Synthesis of asymmetric phosphonate prodrugs

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SYNTHESIS OF ASYMMETRIC PHOSPHONATE PRODRUGS

by

Benjamin John Foust

A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Chemistry in the Graduate College of The University of Iowa

May 2019

Thesis Supervisor: Professor David F. Wiemer
To my family and friends
Everyone has a book inside them, which is exactly where it should, I think in most cases, remain.

Christopher Hitchens
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It has been impossible to compose this thesis without recalling the innumerable instances in which I required the support of those closest to me. My graduate career has been the most transformative period in my life, both professionally and personally, and I have many to thank for their contributions in this respect.

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ABSTRACT

The isoprenoid biosynthetic pathway is an essential metabolic system that is responsible for the production of one of the largest and most diverse ranges of biomolecules ever identified. The termini of this pathway include: fat-soluble vitamins, cholesterol, reproductive hormones, and components of cellular signal transduction and electron transport pathways. With such a diverse set of biologically important metabolites, it has become one of the most targeted pathways for study in human pathology. Earlier pharmaceutical development has yielded clinically relevant classes of compounds that impact specific enzymatically catalyzed stages along the mevalonate pathway. Possibly the most commonly recognizable class would be the statins, which were developed to constrain the production of cholesterol and other sterols via the inhibition of an early stage enzyme HMG-CoA reductase, making them useful in the treatment of cardiovascular disease. Another important class of inhibitors would be nitrogenous bisphosphonates such as Pamidronate and Zoledronate, which have been shown to disrupt the more downstream enzyme farnesyl diphosphate synthase (FDPS). The bisphosphonate core of these compounds helps to impart a high affinity for bone mineral, making them useful in the treatment of bone diseases such as osteoporosis and multiple myeloma.

Noting the success of bisphosphonates in the treatment of certain bone diseases via action on isoprenoid biosynthesis, more recent research has yielded compounds that selectively inhibit the later stage enzyme geranylgeranyl diphosphate synthase (GGDPS). One promising compound is digeranyl bisphosphonate (DGBP) which has been shown to induce apoptosis in some cancer cell lines. Crystallographic data for GGDPS aided in the determination of important structural features that lead to the activity and selectivity of DGBP. The bisphosphonate head group likely coordinates with magnesium cations in the active site of GGDPS, and the long nonpolar side chains
can occupy hydrophobic channels which normally allow the binding of the natural substrates. These data appear to suggest DGBP’s selective binding arises from its capacity to form a ‘V-shaped’ inhibitor with the geranyl groups at the alpha position extending into the hydrophobic regions within the enzyme and the bisphosphonate functionality establishing a strong electrostatic interaction with the magnesium ions.

Noting these features, the development of novel compounds that retain the seemingly important structural features of known inhibitors while modifying certain aspects intended to enhance the biological activity of the resulting compounds was undertaken. These novel compounds were envisioned through a synthetic approach that would incorporate one isoprenoid chain at the α-carbon of the bisphosphonate and a second as a phosphonic ester. The previously studied phosphonate salts also were modified to the corresponding pivaloyl oxymethyl (POM) protected esters. The resulting motif was intended to allow for the liberation of a highly anionic species within a cell, which could more closely resemble the charge of the naturally occurring pyrophosphate substrates while retaining more desirable ADMET properties in the prodrug form. After intracellular formation of the active salt, the isoprenoid chains in the new motif could adopt a structure with more gradual curvature through the central portion making the structure more closely resemble a ‘U-shaped’ inhibitor.

A short synthetic sequence for the production of these new bisphosphonate inhibitors was developed. Each of the triPOM species formed was tested for the ability to disrupt the action of GGDPS in multiple myeloma cells. The compounds showed rather potent activity in cellular bioassays, in the hands of our collaborators in the research group of Dr. Sarah A. Holstein, with EC_{50} values in the single digit micromolar range. These compounds confirmed that the use of POM ester functionality can act as a viable prodrug strategy for the intracellular delivery of
these compounds to the myeloma cells. These studies also demonstrate that the incorporation of a methyl group at the α-carbon results in the enhanced ability of these bisphosphonates to inhibit GGDPS. In general, the low µM range of concentrations required for these compounds to express meaningful cytotoxic biological activity makes them unattractive candidates for further investigation. However, the recognition of improved activity through the use of POM prodrug functionality and alpha methylation led to the development of other compounds that display these features. This has resulted in the synthesis of the most active inhibitors of GGDPS yet reported.

Another focus of research in the Wiemer group is the generation of novel phosphoantigens. Phosphoantigens are small phosphorus containing compounds that are recognized by certain immune cells and stimulate proliferation. The development of drugs that can regulate immune function via immunostimulatory activity is of great interest. The specific target for these phosphoantigens (γδ T cells) has recently been shown to play an important role in native cancer immunosurveillance. Currently the most potent natural phosphoantigen known, (E)-4-hydroxy-3-methyl-but-2-enyl diphosphate (HMBPP), is found in the non-mevalonate isoprenoid biosynthetic pathway of bacterial cells, which may help explain the development of its immunostimulatory effect. However, the inherent lack of metabolic stability of HMBPP, which translates to a short in vivo lifetime, provides a significant challenge to clinical utility. The mechanism for phosphoantigen stimulation of T cell production is not fully characterized, but recent work has demonstrated the importance of intracellular delivery of phosphoantigens that bind to an intracellular domain of the butyrophilin 3A1 protein necessary to induce proliferation.

Noting the potent activity of HMBPP, we have advanced the design and synthesis of structurally similar compounds with potentially enhanced metabolic stability and cellular permeability. Some more recent contributions to this project include the synthesis of tris-
pivaloyloxyethyl prodrug phosphinophosphonates. These phosphinophosphonates were found to produce a strong immunostimulatory cellular response, while demonstrating greater metabolic stability by virtue of the –C-P-C-P linkage replacing the anhydride –O-P-O-P arrangement in HMBPP. The POM prodrug functionality appeared to provide improved cell permeability as reflected in a significant increase in T cell proliferation.

Efforts to develop phosphoantigens with activity that matches or surpasses that of HMBPP, while exhibiting more desirable ADMET properties, are ongoing. The most recently developed compounds vary by the introduction of aryl phosphonic esters. The success of phosphorous-containing prodrugs that incorporate phenolic acid esters such as Sofosbuvir has supported our interest in this area. Preliminary T cell data for the lead compounds show great promise with significant proliferation at nanomolar concentrations. Biological assays for the phosphoantigens developed herein were performed by Dr. Andrew Wiemer and coworkers, and most of the tested compounds were found to be exceptionally active for the stimulated proliferation of Vγ9Vδ2 T cells. Studies are currently being conducted to form novel phosphoantigen prodrugs that express potent activity and desirable pharmacokinetic properties and will no doubt lead to the development of interesting and potentially therapeutically relevant compounds.
PUBLIC ABSTRACT

The isoprenoid biosynthetic pathway (IBP) is responsible for the biosynthesis of a host of metabolites essential for the healthy function of normal cells. Inhibitors of the IBP have been developed to treat a variety of human diseases. Notably statins such as lovastatin (Mevacor ®) which inhibit one of the earliest enzymes in the IBP can lower cholesterol. Other prescription drugs such as risedronate (Actonel®) inhibit a more downstream enzyme and can be used to treat bone conditions like osteoporosis. The early research described herein illustrates the development of a novel set of inhibitors for an enzymatic step that occurs later in the IBP, which may be useful for treatment of multiple myeloma, and provided important information to guide to the synthesis of subsequent generations of compounds.

The human IBP has a parallel in bacterial biosynthesis which is responsible for the production of a crucial intermediate for biosynthesis. The most downstream metabolite that is not shared among the two pathways, known as HMBPP, has been identified for its exceptional capacity to induce the proliferation of a subset of T cells. These T cells provide a potentially viable method for the treatment of any pathogen that they can identify, which would include bacterial infections as well as conditions such as lymphoma. The bioactivity of HMBPP, while impressive, cannot be easily conveyed to pharmaceutical relevance. Significant pharmacokinetic impediments are present and overcoming these barriers through the synthesis of structurally similar analogues provides the basis for the majority of the research described herein.
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CHAPTER 1:

INTRODUCTION TO ISOPRENOID BIOSYNTHESIS, PHOSPHONATE PRODRUGS, AND T CELL IMMUNOSTIMULATION

Isoprenoids, also called terpenoids, are among the oldest and most diverse sets of biomolecules ever to have been identified. Some triterpenoid compounds associated with plasma membrane permeability and rigidity, known as hopanoids, were first isolated from natural resins utilized in varnish for paintings in the early 20th century. However, these hopanoids have been found in sediment depositions as old as 2.5 billion years. Isoprenoids derive their name from the 5-carbon containing parent compound isoprene, which is the repeating unit that determines the carbon framework of resulting terpenoid products. This formation is accomplished through the isoprenoid biosynthetic pathway (IBP), which is active ubiquitously among bacteria, archaea, and eukaryotes. The IBP is responsible for the production of the largest group of contemporary natural products necessary for normal functioning organisms with over 50,000 compounds identified to date.

The linchpin of the IBP is the formation of the five-carbon isoprenoid building block isopentenyl diphosphate (IPP). Both IPP and its isomer dimethylallyl diphosphate (DMAPP) are employed to synthesize larger isoprenoids that contain multiple five-carbon components yielding mono-, sesqui-, di-, etc. terpenoids containing 2, 3, 4, etc. isoprene units. The vast array of polyterpenoid termini of the IBP include: fat-soluble vitamins, cholesterol, photosynthetic pigments, reproductive hormones, and components of cellular signal transduction and electron transport. The formation of IPP may be universal among eukaryotes, archaea, and bacteria, but there are two distinct and independent biosynthetic approaches to this common substrate.
Entry to the mammalian IBP, often referred to as the mevalonic acid pathway (MVA pathway, Figure 1), starts with the rate limiting formation of mevalonate from 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). This is the first committed step of this pathway, once the reaction is catalyzed by the enzyme HMG-CoA reductase. Mevalonate may then be phosphorylated, utilizing a phosphate equivalent originating from adenosine triphosphate (ATP), at the terminal alcohol to give the intermediate mevalonate-5-phosphate through the action of mevalonate-5-kinase. Mevalonate-5-phosphate can be further phosphorylated by ATP and decarboxylated in the formation of the cornerstone IPP. These processes are catalyzed by phosphomevalonate kinase (PMK) and mevalonate-5-diphosphate decarboxylase (MPD), respectively. An equilibrium may then be established between IPP and DMAPP through the action of isopentenyl diphosphate isomerase.² ⁵
Once formed, DMAPP may be condensed with two equivalents of IPP to form the 15-carbon intermediate farnesyl diphosphate (FPP) through the catalytic action of farnesyl diphosphate synthase (FDPS). FPP provides a branching point in the later stages of the isoprenoid pathway (Figure 2). One branch that is vital in normal human metabolism utilizes squalene synthase (SQS) to initiate the formation of squalene and ultimately has cholesterols and steroids as endpoints. Furthermore, FPP also can be utilized by farnesyl transferase (FTase) to add isoprenoid character to a variety of proteins through posttranslational modification, notably the Ras and Rho families of small guanosine triphosphatases (GTPases), which are involved in essential cellular signal transduction and transcription.
Another 5-carbon unit may be added to FPP by geranylgeranyl diphosphate synthase (GGDPS) to provide the 20-carbon compound geranylgeranyl diphosphate (GGPP), which allows further branching in isoprenoid biosynthesis. GGPP may be acted upon by geranylgeranyl transferase I or II (GGTase I and GGTase II, respectively) to alter Rho, Rap, and Rab proteins. These proteins are families of the Ras superfamily of proteins that share a common core G domain, and this addition of a geranylgeranyl unit activates the protein for its use in cellular regulation.6-7

![Figure 2. Later Stage Isoprenoid Biosynthesis](image)

The critical role in cellular function played by the IBP has made it one of the most heavily targeted metabolic pathways for the formulation of therapeutic agents.7 The vast array of products formed through the IBP means that careful manipulation may have utility in the treatment of a
variety of disorders. Pharmaceutical development has yielded clinically relevant classes of compounds that impact specific enzymatically catalyzed steps along the path. Possibly the most commonly proscribed and recognizable class would be the statins (e.g. mevastatin, lovastatin, atorvastatin, and fluvastatin, Figure 3), which bind to and act as reversible competitive inhibitors of HMG CoA reductase in the earliest committed step in the IBP.\textsuperscript{8-9} The inhibition of HMG CoA reductase moderates the formation of mevalonate and this upstream effect is transmitted through the pathway ultimately having the impact of lowering cholesterol levels.\textsuperscript{10} This inhibition also depletes the levels of intermediates like FPP and GGPP, consequently limiting protein prenylation (i.e. farnesylation and geranylgeranylation) and may have interesting consequences for the treatment of certain cancers.\textsuperscript{9,11}
Figure 3. HMG-CoA Reductase Inhibitors

The later stages of the isoprenoid pathway include a variety of compounds that contain diphosphate functionality, often emanating from the most commonly identifiable phosphate containing biomolecule ATP. Utilizing ATP synthase, many energetically demanding processes can couple the hydrolysis of the phosphate bond in ATP with less favorable reactions. This progression may be advantageous in many circumstances that demand chemical energy, but the inherent biological instability of the diphosphate group has made it necessary to turn to more stable analogues when designing pharmaceuticals which act upon enzymes that recognize diphosphates.
Geminal bisphosphonates (Figure 4) have two phosphorus atoms bonded to a central methylene unit, in place of the oxygen present in diphosphates, which provides a chemically similar structure with greatly enhanced metabolic stability.\textsuperscript{14} The addition of the $\alpha$-hydroxy functionality increases the affinity of bisphosphonates for calcium and can naturally allow for bioaccumulation in bone.\textsuperscript{15} This has led to the development of nitrogenous bisphosphonates including zoledronate and risedronate (Figure 4), which are used in the treatment of bone diseases such as osteoporosis, Paget’s disease, and certain bone cancers including multiple myeloma.\textsuperscript{16} These compounds act as inhibitors of FDPS, which in turn restricts the formation of FPP, GGPP, and thereby protein prenylation. Studies suggest that these cellular effects are due to the diminished quantities of GGPP, as the introduction of geranylgeraniol circumvents the action of risedronate and zoledronate on bone resorption.\textsuperscript{17} Considering these data, it may be desirable to synthesize bisphosphonate inhibitors that may promote the desired depletion of GGPP through direct inhibition of GGDPS. This process may provide a useful scaffold for the treatment of multiple myeloma.\textsuperscript{18}

![Figure 4. Diphosphate, Bisphosphonate, and Nitrogenous Bisphosphonates](image-url)
Multiple myeloma is a bone marrow plasma cell cancer that directly affects approximately 100,000 people in the U.S. alone. It is considered treatable but generally incurable, and with a prognosis of approximately 50% five-year survival rate it is a major target for pharmaceutical development.\textsuperscript{14,19} There are several common characteristic features of multiple myeloma: bleeding and infection caused by plasma cell overcrowding, increased blood calcium levels owing to overactive osteoclast function, and kidney damage from the overproduction of antibodies (monoclonal proteins) in malignant plasma cells.\textsuperscript{19}

This last feature was identified as a potential method for differentiation between cancerous and healthy cells. Plasma cells naturally contain the necessary machinery within the endoplasmic reticulum (ER) to excrete volumes of antibodies. However, if protein secretion can be significantly disrupted it would have the maximum effect on cells which produce them at the greatest rate, namely cancerous myeloma cells. Conditions that seriously disrupt ER protein folding triggers the unfolded protein response (UPR) pathway and may lead to a form of programmed cell death known as apoptosis.\textsuperscript{13,20}

Inhibition of GGDPS and, by extension protein prenylation, is one method to disrupt secretion and is therefore a potential strategy to employ in the treatment of multiple myeloma. A variety of non-nitrogenous bisphosphonates have been developed in the Wiemer group with this express purpose.\textsuperscript{21-23} A bisphosphonate containing isoprenoid character emanating from the alpha carbon has been a successful framework for the advancement of GGDPS inhibitors. Both x-ray analysis and modelling of crystallized GGDPS enzyme (Figure 5) can provide an excellent basis for the design of effective competitive inhibitors, as such studies can show important substrate interactions within the active site.\textsuperscript{24}
One of the non-nitrogenous bisphosphonates that shows significant selective inhibitory activity for GGDPS is digeranyl bisphosphonate (DGBP), which displays an IC\textsubscript{50} value of 0.2 μM\textsuperscript{21}. Crystallographic and computational data on DGBP’s interaction with GGDPS (Figure 5) shows the importance of the bisphosphonate’s high anionic charge density, which bears three negative charges at physiological pH and can coordinate well with the two magnesium cations shown in the active site\textsuperscript{24}. The two geranyl chains emanating from the α carbon can occupy hydrophobic channels in a manner similar to the natural substrate and product. An overlay of the natural compounds and the studied inhibitors showed comparable arrangements leading to an electronic distribution resembling a “V-shape”.\textsuperscript{24} This motif has provided compounds that act as inhibitors of GGDPS, but may not represent the only viable arrangement.

Figure 5. GGDPS Active Site Studies\textsuperscript{24}

The bisphosphonate DGBP provides a model for GGDPS inhibitors, as it binds to the active site with reasonable affinity and displays greater metabolic stability when compared with analogous diphosphates, but DGBP does suffer from one notable shortcoming. The high anionic charge density of the free phosphonic salt, which provides necessary complexation with Mg\textsuperscript{2+} ions
in the active site of the target enzyme, does not allow for facile passive diffusion across the lipid bilayer of a cell membrane. To avoid this prohibitive behavior exhibited by necessary functionality it may be deemed necessary to employ a prodrug strategy (Figure 6).

![Figure 6. General Prodrug Strategy](image)

Prodrugs are inactive, or less active, bioreversibly masked derivatives of a compound that must undergo chemical and/or enzymatic transformation \textit{in vivo} to liberate the active pharmacophore. The advantage of this strategy is the capability to manipulate relevant pharmacokinetic properties one must consider in drug formulation: absorption, distribution, metabolism, excretion, and toxicity (A.D.M.E.T.). The prodrug approach can circumvent inherent drawbacks such as: poor solubility, lack of site selectivity, nonspecific toxicity, or chemical instability. In the specific case of phosphonates that display high anionic charge that limits passive cellular absorptivity, phosphonic anions have been masked with pivaloyloxyethyl (POM) ester promoieties. These POM esters have been shown to increase bioavailability of
phosphonates and, once acted upon by so called “non-specific esterases”, they release the parent phosphonate salt with an equivalent of formaldehyde and pivalic acid within the cell.25

One clinically relevant example of the POM prodrug strategy can be seen in the study of the reverse transcriptase inhibitor Adefovir.29 Adefovir is used as an antiretroviral to inhibit the first phosphorylation during the DNA replication of viral propagation, thus limiting the expansion of the hepatitis virus. However, the free phosphonate salt suffers from low bioavailability. Implementation of the POM prodrug strategy allows for the administration of Adefovir to infected cells as the more bioavailable Hepsera ®, and once converted to the active form intracellularly it can provide some relief for chronic hepatitis B sufferers that display active symptoms.30-31

Figure 7. Hepsera ® Prodrug Methodology

While the mevalonate pathway is employed in mammals to generate isoprenoids, there exists a second and distinct pathway employed by Nature to generate IPP and DMAPP. This non-mevalonate pathway, also called the 2-C-methyl-D-erythritol 4-phosphate (MEP) or 1-deoxy-D-xylulose 5-phosphate (DXP) pathway (Figure 8), is initiated by the formation of MEP from DXP by DXP reducto-isomerase (DXR).2-4 MEP may then be converted to ethylerythritol cytidyl diphosphate (CDP-ME) through a coupling with cytidine 5’-triphosphate (CTP) catalyzed by
CDP-ME synthetase (IspD). Then CDP-ME may be phosphorylated utilizing the ATP-dependent enzyme IspE yielding 4-diphosphocytidyl-2-C-methyl-D-erythritol-2-phosphate (CDP-MEP), which can then be cyclized by IspF to 2-C-methyl-D-erythritol-2,4-cyclodiphosphate (MEcPP). This cyclic intermediate may be opened by the enzyme IspG and a reductive dehydration leads to the formation of 4-hydroxy-3-methyl-butenyl 1-diphosphate (HMBPP). To merge with the intermediates of the MVA pathway, HMBPP may be converted to the 5-carbon building blocks IPP and DMAPP through the catalytic action of IspH.\(^4\)

![Figure 8. MEP Pathway](image-url)
The last unique metabolite in the non-mevalonate pathway, before the formation of the central IPP unit, is HMBPP. This intermediate is not shared in the human isoprenoid biosynthetic pathway and it has been suggested that this has allowed for the evolution of a significant immune response upon cellular identification of HMBPP. The HMBPP acts as a small, phosphorus containing, non-peptide ligand of the transmembrane protein butyrophilin 3A1 (BTN3A1, Figure 9).\textsuperscript{32-33} Crystallographic investigation of BTN3A1 highlighted potential binding sites in both the extracellular and intracellular domains that might lead to the measured immunostimulation.\textsuperscript{34-35} This binding allows antigen presenting cells (APC’s) to promote the proliferation of a subset of immune cells known as the $\gamma\delta$ T cells. These T cells naturally have the capacity to detect and eliminate infected cells that express non-self antigen and are therefore potentially useful in the treatment of a variety of pathogens such as certain bacteria, protazoal parasites, and cancerous cells.\textsuperscript{33}
The Vγ9Vδ2 T cells normally account for only a small portion of circulating immune cells. However, once a sufficient concentration of a proliferative agent is identified the population can be greatly expanded. Following this event, circulating Vγ9Vδ2 T cells may mount an immune response to bacterial infection or cancerous cell propagation. Because γδ T cells have been shown to induce T cell mediated lysis of myeloid leukemia cells, they show great promise for this disease and several other cancers.36

Direct intravenous treatment with the most potent identified natural proliferative agent HMBPP, did not prove to be a viable strategy for long lasting proliferative stimulation. Instead HMBPP undergoes rapid metabolism in blood plasma due to the previously mentioned inherent metabolic instability of the diphosphate group.37 This feature, coupled with the high anionic charge

Figure 9. BTN3A1, Internal Binding Site, Apo Form and HMBPP Bound34-35
density that limits cellular absorption, makes these compounds prime targets for the development of more stable ligands and implementation of a prodrug approach.\textsuperscript{38}
CHAPTER 2:

SYNTHESIS AND BIOLOGICAL ACTIVITY OF A NEW MOTIF FOR INHIBITORS OF GERANYLGERANYL DIPHOSPHATE SYNTHASE

Protein prenylation is commonly viewed as one of the later stages of the isoprenoid biosynthetic pathway (IBP). Inhibitors of enzymes along the IBP such as statins, nitrogenous bisphosphonates, and digeranyl bisphosphonate (DGBP) and its analogues, can disrupt the function of HMG-CoA reductase, farnesyl diphosphate synthase (FDPS), and geranylgeranyl diphosphate synthase (GGDPS), respectively. This leads to modulation of protein post-translational prenylation, which makes compounds like lovastatin, risedronate, and DGBP models for the design of structurally similar inhibitors.5

The nitrogenous bisphosphonates zoledronate, risedronate, alendronate, and pamidronate (Figure 10) are used clinically for the treatment of osteoporosis and other bone diseases through their action as FDPS inhibitors. This inhibition diminishes the cellular biosynthesis of farnesyl diphosphate (FPP), and can indirectly decrease the levels of geranylgeranyl diphosphate (GGPP) and consequently geranylgeranylated proteins. The overall impact is a measureable decrease in osteoclast-facilitated bone resorption.14, 19
The minimization of geranylgeranylated Rab proteins has been shown to impact intracellular membrane trafficking and protein secretion. This provides a potential avenue for the treatment of diseases like multiple myeloma, which over-produce monoclonal proteins. If monoclonal protein secretion is disrupted, the resulting cellular stress could selectively induce apoptosis in these over-productive myeloma cells. However, significant suppression of the FDPS enzyme also will alter the normal function of downstream processes such as cholesterol formation. This may have unintended off-target effects on healthy cells. In order to circumvent this negative side effect, the inhibition of a more downstream enzymes like GGDPS, which impact protein geranylgeranylation in a more direct fashion, but do not significantly alter cholesterol formation, may be highly desirable.\(^{39}\)

Previous research in the Wiemer group has led to the development of DGBP analogues that display varying isoprenoid chains at the alpha carbon and retain the bisphosphonate anionic core. Careful crystallographic and computational analysis has elucidated important structural features that allow for binding of these compounds to GGDPS. The bisphosphonate head group that bears high anionic charge can complex with magnesium cations in the active site that likely bind the
diphosphate functionality found in the natural substrates and product during the normal function of this enzyme. The isoprenoid chains may adopt a “V-shaped” orientation to mimic natural binding and once in place may prohibit catalytic action in a competitive fashion.\textsuperscript{24}

Early investigation of DGBP analogues, done in the Wiemer group, led to the synthesis of mono- and dialkyl bisphosphonates (2.01–2.10) with various isoprenoid chain lengths (Figure 11).\textsuperscript{23, 40} The listed compounds were all studied for their capacity to inhibit the IBP, and most were found to be effective inhibitors of protein geranylgeranylation. The most potent inhibitor found at the time was 2\textit{E}, 6\textit{E}-farnesyl bisphosphonate (compound 2.06). The measured half maximal inhibitory concentration (IC\textsubscript{50}) had a value of 0.1 µM for the inhibition of GGDPS. However, this farnesylated bisphosphonate also was found to inhibit squalene synthase (SQS) and farnesyl transferase (FTase) as well as the intended GGDPS target. This lack of selectivity may make this compound less desirable as an inhibitor of protein prenylation, as in the case of the established nitrogenous bisphosphonates. One of the dialkylated bisphosphonates studied was DGBP (2.02), which displayed an IC\textsubscript{50} of 0.2 µM and showed selectivity for GGDPS over FDPS and other more upstream enzymes. The selectivity of the dialkylated DGBP makes it an attractive lead despite its slightly lower inhibitory activity.\textsuperscript{40}
Figure 11. Select Mono- and Dialkyl Bisphosphonate Inhibitors

The set of isoprenoid mono- and di-alkylated bisphosphonate inhibitors of GGDPS was further expanded to include compounds that incorporate slightly longer aliphatic side chains with the synthesis of C-homoalkyl and ether linked O-alkyl analogues (Figure 12). Previous study of bisphosphonates bearing an oxygen at the α carbon shows an inductive electron-withdrawing effect on the phosphonate head group, which results in lowering the pKa values for the corresponding phosphonic acids relative to the parent carbon analogue. This may increase the compounds’ similarity to the natural isoprenoid diphosphate ligand, which naturally has a pKₐ value.
approximating physiological pH, and has been reported to increase the biological activity of bisphosphonates. Within this investigation, the C-geranyl O-citronellyl bisphosphonate (compound 2.10) showed the greatest activity with an IC$_{50}$ value of 82 nM when assayed with GGDPS. This compound also displayed reasonable selectivity for GGDPS, with no activity against FDPS measured at concentrations as high as 10 µM.$^{42}$

Another significant point of interest in this set is the measured difference in activity between pairs of constitutional isomers. Compounds (2.07 and 2.08) have the same molecular formula and functionality, but differ slightly in the connectivity, which makes them constitutional isomers. This seemingly minor difference was noted to have a statistically relevant impact on the measured activity of these compounds for their ability to inhibit GGDPS with the mono-prenyl mono-homogeranyl compound 2.07 showing an IC$_{50}$ of 0.5 ± 0.1 µM and the mono-geranyl mono-homoprenyl compound 2.08 displaying an IC$_{50}$ value of 0.2 ± 0.1 µM.$^{41}$
Another significant advancement in the activity of DGBP analogues can be noted in the implementation of a prodrug strategy for drug delivery. The high anionic charge density that is important for IBP inhibitor binding is incompatible with facile passive cellular absorptivity. It has been shown that highly charged bisphosphonates such as the NBPs require endocytosis for meaningful concentrations to enter the cell.\textsuperscript{43-44} This limitation was sidestepped through the
installation of POM phosphonate prodrug esters, which allow for neutral molecules to traverse the cellular lipid bilayer before being metabolized to the active drug.\textsuperscript{22} This resulted in inhibitors that do not show direct activity for GGDPS inhibition in the POM form, but show increased activity, in every analogue tested, for inhibition of Rap1a geranylgeranylation in intact cells.

All of the dialkylated bisphosphonates discussed thus far have the capacity to exhibit the aforementioned “V-shaped” conformation that was observed in the crystallographic and computational study of GGDPS binding and inhibition. The installation of dialkyl character through modification at the alpha carbon is therefore a reasonable arrangement for the synthesis of inhibitors, but it is not necessarily the optimal motif.

Noting the potential impact of constitutional isomerization and the success in the installation of prodrug functionality, a new motif for bisphosphonate inhibitors was envisioned that could incorporate one of the isoprenoid chains as a phosphonic ester and one or two additional chains emanating from the alpha position. This new scaffold might offer more rotational freedom and more separation between the isoprenoid fragments installed at the alpha position and the phosphorus. If two alkyl chains attached at the $\alpha$ carbon to the bisphosphonate functionality was described as a “V-shaped” arrangement, this new motif might be thought of as a “U-shaped” inhibitor (Figure 13).
Figure 13. V-shaped and U-shaped Inhibitors

The most efficient route to the newly proposed “U-shaped” framework was envisioned through a sequence analogous to the previously established synthesis of bisphosphonate inhibitors. An anion formed at the alpha position of the bisphosphonate may be stabilized through resonance which decreases the pK$_a$ relative to nonfunctionalized hydrocarbons. This has allowed for deprotonation with a strong base and subsequent alkylation with isoprenoid-based alkyl halides, yielding a fast and efficient formation of alkyl bisphosphonates.

The first approach taken in the attempted synthesis of “U-shaped” inhibitors (Figure 14) started with commercially available tetramethyl methylenediphosphonate, which was allowed to react with chloromethyl pivalate (POMCl) in the presence of sodium iodide. A single POM ester was removed from the resulting tetraPOM bisphosphonate in quantitative yield by reaction with
1,4-diazabicyclo[2.2.2]octane (DABCO). The phosphonic salt was then converted to the acid chloride by reaction with oxalyl chloride and catalytic DMF followed by esterification with anhydrous methanol in the presence of triethylamine. Methanol was chosen as the simplest alcohol to establish a model for reactivity. The esterification proceeded in modest yield but did establish proof of concept for the selective manipulation of a single phosphonic ester of the bisphosphonate. This asymmetric bisphosphonate was treated with sodium hydride followed by freshly prepared geranyl bromide (compound 2.14) to introduce an alpha substituent in a similar fashion to previously reported methodology. However, this reaction sequence did not provide the desired geranylated bisphosphonate, but rather gave multiple phosphorus-containing, water soluble products that were likely the result of POM degradation.
Figure 14. First Attempted Synthesis of "U-Shaped" Inhibitor

Noting the sensitivity of the POM functionality, the synthesis was revised to establish the alpha alkyl substituent before the prodrug installation. This attempt utilized the same commercially available starting material, tetramethyl methylenediphosphonate, which was allowed to react with potassium bis(trimethylsilyl)amide (KHMDS). This deprotonation was followed by reaction with
freshly prepared geranyl bromide. The mono-geranylated product 2.20, which was the major product, was further treated with sodium hydride and iodomethane. The mono-geranyl monomethyl bisphosphonate 2.21 was formed in quantitative yield. The tetraPOM prodrug (2.22) functionality was installed again by reaction with chloromethyl pivalate and sodium iodide, and a single POM ester was removed by reaction with one equivalent of DABCO to give compound 2.23. The mono salt formation proceeded in excellent yield. However, subsequent efforts to prepare the acid chloride and then esterify were unsuccessful in establishing a geranylated bisphosphonate ester. This failure to isolate the desired geranylated product was likely due to the inherent sensitivity of allylic phosphonic acid esters. After learning of this sensitivity, as well as the potency of citronellyl ether linked bisphosphonates synthesized by the Wiemer group, substitution of a geranyl phosphonic ester with a citronellyl ester seemed reasonable. The end result would be a constitutional isomer of the newly synthesized, and most potent reported inhibitor, compound 2.10.
With increased respect for the POM prodrug functionality and the desired formation of a citronellyl phosphonate ester in mind, a synthesis was devised that would install the phosphonic acid ester early on and save the POM formation for the final step (Figure 16). The envisioned synthesis started from the commercially available monophosphonate 2.25, dimethyl methylphosphonate, as opposed to the bisphosphonate used in the previous synthesis. Dimethyl
methylphosphonate was treated with \( n \)-butyl lithium and alkylated by reaction with geranyl bromide (2.14) to give the dimethyl homogeranylphosphonate product 2.26 in high yield. The mono methyl ester mono acid chloride then could be obtained by reaction of compound 2.26 with oxalyl chloride and catalytic DMF. In principle, reaction of the resulting acid chloride with a sufficient alcohol could afford a mixed diester, but with respect to the sensitivity of allylic phosphonate esters, \( S \)-(-)citronellol was employed to provide the mixed phosphonate 2.28. Phosphonylation then was carried out by reaction of compound 2.28 with lithium tetramethyl piperidine and dimethyl chlorophosphate in low isolated yield.\(^{45}\)

The resulting asymmetric bisphosphonate (compound 2.29) served as a point of divergence to obtain several products of similar structure. Reaction of the trimethyl bisphosphonate 2.29 directly with POMCl in the presence of sodium iodide gave the desired triPOM compound 2.30. This prodrug installation proceeded in poor yield but was successful in providing the first asymmetric “U-shaped” isoprenoid bisphosphonate. The dealkylation and POM installation appeared to proceed selectively, but not necessarily specifically, at the methyl phosphonic ester. Compound 2.29 also was allowed to react with sodium hydride and several different alkyl halides, namely: iodomethane, allyl bromide, prenyl bromide, and geranyl bromide, to afford compounds with additional alkyl substituents emanating from the \( \alpha \) position. Each of the resulting trimethyl bisphosphonates was treated with POMCl and NaI to impart prodrug functionality.\(^{45}\)
Figure 16. Synthesis of Bisphosphonate Mixed Esters
Each of the aforementioned bisphosphonates was formed as a mixture of stereoisomers. The \((S)\)-citronellyl group was installed as a single enantiomer by virtue of the choice of starting material. However, the stereochemistry at the phosphorus center was not controlled through the listed reaction sequence. This is readily apparent when considering the \(^{31}\text{P}\) NMR spectra of these compounds. In the phosphorus spectrum of the mixed mono-methyl mono-citronellyl monophosphonate \textbf{2.28}, two resonances of equal intensity can be observed, reflecting the formation of two diasteromers after the installation of the citronellyl ester.

The subsequent phosphonylation reaction formed an additional stereogenic center at the carbon \(\alpha\) to the phosphonate functionality and provided a more complex mixture of diastereomers. This complexity can be most easily recognized by the amplified intricacy of the \(^{31}\text{P}\) spectra. The resulting asymmetric bisphosphonate gives a spectrum with two clusters of peaks reflecting both the formation of the new stereogenic center and phosphorus-phosphorus coupling. Similar clusters are observed in most of the subsequent products, with the exception being the bisphosphonate where a second geranyl chain was introduced at the \(\alpha\)-carbon (\textbf{2.34}). This alkylation eliminates the stereocenter and decreases the number of diastereomers formed, thereby diminishing the complexity of the resulting \(^{31}\text{P}\) NMR spectrum.

Assuming the POM products would function as prodrugs, as designed, the phosphorus stereocenter will be destroyed once the POM group of the mixed phosphonate ester is metabolized intracellularly. The stereochemistry at the alpha carbon, at least in the compounds in which it remains a stereocenter, may have a more lasting impact on bioactivity. Even after prodrug hydrolysis, the tested compounds will remain mixtures of diastereomers. In theory, these diastereomers can be separated through traditional methods, but in practice they often require extensive effort. At the time of synthesis, this was deemed inappropriate until the bioactivity of
this new motif could be gauged. The risk of having a greater number of potential variables when attempting to compare the bioactivity of structurally similar compounds was found to be worthwhile in the synthesis of comparable inhibitors of GGDPS at this same time.\textsuperscript{46-47} In the analogous example, a mixture of alkene geometric isomers was first assayed and demonstrated activity that was greater than the individual stereoisomers that were developed through subsequent synthesis.\textsuperscript{48-49}

In order to probe the potential impact that this stereochemical information may have on the biological activity of these compounds, we began to investigate the importance of the citronellyl stereochemistry (Figure 17). A parallel divergent synthesis utilizing the common phosphonic acid chloride \textsuperscript{2.27} as an intermediate, began with treatment of the \textit{R-}(+)-citronellol enantiomer which had been derived from commercially available \textit{R-}(+)-citronellal. The resulting mixed ester compound \textsuperscript{2.40} was then subjected to phosphonylation conditions to afford the asymmetric bisphosphonate compound \textsuperscript{2.41}. This was followed by methylation with iodomethane and POM prodrug installation by reaction with POMCl and NaI. The resulting mixture was a set of diastereomers that would be enantiomeric with the set isomers in of the previously synthesized mixtures. This mixture was then submitted to Professor Sarah A. Holstein, formerly of the Roswell Park Comprehensive Cancer Center, for biological testing.\textsuperscript{45}
Figure 17. Synthesis of \(R-(+)-\text{Citronellol Bisphosphonates}\)
Biological Results

To gauge the impact of this new motif on isoprenoid metabolism, several compounds’ ability to disrupt geranylgeranylation in multiple myeloma cells was determined. All of the tested compounds displayed activity for disruption of post-translational protein geranylgeranylation and the activity was established via two methods: 1) immunoblot analysis of unmodified Rap1a, which is a natural substrate for the later enzyme of the IBP GGTase 1 (Figure 18 A); and 2) ELISA analysis of intracellular lambda light chain which is a marker for disruption of Rab GTPase geranylgeranylation (Figure 18 B). These Rap and Rab proteins normally aid in protein transport after their geranylgeranylation, but with significant disruption their diminished capacity may lead to apoptosis in these cancerous myeloma cells. Of the five compounds of the S-(-)-citronellol ester-containing series examined, the alpha methylated derivative 2.35 was the most potent. Ranked in order from most to least potent: the order is 2.35 > 2.37 > 2.36 ≈ 2.38 > 2.30. The previously discussed HMG-CoA reductase inhibitor lovastatin was utilized to establish a positive control for the inhibition of the synthesis of mevalonate and therefore all downstream isoprenoids.45
Figure 18. New Bisphosphonates Disrupt Protein Geranylgeranylation

A) Immunoblot analysis of Rap1a (unmodified protein) and β-tubulin (loading control)
B) ELISA of intracellular lambda light chain concentrations with lovastatin positive control

(* denotes p < 0.05 per unpaired two-tailed t-test)
The cytotoxic activity of this set of compounds was based on their effect on three human-derived myeloma cell lines. The activity trend seemed to hold for each cell line yielding a small structure-function relationship among the tested compounds, with the methyl derivative \textbf{2.35} displaying the most potent activity and the geranyl derivative \textbf{2.38} showing the least activity. In general, the cytotoxic effect correlated with the potency of these compounds in the disruption of protein geranylgeranylation. The additional alpha isoprenoid character, intended to maximize the molecular interactions of these compounds with the hydrophobic channels in GGDPS, such as in the case of the digeranylated compound \textbf{2.38}, does not seem to enhance the cytotoxic effect of these compounds.

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Table 1. Cytotoxic Activity of the Tested Compounds in Human Cell Lines

(EC\textsubscript{50} values determined by MTT assay)

Once the α-methyl derivative was identified as the most potent member of this family, add-back experiments were performed with isoprenoid intermediates. Addition of either mevalonate or GGDP prevents the ability of lovastatin to disrupt protein geranylgeranylation, but only GGDP
diminishes the effect of compound 2.35. This was observed in both the western analysis (Figure 19 A) and in the ELISA (Figure 19B). These data support the idea that these compounds work to inhibit GGDP as there is a global disruption of geranylgeranylation of both GGTase I and GGtase II substrates and addition of GGDP circumvents these effects.

Figure 19. Novel Bisphosphonate Disrupts Protein Geranylgeranylation by Depletion of GGDP

A) Immunoblot analysis of Rap1a and β-tubulin (loading control)
B) Intracellular lambda light chain concentrations determined via ELISA

(* denotes p < 0.05 per unpaired two-tailed t-test)

Biological assays also were performed to determine the effect that the citronellyl stereochemistry had on the activity on this motif. The (R)-(+)-citronellol analogue was compared directly with the (S)-(−)-citronellol derivative. The activity of the two was very similar, with the (R) stereoisomer showing slightly more activity at lower concentrations based on the levels of unmodified Rap1a (Figure 20 A) and accumulation of intracellular lambda light chain (Figure 20 B). However, these differences were close to the limits imposed by error and further study would be required to ascertain the full effect.
In summary, a small set of compounds that exhibit a novel motif, namely the installation of isoprenoid character through mixed phosphonate ester formation, has been prepared and evaluated for bioactivity as inhibitors of GGDPS. These compounds confirmed that the use of the POM ester functionality can act as a viable prodrug strategy for the delivery of these compounds to multiple myeloma cells. These studies also demonstrate that the incorporation of a methyl group...
at the α-carbon results in enhanced ability of these bisphosphonates for inhibition of GGDPS. In general, the low μM range of concentrations was required for these specific compounds to express meaningful cytotoxic biological activity, which makes them unattractive candidates for further investigation. However, the recognition of improved activity through the use of POM prodrug functionality and alpha methylation led to the development of triazole based compounds, synthesized by others in the Wiemer group, that display these features. This has resulted in the synthesis of the most active inhibitors of GGDPS yet reported.\textsuperscript{50}
CHAPTER 3:

SYNTHESIS AND BIOLOGICAL ACTIVITY OF PHOSPHINOPHOSPHONATES AS ANALOGUES OF HMBPP

There is substantial interest in the development of strategies to alter human immune function via immunostimulatory activity. Among the most promising strategies currently studied is the utilization of an important and unconventional set of immune cells known as $\gamma\delta$ T cells. In coordination with other cell types, $\gamma\delta$ T cells contribute to the innate and adaptive immune responses to infectious diseases. The innate immune system is described as the first line of defense, responding rapidly through a less specific mechanism whereas the adaptive immune system is responsible for antigen-specific immune function after the innate response has run its course. However, $\gamma\delta$ T cells are unique in blurring the lines that distinguish between these two immune strategies.\(^5\)

During the propagation of a general T cell immune response, antigens are taken up by dendritic cells, often at the site of infection. Dendritic cells are immune cells responsible antigen presentation. These cells can then migrate to the lymph nodes to interact with and activate the proliferation of T cells. Dendritic cells often interact with T cells by binding through an antigen-containing major histocompatibility complex protein, but non-traditional T cell activation may also occur through direct antigen recognition without the use of this major histocompatibility complex. The activated T cells can then migrate from the lymph nodes into the blood stream and ultimately stimulate macrophages or directly terminate infected cells.\(^5\)\(^1\)\(^-\)\(^2\)

The $\gamma\delta$ T cell populations constitute approximately 4% of peripheral blood lymphocytes (PBL’s) in healthy adults and their number may be expanded to as much as 60% when combating
a variety of infectious diseases. The expansion of these populations would be a potentially viable approach for the treatment of any pathogens that are recognized by these cells. Most of the expanded γδ T cells that result from infection-driven proliferation express Vγ9 and Vδ2 T cell receptors. Previous study of the in vitro expansion of these cells, through the incubation of PBL with mycobacterial lysates, noted that the stimulatory components of the lysates were protease resistant and phosphatase sensitive. Unlike the αβ T cells that respond to peptides, the Vγ9Vδ2 T cells were found to recognize small phosphorylated non-peptidic molecules that are metabolic intermediates of isoprenoid biosynthesis such as (E)-4-hydroxy-3-methyl-but-2-enyl-diphosphate (HMBPP) and isopentenyl diphosphate (IPP), often referred to in the literature as phosphoantigens. The compound found to have the most significant impact on the proliferation of human Vγ9Vδ2 T cells was HMBPP, which is the last unique metabolite in the non-mevalonate pathway not shared in the human IBP. The unique sensitivity of the T cell proliferative response to HMBPP was likely developed through evolutionary means as a method to fight bacterial infections.

Proliferation of Vγ9Vδ2 T cells may be stimulated through two known methods, direct and indirect action. Indirect action may be understood through the example of the use of nitrogenous bisphosphonates (NBPs), whose clinical relevance was previously discussed. The nitrogenous bisphosphonates’ capacity for direct inhibition of FDPS has the impact of diminishing cellular concentrations of the product FPP, but it is also known to increase the concentration of IPP, the substrate that is naturally consumed during this catalysis. At high enough concentrations, IPP may then act through direct means to stimulate the proliferation of T cells through its function as a phosphoantigen.

Investigation into the direct activation of Vγ9Vδ2 T cells has led to the identification of the vital role of a transmembrane B7 family of proteins. More specifically, the so-called
phosphoantigens such as HMBPP are detected by the Vγ9Vδ2 T cell receptor (TCR) after binding to butyrophilin 3A1 (BTN3A1). This binding occurs through a major histocompatibility complex-independent mechanism that is not fully understood, as binding of phosphoantigens has been reported to occur in both the intracellular and extracellular domains of BTN3A1.55, 54-55 Once binding occurs, activated Vγ9Vδ2 T cells can eliminate phosphoantigen-loaded target cells and recruit other immune cells to assist in pathogen suppression. Therefore, known proliferative agents such as HMBPP, which can bind BTN3A1 in cancerous cells and promote their lysis by Vγ9Vδ2 T cells might be a viable anticancer strategy. However, HMBPP contains chemical features that limit its clinical capacity.56

The diphosphate core functionality present in HMBPP presents the same challenges that were discussed in previous chapters when considering its direct implementation as a pharmaceutical, provided the mechanism of action for these small molecules demands intracellular function. The diphosphate functionality in HMBPP has been noted as exceptionally unstable in blood plasma. One study which measured the in vivo plasma half-life of a structurally similar known proliferative agent, found meaningful concentrations of the compound last only for the time of infusion and were undetectable 30 min after the end of infusion.57 The exceptionally high charge-to-mass ratio present for HMBPP at physiological pH can also diminish the probability of passive cellular diffusion and likely requires endocytic uptake. With this in mind the Wiemer group developed a synthetic route to a POM-protected phosphonate analogue of HMBPP.38

Synthesis of HMBPP analogues in the Wiemer group began with the synthesis of a carbon-phosphorus bond-containing monophosphonate salt (3.04, Figure 21). The monophosphonate was anticipated to provide chemical stability by decreasing the possibility of phosphatase mediated metabolism. The synthesis began with the preparation of prenyl bromide from commercially
available prenol using phosphorus tribromide. The allylic halide was then allowed to react with the lithiate or “lithium anion” of dimethyl methylphosphonate (3.01) to afford a dimethyl homoprenyl phosphonate (3.02). This homoprenyl phosphonate was oxidized upon treatment with selenium dioxide, selectively affording the E alcohol 3.03 after the reduction of any over-oxidized material with sodium borohydride. Hydrolysis of the resulting dimethylphosphonate under standard conditions gave the expected sodium salt 3.04 in good yield. In a divergent fashion, reaction of compound 3.02 could be subjected to previously discussed POMCl and NaI conditions to afford the bis-POM analogue compound 3.05 which could be carried through the selenium dioxide oxidation conditions first advanced through the methyl series.\(^{38,58}\)

![Chemical reaction diagram](image)

**Figure 21. Synthesis of Phosphonate HMBPP Analogaues\(^{38}\)**

The listed monophosphonates 3.03, 3.04, and 3.06 were tested for their capacity to stimulate V\(\gamma\)9V\(\delta\)2 T cell proliferation. Utilizing peripheral blood mononuclear cells (PBMCs) derived from healthy donors, compounds 3.04 and 3.06 were both found to significantly expand
the population of Vγ9Vδ2 T cells. The POM prodrug containing monophosphonate 3.06 was much more potent than the sodium salt 3.04, with measured half maximal effective concentration (EC\textsubscript{50}) values of 0.0054 and 4.0 µM respectively. The bis-POM compound was found to have an approximate potency within an order of magnitude of the most potent naturally occurring compound identified (HMBPP) which was measured to have a 0.00051 µM EC\textsubscript{50} with the same assay. The dimethyl ester intermediate 3.03 from this synthetic sequence was also tested for stimulatory effects. However, dimethyl phosphonates are known to be metabolically stable over normally measured time frames and as predicted the dimethyl intermediate did not show any activity under similar conditions up to a concentration of 10 µM.

Interestingly, the previously mentioned nitrogenous bisphosphonates including risedronate, are known to stimulate Vγ9Vδ2 T cell proliferation. Risedronate can inhibit FDPS, which results in the accumulation of IPP as the direct proliferative agent. To assess the method of activation for the monophosphonate analogues synthesized by the Wiemer group, the activity of compound 3.06 and risedronate was compared in the presence and absence of the HMG-CoA reductase inhibitor lovastatin. Lovastatin inhibits the formation of HMG-CoA and thereby the downstream product IPP. Treatment with lovastatin alone was not found to alter the proliferation of Vγ9Vδ2 T cells. However, in the presence of lovastatin, the stimulatory effect of risedronate on Vγ9Vδ2 T cell proliferation was significantly reduced, whereas the stimulatory effect of compound 3.06 on Vγ9Vδ2 T cell proliferation was not significantly affected in the presence of lovastatin. These data support the view that the monophosphonates are acting as direct activators of T cell proliferation similar to HMBPP and not through altering the mevalonate pathway like the NBPs\textsuperscript{59}.
Confirming that the modification to more metabolically stable phosphonate analogues of HMBPP does not destroy the proliferative activity of similar compounds and noting the increased activity of POM prodrug containing compounds when compared to the salt form of the phosphonates, our group developed a strategy for the synthesis of a phosphinophosphonate motif that was hypothesized to more closely resemble the structure of HMBPP (Figure 22).

![Structural Comparison of HMBPP and Analogue](image)

Figure 22. Structural Comparison of HMBPP and Analogue

The synthesis of this phosphinophosphonate analogue began with the treatment of dimethyl homoprenylphosphonate with oxalyl chloride and catalytic dimethylformamide to afford the desired acid chloride 3.07 (Figure 23). Compound 3.07, without further purification, was allowed to react with the lithium anion of dimethyl methylphosphonate to give the desired phosphinophosphonate in moderate yield. The trimethyl ester phosphinophosphonates 3.08 served as the central intermediate for further transformations. The trisodium salt was made available by hydrolysis through standard conditions involving the use of trimethylsilyl bromide and collidine followed by treatment with NaOH. Allylic oxidation of the common intermediate 3.08 with selenium dioxide gave the expected alcohol 3.10 in low yield, which was carried through a parallel hydrolysis to obtain the hydroxylated trisalt 3.11. The common intermediate 3.08 also was treated with POMCl and NaI to afford the masked phosphinophosphonate 3.12, which was also subjected
to oxidation with SeO₂ and tBuOOH to provide the final target 3.13 selectively as the E-alkene, after NaBH₄ reduction of any overoxidation products.⁶¹

![Chemical diagram showing synthesis of phosphinophosphonate butyrophilin ligands](image)

Figure 23. Synthesis of Phosphinophosphonate Butyrophilin Ligands⁶¹

**Biological Results**

The synthesized phosphinophosphonates were evaluated by our collaborators at the University of Connecticut for their ability to promote proliferation of Vγ9Vδ2 T cells from human
peripheral blood (Figure 24). At a concentration of 0.1 μM, HMBPP and the triPOM prodrug phosphinophosphonate \textbf{3.13} both strongly stimulated proliferation of Vγ9Vδ2 T cells, based on the appearance of a population of cells that was isolated by flow cytometry that stained positive for CD3 and γδ T cell receptor (TCR, Figure 24 A). The corresponding half maximal effective concentrations (EC50’s) tabulated from these data reflect that the tri-sodium salt \textbf{3.11} stimulated proliferation with an EC50 of 26 μM, while no stimulation was observed for the analogous salt that does not contain the omega hydroxyl group, compound \textbf{3.09}. Similarly, the fully methylated species \textbf{3.10} was inactive at concentrations up to 100 μM. Interestingly the tris-POM prodrug \textbf{3.12} was able to stimulate T cell proliferation with an EC50 of 0.041 μM. This is impressive considering that the prodrug is expected to release a metabolite that is essentially compound \textbf{3.09}, which showed a 630-fold decrease in potency with direct treatment.61
Figure 24. Phosphinophosphonate T Cell Proliferation Study

A) Analysis of CD3+/γδ+ T cells by flow cytometry

B) Dose response of compound 3.11 and 3.13.

(\( n = 3. \) * denotes \( p < 0.05 \) per unpaired two-tailed \( t \)-test)

Because compounds such as HMBPP are thought to stimulate \( V\gamma 9V\delta 2 \) T cell proliferation by binding to the butyrophilin protein BTN3A1, isothermal titration calorimetry (ITC) also was utilized in the evaluation of the phosphinophosphonates (Figure 25). To assess the binding, ITC was employed to examine the interaction of the tri-salt compound 3.11 with the intracellular
domain of BTN3A1. The ITC data showed interaction similar to HMBPP with an average enthalpy term $\Delta H$ of -74.7 kJ/mol, and a negative entropy contribution ($T\Delta S = -52$ kJ/mol). Compared to HMBPP the enthalpy term of compound 3.11 shows a more favorable interaction, but the entropy term showed unfavorable changes leading to an overall lower binding affinity. This suggests that modification of the diphosphate functionality to a phosphinophosphonate skeleton results in weaker ligand binding relative to HMBPP due to the detrimental changes in entropy overcoming the positive impact of the favorable enthalpy changes. The ITC data did show that the allylic oxygen plays a significant role in binding, as the analogous phosphinophosphonate without the omega functionality (compound 3.09) was found to show weaker binding when compared to compound 3.11. The ITC data also are consistent with the view of POM functionality acting as a prodrug, as the phosphinophosphonate did not show direct binding to BTN3A1 as the tris-POM species 3.13.\textsuperscript{61}
A) ITC plots for interaction of compound 3.11 (red line) or buffer (black line)

B) ITC plots of HMBPP in absence (red) or presence (gray = 10x, blue = 100x) of 3.09

To form a better understanding of the differences in binding between compound 3.11 and HMBPP, molecular docking analysis was performed using the publicly available crystallographic data for the B30.2 domain of BTN3A1 (Figure 26). Docking predicts several hydrogen bonding interactions between HMBPP and BTN3A1 at the β phosphate, giving support for the value of the phosphinophosphonate skeletal structure that would theoretically allow phosphonate functionality to occupy a similar space within the protein binding site. However, the modelling also suggests an interaction between BTN3A1 and HMBPP at the oxygen atom that links the α and β phosphates.
The phosphinophosphonate that contains a methylene linker in place of the oxygen is incapable of similar hydrogen bonding, resulting in changes to the predicted binding affinity and orientation relative to the HMBPP model. In conjunction with the ITC data, it is likely that even though this specific position is incapable of forming a hydrogen bond, the strength of other interactions contributes to an overall favorable gain in the enthalpy of binding compared to HMBPP.

The difference in binding orientation predicted by the model may be the basis for the entropically unfavorable term found in the ITC analysis. The oxygen that links the prenyl chain to the diphosphate functionality in HMBPP was not predicted to form significant hydrogen bonds to BTN3A1 which may imply that this oxygen is nonessential for strong binding, which in turn would be consistent with the measured activity of the previously discussed monophosphonates. Although, the presence of this oxygen linking the diphosphate may indirectly impact binding through the influence on the pKₐ values of the corresponding acids. If the presence of the oxygen lowers the pKₐ value enough to ensure an anion at physiological pH there may be ionic interactions that contribute to the difference in observed binding affinity, but further study is necessary to elucidate more detail.⁵³, ⁶¹
Figure 26. Molecular Modelling Comparing HMBPP and the Phosphinophosphonate

Rendering was performed using Schrodinger Maestro

The phosphinophosphonates also were tested for their ability to trigger Vγ9Vδ2 T effector cells to lyse cancerous cells. In these experiments, HMBPP triggered lysis of the target K562 cells in a dose-dependent manner with a measured EC\textsubscript{50} value of 0.0016 μM. This was used as a positive control to compare the activity of the phosphinophosphonates which showed EC\textsubscript{50} values of 41
μM for the tri-salt 3.11 and 0.2 μM for the tris-POM species 3.13 representing a 150-fold increase in cellular activity for the prodrug form.

When evaluating all the studies conducted on the phosphinophosphonates it was surprising that a small difference in binding affinity led to such a large difference in observed cellular activity. During analysis of the dose-response data it was noted that stimulation by these phosphoantigens was most consistent with a negatively cooperative event. All the data are suggestive of a homodimer model for the desired protein ligand stimulation (Figure 27) which would require multiple binding events. While these data support this homodimer-phosphoantigen bound mechanism of action, the physiological formation of this dimer is as-yet unproven and further investigation would be required to confirm its existence.61

BTN3A1 homodimer model (2 binding site)

![Diagram of BTN3A1 homodimer model](image)

Figure 27. Binding Model Most Consistent with Data
(Homodimer model of BTN3A1 activation)

To conclude, a small family of phosphoantigens has been synthesized by the Wiemer group and evaluated for biological activity with hopes of utilizing their potential to stimulate the production of cytotoxic T cells that can lyse malignant cancerous cells. The most potent naturally
occurring BTN3A1 agonist, HMBPP, provides a useful model for the synthesis of phosphoantigens, but it contains diphosphate functionality that limits cellular uptake and metabolic stability. To address these shortcomings, a family of phosphoantigens was synthesized containing non-hydrolysable phosphorus-carbon bonds, with the phosphinophosphonate structure designed to interact with phosphate binding sites and avoid the need for cellular phosphorylation. It was hypothesized that the tris-POM phosphinophosphonate prodrugs might increase the stability and cell permeability of these analogues while releasing a metabolite which closely mimics the structure of HMBPP. Significant activity for the proliferation of Vγ9Vδ2 T cells was observed in most compounds tested and the POM prodrug delivery system is indeed effective as it was found to increase cellular proliferation by approximately 630-fold and increase cell lysis by 150-fold in the discussed studies. Continued investigation is required to refine the activity and prodrug functionality of phosphoantigens in order to produce a clinically viable option.
CHAPTER 4:

EARLY WORK IN THE SYNTHESIS AND BIOACTIVITY OF MIXED ARYL PHOSPHONATE PRODRUGS OF A BUTYROPHILIN LIGAND

The synthesis of small organophosphorus compounds designed to bind the signaling protein butyrophilin 3A1 (BTN3A1) has resulted in the production of a number of phosphoantigens that show potent activity. The phosphinophosphonate extended structure does not appear to impart activity that surpasses that of the original carbon framework investigated by the Wiemer group. However, the POM prodrug strategy that has been achieved by the previously discussed syntheses does appear to enhance the activity of phosphoantigens, relative to their corresponding sodium salt or methyl phosphonate ester counterparts. This is what would be expected with the newly developed understanding of these small organophosphorus compounds binding to an intracellular domain of BTN3A1, which was supported by crystallographic techniques as well as isothermal titration calorimetry and NMR studies. With these data in mind, it was deemed necessary to probe what modifications to the prodrug composition may enhance the activity of the phosphonate carbon framework.

Given the growing body of evidence supporting the idea that these phosphoantigens must cross the cell membrane to express biological activity, the high charge-to-mass ratio of HMBPP and the salt forms of synthetic analogues likely reduces effectiveness when extracellularly dosed. The POM prodrug strategy does result in a demonstrated improvement in potency in each case examined, but if these compounds were ultimately advanced to animal studies, concern arose that the pivalic acid that results from POM metabolism may impact carnitine metabolism. The bis-POM species also tends to display short serum half-lives that may limit clinical utility.
One of the simplest and most synthetically accessible ideas for a prodrug motif would be a phosphonate diester derived from a simple alcohol such as methanol or ethanol. However, despite the identification of certain organophosphorus hydrolases found in select bacteria, fast and efficient metabolic cleavage of dialkyl phosphonate esters derived from simple alcohols such as methanol has not been reported in mammalian systems.\textsuperscript{64} Aryl esters, on the other hand, have shown more promise as prodrug candidates with both phenyl and naphthyl systems frequently employed in phosphorus prodrug motifs.\textsuperscript{65} One of the most well-known clinically used examples of aryl-containing phosphorus prodrugs is the aryloxyphoramidate prodrug (ProTide) containing Sofosbuvir (Figure 28).\textsuperscript{66}

The phenyl phosphoramidate prodrug structure of Sofosbuvir is thought to be metabolically activated through a sequence of steps. The mechanism appears to involve hydrolysis of the carboxyl ester group of the amino acid moiety through the intermediacy of esterases such as cathepsin A. This could be followed by an intramolecular cyclization where the internal nucleophilic carboxyl group attacks the phosphorus center to expel the aryloxy functionality, giving the transient formation of a five-membered heterocyclic intermediate. The cyclic mixed anhydride is likely to be hydrolyzed rapidly to the corresponding aminoacyl phosphoramidate, which is believed to undergo P-N bond cleavage mediated by phosphoramidase enzymes (e.g., Hint), but may be the result of simple acid hydrolysis in acidic subcellular environments. If aryloxy-containing phosphorus prodrugs can be successfully metabolized \textit{in vivo} and lead to therapeutically relevant compounds, their implementation into analogues of the bis-POM phosphoantigen that displayed the greatest activity could be an efficacious pursuit.\textsuperscript{67}
Noting the success of this aryl-containing phosphorus prodrug approach, synthesis was first undertaken on a model system to establish that the previous strategy for manipulation of a single phosphonic ester would be viable for the incorporation of aryl phosphonic esters (Figure 29). Dimethyl methylphosphonate 4.06 was chosen as it was the starting material for synthesis of previous monophosphonate analogues and would likely represent the simplest model. Dimethyl methylphosphonate was treated with oxalyl chloride and catalytic DMF to afford the chlorophosphonic acid 4.07 analogous to the previous synthesis. Compound 4.07 was then allowed to react with phenol in the presence of triethylamine. This sequence produced the mixed ester 4.08 in yields that were comparable to those obtained with simpler alcohols.
After successful preparation of the model system of **4.08**, synthesis of mixed aryloxy phosphonates of a known BTN ligand was undertaken (Figure 30). This synthesis began with the reaction of dimethyl methylphosphonate with \( n \)-BuLi followed by alkylation with prenyl bromide. The resulting dimethyl homoprenylphosphonate was then converted to the phosphonic acid chloride **4.10** through treatment with oxalyl chloride and catalytic DMF. The acid chloride was employed in parallel reactions with phenol and 1-naphthol after minimal purification. The mixed aryl methyl phosphonic acid esters **4.11** and **4.12** were obtained in good yield.

To probe the efficacy of mixed aryl phosphonate prodrugs in cellular assays, three derivatives of the phenyl and naphthyl esters were prepared to obtain compounds that might release a biologically active ligand for BTN3A1. The desired compounds were available through a short synthetic sequence. Reaction of the mixed aryl/methyl esters **4.11** and **4.12** with pivaloyloxymethyl chloride (POMCl) in the presence of sodium iodide gave the corresponding mono-POM mono-aryl diesters **4.13** and **4.14** as racemic mixtures based on the newly formed phosphorus stereogenic center. Incorporation of the allylic alcohol functionality was accomplished through oxidation with selenium dioxide and \( tert \)-butyl hydroperoxide followed by brief treatment with sodium borohydride to reduce any aldehyde that resulted from overoxidation. The oxidation
via selenium dioxide proceeded in good yield. The desired products 4.15 and 4.16 were obtained in quantities sufficient for the necessary bioassays.

The parent mono-methyl mono-aryl esters (compounds 4.11 and 4.12) also were used as precursors to other target aryl phosphonate products. Direct allylic oxidation with selenium dioxide gave the expected alcohols although again in poor yield. Fortunately, treatment with sodium iodide in a slight deficiency gave nearly quantitative conversion to the corresponding mono-sodium salts 4.19 and 4.20 with no evidence of undesired aryl cleavage. Through this sequence, the mono-methyl mono-aryl compounds bearing allylic alcohols, compounds 4.17 and 4.18 as well as their mono-sodium salt analogues 4.19 and 4.20, were obtained in amounts sufficient for bioassay.
**Figure 30. Synthesis of Mixed Aryl Phosphonates**

4.09

\[
\begin{align*}
\text{POCH}_3 & \xrightarrow{(COCl)_2, \text{DMF}} \text{POCl} \\
\text{POCH}_3 & \xrightarrow{\text{ArOH, Et}_3\text{N}} \text{POCH}_3
\end{align*}
\]

4.10

\[
\begin{align*}
\text{DCE} & \rightarrow 100 \% \\
\text{POMCl, NaI, ACN} & \rightarrow \text{Ar} = \text{phenyl (14\%)} \\
\text{Ar} = \text{1-naphthyl (32\%)} & \rightarrow \text{4.13} \rightarrow \text{4.14}
\end{align*}
\]

4.11

\[
\begin{align*}
\text{Ar} = \text{phenyl (100\%)} & \rightarrow \text{NaBH}_4 \\
\text{Ar} = \text{1-naphthyl (65\%)} & \rightarrow \text{NaBH}_4 \\
\end{align*}
\]

4.12

\[
\begin{align*}
\text{1) SeO}_2, \text{t-BuOOH} & \rightarrow \text{Nal, ACN} \\
\text{2) NaBH}_4 & \rightarrow \text{Ar} = \text{phenyl (21\%)} \\
\text{Ar} = \text{1-naphthyl (36\%)} & \rightarrow \text{4.17} \rightarrow \text{4.18}
\end{align*}
\]

4.13

\[
\begin{align*}
\text{28\%} & \rightarrow \text{20\%}
\end{align*}
\]

4.14

\[
\begin{align*}
\text{4.15} & \rightarrow \text{4.16}
\end{align*}
\]

4.15

\[
\begin{align*}
\text{4.16} & \rightarrow \text{4.17} \\
\text{4.17} & \rightarrow \text{4.18}
\end{align*}
\]

4.16

\[
\begin{align*}
\text{4.18} & \rightarrow \text{4.19} \\
\text{4.19} & \rightarrow \text{4.20}
\end{align*}
\]

4.17

\[
\begin{align*}
\text{4.18} & \rightarrow \text{4.19} \\
\text{4.19} & \rightarrow \text{4.20}
\end{align*}
\]

4.18

\[
\begin{align*}
\text{4.19} & \rightarrow \text{4.20}
\end{align*}
\]
Biological Results

Each of the resulting phosphonates that bear allylic hydroxyl functionality was assayed by collaborators of the Wiemer group for the ability to stimulate expansion of Vγ9Vδ2 T cells. The mono-phenyl mono-methyl phosphonate compound 4.17 exhibited weak activity with a measured EC$_{50}$ value of 33 μM. Even this weak activity was surprising when compared to the inactivity observed for the dimethyl phosphonates previously investigated and may reflect at least partial metabolism to a charged ligand. The mono-phenyl mono-sodium salt analogue 4.19 showed increased cellular activity, with an EC$_{50}$ of 0.87 μM. This was an important result: considering the suspected challenges associated with cell permeability for the phosphonic acid salts one would expect a minimized value of measured activity when extracellularly dosed. The phenyl/POM compound 4.15 displayed significant potency gains with activity in the low nanomolar range (EC$_{50}$ = 0.014 μM), consistent with the hypothesis that the prodrug approach is superior to the direct extracellular exposure to phosphonic acid salts.$^{69}$

The activity of the phenyl/POM species was quite promising, but still did not surpass that of the bis-POM prodrug analogue. The calculated partition coefficient (cLogP) values of the bis-POM and phenyl/POM species were similar, and therefore it might be assumed that the cellular permeability was likely to be similar as well. The naphthyl analogues were excellent candidates to explore further the potential relationship between LogP and activity of the prodrugs. The naphthyl compounds would be more hydrophobic and, given the increased acidity when compared with phenol, the naphthyl group would likely serve as a better leaving group during metabolism. As hypothesized, each of the analogous compounds containing a naphthyl group (the naphthyl/methyl compound 4.18, the naphthyl/salt compound 4.20, and the naphthyl/POM compound 4.16)
displayed greater activity for the proliferation of T cells than their corresponding phenyl counterparts. Indeed, the naphthyl/POM containing compound 4.16 was the most potent synthetic phosphoantigen reported at that time with an EC$_{50}$ value of 790 pM. This compound was among the first synthetic phosphoantigens that displayed activity within an order of magnitude of the most potent known proliferative agent, the naturally occurring model for phosphoantigens HMBPP which shows activity at 510 pM.$^{69}$

<table>
<thead>
<tr>
<th>Compound</th>
<th>LogP</th>
<th>EC$_{50}$ [µM] (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.04 Na/Na$^{70}$</td>
<td>-0.24</td>
<td>4.0</td>
</tr>
<tr>
<td>3.06 POM/POM$^{70}$</td>
<td>3.42</td>
<td>0.0054</td>
</tr>
<tr>
<td>4.15 Phe/POM (n=3)</td>
<td>3.56</td>
<td>0.014 (0.0040 to 0.051)</td>
</tr>
<tr>
<td>4.17 Phe/Me (n=3)</td>
<td>2.01</td>
<td>33 (7.9 to 140)</td>
</tr>
<tr>
<td>4.19 Phe/Na (n=3)</td>
<td>1.73</td>
<td>0.87 (0.42 to 1.8)</td>
</tr>
<tr>
<td>4.16 Nap/POM (n=3)</td>
<td>4.75</td>
<td>0.00079 (0.00060 to 0.0010)</td>
</tr>
<tr>
<td>4.18 Nap/Me (n=3)</td>
<td>3.19</td>
<td>5.5 (0.029 to 1000)</td>
</tr>
<tr>
<td>4.20 Nap/Na (n=3)</td>
<td>2.92</td>
<td>0.61 (0.14 to 2.6)</td>
</tr>
</tbody>
</table>

Table 2. Activity for Expansion of Vγ9 Vδ2 T Cells from Human PBMC $^{69}$

To elucidate additional binding information for these phosphonate salts, isothermal titration calorimetry (ITC) methods were employed (Figure 31). It remained a possibility that the aryl phosphonic esters were directly influencing BTN3A1 rather than acting as prodrugs of a bioactive form. The ITC data did not show significant binding of the mono-aryl mono-salt forms
of these phosphonates when compared to HMBPP. The more active naphthyl ester was unable to compete with HMBPP for binding to BTN3A1 even at 100-fold greater concentrations. These data were consistent with the ITC studies conducted on the tris-POM phosphinophosphonates that were found to be prodrugs of an actively binding ligand, which suggests by default that the phenyl and naphthyl groups are susceptible to cellular hydrolysis. This metabolic conversion likely yields the same phosphonate dianion as the original bis-POM monophosphonate forwarded by the Wiemer group.

![Graph](image_url)

**Figure 31. Binding Comparison of HMBPP and Aryl Phosphonate Salts**

ITC plots of HMBPP (red), 4.19 (blue), and 4.20 (green) binding the intracellular domain
The phenyl and naphthyl containing phosphonates have shown that aryl phosphonate prodrugs of the homoprenyl phosphonate parent ligand effectively stimulate T cell proliferation. A trend appears to be emerging from our data that suggests the aryl/POM phosphonate prodrugs are more active than the aryl/Na phosphonates, which in turn are much more active than the aryl/Me analogues. The most potent activity within this set was demonstrated by the mono-naphthyl mono-POM species, which displayed an EC$_{50}$ value in the high picomolar range. This potency very nearly approximates that of the most active naturally occurring proliferative agent and model for this study, HMBPP. These results encouraged further investigation of prodrug forms of the parent phosphonate ligand.
CHAPTER 5:

EXPANDING THE STRUCTURE ACTIVITY RELATIONSHIP STUDY OF MIXED ARYL PHOSPHONATE PRODRUGS OF A BUTYROPHILIN LIGAND

After observing the promising results obtained through our initial investigation of aryl phosphonate prodrugs, and with increased evidence that each ultimately yields the same active metabolite, it appeared worthwhile to pursue other compounds that contain similar functionality to develop a better understanding of the relationship between the prodrug and the measured proliferative activity. One avenue for inquiry became apparent after determining the activity of the relevant phenyl and naphthyl phosphonate esters. In each case the naphthyl containing compound displayed enhanced activity when compared with its phenyl containing analogue. Naphthol is known to be more hydrophobic, as noted in the aforementioned calculated partition coefficients (cLogP), which likely impacts the cellular absorptivity of compounds that contain this group when compared with more hydrophilic functionality.\(^{69}\) Naphthol also has a lower pKa value and is therefore more acidic than phenol by approximately a factor of 4.\(^{71}\) This greater acidity may influence the metabolism making the active ligand more readily available. In an attempt to determine which of these factors is exerting the greater influence on the activity of these compounds, synthetic effort was expended to supply analogues for additional comparison.\(^{72}\)

The previously established route to the original set of aryl phosphonate ligands can be shown in a general form (Figure 32). The synthesis in its general form can be thought of as a divergence from the phosphonic acid chloride 4.10, which is amenable to substitution with a variety of nucleophiles. After establishing the mixed aryl phosphonic ester structure, further divergence results in the formation of aryl/salt and aryl/POM phosphoantigen prodrugs.
Figure 32. General Synthetic Scheme for Expansion of Aryl Phosphonate Prodrugs

After comparing phenol and 1-naphthol, it appeared that fitting additions to the set of aryl phosphonate prodrugs would be those that might significantly alter the hydrophobic aromatic character of the protected form. In furtherance of this goal, an anthracene derivative of the parent phosphonate was envisioned. Commercially available 9-anthracencarboxylic acid 5.06 was subjected to treatment with lithium aluminum hydride. The resulting alcohol 5.07 then was allowed to react with the phosphonic acid intermediate (compound 4.10) in an attempt to establish increased aryl character. However, attempted isolation of the desired mixed phosphonic ester 5.08 went unrewarded. During workup of this reaction, the phosphorus containing product of the reaction was found in the aqueous portion which was likely the result of hydrolysis of the anthracene-containing ester. This is consistent with the sensitivity of allylic and benzylic phosphonic esters noted in literature and observed in our previously attempted syntheses.
A second method proposed to convey greater hydrophobic aryl character to the resulting phosphonate involved the installation of two aryl esters through the phosphonate. The previously established synthesis for mono-methyl mono-aryl compounds was undertaken in an iterative fashion for the more active 1-naphthol species (Figure 34). Treatment of the previously synthesized mono-1-naphthyl mono-methyl phosphonate compound 4.12 with oxalyl chloride and catalytic DMF was successful in yielding the intended mono-naphthyl phosphonic acid chloride 5.09, which was allowed to react with 1-naphthol and triethylamine. This process appears selective for methyl ester over aryl ester cleavage and the resulting bis-naphthyl monophosphonate 5.10 was subjected to allylic oxidation by treatment with selenium dioxide and tert-butyl hydroperoxide. As
seen in comparable systems, the allylic oxidation of the phosphonate proceeded in poor yield but was successful in the selective synthesis of the desired $E$ allylic alcohol $5.11$.

![Diaryl Phosphonate Synthesis](image.png)

Figure 34. Diaryl Phosphonate Synthesis

The calculated LogP value of the bis-naphthyl compound $5.11$ is 6.03, which is substantially different from the phenyl/POM $4.15$ at 3.56 and the naphthyl/POM $4.16$ at 4.75. However, preliminary biological activity studies of the bis-naphthyl monophosphonate compound $5.11$ showed a diminished ability to promote $\gamma\delta$ T cell proliferation compared to the monoaryl mono-POM species. The EC$_{50}$ value of compound $5.11$ was found to be 0.27 $\mu$M, which is
less active than the phenyl/POM 4.15 at 0.014 μM and considerably less active than the naphthyl/POM 4.16 at 0.00079 μM. This decrease in activity may be evidence of diminished return for the effect of increased hydrophobicity on the absorptivity and ultimate activity expressed by these phosphonates. However, this may not be the best compound for a direct comparison, as compound 5.11 represents a difference in hydrophobicity as well as a different diaryl prodrug formulation. Simultaneous change may allow for a difference in the metabolism of the prodrug to overshadow the positive effect of the increased hydrophobicity on the activity of this compound. However, the apparent prodrug activity of the mono-aryl mono-salt and especially the mono-aryl mono-methyl species under similar bioassay conditions may justify this comparison. Further investigation that may allow a direct comparison with a single point of difference, such as the installation of an anthracene fragment through aryl alcohol functionality as opposed to benzylic alcohol type esterification, would be desirable to establish more concrete conclusions on the impact of hydrophobicity on the proliferative activity of these ligands.

A parallel path of inquiry was undertaken to more accurately probe the effect of varying the pKa of the aryl fragment. This modification likely affects the leaving group capacity of the aryl group and therefore would likely have consequences for the metabolism of these prodrugs and ultimately their ability to stimulate proliferation. Aryl alcohols that contain para-nitro groups have appreciably different pKa values when compared with their nonfunctionalized analogues. The stabilization of the conjugate base by the nitro substituent results in increased acidity of the aryl alcohol and decreases the measured pKa relative to the parent compound. In the specific case of phenol, incorporation of nitro functionality at the 4-position has a roughly 1000-fold influence on the acidity of the alcohol. Through the established synthesis route, 4-nitro phenol was allowed
to react with the phosphonic acid chloride intermediate 4.10 and the resulting mixed ester was taken to the mono-aryl mono-POM species and ultimately to the allylic alcohol 5.13.\textsuperscript{71, 74}

\begin{align*}
\text{Phenol} \\
pK_a=10
\end{align*}

\begin{align*}
\text{4-Nitro phenol} \\
pK_a=7
\end{align*}

Figure 35. pKa Difference for 4-NitroPhenol\textsuperscript{74}

An alternate synthesis was undertaken to produce a nitro functionalized naphthyl ester derivative (Figure 36). This synthesis utilized the dimethyl homoprenyl phosphonate as with previous synthesis but proceeded through a method developed by Huang et al.\textsuperscript{75} Reaction of the dimethyl species with triflic anhydride and pyridine followed by introduction of 2-nitro naphthol established the desired mixed aryl/methyl functionality 5.14. This reaction sequence required far shorter reaction times and retained high yield, providing the intended intermediate in minutes as opposed to days. This mixed aryl ester monophosphonate was then subjected to the originally employed sequence of POM prodrug installation and allylic oxidation to produce the target compound 5.15.
These nitro containing aryl phosphonates were tested for their ability to stimulate Vγ9Vδ2 T cell proliferation. The para-nitro phenyl/POM prodrug 5.13 displayed a measured EC₅₀ value of 0.0040 μM which is significantly more potent than the previously discussed phenyl/POM 4.15 at 0.014 μM. However, this effect was not observed in the naphthyl case as the activity of the nitro functionalized prodrug species displayed an EC₅₀ value of 0.021 μM compared to 0.00079 μM for the parent naphthyl derivative. Further synthetic and immunological investigation is ongoing and will be necessary to increase our understanding of the influence that aryl functionalization has on the proliferative activity of mixed aryl/POM monophosphonates. An important inclusion to this set would be a compound that exhibits an aryl ester that is functionalized with an electron donating substituent. With respect to synthetic simplicity and structure comparison, an amino substituted aryl fragment might make a sensible candidate for comparison.
Another potentially interesting avenue for exploration would be to probe the difference in activity, or lack thereof, exhibited by different isomers of the mixed aryl prodrugs. One previously unmentioned feature is the choice of naphthol regiochemistry. The 1-naphthol alcohol was used as the starting material based on commercial availability to establish the presence or absence of activity in the resulting compounds. With the potent activity observed for these 1-naphthyl containing prodrugs, it became important to determine what, if any, effect a change in the regiochemistry had on the proliferative activity. Therefore, the general synthesis for these monophosphonates (Figure 32) was replicated with 2-naphthol as the alcohol. The resulting 2-naphthyl/POM derivative displayed similar activity for stimulation of Vγ9Vδ2 T cell proliferation with an EC<sub>50</sub> value of 0.0021 μM. This value was not beyond the confidence intervals established within the assay and cannot be ruled statistically different from the 1-naphthyl/POM compound at 0.00079 μM. Further evaluation through different biological methods may return more conclusive results.

![1-Naphthol and 2-Naphthol](image)

Figure 37. Aryl Regiochemistry

The novel aryl phosphonate prodrugs discussed in this chapter, the 2-naphthol, bis-1-naphthol, 4-nitro phenyl, and 2-nitro-naphthol containing species, have been evaluated for their
ability to stimulate Vγ9Vδ2 T cell proliferation (Figure 38). The resulting EC₅₀ values have already been presented, however another as yet unmentioned important feature of prodrug activity relates to their stability in human plasma.

Figure 38. Examination of Novel Prodrugs Ability to Stimulate T Cell Proliferation

(n = 3–5, error bars mean +/- stdev.)

The natural phosphoantigen HMBPP displays abysmal blood plasma stability as observed in previous studies. The original bis-POM prodrug phosphoantigen developed in the Wiemer group (compound 3.06) also shows extreme sensitivity to metabolism in blood plasma which limits its utility as a prodrug. For this reason, LCMS techniques were employed to evaluate the stability
of the aryl phosphonates discussed in this chapter and to compare them with the bis-POM analogue and the 1-naphyl and phenyl containing phosphonates discussed in Chapter 5 (Figure 39). The results of this study suggest statistically relevant improvement in blood plasma stability for the mixed aryl/POM prodrug motif relative to the bis-POM analogue. Exceptional stability was noted for the bis-1-naphyl monophosphonate, which is consistent with the hypothesis that the lower activity of this compound may be related to a relatively sluggish metabolism which yields the active metabolite. One exceptional result of this study is the unanticipated plasma stability of the 2-napthyl containing phosphonate. This stability coupled with its potent activity may warrant further investigation into structurally similar potential prodrugs.
Each of the aryl/POM prodrug monophosphonates was assayed as a racemic mixture by virtue of the phosphorus stereogenic center having not been controlled through the listed synthesis. While these prodrugs lose stereochemistry upon hydrolysis of either ester, instances of a difference in bioactivity based on the phosphorus stereochemistry have been published, such as the previously discussed example of Sofosbuvir. The difference in measured biological activity could result from the enzymes that are responsible for the metabolism of these compounds naturally displaying stereoselectivity that differentiates between these enantiomers. Careful review of the available
literature led to the development of a potential strategy for the resolution and subsequent divergent synthesis of aryl phosphonate isomers. The envisioned strategy involved the formation of phosphonamidate diastereomers through the establishment of a P-N bond to a chiral amine.

Specifically, (S)-(−)-α-methylbenzylamine was chosen because of its analogous use in previously published resolutions. The crucial P-N bond formation was first investigated with a model system (Figure 40). The parent monophosphonate compound dimethyl methylphosphonate was chosen as a simpler and commercially available monophosphonate. Dimethyl methylphosphonate (4.07) was treated with oxalyl chloride and catalytic DMF to form the desired acid chloride which was subsequently treated with (S)-(−)-α-methylbenzylamine and excess triethylamine to form the desired mixed phosphonamidate compound 5.16.

Figure 40. Model System for Phosphonamidate Formation
After a cursory structure confirmation of the crude model reaction, the sequence was repeated with the dimethyl homoprenyl phosphonate 4.09 which had been employed in the general synthesis. This sequence provided a mixture of diastereomers as the carbon stereocenter was set in the commercially available amine starting material and was retained through the synthesis. The diastereomers gave unique signals in the phosphorus and hydrogen NMR spectra. The mixture was formed in approximately a 1 to 1 ratio based on the NMR data. Unlike enantiomers which display identical physical properties (other than their interaction with plane polarized light), diastereomers can be separated with traditional methods like chromatography, in theory. In practice however, the separation of diastereomers is often a considerable challenge as the difference in stereochemistry may not have a substantial bearing on the relative polarity or other physical properties that are routinely utilized for separations.

![Chemical Structure](image)

**Figure 41. Phosphonamidate Diastereomer Formation**

In this specific instance, iterative regular phase chromatography with silica gel was suitable for appreciable stereo-enrichment of the mixture. The resulting isolated fraction contained a drastically altered mixture whose composition contained the more polar diastereomer as the major component evident through examination of the NMR data. The phosphorus NMR data exhibited a marked transformation in the relative integrations when comparing the two phosphorus singlets in
the crude spectrum (Figure 43 A) and essentially a single peak of the spectrum of the purified material (Figure 43 B). This trend can also be observed in the hydrogen NMR when comparing the signals corresponding to the vinylic position of the two diastereomers (Figure 44 A) compared to the single resonance observed in the spectrum of the purified material (Figure 44 B). This preliminary study has provided considerable evidence that phosphonamidate diasteromers are reasonable candidates for resolution of phosphorus stereoisomers and will likely be the focus of continued research in the Wiemer group.
After continued frustration with the exceptionally low yield observed in the allylic oxidation of mixed aryl phosphonates, an alternative synthesis has been devised that would employ this troublesome oxidation in simpler starting materials (Figure 44). This synthesis incorporates several steps that have well founded literature precedent. Commercially available cyclopropyl
methyl ketone was allowed to react with methyl magnesium bromide and subsequently treated with aqueous sulfuric acid to provide homoprenyl bromide in good yield.\textsuperscript{77-78} This substrate was then subjected to allylic oxidation by treatment with selenium dioxide and tert-butyl hydroperoxide.\textsuperscript{79-80} The desired alcohol was isolated in a moderate 52\% yield, but this far surpassed the single and low double digit yields commonly observed in the original synthesis of mixed aryl phosphonates, where the selenium dioxide oxidation was conducted after introduction of the phosphonate. The alcohol 5.20 then was protected by reaction with tert-butyldimethylsilyl chloride and imidazole to form the desired silyl ether 5.21 in excellent yield.\textsuperscript{81} This bromide was converted to the iodide by reaction with sodium iodide. The primary iodide 5.22 then was allowed to react under Arbuzov conditions by treatment with trimethyl phosphite to obtain the dimethyl phosphonate compound 5.23.\textsuperscript{82} Chlorination of this monophosphonate with oxalyl chloride and catalytic DMF was followed by introduction of 2-naphthol and triethylamine. Unintended loss of the TBS protecting group was observed through this reaction sequence, but this deprotection was to be conducted as the final step of this synthesis. Therefore, this sequence can be seen as the first formal synthesis of a mixed aryl phosphonate ligand that had been previously prepared from prenyl bromide. Despite substantial need for the optimization of this sequence, it is promising as the allylic oxidation of homoprenyl bromide provided greater conversion and an almost trivial separation of the product 5.20 from unreacted starting material 5.19.
Figure 44. Alternate Synthesis of Mixed Aryl Phosphonate Ligands
The exceptionally potent activity of mixed aryl phosphoantigen prodrugs coupled with the observed enhancement in plasma stability when compared to the naturally occurring model HMBPP justifies further synthetic investigations. Several additional compounds proposed in this chapter may aid in the elucidation of structural features that are important for adequate binding to BTN3A1 or for the desired intracellular metabolism of these prodrugs. Namely, the synthesis of an anthracene derivative may be a valuable inclusion to the family of compounds that includes the unsubstituted aryl containing phosphoantigens in order to establish the impact of increasing aryl character and corresponding change in lipophilicity. Potential phosphoantigens that contain an electron donating substituent like the proposed amino functionalized aryl compounds would aid in the investigation of the affect that varying pKa has on proliferative activity. Further investigation into the resolution of phosphorus stereoisomers is also likely to provide an interesting addition to this area. And finally, the revision of the general synthesis of mixed aryl phosphoantigens that might provide optimized yields and more efficient separations throughout the synthesis would be exceptionally useful from a synthetic perspective and will likely increase the efficiency of the development of novel phosphoantigens.
In Chapter 2, methodology for preparation of asymmetric bisphosphonate prodrugs was described. These compounds were evaluated by our collaborators in Dr. Sarah A. Holstein’s lab, formerly at the Roswell Park Comprehensive Cancer Center in Buffalo, New York. The compounds within this set were designed as inhibitors of isoprenoid biosynthesis with the aim of developing a better understanding of structural features that enhance binding to the geranylgeranyl diphosphate synthase (GGDPS) enzyme, which has been identified as a potential strategy for the treatment of conditions that affect the bone such as multiple myeloma. The selective inhibition of GGDPS may allow for the development of treatments that do not significantly alter the other essential functions of the isoprenoid biosynthetic pathway (IBP) in healthy cells.

The compounds within this set display unique functionality that provides a new motif for potential GGDPS inhibitors where one isoprenoid chain is established through a phosphonic acid ester and another emanates from the alpha carbon. While the synthesis of these compounds presented challenges as a result of prodrug and allylic phosphonic ester sensitivity, each of the tested compounds demonstrated some activity for inhibition of GGDPS, with the most potent among them displaying an EC$_{50}$ value in the low micromolar range. While this activity may preclude the direct use of this compound as a pharmaceutical agent there were important lessons learned through this study. The most active member of this family contains a methyl group at the position alpha to the bisphosphonate functionality and incorporates three pivaloyl oxymethyl (POM) phosphonic esters. The use of POM prodrug functionality to increase the relative potency of inhibitors of GGDPS was supported by these findings and has been utilized in subsequent
studies in our group. This POM prodrug strategy, combined with alpha methylation, has since been applied to what was previously the most active inhibitor, which led to the formation of what is currently the most potent bisphosphonate inhibitor of GGDPS to date.

With the successful inclusion of these functional features into previously studied GGDPS inhibitors it appears reasonable to propose their incorporation into inhibitors of more downstream enzymes of the IBP. The Wiemer group has identified several compounds that appear to express selectivity for enzymes that are one step further downstream from the catalytic function of GGDPS. Some of the most promising examples include bisphosphonates and carboxyphosphonates that show some selectivity for GGTase II. If this enzyme is targeted there may be hope for disrupting specifically the geranylgeranylation of proteins necessary for the transport of monoclonal antibodies in cancerous cells without substantial effect on healthy cells.

A reasonable choice for initial investigation might be 3-PEPC (Figure 45). This compound is already known to inhibit GGTase II and would provide literature precedent for biological activity comparison. Minor deviation from the established synthesis of 3-PEPC could result in the introduction of an alpha methyl group. This might proceed through the use of trimethyl 2-phosphonopropionate as a preferred choice in starting material, or a reaction sequence directly analogous to the synthesis discussed in Chapter 2 which includes treatment of the carboxyphosphonate with base and iodomethane. Once the alpha methyl group is in place the 3-PEPC derivative might be converted to the POM prodrug from the methyl ester though already established methods.
Chapter 3 describes the synthesis and biological activity of the first phosphoantigen prodrugs synthesized by our group as well as the phosphinophosphonates. These compounds were developed as mimics of the naturally occurring bacterial metabolite 4-hydroxy-3-methyl-butenyl-1-diphosphate (HMBPP). The phosphinophosphonates were designed with an overall framework that is more directly comparable to HMBPP and may not require intracellular phosphorylation to present an effective ligand for the transmembrane signaling protein butyrophilin 3A1 (BTN3A1). The activation caused by these compounds eventuates in the proliferation of Vγ9Vδ2 T cells which contributes to immunity for a host of different human diseases that can be difficult to treat, notably bacterial infections and cancer. The phosphoantigens prepared by the research group of Dr. David Wiemer at the University of Iowa were evaluated for their capacity to act as T cell proliferative agents by the research group of Dr. Andrew Wiemer at the University of Connecticut.

The parent phosphonate salt analogue of HMBPP (compound 3.04) was synthesized by Dr. Rocky Barney and Dr. Rebekah Shippy. This provided the introduction to phosphoantigen synthesis and was swiftly followed by the introduction of a compound that included POM prodrug.
functionality. This POM prodrug approach was found to enhance greatly the proliferative activity of this phosphoantigen, providing further evidence for intracellular binding of these ligands. The phosphinophosphonate compounds were devised as an extension of these methods that might provide additional binding information and was generally successful. The biological activity of compounds exhibiting this general structure did not surpass that of the original ligand, but these compounds did prove to be useful to our collaborators for additional binding information in the form of modelling and isothermal titration calorimetry studies. From these data it was possible to propose a model of activation involving a cross membrane inside out negatively cooperative signaling event that requires binding of the ligand to the internal domain of BTN3A1 leading to the propagation of T cell immune response.

![Phosphoantigen Prodrug Model](image)

Figure 46. Phosphoantigen Prodrug Model
While the tri-POM prodrug and full salt forms of the phosphinophosphonate motif did not display potency beyond what was observed for the bis-POM prodrug of the original ligand, future studies may be warranted. The original intention to develop a ligand arrangement that more closely mimicked HMBPP was successful based on ITC and modelling data. The main discrepancy is likely due to differences at the alpha position as the phosphinophosphonate does not contain an oxygen that might be beneficial for binding to key residues within BTN3A1 based on the modelling experiments. This difference may also adjust the pKa of the phosphonic acid relative to the phosphoric acid functionality present in the diphosphate HMBPP at physiological pH. This disadvantage might be overcome with the incorporation of an atom (or atoms) at this position that display sufficient electronegativity, which may allow for enhanced binding and lower the pKa of the phosphonic acid. One idea that may offer a worthy inclusion to the study of phosphinophosphonates would be synthesis and biological evaluation of alpha fluorinated phosphinophosphonates. The installation of alpha fluorines through minor deviations from the established synthesis would involve utilizing a starting material such as dimethyl(fluoromethyl)phosphonate or dimethyl(difluoromethyl)phosphonate when establishing the alpha carbon-phosphorus bond (Figure 47). This might then be carried through analogous reaction sequences for the installation of tri-POM prodrug esters. Alternatively, this project could be combined with the method for formation of mixed aryl/POM prodrugs to form a novel phosphinophosphonate with an electronegative atom at the alpha position (Figure 47).
Chapters 4 and 5 are dedicated to the presentation of asymmetric mixed aryl phosphonate prodrugs of the original butyrophilin ligand. Chapter 4 was focused on the first compounds within this family of prodrugs that included phenyl and 1-naphthyl species. A pattern emerged that showed the relative potency for different prodrug combinations where the aryl/POM motif was more active than the aryl/salt combination which in turn was much more active than the aryl/methyl phosphonates. The 1-naphthyl/POM prodrug displayed the most potent activity as a proliferative agent of any synthetic analogues reported at that time, with a measured EC\textsubscript{50} value of 0.00079 μM.

The mixed aryl phosphonate prodrug phosphoantigens still provide several attractive paths of inquiry including: 1) the incorporation of functionality that alters the partition coefficient which would likely modify the cellular absorptivity; and 2) the inclusion of substituted aryl functionality to modify the pKa which would likely change the metabolism of the prodrug. Extended synthetic...
investigation in this area, perhaps utilizing the newly developed alternate synthesis that incorporates allylic hydroxyl functionality early on, would likely result in the development of ligands that surpass the activity of the most potent reported member of this family. The end of Chapter 5 provides several novel compounds that may help elucidate the structure activity relationship between prodrug modifications and the ultimate proliferative activity of these agents.

The phosphaantigen prodrugs discussed to this point have all entailed modifications to the phosphonate terminus and prodrug motif. A significant potential area of future study would be to probe the tolerance of phosphaantigens to alterations of the carbon skeleton. Preliminary investigation has led to the synthesis of diene containing phosphonates (Figure 48). Two parallel syntheses (Figures 48 and 49) have been developed that utilized different protection/deprotections strategies to include allyic hydroxyl functionality early in the synthesis while retaining the ability to selectively manipulate the phosphonate.

The first synthesis (Figure 48) began with commercially available prenyl acetate which was treated with selenium dioxide and tert-butyl hydroperoxide in order to establish the allylic alcohol 6.12. This alcohol was then protected through reaction with tert-butyldimethylsilyl chloride and imidazole to afford the silyl ether 6.13 in excellent yield.84 The acetate functionality was removed through reaction with potassium carbonate to form the free alcohol 6.14 in near quantitative yield.84 The allylic alcohol was then subjected to oxidation conditions by treatment with manganese dioxide to form the unsaturated aldehyde compound 6.15.85 This aldehyde then was allowed to react with the anion of tetramethyl methylenediphosphonate to allow the Horner–Wadsworth–Emmons (HWE) style condensation to the diene framework of compound 6.16.86 Reaction of ester 6.16 with pivaloyl oxymethyl chloride and sodium iodide allowed for successful conversion to the bisPOM prodrug 6.17, which could be assayed directly, or it could be subjected
to silyl ether deprotection conditions by treatment with tetrabutyl ammonium fluoride to form the free alcohol diene analogue to the ligand most studied.

The second synthesis of a diene containing ligand started with commercially available prenol 6.19 which was protected through the use of tert-butyldimethylsilyl chloride (TBSCI) and imidazole to afford the silyl ether 6.20 in excellent yield. Compound 6.20 then was allowed to react with selenium dioxide and tert-butyl hydroperoxide to afford the desired alcohol 6.21 in the highest yield I have observed for this reaction to date at 70%. The newly established allylic alcohol 6.21 was then protected by reaction with acetic anhydride and catalytic 4-dimethylaminopyridine and the TBS group was removed by reaction with tetrabutyl ammonium fluoride to provide a functional group reversed analogue to the previously discussed synthesis.
compound 6.23. The free alcohol was then oxidized with manganese dioxide and the resulting aldehyde was subjected to HWE conditions by reaction with the anion of tetraethyl methylenediphosphonate (TEMBP). While some acetate deprotected product was isolated through the initial study, reaction optimization is likely to provide more desirable results.

**Figure 49. Second Attempted Synthesis of Diene Containing Phosphoantigen Analogue**

With the diethyl monophosphonate 6.25 in hand, formation of mixed aryl phosphonate functionality was accomplished by reaction with triflic anhydride and pyridine followed by introduction of phenol (Figure 50). The resulting asymmetric phosphonate 6.27 will likely provide a reasonable point of divergence for the establishment of other prodrug moieties and is currently
the basis of continued studies. The acetate deprotected diene phosphonate 6.26 was subjected to hydrolysis conditions through the use of trimethyl silyl bromide and subsequent reaction with sodium hydroxide to afford the desired disodium salt analogue 6.31. This provides the first assayable compound of this potential series that might be compared to previously established phosphoantigens. Biological activity evaluations of this compound are currently being pursued.

Figure 50. Actual and Proposed Future Directions

In conclusion, this work reports the synthesis of a novel motif for bisphosphonate inhibitors of geranylgeranyl diphosphate synthase and a variety of small molecule phosphonate ligands of butyrophilin 3A1. The GGDPS inhibitors were evaluated for their bioactivity by our collaborator Dr. Sarah Holstein and coworkers and were found to display moderate activity. Important structural features from this study have been incorporated into subsequent studies to great effect.
Biological assays for the phosphoantigens developed herein were performed by Dr. Andrew Wiemer and coworkers, and these compounds were found to be exceptionally active in their ability to stimulate proliferation of \( \text{V}_\gamma \text{V}_\delta \text{2} \) T cells. Many studies are currently being conducted to form novel phosphoantigen prodrugs that express potent activity and desirable pharmacokinetic properties and these further studies will no doubt lead to the development of interesting and potentially therapeutically relevant compounds.
CHAPTER 7:

EXPERIMENTAL PROCEDURES

General experimental procedures

Both tetrahydrofuran (THF) and diethyl ether (Et₂O) were freshly distilled from sodium and benzophenone, while acetonitrile, methylene chloride (DCM), pyridine, and trimethylamine (Et₃N) were distilled from calcium hydride prior to use. Toluene and DMF were dried over molecular sieves prior to use. Solutions of n-BuLi were purchased from a commercial source and titrated with diphenylacetic acid prior to use. All other reagents and solvents were purchased from commercial sources and used without further purification. All reactions in non-aqueous solvents were conducted in flame or oven dried glassware under a positive pressure of argon and with magnetic stirring. The NMR spectra were obtained at 300, 400, or 500 MHz for ¹H, and 75, 100, or 125 MHz for ¹³C, with internal standards of Si(CH₃)₄ (¹H, 0.00 ppm) or CDCl₃ (¹H, 7.27 ppm; ¹³C, 77.2 ppm) for non-aqueous samples or D₂O (¹H, 4.80 ppm) and 1,4-dioxane (¹³C, 66.6 ppm) for aqueous samples. The ³¹P chemical shifts were reported in ppm relative to 85% H₃PO₄ (external standard). High resolution mass spectra were obtained at the University of Iowa Mass Spectrometry Facility. Silica gel (60 Å, 0.040–0.063 mm) was used for flash chromatography. HPLC separations were carried out using a Beckman System Gold instrument with a model 166 variable wavelength UV detector connected to a 128 solvent module.
Bisphosphonate 2.16

Tetramethylmethylene diphosphonate (2.38 mL, 13.0 mmol.) was added to a solution of acetonitrile (10 mL), sodium iodide (7.70 grams, 52.0 mmol.), and pivaloyloxyethyl chloride (9.62 mL, 66.0 mmol.). The solution was heated to reflux and allowed to react for 48 hours. The solution then was allowed to cool to room temperature, quenched by addition of deionized water (5 mL), and extracted with diethyl ether. The organic portion was dried (Na₂SO₄) and concentrated \textit{in vacuo}. The waxy dark yellow solid that was obtained was ground using a mortar and pestle and washed with cold hexanes (3 × 5 mL). The solid was then dried under reduced pressure for 12 hours. The filtrate also was concentrated and purified via flash chromatography (silica gel, 15% ethyl acetate in hexanes). The product was a pale yellow solid isolated in 43% yield (3.53 grams):

\begin{align*}
^{1}H \text{ NMR (300 MHz, CDCl$_3$)} & \delta 5.71 (m, 8H), 2.67 (t, J_{PH} = 21.6 \text{ Hz}, 2H), 1.24 (s, 36H); \\
^{31}P \text{ NMR (121 MHz, CDCl$_3$)} & \delta +18.0.
\end{align*}
Bisphosphonate 2.17

1,4-Diazabicyclo[2.2.2]octane (0.63g, 5.6 mmol) was added to a solution of bisphosphonate 2.16 (3.53 g, 5.58 mmol) in 50 mL of freshly distilled acetonitrile and placed the solution was placed under argon. The reaction was heated at reflux. After 2 hours, the solution was allowed to cool to room temperature and concentrated in vacuo. The desired product was an extremely viscous clear gel isolated in quantitative yield (4.16 g): $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.58 (d, $J_{PH} = 12.0$ Hz, 4H), 5.44 (d, $J_{PH} = 9.0$ Hz, 2H), 5.18 (s, 2H), 3.40 (t, $J = 6.0$ Hz, 6H), 3.16 (t, $J = 6.0$ Hz, 6H), 2.48 (m, $J_{PH} = 15$ Hz, 2H), 1.19 (s, 9H), 1.14 (s, 27H); $^{31}$P NMR (121 MHz, CDCl$_3$) $\delta$ +25.8 (d, $J_{PP} = 7.3$ Hz), +9.36 (d, $J_{PP} = 7.3$ Hz).
Bisphosphonate 2.18

Compound 2.17 (4.16 g, 5.6 mmol) was added to a solution of toluene (50 mL) and DMF (0.03 mL). Oxalyl chloride (2.16 mL, 25.1 mmol) was added dropwise and allowed to react for 45 minutes. The solution was filtered through celite and Na$_2$SO$_4$ and concentrated in vacuo to provide a yellow oil. This oil was dissolved in THF (60 mL). Triethylamine (0.89 mL, 6.4 mmol) and methanol (0.26 mL, 6.4 mmol) were added to the solution and allowed to react for 16 hours. The reaction mixture was filtered through celite and concentrated to a yellow oil. The resulting material was purified using flash chromatography (silica gel, 30% EtOAc in hexanes). The product was isolated in 42% yield (0.71 g): $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.70 (m, 6H), 3.80 (d, $J_{PH}$ = 11.7 Hz, 3H), 2.62 (t, $J_{PH}$ = 21.7 Hz, 2H), 1.23 (s, 27H); $^{31}$P NMR (121 MHz, CDCl$_3$) $\delta$ +19.5 (d, $J_{PP}$ = 7.7 Hz), +18.7 (d, $J_{PP}$ = 7.7 Hz).
Bisphosphonate 2.20

Tetramethyl methylenediphosphonate (4.8 mL, 25.8 mmol) was dissolved in THF (100 mL) and cooled to 0 °C. NaH (1.03 g, 25.8 mmol, 60% dispersion in mineral oil) was added portion-wise to the solution and allowed to react for 30 minutes. The phosphonate anion was then introduced to a solution of geranyl bromide and allowed to react and reach room temperature overnight. The reaction then was quenched and washed with deionized water (3 × 20 mL). The organic layer was dried (MgSO₄), filtered through celite, and concentrated to give a yellow oil. The material was then purified using flash chromatography (silica gel, 20% acetone in hexanes). The product was isolated in 63% yield (5.47 g): ¹H NMR (300 MHz, CDCl₃) δ 5.23 (t, J = 6.9 Hz, 1H), 5.05 (s, 1H), 3.75 (d, JPH = 10.8 Hz, 12H), 2.60 (m, 2H), 2.34 (tt, JPH = 23.4, J = 6.3 Hz, 1H), 1.99 (m, 4H), 1.63 (s, 3H), 1.60 (s, 3H), 1.56 (s, 3H); ³¹P NMR (121 MHz, CDCl₃) δ +26.09.

Bisphosphonate 2.21

Compound 2.20 (2.00 g, 5.4 mmol) was dissolved in diethyl ether (100 mL), placed under argon, and cooled to 0 °C. After NaH (0.28 g, 7.1 mmol) as a 60% dispersion in mineral oil was added slowly, the reaction was allowed to react for 45 minutes. Methyl iodide (0.44 mL, 7.1 mmol) was introduced dropwise and allowed to react and reach room temperature over 15 hours. The reaction was quenched by addition of 1 M HCl and washed with deionized water (2 × 15 mL). The organic portion was then dried (MgSO₄), filtered through celite, and concentrated in vacuo. The resulting
oil was purified using flash chromatography (silica gel, 3% EtOH in hexanes). The product was isolated in 38% yield (0.77 g; 47% BRSM): $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.33 (t, $J = 7.2$ Hz, 1H), 5.10 (m, 1H), 3.83 (d, $J_{PH} = 10.5$ Hz, 12H), 2.59 (dt, $J_{PH} = 15.6$ Hz, $J = 7.8$, 2H), 2.06 (m, 4H), 1.68 (s, 3H), 1.62 (s, 3H), 1.60 (s, 3H), 1.40 (t, $J_{PH} = 16.2$ Hz, 3H); $^{31}$P NMR (121 MHz, CDCl$_3$) $\delta$ +29.64.

**Bisphosphonate 2.22**

Compound 2.21 (1.06 g, 2.8 mmol) was dissolved in acetonitrile (2 mL) and subsequently concentrated in vacuo three times to remove residual entrapped solvent. The material was then added to a solution of chloromethyl pivalate (2.04 mL, 14.1 mmol) and sodium iodide (1.69 g, 11.3 mmol) in acetonitrile (20 mL). This mixture then was heated at reflux for approximately 12 hours. The reaction then was diluted with Et$_2$O and washed (3 × 10 mL) with brine. The organic layer was then dried (Na$_2$SO$_4$), filtered through celite, and concentrated. This material then was purified using flash chromatography (silica gel, 15% acetone in hexanes) to afford a yellow oil in 30% yield (0.65 g): $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.70 (m, 8H), 5.24 (t, $J = 6.9$ Hz, 1H), 5.05 (m,
1H), 2.60 (dt, $J_{PH} = 16.2$ Hz, $J = 7.5$ Hz, 2H), 2.01 (m, 64), 1.64 (s, 3H), 1.57 (s, 6H), 1.36 (t, $J_{PH} = 16.8$ Hz, 3H), 1.20 (s, 36H); $^{31}$P NMR (121 MHz, CDCl$_3$) δ +25.8.

Bisphosphonate 2.23

1, 4-Diazabicyclo[2.2.2]octane (0.11 g, 0.95 mmol) was added to a solution of compound 2.22 (0.74 g, 0.95 mmol) in 20 mL acetonitrile. The solution was heated at reflux for 2 hours, then allowed to cool to room temperature and concentrated in vacuo. The desired product was utilized without further purification.

(E)-Dimethyl (4,8-dimethylnona-3,7-dien-1-yl)phosphonate (2.26). Dimethyl methylphosphonate (2.56 mL, 22.9 mmol) was added to an oven-dried round bottom flask with THF (100 mL). The solution was cooled in a dry ice/acetone bath for 20 minutes, and then $n$-butyl lithium (10.08 mL, 25.2 mmol) was added as a 2.5 M solution in hexanes. After 1.5
hours freshly prepared geranyl bromide (5.47 g, 25.2 mmol) was added dropwise and the reaction was stirred and allowed to reach room temperature overnight (15 h). The reaction was diluted with Et₂O, quenched by addition of water, and washed with water (3 x 20 mL), and the organic layer was dried (Na₂SO₄). The mixture was vacuum filtered through celite, and the filtrate was concentrated to give a dark yellow oil. Final purification via flash chromatography (EtOAc, silica gel) gave the desired product as a yellow oil (5.67 g, 95%): ¹H NMR (300 MHz, CDCl₃) δ 5.15–5.06 (m, 2H), 3.74 (d, JₚH = 10.8 Hz, 6H), 2.34–2.23 (m, 2H), 2.07–1.98 (m, 4H), 1.86–1.72 (m, 2H), 1.73 (s, 3H), 1.71 (s, 3H), 1.67 (s, 3H); ³¹P NMR (121 MHz, CDCl₃) δ +34.4.

(E)-Methyl (4,8-dimethylnona-3,7-dien-1-yl)phosphonochloridate (2.27).

Compound 2.26 (1.93 g, 7.4 mmol) was diluted with anhydrous toluene and then concentrated under reduced pressure (3 x 3 mL) to remove any residual water. The phosphonate then was dissolved in dichloromethane (25 mL) and dimethyl formamide was added (1 drop). The solution was then cooled on an ice bath for 30 minutes. Oxalyl chloride (1.91 mL, 22.3 mmol) was added, and the solution was allowed to react for 13 hours. The excess oxalyl chloride and solvent were removed in vacuo and the resulting deep red oil was utilized without further purification. The ³¹P NMR spectrum showed nearly complete conversion to product (approximately a 13:1 ratio of product to starting material by integration, or 92% by ³¹P NMR). ³¹P NMR (121 MHz, CDCl₃) δ +46.1 (product peak), +34.8 (residual starting material).
(S)-3,7-Dimethyloct-6-en-1-yl methyl ((E)-4,8-dimethylnona-3,7-dien-1-yl)phosphonate (2.28).

Compound 2.27 (1.963 g, 7.4 mmol) was dissolved in anhydrous toluene (25 mL). After (S)-(−)-citronellol (2.71 mL, 14.8 mmol) and freshly distilled triethylamine (2.07 mL, 14.8 mmol) were added sequentially, the solution was allowed to react overnight. Upon addition of triethylamine formation of a white precipitate and a color change from deep red to dark brown was noted. The reaction was quenched by addition of deionized water (5 mL). A cloudy emulsion formed which clarified after washing with brine (3 x 10 mL). The organic layers were combined, dried (Na₂SO₄), and vacuum filtered through celite, and the filtrate was concentrated in vacuo to afford a dark yellow oil. Final purification by flash chromatography (silica gel, 50% EtOAc in hexanes) gave the desired product 2.28 as a light yellow oil (1.695 g, 59% yield, 64% based on recovered 2.26):

1H NMR (300 MHz, CDCl₃) δ 5.15-5.05 (m, 3H), 4.16–4.01 (m, 2H), 3.73 (d, JₚH = 10.5 Hz, 3H), 2.35-2.23 (m, 2H), 2.05–1.91 (m, 6H), 1.82–1.70 (m, 2H), 1.68 (s, 6H), 1.62 (s, 3H), 1.60 (s, 6H), 1.57–1.13 (m, 5H), 0.92 (d, J = 6.3 Hz, 3H); 13C NMR (75 MHz, CDCl₃) δ 135.9 (d, JCP = 1.2 Hz), 130.8, 130.7, 124.2, 123.8, 122.7 (d, JCP = 16.4 Hz), 63.5, 51.5, 39.2, 37.2 (d, JCP = 5.3 Hz), 36.6, 28.7 (d, JCP = 0.9 Hz), 26.2, 25.1 (d, JCP = 137.9 Hz), 25.3, 25.0, 20.7, 20.5, (d, JCP = 4.6 Hz), 18.9, 17.2, 15.6, 13.8; 31P NMR (121 MHz, CDCl₃) δ +33.11, +33.07; HRMS (ES⁺, m/z) calcd for (M+H)⁺ C₂₂H₉₂O₃P: 385.2872; found: 385.2875.
(S)-3,7-Dimethyloct-6-en-1-yl methyl ((E)-1-(dimethoxyphosphoryl)-4,8-dimethylnona-3,7-dien-1-yl)phosphonate (2.29).

Freshly dried 2,2,6,6-tetramethylpiperidine (5.35 mL, 31.7 mmol) was combined with n-butyllithium (12.68 mL, 31.7 mmol as a 2.46 M solution in hexanes) and THF (50 mL) at dry ice/acetone temperatures. After 15 minutes, compound 2.28 (5.54 g, 14.4 mmol) was added slowly to the solution and allowed to react for 20 minutes. Then the solution was transferred by cannula into a solution of dimethyl chlorophosphate (3.42 mL, 31.7 mmol) in THF (15 mL) and the resulting solution was allowed to react and reach room temperature overnight (9 hours). The reaction was then diluted with Et₂O, quenched by addition of deionized water, dried (Na₂SO₄), and vacuum filtered through celite. The filtrate was concentrated to a yellow oil, which was purified further via flash chromatography (silica gel, 10% EtOH in EtOAc) to give the desired product (2.72 g, 38%, 52% based on recovered 2.28): ¹H NMR (300 MHz, CDCl₃) δ 5.28 (t, J = 6.6 Hz, 1H), 5.11–5.06 (m, 2H), 4.20–4.09 (m, 2H), 3.81 (d, Jₚₙₚ = 11.1 Hz, 9H), 2.72–2.56 (m, 2H), 2.36 (tt, Jₚₙₚ = 24.0 Hz, J = 6.2 Hz 1H), 2.08–1.90 (m, 6H), 1.67 (s, 6H), 1.64 (s, 3H), 1.60 (s, 6H), 1.55–1.30 (m, 4H), 1.24–1.12 (m, 1H), 0.91 (d, J = 6.4 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 137.3, 131.5, 131.4, 124.6, 124.2, 121.6 (t, Jₚₙ₃ = 7.4 Hz), 65.0, 53.4–53.0 (m, 3 C overlap), 40.1 (t, Jₚₙ₃ = 105.8 Hz), 39.8, 38.7, 37.6–37.5 (m), 37.1–36.9 (m), 35.2, 29.1 (d, Jₚₙ₃ = 1.4 Hz), 26.7,
25.8, 25.4, 24.0 (m), 19.4, 17.8, 16.3; $^{31}$P NMR (121 MHz, CDCl$_3$) δ +26.3–26.2 (m), +24.8–24.6 (m); HRMS (ES$^+$, m/z) calcd for (M+H)$^+$ C$_{24}$H$_{47}$O$_6$P$_2$: 493.2848; found: 493.2845.

Compound 2.29 (340 mg, 0.7 mmol) was dissolved in acetonitrile (3 x 3 mL) and then concentrated under reduced pressure to remove any residual water. Compound 2.29 was then added to a solution of chloromethyl pivalate (0.31 mL, 2.2 mmol) and sodium iodide (330 mg, 2.2 mmol) in acetonitrile (5 mL). This reaction mixture was then heated at reflux for approximately 5 hours. The acetonitrile was removed under reduced pressure and the resulting material was dissolved in diethyl ether. The solution was washed with brine (4 x 10 mL) and the organic layer was dried (Na$_2$SO$_4$), vacuum filtered through celite, and concentrated to a yellow oil. Final purification by flash chromatography (silica gel, 25% EtOAc in hexanes + 1% TEA) afforded the desired product 2.30 as a faint yellow oil (60 mg, 11%, 20% based on recovered 2.29): $^1$H NMR (300 MHz, CDCl$_3$) δ 5.71–5.7 (m, 6H), 5.28–5.23 (m, 1H), 5.10–5.06 (m, 2H), 4.20–4.12 (m, 2H), 2.70–2.60 (m, 2H), 2.50 (tt, $J_{PH} = 24.0$ Hz, $J = 6.3$ Hz, 1H), 2.07–1.95 (m, 6H), 1.68 (s, 6H), 1.62 (s, 3H), 1.60 (s, 6H), 1.54–1.29 (m, 5H), 1.24 (s, 27H), 0.91 (d, $J = 6.3$ Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 176.9,
176.8, 176.6, 137.9, 131.5, 131.4, 124.5, 124.0, 120.7, 82.8–81.8 (m, 3 C overlap), 65.2, 39.7, 38.8, 38.7, 37.3–37.2 (m), 37.00, 36.98 29.1, 26.93, 26.91, 26.88, 26.85, 26.81, 26.59, 26.58, 25.70, 25.68, 25.4, 23.71, 23.66, 19.19, 19.14, 17.68, 17.65 16.13, 16.11, 15.3; $^{31}$P NMR (121 MHz, CDCl$_3$) δ +23.1 (d, $J_{PP}$ = 2.9 Hz), +23.0 (d, $J_{PP}$ = 2.8 Hz), +22.4–22.3 (m), +22.2–22.1 (m); HRMS (ES$^+$, m/z) calcd for (M+H)$^+$ C$_{39}$H$_{71}$O$_2$P$_2$: 793.4421; found: 793.4411.

(S)-3,7-Dimethyloct-6-en-1-yl methyl ((E)-2-(dimethoxyphosphoryl)-5,9-dimethyldeca-4,8-dien-2-yl)phosphonate (2.31).

Compound 2.29 (302 mg, 0.6 mmol) was dissolved in THF (5 mL) and the solution was cooled in an ice bath for 15 minutes. After NaH (37 mg, 0.9 mmol, 60% dispersion in mineral oil) was added to the solution, it was allowed to react for 30 minutes. Iodomethane (0.06 mL, 0.9 mmol) was then added and the reaction was allowed to proceed for 1.5 hours. The solution was then diluted with diethyl ether, quenched by addition of deionized water, and washed with water (3 x 5 mL). The organic layer was dried (Na$_2$SO$_4$) and vacuum filtered through celite. The filtrate was concentrated to a yellow oil which was purified by flash chromatography (silica gel, 5% EtOH in EtOAc) yielding the desired compound 2.31 as a yellow oil (0.30 g, 97%): $^1$H NMR (300 MHz, CDCl$_3$) δ 5.34 (t, $J = 7.2$ Hz, 1H), 5.12–5.05 (m, 2H), 4.20–4.12 (m, 2H), 3.75 (d, $J_{PH} = 10.8$ Hz, 3H), 3.74 (d, $J_{PH} = 10.5$ Hz, 3H), 3.73 (d, $J_{PH} = 10.2$ Hz, 3H), 2.60 (td, $J_{PH} = 15.3$ Hz, $J = 7.4$ Hz,
2H), 2.10–1.93 (m, 6H), 1.73–1.70 (m, 2H), 1.67 (s, 6H), 1.61 (s, 3H), 1.60 (s, 6H), 1.39 (t, $J_{\text{PH}}$ = 15.6 Hz, 3H), 1.30–1.12 (m, 3H), 0.92 (d, $J = 6.6$ Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 138.2, 131.4, 131.3, 124.6, 124.3, 118.8 (t, $J_{\text{CP}}$ = 6.9 Hz), 65.0, 53.6–53.2 (m, 3C overlap), 41.5 (t, $J_{\text{CP}}$ = 133.1 Hz), 40.1, 39.7, 37.6 (t, $J_{\text{CP}}$ = 3.6 Hz), 37.1–37.0 (m), 31.1 (t, $J_{\text{CP}}$ = 6.2 Hz), 29.0, 26.7, 25.7, 25.4, 19.3, 19.3, 17.7, 17.7, 16.2; $^{31}$P NMR (121 MHz, CDCl$_3$) $\delta$ +29.8 (d, $J_{\text{PP}}$ = 7.6 Hz), +28.3–28.1 (m); HRMS (ES$^+$, $m/z$) calcd for (M+H)$^+$ C$_{25}$H$_{49}$O$_6$P$_2$: 507.3004; found: 507.3000.

(S)-3,7-Dimethyloct-6-en-1-yl methyl ((E)-4-(dimethoxyphosphoryl)-7,11-dimethyldodeca-1,6,10-trien-4-yl)phosphonate (2.32).

According to the procedure described for preparation of compound 2.31, compound 2.29 (300 mg, 0.6 mmol) was dried, dissolved in THF (8 mL), and treated with NaH (40 mg, 0.9 mmol, 60% dispersion in mineral oil) and allyl bromide (0.08 mL, 0.9 mmol). Standard work-up and concentration in vacuo gave a light-yellow oil. Final purification by flash chromatography (silica gel, 5% EtOH in EtOAc) afforded the desired compound 2.32 (97 mg, 30% yield, 74% based on recovered 10) $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 6.03–5.92 (m, 1H), 5.40–5.36 (m, 1H), 5.11–5.07 (m, 4H), 4.17–4.12 (m, 2H), 3.775 (d, $J_{\text{PH}}$ = 10.5 Hz, 3H), 3.767 (d, $J_{\text{PH}}$ = 10.8 Hz, 3H), 3.760 (d, $J_{\text{PH}}$ = 10.8 Hz, 3H), 2.69–2.58 (m, 4H), 2.10–1.96 (m, 6H), 1.74–1.71 (m, 2H), 1.67 (s, 6H), 1.61 (s, 3H), 1.60 (s, 6H), 1.55–1.30 (m, 3H), 0.91 (d, $J = 6.9$ Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$
137.9, 133.7 (t, $J_{CP} = 7.3$ Hz) 131.5, 131.5, 124.7, 124.4, 118.8 (t, $J_{CP} = 7.7$ Hz), 118.1, 65.1–64.9 (m), 53.6–53.2 (m, 3C overlap), 46.3 (t, $J_{CP} = 130.7$ Hz), 40.2, 37.7–37.6 (m), 37.2–37.1 (m), 35.3–35.2 (m), 29.2, 27.4, 26.7, 25.8, 25.5, 25.1, 19.4–19.3 (m), 17.8, 17.8, 16.5; $^{31}$P NMR (121 MHz, CDCl$_3$) $\delta$ +28.8–28.7 (m), 27.4–27.1 (m); HRMS (ES$^+$, $m/z$) calcd for (M+H)$^+$ C$_{27}$H$_{51}$O$_6$P$_2$: 533.3161; found: 533.3164.

$(S)$-3,7-Dimethyloct-6-en-1-yl methyl (E)-5-(dimethoxyphosphoryl)-2,8,12-trimethyltrideca-2,7,11-trien-5-ylphosphonate (2.33).

According to the procedure described for preparation of compound 2.31, compound 2.29 (360 mg, 0.7 mmol) was dried, dissolved in THF (5 mL), and treated with NaH (40 mg, 1.1 mmol, 60% dispersion in mineral oil) and freshly prepared prenyl bromide (0.13 mL, 1.1 mmol). After stirring overnight, standard work-up gave a faint yellow oil purified further by flash chromatography (silica gel, 5% EtOH in EtOAc) to afford the product 2.33 (270 mg, 65% yield). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.39–5.37 (m, 2H), 5.12–5.10 (m, 2H), 4.14–4.13 (m, 2H), 3.80 (d, $J_{PH} = 11.1$ Hz, 9H), 2.67–2.57 (m, 4H), 2.08–1.97 (m, 6H), 1.73 (s, 3H), 1.69 (s, 6H), 1.63 (s, 6H), 1.61 (s, 6H), 1.58–1.34 (m, 5H), 0.93 (d, $J = 6.6$ Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 137.7, 133.9, 131.5, 131.4, 124.7, 124.4, 119.3–119.0 (m, 2C overlap), 65.0–64.8 (m), 53.5–53.1 (m, 3C overlap), 46.5 (t, $J_{PH} = 130.8$ Hz), 40.2, 37.7, 37.7, 37.2, 37.1, 29.3–29.2 (m), 26.8, 26.3, 25.8, 25.5, 19.4, 19.3,
18.0, 18.0, 17.8, 16.4. $^{31}$P NMR (121 MHz, CDCl$_3$) $\delta$ 29.3–29.3 (m), 27.8–27.6 (m); HRMS (ES$^+$, m/z) calcd for (M+H)$^+$ C$_{29}$H$_{55}$O$_6$P$_2$: 561.3474; found: 561.3479

(S)-3,7-Dimethyloct-6-en-1-yl methyl ((6E,11E)-9-(dimethoxyphosphoryl)-2,6,12,16-tetramethylheptadeca-2,6,11,15-tetraen-9-yl)phosphonate (2.34).

According to the procedure described for preparation of compound 2.31, compound 2.29 (290 mg, 0.6 mmol) was dissolved in THF (5 mL), and treated with NaH (40 mg, 0.9 mmol, 60% dispersion in mineral oil), and freshly prepared geranyl bromide (0.18 mL, 0.9 mmol). After the reaction was allowed to stir overnight, standard work-up gave a light yellow oil (265 mg, 71%): $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.38 (m, 2H), 5.10–5.08 (m, 3H), 4.15–4.13 (m, 2H), 3.78 (d, $J_{PH}$ = 10.8 Hz, 9H), 2.66–2.56 (dt, $J_{PH}$ = 16.2 Hz, $J$ = 8.7 Hz 4H), 2.07–2.04 (m, 10H), 1.67 (s, 9H), 1.62 (s, 6H), 1.60 (s, 9H), 1.48–1.43 (m, 5H), 0.92 (d, $J$ = 6.3 Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 137.6, 131.43, 131.38, 124.7, 124.4, 119.16 (t, $J_{PC}$ = 7.6 Hz), 65.0–64.8 (m), 53.4–53.1 (m), 3 C overlap), 46.5 (t, $J_{PC}$ = 130.7 Hz), 40.2, 37.8–37.7 (m), 37.2–37.1 (m), 34.4, 29.3, 26.7, 25.8, 25.5, 19.4, 19.3, 17.8, 17.7, 16.4; $^{31}$P NMR (121 MHz, CDCl$_3$) $\delta$ 29.4–29.3 (m), +27.8–27.7 (m); HRMS (ES$^+$, m/z) calcd for (M+Na)$^+$ C$_{34}$H$_{62}$O$_6$P$_2$Na: 651.3919; found: 651.3922.
(((E)-2-(((S)-3,7-dimyloct-6-en-1-yl)oxy)((pivaloyloxy)methoxy)phosphoryl)-5,9-dimethyldeca-4,8-dien-2-yl)phosphoryl)bis(oxy))bis(methylene) bis(2,2-dimethylpropanoate) (2.35).

Compound 2.31 (0.12 g, 0.2 mmol) was dissolved in acetonitrile (3 x 3mL) and then concentrated under reduced pressure to remove any residual water. Compound 2.31 was then added to a solution of chloromethyl pivalate (0.11 mL, 0.76 mmol) and sodium iodide (0.11 g, 0.6 mmol) in acetonitrile (5 mL), and this reaction mixture was heated at reflux for approximately 24 hours. The acetonitrile was removed under reduced pressure and the resulting material was dissolved in diethyl ether. The solution was washed with brine (4 x 10 mL) and the organic layer was dried (Na₂SO₄), vacuum filtered through celite, and concentrated to a yellow oil. Final purification by flash chromatography (silica gel, 20% EtOAc in hexanes + 1% TEA) afforded the final product as a faint yellow oil (0.07 g, 34%): ¹H NMR (300 MHz, CDCl₃) δ 5.77–5.66 (m, 6H), 5.29 (t, Jₚ = 7.5 Hz, 1H), 5.08–5.07 (m, 2H), 4.16–4.11 (m, 2H), 2.60 (dt, Jₚ = 15.7, J = 6.9 Hz, 2H), 2.06–1.95 (m, 6H), 1.80–1.71 (m, 2H), 1.70 (s, 3H), 1.67 (s, 6H), 1.59 (s, 6H), 1.54–1.48 (m, 3H), 1.39 (t, Jₚ = 16.5 Hz, 3H), 1.23 (s, 27H), 0.90 (d, J = 6.3 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 176.9–176.8 (m, 3C overlap), 139.2, 131.5, 131.5, 124.6, 124.3, 124.3, 83.0–82.1 (m, 3C overlap), 65.1–65.0 (m), 41.5 (t, Jₚ = 133.3 Hz), 40.2, 38.8, 38.8, 37.4–37.3 (m), 37.11–37.06 (m), 30.6, 30.5, 29.22, 29.18, 27.04, 27.00, 26.99, 26.97, 26.7, 25.8, 25.5, 19.35, 19.31, 19.29, 17.80, 17.76, 16.30,
16.27, 15.7; $^{31}$P NMR (121 MHz, CDCl$_3$) δ +26.8–26.6 (m), +25.9–25.8 (m); HRMS (ES$^+$, m/z) calcd for (M+Na)$^+$ C$_{40}$H$_{72}$O$_2$P$_2$Na: 829.4397; found: 829.4404.

(((E)-4-(((S)-3,7-Dimethyloct-6-en-1-yl)oxy)((pivaloyloxy)methoxy)phosphoryl)-7,11-dimethyldodeca-1,6,10-trien-4-yl)phosphoryl)bis(oxy)bis(methylene) bis(2,2-dimethylpropanoate) (2.36).

According to the procedure described for preparation of compound 2.35, compound 2.32 (97 mg, 0.2 mmol) was added to a solution of chloromethyl pivalate (0.30 mL, 2.0 mmol), sodium iodide (300 mg, 2 mmol), and acetonitrile (5 mL) and the solution was heated at reflux for 8 hours. Standard workup and purification by flash chromatography (silica gel, 15% EtOAc in hexanes + 1% TEA) gave a clear oil (34 mg, 23%). $^1$H NMR (300 MHz, CDCl$_3$) δ 6.01–5.85 (m, 1H), 5.79–5.66 (m, 6H), 5.35–5.31 (m, 1H), 5.10–5.07 (m, 4H), 4.18–4.08 (m, 2H), 2.69–2.57 (m, 4H), 2.08–1.95 (m, 6H), 1.80–1.70 (m, 2H), 1.67 (s, 6H), 1.59 (s, 9H), 1.53–1.45 (m, 3H), 1.23 (s, 27H), 0.92 (d, $J = 6.3$ Hz, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$) δ 176.8–176.7 (m, 3C overlap), 138.5, 138.5, 134.7, 131.4, 131.3, 124.5 124.2, 124.2, 82.9, 82.5, 81.8, 64.8, 47.6, 40.1, 40.0, 38.7, 38.4, 37.3, 37.3, 37.0, 36.3, 29.2, 29.2, 27.1, 27.0, 26.9, 26.9, 26.8, 26.7, 25.7, 25.7, 25.4, 19.2, 19.1, 17.9, 17.7, 17.6, 16.2, 16.2; $^{31}$P NMR (121 MHz, CDCl$_3$) δ +25.5–25.4 (m), 24.8–24.7 (m); HRMS (ES$^+$, m/z) calcd for (M+Na)$^+$ C$_{42}$H$_{74}$O$_{12}$P$_2$Na: 855.4553; found: 855.4559.
(((E)-5-(((S)-3,7-Dimethyloct-6-en-1-yl)oxy)((pivaloyloxy)methoxy)phosphoryl)-2,8,12-trimethyltrideca-2,7,11-trien-5-yl)phosphoryl)bis(oxy))bis(methylene) bis(2,2-dimethylpropanoate) (2.37).

According to the procedure described for preparation of compound 2.35, compound 2.33 (267 mg, 0.41 mmol) was added to a solution of chloromethyl pivalate (0.37 mL, 2.6 mmol), sodium iodide (380 mg, 2.6 mmol), and acetonitrile (7 mL) and the solution was heated at reflux for 8 hours. Standard workup and purification by flash chromatography (silica gel, 15% EtOAc in hexanes + 1% TEA) gave a clear oil (133 mg, 32%): $^1$H NMR (300 MHz, CDCl$_3$) δ 5.78–5.62 (m, 6H), 5.35–5.27 (m, 2H), 5.10–5.02 (m, 2H), 4.20–4.03 (m, 2H), 2.66–2.56 (m, 4H), 2.06–1.94 (m, 6H), 1.81–1.76 (m, 2H), 1.71 (s, 3H), 1.66 (s, 6H), 1.59 (s, 12H), 1.52–1.40 (m, 3H), 1.23 (s, 27H), 0.90 (d, 6.3 Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 176.8–176.7 (m, 3C overlap), 138.5, 138.5, 134.7, 131.4, 131.4, 124.5, 124.2, 124.2, 82.9, 82.6, 81.8, 64.7, 47.6, 40.1, 40.1, 38.7, 38.4, 37.3, 37.2, 37.0, 36.3, 29.2, 29.2, 27.1, 26.9, 26.9, 26.9, 26.9, 26.7, 26.1, 25.7, 25.7, 25.4, 19.2, 19.1, 17.9, 17.7, 17.6, 16.2, 16.2; $^{31}$P NMR (121 MHz, CDCl$_3$) δ +26.0–25.9 (m), 25.3–25.1 (m); HRMS (ES$^+$, m/z) calcd for (M+Na)$^+$ C$_{44}$H$_{78}$O$_{12}$P$_2$Na: 883.4866; found: 883.4872.
(((6E,11E)-9-(((S)-3,7-Dimethyloct-6-en-1-yl)oxy)((pivaloyloxy)methoxy)phosphoryl)-
2,6,12,16-tetramethylheptadeca-2,6,11,15-tetraen-9-yl)phosphoryl)bis(oxy))bis(methylene)
bis(2,2-dimethylpropanoate) (2.38).

According to the procedure described for preparation of compound 2.35, compound 2.34 (265 mg,
0.42 mmol) was added to a solution of chloromethyl pivalate (0.30 mL, 2.1 mmol), sodium iodide
(312 mg, 2.08 mmol), and acetonitrile (5 mL) and the solution was heated at reflux for 10 hours.
Standard workup and flash chromatography (silica gel, 10% EtOAc in hexanes + 1% TEA) gave
a yellow oil (36 mg, 9%): $^1$H NMR (500 MHz, CDCl$_3$) δ 5.78–5.65 (m, 6H), 5.36–5.31 (m, 2H),
5.10–5.08 (m, 3H), 4.22–4.05 (m, 2H), 2.68–2.57 (m, 4H), 2.09–1.95 (m, 10H), 1.80–1.70 (m,
2H), 1.68 (s, 9H), 1.62 (s, 6H), 1.60 (s, 9H), 1.52–1.40 (m, 3H), 1.23 (s, 27H), 0.91 (d, $J = 5.7$ Hz,
3H) $^{13}$C NMR (125 MHz, CDCl$_3$) δ 176.8–176.7 (m, 3C overlap), 138.4, 138.4, 131.4, 131.3,
124.5, 124.2, 82.9, 82.6, 81.8, 64.8, 46.3 (t, $J_{PC} = 130.6$ Hz), 40.2, 40.1, 38.7, 37.5, 37.4, 37.3,
16.3, 16.2; $^{31}$P NMR (202 MHz, CDCl$_3$) δ +26.0 (d, $J_{PP} = 9.9$ Hz), +25.3 (d, $J_{PP} = 9.9$ Hz); HRMS
(ES$^+$, m/z) calcd for (M+Na)$^+$ C$_{49}$H$_{86}$O$_{12}$P$_2$Na: 951.5492; found: 951.5507.
(R)-3,7-Dimethyloct-6-en-1-yl methyl ((E)-4,8-dimethylnona-3,7-dien-1-yl)phosphonate (2.41).

According to the procedure described for preparation of compound 2.28, the acid chloride 2.27 (2.63 g, 9.9 mmol) was allowed to react with (R)-(+)citronellol (1.55 g, 9.9 mmol). Parallel workup and purification provided the mixed ester 2.41 (2.21 g, 58%): \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 5.12-5.08\) (m, 3H), 4.10–4.05 (m, 2H), 3.74 (d, \(J_{PH} = 11.2\) Hz, 3H), 2.30–2.26 (m, 2H), 2.07–1.96 (m, 6H), 1.80–1.70 (m, 2H), 1.67 (s, 6H), 1.61 (s, 3H), 1.60 (s, 6H), 1.59–1.44 (m, 2H), 1.37–1.21 (m, 2H), 1.20–1.17 (m, 1H), 0.92 (d, \(J = 6.3\) Hz, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta 136.4, 131.4, 131.6, 124.5, 124.1, 122.9\) (d, \(J_{CP} = 17.0\) Hz), 64.0 (d, \(J_{CP} = 6.6\) Hz), 52.0 (d, \(J_{CP} = 6.5\) Hz), 39.5, 37.5 (d, \(J_{CP} = 6.7\) Hz), 36.9, 29.0 (d, \(J_{CP} = 2.5\) Hz), 26.5, 25.3 (d, \(J_{CP} = 137.2\) Hz), 25.6, 25.3, 20.9, 20.9, 19.2, (d, \(J_{CP} = 1.7\) Hz), 19.2, 17.6, 15.9; \(^{31}\)P NMR (161 MHz, CDCl\(_3\)) \(\delta +33.25, +33.20\); HRMS (ES+, m/z) calcd. for (M + H\(^+\)) \(C_{22}H_{42}O_3P\): 385.2872; found: 385.2864.

(R)-3,7-Dimethyloct-6-en-1-yl methyl ((E)-1-(dimethoxyphosphoryl)-4,8-dimethylnona-3,7-dien-1-yl)phosphonate (2.42).
According to the procedure described for preparation of compound \(2.29\), the mixed ester \(2.41\) (1.02 g, 2.6 mmol) was treated with a solution of LTMP, formed from 2,2,6,6-tetramethylpiperidine (0.99 mL, 5.8 mmol) and \(n\)-butyl lithium (2.32 mL, 5.8 mmol), followed by reaction with dimethyl chlorophosphate (0.65 mL, 5.8 mmol). Standard work-up and purification gave the desired bisphosphonate \(2.42\) (0.46 g, 36%): \(^1H\) NMR (400 MHz, CDCl\(_3\)) \(\delta 5.20 (t, J = 6.0 \text{ Hz}, 1\text{H}), 5.03–5.00 (m, 2\text{H}), 4.10–4.05 (m, 2\text{H}), 3.73 (d, \(J_{\text{PH}} = 14.8 \text{ Hz}, 9\text{H}), 2.62–2.50 (m, 2\text{H}), 2.29 (tt, \(J_{\text{PH}} = 23.6 \text{ Hz}, J = 6.0 \text{ Hz} \quad 1\text{H}), 2.01–1.85 (m, 6\text{H}), 1.60 (s, 6\text{H}), 1.57 (s, 3\text{H}), 1.53 (s, 6\text{H}), 1.50–1.35 (m, 2\text{H}), 1.32–1.23 (m, 2\text{H}), 1.18–1.06 (m, 1\text{H}), 0.85 (d, \(J = 6.4 \text{ Hz}, 3\text{H})\); \(^{13}\text{C} \) NMR (100 MHz, CDCl\(_3\)) \(\delta 137.1, 131.4, 131.2, 124.5, 124.1, 121.5 (t, \(J_{\text{CP}} = 7.0 \text{ Hz}), 65.0–64.9 (m), 53.2–52.9 (m, 3 \text{C overlap}), 39.6, 36.8 (t, \(J_{\text{CP}} = 132.2 \text{ Hz}), 37.5–37.4 (m), 37.0, 36.9, 28.9 (d, \(J_{\text{CP}} = 2.0 \text{ Hz}), 26.5, 25.7, 25.6, 25.3, 23.9 (t, \(J_{\text{CP}} = 5.1 \text{ Hz}), 19.2, 17.6, 16.0; \(^{31}\text{P} \) NMR (121 MHz, CDCl\(_3\)) \(\delta +26.3, +24.7–24.6 (m); \) HRMS (ES+, m/z) calcd for \((M + H)^+ \text{C}_{24}\text{H}_{47}\text{O}_6\text{P}_2\): 493.2848; found: 493.2850.

(R)-3,7-Dimethyloct-6-en-1-yl methyl ((E)-2-(dimethoxyphosphoryl)-5,9-dimethyldeca-4,8-dien-2-yl)phosphonate (2.43).

According to the procedure described for methylation of compound \(2.31\), the bisphosphonate \(2.42\) (0.160 g, 0.3 mmol) was treated with NaH (20 mg, 0.5 mmol) and methyl iodide (0.05 mL, 0.8 mmol) to give the expected product \(2.43\) (152 mg, 92%): \(^1H\) NMR (300 MHz, CDCl\(_3\)) \(\delta 5.26 (t, J \)
= 6.8 Hz, 1H), 5.03–4.99 (m, 2H), 4.12–4.03 (m, 2H), 3.75 (d, JPH = 10.8 Hz, 3H), 3.74 (d, JPH = 10.8 Hz, 3H), 3.73 (d, JPH = 10.8 Hz, 3H), 2.53 (td, JPH = 15.5 Hz, J = 7.5 Hz, 2H), 2.03–1.86 (m, 6H), 1.69–1.63 (m, 2H), 1.60 (s, 6H), 1.54 (s, 3H), 1.52 (s, 6H), 1.32 (t, JPH = 16.7 Hz, 3H), 1.26–1.07 (m, 3H), 0.85 (d, J = 6.9 Hz, 3H); 13C NMR (75 MHz, CDCl3) δ 138.3, 131.5, 131.4, 124.7, 124.3, 118.8 (t, JCP = 7.3 Hz), 65.1 (m), 53.7–53.3 (m, 3C overlap), 41.6 (t, JCP = 133.3 Hz), 40.2, 38.9, 37.7–37.6 (m), 37.2–37.1 (m), 31.1 (t, JCP = 5.2 Hz), 29.1, 26.7, 25.8, 25.5, 19.4, 19.3, 17.8, 17.7, 16.3; 31P NMR (121 MHz, CDCl3) δ +29.8 (d, JPP = 7.9 Hz), +28.3–28.2 (m); HRMS (ES+, m/z) calcd for (M + H)+ C25H49O6P2: 507.3004; found: 507.3006.

According to the procedure described for preparation of compound 2.35, compound 2.43 (165 mg, 0.3 mmol) was treated with POMCl (0.15 mL, 1.0 mmol) and NaI (151 mg, 1.0 mmol) in acetonitrile at reflux. Standard work-up and purification provided the desired POM ester 2.45 (108 mg, 41%): 1H NMR (300 MHz, CDCl3) δ 5.79–5.66 (m, 6H), 5.29 (t, JPH = 7.5 Hz, 1H), 5.09–5.06 (m, 2H), 4.19–4.08 (m, 2H), 2.61 (dt, JPH = 15.8, J = 7.4 Hz, 2H), 2.08–1.91 (m, 6H), 1.77–1.70 (m, 2H), 1.67 (s, 6H), 1.59 (s, 9H), 1.56–1.47 (m, 3H), 1.39 (t, JPH = 17.3 Hz, 3H), 1.24 (s, 9H),
1.23 (s, 18H), 0.90 (d, \( J = 6.3 \text{ Hz} \), 3H); \(^{13}\text{C} \) NMR (75 MHz, CDCl\(_3\)) \( \delta 176.8–176.7 \) (m, 3C overlap), 139.1, 131.5, 124.5, 124.3, 118.0, 83.1–81.8 (m, 3C overlap), 65.2–64.9 (m), 41.6 (t, \( J_{PC} = 133.4 \) Hz), 40.1, 38.8, 38.8, 37.4–37.3 (m), 37.1–37.0 (m), 30.6, 30.5, 29.21, 29.18, 27.1, 27.00, 26.98, 26.97, 26.6, 25.7, 25.5, 19.4, 19.32, 19.28, 17.79, 17.77, 16.30, 16.27, 15.7; \(^{31}\text{P} \) NMR (121 MHz, CDCl\(_3\)) \( \delta +26.8–26.6 \) (m), +25.9–25.8 (m); HRMS (ES\(^+\), \( m/z \)) calcd for \((\text{M} + \text{Na})^+\) C\(_{40}\)H\(_{72}\)O\(_{12}\)P\(_2\)Na: 829.4397; found: 829.4395.

![Chemical Structure](image)

**(4-Methyl-pent-3-enyl)phosphonic acid dimethyl ester (3.02)**

Dimethyl methylphosphonate (12.08 mL, 108.6 mmol) was dissolved in anhydrous tetrahydrofuran (300 mL) and the solution was cooled to –78 °C for 45 minutes. A solution of 2.5 M \( \text{n–BuLi} \) (43.4 mL, 110 mmol) in hexanes was added dropwise over 1 hr. via cannulation and allowed to react for 30 minutes. A solution of prenyl bromide (11.40 mL, 98.69 mmol) in THF (100 mL) was then cooled to –78 °C and cannulated dropwise and the reaction was stirred overnight and allowed to warm to room temperature. The reaction was quenched by addition of brine and extracted with diethyl ether. The organic portions were combined, dried (Na\(_2\)SO\(_4\)), and filtered over celite. The solution was then heated to 150 °C with a fractional distillation apparatus to remove solvent and volatile impurities. The resulting product was isolated as a yellow oil in a 99% yield (18.78 g.): \(^{1}\text{H} \) NMR (300 MHz, CDCl\(_3\)) \( \delta 5.03 \) (t, \( J = 6.4 \text{ Hz} \), 1H), 3.66 (d, \( J_{PH} = 10.5 \) Hz, 6H), 2.19 (dt, \( J = 5.4 \text{ Hz}, J = 3.0 \text{ Hz} \), 2H), 1.74–1.63 (m, 2H), 1.61 (s, 3H), 1.54 (s, 3H); \(^{31}\text{P} \) NMR (121 MHz, CDCl\(_3\)) \( \delta +34.4 \).
Monophosphonate (3.07)

Oxalyl chloride (0.17 mL, 2.0 mmol) was added dropwise to a solution of 3.02 (0.32 g, 1.65 mmol) dissolved in anhydrous DCM (10 mL) and DMF (0.02 mL) at 0 °C and allowed to warm to room temperature and react for 48 hours. The resulting solution was concentrated fully in vacuo and the resulting red oil was utilized without further purification. $^{31}$P NMR (121 MHz, CDCl$_3$) δ +46.1.

Dimethyl ((methoxy(4-methylpent-3-en-1-yl)phosphoryl)- methyl)phosphonate (3.08).

To a stirred solution of $n$–BuLi (6.11 mL, 15.2 mmol) in toluene at –78 °C, dimethyl methylphosphonate (1.69 mL, 15.2 mmol) was added dropwise. The resulting solution was allowed to stir for 30 minutes and then acid chloride 3.06 (3.11 mmol) was added dropwise. The reaction temperature was held at –78 °C for 1 hour and then allowed to warm unassisted while it stirred overnight. The reaction was quenched by addition of aqueous NH$_4$Cl and extracted with dichloromethane. The combined organic portions were dried (MgSO$_4$) and filtered, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (10% EtOH in hexanes) and the desired product 7 was isolated as a clear oil in 58% yield (0.52 g): $^1$H NMR (500 MHz, CDCl$_3$) δ 5.12–5.06 (m, 1H), 3.78 (d, $J_{PH}$ = 10.1 Hz, 6H), 3.74 (d, $J_{PH}$ = 11.3 Hz, 3H), 2.44–2.34 (m, 2H), 2.33–2.22 (m, 2H), 1.99–1.90 (m, 2H), 1.66 (s, 3H), 1.61 (s, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 133.1 (d, $J_{PC}$ = 1.4 Hz), 122.6 (d, $J_{PC}$ = 15.7 Hz), 52.9 (t, $J_{PC}$ = 6.2 Hz, 2C), 51.4
(d, $J_{PC} = 6.8$ Hz), 29.2 (d, $J_{PC} = 97.9$ Hz), 26.2 (dd, $J_{PC} = 135.6, 76.5$ Hz), 25.5, 20.1 (d, $J_{PC} = 4.2$ Hz), 17.6; $^{31}$P NMR (202 MHz, CDCl$_3$) $\delta$ +48.2 (d, $J_{PP} = 5.0$ Hz), +22.8 (d, $J_{PP} = 5.0$ Hz); HRMS (ES$^+$) calculated for C$_{10}$H$_{23}$O$_5$P$_2$ [M$^+$ + H] 285.1021; found 285.1021.

$$\text{O} \quad \text{P} \quad \text{O} \quad \text{OPOM} \quad \text{OPOM} \quad \text{OPOM}$$

(((4-Methylpent-3-en-1-yl)((pivaloyloxy)methoxy)phosphoryl)-methyl)phosphoryl)bis(oxy))bis(methylene bis(2,2-dimethylpropanoate) (3.12).

The trimethyl ester 3.08 (0.52 g, 1.8 mmol), sodium iodide (1.09 g, 7.30 mmol), and chloromethyl pivalate (1.06 mL, 7.30 mmol) were dissolved in acetonitrile (2 mL) and the solution was heated at reflux overnight. The reaction was quenched by addition of water and extracted with diethyl ether. The combined organic portions were washed with Na$_2$S$_2$O$_3$, dried (Na$_2$SO$_4$), and filtered, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (40% EtOAc in hexanes) and the product 3.12 was isolated as a clear oil in 48% yield (0.51 g): $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 5.76–5.66 (m, 6H), 5.12–5.09 (m, 1H), 2.69–2.52 (m, 2H), 2.40–2.25 (m, 2H), 2.10–1.95 (m, 2H), 1.68 (s, 3H), 1.63 (s, 3H), 1.24 (s, 27H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 177.0, 176.8 (2C), 133.6, 122.3 (d, $J_{PC} = 16.1$ Hz), 81.9 (d, $J_{PC} = 5.6$ Hz, 2C), 81.0 (d, $J_{PC} = 5.6$ Hz), 38.7 (2C), 38.7, 30.3 (d, $J_{PC} = 147.5$ Hz), 29.8 (dd, $J_{PC} = 84.5, 41.0$ Hz), 26.9 (3C), 26.8 (6C), 25.6, 19.9 (d, $J_{PC} = 3.5$ Hz), 17.7; $^{31}$P NMR (202 MHz, CDCl$_3$) $\delta$ +48.9-49.1 (br s), +19.5 (d, $J_{PP} = 3.4$ Hz); HRMS (ES$^+$) calculated for C$_{25}$H$_{47}$O$_{11}$P$_2$ [M$^+$ + H] 585.2594; found 585.2599.
(E)-((((5-Hydroxy-4-methylpent-3-en-1-yl)-
((pivaloyloxy)methoxy)phosphoryl)methyl)phosphoryl)bis(oxy))bis-(methylene) bis(2,2-
dimethylpropanoate) (3.13).

Phosphonate 3.12 (0.51 g, 0.88 mmol), selenium dioxide (49 mg, 0.44 mmol), \( p \)-hydroxybenzonic acid (60 mg, 0.44 mmol), and \( tert \)-butylhydroperoxide (0.30 mL, 2.6 mmol) were dissolved in dichloromethane (5 mL) and the solution was allowed to stir overnight. The reaction was quenched by addition of brine and extracted with diethyl ether. The combined organic portions were washed with \( \text{Na}_2\text{S}_2\text{O}_3 \), dried (\( \text{Na}_2\text{SO}_4 \)), and filtered, and the filtrate was concentrated \textit{in vacuo}. The residue was purified by column chromatography (5\% EtOH in ether) and the product 3.13 was isolated as a clear oil in 34\% yield (179 mg, 45\% yield BRSM): \(^1\text{H NMR (500 MHz, CDCl}_3\)) \( \delta \) 5.77–5.63 (m, 6H), 5.45–5.40 (m, 1H), 3.98 (s, 2H), 3.58 (br s, 1H), 2.69–2.50 (m, 2H), 2.46–2.31 (m, 2H), 2.12–2.02 (m, 2H), 1.67 (s, 3H), 1.23 (s, 27H); \(^{13}\text{C NMR (125 MHz, CDCl}_3\)) \( \delta \) 177.0, 176.9 (d, \( J_{\text{PC}} = 4.1 \text{ Hz}, 2\text{C})\), 136.8, 123.1 (d, \( J_{\text{PC}} = 14.3 \text{ Hz})\), 81.8 (d, \( J_{\text{PC}} = 5.3 \text{ Hz}, 2\text{C})\), 80.9 (d, \( J_{\text{PC}} = 6.3 \text{ Hz})\), 68.2, 38.7–38.6 (m, 3C), 30.0 (d, \( J_{\text{PC}} = 96.7 \text{ Hz})\), 29.1 (dd, \( J_{\text{PC}} = 135.2 \text{ Hz}, 74.9 \text{ Hz})\), 26.8 (3C), 26.8 (6C), 19.6 (d, \( J_{\text{PC}} = 4.4 \text{ Hz})\), 13.6; \(^{31}\text{P NMR (201 MHz, CDCl}_3\)) \( \delta \) +48.4 (d, \( J_{\text{PP}} = 4.0 \text{ Hz})\), +19.3 (d, \( J_{\text{PP}} = 4.0 \text{ Hz})\); HRMS (ES\(^+\)) calculated for C\(_{25}\)H\(_{46}\)O\(_{12}\)P\(_2\) [ M\(^+\) + Na] 623.2362; found 623.2372.
Monophosphonate (4.07)

Dimethyl methylphosphonate (1.00 mL, 9.55 mmol) was dissolved in anhydrous dichloromethane (30 mL) and dimethylformamide (0.04 mL) and the solution was cooled to 0 °C. Oxalyl chloride (2.46 mL, 28.7 mmol) was introduced dropwise and allowed to react for 48 hours. The solution was diluted with anhydrous toluene (3 x 1 mL), and subsequently concentrated in vacuo. The resulting red oil was utilized without further purification. $^{31}$P NMR (201 MHz, CDCl$_3$) $\delta$ +42.1

Monophosphonate 4.08

Compound 4.07 (1.22 g, 9.52 mmol) was dissolved in anhydrous toluene (20 mL). A solution of phenol (1.80 g, 19.1 mmol) in toluene (10 mL) and triethylamine (2.65 mL, 19.11 mmol) was added dropwise and allowed to react for 24 hours. The reaction was quenched by addition of brine and extracted into diethyl ether. The organic portions were then washed 4 times with aqueous NaOH (2 M solution), dried (Na$_2$SO$_4$) and filtered through celite. The filtrate was concentrated in vacuo. The resulting residue was isolated as a red oil in 99% yield (1.75 g): $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.26 (dd, $J = 7.8$ Hz, $J = 7.5$ Hz, 2H), 7.13 (d, $J = 7.8$ Hz, 2H), 7.10 (t, $J = 7.5$ Hz, 1H), 3.73 (d, $J_{PH} = 11.4$ Hz, 3H), 1.55 (d, $J_{PH} = 17.7$ Hz, 3H); $^{31}$P NMR (121 MHz, CDCl$_3$) $\delta$ +28.8.
Methyl phenyl (4-methylpent-3-en-1-yl)phosphonate (4.11).

Phenol (1.25 g, 13.3 mmol) and freshly distilled triethylamine (1.84 mL, 13.3 mmol) were added to a solution of the acid chloride 3.06 (5.3 mmol) in anhydrous toluene (20 mL) and allowed to react overnight at room temperature. The reaction was diluted with diethyl ether (20 mL) and quenched by addition of brine (10 mL). The organic portion was then washed with a 2 M solution of sodium hydroxide (4 x 5 mL), dried (Na₂SO₄), and filtered through celite, and the filtrate was concentrated in vacuo. The crude residue was purified by column chromatography (silica gel, 100% hexanes–30% EtOAc in hexanes) and the resulting product was isolated as a red oil in 100% yield (1.35 g); ¹H NMR (400 MHz, CDCl₃) δ 7.33 (dd, J = 8.0, 7.6 Hz, 2H), 7.21 (dd, J = 8.0, 1.2 Hz 2H), 7.15 (td, J = 7.4, 1.2 Hz, 1H), 5.12 (t, J = 7.1 Hz, 1H), 3.79 (d, JₚH = 11.0, 3H), 2.41–2.32 (m, 2H), 1.96–1.87 (m, 2H), 1.68 (s, 3H), 1.61 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 150.6 (d, JₚC = 8.2 Hz), 133.2, 129.7 (2C), 124.8, 122.7 (d, JₚC = 17.1 Hz), 120.4 (d, JₚC = 4.3 Hz, 2C), 52.7 (d, JₚC S₅ = 7.0 Hz), 25.59, 25.57 (d, JₚC = 137.6 Hz), 21.0 (d, JₚC = 4.7 Hz), 17.7; ³¹P NMR (161 MHz, CDCl₃) δ + 30.1; HRMS (ES⁺, m/z) calcd. for (M+H)⁺ C₁₃H₂₀O₃P: 255.1150; found: 255.1160.

Methyl naphthalene-1-yl (4-methylpent-3-en-1-yl)phosphonate (4.12).

1-Naphthol (1.91 g, 13.3 mmol) was added to anhydrous toluene (10 mL) and stirred for 10 minutes to form a uniform solution. Triethylamine (1.84 mL, 13.3 mmol) was added, the resulting
mixture was added to a solution of the acid chloride 3.06 (5.3 mmol) in toluene (10 mL) and the reaction was allowed to stir overnight at room temperature. The reaction was then diluted with diethyl ether (20 mL) and washed with brine (10 mL) and 2 M NaOH (4 x 5 mL). The organic layer was dried (Na$_2$SO$_4$) and filtered through celite, and the filtrate was concentrated in vacuo. The resulting red oil was purified via flash chromatography (silica, 100% hexanes–40% EtOAc in hexanes) and the resulting product was isolated as a red oil in 65% yield (1.05 g): $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.15 (dd, $J = 7.2, 2.2$ Hz, 1H), 7.84 (dd, $J = 7.2, 2.2$ Hz, 1H), 7.65 (d, $J = 8.2$ Hz, 1H), 7.55–7.50 (m, 3H), 7.40 (dd, $J = 8.2, 7.6$ 1H), 5.12 (tt, $J = 7.2, 1.4$ Hz, 1H), 3.80 (d, $J_{PH} = 11.2$ Hz, 3H), 2.44–2.37 (m, 2H), 2.07–1.98 (m, 2H), 1.66 (s, 3H), 1.58 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 146.6 (d, $J_{PC} = 8.8$ Hz), 134.9, 133.3, 127.9, 126.8, 126.7, 126.4, 125.7, 124.8, 122.8 (d, $J_{PC} = 16.8$ Hz), 121.7, 115.5 (d, $J_{PC} = 1.7$ Hz), 53.0 (d, $J_{PC} = 6.5$ Hz), 25.9 (d, $J_{PC} = 137.6$ Hz), 25.7, 21.2 (d, $J_{PC} = 4.7$ Hz), 17.7; $^{31}$P NMR (121 MHz, CDCl$_3$) $\delta + 30.6$; HRMS (ES$^+$, m/z) calcd. for (M+H)$^+$ C$_{17}$H$_{22}$O$_3$P: 305.1307; found: 305.1306.

(((4-Methylpent-3-en-1-yl)(phenoxy)phosphoryl)oxy)methyl pivalate (4.13).

Mixed ester 4.11 (820 mg, 3.2 mmol) was dissolved in freshly distilled acetonitrile (1 mL), subsequently concentrated in vacuo 2 times, and finally diluted with 5 mL of acetonitrile. Chloromethyl pivalate (0.96 mL, 6.4 mmol) and sodium iodide (720 mg, 4.8 mmol) were then added and the solution was heated at reflux for 1 day. The reaction was diluted with diethyl ether
(5 mL) and washed with brine (4 x 5 mL). The organic portions were dried (Na₂SO₄) and filtered through celite, and the filtrate was concentrated in vacuo. The initial residue was purified via flash chromatography (silica, 100% hexanes–30% EtOAc in hexanes) and the resulting product 4.13 was isolated in 14% yield (163 mg): ¹H NMR (400 MHz, CDCl₃) δ 7.33 (dd, J = 7.6, 7.2 Hz, 2H), 7.21 (d, J = 7.6 Hz, 2H), 7.17 (t, 7.2 Hz, 1H), 5.72 (dd, Jₚₕ = 13.7, 5.2 Hz, 1H), 5.66 (dd, Jₚₖ = 12.5, J = 5.2 Hz, 1H), 5.11 (t, J = 7.2 Hz, 1H), 2.42–2.33 (m, 2H), 2.01–1.90 (m, 2H), 1.67 (s, 3H), 1.61 (s, 3H), 1.18 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 177.2, 150.4 (d, Jₚₖ = 8.7 Hz), 133.6 (2C), 130.0, 125.4 (d, Jₚₖ = 1.5 Hz), 122.7 (d, Jₚₖ = 17.1 Hz), 120.8 (d, Jₚₖ = 4.3 Hz, 2C), 82.0 (d, Jₚₖ = 5.8 Hz), 39.0, 27.1, 26.7 (d, Jₚₖ = 137.7 Hz), 25.9, 21.1 (d, Jₚₖ = 5.1 Hz), 17.9; ³¹P NMR (161 MHz, CDCl₃) δ + 29.6; HRMS (ES⁺, m/z) calcd. for (M+Na)⁺ C₁₈H₂₇NaO₅P: 377.1494; found: 377.1498.

(((4-Methylpent-3-en-1-yl)(naphthalene-1-yloxy)phosphoryl)oxy)methyl pivalate (4.14).

The mixed ester 4.12 (740 mg, 2.4 mmol) was dissolved in freshly distilled acetonitrile (1 mL) and concentrated in vacuo 3 times and then added to a solution of chloromethyl pivalate (0.73 mL, 4.9 mmol) and sodium iodide (550 mg, 3.7 mmol) in acetonitrile (5 mL). The solution was heated to reflux and maintained for 3 days while monitored by TLC analysis. The reaction was then allowed to cool to room temperature, extracted with diethyl ether (~10 mL), and the combined extracts were washed with brine (4 x 5 mL). The organic portions were combined, dried (Na₂SO₄), and
filtered through celite, and the filtrate was concentrated *in vacuo*. The resulting oil was purified via flash chromatography (silica, 100% hexanes–30% EtOAc in hexanes) and the resulting product **4.14** was isolated as a red oil in 32% yield (320 mg): $^1$H NMR (400 MHz, CDCl$_3$) δ 8.12 (dd, $J = 7.2, 2.0$ Hz, 1H), 7.83 (dd, $J = 7.2, 2.0$ Hz, 1H), 7.66 (d, 8.1 Hz, 1H), 7.55–7.49 (m, 3H), 7.40 (dd, $J = 8.1, 7.6$ Hz, 1H), 5.76 (dd, $J_{PH} = 14.0$ Hz, $J = 5.1$ Hz, 1H), 5.66 (dd, $J_{PH} = 12.5$ Hz, $J = 5.1$ Hz, 1H), 5.13 (td, $J = 7.2, 1.6, 1.2$ Hz, 1H), 2.46–2.41 (m, 2H), 2.12–2.04 (m, 2H), 1.66 (s, 3H), 1.58 (s, 3H), 1.11 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 177.1, 146.1 (d, $J_{PC} = 9.1$ Hz), 134.9, 133.5, 127.8, 126.7, 126.6, 126.4, 125.6, 125.0, 122.4 (d, $J_{PC} = 17.7$ Hz), 121.7, 115.7 (d, $J_{PC} = 3.8$ Hz), 81.9 (d, $J_{PC} = 6.1$ Hz), 38.7, 26.8, 26.6 (d, $J_{PC} = 137.0$ Hz), 25.6, 21.0 (d, $J_{PC} = 4.8$ Hz), 17.7; $^{31}$P NMR (161 MHz, CDCl$_3$) δ +29.6; HRMS (ES$^+$, $m/z$) calcd. for (M+Na)$^+$ C$_{22}$H$_{29}$O$_5$NaP: 427.1650; found: 427.1652.

![Chemical structure](image)

**(E)**-(((5-Hydroxy-4-methylpent-3-en-1-yl)(phenoxy)phosphoryl)oxy)methyl pivalate (4.15). Alkene **4.13** (163 mg, 0.5 mmol) was combined with selenium dioxide (38 mg, 0.3 mmol) and a 70% solution of $t$-butyl hydroperoxide (0.19 mL, 1.4 mmol) and allowed to react at room temperature for 4 days. The solution was diluted with dichloromethane (20 mL) and washed with Na$_2$SO$_3$ (4 x 20 mL). The organic layers were combined and dried (Na$_2$SO$_4$) and then concentrated *in vacuo*. The resulting yellow oil was dissolved in freshly distilled THF (20 mL) and treated with sodium borohydride (17 mg, 0.5 mmol) for 2 hr. The reaction then was diluted with diethyl ether
(10 mL), quenched by addition of saturated ammonium chloride (5 mL), and washed with ammonium chloride (4 x 5 mL). The organic layer was dried (Na$_2$SO$_4$) and filtered through celite, and the filtrate was concentrated. The resulting yellow oil was purified by column chromatography (silica, 100% hexanes–75% EtOAc in hexanes) to give the desired product 4.15 as a yellow oil in 28% yield (48 mg): $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.34 (dd, $J = 8.4$, 7.2 Hz, 2H), 7.23–7.18 (m, 3H), 5.72 (dd, $J_{PH} = 13.4$, $J = 5.2$ Hz, 1H), 5.65 (dd, $J_{PH} = 12.4$, $J = 5.2$ Hz, 1H), 5.43 (td, $J = 7.2$, 1.2 Hz, 1H), 4.00 (s, 2H), 2.49–2.40 (m, 2H), 2.07–1.98 (m, 2H), 1.67 (s, 3H), 1.18 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 177.2, 151.1 (d, $J_{PC} = 8.8$ Hz), 137.7, 130.9 (2C), 126.2, 124.2 (d, $J_{PC} = 13.5$ Hz), 121.6 (d, $J_{PC} = 4.3$ Hz, 2C), 82.8 (d, $J_{PC} = 6.2$ Hz), 69.3, 39.8, 27.9 (3C), 27.2 (d, $J_{PC} = 137.0$ Hz), 21.5 (d, $J_{PC} = 4.9$ Hz), 14.7; $^{31}$P NMR (161 MHz, CDCl$_3$) $\delta$ +28.8; HRMS (ES$^+$, m/z) calcd. for (M+H)$^+$ C$_{18}$H$_{27}$O$_6$NaP: 393.1442; found: 393.1418.

(E)-(((5-Hydroxy-4-methylpent-3-en-1-yl)(naphthalene-1-yloxy)phosphoryl)oxy)methyl pivalate (4.16).

The olefin 4.14 (320 mg, 0.8 mmol) was added to a solution of selenium dioxide (66 mg, 0.6 mmol) and a 70% solution of tert-butyl hydroperoxide (0.33 mL, 2.4 mmol). This solution was stirred at room temperature and allowed to react for 3 days. The reaction then was diluted with dichloromethane (20 mL) and washed with Na$_2$SO$_3$ (4 x 5 mL). The extract was dried (Na$_2$SO$_4$) and concentrated $\textit{in vacuo}$. The resulting reddish oil was dissolved in THF (10 mL) and allowed
to react with sodium borohydride (29 mg, 0.8 mmol) for 30 min. The reaction then was diluted
with diethyl ether, quenched by addition of saturated ammonium chloride (5 mL), and washed (4
x 5 mL). The organic layer was dried (Na$_2$SO$_4$) and filtered through celite, and the filtrate was
concentrated in vacuo. The resulting red oil was purified by column chromatography (silica, 100%
hexanes–50% EtOAc in hexanes) to give the desired product 4.16 as a red oil in 20% yield (67
mg): $^1$H NMR (300 MHz, CDCl$_3$) δ 8.11 (dd, $J = 6.8, 2.8$ Hz, 1H), 7.85 (dd, $J = 6.8, 2.8$ Hz, 1H),
7.67 (d, $J = 8.0$ Hz, 1H), 7.54–7.50 (m, 3H), 7.41 (dd, $J = 8.0, 7.6$ Hz, 1H), 5.75 (dd, $J_{PH} = 13.8, J$
= 5.2 Hz, 1H), 5.65 (dd, $J_{PH} = 12.4, J = 5.2$ Hz, 1H), 5.43 (td, $J = 7.2, 1.2$ Hz, 1H), 3.95 (s, 2H),
2.52–2.47 (m, 2H), 2.17–2.07 (m, 2H), 1.63 (s, 3H), 1.11 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ
177.1, 146.0, 136.8 (d, $J_{PC} = 1.5$ Hz), 134.9, 127.9, 126.7, 126.6, 126.5, 125.6, 125.1, 123.1 (d, $J_{PC}$
= 17.0 Hz), 121.6, 115.7 (d, $J_{PC} = 3.7$ Hz), 81.8 (d, $J_{PC} = 6.5$ Hz), 68.2, 38.7, 26.8 (3C), 26.3 (d,
$J_{PC} = 138.0$ Hz), 20.6 (d, $J_{PC} = 4.7$ Hz), 13.6; $^{31}$P NMR (161 MHz, CDCl$_3$) δ +29.2; HRMS (ES$^+$,
m/z) calcd. for (M+H)$^+$ C$_{22}$H$_{29}$O$_6$NaP: 443.1599; found: 443.1601.

![Methyl phenyl (E)-(5-hydroxy-4-methylpent-3-en-1-yl)phosphonate (4.17).](image)

Methyl phenyl (E)-(5-hydroxy-4-methylpent-3-en-1-yl)phosphonate (4.17).

Selenium dioxide (0.12 g, 1.1 mmol) and a 70% solution of tert-butyl hydroperoxide (0.61 mL,
1.5 mmol) were added to a solution of diester 4.11 (0.38 g, 1.5 mmol) in dichloromethane (5 mL).
The solution was stirred at room temperature and allowed to react for 3 days. The reaction was
then diluted with dichloromethane (20 mL) and washed with saturated Na$_2$SO$_3$ (4 x 5 mL). The
organic portions were dried (Na₂SO₄) and filtered, and the filtrate was concentrated fully. The residue was then combine with anhydrous methanol (5 mL) and treated with sodium borohydride (55 mg, 1.5 mmol) for 2 hr. The reaction was then extracted into dichloromethane (20 mL) and washed four times with ammonium chloride (5 mL). The organic layer was then dried (Na₂SO₄) and filtered through celite, and the filtrate was concentrated in vacuo. The resulting yellow oil was purified via column chromatography (silica, 50% EtOAc in hexanes–100% EtOAc) and the product 4.17 was isolated as a yellow oil in 21% yield (82 mg). ¹H NMR (400 MHz, CDCl₃) δ

7.34 (dd, J = 8.0, 7.2 Hz, 2H), 7.20 (dd, J = 8.0, 1.0 Hz, 2H), 7.17 (td, J = 7.2, 1.0 Hz, 1H), 5.43 (dt, J = 7.2, 1.2 Hz, 1H), 3.98 (s, 2H), 3.80 (d, JPH = 11.2 Hz, 3H), 2.47–2.38 (m, 2H), 2.00–1.92 (m, 2H), 1.66 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 150.5 (d, JPC = 8.0 Hz), 136.5, 129.8 (2C), 125.0, 123.44 (d, JPC = 16.0 Hz), 120.4 (d, JPC = 4.2 Hz, 2C), 68.2, 52.8 (d, JPC = 7.2 Hz), 25.3 (d, JPC = 138.6 Hz), 20.6 (d, JPC = 4.3 Hz), 13.7; ³¹P NMR (161 MHz, CDCl₃) δ +29.7; HRMS (ES⁺, m/z) calcd. for (M+Na)⁺ C₁₃H₁₉NaO₄P: 293.0919; found: 293.0920.

Methyl naphthalene-1-yl (E)-(5-hydroxy-4-methylpent-3-en-1-yl)phosphonate (4.18).

Olefin 4.12 (462 mg, 1.5 mmol) was dissolved in freshly distilled dichloromethane (5 mL). Selenium dioxide (126 mg, 1.4 mmol) and a 70% solution of aqueous tert-butyl hydroperoxide (0.62 mL) were added and allowed to react at room temperature for 3 days. The reaction mixture then was diluted with dichloromethane (20 mL) and washed with saturated Na₂SO₃ (4 x 5 mL).
The organic portions were combined, dried (Na$_2$SO$_4$), and filtered, and the filtrate was concentrated in vacuo. The resulting red oil was then dissolved in anhydrous methanol (5 mL) and treated with sodium borohydride (56 mg, 1.5 mmol) for 2 hr. The product then was extracted into dichloromethane (20 mL) and the solution was washed with ammonium chloride (4 x 5 mL). The organic layer was dried (Na$_2$SO$_4$) and filtered through celite, and the filtrate was concentrated in vacuo. The resulting red oil was purified by column chromatography (silica, 40% EtOAc in hexanes–100% EtOAc). The product 4.18 was isolated as a red oil in 36% yield (175 mg): $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.13 (dd, $J = 8.0, 2.3$ Hz, 1H), 7.85 (dd, $J = 8.0, 2.3$ Hz, 1H), 7.66 (d, $J = 8.4$ Hz, 1H), 7.55–7.48 (m, 3H), 7.40 (dd, $J = 8.0, 7.5$ Hz, 1H), 5.41 (t, $J = 7.2$ Hz, 1H), 3.95 (s, 2H), 3.80 (d, $J_{PH} = 11.1$ Hz, 3H), 2.53–2.41 (m, 2H), 2.11–2.00 (m, 2H), 1.61 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 148.5 (d, $J_{PC} = 8.0$ Hz), 136.7, 135.0, 128.1, 126.9, 126.6, 125.9, 125.0, 123.6 (d, $J_{PC} = 15.8$ Hz), 121.8, 115.6 (d, $J_{PC} = 2.8$ Hz), 68.4, 53.2 (d, $J_{PC} = 6.5$ Hz), 25.7 (d, $J_{PC} = 137.9$ Hz), 20.9 (d, $J_{PC} = 4.5$ Hz), 13.8; $^{31}$P NMR (121 MHz, CDCl$_3$) $\delta$ +30.2; HRMS (ES$^+$, $m/z$) calcd. for (M+Na)$^+$ C$_{17}$H$_{21}$NaO$_4$P: 343.1075; found: 343.1077.

Sodium phenyl ($E$)-(5-hydroxy-4-methylpent-3-en-1-yl)phosphonate (4.19).

The methyl phenyl ester 4.17 (161 mg, 0.6 mmol) was dissolved in freshly distilled acetonitrile (5 mL) and combined with flame dried sodium iodide (85 mg, 0.6 mmol). This solution was heated at reflux for 24 hr and then concentrated in vacuo. The yellow solid was dissolved in deionized
water (15 mL) and washed with dichloromethane (4 x 5 mL). The aqueous layer was concentrated under reduced pressure and the product 4.19 was isolated as a faint yellow solid in 90% yield (150 mg): $^1$H NMR (400 MHz, D$_2$O) $\delta$ 7.31 (dd, $J$ = 8.0, 7.6 Hz, 2H), 7.12 (t, $J$ = 7.6 Hz, 1H), 7.09 (d, $J$ = 8.0 Hz, 2H), 5.40 (t, $J$ = 7.0 Hz, 1H), 3.87 (s, 2H), 2.29–2.20 (m, 2H), 1.72–1.62 (m, 2H), 1.56 (s, 3H); $^{13}$C NMR (100 MHz, D$_2$O) $\delta$ 151.5 (d, $J_{PC} = 7.5$ Hz), 134.7, 129.8 (2C) 126.3 (d, $J_{PC} = 16.8$ Hz), 124.2, 120.9 (d, $J_{PC} = 3.8$ Hz, 2C), 67.5, 26.6 (d, $J_{PC} = 134.1$ Hz), 21.3 (d, $J_{PC} = 3.9$ Hz), 12.9; $^{31}$P NMR (161 MHz, D$_2$O) $\delta$ +25.8; HRMS (ES$^-$, m/z) calcd. for (M)$^-$/C$_{12}$H$_{16}$O$_4$P: 255.0786; found: 255.0795.

![Image](image.png)

**Sodium naphthalene-1-yl (E)-(5-hydroxy-4-methylpent-3-en-1-yl)phosphonate (4.20).**

Mixed ester 4.18 (170 mg, 0.5 mmol) and sodium iodide (80 mg, 0.5 mmol) were dissolved in acetonitrile (5 mL) and heated at reflux for 48 hr. The acetonitrile was then removed under reduced pressure and the resulting brown solid was dissolved in deionized water (15 mL) and washed with dichloromethane (4 x 5 mL). The aqueous layer was concentrated fully and the product 4.20 was isolated as a yellow/brown solid in 100% yield (180 mg): $^1$H NMR (400 MHz, D$_2$O) $\delta$ 8.15 (dd, $J$ = 6.8, 2.6 Hz, 1H), 7.86 (dd, $J$ = 7.2, 2.6 Hz, 1H), 7.64 (d, $J$ = 8.0 Hz, 1H), 7.54–7.48 (m, 2H), 7.40 (dd, $J$ = 8.4, 7.6 Hz, 1H), 7.30 (dt, $J$ = 8.0, 1.6 Hz, 1H), 5.34 (td, $J$ = 7.3, 1.2 Hz, 1H), 3.77 (s, 2H), 2.27–2.18 (m, 2H), 1.80–1.72 (m, 2H), 1.42 (s, 3H); $^{13}$C NMR (100 MHz, D$_2$O) $\delta$ 147.6 (d, $J_{PC} = 7.3$ Hz), 134.7, 134.6, 127.8, 127.0 (d, $J_{PC} = 3.9$ Hz), 126.8, 126.4, 126.3, 126.1 (d, $J_{PC} =$
1.0 Hz), 123.7, 122.1, 115.1 (d, $J_{PC} = 3.2$ Hz), 67.5, 26.7 (d, $J_{PC} = 132.6$ Hz), 21.4 (d, $J_{PC} = 3.1$ Hz), 12.8; $^{31}$P NMR (161 MHz, D$_2$O) δ +26.3; HRMS (ES$^-$, m/z) calcd. for (M)$^-$ C$_{16}$H$_{18}$O$_4$P: 305.0943; found: 305.0950.

Phosphonate 5.10

1-Naphthol (833 mg, 5.8 mmol) and triethylamine (0.80 mL, 5.8 mmol) were added to anhydrous THF (25 mL) and stirred for 10 minutes to form a homogenous solution. The resulting material was added to a solution of the acid chloride 5.09 (2.9 mmol) in THF (50 mL) and the reaction was allowed to stir for 8 hrs. The reaction was then diluted with diethyl ether (25 mL) and washed with brine (10 mL) and aqueous K$_2$CO$_3$ (3 x 5 mL). The organic layer was dried (Na$_2$SO$_4$) and filtered through celite, and the filtrate was concentrated in vacuo. The resulting red oil was purified via flash chromatography (silica, 100% hexanes–20% EtOAc in hexanes) and the product 5.10 was isolated as a red oil in 69% yield (831 mg): $^1$H NMR (300 MHz, CDCl$_3$) δ 8.21–8.18 (m, 2H), 7.90–7.87 (m, 2H), 7.64–7.61 (m, 2H), 7.58–7.52 (m, 2H), 7.44–7.39 (m, 4H), 7.37–7.33 (m, 2H), 5.25 (br s, 1H), 2.65–2.55 (m, 2H), 2.42–2.33 (m, 2H), 1.71 (s, 3H), 1.63 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 146.5 (d, $J_{PC} = 8.8$ Hz), 134.9, 133.7, 127.9, 126.7, 126.6, 126.4, 125.7, 125.0, 122.5 (d, $J_{PC} = 16.5$ Hz), 121.6, 115.7, 26.7 (d, $J_{PC} = 137.2$ Hz), 25.7, 21.5 (d, $J_{PC} = 4.8$ Hz), 17.7; $^{31}$P NMR (121 MHz, CDCl$_3$) δ +25.5.
Phosphonate 5.11

Selenium dioxide (166 mg, 1.5 mmol) and a 70% solution of tert-butyl hydroperoxide (0.82 mL, 6.0 mmol) were added to a solution of diester 5.10 (831 mg, 2.0 mmol) in dichloromethane (10 mL). The solution was stirred at room temperature and allowed to react for 3 days. The reaction was then diluted with dichloromethane (20 mL) and washed with saturated Na$_2$SO$_3$ (3 x 5 mL). The organic portions were dried (Na$_2$SO$_4$) and filtered, and the filtrate was concentrated. The resulting yellow oil was purified via column chromatography (silica, 30% EtOAc in hexanes to 100% EtOAc) and the product 5.11 was isolated as a yellow oil in 14% yield (121 mg): $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.11–8.07 (m, 2H), 7.85–7.82 (m, 2H), 7.65 (d, $J$ = 8.1 Hz, 2H), 7.64–7.61 (m, 2H), 7.53–7.47 (m, 4H), 7.36 (dd, $J$ = 7.8 Hz, 2H), 5.44 (t, $J$ = 7.2 Hz, 1H), 3.88 (s, 2H), 2.69–2.57 (m, 2H), 2.40–2.29 (m, 2H), 1.58 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 146.3 (d, $J_{PC}$ = 8.7 Hz), 136.9, 134.8, 127.9, 126.7, 126.5, 126.4, 125.6, 125.0, 122.5 (d, $J_{PC}$ = 16.7 Hz), 121.5, 115.6 (d, $J_{PC}$ = 3.0 Hz), 68.3, 26.4 (d, $J_{PC}$ = 140.4 Hz), 21.1, 13.7; $^{31}$P NMR (121 MHz, CDCl$_3$) $\delta$ +25.5.
Phosphonate 5.12

A solution of 4-nitrophenol (1.48 g, 10.6 mmol) and freshly distilled triethylamine (1.47 mL, 10.6 mmol) in anhydrous toluene (15 mL) was added to a solution of acid chloride 3.07 (5.3 mmol) in toluene (15 mL) and allowed to react overnight at room temperature. The reaction was diluted with diethyl ether (20 mL) and quenched by addition of brine (10 mL). The organic portion was then washed with a 2 M solution of NaOH (3 x 5 mL) and dried (Na$_2$SO$_4$), it was filtered through celite and the filtrate was concentrated \textit{in vacuo}. The residue was purified by column chromatography (silica gel, 50% hexanes in EtOAc–100% EtOAc) and the resulting product 5.12 was isolated as a yellow oil in 35% yield (0.56 g): $^1$H NMR (300 MHz, CDCl$_3$) δ 8.24 (d, $J = 8.7$ Hz, 2H), 7.39 (d, $J = 8.7$ Hz, 2H), 5.12 (br s, 1H), 3.83 (d, $J_{PH} = 11.1$ Hz, 3H), 2.44–2.32 (m, 2H), 2.04–1.93 (m, 2H), 1.69 (s, 3H), 1.62 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 155.7 (d, $J_{PC} = 8.2$ Hz), 144.5, 133.6, 125.6 (2C), 122.2 (d, $J_{PC} = 16.9$ Hz), 120.9 (d, $J_{PC} = 4.5$ Hz, 2C), 53.0 (d, $J_{PC} = 6.9$ Hz), 25.7 (d, $J_{PC} = 138.2$ Hz), 25.5, 20.8 (d, $J_{PC} = 4.8$ Hz), 17.6; $^{31}$P NMR (121 MHz, CDCl$_3$) δ + 31.1.
Phosphonate 5.13

The mixed ester 5.12 (559 mg, 1.6 mmol) was added to a solution of chloromethyl pivalate (0.59 mL, 3.9 mmol) and sodium iodide (355 mg, 2.4 mmol) in acetonitrile (5 mL). The solution was heated at reflux for 24 hrs. The reaction then was allowed to cool to room temperature, extracted with diethyl ether (~10 mL), and the combined extracts were washed with brine (4 x 5 mL). The organic portions were combined, dried (Na$_2$SO$_4$), and filtered through celite, and the filtrate was concentrated in vacuo. The resulting oil was purified via flash chromatography (silica, 100% hexanes–30% EtOAc in hexanes) and the product was isolated as a yellow oil in 28% yield (174 mg).

Selenium dioxide (36 mg, 0.3 mmol) and a 70% solution of tert-butyl hydroperoxide (0.18 mL, 1.3 mmol) were added to a solution of diester (171 mg, 0.4 mmol) in dichloromethane (5 mL). The solution was stirred at room temperature and allowed to react for 2 days. After reaction was diluted with dichloromethane (20 mL), it was washed with brine (3 x 5 mL). The organic portions were dried (Na$_2$SO$_4$) and filtered, and the filtrate was concentrated. The resulting yellow oil was purified via column chromatography (silica, 50% EtOAc in hexanes to 100% EtOAc) and the product 5.13 was isolated as a yellow oil in 9% yield (16 mg): $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.25 (d, $J = 9.0$ Hz, 2H), 7.40 (dd, $J = 9.0$ Hz, $J_{PH} = 1.2$ Hz, 2H) 5.74 (dd, $J_{PH} = 13.5$, $J = 5.4$ Hz, 1H), 5.67 (dd,
$J_{PH} = 12.4$, $J = 5.4$ Hz, 1H), 5.44 (td, $J = 7.1, 1.3$ Hz, 1H), 4.01 (s, 2H), 2.51–2.42 (m, 2H), 2.12–2.03 (m, 2H), 1.68 (s, 3H), 1.17 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 177.0, 155.0, 144.5, 137.0, 125.8 (2C), 122.6 (d, $J_{PC} = 16.6$ Hz), 121.2 (d, $J_{PC} = 4.7$ Hz, 2C), 81.9 (d, $J_{PC} = 6.2$ Hz), 68.2, 38.8, 26.4 (d, $J_{PC} = 138.7$ Hz), 25.7, 20.4 (d, $J_{PC} = 5.0$ Hz), 13.7; $^{31}$P NMR (121 MHz, CDCl$_3$) δ +29.7.

**Phosphonate 5.14**

To a solution of dimethyl phosphonate 4.09 (1.07 g, 5.5 mmol) in freshly distilled DCM (25 mL) was added triflic anhydride (1.02 mL, 6.1 mmol) and pyridine (0.89 mL, 11.1 mmol). The solution was stirred and allowed to react for 15 min followed by addition of 2-nitro-1-naphthol (2.10 g, 11.1 mmol). The reaction was stirred for 30 min and then concentrated in vacuo. The resulting oil was purified via column chromatography (silica, 40% EtOAc in hexanes) and the product 5.14 was isolated as a yellow oil in 95% yield (1.84 g): $^1$H NMR (300 MHz, CDCl$_3$) δ 8.44–8.41 (m, 1H), 7.94 (d, $J = 9.0$ Hz, 1H), 7.91–7.87 (m, 1H), 7.74 (d, $J = 9.0$ Hz, 1H), 7.70–7.67 (m, 2H), 5.19 (tt, $J = 7.0, 1.4$ Hz, 1H), 3.64 (d, $J_{PH} = 11.2$ Hz, 3H), 2.52–2.43 (m, 2H), 2.25–2.13 (m, 2H), 1.71 (s, 3H), 1.66 (s, 3H); $^{31}$P NMR (121 MHz, CDCl$_3$) δ +32.8.
Phosphonate 5.15

According to the procedure described for preparation of compound 5.13, compound 5.14 (707 mg, 2.0 mmol) was treated with POMCl (0.33 mL, 2.2 mmol) and NaI (364 mg, 2.4 mmol) in acetonitrile at reflux. Standard work-up and purification provided the desired POM ester (273 mg, 30%). The POM ester then was allowed to react with selenium dioxide (24 mg, 0.2 mmol) and a 70% solution of tert-butyl hydroperoxide (0.12 mL, 0.9 mmol) in DCM (10 mL). Standard workup and purification provided the desired allylic alcohol compound 5.15 as a yellow oil in 15% yield (20 mg): $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.44–8.42 (m, 1H), 7.98 (d, $J = 9.0$ Hz, 1H), 7.93–7.91 (m, 1H), 7.79 (d, $J = 9.0$ Hz, 1H), 7.74–7.71 (m, 2H), 5.64 (t, $J = 7.5$ Hz, 1H), 5.57 (dd, $J_{PH} = 10.8$ Hz, $J = 5.0$ Hz, 1H), 5.41 (dd, $J_{PH} = 11.2$ Hz, $J = 5.0$ Hz, 1H), 4.25 (s, 2H), 2.73–2.62 (m, 2H), 2.46–2.38 (m, 2H), 1.57 (s, 3H), 1.10 (s, 9H); $^{31}$P NMR (121 MHz, CDCl$_3$) $\delta$ +31.0.

Phosphonamidate 5.17
To a solution of the acid chloride 3.07 (5.3 mmol) in toluene (50 mL) was added (S)-(−)-α-methylbenzylamine (1.37 mL, 10.6 mmol) and triethylamine (1.47, 10.6 mmol) and the mixture was allowed to react for 8 hrs. The reaction then was diluted with diethyl ether (25 mL) and washed with brine (10 mL) and aqueous NH₄Cl (3 x 5 mL). The organic layer was dried (Na₂SO₄) and filtered through celite, and the filtrate was concentrated in vacuo. The resulting oil was purified via column chromatography (silica, 40% EtOAc in hexanes) and the product 5.17 was isolated as a yellow oil in 57% yield (852 mg). The resulting mixture of diastereomers was purified via column chromatography (silica, 100% hexanes – 40% EtOAc in hexanes) and the diastereomerically enriched product 5.17 was isolated as a yellow solid: ¹H NMR (400 MHz, CDCl₃) δ 7.34–7.24 (m, 5H), 5.07–5.05 (m, 1H), 4.37–4.29 (m, 1H), 3.41 (d, JPH = 11.0 Hz, 3H), 2.77 (dd, J = 9.1 Hz, 1H), 2.23 (bs, 2H), 1.76–1.69 (m, 2H), 1.67 (s, 3H), 1.58 (s, 3H), 1.48 (d, 6.8 Hz, 3H); ³¹P NMR (121 MHz, CDCl₃) δ + 34.7.

Bromide 5.19

Cyclopropyl methyl ketone (6.75 mL, 68.2 mmol) was added dropwise to a solution of methylmagnesium bromide in THF (35 mL). The resulting mixture was heated at reflux for 20 minutes then allowed to cool to room temperature. A 2:1 mixture of water: con. H₂SO₄ (45 mL) was added dropwise and allowed to react for 1 hr. The reaction then was diluted with Et₂O (100 mL) and quenched by slow addition of aqueous NaHCO₃. The organic layer was washed with deionized water (3 x 30 mL) and dried (MgSO₄). The material was purified via distillation to give a clear oil 5.19 in 81% yield (9.01g): ¹H NMR (400 MHz, CDCl₃) δ 5.13 (tp, J = 7.2, 1.4 Hz, 1H), 3.33 (t, J = 7.2 Hz, 2H), 2.56 (dt, J = 7.2, 7.2 Hz, 2H), 1.72 (s, 3H), 1.63 (s, 3H).
Alcohol 5.20

Selenium dioxide (142 mg, 1.3 mmol) was added to a 5M solution of tert-butyl hydroperoxide (1.02 mL, 5.2 mmol) in DCM (10 mL) and the solution was allowed to cool to 0 °C for 20 minutes. Bromide 5.19 was added dropwise as a solution in DCM (10 mL) and allowed to react and warm to room temperature over 16 hrs. The reaction was diluted with Et₂O (100 mL) and washed with 1 M aqueous NaOH (10 mL) and brine (2 x 10 mL). The organic portion was dried (Na₂SO₄), filtered through celite, and concentrated in vacuo. The resulting material was purified via flash chromatography (silica gel, 20% EtOAc in hexanes) to give the desired product 5.20 as a yellow oil in 52% yield (238 mg): ¹H NMR (400 MHz, CDCl₃) δ 5.43 (tt, J = 7.2, 1.2 Hz, 1H), 4.01 (s, 2H), 3.39 (t, J = 7.2 Hz, 2H), 2.63 (dt, J = 7.2, 7.2 Hz, 2H), 1.68 (s, 3H).

Protected alcohol 5.21

Imidazole (94 mg, 0.6 mmol) and tert-butyldimethylsilyl chloride (95 mg, 0.6 mmol) were added to a solution of alcohol 5.20 (103 mg, 0.6 mmol) in DMF (1 mL). The reaction was allowed to stir at room temperature for 24 hrs, diluted with Et₂O (10 mL), and washed with brine. The aqueous layer was extracted with Et₂O (2 x 10 mL). The organic portions were combined, dried (Na₂SO₄), filtered through celite, and concentrated in vacuo. The resulting material was isolated as a yellow oil in 99% yield (167 mg): ¹H NMR (400 MHz, CDCl₃) δ 5.48–5.43 (m, 1H), 4.05 (s, 2H), 3.52 (t, J = 6.8 Hz, 2H), 2.56–2.51 (m, 2H), 1.64 (s, 3H), 0.93 (s, 9H), 0.09 (s, 6H).
Iodide 5.22

Sodium iodide (1.76 g, 11.7 mmol) was added to a solution of halide 5.21 (1.15 g, 3.9 mmol) in acetonitrile (10.0 mL) and the solution was heated to reflux at 3 days. The reaction was diluted with Et₂O (20 mL) and quenched by addition of deionized water (10 mL). The organic layer was dried (Na₂SO₄), filtered through celite, and concentrated in vacuo. The resulting product 5.22 was isolated as a light red oil in 99% yield (1.32 g): ¹H NMR (400 MHz, CDCl₃) δ 5.42–5.38 (m, 1H), 4.02 (s, 2H), 3.15 (t, J = 6.8 Hz, 2H), 2.63 (dt, J = 7.2, 6.8 Hz, 2H), 1.68 (s, 3H), 0.91 (s, 9H), 0.07 (s, 6H).

Phosphonate 5.23

Trimethyl phosphite (1.09 mL, 9.2 mmol) was added to a solution of 5.22 (1.05 g, 3.1 mmol) in freshly distilled acetonitrile (10 mL) and the solution was heated at reflux for 3 days. The reaction was then cooled to room temperature, diluted with ether (100 mL), and quenched by addition of water (5 mL). The organic portion was washed with deionized water (3 x 20 mL), dried (MgSO₄), filtered through celite, and concentrated in vacuo. The resulting material was purified via column chromatography (silica, 50% hexanes in EtOAc to 100% EtOAc) and the product was isolated as a yellow oil in 65% yield BRSM (319 mg): ¹H NMR (400 MHz, CDCl₃) δ 5.40 (br s, 1H), 4.00 (s, 2H), 3.74 (d, J_PH = 10.8 Hz, 6H), 2.38–2.34 (m, 2H), 1.84–1.76 (m, 2H), 1.61 (s, 3H), 0.91 (s, 9H), 0.07 (s, 6H).
0.06 (s, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 135.8, 122.6 (d, $J_{PC}$ = 17.2 Hz), 68.1, 52.2 (d, $J_{PC}$ = 6.5 Hz), 25.9 (3C), 24.7 (d, $J_{PC}$ = 138.4 Hz), 20.5 (d, $J_{PC}$ = 4.6 Hz), 18.4, 13.4, 0.0 (2C); $^{31}$P NMR (121 MHz, CDCl$_3$) δ + 34.2.

Phosphonate 5.25

A solution of monophosphonate 5.23 (319 mg, 1.0 mmol) in DCM (10 mL) was treated with oxalyl chloride (0.25 mL, 3.0 mmol) and DMF (3 drops) and allowed to react for 20 hrs. The reaction then was concentrated in vacuo. The resulting material was dissolved in freshly distilled THF (5 mL) and a solution of 2-naphthol (122 mg, 1.1 mmol) in DCM (5 mL) was added dropwise. After the reaction was allowed to proceed overnight, it was diluted with Et$_2$O (50 mL), washed with brine, (4 x 5 mL), dried (MgSO$_4$), filtered through celite, and concentrated in vacuo. The residue was purified via column chromatography (silica, 50% hexanes in EtOAc – 100% EtOAc) and the product 5.25 was isolated in a 24% yield over 2 steps BRSM (75 mg): $^1$H NMR (400 MHz, CDCl$_3$) δ 7.83 (d, $J$ = 9.2 Hz, 1H), 7.81 (dd, $J$ = 9.6, 9.6 Hz, 1H), 7.68 (br s, 1H), 7.52–7.43 (m, 3H), 7.35 (ddd, $J$ = 8.6, 2.4, 1.2 Hz, 1H), 5.55 (t, $J$ = 7.2 Hz, 1H), 3.99 (s, 2H), 3.83 (d, $J_{PH}$ = 11.2 Hz, 3H), 2.52–2.43 (m, 2H), 2.06–1.97 (m, 2H), 1.76 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 148.2 (d, $J_{PC}$ = 9.3 Hz), 133.9, 133.4, 130.9, 130.0, 128.6, 127.7, 127.5, 126.8, 125.5, 120.4 (d, $J_{PC}$ = 4.4 Hz), 116.8 (d, $J_{PC}$ = 4.2 Hz), 53.0 (d, $J_{PC}$ = 6.5 Hz), 51.8, 25.0 (d, $J_{PC}$ = 139.3 Hz), 21.1, 14.2; $^{31}$P NMR (121 MHz, CDCl$_3$) δ + 29.5.
Alcohol 6.12

Prenyl acetate (5.00 mL, 35.9 mmol) was added dropwise to a solution of selenium dioxide (1.99 g, 17.9 mmol) and *tert*-butyl hydroperoxide (10.27 mL, 71.8 mmol) in DCM (80 mL) at 0 °C and allowed to react for 26 hrs while warming to room temperature. The reaction then was washed with 1 M aqueous NaOH (10 mL) and brine (2 x 10 mL). The organic portion was dried (Na₂SO₄), filtered through celite, and concentrated *in vacuo*. The resulting material was purified via flash chromatography (silica gel, 100% hexanes to 30% EtOAc in hexanes) to give the product 6.12 as a yellow oil in 53% yield (2.75 g): ¹H NMR (400 MHz, CDCl₃) δ 5.64–5.60 (m, 1H), 4.64 (d, *J* = 6.8 Hz, 2H), 4.04 (s, 2H), 2.06 (s, 3H), 1.73 (s, 3H).

Silyl ether 6.13

Imidazole (2.51 g, 36.9 mmol) and TBSCl (2.55 g, 16.9 mmol) were added to a solution of alcohol 6.12 (2.21 g, 15.4 mmol) in DMF (30 mL) and allowed to react for 28 hrs. The reaction then was diluted with Et₂O (150 mL) and washed with deionized water (4 x 20 mL). The organic portion was dried (MgSO₄), filtered through celite, and concentrated *in vacuo*. The resulting product was isolated in 98% yield (3.89g) and utilized without further purification: ¹H NMR (400 MHz, CDCl₃) δ 5.60 (tq, *J* = 6.8, 1.2 Hz, 1H), 4.62 (d, *J* = 6.8 Hz, 2H), 4.02 (s, 2H), 2.03 (s, 3H), 1.65 (s, 3H), 0.90 (s, 9H), 0.05 (s, 6H).
Alcohol 6.14

Potassium carbonate (2.12 g, 15.4 mmol) was added to a solution of compound 6.13 in anhydrous methanol (15.00 mL) and allowed to react for 2 hrs at room temperature. The reaction then was diluted with Et₂O (50 mL), washed with deionized water (10 mL), dried (MgSO₄), filtered through celite, and concentrated. The resulting yellow oil 6.14 was isolated in 100% yield (3.31 g): "H NMR (300 MHz, CDCl₃) δ 5.66–5.60 (m, 1H), 4.16 (d, J = 6.9 Hz, 2H), 1.61 (s, H), 0.89 (s, 9H), 0.05 (s, 6H).

Aldehyde 6.15

Manganese dioxide (13.4 g, 153.6 mmol) was added to a solution of alcohol 6.14 (3.31 g, 15.4 mmol) in freshly distilled DCM (100 mL) and allowed to react overnight. The suspension was filtered through celite and concentrated in vacuo. The resulting yellow oil 6.15 was isolated in 72% yield (2.37 g): "H NMR (300 MHz, CDCl₃) δ 10.0 (br s, 1H), 6.17 (br s, 1H), 4.15 (br s, 2H), 2.06 (br s, 3H), 0.90 (br s, 9H), 0.07 (br s, 6H).

Monophosphonate 6.16

Tetramethyl methylenebisphosphonate (601 mg, 2.6 mmol) in THF (10 mL) was added to a suspension of NaH (124 mg, 3.1 mmol) in THF (10 mL) at 0 °C and allowed to react for 15
minutes. A solution of aldehyde \textbf{6.15} (555 mg, 2.6 mmol) in THF (10 mL) was introduced dropwise and allowed to react overnight while it warmed to room temperature. The reaction then was diluted with Et\textsubscript{2}O (50 mL), washed with brine, (4 x 5 mL), dried (MgSO\textsubscript{4}), filtered through celite, and concentrated \textit{in vacuo}. The residue was purified via column chromatography (silica, 20\% hexanes in EtOAc) and the product \textbf{6.16} was isolated as a yellow oil in 72\% yield (598 mg):

\begin{align*}
\text{\textsuperscript{1}H NMR (400 MHz, CDCl}\textsubscript{3}) & \delta 7.48–7.27 (m, 1H), 6.23 (d, \textit{J} = 11.2 \text{ Hz}), 5.58 (dd, \textit{J} = 18.6, 17.8 \text{ Hz}, 1H), 4.10 (s, 2H), 3.73–3.68 (m, 6H), 1.80 (s, 3H), 0.90 (bs, 9H), 0.06 (bs, 6H); \\
\text{\textsuperscript{13}C NMR (100 MHz, CDCl}\textsubscript{3}) & \delta 147.1, 145.5 (\textit{d, J}_{PC} = 7.0 \text{ Hz}), 121.8 (\textit{d, J}_{PC} = 27.1 \text{ Hz}), 113.9 (\textit{d, J}_{PC} = 191.3 \text{ Hz}), 67.1, 52.2 (\textit{d, J}_{PC} = 6.6 \text{ Hz}), 5.9, 18.4, 14.3 -5.4; \\
\text{\textsuperscript{31}P NMR (121 MHz, CDCl}\textsubscript{3}) & \delta +19.3.
\end{align*}

Silyl ether \textbf{6.20}

Imidazole (13.4 g, 197.4 mmol) and TBSCl (16.4 g, 108.6 mmol) were added to a solution of prenol (10.00 mL, 98.7 mmol) in DCM (150 mL) and allowed to react at room temperature for 20 hrs. The reaction was quenched by addition of deionized water (20 mL) and the organic portion was washed with brine (10 mL) and aqueous NH\textsubscript{4}Cl (2 x 10 mL). The organic portion was concentrated \textit{in vacuo} and used without further purification: \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \delta 5.31–5.27 (m, 1H), 4.15 (d, \textit{J} = 6.8 \text{ Hz, 2H}), 1.70 (s, 3H), 1.62 (s, 3H), 0.89 (s, 9H), 0.06 (s, 6H).

Alcohol \textbf{6.21}
Selenium dioxide (5.48 g, 49.3 mmol) and a 70% aqueous solution of t-BuOOH (27.03 mL, 197.4 mmol) were added to DCM (150 mL) at 0 °C and mixed for 30 minutes. Olefin 6.20 (98.7 mmol) in DCM (50 mL) was introduced dropwise and the reaction mixture was allowed to warm to room temperature and react for 3 days. The reaction was quenched by addition of aqueous Na₂SO₃ and extracted with DCM (3 x 50 mL). The organic portions were combined, dried (MgSO₄), filtered through celite, and concentrated in vacuo. The resulting material was dissolved in methanol (200 mL) and allowed to stir at 0 °C for 30 mins before NaBH₄ (1.87 g, 49.3 mmol) was slowly added portion-wise. After 8 hrs, the reaction was quenched by addition of aqueous NH₄Cl, concentrated in vacuo, and diluted with Et₂O (100 mL). The organic solution was washed with brine (3 x 10 mL), dried (MgSO₄), filtered through celite, and concentrated in vacuo. The resulting material was purified via flash chromatography (silica gel, 20% EtOAc in hexanes) to give the product 6.21 as a yellow oil in 70% yield over two steps (10.80 g): ¹H NMR (400 MHz, CDCl₃) δ 5.55 (tt, J = 6.8, 1.2 Hz, 1H), 4.22 (d, J = 6.8 Hz, 2H), 3.98 (s, 2H), 1.64 (s, 3H), 0.89 (s, 9H), 0.06 (s, 6H).

\[
\begin{align*}
\text{Acetate 6.22} \\
\text{Acetic anhydride (8.75 mL, 92.5 mmol) was added dropwise to a solution of alcohol 6.21 (5.01 g, 23.1 mmol) and 4-dimethylaminopyridine (0.28 g, 2.3 mmol) in pyridine (100 mL) and allowed to react for 18 hrs. The reaction was quenched by dropwise addition of aqueous NaHCO₃ and extracted with EtOAc (100 mL). The organic portion was washed with aqueous CuSO₄ and brine (3 x 25 mL), dried (MgSO₄), and concentrated in vacuo. The residue was purified via column}
\end{align*}
\]
chromatography (silica, 10% EtOAc in hexanes) and the product was isolated in 89% yield (5.30 g): $^1$H NMR (400 MHz, CDCl$_3$) $\delta$

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**Aldehyde 6.24**

A 1 M solution of tetrabutylammonium fluoride (22.6 mL, 22.6 mmol) was introduced into a solution of acetate 6.22 (5.30 g, 20.5 mmol) in THF (100 mL) and allowed to react for 3 hrs. The reaction was diluted with Et$_2$O (100 mL) and quenched by addition of brine. The organic portion then was washed with brine (3 x 5 mL), dried (MgSO$_4$), filtered through celite, and concentrated *in vacuo*. The resulting material was utilized without further purification. The free alcohol 6.23 was added to a solution of MnO$_2$ (17.84 g, 205.2 mmol) in DCM (100 mL). After 18 hrs, the reaction was diluted with DCM (50 mL), filtered through celite, and concentrated *in vacuo*. The resulting material was purified by column chromatography (silica, 40% EtOAc in hexanes) to give product 6.24 as a yellow oil in 50% yield over 2 steps (1.45 g): $^1$H NMR (400 MHz, CDCl$_3$) $\delta$

10.05 (dt, $J = 7.6, 2.4$ Hz, 1H), 6.04–6.01 (m, 1H), 4.65 (s, 2H), 2.18 (br s, 3H), 2.15 (s, 3H).

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O   O
O   O
O   O
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**Phosphonates 6.25 and 6.26**

Tetraethyl methylenebisphosphonate (3.52 g, 12.2 mmol) was introduced dropwise to a suspension of 35% KH (1.39 g, 12.2 mmol) in THF (50 mL) at 0 °C and allowed to react for 30 minutes. The
resulting anion was transferred via cannula to a solution of aldehyde 6.24 (1.45 g, 10.2 mmol) in THF (50 mL) at 0 °C and allowed to react for 6 hrs. The reaction was quenched by slow addition of a 1M solution of acetic acid in Et₂O and then concentrated in vacuo. The resulting material was purified via column chromatography (silica, EtOAc) to give the desired phosphonate 6.25 in 57% yield (1.60 g) as well as the deprotected product 6.26: For 6.25:¹H NMR (400 MHz, CDCl₃) δ 7.35–7.22 (m, 1H), 6.07 (d, J = 10.8 Hz, 1H), 5.62 (dd, J = 18.4, 17.6, 1H), 4.47 (s, 2H), 4.04–4.00 (m, 4H), 2.02 (br s, 3H), 1.80 (br s, 3H), 1.26–1.19 (m, 6H). (6.26):¹H NMR (400 MHz, CDCl₃) δ 7.44–7.31 (m, 1H), 6.26 (d, J = 11.2 Hz, 1H), 5.61 (dd, J = 20.4, 16.4, 1H), 4.11 (s, 2H), 4.07 (dq, JₚΗ = 8.0 Hz, J = 7.0 Hz, 4H), 1.84 (s, 3H), 1.33 (t, J = 7.0 Hz, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 147.5, 144.6 (d, J₉C = 6.7 Hz), 120.2 (d, J₉C = 26.9 Hz), 115.2 (d, J₉C = 191.8 Hz), 66.8, 61.7 (d J₉C = 6.0 Hz), 16.3 (d, J₉C = 6.5 Hz), 14.5; ³¹P NMR (121 MHz, CDCl₃) δ + 20.2.

**Phosphonate 6.27**

Triflic anhydride (0.19 mL, 1.1 mmol) and pyridine (0.98 mL, 2.0 mmol) were added to a solution of monophosphonate 6.25 (277 mg, 1.0 mmol) in freshly distilled DCM (10 mL) and allowed to react for 10 mins. Phenol (240 mg, 2.5 mmol) was added and the solution was allowed to react for 30 minutes. After the reaction was concentrated in vacuo, the resulting blackish oil was purified via column chromatography (silica, 20% hexanes in EtOAc) to give the desired product 6.27 in 93% yield (307 mg): ¹H NMR (400 MHz, CDCl₃) δ 7.50–7.37 (m, 1H), 7.31–7.29 (m, 2H), 7.21–
7.18 (m, 2H), 7.16–7.11 (m, 1H), 6.15 (d, $J = 11.2$ Hz, 1H), 5.81 (dd, $J = 19.6, 17.6$ Hz, 1H), 4.55 (s, 2H), 4.24–4.15 (m, 2H), 2.10 (br s, 3H), 1.86 (br s, 3H), 1.36–1.32 (m, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.5, 150.5 (d, $J_{PC} = 7.6$ Hz), 144.7 (d, $J_{PC} = 6.5$ Hz), 141.8, 129.7 (2C), 125.1 (d, $J_{PC} = 27.9$ Hz), 124.8, 120.5 (d, $J_{PC} = 4.2$ Hz, 2C), 116.8 (d, $J_{PC} = 191.7$ Hz), 68.1, 62.5 (d, $J_{PC} = 5.8$ Hz), 20.8, 16.3 (d, $J_{PC} = 6.3$ Hz), 14.9. $^{31}$P NMR (121 MHz, CDCl$_3$) $\delta$ +19.6.
APPENDIX: SELECTED NMR SPECTRA
Figure A 1. $^1$H NMR spectrum of compound 2.16
Figure A 2. $^1$H NMR spectrum of compound 2.17
Figure A 3. $^1$H NMR spectrum of compound 2.20
Figure A 4. $^1$H NMR spectrum of compound **2.21**
Figure A 5. $^1$H NMR spectrum of compound 2.22
Figure A 6. $^{13}$C NMR spectrum of compound 2.22
Figure A 7. $^1$H NMR spectrum of compound 2.26
Figure A 8. $^{31}$P NMR spectrum of compound 2.27
Figure A 9. $^1$H NMR spectrum of compound 2.28
Figure A 10. $^{13}$C NMR spectrum of compound 2.28
Figure A 11. $\textsuperscript{31}$P NMR spectrum of compound 2.28 with expansion
Figure A 12. $^1$H NMR spectrum of compound 2.29
Figure A 13. $^{13}$C NMR spectrum of compound 2.29
Figure A 14. $^{31}$P NMR spectrum of compound 2.29 with expansion
Figure A 15. $^1$H NMR spectrum of compound 2.30
Figure A.16. $^{13}$C NMR spectrum of compound **2.30**
Figure A 17. $^{31}$P NMR spectrum of compound 2.30 with expansion
Figure A 18. $^1$H NMR spectrum of compound 2.31
Figure A 19. $^{13}$C NMR spectrum of compound 2.31
Figure A 20. $^{31}$P NMR spectrum of compound 2.31 with expansion
Figure A 21. $^1$H NMR spectrum of compound 2.32
Figure A 22. $^{13}$C NMR spectrum of compound 2.32
Figure A 23. $^{31}$P NMR spectrum of compound **2.32** with expansion
Figure A 24. $^1$H NMR spectrum of compound 2.33
Figure A 25. $^{13}$C NMR spectrum of compound 2.33
Figure A 26. $^{31}$P NMR spectrum of compound 2.33 with expansion
Figure A 27. $^1$H NMR spectrum of compound 2.34
Figure A 28. $^{13}$C NMR spectrum of compound 2.34
Figure A 29. $^{31}$P NMR spectrum of compound 2.34 with expansion
Figure A 30. $^1$H NMR spectrum of compound 2.35
Figure A 31. $^{13}$C NMR spectrum of compound 2.35
Figure A32. $^{31}$P NMR spectrum of compound 2.35 with expansion
Figure A 33. $^1$H NMR spectrum of compound 2.36
Figure A 34. $^{13}$C NMR spectrum of compound 2.36
Figure A 35. $^{31}$P NMR spectrum of compound 2.36 with expansion
Figure A 36. $^1$H NMR spectrum of compound 2.37
Figure A 37. $^{13}$C NMR spectrum of compound 2.37
Figure A 38. $^{31}$P NMR spectrum of compound 2.37 with expansion
Figure A 39. $^1$H NMR spectrum of compound 2.38
Figure A 40. $^{13}$C NMR spectrum of compound 2.38
Figure A 41. $^{31}$P NMR spectrum of compound 2.38 with expansion
Figure A 42. $^1$H NMR spectrum of compound 2.40
Figure A 43. $^{13}$C NMR spectrum of compound 2.40
Figure A 44. $^{31}$P NMR spectrum of compound 2.40 with expansion
Figure A 45. $^1$H NMR spectrum of compound 2.41
Figure A 46. $^{13}$C NMR spectrum of compound 2.41
Figure A 47. $^{31}$P NMR spectrum of compound 2.41 with expansion
Figure A 48. $^1$H NMR spectrum of compound 2.42
Figure A 49. $^{13}$C NMR spectrum of compound 2.42
Figure A 50. $^{31}$P NMR spectrum of compound $\textbf{2.42}$ with expansion
Figure A 51. $^1$H NMR spectrum of compound 2.43
Figure A 52. $^{13}$C NMR spectrum of compound 2.43
Figure A 53. $^{31}$P NMR spectrum of compound 2.43 with expansion
Figure A 54. $^1$H NMR spectrum of compound 3.08
Figure A 55. $^{13}$C NMR spectrum of compound 3.08
Figure A 56. $^1$H NMR spectrum of compound 3.12
Figure A 57. $^{13}$C NMR spectrum of compound 3.12
Figure A 58. $^1$H NMR spectrum of compound 3.13
Figure A 59. $^{13}$C NMR spectrum of compound 3.13
Figure A 60. $^1$H NMR spectrum of compound 4.11
Figure A 61. $^{13}$C NMR spectrum of compound 4.11
Figure A 62. $^{31}$P NMR spectrum of compound 4.11
Figure A 63. $^1$H NMR spectrum of compound \textbf{4.12}
Figure A 64. $^{13}$C NMR spectrum of compound 4.12
Figure A 65. $^{31}\text{P}$ NMR spectrum of compound 4.12
Figure A 66. $^1$H NMR spectrum of compound 4.13
Figure A 67. $^{13}$C NMR spectrum of compound 4.13
Figure A 68. $^{31}$P NMR spectrum of compound 4.13
Figure A 69. $^1$H NMR spectrum of compound 4.14
Figure A 70. $^{13}$C NMR spectrum of compound 4.14
Figure A 71. $^{31}\text{P}$ NMR spectrum of compound 4.14
Figure A 72. $^1$H NMR spectrum of compound 4.15
Figure A 73. $^{13}$C NMR spectrum of compound 4.15
Figure A 74. $^{31}$P NMR spectrum of compound 4.15
Figure A 75. $^1$H NMR spectrum of compound 4.16
Figure A 76. $^{13}$C NMR spectrum of compound 4.16
Figure A 77. $^{31}$P NMR spectrum of compound 4.16
Figure A 78. $^1$H NMR spectrum of compound 4.17
Figure A 79. $^{13}$C NMR spectrum of compound 4.17
Figure A 80. $^{31}$P NMR spectrum of compound 4.17
Figure A81. $^1$H NMR spectrum of compound 4.18
Figure A 82. $^{13}$C NMR spectrum of compound 4.18
Figure A 83. $^{31}$P NMR spectrum of compound 4.18
Figure A 84. $^1$H NMR spectrum of compound 4.19
Figure A 85. $^{13}$C NMR spectrum of compound 4.19
Figure A 86. $^{31}$P NMR spectrum of compound 4.19
Figure A 87. $^1$H NMR spectrum of compound 4.20
Figure A 88. $^{13}$C NMR spectrum of compound 4.20
Figure A 89. $^{31}$P NMR spectrum of compound 4.20
Figure A 90. $^1$H NMR spectrum of compound 5.10
Figure A 91. $^{13}$C NMR spectrum of compound 5.10
Figure A 92. $^1$H NMR spectrum of compound 5.11
Figure A 93. $^{13}$C NMR spectrum of compound 5.11
Figure A 94. $^1$H NMR spectrum of compound 5.12
Figure A 95. $^{13}$C NMR spectrum of compound 5.12
Figure A 96. $^1$H NMR spectrum of compound 5.13
Figure A 97. $^{13}$C NMR spectrum of compound 5.13
Figure A 98. $^1$H NMR spectrum of compound 5.14
Figure A 99. $^1$H NMR spectrum of compound 5.15
Figure A 100. $^1$H NMR spectrum of compound 5.17
Figure A 101. $^1$H NMR spectrum of compound 5.19
Figure A 102. $^1$H NMR spectrum of compound 5.20
Figure A103. $^1$H NMR spectrum of compound 5.21
Figure A 104. $^1$H NMR spectrum of compound 5.22
Figure A 105. $^1$H NMR spectrum of compound 5.23
Figure A 106. $^{13}$C NMR spectrum of compound 5.23
Figure A 107. $^1$H NMR spectrum of compound 5.25
Figure A 108. $^{13}$C NMR spectrum of compound 5.25
Figure A 109. $^1$H NMR spectrum of compound 6.12
Figure A 110. $^1$H NMR spectrum of compound 6.13
Figure A 111. $^1$H NMR spectrum of compound 6.14
Figure A 112. $^1$H NMR spectrum of compound 6.15
Figure A 113. $^1$H NMR spectrum of compound 6.16
Figure A 114. $^{13}$C NMR spectrum of compound 6.16
Figure A 115. $^1$H NMR spectrum of compound \textbf{6.20}
Figure A 116. $^1$H NMR spectrum of compound 6.21
Figure A 117. $^1$H NMR spectrum of compound 6.24
Figure A 118. $^1$H NMR spectrum of compound 6.25
Figure A 119. $^1$H NMR spectrum of compound 6.26
Figure A 120. $^{13}$C NMR spectrum of compound 6.26
Figure A 121. $^1$H NMR spectrum of compound 6.27
Figure A 122. $^{13}$C NMR spectrum of compound 6.27
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