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Synthesis and biological evaluation of novel phosphonates

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SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL PHOSPHONATES

by

Rocky James Barney

An Abstract

Of 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

December 2010

Thesis Supervisor: Professor David F. Wiemer

ABSTRACT

Phosphonates represent an important class of organophosphorus compounds. Their use as reagents in organic synthesis is prevalent, and there is a plethora of examples of biologically active compounds possessing the phosphonate moiety. To further our exploration of phosphonates as both reagents and biologically active compounds we have developed a one-flask protocol for the direct synthesis of phosphonates from benzylic and allylic alcohols. This transformation is unprecedented and is applicable to a range of substrates. Both electron rich and electron deficient benzylic alcohols react under the conditions developed. Furthermore, good yields are achieved when converting allylic alcohols to the corresponding allylic phosphonates. In at least one case, the one-flask protocol allows for phosphonate formation that was not achievable under the standard conditions.

Bisphosphonates represent a significant subclass of phosphonates. Several nitrogenous bisphosphonates have found use in the clinic as treatments for bone-related disease including osteoporosis, and there is speculation that bisphosphonates that are enzyme-specific inhibitors may be used as cancer therapies. To develop our understanding of isoprenoid metabolism, we have prepared a range of bisphosphonates as potential inhibitors of geranylgeranyl pyrophosphate synthase. After much experimentation, an α-amino analogue of a potent inhibitor of GGDPS has been synthesized and biological data is forthcoming. Furthermore, a new class of aromatic bisphosphonates, analogues of digeranyl bisphosphonic acid, has been synthesized and assayed. The bioassay results indicate that this series of compounds retains its specificity for the GGDPS enzyme, and that the dialkyl analogues retain much of their potency in the assays in spite of the increased steric bulk of the aromatic substructure.

We have also begun the design and synthesis of compounds as potential inhibitors of Rab geranylgeranyl transferase (RGGTase). The lead compound, 3-PEHPC, is documented to inhibit RGGTase selectively, albeit at greater than desirable concentrations. Using 3-PEHPC as the

model compound we have elected to probe the impact of modifications on the hydrophilic "head" portion of the molecule. With the phosphonophosphinate functionality as a surrogate for the phosphonocarboxylate moiety, we have successfully synthesized digeranyl phosphonophosphinate. Initial assay data indicate that this novel phosphonophosphinate does not act upon GGDPS as does the analogous bisphosphonate substructure. Additional bioassay data to probe this compound's impact on RGGTase are forthcoming.

Given the worldwide impact of tuberculosis infection and the emergence of drug-resistant strains of tuberculosis-causing pathogens, new and potent treatments for tuberculosis are necessary. We have engaged in the synthesis of several compounds as inhibitors of Rv2361c, an enzyme key to cell wall biosynthesis in *Mycobacterium tuberculosis*, the principal causative agent of tuberculosis in humans. To probe the impact of modifications at the C-9 position of the most potent of our Rv2361c inhibitors, we have made several analogues having phenyl and indole substituents. The *in vitro* enzyme assay data for the set of compounds have clarified understanding of the essential components of the pharmacophore, and helped to establish the direction for future efforts.

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Graduate College
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Iowa City, Iowa

CERTIFICATE OF APPROVAL

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PH.D. THESIS

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This is to certify that the Ph.D. thesis of

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has been approved by the Examining Committee
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To Orean and Faye Barney

Buy the ticket, take the ride.

Hunter S. Thompson

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My family also has my undying gratitude and love. Rachel has stood by me, through thick and thin. In spite of my shortcomings and the hardships, she has remained my support. I love and cherish her deeply. My children, Elijah James and Chloe Eva, have gone without many of life's comforts in my pursuit of education. Elijah, many times you've went to work with Dad and made my day a little brighter. You've put a smile on my face when I thought there was nothing to smile about. Chloe, not a day passes that I don't look forward to coming home to you and having hugs and kisses for "poppy". Also, my newest son Aaron has enriched my life greatly. Having your kind and gentle personality to smooth out the rough spots has been a real blessing.

I have the greatest mom and dad anyone could hope for. My mother's kindness and gentleness are unprecedented. I wouldn't have been able to go to grad school without all of your support and help. My father has always been someone I look up to. He's supported me whenever I've needed it, and provided me with the confidence to pursue my interests. I know I can always count on you both, and I've never questioned your love for me.

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Finally, Faye and Orean Barney, the grandparents to whom this document is dedicated have been, and will always be the strongest inspiration in my life. Also, my Aunt Cindy has given me more than I could ever thank her for. I don't know if she'll ever know how much her kindness, love, and support have meant to me. I like the man I've become a little better because of you.

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Last but not least, I'm grateful for the friendships I've forged with both past and present members of the Wiemer group. I consider Jeff Neighbors, Jose Yu, and Mona Maalouf true friends, and will cherish their friendship for years to come. Also, the friendship of Jeff Bowles, Rebekah Richardson, John Kodet and Natalie Ulrich will not be forgotten.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

15-crown-5	1,4,7,10,13-pentaoxacyclopentadecane
Ac	acetate
BOC	*tert*-butoxycarbonyl
BP	bisphosphonate
br	broad (NMR)
calcd	calculated
d	doublet (NMR)
DGBP	digeranyl bisphosphonate
DMF	dimethylformamide
DNA	deoxyribonucleic acid
E	entgenen
EMB	ethambutol
equiv	equivalent
ES	electrospray
ESI	electrospray ionization
Et	ethyl
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethyl alcohol
FDPS	farnesyl diphosphate synthase
FPP	farnesyl pyrophosphate
FTase	farnesyl transferase
FTI	farnesyl transferase inhibitor
g	gram

GGDPS	geranylgeranyl diphosphate synthase
GGPP	geranylgeranyl pyrophosphate
GGTase I	geranylgeranyl transferase I
GGTI	geranylgeranyl transferase inhibitor
GI_{50}	50% inhibition of cell growth
hr	hour
HBV	hepatitis B virus
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus
HRMS	high resolution mass spectrometry
HWE	Horner-Wadsworth-Emmons
Hz	hertz
IC_{50}	concentration for 50% enzyme inhibition
INH	isoniazid
IPP	isopentyl pyrophosphate
J	coupling constant
K562	human leukemia cell line
KHMDS	potassium hexamethyldisilazanide
LDA	lithium diisopropylamide
Lov	lovastatin
M	molar
m	multiplet (NMR)
m/z	mass-charge ratio
MDR	Multi-drug resistant
Me	methyl
MeOH	methyl alcohol
mg	milligram

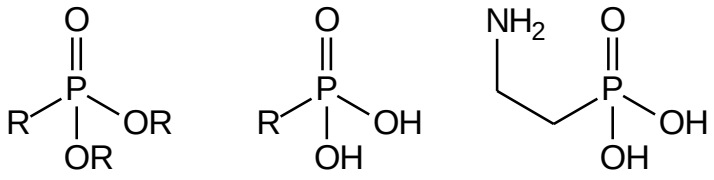
MGBP	monogeranyl bisphosphonate
MHz	megahertz
min	minute
mL	milliliter
mmol	millimol
MOM	methyleneoxymethyl
Ms	methanesulfonyl
N	normal
n-BuLi	*normal*-butyl lithium
NCI	National Cancer Institute
nM	nanomolar
NMR	nuclear magnetic resonance
NtRTI	nucleotide reverse transcriptase inhibitor
PC	phosphonocarboxylate
PEP	phosphonoenolpyruvate phosphono
PMPA	Tenofovir disoproxil fumarate
ppm	parts per million
PZA	pyrazinamide
q	quartet (NMR)
RCM	ring-closing metathesis
REP	Rab escort protein
RGGTase	Rab geranylgeranyl transferase
RIF	rifampicin
rt	room temperature
s	singlet (NMR)
S.M.	starting material
SAR	structure-activity relationship

T	temperature
t	triplet (NMR)
TB	tuberculosis disease
TBSCl	*tert*-butyldimethylsilyl chloride
TEA	triethylamine
TEMBP	tetraethyl methylenebisphosphonate
THF	tetrahydrofuran
TLC	thin-layer chromatography
TMSBr	trimethylsilyl bromide
XDR	extreme-drug resistant
Z	zusammen
δ	chemical shift (NMR)
μM	micromolar

CHAPTER I

AN OVERVIEW OF PHOSPHONATES

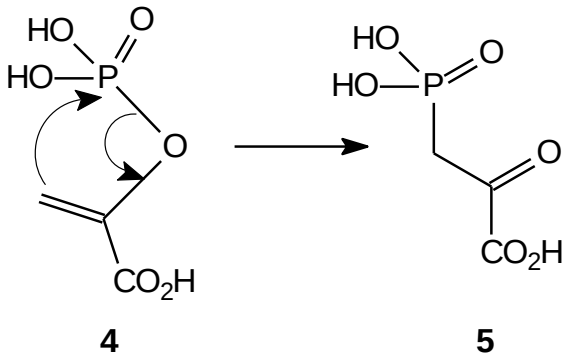
Phosphonates (**1,** Figure 1) and phosphonic acids (**2**, Figure 1) represent an important class of organophosphorus compounds. Their use in a variety of applications is well documented and their importance in a range of fields is only increasing. The first naturally occurring phosphonate described, 2-aminoethane phosphonic acid (**3**), was isolated by Kandatsu and Horiguchi from rumen protozoa in 1959.[1] This led to the accelerated discovery of a multitude of naturally occurring phosphonates from a number of sources including fungi and bacteria as well as higher order organisms.[2,3,4,5] While the biological roles of naturally occurring phosphonates are not understood in their entirety, researchers at Harvard University have isolated one enzyme responsible for C-P bond formation in conversions of compound **4** to **5**. The enzyme is phosphoenolpyruvate phosphonomutase (PEP mutase, Figure 2) from *Tetrahymena pyriformis*. Isolation of this enzyme sheds further light on the processes associated with phosphonate biosynthesis.[5,6]



1 **2** **3**

R = alkyl, aryl

Figure 1. Structures of phosphonates and phosphonic acids.



4 **5**

Figure 2. The reaction catalyzed by PEP mutase.[6]

The applications in which phosphonates have been used are myriad. Industrial applications are abundant, and the function of phosphonates as effective chelating agents, corrosion inhibitors in cooling systems,[7,8,9] and water softeners[10] is well established. Furthermore, the ability to complex metal cations makes phosphonates an excellent choice as stabilizers in oxidation processes associated with the pulp, paper, and textile industries.[11] Phosphonates also play important roles as flame retardants. For example, a leach-resistant, flame retardant oligomeric phosphonate has been developed for the treatment of natural woods, greatly attenuating the combustion characteristics of treated wood compared to those of untreated wood.[12] Furthermore, phosphonates have been used as valuable flame retardants for a range of plastics including polystyrene,[13] and acrylonitrile, methyl methacrylate, and acrylamide[14] polymers.

In addition to their applications in the industrial arena, phosphonates have played roles of key importance as pesticides. Pesticides are defined as "any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest." [15] Herbicides, such as glyphosate (**6**), marketed by Monsanto as "Round Up™ and MCP (**7**), [16] are the most widely used type of pesticides. The fact that agricultural endeavours consume 75% of all pesticides suggests that the food supply in many nations is irrevocably intertwined with the historical

development of phosphonates.[17] In addition to herbicides, there is a range of other phosphonate based pesticides including insecticides such as butonate (**8**) and trichlorfon (**9**),[18] as well as fungicides such as *O*,*O*-bisphenyl methylphosphonate[19] (**10**, Figure 3).

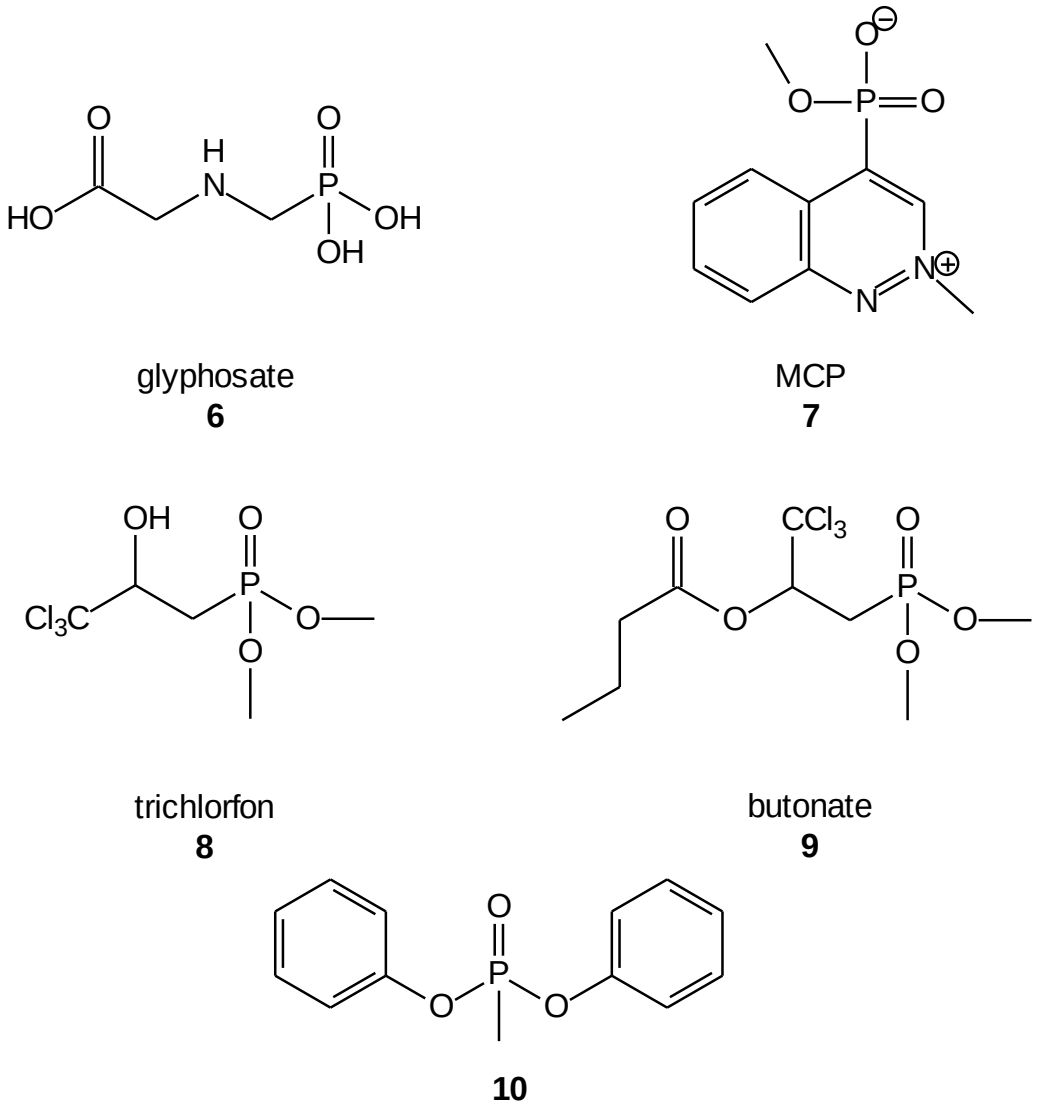


Figure 3. Phosphonate herbicides, insecticides, and fungicide.

It was the development of pesticides that originally led to the interest of the military-industrial complex in phosphonates, and their subsequent development as agents of war. In 1936 the first nerve agent of the "G-agent" class, Tabun, was discovered accidentally by the German scientist Gerhard Schrader.[20] Several related organophosphorus compounds have been developed as chemical warfare agents since, including Sarin (**11**), Cyclosarin (**12**), and Soman (**13**, Figure 4).

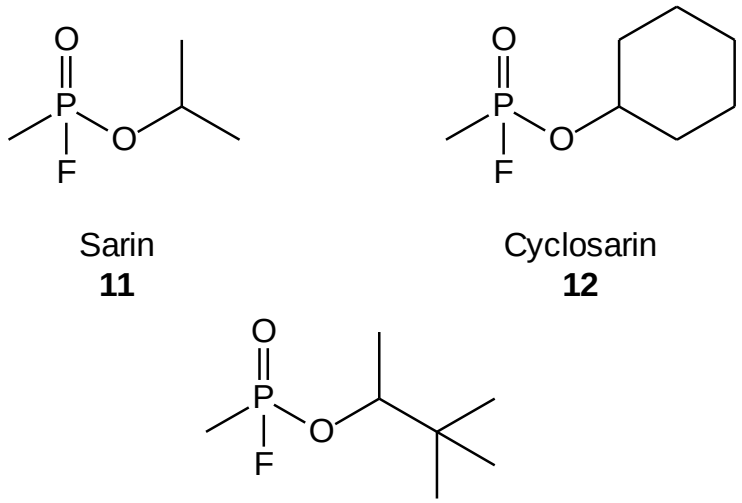


Figure 4. Phosphonate nerve agents.

The phosphonate dimethyl methylphosphonate, as well as other dialkyl methylphosphonates, have been used as a precursor in the synthesis of the Sarin (**11**) nerve agent. The original synthesis of Sarin used the di-di method employing equimolar amounts of difluoromethylphosphonate (**14**) and dichloromethylphosphonate (**15**).[21] More recently, researchers at the Chemistry Section of the Defence Research Board of Canada, Suffield

Experimental Station have been successful in generating radioactive Sarin through a two-step method from diisopropyl methylphosphonate[21] (**17**) via the mono-chloro phosphonate **18** (Figure 5).

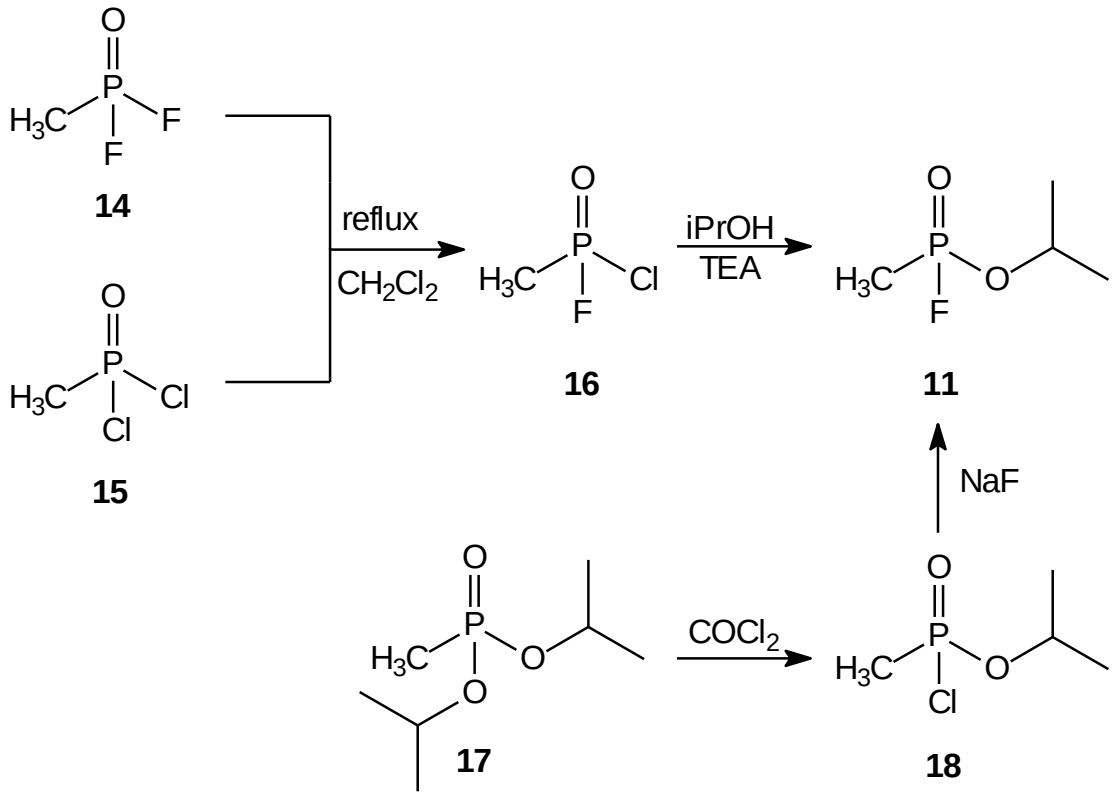


Figure 5. The di-di (top) and direct methods (bottom) of Sarin (**11**) synthesis.

The phosphonate functionality has been incorporated into a range of clinically useful drugs. In Addition, acyclic nucleoside phosphonates have shown potential as therapeutics for pathogenic species.[22] For example, Tenofovir (**19**) disoproxil fumarate (PMPA) is an approved treatment for HIV in humans, and has shown efficacy in the treatment of hepatitis B (HBV).[23] Adefovir, a nucleotide reverse transcriptase inhibitor (NtRTI), has shown activity against

retroviruses including Herpes virus, while the NtRTI Cidofovir (**20**) has shown efficacy in the treatment of DNA viruses such as HPV[23] (Figure 6). In addition, the phosphonate moiety is found in HIV protease inhibitors showing an enhanced resistance profile compared to that of the non-phosphonylated parent,[24] and phosphonate containing protease inhibitors also have shown great potential for the treatment of Hepatitis C virus.[25] Alafosfalin (**21**), an antibiotic with a broad spectrum of activity (Figure 6) has been developed, and demonstrated the ability to inhibit cell wall biosynthesis.[26] It has proved to be potentially useful in the treatment of gastroenteritis and bacterial urinary tract infections.[27]

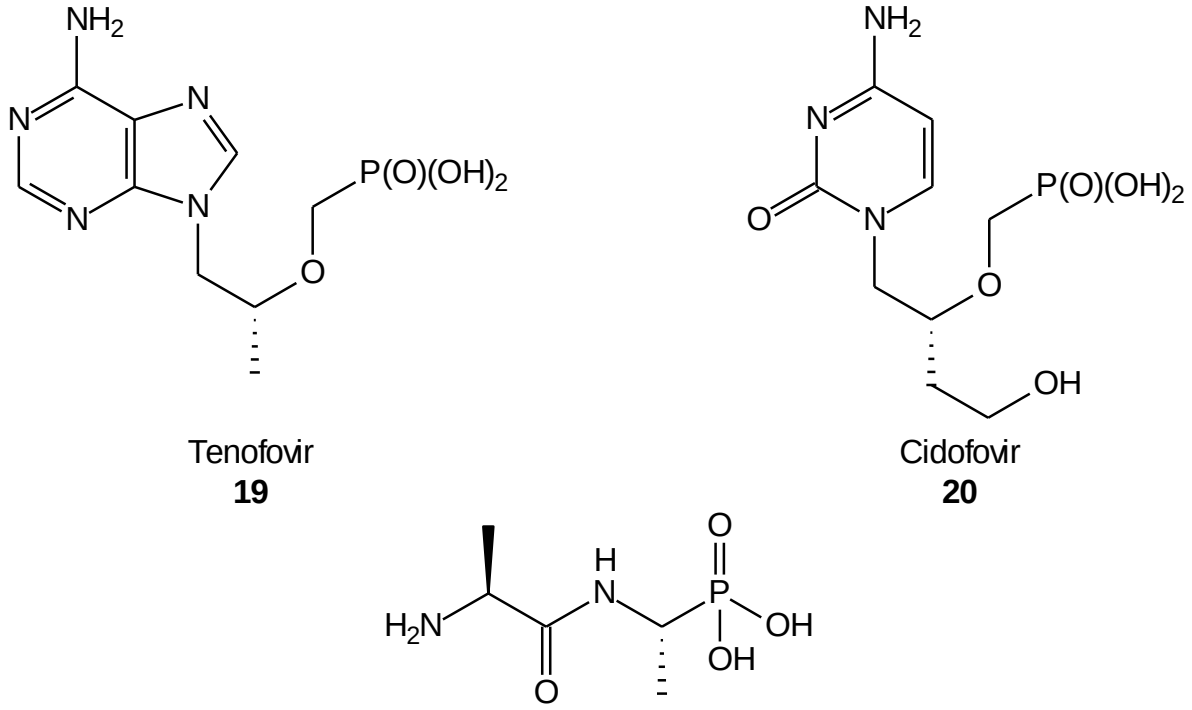
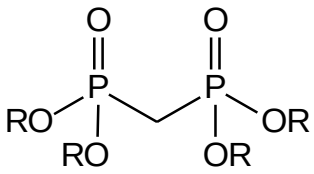


Figure 6. Clinically used phosphonates.

In addition to the antibiotic and antiviral therapies made possible by phosphonates, some have shown potential as cancer therapies[28] and anti-parasitic agents.[29,30] Their ability to inhibit growth of malignant cell lines[31,32] as well as their ability to activate γδ-T cells is documented.[33]

One subset of phosphonates that has been particularly useful in the clinic is the geminal bisphosphonates **22** where two phosphonate moieties are attached to a single carbon (Figure 7). These have found use in the clinic as treatments for bone related disease including osteoporosis[34] and multiple myeloma.[35] In addition; they have shown promise in their ability to chelate radionucleotides in an effort to gain further insight into cancers and their related biological processes.[36]



22

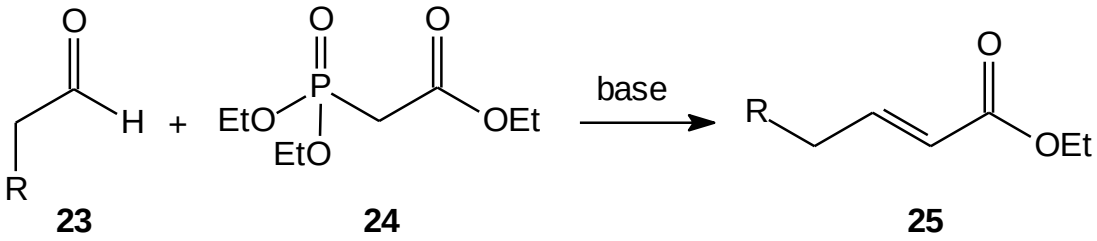
R = aryl, alkyl, H

Figure 7. The bisphosphonate structure.

Phosphonates also have served as essential intermediates in the synthesis of innumerable biologically active compounds including many natural products.[37] Since 1958, when a modified Wittig reaction was first reported by Horner and coworkers[38] a great deal of exploration of this type of olefination has been undertaken. With the additional work of Wadsworth and Emmons, the Horner-Wadsworth-Emmons[39] (HWE) olefination reaction was born (Figure 8).

The HWE reaction is distinct from its Wittig counterpart in that it utilizes a phosphonate-stabilized carbanion to undergo reaction with aldehydes and ketones and provide olefin products.

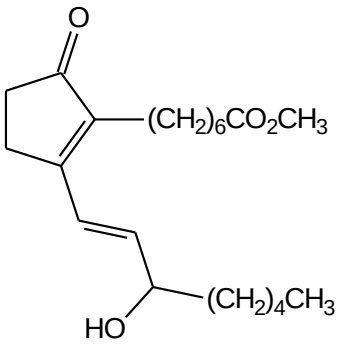
This reaction is generalized in Figure 8 where an aldehyde **23** is treated with the anion of triethyl phosphonoacetate (**24**) to provide the *trans* α,β-unsaturated ester **25**.



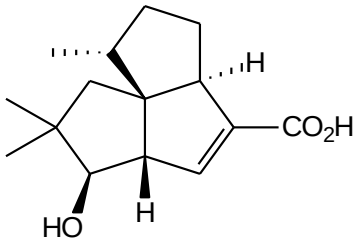
23 **24** **25**

Figure 8. A standard HWE reaction giving rise to a *trans* olefin.

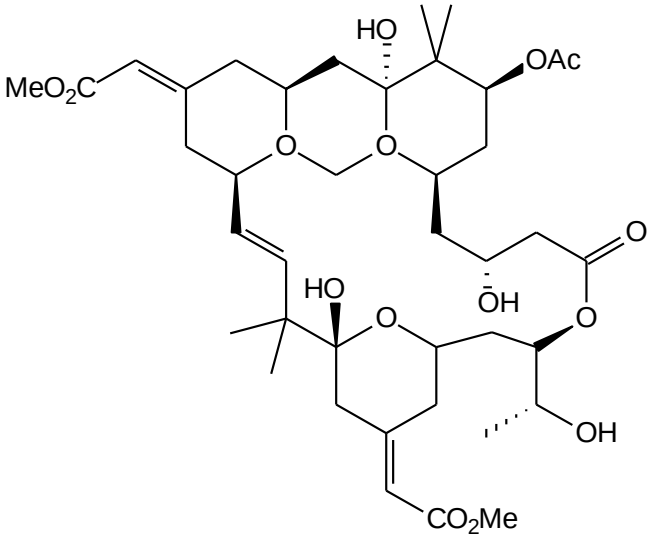
In general, this reaction serves as a complement to the Witting reaction in that it usually provides the *E*-alkene, in contrast to the Wittig reaction's general tendency toward the Z-olefin geometry. Further development of the HWE reaction has allowed for a great deal of versatility in also providing Z-alkenes when desirable through the use of 2,2,2-trifluoroethoxy[40] and aryloxy substituents on the phosphorous atom.[41] The development of asymmetric HWE reactions also has been explored, and the resulting methods have been applied successfully to an assortment of substrates. Examples include prostaglandin methyl ester (**26**), pentalenic acid (**27**), and the complex macrolactone bryostatin 11 (**28**, Figure 9).[42]



(+/-) Prostaglandin B_1 Methyl Ester
26



(+/-)-Pentalenic acid
27



Bryostatin 11
28

Figure 9. Synthetic targets utilizing phosphonate intermediates.

The synthesis of (+/-)-sarkomycin (**34**) serves as a fine example of a phosphonate reagent utilized in the synthesis of a naturally occurring antibiotic. Sarkomycin was first isolated in 1953[43] and its structure was elucidated the following year.[44] Mikolajczyk and coworkers synthesized sarkomycin exploiting the inherent stabilizing capacity of the phosphonate. This was accomplished by first alkylating the β-keto phosphonate **29** to provide compound **30**. The

stabilizing effect of the phosphonate was then exploited to install the diazo-functionality of phosphonate **31** which was subsequently cyclized to give product **32**. The phosphonate **32** was treated under ozonolysis conditions to cleave the olefin and the resulting aldehyde was oxidized to provide carboxylic acid **33**. This was followed with an HWE olefination to provide the target racemic sarkomycin (**34**) in fair yield (Figure 10).[45]

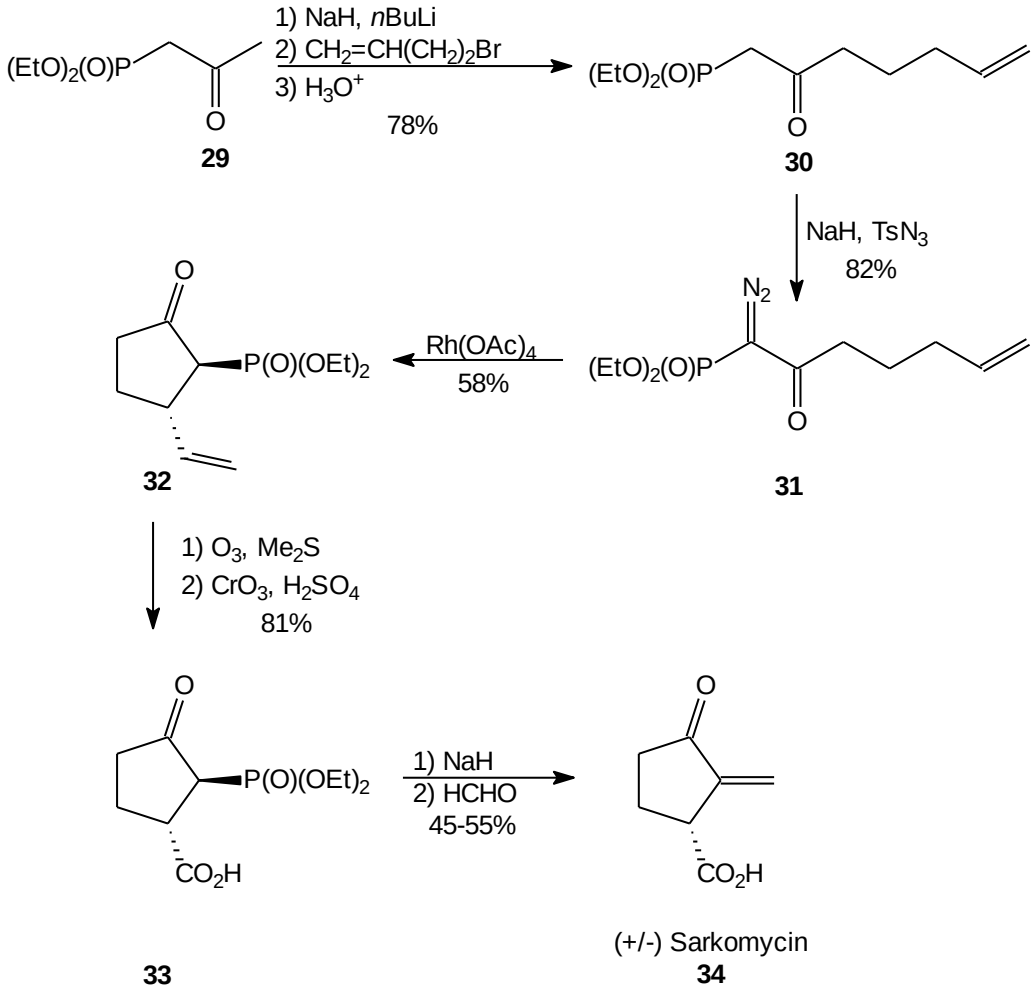


Figure 10. The synthesis of racemic sarkomycin.

Other biologically active natural products, such as the schweinfurthins, also have benefited from the reactivity afforded by the phosphonate moiety. The schweinfurthins were first isolated in 1998 by Beutler and coworkers, and several of the natural schweinfurthins showed promising activity in the NCI 60-cell assay.[46] Wiemer and coworkers have synthesized the stilbene core of a large variety of schweinfurthin analogs [47,48] and more recently have synthesized the natural schweinfurthins B and E exploiting the HWE reaction for the installation of the stilbene core.[49]

Phosphonates also have been used as templates for RCM reactions.[50] Furthermore, Evans and coworkers have utilized α,β-unsaturated acyl phosphonates in enantioselective Diels-Alder reactions.[51] Their work showed that the electron-withdrawing nature of the phosphonate moiety efficiently promoted cycloaddition reactions with electron-rich alkenes. As shown in Figure 11, Evans and coworkers were successful in converting acyl phosphonate **35** to the cyclic vinyl phosphonate **37** via treatment with vinyl ether **36** and catalyst **38**. This strategy provided the cyclic vinyl phosphonate **37** in good yield with excellent stereoselectivity.

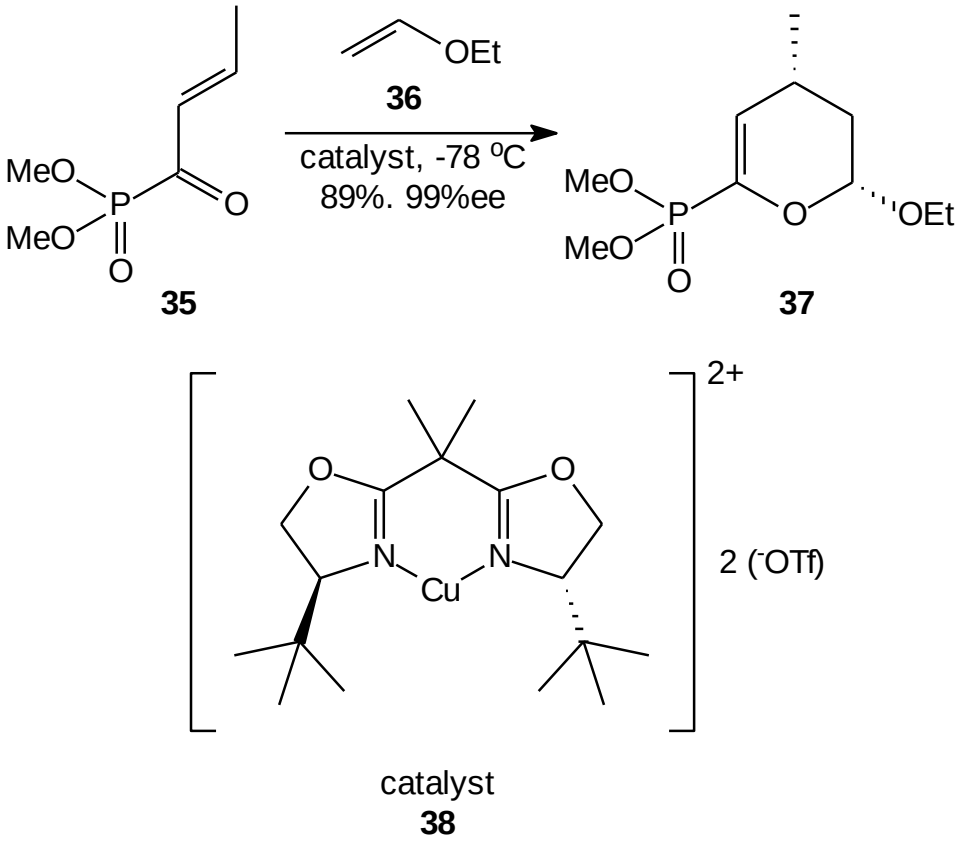


Figure 11. A Diels-Alder reaction incorporating an acyl phosphonate.

In addition to these reactions, a phosphonate-stabilized anion can be trapped through reaction with a variety of electrophiles as portrayed in Figure 12. In general, the anion of phosphonate **39** can be treated with a range of electrophiles to give the corresponding product **40**. For example, the clinically important bisphosphonate moiety can be generated from the phosphonate precursor through treatment with a strong base and reaction with a phosphorus electrophile.[52] Recently, Gibson and coworkers have successfully treated a monophosphonate with LDA, followed by treatment with diethyl chlorophosphate to give the corresponding bisphosphonate in quantitative yield.[53] An impressive range of electrophiles has been incorporated through reaction with a phosphonate stabilized carbanion. Fluorination,[54] bromination,[55] carbonylation,[56] and alkylation[57] all have been performed with great success. In

addition, alpha oxidations and aminations, both racemic and asymmetric, have been developed.[58,59, 60] Finally, trisphosphonates have been synthesized in moderate yield (Figure 13) by treatment of the anion of tetraethyl methylenebisphosphonate (**41**) with the electrophilic phosphorus reagent, diethyl chlorophosphite and subsequent oxidation to the trisphosphonate **42**.[61]

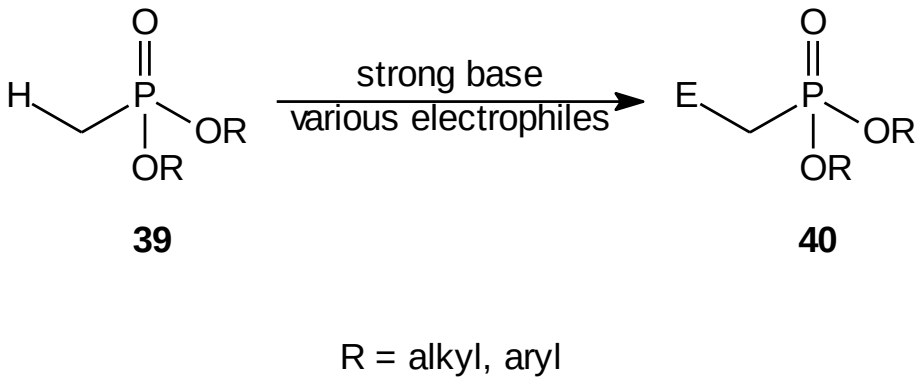


Figure 12. The general reaction for trapping of an electrophile with a phosphonate stabilized anion.

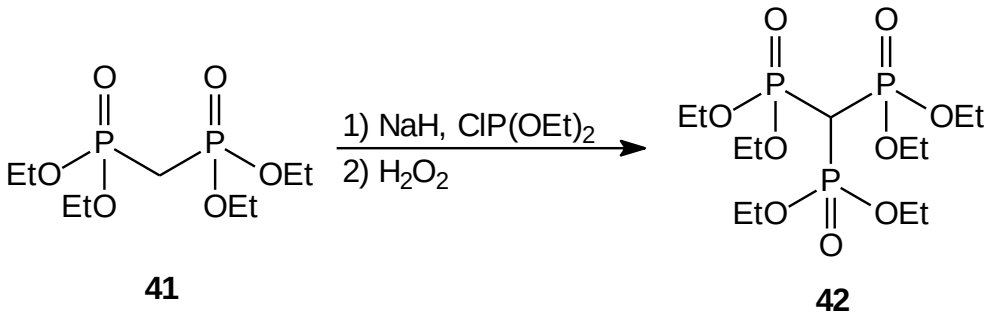


Figure 13. Synthesis of a trisphosphonate.

Despite the abundance of past work on the chemistry and bioactivity of phosphonate-containing compounds, this area remains a vibrant arena of research. In the following chapters, a new method for phosphonate synthesis will be presented, followed by work on the synthesis and biological activity of several groups of new phosphonates.

CHAPTER II

PHOSPHONATE SYNTHESIS: A ZINC IODIDE MEDIATED APPROACH

As described in the preceding chapter, phosphonates serve in a variety of roles in industry[62] and medicine,[63] as well as being useful chemical reagents in a number of synthetic applications.[64] There are several approaches toward the synthesis of phosphonates which focus on the formation of the crucial C-P bond. Of these methods (Figure 14), the Abramov, Pudovik, Michaelis-Becker, and Michaelis-Arbuzov (commonly called the "Arbuzov Reaction") are the most well studied and documented.[65] A search of the literature shows that the Arbuzov reaction is commonly used to form phosphonates, and in fact is the most common method of phosphonylation employed.[66]

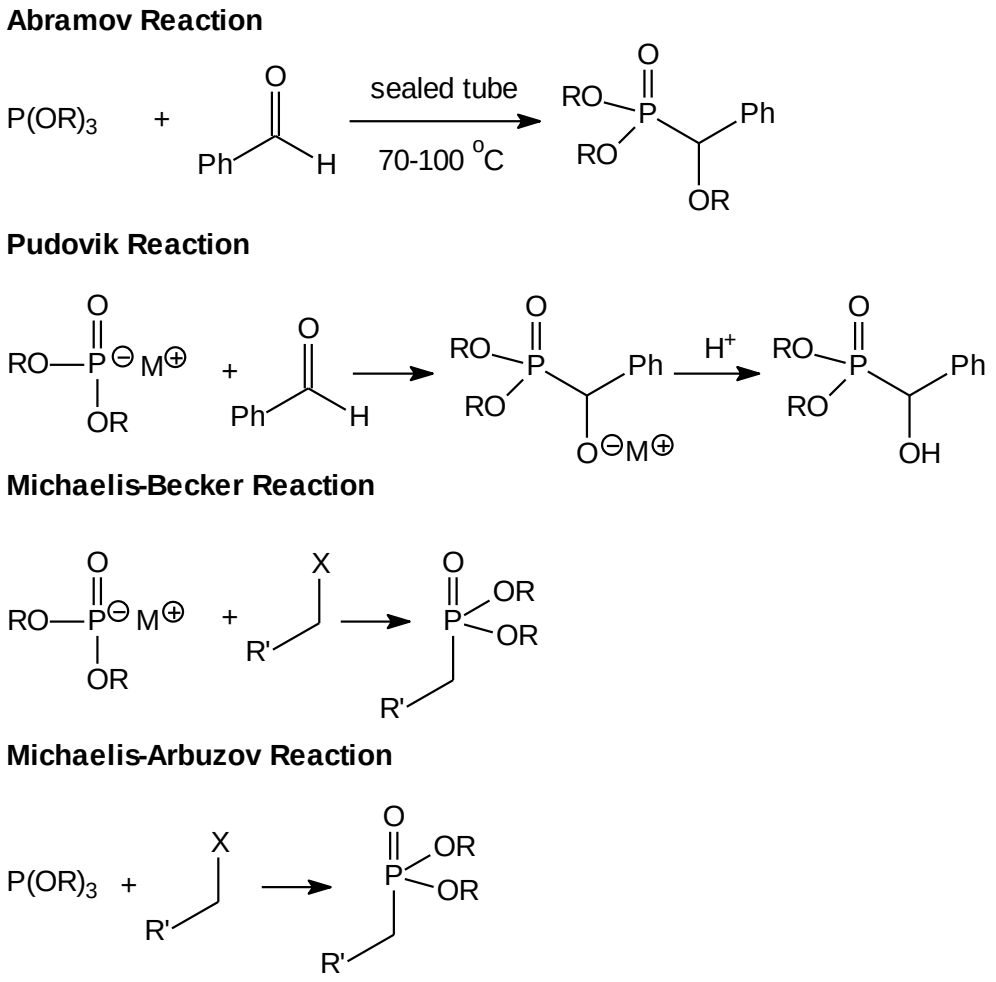
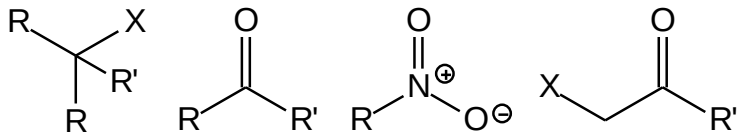


Figure 14. Various reactions resulting in C-P bond formation.

This Michaelis-Arbuzov reaction was first discovered by Michaelis[67] and later explored extensively by Arbuzov and coworkers.[68] The transformation involves treatment of an alkyl halide with a trivalent trialkyl phosphite, which after substitution undergoes rearrangement to provide the pentavalent phosphonate. The Arbuzov reaction has a wide scope but is not without limitations. The alkyl halide is confined to those compounds that can undergo S_N2 attack, and primary alkyl halides are most commonly used because many secondary and tertiary alkyl halides **43** either fail to react or undergo elimination to give the corresponding alkenes. In

addition, functional groups such as carbonyl **44** and nitro **45** groups present further complications. These moieties can be reactive toward the phosphite nucleophile and may give reduced products.[66] Furthermore, saturated α-chloro and α-bromo carbonyl **46** compounds may form the vinyl phosphate (O-P bond formation) selectively over the phosphonate (C-P bond formation, Figure 15). This later phosphate forming sequence is known as the Perkow reaction.[69]



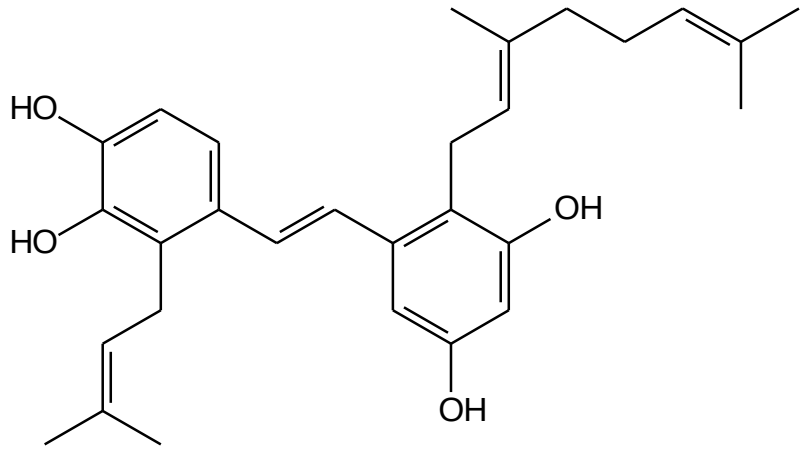
43 **44** **45** **46**

R = C,
R' = C, H, O, N
X = Cl, Br

Figure 15. Structures not well tolerated in the Arbuzov reaction.

The Wiemer group has engaged in the synthesis of numerous natural products containing a *trans*-stilbene core, including the schweinfurthins[46,48,70-73] and pawhuskins[74-76] (e.g. **47**, Figure 16) The previously described HWE olefination reaction has been a generally reliable method for the installation of this moiety. However, synthesis of the requisite phosphonate from the benzylic alcohol precursor most often has required a three-step procedure. The common protocol involves conversion of the alcohol to the mesylate, formation of the corresponding alkyl halide by reaction with an alkali bromide, and finally an Arbuzov reaction[77] to install the required phosphonate functionality. While this procedure is generally applicable to the primary benzylic alcohols, it is time consuming and in some instances has proven to be problematic[78] or

difficult to conduct on a large scale. Continuing in our tradition of the development of carbon-phosphorus bond forming reactions,[79-81] we have sought to contemplate and develop a more direct and efficient route for synthesis of semi-stabilized phosphonates from benzylic and allylic alcohols.



47

Figure 16. The structure of pawhuskin A.

It has been documented that alcohols affording a relatively stable cation, such as tertiary, allylic, and benzylic alcohols, can be activated by treatment with Lewis acids such as the zinc halides. The ensuing species have been trapped via reaction with alcohols,[83] thiols,[84] and hydride,[82] as well as other nucleophiles[85] (Figure 17). A review of the literature confirms that no extensive study of phosphorus as the nucleophile in this type of reaction has been undertaken, and only one example of a phosphorus nucleophile trapping a benzylic cation has been published.[86] This case utilizes cationic quinonoid intermediates and requires the formation of the mesylate followed by trapping of the resulting quinonoid (Figure 18) with trimethyl phosphite to

provide the phosphonate **55** in good yield.[86] Given the documented precedence for activation of stabilized alcohols with zinc halides, further exploration of this type of transformation was pursued, and a portion of this work has been submitted recently for publication.[87]

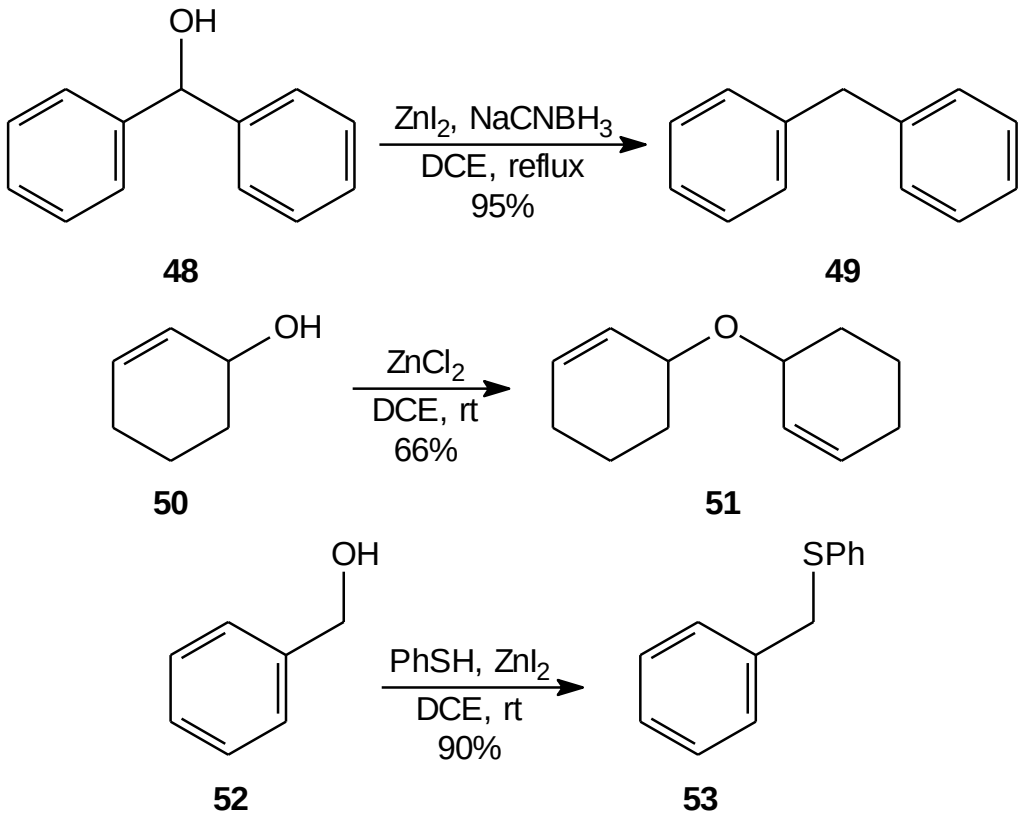


Figure 17. Representative zinc halide mediated transformations.

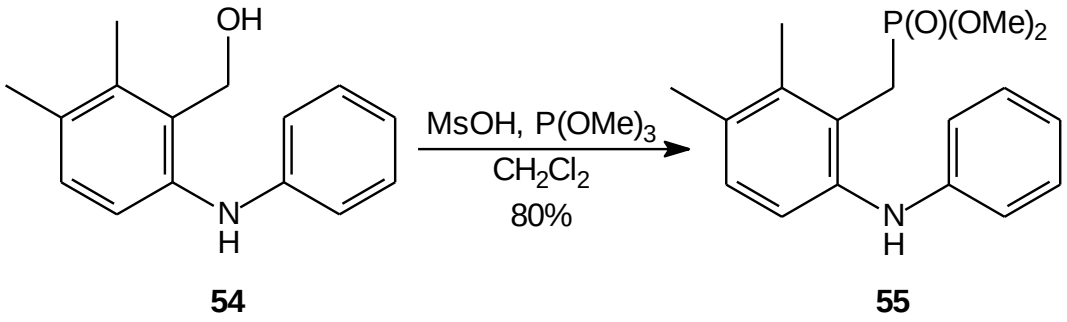


Figure 18: Conversion of a benzylic alcohol to a phosphonate via a quinonoid intermediate.

To assess the viability of a direct conversion of benzylic or allylic alcohols to the corresponding phosphonate via a ZnI_2 mediated reaction, benzyl alcohol (**52**) was treated with zinc iodide under a variety of conditions and the reaction progress was monitored by inspection of the ^{31}P NMR spectrum of the reaction mixture. When the reaction was attempted at room temperature in toluene or methylene chloride, little or no product was detected. Alternatively, when the reaction mixture was heated to reflux in toluene for 24 hours, formation of the diethyl benzylphosphonate[88] (**56**) was evident in the ^{31}P NMR spectrum. After removal of the volatiles in vacuo, dissolution of the residue in diethyl ether, washing with aqueous NaOH, and column chromatography, the desired benzylic phosphonate was isolated in very good yield (Figure 19).

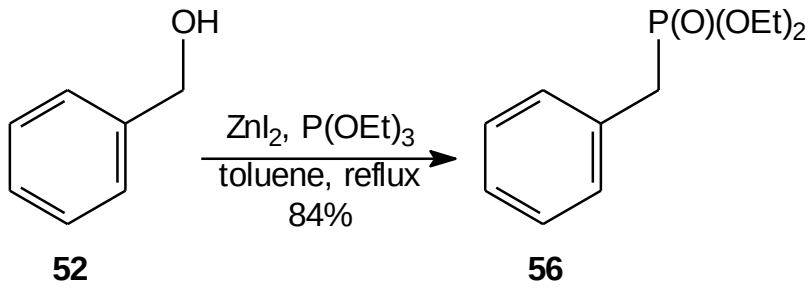


Figure 19: The conversion of benzyl alcohol to diethyl benzylphosphonate (**56**).

To probe further the generality of the reaction, a range of benzylic alcohols was treated with triethyl phosphite and zinc iodide under parallel reaction conditions (Figure 20). In the cases of both 3-bromo- and 3,4-dimethylbenzyl alcohols the reaction proceeded smoothly and gave the desired phosphonates **58**[89] and **60**[90] in acceptable yields. When the conditions were applied to 4-methoxybenzyl alcohol (**61**), the yields were variable and unreliable in toluene, ranging from a high of 76% to a modest yield of 27%. Both the broad range of isolated yields and the general problem of reproducibility in this case might result from a demethylation of the methyl phenyl ether under the reaction conditions to give the free phenol. The phenol presumably would be lost as the sodium salt during the basic work-up. However, when the solvent was changed to THF at reflux the desired phosphonate **62**[91] was consistently formed in good yield.

Parallel results were observed when the reaction was attempted with *para*-nitrobenzyl alcohol (**63**). When the reaction was conducted in toluene at reflux, there was no peak corresponding to the desired phosphonate[92] detectable in the ^{31}P NMR spectrum. However, the desired phosphonate **64** could be isolated when the reaction was conducted in THF at reflux, although the yield was low (15%). This low yield might be attributed to the electron withdrawing nature of the nitro group, but problematic nitrene formation and subsequent reduction even at the attenuated reflux temperature of THF is the more likely culprit.[93] This hypothesis is supported by the success (*vide infra*) with another compound possessing an electron deficient arene.

To probe the impact of a different electron-withdrawing substituent on the scope of the reaction, another electron deficient benzylic alcohol was chosen. The carbomethoxy compound **65** was treated under the standard conditions. In spite of the electron deficient nature of the aromatic moiety, the C-P bond formation was facile, resulting in the desired phosphonate formation. These conditions also induced ester exchange to give the carboethoxy derivative **66**[94] which may have contributed to the modest yield of the isolated product.

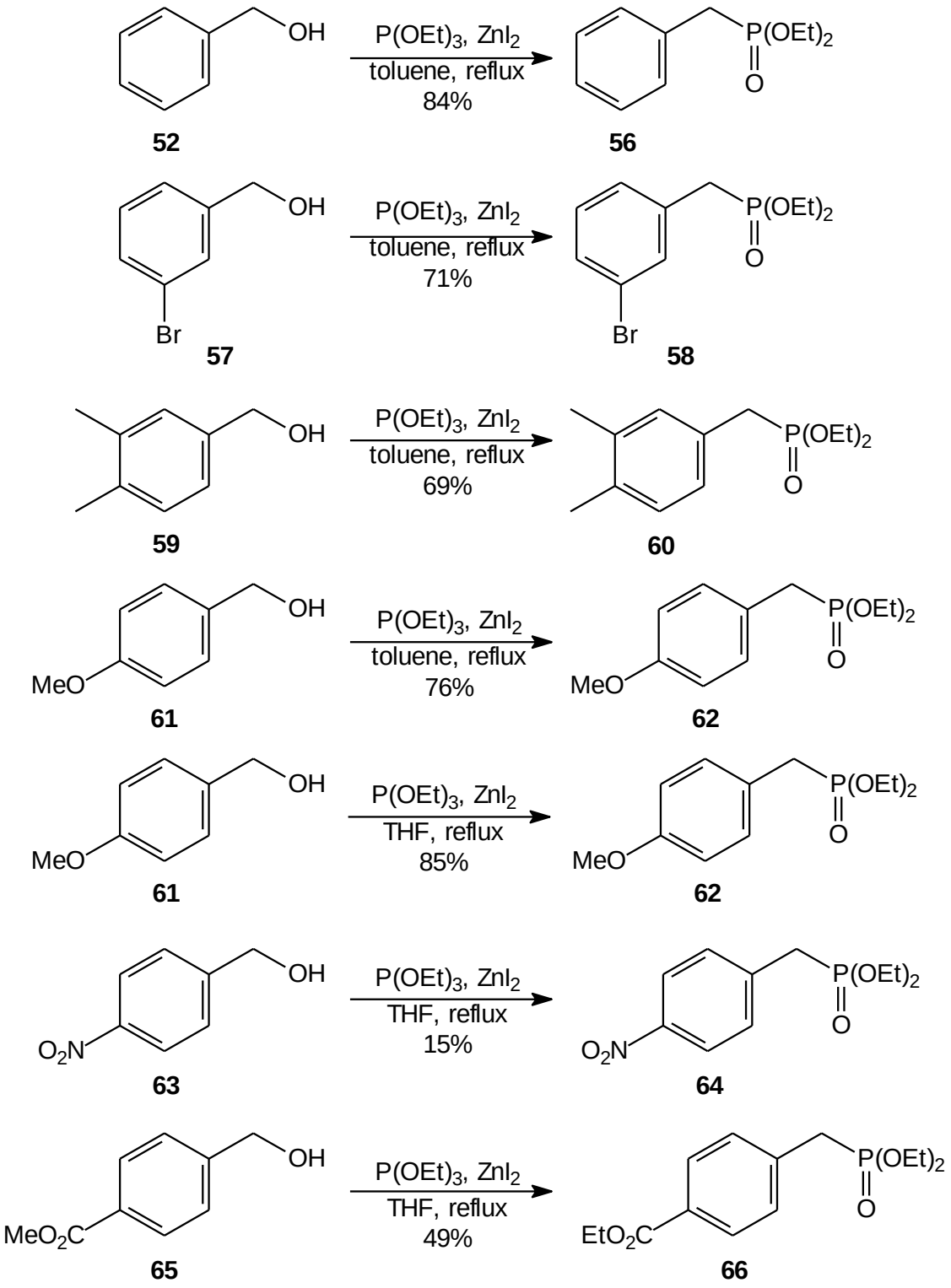


Figure 20. Zinc iodide mediated phosphonylation of benzylic alcohols.

Natural products such as the schweinfurthins and pawhuskins possess a phenolic substructure, and thus application of this methodology to protected phenols is of special interest to our research group. To probe the extent to which this one-flask phosphonylation procedure could be used on protected resorcinols we chose two substrates as models, the 3,5-di-MOM protected benzylic alcohol **67** and the di-TBS protected benzylic alcohol **69**. Both compounds were converted to their respective phosphonates, compounds **68**[95] and **70**,[96] but only in modest yields. In contrast, the mono-protected benzofuran **71** was successfully converted to the corresponding diethyl phosphonate **72** in better yield (64%), suggesting that compounds having only one protected phenol may undergo the desired transformation more effectively than those structures bearing a di-protected resorcinol. Furthermore, preparation of phosphonate **74** had proven problematic using the classical Arbuzov sequence[78] (Figure 8), providing evidence that the ZnI_2 mediated phosphonylation reaction is complementary to the Arbuzov reaction, at least in some instances.

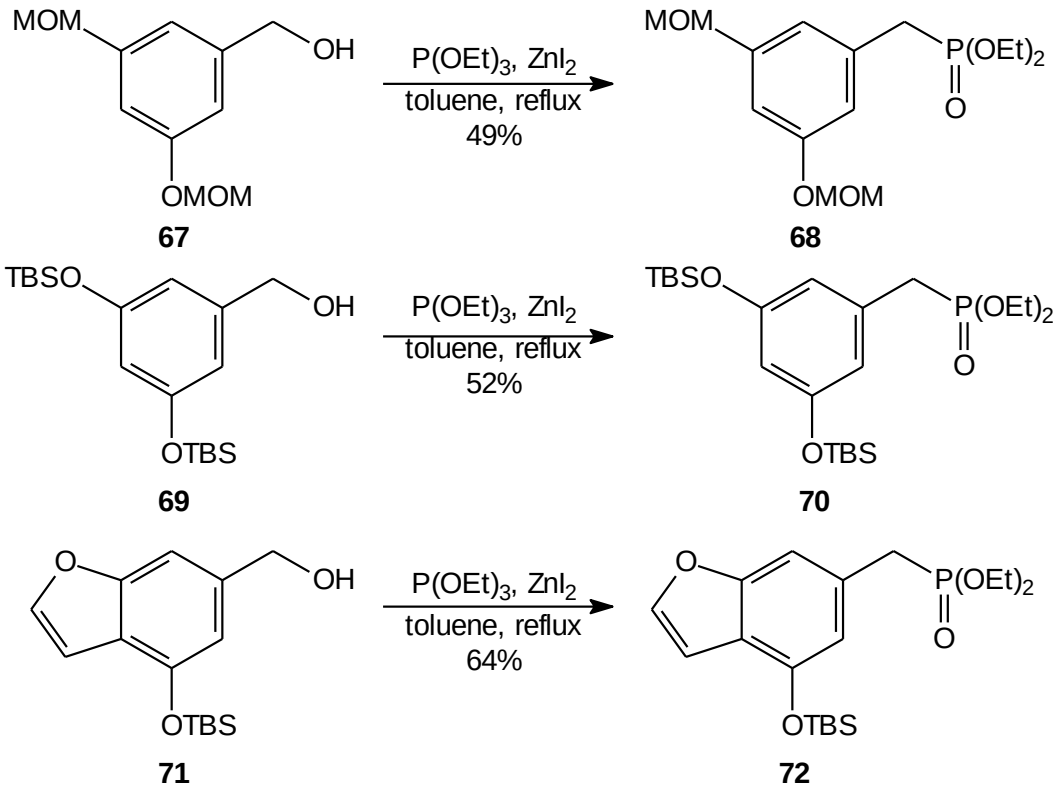


Figure 21. Phosphonylation of resorcinol and benzofuranyl alcohols.

In addition to zinc iodide, other Lewis acids were examined briefly (Figure 22). Sequences using both zinc bromide and aluminum triiodide were investigated. Zinc bromide proved to be effective for the conversion of benzyl alcohol (**52**) to diethyl benzylphosphonate (**56**), although in relation to the ZnI_2 conditions, the reactions were slightly attenuated in yield and longer reaction times were required. Aluminum triiodide was tested as well. It has been reported that allylic, benzylic, and tertiary iodides can be generated from the corresponding alcohols via treatment with AlI_3 in acetonitrile.[97] Unfortunately, attempts at forming the benzylic iodide and using it in the Arbuzov reaction *in situ* proved unsuccessful with the reaction conditions employed. This could be due to either failure to form the corresponding iodide due to

interactions with the phosphite, or if the benzylic iodide was formed, decomposition of the alkyl halide may have occurred more readily than the Arbuzov phosphonylation.

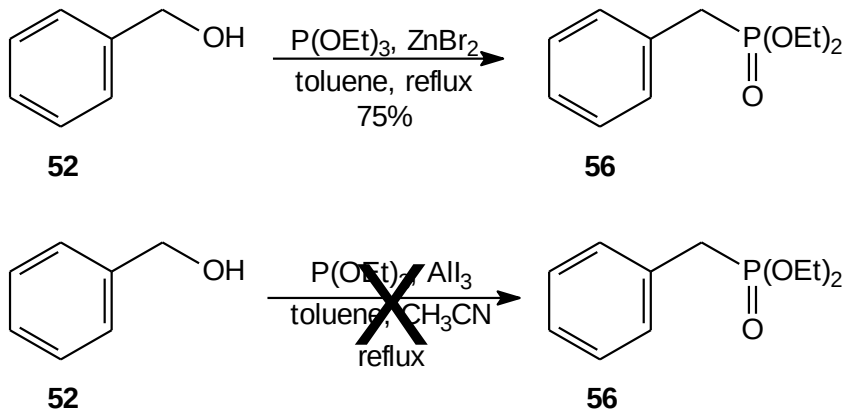


Figure 22. The use of alternative Lewis Acids for phosphonylation.

Given the success and generally attractive yields observed with the benzylic alcohols, exploration of other alcohols that may form relatively stable cationic systems was persued. Several isoprenoid phosphonates have been studied as inhibitors of isoprenoid metabolism,[98] and so the first compounds examined for applicability in this reaction were terpenoid allylic alcohols (Figure 10). Geraniol (**73**) was converted to the corresponding diethyl phosphonate[99] (**74**) in good yield when subjected to the standard reaction conditions. In contrast, the tertiary allylic alcohol linalool (**75**) gave a complex mixture under the standard conditions, although diethyl geranylphosphonate (**74**) could be isolated from the reaction mixture in modest yield. Both perillyl and cinammyl alcohols, **76** and **78** respectively, reacted smoothly under the standard reaction conditions to give the desired phosphonates **77** and **79**[100] in good yields, establishing the efficiency and attractiveness of using the ZnI_2 mediated conditions for phosphonate formation.

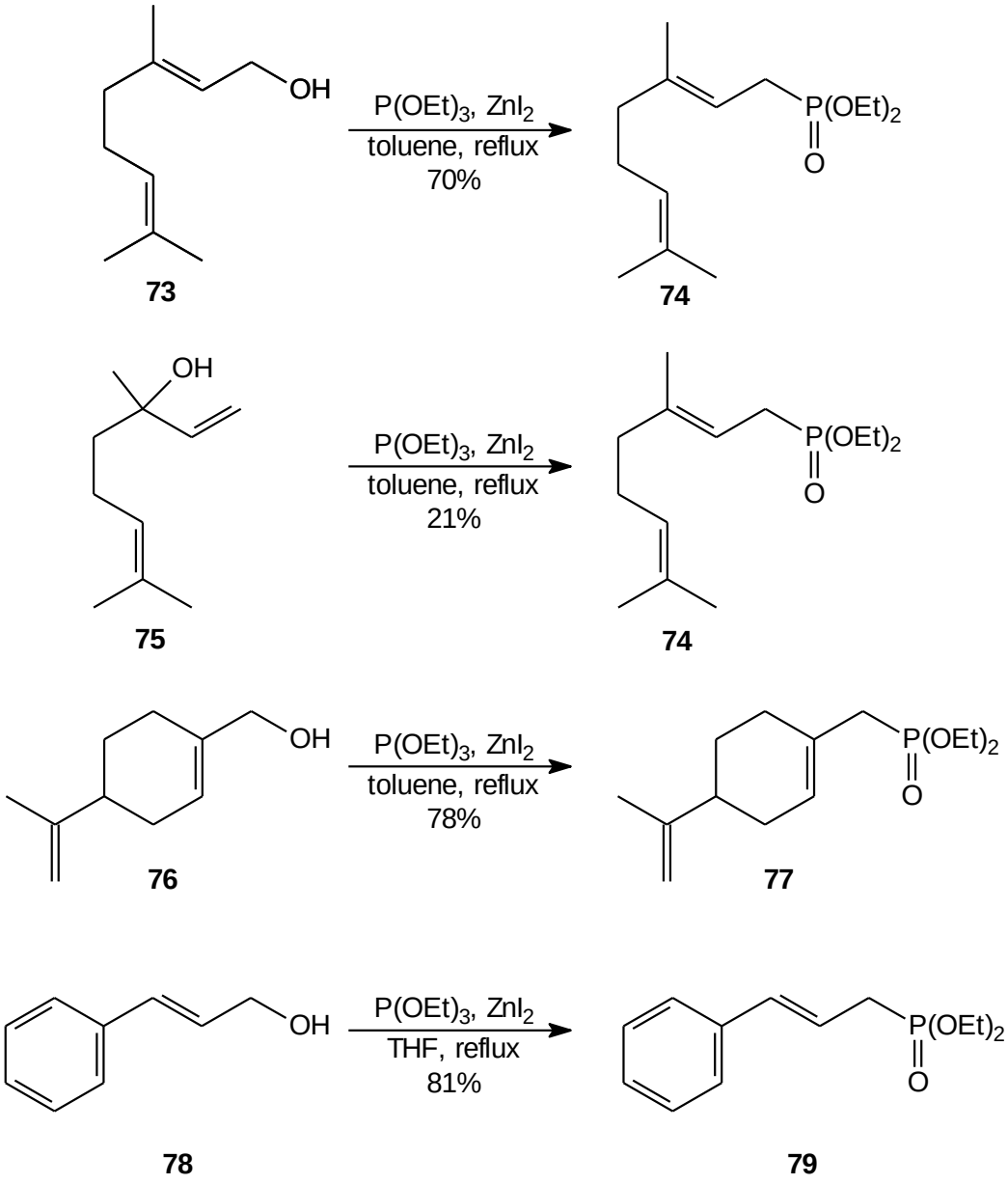


Figure 23. The conversion of allylic alcohols to the corresponding phosphonates.

While there is literature precedent[82-85] for the conversion of benzylic and allylic alcohols to other functional groups through zinc halide mediated reactions, the common assumption is that such transformations are only possible in systems that afford stabilized cationic species. Nevertheless, the simplicity of the reaction conditions employed and the general attractiveness of

the yields observed prompted the examination of simple primary alcohols as possible substrates for the system. Treatment of 3-phenyl propanol (**80**) under the standard reaction conditions did indeed provide a phosphonate product of the desired molecular weight, but the ^{1}H and ^{13}C NMR data proved inconsistent with the expected phosphonate (Figure 24). To resolve this conflict, an authentic sample of compound **83**[101] was prepared (Figure 25) via catalytic hydrogenation of the phosphonate **79**, and the NMR spectral data showed clear differences. The structure of the zinc iodide mediated reaction product of 3-phenyl propanol (**80**) was assigned as a mixed ester of ethyl phosphonate, i. e. compound **83**, on the basis of its spectral data. While the methylene groups of the two esters overlap in the ^{1}H NMR spectrum, the corresponding carbon resonances are distinct in the ^{13}C NMR spectrum, indicative of the absence of symmetry around the central phosphorus atom. Furthermore, the presence of a methylene group directly bonded to phosphorus was apparent in the ^{13}C NMR resonance at 18.3 ppm with a coupling constant of 142 Hz. The dialkyl phosphate **82** also was isolated in a 28% yield from the reaction mixture. Assignment of this structure was made primarily on the basis of the ^{31}P NMR resonance at 7.6 ppm, which is typical for a dialkyl phosphite. Furthermore, inspection of the ^{13}C NMR spectrum indicated the presence of two unsymmetrical carbons coupled to phosphorus at 64.1 and 61.4 ppm.

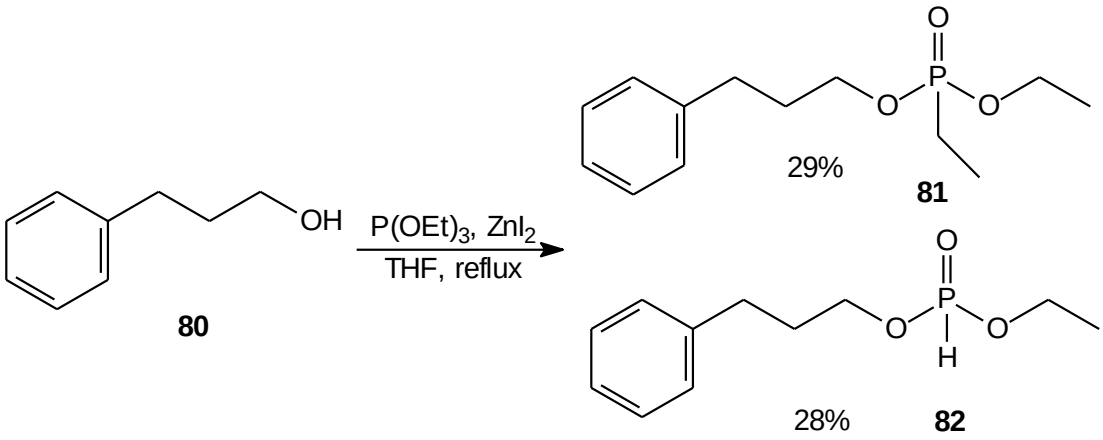


Figure 24. The attempted synthesis of an aliphatic phosphonate.

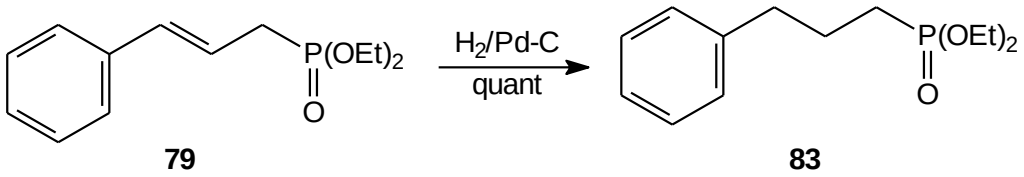


Figure 25. Synthesis of an aliphatic phosphonate via catalytic hydrogenation.

To explore further this transformation, a parallel reaction using citronellol (**84**) as the aliphatic alcohol was attempted. In agreement with the results found with 3-phenylpropanol (**80**) the citronellyl example gave the asymmetric phosphonate **85** as expected. Assignment of this structure was made primarily on the ^{1}H NMR and ^{31}P NMR spectra. The 1H NMR spectra showed a clear doublet of triplets at 1.16 ppm, with a coupling to phosphorus (19.8 Hz) being evident. Furthermore, comparison with the spectral data for diethyl ethylphosphonate showed

clear similarities and the ethylphosphonate portions of both compounds could be clearly identified.

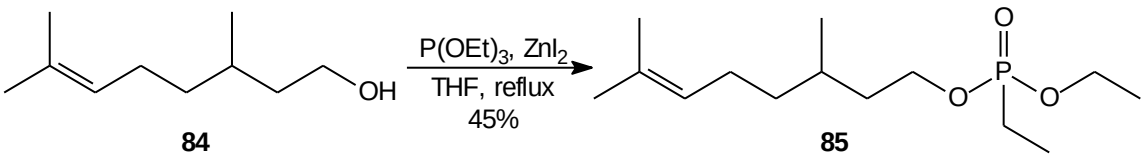


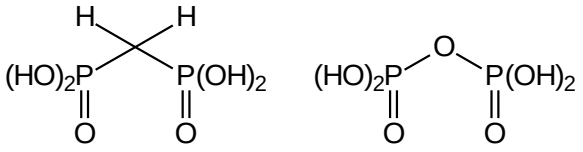
Figure 26. Synthesis of the asymmetric citronellyl phosphonate.

To summarize, a broad range of benzylic and allylic alcohols has been converted to the corresponding phosphonates via reaction with triethyl phosphite and the Lewis acid, zinc iodide. This procedure provides a convenient, one flask alternative to the classical Arbuzov reaction in similar systems. In at least one instance, the reaction conditions have been shown to be effective with a substrate that proved to be difficult to synthesize using the standard Arbuzov conditions. Moreover, zinc bromide also has proven effective as a Lewis acid in the transformation. The comparative ease of the one-flask synthesis coupled with a relatively simple work-up, inexpensive reagents, and the determination that allylic and benzylic systems undergo this transformation in at least modest, and often attractive yields. makes this reaction of potentially broad utility in organic synthesis.

CHAPTER III

BISPHOSPHONATES AS INHIBITORS OF GGDPS: SYNTHESIS AND BIOLOGICAL EVALUATION

Geminal bisphosphonates **86** are an important class of organophosphorus compounds, and have garnered a great deal of attention in the literature. Their roles as structural analogs of pyrophosphate **87** are well documented, and continue to be of interest.[35] The geminal bisphosphonate structure is defined by the formal replacement of the P-O-P bond linkage with a P-C-P bond linkage (Figure 27). The introduction of this methylene linker provides enhanced metabolic stability, as bisphosphonates are not recognized by pyrophosphatase enzymes and are resistant to enzymatic hydrolysis.[102] Furthermore, introduction of the methylene unit allows for structural modifications that would not be possible in the parent pyrophosphate structure.

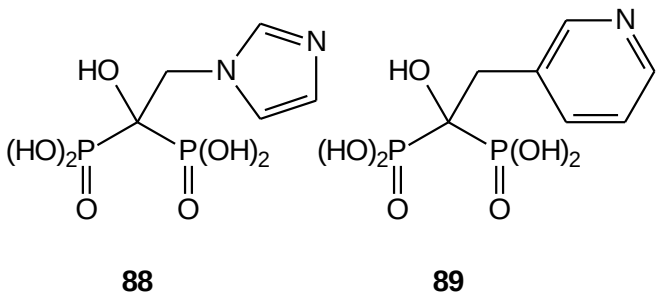


86 **87**

Figure 27. Phosphonate and pyrophosphate structures.

Geminal bisphosphonates have a long history in a variety of applications. Historically, they have been used as chelating agents,[7] corrosion inhibitors,[8,9] water softeners,[10] and as

stabilizers in industrial oxidation processes.[11] More recently bisphosphonates have been used clinically for the treatment of bone-related disease. Examples of clinically useful compounds include the nitrogenous bisphosphonate zoledronate **88** which has been used in the treatment of osteoporosis as well as multiple myeloma and a variety of cancers,[35] and risedronate **89** (Figure 2) which also has been used in the treatment of osteoporosis.[103] In addition to the current clinical applications in which bisphosphonates are employed, specific bisphosphonates have shown promise as potential cancer therapeutics[28] and as anti-parasitic agents.[29,30] Finally, some bisphosphonates have been shown to inhibit the growth of a variety of malignant cell lines[31,32] and to activate γδ-T cells.[33]



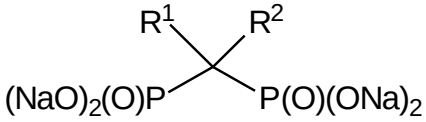
88 **89**

Figure 28. Two clinically implemented nitrogenous bisphosphonates.

There has been speculation that enzyme-specific inhibitors can be designed for key steps in the mevalonate pathway, and indeed bisphosphonates have been developed as enzyme-specific inhibitors of squalene synthase,[104,105] isopentyl pyrophosphate isomerase,[106,107] farnesyl diphosphate synthase,[108,109] and other important enzymes involved in isoprenoid metabolism.[110] The primary enzymatic target of the clinically employed nitrogenous α-hydroxy bisphosphonates has been identified as farnesyl diphosphate synthase (FDPS).[108,109] This specific enzyme is responsible for the cellular production of farnesyl diphosphate, and is a key point of divergence

in the mevalonate pathway. Although the enzymatic target of these nitrogenous α-hydroxy bisphosphonates has been established, the mechanisms leading to the observed cellular effects are not understood completely. Clinical bisphosphonates such as risedronate and zoledronate cause depletion of cellular supplies of farnesyl pyrophosphate through inhibition of FDPS. This attenuation of cellular FPP levels has a direct impact on protein farnesylation, but it also results in a depletion of cellular levels of geranylgeranyl diphosphate (GGPP). It is not known whether the cellular effects observed result from the direct impact on farnesylation, or from the impact on the downstream process of geranylgeranylation.[111-115] Given the large number of cancers that exhibit aberrations in prenylated GTPases,[116] more extensive investigations into the potential of bisphosphonates as anti-cancer therapeutics are warranted.

Our research group has reported the synthesis of a range of mono- and dialkyl isoprenoid bisphosphonates (Figure 29) that exhibit selective inhibition of the enzyme geranylgeranyl diphosphate synthase over the previously discussed enzyme FDPS.[117,118,119] The enzyme GGDPS is responsible for the elaboration of FPP to GGPP. This is an important target because GGPP is the essential precursor for all protein geranylgeranylation, as well as a critical precursor to many larger isoprenoids.



Compound	R^1	R^2	*In vitro* GGDPS IC_{50} (μM)	Cellular GI_{50} (μM)
138	Geranyl	Geranyl	0.2	25
206	Geranyl	H	1	25
54	Neryl	Neryl	6	25
55	Neryl	Geranyl	7	25
171	Neryl	Prenyl	60	>50
166	Neryl	H	>100	>50

Figure 29. GGDPS *in vitro* and cellular activity of selected isoprenoid bisphosphonates.[118]

My research into bisphosphonates as potential inhibitors of GGDPS fall into two arenas, the design and synthesis of α-heteroatomic bisphosphonates and the design and synthesis of bisphosphonates having an aromatic substructure. Each topic will be presented in turn in the following sections.

Synthesis of Heteroatomic Bisphosphonates

The clinically used bisphosphonates such as alendronate **90** and minodronate **91** (Figure 30) possess a hydroxyl functionality that is on the carbon adjacent to the phosphorus atoms. There has been a substantial body of work to probe the importance of this functionality, especially as it relates to the inhibition of FDPS and affinity for the bone matrix.[120] This α-hydroxy functionality, colloquially described as a key feature of the "bone hook", has been

demonstrated to have an important impact on the affinity of a bisphosphonate for bone as demonstrated in hydroxyapatite assays.[121] While the impact of the hydroxyl group has been probed in FDPS enzyme assays, there is still much uncertainty as to how this modification may impact other potential target enzymes in the mevalonate pathway. Much less is known about enzymes in the mevalonate pathway other than FDPS, and it is our intent to further probe the effect of an analogous modification on known GGDPS inhibitors. Specifically, it may be revealing to install a hydrogen bond donor/acceptor or acceptor into the alpha position of the potent and selective GGDPS inhibitor, DGBP (**92**, Figure 30), and to determine the impact of this modification on biological activity.

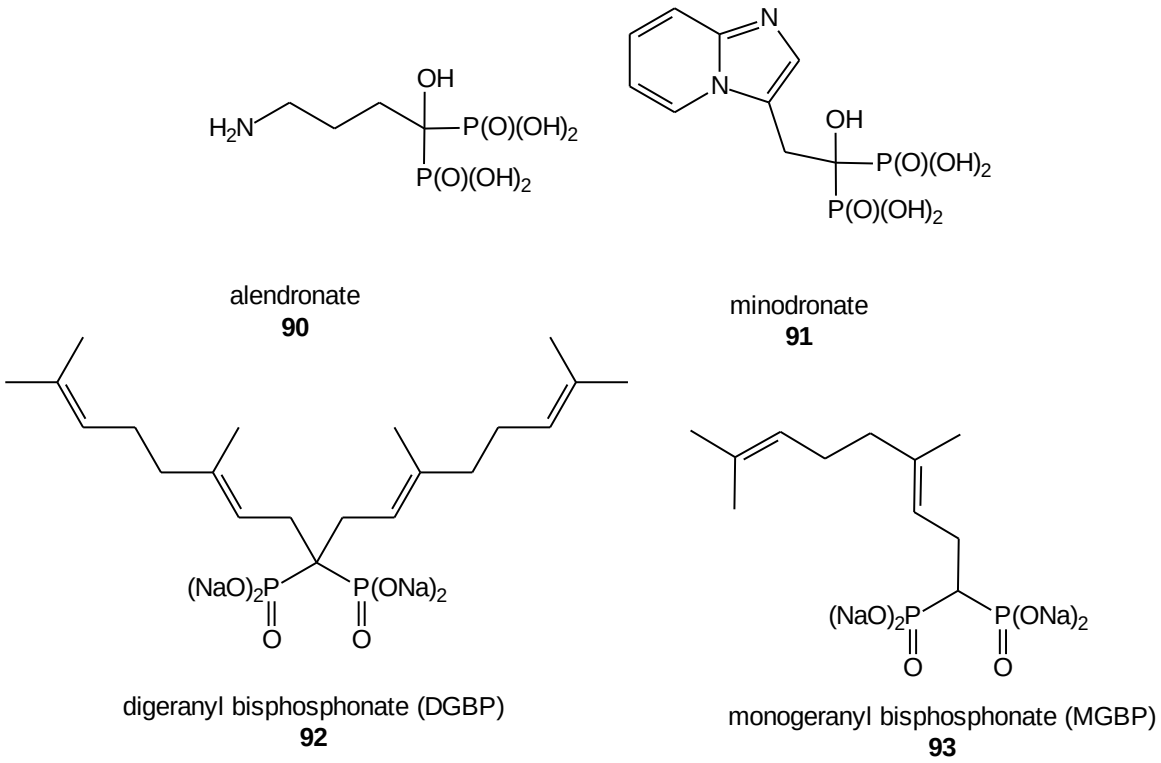


Figure 30. Nitrogenous and non-nitrogenous bisphosphonates.

There are several aspects of the structure activity relationship that can be probed by this modification. First, the effect of the hydrogen bond donor or acceptor on the efficacy of the compound as it relates to the parent GGDPS could be studied. There are a myriad of examples where the presence of a hydrogen bond donor or acceptor imbues or greatly enhances activity of a given compound[122, 123] or improves drug likeness as stated in "Lipinski's rule of five."[124] Second, because the nitrogenous FDPS inhibitors contain a hydrogen bond donor/acceptor at the alpha position, a parallel modification of our GGDPS inhibitor, DGBP (**92**), should shed light on the impact that introduction of the heteroatom would have on selectivity for this enzyme. Third, it has been shown that modification of the alpha carbon has an impact on the pKa of the phosphonic acid.[125-129] Introduction of an electron withdrawing heteroatom also should provide an isoprenoid bisphosphonate with a pKa more closely approximating that of the parent pyrophosphate moiety.[62] This may result in a more pronounced impact on the target enzyme, GGDPS, as well as a potential impact on cellular permeablility.

At first inspection the synthesis of O-geranyl analogs of DGBP should be straightforward. Retrosynthetic analysis would predict that using a Williamson ether synthesis[130] from the geranyl α-hydroxy bisphosphonate using geranyl bromide as the alkylating agent should provide the desired compound directly. Unfortunately, this approach is confounded by the phosphonate-phosphate or P-O rearrangement (Figure 31). Any exposure of the α-hydroxy bisphosphonate to basic conditions would be expected to result in this rearrangement[131-135]and we have observed that even under mildly acidic conditions activated bisphosphonates can give the rearranged product. This is evidenced by our attempted conversion of acyl phosphonate **98** to the corresponding α-hydroxy bisphosphonate, which cleanly gave the rearranged product, phosphonophosphate **99** (Figure 32).

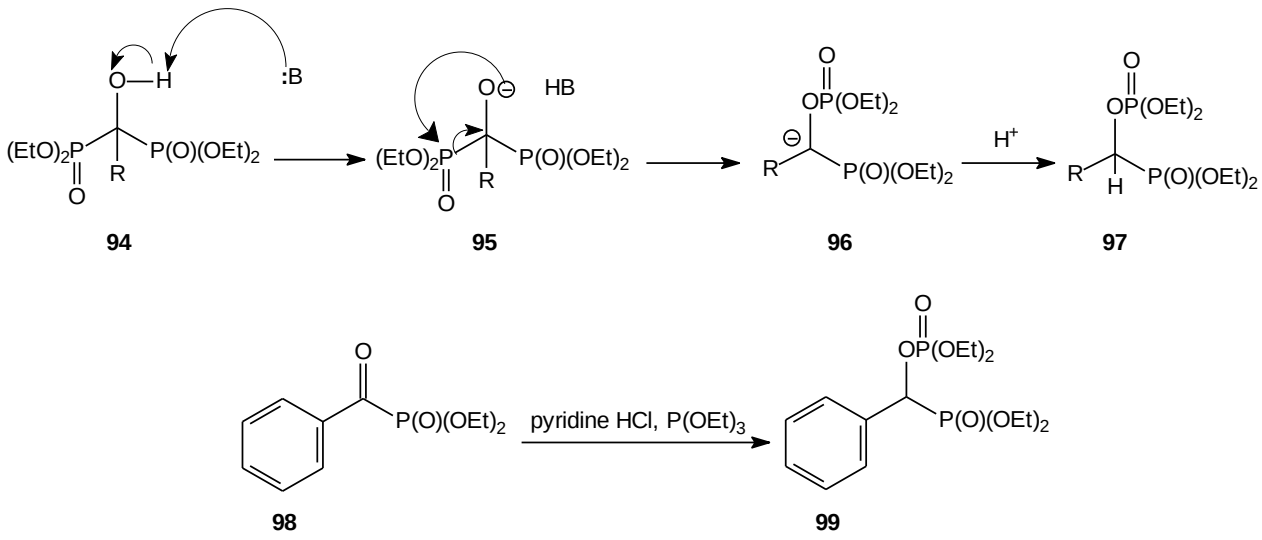


Figure 31. The phosphonate-phosphate rearrangement.

An alternative to this disconnection would be to first install the ether functionality, alkylate the alpha carbon via anion formation, and then conduct a final alkylation with geranyl bromide. This approach also has pitfalls. The Wittig rearrangement (Figure 32) is well documented in a variety of systems having an an allylic ether, including phosphonates.[136,137] After anion formation, the carbon nucleophile can attack the sp^2 hybridized carbon of the allylic alkene providing the C-alkylated rearranged product with a free hydroxyl group (e.g. compound **103**).

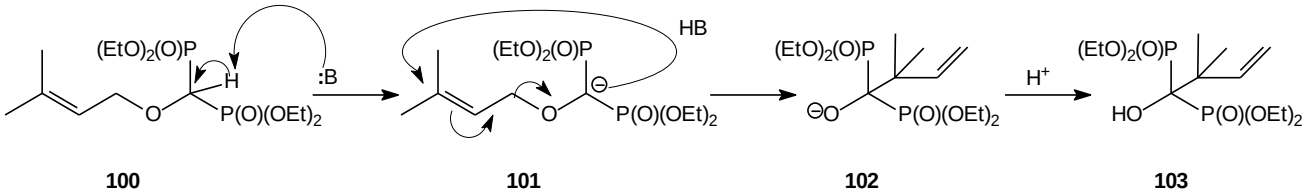
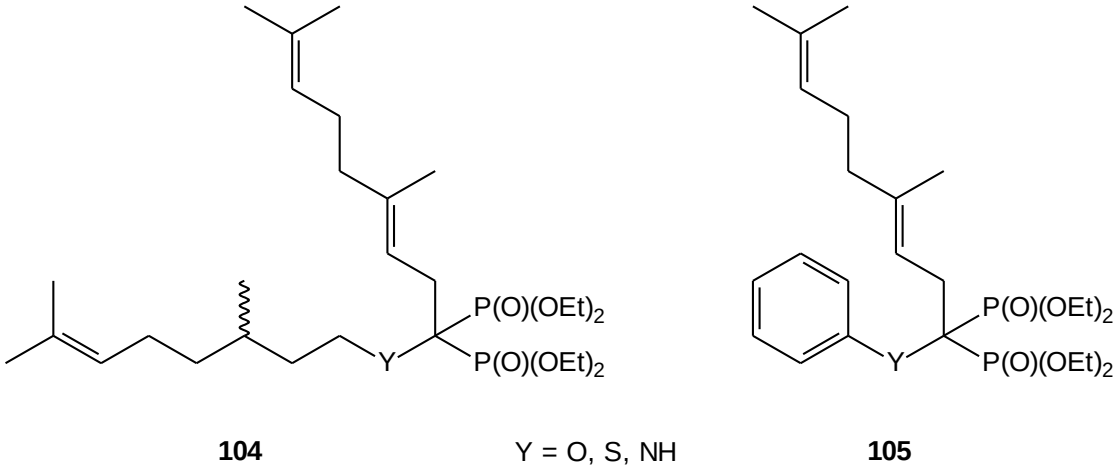


Figure 32. The Wittig rearrangement.

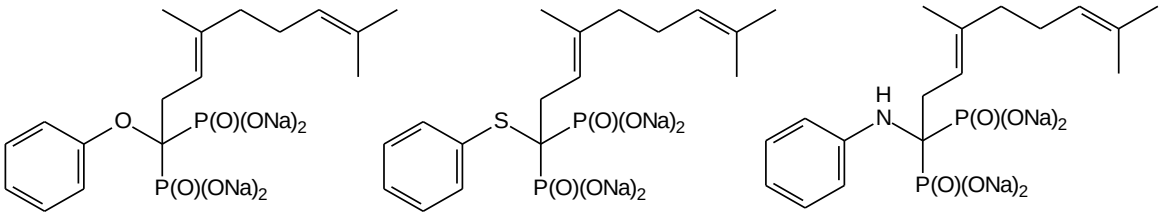
We have actively engaged in synthetic approaches designed to circumvent these complications. For one, the phosphonate-phosphate rearrangement can be sidestepped by changing the order of alkylation. The P-O rearrangement is not observed in ethers. Thus, if the oxygen is alkylated first, this type of rearrangement can be avoided. Next, the Wittig rearrangement can be prevented by two approaches. First, removal of the allylic alkene entirely, as in citronellyl analog **104**, should avoid the functionality needed for the rearrangement to proceed (Figure 33). This is of course, not directly analogous to the digeranyl parent compound, but in theory the geranyl citronellyl bisphosphonate could be synthesized to provide a model for direct comparison. Another approach to circumventing the Wittig rearrangement would be to use an aryl group in place of one of the geranyl chains as in compound **105**. This approach is attractive because the phenyl geranyl analogs should be readily available, making a SAR analysis relatively easy to perform. It is proposed that introduction of the aromatic moiety and the related stability of the aromatic substructure should inhibit the Wittig rearrangement. Given the great energy cost of breaking aromaticity, alkylation of the aromatic ring would be unlikely.



104 Y = O, S, NH **105**

Figure 33. Proposed structures for avoiding rearrangements.

The first series of α-heteroatomic bisphosphonates that were targeted were the phenyl ether **106**, the phenyl thioether **107**, and the aniline derivative **108** (Figure 34). This would seem a reasonable initial goal because it would allow exploration of both hydrogen bond donating and hydrogen bond accepting effects as related to *in vitro* inhibition of GGDPS or *in vivo* cellular assays.



106 **107** **108**

Figure 34. Heteroatomic bisphosphonates.

One approach to the synthesis of the phenyl ether **108** (Figure 35) was begun from the commercially available tosyl chloride (**109**), which upon treatment with sodium azide in acetone/water, provided the known tosyl azide (**110**) in very good yield (92%).[138] Next, tetraethyl methylenebisphosphonate (TEMBP) was treated with base to facilitate anion formation, and subsequently treated with tosyl azide, providing the diazobisphosphonate[139] **111** in acceptable yield. (Care was taken throughout to avoid contact with metals or sharp impact due to the potentially explosive nature of the diazo and azide compounds.) Diazobisphosphonate **111** was treated with phenol and catalytic copper (II) triflate in toluene at reflux overnight,[140] providing the target phenyl ether **112** in modest yield (35%). One might assume that treatment of this material with base followed by addition of geranyl bromide as the reactive electrophile would result in formation of the desired bisphosphonate **113**. Unfortunately, all attempts at this process gave a complex mixture of products that proved difficult to separate and had no clear major component.

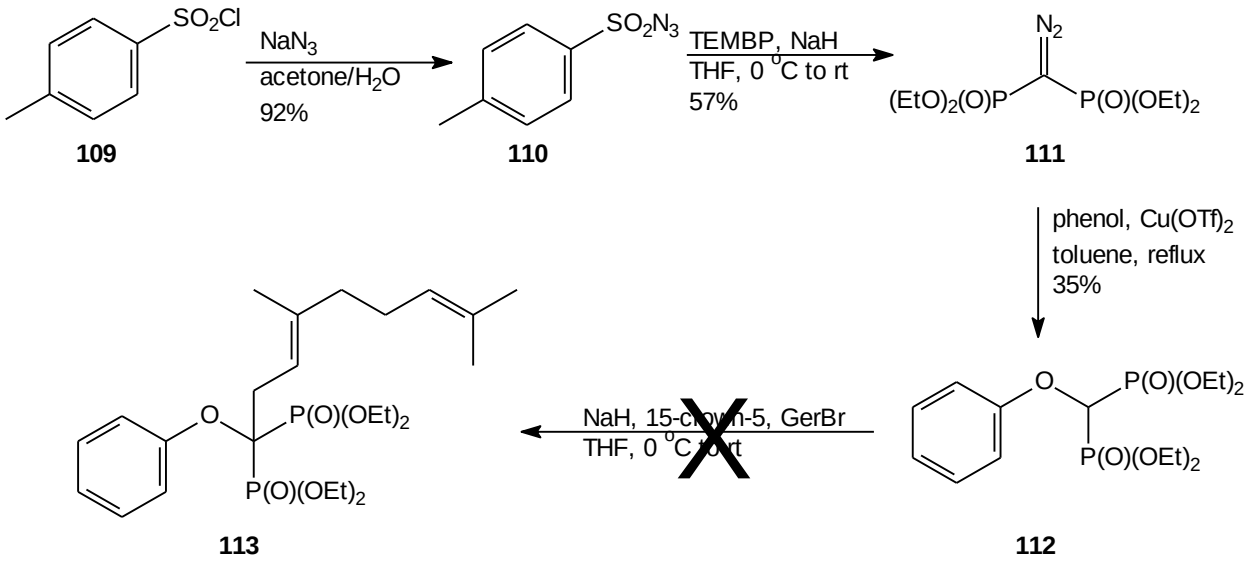


Figure 35. The attempted synthesis of phenyl ether **113**.

Given these results, attention was turned toward the synthesis of the thioether **23** (Figure 10). The thioether would provide a parallel example to that of the phenyl ether and yet would still provide an example that had only hydrogen bond accepting capacity. Tetraethyl methylenebisphosphonate (**114**) was treated with NaH, followed by trapping of the anion with the electrophile diphenylsulfide.[141] This transformation proceeded smoothly and provided the phenyl thioether **115** in good yield. Unfortunately, treatment of the resultant thioether with base and subsequent treatment with geranyl bromide failed to provide the desired compound **116**.

In an effort to circumvent the apparent complications that present themselves when attempting to alkylate bisphosphonates having either an α-alkoxy or α-alkylthio functional group, a new approach was pursued. Given the success with phenyl disulfide as an electrophile in the previous reaction, it seemed reasonable to introduce a thiophenyl group on the previously described mono-alkyl bisphosphonate **117**. Unfortunately, attempts to bring about this transformation failed to provide the desired compound **118**.

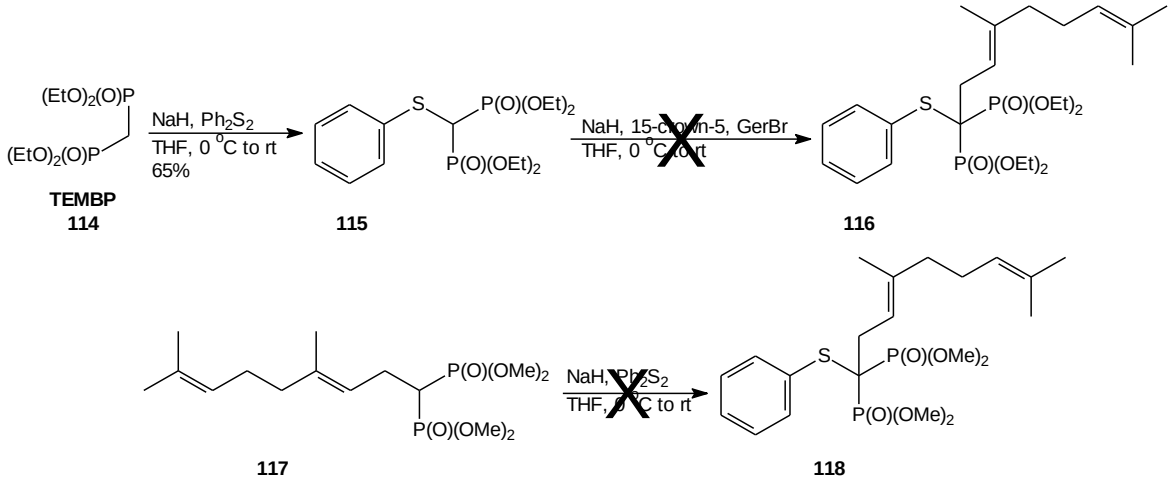


Figure 36. The attempted thioether syntheses.

The synthesis of the aniline derivative also has proven to be elusive (Figure 37). In a reaction sequence parallel to that of the phenyl ether, diazobisphosphonate **111** was treated with aniline (**120**) and catalytic copper (II) triflate in toluene at reflux[140] to provide the aniline bisphosphonate **119** in a manageable yield (57%). Given the expense of the copper reagent, an alternative approach to the synthesis of this compound was implemented utilizing a one-flask condensation[142] of aniline (**120**) and diethyl phosphite with triethyl orthoformate. This reaction gave the desired compound in a superior yield (76%) to that of the carbene insertion reaction, and also avoids use of diazo intermediates. With the aniline derivative **119** in hand, alkylation with geranyl bromide was attempted. The desired compound was not isolated, but instead the phosphonophosphoramidate rearrangement product **122** was isolated in 32% yield. The phosphonophosphoramidate was fully characterized by HRMS, and ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy. The ^{31}P NMR spectrum showed a clear doublet of doublets with matched coupling constants, and the magnitude of the coupling, as well as the chemical shift of the

resonances, were consistent with those reported in similar systems.[143] The phosphonophosphoramidate was subjected to the standard hydrolysis conditions[144] with TMSBr and collidine, followed by aqueous NaOH, but the reaction failed to provide the pure tetrasodium salt.

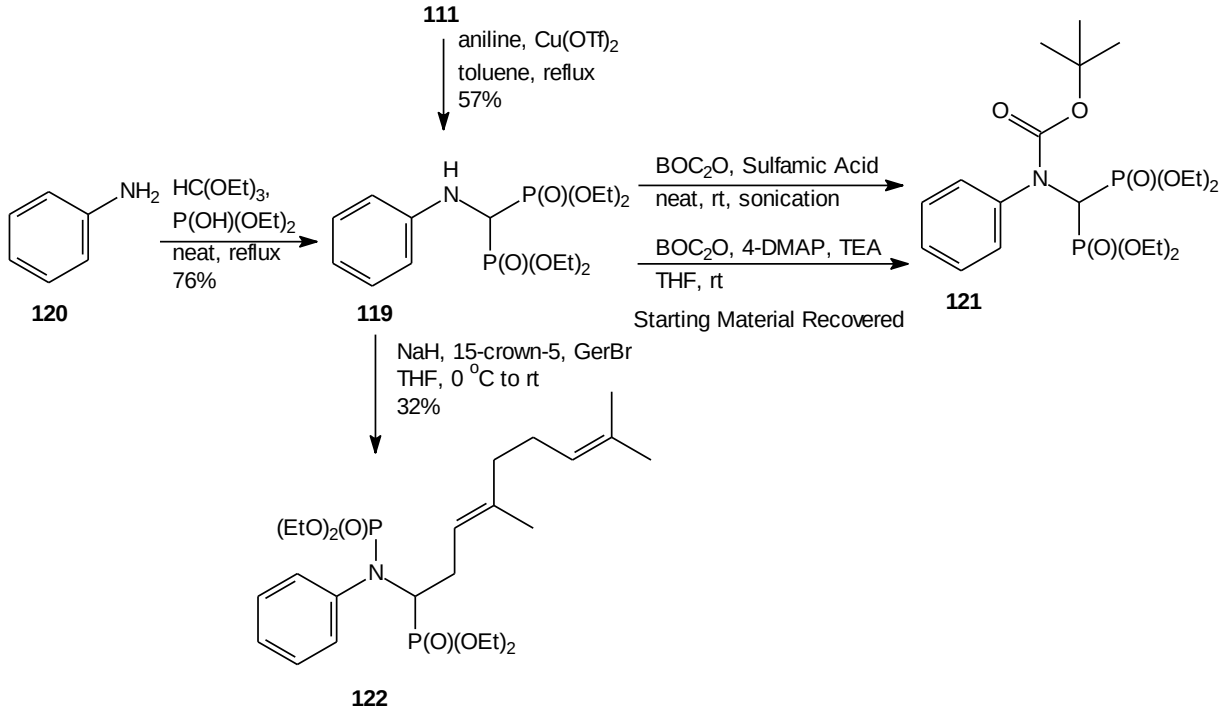
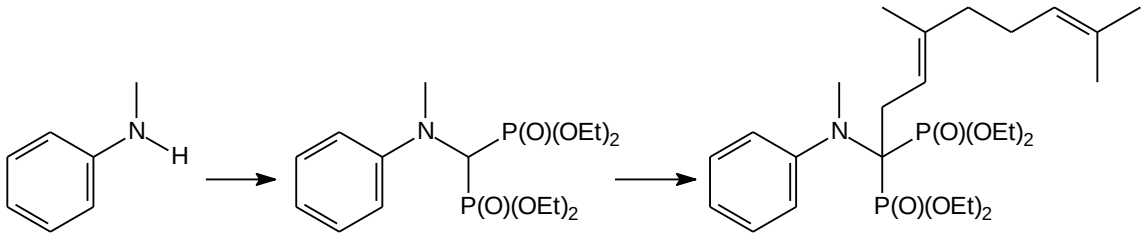


Figure 37. The attempted synthesis of aniline and BOC protected aniline bisphosphonates.

Given the clearly identified rearrangement that occurs when alkylation of the unprotected aniline bisphosphonate is attempted, two approaches were developed to overcome this obstacle. First, BOC protection of the aniline was attempted. Neither the standard protection conditions, nor the more exotic conditions using sulfamic acid[145] gave the desired *N*-BOC protected material **121**. Instead more than 95% of the starting material was recovered in both instances. Second, the N-methyl analog was synthesized using the aforementioned condensation reaction. Starting

with N-methylaniline (**123**), the condensation proceeded as expected to provide the target material **124** in 20% yield. All attempts at alkylation of this bisphosphonate precursor proved unsuccessful (Figure 38). Using several different conditions, alkylation could not be affected and only starting material was recovered.



123 **124** **125**

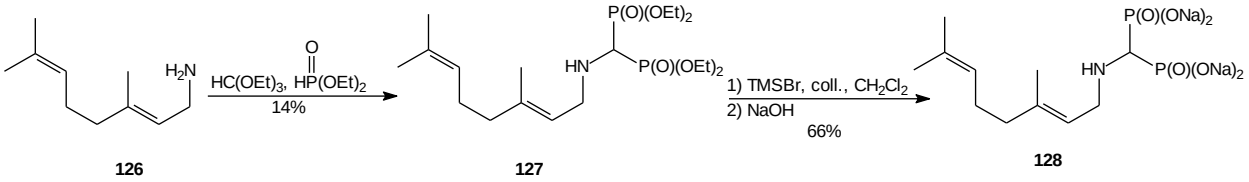
Trial	Base	Solvent	Temperature	Yield
1	KHMDS	Toluene	-78 °C to rt	S. M. recovered
2	NaH	THF	0 °C to rt	S. M. recovered
3	NaH	THF	0 °C to reflux	S. M. recovered
4	NaH	DMF	0 °C to rt	S. M. recovered
5	KHMDS	THF	0 °C to rt	S. M. recovered
6	nBuLi	THF	0 °C to rt	S. M. recovered
7	NaH	THF	Reflux	S. M. recovered

Figure 38. The attempted alkylation of aniline derivative **124**.

As a result of the lack of success and general difficulty encountered in our original approach to the design and synthesis of α-heteroatomic bisphosphonates, an alternative approach was needed. We had initially designed the α-heteroatomic compounds as analogs of the parent

DGBP because of the compound's superior *in vitro* activity and selectivity. Inspection of the data shows that while DGBP is the most active of the series (Figure 40), the monogeranyl bisphosphonate (MGBP) **93** also exhibits pronounced potency and selectivity. As such, we modified our targets toward the synthesis of α-heteroatomic analogs of MGBP **93** (Figure 30).

Given the success of the orthoformate condensation reaction in the work described above it seemed wise to pursue the N-geranyl analog **128** to begin this series. The commercially available geranylamine (**126**) was treated with diethyl phosphite and triethyl orthoformate at reflux to give the tetraethyl bisphosphonate ester **127** in 14% yield. Hydrolysis using standard conditions proved straightforward and provided the tetrasodium salt **128** in fair (66%) yield (Figure 39). This compound has been submitted to our collaborators and is awaiting bioassay. These results should provide insight into the key points of interest established for the project and guide further efforts in the synthesis of α-heteroatomic bisphosphonates.



126 **127** **128**

Figure 39. Synthesis of the α-heteroatomic bisphosphonate **128**.

Aromatic Analogues of Mono- and Digeranyl Bisphosphonate

To explore further the chemistry and biology of isoprenoid bisphosphonates we have designed, synthesized, and assayed a number of aromatic analogs of mono- and digeranyl bisphosphonates[146] (Figure 40). The monoalkyl analogs incorporate a side chain that is a

structural analogue of the monogeranyl compound **93** (pg 30), while the dialkyl analogs of DGBP (**92**) have both a geranyl chain and an aromatic analog of the geranyl substructure.

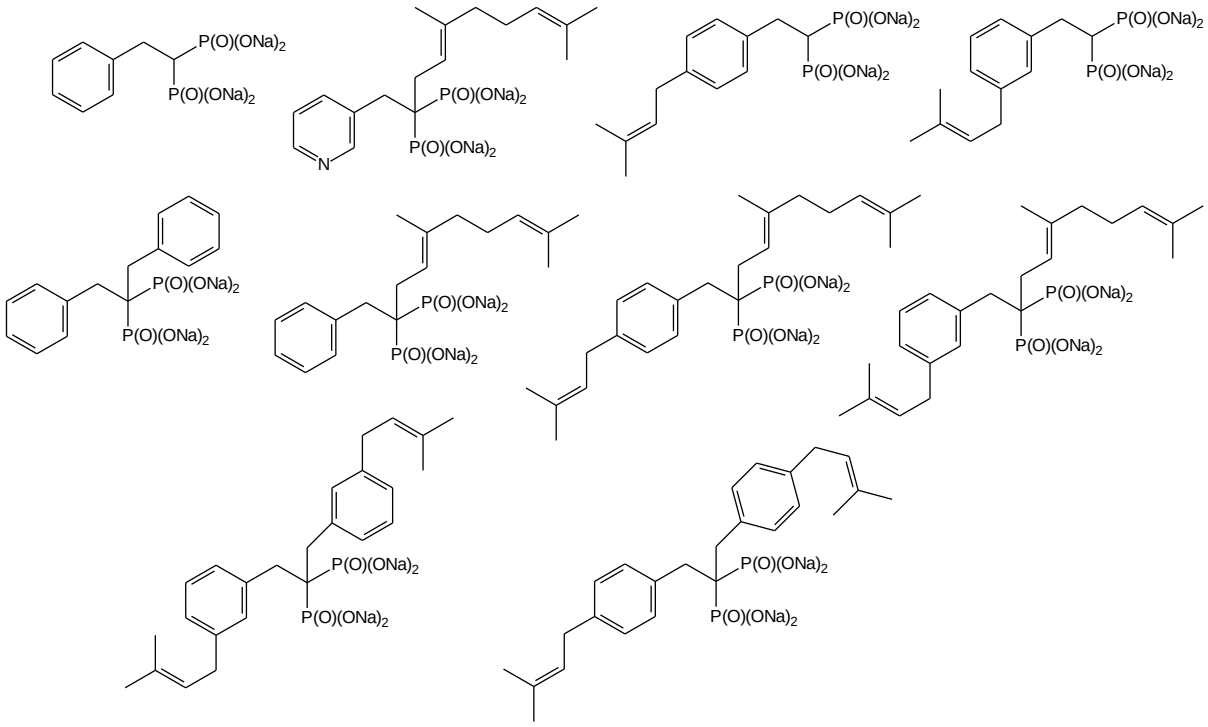


Figure 40: Proposed aromatic analogs of MGBP and DGBP.

There are several motivations for including an aromatic moiety into the structure of these potential inhibitors of GGDPS. First, many of the most important inhibitors of FDPS are nitrogenous bisphosphonates with an aromatic substructure, including zoledronate and risedronate. It is unknown whether the introduction of an aromatic substructure into a potential inhibitor will result in a compound that retains the specificity for the enzyme GGDPS, or provide inhibition of the enzymatic target of the clinical aromatic bisphosphonate, FDPS. Second, it is of interest to probe the impact of the more sterically demanding aromatic moiety on efficacy for inhibition of the enzyme GGDPS. Third, there exists evidence that the high charge-to-mass ratio

in salts of the bisphosphonic acids at physiological pH results in difficulty transversing the cell membrane.[147] The aromatic analogs reported here are more lipophilic than their parent compounds, the mono- and digeranyl bisphosphonates. This increase in lipophilicity may result in the compound crossing the cell membrane more easily, diminishing or eliminating the need for the use of a bisphosphonate prodrug.[148] Fourth, bisphosphonate compounds that contain isoprenoid olefin isomers can display dramatically differing biological activity (Figure 29), a fact well established in the literature.[118] The use of the aromatic moiety in place of the olefin eliminates potential isomerization or transposition of the alkene *in vivo*. Finally, the crystal structure of digeranyl bisphosphonate in the active site of the enzyme provides some indication that the larger π system of an aromatic ring may lead to more favorable stacking interactions with the residues tyrosine 205 and phenylalanine 175 in the active site of GGDPS.[149] If this were the case, an aromatic analogue could be a more potent inhibitor of this enzyme.

This collection of aromatic bisphosphonates was synthesized using parallel reaction sequences. For example, both compounds **133** and **135** were synthesized from commercially available *m*-bromobenzyl alcohol (Figure 41). The alcohol **129** was protected by treatment with TBSCl and imidazole to provide the silyl ether **130** in very good yield (90%). The resulting silyl ether undergoes halogen-metal exchange upon treatment with *n*–BuLi and subsequent reaction with prenyl bromide readily affords the alkylated product. Deprotection upon treatment with TBAF then gives the alcohol **131** in 72% yield over 3 steps. Initial attempts at bromide formation via reaction of alcohol **131** with PBr_3 gave unacceptable results. Instead, bromide formation was achieved cleanly by formation of the mesylate followed by treatment with LiBr to form the target benzylic bromide. Without additional purification this material was moved forward in the synthesis as first isolated. Thus, tetraethyl methylenebisphosphonate was treated with NaH and the resulting anion was treated with the intermediate benzylic bromide to provide the desired bisphosphonate **132** in 47% yield over 3 steps. Hydrolysis of bisphosphonate **132** gave the tetrasodium salt **133** in good yield (90%). Synthesis of the dialkyl bisphosphonate **134** was achieved by alkylation of compound **132** with geranyl bromide. Ester hydrolysis through

reaction with TMSBr to form the tetrasilyl ether followed by final treatment with sodium hydroxide provided the bisphosphonate **135** salt in 88% yield over two steps.

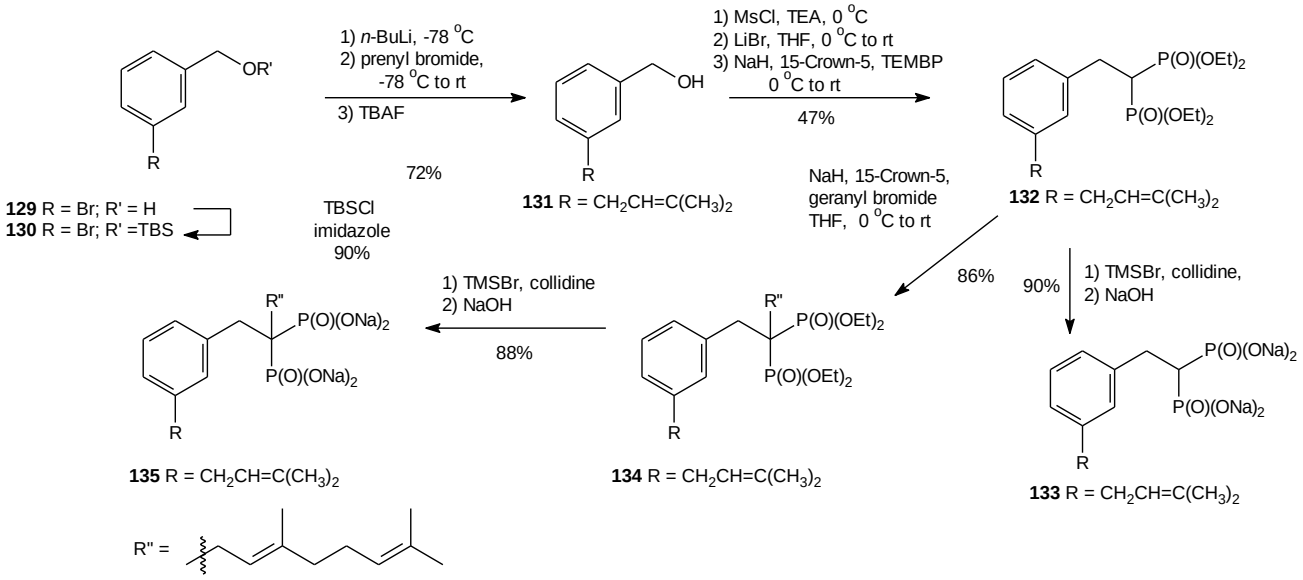


Figure 41: Synthesis of *m*-prenylated analogues.

The isomeric *para*-substituted compounds were obtained through a similar series of reactions as shown in Figure 42. These reactions proceeded in a fashion parallel to those described above, and gave the monoalkyl bisphosphonate salt **140** and the dialkyl bisphosphonate **142** in good yields.

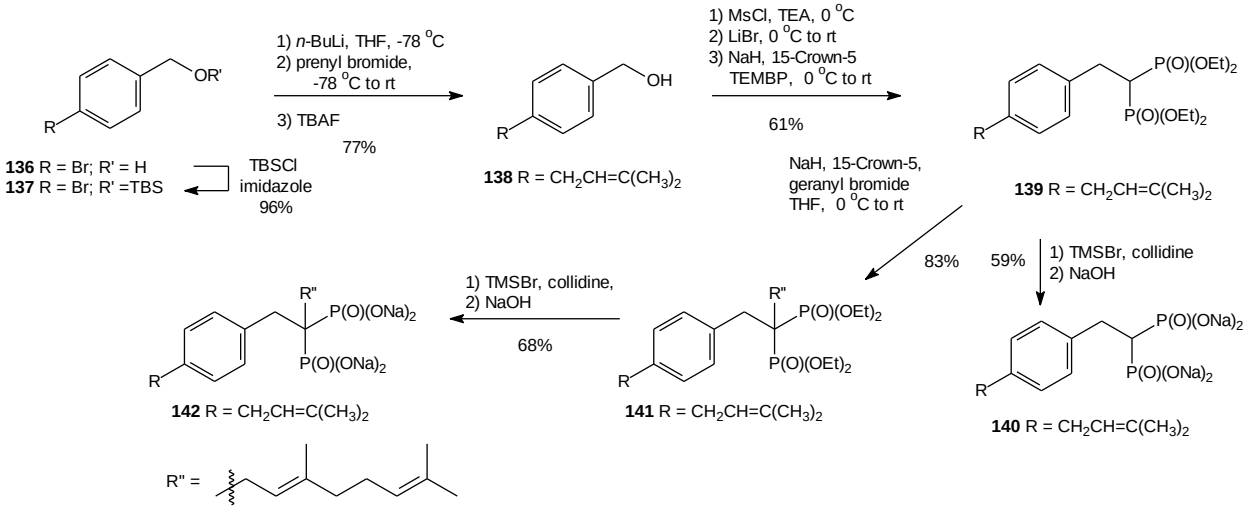


Figure 42: Synthesis of *p*-prenylated aromatic bisphosphonates.

Compound **145**, a bisphosphonate bearing an unsubstituted phenyl ring and geranyl chain, was prepared from tetraethyl methylenebisphosphonate (Figure 43). In this case it was attractive to add the benzyl substituent to the parent bisphosphonate first, followed by alkylation with geranyl bromide to obtain the dialkyl bisphosphonate **144**. This was convenient given that the commercial availability of the benzyl bromide (**143**) allowed the first alkylation to be performed on a larger scale. Without additional purification the monobenzyl and dibenzyl bisphosphonate mixture was treated with base followed by addition of geranyl bromide. The reaction proceeded in an acceptable yield (40%) over two steps and gave better results than the alternative of alkylating first with geranyl bromide followed by alkylation with benzyl bromide. Hydrolysis of bisphosphonate **144** using standard conditions proceeded smoothly, providing the bisphosphonate **145** in good yield. To prepare the corresponding pyridyl compound, an analogue more similar to risedronate (**89**), it was found to be advantageous to add the geranyl chain first and then alkylate the geranyl bisphosphonate with bromide **146**. Complications were encountered when monoalkylation with the TEMBP was attempted using the commercially available hydrobromide salt of pyridyl bromide **146** because it proved difficult to stifle the

dialkylation. Furthermore, the general lack of functionality rendered visualization of TLC analysis difficult, further confounding an already difficult purification. However, alkylating tetraethylmonogeranyl bisphosphonate gave the desired compound **147** cleanly and subsequent hydrolysis was employed to obtain the desired bisphosphonate salt **148**. Finally, the known bisphosphonate **149**[150] was prepared to serve as a control.

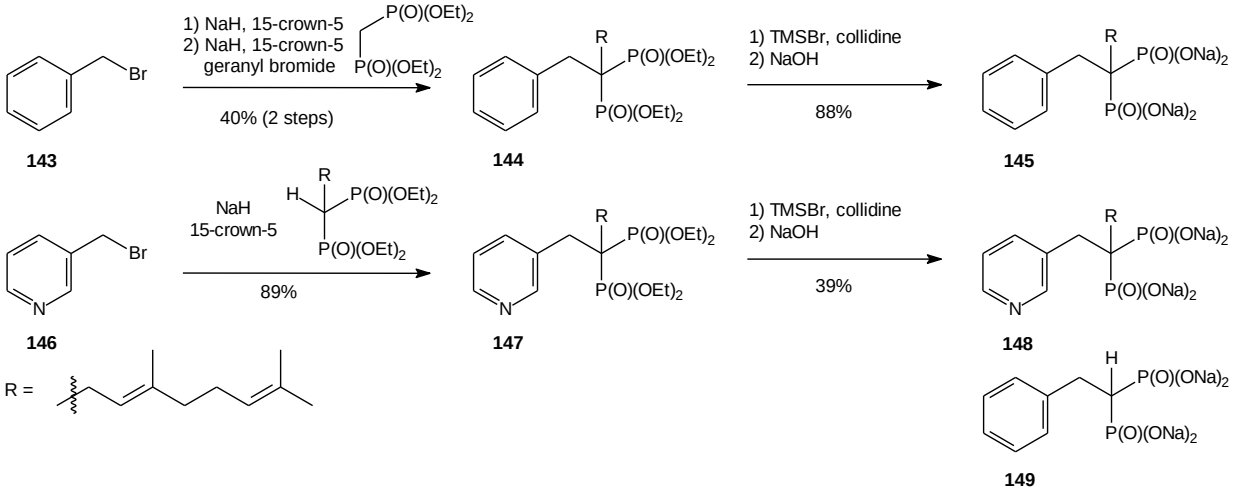


Figure 43. Synthesis of benzyl and pyridiyl analogues.

It should be noted that the isomeric compounds **150** and **151**, each with two aromatic chains, were isolated as minor products in the formation of compounds **132** and **139**. These bisaromatic compounds (Figure 44), as well as the bisbenzyl bisphosphonate **152** (synthesized as a control in the bisaromatic cases) proved difficult to hydrolyze using the conditions routinely employed. Instead of the pure tetrasodium salt, a complex mixture of compounds was formed. These bisaromatic bisphosphonate salts were unyielding to attempts at purification and the complex mixtures were not tested in biological assays.

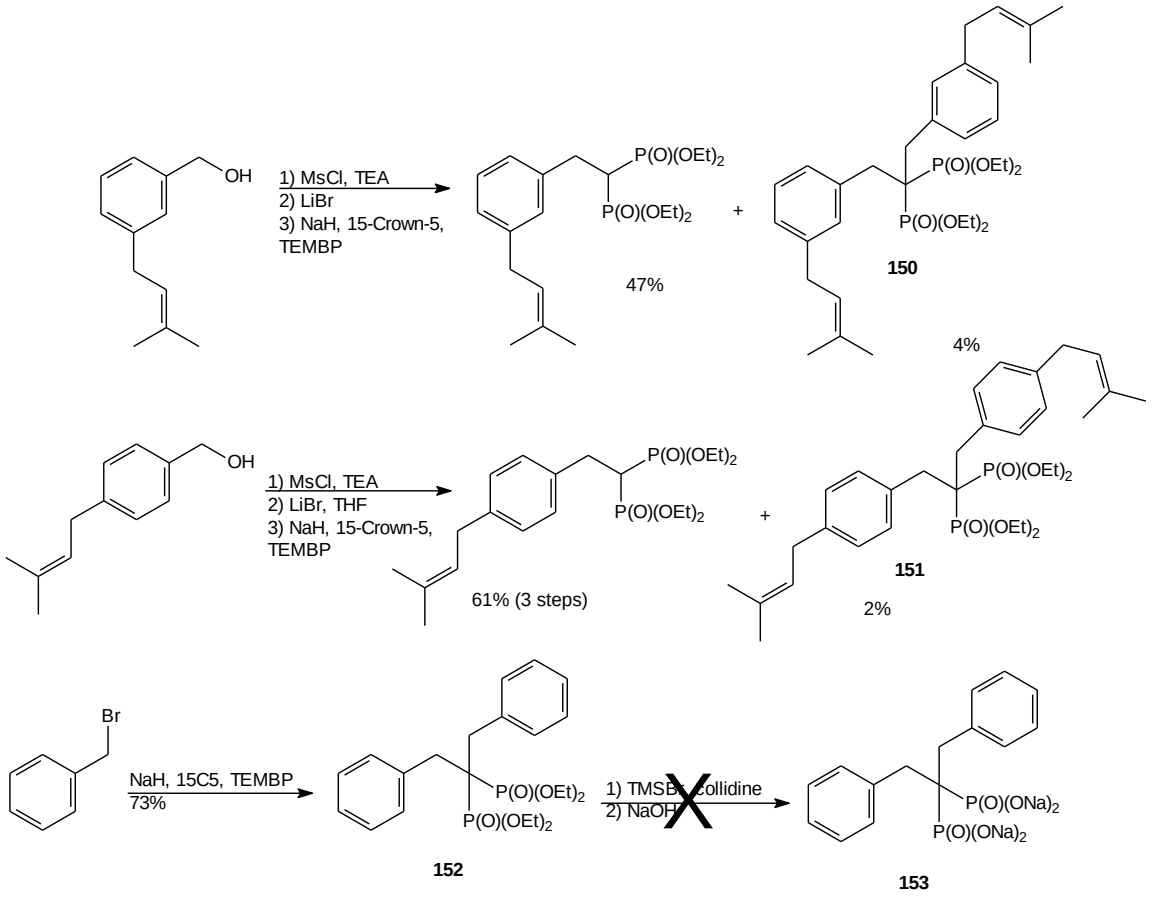


Figure 44. Bisaromatic bisphosphonates.

The aromatic bisphosphonates **135**, **142**, **145**, and **148** described above were evaluated for activity in both enzyme and various whole cell assays. Our laboratory has previously identified numerous mono- and dialkyl bisphosphonates as inhibitors of geranylgeranyl diphosphate synthase (GGDPS) and many of these compounds also have been shown to inhibit protein geranylgeranylation in cellular assays.[118] The new compounds were first screened *in vitro* against recombinantly purified human GGDPS enzyme (Figure 45A). At 10 μM concentrations, the dialkyl bisphosphonates **145**, **135**, **142**, and **148** all displayed various degrees of GGDPS inhibition while the mono alkyl compounds **133**, **140**, and **149** displayed little or no activity. Concentration response curves then were generated to further characterize the

compounds most active in the initial screen. Compounds **135** and **142** were found to display the most potent inhibitory activity with IC_{50} values extrapolated to be 58 and 85, respectively (Figure 45B).

The crystal structure of GGDPS has been solved when complexed with digeranyl bisphosphonate (**92**), which bound to the "inhibitor" binding site[151] in a "V-shaped" conformation occupying portions of both the FPP and GGPP binding sites.[149] Based on the structure of the most new potent compounds reported here, it would be anticipated that these molecules bind to the enzyme GGDPS in a similar manner.

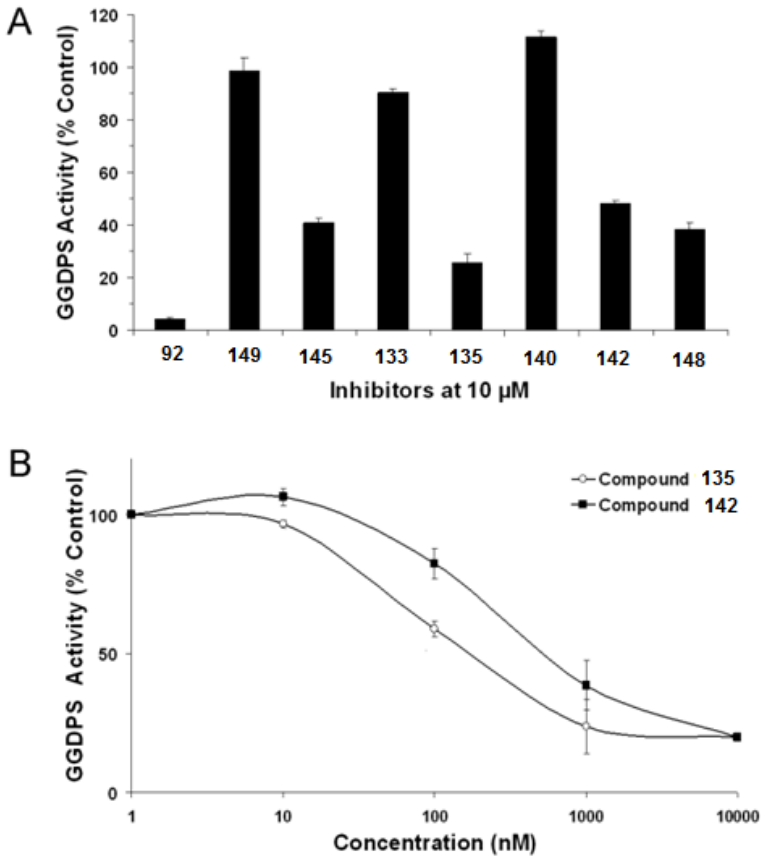
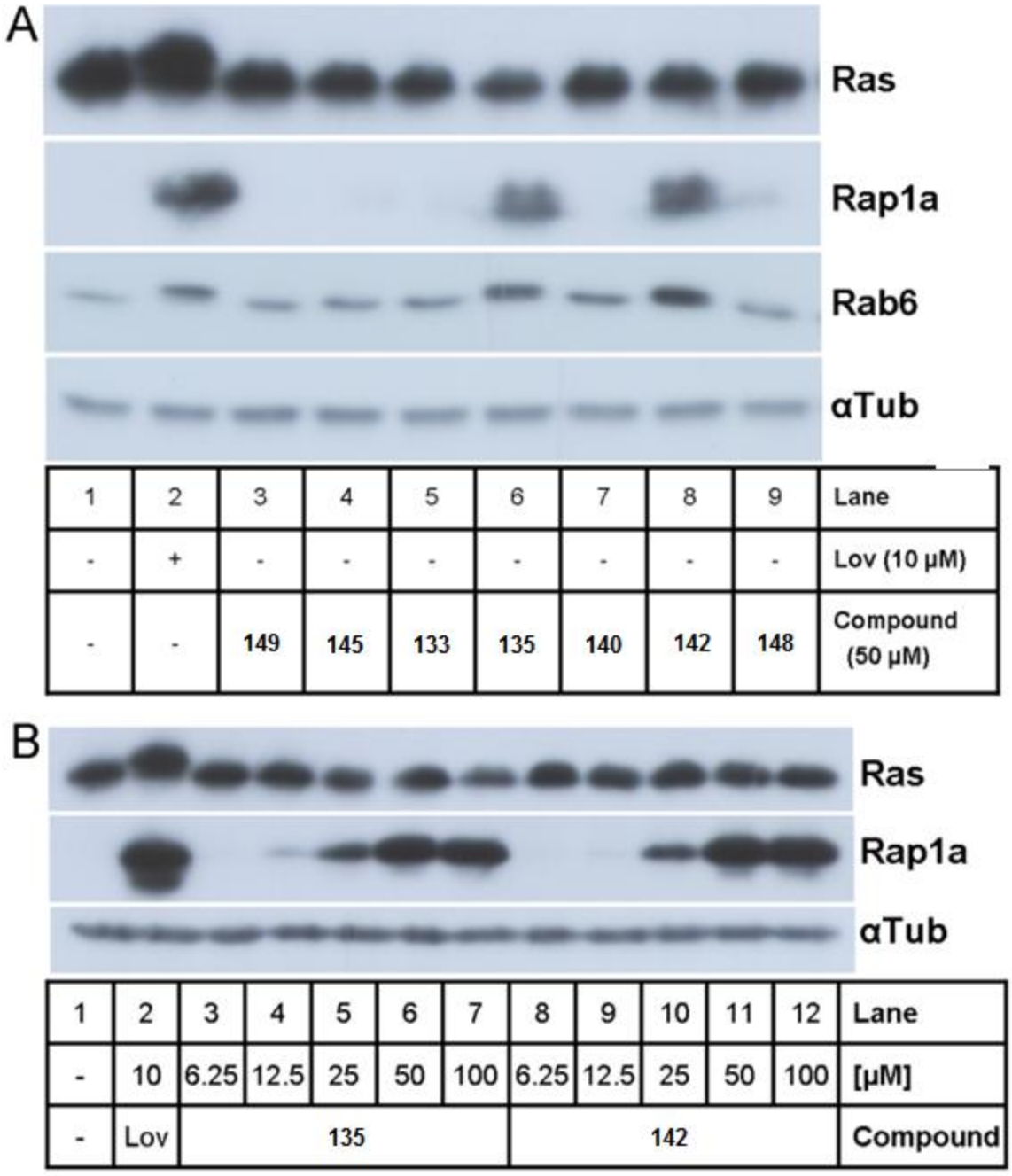


Figure 45. Inhibition of GGDPS *in vitro* by novel bisphosphonates. A. Compound screen at 10 µM of each compound (mean +/- S.D., *n* = 2). B. Concentration response of compounds **135** and **142** (mean +/- S.D., *n* = 2).

This set of compounds then was tested against the K562 human myelogenous leukemia cell line for inhibition of protein prenylation. Western blots were performed and the prenylation status of a panel of proteins was determined (Figure 46A). In these Western blot analyses, interpretation of the data is based on a few key points. The Ras protein is farnesylated and inhibition of farnesylation is made evident by the appearance of a more slowly migrating (upper), unmodified band on the gel. In contrast, Rap1a is geranylgeranylated and the antibody used here detects only the unmodified form of the protein. Inhibition of geranylgeranylation is detected by the appearance of an upper, unmodified band. The addition of lovastatin (Figure 46A, lane 2), an inhibitor of HMG-CoA reductase, depletes mevalonate resulting in an inhibition of farnesylation as well as an inhibition of both types of geranylgeranylation.

After 48 hour incubations, compounds **135** and **142** inhibited geranylgeranylation of both Rap1a and Rab6, while farnesylation of Ras appeared unaffected at this level of detection (Figure 45A). The *in vitro* data correlated well with the cellular data, as the two most potent *in vitro* GGDPS inhibitors were the only compounds to show cellular effects at 25 μM concentrations. Compounds **145**, **135**, **142**, and **148**, all had similar enzyme inhibitory effects on GGDPS (Figure 45A) while only compounds **135** and **142** impaired Rap1a modification in intact cells up to 50 uM concentrations (Figure 46A). Cellular concentration-response assays also were performed with both compounds **135** and **142** (Figure 46B). Inhibition of Rap1a geranylgeranylation was apparent at concentrations as low as 12.5 μM with bisphosphonate **135**, while both compounds **135** and **142** appeared to display maximal inhibition at 50 μM levels.



1	2	3	4	5	6	7	8	9	Lane
-	+	-	-	-	-	-	-	-	Lov (10 μM)
-	-	149	145	133	135	140	142	148	Compound (50 μM)

1	2	3	4	5	6	7	8	9	10	11	12	Lane
-	10	6.25	12.5	25	50	100	6.25	12.5	25	50	100	[μM]
-	Lov	135					142					Compound

Figure 46. Cellular inhibition of protein prenylation.

Finally, cell viability was determined in response to compound treatment by determination of the amount of DNA synthesis with a ^{3}H-thymidine incorporation assay (Figure 47). Bisphosphonates **135** and **142** inhibited DNA synthesis in both concentration and time

dependently, with the meta isomer **135** being the more potent compound. These results suggest that the degree of inhibition of geranylgeranylation correlates with cytotoxicity.

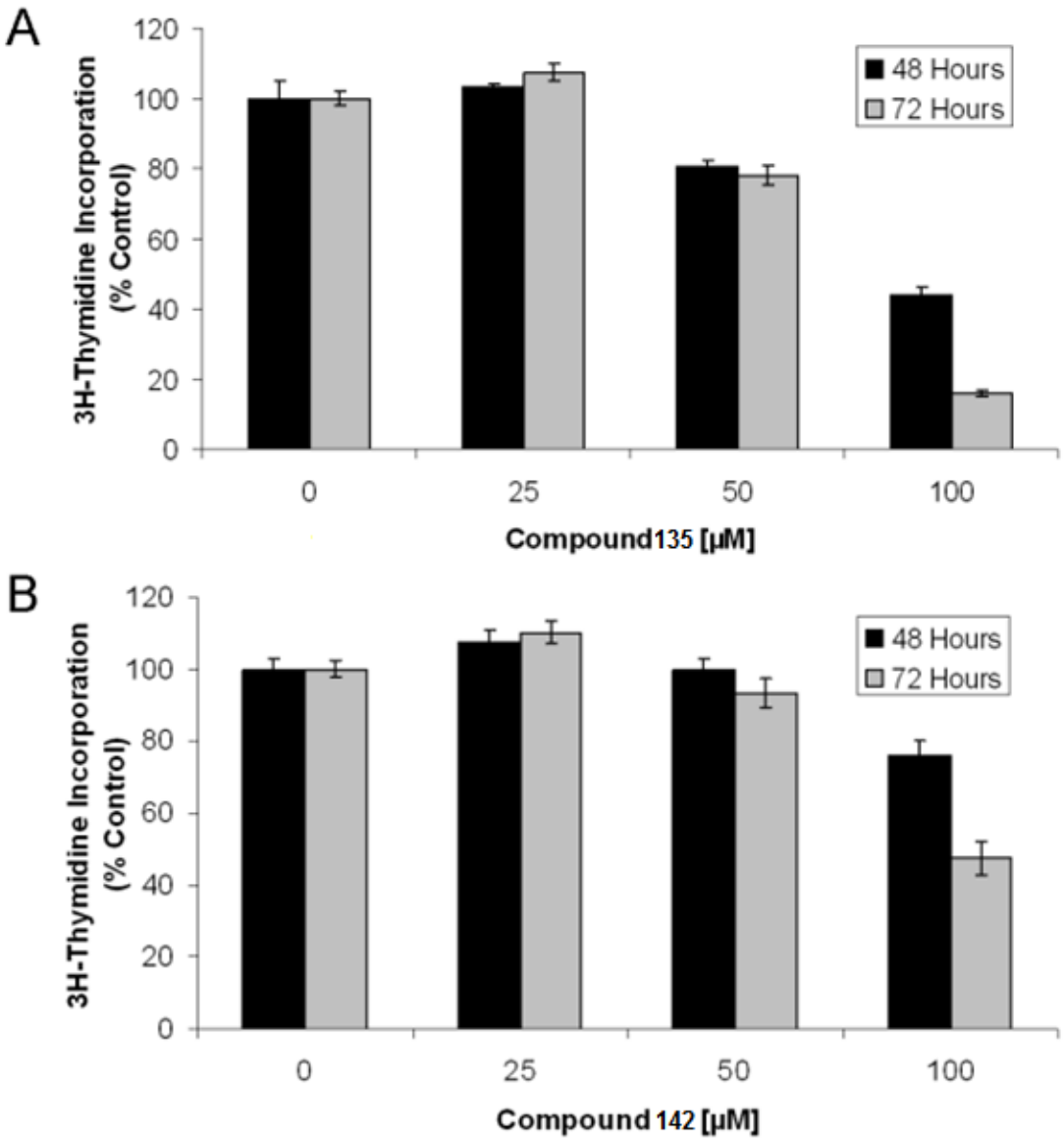


Figure 47. Compounds **135** and **142** inhibited K562 cell proliferation. Cell proliferation as a percentage of untreated cells at 48 and 72 hours was evaluated by [^{3}H]thymidine incorporation (mean +/- S.E., *n* = 4). A. Compound **135**. B. Compound **142**.

Although there has been some pursuit of multi-enzyme (i.e., dual FDPS and GGDPS) inhibitors within the mevalonate pathway as potential anti-cancer agents,[152] specificity remains the ideal for molecular intervention. Compounds with the ability to inhibit a single enzyme are very useful tools as enzymatic probes to study the interrelationships of this complex system, and may have use in anticancer applications in the clinic.[153] The aromatic bisphosphonates reported here, particularly the most potent compounds **135** and **142**, demonstrate selective inhibition of GGDPS over FDPS, and thus expand the list of tools available for manipulation of isoprenoid biosynthesis.

CHAPTER IV

DESIGN AND SYNTHESIS OF POTENTIAL INHIBITORS OF RAB GERANYLGERANYL TRANSFERASE

Rab geranylgeranyl transferase (RGGTase) is an emerging target for the treatment of disease, with special effort being directed toward this enzyme as a target for cancer therapy.[154] The importance of the RGGTase enzyme rests in its key role in the isoprenoid biosynthetic pathway, where it is responsible for a post-translational modification involving the transfer of a geranylgeranyl diphosphate (GGPP) unit to the target proteins. The prenylation of these proteins is essential for both membrane association and specific interactions, and is crucial for them to function in their designated capacity.[155] There are three enzymes responsible for protein prenylation, farnesyl transferase (FTase), geranylgeranyl transferase I (GGTase I), and geranylgeranyl transferase II (RGGTase, also described as GGTase II). Of these three, RGGTase is unique in that it requires participation of an auxiliary protein, the Rab escort protein (REP), to facilitate prenylation of the target protein.[156]

One means of better understanding isoprenoid biosynthesis is to develop enzyme-specific inhibitors for key enzymes in the mevalonate pathway. This not only allows researchers to gain insight into the biological processes associated with an enzyme, but it is also a starting point for developing enzyme inhibitors as drug therapies. A number of potent and effective inhibitors of FTase and GGTase I have been developed, including the peptidomimetic farnesyl transferase inhibitors (FTIs) tipifarnib **154**[157] and FTI-277 **155**,[158] and the geranylgeranyl transferase I inhibitor (GGTI) GGTI-298 **156** (Figure 48).[159] However, much less is known about inhibitors of RGGTase.

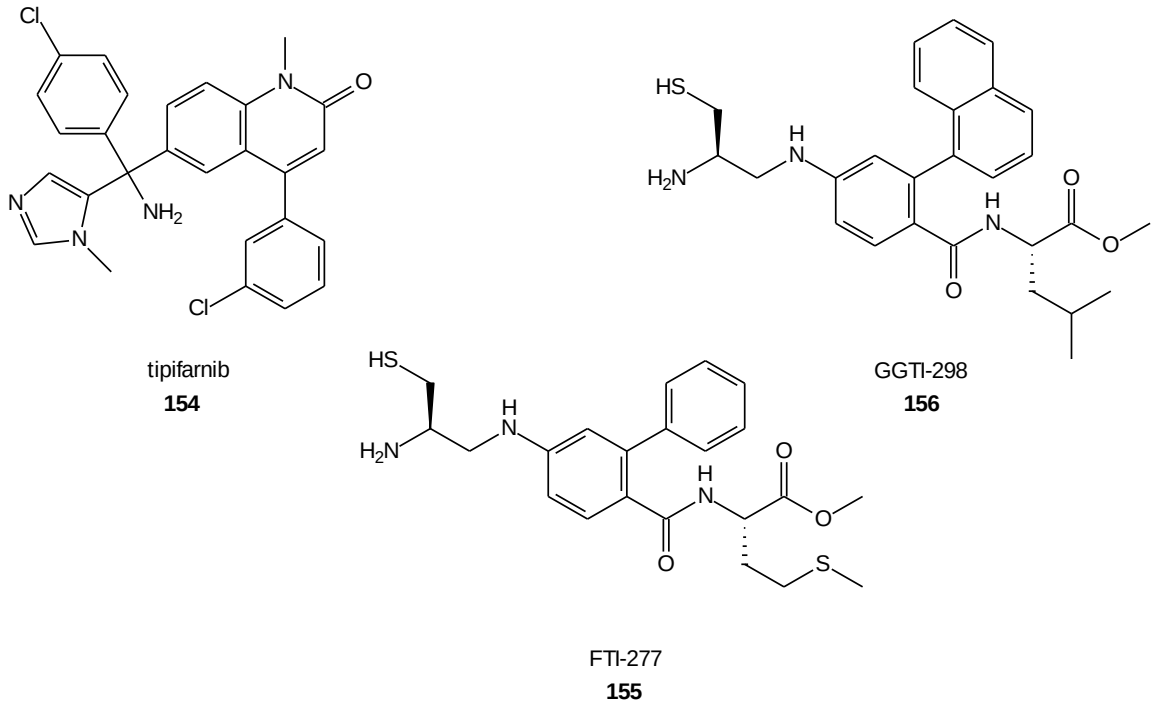


Figure 48. FTase and GGTase I inhibitors.

Recently, the phosphonocarboxylate 3-PEHPC **158** has been synthesized.[160] It was discovered unexpectedly that this compound inhibits specifically the RGGTase enzyme, albeit at very high concentrations[161] for clinical use.

Upon inspection it is evident that 3-PEHPC is a phosphonocarboxylate (PC) analog of risedronate **89**, a nitrogenous bisphosphonate that has found use in the clinic as a treatment for osteoporosis and other bone-related diseases (Figure 2).[103] Risedronate and other nitrogenous bisphosphonates have been shown to derive their activity from the inhibition of the enzyme farnesyl diphosphate synthase (FPPS)[108,109] while phosphonocarboxylates including 3-PEHPC selectively inhibit RGGTase.[161] It would seem reasonable to infer that the pharmacophore of the phosphonocarboxylates would parallel that of the nitrogenous α-hydroxy bisphosphonates, but there is evidence that the SAR between these classes of compounds differ significantly. For example, removal of the α-hydroxy moiety from the parent compounds has little effect on the

phosphonocarboxylate activity for inhibition of RGGTase, but a much more pronounced effect on the inhibition of FPPS in the bisphosphonate series.[162] In spite of this apparent distinction, much of the effort recently devoted to the development of novel inhibitors of RGGTase has focused on the synthesis and biological evaluation of phosphoncarboxylate analogues of established inhibitors of FPPS. For example, McKenna and coworkers have published recently the synthesis and evaluation of the phosphonocarboxylate analog of minodronic acid **157** (Figure 49). While the synthesized analogue **159** was 33–60 fold more potent than 3-PEHPC (**158**) in the assays performed,[163] it is nonetheless based upon an existing FPPS inhibitor, reflecting a relatively limited approach to the development of better enzyme inhibitors of RGGTase.

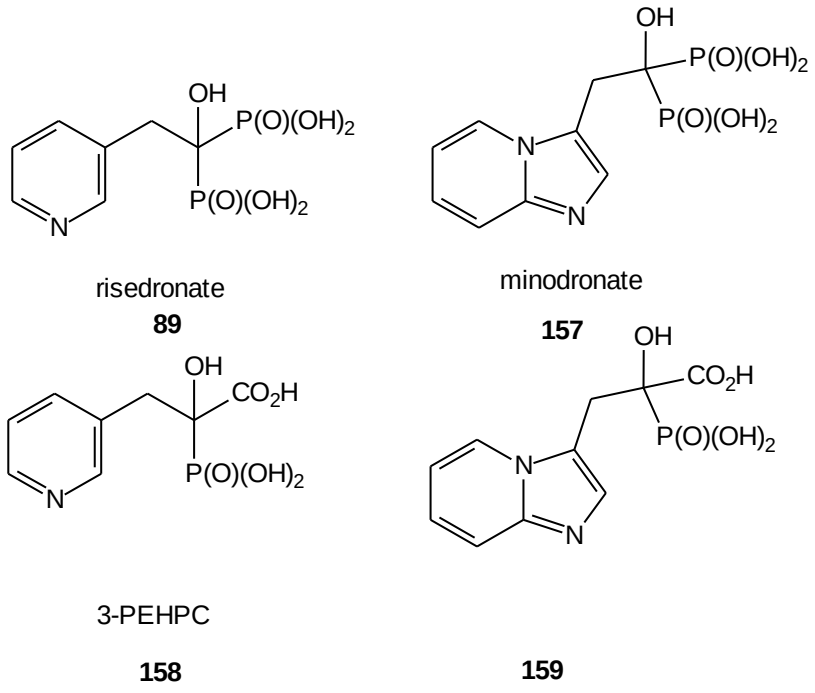


Figure 49. Some bisphosphonates and their phosphonocarboxylate analogues.

We have sought to approach the challenge of designing and synthesizing more potent RGGTase inhibitors from a different perspective. Modification of the polar "head" of the parent compound 3-PEHPC was one of the first variations envisioned. It is reasonable to hypothesize that the enzyme active site is very sensitive to modifications of the phosphonocarboxylate "head" in substrates that bind similarly to the parent compound. The fact that the simple modification of a phosphonocarboxylate (**158**, 3-PEHPC) to a bisphosphonate (**89**, risedronate) completely changes the selectivity and enzymatic target of the compound[162] supports this hypothesis.

To determine functionality parallel to the phosphonocarboxylate, the structure of the natural pyrophosphate substrate **87** was considered. First, when completely deprotonated the charge of the bisphosphonate **86** (-4) and phosphonocarboxylate **160** (-3) differ by a net charge of -1, with the phosphonocarboxylate having a net charge equivalent to that of the parent pyrophosphate (-3) when fully deprotonated (Figure 50). This may have an impact on interactions in the active site, but may also have an impact on the compound's ability to cross cell membranes as evidenced by work on prodrugs of bisphosphonates.[147] In addition to the net charge and cell permeability parameters, atom connectivity also was considered. Inspection of the phosphonocarboxylate core shows a geminal relationship between the carboxylate and phosphonate functional groups. This structural arrangement allows only for alkylation at the bridging methylene carbon. When compared to the natural pyrophosphate structure, it is apparent that the isoprenoid moiety is spatially further removed from the bridging atom. Considering these points, and contemplating potentially similar substructures, the phosphonophosphinate substructure **161** seemed an excellent functionality to explore as a replacement for the phosphonocarboxylate moiety. When fully deprotonated it possesses a net charge identical to that of pyrophosphate. Furthermore, it allows for alkylation and/or functionalization at either the methylene linker, as in the parent phosphonocarboxylate, or alkylation and/or functionalization at the terminal methyl group, parallel to that of the pyrophosphate substrate.

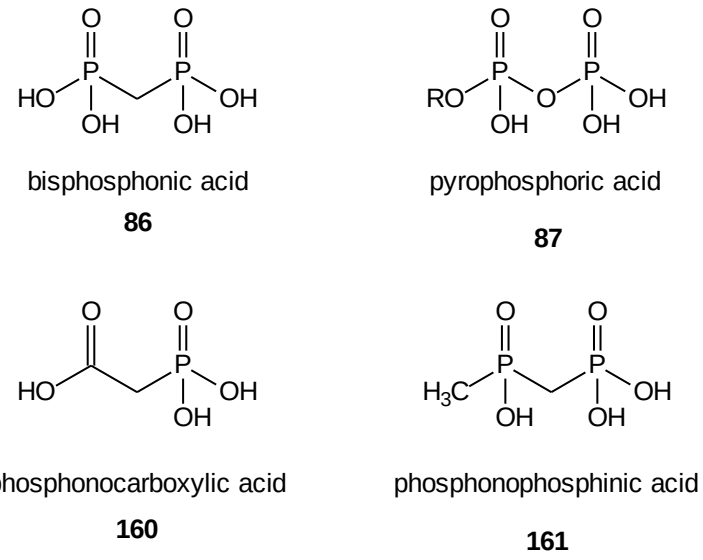
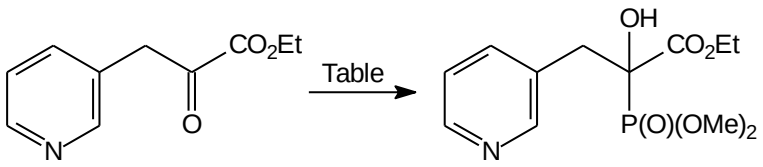


Figure 50. Pyrophosphoric acid and bioisosteres.

Before compounds could be developed for assay, 3-PEHPC (**158**) needed to be prepared as a control compound. The synthesis of 3-PEHPC had not been published in a peer reviewed journal, and only general conditions could be found in the patent literature.[164] Using the patent literature as a guide, the synthesis of 3-PEHPC was attempted but difficulties were soon encountered. The synthesis of the α-keto ester[165] **162** was facilitated by treatment of nicotinaldehyde (**165**) with ethanol and excess sodium hydroxide and *N*,*N*-dimethyl glycine ethylester (**164**) and provided the desired intermediete in modest yield. Next, this material was subjected to non-catalyzed Pudovik reaction[166] under conditions as described in the patent literature. Unfortunately, even under varied conditions (Figure 51) the target compound **163** could not be prepared in any useful yield.

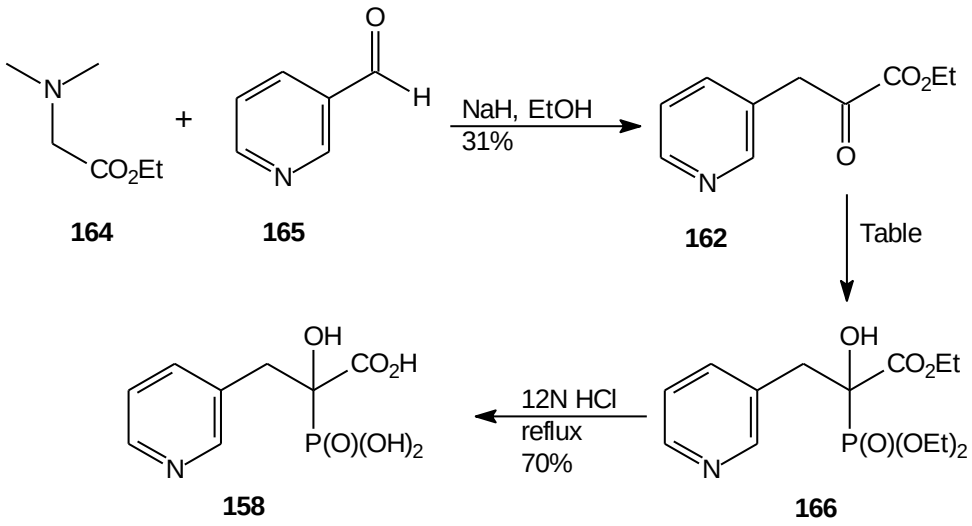


162 **163**

Trial	$P(OH)(OMe)_2$ (eq.)	Time (hrs)	Temperature (°C)	Yield
1	7	72	25	Failed
2	5	72	25	2%
3	11	48	25	Failed
4	6	12	Reflux	failed

Figure 51. Trials for the synthesis of compound **163**.

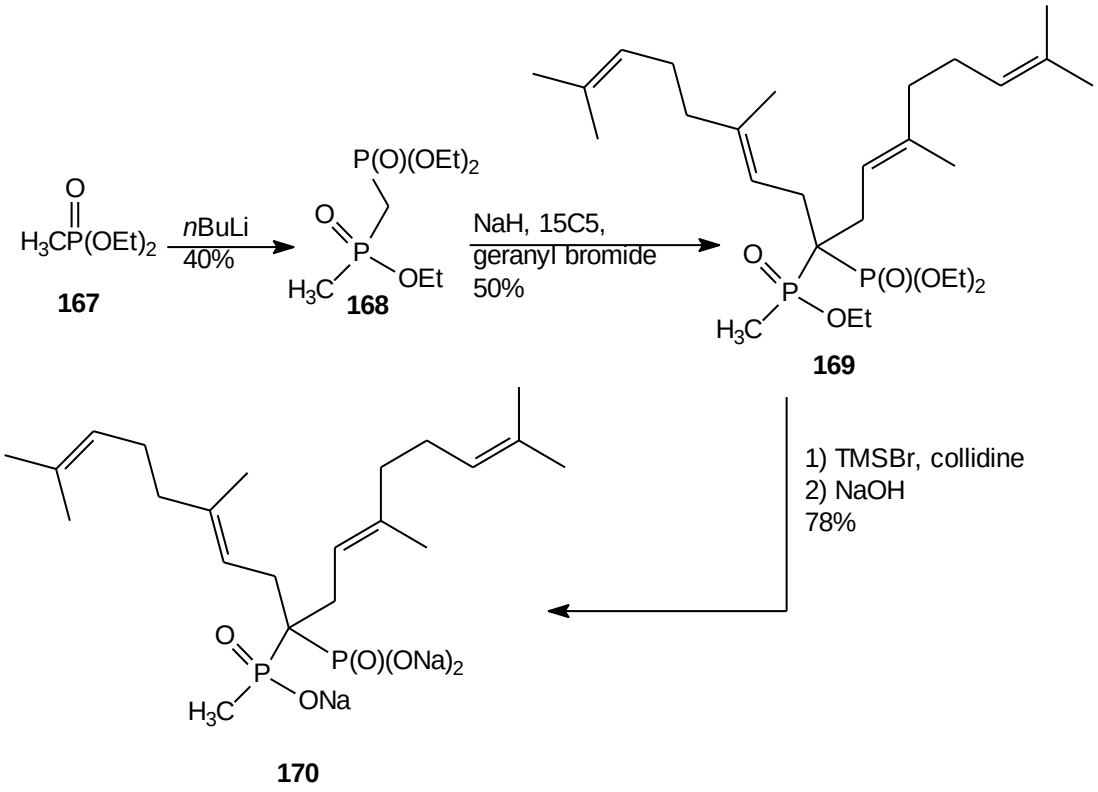
It is documented that dimethyl phosphite can undergo a disproportionation reaction to afford dimethyl methylphosphonate,[167] and it is hypothesized that this competing reaction could be the cause for such miniscule yields in this system. To circumvent complications with disproportionation, we elected to use diethyl phosphite as the phosphorus reagent because it is far less prone to disproportionation. As can be seen in Figure 52, this modification did indeed result in a greatly enhanced yield of the desired phosphonocarboxylate **166**, and the best yields were achieved using the non-catalyzed Pudovik conditions. Hydrolysis through treatment with concentrated HCl proceeded as desired to provide 3-PEHPC (**158**) in 70% yield.



Trial	$P(OH)(OEt)_2$ (eq.)	Time (hrs)	Temperature (°C)	Base	Yield
1	3	12	95 to rt	Alumina	31%
2	3	12	95 to rt	None	78%
3	4	24	95 to rt	None	86%

Figure 152. Synthesis of 3-PEHPC (**158**).

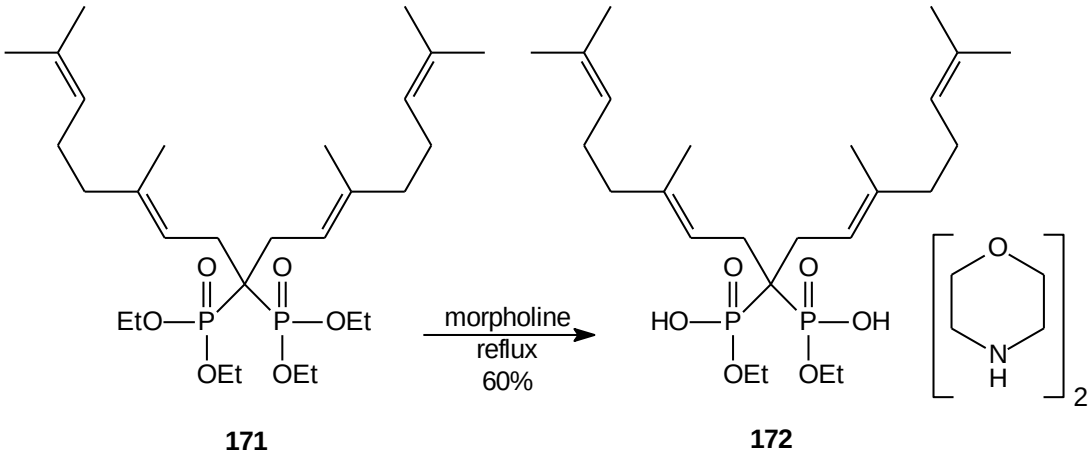
Synthesis of the new phosphonophosphinate **170** began with the known reaction of diethyl methylphosphonate (**167**) with nBuLi under cryogenic conditions to afford the triethyl methylenephosphonophosphinate[168] **168** in an acceptable yield (40%). Even though this yield is modest, the straightforward reaction conditions and facile isolation by column chromatography, make this the preferred route to the phosphonophosphinate **168**. The purified material was next treated with base and alkylated through reaction of the resulting anion with geranyl bromide to provide the digeranylphosphonophosphinate **169**. Hydrolysis through treatment with TMSBr and collidine followed by treatment with NaOH provided the target phosphonophosphinate trisodium salt **170** in good yield (78%, Figure 53).



167 **168** **169** **170**

Figure 53. Synthesis of phosphonophosphinate **170**.

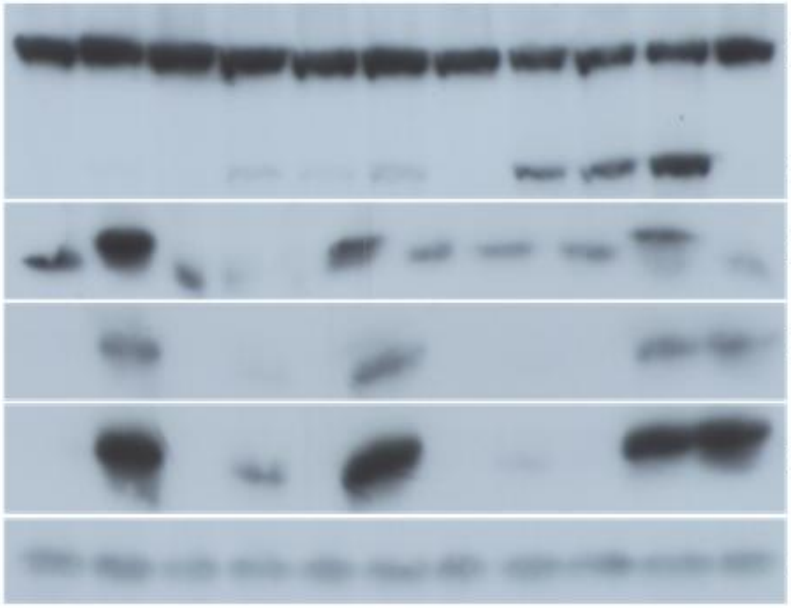
A second strategy for new RGGTase inhibitors was based on partial hydrolysis of a bisphosphonate tetraester. There is evidence that diester diacid bisphosphonate compounds can display activity in some enzyme active sites.[169] Accordingly, the dimorpholino diethyl bisphosphonate **172** was synthesized in one step from the previously described compound **171** by heating a solution at reflux in morpholine.[170] The reaction proceeded as expected and provided the diester **172** in good yield (60%, Figure 54).



171 **172**

Figure 54. Synthesis of dimorpholino bisphosphonate **172**.

Both compounds **170** and **172** were tested in a Western blot analysis to gain some insight into their impact on protein farnesylation and geranylgeranylation. While these results are only preliminary, the Western blot analysis (Figure 55), coupled with the toxicity data (Figure 56) indicate that compound **170** is a potentially potent compound that does not act upon either GGDPS or FDPS exert its observed biological effects (Figure 55). This can be seen in figure 55 where even at 100 μM concentrations, there is no evidence of inhibition of farnesylation or geranylgeranylation yet the MTT assay (Figure 56) shows toxicity. However, it is already clear that isoprenoid phosphonophosphinates represent an intriguing template for synthesis of potential inhibitors of the enzymes of the isoprenoid biosynthetic pathways. Samples of **172** and **170** have been provided to our collaborators and results for the assays of RGGTase inhibition will be forthcoming.



-	-	172	172	172	172	170	170	170	170	-	RJB Compound
-	-	10	100	100	100	10	100	100	100	-	RJB [µM]
-	10	-	-	-	10	-	-	-	10	-	Lov [µM]
-	-	-	-	-	-	-	-	-	-	10	DGBP [µM]
-	-	-	-	20	-	-	-	20	-	-	GGPP [µM]

K562 Cells, 48 Hours

Figure 55. Western blot analysis of compounds **170** and **172**.

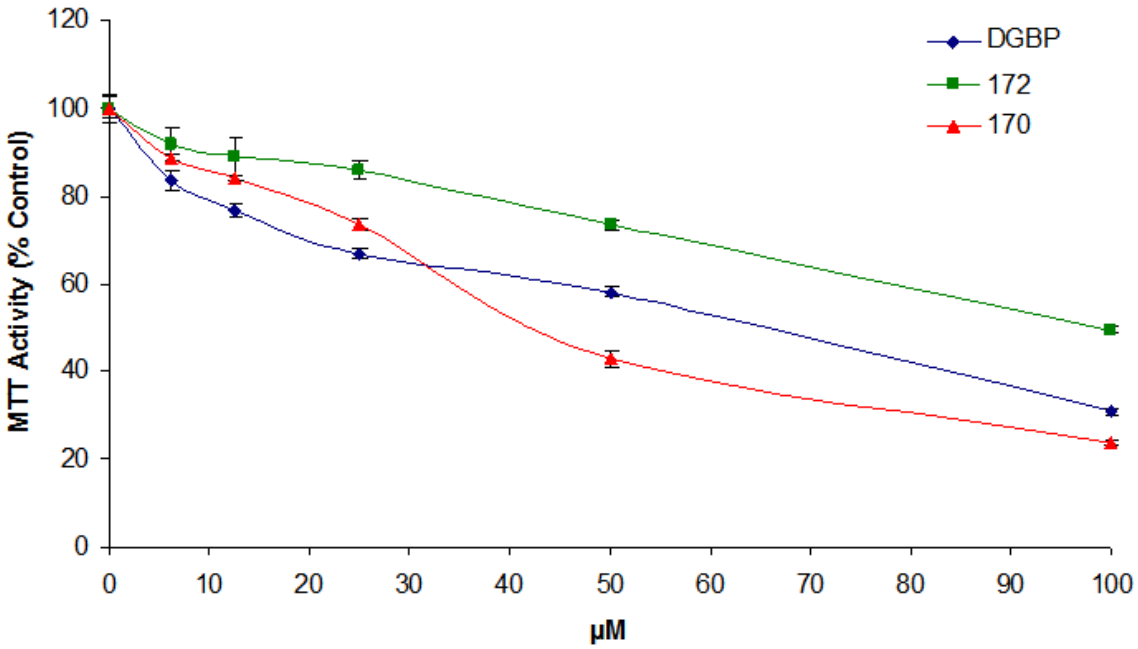


Figure 56. Cell toxicity data for compounds **170** and **172**.

CHAPTER V

CELL WALL BIOSYNTHESIS AS A TARGET FOR TUBERCULOSIS THERAPY

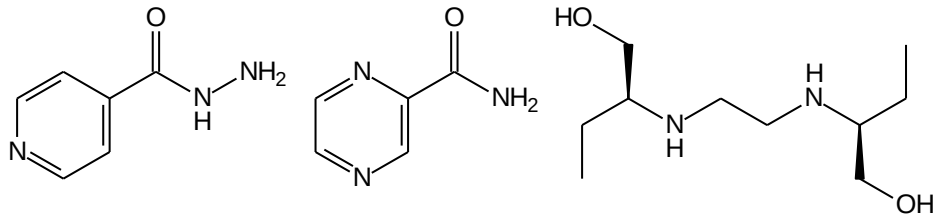
Tuberculosis, often simply called "TB" for tubercles bacillus is an infectious disease having high rates of both mortality and morbidity. The disease is caused in humans primarily by the pathogen *Mycobacterium tuberculosis*, but mycobacteria usually infecting other species, including *Mycobacterium bovis* the causative agent of tuberculosis in cattle, have been known to cross the species barrier and infect humans as well.[171] Tuberculosis has been present in the environment since the distant past. The *Mycobacterium tuberculosis* pathogen has been detected in bison remains more than 17,000 years old.[172] Skeletal remains from a 9000 year old Neolithic man[173] as well as from ancient Egyptian mummies[174] have shown signs of tuberculosis and detectable quantities of *Mycobacterium tuberculosis* complex DNA.

Tuberculosis infection is a global problem with more than one third of the world population exposed and a rate of infection of one new case every second.[175] The World Health Organization has estimated that 5-10% of people that are HIV negative and infected with TB become sick or infectious at some point in their life.[176] It is important to distinguish the groups' HIV status, as HIV positive subjects exhibit much higher rates of mortality from TB infection.

While tuberculosis infection is a worldwide problem, the largest number of new cases occurs in Southeast Asia. However sub-Saharan Africa has the highest incidence per capita, nearly twice that of Southeast Asia.[176] While incidence rates per capita are falling worldwide, sizeable population growth in several affected regions offset any declines in incidence rate resulting in a net increase in the number of new cases.[176]

Tuberculosis is treated using a few key antibiotics. In fact there are only ten drugs currently approved by the U.S. Food and Drug Administration for treatment of the disease.[7] Both latent and active tuberculosis can be treated, but the treatment regiments differ. Latent TB

infections generally are treated with only one antibiotic and the treatment course is much shorter than for active tuberculosis disease. The most common therapy for latent TB infections (Figure 57) is isoniazid (**173**, INH) for 6-9 months. Rifampicin (RIF) alone for 4 months, or less commonly, rifampicin and pyrazinamide (**174**, PZA) for 2 months also have been used effectively.[175] Ethambutol (**175**, EMB) has also been used successfully.[177]



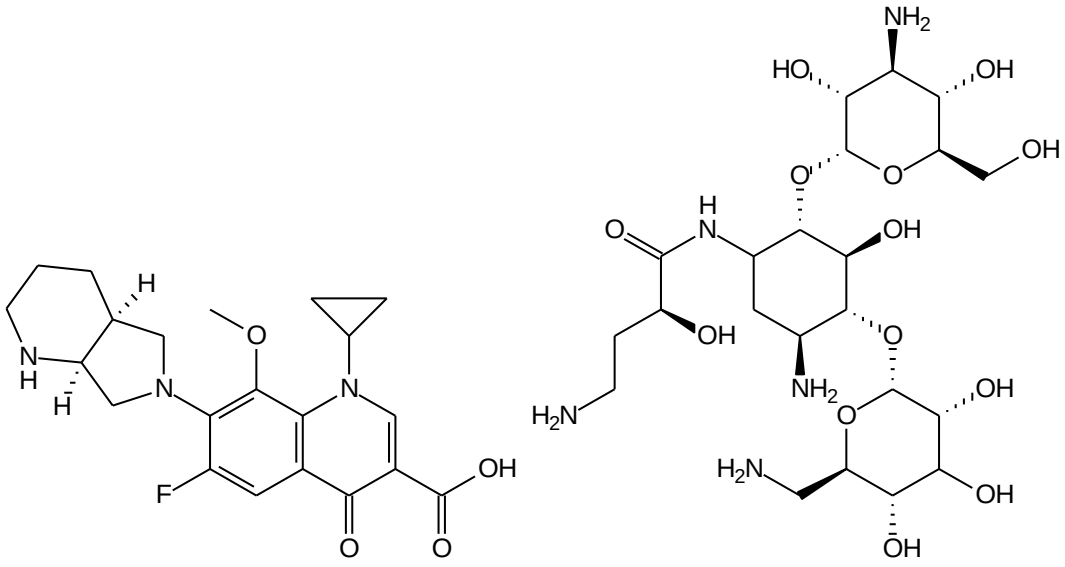
isoniazid **173** pyrazinamide **174** ethambutol **175**

Figure 57. Common first-line drugs for the treatment of tuberculosis.

The current treatment for active TB infections is much longer in duration and much more intensive in the number of drugs used. The preferred regimen is an initial phase of daily INH, RIF, PZA and EMB for 2 months followed by a continuation phase of daily INH and RIF for another 7 months, although treatments requiring as long as two years are not uncommon.[177] One drawback to these long treatment regimens is that it can be difficult to ensure patient compliance, especially in undeveloped areas.

A myriad of drugs is utilized in TB treatments in an attempt to minimize the development of antibiotic resistance in the bacteria.[178] Multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) forms of tuberculosis are rapidly emerging. In 2008 it was estimated that approximately 4% of TB cases globally are of the MDR-TB type.[177] In order to be defined as

MDR-TB, bacteria must be resistant to at least the two most effective tuberculosis drugs, isoniazid and rifampicin. MDR-TB can result from either primary infection, or may develop over the course of treatment, particularly if the treatment regimen is not strictly followed. Similarly, XDR-TB is identified as a form of TB bacteria resistant to the first line drugs as well as any fluoroquinolone such as moxifloxicin (**176**) and any of the second-tier (Figure 58) treatments such as amikacin (**177**) and kanamycin. These resistant forms of TB do not respond to the standard treatment and can take up to two years for treatment with less potent and more toxic and expensive drugs, drugs that often are unavailable in undeveloped regions of the world.[176]



moxifloxicin
176

amikacin
177

Figure 58. Quinololone and aminoglycoside drugs used in the treatment of MDR-TB.

The primary causative agent of TB disease, *Mycobacterium tuberculosis*, is neither defined as gram negative nor gram positive, but instead is described as an acid-fast gram positive bacterium due to its lack of an outer cell membrane. It has an unusually high lipid content in the cell wall, and will not retain any bacterial stain. The atypical structure of the *Mycobacterium tuberculosis* cell wall contributes to its virulence, and also complicates drug therapy. While the cell wall does contain peptidoglycan, over 60% of the cell wall is composed of lipids. This contributes to the bacterium's resistance to dyes and stains, as well as its resistance to antibiotics, acidic and alkaline compounds, lethal oxidations, and survival inside of macrophages.[179] Given the large number of persons infected with TB every year, and the increasing prevalence of MDR and XDR strains of TB, there is an urgent need for the design and development of new drugs and strategies for the treatment of tuberculosis.

One approach to better treatment of TB infections is the targeting of the unique cell wall that imbues *Mycobacterium tuberculosis* with its relative resistance to antibiotic treatment. Disruption of the cell wall may result in cell death, or render the bacterium's natural defenses less useful and allow current or future drugs to work more effectively. This strategy even may allow the host's own immune system an opportunity to better combat the infection. Disruption of cell wall biosynthesis is the approach that we have chosen to pursue.

Cell wall biosynthesis is a reasonable target because it is essential to bacterial survival, and inhibition of key enzymes involved in the biosynthesis of this unique cell wall may provide effective therapies for TB. As stated earlier, mycobacteria are included with gram-positive organisms but in actuality they share features common to both gram-positive and gram-negative bacteria. The cell envelope of *Mycobacterium tuberculosis* is comprised of a few primary components (Figure 59). There is a plasma membrane, a covalently linked mycolic acid network, and an arabinogalactan and peptidoglycan complex (MAPc). In addition, the mycobacterium has a polysaccharide-rich capsule.[180] The fact that the MAPc complex is unique to mycobacteria has led to conjecture that enzymes involved with the biosynthesis of this structure may provide unique drug targets.[181]

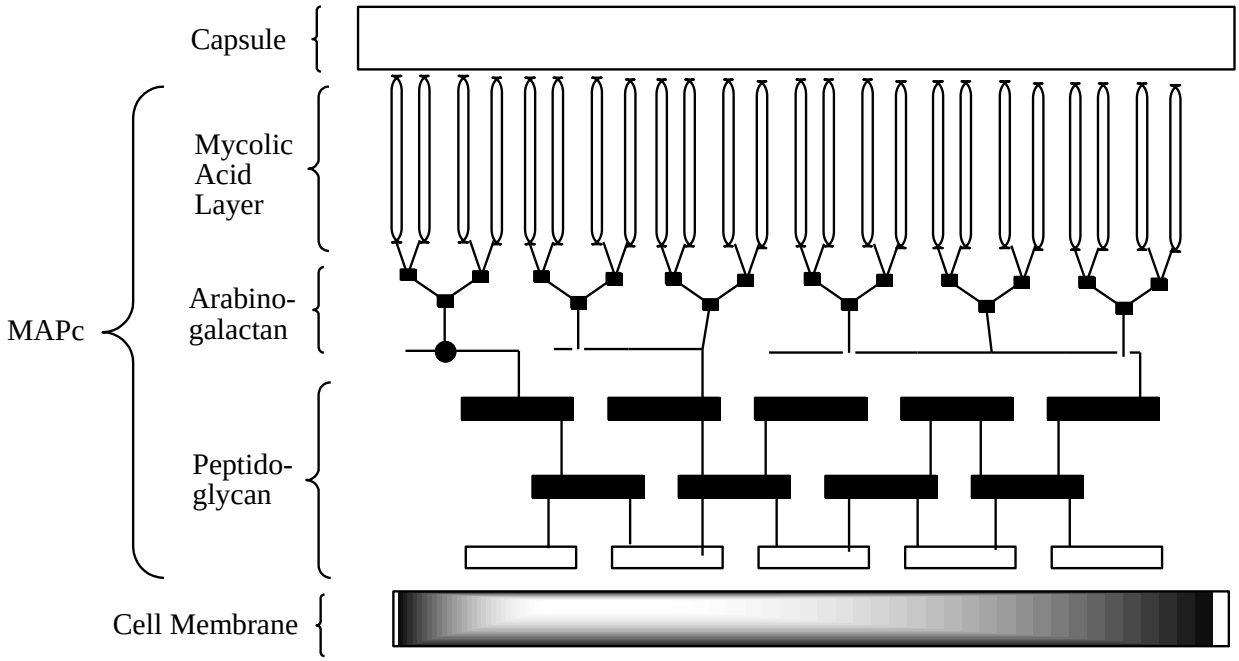


Figure 59. Cross section cartoon of the *Mycobacterium tuberculosis* cell wall.[180]

Isoprenoids often are biosynthesized differently in mammals than in prokaryotes. The primary biosynthetic pathway in prokaryotes is the non-mevalonate, or MEP/DOXP pathway[182] while in mammals isoprenoid biosynthesis occurs via the mevalonate pathway (Figure 60).[183] Given an extensive knowledge of phosphonate synthesis, the enzyme Rv2361c is targeted as one potential site for TB therapies incorporating organophosphorus compounds. The enzyme Rv2361 is a decaprenyl pyrophosphate synthase (DPPS) that utilizes key phosphate structures in the biosynthesis of isomer-specific decaprenylpyrophosphate, a key intermediate in bacterial cell wall biosynthesis. It is thought that if this enzyme could be inhibited, cell wall biosynthesis in mycobacteria could be disrupted, ultimately resulting in bacterial cell death.

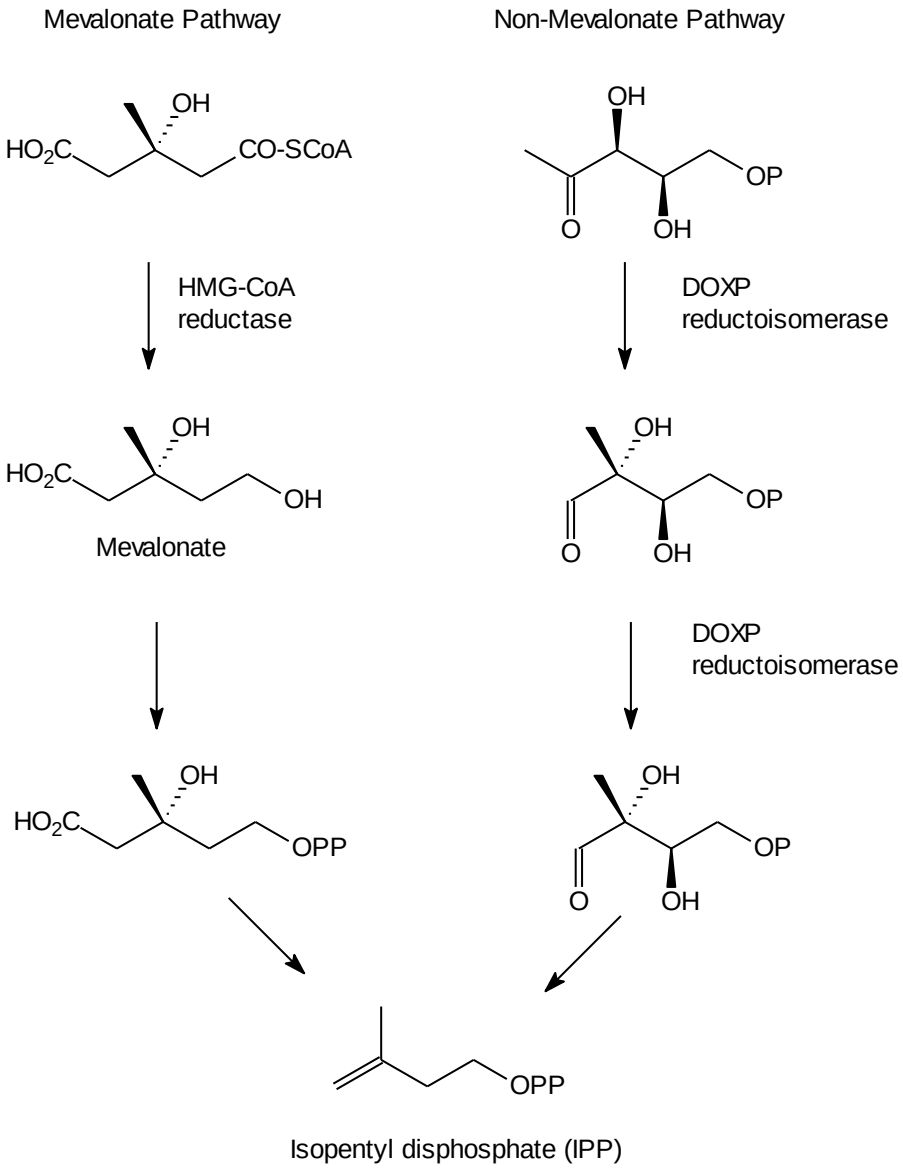


Figure 60. The mevalonate and non-mevalonate pathways of isoprenoid biosynthesis.

Given the substantial library of phosphonate compounds synthesized in the Wiemer lab, our collaborators in the laboratory of Dr. Raymond Hohl undertook a general study of the efficacy of a wide range of phosphonates as related to both inhibition of the enzyme Rv2361 *in vitro*, and growth inhibition of the TB model *Mycobacterium smegmatis in vivo.* Several compounds proved potent in these assays (Figure 61), and based upon the structural and

biological data collected, a series of further compounds was designed as probes and inhibitors of the enzyme Rv2361.

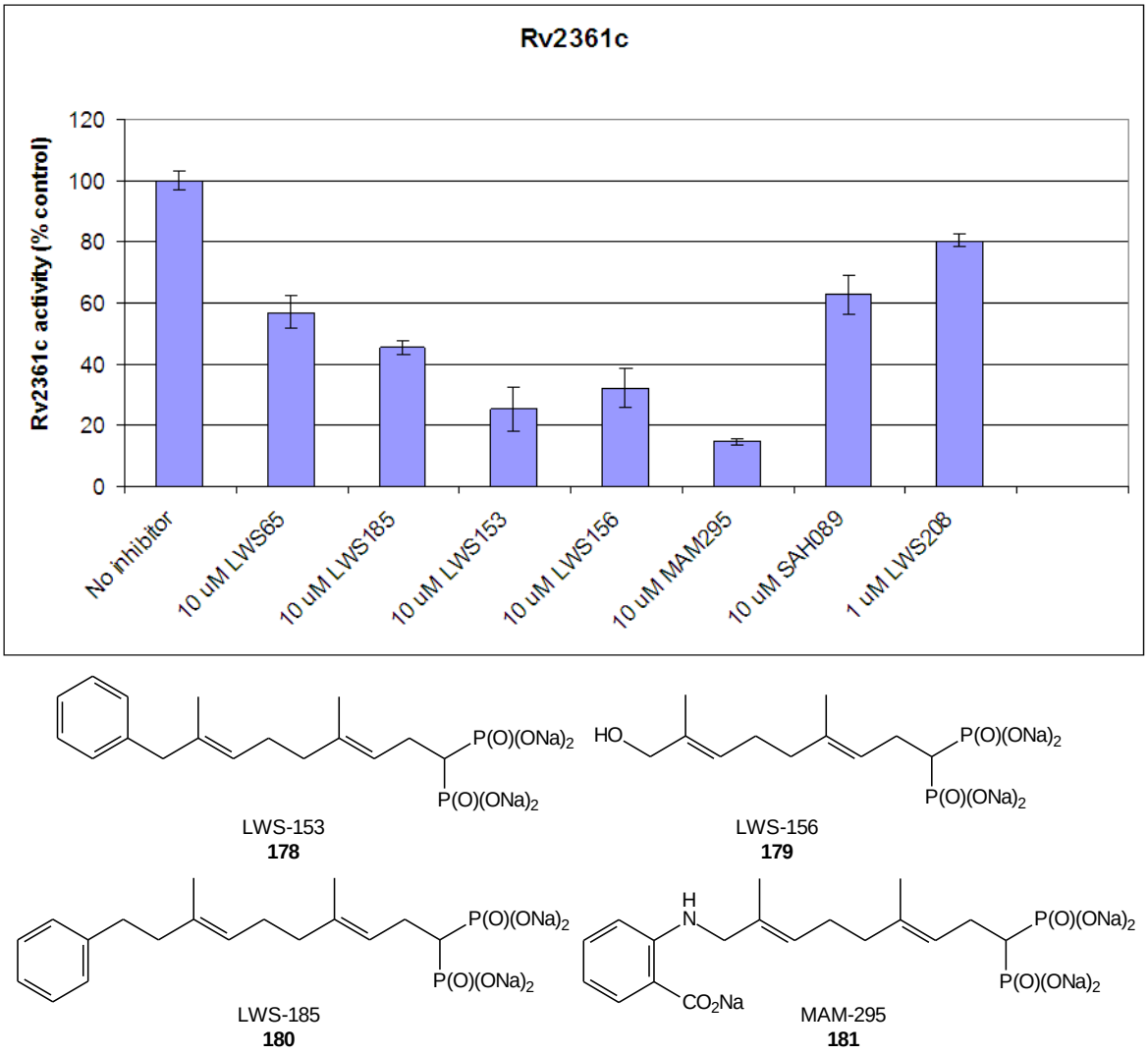


Figure 61. *In vivo* inhibitors of the enzyme Rv2361c.

The most potent compounds identified in the preliminary screening include the 9-phenyl- (**178)**, 9-benzyl (**180)**, 9-hydroxyl (**179)**, and 9-*N*-anthranilogeranyl (**181)** bisphosphonates, with

the 9-*N*-anthrolinogeranylbisphoshonate (**181)** having the greatest activity. A broad range of compounds was tested having various structural motifs. From the preliminary data it appeared that functionalization at the 9-position of monogeranyl- and a small number of dialkyl bisphosphonates (*vide infra*) gave the highest inhibition of the target enzyme.

With this data in hand, several targets were designed to probe the pharmacophore of Rv2361c inhibitors. In light of the fact that all but one of the inhibitors showing activity possess an aromatic moiety at the 9-position, and the fact that a heteroatom may play a role in activity as evidenced by compounds **179** and **181**, the compounds in Figure 62 were proposed as targets. The 9-phenoxy target **182** was deemed reasonable as both the non-oxygenated compound **178** and the non-aromatic hydroxyl compound **179** had activity in the assays. It was hoped that the phenyl ether would maximize interactions present in the benzyl and 9-hydroxyl compounds. The aniline derivative was designed to probe the necessity of the carboxylic acid moiety in the anthranilate compound **181**, while the indole **184** and benzimidazole **185** compounds seemed to retain all of the key components of the most potent inhibitor **181**, while increasing lypophilicity and, in the case of benzimidazole analogue providing another hydrogen bond acceptor/Lewis base site.

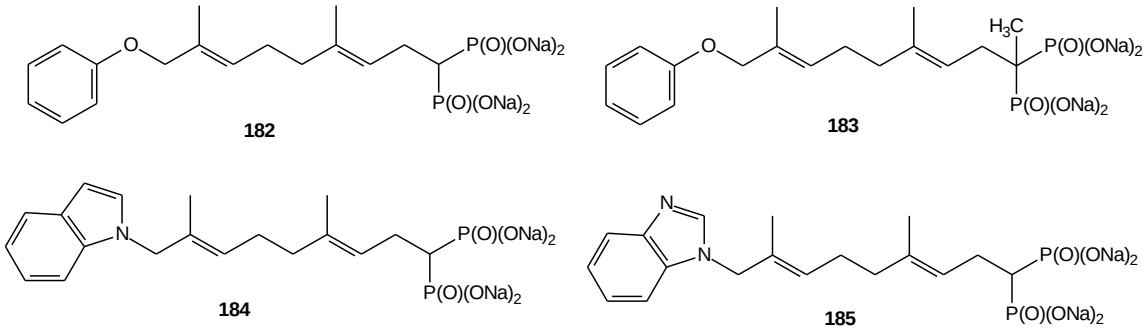


Figure 62. Target inhibitors of the enzyme Rv2361c.

Synthesis of the aniline derivative **188** was straightforward (Figure 63). The previously described compound **186** was treated with MnO_2 to provide the α,β-unsaturated aldehyde **187** in sufficient yield. The aldehyde **187** then was subjected to reductive amination conditions to provide the aniline derivative **188** in good yield.

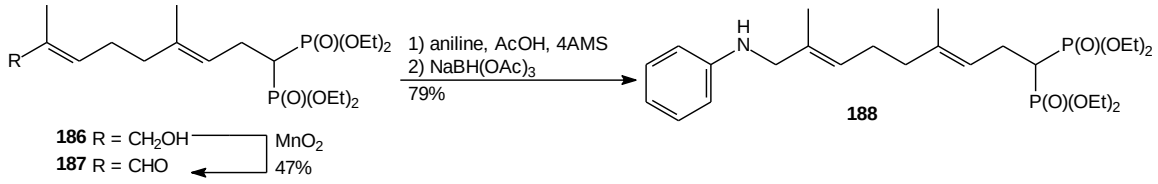


Figure 63. Synthesis of the aniline analogue **188**.

The synthesis of the indole analogue **193** (Figure 64) began with treatment of the monoprotected allylic diol **197** with MsCl followed by treatment with LiBr to provide the reactive bromide **189**. Next, indole was treated with NaH in DMF/THF followed by addition of allylic bromide **189** to provide acetate **190**. It should be noted that yields were much improved in this system when using the mixed DMF/THF solvent system as compared to alkylation in THF alone. The acetate removal was achieved in quantitative yield upon treatment of ester **190** with K_2CO_3 in methanol to provide the target alcohol **191**. The allylic bromide **192** is formed via a sequence parallel to that described later for conversion of alcohol **197** to bromide **189**. This bromide **192** then was allowed to react with the anion of tetraethyl methylenebisphosphonate to provide the 9-substituted geranylbisphosphonate ester **193** in 32% yield over the final three steps.

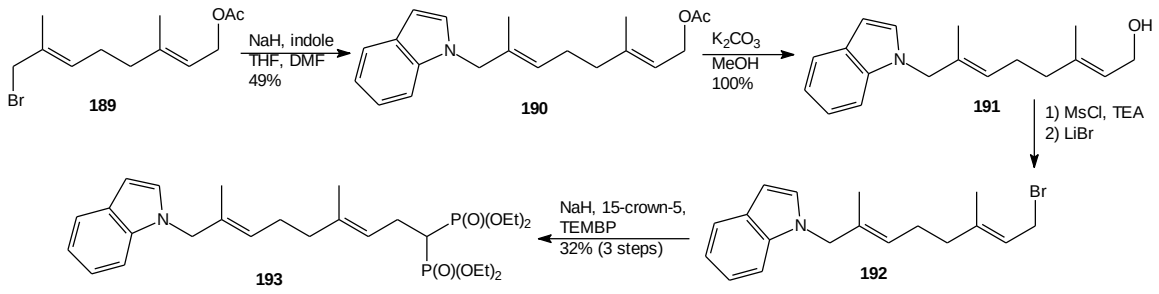


Figure 64. Synthesis of the indole compound **193**.

The attempted synthesis of the benzimidazole derivative **196** (Figure 65) was undertaken via a parallel reaction sequence. Unfortunately, while both alkylation of the benzimidazole analogue and hydrolysis of the acetate **194** proved straightforward, all attempts at preparation and subsequent alkylation of the bromide **195** resulted in the formation of an unreactive polymer. This polymer also failed to provide the target compound when treated with the bisphosphonate anion.

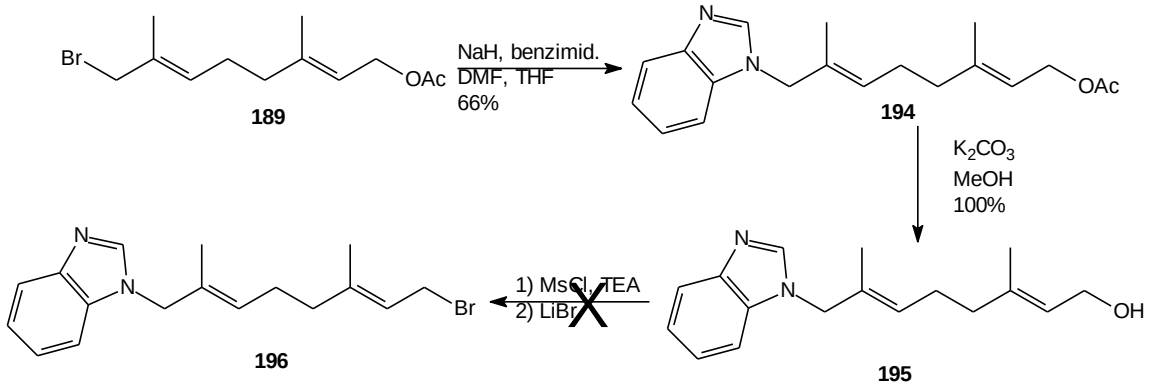


Figure 65. The attempted synthesis of the benzimidazole **185**.

Synthesis of the phenol analogs **200** and **201** proceeded more smoothly (Figure 66). Through a four step sequence from the alcohol **187**, the bromide **189** was prepared as described above. The bromide next was treated with phenol, potassium carbonate, and catalytic potassium iodide at reflux and gave the desired product. When the KI was omitted, the reaction did not proceed nearly as well. It is hypothesized that the allylic bromide undergoes an *in situ* Finkelstein reaction in the presence of KI, forming the more reactive allylic iodide. The intermediate ester was deprotected using K_2CO_3 in MeOH providng the allylic alcohol **198** in good yield (56%) over 4 steps. The allylic alcohol **198** was converted to the bromide through a two step sequence as described previously, and the resulting bromide was treated with the anion of tetraethyl methylenebisphosphonate to provide the target bisphosphonate **200** in 74% yield.

An aliquot of bisphosphonate **200** then was methylated cleanly using NaH and MeI to provide the dialkyl compound **201**. The α-methylated compound was synthesized because there is some evidence that α-methylation provides a more active compound when compared to the parent structure, and the synthesis and subsequent assay of this compound would allow us to probe this possibility further.

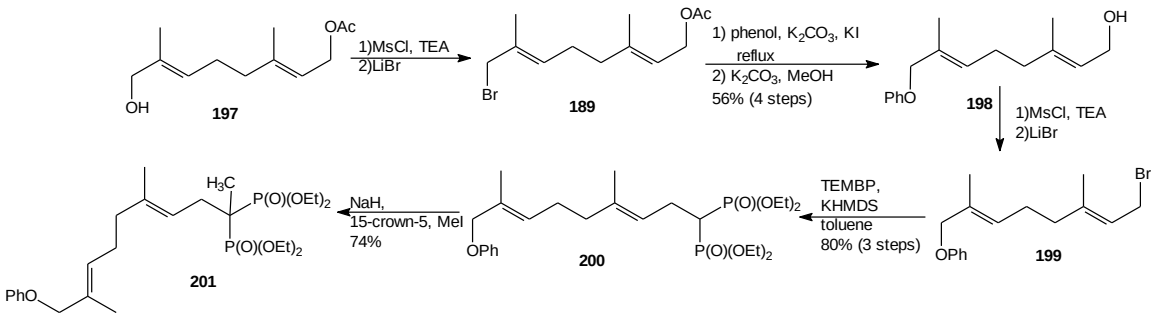


Figure 66. Synthesis of the phenyl ether derivatives **200** and **201**.

The hydrolysis of compounds **193**, **200**, and **201** (Figure 67) proved successful, with compound **188** being the only bisphosphonate that failed to give the desired tetrasodium salt. It should be noted that difficulty was encountered in several of these hydrolyses using the published conditions.[144] However, when the equivalents of TMSBr and collidine were increased, the hydrolyses proceeded cleanly and gave compounds **182**, **183**, and **184** in good yield.

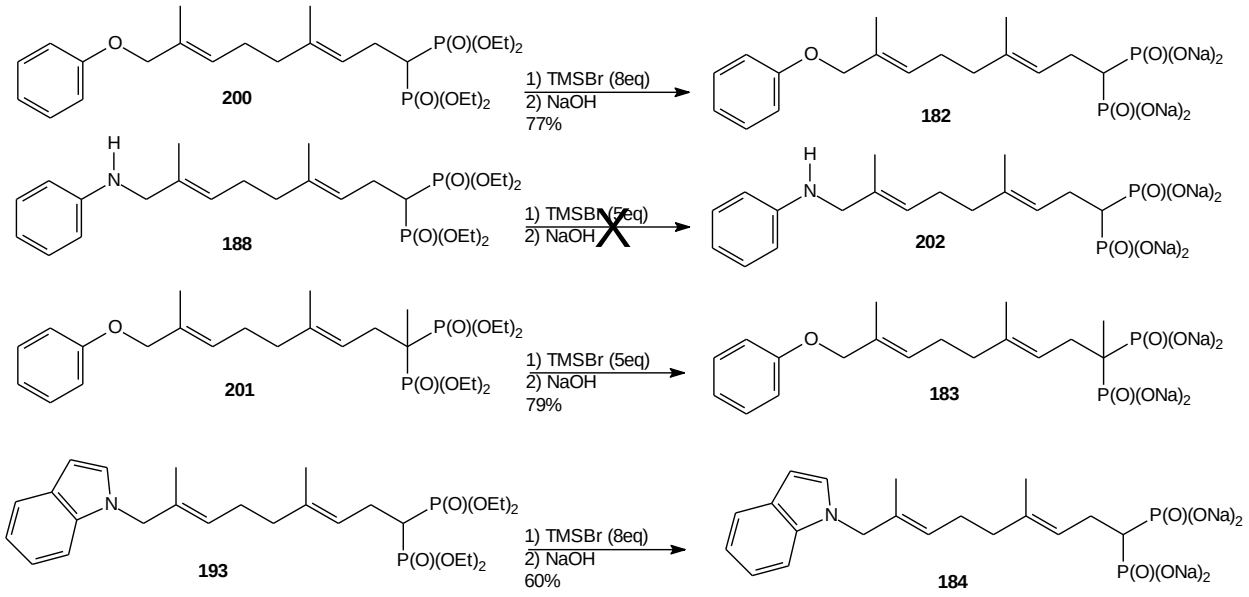


Figure 67. Hydrolysis of key intermediate phosphonate esters.

As detailed in Figure 68, the compounds synthesized as potential inhibitors of Rv2361c have been assayed *in vivo* and show varying degrees of activity. While none of the three new compounds exceeded the potency of the lead compound, **181**, it is noteworthy that methylation of phenyl ether **182** resulted in increased activity in this assay. Further work is needed, but this may indicate that in general methylation of the alpha carbon provides increased potency, and suggests that it would be worthwhile to methylate compound **181**. Furthermore, the assay data

suggests that either or both the carboxylate functionality and/or the hydrogen bond donor capability of the parent compounds aniline hydrogen may be critical for effective inhibition of the enzyme.

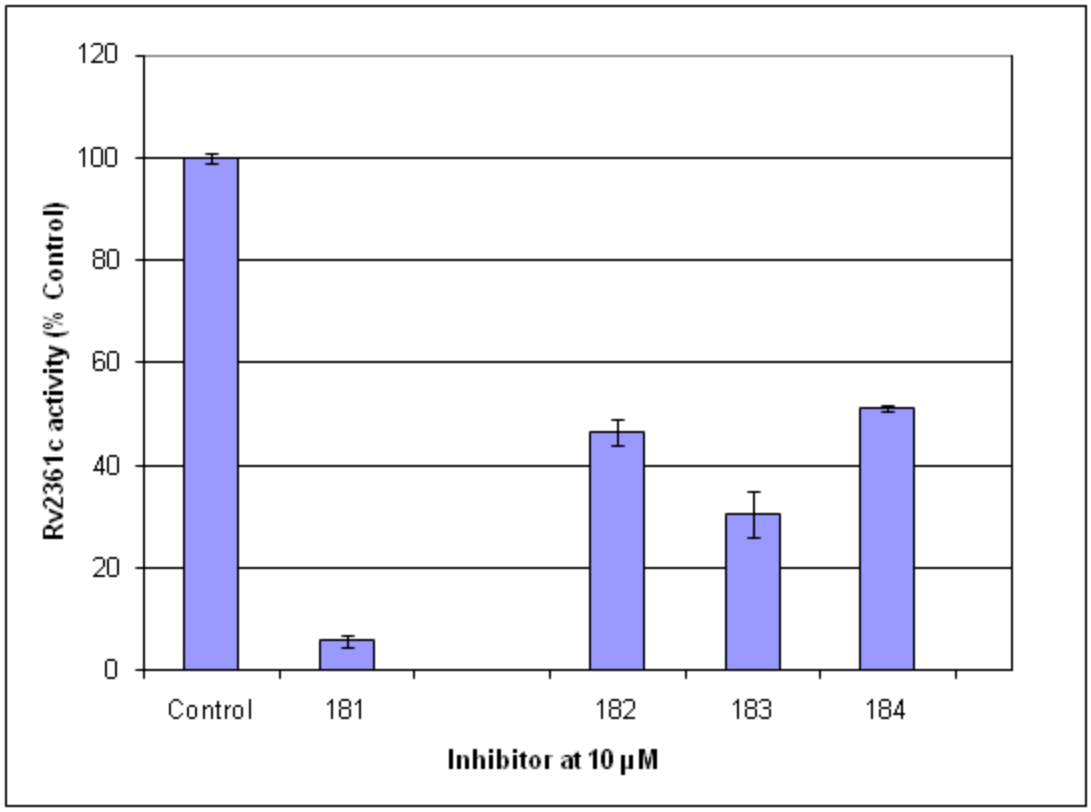


Figure 68. The *in vitro* inhibition of Rv2361.

CHAPTER VI

CONCLUSIONS AND FUTURE WORK

As discussed in Chapter Two, a range of benzylic and allylic phosphonates has been synthesized directly from the alcohol precursors in a one-flask ZnI_2 mediated transformation. Generally, the reactions proceed smoothly and provide the target phosphonates in good yield. We also have been able to demonstrate that the Lewis acid $ZnBr_2$ is able to facilitate the transformation, albeit longer reaction times and slightly attenuated yields are observed. When the direct conversion of aliphatic alcohols was attempted, the reaction did not proceed in the same manner and instead two major products were isolated and identified. Both the unsymmetrical ethylphosphonate **81** and the unsymmetrical dialkylphosphite **82** (the likely precursor to compound **81**) were isolated and characterized by ^{1}H, ^{13}C, ^{31}P NMR and high resolution mass spectroscopy. This process may be a limitation in terms of phosphonate synthesis, but it is an unprecedented reaction and ultimately may provide access to asymmetric phosphonates and phosphites if the need arises.

There is still a great deal of work that can be done to expand the scope of this reaction. Direct conversion of activated functional groups other than alcohols, such as acetals **203** and acetates **204** (Figure 69), may be possible with this system, and this prospect will need to be explored. Furthermore, the nature of the intermediates in the reaction is not completely understood. It would be insightful to run reactions in which only alcohol and ZnI_2 are present to probe whether or not these conditions would provide the allylic and benzylic iodides. Furthermore, identification of the phosphite-zinc complex could be undertaken by NMR experiments. In addition, direct displacement of α-hydroxyphosphonates **205** or α-hydroxy esters **206** (Figure 69) could give rise to bisphosphonate and phosphonocarboxylate compounds whose potential applications have been discussed (*vide supra*). Finally, analogous to the work of

Sarmah and coworkers,[97] it may also be interesting to probe the effect of the zinc iodide system on 1,2 diols (e.g. **207)**.

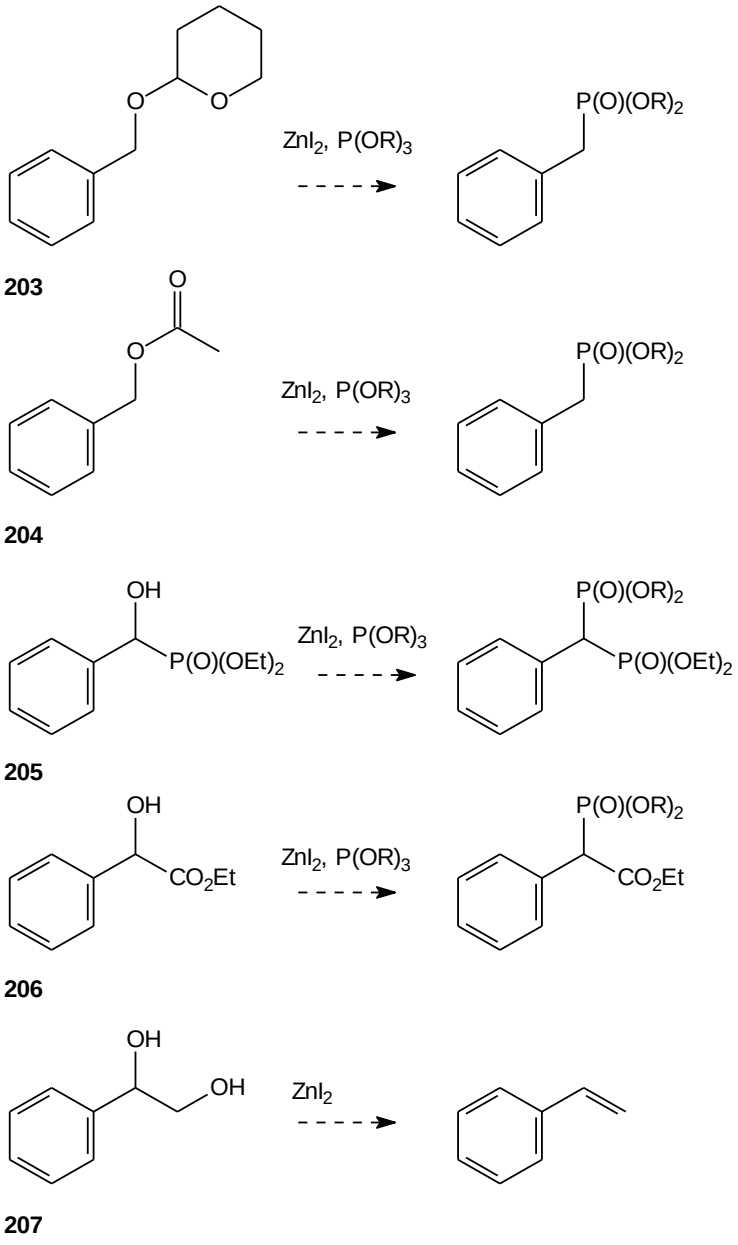


Figure 69. Reactions to be explored using the standard zinc iodide conditions.

In Chapter Three the synthesis of α-heteroatomic bisphosphonates was explored. A majority of the clinically used bisphosphonates possess a heteroatom, usually a hydroxyl group, adjacent to the phosphorus atoms. It has been demonstrated that these clinical bisphosphonates act through inhibition of the enzyme FDPS. While the importance of this functionality has been explored in regard to both bone affinity and impact on FDPS, it is uncertain how this modification will affect inhibitors of GGDPS. To explore the impact of this modification we attempted various routes toward the synthesis of analogs of DGBP having a heteroatom at the α-position. Unfortunately, our efforts at the synthesis of analogues of DGBP were stifled, but to our delight the synthesis of an analog of the potent and selective GGDPS inhibitor MGBP was successfully prepared and has been submitted to our collaborator for biological assay.

Continuing our study of bisphosphonates as inhibitors of GGDPS we successfully designed and synthesized a series of aromatic bisphosphonates. There were several motivations for this undertaking. Many FDPS inhibitors have an aromatic substructure and it was unknown whether introduction of an aromatic moiety to a GGDPS inhibitor would have an effect on enzyme selectivity. Also, the aromatic functionality would allow us to probe the impact of added steric bulk on GGDPS inhibition, and eliminate the potential for olefin isomerization or transposition *in vivo*. Finally, the crystal structure of digeranyl bisphosphonate in the active site of the enzyme gives some indication that an aromatic moiety may maximize favorable π stacking interactions with proximal residues.

Of the series of bisphosphonates synthesized and assayed, the dialkyl bisphosphonates **135**, **142**, **145**, and **148** all showed various degrees of GGDPS inhibition while the monoalkyl compound displayed little or no activity. Compounds **135** and **142** proved to be active with the *meta* substituted analog **135** being the most potent, and they retained both potency and selectivity in regard to inhibition of the enzyme GGDPS. Thus we have been able to show that incorporation of an aromatic moiety into analogues of DGBP allows for retention of specificity and potency for the enzyme GGDPS.

In Chapter Four the enzyme Rab geranylgeranyl transferase (RGGTase) was explored as a promising target for the treatment of disease. The enzyme RGGTase is a key enzyme in the mevalonate pathway and is unique compared to other prenyl transferase proteins in that it requires participation of an auxiliary protein to facilitate geranylgeranylation. While the work discussed in Chapter Four is still in its infancy, inroads have been made. To better understand this enzyme, and ultimately to develop drug therapies exploiting this target protein, we have begun the project of developing potent and selective inhibitors of RGGTase. A survey of the literature provides few examples of selective RGGTase inhibitors, with 3-PEHPC being the most widely recognized. We chose to use 3-PEHPC as the "baseline" for our future inhibitors, and therefore synthesis of this control compound was undertaken. Early on it became evident that although synthesis of 3-PEHPC is described vaguely in patent literature, using this reference as a "road map" proved to be difficult. After exploring several different conditions it was found that synthesis of 3-PEHPC via the previously undescribed triethyl phosphonocarboxylate **166** proved more desirable and gave the target compound in good yields. It is known that dimethyl phosphite can undergo a disproportionation reaction to yield dimethyl methylphosphonate, and it is our hypothesis that this competing reaction is the reason for the unacceptable yield when attempting to make phosphonocarboxylate **163** using dimethyl phosphite. With diethyl phosphite as the phosphorus nucleophile yields were good, allowing us to synthesize the target 3-PEHPC in sufficient quantities to begin biological investigations.

Of the wide range of functionalities that could be explored as inhibitors of RGGTase we have chosen to explore first the phosphonophosphinate moiety. The phosphonophosphinate substructure is attractive as it has a net charge when completely deprotonated identical to 3-PEHPC, but it has a skeleton that allows for additional modifications not possible in a phosphonocarboxylate structure. Furthermore, the geometry of the phosphonophosphinate more closely resembles that of the natural pyrophosphate substructure making it an attractive starting point for the synthesis of RGGTase inhibitors. While there are several methods of synthesizing phosphonophosphinates in the literature, the route we chose was to make the methylene

phosphonophosphinate from the commercially available diethyl methylphosphonate. This was attractive as it allowed us to make a "core" phosphonophosphinate that can be alkylated at either the methyl or methylene carbons presumably with a range of electrophiles. Synthesis of the trisodium phosphonophosphinate salt proceeds smoothly. Initial assays indicate the compound has a toxicity equipotent to our selective and potent GGDPS inhibitor DGBP, but inspection of the Western blot analysis indicates that the compound is not inhibiting geranylgeranylation or farnesylation. These results implicate a target, which if enzymatic, is most likely neither GGDPS nor FDPS. Further bioassay regarding this compound's inhibition of RGGTase is forthcoming.

Regardless of the outcome of the biological assay, the availability of the methylene phosphonophosphinate as a starting material should prove valuable in future work. It is rational to make the phosphonophosphinate analog **208** of 3-PEHPC which should be able to be accomplished from the commercially available bromide. Further analogs (Figure 70), including N-oxide **209**, phosphonophosphinate **210**, substituted pyridine **211**, 2-amino pyridine **212**, and pyridinone **213** should provide additional insights into the impact of structural modifications on activity as enzyme inhibitors.

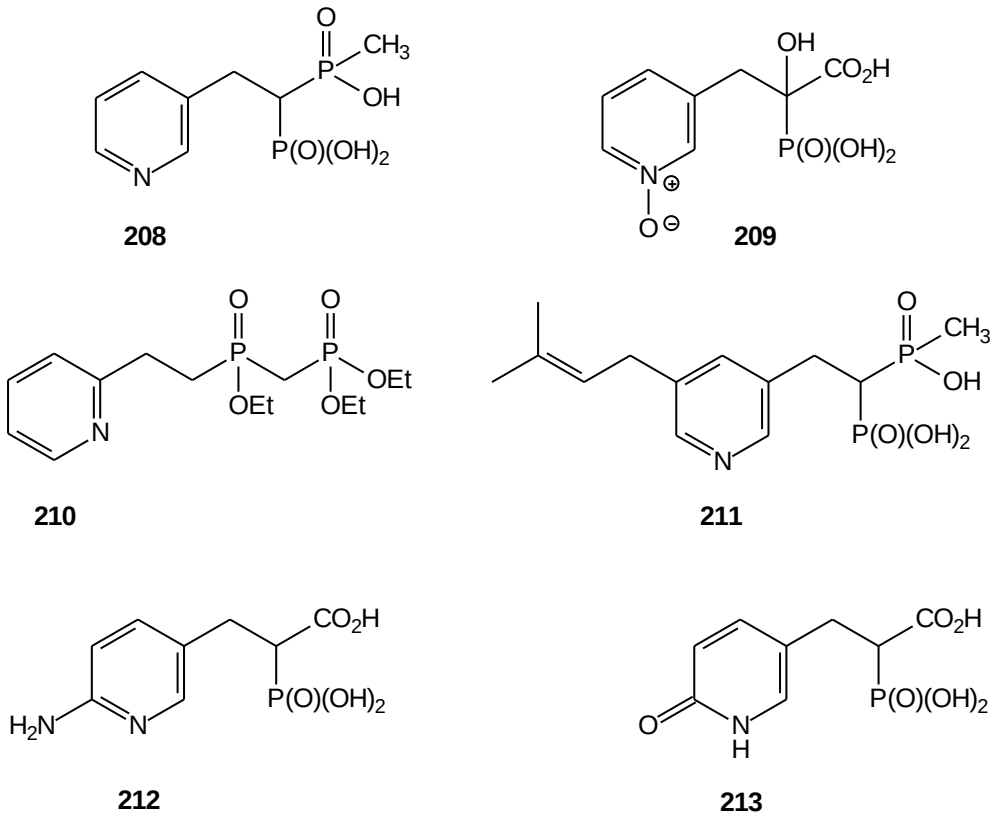


Figure 70. Potential inhibitors of RGGTase.

Tuberculosis is a global problem with more than one third of the world population exposed. The high rates of morbidity and mortality associated with TB, as well as the increasing prevalence of drug resistant strains of the bacteria, make the development of potent new drugs to target TB a critical task.

The *Mycobacterium tuberculosis* bacterium has an atypical cell wall with unusually high lipid content which contributes to its virulence and complicates drug therapy. Our approach to designing drugs as potential TB therapies is focused on disrupting the biosynthesis of this unique feature. Cell wall biosynthesis is a reasonable target because it is essential to bacterial survival. It is our contention that inhibiting key enzymes involved with cell wall biosynthesis will disrupt the process, ultimately leading to bacterial cell death. We have chosen to select the enzyme Rv2361c, thought to be a key enzyme in cell wall biosynthesis, as our initial target for enzymatic

inhibition. Given the substantial library of phosphonate compounds synthesized in the Wiemer labs, our collaborators in the laboratory of Dr Raymond Hohl undertook a general study of a wide range of phosphonates as inhibitors of Rv2361c. The initial results indicated a few key structures in common with all of the most active compounds. In general, the most active compounds were monogeranylated and had functionality at the C-9-postion of the geranyl chain. Of all the compounds tested, the anthranilate derivative was the most potent in the assays performed. Using this set of compounds as a guide, a few new targets as inhibitors of Rv2361 were designed and synthesized. The phenyl ether **182**, α-methyl phenyl ether **183**, and indole analog **184** all were synthesized successfully.

Of the compounds synthesized, the α-methyl phenyl ether **183** was the most active but failed to exceed the activity of the anthranilate analog **181** in the enzyme assay. Nonetheless, these data are helpful because when taken into consideration with earlier data (Figure 61) it appears that the simple modification of introducing an α-methyl group enhances inhibition of the target enzyme, Rv2361c.

There is much work yet to be done on this project. The aniline derivative **203** should be synthesized to establish the importance of the carboxylate functionality in the pharmacophore of compound **181**. Also, the benzimidazole derivative **185** should be revisited. Several modifications at the 9-position, including synthesis of the salicylate analogue **214** as well as modifications to the head as in phosphonosulfonate **215** and phosphonophosphinate **216** also should be explored (Figure 71).

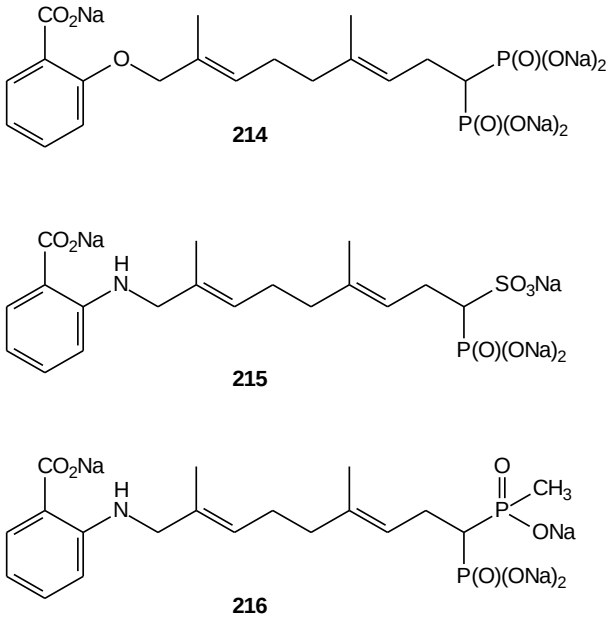


Figure 71. Potential inhibitors of enzyme Rv2361c.

In conclusion, these studies have resulted in a novel one-flask method for the direct conversion of benzylic and allylic alcohols to the corresponding phosphonates. This transformation has proven successful on both electron rich and electron deficient benzylic alcohols as well as allylic alcohols. We have developed a new class of aromatic bisphosphonates and demonstrated their activity and specificity for inhibition of GGDPS. Furthermore, the first α-amino analogue of our GGDPS inhibitor has been synthesized and submitted for biological assay. We also have synthesized the first phosphonophosphinate analog DGBP, setting the stage for future compounds designed as inhibitors of RGGTase. Finally, we have probed the pharmacophore of a potent inhibitor of enzyme Rv2361c through the synthesis and biological assay of a set of C-9-substituted geranyl bisphosphonates, establishing critical structural components for the further design and synthesis of inhibitors of Rv2361c enzyme. Taken together, these studies reconfirm the utility of organophosphorus compounds as inhibitors of different steps in the isoprenoid biosynthetic pathways and initiate several very promising new areas of research.

CHAPTER VII

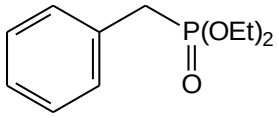
EXPERIMENTAL

General experimental conditions.

Tetrahydrofuran (THF) was distilled from sodium and benzophenone immediately prior to use while toluene was dried over activated molecular sieves. All non-aqueous reactions were done under an argon atmosphere, in oven-dried or flame-dried glassware, and with magnetic stirring. Flash chromatography was done on silica gel with an average particle size of 40-63 μm. The ^{1}H NMR spectra were recorded at 300 MHz (75 MHz for ^{13}C) with $CDCl_3$ as solvent and $(CH_3)_4Si$ as internal standard unless otherwise noted. High resolution (HRMS) mass spectra were obtained at the University of Iowa Mass Spectrometry Facility.

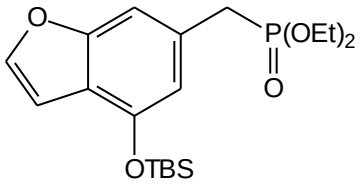
General experimental procedure for the ZnI_2 mediated phosphonylation of benzylic and allylic alcohols.

To a stirred solution of ZnI_2 (1.5 eq) in anhydrous toluene or freshly distilled THF was added $P(OEt)_3$ (3 eq) followed by the benzylic alcohol. The reaction mixture was allowed to stir at reflux overnight (approximately 12 hours). After it had cooled to room temperature, the reaction mixture was immediately placed on a vacuum line to remove volatiles. The residue then was washed with NaOH until the solids dissolved, extracted with ether, dried ($MgSO_4$), and concentrated *in vacuo*. The resulting oil was purified via flash column chromatography to give the desired diethyl phosphonate.



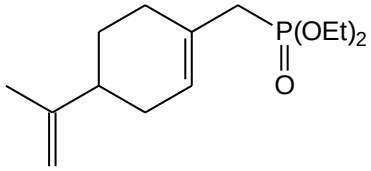
Diethyl benzylphosphonate (52).

To a stirred solution of $ZnBr_2$ (1.63 g. 7.24 mmol, 1.49 eq) in anhydrous toluene (5 mL) was added $P(OEt)_3$ (2.50 mL, 14.6, 3.0 eq) followed by benzyl alcohol (0.5 mL, 4.83 mmol, 1.0 eq). The reaction mixture was allowed to stir at reflux for 24 hours. After it was allowed to cool to room temperature, the reaction mixture was immediately placed on a vacuum line to remove volatiles. The residue then was washed with 2M NaOH until the solids dissolved, extracted with ether, the combined ethereal extracts were dried ($MgSO_4$), and concentrated *in vacuo*. The resulting oil was purified via flash column chromatography (30%EtOAc in hexanes) to give the desired diethyl benzylphosphonate (**56**, 75%, 812 mg). The ^{1}H NMR data correlated to the literature values.[88]



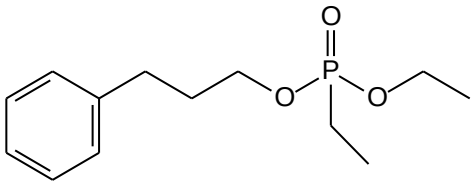
Diethyl phosphonate 72.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (dd, J = 2.3, J_{HP} = 1.0 Hz, 1H), 7.09–7.08 (m, 1H), 6.74 (dd, J = 2.2, J_{HP} = 0.8 Hz, 1H), 6.64 (d, J_{HP} = 2.25 Hz, 1H), 4.04–3.99 (m, 4H), 3.20 (d, J_{HP} = 21.5 Hz, 2H), 1.25 (t, J = 7.2 Hz, 6H), 1.04 (s, 9H), 0.24 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5 (d, J_{CP} = 2.8 Hz), 148.9 (d, J_{CP} = 3.0 Hz), 143.6 (d, J_{CP} = 1.4 Hz), 128.5 (d, J_{CP} = 9.2 Hz), 119.5 (d, J_{CP} = 3.0 Hz), 114.3 (d, J_{CP} = 6.1 Hz), 106.4 (d, J_{CP} = 7.6 Hz), 104.1 (d, J_{CP} = 1.6 Hz), 62.1 (d, J_{CP} = 6.8 Hz, 2C), 33.9 (d, J_{CP} = 138.6 Hz), 25.6 (3C), 18.2, 16.4 (d, J_{CP} = 5.9 Hz, 2C), –4.4 (2C); ^{31}P NMR ($CDCl_3$) δ 26.3. Anal. Calcd for $C_{19}H_{31}O_5PSi$: C, 57.26; H, 7.84. Found: C, 56.96; H, 8.02.



Diethyl phosphonate 77.

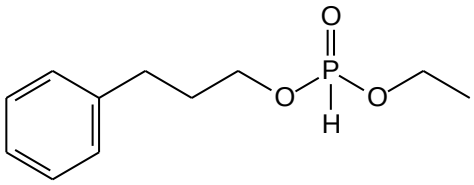
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (br s, 1H), 4.73–4.70 (m, 2H), 4.15–4.05 (m, 4H), 2.52 (d, J_{HP} = 21.8 Hz, 2H), 2.21–2.09 (m, 4H), 2.03–1.92 (m, 1H), 1.87–1.78 (m, 1H), 1.73 (s, 3H), 1.55–1.42 (m, 1H), 1.32 (t, J = 7.1 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.5, 127.9 (d, J_{CP} = 11.0 Hz), 125.8 (d, J_{CP} = 12.5 Hz), 108.5, 61.6 (d, J_{CP} = 1.8 Hz), 61.5 (d, J_{CP} = 1.9 Hz), 40.3, 34.8 (d, J_{CP} = 137.7 Hz), 30.7 (d, J_{CP} = 2.8 Hz), 29.7 (d, J_{CP} = 2.8 Hz), 27.6, 20.6, 16.3 (d, J_{CP} = 6.1 Hz, 2C); ^{31}P NMR ($CDCl_3$) δ 27.9; HRMS (EI^+, *m/z*) calcd for (M^+) $C_{14}H_{25}O_3P$: 272.1541; found: 272.1535.



Ethyl 3-phenylpropyl ethylphosphonate (81).

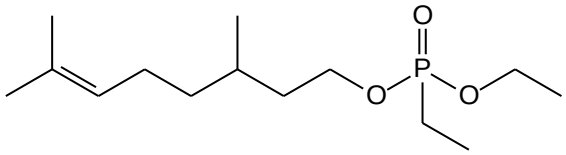
According to the general procedure, 3-phenylpropanol (1.0 mL, 7.35 mmol, 1.0 eq) in toluene was treated with ZnI_2 (3.59 g, 11.3 mmol, 1.5 eq) and $P(OEt)_3$ (3.9 mL, 22.7 mmol, 3.1 eq) and held at reflux for 12 hrs. The resulting solution was allowed to cool to room temperature and the volatiles were removed. After the residue was dissolved in diethyl ether and washed with 2N NaOH, the diethyl ether portion was dried ($MgSO_4$) and concentrated *in vacuo*. Final purification was achieved by flash column chromatography (gradient 25-50% EtOAc in hexanes) to provide phosphonate **81** (530 mg, 28%) along with phosphite **82** (478 mg, 27%): ^{1}H NMR (300 MHz) δ 7.30–7.23 (m, 2H), 7.19–7.16 (m, 3H), 4.14–4.00 (m, 4H), 2.71 (t, J = 7.2 Hz, 2H), 2.03–1.92 (m, 2H), 1.81–1.66 (m, 2H), 1.31 (t, J = 6.6 Hz, 3H), 1.16 (dt, J_{HP} = 19.8, J = 7.8 Hz,

3H); ^{13}C NMR (75 MHz) δ 140.7, 128.1 (4C), 125.6, 64.1 (d, J_{CP} = 6.5 Hz), 61.1 (d, J_{CP} = 6.6 Hz), 31.8 (d, J_{CP} = 6.2 Hz), 31.4, 18.3 (d, J_{CP} = 141.5 Hz), 16.1 (d, J_{CP} = 5.9 Hz), 6.3 (d, J_{CP} = 6.8 Hz); ^{31}P NMR ($CDCl_3$) δ 33.7; HRMS (EI^+, *m/z*) calcd for (M^+) $C_{13}H_{21}O_3P$: 256.1228; found: 256.1220.



Ethyl 3-phenylpropyl phosphite (82).

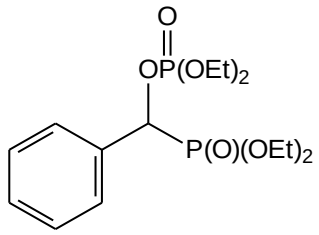
^{1}H NMR δ 7.16 (d, J = 6.9 Hz, 2H), 7.09–7.03 (m, 3H), 6.69 (d, J_{HP} = 693 Hz, 1H), 4.08–3.91 (m, 4H), 2.61 (t, J = 7.5 Hz, 2H), 1.94–1.84 (m, 2H), 1.24 (t, J = 6.9 Hz, 3H); ^{13}C NMR δ 140.3, 128.0 (2C), 127.9 (2C), 125.7, 64.3 (d, J_{CP} = 5.6 Hz), 61.4 (d, J_{CP} = 5.8 Hz), 31.5 (d, J_{CP} = 6.4 Hz), 31.1, 15.9 (d, J_{CP} = 6.1 Hz); ^{31}P NMR δ 7.61; HRMS (EI^+, *m/z*) calcd for (M^+) $C_{11}H_{17}O_3P$: 228.0915; found: 228.0923.



Ethyl citronellyl ethylphosphonate (85).

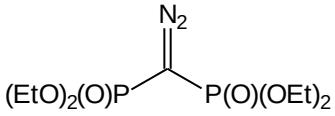
According to the general procedure, citronellol (0.5 mL, 2.75 mmol, 1.0 eq) in toluene was treated with ZnI_2 (1.32 g, 4.13 mmol, 1.5 eq) and $P(OEt)_3$ (1.4 mL, 8.34 mmol, 3.0 eq) for 12 hrs. The resulting solution was allowed to cool to room temperature and the volatiles were removed. The residue was dissolved in diethyl ether and washed with 2N NaOH. The diethyl ether portion was dried ($MgSO_4$) and concentrated *in vacuo*. Final purification was achieved by flash column chromatography (50% EtOAc in hexanes) to provide phosphonate **85** in 45% yield

(342 mg): ^{1}H NMR δ 5.12–5.06 (m, 1H), 4.15–3.96 (m, 4H), 2.01–1.88 (m, 2H), 1.81–1.65 (m, 3H), 1.68 (br s, 3H), 1.60 (br s, 3H), 1.57–1.35 (m, 4H), 1.32 (t, J = 7.5 Hz, 3H), 1.16 (dt, J_{HP} = 19.8, J = 7.5 Hz, 3H), 0.92 (d, J = 6.3 Hz, 3H); ^{31}P NMR δ 33.4 Hz



Phosphonophosphate 99.

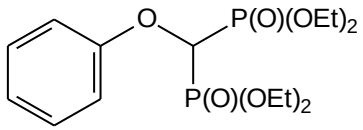
To a stirred solution of diethyl benzylphosphonate (2.0 mL, 9.59 mmol, 1.0 eq) at 0 °C was added triethyl phosphite (2.05 mL, 11.96 mmol, 1.25 eq) and pyridinium chloride (1.38 g, 11.93 mmol, 1.24 eq) and the solution was allowed to stir at room temperature overnight. After NH_4Cl was added, the solution was extracted with CH_2Cl_2, and the combined extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*. Final purification was achieved by flash column chromatography (5% MeOH in Et_2O) to provide compound **99** in good yield (85%, 3.65 g) with ^{1}H NMR data well correlated to the literature values.[184]



Tetraethyl diazobisphosphonate 111.[139]

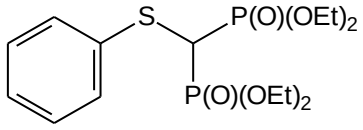
Sodium hydride (60% in mineral oil, 525 mg, 13.1 mmol, 1.26 eq) was placed in a round bottom flask under argon. THF (80 mL) was added, and the slurry was cooled to 0 °C in an ice bath with stirring. Tetraethyl methylenebisphosphonate (3.00 g, 10.4 mmol, 1.00 eq) was added slowly via syringe, and then the suspension was removed from the ice bath and allowed to stir at room temperature for 20 minutes. The suspension was once again cooled to 0 °C and a 0.86 M solution of tosyl azide (14 mL, 12.0 mmol, 1.15 eq) in THF was added slowly. After the

addition was complete, the solution was kept at 0 °C for 30 minutes, then allowed to warm to room temperature and stirred overnight. Water was added, and the resulting emulsion was diluted with diethyl ether. The organic extracts were washed with 1M NaOH, H_2O and brine, and then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Final purification by flash column chromatography (5% MeOH in Et_2O) gave the target compound **111** (1.85 g, 57%): ^{1}H NMR δ 4.16–4.05 (m, 8H), 1.33–1.25 (m, 12H); ^{13}C NMR δ 63.2 (4C), 38.4 (t, J_{CP} = 203.5 Hz), 15.9 (4C); ^{31}P NMR δ 13.9



Tetraethyl phenoxybisphosphonate 112.

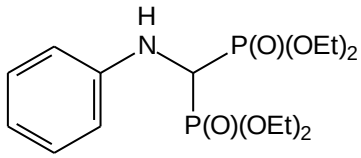
Diazobisphosphonate **111** (501 mg, 1.59 mmol, 1.00 eq) was added via syringe to a suspension of phenol (174 mg, 1.98 mmol, 1.24 eq) and copper (II) triflate (12 mg, 2 mol %) in toluene (24 mL). The flask was fitted with a reflux condenser and heated at reflux overnight. The volatile organics were removed *in vacuo*, and final purification was achieved by flash column chromatography (1:1 acetone:CH_2Cl_2) to afford the target bisphosphonate **112** (199 mg, 33%). ^{1}H, ^{13}C, and ^{31}P NMR data agreed with published values.[140]



Tetraethyl phenylthiobisphosphonate 115.

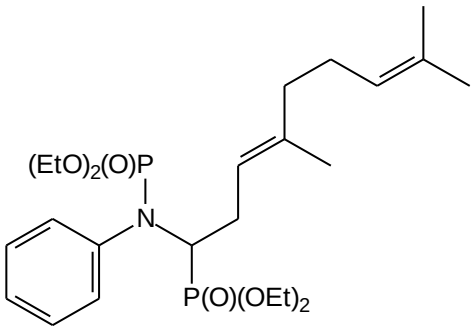
Solid NaH (60% in mineral oil, 272 mg, 6.80 mmol, 1.31 eq) was placed in a round bottom flask, THF was added, and the resulting suspension was cooled to 0 °C in an ice bath. Once the suspension had been thoroughly cooled, tetraethyl methylenebisphosphonate (1.50 g,

5.21 mmol, 1.00 eq) was added slowly via syringe. After the addition was complete the solution was allowed to stir for 30 minutes. Phenyl disulfide (1.47 g, 6.74 mmol, 1.30 eq) was added as a solid and the solution was allowed to warm to room temperature and stirred overnight. After 1N HCl was added to the solution until it was acidic, it was extracted with diethyl ether. The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Final purification by flash column chromatography (gradient 5-8% MeOH in Et_2O) provided the target compound **115** (1.35 g, 65%). The ^{1}H and ^{31}P NMR data corresponded to the published[185] values: ^{13}C NMR δ 134.7 (t, J_{CP} = 3.5 Hz), 131.1 (2C), 128.7 (2C), 127.6, 63.5–63.1 (m, 4C), 42.1 (t, J_{CP} = 137.0 Hz), 16.0–15.7 (m, 4C)



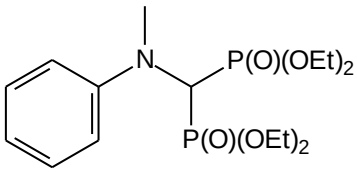
Tetraethyl anilinobisphosphonate 119.

Diazobisphosphonate **111** (509 mg, 1.62 mmol, 1.00 eq) was added via syringe to a suspension of aniline (0.16 mL, 1.75 mmol, 1.08 eq) and copper (II) triflate (12 mg, 2 mol %) in toluene (24 mL). The flask was fitted with a reflux condenser and heated at reflux overnight. The volatile organics were removed *in vacuo*, and final purification was achieved by flash column chromatography (1:1 acetone:CH_2Cl_2) to afford the target bisphosphonate **119** (348 mg, 57%). The ^{31}P NMR data corresponded with published[186] values: ^{1}H NMR δ 7.18 (t, J = 7.5 Hz, 2H), 6.77 (t, J = 7.5 Hz, 1H), 6.71 (d, J = 8.1 Hz, 2H), 4.31–4.07 (m, 9H), 1.29 (t, J = 7.2 Hz, 6H), 1.24 (t, J = 6.9 Hz, 6H); ^{13}C NMR δ 145.8 (t, J_{CP} = 4.0 Hz), 128.8 (2C), 118.6, 113.4 (2C), 63.1 (dt, J_{CP} = 32.6 Hz, J_{CP} = 3.1 Hz, 4C), 49.9 (t, J_{CP} = 146.2 Hz), 15.9 (dt, J_{CP} = 5.6, J_{CP} = 2.9 Hz, 4C); ^{31}P NMR δ 18.1



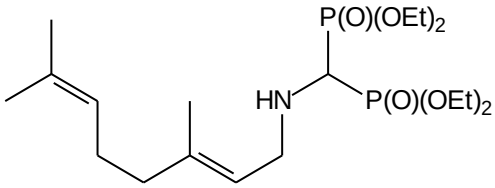
Diethyl phosphonophosphoramidate 122.

A suspension of NaH (60% in mineral oil, 55 mg, 1.38 mmol, 1.05 eq) in THF was cooled to 0 °C in an ice bath, and two drops of 15-crown-5 were added. Bisphosphonate **119** (502 mg, 1.38 mmol, 1.00 eq) was added slowly via syringe, the mixture was allowed to stir for 30 minutes, and then geranyl bromide (296 mg, 1.36 mmol, 1.03 eq) was added. The mixture was immediately removed from the ice bath and allowed to stir at room temperature overnight. Aqueous NH_4Cl was added and the solution was extracted with Et_2O, dried ($MgSO_4$), and concentrated *in vacuo*. Ultimate purification was achieved by flash column chromatography (30% acetone in CH_2Cl_2) to provide the product, **122** (218 mg, 32%): ^{1}H NMR δ 7.43 (d, J = 7.8 Hz, 2H), 7.33–7.25 (m, 3H), 5.44–5.38 (m, 1H), 5.15–5.10 (m, 1H), 4.56–4.41 (m, 1H), 4.24–3.95 (m, 8H), 2.39–2.32 (m, 2H), 2.18–2.04 (m, 4H), 1.70 (s, 3H), 1.62 (s, 3H), 1.57 (s, 3H), 1.30-1.15 (m, 12H); ^{13}C NMR δ 138.1, 137.6 (d, J_{CP} = 2.4 Hz), 131.4, 130.9 (dd, J_{CP} = 1.4, 1.0 Hz, 2C), 128.3 (d, J_{CP} = 4.8 Hz, 2C), 127.2 (d, J_{CP} = 1.5 Hz), 124.1, 121.0 (d, J_{CP} = 15.6 Hz), 62.7 (dd, J_{CP} = 6.2, 5.3 Hz, 2C), 61.8 (dd, J_{CP} = 6.4, 6.4 Hz, 2C), 56.7 (dd, J_{CP} = 154.6, 6.6 Hz), 39.7, 27.0 (d, J_{CP} = 5.3 Hz), 26.5, 25.6, 17.6, 16.3, 17.6–15.9 (m, 4C); ^{31}P NMR δ 25.4 (d, J_{PP} = 13.8 Hz), 5.6 (d, J_{PP} = 13.6 Hz); HRMS (EI$^+$, *m/z*) cald (M^+) $C_{25}H_{43}NO_6P_2$: 515.2566. Found: 515.2574.



Tetraethyl *N*-methyl anilinobisphosphonate 124.

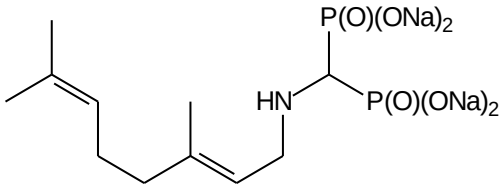
N-methylaniline (3.01 mL, 27.8 mmol, 1.00 eq), triethyl orthoformate (5.55 mL, 33.4 mmol, 1.20 eq), and diethyl phosphate (11.2 mL, 86.9 mmol, 3.13 eq) were combined and the resulting solution was held at reflux overnight. After the solution was allowed to cool to room temperature, the volatiles were removed under an air stream. Final purification by flash column chromatography (gradient, 10-40% acetone in CH_2Cl_2) provided **124** (482 mg, 20%). Both the ^1H and ^{31}P spectra correlated with the published[187] values: ^{13}C NMR δ 149.7 (t, J_{CP} = 3.2 Hz), 129.0 (2C), 118.3, 113.7 (2C), 63.2–62.8 (m, 4C), 56.8 (t, J_{CP} = 145.0 Hz), 35.8, 16.4–16.1 (m, 4C).



Tetraethyl *N*-geranyl bisphosphonate 127.

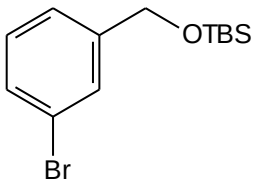
Geranyl amine (1.47 mL, 7.95 mmol, 1.00 eq), triethyl orthoformate (1.62 mL, 9.74 mmol, 1.23 eq), and diethyl phosphate (3.26 mL, 25.3 mmol, 3.18 eq) were combined in a flask fitted with a reflux condenser, and the solution was held at reflux overnight. The resulting solution was allowed to cool to room temperature and the volatiles were evaporated under an air stream. Final purification by flash column chromatography (5% MeOH in Et_2O) gave **127** (482 mg, 14%): ^1H NMR δ 5.23–5.18 (m, 1H), 5.11–5.07 (m, 1H), 4.27–4.16 (m, 8H), 3.47 (d, J = 7.2 Hz, 2H), 3.31 (t, J_{HP} = 22.0 Hz, 1H), 2.12–2.06 (m, 2H), 2.04–1.99 (m, 2H), 1.69 (s, 3H), 1.66 (s, 3H), 1.60 (s, 3H), 1.36 (t, J = 6.8 Hz, 12H); ^{13}C NMR δ 139.4, 131.4, 123.8, 121.6, 62.8 (d, J = 30.2 Hz, 4C), 52.3 (t, J = 144.8 Hz), 46.9 (t, J = 5.3 Hz), 39.5, 26.3, 25.5, 17.5, 16.4–

16.2 (m, 4C), 16.1; ^{31}P NMR δ 20.0; HRMS (EI$^+$, *m/z*) calcd for (M)$^+$ $C_{19}H_{39}NO_6P_2$:439.2273. Found 439.2268.



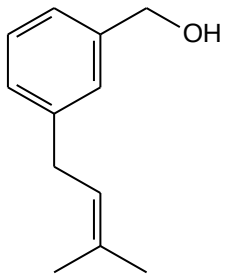
Tetrasodium *N*-geranyl bisphosphonate 128.

To a stirred solution of 2,4,6-collidine (0.48 mL, 3.02 mmol, 9.05 eq) in CH_2Cl_2 at 0 °C was added TMSBr (0.47 mL, 3.62 mmol, 9.05 eq) and the solution was allowed to stir for 10 minutes. After the bisphosphonate **127** (178 mg, 0.40 mmol, 1.0 eq) was added via syringe, the solution was allowed to stir overnight. The volatiles were removed and toluene was added then removed *in vacuo*. The resulting residue was treated with NaOH (1M, 2 mL, 2 mmol, 5.0 eq) and stirred overnight. The liquid was poured into acetone and stored at 4°C for 72 hrs. The resulting precipitate was filtered to provide the target compound in 66% yield (110 mg): ^{1}H NMR 5.35–5.26 (m, 1H), 5.17–5.07 (m, 1H), 3.89 (d, J = 7.5 Hz, 2H), 2.97 (t, J_{HP} = 16.5 Hz, 1H), 2.16–1.99 (m, 4H), 1.70 (br s, 3H), 1.61 (br s, 3H), 1.55 (br s, 3H); ^{13}C NMR δ 147.0, 133.9, 124.1, 114.5, 57.8 (t, J_{CP} = 114.5 Hz), 47.3, 40.0, 25.7, 25.0, 17.1, 16.1; ^{31}P NMR δ 8.6 ppm; HRMS (ESI, *m/z*) cald for (M-H)$^-$ $C_{11}H_{22}NO_6P_2$: 326.0922; found: 326.0923.



(3-bromobenzyloxy)(tert-butyl)dimethylsilane (130).

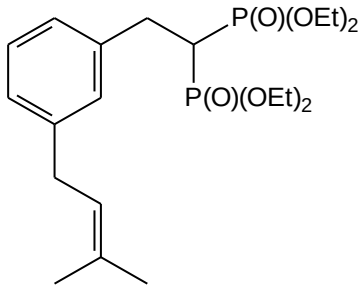
To a solution of 3-bromobenzyl alcohol (4.99 g, 26.7 mmol, 1.0 eq) in CH_2Cl_2 at 0 °C was added imidazole (8.89 g, 130 mmol, 4.90 eq) followed by TBSCl (5.24 g, 34.7 mmol, 1.30 eq). The reaction mixture was allowed to warm to room temperature and left to stir overnight. After the solution was quenched by addition of H_2O, it was extracted with CH_2Cl_2, dried ($MgSO_4$), and concentrated *in vacuo*. Final purification by flash chromatography (8% EtOAc in hexanes) afforded the TBS protected alcohol (7.26 g, 90%). Both the ^1H NMR and ^{13}C NMR data correlated to literature values.[188]



(3-(3-methylbut-2-enyl)phenyl)methanol (131).

A stirred solution of (**130**) (5.89 g, 19.5 mmol, 1.0 eq) in THF was cooled to –78 °C in a dry-ice/acetone bath. Once cooling was complete, a solution of *n*–BuLi in hexanes (10.2 mL, 2.1 M, 21.5 mmol, 1.1 eq.) was added slowly via syringe, and the solution was allowed to stir for 15 minutes. Prenyl bromide (3.82 g, 25.6 mmol, 1.3 eq.) then was added dropwise via syringe. After the solution was held at –78 °C for two hours, it was allowed to warm gradually to room temperature and stirred overnight. The resulting mixture was quenched by addition of H_2O, extracted with diethyl ether, dried ($MgSO_4$) and concentrated *in vacuo*. Without further purification, the initial product was dissolved in THF to make approximately a 2 M solution, cooled to 0 °C, and a 1 M solution of TBAF in THF (23.3 mmol, 1.2 eq) was added dropwise. The reaction was allowed to stir for 4 hours, and then quenched by addition of H_2O. The resulting mixture was extracted with diethyl ether, dried ($MgSO_4$), and concentrated *in vacuo*. Final purification by flash chromatography (10% EtOAc in hexanes) gave compound (**131**) (2.48

g, 72% over two steps): ^{1}H NMR δ 7.20–7.26 (m, 1H), 7.06–7.13 (m, 3H), 5.26-5.44 (m, 1H), 4.54 (s, 2H), 3.31 (d, *J* = 7.2 Hz, 2H), 2.57 (s, 1H), 1.73 (s, 3H),1.70 (s, 3H); ^{13}C NMR δ 142.0, 140.9, 132.4, 128.5, 127.4, 126.9, 124.3, 122.9, 65.0, 34.2, 25.6, 17.7; HRMS (ESI, *m/z*) calcd for $(M)^{+}$ $C_{12}H_{16}O$: 176.1201. Found 176.1204.2023

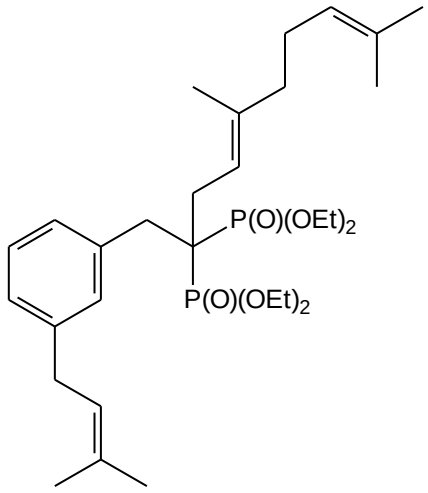


Tetraethyl 3-(prenyl) benzylphosphonate 132.

A stirred solution of (**131**) (2.00 g, 11.4 mmol, 1.0 eq) in CH_2Cl_2 was cooled to 0 °C in an ice bath, and triethylamine (2.01 mL, 14.5 mmol, 1.3 eq) was added followed by dropwise addition of MsCl (1.05 mL, 13.6 mmol, 1.2 eq). After the resulting solution was allowed to stir for 30 minutes, a solution of LiBr (2.52 g, 29.0 mmol, 2.6 eq) in anhydrous THF was added via syringe. The reaction mixture was allowed to stir for 1.5 hours then quenched by addition of H_2O followed by addition of saturated NaCl solution. The resulting solution was extracted with CH_2Cl_2, and the organic extract was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The resulting material was utilized directly in the next reaction without further purification.

To a stirred suspension of NaH (60% in mineral oil, 0.437 g, 10.9 mmol, 1.0 eq) in THF was added 15-crown-5 (0.21 mL, 1.06 mmol, 0.1 eq), and the resulting solution was cooled to 0 °C in an ice bath. Tetraethyl methylenebisphosphonate (3.46 g, 12.0 mmol, 1.1 eq) was added slowly via syringe and the reaction mixture was allowed to stir for 30 minutes. After a solution of the prepared bromide in THF was added slowly via syringe, the mixture was immediately removed from the ice bath and allowed to stir overnight. The resulting solution was filtered through a bed of fluorasil and concentrated *in vacuo*. Final purification by flash column chromatography (6%

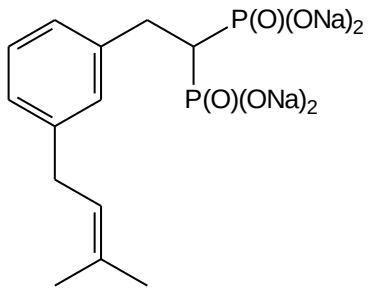
EtOH in hexanes) afforded the target bisphosphonate **132** (2.40 g, 47% overall yield from **131**): ^{1}H NMR δ 7.11 (dd, *J* = 8.1, 7.2 Hz, 1H), 7.04-7.00 (m, 2H), 6.94 (d, *J* = 7.2 Hz, 1H), 5.25-5.16 (m, 1H), 4.12-3.93 (m, 8H), 3.23 (d, *J* = 8.1 Hz, 2H), 3.15 (td, J_{PH} = 16.5, *J* = 6.0 Hz, 2H), 2.69-2.47 (m, 1H), 1.66 (s, 3H), 1.64 (s, 3H), 1.20 (t, *J* = 7.2, 6H), 1.18 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR δ 141.3, 139.3 (t, J_{CP} = 7.3 Hz), 131.9, 128.5, 127.9, 126.1, 125.9, 122.9, 62.1 (dd, J_{CP} = 13.4, 6.5 Hz, 4C), 38.7 (t, J_{CP} = 131.5), 33.9, 30.8 (t, J_{CP} = 4.7 Hz), 25.4, 17.4, 15.9 (d, J_{CP} = 6.4 Hz, 4C); ^{31}P NMR δ 23.6; HRMS (ESI, *m/z*) calcd for $(M)^{+}$ $C_{21}H_{36}O_6P_2$: 446.1987. Found: 446.1991.



Tetraethyl (3-prenyl benzyl) geranyl bisphosphonate 134.

A suspension of NaH (60% in mineral oil, 202 mg, 5.15 mmol, 2.4 eq) in THF was cooled to 0 °C in an ice bath. Once the solution was thoroughly cooled, 15-crown-5 (0.05 mL, 0.3 mmol, 0.1 eq) was added followed by **132** (0.956 g, 2.14 mmol, 1.0 eq) via syringe. The solution was allowed to stir for 30 minutes, then geranyl bromide (0.816 g, 3.76 mmol, 1.8 eq) was added, and the solution was allowed to stir overnight. After the reaction mixture was filtered through a bed of fluorasil, the filtrate was concentrated *in vacuo*. Final purification via flash chromatography (4% EtOH in hexanes) afforded the desired compound **134** (1.08 g, 86% yield): ^{1}H NMR δ 7.15–7.12 (m, 3H), 7.03–6.97 (m, 1H), 5.69–5.64 (m, 1H), 5.34–5.26 (m,

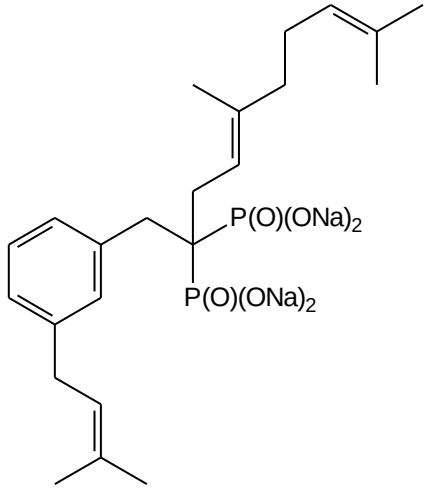
1H), 5.17–5.12 (m, 1H), 4.18–4.02 (m, 8H), 3.32–3.21 (m, 4H), 2.64 (td, J_{PH} = 16.2, J = 5.7 Hz, 2H), 2.16–2.05 (m, 4H), 1.72 (s, 3H), 1.71 (s, 3H), 1.67 (s, 3H), 1.63 (s, 3H), 1.61 (s, 3H), 1.25 (t, J = 6.6 Hz, 6.9 Hz, 6H), 1.21 (t, J = 6.9 Hz, 6H); ^{13}C NMR δ 140.7, 136.8, 136.3 (t, J_{CP} = 7.1 Hz), 132.0, 131.5, 131.3, 128.8, 127.4, 126.3, 124.2, 123.4, 119.2 (t, J_{CP} = 7.6 Hz), 62.4-62.1 (m, 4C), 47.4 (t, J_{CP} = 129.7 Hz), 39.9, 35.1-34.9 (m), 34.3, 28.2-28.0 (m), 26.5, 25.6, 25.5, 17.6, 17.5, 16.3, 16.2-15.8 (m, 4C); ^{31}P NMR δ 26.2; HRMS (ESI, *m/z*) calcd for $(M)^+$ $C_{31}H_{52}O_6P_2$: 582.3239. Found: 582.3235.



Tetrasodium (3-prenyl benzyl) phosphonate 133.

A stirred solution of **132** (0.767 g, 1.72 mmol, 1.0 eq) in CH_2Cl_2 was cooled to 0 °C in an icebath. After 2,4,6-collidine (1.14 mL, 8.60 mmol, 5.0 eq) was added, TMSBr (1.11 mL, 8.58 mmol, 5.0 eq) was added slowly and the reaction mixture was allowed to stir overnight. Toluene was added and removed *in vacuo*. This process was repeated three times. After drying the crude material, the residue was treated with NaOH solution (5 mL, 1.73 M, 8.65 mmol, 5.0 eq) and allowed to stir at room temperature overnight. Acetone was added to the solution and the resulting material was allowed to cool at 3 °C for 72 hr. The solution was filtered, the solid was washed with several portions of cold acetone, and then dried *in vacuo* to provide compound **133** (651 mg, 90%): ^{1}H NMR (D_20) δ7.26–7.14 (m, 3H), 6.99 (d, J = 6.3 Hz, 1H), 5.39–5.34 (m, 1H), 3.29 (d, J = 7.2 Hz, 2H), 3.08 (td, J_{PH} = 15.3 , J = 6.3 Hz, 2H), 2.07 (tt, J_{PH} = 21.9, J = 5.1 Hz, 1H), 1.68 (s, 3H), 1.67 (s, 3H); ^{13}C NMR δ 145.5 (t, J_{CP} = 6.6 Hz), 141.8, 134.2, 129.1,

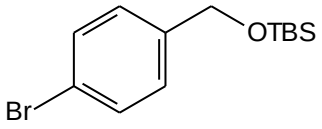
128.6, 126.7, 125.1, 123.7, 42.8 (t, J_{CP} = 117.8 Hz), 33.9, 33.7-33.5 (m), 25.1, 17.3; ^{31}P NMR δ 20.8; HRMS (ESI, *m/z*) calcd for $(M-H)^-$ $C_{13}H_{19}O_6P_2$: 333.0657. Found: 333.0662.



Tetrasodium geranyl (3-prenyl benzyl) bisphosphonate 135.

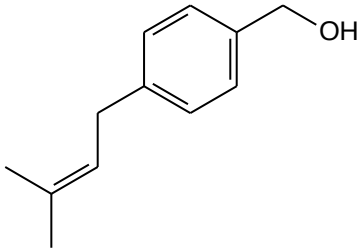
A stirred solution of **134** (70 mg, 0.12 mmol, 1.0 eq) in CH_2Cl_2 was cooled to 0 °C in an icebath. After 2,4,6-collidine (0.12 mL, 0.91 mmol, 7.6 eq) was added, slow addition of TMSBr (0.12 mL, 0.93 mmol, 7.8 eq) was done and the resulting solution was allowed to stir overnight. Toluene was added and removed *in vacuo*, three times. The resulting residue was treated with NaOH solution (1.0 M, 0.9 mL, 0.90 mmol, 7.5 eq) and allowed to stir at room temperature overnight. Acetone was added to the solution and the resulting material was stored at 3 °C for 72 hr. The suspension was filtered and the solid material was washed with several portions of anhydrous acetone. The precipitate then was dissolved in H_2O, filtered, and the aqueous portion was concentrated *in vacuo* to give a fine white powder, **135** (59 mg, 88%): ^{1}H NMR δ 7.36 (d, J = 7.5 Hz, 1H), 7.30–7.12 (m, 2H), 7.02 (d, J = 7.5 Hz, 1H), 5.83–5.78 (m, 1H), 5.41–5.30 (m, 1H), 5.23–5.19 (m, 1H), 3.28 (d, *J* = 6.9 Hz, 2H), 3.11 (t, *J* = 14.1 Hz, 2H), 2.40 (td, J_{PH} = 15.6, *J* = 6.0 Hz, 2H), 2.15–1.96 (m, 4H), 1.69 (s, 3H), 1.67 (s, 3H), 1.61 (s, 3H), 1.59 (s, 3H), 1.14 (s, 3H); ^{13}C NMR 141.0, 140.8 (t, J_{CP} = 7.8 Hz), 135.0, 134.2, 133.6, 131.7, 129.8, 128.2, 125.6, 125.2, 124.0 (t, J_{CP} = 5.9 Hz), 123.8, 45.5 (t, J_{CP} = 109.8 Hz), 39.6, 37.4–37.2 (m), 34.0, 30.9–

30.6 (m), 26.3, 25.2, 25.1, 17.4, 17.2, 15.7; ^{31}P NMR 22.7; HRMS (ESI, *m/z*) calcd for $(M-H)^-$ $C_{23}H_{35}O_6P_2$: 469.1909. Found: 469.1911.



(4-bromobenzyloxy)(tert-butyl)dimethylsilane (137)

Imidazole (4.39 g, 64 mmol, 2.5 eq.) was added to a solution of 4-bromobenzyl alcohol (4.73 g, 25.7 mmol, 1.0 eq.) in CH_2Cl_2. The solution was cooled to 0 °C, TBSCl (4.70 g, 31.2 mmol, 1.2 eq.) was added, and the reaction mixture was allowed to stir overnight. The solution was quenched by addition of H_2O, extracted with CH_2Cl_2, dried ($MgSO_4$), and concentrated *in vacuo*. Final purification by flash chromatography (15% EtOAc in hexanes) afforded the TBS protected alcohol **137** (7.40 g, 96%) with ^{1}H and ^{13}C NMR data corresponding to literature values.[189]

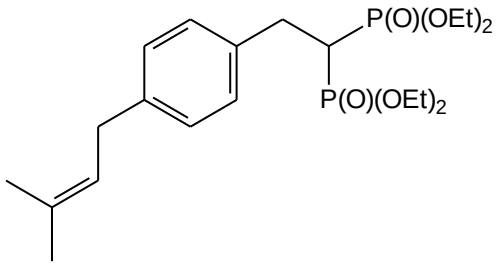


(4-(3-methylbut-2-enyl)phenyl)methanol (138)

A stirred solution of **137** (6.61 g, 21.9 mmol, 1.0 eq.) in THF was cooled to –78 °C in a dry-ice/acetone bath. Once cooling was complete, *n*–BuLi (11.5 mL, 2.1 M in THF, 1.1 eq.) was added slowly via syringe, and the solution was allowed to stir for fifteen minutes. Prenyl bromide (4.26 g, 28.6 mmol, 1.3 eq.) was added dropwise via syringe. The reaction solution was held at -78 °C for two hours, and then allowed to gradually warm to room temperature and stir

overnight. The resulting mixture was quenched by addition of H_2O, extracted with diethyl ether, dried ($MgSO_4$), and concentrated *in vacuo*. The crude mixture was used in the next reaction without additional purification.

A stirred solution of the above material in THF was cooled to 0 °C in an ice bath and a 1M solution of TBAF in THF (27.1 mL, 27.1 mmol, 1.0 eq.) was added dropwise to the reaction vessel. The reaction was allowed to stir for 4 hours, then the solution was diluted with diethyl ether, and finally H_2O was added. The resulting mixture was extracted with diethyl ether, dried ($MgSO_4$), and concentrated *in vacuo*. Final purification by column chromatography (10% EtOAc in hexanes) afforded **138** (2.99 g, 77% over two steps): ^{1}H NMR δ 7.20 (d, *J* = 8.1 Hz, 2H), 7.12 (d, *J* = 8.4 Hz, 2H), 5.25–5.33 (m, 1H), 4.51 (s, 2H), 3.31 (d, *J* = 7.5 Hz, 2H), 2.78 (s, 1H), 1.73 (s, 3H), 1.70 (s, 3H); ^{13}C NMR δ 141.0, 138.2, 132.4, 128.3 (2C), 127.1 (2C), 123.0, 64.7, 33.9, 25.6, 17.7; HRMS (ESI, *m/z*) calcd for $(M)^+$ $C_{12}H_{16}O$: 176.1201. Found: 176.1199.

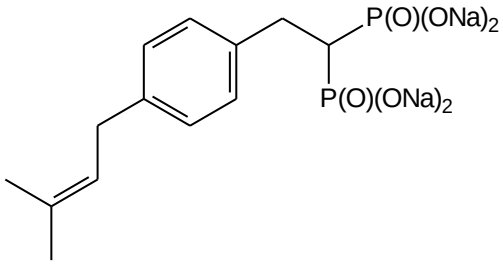


Tetraethyl (4-prenyl) benzylbisphosphonate 139

A stirred solution of (**138**) (4.46 g, 25.3 mmol, 1.0 eq) in CH_2Cl_2 was cooled to 0 °C in an ice bath. To the cooled solution was added anhydrous triethylamine (4.58 mL, 40.0 mmol, 1.6 eq) followed by dropwise addition of MsCl (2.36 mL, 30.5 mmol, 1.2 eq), and the resulting solution was allowed to stir for 30 minutes. After LiBr (5.55 g, 63.9 mmol, 2.5 eq) was dissolved in THF, the solution was transferred via syringe into the reaction vessel. The reaction mixture was allowed to stir for 1.5 hours and then the solution was quenched by addition of H_2O followed by addition of saturated NaCl solution. The resulting solution was extracted with

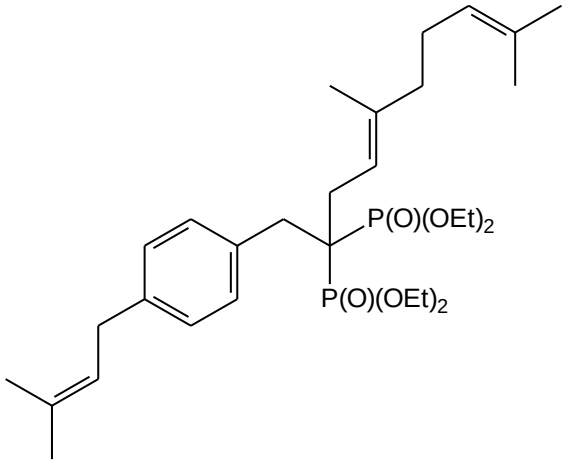
CH_2Cl_2, dried (Na_2SO_4), and concentrated *in vacuo*. Without additional purification, the product was utilized in the synthesis of bisphosphonate **139**.

To a stirred suspension of NaH (60% in mineral oil, 0.932 g, 23.3 mmol, 1.0 eq) in THF was added 15-crown-5 (0.51 mL, 2.58 mmol, 0.1 eq). The solution was cooled to 0 °C in an ice bath. After complete cooling of the solution, tetraethyl methylenebisphosphonate (7.38 g, 25.6 mmol, 1.1 eq) was added slowly via syringe and allowed to stir for 30 minutes to facilitate complete formation of the anion. Next, a solution of the prepared bromide in THF was added slowly via syringe to the reaction vessel. The resulting mixture was immediately removed from the ice bath and allowed to stir overnight. After the solution was filtered through a bed of fluorasil and concentrated *in vacuo*, final purification by flash column chromatography (gradient 6%-8% EtOH in hexanes) gave the target bisphosphonate **139** (6.89 g, 61% over two steps): ^{1}H NMR δ 7.11 (dd, J = 8.3, 2.1 Hz, 2H), 7.01 (dd, J = 7.8, 2.1 Hz, 2H), 5.23-5.16 (m, 1H), 4.10-3.94 (m, 8H), 3.22 (d, J = 7.2 Hz, 2H), 3.09 (td, J_{PH} = 16.5, J = 6.3 Hz, 2H), 2.56 (tt, J_{PH} = 23.7, J = 6.3 Hz, 1H), 1.65 (s, 3H), 1.63 (s, 3H), 1.20 (t, J = 7.2, 6H), 1.17 (t, J = 7.2 Hz, 6H), ^{13}C NMR δ 139.8, 136.7 (t, J_{CP} = 7.4 Hz), 132.1, 128.7 (2C), 127.9 (2C), 123.1, 62.2 (dd, J_{CP} = 13.8, 6.8 Hz, 4C), 38.9 (t, J_{CP} = 131.3 Hz), 33.7, 30.6 (t, J_{CP} = 4.9 Hz), 25.5, 17.6, 16.1 (d, J_{CP} = 7.2 Hz, 4C), ^{31}P NMR δ 23.6; HRMS (ESI, *m/z*) calcd for $(M)^+$ $C_{21}H_{36}O_6P_2$: 446.1987. Found: 446.1989.



Tetrasodium (4-prenyl) benzylbisphosphonate 140.

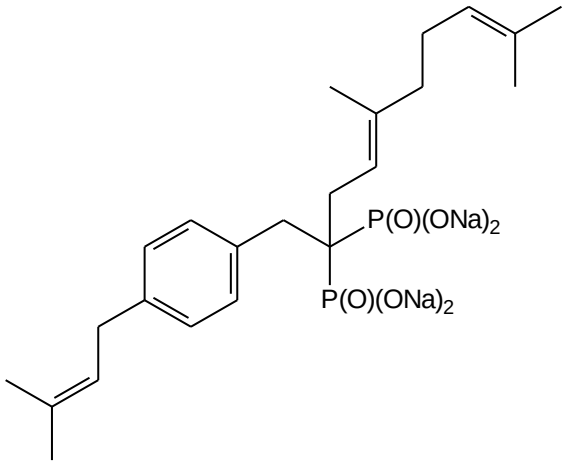
A stirred solution of **139** (289 mg, 0.64 mmol, 1.0 eq) in CH_2Cl_2 was cooled to 0 °C in an ice bath. After 2,4,6-collidine (0.70 mL, 5.28 mmol, 8.3 eq) was added and slow addition of TMSBr (0.69 mL, 5.34 mmol, 8.2 eq) the resulting was allowed to stir overnight. Toluene was added and removed *in vacuo* three times. The remaining residue was treated with NaOH solution (1M, 3.2 mL, 3.20 mmol, 5.0 eq) and allowed to stir at room temperature overnight. Acetone was added to the solution and the resulting suspension was stored at 3 °C for 72 hr. The solution was filtered and the solid was washed with several portions of cold acetone and dried *in vacuo* giving **140** (162 mg, 59%) ^{1}H NMR (D_2O) δ 7.38 (d, J = 7.8 Hz, 2H), 7.21 (d, J = 7.8 Hz, 2H), 5.46–5.37 (m, 1H), 3.35 (d, J = 7.2 Hz, 2H), 3.11 (td, J_{PH} = 15.6, J = 6.3 Hz, 2H), 2.18 (tt, J_{PH} = 21.6, J = 6.3 Hz, 1H), 1.76 (s, 6H); ^{13}C NMR δ 143.6 (t, J_{CP} = 7.3 Hz), 142.0, 136.8, 132.0 (2C), 130.7 (2C), 126.1, 44.6 (t, J_{CP} = 112.4 Hz), 36.0, 34.0–34.7 (m), 27.6, 19.8; ^{31}P NMR δ 20.6; HRMS (ESI, *m/z*) calcd for $(M-H)^-$ $C_{13}H_{19}O_6P_2$: 333.0657. Found: 333.0677.



Tetraethyl (4-prenyl benzyl) geranyl bisphosphonate 141.

A suspension of NaH (60% in mineral oil, 215 mg, 5.37 mmol, 2.4 eq) in THF was cooled to 0 °C in an icebath. Once the solution was thoroughly cooled, 15-crown-5 (0.05 mL, 0.3 mmol, 0.1 eq) was added followed by the addition of **139** (1.02 g, 2.27 mmol, 1.0 eq) via

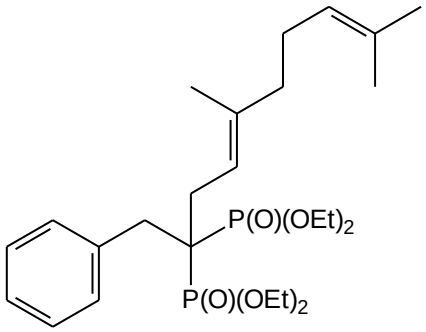
syringe. The resulting solution was allowed to stir for 30 minutes, geranyl bromide (849 mg, 3.91 mmol, 1.7 eq) was added, and the solution was allowed to stir overnight. The reaction mixture was filtered through a bed of fluorasil and the filtrate was concentrated *in vacuo*. Final purification via flash chromatography (4% EtOH in hexanes) afforded the desired compound (1.09 g, 83%): ^{1}H NMR δ 7.23 (d, J = 6.9 Hz, 2H), 7.03 (d, J = 7.2 Hz, 2H), 5.68–5.61 (m, 1H), 5.33-5.24 (m, 1H), 5.18–5.10 (m, 1H), 4.15–4.02 (m, 8H), 3.30–3.21 (m, 4H), 2.62 (td, J_{HP} = 15.9, J = 6.3 Hz, 2H), 2.18-2.01 (m, 4H), 1.72 (s, 3H), 1.70 (s, 3H), 1.67 (s, 3H), 1.62 (s, 6H), 1.24 (t, J = 6.9 Hz, 6H), 1.20 (t, J = 7.2 Hz, 6H); ^{13}C NMR δ 139.7, 136.6, 133.4 (t, J_{CP} = 7.5 Hz), 132.0, 131.3 (2C), 131.1, 127.2 (2C), 124.2, 123.3, 119.1 (t, J_{CP} = 7.7 Hz), 62.3–62.0 (m, 4C), 47.4 (J_{CP} = 129.6 Hz), 39.9, 34.7-34.5 (m), 33.8, 28.2–27.9 (m), 26.4, 25.6 (2C), 17.6, 17.5, 16.3, 16.2–16.0, (m, 4C); ^{31}P NMR δ 25.6; HRMS (ESI, *m/z*) calcd for $(M)^{+}$ $C_{31}H_{52}O_6P_2$: 582.3239. Found: 582.3238.



Tetrasodium (4-prenyl benzyl) geranyl bisphosphonate 142.

A stirred solution of **141** (0.915 g, 1.57 mmol, 1.00 eq) in CH_2Cl_2 was cooled to 0 °C in an ice bath. After 2,4,6-collidine (1.06 mL, 7.99 mmol, 5.09 eq) was added and slow addition of TMSBr (1.01 mL, 7.81 mmol, 4.97 eq) the solution was allowed to stir overnight. Toluene was added and removed *in vacuo*. The resulting residue was treated with NaOH (1.04 M, 6.4 mL,

6.66 mmol, 4.2 eq) and allowed to stir at room temperature overnight. Acetone was added to the solution and the resulting suspension was stored at 3 °C for 72 hr. The suspension was filtered, and the filtrand was washed with several aliquots of anhydrous acetone. The resulting solid was dissolved in H_2O, filtered, and concentrated *in vacuo* to provide compound **142** (598 mg, 68%): ^{1}H NMR δ 7.47 (d, J = 7.8 Hz, 2H), 7.14 (d, J = 7.8 Hz, 2H), 5.88–5.82 (m, 1H), 5.47–5.40 (m, 1H), 5.32–5.25 (m, 1H), 3.35 (d, J = 7.5 Hz, 2H), 3.17 (t, J_{PH} = 14.4 Hz, 2H), 2.47 (td, J_{PH} = 15.9, J = 6.6 Hz, 2H), 2.22–2.03 (m, 4H), 1.76 (s, 6H), 1.69 (s, 3H), 1.67 (s, 3H), 1.54 (s, 3H); ^{13}C NMR δ 141.8, 140.7 (t, J_{CP} = 7.3 Hz), 137.4, 136.7, 136.0, 134.9 (2C), 130.0 (2C), 127.8, 126.9 (t, J_{CP} = 6.8 Hz), 126.2, 48.3 (t, J_{CP} = 110.6 Hz), 42.1, 39.7–39.5 (m, 1C), 36.0, 33.6–33.4 (m, 1C), 28.7, 27.7, 27.6, 19.8 (2C), 18.0; ^{31}P NMR δ 23.4; HRMS (ESI, *m/z*) calcd for $(M-H)^-$ $C_{23}H_{35}O_6P_2$: 469.1909. Found: 469.1912.

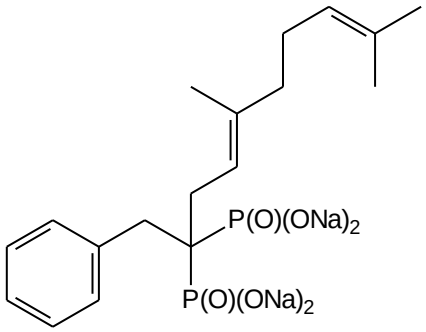


Tetraethyl benzyl geranylbisphosphonate 144.

To a stirred suspension of NaH (498 mg, 12.4 mmol, 1.0 eq) in THF at 0 °C was added 15-crown-5 (0.25 mL, 1.30 mmol, 0.1 eq) followed by dropwise addition of tetraethyl methylenebisphosphonate (3.45 g, 12.0 mmol, 1.0 eq). The resulting solution was allowed to stir for 30 minutes and benzyl bromide (1.49 mL, 12.5 mmol, 1.0 eq) was added dropwise as a neat liquid. The resulting solution was immediately removed from the ice bath and allowed to stir overnight. The solution was filtered through a bed of fluorasil and concentrated *in vacuo*. Final purification by flash column chromatography (gradient 5-10% EtOH in hexanes) gave the target

tetraethyl benzylbisphosphonate (2.07 g, 46%) with ^{1}H NMR data consistent with literature values.[150]

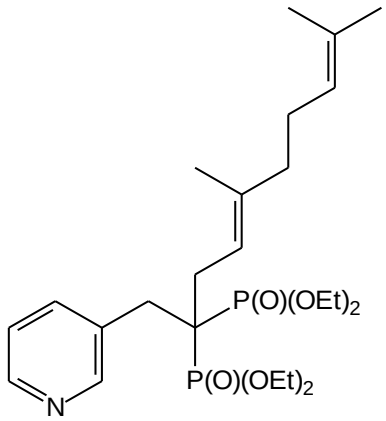
To a stirred suspension of NaH (16.7 mmol, 1.4 eq) in THF at 0 °C was added 15-crown-5 (0.91 mmol, 0.1 eq) followed by dropwise addition of the previously prepared tetraethyl benzylbisphosphonate. The resulting solution was allowed to stir for 30 minutes and geranyl bromide (12.1 mmol, 1.0 eq) was added dropwise as a neat liquid. The resulting solution was allowed to stir overnight, dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo*. Final purification by flash column chromatography (gradient 2-10% MeOH in diethyl ether) gave **144** (1.97 g, 40% over two steps): ^{1}H NMR δ 7.31 (dd, J = 7.8, 1.8 Hz, 2H), 7.25-7.17 (m, 3H), 5.67-5.60 (m, 1H), 5.15-5.08 (m, 1H), 4.17-3.99 (m, 8H), 3.27 (dd, J_{PH} = 16.5, 12.9 Hz, 2H), 2.61 (td, J_{PH} = 15.3, J = 6.0 Hz, 2H), 2.15–2.03 (m, 4H), 1.66 (s, 3H), 1.60 (s, 6H), 1.23 (t, J = 6.9, 6H), 1.19 (t, J = 6.9 Hz, 6H); ^{13}C NMR δ 136.9, 136.4 (t, J_{CP} = 8.6 Hz), 131.5 (2C), 131.4, 127.4 (2C), 126.4, 124.3, 119.2 (t, J_{CP} = 7.7 Hz), 62.5-62.1 (4C, m), 47.4 (t, J_{CP} = 129.6 Hz), 40.0, 35.3–35.1 (m), 28.4–28.2 (m), 26.5, 25.7, 17.7, 16.4, 16.3-16.1 (m, 4C); ^{31}P NMR δ 26.2; HRMS (ES^+, *m/z*) calcd for $(M+Na)^+$ $C_{26}H_{44}O_6P_2Na$: 537.2511. Found: 537.2515.



Tetrasodium benzyl geranylbisphosphonate 145.

To a solution of **144** (1.66 mmol, 1.0 eq) in CH_2Cl_2 at 0 °C, were added collidine (13.4 mmol, 8.09 eq) and TMSBr (13.3 mmol, 8.1 eq) as neat liquids. After 1 hour the solution was allowed to warm gradually to room temperature and stirred overnight. After the solvent was

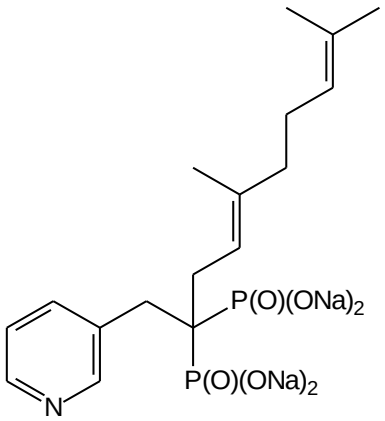
removed *in vacuo*, toluene (15 mL) was added and removed *in vacuo*. An aqueous solution of NaOH (7.4 mL, 0.9M, 6.66 mmol, 4.0 eq) was added, and the mixture was stirred for 1.75 h. The resulting mixture was then poured into acetone and stored at 3 °C for 72 hours. The resulting suspension was filtered and the filtrand was washed with several portions of anhydrous acetone. The remaining solid was dissolved in H_2O, filtered, and the filtrate was concentrated *in vacuo* to provide the desired compound (746 mg, 88% yield): ^{1}H NMR (D_2O) δ 7.48 (dd, *J* =8.1, 1.2, 2H), 7.31-7.16 (m, 3H), 5.79-5.69 (m, 1H), 5.27- 5.18 (m, 1H), 3.15 (t, J_{PH} = 15.0 Hz, 2H), 2.46 (td, J_{PH} = 15.6, *J* = 6.9 Hz, 2H), 2.15-2.01 (m, 4H), 1.64 (s, 3H), 1.61 (s, 3H), 1.55 (s, 3H) ^{13}C NMR δ 139.0 (t, J_{CP} = 7.2 Hz), 137.0, 133.7, 131.8 (2C), 127.8 (2C), 126.3, 125.1, 121.8 (t, J_{CP} = 7.4 Hz), 46.0 (t, J_{CP} = 111.4 Hz), 39.6, 36.5 (br s, 1C), 29.9 (br s, 1C), 26.0, 25.2, 17.2, 15.6; ^{31}P NMR δ 23.3; HRMS (ES^-, *m/z*) calcd for $(M-H)^-$ $C_{18}H_{27}O_6P_2$: 401.1283. Found: 401.1288.



Tetraethyl (3-pyridyl) geranylbisphosphonate 147.

Sodium hydride (60% in mineral oil, 588 mg, 14.7 mmol, 2.5 eq) was placed in a 3-neck flask fitted with two septa and a solid addition tube filled with 3-(bromomethyl)pyridine HBr (1.9 g, 7.76 mmol, 1.3 eq), and the apparatus was purged with argon. Anhydrous THF was added and the solution was cooled to 0 °C in an ice bath. After 15-crown-5 (0.31mL, 1.57 mmol, 0.3 eq) was added, (E)-tetraethyl 4,8-dimethylnona-3,7-dienylbisphosphonate[117] was

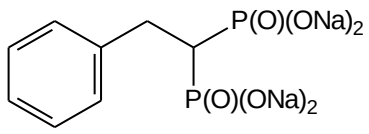
added dropwise as a neat liquid. The solution was allowed to stir for 30 minutes, the pyridine salt was added via the solid addition tube, and the reaction was allowed to stir overnight. After H_2O was added, the aqueous fraction was extracted with diethyl ether, dried (Na_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. Final purification by flash column chromatography (8% MeOH in Et_2O) afforded the desired bisphosphonate (3.01 g, 89%): ^{1}H NMR δ 8.46 (s, 1H), 8.36 (d, *J* = 3.9 Hz, 1H), 7.59 (d, *J* = 7.5 Hz, 1H), 7.10 (dd, *J* = 7.5, 5.1 Hz, 1H), 5.59–5.51 (m, 1H), 5.08-5.01 (m, 1H), 4.13-3.95 (m, 8H), 3.18 (t, J_{PH} = 15.3 , 2H), 2.54 (td, J_{PH} = 15.6, *J* = 6.0 Hz, 2H), 2.09-1.89 (m, 4H), 1.57 (s, 3H), 1.54 (s, 3H), 1.52 (s, 3H), 1.20 (t, *J* = 7.2 Hz, 6H), 1.15 (t, J = 7.2 Hz, 6H); ^{13}C NMR δ 152.2, 147.5, 138.6, 137.5, 132.2 (t, J_{CP} = 7.7 Hz), 131.3, 124.1, 122.3, 118.6 (t, J_{CP} = 7.6 Hz), 62.6–62.3 (m, 4C), 47.0 (t, J_{CP} = 130.2 Hz), 39.9, 32.8 (t, J_{CP} = 4.1 Hz), 28.4 (t, J_{CP} = 4.6 Hz), 26.4, 25.6, 17.5, 16.3, 16.2-16.0 (m, 4C); ^{31}P NMR δ 25.7; HRMS (ES^+, *m/z*) calcd for $(M+Na)^+$ $C_{25}H_{43}NO_6P_2Na$: 538.2463. Found: 538.2468.



Tetrasodium (3-pyridyl) geranylbisphosphonate 148.

A solution of **147** (329 mg, 0.64 mmol, 1.0 eq) in CH_2Cl_2 was cooled to 0 °C in an ice bath. After 2,4,6-collidine (0.63 mL, 4.75 mmol, 7.4 eq) and TMSBr (0.62 mL, 4.79 mmol, 7.5 eq) was added, the reaction mixture was stirred overnight. Toluene was added and removed *in vacuo*, three times. The residue was treated with NaOH solution (1.56 M, 2.45 mL, 6.66 mmol, 6.0 eq) and allowed to stir at room temperature overnight. Acetone was added to the solution

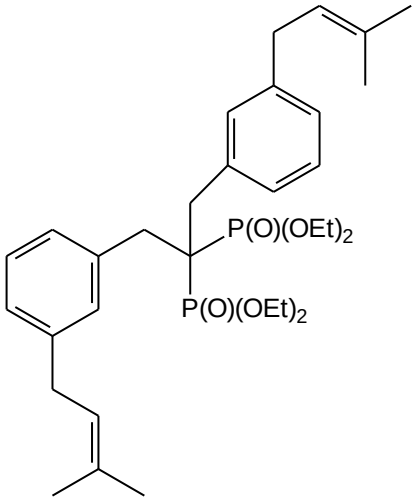
and the resulting material was allowed to stand at 3 °C for 72 hr. The resulting suspension was filtered, and the filtrand was washed with several aliquots of anhydrous acetone. The solids were then dissolved in water, filtered, and the filtrate was concentrated *in vacuo* to provide the target compound (121 mg, 39%): ^{1}H NMR (D_2O) δ 8.44 (d, *J* = 2.1 Hz, 1H), 8.18 (dd, *J* = 5.1, 1.5 Hz, 1H), 7.94 (ddd, *J* = 8.4, 1.8, 1.5 Hz, 1H), 7.21 (dd, *J* = 7.8, 5.1 Hz, 1H), 5.75-5.70 (m, 1H), 5.20-5.14 (m, 1H), 3.07 (t, J_{PH} = 15.0, Hz, 2H), 2.39 (td, J_{PH} = 14.1, *J* = 7.5 Hz, 2H), 2.09–1.92 (m, 4H), 1.56 (s, 6H), 1.44 (s, 3H); ^{13}C NMR δ 153.7, 147.9, 143.0, 139.4 (t, J_{CP} = 6.9 Hz), 138.0, 135.9, 127.5, 126.6 (t, J_{CP} = 6.7 Hz), 125.6, 48.3 (t, J_{CP} = 112.3 Hz), 42.0, 37.5–37.3 (m), 33.4–33.2 (m), 28.5, 27.5, 19.6, 17.8; ^{31}P NMR δ 22.7; HRSM (ESI, *m/z*) calcd for $(M-H)^-$ $C_{17}H_{26}NO_6P_2$: 402.1235. Found: 402.1244.



Tetrasodium benzylbisphosphonate (149).

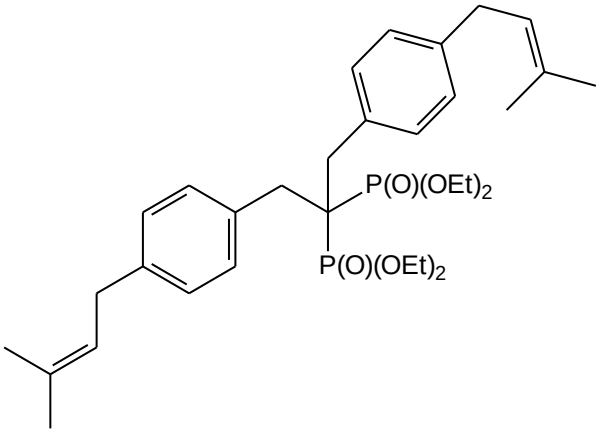
To a stirred solution of tetraethyl benzylbisphosphonate (see compound **144** for synthesis) (942 mg, 2.49 mmol, 1.0 eq) in CH_2Cl_2 at 0 °C was added 2,4,6-collidine (1.65 mL, 12.4 mmol, 5.00 eq) followed by slow addition of TMSBr (1.62 mL, 12.5 mmol, 5.0 eq). The resulting solution was allowed to stir overnight, while gradually warming to ambient temperature. Toluene was added and removed *in vacuo*, and the residue was treated with NaOH (1.7 M, 7.2 mL, 12.2 mmol, 4.9 eq) and allowed to stir for 5 hrs. After acetone was added, the solution was stored at 3 °C for 72 hrs but no precipitate was observed. The acetone/H_2O mixture was concentrated *in vacuo*. The resulting solid was dissolved in a minimum amount of hot water and precipitated by addition of acetone to afford **149** (654 mg, 74%): ^{1}H NMR δ 7.37 (d, *J* = 8.1

Hz, 2H), 7.26 (dd, J = 7.8, 7.2 Hz, 2H), 7.15 (dd, J = 7.5, 6.6 Hz, 1H), 3.05 (td, J_{PH} = 15.3, J = 5.7 Hz, 2H), 2.07 (tt, J_{PH} = 21.9, J = 6.0 Hz, 1H); ^{13}C NMR δ 146.7 (t, J_{CP} = 7.1 Hz), 131.6 (2C), 130.8 (2C), 128.0, 44.9 (t, J_{CP} = 115.7 Hz), 35.3 (br s, 1C); ^{31}P NMR δ 20.8; HRMS (M-H)$^-$ $C_8H_{11}O_6P_2$ cald: 265.0031. Found: 265.0050. ^{31}PNMR data corresponds with literature value.[190]



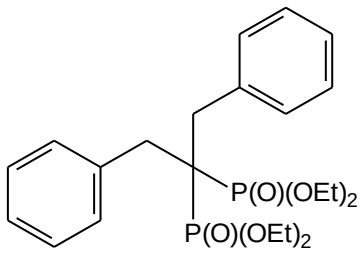
Tetraethyl bis(3-prenyl)benzyl bisphosphonate 150.

Isolated as a minor product in the synthesis of compound **132**: ^{1}H NMR δ 7.28 (d, J = 6.9 Hz, 2H), 7.27 (s, 2H), 7.15 (t, J = 8.1 Hz, 2H), 7.02 (d, J = 7.8 Hz, 2H), 5.35–5.29 (m, 2H), 4.04–3.91 (m, 8H), 3.37–3.26 (m, 8H), 1.72 (s, 6H), 1.70 (s, 6H), 1.13 (t, J = 6.9 Hz, 12H), ^{13}C NMR δ 140.6 (2C), 136.8 (t, J_{CP} = 6.6 Hz, 2C), 132.0 (2C), 131.8 (2C), 129.0 (2C), 127.3 (2C), 126.4 (2C), 123.4 (2C), 62.1–61.9 (m, 4C), 48.9 (t, J_{CP} = 130.4), 37.6 (t, J_{CP} = 5.0 Hz, 2C), 34.3 (2C), 25.7 (2C), 17.7 (2C), 16.0 (t, J_{CP} = 3.2Hz, 4C), ^{31}P NMR δ 25.0; HRMS (ESI, *m/z*) calcd for (M)$^+$ $C_{33}H_{50}O_6P_2$:604.3083. Found 604.3080.



Tetraethyl bis(4-prenyl)benzyl bisphosphonate 151.

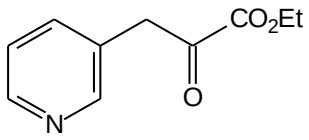
Isolates as a minor product in the synthesis of compound **139**: ^{1}H NMR δ 7.35 (d, *J* = 7.8 Hz, 4H), 7.05 (d, *J* =7.8 Hz, 4H), 5.33–5.25 (m, 2H), 4.04–3.92 (m, 8H), 3.36–3.25 (m, 8H), 1.72 (s, 6H), 1.70 (s, 6H), 1.13 (t, *J* = 6.9 Hz, 12H), ^{13}C NMR δ 139.8 (2C), 133.8 (t, J_{CP} = 6.5 Hz, 2C), 131.8 (2C), 131.5 (4C), 127.0 (4C), 123.3 (2C), 62.0–61.8 (m, 4C), 48.7 (t, J_{CP} = 130.2 Hz), 37.1 (t, J_{CP} = 4.8 Hz, 2C), 33.8 (2C), 25.5 (2C), 17.5 (2C), 15.8 (t, J_{CP} = 3.5 Hz, 4C,), ^{31}P NMR δ 24.5 HRMS (ESI, *m/z*) calcd for $(M)^{+}$ $C_{33}H_{50}O_6P_2$:604.3083. Found 604.3086.



Tetraethyl bisbenzyl bisphosphonate 152.

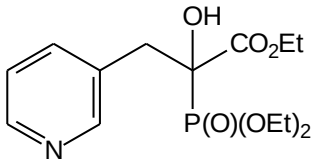
To a stirred solution of NaH (60% in mineral oil, 0.531 g, 13.3 mmol, 2.54 eq) in THF was added 15-crown-5 (0.21 mL, 1.06 mmol, 0.2 eq), and the solution was cooled to 0 °C in an ice bath. After complete cooling of the solution, tetraethyl methylenebisphosphonate (1.51 g, 5.23 mmol, 1.00 eq) was added slowly via syringe and allowed to stir for 30 minutes. Benzyl bromide (1.52 mL, 12.71 mmol, 2.4 eq) then was added via syringe to the reaction vessel. The

mixture was immediately removed from the ice bath and allowed to stir overnight. The solution was filtered through a bed of fluorasil and concentrated *in vacuo*. Final purification was achieved by flash column chromatography (5% EtOH in hexanes) to afford the desired dialkyl bisphosphonate in 74% yield (1.822 g): ^{1}H NMR δ 7.45 (dd, J = 7.8, 1.8 Hz, 4H), 7.27–7.16 (m, 6H), 4.07–3.91 (m, 8H), 3.35 (t, J_{PH} = 16.2 Hz, 4H), 1.13 (t, J_t =7.2 Hz, 12H), ^{13}C NMR δ 136.7 (2C), 131.6 (4C), 127.2 (4C), 126.4 (2C), 62.1 (t, J_{CP} = 3.9 Hz, 4C), 49.7 (t, J_{CP} = 130.6), 37.8 (t, J_{CP} = 4.6 Hz, 2C), 16.0 (4C), ^{31}P NMR δ 24.9



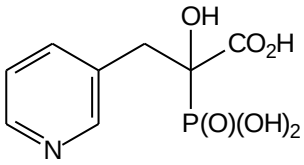
Ketoester 162.

To a stirred solution of NaH (60% in mineral oil, 6.31 g, 157.8 mmol, 1.9 eq) in Et_2O at 0 °C was added EtOH (1.9 mL, 32.3 mmol, 0.38 eq). The solution was allowed to stir for 10 minutes and then *N*,*N*-dimethylglycine ethyl ester (33 mL, 233.5 mmol, 2.7 eq) and nicotinaldehyde (85.2 mmol. 1.0 eq) were added. The solution was allowed to stir for 20 minutes at 0 °C, then removed from the ice bath and allowed to stir overnight. The mixture was diluted with EtOAc and washed with H_2O. The organic layer was extracted with 1N HCl and these aqueous extracts were made basic by addition of $NaHCO_3$ and extracted with EtOAc, dried ($MgSO_4$), and concentrated *in vacuo*. The solid yellow residue was washed with cold EtOAc to provide the previously described[165] compound **162** as a fine white powder in 31% yield (5.03 g): ^{1}H NMR 8.84–8.82 (m, 1H), 8.50–8.47 (m, 1H), 8.25 (dt, J = 8.1, 2.1 Hz, 1H), 7.33–7.26 (m, 1H), 6.49 (s, 1H), 4.38 (q, J = 7.2 Hz, 2H), 4.15 (s, 1H), 1.40 (t, J = 7.2 Hz, 3H)



Triethyl phosphonocarboxylate 166.

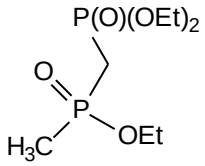
Ester **162** (496 mg, 2.57 mmol, 1.0 eq) was dissolved in an excess of diethyl phosphite (1.3 mL, 10.1 mmol, 3.93 eq) and the resulting solution was heated at 90 °C for one hour in an oil bath. The reaction vessel was removed from the oil bath and allowed to stir at room temperature for an additional 23 hrs. The volatiles were removed *in vacuo* and final purification was achieved by flash column chromatography (5-8% EtOH in EtOAc) to provide the previously described[164] phosphonocarboxylate in 86% yield (733 mg): ^{1}H NMR δ 8.50–8.42 (m, 2H), 7.65 (d, J = 8.1 Hz, 1H), 7.19 (t, J = 6.3 Hz, 1H), 4.31–4.19 (m, 4H), 4.17–4.06 (m, 2H), 3.41 (dd, J_{HP} = 14.1, J = 5.7 Hz, 1H), 3.24 (dd, J_{HP} = 14.1, J = 7.8 Hz, 1H), 2.04 (s, 1H), 1.42–1.23 (m, 9H); ^{13}C NMR δ 170.5 (d, J_{CP} = 3.6 Hz), 150.8, 148.1, 137.9, 130.1 (d, J_{CP} = 14.3 Hz), 122.8, 77.4 (d, J_{CP} = 159.5 Hz), 63.8 (m, 2C), 62.8, 36.6 (d, J_{CP} = 1.4 Hz), 16.3–16.1 (m, 2C), 13.8; ^{31}P NMR δ 16.8 Hz; HRMS (ES^{+}, *m/z*) cald for $(M+H)^{+}$ $C_{14}H_{23}NO_6P$: 332.1263; found: 332.1268.



3-PEHPC (158).

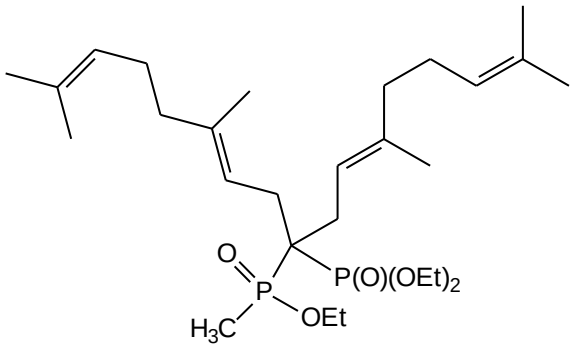
The phosphonocarboxylate **166** (944 mg, 2.85 mmol, 1.0 eq) was dissolved in an excess of concentrated HCl (12 mL) and the solution was heated at reflux overnight. The solvent was removed under a stream of air, the residue was dissolved in H_2O, and the water was subsequently removed *in vacuo*. The residue was recrystallized from H_2O/iPrOH to give the desired compound in 70% yield (704 mg). ^{1}H NMR (D_2O) δ 8.66–8.57 (m, 2H), 8.46 (d, J = 8.7 Hz,

1H), 7.92 (t, J = 6.3 Hz, 1H), 3.59 (dd, J_{HP} = 14.1, J = 5.1, 1H), 3.30 (dd, J_{HP} = 14.1, J = 8.1 Hz, 1H); ^{31}P NMR δ 12.8; HRMS (ES$^-$) calcd for (M-H)$^-$ $C_8H_9NO_6P$: 246.0168; found 246.0160.



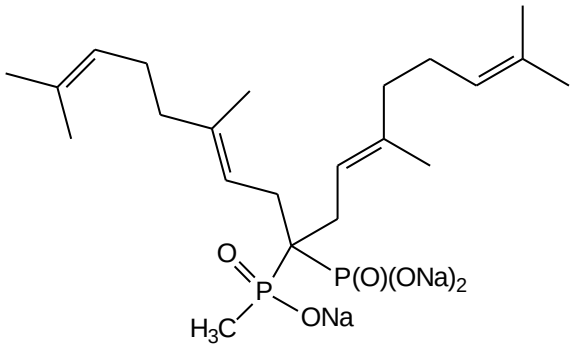
Triethyl methylenephosphonophosphinate 168.

Diethyl methylphosphonate (5.04 g, 32.9 mmol, 1.0 eq) was dissolved in THF and the solution was cooled to –78 °C in a dry ice/acetone bath. Once cooling was complete, nBuLi (2.3 M in hexanes, 15.8 mL, 36.3 mmol, 1.1 eq) was added via syringe with vigorous stirring. A green precipitate formed and an additional 5 mL of THF was added. The solution was held at –78 °C for an additional hour, then removed from the dry ice/acetone bath and allowed to stir at room temperature overnight. Final purification was achieved by flash column chromatography (10% MeOH in Et2O) to provide the phosphonophosphinate in 40% (4.24 g) yield with 1H and 31P NMR data that correlated well with the published[191] values: ^{1}H NMR δ 4.03–3.86 (m, 6H), 2.25 (dd, J_{HP} = 20.4, 17.7 Hz, 2H), 1.51 (d, J_{HP} = 15.3 Hz, 3H), 1.17 (t, J = 6.9 Hz, 9H), ^{31}P NMR δ 44.4 (d, J_{PP} = 3.27 Hz), 19.8 (d, J_{PP} = 3.27 Hz)



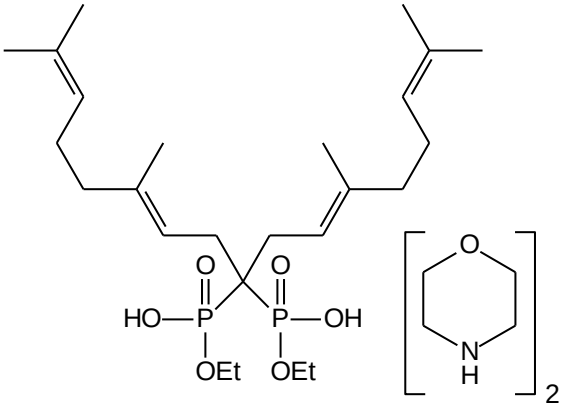
Triethyl digeranylphosphonophosphinate 169.

To a stirred solution of NaH (60% in mineral oil, 234 mg, 5.85 mmol, 2.31 eq) at 0 °C was added 15-crown-5 (0.02 mL, 0.10 mmol, 0.04 eq) and **168** (655 mg, 2.53 mmol, 1.0 eq). After gas evolution had ceased, geranyl bromide (1.27 g, 5.84 mmol, 2.31 eq) was added, the solution was removed from the ice bath and allowed to stir at room temperature for four hours. After ammonium chloride was added, the mixture was extracted with Et_2O and the combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo*. Final purification was achieved by flash column chromatography (8% EtOH in hexanes) to afford the desired phosphonophosphinate in 51% yield (1.34 g) as a pale yellow oil. ^{1}H NMR δ 5.50–5.35 (m, 2H), 5.13–5.05 (m, 2H), 4.24–4.06 (m, 6H), 2.77–2.54 (m, 4H), 2.14–1.96 (m, 8H), 1.73 (d, J_{HP} = 9.6 Hz, 3H), 1.67 (br s, 6H), 1.63 (br s, 6H), 1.59 (br s, 6H), 1.33 (t, J = 6.7 Hz, 9H); ^{13}C NMR δ 137.3 (d, J_{CP} = 8.0 Hz, 2C), 131.2 (d, J_{CP} = 1.9 Hz, 2C), 124.1 (d, J_{CP} = 1.4 Hz, 2C), 119.2–118.8 (m, 2C), 62.1 (dd, J_{CP} = 26.0, 7.3 Hz, 2C), 60.4 (d, J_{CP} = 6.5 Hz), 46.5 (dd, J_{CP} = 130.3, 80.8), 39.9 (d, J_{CP} = 5.0 Hz, 2C), 27.9–27.6 (m, 2C), 26.4 (2C), 25.7 (2C), 17.5 (d, J_{CP} = 1.4 Hz, 2C), 16.5 (d, J_{CP} = 5.6 Hz), 18.3 (dd, J_{CP} = 6.0, 1.5 Hz, 2C), 16.0 (d, J_{CP} = 1.6 Hz, 2C), 14.4 (d, J_{CP} = 90.7 Hz); ^{31}P NMR δ 55.5 (d, J_{PP} = 13.7 Hz), 27.0 (d, J_{PP} = 13.7 Hz)



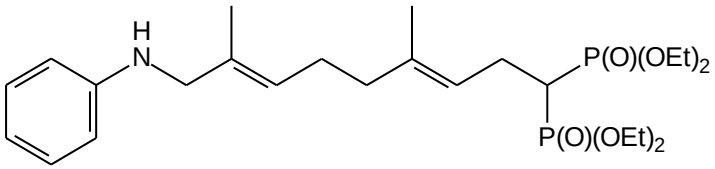
Trisodium digeranyl methylphosphonophosphinate 170.

To a solution of 2,4,6-collidine (1.0 mL, 7.54 mmol, 5.8 eq) in CH_2Cl_2 at 0 °C was added TMSBr (1.02 mL, 7.86 mmol, 6.1 eq) via syringe and the solution was allowed to stir for ten minutes. The phosphonophosphinate ester **169** (690 mg, 1.30 mmol, 1.0 eq) was added and the solution was allowed to stir overnight at room temperature. The volatiles were removed; toluene was added (5mL) and then removed *in vacuo*. This process was repeated once and then aqueous NaOH (1.2 M, 5.4 mL, 5.0 eq) was added and the mixture was allowed to stir overnight. The solution was poured into acetone (5 mL) and stored at 4 °C for 72 hrs over which time a precipitate formed. The precipitate was collected via filtration to provide the target trisodium salt in 78% yield (521 mg). ^{1}H NMR δ 5.67–5.58 (m, 2H), 5.23–5.18 (m, 2H), 2.64–2.40 (m, 4H), 2.18–1.99 (m, 8H), 1.69 (br s, 6H), 1.63 (br s, 12H), 1.38 (d, J_{CP} = 13.5 Hz, 3H); ^{13}C NMR δ 135.7 (2C), 133.6 (2C), 125.1 (2C), 123.1 (t, J_{CP} = 7.6 Hz, 2C), 45.7 (dd, J_{CP} = 115.7, 78.7 Hz), 39.8 (2C), 28.9 (2C), 26.4 (2C), 25.2 (2C), 17.3 (2C), 16.1 (d, J_{CP} = 90.0 Hz), 15.7 (2C); ^{31}P NMR δ 47.0 (d, J_{PP} = 9.1 Hz), 21.5 (d, J_{PP} = 9.1 Hz); HRMS (ES^-, *m/z*) calcd for $(M-H)^-$ $C_{22}H_{39}O_5P_2$: 445.2273; found: 445.2264.



Diethyl bisphosphonate 172.

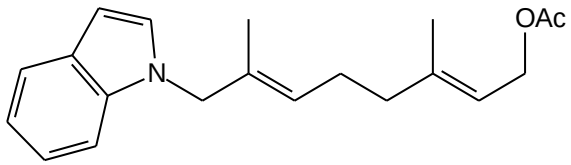
The previously described[117] bisphosphonate **171** (502 mg, 0.90 mmol, 1.0 eq) was dissolved in morpholine (1.5 mL) and held at reflux overnight. The excess morpholine was removed *in vacuo*, the remaining residue was washed with cold Et_2O, dissolved in water, filtered, and the aqueous portion was collected. The water was removed in vacuo to provide compound **172** (366 mg, 60%) as a yellow solid: ^{1}H NMR δ 5.47–5.36 (m, 2H), 5.08–4.95 (m, 2H),3.94–3.77 (m, 12H), 3.31–3.022 (m, 8H), 2.59–2.36 (m, 4H), 2.04–1.80 (m, 8H), 1.62–1.39 (m, 18H), 1.23–1.08 (m, 6H); ^{13}C NMR δ 137.7 (2C), 130.7 (2C), 124.9 (2C), 122.4–122.0 (m, 2C), 63.8 (4C), 60.9–60.7 (m, 2C), 46.0 (t, JCP = 123.4 Hz), 43.2 (4C), 40.2 (2C), 29.2–28.7 (m, 2C), 26.8 (2C), 25.4 (2C), 17.4 (2C), 16.8 (2C), 16.0 (2C); ^{31}P NMR δ 24.2; HRMS (ES$^-$, *m/z*) calcd for $(M–H)^-$ $C_{25}H_{45}O_6P_2$: 503.2691; found: 503.2689.



Tetraethyl bisphosphonate 188.

The previously described aldehyde **187**[192] (420 mg, 0.96 mmol, 1.0 eq), aniline (0.1 mL, 1.10 mmol, 1.15 eq), and glacial acetic acid (0.06 mL, 1.05 mmol, 1.10 eq) were dissolved in 1,2-dichloroethane, and several 4Å molecular sieves were added. The mixture was allowed to

stir for ten minutes. After solid $NaBH(OAc)_3$ (284 mg, 1.34 mmol, 1.40 eq) was added in several portions, the suspension was allowed to stir for 5 hr, followed by addition of aqueous $NaHCO_3$ until gas evolution ceased. The solution was extracted with diethyl ether, dried ($MgSO_4$) and concentrated *in vacuo*. Final purification was achieved by flash column chromatography (12% EtOH in hexanes) to give **188** (392 mg, 79%): ^{1}H NMR δ 7.17–7.11 (m, 2H), 6.69–6.58 (m, 3H), 5.42–5.37 (m, 1H), 5.34–5.29 (m, 1H), 4.22–4.11 (m, 8H), 3.62 (s, 2H), 2.72–2.56 (m, 2H), 2.32 (tt, J_{HP} = 24.0 Hz, J = 5.7 Hz, 1H), 2.17–2.10 (m, 2H), 2.04–1.98 (m, 2H), 1.66 (s, 3H), 1.64 (s, 3H), 1.33 (t, J = 6.6 Hz, 12H); ^{13}C NMR 148.4, 136.3, 132.2, 128.9 (2C), 125.5, 121.8 (t, J_{CP} = 7.4 Hz), 116.8, 112.6 (2C), 62.5–62.2 (m, 4C), 51.5, 39.1, 37.3 (t, J_{CP} = 131.8 Hz), 26.0, 23.9 (t, J_{CP} = 4.9 Hz), 16.3–16.1 (m, 4C), 15.9, 14.5; ^{31}P NMR 23.6; HRMS (EI$^+$, *m/z*) calcd for $(M)^+$ $C_{25}H_{43}NO_6P_2$: 515.2566. Found: 515.2572.

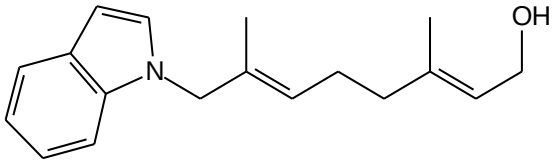


Acetate 190.

The previously reported compound (2E,6E)-8-hydroxy-3,7-dimethylocta-2,6-dienyl acetate[193] (3.93 g, 18.5 mmol, 1.00 eq) was dissolved in THF, cooled to 0 °C in an ice bath, and triethylamine (3.24 mL, 24.5 mmol, 1.32 eq) and mesyl chloride (1.96 mL, 25.3 mmol, 1.37 eq) were added. After stirring for 30 minutes LiBr (4.02 g, 46.3 mmol, 2.50 eq) was added. The suspension was removed from the ice bath and allowed to stir overnight. Water, then brine, was added and the solution was extracted with hexanes. The combined organic extracts were dried (Na_2SO_4), filtered through a bed of basic alumina, and concentrated *in vacuo*. The resulting oil, (2E,6E)-8-bromo-3,7-dimethylocta-2,6-dienyl acetate, was used without additional purification.

Indole (849 mg, 7.25 mmol) was dissolved in anhydrous DMF, the solution was cooled to 0 °C in an ice bath, and solid NaH (60% in mineral oil, 320 mg, 8.00 mmol) was added

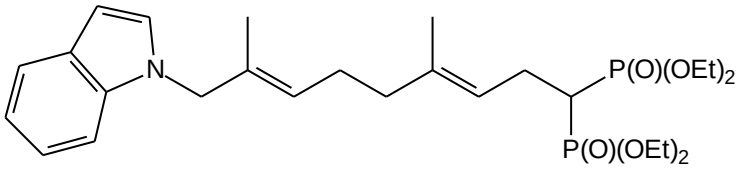
cautiously. Once addition was complete, the solution was allowed to stir vigorously for 30 minutes and then the prepared allylic bromide (2.32 g, 8.43 mmol) in THF (8 mL) was added slowly to the reaction mixture via syringe. The mixture was removed from the ice bath and allowed to stir overnight. Water was added and the mixture was poured into ether. After the solution was extracted with diethyl ether, the extracts were dried ($MgSO_4$) and concentrated *in vacuo*. Final purification by flash column chromatography (10% EtOAc in hexanes) afforded the target compound **190** (1.10 g, 49%): ^{1}H NMR δ 7.62 (d, *J* = 7.8 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.22–7.14 (m, 1H), 7.11–7.04 (m, 1H), 6.49 (d, *J* = 2.7 Hz, 1H), 5.35–5.25 (m, 2H), 4.56 (d, *J* = *7.5* Hz, 2H), 4.48 (s, 2H), 2.21–2.12 (m, 2H), 2.10–2.02 (m, 2H), 2.05 (s, 3H), 1.67 (s, 3H), 1.51 (s, 3H); ^{13}C NMR δ 171.1, 141.5, 136.3, 131.5, 128.5, 128.0, 126.9, 121.2, 120.7, 119.2, 118.7, 109.7, 101.0, 61.2, 54.1, 38.9, 25.7, 21.0, 16.3, 14.0; HRMS (EI$^+$, *m/z*) calcd for (M^+) $C_{20}H_{25}NO_2$: 311.1885. Found 311.1889.



Alcohol 191.

Compound **190** (1.00 g, 3.22 mmol) was dissolved in MeOH, K_2CO_3 (2.5 g, 18.1 mmol) was added, and the mixture was allowed to stir overnight. The mixture was filtered, the filtrate was concentrated until ~80% of the MeOH was removed, and water was added. The aqueous phase was extracted with diethyl ether, and the extracts were dried ($MgSO_4$) and concentrated *in vacuo*. Final purification by flash column chromatography (30% EtOAc in hexanes) afforded the desired alcohol **191** in quantitative yield: ^{1}H NMR δ 7.62 (d, *J* = 7.5 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.20–7.05 (m, 3H), 6.49 (d, *J* = 1.8 Hz, 1H), 5.35–5.23 (m, 2H), 4.59 (s, 2H), 4.09 (d, *J* = 6.6 Hz, 2H), 2.18–2.11 (m, 2H), 2.07–2.01 (m, 2H), 1.63 (s, 3H), 1.53 (s, 3H); ^{13}C NMR δ

138.3, 136.1, 131.2, 128.4, 128.1, 126.9, 123.8, 121.1, 120.7, 119.1, 109.7, 100.7, 59.0, 53.9, 38.8, 25.6, 16.0, 13.9; HRMS (EI^+, *m/z*) calcd for (M^+) $C_{18}H_{23}NO$: 269.1780. Found 269.1770.

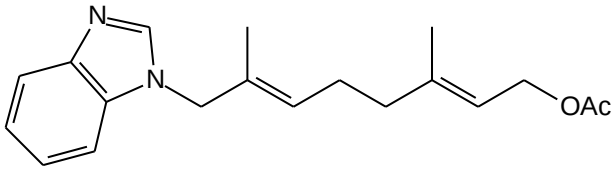


Tetraethyl bisphosphonate 193.

Alcohol **191** was dissolved in THF and the resulting solution was cooled to 0 °C in an ice bath. Triethylamine (0.21 mL, 1.51 mmol, 1.01 eq) and MsCl (0.16 mL, 2.07 mmol, 1.39 eq) were added, the solution was allowed to stir for 1 hr, LiBr (325 mg, 3.74 mmol, 2.51 eq) was added and the solution was stirred overnight. After water was added, the solution was extracted with Et_2O and the combined organic extracts were dried (Na_2SO_4) and concentrated *in vacuo*. The resulting allylic bromide was used in the following reaction without additional purification.

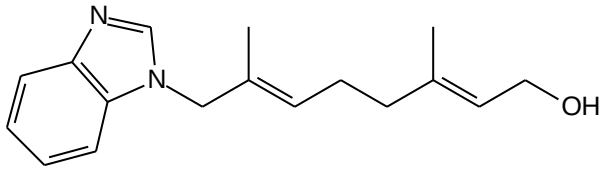
To a suspension of NaH (60% in mineral oil, 150 mg, 3.75 mmol) in THF at 0 °C was added tetraethyl methylenebisphosphonate (1.10 g, 3.81 mmol) via syringe. The resulting mixture was allowed to stir for 20 minutes and the previously prepared allylic bromide (1.24 g, 3.72 mmol) was added. The solution was allowed to warm to room temperature and stirred overnight. After water was added, the solution was extracted with diethyl ether, and the combined extracts were dried ($MgSO_4$) and concentrated *in vacuo*. Final purification by flash column chromatography (8% EtOH in hexanes) afforded the desired bisphosphonate **193** (625 mg, 31%): ^{1}H NMR δ 7.61 (d, J = 7.5 Hz, 1H), 7.33 (d, J = 8.4 Hz, 1H), 7.18 (t, J = 7.2 Hz, 1H), 7.11–7.05 (m, 2H), 6.49 (d, J = 2.4 Hz, 1H), 5.35–5.31 (m, 2H), 4.59 (s, 2H), 4.21–4.13 (m, 8H), 2.73–2.54 (m, 2H), 2.32 (tt, J_{HP} = 23.4 Hz, J = 6.3 Hz, 1H), 2.18–2.09 (m, 2H), 2.05–1.94 (m, 2H), 1.63 (s, 3H), 1.50 (s, 3H), 1.33 (t, J = 6.9 Hz, 12H); ^{13}C NMR 136.2, 136.1, 131.0, 128.5, 128.0, 127.5, 122.1 (t, J_{CP} = 7.3 Hz), 121.2, 120.6, 119.1, 109.6, 100.9, 62.3 (dd, J_{CP} = 8.5, 7.0 Hz, 4C), 54.1, 39.1, 37.3 (t, J_{CP} = 131.8 Hz), 26.1, 23.9 (t, J_{CP} = 4.8 Hz), 16.3 (d, J_{CP} =

7.4 Hz, 4C), 15.9, 13.9; HRMS (EI^+, *m/z*), calcd for (M^+) $C_{27}H_{43}NO_6P_2$: 539.2566. Found 539.2567.



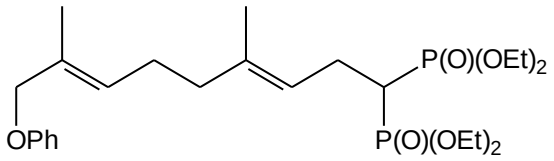
Acetate 194.

Benzimidazole (0.868 g, 7.35 mmol) was dissolved in anhydrous DMF, the solution was cooled to 0 °C in an ice bath, and NaH (60% in mineral oil, 0.321 g, 8.02 mmol) was added cautiously. Once addition was complete, the solution was allowed to stir vigorously for 30 minutes. The previously described compound (2E,6E)-8-bromo-3,7-dimethylocta-2,6-dienyl acetate (see compound **190** for preparation) (2.29 g, 8.31 mmol) was dissolved in THF and this ethereal solution was added slowly via syringe. The resulting solution was removed from the ice bath and allowed to stir overnight. Water was added and the mixture was poured into ether. The aqueous solution was extracted with diethyl ether, and the combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo*. Final purification by flash column chromatography (3% EtOH in EtOAc, 0.5% TEA) afforded the target compound (1.51 g, 66%): ^{1}H NMR δ 7.87 (s, 1H), 7.84–7.80 (m, 1H), 7.38–7.35 (m, 1H), 7.29–7.24 (m, 2H), 5.40–5.31 (m, 2H), 4.62 (br s, 2H), 4.58 (d, *J* = 6.9 Hz, 2H), 2.22–2.15 (m, 2H), 2.13–2.06 (m, 2H), 2.05 (s, 3H), 1.69 (br s, 3H), 1.53 (br s, 3H); ^{13}CNMR δ 170.7, 143.6, 143.0, 140.8, 133.7, 129.6, 128.2, 122.4, 121.6, 119.9, 118.6, 109.9, 60.5, 52.5, 38.4, 25.4, 20.7, 16.0, 13.7; HRMS (EI^+, *m/z*) calcd for (M^+) $C_{19}H_{24}N_2O_2$: 312.1838; found: 312.1841.



Alcohol 195.

Compound **194** (1.41 g, 4.50 mmol) was dissolved in MeOH and K_2CO_3 (3.10 g, 22.4 mmol) was added. The solution was allowed to stir overnight. After TLC analysis indicated the reaction was incomplete, another aliquot of K_2CO_3 (3.72 g, 26.9 mmol) was added and the solution was stirred an additional 7 hrs. The majority of solid K_2CO_3 was removed using gravity filtration and water was added. The solution was concentrated under a stream of air for several hours to remove ~80% of the MeOH and water was added. The aqueous phase was extracted with diethyl ether, and then the extracts were dried ($MgSO_4$) and concentrated *in vacuo*. Analysis of the ^{1}H NMR spectrum indicated the sample needed no further purification. Yield. (1.22 g, 100%): ^{1}H NMR δ 7.78 (s, 1H), 7.72–7.67 (m, 1H), 7.29–7.25 (m, 1H), 7.19–7.14 (m, 2H), 5.31–5.27 (m, 2H), 4.51 (s, 2H), 4.07 (d, J = 6.9 Hz, 2H), 3.19 (bs, 1H), 2.14–2.04 (m, 2H), 1.99–1.94 (m, 2H), 1.53 (bs, 3H), 1.44 (bs, 3H); ^{13}C NMR δ 143.5, 143.2, 137.2, 133.9, 129.5, 128.9, 124.7, 122.7, 121.9, 119.9, 110.0, 58.7, 52.7, 38.6, 25.6, 15.9, 13.9; HRMS $(EI)^+$ calcd for (M^+) $C_{17}H_{22}N_2O$: 270.1732; found: 270.1733.

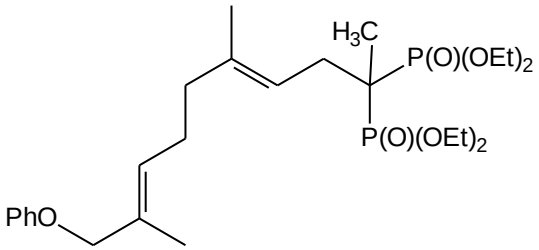


Tetraethyl bisphosphonate 200.

Previously described compound (2E,6E)-3,7-dimethyl-8-phenoxyocta-2,6-dien-1-ol[194] (1.301 g, 5.28 mmol, 1.00 eq) was dissolved in THF, cooled to 0 °C in and ice bath, and triethylamine (0.99 mL, 7.10 mmol, 1.34 eq), and mesyl chloride (0.56 mL, 7.23 mmol, 1.37 eq) were added. After stirring for 4 hr, LiBr (1.143 g, 13.2 mmol, 2.49 eq) was added. The

suspension was removed from the ice bath and allowed to stir overnight. Water, then brine, was added and the solution was extracted with hexanes. The combined organics were dried (Na_2SO_4), filtered through a bed of basic alumina, and concentrated *in vacuo*. The resulting oil was used without additional purification in the synthesis of bisphosphonate **200**.

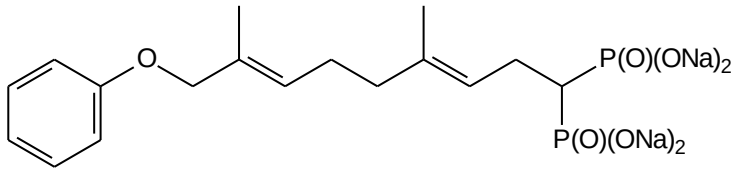
A solution of KHMDS (0.5 M, 10.4 mL, 5.20 mmol, 1.07 eq) in toluene was cooled to –78 °C in a dry ice/acetone bath and tetraethyl methylenebisphosphonate (1.41 g, 4.88 mmol, 1.00 eq) was added cautiously via syringe. The solution was allowed to stir for 30 minutes and the previously prepared bromide (1.50 g, 4.86 mmol, 1.00 eq) was added dropwise over a period of several minutes. The cryogenic conditions were maintained for 4 hr, and then the solution was allowed to warm to room temperature and stir overnight. After water was added, the solution was extracted with Et_2O, dried ($MgSO_4$), and concentrated *in vacuo*. Final purification via flash column chromatography afforded **200** (2.00 g, 80%): ^{1}H NMR δ 7.26 (dd, J = 9.0, 7.5 Hz, 2H), 6.96–6.87 (m, 3H), 5.57–5.50 (m, 1H), 5.39–5.31 (m, 1H), 4.37 (s, 2H), 4.23–4.11 (m, 8H), 2.76–2.51 (m, 2H), 2.32 (tt, J_{HP} = 23.7 Hz, J = 5.7 Hz, 1H), 2.27–2.17 (m, 2H), 2.09–2.01 (m, 2H), 1.73 (s, 3H), 1.66 (s, 3H), 1.34 (t, J = 6.9 Hz, 12H); ^{13}C NMR δ 158.7, 136.0, 130.9, 129.1 (2C), 128.2, 122.0 (t, J_{CP} = 7.5 Hz), 120.3, 114.4 (2C), 73.6, 62.3–62.0 (m, 4C), 38.9, 37.2 (t, J_{CP} = 131.8 Hz), 25.9, 23.8 (t, J_{CP} = 4.9 Hz), 16.3–16.1 (m, 4C), 15.8, 13.6; ^{31}P NMR δ 23.6 Hz; HRMS (EI$^+$, *m/z*) calcd for (M^+) $C_{25}H_{42}O_7P_2$: 516.2406. Found: 516.2414.



Tetraethyl bisphosphonate 201.

A suspension of NaH (60% in mineral oil, 50 mg, 1.25 mmol, 1.43 eq) in THF was cooled to 0 °C in an ice bath, and 15-crown-5 (0.02 mL, 0.10 mmol, 0.11 eq) was added. After bisphosphonate **200** (452 mg, 0.88 mmol, 1.00 eq) was added slowly via syringe, the resulting

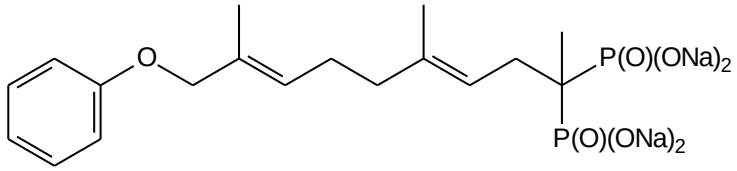
slurry was allowed to stir for 30 minutes, and then iodomethane (0.08 mL, 1.29 mmol, 1.47 eq) was added. The mixture was immediately removed from the ice bath and allowed to stir overnight. Aqueous NH_4Cl was added and the solution was extracted with Et_2O, dried ($MgSO_4$), and concentrated *in vacuo*. Final purification by flash column chromatography (20% EtOH in hexanes) provided **201** (344 mg, 74%): ^{1}H NMR δ 7.29–7.23 (m, 2H), 6.95–6.88 (m, 3H), 5.58–5.52 (m, 1H), 5.45–5.38 (m, 1H), 4.37 (s, 2H), 4.25–4.13 (m, 8H), 2.62 (td, J_{HP} = 15.6 Hz, J = 7.8 Hz, 2H), 2.24–2.16 (m, 2H), 2.13-2.05 (m, 2H), 1.74 (s, 3H), 1.63 (s, 3H), 1.39 (t, J_{HP} = 16.8 Hz, 3H), 1.36–1.30 (m, 12H); ^{13}C NMR 158.7, 137.1, 130.8, 129.1 (2C), 128.4, 120.4, 119.2 (t, J_{CP} = 7.5 Hz), 114.5 (2C), 73.7, 62.4–62.2 (m, 4C), 40.8 (t, J_{CP} = 133.4), 39.2, 30.8 (t, J_{CP} = 4.9 Hz), 26.0, 16.5, 16.3–16.2 (m, 4C), 15.9, 13.7; ^{31}P NMR 27.2; HRMS (EI$^+$, *m/z*) calcd for $(M)^+$ $C_{26}H_{44}O_7P_2$: 530.2562. Found: 530.2579.



Tetrasodium bisphosphonate 182.

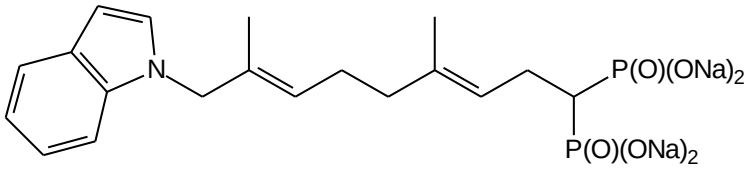
To a stirred solution of **200** (205 mg, 0.40 mmol, 1.00 eq) in CH_2Cl_2 at 0 °C were added 2,4,6-collidine (0.41 mL, 3.09 mmol, 7.78 eq) and TMSBr (0.41 mL, 3.17 mmol, 7.98 eq). The solution was allowed to warm to room temperature and stirred overnight. After the volatiles were removed, toluene was added and the solvent was removed *in vacuo*. The remaining residue was treated with NaOH (1M, 2.0 mL, 2.0 mmol, 5.04 eq), and the suspension was stirred for 5 hours until all the residue had dissolved. The resulting solution was poured into acetone and the mixture was stored at 3 °C for 72 hrs. After the solution was filtered, the solids were washed with several portions of cold acetone and then dissolved in H_2O. The aqueous solution was filtered and concentrated *in vacuo* to afford **182** as a fine white powder (150 mg, 77%); ^{1}H NMR

δ 7.27–7.16 (m, 2H), 6.96–6.81 (m, 3H), 5.56–5.58 (m, 2H), 4.33 (s, 2H), 2.56–2.41 (m, 2H), 2.17–1.79 (m, 5H), 1.59 (br s, 6H); ^{13}C NMR δ 158.2, 136.4, 131.0, 130.3, 129.9 (2C), 124.0 (t, J_{CP} = 8.2 Hz), 121.7, 115.5 (2C), 74.4, 40.0 (t, J_{CP} = 116.1), 38.8, 26.1, 24.2, 15.6, 13.4; ^{31}P NMR δ 20.3; HRMS (ES$^-$) calcd for (M-H)$^-$ $C_{17}H_{25}O_7P_2$: 403.1076; found: 403.1082.



Tetrasodium bisphosphonate 183.

A solution of 2,4,6-collidine (1.0 mL, 7.54 mmol, 7.77 eq) was cooled to 0 °C in an ice bath and TMSBr (1.0 mL, 7.71 mmol, 7.95 eq) was added. The solution was allowed to stir for 10 minutes, the bisphosphonate **201** (516 mg, 0.97 mmol, 1.0 eq) was added, and the solution was stirred overnight. The volatiles were removed, toluene (5 mL) was added and removed *in vacuo*, and the resulting residue was treated with NaOH (1M, 4.1 mL, 4.1 mmol, 4.2 eq) and stirred overnight. The resulting solution was poured into acetone and stored at 4 °C for 72 hrs in which time a white precipitate had formed. The mixture was filtered to give the target tetrasodium salt in 79% yield (389 mg): ^{1}H NMR δ 7.27 (t, J = 7.5 Hz, 2H), 6.98–6.90 (m, 3H), 5.55–5.47 (m, 2H), 4.39 (s, 2H), 2.47–2.34 (m, 2H), 2.15–2.00 (m, 4H), 1.62 (s, 3H), 1.55 (s, 3H), 1.08 (t, J_{HP} = 15.0 Hz, 3H); ^{13}C NMR δ 158.0, 136.4, 130.9, 130.7, 130.0 (2C), 123.6 (t, J_{CP} = 7.9 Hz), 127.7, 155.5 (2C), 74.6, 40.5 (t, J_{CP} = 116.8 Hz), 39.2, 31.7, 26.2, 18.3, 15.5, 13.3; ^{31}P NMR δ 24.9; HRMS (ES$^-$) calcd for (M-H)$^-$ $C_{18}H_{27}O_7P_2$: 417.1232; found: 417.1253.



Tetrasodium bisphosphonate 184.

A solution of 2,4,6-collidine (0.39 mL, 2.94 mmol) in CH_2Cl_2 was cooled to 0 °C in an ice bath and bromotrimethylsilane (0.38 mL, 2.94 mmol) was added. The solution was stirred for 20 minutes and **193** was added via syringe as a neat liquid. The solution was allowed to stir overnight and the volatiles were removed. Toluene was added, and the solvent was removed *in vacuo*. The resulting residue was treated with aqueous NaOH (1M, 1.9 mL, 1.9 mmol) and allowed to stir overnight. The resulting mixture was poured into acetone, held at 3 °C for 72 hrs, and filtered. The solid was dried, dissolved in H_2O, and the aqueous solution was filtered and concentrated *in vacuo* to provide **184** (115 mg, 60%): ^{1}H NMR δ 7.60 (d, J = 7.8 Hz, 1H), 7.39 (d, J = 8.7 Hz, 1H), 7.21–7.12 (m, 2H), 7.06 (dd, J = 7.5, 7.2 Hz, 1H), 6.47 (d, J = 2.7 Hz, 1H), 5.57–5.46 (m, 1H), 5.36–5.30 (m, 1H), 4.57 (s, 2H), 2.51–2.37 (m, 2H), 2.16–1.93 (m, 4H), 1.62 (tt, J_{HP} = 21.6 Hz, J = 5.7 Hz, 1H), 1.57 (s, 3H), 1.38 (s, 3H); ^{13}C NMR δ 136.3, 134.6, 131.8, 129.9, 128.5, 128.2, 127.5 (t, J_{CP} = 8.6 Hz), 121.7, 121.1, 119.7, 110.7, 100.3, 53.9, 41.7 (t, J_{CP} = 115.6), 39.1, 26.3, 26.2, 15.7, 13.3; ^{31}P NMR δ 20.8; HRMS (ESI, *m/z*) calcd for $(M-H)^-$ $C_{19}H_{26}NO_6P_2$: 426.1235. Found: 426.1234.

APPENDIX

SELECTED NMR SPECTRA

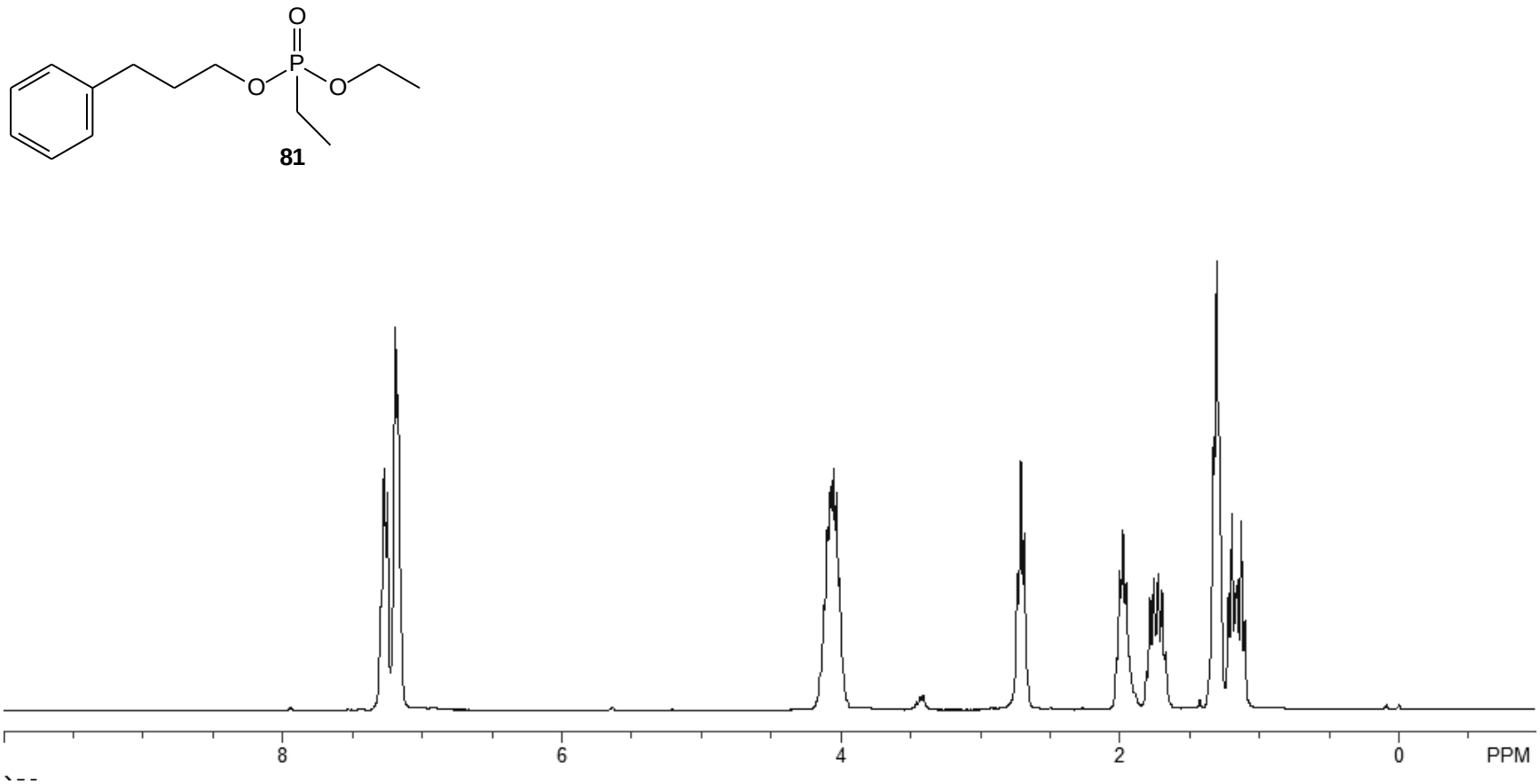


Figure A1. ^{1}H NMR spectrum for phosphonate **81**.

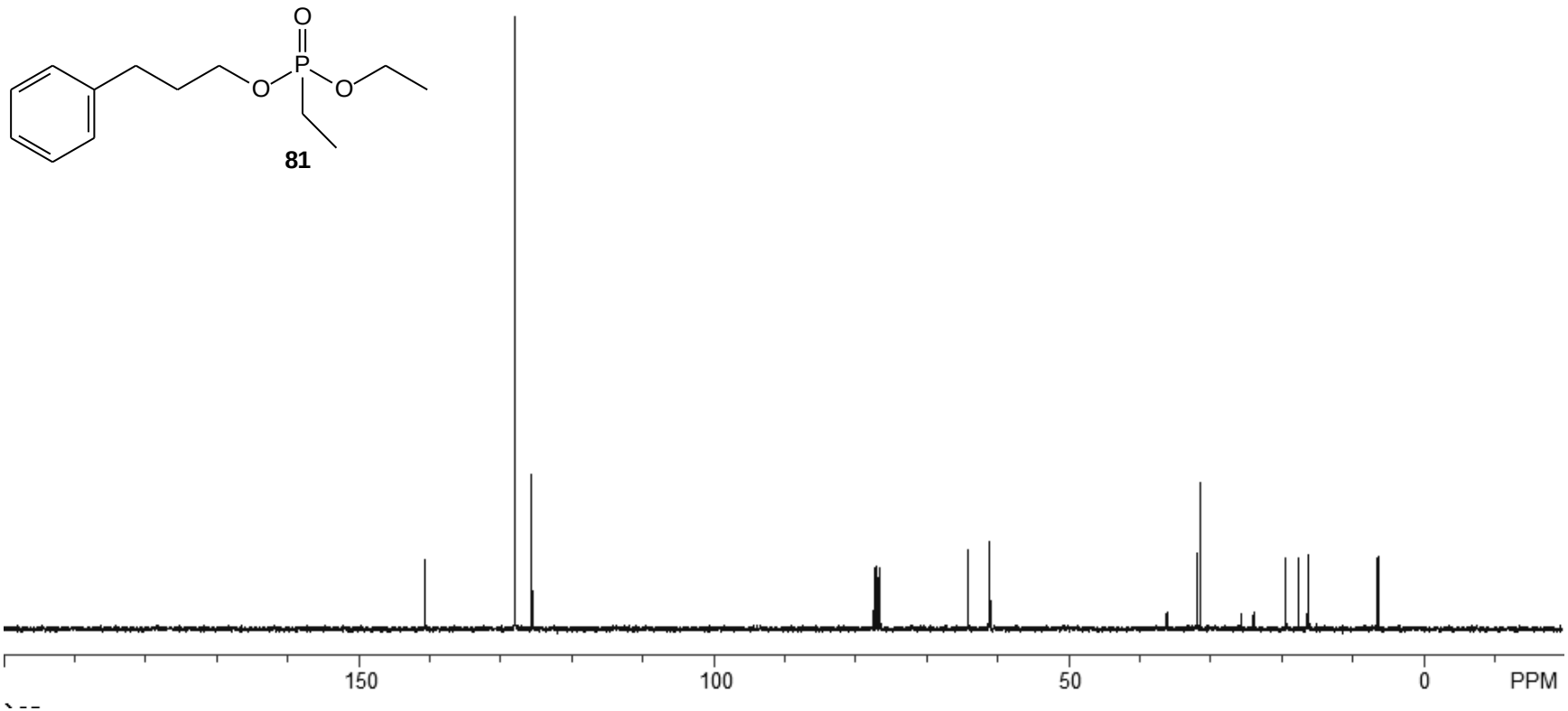
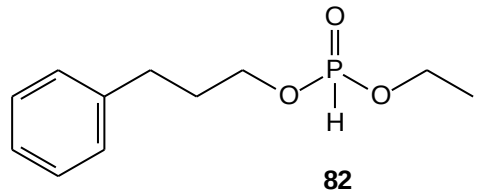


Figure A2. ^{13}C NMR spectrum for phosphonate **81**.



82

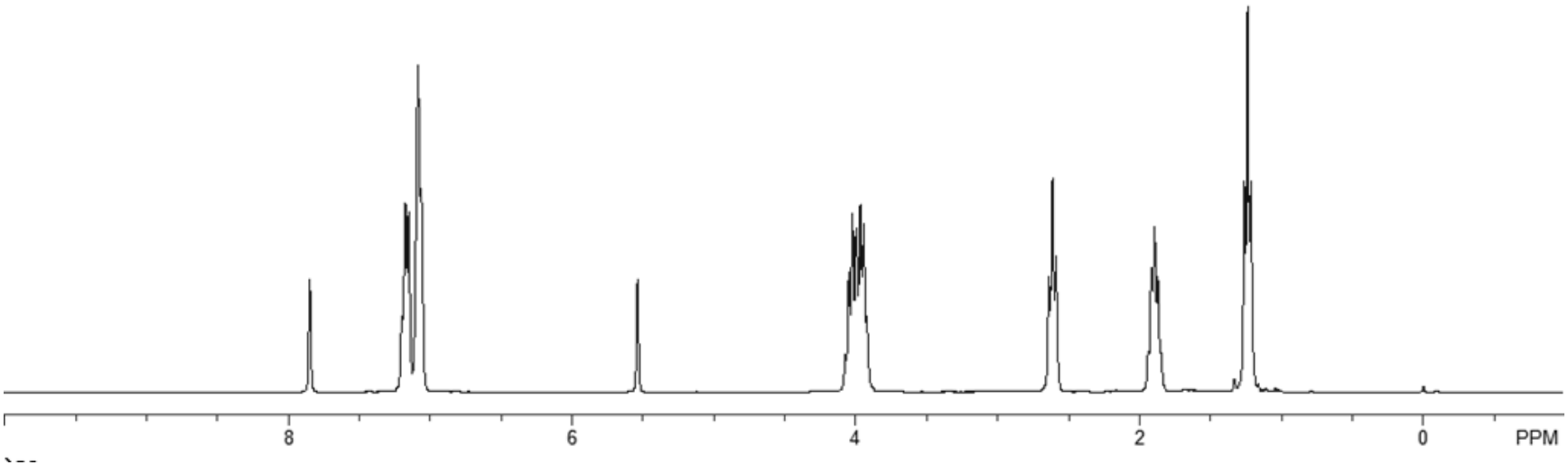


Figure A3. ^{1}H NMR spectrum for phosphite **82**.

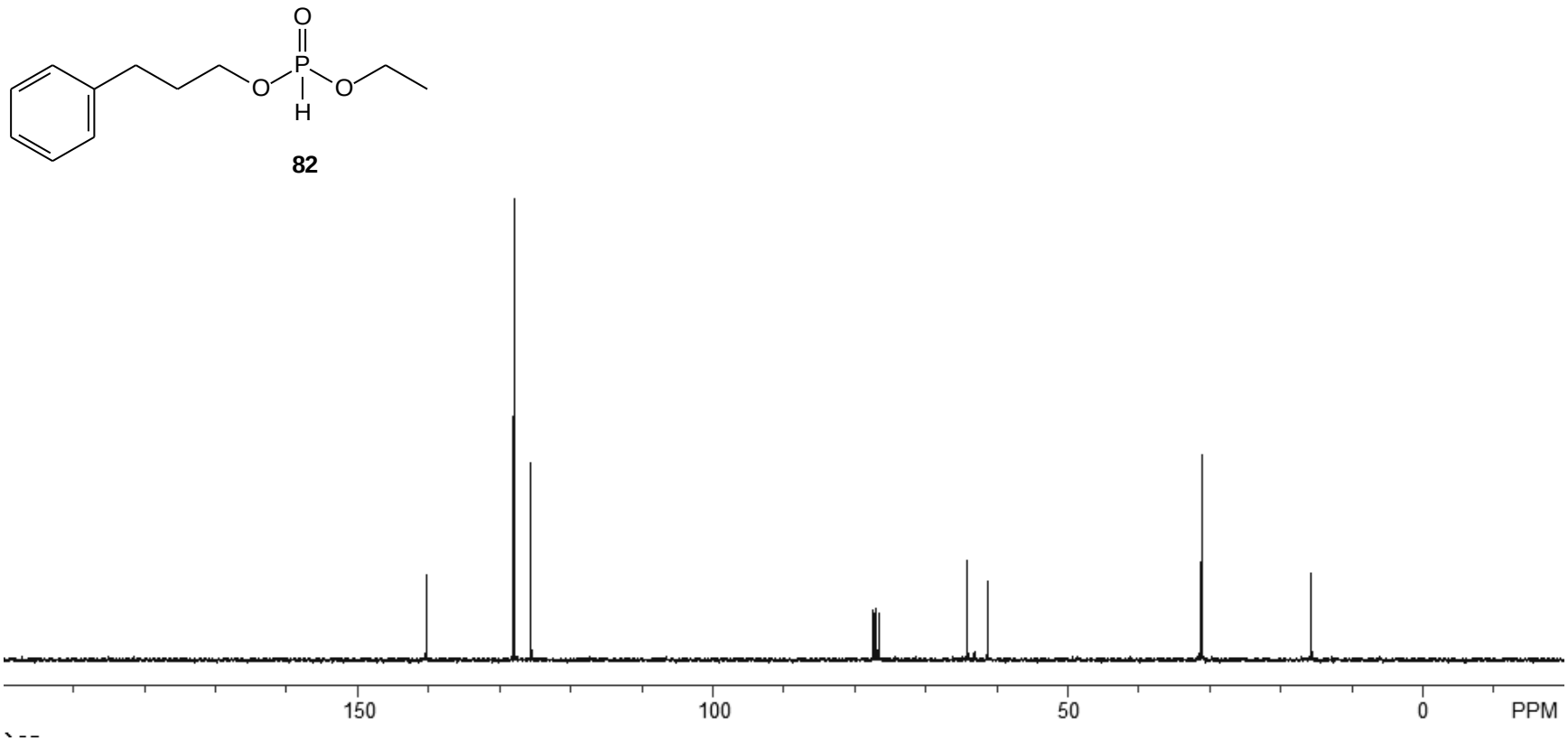


Figure A4. ^{13}C NMR spectrum for phosphite **82**.

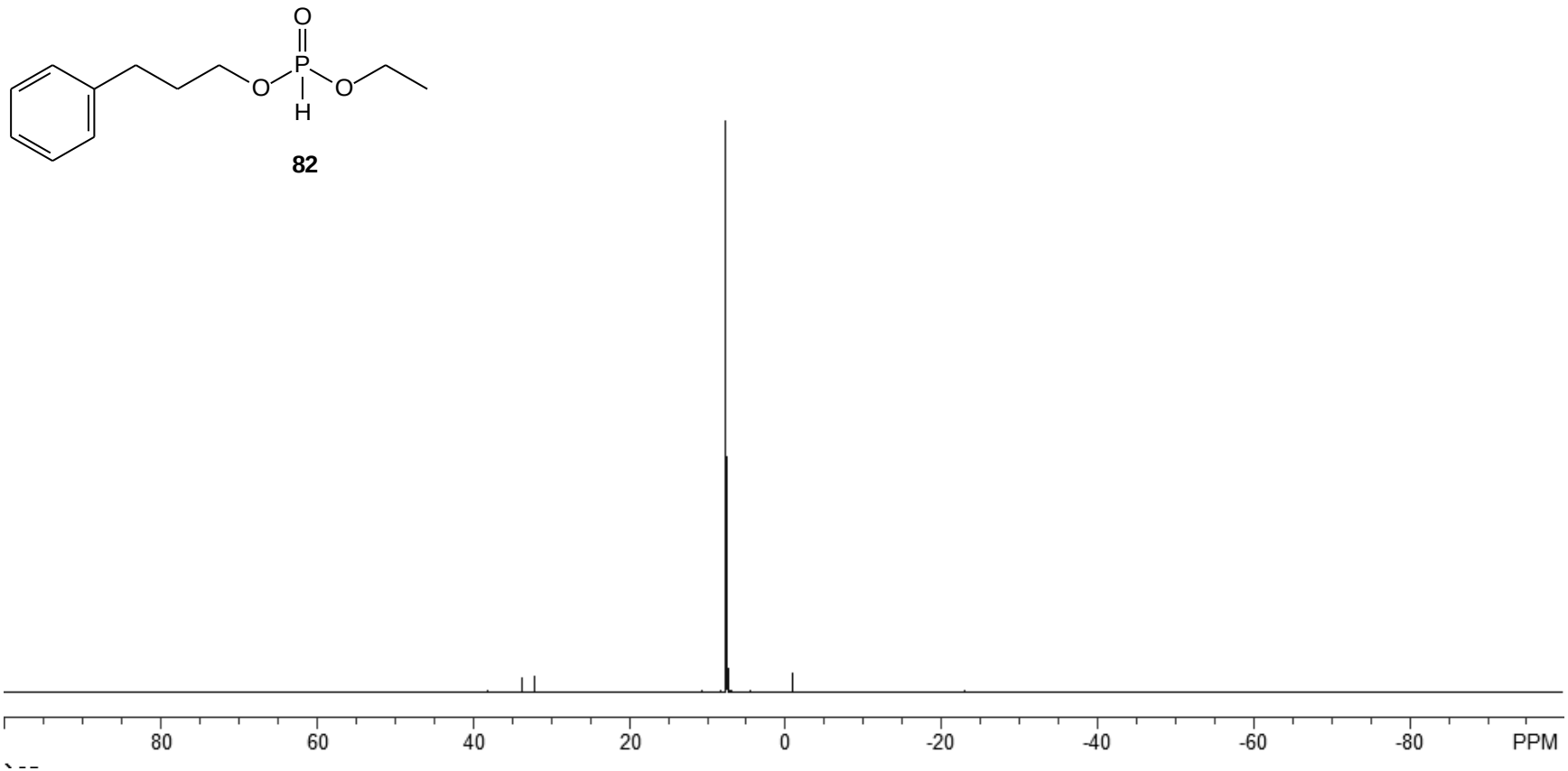
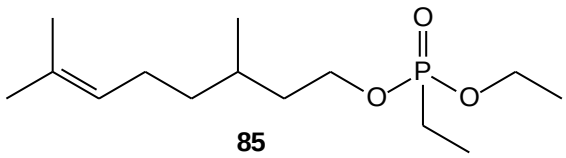


Figure A5. ^{31}P NMR spectrum for phosphite **82**.



85

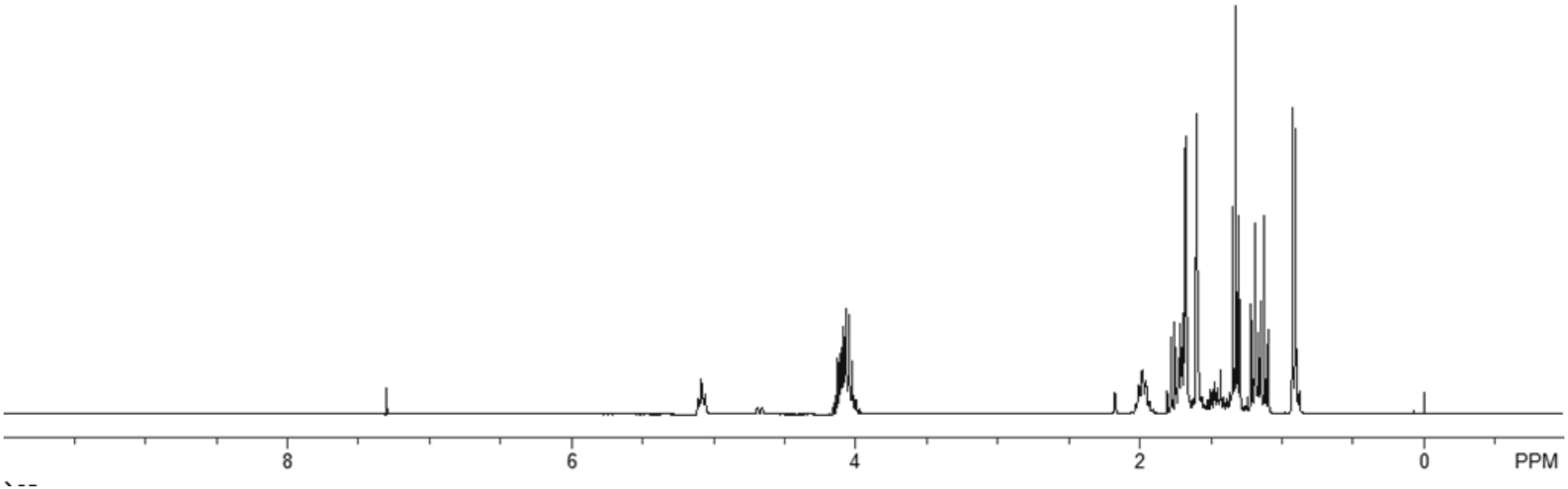


Figure A6. ^{1}H NMR spectrum for phosphonate **85**.

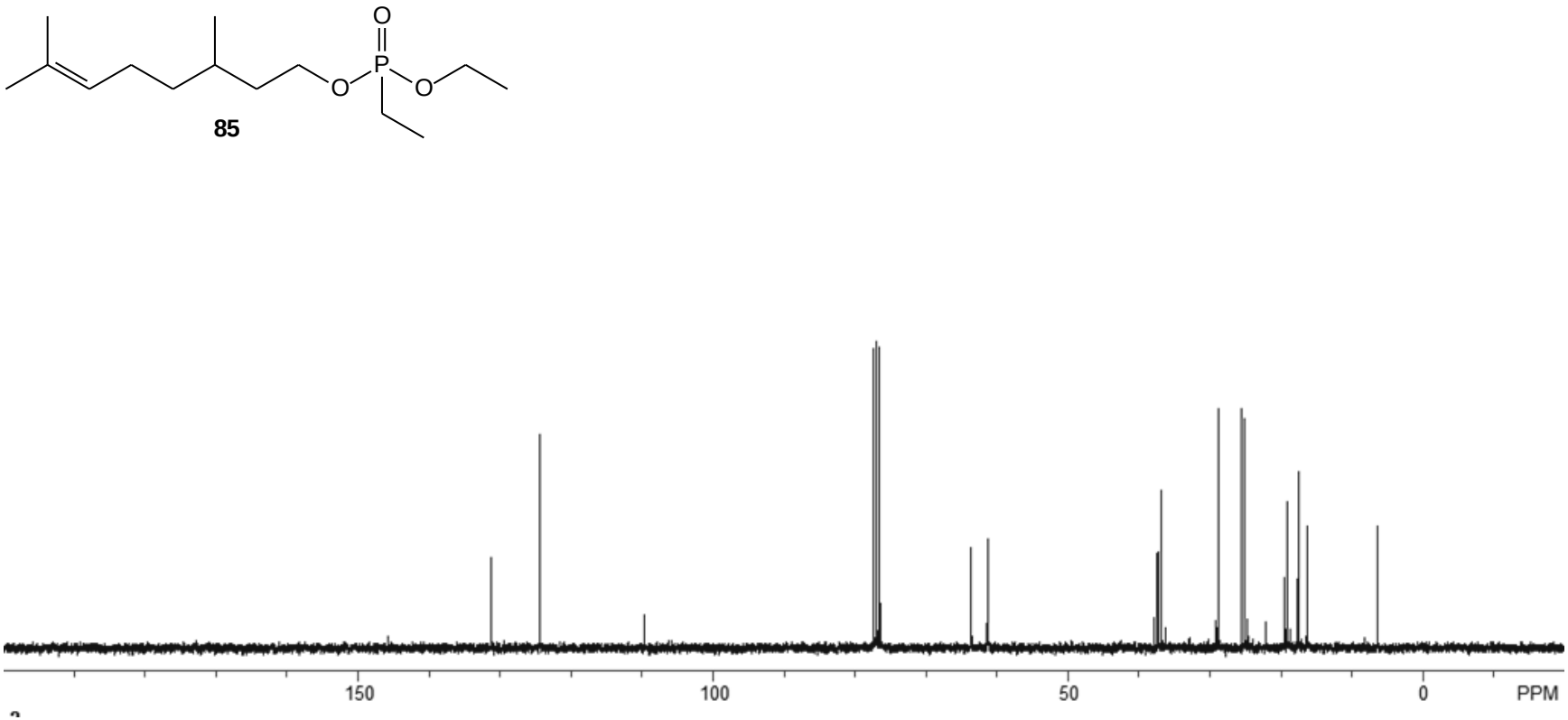


Figure A7. ^{13}C NMR spectrum for phosphonate **85**.

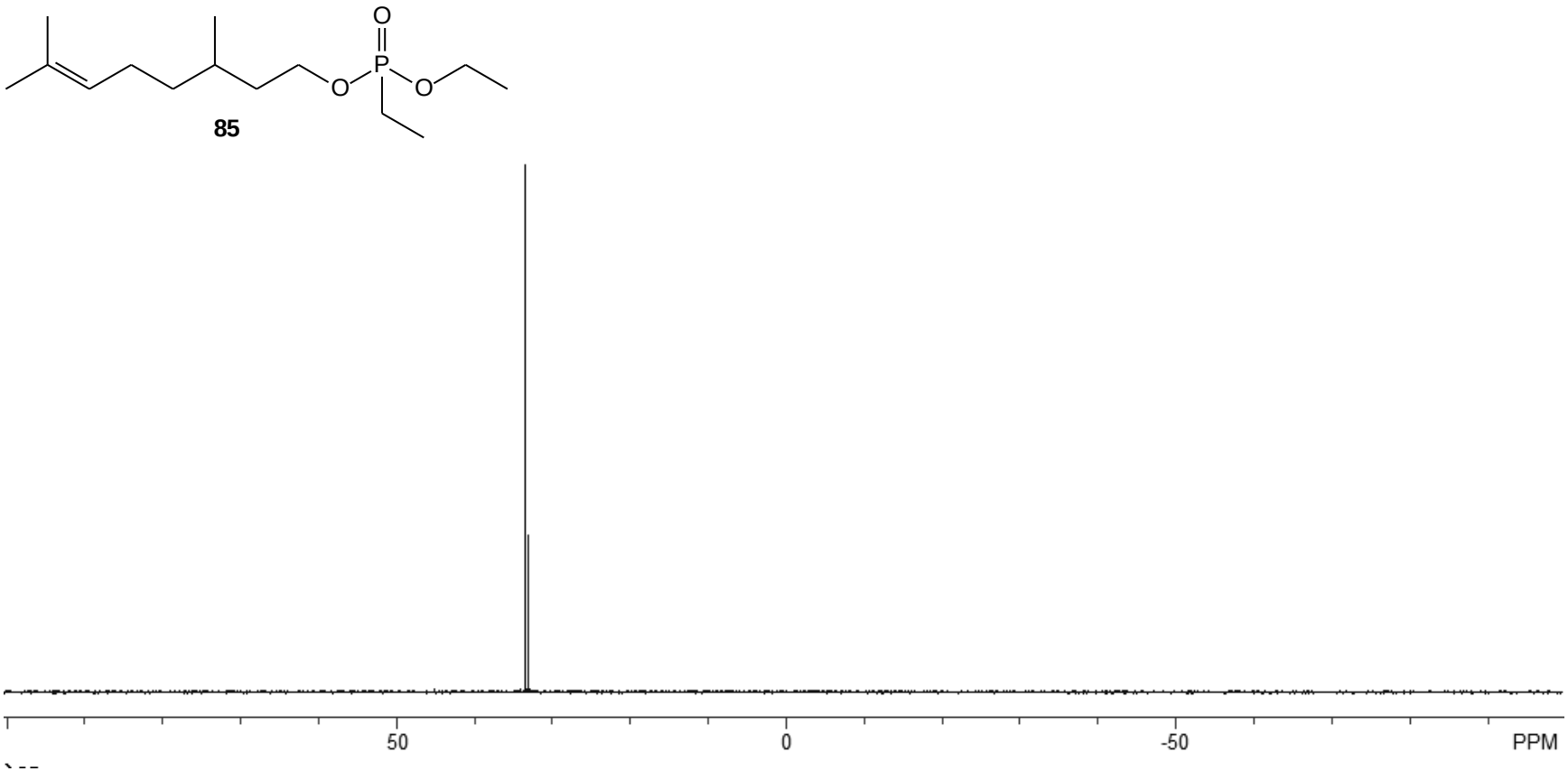
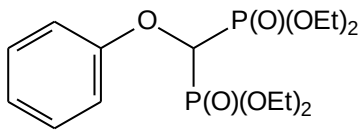


Figure A8. ^{31}P NMR spectrum for phosphonate **85**.



112

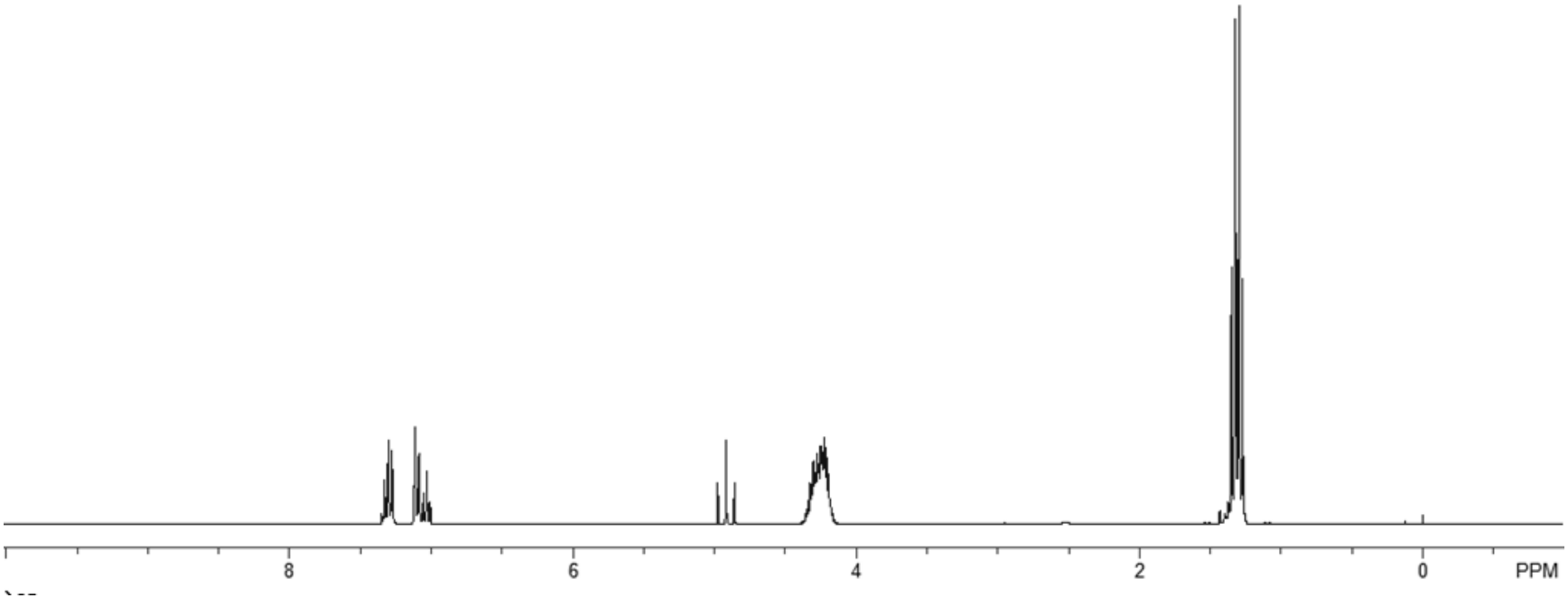
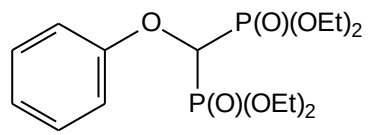


Figure A9. ^{1}H NMR spectrum for bisphosphonate **112**.



112

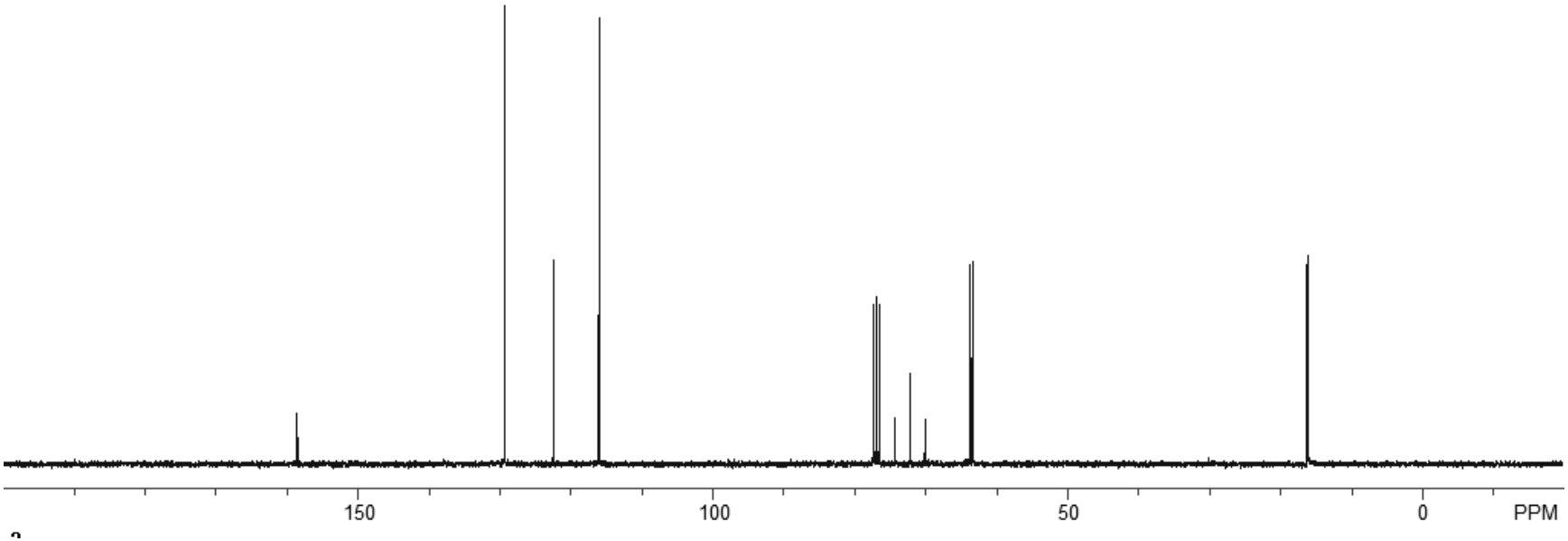


Figure A10. ^{13}C NMR spectrum for bisphosphonate **112**.

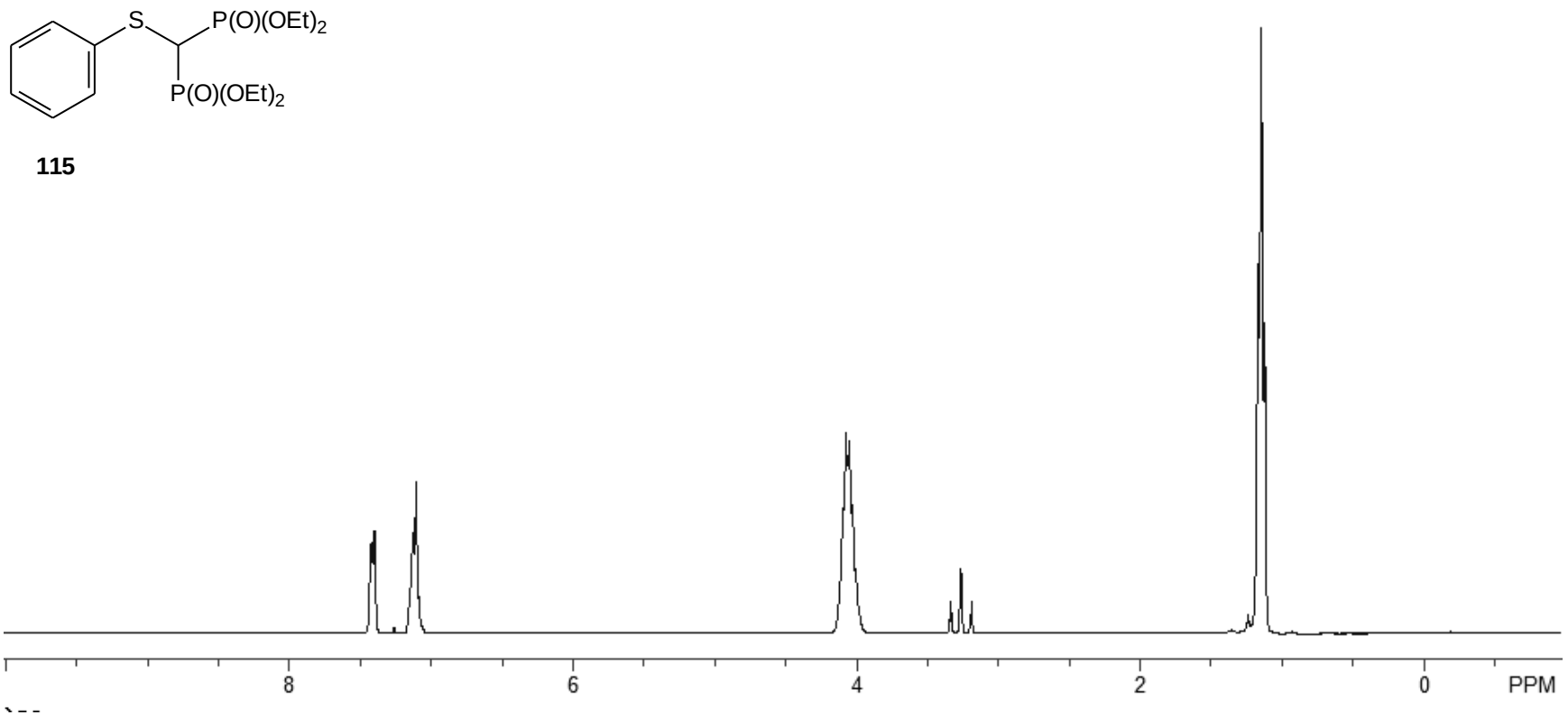


Figure A11. ^{1}H NMR for bisphosphonate **115**.

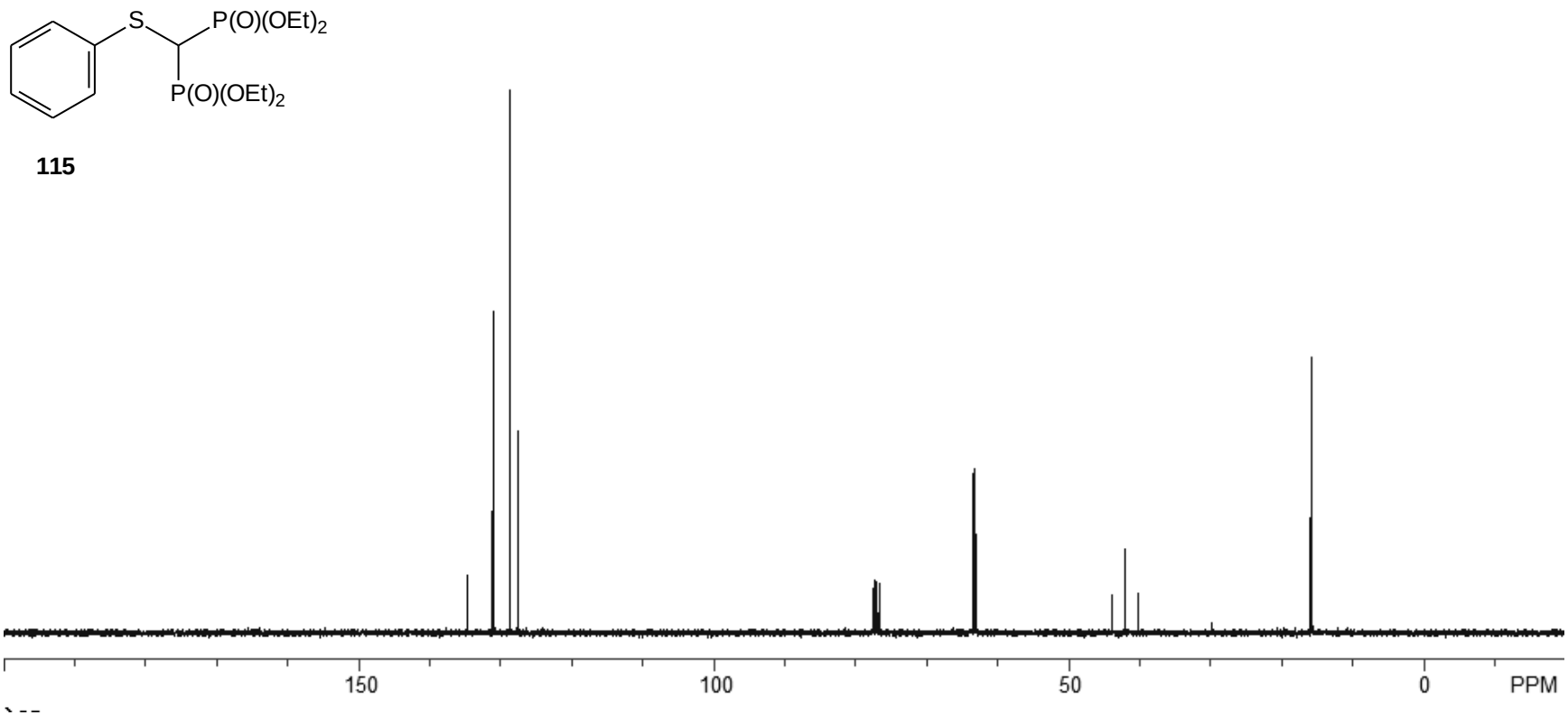


Figure A12. ^{13}C NMR for bisphosphonate **115**.

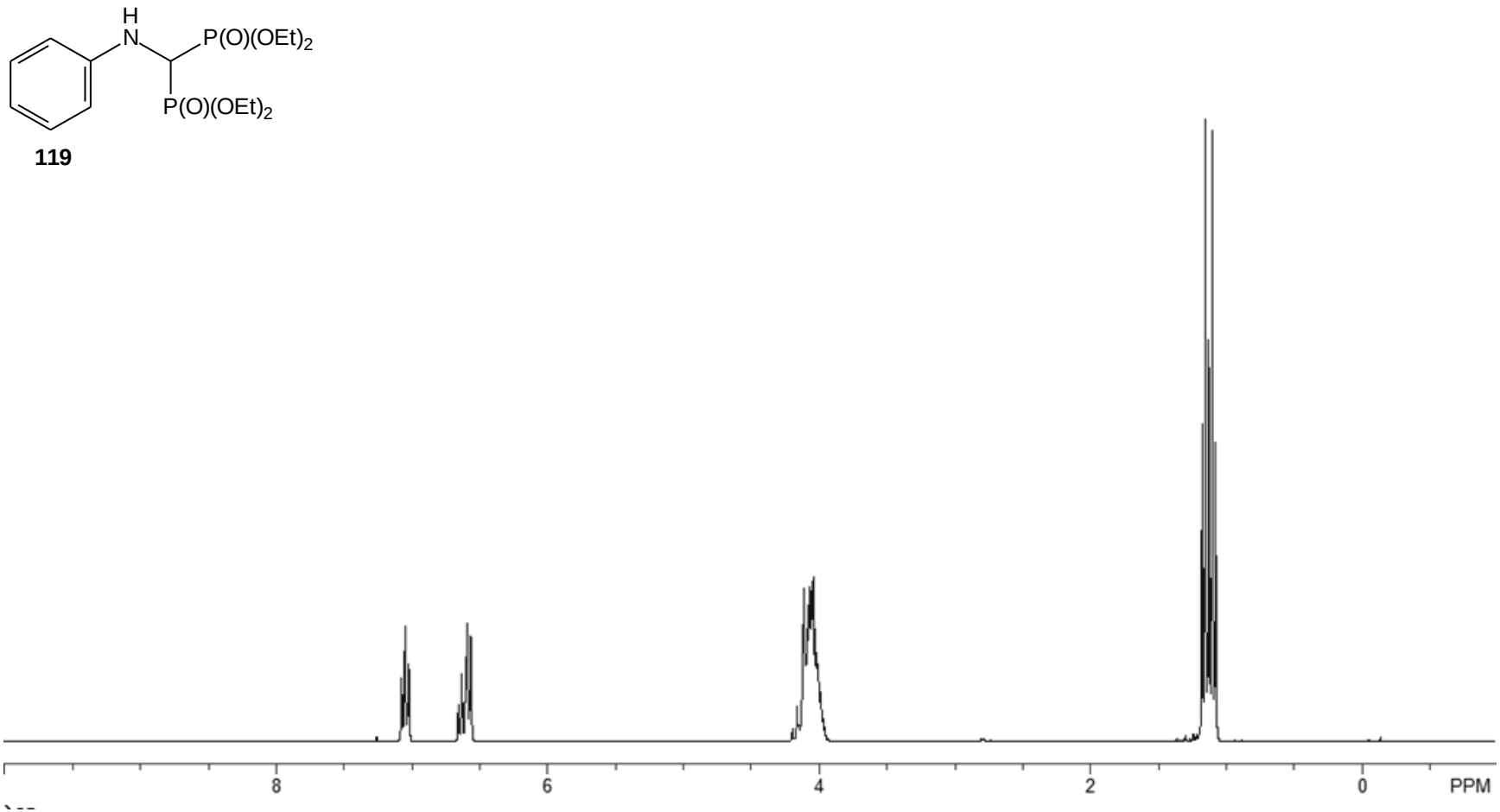


Figure A13. ^{1}H NMR for bisphosphonate **119**.

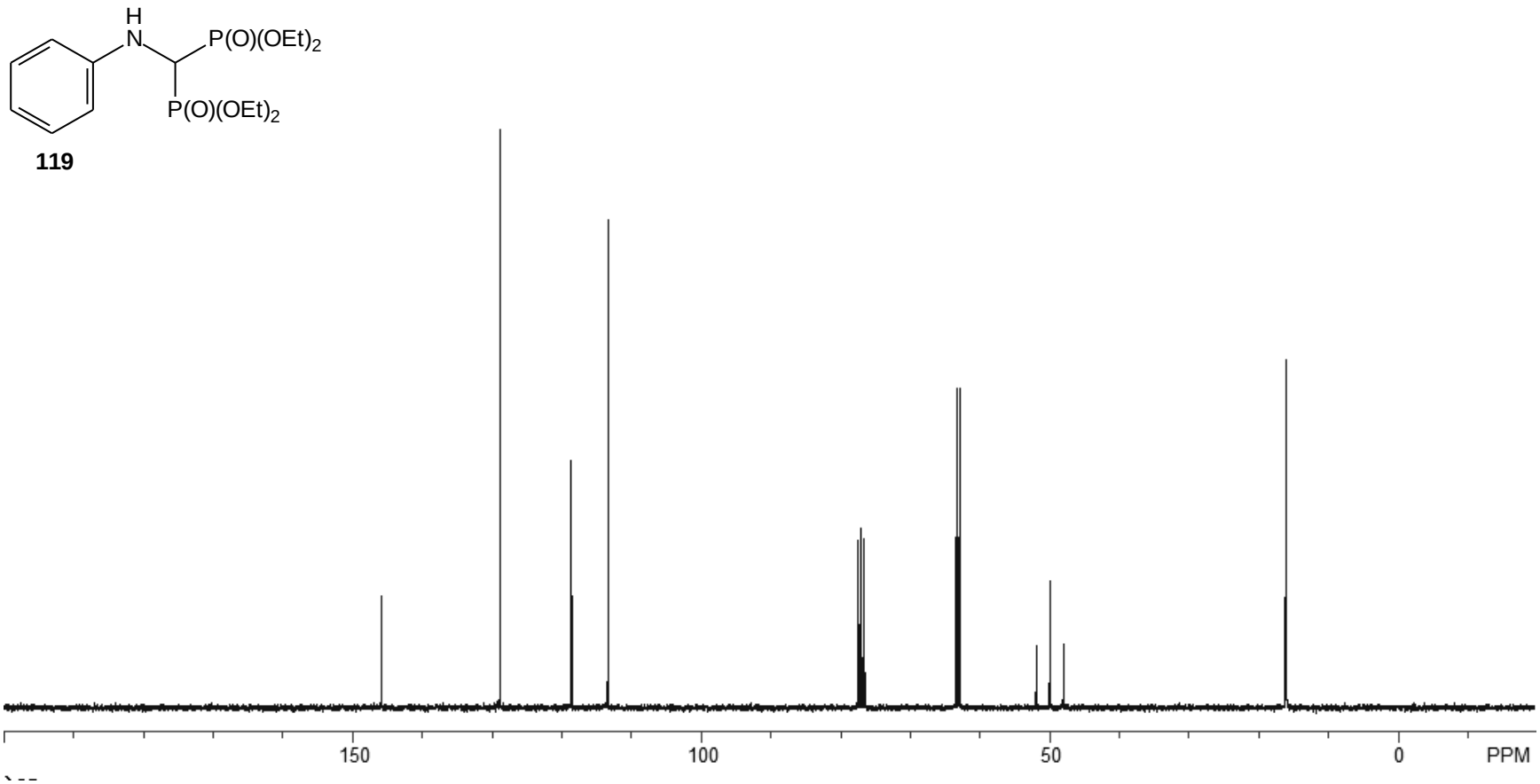
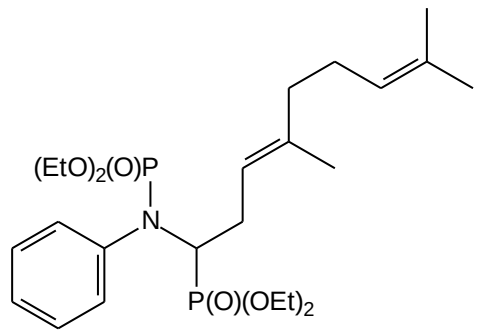


Figure A14. ^{13}C NMR spectrum for bisphosphonate **119**.



122

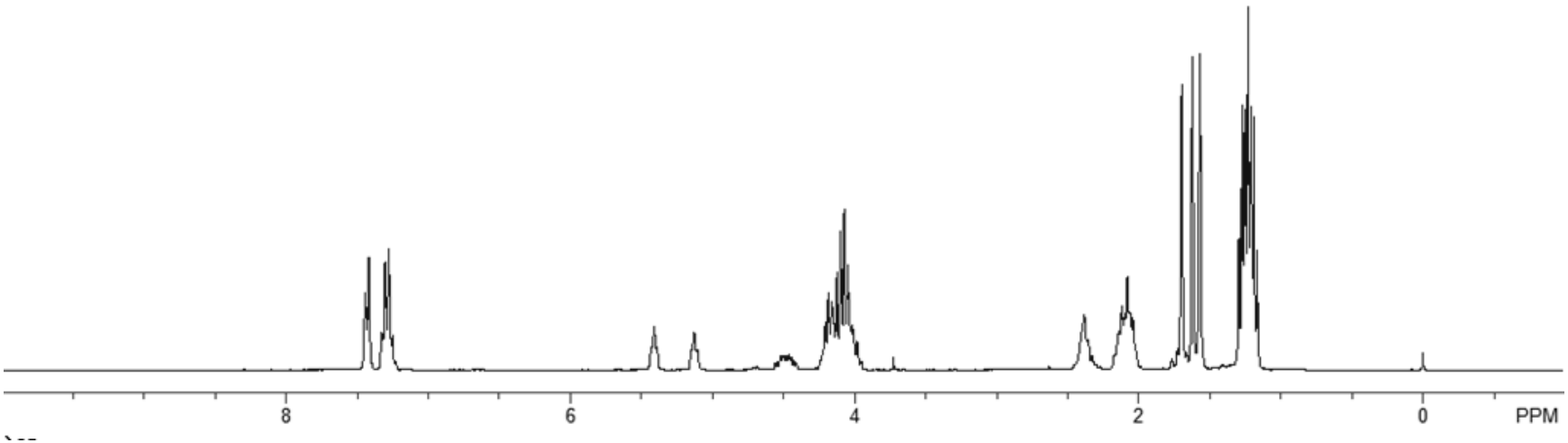


Figure A15. ^{1}H NMR for compound **122**.

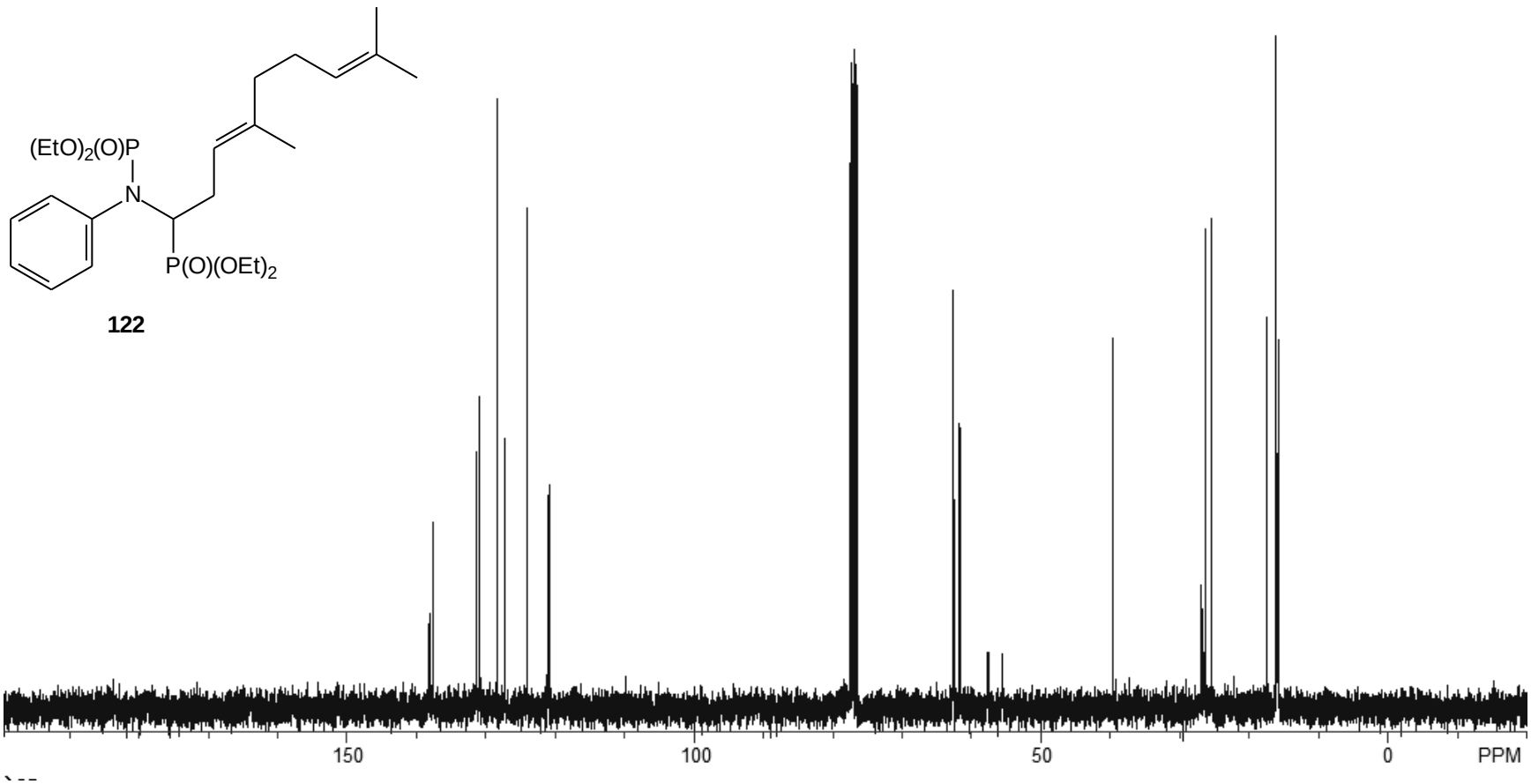


Figure A16. ^{13}C NMR for compound **122**.

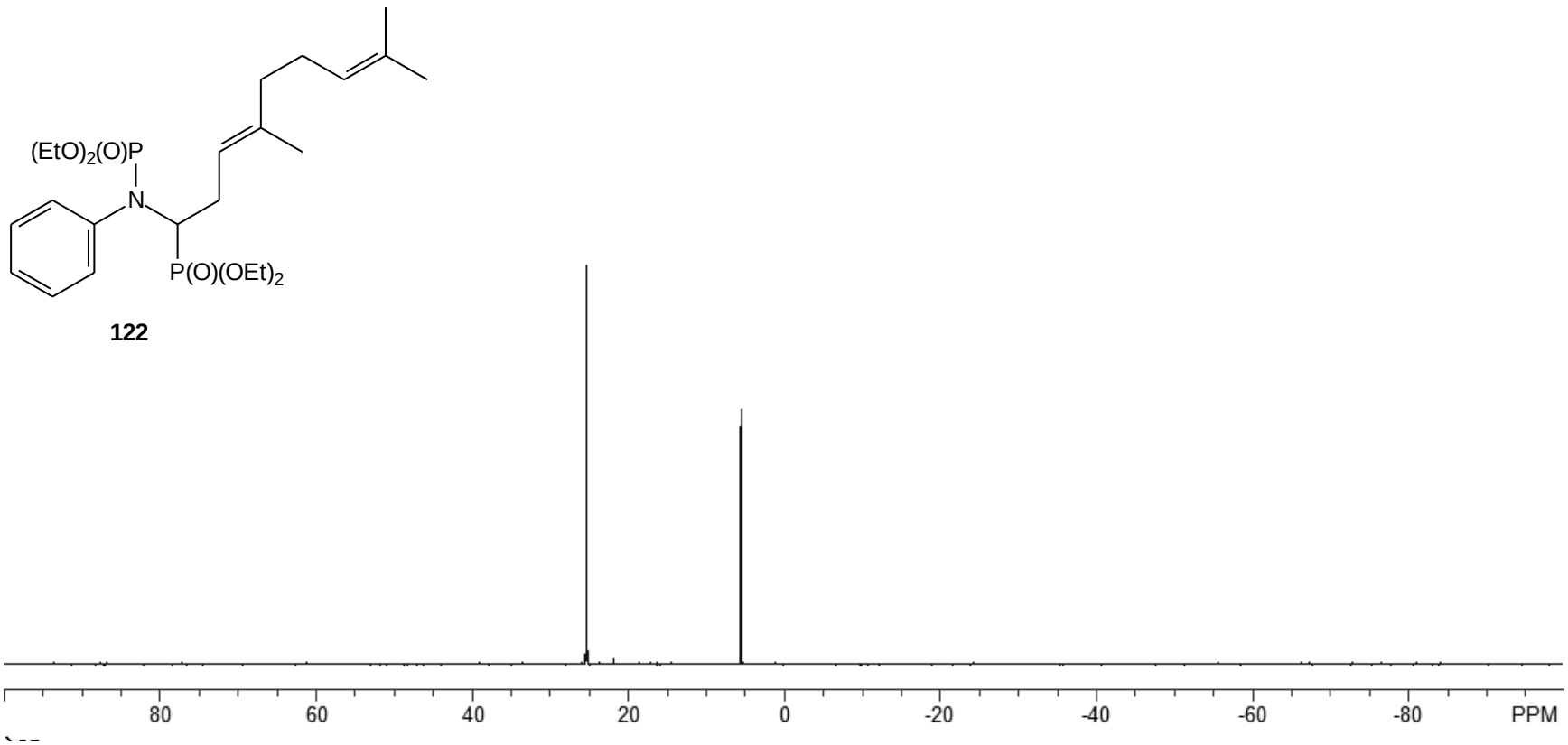
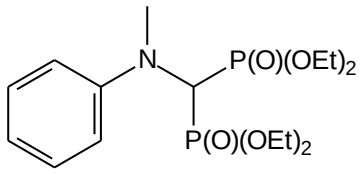


Figure A17. ^{31}P NMR spectrum for compound **122**.



124

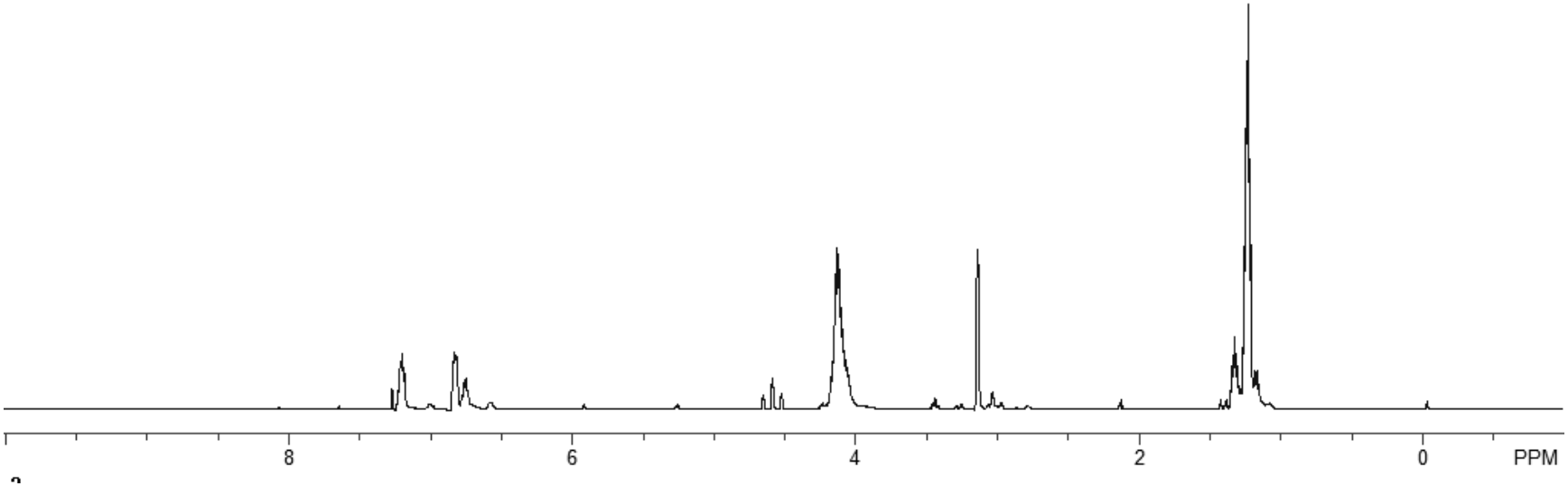


Figure A18. ^{1}H NMR for bisphosphonate **124**.

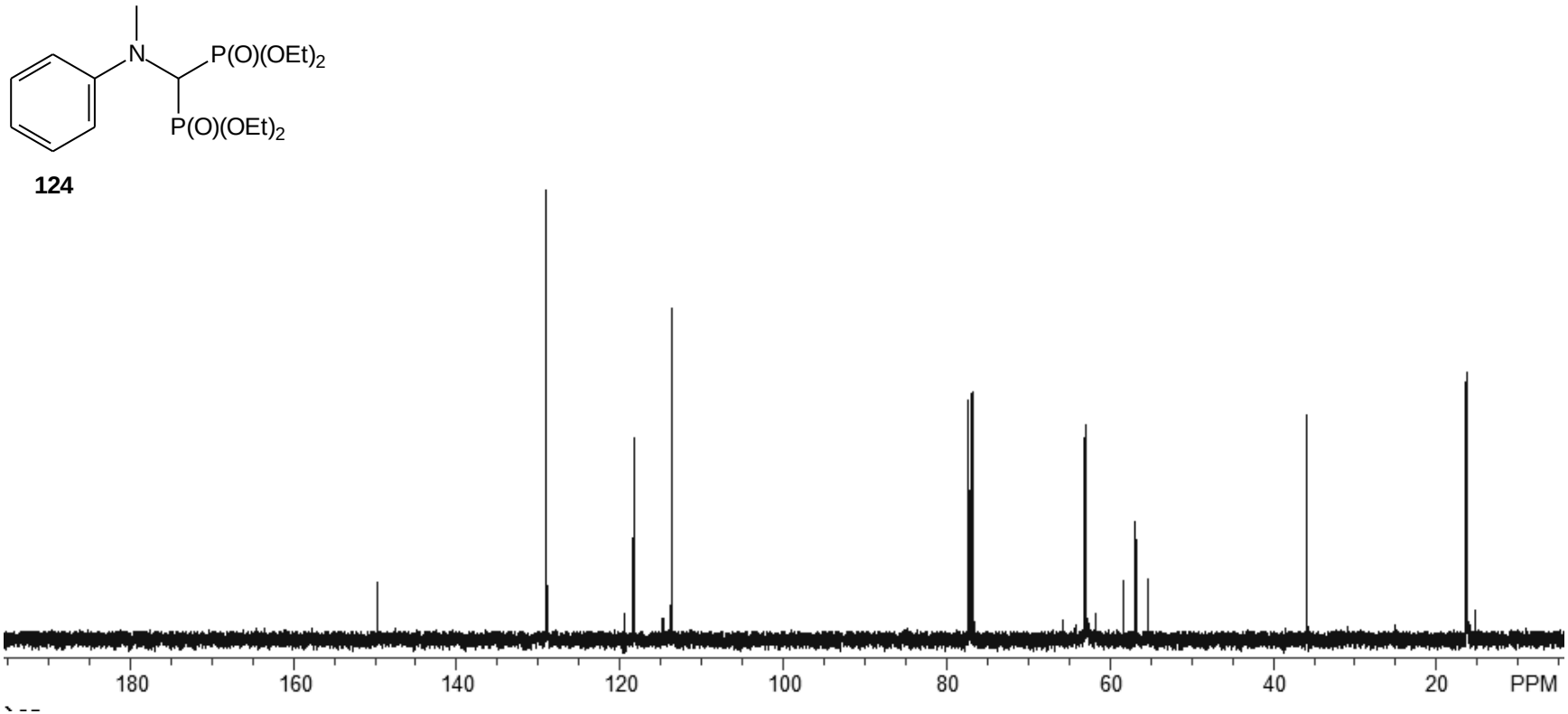


Figure A19. ^{13}C NMR spectrum for bisphosphonate **124**.

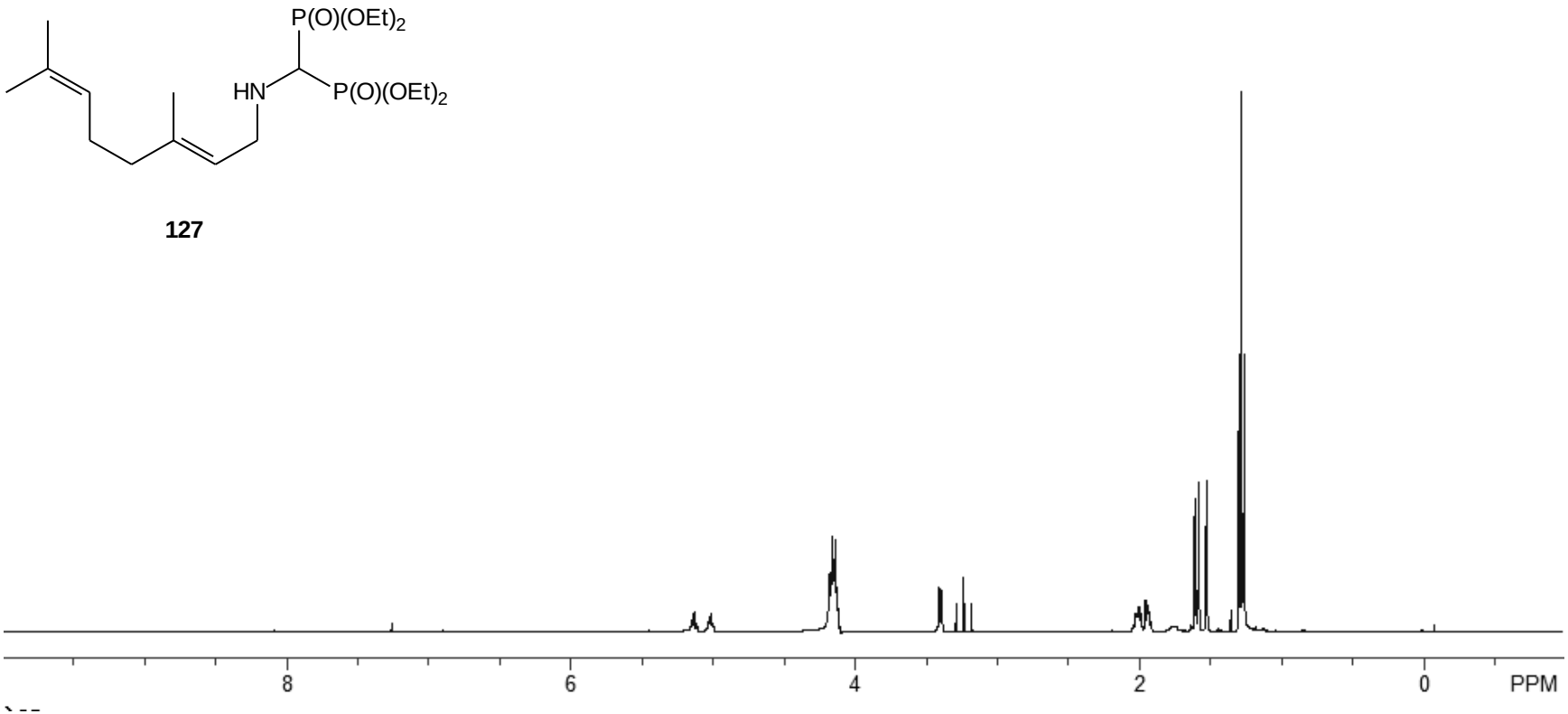


Figure A20. ^{1}H NMR spectrum for bisphosphonate **127**.

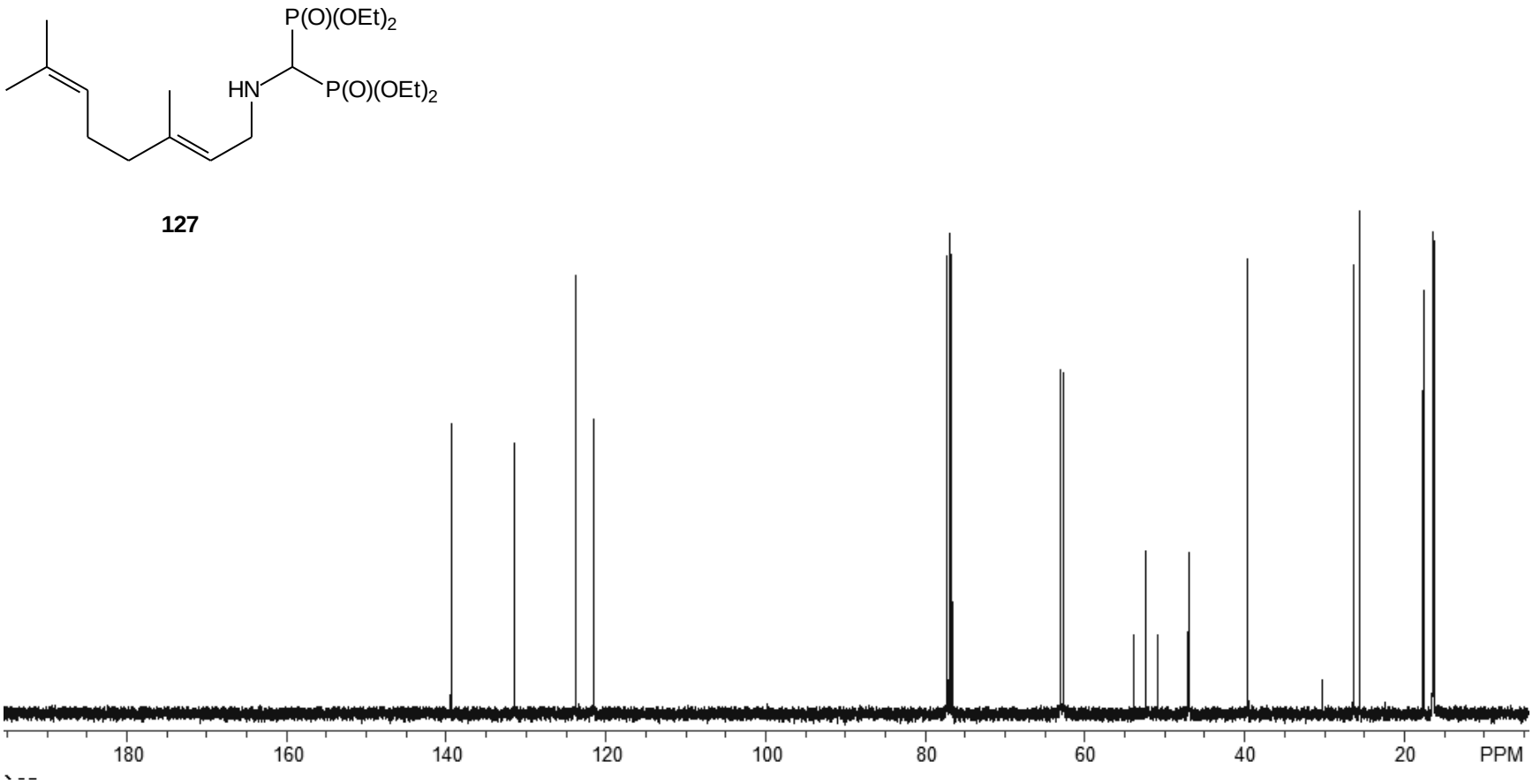


Figure A21. ^{13}C NMR spectrum for bisphosphonate **127**.

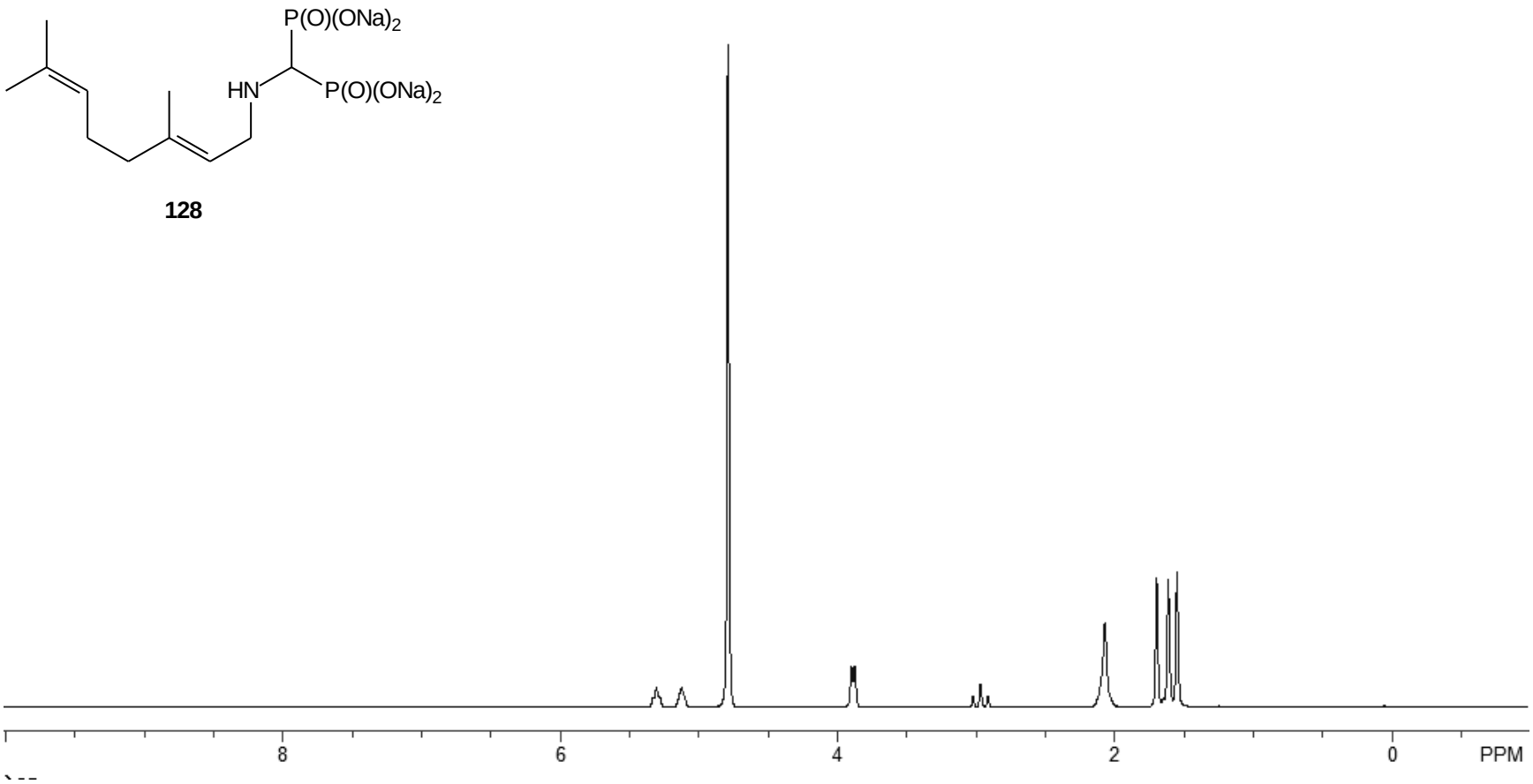
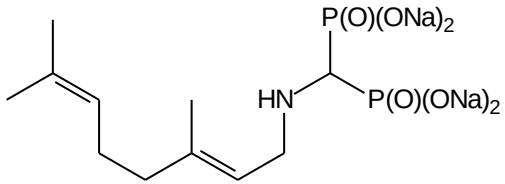


Figure A22. ^{1}H NMR spectrum for bisphosphonate **128**.



128

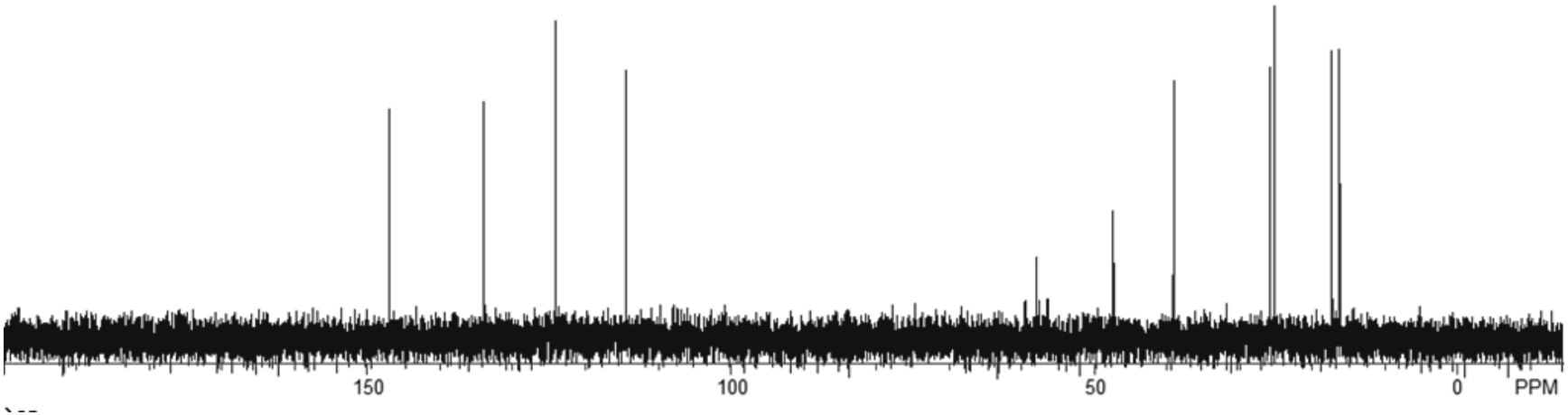


Figure A23. ^{13}C NMR spectrum for bisphosphonate **128**.

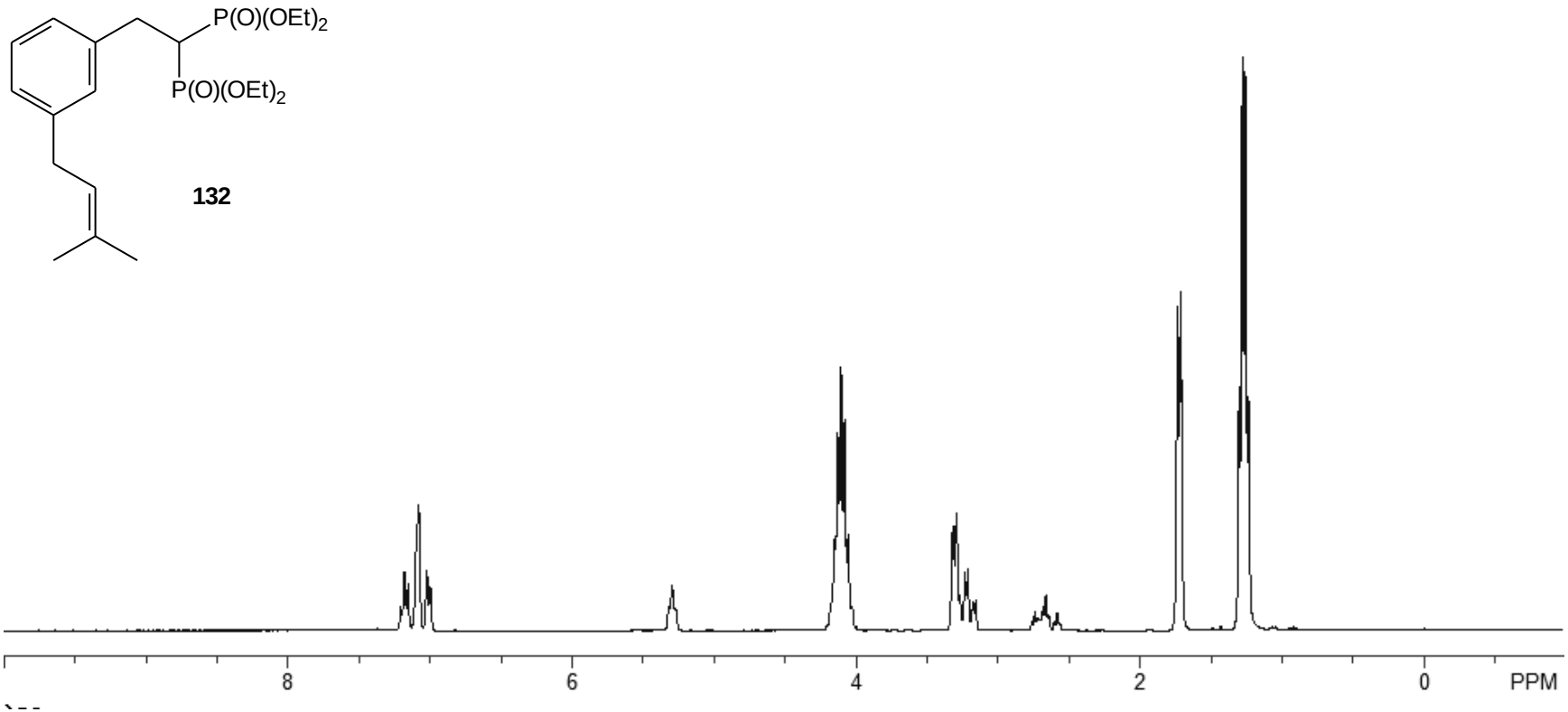


Figure A24. ^{1}H NMR spectrum for bisphosphonate **132**.

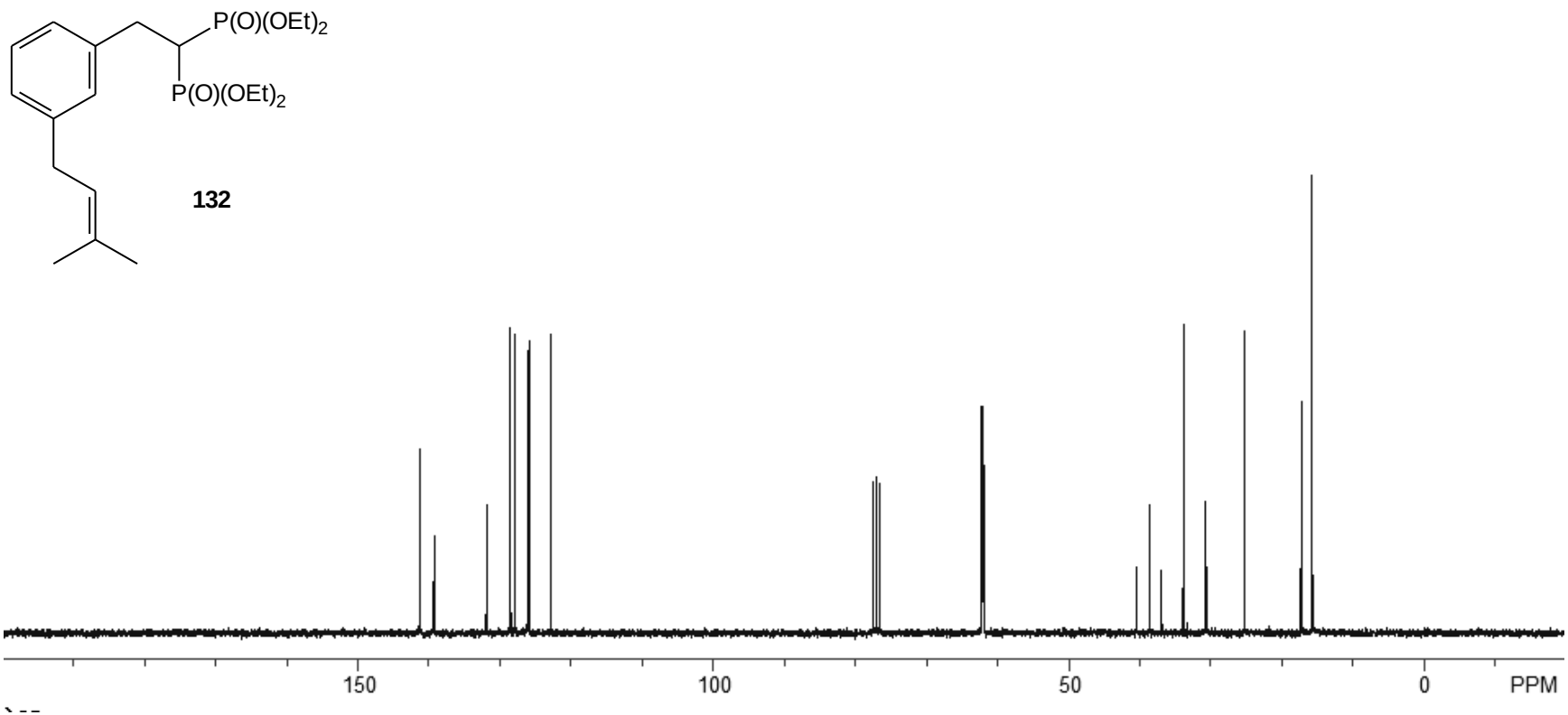


Figure A25. ^{13}C NMR spectrum for bisphosphonate **132**.

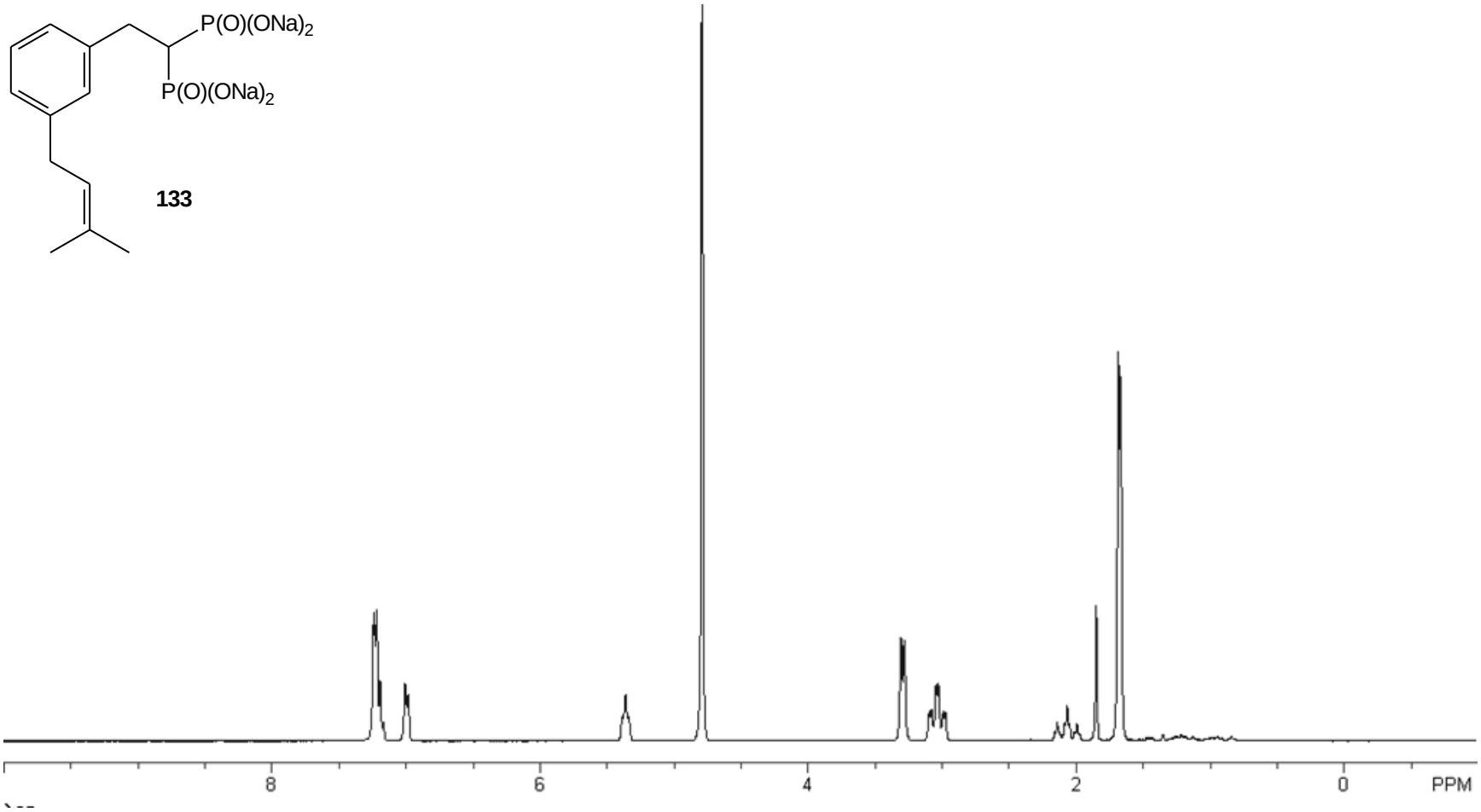
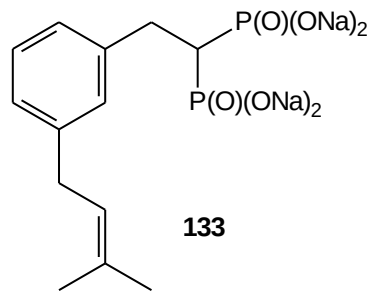


Figure A26. ^{1}H NMR spectrum for bisphosphonate **133**.



133

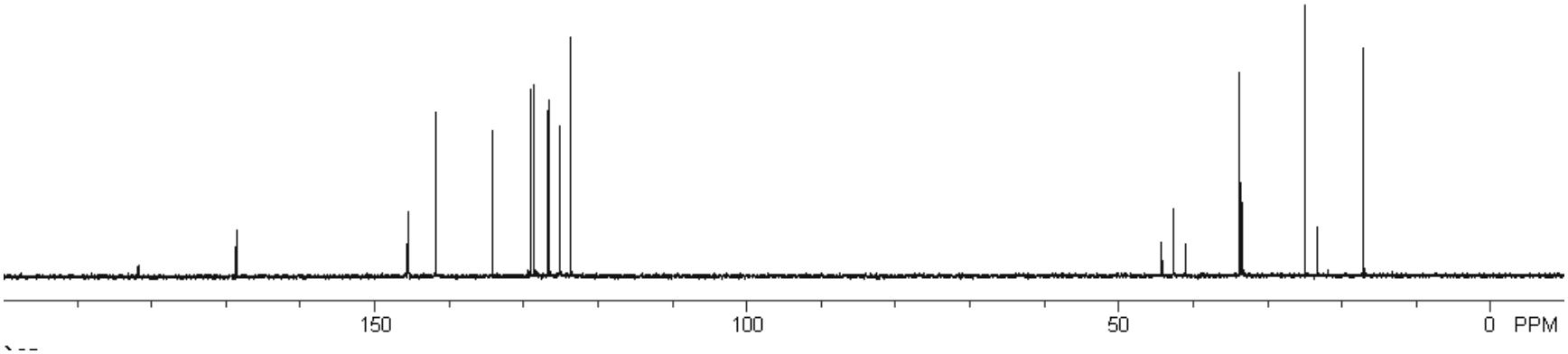


Figure A27. ^{13}C NMR spectrum for bisphosphonate **133**.

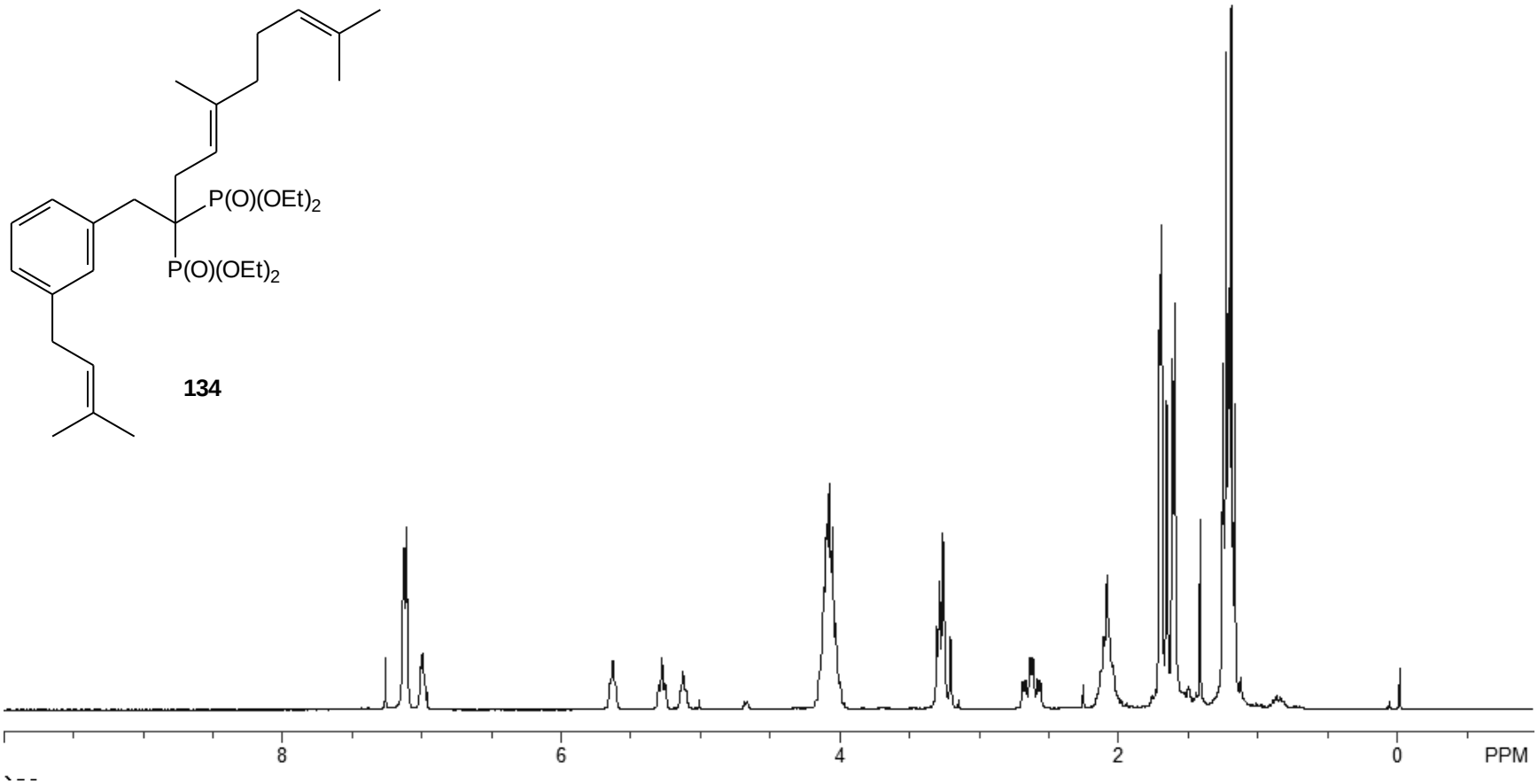


Figure A28. ^{1}H NMR spectrum for bisphosphonate **134**.

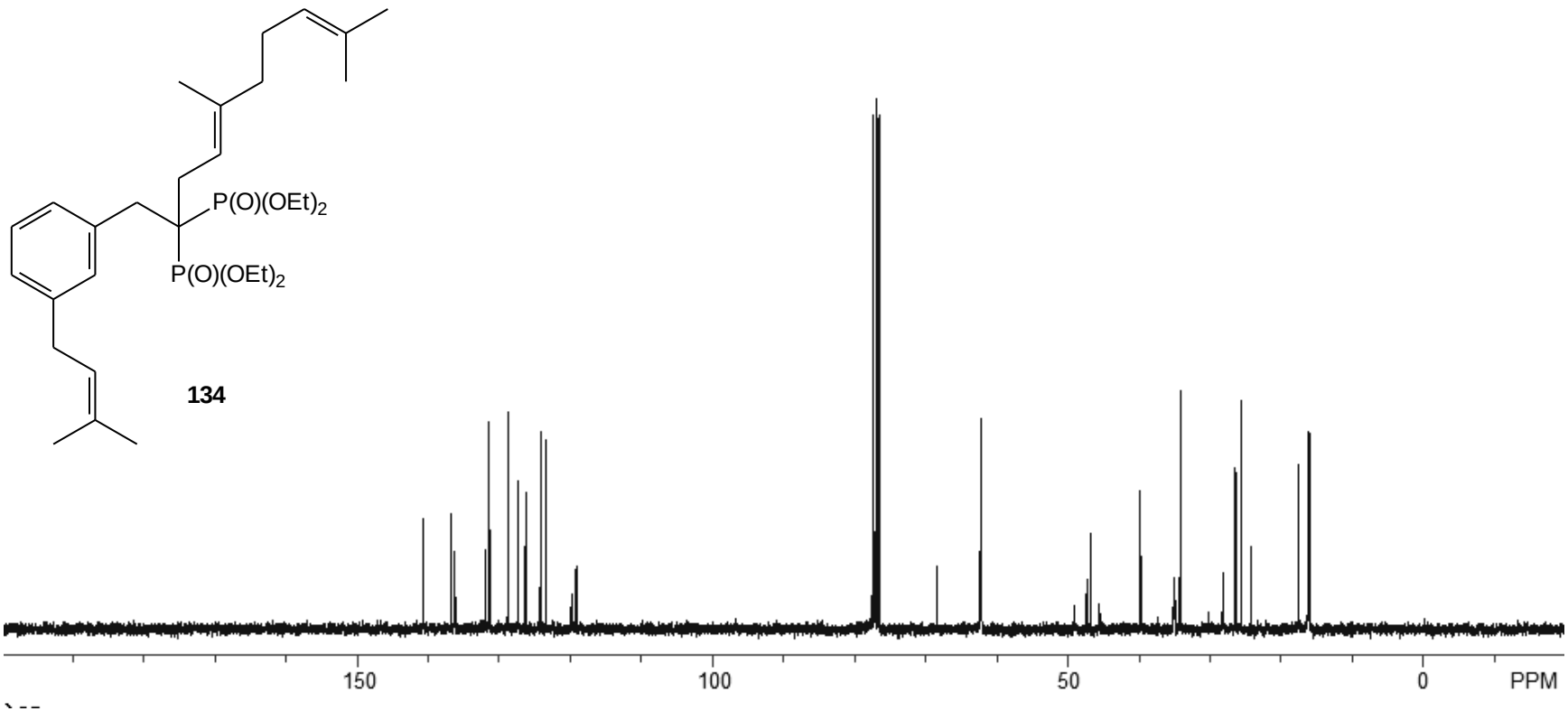


Figure A29. ^{13}C NMR spectrum for bisphosphonate **134**.

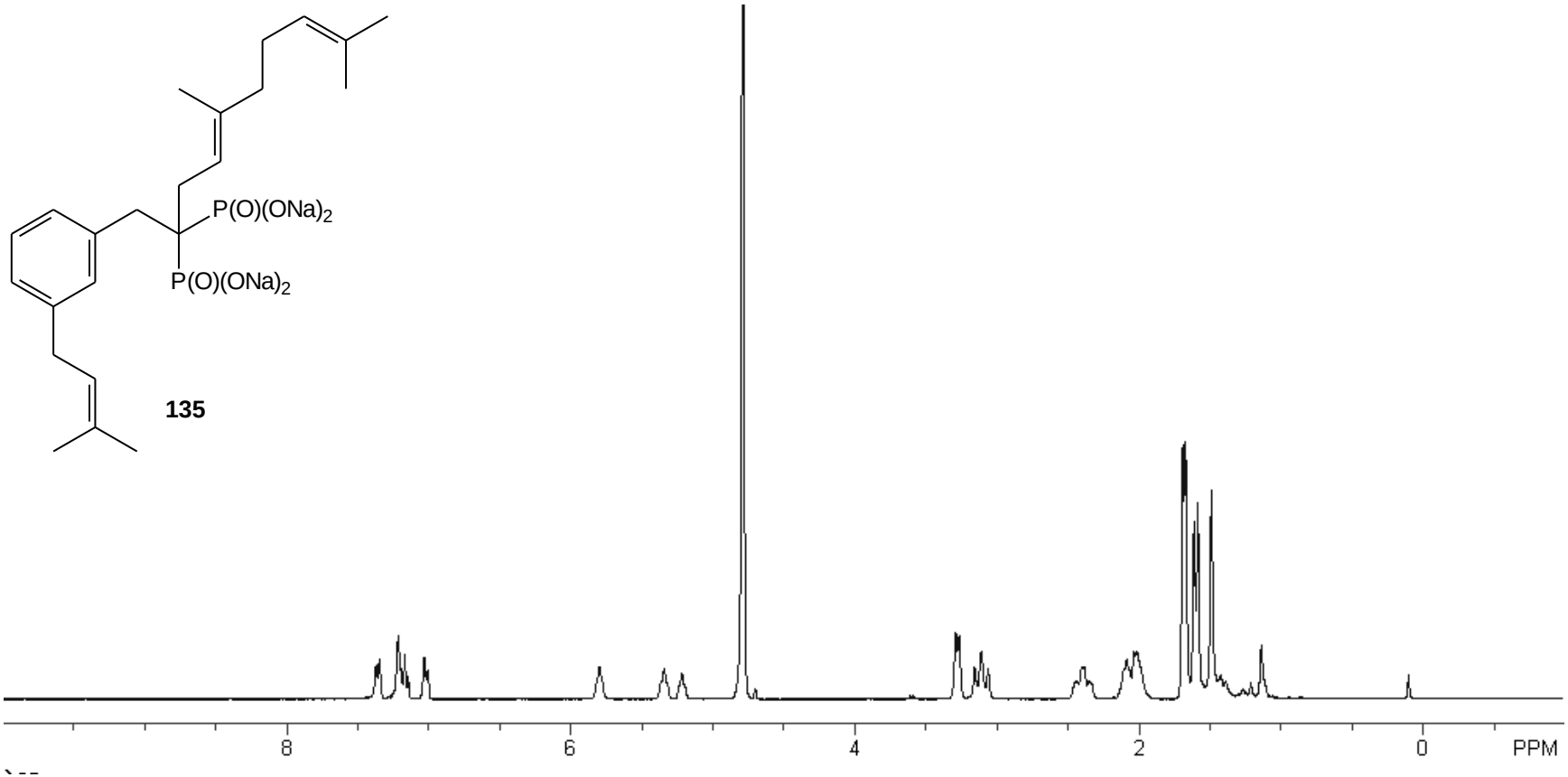


Figure A30. ^{1}H NMR spectrum for bisphosphonate **135**.

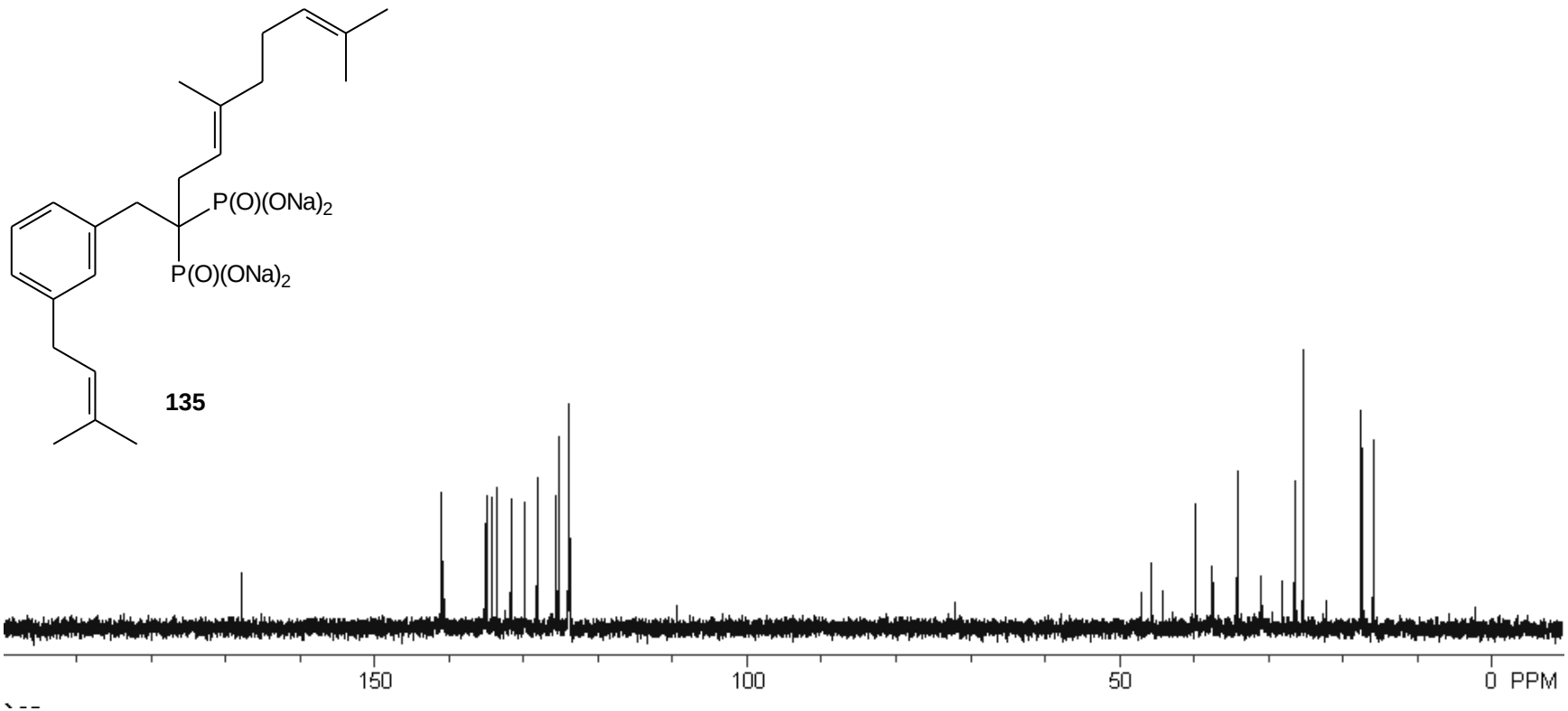
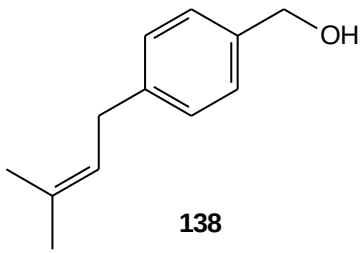


Figure A31. ^{13}C NMR spectrum for bisphosphonate **135**.



138

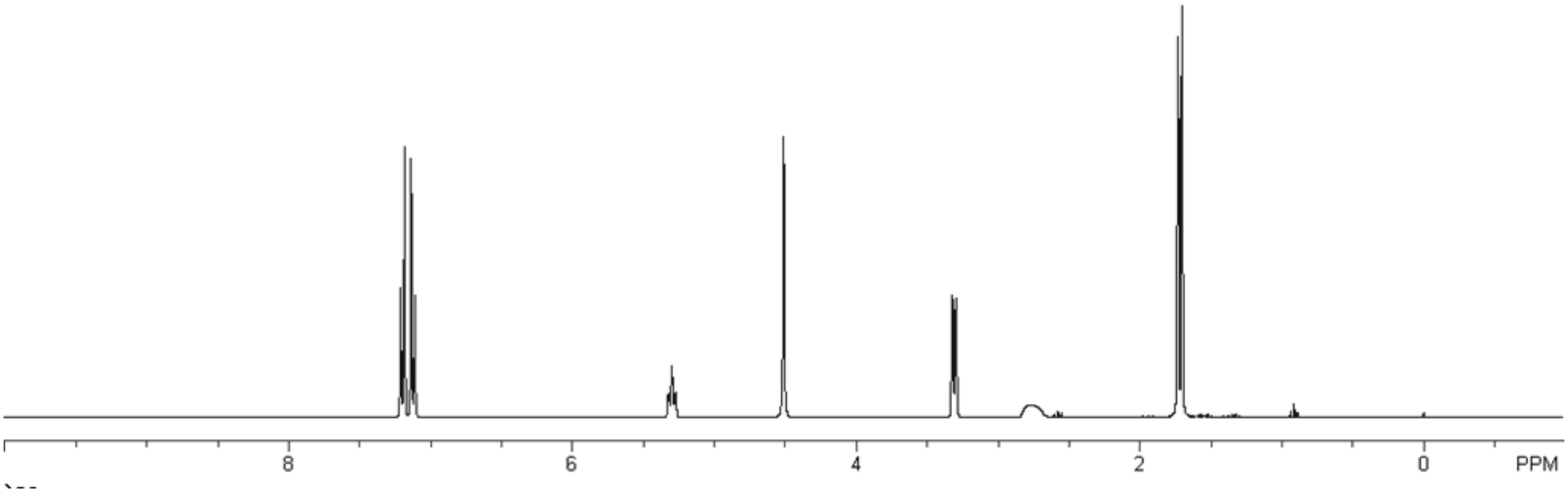


Figure A32. ^{1}H NMR spectrum for alcohol **138**.

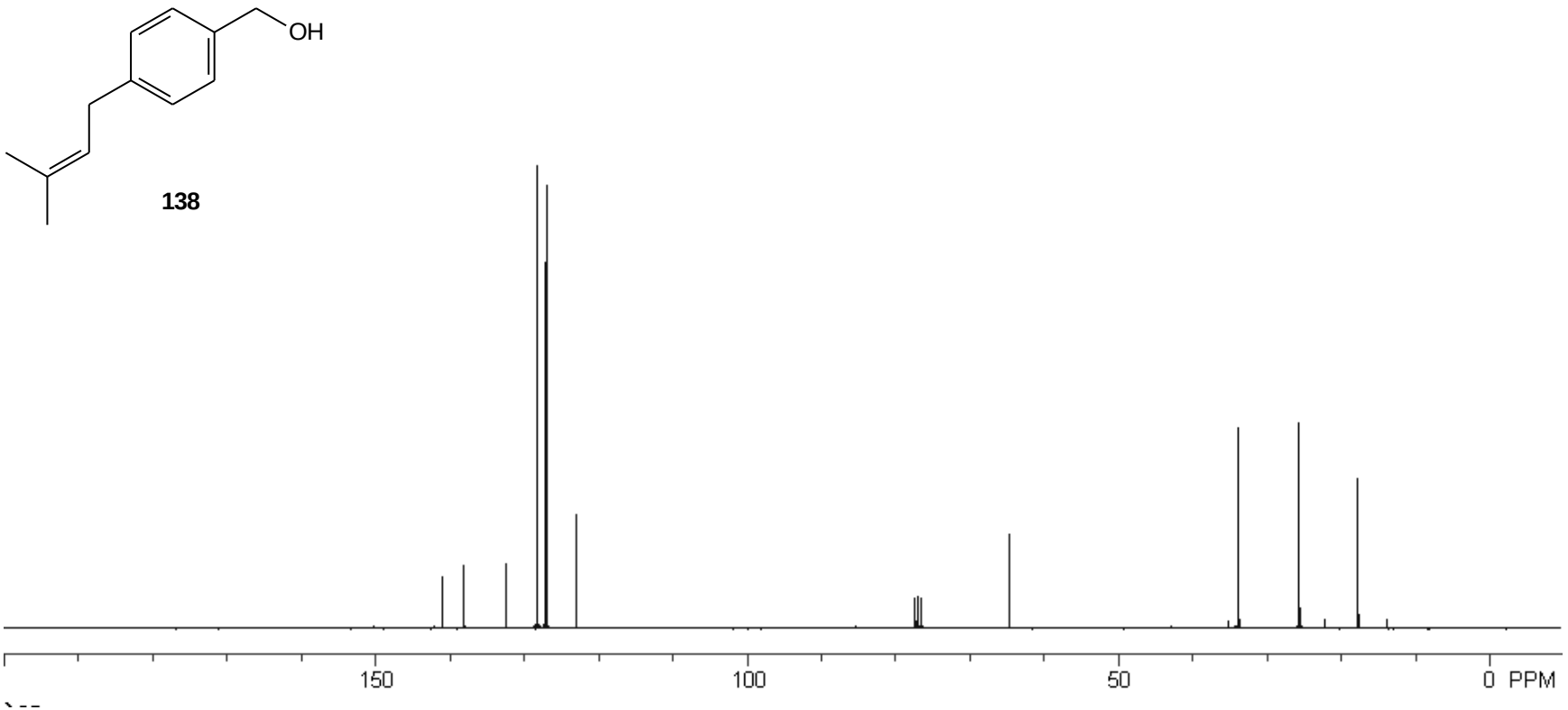


Figure A33. ^{13}C NMR spectrum for alcohol **138**.

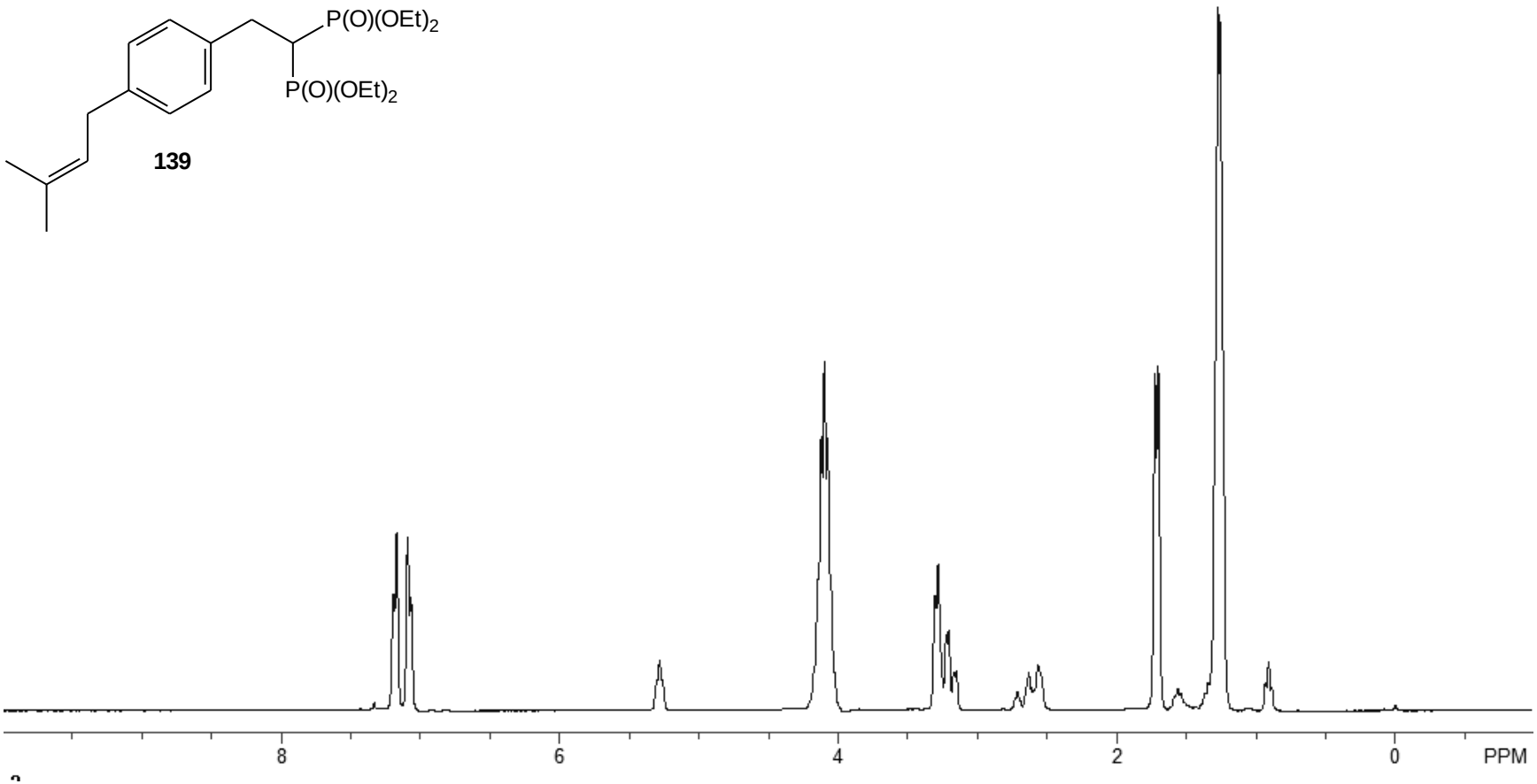


Figure A34. ^{1}H NMR spectrum for bisphosphonate **139**.

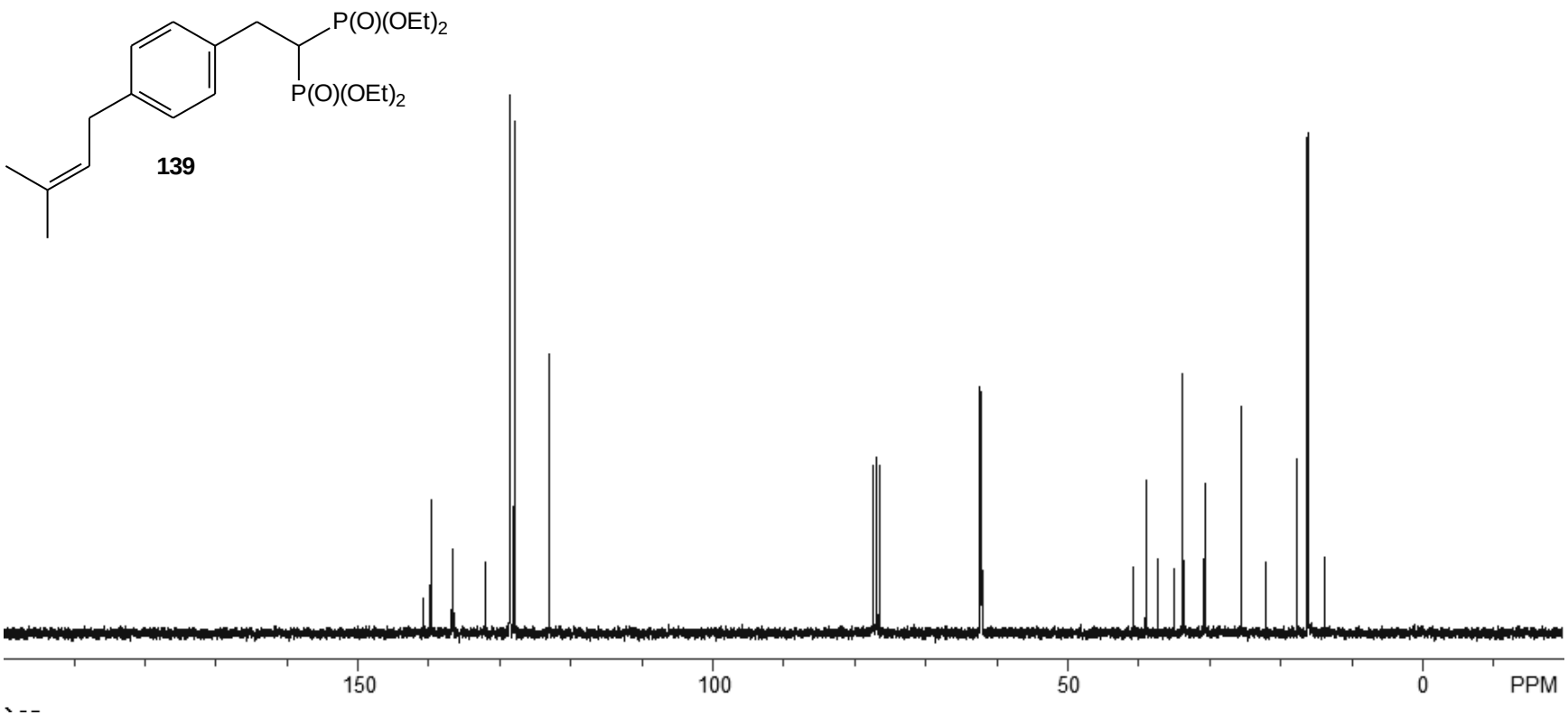


Figure A35. ^{13}C NMR spectrum for bisphosphonate **139**.

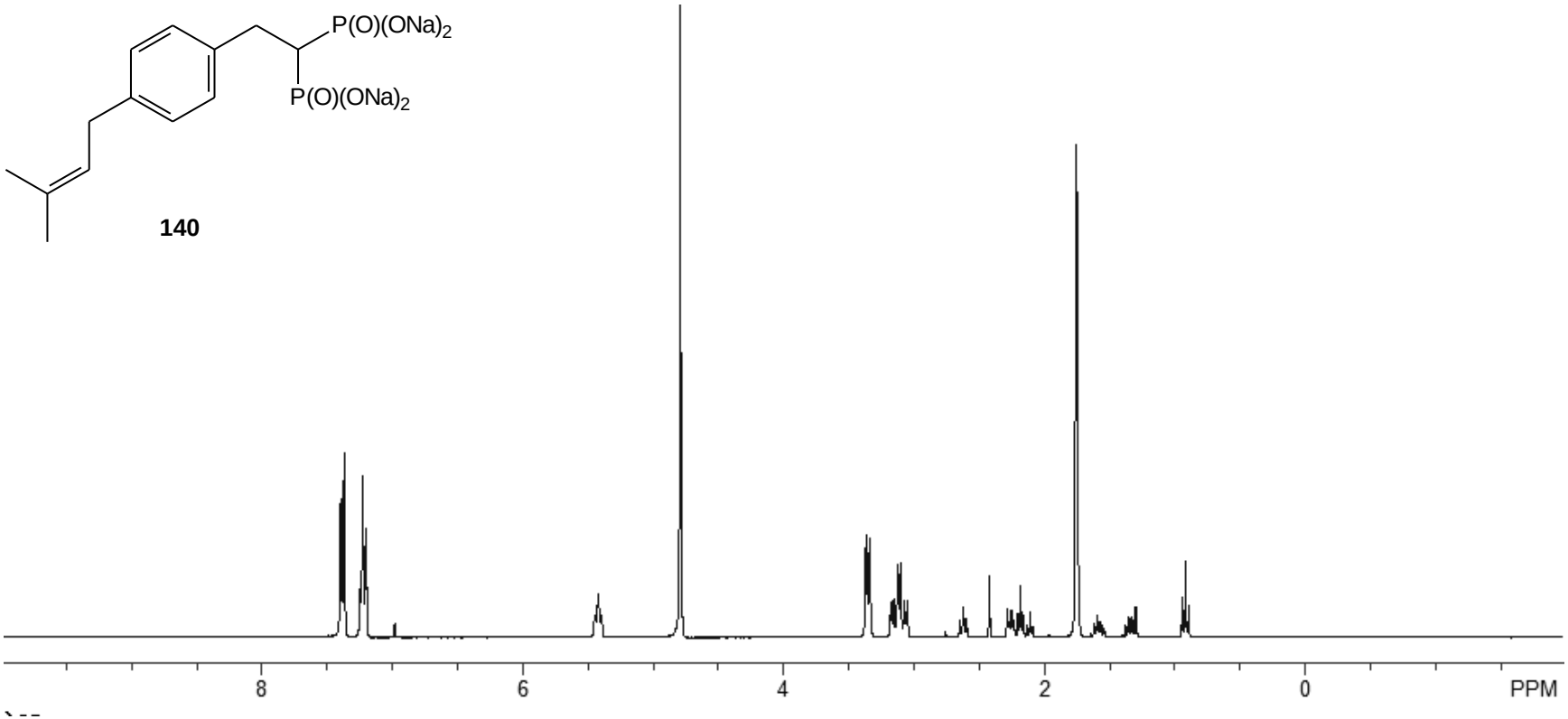
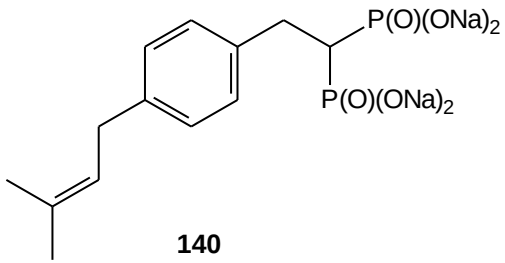


Figure A36. ^{1}H NMR spectrum for bisphosphonate **140**.



140

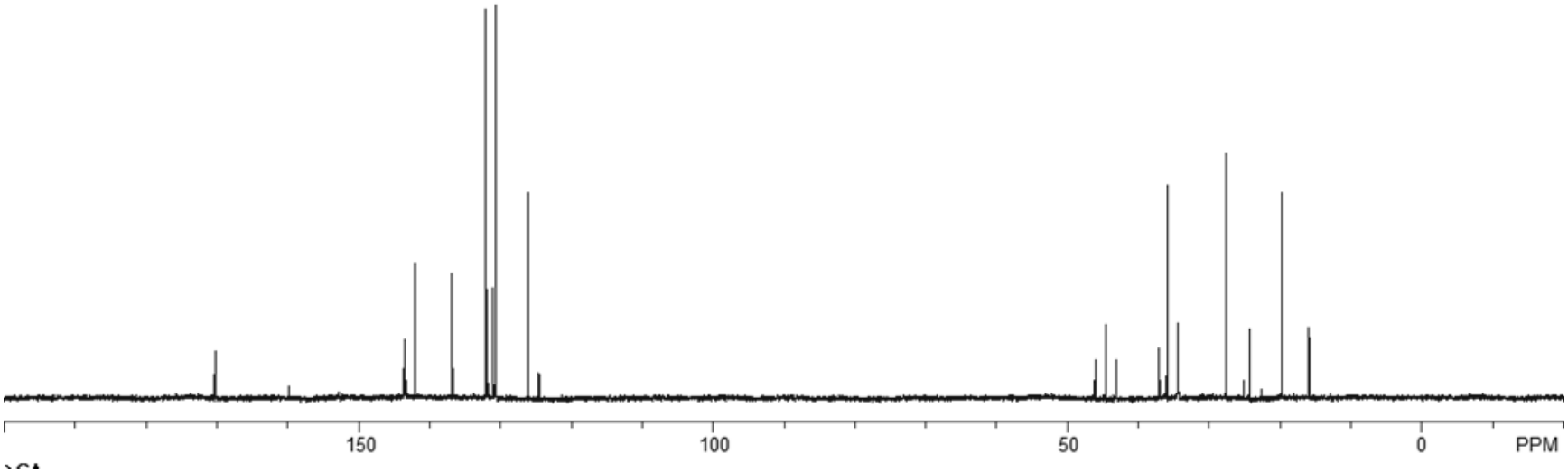


Figure A37. ^{13}C NMR spectrum for bisphosphonate **140**.

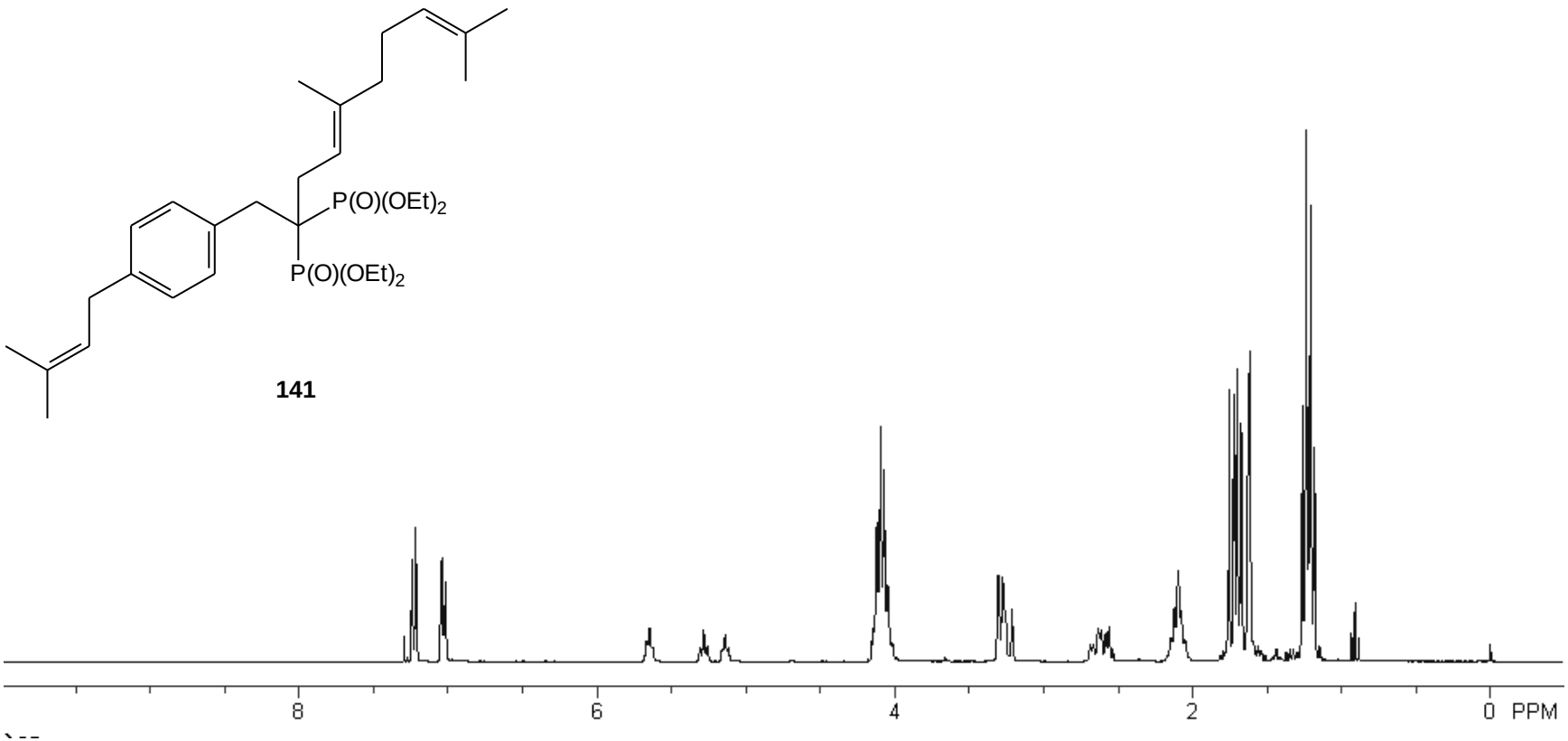


Figure A38. ^{1}H NMR spectrum for bisphosphonate **141**.

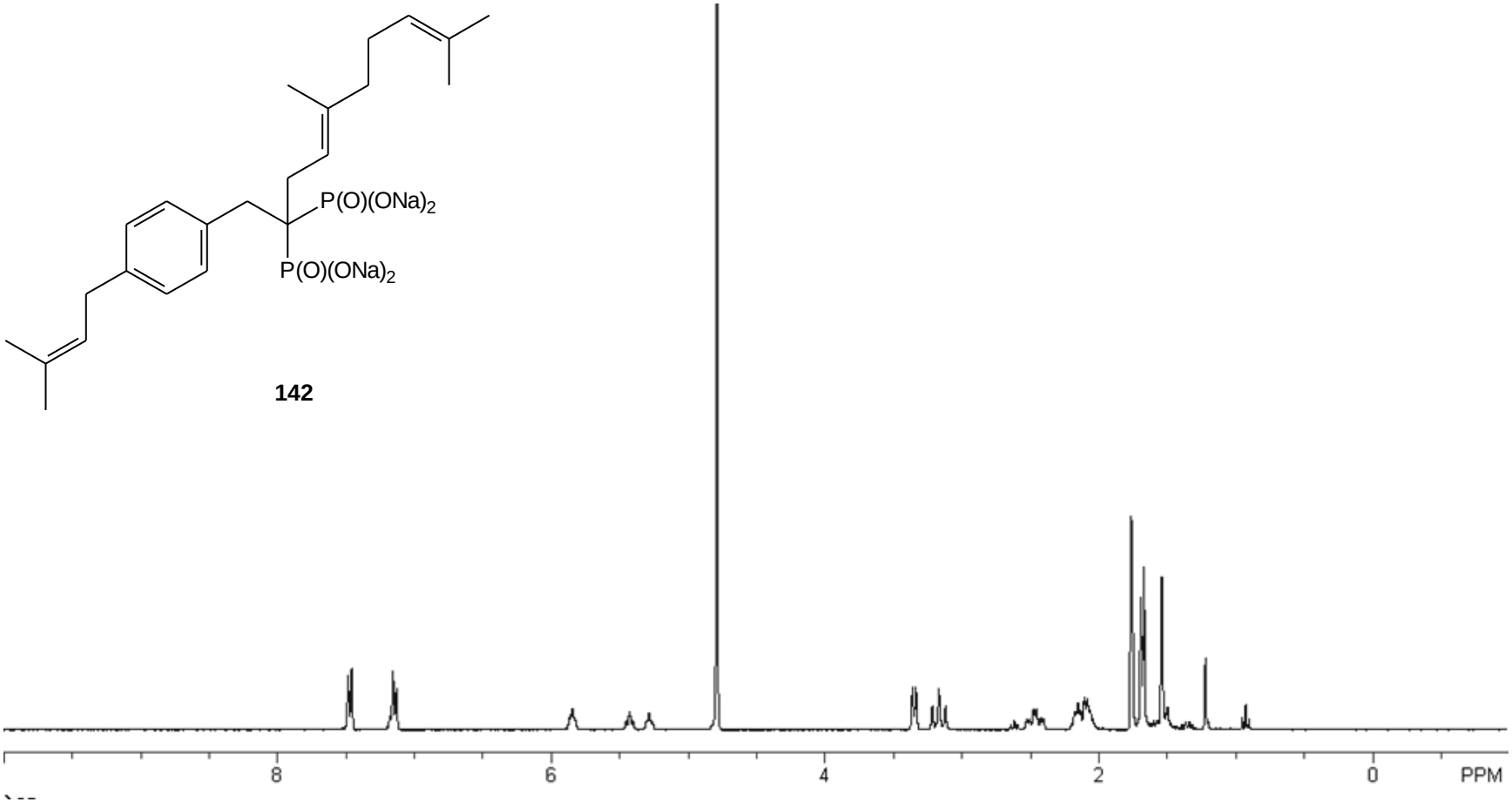


Figure A39. ^{1}H NMR spectrum for bisphosphonate **142**.

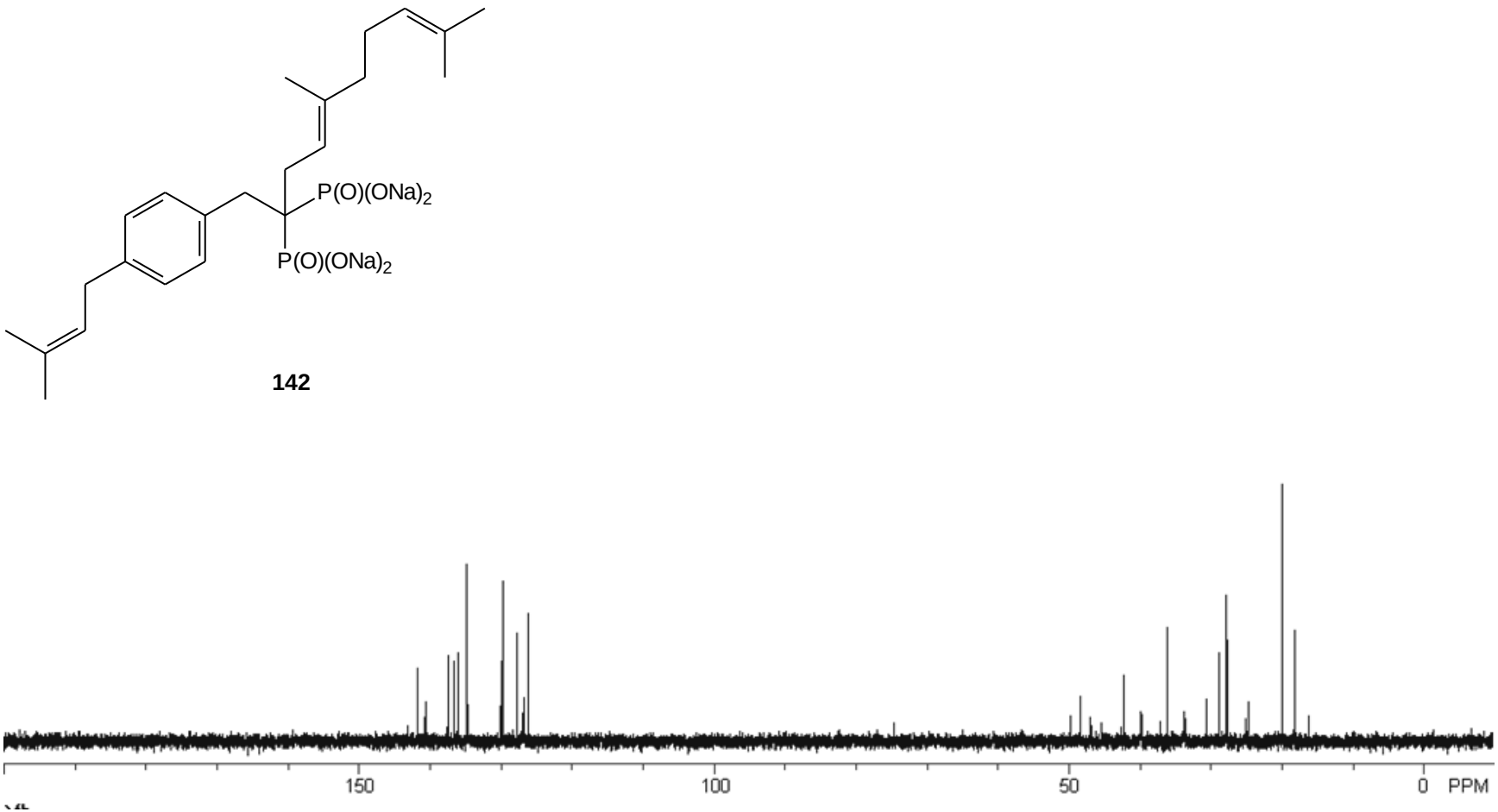


Figure A40. ^{13}C NMR spectrum for bisphosphonate **142**.

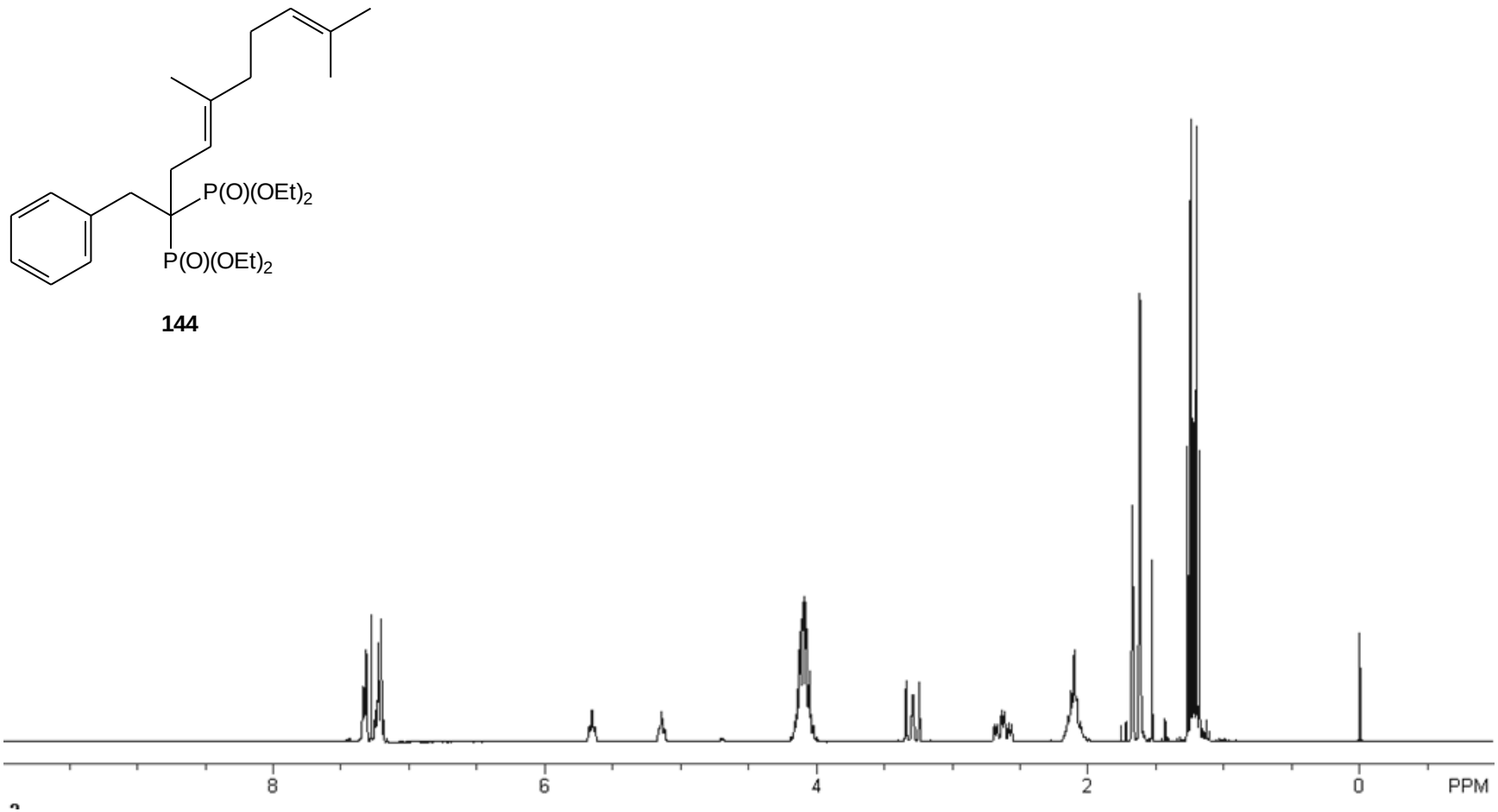


Figure A41. ^{1}H NMR spectrum for bisphosphonate **144**.

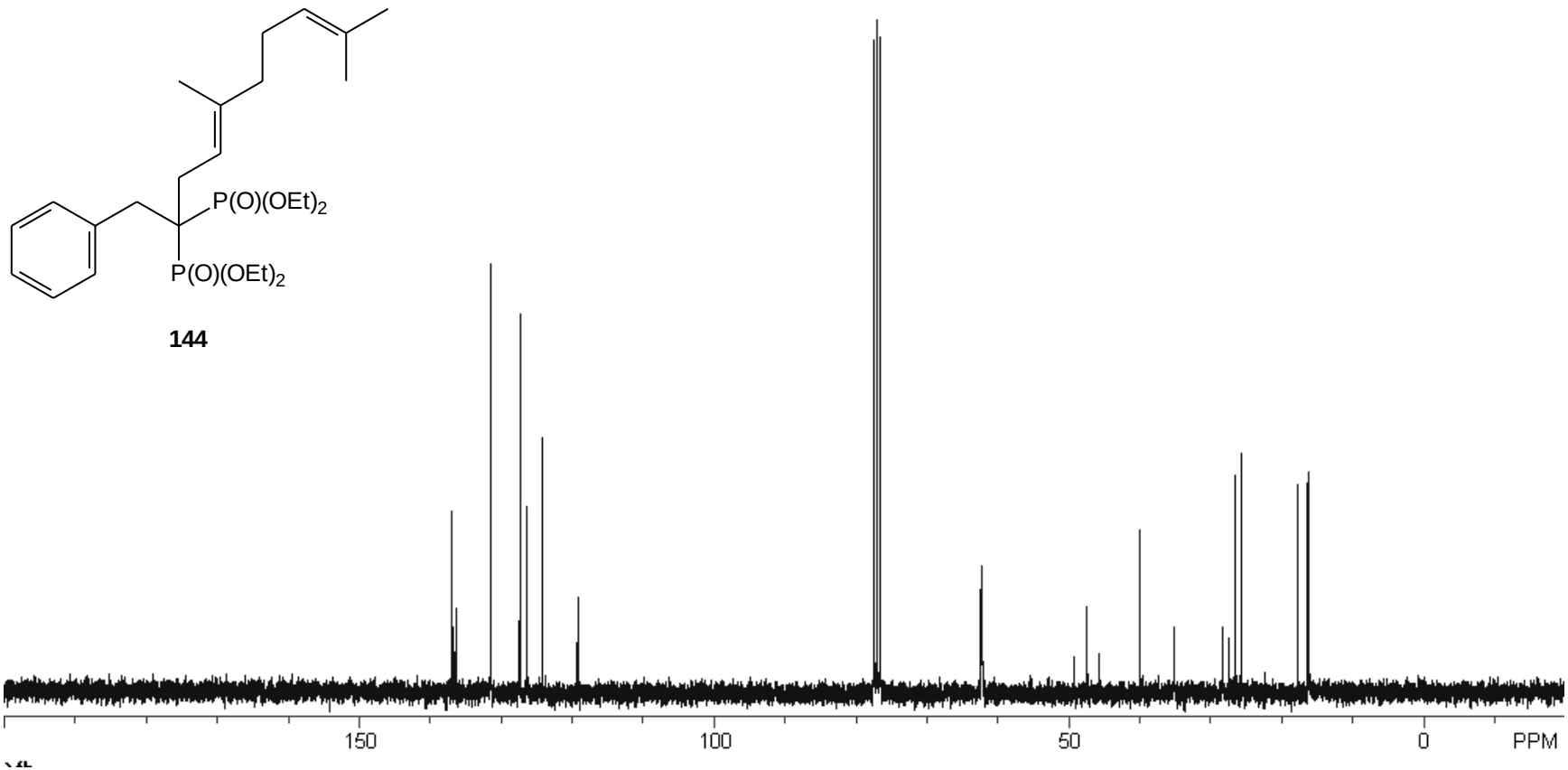


Figure A42. ^{13}C NMR spectrum for bisphosphonate **144**.

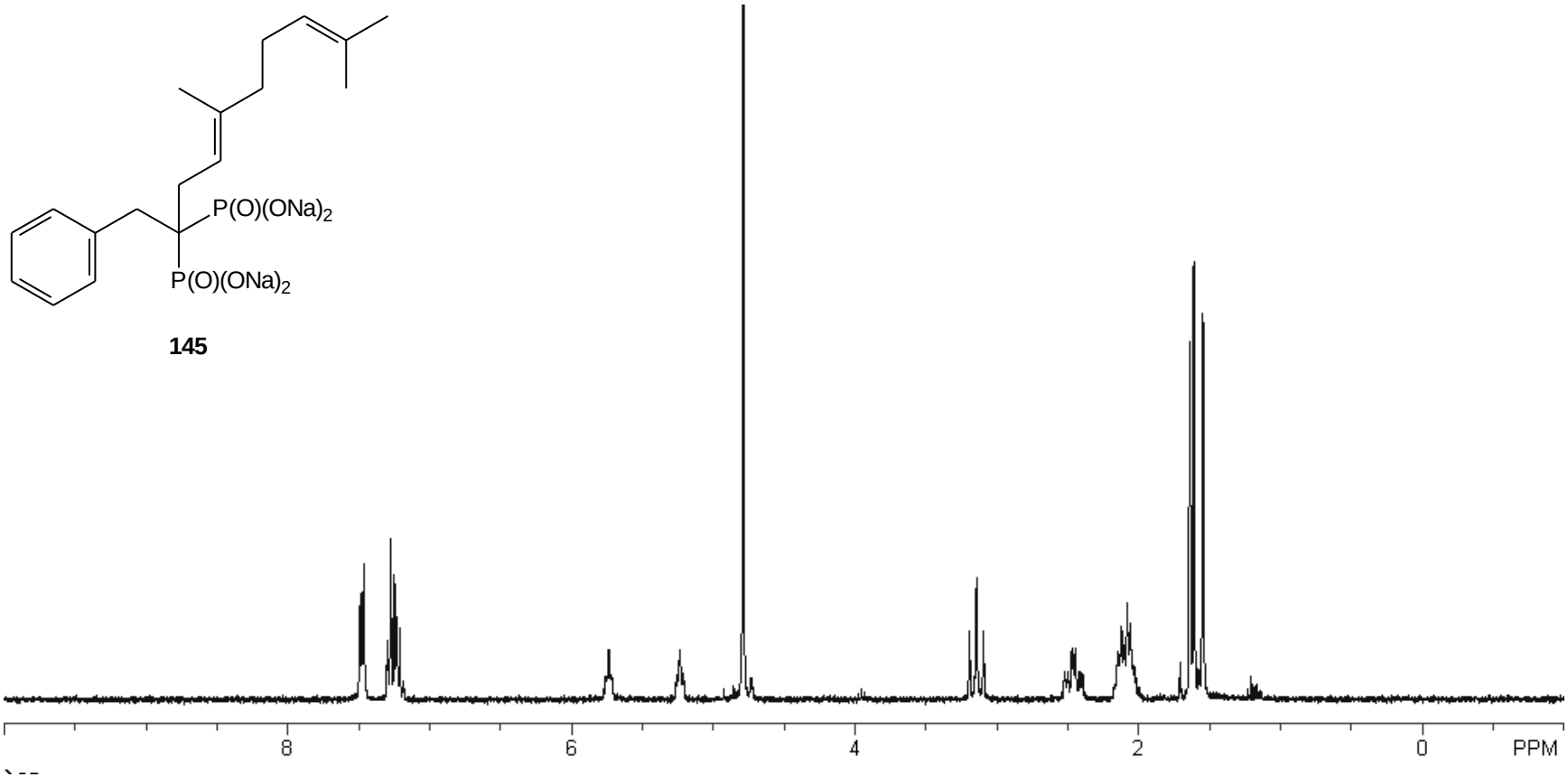


Figure A43. ^{1}H NMR spectrum for bisphosphonate **145**.

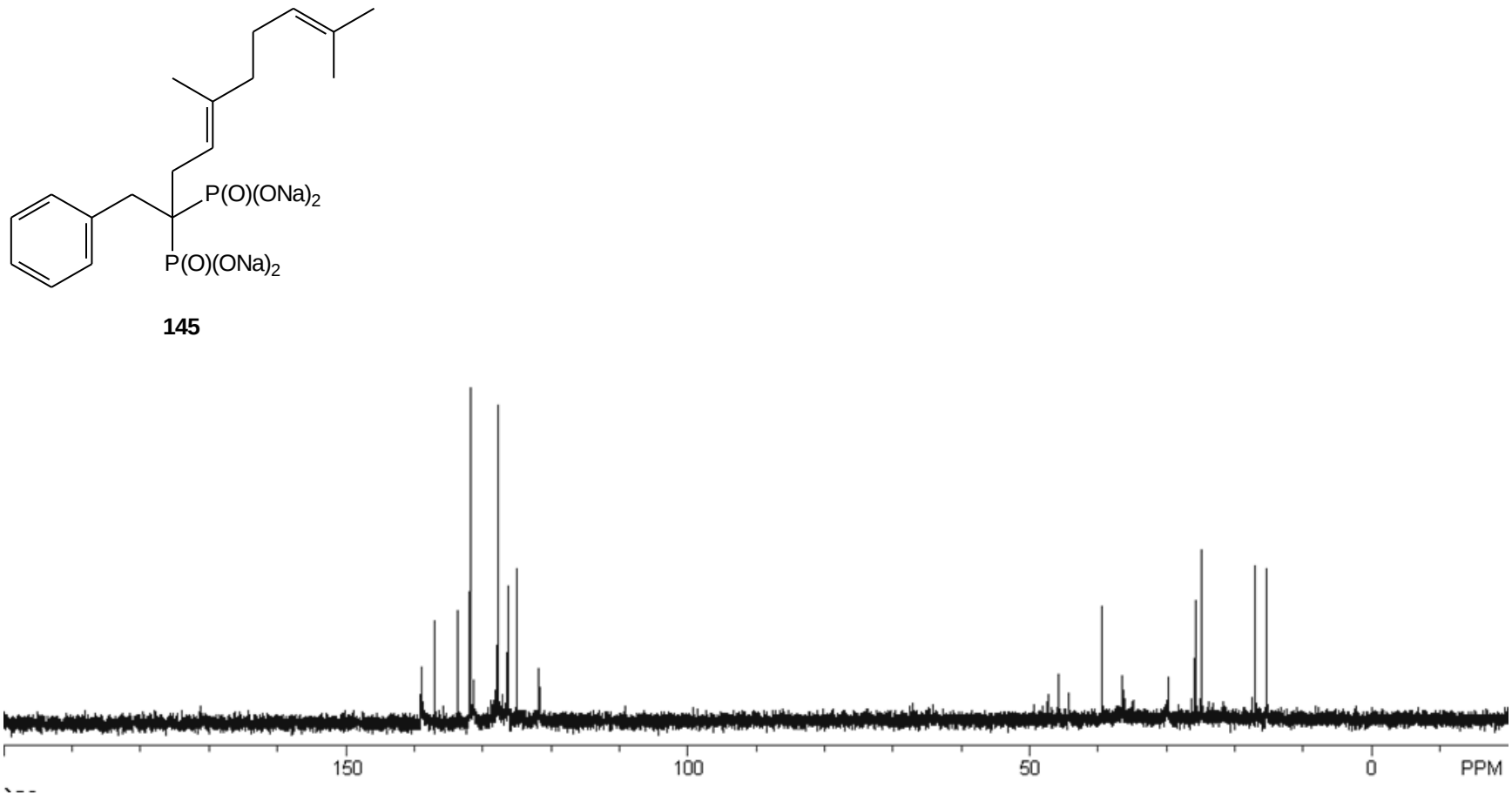


Figure A44. ^{13}C NMR spectrum for bisphosphonate **145**.

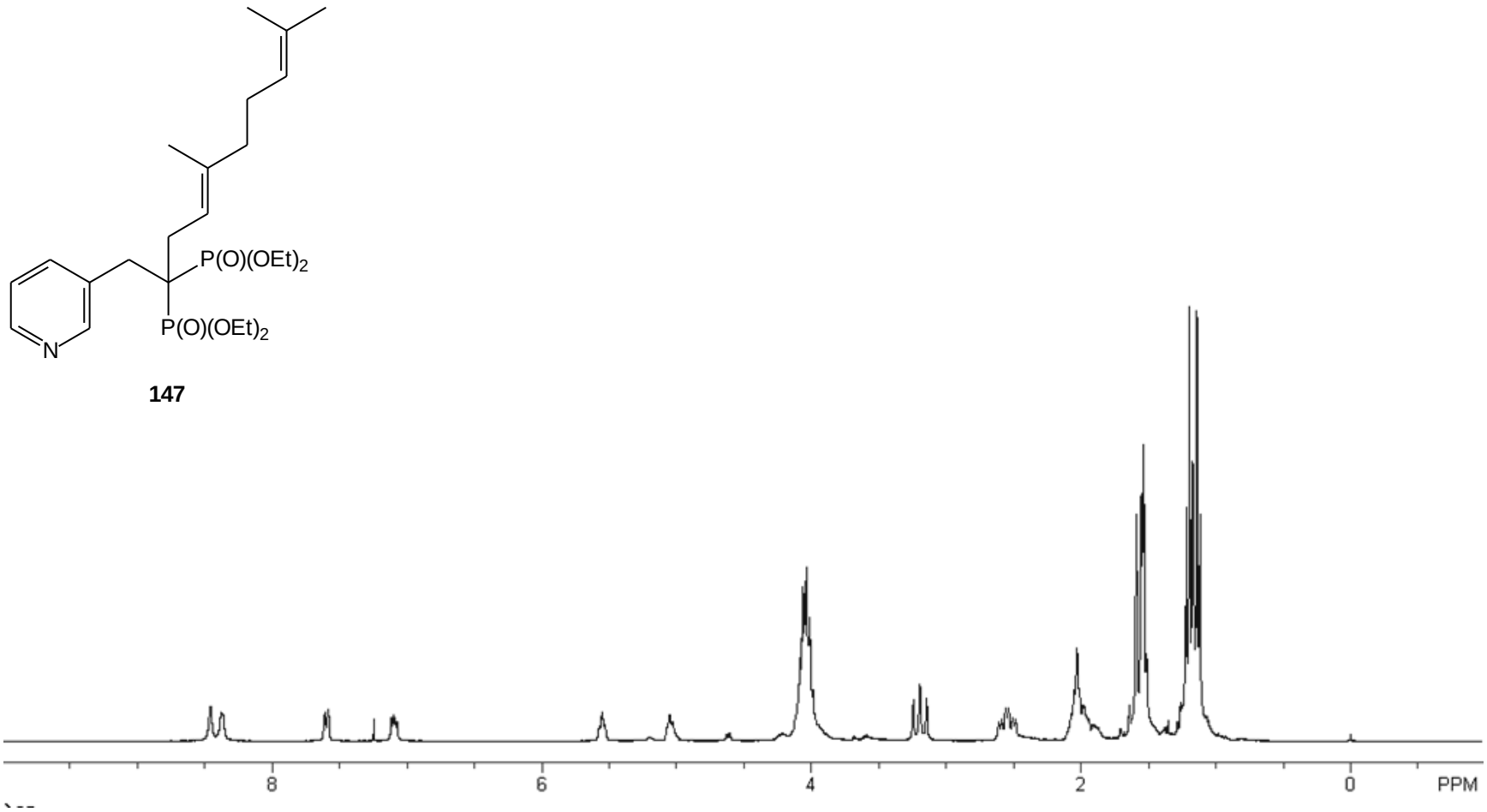


Figure A45. ^{1}H NMR spectrum for bisphosphonate **147**.

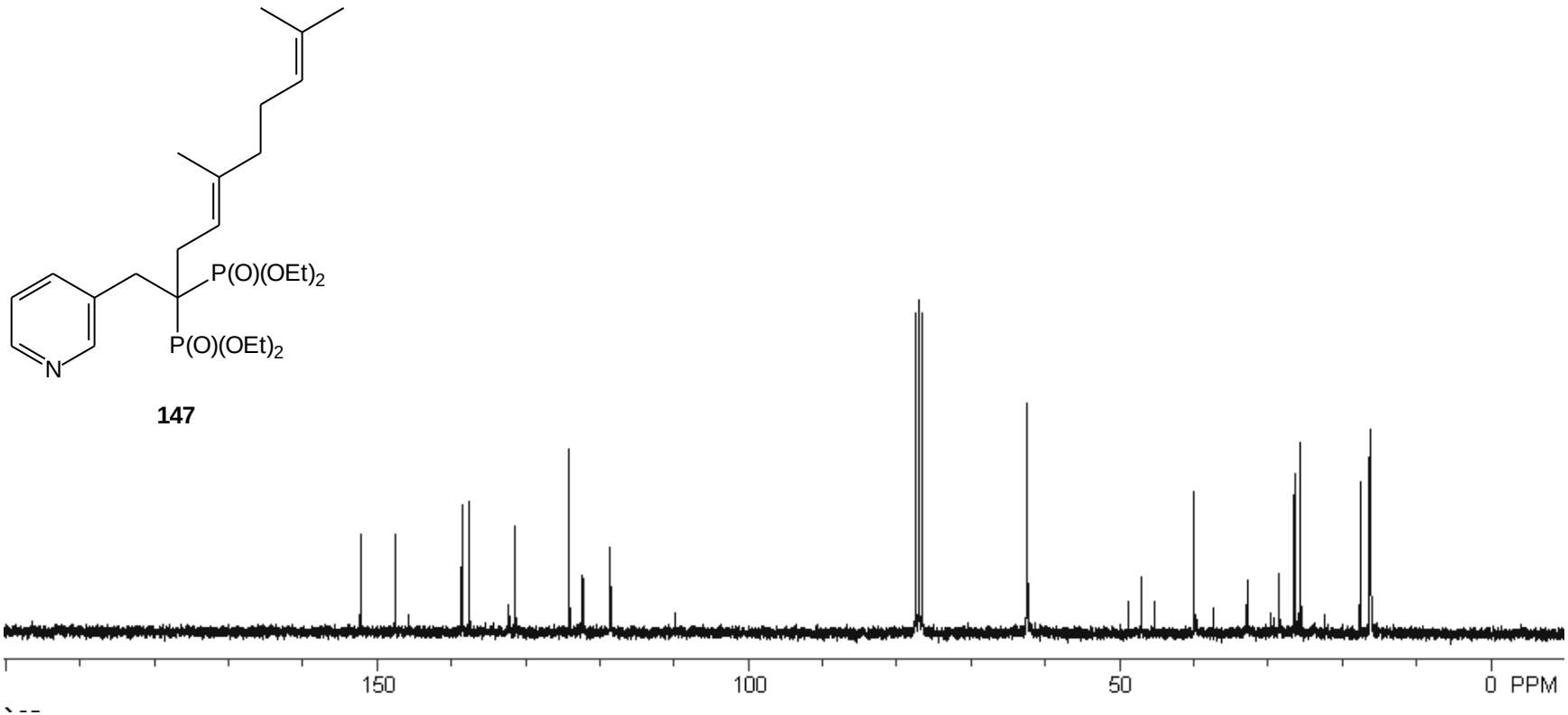


Figure A46. ^{13}C NMR spectrum for bisphosphonate **147**.

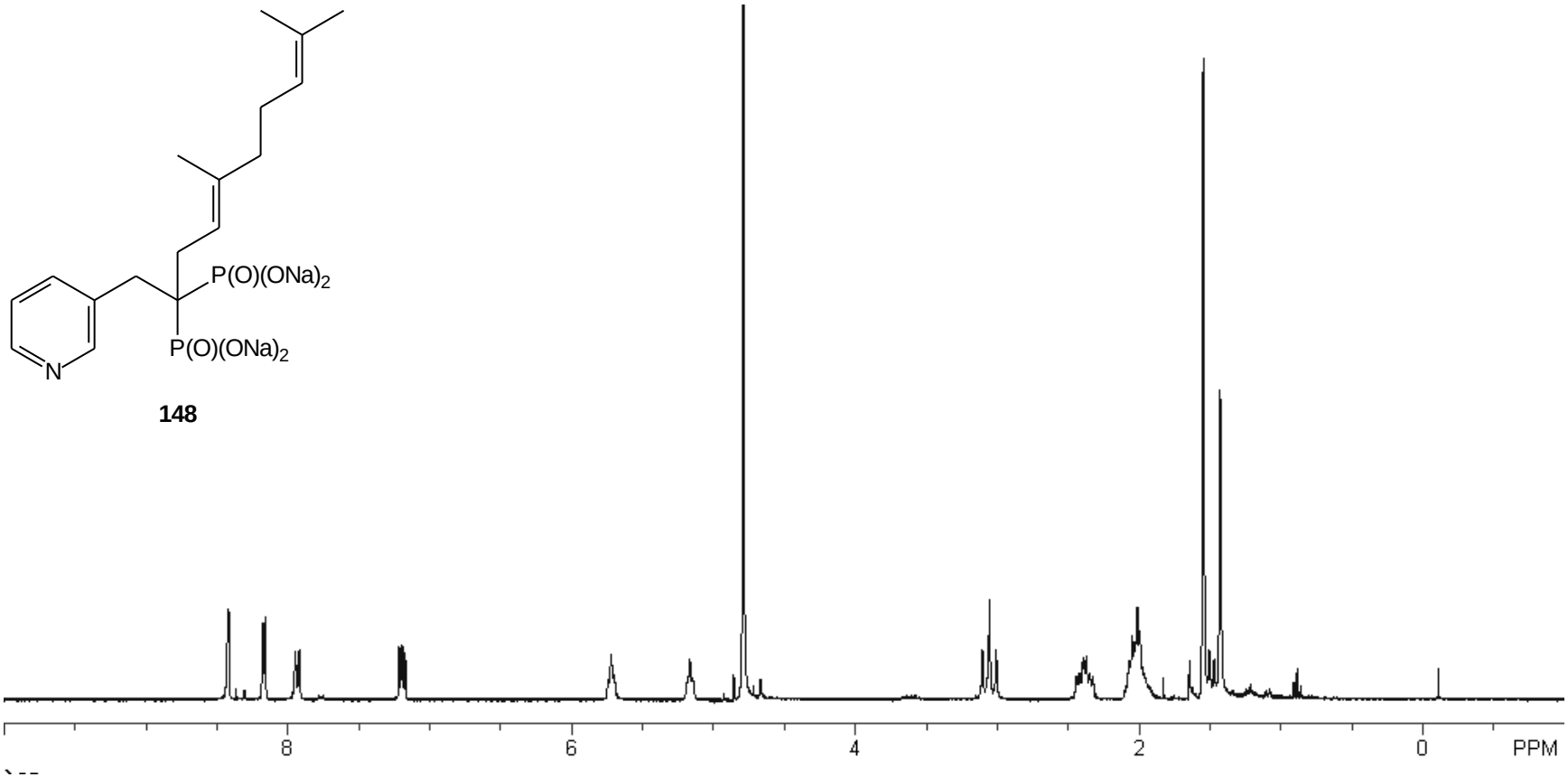
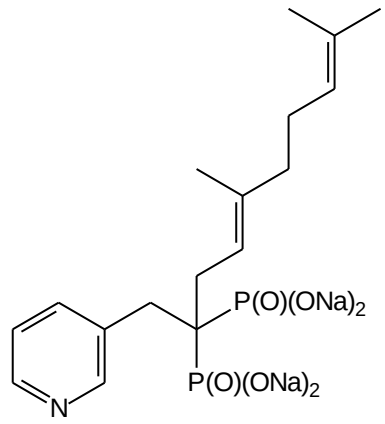


Figure A47. ^{1}H NMR spectrum for bisphosphonate **148**.



148

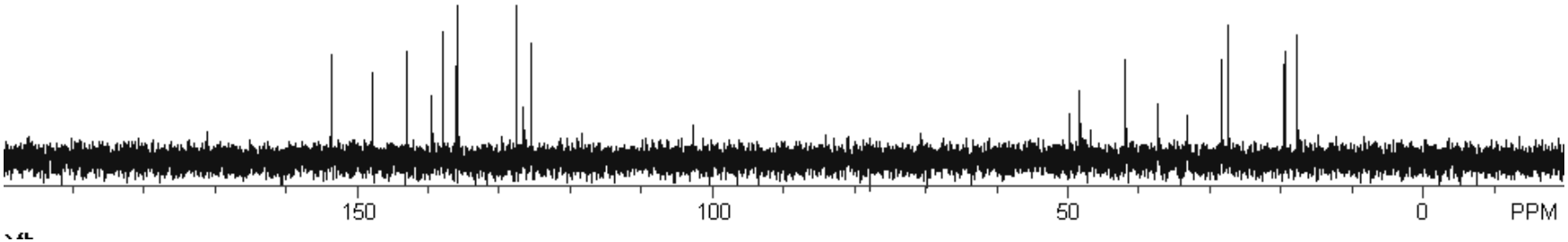


Figure A48. ^{13}C NMR spectrum for bisphosphonate **148**.

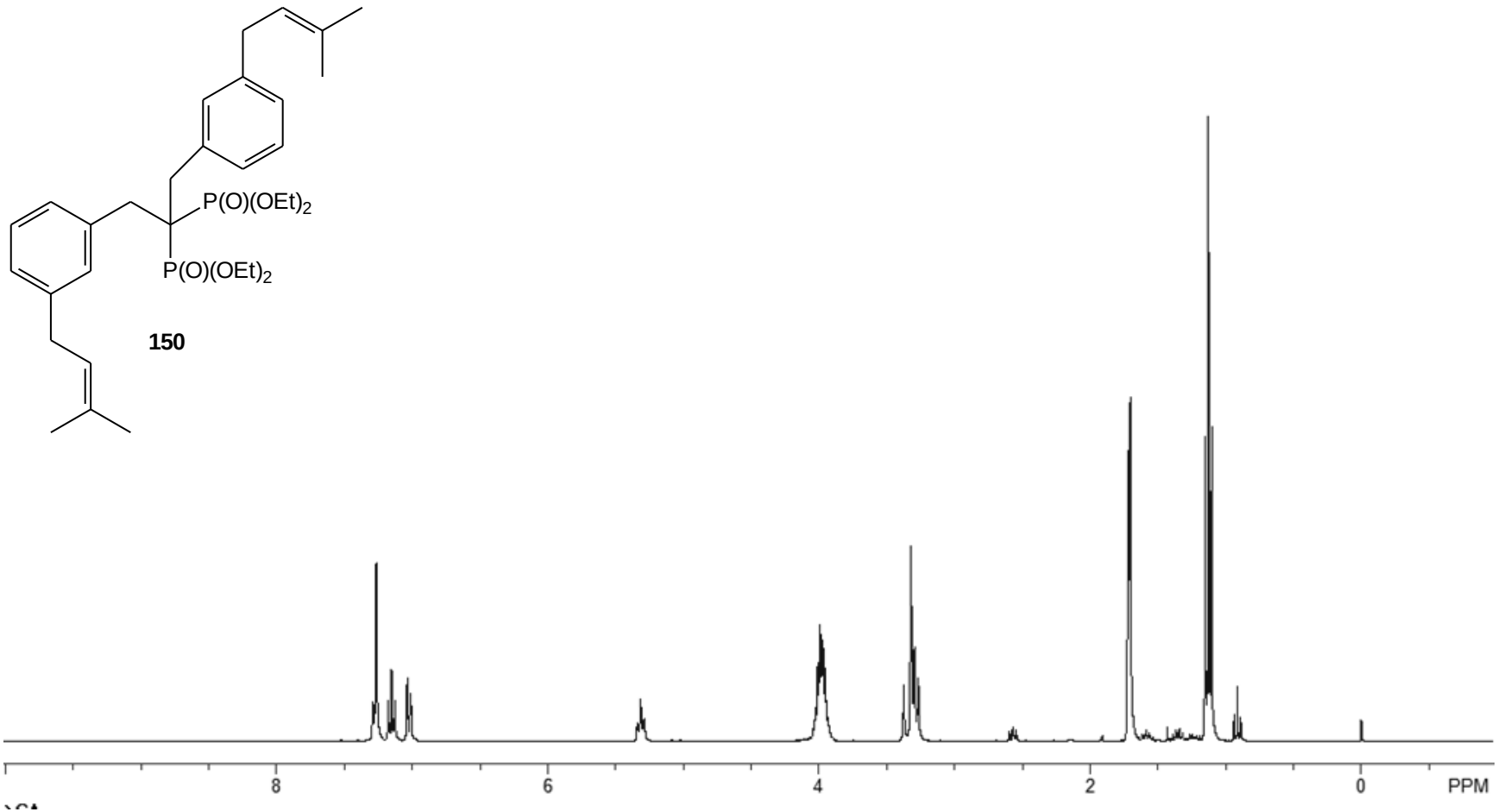


Figure A49. ^{1}H NMR spectrum for bisphosphonate **150**.

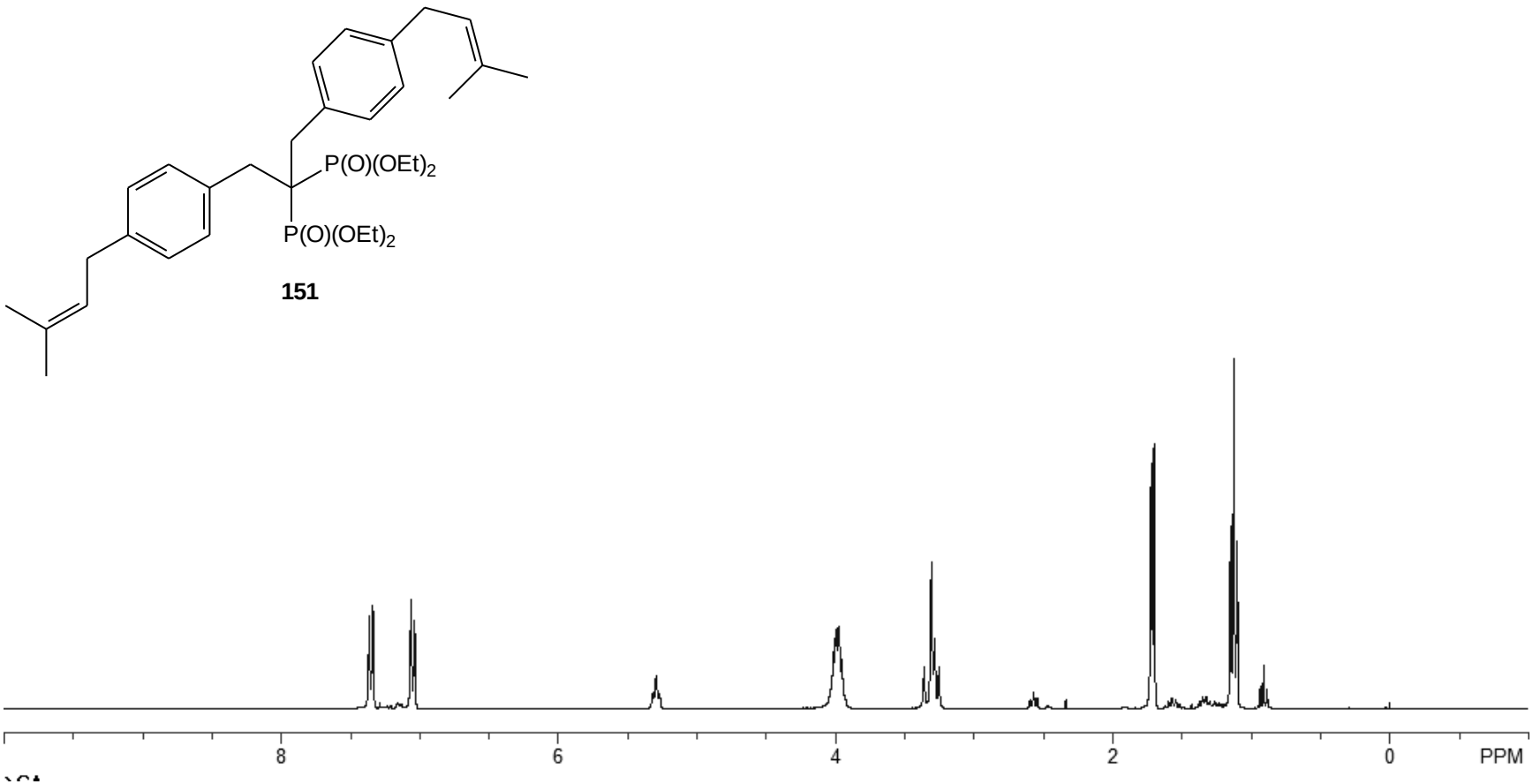


Figure A50. ^{1}H NMR spectrum for bisphosphonate **151**.

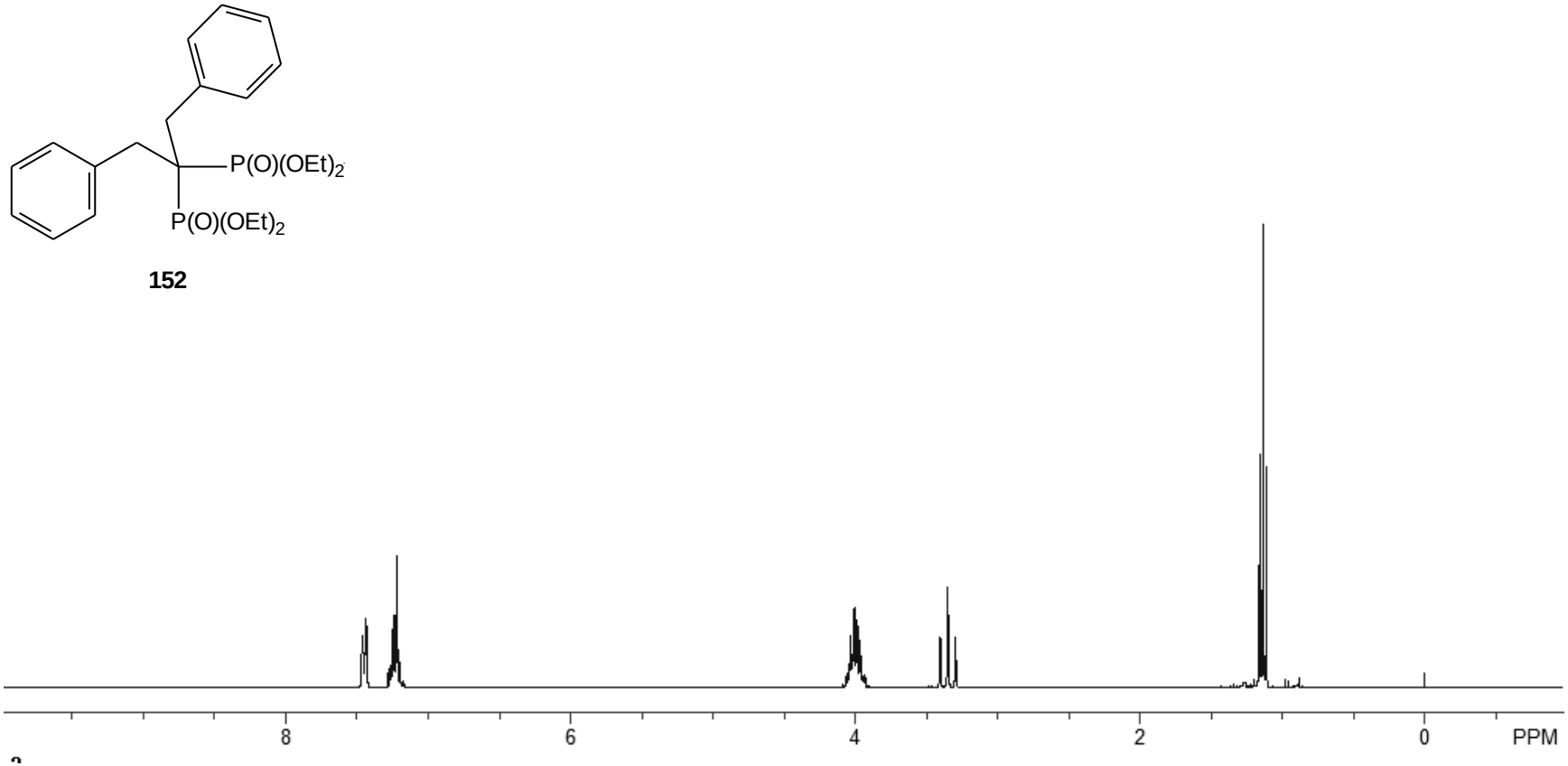


Figure A51. ^{1}H NMR spectrum for bisphosphonate **152**.

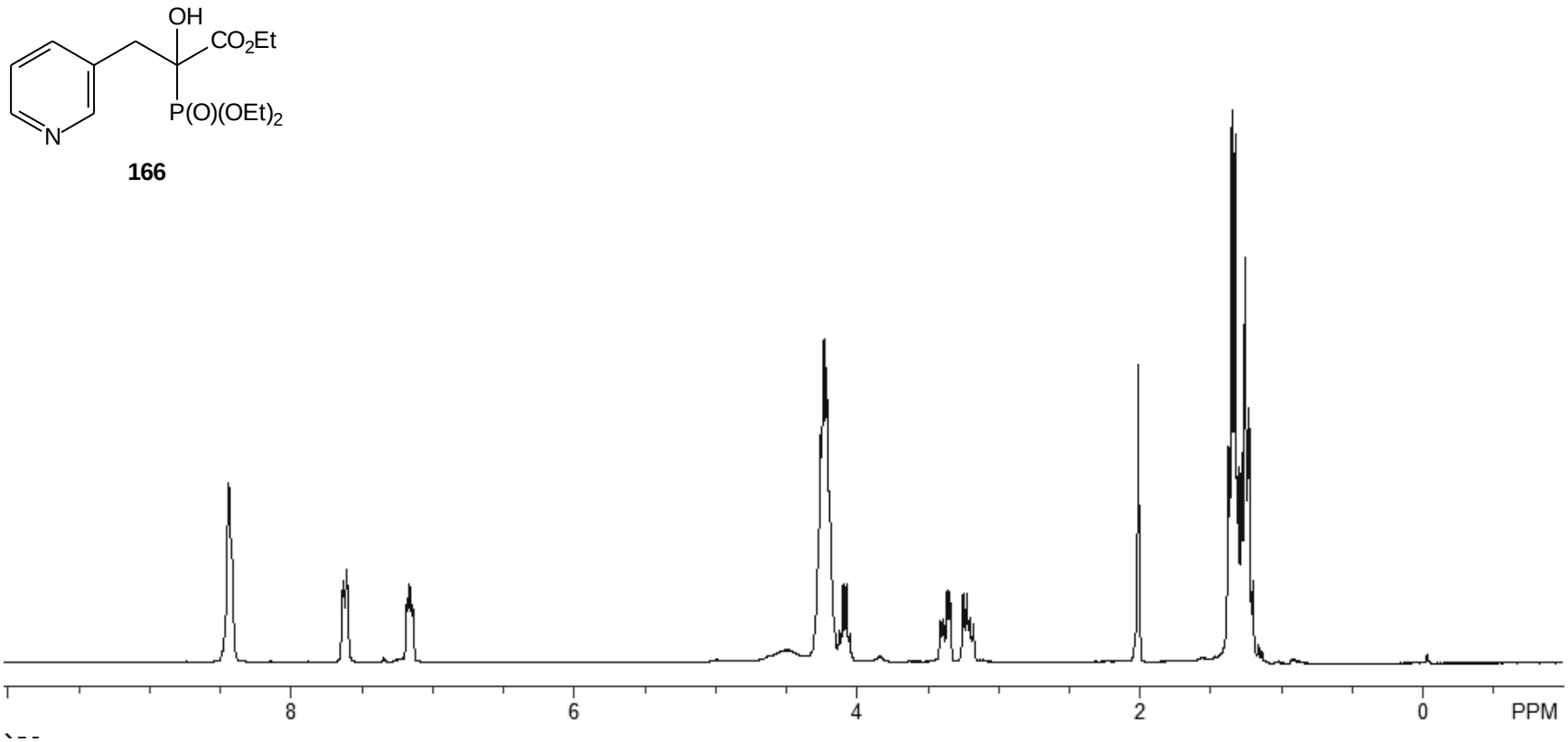
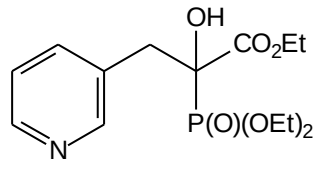


Figure A52. ^{1}H NMR spectrum for phosphonocarboxylate **166**.



166

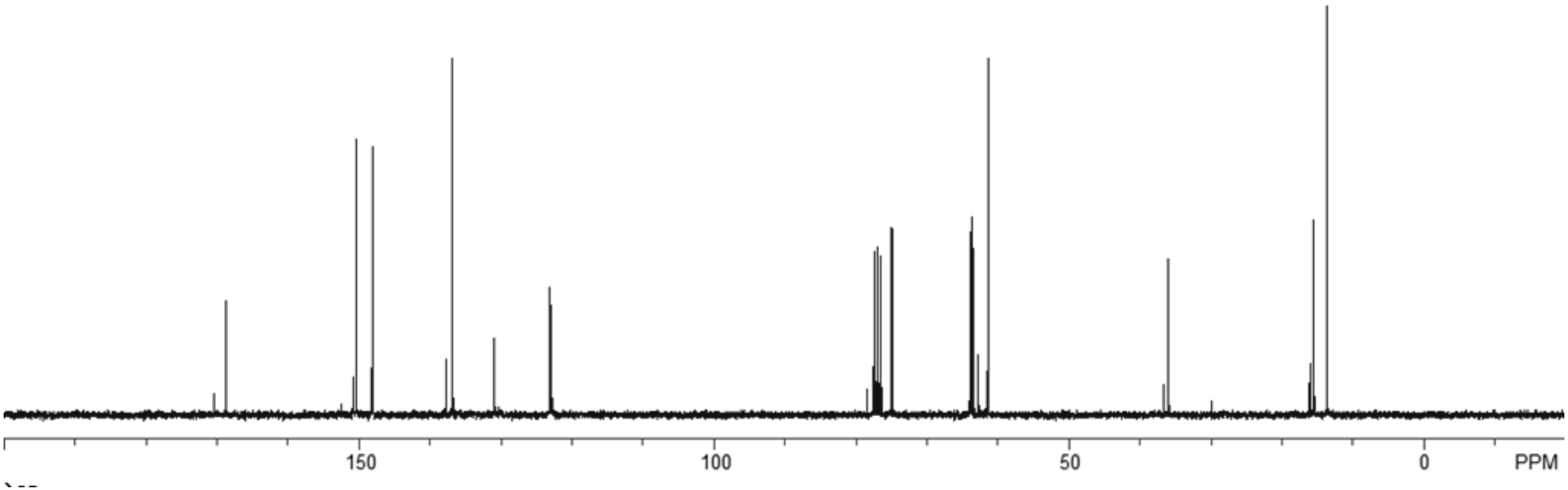


Figure A53. ^{13}C NMR spectrum for phosphonocarboxylate **166**.

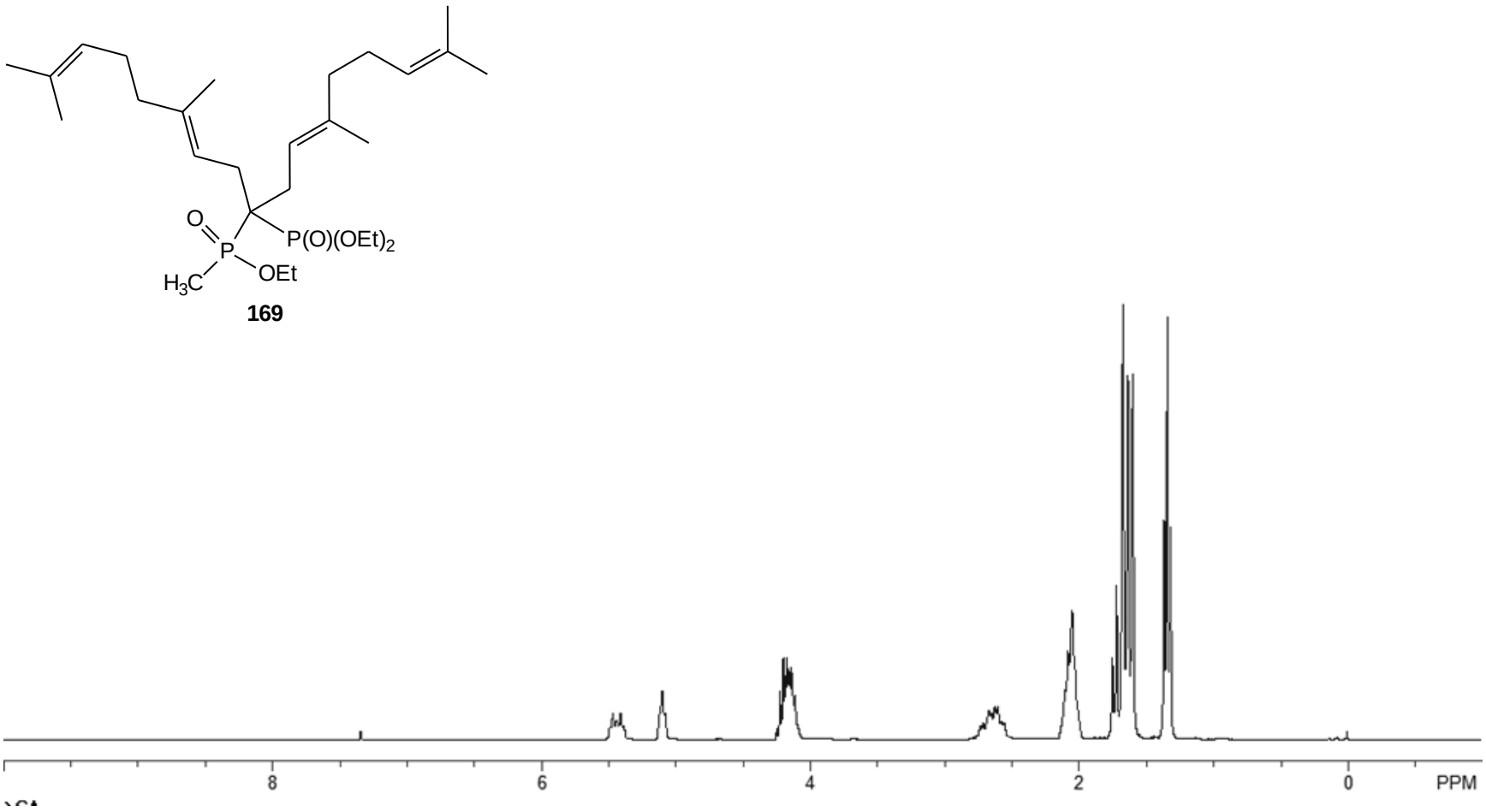


Figure A54. ^{1}H NMR spectrum for phosphonophosphinate **169**.

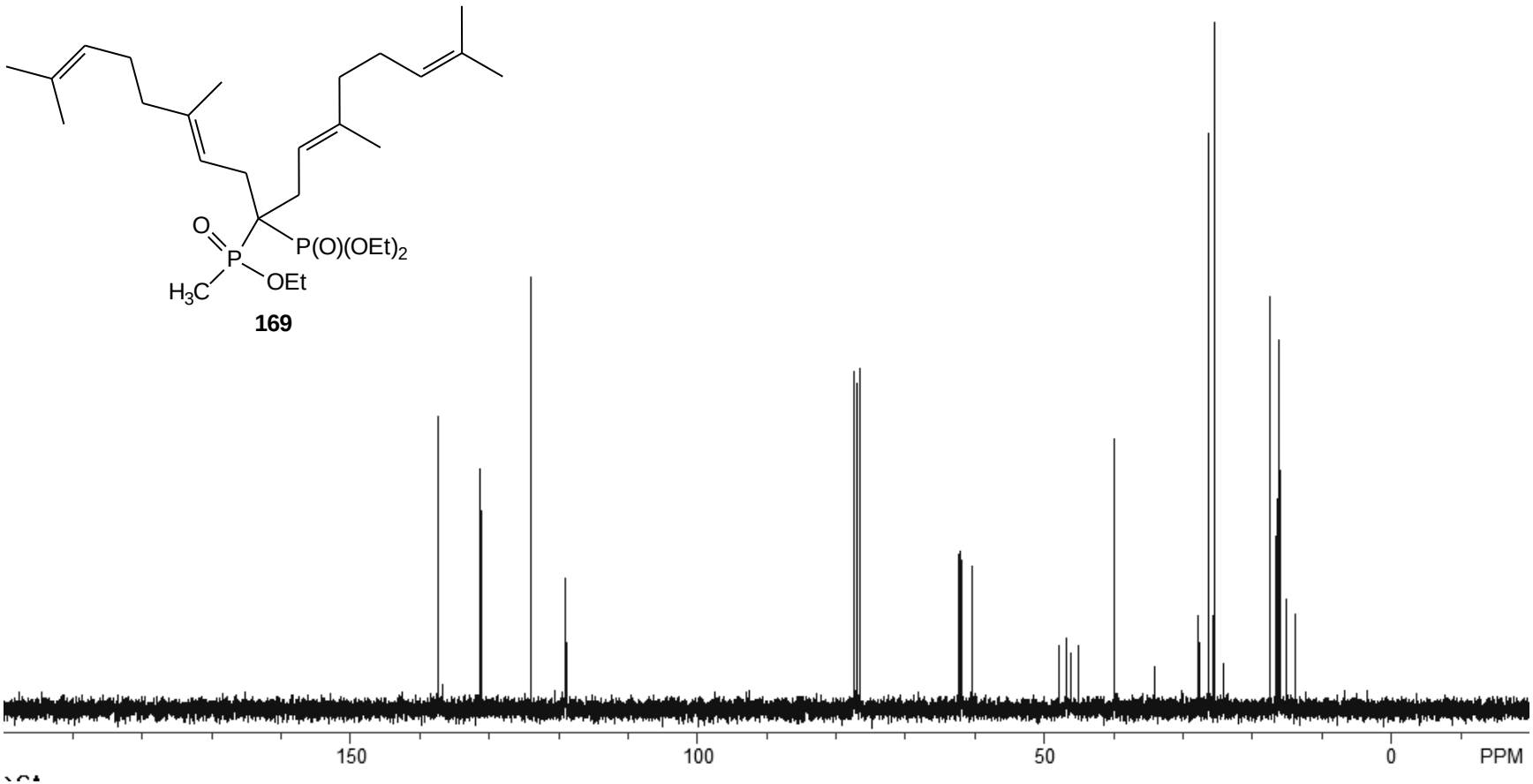


Figure A55. ^{13}C NMR spectrum for phosphonophosphinate **169**.

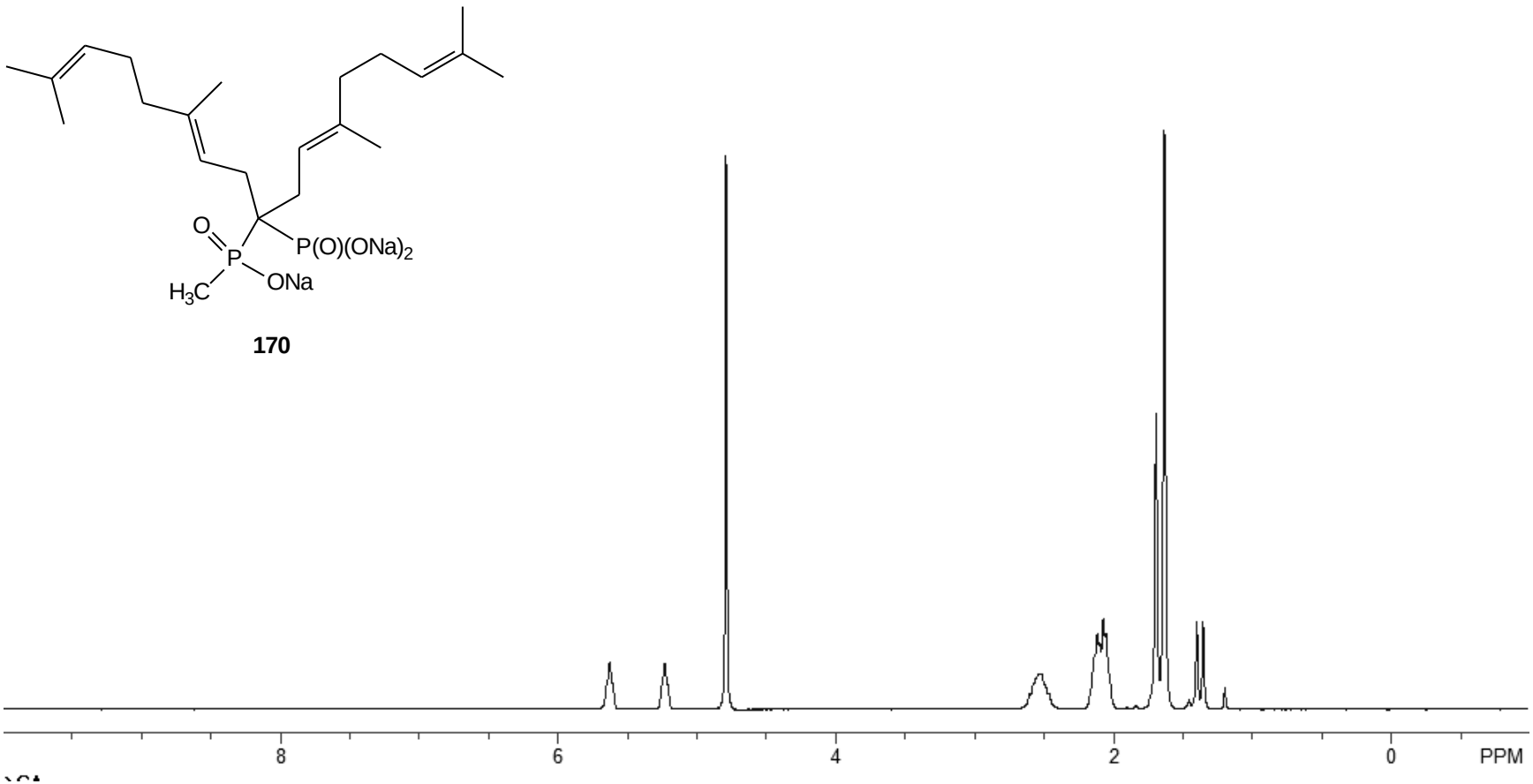
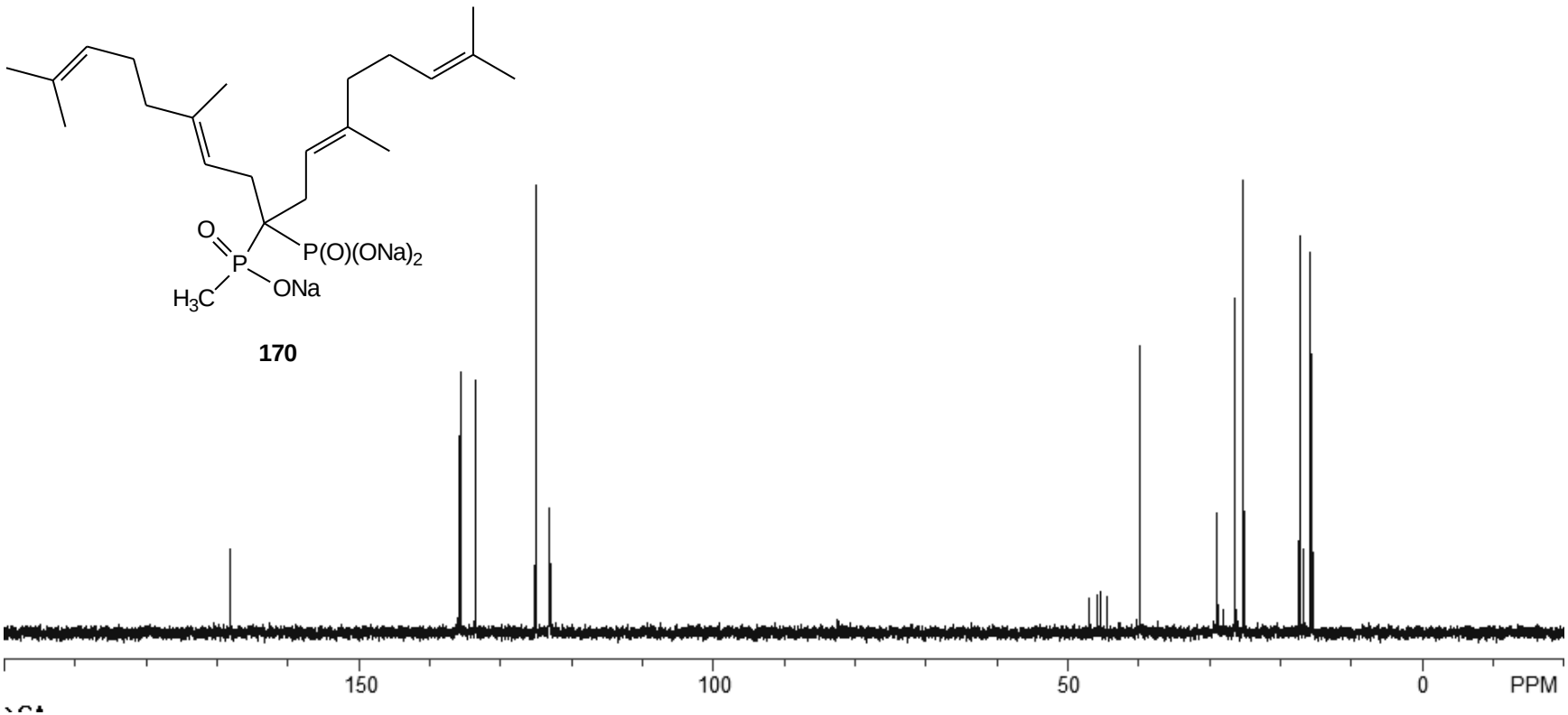


Figure A56. ^{1}H NMR spectrum for phosphonophosphinate **170**.



170

Figure A57. ^{13}C NMR spectrum for phosphonophosphinate **170**.

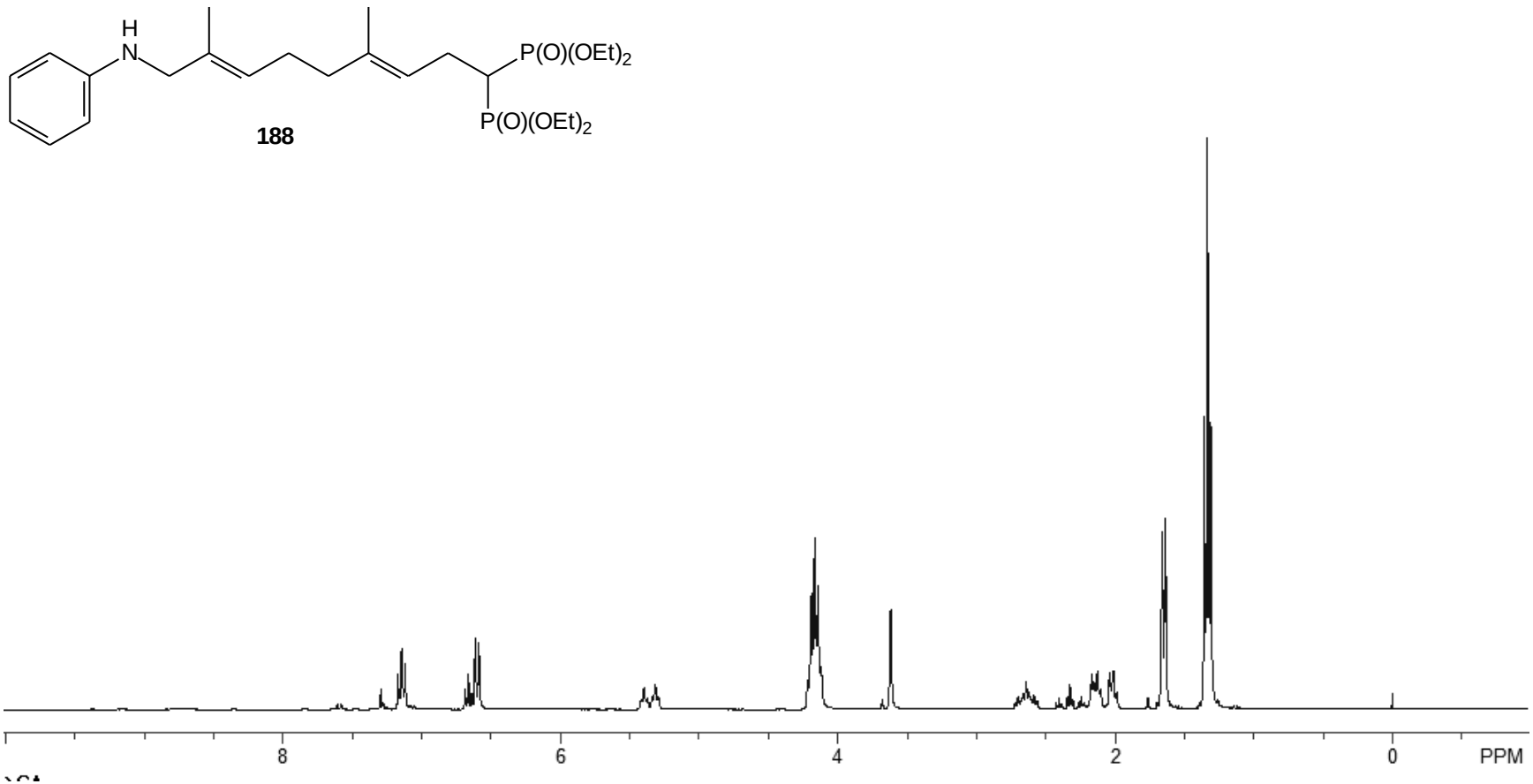


Figure A58. ^{1}H NMR spectrum for bisphosphonate **188**.

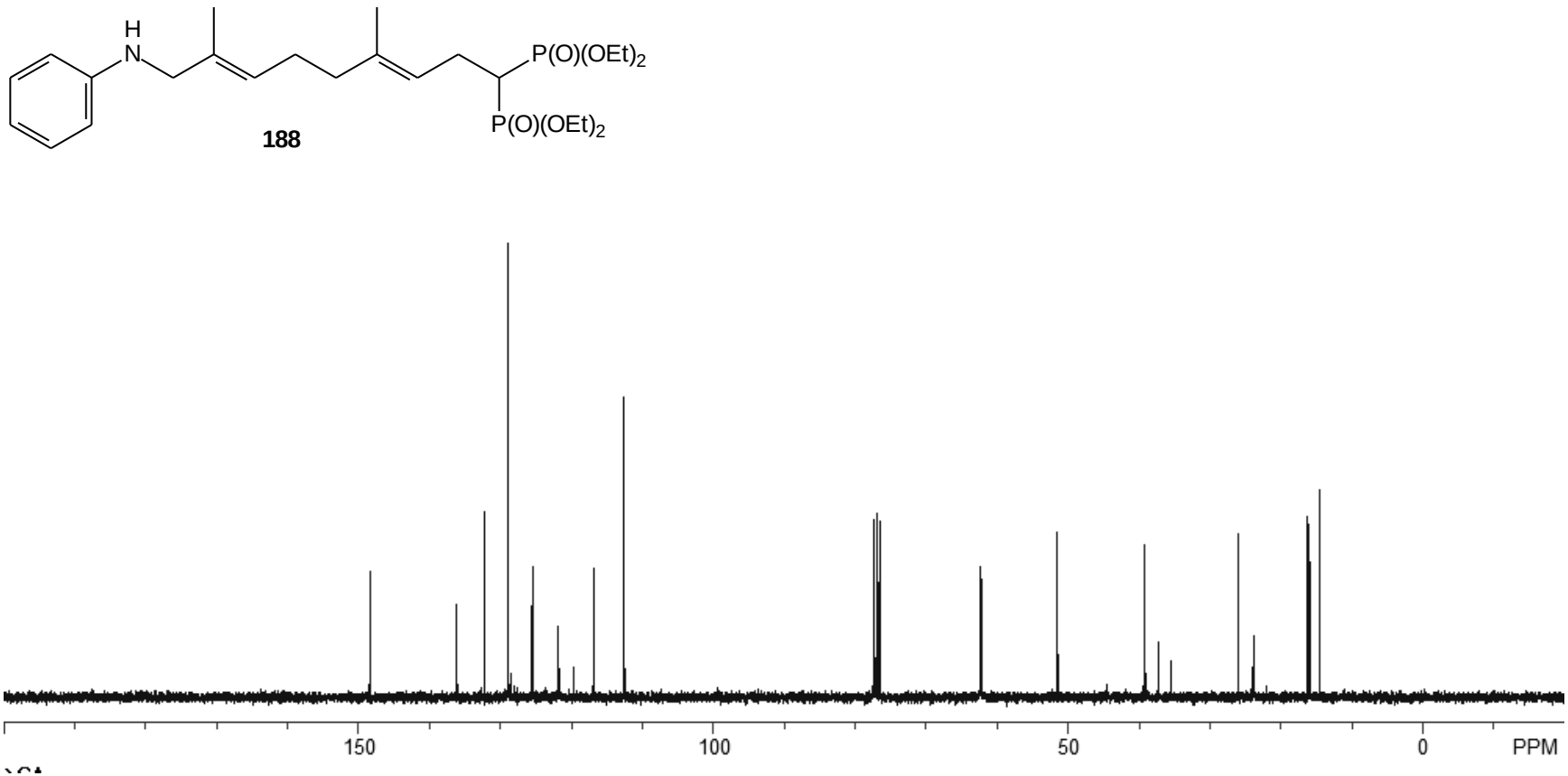


Figure A59. ^{13}C NMR spectrum for bisphosphonate **188**.

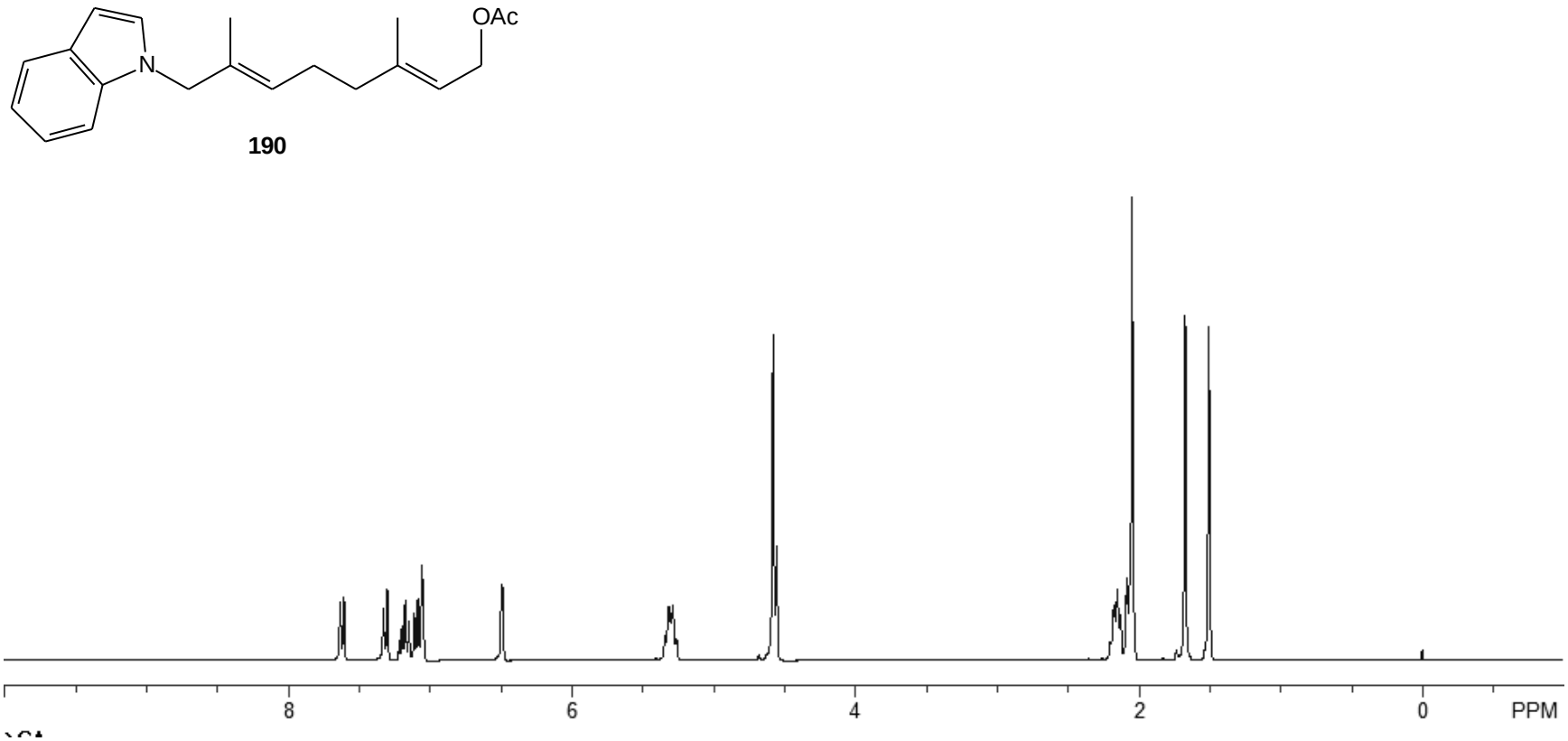
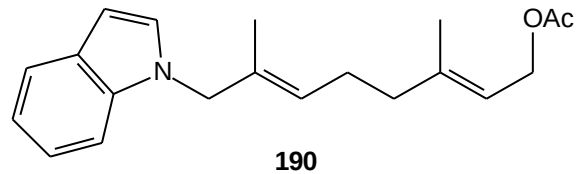


Figure A60. ^{1}H NMR spectrum for acetate **190**.



190

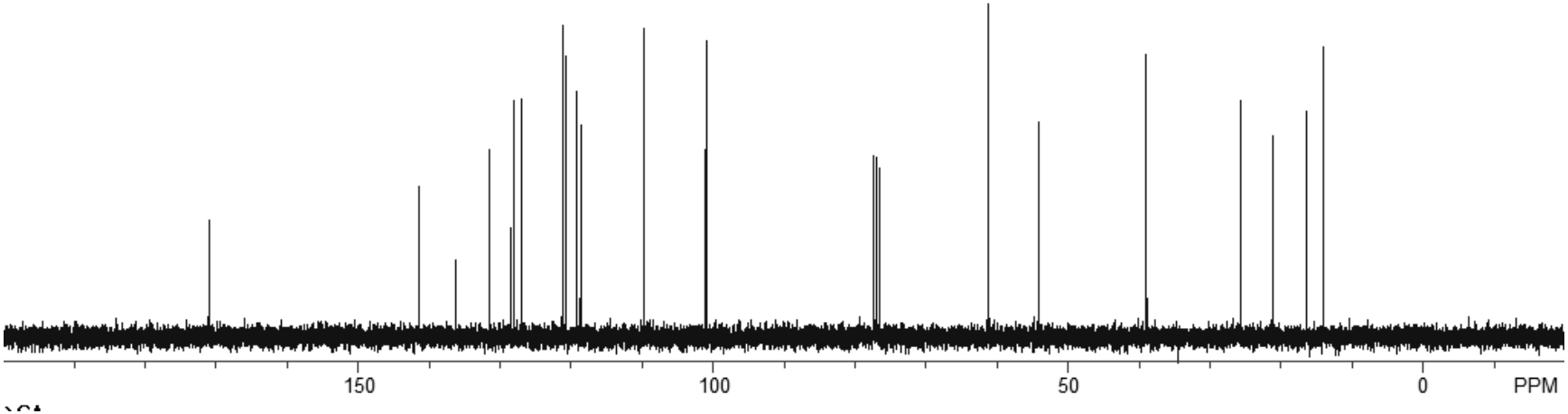


Figure A61. ^{13}C NMR spectrum for acetate **190**.

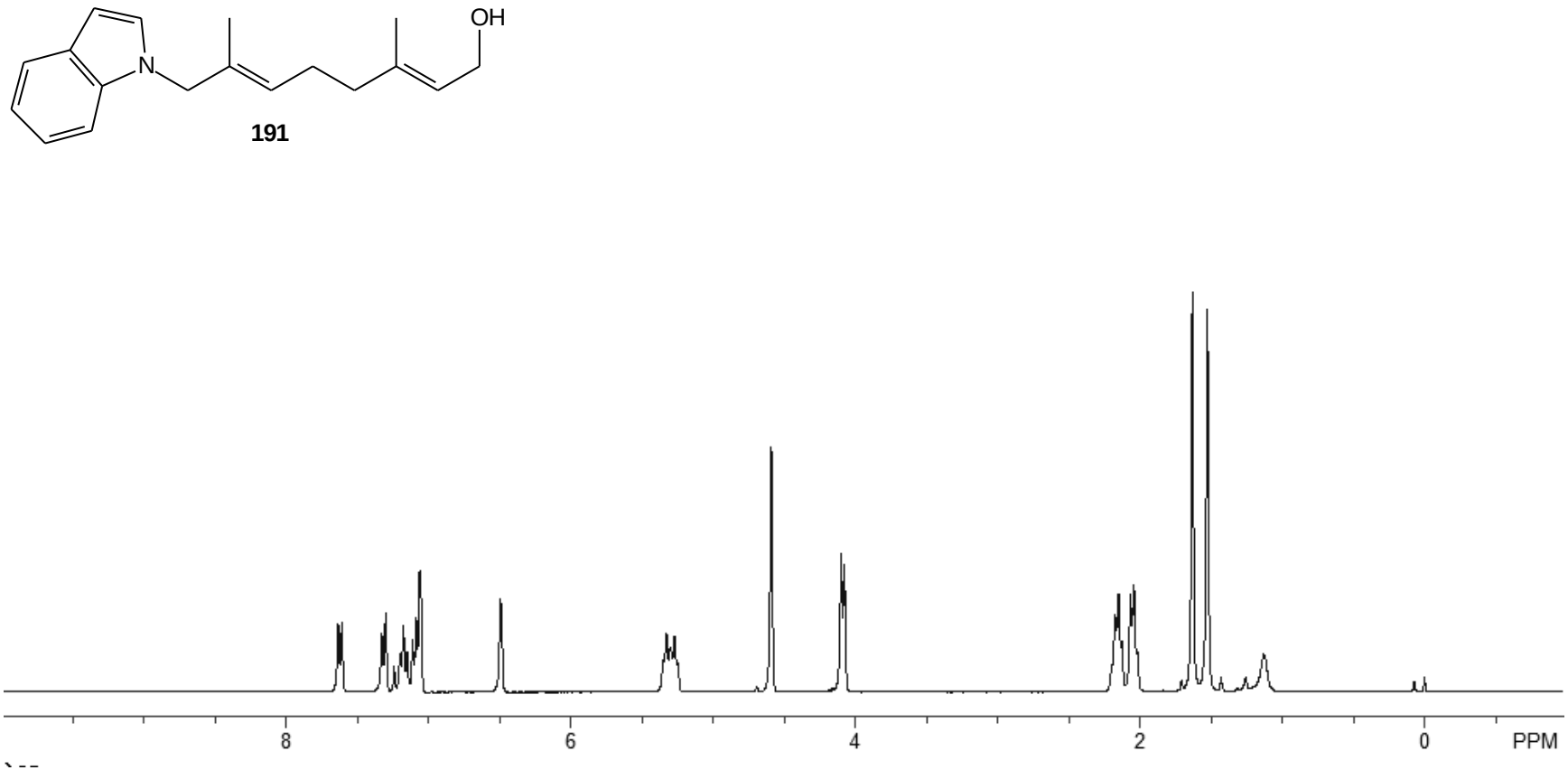


Figure A62. ^{1}H NMR spectrum for alcohol **191**.

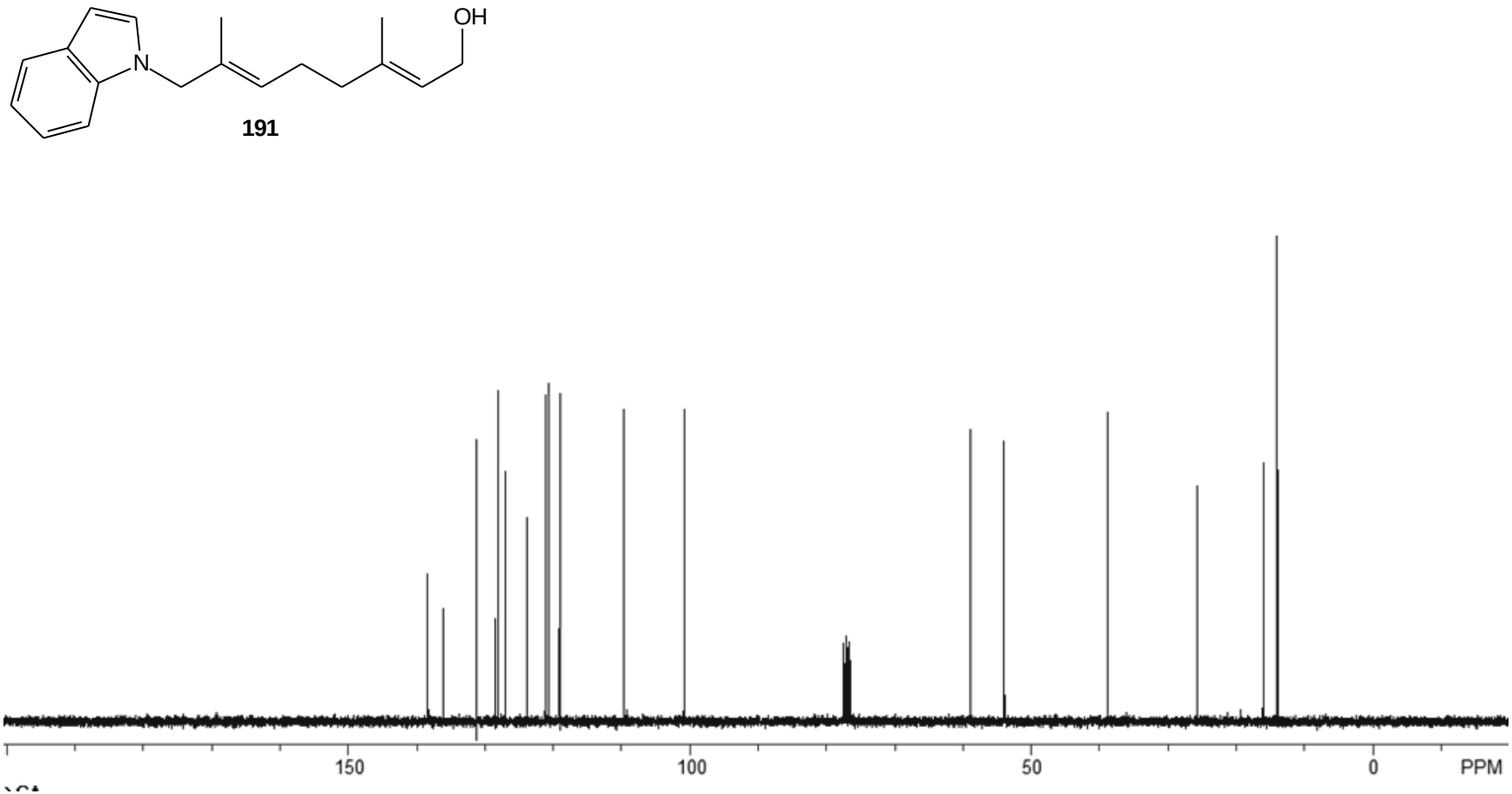


Figure A63. ^{13}C NMR spectrum for alcohol **191**.

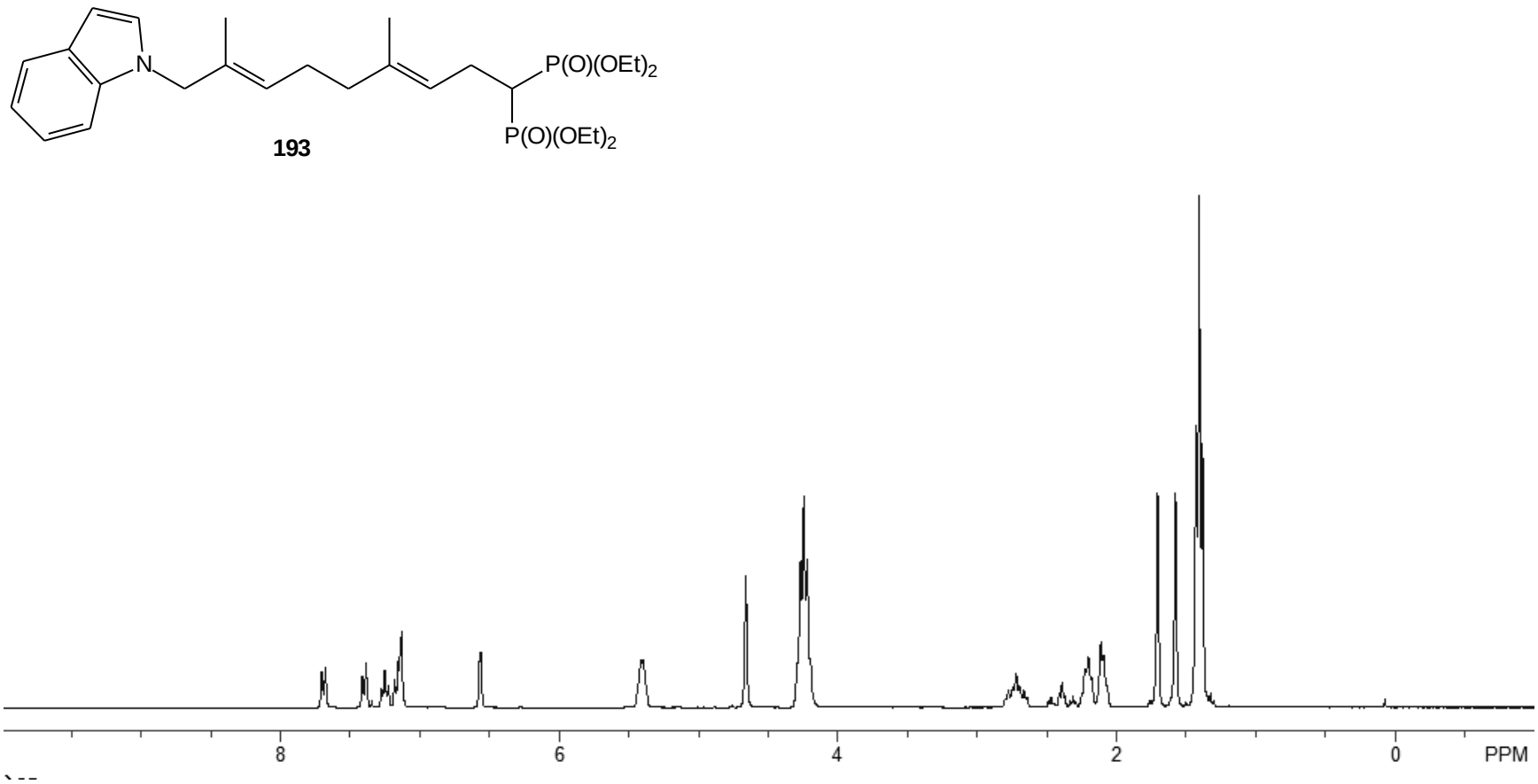
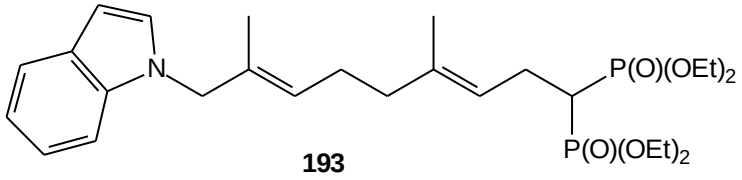


Figure A64. ^{1}H NMR spectrum for bisphosphonate **193**.



193

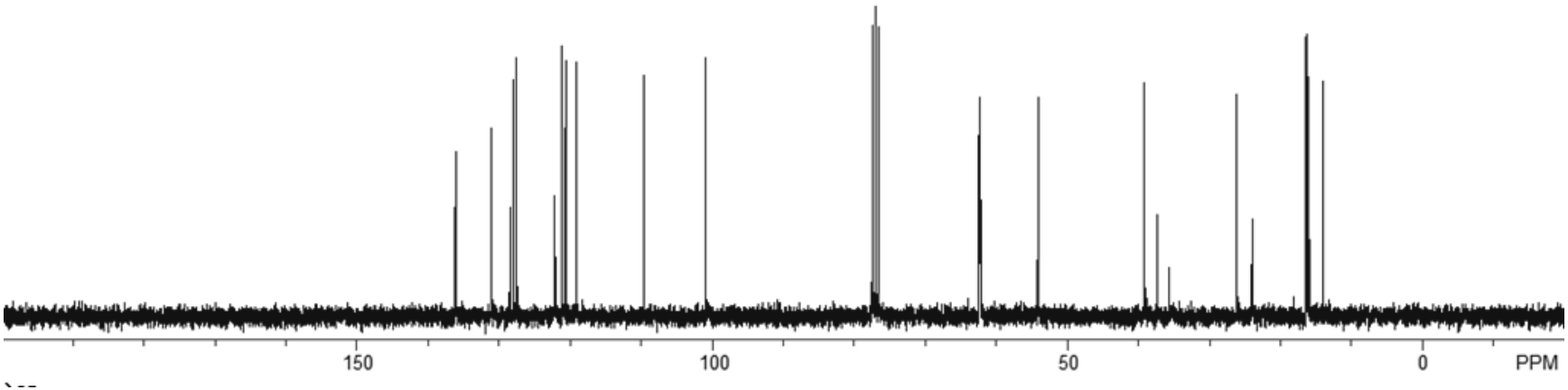


Figure A65. ^{13}C NMR spectrum for bisphosphonate **193**.

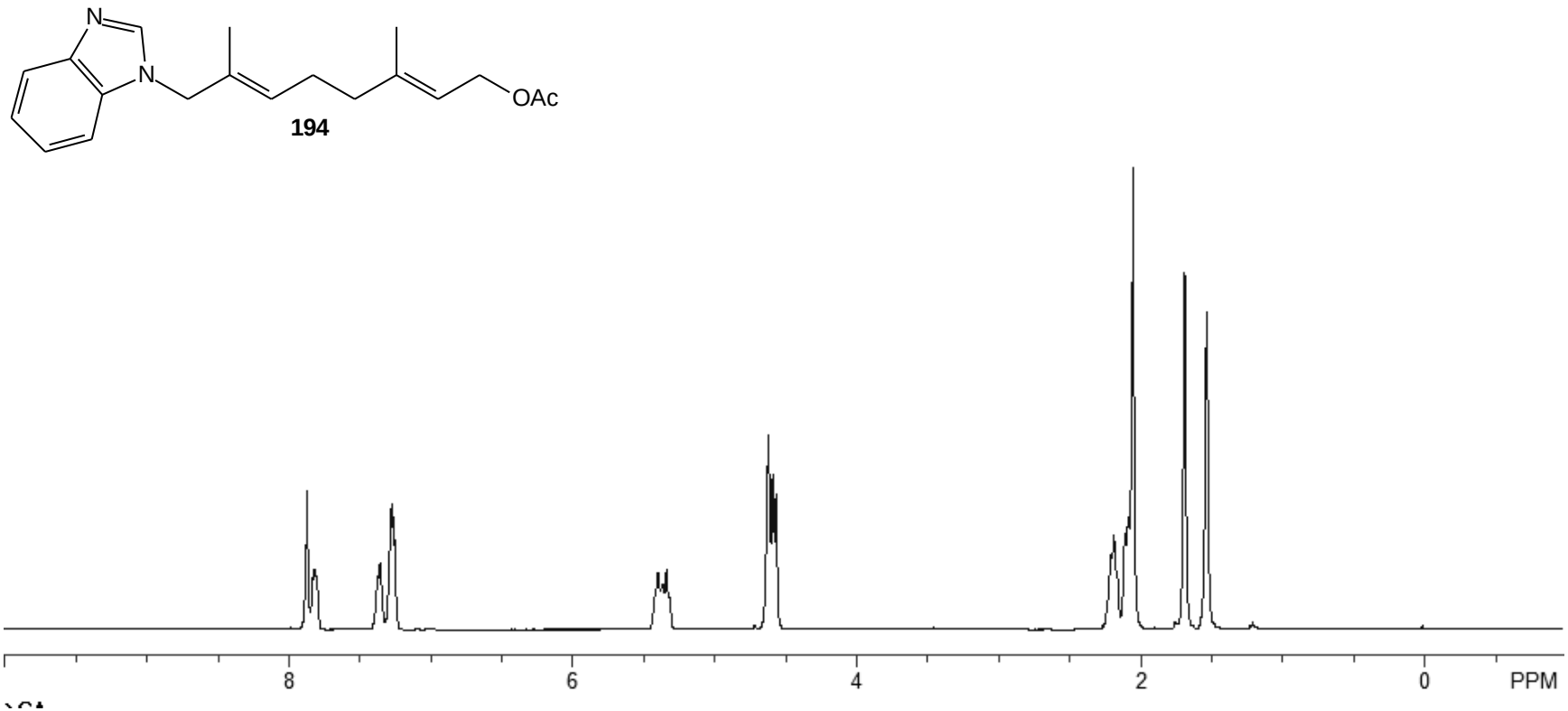


Figure A66. ^{1}H NMR spectrum for acetate **194**.

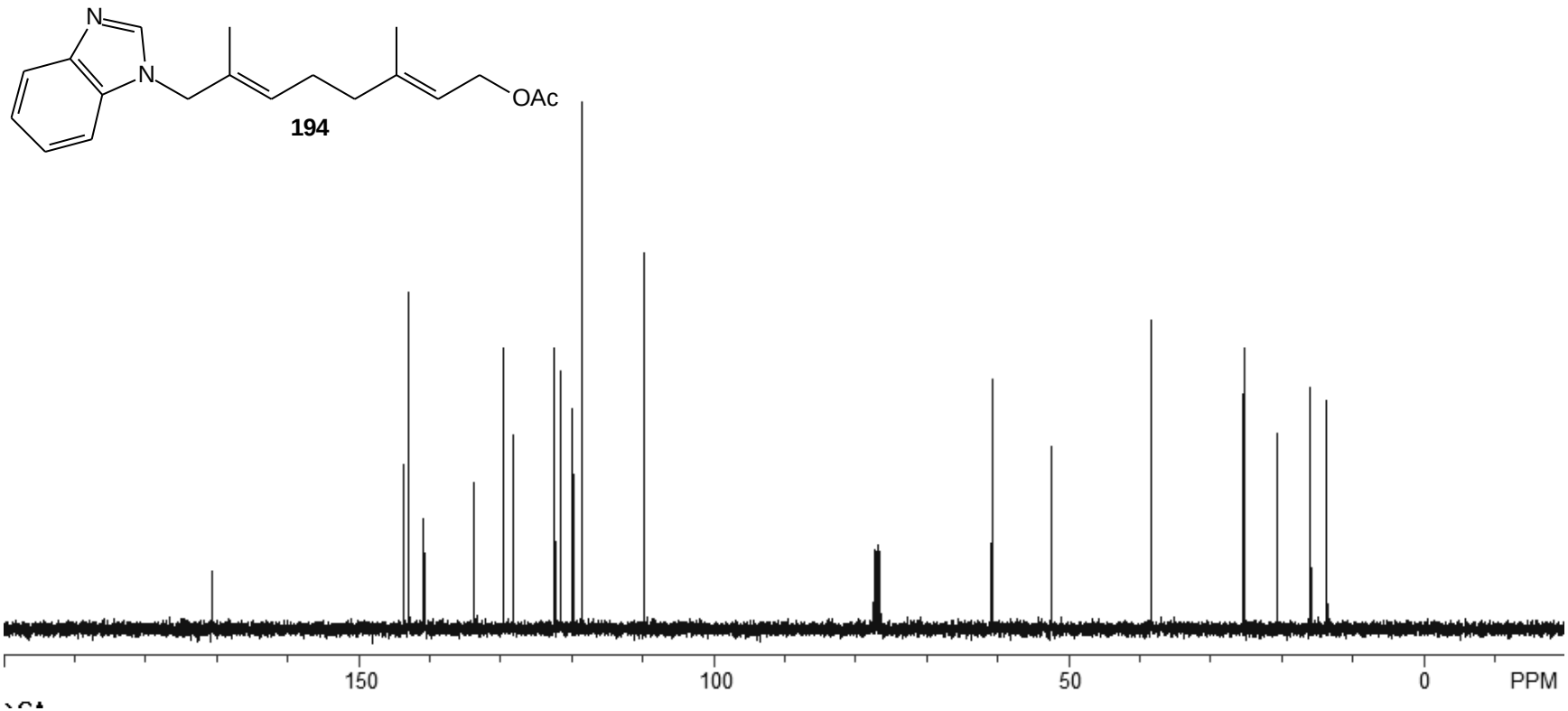


Figure A67. ^{13}C NMR spectrum for acetate **194**.

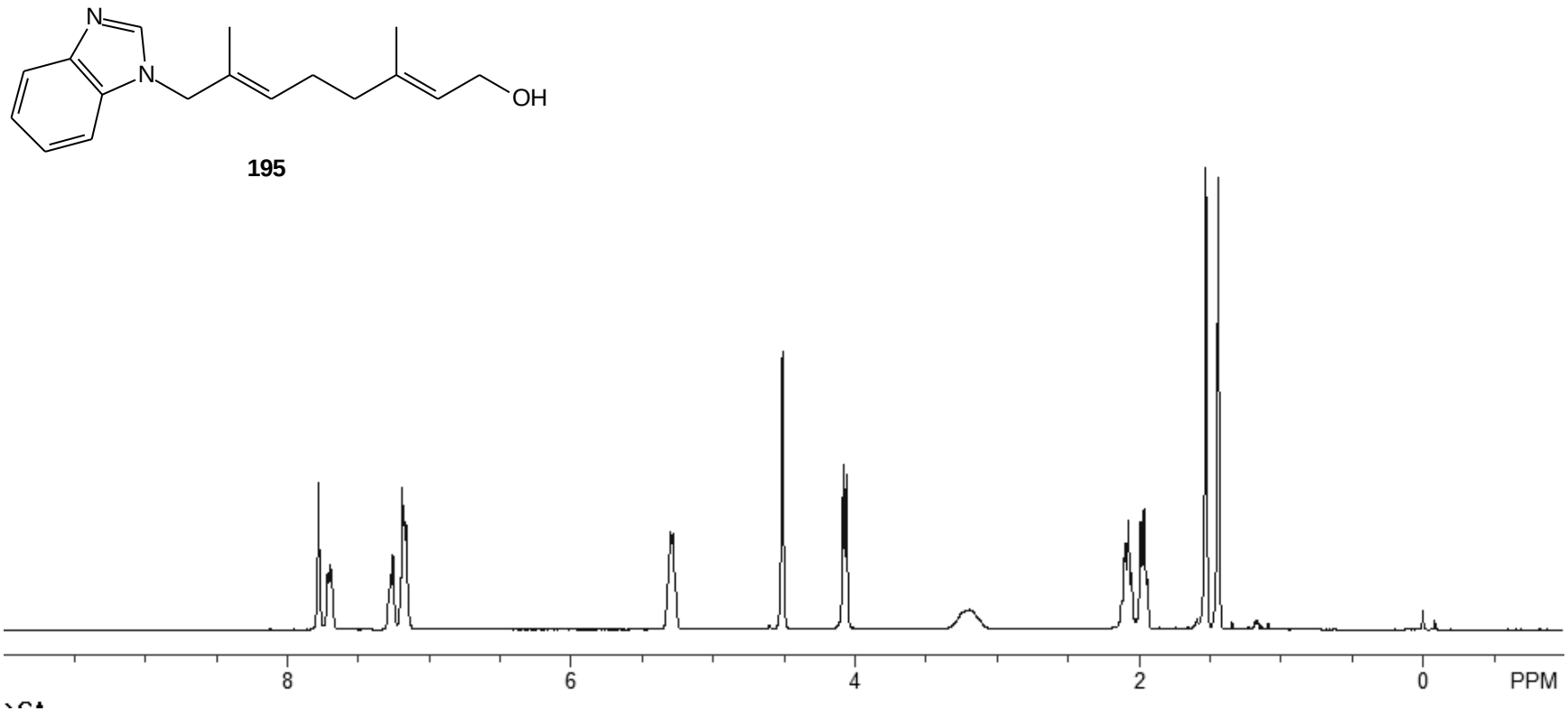


Figure A68. ^{1}H NMR spectrum for alcohol **195**.

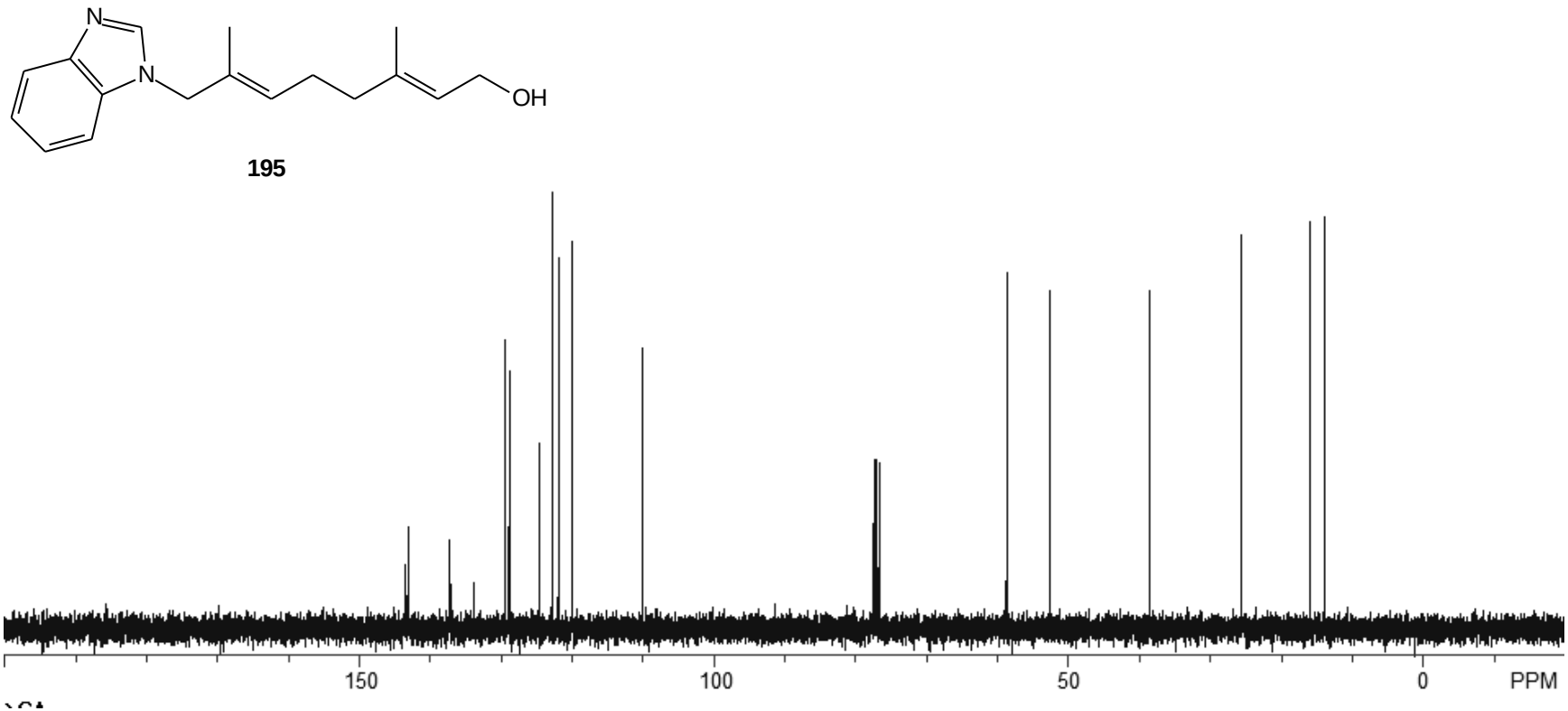


Figure A69. ^{13}C NMR spectrum for alcohol **195**.

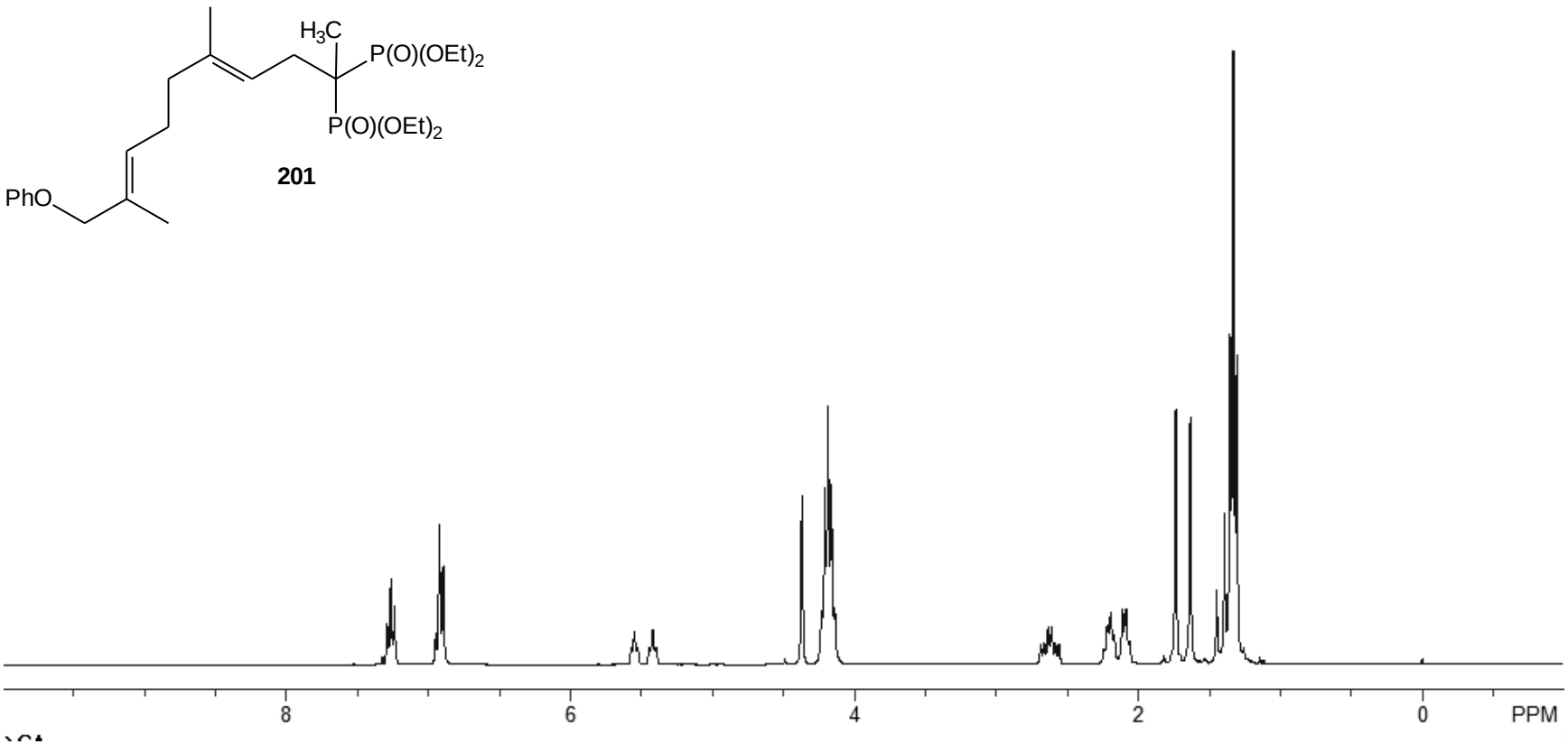


Figure A70. ^{1}H NMR spectrum for bisphosphonate **201**.

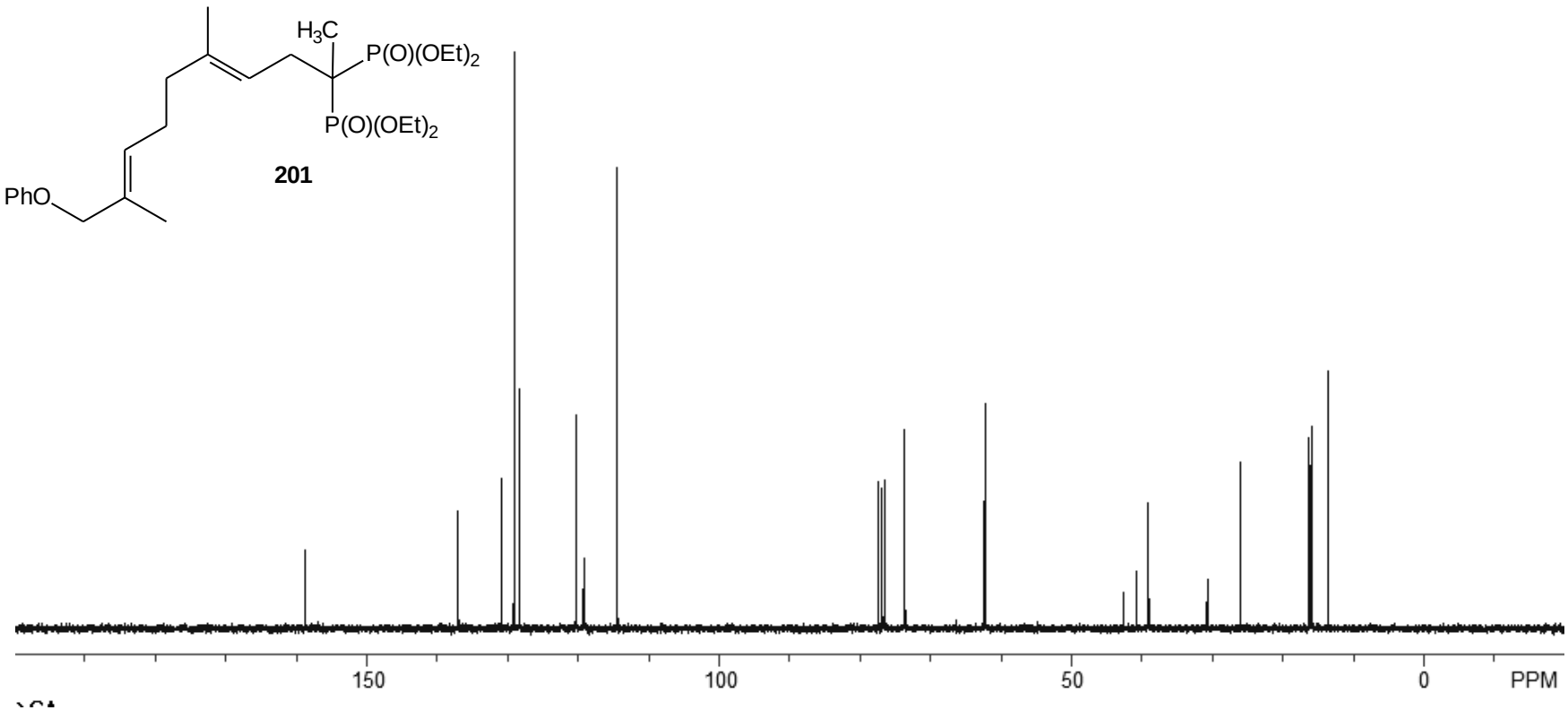


Figure A71. ^{13}C NMR spectrum for bisphosphonate **201**.

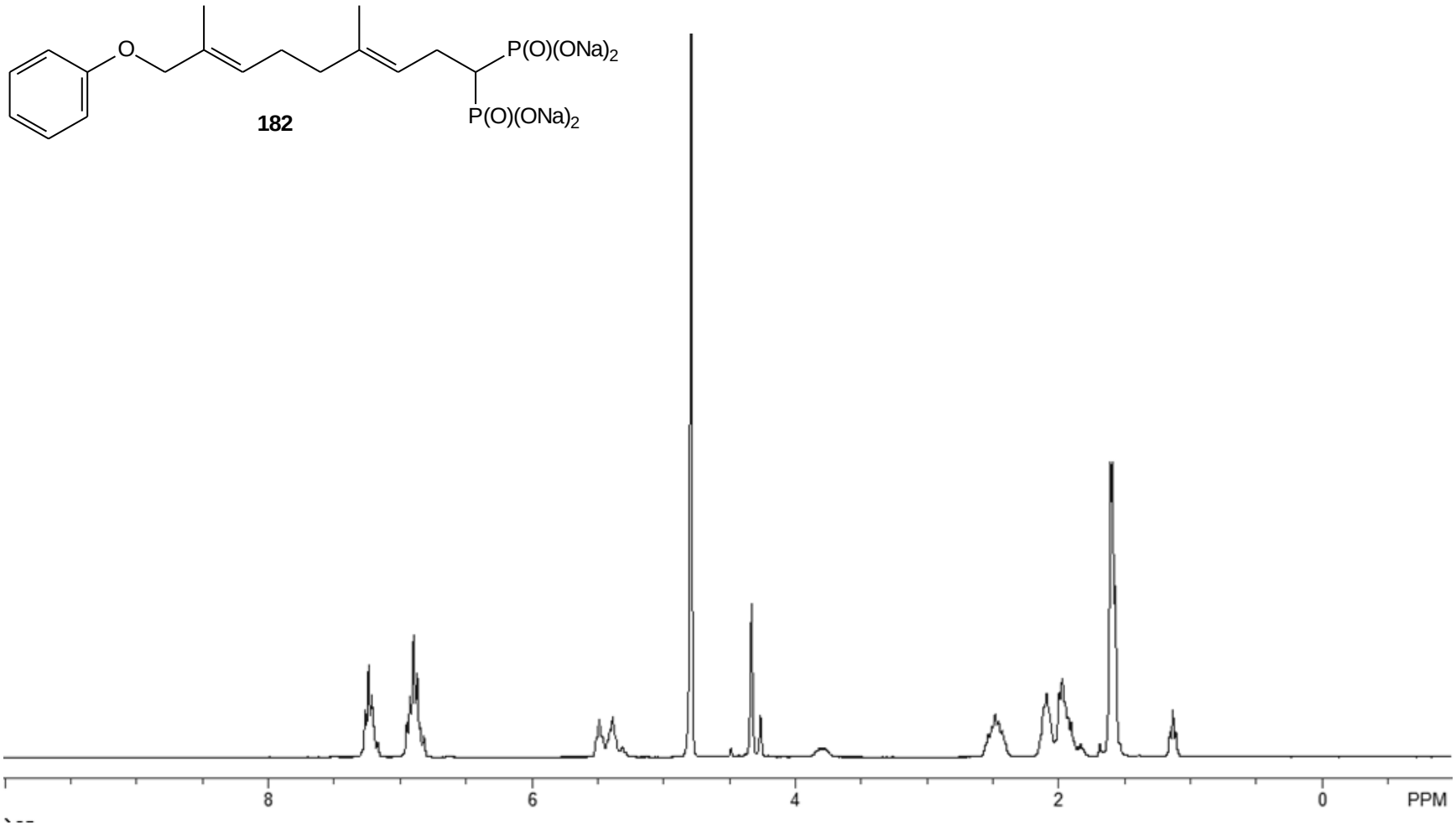


Figure A72. ^{1}H NMR spectrum for bisphosphonate **182**.

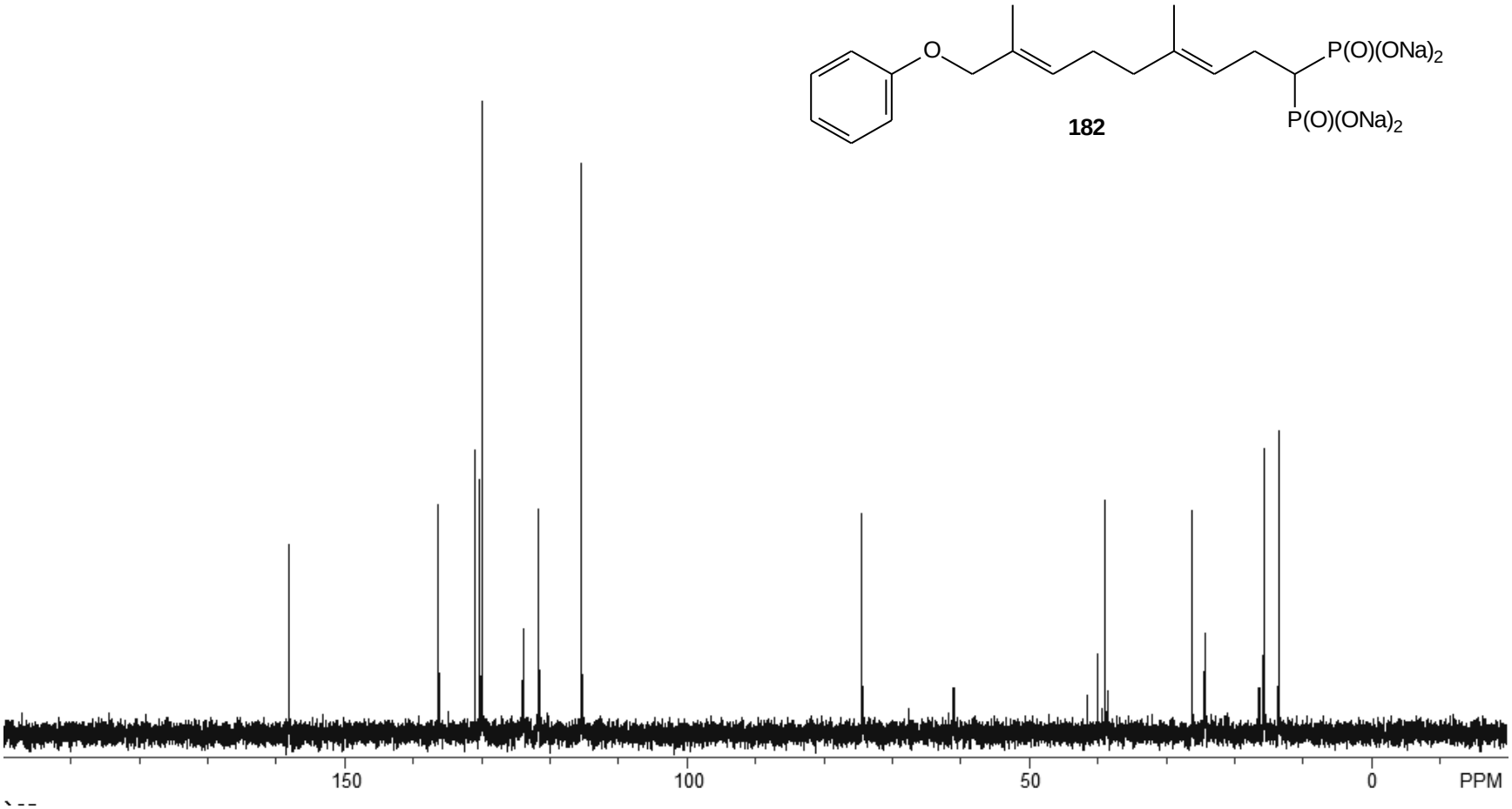


Figure A73. ^{13}C NMR spectrum for bisphosphonate **182**.

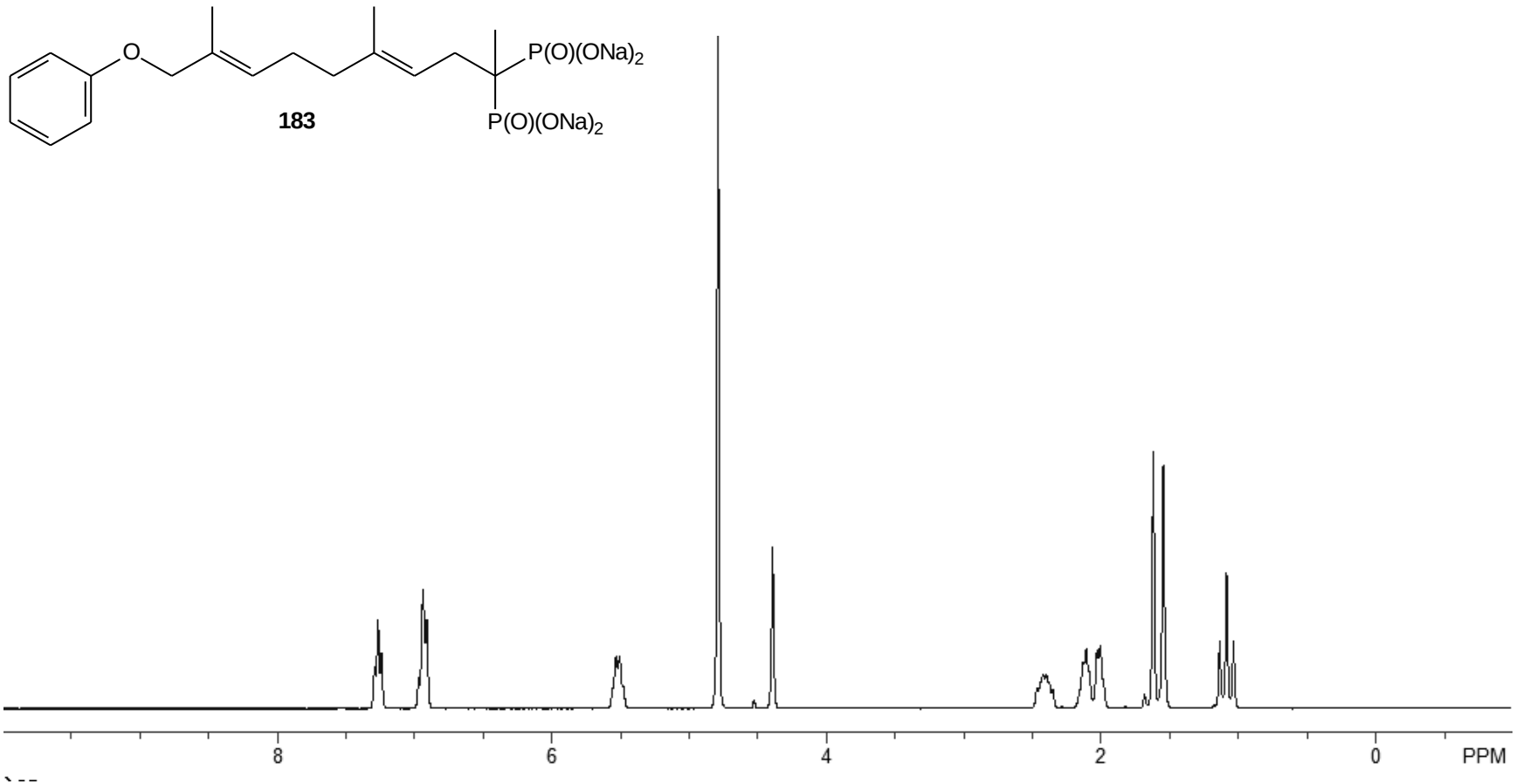


Figure A74. ^{1}H NMR spectrum for bisphosphonate **183**.

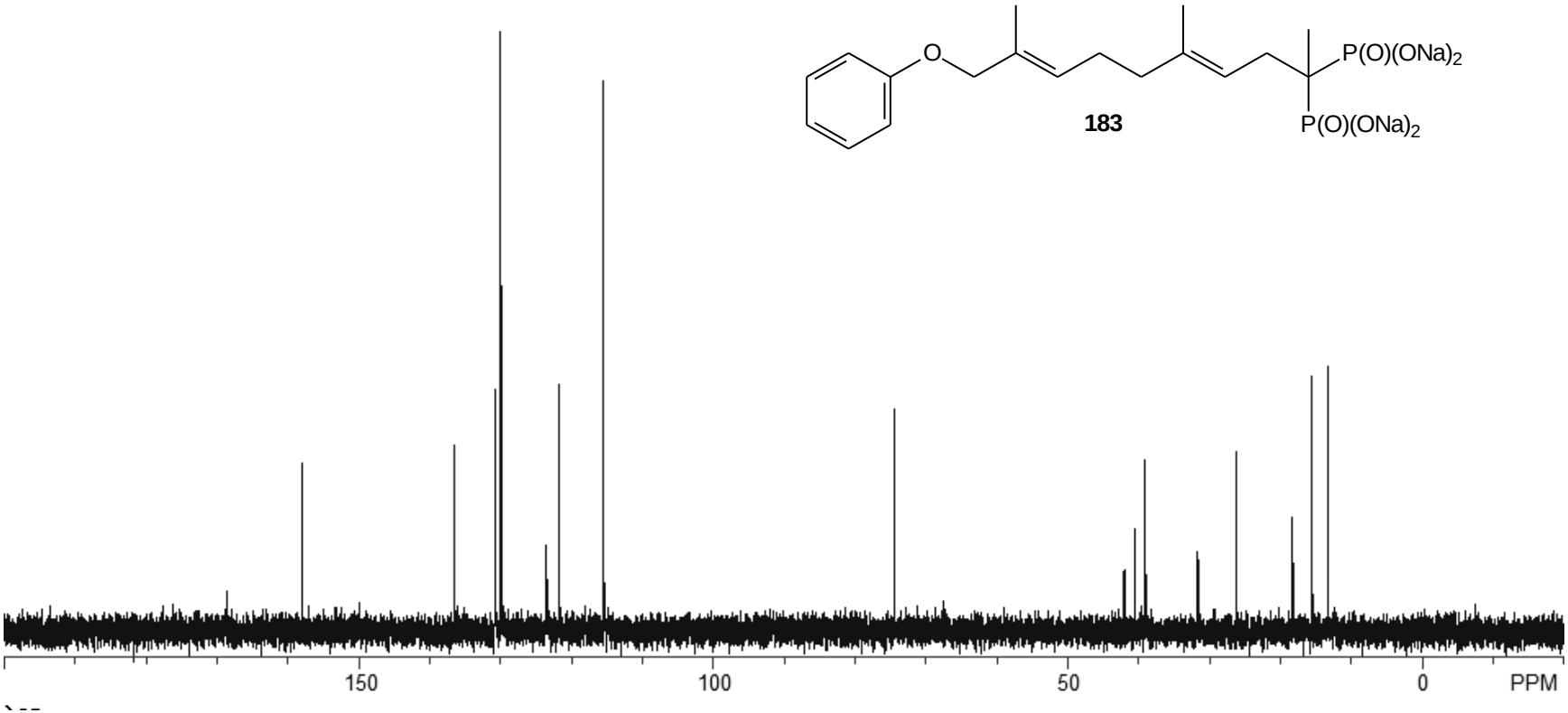


Figure A75. ^{13}C NMR spectrum for bisphosphonate **183**.

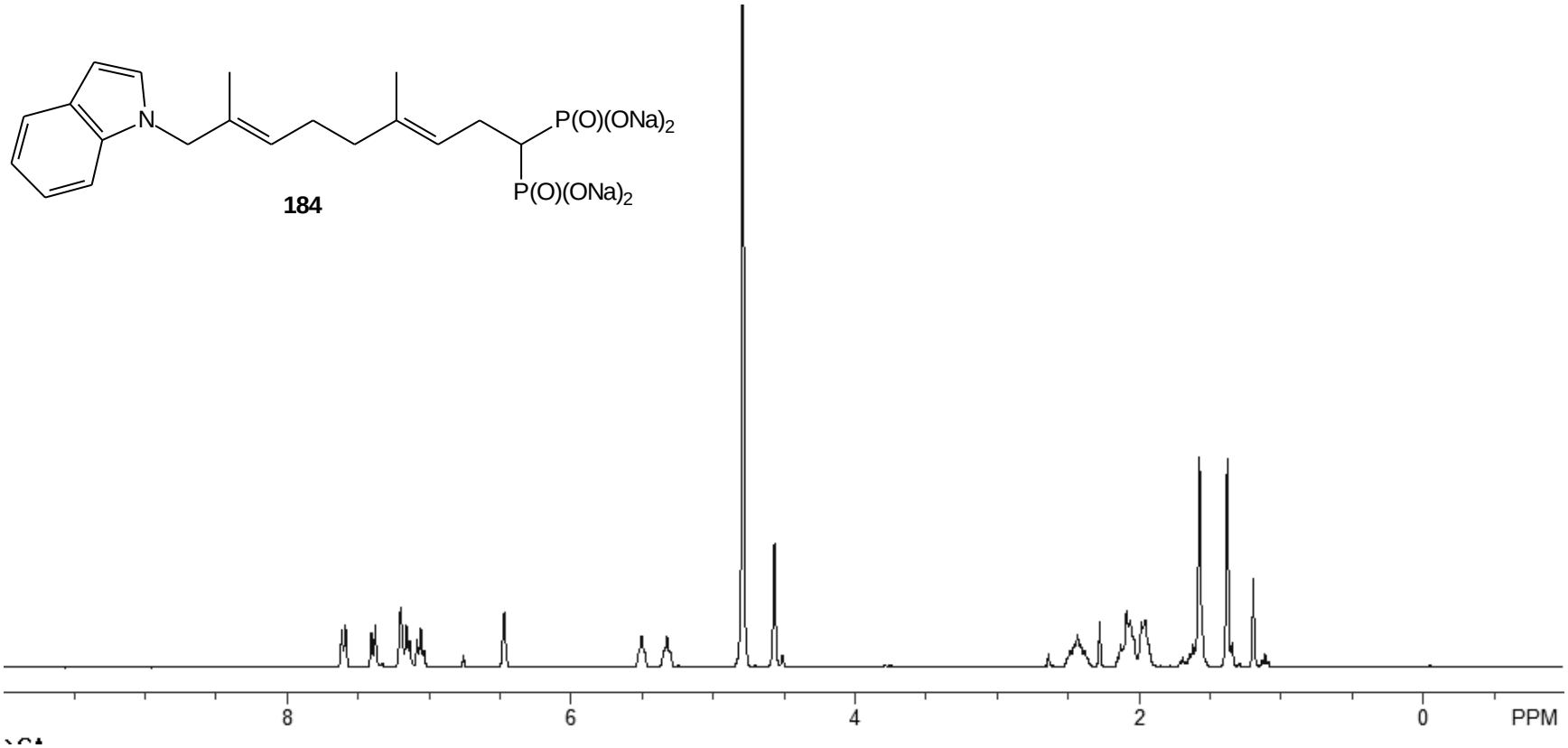
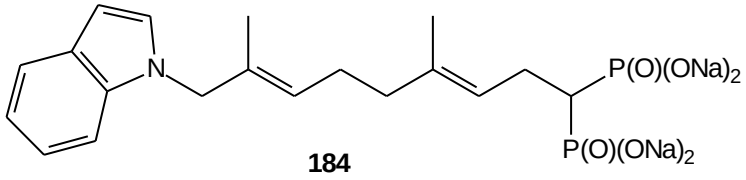


Figure A76. ^{1}H NMR spectrum for bisphosphonate **184**.



184

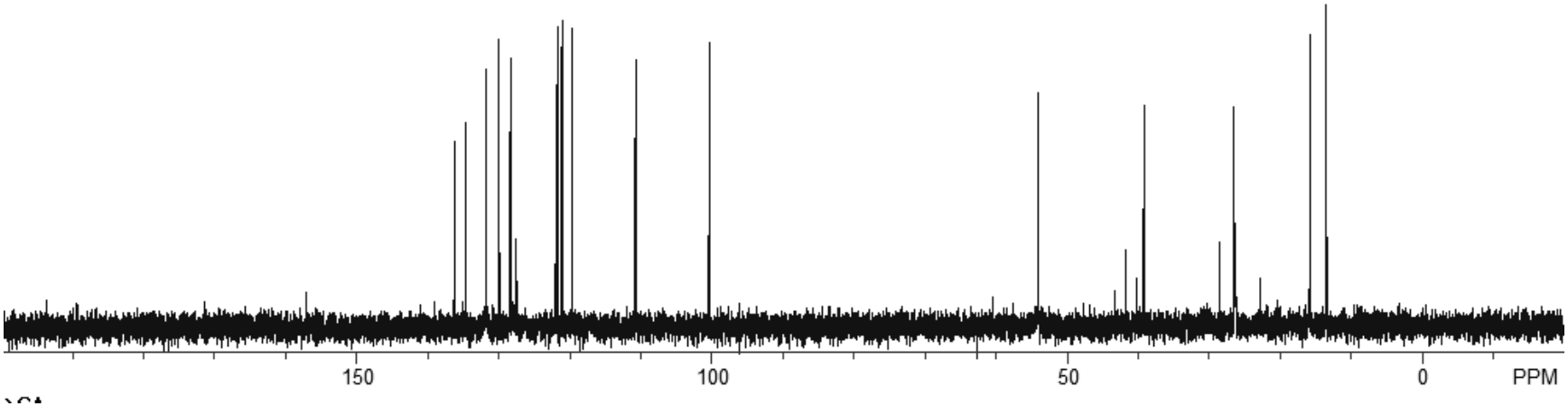


Figure A77. ^{13}C NMR spectrum for bisphosphonate **184**.

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