University of Alberta

Chemical Biology Studies of Prenyltransferase Enzymes

by

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for my Parents, Johannes and Rosario Scholte.

ABSTRACT

Two approaches were undertaken to study the chemistry of prenyltransferase enzymes. Three types of analogs of isopentenyl diphosphate (IPP) were prepared as potential inhibitors or substrates of prenyltransferase enzymes. First, a series of analogs of isopentenyl diphosphate (IPP) (**17-26**) having a dicarboxylate moiety in place of the diphosphate were synthesized and investigated as inhibitors of undecaprenyl diphosphate (UPP) synthase and protein farnesyltransferase (PFTase). PFTase is involved in control of cell proliferation and UPP synthase is a potential target for antimicrobial agents. Their syntheses involved the conjugate addition of an organocuprate to dimethyl acetylenedicarboxylate (DMAD) followed by basic ester hydrolysis. The *E*-pentenylbutanedioic acid, di-lithium salt (**26**) showed inhibition of UPP synthase with an IC_{50} of 135 μM. Compound **25** displays competitive inhibition of PFTase with a K_i of 287 μM. Furthermore, 2-(8-amino-octyl)-but-2-enedioic acid, di-lithium salt (**36**) was shown to be an effective ligand for the binding of PFTase. Three homologs of IPP were prepared as alternative substrates of rubber transferase, a *cis*-prenyltransferase that produces rubber in a variety of plant species. [^{13}C-4]-3-methylene-pentan-1-yl diphosphate (**112**), 3-methylene-4-pentenyl diphosphate (**28**), and 3-methylene-5-hexenyl diphosphate (**29**) were prepared by the palladium catalyzed coupling of an organometallic reagent with 2-(3-iodo-but-3-enyloxy) tetrahydropyran (**109**). Incorporation studies of **112** with rubber transferase indicated that it was not accepted as an alternative substrate of rubber transferase. To investigate the cryptic stereochemistry of rubber transferase, a series of six deuterium-labeled analogs of IPP (**30-35**) were prepared. Incorporation

studies with rubber transferase and subsequent ^{2}H-NMR analysis indicate that the stereochemistry of rubber transferase is similar to that of other related *cis*-prenyltransferases including UPP synthase. Thus, the pro-*S* hydrogen (H_S) is preferentially removed during the polymerization, and *si* face addition to the allylic diphosphate occurs with overall inversion of stereochemistry.

A variety of analytical techniques including ^{13}C-NMR was used to evaluate alternative crops for the production of natural rubber in North America. Two lettuce species, *Lactuca serriola* and *L. sativa* were shown to be excellent candidates for commercialization of natural rubber production. The rubber isolated from both species have been shown to consist of high molecular weight *cis*-polyisoprene, which is similar to that produced by *Hevea brasiliensis*. In addition, the rubber particles, where rubber is compartmentalized, have an average diameter of 6 μm.

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$[\alpha]_D^{26}$	specific rotation
A or Ala	alanine
Ac	acetyl
APP	allylic diphosphate
APT	attached proton test
aq.	aqueous
Ar	aryl
ARS	Agriculture Research Service
Atm	atmosphere
BME	β-mercaptoethanol
Bn	benzyl
bp	boiling point
br	broad
BSA	bovine serum albumin
c	concentration
C or Cys	cysteine
calcd	calculated
Ci	Currie
CoA	coenzyme A
COSY	correlation spectroscopy
CTP	cytosine triphosphate

δ	chemical shift in parts per million downfield from tetramethylsilane
d	doublet
D or Asp	aspartic acid
Da	Dalton
DAP	diaminopimelate
DCC	1,3-dicyclohexylcarbodiimide
DMAD	dimethyl acetylenedicarboxylate
DMAP	4-(dimethylamino)pyridine
DMAPP	dimethylallyl diphosphate
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DTT	dithiothreitol
DW	dry weight
E or Glu	glutamic acid
EDTA	ethylenediaminetetraacetic acid
ee	enantiomeric excess
EI	electron impact
Enz	enzyme
eq	equivalent
ES	electrospray
EST	expressed sequence tag
Et	ethyl

F or Phe	phenylalanine
FPP	farnesyl diphosphate
G or Gly	glycine
GC	gas chromatography
GGPP	geranylgeranyl diphosphate
GPP	geranyl diphosphate
H or His	histidine
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
HMPA	hexamethylphosphoramide
HMQC	heteronuclear multiple quantum coherence
HPLC	high performance liquid chromatography
HRMS	high-resolution mass spectrum
IC_{50}	concentration causing 50% inhibition
IPP	isopentenyl diphosphate
I or Ile	isoleucine
IBX	2-iodoxybenzoic acid
IPA	isopropyl alcohol
IPTG	isopropyl-β-D-thiogalactopyranoside
IR	infrared
J	coupling constant
K or Lys	lysine
K_i	dissociation constant for binding of inhibitor to enzyme
L or Leu	leucine

LC	liquid chromatography
lit.	literature reference
m	multiplet
M or Met	methionine
m/z	mass to charge ratio
MALDI-TOF	matrix-assisted laser desorption / ionization time-of-flight
MAS	magic angle spinning
m-CPBA	*m*-chloroperoxybenzoic acid
Me	methyl
MEP	methylerythritol 4-phosphate
MHz	megahertz
min	minute(s)
M_N	number average molecular weight
mp	melting point
MS	mass spectrometry
MVA	mevalonic acid
MW	molecular weight
M_W	weight average molecular weight
N or Asn	asparagine
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
NaOH	sodium hydroxide
Na_2SO_4	sodium sulfate
NBS	*N*-bromosuccinimide

NMO	4-methylmorpholine *N*-oxide
NMR	nuclear magnetic resonance
nm	nanometer(s)
NOE	nuclear Overhauser effect
OPEC	Organization of the Petroleum Exporting Countries
P or Pro	proline
PAGE	polyacrylamide gel electrophoresis
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
PFTase	protein farnesyltransferase
Ph	phenyl
P_i	phosphate
PP_i	pyrophosphate
PPh_3	triphenylphosphine
ppm	parts per million
PPTS	pyridinium *p*-toluene sulfonate
PTSA	*p*-toluene sulfonic acid
pyr	pyridine
q	quartet
Q or Gln	glutamine
quant.	quantitative yield
qn	quintet
R or Arg	arginine

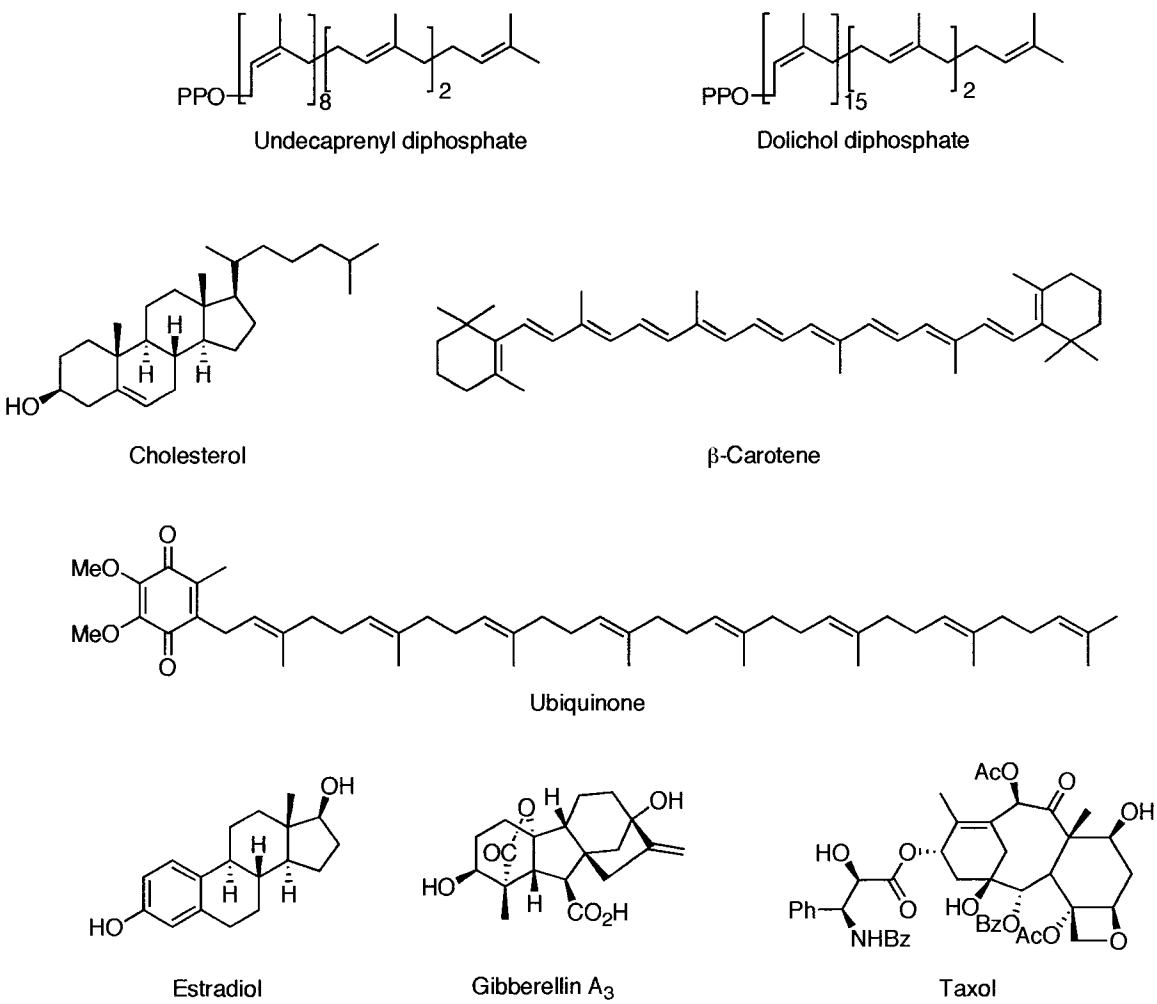
R_f	retention factor
RNA	ribonucleic acid
rt	room temperature
S or Ser	serine
SAM	*S*-adenosylmethionine
SDS	sodium dodecyl sulfate
SEM	scanning electron microscopy
t	triplet
T or Thr	threonine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
THP	tetrahydropyran
TLC	thin layer chromatography
t_R	retention time
TPAP	tetra-*n*-propylammonium perruthenate
Tris	tris-(hydroxymethyl)aminomethane
Ts	*p*-toluenesulfonyl
USDA	United States Department of Agriculture
UPP	undecaprenyl diphosphate
V or Val	valine
W or Trp	tryptophan
WRP	washed rubber particles
Y or Tyr	tyrosine

CHAPTER 1. Introduction: Biosynthesis of Isoprenoids

1.1 INTRODUCTION

Isoprenoids constitute a large family of natural products that encompass more than 36,000 structurally diverse compounds.[1] They are found in all forms of life and their carbon backbone is derived from the branched C_5 skeleton of isoprene.[2] Isoprenoids are known to demonstrate a variety of functions. For example they are involved in the mediation of cell-wall and glycoprotein biosynthesis (undecaprenol and dolichol); electron transport and redox chemistry (ubiquinones); photooxidative protection and photosynthetic light harvesting (carotenoids); assembly of lipid membrane structure (cholesterol in eukaryotes, and archaebacterial lipids); modification of proteins involved in signal transduction (prenylated proteins) to intercellular signaling; and developmental control (estrogens, gibberellins) (Figure 1).[3] However, the functions of the vast majority of isoprenoids remain unknown. Isoprenoids are also exploited as medicinally active molecules, and examples include gingkolides,[4] which act as a cardiovascular agent, and taxoids[5] (Taxol), which act as potent anti-tumor agents.

Figure 1. Structures of important isoprenoids



1.1.1 Isoprenoids

The chemistry of isoprenoids is characterized by a rich history and was initiated in 1887 by Wallach, who made the key discovery that isoprenoids are composed of repeating units of isoprene (C_5).[6] He suggested that head to tail condensations of the branched C_5 unit of isoprene was at the origin of all terpenoids. However, it was

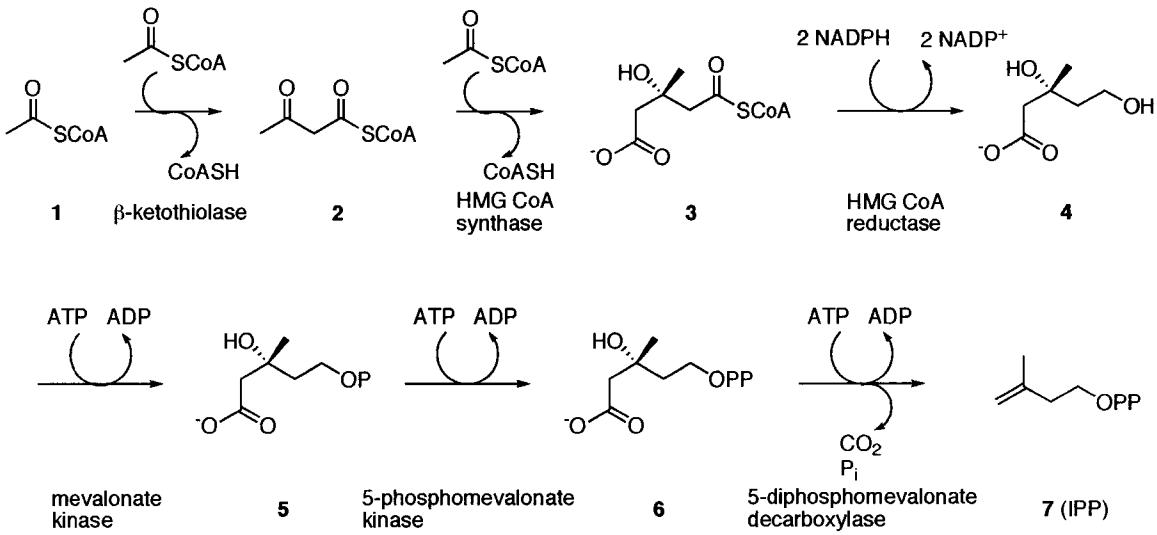
subsequently shown that many isoprenoids do not follow this general rule, and as a result, Ruzicka proposed an extended biogenetic isoprene rule in 1953.[7,8] Ruzicka proposed that the carbon skeleton of terpenes can be deduced from reactions of aliphatic substances such as geraniol, farnesol, geranylgeraniol, and squalene.[9] At the time, the rule was consistent with the known structures, and even predicted all those, which were later discovered.[10] In 1958, the research groups of Lynen[11] and Bloch[12] independently discovered that isopentenyl diphosphate (IPP) was the biologically active isoprene unit.

1.1.2 Biosynthesis of IPP

The elucidation of the biosynthesis of cholesterol in 1957 led to the discovery of the mevalonic acid pathway (MVA), which was accepted as the unique biosynthetic pathway to isoprenoids in all living organisms.[11,12] Recent research, however has proven that an alternative biosynthetic pathway for the formation of IPP exists in many eubacteria, algae, and in the plastids of higher plants.[13] On the MVA pathway (Scheme 1), acetyl-CoA **1** undergoes two consecutive Claisen-type condensations, catalyzed by HMG-CoA synthase, to produce 3-hydroxy-3-methylglutaryl-CoA (**3**, HMG-CoA), which is reduced to mevalonate **4** by HMG-CoA reductase. Mevalonate is the key intermediate in this biosynthetic pathway and no other biological role for mevalonate is currently known.[10] The reaction catalyzed by HMG-CoA reductase, is the first committed step of this pathway, and is a key point in the regulation of isoprenoid biosynthesis, including the formation of a variety of sterols. The primary hydroxyl group is first phosphorylated by mevalonate kinase to give the intermediate mevalonate phosphate **5**,

which undergoes another phosphorylation yielding mevalonate diphosphate **6**. The final step of the mevalonate pathway involves the transformation of mevalonate diphosphate to IPP **7**, by diphosphomevalonate decarboxylase. In an ATP-dependent process, this enzyme catalyzes the phosphorylation of the tertiary alcohol of mevalonate diphosphate as well as the decarboxylation of the resultant β-hydroxy acid.

Scheme 1. Mevalonic acid (MVA) pathway for IPP biosynthesis



Although, the MVA pathway was unanimously accepted as the only pathway for the formation of IPP, certain experimental results in plants and bacteria could not be explained. For example, poor incorporation rates of ^{14}C labeled mevalonate into plant chloroplast isoprenoids (carotenoids) or into monoterpenes were observed, while normal incorporation rates were observed for sterol biosynthesis.[14,15] Other experiments showed that mevinolin, a known inhibitor of HMG-CoA reductase, inhibited the formation of sterols in plant cells, but had no effect on the biosynthesis of carotenoids.[16] Initially, these

odd results were explained on the basis of poor absorption of mevalonate and mevinolin through the chloroplast membrane. Furthermore, cytoplasm-independent IPP biosynthesis was discovered in plant chloroplasts and chromoplasts.[17] In 1993, incorporation studies on the biosynthesis of triterpenoids in bacteria allowed Rohmer and coworkers to identify an alternative pathway for IPP biosynthesis.[18]

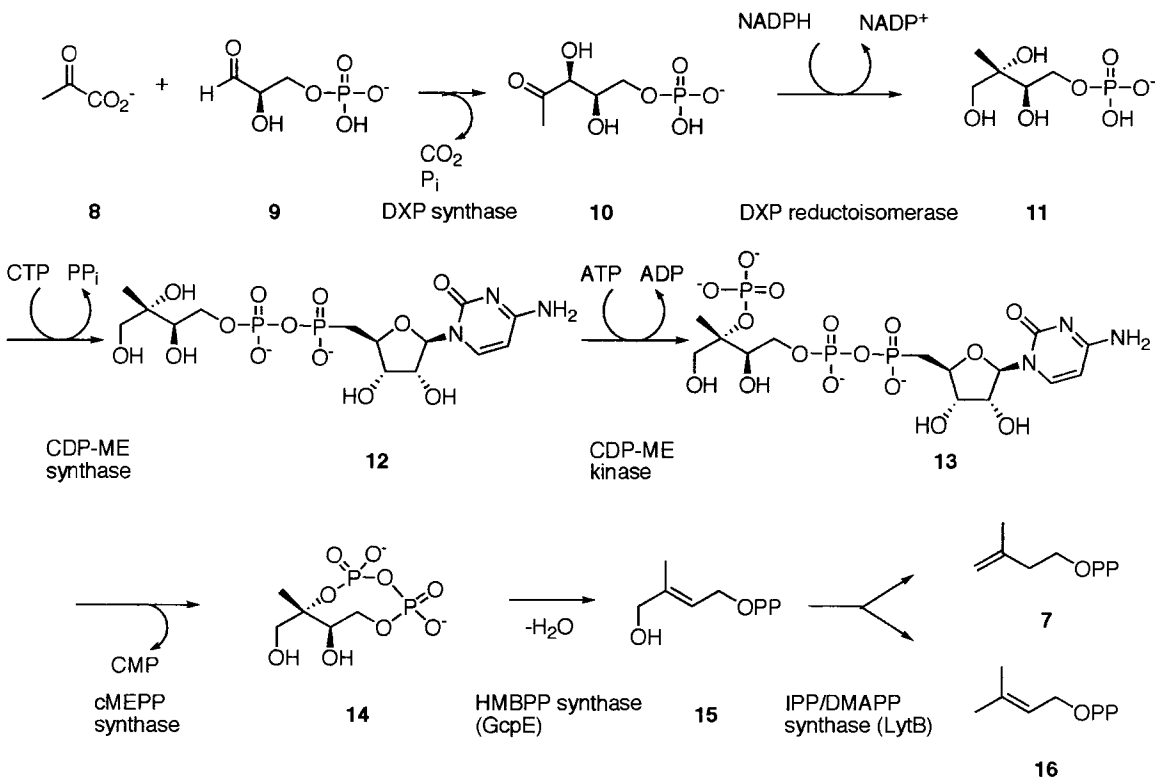
1.1.3 Methylerythritol 4-phosphate (MEP) Pathway

This alternative metabolic route to IPP, designated as the methylerythritol 4-phosphate (MEP) pathway (Scheme 2), operates in eubacteria, algae, and higher plants.[13,18-21] The MVA and MEP pathways can readily be differentiated by the labeling patterns that result from the incorporation of ^{13}C-labeled glucose. At present, the MEP pathway is well understood, although the mechanisms of the last two steps of the pathway remain to be fully elucidated.[22]

The first step of the MEP pathway involves the condensation of pyruvate **8** with glyceraldehyde phosphate **9** to give 1-deoxy-D-xylulose-5-phosphate **10** (Scheme 2). The pentulose intermediate **10** then undergoes rearrangement to the corresponding aldehyde, which is then reduced through an NADPH-dependent process to yield 2-*C*-methyl-D-erythritol 4-phosphate **11** (MEP). Further inspection of this intermediate indicates that the branched skeleton of IPP is largely in place. Reaction of the terminal phosphate group with cytosine triphosphate (CTP) gives the 4-diphosphocytidyl methylerythritol **12**.

Subsequently, the tertiary hydroxyl group of the nucleotide adduct **12** is phosphorylated to give **13** which undergoes cyclization to form methylerythritol 2,4-cyclodiphosphate **14.**

Scheme 2. Methylerythritol 4-phosphate (MEP) pathway for IPP biosynthesis



The last two steps, which involve the transformation of 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate **14** into IPP **7** and dimethylallyl diphosphate (**16**, DMAPP), are still under investigation. From studies in bacteria, GcpE is known to catalyze the reduction of ME 2,4-cyclodiphosphate **14** into (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate (**15**) (HMBPP) by electron transfer, and LytB converts HMBPP into both IPP **7** and DMAPP **16**. Continued research will undoubtedly resolve the remaining questions about this alternative pathway for IPP biosynthesis.

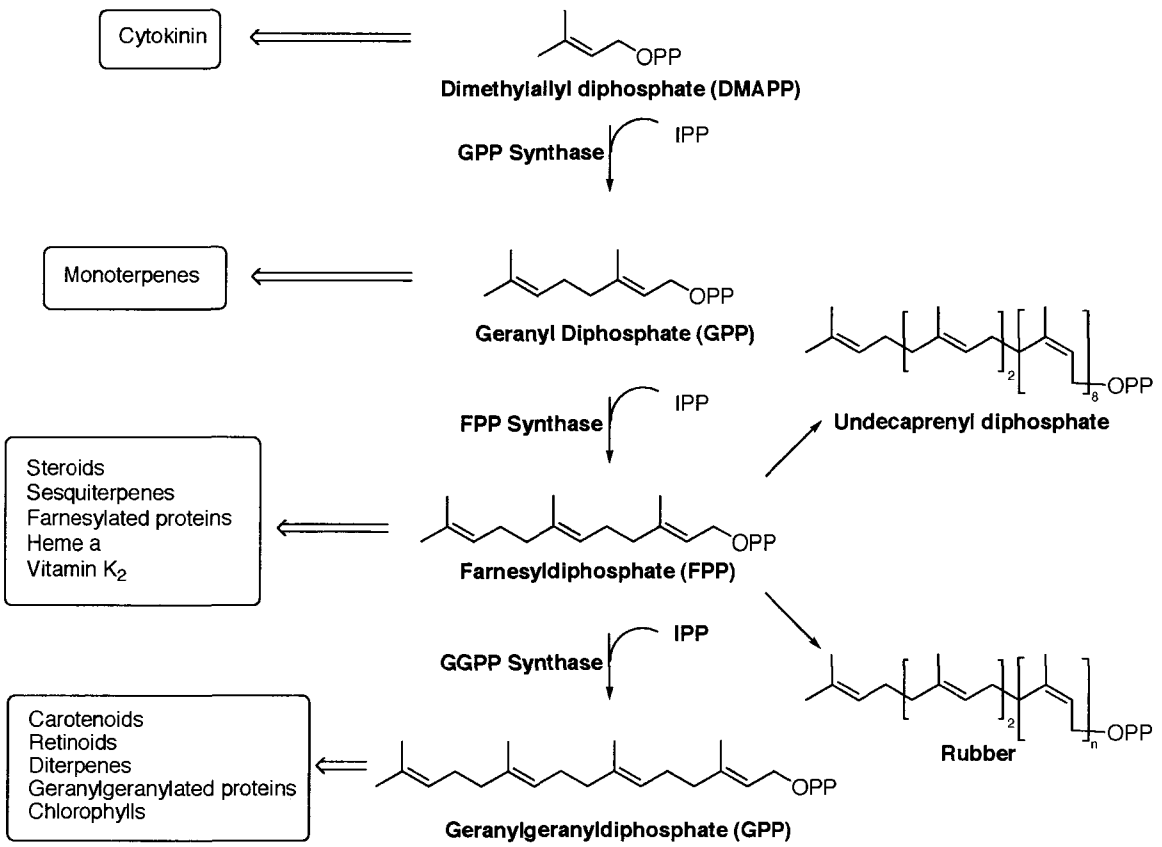
Although both these pathways for IPP biosynthesis are found in plants, they are compartmentalized in different locations of the plant cell. The ubiquitous MVA pathway is located in the cytoplasm of the cell while the MEP pathway is found in the plastid. There is experimental evidence that they cooperate in the formation of certain isoprenoids, probably a consequence of metabolic cross-talk between the pathways via the plastid envelope membrane.[23] For example, experiments using an inhibitor of the MVA pathway provided evidence that the plastidial MVA-independent pathway can compensate for the reduced flux through the cytosolic MVA pathway in the plant *Arabidopsis thaliana*.[24,25] Furthermore, isotope-labeling experiments indicated that only the plastid-localized MEP pathway provides IPP precursors for both plastidial monoterpene and cytosolic sesquiterpene biosynthesis in snapdragon flowers.[26] The trafficking of IPP appears to be unidirectional, from the plastids to the cytosol. Presently, it is unknown what source of IPP is predominately used in the biosynthesis of natural rubber.

1.1.4 Prenyltransferase Enzymes

In the biosynthesis of isoprenoids, the carbon backbones are derived from linear prenyl diphosphates. A family of enzymes called prenyltransferases, which catalyze the head-to-tail condensation of IPP with an allylic diphosphate substrate, produces these prenyl chains. Examples of these allylic substrates and their respective prenyltransferase are shown in Scheme 3. To date, more than 17 types of prenyltransferases with different catalytic functions have been characterized and more than five different prenyltransferase

genes have been cloned.[27,28] Prenyltransferases are classified as either *cis* or *trans* depending on the observed stereochemistry of their products. In addition, there are other types of prenyltransferase enzymes such as protein prenyltransferases, which catalyze the addition of prenyl groups to cysteine residues of proteins. Related enzymes, terpenoid cyclases, catalyze the cyclization of prenyl diphosphates to secondary metabolites.

Scheme 3. Biosynthesis of important linear prenyl chains catalyzed by prenyltransferases

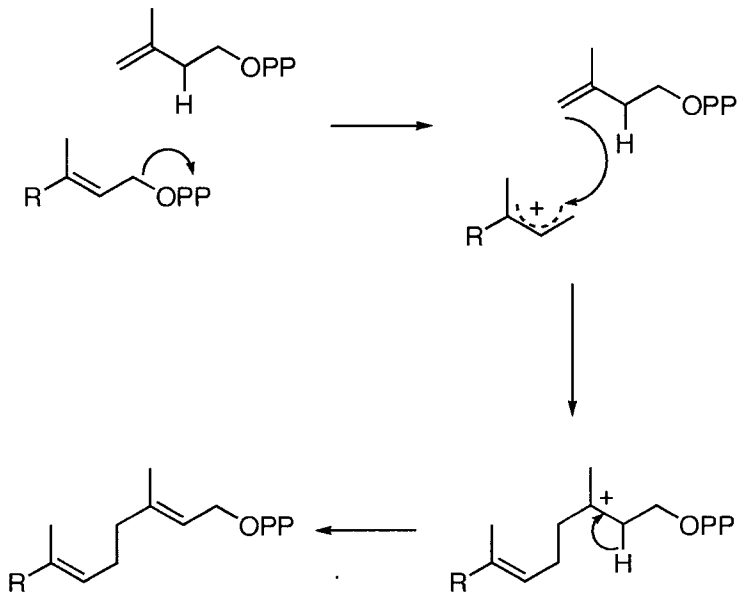


1.1.4.1 *Trans*-prenyltransferases

The molecular mechanism of prenyltransferases that catalyze the elongation of *trans*-type prenyl chains has been studied extensively, in particular FPP synthase (EC 2.5.1.10), which is among the most widely distributed members of this class of enzymes. This enzyme is particularly important because its enzymatic product, FPP, is a key branch point in isoprenoid biosynthesis. FPP is not only a substrate for dimerization to squalene in cholesterol biosynthesis, but is also a key intermediate in the formation of important isoprenoids, including glycosyl carrier lipids, respiratory quinones, heme *a* and prenylated proteins.[27]

Alignment of the amino acid sequences of these *trans*-type enzymes shows the presence of two characteristic aspartate rich DDxxD motifs, which are crucial for both catalytic activity and for substrate binding.[29-31] In addition, analysis of the 3-dimensional structure of FPP synthase indicates that the enzyme uses the Asp residues of the aspartate rich motifs to bind the diphosphate groups of the substrates through Mg^{2+} bridges.[32] The mechanism of prenyltransferases involves the ionization of the allylic diphosphate, followed by electrophilic attack on the resulting allylic cation by C4 of IPP, followed by elimination of the proton on C2 of IPP (Scheme 4).[33-35] The departure of the diphosphate group of the allylic substrate, *e.g.* DMAPP, is facilitated by the metal co-factor Mg^{2+} that is coordinated to the DDxxD motif.[36-38]

Scheme 4. Mechanism of *trans*-prenyltransferases



Site-directed mutagenesis experiments on FPP synthase from *Bacillus stearothermophilus* illustrate how these enzymes determine product length. They show that Tyr81, located at the fifth position of the first conserved aspartate rich domain, is the key residue in determining product chain length.[39] The Y81H mutant is the most effective at increasing production of the C_{20} prenyl chain, GGPP. A systematic study wherein Y81 was replaced with each of the other 19 amino acids shows that Y81A, Y81G and Y81S are able to produce hexaprenyl diphosphate (C_{30}).[39] In the secondary structure of FPP synthase, this tyrosine residue is located 12 Å from the first Asp-rich motif, which is similar to the length of the farnesyl moiety of FPP.

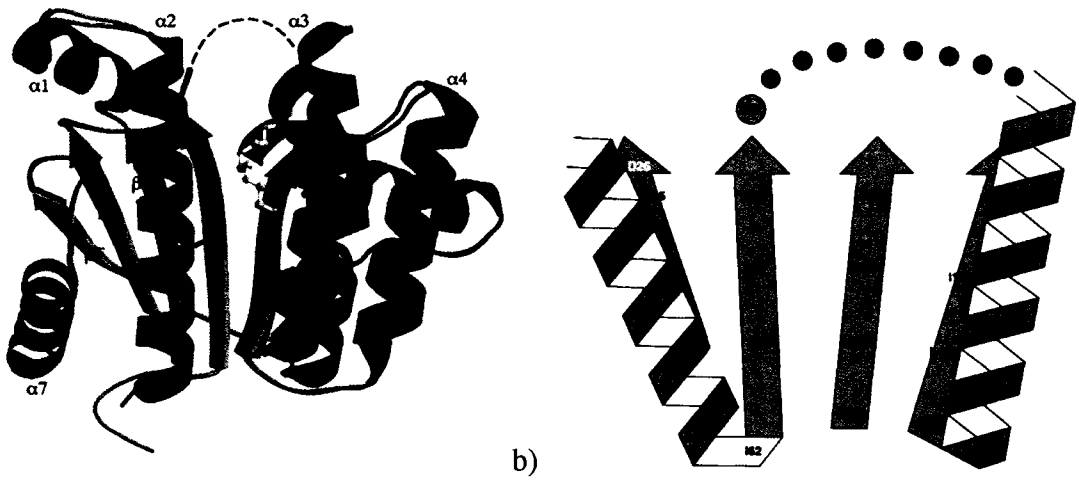
1.1.4.2 *Cis*-prenyltransferases

Unlike *trans*-prenyltransferases, the *cis*-type enzymes lack the DDxxD motifs, although they still require Mg^{2+} for maximum activity. *Cis*- and *trans*-prenyltransferases utilize somewhat different strategies for substrate binding and catalysis but still require an allylic substrate (*e.g.* DMAPP) and a homoallylic substrate (IPP). Lack of sequence similarity between the two types of prenyltransferases was further substantiated by crystal structures of the *cis*-prenyltransferases, octaprenyl diphosphate (OPP) and undecaprenyl diphosphate (UPP) synthase.[36,40-42] In contrast to *trans*-prenyltransferases, *cis*-prenyltransferases do not require Mg^{2+} for binding of the allylic substrate. However, IPP binding and elongation reactions require magnesium catalysis. Single-crystal X-ray studies of UPP synthase indicate that the mechanism of UPP synthase involves binding of FPP substrate first, followed by hydrogen bonding and electrostatic interaction with the amino acid residues Asn28, Gly29, Arg30, Arg39, and His43.[41] Mutagenesis studies on UPP synthase demonstrate the importance of Asp29 for catalysis.[43] For example, a 1000-fold reduction in K_{cat} was observed for the D29A mutant. Without Asp29, the Mg^{2+} remains bound to IPP, and is therefore unable to facilitate the elimination of the pyrophosphate group from the allylic substrate.

Single-crystal X-ray studies of UPP synthase also afford a better understanding of how *cis*-prenyltransferases control product chain length. The 3-dimensional structure of UPP synthase from *Escherichia coli* indicates that the enzyme has a hydrophobic tunnel formed by two α-helices and four β-strands, which is sufficiently large to accommodate

the product, UPP (Figure 2).[40] Large amino acids including I62, L137, V105, and H103 are located at the bottom of this hydrophobic tunnel. Ko *et al.* proposed that L137 plays a key role in determining product length, because L137A mutants are able to produce C_{70} and C_{75} prenyl chains as products.[40] Furthermore, sequence homology with the yeast enzyme Rer2, which synthesizes longer prenyl chain C_{70}-C_{80} products, indicates that this enzyme has an alanine residue at this position instead of leucine. Similar studies with other *cis*-type enzymes may elucidate why rubber transferase, a membrane bound *cis*-prenyltransferase, is capable of producing very long prenyl chains, whereas UPP synthase produces primarily C_{55} prenyl chains.

Figure 2. Hydrophobic tunnel of UPP synthase (adapted from Ko *et al.*[40]). a) ribbon drawing of the tunnel. b) cylindrical projection of the tunnel.



a) b)

1.1.5 Thesis Goals: Chemical Probes of Isoprenoid Biosynthesis

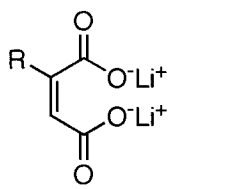
The objective of this research is to apply a variety of chemical biology techniques to study the chemistry of prenyltransferases. Initially small molecule chemistry will be used:

- To investigate the factors that are important for the binding of IPP to prenyltransferases, in particular, the spatial arrangement of the negatively charged oxygens of the diphosphate moiety.
- To compare the substrate specificity of rubber transferase to other *cis*-prenyltransferases.
- To probe stereochemistry of rubber biosynthesis.

Three classes of IPP analogs were chosen as potential substrates or inhibitors of prenyltransferase enzymes (Figure 3). Previous research suggests that dicarboxylates may be structural mimics of the corresponding diphosphates.[44,45] Hence, targets in class **A** (**17-26**) consist of analogs of IPP with the diphosphate moiety replaced with dicarboxylate functionality. Class **B** compounds (**27-29**) consist of 3-alkyl substituted analogs of IPP for comparison of the substrate specificity of rubber transferase to other *cis*-prenyltransferase. Finally, targets in class **C** (**30-35**) probe the stereochemical outcome of rubber biosynthesis. These compounds are analogs of IPP with stereospecific replacement of hydrogen with deuterium.

Figure 3. Dicarboxylate mimics, homologs and ^{2}H analogs of IPP

Class **A**: Dicarboxylate mimics



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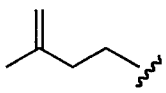

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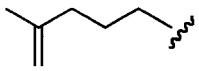

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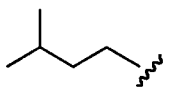

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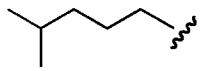

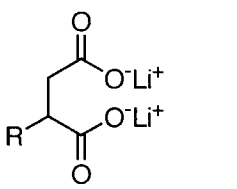

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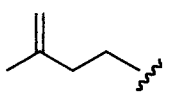

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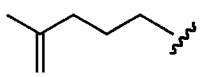

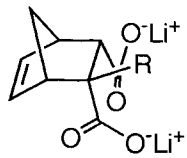

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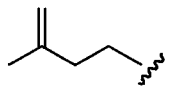

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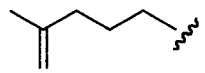

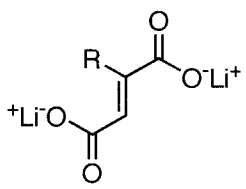

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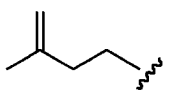

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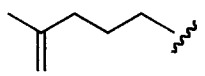

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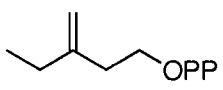

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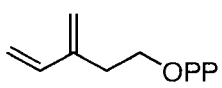

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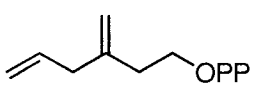

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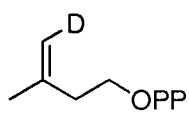
Class **C**: ^{2}H analogs of IPP



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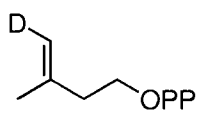

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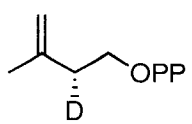

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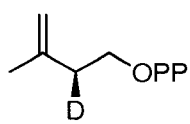

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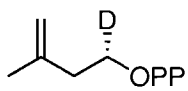

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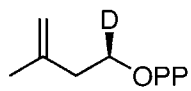
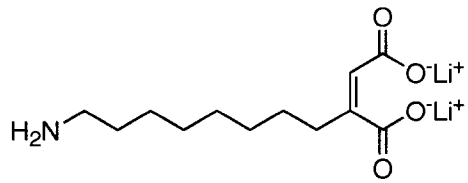


35

Furthermore, the synthesis of a new ligand **36** (Figure 4) and its attachment to solid support could facilitate the purification of prenyltransferases by affinity chromatography.

Figure 4. Structure of affinity ligand **36**



36

CHAPTER 2. Analogs of IPP Containing a Dicarboxylate Moiety as Inhibitors of Prenyltransferase Enzymes

2.1 INTRODUCTION

The discovery of antibiotics for the treatment of bacterial infections has been one of the most significant medical breakthroughs of the past century. Unfortunately, a variety of bacteria (*e.g. Enterococcus faecalis, Mycobacterium tuberculosis, Pseudomonas aeruginosa,* and *Staphylococcus aureus*) have become resistant to antibiotic treatment.[46-49] Penicillin resistant strains of *S. aureus* initially appeared in hospitals shortly after the introduction of penicillin in the 1940's.[50,51] Antibiotic resistance by bacteria has been shown to be mediated by a number of the following mechanisms including:[52-54]

1. Prevention of the drug from reaching its target site of action either by active efflux or by reduced uptake into the cell, as well as by sequestration of the antibiotic by protein binding.
2. Deactivation of the antibiotic by enzymatic modification.
3. Modification of the drug's target, thereby eliminating or reducing the binding of the antibiotic.
4. Metabolic bypass of the inhibited reaction.
5. Overproduction of the antibiotic target.

More than 15 classes of antibiotics that target the essential physiological or metabolic functions of bacteria and some representative examples are shown in Table 1.[46]

Table 1. Major antibiotic families and their respective mode of action.[46]

Mechanism of Action	Antibiotic families
Inhibition of cell wall synthesis	Penicillins; cephalosporins; carbapenems; daptomycin; monobactams; glycopeptides
Inhibition of protein synthesis	Tetracyclines; aminoglycosides; oxazolidinones; streptogramins; ketolides; macrolides; lincosamides
Inhibition of DNA synthesis	Fluoroquinolines
Competitive inhibition of folic acid synthesis	Sulfonamides; trimethoprim
Inhibition of RNA synthesis	Rifampin
Inhibition of DNA repair	Metronidazole

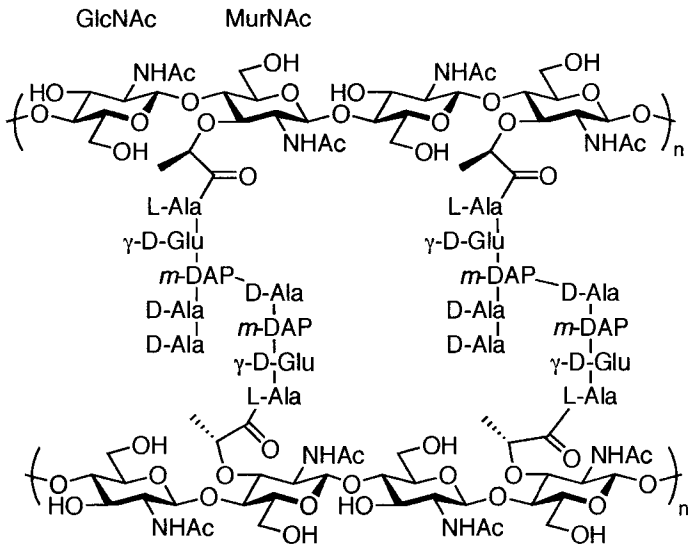
Because an intact cell wall is essential for the survival of bacterial cells, the biosynthesis of peptidoglycan is a major target of several important classes of antibiotics.[55] Enzymes involved in bacterial cell wall biosynthesis, and in particular the peptidoglycan layer, are attractive targets for the development of new antibacterial agents.

2.1.1 Inhibition of Peptidoglycan Biosynthesis

Peptidoglycan or *murein* is a macromolecular net-like structure found on the outside of the cytoplasmic membrane of bacteria. It functions to maintain the defined cellular shape and is intimately involved in cell growth and cell division.[56-58] The molecular structure of peptidoglycan is characterized by a matrix of linear glycan units interlinked by short peptide chains (Figure 5). The glycan units are composed of alternating β-1,4-linked *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc). The carboxyl terminus of the muramic acids are further substituted by a short peptide chain, which is most often L-Ala-γ-D-Glu-X-D-Ala-D-Ala, where X is usually *meso*-diaminopimelate (*m*-DAP) in Gram-negative bacteria or L-lysine in Gram-positive bacteria. The high structural rigidity of peptidoglycan arises from the cross-linking of neighbouring glycan chains via direct peptide bond formation or by short peptide bridges between two peptide subunits.[58]

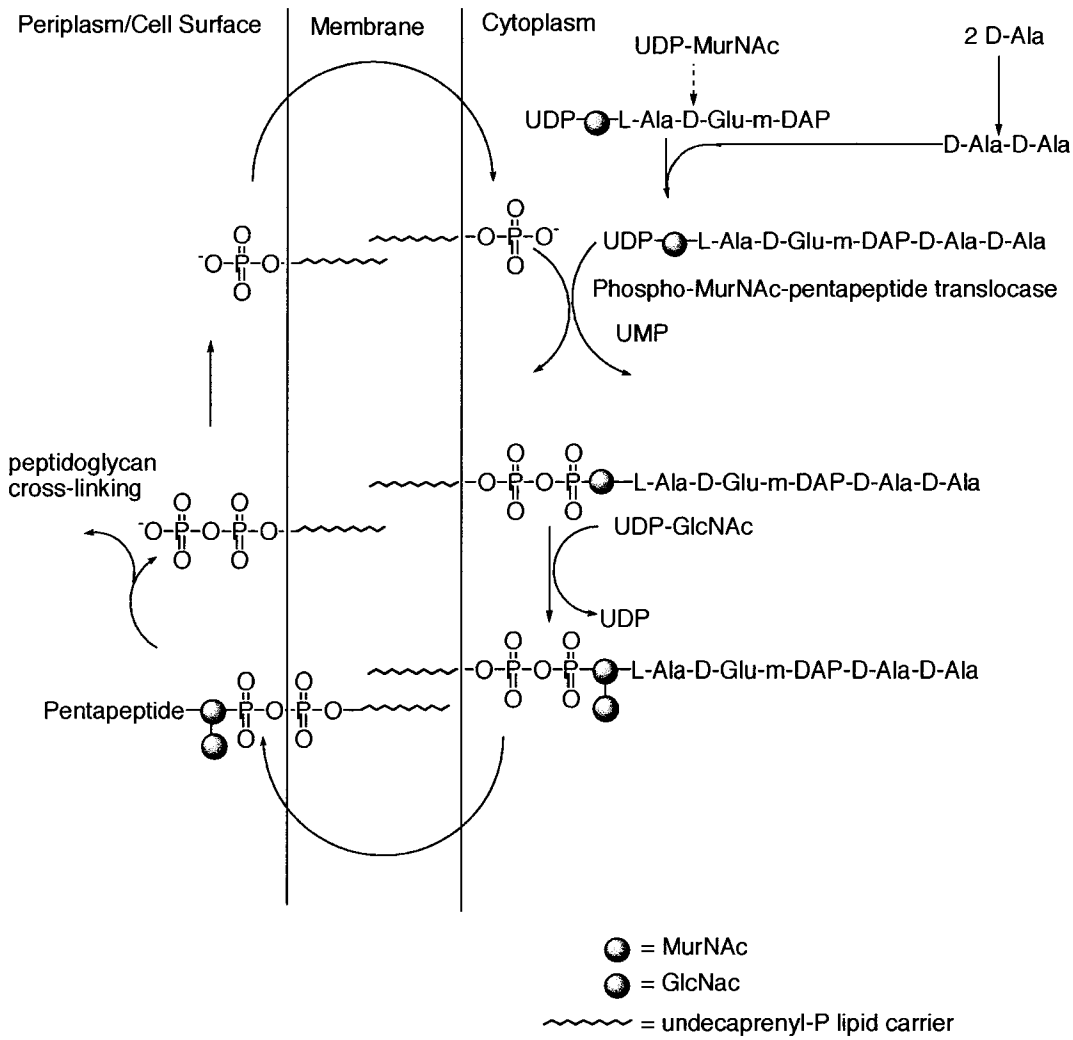
The biosynthesis of bacterial peptidoglycan has been extensively reviewed and involves two stages (Scheme 5).[56,58] The first stage consists of assembly of the peptidoglycan monomer (PGM or Lipid II) units by enzymes located within the cytoplasm or at the inner side of the membrane. This biosynthetic pathway proceeds through a series of enzymatic reactions from UDP-*N*-acetylglucosamine (UDP-GlcNAc) to the final lipid intermediate, disaccharide-(peptide)-diphosphate undecaprenol.

Figure 5. Structure of peptidoglycan in the cell wall of *E. coli*

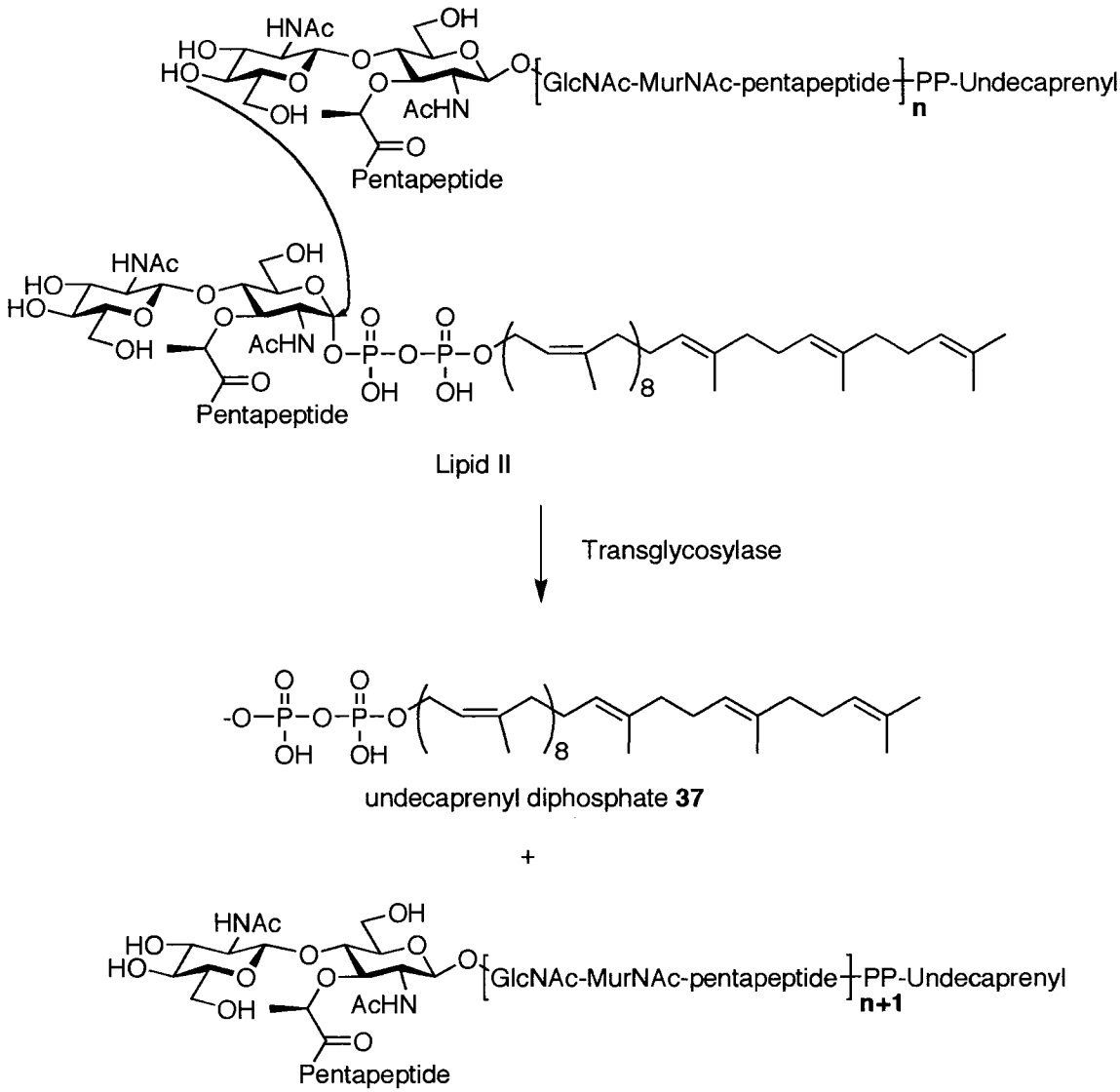


The second stage involves translocation of the peptidoglycan monomer unit through the cytoplasmic membrane and polymerization reactions on the outer side of the cytoplasmic membrane. There are two major types of enzymes that are involved in the polymerization: glycosyltransferases and transpeptidases. Transglycosylation involves extending the linear glycan strands by the creation of a new β-1-4-glycosidic linkage between a MurNAc residue of Lipid II and the terminal GlcNAc residue of the growing polysaccharide chain (Scheme 6).[58] Undecaprenyl diphosphate (**37**) is released during transglycosylation, and is recycled via the undecaprenyl phosphate cycle. Transpeptidases catalyze the formation of the peptide-based cross-links within the structure of peptidoglycan.

Scheme 5. Peptidoglycan biosynthesis (adapted from Bugg and Walsh[59])



Scheme 6. Transglycosylation reaction

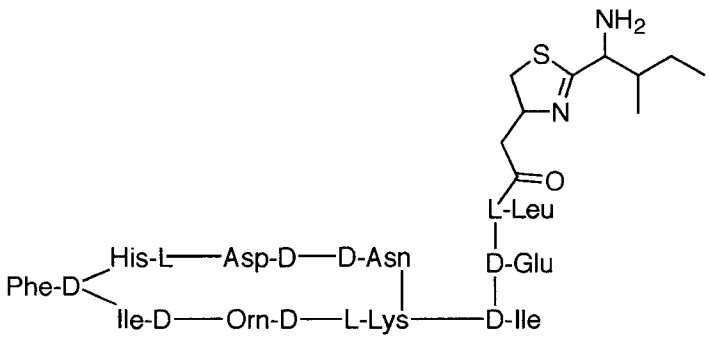


2.1.1.1 Undecaprenyl Phosphate

Undecaprenyl phosphate is a key intermediate not only in the biosynthesis of Lipid I but also for glycosyl carrier lipids involved in the biosynthesis of other cell wall polymers. Undecaprenyl phosphate is produced in bacterial cells by either dephosphorylation of undecaprenyl diphosphate via undecaprenol phosphatase or by

phosphorylation of undecaprenol. Undecaprenyl diphosphate is synthesized by UPP synthase, and is also generated in the course of peptidoglycan biosynthesis when the disaccharide units are polymerized by the transglycosylases.[58] Undecaprenol, undecaprenyl phosphate and UPP are part of the undecaprenyl phosphate cycle. The concentration of undecaprenyl phosphate is critical to the biosynthesis of peptidoglycan as exemplified by the antibiotic action of bacitracin (**38**, Figure 6).[60] This cyclic peptide forms a strong complex with undecaprenyl diphosphate and a divalent cation, thus preventing its dephosphorylation to undecaprenyl phosphate.[61] This process not only lowers the cellular concentration of undecaprenyl phosphate but also inhibits further peptidoglycan biosynthesis, and ultimately leads to bacterial cell death.[62]

Figure 6. Structure of bacitracin

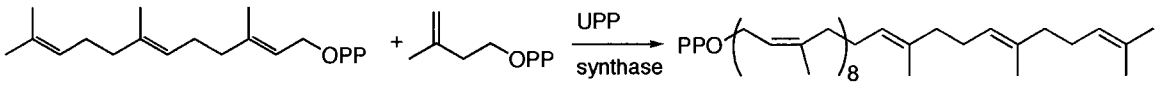


38 Bacitracin

2.1.1.2 Undecaprenyl diphosphate synthase (UPP synthase)

Undecaprenyl diphosphate synthase, a *cis*-prenyltransferase, catalyzes the condensation of eight molecules of isopentenyl diphosphate (IPP, **7**) with farnesyl diphosphate (FPP, **39**) to produce undecaprenyl diphosphate (UPP, **37**) (Scheme 7).[63] Inhibitors of UPP synthase may give some insight on its specificity as well as its molecular mechanism for the formation of UPP. Recently, crystal structures of UPP synthase and a proposed substrate-enzyme binding model were reported.[1,41] Further understanding of this enzyme should provide insight for the development of new antibacterial agents.

Scheme 7. Biosynthesis of undecaprenyl diphosphate



39 FPP **7** IPP **37** UPP

2.1.2 Inhibition of Protein Prenylation

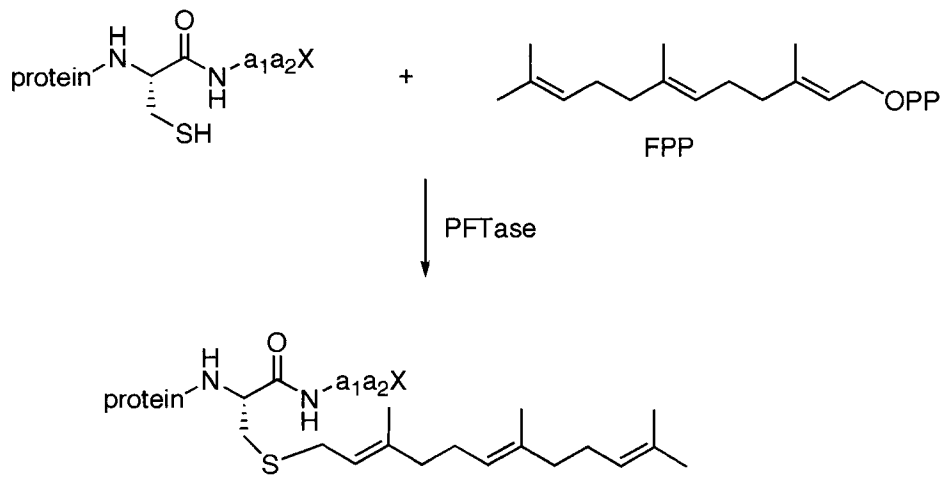
Prenylation of proteins is an important post-translational modification of ribosomally produced proteins that influences cell proliferation.[64] Protein prenylation involves either the addition of farnesyl or geranylgeranyl units to a cysteine residue near

the C-termini of proteins. Examples of proteins that undergo prenylation include a family of small G proteins, in particular Ras oncogenic proteins. These are found in a variety of tumor types, including breast, ovarian and pancreatic cancers.[65] Farnesylation is a critical-post-translational modification that is necessary for the cell-transforming ability of Ras proteins. Therefore, there has been a considerable effort in discovering new inhibitors of Ras farnesylation for investigation of cancer therapy.

2.1.2.1 Protein Farnesyltransferase (PFTase)

Protein farnesyltransferase (PFTase) catalyzes the transfer of the C_{15} farnesyl unit from FPP to the cysteine residue located in a tetrapeptide CAAX (A = aliphatic amino acid, X = methionine or serine) sequence at the carboxyl terminus of proteins (Scheme 8).[66] PFTase has been isolated from variety of sources, and is a heterodimeric protein composed of α and β subunits with molecular weights of 48 and 46 kDa, respectively. PFTase is a metalloenzyme that tightly binds one equivalent of Zn^{2+}.[67] The β-subunit binds the acceptor protein in a zinc dependent manner, whereas the α-subunit complexes to FPP in a magnesium dependent fashion.

Scheme 8. Prenylation of peptides catalyzed by PFTase



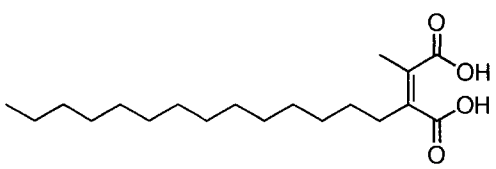
Experiments show that the cysteine residue in the peptide-based substrate is bound to the enzyme as a Zn^{2+} thiolate. Replacement of the cysteine in the CAAX motif with alanine gives a peptide that is neither a substrate nor inhibitor of PFTase.[68] Furthermore, replacement of Zn^{2+} with Cd^{2+} results in a 50% decrease in activity.[69] Site directed mutagenesis experiments also indicate that the enzyme uses a tightly bound zinc not only to enhance substrate binding, but also to enhance the nucleophilicity of the cysteine thiol.[70]

2.1.2.2 Inhibition of PFTase

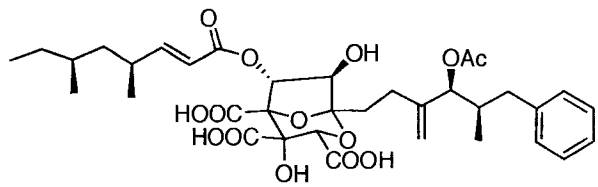
A variety of substances isolated from natural sources are inhibitors of PFTase and are broadly classified based on their mechanism of inhibition. Compounds that are

competitive with the natural substrate farnesyl diphosphate include CP-225,917,[71] oreganic acid,[72] zaragozic acids,[73-76] actinoplanic acids,[77] chaetomellic acids,[78,79] and manumycin analogs.[80] The pepticinnamins[81] encompass a different class of compounds that are competitive with the peptide substrate. Finally, inhibitors that do not obviously resemble either substrate of PFTase include andrastins,[82] cylindrols,[83] preussomerins,[84] fusidienol,[85] gliotoxin,[86] and 10'-desmethoxystreptonigrin.[87] Figure 7 shows the structures of some natural occurring inhibitors of PFTase.

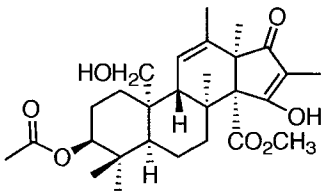
Figure 7. Chemical structures of naturally occurring inhibitors of PFTase



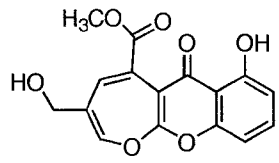
Chaetomellic acid A



Zaragozic Acid A



Andrestatin B

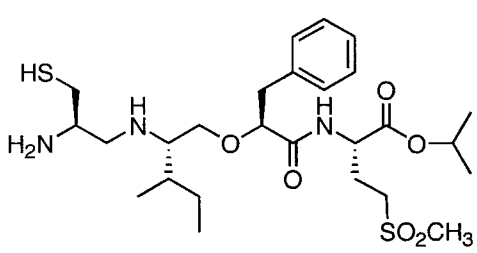


Fusidienol

Considerable research has focused on the design and development of PFTase inhibitors based on one or both of the natural substrates. One group of inhibitors is peptidomimetics that contain the CAAX recognition motif. Replacement of the second aliphatic amino acid with an aromatic amino acid residue results in compounds that are

potent and selective inhibitors of PFTase.[88] For example, compound **40** (Figure 8) developed by researchers at Merck, inhibits Ras PFTase with an IC_{50} of 0.4 nM and is capable of reverting Ras transformed cells back to normal.[89] Although the thiol group is important for substrate recognition by the enzyme, it has the potential for causing toxicity and poor pharmokinetic behaviour due to oxidative metabolism.[90] To circumvent this, inhibitors such as **41,** in which the cysteine group is replaced with an imidazoleacetic acid, were developed. Compound **41** is a highly potent PFTase inhibitor with an IC_{50} of 0.15 nM.[91]

Figure 8. Synthetic inhibitors of PFTase



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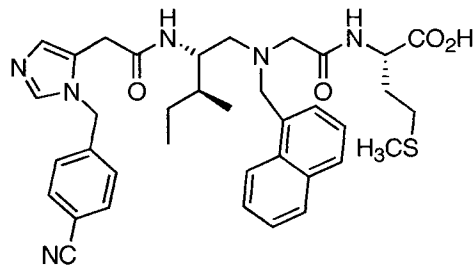

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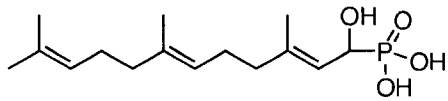


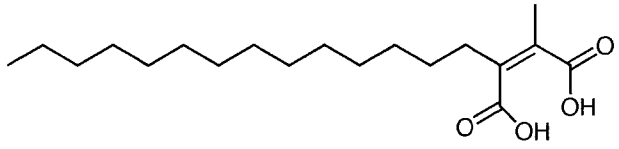
42 (hydroxyfarnesyl)phosphonic acid

Compounds that mimic farnesyl diphosphate are also effective inhibitors of PFTase. A possible disadvantage of utilizing FPP analogs as inhibitors of PFTase is that they could also inhibit other enzymes that are involved in essential cellular functions such as squalene synthase, which is necessary for cholesterol biosynthesis. Recently, (α-hydroxyfarnesyl)phosphonic acid **42** was shown to be a non-hydrolyzable competitive inhibitor of PFTase with an IC_{50} of 30 nM in Ras-transformed cells.[92] Inhibitors based on the natural product chaetomellic acid are also potent inhibitors of PFTase.[44]

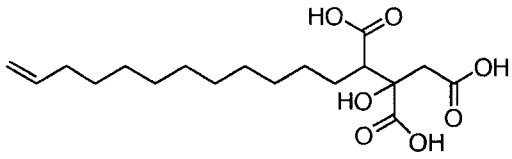
2.1.3 Project Goals: Design and Synthesis of Analogs of IPP as Inhibitors of UPP Synthase, PFTase and Rubber Biosynthesis

Certain alkyl-substituted dicarboxylates are potent inhibitors of protein farnesyltransferase.[77,78,92] For example, chaetomellic acid A (**43**) is a good inhibitor of Ras PFTase and competes for the FPP binding site with an IC_{50} of 17 μM with FPP (Figure 9).[44] Recently, two natural products, CJ-13,981 (**44**) and CJ-13,982 (**45**), containing analogous polyanionic functionality were shown to inhibit squalene synthase, an enzyme that catalyzes the dimerization of two FPP molecules to form squalene, a key precursor for cholesterol biosynthesis.[93] Inhibitors designed as prenyl diphosphate analogs have been employed in substrate specificity studies and may lead to new drugs.[94] Thus, the objective of the first part of this work is to design, synthesize, and examine the interaction of several analogs (**17-26**, see Figure 10) of IPP containing a dicarboxylate functionality as potential inhibitors of prenyltransferases.

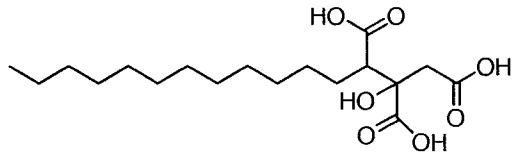
Figure 9. Natural products containing multiple carboxylic acid groups



43 Chaetomellic Acid A

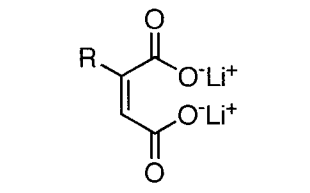


44 CJ-13,981



45 CJ-13,982

Figure 10. Dicarboxylate mimics of IPP **17-26**



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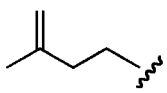

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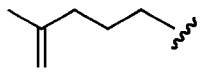

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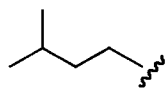

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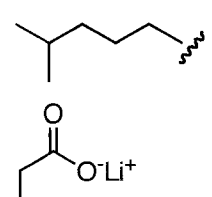

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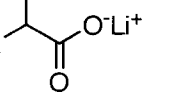

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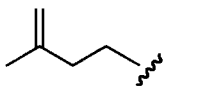

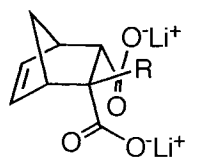

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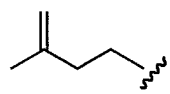

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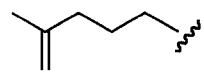

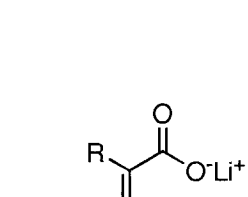

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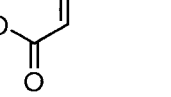

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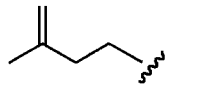
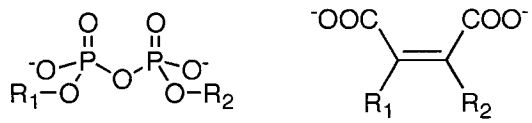


2.2 RESULTS AND DISCUSSION

Initial targets containing maleic acid units and structurally rigid succinic acid units were designed as analogs of IPP. Simple modeling studies suggest that such units can achieve a spacing of negatively charged oxygen atoms that is within 0.1 Å of that in the corresponding diphosphate moiety (Figure 11). Analogs of isopentenyl diphosphate (IPP) containing a dicarboxylate moiety are attractive biochemical tools because of the importance of IPP to the biosynthesis of a variety of isoprenoids, including FPP.

Figure 11. Comparison of diphosphate and maleates

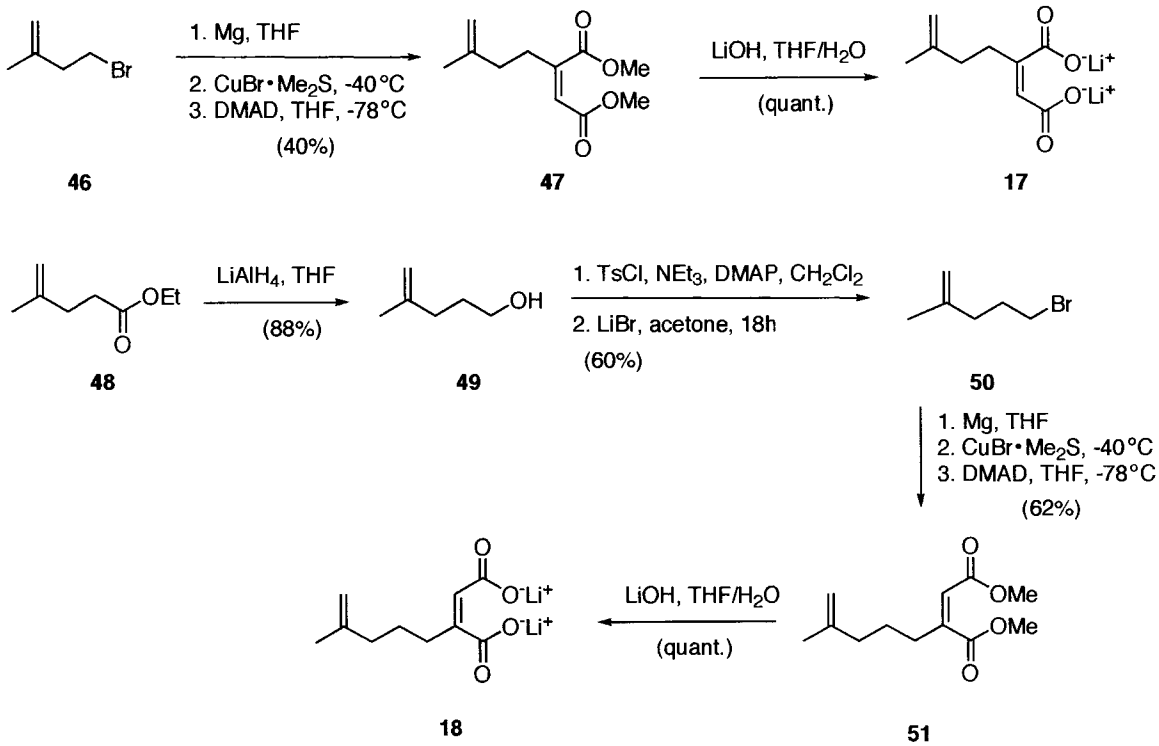


2.2.1 Synthesis of IPP Analogs

As previously mentioned, chaetomellic acid A (**43**), isolated from *Chaetomella acutiseta*, is a competitive inhibitor of Ras PFTase with an IC_{50} of 55 nM.[92] There are several reported syntheses of chaetomellic acid A, including a method developed in our research group by Ratemi *et al.*[44] The key step in this synthetic sequence involves the conjugate addition of an organocuprate to dimethyl acetylenedicarboxylate (DMAD). The use of copper (I) bromide dimethyl sulfide complex ($CuBr•Me_2S$) in Et_2O gives preferential formation of the product with the two esters having a *cis* relationship.

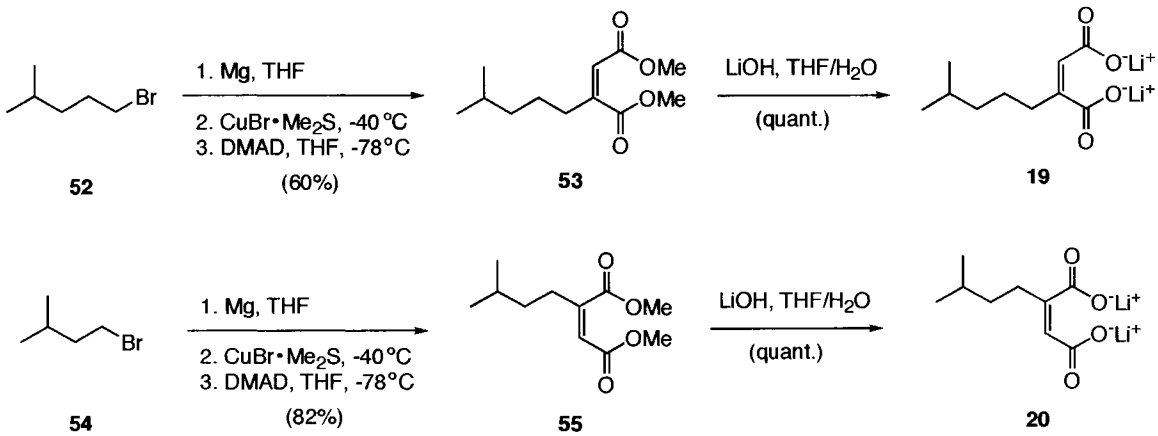
The syntheses of compounds **17** and **18** are shown in Scheme 9. Formation of the Grignard reagent from 3-methyl-3-butenyl bromide (**46**)[95] followed by its addition to a suspension of $CuBr•Me_2S$ in THF gives the corresponding organocuprate, which is subsequently reacted with DMAD to afford the desired diester **47** in moderate yield. Ester hydrolysis using lithium hydroxide generates the desired dicarboxylate **17**. Compound **12** can be prepared in an analogous manner, starting with ethyl 4-methyl-4-pentenoate (**48**). Reduction of the ester with lithium aluminum hydride gives the alcohol **49**,[96] which is then converted to bromide **50** in two steps via the corresponding tosylate.[97] The protected diester **51** can be obtained by the conjugate addition to DMAD. Subsequent base hydrolysis affords **18** in quantitative yield.

Scheme 9. Synthesis of IPP analogs **17** and **18**



To probe the importance of the terminal methylene group for IPP binding to the enzyme active site, saturated analogs of IPP were prepared as shown in Scheme 10. Addition of the organocuprate derived from 1-bromo-3-methyl-butane (**52**) generates the corresponding dimethyl ester **53** in good yield. Its subsequent deprotection with 1.0 M lithium hydroxide affords the lithium dicarboxylate **19**. The preparation of **20** from 1-bromo-4-methyl-4-pentane (**54**) could be achieved using a similar process.

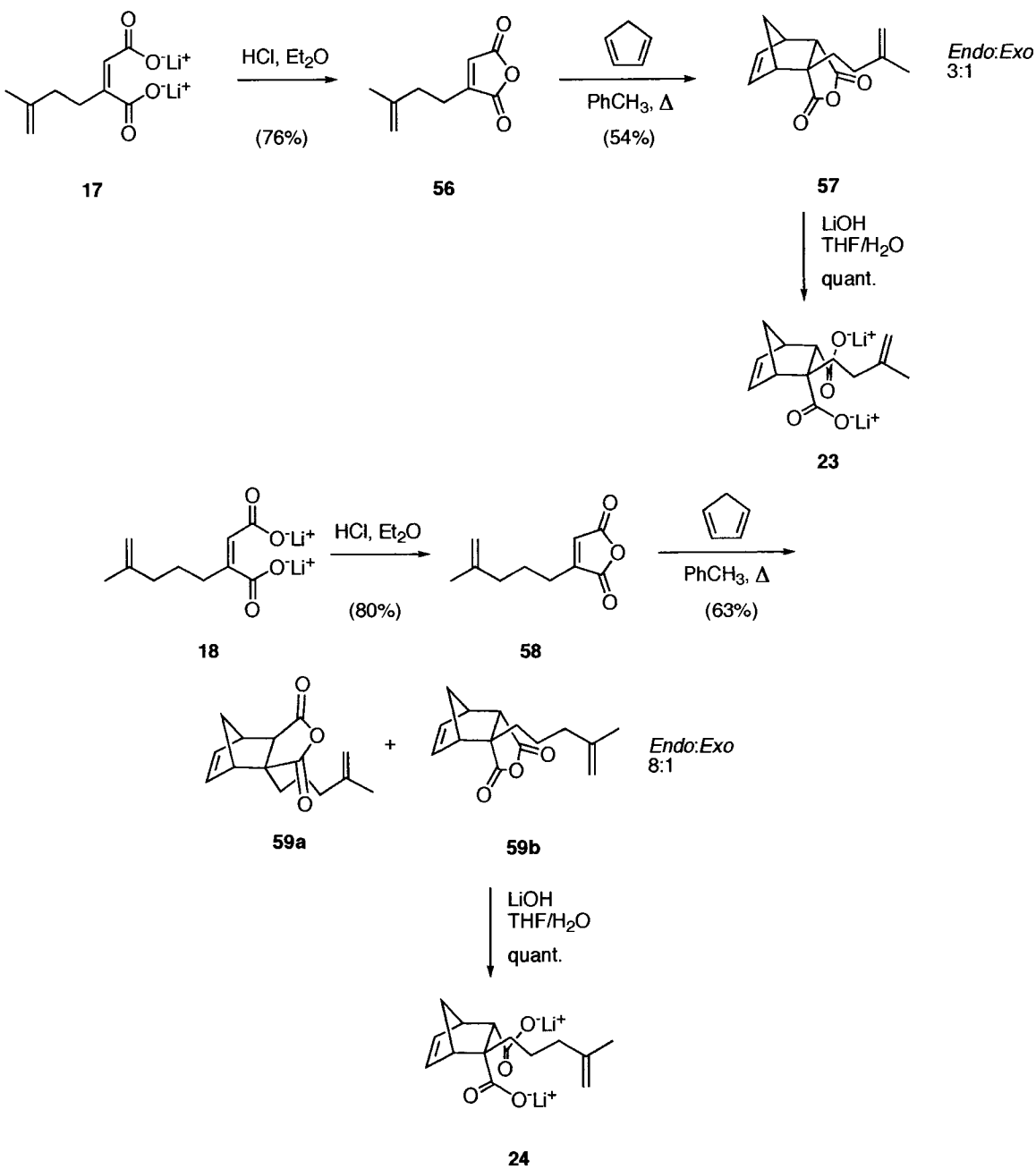
Scheme 10. Synthesis of IPP analogs **19** and **20**



Potential inhibitors based on succinic acid were also prepared in order to determine if selective inhibition of *cis*- or *trans*-prenyltransferases could be achieved. Compounds **23** and **24** were synthesized using a Diels-Alder reaction as the key transformation (Scheme 11). Reaction of the dilithium salt **17** with dilute hydrochloric acid gives the anhydride **56,** which reacts with cyclopentadiene to yield the cycloadduct **57**. ^{1}H-NMR analysis of the reaction mixture indicates preferential formation of the *endo* adduct in a ratio of 3:1 to its *exo* product. Basic hydrolysis of the anhydride **57** produces the desired dicarboxylate **23**. In a similar manner, treatment of the lithium dicarboxylate

18 with dilute HCl gives anhydride **58** in 76% isolated yield. Reaction of **58** with cyclopentadiene generates the bicyclic derivatives **59a** and **59b** in a ratio of 8:1. Base-catalyzed hydrolysis of anhydride **59b** affords the desired compound **24**.

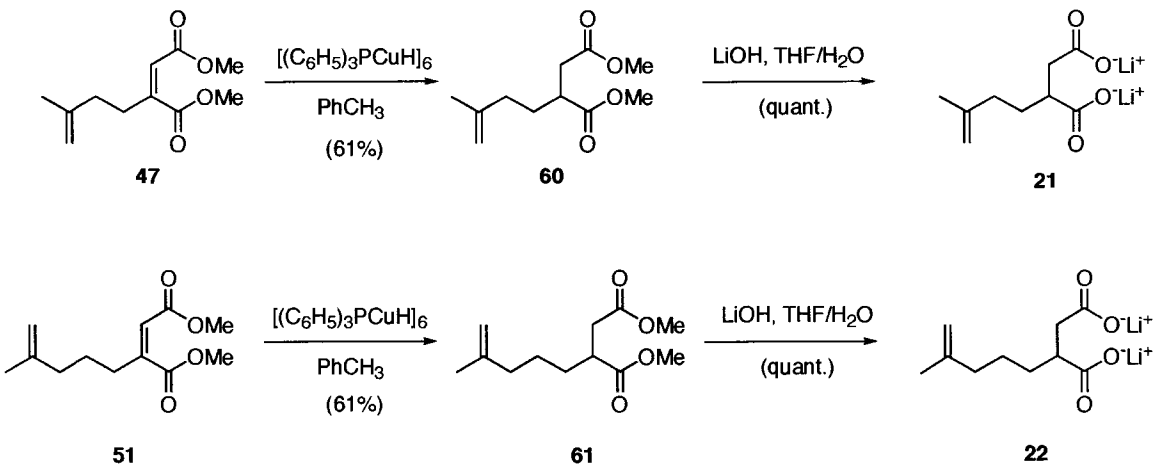
Scheme 11. Synthesis of rigid analogs of IPP **23** and **24**



The presence of a double bond or a bicyclic moiety between the two dicarboxylates restricts the rotational freedom and may not allow the molecule to achieve the correct geometrical or spatial arrangement for efficient binding to the active site of the target enzyme. Hence, compounds **21** and **22** were prepared to investigate the effect of increasing the conformational mobility of the two dicarboxylates.

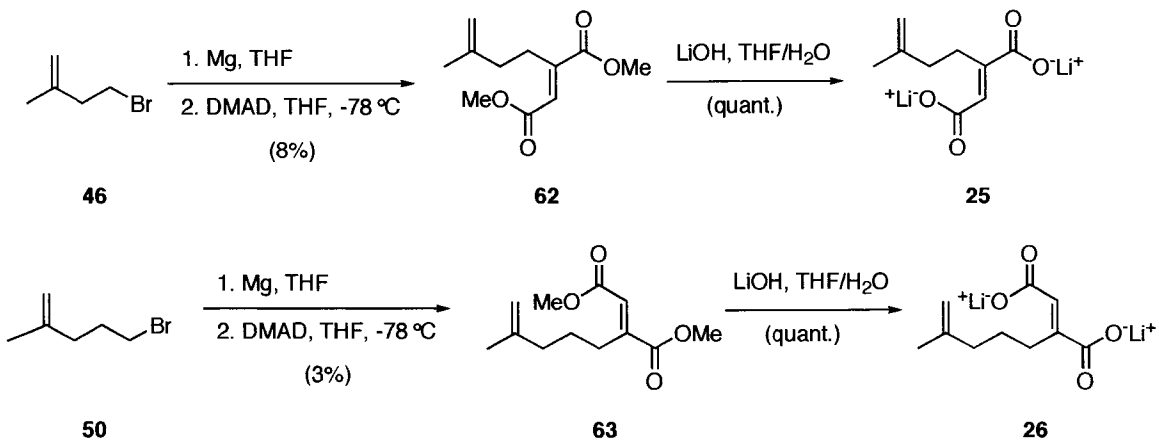
The syntheses of compounds **21** and **22** can be achieved via the reduction of the corresponding substituted dimethyl maleates (Scheme 12). Initial attempts utilizing either dissolving metal reduction conditions (Li and NH_3) or ruthenium-catalyzed reductions using triethylsilane did not yield the desired product. Analysis of the reaction mixtures from both sets of reagents indicated formation of many side products. Copper hydrides such as hexa-μ-hydrohexakis(triphenylphosphine) hexacopper are mild and effective reducing agents for α,β-unsaturated esters, ketones and aldehydes.[98] Treatment of dimethyl (*Z*)-2-(2-methyl)butenylbutenedioate (**47**) with hexa-μ-hydrohexakis(triphenylphosphine) hexacopper gives the desired compound **60** in 61% yield. Hydrolysis of the ester groups with lithium hydroxide generates the desired lithium salt **21**. Compound **51** could be reduced in a similar manner and subsequent base mediated removal of the protective groups gave **22** in quantitative yield.

Scheme 12. Synthesis of flexible analogs of IPP **21** and **22**



To expand the repertoire of possible selective inhibitors, two analogs with *trans* dicarboxylates were prepared (Scheme 13). As previously described, the addition of an organocopper reagent, prepared from CuBr•Me_2S, to DMAD gives primarily *cis* addition. However, it is known that prolonged reaction with DMAD, higher temperature (-40 °C), or use of ether as solvent will usually lead to an increase in *trans* addition.[99] However, no *trans* product was observed when the reaction conditions were changed. The *trans* compounds could be accessed by the addition of the corresponding organomagnesium reagent in the absence of oxygen to DMAD, followed by basic hydrolysis to give the desired compounds **25** and **26**. The *trans* addition may be due to equilibration of the enolate intermediate, resulting from the conjugate addition, which is normally slow with the addition of organocopper reagents but would be faster with the corresponding Grignard reagents. The yields for these compounds are unoptimized.

Scheme 13. Preparation of *trans* analogs of IPP **25** and **26**



2.2.2 Biological Evaluation of the IPP Analogs

The ten analogs of IPP (**17-26**) were tested with PFTase and UPP synthase. Recombinant poly-histidine-tagged protein farnesyltransferase was expressed in *E. coli* and purified on a Ni(II) column to homogeneity.[100] The enzyme activity can be conveniently monitored using a fluorometric assay using Dansyl-Gly-Val-Ile-Ala at an enzyme concentration of 1.0 nM.[101] Most compounds tested are only poor inhibitors of PFTase with IC_{50} values of greater than 1 mM. As shown in Table 2, the bicyclic inhibitors **23** and **24** show improved inhibition with IC_{50} of 812 and 814 μM, respectively. Analog **25**, with the dicarboxylates having a *trans* relationship, displays the best inhibition of PFTase with an IC_{50} of 384 μM. Compound **25** is a competitive inhibitor of PFTase with respect to FPP with a K_i = 287 μM (Figure 12).

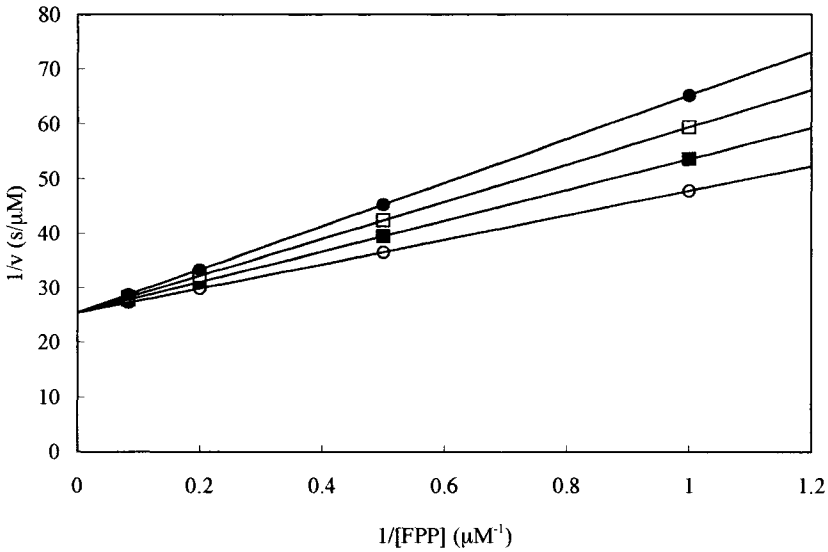
Table 2. Inhibition of protein farnesyltransferase and undecaprenyl diphosphate synthase with IPP analogs

Compound	PFTase IC_{50}, (μM)[a]	UPP Synthase IC_{50}, (μM)[b]
17	>1000	>1000
18	>1000	>1000
19	>1000	>1000
20	>1000	>1000
21	812 ± 28	936 ± 43
22	804 ± 46	>1000
23	>1000	>1000
24	>1000	>1000
25	384 ± 33	492 ± 20
26	>1000	135 ± 18

[a] IC_{50} conditions: 0.1 nM PFTase, 50 mM Tris-HCl, 12 mM $MgCl_2$, 12 μM $ZnCl_2$, 5.8 mM DTT, 0.02% (w/v) *n*-dodecyl-β-D-maltoside, pH 7.0 Dansyl-Gly-Cys-Val-Ile-Ala was the peptide substrate. PFTase (1.0-2.0 nM) was used to initiate reactions.

[b]IC_{50} conditions: 0.3 μM UPPase, 100 mM Tris-HCl pH 8.5, 2 mM $MgCl_2$, 5 μM FPP and 45 μM [1-^{14}C] IPP (55 Ci/mol).

Figure 12. Inhibition of UPP synthase by compound **25**. Double-reciprocal plot with FPP as the varied substrate at fixed concentrations of **25**. Concentrations of compound **25** were 100(●), 200 (□), 300(■), and 400 (○) mM. Dansyl-Gly-Val-Ile-Ala was held constant at 2.4 μM. PFTase (1.0 – 2.0 nM) was used to initiate reactions.



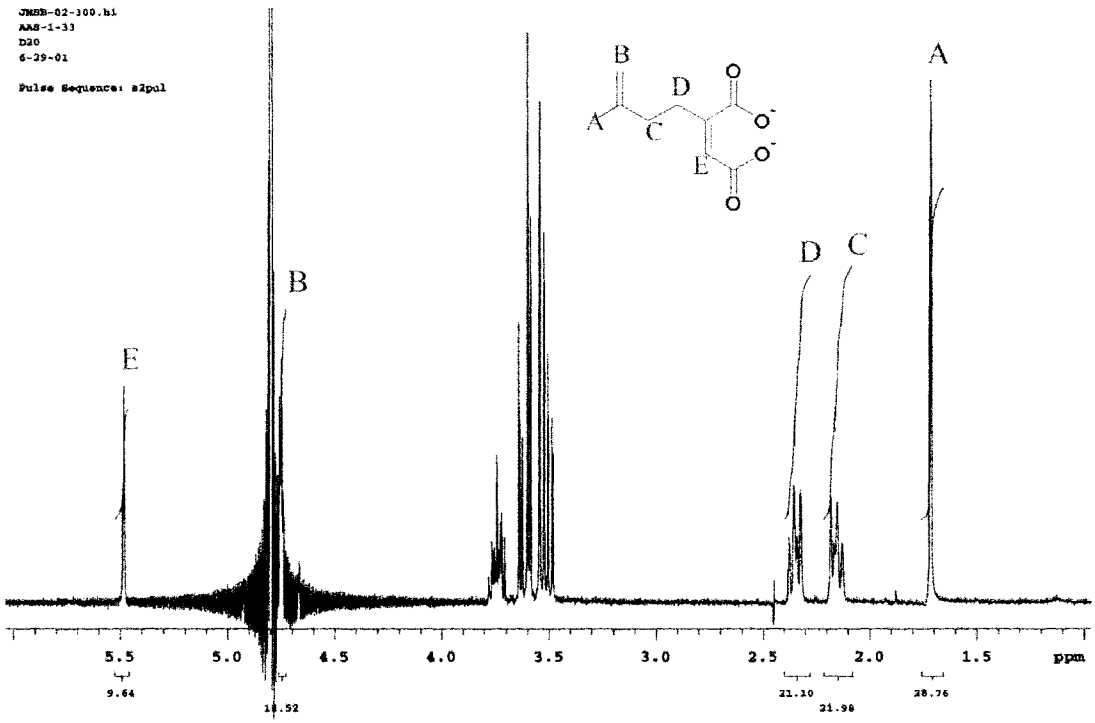
All the analogs of IPP were also tested against UPP synthase from *E. coli*. The enzyme activity can be monitored using a radioactivity-based assay. After incubation of [1-^{14}C] IPP and an inhibitor with UPP synthase, the reaction products were chromatographed on reverse phase silica gel TLC. The plate with radiolabeled products was exposed to a film and the radioactivity distribution was determined by autoradiography. The results demonstrate that the majority of the dicarboxylates show poor inhibition of UPP synthase. Fortunately, compounds **25** and **26** with the *trans* dicarboxylates show modest inhibition with an IC_{50} of 492 and 138 μM, respectively.

IPP isomerase catalyzes the interconversion of IPP and DMAPP. To determine if alternative substrates of this enzyme are accepted, NMR studies with compound **17** were completed by Dr. Sam Barkley (University of Utah). A solution of **17** was analyzed by ^{1}H NMR before and after incubation with IPP isomerase isolated from *Schizosaccharomyces pombe* and the acquired spectra are shown in Figure 13. The ^{1}H NMR resonances of the olefinic protons are obscured by the residual solvent peak. The results indicate that these dicarboxylates are not substrates of IPP isomerase.

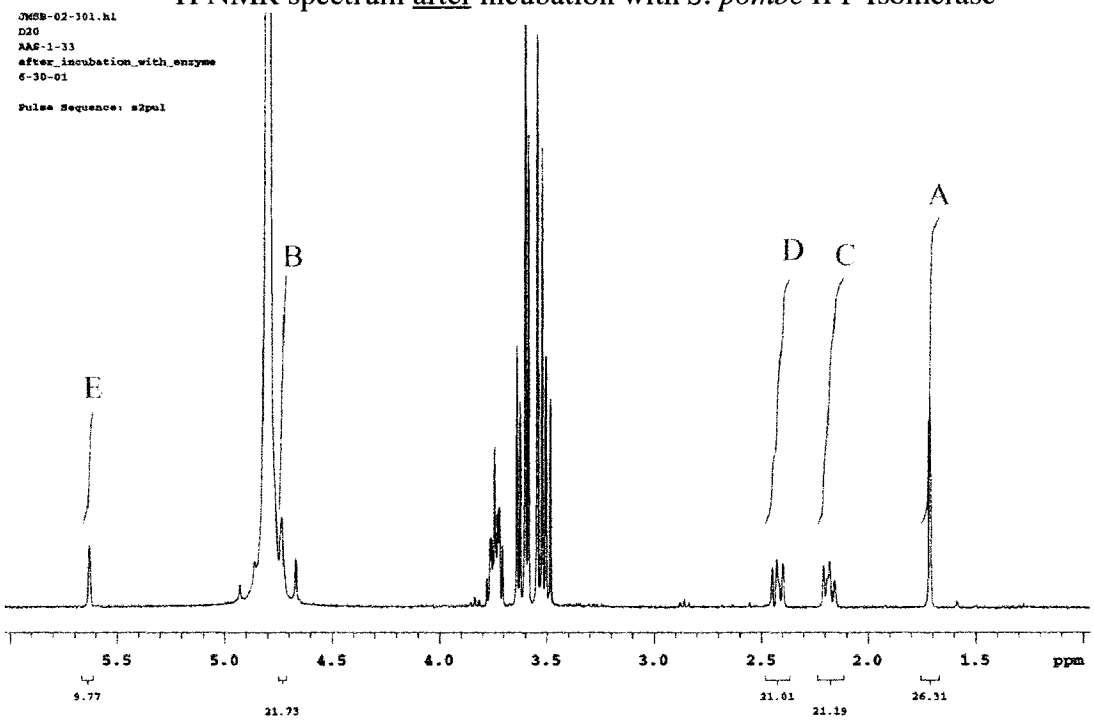
Some of the compounds described were also tested as inhibitors for rubber transferase, a *cis*-prenyltransferase that catalyzes the biosynthesis of natural rubber.[102] Inhibition studies against rubber transferase from *Hevea brasiliensis* and *Parthenium argentatum* were performed using a multiwell filtration system.[103] Similarly to the UPP synthase assay, this assay employs [1-^{14}C]-IPP. Initially, only two of the prepared analogs of IPP (**17** and **18**) were tested. Compound **18** at a concentration of 1 mM causes a 40% decrease in the incorporation of IPP by rubber transferase whereas compound **17** displays even poorer inhibition properties.

Figure 13. ^{1}H NMR spectra of compound **17** with IPP isomerase

^{1}H NMR spectrum before incubation with *S. pombe* IPP Isomerase

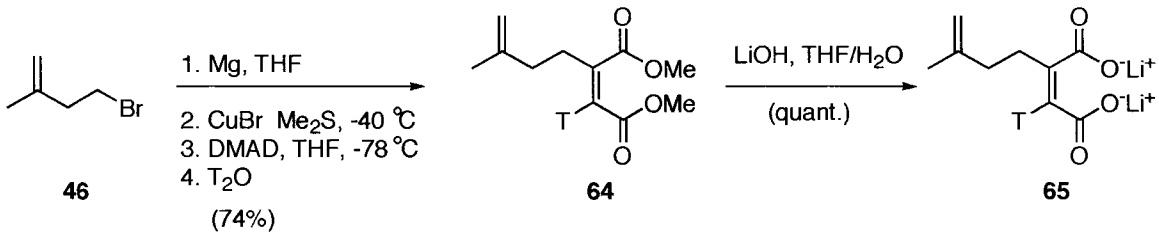


^{1}H NMR spectrum after incubation with *S. pombe* IPP Isomerase



Although these dicarboxylate-based inhibitors are competitive inhibitors of PFTase,[104] their mode of inhibition of rubber transferase is not clear. These compounds could either act as truly competitive inhibitors or as alternative substrates whose incorporation into the growing rubber chain could cause premature termination of biosynthesis. To probe the mode of inhibition, compound **65** was prepared with a tritium label (Scheme 14). The synthesis of the labelled compound was achieved by quenching the alkenylcopper adduct[105] with tritium oxide (*ca.* 5.7 Ci). Tritium labeled compound **65** was prepared at the National Tritium Labelling Facility (NTLF, Lawrence Berkeley Laboratories, Berkeley, CA) with the assistance of P. Williams and H. Morimoto. The tritium labelled analog (36 nCi) was incubated with rubber transferase and the rubber products were analyzed for radioactivity. There was no significant increase in radioactivity in the resulting rubber, which indicates that these compounds inhibit rubber transferase by competing with the natural substrate for the active site.

Scheme 14. Synthesis of ^{3}H analog **65**



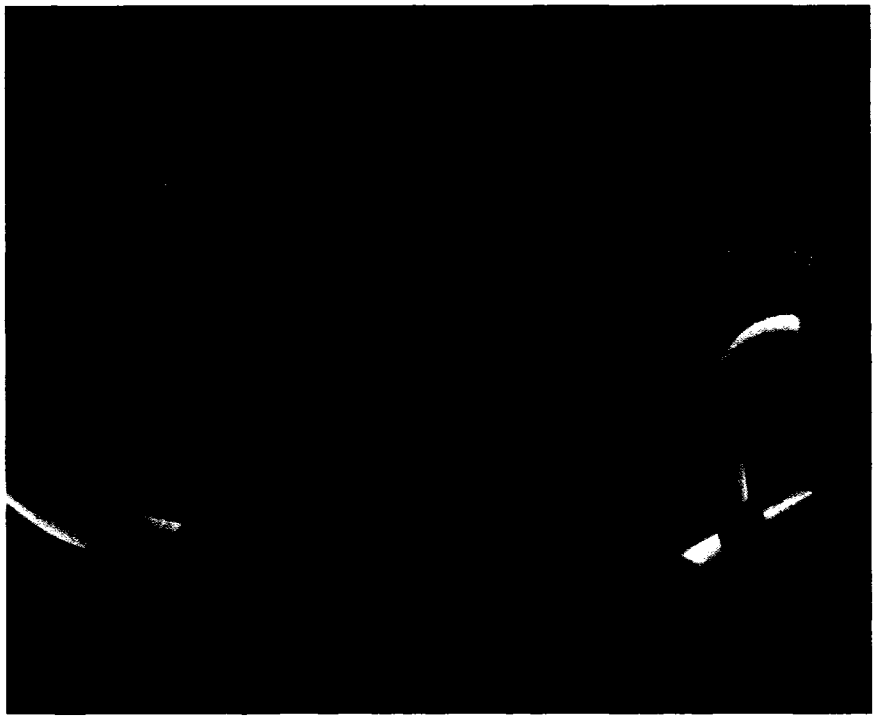
46 64 65

The lack of inhibition of these diphosphate mimics may be due to poor binding of the substrate to the active site of the enzyme. Recently, crystal structures of protein farnesyltransferase indicate that the oxygen atoms of the two phosphates in the bound

FPP molecule do not align and are at slight angles (70 °) with respect to each other.[41,106,107] Modeling studies with some of our dicarboxylate mimics in the active site of UPP synthase by Dr. Kamaljit Kaur indicate that the conformation of these compounds in solution may not allow for efficient binding to the enzyme's active site (Figure 14). These modeling studies illustrate that these inhibitors cannot achieve the same arrangement of the oxygen atoms of the dicarboxylate group as seen in IPP. As a consequence, they show poor inhibition of the enzyme. For binding to the UPP synthase active site, specific spatial arrangement of the diphosphate is likely to be required, and these dicarboxylates can not achieve this arrangement.

Figure 14. Modeling results of UPP synthase (courtesy of Dr. Kamaljit Kaur) a) X-ray structure of IPP bound in active site of UPP synthase b) modeling with compound **26**

a)



b)

2.3 CONCLUSIONS

In summary, several analogs of IPP containing a dicarboxylate moiety (**17-26**) were prepared and were shown to be modest to poor inhibitors of prenyltransferase enzymes. The poor inhibition observed for some of the compounds indicates that the specific spatial arrangement of anionic oxygens of IPP in the active site cannot be achieved by the carboxylate oxygens.

CHAPTER 3. Development of an Affinity Column for Purification of Prenyltransferase Enzymes

3.1 INTRODUCTION

With the advances in genomics and proteomics observed over the last fifteen years, the ability to isolate macromolecules that possess complex bio-molecular structure with high purity has gained great importance. Historically, protein purification is often labor intensive and requires multiple chromatographic steps. Recently, the development of selective adsorbents based on biological specificity has received great attention. New purification methods for the isolation and separation of biological molecules will not only give access to these substances, but will also enable scientists to have a better understanding of their biological and cellular function.

3.1.1 Chromatography

There is a variety of techniques available for the purification of bio-molecules, and these are often dominated by chromatography. Some examples of chromatography employed in protein purification are shown in Table 8. These include adsorption chromatography, ion-exchange chromatography, size exclusion chromatography and normal or reversed phase chromatography.[108] Chromatography of protein or other related biomolecules is characterized by low efficiency but with high selectivity. Typically, the

chromatography involves three distinct steps. First, the protein or analyte is captured onto a stationary phase, next the unbound material is washed out and finally altering the composition of the mobile phase effects the elution of the desired material.

Table 3. Some types of chromatography employed in protein purification

Name	Action Principle	Separation by
Adsorption chromatography	Surface binding	Molecular structure
Ion-exchange chromatography	Ionic binding	Surface charge
Size-exclusion chromatography	Size exclusion	Molecular size and shape
Affinity chromatography	Biospecific adsorption/desorption	Molecular structure
Hydrophobic chromatography	Hydrophobic complex formation	Hydrophobicity and hydrophobic patches
(Metal-) chelate chromatography	Coordination complex	Complex formation with transition metals
Normal-phase chromatography	Poalr complex formation	Polarity
Reversed-phase chromatography	Hydrophobic complex formation	Hydrophobicity

3.1.2 Affinity Chromatography

Affinity chromatography makes use of a bio-molecular recognition event to separate specific components within a sample.[109] Examples of these recognition events include the binding of an enzyme with an inhibitor or of an antibody with an antigen. The basic principle of affinity chromatography involves the immobilization of a ligand to an insoluble and porous support, which can then be used to selectively adsorb the analyte or

protein.[3] The sample of interest is applied to the affinity support under conditions in which the analyte would bind strongly to the immobilized ligand. This step is usually done at a pH and ionic strength that mimic the natural environment of the ligand and/or analyte. Because the ligand has high specificity for the analyte of interest, most other solutes in the sample have less binding affinity and quickly wash through the column. After these non-retained solutes have been removed, the retained analyte is dissociated by changing the pH or buffer composition of the mobile phase, or adding a competing agent to the mobile phase. Once the analyte has been isolated, reapplying the initial buffer can regenerate the column.

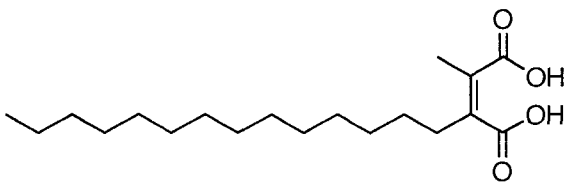
The design of the immobilized ligand is crucial for any affinity chromatographic method.[110] The ligand is designed to possess high selectivity for binding to the biomolecule of interest, while having a low affinity for other solutes in the sample. Furthermore, studies of the purification of β-galactosidase demonstrated the importance of having a hydrocarbon chain between the ligand and the backbone of the solid support.[111] No binding of enzyme was observed with agarose based affinity columns, which had the ligand attached directly to the matrix. Subsequent experiments showed that increased binding of the enzyme was observed as the distance increased between the ligand and the agarose backbone increased. Once the ligand has been selected, it should be immobilized to the solid matrix through a stable covalent bond to avoid progressive leaching and loss of binding capacity.[110]

Historically, research on agarose and cyanogens pioneered by Porath and co-workers gave rise to new chromatographic supports and ligand immobilization chemistry, which were instrumental in the development of affinity chromatography as a technique for the purification of biological macromolecules.[112,113] The first use of affinity chromatography was by Anfinsen, who purified staphylococcal nuclease using porous gel technology.[114] Since then, increasingly sophisticated tools for affinity chromatography have been developed, including more rigid matrices such as cross-linked beaded agarose and cellulose, polymeric supports silica and controlled pore glass.

3.1.3 Project Goals

The advances in proteomic and genomic research have encouraged scientists to discover more efficient methods for the purification of biological macromolecules, specifically enzymes. As discussed earlier, chaetomellic acid A (**43**) (Figure 15) is a inhibitor of PFTase.[44] The goal of this research project is to develop a new affinity column for the purification of prenyltransferase enzymes, for example, Ras protein farnesyltransferase (PFTase), which is an important therapeutic target of anti-cancer drugs.

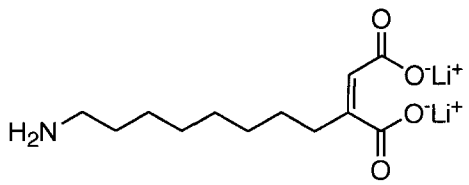
Figure 15. Structure of chaetomellic acid A (**43**)



43 Chaetomellic acid A

Thus, the objective of this project is the preparation of the ligand **36** as a potential moiety for the purification of PFTase (Figure 16).

Figure 16. Dicarboxylate based affinity ligand **36**

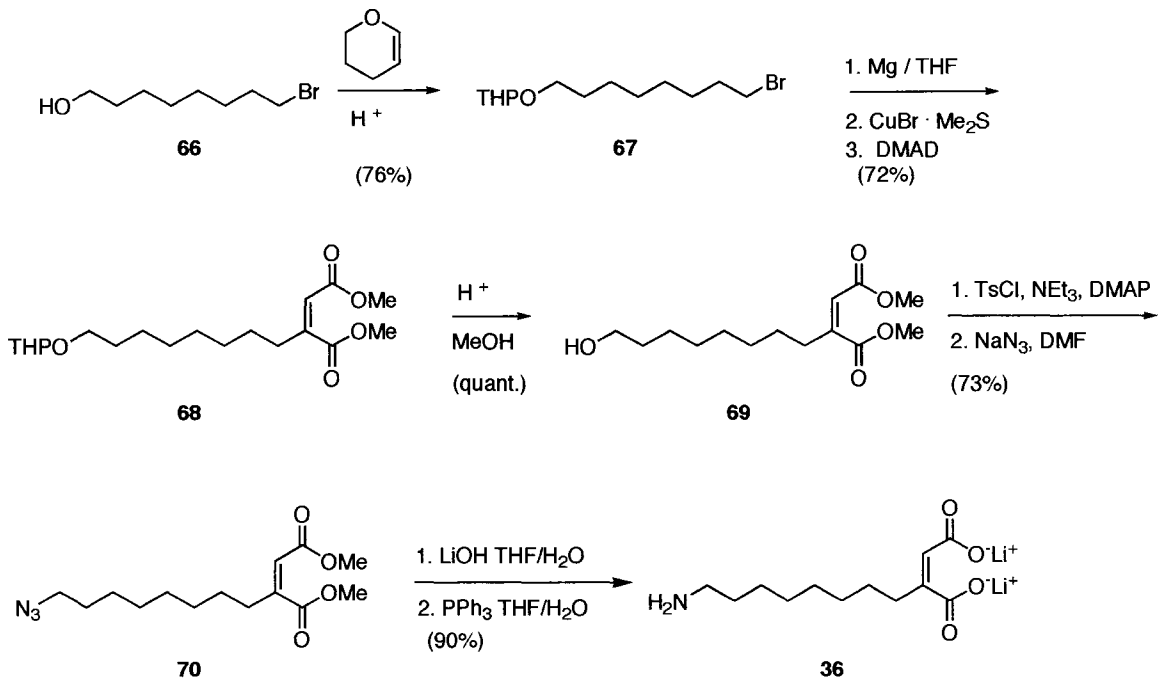


36

3.2 RESULTS AND DISCUSSION

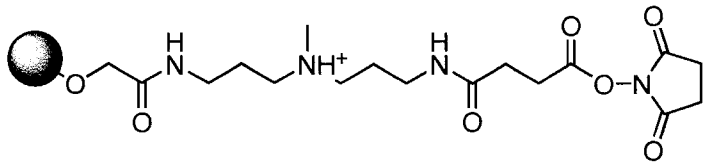
As mentioned previously, chaetomellic acid A (**43**) and a variety of analogs were recently synthesized and were shown to be competitive inhibitors of PFTase.[44] Their syntheses involve the conjugate addition of an organocuprate to dimethyl acetylenedicarboxylate (DMAD), followed by aqueous workup. Therefore, the synthesis of the ligand **36** employs this transformation as a key step (Scheme 15). Treatment of 8-bromooctan-1-ol (**66**) with dihydropyran under proton catalysis provides the THP ether **67**. Formation of the Grignard reagent followed by treatment with copper bromide-dimethyl sulfide complex gives the corresponding organocuprate. Reaction with DMAD provides the substituted maleyl ester **68** in 72% isolated yield. Deprotection of the hydroxyl group by treatment of the THP ether with PTSA yields the alcohol **69**. The maleyl-substituted octanol **69** is transformed into its corresponding azide **70** using a two-step procedure. Base hydrolysis of the di-ester **70** to its di-lithium salt, followed by reaction of the salt with triphenyl phosphine in THF/water gives the desired amine **36**. The overall yield for the entire sequence is 36%.

Scheme 15. Synthesis of ligand **36**



With the synthesis of the ligand completed, the coupling to the affinity support was studied. Affigel-15 (Bio-Rad Laboratories) was selected as the solid support, based on several criteria. This solid support consists of cross-linked agarose beads linked to a positively charged spacer arm through an ether bond (Figure 17). An *N*-hydroxysuccinimide ester of the free end allows acylation of amines such as **36**. Affigel-15 (75-300 μm pore size) is useful as a solid support because its chemical resistance to acid and base virtually eliminates ligand leakage from the column during storage. Furthermore, proteins can be coupled with high efficiency in either aqueous or anhydrous solutions.

Figure 17. Structure of Affigel-15



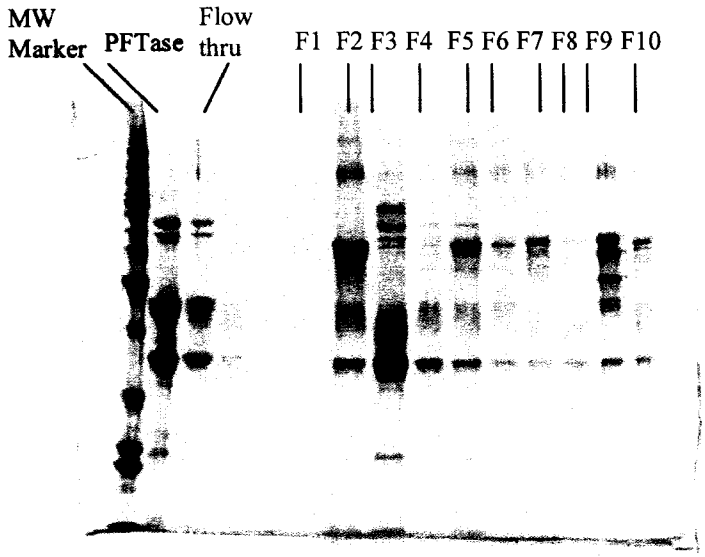
Affigel-15

The ligand was coupled to Affigel-15 using standard coupling conditions. The chaetomellic acid analog was immobilized by treatment of a suspension of Affigel-15 with a solution of the ligand **36** in 0.1 M $NaHCO_3$. In collaboration with Professor Dale Poulter (University of Utah), this affinity support was tested for the purification of PFTase.

A purified sample of PFTase was loaded onto the column and washed with the elution buffer (52 mM Tris pH 7.0, 5.8 mM DTT, 12 mM $MgCl_2$ and 12 μM $ZnCl_2$). Several fractions were collected, and analysis indicates that no active enzyme or protein is present in the eluted fractions. It was possibile that this particular buffer composition was not able to effect dissociation of the enzyme from the affinity support. Therefore, a solution of chaetomellic acid A was employed to elute PFTase, since it is a potent inhibitor of PFTase. Fractions from the elution with chaetomellic acid A were analyzed by SDS-PAGE gel electrophoresis and were shown to contain PFTase (Figure 18). The second lane corresponds to the PFTase sample that was loaded onto the affinity column. Lanes labeled F1 through to F10 correspond to the ten fractions that were collected. Appropriate fractions were dialyzed in buffer containing 50 mM tris and 10 mM β-

mercaptoethanol (BME) and subsequent enzymatic assays indicate that these fractions contain active PFTase. This experiment demonstrates that the dicarboxylate-based ligand **36** is able to bind to PFTase and the binding can be disrupted by treatment with chaetomellic acid A.

Figure 18. SDS-PAGE analysis of affinity column fractions (F1-F10) for presence of PFTase.[a]



[a] Lane 1 is broad range MW marker; Lane 2 is sample of PFTase loaded onto affinity column; Lane 3 is flow thru from column; Lane 6-15 are fractions F1-F10 that were collected from eluent of affinity column.

Because PFTase can bind to the prepared affinity support, the purification of crude enzyme homogenate was attempted. Unfortunately, no binding of PFTase was observed when a crude cellular homogenate of the enzyme was applied to the column. Other impurities found in the crude enzyme preparation may prevent efficient binding of the enzyme to the immobilized ligand.

3.3 CONCLUSION

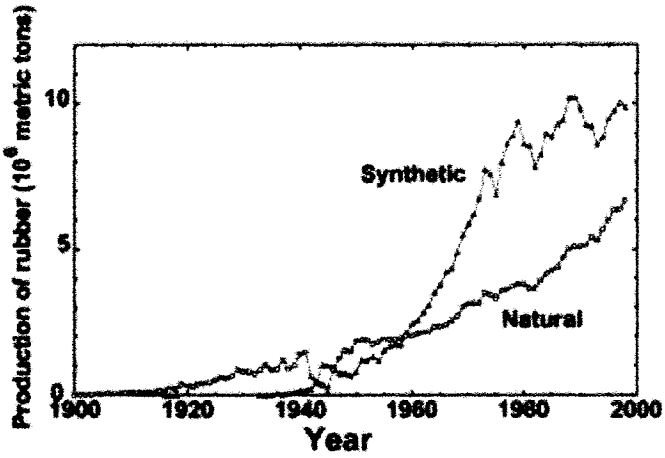
In summary, the chemical synthesis of a dicarboxylate-based ligand **36** for the purification of prenyltransferases is achieved. Although an affinity column consisting of Affigel-15 and the ligand **36** bound can bind PFTase, further work is required for its development as a new method for the purification of prenyltransferase enzymes. Furthermore, this work indicates that compounds consisting of a lipid bearing maleyl dicarboxylate bind to protein prenyltransferase with high affinity.

CHAPTER 4. Rubber Biosynthesis: Development of New Crops for Rubber Production

4.1 INTRODUCTION

Natural rubber, or *caoutchouc* (from Indian: caa = tears; ochu = tree; cahuchu = weeping tree), generally refers to a coagulated or precipitated product obtained from the latex of rubber plants. It forms non-linked, partially vulcanizable isoprene chains having molecular weights up to 10^6 Da that are useful for production of elastomers.[115] Joseph Priestley coined the name rubber, after using it to rub out pencil marks. Rubber is a vital and strategic raw material that is utilized in the manufacturing of more than 40,000 consumer products, including 400 medical devices.[116] The high performance properties of natural rubber such as elasticity, resilience, heat dispersion, and abrasion resistance have not been easily duplicated due to its chemical structure and high molecular weight (> 10^6 Da).[117] The demand for natural rubber has increased throughout the twentieth century, despite the availability of synthetic rubbers (Figure 19). In 2004, the global production of natural rubber was 8.4 million tons, of which 40% was imported by the United States, China and Japan.

Figure 19. Global production of natural and synthetic rubber.[116] Reproduced by permission of The Royal Society of Chemistry



4.1.1 History of Rubber

Throughout civilization, rubber has been an integral part of human history. As early as 1600 BC, the ancient Mesoamerican culture processed latex from the *Castilla elastica* tree using juice from *Ipomoea alba* (morning glory vine).[118] This material was then used to make a variety of rubber products including solid rubber balls, solid and hollow figurines and rubber bands. In addition, they used liquid rubber for medicines, painting, and traditional ritual ceremonies. Indigenous tribes of South and Central America were the first people to produce rubber from the latex of *Hevea brasiliensis*.[119] In 1521, Spanish explorers discovered that rubber was useful for waterproofing utensils and making balls for their ritualistic games. For over 300 years, rubber was used in Europe only as balls or other minor applications.

It was not until 1750 that Francois Fresneau made the first systematic observations on rubber, describing the whereabouts of the *H. brasiliensis* tree and providing methods of latex tapping and rubber preparation.[120] Significant commercial use of rubber did not occur until 1818, when inventor James Hancock developed solvent processing.[121] Soon after, the manufacturing of rubber products began in England. Some early rubber products included the raincoat of Charles Macintosh in 1823 and waterproof shoes. Unfortunately, it was found that these rubber products were inferior because they were unsuitable for the climatic extremes of North America, becoming soft in the summer and brittle in the winter. In a serendipitous discovery by Charles Goodyear, it became known that a mixture of latex, sulfur and lead carbonate, after vulcanization, yields a substance that is firm at normal and high temperatures yet flexible at cold temperatures.[122,123] Vulcanization is a chemical reaction between sulfur and the double bonds found in the hydrocarbon chain of rubber. Alternatively, vulcanization is also achieved by employing organic peroxides or radiation, but these vulcanized materials possess lower long-term stability since the polymer chains are cross-linked solely by carbon bonds.

In 1876, nearly 70,000 seeds from *H. brasiliensis* were smuggled out of Brazil, which held a monopoly of the world's rubber production. The British started a plantation of *H. brasiliensis* in Ceylon (Sri Lanka) and Malaya. In retrospect, this endeavor was fortunate because in the early 1900's the fungus *Microcyclus ulei* destroyed the original plantation of South America.[124] The South American leaf blight (SALB), caused by *M. ulei*, has virtually wiped out all commercial plantations in South America, and as a result, this area has ceased to be a major rubber producer. Presently, annual natural rubber

production in the world is estimated to be 8.6 million tons, with ~78% of this being produced in Thailand, Indonesia and Malaysia.

The first example of synthetic rubber was produced in 1910. German chemistry professor Carl Dietrich found that sodium metal catalyzed the polymerization of 2,3-dimethylbutadiene to a synthetic rubber called "methyl rubber". Later, other synthetic rubbers were developed, including the invention in 1927 by scientists at Bayer of Buna S (butadiene-natrium-styrene) rubber. However, it was not until 1950, after the development of a Ziegler catalyst composed of titanium tetrachloride and alkylaluminum, that polyisoprene could be synthesized stereospecifically, in either the *cis* or *trans* configurations. With current technology, it is possible to produce synthetic polyisoprene that possess physical properties similar to those of natural rubber with a purity of 98 to 99%. These synthetic *cis*-polyisoprenes display different properties from natural rubber, such as stress stability, processability, despite the similarity of its structure and molecular weight to natural rubber.

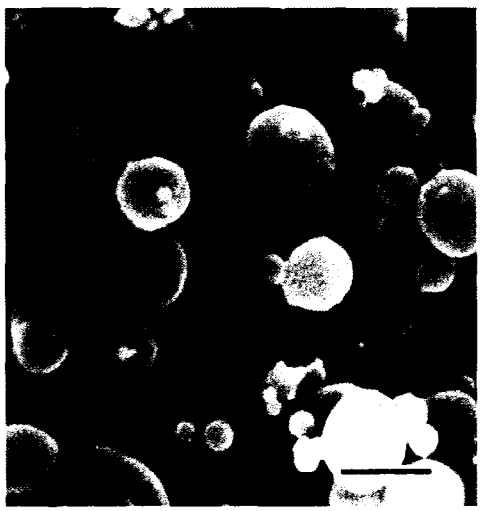
4.1.2 Rubber Biosynthesis

Rubber containing latex is produced in more than 2500 species of plants, including 300 genera of Angiosperms, with varying degrees of quality and quantity.[125] The rubber-producing plant families are *Apocynaceae*, *Asteraceae*, *Asclepiadaceae*, *Euphorbiacae*, *Loranthaceae*, *Moraceae*, and *Sapotaceae.* Rubber, the major component of polyisoprene in latex, has no clear physiological role within the plant and the exact function of latex is

not understood. Several hypotheses have been proposed for the appearance and function of latex and rubber within plants but none have been proven. Rubber does not serve as an energy source, despite tremendous resources being allocated to its formation. Although certain microbes are able to degrade rubber,[115] there is no biochemical evidence that plants possess the enzyme capable of degrading rubber and it appears that once rubber is formed it is sequestered in the cells.[125]

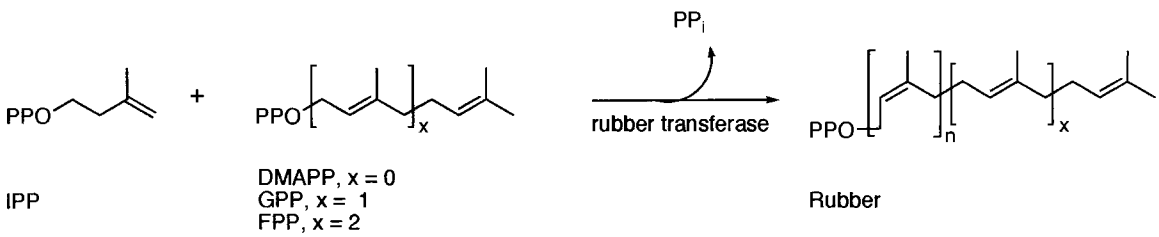
Rubber polymers are compartmentalized within subcellular, membrane bound, rubber particles that are located within the cytosol of cells. The rubber particles can reside in either specialized cells that produce latex (laticifiers), as found in *H. brasiliensis,* or in unspecialized cells, such as the bark parenchyma of *P. argentatum.*[102] The overall structure of rubber particles is similar in all species investigated and consists of a homogenous rubber core surrounded by an intact monolayer membrane.[126] However, there are inter-species variations in the average size of the particles, in fact, differently sized subsets of particles are found in *H. brasiliensis* with mean diameters of 1.0 and 0.2 μm (Figure 20).[127]

Figure 20. SEM of *H. brasiliensis* rubber particles. Magnification bar = 2 μm (from Wood and Cornish[127])



Natural rubber, or *cis*-1,4-polyisoprene, is produced by a rubber transferase (EC 2.5.1.20), a membrane bound *cis*-prenyltransferase.[128,129] This enzyme catalyzes the polymerization of isopentenyl diphosphate (IPP) units with an allylic diphosphate of the growing rubber chain to form rubber (Scheme 16). The particle-bound rubber transferase requires an allylic diphosphate (APP) for initiation of rubber biosynthesis, along with sufficient IPP for elongation and a divalent cation such as Mg^{2+} or Mn^{2+} as an enzyme co-factor.[128,130-134] *In vitro* experiments have shown that a variety of APP's can be accepted as the initiator of rubber polymerization. However, experimental data indicates that FPP is likely the sole initiator *in vivo*, which is directly supported by ^{13}C-NMR data indicating that the molecular structure of natural rubber has a farnesyl tail.[132] Although rubber transferase can use a number of divalent metal ions as cofactors, Mg^{2+} is the primary cofactor *in vivo*, as its physiological concentration is three orders of magnitude higher than Mn^{2+}.[131]

Scheme 16. Rubber biosynthesis



The control of the molecular weight of rubber can be accomplished by alteration of concentration of either IPP or APP.[135,136] For *H. brasiliensis*, *P. argentatum* and *Ficus elastica*, an increase in IPP leads to an increase in molecular weight. However, increasing FPP concentration results in rubber with lower molecular weight at all IPP

concentrations. Furthermore, it has been shown that the metal ion cofactor concentration affects the rate of IPP incorporation. At low concentration, metal ion de-inhibits rubber transferase while at higher concentrations it has an inhibitory effect on rubber transferase activity.[137]

In vitro experiments have indicated that *F. elastica* is able to produce rubber with twice the molecular weight of that produced by *H. brasiliensis* and *P. argentatum,* when using the identical IPP and FPP concentrations.[136] However, *in vivo*, *F. elastica* produces rubber with a significantly lower molecular weight than that of the other rubber producing species, suggesting the existence of another method of controlling molecular weight besides IPP and FPP concentrations. Recently, Keasling and co-workers hypothesized an explanation for this phenomenon.[137] They have shown that the affinity of rubber transferase for IPP varies by more than two orders of magnitude depending on the Mg^{2+} concentration. Therefore, if *H. brasiliensis* is able to regulate cytosolic metal ion concentration *in vivo*, this could be exploited to control both the rate of rubber production and molecular weight of rubber. There is a significant difference in the endogenous Mg^{2+} concentration between rubber producing species *H. brasiliensis* (12 mM) and *F. elastica* (53 mM),[131] which might be one of the reasons that *H. brasiliensis* produces a higher molecular weight rubber *in vivo.*[137] Recent studies on a related prenyltransferase, UPP synthase, support this hypothesis.[41] Moreover, they suggest that Mg^{2+} or Mn^{2+} may act as a regulatory factor to control prenyltransferase activity and product chain length.

4.1.3 Plants Species that Naturally Produce Rubber

Although there are more than 2500 plant species that produce rubber, *H. brasiliensis* is one of the few that produces the high molecular weight rubber required for high quality and high product performance.[138] Since molecular weight is intimately related to quality, *H. brasiliensis* is the primary commercial source of natural rubber. A fundamental problem with the reliance of natural rubber from *H. brasiliensis* is the occurrence of life threatening, IgE-mediated, latex allergy caused by the proteins in the latex.[139-142] Methods to remove these allergenic proteins from the latex were developed to produce condoms and latex gloves with decreased protein contents (less than 20 μg/g of natural rubber). Furthermore, the rubber tree is genetically vulnerable to disease. Plantations around the world were established by clonally propagating a small number of genetically related individuals collected from a single geographic region in the upper Amazon.[143] Trees from this collection are susceptible to the South American leaf blight fungus that effectively eliminated the capability of Central and South America to grow rubber tree plantations. Although leaf blight has not reached Southeast Asia, with the recent increase in global commerce and travel, it is only a matter of time before the pathogen arrives in other rubber producing regions. Another issue regarding *H. brasiliensis* rubber occurred in 2002, where Thailand, Malaysia, and Indonesia, the main rubber producing countries, formed an OPEC-like rubber triumvirate, whose sole purpose is to control rubber production and drive up rubber commodity prices.[144] Hence, there is considerable interest in developing an alternative commercial source of high quality

rubber from other plant species. Table 4 provides an overview of some of the more prominent plants that produce polyisoprene.

Table 4. Prominent plant species that produce rubber (adapted from Mooibroek and Cornish[117])

Species	Common Name	Native or cultivation area	Comments
Asclepias spp.	Milkweeds		
A. linaria	Milkweed	Arizona	MW not determined
A. speciosa	Showy milkweed	Utah	*Cis*-1,4-units: 2.2% of hexane extract (1% of plant)' MW 5.2 x 10^4 Da
A. syriaca	Common milkweed		*Cis*-1,4-units
Castilla spp.	Panama Rubber	South America	10 species, latex bleed usually causes death
Euphorbia spp.	Spurge		13 species
Ficus spp.			
F. elastica	Indian rubber tree	Nigeria	Yield 1,4-polyioprene (w/v) of latex): 24.8 %
F. ovata		Nigeria	Yield 1,4-polyioprene (w/v) of latex): 20.4 %
F. pumila		Nigeria	Yield 1,4-polyioprene (w/v) of latex): 14.7 %
F. volgelii		Nigeria	Yield 1,4-polyioprene (w/v) of latex): 28.1 %
Helianthus annuss	Sunflower	United States	14 subspecies produce rubber in leaves and contain 0.93% DW
H. brasiliensis	Brazilian rubber tree	South-east Asia	Mw: 5x10^5 – 2x10^6 Da; Yield 1,4-polyioprene (w/v) of latex): 44.3 % First species to yield commercial latex for rubber
Landolphia spp.			
L. Dulcis		Nigeria	ND
L. Dulcis		Nigeria	Yield 1,4-polyioprene (w/v) of latex): 24.2 %
Manihot glaziovii	Ceara rubber tree	Brazil	ND
Manilkara zapota	Chicle	Mexico	ND
P. argentatum	Guayule	Chihuahuan desert	MW:>10^6 Da; rubber content 0.7-5.5 mg/g DW from August-December
Solidago spp.	Goldenrod		Successfully applied during WWII
S. altissima	Goldenrod		ND
S. ridellii			ND
Taraxacum kok-saghyz	Russian Dandelion	Russia	Successfully applied during WWII, MW: 3x10^5 Da

Although there are many examples of plants that are able to produce *cis*-polyisoprene, there are only a few plant species that are found to synthesize polyisoprenes in the *trans* configuration. Chicle (*Manikara zapota*), gutta-percha (*Pallaquium gutta*), and balata (*Manikara bidentata*) are typical representatives of *trans*-polyisoprene-synthesizing plants.[115] Gutta-percha and balata produce *trans*-polyisoprenes with high molecular weights (1.4×10^5 to 1.7×10^5).[145,146] The chicle tree is unique, because it produces latex with about equal amounts of *cis*- and *trans*-polyisoprenes.[147]

Besides these higher plants, it is interesting to note that some species of the fungi genera *Lactarius*, *Peziza*, *Russula* and *Hygrophorus* have also been reported to produce latex that contains low molecular weight *cis*-polyisoprene.[148,149] ^{13}C-NMR analysis of rubber isolated from both *Lactarius volemus* and *Lactarius chrysorrheus* indicates it consists of one dimethylallyl group, two *trans*-isoprene units, 160-300 *cis*-polyisoprene units and a terminal hydroxyl or ester group.[150] Presently there are no known bacterial or yeast strains that can synthesize polyisoprene naturally, although many do possess the isoprenoid pathways to produce the rubber precursors, isopentenyl diphosphate (IPP), dimethylallyl diphosphate (DMAPP) and farnesyl diphosphate (FPP).

Since there was a worldwide shortage of natural rubber during World War II, rubber was very strategically important. At that time, *Solidago altissima* (goldenrod) and *Taraxacum kok-saghyz* (Russian dandelion) were used successfully for the production of rubber for military tires.[117] Unfortunately, these plants were not further developed for large-scale rubber production due to their poor agronomic performance. Among the

alternative sources of natural rubber there has been considerable interest in the desert shrub guayule, *Parthenium argentatum*. It is currently under commercial development as a source of hypoallergenic latex but it has two considerable drawbacks. *P. argentatum* produces high quality of rubber in its bark and the entire tree must be processed for processing of its latex. Furthermore, highly specific winter temperatures are required for efficient rubber production. Therefore, it is unlikely that guayule can provide sufficient rubber for domestic uses.[102]

In contrast, lettuce (*Lactuca* spp.) may be an excellent candidate for rubber production. The molecular weight of rubber from lettuce is similar to that of *H. brasiliensis* and the isolation of rubber particles can occur early in development, in addition, lettuce has the advantage of a vast genetic, genomic and transformation systems that allow more efficient modeling, manipulation and selection.[151]

In 1913, Charles Fox reported the analysis of latex from two lettuce species, *L. canadensis* and *L. scariola*.[152] Both species were found to secrete an abundance of latex from all areas of the plant, especially the stem. Rubber content in the latex was determined to be 2.2% in *L. canadensis* and 1.6% in *L. scariola*. In 1942, researchers at the Clemson Agriculture Centre analyzed a variety of plants indigenous to South Carolina for rubber content.[153] In this study, *L. sagittifolia* rubber content was 0.2% of plant dry weight while *L. scariola* had between 0.3 and 0.4% rubber. These reported yields of polyisoprene are unsuitable for comparison due to inconsistency in terminology, tissues used and time of harvest. The variability of rubber production in terms of quantity and

molecular weight differences among lettuce genotypes has been reported in a production report from the Agriculture Research Service of the United States Department of Agriculture.[154] The report cited large differences in rubber content in the latex of *L. sativa* and *L. serriola*, but minimal differences between the percentages of rubber in the whole plants. However, no information was given about the developmental stages of the samples, the *L. sativa* cultivar, and the condition of the tissue previous to extraction. Moreover, they reported higher amounts of rubber in *L. serriola* leaves than stems. Recent collaborative efforts by Dr. Shaun Bushman at the USDA have indicated that there is much more latex in the stems than in the leaves. These discrepancies highlight the need to determine the spatial and temporal variability for rubber production and rubber quantity differences among lettuce genotypes.

All rubber producing species have a rubber transferase enzyme and perhaps a group of other necessary proteins. However, the remainder of rubber particle-associated proteins are considered to be species specific.[102] Through *in vitro* labeling studies, rubber particles removed from the cytoplasm were shown to synthesize rubber if provided with FPP and IPP,[129] suggesting that the enzymes necessary for rubber biosynthesis remain with the rubber particles. The protein fraction of *H. brasiliensis* rubber particles has about 80 proteins, wheras other rubber producing plants are reported to have much less.[116] Biochemical evidence has led to the assumption that the rubber transferase enzyme is the key activity needed for rubber production in plants. However, no rubber transferase encoding genes have been cloned, and the final steps in rubber biosynthesis have not yet been elucidated.

4.1.4 Project Objectives

The first objective of this research is to systematically investigate rubber production in two lettuce species, *L. serriola* and *L. sativa*. This part of the project would determine the following:

- Whether the polyisoprene in lettuce species is truly "natural" rubber (*cis*-polyisoprene) as opposed to *trans*-polyisoprene.
- Rubber content in latex of both lettuce species.
- Molecular weight of the major polyisoprene.

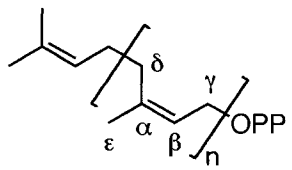
A second objective of this project is the identification of key proteins involved in rubber synthesis in a variety of rubber producing plant species. This portion of the project was completed in collaboration with Dr. Xie Shaung (USDA) and Dr. Shaun Bushman (University of Georgia).

4.2 RESULTS AND DISCUSSION

4.2.1 ^{13}C-NMR Analysis of Rubber

Samples of latex were collected from both *L. serriola* and *L. sativa*. Latex was collected both in collaboration with Dr. Shaun Bushman from the University of Georgia and independently in our own laboratory. The latex was precipitated in an aqueous buffer solution by tapping the lettuce plants and allowing latex to drip into the buffer solution. The samples were centrifuged and the settled material was isolated. Initially, the molecular structure of the major polyisoprene in the latex isolated from each lettuce species was determined by ^{13}C-NMR analysis. *Cis* and *trans*-polyisoprene can be differentiated by the use of ^{13}C-NMR spectroscopy (Table 5).[155] The ^{13}C NMR resonances of the methyl group and the δ carbon of rubber can be used to determine if the double bond geometry of the polyisoprene is *cis* or *trans*.

Table 5. ^{13}C NMR data of *cis* and *trans*-polyisoprene (values in ppm)



	α	β	δ	ε	γ
Cis	135.5	125.3	32.5	23.6	27.0
Trans	135.2	124.6	40.0	16.0	27.1

Initially, the magic angle spinning (MAS) ^{13}C NMR spectra (collected by Dr. Guy Bernard in collaboration with the research group of Professor Rod Wasylishen, University of Alberta) of rubber from both *H. brasiliensis* and *P. argentatum* were recorded (Figure 21). The spectra indicated that the samples did not contain any detectable impurities and the peaks were well resolved. For comparison, the spectrum of synthetic *cis*-polyisoprene is included. However, ^{13}C-NMR analysis of rubber isolated from *L. serriola* indicated that the samples contain a large amount of contaminating material (Figure 22). It was initially proposed that this was due to presence of resin material, which may be isolated during the precipitation of the rubber latex.

4.2.2 Quantification of Rubber

Solution ^{13}C-NMR analysis of rubber in CD_2Cl_2 indicates that there is a large amount of *cis*-polyisoprene along with some resin and other contaminating material (Figure 23). The removal of the resin material from the rubber samples is necessary. It is known that polyisoprene is insoluble in acetone[152] while, plant resin material is soluble in acetone.[156,157] Hence, a purification of the rubber by washing with acetone was attempted. Indeed, washing of the rubber with acetone results in the removal of the majority of contaminating material. ^{13}C-NMR indicates that the remaining material consists primarily of *cis*-polyisoprene. Analysis of rubber isolated from *L. sativa* shows similar results.

Figure 21. ^{13}C NMR magic angle spinning (MAS) spectra (50.331 MHz) of samples of rubber from three samples (a) *H. brasiliensis* (b) *P. argentatum* (c) synthetic *cis*-1,4-polyisoprene. (spectra obtained by Dr. Guy Bernard)

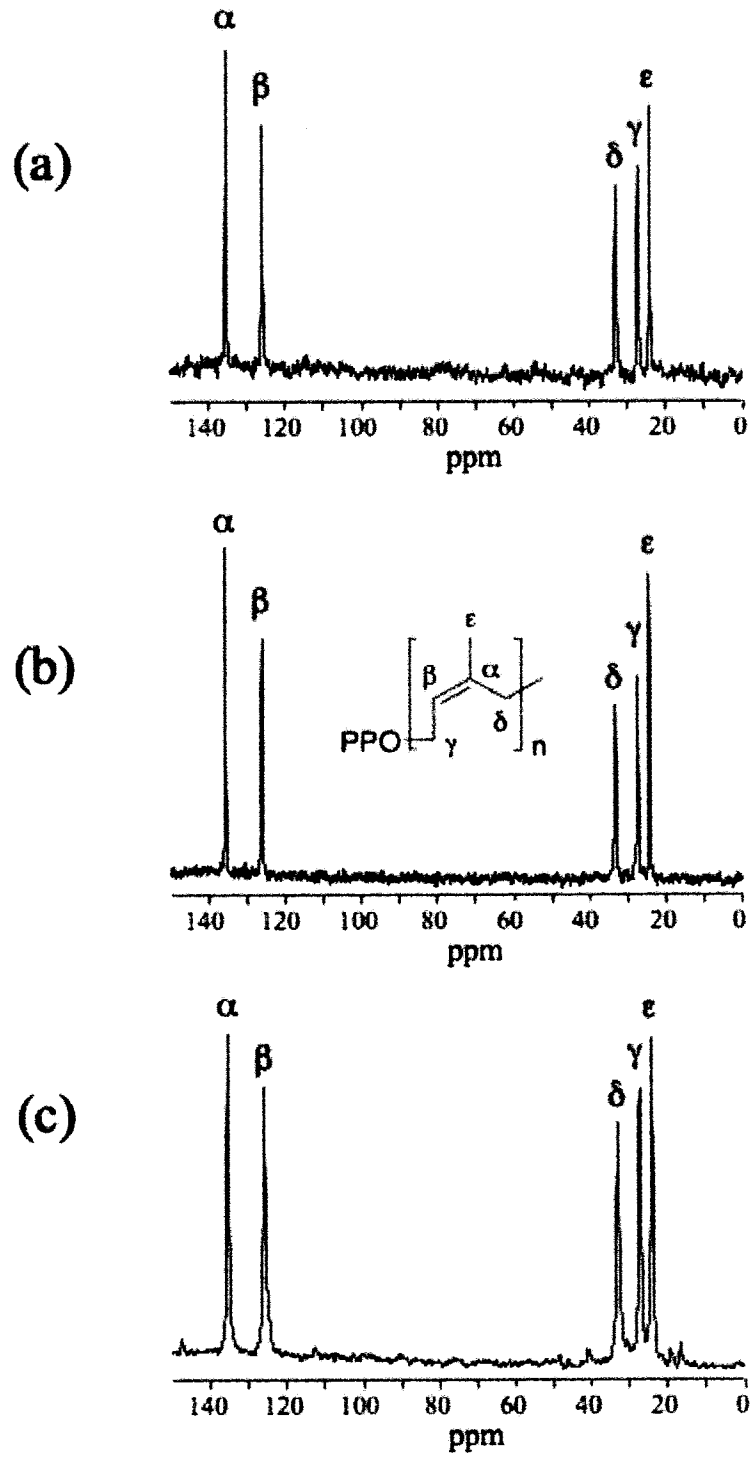


Figure 22. MAS ^{13}C-NMR spectrum of *L. serriola* (spectra obtained by Dr. Guy Bernard)

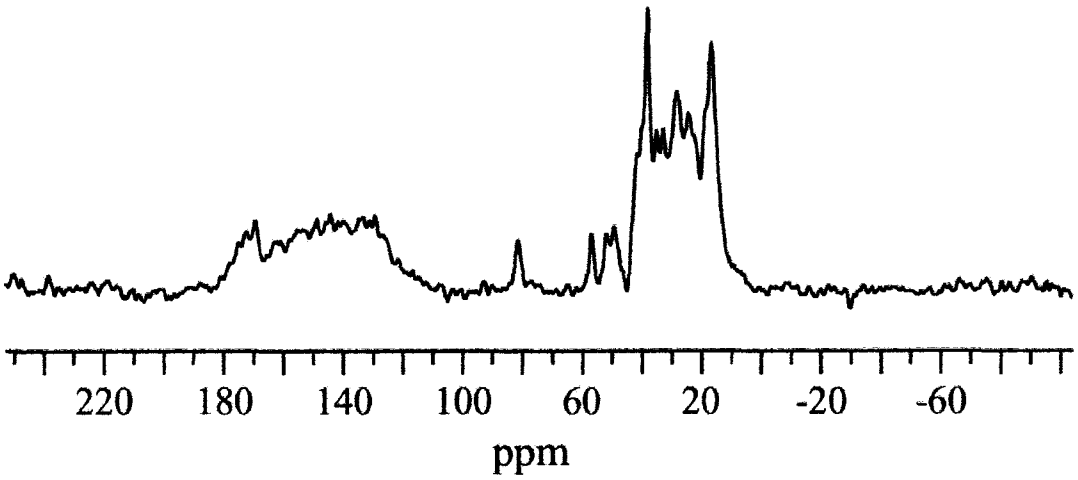


Figure 23. Solution ^{13}C-NMR spectra of rubber from *Lactuca serriola* a) Rubber dissolved in CD_2Cl_2 b) sample washed with acetone

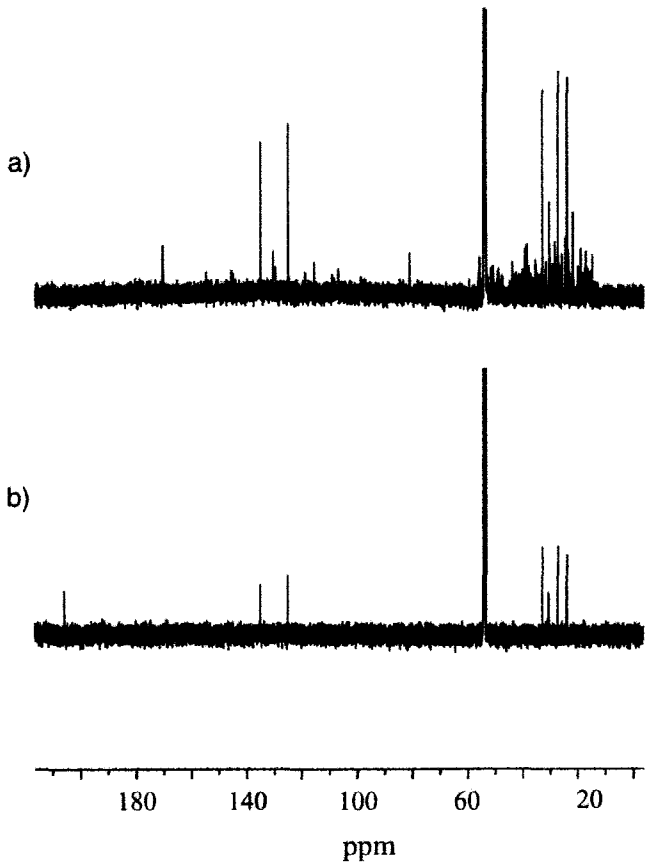
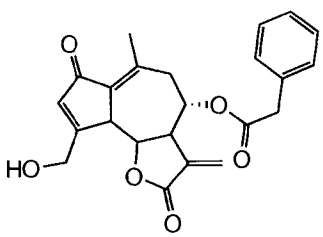


Table 6 shows the relative amounts of rubber and resin in the solid material isolated from both *L. serriola* and *L. sativa*. In comparison to *H. brasiliensis*, which contains about 4% resin, the precipitated rubber material contains much more resin material. Rubber isolated from consumer lettuce (*L. sativa* variant) that would normally be consumed as food contains about 11% rubber with nearly 89% resin. The acetone extracts containing the resin material could also be analyzed by reverse phase HPLC spectrometer coupled to a mass spectral analyzer. Identification of some of the constituents of the resin material show them to be known[158] guaianolide sesquiterpene lactones (Figure 24). In addition to the rubber hydrocarbon, the latex would be typically composed of a variety of proteins, lipids and carbohydrates.[159]

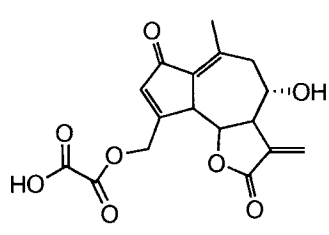
Table 6. Analysis of latex from *L. serriola*, *L. sativa* and edible lettuce for relative amounts of rubber and resin.

Sample	Rubber Percent	Resin Percent
L. serriola	49 ± 5	51 ± 5
L. sativa	47 ± 2	53 ± 2
Edible lettuce	11 ± 2	89 ± 2

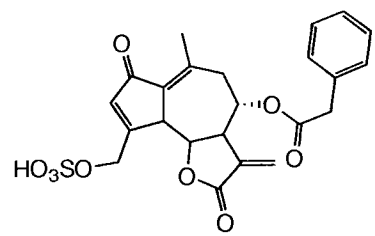
Figure 24. Sesquiterpenes isolated from *L. serriola* and *L. sativa*



lactucopicrin



lactucin-15-oxalate



15-(4-hydroxyphenylacetyl)-lactucin-8-sulfate

4.2.3 Molecular Weight and Particle Size Analysis

In collaboration with the Agriculture Research Service (ARS) at the United States Department of Agriculture (USDA) the molecular weight of rubber and particle size from both lettuce species were determined. The molecular weight of rubber from *L. serriola* and *L. sativa* are shown in Table 7. The data shows that the rubber produced from both species of lettuce is of high quality with molecular weight distributions that are similar to that of *H. brasiliensis* and *P. argentatum*. The data also indicates that the number average molecular weight (M_N) and weight average molecular weight (M_W) of the rubber from each species are very similar and display minimal polydispersity.

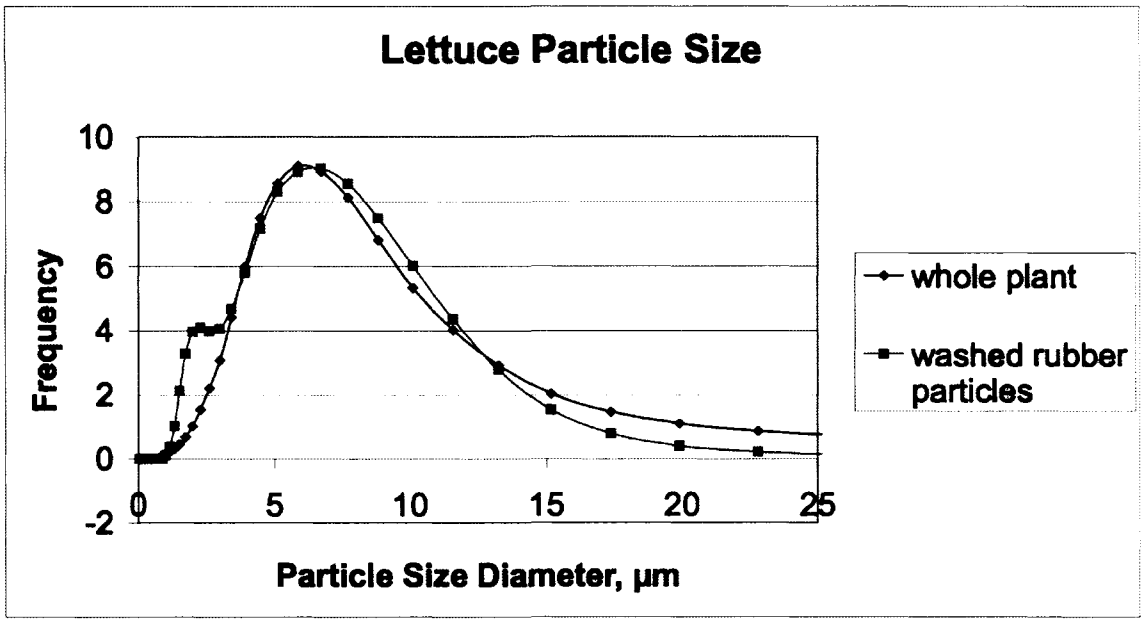
Table 7. Molecular weight data of rubber from *L. serriola* and *L. sativa* (data collected by Ms. Jenny Brichta, USDA)

Species	M_N (Da)	M_W (Da)	M_W / M_N
L. sativa	1.01×10^6	1.12×10^6	1.11
L. serriola	1.27×10^6	1.43×10^6	1.12

The size distribution of the rubber particles from lettuce is shown in Figure 25. The data collected by Ms. Jenny Brichta, a scientist at the USDA, suggests that rubber particles from lettuce have a narrow size distribution with an average diameter of approximately 6 μm. Rubber particles from lettuce are considerable larger than those from *H. brasiliensis*, which has a two subsets of particles with mean diameters of 1.0 and 0.2 μm. Furthermore, washed rubber particles from the latex of *L. serriola* and *L. sativa*

(obtained by Dr. Deborah Scott, USDA) are enzymatically active. More experimentation needs to be completed to determine the kinetic parameters for rubber transferase isolated from these two lettuce species. Currently, in collaboration with Dr. Shaun Bushman from the University of Georgia, the accumulation of rubber during the development of lettuce from seedlings to mature plant is being investigated. These results should provide some details about when rubber production in lettuce is most efficient. Knowledge of this information is critical if lettuce is going to be developed as an alternative source of natural rubber in North America.

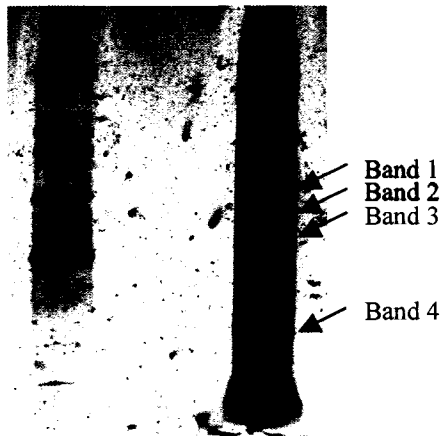
Figure 25. Size distribution of rubber isolated from *L. sativa*. Sample is either whole plant or washed rubber particle (Data obtained by Ms. Jenny Brichta, USDA).



4.2.4 Protein Sequencing

In collaboration with Dr. Shaun Bushman, proteins from *L. serriola* were separated by SDS-PAGE (Figure 26) and sequencing on individual protein bands was attempted. Individual bands from the protein gel were excised and were subjected to tryptic digestion followed by analyses by liquid chromatography-mass spectrometry (LC-MS-MS). Partial sequences of the protein bands were determined by analyzing the fragmentation pattern observed in the MS-MS spectra. The analysis of 4 bands afforded sequences of 2 to 6 amino acids each. The actual data is awaiting USDA approval for release. The sequences were used to search the lettuce expressed sequence tag (EST) database, but no significant homology between the protein sequences and the EST database was observed. The sequences were also analyzed to see if there is any significant homology to known sequences of proteins from other organism but no homology was discovered. Further collaborative research with the USDA may allow us to have a better understanding how rubber is produced in plants and how it is regulated on a biochemical level.

Figure 26. SDS-PAGE of proteins isolated from the latex of *L. serriola.* Indicated bands were partially sequenced by using tryptic digestion of protein followed by LC-MS/MS analysis.



Although several rubber particle proteins have been described that may play a role in rubber biosynthesis, the isolation of a rubber transferase complex from any source has not yet been reported. In order to have a better understanding of rubber biosynthesis, the cellular function of other rubber particle proteins will be critical. Previously, a large hydrophobic glycoprotein with an estimated molecular weight of 376 kDa was isolated and purified from *F. elastica.*[160] This protein was shown to be the most abundant protein in rubber particles from *F. elastica*, and has been named LPR (large protein from rubber particles). Other experiments have indicated that similar large proteins are also present in rubber particles of *H. brasiliensis* and *P. argentatum.* In collaboration with Dr. Katrina Cornish at the USDA, a large protein of molecular weight of 266 kDa was purified from enzymatically active *P. argentatum* rubber particles. Initially, the amino acid composition of this protein was completed and is shown in Table 8. Direct protein sequencing failed probably because many plant proteins are often glycosylated. Tryptic digestion of the

protein followed by analysis of the resulting fragments by LC-MS/MS gave some partial sequence data of the protein (data withheld pending USDA approval). The sequence data was then used for the construction of degenerate DNA probes to enable sequencing via reverse genetics. In addition, methods for its chemical or enzymatic deglycosylation are currently being investigated.

Table 8. Amino acid composition of 266 kDa protein from *P. argentatum*

Amino Acid	Percentage	Amino Acid	Percentage
Aspartic Acid/Asparagine	9.2	Methionine	5.0
Threonine	4.3	Isoleucine	6.9
Serine	11.4	Tyrosine	2.2
Glutamic Acid/Glutamine	13.8	Phenylalanine	2.8
Glycine	16.0	Histidine	2.1
Alanine	6.6	Lysine	4.5
Valine	4.1	Arginine	2.7
Proline	3.9		

In addition, other smaller rubber particle associated proteins were purified from *P. argentatum* and *F. elastica*, and *de novo* protein sequencing was attempted. A small protein, 1.7 kDa in size from *P. argentatum* was sequenced by tryptic digestion and partial sequences were determined (data with held pending USDA approval). Four proteins, 2.0 kDa, 3.5 kDa, 3.7 kDa and 13 kDa were isolated from *F. elastica*. The partial sequence data from these 5 proteins will hopefully allow determination of the complete sequences.

4.3 CONCLUSION

In summary, two lettuce species, *L. serriola* and *L. sativa* are possible commercial sources of rubber that can be cultivated in the temperate climate of North America. The rubber isolated from both species have been shown to consist of high molecular weight *cis*-polyisoprene, which is similar to that produced by *H. brasiliensis*. Furthermore, protein sequencing on proteins from a variety of rubber producing plants including *L. serriola*, *P. argentatum*, and *F. elastica* has been determined. This data may give insight into rubber biosynthesis and may allow isolation of the poorly understood rubber transferase complex. This will undoubtedly give further details on how plants control rubber production *in vivo*.

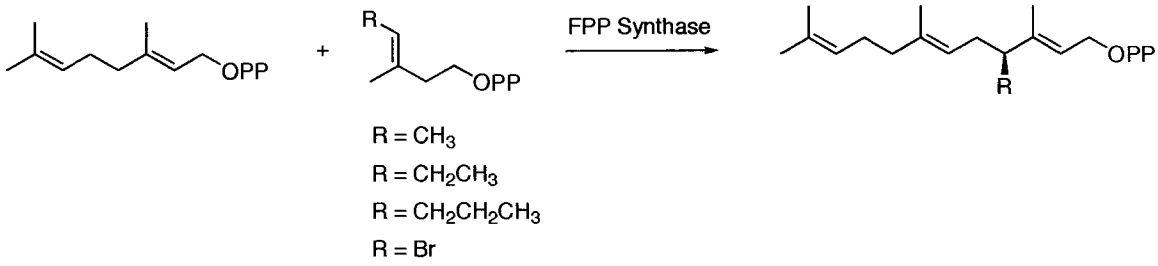
CHAPTER 5. Substrate Specificity of Rubber Transferase

5.1 INTRODUCTION

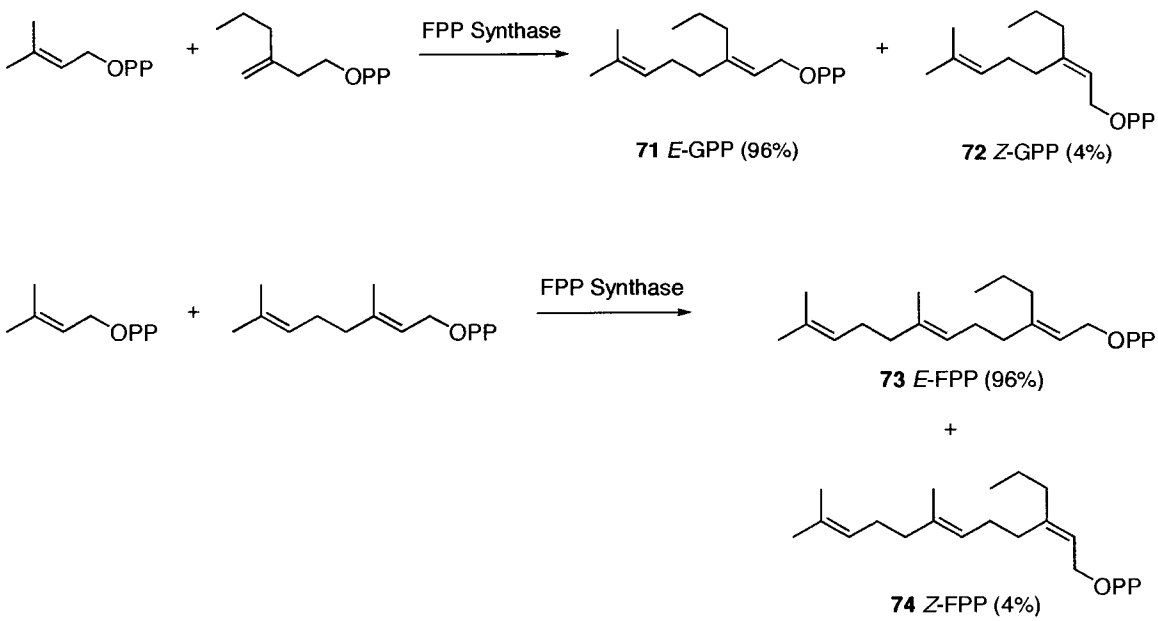
5.1.1 Substrate Specificity of Prenyltransferases

Prenyltransferases are a large family of enzymes that catalyze the head to tail condensation of isopentenyl diphosphate (IPP) with an allylic prenyl diphosphate. These enzymes are highly selective in terms of chain length and the double bond geometry of both substrates and products. For example, farnesyl diphosphate (FPP) synthase, a *trans*-prenyltransferase, catalyzes the condensation of IPP with dimethylallyl diphosphate (DMAPP) or with geranyl diphosphate (GPP) to give FPP. This enzyme can accept a variety of substrates.[161] IPP analogs substituted with alkyl groups at the 4-position are accepted as alternative substrates of FPP synthase (Scheme 17). Surprisingly, incorporation of the propyl analog gave both 2*E* and 2*Z* isomers of GPP (**71** and **72**) and FPP (**73** and **74**) homologs (Scheme 18).[162]

Scheme 17. IPP analogs with FPP synthase



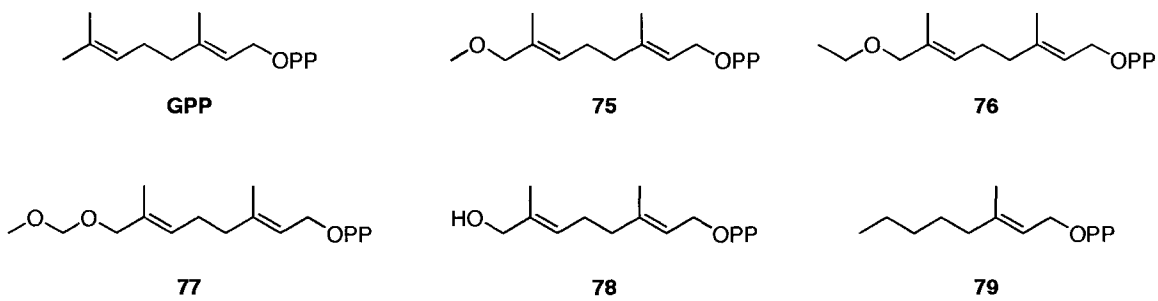
Scheme 18. Incorporation of homologs of IPP with FPP synthase



The basis of the substrate specificity of FPP synthase has been recently examined by Koyama *et al.*, by gene cloning, over-expression and purification of FPP synthase from the thermophilic bacteria *Bacillus stearothermophilus*.[163] Comparison of its primary structure to other *trans*-type prenyltransferases indicates that there are seven conserved amino acid motifs in the amino acid sequence, two of which contain aspartate rich

regions. Through site-directed mutagenesis, Ohnuma *et. al* were able to demonstrate that a tyrosine (Tyr81) residue that is upstream to the first aspartate rich region regulates the size of the prenyl chain in the product.[39] The chain length may be controlled by hydrophobic interactions between the terminus of the reaction product, FPP, and the side chain of tyrosine. Substitution of tyrosine with alanine causes the mutated FPP synthase to produce GPP or even hexaprenyl diphosphate (C_{30}). Furthermore, replacement of the tyrosine with either glycine, arginine, serine or aspartic acid gives a FPP synthase with altered substrate specificities.[164] In particular, homologs of GPP (**75-79**) containing hydrophilic side chains could be accepted as substrates by these mutated enzymes (Figure 27). The amino acid at this position appears to determine the product chain length because all reported *E*-prenyltransferases that catalyze condensation beyond geranylgeranyl diphosphate (GGPP) (C_{20}) have alanine at this position.[165]

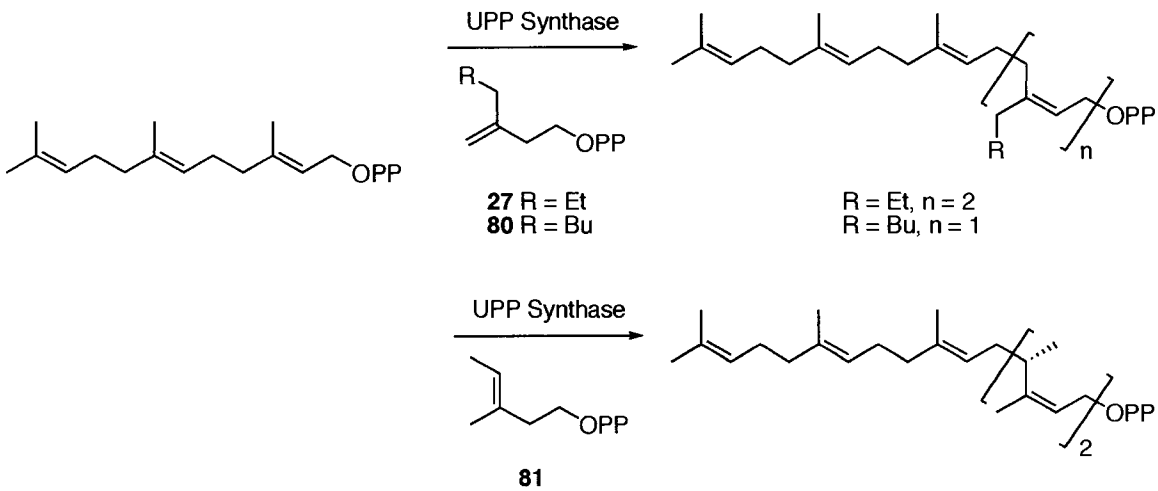
Figure 27. Modified substrates (**75-79**) accepted by mutated FPP synthase



In contrast to *trans*-prenyltransferases (e.g. FPP synthase), the substrate specificity of *cis*-prenyltransferases is less extensively studied. Recently, Koyama and coworkers investigated the substrate specificity of UPP synthases with a series of 3- and

4-alkyl homologs of IPP.[166] Incorporation studies with 3-alkyl homologs of IPP (**27** and **80**) show that the enzyme has greater specificity than the *trans*-prenyltransferase, FPP synthase (Scheme 19). In addition, the incorporation of IPP analogs by UPP synthase stopped only after the first or second stage of prenyl elongation. Studies with 4-alkyl homologs indicate that only *E*-4-methyl-IPP (**81**) can be accepted as an alternative substrate of UPP synthase to produce analogs of C_{20} and C_{25} prenols.

Scheme 19. Incorporation of IPP homologs by UPP synthase



5.1.2 Juvenile Hormones

The substrate specificity studies with FPP synthase and UPP synthase indicate that certain alternative substrates can be accommodated by the enzymes' active sites. Furthermore, homo-IPP **27** is a substrate for isopentenyl diphosphate isomerase and produces a mixture of *E*-3-methyl-3-pentenyl diphosphate (**81**) and *Z*- and *E*-3-methyl-2-

pentenyl diphosphates (**82** and **83**, see Scheme 20). There are also some natural examples of incorporation of homologs of IPP. For example, the juvenile hormones (**JH-0**, **JH-I** and **JH-II**), which are found in a variety of insects, contain an ethyl side chain (Figure 28).[167] It is believed that biosynthesis of these juvenile hormones arises from incorporation of homo-IPP **27**.[168,169]

Scheme 20. Isomerization of homoisopentenyl IPP **27**

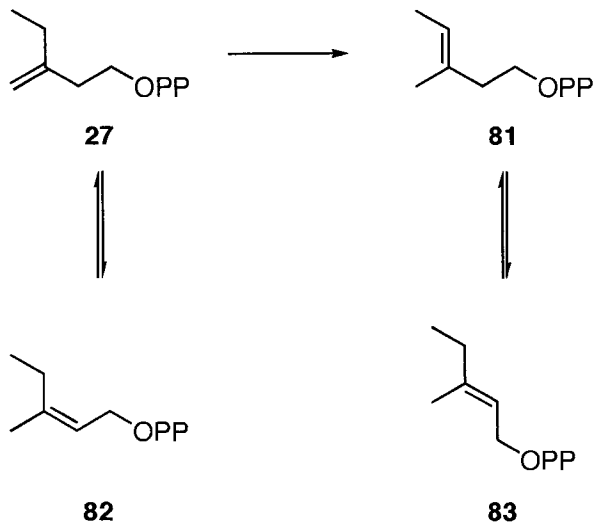
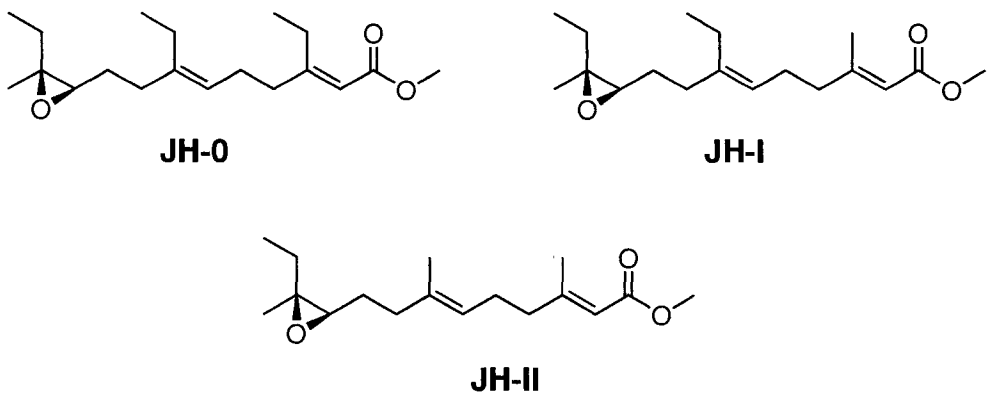
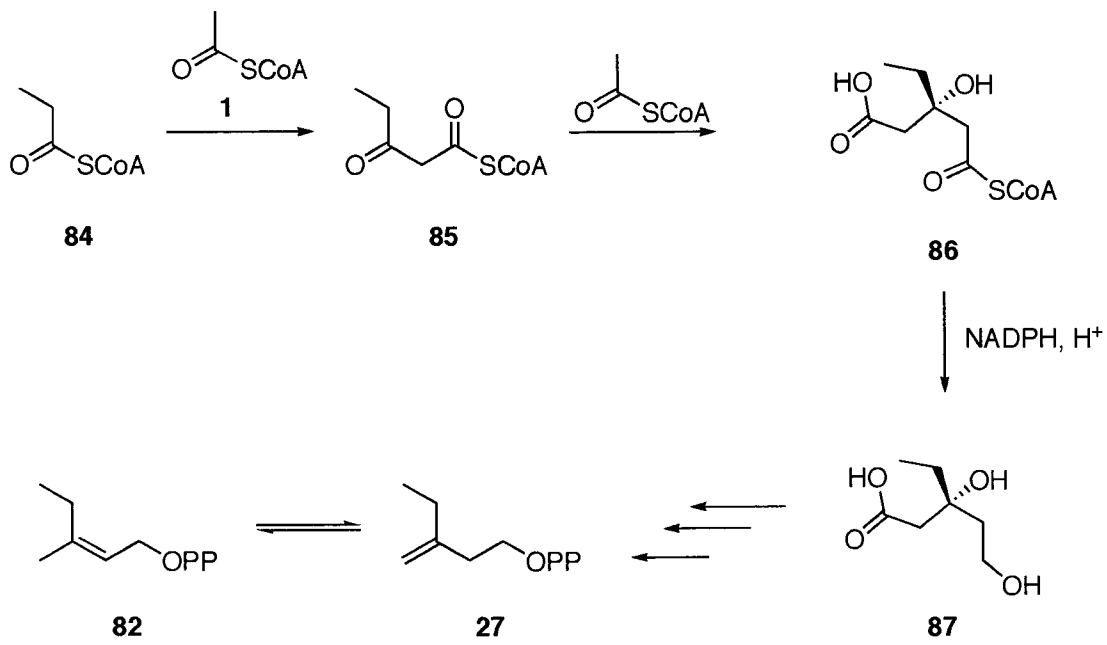


Figure 28. Chemical structures of juvenile hormones from order *Leptidopera*



The ability of insects to produce these sesquiterpenoids with unnatural ethyl side chains is very intriguing. It has been proposed by Schooley and Baker that the ethyl side chain arises either by methylation by a C-1 donor (*e.g. S*-adenosylmethione, SAM), or by condensation of homoisopentenyl IPP **27** derived from one unit of propionyl Co-A **84** plus units of acetyl-CoA **1** via homomevalonate **87** (Scheme 21).[170] Subsequent biosynthetic studies show that [1-^{14}C]-propionate is incorporated efficiently and specifically into the ethyl branches of JH II and/or JH I, via a pathway that is parallel to mevalonate-IPP pathway.[167]

Scheme 21. Biosynthesis of homoisopentenyl IPP **27** via mevalonate pathway

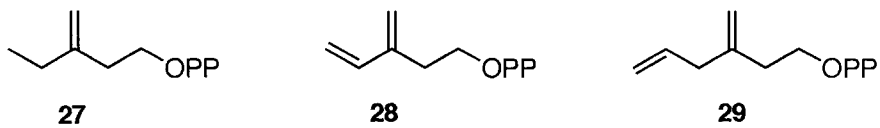


5.1.3 Project Goals: Investigate the Substrate Specificity of Rubber Transferase

The objective of this project is to design, synthesize, and examine unnatural analogs of IPP (**27-29**) as alternative substrates of prenyltransferase enzymes (Figure 29). The investigation of the substrate specificity of rubber transferase may uncover the following:

- Whether rubber transferase can incorporate unnatural analogs of IPP.
- Whether modified rubber possesses physical properties that are useful.
- Whether incorporation of allyl or vinyl side chains allows for increased cross-linking during the vulcanization process.
- Whether the *cis*-prenyltransferases, rubber transferase and UPP synthase, are able to produce rubber products with altered chain length.

Figure 29. Target homologs of IPP **27-29**



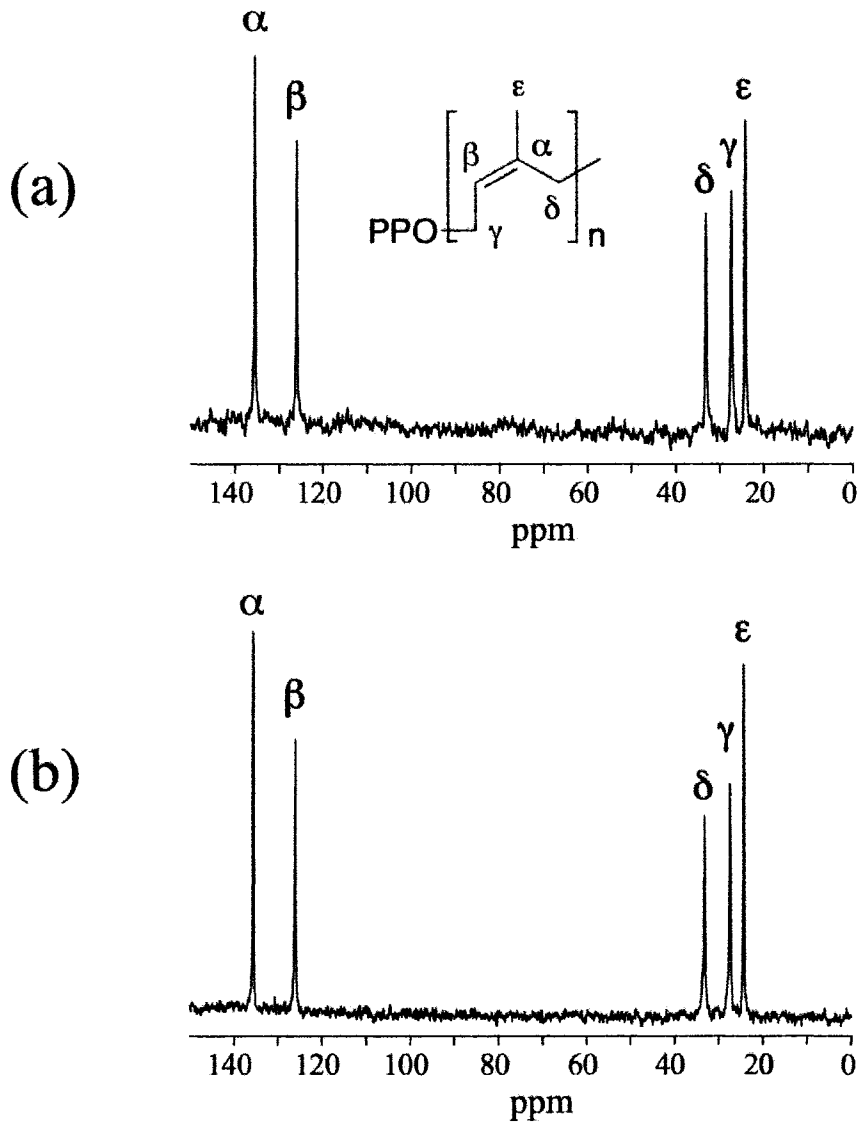
27 **28** **29**

5.2 RESULTS AND DISCUSSION

5.2.1 Synthesis of ^{13}C-IPP

It seemed that ^{13}C-NMR analysis could be used to determine the substrate specificity of the rubber transferase. The ^{13}C NMR spectra recorded from solid-state NMR analysis (obtained in collaboration with Professor Rod Wasylishen, University of Alberta), of natural rubber isolated from both *Hevea brasiliensis* and *Parthenium argentatum* is shown in Figure 30. High-resolution NMR could be used for the analysis of the substrate specificity of rubber transferases if incorporation rates of *ca.* 1% for fully ^{13}C-labeled precursors can be attained. Synthesis of ^{13}C-IPP (**91**) and its subsequent incorporation by rubber transferase was completed to ensure that this mode of analysis could be used to determine the substrate specificity of rubber transferase.

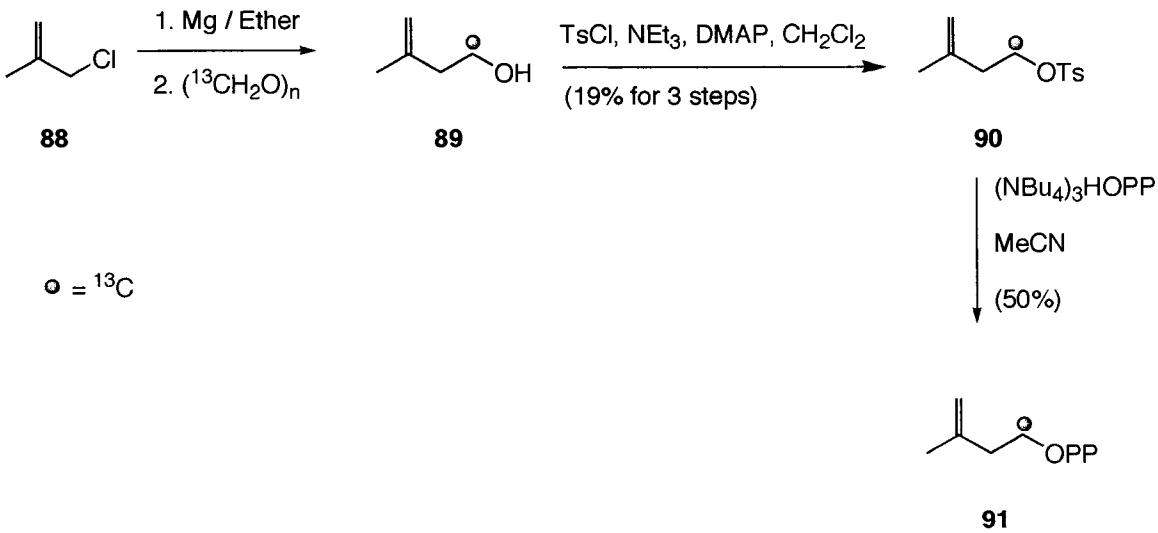
Figure 30. ^{13}C-NMR spectra (50.331 MHz) of magic angle spinning (MAS) samples of rubber from two samples (a) *Hevea brasiliensis* (b) *Parthenium argentatum* (Spectra obtained by Dr. Guy Bernard, University of Alberta)



The synthesis of ^{13}C labeled IPP **91** is shown in Scheme 22, and is based on the preparation of 2-methyl-1-buten-4-ol as described by Paquette *et al.*[171] Treatment of the allyl chloride **88** with magnesium turnings in THF gives the corresponding Grignard reagent, which upon reflux with ^{13}C-paraformaldehyde affords the labeled alcohol **89**.

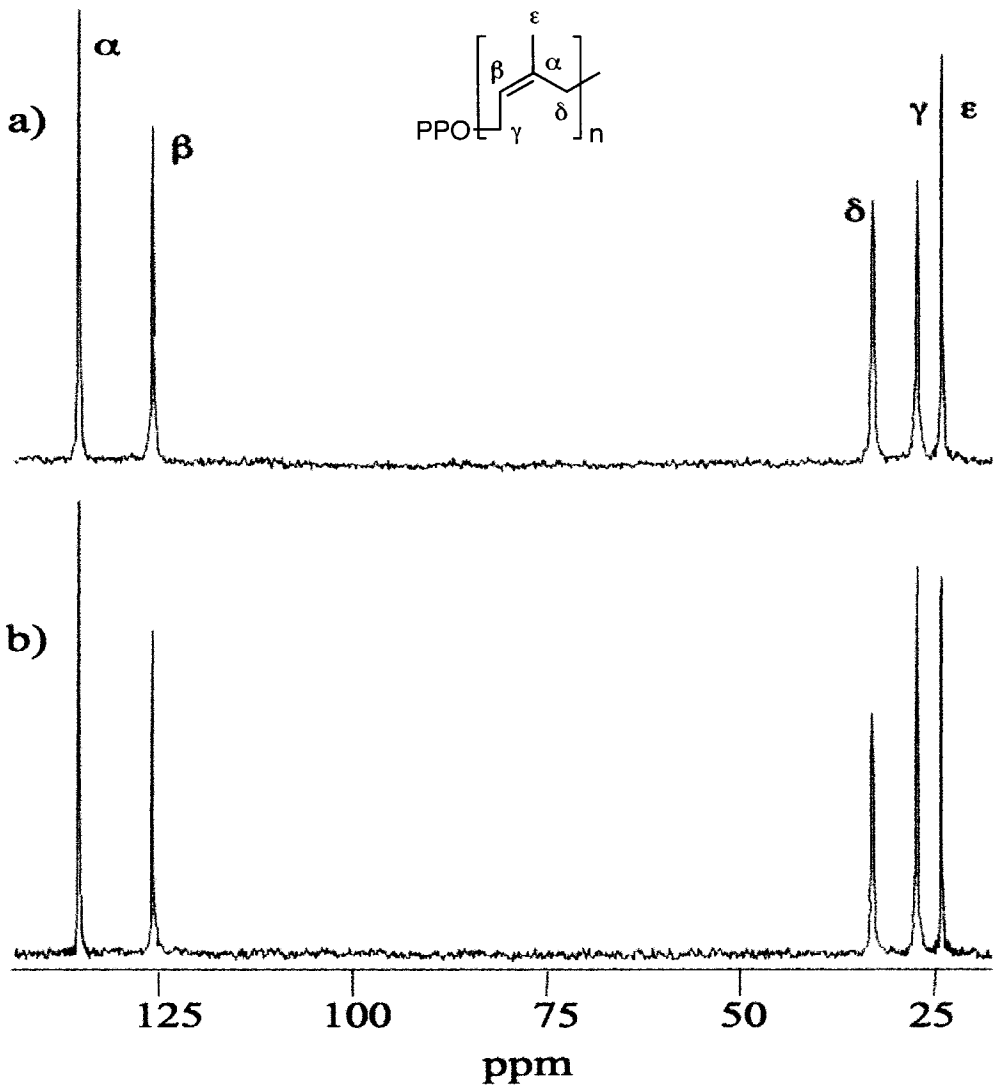
Reaction of the alcohol **89** with *p*-toluenesulfonyl chloride in CH_2Cl_2 provides the tosylate **90**, which is converted to [1-^{13}C]-IPP (**91**) by treatment with tris-*n*-butylammonium hydrogen pyrophosphate in MeCN.

Scheme 22. Synthesis of [1-^{13}C]-IPP (**91**)



With the synthesis of **91** complete, its ability to act as a substrate of rubber transferase was investigated. Incubation of [1-^{13}C]-IPP (**91**) with rubber particles from *H. brasiliensis* for 4 d at 25 °C should give new rubber. Solid state ^{13}C-NMR analysis of the isolated rubber is shown in Figure 31. The top spectrum is that recorded for natural rubber, whereas the lower spectrum is of the rubber isolated after incorporation of [1-^{13}C]-IPP (**91**). The enhancement of the peak corresponding to the γ carbon of the rubber chain shows the specific incorporation of ^{13}C-IPP. The experiment clearly demonstrates that ^{13}C-NMR analysis can be used to detect utilization of labeled analogs of IPP by rubber transferase, provided sufficient levels of incorporation occur.

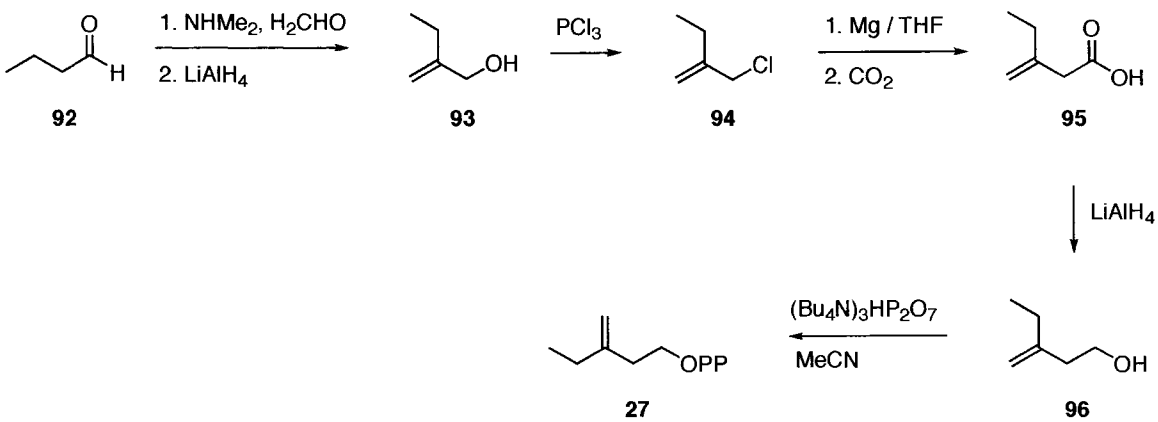
Figure 31. ^{13}C NMR MAS spectra (50.331 MHz) of ^{13}C labeled rubber a) Natural abundance spectrum b) Spectra of rubber with incorporated ^{13}C-IPP **91** (Spectra obtained by Dr. Guy Bernard, University of Alberta)



5.2.2 Synthesis of Homoisopentenyl Diphosphate 27

The synthesis of homoisopentenyl diphosphate **27** has been described by Koyama and is shown in Scheme 23.[172] Starting from *n*-butyraldehyde (**92**) the title compound **27** was synthesized in 7 steps. Although this is a relatively concise synthesis of the desired analog of IPP, it would not be easily applicable to the synthesis of other 3-alkyl substituted analogs of IPP. Therefore, other synthetic strategies for the synthesis of 3-alkyl substituted 3-butenyl alcohols were investigated.

Scheme 23. Koyama's synthesis of homoisopentenyl IPP **27**

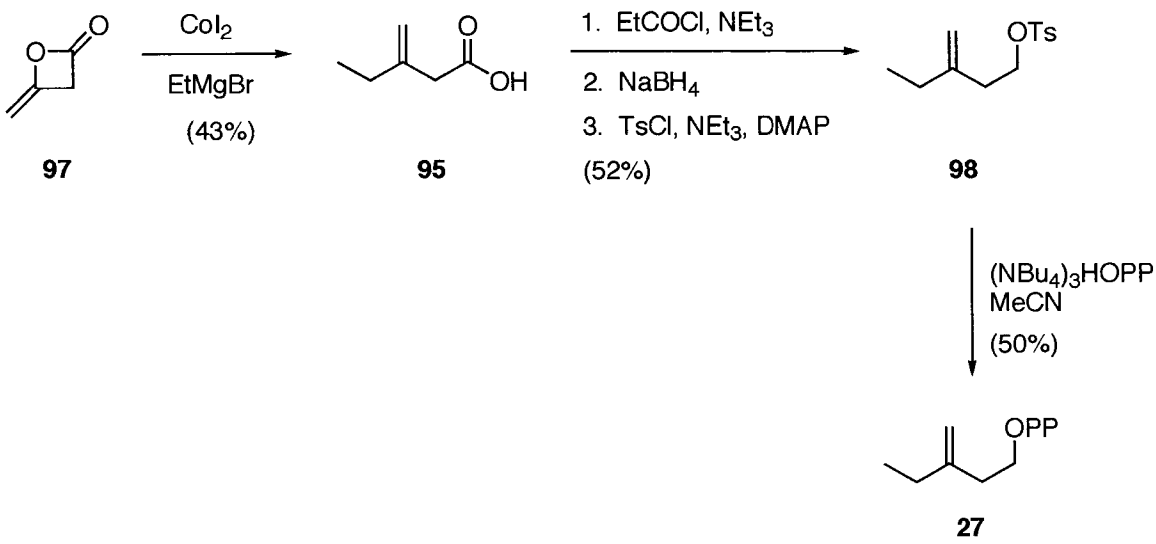


Fujisawa and co-workers have reported the reaction of diketene with Grignard reagents in the presence of cobalt (II) iodide to provide access to 3-substituted but-3-enoic acids.[173] Their reaction conditions were applicable to the use of a variety of Grignard reagents, including methyl, ethyl and butyl, with moderate yields in the range of

66-84%. Thus, this strategy was explored for the synthesis of 3-alkyl substituted analogs of IPP.

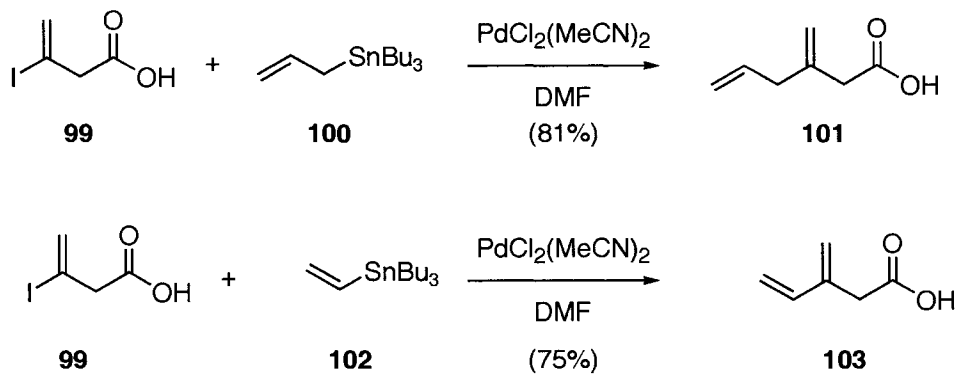
The synthesis of homoisopentenyl diphosphate **27** is shown in Scheme 24. The reaction of ethylmagnesium bromide with diketene (**97**) in the presence of cobalt (II) iodide affords 3-methylenepentanoic acid (**95**) in a moderate yield. Treatment of the acid with ethyl chloroformate and subsequent reduction of the mixed anhydride provides the alcohol, which is then converted to 3-methyl-3-buten-1-yl tosylate (**98**). Reaction of **98** with tris-*n*-butylammonium hydrogen pyrophosphate yields the desired homoisopentenyl diphosphate **27**. With the synthesis of homoisopentenyl diphosphate completed, the syntheses of 3-allyl and 3-vinyl IPP were attempted. However, addition of allyl or vinyl magnesium bromide to diketene (**97**) leads to the formation of polymeric material. Therefore, a new general strategy for the synthesis of 3-substituted but-3-enyl alcohol was designed.

Scheme 24. Synthesis of homoisopentenyl IPP **27**



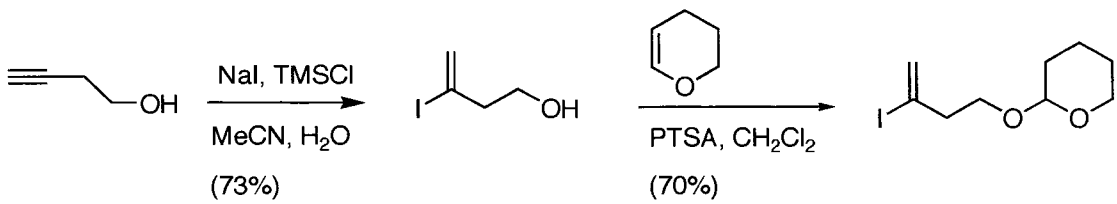
More recently, a report by Abarbri *et al.* described the use of 3-iodobut-3-enoic acid as a precursor for the synthesis of a variety of 3-substituted but-3-enoic acids in good yields (Scheme 25).[174] For example, reaction of 3-iodobut-3-enoic acid (**99**) with ethylzinc bromide under palladium catalysis provided 3-ethylbut-3-enoic acid in 90% yield. One limitation of this reaction, is that no reaction is observed with either allyl or vinyl zinc reagents. This limitation could be overcome by the use of allyltributyltin (**100**) or vinyltributyltin (**102**) with a palladium (II) catalyst. As this synthetic strategy would still require reduction of the resultant acid to the corresponding alcohol, a slightly modified procedure was utilized. The cross coupling partner 3-iodobut-3-enoic acid (**99**) was replaced with the 3-iodo-3-buten-1-yl THP ether **106**. This strategy would give a concise synthesis of the three desired analogs of IPP and should be applicable to other analogs.

Scheme 25. Abarbri's synthesis of 3-alkenylbut-3-enoic acids **101** and **103**



For the incorporation studies of the analogs of IPP, ^{13}C-NMR spectroscopy would be useful to analyze the product. It would be expected that the rubber transferase will produce only a small amount of new rubber against a background of existing rubber in the particle. To make the analysis more sensitive, ^{13}C labeled homoisopentenyl diphosphate was prepared. The required cross coupling partner, 2-(3-iodo-but-3-enyloxy)-tetrahydro-pyran (**106**) was prepared from the commercially available alkynol **104** as shown in Scheme 26. Regioselective addition of HI to 3-butyn-1-ol (**104**) followed by protection of the hydroxyl group as its tetrahydropyran ether gives **106**.

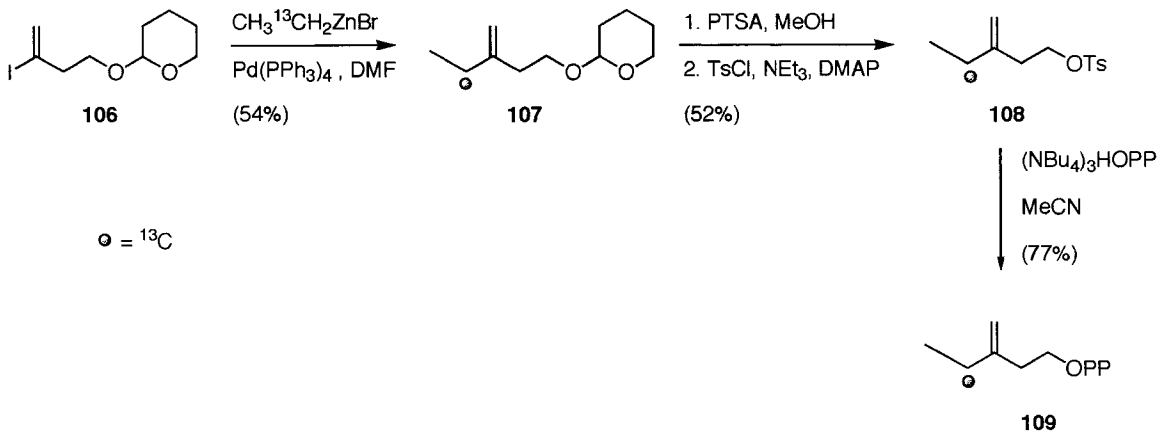
Scheme 26. Synthesis of cross coupling partner **106**



104 **105** **106**

With the required coupling partner in hand, the synthesis of ^{13}C-homoisopentenyl IPP could be completed as shown in Scheme 27. Reaction of vinyl iodide **106** with [1-^{13}C]-ethylzinc bromide in presence of tetrakis(triphenylphosphine)palladium(0) provides the homoallylic ether **107**. Deprotection of the THP ether **107** with *p*-toluenesulfonic acid gives 3-[1-^{13}C]-ethyl-3-buten-ol, which is subsequently transformed into its *p*-toluenesulfonate ester **108** using standard reaction conditions. Treatment of **108** with tris-*n*-butylammonium hydrogen diphosphate gives the desired ^{13}C-homoisopentenyl IPP **109** in good yield.

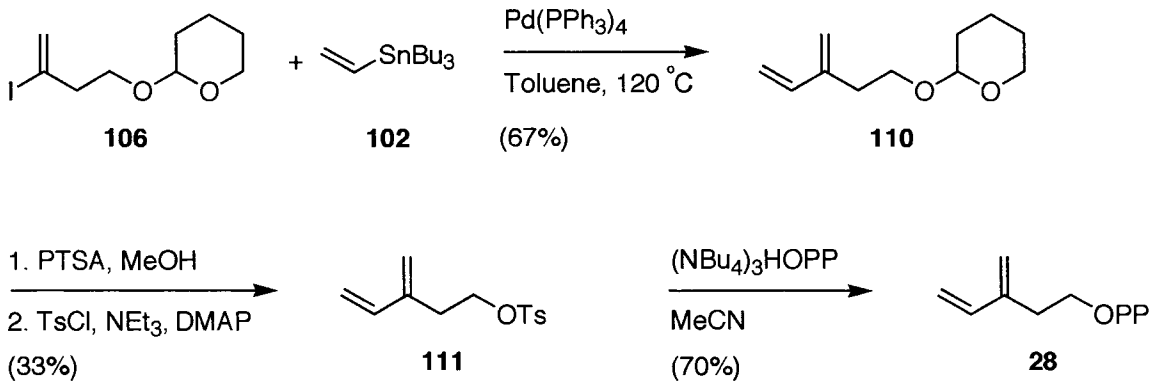
Scheme 27. Synthesis of ^{13}C-homoisopentenyl IPP **109**



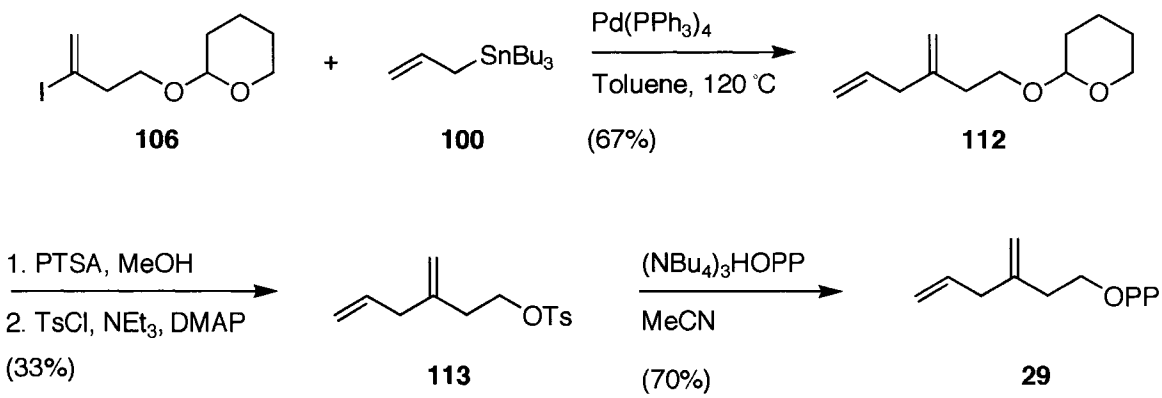
5.2.3 Synthesis of Vinyl and Allyl Substituted IPP

IPP Analogs with allyl and vinyl substitution at the 3-position could be prepared in an analogous fashion. As mentioned previously, the syntheses of these compounds require the use of allyltributyltin and vinyltributyltin, respectively, instead of the corresponding organozinc reagents. Stille cross coupling between vinyltributyltin (**102**) and 2-(3-iodo-but-3-enyloxy)tetrahydropyran (**106**) affords the THP ether **110** (Scheme 28). Deprotection with *p*-toluenesulfonic acid (PTSA) followed by reaction with *p*-toluenesulfonyl chloride yields the tosylate **111**, which is subsequently converted into 3-vinyl IPP **28**. As shown in Scheme 29, the 3-allyl substituted analog of IPP **29** could be made in a similar manner using allyltributyltin (**100**) as the cross coupling partner.

Scheme 28. Synthesis of vinyl-IPP **28**



Scheme 29. Synthesis of allyl-IPP **29**



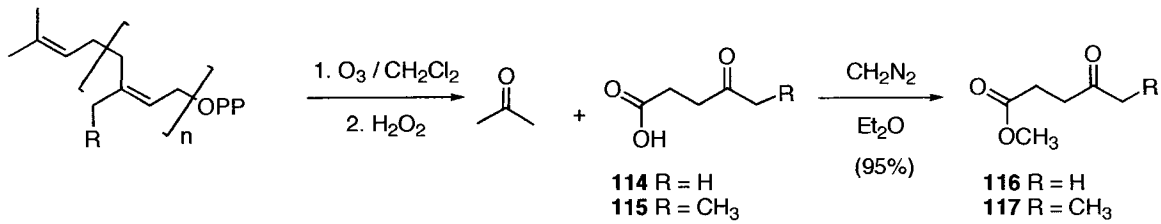
5.2.4 Incorporation Studies

With the synthesis of the three analogs of IPP completed, the transformation of 3-[1-^{13}C]-ethyl IPP (**109**) by rubber transferase was investigated. Initial incorporation experiments used an IPP final concentration of 5 mM and a FPP concentration of 20 μM, which are optimum conditions for incorporation of IPP by rubber transferase.[103,136,144] To ensure maximum incorporation, rubber particles were incubated with the IPP analog at 25

°C for 4 days, and the isolated rubber was analyzed by solid-state ^{13}C-NMR spectroscopy (spectra acquired by Dr. Guy Bernard at the University of Alberta). The incorporation of the [1-^{13}C]-ethyl IPP (**109**) should give rise to an additional peak in the NMR corresponding to the methyl group of the ethyl side chain. The absence of this peak in the NMR spectra indicates that any incorporation of the [1-^{13}C]-ethyl IPP (**109**) is below the detection limit (*ca.* 1%). Previous studies by Koyama showed that UPP synthase is able to add two molecules of 3-ethyl-IPP to FPP before the condensation reaction is terminated.[166] It is possible that rubber transferase successfully incorporates 3-ethyl IPP but ^{13}C-NMR analysis is not sensitive enough to detect its incorporation due to a large background of natural rubber. Therefore, a more rigorous analysis technique for the incorporation of [1-^{13}C]-ethyl IPP (**109**) was developed.

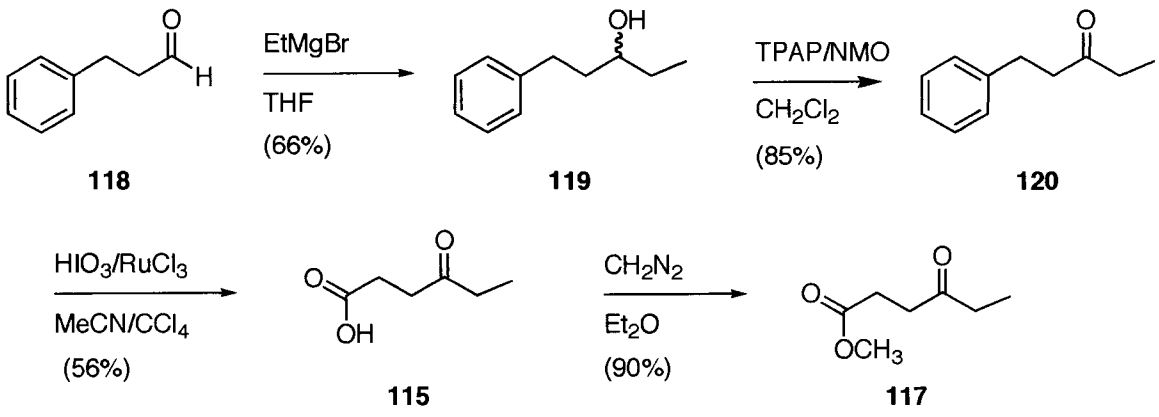
A technique based on the ozonolysis of rubber into levulinic acid was employed.[175] Incorporation of 3-alkyl IPP analogs would give modified alkyl side chains at the 3-position of the polyisoprene backbone. Ozonolysis of the rubber sample followed by oxidative work-up would give a mixture of levulinic acid and a keto acid (Scheme 30). Such acids can then be converted into their corresponding methyl esters and analyzed by GC-MS, which is much more sensitive than ^{13}C-NMR spectroscopy. The difference in the retention times of the two esters should be large enough to allow their facile separation.

Scheme 30. Ozonolysis based analysis for substrate specificity study



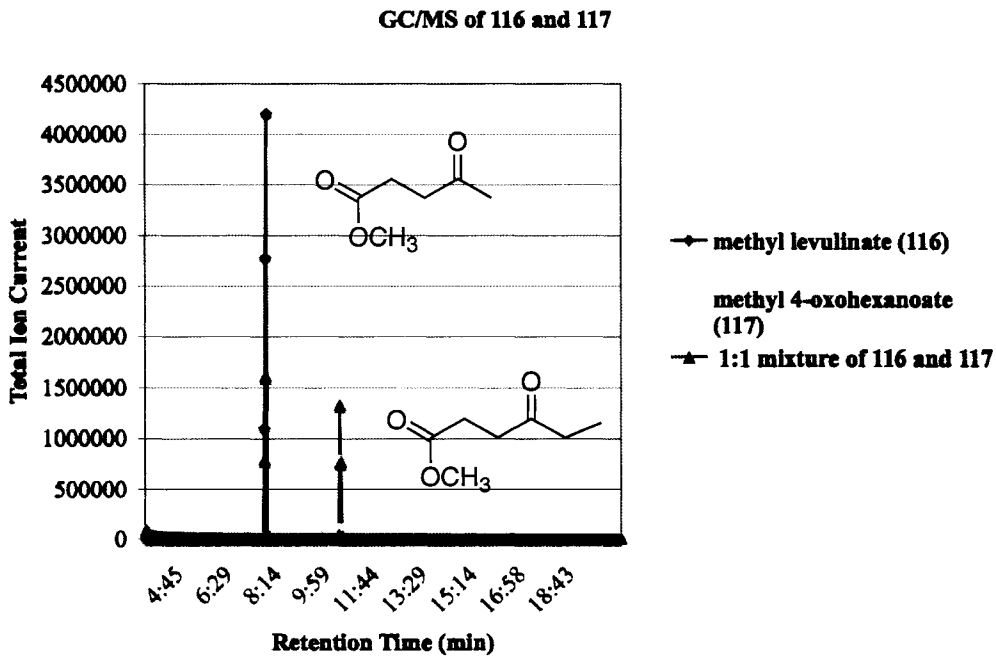
As this assay would require the synthesis of reference standards, the methyl ester of ethyl-levulinic acid or 4-oxo-hexanoic acid (**115**) was prepared as shown in Scheme 31. Addition of ethylmagnesium bromide to hydrocinnamaldehyde (**118**) in THF provides the secondary alcohol **119**, which is readily oxidized to 1-phenyl-pentan-3-one (**120**). Ruthenium catalyzed oxidation of the phenyl ring provides 4-oxohexanoic acid (**115**). Treatment of the acid with an ethereal solution of diazomethane affords the methyl ester **117**.

Scheme 31. Synthesis of methyl 4-oxohexanoate (**117**)



Analysis of the methyl esters of both levulinic acid **116** and 4-oxo-hexanoic acid **117** by GC-MS is shown in Figure 32. The methyl ester of levulinic acid **116** has a retention time of approximately eight minutes, whereas the methyl ester of 4-oxohexanoic acid **117** has a retention time of ten minutes. Furthermore, injection of a sample containing both compounds indicates that separation by gas chromatography is easily achieved. This method of analysis was then applied for the determination of the substrate specificity of rubber transferase.

Figure 32. GC-MS spectrum of methyl levulinate (**116**) and methyl 4-oxohexanoate (**117**)



[1-^{13}C]-Ethyl-IPP (**109**) was incubated with rubber particles isolated from *H. brasiliensis* for 4 days at 25 °C, and the resulting rubber was isolated by filtration. Ozonolysis of the rubber followed by methylation of the resulting acid gave a mixture that was analyzed by GC-MS. Unfortunately, no incorporation of [1-^{13}C]-ethyl-IPP (**109**) was observed, as analysis indicated no methyl 4-oxohexanoate (**117**) in the mixture. Experiments with varying concentrations of [1-^{13}C]-ethyl-IPP (**109**) and the natural substrate **7** were done. However, in every case no incorporation of [1-^{13}C]-ethyl-IPP (**112**) was detected. These results indicate that [1-^{13}C]-ethyl-IPP (**109**) is a very poor substrate for rubber transferase.

5.3 CONCLUSION

In summary three analogs of IPP (**27-29**) were prepared and tested as alternative substrates for rubber transferase. Unfortunately, incorporation experiments with [1-^{13}C]-ethyl-IPP (**109**) indicate that rubber transferase has high specificity and does not widely accept 3-substituted analogs of IPP. Although these results are disappointing, other structural modifications to the natural substrates could be investigated. Previous studies with other prenyltransferases,[166,176,177] suggest that analogs with substitution at the 4 position of IPP may be acceptable substrates for rubber transferase.

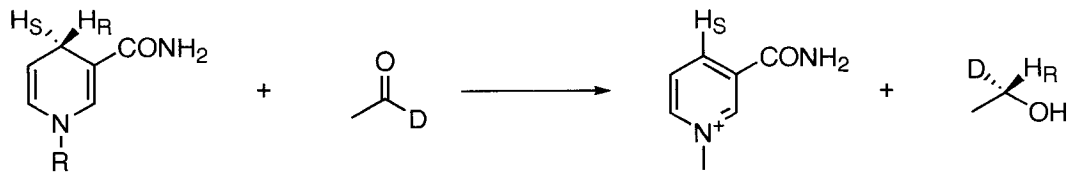
CHAPTER 6. Stereochemical Analysis of Rubber Biosynthesis

6.1 INTRODUCTION

6.1.1 Stereochemical Course of Enzyme-catalyzed Reactions

Determining the stereochemical course of enzyme-catalyzed reactions has been the subject of intense research since the middle of the twentieth century. For example, in the early 1950's the research group of Westheimer was able to determine the stereochemistry of alcohol dehydrogenase, an enzyme that catalyzes the oxidation of ethanol to acetaldehyde, using the coenzyme, nicotinamide dinucleotide (**123**, NAD^+).[178-180] The reduced coenzyme, NADH (**121**), contains a prochiral centre at C-4, the centre at which hydride transfer to the substrate occurs. Through the use of deuterium labeled substrates, the hydride transfer from NADH to acetaldehyde was determined to be stereoselective (Scheme 32).

Scheme 32. Stereochemistry of reaction catalyzed by alcohol dehydrogenase

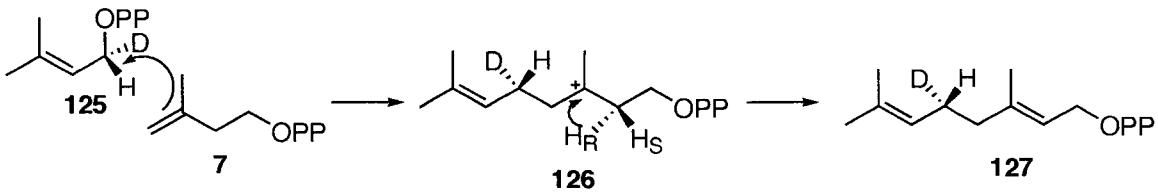


121 **122** **123** **124**

In the 1960's and the 1970's, the research efforts of John Cornforth and coworkers focused on determining the stereochemical outcomes of a variety of

biosynthetic reactions involved in the synthesis of isoprenoids. In 1966, Cornforth delineated the stereochemistry of farnesyl diphosphate (FPP) synthase, the enzyme that synthesizes the C_{15} isoprenoid, farnesol.[181,182] By using a series of deuterium labeled (4*R*) and (4*S*)-mevalonic acid substrates, Cornforth and coworkers were able to show that the reaction between isopentenyl diphosphate (**7**) and (1*R*)-[1-$^2H_1$]-dimethylallyl diphosphate (**125**) involves displacement of the allylic diphosphate with overall inversion of stereochemistry followed by elimination of the pro-*R* proton of IPP to form the *E*-alkene of geranyl diphosphate (**127**) (Scheme 33).[181,182] The ability of FPP synthase to distinguish between the prochiral hydrogens is generally referred as its "cryptic stereochemistry".[183]

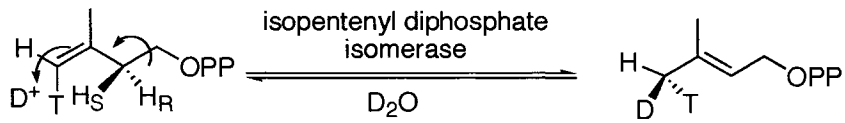
Scheme 33. Cryptic stereochemistry of geranyl diphosphate biosynthesis.



Cornforth and coworkers also investigated the stereochemistry of natural rubber biosynthesis.[184] This experiment involved the feeding of both (4*R*)- and (4*S*)-[4-$^3H_1$] mevalonate to crude plant homogenates. Analysis of the rubber produced showed that the 4-pro-*S* hydrogen was lost during rubber biosynthesis. In addition, Cornforth investigated the stereochemistry of the isomerization of IPP into DMAPP, which is catalyzed by IPP isomerase. It was shown through the use of tritium labeling that the isomerization

involves addition of a proton to the *re* face of [4-$^{3}H_{1}$]-IPP (**128**) to give (4*R*)-[4-$^{2}H_{1}$]-[4-$^{3}H_{1}$]-DMAPP (**129**) (Scheme 34).[185]

Scheme 34. Stereochemistry of isopentenyl diphosphate isomerase



128 **129**

Cornforth first elucidated the stereochemistry of FPP synthase in the 1960's, several investigations into the stereochemical outcome of other prenyltransferases have been reported. More recently, Koyama and co-workers elucidated the stereochemical nature of the formation of undecaprenyl diphosphate (UPP), which is catalyzed by UPP synthase.[186-188] This enzyme, unlike FPP synthase, catalyzes the formation of (*Z*)-prenyl chains. Incorporation studies with deuterium labeled molecules of IPP illustrated that UPP is biosynthesized with elimination of the 2-pro-*S* hydrogen of IPP and that the reaction occurs on the *si* face of the double bond in IPP.[186,189] In addition, it was shown that the stereochemistry of another *trans*-prenyltransferase, heptaprenyl diphosphate synthase proceeds in analogous manner to FPP synthase.[186,189]

It is generally believed that the pro-*R* hydrogen of IPP is eliminated during the biosynthesis of (*E*)-prenyl units while the pro-*S* hydrogen of IPP is eliminated during the biosynthesis of (*Z*)-prenyl units. Although this is often true, there are a few examples of hydrogen elimination that are opposed to Cornforth's original proposal.[181,182] In

particular, studies on the biosynthesis of polyprenols from the plant *Mallotus japonicus* illustrated that the pro-*R* hydrogen was being eliminated during the biological formation of the (*Z*)-prenyl chains of malloprenols.[190] This result is in disagreement with the generally accepted hypothesis that the pro-*S* hydrogen of IPP is eliminated in the biosynthesis of (*Z*)-prenyl chains.

6.1.2 Project Goals: Stereochemical Analysis of Rubber Biosynthesis

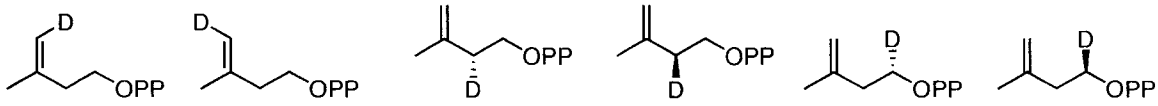
Since there are differences between *cis*-prenyltransferases in the stereochemistry of the hydrogen elimination of IPP, it cannot be stated *a priori* that rubber transferase will proceed via the loss of either the pro-*R* or pro-*S* proton. Moreover, in order to generate a detailed picture of the stereochemical constraints exerted by rubber transferase, two other stereochemical issues must be addressed, namely which face (*re* or *si*) of IPP participates in its addition to the growing rubber chain, and whether this attack of the terminal allylic diphosphate proceeds with retention or inversion of stereochemistry. Furthermore, it has been demonstrated that plants biosynthesize IPP through both the cytosolic mevalonic acid (MVA) pathway and the plastidal methylerythritol 4-phosphate (MEP) pathway.[18,20] Hence, the significance of Cornforth's experiments on rubber formation with mevalonate is unclear.

Therefore, the first objective of this project is to synthesize six deuterium analogs of IPP (**30-35**) that will be used to investigate the cryptic stereochemistry of rubber transferase and to compare it with other (*Z*)-prenyltransferases (Figure 33). This project

aims to elucidate three distinct stereochemical issues involved in prenyltransferase reactions:

- Stereochemistry of hydrogen elimination. Is the pro-*R* or pro-*S* hydrogen eliminated from IPP?
- The displacement of allylic diphosphate. Does it occur with overall inversion or retention of stereochemistry?
- The addition of the allylic diphosphate to IPP. Does the addition occur to the *re* or *si* face of the double bond in IPP?

Figure 33. Deuterium analogs of IPP **30-35**



30 **31** **32** **33** **34** **35**

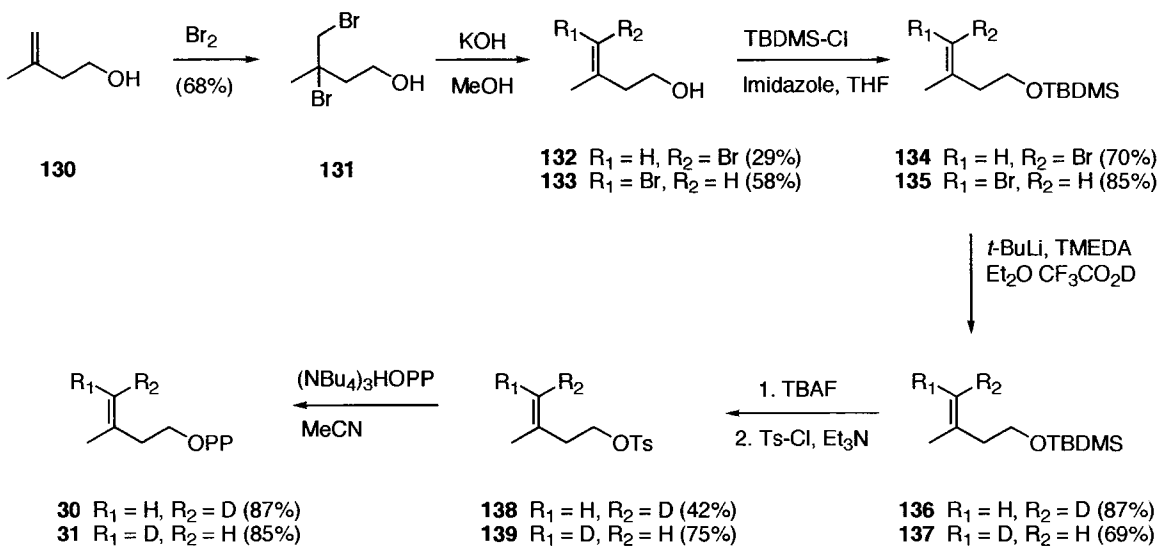
A second objective of this research is to determine if any differences in the stereochemistry of rubber biosynthesis exist between plant species. A detailed understanding of the 3-dimensional requirements of rubber transferase may allow development of new rubber producing crops in the future.

6.2 RESULTS AND DISCUSSION

6.2.1 Synthesis of 4-[$^2H_1$] Analogs of IPP 30 and 31

The synthesis of analogs of IPP, with deuterium labeling at the vinylic position, is outlined in Scheme 35. Following a procedure described by Cane *et al.*,[191] bromination of 3-methyl-3-buten-1-ol (**130**) provides the corresponding dibromide **131**. Debromination of **131** with a 5 M methanolic solution of KOH affords a separable mixture of *E* and *Z*-4-bromo-3-methyl-buten-1-ol, **132** and **133,** respectively. Separation of the isomeric vinyl bromides is accomplished using medium pressure flash chromatography. Compounds **132** and **133** are subsequently transformed into their respective *tert*-butyl-dimethyl silyl ethers **134** and **135** by treatment with *tert*-butyl-dimethyl silyl chloride (TBDMSCl) and imidazole in DMF.

Scheme 35. Synthesis of [4-$^2H_1$] IPP **30** and **31**



Introduction of the deuterium label could be realized through a two-step process. Metalation of **134** with *tert*-butyl lithium at -78°C followed by quenching of the resultant lithium anion with trifluoroacetic acid 1-d (99.8% d) gives the desired deuterated isopentenyl *tert*-butyldimethylsilyl ether **136**. Desilylation of **136** with tetra(*n*-butyl) ammonium fluoride (TBAF), followed by treatment of the corresponding 4-*Z*-deuterated isopentenol with *p*-toluenesulfonyl chloride in CH_2Cl_2 generates **138** in 42% yield from **136**. The reaction of **135** under similar reaction conditions affords **139**. ^{1}H-NMR analysis indicates that deuterium incorporation of compounds **138** and **139** was 95% based on integration of the two olefinic peaks at 4.78 and 4.64 ppm, respectively. The synthesis of 4-*Z*-deuterated IPP **30** is completed by the treatment of **138** with tris(tetra-*n*-butyl)ammonium hydrogen pyrophosphate in MeCN in 87% yield. Likewise, reaction of the *E*-[4-^{2}H$_1$]-isopentenyl tosylate (**139**) with tris(tetra-*n*-butyl)ammonium hydrogen pyrophosphate in MeCN gives **31** in excellent yield.

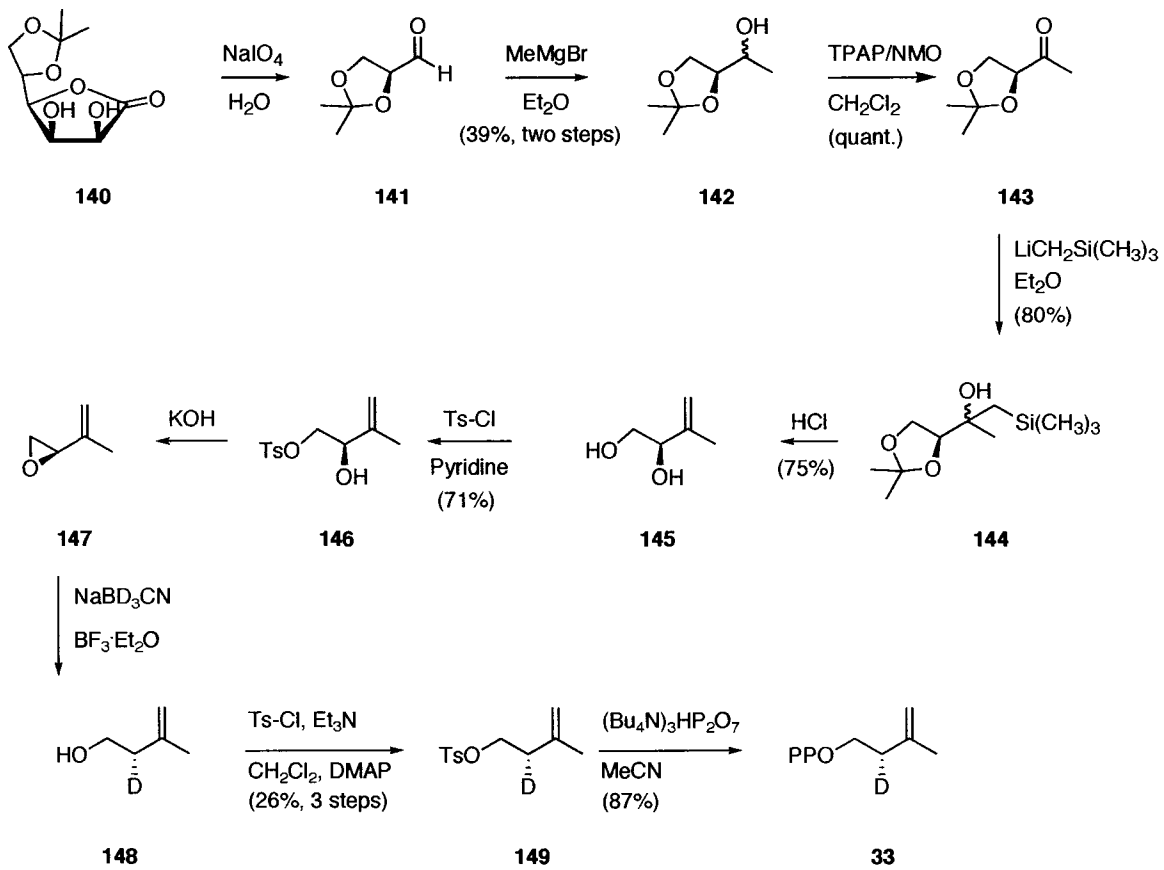
6.2.2 Synthesis of [2-^{2}H$_1$] IPP

There are several reports describing the stereoselective synthesis of (*R*) and (*S*)-[2-^{2}H$_1$] IPP **32** and **33**. These compounds were first synthesized from C4-labelled mevalonate using partially purified enzymes involved in the synthesis of IPP *in vivo*. Purification of the labeled IPP from a complex mixture of products and the lack of commercial preparation of enzymes make this approach undesirable. In 1987, Suga *et al.* described the first stereoselective synthesis of **32** from dimethylallyl alcohol in 5 steps with an overall yield of 15% with 85% *ee*.[192] Since these stereolabeled compounds will

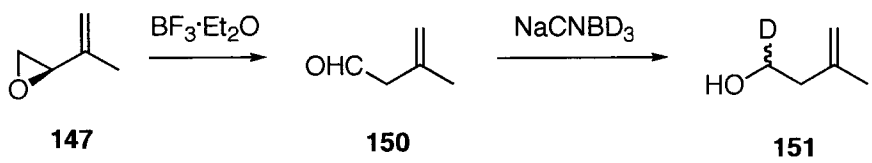
be used to determine the stereochemistry of an enzyme, higher enantiomeric purity would be desirable. In 1999, Poulter reported the synthesis of **32** with >95% enantiomeric purity from commercially available starting material.[193] More recently, Arigoni and co-workers utilized a retro-ene reaction for the synthesis of **33** with 44% *ee*.[194] Thus, of the two recently reported syntheses, the synthesis as described by Poulter was used.

The preparation of (2*S*)-[2-$^2H_1$]-IPP **33** is shown in Scheme 36. Oxidation of 5,6-O-isopropylidene–L-gulono-1,4-lactone (**140**) using a procedure described by Hubschwerlan *et al.*[195,196] provides protected L-glyceraldehyde **141**. Addition of methylmagnesium bromide and subsequent oxidation of the alcohol **142** with TPAP and NMO provides the methyl ketone **143**. Addition of (trimethylsilyl)methyllithium to **143** gives the β-silyl alcohol **144** in 80% isolated yield. Treatment of **144** with HCl in refluxing ethanol affords the enediol **145** which is subsequently treated with *p*-toluenesulfonyl chloride in pyridine at 0 °C to provide **146**. High regioselectivity for reaction of the primary hydroxyl group is observed under these reaction conditions. Reaction of the tosylate **146** with powdered KOH gives the volatile epoxide **147**. Without isolation, the epoxide **147** is treated with sodium borodeuteride in the presence of boron trifluoride–etherate to afford the chiral alcohol **148**. Unfortunately, this product is contaminated with approximately 5% of the racemic [1-$^2H_1$]-isopentenol (**151**), due to a competing BF_3-catalyzed rearrangement of the oxirane **147** to aldehyde **150,** which is subsequently reduced by $NaCNBD_3$ (Scheme 37).

Scheme 36. Synthesis of (2*S*)-[2-$^2H_1$]-IPP (**33**)



Scheme 37. Formation of racemic [1-$^2H_1$]-isopentenol **151**

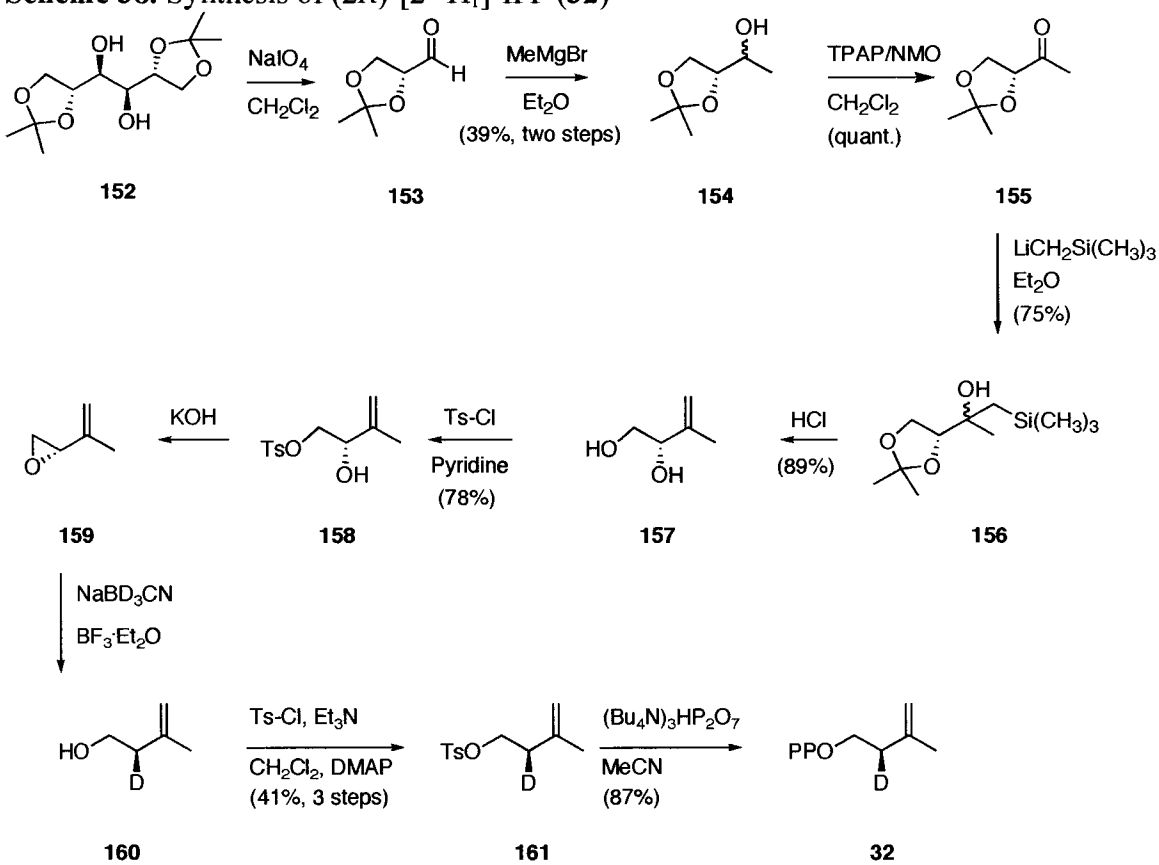


The synthesis of (2*S*)-[2-$^2H_1$]-IPP (**33**) is then completed as follows. Treatment of **148** with *p*-toluenesulfonyl chloride in CH_2Cl_2 affords the chiral tosylate **149**, which is then transformed into **33** by reaction with tris(tetra-*n*-butyl)ammonium hydrogen

pyrophosphate in MeCN. The enantiomeric purity of **149** is determined to be > 95% *ee* by the method of Meddour *et al.*[197] This procedure involves ^{2}H-NMR spectroscopy in a 20% (w/v) liquid crystal of poly-γ-benzyl-L-glutamate (PBLG)/CH_2Cl_2. This technique is sensitive for the enantiotopic discrimination of deuterons in methylene groups. It was previously shown that other methods for determining *ee*'s of chiral deuterio alcohols, such as using chiral derivatization reagents, fail to determine the enantiomeric purity of **149**.

The synthesis of (2*R*)-[2-$^2H_1$]-IPP (**32**) was then realized as shown in Scheme 38. This is similar to the strategy used for the synthesis of **33** except that D-mannitol 1,2:5,6-bis acetonide (**152**) is used as the starting material (Scheme 38).

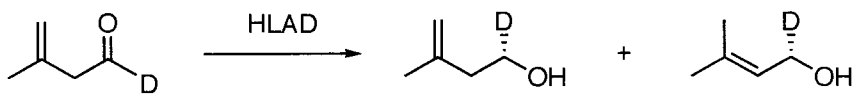
Scheme 38. Synthesis of (2*R*)-[2-$^2H_1$]-IPP (**32**)



6.2.3 Synthesis of [1-$^2H_1$] IPP

Having completed the synthesis of four of the six required deuterium labeled analogs of IPP, the synthetic efforts now focused on the preparation of the enantiomerically enriched [1-$^2H^1$] analogs of IPP **34** and **35**. A key intermediate in the synthesis would be (1*R*) or (1*S*) [1-$^2H^1$]-isopentenol. Historically, chiral 1-deuterio alcohols have been accessed by the reduction of the corresponding deuterio aldehyde. In 1974, Cornforth and coworkers reported the use of horse liver alcohol dehydrogenase to facilitate the chiral reduction of deuterated 3-methylbut-3-enal **162** to give (1*S*)-3-methyl-[1-$^2H^1$]-but-3-en-1ol (**163**) and (1*S*)-3-methyl-[1-$^2H^1$]-but-2-en-1ol (**164**) in a ratio of 6:1, respectively (Scheme 39).[198] Unfortunately, the reaction conditions used in the enzymatic reduction cause isomerization of 3-methylbut-3-enal to the conjugated aldehyde, 3-methylbut-2-enal, which is subsequently reduced to the corresponding alcohol.

Scheme 39. Reduction of aldehyde **162** by liver alcohol dehydrogenase



162 **163** **164**

In addition, various asymmetric reagents have been exploited for the preparation of chiral deuterio alcohols. For example, Mosher and coworkers have reported the use of chiral lithium hydride reagents for the reduction of aliphatic aldehydes to afford chiral alcohols with *ee*'s of 40-60%.[199] More recently the use of organoboranes has enabled

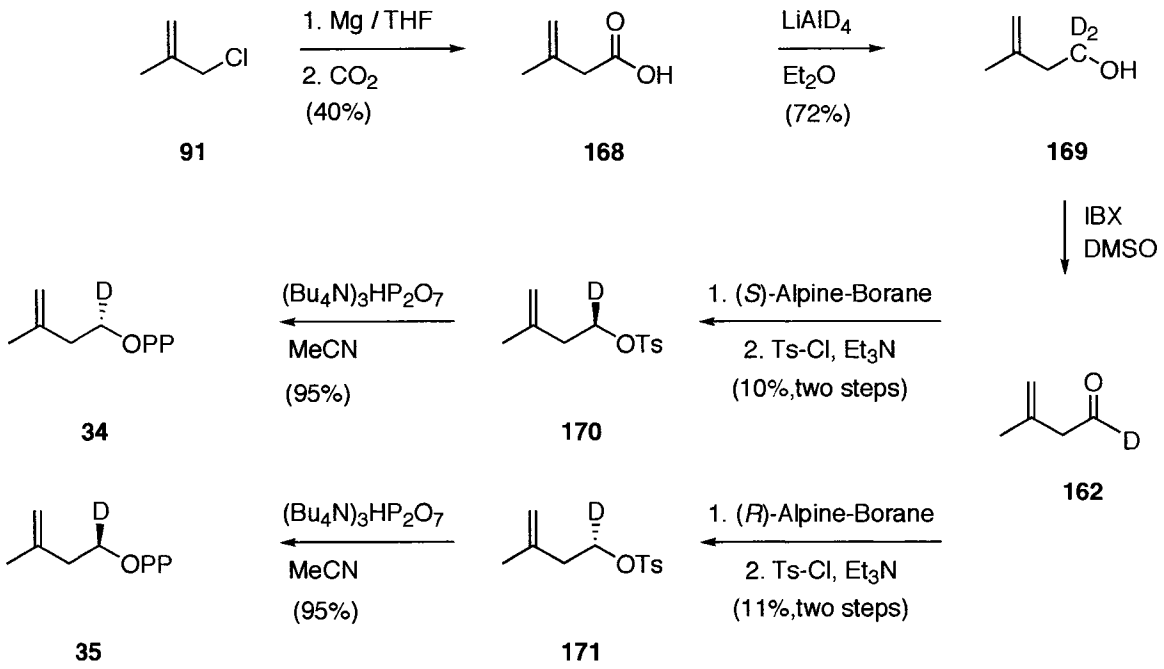
chemists to access chiral alcohols with high enantioselectivity. In 1980, Midland *et al.* discovered that the organoborane, β-isopinocampheyl-9-borabicyclo[3.3.1]nonane (Alpine-Borane®) is an excellent reducing agent for the conversion of aldehydes to alcohols (Scheme 40). This observation made it possible to produce enantiomeric monodeuterated primary alcohols with 100% *ee*.[200,201]

Scheme 40. Alpine-Borane® reduction of deuterated aldehyde **165**

165 **166** **167** 100% *ee*

The synthesis of (1*S*)-isopentenol was attempted based on a report of Cane *et al.* that described the synthesis using a suspension of liver alcohol dehydrogenase.[191] Initial attempts using this procedure failed to produce any significant amounts of desired product; therefore other procedures were investigated. The use of Alpine-Borane® was attractive because it would enable access to both enantiomers of [1-$^{2}H^{1}$]-isopentenol without the use of deuterium labeled reducing agents since both *R*- and *S*-Alpine-Borane® reagents are commercially available. The synthesis of (1*S*)- and (1*R*)-[1-$^{2}H_{1}$]-IPP is shown in Scheme 41.

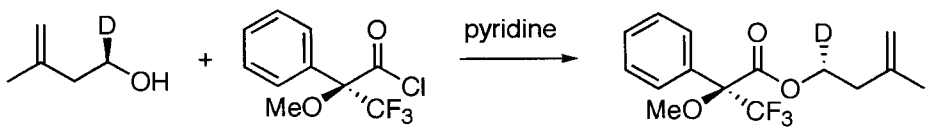
Scheme 41. Synthesis of (1*S*) and (1*R*)-[1-$^2H_1$]-IPP (**34**) and (**35**)



Reaction of chloro-3-methyl-propene **91** with magnesium turnings gives the corresponding Grignard reagent, which is subsequently quenched with CO_2 gas to afford the allylic acid **168**. Reduction of the acid with $LiAlD_4$ provides the deuterio alcohol **169**. With the synthesis of the deuterium labeled precursor completed, its conversion into the corresponding aldehyde **162** was investigated. The procedure by Cane *et al.* using pyridinium chlorochromate (PCC)[202] was attempted, but under these reaction conditions the desired aldehyde is contaminated by the conjugated aldehyde.[203] Other oxidative conditions were tried, including the use of pyridinium dichromate (PDC),[204] Dess-Martin oxidation,[205,206] Swern oxidation[207-209] and IBX.[210,211] Trial reactions with the unlabeled 3-methyl-3-buten-1-ol indicated that only IBX did not cause any isomerization to the conjugated aldehyde at room temperature.

Thus, [1-^{2}H] 3-methylbut-3-en-1-al **162** reacts with (*S*)-Alpine-Borane®, to give (1*R*)-[1-$^2H_1$]-isopentenol (**172**). Typically the enantiomeric purity of alcohols can be determined by conversion to a chiral ester, such as the optically active camphanoyl ester or α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) ester.[212] This technique depends on the chemical shift difference of the α- and β-protons that are now diastereotopic. Treatment of (1*R*)-[1-$^2H_1$]-isopentol (**172**) with (-)-camphanoyl chloride affords its camphanoyl ester. Unfortunately, NMR analysis of this ester in a variety of deuterated NMR solvents did not resolve the α-protons. Reaction of the chiral alcohol **172** with (*R*) α-methoxy-α-(trifluoromethyl)phenylacetyl chloride **173** in pyridine gives the corresponding MTPA ester **174** (see Scheme 42), and ^{2}H-NMR analysis in C_6H_6 indicates that the alcohol **172** has an *ee* of 90%.

Scheme 42. Preparation of MTPA ester of (1*R*)-[1-$^2H_1$]-isopentol (**172**)



172 **173** **174**

Treatment of the chiral alcohol **172** with *p*-toluenesulfonyl chloride affords the tosylate **170**, which is then transformed into the desired (1*S*)-[1-$^2H_1$]-IPP (**34**). ^{2}H-NMR spectral analysis of **170** in a 20% (w/v) liquid crystal of poly-γ-benzyl-L-glutamate (PBLG)/CH_2Cl_2 also indicates that the reduction by (*S*)-Alpine-Borane® proceeds with an *ee* of 90%. Analogously, reduction of the aldehyde **162** with (*R*)-Alpine-Borane®

proceeds with an *ee* of 85%. Reaction of the resulting alcohol with *p*-toluenesulfonyl chloride gives **171**, which is then converted to the desired compound, (1*R*)-[1-$^{2}H_{1}$]-IPP (**35**).

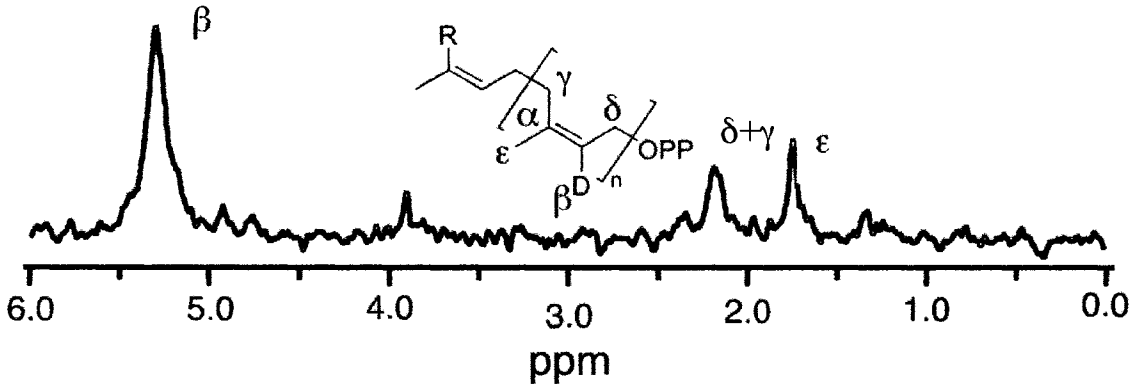
6.2.4 Stereochemistry of Hydrogen Elimination in Rubber Biosynthesis

With the syntheses of the six deuterium labeled analogs of IPP complete, it is possible to probe the stereochemistry of rubber biosynthesis. The stereochemistry of the hydrogen elimination step would be investigated first, since the analysis of the products would be relatively straightforward. It is expected that after incorporation of the specifically deuterated analogs of IPP only one of the two enantiomers should result in retention of deuterium that can be easily detected by ^{2}H-NMR analysis of the rubber products. Thus, each of the stereospecifically deuterated IPP's, (2*R*)-[2-$^{2}H_{1}$]-IPP (**32**) and (2*S*)-[2-$^{2}H_{1}$]-IPP (**33**) were incorporated into rubber by incubation with rubber particles isolated from either *Hevea brasiliensis* or *Parthenium argentatum*. The resulting rubber products could be isolated and subsequently analyzed by ^{2}H NMR.

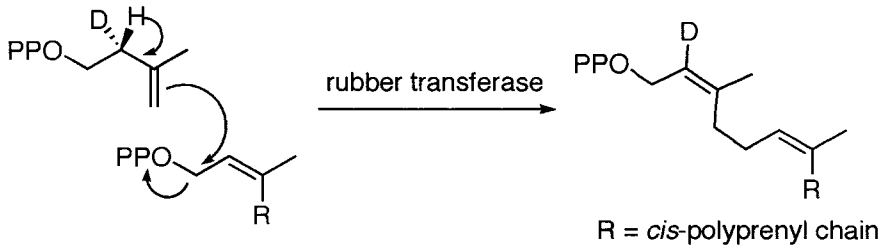
It is expected that incorporation of these labeled IPP analogs would result in incorporation of deuterium at the vinylic position of the *cis*-1,4-polyisoprene chain. Analysis of the rubber product resulting from the incorporation of (2*S*)-[2-$^{2}H_{1}$]-IPP (**33**) results in no increase in the intensity of the olefinic signal at 5.1 ppm. However, ^{2}H-NMR analysis of the rubber product isolated from incorporation of (2*R*)-[2-$^{2}H_{1}$]-IPP (**32**) shows an increase in the intensity of the signal at 5.1 ppm (Figure 34). These results

indicate that the pro-*S* hydrogen is eliminated whereas the pro-*R* hydrogen is retained during rubber biosynthesis (Scheme 43). This observation is in agreement with what is known about other *cis*-prenyltransferases, such as UPP synthase.[186,187]

Figure 34. Solution ^{2}H-NMR (76.5 MHz, $CHCl_3$) spectra of rubber from incorporation of (2*R*)-[2-$^{2}H_1$]-IPP (**32**)



Scheme 43. Analysis of stereochemistry of hydrogen elimination

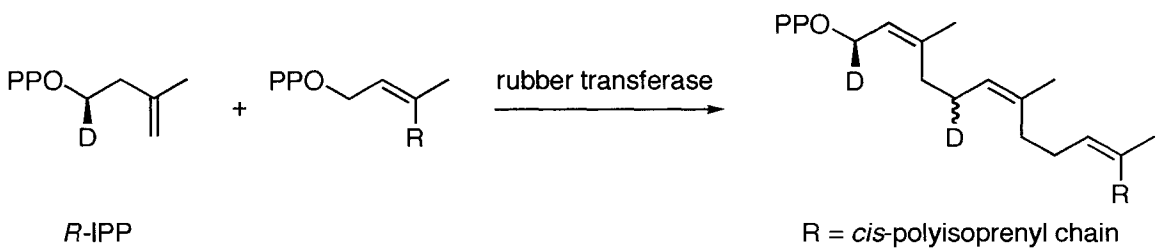


6.2.5 Stereochemistry of Addition of IPP at the Primary Allylic Diphosphate

Analogs of IPP with stereospecific deuterium label at the 1-position can determine the stereochemistry of the addition of IPP to the allylic diphosphate of the

growing chain during rubber biosynthesis (Scheme 44). Incubation of the labeled IPP with rubber particles followed by degradation and spectroscopic analysis of the resulting rubber should establish the relative configuration of deuterium label in the product. Previously, the stereochemical analysis of farnesyl diphosphate synthase involved ozonolysis of the stereolabeled farnesol to give (2*R*)- or (2*S*)-[2-$^{2}H_{1}$]-levulinic acid. The stereochemistry of levulinic acid can be correlated with known optical rotations values for stereospecifically labeled levulinic acids reported by Cornforth and coworkers.[181] This stereochemical analysis requires that the optical rotation value be sufficient to differentiate between enantiomers that are isolated in very small amounts. Since the enzyme preparations used for the biosynthesis of rubber contain a large amount of pre-existing non-labeled rubber, this particular procedure is difficult for stereochemical analysis due to dilution with non-labeled levulinic acid.

Scheme 44. Labeling of rubber by [1-$^{2}H_{1}$]-IPP



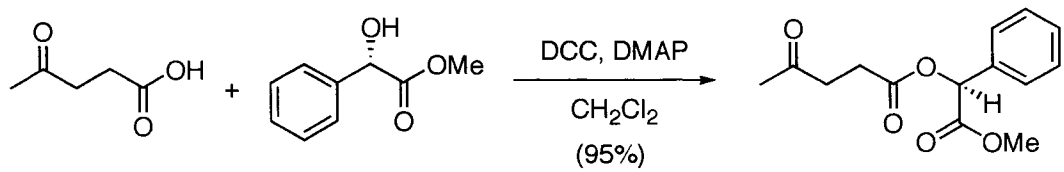
R-IPP

R = *cis*-polyisoprenyl chain

An alternative solution would involve the chemical synthesis of labeled levulinic acid with known configuration followed by derivatization with a chiral reagent so that ^{2}H-NMR could be used to determine the stereochemistry of the levulinic acid isolated from the enzymatic reactions. The ^{1}H-NMR of the methyl mandelate ester of levulinic

acid indicates that the diastereotopic hydrogens at C-2 and C-3 can be differentiated. The chiral ester **176** could be prepared by treatment of levulinic acid (**115**) with (*S*)-methyl mandelate **175** and DCC in CH_2Cl_2 (Scheme 45). Thus, to provide standards for the determination of the stereochemistry of deuterated levulinic acid isolated from enzymatic reactions, the synthesis of (*R*) and (*S*)-[2-$^2H_1$]-levulinic acid **178** and **184** was completed.

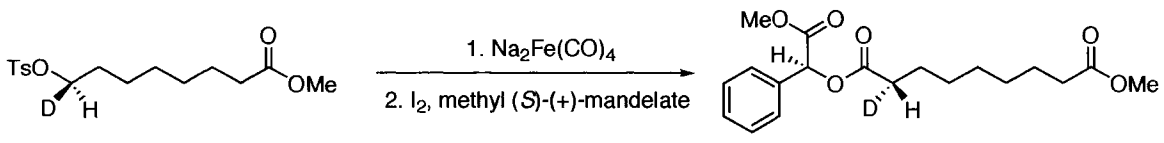
Scheme 45. Synthesis of methyl mandelate ester of levulinic acid **176**



115 **175** **176**

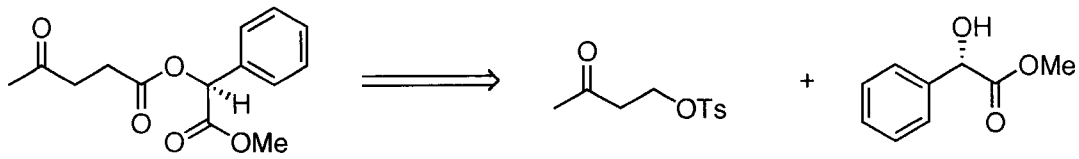
Previous work in our research group has shown that mandelate esters of stereospecifically deuterated esters such as **178** can be made by reacting the corresponding chiral tosylate **177** with disodium tetracarbonylferrate, followed by iodine and methyl (*S*)-(+)-mandelate (**175**, see Scheme 46).[213] Retrosynthetic analysis indicates that the reaction of **179** with disodium tetracarbonylferrate and methyl (*S*)-(+)-mandelate (**175**) should give the desired molecule **176** (Scheme 47). In addition, **179** could be made by ozonolysis of (1*R*)-[1-$^2H_1$]-isopentenyl tosylate (**170**), which is the precursor used for the synthesis of (*1S*)-[1-$^2H_1$] IPP (**34**).

Scheme 46. Reported synthesis of methyl mandelate ester **178**



177 178

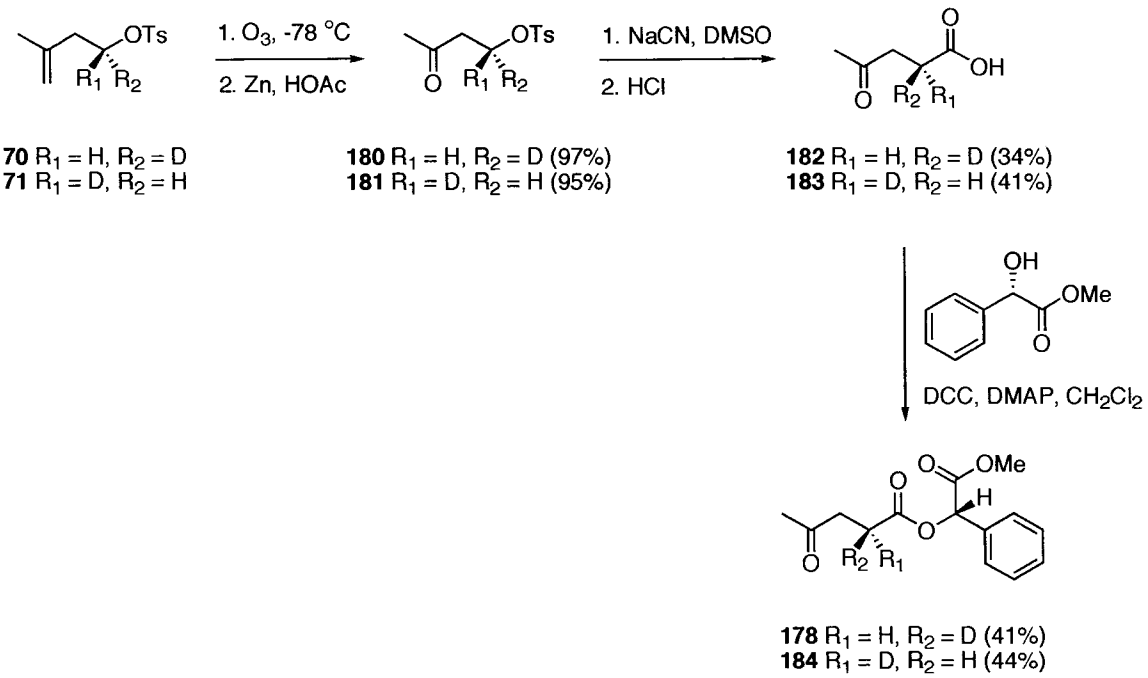
Scheme 47. Retrosynthetic analysis for the preparation of **176**



176 179 175

To ensure that this approach would be suitable, the reaction of unlabeled material was attempted. Unfortunately, the reaction of either 3-methyl-3-buten-1-yl tosylate or 2-butanone-4-yl tosylate (**179**) with disodium tetracarbonylferrate and methyl (*S*)-(+)-mandelate (**175**) did not give any product, only starting material could be isolated. As a result, an alternative synthetic route was designed that is shown in Scheme 48.

Scheme 48. Synthesis of methyl mandelate esters of [2-$^2H_1$] levulinate **178** and **184**



170 R_1 = H, R_2 = D
171 R_1 = D, R_2 = H

180 R_1 = H, R_2 = D (97%)
181 R_1 = D, R_2 = H (95%)

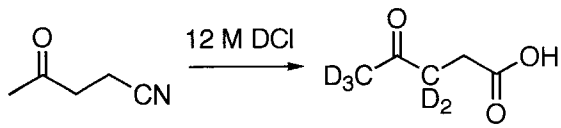
182 R_1 = H, R_2 = D (34%)
183 R_1 = D, R_2 = H (41%)

178 R_1 = H, R_2 = D (41%)
184 R_1 = D, R_2 = H (44%)

Ozonolysis of (1*R*)-[1-$^2H_1$]-3-methyl-3-buten-1yl tosylate (**170**) in CH_2Cl_2 at -78 °C followed by treatment with zinc and acetic acid gives the methyl ketone **180**. Reaction of (4*R*)-[4-$^2H_1$]-2-butanone-4-yl tosylate (**180**) with sodium cyanide in DMSO generates the corresponding nitrile. Model studies of the hydrolysis of the unlabeled nitrile **185** in 12 M DCl indicate that exchange of enolizable hydrogens occurs at the carbons α to the methyl ketone, but that no exchange is observed at the carbon α to the nitrile (Scheme 49). Thus, hydrolysis of the deuterated nitrile with 6 N HCl gives the stereospecifically labeled levulinic acid **182**, which is transformed to its mandelate ester **178** by reaction with methyl (*S*)-(+)-mandelate (**175**). Ester **184** is made analogously from **171**. ^{2}H-NMR analysis of **178** and **184** in C_6H_6 shows that the deuterium in (*R*)-(+)-(methoxycarbonyl)benzyl (2*S*)-[2-$^2H_1$]-levulinate (**178**) has a chemical shift of 2.48 and

that in (*S*)-(+)-(methoxycarbonyl)benzyl (2*R*)-[2-$^2H_1$]-levulinate (**184**) has a chemical shift of 2.44.

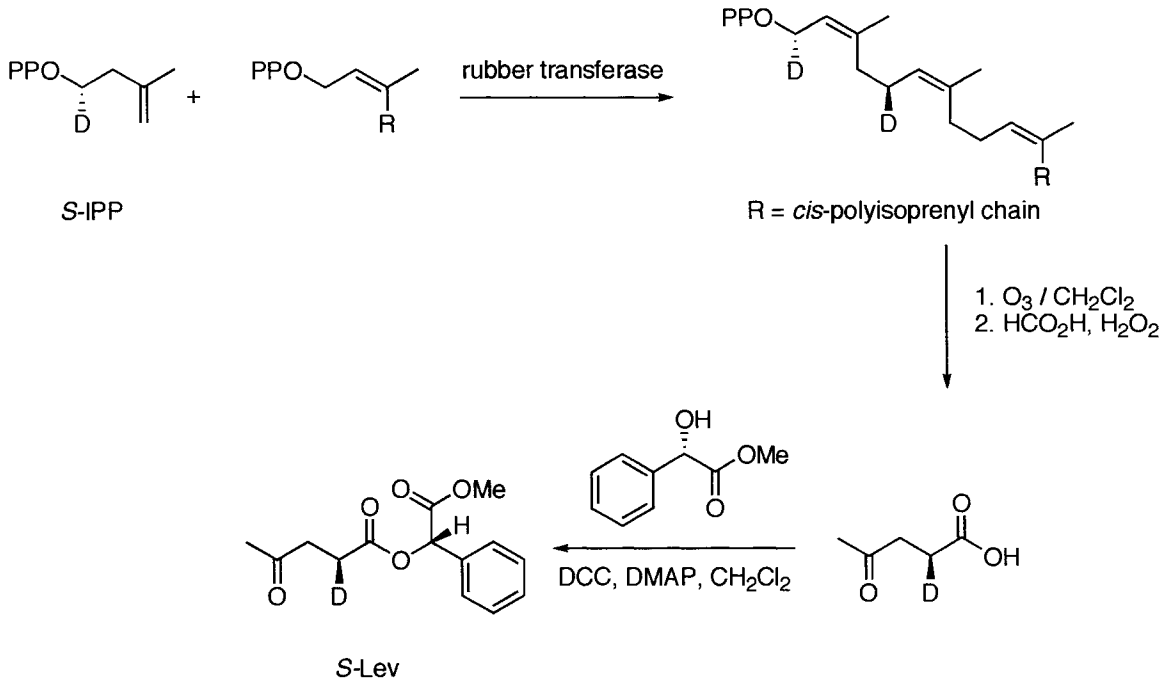
Scheme 49. Hydrolysis of nitrile **182** in 12 M DCl



185 **186**

With the synthesis of the standard methyl mandelate esters of (2*R*)- and (2*S*)-[2-$^2H_1$]-levulinic acid completed, the stereochemistry of displacement of the allylic diphosphate could be investigated. Incorporation of (1*S*)-[1-$^2H_1$] IPP **34** with washed rubber particles from *H. brasiliensis* gave new rubber products. Ozonolysis of the rubber in $CHCl_3$ gives levulinic acid, which is transformed into its mandelate ester. ^{2}H-NMR analysis indicated the incorporated deuterium had a chemical shift of 2.48 ppm, which indicates that the deuterium in the levulinic has an *S* configuration. This demonstrates that displacement occurs with overall inversion of stereochemistry at the primary allylic carbon (Scheme 50). Analysis of the rubber isolated from the incorporation of (1*R*)-[1-$^2H_1$] IPP (**35**) with rubber particles from *H. brasiliensis* indicates that the deuterium in levulinic acid has an *R* configuration, confirming that the displacement occurs with inversion of stereochemistry. Studies with rubber particles isolated from *P. argentatum* give the same experimental outcome.

Scheme 50. Stereochemistry of addition reaction



6.2.6 Stereochemical Direction of Carbon-Carbon Bond Formation

During the biosynthesis of rubber a new carbon-carbon bond is formed between the double bond of IPP and the allylic carbon of the growing rubber chain. The stereochemical direction of this process can be investigated by studying the incorporation of either Z- or *E*-[4-$^2H_1$]-IPP by rubber transferase. Analysis of the isolated rubber from these incorporation studies would establish which surface of the double bond in IPP attacks the incipient cation. Ozonolysis of the rubber products would give (3*R*)- or (3*S*)-levulinic acid, which can be correlated to authentic standards. ^{2}H-NMR analysis of the methyl mandelate esters of levulinic acid would be employed to correlate the observed

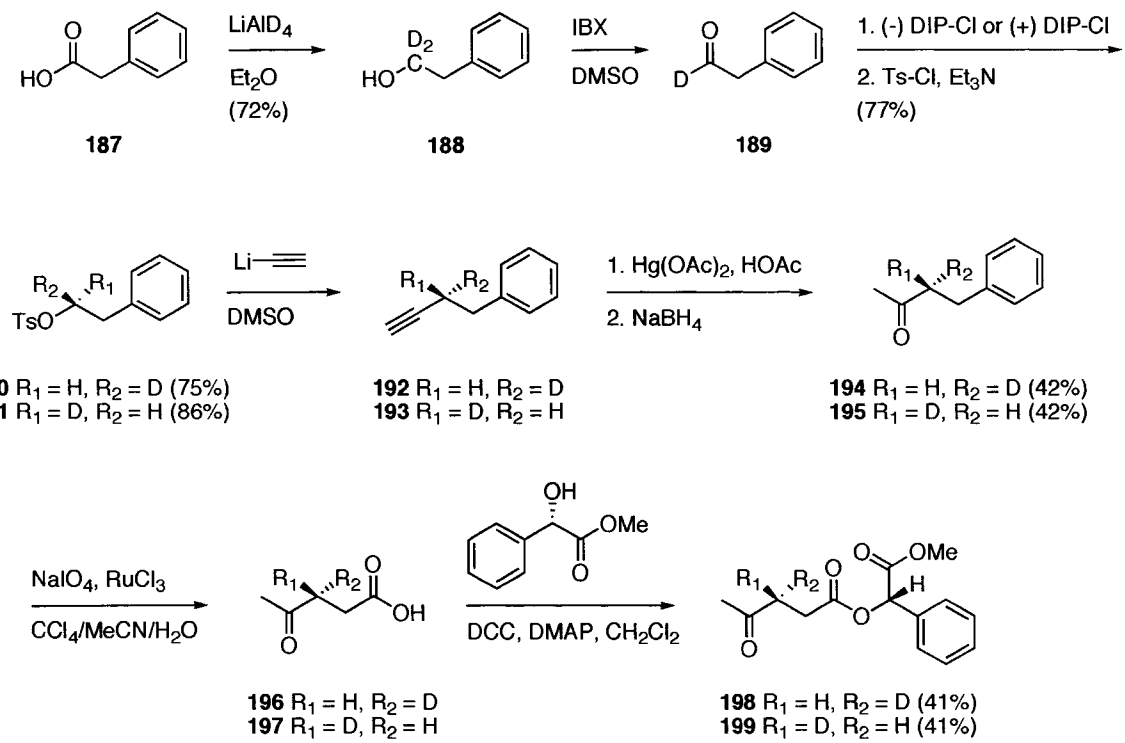
stereochemistry in the enzymatic samples with authentic standards. This stereochemical analysis requires the synthesis of both (3*R*)- and (3*S*)-[3-$^2H_1$]-levulinic acid.

Previously, the synthesis of (2*R*)- and (2*S*)-[2-$^2H_1$]-levulinic acid involved the hydrolysis of the corresponding nitrile to its carboxylic acid. Unfortunately, the reaction conditions used cause exchange of hydrogen at the carbons α to the methyl ketone. Hence, this synthetic strategy would be ineffective for the synthesis of [3-$^2H_1$]-levulinic acid as the deuterium label would be lost due to hydrogen exchange. It is evident that any reaction conditions used would need to be mild to avoid exchange of deuterium with solvent hydrogens. Leslie Crombie and Andrew Heavers have developed reaction sequences that allow the synthesis α-deutero methyl ketones.[214] Their strategy relies on mild oxidative conditions to convert a terminal alkyne to the corresponding methyl ketone without epimerizing the α-carbon. Thus, a synthetic strategy was developed using this methodology as a key step.

The synthesis of (*R*)- and (*S*)-methyl mandelate esters of [3-$^2H_1$]-levulinic acid **198** and **199** is shown in Scheme 51. Reduction of phenylacetic acid (**187**) with lithium aluminum deuteride gives the deutero alcohol **188**, which is treated with IBX to afford [1-$^2H_1$]-phenylethanal (**189**). Reduction of the deuterioaldehyde **189** with (-)-β-chlorodiisopinocampheylborane affords the stereospecifically deuterated alcohol, which is subsequently reacted with *p*-toluenesulfonyl chloride and Et_3N to yield the chiral tosylate **190**. Displacement of the primary sulfonate with lithium acetylide affords the terminal acetylide **192**. Reaction of **192** with stoichiometric amount of mercury (II)

acetate followed by reduction of the carbon-mercury bond with sodium borohydride and oxidation of the secondary alcohol provides the desired methyl ketone **194** with no loss of the deuterium label.

Scheme 51. Synthesis of methyl mandelate esters of [3-$^2H_1$]-levulinate **198** and **199**

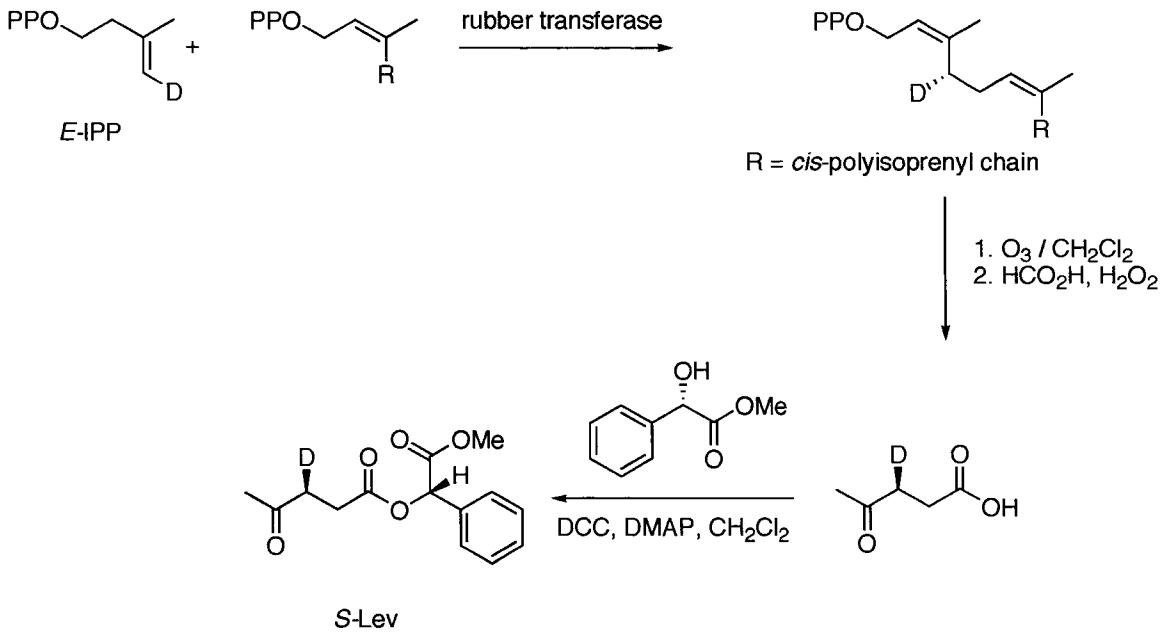


Oxidation of the phenyl group to a carboxylic acid using ruthenium trichloride and periodic acid as the co-oxidant failed to yield any of the desired product with test reactions suggesting that deuterium exchange had occurred. It was discovered that the use of sodium periodate as co-oxidant did not cause exchange of deuterium. Hence, oxidation of (3*R*)-[3-$^2H_1$]-4-phenyl-2-butanone (**194**) with ruthenium trichloride and sodium periodate gives the desired deuterium labeled acid, (3*R*)-[3-$^2H_1$]-levulinic acid

196. Similar reaction conditions were used to synthesize (3*S*)-[3-$^2H_1$]-levulinic acid (**197**) from (1*R*)-[1-^2H]-phenylethan-1-yl tosylate (**191**). Reaction of the acid with methyl (*S*)-(+) mandelate gave the corresponding mandelate ester. ^2H-NMR analysis showed that the deuterium in (*S*)-(+)-(methoxycarbonyl)benzyl (3*R*)-[3-$^2H_1$]-levulinate (**198**) has a chemical shift of 2.01 ppm and that in (*S*)-(+)-(methoxycarbonyl)benzyl (3*S*)-[3-$^2H_1$]-levulinate (**199**) has a chemical shift of 2.21 ppm.

With the synthesis of the methyl mandelate esters of (3*R*)- and (3*S*)-[3-$^2H_1$]-levulinic acid (**198**) and (**199**) completed, the stereochemical course of the carbon-carbon bond formation of rubber biosynthesis could be investigated. Incorporation of *E*-[4-^2H]-IPP (**31**) with washed rubber particles from *H. brasiliensis* gives new rubber. Ozonolysis of the rubber in $CHCl_3$ generates levulinic acid, which is converted into its mandelate ester. ^2H-NMR analysis in C_6H_6 indicates the incorporated deuterium has a chemical of 2.21 ppm, which corresponds to (*S*)-(+)-(methoxycarbonyl)benzyl (3*S*)-[3-$^2H_1$]-levulinate with comparison to authentic standards (Scheme 52). This observation indicates that the *si* face of the double bond in IPP reacts during the formation of rubber. Analysis of the rubber isolated from the incorporation of *Z*-[4-^2H]-IPP (**30**) with rubber particles indicates that the deuterium in levulinic acid has a *R* configuration, confirming that the stereochemical direction of carbon-carbon bond formation occurs from the *si* face of IPP. Incorporation studies with rubber transferase from *P. argentatum* give the same experimental conclusion.

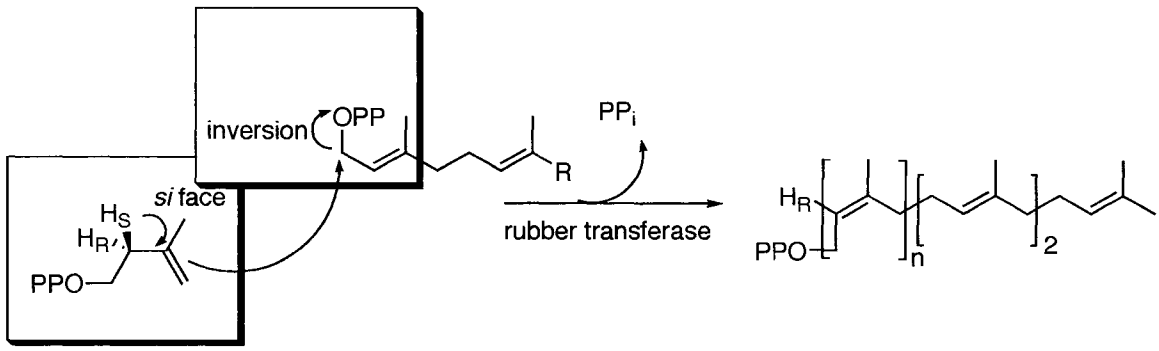
Scheme 52. Stereochemical direction of carbon-carbon bond formation



6.3 CONCLUSION

In summary, six deuterium analogs of IPP were prepared to study the cryptic stereochemistry of rubber biosynthesis in two plant species, *Hevea brasiliensis* and *Parthenium argentatum*. Incorporation studies with corresponding rubber particles and ^{2}H-NMR analysis (after degradation where necessary) indicated that the stereochemistry of rubber transferase is similar to that of certain other *cis*-prenyltransferases including undecaprenyl diphosphate synthase (Scheme 53). Thus, the *pro-S* hydrogen (H_S) is preferentially cleaved during the polymerization, and *si* face addition to the allylic diphosphate occurs with overall inversion of stereochemistry. This project reveals the 3-dimensional arrangement of substrates within the active site of rubber transferase, illustrating the stereochemistry of the enzyme and providing a better mechanistic understanding of how rubber is biosynthesized.

Scheme 53. Cryptic stereochemistry of rubber transferase



Chapter 7. Experimental Procedures

7.1 General Procedures

All non-aqueous reactions involving air or moisture sensitive reactants were performed under an atmosphere of argon using oven-dried glassware. Reagents and solvents were reagent grade and used as supplied unless otherwise stated. Solvents for anhydrous reactions were dried according to Perrin.[215] Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled over sodium and under an argon atmosphere. Acetonitrile (MeCN), dichloromethane (CH_2Cl_2), triethylamine (Et_3N) and pyridine were distilled from calcium hydride. Methanol (MeOH) and ethanol (EtOH) were distilled over magnesium turnings and a catalytic amount of iodine. Water was obtained from a milli-Q reagent water system. "Brine" refers to a saturated aqueous solution of NaCl. Unless otherwise specified, solutions of HCl, NH_4Cl, $NaHCO_3$, KOH and NaOH refer to aqueous solutions. Solvent evaporation was performed under reduced pressure below 40 °C using a Büchi rotary evaporator, followed by evacuation (0.1 torr) to constant sample weight.

All reagents were purchased from Sigma or Aldrich and were used without further purification unless otherwise stated. Copper (I) bromide dimethylsulfide complex was either used fresh from commercial sources or recrystallized from old bottles.[216] Dimethyl acetylenedicarboxylate (DMAD) was distilled at reduced pressure before using (95-98

°C/19 mm Hg). Hexamethylphosphoramide (HMPA) was dried by stirring with calcium hydride under argon for 36 h, followed by distillation at reduced pressure and was stored over molecular sieves.

All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC) using Merck glass-backed plates precoated with normal silica gel (SiO_2, Merck 60F254). One or more of the following methods were used for visualization: UV absorption by fluorescence quenching; iodine staining; phosphomolybdic acid:ceric sulfate:sulfuric acid:H_2O/10 g:1.25 g:12 mL:238 mL spray; and 50% sulfuric acid spray. Flash chromatography was performed according to the method of Still *et al.* using Merck type 60, 230-400 mesh silica gel.[217]

High-pressure liquid chromatography (HPLC) was performed on a Beckman System Gold instrument equipped with a model 166 variable wavelength UV detector and an Altex 210A injector with a 500 or 1000 μL sample loop. The columns used were from Grace-Vydac. All HPLC solvents were prepared fresh daily and filtered with a Millipore filtration system under vacuum before use.

Optical rotations were measured on a Perkin Elmer 241 polarimeter with a microcell (10 cm, 1 mL) at ambient temperature and are reported in units of 10^{-1} deg cm^2 g^{-1}. All optical rotations reported were referenced against air and were measured at the sodium D line and values quoted are valid within ± 1°. Infrared spectra (IR) were recorded on a Nicolet Magna 750 or a 20SX FT-IR. Cast refers to the evaporation of a

solution on a NaCl plate. Mass spectra (MS) were recorded on a Kratos AEIMS-50 high resolution (HMRS), electron impact ionizations (EI), MS-9 (fast atom bombardment with argon (FAB), and Micromass ZabSpec Hybrid Sector-TOF positive mode electrospray ionization ((ES), 0.5% solution of formic acid in MeCN:H_2O/1:1) instruments. Microanalyses were obtained using a Perkin Elmer 240 or Carlo Erba 1108 elemental analyzers.

Solution nuclear magnetic resonance (NMR) spectra were obtained on Bruker AM-300 or Inova Varian 300, 400, 500 and 600 MHz spectrometers. ^{1}H NMR chemical shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS) using the residual proton resonance of solvents as reference: $CDCl_3$ δ 7.24, C_6H_6 δ 7.15, CD_2Cl_2 δ 5.32, D_2O δ 4.72, and CD_3OD δ 3.30. ^{13}C NMR chemical shifts are reported relative to $CDCl_3$ δ 77.0, CD_2Cl_2 δ 53.8, C_6D_6 δ 128.6, and CD_3OD δ 49.0. Selective mononuclear decoupling, attached proton test (APT), ^{1}H-^{1}H, ^{1}H-^{13}C, nuclear Overhauser effect (NOE) correlations, gradient heteronuclear multiple quantum coherence (gHMQC) experiments were occasionally used for signal assignments. ^{1}H NMR data are reported in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; qn, quintet; m, multiplet), number of protons, coupling constant (*J*) in Hertz (Hz) and assignment. When appropriate, the multiplicity is preceded by br, indicating the signal is broad. All literature compounds had IR, ^{1}H NMR, and mass spectra consistent with the assigned structures. More detailed analysis of ^{1}H NMR and ^{13}C NMR was done for all unknown compounds.

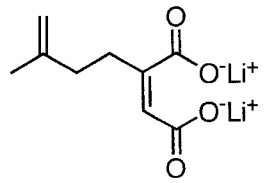
Solid state ^{1}H decoupled ^{13}C NMR spectra were obtained with single-pulse excitation on a CMX Infinity 200 NMR spectrometer operating at 50.33 MHz for ^{13}C; 90 degree pulse widths were 4 us (microseconds); acquisition times were 100 ms and the recycle delay was 5 s. Approximately 4 mg of sample were packed into 4 mm o.d. NMR rotors. NaCl (approximately 80 mg) was added to the rotor above and below the sample to facilitate spinning and to ensure that the sample was centred in the NMR coil. Samples were spun at the magic angle at 5 to 6 kHz. All spectra were obtained by Dr. Guy Bernard in the Department of Chemistry at the University of Alberta.

Radioactivity was determined using standard liquid scintillation procedures in plastic 10 ml scintillation vials with a Beckman Ready-gel scintillation cocktail. The scintillation counter used was a Beckman LS 5000TD with automatic quench control to directly determine decompositions per minute (dpm) in the labelled samples against a quench curve prepared from Beckman ^{14}C quenched standards.

7.2 Experimental Data for Compounds

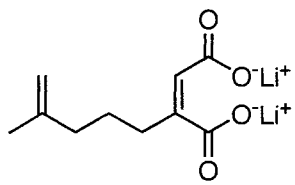
General Procedure for the basic hydrolysis of the dimethyl esters to lithium salts.

To a stirring solution of di-ester in THF-H_2O (2 mL, 1:1) was added a 1.0 M solution of LiOH (3 eq) and the mixture was stirred at 50 °C. The progress of the reaction was monitored by TLC analysis. Upon complete consumption of the starting material, the solvent was removed *in vacuo* and the remaining residue was dissolved in H_2O (4 mL). Lyopholization of the aqueous layer gave the respective lithium salt.



(Z)–2-(2-Methyl)butenylbutenedioic acid, dilithium salt (17).

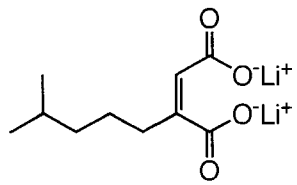
The hydrolysis of ester **47** (100 mg 0.471 mmol) gave salt **17** (90 mg, 99%) as a white powder: IR (μscope) 3379, 3079, 2968, 2937, 1648, 1555, 1419 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 5.51 (m, 1H, C=C<u>H</u>), 4.70 (m, 2H, C=C<u>H</u>$_2$), 2.39 (m, 2H, $CH_2C\underline{H}_2$), 2.23 (m, 2H, $CH_2C\underline{H}_2$), 1.73 (s, 3H, $C{=}CCH_3$); ^{13}C NMR (CD_3OD, 75 MHz) δ 179.0, 174.8, 151.3, 147.2, 120.3, 109.9, 35.5, 33.1, 21.9; HRMS (ES -ve) Calc'd for $[M-H]^-$ $C_9H_{11}O_4$ 183.0657, found 183.0663.



(Z)–2-(2-Methyl)pentenylbutenedioic acid, dilithium salt (18).

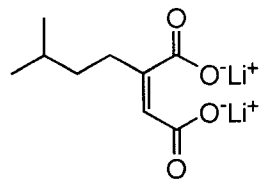
The hydrolysis of ester **51** (98 mg 0.43 mmol) gave salt **18** (89 mg, 99%) as a white powder: IR (μscope) 3379, 3079, 2968, 2937, 1648, 1555, 1419 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 5.48 (m, 1H, C=C<u>H</u>), 4.70 (m, 2H, C=C<u>H</u>$_2$), 2.21 (t, 2H, J = 8.0 Hz, $CH_2C\underline{H}_2$), 2.08 (t, 2H, J = 7.4 Hz, $CH_2C\underline{H}_2$), 1.72 (s, 3H, $C{=}CC\underline{H}_3$), 1.59 (dqn, 2H, J = 1.4 Hz, 7.4 Hz, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (D_2O, 75 MHz) δ 179.9, 175.4, 152.6, 148.6, 120.7, 110.4,

37.4, 34.9, 25.8, 22.3; HRMS (ES -ve) Calc'd for $[M-H]^-$ $C_{10}H_{13}O_4$ 197.0814, found 197.0819.



(Z)–2-(2-Methyl)pentylbutenedioic acid, dilithium salt (19).

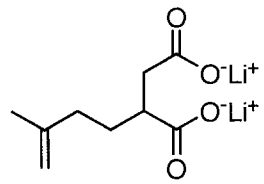
The hydrolysis of ester **53** (100 mg, 0.438 mmol) gave salt **19** (93 mg, 99%) as a white powder: IR (μscope) 3433, 2954, 2870, 2845, 1640, 1586, 1428 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 5.48 (m, 1H, C=CH), 2.21 (m, 2H, C=CC$\underline{H}_2$), 1.45 (m, 3H, C$\underline{H}_2$C$\underline{H}$), 1.23 (m, 2H, $CH_2C\underline{H}_2$), 0.88 (d, 6H, J = 6.6 Hz, C(C$\underline{H}_3$)$_2$); ^{13}C NMR (D_2O, 125 MHz) δ 179.7, 175.2, 152.9, 120.0, 38.4, 35.3, 27.5, 25.4, 22.4 (2C); HRMS (ES -ve) Calc'd for $[M-H]^-$ $C_{10}H_{15}O_4$ 199.0970, found 199.0976.



(Z)–2-(2-Methyl)butylbutenedioic acid, dilithium salt (20).

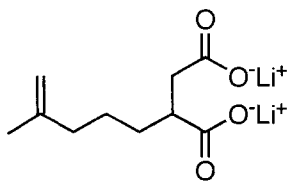
The hydrolysis of ester **55** (99 mg 0.46 mmol) gave salt **20** (102 mg, 94%) as a white powder: IR (μscope) 3409, 2954, 2871, 1638, 1574, 1428 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 5.48 (m, 1H, C=CH), 2.23 (m, 2H, $CH_2C\underline{H}_2$), 1.58 (m, 1H, $CH_2C\underline{H}$), 1.32 (m, 2H, $CHC\underline{H}_2$), 0.87 (d, 6H, J = 6.6 Hz, C(C$\underline{H}_3$)$_2$); ^{13}C NMR (D_2O, 75 MHz) δ 180.0,

175.5, 153.4, 120.2, 37.1, 33.4, 27.9, 22.5 (2C); HRMS (ES -ve) Calc'd for $[M-H]^-$ $C_9H_{13}O_4$ 185.0814, found 185.0819.



2-(3-Methyl-but-3-enyl)-butane dioic acid, dilithium salt (21).

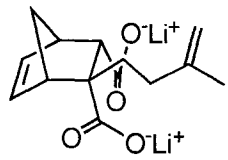
The general procedure was followed. The hydrolysis of ester **60** (21 mg, 98 μmol) gave salt **21** (24 mg, quantitative) as a white powder. IR (μscope) 3372, 2937, 1580, 1433 cm^{-1}; ^1H NMR (D_2O, 500 MHz) δ 4.75 (m, 2H, C=C$\underline{H}_2$), 2.56 (m, 1H, C$\underline{H}$CO), 2.45 (dd, 1H, J = 5.2 Hz, 14.4 Hz, C$\underline{H}_2$CO), 2.18 (dd, 1H, J = 5.2 Hz, 14.4 Hz, C$\underline{H}_2$CO), 2.03 (m, 2H, C$\underline{H}_2$CH), 1.72 (s, 3H, C=CCH_3), 1.59 (m, 2H, C$\underline{H}_2$CH_2); ^{13}C NMR (D_2O, 100 MHz) δ 185.5, 182.6, 148.6, 110.4, 46.8, 42.1, 36.2, 31.1, 22.5; HRMS (ES -ve) Calc'd for $[M-H]^-$ $C_9H_{13}O_4$ 185.0808, found 185.0809.



2-(4-Methyl-pent-4-enyl)-butane dioic acid, dilithium salt (22).

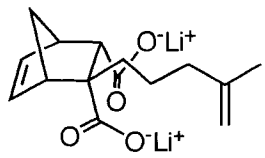
The general procedure was followed. The hydrolysis of ester **61** (21 mg, 98 μmol) gave salt **22** (24 mg, quantitative) as a white powder. IR (μscope) 3356, 2936, 1573, 1427 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ 4.72 (m, 2H, C=C$\underline{H}_2$), 2.54 (m, 1H, C$\underline{H}$CO), 2,41 (dd, 1H, J = 5.4 Hz, 14.3 Hz, C$\underline{H}_2$CO, 2.14 (dd, 1H, J = 9.5 Hz, 14.3 Hz, C$\underline{H}_2$CO), 2.04 (m, 2H,

C<u>H</u>$_2$CH), 1.70 (s, 3H, C=CC<u>H</u>$_3$), 1.42 (m, 4H, C<u>H</u>$_2$C<u>H</u>$_2$) ^{13}C NMR (D_2O, 100 MHz) δ 185.6, 182.6, 148.9, 110.1, 46.9, 42.0, 37.9, 32.5, 25.8, 22.3; HRMS (ES -ve) Calc'd for [M-H]$^-$ $C_{10}H_{15}O_4$ 199.0963 found, 199.0965.



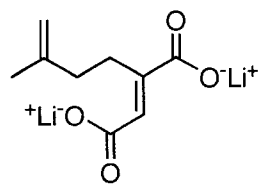
2-(3-Methyl-but-3-enyl)-bicyclo[2.2.1]hept-5-ene-2,3-dioic acid, dilithium salt (23).

The hydrolysis of the anhydride **57** (16 mg, 65 μmol) gave salt **23** (24 mg, 99%) as a white powder. IR (μscope) 3417, 3375, 3078, 2988, 2967, 2884, 1646, 1576, 1469 cm^{-1}; ^{1}H NMR (D_2O, 400 MHz) δ 6.29 (dd, 1H, J = 3.0 Hz, 5.6 Hz, HC=C<u>H</u>), 6.18 (dd, 1H, J = 3.0 Hz, 5.5 Hz, HC=C<u>H</u>), 4.59 (m, 2H, C=C<u>H</u>$_2$), 2.83 (m, 1H, C=CC<u>H</u>) 2.79 (m, 1H, C=CC<u>H</u>), 2.64 (d, 1H, J = 3.1 Hz, C<u>H</u>CO), 2.07 (m, 3H, C<u>H</u>$_2$C=C, CHC<u>H</u>$_2$CH), 1.73 (4H, m, C=CC<u>H</u>$_3$, C<u>H</u>$_2$C=C), 1.57 (d, 1H, J = 8.8 Hz, CH$_2$C<u>H</u>$_2$), 1.28 (td, 1H, J = 1.8 Hz, 8.8 Hz, CH$_2$C<u>H</u>$_2$); ^{13}C NMR (D_2O, 125 MHz) δ 184.3, 183.1, 149.5, 137.8, 136.8, 109.7, 64.4, 61.7, 49.7, 17.6, 47.1, 41.6, 34.9, 22.7; HRMS (ES +ve) Calc'd for [M+Na]$^+$ $C_{14}H_{18}O_4Na$ 273.1097, found 273.1094.



2-(4-Methyl-pent-4-enyl)-bicyclo[2.2.1]hept-5-ene-2,3-dioic acid, dilithium salt (24).

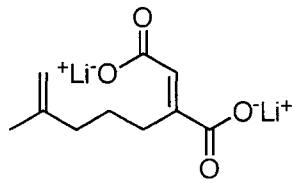
The hydrolysis of the anhydride **59b** (16 mg, 69 μmol) gave salt **24** (25 mg, 99%) as a white powder. IR (μscope) 3373, 3074, 2967, 1704, 1649, 1551, 1416 cm^{-1}; ^1H NMR (D_2O, 500 MHz) δ 6.31 (dd, 1H, J = 5.5 Hz, J = 2.9 Hz, CH=C<u>H</u>), 6.18 (dd, 1H, J = 5.5 Hz, 2.9 Hz, C<u>H</u>=CH), 4.76 (m, 2H, C=C<u>H</u>$_2$), 2.83 (m, 1H, C=CC<u>H</u>), 2.81 (m, 1H, C=CC<u>H</u>), 2.63 (d, 1H, J = 3.1 Hz, C<u>H</u>CO), 2.04 (dt, 2H, J = 3.3 Hz, J = 7.3 Hz, C<u>H</u>$_2$C=C), 1.95 (dt, 1H, J = 4.8 Hz, 12.5 Hz, CH$_2$C<u>H</u>$_2$CH), 1.72 (s, 3H, C=CC<u>H</u>$_3$), 1.51 (m, 4H, CH$_2$C<u>H</u>$_2$CH$_2$, C<u>H</u>$_2$CH$_2$CH$_2$, CHC<u>H</u>$_2$CH), 1.27 (dt, 1H, J = 1.7, 8.7 Hz, C<u>H</u>$_2$CH$_2$CH$_2$); ^{13}C NMR (D_2O, 125 MHz) δ 184.3, 183.3, 149.0, 138.0, 136.6, 110.1, 64.5, 51.9, 49.5, 47.7, 47.2, 42.8, 38.5, 24.5, 22.5; HRMS (ES -ve) Calc'd for $[M-H]^-$ $C_{15}H_{19}O_4$ 263.1278, found 263.1276.



(*E*)–2-(2-Methyl)butenylbutenedioic acid, dilithium salt (25).

The hydrolysis of ester **62** (14 mg, 66 μmol) gave salt **25** (14 mg, 99%) as a white powder: IR (μscope) 3362, 2938, 1648, 1567, 1390 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 6.34 (s, 1H, C=C<u>H</u>), 4.71 (s, 1H, C=C<u>H</u>$_2$), 4.69 (s, 1H, C=C<u>H</u>$_2$), 2.59 (t, 2H, J = 8.1 Hz,

$CH_2C\underline{H}_2$), 2.09 (t, 2H, J = 7.5 Hz, $CH_2C\underline{H}_2$), 1.69 (s, 3H, $C{=}CC\underline{H}_3$); ^{13}C NMR (D_2O, 75 MHz) δ 178.1, 177.2, 147.8, 144.4, 129.4, 110.7, 37.3, 28.0, 22.3; HRMS (ES -ve) Calc'd for $[M-H]^-$ $C_9H_{11}O_4$ 183.0652, found 183.0653.



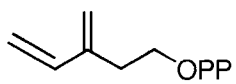
(*E*)–2-(2-Methyl)pentenylbutenedioic acid, dilithium salt (26).

The hydrolysis of ester **63** (15 mg, 66 μmol) gave salt **26** (15 mg, 99%) as a white powder: IR (μscope) 3328, 2935, 1566, 1502, 1428 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ 6.29 (s, 1H, $C{=}C\underline{H}$), 4.69 (m, 2H, $C{=}C\underline{H}_2$), 2.40 (t, 2H, J = 7.8 Hz, $C\underline{H}_2CH_2CH_2$), 1.98 (t, 2H, J = 7.5 Hz, $CH_2CH_2C\underline{H}_2$), 1.66 (s, 3H, $C{=}CCH_3$), 1.47 (qn, 2H, J = 7.7 Hz, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (CD_3OD, 75 MHz) δ 178.4, 177.3, 148.7, 144.8, 129.0, 110.3, 37.7, 29.1, 27.1, 22.3; HRMS (ES -ve) Calc'd for $[M-H]^-$ $C_{10}H_{13}O_4$ 197.0808, found 197.0806.

General Procedure for the preparation of diphosphates.

The general procedure for the preparation of diphosphates is similar to that described by Poulter and coworkers.[218,219] A solution of tosylate in MeCN (5 mL) was treated with tris-*n*-butylammonium hydrogen diphosphate (3 equiv) and stirred for 18 h at room temperature. Solvent was evaporated and residue was dissolved in a minimal volume of a solution of isopropanol:25 mM NH_4HCO_3 (1:49). The solution was passed through an

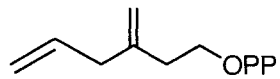
ion exchange column containing DOWEX AG 50W X8 (100-200 mesh, 15 mequiv) cation exchange resin (ammonium form). The clear eluent was lypholized to dryness to give a white solid. ^{1}H NMR analysis indicated that the tetra-*n*-butyl ammonium ion had been successfully exchanged for an ammonium ion. The residue was dissolved in 100 mM NH_4HCO_3 (3 mL) and transferred to centrifuge tube. A solution of MeCN:isopropanol (4 mL, 1:1) was added, and the contents of the tube were mixed on a vortex mixer. The suspension was cleared by centrifugation for 5 min at 2000 rpm. The supernatant was removed and the extraction process was repeated 3 times. The supernatants were combined, concentrated and lypholized. Purification of the crude product using HPLC (Vydac 259VHP reverse phase polymer; 4.6 mm x 150 mm; 10% MeCN, 90% 100 mM NH_4HCO_3 20 min, 10-50% MeCN over 5 min, 50% MeCN 10 min, 50-90% MeCN over 1 min, 90% MeCN 20 min, t_R 44.0 min) afforded the diphosphate as a white solid.



3-Methylene-4-pentenyl diphosphate (28).

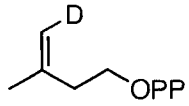
Following the general procedure described, treatment of a solution of 3-methylene-4-pentenyl tosylate (**111**) (53 mg, 0.21 mmol) in MeCN (5 mL) with tris-*n*-butylammonium hydrogen diphosphate (570 mg, 0.63 mmol) gave the crude labeled IPP. Purification of the crude product using HPLC (Vydac 259VHP reverse phase polymer; 4.6 mm x 150 mm; linear gradient elution over 40 minutes of 30% MeCN in 100 mM NH_4HCO_3, t_R 28 min) afforded **28** as a white solid (38 mg, 70%). ^{1}H NMR (D_2O/ND_4OD, 500 MHz) δ

6.47 (1H, dd, J = 10.5 Hz, 17.4 Hz, C<u>H</u>CH$_2$), 5.37 (d, 1H, J = 7.6 Hz, CHC<u>H</u>$_2$), 5.18 (m, 1H, CHC<u>H</u>$_2$), 5.16 (m, 1H, C=C<u>H</u>$_2$), 5.14 (m, 1H, C=C<u>H</u>$_2$), 4.07 (dt, 2H, J = 7.0 Hz, 7.0 Hz, C<u>H</u>$_2$OP), 2.61 (t, 2H, J = 7.0 Hz, C<u>H</u>$_2$CH$_2$); ^{13}C NMR (D_2O/ND_4OD, 125 MHz) δ 144.2, 139.9, 118.9, 115.5, 65.5 (d, J = 5.6 Hz), 33.1; ^{31}P NMR (D_2O/ND_4OD, 162 MHz) δ -9.00 (m, 1P), -5.10 (m, 1P); HRMS (ES -ve) Calc'd for [M-H]$^-$ $C_6H_{11}O_7P_2$ 256.9975, found 256.9971.



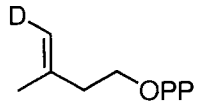
3-Methylene-5-hexenyl diphosphate (29).

Following the general procedure described, treatment of a solution of 3-methylene-5-hexenyl tosylate (**113**) (110 mg, 0.41 mmol) in MeCN (10 mL) with tris-*n*-butylammonium hydrogen diphosphate (1.12 g, 1.24 mmol) gave the crude labeled IPP. Purification of the crude product using HPLC (Vydac 259VHP reverse phase polymer; 4.6 mm x 150 mm; linear gradient elution over 40 minutes of 30% MeCN in 100 mM NH_4HCO_3, t_R 28 min) afforded **29** as a white solid (76 mg, 68%). IR (μscope) 3031 (b), 1640, 1452, 1083 cm^{-1}; ^{1}H NMR (D_2O/ND_4OD, 400 MHz) δ 6.21 (m, 1H, C<u>H</u>=CH$_2$), 5.42 (m, 1H, C<u>H</u>$_2$=CH), 5.41 (m, 1H, C<u>H</u>$_2$=CH) 5.22 (m, 1H, C=CC<u>H</u>$_2$), 5.19 (m, 1H, C=CC<u>H</u>$_2$), 4.33 (dt, 2H, J = 6.7 Hz, 6.7 Hz C<u>H</u>$_2$OP), 3.13 (d, 2H, J = 7.1 Hz, C<u>H</u>$_2$CH), 2.69 (t, 2H, J = 6.7 Hz, C<u>H</u>$_2$CH$_2$); ^{13}C NMR (D_2O/ND_4OD, 100 MHz) δ 146.4, 137.3, 116.7, 122.2, 64.6 (d, J = 5.6 Hz), 40.8, 36.9; ^{31}P NMR (D_2O/ND_4OD, 162 MHz) δ -9.12 (m, 1P), -5.10 (m, 1P); HRMS (ES -ve) Calc'd for [M-H]$^-$ $C_7H_{13}O_7P_2$ 271.0215, found 271.0210.



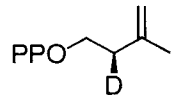
Z-[4-^{2}H]-3-Methyl-3-buten-1-yl diphosphate (30).

The known compound **30**[186,187,191,220,221] was prepared by following the general procedure. Treatment of a solution of Z-[4-^{2}H]-3-methyl-3-buten-1-yl tosylate (**138**) (10 mg, 41 μmol) in MeCN (10 mL) with tris-*n*-butylammonium hydrogen diphosphate (111 mg, 0.123 mmol) gave the crude labeled IPP. The reaction was worked up as previously described. Purification of the crude product using HPLC (Vydac 259VHP reverse phase polymer; 4.6 mm x 150 mm; linear gradient elution over 40 minutes of 30% MeCN in 100 mM NH_4HCO_3, t_R 28 min) afforded **30** as a white solid (11 mg, 87%). IR (μscope) 3020 (b), 1608, 1559, 1405 cm^{-1}; ^{1}H NMR (D_2O/ND_4OD, 76.5 MHz) δ 4.84 (s, 1H, C=C<u>H</u>), 4.04 (dt, 2H, J = 6.7 Hz, 6.7 Hz, C<u>H</u>$_2$OP), 2.38 (t, 2H, J = 6.7 Hz, C<u>H</u>$_2$CH$_2$), 1.76 (s, 3H, C=CC<u>H</u>$_3$); ^{2}H NMR (H_2O, 76.5 MHz) δ 4.80; ^{13}C NMR (D_2O/ND_4OD, 125 MHz) δ 144.3, 111.6 (t, $^1J_{C\text{-}D}$ = 23.6 Hz), 66.4 (d, $^2J_{C\text{-}P}$ = 5.6 Hz), 38.3, 22.1; ^{31}P NMR (D_2O/ND_4OD, 162 MHz) δ -9.04 (m, 1P), - 5.01 (m, 1P,); HRMS (ES -ve) Calc'd for [M-H]$^-$ $C_5H_{10}DO_7P_2$ 246.0037, found 246.0037.



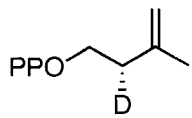
_E_-[4-^{2}H]-3-Methyl-3-buten-1-yl diphosphate (31).

The known compound **31**[186,187,191,220,221] was prepared by following the general procedure described. Treatment of a solution of _E_-[4-^{2}H]-3-methyl-3-buten-1-yl tosylate (**139**) (85 mg, 0.35 mmol) in MeCN (10 mL) with tris-_n_-butylammonium hydrogen diphosphate (1.07 g, 1.06 mmol) gave the crude labeled IPP. The reaction was worked up as previously described. Purification of the crude product using HPLC (Vydac 259VHP reverse phase polymer; 4.6 mm x 150 mm; linear gradient elution over 40 minutes of 30% MeCN in 100 mM NH_4HCO_3, t_R 28 min) afforded **31** as a white solid (88 mg, 85%). IR (μscope) 3141 (b), 3045, 2919, 1405 cm^{-1}; ^{1}H NMR (D_2O/ND_4OD, 500 MHz) δ the vinylic proton was obscured by solvent, 4.04 (dt, 2H, J = 6.7 Hz, 6.7 Hz C$\underline{H}_2$OP), 2.38 (t, 2H, J = 6.7 Hz, C$\underline{H}_2$CH_2), 1.76 (s, 3H, C=CC$\underline{H}_3$); ^{2}H NMR (H_2O, 61.4 MHz) δ 4.73; ^{13}C NMR (D_2O/ND_4OD, 125 MHz) δ 144.6, 111.6 (t, $^1J_{C\text{-}D}$ = 23.6 Hz), 64.7 (d, $^2J_{C\text{-}P}$ = 5.6 Hz), 38.5, 22.1; ^{31}P NMR (D_2O/ND_4OD, 162 MHz) δ -8.80 (m, 1P), - 5.10 (m, 1P,); HRMS (ES -ve) Calc'd for $[M\text{-}H]^-$ $C_5H_{10}DO_7P_2$ 246.0037, found 246.0035.



(2*R*)-[2-^{2}H]-Isopentenyl diphosphate (32).

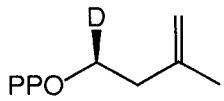
The known compound **32**[192,193] was prepared by following the general procedure. Treatment of a solution of (2*R*)-[2-^{2}H]-isopentenyl tosylate (**161**) (10 mg, 41 μmol) in MeCN (2 mL) with tris-*n*-butylammonium hydrogen diphosphate gave the crude diphosphate. Purification of the crude product by HPLC (Vydac 259VHP reverse phase polymer; 4.6 mm x 150 mm; linear gradient elution over 40 minutes of 30% MeCN in 100 mM NH_4HCO_3, t_R 28 min) afforded **32** as a white solid (15 mg, 87%). IR (μscope) 2722 (b), 1685, 1431 cm^{-1}; ^{1}H NMR (D_2O/ND_4OD, 500 MHz) δ 4.79 (m, 1H, C=C<u>H</u>), the other vinylic protone was obscured by solvent, 3.97 (m, 2H, C<u>H</u>$_2$OP), 2.30 (m, 1H, C<u>H</u>DCH$_2$), 1.68 (s, 3H, C=CC<u>H</u>$_3$); ^{13}C NMR (D_2O/ND_4OD, 100 MHz) δ 145.2, 112.8, 67.1 (d, $^2J_{P\text{-}P}$ = 5.8 Hz), 39.2, 23.1; ^{31}P NMR (D_2O/ND_4OD, 162 MHz) δ -9.00 (m, 1P), -5.10 (m, 1P); HRMS (ES -ve) Calc'd for [M-H]$^-$ $C_5H_{10}DO_7P_2$ 246.0037, found 246.0032.



(2*S*)-[2-^{2}H]-Isopentenyl diphosphate (33).

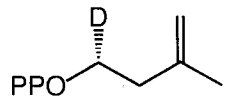
The known compound **33**[192] was prepared by following the general procedure described. Treatment of a solution of (2*S*)-[2-^{2}H]-isopentenyl tosylate (**149**) (10 mg, 41 μmol) in MeCN (2 mL) with tris-*n*-butylammonium hydrogen diphosphate gave the crude labeled IPP **33**. Purification of the crude product using HPLC (Vydac 259VHP reverse phase polymer; 4.6 mm x 150 mm; linear gradient elution over 40 minutes of 30% MeCN in

100 mM NH_4HCO_3, t_R 28 min) afforded **33** as a white solid (15 mg, 87%). IR (μscope) 2707 (b), 1685, 1430 cm^{-1}; ^1H NMR (D_2O/ND_4OD, 400 MHz) 4.99 (m, 2H, C=C$\underline{H}_2$), 4.18 (m, 2H, C$\underline{H}_2$OP), 2.53 (m, 1H, C$\underline{H}$DCH_2), 1.91 (3H, s, C=CC$\underline{H}_3$); ^{13}C NMR (D_2O/ND_4OD, 100 MHz) δ 145.2, 112.8, 67.1 (d, $^2J_{P-P}$ = 5.8 Hz), 39.2, 23.1; ^{31}P NMR (D_2O/ND_4OD, 162 MHz) δ -9.05 (m, 1P), -5.05 (m, 1P); HRMS (ES -ve) Calc'd for [M-H]$^-$ $C_5H_{10}DO_7P_2$ 246.0037, found 246.0036.



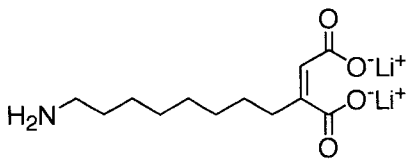
(*1S*)-[1-^{2}H]-Isopentenyl diphosphate (34).

Following the general procedure described, treatment of (1*R*)-[1-^{2}H]-isopentenyl tosylate (**170**) (10.5 mg, 0.0435 mmol) in MeCN (2 mL) with tris-*n*-butylammonium hydrogen diphosphate (118 mg, 0.131 mmol) gave the crude labeled IPP. Purification of the crude product using HPLC (Vydac 259VHP reverse phase polymer; 4.6 mm x 150 mm; linear gradient elution over 40 minutes of 30% MeCN in 100 mM NH_4HCO_3, t_R 28 min) afforded **34** as a white solid (15 mg, 87%). IR (μscope) 2722 (b), 1685, 1431 cm^{-1}; ^1H NMR (D_2O/ND_4OD, 500 MHz) δ 5.10 (m, 1H, C=C$\underline{H}$), 5.08 (m, 1H, C=C$\underline{H}$), 4.27 (dt, 1H, J = 6.8 Hz, 6.8 Hz, C$\underline{H}$DOP), 2.62 (dt, 2H, J = 6.8 Hz, 6.8 Hz, C$\underline{H}_2$CHD), 2.02 (s, 3H, C=CC$\underline{H}_3$); ^{13}C NMR (D_2O/ND_4OD, 125 MHz) δ 144.6, 112.2, 64.4 (dt, J_{C-D} = 22.9 Hz, J_{C-P} = 5.5 Hz), 38.4, 22.3; ^{31}P NMR (D_2O/ND_4OD, 162 MHz) δ -9.00 (m, 1P), -5.10 (m, 1P); HRMS (ES -ve) Calc'd for [M-H]$^-$ $C_5H_{10}DO_7P_2$ 246.0037, found 246.0035.



(1*R*)-[1-^{2}H]-Isopentenyl diphosphate (35).

Following the general procedure described, treatment of (*1S*)-[2-^{2}H]-isopentenyl tosylate (**171**) (11.0 mg, 0.0456 mmol) in MeCN (2 mL) with tris-*n*-butylammonium hydrogen diphosphate (123 mg, 0.137 mmol) gave the crude labeled IPP. Purification of the crude product using HPLC (Vydac 259VHP reverse phase polymer; 4.6 mm x 150 mm; linear gradient elution over 40 minutes of 30% MeCN in 100 mM NH_4HCO_3, t_R 28 min) afforded **35** as a white solid (12 mg, 86%). IR (μscope) 2846 (b), 1652, 1436 cm^{-1}; ^{1}H NMR (D_2O/ND_4OD, 500 MHz) δ 5.10 (m, 1H, C=C<u>H</u>), 5.08 (m, 1H, C=C<u>H</u>), 4.26 (m, 1H, C<u>H</u>DOP), 2.61 (d, 2H, J = 6.8 Hz, C<u>H</u>$_2$CHD), 2.00 (s, 3H, C=CC<u>H</u>$_3$); δ ^{13}C NMR (D_2O/ND_4OD, 125 MHz) δ 144.6, 112.2, 64.4 (dt, $J_{C\text{-}D}$ = 22.9 Hz, $J_{C\text{-}P}$ = 5.5 Hz), 38.4, 22.6; ^{31}P NMR (D_2O/ND_4OD, 162 MHz) δ -9.00 (m, 1P), -5.10 (m, 1P); HRMS (ES -ve) Calc'd for [M-H]$^-$ $C_5H_{10}DO_7P_2$ 246.0037, found 246.0038.



2-(8-Amino-octyl)-but-2-enedioic acid, dilithium salt (36).

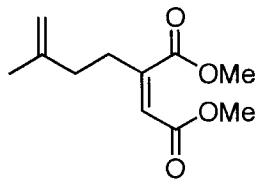
A stirring solution of 2-(8-azido-octyl)-but-2-enedioic acid, dilithium salt (190 mg, 0.68 mmol) in THF-H_2O (20 mL, 1:1) was treated with triphenylphosphine (268 mg, 1.02 mmol). The resulting reaction mixture was stirred at reflux for 48 h, at which point the solution was cooled to room temperature and the solvent removed *in vacuo*. The residue

was dissolved in water (10 mL) and washed with CH_2Cl_2 (2 x 15 mL) to remove triphenylphoshine impurities. The aqueous layer was freeze dried to afford **36** as a white solid (183 mg, 98%). IR (μscope) 3246 (b), 3054, 2926, 2854, 1725, 1651, 1436, 1197 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 5.45 (m, 1H, C=C<u>H</u>), 2.73 (t, 2H, J = 7.2 Hz, C<u>H</u>$_2$N), 2.20 (t, 2H, J = 7.6 Hz, C<u>H</u>$_2$C=C), 1.50 (m, 2H, C<u>H</u>$_2$CH$_2$), 1.42 (m, 2H, C<u>H</u>$_2$CH$_2$), 1.26-1.34 (m, 8H, C<u>H</u>$_2$C<u>H</u>$_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 179.5, 174.9, 152.5, 119.8, 40.4, 34.8, 29.8, 28.5 (2C), 28.4, 27.1, 26.0; HRMS (ES -ve) Calc'd for $[M-H]^-$ $C_{12}H_{20}NO_4$ 242.1387, found 242.1385.

General procedure for the preparation of *cis*-maleyl substituted dicarboxylates

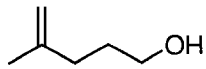
To a flame dried, argon flushed, round-bottom flask was added freshly ground magnesium turnings (1.2 equiv) and THF (3 mL). To this suspension, a solution of bromide (1.0 equiv) in THF (5 mL) was added dropwise followed by a crystal of iodine. The reaction mixture was refluxed for 2 h and cooled to room temperature. The prepared Grignard reagent was then added dropwise to a suspension of copper bromide-dimethyl sulfide complex (1.2 equiv) in THF (10 mL) at –40 °C. The resulting solution was stirred at –40 °C for 2 h and then cooled to –78 °C, and freshly distilled DMAD (1 equiv) in THF (5 mL) was added dropwise to give a dark red-brown mixture. After 1 h, the reaction mixture was quenched with a saturated solution of ammonium chloride (5 mL, adjusted to pH 8 with 10% ammonia) and allowed to warm to room temperature. After 30 min, the mixture is partitioned between water and Et_2O. The aqueous layer was extracted with Et_2O (3 x 15 mL) and the combined organic extracts were washed with saturated aqueous NH_4Cl solution (25 mL) and brine (25 mL). Drying over $MgSO_4$ and

concentration *in vacuo* gave an amber oil. Purification by flash column chromatography gave the diester.



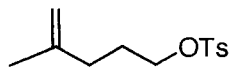
Dimethyl (*Z*)–2-(2-methyl)butenylbutenedioate (47).

Following the general procedure described. Thus, 4-bromo-2-methyl-1-butene (**46**) (1.00 g, 6.71 mmol), magnesium turnings (300 mg, 12.08 mmol), CuBr•Me_2S (1.38 g, 6.71 mmol), and DMAD (800 mg, 5.6 mmol) were reacted. Purification of the crude product by flash column chromatography (SiO_2, petroleum ether:Et_2O, 9:1) gave **47** as an oil (490 mg, 41%). IR (film) 3077, 2953, 1732, 1651, 1436 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.82 (m, 1H, C=C<u>H</u>), 4.76 (m, 1H, C=C<u>H</u>$_2$) 4.70 (m, 1H, C=C<u>H</u>$_2$), 3.82 (s, 3H, OC<u>H</u>$_3$), 3.71 (s, 3H, OC<u>H</u>$_3$), 2.49 (m, 2H, C<u>H</u>$_2$CH$_2$), 2.19 (m, 2H, CH$_2$C<u>H</u>$_2$), 1.72 (s, 3H, C=CC<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.5, 165.4, 150.0, 143.5, 119.6, 111.2, 52.4, 51.9, 34.9, 32.5, 22.4; HRMS (ES +ve) Calc'd $[M+Na]^+$ for $C_{11}H_{16}O_4Na$ 235.0946, found 235.0944.



4-Methyl-4-penten-1-ol (49).

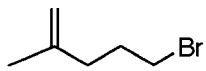
The known alcohol **49**[222] was prepared using the following procedure. To a stirring solution of ethyl 4-methyl-pentenoate (**48**) (10.0 g, 70.3 mmol) in THF (100 mL) a solution of lithium aluminum (35.2 mL, 35.2 mmol) was added dropwise at 0°C. The resulting reaction mixture was stirred with warming to room temperature for 1 h. Solid $Na_2SO_4 \cdot 10H_2O$ was added to quench reaction and solid material was removed by filtration through a pad of Celite®. Evaporation of the filtrate *in vacuo* afforded the alcohol **49** as an oil (6.22 g, 88%), which was essentially pure by ^{1}H-NMR analysis. IR (film) 3332, 3075, 2939, 2875, 1650, 1374, 1034 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.77 (m, 1H, C=C$\underline{H}_2$), 4.72 (m, 1H, C=C$\underline{H}_2$), 3.42 (t, 2H, J = 7.5 Hz, C$\underline{H}_2$OH) 2.16 (t, 2H, J =7.5 Hz, C=CC$\underline{H}_2$) 2.00 (m, 2H, $CH_2C\underline{H}_2CH_2$) 1.73 (s, 3H, C=CC$\underline{H}_3$) ; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 145.5, 110.1, 62.5, 34.0, 30.5, 22.3; HRMS (EI) Calc'd for [M+] $C_6H_{12}O$ 100.0888, found 100.0890.



4-Methyl-4-penten-yl tosylate.

The known compound[223-225] was made using the following procedure. A stirring solution of 4-methyl-4-penten-1-ol (**49**) (500 mg, 4.49 mmol) in CH_2Cl_2 (15 mL) was treated with *p*-toluenesulfonyl chloride (942 mg, 4.94 mmol), triethylamine (1.25 mL, 8.98 mmol) and DMAP (10 mg, 0.08 mmol). The resulting reaction mixture was stirred at room temperature for 18 h, at which time the mixture was diluted with water (5 mL) and

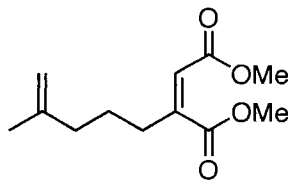
extracted with CH_2Cl_2. Combine organic extracts were washed with water, brine, dried over $MgSO_4$ and evaporated *in vacuo*. The crude residue was purified by flash column chromatography (SiO_2, hexane:EtOAc, 10:1) to afford tosylate as an oil (1.06 g, 93%). IR ($CHCl_3$ cast) 3074, 2966, 1651, 1598, 1495, 1361, 1210, 1177 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.77 (m, 2H, Ar), 7.33 (m, 2H, Ar) 4.67 (m, 1H, $CH_2C=C$), 4.57 (m, 1H, $CH_2C=C$), 4.04 (t, 2H, J = 7.1 Hz, CH_2OS), 2.43 (s, 3H, $ArCH_3$), 2.01 (t, 2H, J = 7.6 Hz, $CH_2CH_2CH_2$), 1.76 (m, 2H, $CH_2CH_2CH_2$), 1.64 (s, 3H, $C=CCH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 144.7, 143.9, 133.3, 129.9, 127.9, 110.9, 70.1, 33.4, 26.8, 22.3, 21.7; HRMS (ES +ve) Calc'd for $[M+Na]^+$ $C_{13}H_{18}O_3SNa$ 277.0874, found 277.0875



1-Bromo-4-methyl-4-pentene (50).

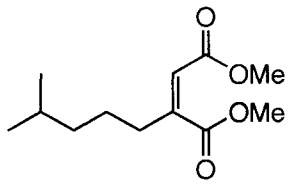
The known bromide **50**[223,225-227] was prepared using the following procedure. To a stirring solution of 4-methyl-4-penten-yl tosylate[223,225] (14.5 g, 57.1 mmol) in acetone (200 mL) was added a solution of lithium bromide (9.89 g, 0.114 mol) in acetone (20 ml) dropwise. The resulting reaction mixture was stirred at reflux for 18 h. The mixture was cooled to room temperature and poured into ice-water (25 mL), and extracted with Et_2O (3 x 25 mL). Combined ethereal extracts were washed with water, brine, and dried over $MgSO_4$. Evaporation of solvent *in vacuo* gave crude bromide. Crude material was purified by distillation to afford **50** as a colorless oil (6.22 g, 67%). IR (film) 3074, 2964, 2936, 1650, 1438, 1375, 1267, 1205 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.74 (m, 1H, $CH_2C=C$), 4.70 (m, 1H, $CH_2C=C$), 3.39 (t, 2H, J = 6.7 Hz, CH_2Br), 2.15 (t, 2H, J = 7.9

Hz, C$\underline{H}_2$CH$_2$CH$_2$), 1.98 (m, 2H, CH$_2$C$\underline{H}_2$CH$_2$), 1.71 (s, 3H, C=CC$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 143.9, 111.0, 36.1, 33.3, 30.6, 22.3; HRMS (EI) Calc'd for [M+·] $C_6H_{11}Br$ 162.0, found 162.0 (low-resolution MS).



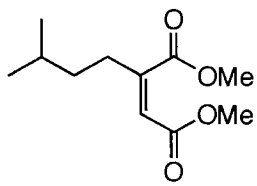
Dimethyl (*Z*)–2-(2-methyl)pentenylbutenedioate (51).

The general procedure was followed. Thus, 1-bromo-4-methyl-4-pentene (**50**) (1.00 g, 6.13 mmol), magnesium turnings (298 mg, 12.26 mmol), CuBr•Me_2S (1.63 g, 6.13 mmol), and DMAD (0.63 mL, 4.85 mmol) were reacted as before. Purification of the crude product by flash column chromatography (SiO_2, Petroleum ether:Et_2O, 9:1) gave **51** (680 mg, 62%) as an oil. IR (film) 3074, 2951, 1728, 1649, 1436 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.80 (t, 1H, J = 1.5 Hz, C=C$\underline{H}$), 4.71 (m, 1H, C=C$\underline{H}_2$), 4.65 (m, 1H, C=C$\underline{H}_2$), 3.80 (s, 3H, OC$\underline{H}_3$), 3.70 (s, 3H, OC$\underline{H}_3$), 2.32 (m, 2H, C=CC$\underline{H}_2$), 2.03 (m, 2H, CH$_2$CH$_2$C$\underline{H}_2$), 1.67 (s, 3H, C=CC$\underline{H}_3$), 1.58 (m, 2H, CH$_2$C$\underline{H}_2$CH$_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.4, 165.4, 150.7, 144.6, 119.4, 110.8, 52.3, 51.8, 36.8, 33.5, 24.8, 22.2; HRMS (ES +ve) Calc'd for [M+Na]$^+$ $C_{12}H_{18}O_4Na$ 249.1103, found 249.1108.



Dimethyl (*Z*)–2-(2-methyl)pentylbutenedioate (53).

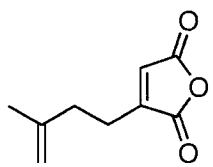
The general procedure was followed. Thus, 1-bromo-4-methylpentane (**52**) (1.00 g, 6.06 mmol), magnesium turnings (300 mg, 12.12 mmol), CuBr•Me_2S (1.25 g, 6.06 mmol), and DMAD (0.63 mL, 4.9 mmol) was reacted as before. Purification of the crude product by flash column chromatography (SiO_2, petroleum ether:Et_2O, 9:1) gave **53** (830 mg, 60%) as an oil. IR (film) 2954, 2870, 1728, 1651, 1436 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.79 (t, 1H, J = 1.5 Hz, C=C<u>H</u>), 3.81 (s, 3H, OC<u>H</u>$_3$), 3.70 (s, 3H, OC<u>H</u>$_3$), 2.32 (m, 2H, C<u>H</u>$_2$CH$_2$), 1.50 (m, 3H, CH$_2$C<u>H</u>$_2$CH$_2$, C<u>H</u>(CH$_3$)$_2$), 1.19 (m, 2H, CHC<u>H</u>$_2$), 0.85 (d, 6H, J = 6.6 Hz, CH(C<u>H</u>$_3$)$_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.5, 165.5, 151.1, 119.0, 52.3, 51.8, 38.1, 34.6, 27.8, 24.8, 22.5 (2C); HRMS (ES +ve) Calc'd for $[M+Na]^+$ $C_{12}H_{20}O_4Na$ 251.1259, found 251.1262.



Dimethyl (*Z*)–2-(2-methyl)butylbutenedioate (55).

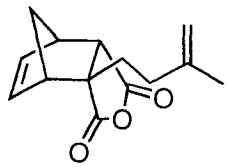
The general procedure was followed. Thus, 1-bromo-3-methylbutane (**54**) (1.0 g, 6.62 mmol), magnesium turnings (322 mg, 13.2 mmol), CuBr•Me_2S (1.76 g, 6.62 mmol), and DMAD (784 mg, 5.52 mmol) were reacted as before. Purification of the crude product by flash column chromatography (SiO_2, petroleum ether:Et_2O, 9:1) gave **55** (970 mg, 82%)

as an oil. IR (film) 2955, 2871, 1731, 1635, 1436 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.79 (t, 1H, J = 1.4 Hz, C=C<u>H</u>), 3.81 (s, 3H, OC<u>H</u>$_3$), 3.69 (s, 3H, OC<u>H</u>$_3$), 2.33 (m, 2H, C=CC<u>H</u>$_2$), 1.56 (m, 1H, CH_2CH_2C<u>H</u>), 1.35 (m, 2H, C<u>H</u>$_2$CH) 0.87 (d, 6H, J = 8.7 Hz, CH(C<u>H</u>$_3$)$_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.5, 165.5, 151.3, 118.9, 52.3, 51.8, 35.9, 32.4, 27.5 22.3 (2C); HRMS (ES +ve) Calc'd for $[M+Na]^+$ $C_{11}H_{18}O_4Na$ 237.1103, found 237.1096.



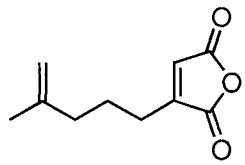
(*Z*)–2-(2-Methyl)butenylbutenedioic anhydride (56).

A stirring solution of (*Z*)–2-(2-methyl)butenylbutenedioic acid, dilithium salt (**17**) (100 mg, 0.471 mmol) in water (5 mL) was treated with 1.0 N HCl at 0°C. After stirring for 30 min the solution was extracted with Et_2O. The combined ethereal extracts were washed with water, brine, dried over $MgSO_4$ and concentrated *in vacuo* to give the crude anhydride. Purification by column chromatography afforded **56** as a colorless oil (59 mg, 76%). IR (CH_2Cl_2 cast) 2916, 1697, 1648, 1424, 1380 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.90 (s, 1H, C=C<u>H</u>), 4.78 (s, 1H, C=C<u>H</u>$_2$), 4.71 (s, 1H, C=C<u>H</u>$_2$), 2.54 (m, 2H, C=CC<u>H</u>$_2$), 2.40 (t, 2H, J = 6.9 Hz, C=CC<u>H</u>$_2$), 1.73 (s, 3H, OC<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.9, 170.5, 150.4, 143.2, 120.4, 111.5, 34.9, 32.5, 22.4; HRMS (EI) Calc'd for $[M^+]$ $C_9H_{10}O_3$ 166.0630, found 166.0628.



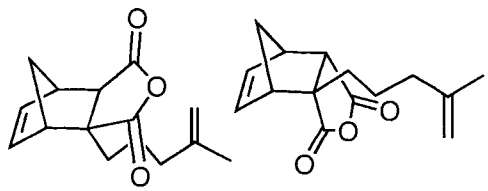
2-(3-Methyl-but-3-enyl)-bicyclo[2.2.1]hept-5-ene-2,3-dicarbonic anhydride (57).

To a stirring solution (Z)–2-(3-methyl-but-3-enyl)-butenedioic anhydride (**56**) (80 mg, 0.48 mmol) in toluene (2 mL) was added cyclopentadiene (0.18 mL, 2.4 mmol). The resulting reaction mixture was stirred at reflux for 5 hours. The solution was cooled to room temperature and solvent was removed *in vacuo*. Purification by flash column chromatography (SiO_2, hexane:EtOAc, 15:1) gave **57** (60 mg, 54%) as a colorless oil. IR (CH_2Cl_2 cast) 3073, 2952, 1779, 1649, 1457 cm^{-1}; ^1H NMR (CD_2Cl_2, 500 MHz) δ 6.36 (dd, 1H, J = 5.7 Hz, 3.0 Hz, CH=C<u>H</u>), 6.29 (dd, 1H, J = 5.7 Hz, 2.9 Hz, CH=C<u>H</u>), 4.77 (m, 1H, C=C<u>H</u>$_2$), 4.72 (m, 1H, C=C<u>H</u>$_2$), 3.44 (m, 1H, C=CC<u>H</u>), 3.24 (d, 1H, J = 4.8 Hz, C=CC<u>H</u>), 3.06 (m, 1H, C<u>H</u>CO), 2.30 (ddd, 1H, J = 13.8 Hz, 10.7 Hz, 5.5 Hz, C<u>H</u>$_2$CH$_2$), 2.16 (m, 1H, C<u>H</u>$_2$CH$_2$), 2.07 (m, 1H, C<u>H</u>$_2$CH$_2$), 1.81 (m, 3H, C<u>H</u>$_2$CH, C<u>H</u>$_2$CH$_2$), 1.74 (s, 3H, C=CC<u>H</u>$_3$). ^{13}C NMR (CD_2Cl_2, 125 MHz) δ 174.6, 171.3, 144.4, 137.5, 135.9, 111.3, 59.5, 52.3, 51.4, 51.1, 47.4, 34.6, 34.0, 22.4; HRMS (EI) Calc'd for [M+·] $C_{14}H_{16}O_3$ 232.1099, found 232.1096.



(*Z*)–2-(2-Methyl)pentenylbutenedioic anhydride (58).

A similar procedure was employed as that described for the preparation of **56**. Acidification of (*Z*)–2-(2-methyl)pentenylbutenedioic acid, dilithium salt (**18**) (100 mg, 0.471 mmol) with 1.0 N HCl gave anhydride **58** (68 mg, 80%) as a colorless oil. IR (CH_2Cl_2 cast) 2939, 1843, 1772, 1700, 1652, 1436, 1376 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.88 (s, 1H, C=C<u>H</u>), 4.74 (m, 1H, C=C<u>H</u>$_2$), 4.68 (m, 1H, C=C<u>H</u>$_2$), 2.40 (t, 2H, *J* = 7.0 Hz, C=CC<u>H</u>$_2$), 2.06 (t, 2H, *J* = 7.5 Hz, C=CC<u>H</u>$_2$), 1.70 (m, 5H, $CH_2C\underline{H}_2$, C=CC<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.3, 170.5, 150.9, 144.3, 119.9, 110.9, 36.8, 33.8, 24.8, 22.2; HRMS (EI) Calc'd for [M+·] $C_{10}H_{12}O_3$ 180.0786, found 180.0781.



59a **59b**

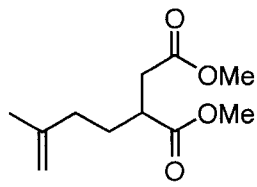
2-(4-Methyl-pent-4-enyl)-bicyclo[2.2.1]hept-5-ene-2,3-dicarbonic anhydride (59a and 59b).

A similar procedure was employed as that described for the preparation of **57**. Reaction of (*Z*)–2-(3-methyl-pent-3-enyl)-butenedioic anhydride (**58**) (50 mg, 0.28 mmol) and cyclopentadiene (0.11 mL, 1.39 mmol) in toluene (2 mL) gave the crude cycloadduct.

Purification of the crude product by flash column chromatography (SiO_2, hexane:EtOAc, 10:1) afforded **59a** (5 mg, 7%) and **59b** (40 mg, 59%) as colorless oils.

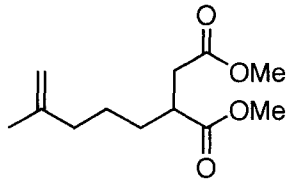
Data for 59a: ^1H NMR (CD_2Cl_2, 500 MHz) δ 6.38 (dd, 1H, *J* = 5.7 Hz, 3.1 Hz, CH=C<u>H</u>), 6.33 (dd, 1H, *J*= 5.7, 3.1 Hz, CH=C<u>H</u>), 4.71 (m, 1H, C=C<u>H</u>$_2$), 4.64 (m, 1H, C=C<u>H</u>$_2$), 3.37 (m, 1H, C=CC<u>H</u>), 3.17 (m, 1H, C=CC<u>H</u>), 2.49 (m, 1H, C<u>H</u>CO), 1.99 (t, 2H, *J* = 7.3 Hz, C<u>H</u>$_2$C=C), 1.78 (dt, 1H, *J* = 13.1 Hz, 4.6 Hz, C<u>H</u>$_2$CH$_2$CH$_2$), 1.67 (s, 3H, C=CC<u>H</u>$_3$), 1.65 (m, 1H, CH$_2$C<u>H</u>$_2$CH$_2$), 1.51 (m, 2H, CH$_2$C<u>H</u>$_2$CH$_2$), 1.43 (m, 1H, CHC<u>H</u>$_2$CH), 1.30 (dt, 1H, *J* = 13.1 Hz, 4.5 Hz, C<u>H</u>$_2$CH$_2$CH$_2$); ^{13}C NMR (CD_2Cl_2, 75 MHz) δ 175.8, 171.9, 145.1, 138.0, 136.9, 110.7, 60.9, 52.0, 50.9, 48.6, 46.0, 38.0, 34.8, 23.8, 22.4.

Data for 59b: IR (cast CH_2Cl_2) 3073, 2943, 1771, 1650, 1459 cm^{-1}; ^1H NMR (CD_2Cl_2, 500 MHz) δ 6.34 (dd, 1H, *J* = 5.7 Hz, 2.9 Hz, CH=C<u>H</u>), 6.26 (dd, 1H, *J* = 5.7, 2.9 Hz, CH=C<u>H</u>), 4.72 (m, 1H, C=C<u>H</u>$_2$), 4.66 (m, 1H, C=C<u>H</u>$_2$), 3.42 (m, 1H, C=CC<u>H</u>), 3.19 (d, 1H, *J* = 4.6 Hz, C=CC<u>H</u>), 3.03 (m, 1H, C<u>H</u>CO), 2.07 (m, 3H, C=CC<u>H</u>$_2$, CH$_2$CH$_2$C<u>H</u>$_2$), 1.78 (m, 2H, CH$_2$C<u>H</u>$_2$CH$_2$), 1.69 (s, 3H, C=CC<u>H</u>$_3$), 1.64 (td, 1H, *J* = 13.3 Hz, 4.3 Hz, CH$_2$CH$_2$C<u>H</u>$_2$), 1.56 (m, 1H, C<u>H</u>$_2$CH$_2$CH$_2$), 1.45 (m, 1H, C<u>H</u>$_2$CH$_2$CH$_2$); ^{13}C NMR (CD_2Cl_2, 75 MHz) δ 174.8, 171.4, 145.1, 137.8, 135.9, 110.9, 59.7, 51.9, 51.3 (2C), 47.2, 38.0, 35.5, 24.2, 22.2; HRMS (EI) Calc'd for [M+] $C_{15}H_{18}O_3$ 246.1256, found 246.1254.



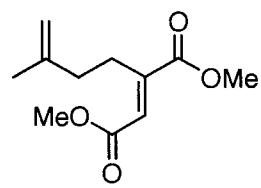
Dimethyl 2-(3-methyl-but-3-enyl)-butanedioate (60).

A flame dried, argon flushed round-bottom flask was charged with dimethyl (*Z*)–2-(2-methyl)butenylbutanedioate (**47**) (25 mg, 0.12 mmol). In an inert atmosphere, $[(Ph_3P)CuH]_6$ (115 mg, 58.6 μmol) was weighed and added to the round bottom flask under a positive N_2 pressure. Deoxygenated toluene (2 mL) containing water (50 μL) was added, and the resultant red solution was stirred at room temperature for 2 h. The cloudy red-brown reaction mixture was opened to air, and stirring was continued for 1 h. Filtration of mixture through Celite® and concentration of the resulting filtrate *in vacuo* gave the crude product. Purification of the crude product by flash column chromatography (SiO_2, hexane:EtOAc, 10:1) gave **60** (17 mg, 67%) as an oil. IR (CH_2Cl_2 cast) 3075, 2952, 1738, 1650, 1437 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.71 (m, 1H, C=C$\underline{H}_2$), 4.65 (m, 1H, C=C$\underline{H}_2$), 3.68 (s, 3H, OC$\underline{H}_3$), 3.65 (s, 3H, OC$\underline{H}_3$), 2.82 (m, 1H, C$\underline{H}$CO), 2.70 (dd, 1H, J = 9.1 Hz, 16.3 Hz, C$\underline{H}_2$CO), 2.44 (dd, 1H, J = 5.1 Hz, 16.2 Hz, C$\underline{H}_2$CO), 2.0 (m, 2H, C$\underline{H}_2$CH), 1.79 (m, 1H, $CH_2C\underline{H}_2$), 1.68 (s, 3H, C=CC$\underline{H}_3$), 1.63 (m, 1H, $CH_2C\underline{H}_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.3, 172.3, 144.5, 110.7, 51.9, 51.7, 40.7, 35.8, 35.0, 29.8, 22.3; HRMS (EI) Calc'd for [M+] $C_{11}H_{18}O_4$ 214.1205, found 214.1204.



Dimethyl 2-(4-methyl-4-pentenyl)-butanedioate (61).

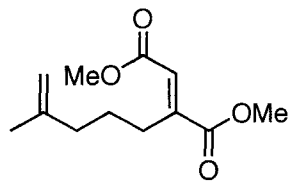
A similar procedure was employed as that described for the preparation of **60**. Reaction of dimethyl (Z) –2-(2-methyl)pentenylbutenedioate (**51**) (100 mg, 0.442 mmol) and $[(Ph_3P)CuH]_6$ (350 mg, 0.178 mol) in toluene gave the crude diester. Purification by flash column chromatography (SiO_2, hexane:EtOAc, 10:1) gave **61** (62 mg, 61%) as an oil. IR (CH_2Cl_2 cast) 3074, 2951, 1738, 1650, 1437 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.68 (m, 1H, C=C$\underline{H}_2$), 4.63 (m, 1H, C=C$\underline{H}_2$), 3.67 (s, 3H, OC$\underline{H}_3$), 3.64 (s, 3H, OC$\underline{H}_3$), 2.84 (m, 1H, C$\underline{H}$CO), 2.70 (ddd, 1H, J = 2.3 Hz, 9.2 Hz, 16.5 Hz, C$\underline{H}_2$CO), 2.41 (ddd, 1H, J = 2.1 Hz, 5.2 Hz, 16.4 Hz, C$\underline{H}_2$CO), 1.98 (m, 2H, C$\underline{H}_2$CH), 1.66 (s, 3H, C=CC$\underline{H}_3$), 1.60 (m, 1H, $CH_2C\underline{H}_2CH_2$), 1.42 (m, 3H, C$\underline{H}_2C\underline{H}_2CH_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.3, 172.3, 145.0, 110.2, 51.7 (2C), 41.0, 37.3, 35.8, 31.4, 24.7, 22.2; HRMS (ES +ve) Calc'd for $[M+Na]^+$ $C_{12}H_{20}O_4Na$ 251.1254, found 251.1256.



Dimethyl (*E*)–2-(2-methyl)butenylbutenedioate (62).

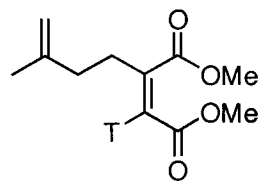
To a flame dried, argon flushed round-bottom flask was added freshly ground magnesium turnings (245 mg, 10.1 mmol) and THF (3 mL). To this suspension was added a solution of 4-bromo-2-methyl-1-butene (**46**) (750 mg, 5.03 mmol) in THF (5 mL) dropwise, and a

crystal of iodine. The reaction mixture was heated to reflux for 2 h and cooled to room temperature. The prepared Grignard was then added dropwise to a stirring solution of freshly distilled DMAD (0.56 ml, 4.6 mmol) at –78 °C to give a dark brown-red mixture. After 1 h, the reaction mixture was quenched with a saturated solution of ammonium chloride (5 mL, adjusted to pH 8 with 10% ammonia) and allowed to warm to room temperature. After 30 min, the mixture was partitioned between water and Et_2O. The aqueous layer was extracted with Et_2O (3 x 15 mL), and the combined organic extracts were washed with saturated NH_4Cl solution (25 mL) and brine (25 mL). Drying over $MgSO_4$ and concentration *in vacuo* gave crude diester. Purification of the crude product by flash column chromatography (SiO_2, hexane:EtOAc, 10:1) gave **62** as an oil (78 mg, 8%). IR (film) 3075, 2953, 1725, 1645, 1436 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.73 (s, 1H, C=C$\underline{H}$), 4.70 (m, 1H, C=C$\underline{H_2}$), 4.67 (m, 1H, C=C$\underline{H_2}$), 3.78 (s, 3H, OC$\underline{H_3}$), 3.74 (s, 3H, OC$\underline{H_3}$), 2.91 (m, 2H, C$\underline{H_2}$CH_2), 2.13 (m, 2H, CH_2C$\underline{H_2}$), 1.74 (s, 3H, C=CC$\underline{H_3}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.2, 165.9, 147.8, 144.8, 126.5, 110.7, 52.6, 51.8, 37.2, 26.7, 22.3; HRMS (ES +ve) Calc'd for $[M+H]^+ C_{11}H_{17}O_4$ 213.1121, found 213.1122.



Dimethyl (*E*)–2-(2-methyl)pentenylbutenedioate (63).

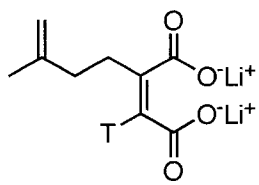
A similar procedure was employed as that described for the preparation of **62**. Reaction of 1-bromo-4-methyl-4-pentene (**50**) (750 mg, 4.60 mmol), magnesium turnings (223 mg, 9.20 mmol), and DMAD (0.38 mL, 2.8 mmol) gave the crude product. Purification by flash column chromatography (SiO_2, hexane:EtOAc, 10:1) gave **63** as an oil (30 mg, 3%). IR (film) 3073, 2929, 1725, 1649, 1436, 1373 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.74 (s, 1H, C=C<u>H</u>), 4.71 (m, 1H, C=C<u>H</u>$_2$), 4.68 (m, 1H, C=C<u>H</u>$_2$), 3.79 (s, 3H, OC<u>H</u>$_3$), 3.76 (s, 3H, OC<u>H</u>$_3$), 2.74 (m, 2H, C<u>H</u>$_2$CH$_2$), 2.07 (t, 2H, J = 7.6 Hz, CH$_2$C<u>H</u>$_2$), 1.70 (s, 3H, C=CC<u>H</u>$_3$), 1.58 (m, 2H, CH$_2$C<u>H</u>$_2$CH$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.3, 166.0, 148.1, 145.3, 126.3, 110.1, 52.4, 51.6, 37.7, 27.6, 26.9, 22.2; HRMS (EI) Calc'd for [M+·]$C_{12}H_{18}O_4$ 226.1205, found 226.1201.



[3-$^3H_1$]-Dimethyl (*Z*)–2-(2-methyl)butenylbutenedioate (64).

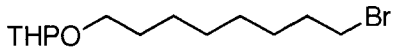
This procedure was done at the National Tritum Labeling Facility (Lawrence Berkeley Laboratory, Berkeley CA, USA) with the supervision of H. Morimoto and P. Williams. The tritium labeled analog **64** was prepared using a modification of the general procedure. Thus, 4-bromo-2-methyl-1-butene (**46**) (125 mg, 0.838 mmol), magnesium

turnings (41.0 mg, 1.68 mmol), $CuBr•Me_2S$ (154 mg, 0.749 mmol), and DMAD (71 mg, 0.50 mmol) were reacted as before. Reaction was quenched with a solution of tritiated water (0.1 mmol, 2 μL, *ca.* 5.7 Ci) in THF (0.2 mL) that was prepared prior to the reaction.[228] After stirring for 5 min, a saturated solution of saturated NH_4Cl (5 mL) was added to ensure reaction was completely quenched. After work-up, the crude product was purified by flash column chromatography (SiO_2, hexane:EtOAc, 15:1) and evaporation of appropriate fractions gave **64** as an oil (78 mg, 74%). No spectra data was recorded for this intermediate. Chromatographic properties were identical to **47**.



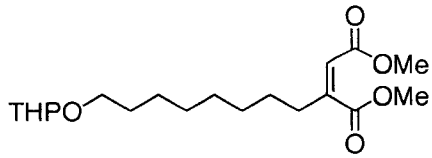
[3-$^3H_1$]-(Z)–2-(2-Methyl)butenylbutenedioic acid, dilithium salt (65).

This procedure was done at the National Tritium Labeling Facility (see previous entry). The hydrolysis of ester **64** (78 mg, 0.37 mmol) with 1.0 M LiOH (1.1 mL, 1.1 mmol) gave **65** (74 mg, 99%) as a white powder: The triated dilithium salt **65** has a specific activity of 607 mCi/mmol and a total activity of 224 mCi. ^1H NMR (D_2O, 300 MHz) δ 5.39 (m, 1H, C=C<u>H</u> (from unlabeled material)), 4.60 (m, 2H, C=C<u>H_2</u>), 2.19 (m, 2H, $CH_2C\underline{H_2}$), 1.97 (m, 2H, $CH_2C\underline{H_2}$), 1.53 (s, 3H, $C=CCH_2$); ^3H NMR (D_2O, 320 MHz) δ 5.39 (s, 1H, C=C<u>^{3}H</u>).



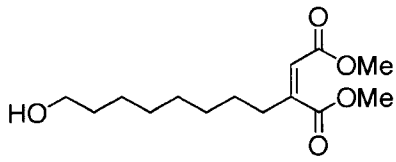
2-(8-Bromo-octyloxy) tetrahydropyran (67).

To a stirring solution of 8-bromo-1-octanol (**66**) (5.3 mL, 30.1 mmol) in CH_2Cl_2 (75 mL) was added dihydropyran (4.0 mL, 44 mmol) and pyridinum *p*-toluenesulfonate (730 mg, 2.90 mmol). The resulting reaction mixture was stirred at room temperature for 12 h at which point the solution was diluted with water (10 mL) and extracted with EtOAc. Combined organic extracts were washed with water, brine, dried over $MgSO_4$ and evaporated *in vacuo*. Purification of the crude product by flash column chromatography (SiO_2, hexane:EtOAc, 25:1) afforded **67** as a colorless oil (6.73 g, 76%). IR (neat film) 2934, 2855, 1465, 1200 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.55 (dd, 1H, J = 4.4 Hz, 2.6 Hz, OC<u>H</u>CH_2), 3.85 (m, 1H, C<u>H</u>$_2$OC), 3.71 (m, 1H, C<u>H</u>$_2$OC), 3.48 (m, 1H, C<u>H</u>$_2$OC), 3.37 (m, 3H, C<u>H</u>$_2$OC, C<u>H</u>$_2$Br), 1.82 (m, 3H, C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$), 1.69 (m, 1H, C<u>H</u>$_2$C<u>H</u>$_2$), 1.54 (m, 6H, C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$), 1.41 (m, 2H, C<u>H</u>$_2$C<u>H</u>$_2$), 1.31 (m, 6H, C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 98.8, 67.6, 62.4, 34.0, 32.9, 30.9, 29.8, 29.3, 28.8, 28.2, 26.2, 25.6, 19.8; HRMS (EI) Calc'd for [M+·] $C_{13}H_{25}{}^{81}BrO_2$ 293.0939, found 293.0938.



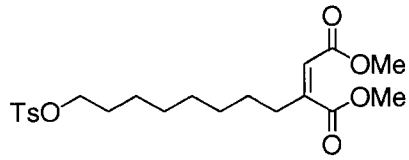
2-[8-(Tetrahydropyran-2-yloxy)-octyl]-but-2-enedioic acid dimethyl ester (68).

A similar procedure was employed as that described for the preparation of **47**. Treatment of a solution of 2-(8-bromo-octyloxy)-tetrahydropyran (**67**) (1.00 g, 3.41 mmol) in THF (10 mL) with magnesium turnings (166 mg, 6.82 mmol) provided the Grignard reagent. Reaction of the Grignard reagent with copper bromide-dimethyl sulfide complex (907 mg, 3.41 mmol) followed by addition of DMAD (0.35 mL, 2.8 mmol) gave the crude diester. Purification using flash column chromatography (SiO_2, hexane:EtOAc, 4:1) afforded **68** as a colorless oil (730 mg, 72%). IR (CH_2Cl_2, cast) 2934, 2856, 1730, 1650, 1436, 1168 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.78 (t, 1H, J = 1.4 Hz, C=C<u>H</u>), 4.55 (m, 1H, C<u>H</u>(O)$_2$), 3.81 (m, 4H, OC<u>H</u>$_3$, C<u>H</u>$_2$O), 3.70 (m, 4H, OC<u>H</u>$_3$, C<u>H</u>$_2$O), 3.48 (m, 1H, C<u>H</u>$_2$O), 3.36 (m, 1H, C<u>H</u>$_2$O), 2.32 (m, 2H, C<u>H</u>$_2$C=C), 1.80 (m, 1H, C<u>H</u>$_2$CH$_2$), 1.69 (m, 1H, C<u>H</u>$_2$CH$_2$), 1.40-1.60 (m, 8H, C<u>H</u>$_2$C<u>H</u>$_2$ x 2), 1.20-1.35 (m, 8H, C<u>H</u>$_2$C<u>H</u>$_2$ x 2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.2, 165.2, 150.8, 118.9, 98.8, 67.6, 62.4, 52.3, 51.8, 34.4, 30.8, 29.8, 29.3, 29.2, 28.9, 27.0, 26.2, 25.6, 19.8; HRMS (EI) Calc'd for [M+·] $C_{19}H_{32}O_6$ 356.2199, found 356.2209.



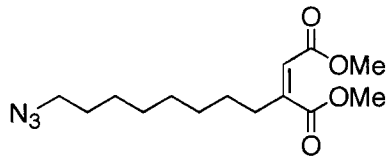
2-(8-Hydroxy-octyl)-but-2-enedioic acid dimethyl ester (69).

To a stirring solution of 2-[8-(tetrahydropyran-2-yloxy)-octyl]-but-2-enedioic acid dimethyl ester (**68**) (200 mg, 0.56 mmol) in MeOH (5 mL) was added *p*-toluenesulfonic acid (11 mg, 56 μmol). The resulting reaction mixture was stirred at room temperature for 2 h at which point the solution was extracted with EtOAc (3 x 25 mL). The combined organic extracts were washed with a saturated solution of $NaHCO_3$, water, brine dried over MgSO4 and evaporated *in vacuo* to give the crude alcohol. Purification using flash column chromatography (SiO_2, hexane:EtOAc, 10:1) afforded **69** as a colorless oil (152 mg, 99%). IR (CH_2Cl_2, cast) 3434, 2930, 2856, 1728, 1650, 1436, 1170 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.78 (t, 1H, J = 1.4 Hz, C=C<u>H</u>), 3.80 (s, 3H, OC<u>H</u>$_3$), 3.70 (s, 3H, OC<u>H</u>$_3$), 3.63 (t, 2H, J = 6.0 Hz, C<u>H</u>$_2$OH), 2.32 (m, 2H, C<u>H</u>$_2$C=CH), 1.54 (m, 2H, C<u>H</u>$_2$CH$_2$), 1.46 (m, 2H, C<u>H</u>$_2$CH$_2$), 1.26-1.34 (m, 8H, C<u>H</u>$_2$C<u>H</u>$_2$ x 2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.2, 165.3, 150.8, 119.0, 63.0, 52.3, 51.8, 34.4, 32.8, 29.3 (2C), 29.2, 28.9, 27.0; HRMS (EI) Calc'd for [M+·] $C_{14}H_{24}O_5$ 272.1624, found 272.1626.



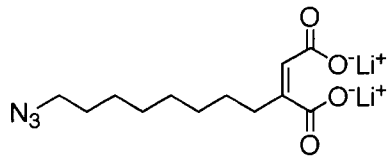
2-[8-(Toluene-4-sulfonyloxy)-octyl]-but-2-enedioic acid dimethyl ester.

A solution of 2-(8-hydroxy-octyl)-but-2-enedioic acid dimethyl ester (**69**) (175 mg, 0.643 mmol) in CH_2Cl_2 (10 mL) was treated with *p*-toluenesulfonyl chloride (172 mg, 0.902 mmol), triethylamine (0.25 mL, 1.8 mmol) and DMAP (10.0 mg, 0.04 mmol). The resulting reaction mixture was stirred at room temperature for 18 h, at which time the solution was diluted with water (5 mL) and extracted with CH_2Cl_2 (3 x 25 mL). The combined organic extracts were washed with water, brine, dried over $MgSO_4$ and evaporated *in vacuo* to give the crude product. Purification using flash column chromatography (SiO_2, hexane:EtOAc, 4:1) afforded 2-[8-(toluene-4-sulfonyloxy)-octyl]-but-2-enedioic acid dimethyl ester as a colorless oil (247 mg, 90%). IR (CH_2Cl_2, cast) 3434, 2930, 2856, 1728, 1650, 1436, 1170 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.77 (m, 2H, Ar), 7.32 (m, 2H, Ar), 5.78 (t, 1H, J = 1.5 Hz, C=CH), 3.99 (t, 2H, J = 6.5 Hz, CH_2OS) 3.81 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 2.43 (s, 3H, $ArCH_3$), 2.31 (m, 2H, $CH_2C=C$), 1.61 (m, 2H, CH_2CH_2), 1.44 (m, 2H, CH_2CH_2), 1.20-1.32 (m, 8H, CH_2CH_2 x 2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.2, 165.3, 150.7, 144.5, 129.7 (2C), 127.8 (3C), 119.0, 70.6, 52.3, 51.8, 34.4, 29.1, 28.9, 28.8 (2C), 26.9, 25.4, 21.7; HRMS (EI) Calc'd for [M+·] $C_{21}H_{30}O_7S$ 426.1712, found 426.1705.



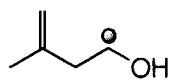
2-(8-Azido-octyl)-but-2-enedioic acid dimethyl ester (70).

To a stirring solution of 2-[8-(toluene-4-sulfonyloxy)-octyl]-but-2-enedioic acid dimethyl ester (**69**) (236 mg, 0.553 mmol) in DMF (5 mL) was added sodium azide (90 mg, 1.4 mmol). The resulting reaction mixture was stirred at room temperature for 18 h. The solution was diluted with water (10 mL) and extracted with EtOAc. The combined organic extracts were washed with water, brine, dried over $MgSO_4$ and the solvent evaporated *in vacuo* to give **70** as an oil (140 mg, 85%). The azide was used without further purification for preparation of **36**. IR (CH_2Cl_2, cast) 2932, 2858, 2096, 1729, 1650, 1436, 1169 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.78 (t, 1H, J = 1.5 Hz, C=C$\underline{H}$), 3.81 (s, 3H, OC$\underline{H}_3$), 3.70 (s, 3H, OC$\underline{H}_3$), 3.23 (t, 2H, J = 6.9 Hz, C$\underline{H}_2$N$_3$), 2.33 (m, 2H, C$\underline{H}_2$C=C), 1.56 (m, 2H, C$\underline{H}_2$CH$_2$), 1.46 (m, 2H, C$\underline{H}_2$CH$_2$), 1.23-1.37 (m, 8H, C$\underline{H}_2$C$\underline{H}_2$ x 2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.3, 165.3, 150.8, 119.0, 52.4, 51.9, 51.5, 34.4, 29.2, 29.0, 28.9, 28.8, 27.0, 26.7; HRMS (ES +ve) Calc'd for $[M+Na]^+$ $C_{14}H_{23}N_3O_4Na$ 320.1586, found 320.1590.



2-(8-Azido-octyl)-but-2-enedioic acid, dilithium salt.

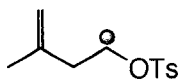
A solution of 2-(8-azido-octyl)-but-2-enedioic acid dimethyl ester (**70**) (200 mg, 0.669 mmol) in THF-H_2O (10 mL, 1:1) was treated with 1.0 N LiOH (3 eq) and the mixture was stirred refluxed for 5 h. The solvent was removed *in vacuo* and the remaining residue was dissolved in H_2O (4 mL). Freeze-drying of the aqueous layer gave the desired lithium salt as a white solid (130 mg, 67%). IR (μscope) 3415(b), 2928, 2855, 2498, 2096, 1641, 1573, 1422, 1294 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 5.45 (s, 1H, C=C<u>H</u>), 3.29 (t, 2H, J = 6.5 Hz, C<u>H</u>$_2$N), 2.20 (t, 2H, J = 6.9 Hz, C<u>H</u>$_2$C=C), 1.58 (m, 2H, C<u>H</u>$_2$CH$_2$), 1.42 (m, 2H, C<u>H</u>$_2$CH$_2$), 1.26-1.35 (m, 8H, C<u>H</u>$_2$C<u>H</u>$_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 180.1, 175.5, 153.1, 120.3, 52.1, 35.3, 29.2 (2C), 29.0, 28.8, 27.8, 26.7; HRMS (ES -ve) Calc'd for $[M-H]^-$ $C_{12}H_{18}N_3O_4$ 268.1292, found 268.1288.



[1-^{13}C]-3-Methyl-3-buten-1-ol (89).

This compound was prepared using a modified procedure of Paquette *et al.*.[171] To a flame dried, argon flushed round-bottom flask were added freshly ground magnesium turnings (1.21 g, 22.1 mmol) and Et_2O (5 mL). To this suspension was added a solution of methylallyl chloride (**88**) (1.0 g, 11.04 mmol) in Et_2O (10 mL) dropwise, followed by a crystal of iodine. The reaction mixture was heated to reflux for 2 h and then [^{13}C]-

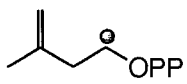
paraformaldehyde (330 mg, 10.6 mmol) was added, and refluxing was continued for a further 12 h. After cooling to room temperature the solvent was evaporated *in vacuo*. The residue was dissolved in Et_2O (25 mL) and poured into a solution of cold 1.2 N HCl (100 mL) and extracted with Et_2O. The combined ethereal extracts were washed with saturated $NaHCO_3$, water, brine and dried over $MgSO_4$. Evaporation of the solvent *in vacuo* followed purification using flash column chromatography (SiO_2, pentane:Et_2O, 4:1) afforded alcohol **89** as a liquid (450 mg, 42%). Due to its volatile nature no spectra was obtained on this labeled intermediate.



[1-^{13}C]-3-Methyl-3-buten-1-yl tosylate (90).

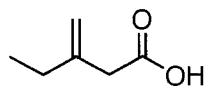
To a stirring solution of [1-^{13}C]-3-methyl-3-buten-1-ol (**89**) (400 mg, 4.64 mmol) in CH_2Cl_2 (10 mL) was added *p*-toluenesulfonyl chloride (884 mg, 4.64 mmol), triethylamine (0.65 mL, 4.64 mmol) and DMAP (10.0 mg, 0.04 mmol). The resulting reaction mixture was stirred at room temperature for 18 h, before being poured into water and extracted with CH_2Cl_2. The combined organic extracts were washed with saturated $NaHCO_3$ solution (25 mL), water (25 mL), brine (25 mL), dried over $MgSO_4$ and evaporated. Purification using flash column chromatography (SiO_2, hexane:EtOAc, 4:1) afforded **90** as a colorless oil (430 mg, 38%). IR (CH_2Cl_2, cast) 3078, 2971, 1652, 1448, 1359, 1177 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.77 (m, 2H, Ar), 7.32 (m, 2H, Ar), 4.77 (m, 1H, C=C$\underline{H}_2$), 4.65 (m, 1H, C=C$\underline{H}_2$), 4.10 (dt, 2H, $^1J_{13C\text{-}H}$ = 50 Hz, J = 6.8 Hz, $^{13}C\underline{H}_2OS$), 2.43 (s, 3H, ArC$\underline{H}_3$), 2.33 (m, 2H, $^{13}CH_2C\underline{H}_2$), 1.64 (s, 3H, C=CC$\underline{H}_3$); ^{13}C

NMR ($CDCl_3$, 75 MHz) δ 144.8, 140.2, 133.3, 129.8 (2C), 128.0 (2C), 133.1, 68.6, 36.8 (d, J = 39 Hz), 22.4, 21.7; HRMS (EI) Calc'd for [M+·] $^{13}CC_{11}H_{16}SO_3$ 241.0854, found 241.0856.



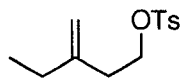
[1-^{13}C]-3-Methyl-3-buten-1-yl diphosphate (91).

The diphosphate **91** was prepared using the general procedure described for **28**. Treatment of a solution of [1-^{13}C]-3-methyl-3-buten-1-yl tosylate (**90**) (108 mg, 0.448 mmol) in MeCN (5 mL) with tris-*n*-butylammonium hydrogen diphosphate (1.21 g, 1.34 mmol) gave the crude diphosphate. Purification of the crude product using HPLC (Vydac 259VHP reverse phase polymer; 4.6 mm x 150 mm; linear gradient elution over 40 minutes of 30% MeCN in 100 mM NH_4HCO_3, t_R 28 min) afforded **91** as a white solid (60 mg, 55%). IR (μscope) 3141 (b), 3045, 2919, 1405 cm^{-1}; ^{1}H NMR (D_2O/ND_4OD, 500 MHz) δ 4.94 (m, 1H, C=CC<u>H</u>$_2$), 4.92 (m, 1H, C=CC<u>H</u>$_2$), 4.12 (ddt, 2H, J = 147 Hz, 6.6 Hz, 6.6 Hz, C<u>H</u>$_2$OP), 2.47 (m, 2H, $^{13}CH_2C\underline{H}_2$), 1.85 (3H, s, C=CC<u>H</u>$_3$); ^{13}C NMR (D_2O/ND_4OD, 125 MHz) δ 144.8, 112.3, 64.9 (d, $J_{C\text{-}P}$ = 5.8), 38.9 (d, J = 38.9 Hz), 22.7; ^{31}P NMR (D_2O/ND_4OD, 200 MHz) δ -9.28 (d, 1P, J = 20.8 Hz), -5.17 (m, 1P); HRMS (ES -ve) Calc'd for [M-H]$^-$ $C_4{}^{13}CH_{11}O_7P_2$ 246.0008, found 246.0008.



3-Ethylbut-3-enoic acid (95).

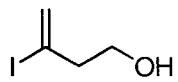
This known acid[173,174] was prepared using a procedure described by Fujisawa and coworkers.[173] To a stirring solution of diketene (**97**) (1.09 g, 13.0 mmol) in Et_2O (25 mL) was added cobalt (II) iodide and the solution was cooled to -78°C. A 3.0 M solution of ethylmagnesium bromide (4.73 mL, 14.2 mmol) was then added dropwise and the resulting reaction mixture was stirred at -78 °C for 6 h. The reaction was quenched by the addition of 6 M HCl (10 mL) and the solution was warmed to room temperature and then extracted with Et_2O (3 x 15 mL). Combined ethereal extracts were then extracted with 3 M NaOH (3 x 10 mL). The aqueous extracts were cooled to 0 °C and neutralized with dropwise addition of 6 M HCl and extracted with Et_2O (3 x 25 mL). The combined ethereal extracts were washed with brine, dried over $MgSO_4$ and evaporated *in vacuo*. The crude acid was purified by Kugelohr distillation to give **95** as an oil (640 mg, 43%). IR (CH_2Cl_2, cast) 3088, 2969, 2978, 1711, 1651, 1409, 1294 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.94 (m, 1H, C=C$\underline{H}_2$), 4.91 (m, 1H, C=C$\underline{H}_2$), 3.07 (s, 2H, $C\underline{H}_2CO_2H$), 2.12 (m, 2H, $C\underline{H}_2CH_3$), 1.04 (t, 3H, J = 7.4 Hz, $CH_2C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.5, 143.4, 113.0, 41.9, 28.8, 12.1; HRMS (EI) Calc'd for [M+·] $C_6H_{10}O_2$ 114.0681, found 114.0675.



3-Ethyl-3-butenyl tosylate (98).

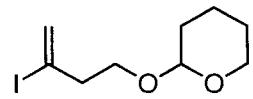
The known tosylate **98**[229] was prepared using the following procedure. To a stirring solution of 3-ethylbut-3-enoic acid (**95**) (100 mg, 0.877 mmol) in THF (5 mL) and triethylamine (0.15 mL, 1.06 mol) was added ethyl chloroformate (0.93 mL, 0.97 mmol) at 0 °C. After stirring at 0 °C for 3 h, the solution was filtered to remove solid material, and the filtrate was treated with a solution sodium borohydride (73 mg, 1.9 mmol) in water (2 mL). The resulting reaction mixture was stirred at room temperature for 18 h. Mixture was quenched with 3 M HCl and extracted with Et_2O (3 x 15 mL). Combined ethereal extracts were washed with 1N NaOH, water, brine dried over $MgSO_4$ and evaporated *in vacuo* to give crude alcohol. A solution of the alcohol in CH_2Cl_2 (5 mL) was treated with *p*-toluenesulfonyl chloride (252 mg, 1.32 mmol), triethylamine (0.37 mL, 2.6 mmol), and DMAP (10 mg, 0.08 mmol). The resulting reaction mixture was stirred at room temperature for 18 h and then diluted with water (5 mL) and extracted with Et_2O (3 x 20 mL). The combined ethereal extracts were washed with water, brine, dried over $MgSO_4$ and evaporated *in vacuo*. Purification of the crude product using flash column chromatography (SiO_2, hexane:EtOAc, 4:1) afforded **98** as a colorless oil (115 mg, 52%) IR (CH_2Cl_2, cast) 2966, 1648, 1598, 1360, 1176 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.77 (m, 2H, Ar), 7.32 (m, 2H, Ar), 4.77 (m, 1H, C=CH_2), 4.67 (m, 1H, C=CH_2), 4.10 (t, 2H, J = 7.1 Hz, CH_2OS), 2.43 (s, 3H, ArCH_3), 2.34 (t, 2H, J = 7.0 Hz, CH_2CH_2), 1.93 (q, 2H, J = 7.3 Hz, CH_2CH_3), 0.95 (t, 3H, J = 7.4 Hz, CH$_2$CH_3); ^{13}C NMR ($CDCl_3$,

100 MHz) δ 144.6, 134.6, 129.7 (2C), 127.8 (2C), 122.1, 110.7, 68.9, 35.4, 29.0, 21.7, 12.1; HRMS (ES +ve) Calc'd for $[M+Na]^+$ $C_{13}H_{18}O_3SNa$ 277.0869, found 277.0869.



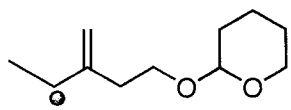
3-Iodo-3-buten-1-ol (105).

The known compound **105**[230,231] was prepared using the procedure described by Ishii and coworkers.[232,233] Sodium iodide (43.0 g, 0.285 mol) was added to MeCN (60 mL) and stirred until homogeneity was achieved. To this solution was added trimethylsilyl chloride (36.2 mL, 0.285 mol) followed by water (0.76 mL, 0.043 mol). The resulting mixture was stirred for 10 min at room temperature at which point a solution of 3-butyn-1-ol (**104**) (10.0 g, 0.143 mol) in MeCN was added. After stirring was continued for 2 h, and the reaction was quenched by the addition of water (10 mL) and the mixture was extracted with Et_2O. The combined ethereal extracts were washed with water, brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude alcohol was purified by flash column chromatography (SiO_2, hexanes:EtOAc, 4:1) to afford **105** as an oil (20.4 g, 73%). IR (CH_2Cl_2, cast) 3329, 2939, 2879, 1616, 1414, 1193 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.16 (m, 1H, C=C$\underline{H}_2$), 5.84 (m, 1H, C=C$\underline{H}_2$), 3.74 (t, 2H, J = 5.8 Hz, C$\underline{H}_2$OH), 2.62 (m, 2H, $CH_2C\underline{H}_2$), 1.45(s, 1H, O$\underline{H}$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 128.3, 107.3, 60.9, 48.1; HRMS (EI) Calc'd for $[M^+]$ C_4H_7IO 197.9542, found 197.9533.



2-(3-Iodo-but-3-enyloxy) tetrahydropyran (106).

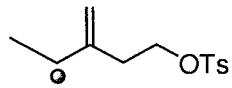
A stirring solution of 3-iodo-3-buten-1-ol (**105**) (1.00 g, 5.05 mmol) in CH_2Cl_2 (10 mL) was treated with dihydropyran (0.75 mL, 8.2 mmol) and pyridinium *p*-toluenesulfonate (138 mg, 0.550 mmol). The resulting reaction mixture was stirred at room temperature for 18 h. The reaction mixture was then diluted with water and extracted with Et_2O. The combined ethereal extracts were washed with saturated $NaHCO_3$, water, brine and dried over $MgSO_4$. Evaporation of the solvent *in vacuo* followed by purification by flash column chromatography afforded **106** as a colorless oil (1.1 g, 80%). IR (CH_2Cl_2, cast) 3077, 2941, 2871, 1642, 1453, 1137 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.11 (m, 1H, C=C$\underline{H}_2$), 5.76 (m, 1H, C=C$\underline{H}_2$), 4.60 (t, 1H, J = 3.4 Hz, C$\underline{H}$(O)$_2$), 3.84 (m, 2H, C$\underline{H}_2$OC), 3.51 (m, 2H, C$\underline{H}_2$OC), 2.67 (m, 2H, C$\underline{H}_2$C=C), 1.78 (m, 1H, $CH_2C\underline{H}_2CH$), 1.68 (m, 1H, $CH_2C\underline{H}_2CH$), 1.46-1.60 (m, 4H, $C\underline{H}_2C\underline{H}_2CH_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 127.2, 107.3, 98.8, 65.9, 62.3, 45.4, 30.5, 25.4, 19.4; HRMS (EI) Calc'd for [M+·] $C_9H_{15}IO_2$ 282.0117, found 282.0110.



[4-^{13}C]-2-(3-Ethyl-but-3-enyloxy) tetrahydropyran (107).

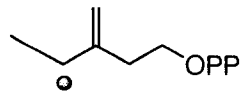
This transformation was performed using an adaptation of the method by Abarbri *et al.*.[174] To a flame dried, argon flushed round-bottom flask was added freshly ground

magnesium turnings (442 mg, 18.2 mmol) and Et_2O (5 mL). To this suspension, a solution of [^{13}C]-bromoethane (1.00 g, 9.09 mmol) chloride in Et_2O (10 mL) was then added dropwise and the resultant mixture was refluxed for 2 h. After cooling to room temperature, the Grignard reagent was added dropwise to a suspension of zinc chloride (1.24 g, 9.09 mmol) in Et_2O at -78 °C. After warming to room temperature DMF (10 mL) was added and a white solid formed. To this suspension, a solution of 2-(3-iodo-but-3-enyloxy)-tetrahydropyran (**106**) (1.71 g, 6.06 mmol) was added followed by tetrakis(triphenylphosphine)palladium(0) (350 mg, 0.303 mmol). The resulting reaction mixture was stirred at room temperature for 3 h. The reaction was quenched by the addition of 1.0 N HCl (10 mL) and then extracted with Et_2O. The combined ethereal extracts were washed with saturated NH_4Cl, brine and dried over $MgSO_4$. Evaporation of the solvent *in vacuo* followed by purification using flash column chromatography (SiO_2, hexane:EtOAc, 10:1) afforded **107** as a colorless oil (600 mg, 54%). IR (CH_2Cl_2, cast) 2958, 2627, 1646, 1461, 1378, 1364, 1122 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.75 (m, 2H, C=C$\underline{H}_2$), 4.58 (m, 1H, C$\underline{H}$(O)$_2$), 3.83 (m, 2H, C$\underline{H}_2$OC), 3.48 (m, 2H, C$\underline{H}_2$OC), 2.32 (m, 2H, C=CC$\underline{H}_2$), 2.03 (m, 2H, C=C^{13}C$\underline{H}_2$), 1.80 (m, 1H, C$\underline{H}_2$CH$_2$O), 1.68 (m, 1H, C$\underline{H}_2$CH$_2$O), 1.45-1.59 (m, 4H, C$\underline{H}_2$C$\underline{H}_2$CH$_2$), 1.01 (dt, 3H, J = 3.0 Hz, 10.4 Hz, C$\underline{H}_3$^{13}CH$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.2, 109.0, 98.8, 66.4, 62.3, 36.2, 30.7, 29.2, 25.2, 19.6, 12.3 (d, J = 32 Hz); HRMS (EI) Calc'd for [M+·] $C_{10}{}^{13}CH_{20}O_2$ 185.1497, found 185.1493.



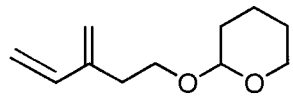
[^{13}C-4]-3-Methylene-pentan-yl tosylate (108).

A stirring solution of of [4-^{13}C]-2-(3-ethyl-but-3-enyloxy) tetrahydropyran (**107**) (200 mg, 1.08 mmol) in MeOH (5 mL) was treated with *p*-toluenesulfonic acid (21 mg, 0.11 mmol). The resulting reaction mixture was stirred for 4 h, after which time it was diluted with water and extracted with Et_2O. The combined ethereal extracts washed with saturated solution of $NaHCO_3$, water, brine, and dried over $MgSO_4$. Careful evaporation of solvent at 0 °C gave the desired alcohol. To a stirring solution of the crude alcohol in CH_2Cl_2 (10 mL) was added *p*-toluenesulfonyl chloride (309 mg, 1.62 mmol), triethylamine (0.23 mL, 1.62 mmol) and DMAP (10 mg, 82 μmol) gave crude product. Purification using flash column chromatography (SiO_2, hexane:EtOAc, 4:1) afforded **108** as a colorless oil (144 mg, 52%). IR (CH_2Cl_2, cast) 2966, 1648, 1598, 1360, 1176 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.77 (m, 2H, Ar), 7.32 (m, 2H, Ar), 4.77 (m, 1H, C=C$\underline{H}_2$), 4.67 (m, 1H, C=C$\underline{H}_2$), 4.10 (t, 2H, J = 13.0 Hz, C$\underline{H}_2$OS), 2.43 (s, 3H, ArC$\underline{H}_3$), 2.35 (dt, 2H, J = 3.8 Hz, 7.0 Hz, CH_2CH_2), 1.93 (dq, 2H, J = 7.4 Hz, $^1J_{C\text{-}H}$ = 125.4 Hz, ^{13}C$\underline{H}_2$CH$_3$), 0.95 (dt, 3H, J = 4.3 Hz, 7.4 Hz, $^{13}CH_2C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 145.5 (d, J = 40 Hz), 134.6, 129.7 (2C), 127.8 (2C), 122.1, 110.7, 68.9, 35.4, 29.0, 21.7, 12.1 (d, J = 33 Hz); HRMS (ES +ve) Calc'd for $[M+Na]^+$ $C_{12}{}^{13}CH_{18}O_3SNa$ 278.0908, found 278.0906.



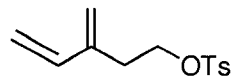
[^{13}C-4]-3-Methylene-pentyl diphosphate (109).

A similar procedure was employed as that described for the preparation of **28**. Reaction of [^{13}C-4]-3-methylene-pentyl tosylate (**108**) (130 mg, 0.510 mmol) in MeCN (10 mL) with tris-*n*-butylammonium hydrogen diphosphate (1.38 g, 1.53 mmol) gave the crude labeled diphosphate. The reaction was worked up as previously described. Purification of the crude product using HPLC (Vydac 259VHP reverse phase polymer; 4.6 mm x 150 mm; linear gradient elution over 40 minutes of 30% MeCN in 100 mM NH_4HCO_3, t_R 28 min) afforded **109** as a white solid (122 mg, 77%). ^{1}H NMR (D_2O/ND_4OD, 300 MHz) δ 4.88 (m, 2H, C=CC$\underline{H}_2$), 4.05 (dt, 2H, J = 7.3 Hz, 7.3 Hz, C$\underline{H}_2$OP,), 2.41 (m, 2H, C$\underline{H}_2$CH$_2$), 2.08 (dq, 2H, J = 7.7 Hz, $^1J_{13C\text{-}H}$ = 125.6 Hz, C=CC$\underline{H}_2$), 1.01 (m, 3H, ^{13}CH$_2$C$\underline{H}_3$); ^{13}C NMR (D_2O/ND_4OD, 125 MHz) δ 150.0 (d, J = 42.7 Hz), 110.1, 65.8, 34.5, 29.2, 12.5 (d, J = 15.3 Hz); ^{31}P NMR (D_2O/ND_4OD, 162 MHz) δ -9.00 (m, 1P), -5.10 (m, 1P); HRMS (ES -ve) Calc'd for $[M\text{-}H]^-$ $^{13}CC_5H_{13}O_7P_2$ 260.0165, found 260.0164.



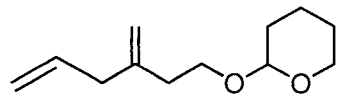
2-(3-Methylene-pent-4-enyloxy) tetrahydropyran (110).

This compound was prepared by a modified procedure of Abarbri *et al.*.[174] A flame dried 3-neck flask was charged with tetrakis(triphenylphosphine)palladium(0) (406 mg, 0.352 mmol). To this was then added 2-(3-iodo-but-3-enyloxy) tetrahydropyran (**106**) (1.00 g, 3.52 mmol) in toluene (25 mL) and vinyltributyltin (**102**) (2.16 mL, 7.39 mmol). The resulting reaction mixture was heated under reflux for 5 h and then cooled to room temperature. The reaction was then treated with saturated solution of KF (5 mL) and filtered through a pad of Celite®. The filtrate was extracted with Et_2O (3 x 15 mL). The combined ethereal extracts were washed with water, brine and dried over $MgSO_4$. Evaporation of solvent *in vacuo* followed by purification using flash column chromatography (SiO_2, hexane:EtOAc, 20:1) afforded **110** as a colorless oil (430 mg, 67%). IR (CH_2Cl_2, cast) 3088, 2941, 2871, 1595, 1465, 1200 cm^{-1} ^1H NMR ($CDCl_3$, 300 MHz) δ 6.36 (dd, 1H, J = 10.9 Hz, 17.7 Hz, C$\underline{H}$=CH_2), 5.25 (d, 1H, J = 17.7 Hz, CH=C$\underline{H_2}$), 5.07 (m, 1H, CH=C$\underline{H_2}$), 5.04 (m, 2H, C=C$\underline{H_2}$), 4.59 (m, 1H, C$\underline{H}$OCH_2), 3.86 (m, 2H, C$\underline{H_2}$OC), 3.52 (m, 2H, C$\underline{H_2}$OC), 2.52 (t, 2H, J = 7.3 Hz, C$\underline{H_2}CH_2$), 1.80 (m, 1H, $CH_2CH_2C\underline{H_2}$), 1.70 (m, 1H, $CH_2CH_2C\underline{H_2}$), 1.47-1.60 (m, 4H, C$\underline{H_2}C\underline{H_2}CH_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 143.1, 138.8, 116.9, 113.4, 98.8, 66.2, 62.2, 31.6, 30.6, 25.4, 19.5; HRMS (EI) Calc'd for [M+·] $C_{11}H_{18}O_2$ 182.1307, found 182.1302.



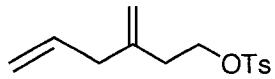
3-Methylene-4-pentenyl tosylate (111).

This compound was prepared using a similar procedure as described for **108**. Thus, treatment of a solution of 2-(3-methylene-pent-4-enyloxy) tetrahydropyran (**110**) (200 mg, 1.10 mmol) in MeOH (5 mL) with *p*-toluenesulfonic acid (19 mg, 0.102 mmol) gave the corresponding alcohol. Reaction of the crude alcohol with *p*-toluenesulfonyl chloride (420 mg, 2.20 mmol), triethylamine (0.31 mL, 2.20 mmol) and DMAP (10.0 mg, 8.2 μmol) in CH_2Cl_2 (10 mL) gave the crude product. Purification using flash column chromatography (SiO_2, hexane:EtOAc, 4:1) afforded **111** as a colorless oil (278 mg, 33%). IR (CH_2Cl_2, cast) 3088, 2962, 1597, 1359, 1176 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.76 (m, 2H, Ar), 7.32 (m, 2H, Ar), 6.27 (dd, 1H, J = 10.8 Hz, 17.6 Hz, C<u>H</u>=CH_2), 5.10 (d, 1H, J = 17.6 Hz, CH=C<u>H</u>$_2$), 5.06 (m, 1H, C=C<u>H</u>$_2$), 5.02 (d, 1H, J = 10.8 Hz, CH=C<u>H</u>$_2$), 4.97 (m, 1H, C=C<u>H</u>$_2$), 4.12 (t, 2H, J = 8.7 Hz, C<u>H</u>$_2$OS), 2.43 (s, 3H, ArC<u>H</u>$_3$), 2.56 (t, 2H, J = 8.7 Hz, C<u>H</u>$_2$CH$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.6, 140.6, 137.8, 133.1, 129.7 (2C), 127.8 (2C), 118.3, 113.8, 68.7, 31.0, 12.7; HRMS (EI) Calc'd for [M+·] $C_{13}H_{16}O_3S$ 252.0820, found 252.0815.



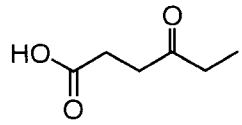
2-(3-Methylene-hex-5-enyloxy) tetrahydropyran (112).

A similar procedure was employed as that used for the preparation of **110**. Reaction of 2-(3-iodo-but-3-enyloxy) tetrahydropyran (**106**) (100 mg, 0.352 mmol), tetrakis(triphenylphosphine)palladium(0) (41 mg, 35 μmol) and allyltributyltin (**100**) (0.22 mL, 0.704 mmol) gave the crude diene. Purification by flash column chromatography (SiO_2, hexane:EtOAc, 25:1) afforded **112** as a colorless oil (36 mg, 53%). IR (CH_2Cl_2, cast) 3088, 2941, 2871, 1595, 1465, 1200 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.79 (ddt, 1H, J = 13.8 Hz, 7.0 Hz, 7.0 Hz, C<u>H</u>=CH_2), 5.05 (m, 1H, CH=C<u>H</u>$_2$), 5.01 (m, 2H, CH=C<u>H</u>$_2$), 4.80 (m, 1H, C=C<u>H</u>$_2$), 4.58 (m, 1H, C<u>H</u>$(O)_2$), 3.83 (m, 2H, C<u>H</u>$_2$OC), 3.49 (m, 2H, C<u>H</u>$_2$OC), 2.78 (d, 2H, J = 6.9 Hz, C<u>H</u>$_2$CH=CH_2), 2.31 (t, 2H, J = 7.2 Hz, C<u>H</u>$_2$C=CH_2), 1.79 (m, 1H, CH_2CH_2C<u>H</u>$_2$), 1.68 (m, 1H, CH_2CH_2C<u>H</u>$_2$), 1.45-1.59 (m, 4H, C<u>H</u>$_2$C<u>H</u>$_2CH_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 145.2, 136.2, 116.3, 111.5, 98.8, 66.3, 62.4, 41.3, 36.0, 30.9, 25.6, 19.7; HRMS (EI) Calc'd for [M+] $C_{12}H_{20}O_2$ 196.1463, found 196.1440.



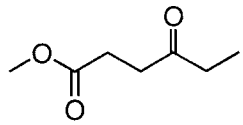
3-Methylene-5-hexenyl tosylate (113).

This compound was prepared using a similar procedure as described for **108**. Thus, treatment of a solution of 2-(3-methylene-hex-5-enyloxy) tetrahydropyran (**112**) (200 mg, 1.02 mmol) in MeOH (5 mL) with *p*-toluenesulfonic acid (19 mg, 0.10 mmol) gave the corresponding alcohol. Reaction of the crude alcohol with *p*-toluenesulfonyl chloride (389 mg, 2.04 mmol), triethylamine (0.29 mL, 2.0 mmol) and DMAP (10 mg, 8.2 μ mol) in CH_2Cl_2 (10 mL) gave the crude product. Purification using flash column chromatography (SiO_2, hexane:EtOAc, 4:1) afforded **113** as a colorless oil (90 mg, 33%). IR (CH_2Cl_2, cast) 3088, 2962, 1597, 1359, 1176 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.77 (m, 2H, Ar), 7.32 (m, 2H, Ar), 5.68 (ddt, 1H, J = 10.2 Hz, 6.8 Hz, 6.8 Hz, C$\underline{H}$=CH_2), 5.00 (m, 2H, CH=C$\underline{H_2}$), 4.82 (m, 1H, C=C$\underline{H_2}$), 4.74 (m, 1H, C=C$\underline{H_2}$), 4.10 (t, 2H, J = 6.9 Hz, C$\underline{H_2}$OS), 2.66 (d, 2H, J = 6.8 Hz, C$\underline{H_2}$C=CH_2), 2.43 (s, 3H, ArC$\underline{H_3}$), 2.34 (t, 2H, J = 6.9 Hz, C$\underline{H_2}$C=CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.7, 142.3, 135.4, 133.1, 129.8 (2C), 127.9 (2C), 116.8, 113.2, 68.5, 40.7, 34.8, 21.6; HRMS (ES +ve) Calc'd for [M+Na^+] $C_{14}H_{18}O_3SNa$ 289.0874, found 289.0874.



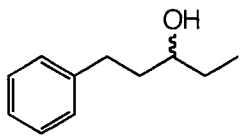
4-Oxohexanoic acid (115).

The known compound **115**[234,235] was prepared by procedure of Nunez and Martin.[234] To a stirring solution of 5-phenyl-3-pentanone (**120**) (3.08 mmol) in a CCl_4:MeCN solution (1:1, 14 mL) was added water 10 (mL) and periodic acid (10.0 g, 43.8 mmol). The resulting mixture was stirred until the solution was clear. At this time, ruthenium trichloride (13 mg, 0.062 mmol) was added and stirred at room temperature for 10 h. The solution was the cooled to 0 °C and extracted with Et_2O. Combined ethereal extracts were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude residue was distilled under reduced pressure (Kugelohr) to give **115** as an oil (225 mg, 56%). IR (CH_2Cl_2, cast) 2979, 2942, 1713, 1416, 1244 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.71 (m, 2H, $C\underline{H}_2CH_2$), 2.63 (m, 2H, $C\underline{H}_2CH_2$), 2.46 (q, 2H, J = 7.3 Hz, $C\underline{H}_2CH_3$), 1.06 (t, 3H, J = 7.3 Hz, $CH_2C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 209.4, 178.1, 36.2, 35.9, 27.8, 7.8; HRMS (EI) Calc'd for [M+·] $C_6H_{10}O_3$ 130.0629, found 130.0629.



4-Oxohexanoic acid methyl ester (117).

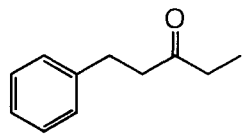
A solution of 4-oxohexanoic acid (**115**) (250 mg, 2.15 mmol) in Et_2O (5 mL) was poured into an ethereal solution of diazomethane (20 mL, *ca.* 5 mmol) at 0 °C. After stirring for a further 2 h at room temperature, excess diazomethane was quenched with addition of a solution of 10% acetic acid. The solution was diluted with Et_2O and washed with saturated $NaHCO_3$, brine, and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give **117** as an oil (265 mg, 95%). IR (CH_2Cl_2, cast) 2978, 2953, 1739, 1717, 1438, 1210, 1172 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.66 (s, 3H, $OC\underline{H}_3$), 2.71 (t, 2H, J = 6.8 Hz, $C\underline{H}_2CH_2$), 2.59 (t, 2H, J = 6.8 Hz, $C\underline{H}_2CH_2$), 2.46 (q, 2H, J = 7.3 Hz, $C\underline{H}_2CH_3$), 1.06 (t, 3H, J = 7.3 Hz, $CH_2C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 209.5, 173.4, 51.8, 36.6, 35.9, 27.8, 7.8; HRMS (EI) Calc'd for [M+·] $C_7H_{12}O_3$ 144.0786, found 144.0786.



1-Phenylpentan-3-ol (119).

The known compound **119**[236-238] was made using a modified procedure of Hoveyda and coworkers.[239] To a flame dried, argon flushed round-bottom flask was added freshly ground magnesium turnings (1.36 g, 55.8 mmol) and THF (10 mL). To this suspension, a solution of bromoethane (4.05 g, 37.2 mmol) chloride in THF (10 mL) was then added

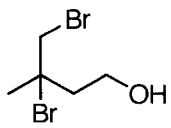
dropwise and refluxed for 2 h. After initial cooling to room temperature, the Grignard reagent was cooled to 0°C and was treated with a solution of hydrocinnamaldehyde (**118**) (2.50 g 18.6 mmol) in THF (20 mL). The resulting reaction mixture was stirred for 3 h with warming to room temperature. The reaction was quenched by the addition of a saturated solution of NH_4Cl (10 mL) and extracted with Et_2O. Combined ethereal extracts were washed with saturated NH_4Cl, brine and dried over $MgSO_4$. Evaporation of solvent *in vacuo* followed by purification using flash column chromatography (SiO_2, hexane:EtOAc, 4:1) afforded **119** as a colorless oil (3.05 g, 66%). IR (CH_2Cl_2, cast) 3360, 3062, 2962, 2876, 1603, 1496, 1254 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.26 (m, 2H, Ar), 7.18 (m, 3H, Ar) 3.55 (m, 1H, CHOH), 2.79 (m, 1H, CH_2CH), 2.66 (m, 1H, CH_2CH), 1.75 (m, 2H, CH_2CH_2), 1.49 (m, 3H, CH_2CH_3, OH), 0.93 (t, 3H, J = 7.4 Hz, CH_2CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 142.2, 128.4 (4C), 125.8, 72.7, 38.6, 32.1, 30.3, 9.8; HRMS (EI) Calc'd for [M+·] $C_{11}H_{16}O$ 164.1201, found 164.1201.



1-Phenylpentan-3-one (120).

The known ketone **120**[234] was prepared using the following procedure. A solution of *N*-methylmorpholine *N*-oxide (2.03 g, 11.6 mmol) in CH_2Cl_2 (20 mL) was treated with $MgSO_4$ and stirred at room temperature for 20 min. After removal of the drying agent by gravity filtration, molecular sieves were added, followed by a solution of 1-phenylpentan-3-ol (**119**) (1.90 g, 11.6 mmol) in CH_2Cl_2 (10 mL). The solution was stirred for 15 min prior to the addition of tetra-*n*-propylammonium perruthenate (50 mg, 0.14 mmol). The

reaction was stirred for 12 h at room temperature at which point the solution was filtered through a plug of silica gel to remove the ruthenium catalyst. The eluent was washed with a saturated solution of $CuSO_4$, water, brine, and dried over $MgSO_4$. Evaporation of solvent *in vacuo* followed by purification using flash column chromatography gave **120** as a colorless oil (1.60 g, 85%). IR (CH_2Cl_2, cast) 3062, 2976, 2937, 1714, 1603, 1496, 1271 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.26 (m, 2H, Ar), 7.17 (m, 3H, Ar), 2.88 (t, 2H, J = 7.9 Hz, $C\underline{H}_2CH_2$), 2.71 (t, 2H, J = 7.9 Hz, $C\underline{H}_2CO$), 2.39 (q, 2H, J = 7.3 Hz, $C\underline{H}_2CH_3$), 1.03 (t, 3H, J = 7.3 Hz, $CH_2C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 210.7, 141.2, 128.5 (2C), 128.4 (2C), 126.1, 43.9, 36.1, 29.9, 7.8; HRMS (EI) Calc'd for [M+] $C_{11}H_{14}O$ 162.1045, found 162.1046.



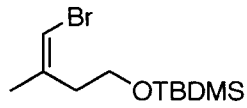
3,4-Dibromo-3-methyl-1-butanol (131).

This known compound **131**[186,187,191,220,221] was prepared using the procedure of Koyama and co-workers.[186] A solution of 3-methyl-3-buten-ol (**130**) (10.0 g, 116 mmol) in carbon tetrachloride (30 mL) was cooled to 0 °C and treated with a solution of bromine (18.6 g, 116 mmol) in CCl_4 (50 mL). The resulting mixture was stirred at room temperature for 30 min at which point the solvent was evaporated to give the crude alcohol **131**. The crude compound was used directly in next reaction with no further purification. ^1H NMR ($CDCl_3$, 300 MHz) δ 3.95 (m, 2H, $C\underline{H}_2Br$), 3.85 (m, 2H, $C\underline{H}_2OH$), 2.25 (dt, 2H, J = 2.2

Hz, 6.6 Hz, C$\underline{H}_2$CBr), 1.90 (s, 3H, C$\underline{H}_3$CBr); HRMS (EI) Calc'd for [M+·] $C_5H_{10}{}^{79}Br^{81}BrO$ 245.9078, found 245.9080.

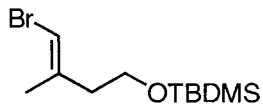
_Z_ and _E_-4-Bromo-3-methyl-3-buten-1-ol (132 and 133).

These known compounds **132** and **133**[186,187,191,220,221] were prepared using the method of Koyama and coworkers.[186] To a stirring solution of 3,4-dibromo-3-methyl-1-butanol (**131**) (3.94 g, 16.0 mmol) in MeOH (10 mL) was added 5 M KOH (15 mL solution in MeOH) at 0 °C. The reaction mixture was stirred for 18 h with warming to room temperature, then added Et_2O (10 mL) and precipitates were removed by filtration. The filtrate was washed with water, brine, dried over $MgSO_4$ and evaporated *in vacuo*. The crude mixture of alcohols were purified using flash column chromatography (SiO_2, 2:1 hexane:EtOAc). The *Z* isomer **132** was obtained as an oil (965 mg, 29%). IR (CH_2Cl_2, cast) 3335, 3069, 2953, 2881, 1631, 1471, 1050 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.01 (s, 1H, C=C$\underline{H}$), 3.77 (t, 2H, J = 6.7 Hz, C$\underline{H}_2$O), 2.53 (t, 2H, J = 6.7 Hz, C$\underline{H}_2$CH$_2$), 1.85 (s, 3H, C=CC$\underline{H}_3$), 1.40 (b, 1H, O$\underline{H}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.4, 103.1, 59.9, 41.2, 19.0; HRMS (EI) Calc'd for [M+·] $C_5H_9{}^{79}BrO$ 163.9837 found 163.9832. The *E* isomer **133** was obtained as an oil (1.93g, 58%). IR (CH_2Cl_2, cast) 3334, 3074, 2942, 4632, 1435, 1048 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.99 (1H, s, C=C$\underline{H}$), 3.73 (t, 2H, J = 6.2 Hz, C$\underline{H}_2$OH), 2.39 (t, 2H, J = 6.2 Hz, C$\underline{H}_2$CH$_2$), 1.84 (3H, s, C=CC$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.4, 102.8, 61.2, 37.5, 22.5; HRMS (EI) Calc'd for [M+·] $C_5H_9{}^{79}BrO$ 163.9837, found 163.9839.



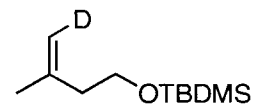
Z-4-Bromo-3-methyl-3-buten-1-yl-tert-butyldimethylsilyl ether (134).

The known vinyl bromide **134**[186,187,191,220,221] was synthesized using the procedure of Koyama and coworkers.[186] To a stirring solution of *Z*-4-bromo-3-methyl-3-buten-1-ol (**132**) (350 mg, 2.12 mmol) in DMF (50 mL) was added tert-butyldimethyl silyl chloride (640 mg, 4.24 mmol) and imidazole (722 mg, 10.6 mmol). The resulting reaction mixture was stirred at room temperature for 18 h at which time the solution was extracted with Et_2O (3 x 25 mL). The combined ethereal extracts were washed with water, brine dried over $MgSO_4$ and evaporated. The crude silyl ether was purified using flash column chromatography (SiO_2, 100:1 hexane:EtOAc) to afford **134** as a colorless oil (410 mg, 70%). IR (CH_2Cl_2, cast) 3069, 2955, 2857, 1632, 1472, 1101 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.93 (s, 1H, C=C<u>H</u>), 3.73 (t, 2H, *J* = 7.0 Hz, C<u>H</u>$_2$OSi), 2.45 (t, 2H, *J* = 7.0 Hz, $CH_2C\underline{H}_2$), 1.83 (s, 3H, C=CC<u>H</u>$_3$), 0.90 (s, 9H, C(C<u>H</u>$_3$)$_3$), 0.04 (s, 6H, Si(C<u>H</u>$_3$)$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 139.3, 101.8, 60.7, 37.8, 25.9, 23.1, 18.3 (3C), -5.4 (2C); HRMS (ES +ve) $[M+H]^+$ Calc'd for $C_{11}H_{24}{}^{79}BrOSi$ 279.0774, found 279.0776.



_E_-4-Bromo-3-methyl-3-buten-1-yl-tert-butyldimethylsilyl ether (135).

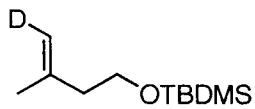
A similar procedure was employed as that described for the preparation of the known compound **134**[186,187,191,220,221]. Reaction of *E*-4-bromo-3-methyl-3-buten-1-ol (**133**) (4.26 g, 25.8 mmol) with imidazole (4.37 g, 64.3 mmol) and *tert*-butyldimethyl silyl chloride (2.12 g, 12.9 mmol) gave the crude product. The crude silyl ether was purified using flash column chromatography (SiO_2, 100:1 hexane:EtOAc) to afford **135** as an oil (3.04 g, 85%). IR (CH_2Cl_2, cast) 3074, 1954, 2858, 1634, 1472, 1104 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.94 (m, 1H, C=C<u>H</u>), 3.69 (t, 2H, J = 6.6 Hz, C<u>H</u>$_2$OSi), 2.32 (td, 2H, J = 6.6 Hz, 0.9 Hz, C<u>H</u>$_2$CH$_2$), 1.81 (d, 3H, J = 0.7 Hz C=CC<u>H</u>$_3$), 0.89 (s, 9H, C(CH$_3$)$_3$), 0.04 (s, 6H, Si(C<u>H</u>$_3$)$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.9, 102.6, 61.2, 41.4, 25.8, 19.4, 18.2 (3C), -5.4 (2C); HRMS (ES +ve) Calc'd for $[M+H]^+$ $C_{11}H_{24}{}^{79}BrOSi$ 279.0774, found 279.0775.



_Z_-[4-^{2}H]-3-Methyl-3-buten-1-yl-tert-butyldimethylsilyl ether (136).

The known compound **136**[186,187,191,220,221] was prepared using a procedure described by Koyama and coworkers.[186] A solution of *Z*-4-bromo-3-methyl-3-buten-1-yl-tert-butyldimethylsilyl ether (**134**) (390 mg, 1.40 mmol) in Et_2O (5 mL) was treated with $MgSO_4$ for 1 h and filtered to remove drying agent. The filtrate was transferred to 50 mL round bottom flash, dry *N,N,N',N'*-tetraethylenediamine (TMEDA, 0.85 mL, 5.6 mmol) was added and cooled to -78°C. To this solution, tert-butyl lithium (1.9 mL of 1.7 M

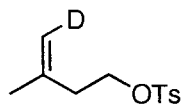
solution in pentanes, 3.2 mmol) was then added dropwise over 5 minutes. The resulting solution was stirred for 30 min, at which time the reaction was quenched by the addition of triflouroacetic acid-d_1 and stirred for 5 minutes. The solution was warmed to 0 °C and $NaHPO_4$ buffer (5 mL, 1.0 M, pH 7.0) was added. The reaction mixture was warmed to room temperature and extracted with Et_2O (3 x 15 mL). The combined organic extracts were washed with water, brine, dried over $MgSO_4$ and evaporated. The crude product was purified by flash column chromatography (SiO_2, 100:1 hexane:EtOAc) to afford **136** as colorless oil (245 mg, 87%). IR (CH_2Cl_2, cast) 3042, 2956, 2858, 1631, 1472, 1101 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.74 (s, 1H, C=C<u>H</u>), 3.71 (t, 2H, J = 7.1 Hz, C<u>H</u>$_2$OSi), 2.24 (t, 2H, J = 7.1 Hz, C<u>H</u>$_2$CH$_2$), 1.74 (s, 3H, C=CC<u>H</u>$_3$), 0.89 (s, 9H, C(C<u>H</u>$_3$)$_3$), 0.05 (s, 6H, Si(C<u>H</u>$_3$)$_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 142.9, 111.1 (t, $^1J_{C\text{-}D}$ = 23.4 Hz), 62.1, 41.1, 25.9, 22.8, 18.3 (3C), -5.3 (2C); HRMS (EI) Calc'd for [M+·] $C_{11}H_{23}DOSi$ 201.1659, found 201.1652.



_E_-[4-^{2}H]-3-Methyl-3-buten-1-yl-tert-butyldimethylsilyl ether (137).

A similar procedure was employed as that described for the preparation of **136**. The reaction of _E_-4-bromo-3-methyl-3-buten-1-yl-tert-butyldimethylsilyl ether (**135**) (200 mg, 0.72 mmol) with TMEDA (0.44 mL, 2.88 mmol) and tert-butyl lithium (1.6 mmol of 1.7 M solution in pentanes) followed by quenching with trifluoroacetic acid 1-d (0.5 mL, 5.6 mmol) gave the crude silyl ether. Purification by flash column chromatography (SiO_2, 100:1 hexane:EtOAc) afforded **137** as a colorless oil (100 mg, 69%). IR (CH_2Cl_2, cast)

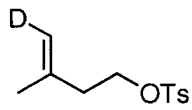
3042, 2956, 2858, 1631, 1472, 1101 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.68 (s, 1H, C=C<u>H</u>), 3.71 (t, 2H, J = 7.0 Hz, C<u>H</u>$_2$OSi), 2.24 (t, 2H, J = 7.1 Hz, C<u>H</u>$_2$CH$_2$), 1.74 (s, 3H, C=CC<u>H</u>$_3$), 0.89 (s, 9H, C(C<u>H</u>$_3$)$_3$), 0.05 (s, 6H, Si(CH$_3$)$_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 143.0, 111.1 (t, J = 23.4 Hz), 62.1, 41.1, 25.9, 22.8, 18.3 (3C), -5.3 (2C); HRMS (EI) Calc'd for [M+·] $C_{11}H_{23}DOSi$ 201.1659, found 201.1652.



Z-[4-^{2}H]-3-Methyl-3-buten-1-yl tosylate (138).

The known tosylate **138**[186,187,191,220,221] was made using a modified procedure described by Koyama and coworkers.[186] A solution of Z-[4-^{2}H]-3-methyl-3-buten-1-yl-tert-butyldimethylsilyl ether (**136**) (140 mg, 0.695 mmol) in THF (5 mL) was treated with a 1.0 M solution of tetrabutylammonium fluoride (1.39 mL, 1.39 mmol). The resulting reaction mixture was stirred at room temperature for 2 h and extracted with CH_2Cl_2 (3 x 5 mL). The combined organic extracts (5 mL) were washed with brine, dried over $MgSO_4$ and evaporation *in vacuo* to provide the crude product. To a solution of the crude alcohol in CH_2Cl_2 (10 mL) was added *p*-toluenesulfonyl chloride (400 mg, 2.09 mmol), triethylamine (0.29 mL, 2.1 mmol) and DMAP (5 mg, 41 μmol). The mixture was stirred at room temperature for 18 h at which point, it was poured onto ice/water (10mL) and extracted with Et_2O. The combined ethereal extracts were washed with saturated $NaHCO_3$ solution (15 mL), brine (15 mL), dried over $MgSO_4$ and evaporated. The crude tosylate was purified using flash column chromatography (SiO_2, 10:1 hexane:EtOAc) to afford **138** as an oil (70 mg, 42% over two steps). IR (CH_2Cl_2, cast) 3038, 2921, 1631,

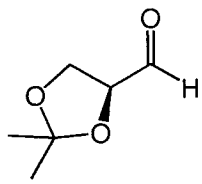
1495, 1176 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.80 (d, 2H, J = 8.3 Hz, Ar), 7.35 (d, 2H, J = 8.3 Hz, Ar), 4.78 (s, 1H, C=CH), 4.13 (t, 2H, J = 6.8 Hz, CH_2OS), 2.46 (s, 3H, $ArCH_3$), 2.36 (td, 2H, J = 6.8 Hz, 1.1 Hz CH_2CH_2), 1.66 (s, 3H, $C=CCH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.7, 140.0, 133.2, 129.8 (2C), 127.9 (2C), 112.8 (t, $^1J_{C\text{-}D}$ = 23.7 Hz), 68.5, 36.7, 22.3, 21.6; HRMS (ES +ve) Calc'd for $[M+Na]^+$ $C_{12}H_{15}DO_3SNa$ 264.0775, found 264.0778.



***E*-[4-^{2}H]-3-Methyl-3-buten-1-yl tosylate (139).**

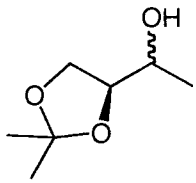
The known tosylate **139**[186,187,191,220,221] was made using similar procedure was employed as that described for the preparation of **138**. Reaction of *E*-[4-^{2}H]-3-methyl-3-buten-1-yl-tert-butyldimethylsilyl ether (0.85 mg, 0.42 mmol) (**137**) in THF (5 mL) with tetrabutylammonium fluoride (0.85 ml of 1.0 M solution in THF, 0.85 mmol) gave the crude alcohol. A solution of the crude alcohol in CH_2Cl_2 (10 mL) was treated with *p*-toluenesulfonyl chloride (410 mg, 2.15 mmol), triethylamine (0.30 mL, 2.2 mmol) and DMAP (5 mg, 4.1 μmol) to give the crude product. The crude tosylate was purified using flash column chromatography (SiO_2, 10:1 hexane:EtOAc) to afford **139** as an oil (130 mg, 75% over two steps). IR (CH_2Cl_2, cast) 3038, 2921, 1631, 1495, 1176 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.80 (m, 2H, Ar), 7.35 (m, 2H, Ar), 4.64 (m, 1H, C=CH), 4.13 (t, 2H, J = 6.8 Hz, CH_2OS), 2.46 (s, 3H, $ArCH_3$), 2.36 (td, 2H, J = 6.8 Hz, 1.1 Hz, CH_2CH_2), 1.66 (s, 3H, $C=CCH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.7, 140.0, 133.2, 129.8, 127.8,

112.8 (t, $^1J_{C\text{-}D}$ = 24 Hz), 68.5, 36.7, 22.2, 21.6; HRMS (EI) Calc'd for [M+] $C_{12}H_{15}DO_3S$ 241.0883, found 241.0887.



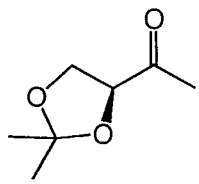
(*S*)-Glyceraldehyde acetonide (141).

The known aldehyde **141**[195,196] was prepared using the method described by Hubschwerlen *et al.*.[195] Sodium periodate (9.80 g, 45.8 mmol) was added portionwise to a stirring suspension of 5,6-O-isopropylidene-L-gulonolactone (**140**) (5.00 g, 22.9 mmol) in water at 0 °C over 30 minutes. The pH of this solution was kept constant with the addition of 3 M NaOH. The suspension was stirred at room temperature for 2 h, then saturated with solid sodium chloride, and filtered. The filtrate was extracted with CH_2Cl_2 (6 x 25 mL) and Et_2O (2 x 30 mL). The combined organic extracts were dried with $MgSO_4$ and evaporation at 0 °C gave **141** as a colorless liquid. The aldehyde was partially characterized and used directly for the preparation of (2*S*)-1,2,3-butanetriol-1,2-acetonide (**142**). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.73 (d, 1H, *J* = 1.9 Hz, C<u>H</u>O), 4.36 (ddd, 1H, *J* = 7.3 Hz, 4.8 Hz, 1.8 Hz, C<u>H</u>OC), 4.15 (1H, dd, *J* = 8.8 Hz, 7.3 Hz, C<u>H</u>$_2$OC), 4.09 (dd, 1H, *J* = 8.8 Hz, 4.8 Hz, C<u>H</u>$_2$OC), 1.47 (s, 3H, C(C<u>H</u>$_3$)$_2$), 1.40 (s, 3H, C(C<u>H</u>$_3$)$_2$).



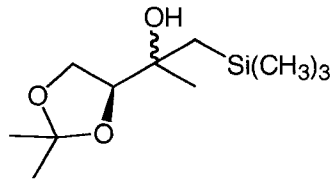
(2*S*)-1,2,3-Butanetriol-1,2-acetonide (142).

The compound **142** was prepared using a the method described by Poulter and coworkers.[193] Methylmagnesium bromide (11.5 mL of a 3.0 M solution in Et_2O, 34.5 mmol) was added dropwise to a 0°C stirring solution of (*S*)-glyceraldehyde acetonide (**141**) (22.9 mmol) in Et_2O (15 mL). The ice bath was removed and stirring was continued at room temperature overnight. The reaction was poured onto ice/saturated NH_4Cl solution and the aqueous layer was extracted with Et_2O (3 x 25 mL). The combined ethereal extracts were washed with water, brine, dried over Na_2SO_4 and evaporated. Purification of the crude product by flash column chromatography (SiO_2, pentanes:ether, 1:1) afforded **142** as a 1:1 mixture of diastereomers (1.3 g, 39% over two steps). IR (CH_2Cl_2, cast) 3455, 2986, 2886, 1669, 1456, 1214 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.88-4.05 (m, 3H, C$\underline{H}_2$CH, C$\underline{H}$OH), 3.70 (dd, 1H, J = 6.2 Hz, 7.9 Hz, C$\underline{H}$OC), 2.01 (b, 1H, O$\underline{H}$) 1.43 (s, 3H, C(C$\underline{H}_3$)$_2$), 1.37 (s, 3H, C(C$\underline{H}_3$)$_2$), 1.16 (d, 3H, J = 6.5 Hz, CHC$\underline{H}_3$), 1.15 (d, 3H, J = 6.4 Hz, CHC$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 109.5, 109.1, 80.4, 79.4, 68.8, 66.8, 66.1, 64.5, 26.7, 26.5, 25.3, 25.2, 18.9, 18.3; HRMS (ES +ve) Calc'd for $[M+Na]^+$ $C_7H_{14}O_3Na$ 169.0835, found 169.0837.



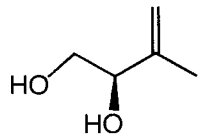
(*S*)-3,4-Dihydroxybutanone acetonide (143).

The compound **143** was prepared using the method described by Poulter and coworkers.[193] A solution of *N*-methylmorpholine *N*-oxide (1.57 g, 13.4 mmol) in CH_2Cl_2 (20 mL) was treated with $MgSO_4$ and stirred at room temperature for 20 min. After removal of the drying agent by gravity filtration, molecular sieves were added followed by a solution of (2*R*)-1,2,3-butanetriol-1,2-acetonide (**142**) (1.29 g, 8.95 mmol) in CH_2Cl_2 (10 mL). The solution was stirred for 15 min prior to the addition of tetra-*n*-propylammonium perruthenate (50 mg, 0.14 mmol). The reaction mixture was stirred for 6 h at room temperature at which point the solution was filtered through a plug of silica gel to remove the ruthenium catalyst. The eluent was washed with a saturated solution of $CuSO_4$, water, and brine. The organic layer was dried over $MgSO_4$ and evaporation of the solvent *in vacuo* gave **143** as an oil (1.28 g, quantitative). $[\alpha]_D^{26}$ = -57.0° (*c* 1.4, CH_2Cl_2); IR (CH_2Cl_2, cast) 2990, 2938, 1720, 1420; cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.39 (dd, 1H, *J* = 7.7 Hz, 5.6 Hz, C<u>H</u>OC), 4.18 (dd, 1H, *J* = 8.6 Hz, 7.8 Hz, C<u>H</u>$_2$OC), 3.98 (dd, 1H, *J* = 8.6 Hz, 5.6 Hz, C<u>H</u>$_2$OC), 2.23 (3H, s, C<u>H</u>$_3$CO), 1.48 (s, 3H, C(C<u>H</u>$_3$)$_2$), 1.38 (s, 3H, C(C<u>H</u>$_3$)$_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 209.2, 111.1, 80.5, 66.5, 26.3, 26.1, 25.1; HRMS (EI) Calc'd for [M+·] $C_7H_{12}O_3$ 144.0786, found 144.0789.



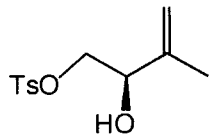
(2*S*)-3-Trimethylsilyl)methyl-1,2,3-butanetriol-1,2-acetonide (144).

The compound **144** was prepared using a procedure described by Poulter and coworkers.[193] To a vigorously stirring solution of (*S*)-3,4-dihydroxybutanone acetonide (**143**) (1.30 g, 9.14 mmol) in Et_2O (50 mL) at -78 °C was added (trimethylsilyl)methyllithium (15.5 mL of 1.0 M solution in pentanes, 15.5 mmol) dropwise. The resulting solution was stirred at -78°C for 1 h at which point a solution of $NH_4Cl/NaHCO_3$ (20 mL) was carefully added to quench reaction. The aqueous layer was separated and extracted with Et_2O (3 x 15 mL). The combined ethereal extracts were washed with water, brine, dried over $MgSO_4$, and evaporated. Purification of the crude alcohol using flash column chromatography (SiO_2, pentanes: Et_2O, 10:1) afforded **144** as a 1:1 mixture of diastereomers (2.13 g, 80%). IR (CH_2Cl_2, cast) 3490, 2986, 2953, 2892, 1456, 1418 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.81-3.95 (3H, m, C$\underline{H}_2$OC + C$\underline{H}$OC), 1.44 (3H, s, C(C$\underline{H}_3$)$_2$), 1.38 (3H, s, C(C$\underline{H}_3$)$_2$), 1.14 (3H, s, C$\underline{H}_3$COH) 1.07 (1H, d, J = 14.7 Hz, C$\underline{H}_2$Si), 0.93 (1H, d, J = 14.7 Hz, C$\underline{H}_2$Si), 0.87 (1H, d, J = 14.7 Hz, C$\underline{H}_2$Si), 0.72 (1H, d, J = 14.7 Hz, C$\underline{H}_2$Si), 0.1 (9H, s, Si(C$\underline{H}_3$)$_3$), 0.07 (9H, s, Si(C$\underline{H}_3$)$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 109.4, 109.3, 83.3, 83.2, 72.2, 65.3 (2C), 30.2, 27.4, 26.7, 26.5, 25.5, 25.4, 24.5, 0.6 (3C), 0.5 (3C); HRMS (ES +ve) Calc'd for $[M+Na]^+$ $C_{11}H_{24}O_3SiNa$ 255.1387, found 255.1386.



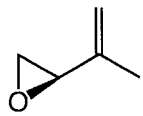
(2*R*)-3-Methyl-3-butene-1,2-diol (145).

This diol **145** was prepared using the procedure described by Poulter and coworkers.[193] A stirring solution of (2*R*)-3-trimethylsilyl)methyl-1,2,3-butanetriol-1,2-acetonide (**144**) (1.19 g, 5.12 mmol) in EtOH (16 mL) was treated with 3N HCl (0.5 mL) and the mixture was refluxed for 2 h. After cooling to room temperature, the solution was neutralized by the slow addition of solid $NaHCO_3$. The precipitated solids were filtered, washed with EtOH, and the filtrate was concentrated *in vacuo*. The resulting residue was re-dissolved in MeOH and treated with Et_2O (50 mL). The precipitated solids were filtered and the solvent was concentrated *in vacuo* to afford **145** as a colorless oil (390 mg, 75%). $[\alpha]_D^{26}$ = -15.7 ° (*c* 1.4, CH_2Cl_2); IR (CH_2Cl_2, cast) 3373, 3075, 2970, 2879, 1652, 1444; cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.06 (m, 1H, C=C$\underline{H}_2$), 4.96 (m, 1H, C=C$\underline{H}_2$), 4.18 (dd, 1H, *J* = 7.2 Hz, 3.4 Hz, C$\underline{H}$OH), 3.70 (dd, 1H, *J* = 11.2 Hz, 3.5 Hz, C$\underline{H}_2$OH), 3.55 (dd, 1H, *J* = 11.2 Hz, 7.3 Hz, C$\underline{H}_2$OH), 2.58 (b, 2H, O$\underline{H}$), 1.75 (m, 3H, C=CC$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 144.1, 112.0, 75.7, 65.2, 18.9; HRMS (EI) Calc'd for $C_5H_{10}O_2$ [M+·] 102.0681, found 102.0679.



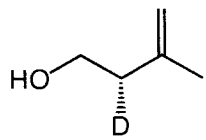
(2*R*)-3-Methyl-3-butene-1,2-diol-1-yl tosylate (146).

The compound **146** was prepared using a procedure described by Poulter and coworkers.[193] To a stirring solution of (2*R*)-3-methyl-3-butene-1,2-diol (**145**) (225 mg, 2.20 mmol) in pyridine (5 mL) at 0 °C was added freshly recrystallized *p*-toluenesulfonyl chloride (462 mg, 2.42 mmol) in pyridine (10 mL) and stirred at 0 °C for 18 h. The solution was then poured onto an ice-water mixture and extracted with Et_2O (3 x 15 mL). The combined ethereal extracts washed with 1N HCl followed by saturated $NaHCO_3$ solution, water, brine and dried over $MgSO_4$. Evaporation of the solvent and purification of the crude product by flash column chromatography (SiO_2, hexane:EtOAc, 4:1) afforded the tosylate **146** as a colorless oil (564 mg, 71%). $[\alpha]_D^{26} = -13.3°$ (*c* 1.2, CH_2Cl_2); IR (CH_2Cl_2, cast) 3533, 3069, 2951, 2921, 1652, 1495, 1190 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.81 (m, 2H, Ar), 7.36 (m, 2H, Ar), 5.06 (m, 1H, C=CH_2), 4.97 (m, 1H, C=CH_2), 4.31 (dd, 1H, *J* = 7.6 Hz, 3.2 Hz, CHOH), 4.12 (dd, 1H, *J* = 10.6 Hz, 3.3 Hz, CH_2OS), 3.97 (dd, 1H, *J* = 10.3 Hz, 7.7 Hz, CH_2OS), 2.46 (s, 3H, ArCH_3), 2.06 (b, 1H, OH) 1.70 (m, 3H, C=CCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 145.1, 142.0, 132.8, 129.9 (2C), 128.0 (2C), 113.7, 73.0, 72.4, 21.7, 18.6; HRMS (ES +ve) Calc'd for $[M+Na]^+$ $C_{12}H_{16}O_4SNa$ 279.0662, found 279.0660.



(*R*)-2-Isopropyledene oxirane (147).

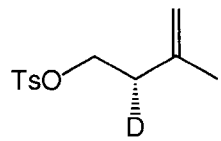
The known compound **147**[240] was prepared using a modified procedure described by Crawford and coworkers.[240] Finely powdered KOH (1.00 g, 17.8 mmol) was added to neat (*S*)-3-methyl-3-butene-1,2-diol-1-yl tosylate (**146**) (325 mg, 1.27 mmol) and heated to 60 °C for 1 h. After cooling to room temperature, the residue was dissolved in Et_2O and filtered through a mini-pad of Celite® to remove solid impurities. This solution was partially characterized and used without further purification in the next reaction. ^1H NMR ($CDCl_3$, 300 MHz) δ 5.18 (m, 1H, C=C$\underline{H}_2$), 5.03 (m, 1H, C=C$\underline{H}_2$), 2.88 (t, 1H, J = 4.2 Hz, C$\underline{H}$CH$_2$), 2.73 (dd, 1H, J = 5.3 Hz, 2.7 Hz, CHC$\underline{H}_2$), 2.47 (m, 1H, CHC$\underline{H}_2$), 1.64 (m, 3H, C=CC$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 141.5, 114.6, 54.5, 46.8, 16.2; HRMS (EI) Calc'd for [M+·] C_5H_8O 84.0575, found 84.0563.



(2*S*)-[2-^{2}H]-Isopentenol (148).

The known alcohol **148**[192-194] was prepared using similar procedure that was described by Poulter and coworkers.[193] To a stirring solution of (*R*)-2-isopropyledene oxirane (**147**) (1.27 mmol), sodium cyanoborodeuteride (167 mg, 2.54 mmol) and a small amount bromocresol green (5 mg) in Et_2O (5 ml) was added dropwise a $BF_3{\cdot}Et_2O$ solution until the solution was observed to be yellow in color. The resulting mixture was stirred at room

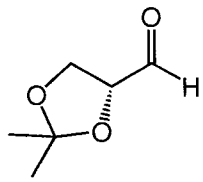
temperature for 5 h and additional amounts of $BF_3 \cdot Et_2O$ were added periodically to keep the solution acidic. The solution was diluted with brine and extracted with Et_2O (3 x 15 mL). The combined ethereal extracts were dried over $MgSO_4$ and evaporation of the solvent *in vacuo* at 0°C gave **148** as a colorless liquid. The crude alcohol was partially characterized and used without further purification for the preparation of (2*S*)-[2-^{2}H]-isopentenyl tosylate (**149**). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.87 (m, 1H, C=C$\underline{H}_2$), 4.79 (m, 1H, C=C$\underline{H}_2$), 3.72 (m, 2H, C$\underline{H}_2$OH), 2.29 (m, 1H, C$\underline{H}$DCH$_2$), 1.76 (m, 3H, C=CC$\underline{H}_3$).



(2*S*)-[2-^{2}H]-Isopentenyl tosylate (149).

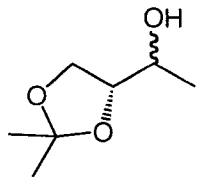
The known tosylate **149**[192,193] was made by a similar procedure described by Poulter and coworkers.[193] A stirring solution of (2*S*)-[2-^{2}H]-isopentenol (**148**) (0.67 mmol) in CH_2Cl_2 (10 mL) was treated with *p*-toluenesulfonyl chloride (193 mg, 1.01 mmol), triethylamine (0.27 mL, 1.91 mmol) and DMAP (10 mg, 8.2 μmol). The resulting mixture was stirred at room temperature for 12 hour, at which time the mixture was diluted with water (10 mL) and extracted with CH_2Cl_2. Combined organic extracts were washed with water, brine dried over $MgSO_4$ and evaporated *in vacuo*. Purification of the crude tosylate using flash column chromatography (SiO_2, hexane:EtOAc, 4:1) afforded **149** as a colorless oil (80 mg, 26% for three steps). $[\alpha]_D^{26}$ = -0.4° (*c* 1.0, CH_2Cl_2); IR (CH_2Cl_2, cast) 3078, 2971, 1652, 1495, 1177 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.79 (m, 2H, Ar), 7.34 (m, 2H, Ar), 4.79 (m, 1H, C=CC$\underline{H}_2$), 4.68 (m, 1H, C=CC$\underline{H}_2$), 4.12 (m, 2H, C$\underline{H}_2$OS), 2.45 (s, 3H,

ArC$\underline{H}_3$), 2.34 (m, 1H, C$\underline{H}$DCH$_2$), 1.66 (m, 3H, C=CC$\underline{H}_3$); ^{2}H NMR ($CHCl_3$, 61.4 MHz) δ 2.36; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 144.8, 140.1. 133.2, 129.8 (2C), 127.9 (2C), 113.2, 66.8, 36.4 (t, J = 19.5 Hz), 22.3, 21.7; HRMS (EI) Calc'd for [M+·] $C_{12}H_{15}DO_3S$ 241.0883, found 241.0877.



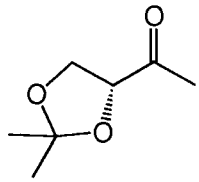
D-Glyceraldehyde acetonide (153).

The known compound **153**[193,241-243] was prepared using an adaptation of the method described by Cardillo and co-workers.[242] Sodium periodate (16.4 g, 76.8 mmol) was added portionwise over 30 minutes at 0 °C to a vigorously stirring solution of D-mannitol-1,2:5,6-bis-acetonide (**152**) (15.5 g, 59.0 mmol) and $NaHCO_3$ (13.0 g, 154 mmol) in CH_2Cl_2 (210 mL) and water (120 mL). After stirring for 2 h at room temperature, the reaction mixture was filtered through a pad of Celite®, and the filtrate was extracted with Et_2O. The combined ethereal extracts were dried over Na_2SO_4 and careful evaporation of solvent afforded **153** as an oil. The aldehyde **153** was partially characterized and used directly for the preparation of (2*R*)-1,2,3-butanetriol-1,2-acetonide (**154**). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.70 (d, 1H, J = 1.9 Hz, C$\underline{H}$CO), 4.38 (ddd, 1H, J = 7.3 Hz, 4.8 Hz, 1.9 Hz, C$\underline{H}$OC), 4.14 (1H, dd, J = 8.8 Hz, 7.3 Hz, C$\underline{H}_2$OC), 4.09 (1H, dd, J = 8.8 Hz, 4.8 Hz, C$\underline{H}_2$OC), 1.47 (s, 3H, C(C$\underline{H}_3$)$_2$), 1.40 (s, 3H, C(C$\underline{H}_3$)$_2$).



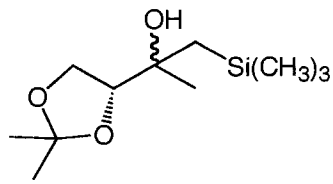
(2*R*)-1,2,3-Butanetriol-1,2-acetonide (154).

The known compound **154**[193,243] was made using a similar procedure described for the preparation of **142**. Methylmagnesium bromide (2.15 mL of a 3.0 M solution in Et_2O, 6.45 mmol) was added dropwise to a 0°C stirring solution of D-glyceraldehyde acetonide (**153**) in Et_2O (15 mL). The ice bath was removed and stirring was continued at room temperature overnight. The reaction mixture was poured onto ice/saturated NH_4Cl solution and the aqueous layer was extracted with Et_2O. The combined ethereal extracts were washed with water, brine, dried over Na_2SO_4 and evaporated. Purification of the crude product using flash column chromatography (SiO_2, pentanes:Et_2O, 10:1) gave **154** as a 1:1 mixture of diastereomers (1.3 g, 39% over two steps). IR (CH_2Cl_2, cast) 3455, 2986, 2886, 1669, 1456, 1214 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.88-4.05 (m, 3H, C$\underline{H}_2$CH, C$\underline{H}$OH), 3.70 (m, 1H, J = 6.2 Hz, 7.9 Hz, C$\underline{H}$OC), 1.93 (b, 1H, O$\underline{H}$) 1.43 (s, 3H, C(C$\underline{H}_3$)$_2$), 1.37 (s, 3H, C(C$\underline{H}_3$)$_2$), 1.16 (d, 3H, J = 6.5 Hz, CHC$\underline{H}_3$), 1.15 (3H, d, J = 6.4 Hz, CHC$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 109.5, 109.1, 80.4, 79.4, 68.8, 66.1, 64.5, 26.7, 26.5, 25.3, 25.2, 18.9, 18.3; HRMS (ES +ve) Calc'd for $[M+Na]^+$ $C_7H_{14}O_3Na$ 169.0835, found 169.0837.



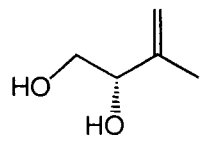
(*R*)-3,4-Dihydroxybutanone acetonide (155).

The known ketone **155**[193,243] was made using a similar procedure described for the preparation of **143**. A solution of *N*-methylmorpholine *N*-oxide (2.07 g, 17.7 mmol) in CH_2Cl_2 (20 mL) was treated with $MgSO_4$ and stirred at room temperature for 20 min. After removal of the drying agent by gravity filtration, molecular sieves were added followed by a solution of (2*R*)-1,2,3-butanetriol-1,2-acetonide (**154**) (1.70 g, 11.8 mmol) in CH_2Cl_2 (10 mL). The resulting solution was stirred for 15 min prior to the addition of tetra-*n*-propylammonium perruthenate (66 mg, 0.19 mmol). The reaction mixture was stirred for 6 h at room temperature at which point the solution was filtered through a plug of silica gel to remove the ruthenium catalyst. The filtrate was washed with a saturated solution of $CuSO_4$, water, and brine. The organic layer was dried over $MgSO_4$ and evaporation of the solvent *in vacuo* gave **155** as a colorless oil (1.70 g, quantitative). IR (CH_2Cl_2, cast) 2990, 2938, 1720, 1420; cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.39 (dd, 1H, J = 7.7 Hz, 5.6 Hz, C<u>H</u>OC), 4.18 (dd, 1H, J = 8.6 Hz, 7.8 Hz, C<u>H</u>$_2$OC), 3.98 (dd, 1H, J = 8.6 Hz, 5.6 Hz, C<u>H</u>$_2$OC), 2.23 (3H, s, C<u>H</u>$_3$CO), 1.48 (s, 3H, C(C<u>H</u>$_3$)$_2$), 1.38 (s, 3H, C(C<u>H</u>$_3$)$_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 209.2, 111.1, 80.5, 66.5, 26.3, 26.1, 25.1; HRMS (EI) Calc'd for [M+·] $C_7H_{12}O_3$ 144.0786, found 144.0781.



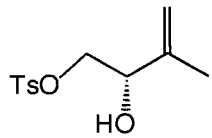
(2*R*)-3-Trimethylsilyl)methyl-1,2,3-butanetriol-1,2-acetonide (156).

The known compound **156**[193,243] was made using a similar procedure described for the preparation of **144**. To a vigorously stirring solution of (*R*)-3,4-dihydroxybutanone acetonide (**155**) (1.82 g, 12.8 mmol) in Et_2O (75 mL) at -78°C was added (trimethylsilyl)methyllithium (21.8 mL of a 1.0 M solution in pentanes, 21.8 mmol) dropwise. The resulting solution was stirred for 1 h at -78°C at which time the acetone/dry ice bath was removed and the reaction was quenched by carefully adding a 25 mL saturated NH_4Cl/NaCl solution. The resulting solution was warmed to room temperature and the aqueous layer was extracted with Et_2O. The combined ethereal extracts were washed with water, brine, dried over $MgSO_4$ and evaporated *in vacuo*. Purification of the crude product using flash column chromatography (SiO_2, pentanes:Et_2O, 10:1) gave **156** as a 1:1 mixture of diastereomers (2.22 g, 75%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.81-3.95 (m, 3H, C<u>H</u>C<u>H</u>$_2$OC), 1.44 (s, 3H, C(C<u>H</u>$_3$)$_2$), 1.38 (s, 3H, C(C<u>H</u>$_3$)$_2$), 1.14 (s, 3H, C<u>H</u>$_3$COH) 1.07 (d, 1H, J = 14.7 Hz, C<u>H</u>$_2$Si), 0.93 (d, 1H, J = 14.7 Hz, C<u>H</u>$_2$Si), 0.87 (d, 1H, J = 14.7 Hz, C<u>H</u>$_2$Si), 0.72 (d, 1H, J = 14.7 Hz, C<u>H</u>$_2$Si), 0.1 (9H, s, C<u>H</u>$_3$Si), 0.07 (9H, s, C<u>H</u>$_3$Si); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 109.4, 109.3, 83.3, 83.2, 72.2, 65.3 (2C), 30.2, 27.4, 26.7, 26.5, 25.5, 25.4, 24.5, 0.6 (3C), 0.5 (3C); HRMS (ES +ve) Calc'd for $[M+Na]^+$ $C_{11}H_{24}O_3SiNa$ 255.1387, found 255.1385.



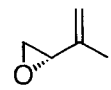
(*S*)-3-Methyl-3-butene-1,2-diol (157).

This known compound **157**[193,243] was made using a similar procedure described for the preparation of **145**. A stirring solution of (2*R*)-3-trimethylsilyl)methyl-1,2,3-butanetriol-1,2-acetonide (**156**) (425 mg, 1.83 mmol) in EtOH (10 mL) was treated with 3N HCl (1.2 mL) and the mixture was refluxed for 2 h. After cooling to room temperature the solution was neutralized by the slow addition of solid $NaHCO_3$. The precipitated solids were filtered, washed with EtOH, and the filtrate was concentrated *in vacuo*. The resulting residue was re-dissolved in MeOH and treated with Et_2O (50 mL). The precipitated solids were filtered and the solvent was concentrated *in vacuo*, which afforded **157** as a colorless oil (170 mg, 89%). IR (CH_2Cl_2, cast) 3373, 3075, 2970, 2879, 1652, 1444; cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.06 (m, 1H, C=C$\underline{H}_2$), 4.96 (m, 1H, C=C$\underline{H}_2$), 4.18 (dd, 1H, J = 7.2 Hz, 3.4 Hz, C$\underline{H}$OH), 3.70 (dd, 1H, J = 11.2 Hz, 3.5 Hz, C$\underline{H}_2$OH), 3.55 (dd, 1H, J = 11.2 Hz, 7.3 Hz, C$\underline{H}_2$OH), 2.58 (b, 2H, O$\underline{H}$), 1.75 (m, 3H, C=CC$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 144.1, 112.0, 75.7, 65.2, 18.9; HRMS (EI) Calc'd for [M+·] $C_5H_{10}O_2$ 102.0681, found 102.0680.



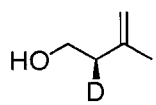
(*S*)-3-Methyl-3-butene-1,2-diol-1-yl tosylate (158).

This known compound **158**[193,243] was made using a similar procedure decribed for the preparation of **146**. To a stirring solution of (*S*)-3-methyl-3-butene-1,2-diol (**157**) (400 mg, 3.92 mmol) in dry pyridine (10 mL) was added a solution of *p*-toluenesulfonyl chloride (1.57 g, 8.23 mmol) in pyridine (5 mL). The resulting mixture was stirred at 4 °C for 18 h, after which time it was poured onto a mixture of ice-water and extracted with Et_2O (3 x 15 mL). The combined ethereal extracts were washed with 1N HCl, water, brine, dried over $MgSO_4$ and evaporation *in vacuo*. Purification of the crude product by flash column chromatography (SiO_2, pentanes:Et_2O, 10:1) gave **158** as a colorless oil (781 mg, 78%). $[\alpha]_D^{26} = +12.0°$ (*c* 1.2, CH_2Cl_2). IR (CH_2Cl_2, cast) 3533, 3069, 2951, 2921, 1652, 1495, 1190 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.81 (d, 2H, *J* = 8.3 Hz, Ar), 7.36 (d, 2H, *J* = 8.6 Hz, Ar), 5.06 (m, 1H, C=CH_2), 4.97 (m, 1H, C=CH_2), 4.31 (dd, 1H, *J* = 7.6 Hz, 3.2 Hz, CHOH), 4.12 (dd, 1H, *J* = 10.6 Hz, 3.3 Hz, CH_2OS), 3.97 (dd, 1H, *J* = 10.3 Hz, 7.7 Hz, CH_2OS), 2.46 (s, 3H, ArCH_3), 2.06 (b, 1H, OH) 1.70 (m, 3H, C=CCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 145.1, 142.0, 132.8, 129.9, 128.0, 113.7, 73.0, 72.4, 21.7, 18.6; HRMS (EI) [M^+] Calc'd for $C_{12}H_{16}O_4S$ 256.0769, found 256.0770



(*S*)-2-Isopropyledene oxirane (159).

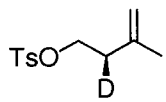
The known compound **159**[193,240,243] was made using a similar procedure described for the preparation of **147**. Finely powdered KOH (1.0 g, 17.8 mmol) was added to neat (*S*)-3-methyl-3-butene-1,2-diol-1-yl tosylate (**158**) (325 mg, 1.27 mmol) and heated to 60°C for 1h. After cooling to room temperature, the residue was dissolved in Et_2O and filtered through a mini-pad of Celite® to remove solid impurities. Careful evaporation of solvent *in vacuo* afforded **159** as an oil, which was partially characterized and used without further purification for the preparation of (2*R*)-[2-^{2}H]isopentenol (**160**). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.18 (m, 1H, C=CH_2), 5.03 (m, 1H, C=CH_2), 2.88 (dd, 1H, J = 5.3 Hz, 4.2 Hz, C$\underline{H}_2$OC), 2.73 (dd, 1H, J = 5.3 Hz, 2.7 Hz, C$\underline{H}_2$OC), 2.47 (m, 1H, C$\underline{H}$OC), 1.64 (m, 3H, C=CC$\underline{H}_3$);



(2*R*)-[2-^{2}H]-Isopentenol (160).

The known compound **160**[193,194] was made using a similar procedure described for the preparation of **148**. To a stirring solution of (*S*)-2-isopropyledene oxirane (**159**), sodium cyanoborodeuteride (103 mg, 1.56 mmol) and a small amount bromocresol green (5 mg) in Et_2O (5 ml) was added dropwise a $BF_3{\cdot}Et_2O$ solution until the solution was observed to be yellow in color. The resulting mixture was stirred at room temperature for 5 h and additional amounts of $BF_3{\cdot}Et_2O$ were added periodically to keep the solution acidic. The

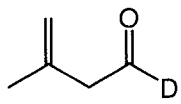
solution was diluted with brine and extracted with Et_2O (3 x 15 mL). The combined ethereal extracts were dried over $MgSO_4$ and evaporation of the solvent *in vacuo* at 0°C gave **160** as a colorless liquid. Crude alcohol was partially characterized and used without further purification for the preparation of (2*R*)-[2-^{2}H]-isopentenyl tosylate (**161**). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.87 (1H, m, C=C$\underline{H}_2$), 4.79 (1H, m, C=C$\underline{H}_2$), 3.71 (2H, m, C$\underline{H}_2$OH), 2.28 (1H, m, C$\underline{H}$DCH$_2$), 1.76 (3H, m, C=CC$\underline{H}_3$).



(2*R*)-[2-^{2}H]-Isopentenyl tosylate (161).

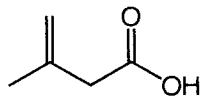
The known tosylate **161**[192,193] was made using a similar procedure described for the preparation of **149**. A stirring solution of (2*R*)-[2-^{2}H]-isopentenol (**160**) and *p*-toluenesulfonyl chloride (193 mg, 1.01 mmol) in CH_2Cl_2 (5 mL) was treated with DMAP (136 mg, 1.11 mmol). The resulting mixture was stirred at room temperature for 12 hour, at which time Et_2O (50 mL) was added. Precipitated solids were removed by filtration, and the filtrate was concentrated *in vacuo*. Purification of the crude tosylate by flash column chromatography (SiO_2, hexane:EtOAc, 10:1) afforded **161** as an oil (125 mg, 41% for three steps from **158**). IR (CH_2Cl_2, cast) 3077, 2924, 2200, 1652, 1598, 1364, 1120 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.79 (m, 2H, $\underline{Ar}$), 7.34 (2H, m, $\underline{Ar}$), 4.79 (m, 1H, C=C$\underline{H}_2$), 4.68 (m, 1H, C=C$\underline{H}_2$), 4.12 (m, 2H, C$\underline{H}_2$OS), 2.45 (s, 3H, ArC$\underline{H}_3$), 2.34 (m, 1H, C$\underline{H}$DCH$_2$), 1.66 (m, 3H, C=CC$\underline{H}_3$); ^{2}H NMR ($CHCl_3$, 61.4 MHz) δ 2.34; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.8, 140.2, 134.1, 130.1 (2C), 127.6 (2C) 113.1, 68.3, 36.5 (t, *J* =

19.6 Hz), 22.4, 21.7; HRMS (EI) Calc'd for [M+] $C_{12}H_{15}DO_3S$ 241.0883, found 241.0871.



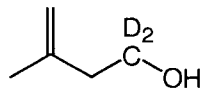
[1-^{2}H] 3-Methylbut-3-en-1-al (162).

The known aldehyde **162**[203] was prepared using the following procedure. To a stirring solution of IBX (4.88 g, 17.4 mmol) in DMSO (40 mL) was added [1,1-$^2H_2$]-3-methyl-3-buten-1-ol (**169**) (500 mg, 5.67 mmol) and stirred at room temperature for 18 h. The solution was cooled to 0 °C and added 5 mL H_2O and stirred for an additional 5 min at 0 °C. Filtered suspension through a pad of Celite® and the filtrate was extracted exhaustively with Et_2O. The combined ethereal extracts were washed with brine, dried over $MgSO_4$ and evaporated *in vacuo* to afford the aldehyde **162** as colorless oil. The crude aldehyde was used immediately for the preparation of **170** and **171** as it is very unstable to heat or base, and isomerization to the conjugated aldehyde is observed during storage. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.00 (m, 1H, C=C$\underline{H}_2$), 4.84 (m, 1H, C=C$\underline{H}_2$), 3.06 (s, 2H, C$\underline{H}_2$CDO), 1.78 (m, 3H, C=CC$\underline{H}_3$).



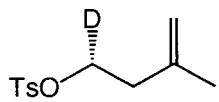
3-Methyl-3-butenoic acid (168).

The known comound **168**[244] was prepared using a method described by Wang and coworkers.[244] To a flame dried, argon flushed round-bottom flask were added freshly ground magnesium turnings (4.98 g, 205 mmol) and THF (75 mL). To this suspension a solution of 3-chloro-2-methyl-propene (**91**) (10.0 g, 221 mmol) in THF (25 mL) was then added dropwise, and a crystal of iodine. After the addition was complete the reaction mixture was refluxed for 2 h and cooled to room temperature. The reaction mixture was stirred for a further 30 min and then cooled to -78 °C with a dry ice/acetone bath. At this point CO_2 gas was bubbled though the solution for 1 h, and then the temperature was slowly allowed to increase to 0 °C by removal of the ice bath. Once the reaction reached 0 °C, it was cooled to -78 °C with bubbling of CO_2 through solution for ten minutes. Then the reaction mixture was allowed to warm to 10 °C. The solution was adjusted to pH 10 with cold 2 M NaOH and extracted with Et_2O (3 x 50 mL). The aqueous layer was then acidified with cold 4 M HCl to pH 2 and extracted with Et_2O (3 x 75 mL). The combined ethereal extracts were washed with brine, dried oved $MgSO_4$ and evaporated to give **168** as a colorless oil (4.0 g, 39%). IR (CH_2Cl_2, cast) 3083, 2978, 1711, 1651, 1413 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 10.0 (b, 1H, $CO_2\underline{H}$), 4.97 (m, 1H, $C{=}C\underline{H}_2$), 4.90 (m, 1H, $C{=}C\underline{H}_2$), 3.09 (d, 2H, J = 1.0 Hz, $C\underline{H}_2$), 1.85 (m, 3H, $C{=}CC\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.7, 137.9, 115.3, 43.1, 22.4; HRMS (EI) Calc'd for [M+·] $C_5H_8O_2$ 100.0524, found 100.0521.



[1,1-$^2H_2$]-3-Methyl-3-buten-1-ol (169).

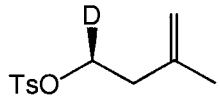
The known alochol **169**[203] was made using a modified procedure described by Cane *et al.*.[203] A solution of 3-methyl-3-butenoic acid (**168**) (1.00 g, 10.0 mmol) in Et_2O (15 mL) was treated with a solution of lithium aluminum deuteride (5.0 ml of 1.0 **M** solution in Et_2O, 5.0 mmol) at 0°C. The resulting reaction mixture was stirred with warming to room temperature for 4 h. The reaction was quenched by the addition of solid $Na_2SO_4 10H_2O$. Solid material was removed by filtration and filtrate was concentrated *in vacuo* to afford alcohol **169** (0.88 g, 70%). IR (CH_2Cl_2, cast) 3346, 3076, 2917, 2849, 2208, 1651, 1455 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.86 (m, 1H, C=CC$\underline{H}_2$), 4.78 (m, 1H, C=CC$\underline{H}_2$), 2.28 (s, 2H, C$\underline{H}_2CD_2$), 1.75 (m, 3H, C=CC$\underline{H}_3$); ^2H NMR ($CHCl_3$, 61.4 MHz) δ 3.68; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.2, 112.7, 59.5 (qn, $^1J_{C-D}$ = 22 Hz), 40.7, 22.2; HRMS (EI) Calc'd for [M+·] $C_5H_8D_2O$ 88.0857, found 88.0854.



(1*R*)-[1-^{2}H]Isopentenyl tosylate (170).

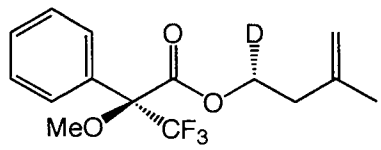
The tosylate **170** was prepared using a similar procedure described by Vederas and coworkers.[213] To a stirring solution of [1-$^2H_1$] 3-methylbut-3-en-1-al (**162**) (5.67 mmol) in Et_2O (10 mL) at 0 °C was added a solution of (*S*)-Alpine-Borane® (13.6 mL, 6.80 mmol) dropwise over 20 min. After the addition was complete, the reaction mixture was warmed to room temperature and stirred for 18 h. Acetaldehyde (0.38 mL, 0.79 mmol) was added, and the mixture was stirred for 5 minutes, at which time the volatiles were removed by evaporation (40 °C, 0.05 mm Hg). The resulting residue was dissolved in Et_2O and was

treated with ethanolamine (0.41 mL, 6.80 mmol) and filtered. Filtrate was concentrated and purification of the crude alcohol by flash column chromatography (SiO_2, pentanes:Et_2O, 4:1) afforded the alcohol **172**. The alcohol **172** was partially characterized and used in next step. ^1H NMR ($CDCl_3$, 300 MHz) δ 4.87 (m, 1H, C=C$\underline{H}_2$), 4.79 (m, 1H, C=C$\underline{H}_2$), 3.71 (m, 1H, C$\underline{H}$DOH), 2.29 (d, 2H, J = 6.5 Hz, C$\underline{H}_2$CHD), 1.56 (3H, m, C=CC$\underline{H}_3$). A solution of the crude alcohol **172** in CH_2Cl_2 (10 mL) was treated with *p*-toluenesulfonyl chloride (1.19 g, 6.23 mmol) and DMAP (832 mg, 6.80 mmol). The resulting reaction mixture was stirred at room temperature for 18 h and solid material was removed by filtration. Concentration of filtrate *in vacuo* gave the crude tosylate, which was purified using flash column chromatography (SiO_2, hexane:EtOAc, 10:1) to afford **170** as a colorless oil (131 mg, 10%). $[\alpha]_D^{26}$ = +0.9 ° (*c* 1.5, CH_2Cl_2); IR (CH_2Cl_2, cast) 2927, 1649, 1364, 1177 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.76 (m, 2H, $\underline{Ar}$), 7.32 (m, 2H, $\underline{Ar}$), 4.76 (m, 1H, C=C$\underline{H}_2$), 4.65 (m, 1H, C=C$\underline{H}_2$), 4.11 (tt, 1H, J = 6.9 Hz, 1.2 Hz C$\underline{H}$DOS), 2.45 (s, 3H, ArC$\underline{H}_3$), 2.32 (d, 2H, J = 6.9 Hz, C$\underline{H}_2$CHD), 1.63 (m, 3H, C=CC$\underline{H}_3$); ^2H NMR ($CHCl_3$, 76.5 MHz) δ 4.11; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.7, 140.2, 133.3, 129.9 (2C), 128.0 (2C), 113.1, 68.3 (t, J = 22.9 Hz), 36.7, 22.4, 21.7; HRMS (ES +ve) Calc'd for $[M+Na]^+$ $C_{12}H_{15}DO_3SNa$ 264.0775, found 264.0773.



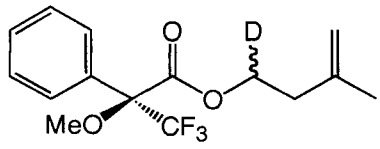
(1*S*)-[1-^{2}H]Isopentenyl tosylate (171).

A similar procedure was employed as that described for the preparation of **170**. Reaction of [1-^{2}H] 3-methylbut-3-en-1-al (**162**) (5.67 mmol) and (*R*)-Alpine-Borane® (13.6 mL, 6.80 mmol) gave the crude alcohol. Purification of the crude product using flash column chromatography (SiO_2, Pentanes:Et_2O, 4:1) gave alcohol. A solution of the alcohol in CH_2Cl_2 (10 mL) was reacted with *p*-toluenesulfonyl chloride (1.19 g, 6.23 mmol) and DMAP (832 mg, 6.80 mmol) to give crude tosylate. Purification using flash chromatography (SiO_2, hexane:EtOAc, 4:1) afforded **171** as an oil (131 mg, 10% over three steps). $[\alpha]_D^{26}$ = -1.8 ° (*c* 0.5, CH_2Cl_2); IR (CH_2Cl_2, cast) 3078, 2926, 2200, 1653, 1598, 1365 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.76 (2H, m, Ar), 7.32 (2H, m, Ar), 4.76 (m, 1H, C=CH_2), 4.65 (m, 1H, C=CH_2), 4.11 (tt, 1H, *J* = 7.0 Hz, 1.2 Hz, CHDOS), 2.45 (s, 3H, ArCH_3), 2.32 (d, 2H, *J* = 6.8 Hz, CHDCH_2), 1.63 (m, 3H, C=CCH_3); ^{2}H NMR ($CHCl_3$, 76.5 MHz) δ 4.11; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.8, 140.1, 133.3, 129.9 (2C), 128.0 (2C), 133.1, 68.3 (t, $^1J_{C\text{-}D}$ = 23.0 Hz), 36.7, 22.4, 21.7; HRMS (ES +ve) Calc'd for $[M+Na]^+$ $C_{12}H_{15}DO_3SNa$ 264.0775, found 264.0773.



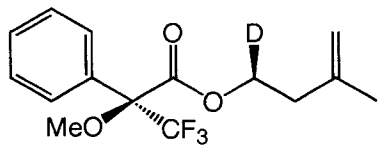
(2*R*)-3,3,3-Trifluoro-2-methoxy-2-phenylpropionic acid (1*R*)-[1-$^2H_1$]-3-methyl-but-3-enyl ester (174).

To a solution of (1*R*)-[1-^{2}H]-3-methylbut-3-en-1-ol (**172**) (0.01 mL, 0.1 mmol) in pyridine (1 mL) at 0 °C was added (*R*)-(-)-α-methoxy-α(-trifluoromethyl)phenylacetyl chloride (0.037 mL, 0.20 mmol). The resulting mixture was stirred with warming to room temperature for 1 h. The solution was diluted with water and extracted with Et_2O. Combined ethereal extracts were washed with a saturated solution of $CuSO_4$, water, brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by preparative chromatography (SiO_2, hexane:EtOAc, 25:1) to afford **174** as an oil (13 mg, 43%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.48-7.52 (m, 2H, Ar), 7.34-7.40 (m, 3H, Ar), 4.78 (1H, m, C=CH_2), 4.70 (1H, m, C=CH_2), 4.43 (dd, 1H, J = 6.8 Hz, 1.3 Hz, CHDOCO), 3.52 (m, 3H, OCH_3), 2.39 (d, 2H, J = 6.8 Hz, CH_2CHD), 1.72 (s, 3H, C=CCH_3); ^{2}H NMR (C_6H_6, 76.5 MHz) δ 4.06. HRMS (EI) Calc'd for [M+] $C_{15}H_{16}F_3O_3D$ 303.1192, found 303.1198.



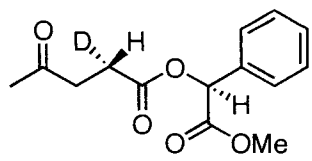
(2*R*)-3,3,3-Trifluoro-2-methoxy-2-phenylpropionic acid [1-$^2H_1$]-3-methyl-but-3-enyl ester.

A similar procedure was employed as that described for the preparation of **174**. To a solution of [1-$^2H_1$]-3-methylbut-3-en-1-ol (**169**) (0.01 mL, 0.010 mmol) in pyridine (1 mL) at 0 °C was added (*R*)-(-)-α-methoxy-α(-trifluoromethyl)phenylacetyl chloride (0.037 mL, 0.20 mmol). The resulting mixture was stirred with warming to room temperature for 1 h. The solution was diluted with water and extracted with Et_2O. Combined ethereal extracts were washed with a saturated solution of $CuSO_4$, water, brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by preparative chromatography (SiO_2, hexane:EtOAc, 25:1) to afford the ester as an oil (11.5 mg, 39%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.49-7.52 (m, 2H, Ar), 7.36-7.39 (m, 3H, Ar), 4.79 (m, 1H, C=CH_2), 4.70 (m, 1H, C=CH_2), 4.42 (m, 1H, CHDOCO), 3.52 (s, 3H, OCH_3), 2.39 (m, 2H, CH_2CHD), 1.72 (s, 3H, C=CCH_3); ^{2}H NMR (C_6H_6, 76.5 MHz) δ 4.05, 4.02; ^{13}C NMR ($CDCl_3$, 300 MHz) δ 166.5, 129.5, 128.3, 127.3, 112.9 (2C), 84.7, 64.6, 64.3 (t, $^1J_{C\text{-}D}$ = 23.0 Hz), 55.4 (2C), 36.4, 36.3, 22.3; HRMS (EI) Calc'd for [M+] $C_{15}H_{16}F_3O_3D$ 303.1192, found 303.1179.



(2*R*)-3,3,3-Trifluoro-2-methoxy-2-phenylpropionic acid (1*S*)-[1-$^2H_1$]-3-methyl-but-3-enyl ester.

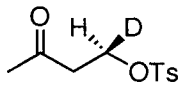
A similar procedure was employed as that described for the preparation of **174**. Reaction of (1*S*)-[1-^{2}H] 3-methylbut-3-en-1-ol[203] (0.01 mL, 0.1 mmol) in pyridine (1 mL), with (*R*)-(-)-α-methoxy-α(-trifluoromethyl)phenylacetyl chloride (0.021 mL, 0.20 mmol) gave the crude ester. Purification using preparative chromatography (SiO_2, hexane:EtOAc, 25:1) afforded the ester as an oil (12 mg, 40%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.51-7.56 (m, 2H, Ar), 7.38-7.44 (m, 3H, Ar), 4.79 (m, 1H, C=C$\underline{H}_2$), 4.71 (m, 1H, C=C$\underline{H}_2$), 4.42 (dd, 1H, J = 6.8 Hz, 1.5 Hz, C$\underline{H}$DOCO), 3.53 (s, 3H, OC$\underline{H}_3$), 2.39 (d, 2H, J = 6.8 Hz, C$\underline{H}_2$CHD), 1.72 (s, 3H, C=CC$\underline{H}_3$); ^{2}H NMR (C_6H_6, 76.5 MHz) δ 4.02; HRMS (EI) Calc'd for [M+] $C_{15}H_{16}F_3O_3D$ 303.1192, found 303.1198.



(2*S*)-(+)-(Methoxycarbonyl)benzyl (2*S*)-[2-$^2H_1$]-levulinate (178).

To a stirring solution of (2*S*)-[2-$^2H_1$]-levulinic acid (**182**) (18 mg, 0.15 mmol) in CH_2Cl_2 (5 mL) was added (*S*)-(+)-mandelic acid methyl ester (**175**) (28 mg, 0.17 mmol), DCC (39 mg, 0.19 mmol) and DMAP (2 mg, 0.02 mmol). The resulting reaction mixture was

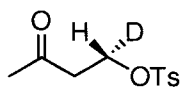
stirred at room temperature for 18 h and filtered through a sintered glass funnel to remove solid impurities. The crude ester was purified by flash column (SiO_2, hexane:EtOAc, 4:1) to afford (*S*)-(+)-(methoxycarbonyl)benzyl (2*S*)-[2-$^2H_1$]-levulinate **178** (16 mg, 41%) as a colorless oil. For unlabeled material: IR (CH_2Cl_2, cast) 3034, 2954, 1745, 1719, 1587, 1364, 1152 cm^{-1}; ^1H NMR (C_6D_6, 300 MHz) δ 7.42 (m, 2H, Ar), 7.04 (m, 3H, Ar), 6.05 (s, 1H, C<u>H</u>CO_2Me), 3.18 (s, 3H, OC<u>H</u>$_3$), 2.50 (m, 2H, J = 7.0 Hz, C<u>H</u>$_2CO_2$R), 2.21 (td, 1H, J = 6.3 Hz, 18.2 Hz, C<u>H</u>$_2$CO), 2.01 (td, 1H, J = 6.3 Hz, 18.2 Hz, C<u>H</u>$_2$CO), 2.01 (s, 3H, C<u>H</u>$_3$CO); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 206.1, 172.1, 169.2, 133.7, 129.3, 128.8 (2C), 127.6 (2C), 74.6, 52.6, 37.8, 29.8, 27.8; HRMS (EI) Calc'd for [M+·] $C_{14}H_{16}O_5$ 264.0998, found 264.0989. The (*S*)-(+)-(methoxycarbonyl)benzyl (2*S*)-[2-$^2H_1$]-levulinate **178** showed identical chromatographic properties and displayed similar spectral properties except for the following: ^2H NMR (C_6H_6, 76.5 MHz) δ 2.48.



(4*R*)-[4-$^2H_1$]-2-Butanone-4-yl tosylate (180).

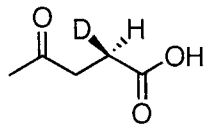
The methyl ketone **180** was prepared using the method of Sih and coworkers.[245] (1*R*)-[1-^{2}H]-Isopentenyl tosylate (**170**) (200 mg, 0.832 mmol) was dissolved in CH_2Cl_2 (10 mL) and cooled to -78 °C in a dry ice/acetone bath. Through this solution was bubbled O_3 for 20 minutes at which time the solution was purple in color. The excess O_3 was removed by bubbling O_2 through solution for 5 min. The solution was warmed to room temperature and then zinc (109 mg, 1.67 mmol) was added, followed by acetic acid (0.26

mL, 4.50 mmol) and stirred at room temperature for 2 h. The reaction mixture was diluted with H_2O and extracted with Et_2O. The combined ethereal extracts washed with saturated solution of $NaHCO_3$, water, brine, dried over $MgSO_4$ and evaporated *in vacuo* to give the crude tosylate **180** (195 mg, 97%). This was used without purification for the preparation of (2*S*)-[2-$^2H_1$]-levulinic acid (**182**). For unlabeled material: IR (CH_2Cl_2, cast) 2921, 1719, 1359, 1176 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.77 (m, 2H, Ar), 7.33 (m, 2H, Ar), 4.24 (t, 2H, J = 6.4 Hz, CH_2OS), 2.81 (t, 2H, J = 6.4 Hz, CH_2CO), 2.43 (s, 3H, $ArCH_3$) 2.13 (s, 3H, CH_3CO); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 204.3, 144.9, 132.7, 129.9, 128.0, 64.9, 42.2, 30.3, 21.7; HRMS (EI) Calc'd for [M+·] $C_{11}H_{14}O_4S$ 242.0613, found 242.0619. The (4*R*)-[4-$^2H_1$]-2-butanone-4-yl tosylate showed identical chromatographic properties and displayed similar spectral properties except for the following: ^1H NMR ($CDCl_3$, 300 MHz) δ 7.77 (m, 2H, Ar), 7.33 (m, 2H, Ar), 4.24 (m, 1H, $CHDCH_2$), 2.82 (d, 2H, J = 6.3 Hz, CH_2CHD), 2.43 (s, 3H, $ArCH_3$) 2.13 (s, 3H, CH_3CO); ^2H NMR (C_6H_6, 76.5 MHz) δ 4.24.



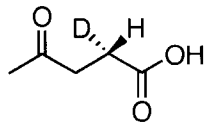
(4*S*)-[4-$^2H_1$]-2-Butanone-4-yl tosylate (181).

Substitution of (1*S*)-[1-^2H]-isopentenyl tosylate (**171**) (62 mg, 0.26 mmol) for (1*R*)-[1-^2H]-isopentenyl tosylate **170** in the procedure used above to prepare the enantiomeric tosylate **180** gave the desired compound **181** (60 mg, 95%). Chromatographic properties and spectral data were identical.



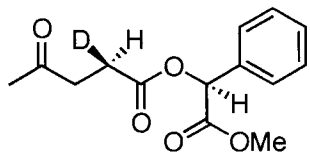
(2*S*)-[2-$^2H_1$]-Levulinic acid (182).

A stirring solution of (4*R*)-[4-$^2H_1$]-2-butanone-4-yl tosylate (**180**) (92 mg, 0.38 mmol) in DMSO (5 mL) was treated with sodium cyanide (56 mg, 1.1 mmol) and stirred at 60 °C for 12 h. The reaction mixture was cooled to room temperature and extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried over $MgSO_4$ and evaporated *in vacuo*. To the crude nitrile was added 6 N HCl (1.5 mL, 1.5 mmol) and the resulting reaction mixture heated to reflux for 5 h. The reaction mixture was cooled to room temperature and extracted with CH_2Cl_2. Combined organic extracts were washed with brine, dried over $MgSO_4$ and evaporated *in vacuo* to give crude acid **182** (15 mg, 34%). This was used without further purification for the preparation of (*S*)-(+)-(methoxycarbonyl)benzyl (2*S*)-[2-$^2H_1$]-levulinate (**178**). For unlabeled material: IR (CH_2Cl_2, cast) 3111, 1716, 1402, 1369, 1165 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.73 (m, 2H, C<u>H</u>$_2$CO), 2.61 (m, 2H, C<u>H</u>$_2$COOH), 2.17 (s, 3H, C<u>H</u>$_3$CO); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 206.9, 178.4, 37.7, 29.7, 27.8; HRMS (EI) Calc'd for [M+·] $C_5H_8O_3$ 116.0474, found 116.0472. The (2*S*)-[2-$^2H_1$]-levulinic acid **182** showed identical chromatographic and spectral properties except for the following: ^2H NMR (61.4 MHz C_6H_6) δ 2.61.



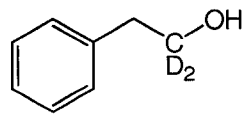
(2*R*)-[2-$^2H_1$]-Levulinic acid (183).

Substitution of (4*S*)-[4-$^2H_1$]-2-butanone-4-yl tosylate (**181**) (77 mg, 0.32 mmol) in the procedure above was used to prepare the enantiomeric acid **183** (15 mg, 41%). Chromatographic properties and spectral data were identical.



(*S*)-(+)-(Methoxycarbonyl)benzyl (2*R*)-[2-$^2H_1$]-levulinate (184).

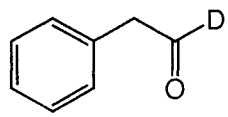
A procedure similar to that used for the preparation of (*S*)-(+)-(Methoxycarbonyl)benzyl (2*S*)-[3-$^2H_1$]-levulinate (**178**) was employed except that (2*R*)-[2-$^2H_1$]-levulinic acid (**183**) (15 mg, 0.13 mmol) was used as the starting material to give **184** as an oil (15 mg, 44%). Spectral data was similar to those of **178** except for ^2H NMR (C_6H_6, 76.5 MHz) δ 2.44.



[1,1-$^2H_2$]-Phenylethanol (188).

A solution of lithium aluminum deuteride (12.3 ml of 1.0 M solution in Et_2O, 12.3 mmol) was added dropwise a stirring solution of phenylacetic acid (**187**) (5.00 g, 36.7 mmol) in

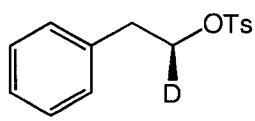
Et_2O at 0 °C. The ice bath was removed and stirring was continued for 3 h at room temperature. The solution was then cooled to 0 °C and quenched by the slow addition of solid sodium sulphate decahydrate and the resulting suspension was stirred for 30 minutes. The precipitated aluminum salts were removed by filtration and the filtrate was concentrated to give **188** as a colorless liquid (1.25 g, 27%) IR (CH_2Cl_2, cast) 3311, 3086, 3062, 3028, 2932, 2213, 1603, 1496 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.36-7.21 (5H, m, Ar), 2.87 (2H, s, CH_2); ^2H NMR ($CHCl_3$, 76.5 MHz) δ 3.86; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.4, 129.0 (2C), 128.6 (2C), 126.5, 63.0 ($^1J_{C\text{-}D}$ = 23 Hz), 39.0; HRMS (EI) Calc'd for [M+·] $C_8H_8D_2O$ 124.0855, found 124.0857.



[1-$^2H_1$]-Phenylethanal (189).

To a homogenous stirring solution of IBX, 1-hydroxy-1,2-benziodoxol-3(1H)-one 1-oxide (1.69 g, 6.05 mmol) in DMSO (15 mL) was added [1,1-$^2H_2$]-phenylethanol (**188**) (500 mg, 4.03 mmol) and the resulting reaction mixture was stirred at room temperature for 4 h. The solution was then cooled to 0 °C and water (5 mL) was added. Stirring was continued for ten additional minutes at which point the mixture was filtered through a pad of Celite® and filtrate was extracted with Et_2O. The combined ethereal extracts were washed with water, brine, dried over $MgSO_4$ and evaporated to afford **189** as a colorless oil (450 mg, 91%). The crude aldehyde was only partially characterized as it is unstable at room temperature. Data for unlabeled material: IR (CH_2Cl_2, cast) 3086, 3062, 3028, 1723, 1603, 1453 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 9.76 (t, 1H, J = 2.4 Hz, CHO),

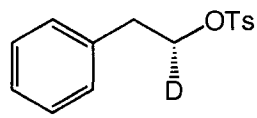
7.42-7.21 (m, 5H, Ar), 3.70 (d, 2H, J = 2.4 Hz, CH_2CHO); HRMS (EI) Calc'd for [M+] C_8H_8O 120.0575, found 120.0574. The labeled aldehyde **189** had similar data that of the unlabeled material except for the following: ^1H NMR ($CDCl_3$, 300 MHz) δ 7.21-7.42 (5H, m, Ar), 3.70 (2H, s, CH_2).



(1*S*)-[1-^{2}H]-Phenylethan-1-yl tosylate (190).

The compound **190** was prepared using a variation of the method of Brown *et al.*.[246] An oven dried 100 mL round bottom flask was charged with (-)-β-chlorodiisopinocampheylborane (1.55 g, 4.84 mmol) in an inert atmosphere and dissolved in THF (10 mL). The solution was cooled to -78 °C and to this cold solution was added dropwise a solution of [1-^{2}H]-phenylethanal (**189**) (4.03 mmol) and stirred with warming to room temperature for 18 h. The solvent was removed *in vacuo* and α-pinene was removed by high vacuum for 8 h. The residue was dissolved in Et_2O and to this stirring solution was added diethanolamine (1.02 ml, 10.7 mmol). The resulting precipitate was removed by filtration through a pad of Celite® and filtrate was concentrated *in vacuo*. Purification of the crude alcohol using flash column chromatography (SiO_2, hexane:EtOAc, 4:1) gave (1*S*)-[1-^{2}H]-phenylethanol as a colorless oil (320 mg, 65%). IR (CH_2Cl_2, cast) 3335, 3085, 3062, 2932, 2158, 1603, 1496 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.29-7.36 (m, 2H, Ar), 7.21-7.27 (m, 3H, Ar), 3.86 (dt, 1H, J = 6.6 Hz, 1.5 Hz, $CHDCH_2$), 2.88 (d, 2H, J = 6.3 Hz, CH_2CHD); ^{13}C NMR

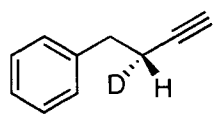
($CDCl_3$, 125 MHz) δ 138.5, 129.0 (2C), 128.6 (2C), 126.5, 63.3 (t, $^1J_{C\text{-}D}$ = 22 Hz), 39.1; HRMS (EI) Calc'd for [M+·] C_8H_9DO 123.0793, found 123.0795. The reaction of the alcohol (107 mg, 0.883 mmol) in CH_2Cl_2 (10 mL) with *p*-toluenesulfonyl chloride (202 mg, 1.06 mmol), triethylamine (0.16 mL, 1.16 mmol) and DMAP (10 mg, 0.08 mmol) provided the crude tosylate. Purification by flash column chromatography (SiO_2, hexane:EtOAc, 10:1) afforded **190** as an oil (184 mg, 75%). IR (CH_2Cl_2, cast) 3029, 2924, 2193, 1597, 1362, 1178 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.68 (m, 2H, Ar), 7.19-7.29 (m, 5H, Ar), 7.09 (2H, m, Ar), 4.18 (m, 1H, C<u>H</u>DCH$_2$), 2.93 (d, 2H, *J* = 7.1 Hz, C<u>H</u>$_2$CHD), 2.41 (s, 3H, ArC<u>H</u>$_3$); ^2H NMR (CH_2Cl_2, 76.5 MHz) δ 4.23; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 144.6, 136.2, 133.0, 129.8 (2C), 128.8 (2C), 128.6 (2C), 126.8 (2C), 126.9, 70.3, (t, $^1J_{C\text{-}D}$ = 23 Hz), 35.3, 21.6; HRMS (EI) Calc'd for [M+·] $C_{15}H_{15}DO_3S$ 277.0883, found 277.0884.



(1*R*)-[1-^{2}H]-Phenylethan-1-yl tosylate (191).

A similar procedure was employed as that described for the preparation of **190**. Treatment of [1-^{2}H]-phenylethanal (**189**) (4.03 mmol) in THF (10 mL) with (+)-β-chlorodiisopinocampheylborane (1.55 g, 4.84 mmol) gave the crude alcohol. Purification using flash column chromatography (SiO_2, hexane:EtOAc, 4:1) afforded (1*R*)-[1-^{2}H]-phenylethanol as a colorless oil (200 mg, 40%). IR (CH_2Cl_2, cast) 3335, 3085, 3062, 2932, 2158, 1603, 1496 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.29-7.36 (m, 2H, Ar), 7.21-

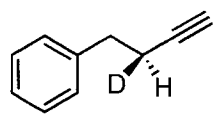
7.27 (m, 3H, Ar), 3.86 (dt, 1H, J = 6.6 Hz, 1.5 Hz, C<u>H</u>DCH$_2$), 2.88 (d, 2H, J = 6.3 Hz, C<u>H</u>$_2$CHD); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 138.5, 129.0 (2C), 128.6 (2C), 126.5, 63.3 (t, $^1J_{C\text{-}D}$ = 22 Hz), 39.1; HRMS (EI) Calc'd for [M+·] C_8H_9DO 123.0793, found 123.0795. The reaction of alcohol (250 mg, 2.06 mmol) in CH_2Cl_2 (15 mL) with *p*-toluenesulfonyl chloride (471 mg, 2.47 mmol), triethylamine (0.38 mL, 2.72 mmol) and DMAP (10 mg, 0.08 mmol) provided the crude tosylate. Purification by flash column chromatography (SiO_2, hexane:EtOAc, 10:1) afforded **191** as an oil (490 mg, 86%). IR (CH_2Cl_2, cast) 3030, 2925, 2195, 1653, 1598, 1362, 1176 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.67 (m, 2H, Ar), 7.19-7.29 (m, 5H, Ar), 7.09 (m, 2H, Ar), 4.18 (m, 1H, C<u>H</u>DCH$_2$), 2.93 (d, 2H, J = 7.1 Hz, C<u>H</u>$_2$CHD), 2.41 (s, 3H, ArC<u>H</u>$_3$); ^{2}H NMR (CH_2Cl_2, 76.5 MHz) δ 4.23; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 144.6, 136.2, 133.0, 129.8 (2C), 128.8 (2C), 128.6 (2C), 127.8 (2C), 126.9, 70.3 (t, $^1J_{C\text{-}D}$ = 23 Hz), 35.3, 21.6; HRMS (EI) Calc'd for [M+·] $C_{15}H_{15}DO_3S$ 277.0883, found 277.0884.



(3*R*)-[3-^{2}H$_1$]-4-Phenylbutyne (192).

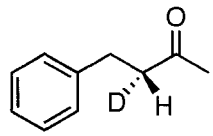
The alkyne **192** was made using a modified procedure described by Crombie and Heavers.[214] To a stirring solution of (1*S*)-[1-^{2}H]-phenylethan-1-yl tosylate (**190**) (200 mg, 0.721 mmol) in DMSO (5 mL) was added lithium acetylide, ethylenediamine complex (199 mg, 2.16 mmol). The resulting reaction mixture was stirred at room temperature for 2 h and then poured into a mixture of cold 1N HCl to quench reaction. The insoluble

material was removed by filtration through a pad of Celite® and the filtrate was extracted with Et_2O. The combined ethereal extracts were washed with water, brine, dried over $MgSO_4$ and evaporated *in vacuo* to give the crude product. The crude alkyne **192** was used without any further purification for the preparation of (3*R*)-[3-$^2H_1$]-4-phenyl-2-butanone (**194**). IR (CH_2Cl_2, cast) 3304, 3078, 3025, 2976, 2258, 1601, 1494, 1079 cm^{-1}; ^1H NMR (CD_2Cl_2, 400 MHz) δ 7.37-7.40 (m, 2H, Ar), 7.20-7.33 (m, 3H, Ar), 2.83 (d, 2H, J = 7.1 Hz, CHDC$\underline{H}_2$), 2.48 (m, 1H, C$\underline{H}$DCH_2), 1.98 (d, 1H, J = 2.6 Hz, C≡C$\underline{H}$); ^2H NMR (CH_2Cl_2, 76.5 MHz) δ 2.47; ^{13}C NMR (CD_2Cl_2, 125 MHz) δ 136.8, 128.5 (2C), 127.8 (2C), 126.3, 83.8, 68.9, 34.8, 20.3 (t, $^1J_{C\text{-}D}$ = 20.0 Hz); HRMS (EI) Calc'd for [M+·] $C_{10}H_9D$ 131.0845, found 131.0846.



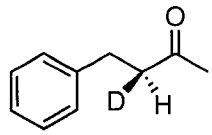
(3*S*)-[3-$^2H_1$]-4-Phenylbutyne (193).

A similar procedure was employed as that described for the preparation of **192**. The reaction of (1*R*)-[1-^{2}H]-phenylethan-1-yl tosylate (**191**) (200 mg, 0.721) in DMSO (5 mL) with lithium acetylide, ethylenediamine complex (199 mg, 2.16 mmol) gave crude (3*S*)-[3-$^2H_1$]-4-phenyl butyne. The crude alkyne **193** was used without any further purification for the preparation of (3*S*)-[3-$^2H_1$]-4-phenyl-2-butanone (**195**). The enantiomeric alkyne had identical spectral properties to that of (3*R*)-[3-2H1]-4-phenylbutyne.



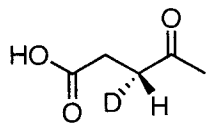
(3*R*)-[3-$^2H_1$]-4-Phenyl-2-butanone (194).

The ketone **194** was prepared using a modified procedure described by Heavers and Crombie.[214] To a stirring solution of (3*R*)-[3-$^2H_1$]-4-phenylbutyne (**192**) (0.721 mmol) in acetic acid (6 mL) and water (1 mL) was added mercuric acetate (919 mg, 2.88 mmol). The resulting reaction mixture was stirred at 70 °C for 3 h at which time water (30 mL) was added. The pH of the solution was controlled by the addition of sodium acetate. The mixture was cooled to 0 °C in an ice-water bath and $NaBH_4$ (202 mg, 5.34 mmol) was added carefully. The mixture was stirred for 30 min, filtered through a pad of Celite® and the filtrate was extracted with Et_2O. The combined ethereal extracts were washed with water, brine, dried over $MgSO_4$ and evaporated *in vacuo*. Crude residue was dissolved in CH_2Cl_2 and treated with PDC (298 mg, 0.792 mmol) and stirred for 3 h at room temperature. Filtered through a pad of Celite® and the filtrate was evaporated *in vacuo* to give the crude ketone. Purification by flash chromatography (SiO_2, pentanes:Et_2O, 10:1) to afford (3*R*)-[3-$^2H_1$]4-phenyl-2-butanone (**194**) as a colorless liquid (44 mg, 42% over two steps). IR (CH_2Cl_2, cast) 3027, 2956, 2871, 1717, 1602, 1497, 1162 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.15-7.29 (m, 5H, Ar), 2.86 (d, 2H, J = 7.0 Hz, CH_2CHD), 2.74 (m, 1H, CH_2CHD), 2.12 (s, 3H, CH_3CO); ^2H NMR ($CHCl_3$, 76.5 MHz) δ 2.74; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 207.8, 141.0, 128.5 (2C), 128.3 (2C), 126.1, 45.2, 30.1 (t, $^1J_{C\text{-}D}$ = 29 Hz), 29.8; HRMS (EI) Calc'd for [M+·] $C_{10}H_{11}DO$ 149.0951, found 149.0944.



(3*S*)-[3-$^2H_1$]-4-Phenyl-2-butanone (195).

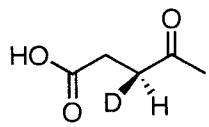
A similar procedure was employed as that described for the preparation of **194**. Treatment of a solution of of (3*S*)-[3-$^2H_1$]-4-phenylbutyne (**193**) (0.721 mmol) in acetic acid (6.0 mL) and water (1.0 mL) with mercuric acetate (919 mg, 2.88 mmol) gave the mercuric salt. Addition of $NaBH_4$ (202 mg, 5.34 mmol) and oxidation by PDC (220 mg, 0.584 mmol) afforded (3*R*)-[3-$^2H_1$]4-phenyl-2-butanone. Purification by flash column chromatography (SiO_2, pentanes:Et_2O) provided the ketone **195** (45 mg, 42% over two steps). The enantiomeric ketone had identical spectral properties to that of (3*R*)-[3-$^2H_1$]-4-Phenyl-2-butanone (**195**).



(3*R*)-[3-$^2H_1$]-Levulinic acid (196).

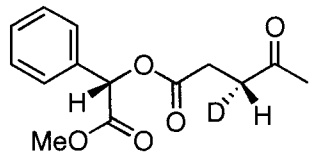
The known acid **196**[186,187,220,221] was prepared using a modified procedure described by Greene and coworkers.[247] A solution of (3*R*)-[3-$^2H_1$]-4-phenyl-2-butanone (**194**) (106 mg, 0.721 mmol) in CCl_4 (4 mL), MeCN (4 mL) and water (6 mL) was treated with sodium periodate (1.54 g, 7.21 mmol) and ruthenium trichloride (3 mg, 1 μmol). The resulting reaction mixture was stirred at room temperature for 18 h. The solution was diluted with water and extracted with CH_2Cl_2. The combined organic extracts were dried with $MgSO_4$ and evaporated to give the crude product. The crude acid was used for the preparation of (*S*)-(+)-(methoxycarbonyl)benzyl (3*R*)-[3-$^2H_1$]-levulinate (**198**) without

further purification. For unlabeled material: IR (CH_2Cl_2, cast) 3111, 1716, 1402, 1369, 1165 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.73 (m, 2H, $C\underline{H}_2CO$), 2.61 (m, 2H, $C\underline{H}_2COOH$), 2.17 (s, 3H, $C\underline{H}_3CO$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 206.9, 178.4, 37.7, 29.7, 27.8; HRMS (EI) Calc'd for [M+] $C_5H_8O_3$ 116.0472, found 116.0474. The (3*R*)-[3-$^2H_1$]-levulinic acid (**196**) showed identical chromatographic and spectral properties except for the following: ^2H NMR (C_6H_6, 61.4 MHz) δ 2.73.



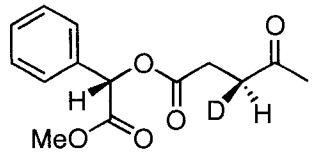
(3*S*)-[3-$^2H_1$]-Levulinic acid (197).

The known acid **197**[186,187,220,221] was prepared using a similar procedure employed as that described for the preparation of **196**. Treatment of (3*S*)-[3-$^2H_1$]-4-phenyl-2-butanone (**195**) (106 mg, 0.721 mmol) in CCl_4 (4 mL), MeCN (4 mL) and H_2O (6 mL) with sodium periodate (1.54 g, 7.21 mmol) and ruthenium trichloride (3 mg, 1 μmol) gave (3*S*)-[3-$^2H_1$] levulinic acid as crude mixture. The acid **197** was used without further purification for the preparation of (*S*)-(+)-(methoxycarbonyl)benzyl (3*S*)-[3-$^2H_1$]-levulinate (**199**). Spectral data was identical to those of **194.**



(*S*)-(+)-(Methoxycarbonyl)benzyl (3*R*)-[3-$^2H_1$]-levulinate (198).

To a stirring solution of (3*R*)-[2-$^2H_1$]-levulinic acid (**196**) (18 mg, 0.154 mmol) in CH_2Cl_2 (5 mL) was added (*S*)-(+)-mandelic acid methyl ester (28 mg, 0.17 mmol), DCC (39 mg, 0.19 mmol) and DMAP (2 mg, 20 μmol). The resulting reaction mixture was stirred at room temperature for 18 h and filtered through a sintered glass funnel to remove solid impurities. The crude ester was purified by flash column (SiO_2, hexane:EtOAc, 4:1) to afford (*S*)-(+)-(methoxycarbonyl)benzyl (3*R*)-[2-$^2H_1$]-levulinate (**198**) (16 mg, 41%) as a colorless oil. For unlabeled material: IR (CH_2Cl_2, cast) 3034, 2954, 1745, 1719, 1587, 1364, 1152 cm^{-1}; ^1H NMR (C_6D_6, 300 MHz) δ 7.42 (m, 2H, Ar), 7.04 (m, 3H, Ar), 6.05 (s, 1H, C<u>H</u>CO_2Me), 3.18 (s, 3H, $OC\underline{H}_3$), 2.50 (m, 2H, J = 7.0 Hz, $C\underline{H}_2CO_2R$), 2.21 (td, 1H, J = 6.3 Hz, 18.2 Hz, $C\underline{H}_2CO$), 2.01 (td, 1H, J = 6.3 Hz, 18.2 Hz, $C\underline{H}_2CO$), 2.01 (s, 3H, $C\underline{H}_3CO$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 206.1, 172.1, 169.2, 133.7, 129.3, 128.8 (2C), 127.6 (2C), 74.6, 52.6, 37.8, 29.8, 27.8; HRMS (EI) Calc'd for [M+·] $C_{14}H_{16}O_5$ 264.0998, found 264.0989. The (*S*)-(+)-(methoxycarbonyl)benzyl methyl (3*R*)-[3-$^2H_1$]-levulinate showed identical chromatographic properties and displayed similar spectral properties except for the following: ^2H NMR (C_6H_6, 76.5 MHz) δ 2.01.



(*S*)-(+)-(Methoxycarbonyl)benzyl (3*S*)-[3-$^2H_1$]-levulinate (199).

A procedure similar to that used for the preparation of (*R*)-(+)-(methoxycarbonyl)benzyl (3*R*)-[3-$^2H_1$]-levulinate (**198**) was employed except that (3*S*)-[2-$^2H_1$] levulinic acid **195** was used as the starting material. Spectral data were similar to those of **198** except for ^2H NMR (C_6H_6, 76.5 MHz) δ 2.21.

Coupling of 36 to *Affi-gel* 10

A solution of 2-(8-amino-octyl)-but-2-enedioic acid, dilithium salt (**36**) (40 mg, 0.113 mmol) was dissolved in an aqueous $NaHCO_3$ buffer (pH 8) and was added to a suspension of Affi-gel 10 (5.0 mL, 0.075 mmol). The resulting suspension was stirred for 16 h at 4 °C. To this suspension was then added a 1.0 M solution of glycine ethyl ester (0.1 mL, 0.1 mmol) and the resulting reaction mixture was stirred for 12 h at 4 °C. The prepared affinity support was filtered through a sintered glass funnel and washed with water and stored in 0.2% NaN_3.

Affinity chromatography

Buffer Solutions

Standard Buffer: 52 mM Tris-HCl pH 7.0, 5.8 mM DTT, 12 mM $MgCl_2$, 12 μM $ZnCl_2$

Elution Buffer: 52 mM Tris-HCl pH 7.0, 5.8 mM DTT, 5 mM chaetomellic acid A

Dialysis Buffer: 52 mM Tris-HCl pH 7.0, 10 mM BME

PFTase (crude or previously purified) was dissolved in 150 μL of standard buffer and was pre-incubated with 500 μL of affinity support. After incubation, the suspension was transferred to a glass pipette, which was plugged at one end with glass wool. The solution was allowed to flow through column and three column volumes of the standard buffer was passed through the column to wash unbound enzyme through. The elution buffer was passed through the column and the fractions were collected. Fractions were analyzed by SDS-PAGE electrophoresis (12% SDS) and fractions indicating presence of PFTase were dialyzed and analyzed for activity.

Inhibition studies with protein farnesyltranferase

Recombinant yeast PFTase was produced in *Escherichia coli* (and purified by chromatography on a Ni(II) column as previously described.[100] Catalytic rate constants were measured using a fluorescence assay, that continuously monitored farnesylation of dansylated pentapeptide using a Spex Fluoromax model spectrofluorimeter with $\lambda_{ex} = 340$ (slit width = 5.1 nm) and $\lambda_{em} = 486$ nm (slit width = 5.1 nm) and 3 mm square cuvettes. For PFTase, assays (250 μL) were conducted at 30 °C in 50 mM TrisHCl, 12 mM $MgCl_2$, 12 μM $ZnCl_2$, 5.8 mM DTT, 0.04% (w/v) *n*-dodecyl-β-D-maltoside, pH 7.0. Dansyl-Gly-Cys-Val-Ile-Ala was the peptide substrate. The reaction mixture was preincubated at 30 °C for 5 minutes before the reaction was initiated by PFTase previously diluted with an assay buffer containing 1 mg/mL bovine serum albumin. Initial rates were measured from the linear region of each run, and all measurements were made in duplicate. Rates were measured in counts/second per second and converted to units of s^{-1} using a conversion factor calculated from the slope of a line generated in a plot of concentration of synthetic dansyl-Gly-((*S*)-farnesyl)Cys-Val-Ile-Ala versus fluorescence intensity.

Inhibition studies with undecaprenyl diphosphate synthase

The assay with UPP synthase was performed by Dr. Lisa Eubanks at the University of Utah. Recombinant yeast his_6-tagged UPP synthase was produced in *Escherichia coli* and purified by Ni(II) affinity chromatography to homogeniety as previously described.[248] The catalytic rate constants were measured in duplicate using a radioactive assay that employed [1-^{14}C] IPP and the radioactivity quantified with a Molecular Dynamics PhosphorImager (Amersham Biosciences). For UPP synthase, assays (250 μL) were conducted at 37 °C in 100 mM TrisHCl pH 8.5, 2 mM $MgCl_2$, 5 μM FPP, 45 μM ^{14}C IPP (55 Ci/mol), and 0.3 μM UPP synthase. After incubation at 37 °C the mixture was quenched and the reaction products were analyzed on reverse phase TLC. The plate with the radiolabeled products was exposed to a film and the radioactivity was quantified.

Rubber transferase assays

The rubber transferase assays were performed in triplicate as previously described.[103] Concentrated stock solutions (1mM) of ispentenyl diphosphate (IPP) analogs were prepared in water, the typical reaction assay (50 μL) contained [1-^{14}C]-IPP (1mM, 0.19 mCi/mmol), $MgSO_4$ (1.25 mM), DTT (5 mM), and Tris pH 7.5 (100 mM). The reactions were incubated for 4 h at 16 °C for *P. argentatum* WRP (0.25 mg) and at 25 °C for *H. brasilensis* WRP (0.25 mg), and were stopped by addition of EDTA (0.5 M, pH 8.0) to a final concentration of 20 mM. The incorporated ^{14}C was measured by liquid

scintillation counting if the newly synthesized rubber that had been trapped on filters and subsequently washed to remove unincorporated ^{14}C-IPP.

Incorporation studies with rubber transferase.

Incorporation assays were performed in duplicate and using a modification of the method by Cornish *et al.*.[129] The total reaction volume was typically 250 μL (100 mM Tris-HCl pH 7.5; 5mM DTT, 20 μM FPP, 1.25 mM $MgSO_4$, 11 mg WRP, and 5 mM of deuterated IPP analog). The reactions were incubated for 4 d at 27 °C for *H. brasiliensis* or at 16 °C for *P. argentatum*, and were stopped by the addition of EDTA (0.5 M, pH 8.0). The reaction mixtures were then filtered through 0.22 μm nitrocellulose filters to trap the rubber particles. The rubber was then washed successively with water (2 x 1 mL), 1.0 N HCl (3 mL) and EtOH (3 x 4 mL). The newly synthesized rubber was then removed from the filter paper and analyzed by either ^{2}H NMR or ozonolysis.

Ozonolysis of rubber.

Rubber isolated from incorporation experiments was dissolved in 10 mL of CH_2Cl_2 and cooled to -40 °C. Through this solution was then bubbled O_3 for 20 minutes and at which time excess O_3 was purged by bubbling with O_2. The solvent was evaporated *in vacuo* and to the resulting residue was added formic acid (1 mL) and H_2O_2 (30%, 0.3 mL). After stirring at room temperature for 4 h, the excess peroxides were decomposed by the addition of solid $FeSO_4$. The solution was diluted with Et_2O (5 mL)

and extracted with Et_2O (2 x 5 mL). The combined ethereal extracts were washed with brine, dried over $MgSO_4$ and evaporated to give levulinic acid.

Derivatization of enzymatically synthesized levulinic acid as its methyl ester.

A solution of levulinic acid in Et_2O (5 mL), which was obtained from the ozonolysis of rubber, was poured into an ethereal solution of diazomethane (20 mL, *ca.* 5 mmol) at 0 °C. After stirring for a further 2 h at room temperature, excess diazomethane was quenched with addition of a solution of 10% acetic acid. The solution was diluted with Et_2O and washed with saturated $NaHCO_3$, brine, and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to afford the methyl ester.

Gas chromatography analysis of methyl levulinate.

Methyl levulinate ester was analyzed on by gas chromatography – mass spectrometry (GC-MS) on a VG7070E double focusing sector mass spectrometer. 1 μL CH_2Cl_2 solution of methyl levulinate (diluted 200 fold) was injected at 250 °C onto a 5% phenyl dimethyl polysiloxane (DB-5) GC column with dimensions of 30 m x 0.25 mm, 0.25 μm film. The column was heated at 50 °C for three min and then the heat was increased at a a rate of 10 °C/min until the column reached a temperature of 300 °C.

Derivatization of enzymatically synthesized levulinic acid.

Crude levulinic acid from ozonolysis of rubber was dissolved in CH_2Cl_2 (10 mL) and to this was added was added (*S*)-(+)-mandelic acid methyl ester (28 mg, 0.17 mmol), DCC (39 mg, 0.19 mmol) and DMAP (2 mg, 0.02 mmol). The resulting reaction mixture was stirred at room temperature for 18 h and filtered through a sintered glass funnel to remove solid impurities. The crude ester was purified by preparative thin layer chromatography (SiO_2, Hexane:EtOAc, 4:1, $R_f = 0.21$) to afford desired ester.

Extraction of natural rubber

A sample of latex suspended in buffer was filtered using centrifugal filtration device (Ultrafree-MC, 0.22 mm, Millipore, Bedford, MA). Samples were spun at 10,000 rpm (Denver Instruments microcentrifuge) for ten minutes to collect solid. Filtered solid was extracted twice with acetone and acetone extracts were combined. The residue was then extracted twice with CH_2Cl_2 and the organic extracts were combined and evaporated *in vacuo*. The percentage of rubber in the plants was determined by weighing the residues from the CH_2Cl_2 extraction. The percentage of resin in the latex was determined by weighing the residue from the acetone extraction.

Tapping of latex from lettuce

This experiment was performed independently by both Dr. Shaun Bushman (University of Georgia) and Andrew Scholte (University of Alberta). Stems of *L. serriola* or *L. sativa* were nicked with a scalpel and the latex was collected in microcentrifuge tube containing sucrose wash buffer (100 mM Tris-HCl (pH 7.5), 5 mM $MgSO_4$, 5 mM DTT, 35% sucrose). After sufficient latex is collected, the tube is spun at 4°C, 9000 rpm for 10 minutes (Eppendorf 5415 D microcentrifuge). Latex that is floating on top of the solution is scraped and suspended in a standard wash buffer (100 mM Tris-HCl pH 7.5, 5 mM $MgSO_4$, 5mM DTT). This is repeated and the latex is collected as before. The collected latex is re-suspended in wash buffer.

Protein extraction of *L. serriola* and *L. sativa*

This experiment was performed by Dr. Shaun Bushman (University of Georgia). Latex isolated from *L. serriola* and *L. sativa* was extracted using this standard procedure. To the latex was added 800 μL extraction buffer (500 μL standard wash buffer, 5 mM DTT, 0.4 mM Pefabloc, 10% Triton X-100) and the resulting solution was placed on a shaker for 1 hr, then spun at 12,000 rpm for 5 min (Eppendorf 5415 D microcentrifuge). The supernatant was removed and placed into 0.5 mL microcentrifuge tube. To the isolated supernatant was added 50 μL solution of deoxycholate (1.5 mg/mL) and set the mixture aside for 10 min at room temperature. A solution of 72% TCA (50 μL) was then added and the mixture was spun at 12,000 for 10 minutes and the supernatant was

removed. The pellet was washed with ice-cold acetone (500 μL) and the supernatant was removed. The remaining pellet was re-suspended in a SDS suspension buffer (5% SDS, 0.1 M NaOH). Protein solution was analyzed by SDS-PAGE electrophoresis and protein bands were sequenced.

Protein sequencing (Edman degradation)

This experiment was performed by Michael Carpenter of the Alberta Protein Institute. Samples were subjected to standard Edman degradation chemistry in an HP model G1105A sequencing system (Routine 3.0) with all sequencer reagents and HPLC separation solvents purchased from Agilent Technologies. Separation and quantification of 3-phenyl-2-thiohydantoin amino acid (PTH) derivatives was made with an HP 1090 liquid chromatographer at 40 °C on a 2.1 x 250 mm Hypersil ODS 5 μm column. A two-component eluent was employed, component A (15% MeCN, 0.1% TFA) and component B (31% isopropanol, 0.1% TFA) at a flow rate of 300 μl/min with a 16 min linear gradient from 0-100% of component B and PTH-derivative detection at 269 nm.

Protein sequencing (tryptic digestion)

This experiment was performed by Mr. Paul Semchuk of the Institute for Biomolecular Design at the University of Alberta. The excised gel pieces were de-stained, reduced (DTT), alkylated (Iodoacetamide), digested with trypsin (Promega Sequencing Grade Modified) and the resulting peptides extracted from the gel and

analyzed via LC/MS. After enzymatic digestion, the tryptic peptides were subjected to LC/MS/MS analysis on a Micromass Q-ToF-2 mass spectrometer (Micromass, Manchester, UK) coupled with a Waters CapLC capillary HPLC (Waters Corp., USA). This procedure involved separation on a PicoFrit capillary reversed-phase column (5 micron BioBasic C18, 300 Å pore size, 75 micron ID x 10 cm, 15 micron tip (New Objectives, MA, USA), using a linear water/MeCN gradient (0.2% formic acid), with a 300 μm x 5 mm PepMap C18 column, 5 micron particle size, (LC Packings, CA, USA) used as a loading/desalting column. The eluent was introduced directly to the mass spectrometer by electrospray ionization at the tip of the capillary column. Data dependent MS/MS acquisitions were performed on detected peptides with a charge state of 2 or 3.

Protein identification from the generated MS/MS data was done by searching the NCBI non-redundant database using Mascot Daemon (Matrix Science, UK). Search parameters included carbamidomethylation of cysteine, possible oxidation of methionine and one missed cleavage per peptide.

Amino acid analysis

This experiment was performed by Mr. Michael Carpenter (Alberta Peptide Institute). Proteins and/or polypeptides were hydrolyzed at 160 °C with 100 mL, 5.7 M HCl containing 0.1% phenol (w/v) in sealed, evacuated ampoules for 1 h. Following hydrolysis the acid was removed by drying under vacuum in a speedVac (Savant Instruments) and the hydrolysates were re-dissolved in a sample buffer pH 2.0 (Beckman

Instruments buffer S) prior to injection on a Beckman 6300 amino acid analyzer. Separation of the hydrosylate amino acids was made by elution with sodium citrate buffers E, F and D (Beckman Instruments) of pH 3.3, 4.3 and 6.3 for 26.0, 10.7 and 30 min respectively and temperature stepped from 40 °C to 73 °C at 11 min. with a flow of 14 mL/h through a 12 cm sodium cation exchange column (Beckman Instruments), with post-column detection of the separated amino acids by reaction with a 7 ml/h flow of ninhydrin solution at 135 °C. Calibration against a standard amino acid mixture (Sigma Chemicals) and subsequent identification and quantitation of peaks was made with Beckman System Gold software (v 8.1).

Molecular weight determination of washed rubber particles

Experiments were performed by Ms. Jenny Brichta at the United States Department of Agriculture, Albany CA.

Sample Preparation - Approximately 2 mg of coagulate was dissolved in 0.2 μm filtered tetrahydrofuran (THF) in 8 ml borosilicate vials with Teflon coated lids overnight. Next day, sample solution was filtered through 1.6 m GF/A w/GMF Whatman syringe filter into 12 x 32 mm clear borosilicate screw-cap vials w/ PTFE septa.

Run Specifications - Equipment consists of HP 1100 series HPLC connected to two downstream detectors. The first detector was a DAWN DSP Laser Photometer (Wyatt Technologies). The second detector was an HP 1047 Refractive Index detector. The

stainless steel tubing connecting the flow cell of the DAWN to the RI detector has a delay volume of 0.208 ml. Sample vials are loaded onto the autosampler tray. A 50 μL solution of sample was injected and run through a Phenogel 5 μm Linear/Mixed Guard Column (Phenomenex) and a PLgel 10 μm mixed-B exclusion column (300 mm x 7.5 mm). Flow rate was 1 mL/min. Column temperatures was 35°C.

Analysis - Chromatograms were analyzed using Astra version 4.73 software (Wyatt Technologies). Analysis includes the number average molecular weight (M_N), weight average molecular weight (M_W), measure of polydispersity (M_W/M_N), root mean square (RMS) radius that provides the size of the molecule in nm (R_z) and the measure of the molecular conformation (RMS versus Molar Mass) where 0.33 = sphere, 0.5-0.6=random coil, 1.0=rod.

CHAPTER 8. References

1. Fujihashi, M.; Zhang, Y. W.; Higuchi, Y.; Li, X. Y.; Koyama, T.; Miki, K. *Proc. Nat. Acad. Sci. U.S.A.* **2001**, *98*, 4337-4342.
2. Glasby, J. S. *Encylopedia of Terpenoids.* Wiley: Chichester, 1982.
3. Cane, D. E. Isoprenoid Biosynthesis: Overview. In *Comprehensive Natural Products Chemistry*, Barton, D.; Nakanishi, K., Eds. Elsevier: New York, 1999; Vol. 2, 1-13.
4. Braquet, P.; Esanu, A.; Buisine, E.; Hosford, D.; Broquet, C.; Koltai, M. *Med. Chem. Rev.* **1991**, *11*, 295-355.
5. Kingston, D. G. I. *Pure Appl. Chem.* **1998**, *70*, 331-334.
6. Wallach, O. *Liebigs Ann. Chem.* **1887**, *239*, 1-54.
7. Ruzicka, L. *P. Chem. Soc. Lond.* **1959**, 341-360.
8. Ruzicka, L. *Experientia* **1953**, *9*, 357-367.
9. Ruzicka, L. *Annu. Rev. Biochem* **1973**, *42*, 1-20.
10. Rohmer, M.; Seemann, M.; Grosdemange-Billiard, C. Biosynthetic Routes to the Building Blocks of Isoprenoids. In *Biopolymers*, Koyama, T.; Steinbuchel, A., Eds. Wiley-VCH: New York, 2001; Vol. 2, 49-72.
11. Lynen, F.; Eggerer, H.; Henning, U.; Kessel, I. *Angew. Chem. Int. Ed.* **1958**, *70*, 738-742.
12. Chaykin, S.; Law, J.; Phillips, A. H.; Tchen, T. T.; Bloch, K. *Proc. Nat. Acad. Sci. U.S.A.* **1958**, *44*, 998-1004.
13. Rohmer, M. *Pure Appl. Chem.* **2003**, *75*, 375-387.

14. Treharne, K. J.; Mercer, E. I.; Goodwin, T. W. *Biochem. J.* **1966**, *99*, 239-245.

15. Banthorpe, D. V.; Charlwood, B. V.; Francis, M. J. *Chem. Rev.* **1972**, *72*, 115-155.

16. Bach, T. J.; Motel, A.; Weber, T. Some Properties of Enzymes Involved in the Biosynthesis and Metabolism of 3-Hydroxy-3-methylglutararyl-CoA in Plants. In *Biochemistry of the Mevalonic Acid Pathway to Terpenoids*, Towers, N. G. H.; Stafford, H. A., Eds. Plenum Press: New York, 1990; Vol. 24, 1-82.

17. Lutkebrinkhaus, F.; Kleinig, H. *Planta* **1987**, *171*, 406-411.

18. Flesch, G.; Rohmer, M. *Eur. J. Biochem.* **1988**, *175*, 405-411.

19. Hoeffler, J. F.; Tritsch, D.; Grosdemange-Billiard, C.; Rohmer, M. *Eur. J. Biochem.* **2002**, *269*, 4446-4457.

20. Rohmer, M. *Nat. Prod. Rep.* **1999**, *16*, 565-574.

21. Wolff, M.; Seemann, M.; Tse Sum Bui, B.; Frapart, Y.; Tritsch, D.; Garcia Estrabot, A.; Rodriguez-Concepcion, M.; Boronat, A.; Marquet, A.; Rohmer, M. *FEBS Lett.* **2003**, *541*, 115-120.

22. Seemann, M.; Wegner, P.; Schunemann, V.; Bui, B. T.; Wolff, M.; Marquet, A.; Trautwein, A. X.; Rohmer, M. *J. Biol. Inorg. Chem.* **2005**, *10*, 131-137.

23. Enfissi, E. M. A.; Fraser, P. D.; Lois, L. M.; Boronat, A.; Schuch, W.; Bramley, P. M. *Plant Biotech. J.* **2005**, *3*, 17-27.

24. Bick, J. A.; Lange, B. M. *Arch. Biochem. Biophys.* **2003**, *415*, 146-154.

25. Laule, O.; Furholz, A.; Chang, H. S.; Zhu, T.; Wang, X.; Heifetz, P. B.; Gruissem, W.; Lange, B. M. *Proc. Nat. Acad. Sci. U.S.A.* **2003**, *100*, 6866-6871.

26. Dudareva, N.; Andersson, S.; Orlova, I.; Gatto, N.; Reichelt, M.; Rhodes, D.; Boland, W.; Gershenzon, J. *Proc. Nat. Acad. Sci. U.S.A.* **2005**, *102*, 933-938.

27. Koyama, T.; Ogura, K. Isopentenyl Diphosphate Isomerase and Prenyltransferases. In *Comprehensive Natural Products Chemistry*, Barton, D.; Nakanishi, K., Eds. Elsevier: New York, 1999; Vol. 2, 69-96.

28. Wang, K. C.; Ohnuma, S. B. B. A. B. A. *Biochim. Biophys. Acta* **2000**, *1529*, 33-48.

29. Song, L. S.; Poulter, C. D. *Proc. Nat. Acad. Sci. U.S.A.* **1994**, *91*, 3044-3048.

30. Joly, A.; Edwards, P. A. *J. Biol. Chem.* **1993**, *268*, 26983-26989.

31. Marrero, P. F.; Poulter, C. D.; Edwards, P. A. *J. Biol. Chem.* **1992**, *267*, 21873-21878.

32. Tarshis, L. C.; Proteau, P. J.; Kellogg, B. A.; Sacchettini, J. C.; Poulter, C. D. *Proc. Nat. Acad. Sci. U.S.A.* **1996**, *93*, 15018-15023.

33. Poulter, C. D.; Rilling, H. C. *Acc. Chem. Res.* **1978**, *11*, 307-313.

34. Poulter, C. D.; Satterwhite, D. M.; Rilling, H. C. *J. Am. Chem. Soc.* **1976**, *98*, 3376-3377.

35. Poulter, C. D.; Rilling, H. C. *Biochemistry* **1976**, *15*, 1079-1083.

36. Joly, A.; Edwards, P. A. *J. Biol. Chem.* **1993**, *268*, 26983-26989.

37. Hosfield, D. J.; Zhang, Y.; Dougan, D. R.; Broun, A.; Tari, L. W.; Swanson, R. V.; Finn, J. *J. Biol. Chem.* **2004**, *279*, 8526-8529.

38. Chen, A.; Kroon, P. A.; Poulter, C. D. *Protein Sci.* **1994**, *3*, 600-607.

39. Ohnuma, S.; Narita, K.; Nakazawa, T.; Ishida, C.; Takeuchi, Y.; Ohto, C.; Nishino, T. *J. Biol. Chem.* **1996**, *271*, 30748-30754.

40. Ko, T. P.; Chen, Y. K.; Robinson, H.; Tsai, P. C.; Gao, Y. G.; Chen, A. P.; Wang, A. H.; Liang, P. H. *J. Biol. Chem.* **2001**, *276*, 47474-47482.

41. Guo, R. T.; Ko, T. P.; Chen, A. P.; Kuo, C. J.; Wang, A. H.; Liang, P. H. *J. Biol. Chem.* **2005**, *280*, 20762-20774.

42. Guo, R. T.; Kuo, C. J.; Chou, C. C.; Ko, T. P.; Shr, H. L.; Liang, P. H.; Wang, A. H. *J. Biol. Chem.* **2004**, *279*, 4903-4912.

43. Pan, J. J.; Yang, L. W.; Liang, P. H. *Biochemistry* **2000**, *39*, 13856-13861.

44. Ratemi, E. S.; Dolence, J. M.; Poulter, C. D.; Vederas, J. C. *J. Org. Chem.* **1996**, *61*, 6296-6301.

45. Mau, C. J.; Garneau, S.; Scholte, A. A.; Van Fleet, J. E.; Vederas, J. C.; Cornish, K. *Eur. J. Biochem.* **2003**, *270*, 3939-3945.

46. Levy, S. B.; Marshall, B. *Nat. Med.* **2004**, *10*, S122-129.

47. Harris, C. R.; Thorarensen, A. *Curr. Med. Chem.* **2004**, *11*, 2213-2243.

48. Levy, S. B. *J. Antimicrob. Chemother.* **2002**, *49*, 25-30.

49. Davies, J. *Nature* **1996**, *383*, 219-220.

50. Kirby, W. M. M. *Science* **1944**, *99*, 452.

51. Barber, M.; Rozwadowskadowzenko, M. *Lancet* **1948**, *255*, 641-644.

52. Ritter, T. K.; Wong, C. H. *Angew. Chem. Int. Ed.* **2001**, *40*, 3508-3533.

53. Davies, J. *Science* **1994**, *264*, 375-382.

54. Walsh, C. *Nature* **2000**, *406*, 775-781.

55. Lazar, K.; Walker, S. *Curr. Opin. Chem. Biol.* **2002**, *6*, 786-793.

56. van Heijenoort, J. *Nat. Prod. Rep.* **2001**, *18*, 503-519.

57. Rogers, H. J.; Perkins, H. R.; Ward, J. B. Biosynthesis of peptidoglycan. In *Microbial Cell Walls and Membrane*, J., R. H., Ed. Chapman and Hall: London, 1980; 239-297.

58. van Heijenoort, J. *Glycobiology* **2001**, *11*, 25R-36R.

59. Bugg, T. D. H.; Walsh, C. T. *Nat. Prod. Rep.* **1992**, *9*, 199-215.

60. Siewert, G.; Strominger, J. L. *Proc. Nat. Acad. Sci. U.S.A.* **1967**, *57*, 767-773.

61. Storm, D. R.; Strominger, J. L. *J. Biol. Chem.* **1973**, *248*, 3940-3945.

62. Bernard, R.; El Ghachi, M.; Mengin-Lecreulx, D.; Chippaux, M.; Denizot, F. *J. Biol. Chem.* **2005**.

63. Allen, C. M. *Essentials of Carbohydrate Chemistry*. Springer-Verlag Inc.: New York, p 305-318.

64. Leonard, D. M. *J. Med. Chem.* **1997**, *40*, 2971-2990.

65. Bis, J. L. *Cancer Res.* **1989**, *49*, 4682-4689.

66. Adjei, A. A. *Drugs Fut.* **2000**, *25*, 1069-1079.

67. Rozema, D. B.; Poulter, C. D. *Biochemistry* **1999**, *38*, 13138-13146.

68. Goldstein, J. L.; Brown, M. S.; Stradley, S. J.; Reiss, Y.; Gierasch, L. M. *J. Biol. Chem.* **1991**, *266*, 15575-15578.

69. Zhang, F. L.; Fu, H. W.; Casey, P. J.; Bishop, W. R. *Biochemistry* **1996**, *35*, 8166-8171.

70. Rozema, D. B.; Phillips, S. T.; Poulter, C. D. *Org. Lett.* **1999**, *1*, 815-817.

71. Dabrah, T. T.; Kaneko, T.; Massefski, W.; Whipple, E. B. *J. Am. Chem. Soc.* **1997**, *119*, 1594-1598.

72. Jayasuriya, H.; Bills, G. F.; Cascales, C.; Zink, D. L.; Goetz, M. A.; Jenkins, R. G.; Silverman, K. C.; Lingham, R. B.; Singh, S. B. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2081-2084.

73. Evans, D. A.; Barrow, J. C.; Leighton, J. L.; Robichaud, A. J.; Sefkow, M. *J. Am. Chem. Soc.* **1994**, *116*, 12111-12112.

74. Nicolaou, K. C.; Yue, E. W.; Naniwa, Y.; Dericcardis, F.; Nadin, A.; Leresche, J. E.; Lagreca, S.; Yang, Z. *Angew. Chem. Int. Ed.* **1994**, *33*, 2184-2187.

75. Nicolaou, K. C.; Nadin, A.; Leresche, J. E.; Yue, E. W.; Lagreca, S. *Angew. Chem. Int. Ed.* **1994**, *33*, 2190-2191.

76. Dufresne, C.; Wilson, K. E.; Singh, S. B.; Zink, D. L.; Bergstrom, J. D.; Rew, D.; Polishook, J. D.; Meinz, M.; Huang, L. Y.; Silverman, K. C.; Lingham, R. B.; Mojena, M.; Cascales, C.; Pelaez, F.; Gibbs, J. B. *J. Nat. Prod.* **1993**, *56*, 1923-1929.

77. Singh, S. B.; Liesch, J. M.; Lingham, R. B.; Silverman, K. C.; Sigmund, J. M.; Goetz, M. A. *J. Org. Chem.* **1995**, *60*, 7896-7901.

78. Lingham, R. B.; Silverman, K. C.; Bills, G. F.; Cascales, C.; Sanchez, M.; Jenkins, R. G.; Gartner, S. E.; Martin, I.; Diez, M. T.; Pelaez, F.; Mochales, S.; Kong, Y. L.; Burg, R. W.; Meinz, M. S.; Huang, L.; Nallinomstead, M.; Mosser, S. D.; Schaber, M. D.; Omer, C. A.; Pompliano, D. L.; Gibbs, J. B.; Singh, S. B. *Appl. Microbiol. Biot.* **1993**, *40*, 370-374.

79. Singh, S. B.; Zink, D. L.; Liesch, J. M.; Goetz, M. A.; Jenkins, R. G.; Nallinomstead, M.; Silverman, K. C.; Bills, G. F.; Mosley, R. T.; Gibbs, J. B.; Albersschonberg, G.; Lingham, R. B. *Tetrahedron* **1993**, *49*, 5917-5926.

80. Hara, M.; Akasaka, K.; Akinaga, S.; Okabe, M.; Nakano, H.; Gomez, R.; Wood, D.; Uh, M.; Tamanoi, F. *Proc. Nat. Acad. Sci. U.S.A.* **1993**, *90*, 2281-2285.

81. Shiomi, K.; Yang, H.; Inokoshi, J.; Van der Pyl, D.; Nakagawa, A.; Takeshima, H.; Omura, S. *J. Antibiot.* **1993**, *46*, 229-234.

82. Uchida, R.; Shiomi, K.; Inokoshi, J.; Sunazuka, T.; Tanaka, H.; Iwai, Y.; Takayanagi, H.; Omura, S. *J. Antibiot.* **1996**, *49*, 418-424.

83. Singh, S. B.; Ball, R. G.; Bills, G. F.; Cascales, C.; Gibbs, J. B.; Goetz, M. A.; Hoogsteen, K.; Jenkins, R. G.; Liesch, J. M.; Lingham, R. B.; Silverman, K. C.; Zink, D. L. *J. Org. Chem.* **1996**, *61*, 7727-7737.

84. Singh, S. B.; Zink, D. L.; Liesch, J. M.; Ball, R. G.; Goetz, M. A.; Bolessa, E. A.; Giacobbe, R. A.; Silverman, K. C.; Bills, G. F.; Pelaez, F.; Cascales, C.; Gibbs, J. B.; Lingham, R. B. *J. Org. Chem.* **1994**, *59*, 6296-6302.

85. Singh, S. B.; Jones, E. T.; Goetz, M. A.; Bills, G. F.; Nallinomstead, M.; Jenkins, R. G.; Lingham, R. B.; Silverman, K. C.; Gibbs, J. B. *Tetrahedron Lett.* **1994**, *35*, 4693-4696.

86. Van der Pyl, D.; Inokoshi, J.; Shiomi, K.; Yang, H.; Takeshima, H.; Omura, S. *J. Antibiot.* **1992**, *45*, 1802-1805.

87. Liu, W. C.; Barbacid, M.; Bulgar, M.; Clark, J. M.; Crosswell, A. R.; Dean, L.; Doyle, T. W.; Fernandes, P. B.; Huang, S.; Manne, V.; et al. *J. Antibiot.* **1992**, *45*, 454-457.

88. Crul, M.; de Klerk, G. J.; Beijnen, J. H.; Schellens, J. H. *Anticancer Drugs* **2001**, *12*, 163-184.

89. Prendergast, G. C.; Davide, J. P.; deSolms, S. J.; Giuliani, E. A.; Graham, S. L.; Gibbs, J. B.; Oliff, A.; Kohl, N. E. *Mol. Biol. Cell* **1994**, *14*, 4193-4202.

90. Dinsmore, C. J.; Bell, I. M. *Curr. Top. Med. Chem.* **2003**, *3*, 1075-1093.

91. Anthony, N. J.; Gomez, R. P.; Schaber, M. D.; Mosser, S. D.; Hamilton, K. A.; O'Neil, T. J.; Koblan, K. S.; Graham, S. L.; Hartman, G. D.; Shah, D.; Rands, E.; Kohl, N. E.; Gibbs, J. B.; Oliff, A. I. *J. Med. Chem.* **1999**, *42*, 3356-3368.

92. Gibbs, J.; Pompliano, D.; Mosser, S.; Rands, E.; Lingham, R.; Singh, S.; Scolnick, E.; Kohl, N.; Oliff, A. *J. Biol. Chem.* **1993**, *268*, 7617-7620.

93. Watanabe, S.; Hirai, H.; Kambara, T.; Kojima, Y.; Nishida, H.; Sugiura, A.; Yamauchi, Y.; Yoshikawa, N.; Harwood, H. J., Jr.; Huang, L. H.; Kojima, N. *J. Antibiot.* **2001**, *54*, 1025-1030.

94. Turek-Etienne, T. C.; Strickland, C. L.; Distefano, M. D. *Biochemistry* **2003**, *42*, 3716-3724.

95. Pattenden, G.; Teague, S. J. *J. Chem. Soc., Perkin Trans. 1* **1988**, 1077-1083.

96. Snider, B. B.; Merrit, J. E. *Tetrahedron* **1991**, *47*, 8663-8678.

97. van der Gen, A.; Wiedhaup, K.; Swoboda, J. J.; Dunathan, H. C.; Johnson, W. S. *J. Am. Chem. Soc.* **1973**, *95*, 2656.

98. Mahoney, W. S.; Brestensky, D. M.; Stryker, J. M. *J. Am. Chem. Soc.* **1988**, *110*, 291-293.

99. Ratemi, S. E. Synthetic Studies on Serine and Threonine B-Lactones and the Design and Synthesis of Pyrophosphate Mimics University of Alberta, Edmonton, 1997.

100. Harris, C. M.; Derdowski, A. M.; Poulter, C. D. *Biochemistry* **2002**, *41*, 10554-10562.

101. Cassidy, P. B.; Dolence, J. M.; Poulter, C. D. *Methods Enzymol.* **1995**, *250*, 30-43.

102. Cornish, K. *Phytochemistry* **2001**, *57*, 1123-1134.

103. Mau, C. J. D.; Scott, D. J.; Cornish, K. *Phytochem. Anal.* **2000**, *11*, 356-361.

104. Scholte, A. A.; Eubanks, L. M.; Poulter, C. D.; Vederas, J. C. *Bioorg. Med. Chem.* **2004**, *12*, 763-770.

105. Nilsson, K.; Andersson, T.; Ullenius, C.; Gerold, A.; Krause, N. *Chem. Eur. J.* **1998**, *4*, 2051-2058.

106. Long, S. B.; Casey, P. J.; Beese, L. S. *Biochemistry* **1998**, *37*, 9612-9618.

107. Strickland, C. L.; Windsor, W. T.; Syto, R.; Wang, L.; Bond, R.; Wu, R.; Schwartz, J.; Le, H. V.; Beese, L. S.; Weber, P. C. *Biochemistry* **1998**, *37*, 16601-16611.

108. Jungbauer, A. *J. Chromatogr. A* **2005**, *1065*, 3-12.

109. Cuatreca, P.; Anfinsen, C. B. *Annu. Rev. Biochem* **1971**, *40*, 259-278.

110. Jones, C.; Patel, A.; Griffin, S.; Martin, J.; Young, P.; O'Donnell, K.; Silverman, C.; Porter, T.; Chaiken, I. *J. Chromatogr. A* **1995**, *707*, 3-22.

111. Steers, E., Jr.; Cuatrecasas, P.; Pollard, H. B. *J. Biol. Chem.* **1971**, *246*, 196-200.

112. Porath, J.; Axen, R.; Ernback, S. *Nature* **1967**, *215*, 1491.

113. Axen, R.; Porath, J.; Ernback, S. *Nature* **1967**, *214*, 1302.

114. Cuatreca, P.; Wilchek, M.; Anfinsen, C. B. *Proc. Nat. Acad. Sci. U.S.A.* **1968**, *61*, 636-643.

115. Rose, K.; Steinbuchel, A. *Appl. Environ. Microbiol.* **2005**, *71*, 2803-2812.

116. Cornish, K. *Nat. Prod. Rep.* **2001**, *18*, 182-189.

117. Mooibroek, H.; Cornish, K. *Appl. Microbiol. Biot.* **2000**, *53*, 355-365.

118. Hosler, D.; Burkett, S. L.; Tarkanian, M. J. *Science* **1999**, *284*, 1988-1991.

119. Schurer, H. *Rubber J.* **1957**, *132*, 543-549.

120. Sethuraj, M. R.; Mathew, N. M. Natural Rubber: biology, cultivation and technology. In *Developments in Crop Science*, R., S. M.; Mathew, N. M., Eds. 1992; Vol. 23, p 610.

121. Schurer, H. *Rubber Plant Weekly* **1962**, *143*, 943-949.

122. Barker, P. W. *Rubber Age* **1938**, *43*, 229-230.

123. E., S. H. *India Rubber* **1939**, *101*.

124. Davis, W. *Fortune* **1997**, *136*, 86.

125. Archer, B. L.; Audley, B. G. Rubber, gutta percha and chicle. In *Phytochemistry*, Miller, L. P., Ed. Van Nostrand Reinhold: New York, 1973; Vol. 2, 310-343.

126. Cornish, K.; Wood, D. F.; Windle, J. J. *Planta* **1999**, *210*, 85-96.

127. Wood, D. F.; Cornish, K. *Int. J. Plant. Sci.* **2000**, *161*, 435-445.

128. Cornish, K. *Eur. J. Biochem.* **1993**, *218*, 267-271.

129. Cornish, K.; Backhaus, R. A. *Phytochemistry* **1990**, *29*, 3809-3813.

130. Archer, B. L.; Audley, B. G. *Bot. J. Linn. Soc.* **1987**, *94*, 181-196.

131. Scott, D. J.; da Costa, B. M.; Espy, S. C.; Keasling, J. D.; Cornish, K. *Phytochemistry* **2003**, *64*, 123-134.

132. Tanaka, Y.; AikHwee, E.; Ohya, N.; Nishiyama, N.; Tangpakdee, J.; Kawahara, S.; Wititsuwannakul, R. *Phytochemistry* **1996**, *41*, 1501-1505.

133. Benedict, C. R.; Madhavan, S.; Greenblatt, G. A.; Venkatachalam, K. V.; Foster, M. A. *Plant Physiol.* **1990**, *92*, 816-821.

134. Madhavan, S.; Greenblatt, G. A.; Foster, M. A.; Benedict, C. R. *Plant Physiol.* **1989**, *89*, 506-511.

135. Castillon, J.; Cornish, K. *Phytochemistry* **1999**, *51*, 43-51.

136. Cornish, K.; Castillon, J.; Scott, D. J. *Biomacromolecules* **2000**, *1*, 632-641.

137. da Costa, B. M.; Keasling, J. D.; Cornish, K. *Biomacromolecules* **2005**, *6*, 279-289.

138. Swanson, C. L.; Buchanan, R. A.; Otey, F. H. *J. Appl. Polym. Sci.* **1979**, *23*, 743-748.

139. Slater, J. E. *Clin. Allergy Immunol.* **2004**, *18*, 369-386.

140. Slater, J. E. *J. Allergy Clin. Immun.* **1994**, *94*, 139-149.

141. Slater, J. E.; Mostello, L. A.; Shaer, C.; Honsinger, R. W. *Ann. Allergy* **1990**, *65*, 411-414.

142. Tomazic, V. J.; Withrow, T. J.; Fisher, B. R.; Dillard, S. F. *Clin. Immunol. Immunopathol.* **1992**, *64*, 89-97.

143. Polhamus, L. G. *Rubber (Botany, Production, and Utilization)*. Interscience Publishers Inc.: New York, 1962.

144. Cornish, K.; Scott, D. J. *Ind. Crop Prod.* **2005**, *22*, 49-58.

145. Tanaka, Y.; Mori, M.; Takei, A.; Boochatum, P.; Sato, Y. *J. Nat. Rubber Res.* **1990**, *5*, 241-245.

146. Tanaka, Y. *J. Appl. Polym. Sci. Appl. Polym. Symp.* **1989**, *44*, 1-9.

147. Tanaka, Y.; Nunogaki, K.; Kageyu, A.; Mori, M.; Sato, Y. *J. Nat. Rubber Res.* **1988**, *3*, 177-183.

148. Ohya, N.; Takizawa, J.; Kawahara, S.; Tanaka, Y. *Phytochemistry* **1998**, *48*, 781-786.

149. Stewart, W. D.; Wachtel, W. L.; Shipman, J. J.; Yanko, J. A. *Science* **1955**, *122*, 1271-1272.

150. Tanaka, Y.; Mori, M.; Ute, K.; Hatada, K. *Rubb. Chem. Technol.* **1990**, *63*, 1-7.

151. Ryder, E. J. *Lettuce, endive and chicory*. CABI Publishing: New York, 1999.

152. Fox, C. P. *J. Ind. Eng. Chem.* **1913**, *5*, 477-478.

153. Mitchell, J. H.; Rice, M. A.; Roderick, D. B. *Science* **1942**, *95*, 624-625.

154. Anonymous, Rubber content of miscellaneous plants. Production Report No. 10. In Service, U.-A. R., Ed. US Government Printing Office: O-408250: 1956.

155. Duch, M. W.; Grant, D. M. *Macromolecules* **1970**, *3*, 165-174.

156. Buchanan, R. A.; Cull, I. M.; Otey, F. H.; Russell, C. R. *Econ. Bot.* **1978**, *32*, 131-145.

157. Turrall, W. T.; Klassen, J.; Smedley, H. *Can. J. Res.* **1943**, *21*, 195-201.

158. Sessa, R. A.; Bennett, M. H.; Lewis, M. J.; Mansfield, J. W.; Beale, M. H. *J. Biol. Chem.* **2000**, *275*, 26877-26884.

159. Wititsuwannakul, D.; Wititsuwannakul, R. Biochemistry of Natural Rubber and Structure of Latex. In *Biopolymers*, Koyama, T.; Steinbuchel, A., Eds. Wiley-VCH: New York, 2001; Vol. 2, 151-201.

160. Siler, D. J.; Cornish, K. *Phytochemistry* **1993**, *32*, 1097-1102.

161. Nagaki, M.; Yamamoto, H.; Takahashi, A.; Maki, Y.; Ishibashi, J.; Nishino, T.; Koyama, T. *J. Mol. Catal. B-Enzym.* **2002**, *17*, 81-89.

162. Nagaki, M.; Kannari, H.; Ishibashi, J.; Maki, Y.; Nishino, T.; Ogura, K.; Koyama, T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2549-2554.

163. Koyama, T.; Obata, S.; Osabe, M.; Takeshita, A.; Yokoyama, K.; Uchida, M.; Nishino, T.; Ogura, K. *J. Biochem.* **1993**, *113*, 355-363.

164. Maki, Y.; Komabayashi, M.; Gotoh, Y.; Ohya, N.; Hemmi, H.; Hirooka, K.; Nishino, T.; Koyama, T. *J. Mol. Catal. B-Enzym.* **2002**, *19*, 431-436.

165. Ohnuma, S.; Hirooka, K.; Tsuruoka, N.; Yano, M.; Ohto, C.; Nakane, H.; Nishino, T. *J. Biol. Chem.* **1998**, *273*, 26705-26713.

166. Nagaki, M.; Sato, S.; Maki, Y.; Nishino, T.; Koyama, T. *J. Mol. Catal. B-Enzym.* **2000**, *9*, 33-38.

167. Brindle, P. A.; Schooley, D. A.; Tsai, L. W.; Baker, F. C. *J. Biol. Chem.* **1988**, *263*, 10653-10657.

168. Lee, E.; Schooley, D. A.; Hall, M. S.; Judy, K. J. *J. Chem. Soc., Chem. Commun.* **1978**, 290-292.

169. Schooley, D. A.; Judy, K. J.; Bergot, B. J.; Hall, M. S.; Siddall, J. B. *Proc. Nat. Acad. Sci. U.S.A.* **1973**, *70*, 2921-2925.

170. Schooley, D. A., Baker, F.C. *Juvenile Hormone Biosynthesis*. Pergamon Press: 1985; Vol. 7, p 363-389.

171. Paquette, L. A. S., Daryl R. ; Cleary, Darryl G. ; Kinsella, Mary A. ; Blackwell, Christopher M. *J. Am. Chem. Soc.* **1992**, *114*, 7375.

172. Koyama, T.; Ogura, K.; Seto, S. *J. Biol. Chem.* **1973**, *248*, 8043-8051.

173. Fujisawa, T.; Sato, T.; Gotoh, Y.; Kawashima, M.; Kawara, T. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 3555-3559.

174. Abarbri, M.; Parrain, J. L.; Kitamura, M.; Noyori, R.; Duchene, A. *J. Org. Chem.* **2000**, *65*, 7475-7478.

175. Anachkov, M. P.; Rakovski, S. K.; Stefanova, R. V. *Polym. Degrad. Stabil.* **2000**, *67*, 355-363.

176. Ohnuma, S.; Ito, M.; Koyama, T.; Ogura, K. *Tetrahedron* **1989**, *45*, 6145-6160.

177. Koyama, T.; Saito, A.; Ogura, K.; Seto, S. *J. Am. Chem. Soc.* **1980**, *102*, 3614-3618.

178. Levy, H. R.; Loewus, F. A.; Vennesland, B. *J. Am. Chem. Soc.* **1957**, *79*, 2949-2953.

179. Loewus, F. A.; Westheimer, F. H.; Vennesland, B. *J. Am. Chem. Soc.* **1953**, *75*, 5018-5023.

180. Fisher, H. F.; Conn, E. E.; Vennesland, B.; Westheimer, F. H. *J. Biol. Chem.* **1953**, *202*, 687-697.

181. Cornforth, J. W.; Cornforth, R. H.; Popjak, G.; Yengoyan, L. *J. Biol. Chem.* **1966**, *241*, 3970-3987.

182. Cornforth, J. W.; Cornforth, R. H.; Donninger, C.; Popjak, G. *Proc. R. Soc. London, Ser. B* **1966**, *163*, 492-514.

183. Benner, S. A.; Glasfeld, A.; Piccirilli, J. A. *Top. Stereochem.* **1989**, *19*, 127.

184. Archer, B. L.; Barnard, D.; Cockbain, E. G.; Cornforth, J. W.; Cornforth, R. H.; Popjak, G. *Proc. R. Soc. London, Ser. B* **1966**, *163*, 519-523.

185. Clifford, K. H.; Cornforth, J. W.; Donninger, C.; Mallaby, R. *Eur. J. Biochem.* **1972**, *26*, 401-406.

186. Ito, M.; Kobayashi, M.; Koyama, T.; Ogura, K. *Biochemistry* **1987**, *26*, 4745-4750.

187. Kobayashi, M.; Ito, M.; Koyama, T.; Ogura, K. *J. Am. Chem. Soc.* **1985**, *107*, 4588-4589.

188. Takahashi, I.; Ogura, K. *J. Biochem.* **1982**, *92*, 1527-1537.

189. Takahashi, I.; Ogura, K.; Seto, S. *J. Biol. Chem.* **1980**, *255*, 4539-4543.

190. Suga, T.; Hirata, T.; Aoki, T.; Kataoka, T. *J. Am. Chem. Soc.* **1986**, *108*, 2366-2371.

191. Cane, D. E.; Weiner, S. W. *Can. J. Chem.* **1994**, *72*, 118-127.

192. Suga, T.; Ohta, S.; Ohmoto, T. *J. Chem. Soc., Perkin Trans. 1* **1987**, 2845-2848.

193. Leyes, A. E.; Poulter, C. D. *Org. Lett.* **1999**, *1*, 1067-1070.

194. Giner, J. L.; Arigoni, D. *Chem. Comm.* **2002**, 1388-1389.

195. Hubschwerlen, C. *Synthesis-Stuttgart* **1986**, 962-964.

196. Hubschwerlen, C.; Specklin, J. L.; Higelin, J. *Org. Syn.* **1995**, *72*, 1-5.

197. Meddour, A.; Canet, I.; Loewenstein, A.; Pechine, J. M.; Courtieu, J. *J. Am. Chem. Soc.* **1994**, *116*, 9652-9656.

198. Cornforth, J. W.; Ross, F. P.; Wakselman, C. *J. Chem. Soc., Perkin Trans. 1* **1975**, 429-432.

199. Reich, C. J.; Sullivan, G. R.; Mosher, H. S. *Tetrahedron Lett.* **1973**, 1505-1508.

200. Midland, M. M.; McDowell, D. C.; Hatch, R. L.; Tramontano, A. *J. Am. Chem. Soc.* **1980**, *102*, 867-869.

201. Midland, M. M.; Greer, S.; Tramontano, A.; Zderic, S. A. *J. Am. Chem. Soc.* **1979**, *101*, 2352-2355.

202. Piancatelli, G.; Scettri, A.; D'Auria, M. *Synthesis-Stuttgart* **1982**, 245-258.

203. Cane, D. E.; Iyengar, R.; Shiao, M. S. *J. Am. Chem. Soc.* **1981**, *103*, 914-931.

204. Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, 399-402.

205. Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277-7287.

206. Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155-4156.

207. Mancuso, A. J.; Swern, D. *Synthesis-Stuttgart* **1981**, 165-185.

208. Omura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651-1660.

209. Mancuso, A. J.; Huang, S. L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480-2482.

210. Frigerio, M.; Santagostino, M.; Sputore, S. *J. Org. Chem.* **1999**, *64*, 4537-4538.

211. Frigerio, M.; Santagostino, M. *Tetrahedron Lett.* **1994**, *35*, 8019-8022.

212. Parker, D. *Chem. Rev.* **1991**, *91*, 1441-1457.

213. Rawlings, B. J.; Reese, P. B.; Ramer, S. E.; Vederas, J. C. *J. Am. Chem. Soc.* **1989**, *111*, 3382-3390.

214. Crombie, L.; Heavers, A. D. *J. Chem. Soc., Perkin Trans. 1* **1992**, 1929-1937.

215. Perrin, D. D.; Amarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*. 2nd ed.; Pergamon Press: New York, 1980.

216. House, H. O.; Chu, C. Y.; Wilkins, J. M.; Umen, M. J. *J. Org. Chem.* **1975**, *40*, 1460-1469.

217. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925.

218. Davisson, V. J.; Woodside, A. B.; Poulter, C. D. *Methods Enzymol.* **1985**, *110*, 130-144.

219. Davisson, V. J.; Woodside, A. B.; Neal, T. R.; Stremler, K. E.; Muehlbacher, M.; Poulter, C. D. *J. Org. Chem.* **1986**, *51*, 4768-4779.

220. Suga, T.; Zheng, R.-Y.; Ohta, S.; Yoshioka, T.; Hirata, T. *Chem. Lett.* **1987**, 497-500.

221. Ito, M.; Koyama, T.; Ogura, K. *Chem. Lett.* **1986**, 101-104.

222. Boyer, F.; Ducrot, P.-H. *Eur. J. Org. Chem.* **1999**, *1999*, 1201-1211.

223. Jung, J. C.; Kache, R.; Vines, K. K.; Zheng, Y. S.; Bijoy, P.; Valluri, M.; Avery, M. A. *J. Org. Chem.* **2004**, *69*, 9269-9284.

224. Larock, R. C.; Yang, H.; Weinreb, S. M.; Herr, R. J. *J. Org. Chem.* **1994**, *59*, 4172-4178.

225. Mazzocchi, P. H.; Wilson, P.; Khachik, F.; Klingler, L.; Minamikawa, S. *J. Org. Chem.* **1983**, *48*, 2981-2989.

226. Negishi, E.; Nguyen, T.; Boardman, L. D.; Sawada, H.; Morrison, J. A. *Heteroatom Chem.* **1992**, *3*, 293-302.

227. Becker, D.; Haddad, N. *Tetrahedron* **1993**, *49*, 947-964.

228. Morimoto, H.; Williams, P. G. *Fusion Technol.* **1992**, *21*, 256-261.

229. Tamai, Y.; Mizutani, Y.; Uda, H.; Harada, N. *J. Chem. Soc., Chem. Commun.* **1983**, 114-116.

230. Sugiyama, H.; Yokokawa, F.; Shioiri, T. *Org. Lett.* **2000**, *2*, 2149-2152.

231. Larock, R. C.; He, Y.; Leong, W. W.; Han, X.; Refvik, M. D.; Zenner, J. M. *J. Org. Chem.* **1998**, *63*, 2154-2160.

232. Kamiya, N.; Chikami, Y.; Ishii, Y. *Synlett* **1990**, 675-676.

233. Irifune, S.; Kibayashi, T.; Ishii, Y.; Ogawa, M. *Synthesis-Stuttgart* **1988**, 366-369.

234. Nunez, M. T.; Martin, V. S. *J. Org. Chem.* **1990**, *55*, 1928-1932.

235. McCaffery, E. L.; Shalaby, S. W. *J. Organomet. Chem.* **1967**, *8*, 17-27.

236. Liang, X. F.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2002**, 503-508.

237. Castellnou, D.; Sola, L.; Jimeno, C.; Fraile, J. M.; Mayoral, J. A.; Riera, A.; Pericas, M. A. *J. Org. Chem.* **2005**, *70*, 433-438.

238. Abe, N.; Hanawa, H.; Maruoka, K.; Sasaki, M.; Miyashita, M. *Tetrahedron Lett.* **1999**, *40*, 5369-5372.

239. Cesati, R. R.; de Armas, J.; Hoveyda, A. H. *Org. Lett.* **2002**, *4*, 395-398.

240. Crawford, R. J.; Lutener, S. B.; Cockcroft, R. D. *Can. J. Chem.* **1976**, *54*, 3364-3376.

241. Schmid, C. R.; Bryant, J. D.; Dowlatzedah, M.; Phillips, J. L.; Prather, D. E.; Schantz, R. D.; Sear, N. L.; Vianco, C. S. *J. Org. Chem.* **1991**, *56*, 4056-4058.

242. Cardillo, G.; Gentilucci, L.; De Matteis, V. *J. Org. Chem.* **2002**, *67*, 5957-5962.

243. Wistuba, D.; Weigand, K.; Peter, H. *Chem. Res. Toxicol.* **1994**, *7*, 336-343.

244. Andreana, P. R.; McLellan, J. S.; Chen, Y.; Wang, P. G. *Org. Lett.* **2002**, *4*, 3875-3878.

245. Patel, D. V.; Vanmiddlesworth, F.; Donaubauer, J.; Gannett, P.; Sih, C. J. *J. Am. Chem. Soc.* **1986**, *108*, 4603-4614.

246. Brown, H. C.; Chandrasekharan, J.; Ramachandran, P. V. *J. Am. Chem. Soc.* **1988**, *110*, 1539-1546.

247. de Azevedo, M. B. M.; Murta, M. M.; Greene, A. E. *J. Org. Chem.* **1992**, *57*, 4567-4569.

248. Kadrmas, J. L., and Poulter, C. D., unpublished work.