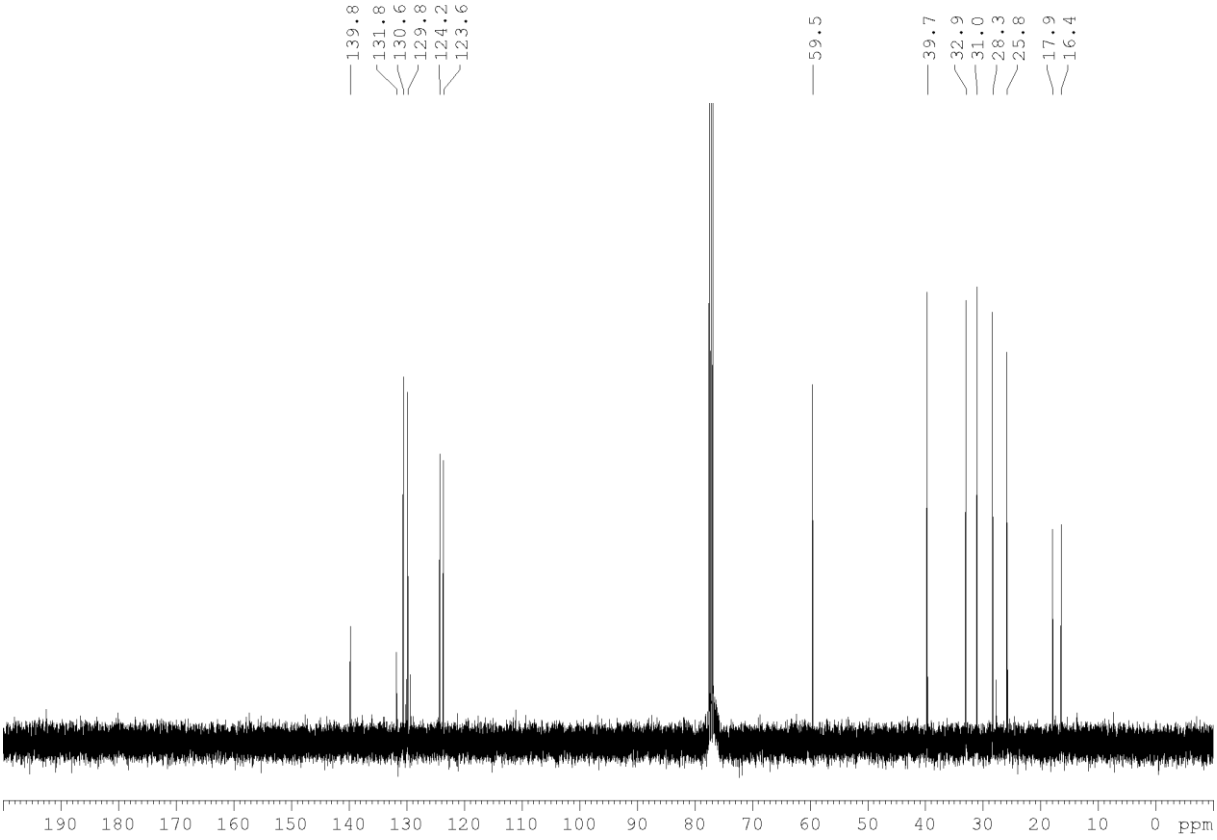
164

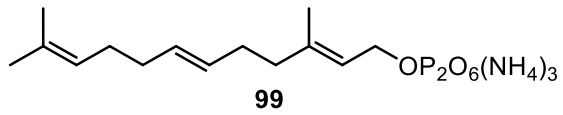

TBSO
OAc
161
3.6 ppm
1.7
1.6
1.5 ppm
5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 ppm
2.89
2.00
2.00
9.34
3.01
2.46
9.60
6.36
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
171.3
142.2
130.4
129.8
118.5
62.7
61.5
39.7
32.8
30.9
28.9
26.1
21.2
16.6
-5.1
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


OH

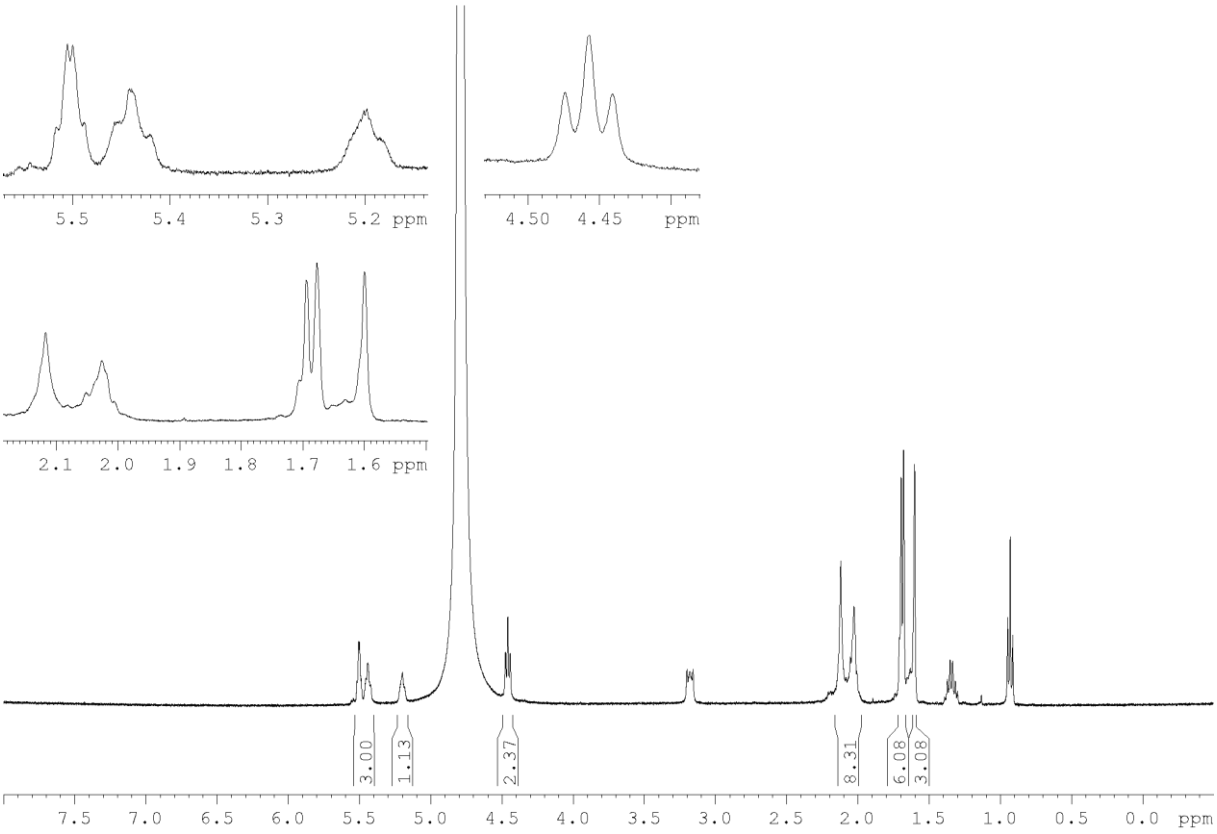
182

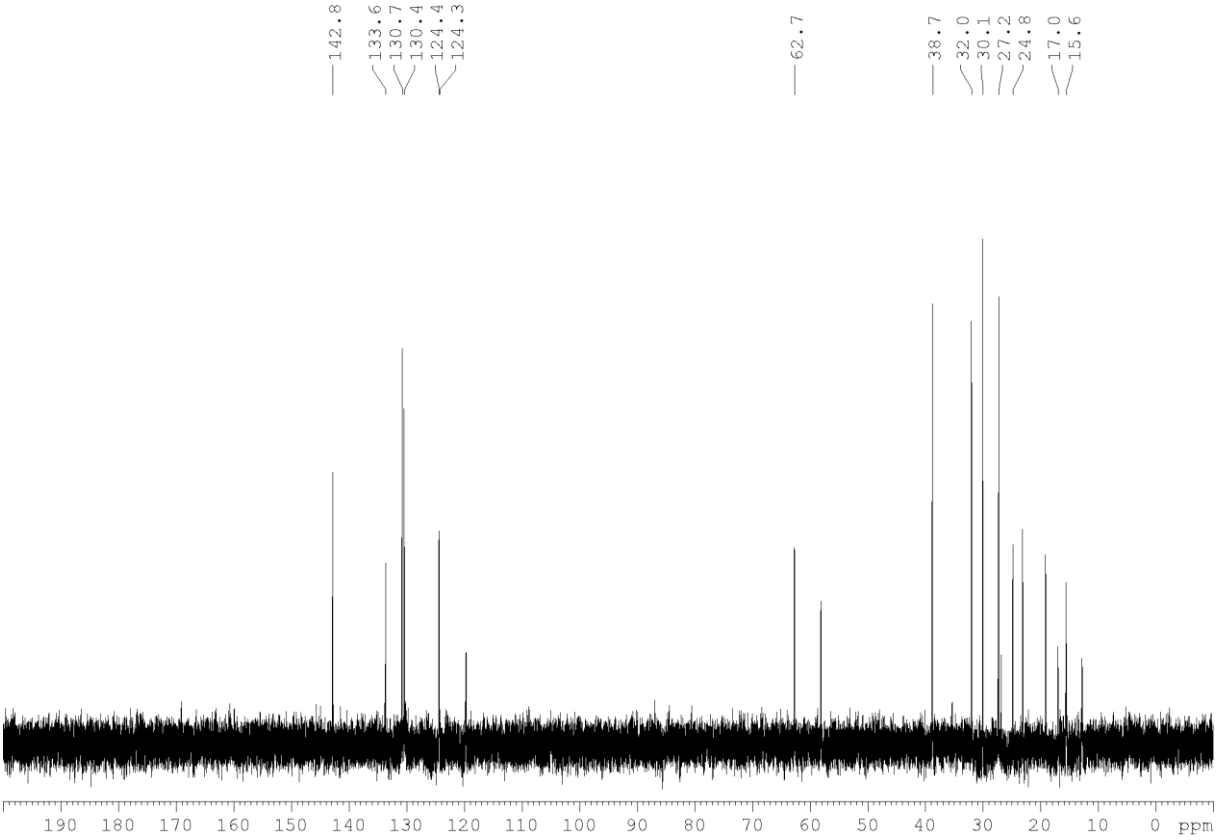

5.4 5.2 5.0 4.8 4.6 4.4 ppm
2.0 1.8 ppm
2.92
0.99
2.00
8.03
6.02
3.01
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

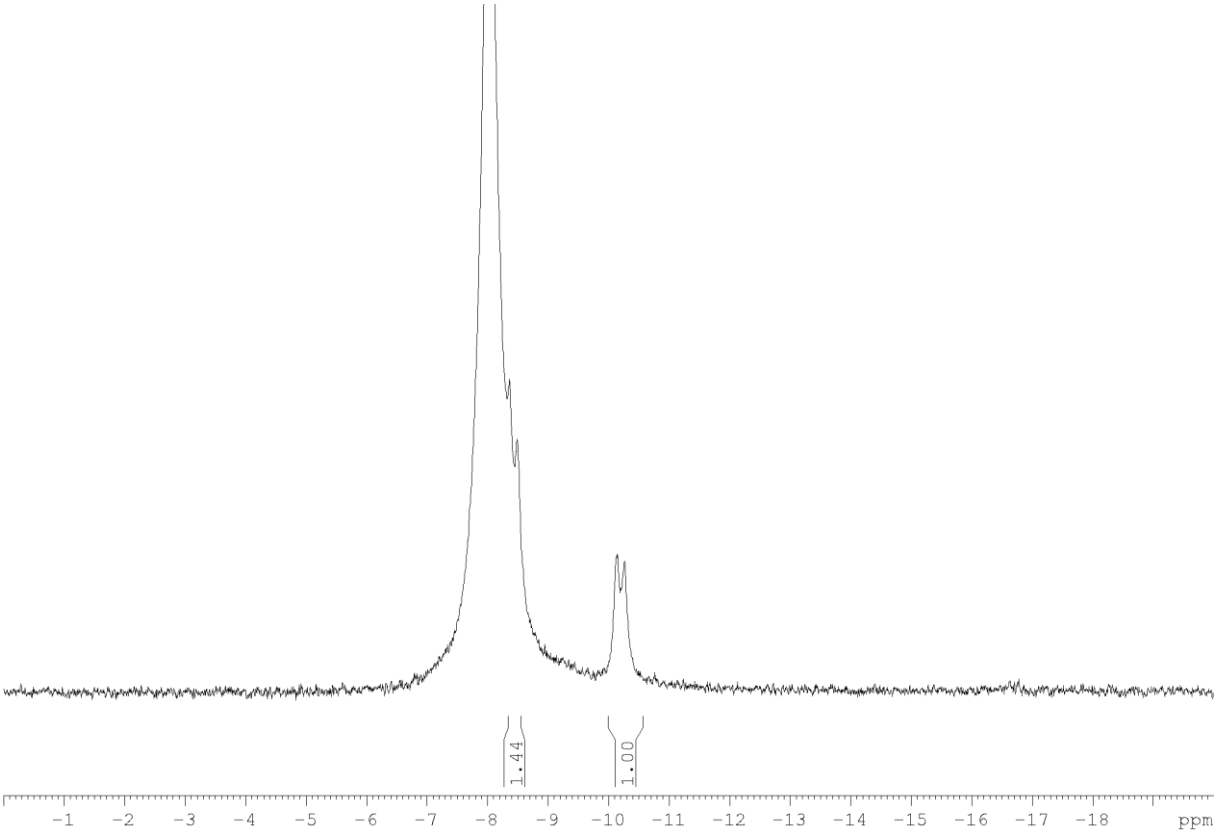

139.8
131.8
130.6
129.8
124.2
123.6
59.5
39.7
32.9
31.0
28.3
25.8
17.9
16.4
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

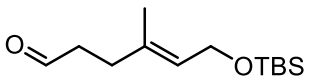

OP2O6(NH4)3

99

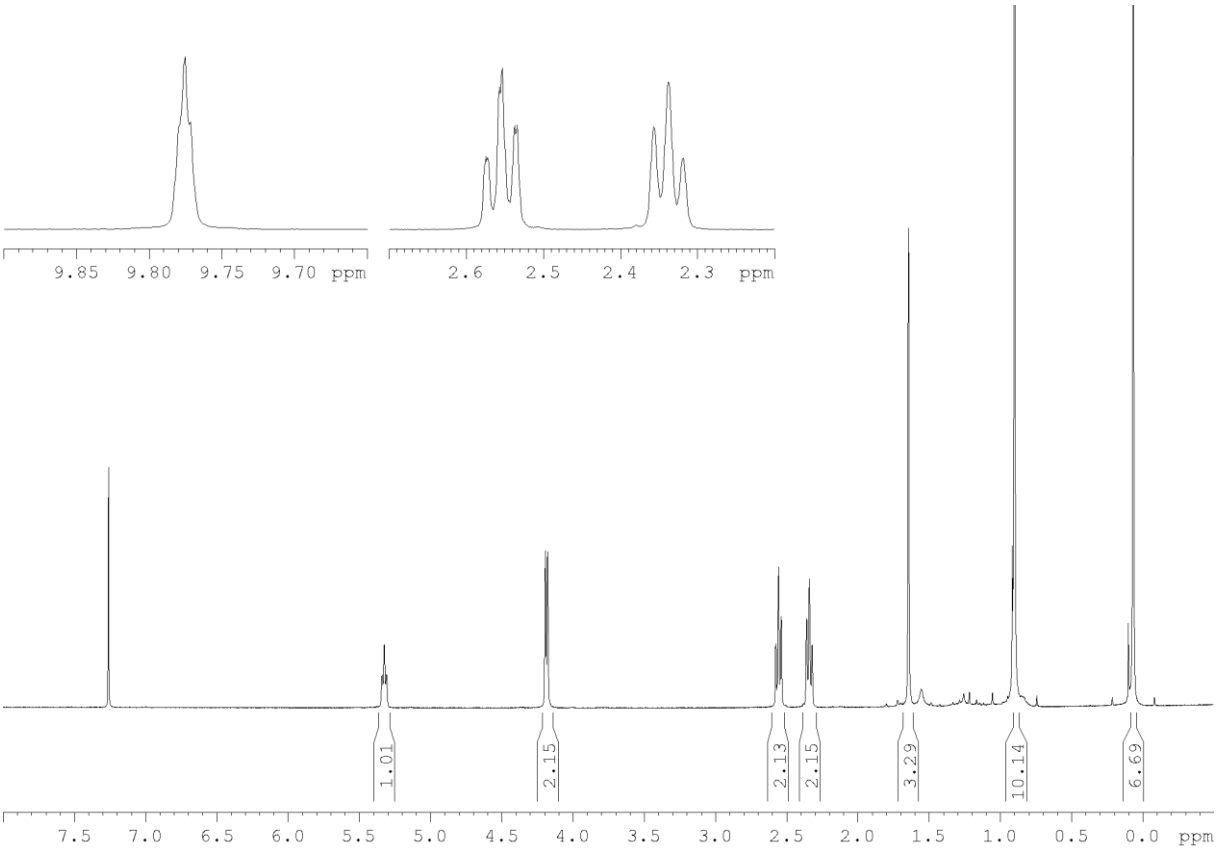

5.5 5.4 5.3 5.2 ppm
4.50 4.45 ppm
2.1 2.0 1.9 1.8 1.7 1.6 ppm
3.00
1.13
2.37
8.31
6.08
3.08
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

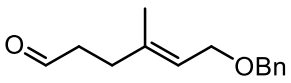

142.8
133.6
130.7
130.4
124.4
124.3
62.7
38.7
32.0
30.1
27.2
24.8
17.0
15.6
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


1.44
1.00
-1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18
ppm


O
OTBS

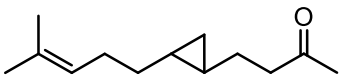
193


9.85 9.80 9.75 9.70 ppm
2.6 2.5 2.4 2.3 ppm
1.01
2.15
2.13
2.15
3.29
10.14
6.69
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

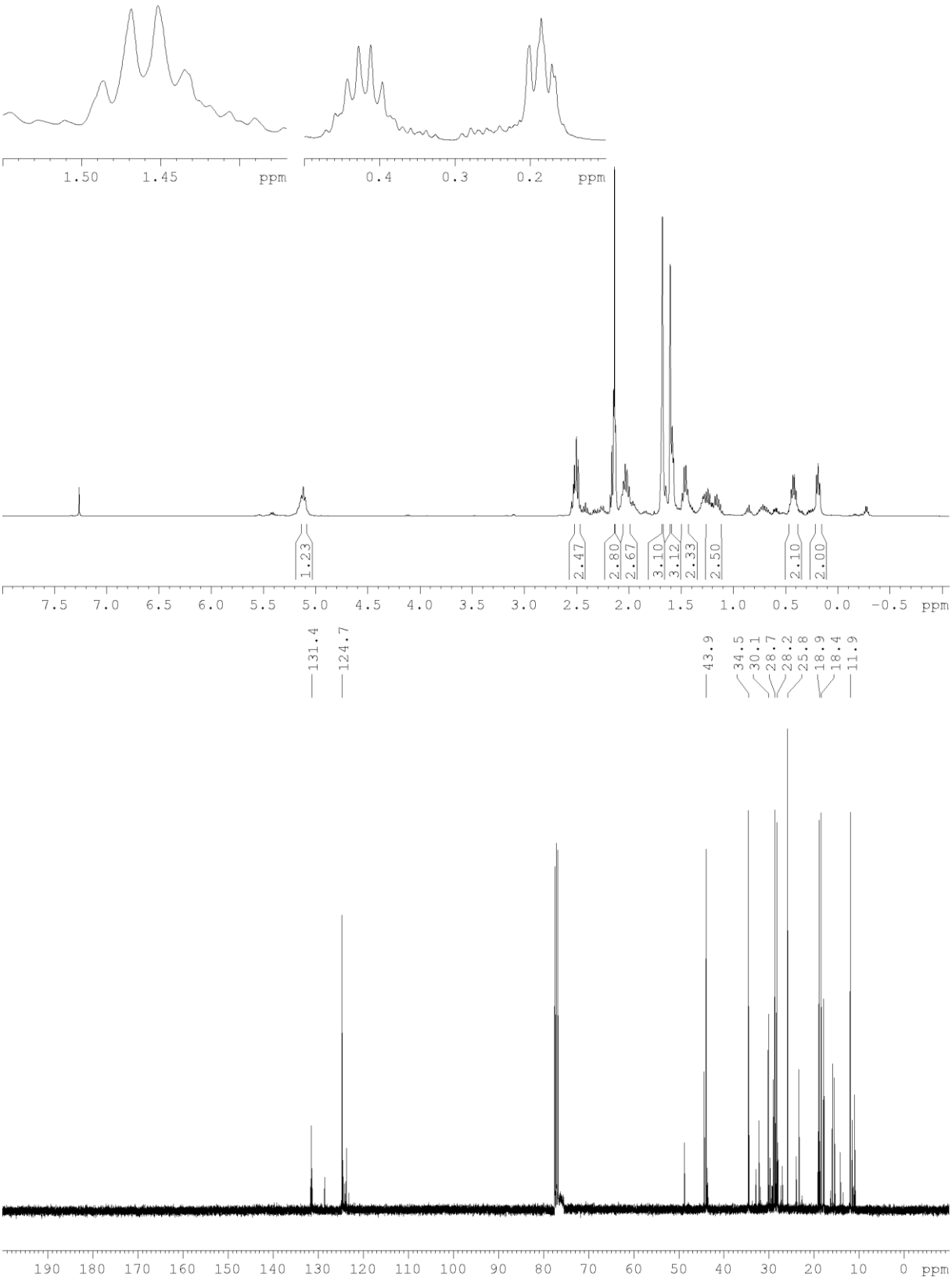

O
OBn

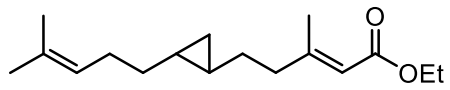
190

9.79 9.78 9.77 ppm
5.45 5.40 ppm
5.51
1.07
2.17
2.14
2.04
1.99
3.20
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

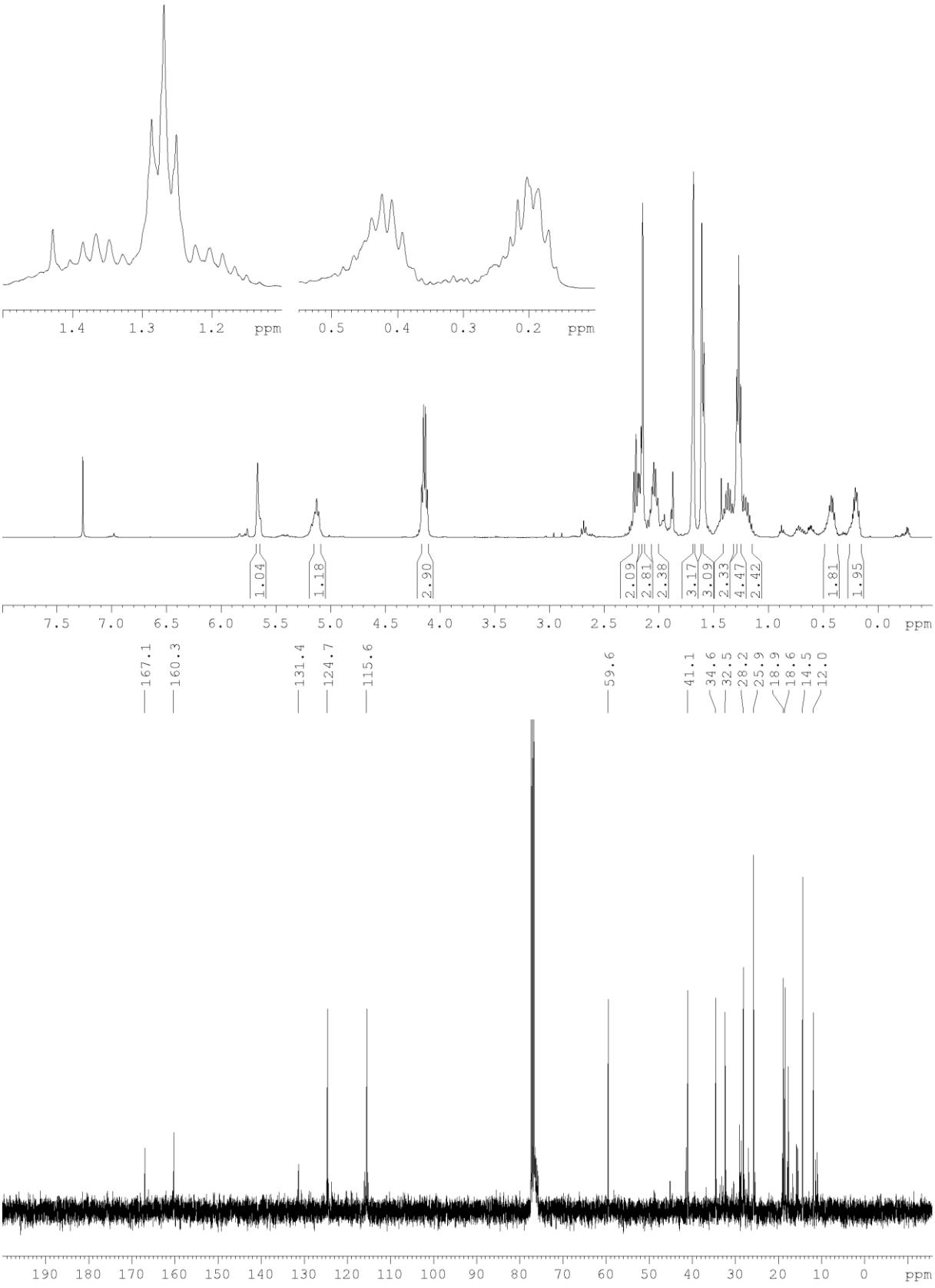


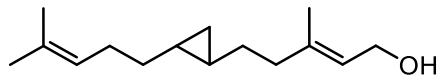
195


1.50
1.45
ppm
0.4
0.3
0.2
ppm
1.23
2.47
2.80
2.67
3.10
3.12
2.33
2.50
2.10
2.00
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 ppm
131.4
124.7
43.9
34.5
30.1
28.7
28.2
25.8
18.9
18.4
11.9
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

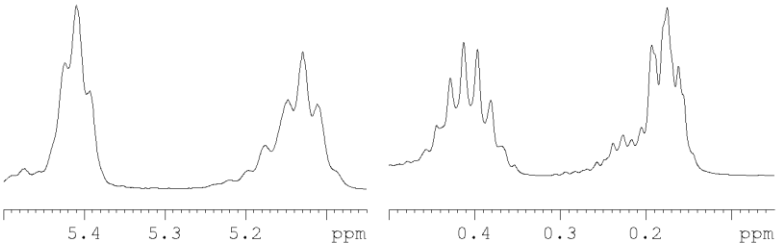

O
OEt

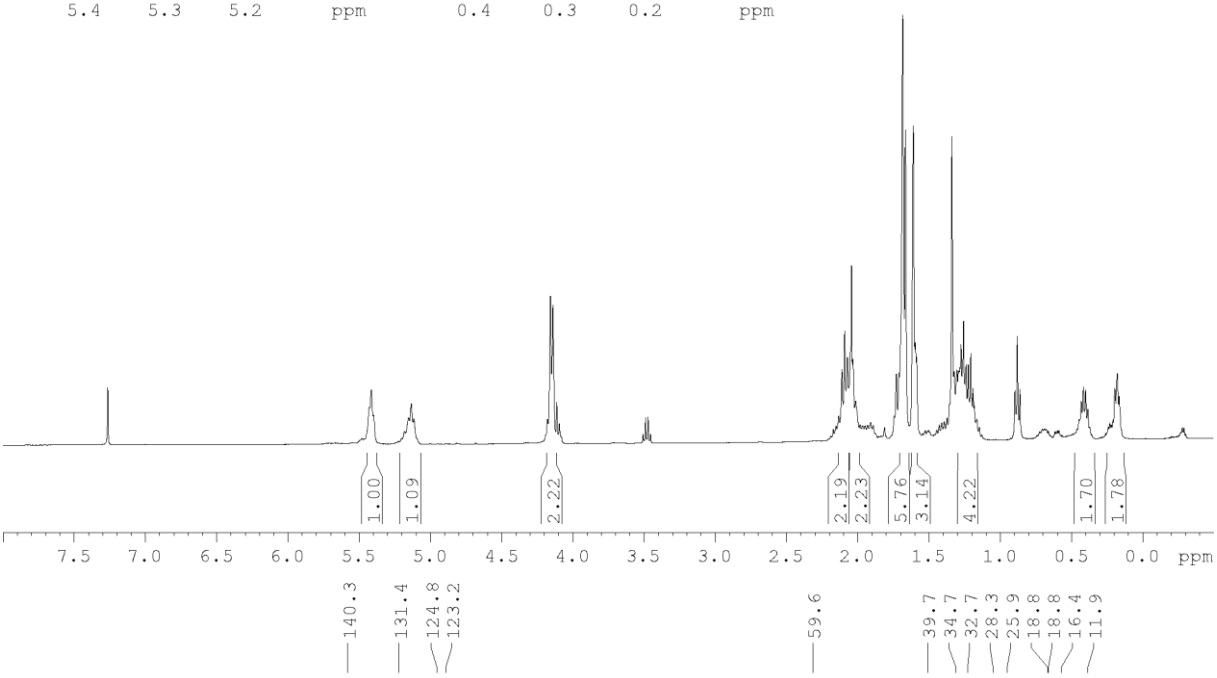
196

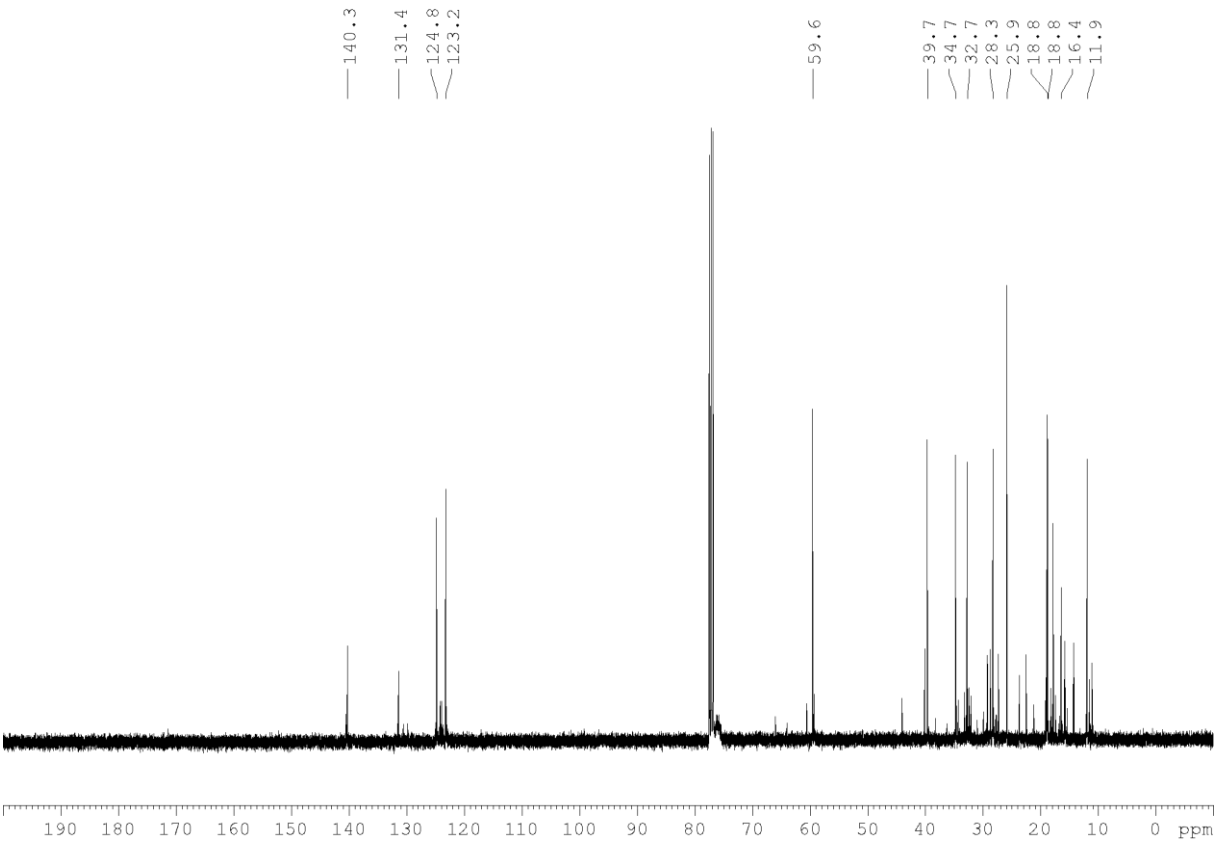

1.4 1.3 1.2 ppm
0.5 0.4 0.3 0.2 ppm
1.04 1.18 2.90 2.09 2.81 2.38 3.17 3.09 2.33 4.47 2.42 1.81 1.95
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
167.1 160.3 131.4 124.7 115.6 59.6 41.1 34.6 32.5 28.2 25.9 18.9 18.6 14.5 12.0
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

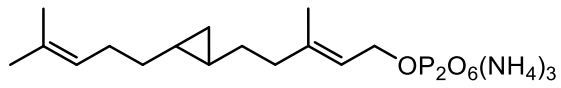

OH

121

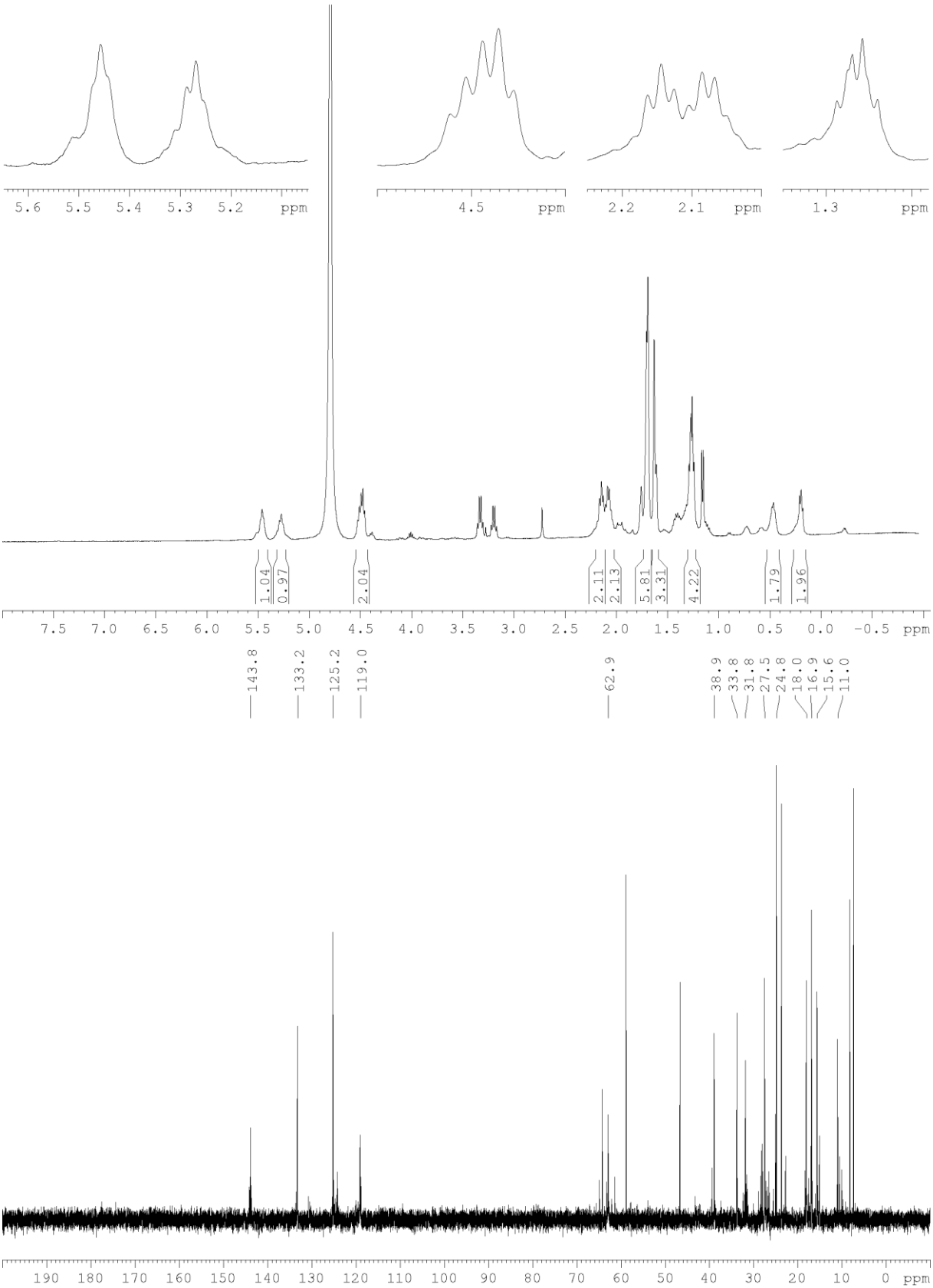

5.4
5.3
5.2
ppm
0.4
0.3
0.2
ppm

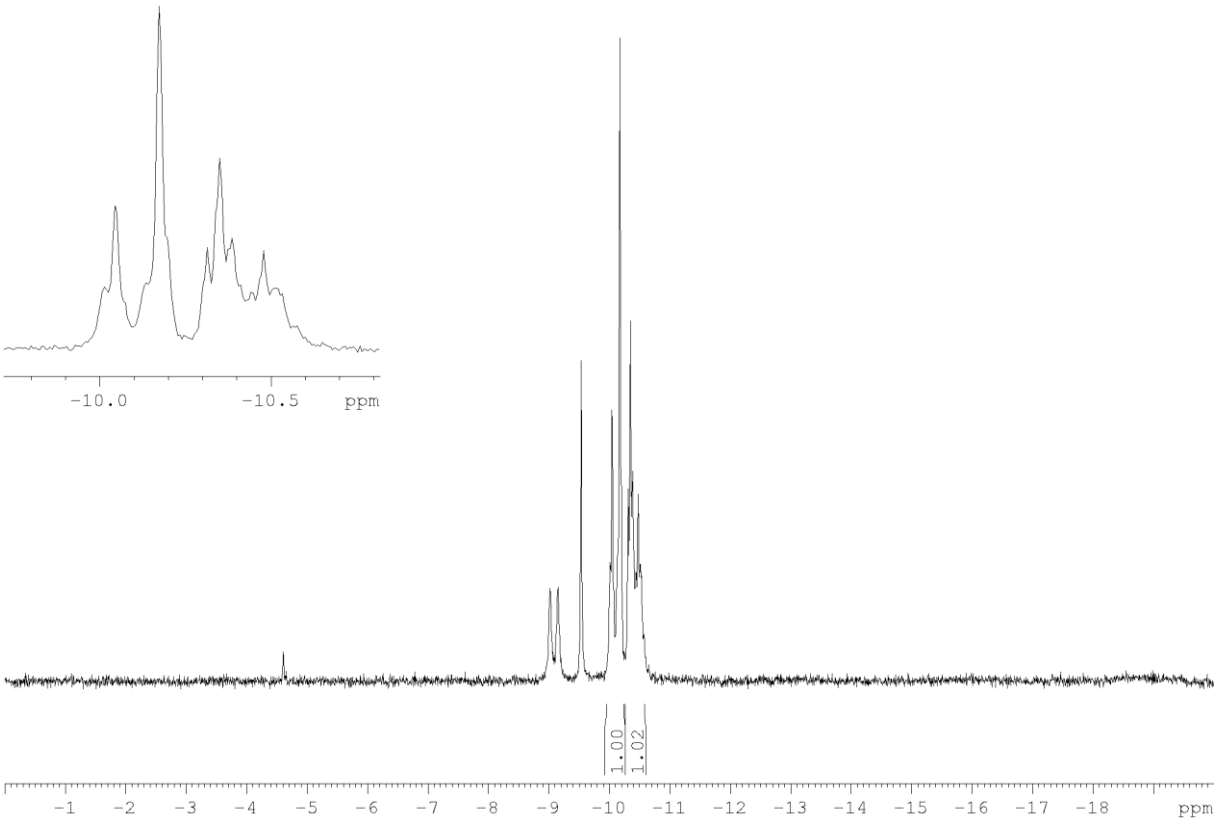

1.00
1.09
2.22
2.19
2.23
5.76
3.14
4.22
1.70
1.78
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

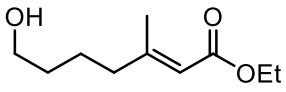

140.3
131.4
124.8
123.2
59.6
39.7
34.7
32.7
28.3
25.9
18.8
18.8
16.4
11.9
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


OP2O6(NH4)3

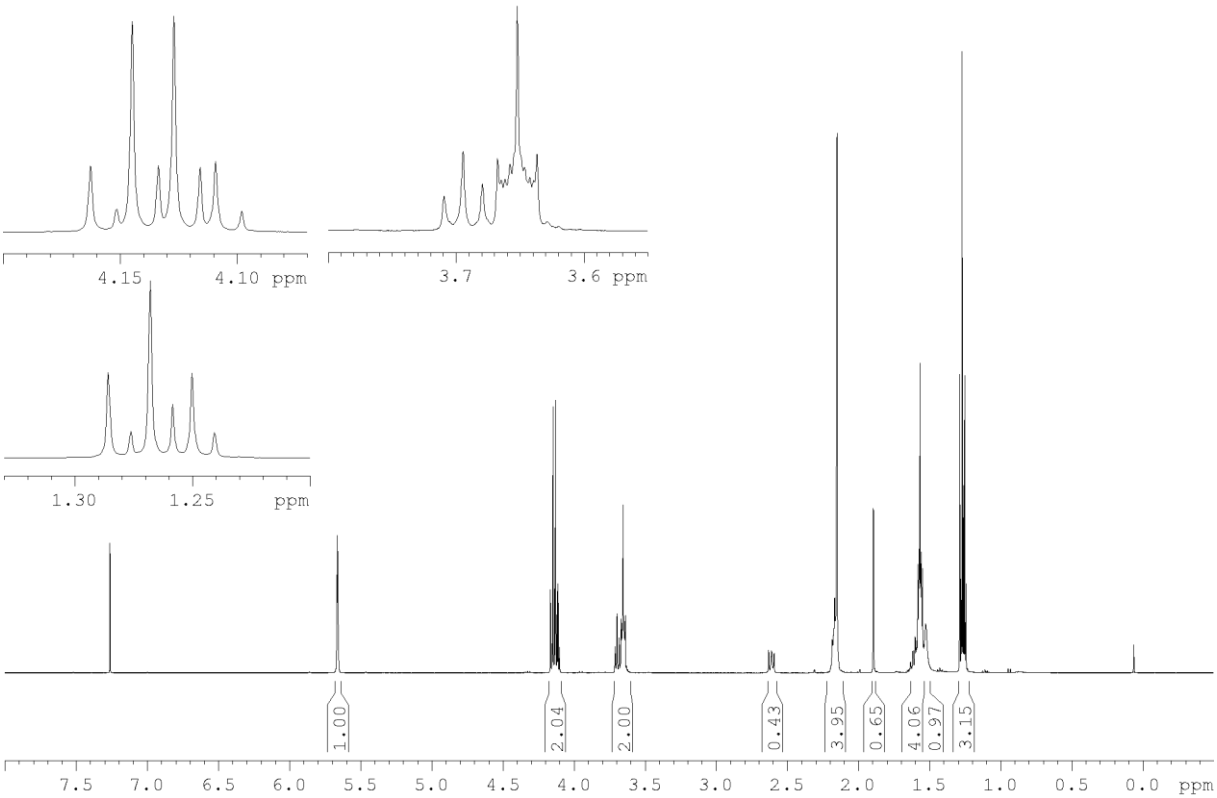
88

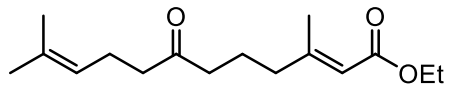

5.6 5.5 5.4 5.3 5.2 ppm
4.5 ppm
2.2 2.1 ppm
1.3 ppm
1.04 0.97 2.04 2.11 2.13 5.81 3.31 4.22 1.79 1.96
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 ppm
143.8 133.2 125.2 119.0 62.9 38.9 33.8 31.8 27.5 24.8 18.0 16.9 15.6 11.0
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


-10.0
-10.5
ppm
1.00
1.02
-1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18
ppm

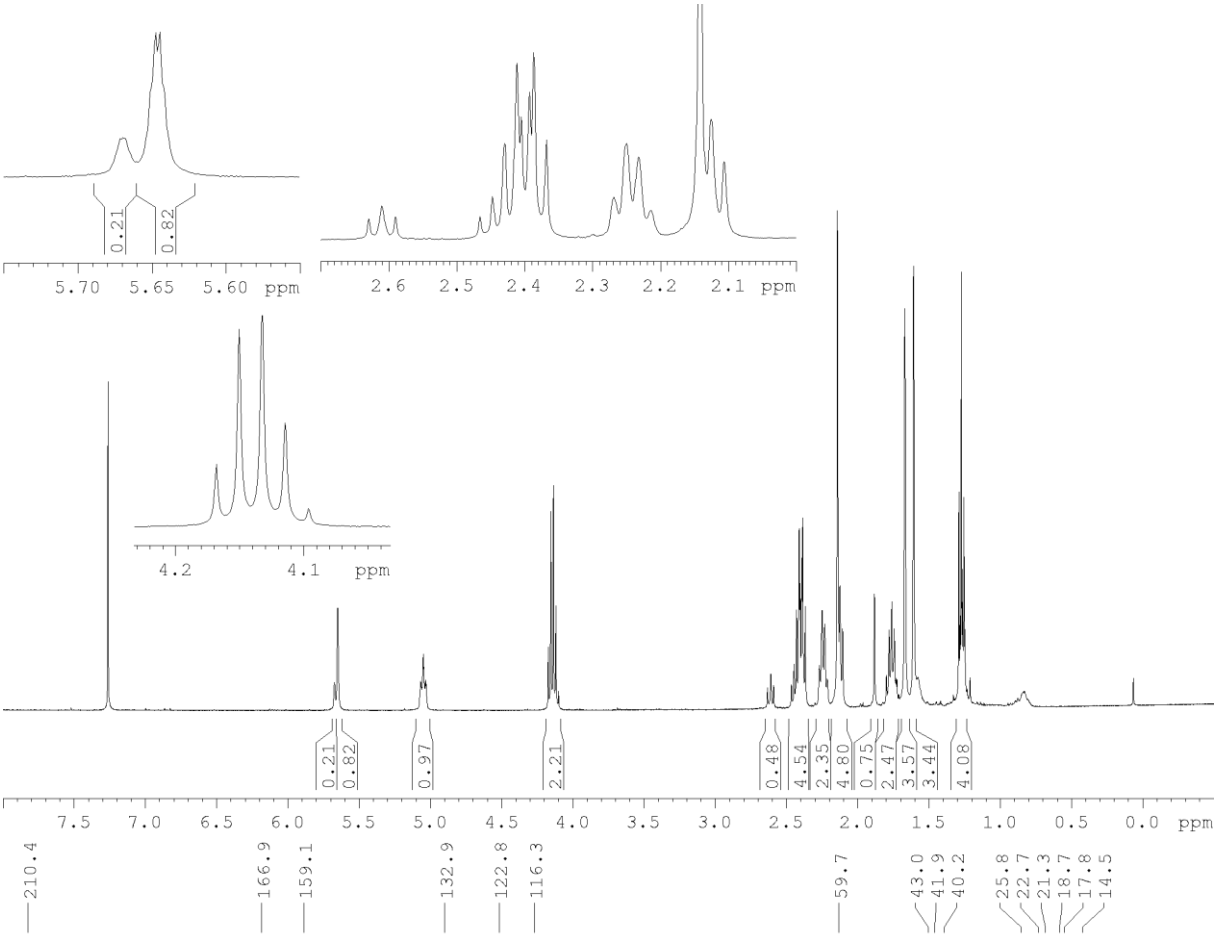

OH
O
OEt

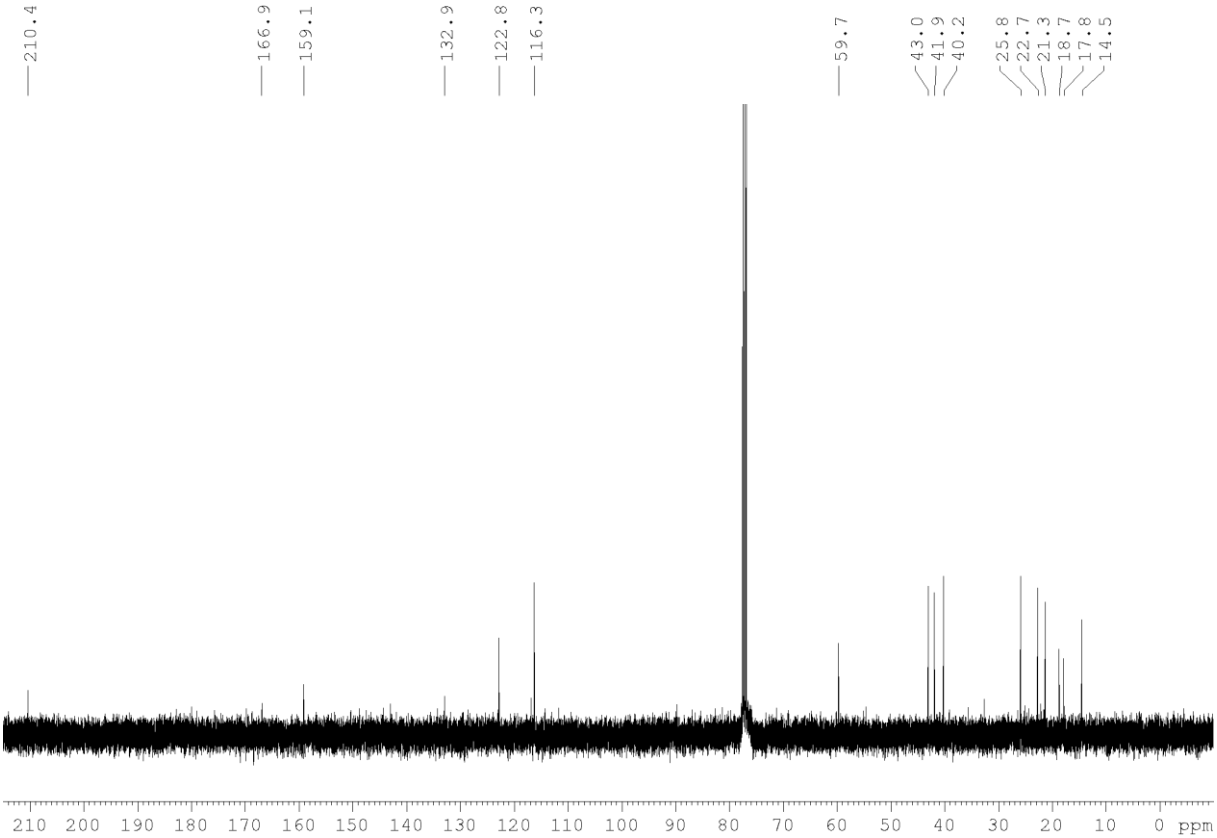
229

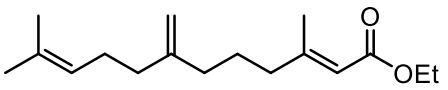

4.15
4.10 ppm
3.7
3.6 ppm
1.30
1.25
ppm
1.00
2.04
2.00
0.43
3.95
0.65
4.06
0.97
3.15
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
ppm


O
O
OEt

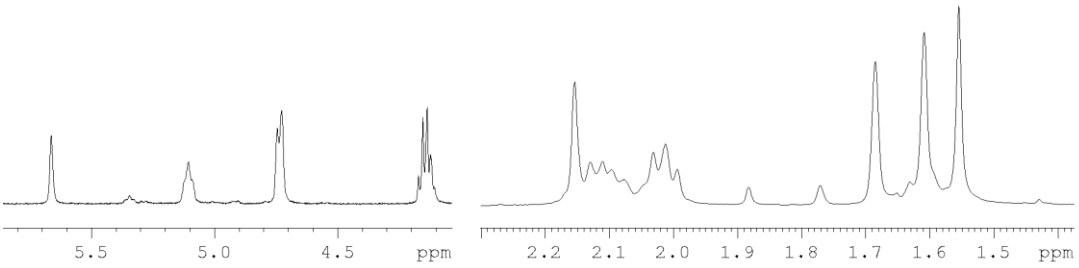
222

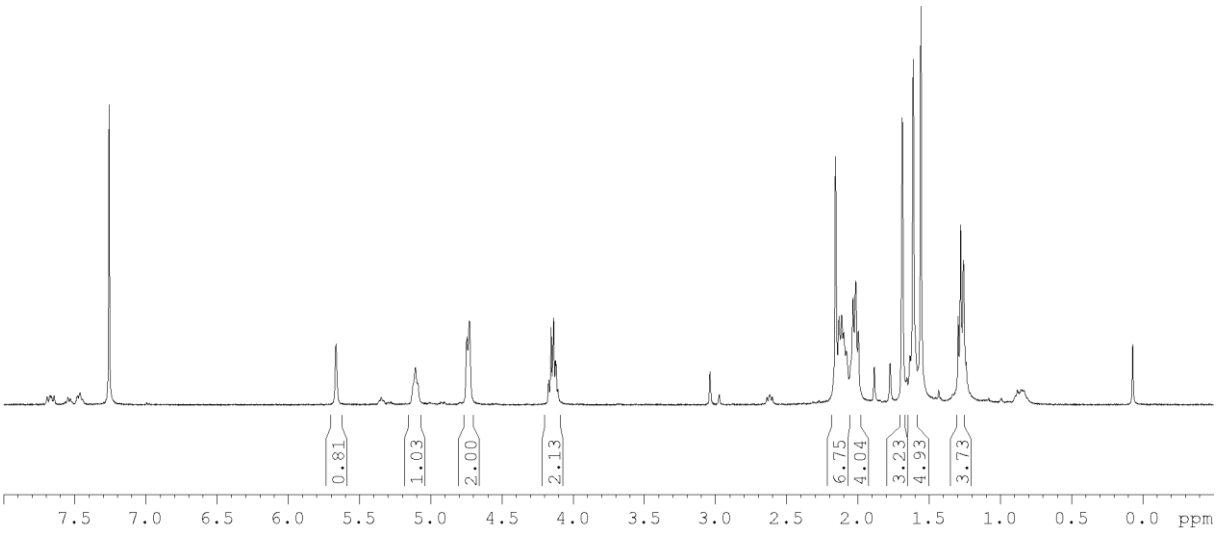

0.21
0.82
5.70 5.65 5.60 ppm
2.6 2.5 2.4 2.3 2.2 2.1 ppm
4.2 4.1 ppm
0.21
0.82
0.97
2.21
0.48
4.54
2.35
4.80
0.75
2.47
3.57
3.44
4.08
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm


210.4
166.9
159.1
132.9
122.8
116.3
59.7
43.0
41.9
40.2
25.8
22.7
21.3
18.7
17.8
14.5
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

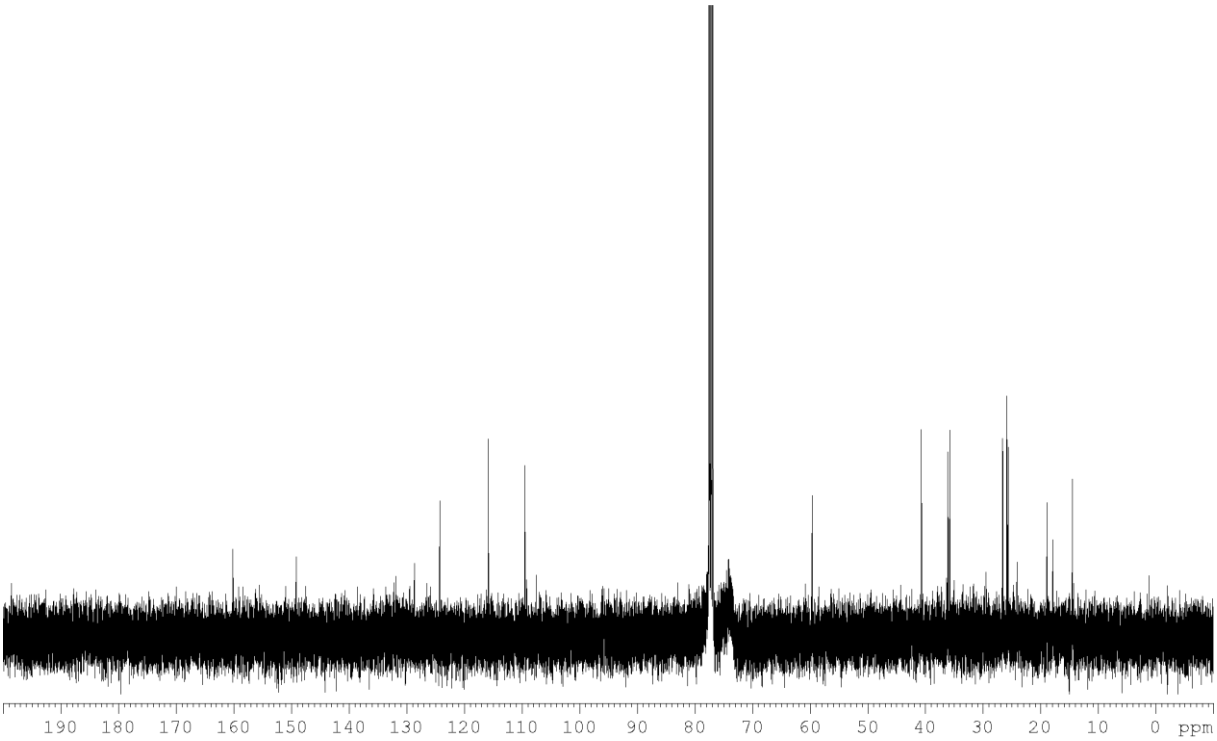

O
OEt

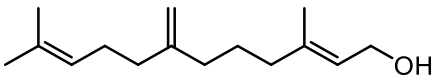
221


5.5
5.0
4.5
ppm
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
ppm

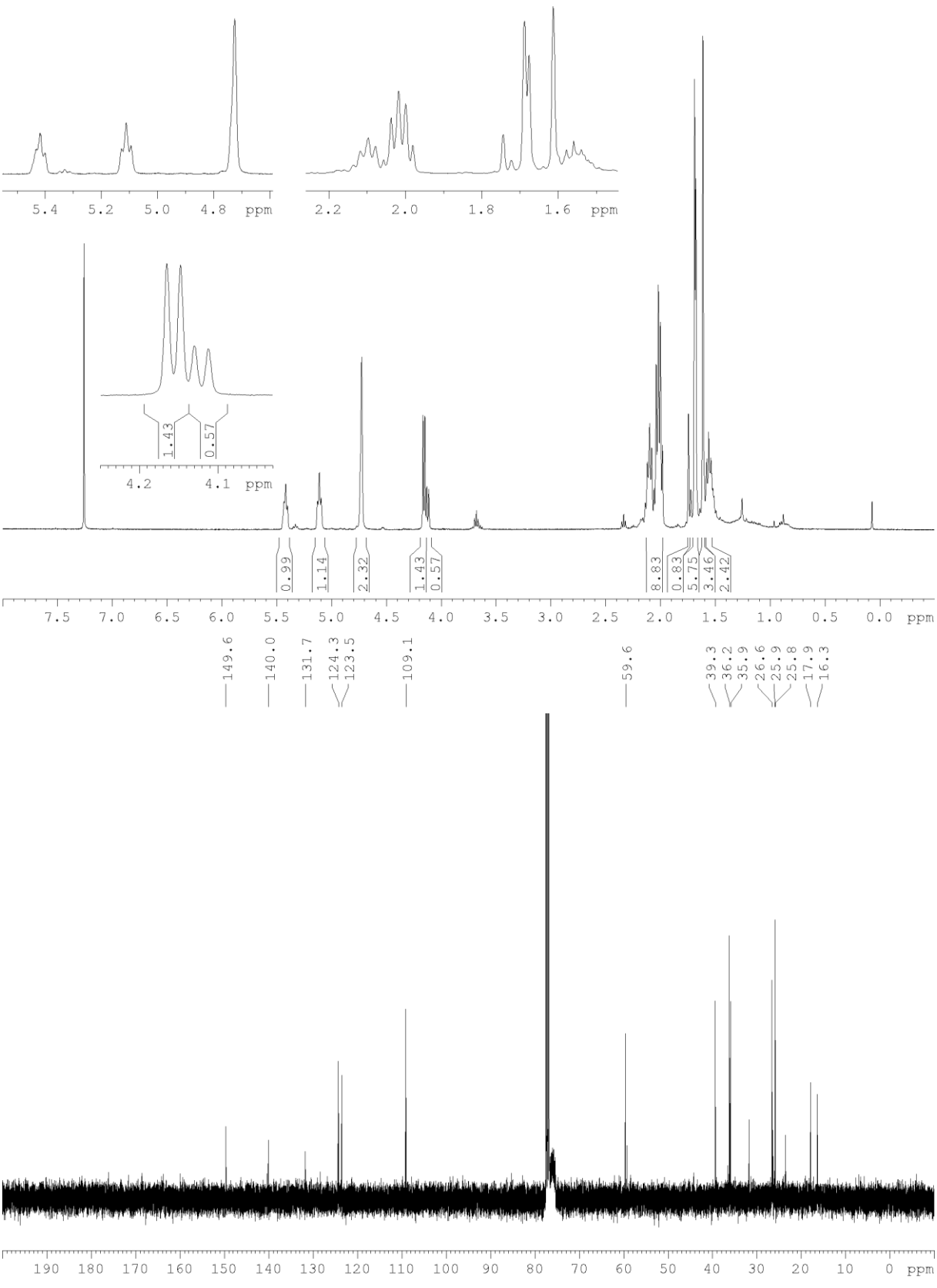

0.81
1.03
2.00
2.13
6.75
4.04
3.23
4.93
3.73
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

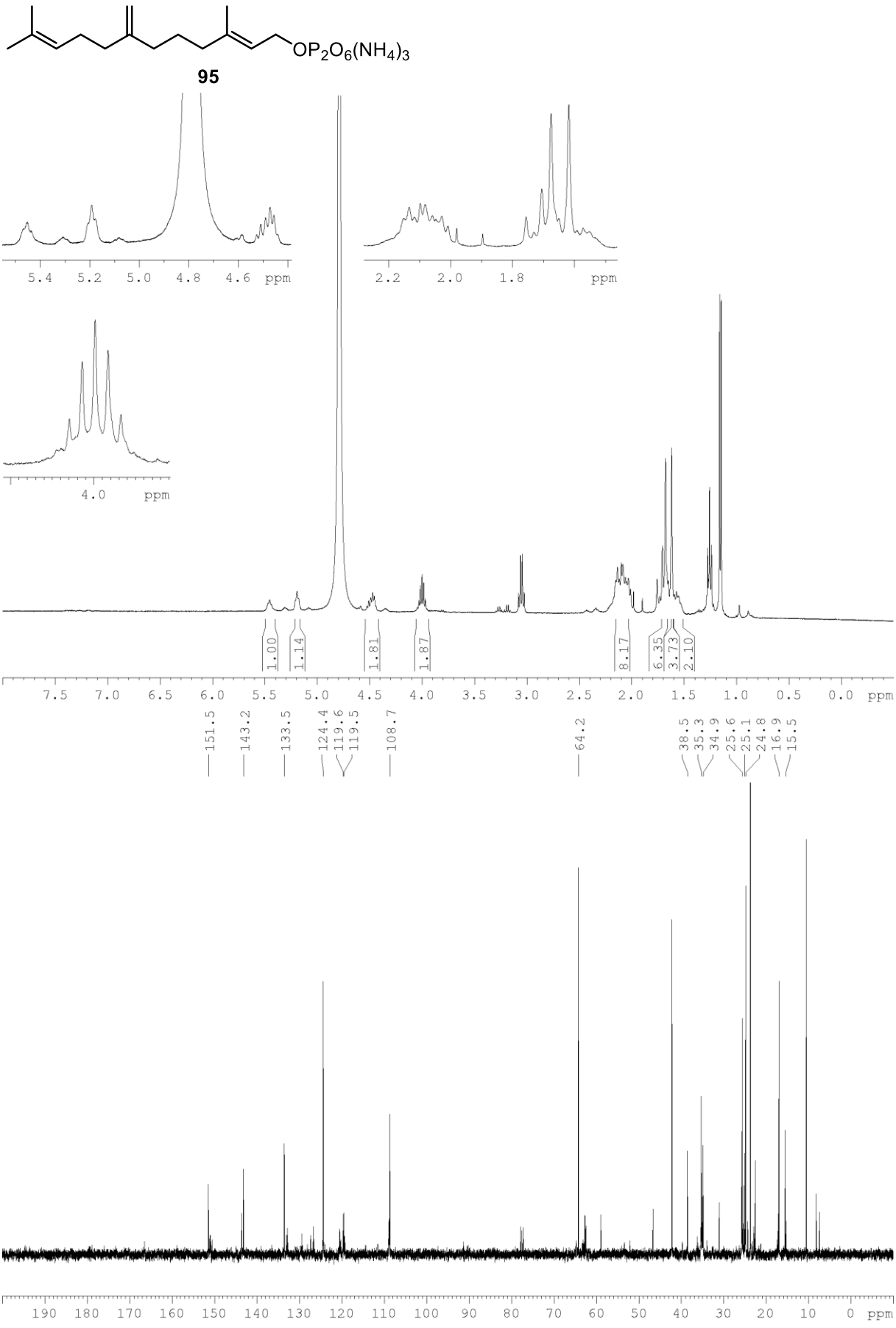

160.1
149.1
128.6
124.2
115.8
109.5
59.6
40.6
36.1
35.7
26.5
25.8
25.6
18.9
17.9
14.5

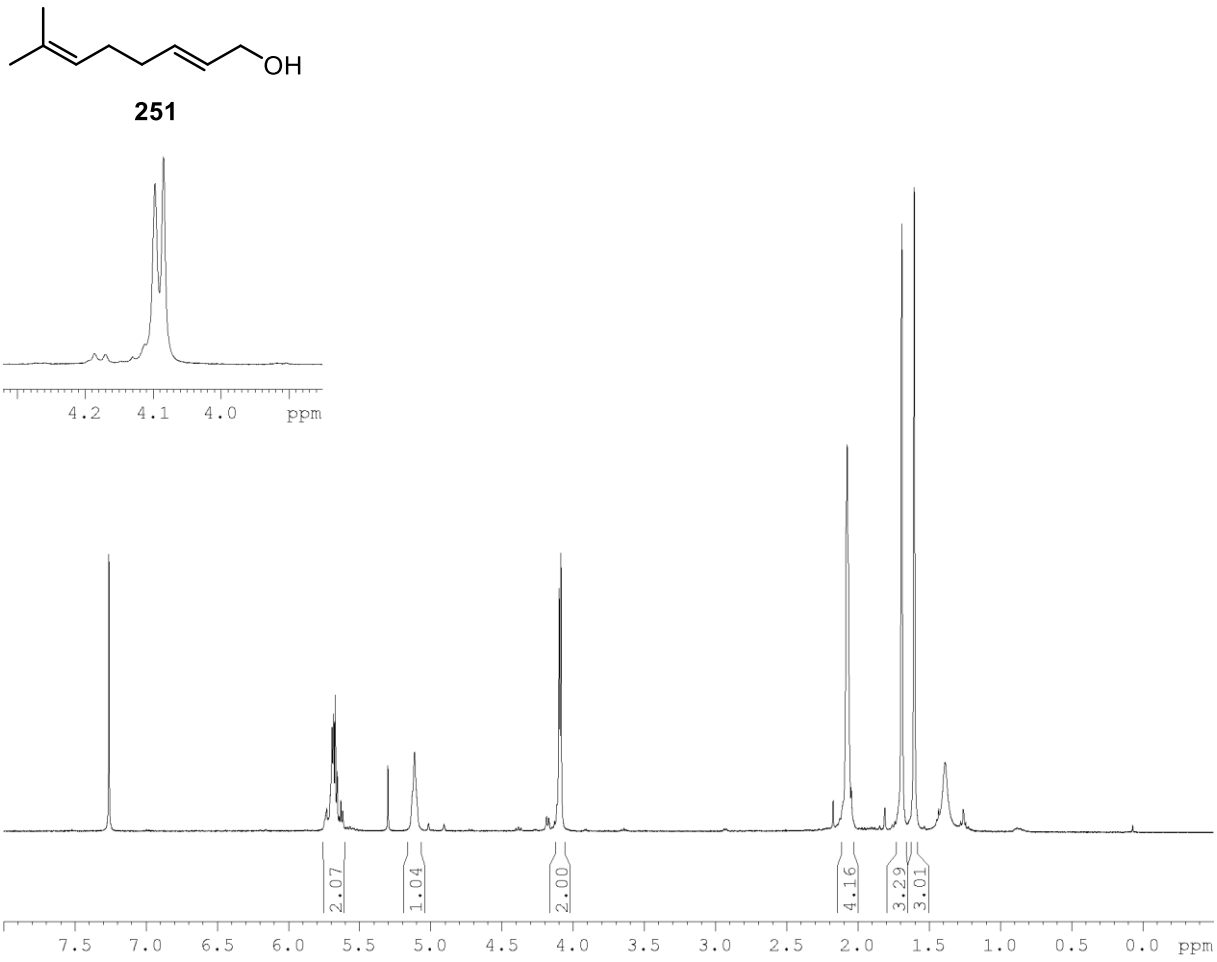

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

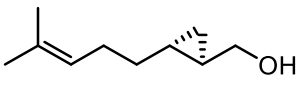

OH

220

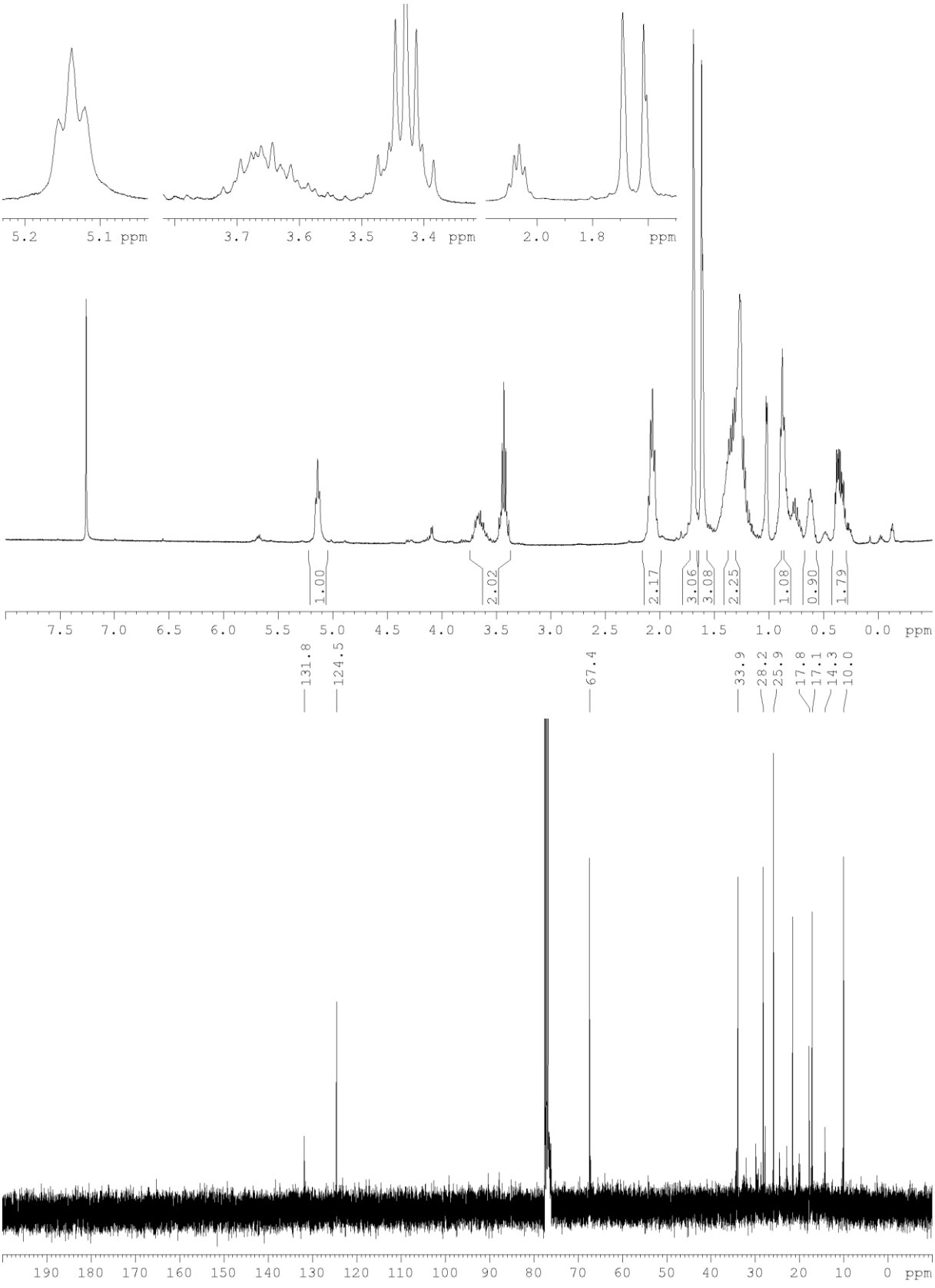

5.4 5.2 5.0 4.8 ppm
2.2 2.0 1.8 1.6 ppm
4.2 4.1 ppm
1.43
0.57
0.99
1.14
2.32
1.43
0.57
8.83
0.83
5.75
3.46
2.42
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
149.6
140.0
131.7
124.3
123.5
109.1
59.6
39.3
36.2
35.9
26.6
25.9
25.8
17.9
16.3
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

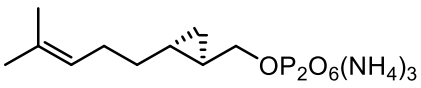

OP2O6(NH4)3
95
5.4 5.2 5.0 4.8 4.6 ppm
2.2 2.0 1.8 ppm
4.0 ppm
1.00 1.14 1.81 1.87 8.17 6.35 3.73 2.10
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
151.5 143.2 133.5 124.4 119.6 119.5 108.7 64.2 38.5 35.3 34.9 25.6 25.1 24.8 16.9 15.5
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


OH
251
4.2
4.1
4.0
ppm
2.07
1.04
2.00
4.16
3.29
3.01
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

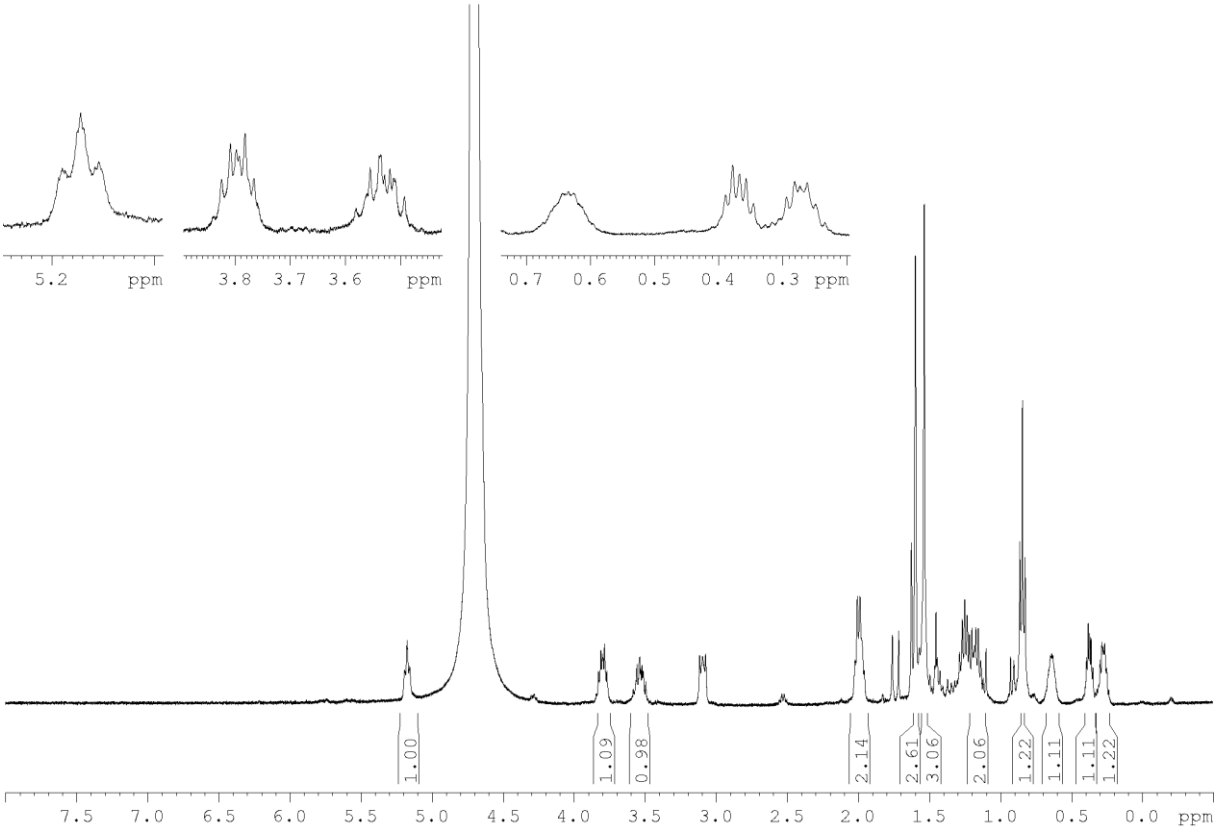

OH

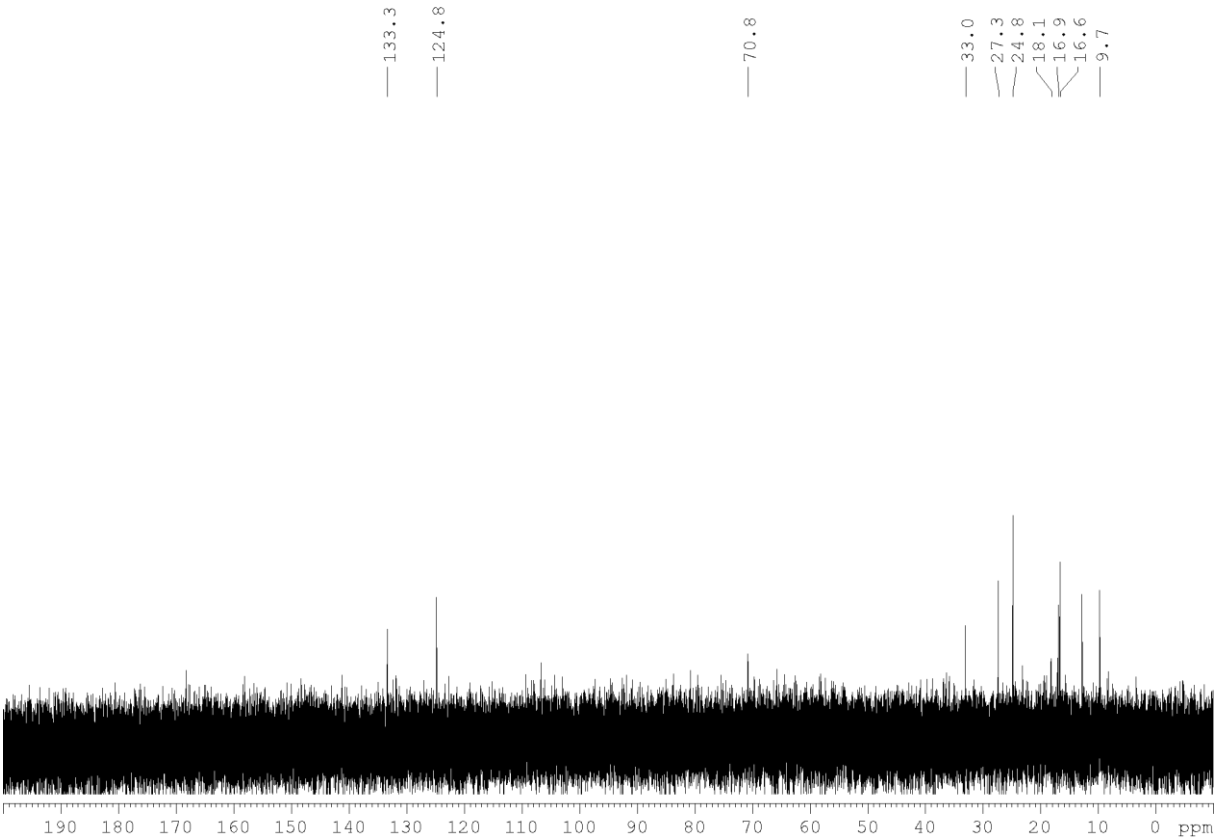
252

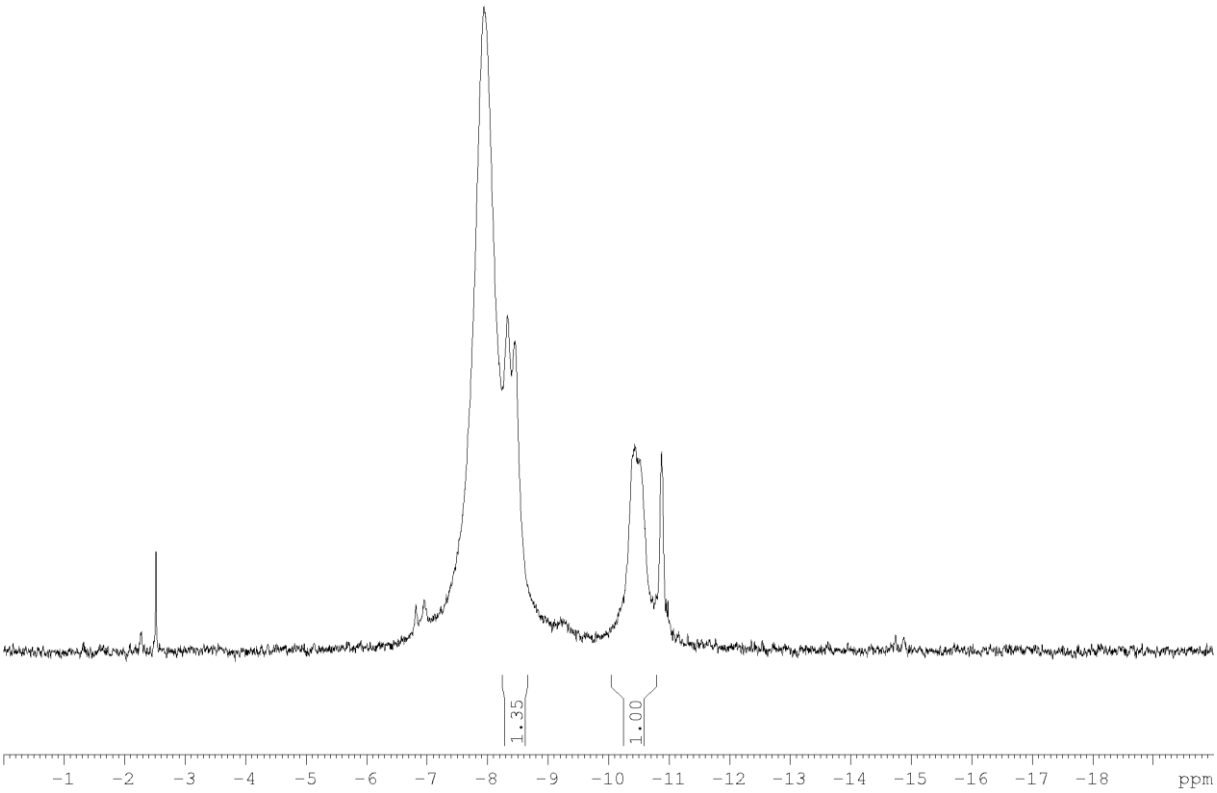

5.2
5.1 ppm
3.7
3.6
3.5
3.4 ppm
2.0
1.8
ppm
1.00
2.02
2.17
3.06
3.08
2.25
1.08
0.90
1.79
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0 ppm
131.8
124.5
67.4
33.9
28.2
25.9
17.8
17.1
14.3
10.0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0 ppm

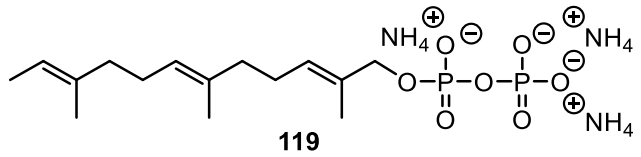

OP2O6(NH4)3

254

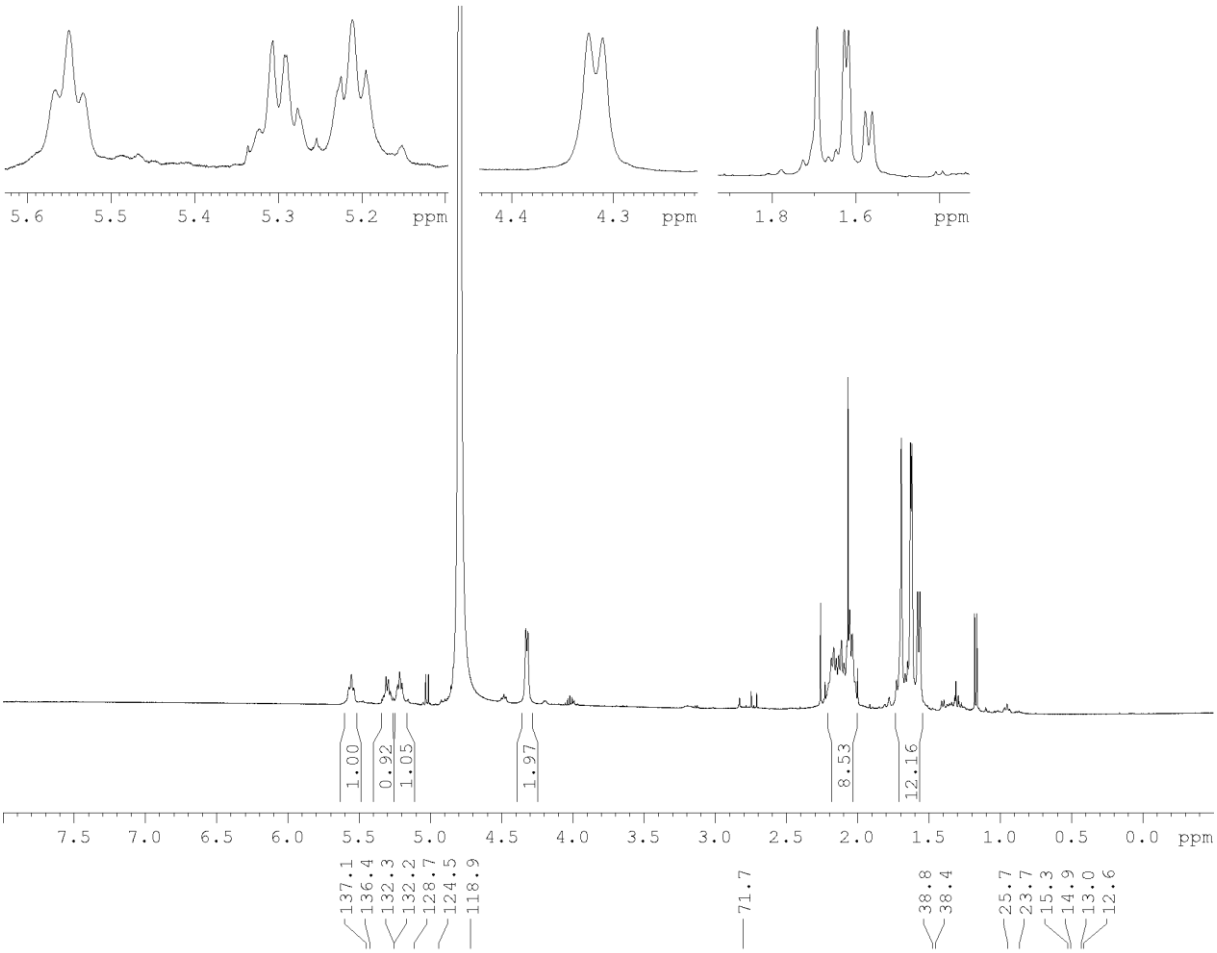

5.2 ppm
3.8 3.7 3.6 ppm
0.7 0.6 0.5 0.4 0.3 ppm
1.00
1.09
0.98
2.14
2.61
3.06
2.06
1.22
1.11
1.11
1.22
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

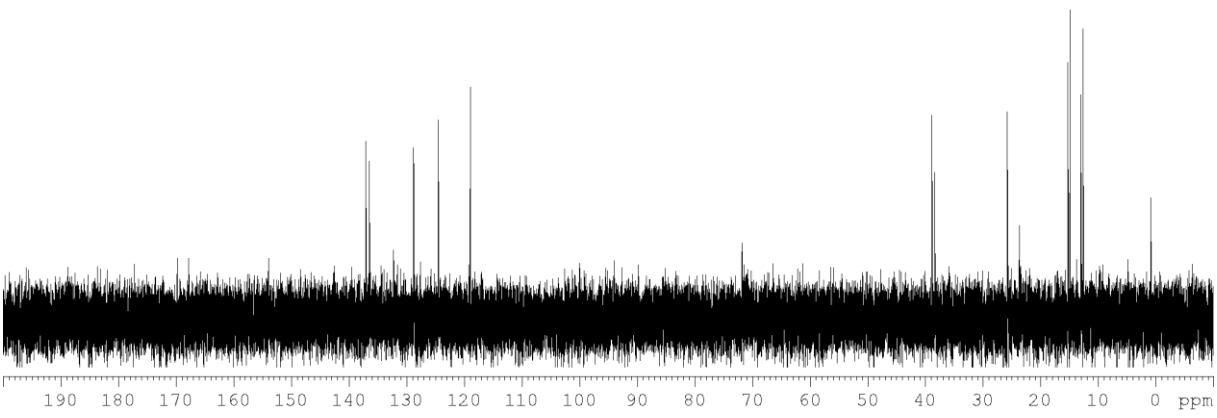

133.3
124.8
70.8
33.0
27.3
24.8
18.1
16.9
16.6
9.7
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

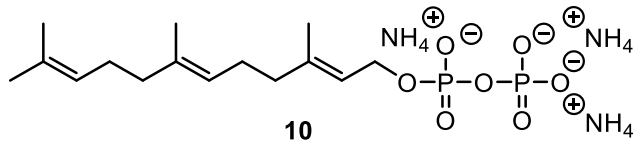

1.35
1.00
-1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18
ppm


NH4
O
O
P
O
O
P
O
O
NH4
NH4
NH4

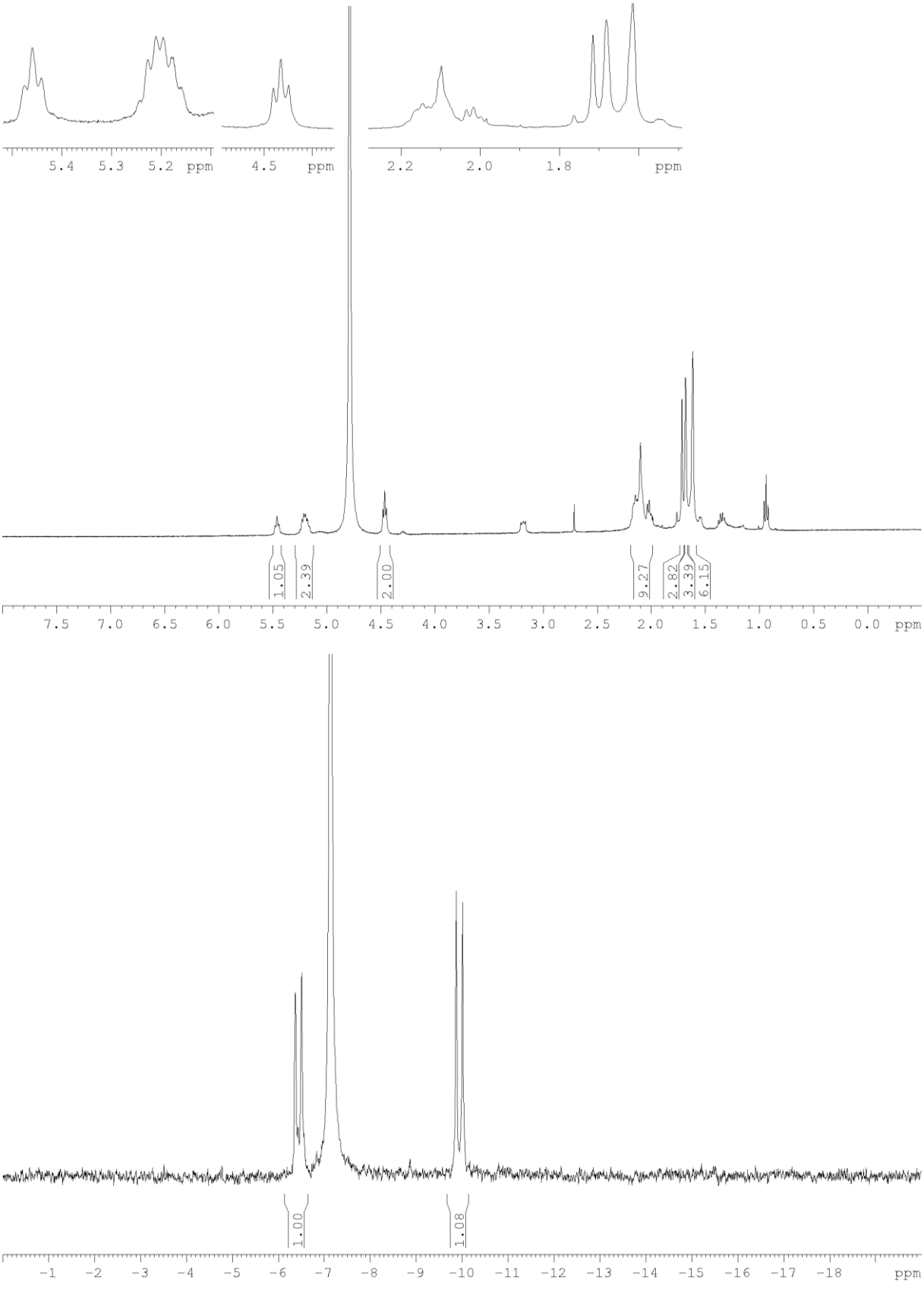
119

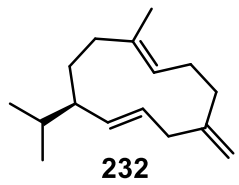

5.6 5.5 5.4 5.3 5.2 ppm
4.4 4.3 ppm
1.8 1.6 ppm
1.00
0.92
1.05
1.97
8.53
12.16
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
137.1
136.4
132.3
132.2
128.7
124.5
118.9
71.7
38.8
38.4
25.7
23.7
15.3
14.9
13.0
12.6


190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

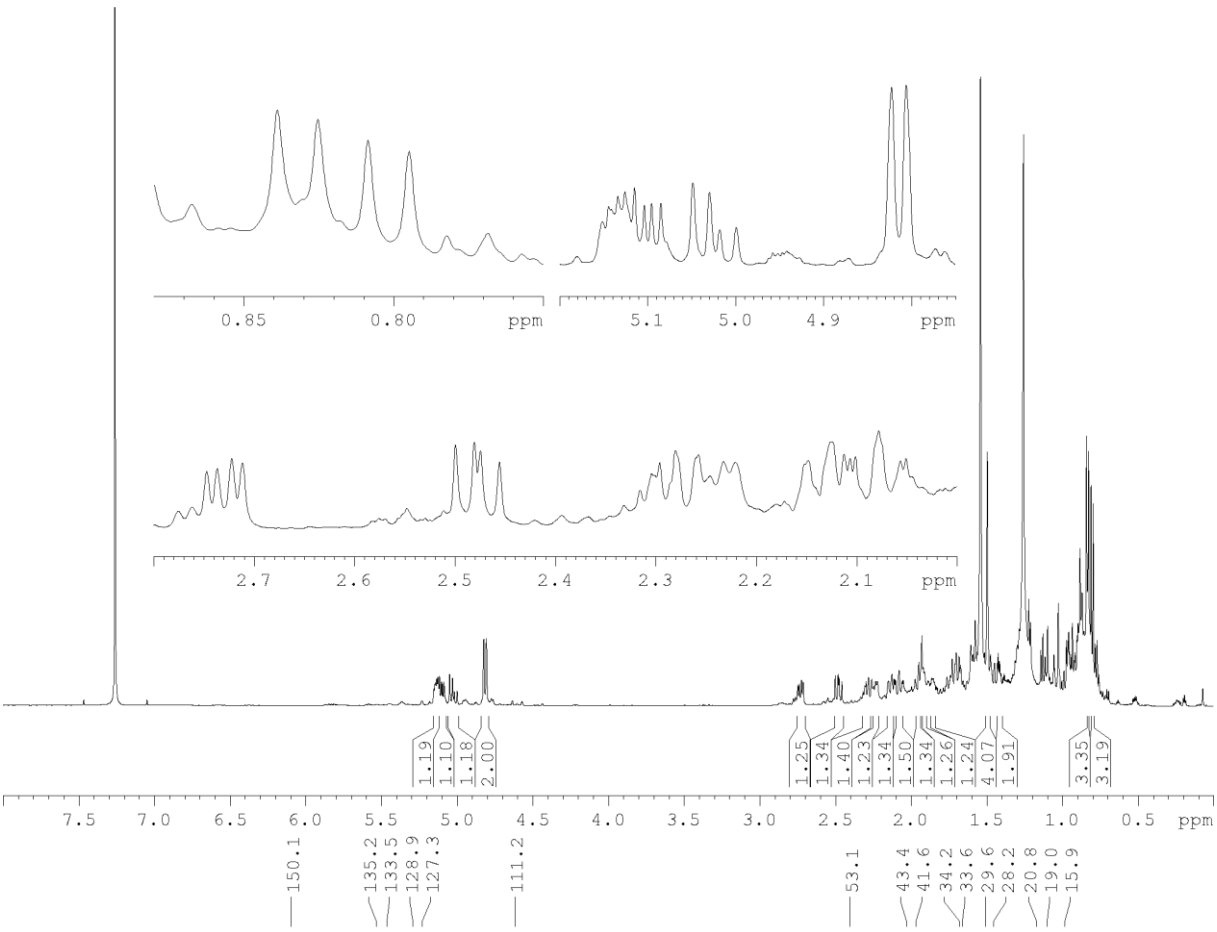

NH4
O
O
P
O
O
P
O
O
NH4
NH4
NH4

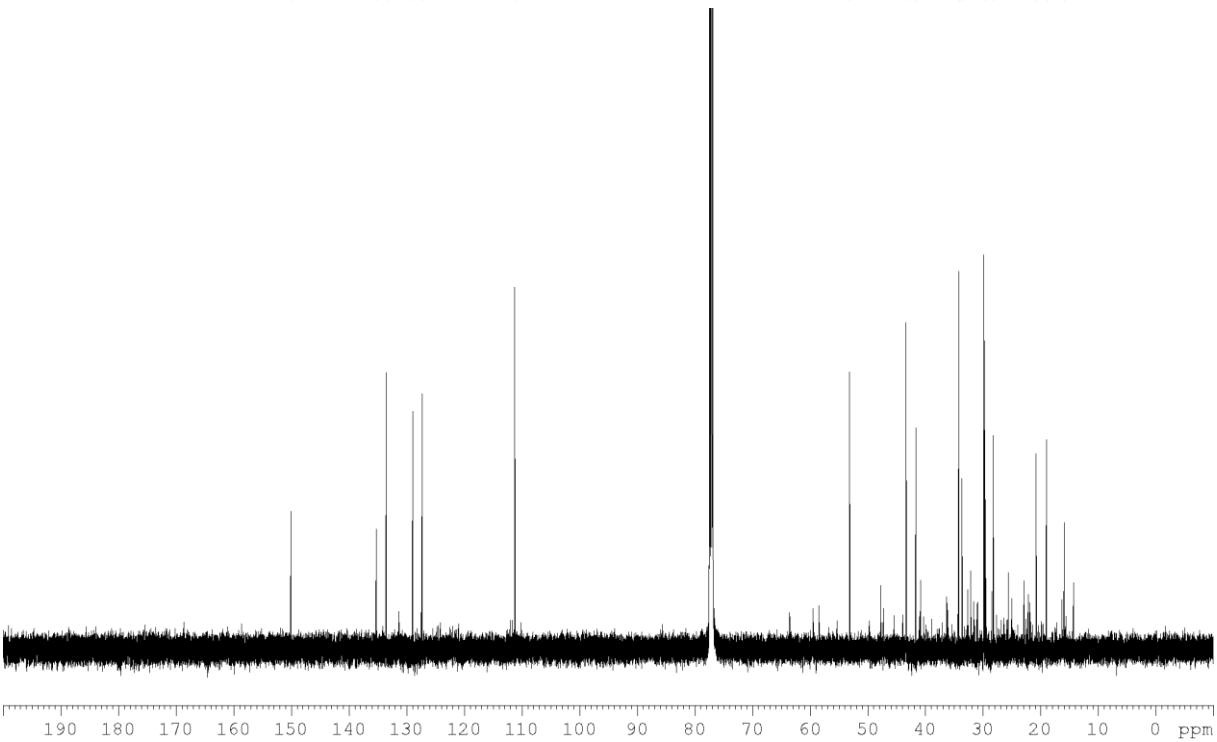
10

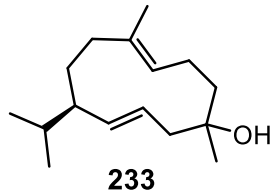

5.4
5.3
5.2
ppm
4.5
ppm
2.2
2.0
1.8
ppm
1.05
2.39
2.00
9.27
2.82
3.39
6.15
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
1.00
1.08
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
-13
-14
-15
-16
-17
-18
ppm



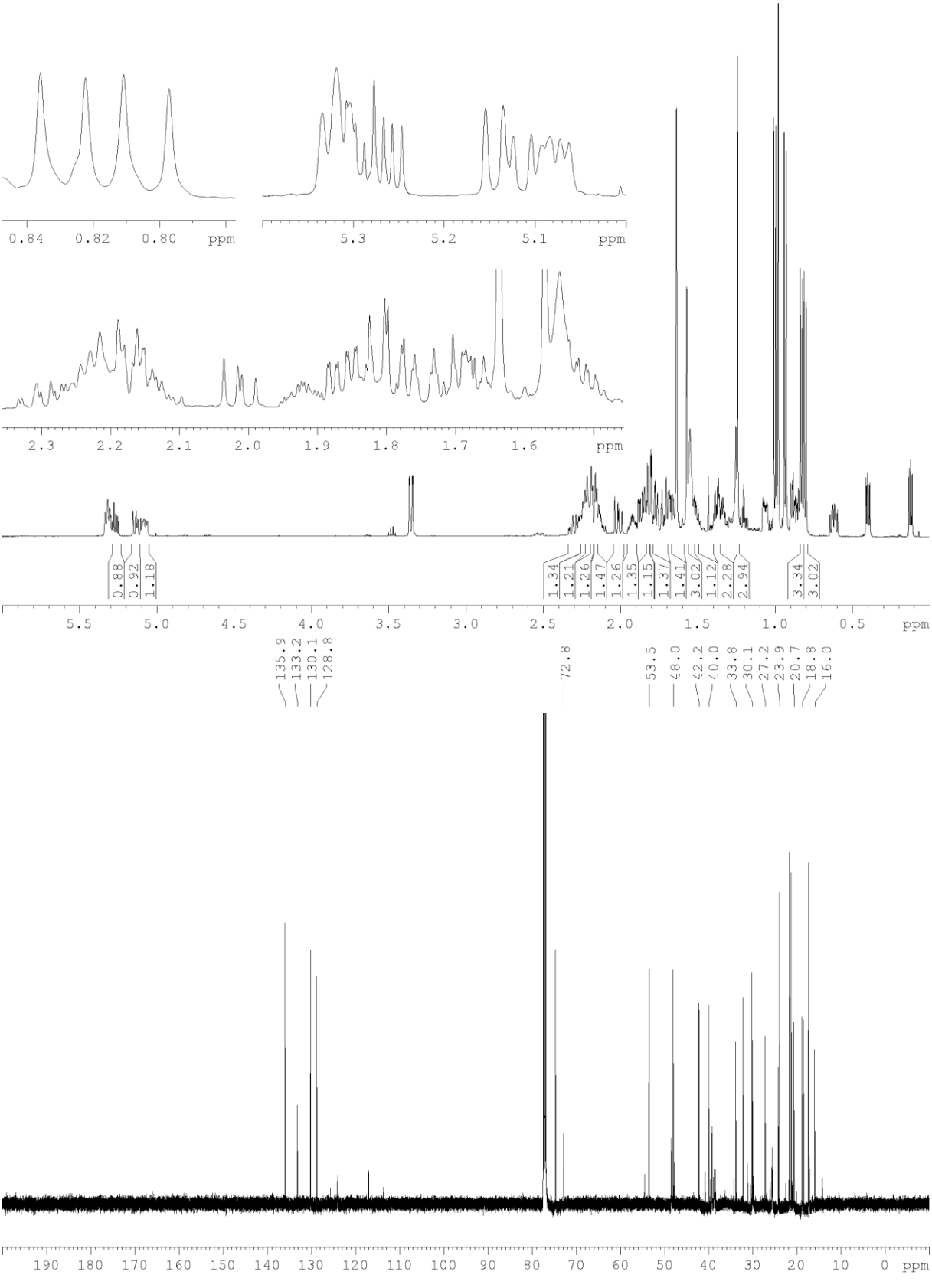
232

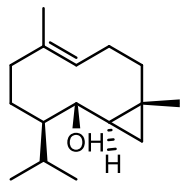

0.85
0.80
ppm
5.1
5.0
4.9
ppm
2.7
2.6
2.5
2.4
2.3
2.2
2.1
ppm
1.19
1.10
1.18
2.00
1.25
1.34
1.40
1.23
1.34
1.50
1.34
1.26
1.24
4.07
1.91
3.35
3.19
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm


150.1
135.2
133.5
128.9
127.3
111.2
53.1
43.4
41.6
34.2
33.6
29.6
28.2
20.8
19.0
15.9
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

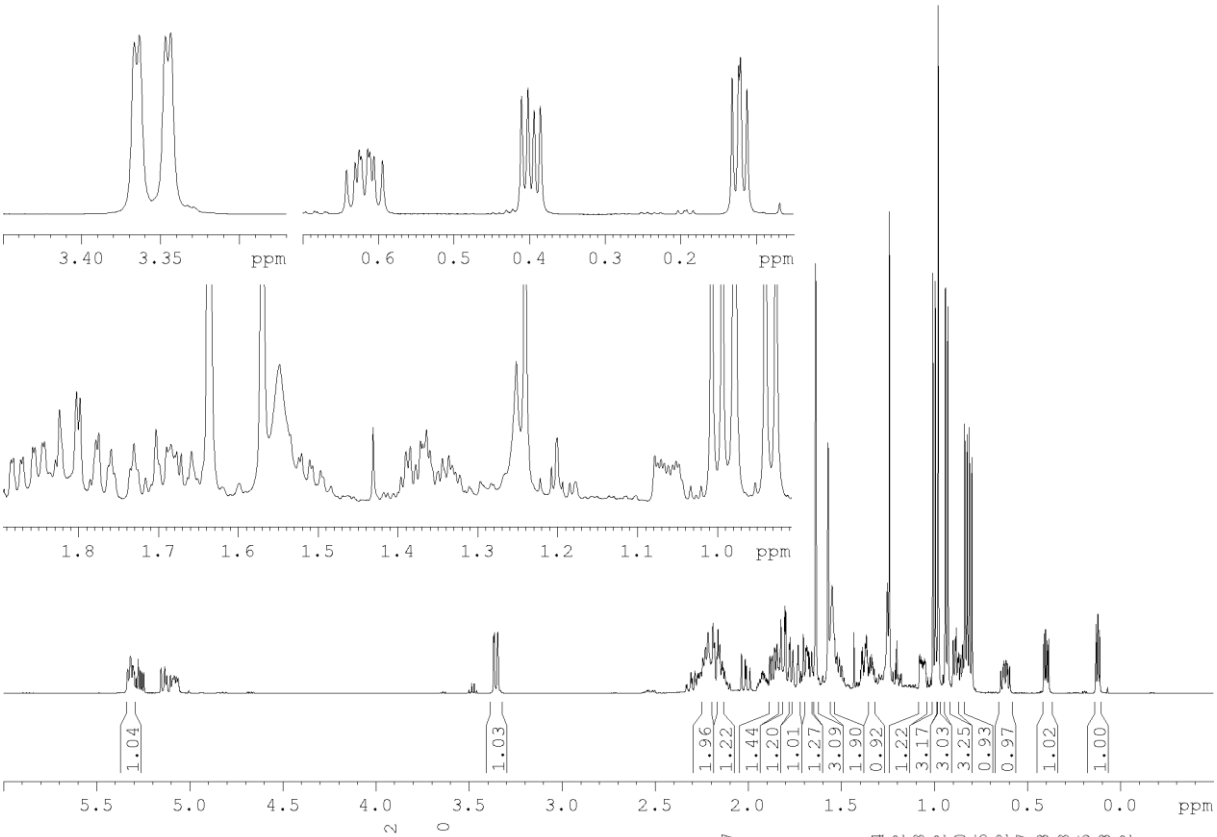

OH

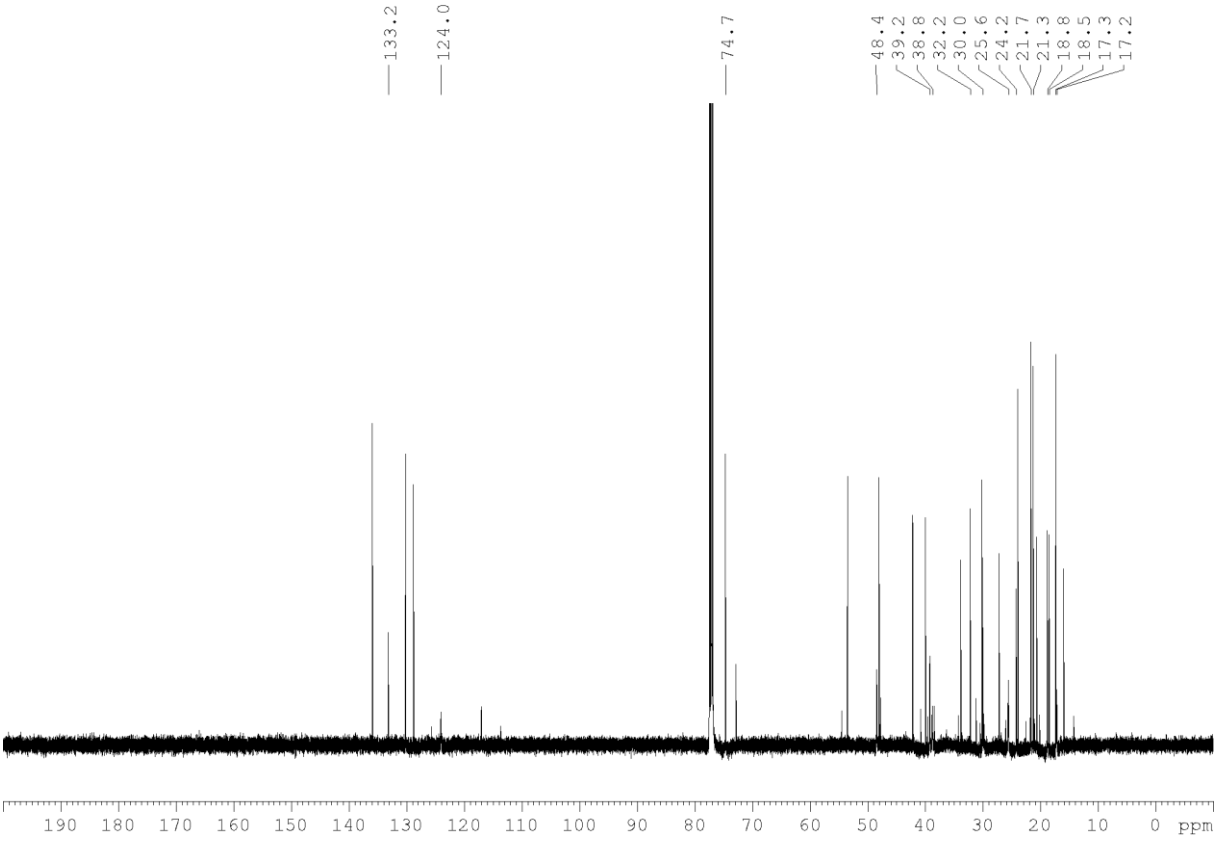
233


0.84
0.82
0.80
ppm
5.3
5.2
5.1
ppm
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
ppm
0.88
0.92
1.18
1.34
1.21
1.26
1.47
1.26
1.35
1.15
1.37
1.41
3.02
1.12
2.28
2.94
3.34
3.02
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
135.9
133.2
130.1
128.8
72.8
53.5
48.0
42.2
40.0
33.8
30.1
27.2
23.9
20.7
18.8
16.0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


OH
H

234


3.40 3.35 ppm
0.6 0.5 0.4 0.3 0.2 ppm
1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 ppm
1.04 1.03 1.96 1.22 1.44 1.20 1.01 1.27 3.09 1.90 0.92 1.22 3.17 3.03 3.25 0.93 0.97 1.02 1.00
5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm


133.2
124.0
74.7
48.4
39.2
38.8
32.2
30.0
25.6
24.2
21.7
21.3
18.8
18.5
17.3
17.2
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

8.2 GC-MS DATA

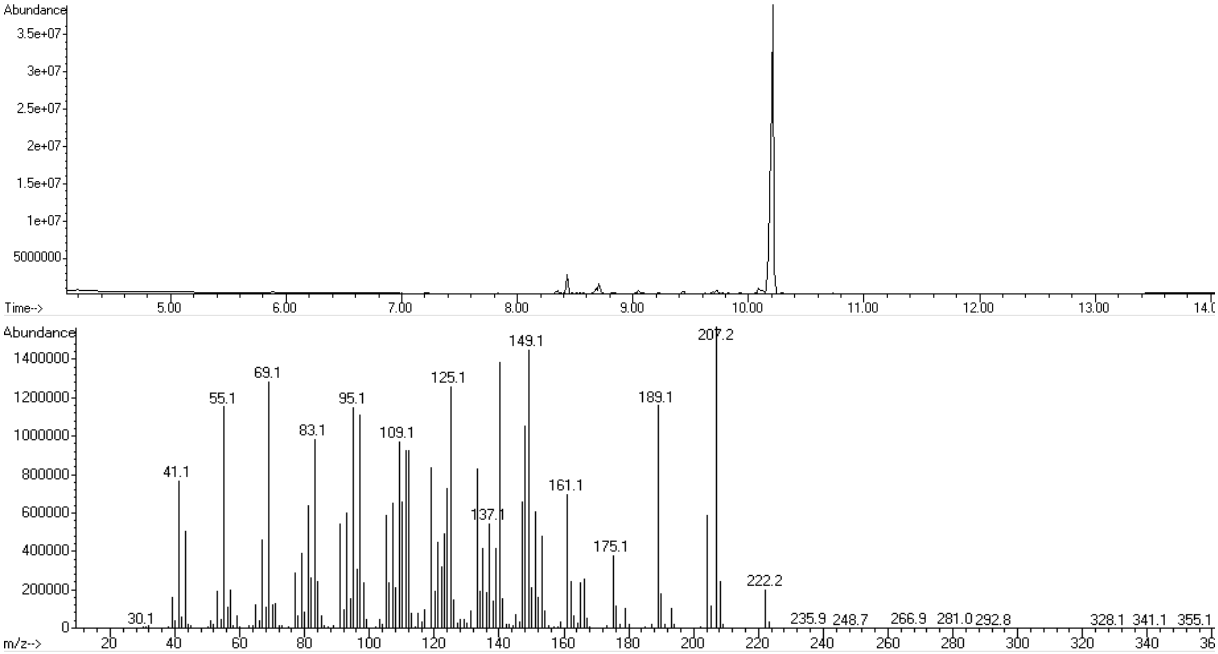
Remarks

- Chromatograms of the positive controls and MS data of all respective products formed are shown. In addition, GC-MS data of noteworthy biotransformations of unnatural substrates, as highlighted in the discussion section (p. 49), are presented.

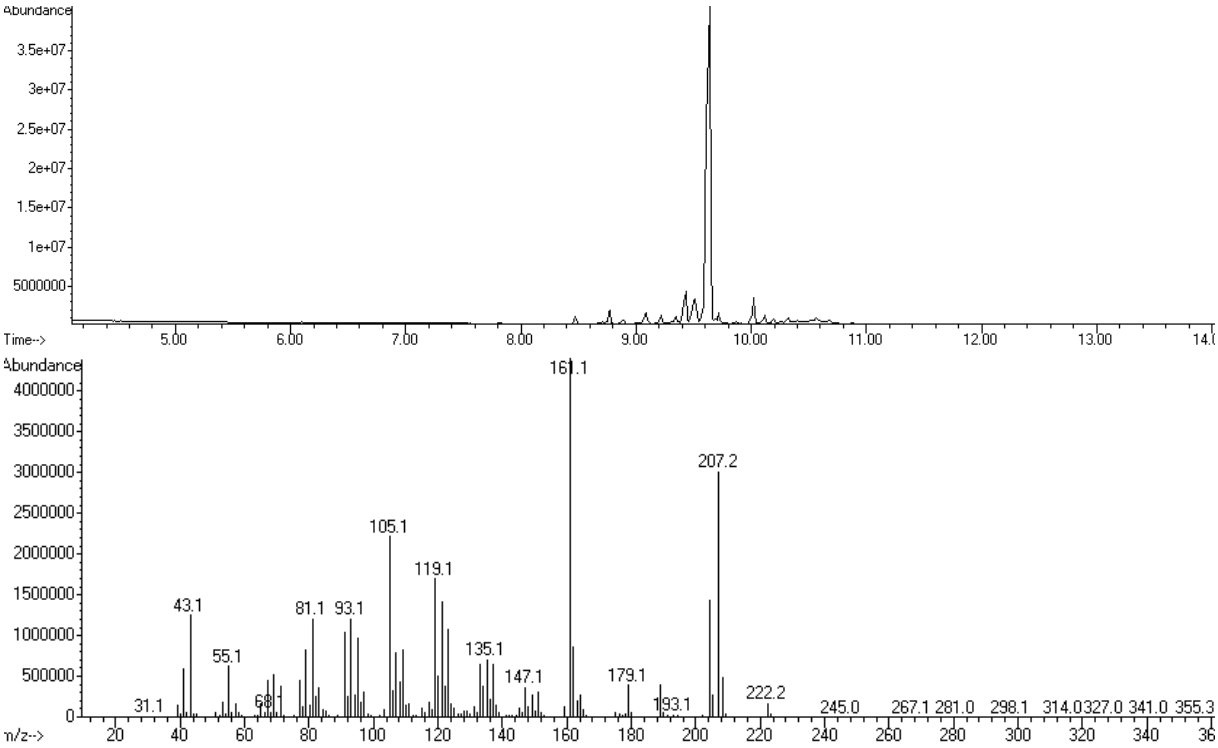
8.2.1 POSITIVE CONTROLS

Sought for all positive controls: m/z = 204, 222.

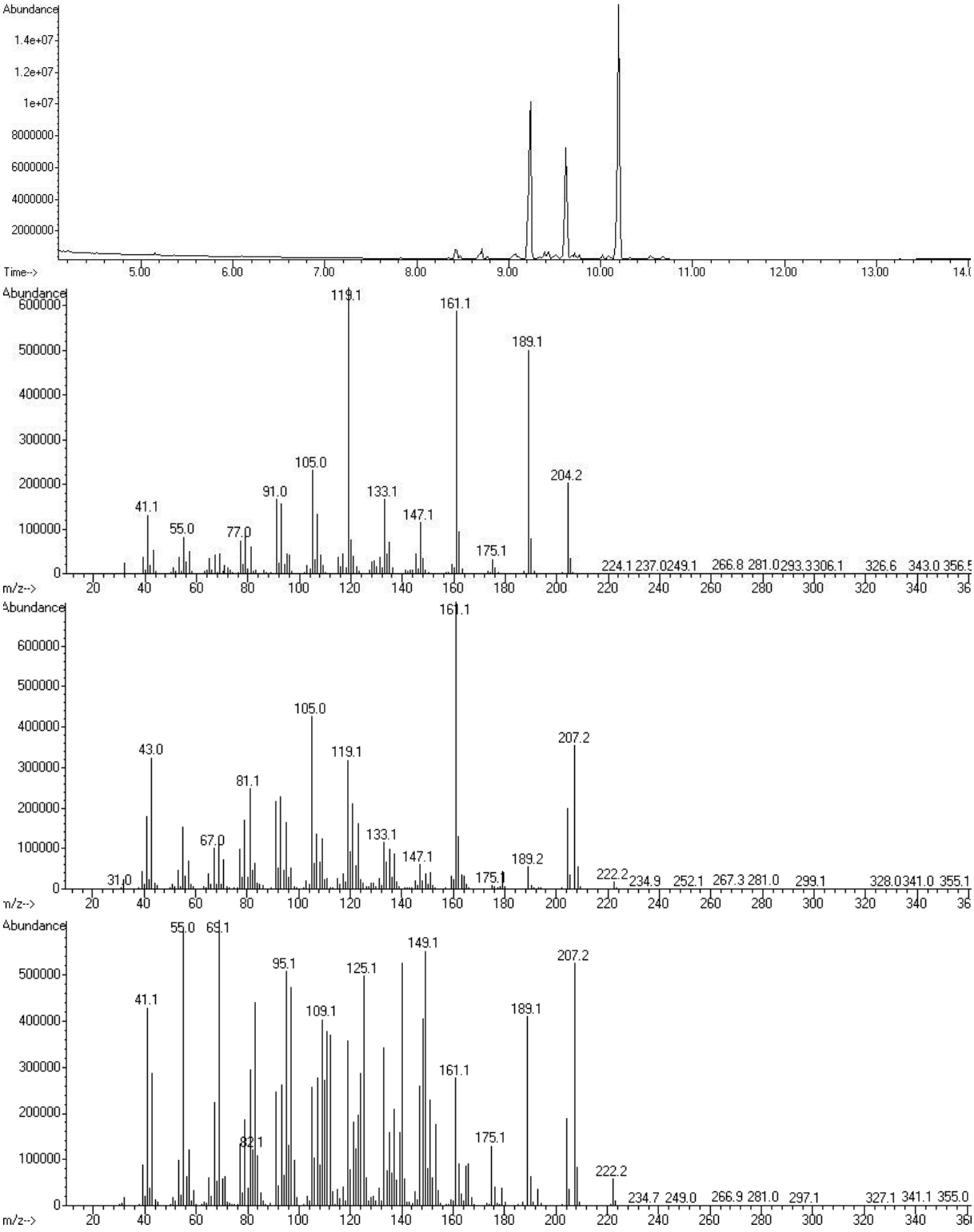
FPP (10) + BcBot2 – m/z at 10.21 min (presilphipherfolan-8-β-ol)



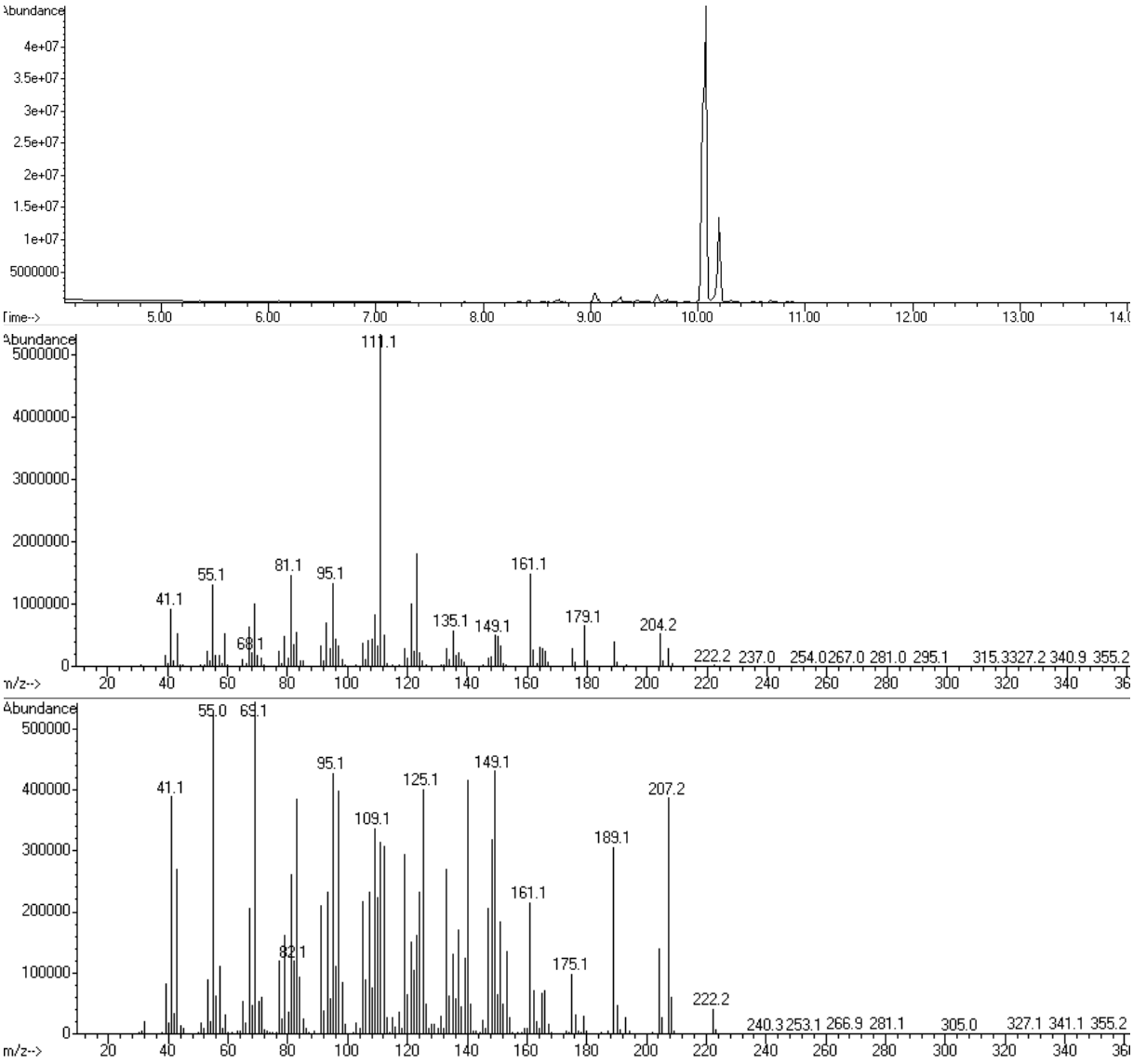
FPP (10) + Cop4 – m/z at 9.64 min (cubebol)



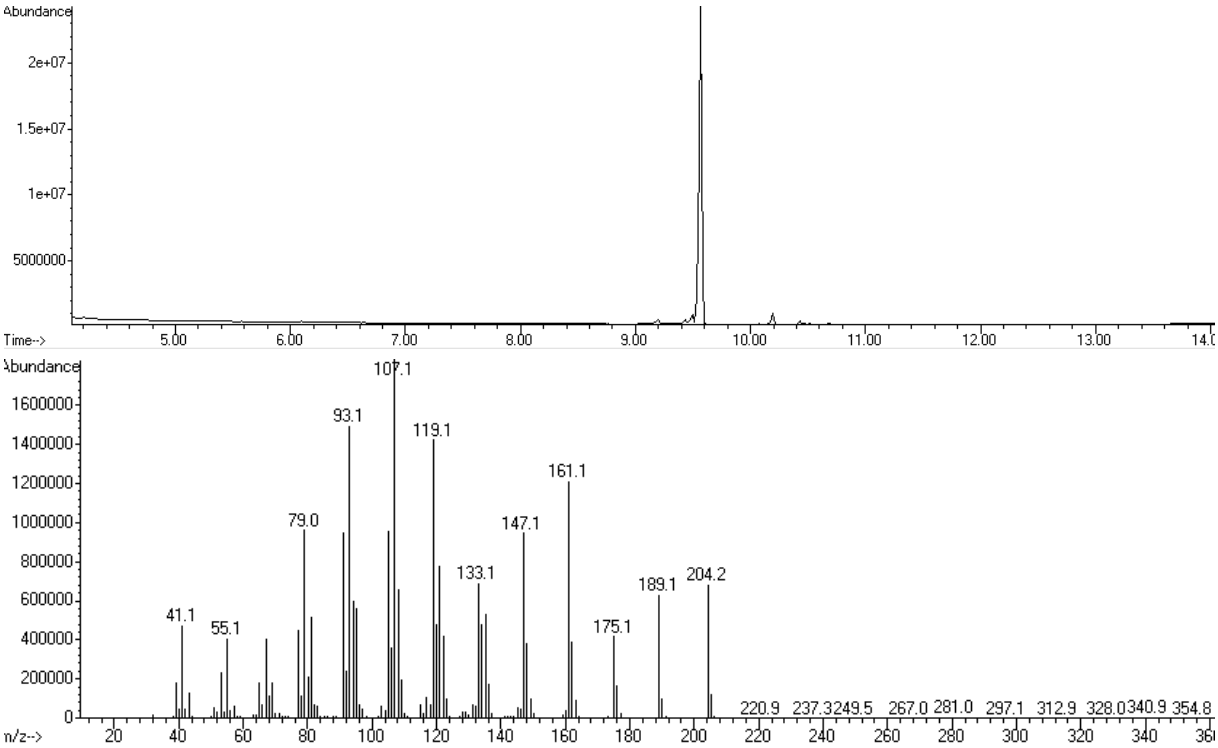
FPP (10) + Cyc1 – m/z at 9.24 (*epi*-isozizaene), 9.63 (cubebol), and 10.20 min (presilphipherfolan-8-β-ol)



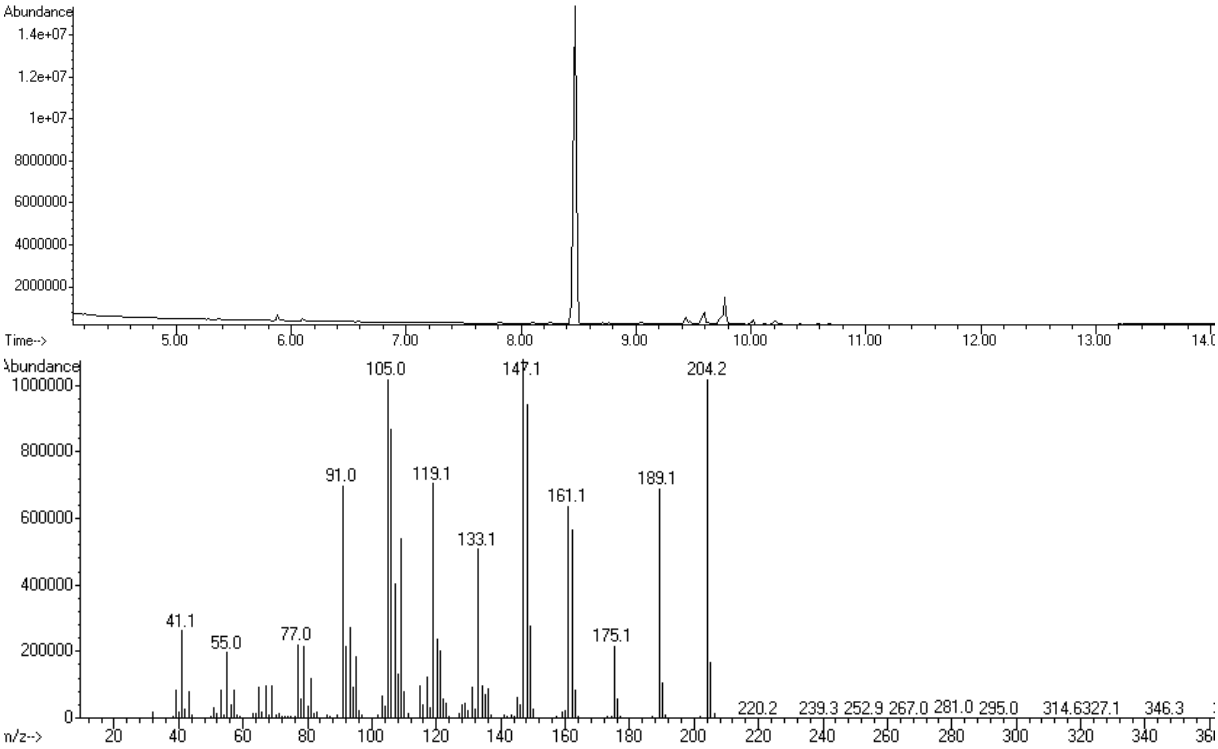
10 + GcoA– m/z at 10.08 [(+)-caryolan-1-ol] and 10.20 min (presilphipherfolan-8-β-ol)



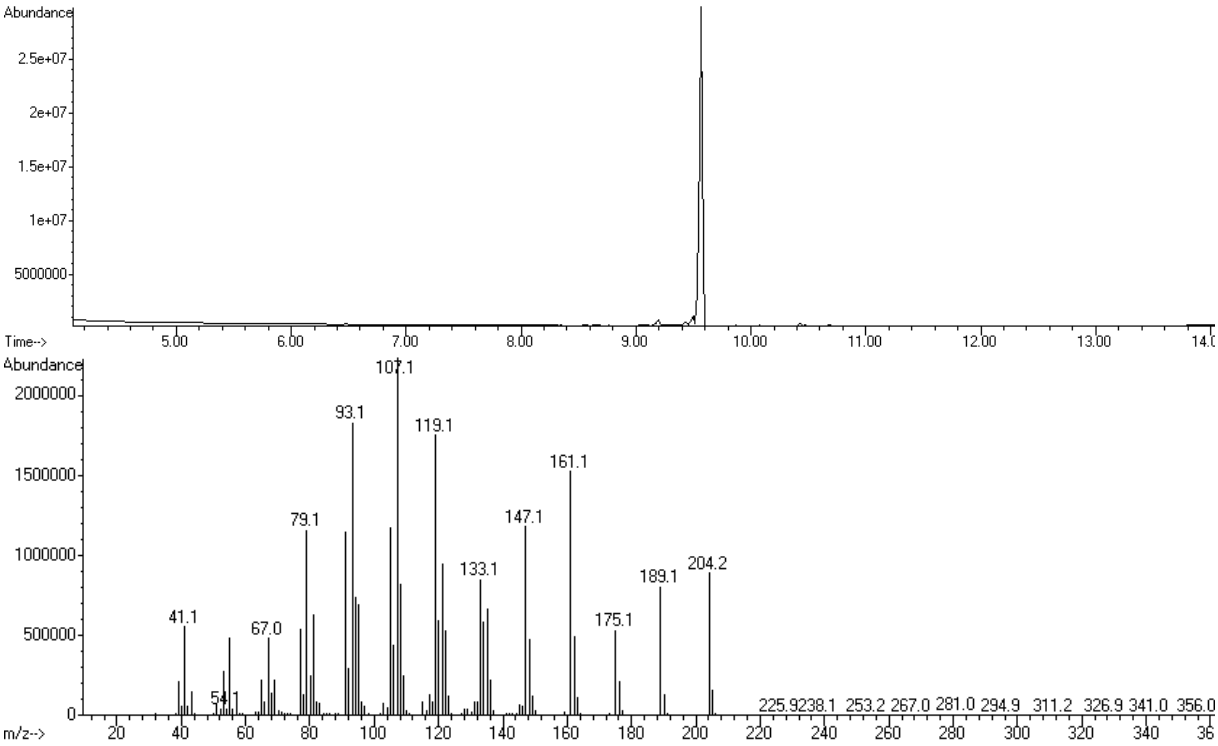
FPP (10) + Hvs1– m/z at 9.57 min (vetispiradiene)



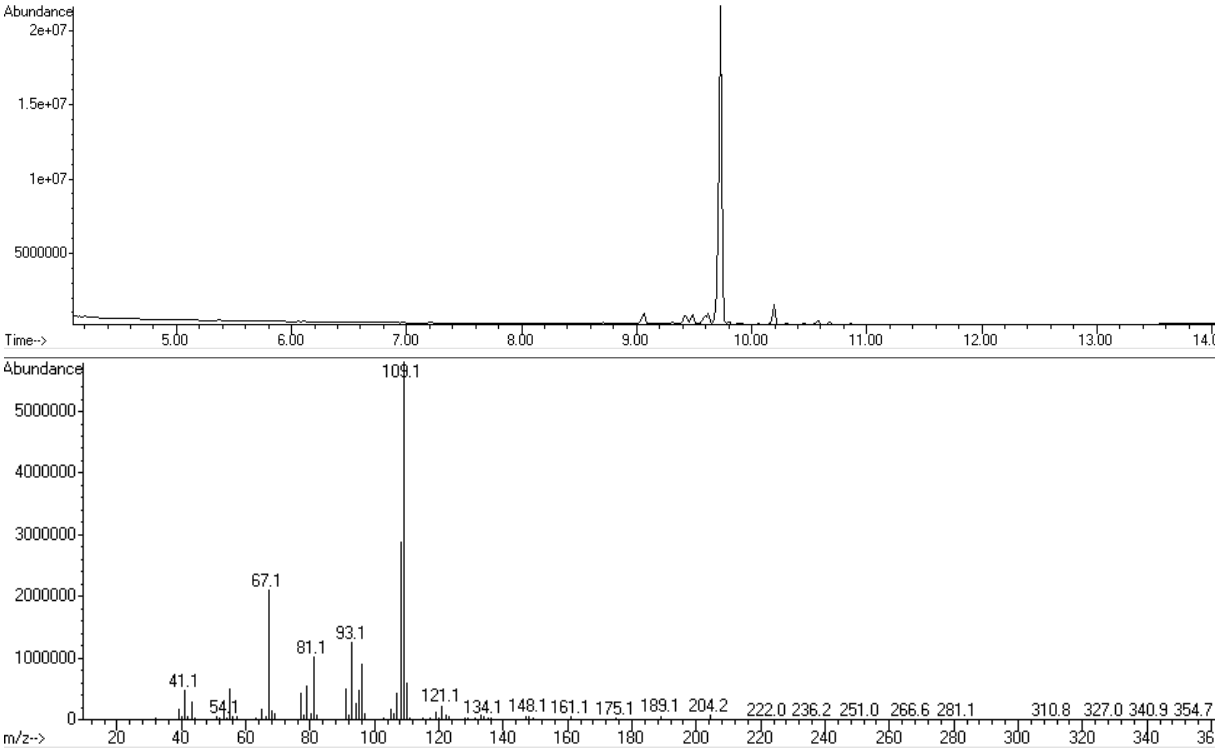
FPP (10) + PenA– m/z at 8.48 min (pentalenene)



FPP (10) + Tps32– m/z at 9.57 min (vetispiradiene)

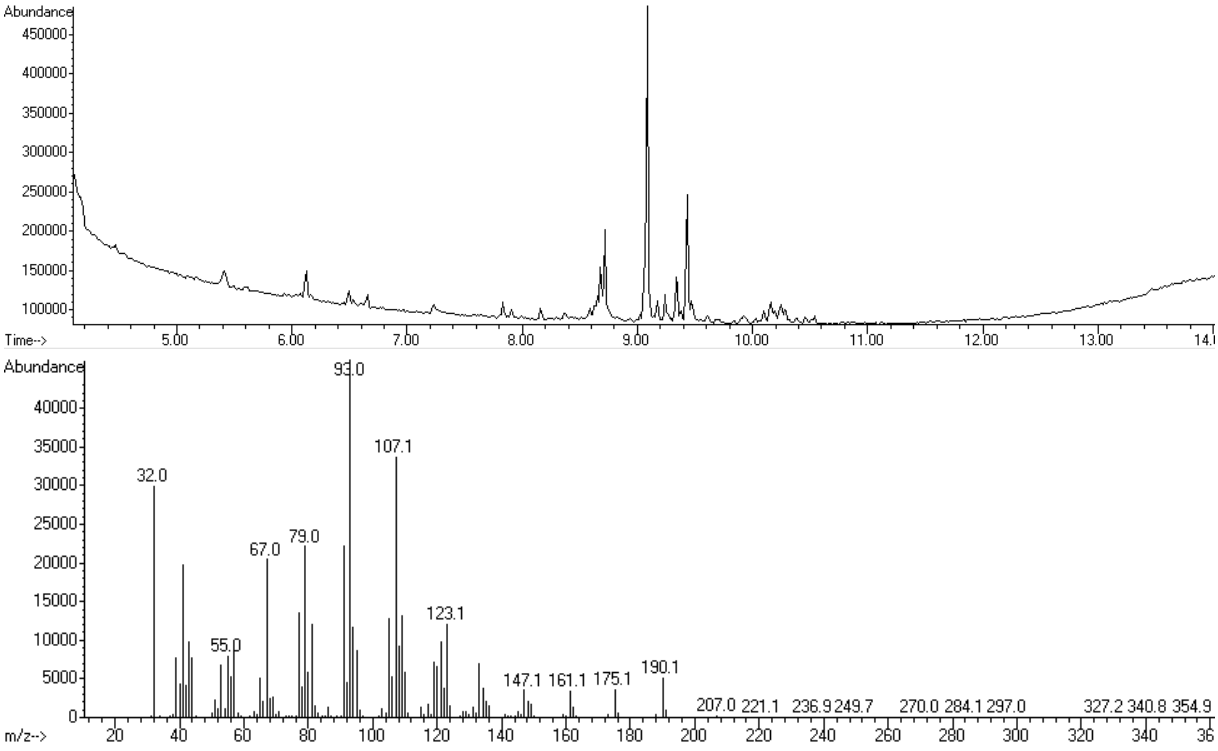


FPP (10) + Tri5 – m/z at 9.74 min (trichodiene)

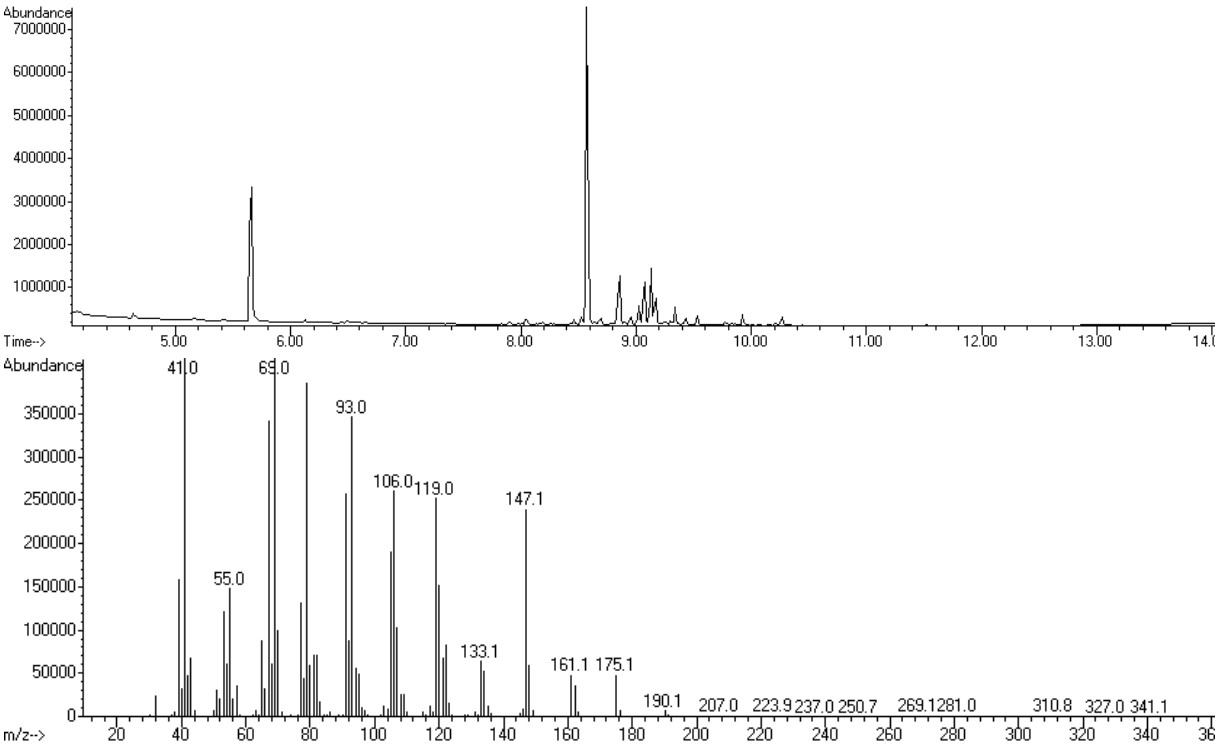


8.2.2 BIOTRANSFORMATIONS OF UNNATURAL DERIVATIVES

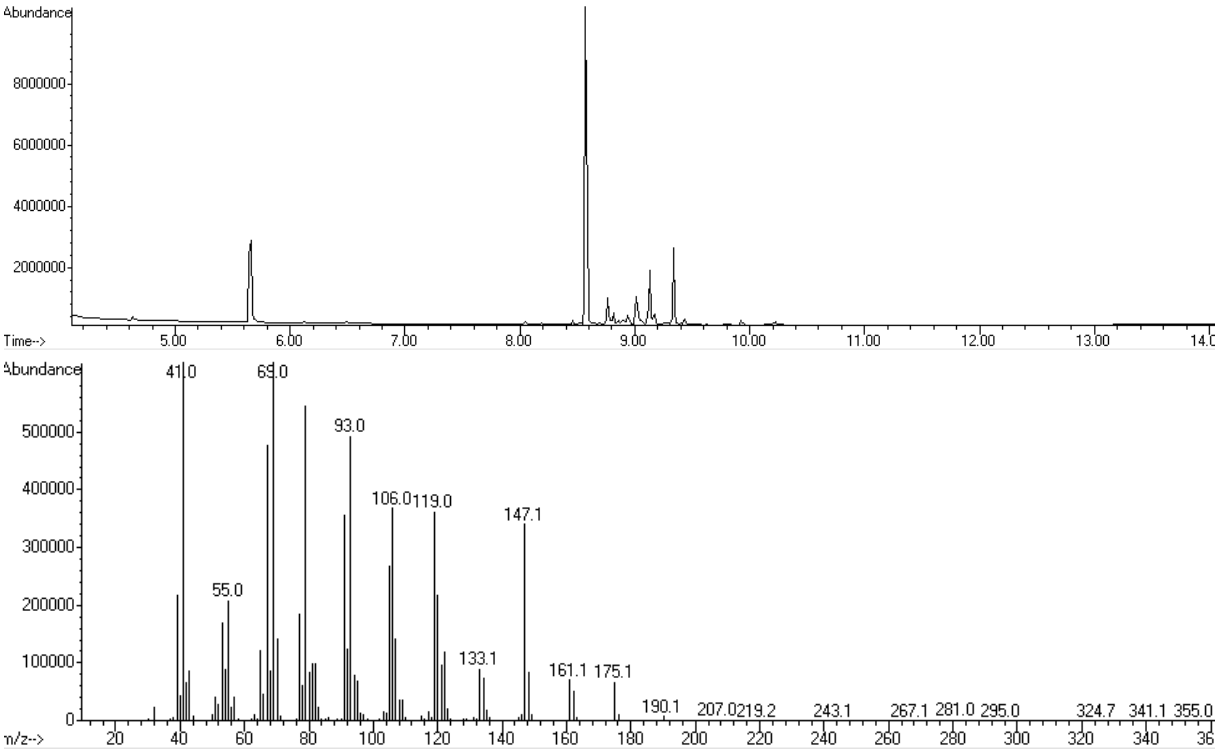
98 + BcBot2 – m/z at 9.10 min (sought: m/z = 190, 208)



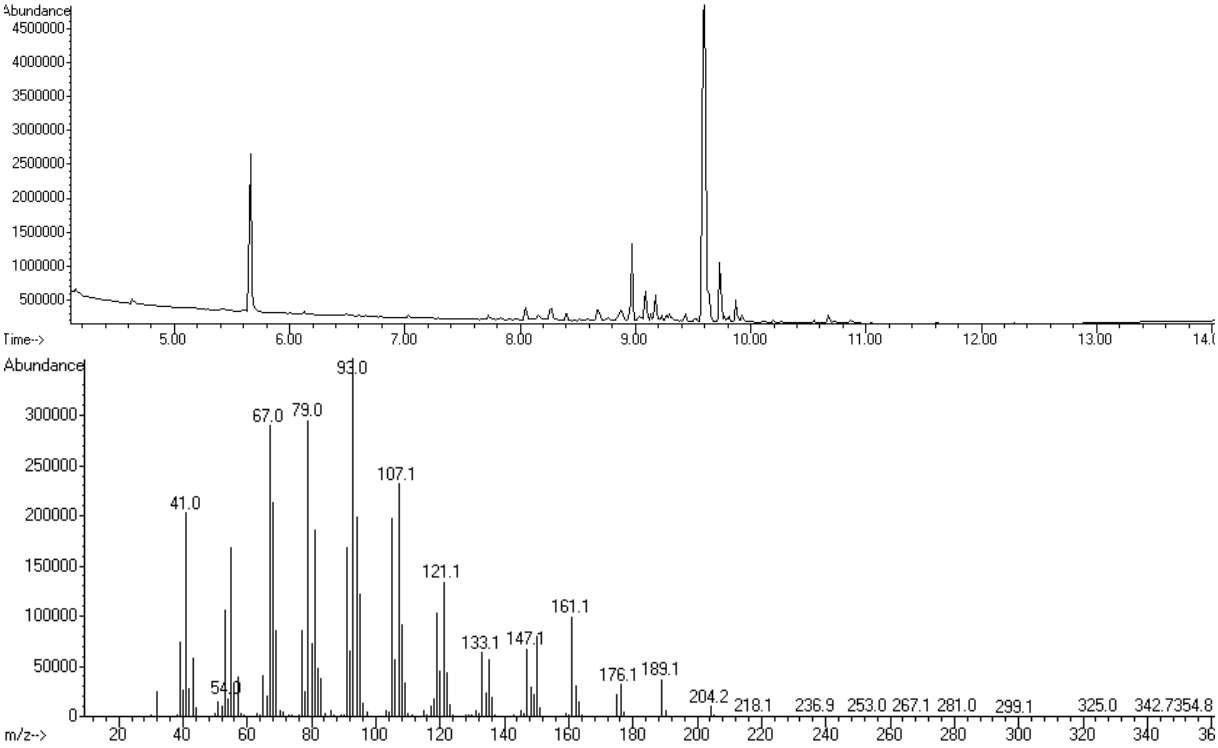
99 + Cyc1 – m/z at 8.58 min (sought: m/z = 190, 208)



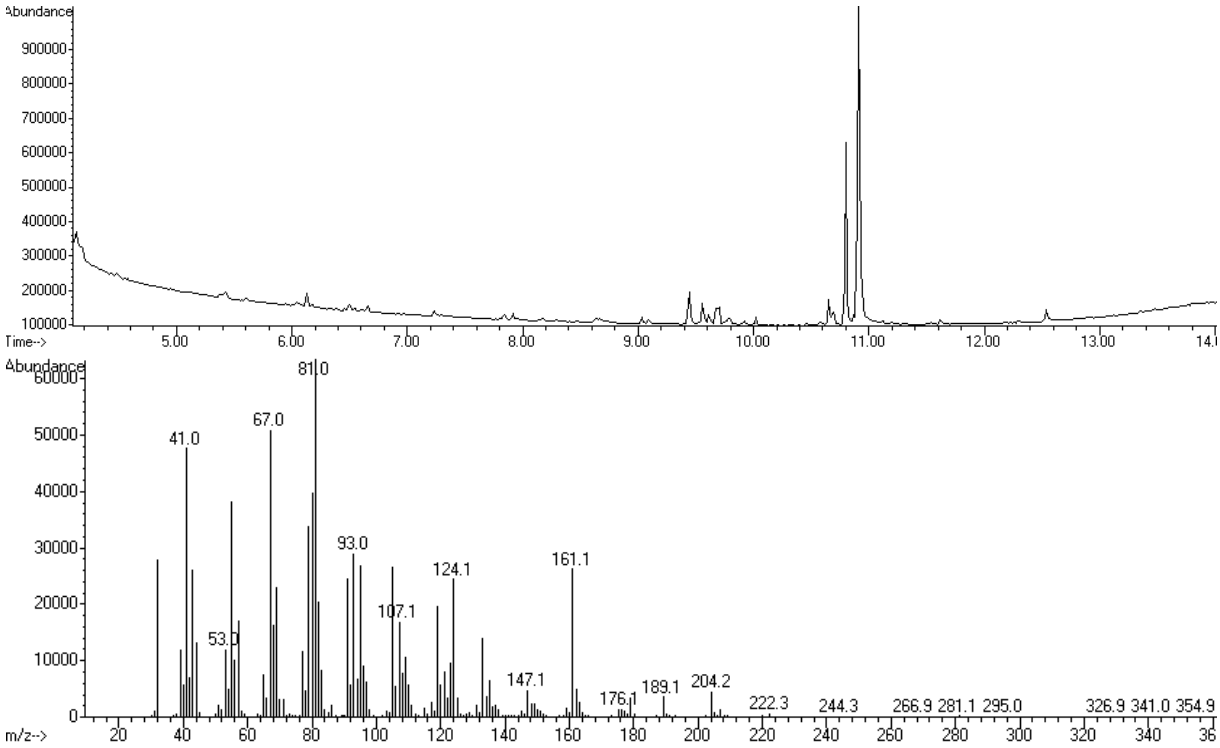
99 + Tri5 – m/z at 8.58 min (sought: m/z = 190, 208)



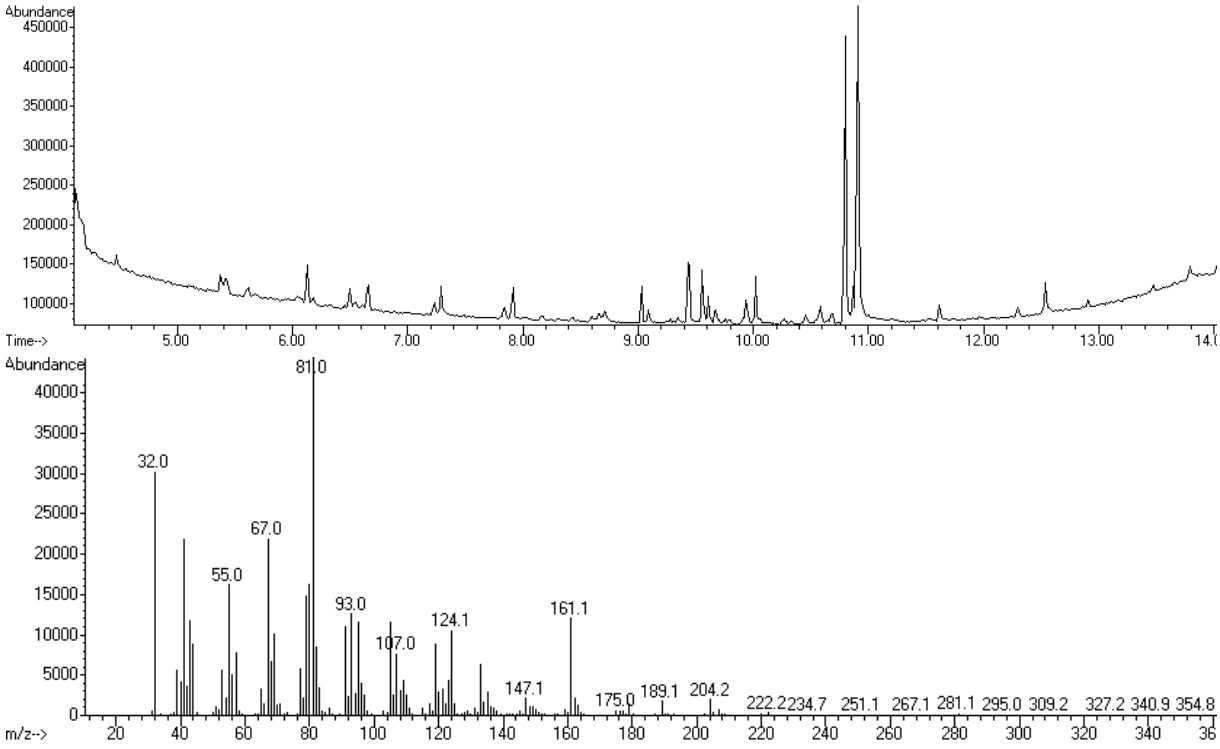
88 + Tps32 – m/z at 9.60 min (sought: m/z = 204, 222)



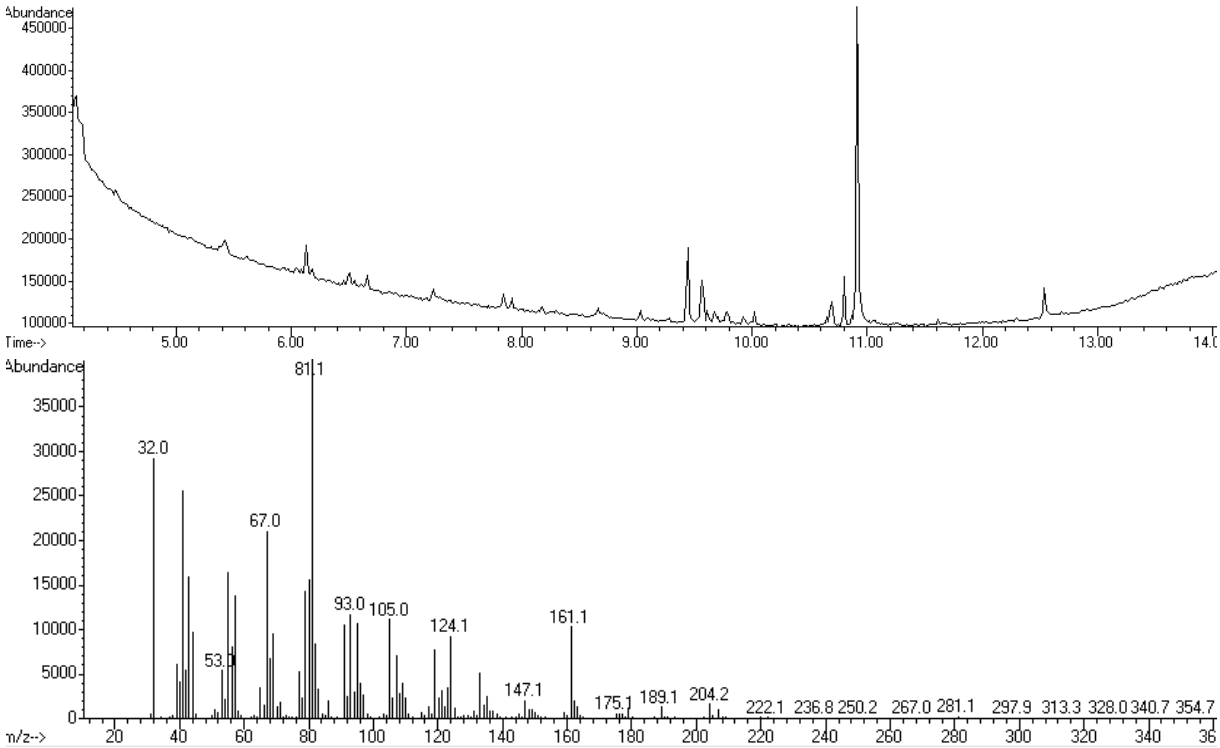
89S + BcBot2 – m/z at 10.92 min (sought: m/z = 204, 222)



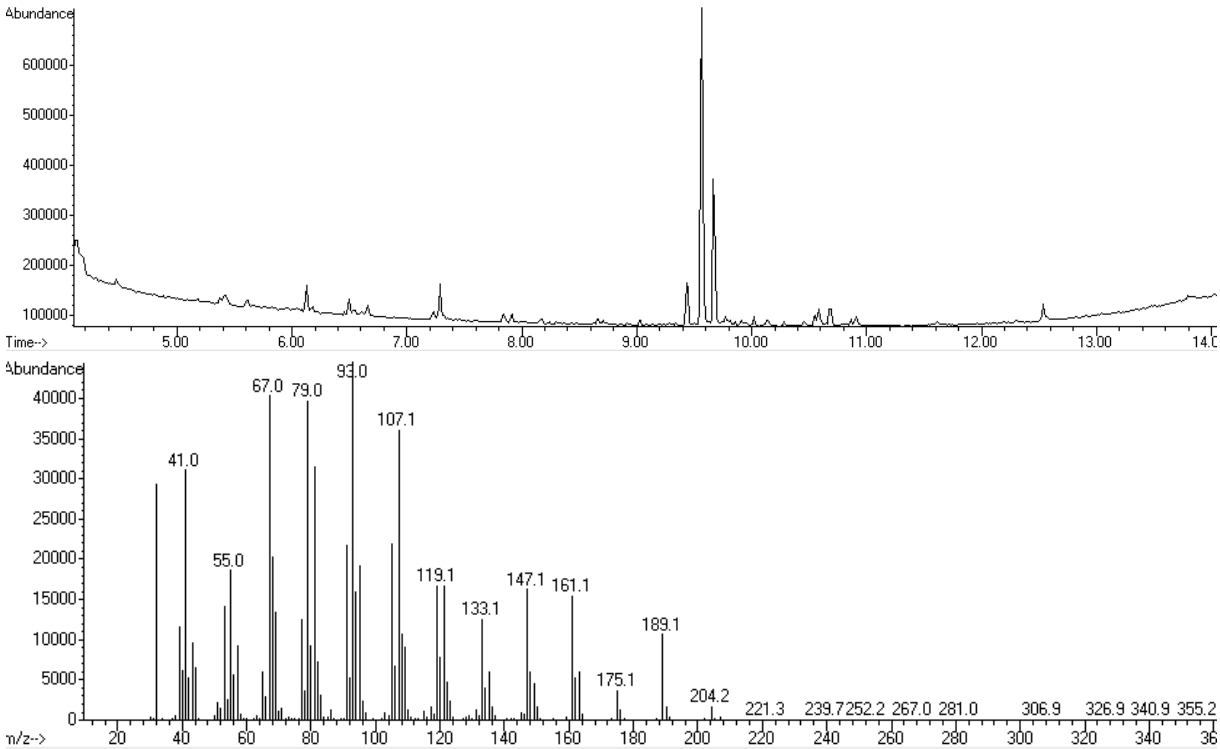
89S + GcoA – m/z at 10.91 min (sought: m/z = 204, 222)



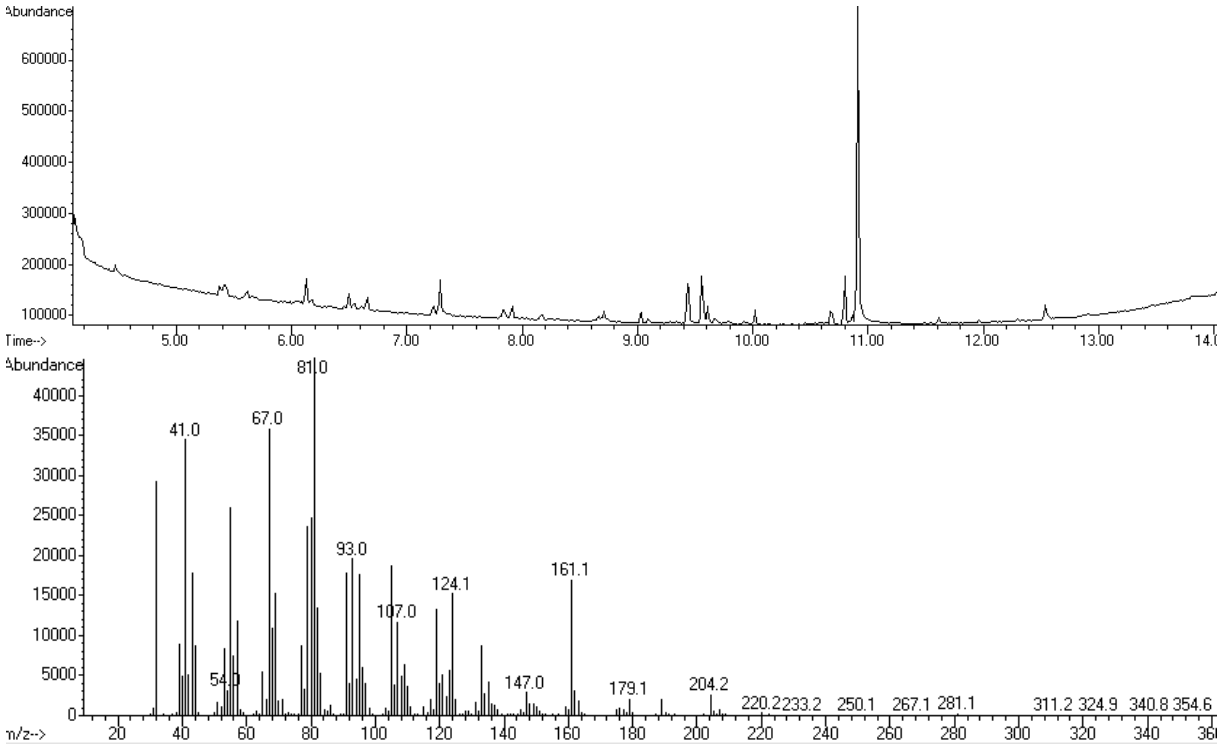
89R + BcBot2 – m/z at 10.92 min (sought: m/z = 204, 222)



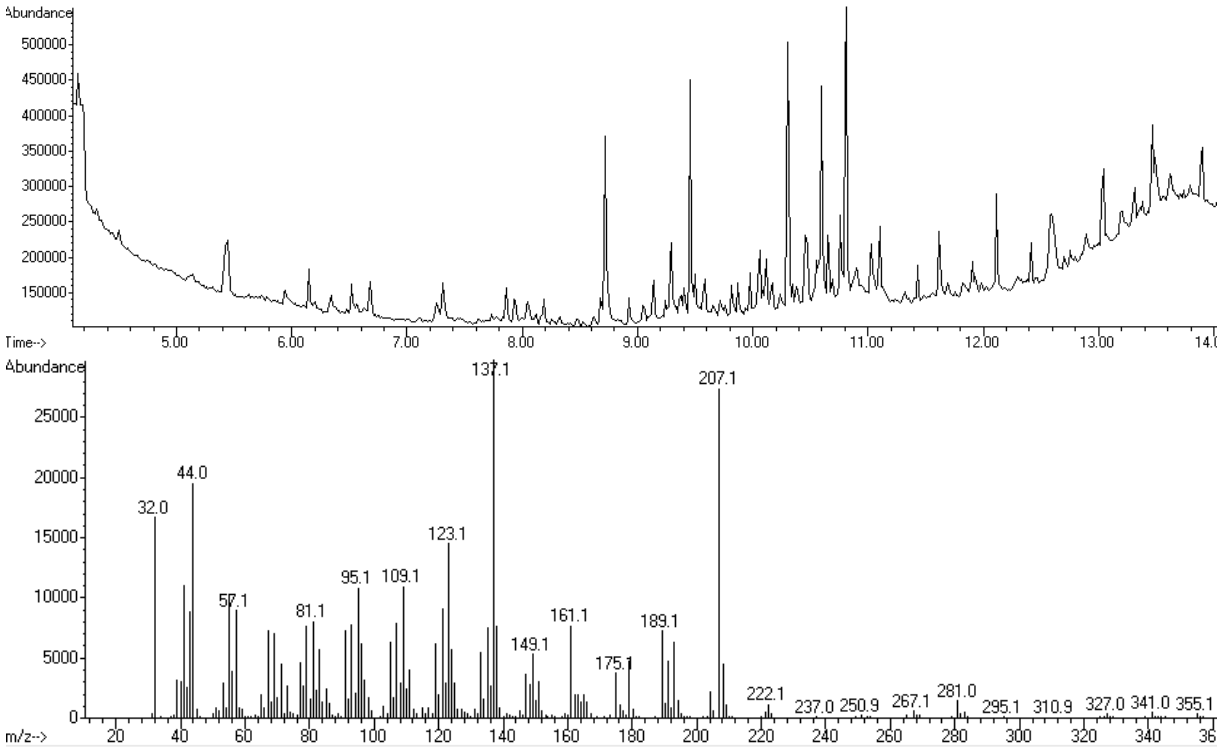
89R + Cop4 – m/z at 9.57 min (sought: m/z = 204, 222)



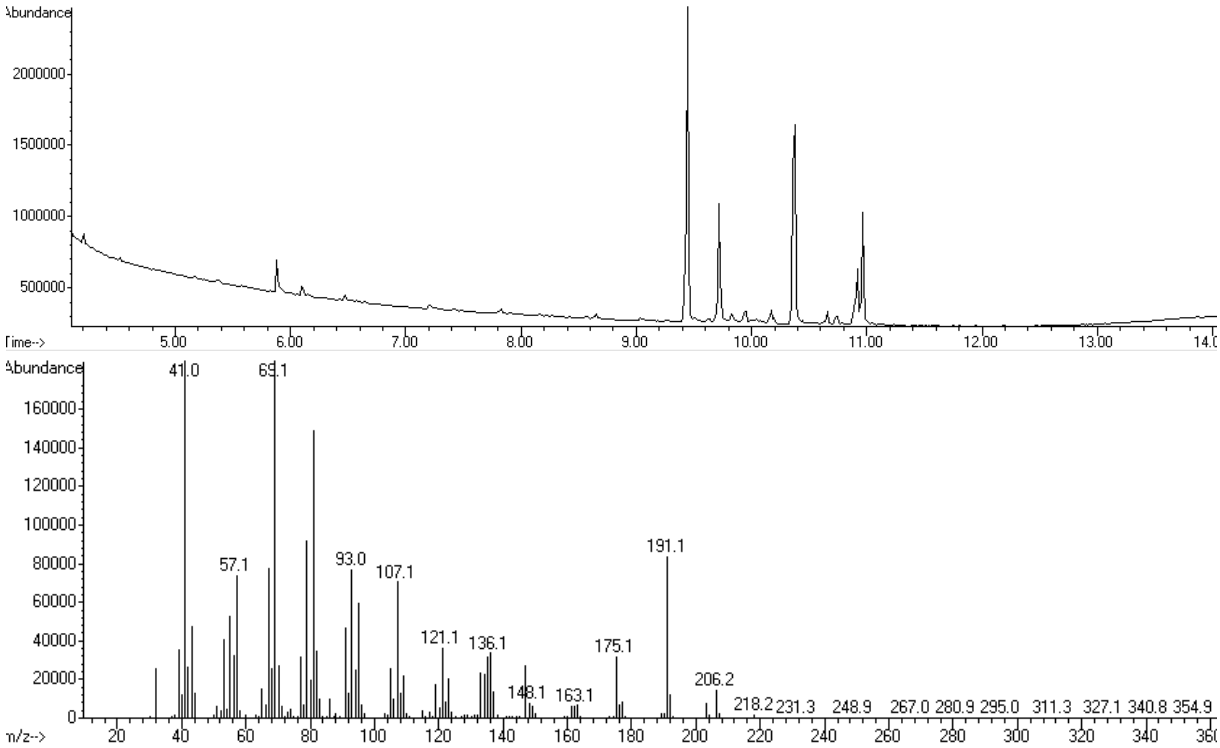
89R + GcoA – m/z at 10.92 min (sought: m/z = 204, 222)



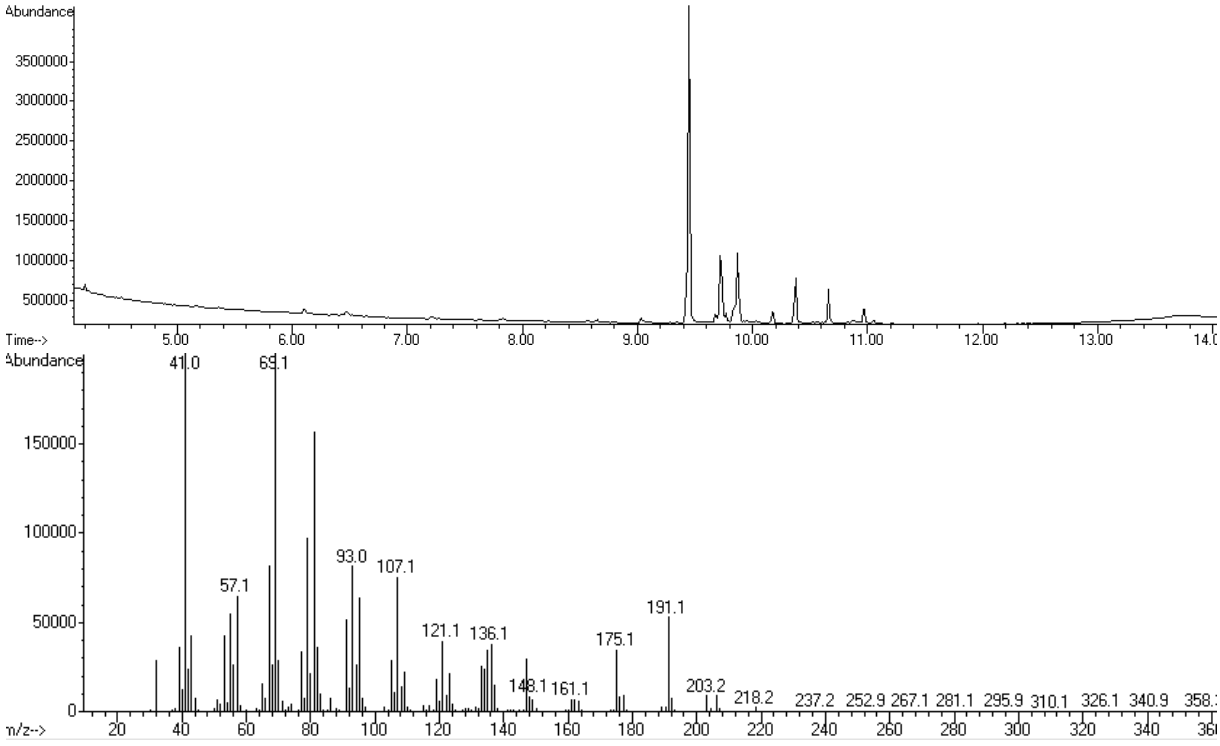
95 + BcBot2 – m/z at 10.31 min (sought: m/z = 204, 222)



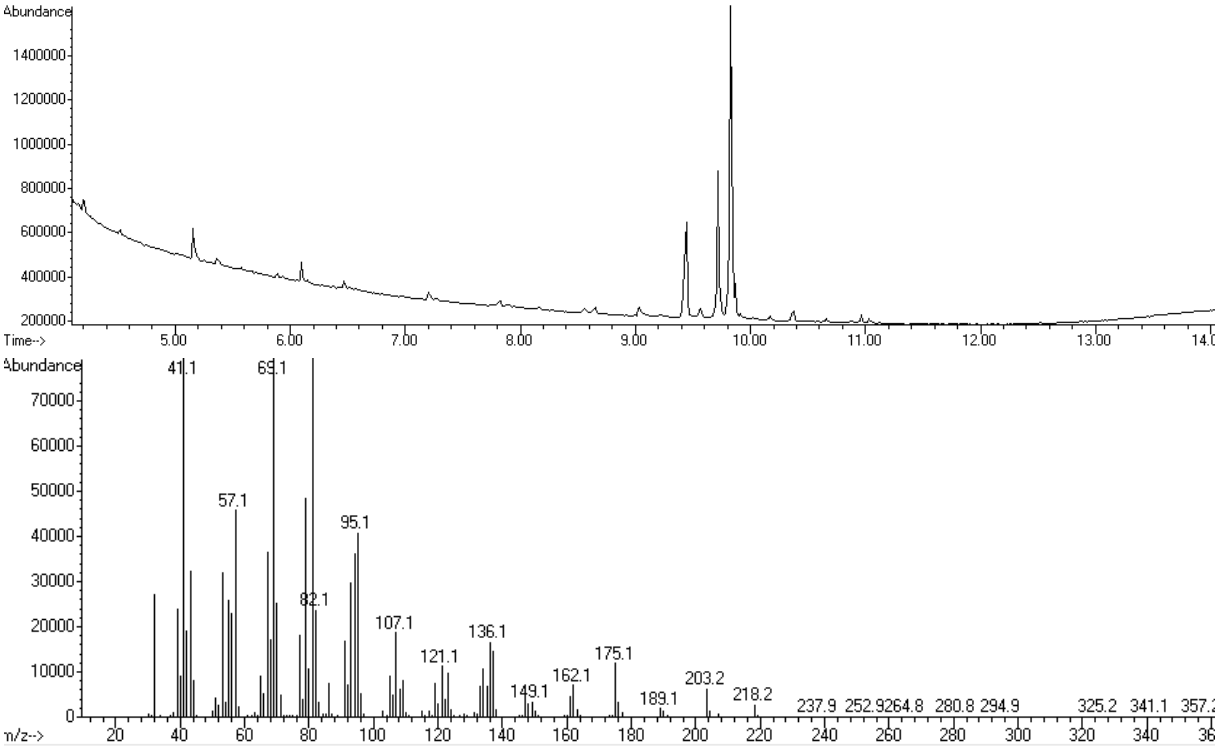
90S + BcBot2 – m/z at 9.45 min (sought: m/z = 218, 236)



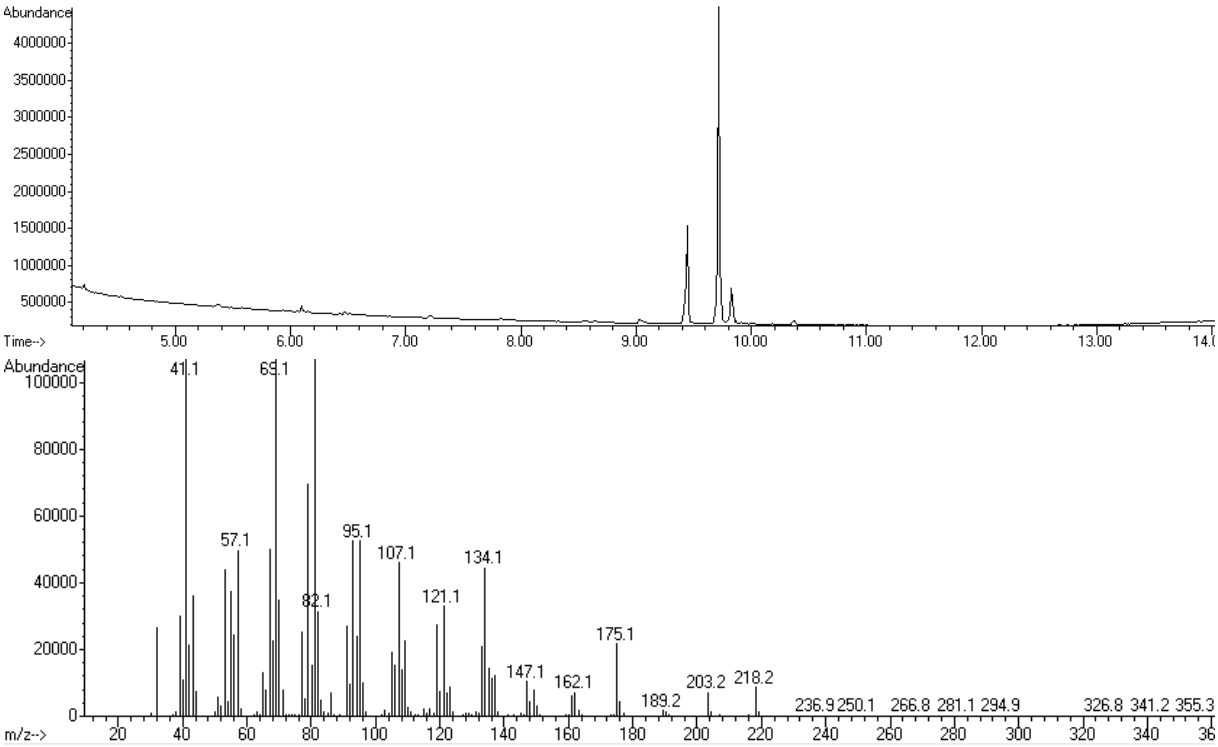
90S + Cop4 – m/z at 9.45 min (sought: m/z = 218, 236)



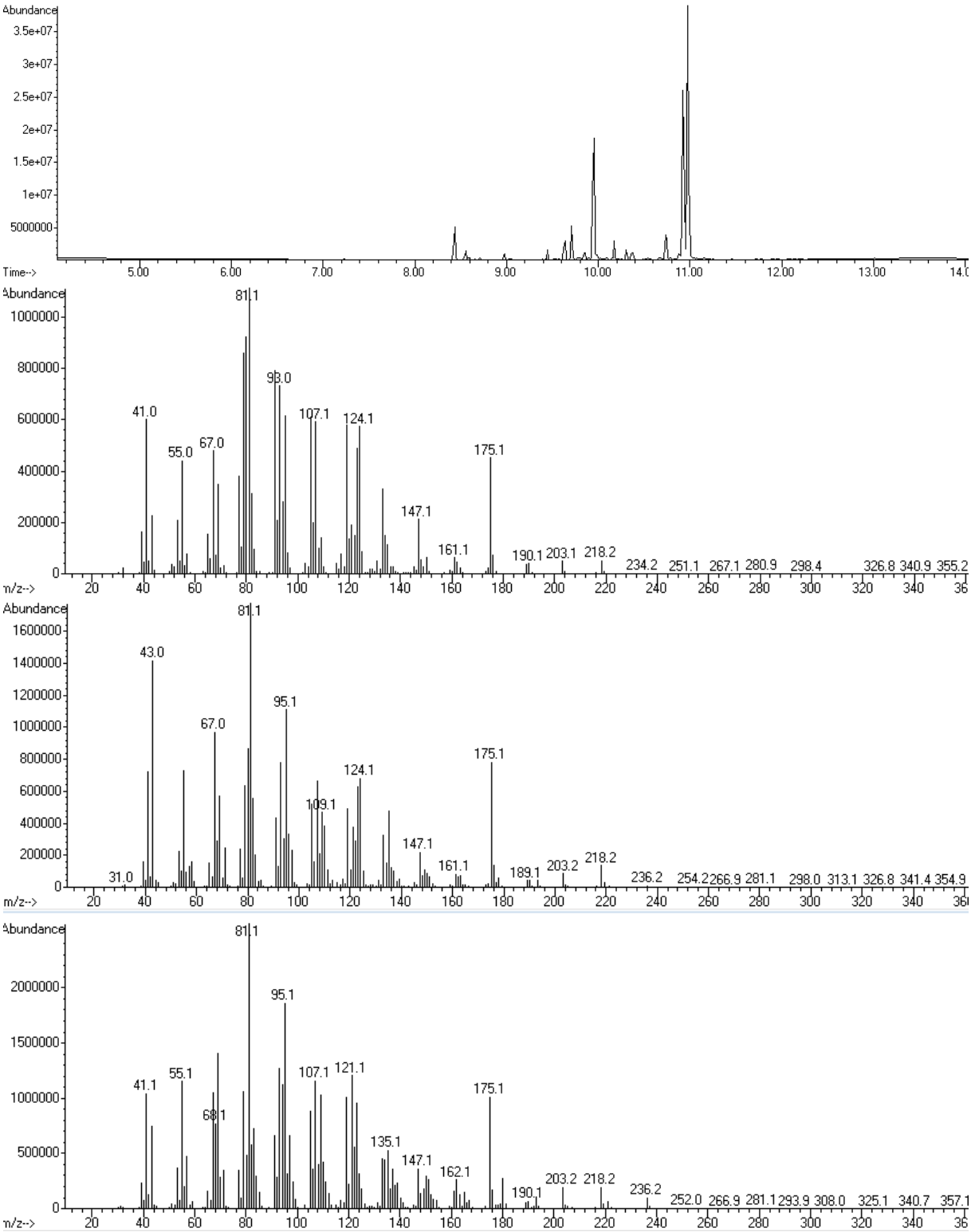
90S + Cyc1 – m/z at 9.84 min (sought: m/z = 218, 236)



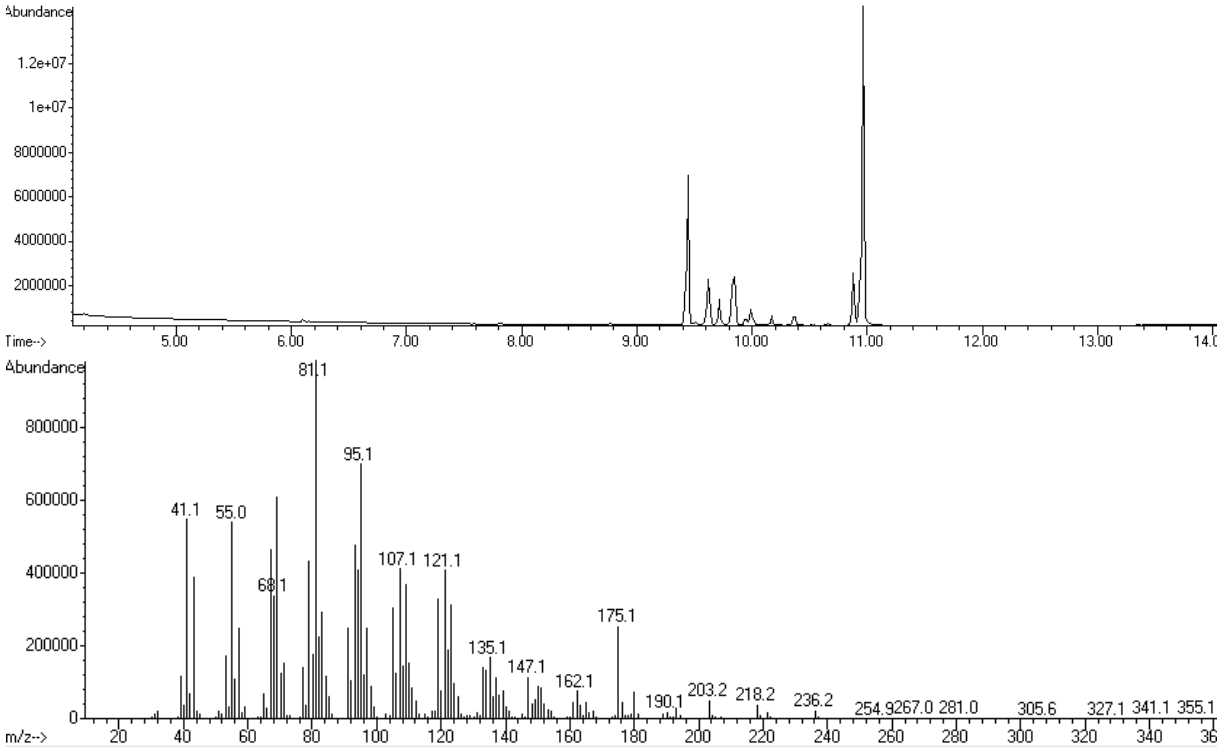
90S + Tri5 – m/z at 9.73 min (sought: m/z = 218, 236)



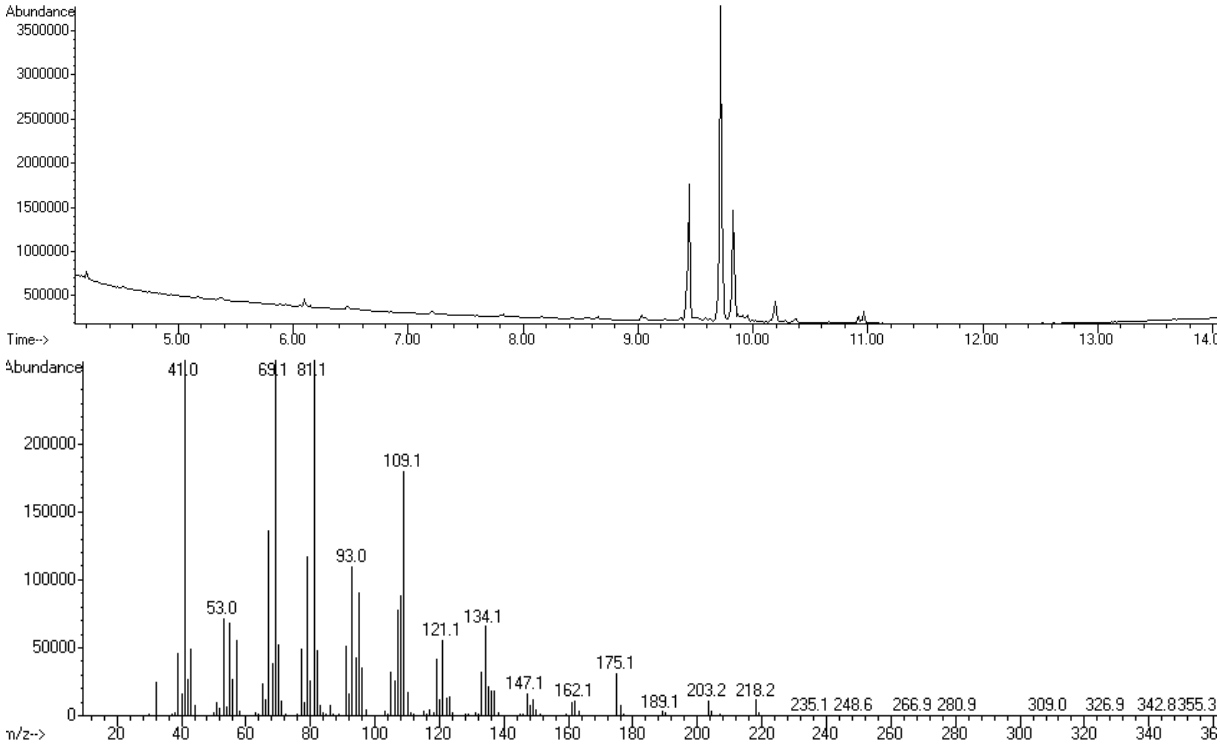
90R + BcBot2 – m/z at 9.95 (232), 10.93 (233/234), and 10.98 min (233/234); (sought: m/z = 218, 236)



90R + Cop4 – m/z at 10.97 min (233/234); (sought: m/z = 218, 236)



90R + Tri5 – m/z at 9.73 min (sought: m/z = 218, 236)



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